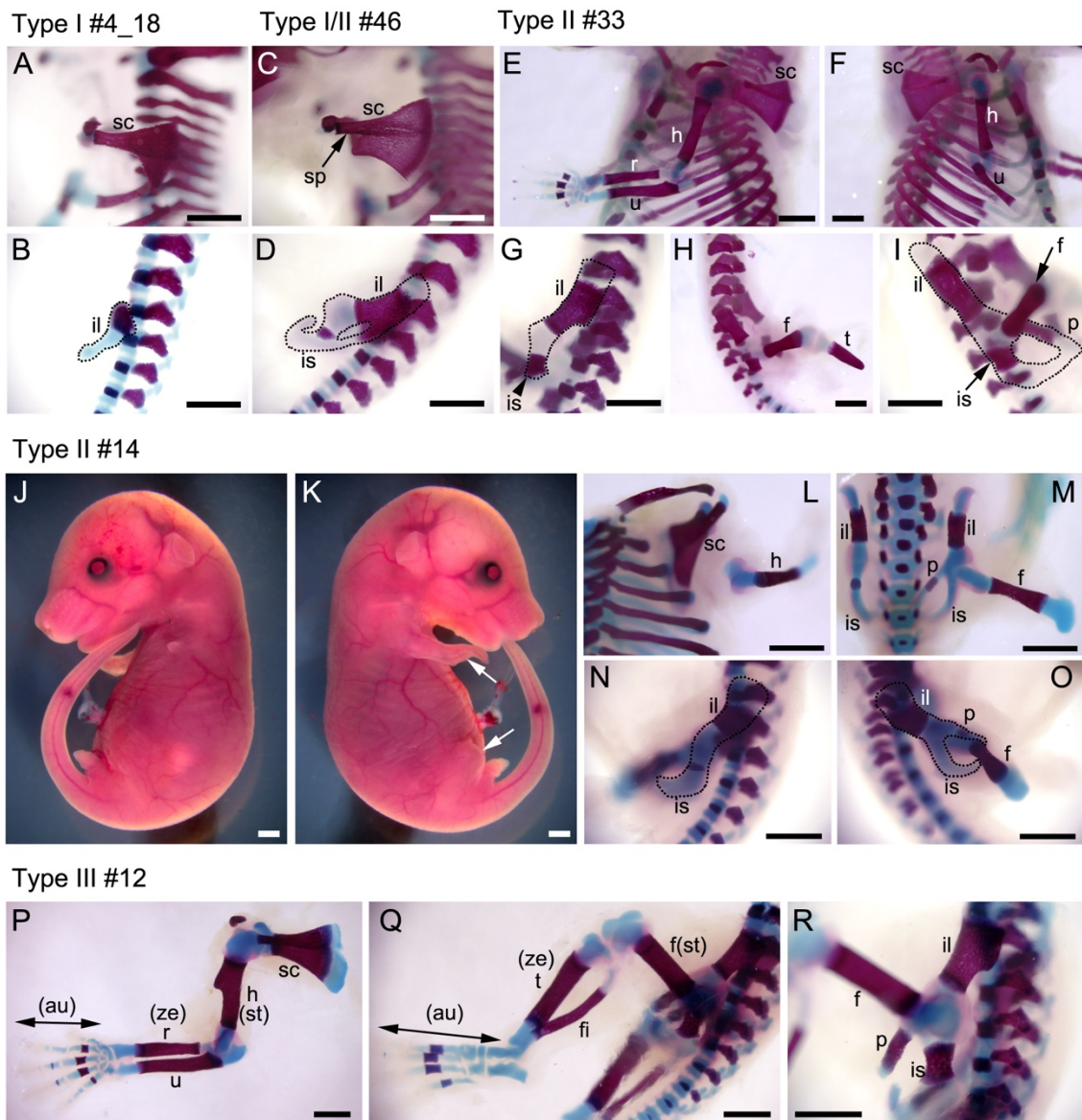# Fig 1. Cartilage and bone staining to reveal limb skeletal structures.

A-I, L-R, left or right lateral views of limb and girdle regions (at E18.5) are shown. Cartilage is stained with Alcian blue and bone is stained with Alizarin red. **A, B,** type I #4_18 embryo. **C, D,** type I/II #46 embryo. **E-I,** type II #33 embryo. **L-O,** type II #14 embryo. **P-R,** type III #12 embryo. **J, K,** Lateral views of type II #14 embryo are shown. Arrows in (**K**) show truncated limbs. 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.

