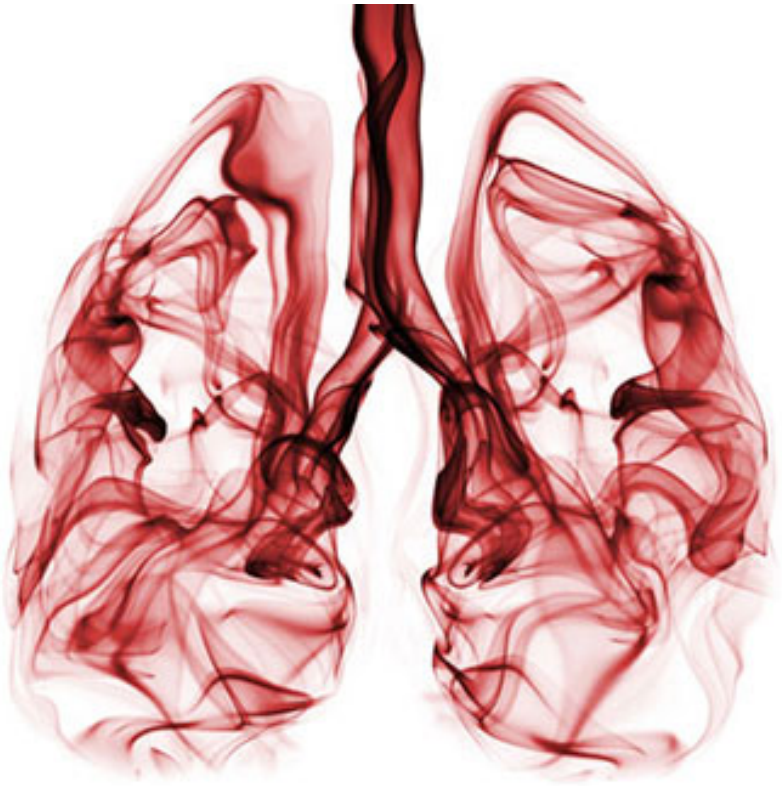
Generation of pulmonary neuroendocrine cells and tumors resembling small cell lung cancers from human embryonic stem cells

Weill Cornell Medicine
Joyce Chen
Arun Unni
Harold Varmus

Asaf Poran
Olivier Elemento

Columbia College of P&S
Sarah Huang
Hans Snoeck



bioRxiv 261461; doi: https://doi.org/10.1101/261461

Initiate oncogenesis in human ESC-derived differentiated cells to explore relationship between cell type and oncogenic genotype

--differentiate RUES-2 cells to lung lineage
(with Hans Snoeck's lab at Columbia)

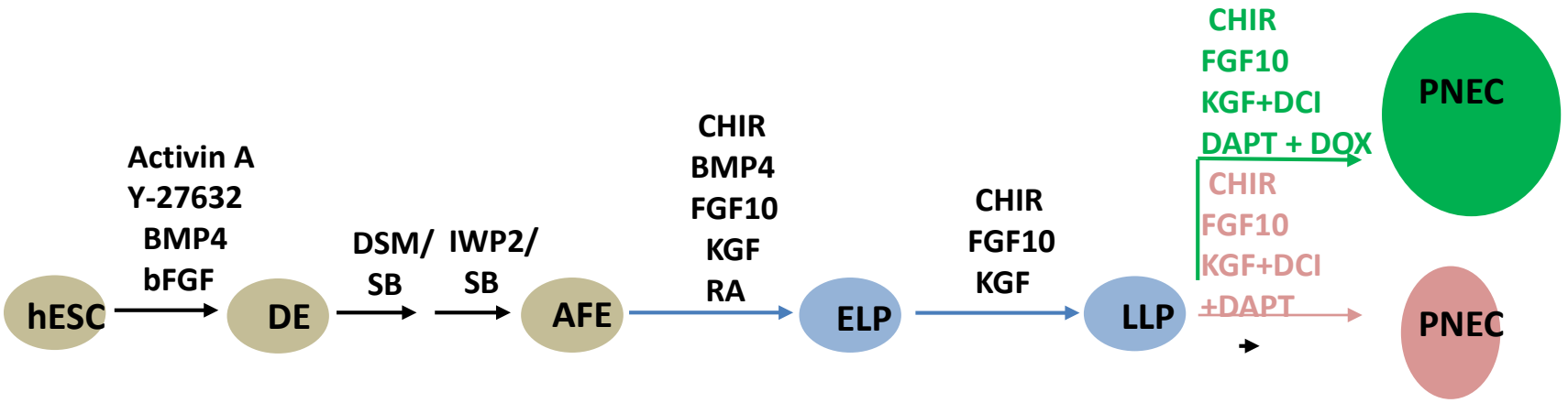
--characterize cells for differentiation markers
and for single cell transcriptomes
(with Olivier Elemento's lab at WCM)

--activate or induce mutations characteristic
of common lung cancer types
(LUAD, LUSC, SCLC)

--focus first on SCLC (neuro-endocrine cells,
loss of RB1 and p53)

Joyce Chen

INDUCING PULMONARY NEUROENDOCRINE CELLS (PNECs) BY INHIBITION OF NOTCH AND RB DURING LUNG DIFFERENTIATION FROM A HUMAN ESC LINE



