SAHLGRENSKA CANCER CENTER

GÖTEBORGS UNIVERSITET

Mouse Models of K-RAS- and B-RAF-induced Cancer

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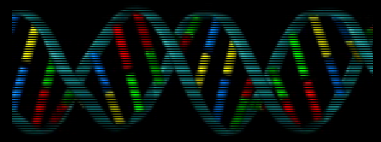
Why models? Why mice?

- Critical to understanding pathogenesis and identifying and testing new therapies
- Mice: mammals, good size, short reproductive cycle, tumor histology similar to human...

Mouse = Human

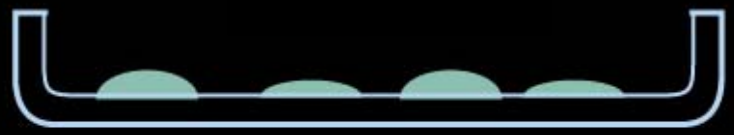
DNA in embryonic stem (ES) cells are altered by homologous recombination

DNA – part of a gene with engineered mutation



+ 40 mA

ES cells

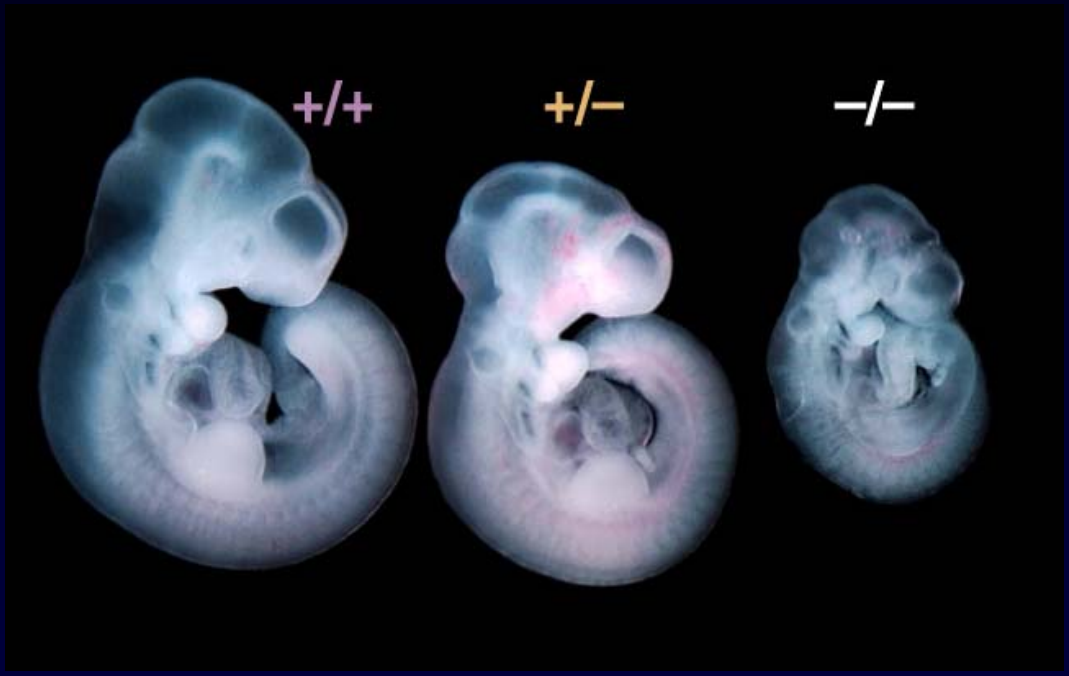


DNA also contains drug resistance selection markers and clones of resistant ES cells grow up in a few weeks

Mutant ES cells are injected into eggs and transplanted into a pregnant female who gives birth to chimeric mice

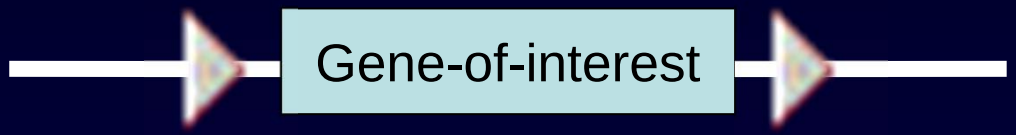
If mutant ES cells gave rise to gonads and sperm of the chimeric mouse, its offspring can inherit the mutation

Embryonic lethality is a common problem in conventional gene-targeting experiments



Advantagous if mutation could be switched on conditionally in adult mice or in specific cell types

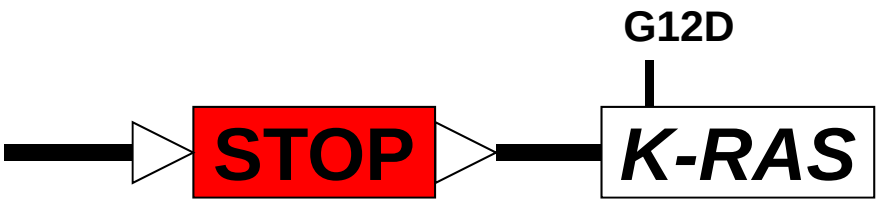
Conditional gene targeting using the Cre/loxP system



The CRE enzyme excises DNA sequences between two *loxP* sites

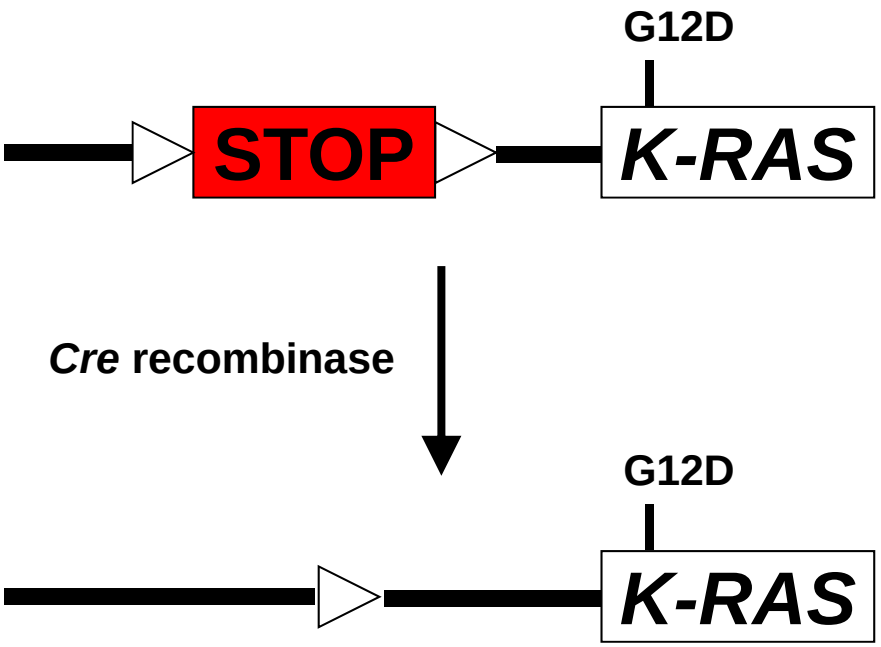
CRE can be expressed at will in specific cell types at different stages of development

