



Animal Model Workshop

Creating Mouse ccRCCs Using CRISPR

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September 11-13, 2025

Session 5: Somatic Models



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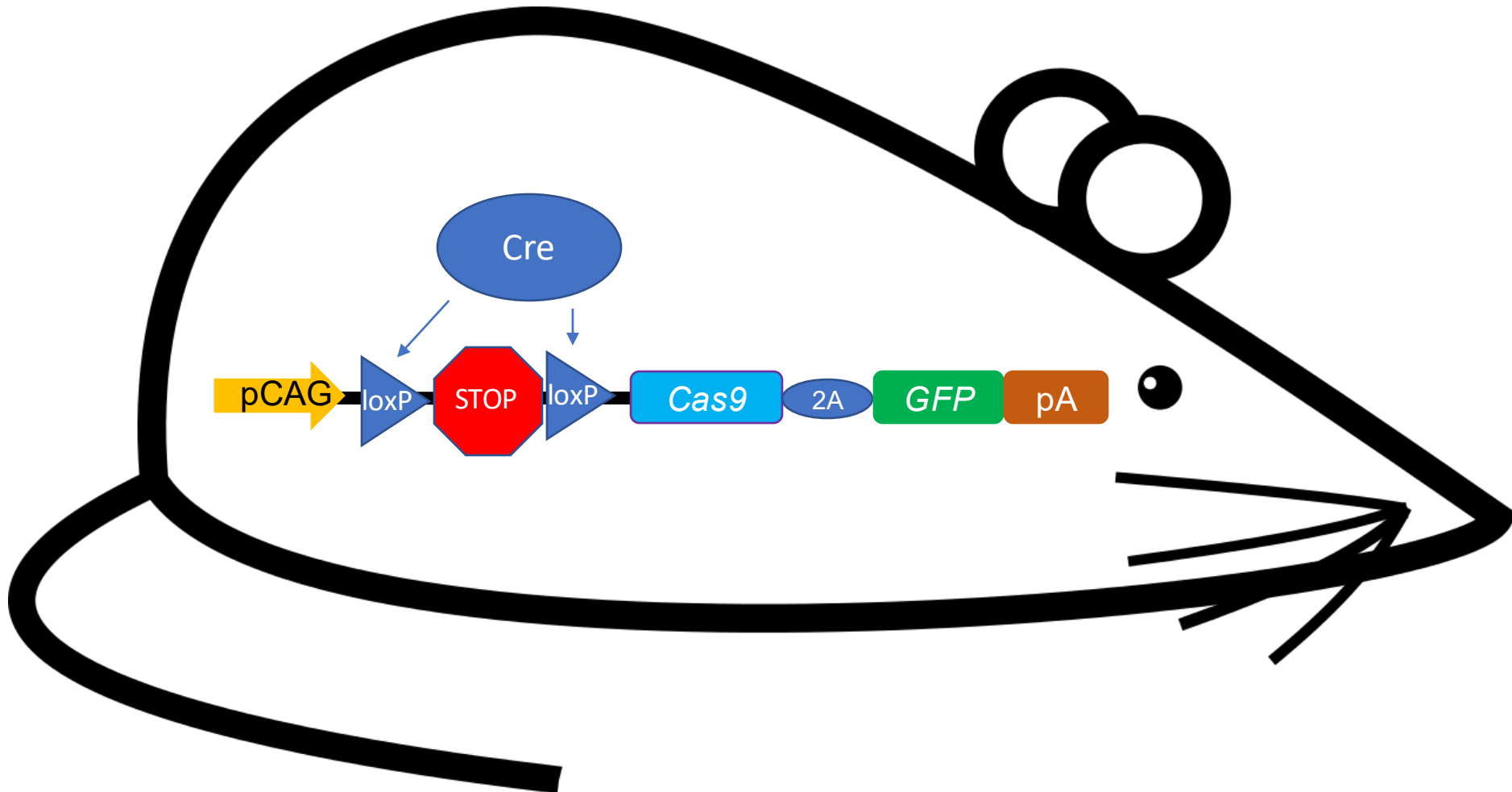
Toward a CRISPR-based mouse model of *Vhl*-deficient clear cell kidney cancer: Initial experience and lessons learned

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Affiliations are included on p. 10.

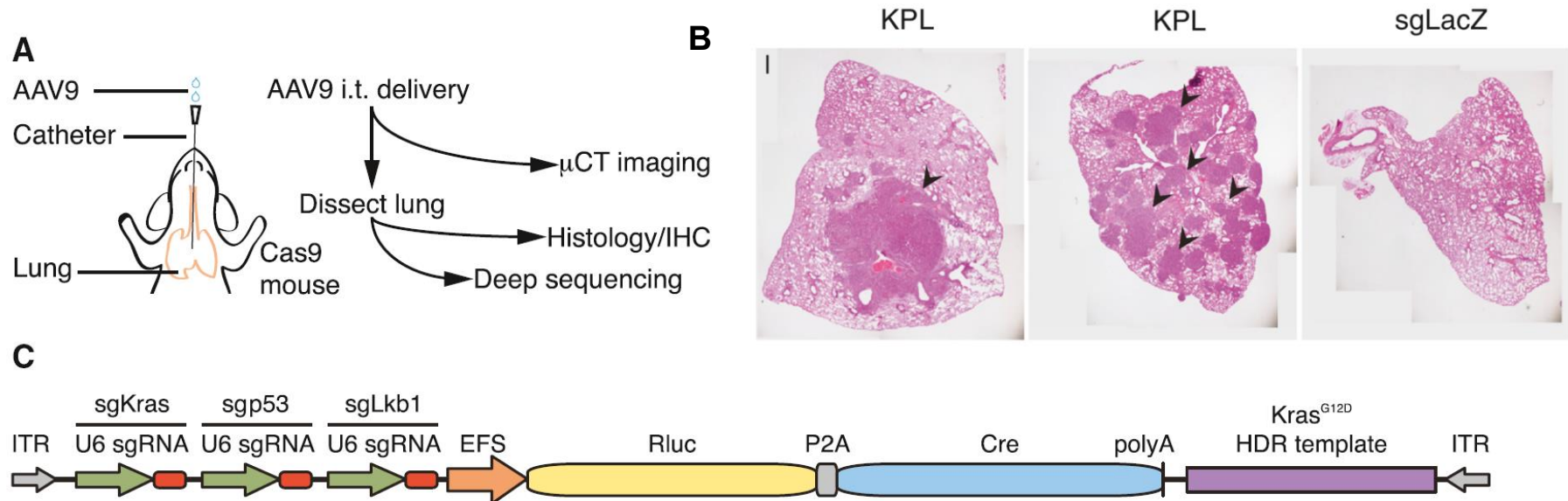
Contributed by William G. Kaelin, Jr.; received May 3, 2024; accepted September 3, 2024; reviewed by M. Celeste Simon and Arul M. Chinnaiyan

