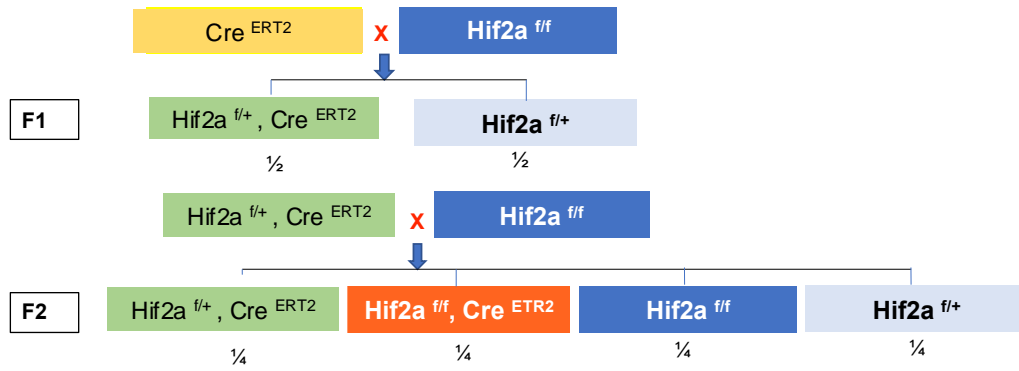
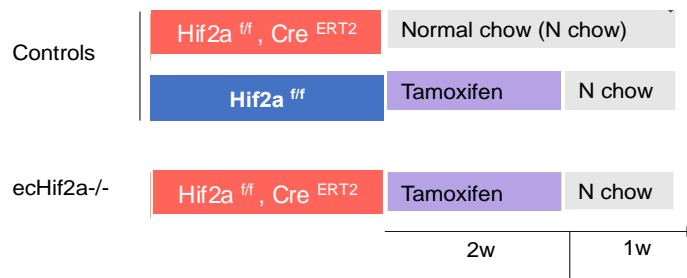