Inducible oncogenic K-RAS allele driven by the endogenous promoter

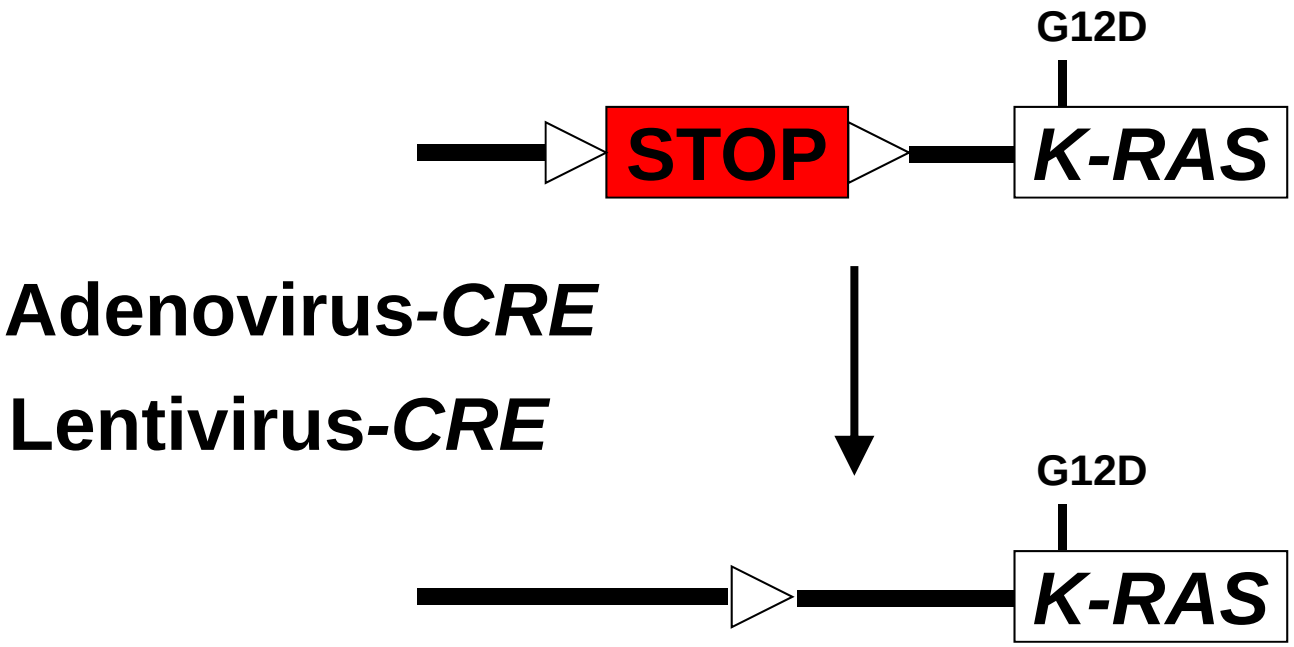


Tyler Jacks' model

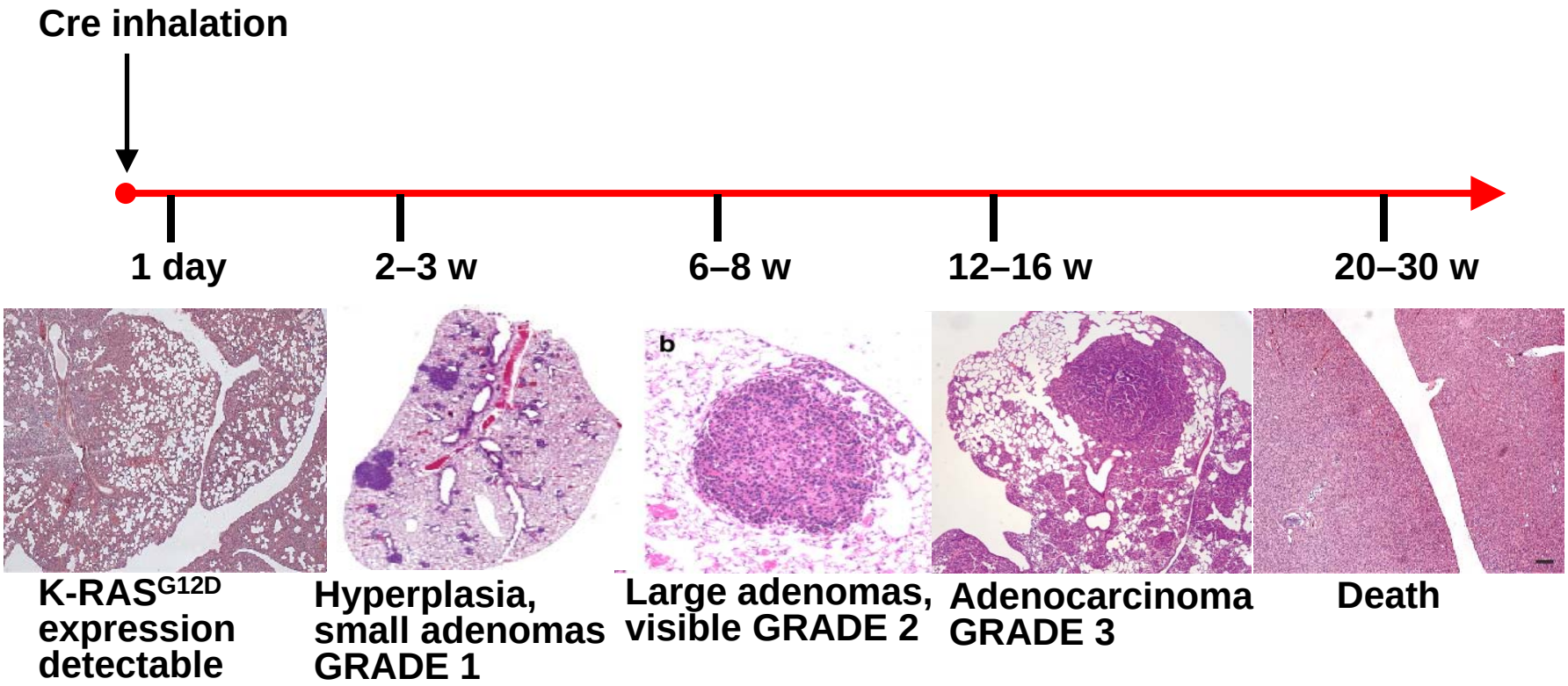
Inducible oncogenic K-RAS allele driven by the endogenous promoter



CRE expression triggered by inhalation of viruses with different properties

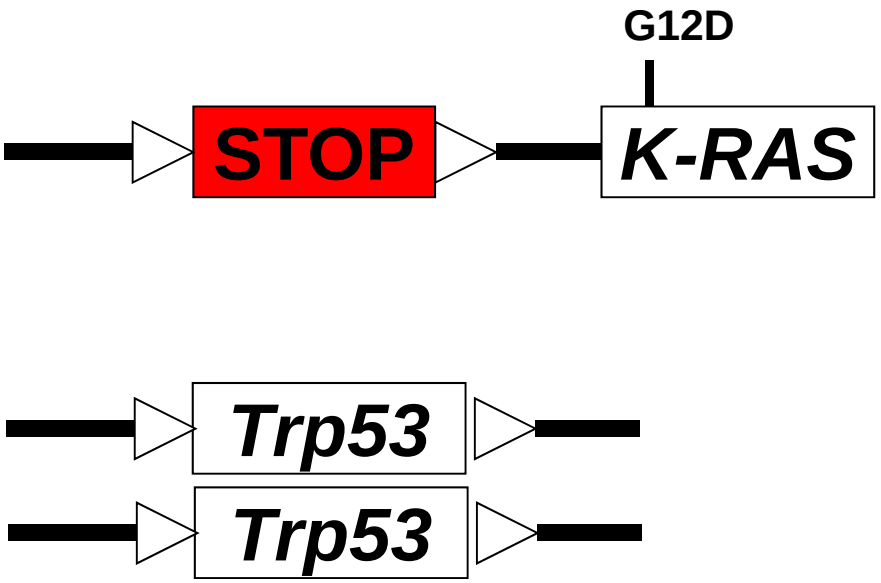


Tumor progression K-RAS^{G12D} alone: from hyperplasia to adenocarcinoma

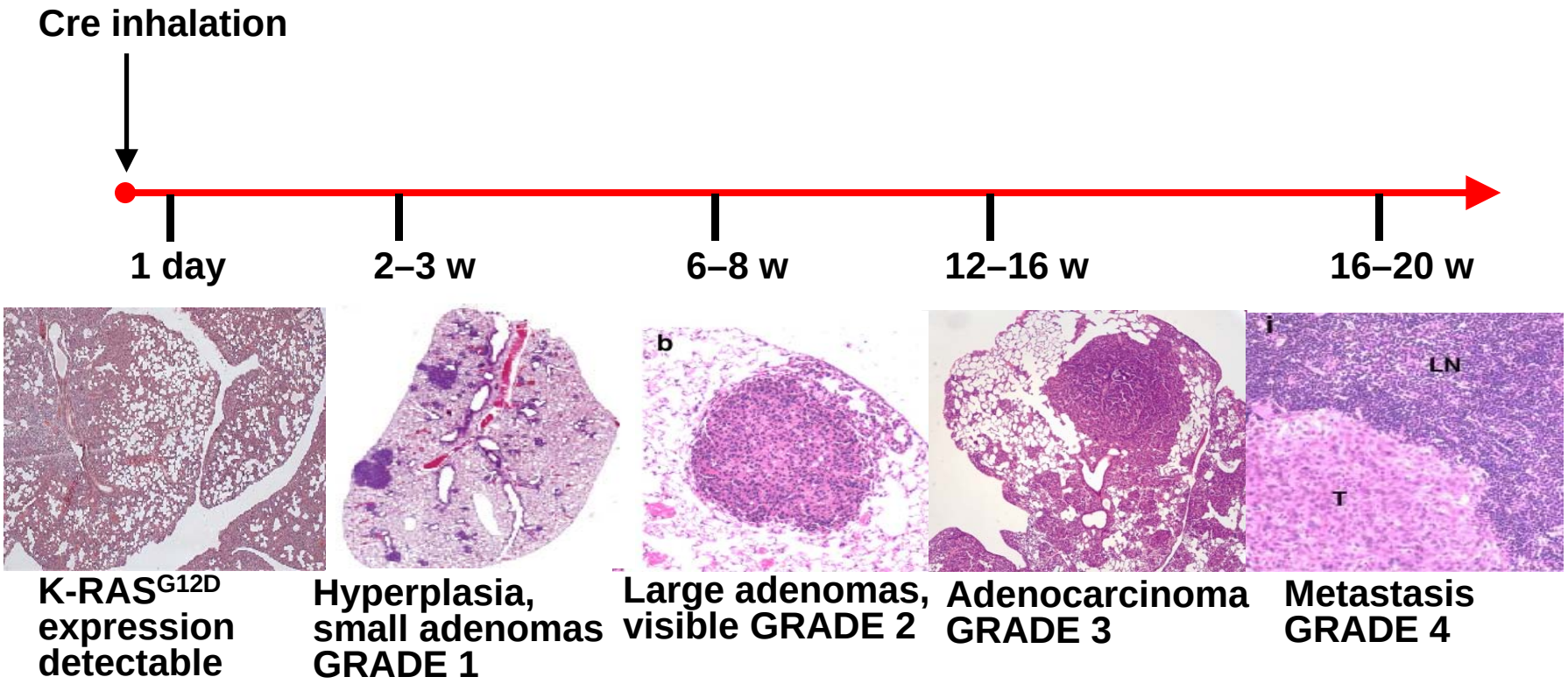


DuPage *et al. Nat Protocols. 2009*
Sjogren *et al. J. Clin. Invest.* 2007
Liu *et al. PNAS* 2010

Rapidly progressing metastatic K-RAS-induced lung cancer with p53 deficiency



K-RAS^{G12D} activation and simultaneous p53 inactivation: hyperplasia to metastatic adenocarcinoma



