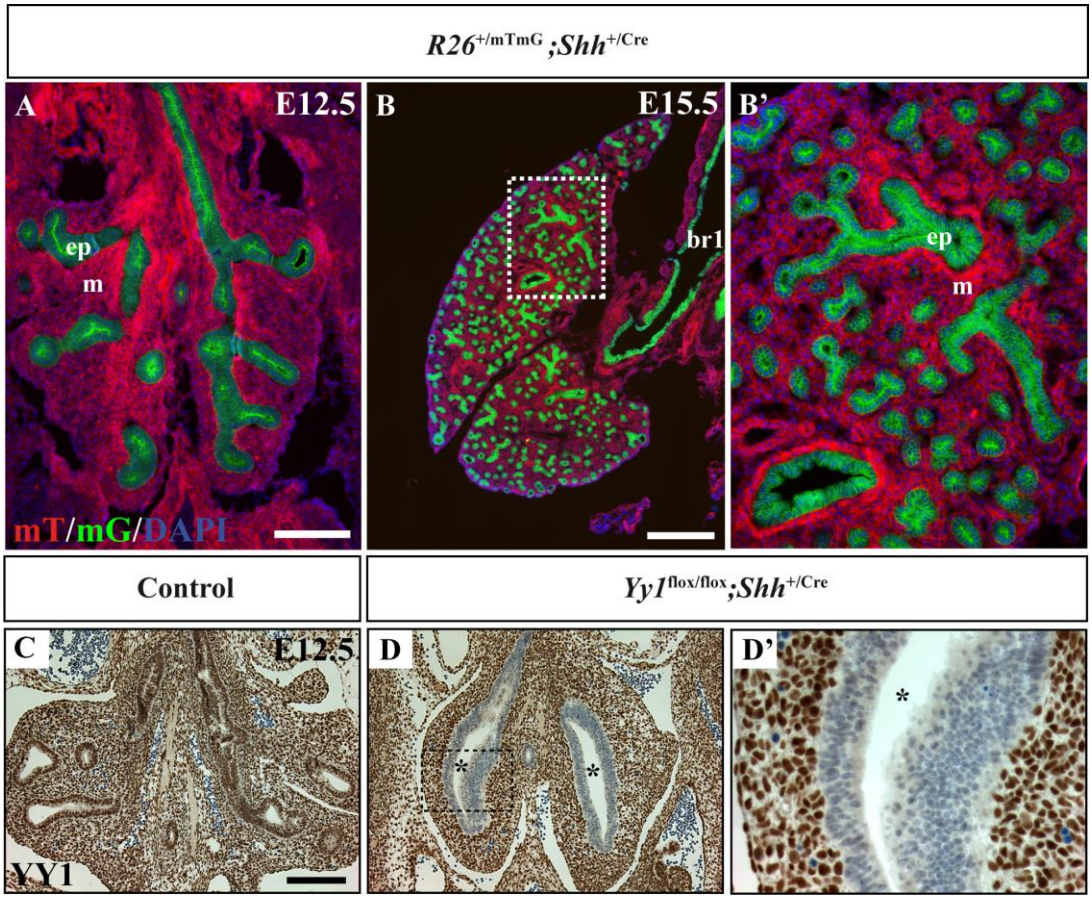

Figure S1. Cre recombinase specificity and efficiency of the *Yy1* deletion by the *Shh*^{Cre} allele in the developing lung. (A,B) Validation of Cre efficiency was assessed by breeding *Shh*$^{+/Cre}$ mice with *R26*$^{mTmG/mTmG}$ reporter mice expressing the membrane-targeted tandem dimer Tomato (mT) prior Cre-mediated excision and membrane-targeted green fluorescent protein (mG) after excision. At E12.5 (A) and E15.5 (B), GFP expression was detected in the respiratory epithelium. No Cre activity was detected in lung mesenchyme. (C,D) YY1 IHC experiments demonstrated the loss of YY1 expression in the respiratory epithelium of E12.5 *Yy1*$^{flox/flox}$;*Shh*$^{+/Cre}$ embryos. Boxed areas in B and D are magnified in B' and D', respectively. Br1, primary bronchi; ep, epithelium; m, mesenchyme. Asterisks indicate cysts. Scale bars: 200 μm (A,C,D), 300 μm (B).

