Animal Model Workshop

In Vivo CRISPR Engineering of Renal Tumors



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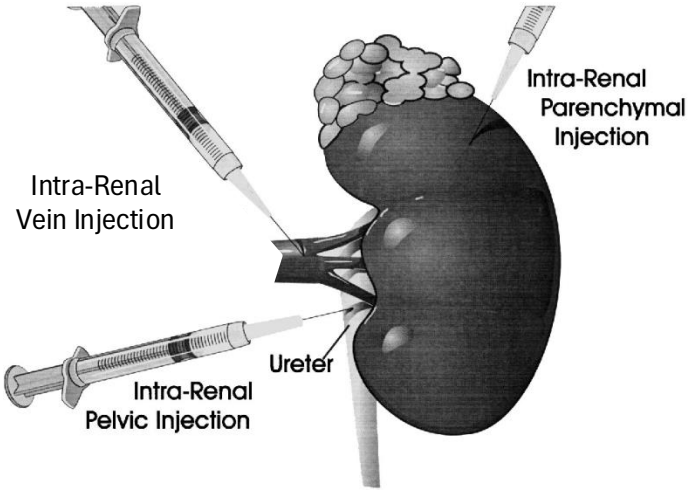
Session 5

Kidney Cancer Association® IN PARTNERSHIP WITH UT Southwestern Medical Center
Kidney Cancer Program

Optimizing AAV9 Delivery

AAV9 (mCherry)

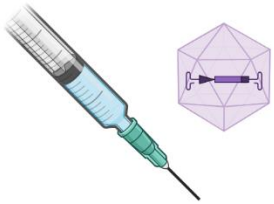


Pax8-Cre

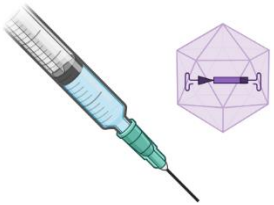
X

LSL-Cas9 (ROSA26)

No tumors



No tumors

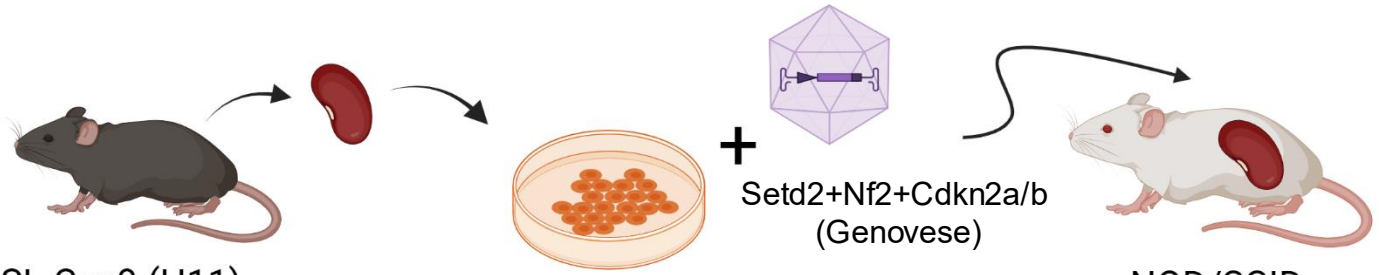


No tumors*

- Bap1+Tp53+Cdkn2a (n=23)
- Bap1+Tp53+Cdkn2a/b (n=21)
- Setd2+Nf2+Cdkn2a/b (n=6)
- Pbrm1+Setd2+Bap1+Cdkn2a (n=18)

*One 1.5 mm tumor

Alt #1: *Ex vivo* transduction

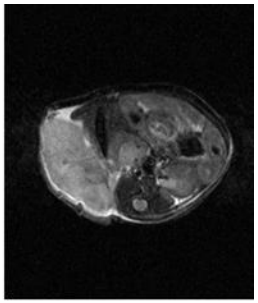


UTSW-KCP-1

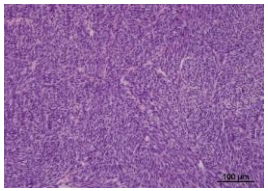
UTKB6F(P8C9)Ki>CL+A9(SNC$^{2a/2b}$)>NODS(sc)>Tu>CL(NC$^{2a/2b}$)

