ADVANCED SCIENCE
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# Supporting Information

for *Adv. Sci.*, DOI: 10.1002/advs.201900386

## CRISPR/Cas9 Delivery Mediated with Hydroxyl-Rich Nanosystems for Gene Editing in Aorta

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# Supporting Information

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**Table S1 |** PDI values of complexes

| Samples | Average PDI[a] (at different N/P ratios) | | | |
|---|---|---|---|---|
| | **5** | **10** | **15** | **20** |
| PEI/pCas9-sg*Fbn1* | | 0.233 | | |
| CHO-PGEA/pCas9-sg*Fbn1* | 0.229 | 0.158 | 0.150 | 0.137 |

[a]Determined from DLS results. PDI =Polydispersity Index.

**Table S2.** Sequences of sgRNA oligos

| sgRNA | Oligo F | Oligo R |
|---|---|---|
| sgRNA1 | 5’CACCGGTTGCGATCTTGGC CGGTGC3’ | 5’AAACGCACCGGCCAAGATC GCAACC3’ |
| sgRNA2 | 5’CACCGATGCAGTGCTGTTG CGATCT3’ | 5’AAACAGATCGCAACAGCAC TGCATC3’ |
| sgRNA3 | 5’CACCGAGTGCTGTTGCGAT CTTGGC3’ | 5’AAACGCCAAGATCGCAACA GCACTC3’ |

**Table S3.** Oligo sequences for off-target detection

| Gene name | Sequence 5’-3’ | Primer F 5’-3’ | Primer R 5’-3’ |
|---|---|---|---|
| *Neblin* | GTTGCTCTTGTGA | GCCACCTCATTCT | GGTGCAGTACGTGA |
| | TCTTGGCCAG | GTCACCTACCAC | GAGACTTACGTCAC |
| *Nav* | GCTGCTGTTGCG | TCAGTCACCTTAG | GTCGGAAATGTGCC |
| | ATCCTGGGTGG | GAGTACACCGCAC | TTTCAGACAGC |
| *Ndu* | TGTGCTGTTCCG | GAAGGAATATGCC | CGTGGGAGACAAAT |
| | ATCCGGGCCGG | GTCCGCTAC | GAGGCGAA |
| *Ttl* | AGTGCTGTGGAG | TTCGTCAAGCTGT | CATCCAAGGCCAGA |
| | ACCTTGGGAAG | GAAGCACCGGA | AACCGCTCAAAG |

**Table S4.** The primer sequences for real-time PCR

| Gene name | Primer F | Primer R |
|---|---|---|
| *GAPDH* | 5’TGATGACATCAAGAAGGTG GTGAAG3’ | 5’TCCTTGGAGGCCATGTA GGCCAT3’ |
| *Mmp-2* | 5’TGGAATGCCATCCCTGATAA 3’ | 5’AGCCCAGCCAGTCTGAT TTG3’ |
| *Ctgf* | 5’TTGAAGTCTCAGAAGGTGG AT3’ | 5’GCAGGAGGTCGTAGGT CAC3’ |
| *Cas9* | 5’ATGGACTATAAGGACCACGA CGGAGAC3’ | 5’CGGTGTTGCCCAGCACC TTGAAT3’ |

