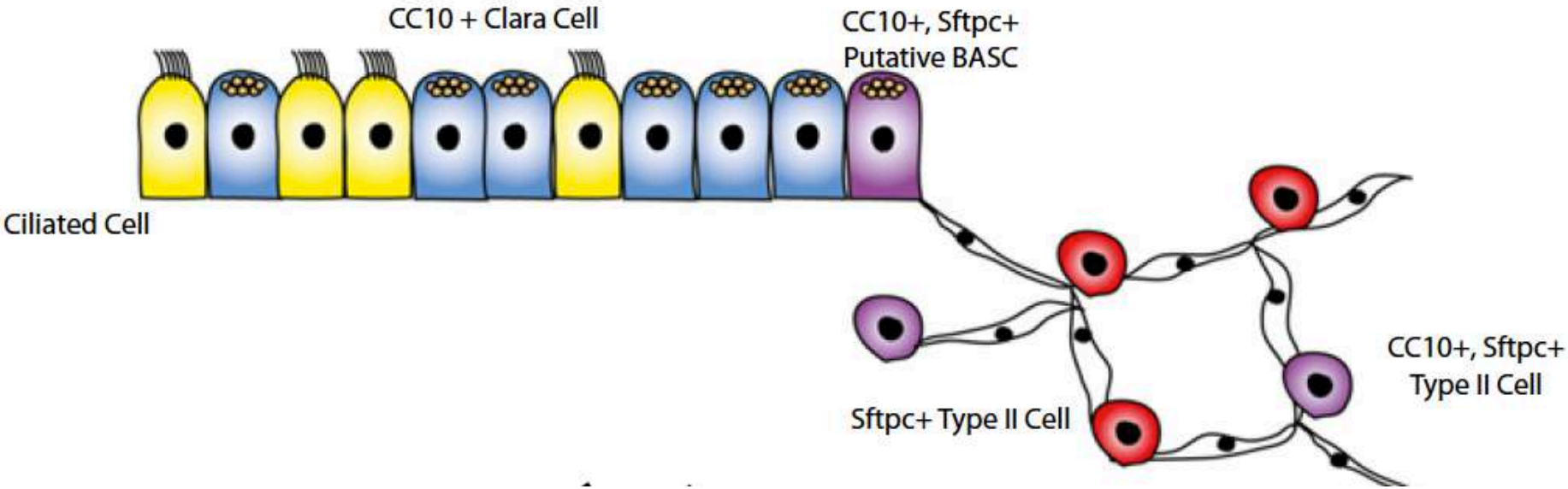
Proximal Differentiation Therapy for KRAS Mutant Lung Adenocarcinoma

November 13, 2017

Questions

- What is the cell of origin of lung adenocarcinoma?
- Are distal adenocarcinomas similar to distal lung progenitors?
- How "plastic" are respiratory epithelial cells?

Distal Epithelium



Differences from intestinal epithelium

- very slow turnover at steady state
- multiple progenitors at steady-state and after injury
  - basal cell, Clara cell, Type II cell
- bronchioalveolar stem cell (BASC) has been described as a stem cell for bronchioles and alveoli and as the cell-of-origin of K-RasG12D-induced adenocarcinoma

Putative BASCs

Identification of Bronchioalveolar Stem Cells in Normal Lung and Lung Cancer

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[1]Center for Cancer Research and Department of Biology
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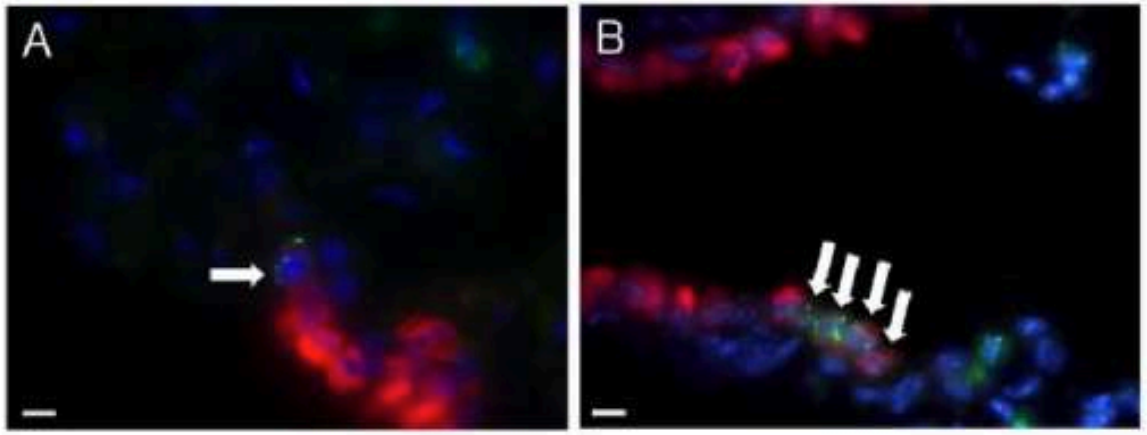
Massachusetts Institute of Technology
Cambridge, Massachusetts 02139

Cell, Vol. 121, 823–835, June 17, 2005,

Adenoviral Cre delivered by inhalation



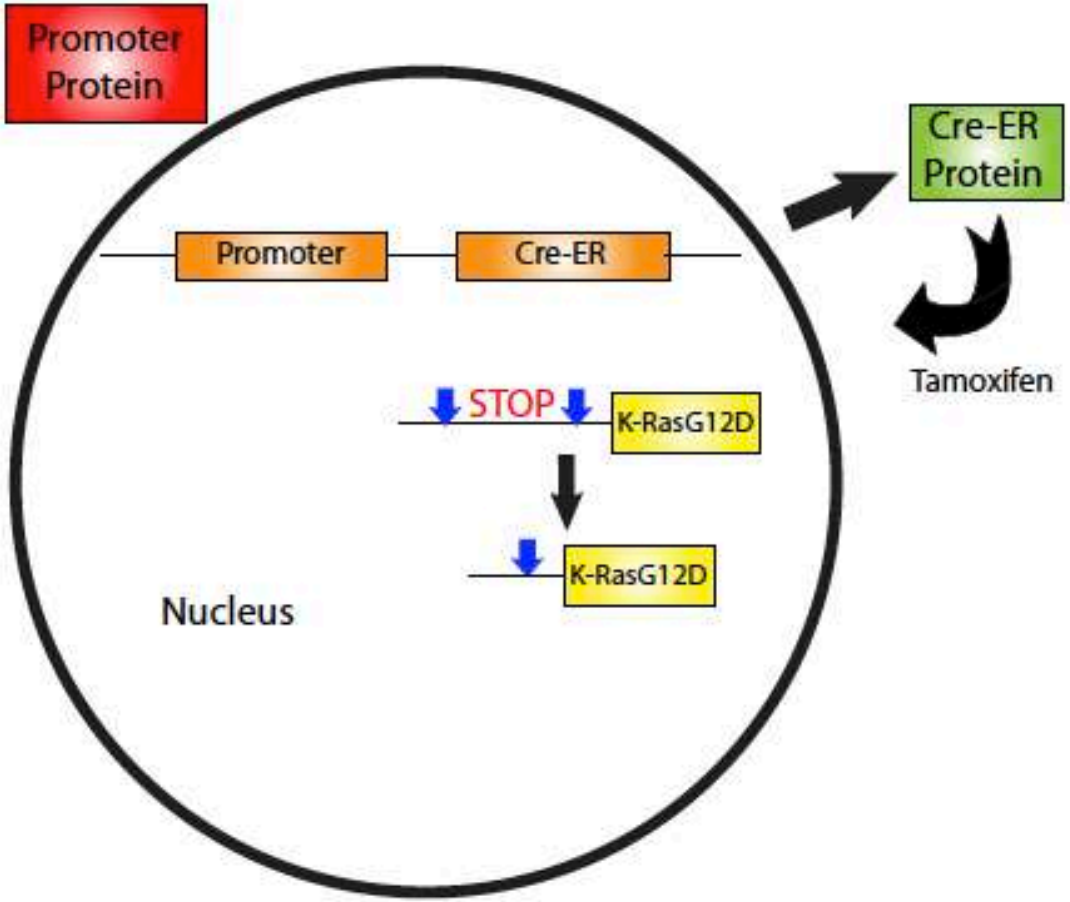
But...

- Lineage tracing using a Clara cell specific mouse demonstrates *NO* alveolar contribution at steady state or after injury (Rawlins et al. 2009 *Cell Stem Cell*, 4:525-34).
- Human K-Ras-mutant tumors are exclusively alveolar (Cooper et al. 1997 *J Pathol*, 181: 401-4).
- A/J mouse chemically-induced tumors (K-Ras) resemble Type II cells via electron microscopy (Belinsky et al 1992 *Cancer Res*, 52: 3164-73).

Hypothesis

Type II cells are another cell-of-origin for K-Ras-induced lung adenocarcinoma

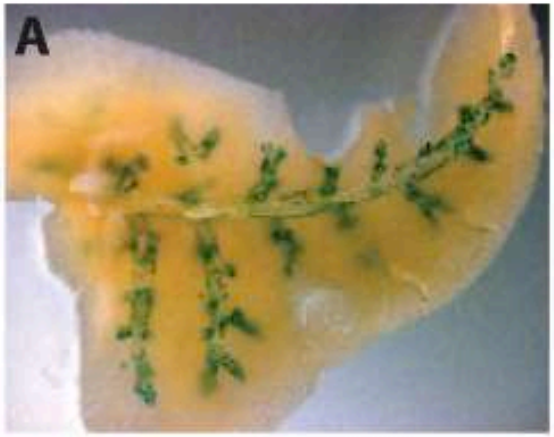
CreER-loxP system: Controlling expression of genes in time and space