Inducible Cas9-Mouse

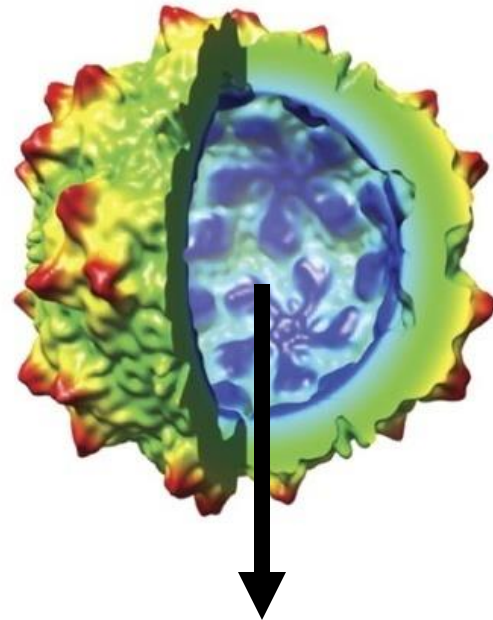


Platt et al. (2014), *Cell*, 159: 440-455 (Feng Zhang Group)

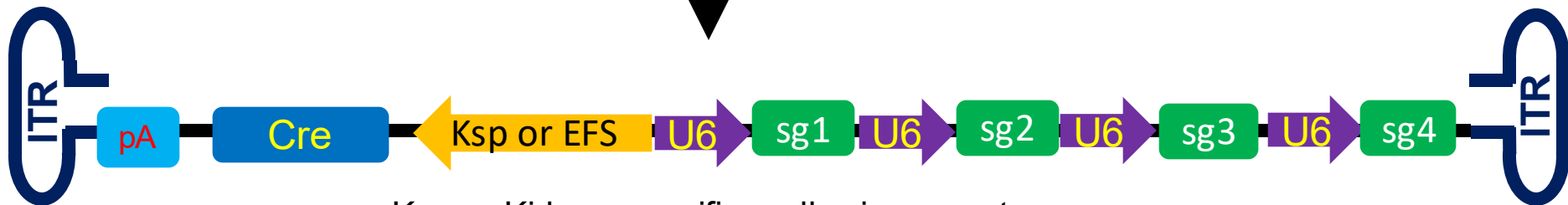
Use of AAV9 and LSL-Cas9 Knockin Mouse To Generate Lung Cancer Model



AAV-9 Expressing Both Cre and sgRNA Cassettes



AAV-9

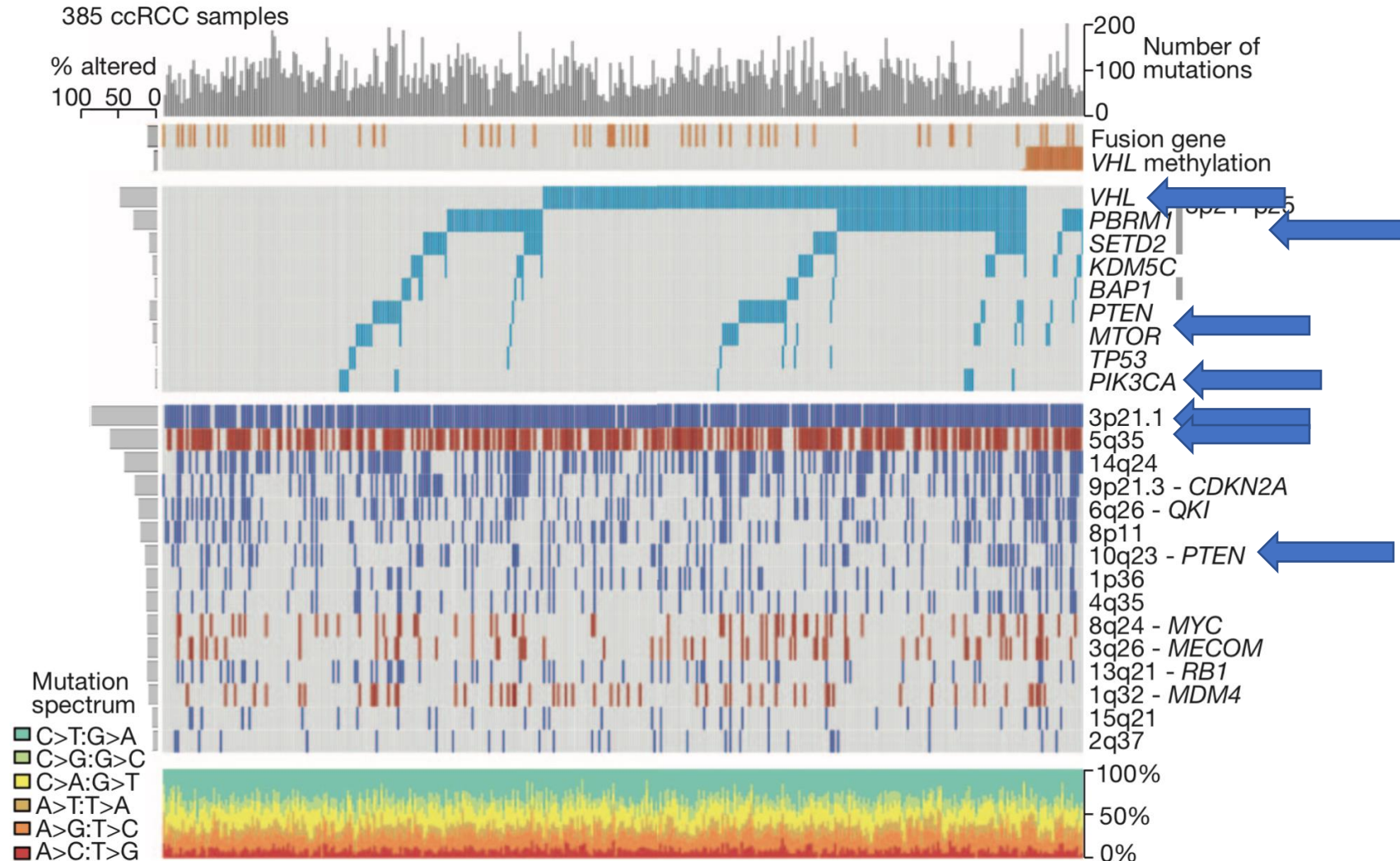


Ksp: a Kidney specific cadherin promoter

~~EFS: a strong and small ubiquitous promoter~~

Which genes to target?