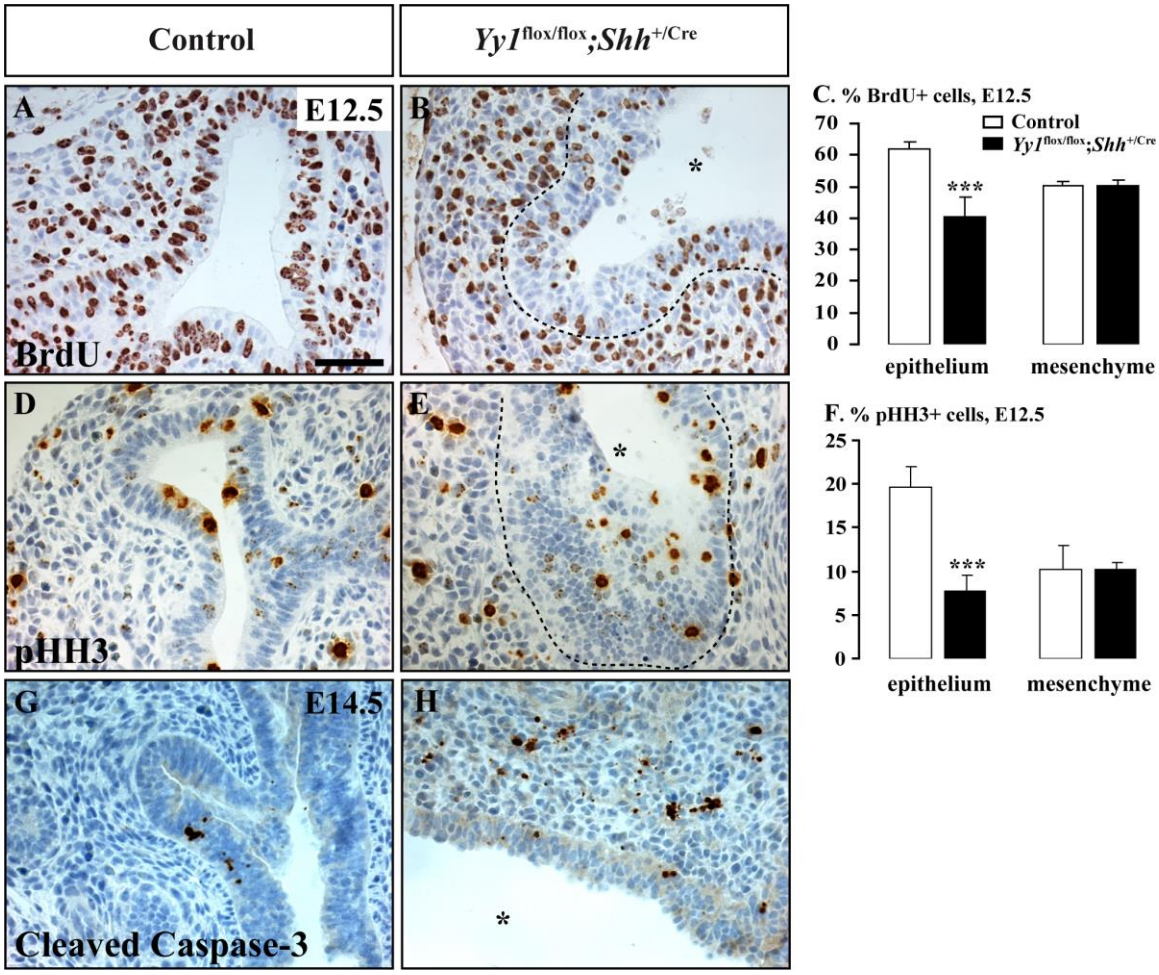

Figure S2. Reduced epithelial proliferation and increased apoptosis in lungs from *Yy1*$^{flox/flox}$;*Shh*$^{+/Cre}$ mutants. (A-C) The number of cells in S-phase, as determined by BrdU pulse-labeling assay, was significantly decreased in lung epithelium from *Yy1*$^{flox/flox}$;*Shh*$^{+/Cre}$ mutants while no change was seen in the mesenchyme. (D-F) Reduced proliferation of lung epithelial cells was also demonstrated by pHH3 immunostaining, a marker for cells in late G2 and mitosis. Thus, *Yy1* is essential for the control of lung epithelial cell proliferation throughout the cell cycle. (G,H) Cleaved caspase-3 immunostaining revealed increased apoptosis in lung mesenchyme of E14.5 *Yy1*$^{flox/flox}$;*Shh*$^{+/Cre}$ specimens. Asterisks indicate cysts. **P<0.01. Scale bar: 50 μm.

