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(54) **TREATMENT OF FIBROSIS WITH GENETICALLY-ENGINEERED MACROPHAGES**

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(52) **U.S. Cl.**

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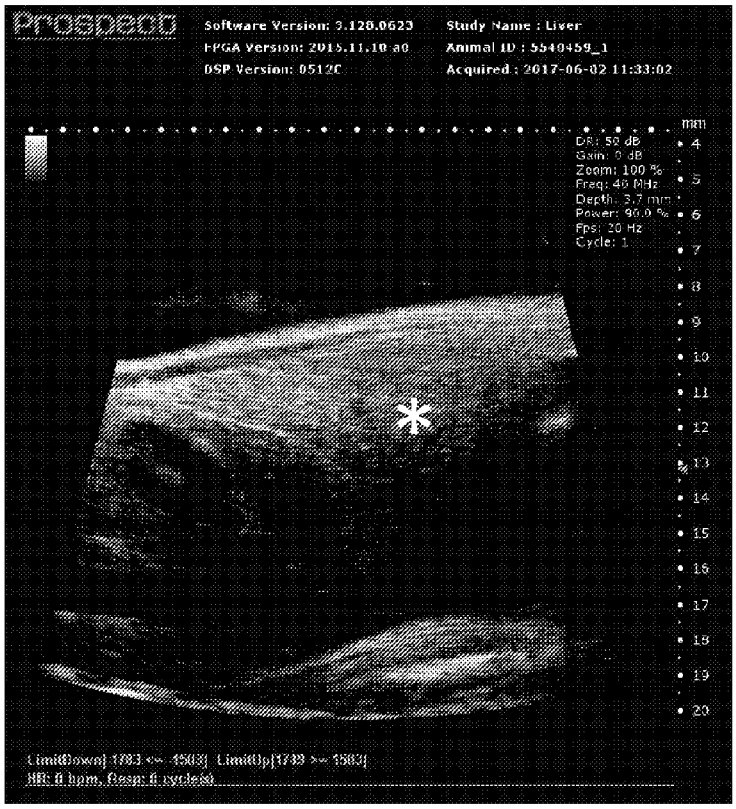
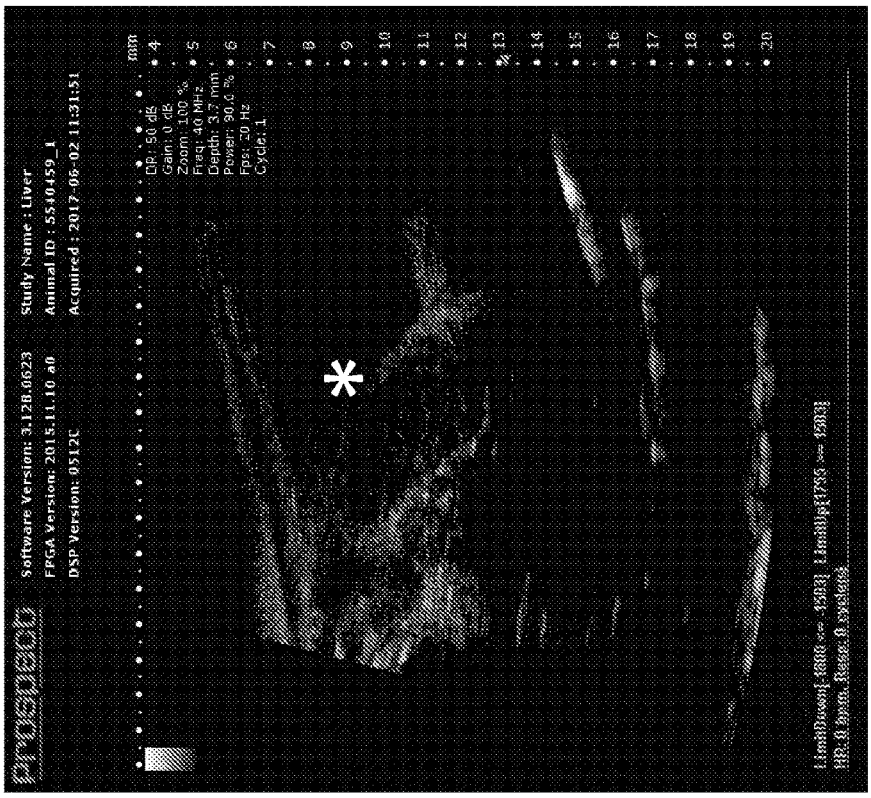
(57) **ABSTRACT**

Provided herein are macrophages engineered for treating fibrosis and ameliorating the effects of fibrotic lesions in various organs and tissues. Certain embodiments are directed to genetically-engineered macrophages capable of treating fibrosis or reducing fibrotic lesions. In certain aspects macrophages can be genetically-engineered to (1) target extracelluar matrix (ECM) or components thereof, (2) enhance degradation of ECM, or (3) target ECM and enhance degradation of ECM. Further provided is a cellular therapy product comprising a genetically-engineered macrophage comprising at least one of a recombinant targeting protein and a recombinant catalytic enzyme. Further provided is a method of treating an individual for fibrosis comprising administering the cellular therapy product.

Specification includes a Sequence Listing.



(A)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FPGA Version: 2015.11.10 a0
DSP Version: 0512C
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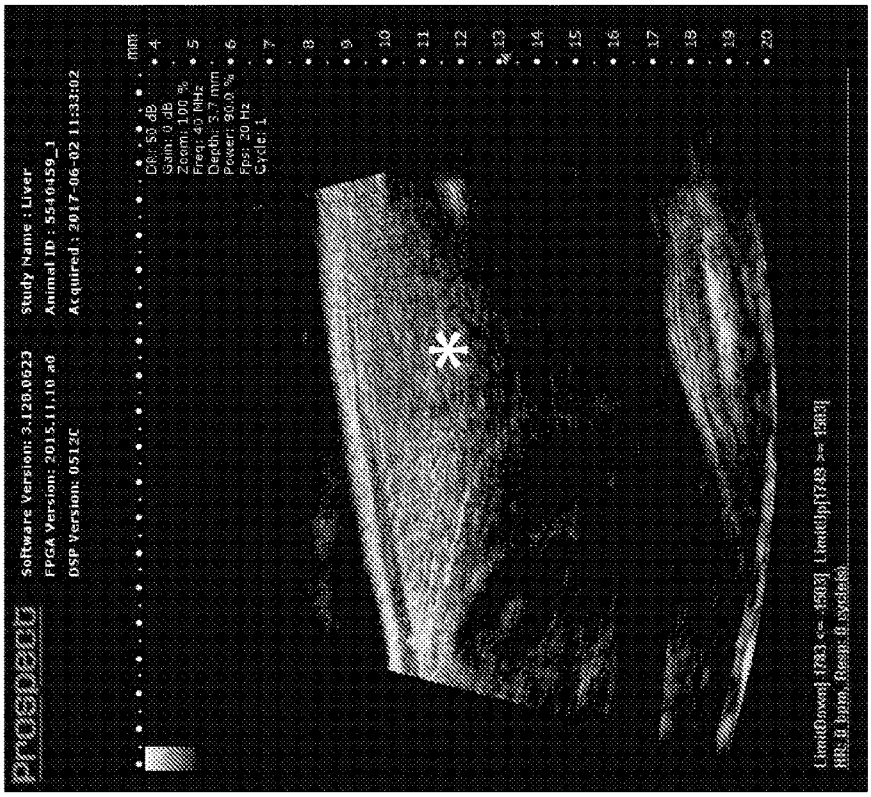
Gain: 0 dB
Zoom: 100 %
Freq: 40 MHz
Depth: 3.7 mm
Power: 90.0 %
Fps: 20 Hz
Cycle: 1
mm
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20

(B)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Fps: 20 Hz
Cycle: 1
mm
4
5
6
7
8
9
10
11
12
13
14
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16
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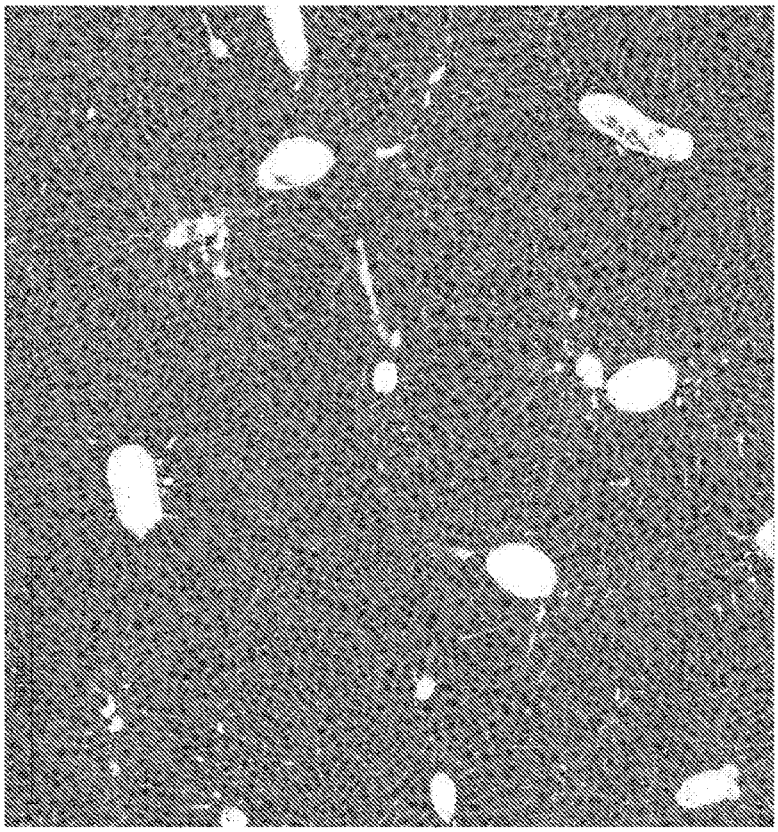
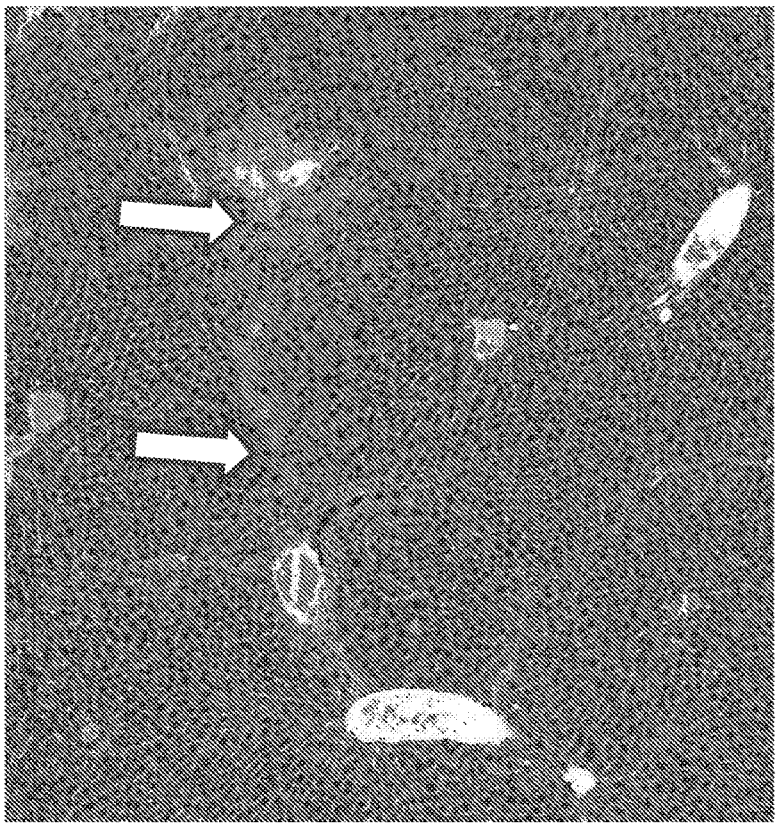
18
19
20

(A)

FIGS. 1A-B

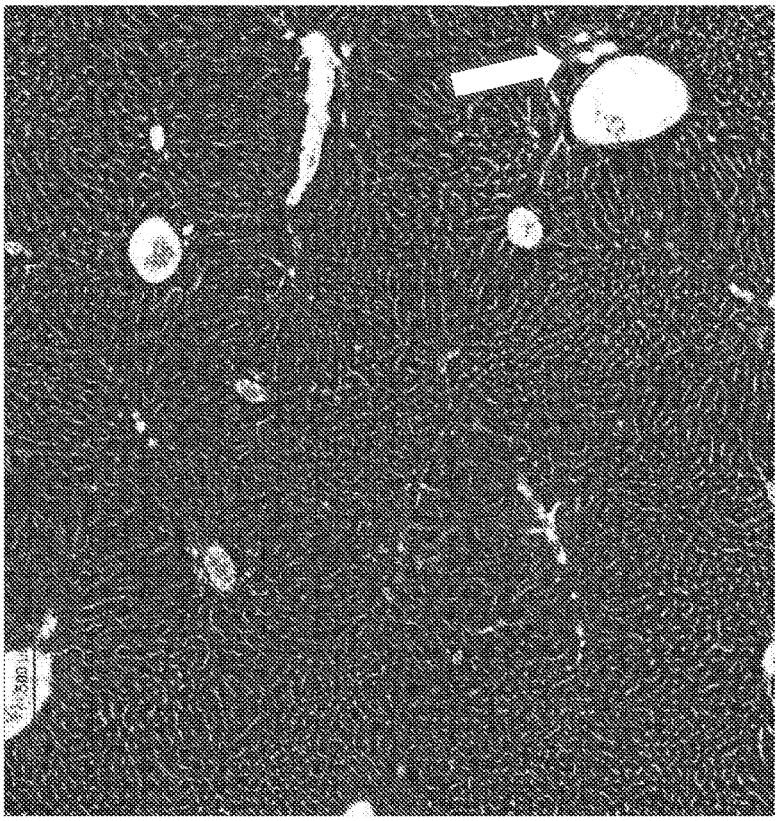


(B)

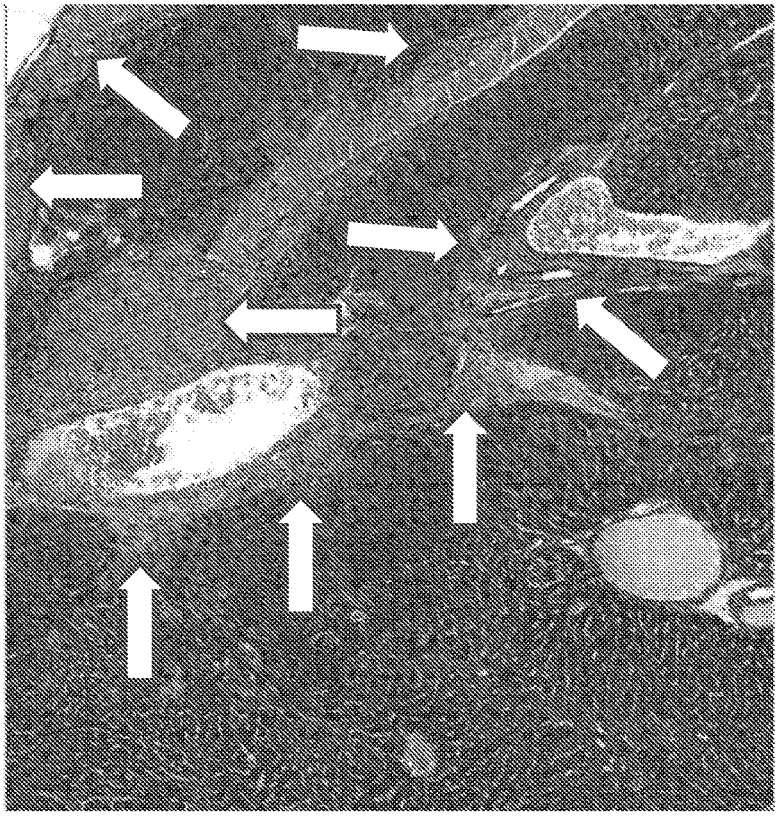


(A)

FIGs. 2A-B

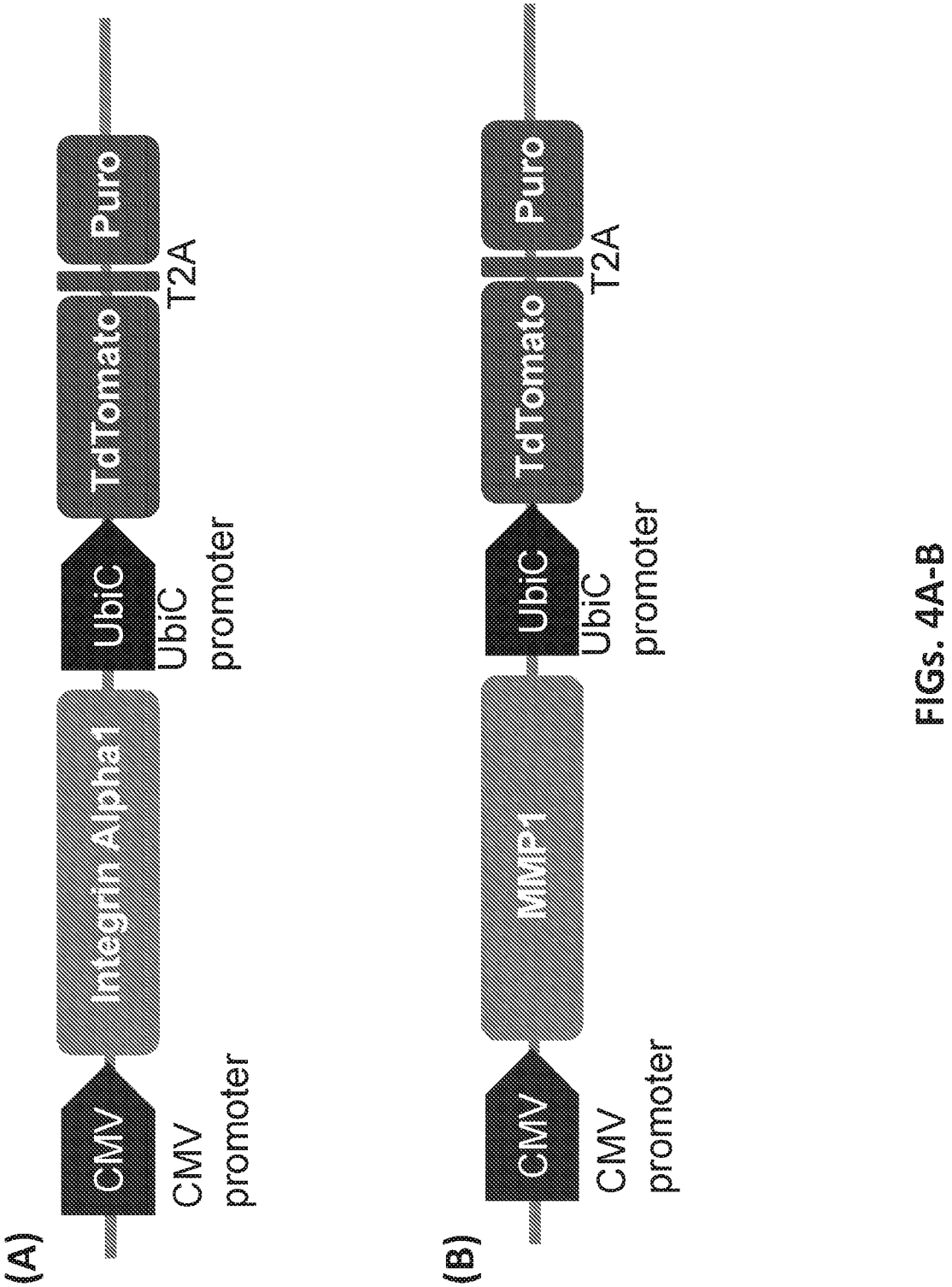


(B)

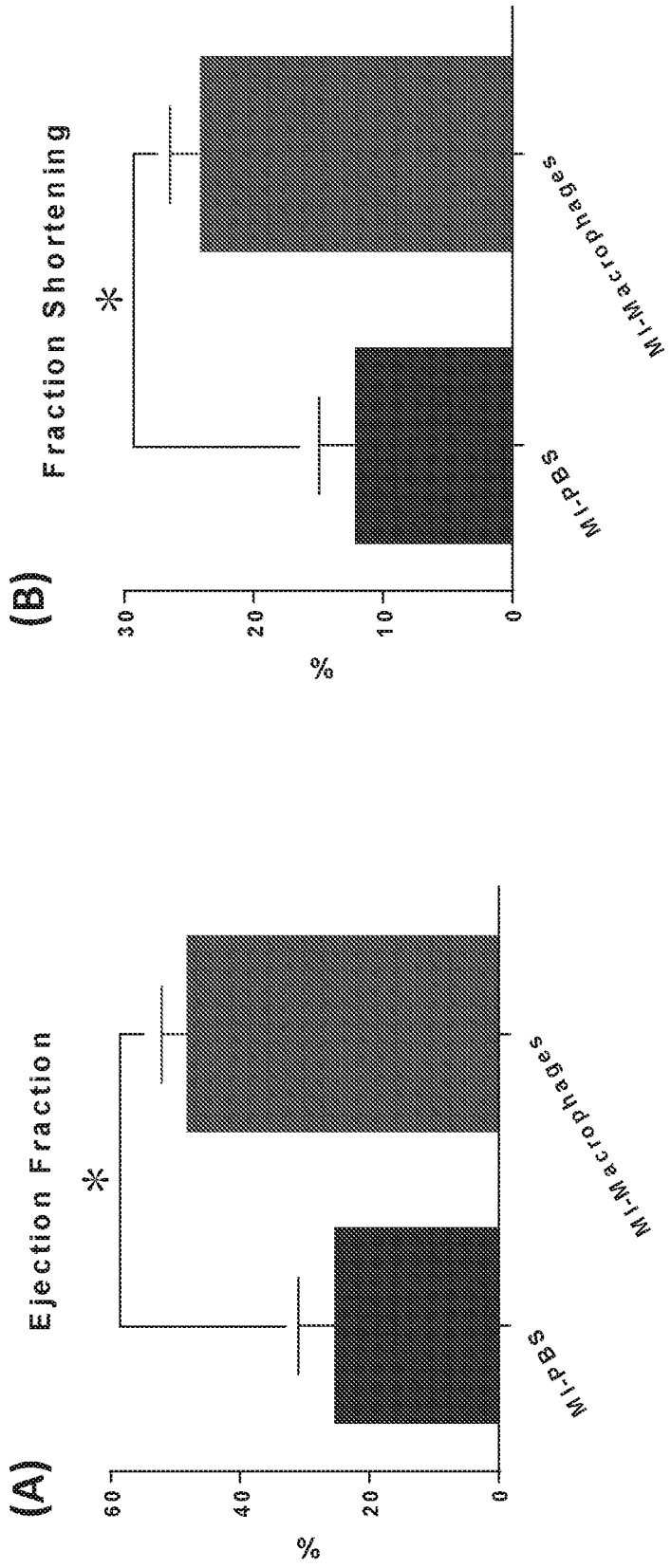


(A)

