Gao et al., http://www.jcb.org/cgi/content/full/jcb.201506014/DC1

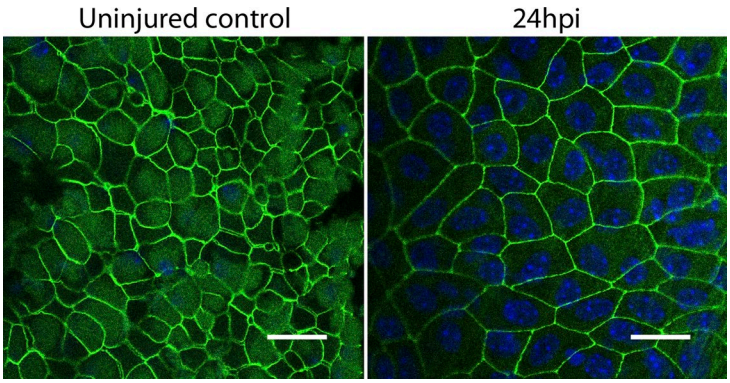

Figure S1. **ZO1 is localized between tracheal BCs early during regeneration after loss of luminal cells.** Tracheas from uninjured *Tjp1*^{tm1Ich} mice and mice 24 h after exposure to SO_2 were fixed and examined by confocal microscopy without further manipulation. Image is a single optical section near the apical surface. At 24 hpi, many of the squamous epithelial cells express Trp63, a marker of BCs. Bars, 20 µm.

