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Supporting Information

for *Adv. Sci.*, DOI 10.1002/advs.202409744

Localized COUP-TFII pDNA Delivery Modulates Stem/Progenitor Cell Differentiation to Enhance Endothelialization and Inhibit Calcification of Decellularized Allografts

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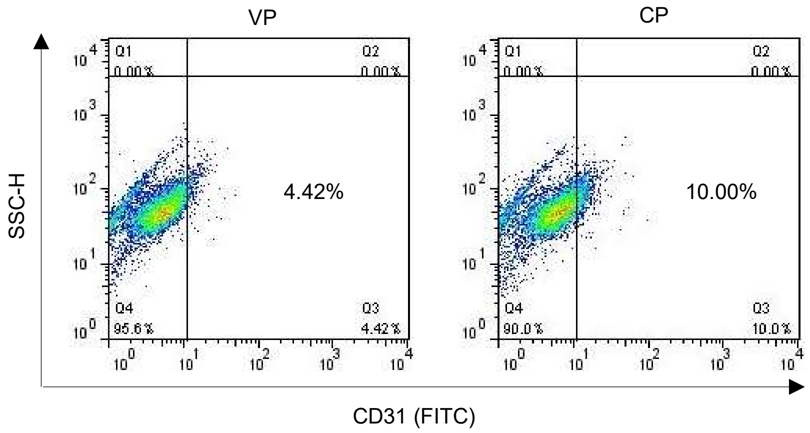
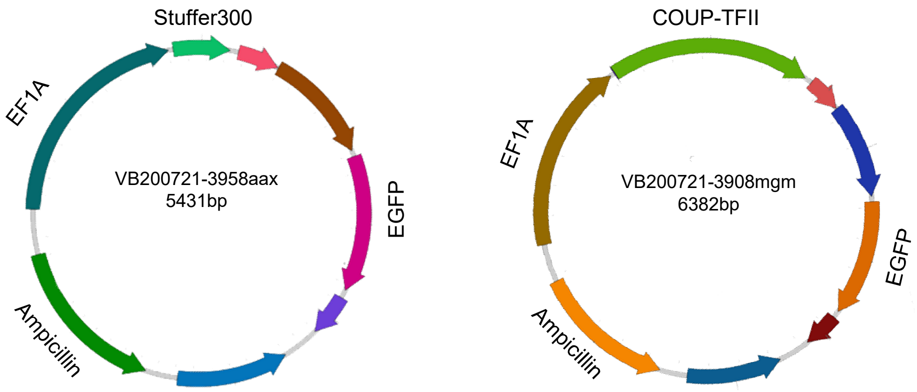


Figure S1. Flow cytometry analysis shows the differentiation of the SPCs overexpressed COUP-TFII.

A



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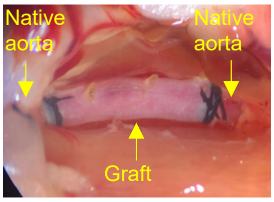

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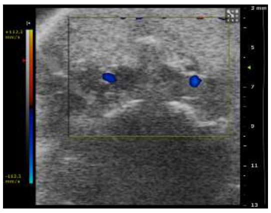

D

Patency rate	VP	CP
1 month	8/8	8/8
3 months	7/7	7/7

E

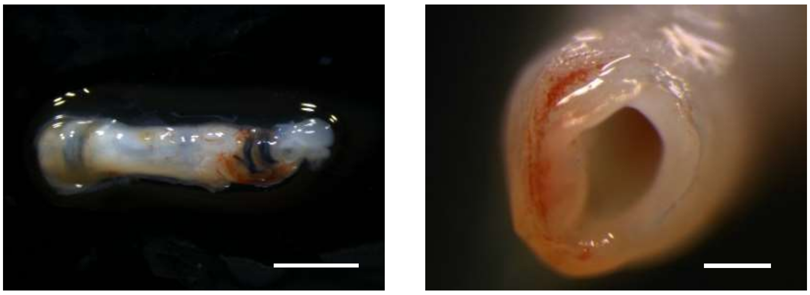

Figure S2. (A) Shown is the map of pDNA used in mouse carotid artery replacement model. **(B)** Representative image shows the graft after implantation. **(C)** Ultrasound image of the implanted graft is shown. **(D)** Statistic analysis of the patency rates of implanted vascular grafts. **(E)** Stereoscopic images shows the grafts explanted at 1 month. Scale bars: 2 mm (Left) or 500 μm (Right).

