Animal Model Workshop

Organoid Models of Renal Cancer: From Culture Optimization to Therapeutic Insights

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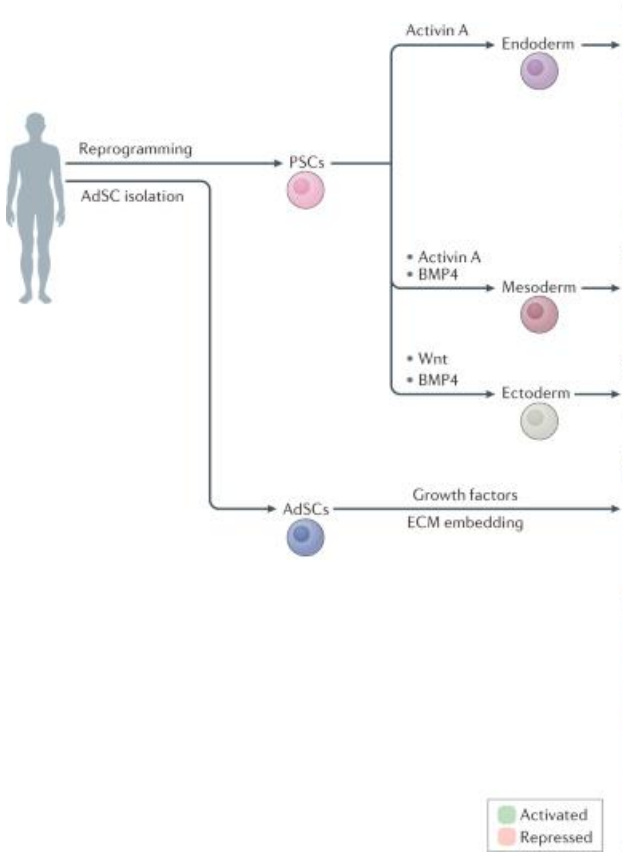
September 11-13, 2025

Organoid Models of Renal Cancer: From Culture Optimization to Therapeutic Insights

- Introduction
- Characterizing and targeting a stem cell phenotype in RCC PDO
- Challenges in establishing RCC PDO
    - How can we improve efficiency in PDO establishement
    - What clones are selected in culture?
    - How can we model early tumorigenesis and tumor evolution?
    - How can we incorporate the immune system?
- Conclusions

What are organoids?



Kim, J., et al. *Nature Reviews Molecular Cell Biology* **21**, 571-584 (2020).

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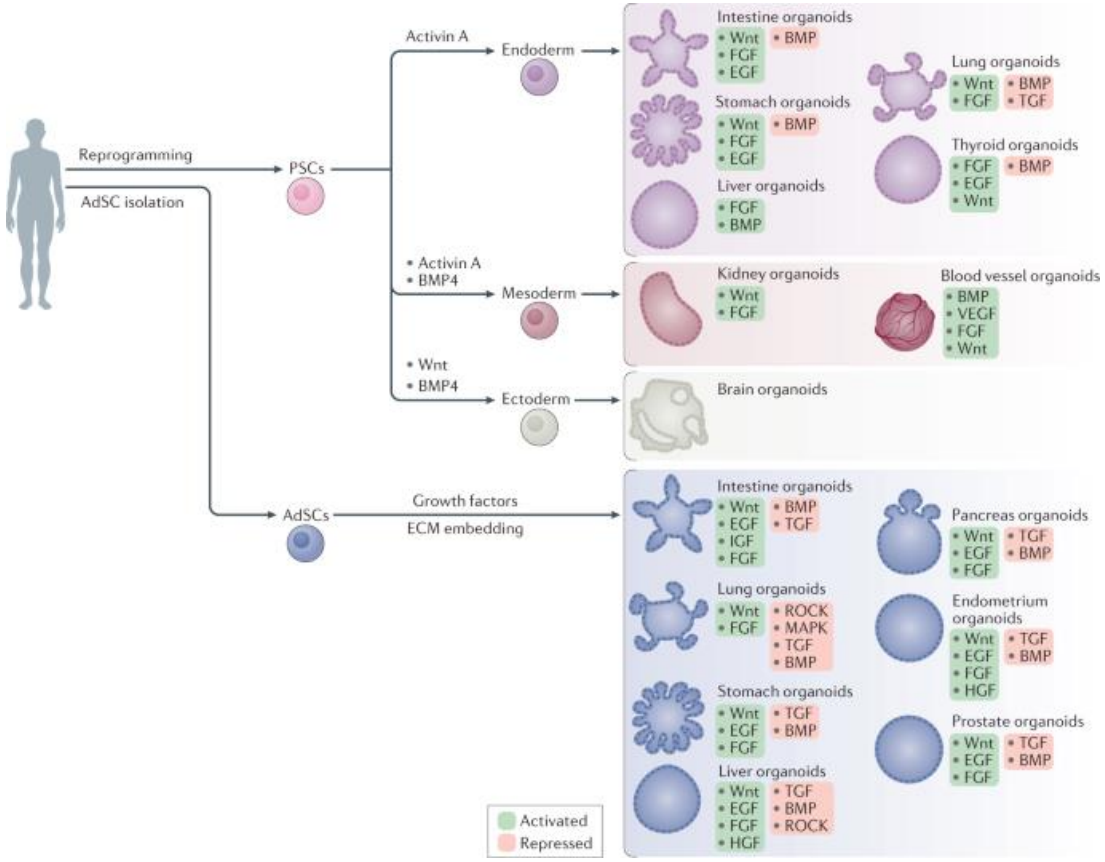

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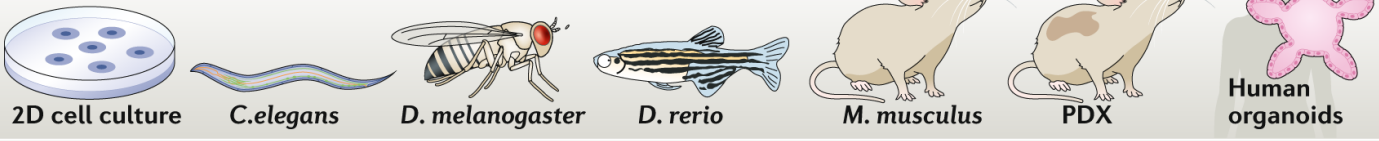
How do PDO compare to other model systems

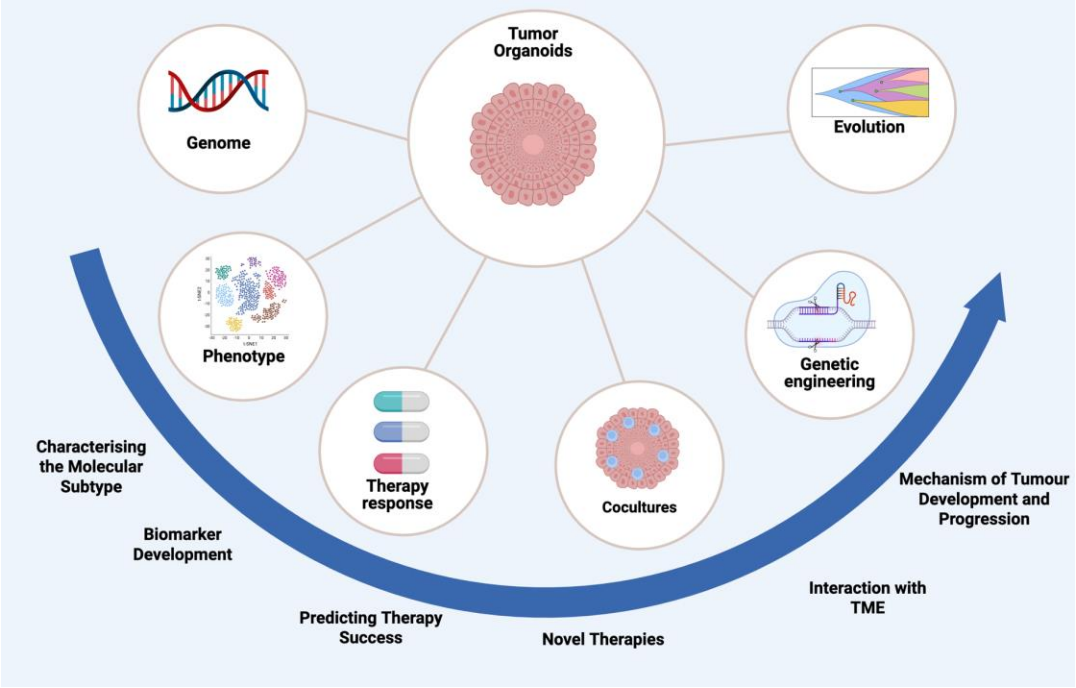


	2D cell culture	*C.elegans*	*D. melanogaster*	*D. rerio*	*M. musculus*	PDX	Human organoids
Ease of establishing system	✓/✗	✓	✓	✓	✓	✓	✓
Ease of maintenance	✓	✓	✓	✓	✓	✓	✓
Recapitulation of developmental biology	✗	✓	✓	✓	✓	✗	✓
Duration of experiments	✓	✓	✓	✓	✓	✓	✓
Genetic manipulation	✓	✓	✓	✓	✓	✗	✓
Genome-wide screening	✓	✓	✓	✓	✗	✗	✓
Physiological complexity	✗	✓	✓	✓	✓	✓	✓
Relative cost	✓	✓	✓	✓	✓	✓	✓
Recapitulation of human physiology	✓	✓	✓	✓	✓	✓	✓