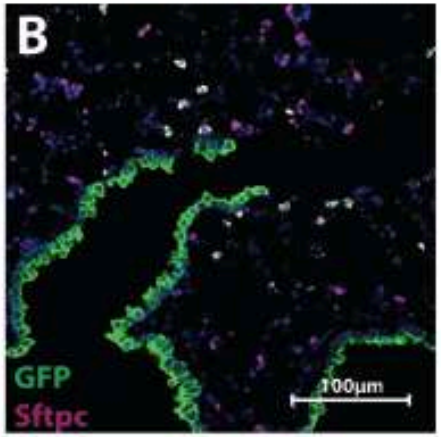


We have CC10-CreER and Sftpc-CreER "knock-in" mouse lines

CC10CreER expresses throughout airway and in a subset of Type II cells

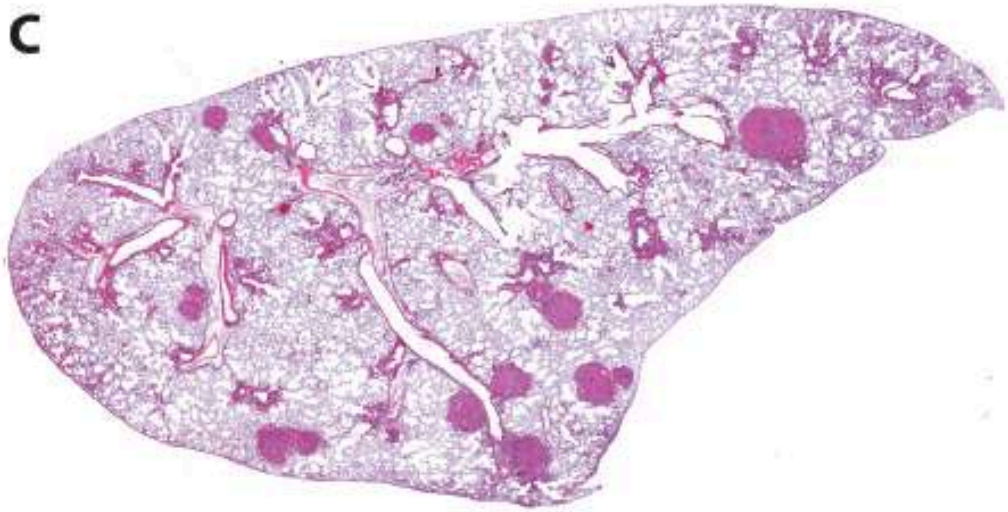
CC10CreER;
lsl LacZ



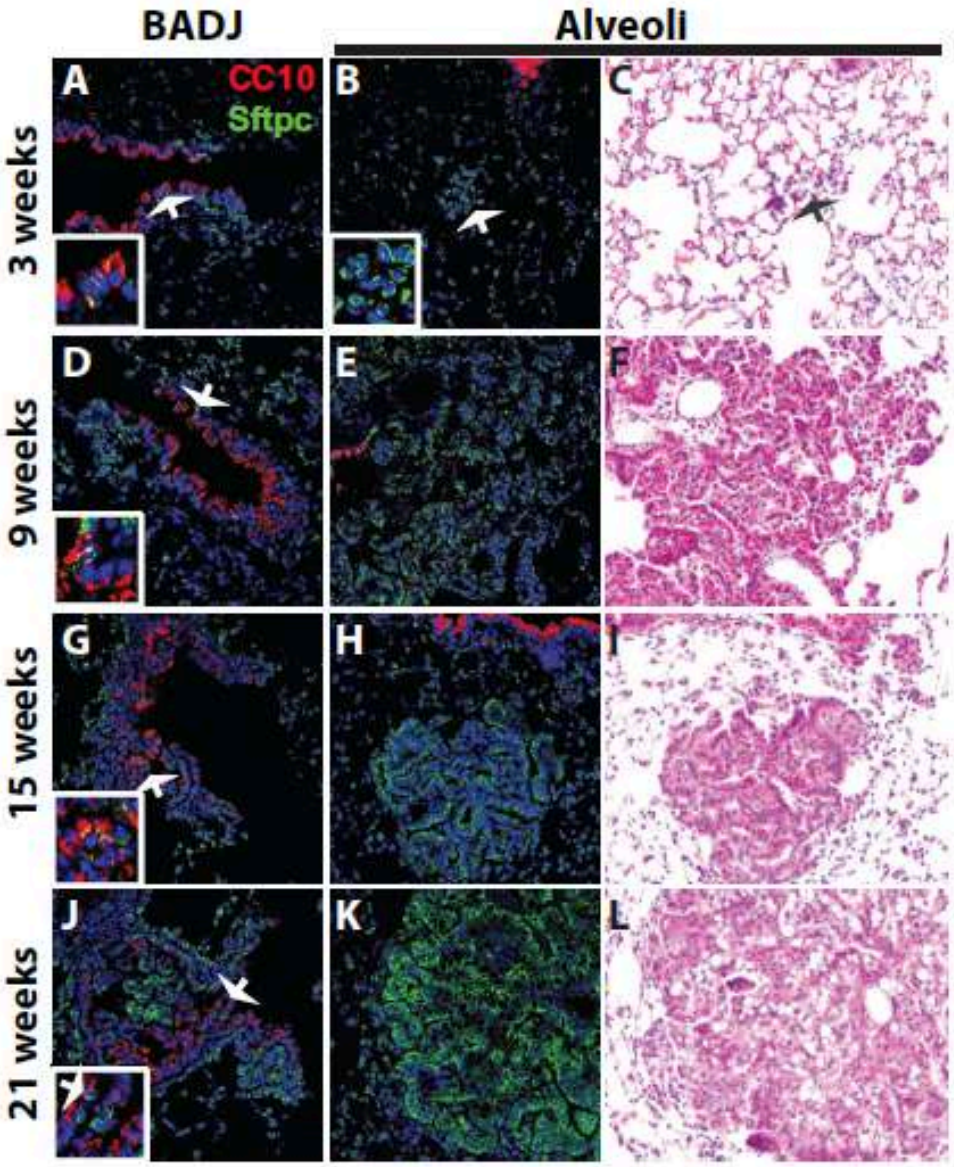


CC10CreER;
lsl fGFP

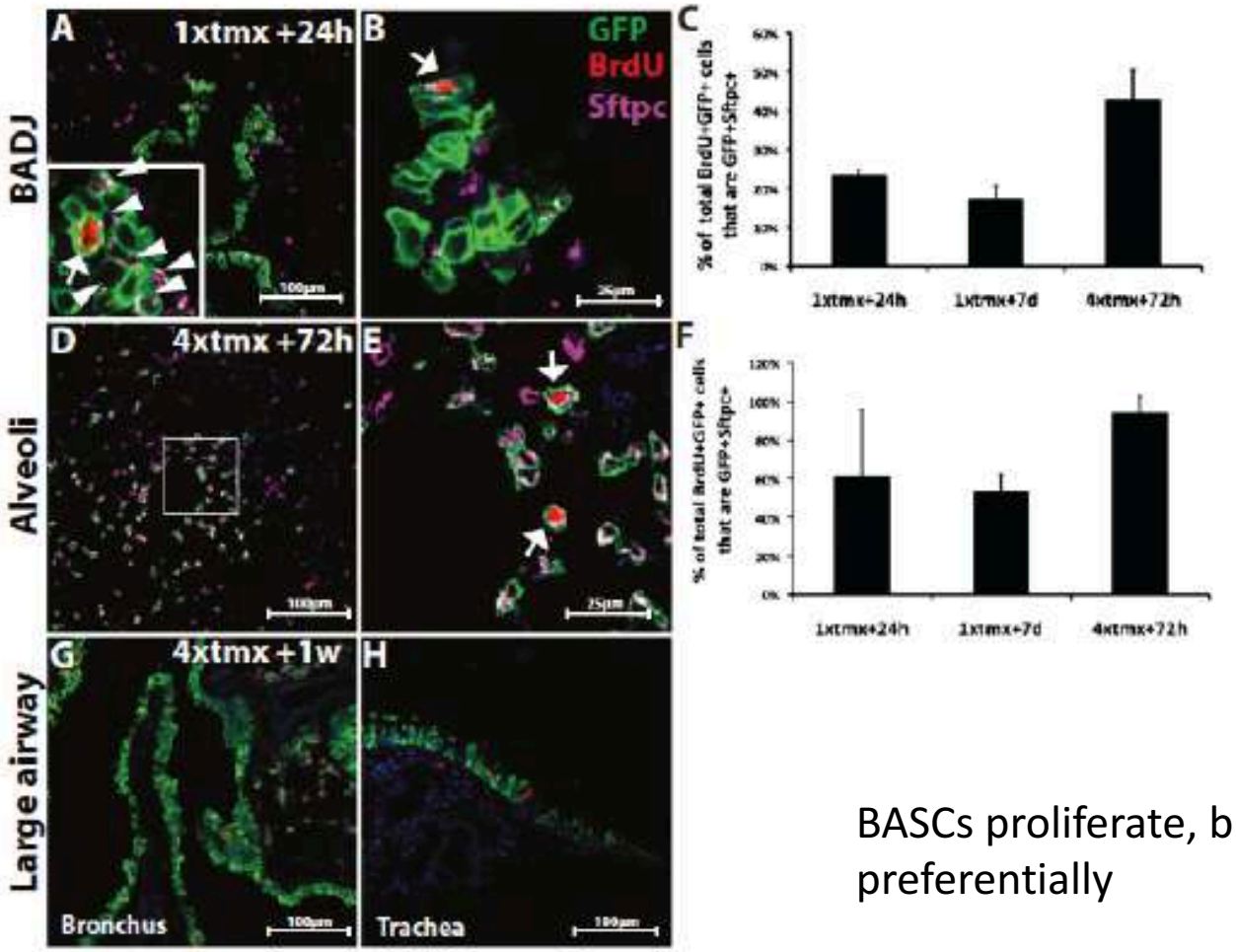
CC10CreER;
lsl K-RasG2D



Adenoma/Adenocarcinoma Sequence

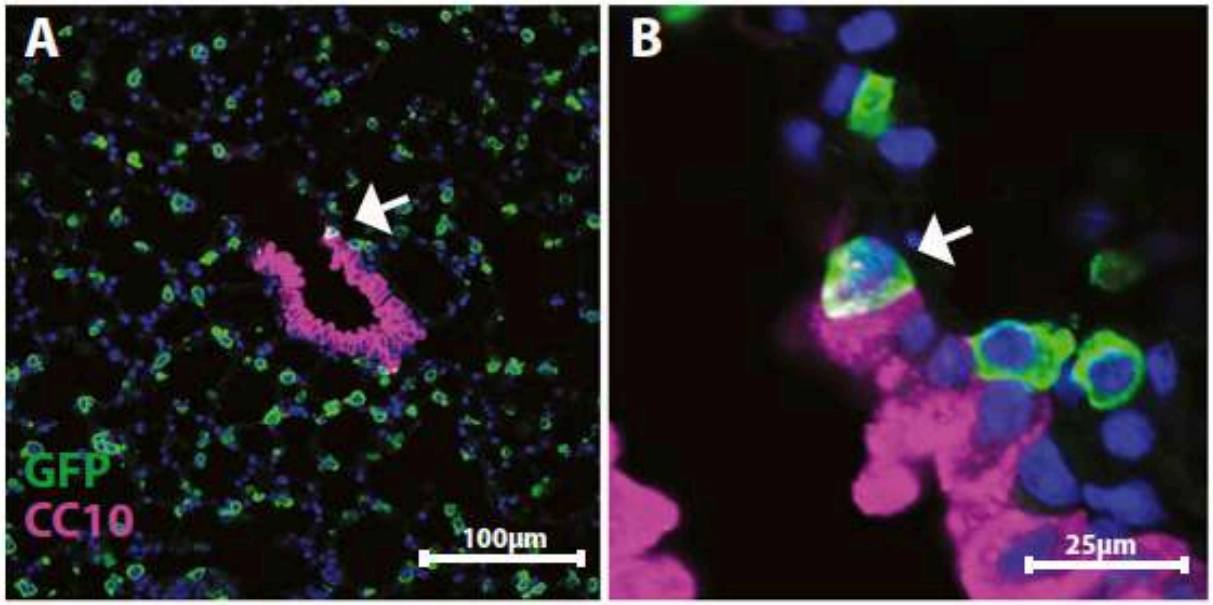


Lineage Tracing of CC10-Expressing Cells upon K-RasG12D Induction–CC10CreER; lsl K-RasG12D; lsl fGFP Mouse

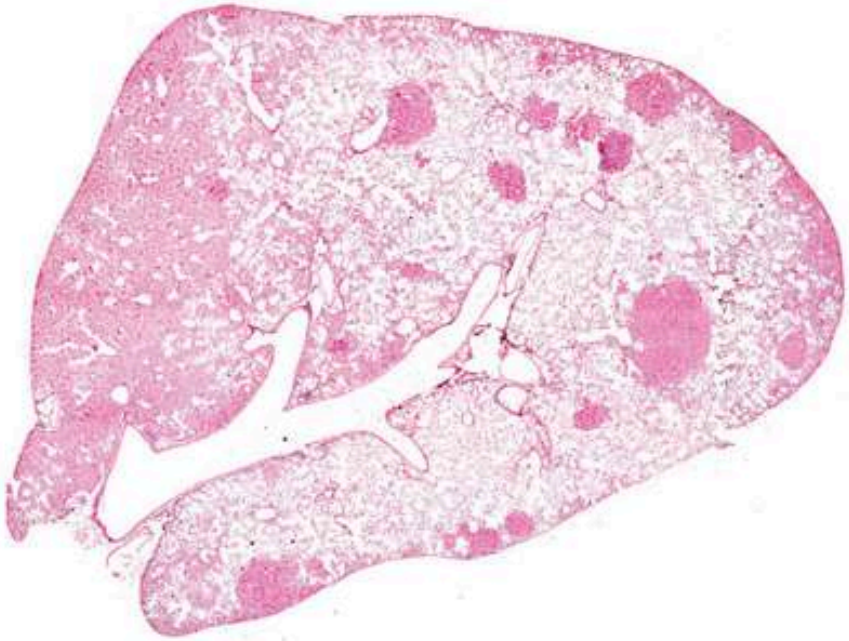


BASCs proliferate, but not preferentially

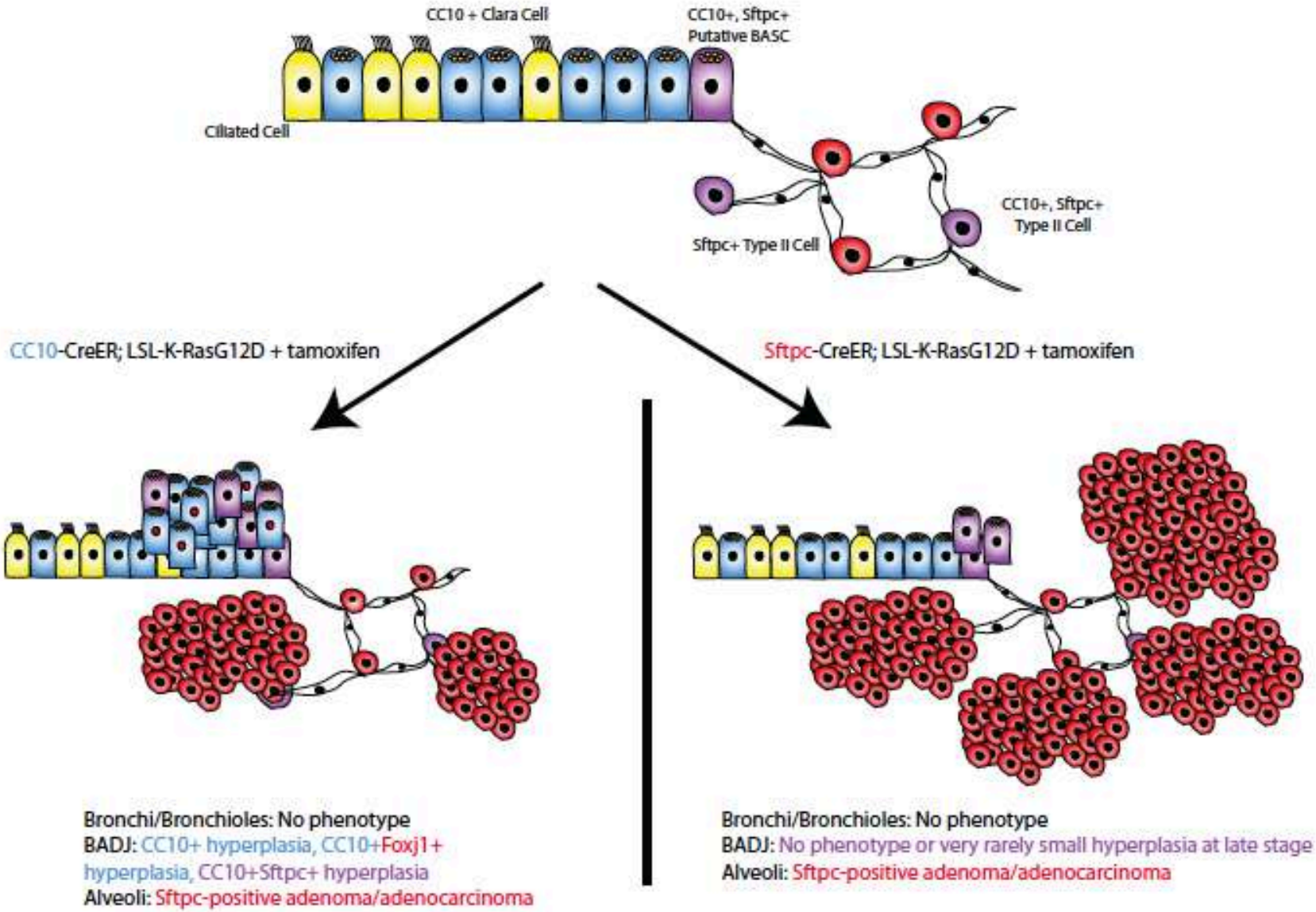
Induction of K-RasG12D using SpcCreER



C



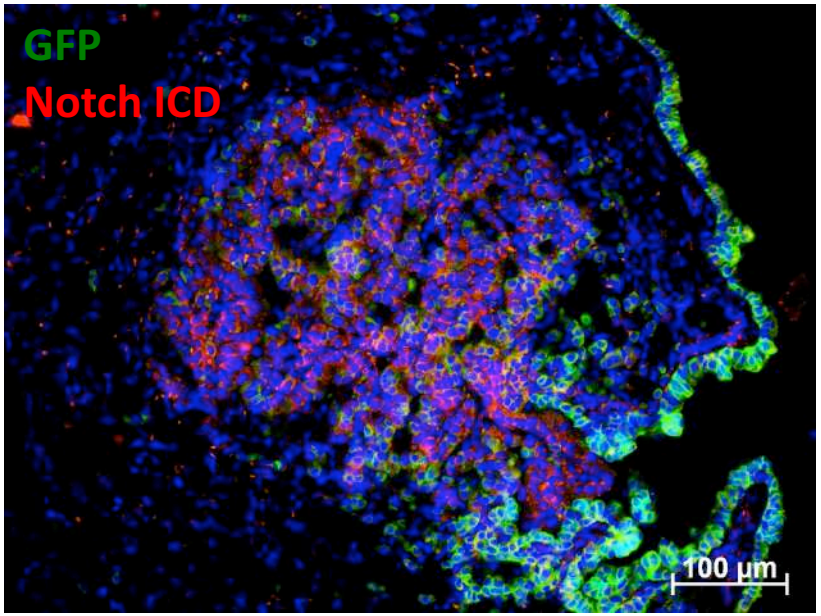
Summary


CC10 + Clara Cell
CC10+, Sftpc+
Putative BASC
Ciliated Cell
CC10+, Sftpc+
Type II Cell
Sftpc+ Type II Cell
CC10-CreER; LSL-K-RasG12D + tamoxifen
Sftpc-CreER; LSL-K-RasG12D + tamoxifen
Bronchi/Bronchioles: No phenotype
BADJ: CC10+ hyperplasia, CC10+Foxj1+ hyperplasia, CC10+Sftpc+ hyperplasia
Alveoli: Sftpc-positive adenoma/adenocarcinoma
Bronchi/Bronchioles: No phenotype
BADJ: No phenotype or very rarely small hyperplasia at late stage
Alveoli: Sftpc-positive adenoma/adenocarcinoma

