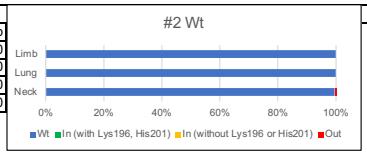
S1 Table. List of reagents and equipment used in this study.

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Chemicals		
Anti-human prosurfactant protein C	Abcam	ab90716
Cas9 protein	Integrated DNA Technologies	Cat# 1074181
Dako REAL Target Retrieval Solution (10x)	Agilent Technologies	S2031
Di-N Butyl-Phthalate in xylene, DPX	Merck	Cat# 1.01979.0500
Eosin	Merck	Cat# 1.09844.1000
EtOH, General alcohol 99 degree, synthetic	Japan Alcohol Trading Company Limited	
EtOH, Special grade, 99.5	SIGMA-ALDRICH	Code No. 09-0770-4
Hematoxylin Solution, Mayer's	Merck	Cat# 1.09249.0500
Paraffin	Thermo Fisher Scientific	Item Number: 6774060
PBS (Phosphate Buffered Salts) Tablet	Takara Bio	Cat# T900
Xylene	Nacalai tesque	Product code: 36611-45
Critical Commercial Assays		
BigDye Terminator v1.1 Cycle Sequencing Kit	Thermo Fisher Scientific	Product No.: 4337450
BrightGreen 2X qPCR MasterMix-No Dye	Applied Biological Materials	MasterMix-S
DNeasy Blood & Tissue Kit	QIAGEN	Cat# 69504
FastGene cDNA Synthesis 5x ReadyMix	Nippon Genetics	NE-LS64
Guide-it Mutation Detection Kit	Takara Bio	Product code: 631448
ImmPRESS Polymer Detection Kit for IHC	Vector Labs	Cat# MP-7401
NucleoZOL	Macherey-Nagel	REF 740404.200
RNAlater	Thermo Fisher Scientific	AM7020
DNA polymerase		
PrimeSTAR Max DNA polymerase	Takara Bio	Product code: R045A
Oligonucleotides		
Fgf10 Ex3F (5'-TGACTCTTCT GTTGTTAGCGT TG-3')	Eurofins Genomics	Yasue et al. 2014 [14]
Fgf10 Ex3R (5'-ACATCCAAAG CCTTCCTTCC-3')	Eurofins Genomics	Yasue et al. 2014 [14]
Fgf10 primer for MiSeq Fw (5'-GGATACAACA CCTATGCATC T-3')	Eurofins Genomics	This paper
Fgf10 primer for MiSeq Rv (5'-GTCTTTGCCT TTTGAGCTAC-3')	Eurofins Genomics	This paper
Machines or Instruments		
Digital camera system	Nikon	DS-Fi1
Digital camera system	Leica Microsystems	DFC310FX
DNA sequencer	Thermo Fisher Scientific	ABI PRISM 3100
DNA sequencer	Illumina	MiSeq
Electrophoresis	Agilent Technologies	TAPEStation 4200
Industrial blade	FEATHER Safety Razor	Item No.: 990077
Micro-scissors	Natsume Seisakusho	MB-54-2
Microscope	Leica Microsystems	DM5000B
Microscope	Leica Microsystems	M165FC
NanoDrop	Thermo Fisher Scientific	ND-1000
Paraffin extender	Sakura Seiki	PS-52
Paraffin extender	Sakura Finetek Japan	PS-53
Rotary microtome	Leica Biosystems	RM-2145
Superfrost Plus Microscope Slides	Thermo Fisher Scientific	Cat# 12-550-15
Thermal cycler	BIO-RAD	T100
Thermal cycler	Biometra	Tgradient
Thermal cycler	Roche	LightCycler Nano system
Tweezers	FONTAX	KN3345108

S2 Table. Summary of deep sequencing data on DNA from different tissues of E18.5 embryos.

#2 Wild type

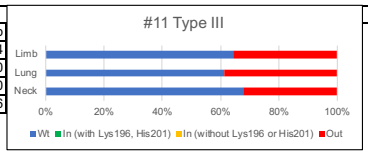
	Neck	Lung	Limb
Wt	99.6	100	100
In (with Lys196, His201)	0	0	0
In (without Lys196 or His201)	0	0	0
Out	0.37	0	0



p-value: N.A.