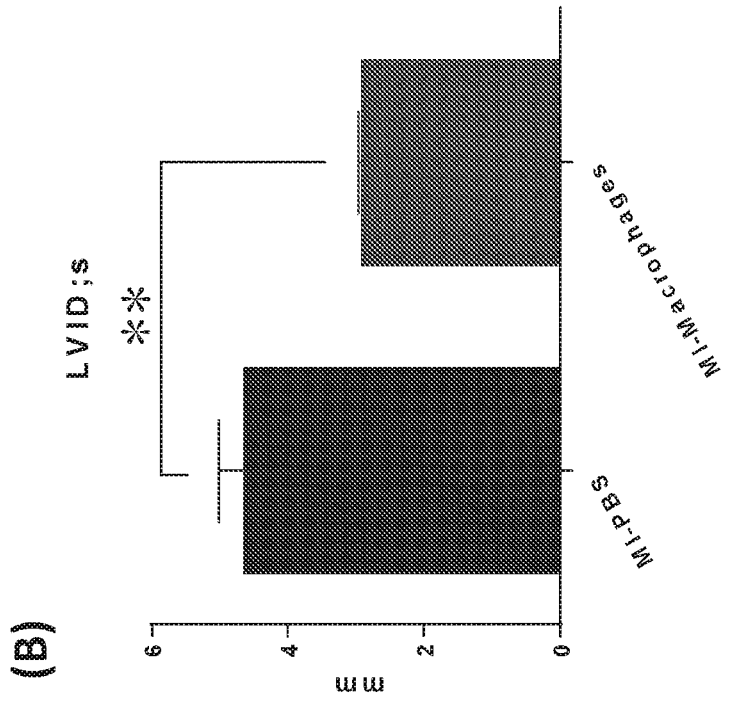
FIGs. 3A-B

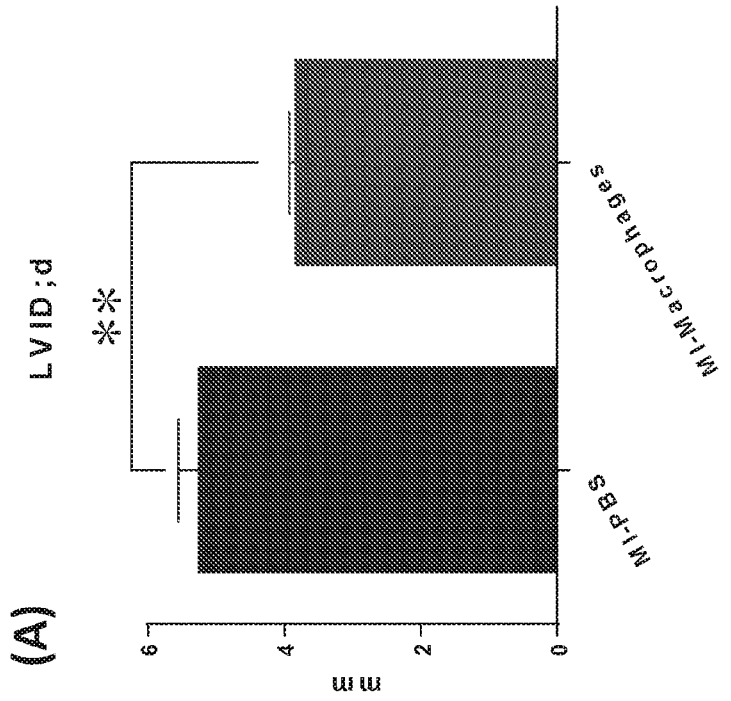

(A)
CMV
CMV promoter
Integrin Alpha1
UbiC
UbiC promoter
TdTomato
T2A
Puro
(B)
CMV
CMV promoter
MMP1
UbiC
UbiC promoter
TdTomato
T2A
Puro

FIGs. 4A-B

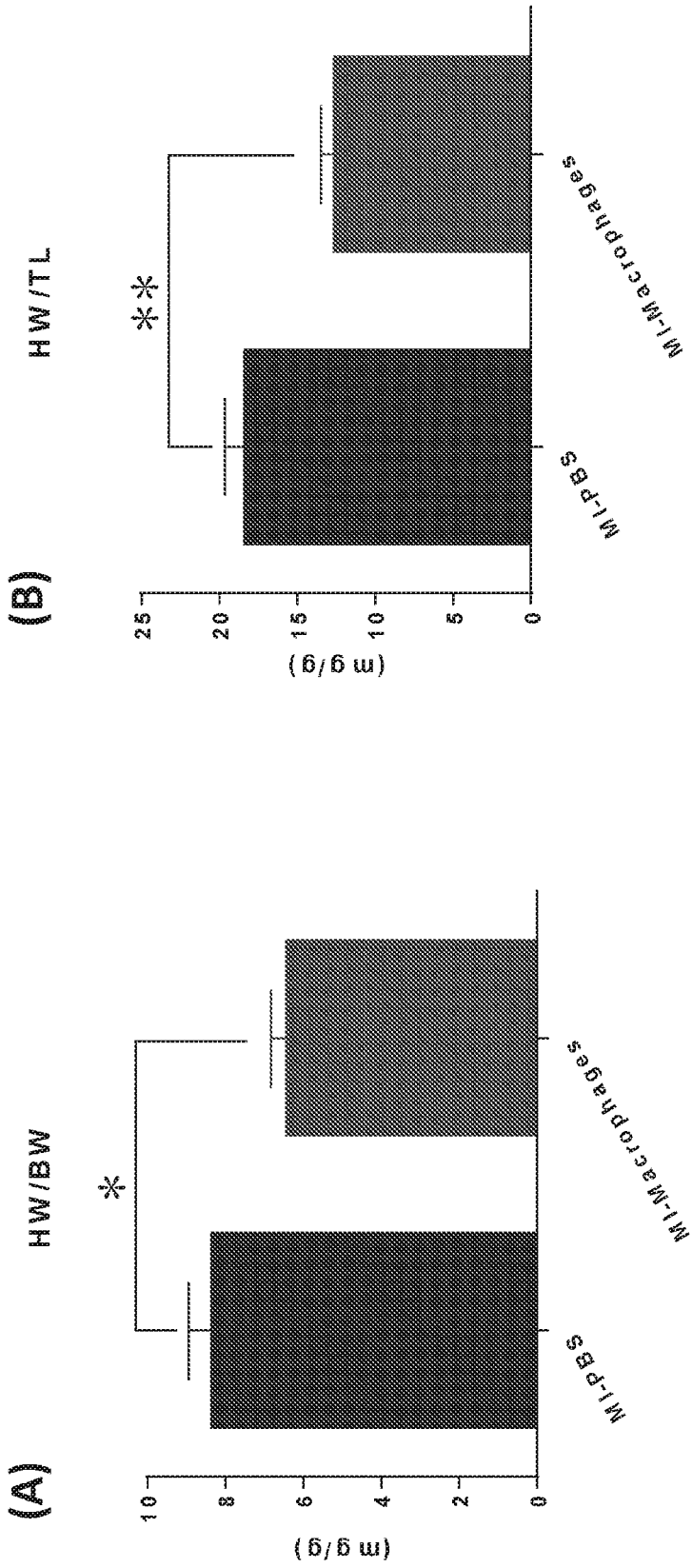

(A)
Ejection Fraction
*
60
40
20
0
%
MI-PBS
MI-Macrophages
(B)
Fraction Shortening
*
30
20
10
0
%
MI-PBS
MI-Macrophages

FIGS. 5A-B


(B)
LVID;s
**
M1-Macrophages
M1-PBS
0
2
4
6
mm


(A)
LVID;d
**
M1-Macrophages
M1-PBS
0
2
4
6
mm

FIGs. 6A-B


(A)
HW/BW
*
(mg/g)
10
8
6
4
2
0
M1-PBS
M1-Macrophages
(B)
HW/TL
**
(mg/g)
25
20
15
10
5
0
M1-PBS
M1-Macrophages

FIGS. 7A-B

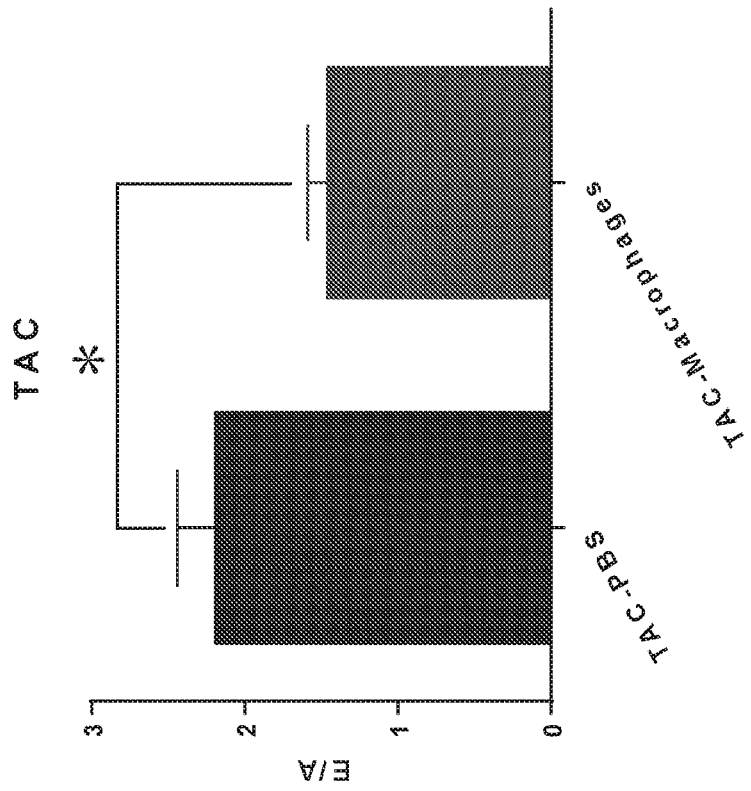
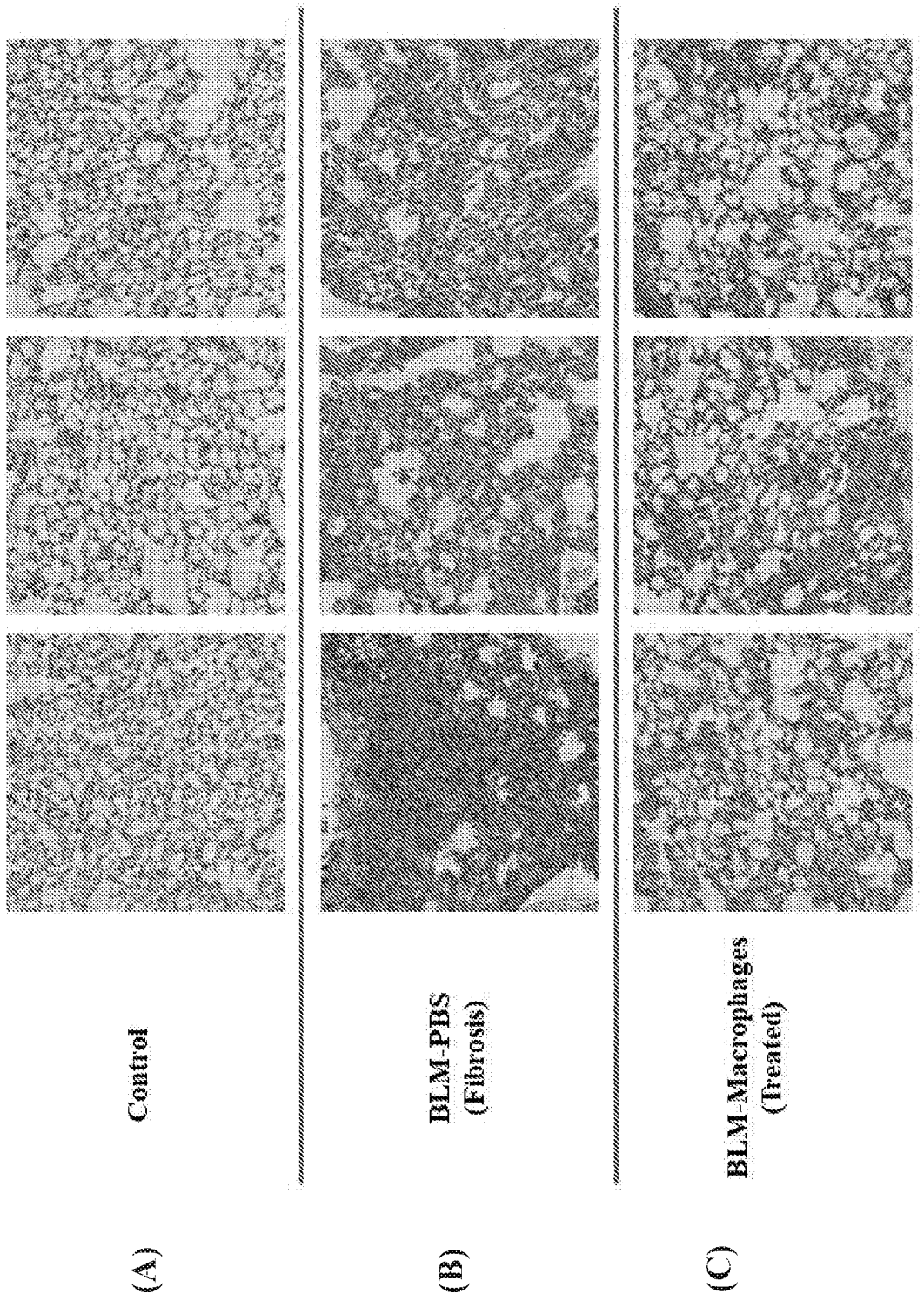


FIG. 8



FIGs. 9A-C

TREATMENT OF FIBROSIS WITH GENETICALLY-ENGINEERED MACROPHAGES

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of priority of U.S. Provisional Patent Application No. 62/598,894 filed Dec. 14, 2017, which is hereby incorporated by reference in its entirety.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH

[0002] This invention was made with government support under grant numbers R01 OD023700 and R01 DK102960 each awarded by the National Institutes of Health. The government has certain rights in the invention.

BACKGROUND OF THE INVENTION

Field of the Invention

[0003] This disclosure relates generally to the fields of molecular biology and medicine; in particular to genetically-engineered macrophages and their use in the treatment of fibrosis.

[0004] Description of Related Art

[0005] Fibrosis is the common scarring reaction associated with chronic injury that results from prolonged parenchymal cell injury and/or inflammation that may be induced by a wide variety of agents, e.g., drugs, toxins, radiation, any process disturbing tissue or cellular homeostasis, toxic injury, altered blood flow, infections (viral, bacterial, spirochetal, and parasitic), storage disorders, and disorders resulting in the accumulation of toxic metabolites. Fibrosis is most common in the liver, heart, lung, peritoneum, and kidney.

[0006] For instance, hepatic fibrosis (liver fibrosis) results from an altered wound healing response that is characterized by increased production of matrix proteins and decreased matrix remodeling. Normal structural elements of tissues are replaced with excessive amounts of non-functional scar tissue. Hepatic fibrosis is a common pathological consequence of chronic liver diseases. In a number of patients, fibrosis ultimately leads to cirrhosis, a condition defined by an abnormal liver architecture, with fibrotic septa surrounding regenerating nodules and altered vacularization. Due to decreased functional parenchymal reserve and altered hepatic blood flow, cirrhosis is associated with the life-threatening complications of liver failure including hepatic encephalopathy, coagulation disorders and bacterial infections, and complications of portal hypertension such as ascites, variceal rupture and hepatorenal syndrome. In addition, the cirrhotic liver is a precancerous state, and thus requires the systematic screening for hepatocellular carcinoma. Several clinical reports have documented that regression of liver fibrosis occurs in a substantial proportion of patients, provided that the factor responsible for liver insult is eradicated or controlled. Consistent with this observation, studies in rodents have also documented regression of fibrosis or early stage cirrhosis within weeks following eradication of the toxic insult. The potential for reversibility of fibrosis declines at advanced stages. It is imperative to treat fibrosis in the early stages of reversible liver scarring so that irreversible cirrhosis can be prevented.

[0007] There remains a need for additional and/or improved therapies to reverse fibrosis in individuals suffering from fibrotic conditions.

SUMMARY OF THE INVENTION

[0008] Embodiments described herein provide macrophages engineered for treating fibrosis and ameliorating the effects of fibrotic lesions in various organs and tissues. Certain embodiments are directed to genetically-engineered macrophages capable of treating fibrosis or reducing fibrotic lesions. In certain aspects macrophages can be genetically-engineered to (1) target extracelluar matrix (ECM) or components thereof, (2) enhance degradation of ECM, or (3) target ECM and enhance degradation of ECM. Macrophages can be engineered to target ECM by expressing one or more cell surface receptors (e.g., a collagen receptor) that bind one or more component of the ECM (e.g., collagen). In addition, macrophages can be engineered for enhance degradation of ECM by expression of a protease or other enzyme that cleaves or degrades one or more ECM component (e.g., matrix metalloprotease, MMP).

[0009] In a first aspect, a genetically-engineered macrophage can include or express a recombinant targeting protein and/or a recombinant catalytic enzyme. A recombinant targeting protein can include a protein that binds an ECM component, e.g., collagen. In one embodiment, the recombinant targeting protein is a collagen receptor or a subunit thereof. Collagen receptors are membrane proteins that bind the extracellular matrix protein collagen. In one embodiment, the collagen receptor or a subunit thereof comprises one or more of an integrin, a discoidin domain receptor, a mannose family receptor, and/or an immunoglobulin-like receptor. The integrin can be a $\alpha 1\beta 1$, $\alpha 2\beta 1$, $\alpha 10\beta 1$, and/or $\alpha 11\beta 1$ integrin. In one aspect, the discoidin domain receptor (DDR) can be DDR1 and/or DDR2. In one aspect, the mannose family receptor can be M-phospholipase A2 receptor and/or Endo180. In one aspect, the immunoglobulin-like receptor can be glycoprotein VI.

[0010] In one aspect, the recombinant targeting protein is Integrin Alpha 1 (ITGA-1) (SEQ ID NO: 1 or SEQ ID NO: 3, mouse (e.g., GenBank accession number NP_001028400.2) and human (e.g., GenBank accession number NP_852478.1, respectively). In certain aspects a macrophage can express a nucleic acid that is or is at least 70, 75, 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% identical to SEQ ID NO:1 or SEQ ID NO:3 (or any range derivable therein), or a segment thereof. In other aspects a macrophage can express a polypeptide that is or is at least 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% identical to SEQ ID NO:2 or SEQ ID NO:4 (or any range derivable therein), or a functional variant or segment thereof.

[0011] SEQ ID NO:1 provides the full length coding sequence of mouse ITGA-1 that encodes the amino acid sequence of SEQ ID NO:2. SEQ ID NO:2 is a 1179 amino acid protein having a signal peptide from amino acid 1 to 28 (mature protein comprising amino acids 29 to 1179 of SEQ ID NO:2) and a transmembrane region from approximately amino acid 1142 to 1164 of SEQ ID NO:2. In certain aspects a segment of SEQ ID NO:2 can be expressed by an engineered macrophage, the segment comprising, comprising at least, or comprising at most 500, 501, 502, 503, 504, 505, 506, 507, 508, 509, 510, 511, 512, 513, 514, 515, 516, 517, 518, 519, 520, 521, 522, 523, 524, 525, 526, 527, 528, 529,

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[0012] SEQ ID NO:3 provides the full length coding sequence of human ITGA-1 that encodes the amino acid sequence of SEQ ID NO:4. SEQ ID NO:4 is a 1179 amino acid protein having a signal peptide from amino acid 1 to 28 (mature protein comprising amino acids 29 to 1179 of SEQ ID NO:4) and a transmembrane region from approximately amino acid 1142 to 1164 of SEQ ID NO:4. In certain aspects a segment of SEQ ID NO:4 can be expressed by an engineered macrophage, the segment comprising, comprising at least, or comprising at most 500, 501, 502, 503, 504, 505, 506, 507, 508, 509, 510, 511, 512, 513, 514, 515, 516, 517, 518, 519, 520, 521, 522, 523, 524, 525, 526, 527, 528, 529, 530, 531, 532, 533, 534, 535, 536, 537, 538, 539, 540, 541, 542, 543, 544, 545, 546, 547, 548, 549, 550, 551, 552, 553, 554, 555, 556, 557, 558, 559, 560, 561, 562, 563, 564, 565, 566, 567, 568, 569, 570, 571, 572, 573, 574, 575, 576, 577, 578, 579, 580, 581, 582, 583, 584, 585, 586, 587, 588, 589, 590, 591, 592, 593, 594, 595, 596, 597, 598, 599, 600, 601, 602, 603, 604, 605, 606, 607, 608, 609, 610, 611, 612, 613, 614, 615, 616, 617, 618, 619, 620, 621, 622, 623, 624, 625, 626, 627, 628, 629, 630, 631, 632, 633, 634, 635, 636, 637, 638, 639, 640, 641, 642, 643, 644, 645, 646, 647, 648, 649, 650, 651, 652, 653, 654, 655, 656, 657, 658, 659, 660, 661, 662, 663, 664, 665, 666, 667, 668, 669, 670, 671, 672, 673, 674, 675, 676, 677, 678, 679, 680, 681, 682, 683, 684, 685, 686, 687, 688, 689, 690, 691, 692, 693, 694, 695, 696, 697, 698, 699, 700, 700, 701, 702, 703, 704, 705, 706, 707, 708, 709, 710, 711, 712, 713, 714, 715, 716, 717, 718, 719, 720, 721, 722, 723, 724, 725, 726, 727, 728, 729, 730, 731, 732, 733, 734, 735, 736, 737, 738, 739, 740, 741, 742, 743, 744, 745, 746, 747, 748, 749, 750, 751, 752, 753, 754, 755, 756, 757, 758, 759, 760, 761, 762, 763, 764, 765, 766, 767, 768, 769, 770, 771, 772, 773, 774, 775, 776, 777, 778, 779, 780, 781, 782, 783, 784, 785, 786, 787, 788, 789, 790, 791, 792, 793, 794, 795, 796, 797, 798, 799, 800, 801, 802, 803, 804, 805, 806, 807, 808, 809, 810, 811, 812, 813, 814, 815, 816, 817, 818, 819, 820, 821, 822, 823, 824, 825, 826, 827, 828, 829, 830, 831, 832, 833, 834, 835, 836, 837, 838, 839, 840, 841, 842, 843, 844, 845, 846, 847, 848, 849, 850, 851, 852, 853, 854, 855, 856, 857, 858, 859, 860, 861, 862, 863, 864, 865, 866, 867, 868, 869, 870, 871, 872, 873, 874, 875, 876, 877, 878, 879, 880, 881, 882, 883, 884, 885, 886, 887, 888, 889, 890, 891, 892, 893, 894, 895, 896, 897, 898, 899, 900, 901, 902, 903, 904, 905, 906, 907, 908, 909, 910, 911, 912, 913, 914, 915, 916, 917, 918, 919, 920, 921, 922, 923, 924, 925, 926, 927, 928, 929, 930, 931, 932, 933, 934, 935, 936, 937, 938, 939, 940, 941, 942, 943, 944, 945, 946, 947, 948, 949, 950, 951, 952, 953, 954, 955, 956, 957, 958, 959, 960, 961, 962, 963, 964, 965, 966, 967, 968, 969, 970, 971, 972, 973, 974, 975, 976, 977, 978, 979, 980, 981, 982, 983, 984, 985, 986, 987, 988, 989, 990, 991, 992, 993, 994, 995, 996, 997, 998, 999, 1000, 1001, 1002, 1003, 1004, 1005, 1006, 1007, 1008, 1009, 1010, 1011, 1012, 1013, 1014, 1015, 1016, 1017, 1018, 1019, 1020, 1021, 1022, 1023, 1024, 1025, 1026, 1027, 1028, 1029, 1030, 1031, 1032, 1033, 1034, 1035, 1036, 1037, 1038, 1039, 1040, 1041, 1042, 1043, 1044, 1045, 1046, 1047, 1048, 1049, 1050, 1051, 1052, 1053, 1054, 1055, 1056, 1057, 1058, 1059, 1060, 1061, 1062, 1063, 1064, 1065, 1066, 1067, 1068, 1069, 1070, 1071, 1072, 1073, 1074, 1075, 1076, 1077, 1078, 1079, 1080, 1081, 1082, 1083, 1084, 1085, 1086, 1087, 1088, 1089, 1090, 1091, 1092, 1093, 1094, 1095, 1096, 1097, 1098, 1099, 1100, 1101, 1102, 1103, 1104, 1105, 1106, 1107, 1108, 1109, 1110, 1111, 1112, 1113, 1114, 1115, 1116, 1117, 1118, 1119, 1120, 1121, 1122, 1123, 1124, 1125, 1126, 1127, 1128, 1129, 1130, 1131, 1132, 1133, 1134, 1135, 1136, 1137, 1138, 1139, 1140, 1141, 1142, 1143, 1144, 1145, 1146, 1147, 1148, 1149, 1150, 1151, 1152, 1153, 1154, 1155, 1156, 1157, 1158, 1159, 1160, 1161, 1162, 1163, 1164, 1165, 1166, 1167, 1168, 1169, 1170, 1171, 1172, 1173, 1174, 1175, 1176, 1177, 1178, 1179 amino acids of SEQ ID NO:4 (or any range derivable therein) starting at amino acid 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52. 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96. 97, 98, 99, 100, 101,