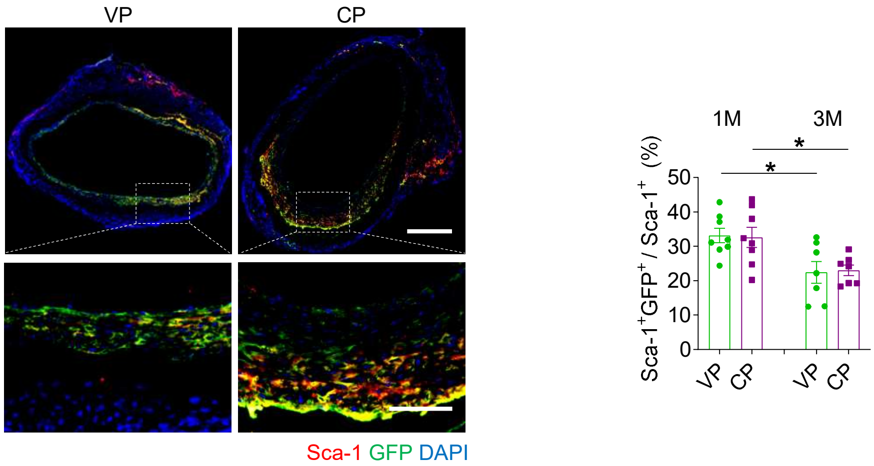

Figure S3. Shown is co-immunofluorescence staining for Sca-1 and GFP and quantification of the transfection efficiency (n=8 for 1 month; n=7 for 3 months). Scale bar: 100 μm. All data are presented as the means ± SEM. *: $p < 0.05$.