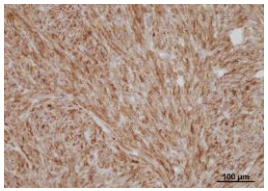
UTSW-KCP-1

(UTKB6F(P8C9)Ki>CL +A9(SNC$^{2a/2b}$)>NODS(sc)>Tu>CL(NC$^{2a/2b}$)

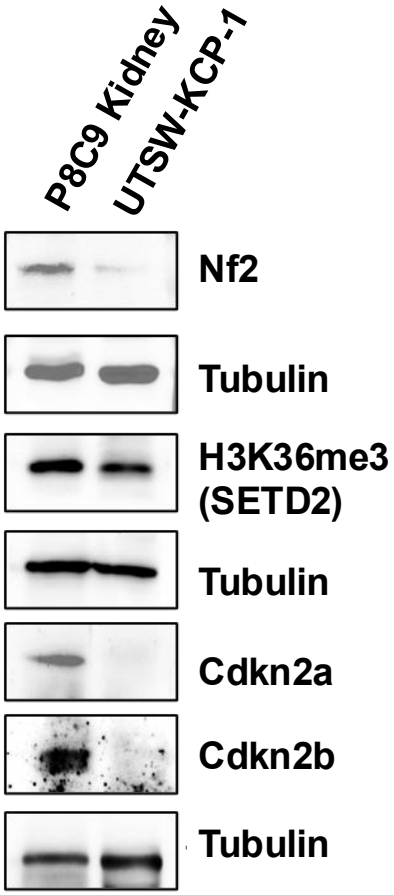


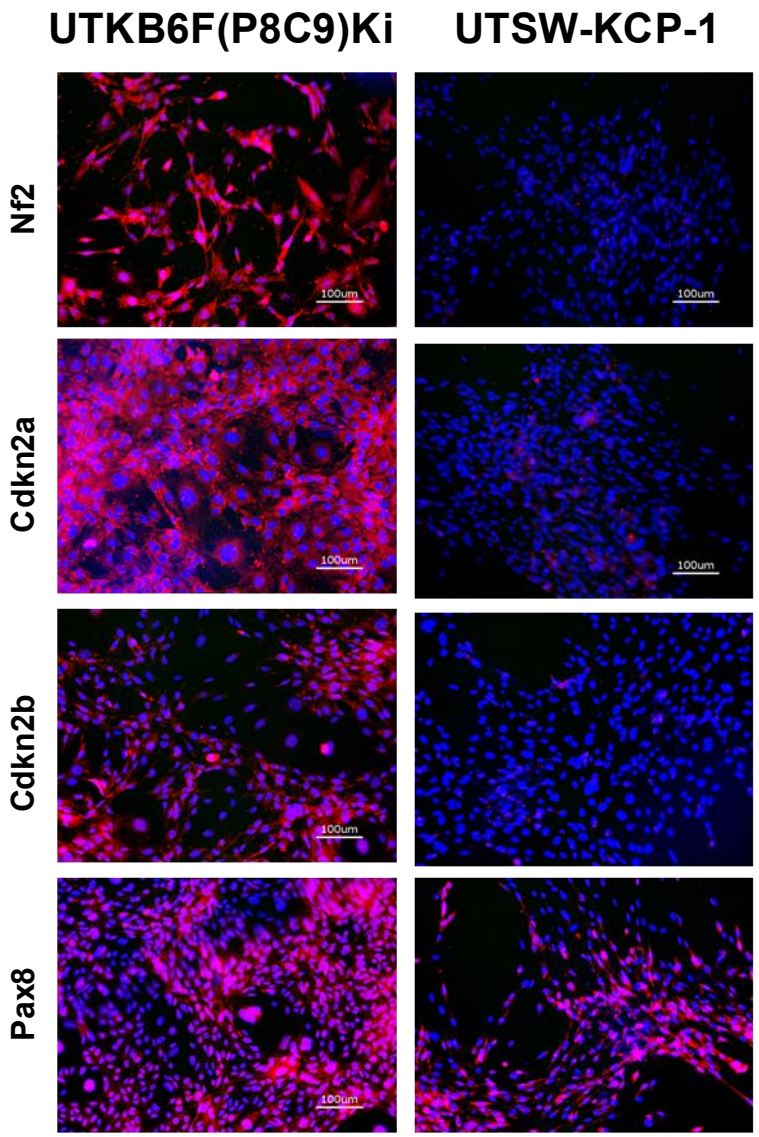
CD10





Sarcomatoid (PK)