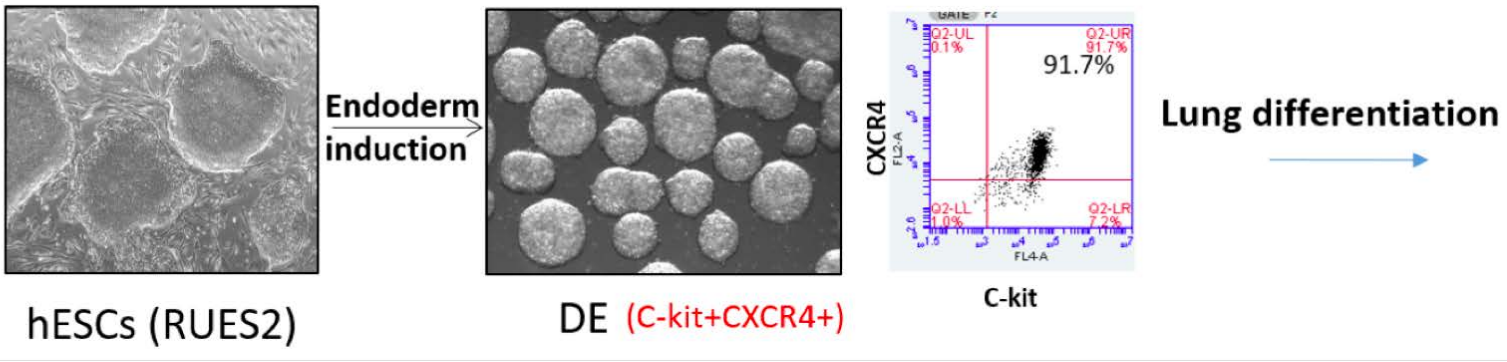
--LUNG DIFFERENTIATION VIA SNOECK PROTOCOL

--INHIBIT CLEAVAGE OF NOTCH RECEPTOR WITH DAP

--KNOCK DOWN RB EXPRESSION WITH DOX INDUCED shRNA for RB1

DIFFERENTIATION OF RUES-2

A



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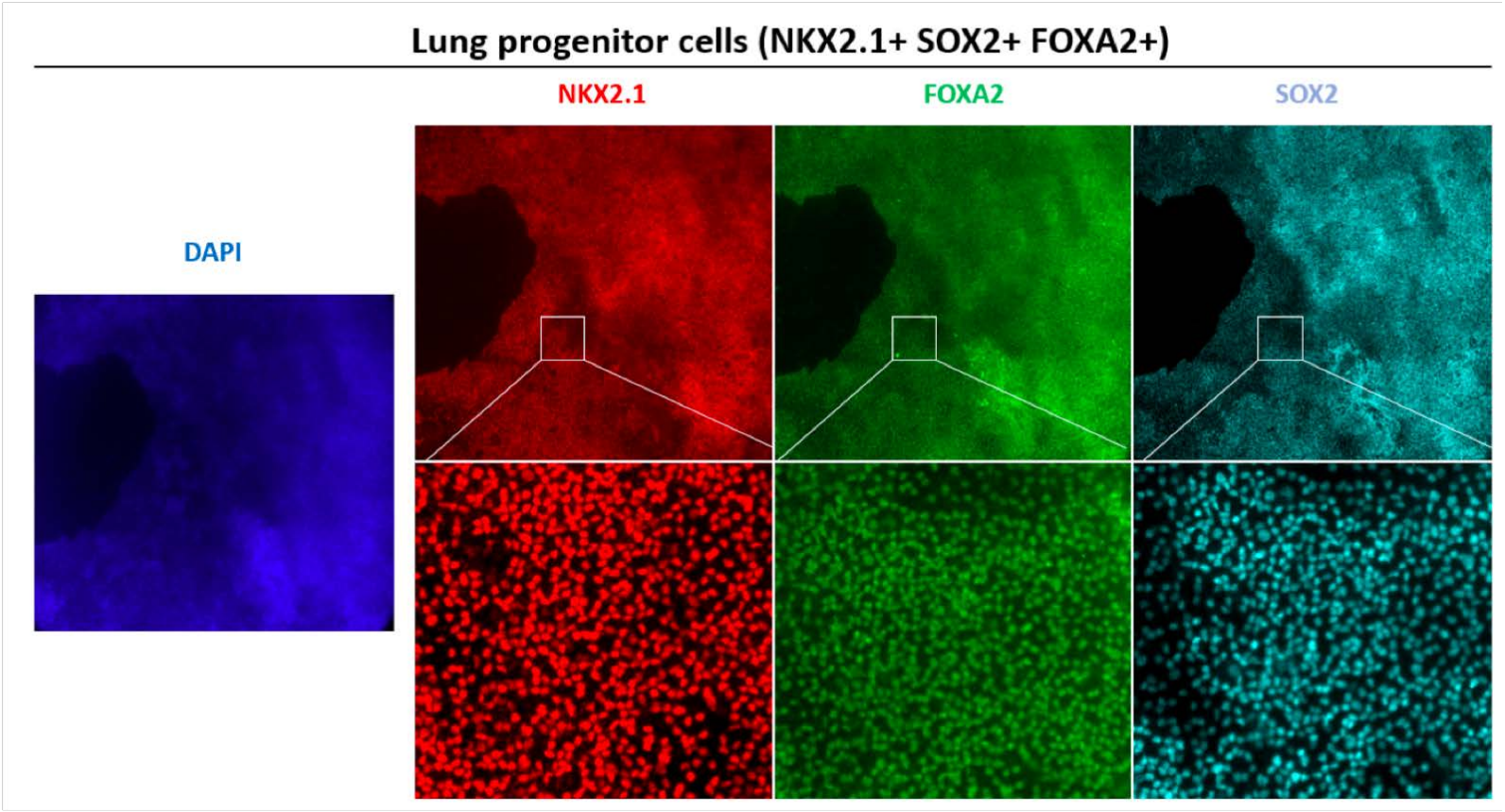
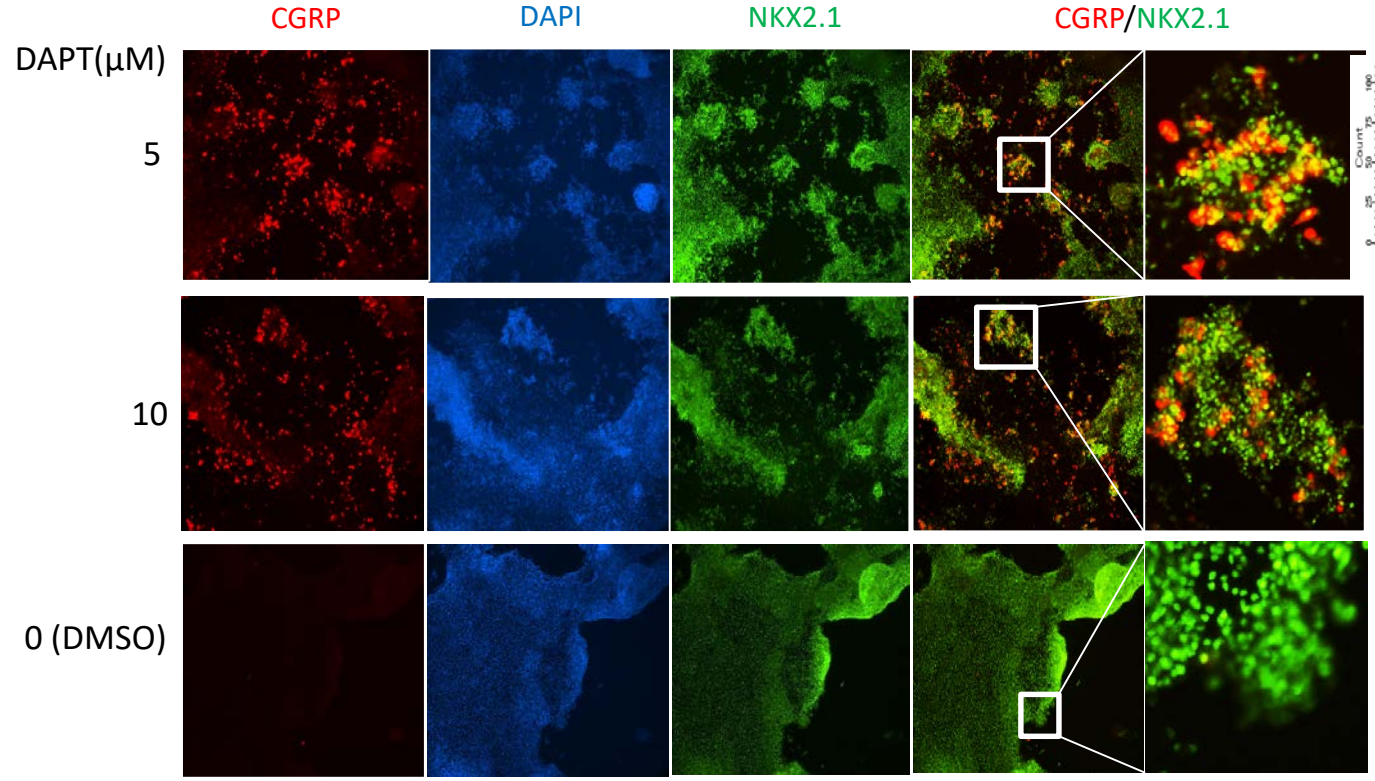
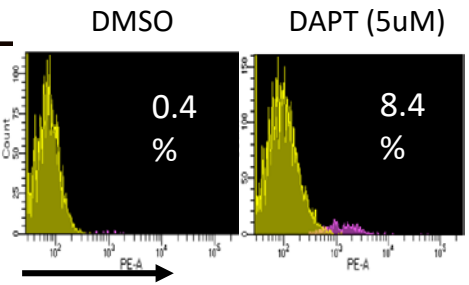