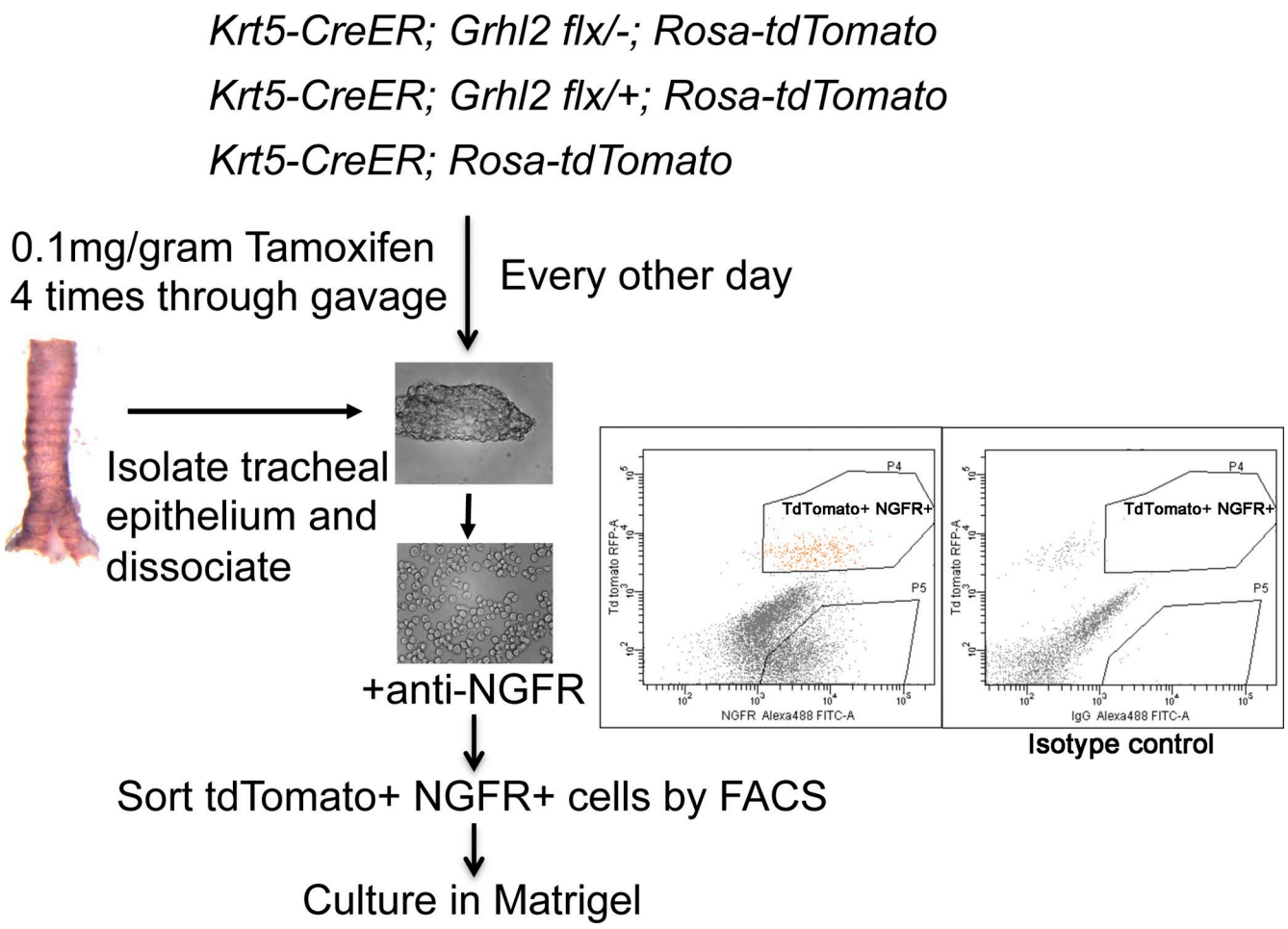

Figure S2. **Isolation of tracheal BCs.** Schematic for timing and dose of Tmx treatment, isolating tracheal epithelium, and FACS sorting lineage-labeled NGFR$^+$ BCs. Right panel is isotype control for anti-Ngfr.

