

Table S1. Sequencing results of the *TTF-1* gene of the mouse embryos edited by four sgRNA-expressing plasmids *in vivo*.

In the TTF-1 sequences, “TGACATCTTGAGTcccCTGG” is the target of sgRNA1, “CTGGAGGaaagctacaagaa” is the target of sgRNA2,

“**CGCCTACCACATGACGGCGG**” is the target of sgRNA3, “**ctctcgactccgcgtgg**” is the target of sgRNA4.

Table S2. Comparison of *TTF-1* gene editing activity of four sgRNA-expressing plasmids *in vivo*.

No. of Embryos analyzed	No. of Embryos with <i>TTF-1</i> gene edited (editing activity%)			
	sgRNA1 targeted	sgRNA2 targeted	sgRNA3 targeted	sgRNA4 targeted
9	8 (88.89%)	6 (66.67%)	9 (100%)	0

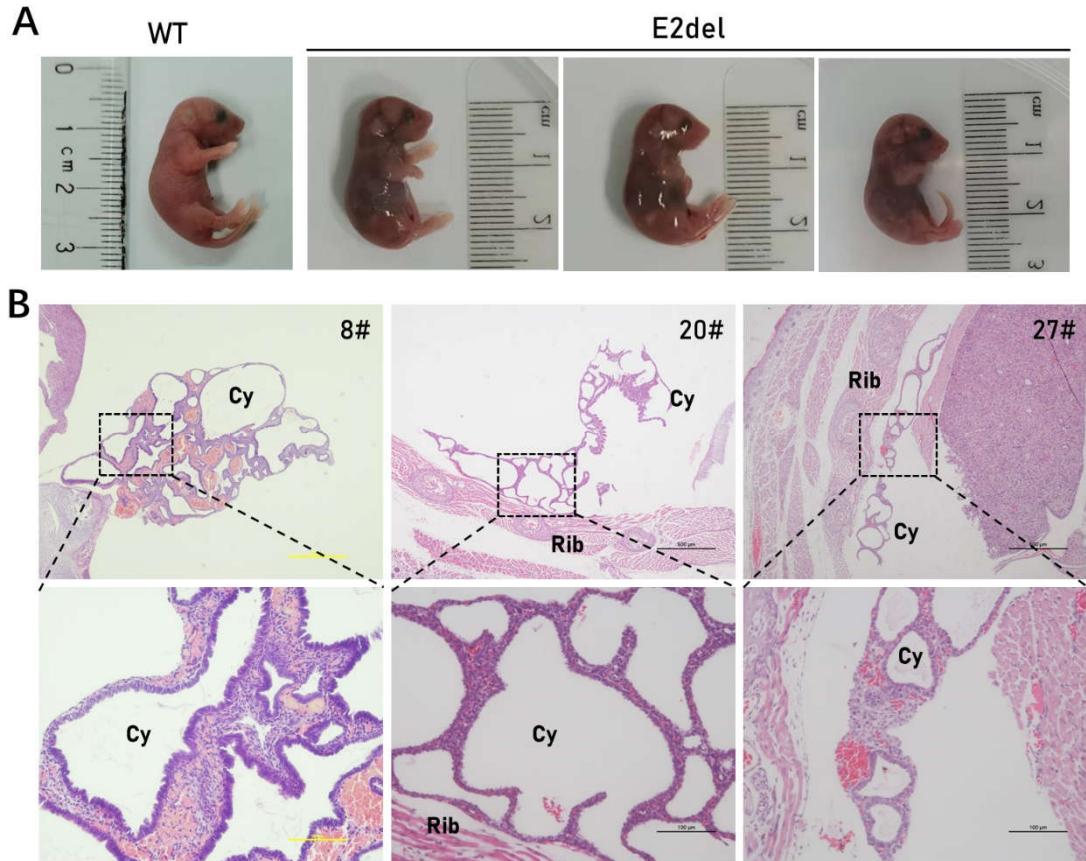


Figure S1. Gross morphology of the E2del mice and their lungs. (A) Entirety morphology of the E2del mice at E19 as compared with the age-matched wild-type control. (B) Supplemental H&E staining results of lung sections in the E2del mouse embryos. Scale bar: primitive is 500 μm , enlarged is 100 μm . Cy, cyst.