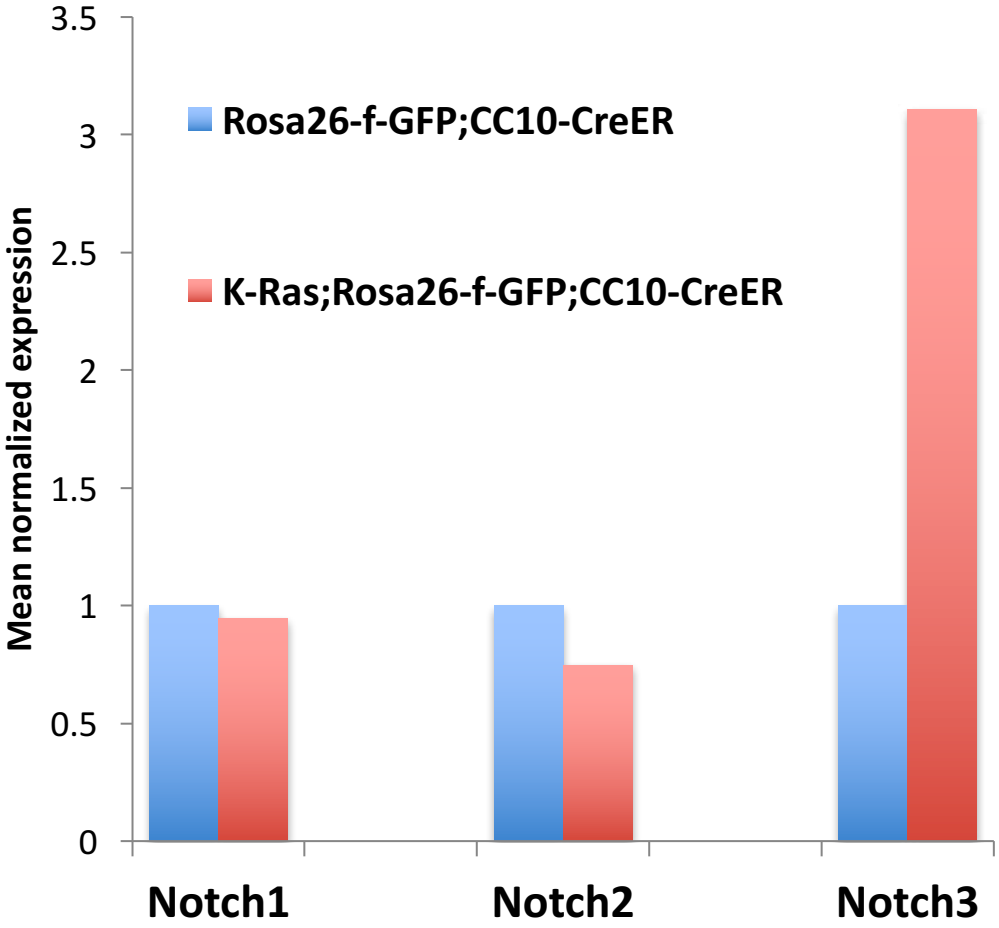
Next questions

- Why are Type II cells uniquely able to form tumors when K-Ras is activated?
- Why are bronchial epithelial cells protected?

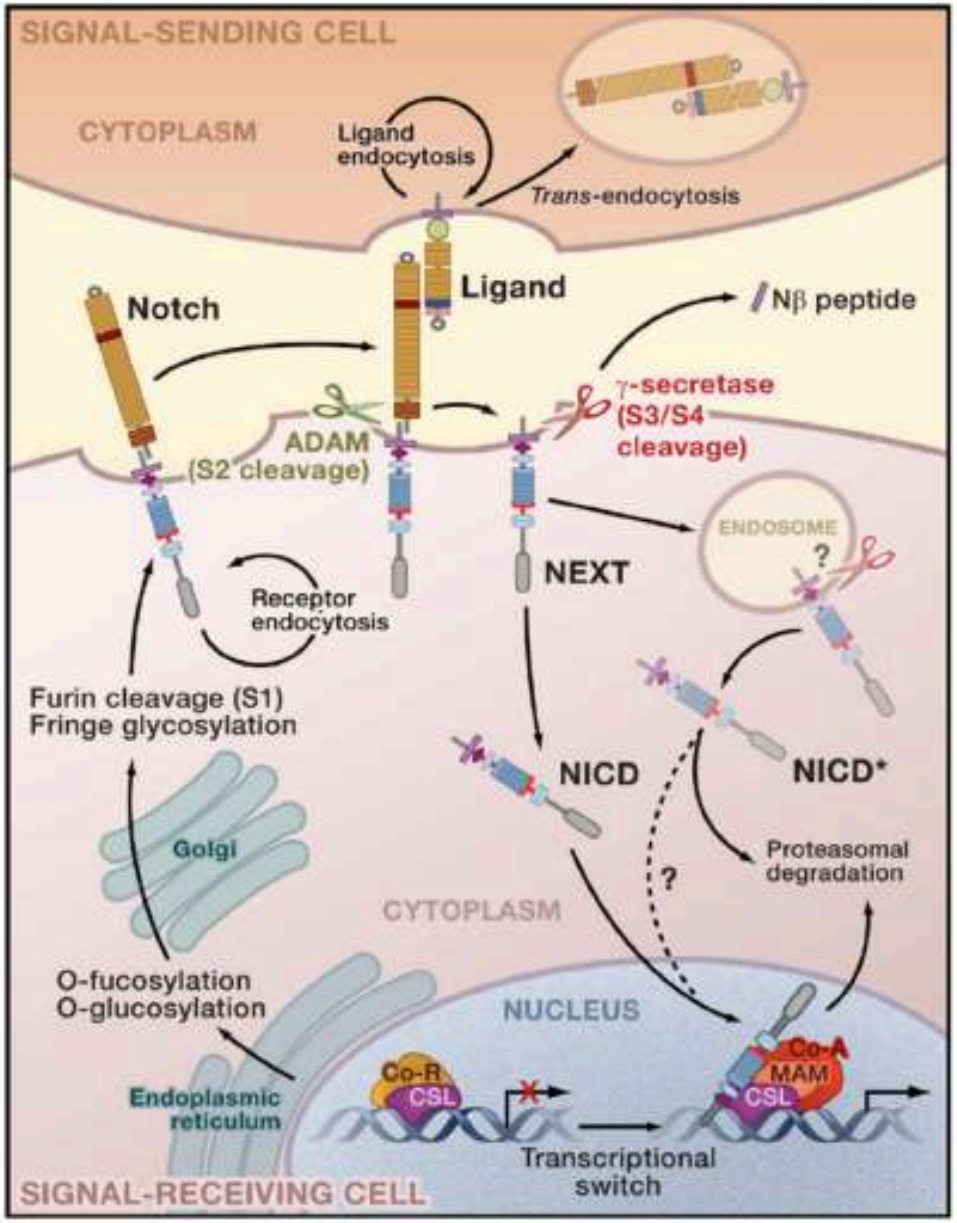
K-Ras activation leads to upregulation of Notch



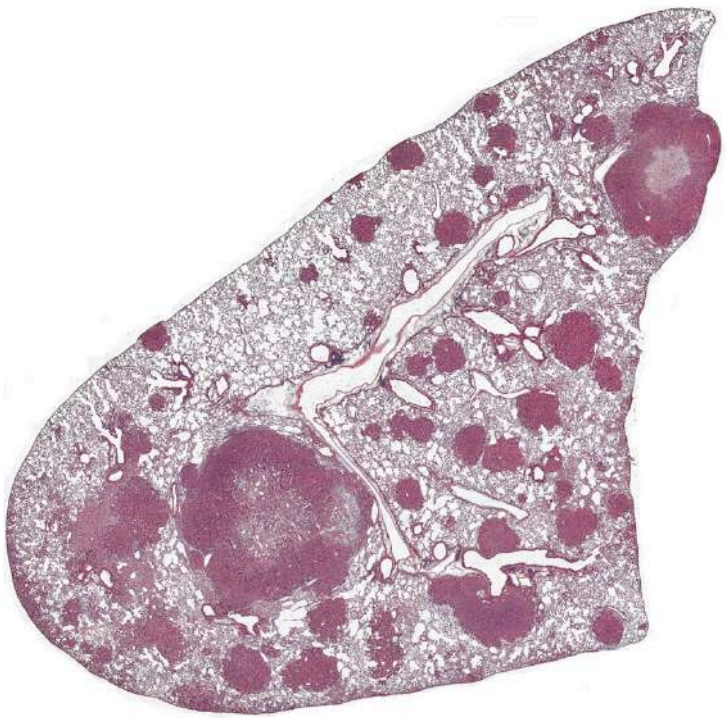
CC10-CreER;LSL-K-RasG12D;LSL-f-GFP

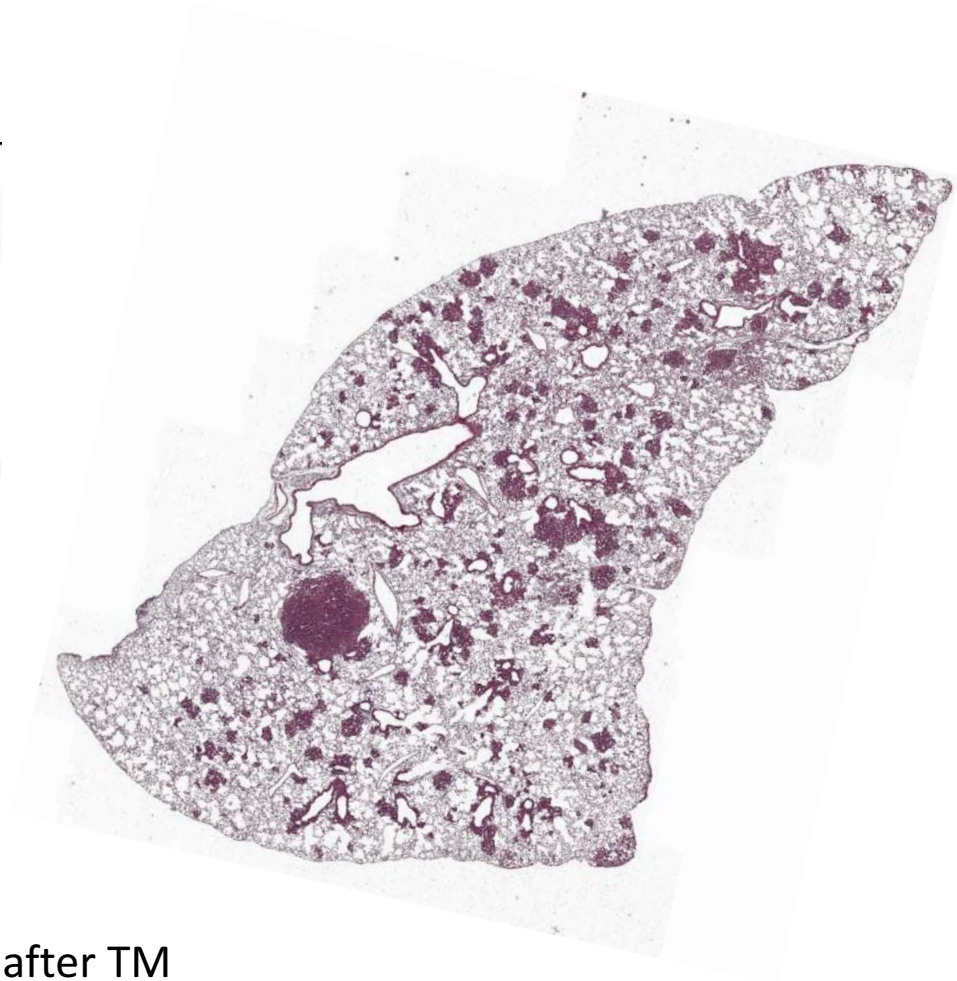


Notch Pathway



Inhibition of Notch by dominant negative DNMAML1 reduces K-RasG12D induced tumorigenesis in CC10-CreER mice