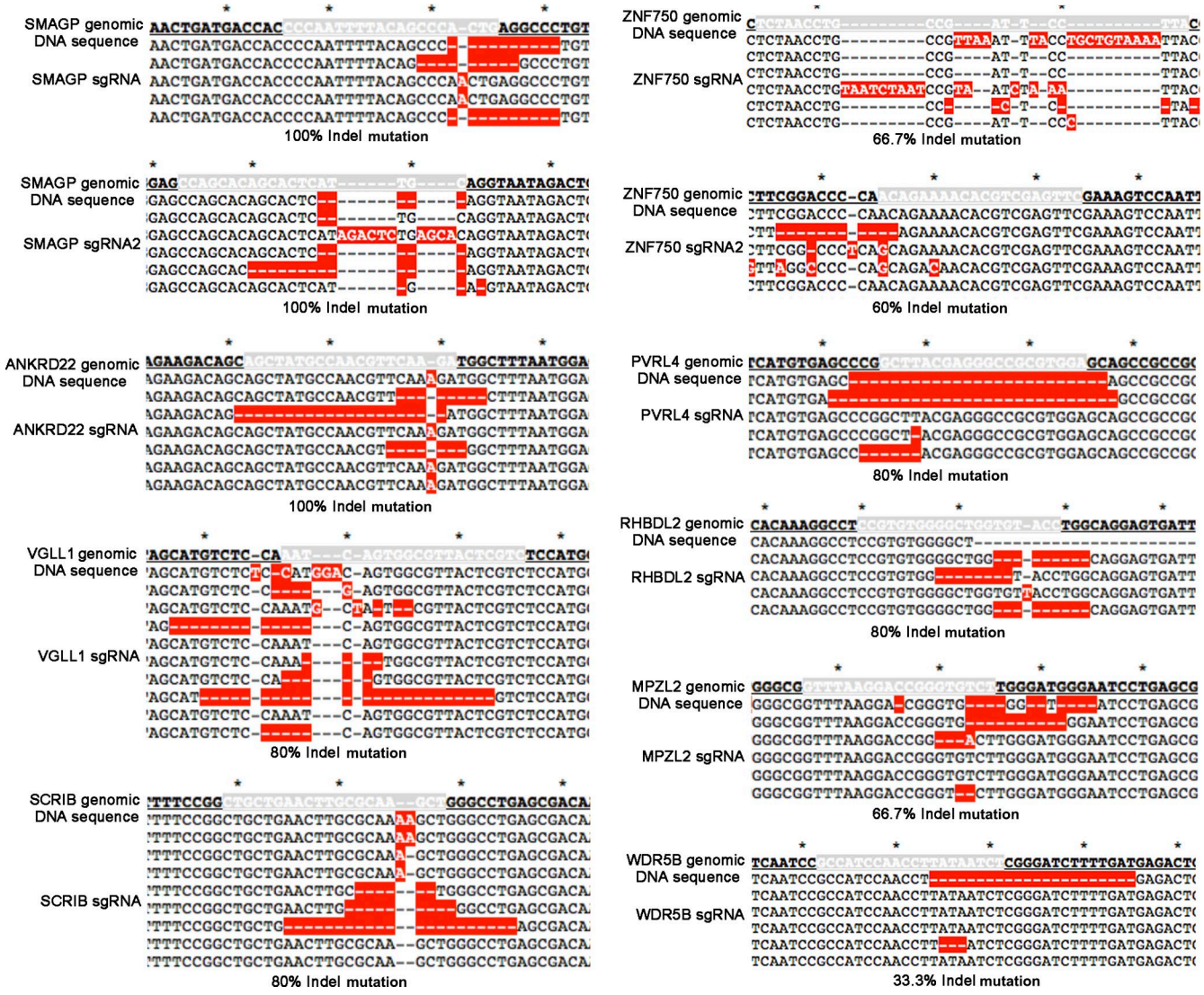

Figure S3. **Mutation of potential Grhl2 target genes in HBE cells using CRISPR/Cas9.** Cells were transfected with the lentiCRISPR virus–specific target for different genes and selected in puromycin, and genomic DNA was extracted and analyzed as described in Materials and methods (section CRISPR/Cas9 genome editing). The sgRNA region was PCR amplified (for primers, see Table S3) and cloned into a sequencing vector. The red highlights are mismatched, missing, or inserted nucleotides. The asterisks indicate every 10 nucleotides of the genomic sequence.

