Animal Model Workshop

The TFEB-mTORC1 axis in kidney cancer

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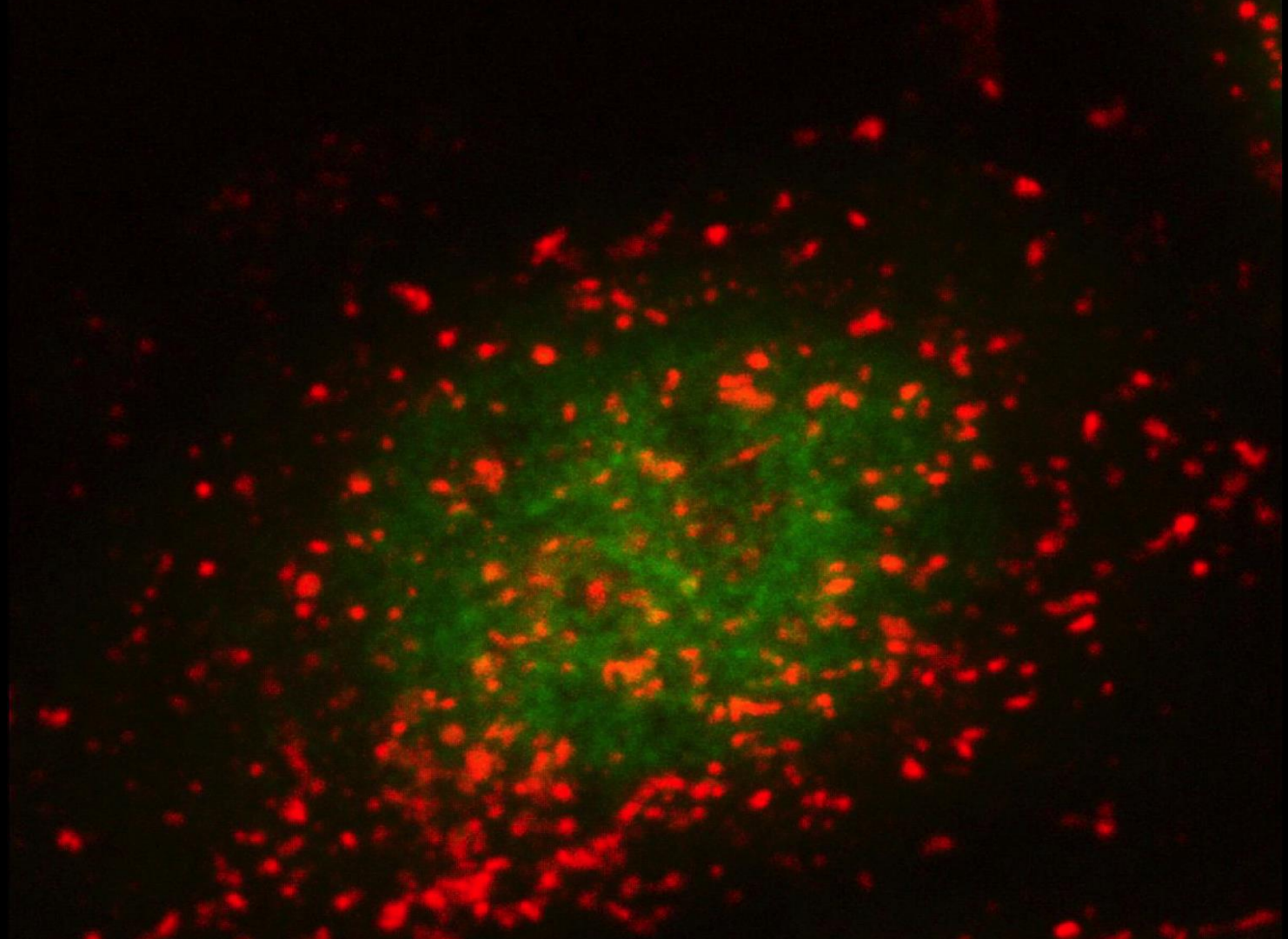
September 11-13, 2025



A MASTER REGULATOR OF LYSOSOMAL BIOGENESIS AND AUTOPHAGY

Lysosomal biogenesis

Autophagy

Science 325: 473-476, 2009

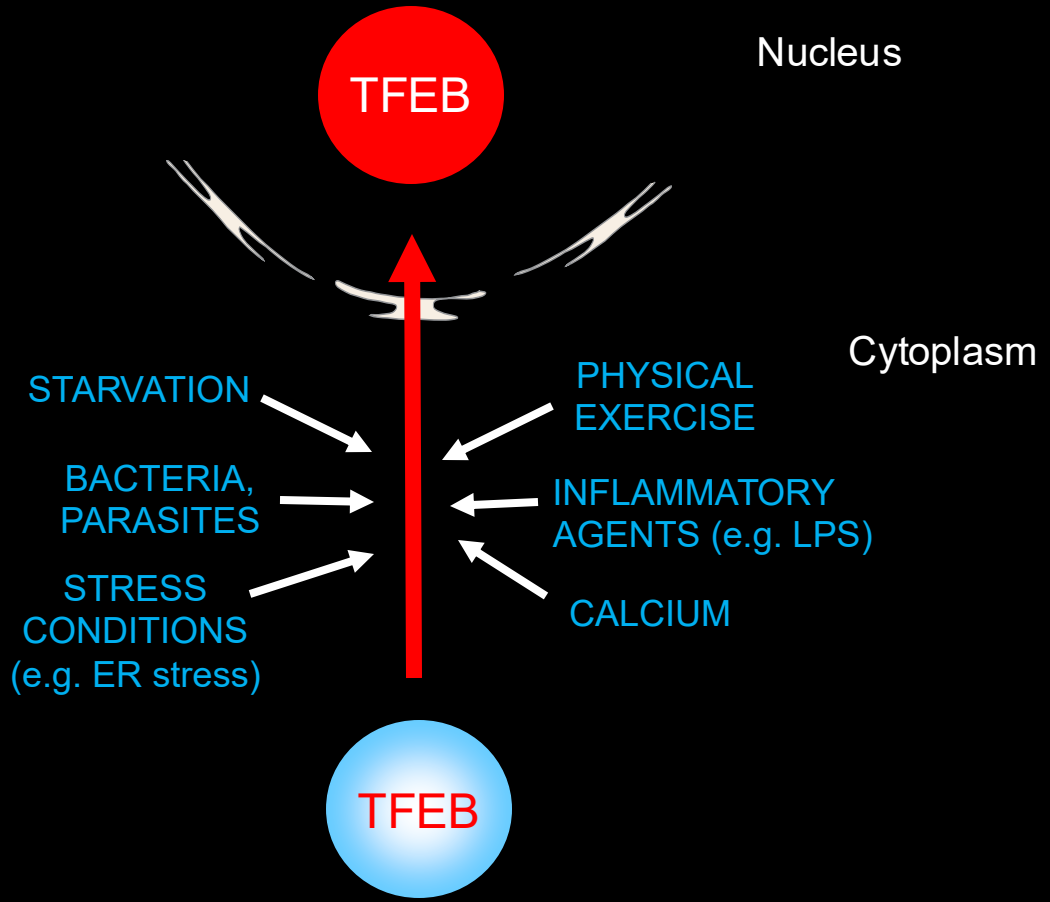
Science *332: 1429-1433, 2011*

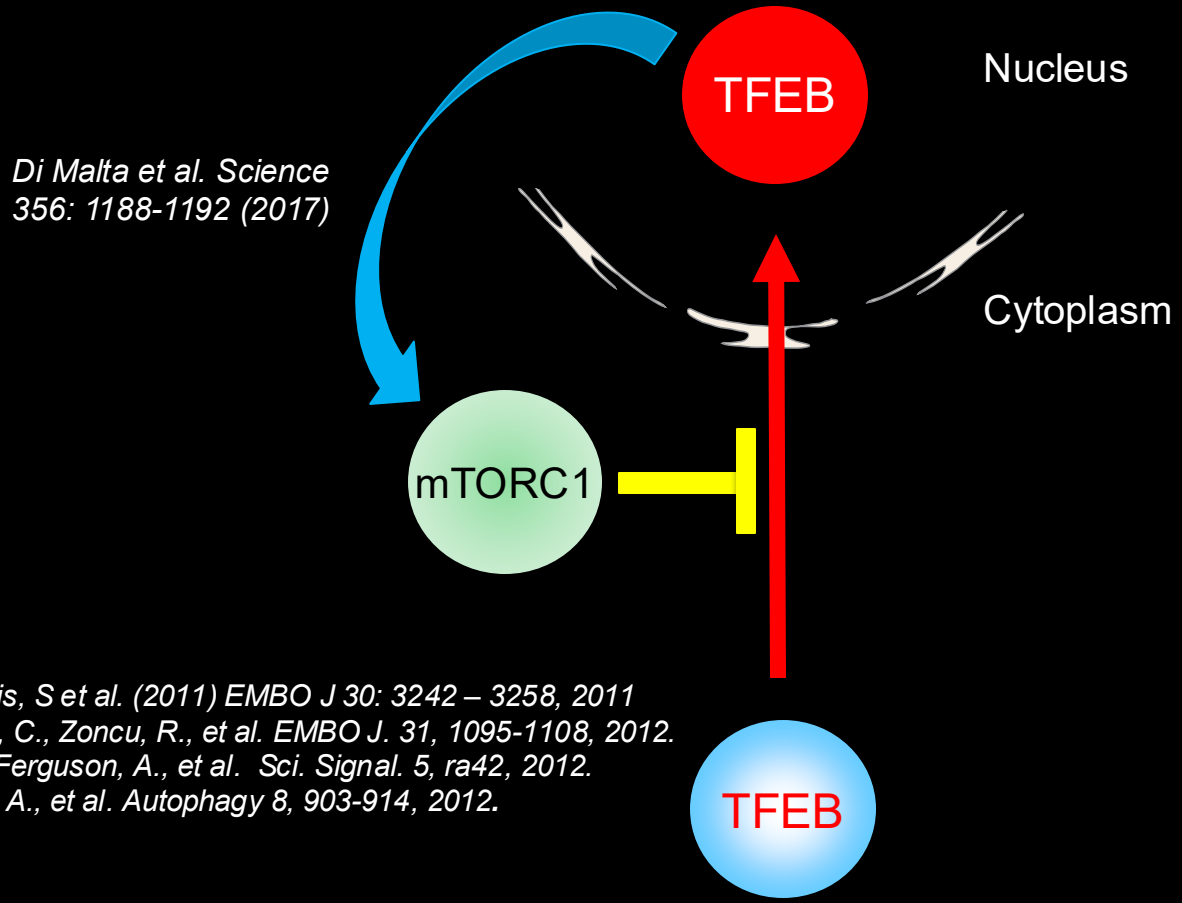
TFEB and the MiT-TFE family

A subfamily of the Helix-Loop-Helix Leucine Zipper family of transcription factors (Hamesath et al. Genes Dev. 8: 2770-2780, 1994)

It includes 4 members (MITF, TFEB, TFE3 and TFEC), which can both homo- and hetero-dimerize, bind to a similar set of promoters, and are post-translationally regulated in a similar manner.