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906, 907, 908, 909, 910, 911, 912, 913, 914, 915, 916, 917, 918, 919, 920, 921, 922, 923, 924, 925, 926, 927, 928, 929, 930, 931, 932, 933, 934, 935, 936, 937, 938, 939, 940, 941, 942, 943, 944, 945, 946, 947, 948, 949, 950, 951, 952, 953, 954, 955, 956, 957, 958, 959, 960, 961, 962, 963, 964, 965, 966, 967, 968, 969, 970, 971, 972, 973, 974, 975, 976, 977, 978, 979, 980, 981, 982, 983, 984, 985, 986, 987, 988, 989, 990, 991, 992, 993, 994, 995, 996, 997, 998, 999, 1000, 1001, 1002, 1003, 1004, 1005, 1006, 1007, 1008, 1009, 1010, 1011, 1012, 1013, 1014, 1015, 1016, 1017, 1018, 1019, 1020, 1021, 1022, 1023, 1024, 1025, 1026, 1027, 1028, 1029, 1030, 1031, 1032, 1033, 1034, 1035, 1036, 1037, 1038, 1039, 1040, 1041, 1042, 1043, 1044, 1045, 1046, 1047, 1048, 1049, 1050, 1051, 1052, 1053, 1054, 1055, 1056, 1057, 1058, 1059, 1060, 1061, 1062, 1063, 1064, 1065, 1066, 1067, 1068, 1069, 1070, 1071, 1072, 1073, 1074, 1075, 1076, 1077, 1078, 1079, 1080, 1081, 1082, 1083, 1084, 1085, 1086, 1087, 1088, 1089, 1090, 1091, 1092, 1093, 1094, 1095, 1096, 1097, 1098, 1099, 1100, 1101, 1102, 1103, 1104, 1105, 1106, 1107, 1108, 1109, 1110, 1111, 1112, 1113, 1114, 1115, 1116, 1117, 1118, 1119, 1120, 1121, 1122, 1123, 1124, 1125, 1126, 1127, 1128, 1129, 1130, 1131, 1132, 1133, 1134, 1135, 1136, 1137, 1138, 1139, 1140, 1141, 1142, 1143, 1144, 1145, 1146, 1147, 1148, 1149, 1150, 1151, 1152, 1153, 1154, 1155, 1156, 1157, 1158, 1159, 1160, 1161, 1162, 1163, 1164, 1165, 1166, 1167, 1168, 1169, 1170, 1171, 1172, 1173, 1174, 1175, 1176, 1177, 1178, or 1179 of SEQ ID NO:4 (or any range of positions therein).

[0013] In one embodiment, the recombinant catalytic enzyme is a protease. In certain aspects the protease is a matrix metalloproteinase (MMP). In one embodiment, the matrix metalloproteinase is MMP1 (e.g., NP_002412), MMP1a (e.g., NP_114395.1), MMP2 (e.g., NP_001121363), MMP3 (e.g., NP_002413), MMPI (e.g., NP_002414), MMP8 (e.g., NP_001291370), MMP9 (e.g., NP_004985), MMP10 (e.g., NP_002416), MMP12 (e.g., NP_002417), MMP13 (e.g., NP_002418), MMP14 (e.g., NP_004986), MMP17 (e.g., NP_057239), MMP19 (e.g., NP_001259030), MMP20 (e.g., NP_004762), MMP21 (e.g., NP_671724), MMP22 (NP_008914.1), MMP24 (e.g., NP_006681), MMP25 (e.g., NP_071913), MMP26 (e.g., NP_068573), MMP2? (e.g., NP_071405), and/or MMP28 (e.g., NP_001027449). In some aspects, one or more of these may be excluded as an embodiment.

[0014] In certain embodiments, the matrix metalloproteinase is MMP1a. In certain embodiments, the macrophage is an M2-specific macrophage. In one embodiment, the recombinant targeting protein is a human integrin α1 encoded by SEQ ID NO: 3, the recombinant catalytic enzyme is a human MMP1 encoded by SEQ ID NO: 5, and wherein the macrophage is a human M2-specific macrophage. In certain aspects a macrophage can express a nucleic acid that is or is at least 70, 75, 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% identical (or any range derivable therein) to SEQ ID NO:5 or SEQ ID NO:7, or a segment thereof. In other aspects a macrophage can express a polypeptide that is 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% identical to SEQ ID NO:6 or SEQ ID NO:8, or a functional variant or segment thereof.

[0015] SEQ ID NO:5 provides the full length coding sequence of human MMP1 that encodes the amino acid sequence of SEQ ID NO:6. SEQ ID NO:6 is a 469 amino acid protein having a signal peptide from amino acid 1 to 17 (mature protein comprising amino acids 18 to 469 of SEQ ID NO:6) and a metalloprotease region from approximately amino acid 98 to 276 of SEQ ID NO:6. In certain aspects a segment of SEQ ID NO:6 can be expressed by an engineered macrophage, the segment comprising or comprising at least 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96. 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 251, 252, 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453, 454, 455, 456, 457, 458, 459, 460, 461, 462, 463, 464, 465, 466, 467, 468 amino acids of SEQ ID NO:6 (or any range derivable therein) starting at amino acid 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 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390, 391, 392, 393, 394 of SEQ ID NO:6 (or any range of positions therein). and ending at amino acid 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 251, 252, 253, 254, 255, 256, 257, 258, 259, 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460, 461, 462, 463, 464, 465, 466, 467, 468, 469 of SEQ ID NO:6 (or any range of positions therein).

[0016] SEQ ID NO:7 provides the full length coding sequence of mouse MMP1a that encodes the amino acid sequence of SEQ ID NO:8. SEQ ID NO:8 is a 464 amino acid protein having a signal peptide from amino acid 1 to 17

(mature protein comprising amino acids 18 to 464 of SEQ ID NO:8) and a metalloprotease region from approximately amino acid 95 to 274 of SEQ ID NO:8. In certain aspects a segment of SEQ ID NO:8 can be expressed by an engineered macrophage, the segment comprising or comprising at least 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 251, 252, 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453, 454, 455, 456, 457, 458, 459, 460, 461, 462, 463, 464, 465, 466, 467, 468 amino acids of SEQ ID NO:8 (or any range derivable therein) starting at amino acid 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 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of SEQ ID NO:8 (or any range of positions therein), and ending at amino acid 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 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464 of SEQ ID NO:8 (or any range of positions therein).

[0017] In a second aspect, a population of cells is contemplated that includes the genetically-engineered macrophage according to the first aspect and embodiments thereof.

[0018] In a third aspect, a cellular therapy product includes a genetically-engineered macrophage comprising at least one of a recombinant targeting protein and a recombinant catalytic enzyme. In one embodiment of the third aspect, the cellular therapy product further includes one or more cell media components and/or therapeutic compounds. In another embodiment of the third aspect, the cellular therapy product further includes an effective amount of one or more of α-tocopherol, interferon-γ, quercetin, an ACE inhibitor, and PPAR-δ. In another embodiment of the third aspect, the cellular therapy product further includes a pharmaceutical reagents and/or excipients suitable for therapeutic application.

[0019] The term "effective amount" means an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic or prophylactic result. In regard to liver, cardiac, or lung fibrosis, an effective amount is a dose sufficient to prevent advancement, delay progression, or to cause regression of liver, cardiac, or lung fibrosis, or which is capable of reducing symptoms caused by the disease. In one example, an effective amount is an amount

of a therapy sufficient to reduce inflammation in the liver, reduce liver enzyme levels (such as AST, ALT, and/or AP) and/or reduce scarring of the liver by at least 10%, at least 20%, at least 50%, at least 70%, or at least 90%. In one example, a effective amount is an amount of a therapy sufficient to increase liver, cardiac, or lung function in a fibrotic liver, heart, or lung, for example an increase of at least 10%. at least 20%, at least 50%, at least 70%, or at least 90% as compared to an absence of therapy. In certain aspects and effective amount of macrophages can include or include at least or at most 10, 100, 1000, 1×10^4, 1×10^5, 1×10^6, 1×10^7 1×10^8, 1×10^9, 1×10^{10} macrophages, including all values and ranges there between.

[0020] In a fourth aspect, a method of treating an individual for fibrosis includes administering the cellular therapy product according to the third aspect and embodiments thereof. In one embodiment, the cellular therapy product is administered by injection to the individual (e.g., systemic or local injection). In certain aspects the injection is by intravenous injection. In other aspects the injection into or around (within 1 to 10 cm) of a fibrotic lesion or potential fibrotic area. When administering the cellular therapy product by injection, the administration may be by continuous infusion or by single or multiple boluses. In one embodiment, the cellular therapy product comprises of genetically-engineered macrophages derived from the individual being treated (i.e., autologous cells).

[0021] In a fifth aspect, a method of reversing or tretaing fibrosis in an individual in need thereof includes administering to the individual a genetically-engineered M2 macrophage capable of expressing recombinant ITGA-1 and MMP1 or MMP1a, targeting the macrophage to the fibrotic area of the individual, and reversing fibrosis within the targeted area.

[0022] In a sixth aspect, genetically engineered macrophages are made by transfecting M2-specific macrophages with one or more expression vector. e.g., lentiviral constructs, and selected for incorporation of the expression vector(s) and expression of the recombinant genes. Recombinant M2-specific recombinant macrophages expressing integrin A1, MMP1 or MMP1a, or both integrin A1 and MMP1 or MMP1a can be selected. The selected cell can be introduced into an individual as a novel therapeutic approach for liver, cardiac, lung fibrosis and other fibrotic diseases.

[0023] All publications, patents, and patent applications cited herein are hereby-expressly incorporated by reference in their entirety for all purposes.

[0024] As used herein, the singular forms "a," "an," and "the" include plural referents unless the context clearly dictates otherwise. For example, reference to "a metabolite" means one or more metabolites.

[0025] It is noted that terms like "preferably," "commonly," and "typically" are not utilized herein to limit the scope of the claimed invention or to imply that certain features are critical, essential, or even important to the structure or function of the claimed invention. Rather, these terms are merely intended to highlight alternative or additional features that can or cannot be utilized in a particular embodiment of the present invention.

[0026] For the purposes of describing and defining the present invention it is noted that the term "substantially" as used herein represents the inherent degree of uncertainty that can be attributed to any quantitative comparison, value, measurement, or other representation. The term "substantially" is also used herein to represent the degree by which a quantitative representation can vary from a stated reference without resulting in a change in the basic function of the subject matter at issue.

[0027] Methods well known to those skilled in the art can be used to construct genetic expression constructs, targeting vectors, and genetically-engineered cells according to this invention. These methods include in vitro recombinant DNA techniques, synthetic techniques, in vivo recombination techniques, polymerase chain reaction (PCR) techniques, and others. See, for example, techniques as described in Green & Sambrook, 2012, MOLECULAR CLONING: A LABORATORY MANUAL, Fourth Edition, Cold Spring Harbor Laboratory, New York; Ausubel et al., 1989, CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, Greene Publishing Associates and Wiley Interscience, New York, and PCR Protocols: A Guide to Methods and Applications (Innis et al., 1990, Academic Press, San Diego, Calif.).

[0028] As used herein, the terms "polynucleotide," "nucleotide," "oligonucleotide," and "nucleic acid" can be used interchangeably to refer to nucleic acid comprising DNA, RNA, derivatives thereof, or combinations thereof.

[0029] As used herein, the term "genetically-engineered" refers to the genetic manipulation of one or more cells, whereby the genome of the one or more cells has been augmented by at least one DNA sequence. Candidate DNA sequences include but are not limited to genes that are not naturally present, DNA sequences that are not normally transcribed into RNA or translated into a protein ("expressed"), and other genes or DNA sequences which one desires to introduce into the one or more cells. It will be appreciated that typically the genome of genetically-engineered cells described herein is augmented through transient or stable introduction of one or more recombinant genes.

[0030] Generally, introduced DNA is not originally resident in the cell that is the recipient of the DNA, but it is within the scope of this disclosure to isolate a DNA segment from a given genetically-engineered cell, and to subsequently introduce one or more additional copies of that DNA into the same genetically-engineered cell, e.g., to enhance production of the product of a gene or alter the expression pattern of a gene. In some instances, the introduced DNA will modify or even replace an endogenous gene or DNA sequence by, e.g., homologous recombination, site-directed mutagenesis, and/or genome editing technology, including CRISPR (clustered regularly-interspaced short palindromic repeats), and/or mammalian transposon technology, such as by using the piggyBac™ transposon. In some instances, the introduced DNA is introduced into the recipient via viral vectors, including vectors derived from retrovirus, lentivirus, and adeno-associated virus. In some instances, the introduced DNA is introduced into the recipient cell directly with electroporation.

[0031] As used herein, the term "recombinant gene" refers to a gene or DNA sequence that is introduced into a genetically-engineered cell. regardless of whether the same or a similar gene or DNA sequence may already be present in such a host. "Introduced," or "augmented" in this context, is known in the art to mean introduced or augmented by the hand of man. Thus, a recombinant gene can be a DNA sequence from another species, or can be a DNA sequence that originated from or is present in the same species. but has

been incorporated into a cell by methods to form a genetically-engineered cell. It will be appreciated that a recombinant gene that is introduced into a cell can be identical to a DNA sequence that is normally present in the cell being transformed, and is introduced to provide one or more additional copies of the DNA to thereby permit overexpression or modified expression of the gene product of that DNA. Recombinant genes can also be introduced with different driving promoters or associated sequences that can alter the gene's expression level or pattern. Such recombinant genes are particularly-encoded by cDNA. Non-coding sequences, such as short hairpin RNAs, microRNAs, or long non-coding RNAs, may also be included.

[0032] It is further contemplated that recombinant genes can be codon optimized to maximize protein expression in genetically-engineered cells by increasing the translation efficiency of a particular gene. Codon optimization can be achieved, for example, by transforming nucleotide sequences of one species into the genetic sequence of a different species. Optimal codons help to achieve faster translation rates and high accuracy. As a result of these factors, translational selection is expected to be stronger in highly-expressed genes. However, while optimal codon usage is contemplated herein for expression of disclosed proteins, all possible codons are contemplated for use herein for nucleic acids encoding any disclosed protein.

[0033] As used herein, the term "cellular therapy product" refers to a population of cells including one or more cells that has been genetically engineered to at least one of target a desired location within an individual and have a physiologically relevant effect at the desired location. For example, a cellular therapy product can be a population of cells including a genetically-engineered macrophage that can degrade collagen. The population of cells can be homogeneous (i.e., including only genetically-engineered macrophages) or heterogeneous (including genetically-engineered macrophages, non-genetically engineered macrophages, and other cell types whether genetically-engineered or not). A cellular therapy product can further include one or more cell media components (e.g., buffers, antibiotics, salts, vitamins, growth factors, amino acids, etc.) and/or therapeutic compounds to maintain the population of cells and/or treat a disease. For example, a cellular therapy product can include a genetically-engineered macrophage and an antibiotic. Cellular therapy products can further include additional therapeutic agents, such as one or more of α-tocopherol, interferon-γ, quercetin, an ACE inhibitor, and PPAR-δ. Additional therapeutic agents and pharmaceutical reagents and/or excipients suitable for therapeutic application can also be included in contemplated cellular therapy products. Additional reagents are contemplated for inclusion in cellular therapy products.

[0034] The terms "treating" or "treatment" refer to any success or indicia of success in the attenuation or amelioration of an injury, pathology or condition, including any objective or subjective parameter such as abatement, remission, diminishing of symptoms or making the injury, pathology, or condition more tolerable to the patient, slowing in the rate of degeneration or decline, making the final point of degeneration less debilitating, improving a subject's physical or mental well-being, or prolonging the length of survival. The treatment or amelioration of symptoms can be based on objective or subjective parameters; including the results of a physical examination, neurological examination, and/or psychiatric evaluations.

[0035] As used herein, the terms "or" and "and/or" are utilized to describe multiple components in combination or exclusive of one another. For example, "x, y, and/or z" can refer to "x" alone, "y" alone, "z" alone, "x, y, and z," "(x and y) or z," "x or (y and z)," or "x or y or z." Is is specifically contemplated that x, y, or z may be specifically excluded from an embodiment.

[0036] Throughout this application, the term "about" is used according to its plain and ordinary meaning in the area of cell biology to indicate that a value includes the standard deviation of error for the device or method being employed to determine the value.

[0037] The term "comprising," which is synonymous with "including," "containing," or "characterized by," is inclusive or open-ended and does not exclude additional, unrecited elements or method steps. The phrase "consisting of" excludes any element, step, or ingredient not specified. The phrase "consisting essentially of" limits the scope of described subject matter to the specified materials or steps and those that do not materially affect its basic and novel characteristics. It is contemplated that embodiments described in the context of the term "comprising" may also be implemented in to context of the term "consisting of" or "consisting essentially of."

[0038] It is specifically contemplated that any limitation discussed with respect to one embodiment of the invention may apply to any other embodiment of the invention. Furthermore, any composition of the invention may be used in any method of the invention, and any method of the invention may be used to produce or to utilize any composition of the invention. Aspects of an embodiment set forth in the Examples are also embodiments that may be implemented in the context of embodiments discussed elsewhere in a different Example or elsewhere in the application, such as in the Summary of Invention, Detailed Description of the Embodiments, Claims, and description of Figure Legends.

[0039] These and other features and advantages of the present invention will be more fully understood from the following detailed description taken together with the accompanying claims. It is noted that the scope of the claims is defined by the recitations therein and not by the specific discussion of features and advantages set forth in the present description.

DESCRIPTION OF DRAWINGS

[0040] FIGS. **1**A and **1**B illustrate the effectiveness of anti-inflammatory M2-specific macrophage treatment against CCl_4-mediated liver fibrosis in mice. FIG. **1**A shows an ultrasound scan of a mouse liver after 10 weeks of CCl_4 treatment only. FIG. **1**B shows an ultrasound scan of a mouse liver after 10 weeks of CCl_4 treatment followed by treatment with anti-inflammatory M2-specific macrophages, which can promote tissue repair and regeneration. Asterisks in each figure designate liver lobes, and signal intensity (brightness) indicates liver texture hardness, which correlates with fibrosis. The notable lesser intensity (brightness) in FIG. **1**B compared to FIG. **1**A indicates the effectiveness of the inventive anti-inflammatory M2-specific macrophage cell treatment in removing liver fibrosis.

[0041] FIGS. **2**A and **2**B show histochemical analyses of CCl_4-treated mouse livers. Treated mice were sacrificed and their livers removed, sectioned, and stained with hematoxy-

lin and eosin. FIG. **2**A shows a section of mouse liver after 10 weeks of CCl_4 treatment only. Inflammation, fibrotic lesions, and necrotic lesions are evident. FIG. **2**B shows a section of mouse liver after 10 weeks of CCl_4 treatment followed by treatment with anti-inflammatory M2-specific macrophages. Marked reductions in inflammation and fibrotic lesions are evident in FIG. **2**B compared to FIG. **2**A (arrows). For each figure, the scale bar indicates 500 μm. Pathological evaluations are shown in Table No. 1.

[0042] FIGS. **3**A and **3**B show histochemical analyses of CCl_4-treated mouse livers. Treated mice were sacrificed and their livers removed, sectioned, and stained with trichrome staining for collagenous fibers. Arrows indicate areas of fibrotic lesions. FIG. **3**A shows a section of mouse liver after 10 weeks of CCl_4 treatment only. Several areas of fibrosis are evident. FIG. **3**B shows a section of mouse liver after 10 weeks of CCl_4 treatment followed by treatment with anti-inflammatory M2-specific macrophages. Marked reductions in fibrotic lesions are noted in FIG. **3**B compared to FIG. **3**A (arrows). For each figure, the scale bar indicates 500 μm.

[0043] FIGS. **4**A and **4**B show lentiviral constructs for the expression of integrin A1 (FIG. **4**A) or MMP1 (FIG. **4**B). Each vector encodes integrin or MMP1 driven by a CMV promoter and a selection marker (fluorescence protein tdTomato and puromycin resistant gene, Puro) driven by a constitutive promoter UbiC (Ubiquitin C promoter). TdTomato and Puro are separated by a self-cleavable peptide T2A.

[0044] FIGS. **5**A and **5**B show engraftment of engineered macrophages partially prevented MI-induced systolic dysfunction in left ventricle. There were marked deteriorations in Ejection Fraction (EF)(FIG. **5**A) and Fraction Shortening (FS)(FIG. **5**B) in MI mice receiving PBS injections, indicating an impaired systolic function/heart failure induced by LAD surgery. Ejection Fraction (EF) and Fraction Shorting (FS) in mice received engineered macrophage were higher than those received PBS, showing cardioprotective effect of engineered macrophage in post-MI heart.

[0045] FIGS. **6**A and **6**B show that cellular therapy using engineered macrophages prevented MI-induced LV dilation. Enlargement of LV chamber size was observed following surgical ligation of the LAD in PBS group. Engraftment of engineered macrophages prevented LV from MI-induced dilation. FIG. **6**A LVID;d and FIG. **6**B LVID;s.

[0046] FIGS. **7**A and **7**B show cellular therapy using engineered macrophages prevented ischemic myocardium remodeling. Myocardial infarction induced myocardium remodeling in PBS group, evidenced by an increase in heart weight. Lower heart weight in the engineered macrophages group indicates the cellular therapy regressed the remodeling progress. FIG. **7**A HW/BW and FIG. **7**B HW/T.

[0047] FIG. **8** shows cellular therapy using engineered macrophages prevented TAC-induced LV diastolic dysfunction. Increasing of E/A was observed following surgical constraining of the aorta in PBS group. Engraftment of engineered macrophages prevented LV from TAC-induced diastolic dysfunction.

