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## Supplemental information

# Robust genome editing in adult vascular endothelium by nanoparticle delivery of CRISPR-Cas9 plasmid DNA

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# SUPPLEMENTAL MATERIALS

## Robust Genome Editing in Adult Vascular Endothelium by Nanoparticle Delivery of CRISPR/Cas9 Plasmid DNA

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**Supplemental Tables**

**Table S1. gRNA sequences against mouse *Pik3cg* or *Vegfr2*, Related to the Star Methods**

| **Name** | **Sequence (5'→ 3')** |
|---|---|
| Pik3cg-gRNA-1* | ACCGTACCACGACAGTGCGC |
| Pik3cg-gRNA-2 | ATCTGGCCAGCGCACTGTCG |
| Pik3cg-gRNA-3 | AGCCTCGCAGGTACGCCTCC |
| Pik3cg-gRNA-4 | ACTAAAAGCCGGTACCCTGG |
| Vegfr2-gRNA-1 | GTCCCGGTACGAGCACTTGT |
| Vegfr2-gRNA-2 | TGATGTACACGATGCCATGC |
| Vegfr2-gRNA-3* | CAACCCTTCAGATTACTTGC |
| Vegfr2-gRNA-4 | GAGCCTACAAGTGCTCGTAC |

* indicates the potent gRNA

**Table S2. QPCR primer sequences for genome editing analysis, Related to the Star Methods**

| **Name** | **Forward (5'→3')** | **Reverse (5'→ 3')** |
|---|---|---|
| Pik3cg-gRNA1 | TTGAACCGTACCACGACAGTG | ACCAGAACAAGAAGTGACCGAT |
| Pik3cg-gRNA2 | GAACTGTGGGTTTCCCCCAT | GAATCTGGCCAGCGCACTG |
| Pik3cg-gRNA3 | TTGAACCGTACCACGACAGTG | AGCCTCGCAGGTACGCC |
| Pik3cg-gRNA4 | TGGACTAAAAGCCGGTACCC | TGGTGCTAGTGATGAGAGGGT |
| | | |
| Vefgr2-gRNA1 | GGCGGTGGTGACAGTATCTT | CGTCCCGGTACGAGCACT |
| Vefgr2-gRNA2 | GATGTCCGCATTCATGCAAGT | CGGTGATGTACACGATGCCA |
| Vefgr2-gRNA3 | AGTGGAAATTGTTGTGACCTCAG | AGTGGAAATTGTTGTGACCTCAG |
| Vefgr2-gRNA4 | ACTGGAGCCTACAAGTGCTCG | ACGACATTGGAAGCAGACGG |

**Table S3. Primer sequences for quantitative RT-PCR analysis of gene expression, Related to the Star Methods**

| **Name** | **Forward (5' → 3')** | **Reverse (5' → 3')** |
|---|---|---|
| *Tnfa* | CCCTCACACTCAGATCATCTTC | GTTTGCTACGACGTGGGCTACA |
| *Il6* | GGATACCACTCCCAACAGACC | CTGCAAGTGCATCATCGTTGT |
| *Icam1* | AGGGTGGCGGGAAAGTTCCTG | CGTCTGCAGGTCATCTTAGGAG |
| *Ccna2* | TGCAGCTGTCTCTTTACCCGCA | CTCCATTTCCCTAAGGTACGTG |
| *Ccnb1* | ACCAGAGGTGGAACTTGCTGA | ATGTTTCCATCGGGCTTGGAGA |
| *Foxm1* | CACTTGGATTGAGGACCACTT | GGTCGTTTCTGCTGTGATTCCA |
| *Ppia* | TCTTGTCCATGGCAAATGCTG | TGATCTTCTTGCTGGTCTTGC |

