SUPPLEMENTAL FIGURES

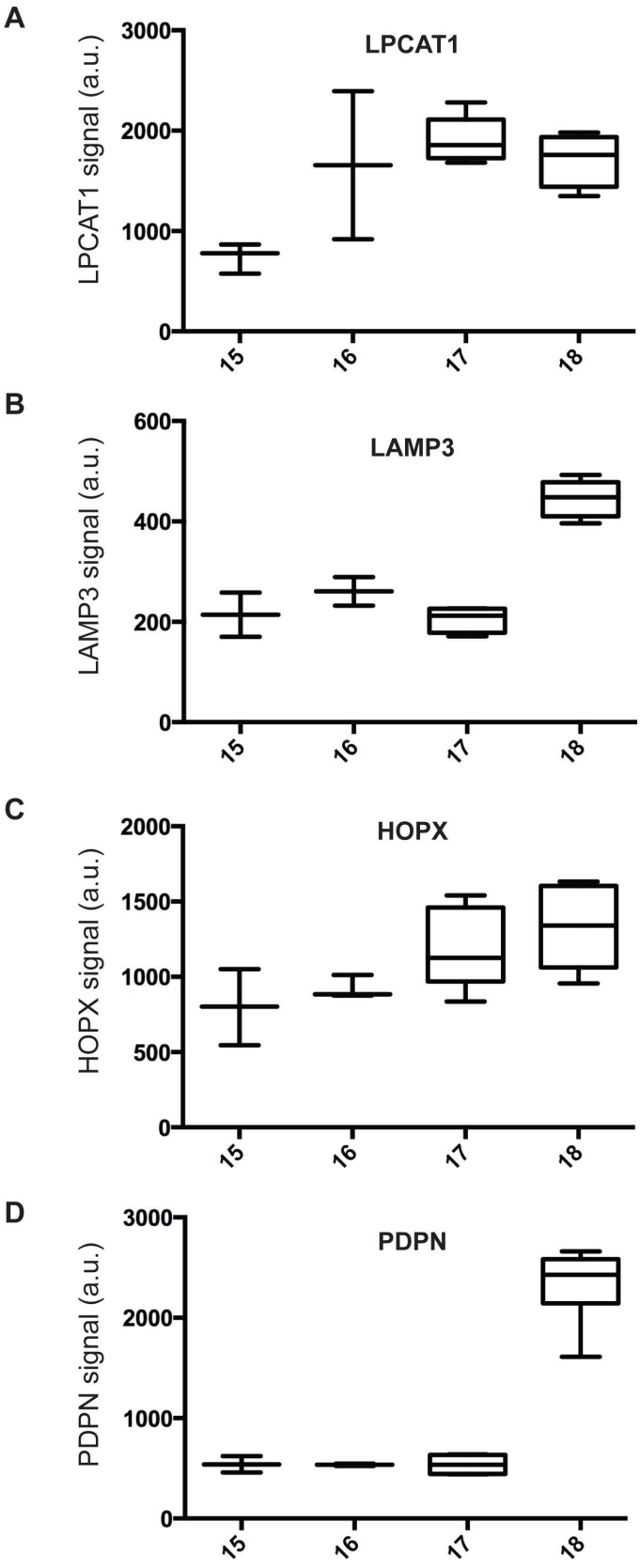


Figure S1. Relative expression level of alveolar epithelial fate markers in normal lung development over time. Bar and whisker plots showing relative expression levels of alveolar fate markers analysed in Figure 1 in arbitrary units normalized to the total cell area sampled. A. LPCAT1 (raw data from images underpinning Fig. 1D). B. LAMP3 (raw data from images underpinning Fig 1D). C. HOPX (raw data from images underpinning Fig. 1F). D. PDPN (raw data from images underpinning Fig. 1D).