#11 Type III

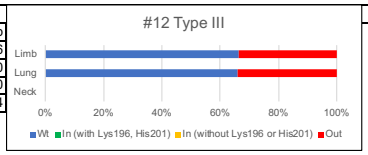
	Neck	Lung	Limb
Wt	67.8	61	64
In (with Lys196, His201)	0	0	0
In (without Lys196 or His201)	0	0	0
Out	32.2	39	36



p-value: 3.34E-29

#12 Type III

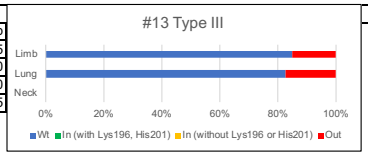
	Neck	Lung	Limb
Wt	N.D.	66	66
In (with Lys196, His201)		0	0
In (without Lys196 or His201)		0	0
Out		34	34



p-value: 0.751529

#13 Type III

	Neck	Lung	Limb
Wt	N.D.	83	85
In (with Lys196, His201)		0	0
In (without Lys196 or His201)		0	0
Out		17	15

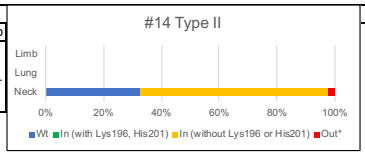


p-value: 4.02E-05

#14 Type II

	Neck	Lung	Limb
Wt	33	N.D.	N.D.
In (with Lys196, His201)	0		
In (without Lys196 or His201)	64.7		
Out*	2.3		

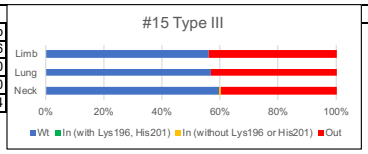
*includes a large deletion



p-value: N.A.

#15 Type III

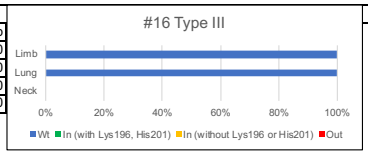
	Neck	Lung	Limb
Wt	59.8	57	56
In (with Lys196, His201)	0	0	0
In (without Lys196 or His201)	0.39	0	0
Out	39.8	43	44



p-value: 2.90E-11

#16 Type III

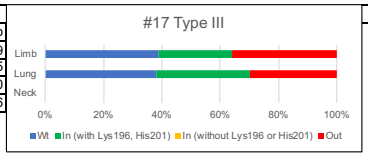
	Neck	Lung	Limb
Wt	N.D.	100	100
In (with Lys196, His201)		0	0
In (without Lys196 or His201)		0	0
Out		0	0



p-value: N.A.

#17 Type III

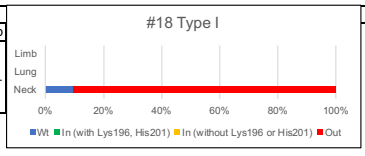
	Neck	Lung	Limb
Wt	N.D.	38	39
In (with Lys196, His201)		32	25
In (without Lys196 or His201)		0	0
Out		30	36



p-value: 3.38E-50

#18 Type I

	Neck	Lung	Limb
Wt	9.27	N.D.	N.D.
In (with Lys196, His201)	0		
In (without Lys196 or His201)	0		
Out	90.7		

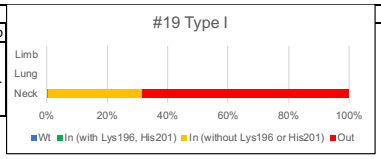


p-value: N.A.

S2 Table (continued)

#19 Type I

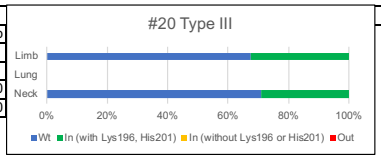
	Neck	Lung	Limb
Wt	0.5	N.D.	N.D.
In (with Lys196, His201)	0		
In (without Lys196 or His201)	31.2		
Out	68.3		



p-value: N.A.

#20 Type III

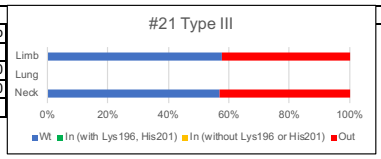
	Neck	Lung	Limb
Wt	71.1	N.D.	67.7
In (with Lys196, His201)	28.9		32.3
In (without Lys196 or His201)	0		0
Out	0		0



p-value: 1.89E-09

#21 Type III

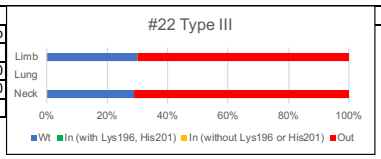
	Neck	Lung	Limb
Wt	56.9	N.D.	57.5
In (with Lys196, His201)	0		0
In (without Lys196 or His201)	0		0
Out	43.1		42.5



p-value: 0.17603

#22 Type III

	Neck	Lung	Limb
Wt	28.9	N.D.	29.9
In (with Lys196, His201)	0		0
In (without Lys196 or His201)	0		0
Out	71.1		70.1

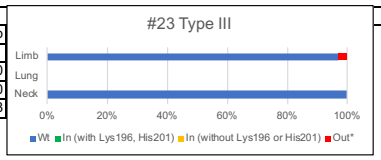


p-value: 0.271069

#23 Type III

	Neck	Lung	Limb
Wt	100	N.D.	97.2
In (with Lys196, His201)	0		0
In (without Lys196 or His201)	0		0
Out*	0		2.8

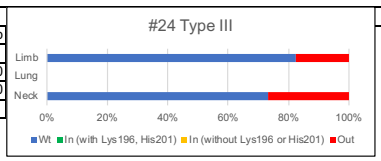
*including large deletion



p-value: N.A.