## Supplemental Figures and Figure Legends

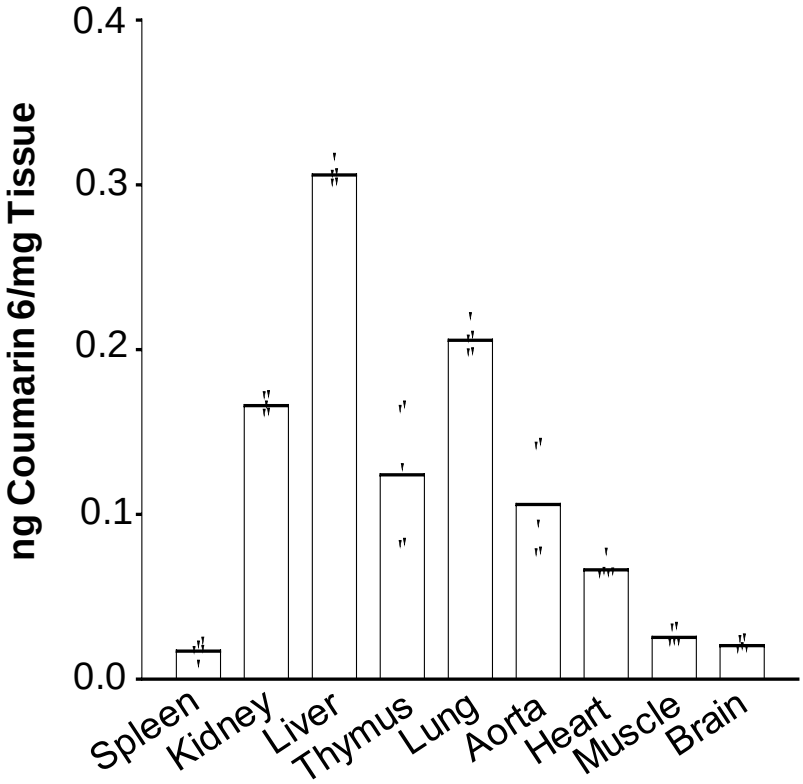


**Figure S1. Biodistribution of PP/PEI nanoparticles.** Related to Figure 1. Coumarin 6-loaded PP/PEI nanoparticles were administered to adult mice retro-orbitally. 8h later, various organ tissues were collected after bloodletting through abdominal aorta for homogenization and determination of Coumarin 6 fluorescent intensity. The amount of Coumarin 6 was calculated using a Coumarin 6 standard curve and normalized according to the tissue weight (ng/mg tissue) (n=5).