15 weeks after TM

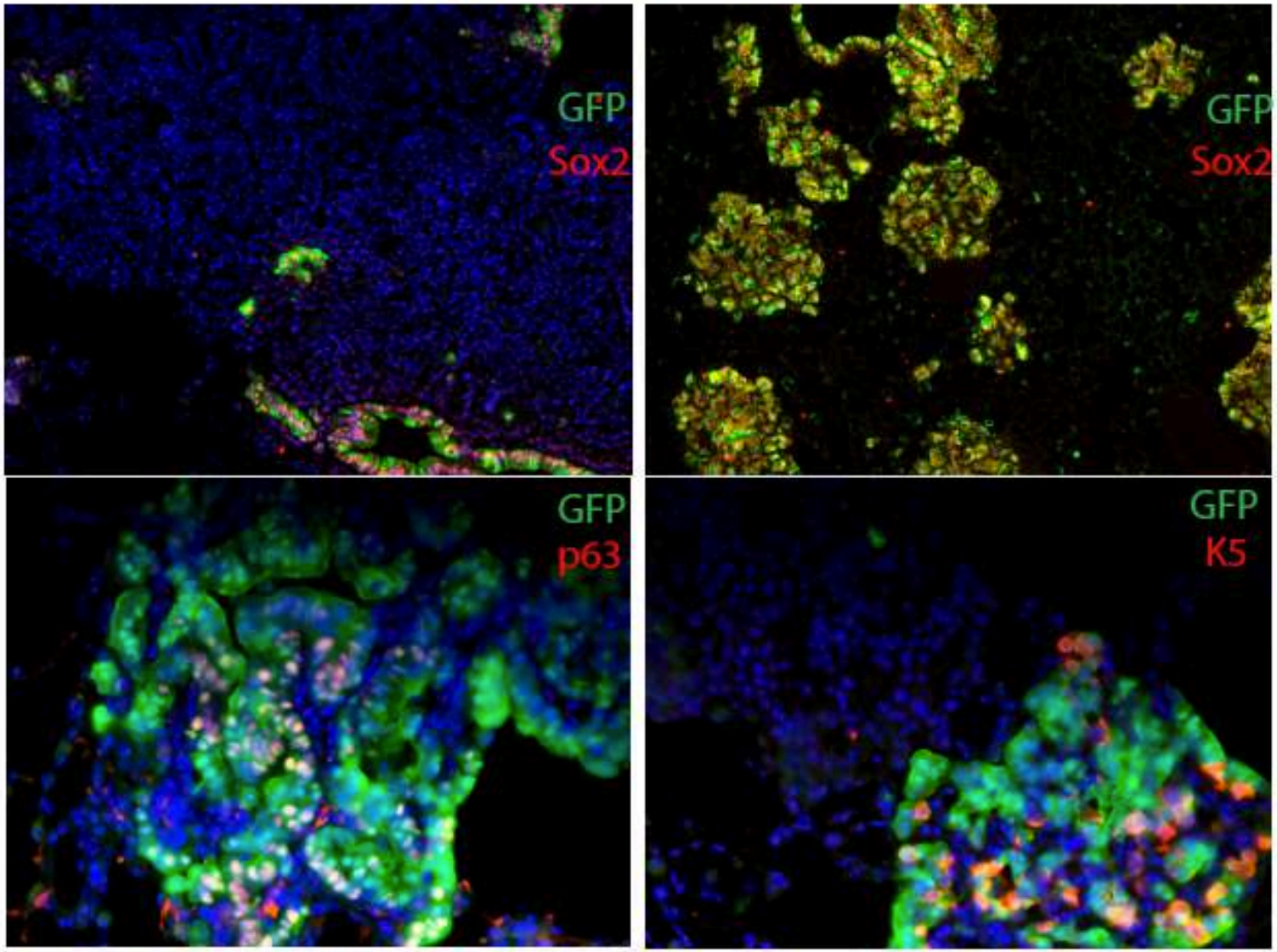
K-RasG12D; Rosa26-f-GFP;CC10-CreER

K-RasG12D; Rosa26-DNMAML1-GFP;CC10-CreER

Immunohistochemical analysis

CC10CreER; KRasG12D; DNMAML-GFP

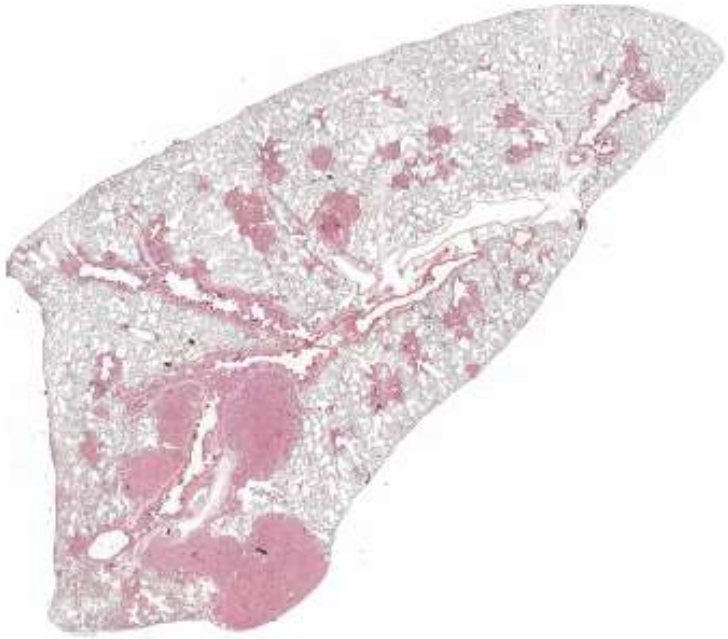
Large tumors contain no DNMAML-GFP

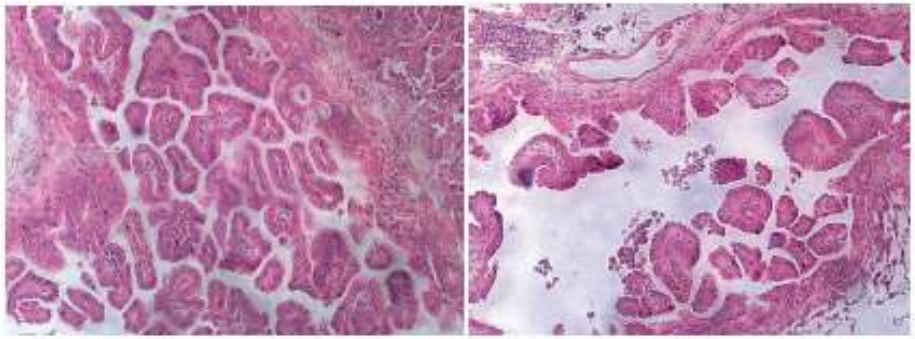


Small DNMAML-GFP-positive tumors express squamous markers

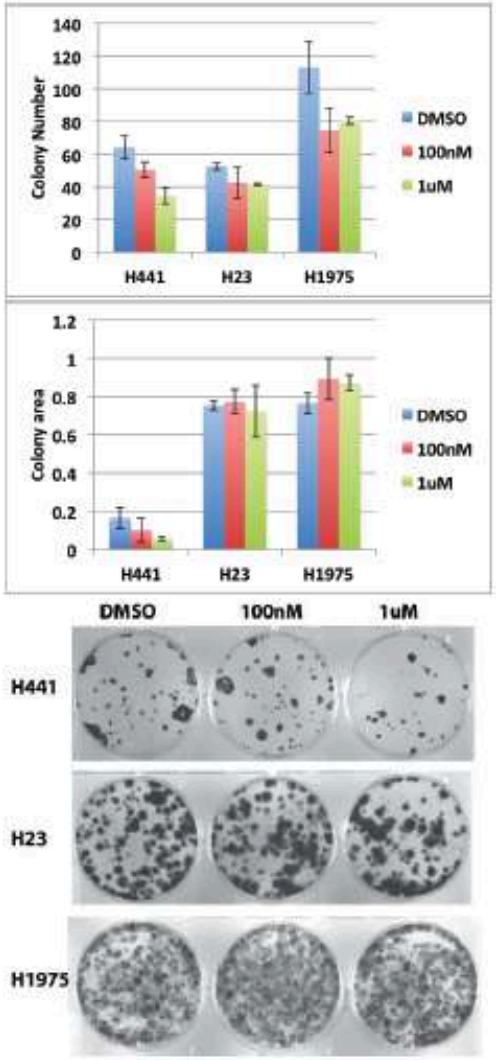
Notch Overexpression with K-RasG12D

CC10-CreER; lsl-K-RasG12D; ROSA Notch mice





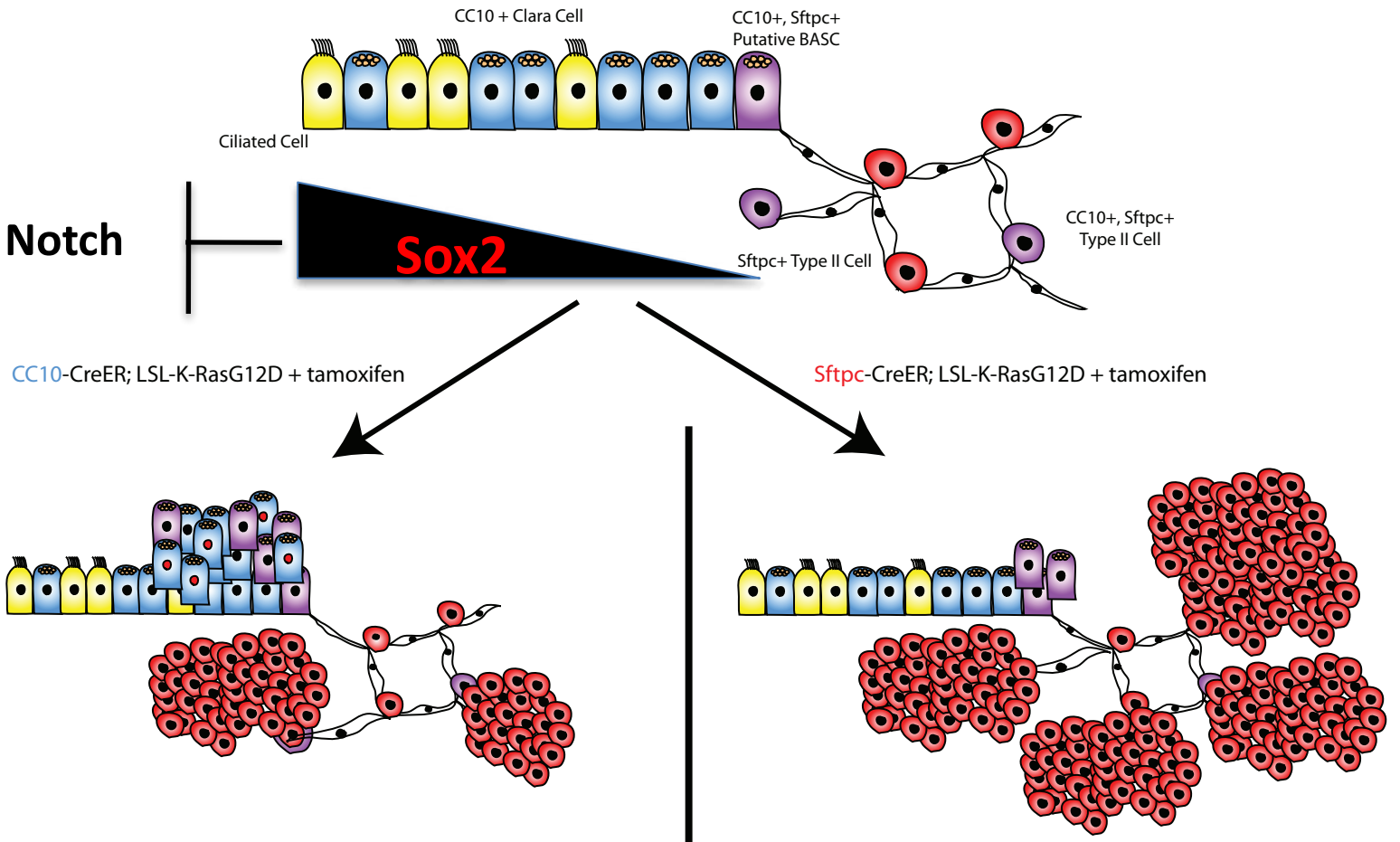
Gamma secretase inhibition affects K-Ras-addicted lung cancer cells



What is regulating Notch?

- SOX2 ChIP-seq in H520 cells: Notch 1-4 are direct SOX2 targets
- In microarrays of CC10-CreER; KRas and Sftpc-CreER; Kras tumors, Sox2 is one of the 15 differentially-regulated genes
- Sox2 expression is highest in the proximal airway and lowest in the alveoli

Sox2/Notch interaction



Bronchi/Bronchioles: No phenotype
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BADJ: No phenotype or very rarely small hyperplasia at late stage
Alveoli: Sftpc-positive adenoma/adenocarcinoma

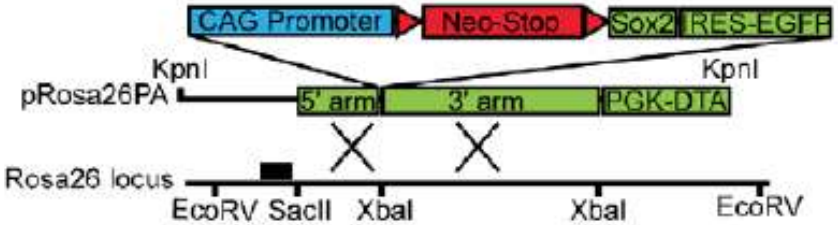
Rosa lsl-Sox2-IRES-GFP mouse

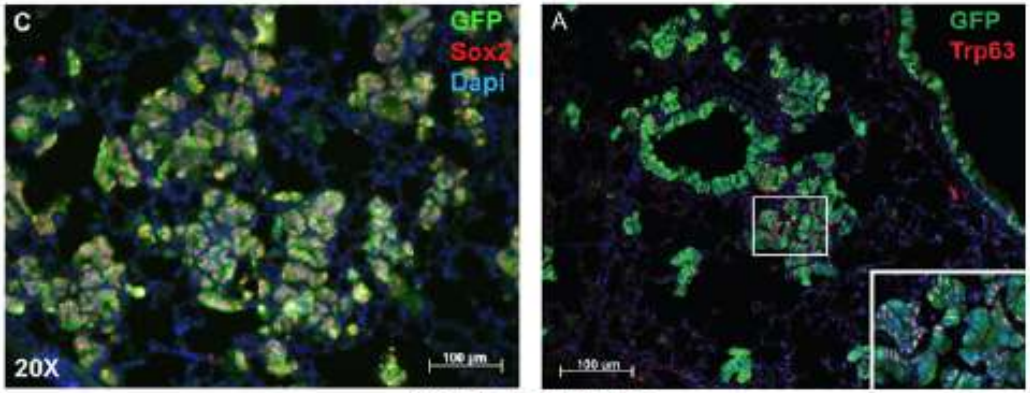
Evidence That SOX2 Overexpression Is Oncogenic in the Lung

Yun Lu[1], Christopher Futtner[1], Jason R. Rock[2], Xia Xu[1], Walter Whitworth[1], Brigid L. M. Hogan[2], Mark W. Onaitis[1]*