PROTEIN LOCALIZATION





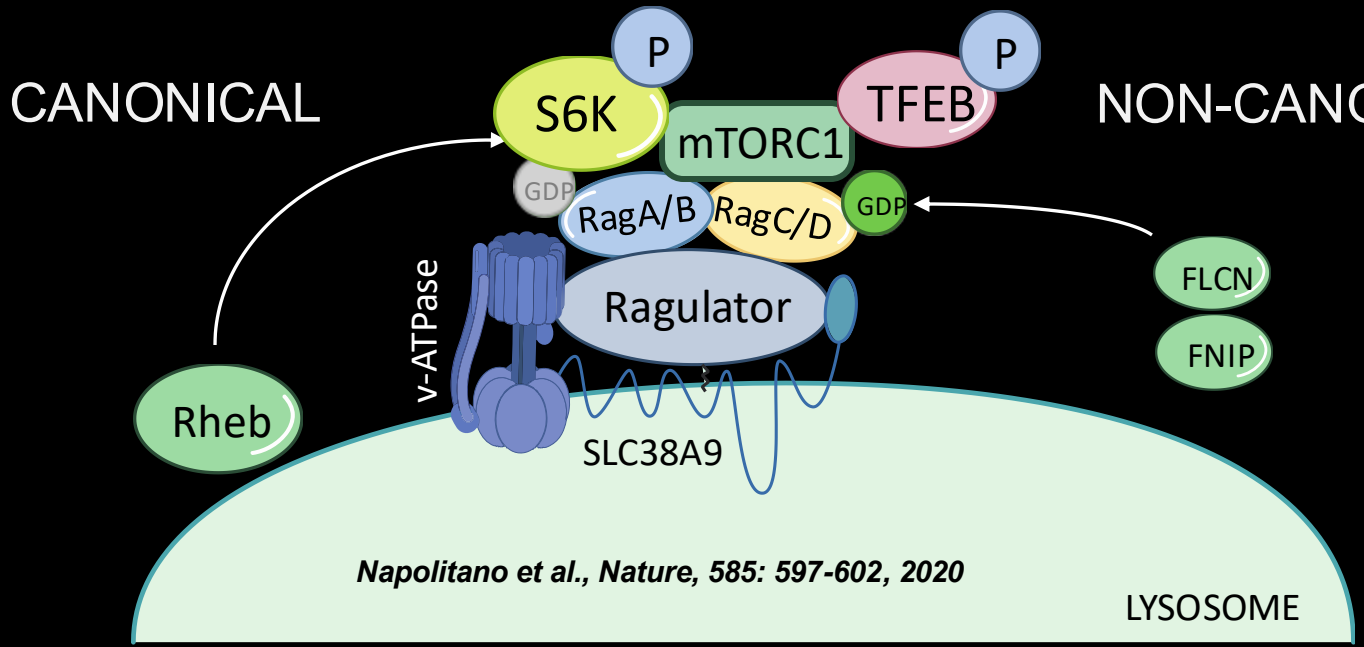
Di Malta et al. Science 356: 1188-1192 (2017)

Pena-Llopis, S et al. (2011) EMBO J 30: 3242 – 3258, 2011
Settembre, C., Zoncu, R., et al. EMBO J. 31, 1095-1108, 2012.
Roczniak-Ferguson, A., et al. Sci. Signal. 5, ra42, 2012.
Martina, J. A., et al. Autophagy 8, 903-914, 2012.

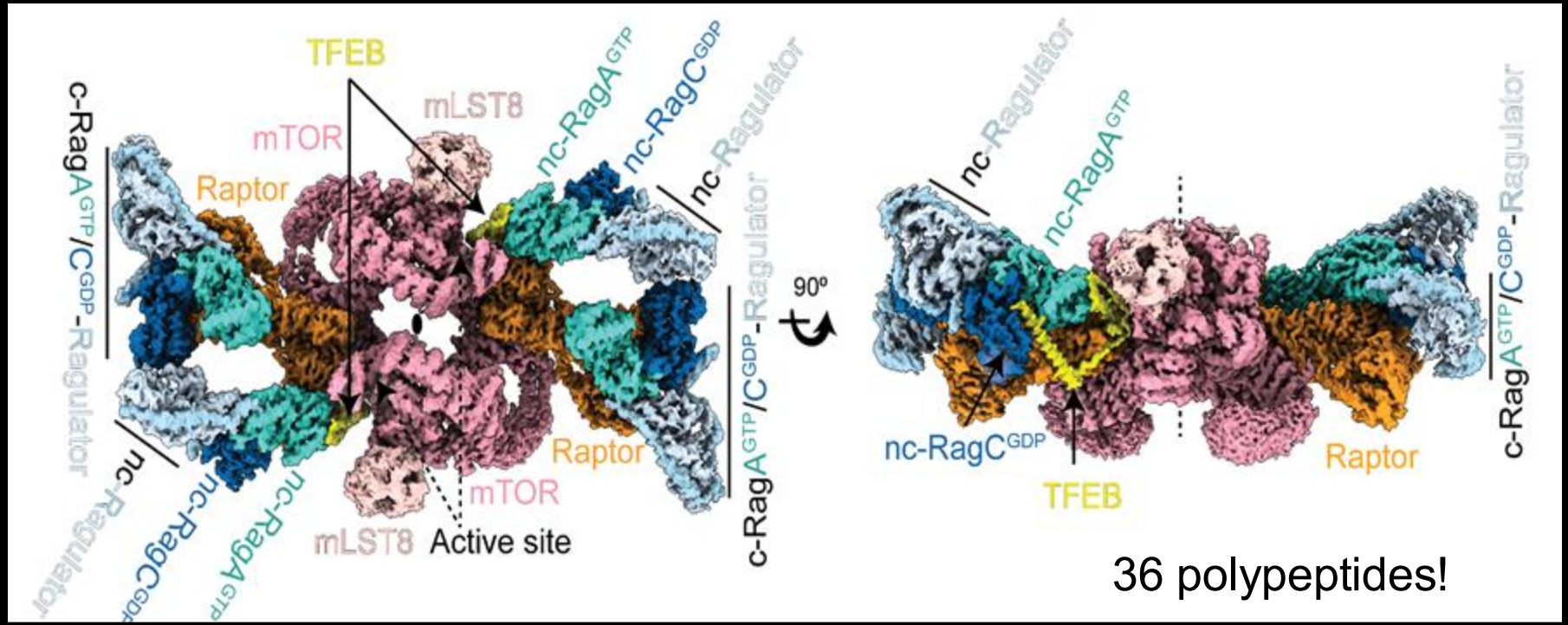
mTORC1 activity can be substrate-specific

Gennaro Napolitano

Two distinct TORC1 signaling pathways



Structure of the mTORC1-TFEB-Rags-Ragulator megacomplex



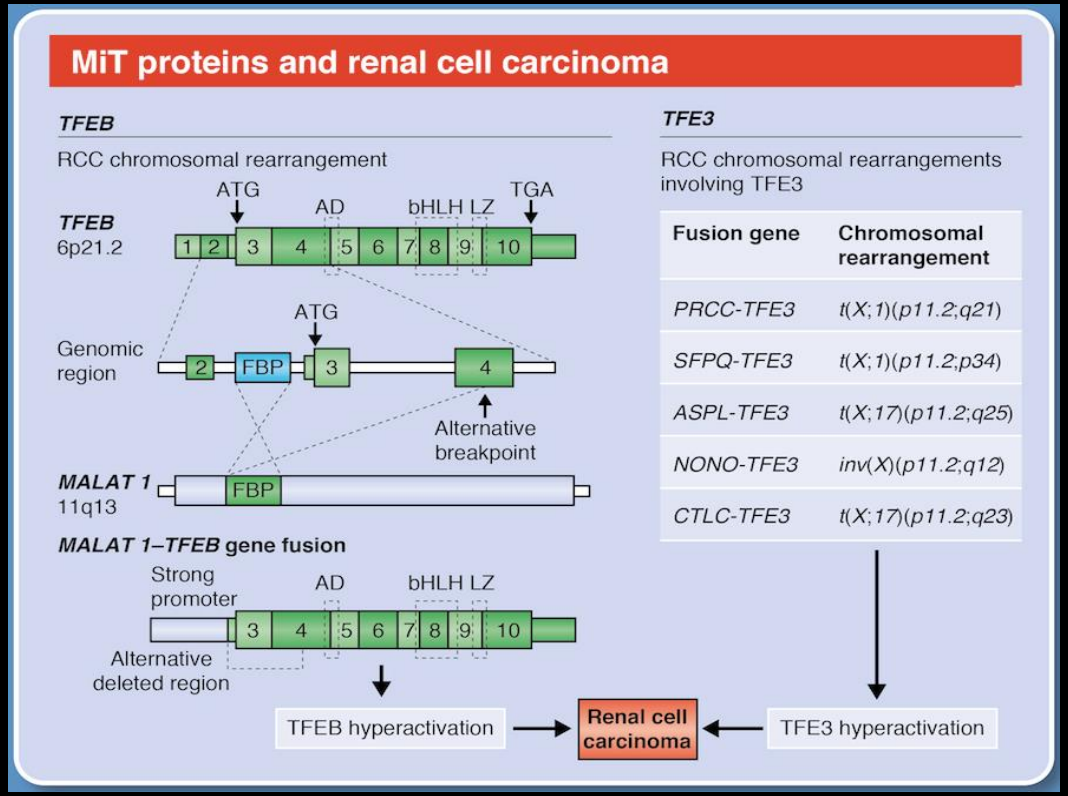
36 polypeptides!