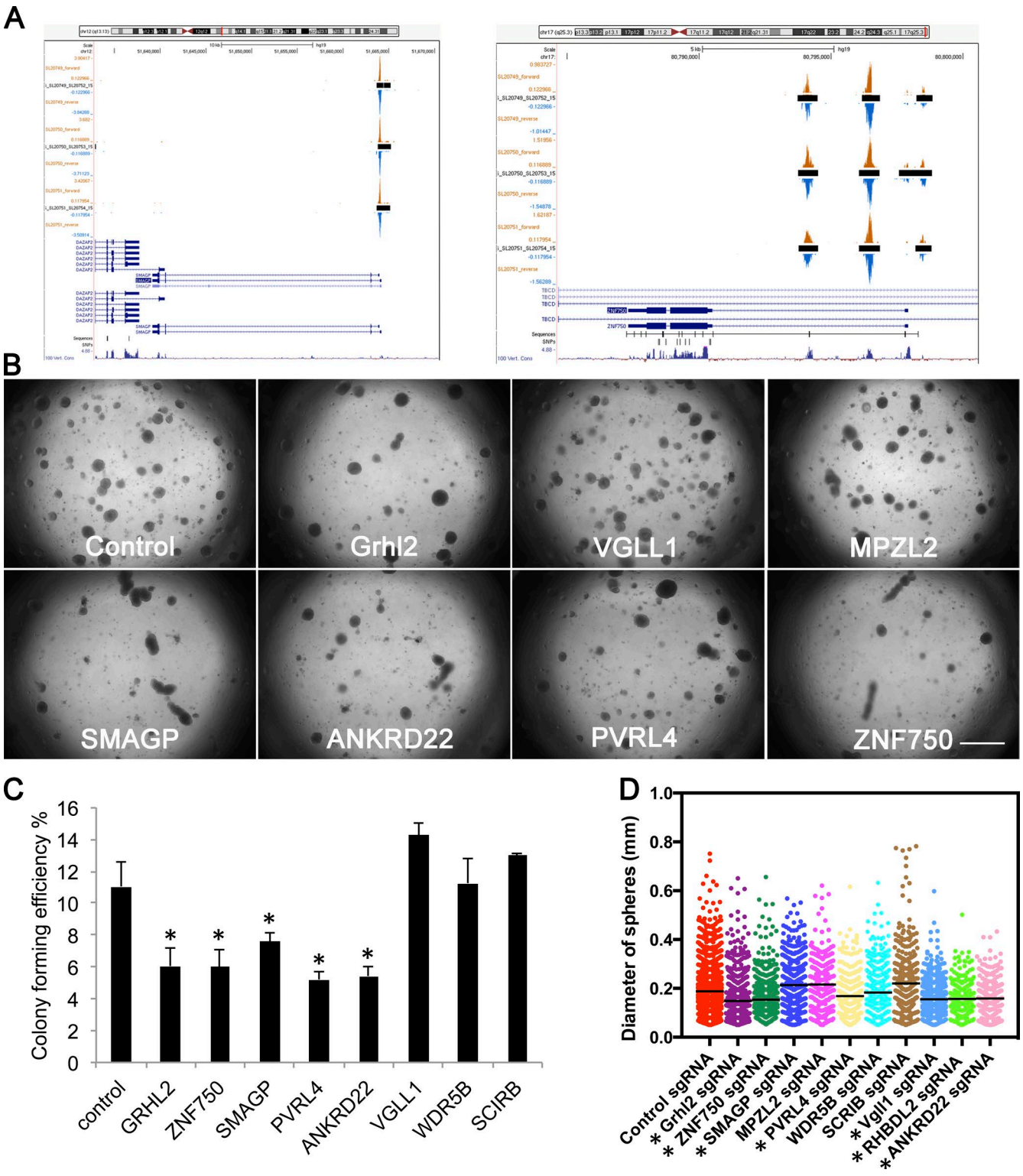

Figure S4. **Functional screening of potential Grhl2 target genes in HBE cells.** (A) Location of Grhl2-binding sites by ChIP-Seq in *SMAG* and *ZNF750* genes in primary HBE cells from three donors (Gao et al., 2013; database located at http://genome.ucsc.edu/cgi-bin/hgTracks?db=hg19&position =chr7%3A73236989-73255218&hgsid=442126887_T1bUzLD4IUdWAvl34kwvYM0T85zA). The sgRNAs for ZNF750 are located in exon 2. This is also in the intron of the *TBCD* gene on the opposite strand. Control qRT-PCR experiments showed no change in the expression of this gene in sgGRLH2 mutant cells. (B) Representative DIC microscopy images of spheres formed in Matrigel after 21 d from BCs transfected with lentiCRISP-sgRNA virus. (C and D) The quantification of CFE (C) and the diameters of control and mutant spheres (D) is described in Materials and methods (section HBE cell culture). Spheres were analyzed in triplicate wells from two to three different donors. *, $P < 0.05$. Bar, 2 mm. Horizontal bars represent mean. Data are reported as mean ± SEM.