# Supplemental Figures

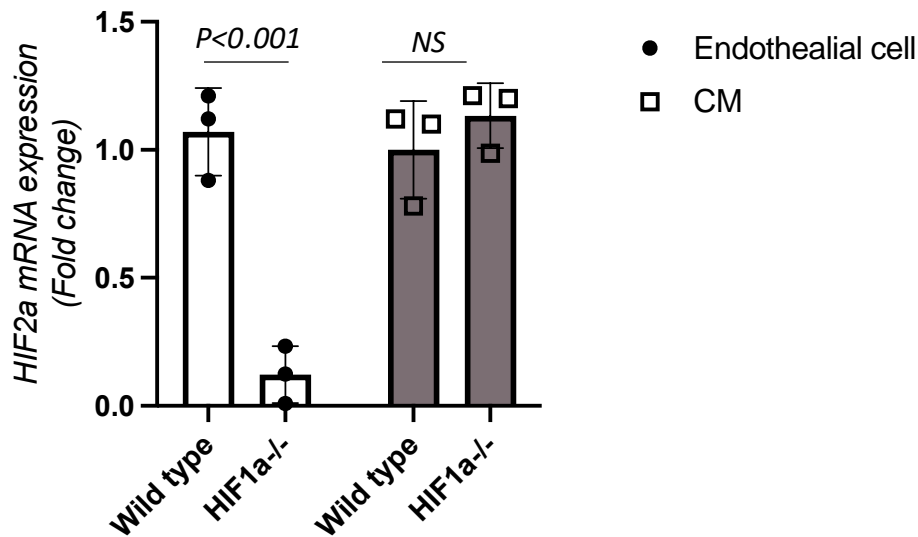
**A**



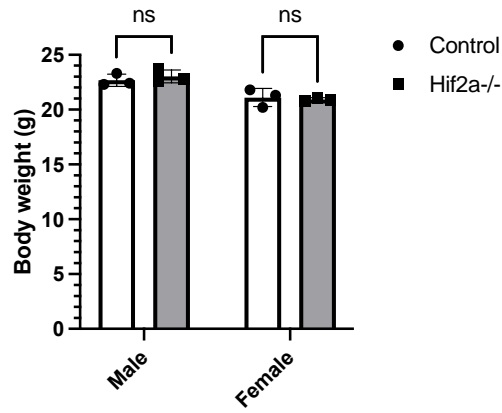
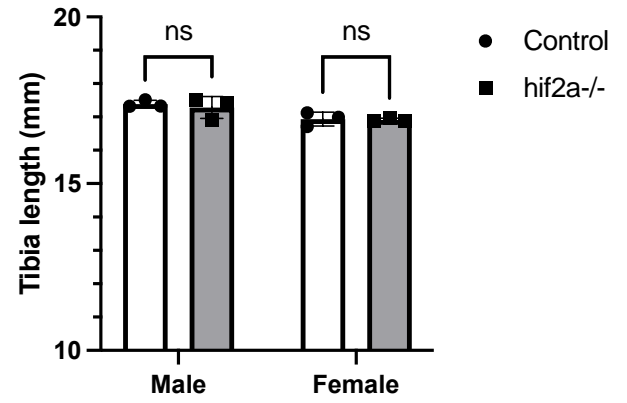
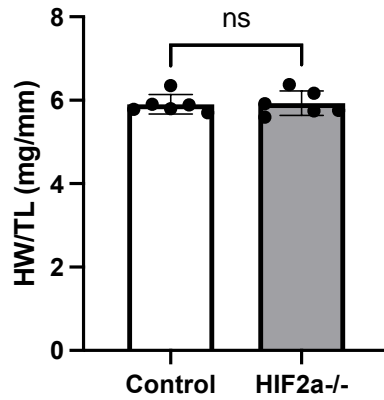
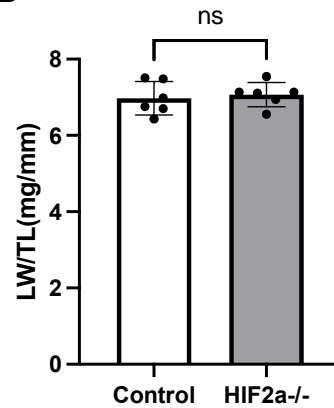
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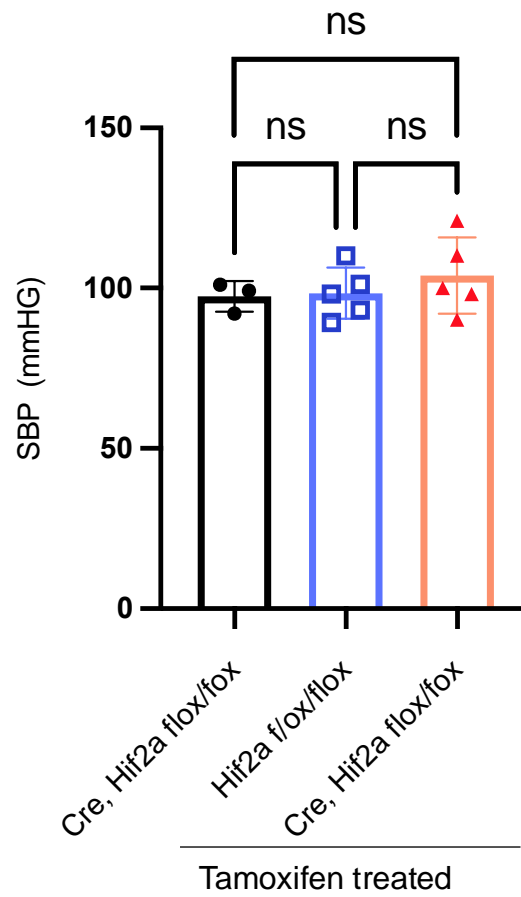
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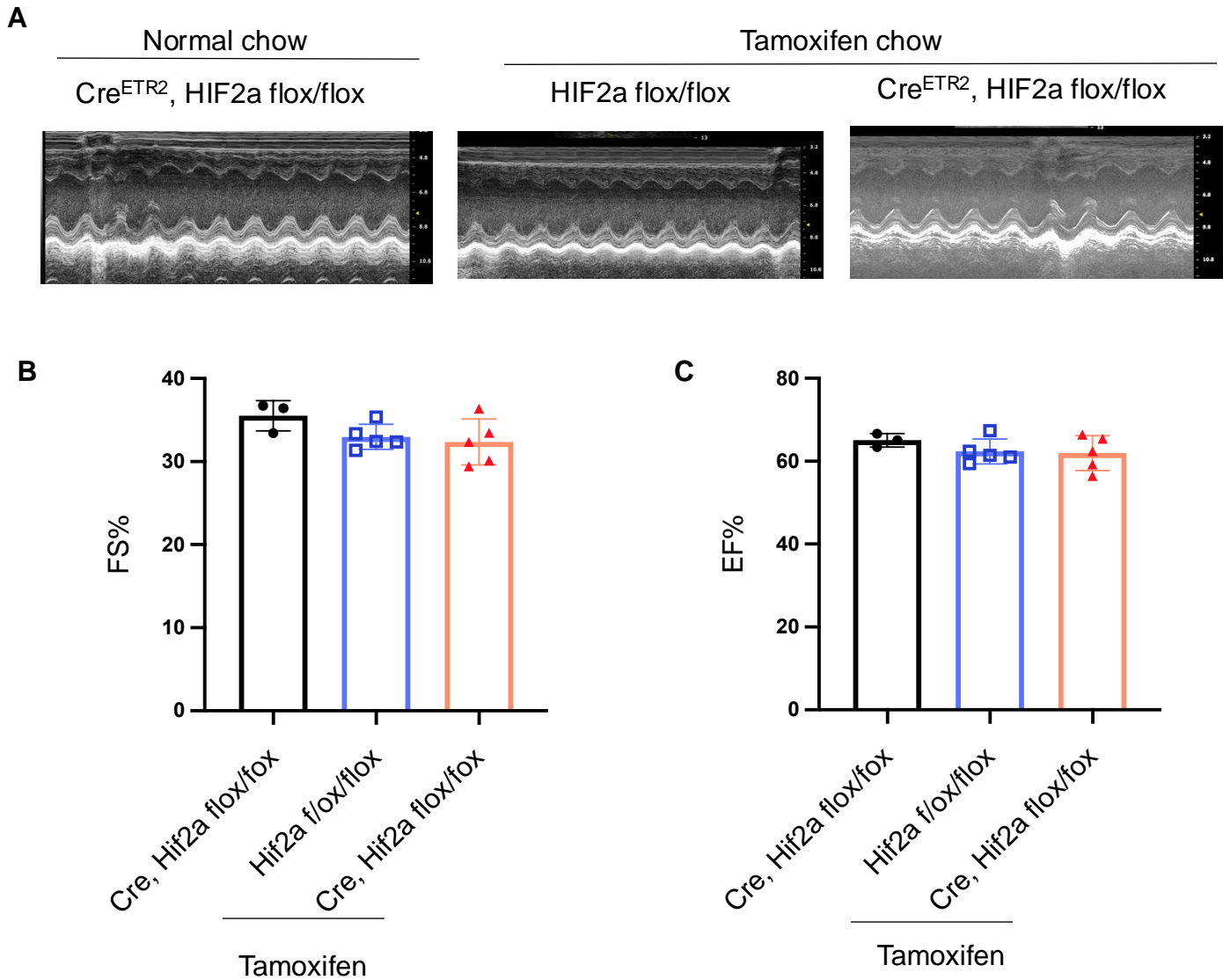
**Supplemental Fig 1:** Schematic Diagram of Generating Adult Inducible ecHIF2α<sup>-/-</sup> Mice and confirmation of HIF2α gene deletion . A) Breeding Strategy for Achieving Hif2a Deletion After Tamoxifen Induction. The breeding plan illustrates the generation of different genotypes. All possible genotypes are indicated for both the F1 (first filial generation) and F2 (second filial generation obtained by crossing the F1 generation). B) Schematic Representation of Tamoxifen Treatment in Each Experimental Group. The figure outlines the tamoxifen treatment regimen for each experimental group, facilitating the induction of Hif2a deletion in adult inducible ecHIF2α<sup>-/-</sup> mice. C) HIF2α mRNA expression from primary isolated endothelial cell and cardiomyocytes.(CM) Data are presented as mean ± SEM (n=3 mice) for each group.

**A****B****C****D**

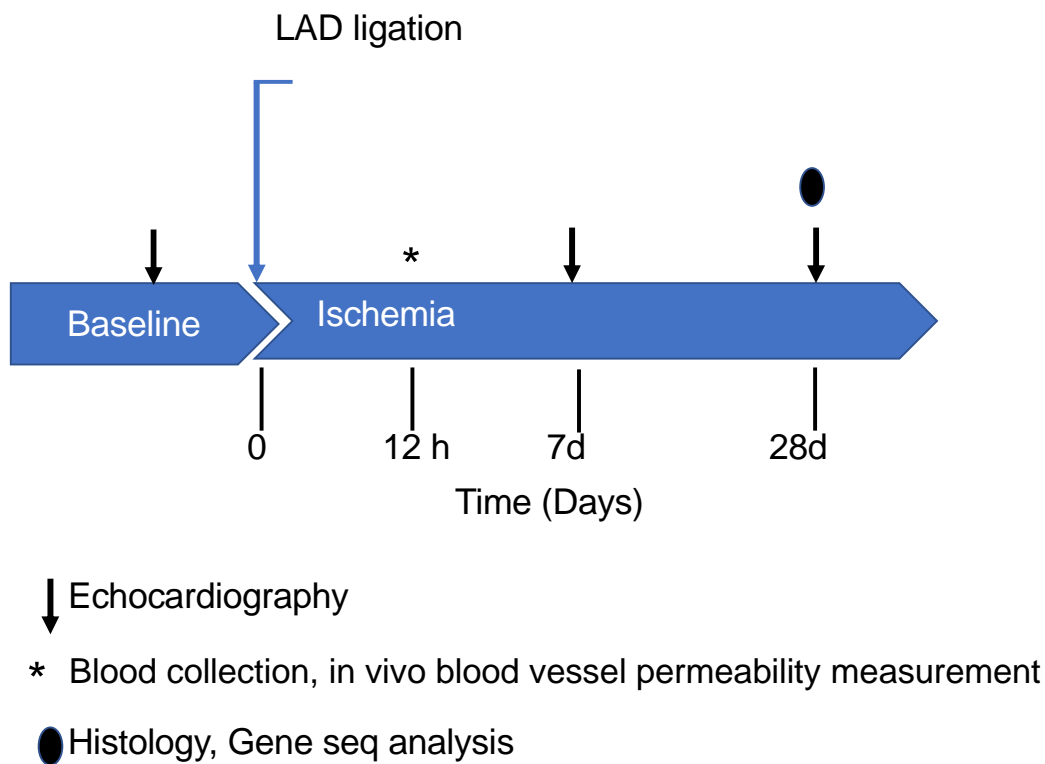
**Supplemental Fig 2:** Absence of Phenotypic Changes One Month After Tamoxifen-Induced Hif2a Deletion. A. Comparison of Body Weight between Female and Male Control and Hif2a<sup>-/-</sup> Mice at the Age of 3 Months. B. Assessment of Tibia Length in Various Groups of Animals. C. Calculation of the Heart Weight to Tibia Length Ratio. D. Determination of the Lung Weight to Tibia Length Ratio. Data are presented as mean  $\pm$  SEM (n=3-6) for each group.