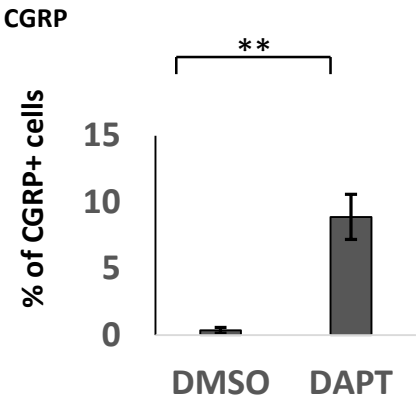
Generation of PNEC-like cells with a NOTCH inhibitor (DAPT) that blocks gamma-secretase

PNEC differentiation

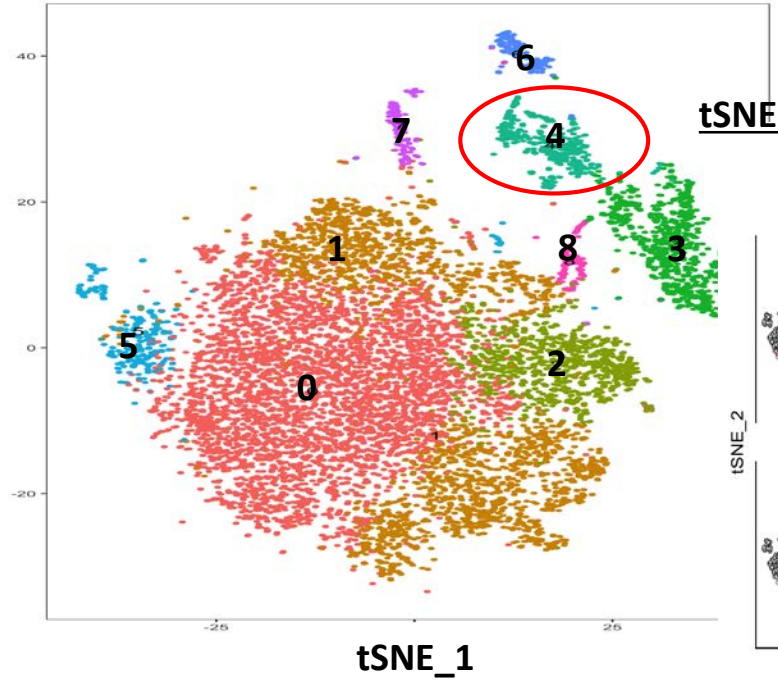
hESC → DE/AFE → lung progenitor (d15) → lung progenitor (d25) → (+ DAPT) → Mature lung cells (d50)



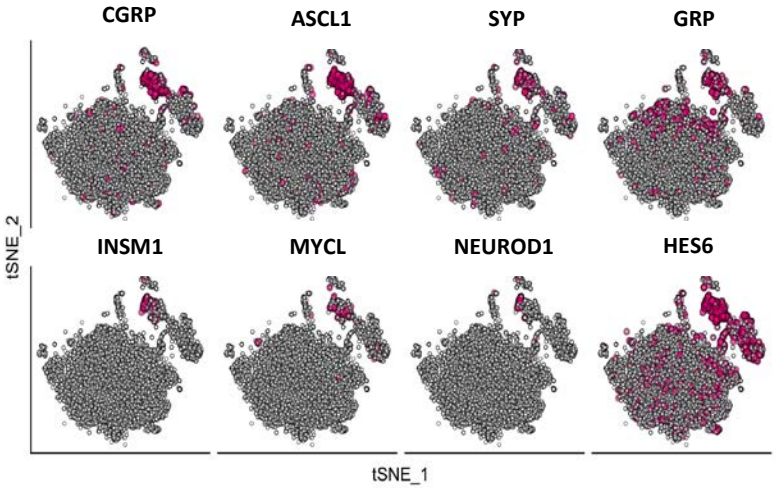




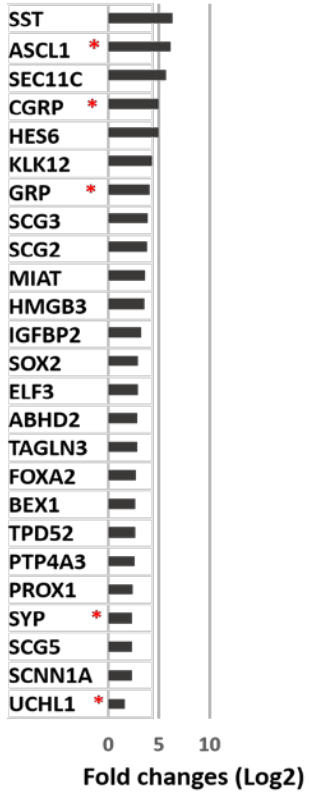
Clustering single lung cells by gene expression profiles



tSNE of individual cells positive for PNEC markers



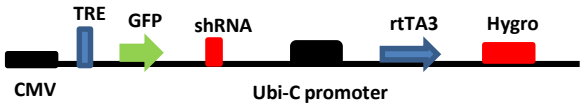
Putative Marker Genes

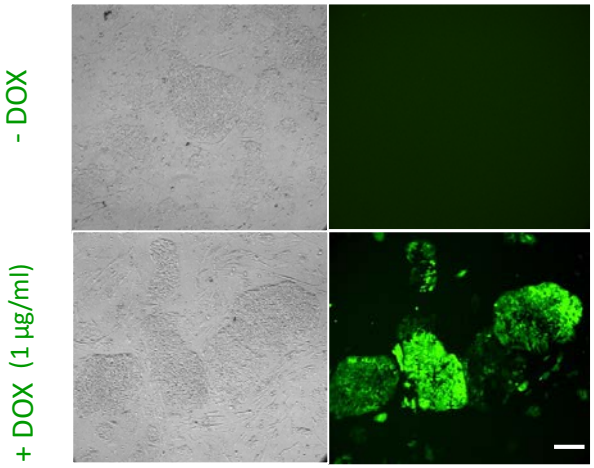


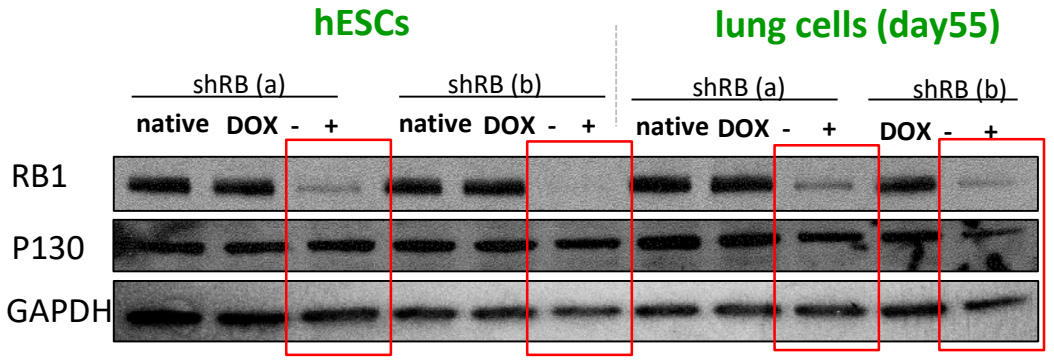
- *tSNE: t-Distributed Stochastic Neighbor Embedding (tSNE) analyses to divide1,200 single cells into distinct cell populations.*