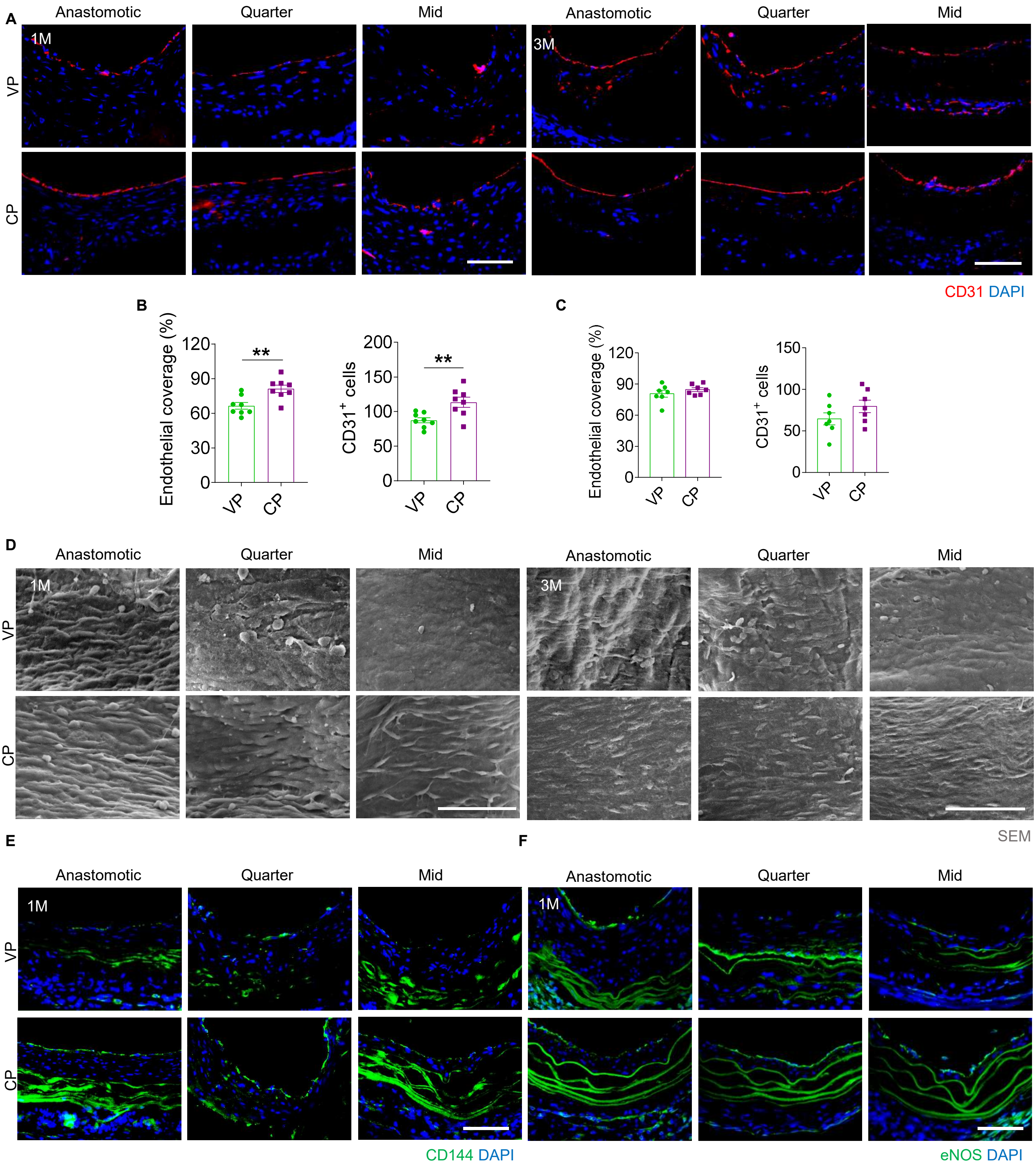

Figure S4. (A) Immunofluorescence staining for CD31 of vascular grafts is shown. Scale bar: 50 μm. **(B,C)** The endothelial coverage fraction and the number of CD31⁺ cells at 1 and 3 months were quantified, respectively (n=8 for 1 month, n=7 for 3 months). **(D)** SEM images shows the spindle ECs lining on the lumen of grafts. Scale bar: 50 μm. **(E)** Shown is immunofluorescence staining for CD144 of vascular grafts. Scale bar: 50 μm. **(F)** Immunofluorescence staining for eNOS of the explanted grafts is shown. Scale bar: 50 μm. All data are presented as the means ± SEM. **: $p < 0.01$.

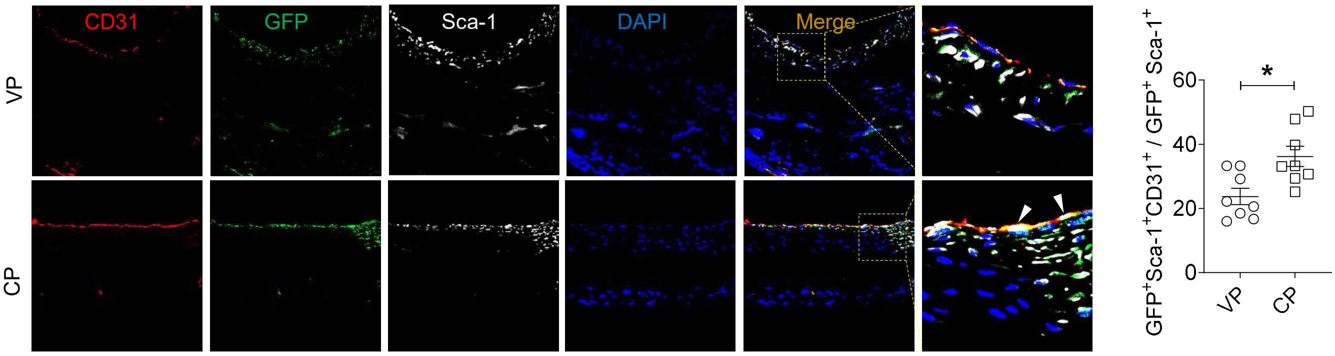

Figure S5. Shown is co-immunofluorescence staining for CD31, GFP and Sca-1 of the explanted grafts at 1 month. Scale bar: 50 μm. White arrows indicate the differentiated SPCs. The ratio of $GFP^{+}Sca\text{-}1^{+}CD31^{+}$ to $GFP^{+}Sca\text{-}1^{+}$ cells was further quantified (n=8). All data are presented as the means ± SEM. *: $p < 0.05$.

