Supporting Information

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SI Methods

The following antibodies were used for immunostaining: Cre (1:30,000, no. 69050–3, Novagen); KI-67 (1:500, clone Tec-3, Dako); pro-SP-C (1:1,500, AB-3428, Chemicon International); SP-B (1:1,500, generated in lab of J. A. Whitsett); T1-α (1:1,000, 8.1.1., University of Iowa Hybridoma bank); TTF-1 (1:3,000, gift of R. DiLauro, Stazione Zoologica, Naples, Italy); Foxj1 (1:5,000, generated in lab of J. A. Whitsett); CCSP (1:1,000, a gift from B. Stripp, Duke University); and Pecam-1 (1:50, clone MEC 13.3, PharMingen), activated caspase 3 (1:200, 5A1, Cell Signaling), and Foxa2 (1:16,000, Seven Hills Bioreagents). Antibody–antigen complexes were detected using biotinylated secondary antibody followed by avidin–horseradish peroxidase (HRP) complex and DAB substrate (all from Vector Labs). Sections were counterstained with nuclear fast red (Vector Labs).

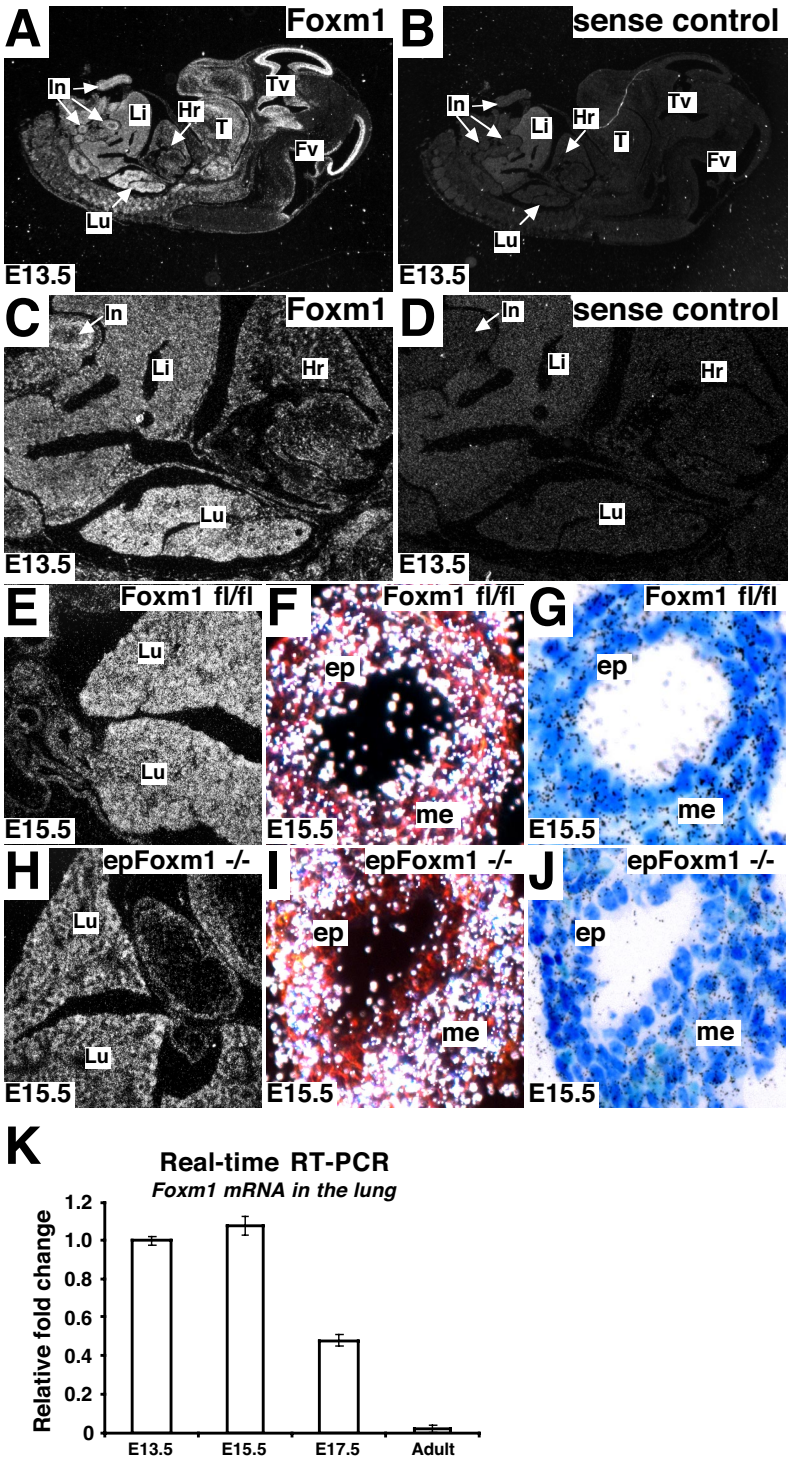