Fig 2. Genomic analysis of the *Fgf10*-CRISPR F0 embryos at E16.5 as revealed by deep sequencing.

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                                                                  sequence                                                           Type
         WT    # of reads  % of reads  indel  In-/Out- of frame 196-Lys 201-His ProArgArgGlyGlnLysThrArgArgLysAsnThrSerAlaHisPheLeu   WT
               9188        100         0      WT                                CCCAGGAGAGGACAAAAACAAGAAGGAAAAACACCTCTGCTCACTTCCTC

               # of reads  % of reads  indel  In-/Out- of frame 196-Lys 201-His sequence                                             Type
 E16.5  #1     494         27.76840922  0     WT                                CCCAGGAGAGGACAAAAACAAGAAGGAAAAACACCTCTGCTCACTTCCTC   III
               407         22.87802136  -8    Out                               CCCAGGAGAGGACAAAA--------GGAAAACACCTCTGCTCACTTCCTC
               327         18.38111298  +4    Out                               CCCAGGAGAGGACAAAAAANAAGGAAGAAGGAAAAACACCTCTGCTCACTT
               268         15.06464306  +1    Out                               NCCAGGAGAGGACAAAAAACAAAGAAGGAAAAACACCTCTGCTCACTTCCT
               268         15.06464306  +9    In                +        +      CCCAGGAGAGGACAAAAAANGAAGGAAAAAGAAGGAAAAACACCTCTGCT
               15          0.84317032   -2    Out                               CCCAGGAGAGGACAAAAAA--AGAAGGAAAAACACCTCTGCTCACTTCCTC

        #2     5019        38.83472609  0     WT                                CCCAGGAGAGGACAAAAACAAGAAGGAAAAACACCTCTGCTCACTTCCTC   III
               3950        30.5632931   -4    Out                               CCCAGGAGAGGACAAAAA----GAAGGAAAAACACCTCTGCTCACTTCCTC
               2904        22.46982358  -28   Out                               CCCAGGAGAGGACAAAAACA-------------------------TC
               1051        8.132157227  -6    In                +        +      CCCAGGAGAGGACAAAAA------GGAAAACACCTCTGCTCACTTCCTC

        #3     2929        34.49941107  -15   In                -        +      CCCAGGAGAGGACAAAA---------------CACCTCTGCTCACTTCCTC   II
               1552        18.2803298   0     WT                                CCCAGGAGAGGACAAAAACAAGAAGGAAAAACACCTCTGCTCACTTCCTC
               1540        18.13898704  -5    Out                               CCCAGGAGAGGACAAAA-----GAAGGAAAAACACCTCTGCTCACTTCCTC
               1104        13.00353357  -4+332 Out                              CCCAGGAGAGGACAAAAGCAGCAGCAGCAGTGGAGTAGAGGCATCTGGAGC
               604         7.114252061  +3    In                +        +      CCCAGGAGAGGACAAAAAAACACCCAGNNGGAAAAACACCTCTGCTCACTTC
               384         4.522968198  +1    Out                               CCCAGGAGAGGACAAAAANCNNGAAGGAAAAACACCTCTGCTCACTTCCT
               377         4.440518257  -14   Out                               CCCAGGAGAGGACAAAA--------------CACCTCTGCTCACTTCCTC

        #4     172         66.92607004  +1    Out                               CCCAGGAGAGGACAAAAAACAAGAGAAGGAAAAACACCTCTGCTCACTTCCT  II
               75          29.18287938  0     WT                                CCCAGGAGAGGACAAAAAACAAGAAGGAAAAACACCTCTGCTCACTTCCTC
               5           1.945525292  -13   Out                               CCCAGGAGAGGACAAAAAACA-------------CCTCTGCTCACTTCCTC
               3           1.167315175  -7    Out                               CCCAGGAGAGGACAAAA-------GGAAAAACACCTCTGCTCACTTCCTC
               2           0.778210117  -3    In                +        +      CCCAGGAGAGGACNAAAAA---GAAGGAAAAACACCTCTGCTCACTTCCTC