in collaboration with Jim Hurley (Berkeley) and Lukas Huber (Innsbruck)

Cui et al., Nature, 614:572-579, 2023

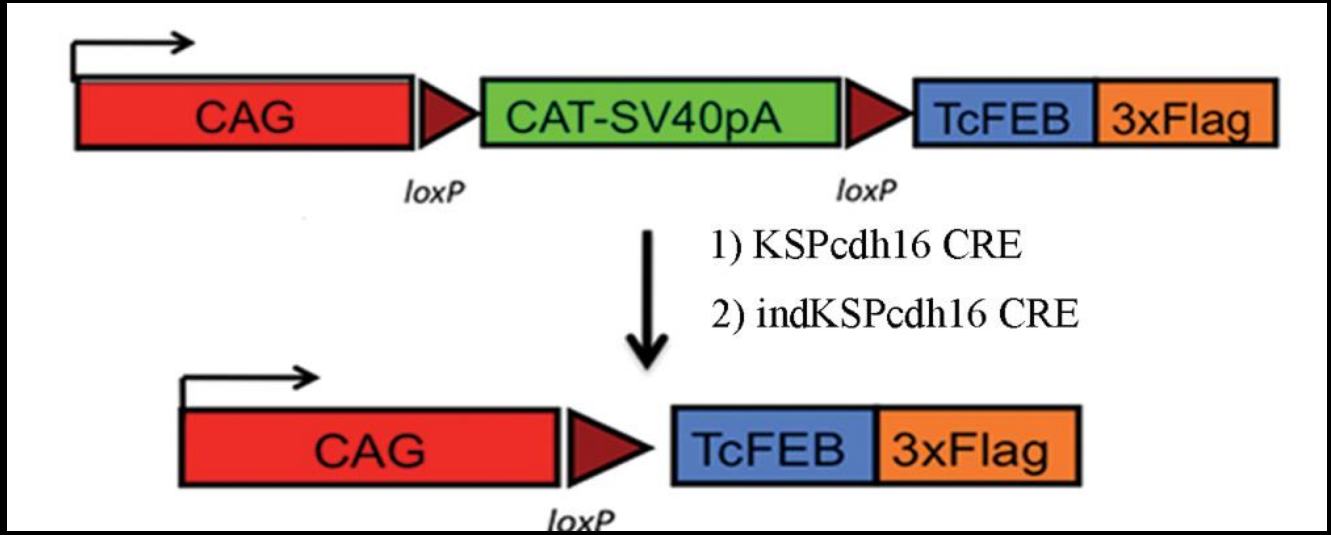
MiT-TFE genes in cancer

MiT-TFE family Translocation Renal Cell Carcinoma



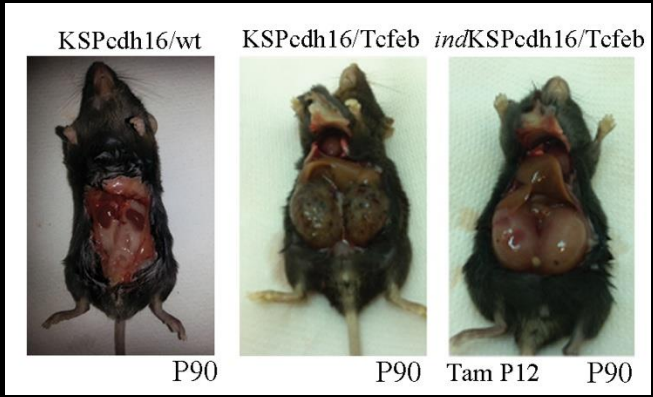
Napolitano and Ballabio, J. of Cell Sci. (2016) 129, 2475-2481

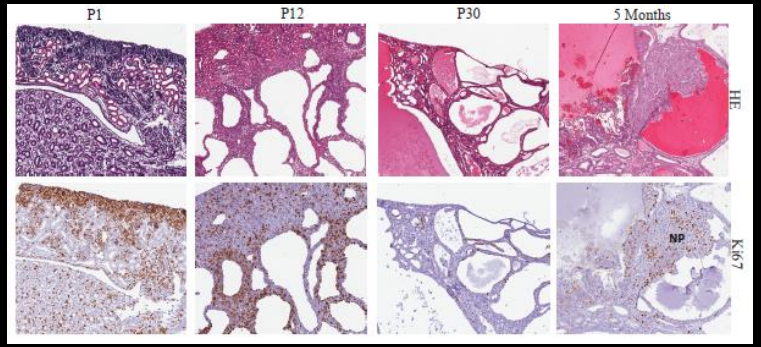
Kidney-specific cadherin 16 (Ksp-CDH16)-CRE Tcfeb overexpressing conditional mouse lines (*Constitutive* and *Inducible*)

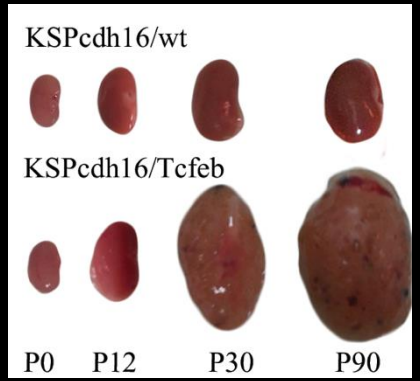


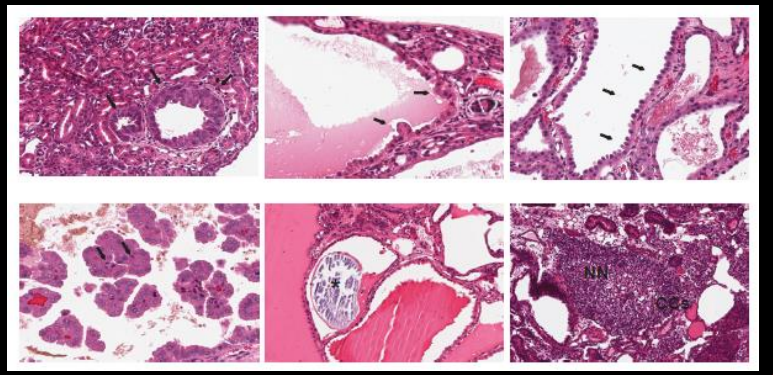
Calcagni' et al. eLife *2016;5:e17047.*

Kidney-specific TFEB-overexpressing mice develop renal cysts and cancer







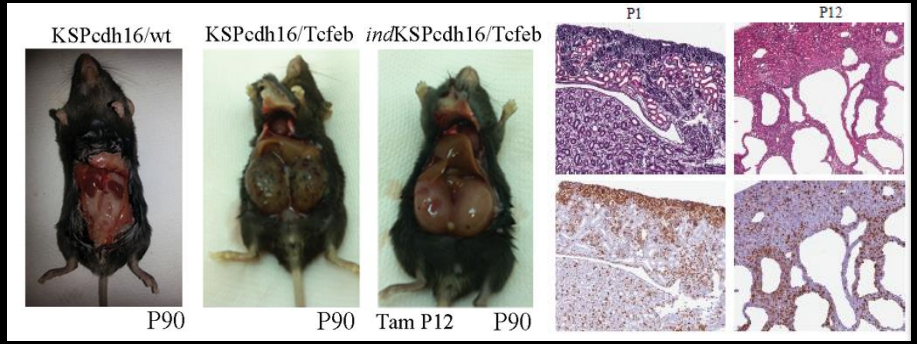


Calcagnı` et al. eLife 2016;5:e17047.

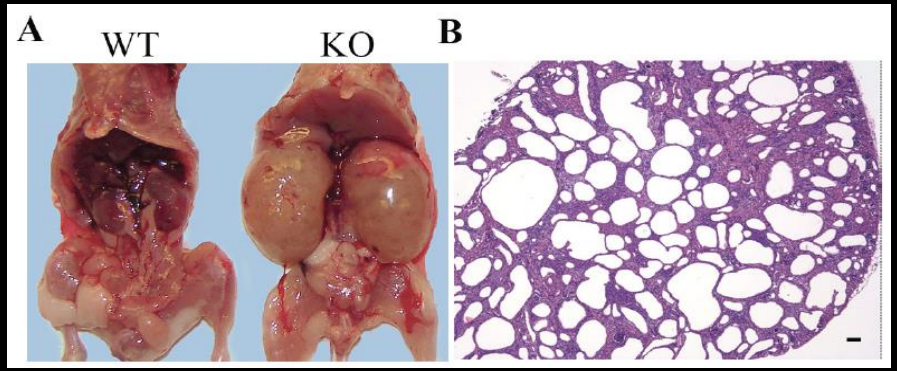
Striking similarities between TFEB overexpressing mice and FLCN KO mice