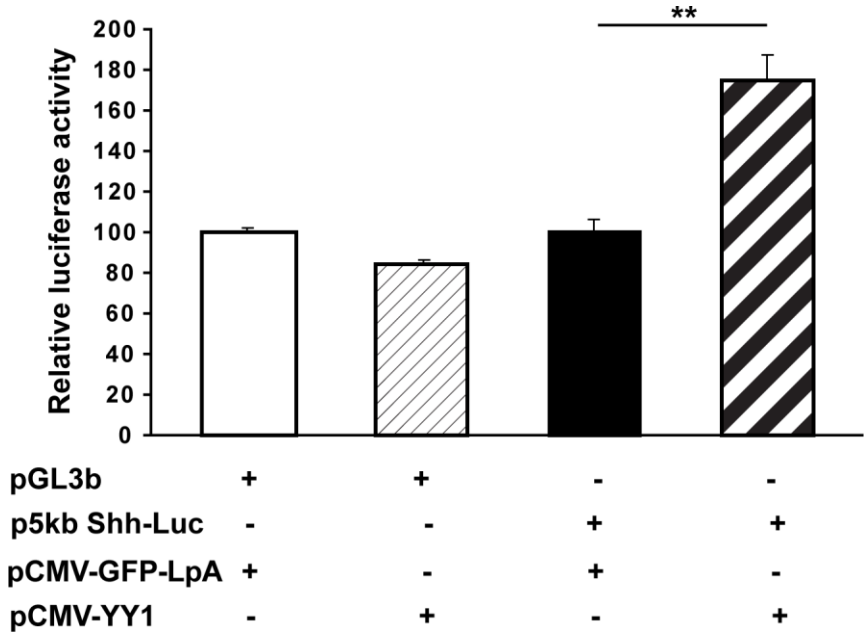

Figure S3. YY1 can activate *Shh* expression in transactivation assays. In a transient transfection assay in HEK293 cells, human YY1 protein upregulated transcription of a luciferase reporter construct containing a 5-kb genomic fragment encompassing *Shh* promoter and upstream sequences. Data from a representative experiment are presented as the fold induction±s.d. of normalized relative luciferase activity. ***P*<0.01

