Animal Model Workshop

Building ccRCC models with non-viral *in vivo* somatic cell editing methods

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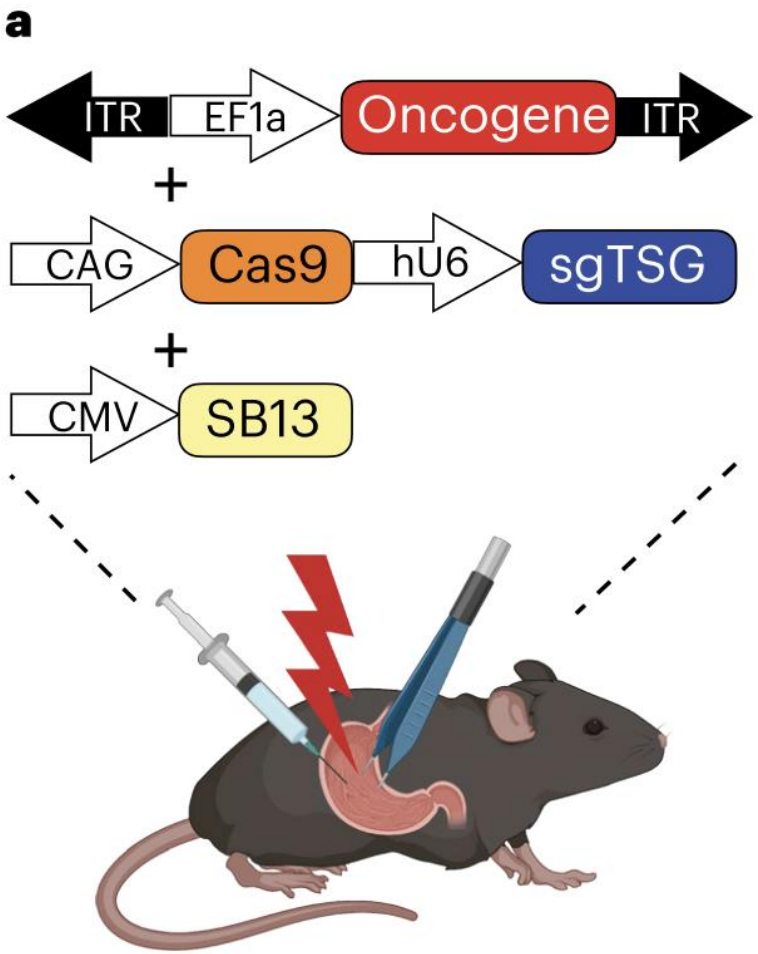
Session 5: Somatic models

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

Limitations of germline GEM models

1. Extensive breeding times to achieve multi-allele homozygous editing
2. Long tumor latency
3. Multifocal tumors
4. Low rates of metastases
5. Mixed strain background

Electroporation-based (EPO)-GEMMs

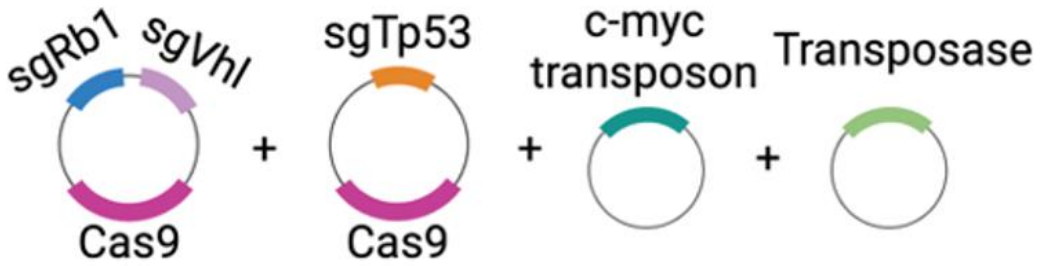


Cancer	Genes	Penetrance	Ref
Pancreatic	Ptf1a$^{Cre/+}$ KRAS$^{LSL-G12D/+}$	~50% d20-168	Maresch et al, Nat Comms 2016
Liver	p19Arf-/- mice c-Myc + NRAS^{G12V} c-Myc + AKT1	Not reported	Seehawer et al, Nature 2018
Ovarian	c-Myc + sg-p53 sg-p53 + sg-Pten sg-p53 + sg-Pten + sg-Rb1	~40-100% d40-400	Paffenholz et al, PNAS 2022
Prostate	c-Myc + sg-p53 c-Myc + sg-Pten	~75% d50-200	Leibold et al, Cancer Discovery 2020
Gastric	c-Myc + sg-p53 c-Myc + sg-Apc c-Myc + sg-p53 + sgMsh2	100% d30-150	Leibold et al, Nature Cancer 2024

Rapid, flexible, limited/no breeding

Leibold et al, Nature Cancer 2024

Electroporation-derived tumors

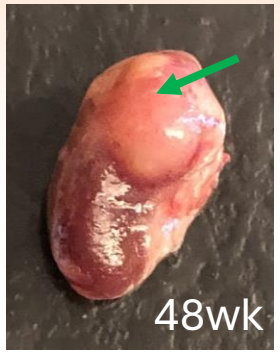


	n	tumors	cysts
VPR	49	0	3
VPRM	50	5	9

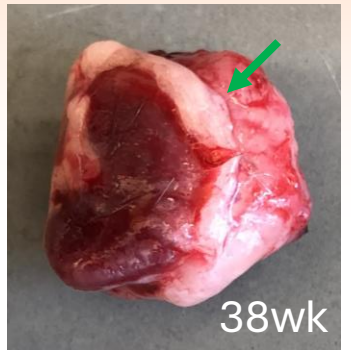
Followed up to 18 mo

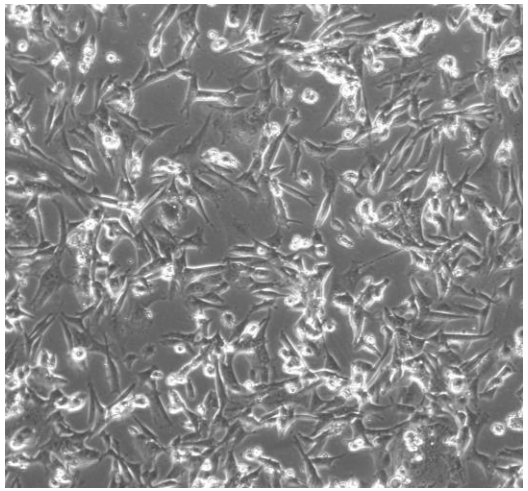
1 osteosarcoma
2 mccRCC (no viable tissue)
2 primary ccRCC

