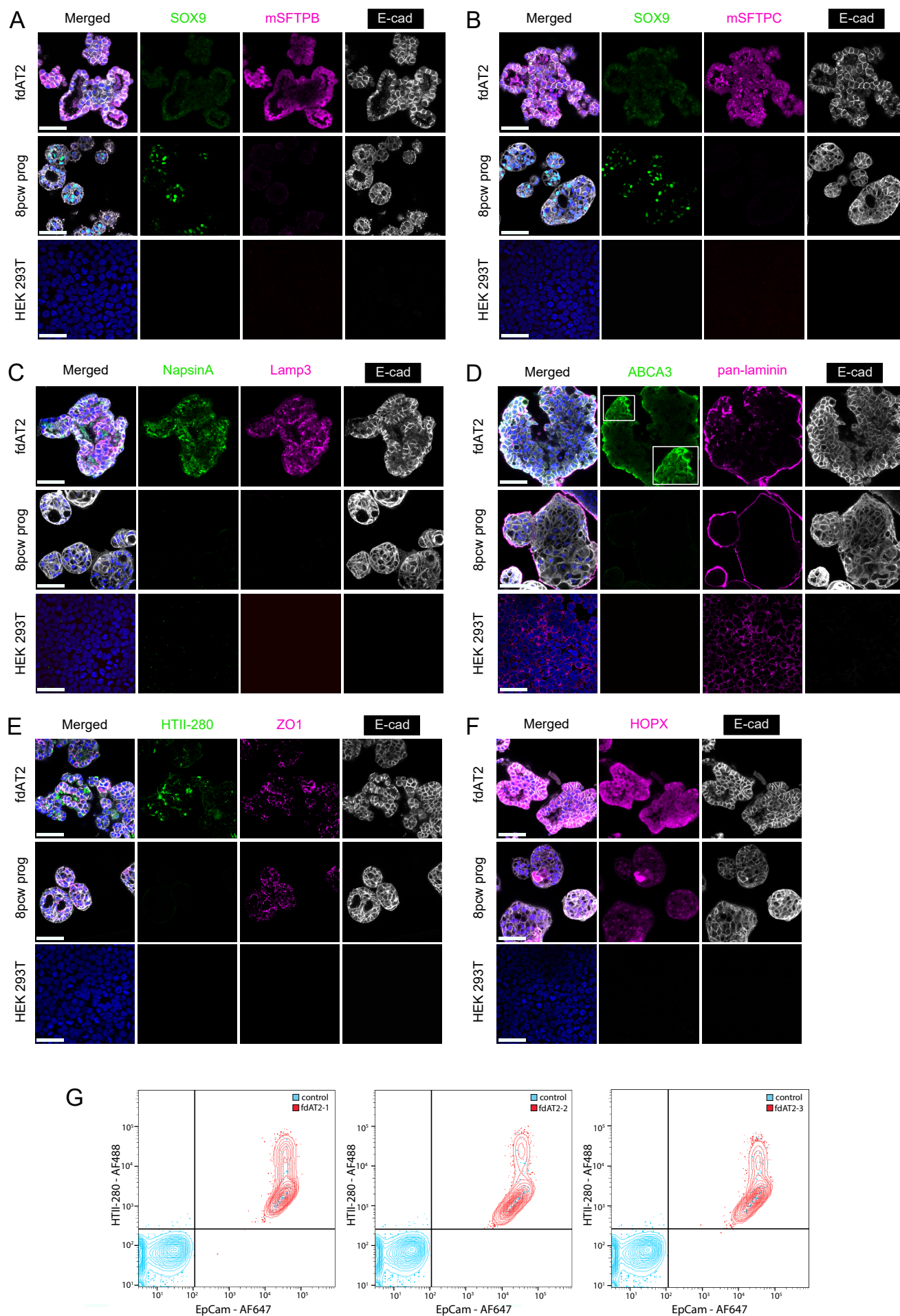