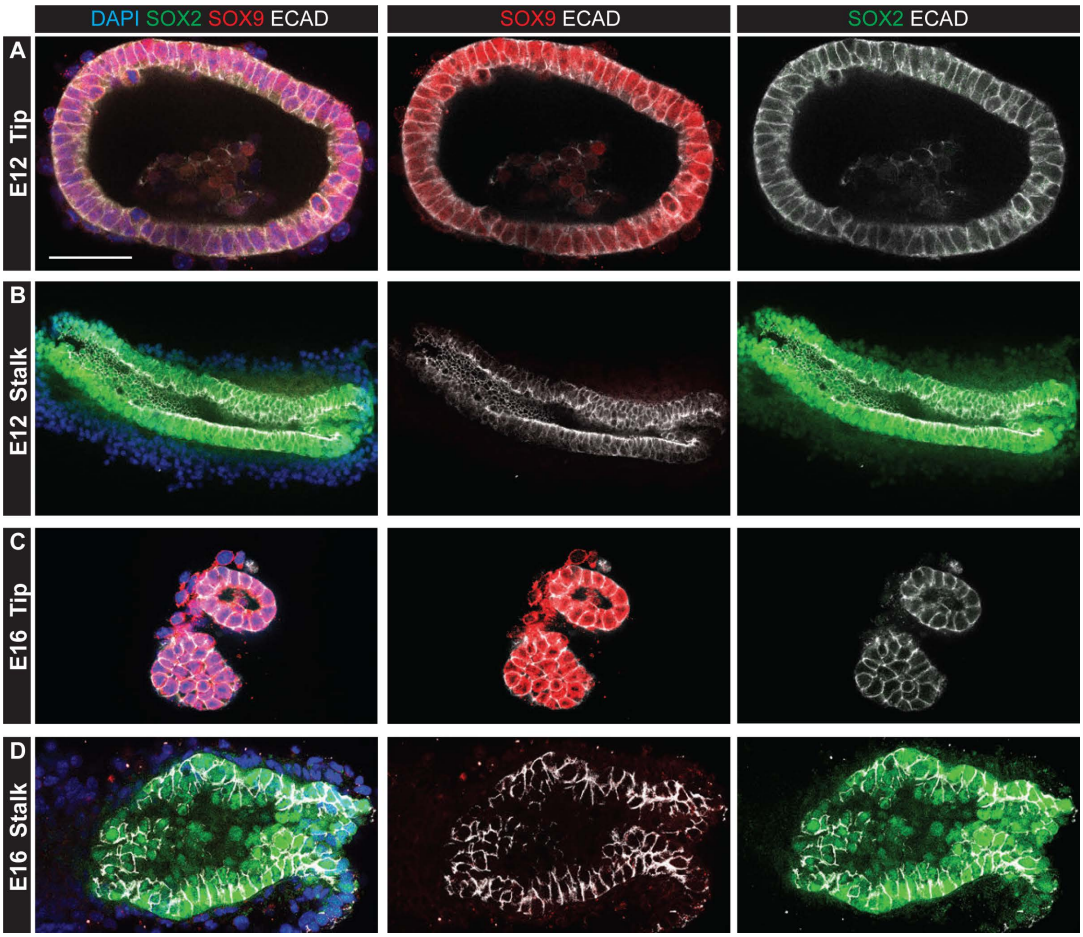


Figure S2. Microdissected tip and stalk cells. Examples of wholemount stained microdissected tip and stalk cells. Green: SOX2 (stalk/differentiating bronchioles); red: SOX9 (tip); white: E-CAD (epithelium); blue: DAPI (nuclei). A. E12.5 tip. B. E12.5 stalk. C. E16.5 tip. D. E16.5 stalk. Bars = 50 μm A,C,D; 100 μm B.

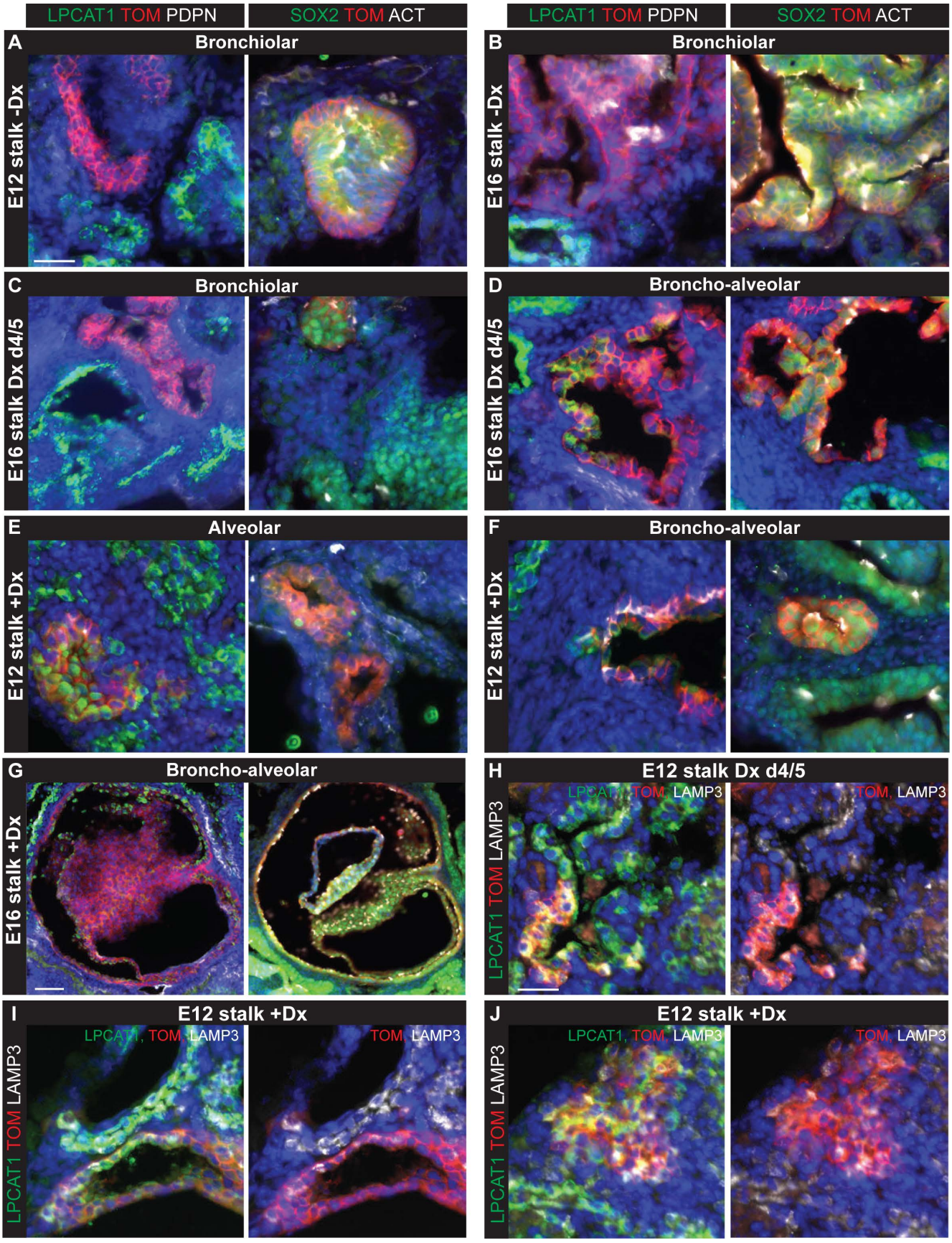
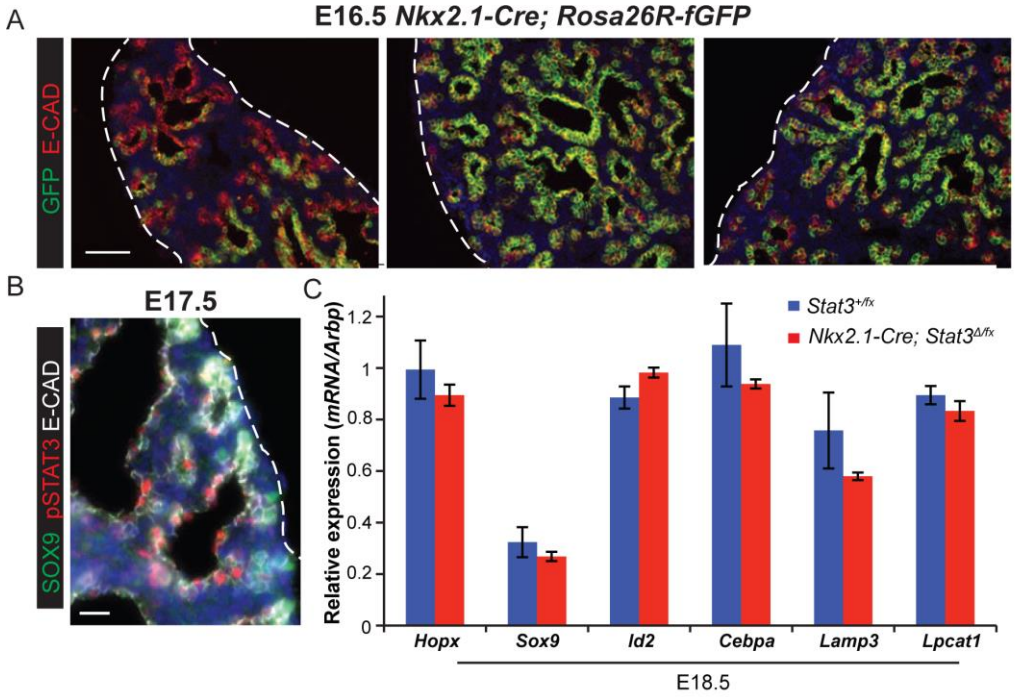
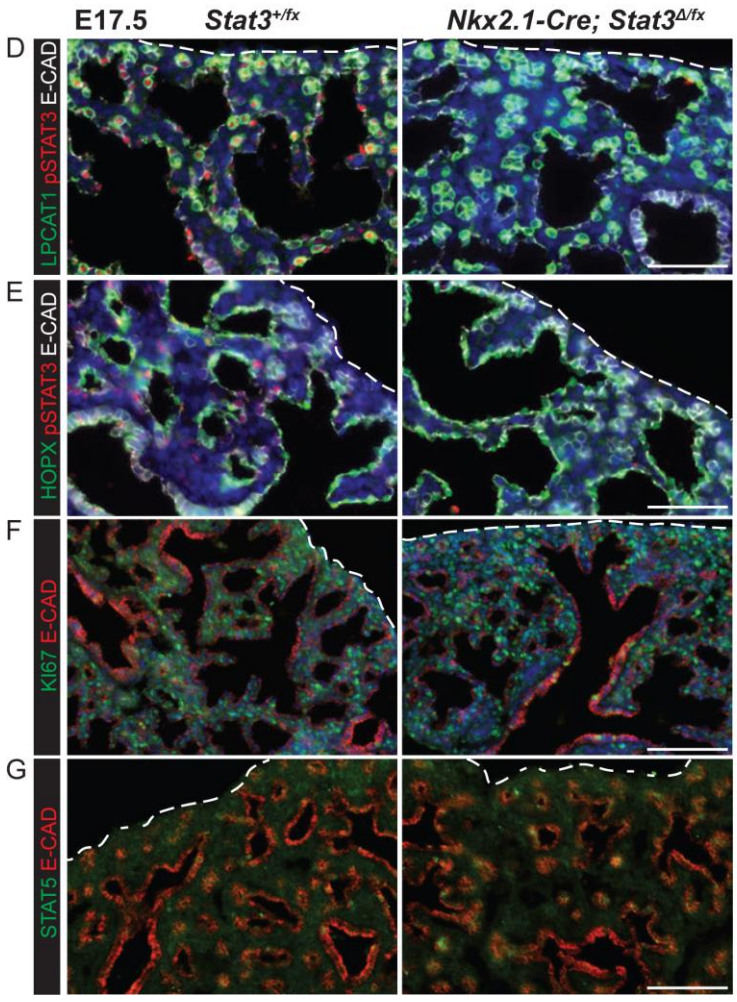