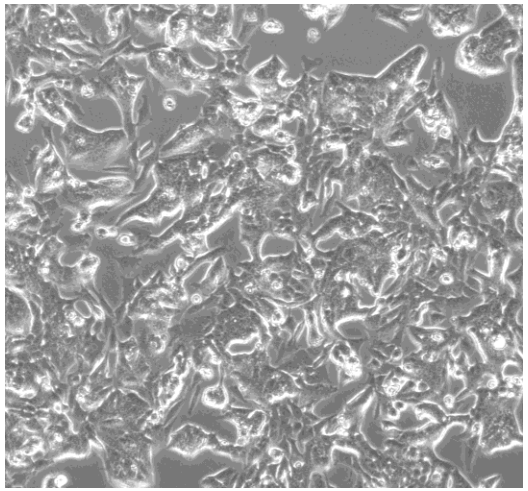
LVRCC67



LVRCC88



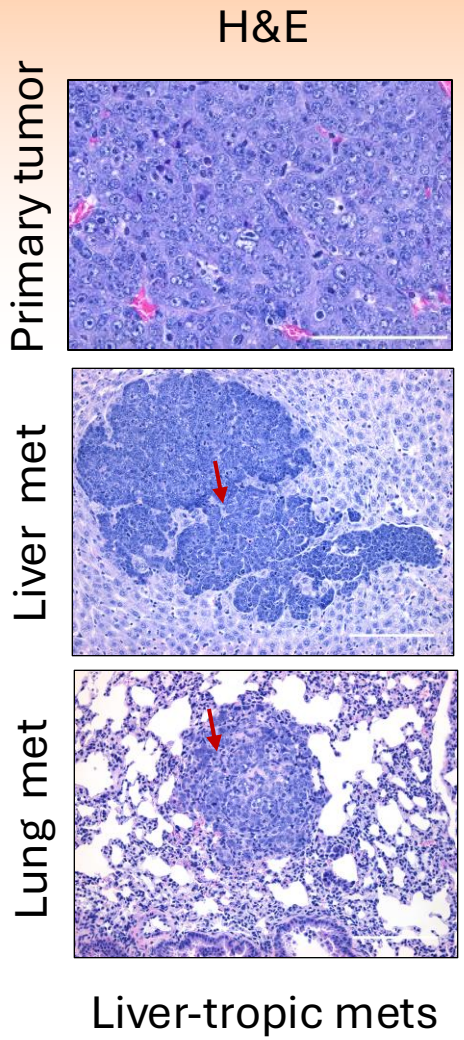




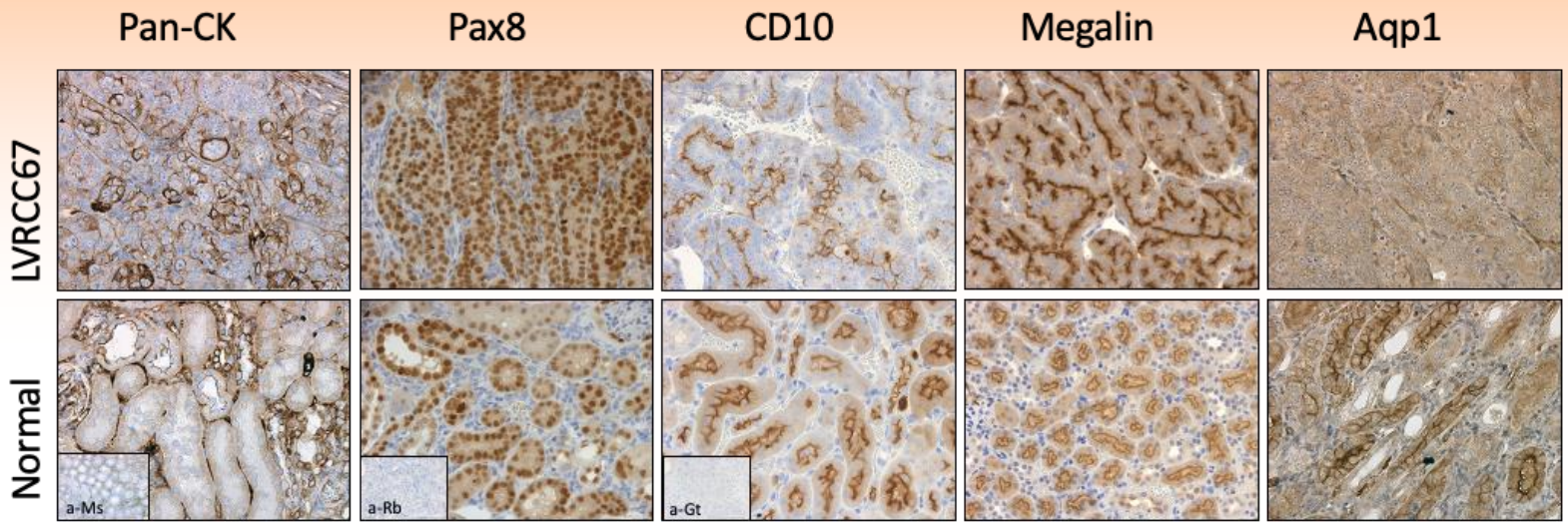
Rappold & Vuong et al
Cancer Discovery 2022