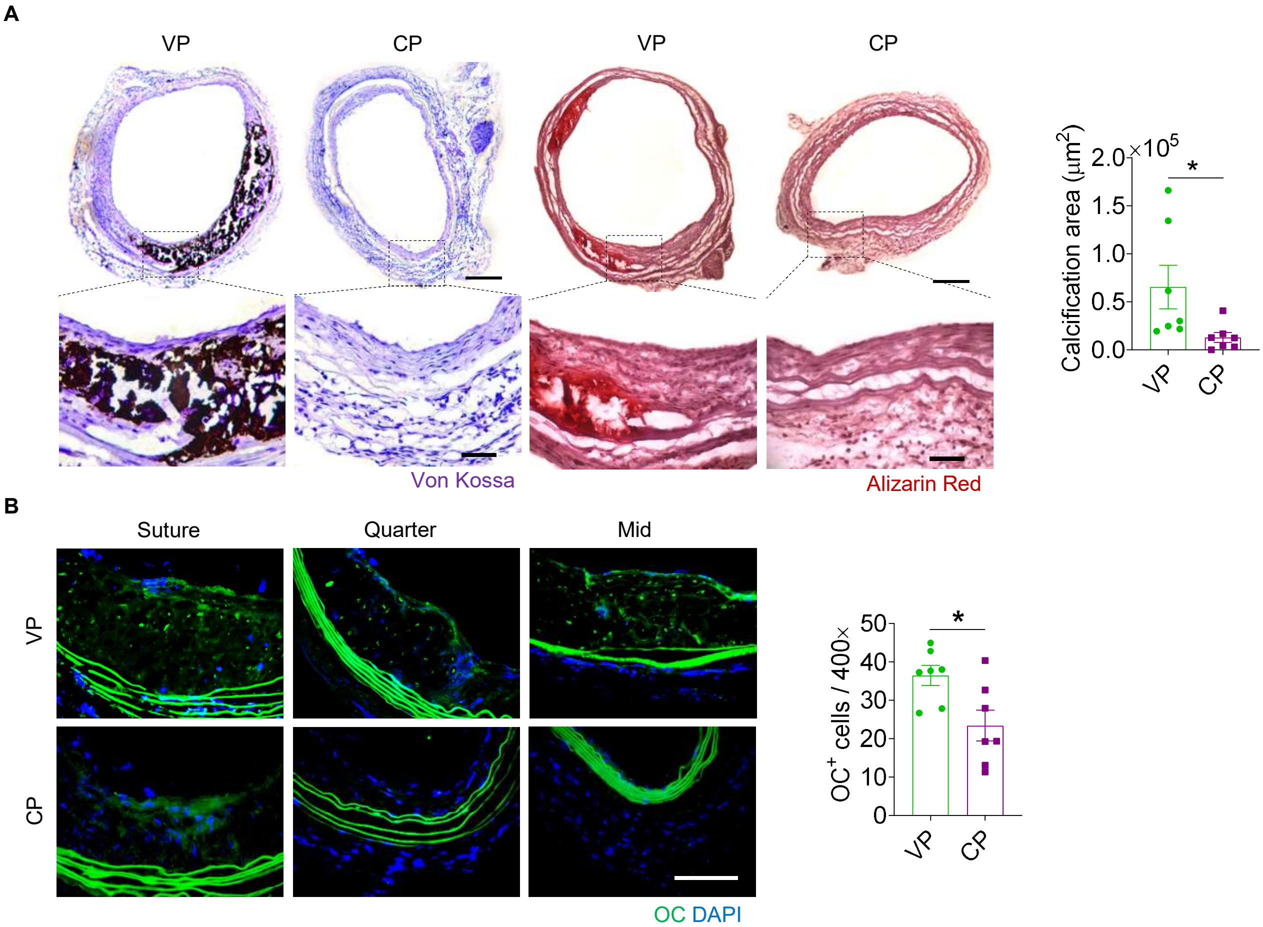

Figure S6. (A) Von Kossa and Alizarin Red staining of grafts explanted at 3 months are shown, and the calcification area was quantified according to Von Kossa staining (n=7). Scale bars: 200 μm or 50 μm (magnified images). **(B)** Shown is immunofluorescence staining for OC of grafts explanted at 3 months and quantification of the number of OC^+ cells (n=7). Scale bar: 50 μm. All data are presented as the means ± SEM. *: $p < 0.05$.