Figure S3. Grafted stalk cells can respond to the host environment and alter progeny cell fate. Examples of grafted stalk cells following 8 days of culture in various conditions. A-G. Two images of each graft are shown taken from adjacent slides stained to detect alveolar fate: green: LPCAT1 (alveolar fate); red: Tomato fluorescence (grafted cells); white: PDPN (basal and type 1 cells), or bronchiolar fate: green: SOX2 (bronchiolar fate); red: RFP (Tomato^{+} graft); white: acetylated tubulin (cilia) to determine graft fate. A. E12.5 stalk, no Dx, bronchiolar-fated. B. E16.5 stalk, no Dx, bronchiolar-fated. C. E16.5 stalk, Dx day4/5, bronchiolar fated. D. E16.5 stalk, Dx day4/5, mixed broncho-alveolar fated. E. E12.5 stalk, Dx throughout, alveolar fated. F. E12.5 stalk, Dx throughout, mixed broncho-alveolar fated. G. E16.5 stalk, Dx throughout, mixed broncho-alveolar fated. H-J. Grafts are stained to detect alveolar markers. Green: LPCAT1 (alveolar fate); red: Tomato fluorescence (grafted cells); white: LAMP3 (differentiating AT2 cells). Right panel has the green channel removed to show the Tomato/LAMP3 co-localisation clearly. H. E12.5 stalk, Dx day4/5. I, J. E12.5 stalk, Dx throughout. Bars = 50 μm C left panel; 100 μm all other panels.





H

% alveolar epithelial cells expressing LPCAT1

	Stat3$^{+/fx}$	*Nkx2.1-Cre; Stat3*$^{\Delta/fx}$
E17.5	79 +/- 2.3%	81 +/- 2.2%
E18.5	70 +/- 0.5%	71 +/- 2.6%

% alveolar epithelial cells expressing HOPX

	Stat3$^{+/fx}$	*Nkx2.1-Cre; Stat3*$^{\Delta/fx}$
E17.5	69 +/- 1.3%	67 +/- 2%
E18.5	52 +/- 1.1%	57 +/- 2.1%

Figure S4. Alveolar maturation ultimately occurs in the *Stat3* cKO lungs. A. Sections of E16.5 *Nkx2.1-Cre; Rosa26R-fGFP* lungs illustrating variation in the extent of recombination. Green: eGFP (reporter); red: E-CAD (epithelium). B. Section of E17.5 wild-type lung illustrating that pSTAT3 is predominantly seen in differentiating cells that have exited the tip. Green: SOX9 (tip); red: pSTAT3; white: E-CAD (epithelium). C. RT-qPCR of progenitor and alveolar markers from *Nkx2.1-Cre; Stat3*$^{\Delta/fx}$ and sibling *Stat3*$^{fx/+}$ control E18.5 lungs. Error bars = s.e.m. D, E. Sections of *Stat3* cKO and sibling lungs. D. Green: LPCAT1 (late tip and AT2 cells); red: pSTAT3; white: E-CAD (epithelium). E. Green: HOPX (differentiating AT1 cells); red: pSTAT3; white: E-CAD (epithelium). F: Green: Ki67 (proliferating cells); red: E-CAD (epithelium); blue: Dapi. G. Green: STAT5; red: E-CAD (epithelium). H. Quantification of LPCAT1 and HOPX staining in E17.5 and E18.5 *Nkx2.1-Cre; Stat3*$^{\Delta/fx}$ and sibling *Stat3*$^{fx/+}$ control. Mean and s.e.m. are displayed for 3 independent samples. Bar = 50 µm A, D, E; 25 µm B; 100 µm F, G.