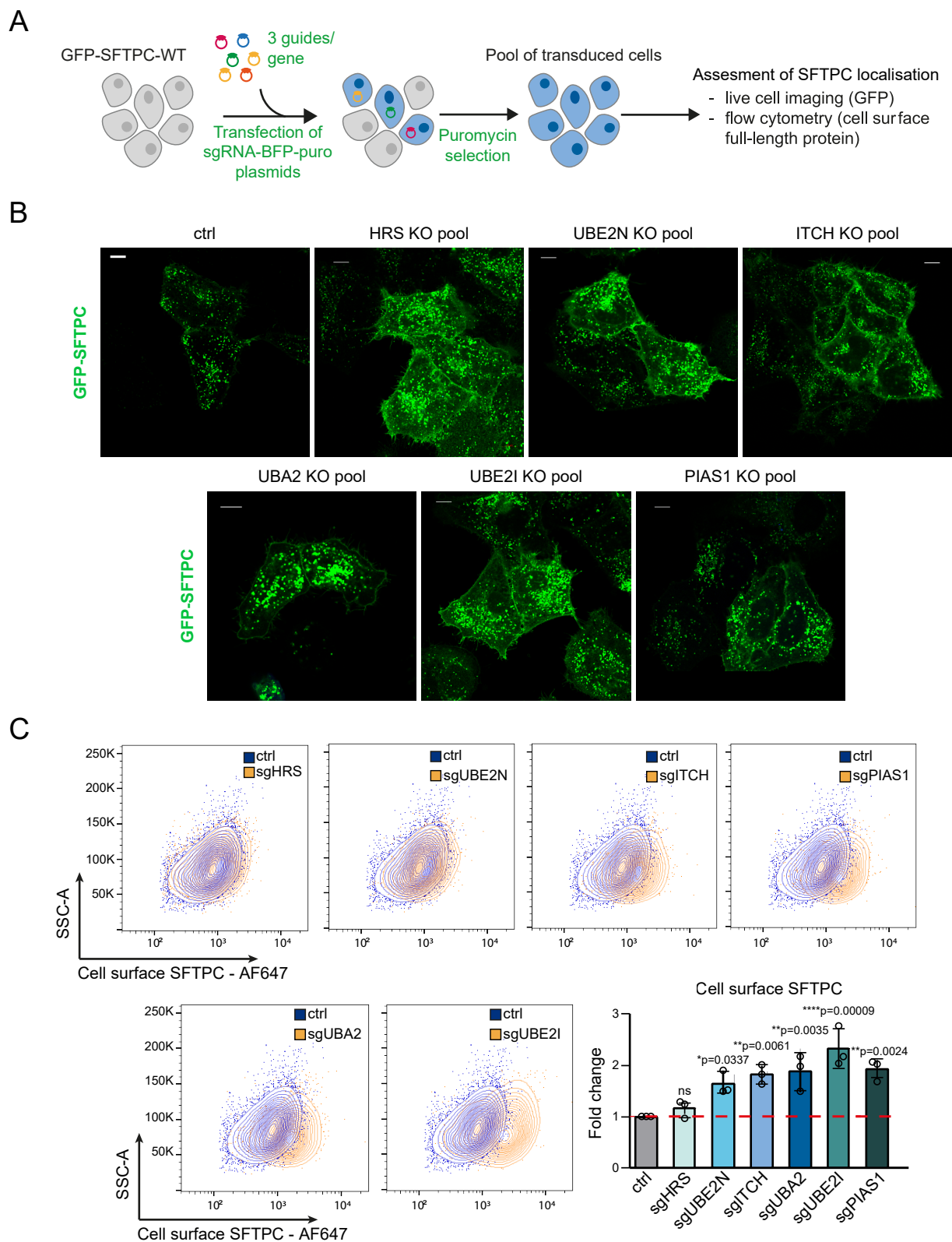