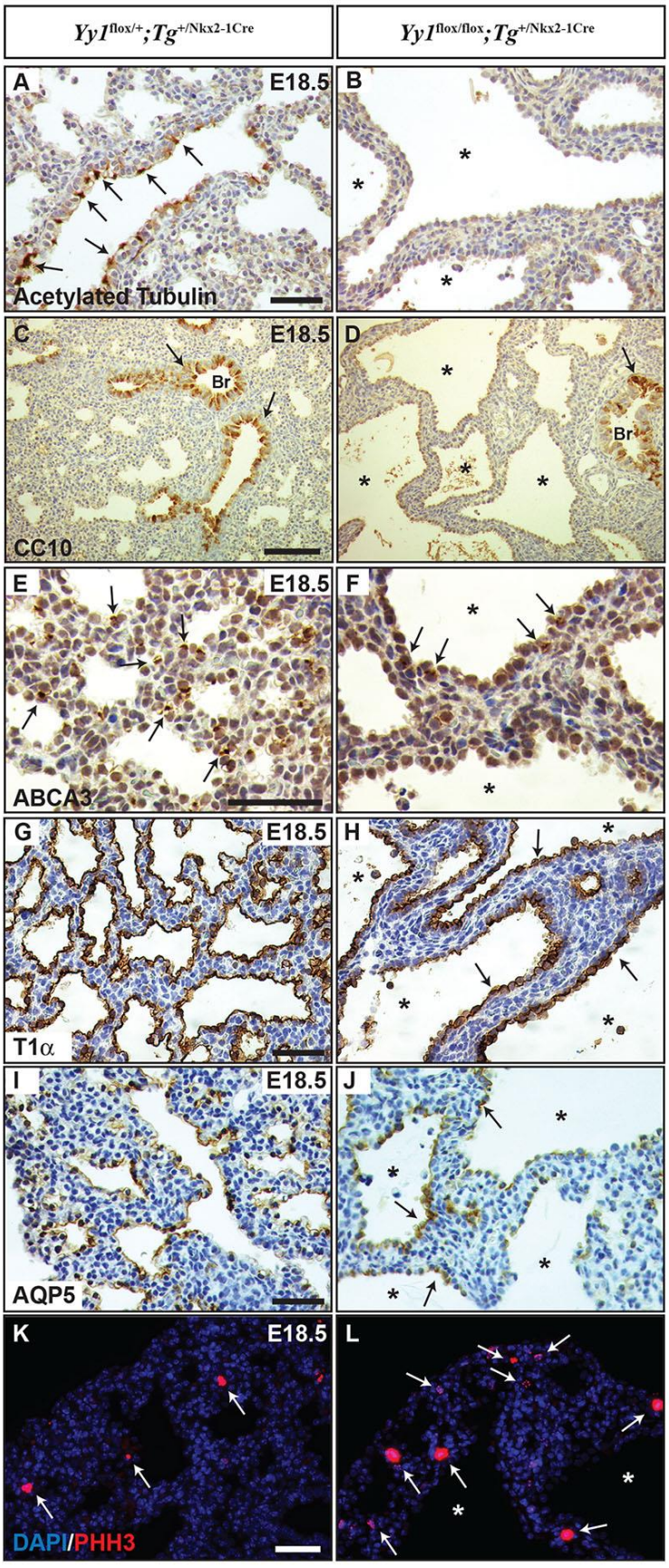
Yy1flox/+;Tg+/Nkx2-1Cre
Yy1flox/flox;Tg+/Nkx2-1Cre
A
E18.5
Acetylated Tubulin
B
C
E18.5
Br
CC10
D
Br
E
E18.5
ABCA3
F
G
E18.5
T1α
H
I
E18.5
AQP5
J
K
E18.5
DAPI/PHH3
L

Figure S4. Abnormal epithelial differentiation and increased cell proliferation in lungs from E18.5 *Yy1*$^{flox/flox}$;*Tg*$^{+/Nkx2\text{-}1Cre}$ embryos. (A-D) Neither ciliated nor club cells, as detected by IHC with acetylated tubulin and CC10 specific markers, respectively, were observed in cyst epithelium of E18.5 *Yy1*$^{flox/flox}$;*Tg*$^{+/Nkx2\text{-}1Cre}$ mutants. (E-J) Cysts were lined by Type II and Type I pneumocytes as revealed by ABCA3 and T1α/AQP5 specific markers, respectively (arrows). (K,L) pHH3-positive cells (arrows) revealed increased proliferation in lungs from E18.5 *Yy1*$^{flox/flox}$; *Tg*$^{+/ Nkx2\text{-}1Cre}$. Br, bronchi. Asterisks indicate cysts. Scale bars: 50 μm (A,B,E-L), 100 μm (C,D).