Fig. S1. Foxm1 expression during mouse embryonic development. (*A–J*) Foxm1 is expressed in mesenchyme and epithelium of the lung and other mouse organs. Paraffin E13.5 (*A–D*) and E15.5 (*E–J*) sections were used for *in situ* hybridization with ^{35}S-labeled antisense riboprobe specific to mouse Foxm1. Sense probe was used as a control (*B* and *D*). E15.5 lung sections were counterstained with toluidine blue [blue staining in bright-field images (*G* and *J*) and red fluorescence in dark-field images (*F* and *I*)]. Foxm1 mRNA (white dots) was present in both epithelium (ep) and mesenchyme (me) of control *Foxm1*$^{fl/fl}$ lungs (*F* and *G*). Foxm1 expression was selectively decreased in *epFoxm1*$^{-/-}$ lung epithelium (*I* and *J*). (Magnification: *A* and *B*, ×25; *C*, *D*, *E*, and *H*, ×100; *F*, *G*, *I*, and *J*, ×600.) (*K*) Quantitative real-time RT–PCR of total lung RNA from E13.5, E15.5, and E17.5 embryos and adult mice. WT mice were used for qRT–PCR. Foxm1 mRNA was analyzed in triplicates and expression levels were normalized to β-actin. Lu, lung; Hr, heart; In, intestine; Li, liver, T, tongue; Tv, third ventricle; Fv, fourth ventricle.