> 100 different *CAAX* Proteins in mammalian cells

CAAX

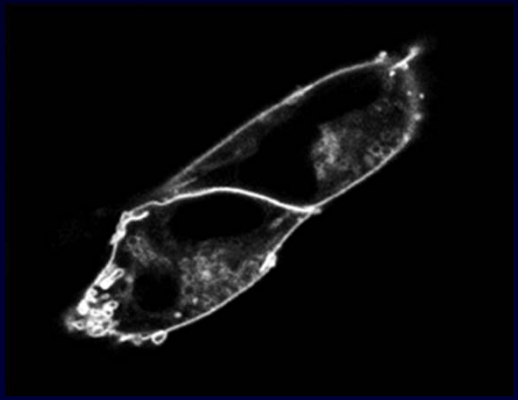
H-RAS
Prelamin A
Lamin B
HDJ2

N-RAS
K-RAS
RHEB

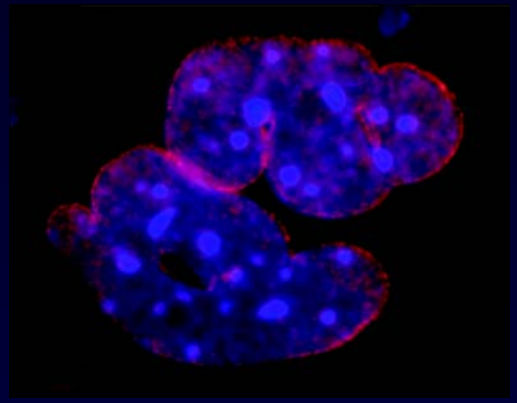
RHOA
RHOB
CDC42
RAC1
RAP1

CAAX Proteins are targeted to membrane surfaces…

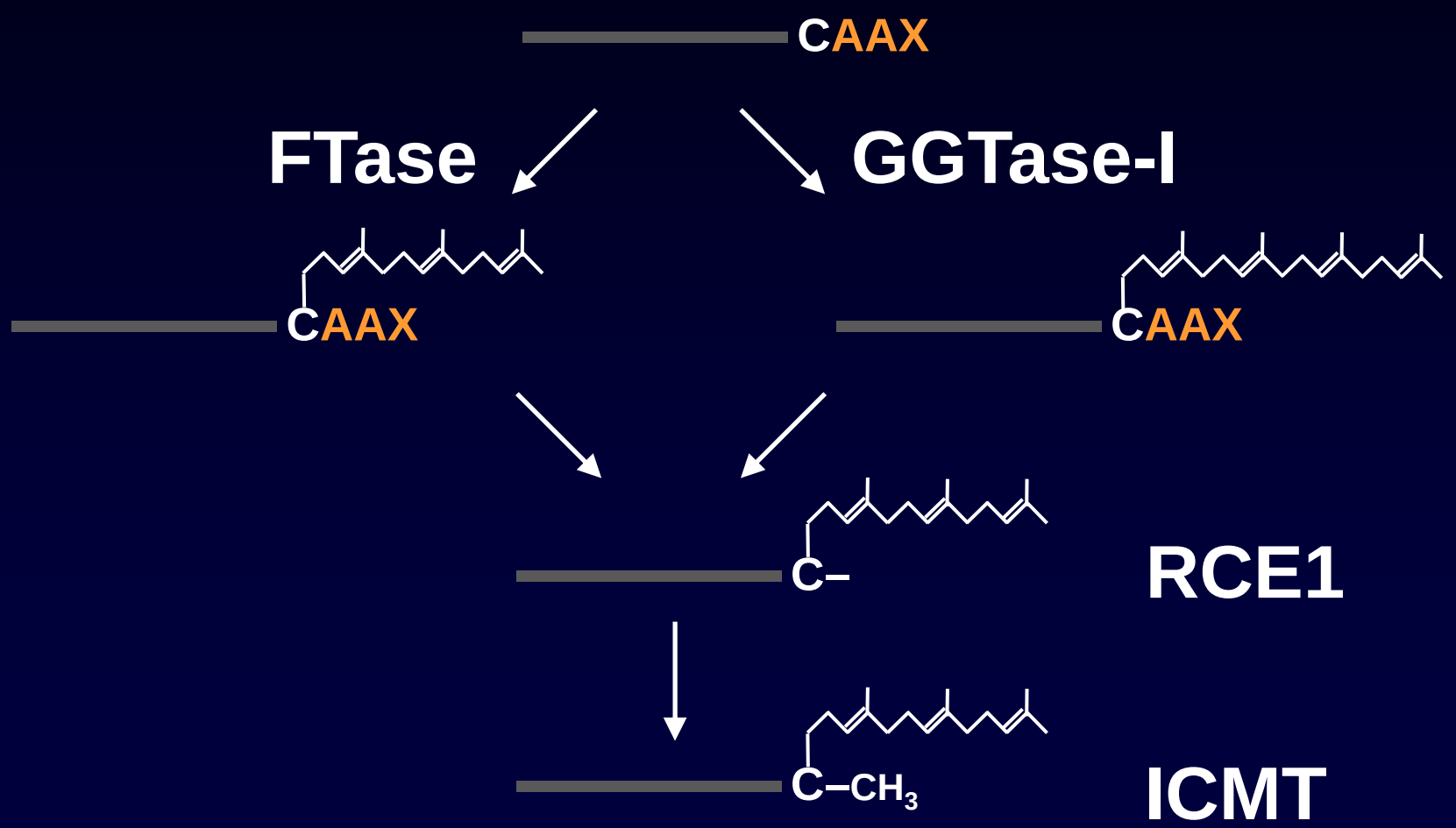
K-RAS



Prelamin A

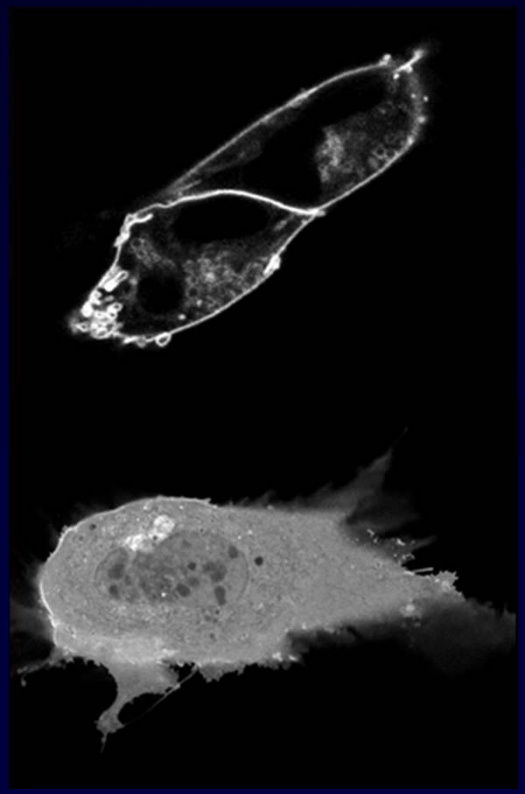


...by posttranslational processing of the *CAAX* motif



The posttranslational processing steps are important for membrane targeting

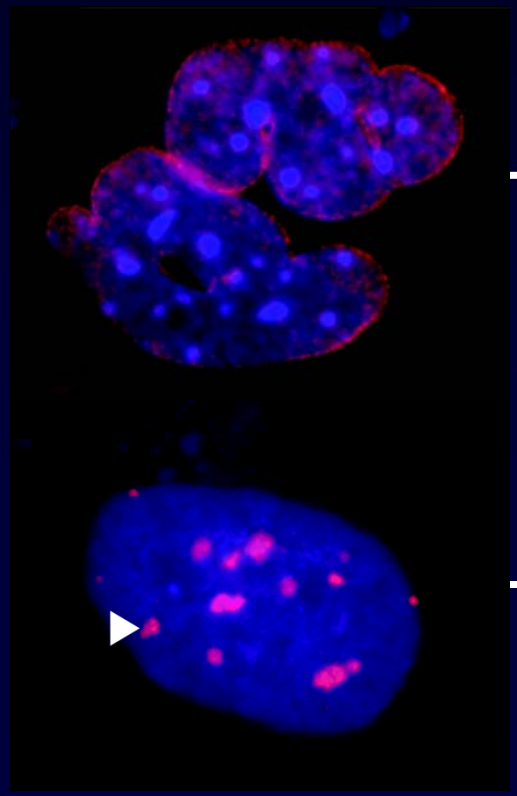
K-RAS



+ ICMT

– ICMT

Prelamin A



+ FTase

– FTase

FTase and GGTase-I have unique and overlapping *CAAX* protein substrates

CAAX

FTase

H-RAS
Prelamin A
Lamin B
HDJ2

N-RAS