TFEB overexpressing
ONCOGENE

TUMOR
SUPPRESSOR
FLCN KO

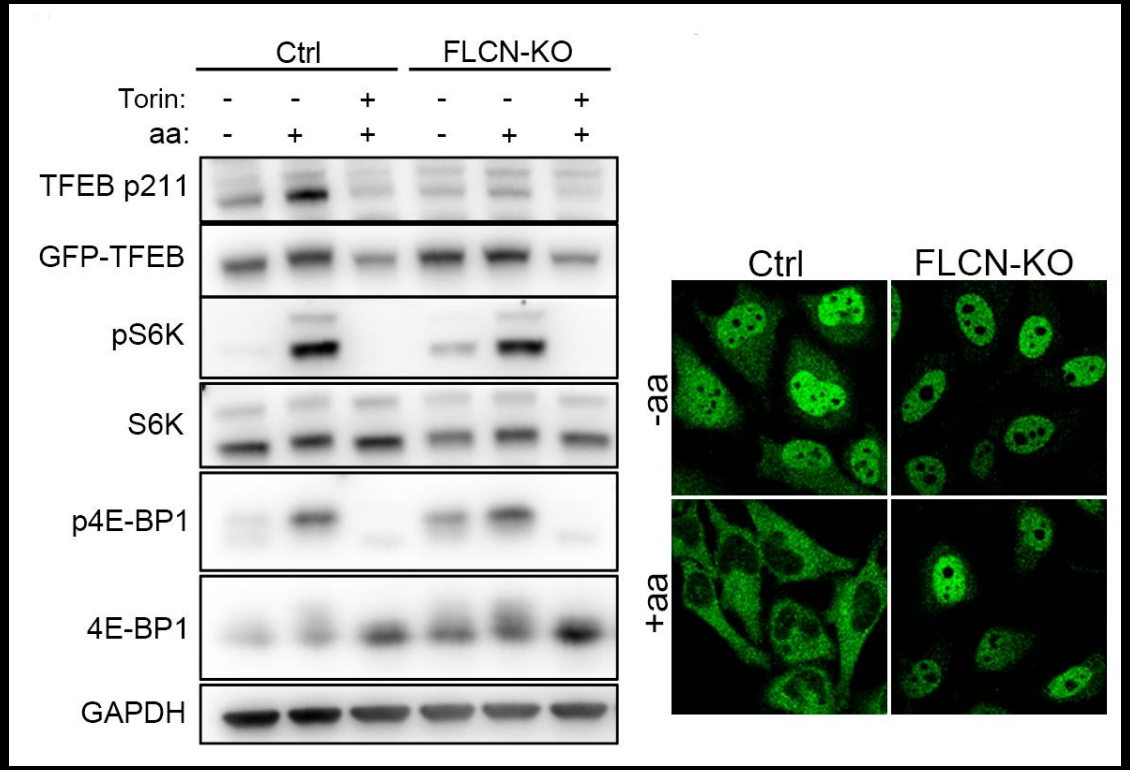


***Calcagni' et al. eLife** 2016;5:e17047.*



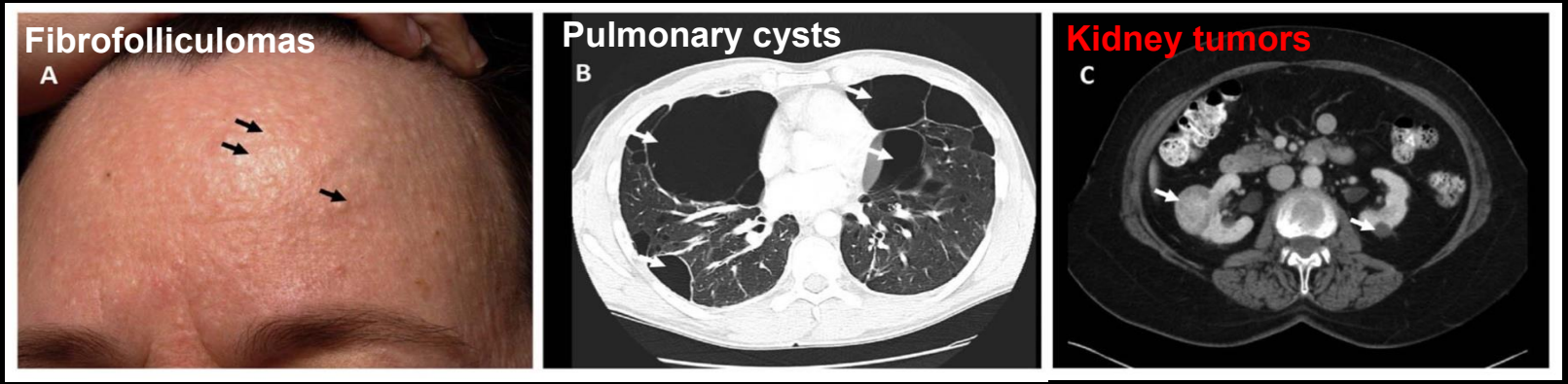
Wu M. et al. Oncotarget. 2015; 6: 32761-32773.

TFEB PHOSPHORYLATION (UNLIKE S6K AND 4E-BP1) REQUIRES FLCN

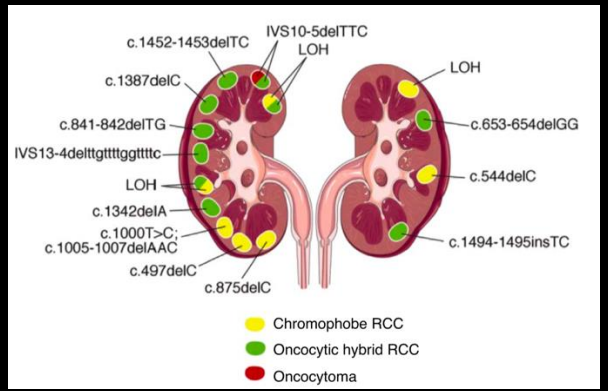


Napolitano, Di Malta* et al., Nature, 585: 597-602, 2020*

Birt-Hogg-Dubé (BHD) syndrome



Autosomal dominant, caused by mutations in the *FLCN* gene. LOH in tumors.



Schmidt et al., *Gene* 2018

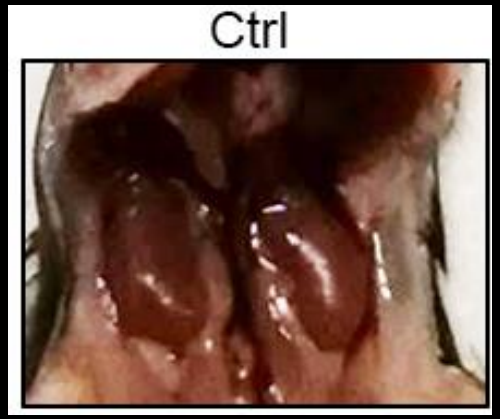
HYPOTHESIS

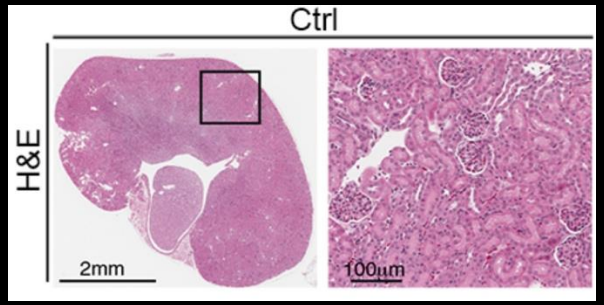
Chiara Di Malta

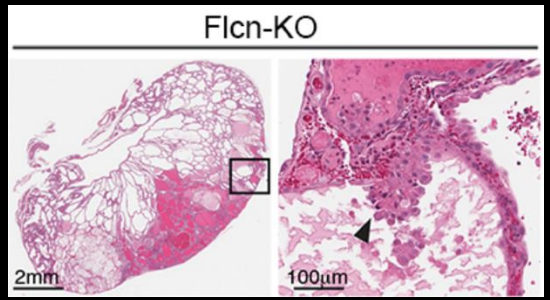
Is BHD syndrome driven by TFEB activation (due to loss of FLCN, a TFEB inhibitor)?

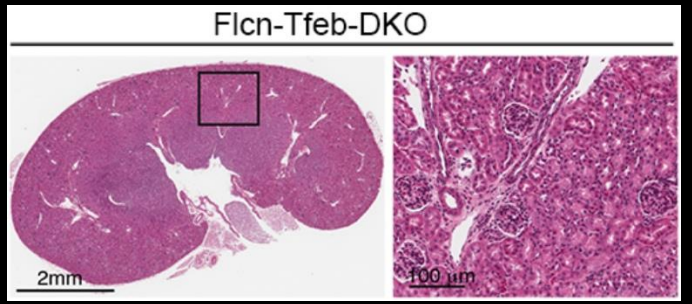
We tested this hypothesis by generating kidney-specific FLCN/TFEB double KO mice