✓ Best ✓ Good ✓ Partly suitable ✗ Not suitable

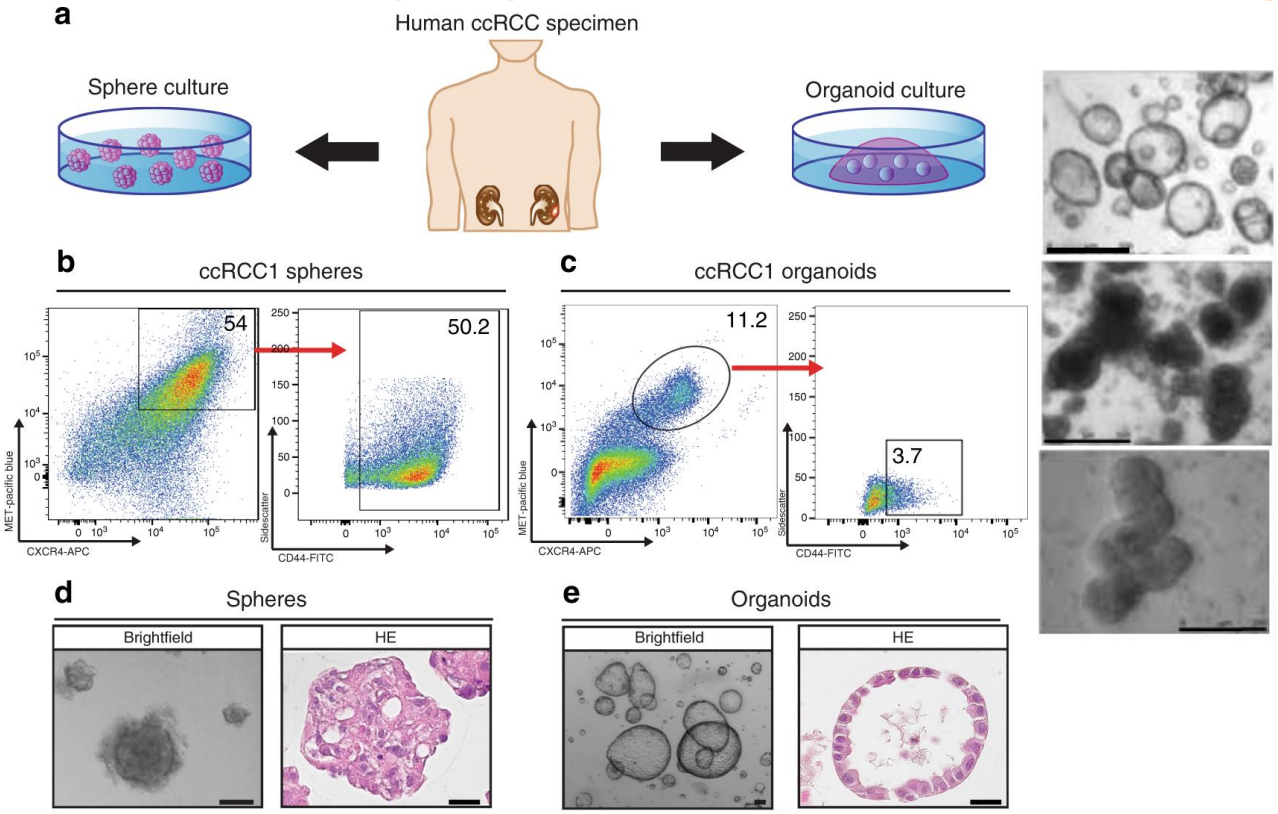
Kim, J., et al. *Nature Reviews Molecular Cell Biology* **21**, 571-584 (2020).

Applications of PDO



Fendler et al. Nature Communications, 2020

RCC cells display differentiation in organoid cultures



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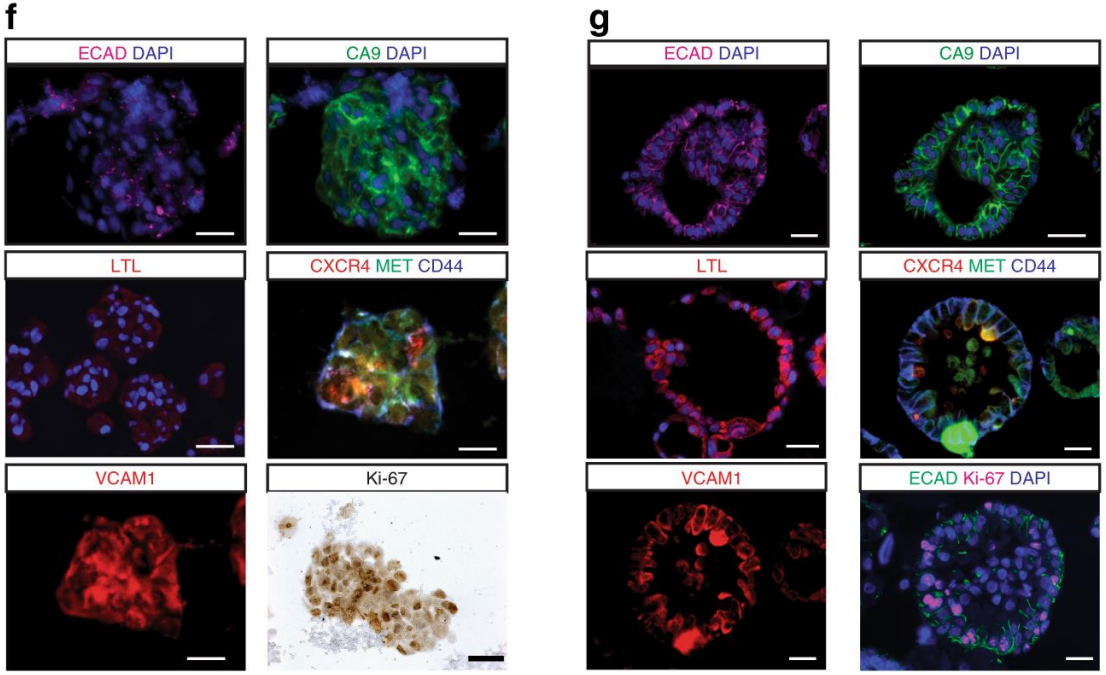

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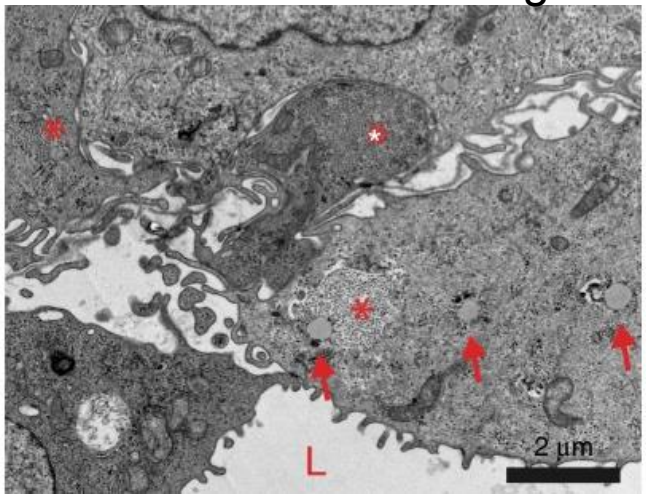
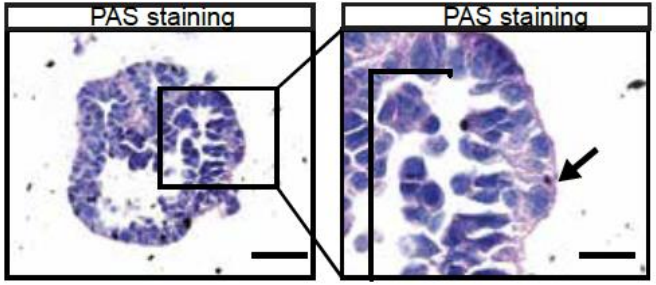
Fendler et al. Nature Communications, 20

RCC cells display differentiation in organoid cultures

Glycogen and lipid deposits indicate metabolic changes





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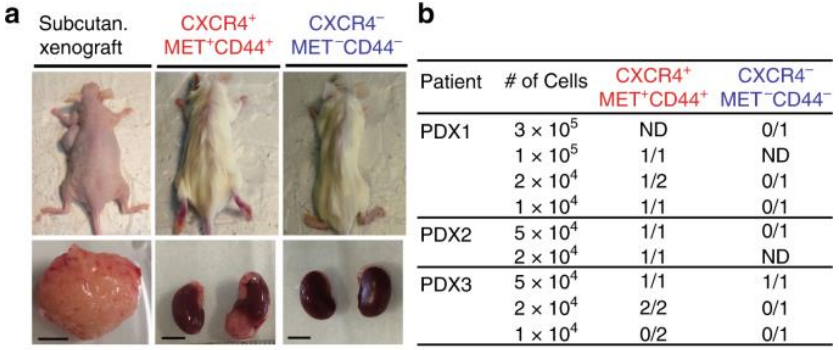
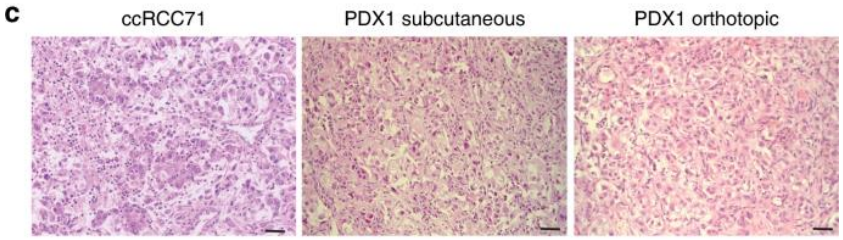
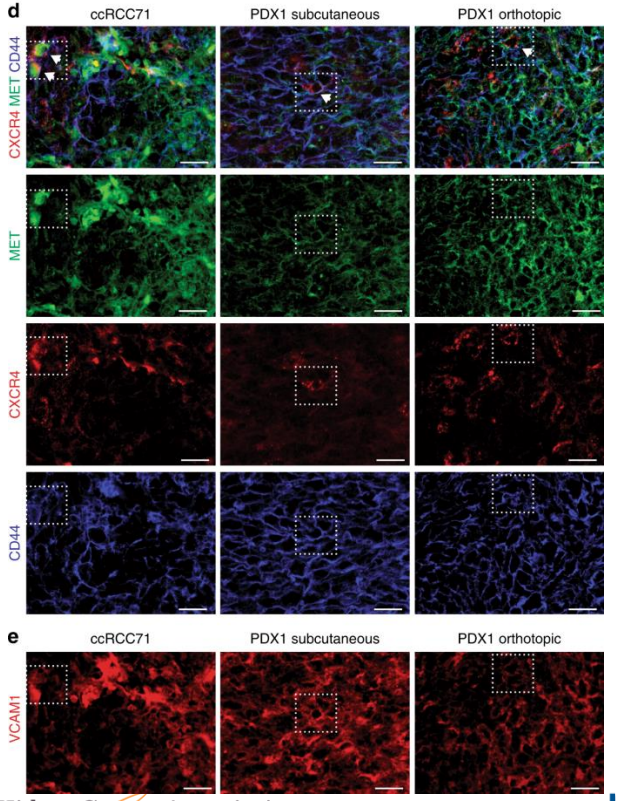
Cells with a regenerative phenotype can reseed tumors in PDX