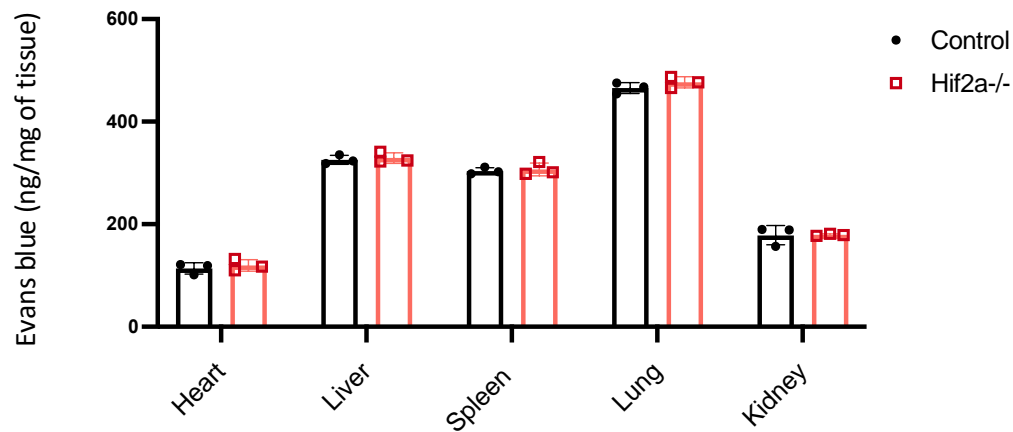
**Supplemental Fig 3.** Measurement of systolic blood pressure using the tail-cuff method. No significant difference was observed among the study groups. Data is shown as mean  $\pm$  SEM in mmHg. (n=3-6).



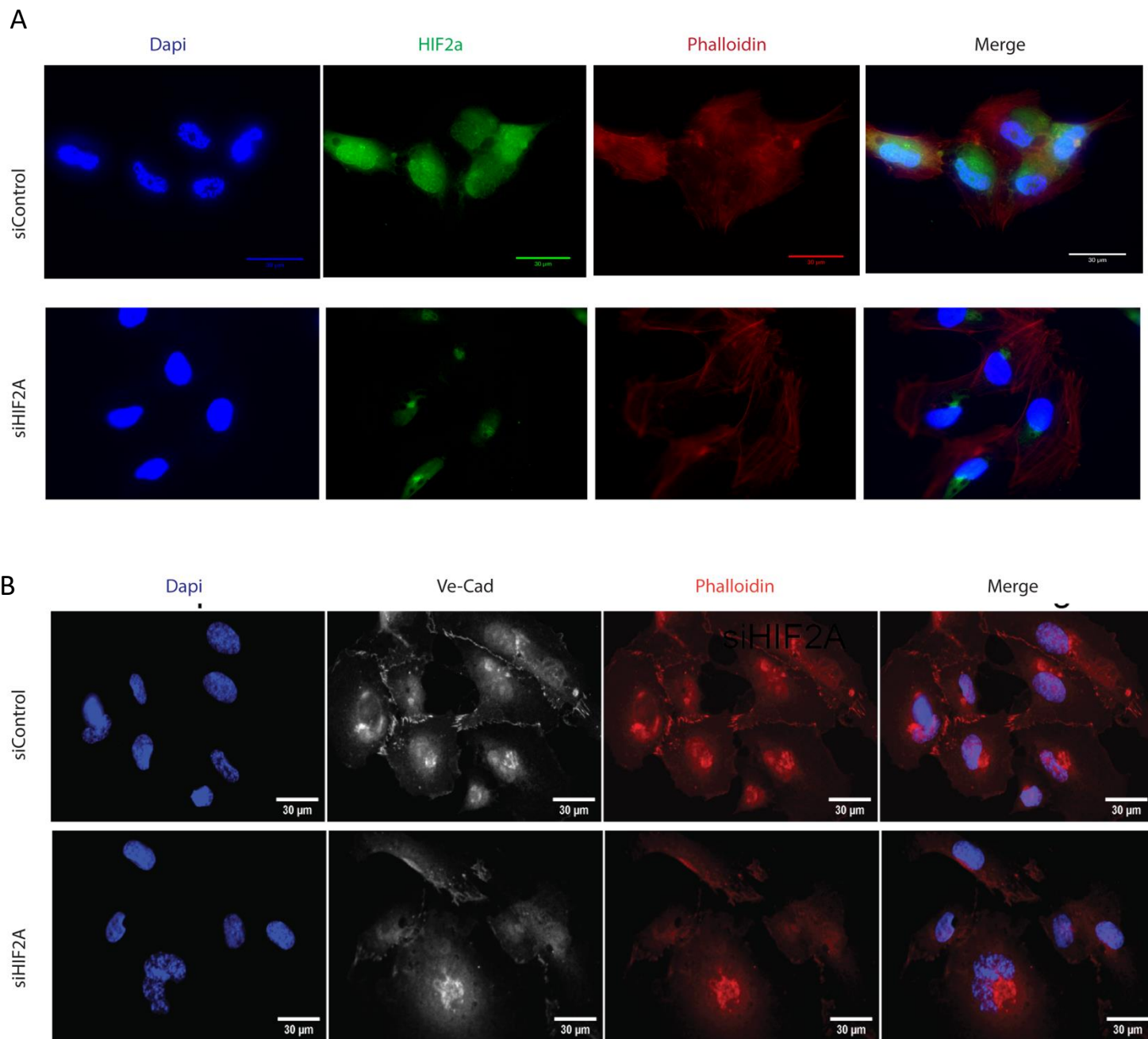
**Supplemental Fig 4.** Echocardiographic Assessment of Cardiac Function in Different Experimental Mouse Groups: (A) Representative M-mode echocardiographic images from the indicated mouse groups, 2 weeks post-tamoxifen administration. (B) Fractional shortening (FS%) and (C) Ejection fraction (EF%). Data are presented as mean ± SEM, n=3-6.



**Supplemental Fig 5.** Schematic Overview of the Myocardial Infarction (MI) Experimental Protocol: MI was induced by permanent ligation of the left anterior descending (LAD) coronary artery. Subsequent assessments, including echocardiography, blood sample collection, in vivo blood vessel permeability tests, histological examinations, and gene sequencing analyses from the mouse hearts, were conducted at the specified time points as indicated.