TFEB KO rescues the kidney phenotype of *Flcn* KO mice



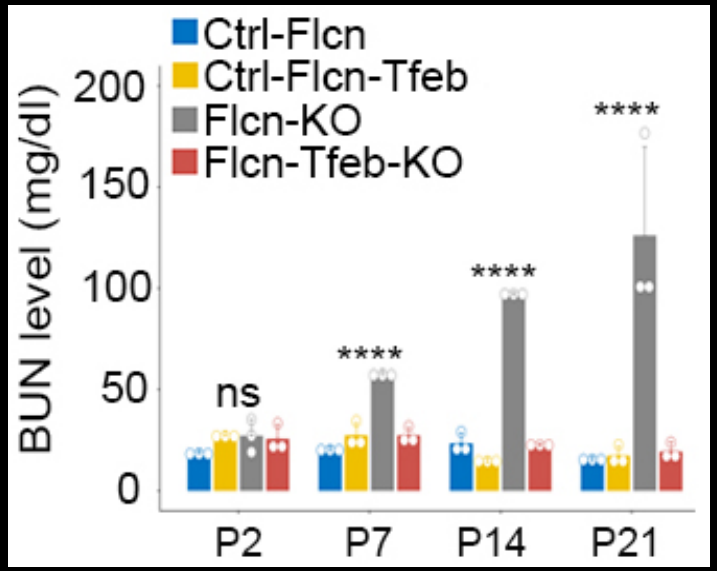


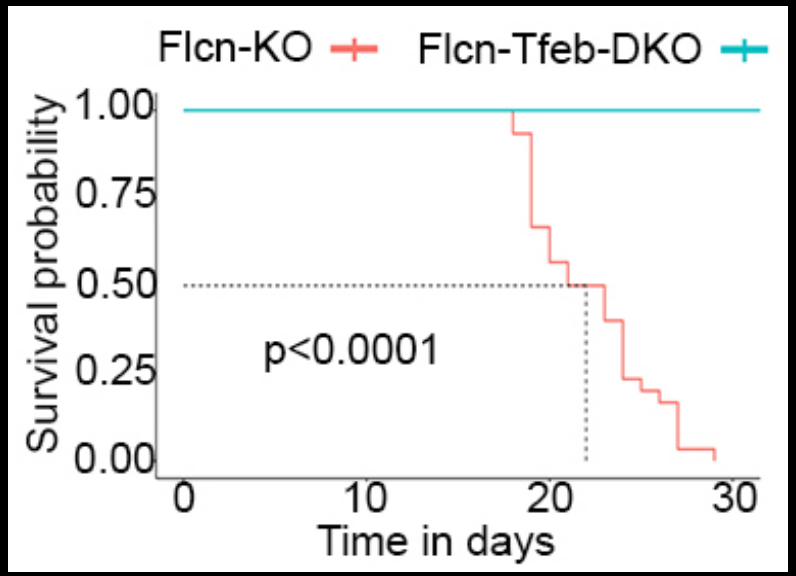




Napolitano, Di Malta* et al., Nature, 585: 597-602, 2020*

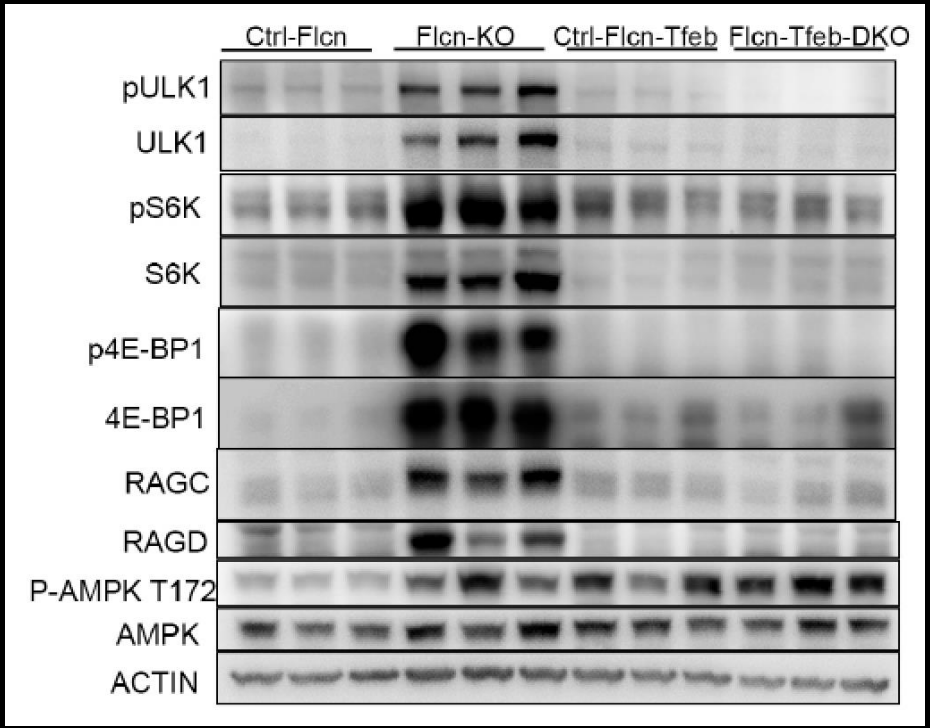
TFEB DEPLETION RESCUES KIDNEY DYSFUNCTION AND LETHALITY IN FLCN KO MICE





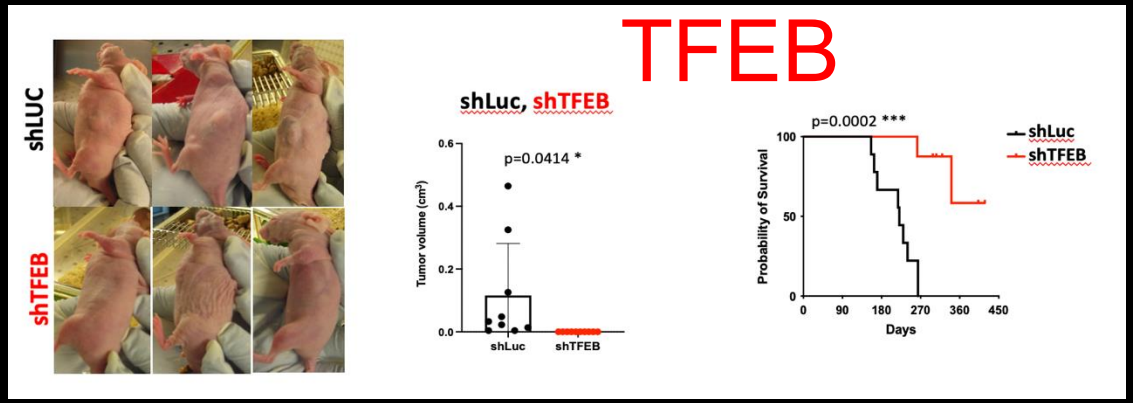
Napolitano, Di Malta* et al., Nature, 585: 597-602, 2020*

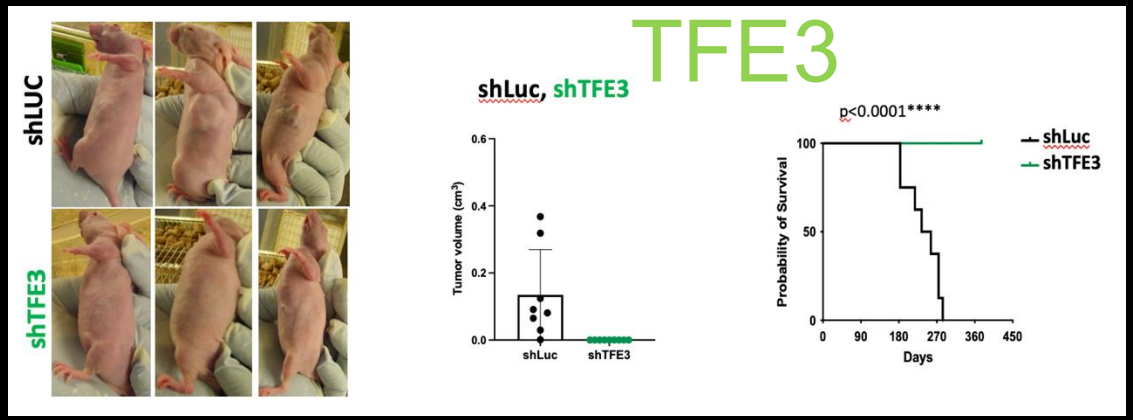
TFEB KO RESCUES mTORC1 HYPERACTIVITY ASSOCIATED WITH LOSS OF FLCN



Napolitano, Di Malta* et al., Nature, 585: 597-602, 2020*

BOTH TFEB AND TFE3 PROMOTE TUMORIGENESIS IN BHD SYNDROME



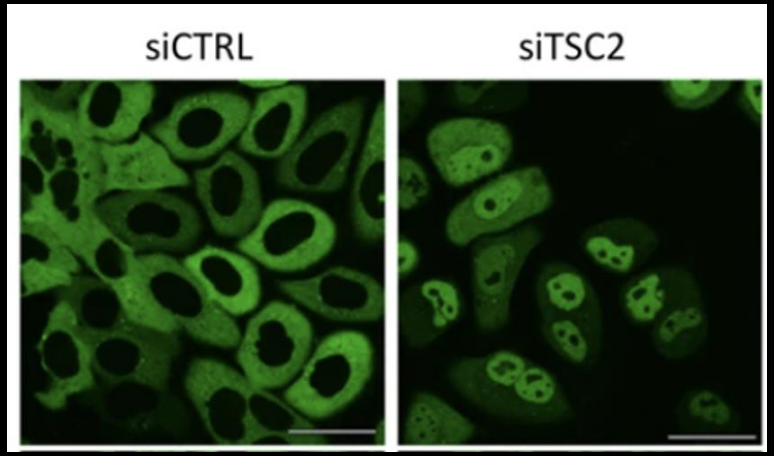


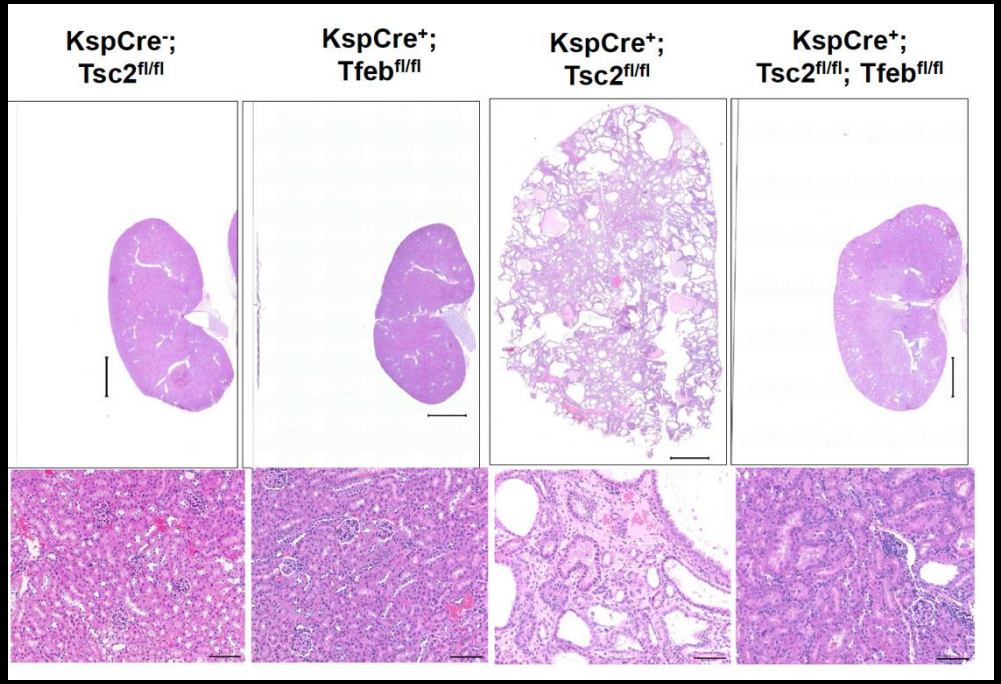
Silencing of TFEB and TFE3 in mouse xenografts generated using a cell line (UOK-257) from a kidney tumor of a patient with BHD syndrome (collaboration with L. Schmidt and M. Linehan, NIH).