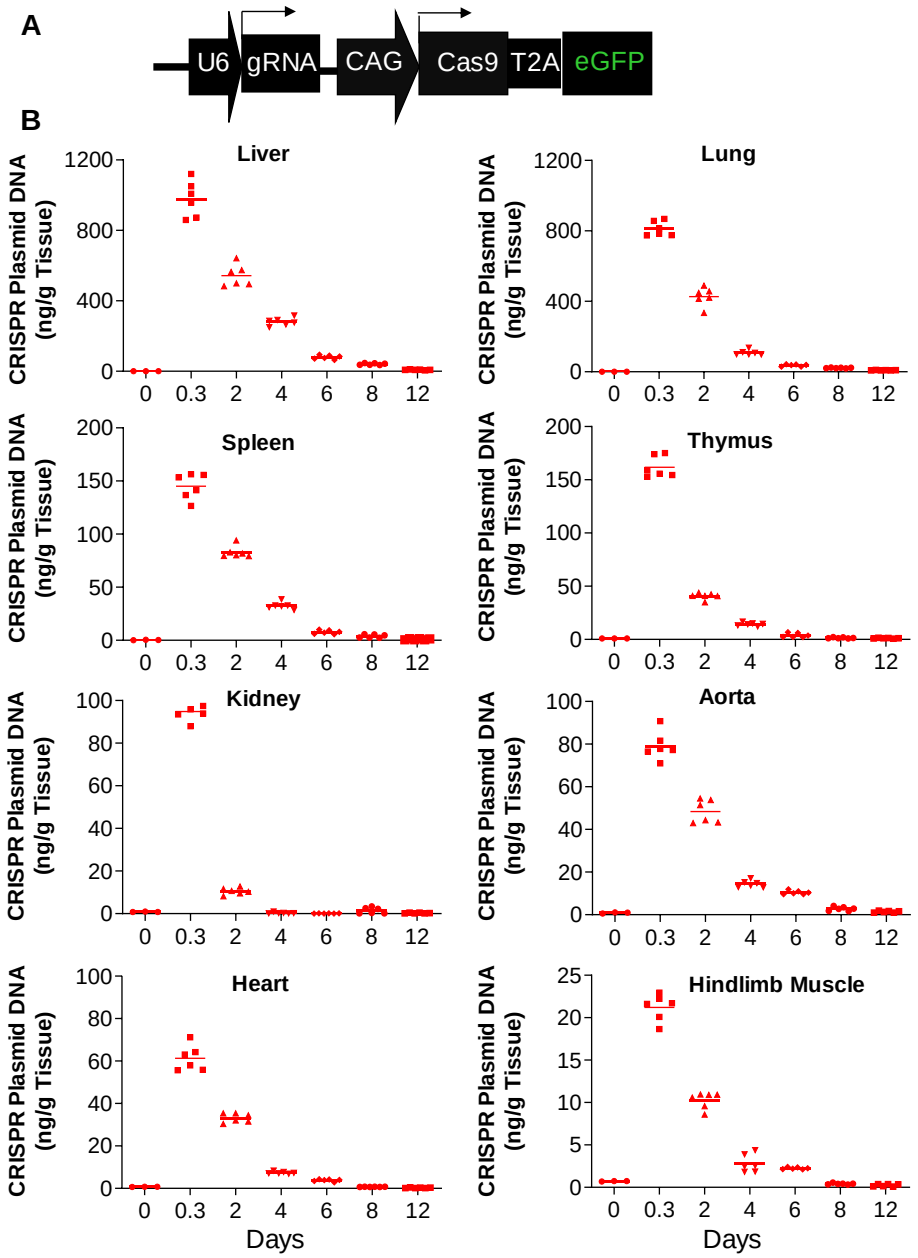


**Figure S2. Pharmacokinetics of CRISPR plasmid DNA in various organs in adult mice**. Related to Figure 1. (**A**) Schematic diagram of the all-in-one CRISPR$^{CAG}$ plasmid DNA expressing Cas9 under the control of *CAG* promoter and gRNA driven by *U6* promoter. (**B**) Time course of plasmid DNA accumulation in various organs. The CRISPR plasmid DNA (40 μg/mouse) were delivered into adult mice by PP/PEI nanoparticles through retro-orbital injection. Tissues were collected after bloodletting through abdominal aorta at the indicated times after delivery. Plasmid DNA in each organ tissue was extracted after proteinase K digestion. The amount of Cas9 DNA was determined by QPCR analysis, and calculated using a standard curve generated from the CRISPR plasmid DNA. The amount of Cas9 DNA was normalized according to the tissue weight (ng/g tissue) (n=6). Bars represent means.

