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Supplementary Materials for

Engineered bridge protein with dual affinity for bone morphogenetic protein-2 and collagen enhances bone regeneration for spinal fusion

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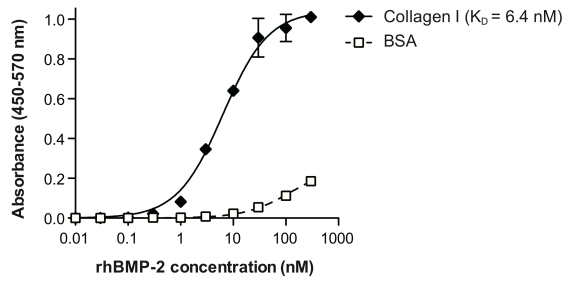
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Tables S1 and S2

A. rhBMP-2 binding to bovine collagen I



B. Competition binding of rhBMP-2 and FabCol to collagen I

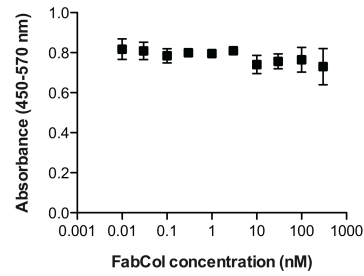
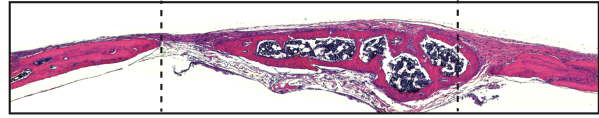
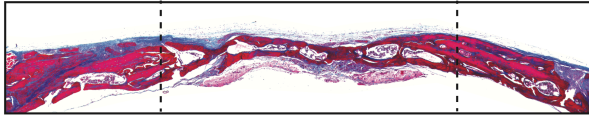


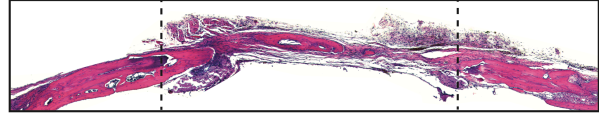
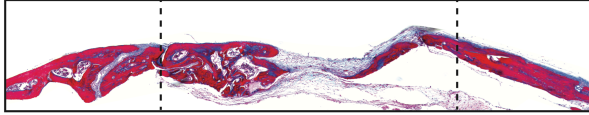
Figure S1. FabCol binding to collagen I does not compete with the natural interaction of rhBMP-2 to collagen I. (A) RhBMP-2 binding to bovine type I collagen. ELISA plates were coated with collagen I and probed with various concentrations of rhBMP-2, further detected with anti-BMP-2. BSA coated plates were used as control for unspecific binding. Specific affinity of rhBMP-2 to collagen was observed with a low dissociation constant of 6.4 nM. (B) Competitive binding of rhBMP-2 and FabCol to collagen I. Binding of 100 nM of rhBMP-2 to collagen I-coated plates was assessed in presence of various concentration of FabCol, and detected using anti-BMP-2. Binding of rhBMP-2 was not inhibited by the presence of the FabCol domain, suggesting that both ligands binds to different sites on the collagen helix.

A.

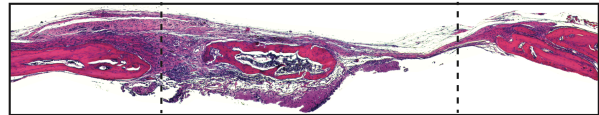
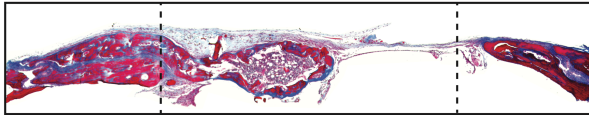
FabCol-LamLG4 + rhBMP-2



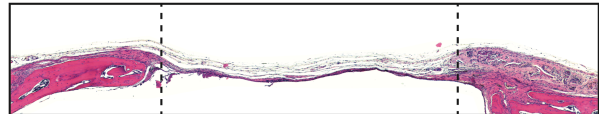
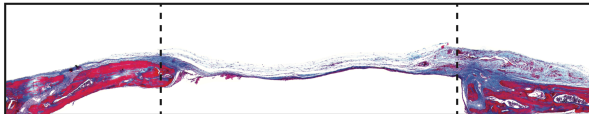
FabCol-FgHBD + rhBMP-2