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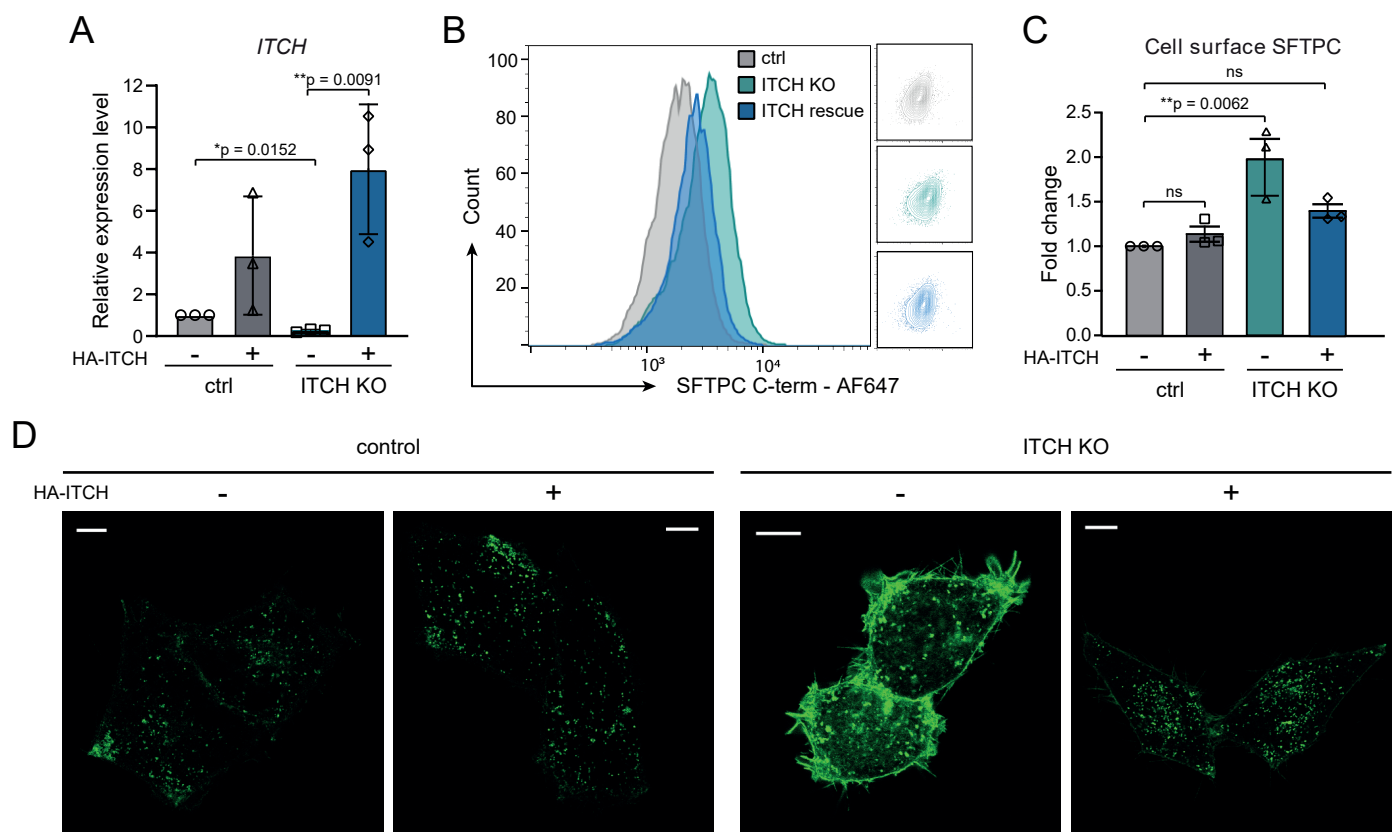
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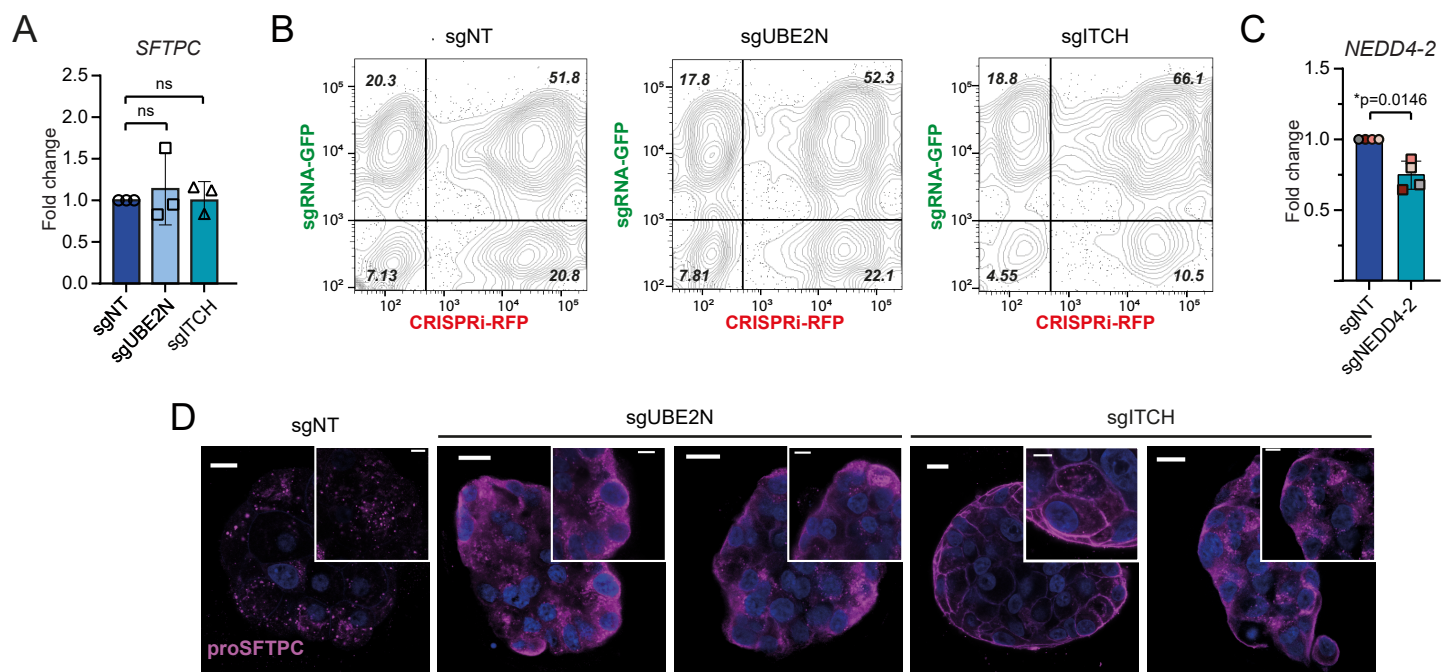
Appendix Figure S1. Control antibody staining comparing intensity levels in fetal-derived AT2 organoids, 8 pcw tip progenitors and HEK 293T cells. FdAT2 organoids (passage 19 or 20), 8 pcw tip progenitor organoids (passage 10 or 11) and Human embryonic kidney 293 (HEK 293T) cells were immunostained. Images are shown as individual z planes. (A) SOX9 (green), mature SFTPB (mSFTPB, magenta) and E-cadherin (grey). (B) SOX9 (green), mature SFTPC (mSFTPC, magenta) and E-cadherin (grey). (C) NapsinA (green), LAMP3 (magenta) and E-cadherin (grey). (D) ABCA3 (green), pan-Laminin (magenta) and E-cadherin (grey). (E) HTII-280 (green), ZO1 (magenta) and E-cadherin (grey). (F) HOPX (magenta) and E-cadherin (grey). Scale bar, 50µm. (G) Flow cytometry analysis of three independent fdAT2 lines (fdAT2-(1-3)) for cell surface expression of EpCam and HTII-280 (control = fdAT2 stained with secondary antibody only).