        #5     1106        23.92385897  -13   Out                               CCCAGGAGAGGACAAAAAACA-------------CCTCTGCTCACTTCCTC   I
               1025        22.17174995  -16   Out                               CCCAGGAGAGGACAAA----------------CACCTCTGCTCACTTCCTC
               994         21.5011897   -10   Out                               CCCAGGAGAGGA----------GAAGGAAAAACACCTCTGCTCACTTCCTC
               675         14.6009085   -14   Out                               CCCAGGAGAGGACAAAAA--------------CACCTCTGCTCACTTCCTC
               401         8.674021198  -10   Out                               CCCAGGAGAGGACAA----------GGAAAAACACCTCTGCTCACTTCCTC
               214         4.629028769  0     WT                                CCCAGGAGAGGACAAAAAACAAGAAGGAAAAACACCTCTGCTCACTTCCTC
               208         4.499242916  -1    Out                               CCCAGGAGAGGACAANNANNA-GAAGGAAAAACACCTCTGCTCACTTCCTC

        #7     3879        44.20512821  -7    Out                               CCCAGGAGAGGACAAAAA-------GGAAAAACACCTCTGCTCACTTCCTC   I
               3110        35.44159544  -13   Out                               CCCAGGAGAGGACAAAAAACA-------------CCTCTGCTCACTTCCTC
               1068        12.17094017  -3    In                +        +      CCCAGGAGAGGACAAAAA---GAAGGAAAAACACCTCTGCTCACTTCCTC
               718         8.182336182  +9    In                +        +      CCCAGGAGAGGACAAAAAACAAGNAAAAACTGTAGGAAAAACACCTCTGCT

        #8     1585        26.94203638  +1    Out                               CCCAGGAGAGGACAAAAAACAAAGAAGGAAAAACACCTCTGCTCACTTCCT  III
               1483        25.2082271   0     WT                                CCCAGGAGAGGACAAAAAACAAGAAGGAAAAACACCTCTGCTCACTTCCTC
               1419        24.12034676  -3    In                +        +      CCCAGGAGAGGACAAAAA---GAAGGAAAAACACCTCTGCTCACTTCCTC
               1396        23.72938977  -6    In                +        +      CCCAGGAGAGGACAAA------GAAGGAAAAACACCTCTGCTCACTTCCTC

        #9     1193        45.01886792  -1    Out                               CCCAGGAGAGGACAAAAACA-GAAGGAAAAACACCTCTGCTCACTTCCTC   I
               1065        40.18867925  -30   In                -        +      CCCAGGAGAG-------------------------CTCACTTCCTC
               301         11.35849057  -33   In                -        +      CCC-------------------------------TCTGCTCACTTCCTC
               91          3.433962264  +1    Out                               CCCAGGAGAGGACAAAAAACAAAGAAGGAAAAACACCTCTGCTCACTTCCT

        #11    3304        79.40398943  -13   Out                               CCCAGGAGAGGACAAAAAACA-------------CCTCTGCTCACTTCCTC   II
               857         20.59601057  0     WT                                CCCAGGAGAGGACAAAAACAAGAAGGAAAAACACCTCTGCTCACTTCCTC
```

The target nucleotide sequence is highlighted in yellow. Proto-spacer adjacent motif sequence is shown in blue. Insertion and deletion sequences are shown in red.

Fig 3. Deduced amino acids for in-frame mutations after deep sequencing.

```
WT                    MWKWILTHCASAFPHLPGCCCCFLLLFLVSSFPVTCQALGQDMVSQEATNCSSSSSSFSSPSSAGR
                      HVRSYNHLQGDVRWRRLFSFTKYFLTIEKNGKVSGTKNEDCPYSVLEITSVEIGVVAVKAINSNYY
                      LAMNKKGKLYGSKEFNNDCKLKERIEENGYNTYASFNWQHNGRQMYVALNGKGAPRRGQKTRRKNT    Phenotype
                      SAHFLPMTIQT*

#1       -9           MWK...GAPRRGQKRKEKRRKNTSAHFLPMTIQT*                                    Type Ⅲ

#2       -6           MWK...GAPRRGQK--RKNTSAHFLPMTIQT*                                       Type Ⅲ

#3       -15          MWK...GAPRRGQ-----NTSAHFLPMTIQT*                                       Type Ⅱ
         +3           MWK...GAPRRGQKTPRWKNTSAHFLPMTIQT*

#4       -3           MWK...GAPRRGXK-RRKNTSAHFLPMXIQT*                                       Type Ⅱ

#7       -3           MWK...GAPRRGQK-RRKNTSAHFLPMTIQT*                                       Type Ⅰ
         +9           MWK...GAPRRGQKTRKNCRKNTSAHFLPMTIQT*

#8       -3           MWK...GAPRRGQK-RRKNTSAHFLPMTIQT*                                       Type Ⅲ
         -6           MWK...GAPRRGQ--RRKNTSAHFLPMTIQT*