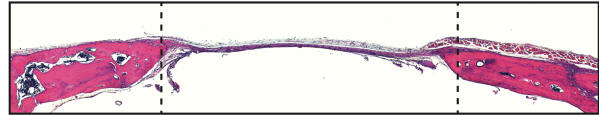
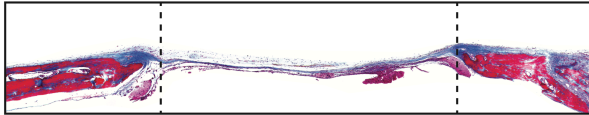
rhBMP-2



FabCol-LamLG4



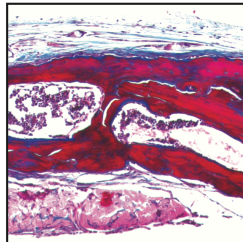
PBS (collagen sponge only)



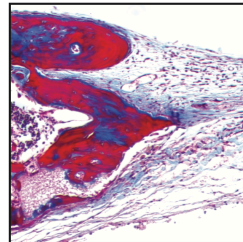
500 μ m

B.

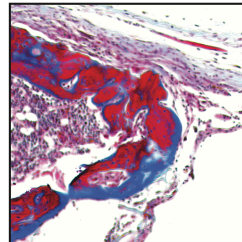
FabCol-LamLG4 + rhBMP-2



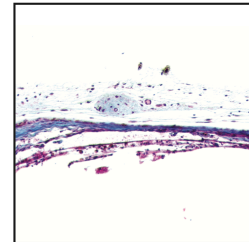
FabCol-FgHBD + rhBMP-2



rhBMP-2



PBS



250 μ m

Figure S2. Histology of the regenerated bone 2 months after the delivery rhBMP-2 in the presence of bridge proteins in the calvarial defect model in mice. **(A)** Representative histology stained with Masson-Trichrome (left images: mineralized mature bone in red, collagenous connective tissues in blue) or with H&E (right images) showing bone regenerated in the defect upon treatment with 50 ng of rhBMP-2 with or without bridge proteins (the space between the dashed lines represents the initial defect size). **(B)** High magnification images of regenerated bone in the defects stained with Masson-Trichrome.

Bone regeneration over time in the calvarial defect model upon delivery of 50 ng of BMP-2

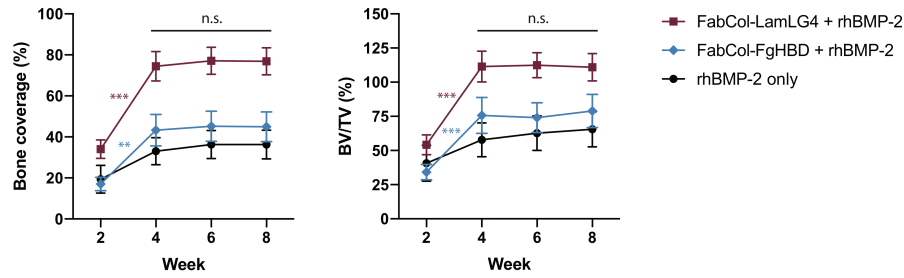


Figure S3. Bone regeneration over time upon delivery of rhBMP-2 in presence of bridge proteins in the calvarial defect model in mice. Defect coverage (left panel) and regenerated bone volume (right panel) was quantified every 2 weeks upon treatments with 50 ng of rhBMP-2 in presence of the bridge proteins. In this model, bone regenerates during the 4 first weeks following treatments, but not at later time (repeated-measurements two-way ANOVA with Sidak's post-test: ** $p \leq 0.01$, *** $p \leq 0.001$, n.s. not significant).