Appendix Figure S2. Initial validation of screen hits. (A) Schematic of screen hit validation strategy. (B) Relocalisation of SFTPC as assessed by live cell confocal microscopy in HeLa cells depleted of specific proteins of interest. Scale bar, 10µm. (C) Knockout pools were assessed for cell surface full length SFTPC enrichment by flow cytometry as measured by C-terminal antibody binding and mean fluorescence intensity quantified; mean $\pm$ SD, n=3 independent repeats (one-way ANOVA with Dunnetts multiple comparison test).



Appendix Figure S3. Restoration of ITCH expression in knockout cells reverses SFTPC mislocalisation. Control and ITCH knockout HeLa cells transfected with HA-ITCH were assessed for relative ITCH mRNA expression (A) and cell surface full-length SFTPC protein by flow cytometry (B&C); mean $\pm$ SD, n=3 independent repeats (one-way ANOVA with Tukey multiple comparison post-test);. (D) GFP-SFTPC localisation following ITCH depletion and rescue. Scale bar, 10µm.



Appendix Figure S4. FdAT2 CRISPRi additional data. (A) Flow cytometry dot plots of sgRNA-GFP positive (y-axis) and CRISPRi-RFP (x-axis) positive populations in fdAT2 organoids transduced with sg non-targeting (NT), sgUBE2N or sgITCH at cell harvesting (5 days post induction of CRISPRi system). Dual positive populations varied by organoid line and ranged between 50 and 80%. (B) Relative SFTPC expression in fdAT2 depleted of UBE2N or ITCH; mean $\pm$ SD, n=3 biologically independent organoid lines repeats (paired two-tailed Student's t-test). (C) Relative NEDD4-2 expression in fdAT2 depleted of NEDD4-2; mean $\pm$ SD, n=3 biologically independent organoid lines repeats (one sample t-test). (D) Additional representative images of SFTPC localisation and intensity in fdAT2 organoids depleted of ITCH or UBE2N with exposure optimised to show SFTPC subcellular localisation. Scale bar, 10 μ m / 5 μ m in zoomed inserts.

Appendix Table S1: qRT-PCR primer sequences

Gene assession number	Gene name	Forward (5'-3')	Reverse (5'-3')
NM_001101	<i>ACTB</i>	TGCCCTCAACGACCACTTTG	GGGTCTCTCTCTCCTCTTG TGCT
NM_001172357	<i>SFTPC</i>	CTCCACATGAGCCAGAAAC ACAC	GTAGTCATACACCACGAGG CCAGT
NM_003317	<i>NKX2.1</i>	CGTACCAGGACACCATGA GGAA	CGTCGCTCCAGCTCGTACA C
NM_001089	<i>ABCA3</i>	CACCTCAAGAGCAAGTTCG GCAG	GGACCATGCCTTGGTGCTC ATC
NM_014398	<i>LAMP3</i>	AACAACCGATGTCCAACCTT CAAGCC	CCCATACCCATAAGGCAGA GACCAA
NM_001172357	<i>SFTPC</i>	GTCCTCATCGTCGTGGTGA TTG	AGAAGGTGGCAGTGGTAAC CAG
NM_002046	<i>GAPDH</i>	GAAGGTGAAGGTCGGAGT C	GAAGATGGTGATGGGATTTC
NM_001257137	<i>ITCH</i>	GAGGTCACAGTAGATGGAC AG	GCAGTTCCCAACAAAACAT CAG
NM_003348	<i>UBE2N</i>	AGGATCATCAAGGAAACCC AG	CAAGTTTAAAAGTCCCTCCC TC

Appendix Table S2: gRNA sequences

Forward genetic screen validation	
ITCH (guide 1)	GAACGGCGGGTTGACAACAT
ITCH (guide 2)	ATTGTGTAATTCGTGTGTTG
ITCH (guide 3)	AATACAAACCTGGTCTACGT
UBE2N (guide 1)	CTGTTGCCTTCATAGATAAG
UBE2N (guide 2)	CCTCAAAGGGGAATCCTGA
UBE2N (guide 3)	CCTTCCAGAAGAATACCCAA
HRS (guide 1)	GTTGCGGGTGATGACCCGTA
HRS (guide 2)	CTTGGGGTACGAAGTGTACG
HRS (guide 3)	GCACGCAGCCCCACCAACG
UBA2 (guide 1)	TGACAATGGACAACTATGG
UBA2 (guide 2)	ACAGCCAGGAAAGGTTCTCT
UBA2 (guide 3)	TTGTAGCCCTGACTATAATG
UBE2I (guide 1)	ACAGGCACACTGTCCCCGAA
UBE2I (guide 2)	AAGCCCTCCTTACAAATGGG
UBE2I (guide 3)	AAGGAGGCTTGTTTAACTA
PIAS1 (guide 1)	ACGCAAGATGGCGGACAGTG
PIAS1 (guide 2)	CTTAAAGCTCTAGAATGATC
PIAS1 (guide 3)	CCTCTGAAGAAGAACTGTTG
CRISPRi	
ITCH	GGGAGTGGGACGCGCGGTTC
UBE2N	GACGCACGAGTGGAAGTCCC
Non-targeting	GCTGATCTATCGCGGTCGTC

Appendix Table S3: Sample information for RNA sequencing

Human fetal lung_ID	Developmental age	Sample name	Early passage bulk RNAseq	Late passage bulk RNAseq	Single cell RNA seq
HDBR-L 15934	17 pcw	AT2_1	P7	P16	P16
HDBR-L 16011	21 pcw	AT2_2	P6	P17	P11
HDBR-L 16392	17 pcw	AT2_3	P1	P12	P15
HDBR-L 16402	20 pcw	AT2_4	P4	P13	P16