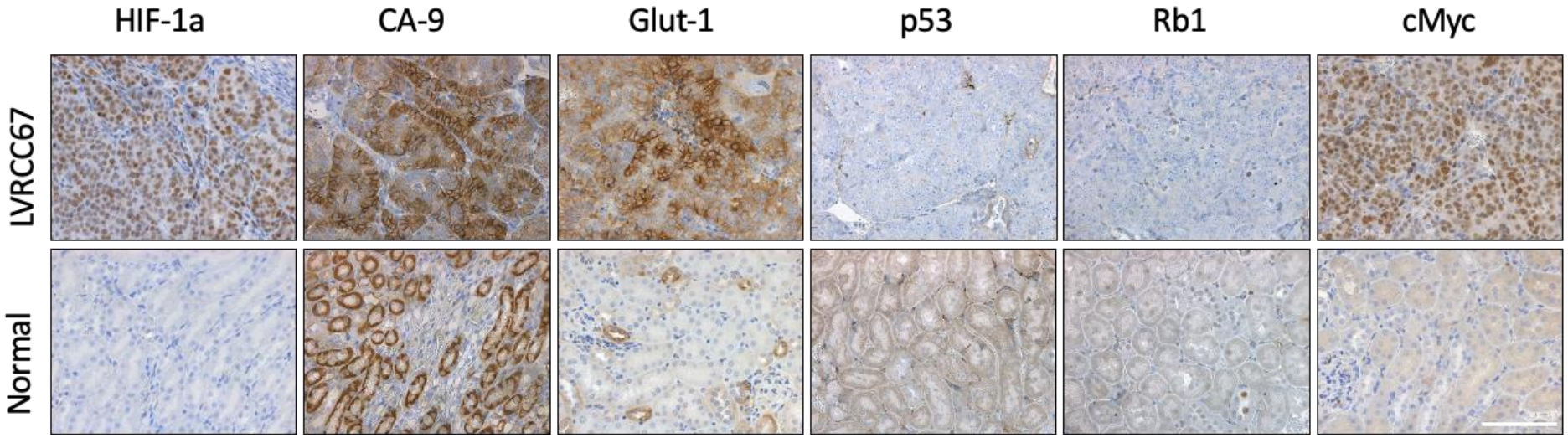
Unpublished

EP-derived syngeneic model histological characterisation



Liver-tropic mets



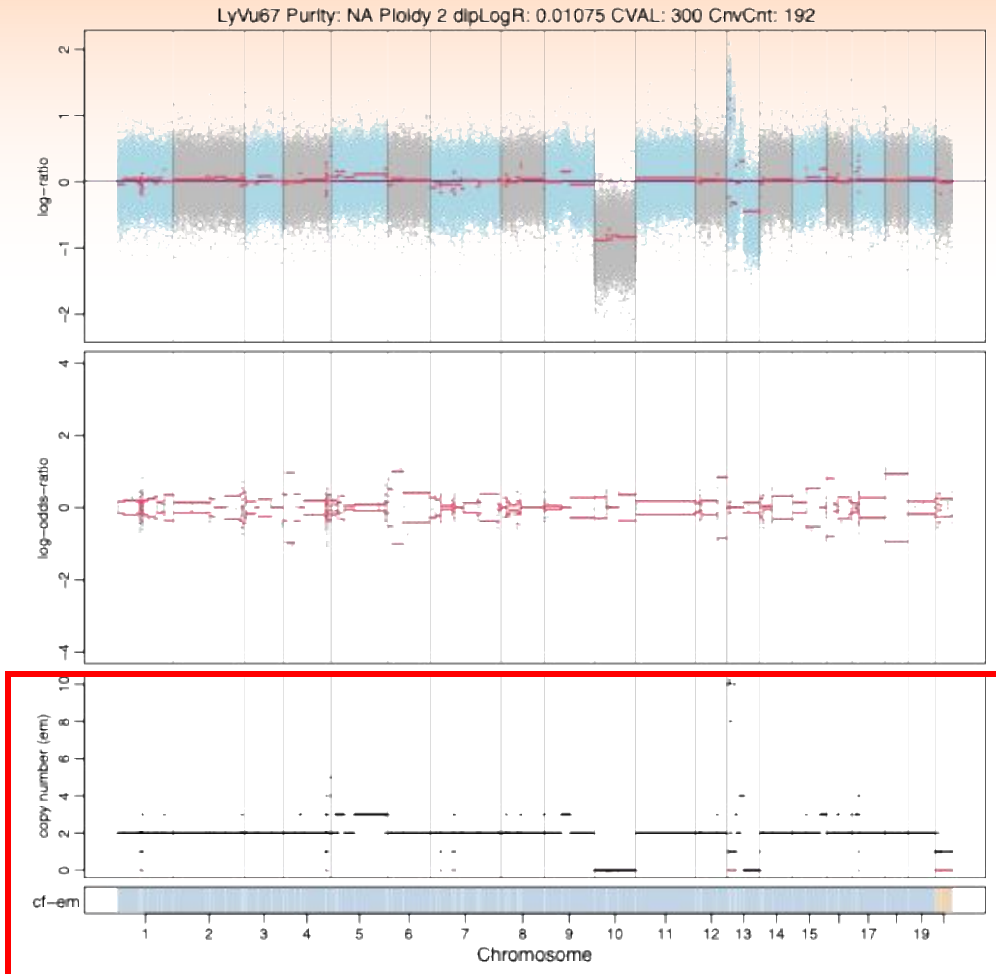


Rappold & Vuong et al 2022 Cancer Discovery

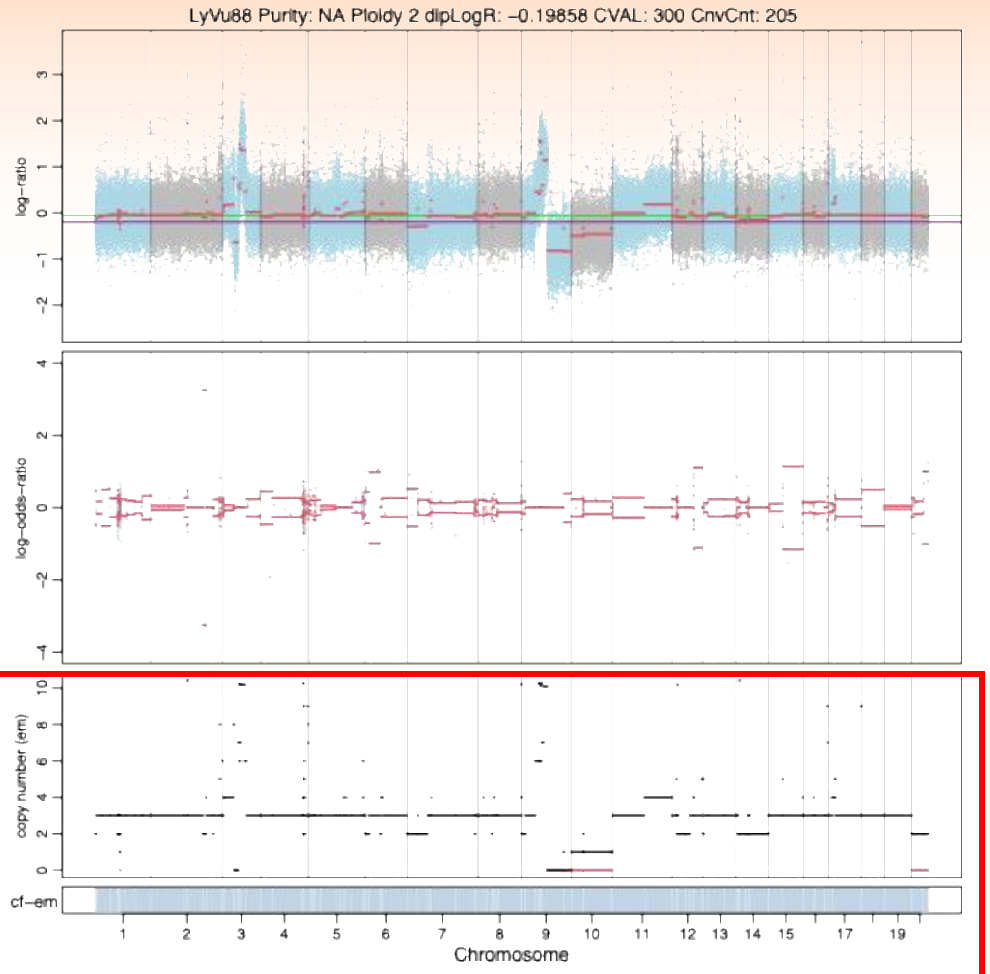
LVRCC67 shown but LVRCC88 v. similar

Whole exome sequencing

LVRCC67