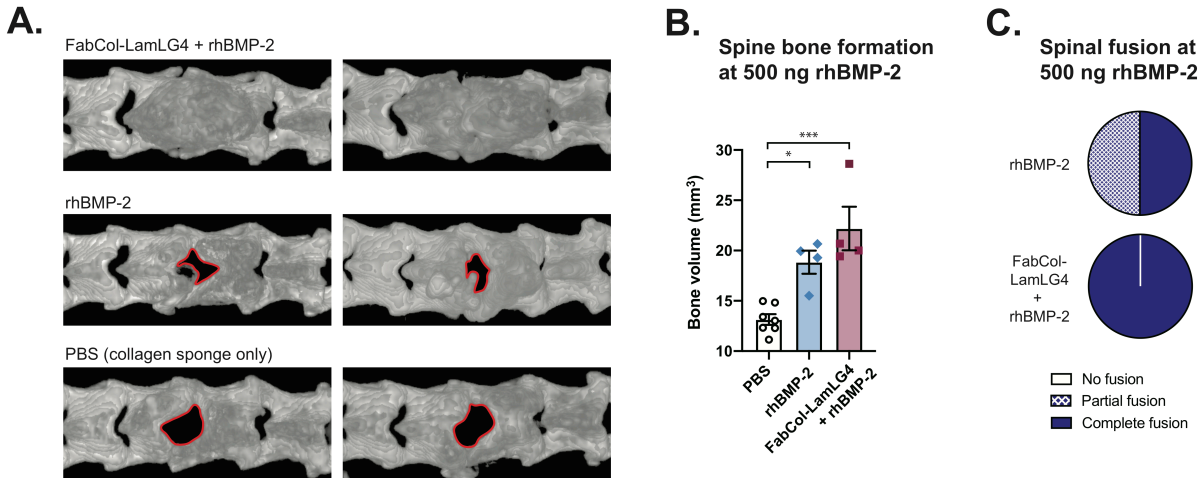


Figure S4. Bone formation and spinal fusion rate upon delivery of rhBMP-2 in presence of FabCol-LamLG4 in the intervertebral defect model in mice. (A) High magnification μ CT scans of the bone regenerated at week 4 after treatment with 100 ng of rhBMP-2 with or without FabCol-LamLG4 in the intervertebral defects in mice (mineralized bone in gray, bone defect circled in red). (B) Quantification of the bone volume formed 4 weeks after treatments with 500 ng of rhBMP-2 \pm FabCol-LamLG4. Both rhBMP-2 and FabCol-LamLG4 + rhBMP-2 regenerated better than the PBS control group (mean \pm SEM, $N \geq 4$, one-way ANOVA with Tukey's post-test: $*p \leq 0.05$, $***p \leq 0.001$). (C) Corresponding proportion of spinal fusions upon treatments with rhBMP-2 \pm FabCol-LamLG4 ($N=4$ per group).

Table S1. Sequences of the bridge proteins FabCol-LamLG4 and FabCol-FgHBD. Both fusion bridge proteins are composed of the variable regions of the light and a heavy chain from the anti-collagen Fab attached to a Fab human constant regions, fused at the C-terminus with one or multiple repeats of the growth factor-binding sites LamLG4 and FgHBD respectively (italicized sequences are the signal peptides, underlined sequences are the light and heavy chains of the FabCol domain, regular sequences are the Fab human constant regions, and bold sequences are the LamLG4 (including the Laminin $\alpha 3$ linker domain) or FgHBD with GlySer linkers, asterisks are stop codon).

