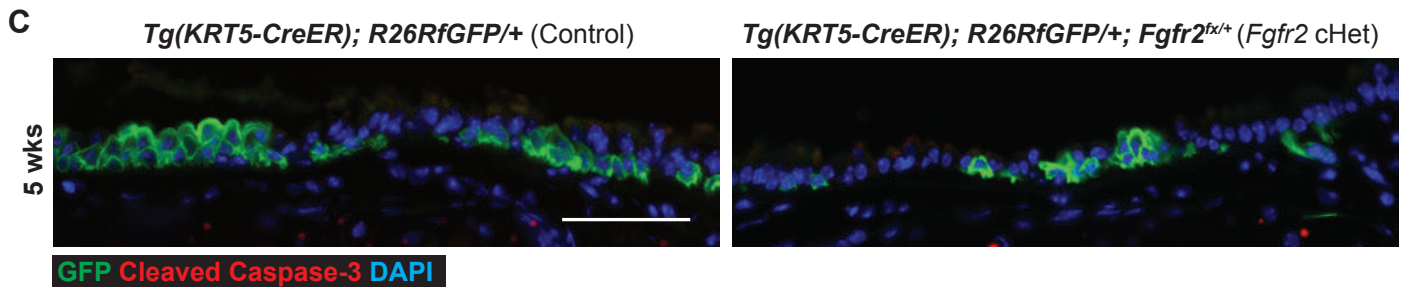
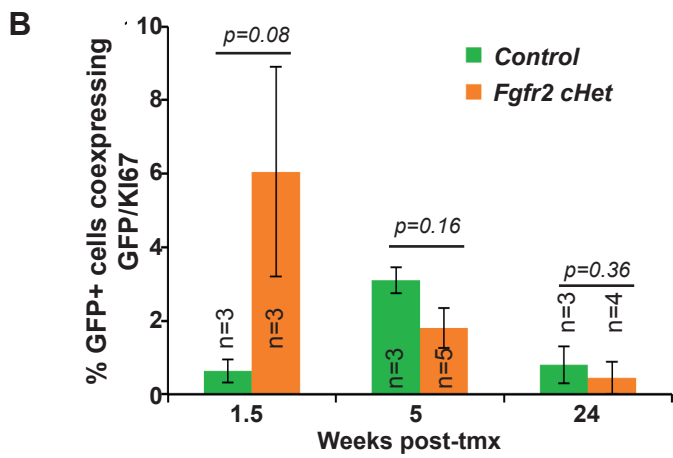
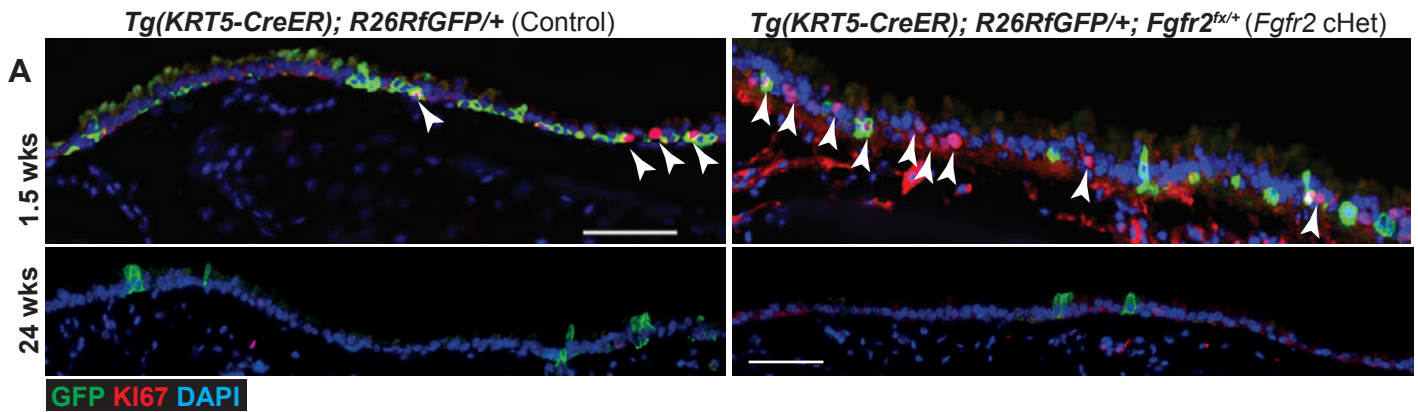


Figure S1. No evidence for cell competition between *Fgfr2^{A/+}* and *Fgfr2^{+/+}* basal cells in vitro. (A) Experimental set-up in B. Freshly isolated basal cells were mixed at a 1:2 ratio, grown to confluence on cell culture inserts and imaged at intervals for 10 days. In cultures with no competition both cell populations will continue at the same ratio, whereas in cultures with competition the patch size of the “loser” cell population will decrease over time. (B) 0 and 240 hour frames from phase contrast/red channel time-lapse experiments. Upper panel: control experiment, red cells: *Fgfr2^{A/+}*, unlabelled cells: *Fgfr2^{A/+}*. Lower panel: competition experiment, red: *Fgfr2^{A/+}*, unlabelled: *Fgfr2^{+/+}*. No evidence for competition was observed. (C) Experimental set-up in D. Freshly isolated basal cells were mixed at a 1:2 ratio, grown to confluence on cell culture inserts, mechanically wounded using a pipette tip and imaged at intervals for 5 days. In cultures with no competition labelled and unlabelled cells will contribute approximately equally to wound closure. In cultures with competition, the “loser” cell population will contribute less to wound closure. (D) 0 hour and 114 hour frames from phase contrast/red channel time-lapse experiments. Upper panel: control experiment, red cells: *Fgfr2^{A/+}*, unlabelled cells: *Fgfr2^{A/+}*. Lower panels: competition experiment, red cells: *Fgfr2^{A/+}*, unlabelled cells: *Fgfr2^{+/+}*. No evidence for competition was observed. Bar = 0.5 mm in all panels. See also movies 1-5.