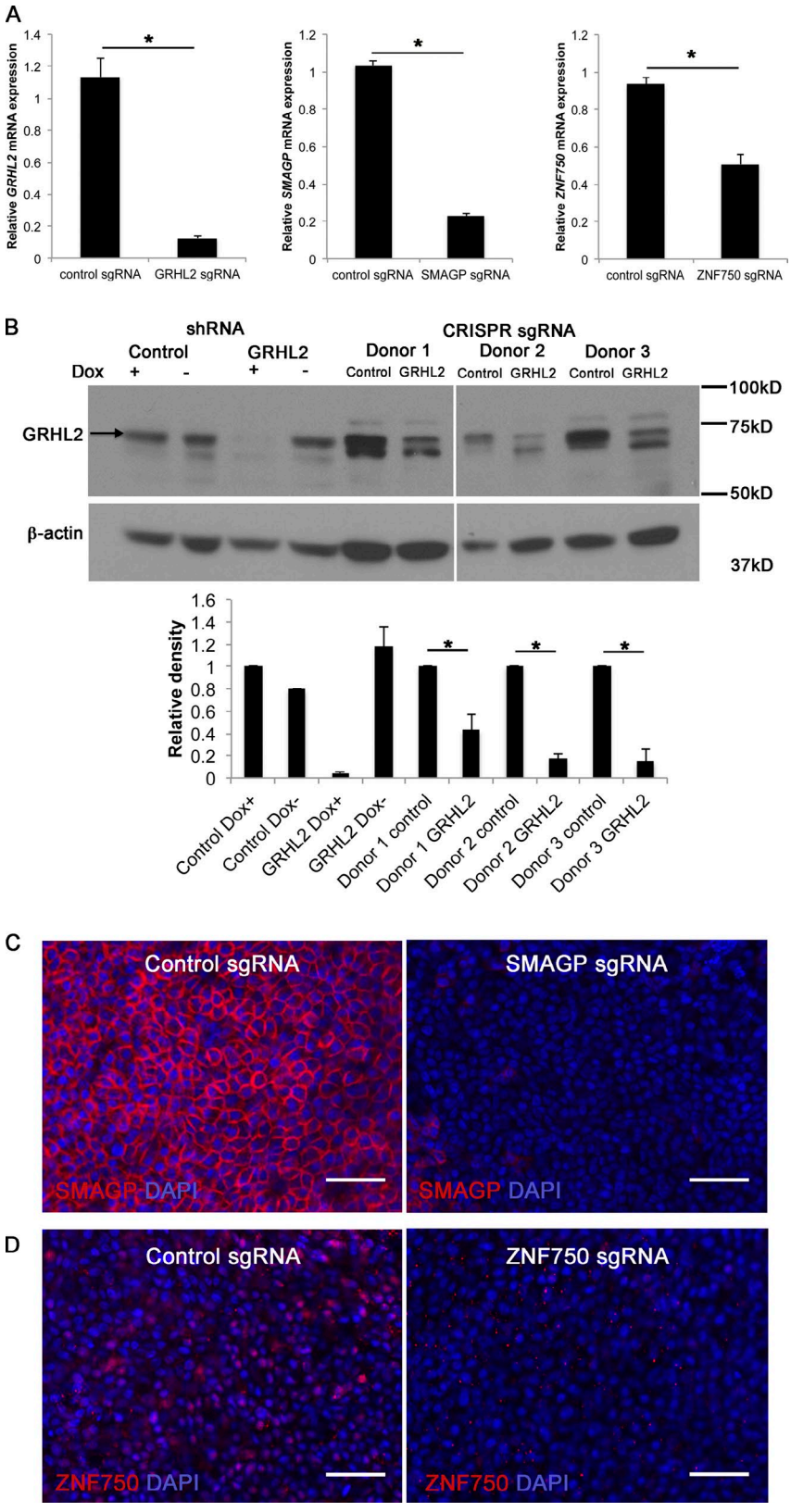

Figure S5. **Evidence that CRISPR/Cas9-induced mutations reduce gene expression levels in HBE cells.** (A) qRT-PCR analysis of *GRHL2*, *ZNF750*, and *SMAGP* RNA in control and mutant cells cultured in ALI for 21 d. Data are averaged from triplicate wells. Data are SEM of triplicates from triplicate samples. *, $P < 0.05$. (B, top) Western blot of extracts of HBE cells from three different donors infected with lentiCRISPR-control and GRHL2 sgRNA virus. HBE cells infected with a Dox-inducible GRHL2 shRNA virus were used as a positive control. ACTB is β-actin loading control. (Bottom) The quantification of Western blot (band marked by an arrow). Values are from duplicate blots. *, $P < 0.05$. On average, Grhl2 protein was reduced 75%. Data are SEM of duplicate experiments. (C) Whole-mount IHC of control and SMAGP mutant cultures at ALI culture 21 d for SMAGP and DAPI. (D) Whole-mount IHC of control and ZNF750 mutant cultures at ALI culture 21 d for ZNF750 and DAPI. Bars, 50 µm.