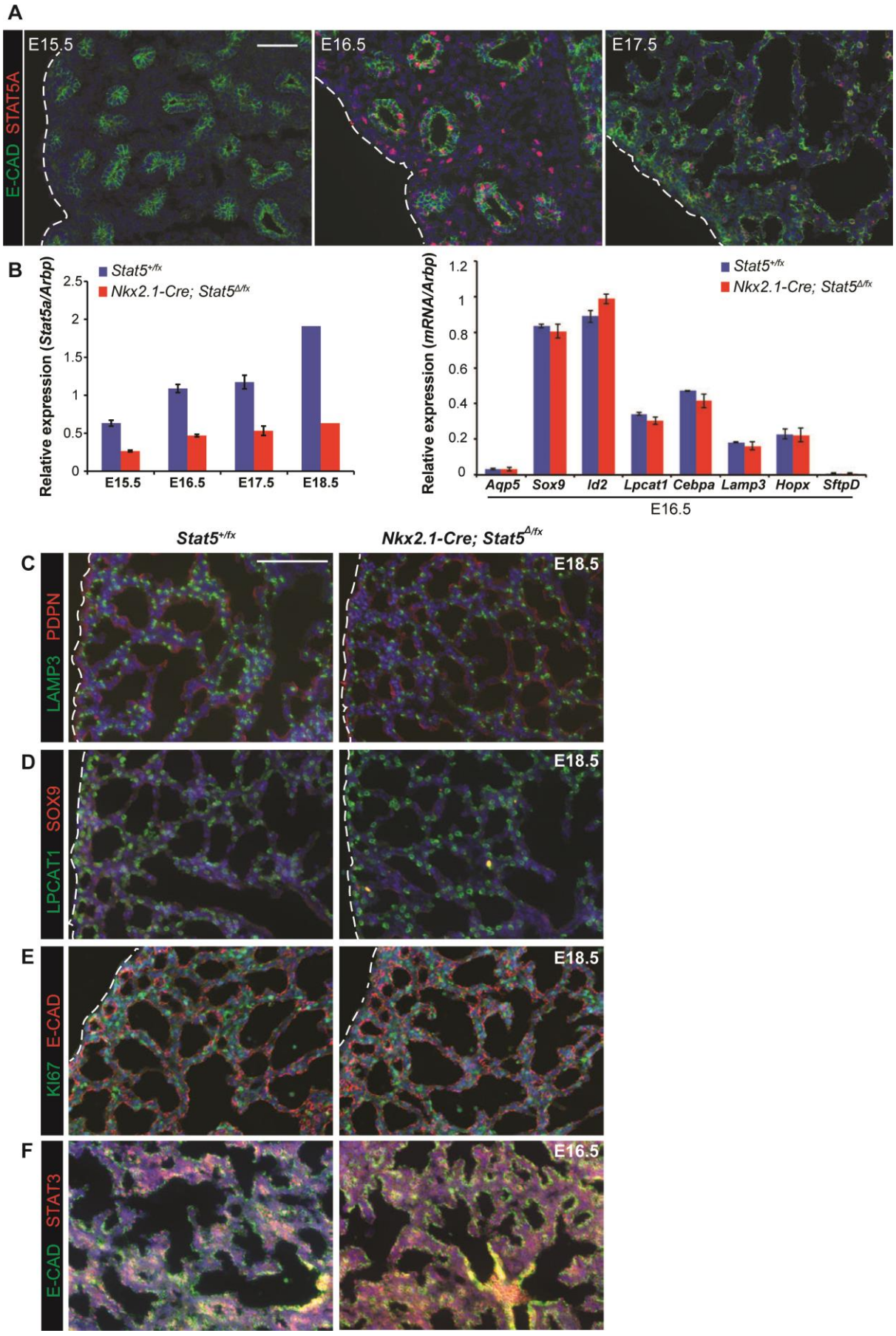


Figure S5. Lung epithelial specific knock-out of *Stat5a/b* does not result in alveolar differentiation phenotypes. A. Wild-type E15.5, 16.5, 17.5 lung sections. Green: E-CAD (epithelium); Red: STAT5A. B. RT-qPCR of *Stat5a* and progenitor/alveolar markers from *Nkx2.1-Cre; Stat5*$^{\Delta/fx}$ and sibling *Stat5*$^{fx/+}$ control lungs. Error bars = s.e.m. C-F. Sections of *Stat5* cKO and sibling control lungs. C. E18.5. Green: LAMP3 (differentiating AT2 cells); red: PDPN (AT1 cells). D. E18.5. Green: LPCAT1 (AT2 cells); red: SOX9 (progenitor cells). E. E18.5. Green: KI67 (proliferating cells); red: E-CAD (epithelium). F. E16.5. Green: E-CAD (epithelium); red: STAT3. At each time-point, three independent litters were collected and stained. Blue: Dapi. Bar = 50 μm A, E, F; 100 μm C,D.