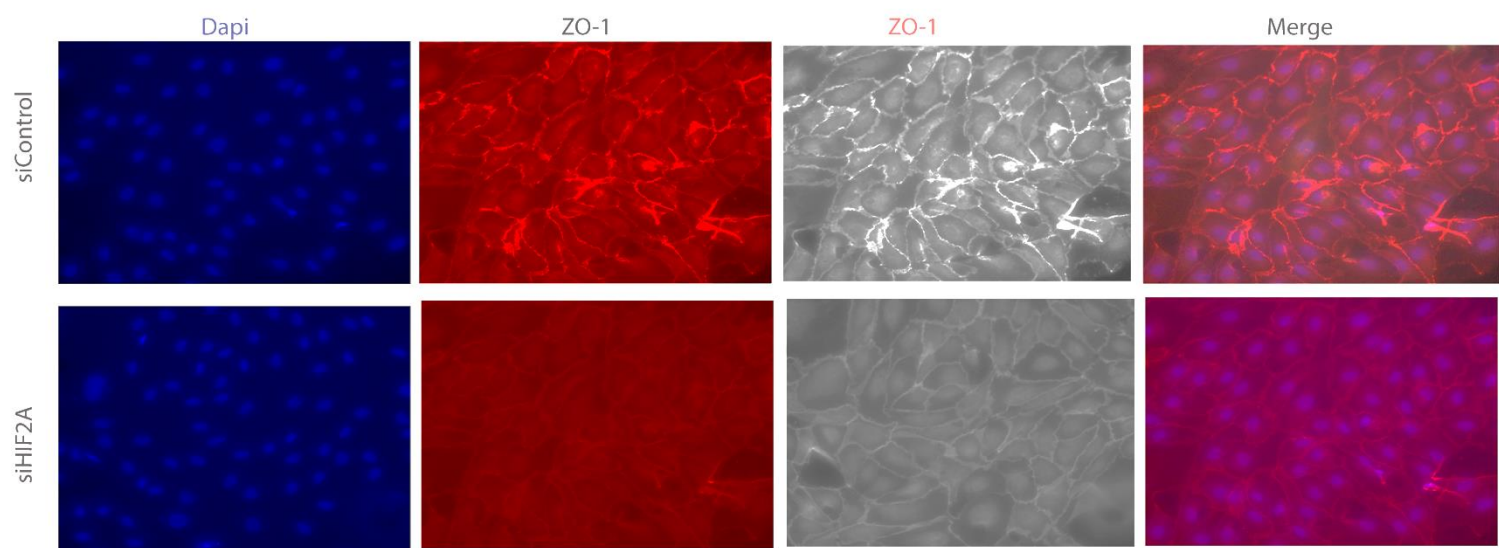


**Supplemental Fig 6.** Quantification of Evans Blue extravasation in different mouse tissues under normoxic conditions. Mice were either control (Hif2 $\alpha$  flox/flox) or underwent hif2 $\alpha$  deletion for two weeks. Post 0.5% Evans Blue injection (30-min duration), mice were euthanized via cervical dislocation. Tissue samples (50-100 mg) were treated with formamide to extract the extravasated Evans Blue. The optical density was measured at 610 nm, with values converted into ng of Evans Blue dye per mg of tissue. Data are presented as mean  $\pm$  SEM (n=3)

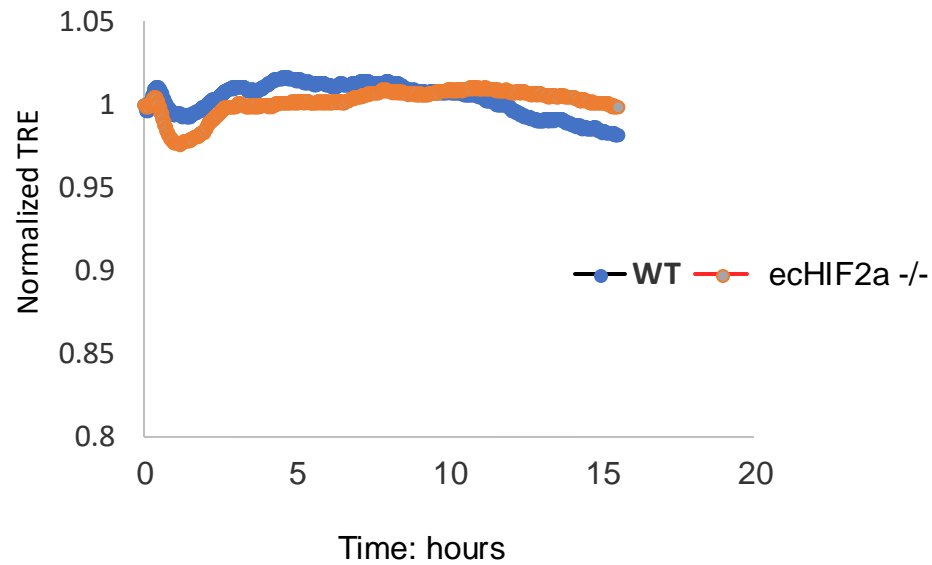


**Supplemental Fig 7. Immunofluorescent stating of HIF2A and Ve-Cadherin (Ve-Cad) expression in primary HMVECs.** (A) Immunofluorescent staining of 4% PFA fixed human microvascular endothelial cells transfected with either control siRNA or HIF2A siRNA. Cells were exposed to 1% oxygen for overnight and analyzed with indicated antibodies. Anti-HIF2a (green), DAPI (blue), and phalloidin (red) are shown. Scale bar is 30 uM. (B) Immunofluorescent staining of 4% PFA fixed HUVECs transfected with either control or HIF2A siRNA. Cells were stained with anti-VE cadherin (white), DAPI (blue), and phalloidin (red). The scale bar is 30 uM.

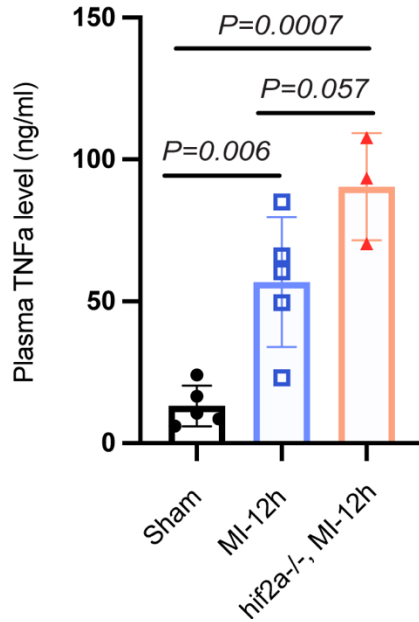
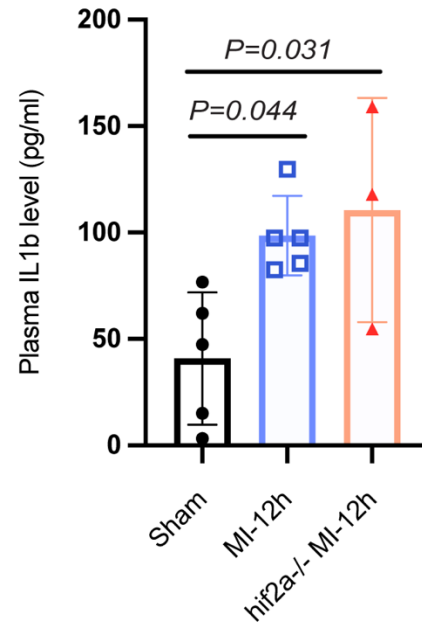