Table S1. **The sequences of sgRNA in lentiCRISPR vectors**

Gene names	sgRNA sequences (5′ to 3′)
Random control	ACGGAGGCTAAGCGTCGCAA
GRHL2	ATCAAACATGTCACAAGAGT
GRHL2 sgRNA2	TCCAGGTTCCTCGAGACAAG
SMAGP	CCCAATTTTACAGCCCACTG
SMAGP sgRNA2	GCCAGCACAGCACTCATTGC
ZNF750	TCTAACCTGCCGATTCCTTA
ZNF750 sgRNA2	GAACTCGACGTGTTTTCTGT
ANKRD22	AGCTATGCCAACGTTCAAGA
MPZL2	GGTTTAAGGACCGGGTGTCT
SCRIB	CTGCTGAACTTGCGCAAGCT
WDR5B	GCCATCCAACCTTATAATCT
VGLL1	GACGAGTAACGCCACTGATT
PVRL4	TCCACGCGGCCCTCGTAAGC
RHBDL2	CCGTGTGGGGCTGGTGTACC

Table S2. **Primers used for qRT-PCR**

Genes	Forward primer sequence (5′ to 3′)	Reverse primer sequence (5′ to 3′)
GAPDH	AGGTCGGTGTGAACGGATTTG	TGTAGACCATGTAGTTGAGGTCA
Grhl1	GCTGAGACACTGGAAGTACTG	CGTGAAGGAAATGGCGTTATAAG
Grhl2	AAGGAGAGCTTCAACACCATC	TGTACTCAGGCAATTCACGG
Grhl3	CTCTGCTCTTCCCTGATATTCTG	TGACTCCCCTGCTTTGATG
Trp63	CCCACAGACTGCAGCATTG	GAGATGAGGAGGTGAGGAGAAG
Foxj1	CATCTACAAGTGGATCACGGAC	GAGCAGGCGCTCTGCGTACTG
Scgb3a2	CCAAAGTCCCGGAAAACATC	AGGGCAGTGGCAGAATAACC
Mcidas	CCAGCTCTCACAACCATAGAC	GCATCTCTGAAATTCTGCAGG
Rfx2	TCTCTGTGTAAATGTGAGCCG	TCTTGGCGAAGTTCCTGATG
Myb	CAAGGGAAGAGGATGAGAAGC	ATGAGTTCAGGGTTCAGCAC
Ccnd1	GCCCTCCGTATCTTACTTCAAG	GCGGTCCAGGTAGTTCATG
Notch1	TCAGGGTGTCTTCCAGATCC	CAGCATCCACATTGTTCACC
Notch2	ATGTGGACGAGTGTCTGTTGC	GGAAGCATAGGCACAGTCATC
Notch3	TGCCAGGGAATTTCAGGTG	AGGCAAGAACAGGAAAAGGAG
Dll1	CAGGACCTTCTTTCGCGTATG	AAGGGGAATCGGATGGGGTT
Dll3	CTGGTGTCTTCGAGCTACAAAT	TGCTCCGTATAGACCGGGAC
Dll4	TTCCAGGCAACCTTCTCCGA	ACTGCCGCTATTCTTGTCCC
Jag1	CCTCGGGTCAGTTTGAGCTG	CCTTGAGGCACACTTTGAAGTA
Jag2	CAATGACACCACTCCAGATGAG	GGCCAAAGAAGTCGTTGCG
Hay1	TACCCAGTGCCTTTGAGAAG	TCCGATAGTCCATAGCCAGG
Hay2	AGCGAGAACAATTACCCTGG	TTTATTCGATCCCGACGCC
HayL	CGCAGAGGGATCATAGAGAAAC	CAAGACCTCAGCTTTCTCCAG
Hes1	GGCGAAGGGCAAGAATAAATG	GTGCTTCACAGTCATTTCCAG
Homo *TBCD*	GATCAGACATCTCCAGCTTCC	ATCTAAAACAGGCTCTACATCGG
Homo *GRHL2*	ATCAAACATGTCACAAGAGTCGG	CAAGTATGACTTCCAGGCTTC
Homo *ZNF750*	TCTAACCTGCCGATTCCTTACGG	TGGATAGACCAGGGTGGCTTCT
Homo *SMAGP*	CCCAATTTTACAGCCCACTG	TGCCTTTGTTCTTGTACAGGT
Homo *FoxJ1*	ACGGACAACTTCTGCTACTTC	TTGTTCAGAGACAGGTTGTGG
Homo *Rfx2*	CCAGTTCCACTACATCGAGAAG	CTGACACAGGGAGATAAGCTTG