#24 Type III

	Neck	Lung	Limb
Wt	73.1	N.D.	82.3
In (with Lys196, His201)	0		0
In (without Lys196 or His201)	0		0
Out	26.9		17.7

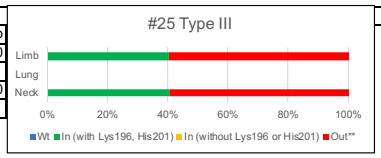


p-value: 1.18E-26

#25 Type III

	Neck	Lung	Limb
Wt	0	N.D.	0
In (with Lys196, His201)	40.7		40.2
In (without Lys196 or His201)	0		0
Out**	59.3		59.8

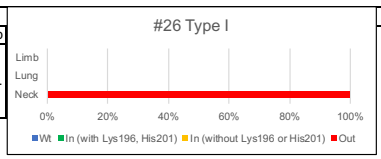
**including large insertion



p-value: 0.139527

#26 Type I

	Neck	Lung	Limb
Wt	0.45	N.D.	N.D.
In (with Lys196, His201)	0		
In (without Lys196 or His201)	0		
Out	99.5		



p-value: N.A.

The percentage of sequence reads for each genotype category is shown in contingency tables. Wt, wild type *Fgf10* genotype; In, in-frame mutations in the *Fgf10* gene; Out, frameshift mutations in the *Fgf10* gene; N.D., not done; N.A., not applicable. *p*-values of Chi-square test (see Table 4) are shown for reference.

S3 Table. Summary of type II and type III embryos examined for E18.5 lungs by immunohistochemistry, shown in Fig 6 and Fig S5.

Type	Embryo No.	Forelimb		Hindlimb		SPC (+) cells/total cells (mean±SEM %)	Wt genotype (%) in neck DNA	Wt genotype (mean± SEM %) in neck DNA	Figure
		Left	Right	Left	Right				
II	#11_2*	Truncated	Normal	Truncated	Truncated	10.5 ± 1.1	27.78	15.7 ± 6.0	Fig S5; Fig 6A,D,G
	#24_2	Only 3 digits	Normal	Only 3 digits	Normal	14.8 ± 0.4	9.75		Fig S5
	#25_2	Only 2 digits	Truncated	Truncated	Truncated	10.9 ± 0.7	9.53		Fig S5
III	#8_2	Normal	Normal	Normal	Normal	17.0 ± 0.8	62.40	51.8 ± 6.9	Fig S5
	#21_2					19.5 ± 0.8	54.17		Fig S5
	#22_2*					14.9 ± 0.8	38.80		Fig S5
WT	#2_18	Normal	Normal	Normal	Normal	N.D.	N.D.	N.D.	Fig 6C,F,I

*Approximately 295- (#11_2) and 79- (#22_2) bp insertion were detected by microchip electrophoresis and the number of sequence reads in deep sequencing was corrected accordingly (see Materials and methods). N.D., not determined.

S4 Table. Primers used for quantitative PCR (qPCR) analysis.

Gene name	Primer name	Nucleotide sequence of PCR primers	Amplicon size (bp)	GC content (%)	References
Fgf10	mmu_fgf10_qPCR_Fw	AGCGGGACCAAGAATGAAGACT	91	49.5	This study
	mmu_fgf10_qPCR_Rv	TGCTGTTGATGGCTTTGACGG			
Hopx	mmu_hopx_qPCR_Fw	TTCAACAAGGTCAACAAGCACC	106	61.3	This study
	mmu_hopx_qPCR_Rv	CCAGGCGCTGCTTAAACCAT			
Sftpc	mmu_sftpc_qPCR_Fw	CACCTCAAACGCCTTCTCATCG	147	52.4	This study
	mmu_sftpc_qPCR_Rv	TTTCTGAGTTTCCGGTGCTCC			
Vegfa	mmu_vegfa_qPCR_Fw	GCTGTACCTCCACCATGCCA	131	56.5	This study
	mmu_vegfa_qPCR_Rv	CCACCAGGGTCTCAATCGGA			
Pdgfa	mmu_pdgfa_qPCR_Fw	CGTCAAGTGCCAGCCTTCAC	148	54.7	This study
	mmu_pdgfa_qPCR_Rv	TGGGTTCAGGTTGGAGGTCG			
Plin2	mmu_plin2_qPCR_Fw	AAGAGAAGCATCGGCTACGA	78	55.1	Shaul et al. 2010 [1]
	mmu_plin2_qPCR_Rv	GGCGATAGCCAGAGTACGTG			
Pdgfra	mmu_pdgfra_qPCR_Fw	TCCTTCTACCACCTCAGCGAG	103	47.6	Awuah et al. 2013 [2]
	mmu_pdgfra_qPCR_Rv	CCGGATGGTCACTCTTTAGGAAG			
Acta2	mmu_acta2_qPCR_Fw	GTCCCAGACATCAGGGAGTAA	102	52.9	Hecker et al. 2009 [3]
	mmu_acta2_qPCR_Rv	TCGGATACTTCAGCGTCAGGA			
Gapdh	GAPDH_qPCRFw	AGGTTGTCTCCTGCGACTTCA	184	52.2	Ning et al. 2013 [4]
	GAPDH_qPCRRv	TGGTCCAGGGTTTCTTACTCC			