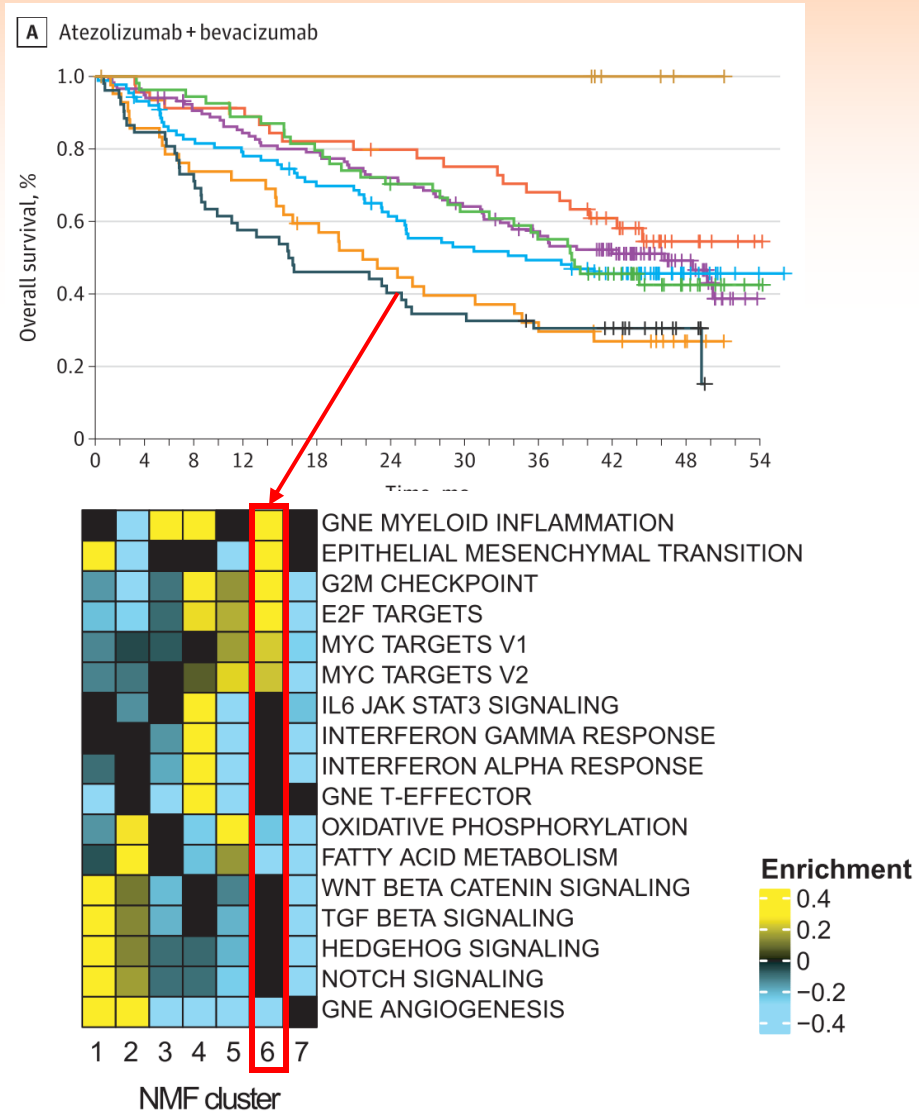
LVRCC88



Biallelic gene edits confirmed

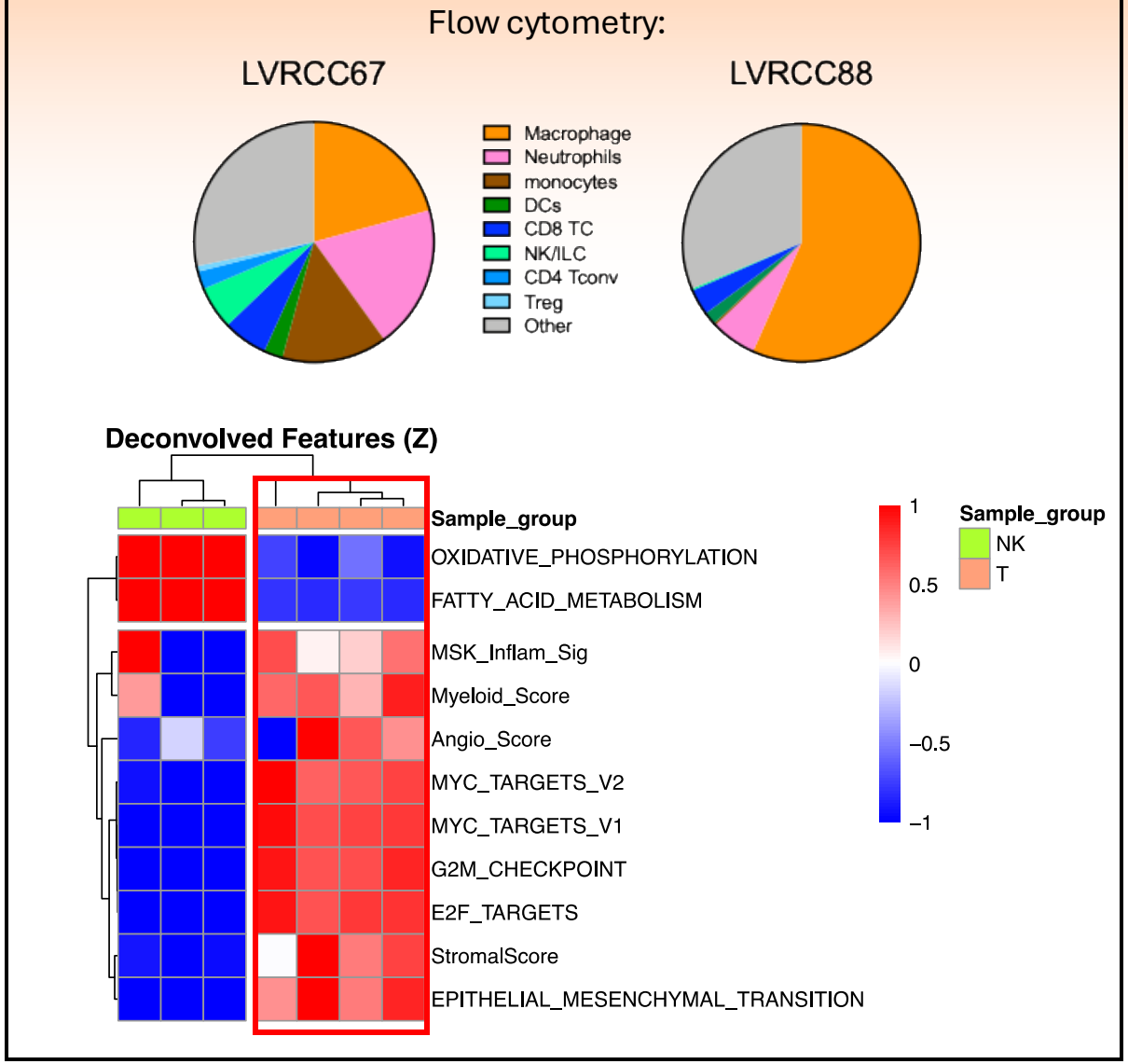
Tumors resemble human stromal/proliferative ccRCC molecular subtype

IMMOTION151 molecular subtypes



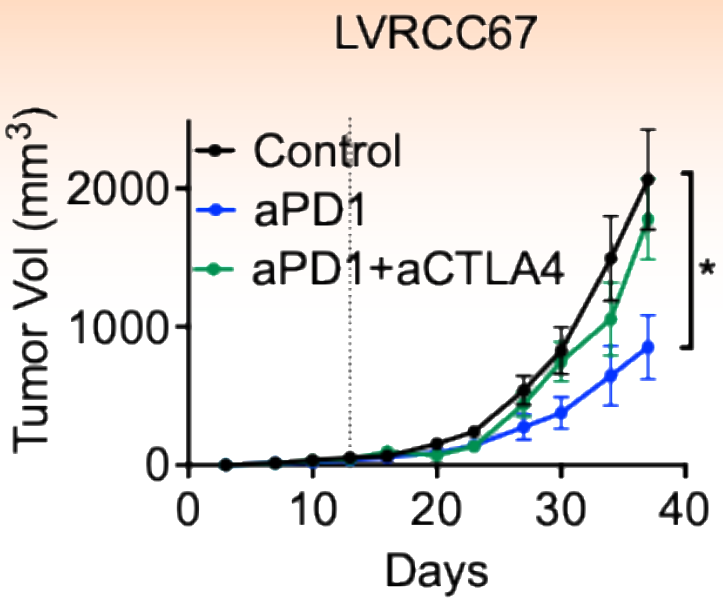
Motzer et al 2020 Cancer Cell, Motzer et al 2022 JAMA Oncol