1 Department of Surgery, Duke University Medical Center, Durham, North Carolina, United States of America, 2 Department of Cell Biology, Duke University Medical Center, Durham, North Carolina, United States of America

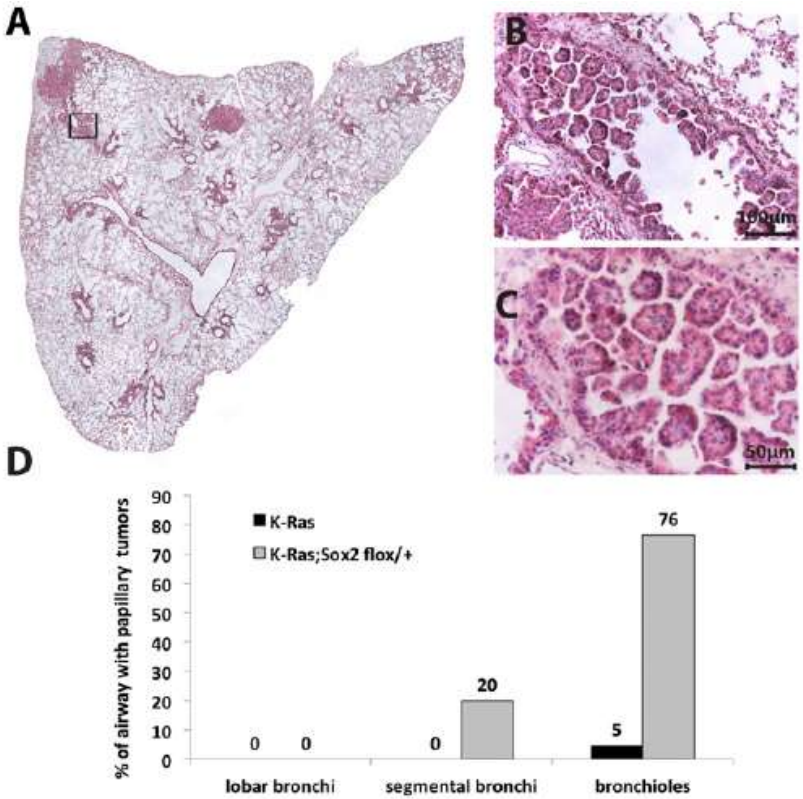
PLoS ONE June 2010 | Volume 5 | Issue 6



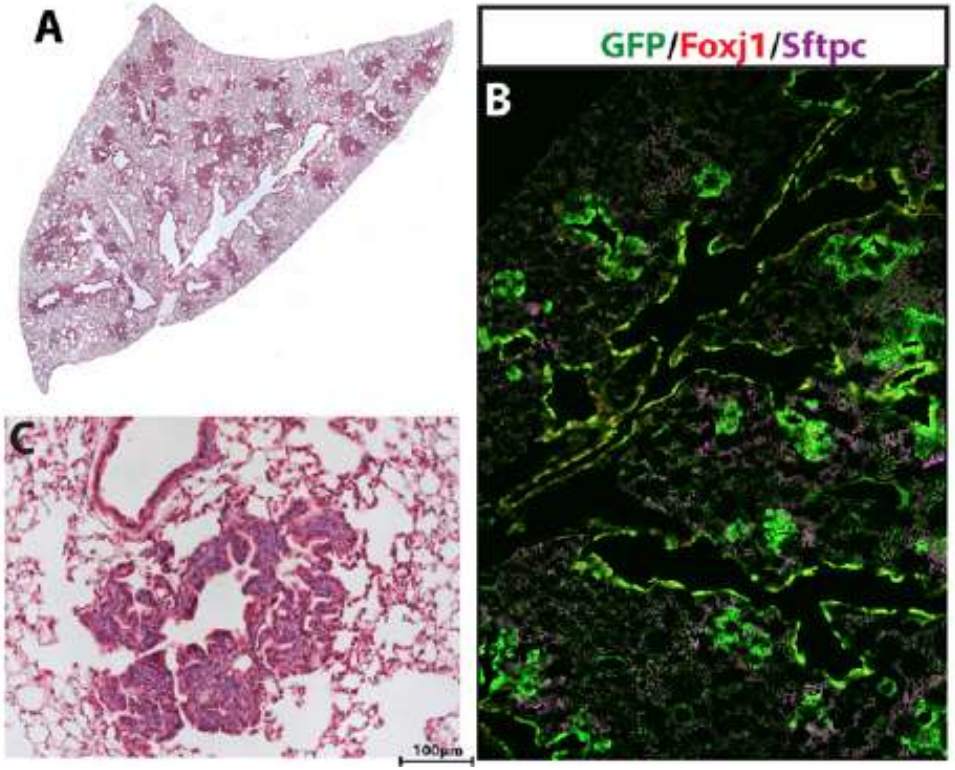


CC10CreER; lsl Sox2-IRES GFP

Modulation of Sox2

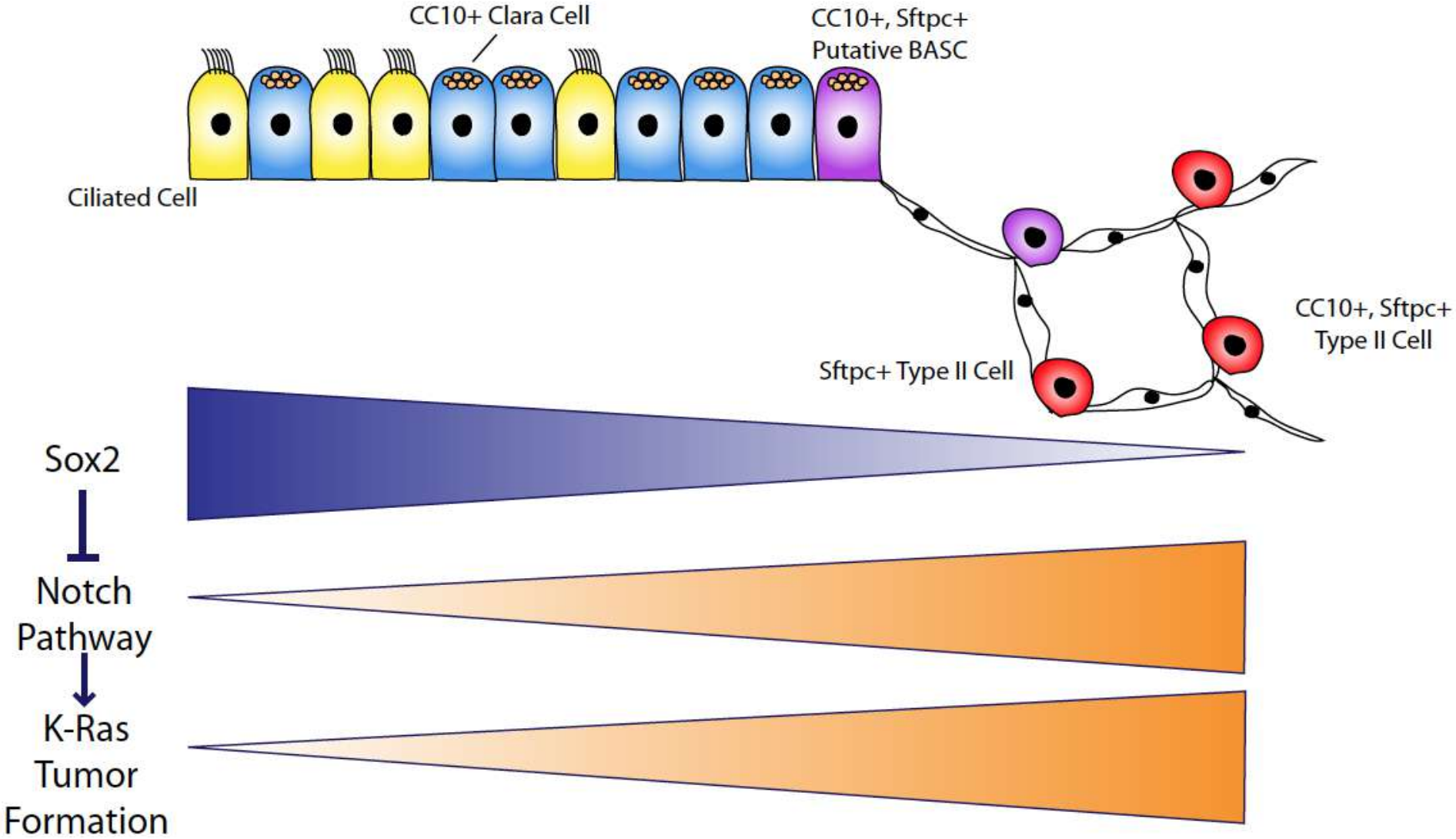


CC10CreER; KRasG12D; low Sox2 (Sox2$^{fl/+}$)



CC10CreER; KRasG12D; high Sox2 (lsl Sox2)

Model



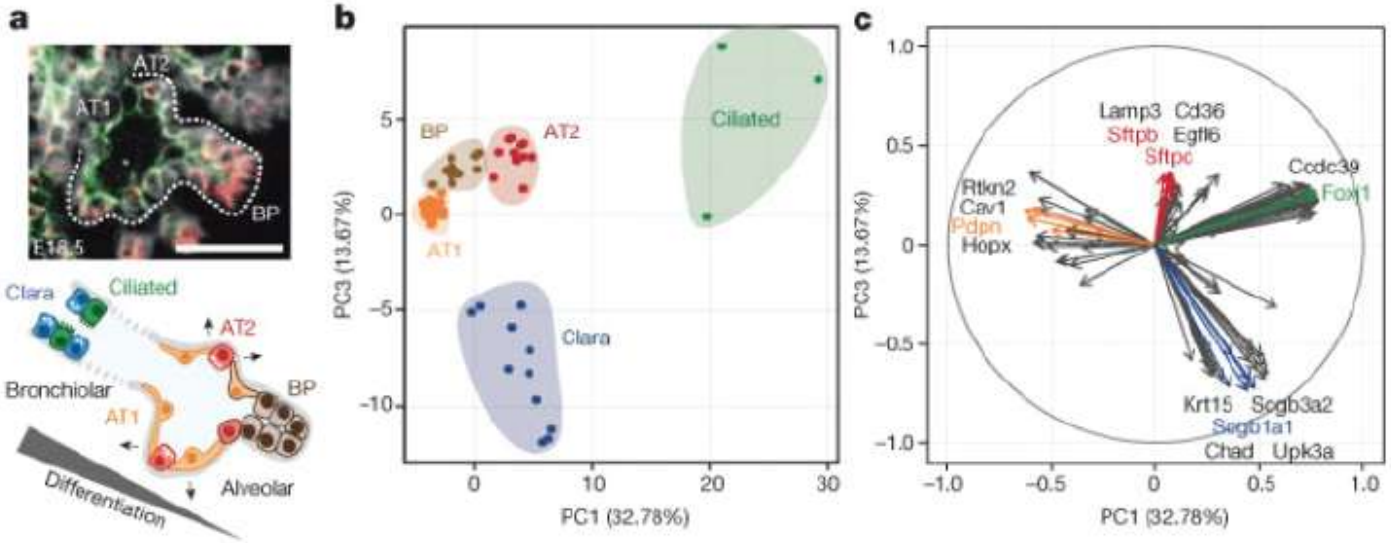
GENES & DEVELOPMENT 28:1929–1939

Lung Development–Bipotent Progenitor

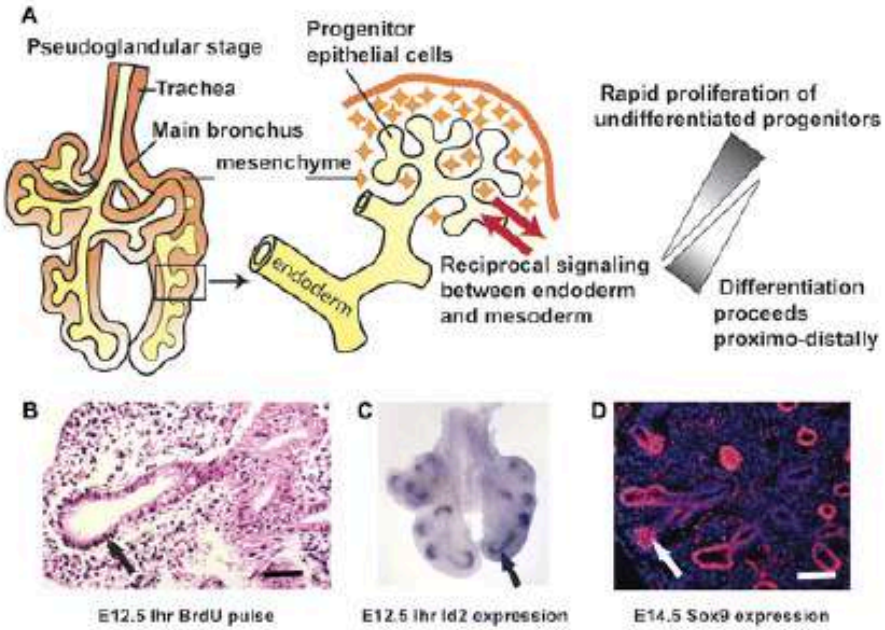
Reconstructing lineage hierarchies of the distal lung epithelium using single-cell RNA-seq

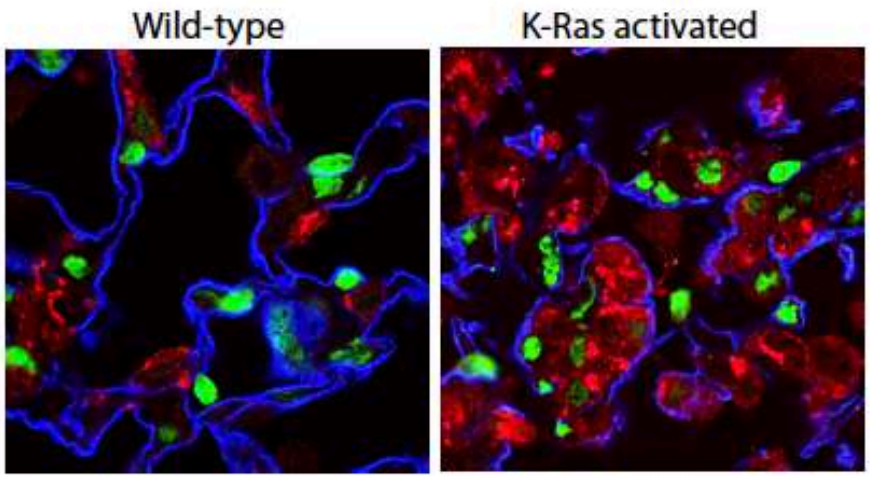
Barbara Treutlein[1]*, Doug G. Brownfield[2]*, Angela R. Wu[1], Norma F. Neff[1], Gary L. Mantalas[1], F. Hernan Espinoza[2], Tushar J. Desai[3], Mark A. Krasnow[2] & Stephen R. Quake[1]