Di Malta et al. EMBO Mol Med (2023) 15: e16877

TFEB KO rescues the kidney phenotype of *Tsc2* KO mice

(collaboration with Lisa Henske's group, Harvard)





Alesi et al. Nat Commun. 15: 406. doi: 10.1038/s41467-023-44229-4

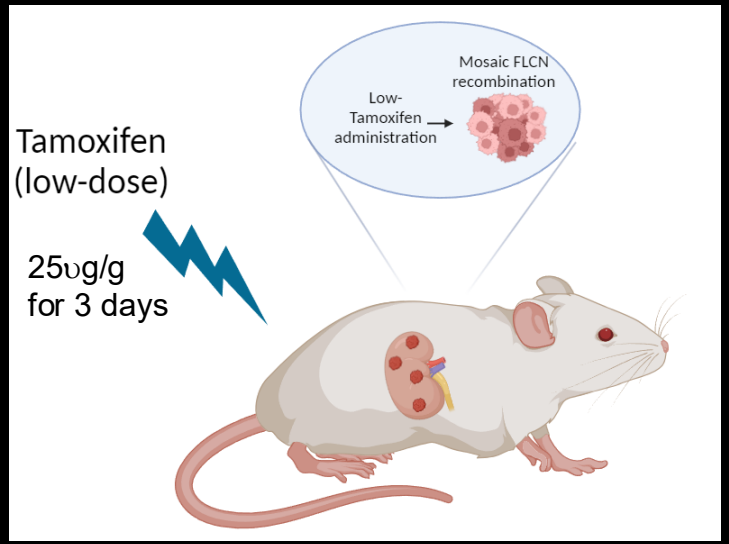
The phenotype of commonly used mouse models of BHD syndrome is too aggressive and too diffuse, not mimicking the human disease

A new mouse model to mimic the multi-focal nature of the kidney phenotype of BHD syndrome

Alessia Calcagni'

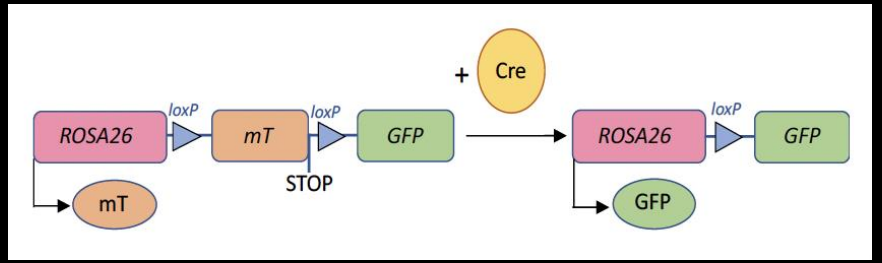
A new mouse model for BHD syndrome

Flcn$^{fl/fl}$;LOW TAM indKSP-CRE$^{tg/wt}$ mice



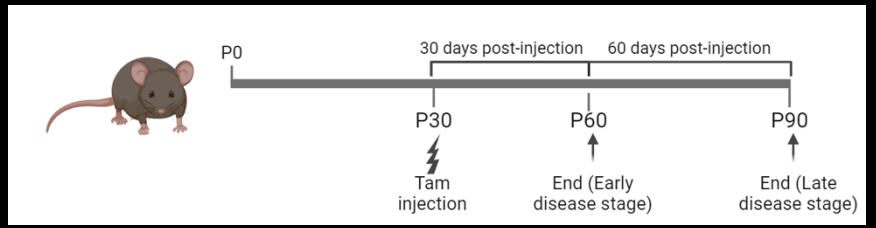
X

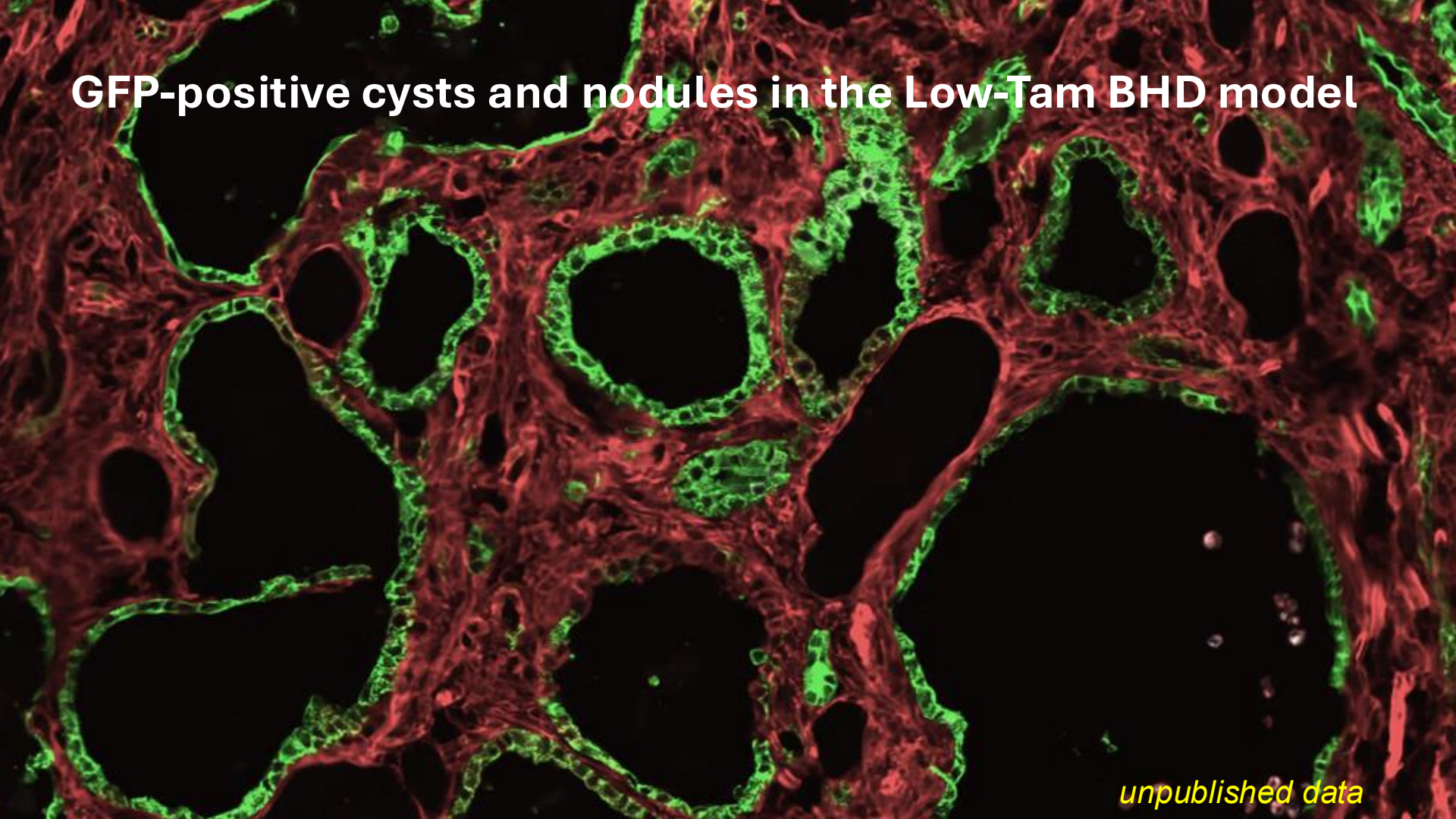
mT/mG CRE reporter



Muzumdar et al. Genesis 45: 593–605 (2007)