Genomic Landscape of ccRCC



SQSTM1 Is a Pathogenic Target of 5q Copy Number Gains in Kidney Cancer

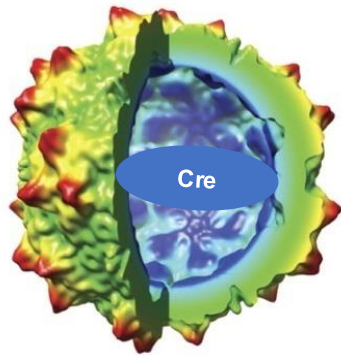
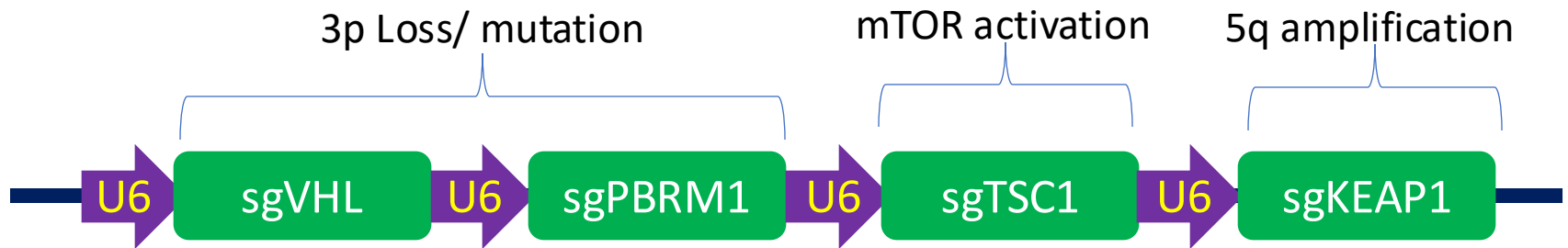
Lianjie Li,¹ Chuan Shen,¹ Eijiro Nakamura,¹ Kiyohiro Ando,¹ Sabina Signoretti,^{1,3} Rameen Beroukhi,^{1,2,4,5} Glenn S. Cowley,⁵ Patrick Lizotte,⁵ Ella Liberzon,^{1,11} Steven Bair,¹ David E. Root,⁵ Pablo Tamayo,⁵ Aviad Tsherniak,⁵ Su-Chun Cheng,⁶ Barbara Tabak,^{2,5} Anders Jacobsen,⁷ A. Ari Hakimi,⁸ Nikolaus Schultz,⁷ Giovanni Ciriello,⁷ Chris Sander,⁷ James J. Hsieh,^{9,10} and William G. Kaelin, Jr.^{1,4,5,11,*}

SQSTM1 encodes p62

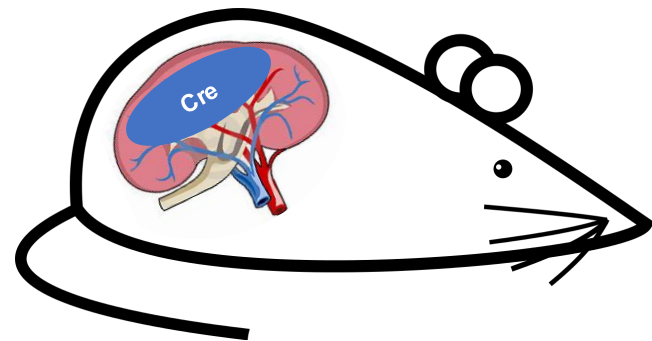
P62 activates mTOR and NRF2

KEAP1 degrades NRF2

Kidney-Specific Cas9 Activation and Target Gene Knockout in LSL-Cas9 Mouse



Ksp-Cre delivered in the AAV
LSL-Cas9-P2A-GFP mice

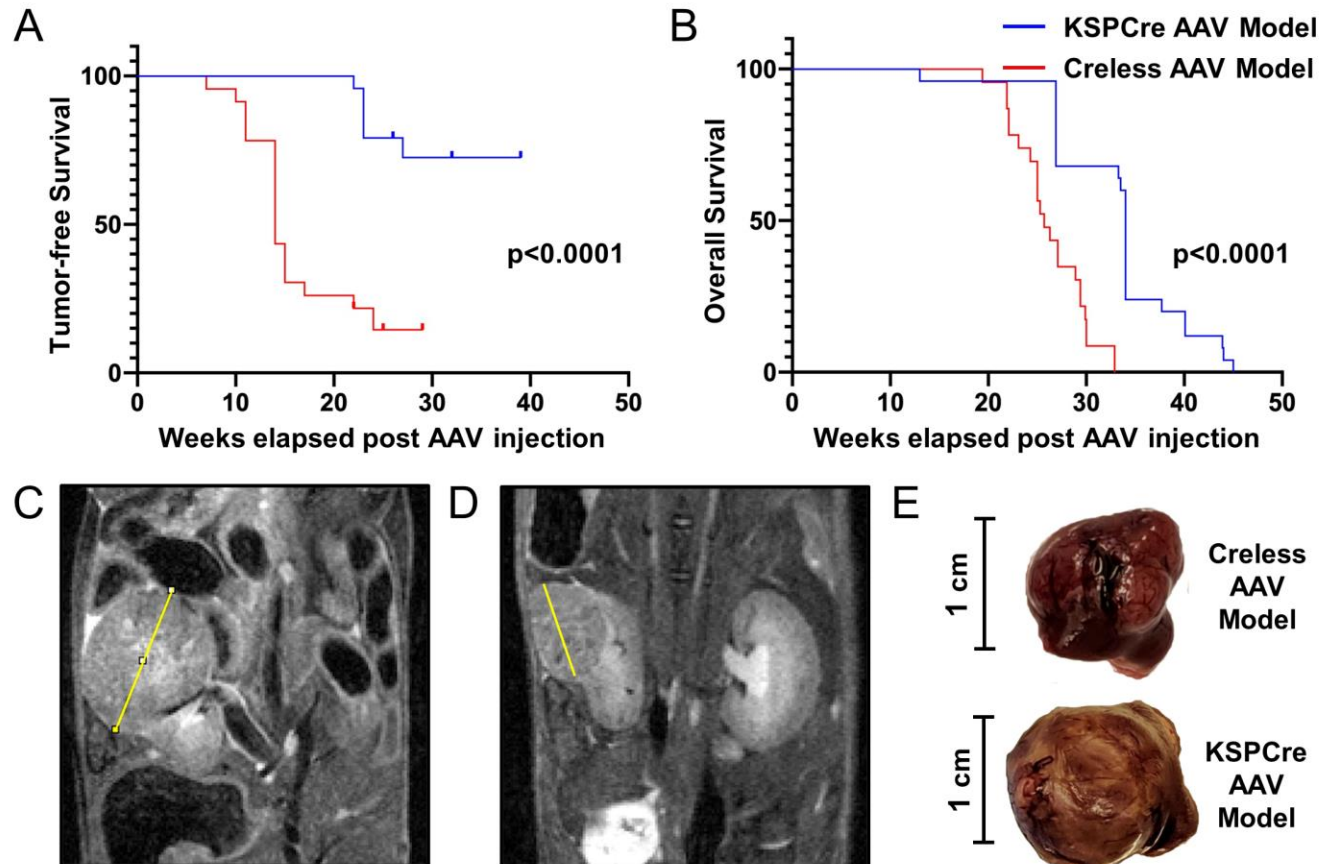


Transgenic Pax8-Cre, LSL-Cas9-P2A-GFP mice
Creless AAV

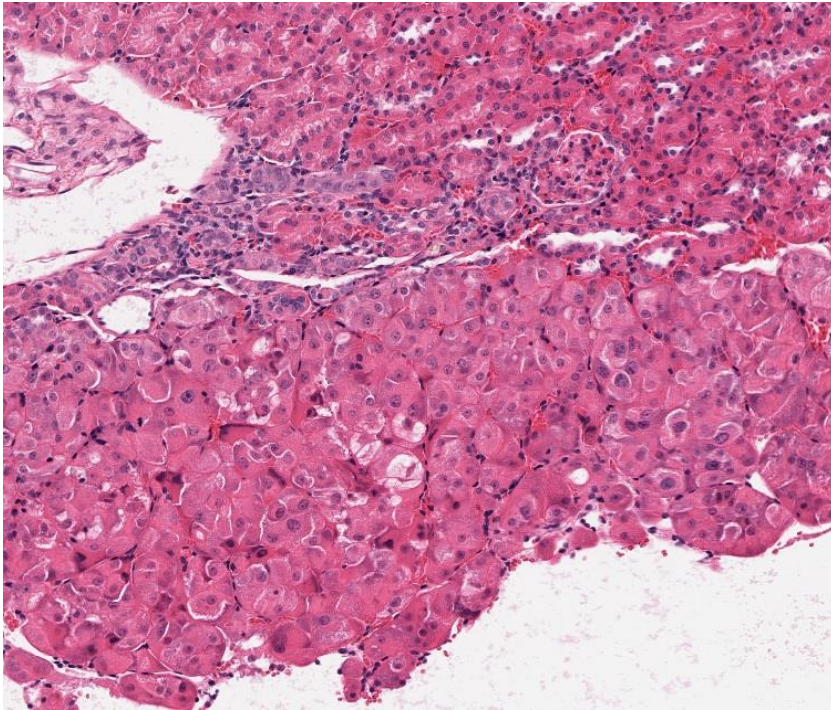
Examples of Tumor Suppressor Gene Combinations Tested in Pilot Experiments