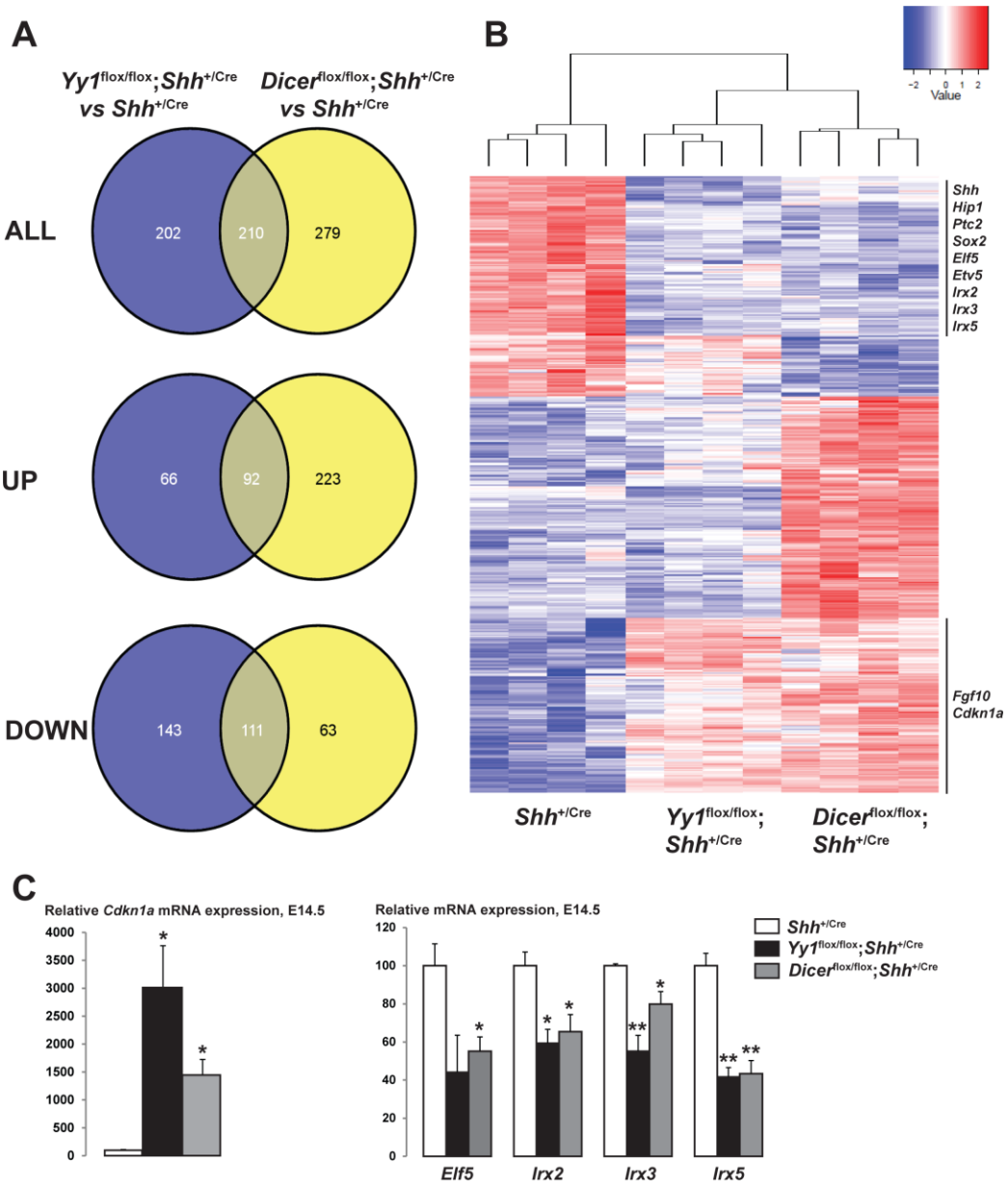

Figure S5. Differential gene expression in lungs from E14.5 *Yy1*$^{flox/flox}$;*Shh*$^{+/Cre}$ and *Dicer*$^{flox/flox}$;*Shh*$^{+/Cre}$ embryos. (A) Venn diagrams representing the overlap of genes differentially expressed (≥1.5-fold change; Benjamini-Hochberg-adjusted $P<0.05$) between genotypes. (B) Heat map analysis displaying deregulated genes (≥1.5-fold; $P<0.05$). The length of the arms of the dendogram reflects the degree of correlation

between the samples. Genes mentioned in the text are indicated on the right. (C) qRT-PCR analyses for *Cdkn1a*, *Elf5*, *Irx2*, *Irx3*, and *Irx5* were performed on biological replicates and confirmed the microarray data. Values are expressed as mean±s.e.m. *P<0.05, **P<0.01.

Table S1. General characteristics of cases

Nº Patient	Final Diagnosis	Age at tissue collection (months)	Localization
1	sudden infant death	4	n.a.
2	sudden infant death	7	n.a.
3	sudden infant death	12	n.a.
4	sudden infant death	10	n.a.
5	sudden infant death	7	n.a.
6	CCAM type I	18	Left lower lobe
7	CCAM type I	1.5	Left superior lobe
8	CCAM type I	11.5	Right lower lobe
9	CCAM type II	16	Left lower lobe
10	CCAM type I	18	Right basal pyramid
11	CCAM type I	22	Left lower lobe
12	CCAM type I	18	Right lower lobe
13	CCAM type I	21	Left lower lobe
14	CCAM type I	20	Left lower lobe
15	PPB type I DICER1 status: unknown	36	Left lower lobe
16	PPB type I DICER1 status: unknown	3	Left lung
17	PPB type I DICER1: c.3293G>A	1.5	Left lingula
18	PPB type II DICER1: c.2379T>G	24	Right lung
19	PPB type II DICER1: c.5392delA	36	Left lower lobe
20	PPB type III DICER1: c.2040+1G>T	36	Right lower lobe
21	PPB type III DICER1: c.4407_4410delTTCT	48	Right lower lobe