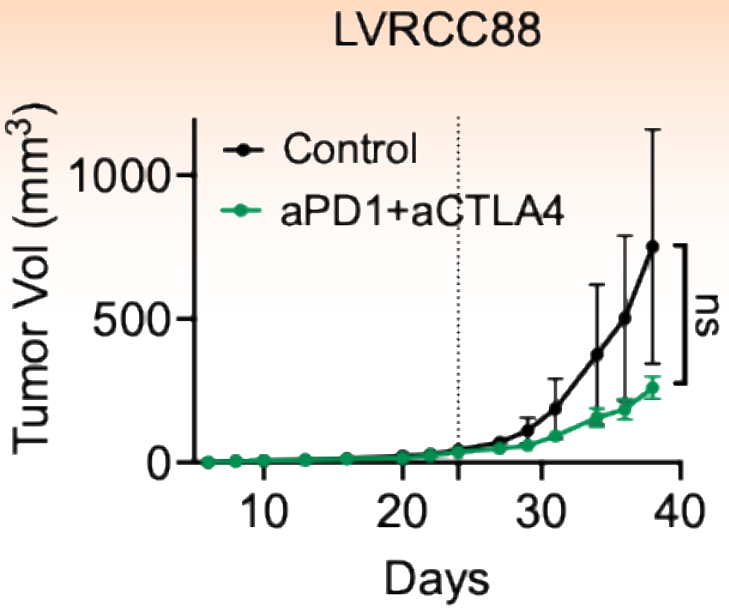
LVRCC67 syngeneic model

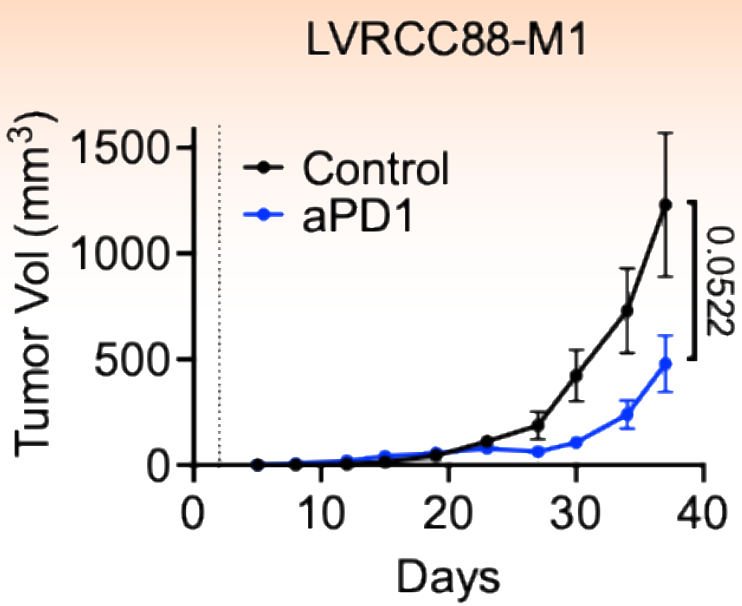


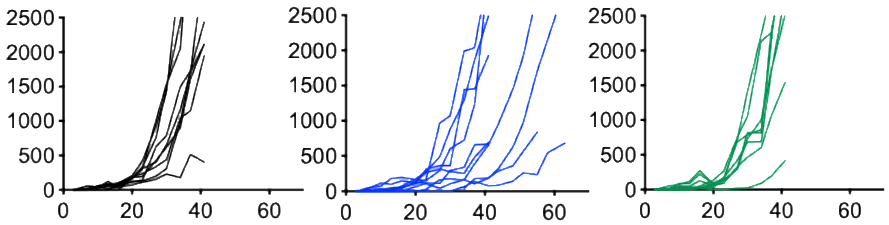
Rappold & Vuong et al 2022 Cancer Discovery

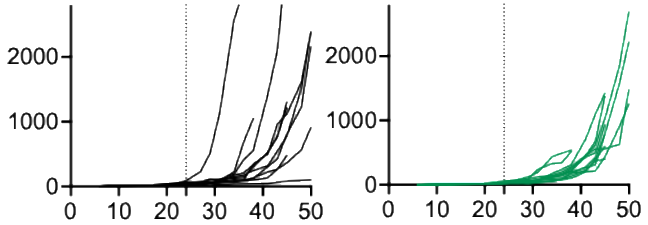
Response to immune checkpoint inhibitors

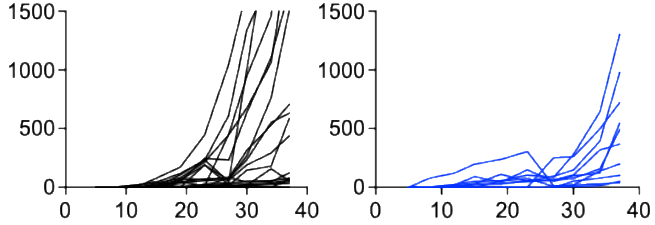










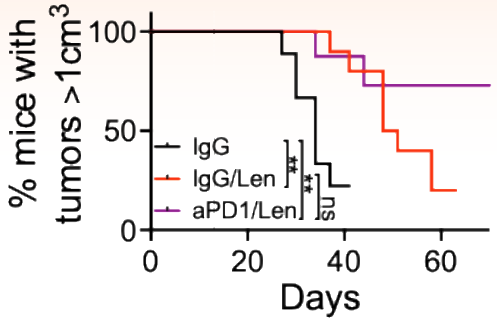


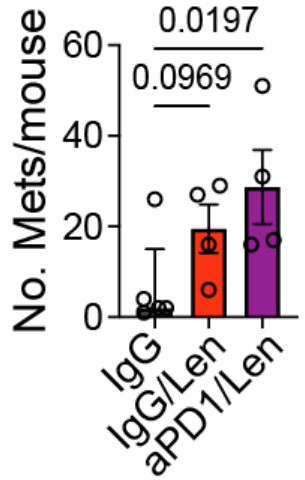
Variable and heterogeneous response across experiments