K-RAS
RHEB

GGTase-I

RHOA
RHOB
CDC42
RAC1
RAP1

FTase and GGTase-I inhibitors (FTI, GGTI) were developed to block oncogenic RAS

CAAX

FTI ⊣ **FTase**

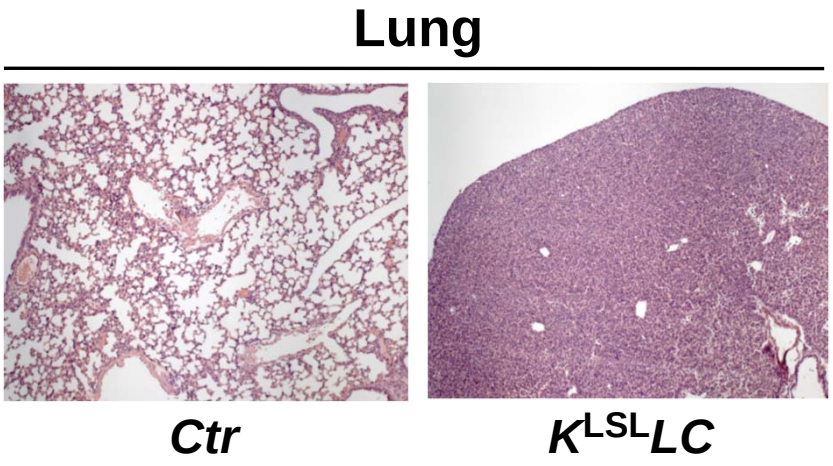
H-RAS
Prelamin A
Lamin B
HDJ2

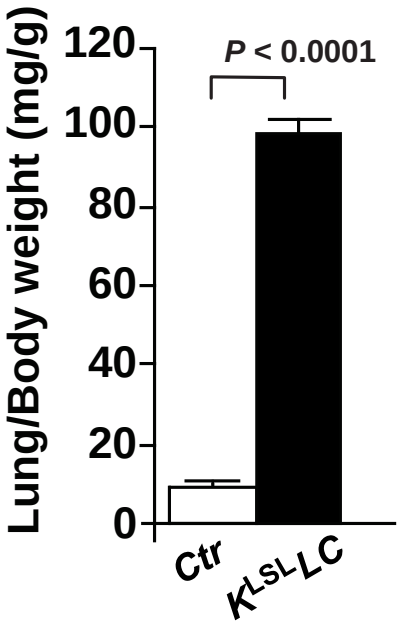
N-RAS
K-RAS
RHEB

GGTI ⊣ **GGTase-I**

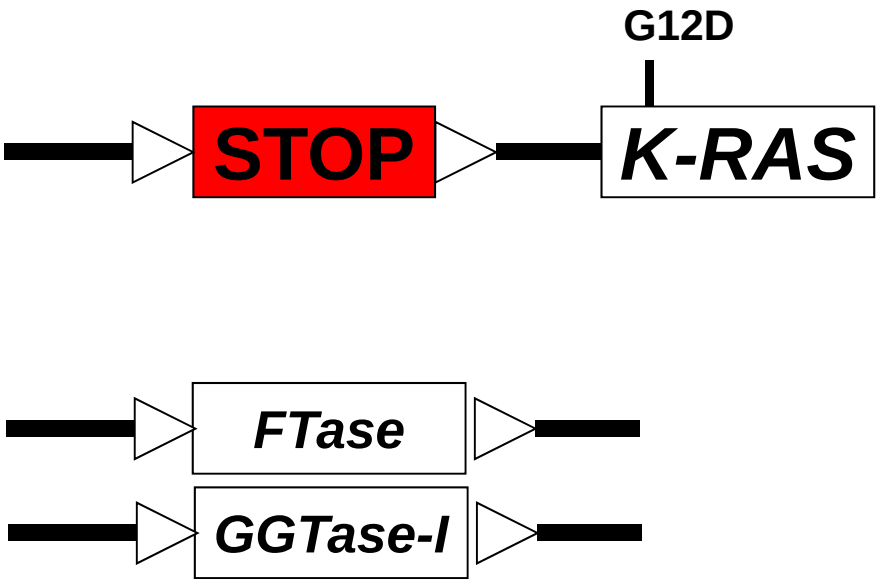
RHOA
RHOB
CDC42
RAC1
RAP1

A “quick” robust model: activating K-RAS^{G12D} expression in type-2 pneumocytes

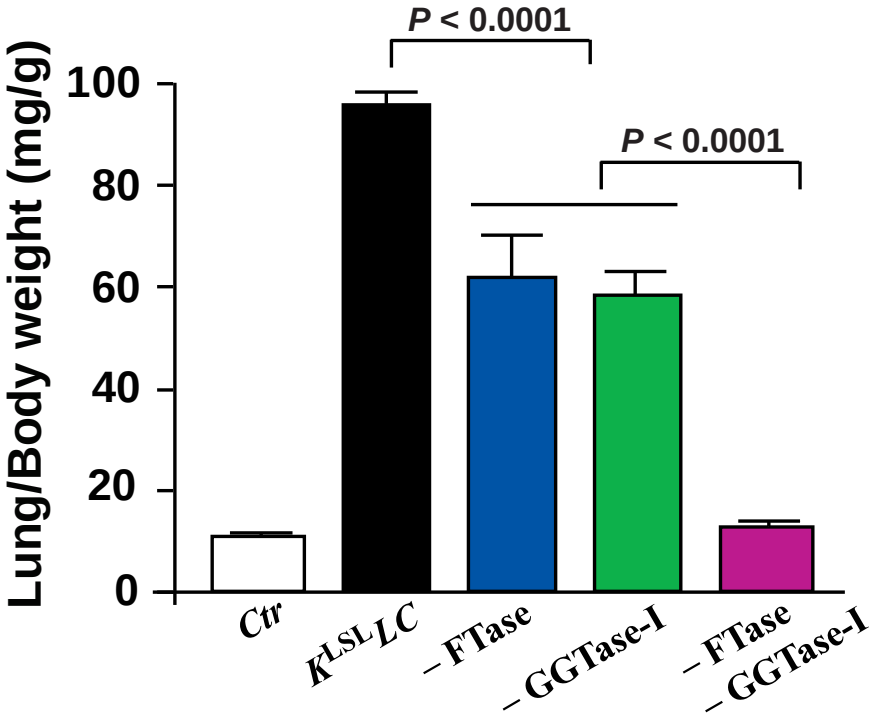




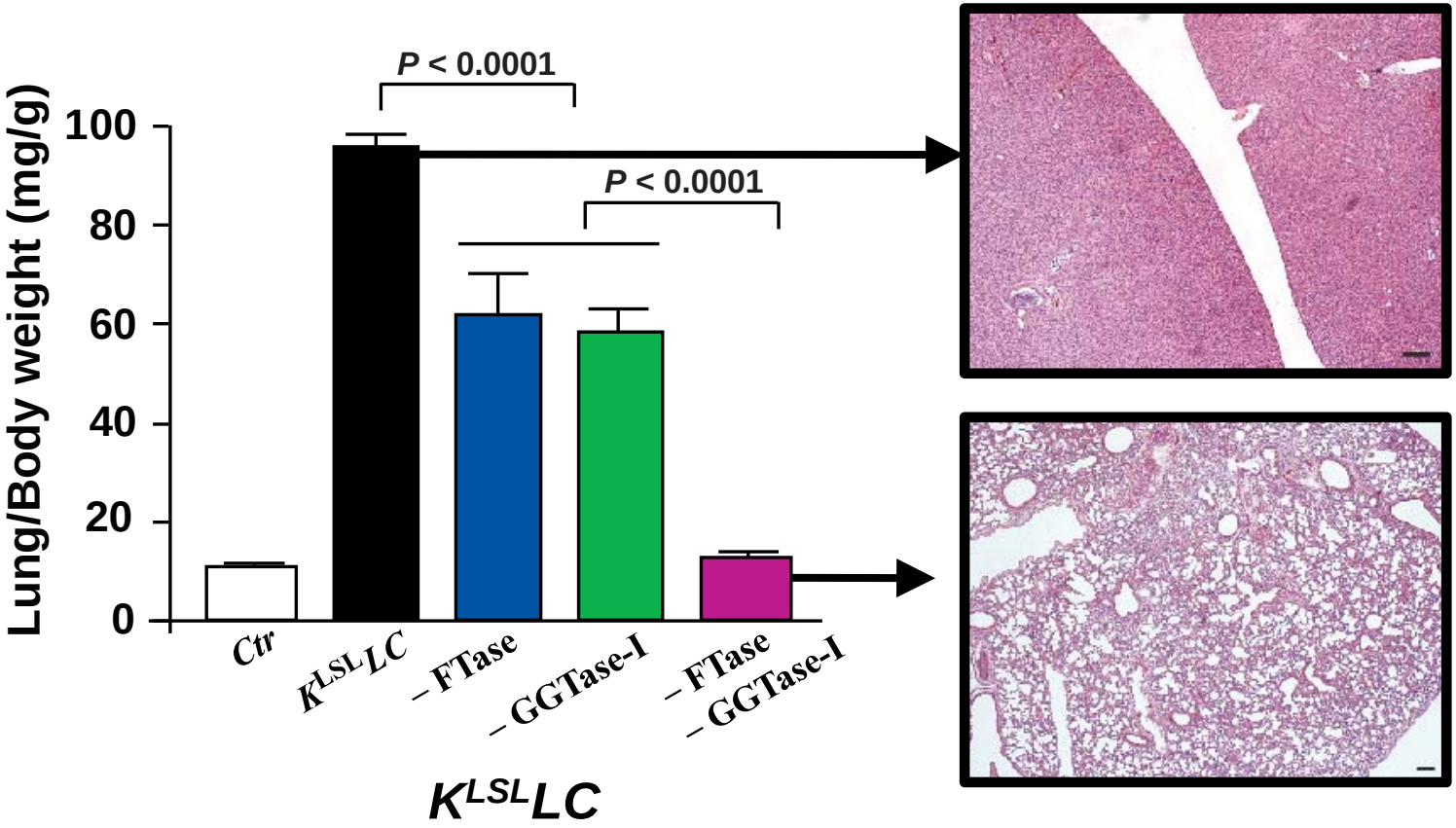
Using CRE to activate K-RAS and simultaneously inactivate FTase or GGTase-I, or both enzymes



Inactivation of FTase and/or GGTase-I Reduced Lung Weight of 3-week-old *K*^{LSL}*LC* Mice