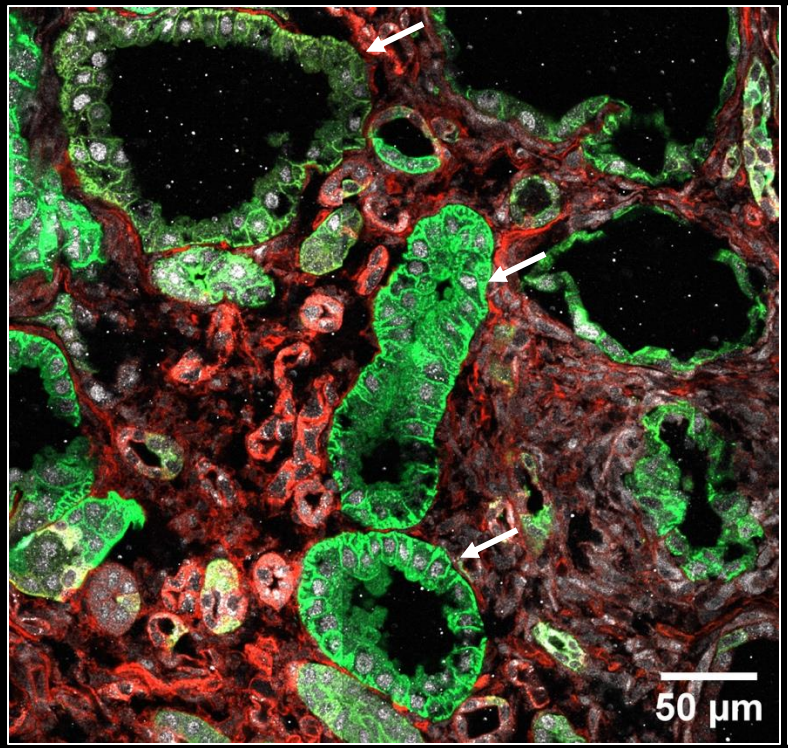
Experimental design

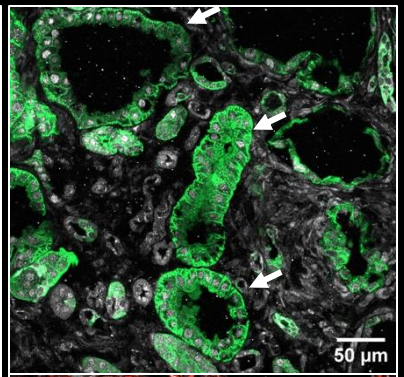


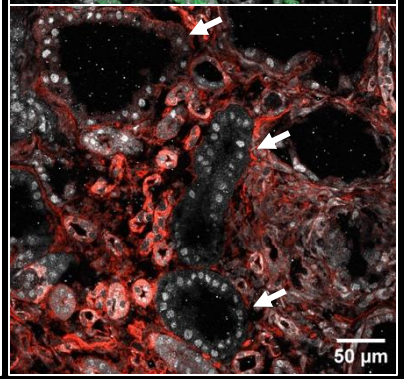

GFP-positive cysts and nodules in the Low-Tam BHD model
unpublished data

TFEB nuclear localization in GFP+ cells

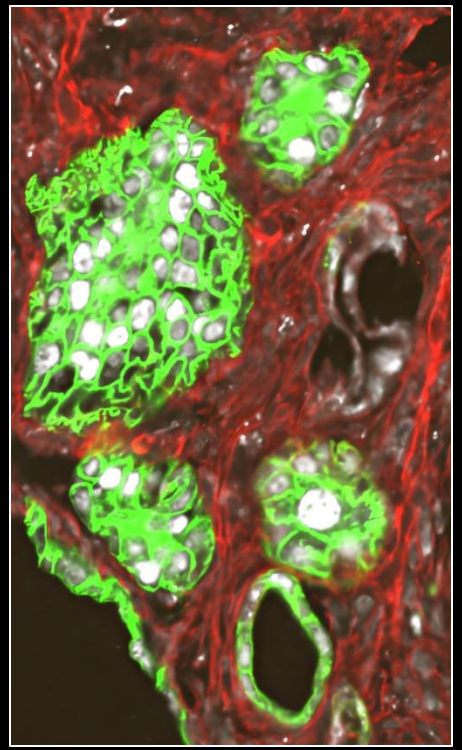
GFP (CRE pos)
Tom (CRE neg)
TFEB





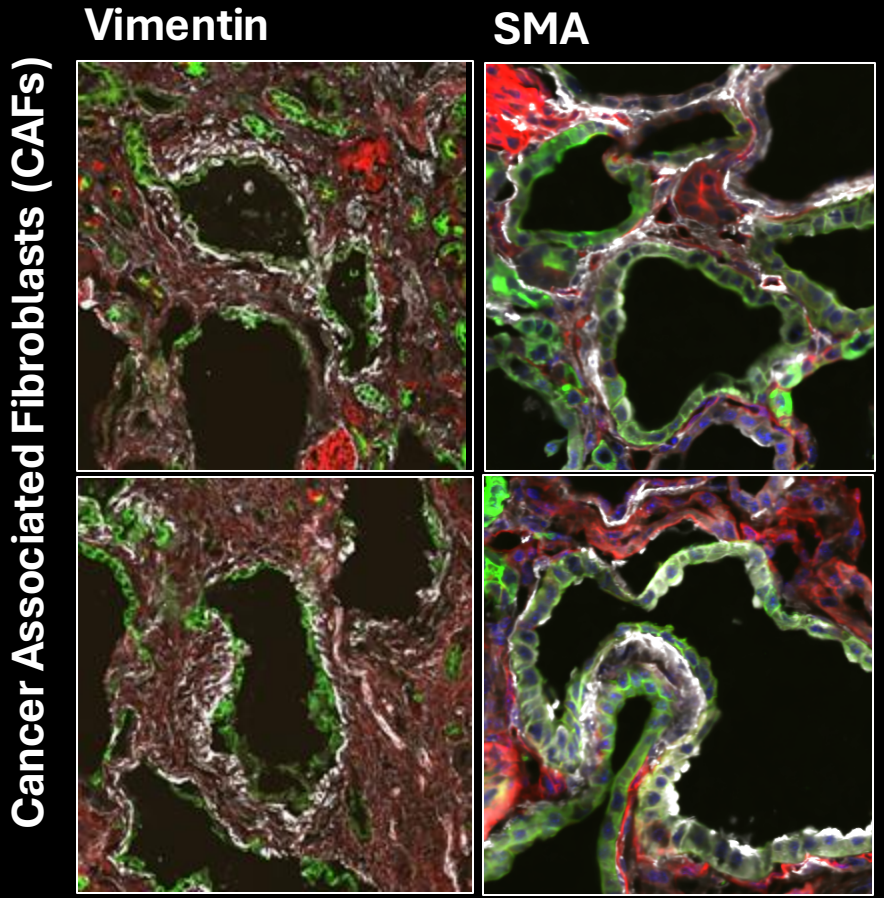


Nuclear TFEB in GFP+ neoplastic nodules



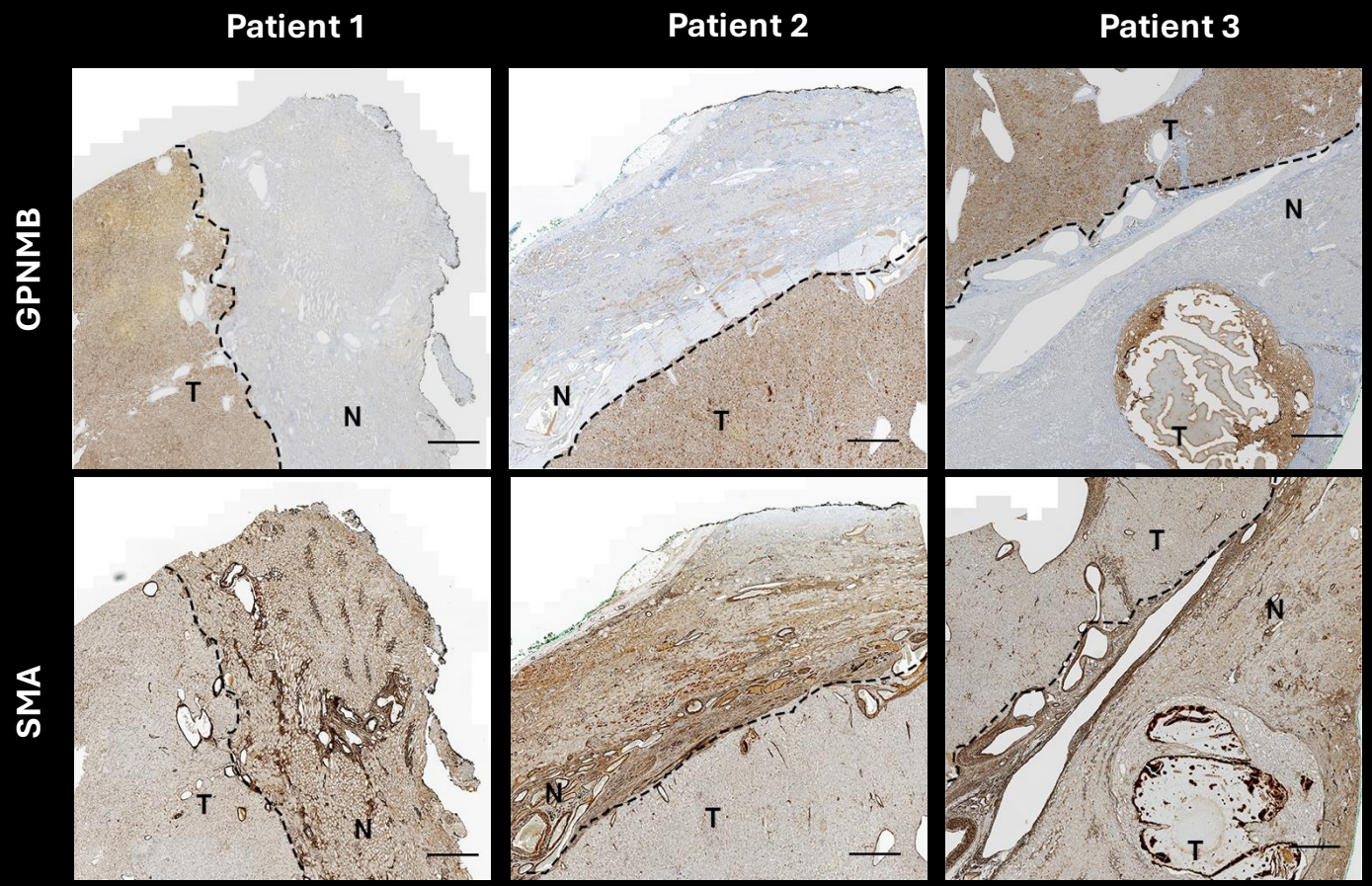
unpublished data

Fibrosis in Tom+ cells



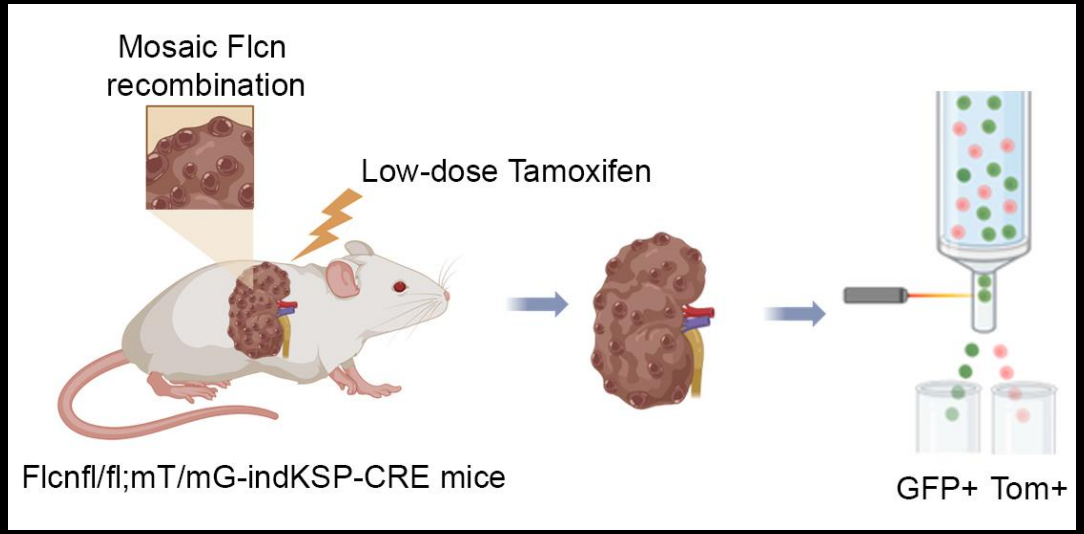
unpublished data

FIBROSIS IN NORMAL TISSUE ADJACENT TO BHD-ASSOCIATED KIDNEY TUMOURS

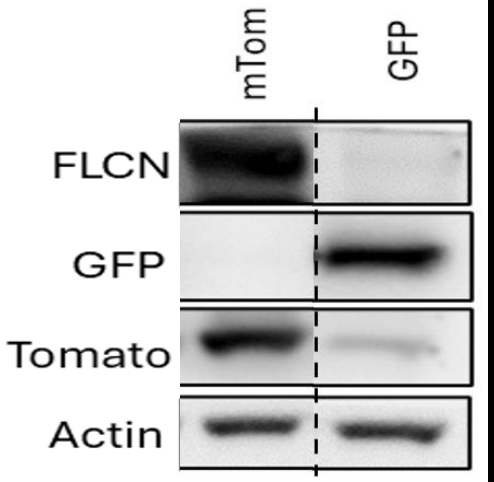


unpublished data

FACS allows isolation of GFP and Tom cell populations from BHD mice

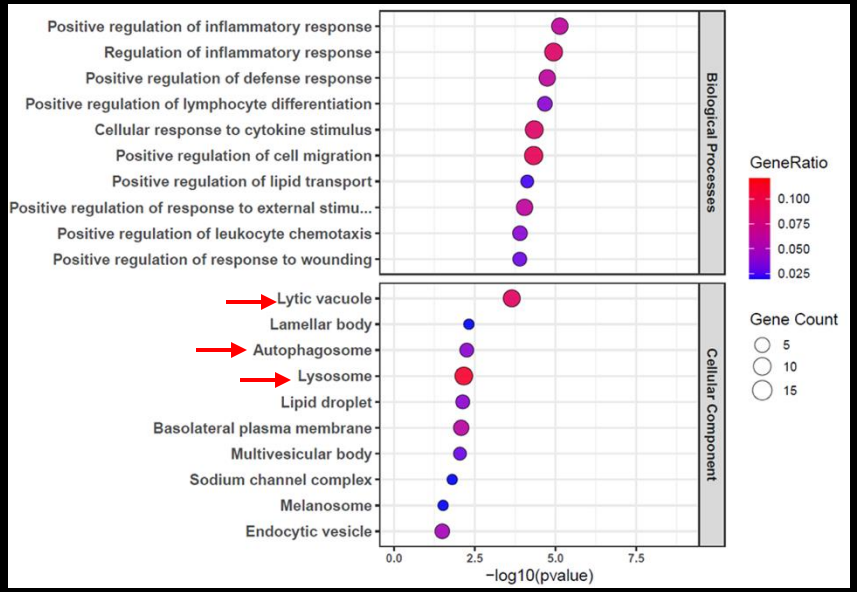


FACS-sorted
mTom+ and GFP+ cells

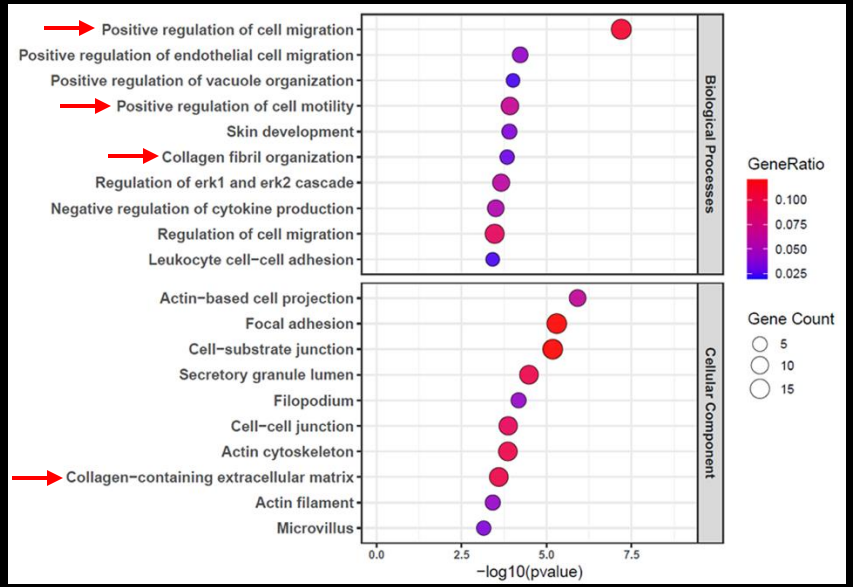


RNAseq analysis of sorted GFP and Tom cells from BHD mice

GO analysis: FLCN-GFP vs WT-GFP



GO analysis: FLCN-Tom vs WT-Tom