Response to VEGFR TKI Lenvatinib

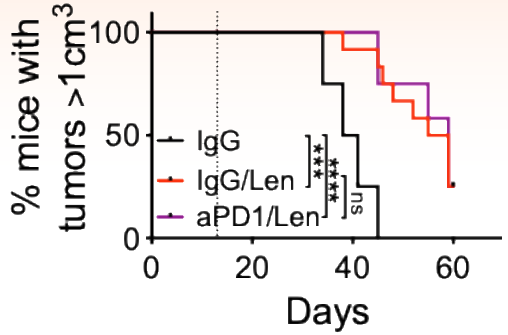
Subcutaneous

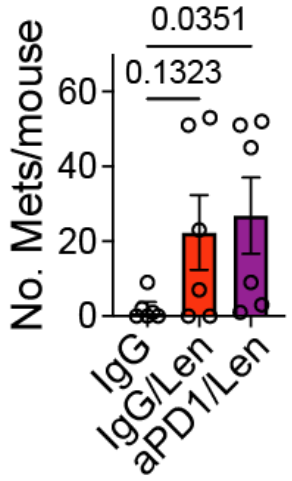
LVRCC67





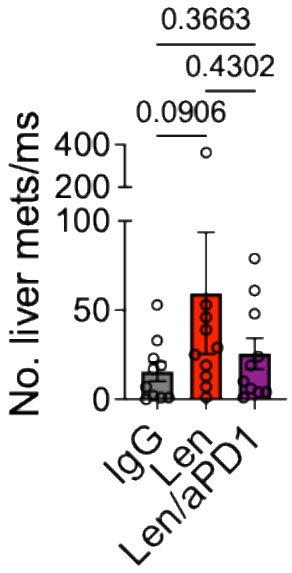
LVRCC88





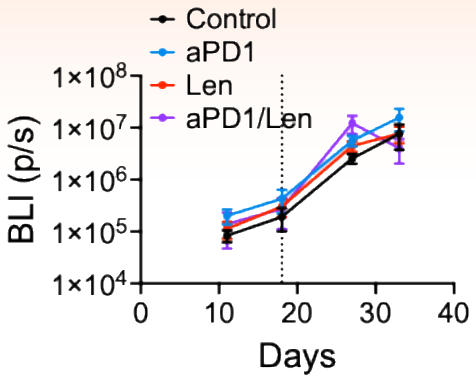
Intravenous

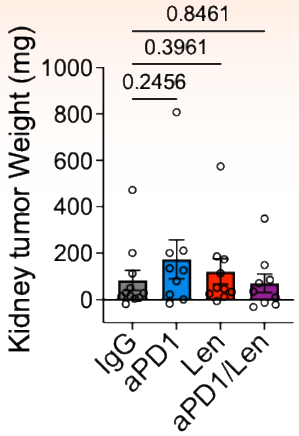
LVRCC67-M2

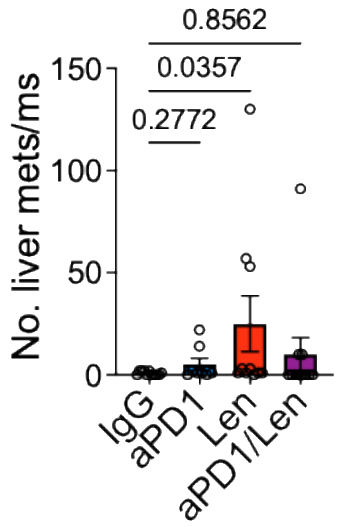


Orthotopic

LVRCC67-M (luciferase)







- Luciferase/GFP immune rejection
- Tdt better than zsgreen/GFP (.D Schoenfeld)
- Tumor calcification: unreliable BLI, ultrasound, weight
- Peritoneal & gastric mets- injection leakage?

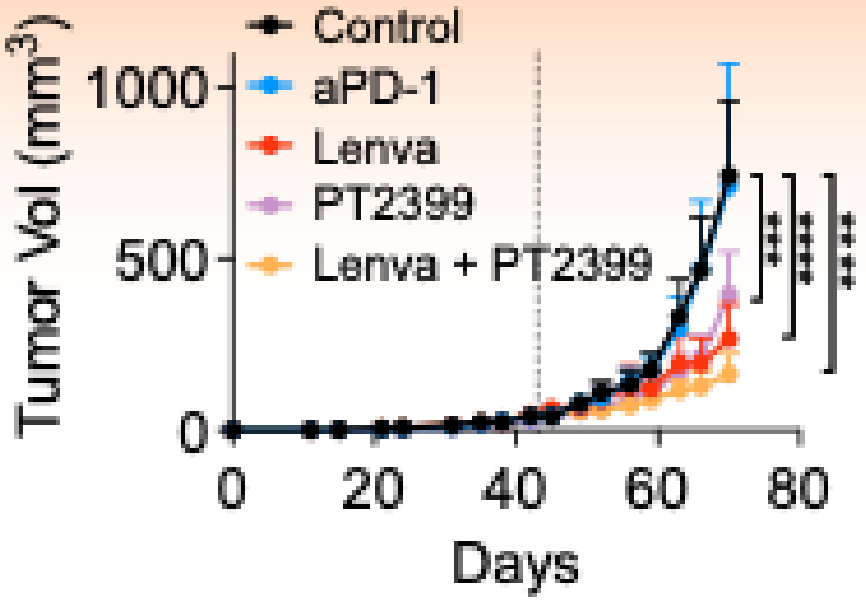
Primary tumors

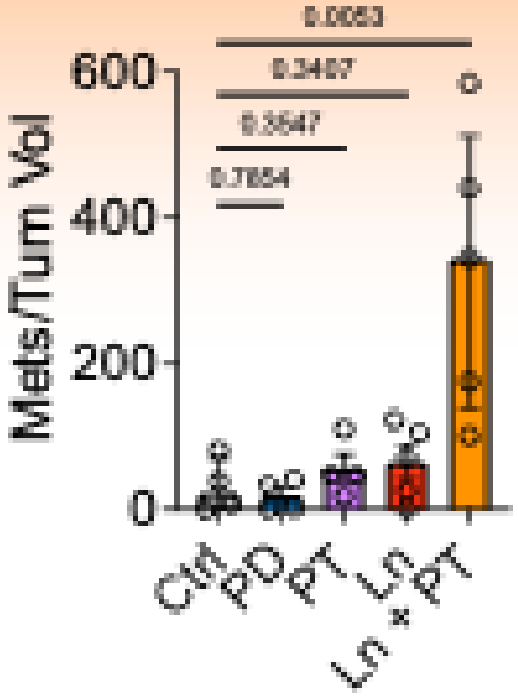
Metastases

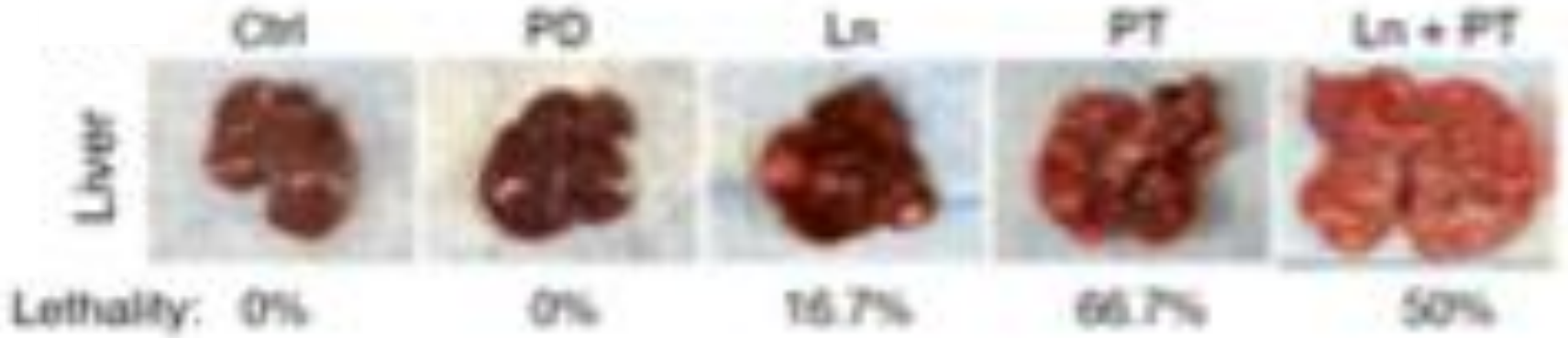
Unpublished, PLEASE DO NOT POST

LVRCC88-M1 OT (TBD)

Response to HIF2a inhibitor PT2399







Summary

- Failed to create bonafide EPO-GEMM
- 2 ccRCC syngeneic lines created – LVRCC67 sent to 54 labs globally!