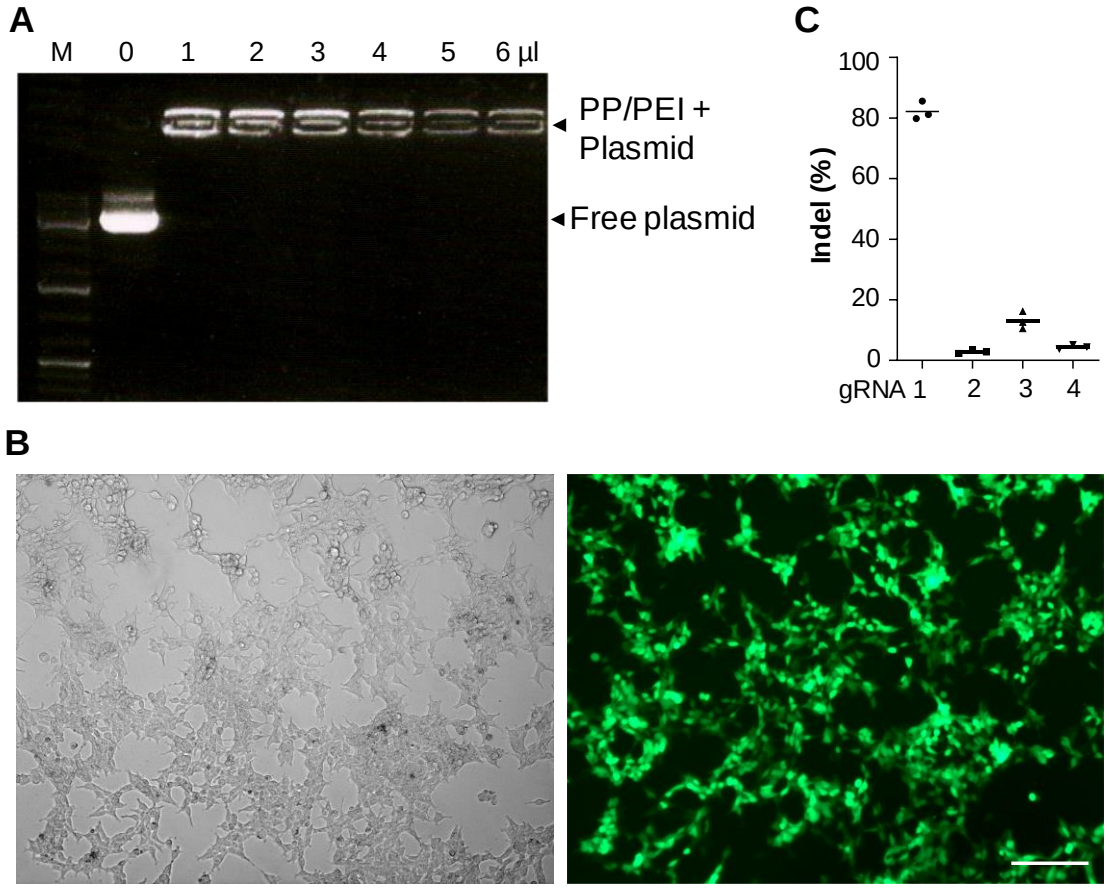


**Figure S3. Identification of a potent *Pik3cg* gRNA in cultured cells.** Related to Figure 2 and STAR Methods "In vitro Identification of potent gRNA…". (**A**) DNA electrophoresis demonstrating that PP/PEI nanoparticles neutralized the negative charge of CRISPR plasmid DNA and thus blocked their movement in the gel. 1 µg CRISPR$^{CAG}$ plasmid DNA was mixed with various amount of PP/PEI (0-6 µl) nanoparticles and then loaded to 1% agarose gel for electrophoresis. M, molecular weight marker. (**B**) Representative micrographs of phase contrast and fluorescent microscopy demonstrating highly efficient transfection in cultured Hepa1c1c7 cells. Complexes of PP/PEI nanoparticle:CRISPR$^{CAG}$ plasmid DNA were added to Hepa-1c1c7 cells for 48 h. Expression of GFP (green) was detected using fluorescent microscopy. Phase contrast image was shown to demonstrate the cell density. Scale bar, 100 µm. (**C**) Indel analysis identifying the potent gRNA. CRISPR$^{CAG}$ plasmid DNA expressing gRNA specific for mouse *Pik3cg* gene was mixed with PP/PEI nanoparticles for 10 min at room temperature then transfected to subconfluent Hepa-1c1c7 cells. 48 h later, genomic DNA was extracted for Sanger sequencing decomposition analysis using TIDE software. gRNA1 is a potent gRNA which induced 80% genome editing efficiency (n=3).