[0048] FIGS. **9**A, **9**B, and **9**C show the effect of Macrophage engraftment on BLM-induced lung injury in mice. H&E staining on tissue sections prepared from the lungs of C57BL6 mice 14 days after PBS/BLM exposure. (FIG. **9**A): Control mice exposed to PBS and injected with PBS. (FIG. **9**B): Mice in fibrosis group exposed to BLM then injected with PBS. (FIG. **9**C). Macrophages treatment via tail vein injection reduced the fibrosis and the degree of inflammation in lungs of mice challenged with BLM.

DETAILED DESCRIPTION

[0049] Embodiments described herein are directed to genetically-engineered macrophages capable of removing fibrotic scarring, for example, in liver, cardiac, or lung fibrosis. This disclosure is further directed to a cellular therapy product, such as an enriched population of genetically-engineered macrophages. Still further, this disclosure is directed to novel therapeutic approaches to enhance decomposition of fibrotic tissue and induce regeneration of functional hepatocytes by delivery of genetically-engineered macrophages to damaged liver. Additional characteristics and advantages of certain embodiments are described below.

Cell Selection and Growth

[0050] Suitable cells that can be used in the present disclosure include, but are not limited to, macrophages. In one specific embodiment, contemplated cells for use herein include M2 macrophages that can turn off inflammatory responses and promote tissue wound repair, termed "anti-inflammatory M2-specific macrophages."

[0051] In some embodiments, cells can be taken from an individual (autologous source) to be treated, genetically-modified, and introduced (e.g., by injection) back into the individual to remove fibrotic scars in the individual's liver, heart, lung, or other tissue or organ. In one embodiment, such a cellular therapy product can be derived from an apheresis product taken from the individual. In another embodiment, a cellular therapy product intended for an individual can be derived from an apheresis product taken from another individual (heterologous source) or from another cell source. In one embodiment, a suitable autologous macrophage population can be produced as described in Fraser et al. (Development, functional characterization and validation of methodology for GMP-compliant manufacture of phagocytic macrophages: A novel cellular therapeutic for liver cirrhosis. *Cytotherapy* 2017 September; 19(9):1113-1124).

[0052] The methods for the treatment of fibrosis in a human or other mammalian subject by administering engineered M2 macrophages to the subject at the site of fibrosis. The source of macrophages can be peripheral blood or tissue at or near the site of inflammation. The source of macrophages may be an isolated source, which comprises an ex-vivo composition comprising macrophages. Such a composition may be a culture of macrophages, a macrophage-containing tissue obtained from a subject (which may be the subject to be treated), or a culture, such as a culture comprising monocytes.

[0053] The source of macrophages may be a concentrated macrophage solution generated by fractionating peripheral blood obtained from the patient. Fractionating peripheral blood comprises preparing a suspension of peripheral blood mononuclear cells (PBMCs) and inducing the PBMCs to differentiate into macrophages. Preparing a suspension of PBMCs from peripheral blood can be performed by any method commonly known in the art. As a non-limiting example, PBMCs can be prepared by Ficoll gradient centrifugation. Ficoll gradient centrifugation includes transferring a volume of Ficoll in a tube, such as a test tube. Whole blood is then gently overlayed onto the Ficoll and the tube

is centrifuged for from about 15 minutes to about 60 minutes at from about 175 g to about 225 g at room temperature. In a preferred embodiment, the tube is centrifuged for 45 minutes at 200 g. After centrifugation, there remains a pellet of red blood cells, a Ficoll layer, a white layer comprising PBMCs, and a plasma layer. The white layer comprising PBMCs can then be removed from the tube. Because the PBMCs include monocytes and lymphocytes, the PBMCs can be processed to isolate the monocytes. For example, an Anti-CX3CR1 MicroBeads Kit (Miltenyi Biotec Inc., Auburn, Calif.) can be used to specifically bind monocytes to magnetic beads, which can then be separated from the lymphocytes. Alternatively, the PBMCs can be separated from lymphocytes by flow cytometry techniques, such as fluorescence-activated cell sorting (FACS). After isolation, PBMCs can be cultured in Macrophage Base Medium DXF (PrmoCell), which does not induce differentiation. Differentiation of PBMCs or isolated monocytes into macrophages can be induced by culturing the PBMCs or isolated monocytes, for example, in the presence of differentiation medium containing macrophage colony-stimulating factor (M-CSF) or granulocyte-macrophage colony-stimulating factor (GM-CSF). In various embodiments, a differentiation medium is Macrophage Base Medium DXF (Promocell, Heidelberg, Germany). Once differentiated into macrophages, the macrophages can be suspended in a medium to generate the concentrated macrophage solution. The M2 macrophages can then be manipulated, e.g., transfected and engineered, to produce the targeted macrophages described herein.

[0054] Culturing Process. The culture medium to be used may be a basic culture medium containing components (inorganic salts, carbohydrates, hormones, essential amino acids, non-essential amino acids, and vitamins) and the like required for the cell's viable growth. Examples of the culture medium include Dulbecco's Modified Eagle's Medium (DMEM), Minimum Essential Medium (MEM), Basal Medium Eagle (BME), Dulbecco's Modified Eagle's Medium: Nutrient Mixture F-12 (DMEM/F-12), Glasgow Minimum Essential Medium (Glasgow MEM), Gibco® RPMI 1640 culture medium (manufactured by Life Technologies), HL-1 known composition, serum-free culture medium (manufactured by Lonza Inc.), and the like. In the culturing process, the culture medium may be suitably replaced with a new one according to the growth rate of the cells.

[0055] In addition, a compound inducing the differentiation or trait of the macrophage may be added to the culture medium to be used. By adding the compound, the rate of differentiation or trait change can be further accelerated, and differentiation or trait can be controlled in a certain direction. Examples of compounds that trait-induce the macrophage into the M1 macrophage include Th1 cytokines such as interferon (IFN)-γ, tumor necrosis factor (TNF)-α, lipopolysaccharide (LPS) and the like, and two or more of these compounds may be used in combination. In addition, examples of compounds that trait-induce the macrophage into the M2 macrophage include Th2 cytokines such as interleukin (IL)-4 and IL-13, and two or more of these compounds may be used in combination. In addition, the compounds trait-inducing into the M1 macrophage and the compounds trait-inducing into the M2 macrophage may be used in combination.

[0056] The concentration of the compounds that induce the macrophage differentiation is not particularly limited, and may be 1 nM or more and 1 μM or less, and may be 5 nM or more and 100 nM or less. Within the above range, it is possible to more efficiently induce the trait from the macrophage into the M1 or M2 macrophage.

[0057] Culture conditions are not particularly limited as long as it is a method suitable for culturing the macrophage, for example, the density of seeding the macrophage in the culture medium is preferably 1×10^0 to 1×10^7 cells/mL, and more preferably 1×10^2 to 1×10^6 cells/mL. The culture temperature is preferably 25° C. or more and 40° C. or less, more preferably 30° C. or more and 39° C. or less, and further preferably 35° C. or more and 39° C. or less. The culturing time can be appropriately set depending on the growth state of the macrophage, and it is preferably 1 hour or more and 100 hours or less. The culture environment is preferably cultured under CO_2 conditions through approximately 5% carbon dioxide.

Genetic Constructs

[0058] In some embodiments, genetically-engineered macrophages of the present invention can include one or more recombinant genes. Genetic constructs contemplated for use herein can be transiently expressed or permanently expressed in a recombinant host cell. In one particular embodiment, a genetically-engineered macrophage can include one or more genes that can be used to target the cell (e.g.. a macrophage) to a desired location, such as the liver, heart, lung or specifically to a fibrotic scar. For example, a genetically-engineered macrophage can include one or more recombinant collagen receptors or subunits thereof. Examples of contemplated collagen receptors useful herein include, but are not limited to, integrins. In one embodiment, genetically-engineered macrophages include one or more of subunits of α1β1, α2β1, α10β1, and/or α11β1 integrins. Specific examples include integrin A1 or α1 (ITGA-1), such as shown in SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, and/or SEQ ID NO:4. Other contemplated collagen receptors include discoidin domain receptors, such as DDR1 (e.g., NP_001189450) and/or DDR2 (e.g., NP_001014796), mannose family receptors, such as M-phospholipase A2 receptor (e.g., NP_001007268 and Endo180 receptor (e.g., P22897), and immunoglobulin-like receptors, such as glycoprotein VI (e.g. NP_001077368). In one particular embodiment, a genetically-engineered macrophage includes and expresses ITGA-1 (integrin a subunit 1). While not wishing to be bound by theory, it is believed that expression of one or more targeting proteins, such as a collagen receptor or subunit thereof, will not only augment targeting of genetically-engineered macrophages to the liver, heart, lung or other tissue, but will also cause the macrophages to be retained at the site of damage (a collagen-rich environment) for a longer period of time and thereby increase their efficacy, specificity, and safety for treating fibrosis.

[0059] In another embodiment, a genetically-engineered macrophage of the present invention can include one or more genes that enhance fibrosis (e.g., liver, cardiac, or lung) degradation. For example, a genetically-engineered macrophage of the present invention can include one or more collagenases. In one particular example contemplated herein, genetically-engineered macrophages described herein include and express one or more matrix metalloproteinases (MMPs). Examples of contemplated MMPs

include, but are not limited to, MMP1, MMP1a, MMP2, MMP3, MMP7, MMP8, MMP9, MMP10, MMP12, MMP13, MMP14, MMP17, MMP19, MMP20, MMP21, MMP22, MMP24, MMP25, MMP26, MMP27, and MMP28 (Caley et al. *Adv. Wound Care (New Rochelle)* 2015, 4:225-34). In some embodiments, one or more MMPs may be excluded.

[0060] It is further contemplated that genetically-engineered macrophages of the present invention can include other organ or tissue-specific targeting proteins, peptides, and/or molecules and/or other catalytic enzymes or substances to remove fibrotic scars from an afflicted individual.

Treatment Methodologies

[0061] In some embodiments of the present invention, methods of treating an individual for fibrosis are contemplated. Examples of conditions that can be treated include liver fibrosis, cardiac fibrosis, pulmonary fibrosis, arthrofibrosis, myelofibrosis, mediastinal fibrosis, retroperitoneal fibrosis, nephrogenic systemic fibrosis, as well as keloids, Crohn's disease, fibrocystic breasts, and Peyronie' s disease, among others. In one specific embodiment, a method of treating an individual for liver, cardiac, or lung fibrosis includes acquiring a population of macrophages, genetically-engineering the population of macrophages to express a fibrosis targeting protein, and administering the population of genetically-engineered macrophages to the individual.

[0062] Genetically-engineered macrophages of the present invention can be prepared and used immediately to treat an individual in need thereof. Alternatively, a population of genetically-engineered macrophages can be prepared and frozen for later use.

[0063] Administration of genetically-engineered macrophages can be through any means generally accepted for the administration of cells to an individual (e.g., intravenously). In some embodiments, genetically-engineered macrophages can be introduced into an individual in need thereof by portal vein injection, intracardiac injection, or intravenous (IV) injection.

[0064] Liver fibrosis. Liver fibrosis or fibrotic scarring of the liver often occurs in patients with chronic liver disease. Diseases such as hepatitis infection (via hepatitis B virus or hepatitis C virus), Wilson's disease. blocked bile duct, non-alchoholic fatty liver and alcohol abuse (such as alcohol use disorder or "AUD") commonly lead to the development of liver fibrosis, though exposure to toxins and trauma have also been associated with the condition. Liver fibrosis is the result of excessive accumulation of extracellular matrix (ECM) proteins, especially α1 collagen, produced by cells such as hepatic stellate cells (HSCs) responding to liver injury (i.e., chronic activation of the wound-healing reaction).

[0065] Typically, at least several months to years of ongoing liver injury are required to cause fibrosis. Advanced liver fibrosis can lead to cirrhosis, hepatic insufficiency, portal hypertension, and liver failure. There are few treatment options for patients with end-stage chronic liver disease with liver transplantation being the last resort for those whose liver has been damaged beyond its capacity to regenerate. However, liver transplantation is an extremely invasive and risky medical intervention. As well, patients with end-stage liver disease are often not eligible for transplantation. Moreover, liver transplantation is extremely-expensive, and can cost in excess of $600,000 in the United States. Therefore, new treatment options are needed for individuals with liver fibrosis.

[0066] One potential treatment option is to reverse liver fibrosis. Approaches to reversing liver fibrosis have been under investigation for nearly 50 years. Even so, the best line of attack for reversing liver fibrosis remains to be attempting to remove the primary disease causing the fibrosis and allowing the liver to regenerate. Even so, liver regeneration cannot always fully reverse liver fibrosis, and the ability of the liver to regenerate is progressively lost in individuals with advancing liver disease. Therefore, ongoing therapeutic investigations are developing an antifibrotic armamentarium of chemical compounds aimed at various molecular and cellular targets to prevent or slow fibrosis. Examples of antifibrotic chemical candidates include α-tocopherol (inhibits HSC activation), interferon-γ (inhibits ECM synthesis in HSCs), quercetin (antioxidant), ACE inhibitors (inhibit HSC proliferation). and PPAR-δ (see Houlum et al. *Gastroenterology* 1997, 113:1069-73; Rockey-et al. *J Investig Med.* 1994, 42:660-70; Pavanato et al. *Dig Dis Sci.* 2003, 48:824-9; Warner et al. *Clin Sci (Loud)* 2007, 113:109-18; Marra et al. *Gastroenterology* 2000, 119:466-78). However, many of these nascent therapeutic candidates apparently function by preventing development of liver fibrosis (inhibiting chronic wound healing) rather than by removing existing fibrotic scarring. There are alternative approaches for reversing liver fibrosis.

[0067] One alternative approach for treating liver disease is being explored that utilizes bone marrow cell therapy for improving liver fibrosis. Using animal models of experimental liver damage has shown that macrophages can play a key role in the control and repair of fibrotic liver disease (Ramachandran et al. *Proc Natl Acad Sci USA* 2012, 109: E3186-95). Indeed, some studies of bone marrow cell therapy for liver cirrhosis have shown improvements in several clinical parameters in experimental chronic liver injury. (Thomas et al. *Hepatology* 2011, June; 53(6):2003-15). However, existing cell-based approaches have limited efficacy.

[0068] Cardiac fibrosis. Cardiac fibrosis, a hallmark of heart disease, is thought to contribute to sudden cardiac death, ventricular tachyarrhythmia, left ventricular (LV) dysfunction, and heart failure. Cardiac fibrosis is characterized by a disproportionate accumulation of fibrillated collagen that occurs after myocyte death, inflammation, enhanced workload, hypertrophy, and stimulation by a number of hormones. cytokines, and growth factors.

[0069] Cardiac fibrosis may also refer to an abnormal thickening of the heart valves due to inappropriate proliferation of cardiac fibroblasts but more commonly refers to the proliferation of fibroblasts in the cardiac muscle. Fibrocyte cells normally secrete collagen, and function to provide structural support for the heart. When over-activated this process causes thickening and fibrosis of the valve, with white tissue building up primarily on the tricuspid valve, but also occurring on the pulmonary valve. The thickening and loss of flexibility eventually may lead to valvular dysfunction and right-sided heart failure.

[0070] The most obvious treatment for cardiac valve fibrosis or fibrosis in other locations, consists of stopping the stimulatory drug or production of serotonin. Surgical tricuspid valve replacement for severe stenosis (blockage of blood flow) has been necessary in some patients. Also, a com-

pound found in red wine, resveratrol, has been found to slow the development of cardiac fibrosis. (Olson et al. (2005) *American journal of physiology. Heart and circulatory physiology* 288(3):H1131-8; Aubin, et al. (2008) *The Journal of Pharmacology and Experimental Therapeutics* 325 (3):961-8). More sophisticated approaches of countering cardiac fibrosis like microRNA inhibition (miR-21, for example) are being tested in animal models.

[0071] Heart disease is the major cause of mortality in developed countries, accounting for an annual death of about 800,000 in United States alone. Numerous forms of cardiovascular disease exist that have differential pathological observations. Most cardiac diseases are associated with cardiac fibrosis that refers to an abnormal scarring process of heart valves caused by inappropriate proliferation of myofibroblast and excessive deposition of extracellular matrix (ECM) proteins in cardiac muscle. Myofibroblasts are principally responsible for deposition of the excessive fibrotic ECM. (Travers et al. *Circ Res,* 2016, 118(6):1021-40).

[0072] Activation of cardiac fibrosis has been extensively studied in the past few decades. In response to acute cardiac injury like ischemia or myocardium infarction, or chronic disease like hypertension, diabetic cardiomyopathy, Cardiac Fibroblast (CFs) within the connective tissue in the heart is activated and transformed to myofibroblasts, which induce excessive extracellular matrix (ECM) deposition.(Liu et al. *Front Physiol.,* 2017, 8:238; Tian et al. *Exp Ther Med* 2017, 13(5):1660-4).

[0073] There are two most common types of cardiac fibrosis, Reactive Interstitial Fibrosis (RIF) and Replacement Fibrosis (RF). RIF is often induced by one or multiple progressive chronic courses (e.g., diabetics and hypertension) that is characterized by diffused deposition of collagen protein (a type of ECM) and increased interstitial compartment volume. RF occurs after acute injury while the expansion of ECM and elevated collagen I deposition replace the dead cardiomyocyte in order to prevent the infarcted myocardium from rupture. In general, the increased cardiac fibrosis leads to distorted organ architecture and function that results in heart failure. (McLenachan and Dargie, *Am J Hypertens* 1990, 3(10):735-40; Krenning et al. *J Cell Physiol* 2010, 225(3):631-7; Mewton et al., *J Am Coll Cardiol* 2011, 57(8):891-903).

[0074] During the pathological process of cardiac fibrosis, the necrotic and apoptotic cardiomyocytes trigger the excessive accumulation of ECM proteins in both RIF and RF. Thus it is hypothesized that, using macrophage subsets with anti-inflammatory properties may have direct anti-fibrotic effects by clearing necrotic and apoptotic cells and suppressing fibroblast activation. In this studies described below, macrophages were injected directly into ischemic mouse heart and monitored the cardiac function to investigate the anti-fibrotic potential of the cellular therapy.

[0075] Current clinical therapies for cardiac fibrosis mainly rely on established pharmacological agents. ACE inhibitors, statins and aldosterone antagonists are among the drugs that have been shown to exert beneficial effects on cardiac fibrosis. ACE inhibitors like Lisinopril regress cardiac fibrosis and improve LV function in patients with hypertension. Statins treatment with Atorvastatin reduces fibrotic biomarker in heart failure patients. Spironolactone, an aldosterone antagonist, can reduce cardiac fibrosis in cardiomyopathy. Nevertheless, existing treatments have several major shortcomings: (1)These drugs can only moderately improve the heart functions; (2)What is more problematic is none of the existing therapies exclusively treats fibrosis in the heart; (3) these treatments target the causes or symptoms but fail to effectively inhibit myocardial scar formation, which leaves the patients with severe cardiac fibrosis with little options. New compounds targeting key components of pro-fibrotic pathways are being tested on animal models and pre-clinical trials. but so far the results are mixed and clinical translations are very limited. Lack of effective clinical treatment for cardiac fibrosis brings an urgent need for developing novel, tissue-specific and effective therapeutic approaches using unconventional strategy like cellular therapy with engineered macrophages.

[0076] The methods described herein are suitable for treating an individual who has been diagnosed with a disease related to progressive cardiac fibrosis, who is suspected of having a disease related to progressive cardiac fibrosis, who is known to be susceptible and who is considered likely to develop a disease related to progressive cardiac fibrosis, or who is considered likely to develop a recurrence of a previously treated disease relating to progressive cardiac fibrosis.

[0077] Existing evidence demonstrates the association of fibrosis with the heart failure process in a variety of heart diseases, including those associated with both volume and pressure overload (Maron et al, *Am. J. Cardiol.,* 35:725-39 (1975); Schwarz et al, *Am. J. Cardiol.,* 42:661-69 (1978); Fuster et al, *Circ.,* 55:504-08 (1976); Bartosova et al, *J. Physiol.,* 200:285-95 (1969); Weber et al, *Circ.,* 83:1849-65 (1991); Schaper et al, *Basic Res. Cardiol.,* 87:S1303-S1309 (1992); Boluyt et al, *Circ. Res.,* 75:23-32 (1994); and Bishop et al, *J. Mol. Cell Cardiol.,* 22:1157-65 (1990)). In the setting of heart failure, fibrosis involves an increase in both fibroblast number and matrix deposition (Morkin et al, *Am. J. Physiol.,* 215:1409-13 (1968); Skosey et al, *Circ. Res.,* 31:145-57 (1972); and Booz et al, *Cardiovasc. Res.,* 30:537-43 (1995)), suggesting the importance of the fibroblast in the development of this condition. Cardiac fibroblasts are also the predominant source of synthesis of interstitial proteins and other myocardial components which have been implicated in heart failure by their effects on diastolic function and, indirectly, by effects on cardiac myocytes to cause or potentiate systolic dysfunction (Hess et al, *Circ.,* 63:360-71 (1981); Villari et al, *Am J. Cardiol.,* 69:927-34 (1992); Villari et al, *JACC,* 22:1477-84 (1993); Brilla et al, *Circ. Res.,* 69:107-15 (1991); and Sabbah et al, *Mol. & Cell Biochem.,* 147:29-34 (1995)).

[0078] The treatment of the fibrotic cardiac disease state can be determined by measuring one or more diagnostic parameters indicative of the course of the disease, compared to a suitable control. For comparison with animal models, a "suitable control" is an animal not treated with relaxin, or treated with the pharmaceutical formulation without relaxin. In the case of a human subject, a "suitable control" may be the individual before treatment, or may be a human (e.g., an age-matched or similar control) treated with a placebo.

[0079] Cardiac fibrosis to be treated by the methods of the present invention may be due to a variety of diseases associated with cardiac fibroblast proliferation or the activation of extracellular matrix protein synthesis by cardiac fibroblasts. These diseases may be effectively treated in the present invention. Such diseases include aortic and mitral valvular regurgitation. In addition, cardiac hypertrophy, which is associated with many cardiac diseases, and often

involves myocyte and fibroblast components, may be effectively treated in the present invention.

[0080] Heart failure is defined as the inability of the cardiac pump to move blood as needed to provide for the metabolic needs of body tissue. Decreases in pumping ability arise most often from loss or damage of myocardial tissue. As a result, ventricular emptying is suppressed which leads to an increase in ventricular filling pressure and ventricular wall stress, and to a decrease in cardiac output. As a physiological response to the decrease in cardiac output, numerous neuroendocrine reflexes are activated which cause systemic vasoconstriction, sympathetic stimulation of the heart and fluid retention. Although these reflex responses tend to enhance cardiac output initially, they are detrimental in the long term. The resulting increases in peripheral resistance increase the afterload on the heart and the increases in blood volume further increase ventricular filling pressure. These changes, together with the increased sympathetic stimulation of the heart, lead to further and often decompensating demands on the remaining functional myocardium.

[0081] Congestive heart failure, which is a common end point for many cardiovascular disorders, results when the heart is unable to adequately perfuse the peripheral tissues. According to recent estimates, there are about 4 million people in the United States diagnosed with this disease, and more than 50% of these cases are fatal within 5 years of diagnosis (Taylor et al., *Annual Reports in Med. Chem.* 22, 85-94 (1987)).