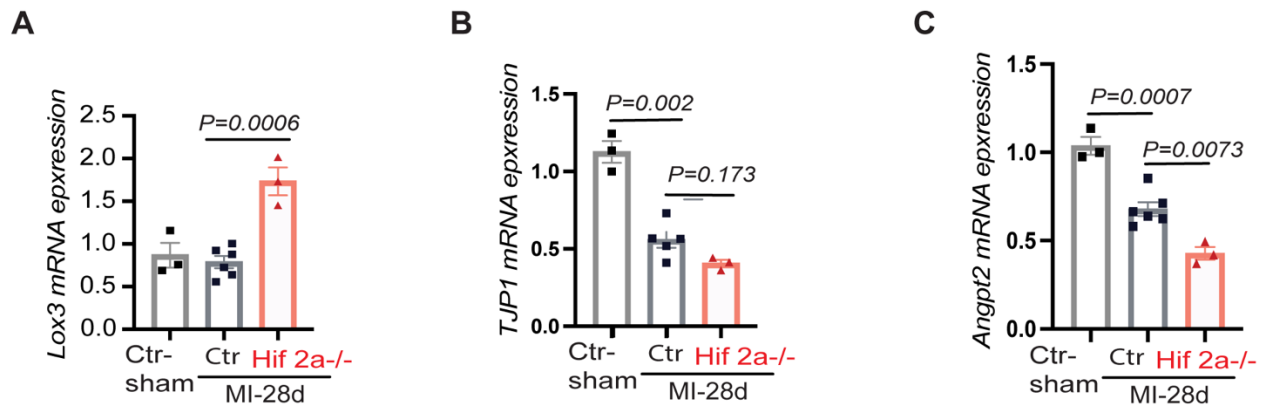
**Supplemental Figure 8:** Immunofluorescent stating of ZO-1 in HMVECs transfected with or without siHIF2A and exposed to 1% oxygen for overnight. Nucleus were stained with Dapi (Blue) and, ZO-1 (Red). The scale bar denotes 30µM



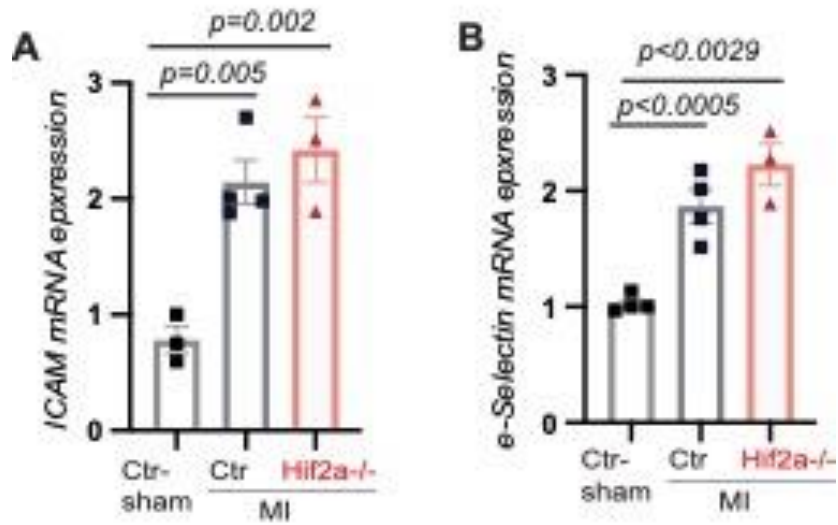
**Supplemental Fig 9:** Assessment of Endothelial Barrier Integrity: Endothelial barrier function was evaluated using primary cardiac microvascular endothelial cells (CMVECs) isolated from control wild-type and ecHIF2a  $-/-$  mice under normoxic conditions. Trans-endothelial Electrical Resistance (TER) was normalized and measured using the Electric Cell-substrate Impedance Sensing (ECIS) system. Data represent findings from three independent experiments (n=3).

**A****B**

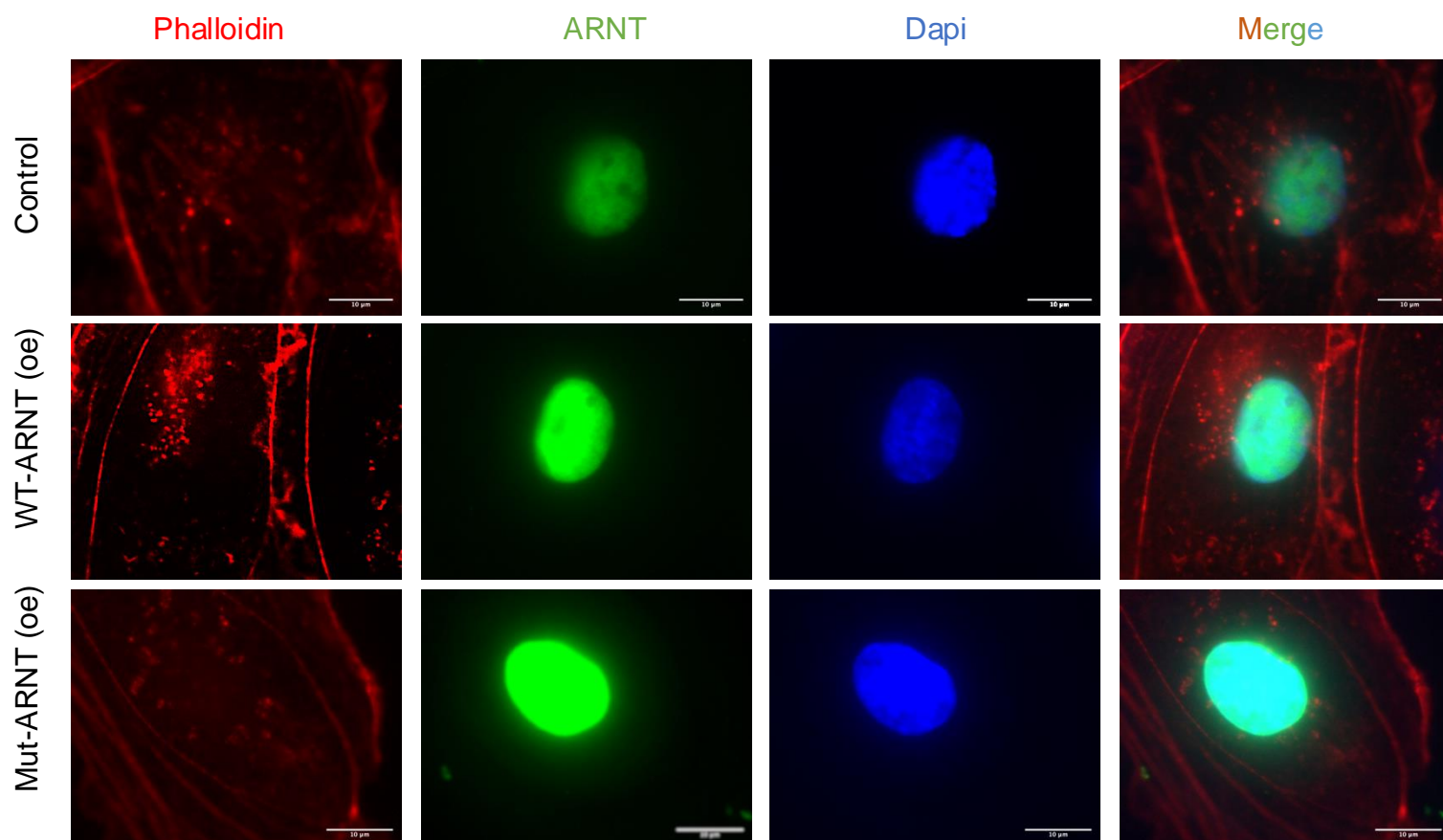
**Online Supplemental Figure 10.** ELISA of Mouse plasma TNF- $\alpha$  (A) and IL1b(B) levels.. 50  $\mu$ L of blood was collected from the tail vein 12h after surgery in different experimental groups as indicated. Plasma harvested from sham operated on HIF2a f/f mice served as control. The levels of TNF- $\alpha$  and IL-1b in the plasma of both MI groups were significantly higher than in sham. Data is shown as mean  $\pm$  SEM. n=3-5



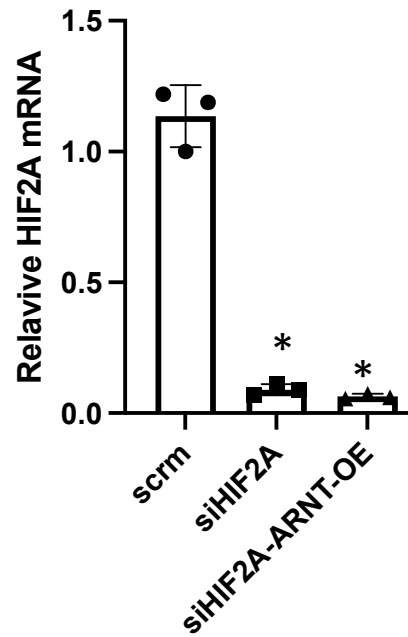
**Supplemental Fig11:** qPCR analysis showing the mRNA levels of Lox3, TJP1, and Angpt2 obtained from isolated RNA samples derived from various mouse hearts. The data are expressed as the mean  $\pm$  SEM (standard error of mean), with the number of samples ranging from 3 to 6.