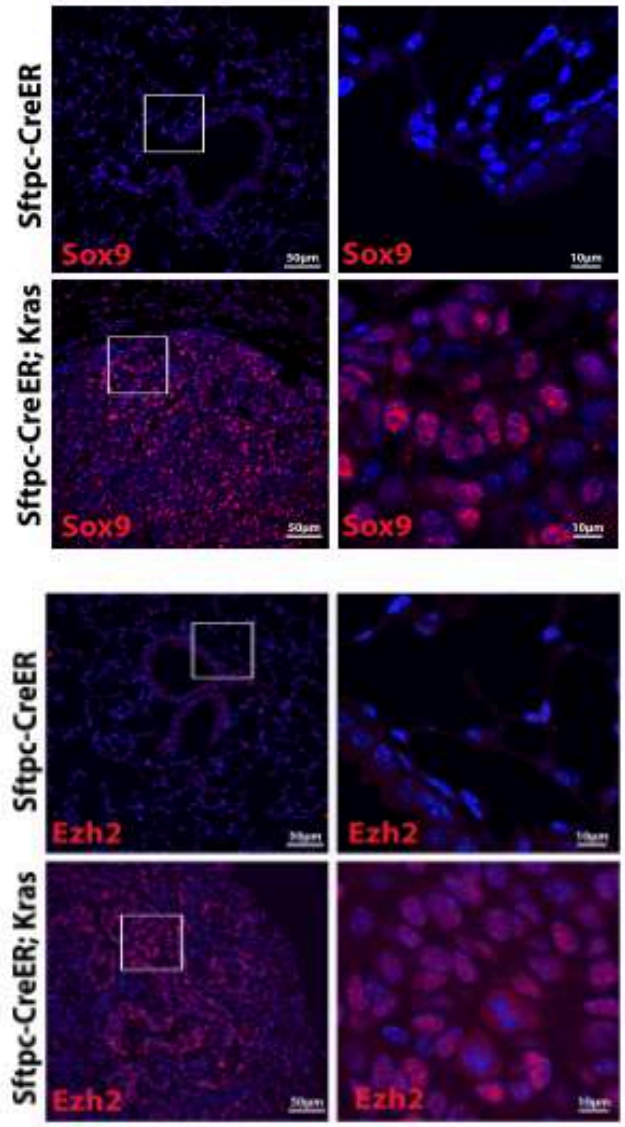
15 MAY 2014 | VOL 509 | NATURE



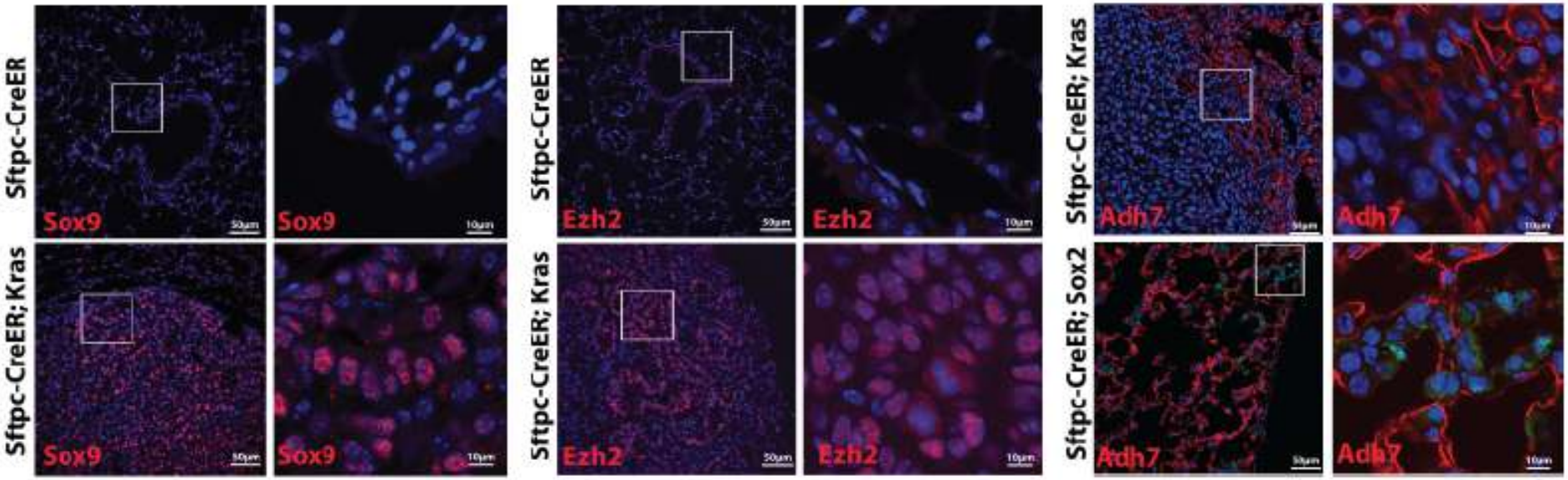
Type I/Type II cell marker expression







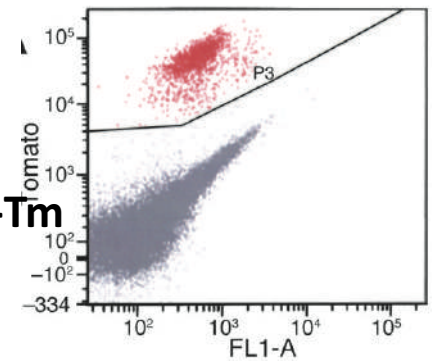
K-Ras-induced Dedifferentiation

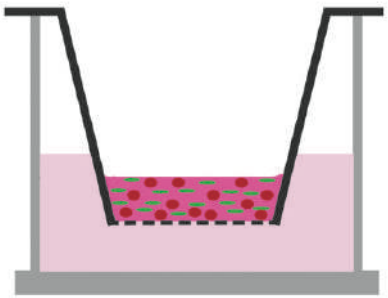


3D co-culture of K-Ras;Spc-CreER;Tdtm

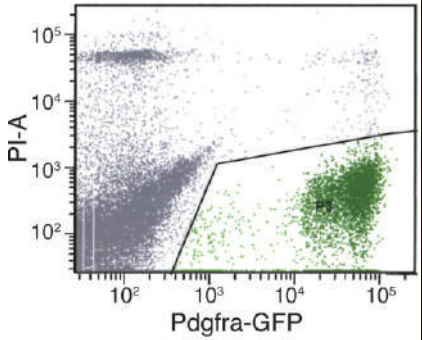
Sftpc-CreER;Rosa-Tm

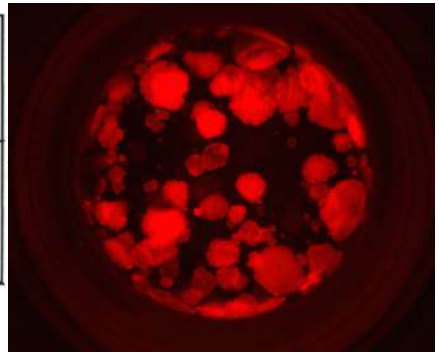
Sftpc-CreER;Kras;Rosa-Tm



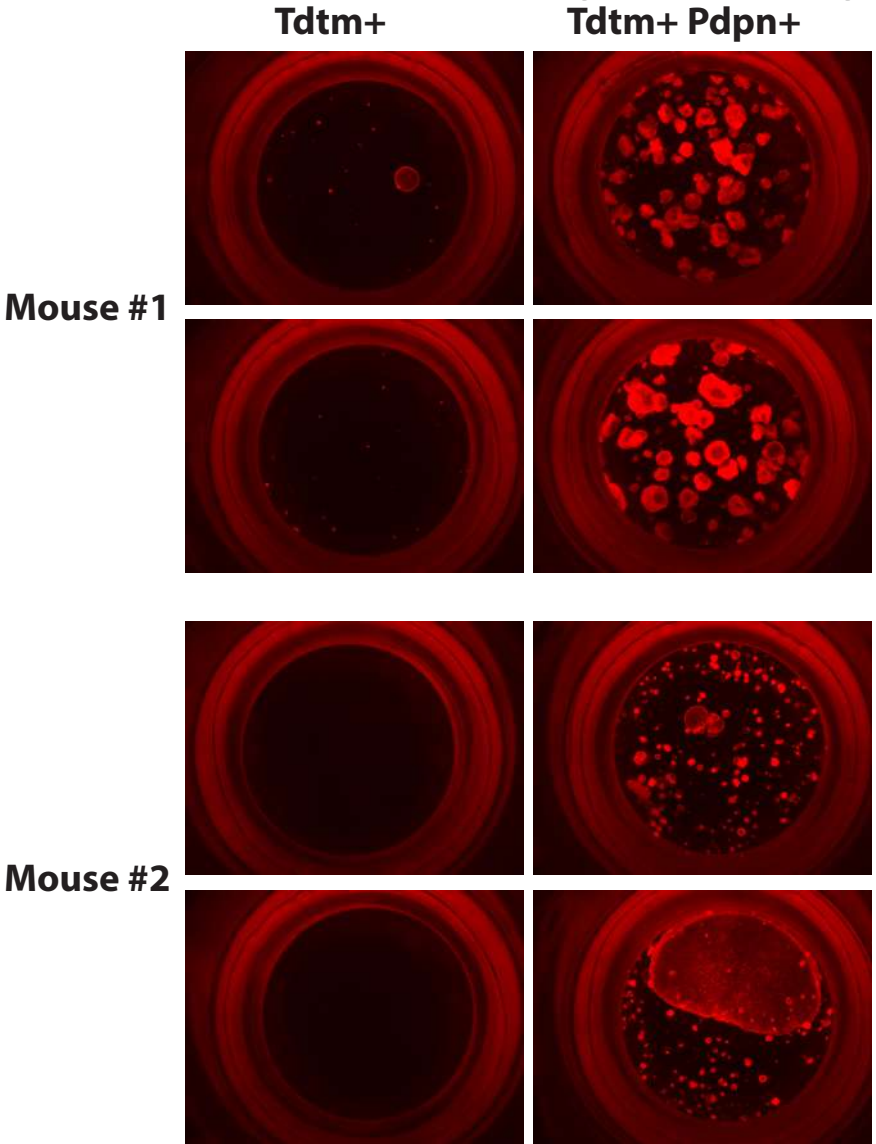


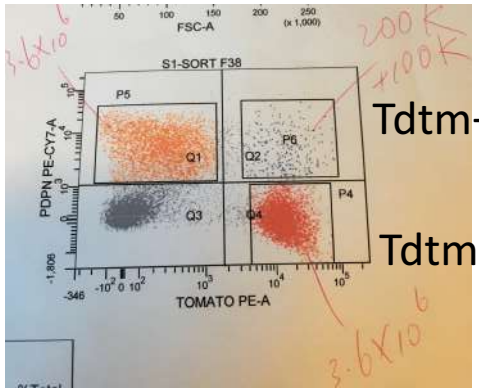
Pdgfra-H2B:GFP



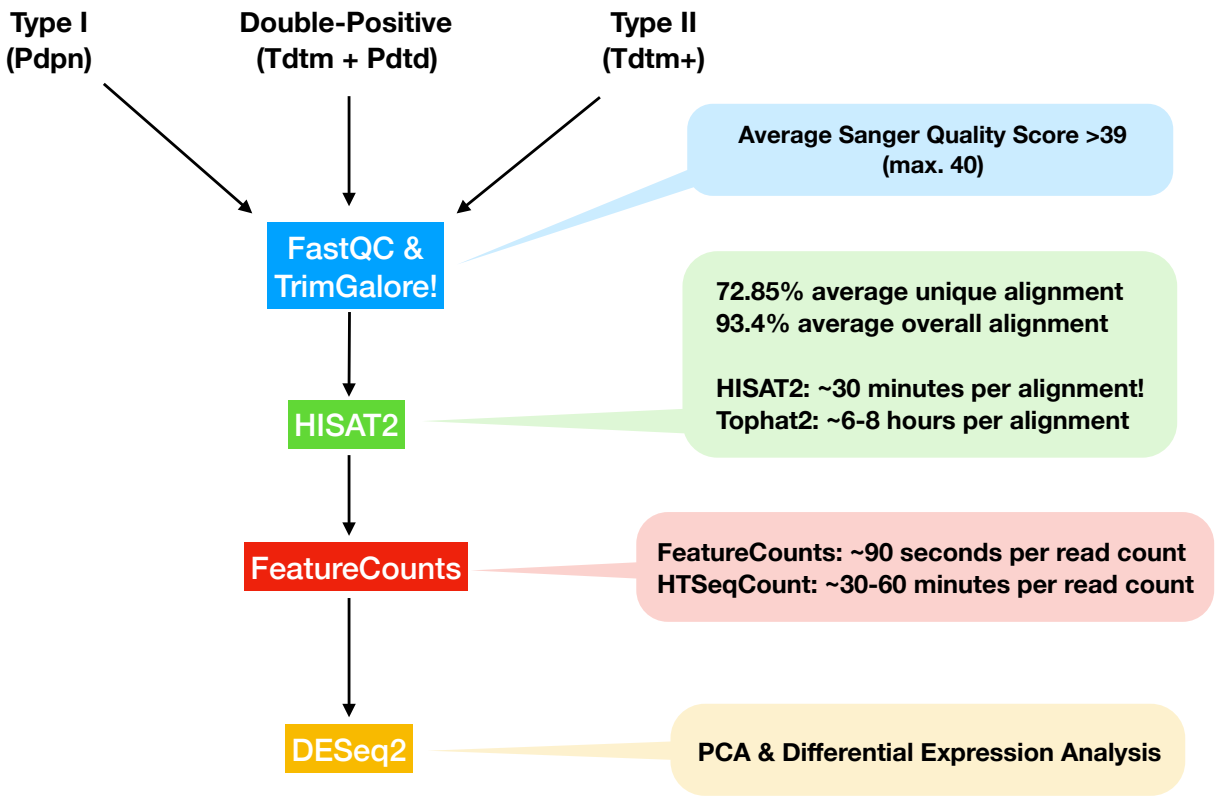


Increased tumor sphere formation in Spc+Pdpn+ cells



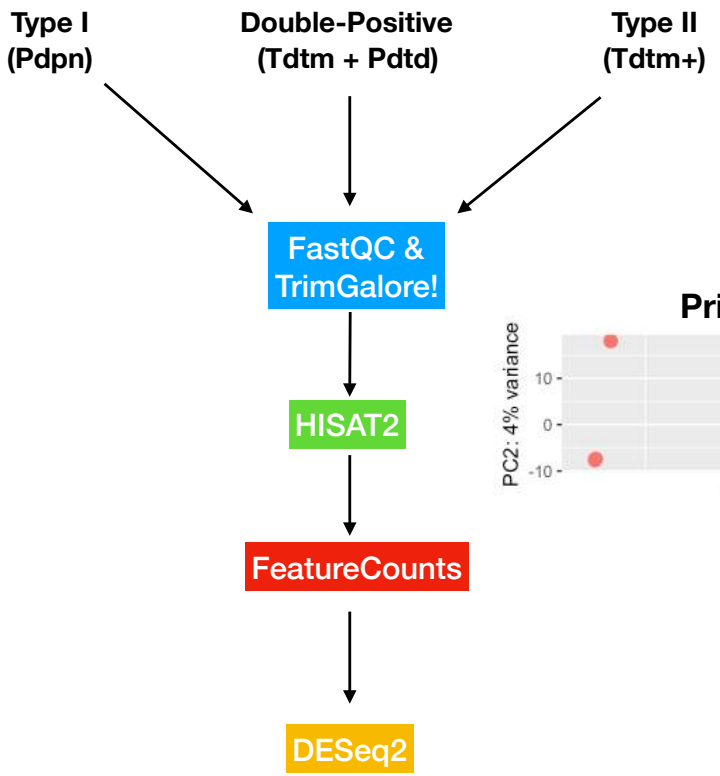


RNAseq analysis for Xia

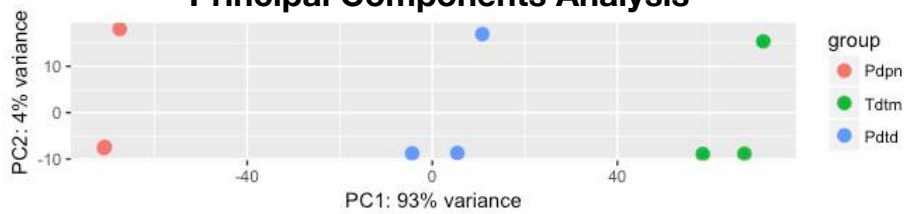


All analysis done locally on my laptop!

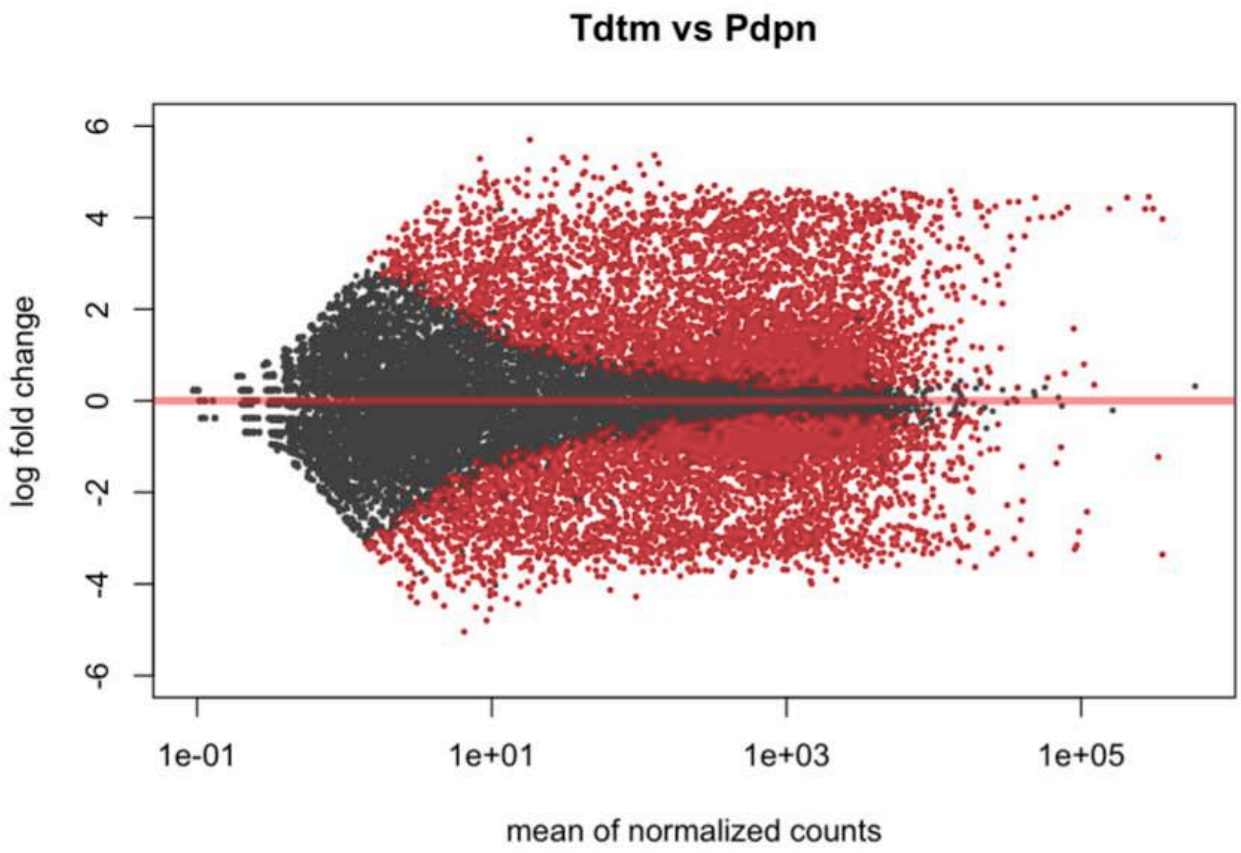
RNAseq analysis for Xia



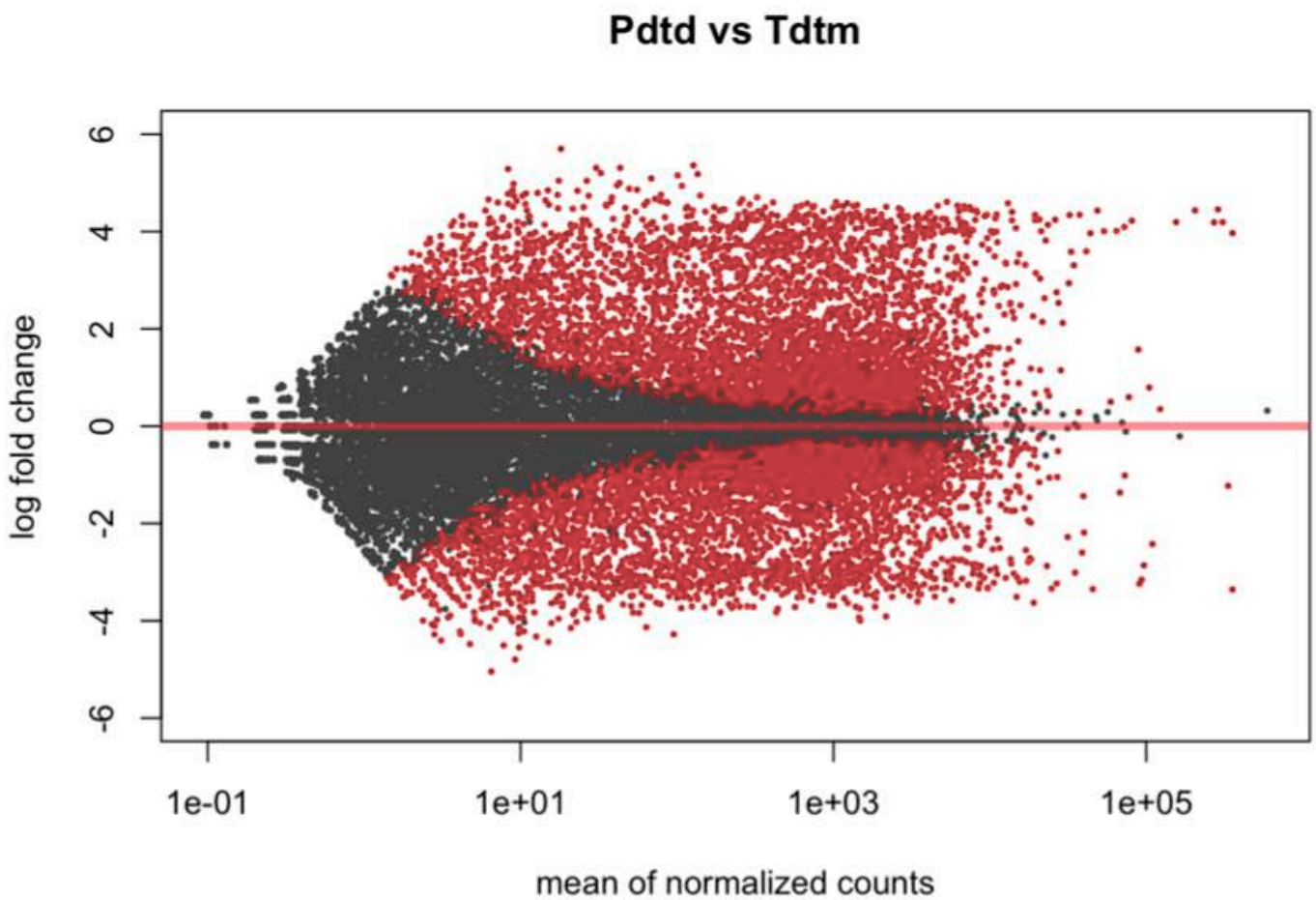
Principal Components Analysis



Tdtm vs Pdpn


Tdtm vs Pdpn
log fold change
6
4
2
0
-2
-4
-6
1e-01
1e+01
1e+03
1e+05
mean of normalized counts

DP vs Tdtm


Pdtd vs Tdtm
log fold change
6
4
2
0
-2
-4
-6
1e-01
1e+01
1e+03
1e+05
mean of normalized counts

DP vs Tdtm

- Top 20 positive signatures from GSEA ***Hallmark*** Database
  - In other words, those signatures which are highly ***enriched*** in the Pdtd cells
- NES = Normalized Enrichment Score

NAME	NES	NOM p-val	FDR q-val	FWER p-val
HALLMARK_ALLOGRAFT_REJECTION	2.2741482	0	0	0
HALLMARK_EPITHELIAL_MESENCHYMAL_TRANSITION	2.2713459	0	0	0
HALLMARK_TNFA_SIGNALING_VIA_NFKB	2.150286	0	0	0
HALLMARK_E2F_TARGETS	2.1433797	0	0	0
HALLMARK_INFLAMMATORY_RESPONSE	2.124931	0	0	0
HALLMARK_KRAS_SIGNALING_UP	2.083865	0	0	0
HALLMARK_COMPLEMENT	2.0676389	0	0	0
HALLMARK_G2M_CHECKPOINT	2.0622153	0	0	0
HALLMARK_INTERFERON_GAMMA_RESPONSE	2.017774	0	0	0
HALLMARK_COAGULATION	2.0103047	0	0	0
HALLMARK_IL6_JAK_STAT3_SIGNALING	1.9446948	0	0	0