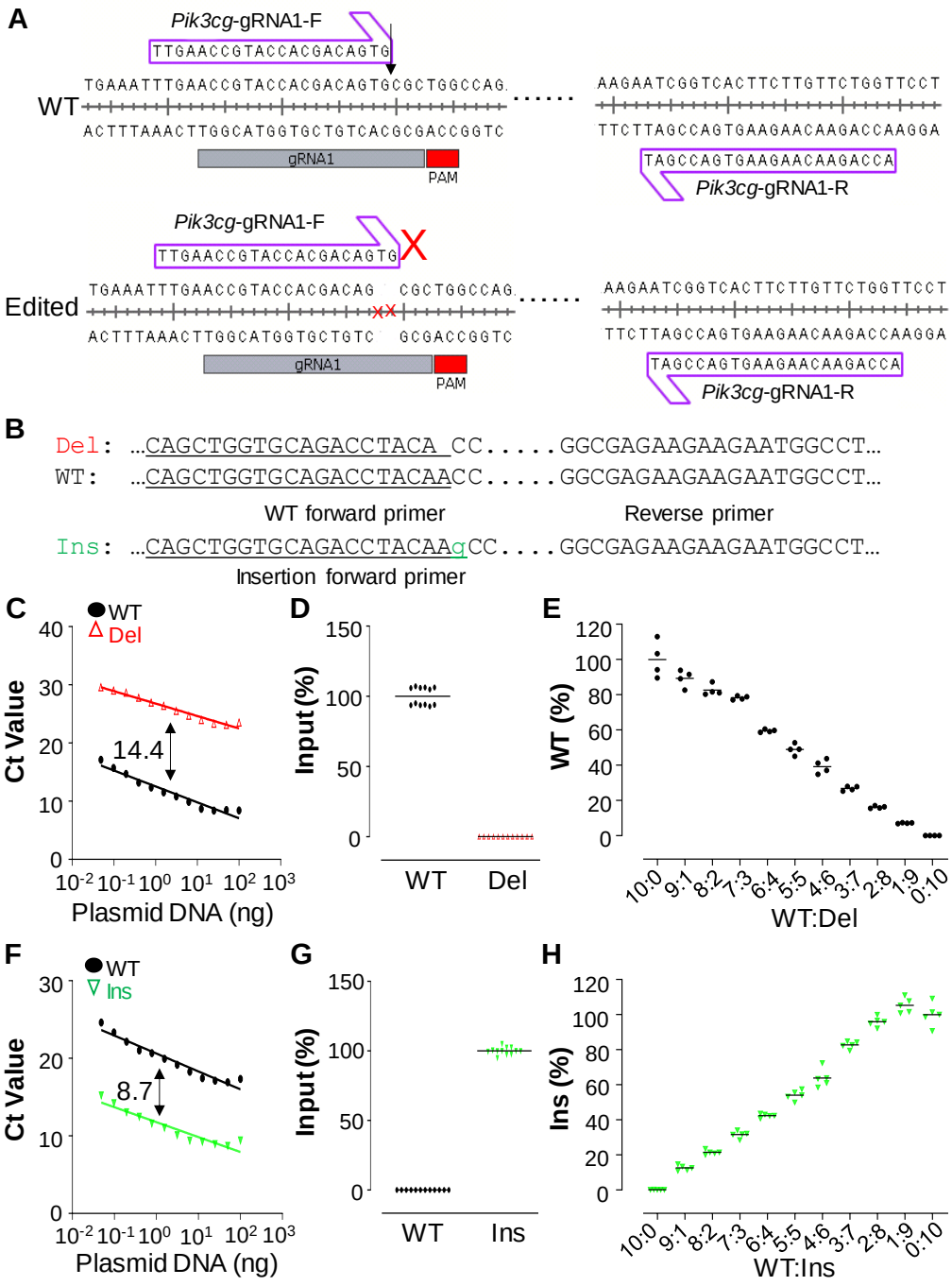


**Figure S4. QPCR efficiently distinguished single base deletion or insertion from wild-type DNA.** Related to Figure 2. (**A**) Schematic diagram of the strategy to design primers for QPCR analysis of indels. Arrow point to the predicted cleavage site. PAM, Protospacer Adjacent Motif sequence. (**B**) Diagram showing the single base deletion/insertion and the primers. (**C-E**) The WT forward primer could not amplify the fragment in plasmid DNA$^{Del}$ with 1bp deletion (Del). (**F-H**) Quantitative detection of 1bp insertion in plasmid DNA$^{Ins}$ with the Insertion forward primer which could not amplify the plasmid DNA$^{WT}$ due to the 3' mismatch. n=12 (**D**, **G**), n=5 (**E, H**).

