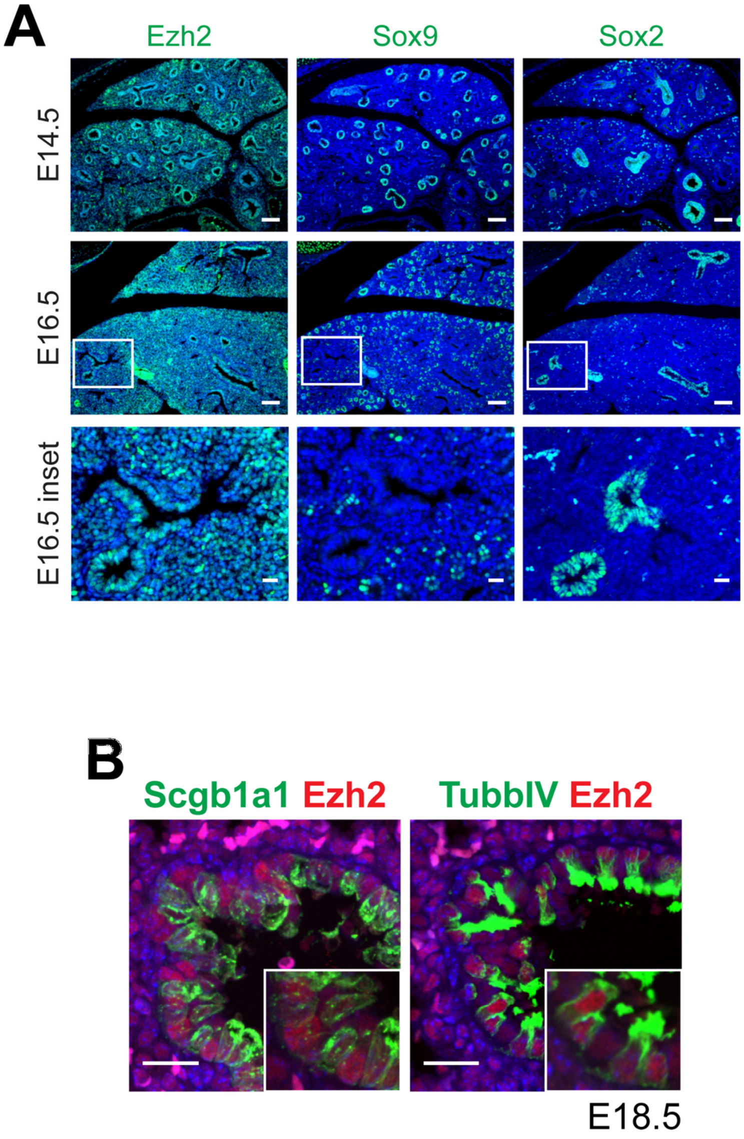
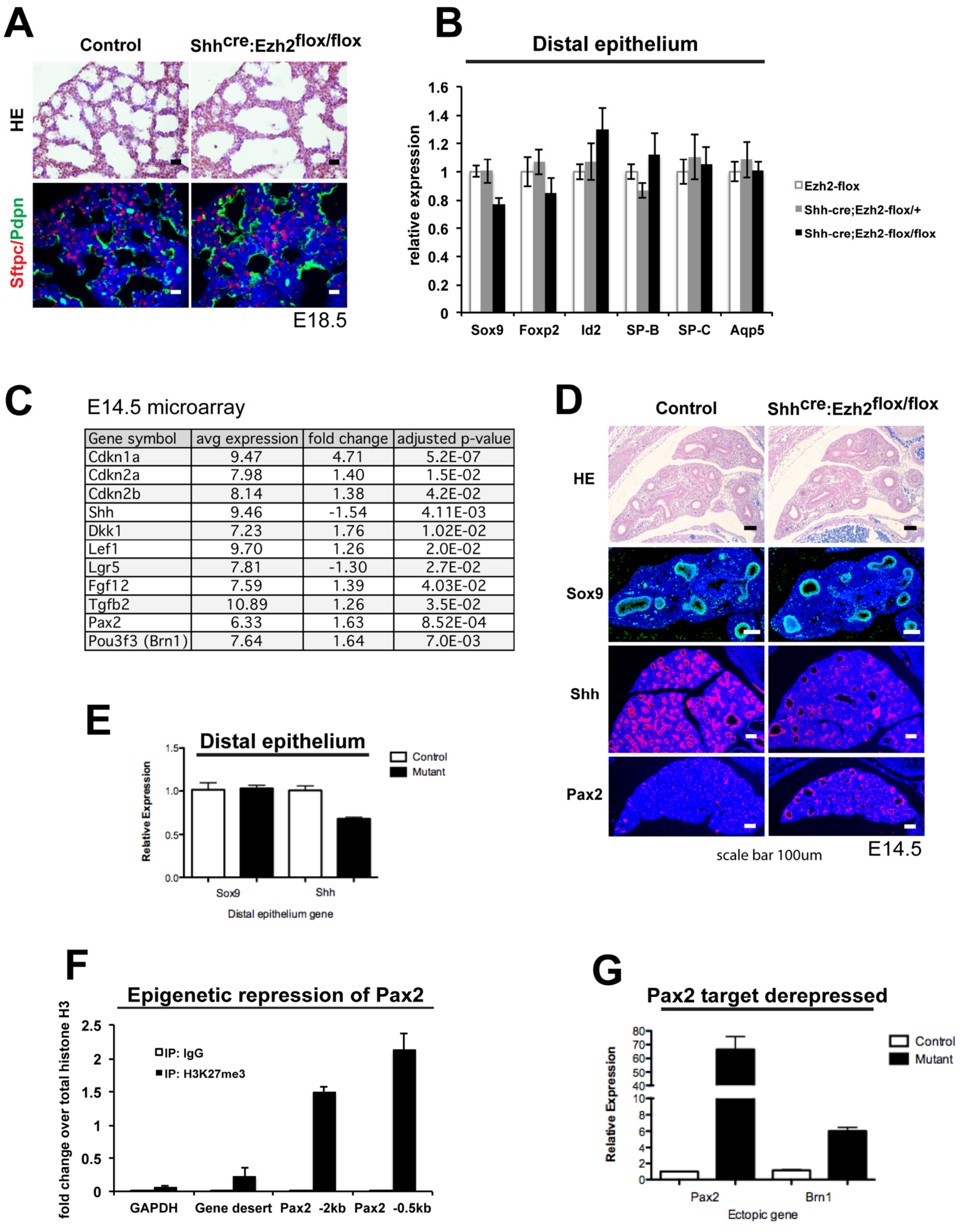