[0082] Lung fibrosis and Pulmonary Fibrosis Diseases. Pulmonary fibrosis disease is a devastating chronic lung disease resulting in scarring (fibrosis) of the lungs. Over time, the scarring gets worse and it becomes hard to take in a deep breath and the lungs cannot take in enough oxygen. Lung function decline is gradual, with the potential for intermittent, unpredictable, acute exacerbations and the development of associated pulmonary hypertension. Sometimes doctors can identify the cause of the fibrosis, but in most cases, they cannot. They call these cases idiopathic pulmonary fibrosis (IPF).

[0083] Pulmonary fibrosis disease primarily affect middle aged and older adults. About 50,000 people in the U.S. have idiopathic pulmonary fibrosis and an estimated 15,000 new cases develop each year. According to NIH/National Heart Lung, and Blood Institute, currently, no medicines are proven to slow the progression of IPF. Prednisone, azathioprine and N-acetylcysteine have been used to treat IPF, either alone or in combination. However, experts have not found enough evidence to support their use.

Cotherapies

[0084] In some embodiments, cotherapies are envisioned in the present application. For example, a method of treating an individual with liver fibrosis can include introducing a cellular therapy product including a genetically-engineered macrophage into the individual and administering to the individual an effective amount of one or more of α-tocopherol, interferon-γ, quercetin, an ACE inhibitor, and PPAR-δ.

[0085] In certain instances method of treating may further involve performing surgery on the patient, such as by resecting all or part of the liver or fibrotic regions of the liver. Cellular therapy product may be administered to the patient before, after, and/or at the same time as surgery. In certain aspects the methods can be used to ameliorate fibrosis resulting from surgery and assist in regeneration. In other aspects, the methods can be used treat or reducing fibrotic areas not removed by surgery.

Polypeptide Composition

[0086] "Polypeptide" refers to any peptide or protein comprising amino acids joined by peptide bonds or modified peptide bonds. "Polypeptide" can include short chain polypeptides, including peptides, oligopeptides or oligomers, and longer chain polypeptides, including proteins. Polypeptides may contain amino acids other than the 20 gene-encoded amino acids. "Polypeptides" include amino acid sequences modified either by natural processes, such as post-translational processing, or by chemical modification or other synthetic techniques well known in the art. Modifications can occur anywhere in a polypeptide, including the peptide backbone, the amino acid side-chains, and the amino terminus or the carboxy terminus. It will be appreciated that the same type of modification may be present in the same or varying degrees at several sites in a given polypeptide. Also, a given polypeptide may contain many types of modifications. Modifications include terminal fusion (N- and/or C-terminal), acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphotidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cystine, formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination.

[0087] The term "isolated" can refer to a nucleic acid or polypeptide that is substantially free of cellular material, bacterial material, viral material, or culture medium (when produced by recombinant DNA techniques) of their source of origin, or chemical precursors or other chemicals (when chemically synthesized). Moreover, an isolated polypeptide refers to one that can be administered to a subject as an isolated polypeptide; in other words, the polypeptide may not simply be considered "isolated" if it is adhered to a column or embedded in a gel. Moreover, an "isolated nucleic acid fragment" or "isolated peptide" is a nucleic acid or protein fragment that is not naturally occurring as a fragment and/or is not typically in the functional state.

[0088] The term "amino acid" or "residue" should be understood to mean a compound containing an amino group (NH_2), a carboxylic acid group (COOH), and any of various side groups, that have the basic formula $NH_2CHRCOOH$, and that link together by peptide bonds to form proteins. Amino acids may, for example, be acidic, basic, aromatic, polar or derivatized. Non-standard amino acids may be referred to as "non-canonical" amino acids. Amino acids are naturally found in the α- and L-form, however, β- and D-form amino acids can also be prepared.

[0089] A one-letter abbreviation system is frequently applied to designate the identities of the twenty "canonical" amino acid residues generally incorporated into naturally occurring peptides and proteins, these designation are well known in the art. Such one-letter abbreviations are entirely

interchangeable in meaning with three-letter abbreviations, or non-abbreviated amino acid names. The canonical amino acids and their three letter and one letter codes include Alanine (Ala) A, Glutamine (Gln) Q, Leucine (Leu) L, Serine (Ser) S, Arginine (Arg) R, Glutamic Acid (Glu) E, Lysine (Lys) K, Threonine (Thr) T, Asparagine (Asn) N, Glycine (Gly) G, Methionine (Met) M, Tryptophan (Trp) W, Aspartic Acid (Asp) D. Histidine (His) H, Phenylalanine (Phe) F, Tyrosine (Tyr) Y, Cysteine (Cys) C, Isoleucine (Ile) I, Proline (Pro) P, and Valine (Val) V.

[0090] Certain embodiments also include variants of the polypeptides described herein. Variants of the disclosed polypeptides may be generated by making amino acid additions or insertions, amino acid deletions, amino acid substitutions, and/or chemical derivatives of amino acid residues within the polypeptide sequence. Desired amino acid substitutions (whether conservative or non-conservative) can be determined by those skilled in the art in accordance with guidance provided herein for increasing stability, while maintaining or enhancing potency of the polypeptides. In certain embodiments, conservative amino acid substitutions can encompass non-naturally occurring amino acid residues which are typically incorporated by chemical peptide synthesis rather than by synthesis in biological systems.

[0091] Conservative modifications can produce peptides having functional, physical, and chemical characteristics similar to those of the peptide from which such modifications are made. In contrast, substantial modifications in the functional and/or chemical characteristics of peptides may be accomplished by selecting substitutions in the amino acid sequence that differ significantly in their effect on maintaining (a) the structure of the molecular backbone in the region of the substitution, for example, as an α-helical conformation, (b) the charge or hydrophobicity of the molecule at the target site, or (c) the size of the molecule. For example, a "conservative amino acid substitution" may involve a substitution of a native amino acid residue with a non-native residue such that there is little or no effect on the polarity or charge of the amino acid residue at that position.

[0092] Recombinant DNA- and/or RNA-mediated protein expression and protein engineering techniques, or any other methods of preparing peptides, are applicable to the making of the polypeptides disclosed herein or expressing the polypeptides disclosed herein in a target cell or tissue. The term "recombinant" should be understood to mean that the material (e.g., a nucleic acid or a polypeptide) has been artificially or synthetically (i.e., non-naturally) altered by human intervention. The alteration can be performed on the material within, or removed from, its natural environment or state. For example, a "recombinant nucleic acid" is one that is made by recombining nucleic acids, e.g., during cloning, DNA shuffling or other well-known molecular biological procedures. Examples of such molecular biological procedures are found in Maniatis et al., *Molecular Cloning. A Laboratory Manual.* Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 1982. A "recombinant DNA molecule," is comprised of segments of DNA joined together by means of such molecular biological techniques. The term "recombinant protein" or "recombinant polypeptide" as used herein refers to a protein molecule which is expressed using a recombinant DNA molecule. A "recombinant host cell" is a cell that contains and/or expresses a recombinant nucleic acid.

[0093] The polypeptides can be made in transformed host cells according to methods known to those of skill in the art. Briefly, a recombinant DNA molecule, or construct, coding for the peptide is prepared. Methods of preparing such DNA molecules are well known in the art. For instance, sequences encoding the peptides can be excised from DNA using suitable restriction enzymes. Any of a large number of available and well-known host cells may be used in the practice of various embodiments. The selection of a particular host is dependent upon a number of factors, which include, for example, compatibility with the chosen expression vector, toxicity of the polypeptides encoded by the DNA molecule, rate of transformation, ease of recovery of the polypeptides, expression characteristics, bio-safety, and costs. A balance of these factors should be struck with the understanding that not all hosts may be equally effective for the expression of a particular DNA sequence. Within these general guidelines, useful microbial host cells in culture include bacteria (such as *Escherichia coli* sp.), yeast (such as *Saccharomyces* sp.) and other fungal cells, insect cells, plant cells, mammalian (including human) cells, e.g., CHO cells and HEK293 cells. Modifications can be made at the DNA level, as well. The peptide-encoding DNA sequence may be changed to codons more compatible with the chosen host cell. For *E. coli,* optimized codons are known in the art. Codons can be substituted to eliminate restriction sites or to include silent restriction sites, which may aid in processing of the DNA in the selected host cell. Next, the transformed host is cultured and purified. Host cells may be cultured under conventional fermentation conditions so that the desired polypeptides are expressed. In addition, the DNA optionally further encode, 5' to the coding region of a fusion protein, a signal peptide sequence (e.g., a secretory signal peptide) operably linked to the expressed polypeptide.

Expression and Expression Vectors

[0094] The nucleic acids encoding any polypeptide(s) described herein can be inserted into or employed with any suitable expression system. Recombinant expression can be accomplished using a vector, such as a plasmid, virus, etc. The vector can include a promoter operably linked to nucleic acid encoding one or more polypeptides. The vector can also include other elements required for transcription and translation. As used herein, vector refers to any carrier containing exogenous DNA. Thus, vectors are agents that transport the exogenous nucleic acid into a cell without degradation and include a promoter yielding expression of the nucleic acid in the cells into which it is delivered. Vectors include but are not limited to plasmids, viral nucleic acids, viruses, phage nucleic acids, phages, cosmids, and artificial chromosomes. A variety of prokaryotic and eukaryotic expression vectors suitable for carrying, encoding and/or expressing nucleic acids encoding proteases can be produced. Such expression vectors include, for example, pET, pET3d, pCR2.1, pBAD, pUC, and yeast vectors. The vectors can be used, for example, in a variety of in vivo and in vitro situations. The vector may be a gene therapy vector, for example an adenovirus vector, a lentivirus vector or a CRISP-R vector.

[0095] The expression cassette, expression vector, and sequences in the cassette or vector can be heterologous. As used herein, the term "heterologous" when used in reference to an expression cassette, expression vector, regulatory sequence, promoter, or nucleic acid refers to an expression cassette, expression vector, regulatory sequence, or nucleic

acid that has been manipulated in some way. For example, a heterologous promoter can be a promoter that is not naturally linked to a nucleic acid to be expressed, or that has been introduced into cells by cell transformation procedures. A heterologous nucleic acid or promoter also includes a nucleic acid or promoter that is native to an organism but that has been altered in some way (e.g., placed in a different chromosomal location, mutated, added in multiple copies, linked to a non-native promoter or enhancer sequence, etc.). Heterologous nucleic acids may comprise sequences that comprise cDNA. Heterologous coding regions can be distinguished from endogenous coding regions, for example, when the heterologous coding regions are joined to nucleotide sequences comprising regulatory elements such as promoters that are not found naturally associated with the coding region, or when the heterologous coding regions are associated with portions of a chromosome not found in nature (e.g., genes expressed in loci where the protein encoded by the coding region is not normally expressed). Similarly, heterologous promoters can be promoters that are linked to a coding region to which they are not linked in nature.

[0096] Viral vectors that can be employed include those relating to lentivirus, adenovirus, adeno-associated virus, herpes virus, vaccinia virus, polio virus, AIDS virus, neuronal trophic virus, Sindbis and other viruses. Also useful are any viral families which share the properties of these viruses which make them suitable for use as vectors. Retroviral vectors that can be employed include those described in by Verma, I. M., Retroviral vectors for gene transfer. In Microbiology-1985, American Society for Microbiology, pp. 229-232, Washington, (1985). For example, such retroviral vectors can include Murine Maloney Leukemia virus, MMLV, and other retroviruses that express desirable properties. Typically, viral vectors contain, nonstructural early genes, structural late genes, an RNA polymerase III transcript, inverted terminal repeats necessary for replication and encapsidation, and promoters to control the transcription and replication of the viral genome. When engineered as vectors, viruses typically have one or more of the early genes removed and a gene or gene/promoter cassette is inserted into the viral genome in place of the removed viral nucleic acid.

[0097] A variety of regulatory elements can be included in the expression cassettes and/or expression vectors, including promoters, enhancers, translational initiation sequences, transcription termination sequences and other elements. A "promoter" is generally a sequence or sequences of DNA that function when in a relatively fixed location in regard to the transcription start site. For example, the promoter can be upstream of the nucleic acid segment encoding a protease. A "promoter" contains core elements required for basic interaction of RNA polymerase and transcription factors and can contain upstream elements and response elements. "Enhancer" generally refers to a sequence of DNA that functions at no fixed distance from the transcription start site and can be either 5' or 3' to the transcription unit. Furthermore, enhancers can be within an intron as well as within the coding sequence itself. They are usually between 10 and 300 nucleotides in length, and they function in cis. Enhancers function to increase transcription from nearby promoters. Enhancers, like promoters, also often contain response elements that mediate the regulation of transcription. Enhancers often determine the regulation of expression.

[0098] Expression vectors used in eukaryotic host cells (yeast, fungi, insect, plant, animal, human or nucleated cells) can also contain sequences necessary for the termination of transcription which can affect mRNA expression. These regions are transcribed as polyadenylated segments in the untranslated portion of the mRNA encoding tissue factor protein. The 3' untranslated regions also include transcription termination sites. It is preferred that the transcription unit also contains a polyadenylation region. One benefit of this region is that it increases the likelihood that the transcribed unit will be processed and transported like mRNA. The identification and use of polyadenylation signals in expression constructs is well established. It is preferred that homologous polyadenylation signals be used in the expression constructs.

[0099] The expression of one or more protease from an expression cassette or expression vector can be controlled by any promoter capable of expression in prokaryotic cells or eukaryotic cells. Examples of prokaryotic promoters that can be used include, but are not limited to, SP6, T7, T5, tac, bla, trp, gal, lac, or maltose promoters. Examples of eukaryotic promoters that can be used include, but are not limited to, constitutive promoters, e.g., viral promoters such as CMV, SV40 and RSV promoters, as well as regulatable promoters, e.g., an inducible or repressible promoter such as the tet promoter, the hsp70 promoter and a synthetic promoter regulated by CRE. Vectors for bacterial expression include pGEX-5X-3, and for eukaryotic expression include pCIneo-CMV.

[0100] The expression cassette or vector can include nucleic acid sequence encoding a marker product. This marker product is used to determine if the gene has been delivered to the cell and once delivered is being expressed. Preferred marker genes are the *E. coli* lacZ gene which encodes β-galactosidase and green fluorescent protein. In some embodiments the marker can be a selectable marker. When such selectable markers are successfully transferred into a host cell, the transformed host cell can survive if placed under selective pressure. There are two widely used distinct categories of selective regimes. The first category is based on a cell's metabolism and the use of a mutant cell line which lacks the ability to grow independent of a supplemented media. The second category is dominant selection which refers to a selection scheme used in any cell type and does not require the use of a mutant cell line. These schemes typically use a drug to arrest growth of a host cell. Those cells which have a novel gene would express a protein conveying drug resistance and would survive the selection. Examples of such dominant selection use the drugs neomycin (Southern and Berg, *Molec. Appl. Genet.* 1: 327 (1982)), mycophenolic acid, (Mulligan and Berg, *Science* 209: 1422 (1980)) or hygromycin, (Sugden et al., *Mol. Cell. Biol.* 5: 410-13 (1985)).

[0101] Gene transfer can be obtained using direct transfer of genetic material, in but not limited to, plasmids, viral vectors, viral nucleic acids, phage nucleic acids, phages, cosmids, and artificial chromosomes, or via transfer of genetic material in cells or carriers such as cationic liposomes or viruses. Such methods are well known in the art and readily adaptable for use in the method described herein. Transfer vectors can be any nucleotide construction used to deliver genes into cells (e.g., a plasmid), or as part of a general strategy to deliver genes, e.g., as part of recombinant retrovirus or adenovirus (Ram et al. *Cancer Res.* 53:83-88,

(1993)). Appropriate means for transfection, including viral vectors, chemical transfectants, or physico-mechanical methods such as electroporation and direct diffusion of DNA, are described by, for example, Wolff et al., *Science,* 247, 1465-1468, (1990); and Wolff, *Nature,* 352, 815-818, (1991).

[0102] For example, the nucleic acid molecule, expression cassette and/or vector encoding a protease can be introduced to a cell by any method including, but not limited to, calcium-mediated transformation, electroporation, microinjection, lipofection, particle bombardment and the like. The cells can be expanded in culture and then administered to a subject, e.g., a mammal such as a human. The amount or number of cells administered can vary but amounts in the range of about 10^6 to about 10^9 cells can be used. The cells are generally delivered in a physiological solution such as saline or buffered saline. The cells can also be delivered in a vehicle such as a population of liposomes, exosomes or microvesicles.

EXAMPLES

[0103] The Examples that follow are illustrative of specific embodiments of the invention, and various uses thereof. They are set forth for explanatory purposes only and are not taken as limiting the invention.

Example 1

Reduction of Liver Fibrosis in an Animal Model of Cirrhosis

[0104] In this example, M2-specific macrophages were used to treat an animal model of cirrhosis to demonstrate the ability of the macrophages to reverse liver fibrosis.

[0105] Animals. Six to eight week old male C57BJ/6 mice were purchased from the Jackson Lab and housed under specific pathogen-free conditions in the University of Chicago animal core facility. Animals consumed a standard sterile diet and filtered water ad libitum under a 12 hr light-dark cycle. The experimental protocol was approved by the Animal Care and Use Committee and the Ethics Committee of University of Chicago.

[0106] Induction of cirrhosis. Mice were intraperitoneally injected with 20% CCl_4 in corn oil at a dose of 0.1 mL/10 g body weight for 6-8 weeks to induce cirrhosis.

[0107] Treatment. Sedated mice were placed in a supine position with abdomen exposed and disinfected. Buprenorphine was subcutaneously given at a dose of 0.1 mg/kg before surgery. After a single 1.5 cm incision was made along the middle line by starting below the diaphragm, surgically exposing the portal vein without damaging intestines, liver, or diaphragm, 3.0×10^6 M2-specific macrophages were collected in 100 μL PBS and slowly injected into the portal vein towards the liver mass. Incisions were closed using Nylon sutures. One hundred microliters of bupivacaine (5 mg/mL) were injected along the incision site for local pain management. One half milliliter of sterile saline was injected subcutaneously for hydration. Buprenorphine was re-administered every 12 hours for up to 72 hrs.

[0108] Ultrasonic Scan. At week 10 post CCl_4 treatment, sedated mice were placed in a supine position with abdomen hair removed. Due to the disproportional ratio of the ultrasonic probe to mouse body size, only longitudinal scans from the outer margin of the left side lobe to the outer margin of the far right side lobe were conducted for images of liver tissue texture reflection. Fibrotic tissues gave relatively stronger echo signals.

[0109] Histology. Subsequent to ultrasonic liver scans of the treated mice, liver lobes were collected and fixed in 10% formalin for histology. Trichrome and hematoxylin and eosin staining were performed on the fixed liver tissue samples. Hematoxylin and eosin staining was for general pathological evaluation and trichrome staining highlights collagen fibers. Histological evaluation for each group was performed by following HAI-Knodell Score system, one of the most recognized numeric scoring systems for pathologists to evaluate acute and chronic hepatic conditions in terms of liver parenchymal damage, inflammation, and fibrotic lesions. As shown in Table 1, all the listed aspects of hepatic pathological appearance were examined and scored with various weights. The total scores of each sample indicate the severity of liver damage and the efficacy of the treatment in a semi-quantitative way.

[0110] The results from this study demonstrate that the M2-specific macrophages significantly reversed established liver fibrosis in a mouse model of cirrhosis. As can be seen in FIG. **1**, a marked reduction in liver fibrosis is evident based on ultrasonic scans. Treatment of cirrhotic mice with M2-specific macrophages led to marked reductions in inflammation and fibrotic lesions (see FIG. **2**B compared to FIG. **2**A). The reversal of liver fibrosis is further highlighted by the considerable reduction in number and size of liver fibrotic lesions in treated mice shown in FIG. **3**B.

[0111] Further, a histology index was employed based on a previously reported index (see Knodell RG, et al. *Hepatology* 1981,1(5):431-5). Results of the histological assessment shown in Table 1 strongly suggest that portal vein delivery of macrophages significantly reverses liver damage by reducing fibrosis.

TABLE 1

Histology Index (HAI-Knodell Score)					
	Score	Ctrl 1	Ctrl 2	Treated #1	Treated #2
Periportal ± Bridging Necrosis					
None	0				
Mild piecemeal necrosis	1			1	1
Moderate piecemeal necrosis (involves <50% of the circumference of most portal tracts)	3	3			
Marked piecemeal necrosis (involves >50% of the circumference of most portal tracts)	4		4		
Moderate piecemeal necrosis plus bridging necrosis	5				
Marked piecemeal necrosis plus bridging necrosis	6				
Multilobular necrosis	10				
Intralobular Degeneration and Focal Necrosis					
None	0				
Mild (acidophilic bodies, ballooning degeneration and/or scattered foci of hepatocellular necrosis in 1/3 of lobules or nodules)	1			1	1
Moderate (involvement of 1/3-2/3 of lobules or nodules)	3	3	3		
Marked (involvement of >2/3 of lobules or nodules)	4				

TABLE 1-continued

Histology Index (HAI-Knodell Score)					
	Score	Ctrl 1	Ctrl 2	Treated #1	Treated #2
Portal Inflammation					
No portal inflammation	0				
Mild (sprinkling of inflammatory cells in <1/3 of portal tracts)	1			1	1
Moderate (increased inflammatory cells in 1/3-2/3 of portal tracts)	3	3	3		
Marked (dense packing of inflammatory cells in >2/3 of portal tracts)	4				
Fibrosis					
No fibrosis	0				
Fibrous portal expansion	1			1	1
Bridging Fibrosis (portal-portal or portal-central linkage)	3	3	3		
Cirrhosis	4				
Total		12	13	4	4

[0112] A novel therapeutic approach was developed that enhanced decomposition of fibrotic tissue and induced regeneration of functional hepatocytes in liver by delivery of M2-specific macrophages into damaged liver. Through portal vein injection of M2 macrophages, which can turn off inflammatory responses by producing various anti-inflammatory cytokines and function in wound healing and tissue repair, significant effects in reduction of liver fibrosis were observed using a well-established carbon tetrachloride administration model. The results of this study demonstrate the utility of administration of M2-specific macrophages to cirrhotic liver to reverse liver fibrosis in afflicted individuals compared to other macrophage types.

Example 2

Genetically-Engineered Macrophages

[0113] Genetically-engineered M2-specific macrophages are constructed to augment their ability to reverse fibrosis.

[0114] To further increase the efficacy of the approach shown in Example 1, M2-specific macrophages are augmented by exogenous expression of collagen targeting agents or collagen receptors, such ITGA-1. Normal M2-specific macrophages are otherwise incapable of attachment or homing to the collagen-rich environment in fibrotic tissue, and expression of ITGA-1 or other collagen targeting agent will likely greatly enhance the retention of the cells to fibrotic tissues and increase the specificity and safety of the approach. Additionally, expression of collagenase (MMP1) in M2-specific macrophages increases the capability of engineered M2 cells to degrade surrounding abnormal collagen matrices and enhance tissue regeneration. MMP1a is not present in the unmodified M2 cells, and it is the major enzyme that degrades collagen in vivo.