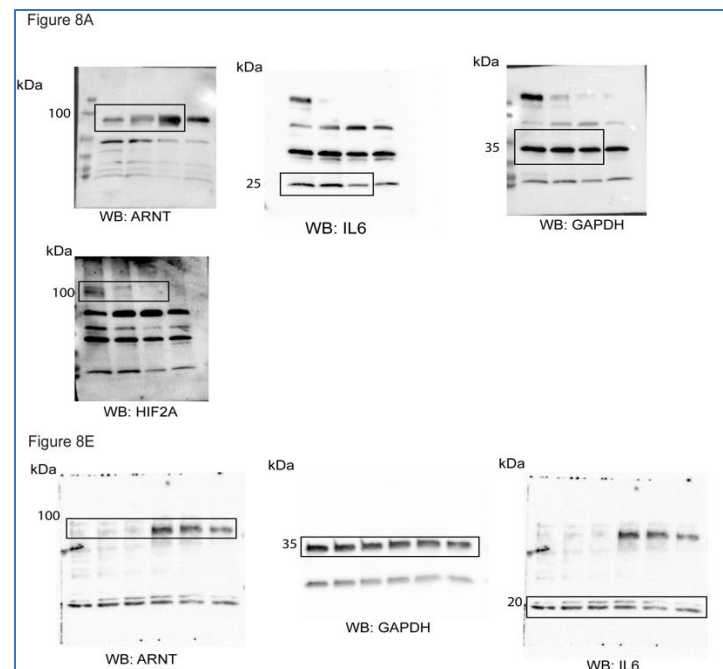
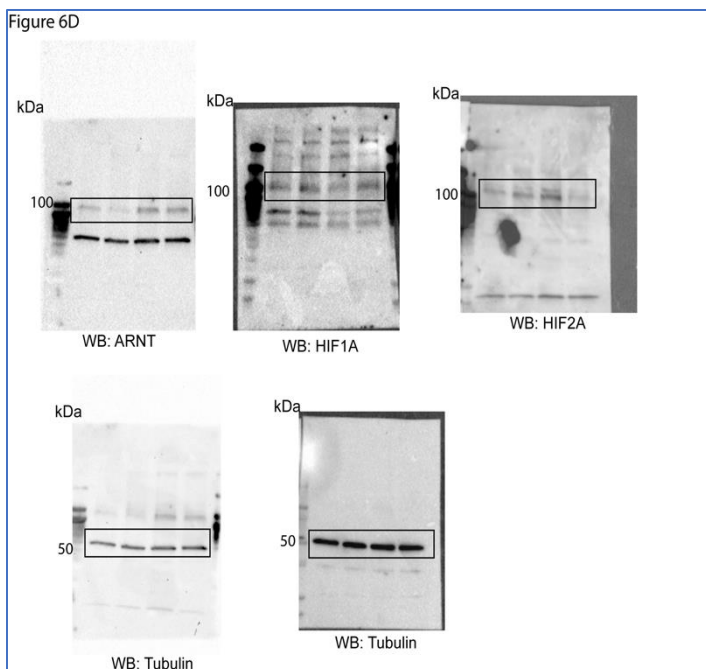
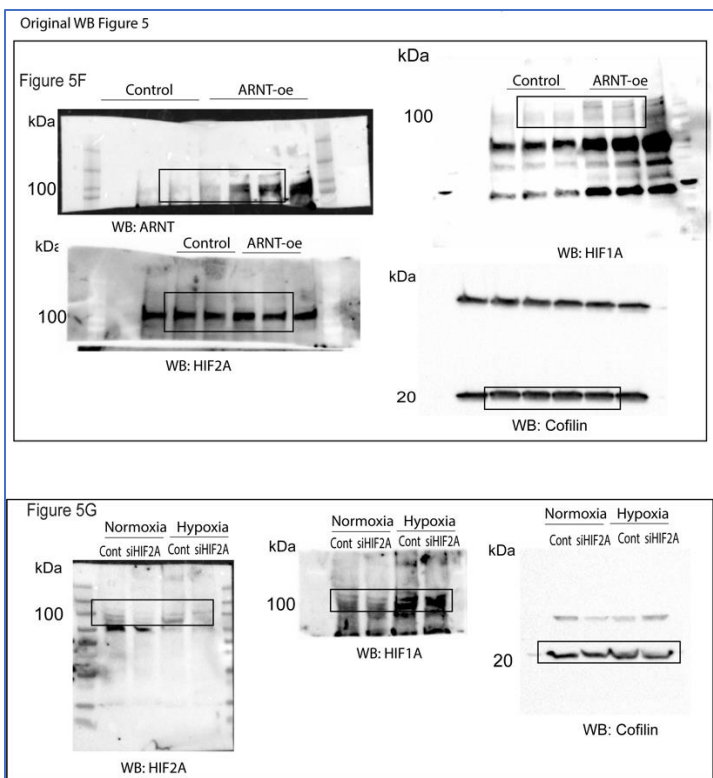
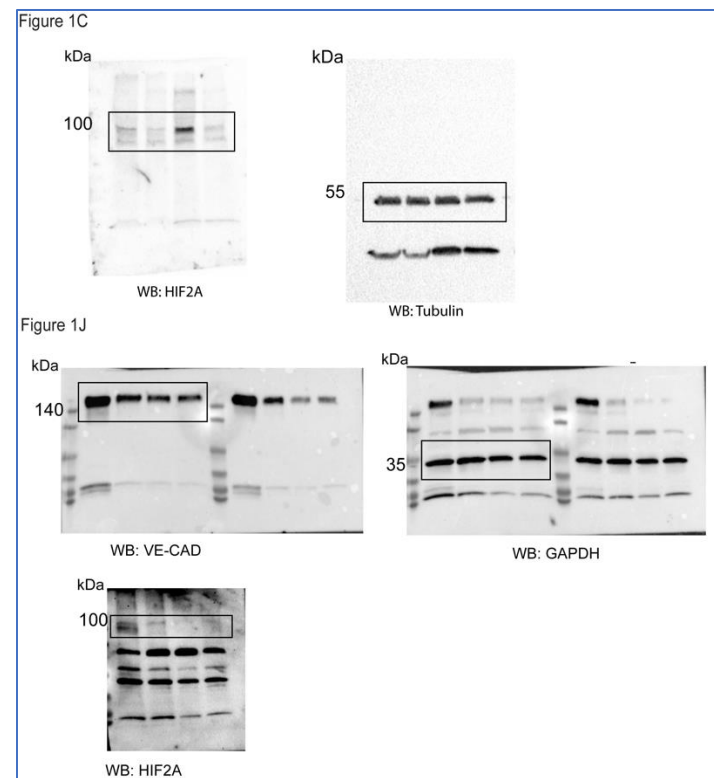
**Online Supplemental Figure 12:** mRNA levels of inflammatory markers, ICAM and e-Selectin, derived from mouse hearts after 28 days post-myocardial infarction. The data are presented as mean ± SEM, n=3-4..