Combo	Mouse Strain	Cre	Guide 1	Guide 2	Guide 3	Guide 4	Mice Injected	Kidney Tumors
1	B6 LSL-Cas9	Ksp-Cre (AAV)	VHL	TSC1	SETD2	PBRM1	25	0
2	B6,129 PBRM1 f/f, LSL-Cas9	Ksp-Cre (AAV)	VHL	TSC1	SETD2	CDKN2A	5	0
3	B6,129 PBRM1 f/f, LSL-Cas9	Ksp-Cre (AAV)	VHL	TSC1	SETD2	NT Control	9	0
4	B6,129 PBRM1 f/f, LSL-Cas9	Ksp-Cre (AAV)	VHL	TSC1	SETD2	HIF1a	10	0
5	B6,129 PBRM1 f/f, LSL-Cas9	Ksp-Cre (AAV)	VHL	TSC1	SETD2	EPAS1	7	0
6	B6 LSL-Cas9, Pax8-Cre	Pax8-Cre (Tg)	VHL	TSC1	SETD2	NT Control	7	0
7	B6 LSL-Cas9, Pax8-Cre	Pax8-Cre (Tg)	VHL	TSC1	SETD2	PBRM1	7	0
8	B6 LSL-Cas9, Pax8-Cre	Pax8-Cre (Tg)	VHL	TSC1	SETD2	BAP1	7	0
9	B6 LSL-Cas9, Pax8-Cre	Pax8-Cre (Tg)	VHL	TSC1	PBRM1	NT Control	25	0
10	B6 LSL-Cas9, Pax8-Cre	Pax8-Cre (Tg)	VHL	KEAP1	PBRM1	NT Control	25	0
11	B6 LSL-Cas9, Pax8-Cre	Pax8-Cre (Tg)	VHL	KEAP1	NT Control	NT Control	25	1
12	B6 LSL-Cas9, Pax8-Cre	Pax8-Cre (Tg)	VHL	KEAP1	TSC1	NT Control	25	24

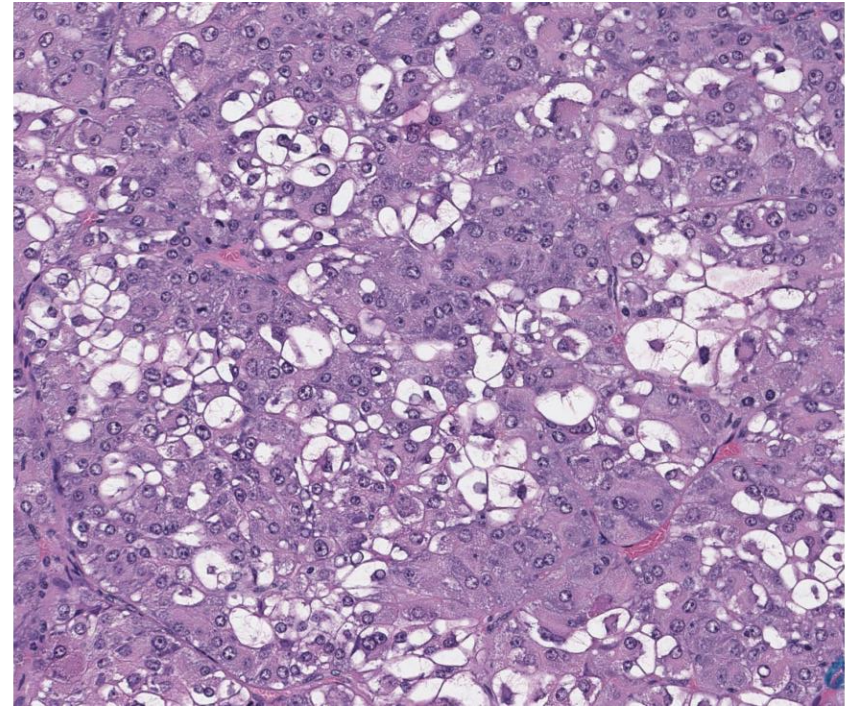
Combined Loss of *Vhl*, *Pbrm1*, *Keap1* and *Tsc1* Sufficient to Drive ccRCC Development



Combined Loss of *VHL*, *PBRM1*, *KEAP1*, and *TSC1* is Sufficient to Drive ccRCC Development

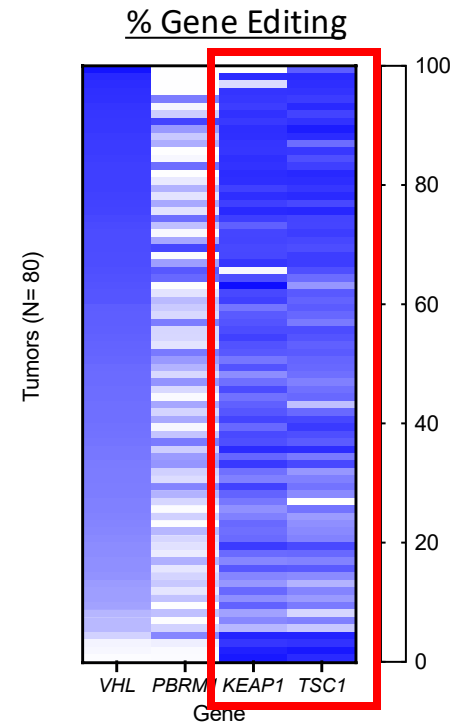
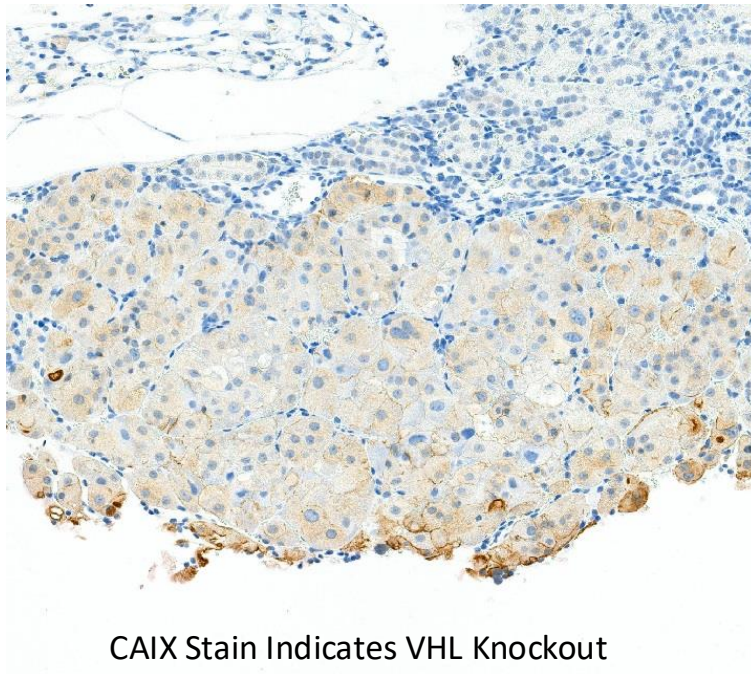