[0115] Genetic Constructs. Lentiviral constructs are assembled for the expression of integrin A1 (SEQ ID NO: 1 or SEQ ID NO: 2, FIG. **4**A) or MMP1 (SEQ ID NO: 3 or SEQ ID NO: 4, FIG. **4**B). Each vector encodes integrin A1 or MMP1 (or MMP1a) driven by a CMV promoter and a selection marker (fluorescence protein tdTomato and puromycin resistant gene, Puro) driven by a constitutive promoter UbiC (Ubiquitin C promoter). TdTomato and Puro are separated by a self-cleavable peptide T2A.

[0116] Macrophages. M2-specific macrophages are transfected with one or both lentiviral constructs and selected for incorporation of the expression vector(s) and expression of the recombinant genes.

[0117] It is contemplated that recombinant M2-specific recombinant macrophages expressing integrin A1, MMP1 or MMP1a, or both integrin A1 and MMP1 or MMP1a can be introduced into an individual as a novel therapeutic approach for liver fibrosis and other fibrotic diseases. Once introduced, the integrin A1-expressing M2-specific macrophages are localized to the fibrotic lesions with greater specificity and are retained longer than in other tissues due to integrin A1 expression. The MMP1- or MMP1a-expressing recombinant M2-specific macrophages reduce fibrotic lesions at a greater rate than non-recombinant M2-specific macrophages. Integrin A1 and MMP1 expressing M2-specific macrophages demonstrate greater fibrotic lesion removal than either of the singly recombinant M2-specific macrophages and greater than non-recombinant M2-specific macrophages. Such recombinant M2-specific macrophages are useful as cellular therapy products for treating fibrotic diseases.

[0118] Having described the invention in detail and by reference to specific aspects and/or embodiments thereof, it will be apparent that modifications and variations are possible without departing from the scope of the invention defined in the appended claims. More specifically, although some aspects of the present invention may be identified herein as particularly advantageous, it is contemplated that the present invention is not limited to these particular aspects of the invention.

Example 3

Reduction of Cardiac Fibrosis in an Animal Model

[0119] In this example, M2-specific macrophages were used to treat an animal model of cardiac fibrosis to demonstrate the ability of the macrophages to ameliorate cardiac fibrosis.

[0120] Animal: 12-week old male C57/BL6 mice.

[0121] Myocardial Infarction(MI): MI was induced through thoracotomy following permanent ligation of left anterior descending (LAD) coronary artery using a 7-0 suture following the procedure as previously described (19).

[0122] Engineered Macrophages Engraftment: 5×10^5 bone marrow derived M0 macrophage (21) in 0.1 ml PBS were directly injected with a 28-gauge syringe to the border-zone of the infarct site immediately after the ligation. Infarct site was identified by the blanching of left ventricle. Control group was injected with PBS only.

[0123] Echocardiography: Echocardiography was performed at 7, 14, and 21 days post operations using a VisualSonic Vevo770 High Resolution Ultrasound System. M-Mode was recorded and echocardiographic parameters were calculated using the pre-installed software in the Vevo770 system.

[0124] Tissue Collection: Mice were sacrificed 21 days post surgery. Heart weight and tibia length were measured.

[0125] The studies to investigate the engineered macrophages in the treatment of cardiac fibrosis involves 5 steps: (i) Generation of murine myocardial infarction(MI)-induced cardiac fibrosis model using LAD; (ii) Differentiation of

bone marrow monocytes into M0 macrophages; (iii) On-site injection of the resulting macrophages into border zone of the infarcted myocardium; (iv) Evaluation of cardiac functions using echocardiography by measuring following parameters: (a) Ejection Fraction (EF)—For left ventricular systolic function, (b) Fraction Shortening (FS) for left ventricular diastolic function, (c) Left Ventricular Internal Dimension at End-diastole (LVID;d) for left ventricular chamber size and myocardium remodeling, (d) Left Ventricular Internal Dimension at End-systole (LVID;s) for left ventricular chamber size and myocardium remodeling; (v) Examination of hypertrophy/myocardium remodeling by measuring heart weight.

[0126] The data showed that engineered macrophages successfully improved cardiac performance in mice with myocardial infarction (MI), indicating a cardioprotective effect of engineered macrophages in treating MI-induced cardiac fibrosis and heart failure. The study demonstrated that the engineered macrophages treatment has various advantages over existing therapies: (1) Effectively repressing the development of cardiac fibrosis evidenced by the improved cardiac functions; (2)Developing a novel tissue-specific strategy by using a direct and localized delivery method; and (3) Avoiding side effects induced by existing pharmacological agents.

[0127] FIG. **5** shows engraftment of engineered macrophages partially prevented MI-induced systolic dysfunction in left ventricle. There were marked deteriorations in Ejection Fraction(EF) and Fraction Shortening(FS) in MI mice received PBS injections, indicating an impaired systolic function/heart failure induced by LAD surgery; Ejection Fraction (EF) and Fraction Shorting (FS) in mice received engineered macrophage were higher than those received PBS, showing cardioprotective effect of engineered macrophage in post-MI heart. Ejection Fraction(EF) and Fraction Shortening(FS) are the two key parameters that measures the percentage of blood pumped out of a filled ventricle with each heartbeat. Decrease in EF and FS indicates the left ventricle loses its ability to distribute enough blood flow to meet the body's needs, a symptom that is clinically defined as "systolic dysfunction", which ultimately leads to heart failure without effective intervention.

[0128] FIG. **6**. shows that cellular therapy using engineered macrophages prevented MI-induced LV dilation. Enlargement of LV chamber size was observed following surgical ligation of the LAD in PBS group. Engraftment of engineered macrophages prevented LV from MI-induced dilation. LVID;d and LVID;s are parameters used to measure the internal dimension of the left ventricle at end-diastolic or end-systolic stage of a heart beating cycle. Increase of these two parameter indicates a enlarged left ventricle in a dilated heart caused by pathological myocardium re-construction.

[0129] FIG. **7**. Shows that cellular therapy using engineered macrophages prevented ischemic myocardium remodeling. Myocardial infarction induced myocardium remodeling in PBS group, evidenced by an increase in heart weight. Lower heart weight in the engineered macrophages group indicates the cellular therapy regressed the remodeling progress. Measurements of "heart weight/body weight" or "heart weight/tibia length" both serve as markers for cardiac fibrosis-induced hypertrophy, as the heart mass increases during the remodeling process.

[0130] FIG. **8**. Shows that cellular therapy using engineered macrophages prevented TAC-induced LV diastolic dysfunction. Increasing of E/A was observed following surgical constraining of the aorta in PBS group. Engraftment of engineered macrophages prevented LV from TAC-induced diastolic dysfunction. E/A is a key parameters used to evaluate the diastolic function of the left ventricle by measuring the peak velocity of mitral annular motion ratio. Increase of this parameter indicates a fibrosis-induced diastolic dysfunction.

[0131] In this study, the effectiveness of engineered macrophages has been validated in treating cardiac fibrosis, making this therapeutic approach a competitive candidate that will likely have tremendous potential for clinical applications. The animal results demonstrates a proof-of-principle for the use of engineered macrophages for treating cardiac fibrosis.

Example 4

Reduction of Lung Fibrosis in an Animal Model

[0132] In this example, M2-specific macrophages were used to treat an animal model of lung fibrosis to demonstrate the ability of the macrophages to ameliorate lung fibrosis.

[0133] Bleomycin(BLM)-induced mouse IPF model. The model of BLM-induced lung fibrosis represents the most commonly applied experimental model. BLM is a chemotherapeutic antibiotic that has been identified as a profibrotic agent when lymphoma patients developed pulmonary fibrosis after intravenous administration of BLM. The recognition that bleomycin could result in pulmonary fibrosis in humans led to its use in experimental models, and for four decades it has been the most commonly applied model of experimental lung fibrosis. It is believed that BLM acts by causing single and double-strand DNA breaks in tumor cells and thereby interrupting cell cycle leading to apoptosis.

[0134] Animal: 10-week old male C57/BL6 mice.

[0135] Generating murine pulmonary fibrosis model: Mice were anesthetized using isoflurane inhalation, then were exposed to bleomycin(BLM) via intratracheal delivery at a dose of 3 U/kg. Control group were administrated with PBS instead.

[0136] Isolation and culturing of macrophages: Isolate then differentiate of mouse bone marrow monocytes into M0 macrophages.

[0137] Engineered Macrophages Engraftment: 5×10^6 bone marrow derived M0 macrophage in 0.1 ml PBS were directly injected with an 1 ml insulin syringe via tail vein 7 days after the BLM exposure. Control group was injected with PBS only.

[0138] Tissue Collection and Histology Analysis: Mice were sacrificed 14 days post BLM exposure. The lung tissues were fixed for 2 h by the intratracheal instillation of 10% neutral formalin and then removed and continuously fixed for 24 h. Then the tissues were embedded with paraffin and subjected to H&E staining.

[0139] The histology analysis showed that Engraftment of macrophages reduced the BLM-induced lung fibrosis and inflammation, and partially preserved structure of pulmonary vesicles.

[0140] In this study, a mouse pulmonary fibrosis model is established through intratracheal delivery of bleomycin (BLM). 14 days post the original exposure of BLM, lung tissue affected with inflammatory reactions and suffered a severe destruction of basic structure of pulmonary vesicles.

[0141] Macrophages treatment via tail vein injection reduced the fibrosis and the degree of inflammation in lungs of mice challenged with BLM. Our data indicate that treatment of macrophages constitute an effective cellular vehicle for the treatment of fibrotic lung disease and present a novel therapeutic approach. The effect of macrophage engraftment on BLM-induced lung injury in mice is shown in FIG. **9**. H&E staining on tissue sections prepared from the lungs of C57BL6 mice 14 days after PBS/BLM exposure. FIG. **9**A shows the histology of control mice exposed to PBS and injected with PBS. FIG. **9**B shows the histology of mice in the fibrosis group exposed to BLM then injected with PBS. FIG. **9**C shows the histology of mice in treatment group exposed to BLM then injected with macrophages.

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465                 470                 475                 480

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                485                 490                 495

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aac aag aat gag ccc tgt ggg gcc cgt ttt gga aca gca gtt gct gct     1728
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gaa aat gca att att cat tta agt gca aca agt gac agt gaa gag ccc     2784
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ctg gaa tct ctt tac gat aat gaa gta aat att tcc atc ccc gta aaa     2832
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Lys Ala Leu Gly Glu Arg Ile Phe Ala Leu Glu Ala Thr Ala Asp Gln
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Trp Asn Gly Thr Val Val Met Gln Lys Ala Asn Gln Ile Val Ile Pro
                405                 410                 415

His Asn Thr Thr Phe Gln Thr Glu Pro Thr Lys Met Asn Glu Pro Leu
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Ala Ser Tyr Leu Gly Tyr Thr Val Asn Ser Ala Thr Ile Pro Gly Asp
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Val Leu Tyr Ile Ala Gly Gln Pro Arg Tyr Asn His Thr Gly Gln Val
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Val Ile Tyr Lys Met Glu Asp Gly Asp Val Asn Ile Leu Gln Thr Leu
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Ser Gly Glu Gln Ile Gly Ser Tyr Phe Gly Ser Val Leu Thr Thr Ile
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Asp Ile Asp Lys Asp Ser Tyr Thr Asp Leu Leu Leu Val Gly Ala Pro
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Pro Leu Glu Asp Asp His Ala Gly Ala Val Tyr Ile Tyr His Gly Ser
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Asp Gly Lys Thr Leu Lys Phe Phe Gly Gln Ser Ile His Gly Glu Met
625                 630                 635                 640

Asp Leu Asn Gly Asp Gly Leu Thr Asp Val Thr Ile Gly Gly Leu Gly
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Val Lys Leu Lys Ser Lys Glu Asp Ser Val Tyr Glu Ala Asp Leu Gln
705                 710                 715                 720

Tyr Arg Val Thr Leu Asp Ser Leu Arg Gln Ile Ser Arg Ser Phe Phe
                725                 730                 735

Ser Gly Thr Gln Glu Arg Arg Ile Gln Arg Asn Leu Thr Val Arg Glu
            740                 745                 750

Ser Glu Cys Ile Arg His Ser Phe Tyr Met Leu Asp Lys His Asp Phe
        755                 760                 765

Gln Asp Ser Val Arg Val Thr Leu Asp Phe Asn Leu Thr Asp Pro Glu
    770                 775                 780

Asn Gly Pro Val Leu Asp Asp Ala Leu Pro Asn Ser Val His Gly His
785                 790                 795                 800

Ile Pro Phe Ala Lys Asp Cys Gly Asn Lys Glu Arg Cys Val Ser Asp
                805                 810                 815

Leu Thr Leu Asp Val Ser Thr Thr Glu Lys Asn Leu Leu Ile Val Arg
            820                 825                 830

Ser Gln Asn Asp Lys Phe Asn Val Ser Leu Thr Val Lys Asn Lys Gly
        835                 840                 845

Asp Ser Ala Tyr Asn Thr Arg Thr Val Val Gln Tyr Ser Pro Asn Leu
    850                 855                 860

Ile Phe Ser Gly Ile Glu Glu Ile Gln Lys Asp Ser Cys Glu Ser Asn
865                 870                 875                 880

Gln Asn Ile Thr Cys Arg Val Gly Tyr Pro Phe Leu Arg Thr Gly Asp
                885                 890                 895

Val Val Asn Phe Lys Ile Ile Phe Gln Phe Asn Thr Ser His Leu Ser
            900                 905                 910

Glu Asn Ala Ile Ile His Leu Ser Ala Thr Ser Asp Ser Glu Glu Pro
        915                 920                 925

Leu Glu Ser Leu Tyr Asp Asn Glu Val Asn Ile Ser Ile Pro Val Lys
    930                 935                 940

Tyr Glu Val Gly Leu Gln Phe Tyr Ser Ser Ala Ser Glu His His Ile
945                 950                 955                 960

Ser Val Ala Ala Asn Glu Thr Val Pro Glu Leu Ile Asn Ser Thr Lys
                965                 970                 975

Asp Ile Gly Asp Glu Ile Asn Val Phe Tyr Thr Ile Arg Lys Arg Gly
            980                 985                 990

His Phe Pro Met Pro Glu Leu Arg  Leu Ala Ile Ser Phe  Pro Asn Leu
        995                 1000                1005

Thr Ser  Asp Gly Tyr Pro Val  Leu Tyr Pro Thr Gly  Trp Ser Ser
    1010                1015                1020

Ser Asp  Asn Val Asn Cys Arg  Pro Arg Ser Leu Glu  Asp Pro Leu
    1025                1030                1035

Gly Ile  Asn Ser Gly Lys Lys  Met Thr Ile Ser Lys  Ser Glu Val
    1040                1045                1050

Leu Lys  Arg Gly Thr Ile Gln  Asp Cys Ser Thr Cys  Lys Ile Ala
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    1085                1090                1095

Ser Ser  Leu Asn Leu Thr Ile  Arg Gly Glu Leu Gln  Ser Glu Asn
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Ser Ser  Leu Thr Leu Ser Ser  Ser Asn Arg Lys Arg  Glu Leu Ala
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Ile Gln  Ile Ser Lys Asp Gly  Leu Pro Gly Arg Val  Pro Leu Trp
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Val Ile  Leu Leu Ser Ala Phe  Ala Gly Leu Leu Leu  Leu Met Leu
    1145                1150                1155

Leu Ile  Leu Ala Leu Trp Lys  Ile Gly Phe Phe Lys  Arg Pro Leu
    1160                1165                1170

Lys Lys  Lys Met Glu Lys
    1175
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<210> SEQ ID NO 3
<211> LENGTH: 3537
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (1)..(3537)

<400> SEQUENCE: 3

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atg gcc cct cgg ccc cgc gcc cgc cca ggg gtc gct gtc gcc tgc tgc       48
Met Ala Pro Arg Pro Arg Ala Arg Pro Gly Val Ala Val Ala Cys Cys
1               5                  10                  15

tgg ctc ctc act gtt gtt cta cgc tgc tgc gta tca ttc aat gtt gat       96
Trp Leu Leu Thr Val Val Leu Arg Cys Cys Val Ser Phe Asn Val Asp
            20                  25                  30

gtg aaa aat tca atg act ttc agc ggc ccg gtg gaa gac atg ttt gga      144
Val Lys Asn Ser Met Thr Phe Ser Gly Pro Val Glu Asp Met Phe Gly
        35                  40                  45

tat act gtt caa caa tat gaa aat gaa gaa gga aaa tgg gtg ctt att      192
Tyr Thr Val Gln Gln Tyr Glu Asn Glu Glu Gly Lys Trp Val Leu Ile
    50                  55                  60

ggt tct ccg tta gtt ggc caa ccc aaa aac aga act gga gat gtc tat      240
Gly Ser Pro Leu Val Gly Gln Pro Lys Asn Arg Thr Gly Asp Val Tyr
65                  70                  75                  80

aag tgt cca gtt ggg aga ggt gaa tca tta cct tgt gta aag ttg gat      288
Lys Cys Pro Val Gly Arg Gly Glu Ser Leu Pro Cys Val Lys Leu Asp
                85                  90                  95

cta cca gtt aat aca tca att ccc aat gtc aca gaa gta aag gag aac      336
Leu Pro Val Asn Thr Ser Ile Pro Asn Val Thr Glu Val Lys Glu Asn
            100                 105                 110

atg aca ttt gga tca act tta gtc acc aac cca aat gga gga ttt ctg      384
Met Thr Phe Gly Ser Thr Leu Val Thr Asn Pro Asn Gly Gly Phe Leu
        115                 120                 125

gct tgt ggg ccc tta tat gcc tat aga tgt gga cat ttg cat tac aca      432
Ala Cys Gly Pro Leu Tyr Ala Tyr Arg Cys Gly His Leu His Tyr Thr
    130                 135                 140

act gga atc tgt tct gac gtc agc ccc aca ttt caa gtc gtg aat tcc      480
Thr Gly Ile Cys Ser Asp Val Ser Pro Thr Phe Gln Val Val Asn Ser
145                 150                 155                 160

att gcc cct gta caa gaa tgc agc act caa ctg gac ata gtc ata gtg      528
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Ile Ala Pro Val Gln Glu Cys Ser Thr Gln Leu Asp Ile Val Ile Val
                165                 170                 175

ctg gat ggt tcc aac agt att tac cca tgg gac agt gtt aca gct ttt      576
Leu Asp Gly Ser Asn Ser Ile Tyr Pro Trp Asp Ser Val Thr Ala Phe
            180                 185                 190

tta aat gac ctt ctt gaa aga atg gat att ggt cct aaa cag aca cag      624
Leu Asn Asp Leu Leu Glu Arg Met Asp Ile Gly Pro Lys Gln Thr Gln
        195                 200                 205

gtt gga att gta cag tat gga gaa aac gtg acc cat gag ttc aac ctc      672
Val Gly Ile Val Gln Tyr Gly Glu Asn Val Thr His Glu Phe Asn Leu
    210                 215                 220

aat aag tat tct tcc acc gaa gag gta ctt gtt gca gca aag aaa ata      720
Asn Lys Tyr Ser Ser Thr Glu Glu Val Leu Val Ala Ala Lys Lys Ile
225                 230                 235                 240

gtc cag aga ggt ggc cgc cag act atg aca gct ctt gga ata gac aca      768
Val Gln Arg Gly Gly Arg Gln Thr Met Thr Ala Leu Gly Ile Asp Thr
                245                 250                 255

gca aga aag gag gca ttc acg gaa gcc cgg ggt gcc cga aga gga gtt      816
Ala Arg Lys Glu Ala Phe Thr Glu Ala Arg Gly Ala Arg Arg Gly Val
            260                 265                 270

aaa aaa gtc atg gtt att gtg aca gat gga gag tct cat gac aat cat      864
Lys Lys Val Met Val Ile Val Thr Asp Gly Glu Ser His Asp Asn His
        275                 280                 285

cga ctg aag aag gtc atc caa gac tgt gaa gat gaa aac att caa cgg      912
Arg Leu Lys Lys Val Ile Gln Asp Cys Glu Asp Glu Asn Ile Gln Arg
    290                 295                 300

ttt tcc ata gct att ctt ggc agc tat aac cga gga aat tta agc act      960
Phe Ser Ile Ala Ile Leu Gly Ser Tyr Asn Arg Gly Asn Leu Ser Thr
305                 310                 315                 320

gaa aaa ttt gtg gag gaa ata aaa tca att gca agt gaa ccc act gaa     1008
Glu Lys Phe Val Glu Glu Ile Lys Ser Ile Ala Ser Glu Pro Thr Glu
                325                 330                 335

aag cat ttc ttc aat gtc tct gat gaa ttg gct cta gtc acc att gtt     1056
Lys His Phe Phe Asn Val Ser Asp Glu Leu Ala Leu Val Thr Ile Val
            340                 345                 350

aaa act ctg gga gaa aga ata ttt gcc ctg gaa gcc aca gct gac cag     1104
Lys Thr Leu Gly Glu Arg Ile Phe Ala Leu Glu Ala Thr Ala Asp Gln
        355                 360                 365

tca gca gct tca ttt gaa atg gaa atg tct cag act ggc ttc agt gct     1152
Ser Ala Ala Ser Phe Glu Met Glu Met Ser Gln Thr Gly Phe Ser Ala
    370                 375                 380

cat tat tca cag gac tgg gtc atg ctt gga gca gta gga gcc tat gat     1200
His Tyr Ser Gln Asp Trp Val Met Leu Gly Ala Val Gly Ala Tyr Asp
385                 390                 395                 400

tgg aat gga aca gtt gtc atg cag aag gct agt caa atc ata atc cct     1248
Trp Asn Gly Thr Val Val Met Gln Lys Ala Ser Gln Ile Ile Ile Pro
                405                 410                 415

cga aac aca acc ttt aat gtt gag tct acc aaa aag aat gaa ccg ctt     1296
Arg Asn Thr Thr Phe Asn Val Glu Ser Thr Lys Lys Asn Glu Pro Leu
            420                 425                 430

gct tct tat tta ggt tac act gta aac tct gct act gct tct tct gga     1344
Ala Ser Tyr Leu Gly Tyr Thr Val Asn Ser Ala Thr Ala Ser Ser Gly
        435                 440                 445

gat gtg ctc tat att gct gga cag cct cgg tac aat cat aca ggc cag     1392
Asp Val Leu Tyr Ile Ala Gly Gln Pro Arg Tyr Asn His Thr Gly Gln
    450                 455                 460

gtc att atc tac agg atg gaa gat gga aac atc aaa att ctc cag acg     1440
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Val Ile Ile Tyr Arg Met Glu Asp Gly Asn Ile Lys Ile Leu Gln Thr
465                 470                 475                 480

ctc agt gga gaa cag att ggt tcc tac ttt ggc agt att tta aca aca     1488
Leu Ser Gly Glu Gln Ile Gly Ser Tyr Phe Gly Ser Ile Leu Thr Thr
                485                 490                 495

act gac att gac aag gat tct aat act gac att ctt cta gtc gga gcc     1536
Thr Asp Ile Asp Lys Asp Ser Asn Thr Asp Ile Leu Leu Val Gly Ala
            500                 505                 510

cct atg tac atg gga aca gag aag gag gag caa gga aaa gtg tat gtg     1584
Pro Met Tyr Met Gly Thr Glu Lys Glu Glu Gln Gly Lys Val Tyr Val
        515                 520                 525

tat gct ctc aat cag aca agg ttt gaa tat caa atg agc ctg gaa cct     1632
Tyr Ala Leu Asn Gln Thr Arg Phe Glu Tyr Gln Met Ser Leu Glu Pro
    530                 535                 540