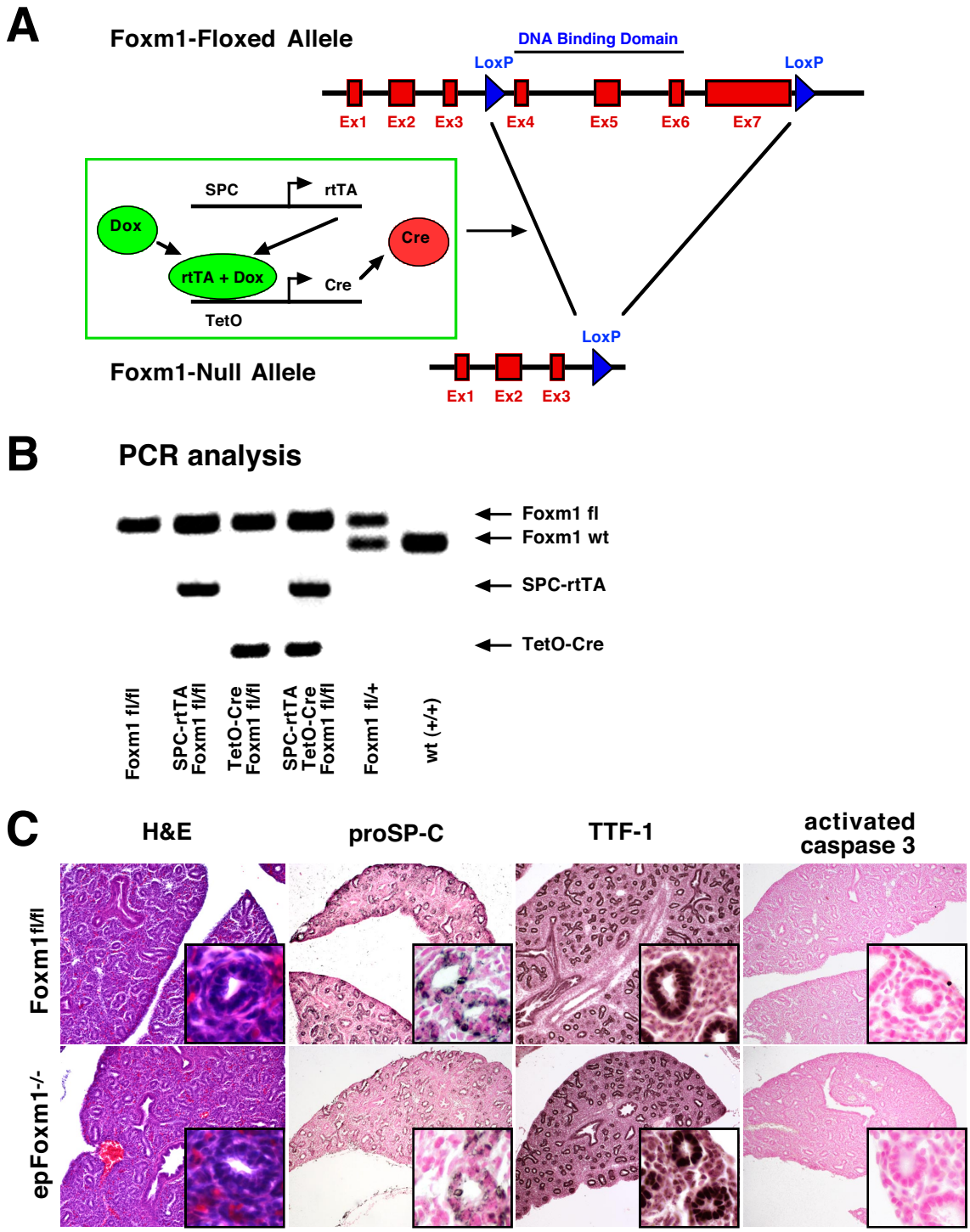

Fig. S2. Deletion of Foxm1 in the respiratory epithelium. (*A*) Schematic drawing of Cre-mediated deletion in the Foxm1-floxed gene. To delete Foxm1 in respiratory epithelium, *Foxm1*$^{fl/fl}$ mice were bred with *SP-C–rtTA*$^{tg/-}$/*TetO-Cre*$^{tg/-}$ mice to generate *SP-C–rtTA*$^{tg/-}$/*TetO-Cre*$^{tg/-}$*Foxm1*$^{fl/fl}$ triple transgenic mice. Doxycycline (Dox) was given to the dam in water from E7.5 to E14.5, resulting in deletion of exons 4–7 of the mouse Foxm1 gene (*epFoxm1*$^{-/-}$ mice). (*B*) PCR analysis of genomic DNA identified the Foxm1-floxed allele as well as *SP-C-rtTA* and *TetO-Cre* transgenes. (*C*) Lungs from Dox-treated (E7.5–E14.5) *epFoxm1*$^{-/-}$ and control *Foxm1*$^{fl/fl}$ E15.5 embryos were fixed, paraffin embedded, sectioned, and then stained with either hematoxylin and eosin (H&E) or antibody against Cre, proSP-C, TTF-1, or activated caspase 3. Lung sections were counterstained with nuclear fast red. (Magnifications, ×50 and ×400.)