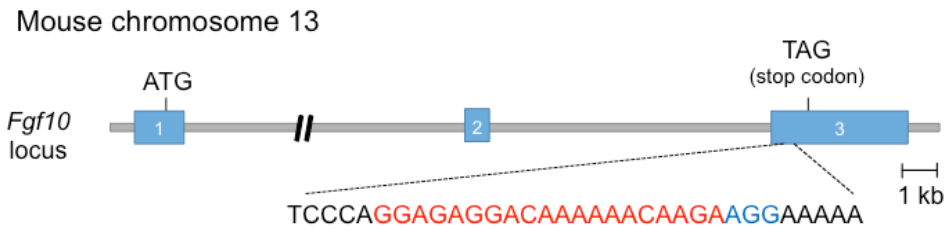
References

1. Shaul ME, Bennett G, Strissel KJ, Greenberg AS, Obin MS. Dynamic, M2-like remodeling phenotypes of CD11c+ adipose tissue macrophages during high-fat diet-induced obesity in mice. Diabetes. 2010; 59(5): 1171-81. doi: 10.2337/db09-1402.
2. Awuah PK, Nejak-Bowen KN, Monga SPS. Role and regulation of PDGFRα signaling in liver development and regeneration. Am J Pathol. 2013; 182(5):1648-58. doi: 10.1016/j.ajpath.2013.01.047.
3. Hecker L, Vittal R, Jones T, Jagirdar R, Luckhardt TR, Horowitz JC, et al. NADPH oxidase-4 mediates myofibroblast activation and fibrogenic responses to lung injury. Nat Med. 2009;15(9):1077-81. doi: 10.1038/nm.2005.
4. Ning YL, Yang N, Chen X, Xiong RP, Zhang XZ, Li P, et al. Adenosine A2A receptor deficiency alleviates blast-induced cognitive dysfunction. J Cereb Blood Flow Metab. 2013;33(11):1789-98. doi: 10.1038/jcbfm.2013.127.

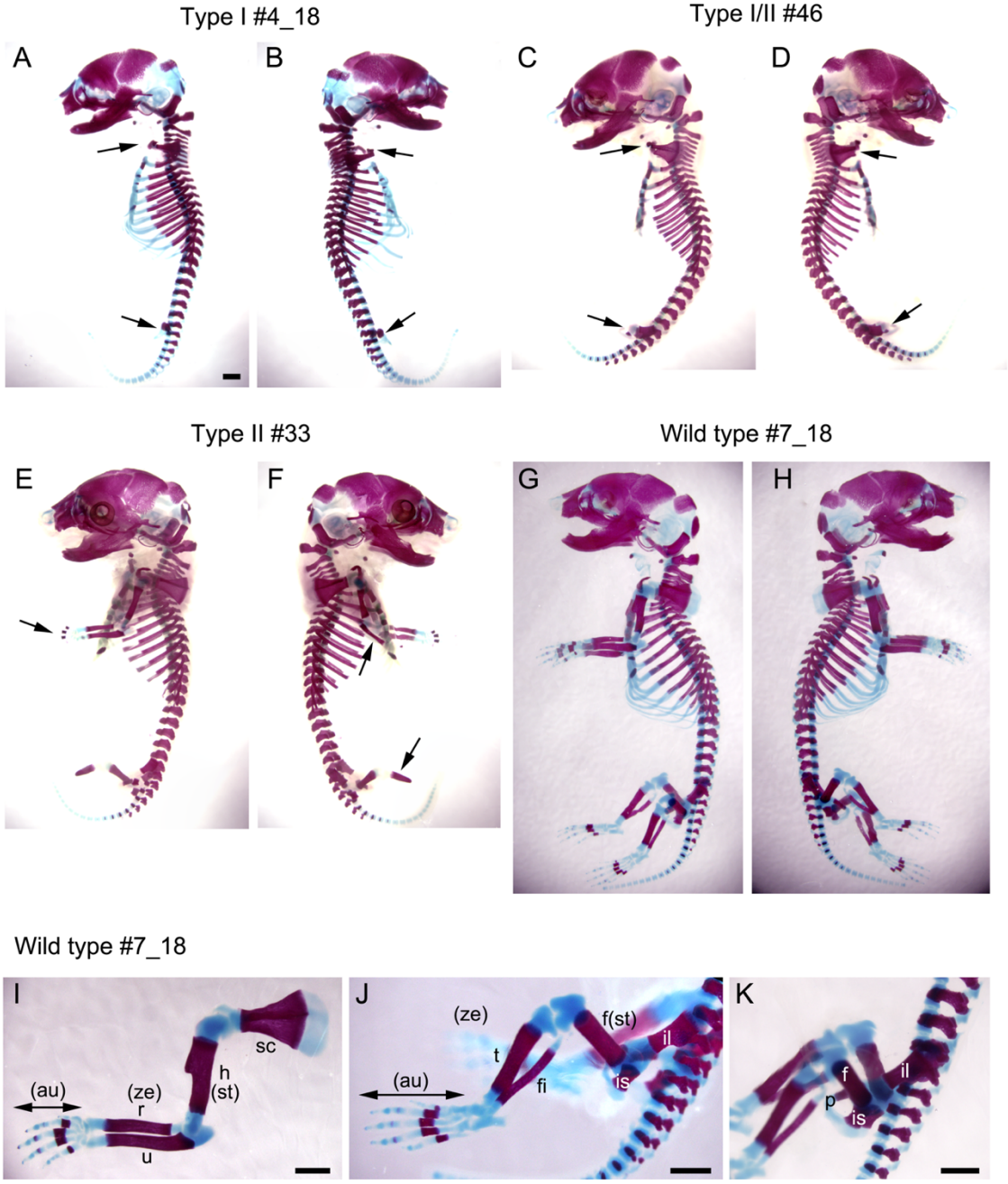
Summary of the gene profiles examined by qPCR