FabCol (light chain)	<i>MRAWIFFLLCLAGRALAEIVLTQSPGTLSPGERATLSCRASQSVSSSYLAWYQ</i> <u>QKPGQAPRLLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQA</u> <u>I</u> <u>GFPQTFGQG</u> <u>TKVEIK</u> <u>RTVAAPS</u> <u>VFIFPPSDEQLKSGTASVVCLLNNFY</u> <u>P</u> <u>REAKV</u> <u>QWKVDNALQSGNSQESVTEQDSK</u> <u>STYSL</u> <u>SSTLTLSKADYEKHKVYACEVTH</u> <u>QGLSSPVTKSFNRGEC</u> *
FabCol (heavy chain)	<i>MRAWIFFLLCLAGRALAEVQLLES</i> <u>GGGLVQPGGSLRLS</u> <u>CAASGFTFSSYAMSWV</u> <u>RQAPGKGLEQVSAISGSGGSTYYADSVKGRFTISRDN</u> <u>SKNTLYLQMN</u> <u>SLRAED</u> <u>TAVYYCAKTLAAFDYWGG</u> <u>QTLTV</u> <u>VSSASTKGPSVFPLAPSSKSTSGGTAALG</u> <u>CLVKDYFPEP</u> <u>TVSWNSGALTSGVHTFPAVLQSSGLYSLSSV</u> <u>TV</u> <u>PSSSLGTQT</u> <u>YICNVNHKPSNTKVDKRVEPKSCGS</u> *
FabCol- LamLG4 (light chain)	<i>MRAWIFFLLCLAGRALAEIVLTQSPGTLSPGERATLSCRASQSVSSSYLAWYQ</i> <u>QKPGQAPRLLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQA</u> <u>I</u> <u>GFPQTFGQG</u> <u>TKVEIK</u> <u>RTVAAPS</u> <u>VFIFPPSDEQLKSGTASVVCLLNNFY</u> <u>P</u> <u>REAKV</u> <u>QWKVDNALQSGNSQESVTEQDSK</u> <u>STYSL</u> <u>SSTLTLSKADYEKHKVYACEVTH</u> <u>QGLSSPVTKSFNRGEC</u> *
FabCol- LamLG4 (heavy chain)	<i>MRAWIFFLLCLAGRALAEVQLLES</i> <u>GGGLVQPGGSLRLS</u> <u>CAASGFTFSSYAMSWV</u> <u>RQAPGKGLEQVSAISGSGGSTYYADSVKGRFTISRDN</u> <u>SKNTLYLQMN</u> <u>SLRAED</u> <u>TAVYYCAKTLAAFDYWGG</u> <u>QTLTV</u> <u>VSSASTKGPSVFPLAPSSKSTSGGTAALG</u> <u>CLVKDYFPEP</u> <u>TVSWNSGALTSGVHTFPAVLQSSGLYSLSSV</u> <u>TV</u> <u>PSSSLGTQT</u> <u>YICNVNHKPSNTKVDKRVEPKSCGSGGGSGGSLNKPPFLMLLKGSTRFNKT</u> KTFRINQLLQDTPVASPRSVK VWQDAC SPLPKTQANHGALQFGDIPTSHL LFKLPQELLKPRSQFAVDMQTTSSRGLVFHTG TKN SFMALYLSKGR LVFA L GT DG KKLRIKSKEK CNDGK WHTV VFGHDGEK GRLVVDGLRAREGSLP GNSTISIRAPVYL GSPPSGKPKSLPTNSFVGCLKNFQLDSKPLYTPSSSFGVS SCTG *
FabCol-FgHBD (light chain)	<i>MRAWIFFLLCLAGRALAEIVLTQSPGTLSPGERATLSCRASQSVSSSYLAWYQ</i> <u>QKPGQAPRLLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQA</u> <u>I</u> <u>GFPQTFGQG</u> <u>TKVEIK</u> <u>RTVAAPS</u> <u>VFIFPPSDEQLKSGTASVVCLLNNFY</u> <u>P</u> <u>REAKV</u> <u>QWKVDNALQSGNSQESVTEQDSK</u> <u>STYSL</u> <u>SSTLTLSKADYEKHKVYACEVTH</u> <u>QGLSSPVTKSFNRGEC</u> GAGGGSGGGHRPLDKKREEAPSLRPAPPISGGGY

	RARPAKAAATQKKVERKAPDAGGSGGGSGGGHRPLDKKREEAPSLRPA PPISGGGYRARPAKAAATQKKVERKAPDAGGSGGGSGGGHRPLDKKR EEAPSLRPAPPPISGGGYRARPAKAAATQKKVERKAPDAGGGT*
FabCol-FgHBD (heavy chain)	<i><u>MRAWIFLLCLAGRALA</u>EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWV RQAPGKGLEQVSAISGSGGSTYYADSVKGRFTISRDN SKNTLYLQMNSLRAED <u>TAVYYCAKTLAAFDYWGQGL</u>LVTVSSASTKGPSVFPLAPSSKSTSGGTAALG CLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQT YICNVNHKPSNTKVDKRVEPKSCGSGGGSGGGHRPLDKKREEAPSLRPAPP PISGGGYRARPAKAAATQKKVERKAPDAGGSGGGSGGGHRPLDKKREE APSLRPAPPPISGGGYRARPAKAAATQKKVERKAPDAGGSGGGSGGGH RPLDKKREEAPSLRPAPPPISGGGYRARPAKAAATQKKVERKAPDAGGTG *</i>

Table S2. Sequences of the LamLG4 and FgHBD peptides. LamLG4 and FgHBD peptides are used for conjugation to FabCol and as experimental negative controls in Fig. 2. An additional cysteine residue (underlined) has been added for conjugation purpose.

LamLG4 peptide	<u>C</u> GGGRLVFALGTDGKKLRIKSKEKSNDGK
FgHBD peptide	G <u>C</u> GGSLRPAPPPISGGGYRARPAKAAATQKKVERKAPDA