#9       -30          MWK...GAPRR----------AHFLPMTIQT*                                       Type Ⅰ
         -33          MWK...GAP----------SAHFLPMTIQT*

#11      -12          MWK...GAPRRGQ----KNTSAHFLPMTIQT*                                       Type Ⅱ
```

Wild type (WT) sequences are shown on the top. The embryo (E16.5) number (#), nucleotide number of small insertion (+) or deletion (-) (Indels) are shown on the left. Classified "types" are shown on the right. Amino acids corresponding to the guide RNA sequence are underlined. Deleted and altered amino acids are indicated in red.

Fig 4. Schematic representation of genomic analysis of *Fgf10*-CRISPR F0 embryos at E16.5 by deep sequencing.
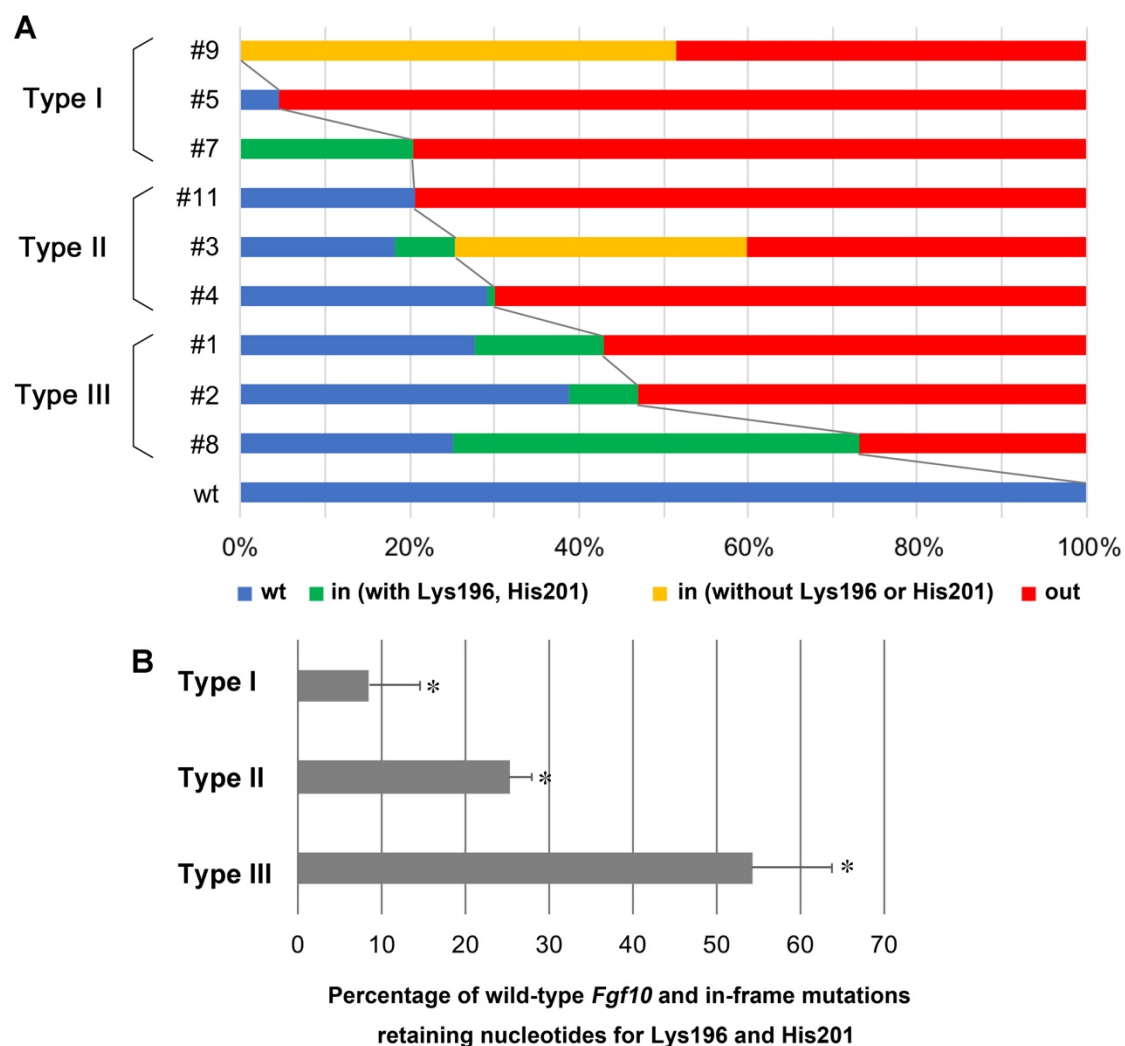
A, percentage of total reads for the *Fgf10* crispants. wt, wild type *Fgf10* target nucleotide sequence; in, in-frame mutations by small insertion or deletion; out, frameshift mutations. Among in-frame mutations, the percentage of those that preserve the codons for Lys196 and His201 is shown in green, while the percentage that eliminate either of them is shown in yellow. **B**, percentage of wild type and in-frame