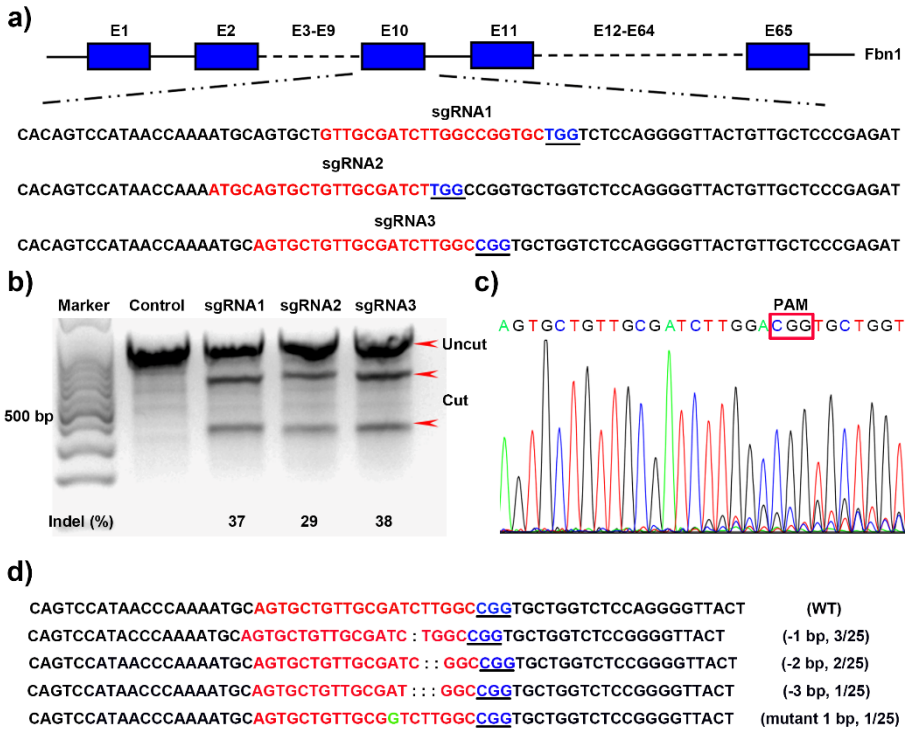


**Figure S1.** a) Schematic diagram of the candidate sgRNAs targeting *Fbn1* locus. The sgRNA pairs with the 20-bp DNA target (Red), upstream of 5'-NGG adjacent motif PAM (blue) and guides Cas9 nuclease to mediate a break 3 bp upstream of the PAM. b) Detection of Cas9/sgRNA-mediated cleavage of *Fbn1* loci by T7 endonuclease I -based assay. c) Sanger sequencing of the PCR amplicon of the targeted sites in the N2a cells transfected with pCas9-sg*Fbn1* by Lipofectamine 3000. d) Representative sequences of *Fbn1* locus in pCas9-sg*Fbn1* transfected N2a cells.

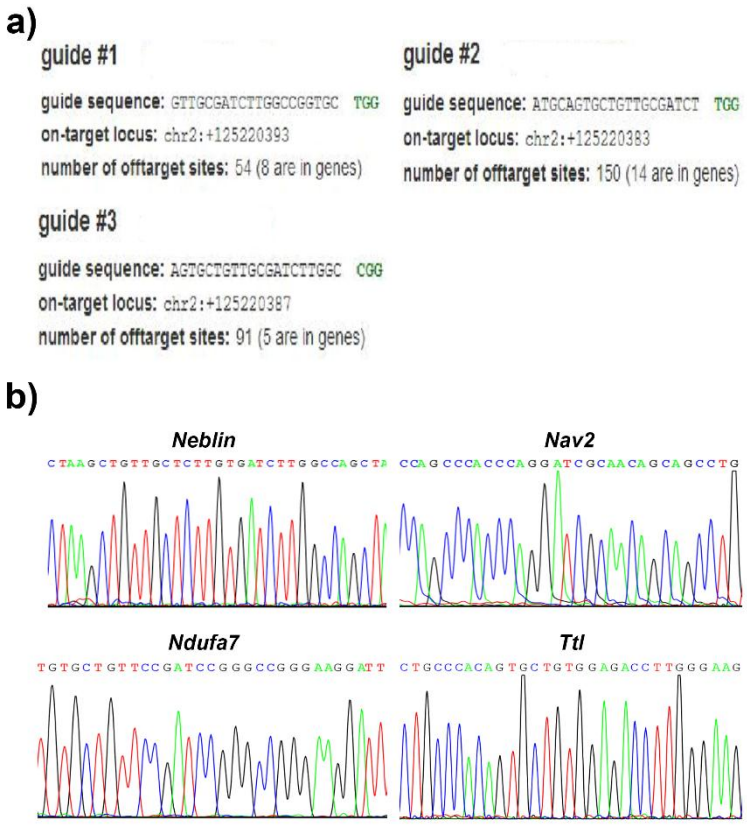


**Figure S2.** a) The screenshot of the number of off-target sites for the three sgRNAs predicted by the CRISPR design website: http://crispr.mit.edu/. b) Sanger sequencing of the potential off-targeted sites of sg*Fbn1* that are in open reading frames of *Neblin*, *Nav2*, *Ndufa7* and *Ttl* genes after pCas9-sg*Fbn1* transfection.