Mouse strain: C57BL/6 LSL-Cas9-P2A-GFP
AAV: Ksp-Cre, sgVHL, sgPBRM1, sgKEAP1, sgTSC1

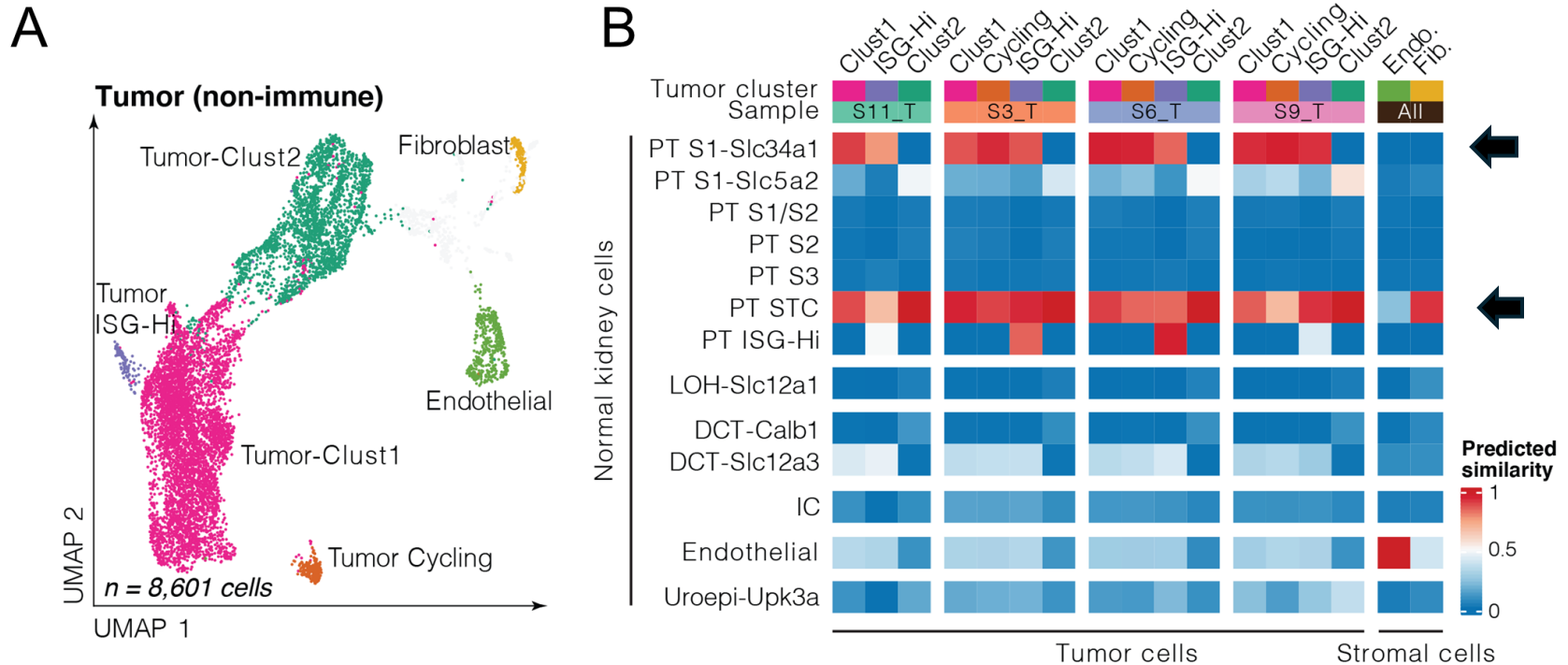


Mouse strain: C57BL/6 LSL-Cas9-P2A-GFP: Pax8-Cre
AAV: Creless sgVHL, sgPBRM1, sgKEAP1, sgTSC1

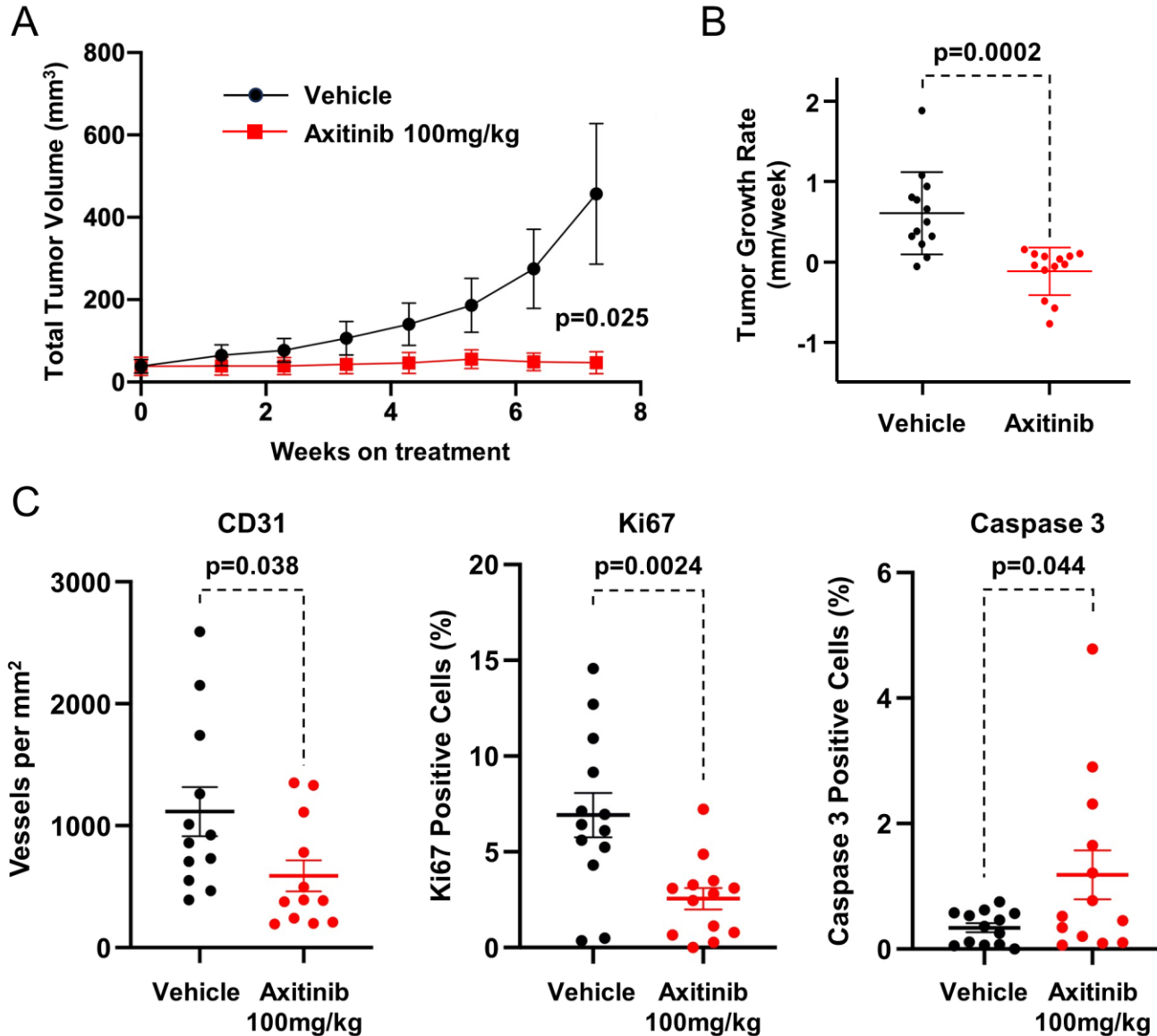
Successful Inactivation of Targeted Genes Validated by IHC and Genomic Sequencing