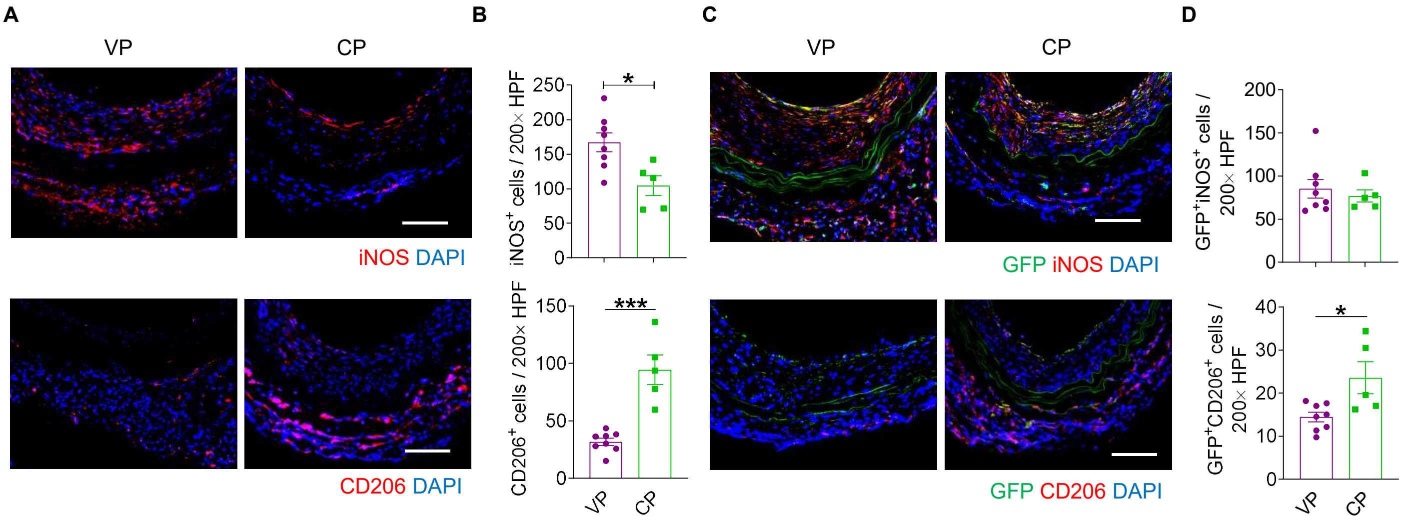

Figure S7. (A) Immunofluorescence staining for iNOS and CD206 of grafts explanted at 1 month are shown. Scale bar: 100 μm. **(B)**The number of iNOS⁺ and CD206⁺ cells were quantified (n=8). **(C)** Co-immunofluorescence staining for GFP and iNOS, GFP and CD206 of grafts explanted at 1 month are shown. Scale bar: 100μm. **(D)** The number of GFP^+iNOS^+ cells and GFP^+CD206^+ cells were quantified (n=8). All data are presented as the means ± SEM. *: $p < 0.05$, ***: $p < 0.001$.