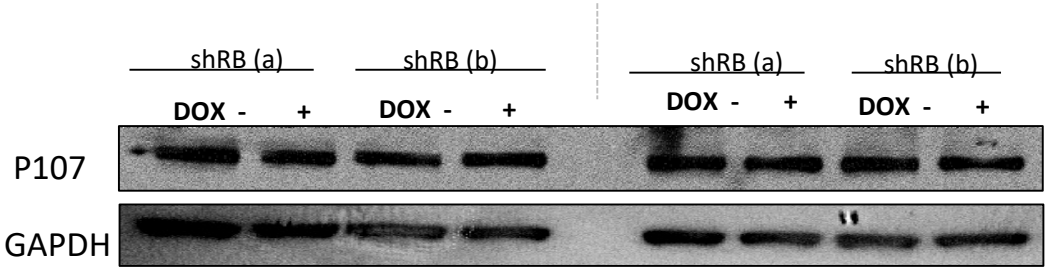
Knocking down RB with TetO-regulated shRNA

RUES2 TetO-shRNA-RB

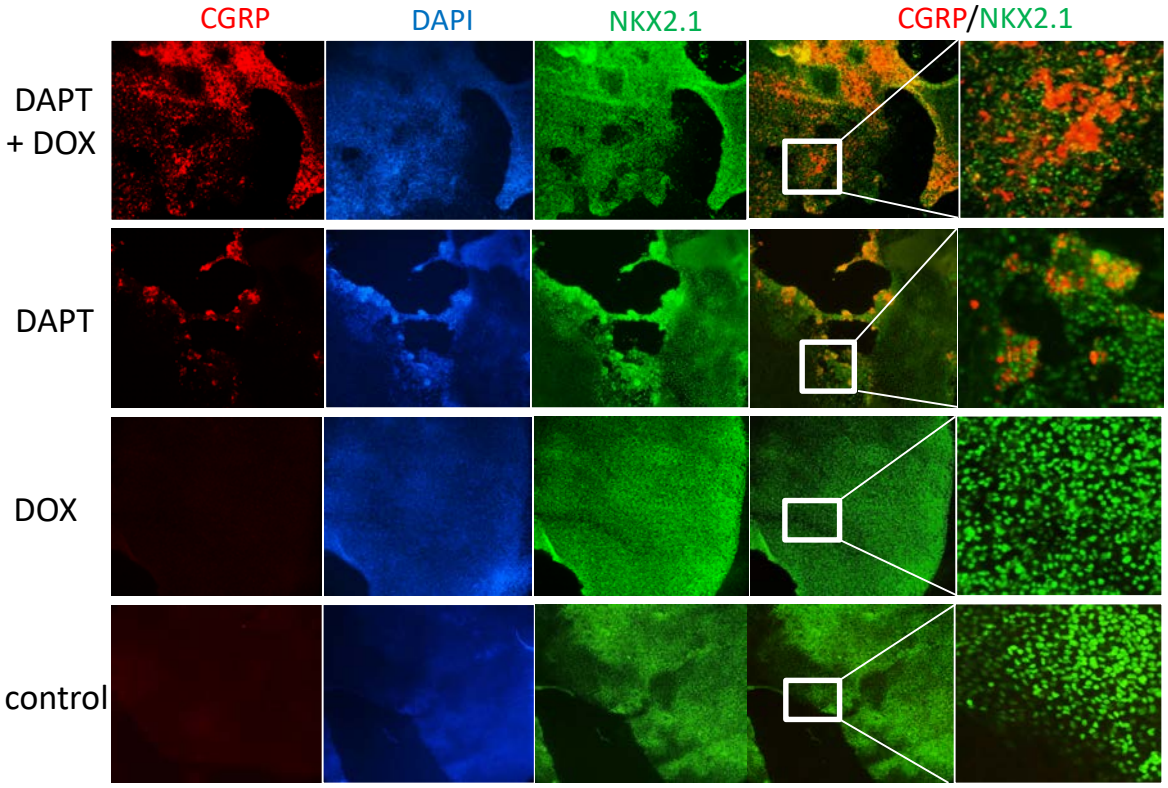


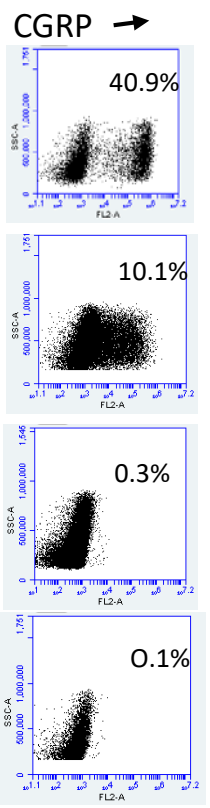


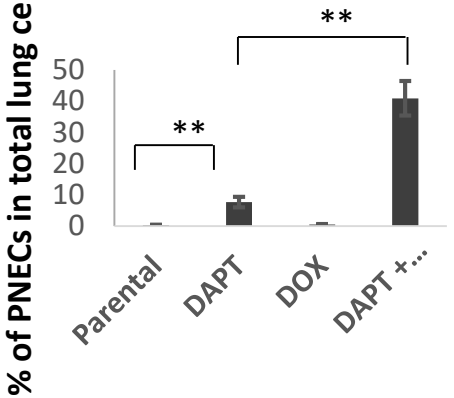




Knocking down RB increases the percentage of PNEC





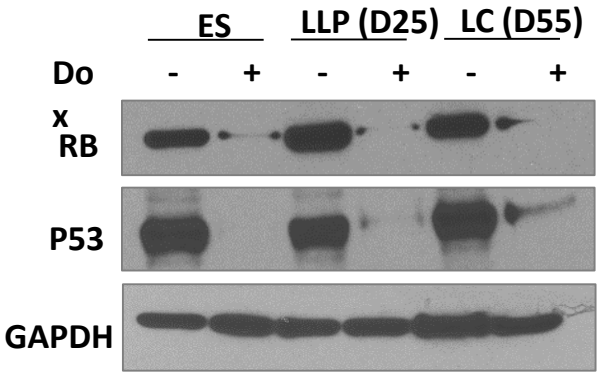


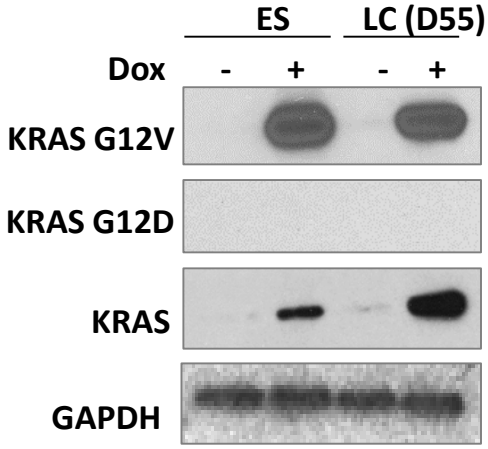
Reduced P53 or Mutated KRAS or EGFR do not induce or change percentage of PNECs