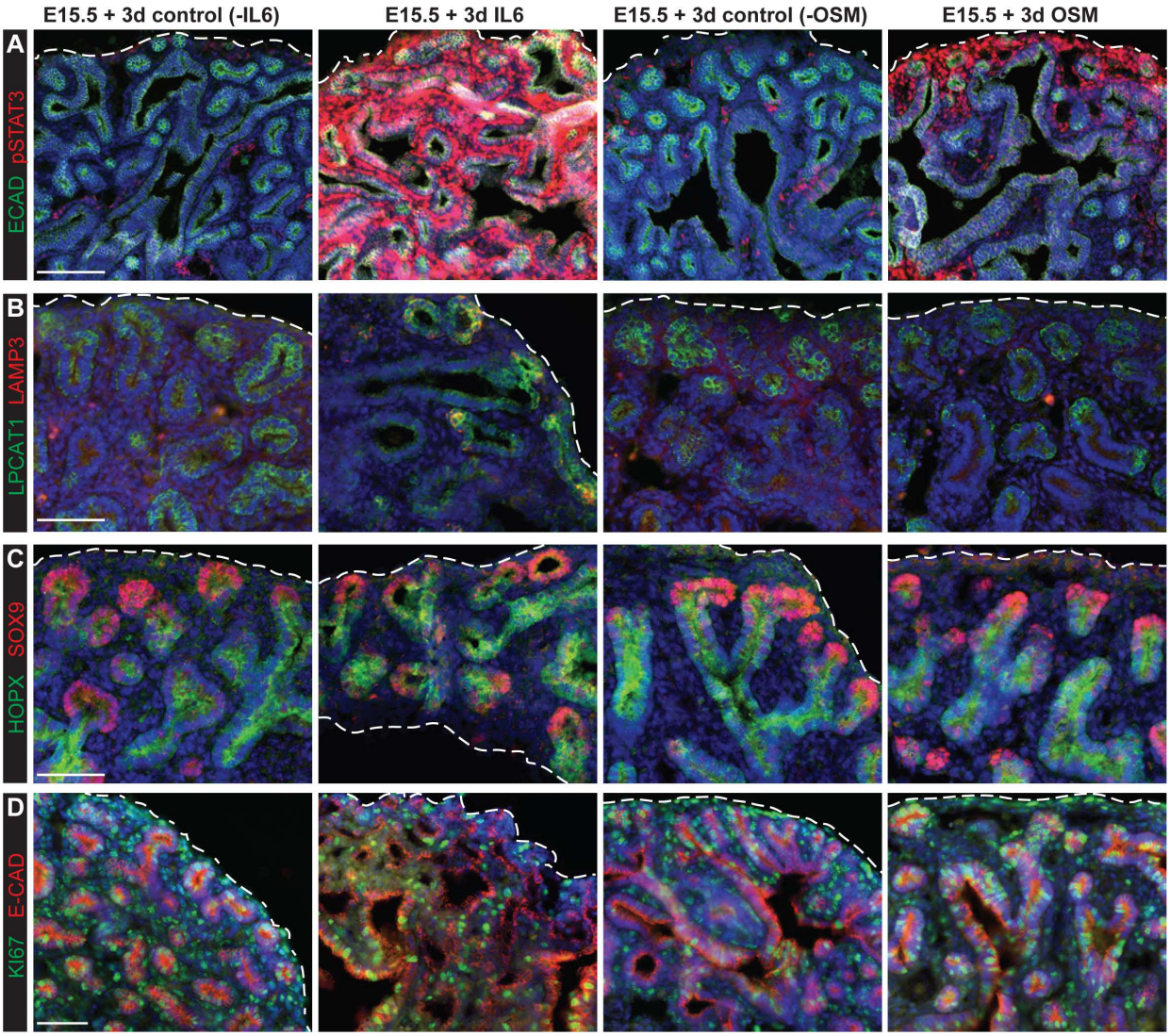


Figure S6. Activating pSTAT3 via OSM is not sufficient to accelerate AT2 differentiation. Sections of E15.5 lung slices cultured for 3 days +/- 10 ng/ml IL6, or +/- 25 ng/ml OSM. A. Green: E-CAD (epithelium); red (pSTAT3). B. Green: LPCAT1 (late tip and AT2); red: LAMP3 (AT2). C. Green: HOPX (stalk cells from E16.5, AT1 cells); red: SOX9. D. Green: KI67 (proliferating cells); red: E-CAD (epithelium); Blue: Dapi. Bar = 100 μm A; 50 μm B-D.