Pros

- High take-rate, rapid (no breeding), metastatic, immunocompetent
- Vhl loss – response to VEGFR TKIs
- Resembles aggressive treatment-refractory myeloid dominant ccRCC – study treatment resistance

Cons

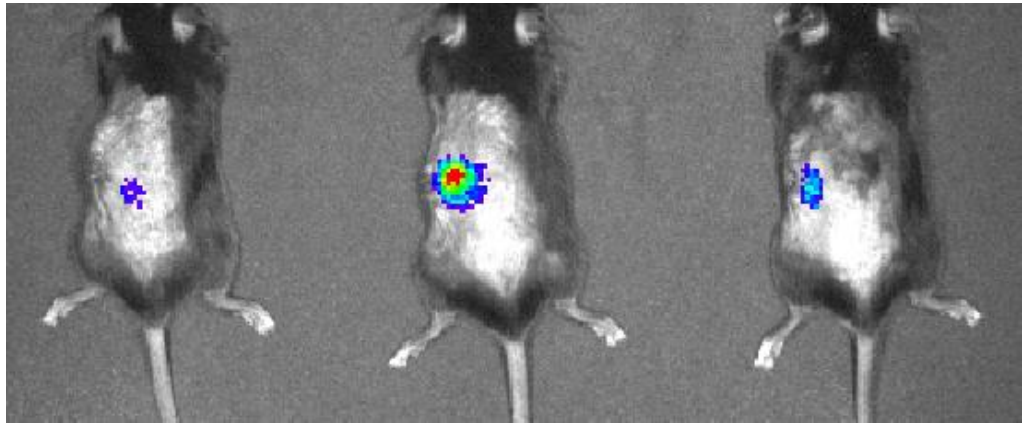
- Dedifferentiated histology, Rb1 loss not typical
- Heterogeneous growth and response
- Inconsistent/mostly poor response to ICB
- Real time tracking of orthotopic tumors challenging
- Difficult to process tumors due to calcifications

- Sensitive to inbred strain rejection- need to be grown in JAX lab B6 mice
- Do not grow well in females

Electroporation is variable, inefficient and damages kidney

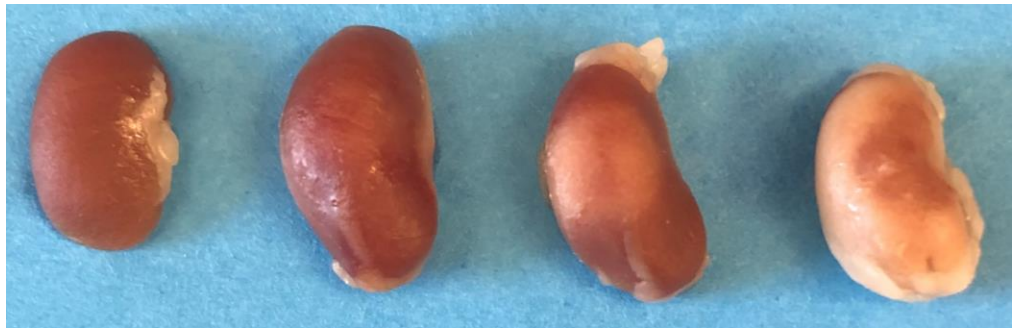
Original KVPR GEMM: ~87% penetrance at 25-40 wks
Reasons for EPO-GEMM failure?

Bioluminescence 24h post-EP:



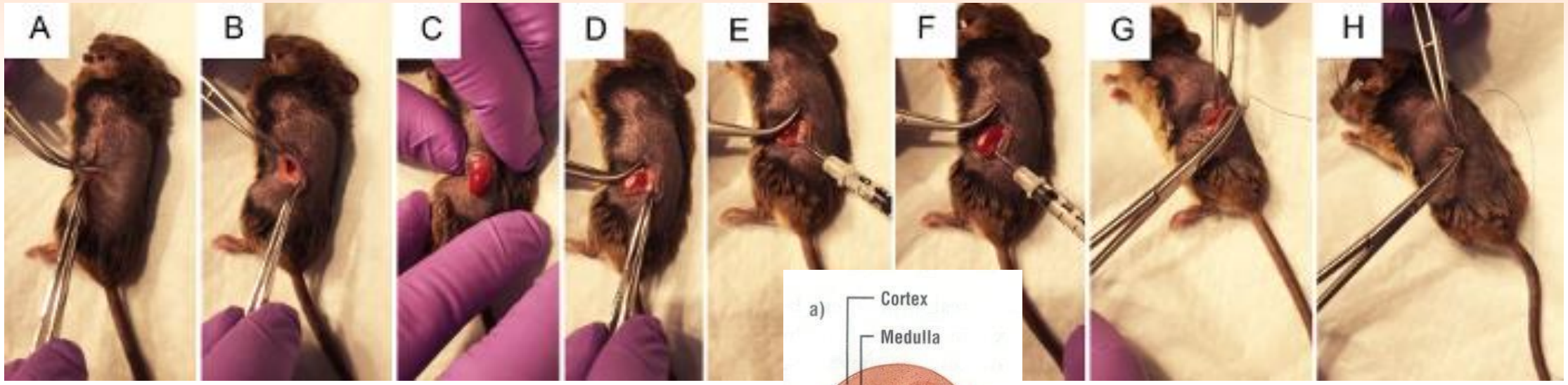
Damage 24h post-EP:

Normal Low Moderate High

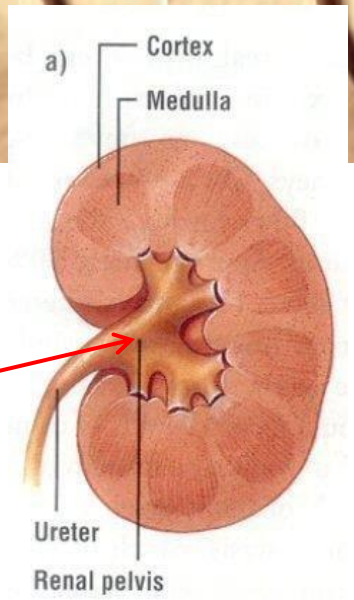


Max few hundred cells survive long term (2-7 days) post-EP
(Maresch et al 2016 Nat Comm)

Hydrodynamic Renal Pelvis Injections as an alternative transgene delivery route?