b

Patient	# of Cells	CXCR4+ MET+CD44+	CXCR4− MET−CD44−
PDX1	3×10^5	ND	0/1
	1×10^5	1/1	ND
	2×10^4	1/2	0/1
	1×10^4	1/1	0/1
PDX2	5×10^4	1/1	0/1
	2×10^4	1/1	ND
PDX3	5×10^4	1/1	1/1
	2×10^4	2/2	0/1
	1×10^4	0/2	0/1





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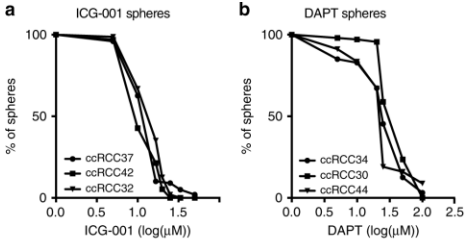
Personalised treatment response in organoids

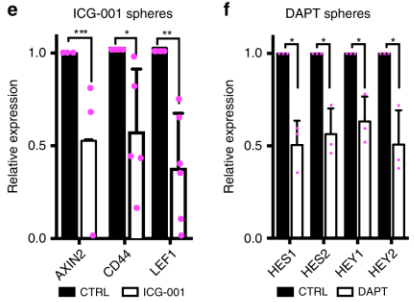


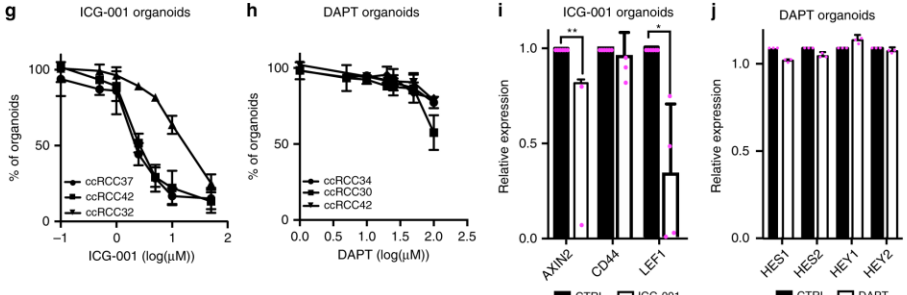
c

		DAPT		
		Responders	Non-responders	
ICG-001	Responders	19 (46%)	10 (24%)	29 (71%)
	Non-responders	7 (17%)	5 (12%)	12 (29%)
		26 (63%)	15 (37%)	41 (100%)

d

		% of spheres	Range (min–max)
Responders	Ctrl	100	98.0–102.0
	ICG-001	42.30	29.4–52.9
	DAPT	51.02	46.2–57.5
	ICG-001 + DAPT	9.55	0.0–25.4
Non-responders	Ctrl	100.00	95.6–104.1
	ICG-001	88.82	75.2–103.4
	DAPT	102.04	88.1–110.1
	ICG-001 + DAPT	70.42	42.0–88.1





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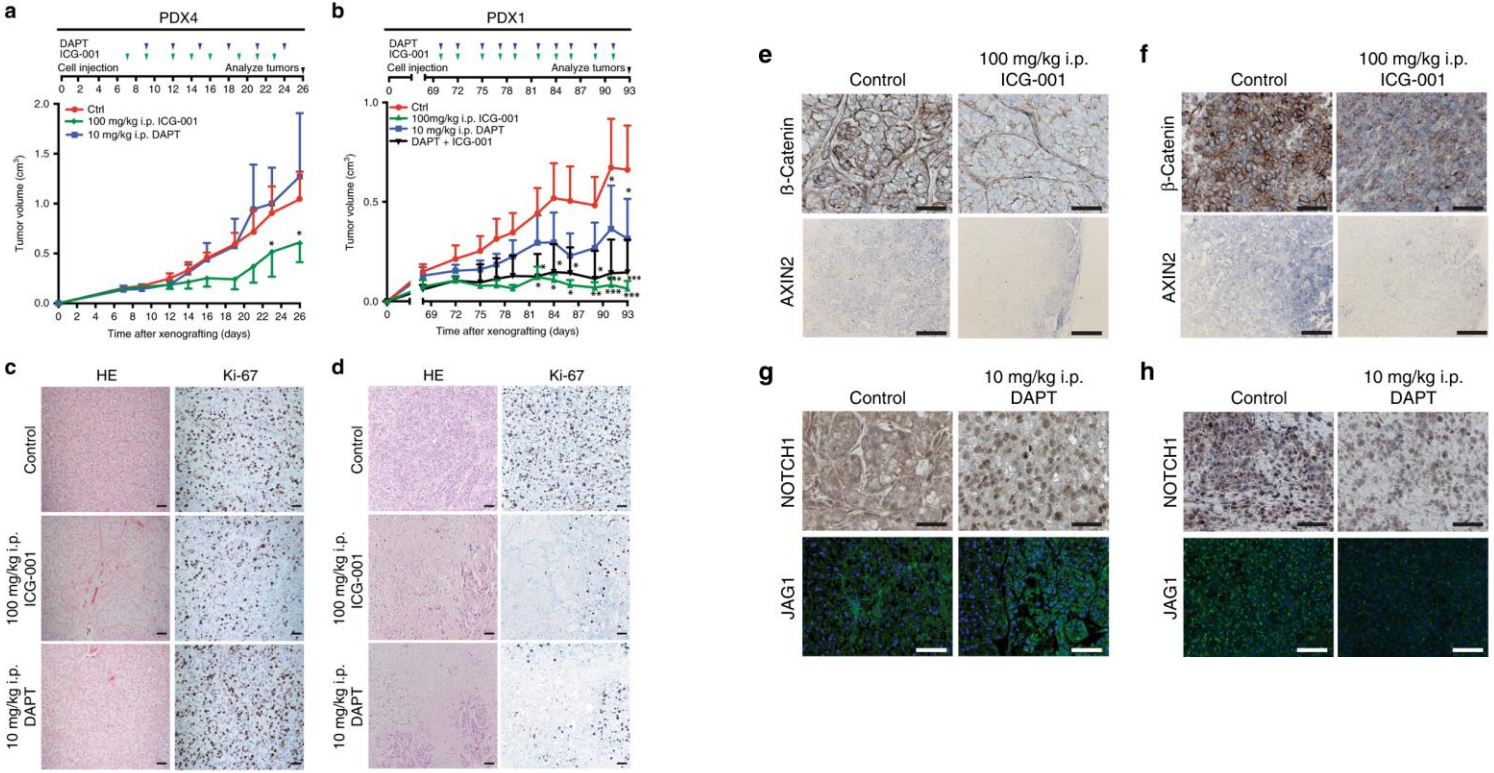
Outcomes in organoids reflect outcomes in PDX