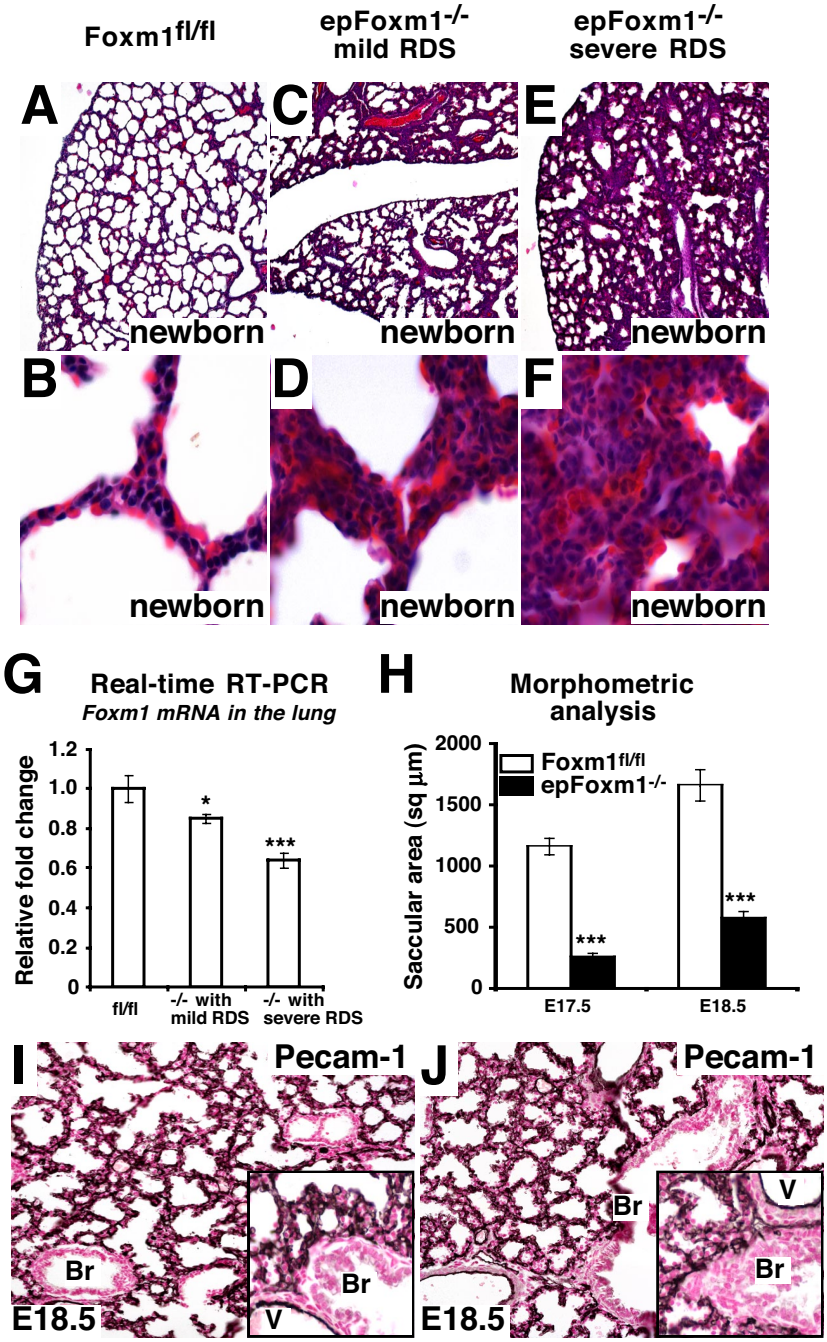

Fig. S3. Foxm1 is critical for perinatal survival. (*A–F*) Pulmonary congestion and RDS in a subset of *epFoxm1*$^{-/-}$ newborn mice. Embryonic lungs from Dox-treated (E7.5–E14.5) *epFoxm1*$^{-/-}$ and control *Foxm1*$^{fl/fl}$ newborn mice were fixed, paraffin embedded, sectioned, and stained with hematoxylin and eosin (H&E). Pulmonary congestion and lung atelectasis were observed in *epFoxm1*$^{-/-}$ mice with severe RDS at birth (*E* and *F*). Surviving *epFoxm1*$^{-/-}$ mice displayed mild RDS with focal atelectasis and mesenchymal thickening (*C* and *D*). Normal lung sacculation was observed in control *Foxm1*$^{fl/fl}$ newborn mice (*A* and *B*). (*G*) Quantitative real-time RT–PCR of total lung RNA from newborn mice. Foxm1 mRNA was normalized to β-actin. Newborn *epFoxm1*$^{-/-}$ mice with severe RDS displayed a significantly decreased Foxm1 mRNA compared to either *Foxm1*$^{fl/fl}$ mice or *epFoxm1*$^{-/-}$ mice with mild RDS. $*P < 0.05$, $***P < 0.001$. (*H*) Morphometric analysis of E17.5 and E18.5 lungs. Area of a single pulmonary saccule (mean ± SD) was measured in 10 random ×400 microscope fields of Dox-treated *epFoxm1*$^{-/-}$ and control *Foxm1*$^{fl/fl}$ lungs (five embryos in each group). (*I* and *J*) E18.5 lung sections were stained with endothelial-specific Pecam-1 antibodies. Similar Pecam-1 staining was detected in *epFoxm1*$^{-/-}$ and control *Foxm1*$^{fl/fl}$ lungs. (Magnifications: *A*, *C*, and *E*, ×50; *B*, *D*, and *F*, ×400; *I* and *J*, ×200.)