Supplemental Figure 1. Expression of Ezh2 becomes restricted during late lung development. (A) IHC for Ezh2, proximal airway epithelium marker Sox2, and distal epithelium marker Sox9 at E14.5 and E16.5. Note that Ezh2 appears in both Sox2 and Sox9-positive epithelium at these stages. (B) IHC for Ezh2 and mature proximal epithelium markers Scgb1a1 and TubbIV. Note that Ezh2 is more highly expressed in TubbIV-positive multi-ciliated cells than in Scgb1a1-positive club cells. Scale bar: E14.5 and E16.5=100μm; E16.5 inset and E18.5=20μm.

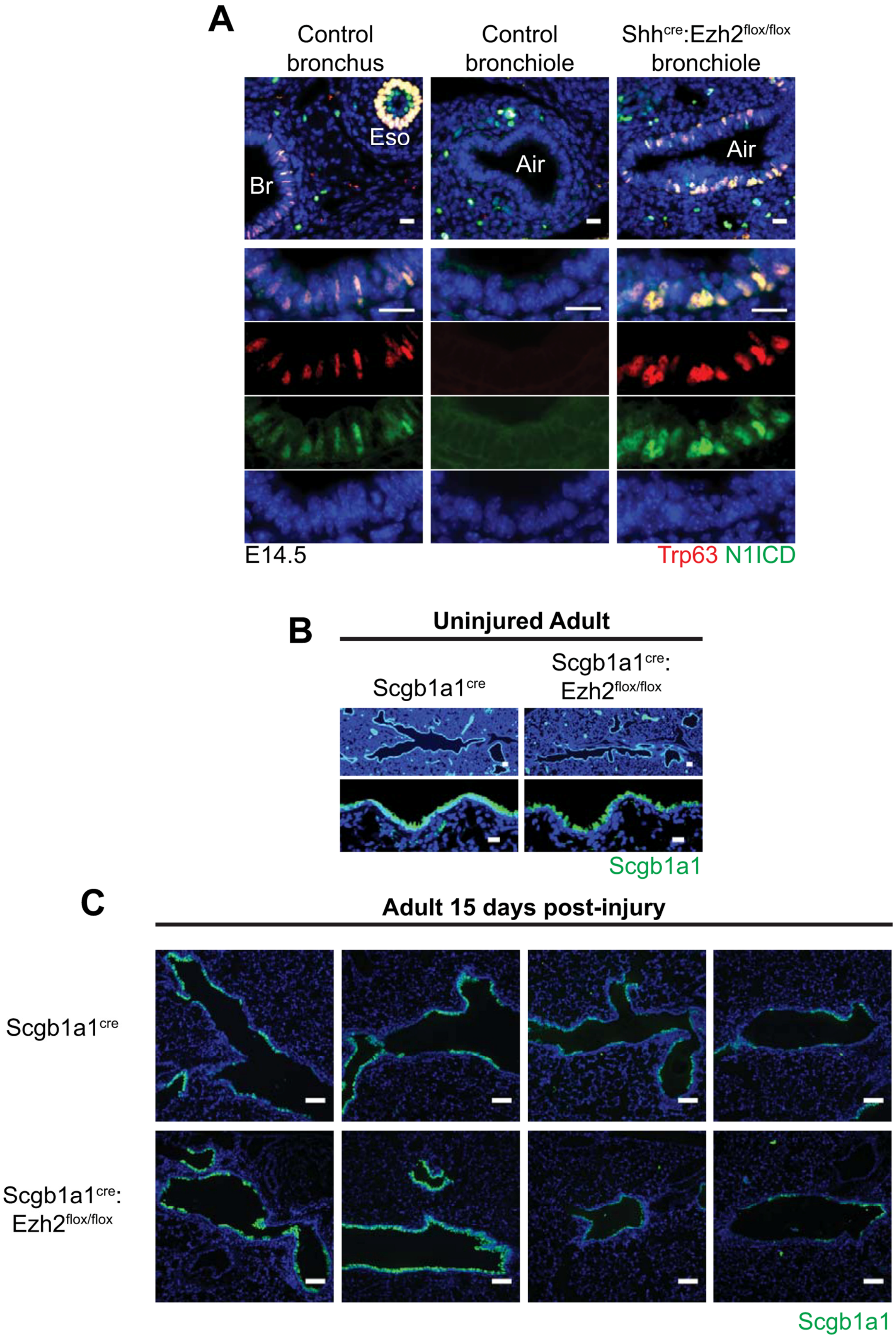


E14.5 microarray

Gene symbol	avg expression	fold change	adjusted p-value
Cdkn1a	9.47	4.71	5.2E-07
Cdkn2a	7.98	1.40	1.5E-02
Cdkn2b	8.14	1.38	4.2E-02
Shh	9.46	-1.54	4.11E-03
Dkk1	7.23	1.76	1.02E-02
Lef1	9.70	1.26	2.0E-02
Lgr5	7.81	-1.30	2.7E-02
Fgf12	7.59	1.39	4.03E-02
Tgfb2	10.89	1.26	3.5E-02
Pax2	6.33	1.63	8.52E-04
Pou3f3 (Brn1)	7.64	1.64	7.0E-03

Supplemental Figure 2. Alveolar defects include de-repression of Ezh2 target genes and altered expression of signaling pathways. (A) H+E and IHC for Sftpc and Pdpn on E18.5 control and *Shh*^{cre}*:Ezh2*$^{flox/flox}$ mutant lungs.

(B) qPCR for the indicated distal epithelial marker genes at E18.5. (C) Subset of gene expression changes that could be related to defects in distal epithelial differentiation in $Shh^{cre}:Ezh2^{flox/flox}$ mutant lungs. (D) H+E, IHC