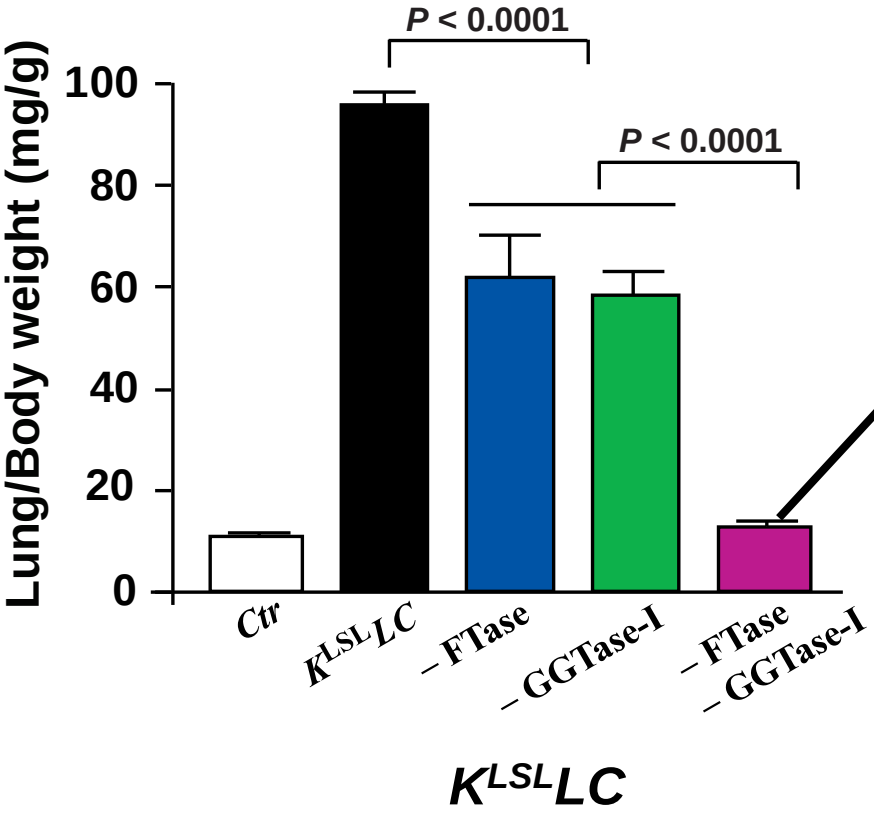


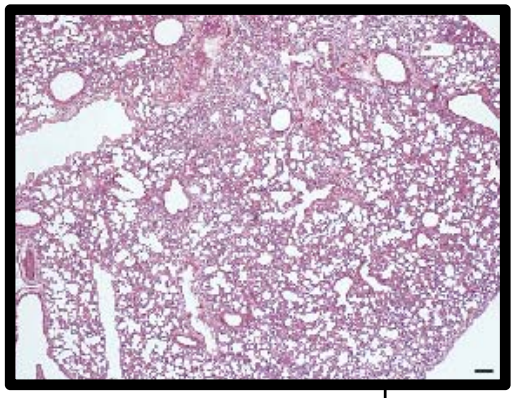
Inactivation of both *Fntb* and *Pggt1b* Normalized Lung Weight and Histology of $K^{LSL}LC$ Mice

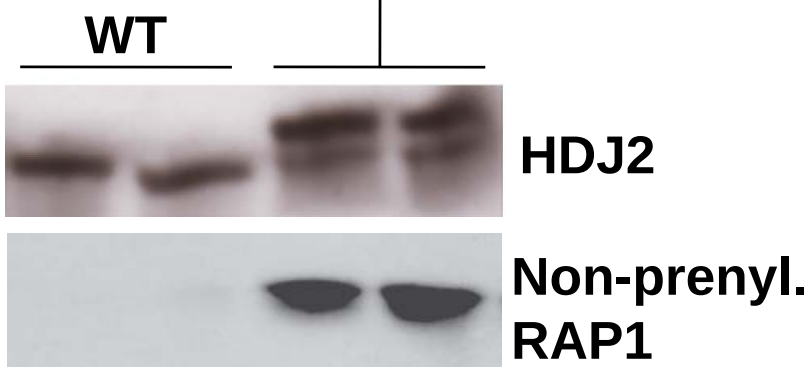


Normal histology and apparent lung function despite wide-spread expression of K-RAS^{G12D}

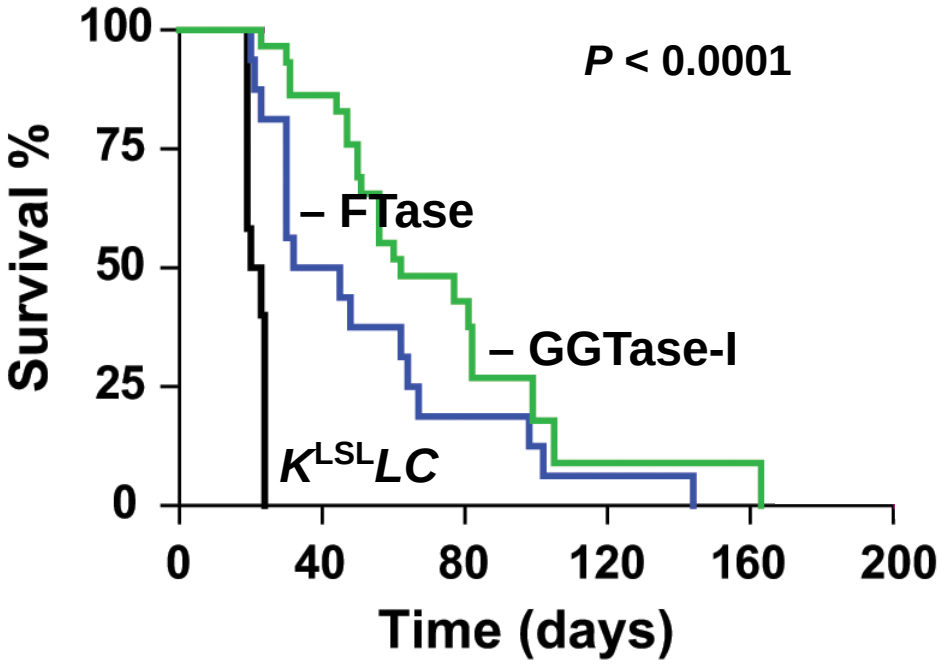
Detection of Markers for Absent FTase and GGTase-I Activities in Lung Extracts