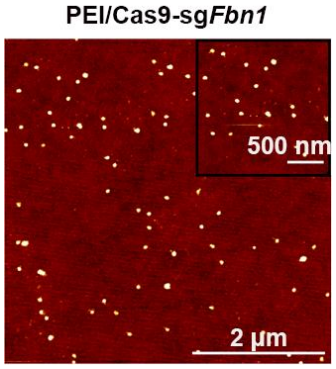


**Figure S3.** Morphology of PEI/pCas9-sg*Fbn1* nanoparticles at N/P=10 measured by atomic force microscope (AFM).

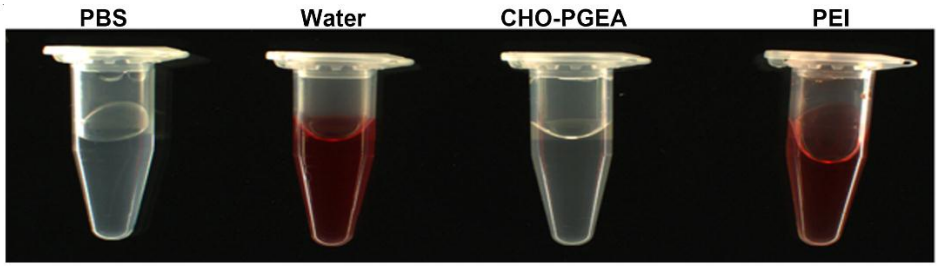


**Figure S4.** Images of RBCs treated with PBS, water, PEI/pCas9-sg*Fbn1* and CHO-PGEA/pCas9-sg*Fbn1* nanoparticles at their respective N/P ratios at the concentration of 1 mg/mL, where deionized water and PBS were used as the positive and negative controls.

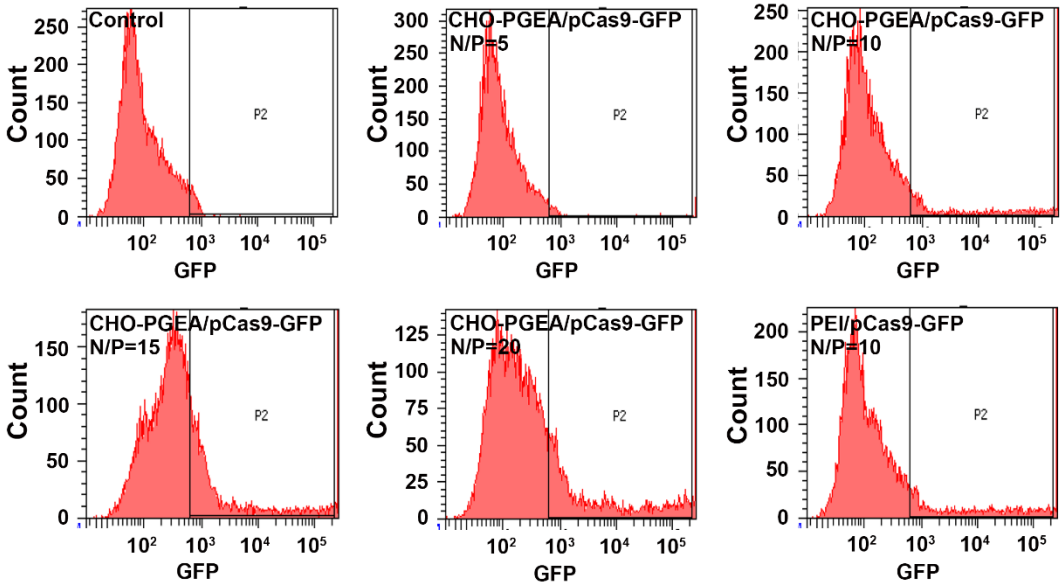


**Figure S5.** Transfection efficiencies of CHO-PGEA/pCas9-GFP at different N/P ratios and PEI/pCas9-GFP at the N/P ratio of 10 in mouse vascular smooth muscle cells (VSMCs).

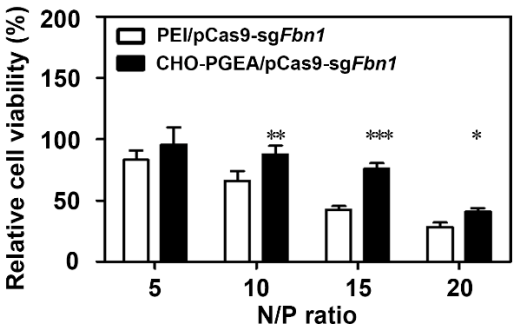


**Figure S6.** Cell viabilities of CHO-PGEA/pCas9-sg*Fbn1* and PEI/pCas9-sg*Fbn1* nanoparticles at various N/P ratios in VSMCs. Error bars represent the standard deviation of six measurements (**P* < 0.05, ***P* < 0.01, ****P* < 0.001).