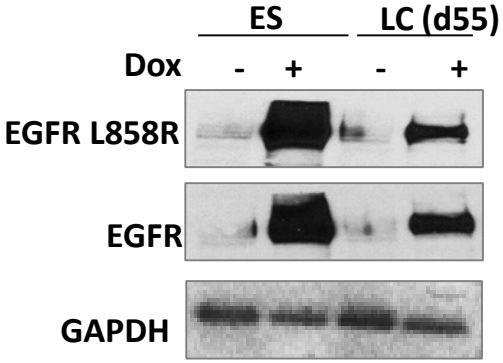
- *TetO- shP53 transgenic RUES2 lines*
- *TetO-KRAS(G12V) or TetO-EGFR(L858R) transgenic RUES2 lines*

1. DAPT
2. DOX (shP53/KRAS G12V / EGFR L858R)
3. DAPT+ DOX

hESCs → DE/AFE → Early progenitor (d15) → late progenitor (d25) → Lung cells (d55)







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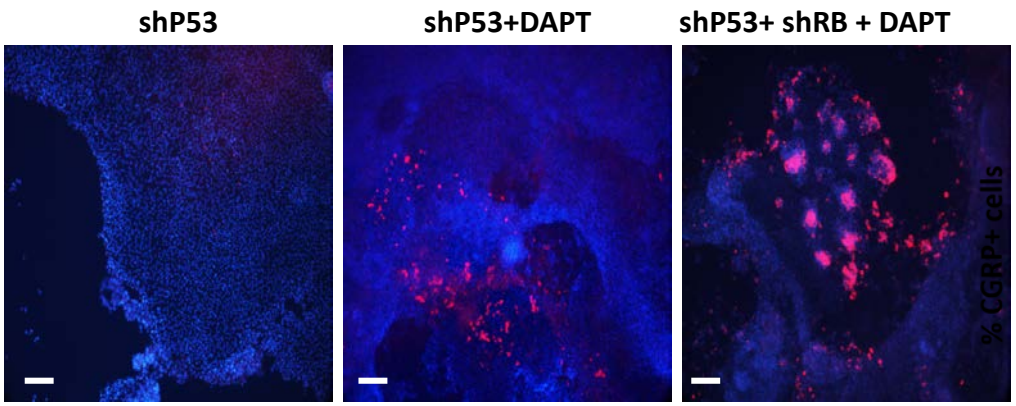
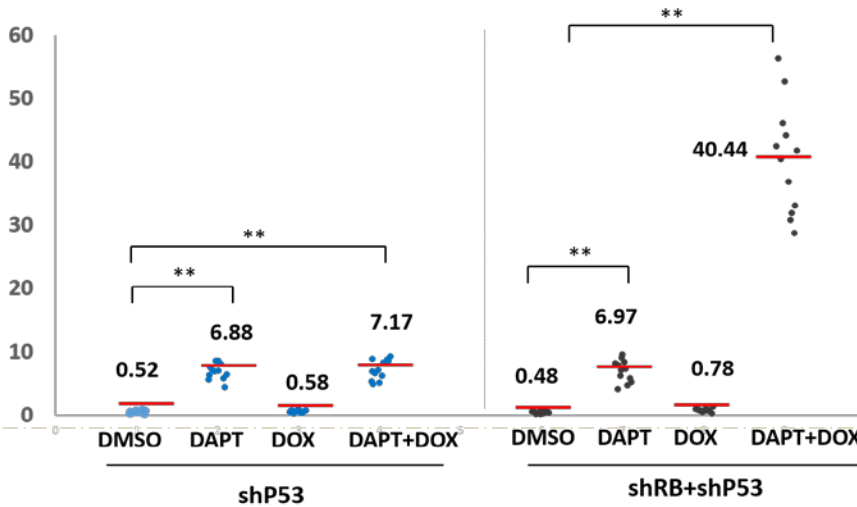
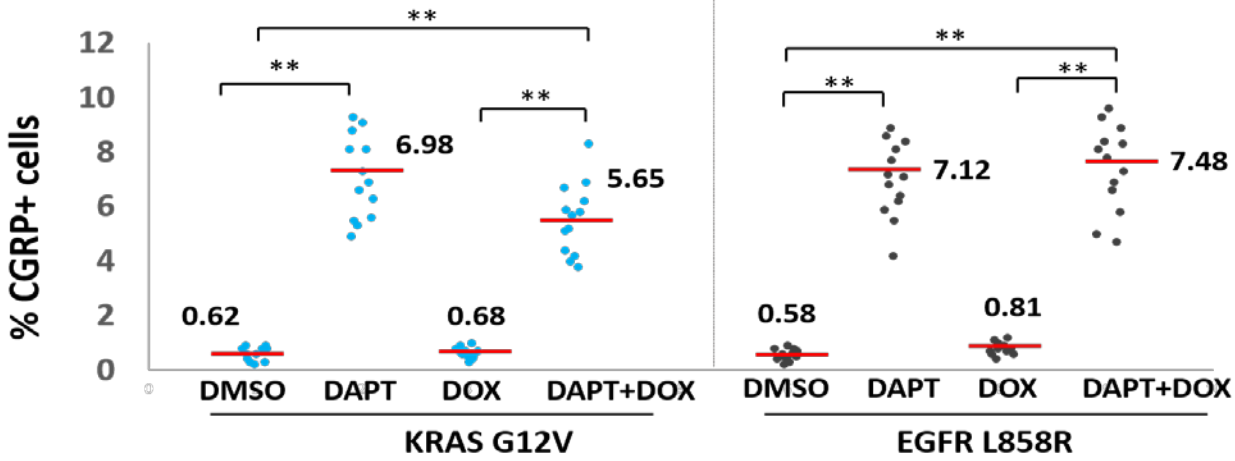
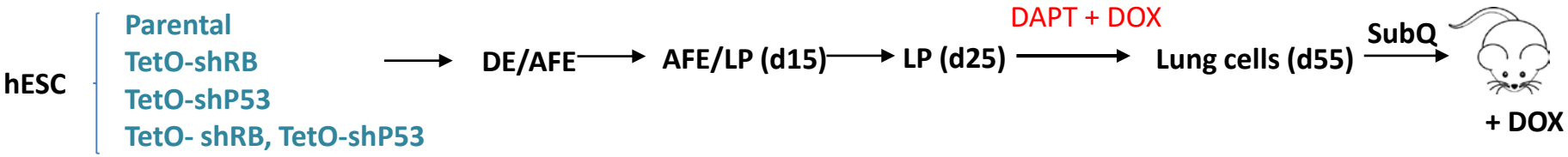