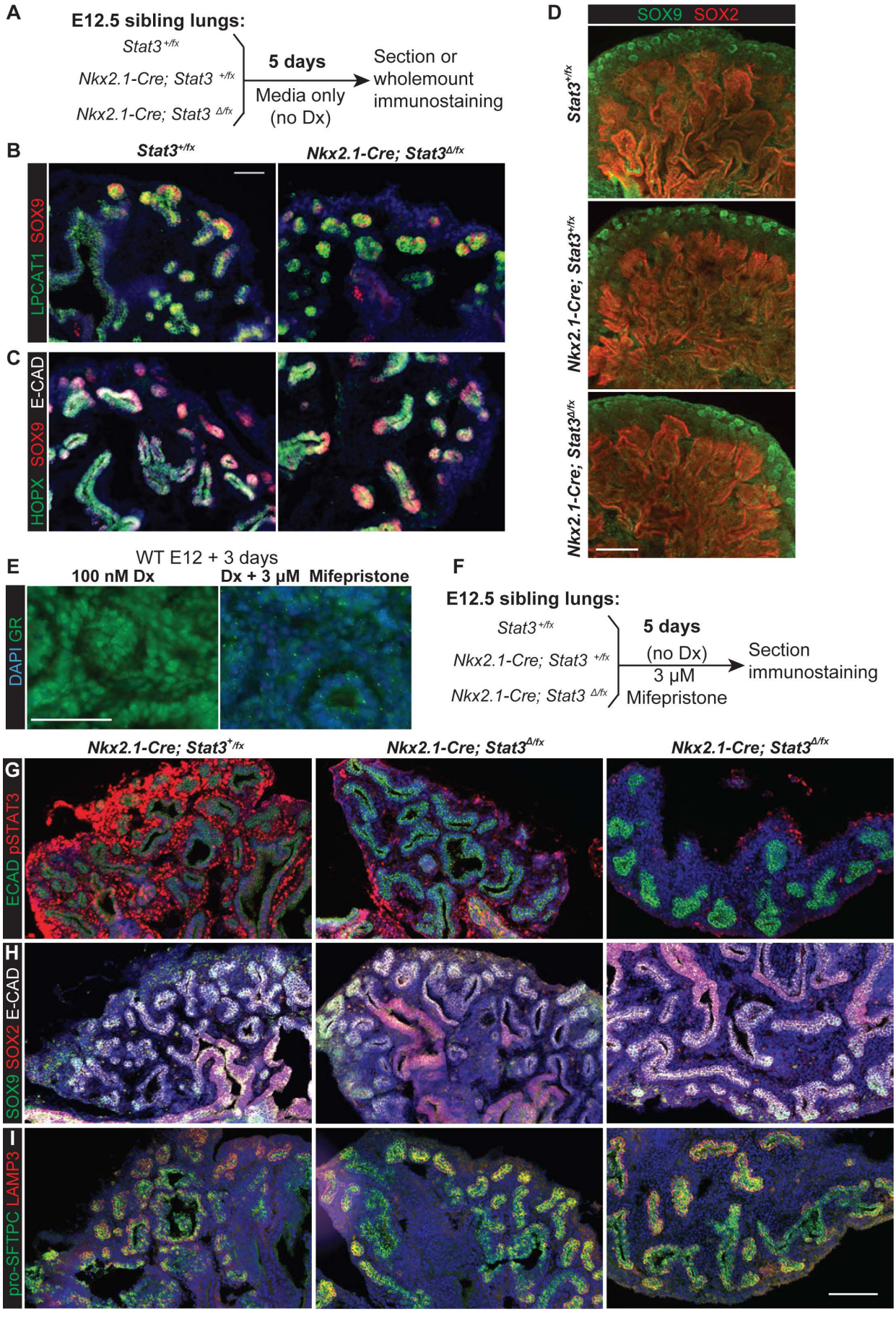


Figure S7. No evidence for redundant Glucocorticoid and STAT3 activity in alveolar development. A. Experimental scheme: E12.5 *Stat3* cKO and sibling control lungs were cultured for 5 days in the absence of exogenous glucocorticoid. B, C. E12.5 + 5 day sections (n=6 *Stat3* cKO lungs in two independent experiments). B. Green: LPCAT1; red: SOX9. C. Green: HOPX; red: SOX9; white: E-CAD. D. Confocal z-stack projections of E12.5 + 5 day wholemount immunostaining (n=3 *Stat3* cKO lungs). Green: SOX9; red: SOX2. Blue: Dapi. E. Sections of wild-type E12.5 lungs cultured for 3 days in 100nm Dx (left panel), or 100 nM Dx with 3 μM Mifepristone (right panel). Green: GR. GR staining is reduced in the presence lungs were cultured for 5 days in the presence of 3 μM Mifepristone, a Glucocorticoid inhibitor. G-I. Sections of *Stat3* cKO and sibling control lungs E12.5 + 5 days culture in Mifepristone. Two independent *Stat3* cKO lungs are shown (n=6 *Stat3* cKO lungs in two independent experiments). G. Green: E-CAD (epithelium); red: pSTAT3. H. Green: SOX9; red: SOX2; white: E-CAD. I. Green: pro-SFTPC; red: LAMP3. Bar = 50 μm B, C, E; 250 μm D; 100 μm G-I.

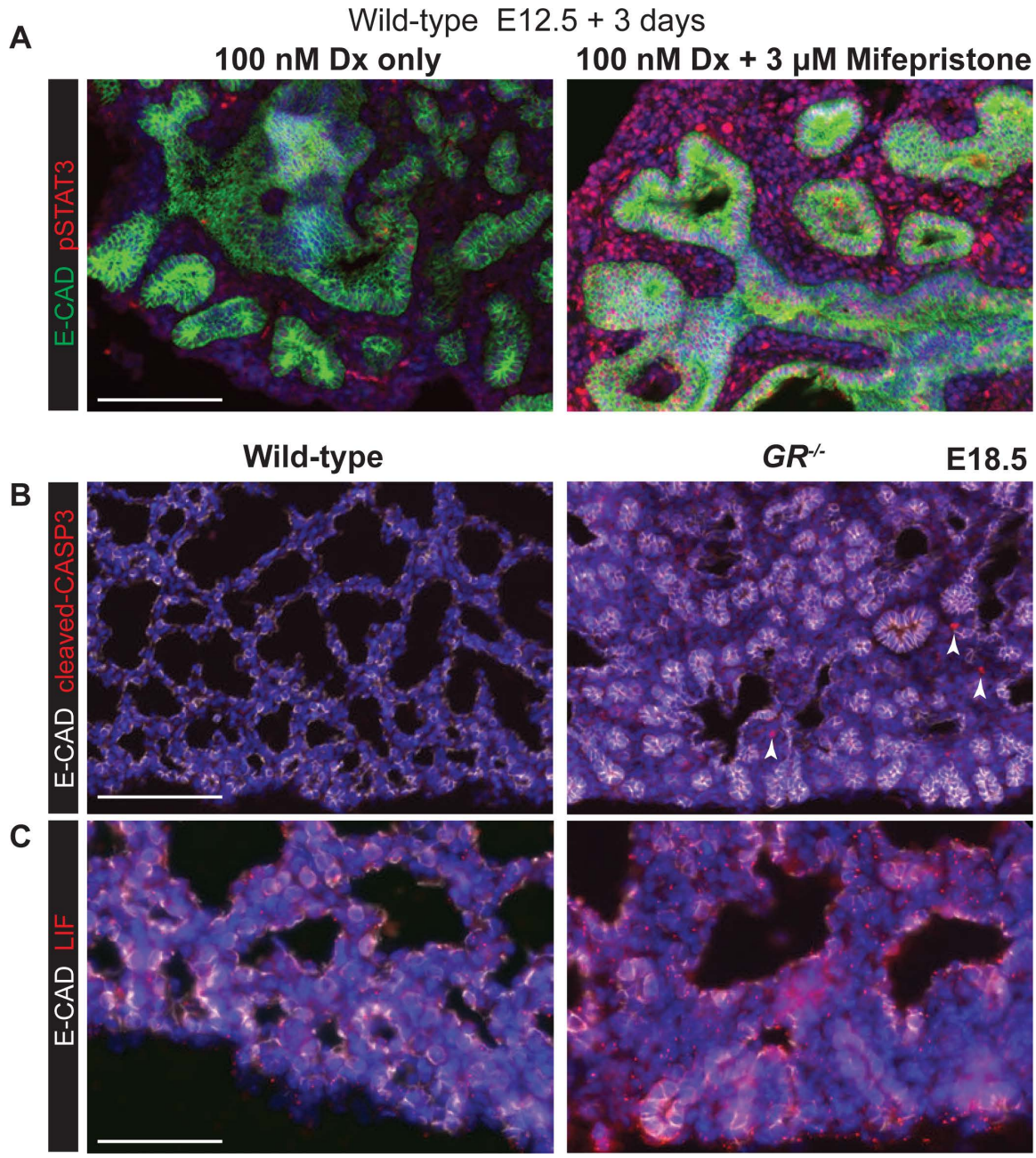


Figure S8. STAT3 pathway activity in GR abrogated lungs. A. Sections of wild-type E12.5 lungs cultured for 3 days in 100nm Dx (left panel), or 100 nM Dx with 3 µM Mifepristone (right panel). Green: E-CAD (epithelium); red: pSTAT3. B, C. Sections of *GR*$^{-/-}$ and sibling *GR*$^{+/+}$ control lungs. B. Red: cleaved-Caspase 3 (apoptotic cells); white: E-CAD (epithelium). Arrowheads mark a small number of apoptotic cells in the *GR*$^{-/-}$ lungs. C. Red: LIF; white: E-CAD (epithelium). Blue: Dapi. Bar = 100 µm A, B; 50 µm C.