att aag cag acg tgc tgt tca tct cgg cag cac aat tca tgc aca aca     1680
Ile Lys Gln Thr Cys Cys Ser Ser Arg Gln His Asn Ser Cys Thr Thr
545                 550                 555                 560

gaa aac aaa aat gag cca tgc ggg gct cgt ttt gga act gca att gct     1728
Glu Asn Lys Asn Glu Pro Cys Gly Ala Arg Phe Gly Thr Ala Ile Ala
                565                 570                 575

gct gta aaa gac ctc aat ctt gat gga ttt aat gac atc gtg ata gga     1776
Ala Val Lys Asp Leu Asn Leu Asp Gly Phe Asn Asp Ile Val Ile Gly
            580                 585                 590

gct ccg ctg gaa gat gat cac ggg gga gct gtg tac att tat cat gga     1824
Ala Pro Leu Glu Asp Asp His Gly Gly Ala Val Tyr Ile Tyr His Gly
        595                 600                 605

agt ggc aag act ata agg aaa gag tat gca caa cgt att cca tca ggt     1872
Ser Gly Lys Thr Ile Arg Lys Glu Tyr Ala Gln Arg Ile Pro Ser Gly
    610                 615                 620

ggg gat ggt aag aca ctg aaa ttt ttt ggc cag tct atc cac gga gaa     1920
Gly Asp Gly Lys Thr Leu Lys Phe Phe Gly Gln Ser Ile His Gly Glu
625                 630                 635                 640

atg gat tta aat ggt gac ggt ctg aca gat gtg act att ggg ggc ctt     1968
Met Asp Leu Asn Gly Asp Gly Leu Thr Asp Val Thr Ile Gly Gly Leu
                645                 650                 655

ggt ggt gct gcc ctc ttc tgg tcc cga gat gtg gcc gta gtt aaa gtg     2016
Gly Gly Ala Ala Leu Phe Trp Ser Arg Asp Val Ala Val Val Lys Val
            660                 665                 670

acc atg aat ttt gag cca aat aaa gtg aat att caa aag aaa aac tgc     2064
Thr Met Asn Phe Glu Pro Asn Lys Val Asn Ile Gln Lys Lys Asn Cys
        675                 680                 685

cat atg gag gga aag gaa aca gta tgc ata aat gct aca gtg tgt ttt     2112
His Met Glu Gly Lys Glu Thr Val Cys Ile Asn Ala Thr Val Cys Phe
    690                 695                 700

gat gtg aaa tta aag tct aaa gaa gac acg att tat gaa gct gat ttg     2160
Asp Val Lys Leu Lys Ser Lys Glu Asp Thr Ile Tyr Glu Ala Asp Leu
705                 710                 715                 720

cag tac cgt gtc acc cta gat tca cta aga caa ata tca cga agt ttt     2208
Gln Tyr Arg Val Thr Leu Asp Ser Leu Arg Gln Ile Ser Arg Ser Phe
                725                 730                 735

ttc tct gga act caa gag aga aag gtt caa agg aac atc aca gtt cga     2256
Phe Ser Gly Thr Gln Glu Arg Lys Val Gln Arg Asn Ile Thr Val Arg
            740                 745                 750

aaa tca gaa tgc act aag cac tcc ttc tac atg ttg gac aag cat gac     2304
Lys Ser Glu Cys Thr Lys His Ser Phe Tyr Met Leu Asp Lys His Asp
        755                 760                 765

ttt cag gac tct gtg aga ata acg ttg gac ttt aat ctt acc gat cca     2352
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Phe Gln Asp Ser Val Arg Ile Thr Leu Asp Phe Asn Leu Thr Asp Pro
    770                 775                 780

gaa aat ggg cct gtt ctt gat gat tct cta cca aac tca gta cat gaa     2400
Glu Asn Gly Pro Val Leu Asp Asp Ser Leu Pro Asn Ser Val His Glu
785                 790                 795                 800

tat att ccc ttt gcc aaa gat tgt gga aat aag gaa aaa tgt atc tca     2448
Tyr Ile Pro Phe Ala Lys Asp Cys Gly Asn Lys Glu Lys Cys Ile Ser
                805                 810                 815

gac ctc agc ctg cat gtc gcc acc act gaa aag gac ctg ctg att gtc     2496
Asp Leu Ser Leu His Val Ala Thr Thr Glu Lys Asp Leu Leu Ile Val
            820                 825                 830

cga tcc cag aat gat aag ttc aac gtt agc ctc aca gtc aaa aat aca     2544
Arg Ser Gln Asn Asp Lys Phe Asn Val Ser Leu Thr Val Lys Asn Thr
        835                 840                 845

aag gac agt gcc tat aac acc agg aca ata gtg cat tat tct cca aat     2592
Lys Asp Ser Ala Tyr Asn Thr Arg Thr Ile Val His Tyr Ser Pro Asn
    850                 855                 860

cta gtt ttt tca gga att gag gct atc caa aaa gac agt tgt gaa tct     2640
Leu Val Phe Ser Gly Ile Glu Ala Ile Gln Lys Asp Ser Cys Glu Ser
865                 870                 875                 880

aat cat aat atc aca tgt aaa gtt gga tat ccc ttc ctg aga aga gga     2688
Asn His Asn Ile Thr Cys Lys Val Gly Tyr Pro Phe Leu Arg Arg Gly
                885                 890                 895

gag atg gta act ttc aaa ata ttg ttt cag ttt aac aca tcc tat ctc     2736
Glu Met Val Thr Phe Lys Ile Leu Phe Gln Phe Asn Thr Ser Tyr Leu
            900                 905                 910

atg gaa aat gtg acc att tat tta agt gca aca agt gac agc gaa gaa     2784
Met Glu Asn Val Thr Ile Tyr Leu Ser Ala Thr Ser Asp Ser Glu Glu
        915                 920                 925

cct cct gaa acc ctt tct gat aat gta gta aac att tct atc ccg gta     2832
Pro Pro Glu Thr Leu Ser Asp Asn Val Val Asn Ile Ser Ile Pro Val
    930                 935                 940

aaa tat gaa gtt gga cta cag ttt tac agc tct gca agt gaa tac cac     2880
Lys Tyr Glu Val Gly Leu Gln Phe Tyr Ser Ser Ala Ser Glu Tyr His
945                 950                 955                 960

att tca att gct gcc aat gag aca gtc cct gaa gtt att aat tct act     2928
Ile Ser Ile Ala Ala Asn Glu Thr Val Pro Glu Val Ile Asn Ser Thr
                965                 970                 975

gag gac att gga aat gaa att aat atc ttc tac ttg att aga aaa agt     2976
Glu Asp Ile Gly Asn Glu Ile Asn Ile Phe Tyr Leu Ile Arg Lys Ser
            980                 985                 990

gga tct ttt cca atg cca gag ctt  aag ctg tca att tca  ttc ccc aat   3024
Gly Ser Phe Pro Met Pro Glu Leu  Lys Leu Ser Ile Ser  Phe Pro Asn
        995                 1000                1005

atg aca  tca aat ggt tac cct  gtg ctg tac cca act  gga ttg tca      3069
Met Thr  Ser Asn Gly Tyr Pro  Val Leu Tyr Pro Thr  Gly Leu Ser
    1010                1015                1020

tct tct  gag aat gca aac tgc  aga ccc cat atc ttt  gag gat cct      3114
Ser Ser  Glu Asn Ala Asn Cys  Arg Pro His Ile Phe  Glu Asp Pro
    1025                1030                1035

ttc agt  atc aac tct gga aag  aaa atg act aca tca  act gac cat      3159
Phe Ser  Ile Asn Ser Gly Lys  Lys Met Thr Thr Ser  Thr Asp His
    1040                1045                1050

ctc aaa  cga ggc aca att ctg  gac tgc aat aca tgt  aaa ttt gct      3204
Leu Lys  Arg Gly Thr Ile Leu  Asp Cys Asn Thr Cys  Lys Phe Ala
    1055                1060                1065

acc atc  aca tgt aat ctc act  tct tct gac atc agc  caa gtc aat      3249
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Thr Ile  Thr Cys Asn Leu Thr  Ser Ser Asp Ile Ser  Gln Val Asn
    1070                 1075                 1080

gtt tcg  ctt atc ttg tgg aaa  cca act ttt ata aaa  tca tat ttt      3294
Val Ser  Leu Ile Leu Trp Lys  Pro Thr Phe Ile Lys  Ser Tyr Phe
    1085                 1090                 1095

tcc agc  tta aat ctt act ata  agg gga gaa ctt cgg  agt gaa aat      3339
Ser Ser  Leu Asn Leu Thr Ile  Arg Gly Glu Leu Arg  Ser Glu Asn
    1100                 1105                 1110

gca tct  ctg gtt tta agt agc  agc aat caa aaa aga  gag ctt gct      3384
Ala Ser  Leu Val Leu Ser Ser  Ser Asn Gln Lys Arg  Glu Leu Ala
    1115                 1120                 1125

att caa  ata tcc aaa gat ggg  cta ccg ggc aga gtg  cca tta tgg      3429
Ile Gln  Ile Ser Lys Asp Gly  Leu Pro Gly Arg Val  Pro Leu Trp
    1130                 1135                 1140

gtc atc  ctg ctg agt gct ttt  gcc gga ttg ttg ctg  tta atg ctg      3474
Val Ile  Leu Leu Ser Ala Phe  Ala Gly Leu Leu Leu  Leu Met Leu
    1145                 1150                 1155

ctc att  tta gca ctg tgg aag  att gga ttc ttc aaa  aga cca ctg      3519
Leu Ile  Leu Ala Leu Trp Lys  Ile Gly Phe Phe Lys  Arg Pro Leu
    1160                 1165                 1170

aaa aag  aaa atg gag aaa                                         3537
Lys Lys  Lys Met Glu Lys
    1175
```

<210> SEQ ID NO 4
<211> LENGTH: 1179
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 4

```
Met Ala Pro Arg Pro Arg Ala Arg Pro Gly Val Ala Val Ala Cys Cys
1               5                  10                  15

Trp Leu Leu Thr Val Val Leu Arg Cys Cys Val Ser Phe Asn Val Asp
            20                  25                  30

Val Lys Asn Ser Met Thr Phe Ser Gly Pro Val Glu Asp Met Phe Gly
        35                  40                  45

Tyr Thr Val Gln Gln Tyr Glu Asn Glu Glu Gly Lys Trp Val Leu Ile
    50                  55                  60

Gly Ser Pro Leu Val Gly Gln Pro Lys Asn Arg Thr Gly Asp Val Tyr
65                  70                  75                  80

Lys Cys Pro Val Gly Arg Gly Glu Ser Leu Pro Cys Val Lys Leu Asp
                85                  90                  95

Leu Pro Val Asn Thr Ser Ile Pro Asn Val Thr Glu Val Lys Glu Asn
            100                 105                 110

Met Thr Phe Gly Ser Thr Leu Val Thr Asn Pro Asn Gly Gly Phe Leu
        115                 120                 125

Ala Cys Gly Pro Leu Tyr Ala Tyr Arg Cys Gly His Leu His Tyr Thr
    130                 135                 140

Thr Gly Ile Cys Ser Asp Val Ser Pro Thr Phe Gln Val Val Asn Ser
145                 150                 155                 160

Ile Ala Pro Val Gln Glu Cys Ser Thr Gln Leu Asp Ile Val Ile Val
                165                 170                 175

Leu Asp Gly Ser Asn Ser Ile Tyr Pro Trp Asp Ser Val Thr Ala Phe
            180                 185                 190

Leu Asn Asp Leu Leu Glu Arg Met Asp Ile Gly Pro Lys Gln Thr Gln
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        195                 200                 205

Val Gly Ile Val Gln Tyr Gly Glu Asn Val Thr His Glu Phe Asn Leu
    210                 215                 220

Asn Lys Tyr Ser Ser Thr Glu Glu Val Leu Val Ala Ala Lys Lys Ile
225                 230                 235                 240

Val Gln Arg Gly Gly Arg Gln Thr Met Thr Ala Leu Gly Ile Asp Thr
                245                 250                 255

Ala Arg Lys Glu Ala Phe Thr Glu Ala Arg Gly Ala Arg Arg Gly Val
            260                 265                 270

Lys Lys Val Met Val Ile Val Thr Asp Gly Glu Ser His Asp Asn His
        275                 280                 285

Arg Leu Lys Lys Val Ile Gln Asp Cys Glu Asp Glu Asn Ile Gln Arg
    290                 295                 300

Phe Ser Ile Ala Ile Leu Gly Ser Tyr Asn Arg Gly Asn Leu Ser Thr
305                 310                 315                 320

Glu Lys Phe Val Glu Glu Ile Lys Ser Ile Ala Ser Glu Pro Thr Glu
                325                 330                 335

Lys His Phe Phe Asn Val Ser Asp Glu Leu Ala Leu Val Thr Ile Val
            340                 345                 350

Lys Thr Leu Gly Glu Arg Ile Phe Ala Leu Glu Ala Thr Ala Asp Gln
        355                 360                 365

Ser Ala Ala Ser Phe Glu Met Glu Met Ser Gln Thr Gly Phe Ser Ala
    370                 375                 380

His Tyr Ser Gln Asp Trp Val Met Leu Gly Ala Val Gly Ala Tyr Asp
385                 390                 395                 400

Trp Asn Gly Thr Val Val Met Gln Lys Ala Ser Gln Ile Ile Ile Pro
                405                 410                 415

Arg Asn Thr Thr Phe Asn Val Glu Ser Thr Lys Lys Asn Glu Pro Leu
            420                 425                 430

Ala Ser Tyr Leu Gly Tyr Thr Val Asn Ser Ala Thr Ala Ser Ser Gly
        435                 440                 445

Asp Val Leu Tyr Ile Ala Gly Gln Pro Arg Tyr Asn His Thr Gly Gln
    450                 455                 460

Val Ile Ile Tyr Arg Met Glu Asp Gly Asn Ile Lys Ile Leu Gln Thr
465                 470                 475                 480

Leu Ser Gly Glu Gln Ile Gly Ser Tyr Phe Gly Ser Ile Leu Thr Thr
                485                 490                 495

Thr Asp Ile Asp Lys Asp Ser Asn Thr Asp Ile Leu Leu Val Gly Ala
            500                 505                 510

Pro Met Tyr Met Gly Thr Glu Lys Glu Glu Gln Gly Lys Val Tyr Val
        515                 520                 525

Tyr Ala Leu Asn Gln Thr Arg Phe Glu Tyr Gln Met Ser Leu Glu Pro
    530                 535                 540

Ile Lys Gln Thr Cys Cys Ser Ser Arg Gln His Asn Ser Cys Thr Thr
545                 550                 555                 560

Glu Asn Lys Asn Glu Pro Cys Gly Ala Arg Phe Gly Thr Ala Ile Ala
                565                 570                 575

Ala Val Lys Asp Leu Asn Leu Asp Gly Phe Asn Asp Ile Val Ile Gly
            580                 585                 590

Ala Pro Leu Glu Asp Asp His Gly Gly Ala Val Tyr Ile Tyr His Gly
        595                 600                 605
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Ser Gly Lys Thr Ile Arg Lys Glu Tyr Ala Gln Arg Ile Pro Ser Gly
    610                 615                 620

Gly Asp Gly Lys Thr Leu Lys Phe Phe Gly Gln Ser Ile His Gly Glu
625                 630                 635                 640

Met Asp Leu Asn Gly Asp Gly Leu Thr Asp Val Thr Ile Gly Gly Leu
                645                 650                 655

Gly Gly Ala Ala Leu Phe Trp Ser Arg Asp Val Ala Val Val Lys Val
            660                 665                 670

Thr Met Asn Phe Glu Pro Asn Lys Val Asn Ile Gln Lys Lys Asn Cys
        675                 680                 685

His Met Glu Gly Lys Glu Thr Val Cys Ile Asn Ala Thr Val Cys Phe
    690                 695                 700

Asp Val Lys Leu Lys Ser Lys Glu Asp Thr Ile Tyr Glu Ala Asp Leu
705                 710                 715                 720

Gln Tyr Arg Val Thr Leu Asp Ser Leu Arg Gln Ile Ser Arg Ser Phe
                725                 730                 735

Phe Ser Gly Thr Gln Glu Arg Lys Val Gln Arg Asn Ile Thr Val Arg
            740                 745                 750

Lys Ser Glu Cys Thr Lys His Ser Phe Tyr Met Leu Asp Lys His Asp
        755                 760                 765

Phe Gln Asp Ser Val Arg Ile Thr Leu Asp Phe Asn Leu Thr Asp Pro
    770                 775                 780

Glu Asn Gly Pro Val Leu Asp Asp Ser Leu Pro Asn Ser Val His Glu
785                 790                 795                 800

Tyr Ile Pro Phe Ala Lys Asp Cys Gly Asn Lys Glu Lys Cys Ile Ser
                805                 810                 815

Asp Leu Ser Leu His Val Ala Thr Thr Glu Lys Asp Leu Leu Ile Val
            820                 825                 830

Arg Ser Gln Asn Asp Lys Phe Asn Val Ser Leu Thr Val Lys Asn Thr
        835                 840                 845

Lys Asp Ser Ala Tyr Asn Thr Arg Thr Ile Val His Tyr Ser Pro Asn
    850                 855                 860

Leu Val Phe Ser Gly Ile Glu Ala Ile Gln Lys Asp Ser Cys Glu Ser
865                 870                 875                 880

Asn His Asn Ile Thr Cys Lys Val Gly Tyr Pro Phe Leu Arg Arg Gly
                885                 890                 895

Glu Met Val Thr Phe Lys Ile Leu Phe Gln Phe Asn Thr Ser Tyr Leu
            900                 905                 910

Met Glu Asn Val Thr Ile Tyr Leu Ser Ala Thr Ser Asp Ser Glu Glu
        915                 920                 925

Pro Pro Glu Thr Leu Ser Asp Asn Val Val Asn Ile Ser Ile Pro Val
    930                 935                 940

Lys Tyr Glu Val Gly Leu Gln Phe Tyr Ser Ser Ala Ser Glu Tyr His
945                 950                 955                 960

Ile Ser Ile Ala Ala Asn Glu Thr Val Pro Glu Val Ile Asn Ser Thr
                965                 970                 975

Glu Asp Ile Gly Asn Glu Ile Asn Ile Phe Tyr Leu Ile Arg Lys Ser
            980                 985                 990

Gly Ser Phe Pro Met Pro Glu Leu  Lys Leu Ser Ile Ser  Phe Pro Asn
        995                 1000                1005
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Met Thr  Ser Asn Gly Tyr Pro  Val Leu Tyr Pro Thr  Gly Leu Ser
    1010                1015                1020

Ser Ser  Glu Asn Ala Asn Cys  Arg Pro His Ile Phe  Glu Asp Pro
    1025                1030                1035

Phe Ser  Ile Asn Ser Gly Lys  Lys Met Thr Thr Ser  Thr Asp His
    1040                1045                1050

Leu Lys  Arg Gly Thr Ile Leu  Asp Cys Asn Thr Cys  Lys Phe Ala
    1055                1060                1065

Thr Ile  Thr Cys Asn Leu Thr  Ser Ser Asp Ile Ser  Gln Val Asn
    1070                1075                1080

Val Ser  Leu Ile Leu Trp Lys  Pro Thr Phe Ile Lys  Ser Tyr Phe
    1085                1090                1095

Ser Ser  Leu Asn Leu Thr Ile  Arg Gly Glu Leu Arg  Ser Glu Asn
    1100                1105                1110

Ala Ser  Leu Val Leu Ser Ser  Ser Asn Gln Lys Arg  Glu Leu Ala
    1115                1120                1125

Ile Gln  Ile Ser Lys Asp Gly  Leu Pro Gly Arg Val  Pro Leu Trp
    1130                1135                1140

Val Ile  Leu Leu Ser Ala Phe  Ala Gly Leu Leu Leu  Leu Met Leu
    1145                1150                1155

Leu Ile  Leu Ala Leu Trp Lys  Ile Gly Phe Phe Lys  Arg Pro Leu
    1160                1165                1170

Lys Lys  Lys Met Glu Lys
    1175


<210> SEQ ID NO 5
<211> LENGTH: 1407
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (1)..(1407)