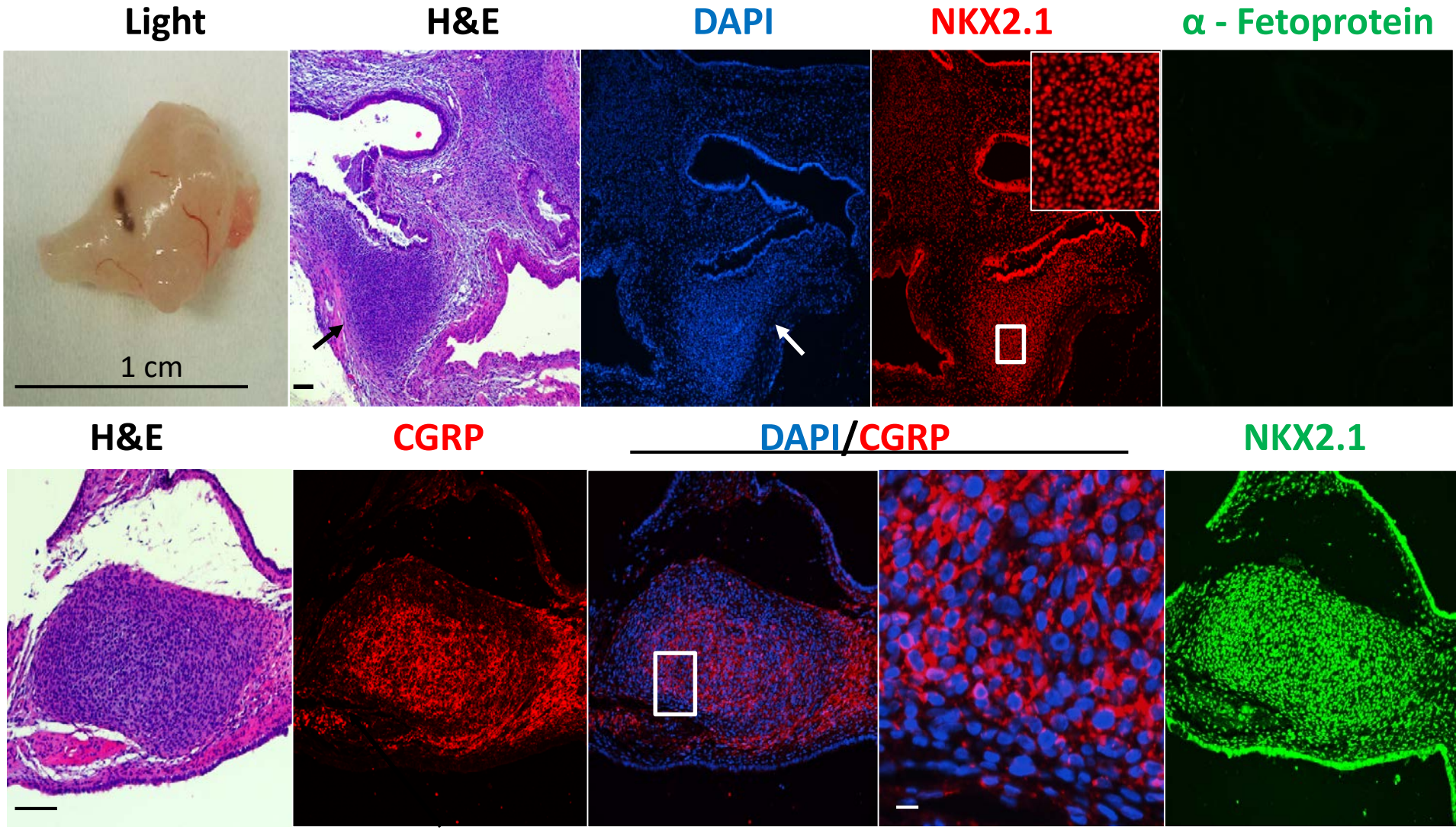
After inhibition of NOTCH, RB, and P53, hESC-derived lung cells form tumors in mice



Tumor formation SubQ in immunodeficient mice

Cells	% CGRP+ cells in total injected cells	Tumors / injection (≥ 250mm^3)
Parental (DAPT alone)	7.6 ± 1.0	0/12
DAPT+shRB	39.6 ± 4.4	0/14
DAPT+shP53	8.0 ± 1.3	0/11
DAPT+shRB+shP53	41.9 ± 4.6	14/19 **

After inhibition of NOTCH, RB, and P53, hESC-derived lung cells form small SCLC-like tumors in mice sub Q



SOME QUESTIONS:

--What accounts for increased proportion of PNECs after lowering RB levels? Replication vs differentiation?

--How similar are the tumors to clinical SCLC?

--Can additional genetic changes cause tumor progression?

--Are some PNECs more tumorigenic than others?

--Better tumorigenesis assays than xenografting subQ?

--What strategies can produce LUAD or LUSC from hESCs?

--Are other cells in the lung lineage or other lung cancer ce susceptible to changes that produce SCLC in PNECs?

THREE GENERAL OBSERVATIONS

- Then vs Now: transformation assays are still useful, but more sophisticated

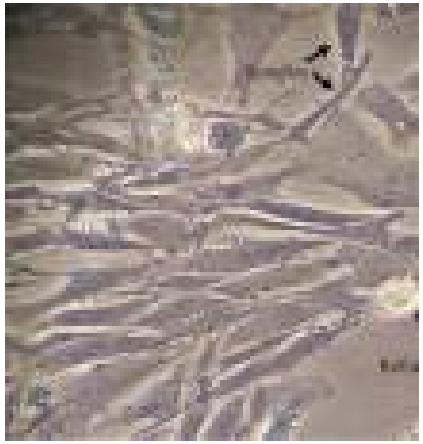
THEN: HOW DOES A NORMAL CELL BECOME A CANCER CELL

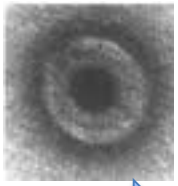
LATE STAGE CHICKEN EMBRYO

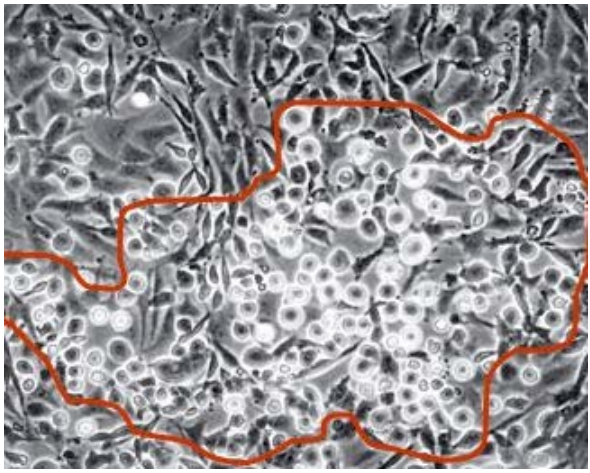
FIBROBLASTS IN CULTURE DISH

INFECT WITH RSV

TRANSFORMED CELLS (SARCOMA)



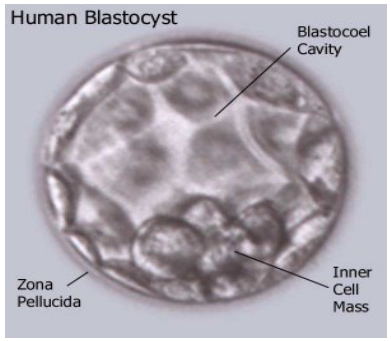




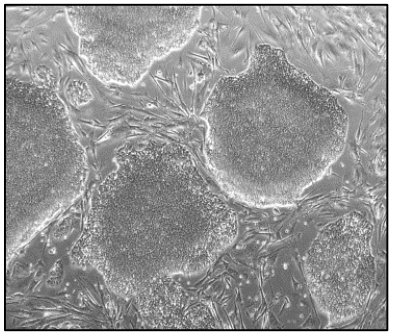
TEMIN AND RUBIN 1957

NOW: HOW DOES A NORMAL CELL BECOME A CANCER CELL?