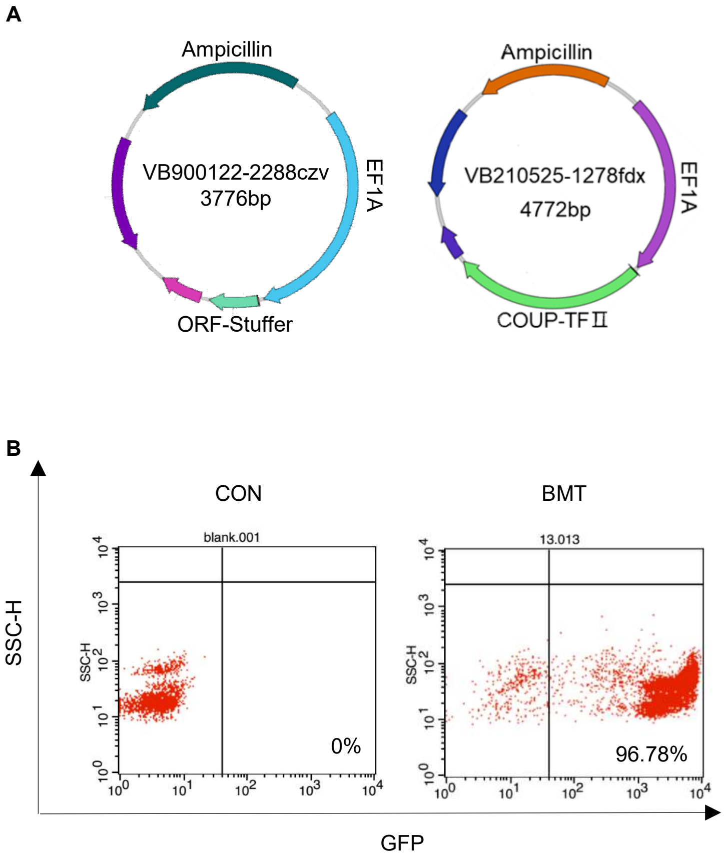

Figure S8. (A) Shown is the map of pDNA used in reconstituted bone marrow chimera mouse carotid artery replacement model. **(B)** Flow cytometry analysis shows the blood reconstruction efficiency after bone marrow transplantation.