<400> SEQUENCE: 5

atg cac agc ttt cct cca ctg ctg ctg ctg ctg ttc tgg ggt gtg gtg       48
Met His Ser Phe Pro Pro Leu Leu Leu Leu Leu Phe Trp Gly Val Val
1               5                   10                  15

tct cac agc ttc cca gcg act cta gaa aca caa gag caa gat gtg gac       96
Ser His Ser Phe Pro Ala Thr Leu Glu Thr Gln Glu Gln Asp Val Asp
            20                  25                  30

tta gtc cag aaa tac ctg gaa aaa tac tac aac ctg aag aat gat ggg      144
Leu Val Gln Lys Tyr Leu Glu Lys Tyr Tyr Asn Leu Lys Asn Asp Gly
        35                  40                  45

agg caa gtt gaa aag cgg aga aat agt ggc cca gtg gtt gaa aaa ttg      192
Arg Gln Val Glu Lys Arg Arg Asn Ser Gly Pro Val Val Glu Lys Leu
    50                  55                  60

aag caa atg cag gaa ttc ttt ggg ctg aaa gtg act ggg aaa cca gat      240
Lys Gln Met Gln Glu Phe Phe Gly Leu Lys Val Thr Gly Lys Pro Asp
65                  70                  75                  80

gct gaa acc ctg aag gtg atg aag cag ccc aga tgt gga gtg cct gat      288
Ala Glu Thr Leu Lys Val Met Lys Gln Pro Arg Cys Gly Val Pro Asp
                85                  90                  95

gtg gct cag ttt gtc ctc act gag ggg aac cct cgc tgg gag caa aca      336
Val Ala Gln Phe Val Leu Thr Glu Gly Asn Pro Arg Trp Glu Gln Thr
            100                 105                 110

cat ctg acc tac agg att gaa aat tac acg cca gat ttg cca aga gca      384
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His Leu Thr Tyr Arg Ile Glu Asn Tyr Thr Pro Asp Leu Pro Arg Ala
        115                 120                 125

gat gtg gac cat gcc att gag aaa gcc ttc caa ctc tgg agt aat gtc      432
Asp Val Asp His Ala Ile Glu Lys Ala Phe Gln Leu Trp Ser Asn Val
    130                 135                 140

aca cct ctg aca ttc acc aag gtc tct gag ggt caa gca gac atc atg      480
Thr Pro Leu Thr Phe Thr Lys Val Ser Glu Gly Gln Ala Asp Ile Met
145                 150                 155                 160

ata tct ttt gtc agg gga gat cat cgg gac aac tct cct ttt gat gga      528
Ile Ser Phe Val Arg Gly Asp His Arg Asp Asn Ser Pro Phe Asp Gly
                165                 170                 175

cct gga gga aat ctt gct cat gct ttt caa cca ggc cca ggt att gga      576
Pro Gly Gly Asn Leu Ala His Ala Phe Gln Pro Gly Pro Gly Ile Gly
            180                 185                 190

ggg gat gct cat ttt gat gaa gat gaa agg tgg acc aac aat ttc aga      624
Gly Asp Ala His Phe Asp Glu Asp Glu Arg Trp Thr Asn Asn Phe Arg
        195                 200                 205

gag tac aac tta cat cgt gtt gca gct cat gaa ctc ggc cat tct ctt      672
Glu Tyr Asn Leu His Arg Val Ala Ala His Glu Leu Gly His Ser Leu
    210                 215                 220

gga ctc tcc cat tct act gat atc ggg gct ttg atg tac cct agc tac      720
Gly Leu Ser His Ser Thr Asp Ile Gly Ala Leu Met Tyr Pro Ser Tyr
225                 230                 235                 240

acc ttc agt ggt gat gtt cag cta gct cag gat gac att gat ggc atc      768
Thr Phe Ser Gly Asp Val Gln Leu Ala Gln Asp Asp Ile Asp Gly Ile
                245                 250                 255

caa gcc ata tat gga cgt tcc caa aat cct gtc cag ccc atc ggc cca      816
Gln Ala Ile Tyr Gly Arg Ser Gln Asn Pro Val Gln Pro Ile Gly Pro
            260                 265                 270

caa acc cca aaa gcg tgt gac agt aag cta acc ttt gat gct ata act      864
Gln Thr Pro Lys Ala Cys Asp Ser Lys Leu Thr Phe Asp Ala Ile Thr
        275                 280                 285

acg att cgg gga gaa gtg atg ttc ttt aaa gac aga ttc tac atg cgc      912
Thr Ile Arg Gly Glu Val Met Phe Phe Lys Asp Arg Phe Tyr Met Arg
    290                 295                 300

aca aat ccc ttc tac ccg gaa gtt gag ctc aat ttc att tct gtt ttc      960
Thr Asn Pro Phe Tyr Pro Glu Val Glu Leu Asn Phe Ile Ser Val Phe
305                 310                 315                 320

tgg cca caa ctg cca aat ggg ctt gaa gct gct tac gaa ttt gcc gac     1008
Trp Pro Gln Leu Pro Asn Gly Leu Glu Ala Ala Tyr Glu Phe Ala Asp
                325                 330                 335

aga gat gaa gtc cgg ttt ttc aaa ggg aat aag tac tgg gct gtt cag     1056
Arg Asp Glu Val Arg Phe Phe Lys Gly Asn Lys Tyr Trp Ala Val Gln
            340                 345                 350

gga cag aat gtg cta cac gga tac ccc aag gac atc tac agc tcc ttt     1104
Gly Gln Asn Val Leu His Gly Tyr Pro Lys Asp Ile Tyr Ser Ser Phe
        355                 360                 365

ggc ttc cct aga act gtg aag cat atc gat gct gct ctt tct gag gaa     1152
Gly Phe Pro Arg Thr Val Lys His Ile Asp Ala Ala Leu Ser Glu Glu
    370                 375                 380

aac act gga aaa acc tac ttc ttt gtt gct aac aaa tac tgg agg tat     1200
Asn Thr Gly Lys Thr Tyr Phe Phe Val Ala Asn Lys Tyr Trp Arg Tyr
385                 390                 395                 400

gat gaa tat aaa cga tct atg gat cca ggt tat ccc aaa atg ata gca     1248
Asp Glu Tyr Lys Arg Ser Met Asp Pro Gly Tyr Pro Lys Met Ile Ala
                405                 410                 415

cat gac ttt cct gga att ggc cac aaa gtt gat gca gtt ttc atg aaa     1296
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His Asp Phe Pro Gly Ile Gly His Lys Val Asp Ala Val Phe Met Lys
            420                 425                 430

gat gga ttt ttc tat ttc ttt cat gga aca aga caa tac aaa ttt gat     1344
Asp Gly Phe Phe Tyr Phe Phe His Gly Thr Arg Gln Tyr Lys Phe Asp
        435                 440                 445

cct aaa acg aag aga att ttg act ctc cag aaa gct aat agc tgg ttc     1392
Pro Lys Thr Lys Arg Ile Leu Thr Leu Gln Lys Ala Asn Ser Trp Phe
    450                 455                 460

aac tgc agg aaa aat                                                 1407
Asn Cys Arg Lys Asn
465


<210> SEQ ID NO 6
<211> LENGTH: 469
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 6

Met His Ser Phe Pro Pro Leu Leu Leu Leu Leu Phe Trp Gly Val Val
1               5                   10                  15

Ser His Ser Phe Pro Ala Thr Leu Glu Thr Gln Glu Gln Asp Val Asp
            20                  25                  30

Leu Val Gln Lys Tyr Leu Glu Lys Tyr Tyr Asn Leu Lys Asn Asp Gly
        35                  40                  45

Arg Gln Val Glu Lys Arg Arg Asn Ser Gly Pro Val Val Glu Lys Leu
    50                  55                  60

Lys Gln Met Gln Glu Phe Phe Gly Leu Lys Val Thr Gly Lys Pro Asp
65                  70                  75                  80

Ala Glu Thr Leu Lys Val Met Lys Gln Pro Arg Cys Gly Val Pro Asp
                85                  90                  95

Val Ala Gln Phe Val Leu Thr Glu Gly Asn Pro Arg Trp Glu Gln Thr
            100                 105                 110

His Leu Thr Tyr Arg Ile Glu Asn Tyr Thr Pro Asp Leu Pro Arg Ala
        115                 120                 125

Asp Val Asp His Ala Ile Glu Lys Ala Phe Gln Leu Trp Ser Asn Val
    130                 135                 140

Thr Pro Leu Thr Phe Thr Lys Val Ser Glu Gly Gln Ala Asp Ile Met
145                 150                 155                 160

Ile Ser Phe Val Arg Gly Asp His Arg Asp Asn Ser Pro Phe Asp Gly
                165                 170                 175

Pro Gly Gly Asn Leu Ala His Ala Phe Gln Pro Gly Pro Gly Ile Gly
            180                 185                 190

Gly Asp Ala His Phe Asp Glu Asp Glu Arg Trp Thr Asn Asn Phe Arg
        195                 200                 205

Glu Tyr Asn Leu His Arg Val Ala Ala His Glu Leu Gly His Ser Leu
    210                 215                 220

Gly Leu Ser His Ser Thr Asp Ile Gly Ala Leu Met Tyr Pro Ser Tyr
225                 230                 235                 240

Thr Phe Ser Gly Asp Val Gln Leu Ala Gln Asp Asp Ile Asp Gly Ile
                245                 250                 255

Gln Ala Ile Tyr Gly Arg Ser Gln Asn Pro Val Gln Pro Ile Gly Pro
            260                 265                 270

Gln Thr Pro Lys Ala Cys Asp Ser Lys Leu Thr Phe Asp Ala Ile Thr
        275                 280                 285
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Thr Ile Arg Gly Glu Val Met Phe Phe Lys Asp Arg Phe Tyr Met Arg
    290                 295                 300

Thr Asn Pro Phe Tyr Pro Glu Val Glu Leu Asn Phe Ile Ser Val Phe
305                 310                 315                 320

Trp Pro Gln Leu Pro Asn Gly Leu Glu Ala Ala Tyr Glu Phe Ala Asp
                325                 330                 335

Arg Asp Glu Val Arg Phe Phe Lys Gly Asn Lys Tyr Trp Ala Val Gln
            340                 345                 350

Gly Gln Asn Val Leu His Gly Tyr Pro Lys Asp Ile Tyr Ser Ser Phe
        355                 360                 365

Gly Phe Pro Arg Thr Val Lys His Ile Asp Ala Ala Leu Ser Glu Glu
    370                 375                 380

Asn Thr Gly Lys Thr Tyr Phe Phe Val Ala Asn Lys Tyr Trp Arg Tyr
385                 390                 395                 400

Asp Glu Tyr Lys Arg Ser Met Asp Pro Gly Tyr Pro Lys Met Ile Ala
                405                 410                 415

His Asp Phe Pro Gly Ile Gly His Lys Val Asp Ala Val Phe Met Lys
            420                 425                 430

Asp Gly Phe Phe Tyr Phe Phe His Gly Thr Arg Gln Tyr Lys Phe Asp
        435                 440                 445

Pro Lys Thr Lys Arg Ile Leu Thr Leu Gln Lys Ala Asn Ser Trp Phe
    450                 455                 460

Asn Cys Arg Lys Asn
465


<210> SEQ ID NO 7
<211> LENGTH: 1392
<212> TYPE: DNA
<213> ORGANISM: Mus musculus
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (1)..(1392)

<400> SEQUENCE: 7

atg cct agc ctt cct ttg ctg ttg ctt ctc tgg gct gct agc tca tac       48
Met Pro Ser Leu Pro Leu Leu Leu Leu Leu Trp Ala Ala Ser Ser Tyr
1               5                   10                  15

agt ttc cct gtg ttt cac aac gga gac cgg caa aat gtg gag aca gtc       96
Ser Phe Pro Val Phe His Asn Gly Asp Arg Gln Asn Val Glu Thr Val
            20                  25                  30

tgg aaa tac ctg gaa aac tac tac aac ttg ggc aaa aac atg caa gct      144
Trp Lys Tyr Leu Glu Asn Tyr Tyr Asn Leu Gly Lys Asn Met Gln Ala
        35                  40                  45

aaa aac gtg aat ggc aag gag atg atg gct gaa aag ctg agg caa atg      192
Lys Asn Val Asn Gly Lys Glu Met Met Ala Glu Lys Leu Arg Gln Met
    50                  55                  60

cag cag tta ttt ggg ctg aaa gtg act gga aat tca gat cct gaa acc      240
Gln Gln Leu Phe Gly Leu Lys Val Thr Gly Asn Ser Asp Pro Glu Thr
65                  70                  75                  80

ctg aga gct atg aag aag ccc agg tgt ggg gtg cct gat gtg gcc cca      288
Leu Arg Ala Met Lys Lys Pro Arg Cys Gly Val Pro Asp Val Ala Pro
                85                  90                  95

tat gcc att act cac aac aat cct cgt tgg acc aaa aca cat ctg aca      336
Tyr Ala Ile Thr His Asn Asn Pro Arg Trp Thr Lys Thr His Leu Thr
            100                 105                 110
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tac agc att tta aac tac aca cca tat ttg cca aaa gca gtt gtg gaa      384
Tyr Ser Ile Leu Asn Tyr Thr Pro Tyr Leu Pro Lys Ala Val Val Glu
        115                 120                 125

gat gcc atc gcg aga gcc ttt aga gtc tgg agt gat gtg aca cca ctt      432
Asp Ala Ile Ala Arg Ala Phe Arg Val Trp Ser Asp Val Thr Pro Leu
    130                 135                 140

acg ttc caa aga gtc ttt gag gag gaa ggc gat att gtg ctc tcc ttc      480
Thr Phe Gln Arg Val Phe Glu Glu Glu Gly Asp Ile Val Leu Ser Phe
145                 150                 155                 160

cac aga gga gac cat ggt gac aac aac cca ttt gat gga cct aac tat      528
His Arg Gly Asp His Gly Asp Asn Asn Pro Phe Asp Gly Pro Asn Tyr
                165                 170                 175

aag ctt gct cac act ttc cag cca ggc cca ggt ttg ggg ggt gat gtt      576
Lys Leu Ala His Thr Phe Gln Pro Gly Pro Gly Leu Gly Gly Asp Val
            180                 185                 190

cat tat gac ctt gat gag acg tgg acc aac agc agt gaa aat ttc aac      624
His Tyr Asp Leu Asp Glu Thr Trp Thr Asn Ser Ser Glu Asn Phe Asn
        195                 200                 205

ttg ttc tat gtt acg gct cat gaa ctg ggt cac tcc ctt ggg ctc act      672
Leu Phe Tyr Val Thr Ala His Glu Leu Gly His Ser Leu Gly Leu Thr
    210                 215                 220

cat tct agt gat ata gga gca cta atg ttc ccc agt tac acg tgg tac      720
His Ser Ser Asp Ile Gly Ala Leu Met Phe Pro Ser Tyr Thr Trp Tyr
225                 230                 235                 240

act gaa gac ttt gtg cta aac cag gat gat att aat cgc atc cag gac      768
Thr Glu Asp Phe Val Leu Asn Gln Asp Asp Ile Asn Arg Ile Gln Asp
                245                 250                 255

tta tat gga cct tcc cca aat ccc atc cag cca aca ggt gca aca aca      816
Leu Tyr Gly Pro Ser Pro Asn Pro Ile Gln Pro Thr Gly Ala Thr Thr
            260                 265                 270

cca cat cca tgt aat ggt gat cta acc ttt gat gct ata act aca ttt      864
Pro His Pro Cys Asn Gly Asp Leu Thr Phe Asp Ala Ile Thr Thr Phe
        275                 280                 285

agg gga gag gtg ttt ttc ttc aaa ggc agg ttc tac att cgg gta aat      912
Arg Gly Glu Val Phe Phe Phe Lys Gly Arg Phe Tyr Ile Arg Val Asn
    290                 295                 300

aga ttc atg cca gaa cct gag ctc aat tta ata ggt att ctc tgg cca      960
Arg Phe Met Pro Glu Pro Glu Leu Asn Leu Ile Gly Ile Leu Trp Pro
305                 310                 315                 320

aat ctt cca gtt aaa ctt gac gct gct tat gaa gct agt atg ata gat     1008
Asn Leu Pro Val Lys Leu Asp Ala Ala Tyr Glu Ala Ser Met Ile Asp
                325                 330                 335

caa gtc cgc tat ttc aaa ggc agc aaa gta tgg gct gtt caa gag cag     1056
Gln Val Arg Tyr Phe Lys Gly Ser Lys Val Trp Ala Val Gln Glu Gln
            340                 345                 350

agt gta ctg aga gga ttc ccc aga gac atc cac agt ttc ttt ggc ttc     1104
Ser Val Leu Arg Gly Phe Pro Arg Asp Ile His Ser Phe Phe Gly Phe
        355                 360                 365

cct agc aat gtg aca cac att gat gct gct gtt tgt gag gaa gag act     1152
Pro Ser Asn Val Thr His Ile Asp Ala Ala Val Cys Glu Glu Glu Thr
    370                 375                 380

gga aaa aca tac ttc ttt gtt gac cac atg tac tgg agg tat gat gaa     1200
Gly Lys Thr Tyr Phe Phe Val Asp His Met Tyr Trp Arg Tyr Asp Glu
385                 390                 395                 400

aat aca cag tct atg gat cca ggt tat ccc aga tta aca gca gaa gac     1248
Asn Thr Gln Ser Met Asp Pro Gly Tyr Pro Arg Leu Thr Ala Glu Asp
                405                 410                 415
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ttc cct gga att gat gat aaa gtt gat gat gtt ttc caa aaa gga gaa     1296
Phe Pro Gly Ile Asp Asp Lys Val Asp Asp Val Phe Gln Lys Gly Glu
            420                 425                 430

aat ttc tat ttc ttt cac caa tca gtt caa cac aga ttt aac ctc caa     1344
Asn Phe Tyr Phe Phe His Gln Ser Val Gln His Arg Phe Asn Leu Gln
        435                 440                 445

ata aga aga gtt gat gat tcc cgt gat tct agt aca tgg ttc aac tgc     1392
Ile Arg Arg Val Asp Asp Ser Arg Asp Ser Ser Thr Trp Phe Asn Cys
    450                 455                 460


<210> SEQ ID NO 8
<211> LENGTH: 464
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 8

Met Pro Ser Leu Pro Leu Leu Leu Leu Leu Trp Ala Ala Ser Ser Tyr
1               5                   10                  15

Ser Phe Pro Val Phe His Asn Gly Asp Arg Gln Asn Val Glu Thr Val
            20                  25                  30

Trp Lys Tyr Leu Glu Asn Tyr Tyr Asn Leu Gly Lys Asn Met Gln Ala
        35                  40                  45

Lys Asn Val Asn Gly Lys Glu Met Met Ala Glu Lys Leu Arg Gln Met
    50                  55                  60

Gln Gln Leu Phe Gly Leu Lys Val Thr Gly Asn Ser Asp Pro Glu Thr
65                  70                  75                  80

Leu Arg Ala Met Lys Lys Pro Arg Cys Gly Val Pro Asp Val Ala Pro
                85                  90                  95

Tyr Ala Ile Thr His Asn Asn Pro Arg Trp Thr Lys Thr His Leu Thr
            100                 105                 110

Tyr Ser Ile Leu Asn Tyr Thr Pro Tyr Leu Pro Lys Ala Val Val Glu
        115                 120                 125

Asp Ala Ile Ala Arg Ala Phe Arg Val Trp Ser Asp Val Thr Pro Leu
    130                 135                 140

Thr Phe Gln Arg Val Phe Glu Glu Glu Gly Asp Ile Val Leu Ser Phe
145                 150                 155                 160

His Arg Gly Asp His Gly Asp Asn Asn Pro Phe Asp Gly Pro Asn Tyr
                165                 170                 175

Lys Leu Ala His Thr Phe Gln Pro Gly Pro Gly Leu Gly Gly Asp Val
            180                 185                 190

His Tyr Asp Leu Asp Glu Thr Trp Thr Asn Ser Ser Glu Asn Phe Asn
        195                 200                 205

Leu Phe Tyr Val Thr Ala His Glu Leu Gly His Ser Leu Gly Leu Thr
    210                 215                 220

His Ser Ser Asp Ile Gly Ala Leu Met Phe Pro Ser Tyr Thr Trp Tyr
225                 230                 235                 240

Thr Glu Asp Phe Val Leu Asn Gln Asp Asp Ile Asn Arg Ile Gln Asp
                245                 250                 255

Leu Tyr Gly Pro Ser Pro Asn Pro Ile Gln Pro Thr Gly Ala Thr Thr
            260                 265                 270

Pro His Pro Cys Asn Gly Asp Leu Thr Phe Asp Ala Ile Thr Thr Phe
        275                 280                 285

Arg Gly Glu Val Phe Phe Phe Lys Gly Arg Phe Tyr Ile Arg Val Asn
    290                 295                 300
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Arg Phe Met Pro Glu Pro Glu Leu Asn Leu Ile Gly Ile Leu Trp Pro
305                 310                 315                 320

Asn Leu Pro Val Lys Leu Asp Ala Ala Tyr Glu Ala Ser Met Ile Asp
                325                 330                 335

Gln Val Arg Tyr Phe Lys Gly Ser Lys Val Trp Ala Val Gln Glu Gln
            340                 345                 350

Ser Val Leu Arg Gly Phe Pro Arg Asp Ile His Ser Phe Phe Gly Phe
        355                 360                 365

Pro Ser Asn Val Thr His Ile Asp Ala Ala Val Cys Glu Glu Glu Thr
    370                 375                 380

Gly Lys Thr Tyr Phe Phe Val Asp His Met Tyr Trp Arg Tyr Asp Glu
385                 390                 395                 400

Asn Thr Gln Ser Met Asp Pro Gly Tyr Pro Arg Leu Thr Ala Glu Asp
                405                 410                 415

Phe Pro Gly Ile Asp Asp Lys Val Asp Asp Val Phe Gln Lys Gly Glu
            420                 425                 430

Asn Phe Tyr Phe Phe His Gln Ser Val Gln His Arg Phe Asn Leu Gln
        435                 440                 445

Ile Arg Arg Val Asp Asp Ser Arg Asp Ser Ser Thr Trp Phe Asn Cys
    450                 455                 460
```

1. A genetically-engineered macrophage, comprising:

a recombinant extracellular matrix (ECM) targeting protein; and/or

a recombinant protease.

2. The genetically-engineered macrophage of claim **1**, wherein the recombinant targeting protein is a collagen receptor or a subunit thereof.

3. The genetically-engineered macrophage of claim **2**, wherein the collagen receptor or a subunit thereof comprises one or more of an integrin, a discoidin domain receptor, a mannose family receptor, and an immunoglobulin-like receptor.

4. The genetically-engineered macrophage of claim **3**, wherein the integrin is an $\alpha 1\beta 1$, $\alpha 2\beta 1$, $\alpha 10\beta 1$, and/or $\alpha 11\beta 1$ integrin.

5. The genetically-engineered macrophage of claim **3**, wherein the discoidin domain receptor is DDR1 and/or DDR2.

6. The genetically-engineered macrophage of claim **3**, wherein the mannose family receptor is M-phospholipase A2 receptor and/or Endo180.

7. The genetically-engineered macrophage of claim **3**, wherein the immunoglobulin-like receptor is glycoprotein VI.

8. The genetically-engineered macrophage of claim **1**, wherein the recombinant targeting protein is ITGA-1.

9. The genetically-engineered macrophage of claim **1**, wherein the recombinant protease is a matrix metalloproteinase (MMP).

10. The genetically-engineered macrophage of claim **9**, wherein the matrix metalloproteinase is MMP1, MMP1a, MMP2, MMP3, MMP7, MMP8, MMP9, MMP10, MMP12, MMP13, MMP14, MMP17, MMP19, MMP20, MMP21, MMP22, MMP24, MMP25, MMP26, MMP27, and/or MMP28.

11. The genetically-engineered macrophage of claim **10**, wherein the matrix metalloproteinase is MMP1a.

12. The genetically-engineered macrophage of claim **1**, wherein the macrophage is an M2-specific macrophage.

13. The genetically-engineered macrophage of claim **1**, wherein the recombinant targeting protein is a human integrin $\alpha 1$ encoded by SEQ ID NO: 3, the recombinant catalytic enzyme is a human MMP1 encoded by SEQ ID NO: 5, and wherein the macrophage is a human M2-specific macrophage.

14. A population of cells comprising the genetically-engineered macrophage of any of the preceding claims.

15. A cellular therapy product, comprising:

a genetically-engineered macrophage comprising at least one of a recombinant extracellular matrix (ECM) targeting protein and a recombinant protease.

16. The cellular therapy product of claim **15** further comprising one or more cell media components and/or therapeutic compounds.

17. The cellular therapy product of claim **16** further comprising an effective amount of one or more of α-tocopherol, interferon-γ, quercetin, an ACE inhibitor, and PPAR-δ.

18. The cellular therapy product of claim **17** further comprising a pharmaceutical reagents and/or excipients suitable for therapeutic application.

19. A method of treating an individual for fibrosis, comprising administering the cellular therapy product according to any of claims **15-18**.

20. The method of claim **19**, wherein the fibrosis is liver fibrosis, cardiac fibrosis, or lung fibrosis.

21. The method of claim **19**, wherein the cellular therapy product is administered by injection to the individual.

22. The method of claim **19**, wherein the cellular therapy product is injected in a fibrotic lesion.

23. The method of claim **21**, wherein the cellular therapy product comprises of genetically-engineered macrophages was derived from the individual.

24. A method of reversing liver fibrosis in an individual in need thereof, comprising:

administering to the individual a genetically-engineered M2 macrophage capable of expressing recombinant ITGA-1 and MMP1 or MMP1a;

targeting the macrophage to the liver of the individual; and

reversing fibrosis within the liver.

25. A method of treating cardiac fibrosis in an individual in need thereof, comprising:

administering to the individual a genetically-engineered M2 macrophage capable of expressing recombinant ITGA-1 and MMP1 or MMP1a;

targeting the macrophage to the cardiac fibrosis of the individual; and

ameliorating fibrosis within the cardiac tissue.

26. A method of treating lung fibrosis in an individual in need thereof, comprising:

administering to the individual a genetically-engineered M2 macrophage capable of expressing recombinant ITGA-1 and MMP1 or MMP1a;

targeting the macrophage to lung fibrosis of the individual; and

ameliorating fibrosis within the lung tissue.

* * * * *