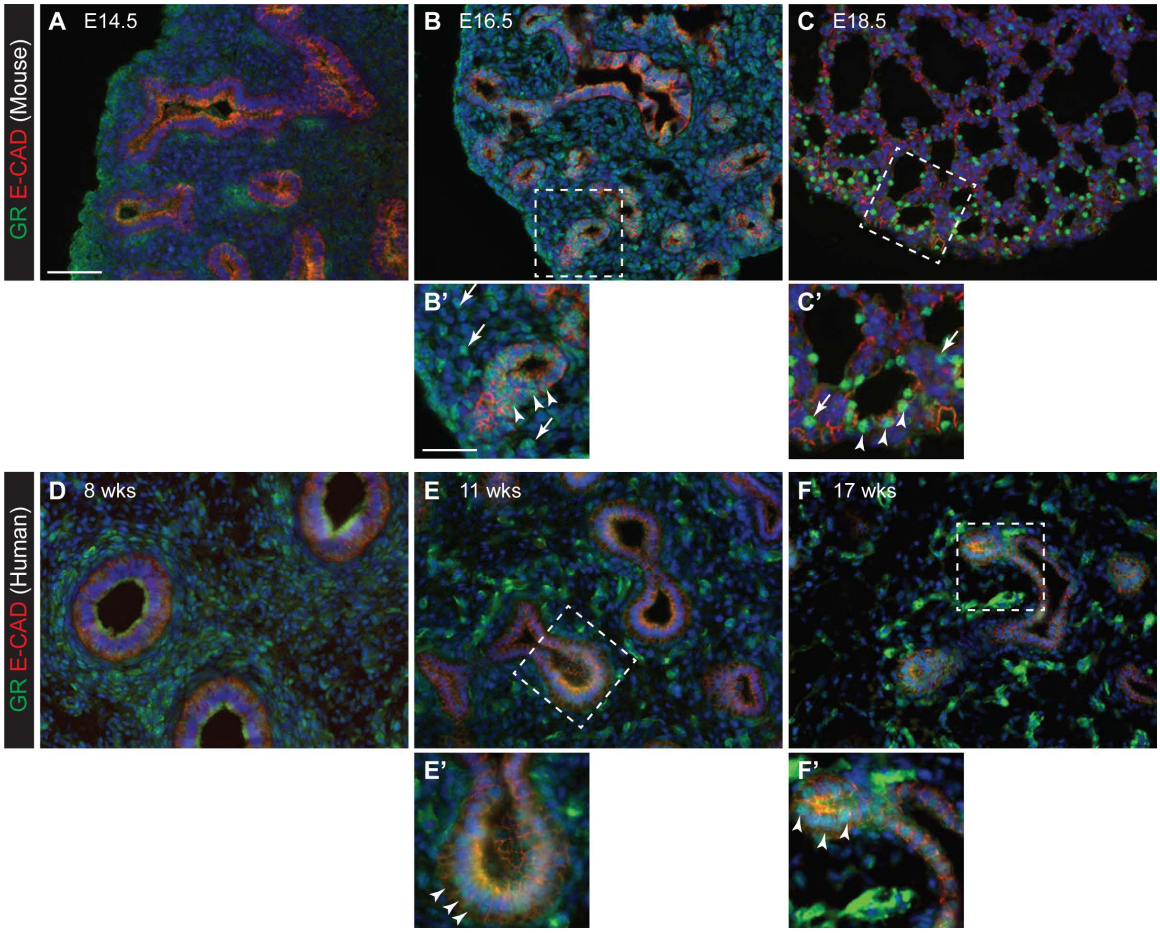


Figure S9. GR staining in embryonic mouse and human lungs. Sections of embryonic lungs stained for green: GR; red: E-CAD (epithelium); blue: Dapi. A-C. Mouse E14.5, 16.8 and 18.5. D-F. Human 8, 11 and 17 pcw. Boxed regions are blown up in B', C', E', F'. Arrow heads = GR^+ epithelial cells; arrows = GR^+ mesenchymal cells. Bar = 100 μm; 50 μm insets.

Table S1. Raw data accompanying Fig. 4.