D E18.5 *GR*^{-/-} lung (Positive control for Caspase-3 staining)

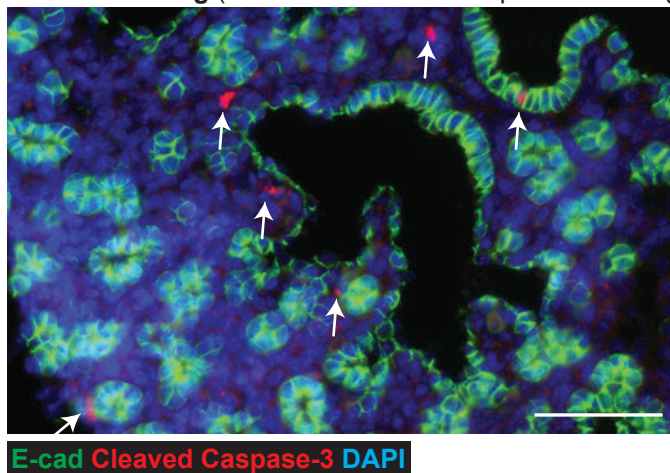


Figure S2. *Fgfr2* conditional heterozygous basal cells can proliferate and show no evidence of apoptosis. (A) Sections from control *Tg(KRT5-CreER); Rosa26R^{fGFP/+}* and cHet *Tg(KRT5-CreER); Rosa26R^{fGFP/+}; Fgfr2^{fx/+}* tracheae at 1.5 and 24 weeks post-tmx. Green: GFP (*Rosa* reporter); red: KI67 (proliferating cells); blue: DAPI (nuclei). Arrowheads mark KI67 positive cells. (B) Quantitation of the percentage of GFP⁺ cells that co-express KI67 throughout the experimental timecourse. Error bars = sem. (C) Sections from control *Tg(KRT5-CreER); Rosa26R^{fGFP/+}* and cHet *Tg(KRT5-CreER); Rosa26R^{fGFP/+}; Fgfr2^{fx/+}* tracheae at 5 weeks post-tmx. Green: GFP (*Rosa* reporter); red: Cleaved Caspase-3 (apoptotic cells); blue: DAPI (nuclei). (D) Section of E18.5 Glucocorticoid receptor null lung (GR^{-/-}, also known as *Nr3c1*) as a positive control for Cleaved Caspase-3 staining. Green: E-cadherin (lateral membranes); red: Cleaved Caspase-3 (apoptotic cells); blue: DAPI (nuclei). Scale bar = 50 μ m in all panels.

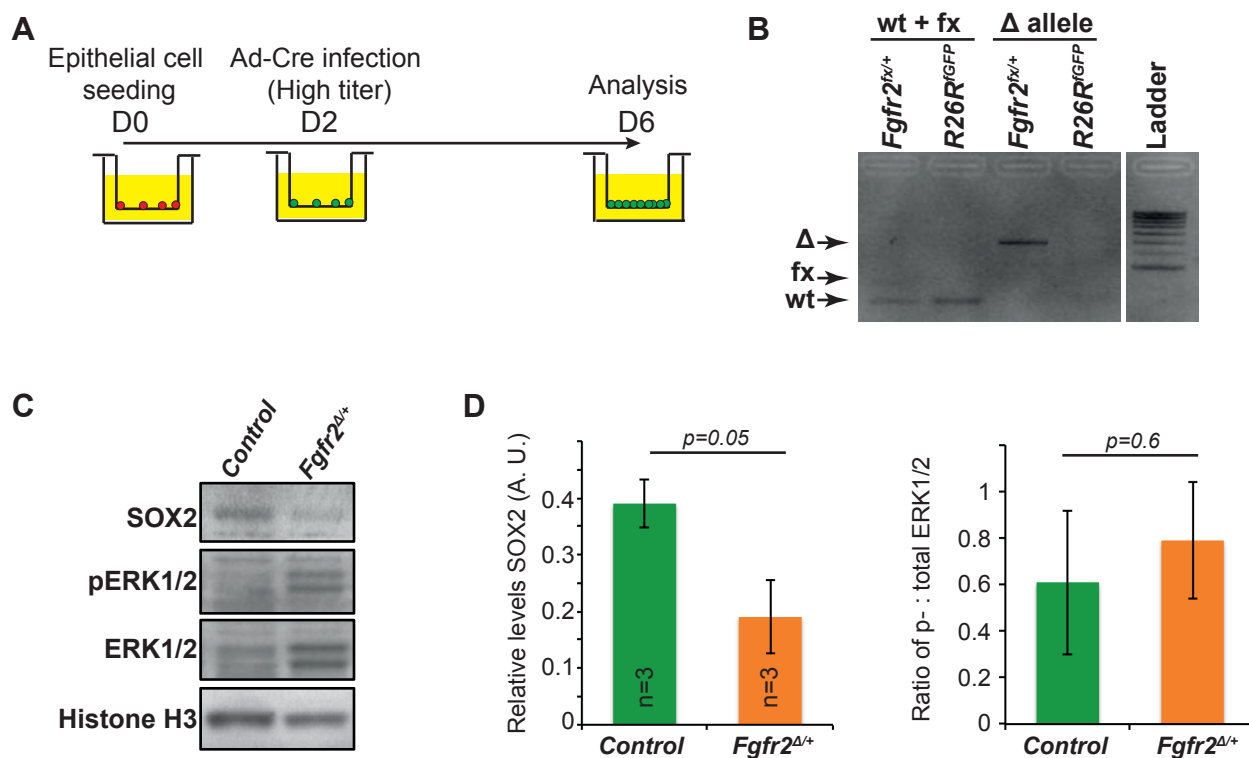


Figure S3. *Fgfr2* conditional heterozygous tracheal cells fail to terminally differentiate and self-renew in vitro