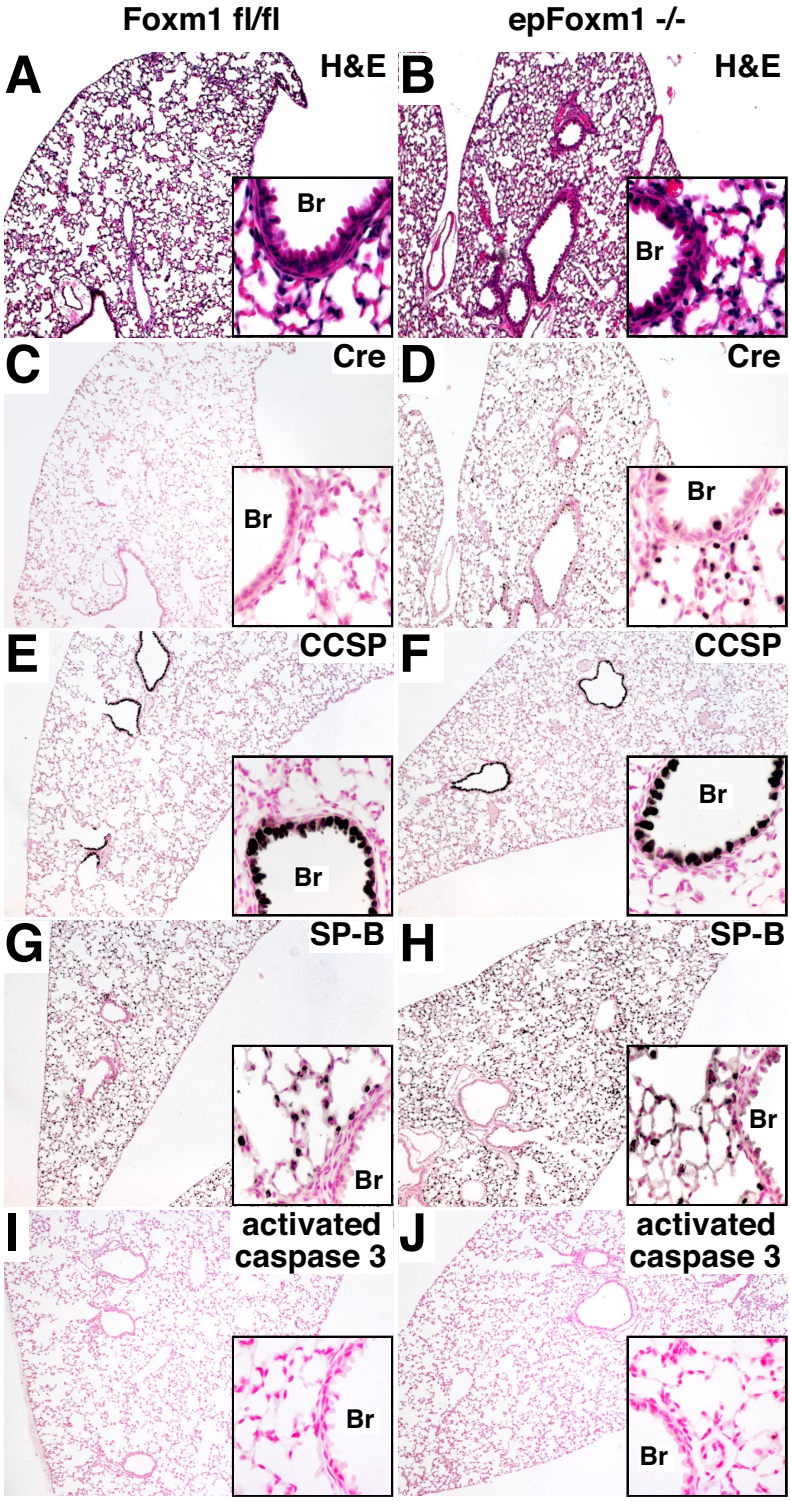

Fig. S4. Postnatal Foxm1 deletion did not influence overall lung morphology or expression of epithelial marker proteins. *epFoxm1*$^{-/-}$ and control *Foxm1*$^{fl/fl}$ mice were treated with Dox from postnatal day 3 (P03) to P30. Lungs were fixed, paraffin embedded, sectioned, and then stained with either hematoxylin and eosin (H&E, *A* and *B*) or antibody against Cre (*C* and *D*), CCSP (*E* and *F*), SP-B (*G* and *H*), or activated caspase 3 (*I* and *J*). Lung sections were counterstained with nuclear fast red. Br, bronchioles. (Magnifications, ×50 and ×400.)