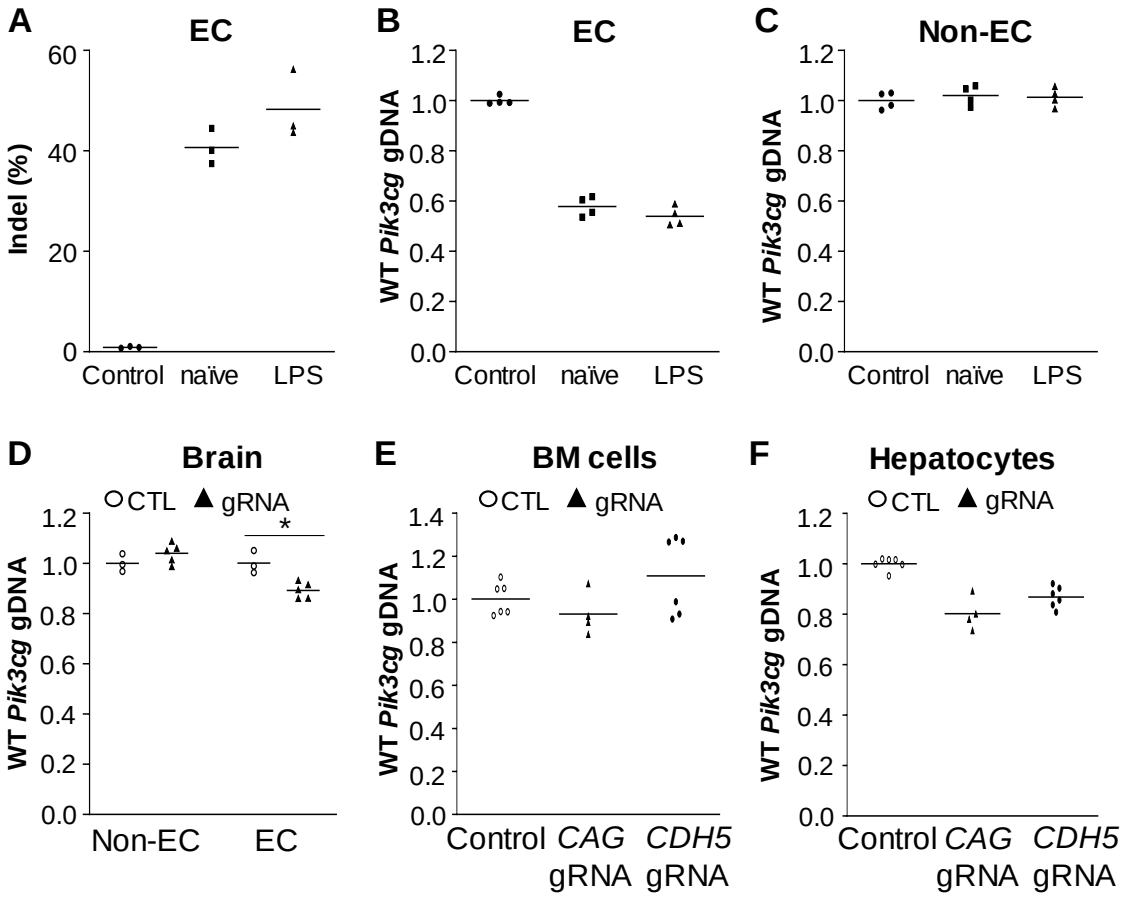


**Figure S5. CRISPR$^{CDH5}$ plasmid delivered by PP/PEI nanoparticles had comparable genome editing efficiency in lung ECs under conditions with and without inflammation.** Related to Figure 3. (**A**) Next generation sequencing demonstrating similar efficiency of genome editing in lung ECs in naïve mice and septic mice. Same amount of CRISPR$^{CDH5}$ plasmid DNA expressing gRNA1 (40μg/mouse) was delivered to wild-type mice at basal (naive) or 20h post-LPS challenge (2.5 mg/kg i.p.) (LPS) by retro-orbital injection with PP/PEI nanoparticles. Seven days after nanoparticle administration, lung tissues were collected for EC and non-EC isolation followed by indel analyses. Control, mice without nanoparticle delivery and LPS challenge (n=3). (**B**, **C**) QPCR analysis of wild-type (WT) genomic DNA of the genome editing region demonstrating similar efficiency of genome editing in lung ECs in naïve mice and septic mice (n=4). (**D-F**) Genome editing efficiency in brain ECs, bone marrow cells and hepatocytes in naïve mice. Small but significant genomic editing in cerebral vascular ECs (**D**). There was little genomic editing in bone marrow cells with either CAG or CDH5 promoter (**E**) whereas 20% genomic editing in hepatocytes in mice transduced the CRISPR$^{CAG}$ plasmid DNA but not the CRISPR$^{CDH5}$ plasmid DNA. n=3-6. * $P < 0.05$. Student's t test.