EARLY STAGE HUMAN EMBRYO

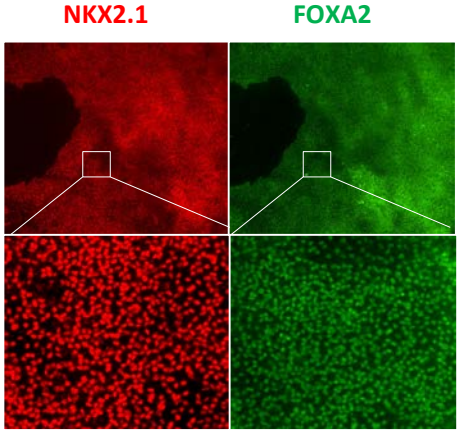


EMBRYO STEM CELLS IN CULTURE DISH



HORMONES

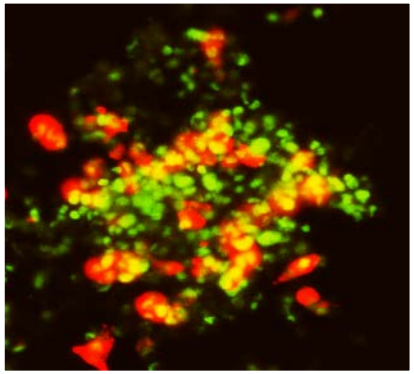
HUMAN LUNG CELL PROGENITORS



LUNG NEURO-ENDOCRINE CELLS

CGRP/NKX2.1

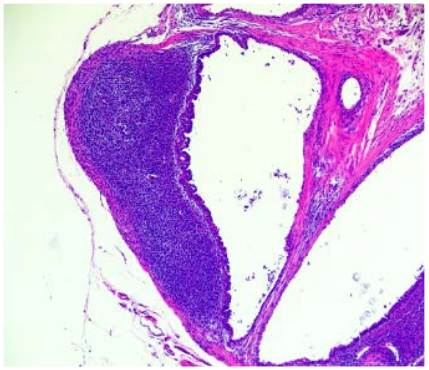
BLOCK NOTCH SIGNALING



INACTIVATE TUMOR SUPPRESSOR GENES

INJECT CELLS UNDER MOUSE SKIN

SMALL CELL LUNG CANCER IN MOUSE



CHEN ET AL 2018

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- Then vs Now: transformation assays are still useful, but more sophisticated

- **Developmental biology and cancer biology are increasingly intertwined and influenced by single cell biolo**

THREE GENERAL OBSERVATIONS

- Then vs Now: transformation assays are still useful, but more sophisticated

- Developmental biology and cancer biology are increasingly intertwined and influenced by single cell biolo

- **Internet-based communication of scientific results can (and should) be accelerated by pre-print servers**

Not yet in a peer-reviewed journal, but available to all:

Generation of pulmonary neuro-endocrine cells and tumors resembling small cell lung cancers from human embryonic stem cells

Joyce Chen, Asaf Poran, Arun Unni, Sarah Huang, Olivier Elemento, Hans-Willem Snoeck, Harold Varmus

bioRxiv 261461;
doi: https://doi.org/10.1101/261461

Encourage (or mandate) posting of preprints by members of the SCLC Consortium with alerts via email and web site

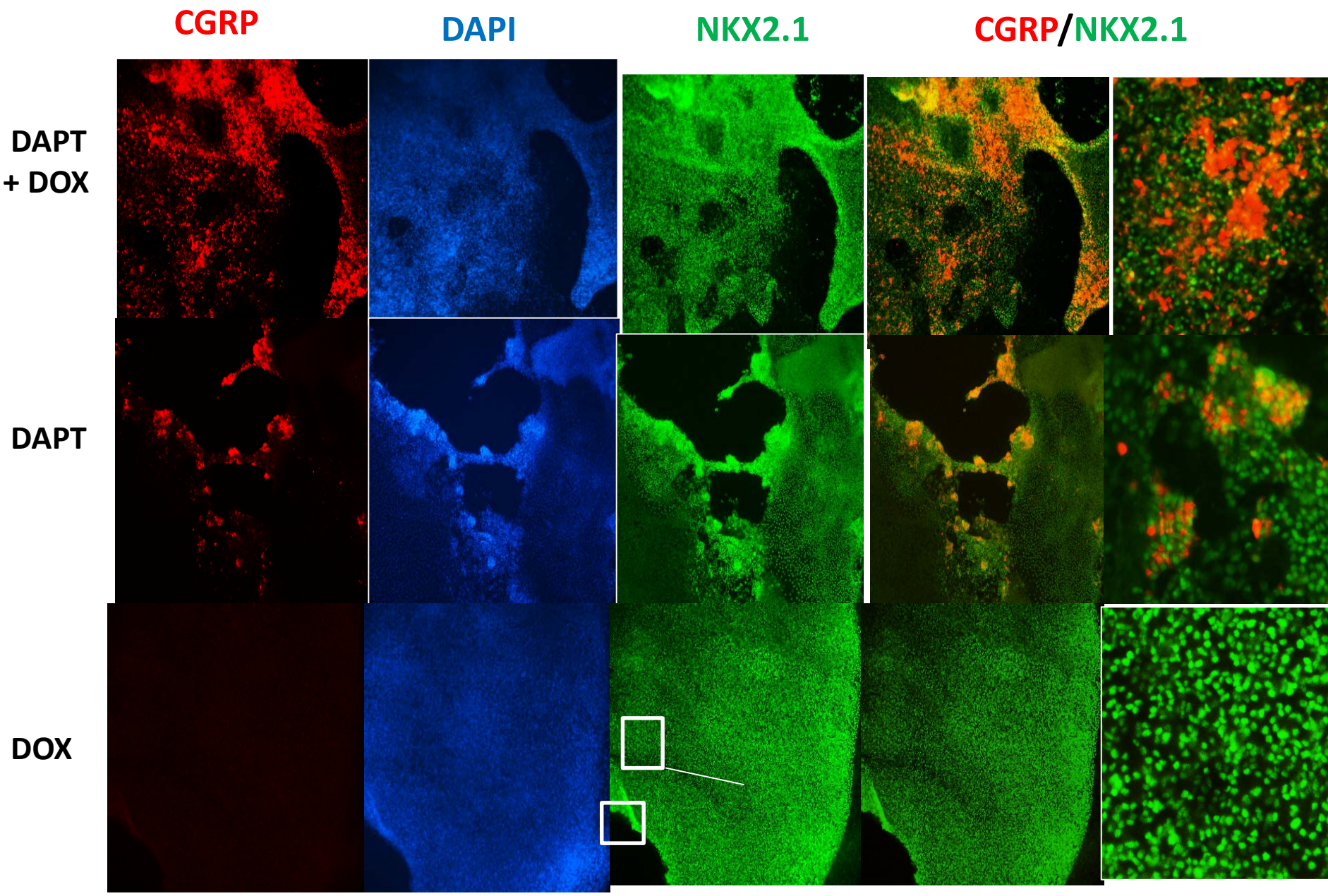
cc

cc

REMINDERS ABOUT LUNG CANCER, ESPECIALLY SCLC

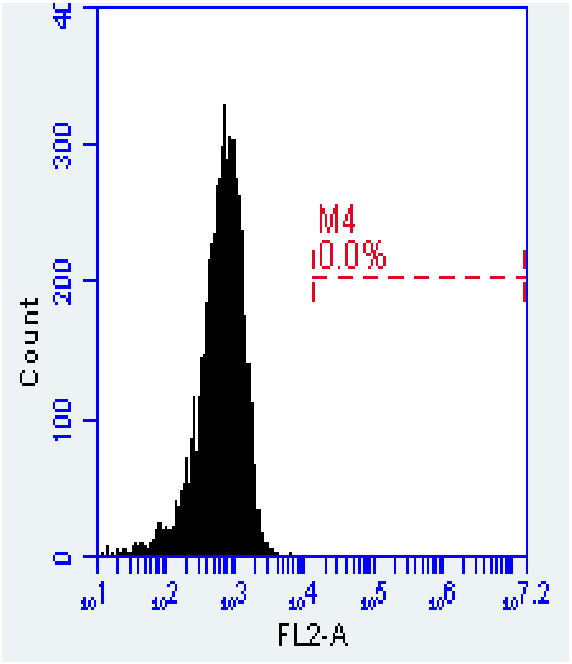
- Most common cause of cancer death worldwide
- Risks markedly increased by tobacco smoking
- Generally high mutation rate
- Three major forms---adeno CA, squamous CA, small cell (neuroendocrine) CA (SCLC)
- Characteristic genotypes
- Mouse models (Berns, Jacks) for SCLC