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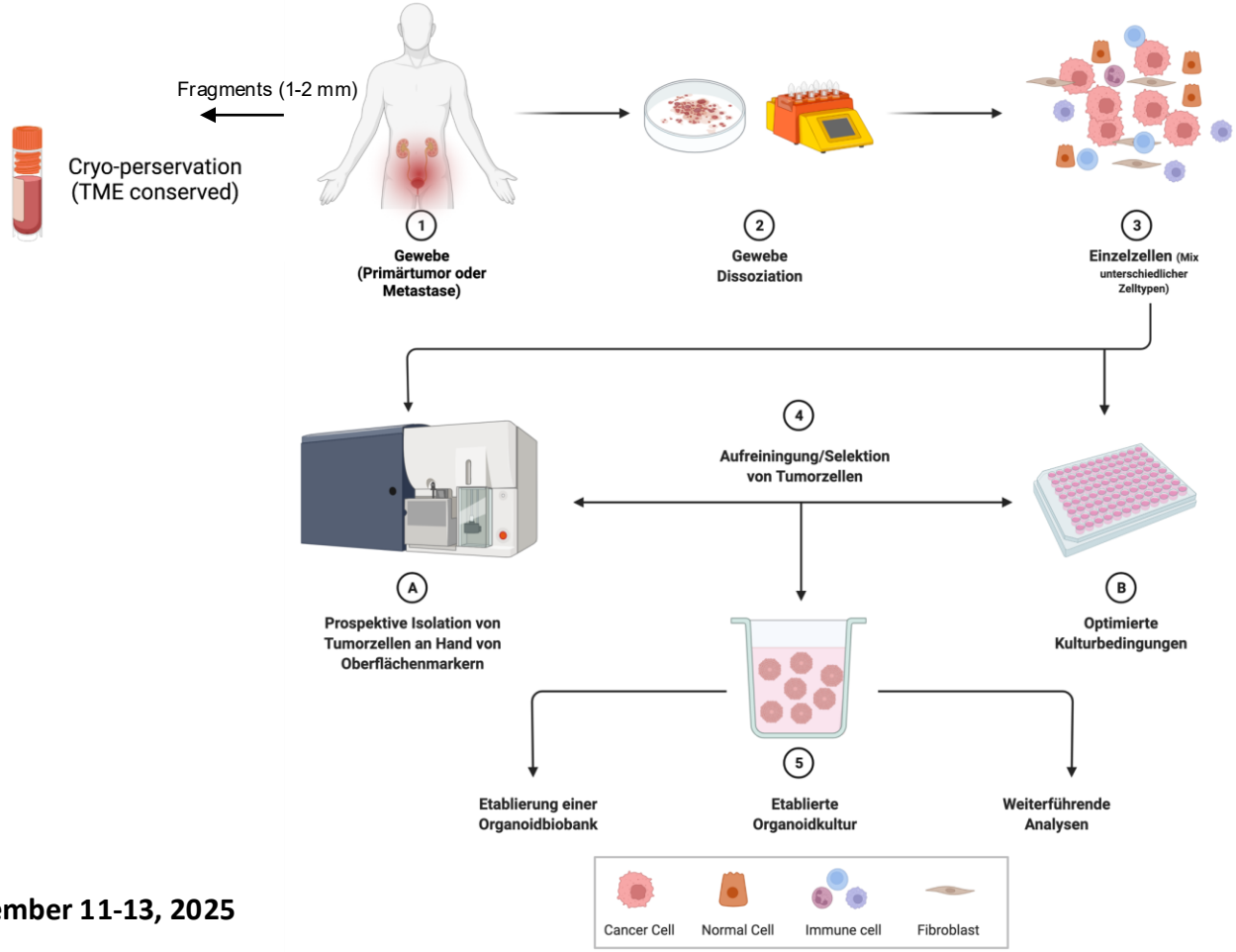
What are the limitations for kidney PDO

1. How can we improve efficiency in PDO establishement
2. What clones are selected in culture?
3. How can we model early tumorigenesis and tumor evolution?
4. How can we incorporate the immune system?

Workflow to establish patient-derived RCC organoids



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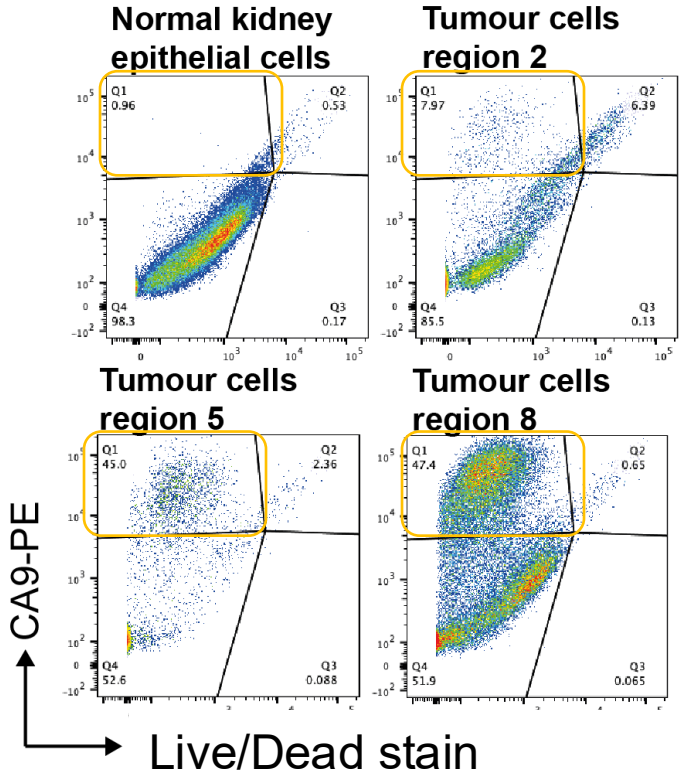
1. Efficiency of PDO formation

Proportion of tumor cells is variable in different biopsies

- some biopsies have low tumor cell content
- failure to grow
- risk of other cell types outgrowing the tumor cells

Solution:

- Depletion of other cell types
- Positive selection of tumor cells



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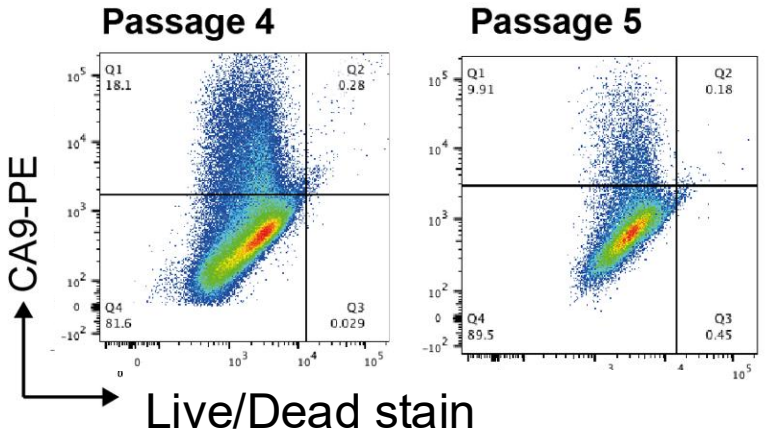
1. Efficiency of PDO formation

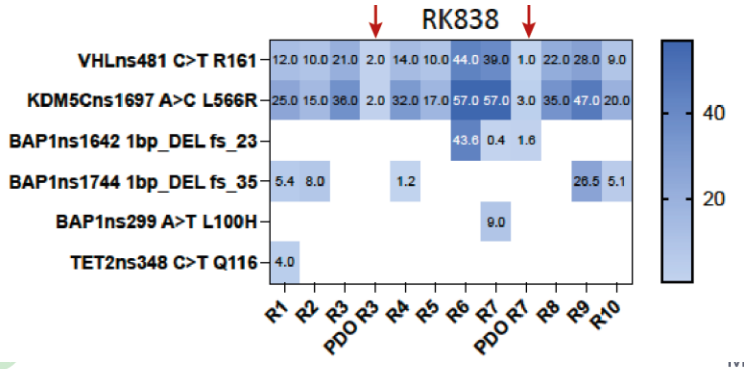
Tumour cells get depleted over time

- The proportion of tumor cells decreases with each passage
- Genomic alterations are representative of primary region but at lower AF

Solution:

- Depletion of other cell types
- Positive selection of tumour cells
- Improve culture conditions





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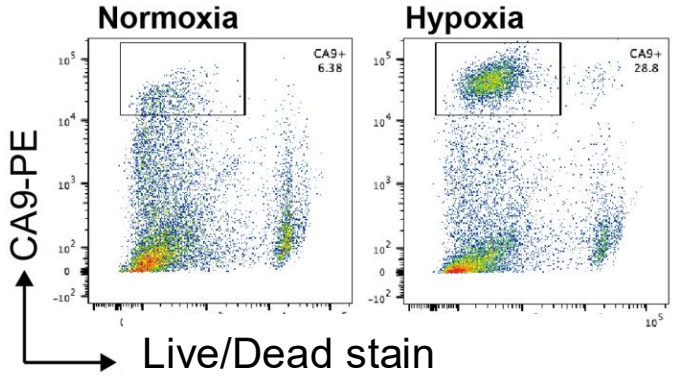
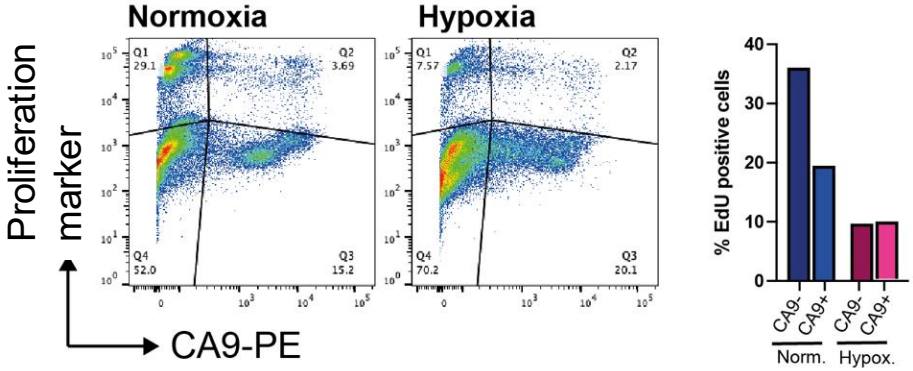
1. Efficiency of PDO formation

RCC tumor cell proliferation can be maintained in hypoxic growth conditions

No depletion of tumor cells in hypoxia



Proliferation changes in favor of tumor cells



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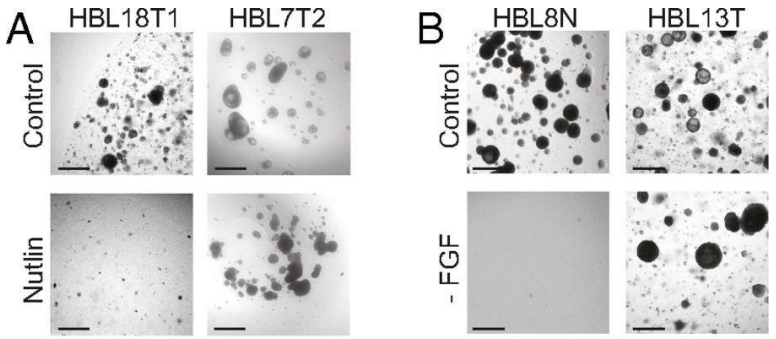
1. Efficiency of PDO formation

Optimize growth conditions

- Add growth factors
- Withdraw growth factors to enrich for specific driver genes