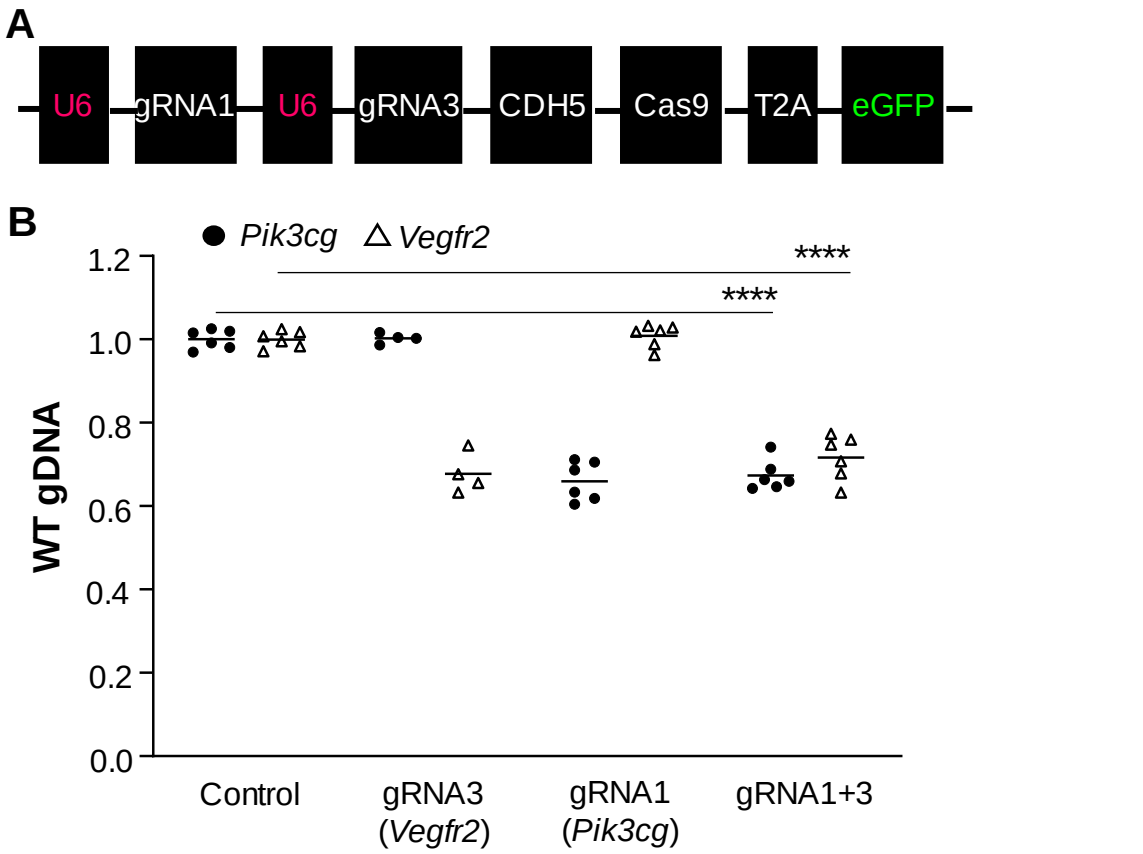


**Figure S6. Simultaneous genomic editing of 2 genes in lung ECs with one plasmid DNA.** Related to Figures 3 and 7. (**A**) Diagram presentation showing the CRISPRCDH5 plasmid expressing 2 gRNA against 2 different genes (gRNA1-*Pik3cg* and gRNA3-*Vegfr2*). (**B**) QPCR analysis showing similar efficiency of genome editing in lung ECs of mice transduced the CRSIPR plasmid DNA expressing single gRNA or 2 gRNAs. Each mouse was delivered 40 μg plasmid DNA by PP/PEI nanoparticles and lung tissues were collected at 7 days later for EC isolation followed by QPCR analysis with wild-type primers for Pik3cg and Vegfr2, respectively (n=4 or 6). **** $P < 0.0001$. Student's *t* test.