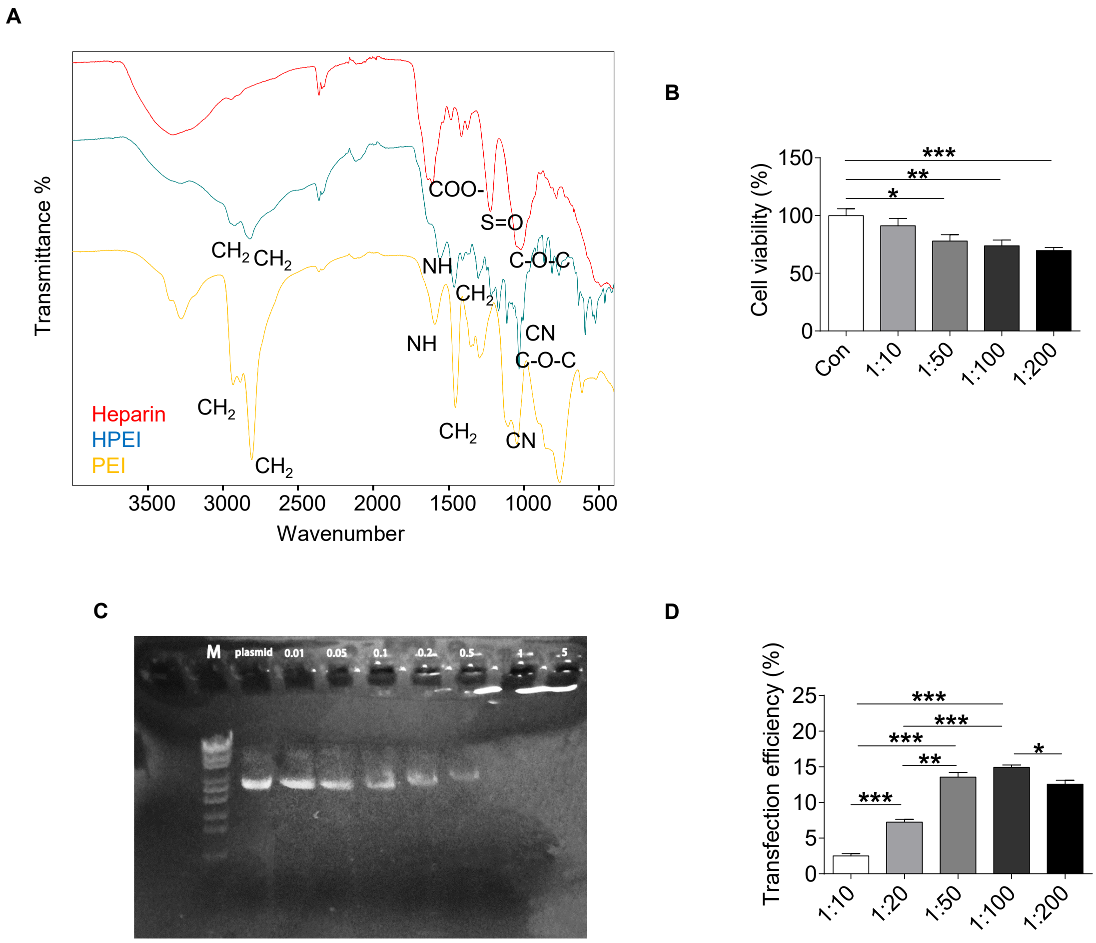

Figure S9. (A) Infrared spectroscopy of the heparin, PEI and HPEI nanoparticles is shown. **(B)** CCK8 assay shows the cytotoxicity of HPEI nanoparticles. **(C)** Shown is the ability of the HPEI nanoparticles absorb plasmid DNA. **(D)** The transfection efficiency was quantified by flow cytometry. All data are presented as the means ± SEM. *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$.