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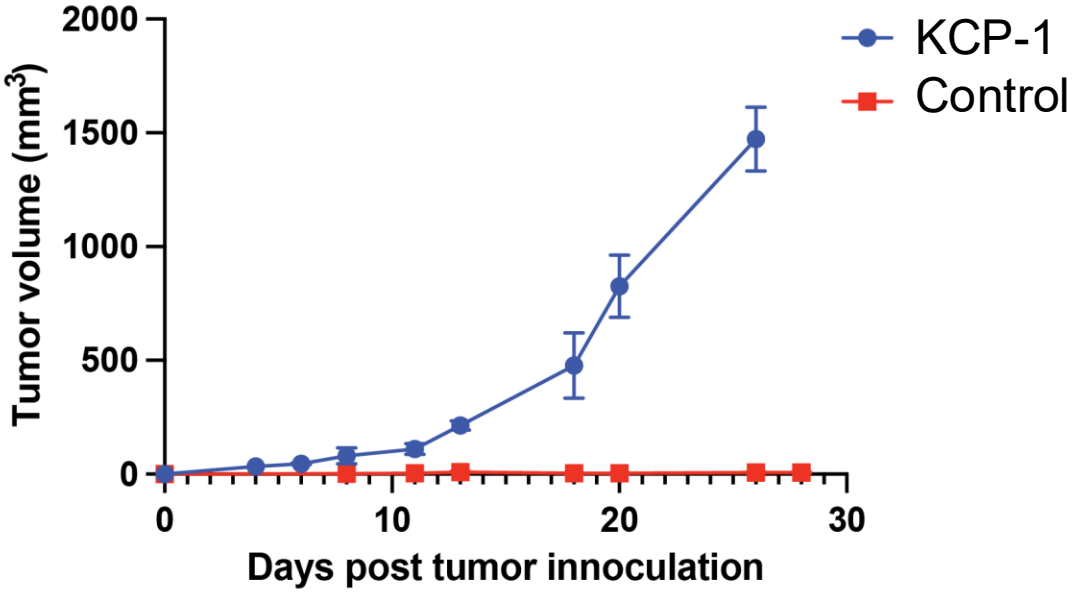
KCP-1 growth in C57BL/6 mice

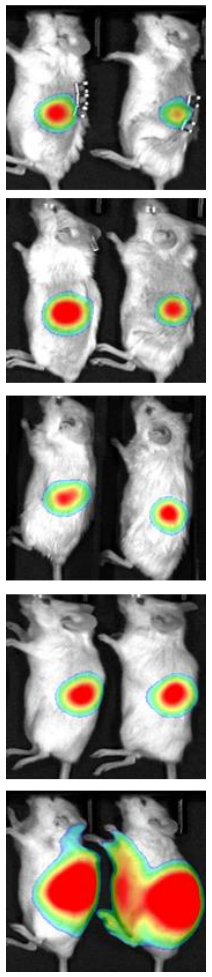


(Independent assessment)

Immunophenotype of KCP-1 cell line derived tumors

UTSW-KCP-1	Tumor 1	Tumor 2	LVRCC (Hakimi)
CD45+ immune cells (% of live cells)	5.70%	3.90%	20%
NK cells (% of CD45+)	0.62%	0.93%	1%
CD8+ (% of CD45+)	1.93%	2.69%	6
PD1+ CD8+ T cells (% of CD8+)	41.30%	41.98%	17%
Tim3+ CD8+ T cells (% of CD8+)	17.90%	7.68%	N/A
CD4+ (% of CD45+)	0.93%	1.47%	5%
CD25+ Foxp3+ CD4+ Treg (% of CD4+)	26.50%	37.50%	30%
Macrophages (% of CD45+)	55.60%	54.90%	45%
M1-like (% of Macrophages)	18.70%	45.10%	30%
M2 like (% of Macrophages)	15.50%	10.30%	30%
Monocytes (% of CD45+)	19.30%	23.20%	5%
DCs (% of CD45+)	2.10%	3.60%	N/A
Neutrophil (% of CD45+)	17.60%	21.30%	6%
APCAF (% of CD45-)	0.87%	1.80%	N/A