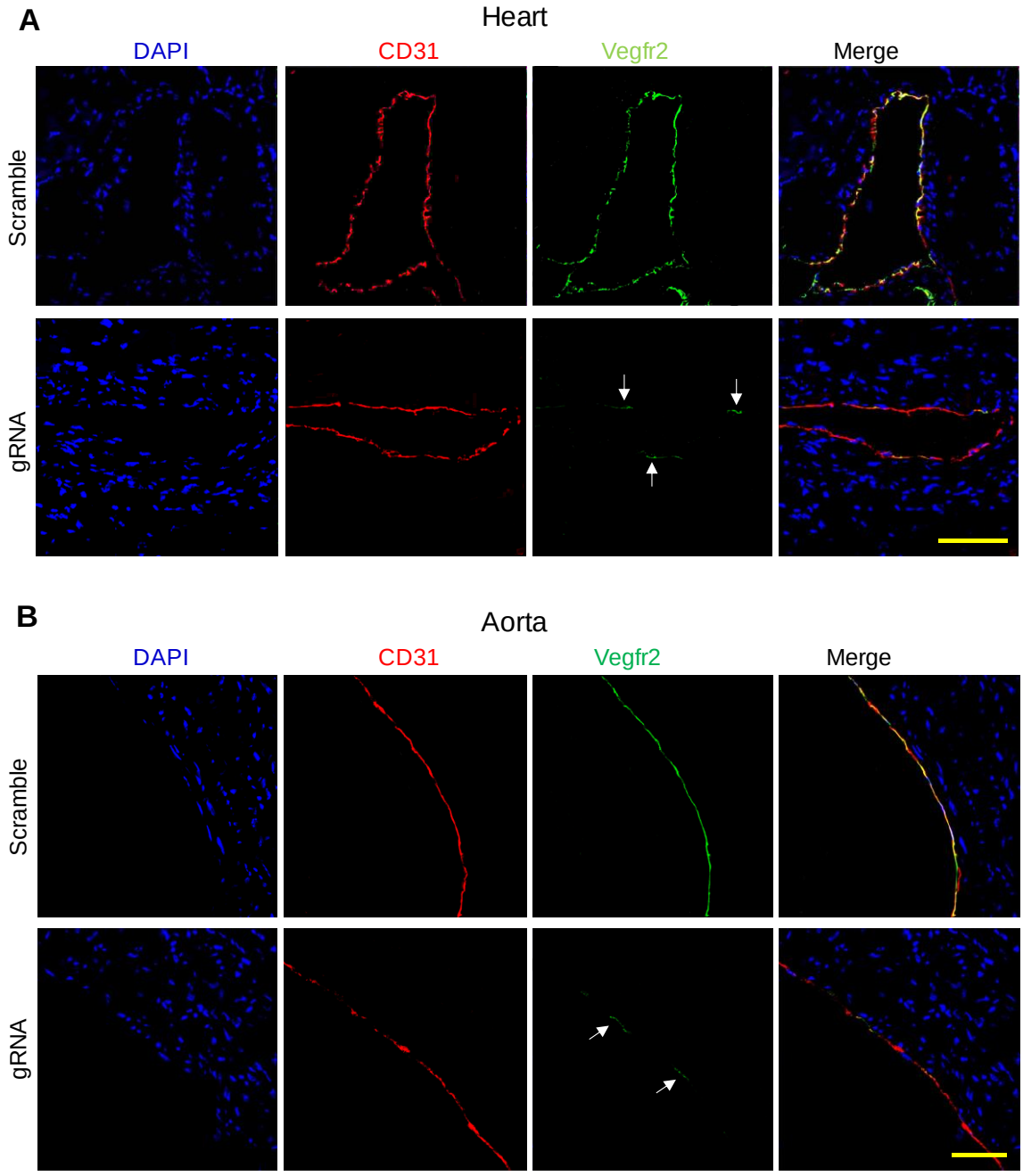


**Figure S7. Representative micrographs of immunostaining showing diminished Vegfr2 expression *Vegfr2* gRNA-CRISPR$^{CDH5}$ plasmid DNA-transduced mice.** Related to Figure 7. A mixture of PP/PEI nanoparticles:CRISPR$^{CDH5}$ plasmid DNA expressing *Vegfr2* gRNA or scramble RNA was administered to adult WT mice (40μg/mouse) retro-orbitally. 7 days later, heart and aorta tissues were collected for cryosectioning and immunostaining with anti-Vegfr2 (green) and anti-CD31 (red). Nuclei were counter-stained with DAPI (blue). Arrows point to ECs with less efficient knockdown of Vegfr2. Scale bars, 50μm.