(A) Experimental schematic. Control (*Rosa26R^{fGFP/fGFP}*) and cHet (*Rosa26R^{fGFP/+}; Fgfr2^{fx/+}*) tracheal epithelial cells were seeded in BC expansion conditions and infected with Ad-Cre at day 2. On day 4 BCs were passaged onto new collagen-coated inserts for further expansion and ALI differentiation. (B) cHet BCs attach and proliferate post-passaging on collagen-coated inserts. (C, D) Control cultures form fully-differentiated monolayers containing multiciliated cells (C) and differentiated BCs (D) by 12 days post-seeding, but cHet BCs do not reach confluence and do not express differentiated markers in vitro. Arrows: fragmented nuclei, or multi-nucleate cells, seen in cHet cultures, but not controls. (E) Experimental schematic. Control (*Rosa26R^{fGFP/fGFP}*) and cHet (*Rosa26R^{fGFP/+}; Fgfr2^{fx/+}*) tracheal epithelial cells were seeded in BC expansion conditions and infected with Ad-Cre at day 2. On day 4 BCs were passaged into matrigel for sphere-forming assays. (F) Representative confocal sections of control and *Fgfr2* cHet cultures 2 days post-seeding in matrigel. Green: KRT8; red: KRT5. (G) Images of control and *Fgfr2* cHet tracheospheres 9 days post-seeding in matrigel. (H) Tracheosphere diameter, arbitrary units. Scale bars = 100 μ m (B-D, G); 5 μ m (F).

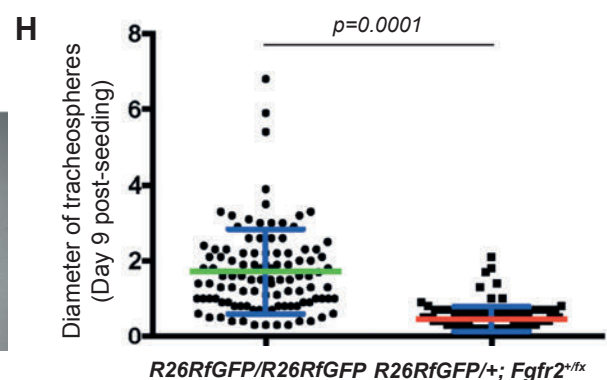
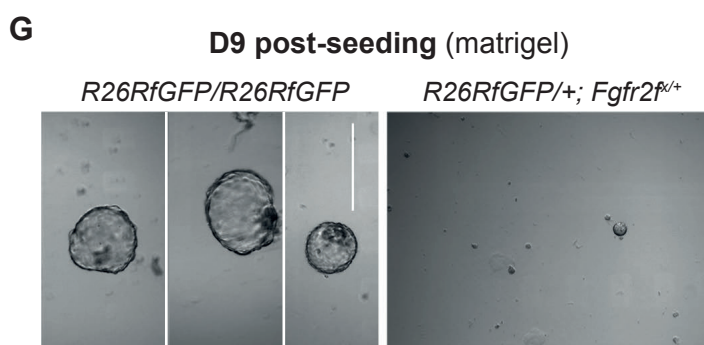
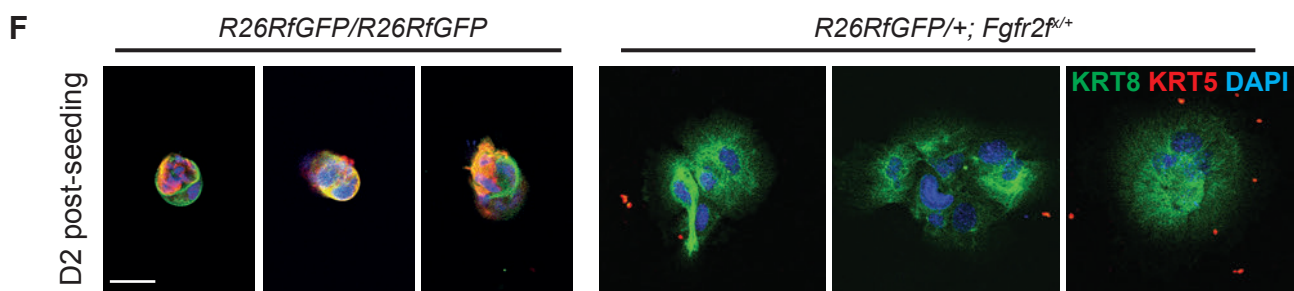
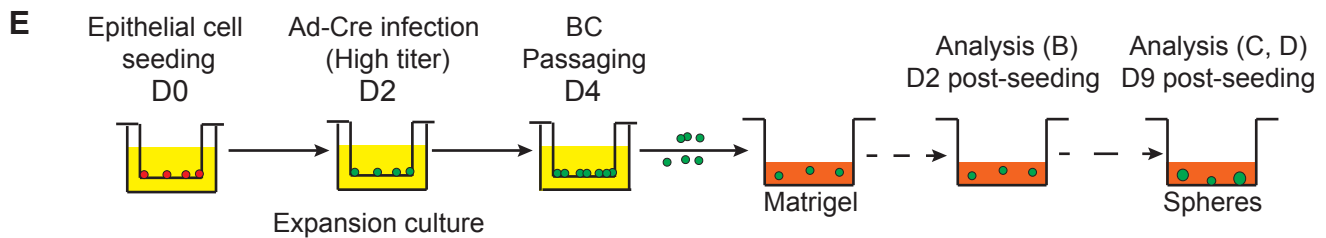
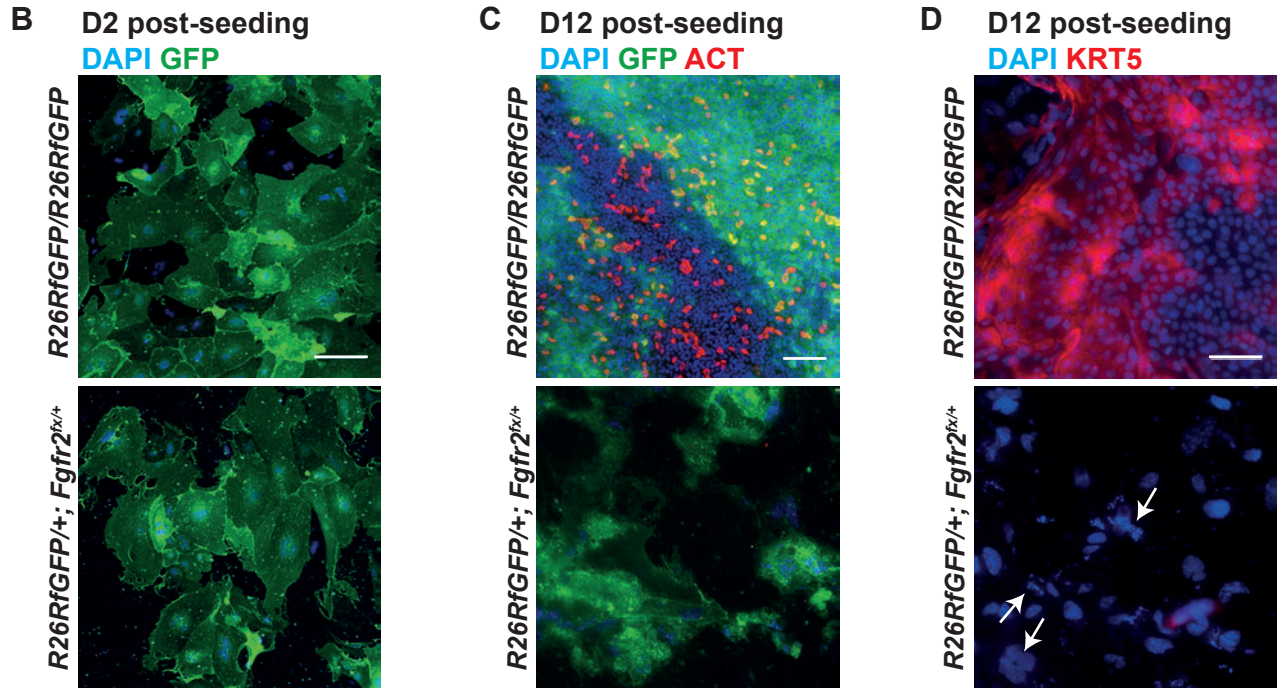
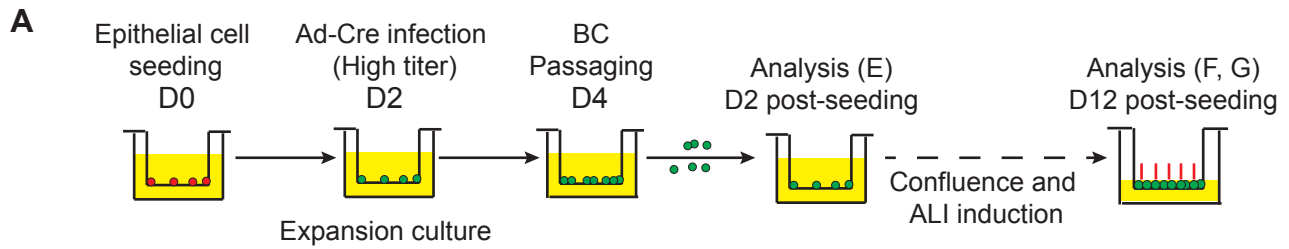