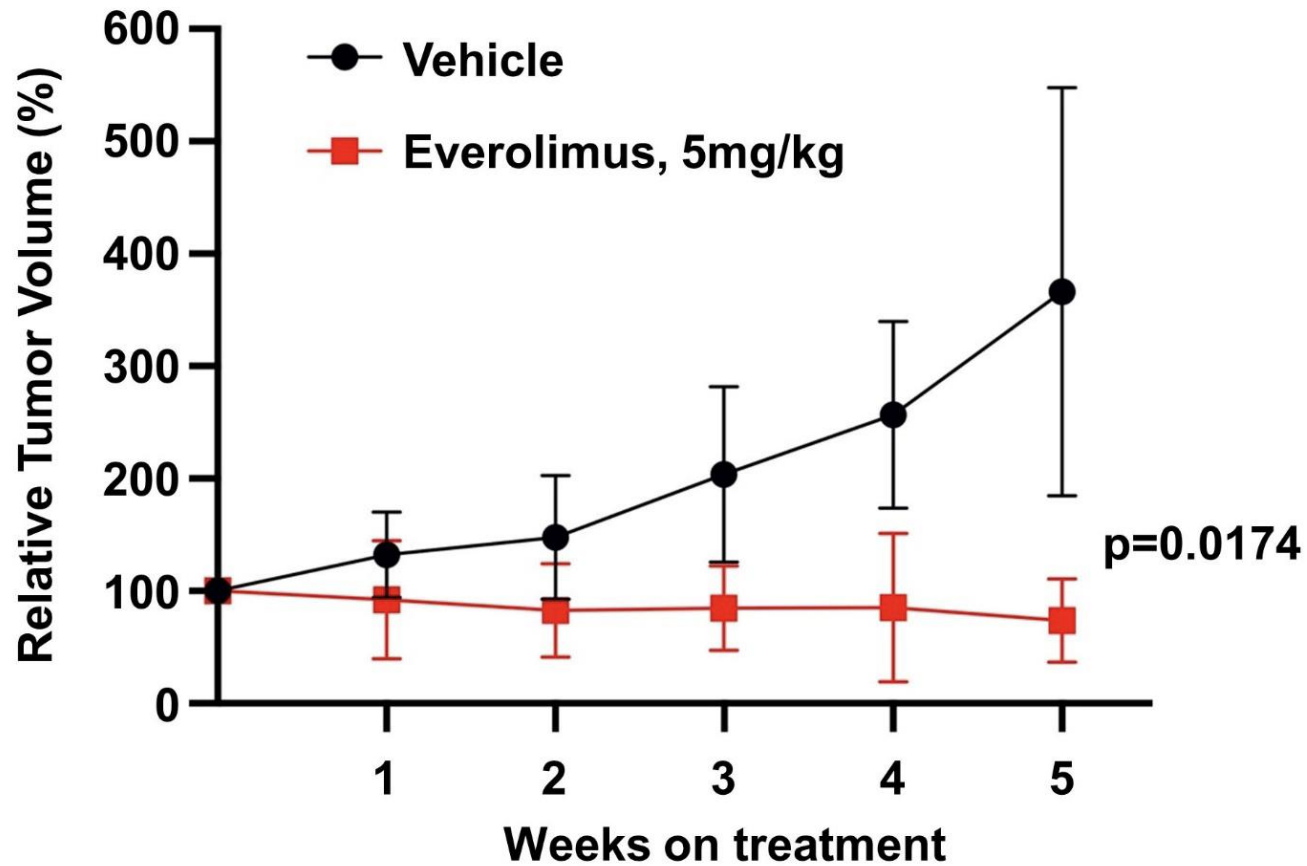
Tumors Arise from Proximal Tubule Cells Based on scRNA-seq