Table S3. **Primers used for amplifying the mutation regions of genomic DNA**

Genes	Forward primer sequence (5′ to 3′)	Reverse primer sequence (5′ to 3′)
GRHL2 sgRNA	CCGGGATATCGTCGACATCCCACCTTTCCGGCTAGGTG	TACTCTAGAGCGGCCGCTTCTCCCCTTAATCAGGGTAGA
GRHL2 sgRNA2	CCGGGATATCGTCGACATGAGAGGTGCTCGGGTCCA	TACTCTAGAGCGGCCGCCTTCTTGACCCCATCTCCTC
SMAGP sgRNA and sgRNA2	CCGGGATATCGTCGACTTGTCTTTGGGTGGTGGTTTA	TACTCTAGAGCGGCCGCCATTTACTGGTAGTGCCTCTG
ZNF750 sgRNA	CCGGGATATCGTCGACCACTTTTACACAGAGCACGGG	TACTCTAGAGCGGCCGCCCAGCCTTGGAGGAGTCTTTA
ZNF750 sgRNA2	CCGGGATATCGTCGACGGAACCCAAGACCCGAGACA	TACTCTAGAGCGGCCGCGCCTTGTTGGAGAGGTCGTA
MZPL2	CCGGGATATCGTCGACGAAAGCAAGTGACAGAGCTGC	TACTCTAGAGCGGCCGCGCCTTAACAAAGATTGCCCCT
SCIRB	CCGGGATATCGTCGACGGCGTGTGTACCGTGTGTTTC	TACTCTAGAGCGGCCGCGAACTTGATGCTCTCCGGGAT
WDR5B	CCGGGATATCGTCGACATTTGGGGAGCATATGATGGA	TACTCTAGAGCGGCCGCAGGAGGGTTATCGTCATCAAC
VGLL1	CCGGGATATCGTCGACCTAGAAACAGTACACCTACGG	TACTCTAGAGCGGCCGCGAAGGGATGAGAGTAGCCAGG
PVRL4	CCGGGATATCGTCGACCAGACGTGGTAACTGTGGTGC	TACTCTAGAGCGGCCGCCTACCTCTGCCCCATCCATCT
RHBDL2	CCGGGATATCGTCGACAAAGTCTGTTCATCTCCCA	TACTCTAGAGCGGCCGCGAGCGCCAGGCATACTTGCAA
ANKRD22	CCGGGATATCGTCGACTTTCCCGACCCAGCCCATCTG	TACTCTAGAGCGGCCGCTGTCCTTGGTATGTGTCAGTC

Reference

Gao, X., C.M. Vockley, F. Pauli, K.M. Newberry, Y. Xue, S.H. Randell, T.E. Reddy, and B.L. Hogan. 2013. Evidence for multiple roles for grainyhead-like 2 in the establishment and maintenance of human mucociliary airway epithelium. *Proc. Natl. Acad. Sci. USA.* 110:9356–9361. http://dx.doi.org/10.1073/pnas.1307589110