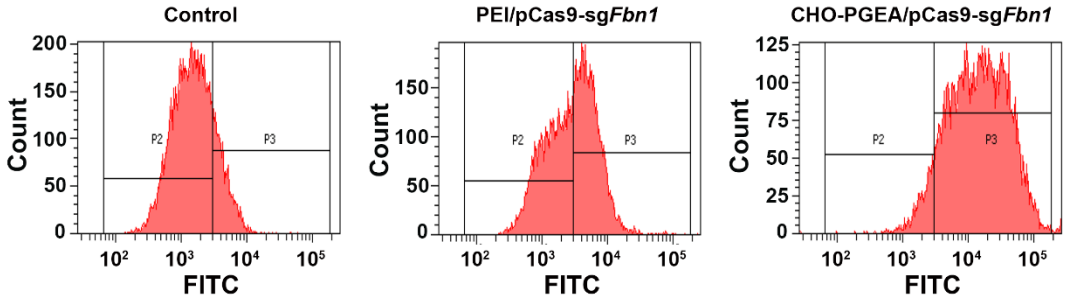


**Figure S7.** Flow cytometry analysis for cellular uptake of CHO-PGEA and PEI at their optimal N/P ratios.

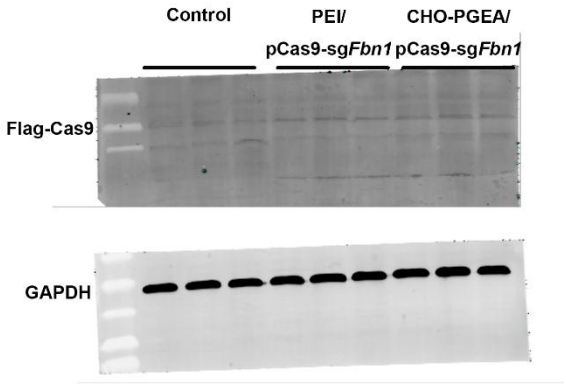


**Figure S8.** Raw data of western blot analysis of Cas9 protein expression in VSMCs incubated with nanoparticles with an anti-Flag antibody.

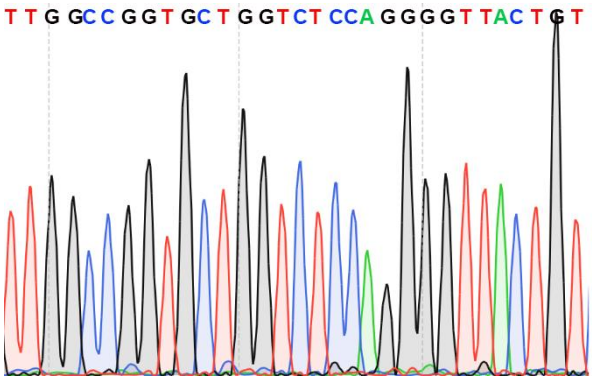


**Figure S9.** Sanger sequencing of the PCR amplicon of the targeted *Fbn1* locus in CHO-PGEA/pCas9-sgnull treated VSMCs.

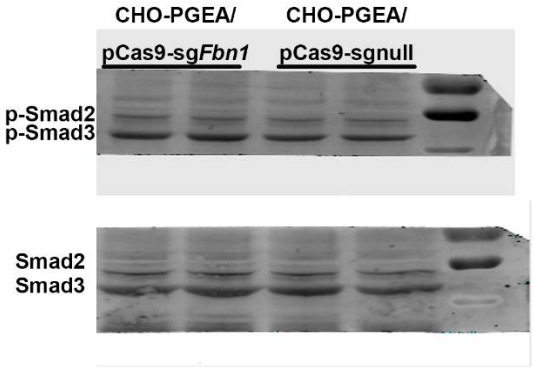


**Figure S10.** Raw data of western blot analysis of p-Smad2/3 and Smad2/3 expression in the VSMCs after CHO-PGEA/pCas9-sgnull and CHO-PGEA/pCas9-sg*Fbn1* treatment.

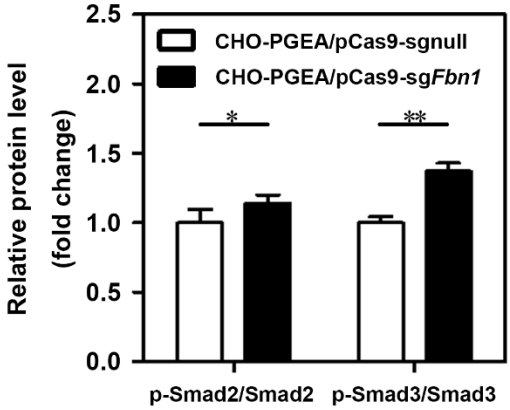


**Figure S11.** Quantification of relative protein levels (calculated from the relative density of the western blot bands) of p-Smad2/3 and Smad2/3 in the VSMCs after CHO-PGEA/pCas9-sgnull and CHO-PGEA/pCas9-sg*Fbn1* treatment. (*$P < 0.05$, **$P < 0.01$).