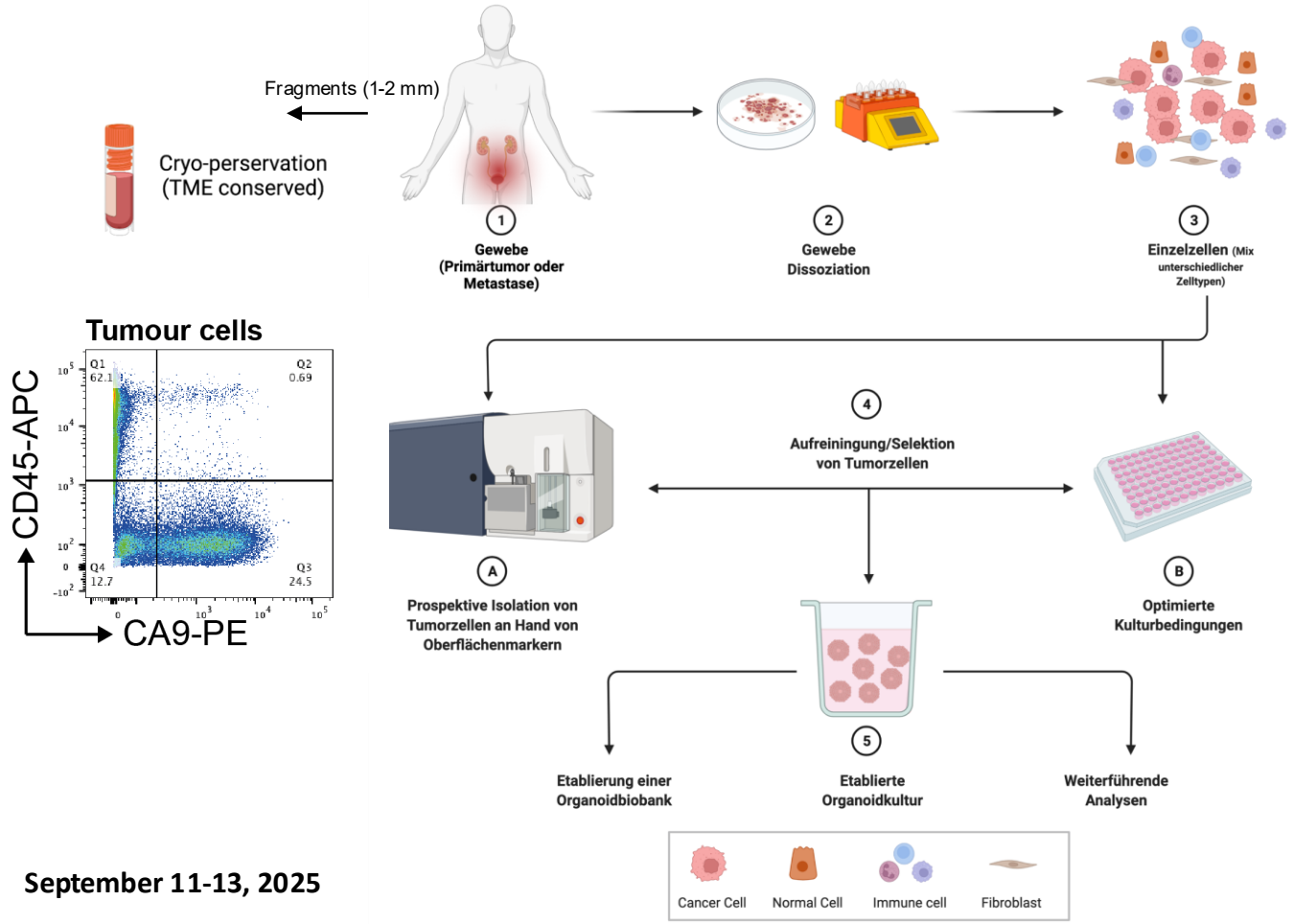
But

- Challenge to identify growth factors with consistent growth advantaged
- Need for prior characterization if patient specific



Mullenders, J. *et al*. *PNAS* **116**, 4567-4574 (2019).

Workflow to establish patient-derived RCC organoids



→ Hypoxia

→ Additional growth factors?
Specific for molecular subtype?

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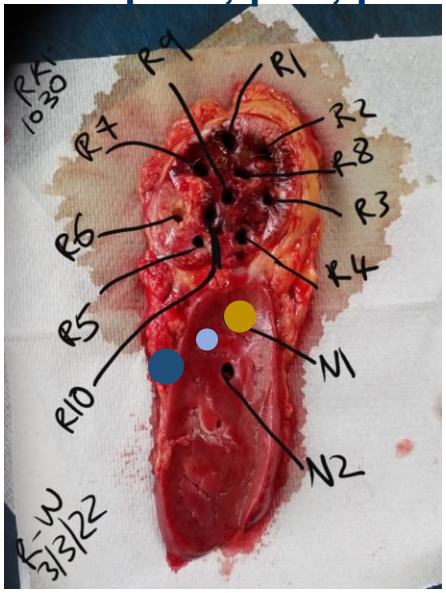
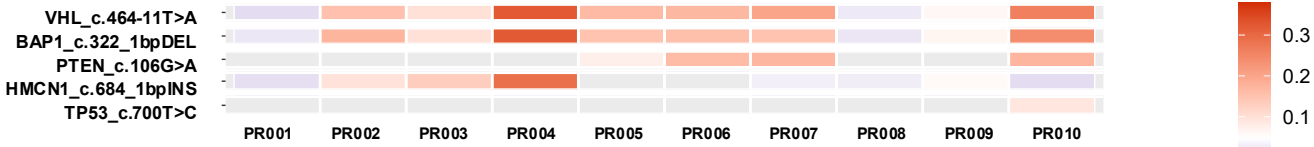
2. What clones are selected in culture?

G4 pT3a; pNx; pMx



VHL, BAP1 clonal mutations



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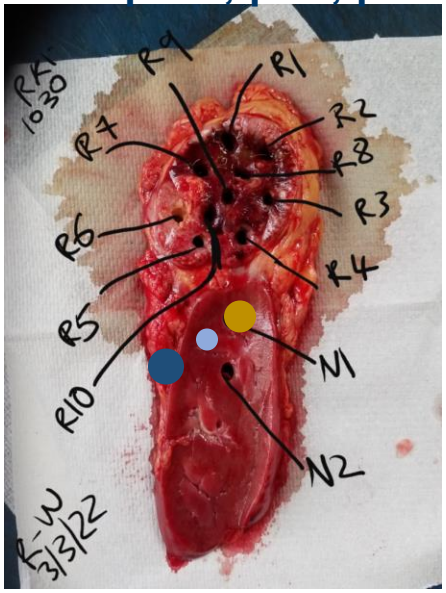

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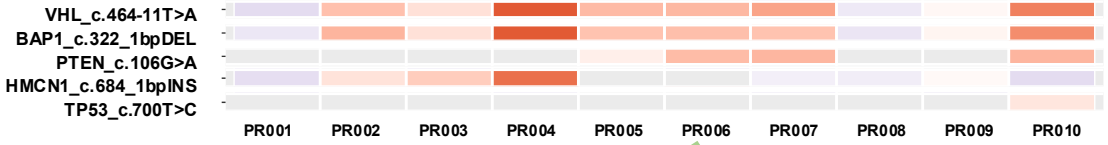

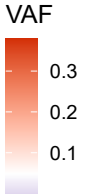

	GB1		GB2	
	Passage 3	Passage 9	Passage 10	Passage 13
VHL	0.98	0.98	0.99	0.97
BAP1	0.96	0.95	0.92	0.95
PTEN	0.97	0.99	0	0
HMCN1	0	0	0	0
TP53	0	0	0.24	0.12

present in adjacent PR010

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2. What clones are selected in culture?

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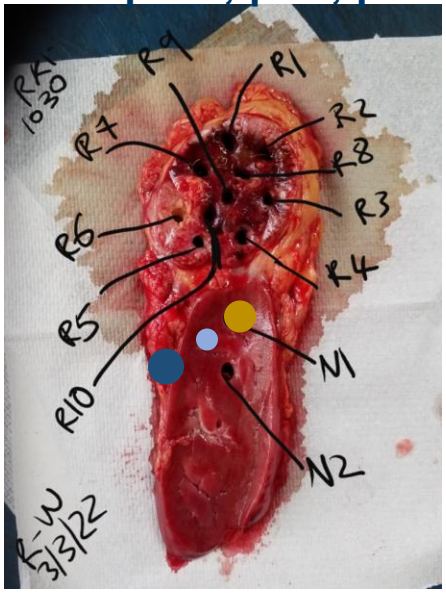

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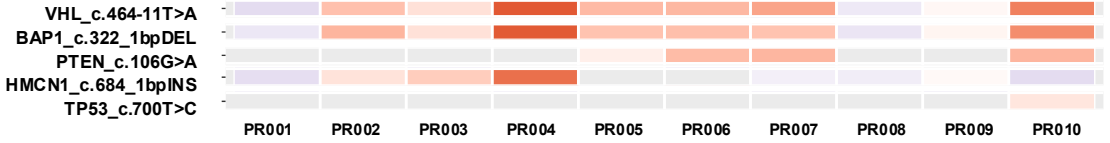