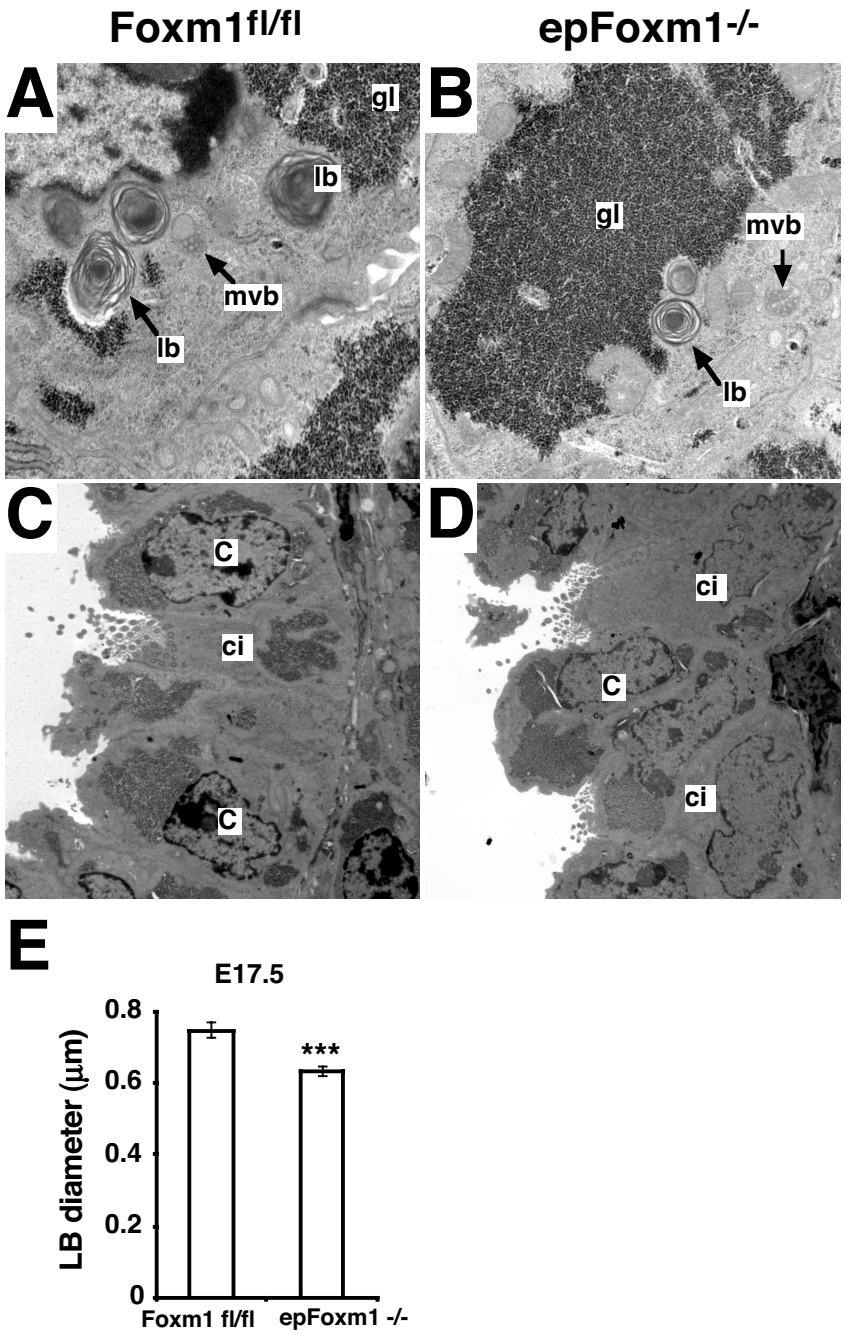

Fig. S5. Decreased size of lamellar bodies in *epFoxm1*$^{-/-}$ type II epithelial cells. (*A–D*) Transmission electron microscopy (TEM) of *epFoxm1*$^{-/-}$ E17.5 lungs. Lamellar bodies (lb), multivesicular bodies (mvb), and glycogen (gl) were observed in *epFoxm1*$^{-/-}$ and *Foxm1*$^{fl/fl}$ type II epithelial cells at E17.5 (*A* and *B*). Similar ultrastructure was observed in ciliated (ci) and Clara cells (C) of *epFoxm1*$^{-/-}$ and *Foxm1*$^{fl/fl}$ lungs (*C* and *D*). (Magnifications: *A* and *B*, ×30,000; *C* and *D*, ×15,000.) (*E*) Decreased diameter of lamellar bodies in *epFoxm1*$^{-/-}$ E17.5 lungs. Diameters of 100 lamellar bodies and composite bodies (immature lamellar bodies) were measured in ×30,000 TEM images and presented as mean ± SD. ***$P < 0.05$.

Table S1. Breeding data for *epFoxm1*$^{-/-}$ mice

Developmental period	Total no. of mice	Expected % of *epFoxm1*$^{-/-}$ mice	Experimental % (no.) of *epFoxm1*$^{-/-}$ mice	% mortality in *epFoxm1*$^{-/-}$ mice
E17.5–E18.5	91	25	24% (22) (all alive)	0