LUNG NE CELLS INDUCED BY INHIBITING NOTCH AND

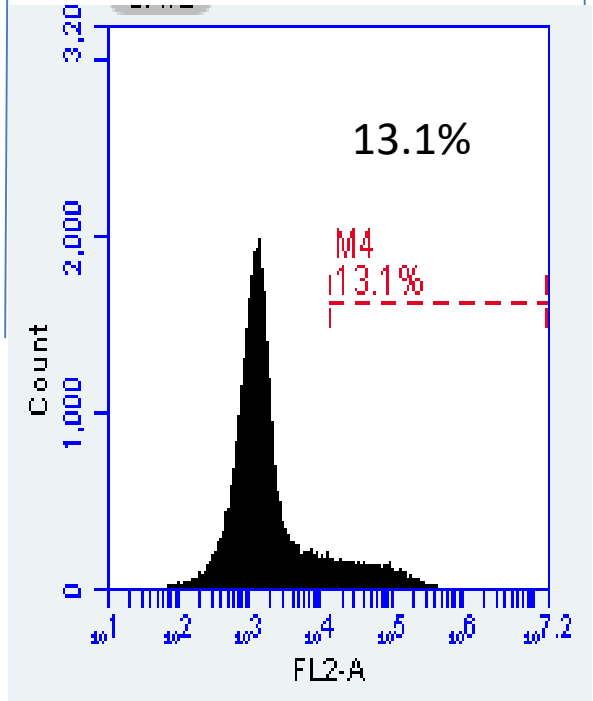


Percentage of PNEC like (CGRP+) cells increased by blocking NOTCH signaling, augmented by knocking down RB1 RNA

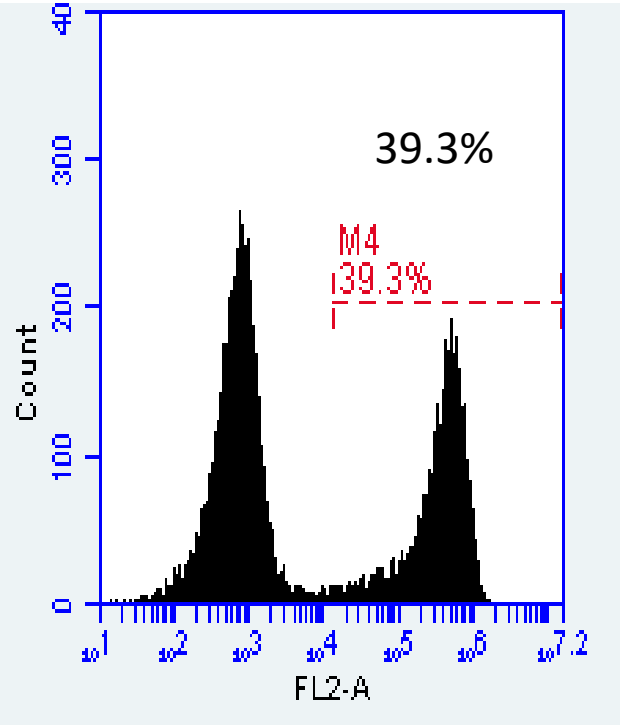
Control



NOTCH inhibition (DAPT)



NOTCH inhibition + reduction of RB (DAPT + Dox)



PUZZLES POSED BY GENOMIC RESULTS (& HOW USEFUL ARE MICE FOR SOLVING THEM

Why are some mutations mutually exclusive?

Example: KRAS + EGFR in lung adenocarcinomas (LUA

Why are some unexpected mutations oncogenic?

Example: splicing factor mutations in myeloid neoplasm

Why are certain patterns of mutations associated with cancers in certain lineages?

Examples: RB + P53 in small cell lung cancers (SCLC)
KRAS pathway in LUAD

THREE EXAMPLES: THEN VS NOW

THEN = 20th Century (1970 on)
vs.
NOW = 21st Century (so far)

Most changes driven by technology, not new questions

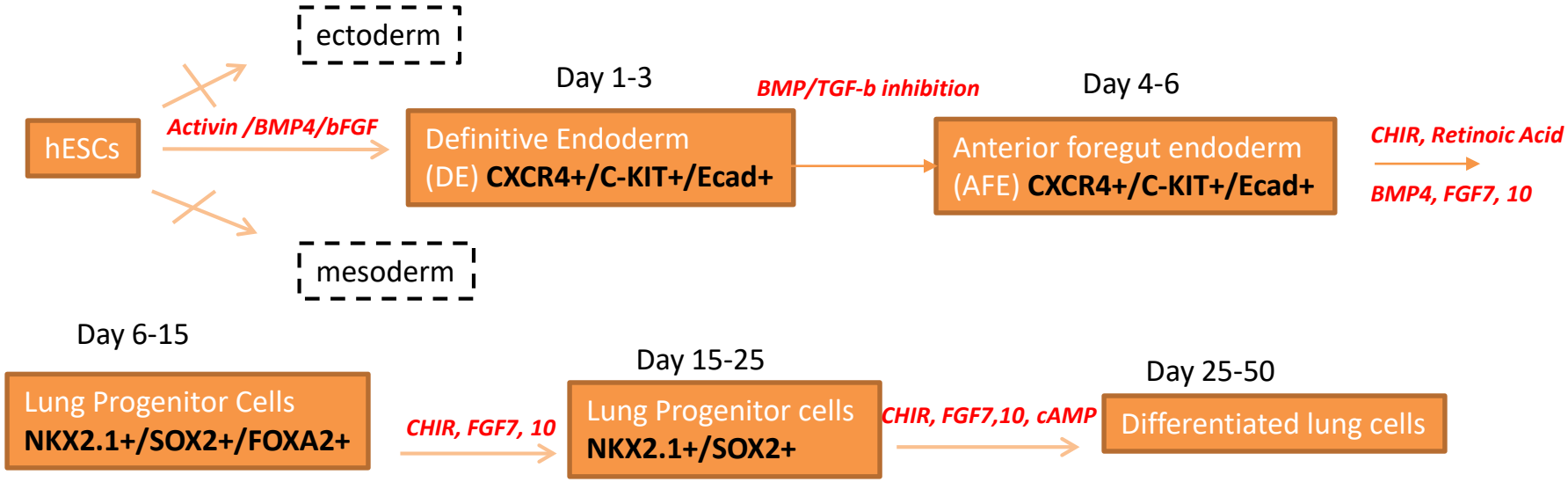
Goals: (1) Identify and understand cancer genes

(2) Find meaning in mutational combinations

(3) Define conditions for making cancer cells in cultu

Generation of lung cells by directed differentiation of human embryonic stem cells (hESCs) – RUES2 line

- **Lung differentiation**



Snoeck et.al, Nat Biotechnol, 2014.
Snoeck et.al, Nat Protoc, 2015.

Themes…

Transformation assays

Development and cancer genotypes

Information exchange

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