Gene name	Expressed by/in	Role
Hopx	AECI	
Sftpc	AECII	
Vegfa	Distal lung epithelium	Stimulates endothelial proliferation
Pdgfa	Distal lung epithelium	Stimulates proliferation of alveolar smooth muscle cell progenitors
Plin2	Lipofibroblast	
Pdgfra	Alveolar smooth muscle cell progenitors	
Acta2	Smooth muscle cell	

S1 Fig. Structure of the mouse *Fgf10* gene indicating the target sites for CRISPR/Cas9 system.

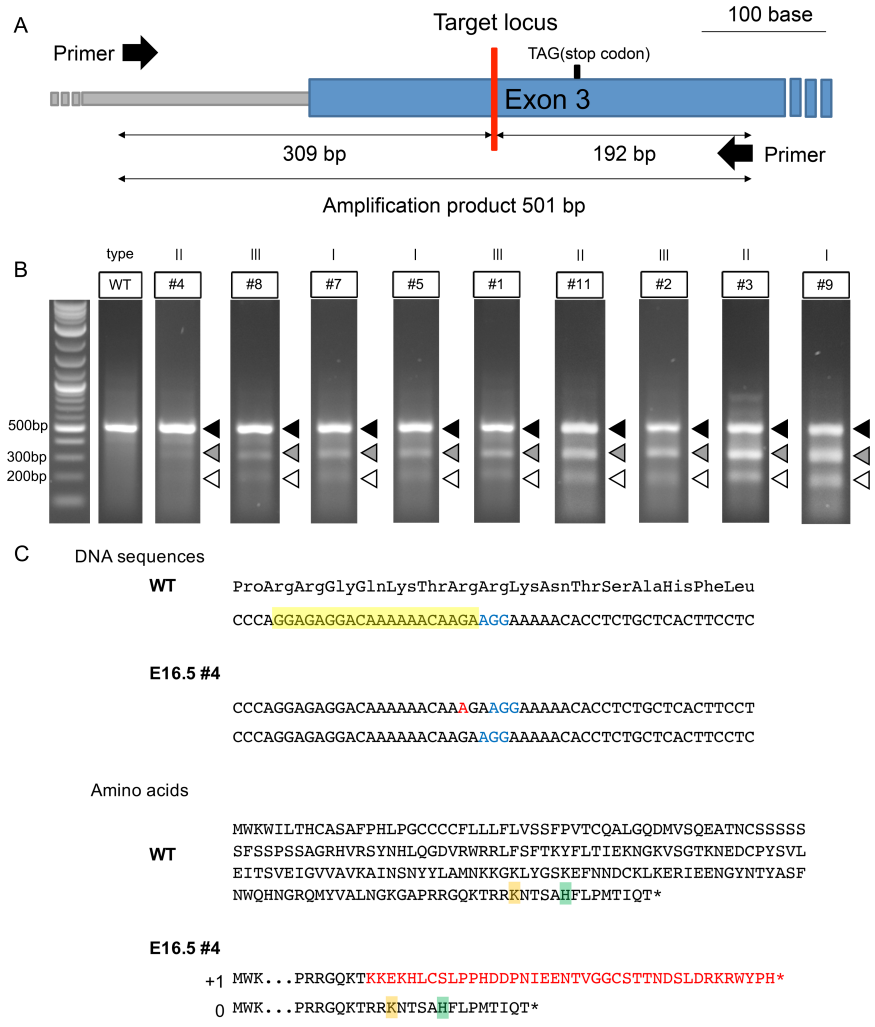


S2 Fig. Cartilage and bone staining to reveal skeletal structures.