**Supplemental Figure 13:** Immunofluorescent staining of ARNT expression in HMVECs. ARNT overexpression was induced via lentiviral transduction in HMVECs for a period of 48-72 hours, followed by exposure to 1% hypoxia overnight. The respective stains were: ARNT (green), DAPI (blue), and Phalloidin (red).



**Supplemental Figure 14:** *HIF2A* mRNA Expression Analysis in HMVECs by qPCR. Human microvascular endothelial cells (HMVECs) were subjected to various experimental conditions. These included transfection with scrambled siRNA (scrm), transfection with HIF2 $\alpha$  siRNA, and co-transfection with either an empty vector or a vector encoding human recombinant ARNT (ARNT-oe). Following these procedures, cells were exposed to 1% oxygen overnight. Results are presented as mean  $\pm$  SEM. \*P < 0.001 when compared to the Scrambled (scrm) group.



## Supplementary Figure 15. Uncropped Western Blot Images Corresponding to Main Figures

This figure presents the uncropped Western blot images corresponding to the blots displayed in the main figures. (A) Uncropped Western blot images for Figure 1C, showing HIF-2 $\alpha$  expression with Tubulin as a loading control. (B) Uncropped Western blot images for Figure 1J, displaying VE-Cadherin, GAPDH, and HIF-2 $\alpha$  expression. (C) Uncropped Western blot images for Figure 6D, showing ARNT, HIF-1 $\alpha$ , HIF-2 $\alpha$ , and Tubulin expression. (D) Uncropped Western blot images for Figure 5F, depicting ARNT, HIF-1 $\alpha$ , HIF-2 $\alpha$ , and Cofilin expression in control and ARNT-overexpressing conditions. (E) Uncropped Western blot images for Figure 5G, showing HIF-1 $\alpha$ , HIF-2 $\alpha$ , and Cofilin expression under normoxia and hypoxia conditions. (F) Uncropped Western blot images for Figure 8A, demonstrating ARNT, IL-6, HIF-2 $\alpha$ , and GAPDH expression. (G) Uncropped Western blot images for Figure 8E, displaying ARNT, IL-6, and GAPDH expression. Molecular weight markers (kDa) are indicated on the left of each blot.



Supplementary Table 1 . DEG of HIF2a<sup>-/-</sup> vs WT after MI-28 d

	logFC	logCPM	LR	PValue	FDR
Col13a1	2.55067543	1.22925484	26.0651585	3.30E-07	0.00015422
Esm1	2.52793941	1.84464138	18.3599412	1.83E-05	0.00452315
Car9	2.01142864	1.01873362	16.347044	5.27E-05	0.01057446
Slco2a1	1.93163626	3.81903308	68.1618213	1.51E-16	6.33E-13
Exoc3l2	1.8974996	2.59713576	18.2646215	1.92E-05	0.00457578
Piezo2	1.79996398	3.64868569	67.0931664	2.59E-16	8.17E-13
Plvap	1.76701693	5.21075091	104.536545	1.54E-24	1.95E-20
F19Rik	1.71186239	2.61711115	13.1352164	0.0002898	0.03248766
Jchain	1.70318527	6.59792369	19.0754398	1.26E-05	0.00337254
Snca	1.66497252	1.19695183	16.0724049	6.10E-05	0.01149653
Msln	1.65040818	3.09611165	12.1693304	0.00048582	0.04750842
Alas2	1.64240039	4.31734513	77.9459625	1.06E-18	6.68E-15
Ces2e	1.6066815	2.23073148	24.3841465	7.89E-07	0.00034323
Stc1	1.47888144	3.26425556	17.9590513	2.26E-05	0.00508449
Igfbp3	1.34372331	6.08102208	45.7751834	1.33E-11	1.86E-08
Tent5c	1.2547628	3.14386982	26.836767	2.21E-07	0.00011171
Kif21b	1.25391684	2.75690013	15.6252383	7.72E-05	0.01334374
Myoc	1.2417222	4.45780477	35.3173635	2.80E-09	2.52E-06
Myh7	1.23523556	11.8126282	15.7166193	7.36E-05	0.01289093
Emcn	1.21882606	5.38875445	39.5667921	3.17E-10	3.22E-07
Hba-a1	1.2188222	8.99525322	51.7165739	6.41E-13	1.35E-09
Hbb-bs	1.20250512	9.3344451	48.0480602	4.16E-12	6.56E-09
Hba-a2	1.20121413	8.87586025	49.455027	2.03E-12	3.66E-09
Enpp6	1.17700114	3.19966799	24.0547444	9.36E-07	0.00036913
Hbb-bt	1.17303284	8.51529643	44.1583905	3.03E-11	3.82E-08
Lars2	1.16357754	9.63265117	29.4802288	5.65E-08	3.39E-05
Runx3	1.15929048	2.72725274	14.8686166	0.00011526	0.01690773
Sh3rf3	1.13932259	2.90414283	18.3814374	1.81E-05	0.00452315
Ccn5	1.08762603	7.29129991	34.7169216	3.81E-09	3.21E-06
C7	1.06887614	4.66649465	12.0835378	0.00050869	0.04936248
Capn5	1.06865929	2.82968401	16.3910847	5.15E-05	0.01057446
Egln3	1.06273241	6.66733626	22.830674	1.77E-06	0.00065642
Cilp	1.00894224	8.97275801	28.9723514	7.34E-08	4.16E-05
Lgals3	0.99519935	4.72889396	30.2132455	3.87E-08	2.44E-05

Supplementary Table 1 (continued). DEG of HIF2a<sup>-/-</sup> vs WT after MI-28 d

	logFC	logCPM	LR	PValue	FDR
Slit3	0.96581333	5.47791007	24.0870475	9.21E-07	0.00036913
Rnf152	0.9616726	3.23492763	15.9591599	6.47E-05	0.01200722
Col13a2	0.76209899	6.90714998	23.6070053	5.18E-05	0.00644727
Esm2	0.72649619	7.01867232	23.1656048	5.18E-05	0.0064334
Car10	0.6908934	7.13019466	22.7242044	5.17E-05	0.00641952
Slco2a2	0.6552906	7.241717	22.2828039	5.17E-05	6.41E-03
Exoc3l3	0.6196878	7.35323935	21.8414034	5.17E-05	0.00639177
Piezo3	0.58408501	7.46476169	21.4000029	5.17E-05	6.38E-03
Plvap	0.54848221	7.57628403	20.9586024	5.17E-05	6.36E-03
9330159F19Rik	0.51287941	7.68780637	20.5172019	5.166E-05	0.00635015
Jchain	0.47727662	7.79932871	20.0758015	5.16E-05	0.00633627
Snca	0.44167382	7.91085106	19.634401	5.16E-05	0.0063224
Msln	0.40607102	8.0223734	19.1930005	5.1607E-05	0.00630852
Alas3	0.37046823	8.13389574	18.7516	5.16E-05	6.29E-03
Lox	0.81714984	7.27597884	20.963076	4.68E-06	0.00146163
Itgbl1	0.80462595	7.34989121	20.958131	4.69E-06	0.00146163
Pdgfrl	0.80428732	5.10176248	19.5061013	1.00E-05	0.00277492
Ptgs1	0.78183174	4.25744613	15.7175662	7.35E-05	0.01289093
Scarf2	0.77810728	4.23148374	13.6194805	0.00022385	0.02715265
Dennd4a	0.77674329	5.45662555	20.0433934	7.57E-06	0.00217049
Gucy1a1	0.76610019	5.42728671	12.6769619	0.00037019	0.03766072
Setbp1	0.75646676	4.78986256	15.1878484	9.73E-05	0.01554163
Spon1	0.75603503	5.95435382	14.2138257	0.00016317	0.02122015
Rai14	0.73572558	4.49008574	13.6858455	0.00021608	0.02646418
Ahnak2	0.73217068	5.67500665	18.0544293	2.15E-05	0.00492395
Ddah1	0.72883449	5.0957387	14.3383523	0.00015272	0.02006858
Errfi1	0.72074541	5.93093728	15.0742212	0.00010337	0.01629941
Cybb	0.7207042	4.72432158	13.9960554	0.00018319	0.02301031
Pfkfb3	0.72050776	5.0066907	16.786553	4.18E-05	0.00909773
Lbp	0.71858161	4.4865724	14.4344749	0.00014512	0.01927057
P3h2	0.71759118	3.95445544	12.1771252	0.00048379	0.04750842
Gja4	0.71161634	4.05693967	12.8876648	0.00033075	0.03477061
Eln	0.71098612	8.22959671	18.1325725	2.06E-05	0.00481348
Kcne4	0.70995039	4.20680353	13.947968	0.00018794	0.02324384
Cyp2d22	0.70781119	4.58769398	14.9416521	0.00011089	0.01690773