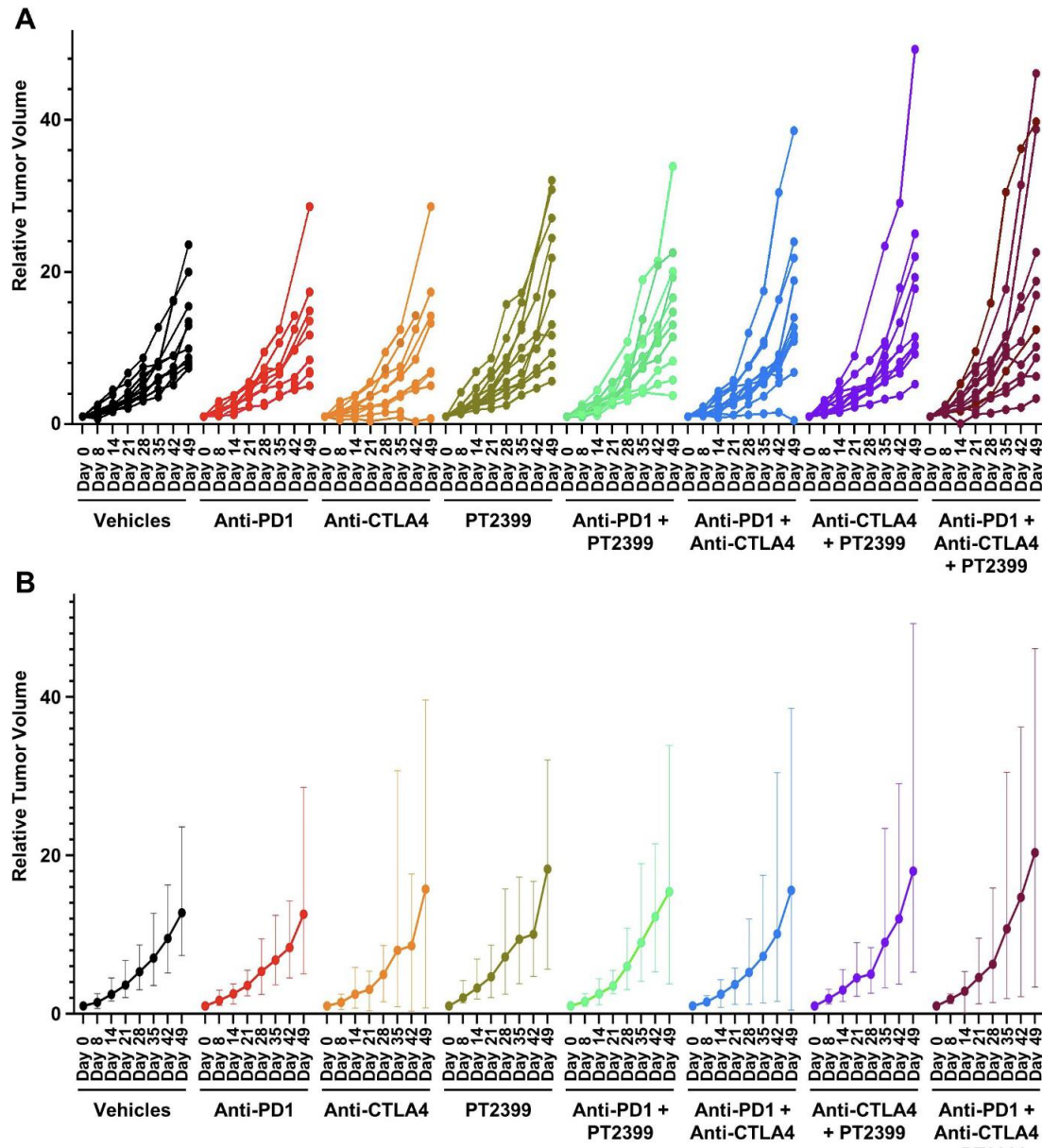
Tumors Respond to Axitinib



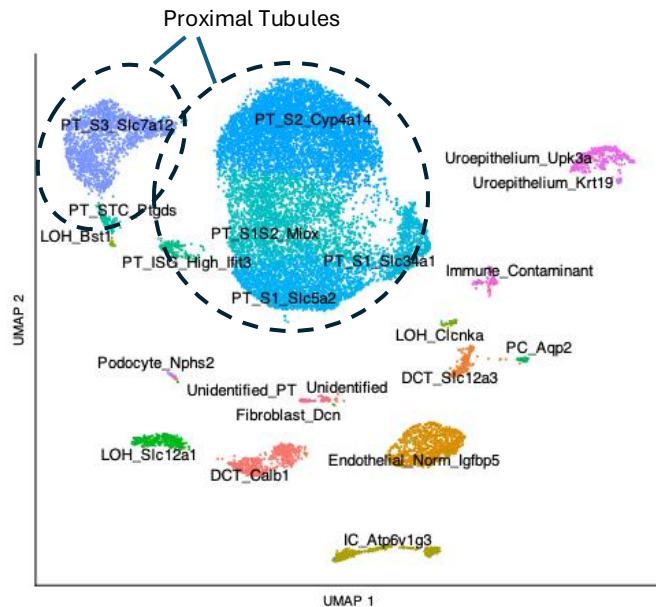
Tumors Respond to Everolimus



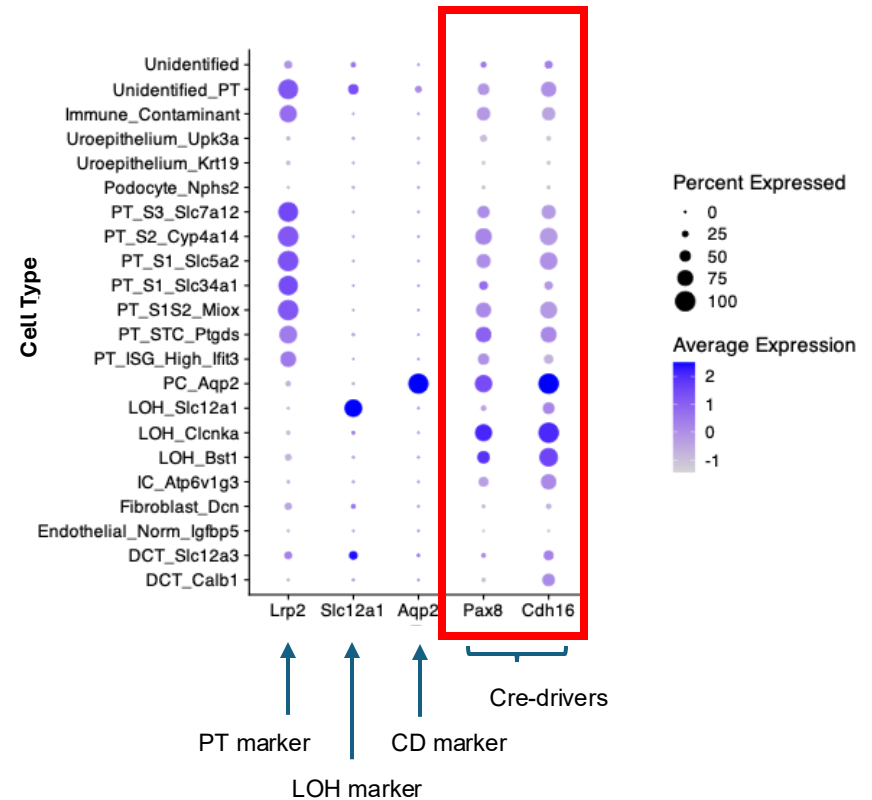
Tumors DON'T Respond to HIF2i or ICB



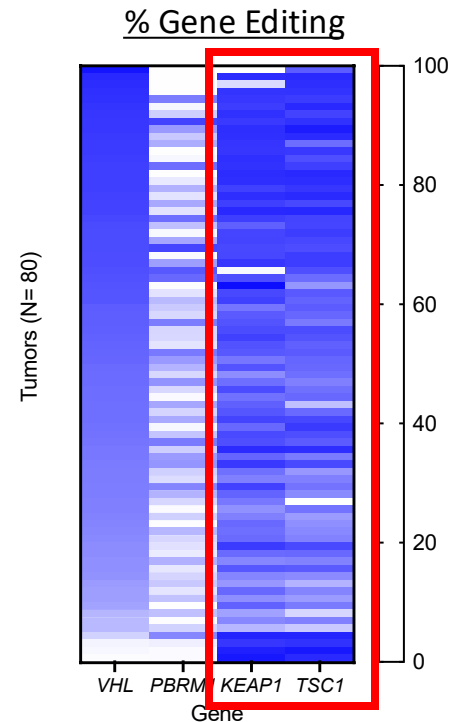
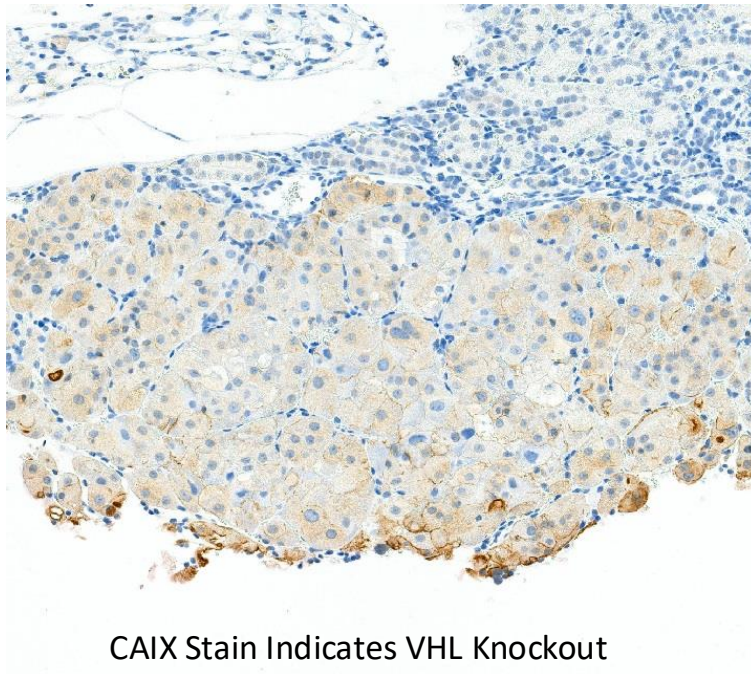
The CRE Drivers We Used Target Many Different Kidney Cell Types