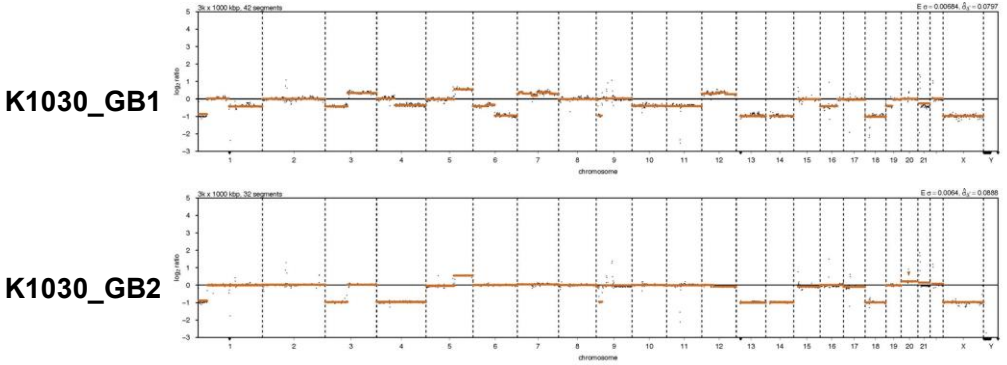

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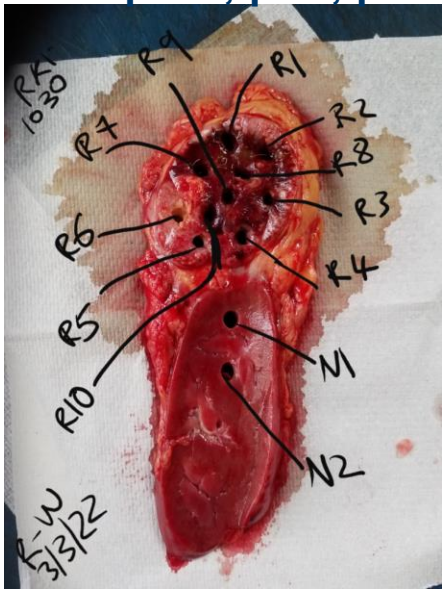

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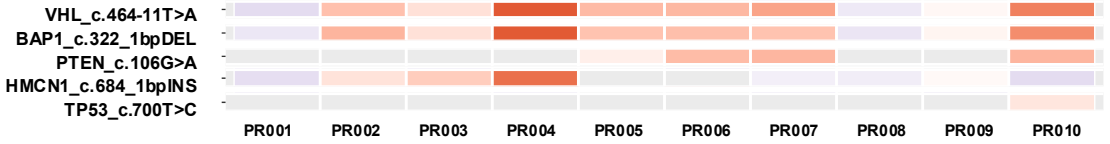

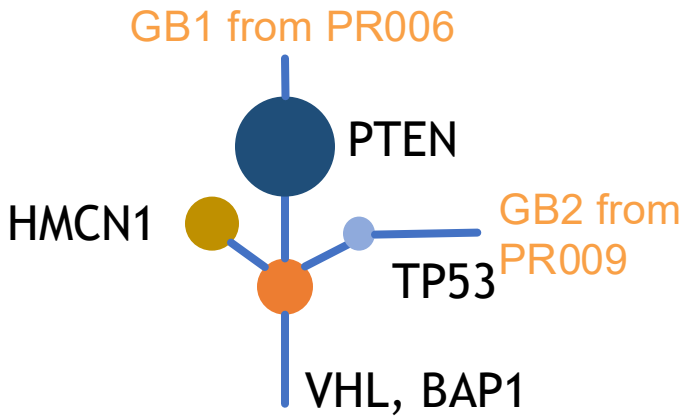

PDO can maintain tumor subcloneswhich are representative of the primary tumor region

BUT: Selective advantage may differ

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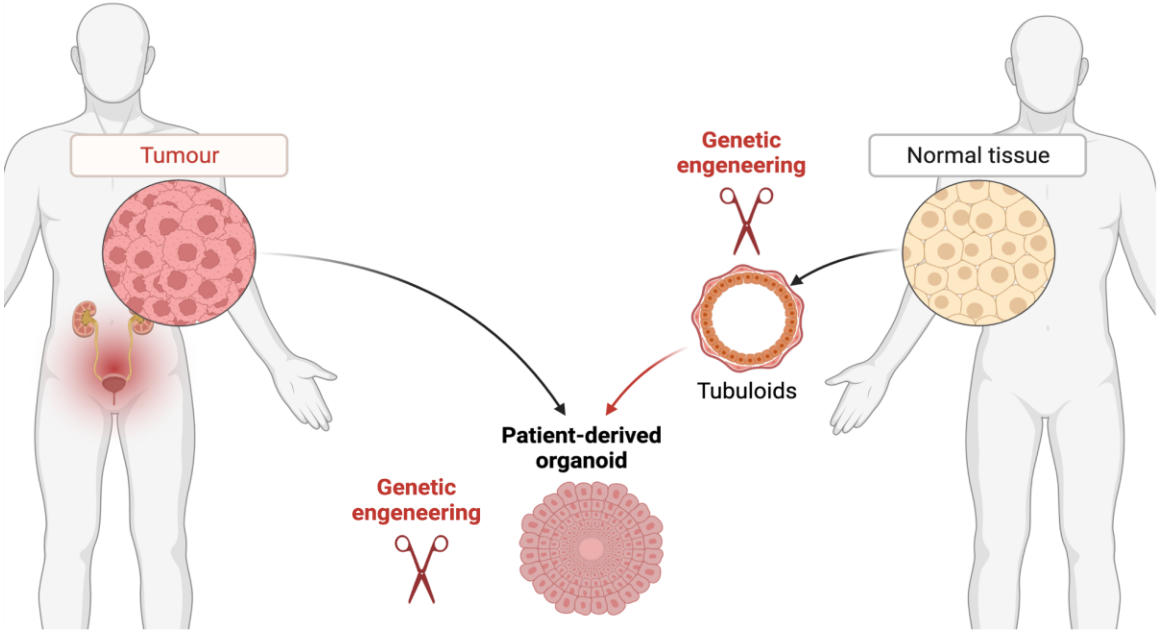
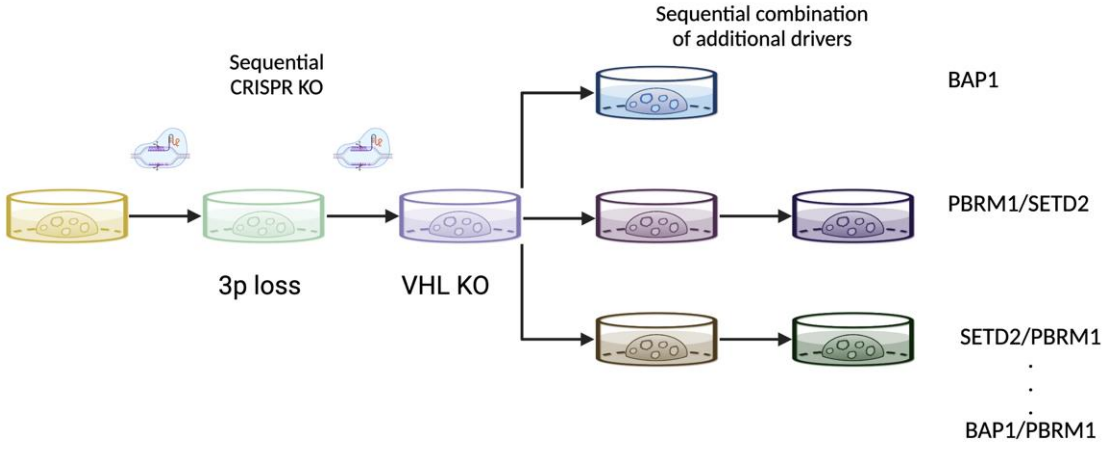
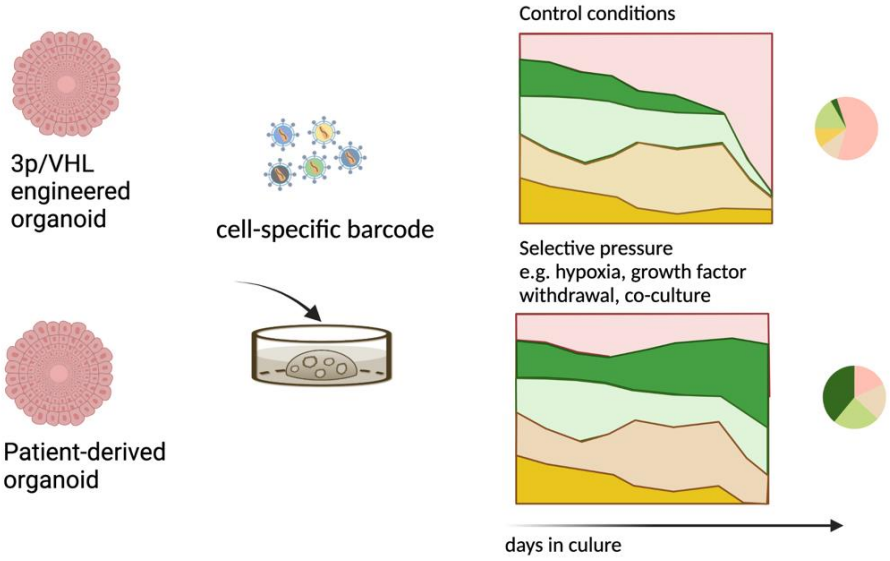
Patient-derived organoid cultures



Evolutionary replay: Understanding early stages of tumorigenesis



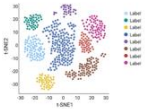
Evolutionary forward play: Understanding how tumours will continue to evolve