Table S2. List of primary and secondary antibodies used for IHC, IF and ChIP

Antigen	Antibody/clone	Reference	Source	Dilution
IHC and IF analyses				
ABCA3	rabbit polyclonal	WRAB-ABCA3	Seven Hills Bioreagents, Cincinnati, OH, USA	1:500
Acetylated tubulin	mouse monoclonal/ clone 6-11B-1	T6793	Sigma Aldrich, Oakville, ON, Canada	1:2000
Active-caspase3	rabbit polyclonal	9661	Cell Signaling, Danvers, MA, USA	1:200
Aquaporin5	rabbit polyclonal	AB78486	Abcam, Toronto, ON, Canada	1:500
αSMA	rabbit polyclonal	AB5694	Abcam, Toronto, ON, Canada	1:300
BrdU	mouse monoclonal/ clone 131-14871	MAB4072	Millipore, Billerica, MA, USA	1:1000
CC10	goat polyclonal		Dr. G. Singh, Georgia Regents University Medical Center, GA, USA	1:500
Cyclin D1	rabbit monoclonal/ clone SP4	RM-9104-S1	Thermo Fisher Scientific, Waltham, MA, USA	1:200
E-cadherin	mouse monoclonal	36/E-Cadherin	BD Biosciences Pharmingen, Franklin Lakes, NJ, USA	1:200
FOXA2	rabbit polyclonal	WRAB-FOXA2	Seven Hills Bioreagents, Cincinnati, OH, USA	1:2000
Ki67	mouse monoclonal/ clone MM1	NCL-L-Ki67-MM1	Leica Biosystems, Concord, ON, Canada	1:200
NKX2-1	rabbit polyclonal	WRAB-TTF1	Seven Hills Bioreagents, Cincinnati, OH, USA	1:3000
p63	mouse monoclonal clone 4A4	sc-8431	Santa Cruz, Santa Cruz, CA, USA	1:100
PECAM-1	rat monoclonal/ clone 13.3	557355	BD Biosciences Pharmingen, Franklin Lakes, NJ, USA	1:75
pERK	rabbit monoclonal	4370	Cell Signaling, Danvers, MA, USA	1:200
pHH3	rabbit monoclonal	9701	Cell Signaling, Danvers, MA, USA	1:200
proSP-C	rabbit polyclonal	AB3786	Millipore, Billerica, MA, USA	1:1500
SOX2	rabbit monoclonal	3728	Cell Signaling, Danvers, MA, USA	1:150
SOX9	rabbit polyclonal	sc-20095	Santa Cruz, Santa Cruz, CA, USA	1:100
T1α	syrian-hamster/ clone 8.1.1.		Hybridoma Bank, IO, USA	1:1000
Vimentin	rabbit monoclonal/ clone D21H3	5741	Cell Signaling, Danvers, MA, USA	1:200
YY1	rabbit polyclonal	sc-1703	Santa Cruz, Santa Cruz, CA, USA	1:300
Biotinylated goat anti-rabbit		BA-1000	Vector Laboratories, Burlington, ON, Canada	1:300
Biotinylated swine anti-goat		CLCC50015	Cedarlane, Burlington, ON, Canada	1:300
Biotinylated goat anti-syrian hamster		107-065-142	Cedarlane, Burlington, ON, Canada	1:300
Biotinylated goat anti-rat		112-065-062	Cedarlane, Burlington, ON, Canada	1:500
Biotinylated goat anti-mouse		115-065-003	Cedarlane, Burlington, ON, Canada	1:500
Donkey anti-rabbit, Alexa Fluor 594 conjugate			Molecular Probes, Eugene, OR, USA	1:250
ChIP assay				
YY1	rabbit polyclonal	sc-1703	Santa Cruz, Santa Cruz, CA, USA	
Histone H3	rabbit polyclonal	ab1791	Abcam, Toronto, ON, Canada	
IgG	-	sc-2027	Santa Cruz, Santa Cruz, CA, USA	