Supplementary Table 1 (continued). DEG of HIF2a<sup>-/-</sup> vs WT after MI-28 d

	logFC	logCPM	LR	PValue	FDR
Cygb	0.70726588	7.37795793	18.602616	1.61E-05	0.00414491
Thbs3	0.7068232	4.94627804	12.5028317	0.00040634	0.04068194
Rbp1	0.70484566	4.77743201	15.3761292	8.81E-05	0.01461876
Serping1	0.69262027	8.38559646	15.3909249	8.74E-05	0.01461876
Fxyd6	0.67578739	6.44452933	14.8975795	0.00011351	0.01690773
Thbs2	0.67097968	6.70869351	15.7990119	7.04E-05	0.01269417
Npr3	0.66574252	5.09744304	14.5098918	0.00013943	0.01927057
Tmem176a	0.6557902	4.6799927	12.9282203	0.00032367	0.03431125
Itgb5	0.64786266	8.04085376	14.5187335	0.00013877	0.01927057
Kirrel	0.64345879	5.43138351	14.6634278	0.00012852	0.01842303
Dpep1	0.64243188	6.1937619	15.3566693	8.90E-05	0.01461876
Loxl3	0.63239295	5.89704709	14.6191619	0.00013157	0.01864893
Ptprj	0.63228891	5.18766243	13.4164432	0.00024943	0.0291346
C1qtnf6	0.62952098	5.08497225	13.1273818	0.00029101	0.03248766
Col8a2	0.62576279	6.06994926	13.1885791	0.00028166	0.03230132
Prss23	0.6222481	5.5205872	14.0059914	0.00018223	0.02301031
Timp4	-0.6188735	5.87820593	12.7529702	0.00035544	0.03698951
Ppif	-0.6297298	6.95944802	13.0952569	0.00029604	0.03268498
Gpam	-0.6406637	7.84370177	12.7409992	0.00035773	0.03698951
Hspa4l	-0.6841529	6.12491124	15.2789267	9.27E-05	0.01499979
Fabp4	-0.7185635	8.21414492	16.2124039	5.66E-05	0.01115198
Myh7b	-0.7767873	4.86164366	14.8805333	0.00011454	0.01690773
Fkbp4	-0.7887051	7.26876334	13.5709703	0.00022971	0.02759809
C1qtnf9	-0.7962827	5.1018469	15.3519111	8.92E-05	0.01461876
Sox7	-0.8068756	4.43318874	17.1256659	3.50E-05	0.00774302
Dhrs7c	-0.838604	5.47265022	13.3473177	0.00025879	0.02995108
Mdga1	-0.8546905	3.49816875	12.5245155	0.00040165	0.04053422
Hopx	-0.8844896	6.62400382	16.0694978	6.11E-05	0.01149653

Supplementary Table 1 (continued). DEG of HIF2a<sup>-/-</sup> vs WT after MI-28 d

	logFC	logCPM	LR	PValue	FDR
Cygb	-1.067621	5.65021975	14.1323203	2.06E-04	0.02475167
Zfhx2	-0.8962505	4.00893165	16.368768	5.21E-05	0.01057446
Meox2	-0.899809	3.53341272	12.9953625	0.00031226	0.03361101
Slc28a2	-0.901964	4.44842764	20.383295	6.34E-06	0.00185941
Adam11	-0.9170832	3.71673908	13.5432066	0.00023313	0.02774511
Hr	-0.9562224	5.27541117	25.6021674	4.20E-07	0.00018903
Ppargc1a	-1.0062119	7.97256912	20.7698961	5.18E-06	0.00155557
Folr2	-1.0075601	3.21271463	15.0497066	0.00010472	0.01630863
Mpp7	-1.0110908	3.09574521	13.4776477	0.00024142	0.02846301
Aqp7	-1.0320828	3.90541618	14.4841663	0.00014134	0.01927057
Myl1	-1.0400831	4.97129982	26.6787334	2.40E-07	0.00011657
Aqp1	-1.0701726	8.40026378	34.5558167	4.14E-09	3.27E-06
Depp1	-1.080029	5.96423715	32.0339082	1.52E-08	1.06E-05
Ano4	-1.1018567	2.66785527	13.0307358	0.00030642	0.03332319
Pdp2	-1.1126567	4.63770454	14.4348312	0.00014509	0.01927057
Cyp4b1	-1.1147587	4.06848195	24.3191136	8.16E-07	0.00034323
Car4	-1.2279705	4.05862298	30.3933275	3.53E-08	2.34E-05
Mylk4	-1.2531217	7.6764429	21.8620131	2.93E-06	0.00097261
Metrn	-1.2672435	3.9006579	27.6194851	1.48E-07	7.76E-05
Pdk4	-1.2989933	10.3626438	39.4760747	3.32E-10	3.22E-07
Scn4b	-1.3213515	5.57239223	23.4282024	1.30E-06	0.00049567
Stmn1	-1.3559787	3.89583839	22.6392087	1.95E-06	0.0006664
Ucp3	-1.4062228	5.75234423	19.4888938	1.01E-05	0.00277492
Adora2a	-1.4962434	2.6214644	12.7178636	0.00036218	0.03714546
Arc	-1.6303966	1.86735476	14.0319633	0.00017973	0.02301031
Ano5	-1.7307574	1.88655154	14.4514116	0.00014382	0.01927057
Ush1c	-1.7385907	1.59504638	14.9092168	0.00011281	0.01690773
Gfra2	-1.7946495	1.26594621	13.9854745	0.00018423	0.02301031
Cdh23	-1.9302848	2.70195947	34.4096851	4.46E-09	3.31E-06
Nrn1	-1.9395085	3.07167424	40.8213526	1.67E-10	1.91E-07
Angptl4	-2.0929161	3.91272425	53.2661842	2.91E-13	7.35E-10

RNA-Seq analysis: Reactome analysis of 130 differentially expressed genes between control and ecHIF2a<sup>-/-</sup> after MI-28 days revealing that the majority of these genes are annotated to the regulation of extracellular matrix and collagen pathways.