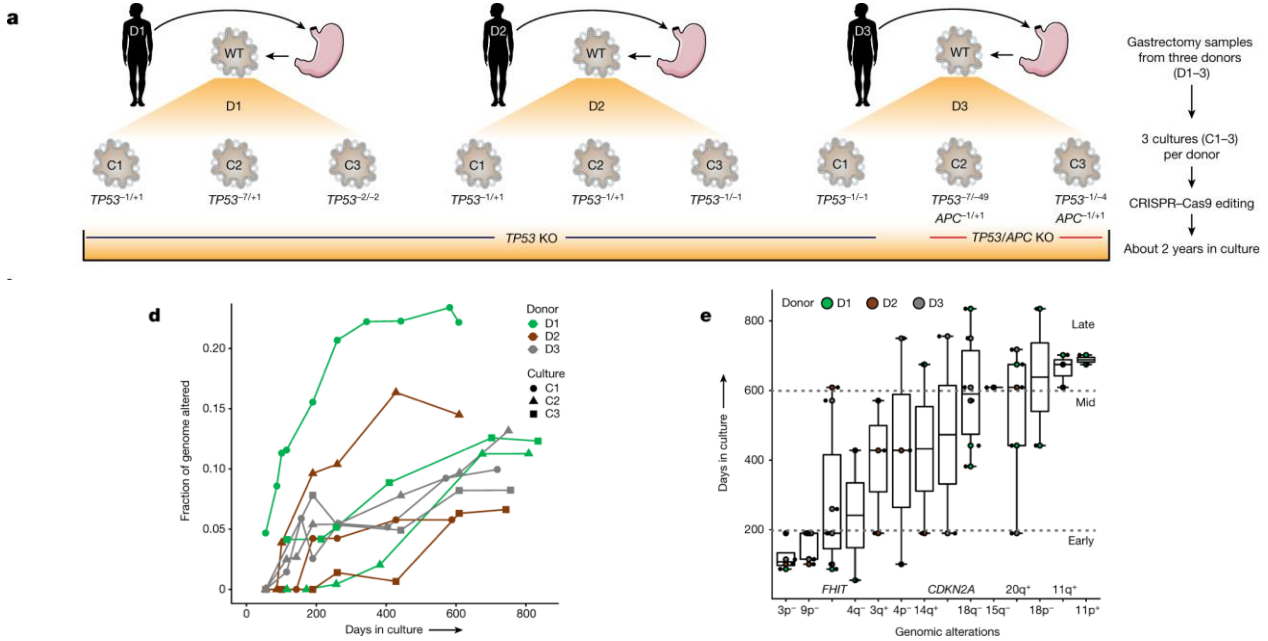

scSeq, shallow whole genome


Population dynamics
Clone dominance
Clone distinction


Genome evolution
Cell lineage


Single cell transcriptomic patterns and trajectories

Gastric organoids acquire copy number alterations over time



Karlsson, K. *et al*. *Nature* **618**, 383-393 (2023).

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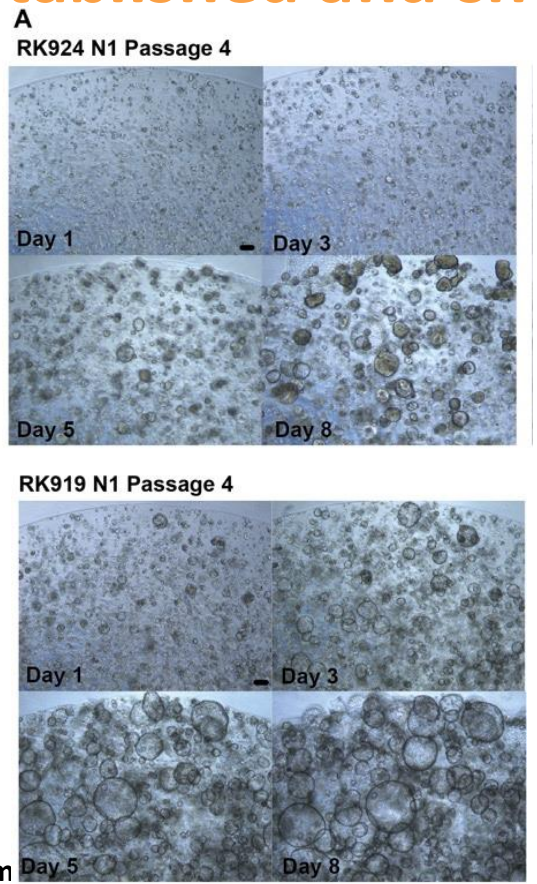
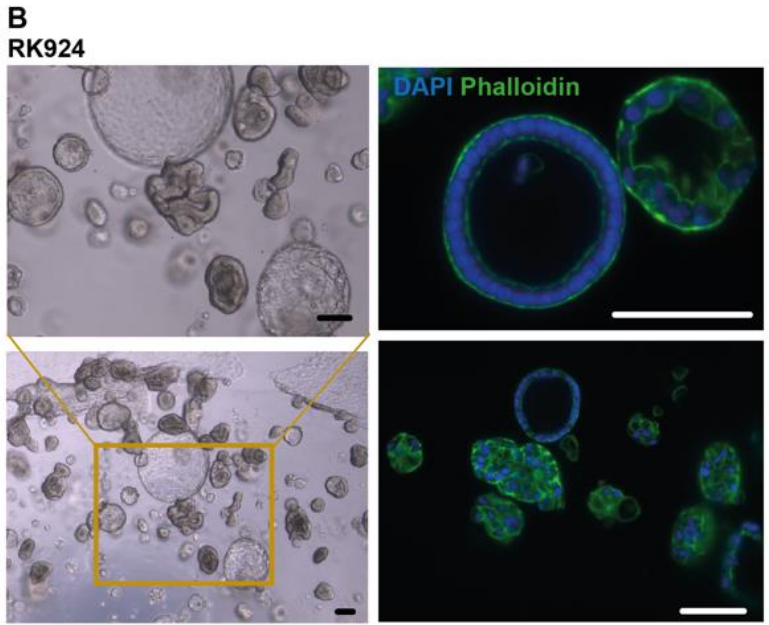
Normal organoid cultures can be successfully established and show heterogeneous growth patterns





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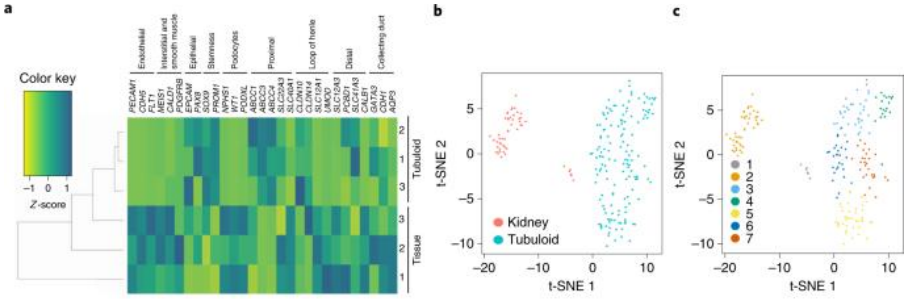
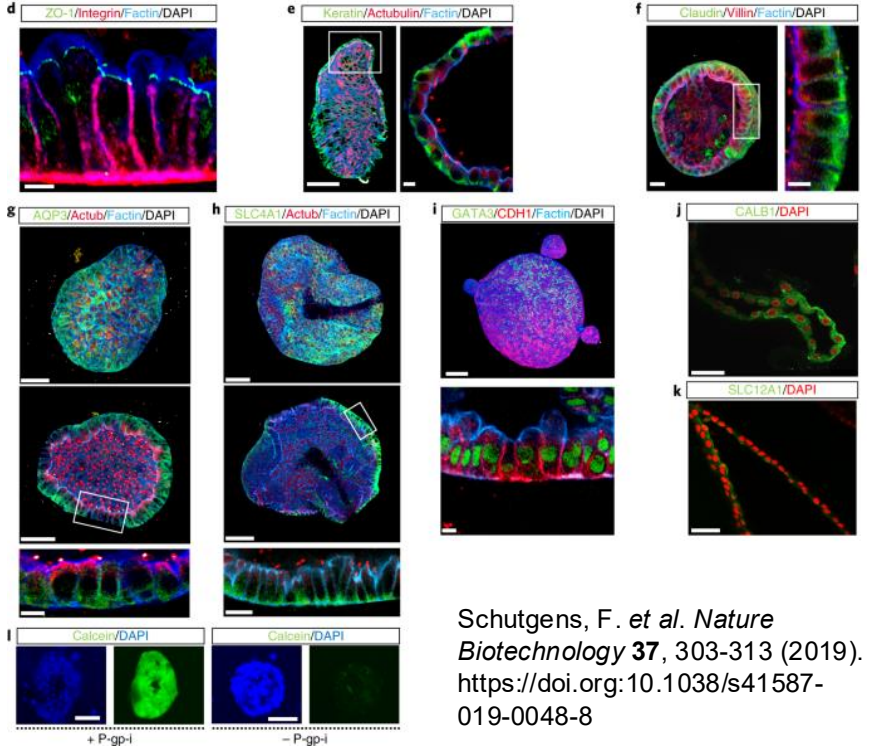
Normal organoid cultures can be successfully established and show heterogeneous growth patterns

- Mainly represent are regenerative phenotype
- including proximal tubules and collecting duct
- Markers are distributed in an organoid-specific fashion





Schutgens, F. *et al*. *Nature Biotechnology* **37**, 303-313 (2019). https://doi.org:10.1038/s41587-019-0048-8