Alt #2: Vhl/Myc kidney transplantation

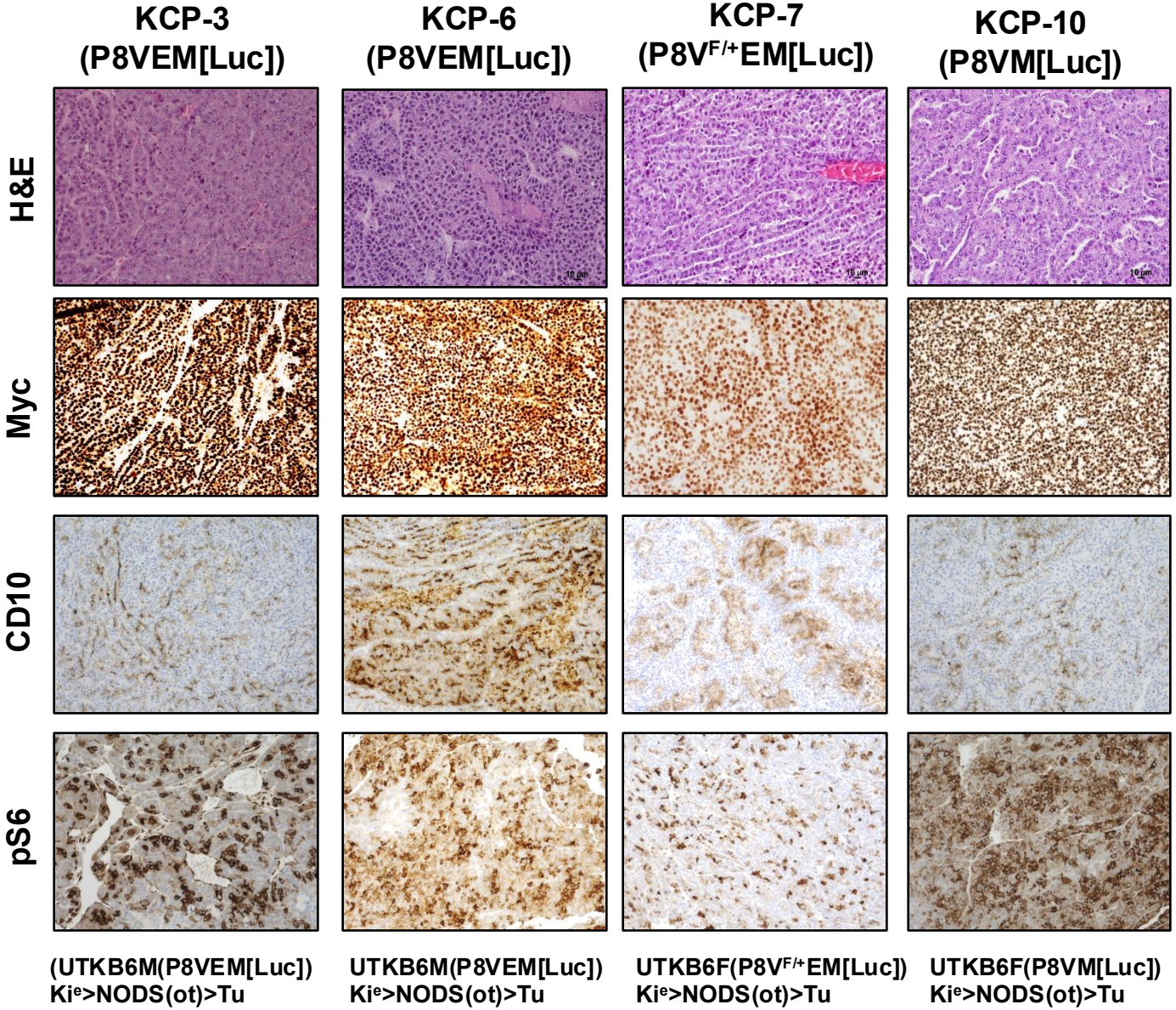


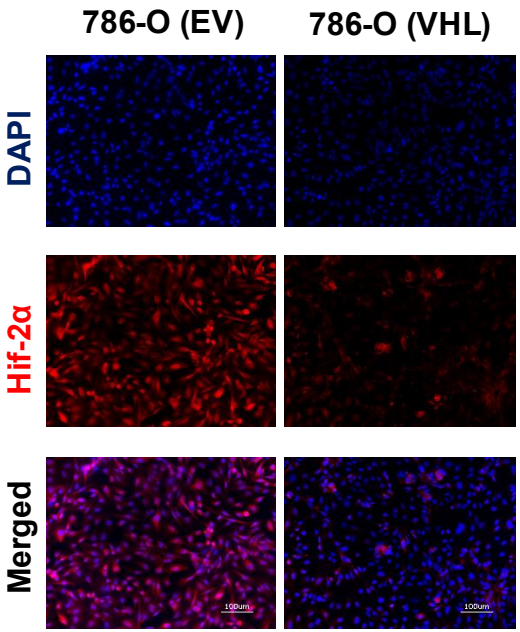
Cell Lines
UTSW-KCP-3
UTSW-KCP-6
UTSW-KCP-7
UTSW-KCP-10