HALLMARK_APICAL_JUNCTION	1.9340765	0	0	0
HALLMARK_APOPTOSIS	1.9092989	0	0	0
HALLMARK_IL2_STAT5_SIGNALING	1.8502016	0	0	0

DP vs Tdtm

- Top 20 positive signatures from GSEA ***Curated*** Database
  - In other words, those signatures which are highly ***enriched*** in the Pdtd cells
- NES = Normalized Enrichment Score

NAME	NES	NOM p-val	FDR q-val	FWER p-val
ROSTY_CERVICAL_CANCER_PROLIFERATION_CLUSTER	2.5073776	0	0	0
LIAN_LIPA_TARGETS_3M	2.4375947	0	0	0
LIAN_LIPA_TARGETS_6M	2.4366956	0	0	0
KOBAYASHI_EGFR_SIGNALING_24HR_DN	2.4295037	0	0	0
CROONQUIST_NRAS_SIGNALING_DN	2.4169986	0	0	0
POOLA_INVASIVE_BREAST_CANCER_UP	2.4161587	0	0	0
MCLACHLAN_DENTAL_CARIES_UP	2.4098496	0	0	0
SOTIRIOU_BREAST_CANCER_GRADE_1_VS_3_UP	2.4032764	0	0	0
CHANG_CYCLING_GENES	2.40299	0	0	0
HESS_TARGETS_OF_HOXA9_AND_MEIS1_DN	2.3621871	0	0	0
KANG_DOXORUBICIN_RESISTANCE_UP	2.353079	0	0	0
GRAHAM_NORMAL_QUIESCENT_VS_NORMAL_DIVIDING_DN	2.3498018	0	0	0
VERHAAK_GLIOBLASTOMA_MESENCHYMAL	2.3442886	0	0	0

Pdtd vs Tdtm

- Top 20 positive signatures from GSEA ***Gene Ontology*** Database
  - In other words, those signatures which are highly ***enriched*** in the Pdtd cells
- NES = Normalized Enrichment Score

NAME	NES	NOM p-val	FDR q-val	FWER p-val
GO_MYELOID_LEUKOCYTE_ACTIVATION	2.2205086	0	0	0
GO_REGULATION_OF_LEUKOCYTE_CHEMOTAXIS	2.209167	0	0	0
GO_REGULATION_OF_LEUKOCYTE_MIGRATION	2.1804385	0	0	0
GO_LEUKOCYTE_MIGRATION	2.179667	0	0	0
GO_INFLAMMATORY_RESPONSE	2.172299	0	0	0
GO_POSITIVE_REGULATION_OF_LEUKOCYTE_MIGRATION	2.1706402	0	0	0
GO_CELL_CHEMOTAXIS	2.1698291	0	0	0
GO_LEUKOCYTE_CHEMOTAXIS	2.159894	0	0	0
GO_REGULATION_OF_CYTOKINE_SECRETION	2.1585002	0	0	0
GO_LEUKOCYTE_ACTIVATION	2.15269	0	0	0
GO_CHEMOKINE_MEDIATED_SIGNALING_PATHWAY	2.1525762	0	0	0
GO_POSITIVE_REGULATION_OF_CYTOKINE_SECRETION	2.1524837	0	0	0
GO_CELL_ACTIVATION	2.1472375	0	0	0
GO_POSITIVE_REGULATION_OF_LEUKOCYTE_CHEMOTAXIS	2.1330056	0	0	0
	2.120372			