Figure S4. Decrease in FGFR2 signalling in vitro does not affect levels of MEK-ERK signalling. (A) Schematic of in vitro experimental time-course. (B) Representative genotyping (gDNA) PCR from *Rosa26R^{fGFP/fGFP}* and *Rosa26R^{fGFP/+}; Fgfr2^{fx/+}* viral-infected cells at day 6. Note that the cHet cells have efficient amplification of the wild-type (wt) and deleted (Δ) alleles, but very little amplification of the floxed (fx) allele indicating high levels of recombination in vitro. (C) Representative western blots from control and *Fgfr2* cHet day 6 basal cells showing levels of SOX2, pERK1/2, total ERK and Histone H3. (F) Quantification of protein levels in (E).

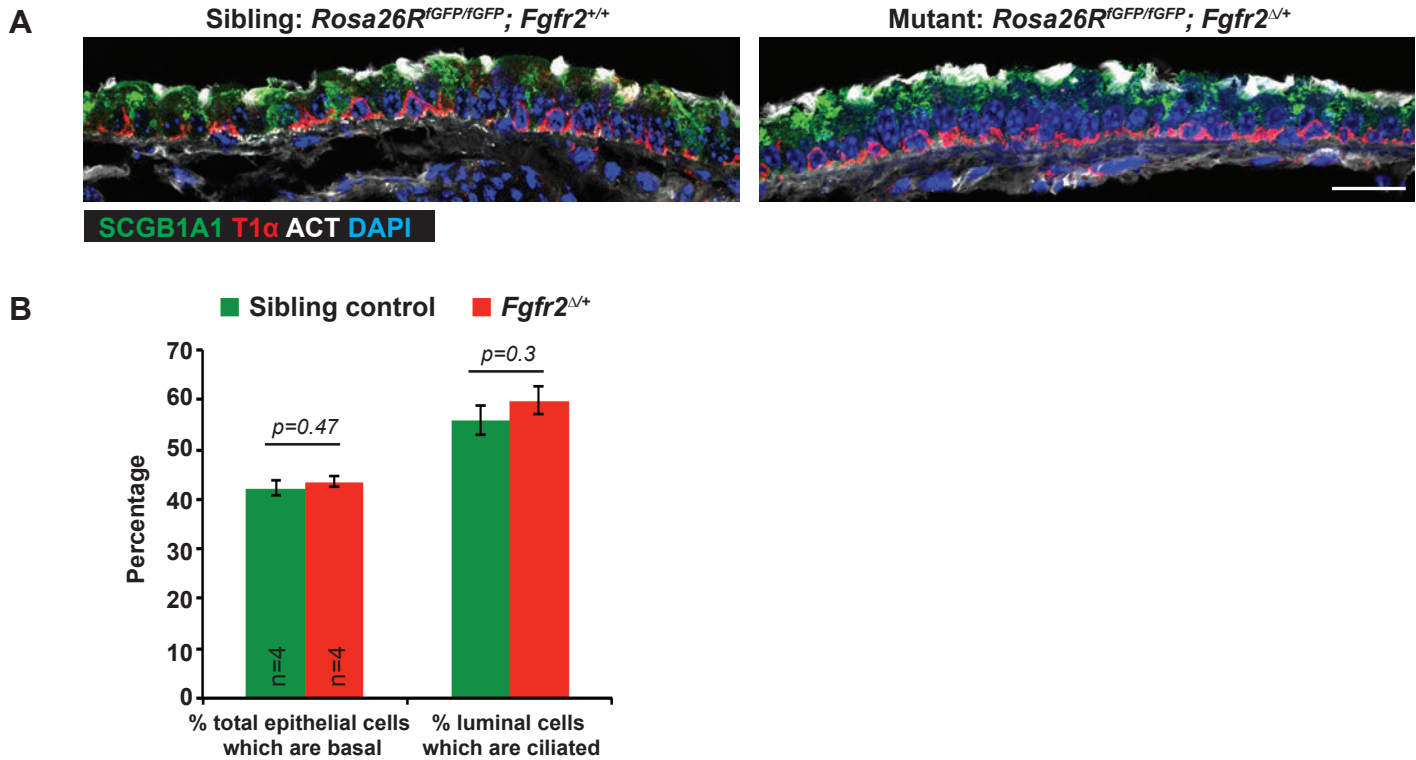
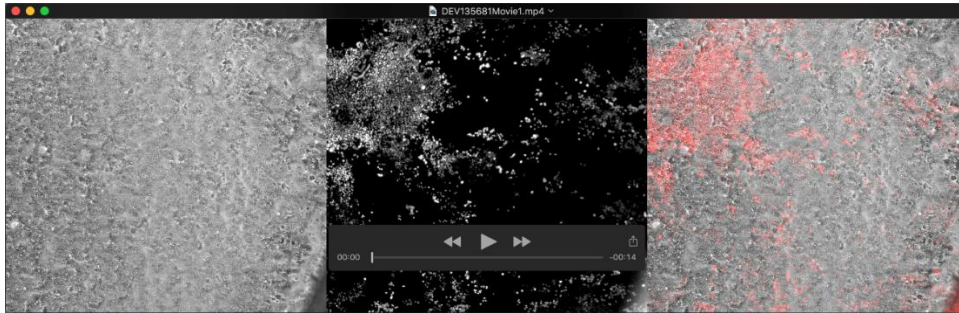


Figure S5. *Fgfr2^{Δ/+}* adult mice have a normal tracheal epithelium. (A) Representative sections from control *Rosa26R^{fGFP/fGFP}* and sibling *Rosa26R^{fGFP/fGFP}; Fgfr2^{Δ/+}* tracheae. Green: SCGB1A1 (secretory cells); red: T1α (basal cells); white: acetylated tubulin (cilia); blue: DAPI. (B) Quantitation of the percentage of epithelial cells which are basal, and luminal cells which are ciliated in the two genotypes. Error bars = sem. Scale bar = 20 μm.