Table S3. List of primer sequences

(A) qRT-PCR		
Gene	**Sequence (5'-3')**	**Fragment size (bp)**
Bmp4	F- AGCGTCCCGCCAGCCGA R- CGGAGCTCTGCCGAGGAG	148
Cdkn1a	F- TCTCAGGGCCGAAAACGG R- ACTTCAGGGTTTTCTCTTGCAG	90
Dicer	F- CGCCTCCTACCACTACAACA R- AGAGCGCAAGTCAGTCAAGA	145
Elf5	F- ACATTCGCTCGCAAGGTTAC R- GTTCGGCTGTGACAGTCTTG	127
Etv4	F- GTCACTTCCAAGAGACGTGG R- GGGGCTATGGAAAGAGTTTTCTG	98
Etv5	F- TGAGAGGCGGGTATTTCTCC R- CCCTCTCGATACATGGTGGG	139
Fgf9	F- TATCCAGGGAACCAGGAAAGAC R- CAGGCCCACTGCTATACTGATAAA	70
Fgf10	F- TCAAAGCCATCAACAGCAACTATT R- CTCTTTCAGCTTACAGTCGTTGTTAAA	95
Foxf1	F- AGCAGCCATACCTTCACCAA R- CTGGGCGACTGTGAGTGATA	82
Foxp1	F- AAAAGACAAAGAGCGCCTGC R- CAGACTTGGAGAGGGTGACA	116
Foxp2	F- ATGCATTGGATGACCGAAGC R- AGTGGGTCATCATCGCTTGA	114
Foxp4	F- GCAGCTGACGCTAAATGAGA R- TCCACACGGACGAAACACTT	129
Hdac1	F- CGAATCCGCATGACTCACAA R- GTCATCTCCTCAGCATTGGC	95
Hdac2	F- ATGGCGTACAGTCAAGGAGG R- GGGATGACCCTGGCCATAAT	90
Hip1	F- CACTTCAACAGCACCAACCA R- AGTAGGATGTCGATCCACGG	87
Hoxa5	F- CCCAGATCTACCCCTGGATG R- GGCATGAGCTATTTCGATCCT	173
Hoxb5	F- TATTCCCCTGGATGAGGAAG R- GGGTCAGGTAGCGATTGAAG	135
Irx2	F- CCGTCCTACGTGGGCTC R- GGTCGTTGAGCTGGTATGGA	106
Irx3	F- GGTTACGGCGCCTTCCT R- AGGGCTGTCCTTCAGCTC	84
Irx5	F- AACTCGCACCTCCAGTACG R- TATCCCAAGGAACCTGCCAT	110
Ptc1	F- GCCTCATTGGGATCAAGCTG R- GCATAGCCCTGTGGTTCTTG	135
Rpl19	F- GATCATCCGCAAGCCTGTGA R- GCATCCGAGCATTGGCAGTA	122
Shh	F- TGACTCAGAGGTGCAAAGACA R- ACTCCTCTGAATGATGGCCG	120

Spry2	F- AAGCCGCGATCACGGAGTTCA R- CTGCAGCAAAGGCTGCGACC	113
Yy1	F- CATGTGGTCCTCGGATGAAA R- GGGAGTTTCTTGCCTGTCATA	117

(B) ChIP		
Gene	**Sequence (5'-3')**	**Fragment size (bp)**
Sfrs10	F-TTTCTCCGCTTCACCCTTGGA R-AACGGTATCTTCTTTCGCCGTTGGA	175
Rcor3	F-GTCTCAGCTGAAGGAATTTGGCCT R-GCCATCCTCATAGCTCCTGTCAAA	157
Shh #1	F-CTGGAGAGCTTGTGAGACAG R-AGTCTTTCTCAGGGTTAACATCA	113
Shh #2	F-CGAAGACCAAATAAGAGCCAGA R-CTGGATCAAGAACAGTGGGT	104
Shh #3	F-CCTAAGCTGCCAGTGTTCTATG R-GAACCAAGTCACCTCCTTCTTC	97
Shh #4	F-CCCTGGAGGCATAGTCCTG R-CCGAAGGCAGAGTGAGGA	79
Shh #5	F-CTGTCCAGAGTGAGCACAAG R-CTCCATTTCCCAGTGTGAGG	103
ctl locus	F-AGACCTGGATATAGTTAAGATGCG R-ACCCTGTGTTCCATCCAATAG	83