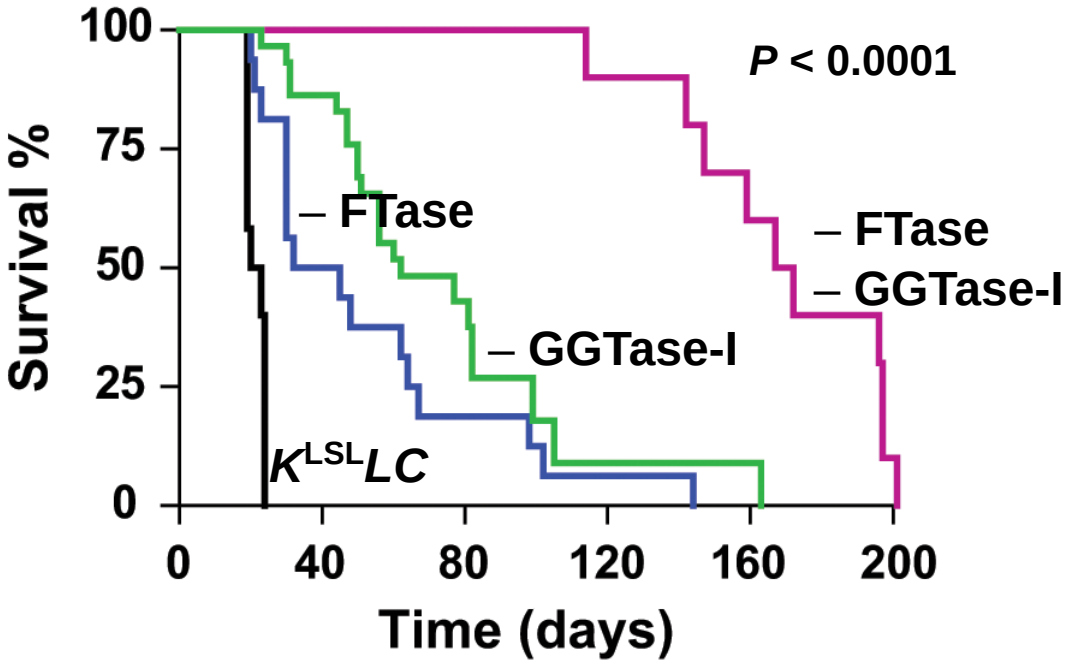




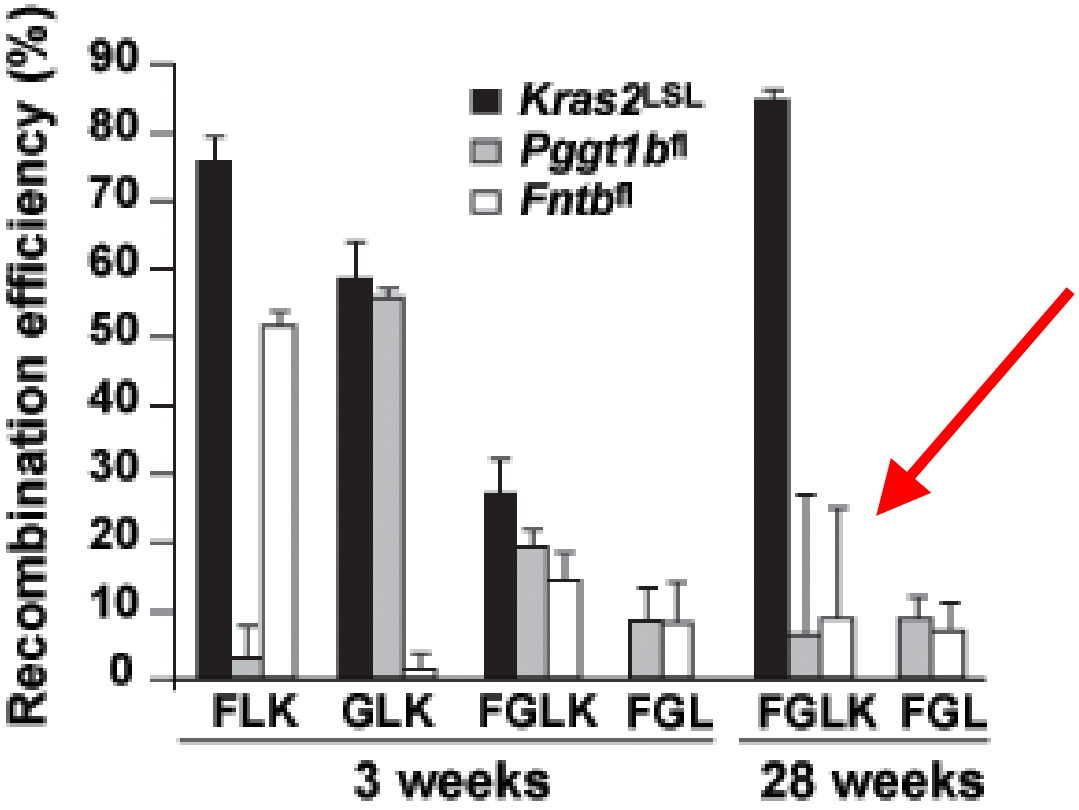
Inactivating FTase Improved Survival to a Similar Extent as Inactivating GGTase-I



Simultaneous Inactivation of FTase and GGTase-I Further Extended Lifespan of *K*^{LSL}*LC* mice



Tumors in 28-week-old FGLK mice show incomplete *Cre* recombination



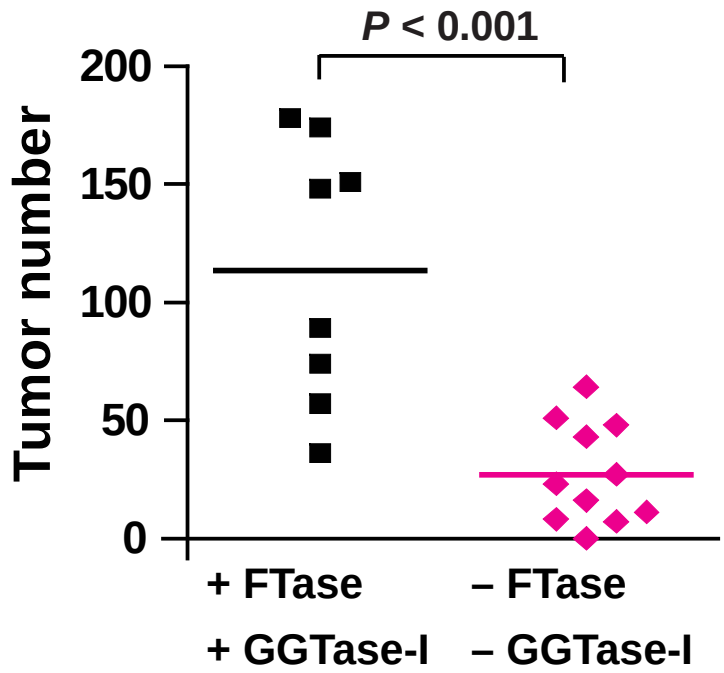
Potential drawback of approach: *in vivo* slection of tumor cells with incomplete recombination

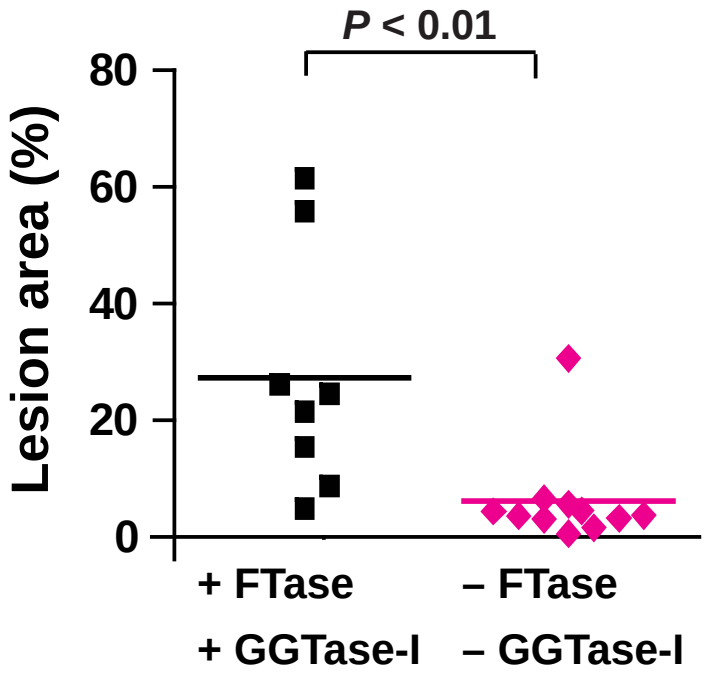
Induction of Lung Tumors By *Cre*-adenovirus Inhalation: Impact of FTase/GGTase Deficiency

K-RAS^{G12D} **Wild-type**

Control **FTase/GGTase-I knockout**

Inactivation of FTase and GGTase-I Reduced K-RAS-induced Lung Tumors



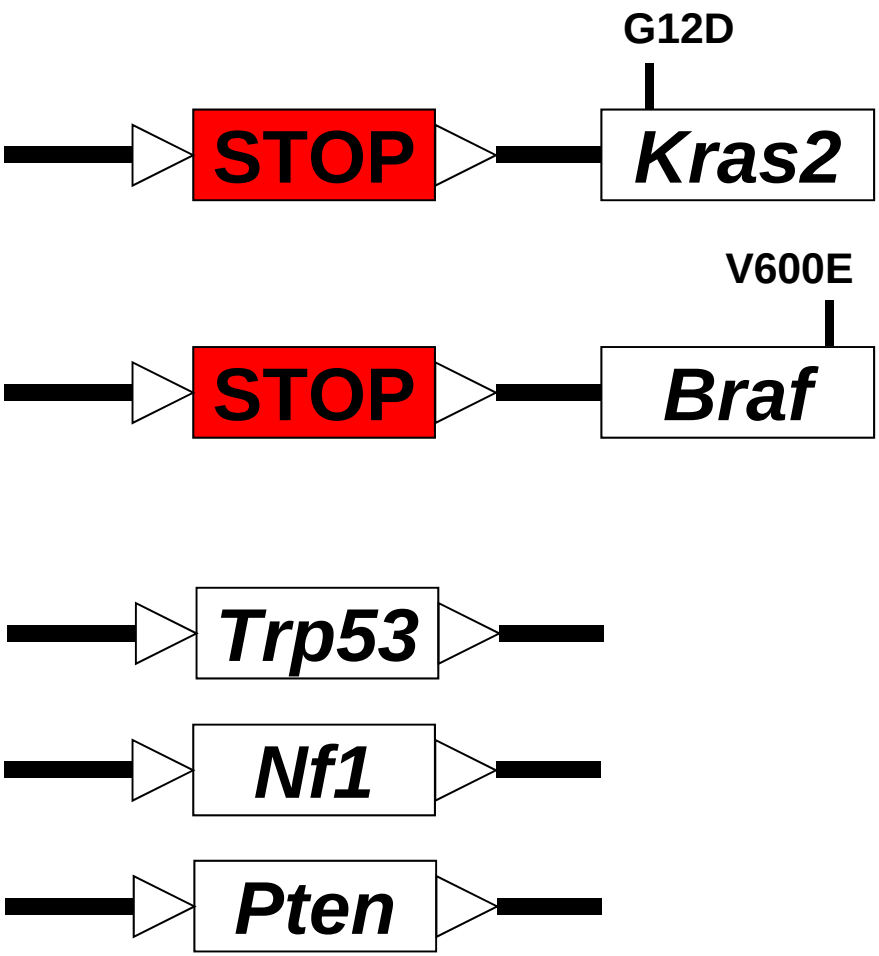


Targeting FTase and GGTase-I in the treatment of RAS-induced lung cancer

- **Reduced lung tumors and improved survival in mice with K-RAS-induced lung cancer**
- **K-RAS mislocalized away from plasma membrane**
- **No toxicity from lack of enzymes in the lung**
- **Toxicity might be a problem in other tissues**

Khan *et al*. *J. Clin. Invest.* 2011
Sjogren *et al*. *J. Clin. Invest.* 2007
Liu *et al*. *PNAS* 2010