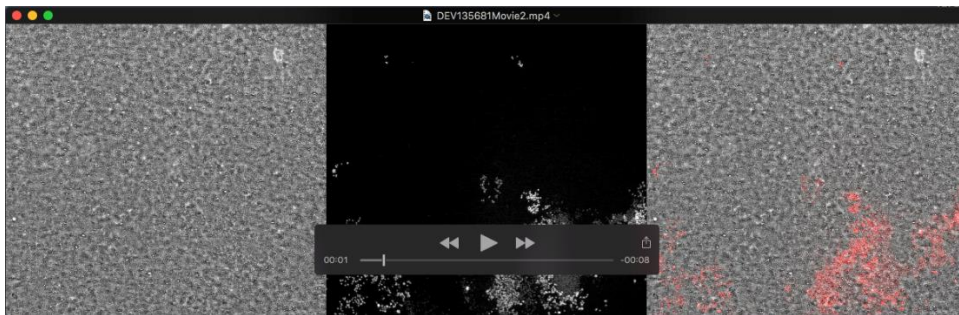
Fig. S6. Raw cell counts

Fgf2 conditional heterozygous cell counts					Figure S2					Figure 1					Figure 2					Figure 2				
3.5 weeks post-tmx	Total DAPI+ cells	Total GFP+ cells	Ki67+, GFP+ cells	Ki67+, GFP+ cells	% of total GFP+ cells that are dual GFP+, Ki67+	Total DAPI+ cells	Tia+, GFP+ cells	Tia2+GFP+ cells	Tia+, GFP+ cells	% of total Tia+ basal cells that are dual GFP+, Tia+	% of total luminal Tia+ cells that are GFP+	GFP+, Ki67+ basal cells	GFP+, Ki67+ luminal cells	Ki67+ cells	Ki67+ cells	% GFP+ columnar cells from total GFP+	GFP+, SCGB1A1+ sub cells	GFP+, MUC5AC+ goblet cells	GFP+, MUC5AC+ cells	GFP+, SCGB1A1+, MUC5AC+ cells	% GFP+ cells which are GFP+, SCGB1A1+ sub cells	% GFP+ cells which are GFP+, MUC5AC+ cells	% GFP+ cells which are GFP+, SCGB1A1+, MUC5AC+	
1	1033	64	6	0	0.0	1164	564	80	1	14.8	0.17	291	27	0	10	10	196	0	259	0	43.1	56.9	0	
2	1469	96	24	1	1.0	1007	934	201	5	21.92	0.61	227	46	0	2	10.2	139	0	238	0	36.9	63.1	0	
3	1024	228	6	2	0.9	1573	789	307	7	38.91	0.89	271	18	0	5	11	75	0	184	0	29.2	70.8	0	
4	KRT5-CreER/+; R26-RFP/+; Fgf2 D/+	216	25	3	1	4.0	648	238	82	493	34.45	159	32	0	8	14.3	31	0	82	0	27.4	72.6	0	
5	KRT5-CreER/+; R26-RFP/+; Fgf2 D/+	1523	203	87	18	8.8	1607	677	221	325	32.64	136	22	0	6	22.2	30	0	44	0	40.5	59.5	0	
6	KRT5-CreER/+; R26-RFP/+; Fgf2 D/+	1523	285	48	9	1.2	1472	752	241	341	32.05	133	24	0	9	16.2	119	0	109	0	52.2	47.8	0	
7	KRT5-CreER/+; R26-RFP/+; Fgf2 D/+	1001	263	20	9	2.4	1451	529	212	357	40.08	267	55	0	20	24.6	63	0	94	1	39.9	59.5	0.6	
8	KRT5-CreER/+; R26-RFP/+; Fgf2 D/+	1241	429	8	2	0.5	1195	685	301	384	43.84	190	5	0	20	24.6	139	0	135	2	50.4	49.9	0.7	
9	KRT5-CreER/+; R26-RFP/+; Fgf2 D/+	1171	408	22	11	2.7	1283	583	234	349	40.14	159	32	0	8	14.3	31	0	82	0	27.4	72.6	0	
10	KRT5-CreER/+; R26-RFP/+; Fgf2 D/+	1072	218	9	2	0.9	1149	441	151	290	34.24	136	22	0	6	22.2	30	0	44	0	40.5	59.5	0	
11	KRT5-CreER/+; R26-RFP/+; Fgf2 D/+	970	347	21	6	1.7	1505	733	276	457	37.65	164	55	0	14	28.9	119	0	109	0	52.2	47.8	0	
12	KRT5-CreER/+; R26-RFP/+; Fgf2 D/+	1001	263	20	9	2.4	1451	529	212	357	40.08	267	55	0	20	24.6	63	0	94	1	39.9	59.5	0.6	
13	KRT5-CreER/+; R26-RFP/+; Fgf2 D/+	1241	429	8	2	0.5	1195	685	301	384	43.84	190	5	0	20	24.6	139	0	135	2	50.4	49.9	0.7	
14	KRT5-CreER/+; R26-RFP/+; Fgf2 D/+	1171	408	22	11	2.7	1283	583	234	349	40.14	159	32	0	8	14.3	31	0	82	0	27.4	72.6	0	
15	KRT5-CreER/+; R26-RFP/+; Fgf2 D/+	1072	218	9	2	0.9	1149	441	151	290	34.24	136	22	0	6	22.2	30	0	44	0	40.5	59.5	0	
16	KRT5-CreER/+; R26-RFP/+; Fgf2 D/+	970	347	21	6	1.7	1505	733	276	457	37.65	164	55	0	14	28.9	119	0	109	0	52.2	47.8	0	
17	KRT5-CreER/+; R26-RFP/+; Fgf2 D/+	1001	263	20	9	2.4	1451	529	212	357	40.08	267	55	0	20	24.6	63	0	94	1	39.9	59.5	0.6	
18	KRT5-CreER/+; R26-RFP/+; Fgf2 D/+	1241	429	8	2	0.5	1195	685	301	384	43.84	190	5	0	20	24.6	139	0	135	2	50.4	49.9	0.7	
19	KRT5-CreER/+; R26-RFP/+; Fgf2 D/+	1171	408	22	11	2.7	1283	583	234	349	40.14	159	32	0	8	14.3	31	0	82	0	27.4	72.6	0	
20	KRT5-CreER/+; R26-RFP/+; Fgf2 D/+	1072	218	9	2	0.9	1149	441	151	290	34.24	136	22	0	6	22.2	30	0	44	0	40.5	59.5	0	
21	KRT5-CreER/+; R26-RFP/+; Fgf2 D/+	970	347	21	6	1.7	1505	733	276	457	37.65	164	55	0	14	28.9	119	0	109	0	52.2	47.8	0	
22	KRT5-CreER/+; R26-RFP/+; Fgf2 D/+	1001	263	20	9	2.4	1451	529	212	357	40.08	267	55	0	20	24.6	63	0	94	1	39.9	59.5	0.6	
23	KRT5-CreER/+; R26-RFP/+; Fgf2 D/+	1241	429	8	2	0.5	1195	685	301	384	43.84	190	5	0	20	24.6	139	0	135	2	50.4	49.9	0.7	