⇨ **C57BL/6**

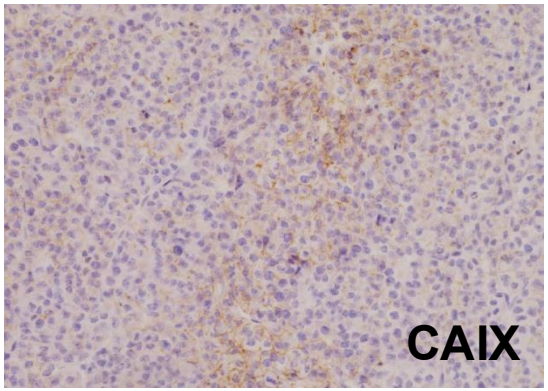
UTSW RCC Cell Lines

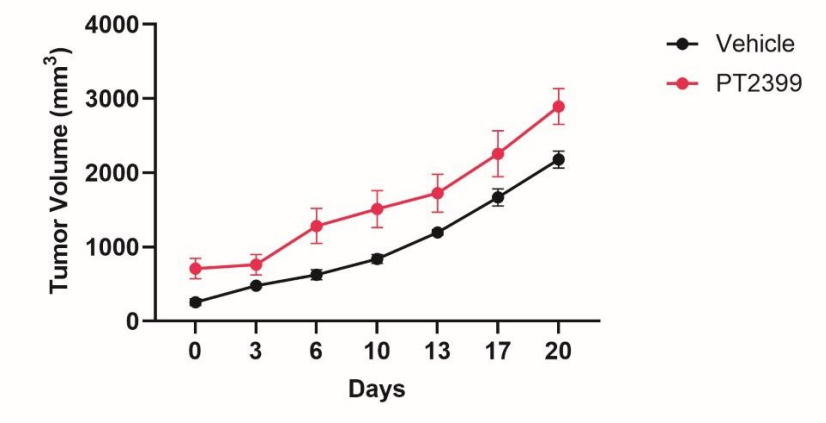
Cell line	Somatic (failed)
KCP-1	Setd2





KCP-3 (NOD/SCID)