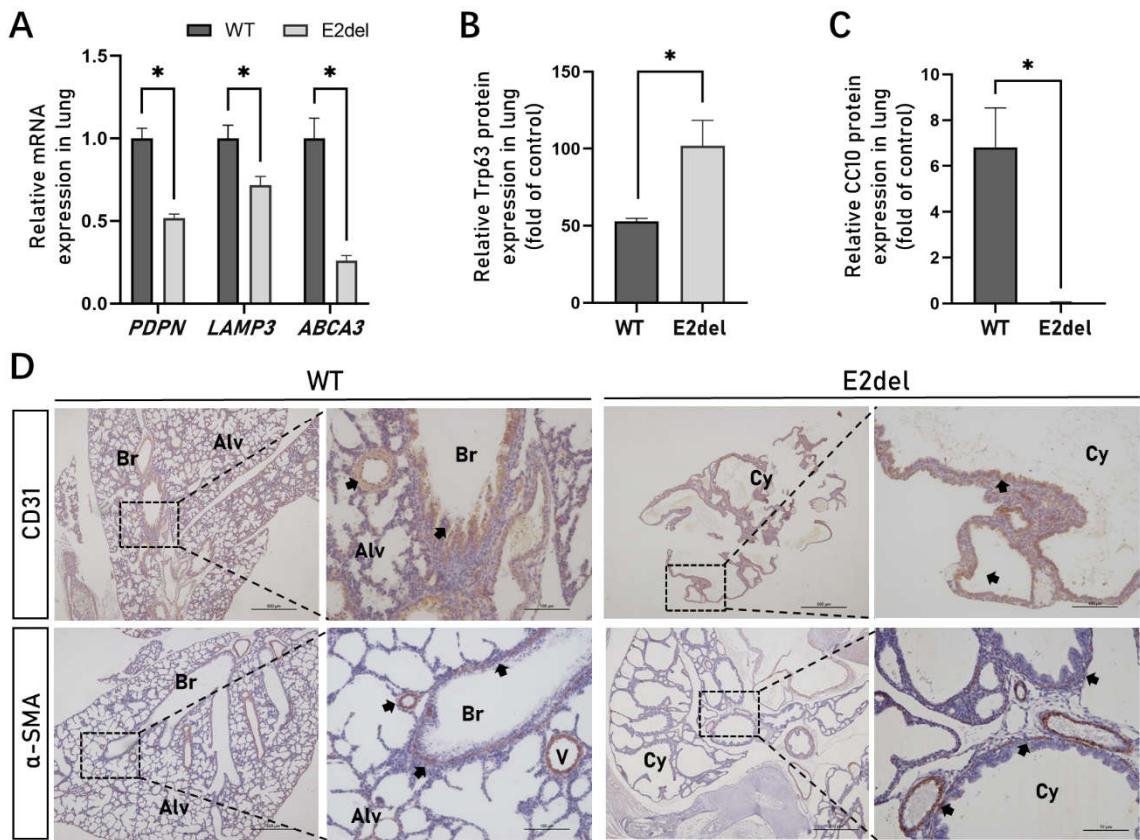


Figure S2. The specific gene and protein expression in the E2del lungs. (A) The relative mRNA expression showed that specific PDPN gene of alveolar type I cells and TTF-1-regulated gene *LAMP3* and *ABCA3* were inhibited in the E2del lungs compared to the wild-type lungs. (B) The means of data of Trp63-positive area as in figure 4E. (C) The means of data of CC10-positive area as in figure 5E. (D) The expressions of CD31 and α -SMA protein in the wild-type and E2del lungs at E19 as determined by IHC staining. Scale bar: primitive is 500 μ m, enlarged is 100 μ m. Br, bronchus; Alv, alveoli; Cy, cyst; V, blood vessel. The arrowheads indicate protein-positive area. The data are presented as mean \pm SEM. * $p < 0.05$, via paired student's *t* test.

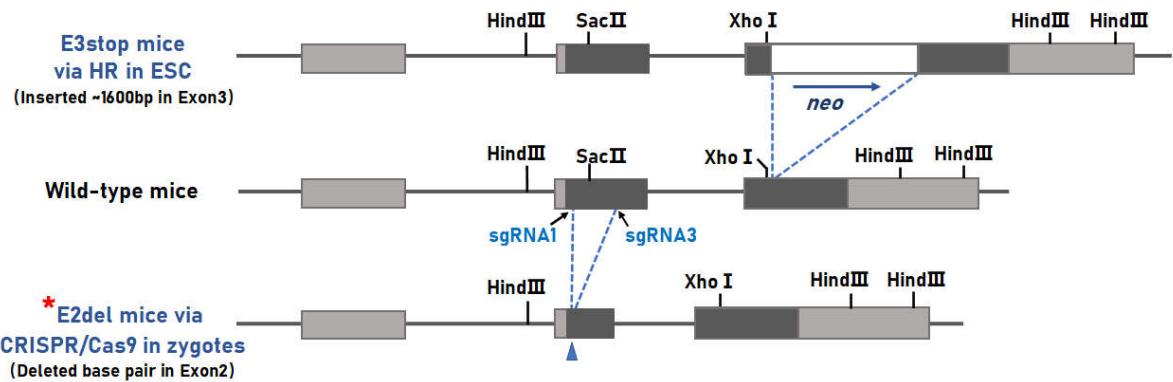


Figure S3. Schematic diagram of *TTF-1* gene in the E3stop mice, wild-type mice and E2del mice. The *TTF-1* gene of the E3stop mice was inserted the ~1600bp *neo* (neomycin) sequence in Exon 3 via HR (homologous recombination) in ESC (embryonic stem cell). The *TTF-1* gene of the E2del mice in this study (Red asterisk) was deleted base pair among the targeting sites of TTF1-sgRNA1 and TTF1-sgRNA3 in Exon 2 via CRISPR/Cas9 in zygotes.