24	KRT5-CreER/+; R26-RFP/+; Fgf2 D/+	1171	408	22	11	2.7	1283	583	234	349	40.14	159	32	0	8	14.3	31	0	82	0	27.4	72.6	0	
25	KRT5-CreER/+; R26-RFP/+; Fgf2 D/+	1072	218	9	2	0.9	1149	441	151	290	34.24	136	22	0	6	22.2	30	0	44	0	40.5	59.5	0	
26	KRT5-CreER/+; R26-RFP/+; Fgf2 D/+	970	347	21	6	1.7	1505	733	276	457	37.65	164	55	0	14	28.9	119	0	109	0	52.2	47.8	0	
27	KRT5-CreER/+; R26-RFP/+; Fgf2 D/+	1001	263	20	9	2.4	1451	529	212	357	40.08	267	55	0	20	24.6	63	0	94	1	39.9	59.5	0.6	
28	KRT5-CreER/+; R26-RFP/+; Fgf2 D/+	1241	429	8	2	0.5	1195	685	301	384	43.84	190	5	0	20	24.6	139	0	135	2	50.4	49.9	0.7	
29	KRT5-CreER/+; R26-RFP/+; Fgf2 D/+	1171	408	22	11	2.7	1283	583	234	349	40.14	159	32	0	8	14.3	31	0	82	0	27.4	72.6	0	
30	KRT5-CreER/+; R26-RFP/+; Fgf2 D/+	1072	218	9	2	0.9	1149	441	151	290	34.24	136	22	0	6	22.2	30	0	44	0	40.5	59.5	0	
31	KRT5-CreER/+; R26-RFP/+; Fgf2 D/+	970	347	21	6	1.7	1505	733	276	457	37.65	164	55	0	14	28.9	119	0	109	0	52.2	47.8	0	
32	KRT5-CreER/+; R26-RFP/+; Fgf2 D/+	1001	263	20	9	2.4	1451	529	212	357	40.08	267	55	0	20	24.6	63	0	94	1	39.9	59.5	0.6	
33	KRT5-CreER/+; R26-RFP/+; Fgf2 D/+	1241	429	8	2	0.5	1195	685	301	384	43.84	190	5	0	20	24.6	139	0	135	2	50.4	49.9	0.7	
34	KRT5-CreER/+; R26-RFP/+; Fgf2 D/+	1171	408	22	11	2.7	1283	583	234	349	40.14	159	32	0	8	14.3	31	0	82	0	27.4	72.6	0	
35	KRT5-CreER/+; R26-RFP/+; Fgf2 D/+	1072	218	9	2	0.9	1149	441	151	290	34.24	136	22	0	6	22.2	30	0	44	0	40.5	59.5	0	
36	KRT5-CreER/+; R26-RFP/+; Fgf2 D/+	970	347	21	6	1.7	1505	733	276	457	37.65	164	55	0	14	28.9	119	0	109	0	52.2	47.8	0	
37	KRT5-CreER/+; R26-RFP/+; Fgf2 D/+	1001	263	20	9	2.4	1451	529	212	357	40.08	267	55	0	20	24.6	63	0	94	1	39.9	59.5	0.6	
38	KRT5-CreER/+; R26-RFP/+; Fgf2 D/+	1241	429	8	2	0.5	1195	685	301	384	43.84	190	5	0	20	24.6	139	0	135	2	50.4	49.9	0.7	
39	KRT5-CreER/+; R26-RFP/+; Fgf2 D/+	1171	408	22	11	2.7	1283	583	234	349	40.14	159	32	0	8	14.3	31	0	82	0	27.4	72.6	0	
40	KRT5-CreER/+; R26-RFP/+; Fgf2 D/+	1072	218	9	2	0.9	1149	441	151	290	34.24	136	22	0	6	22.2	30	0	44	0	40.5	59.5	0	
41	KRT5-CreER/+; R26-RFP/+; Fgf2 D/+	970	347	21	6	1.7	1505	733	276	457	37.65	164	55	0	14	28.9	119	0	109	0	52.2	47.8	0	
42	KRT5-CreER/+; R26-RFP/+; Fgf2 D/+	1001	263	20	9	2.4	1451	529	212	357	40.08	267	55	0	20	24.6	63	0	94	1	39.9	59.5	0.6	
43	KRT5-CreER/+; R26-RFP/+; Fgf2 D/+	1241	429	8	2	0.5	1195	685	301	384	43.84	190	5	0	20	24.6	139	0	135	2	50.4	49.9	0.7	
44	KRT5-CreER/+; R26-RFP/+; Fgf2 D/+	1171	408	22	11	2.7	1283	583	234	349	40.14	159	32	0	8	14.3	31	0	82	0	27.4	72.6	0	
45	KRT5-CreER/+; R26-RFP/+; Fgf2 D/+	1072	218	9	2	0.9	1149	441	151	290	34.24	136	22	0	6	22.2	30	0	44	0	40.5	59.5	0	
46	KRT5-CreER/+; R26-RFP/+; Fgf2 D/+	970	347	21	6	1.7	1505	733	276	457	37.65	164	55	0	14	28.9	119	0	109	0	52.2	47.8	0	
47	KRT5-CreER/+; R26-RFP/+; Fgf2 D/+	1001	263	20	9	2.4	1451	529	212	357	40.08	267	55	0	20	24.6	63	0	94	1	39.9	59.5	0.6	
48	KRT5-CreER/+; R26-RFP/+; Fgf2 D/+	1241	429	8	2	0.5	1195	685	301	384	43.84	190	5	0	20	24.6	139	0	135	2	50.4	49.9	0.7	
49	KRT5-CreER/+; R26-RFP/+; Fgf2 D/+	1171	408	22	11	2.7	1283	583	234	349	40.14	159	32	0	8	14.3	31	0	82	0	27.4	72.6	0	
50	KRT5-CreER/+; R26-RFP/+; Fgf2 D/+	1072	218	9	2	0.9	1149	441	151	290	34.24	136	22	0	6	22.2	30	0	44	0	40.5	59.5	0	
51	KRT5-CreER/+; R26-RFP/+; Fgf2 D/+	970	347	21	6	1.7	1505	733	276	457	37.65	164	55	0	14	28.9	119	0	109	0	52.2	47.8	0	
52	KRT5-CreER/+; R26-RFP/+; Fgf2 D/+	1001	263	20	9	2.4	1451	529	212	357														