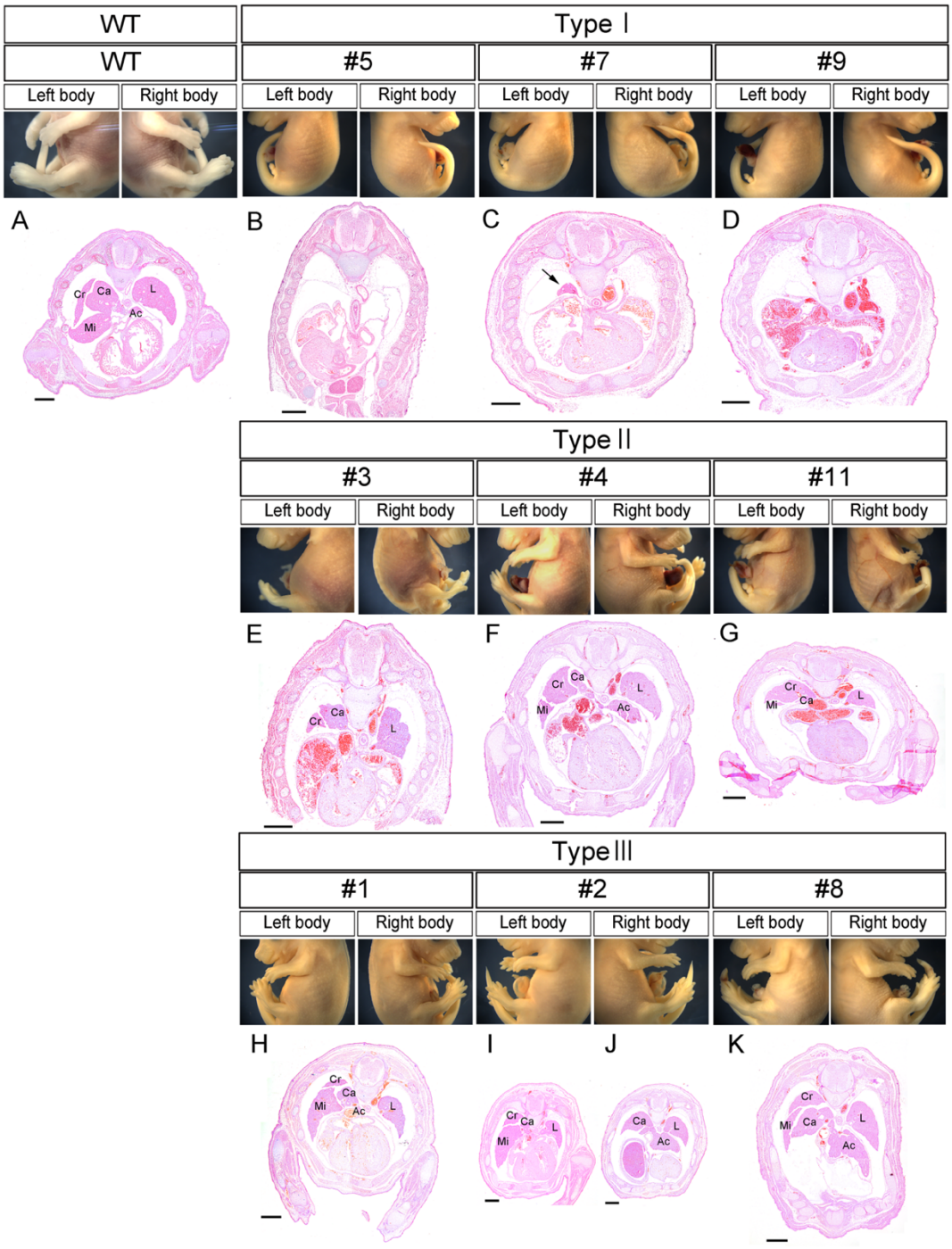
A-H, whole mount staining. Left (A, C, E, G) and right (B, D, F, H) lateral views are shown. Arrows show truncated limb and girdle bones. I-K, wild type skeletal structures, showing scapula and forelimb (I), hindlimb (J), and pelvic girdle (K). au, autopod; f, femur; fi, fibula; h, humerus; il, ilium; is, ischium; p, pubis; r, radius; sc, scapula; sp, spine; st, stylopod; t, tibia; u, ulna; ze, zeugopod. Scale bars: 1 mm.