Single-cell RNA-seq on
Healthy mouse kidney from
Stransky et. al. (2024)

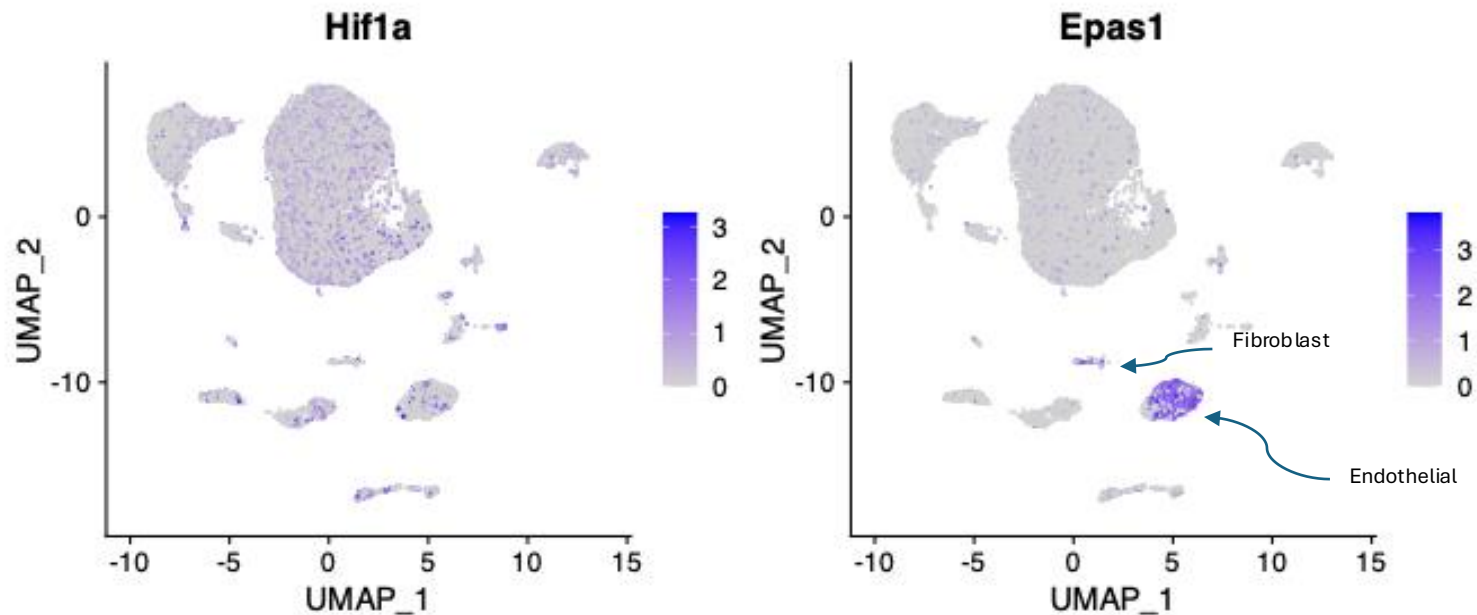


Potential Problem



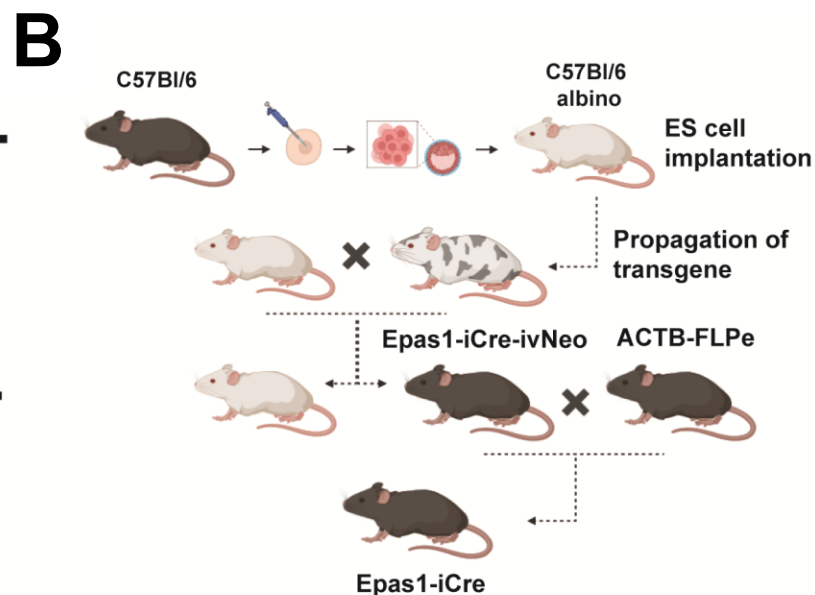
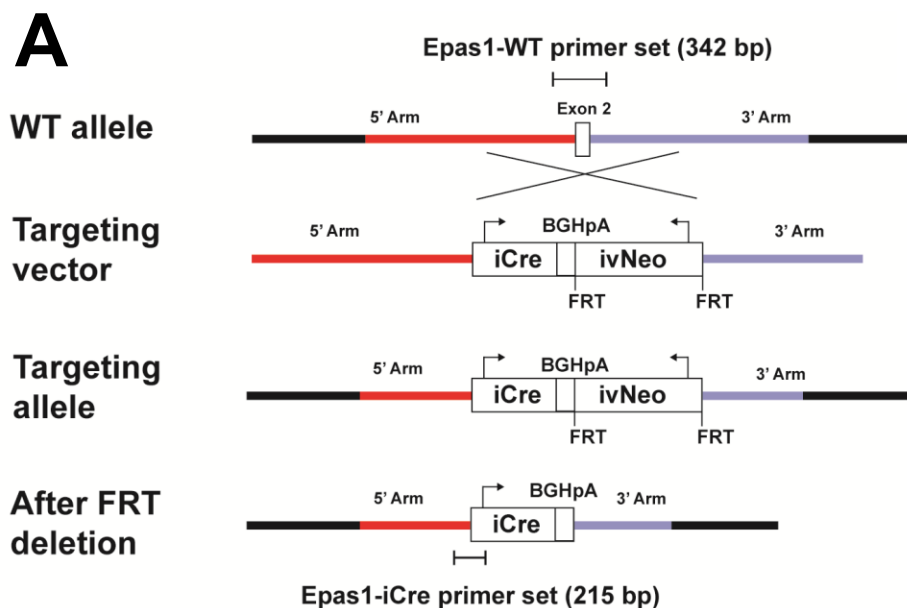
Tsc Loss Causes ccRCC in Rats (Eker Rat) and Mice!

Epas1 (Hif2a) is Sparsely Expressed, But Not Absent, In kidney epithelium



Gene	% Epithelial Cells Expressing Gene
Hif1a	39%
Epas1	18.4%
Lrp2	86%

Possible Solution: Make an Epas1-Cre Mouse to Specifically Target Epas1-Expressing Cells



Greg Wyant and VJ Ramesh