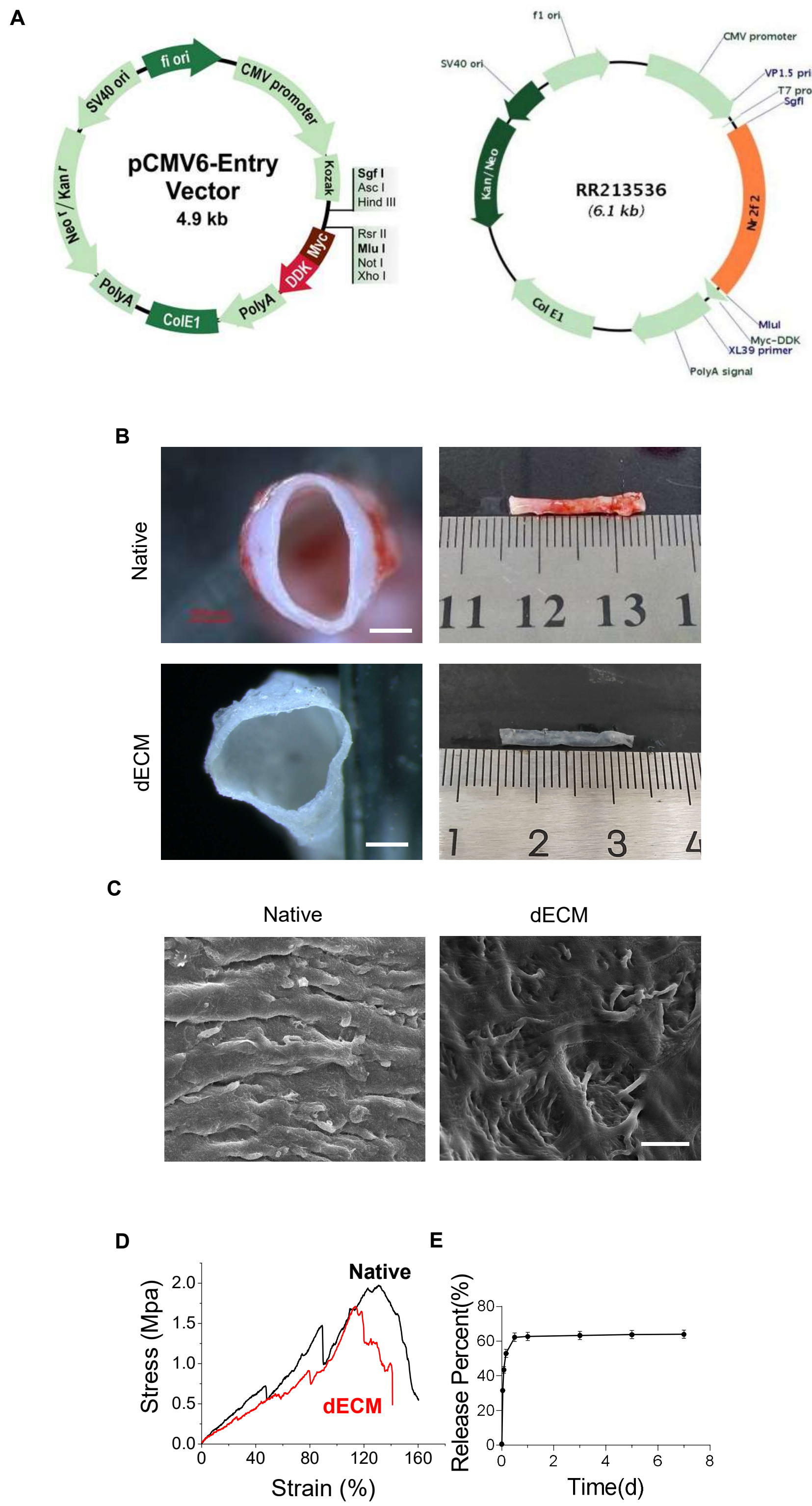

Figure S10. (A) Shown is the map of plasmids used in rat abdominal artery replacement model. (B) Stereoscopic images show the allograft before and after decellularization. Scale bar: 500 μm. (C) The SEM images of the allografts are shown. Scale bar: 20 μm. (D) Representative stress-strain curves are shown of the allograft before and after decellularization (n=3). (E) *In vitro* release of the COUP-TFII@HPEI nanoparticles from the decellularized allograft is shown (n=3).

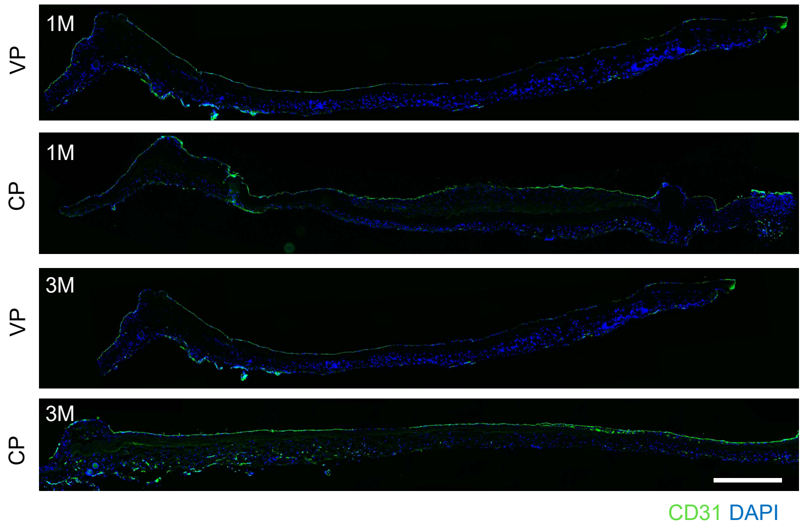

Figure S11. Immunofluorescence staining of longitudinal section is shown for CD31. Scale bar: 50 μm.

Table S1. Real-time qPCR primer sequences.

Genes	species	Forward primer	Reverse primer
Nr2f2	rats	5'-TCAGATGCCTGTGGTCTGTC-3'	5'-AAAGCTTTCCGAACCGTGT-3'
Pecam1	rats	5'-CTGGGAGGTATCGAATGGGC-3'	5'-GTGCATTTGTACTTCCCGGC-3'
Tek	rats	5'-AAGAGCGAGTAGACCATGCG-3'	5'-ACTAGTCCATAAAGGAGCAAGC-3'
Angpt1	rats	5'-AGCATGTGATGGAAAATTATACT-3'	5'-AGTACCTGGGTCTCCACATC-3'
Acta2	rats	5'-CTGCCTTGGTGTGTGACAATGG-3'	5'-CGGGTACTTCAGGGTCAGGATTC-3'
Cnn1	rats	5'-ACCAAGCGGCAGATCTTTGA-3'	5'-CATCTGCAAGCTGACGTTGA-3'
Tagln	rats	5'-TTCTGCCTCAACATGGCCAAC-3'	5'-CACCTTCACTGGCTTGGATC-3'
Myh11	rats	5'-GCACAAGAAGAAGAAGCTGGA-3'	5'-TTGAGCATGCCTGTGACACT-3'
Gapdh	rats	5'-GTCGGTGTGAACGGATTTG-3'	5'-TCCCATTCTCAGCCTTGAC-3'