mutations in which nucleotides for both Lys196 and His201 are retained: $8.3 \pm 6.2\%$ for type I, $25.3 \pm 2.7\%$ for type II, and $54.3 \pm 9.5\%$ for type III. Data are presented as means ± SEM. *$p < 0.01$ ($p = 0.008$).

Fig 5. Limb phenotypes and lung histology of *Fgf10*-CRISPR F0 embryos at E16.5.
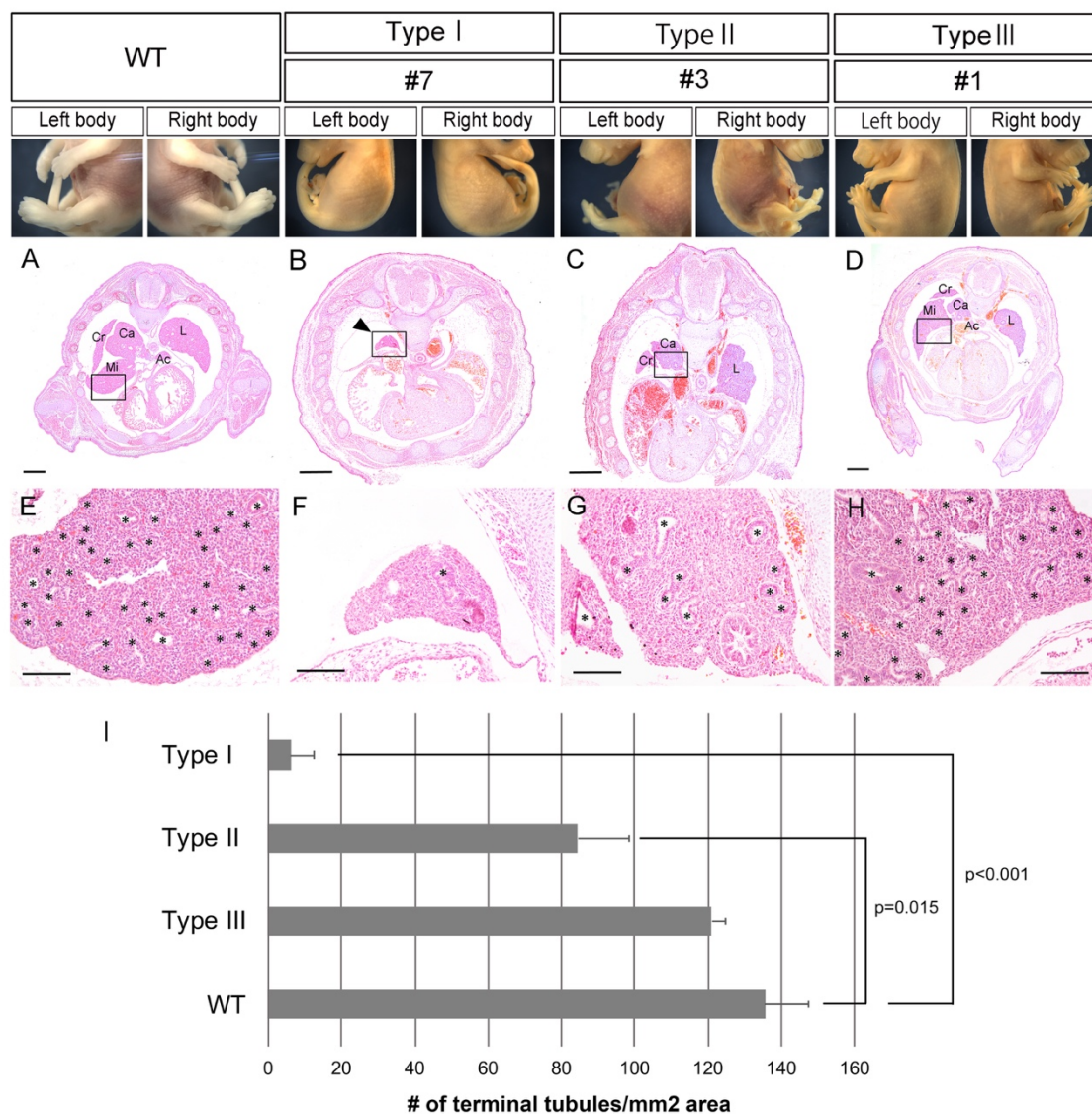
Representative embryos are shown for wild type (WT), type I (embryo #7), type II (#3), and type III (#1). **A–D**, transverse section of the embryonic chest region. **E–H**, close-up of the embryonic lung (boxed area) shown in (**A–D**), respectively. Asterisks show putative terminal tubules in the lung. **I**, the number of lung terminal tubules per unit area.

In type I and type II embryos, there is a significant decrease in the number compared with that of wild type. Data are presented as means ± SEM. Source data are available in Table 3. Ac, accessory lobe; Ca, caudal lobe; Cr, cranial lobe; L, left lobe; Mi, middle lobe. Scale bars: 500 μm in (**A–D**), and 100 μm in (**E–H**).

Fig 6. Immunohistochemistry of the lung in type II and type III *Fgf10*-crispants and wild type embryos at E18.5.