Postnatal day 1	102	25	4% alive (4) 22% dead (22)	82

Breeding data from SP-C–rtTA$^{tg/-}$ Foxm1$^{fl/fl}$ × TetO-Cre$^{tg/-}$ Foxm1$^{fl/fl}$ mouse crosses are shown as a frequency of occurrence of SP-C–rtTA$^{tg/-}$/TetO-Cre$^{tg/-}$ Foxm1$^{fl/fl}$ mice (epFoxm1$^{-/-}$). Doxycycline was given to the dam in water from E7.5 to E14.5.

Table S2. TaqMan gene expression assays (Applied Biosystems) were used for qRT–PCR analysis

Mouse *TaqMan* gene expression assays	Catalog no.
Sftpa	Mm00499170_m1
Sftpb	Mm00455681_m1
Sftpc	Mm00488144_m1
Sftpd	Mm00486060_m1
β-actin	Mm00607939_s1
T1α	Mm00494716_m1
TTF-1	Mm00447558_m1
Aquaporin 5	Mm00437578_m1
Cdc25B	Mm00499136_m1
Cyclin B1	Mm00838401_g1
Foxa2	Mm00839404__mH

Table S3. PCR oligonucleotides for ChIP assays

Promoter region	Forward primer (5′-3′)	Reverse primer (5′-3′)
Sftpa (−330/−203)	GCTTTCTTTTCTGAATGGCAGTGC	TGTGGGAAGGGAATCTCTCTCTTG
Sftpb (−844/−660)	CATTTATCAGCGGAGACAGCAAG	AGAGCCATTTACTGCCTACTCGG
Sftpc (−682/−547)	CGCCAAAGCAAAGAGTGAAACG	TTTACCCCACCTTCTCCCACAC
Sftpd (−543/−406)	CTCACAGGTTGCTGCCTTTGTAG	GGTAGAATCCATTGCTTTGTCCAC