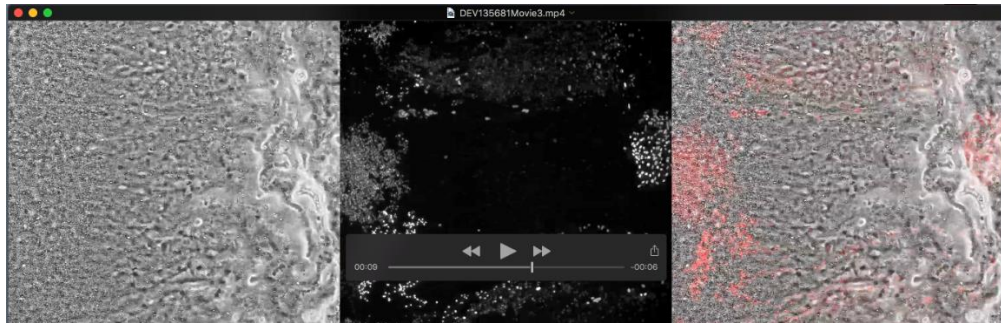
Movies



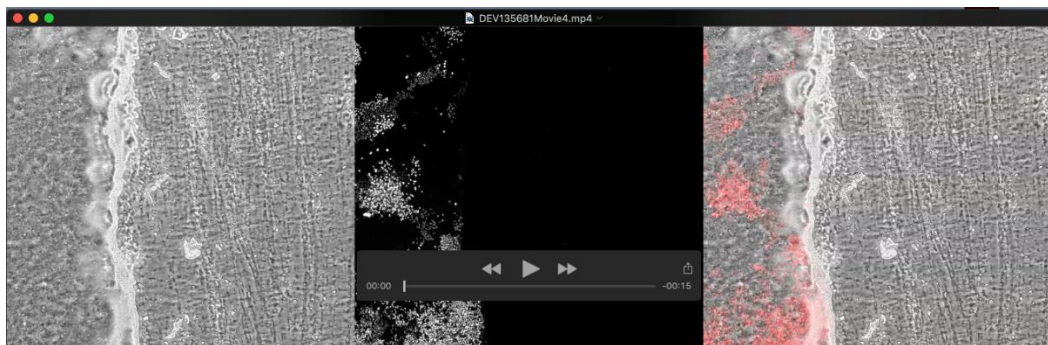
Movie 1. In vitro cell competition at confluence, control culture. Red cells: *Fgfr2^{Δ/+}*, unlabelled cells: *Fgfr2^{Δ/+}*. Confluent culture imaged every 4 hours for 10 days in a Nikon Biostation. Cell clones do not change in size; no evidence for cell competition.



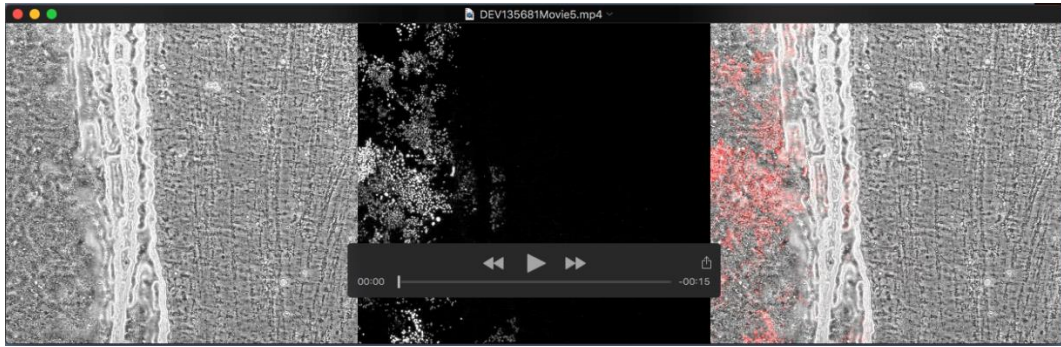
Movie 2. In vitro cell competition at confluence, experimental culture. Red cells: *Fgfr2^{Δ/+}*, unlabelled cells: *Fgfr2^{+/+}*. Confluent culture imaged every 4 hours for 10 days in a Nikon Biostation. Cell clones do not change in size; no evidence for cell competition.



Movie 3. In vitro cell competition following wounding, control culture. Red cells: *Fgfr2*^{Δ/+}, unlabelled cells: *Fgfr2*^{Δ/+}. Confluent culture was wounded and then imaged every 2 hours for 5 days in a Nikon Biostation. Both cell populations expand into the wound equally; no evidence for cell competition.



Movie 4. In vitro cell competition following wounding, experimental culture 1. Red cells: *Fgfr2*^{Δ/+}, unlabelled cells: *Fgfr2*^{+/+}. Confluent culture was wounded and then imaged every 2 hours for 5 days in a Nikon Biostation. Both cell populations expand into the wound equally; no evidence for cell competition.



Movie 5. In vitro cell competition following wounding, experimental culture 2. Red cells: *Fgfr2^{Δ/+}*, unlabelled cells: *Fgfr2^{+/+}*. Confluent culture was wounded and then imaged every 2 hours for 5 days in a Nikon Biostation. Both cell populations expand into the wound equally; no evidence for cell competition.

Table S1. Antibodies**Primary antibodies used for immunostaining on tissue sections or cells**

Protein	Species	Dilution Factor	Antigen Retrieval*	Company	Order number/clone
Acetylated tubulin	Mouse	1:3000	No	Sigma	T7451
Cleaved Caspase-3	Rabbit	1:100	No	AbCam	ab2302
E-cadherin	Rat	1:3000	No	ThermoFisher	13-1900
FGFR2	Rabbit	1:200	No	Santa Cruz	sc-122
GFP	Chick	1:1000	No	AbCam	AB13970
Keratin5	Rabbit	1:500	No	Covance	PRB-160P
Keratin8	Rat	1:200	No	DSHB	TROMA-1
KI67	Mouse	1:200	Yes	BD	550609
MUC5AC	Mouse	1:500	No	ThermoFisher	MS-145P0
SCGB1A1	Goat	1:400	No	Santa Cruz	sc9772
SOX2	Goat	1:200	No	Santa Cruz	clone Y-17
T1 α	Hamster	1:1000	No	DSHB	8.1.1

*Antigen retrieval by boiling tissue sections in 10 mM sodium citrate, pH 8 for Ki67.

Primary antibodies used for western blot

Protein	Dilution Factor	Company	Order number/clone
p-Akt(S473)	1:3000	Cell Signalling	3787
Akt (pan)	1:1000	Cell Signalling	4691
dpErk1/2	1:300	Cell Signalling	4370
Erk1/2 (total)	1:300	Cell Signalling	4695
SOX2	1:3000	AbCam	ab97959
Histone H3	1:10000	AbCam	ab39655
β -actin	1:50000	Sigma	A3854

Fluorescent secondary antibodies

All at 1:2000 from ThermoFisher Scientific (Molecular Probes)

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