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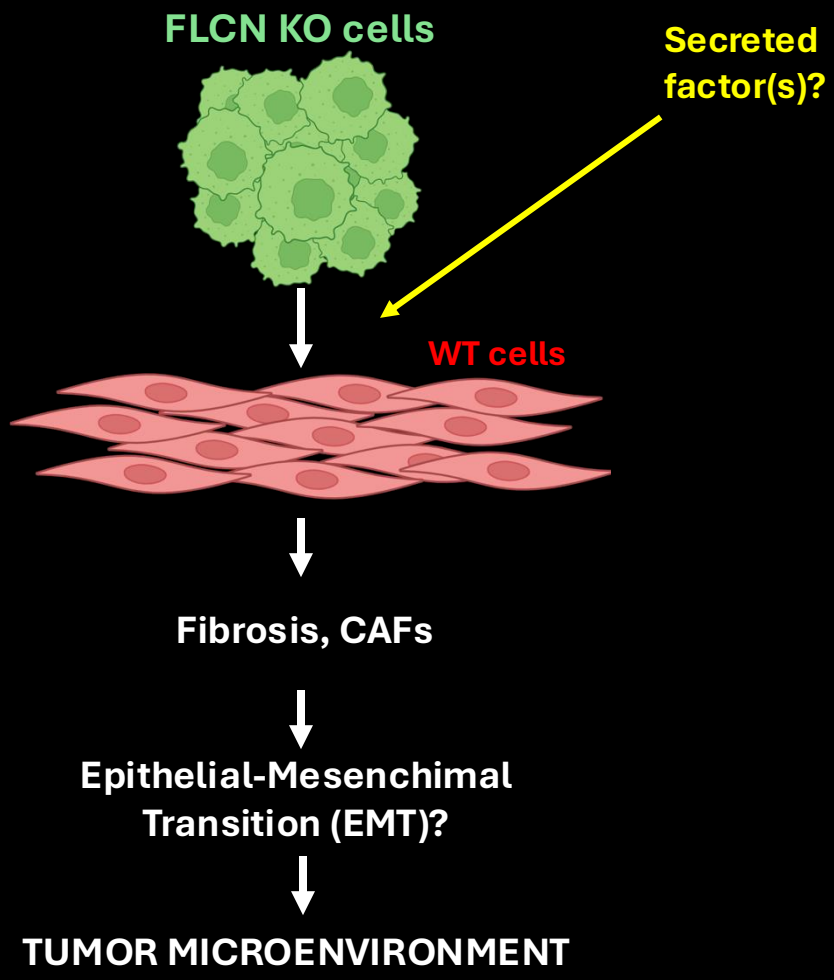
FLCN KO cells
Secreted factor(s)?
WT cells
Fibrosis, CAFs
Epithelial-Mesenchimal Transition (EMT)?
TUMOR MICROENVIRONMENT


Fabrizio Andreone
Alessia Calcagni'
Francesca Carriero
Marcella Cesana
Marco Ferniani
Floriana Forzati
Maria Lambiase
Jlenia Monfregola
Rossella Pennella
Barbara Rossi
Irene Sambri
Lucia Vittoria Sepe
Gennaro Tufano
Claudia Vilardo
Gennaro Napolitano
Alessandra Esposito
Chiara Di Malta
Paolo Grumati
Helene Knaevelsrud
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Lisa Henske
Nicola Alesi
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Boston MA
Marston Linehan
Laura Schmidt
NIH, Bethesda,
Lukas Huber
Mariana De Araujo
Medical Univ. of
Innsbruck, Austria
Luisa Lanfrancone
Giovanni Bertalot
IEO, Milan, Italy
Jim Hurley
Zhicheng Cui
Berkeley Univ., CA
Roberto Zoncu and
Rushika Perera
Univ. Berkeley and UCSF
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