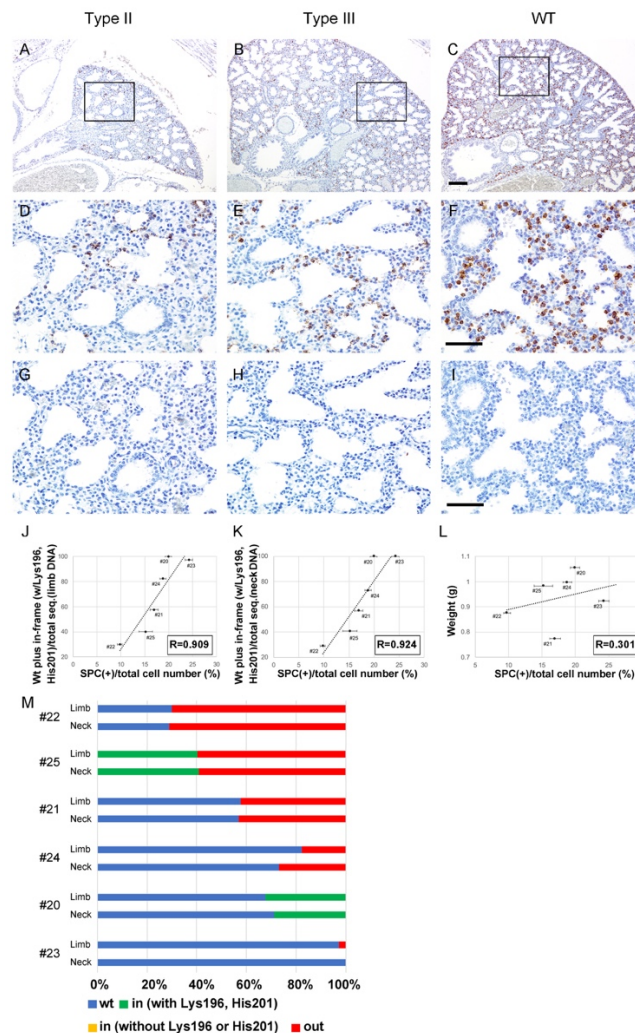
Representative data are shown. Nuclei are stained with Hematoxylin. **A-C**, Localization of Surfactant protein C (SPC) (alveolar type 2 epithelial cells) is indicated by brown staining. **D-F**, close-up of the boxed area shown in (**A–C**), respectively. **G-I**, negative control, using normal rabbit IgG instead of anti-SPC antibody. Scale bars: 100 μm in (**A-C**); 50 μm in (**D-I**). **J**, **K**, the number of SPC-positive cells in type III embryos is correlated to the percentage of functional *Fgf10* genotypes in limb (**J**) or neck DNA (**K**). **L**, the number of SPC-positive cells in type III embryos is not to correlated to their weight (**K**). Source data for (**J-L**) are available in Table 1. **M**, Schematic drawings to show percentage of total reads for the type III *Fgf10* crispants. wt, wild type *Fgf10* target nucleotide sequence; in, in-frame mutations by small insertion or deletion; out, out-of-frame mutations. Among in-frame mutations, the percentage of those that preserve the codons for Lys196 and His201 is shown in green, while the percentage that eliminate either of them is shown in yellow.