Pdtd vs Tdtm

- Top positive signatures from GSEA ***Oncogenic Signatures*** Database
  - In other words, those signatures which are highly ***enriched*** in the Pdtd cells
- NES = Normalized Enrichment Score

NAME	NES	NOM p-val	FDR q-val	FWER p-val
RPS14_DN.V1_UP	2.3021746	0	0	0
CORDENONSI_YAP_CONSERVED_SIGNATURE	2.2323203	0	0	0
SNF5_DN.V1_UP	2.109282	0	0	0
ESC_V6.5_UP_EARLY.V1_DN	2.108703	0	0	0
STK33_SKM_UP	2.0985887	0	0	0
RB_P107_DN.V1_UP	2.0734408	0	0	0
CSR_LATE_UP.V1_UP	2.0404038	0	0	0
BMI1_DN_MEL18_DN.V1_UP	2.02171	0	0	0
LEF1_UP.V1_UP	1.9952567	0	0	0
P53_DN.V1_DN	1.9363867	0	0	0
ATF2_S_UP.V1_UP	1.934966	0	0	0
TGFB_UP.V1_UP	1.92077	0	0	0
MEL18_DN.V1_UP	1.9112036	0	0	0
BRCA1_DN.V1_UP	1.8931711	0	0	0
HOXA9_DN.V1_UP	1.8710018	0	0	0
STK33_UP	1.8638195	0	0	0

SOX2 Mimetic

A Small-Molecule Inhibitor of Tgf-β Signaling Replaces *Sox2* in Reprogramming by Inducing *Nanog*

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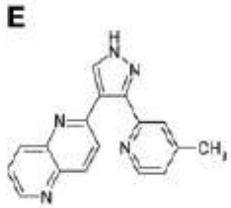
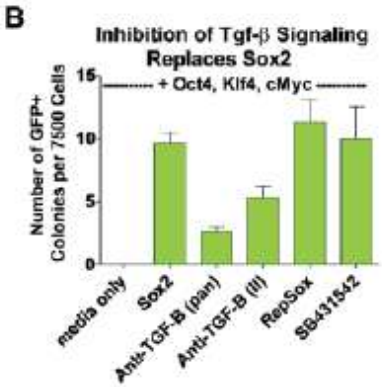
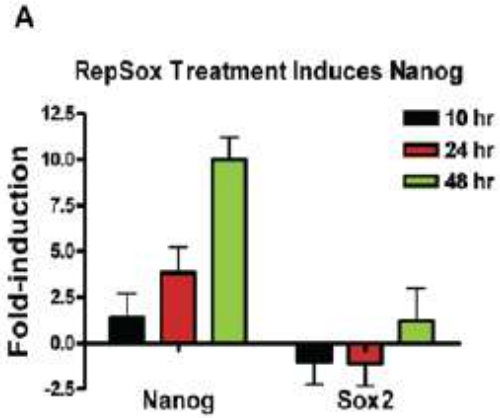
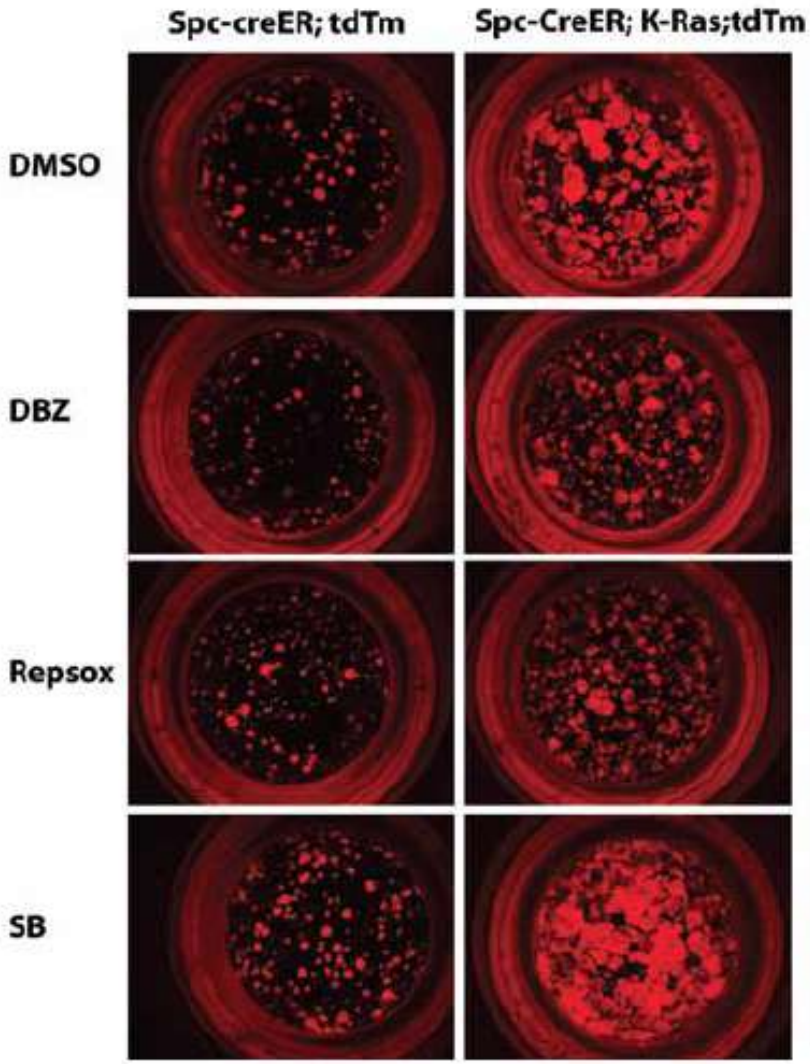


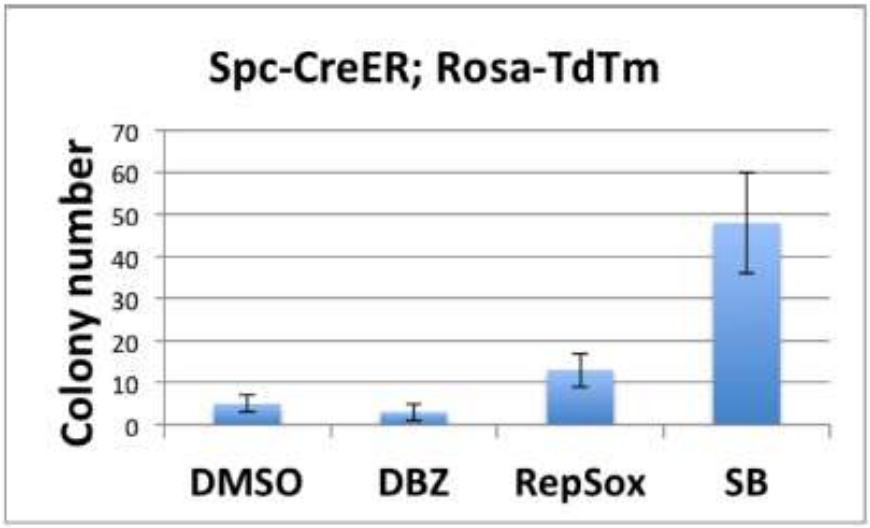
E-616452
(RepSox)
(Tgfbr1 inhibitor)
25 μM

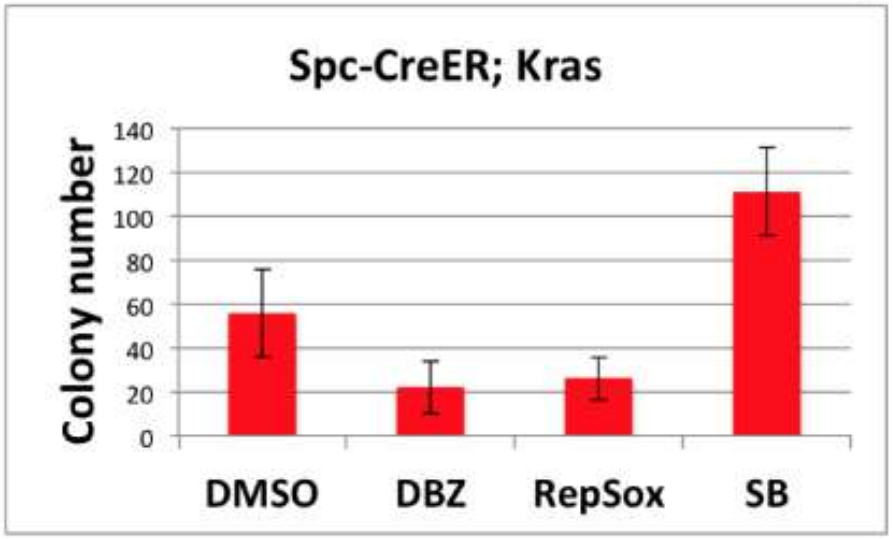




In vitro culture system

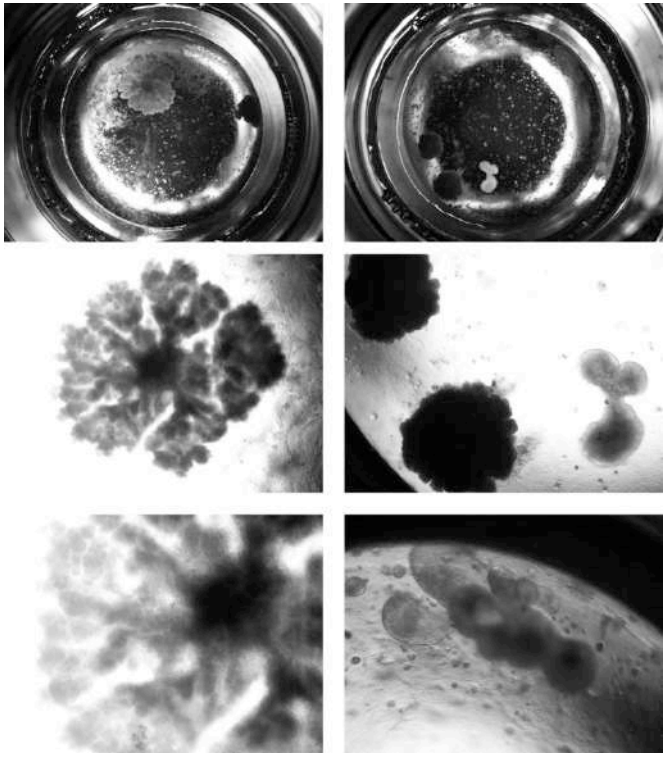




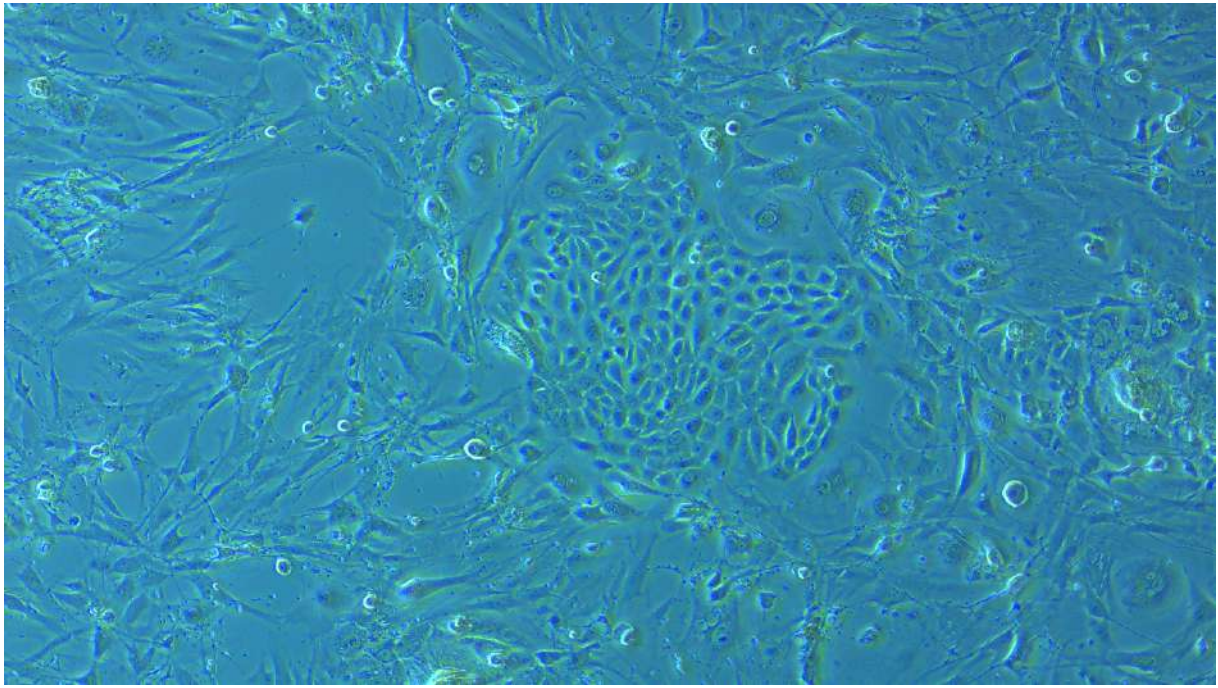


SOX2 Mimetic In Vivo

In vitro culture of human cells



Type II cells (3D)



Tumor (2D on feeder layer
with ROCK1 inhibitor)

Summary/Conclusions

- Active Notch is necessary for K-RasG12D-induced lung adenocarcinoma
- Inhibition of Notch signaling in K-RasG12D-expressing cells leads to squamous differentiation
- Activation of Notch along with K-RasG12D in bronchial/bronchiolar cells leads to papillary adenocarcinoma
- Sox2 activation determines K-Ras-induced tumor location by down regulating Notch
- Both Sox2 over expression and Notch inhibition proximally differentiate Type II cells such that K-Ras activation is not transforming