Mouse Cancer Models



Lenti-*GFP-Cre*

Tyr-ER-*Cre:* Melanocytes

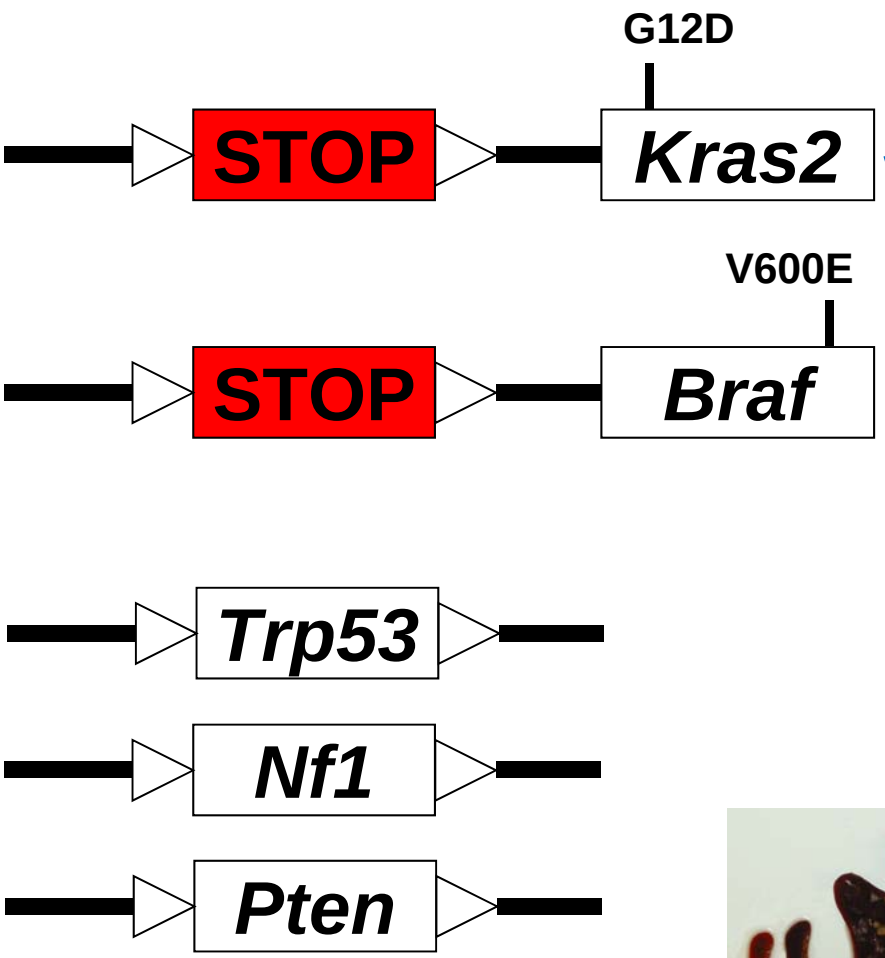
Albumin-*Cre*: Hepatocytes

Col1a1-*Cre*: Osteoblasts

Mx1-*Cre*: BM progenitors

Pdx1-*Cre*: Pancreas

K-RAS-induced Leukemia



Lenti-*GFP-Cre*

Tyr-ER-*Cre:* Melanocytes

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Col1a1-*Cre*: Osteoblasts

Mx1-*Cre*: BM progenitors

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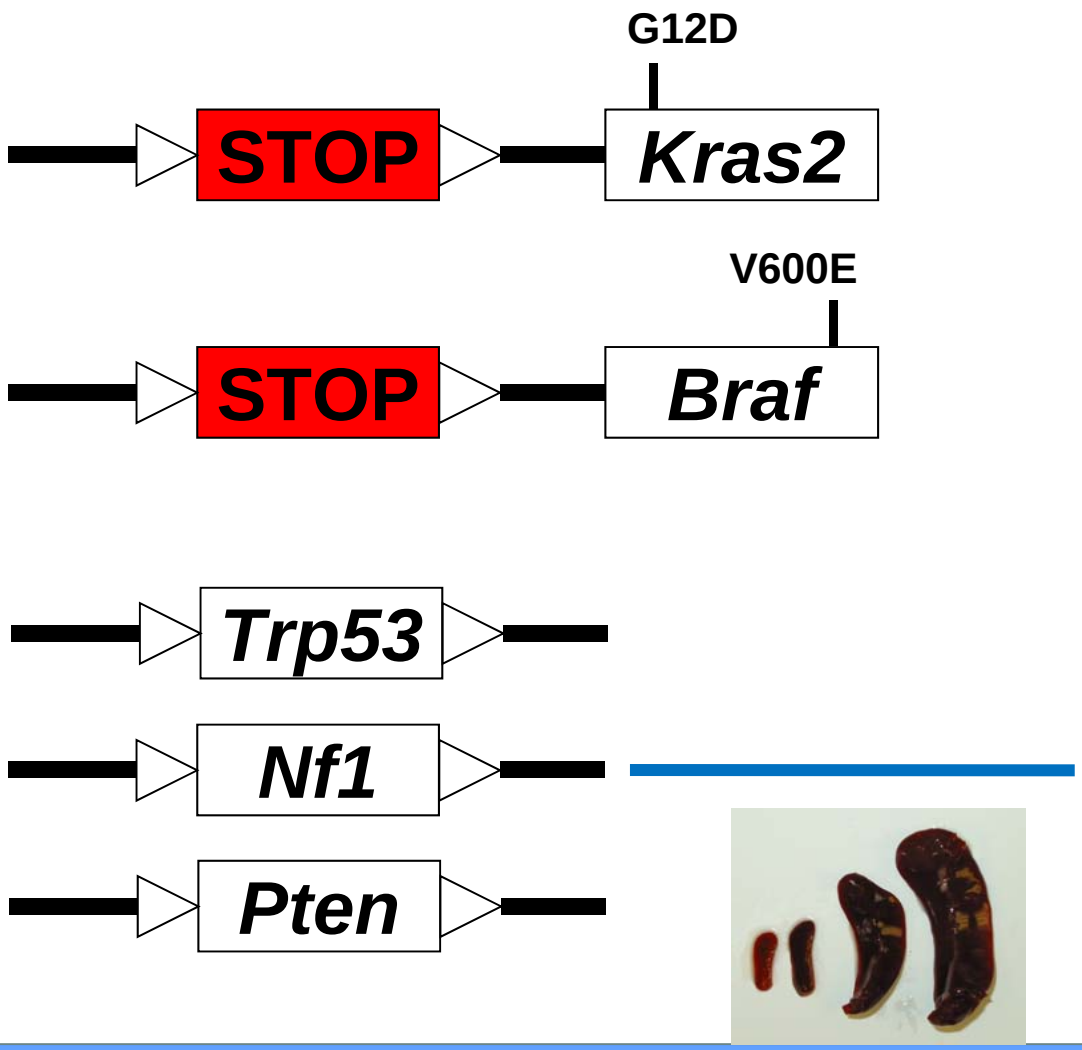
Slowly progressing, lethal myeloproliferative disease; leukocytosis, hepatosplenomegaly