S3 Fig. Mismatch cleavage assay.



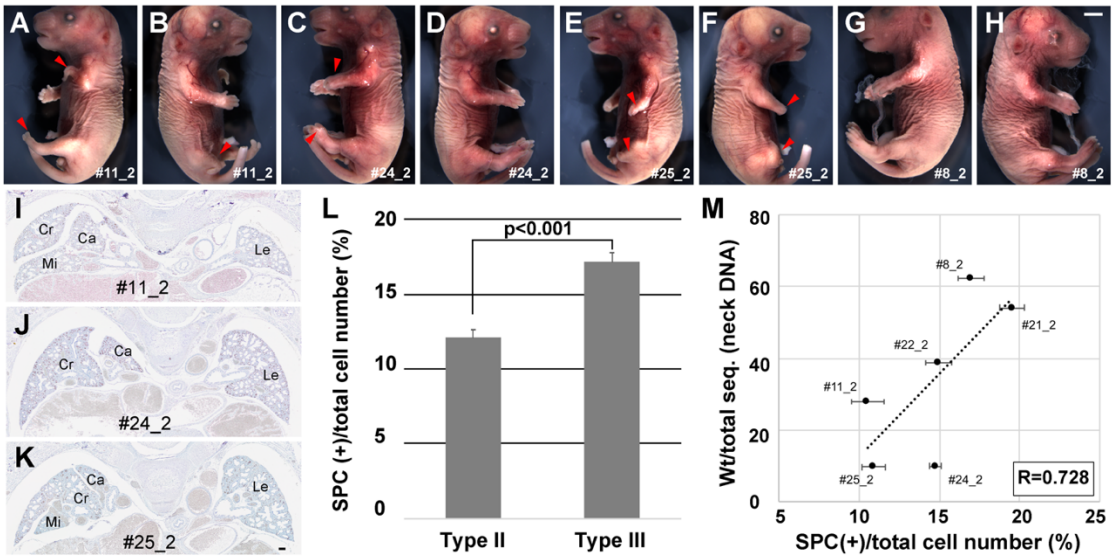
A, PCR primers were designed in the upstream region of the exon 3 and in the exon 3, giving rise to a PCR amplicon size of 501 bp. DNA fragments of 309 bp and 192 bp are generated by the Resolvase when the Fgf10 genome has been cleaved by Cas9 and non-homologous end joining has been achieved. B, Electrophoresis of the enzyme- treated mouse genomic DNA from the Fgf10-CRISPR F0 embryonic necks. The DNA ladder for DNA size reference and a result of DNA from a wild type (WT) mouse are shown on the left. Three DNA fragments of approximately 500 bp (▼), 300 bp (5 in gray), and 200 bp (5) are seen in all the lanes except for the wild type and #4 lanes. In embryo #3, an extra band for large insertion (328 base) is shown (see Fig 2). C, Genomic analysis of the #4 embryo as revealed by Sanger sequencing. Deduced amino acid sequences are also shown. Lys-196 and His-201 are highlighted in yellow and green, respectively. Altered amino acids are indicated in red. Asterisks indicate stop codons.

S4 Fig. Limb phenotypes and lung histology of all embryos examined at E16.5 as summa- rized in Table 3.



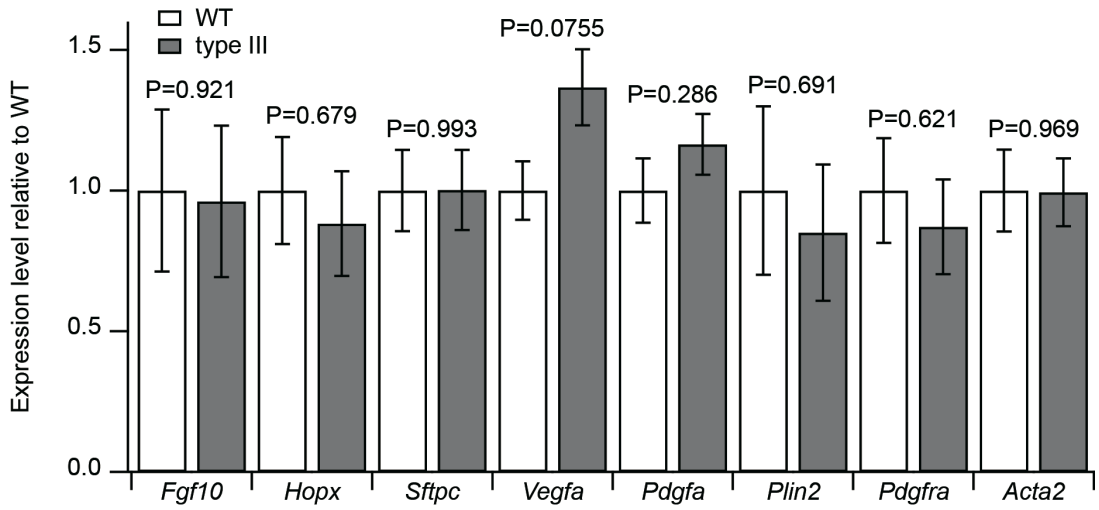
Scale bars: 500 μm.