Virus	Experiment number	Lung number	Total bronchiolar cells	GFP+ bronchiolar cells	Total alveolar cells	GFP+ alveolar cells	GFP+ bronchiolar cells / total bronchiolar cells (column E/ column D)	GFP+ alveolar cells / total alveolar cells (column G/column F)	Ratio GFP+ alveolar : bronchiolar (column I/column J)	Mean ratio GFP+ alveolar : bronchiolar	Standard deviation	2-tailed T test versus GFP	Total E-CAD+ cells (column D + column F)	GFP+ E-CAD cells (column E + column G)	% GFP+ E-CAD cells (column P/column O *100)
GFP	ALC8	1	323	34	901	89	0.105263158	0.098779134	0.938401776				1224	123	10.04901961
	ALC8	2	161	14	665	81	0.086956522	0.121804511	1.40075188				826	95	11.50121065
	ALC10	3	478	29	953	79	0.060669456	0.082896118	1.366356696				1431	108	7.547169811
	ALC15	4	373	108	542	144	0.289544236	0.265682657	0.917589176				915	252	27.54098361
	ALC15	5	189	31	783	131	0.164021164	0.167305236	1.020022247				972	162	16.66666667
	ALC15	6	258	62	595	118	0.240310078	0.198319328	0.825264299	1.078064346	0.244857		853	180	21.10199297
NOTCH	ALC8	1	240	14	594	40	0.058333333	0.067340067	1.154401154				834	54	6.474820144
	ALC10	2	482	24	404	22	0.049792531	0.054455446	1.093646865				886	46	5.191873589
	ALC16	3	192	21	582	174	0.109375	0.298969072	2.733431517				774	195	25.19379845
	ALC16	4	274	44	239	76	0.160583942	0.317991632	1.980220616				513	120	23.39181287
	ALC16	5	377	64	578	74	0.169761273	0.128027682	0.754163062				955	138	14.45026178
	ALC16	6	279	38	1121	169	0.136200717	0.150758252	1.106882952				1400	207	14.78571429
	ALC16	7	596	88	301	34	0.147651007	0.112956811	0.765025672	1.369681691	0.72671	0.370618892	897	122	13.60089186
CEBPA	ALC19	1	133	35	587	97	0.263157895	0.165247019	0.627938671				720	132	18.33333333
	ALC19	2	211	83	333	167	0.393364929	0.501501502	1.274901407				544	250	45.95588235
	ALC19	3	231	52	713	187	0.225108225	0.26227209	1.165093322				944	239	25.31779661
	ALC19	4	368	105	524	227	0.285326087	0.433206107	1.51828426				892	332	37.21973094
	ALC21	5	305	46	373	84	0.150819672	0.225201072	1.493181023				678	130	19.1740413
	ALC21	6	299	40	380	63	0.133779264	0.165789474	1.239276316				679	103	15.16936672
	ALC21	7	322	47	289	64	0.145962733	0.221453287	1.517190606				611	111	18.16693944
	ALC21	8	398	58	441	87	0.145728643	0.197278912	1.353741497	1.273700888	0.293666	0.211391815	839	145	17.28247914
CITED2	ALC8	1	176	4	304	12	0.022727273	0.039473684	1.736842105				480	16	3.333333333
	ALC8	2	465	18	970	105	0.038709677	0.108247423	2.796391753				1435	123	8.571428571
	ALC17	3	58	5	1007	156	0.086206897	0.154915591	1.797020854				1065	161	15.11737089
	ALC17	4	685	50	553	97	0.072992701	0.175406872	2.403074141				1238	147	11.87399031
	ALC17	5	191	46	560	206	0.240837696	0.367857143	1.527406832	2.052147137	0.52842	0.002865192	751	252	33.55525965
STAT3.1	ALC14	1	390	72	1220	403	0.184615385	0.330327869	1.789275956				1610	475	29.50310559
	ALC14	2	452	89	628	94	0.196902655	0.149681529	0.760180348				1080	183	16.94444444
	ALC20	3	283	25	477	118	0.088339223	0.247379455	2.80033543				760	143	18.81578947
	ALC20	4	148	14	254	86	0.094594595	0.338582677	3.579302587				402	100	24.87562189
	ALC20	5	85	15	396	107	0.176470588	0.27020202	1.531144781				481	122	25.36382536
	ALC20	6	61	11	322	110	0.180327869	0.341614907	1.894409938				383	121	31.5926893
	ALC22	7	283	25	477	118	0.088339223	0.247379455	2.80033543				760	143	18.81578947
	ALC22	8	148	14	254	86	0.094594595	0.338582677	3.579302587	2.341785882	1.010478	0.01169454	402	100	24.87562189
STAT3.2	ALC20	1	219	26	466	107	0.118721461	0.229613734	1.934054143				685	133	19.41605839
	ALC20	2	259	15	292	44	0.057915058	0.150684932	2.601826484				551	59	10.70780399
	ALC22	3	318	24	475	86	0.075471698	0.181052632	2.398947368				793	110	13.87137453
	ALC22	4	812	54	335	45	0.066502463	0.134328358	2.019900498				1147	99	8.631211857
	ALC22	5	420	36	474	82	0.085714286	0.172995781	2.018284107	2.19460252	0.290294	0.000142186	894	118	13.19910515
STAT5A.1	ALC12	1	409	7	739	43	0.017114914	0.058186739	3.399768026				1148	50	4.355400697
	ALC12	2	100	1	539	19	0.01	0.035250464	3.525046382				639	20	3.129890454
	ALC12	3	302	9	971	43	0.029801325	0.044284243	1.485982378				1273	52	4.084838963
	ALC17	4	664	88	912	270	0.13253012	0.296052632	2.233851675				1576	358	22.71573604
	ALC17	5	367	53	1034	244	0.144414169	0.235976789	1.634027955				1401	297	21.19914347
	ALC17	6	190	12	557	76	0.063157895	0.136445242	2.160383004	2.406509903	0.868582	0.004801348	747	88	11.78045515
STAT5A.2	ALC12	1	570	23	808	71	0.040350877	0.087871287	2.177679724				1378	94	6.821480406
	ALC12	2	388	15	489	64	0.038659794	0.130879346	3.385412406				877	79	9.007981756
	ALC18	3	615	89	942	261	0.144715447	0.277070064	1.914585272				1557	350	22.47912653
	ALC18	4	617	98	561	223	0.158833063	0.397504456	2.502655608	2.495083253	0.640434	0.001027725	1178	321	27.24957555
STAT6	ALC19	1	97	12	476	105	0.12371134	0.220588235	1.783088235				573	117	20.41884817
	ALC19	2	182	47	345	103	0.258241758	0.298550725	1.15609004				527	150	28.4629981
	ALC19	3	452	56	542	115	0.123893805	0.212177122	1.712572483				994	171	17.20321932
	ALC19	4	313	20	416	64	0.063897764	0.153846154	2.407692308	1.764860766	0.512152	0.020296067	729	84	11.52263374

Table S2. Secondary antibodies

Species and fluorophore	Catalogue code
Donkey anti-mouse 488	A21202
Goat anti-chick 488	A11039
Donkey anti-goat 488	A11055
Donkey anti-rabbit 488	A21206
Donkey anti-mouse 546	A10036
Donkey anti-rabbit 546	A10040
Donkey anti-goat 555	A21432
Goat anti-hamster 568	A21112
Donkey anti-rat 594	A21209
Donkey anti-mouse 647	A31571
Donkey anti-rabbit 647	A31573
Goat anti hamster 647	A21451
Goat anti-rat 647	A21247

Supplementary Materials and Methods

Measurement of protein expression levels

A custom macro (below) for ImageJ was used for quantification of protein expression. To install the plugin, put the file in the `Fiji.app/Plugins/' folder and restart Fiji or run `Help/Refresh' menus. To access the relevant part of the filesystem under MacOS, Ctrl-click on the Fiji application icon to open the contextual menu and click `Show Package Contents'.

Click here to Download macro file