for Sox9, and ISH for Shh and Pax2 on control and $Shh^{cre}:Ezh2^{flox/flox}$ mutant lungs at E14.5. (E) Q-PCR for Sox9 and Shh at E14.5. (F) ChIP-qPCR for H3K27me3 marks on the *Pax2* promoter and an unrelated gene desert region as well as the *Gapdh* promoter at E12.5. (G) qPCR for Pax2 and Brn2 at E14.5. Scale bar: E18.5=20μm; E14.5=100μm.


A
Control bronchus
Control bronchiole
Shh^{cre}:Ezh2$^{flox/flox}$ bronchiole
Eso
Br
Air
Air
E14.5
Trp63 N1ICD
B
Uninjured Adult
Scgb1a1^{cre}
Scgb1a1^{cre}: Ezh2$^{flox/flox}$
Scgb1a1
C
Adult 15 days post-injury
Scgb1a1^{cre}
Scgb1a1^{cre}: Ezh2$^{flox/flox}$
Scgb1a1

Supplemental Figure 3. Ezh2 loss inhibits club cell development, but not postnatal homeostasis or regeneration. (A) IHC for Trp63 and NICD shows co-expression in the control bronchus and the *Shh*^{cre}*:Ezh2*$^{flox/flox}$ mutant bronchioles, but not the control bronchiole. (B) *Scgb1a1*^{cre}*:Ezh2*$^{flox/flox}$ adult mutant lungs contain normal numbers of Scgb1a1-positive secretory cells. (C) Club cell regeneration after naphthalene-induced injury is unaffected in *Scgb1a1*^{cre}*:Ezh2*$^{flox/flox}$ lungs as compared to control lungs. Scale bar: (A)=20μm; (B upper row)=100μm; (B lower row)=20μm; (C)=100μm.

Table S1. Microarray data for *Ezh2* knockout

Click here to Download Table S1

Table S2. PCR primers

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