S5 Fig. Limb phenotypes, lung histology, and the number of SPC-positive cells per total cell number of type II and type III embryos at E18.5.

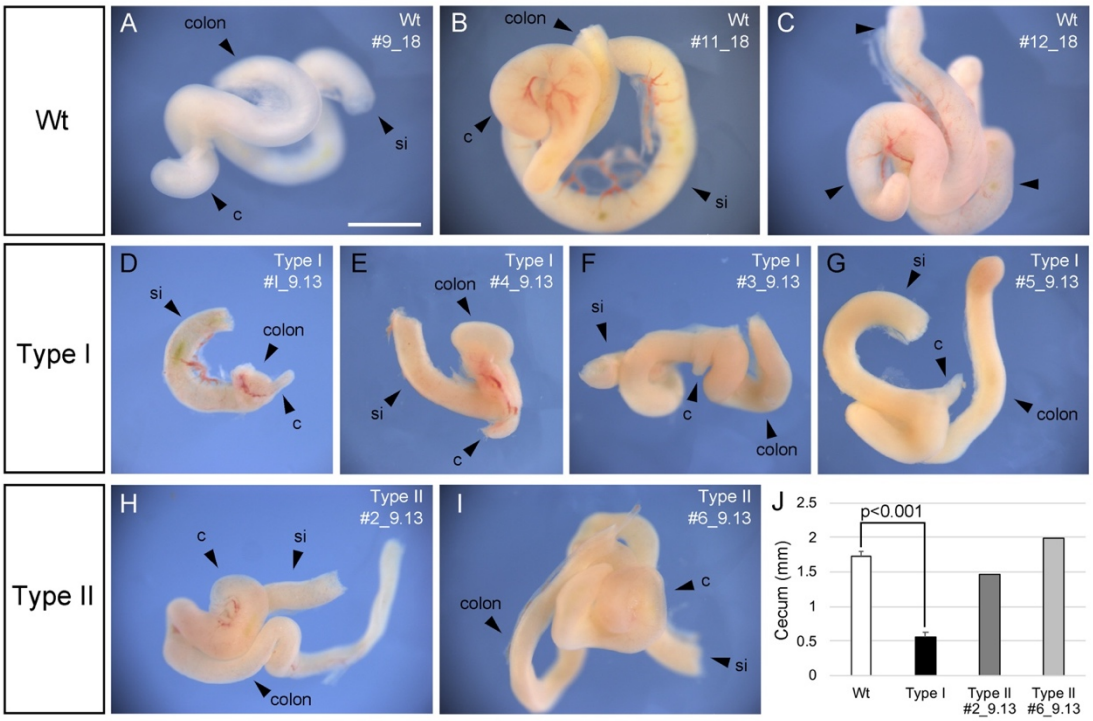


Source data for (L, M) are available in S3 Table. Lateral views of type II (A-F), and type III (G, H) embryos at E18.5. Arrowheads show limb defects. (I-K) In all three type II embryos, the accessory lobe was lost. In embryo #24_2, (J), the middle lobe (Mi) was also undetectable. Data in (L) are presented as means ± SEM. (M) In these embryos, the number of SPC-positive cells was more correlated to the percentage of wild type Fgf10 genotype (correlation coefficient [R] was 0.728 for neck DNA) than that including in-frame mutations with Lys196 and His201 retained (R = 0.334). Scale bar: 2 mm (A-H).

S6 Fig. Quantitative PCR analysis for mRNA expression of mesenchymal and its related marker genes in wild type and type III lungs at E18.5.



S7 Fig. Cecum and colons of the wild type and Fgf10-CRISPR F0 (type I and type II) embryos at E18.5.



A-C, wild type (Wt) cecum (c), colon, and small intestine (si) are shown from three embryos examined. Ileum and colon were cut at dissection. D-G, type I cecum is reduced compared with the wild type. Whether the cecum epithelium is absent or not cannot be identified from these photos. Type I embryos show an atresia of the colon, but the length varies depending upon the embryos. H-I, type II embryos examined (n = 2) do not exhibit a reduced cecum or an atresia of the colon. The colons presented here were cut as distally as possible. J, the approximate length of the cecum. The length of type I cecum is significantly decreased compared with the wild type. The length of two type II embryos examined is also shown for reference. Scale bar: 1 mm (in A for all to scale).