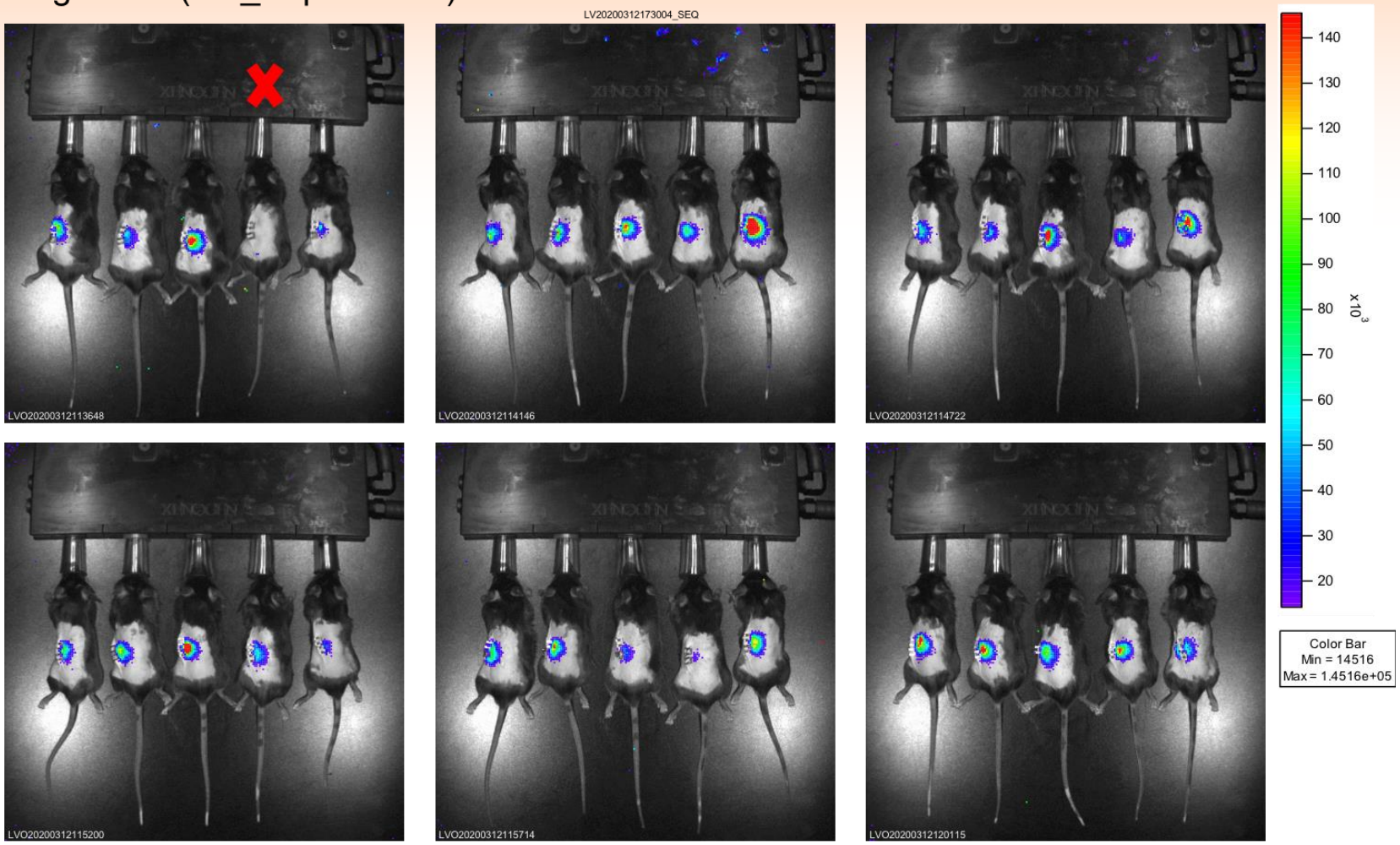
Rapid < 3secs injection of 100 ul plasmid into renal pelvis



Woodard LE et al J Vis Exp 2018

Hydrodynamic Renal Pelvis Injections as an alternative transgene delivery route?

Cage 7-12 (Vhl_Bap1 + HLZ)



Less technical error prone.
Kidneys macroscopically normal, histologically no necrosis, no inflammation, calyxes expanded in some but no visible damage around them.

Hydrodynamic Renal Pelvis Injections as an alternative transgene delivery route?

Genes targeted	n	tumors	cysts	hydronephrosis
Vhl Rb1 P53	28	0	0	10
Vhl Bap1	29	0	0	12
Vhl Bap1 Cdkn2a/Arf Cdkn2b	30	0	0	11
Vhl Pbrm1 Setd2	29	0	0	9
Luciferase only control	5	0	0	1

Followed for ~2 years

Work in progress: Transposon-mediated sgRNA genome integration

- Breeding Ksp-cre LSL-cas9 mice or H11-cas9 mice
- Cloned sgRNAs into sleeping beauty transposon system
- VPRM as positive control
- VBCC as test

Acknowledgements

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Ming Li lab

Jing Zhang

David Solit lab

Aphrothiti Hanharan

Scott Lowe lab

Josef Liebold

Pathology

Yingbei Chen

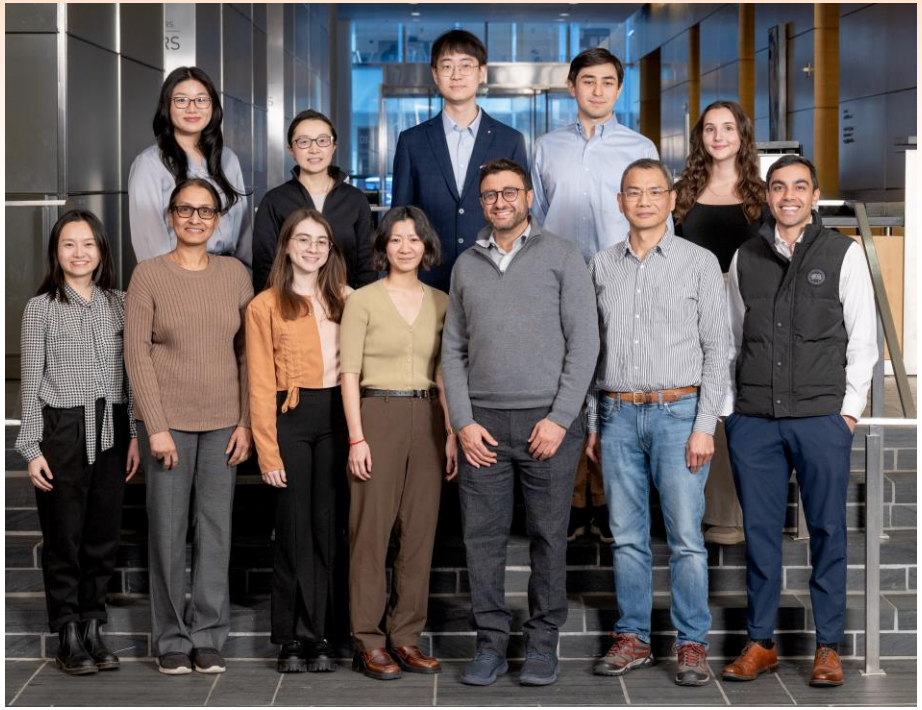
MSKCC core facilities

Small Animal Imaging

Flow Cytometry

Molecular Cytology

All the mice!



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