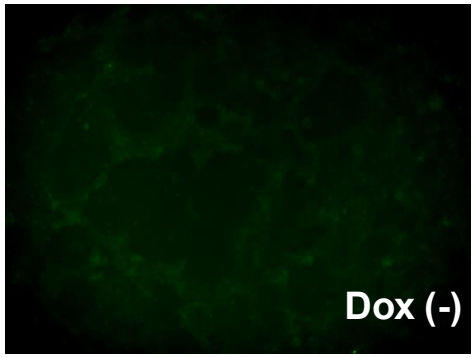


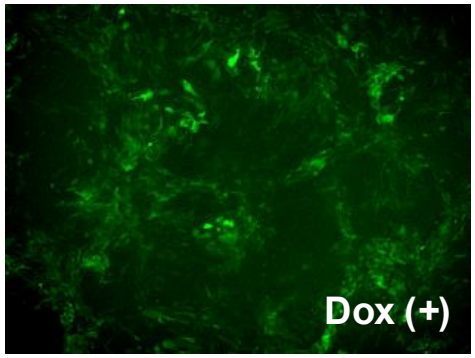


Evaluating the role of Vhl and Myc

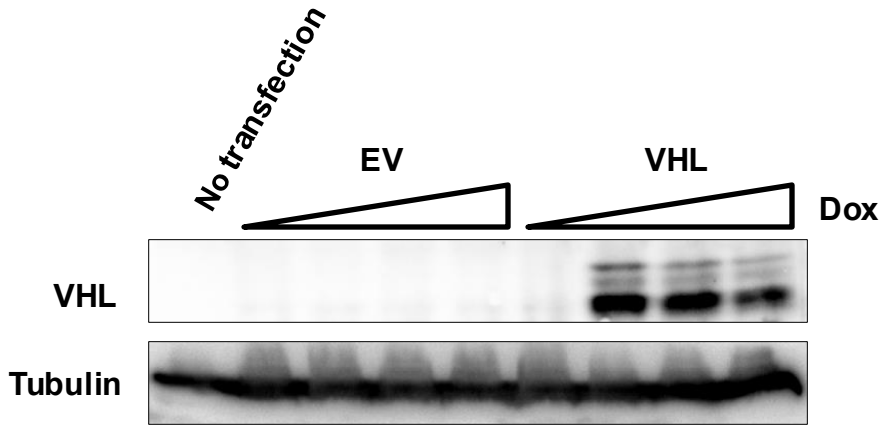
UTSW KCP-6 (UTKB6M(P8VEM[Luc])Ki^{e}>NODS(ot)>CL)

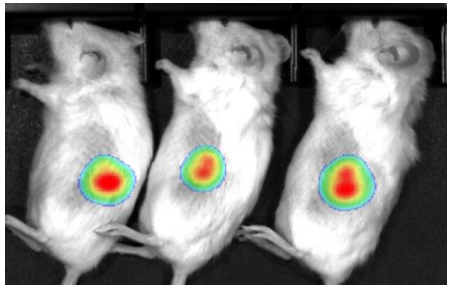
Tet-on inducible system (GFP):
1. VHL reconstitution
2. Myc KD

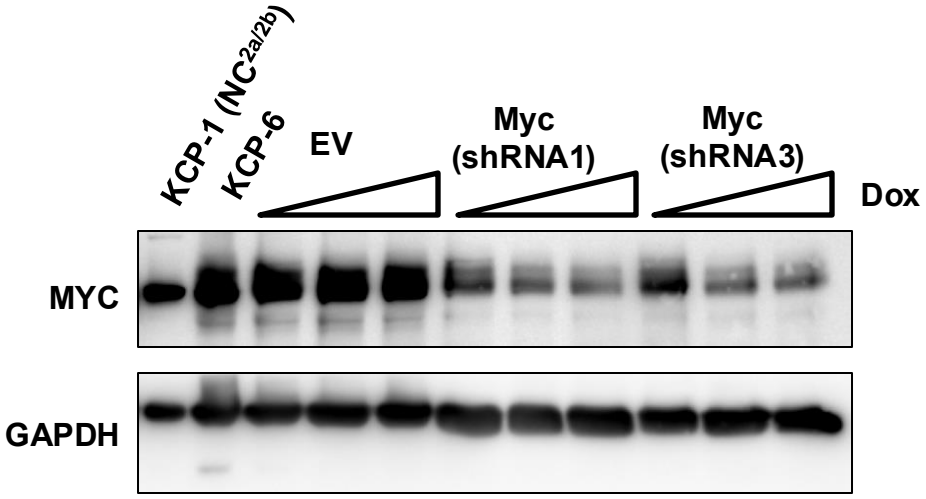


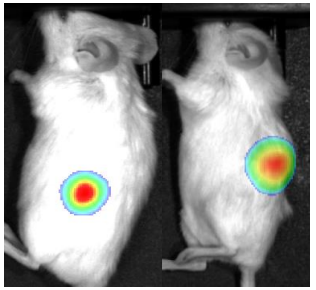


VHL(GFP) expression









Considerations

- While targeting Vhl+Pbrm1 and Vhl+Bap1 in the Pax8 lineage in GEMM results in tumors with 100% penetrance, similar somatic targeting strategies have largely failed
  - Targeting the right cell type?
  - Sequencing?
  - Latency?
- *Ex vivo* approaches have the potential to introduce confounding factors.
- While simultaneous disruption of different genes may result in tumor formation, this does not mean that each gene is required.
  - Potent oncogenes (Myc).
  - Multiple gene targeting.
- Validation approaches should go beyond the role of the particular genes and pathways (i.e. HIF2α).

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