Leukemia induced by *Nf1* deficiency



Lenti-*GFP-Cre*

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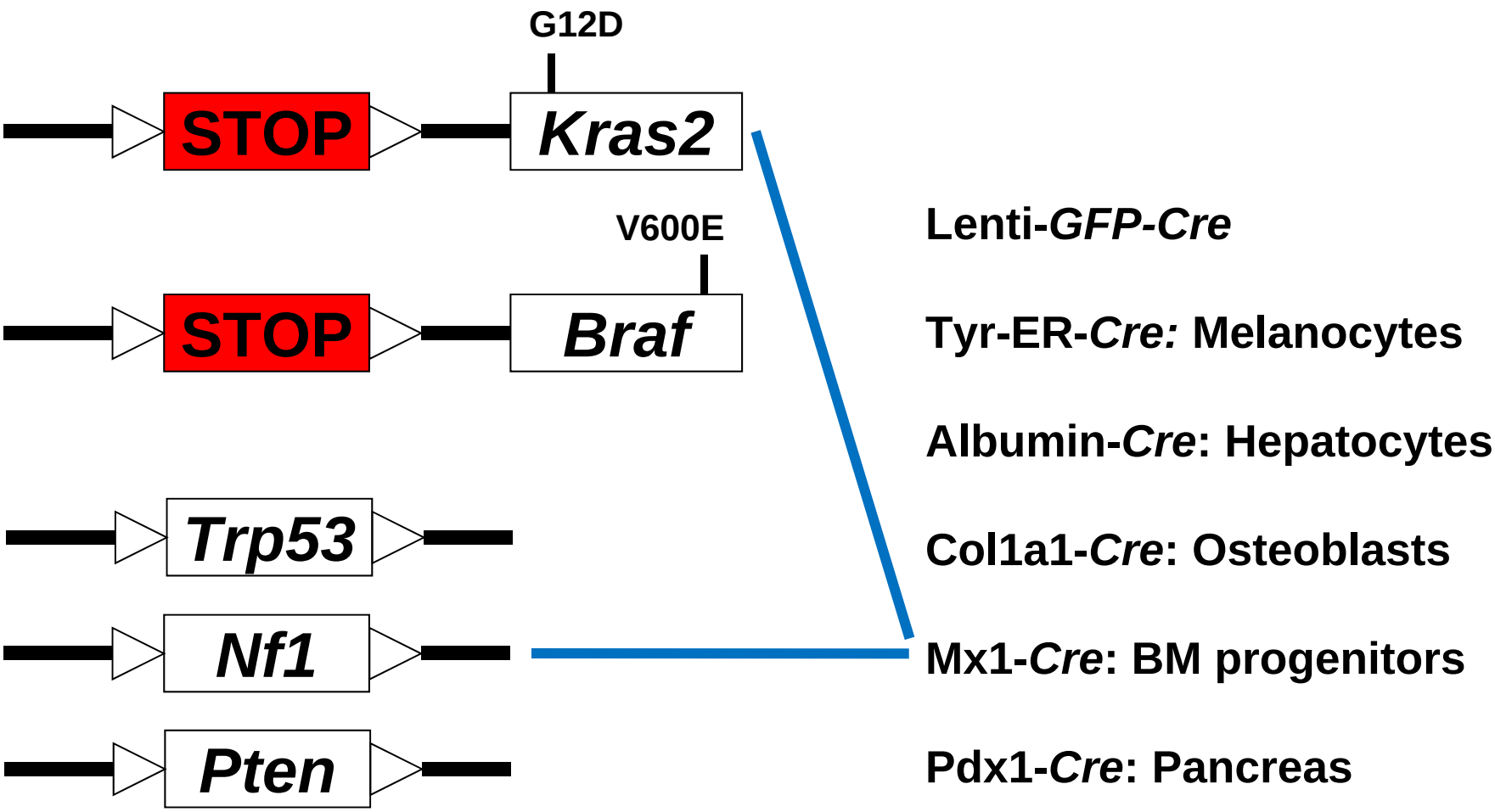
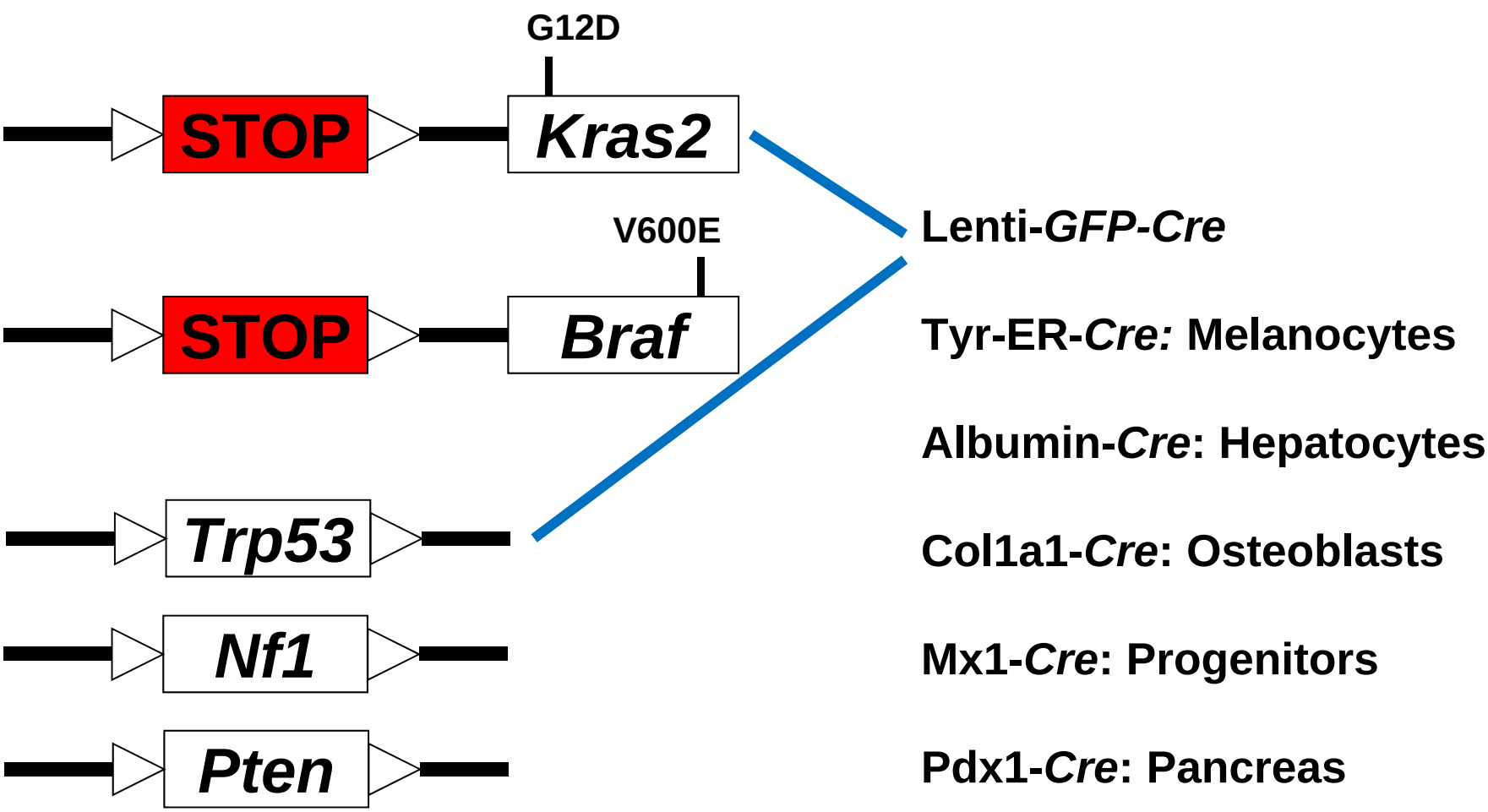
Slowly progressing myeloproliferative disease; leukocytosis, hepatosplenomegaly

Acute Leukemia in K-RAS:*Nf1* double-mutant mice



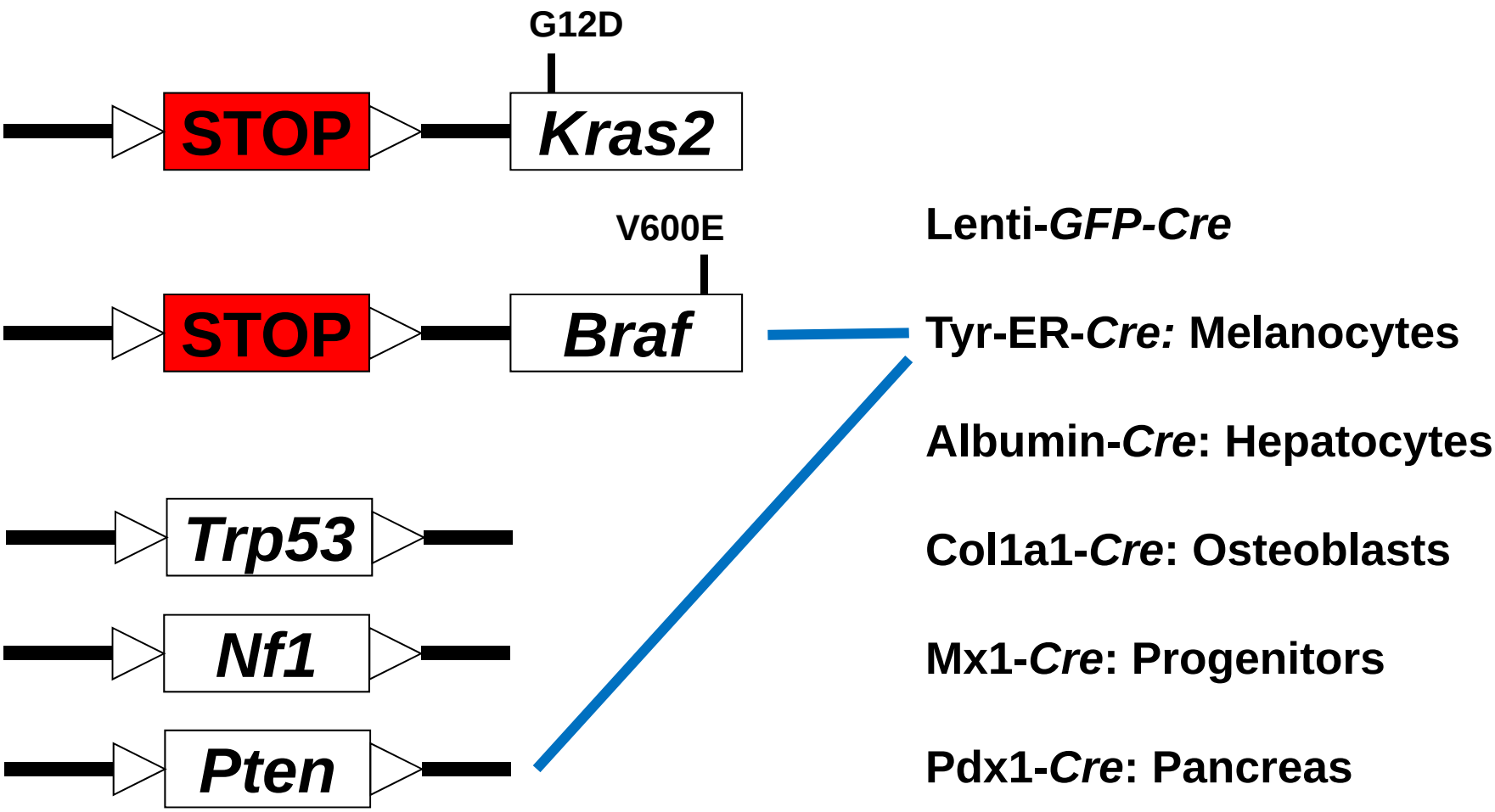
High levels of myeloblasts in bone marrow, transplantable to sublethally irradiated secondary mice

Metastasizing lung cancer



Local and distant metastases to lymph nodes, kidney, liver, premature death

Metastasizing malignant melanoma



Dankort and McMahon's model

Mouse models of RAS- and B-RAF-induced cancer

- **Activation of one oncogene: nonmetastatic cancer**
- **Inactivation of a tumor suppressor: mild form of adenoma, myeloid leukemia, local melanoma skin tumors**
- **Combine an oncogene and a tumor suppressor: metastatic invasive cancer**

Mouse ≠ Human

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Ella Äng (amanuens)

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