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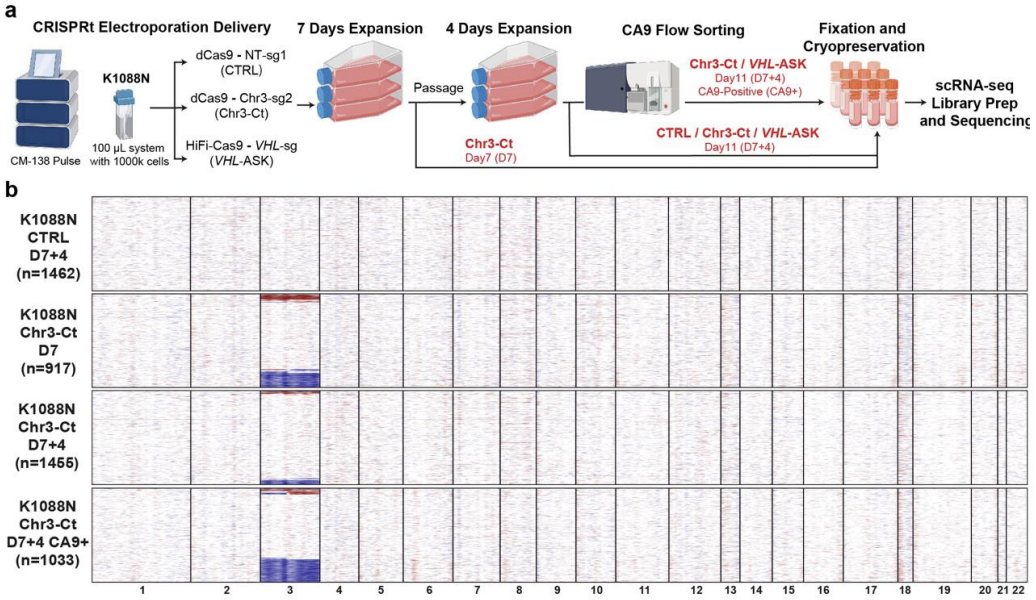
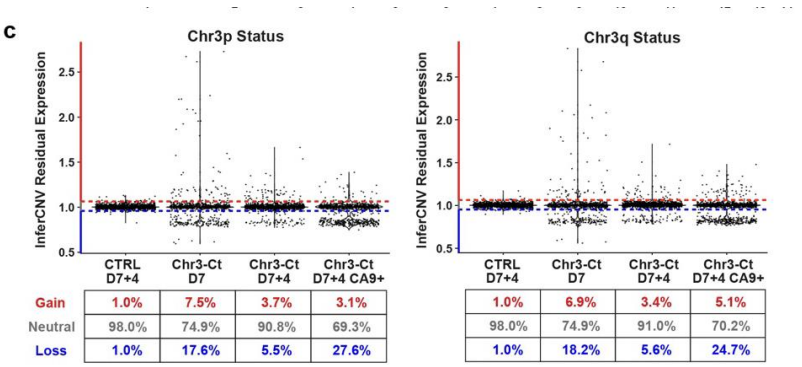
Genetic engineering of normal organoids



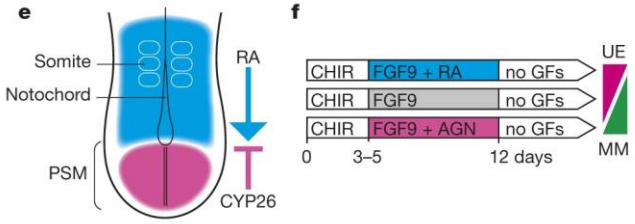


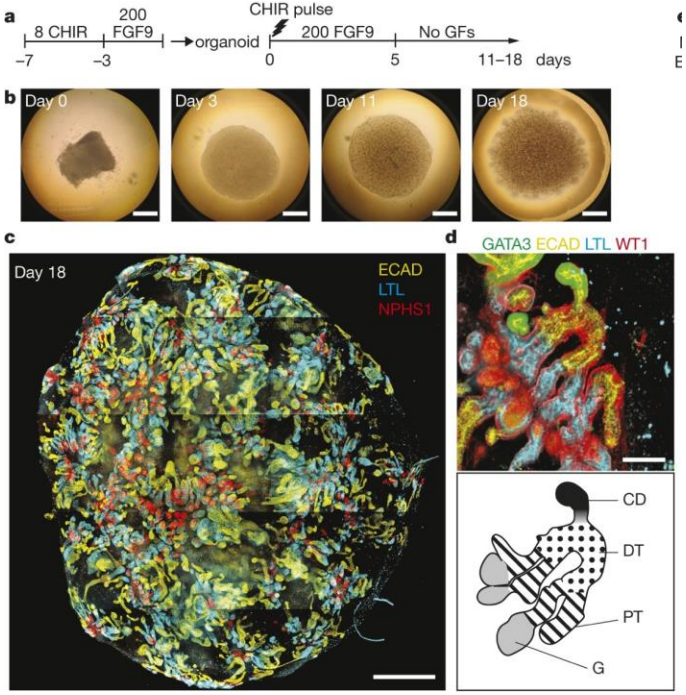
	CTRL D7+4	Chr3-Ct D7	Chr3-Ct D7+4	Chr3-Ct D7+4 CA9+
Gain	1.0%	7.5%	3.7%	3.1%
Neutral	98.0%	74.9%	90.8%	69.3%
Loss	1.0%	17.6%	5.5%	27.6%

	CTRL D7+4	Chr3-Ct D7	Chr3-Ct D7+4	Chr3-Ct D7+4 CA9+
	1.0%	6.9%	3.4%	5.1%
	98.0%	74.9%	91.0%	70.2%
	1.0%	18.2%	5.6%	24.7%

Feng, H. *et al. Chromosome-Specific Aneuploidy Engineering via dCas9-Induced Centromeric Chromatin Relaxation* (Cold Spring Harbor Laboratory, 2025).

Modelling kidney development from iPSC

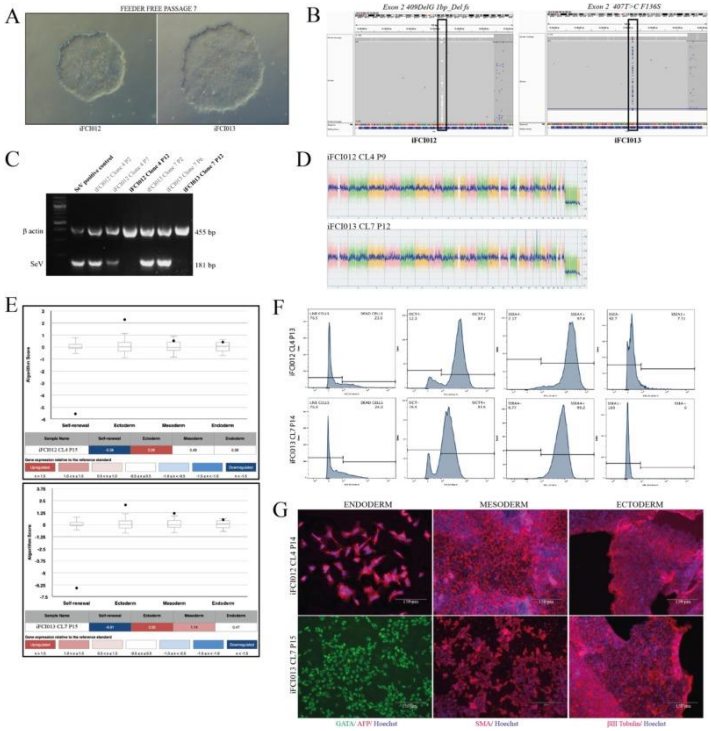




- iPSC generated from patients
- Modelling heriditary disease, e.g. vHL

Takasato, M. *et al*. *Nature* **526**, 564-568 (2015).

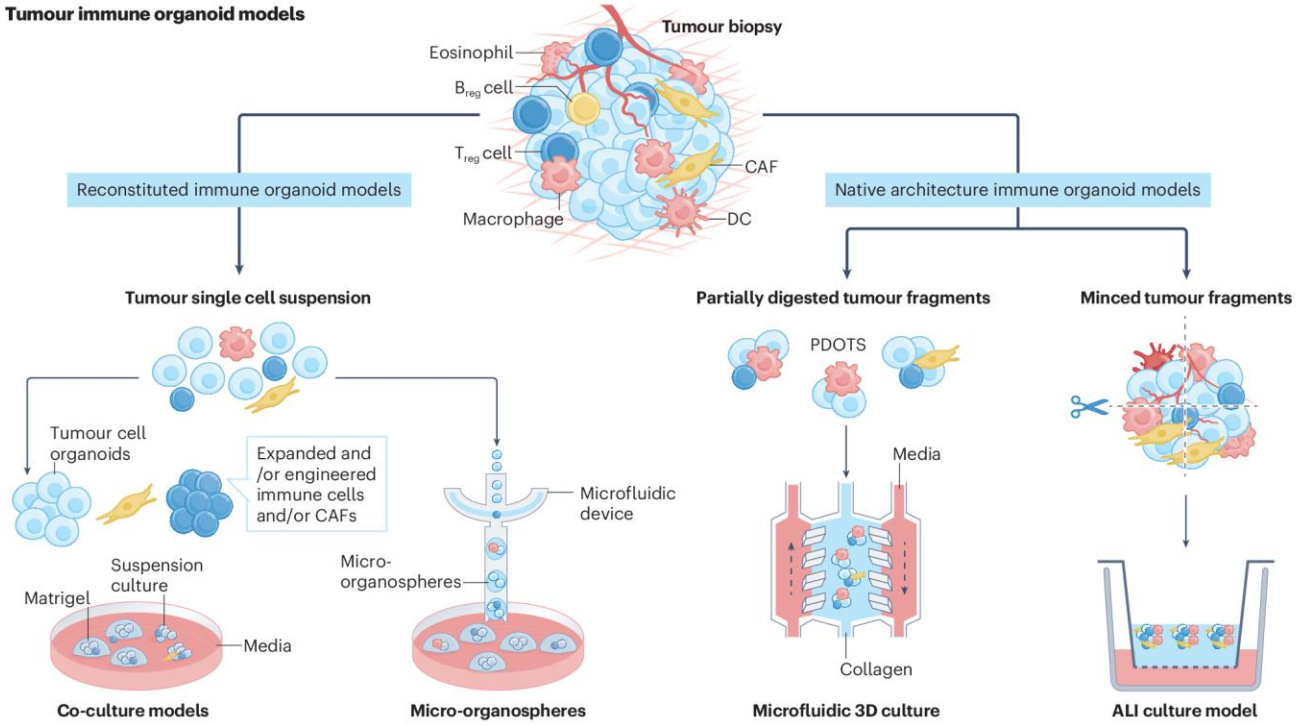
Generation of iPSC lines from patients with VHL disease



- Functional effect of specific VHL mutations
- Differentiation to different lineages possible
- Genetic engineering (loss of second allele, loss of 3p)

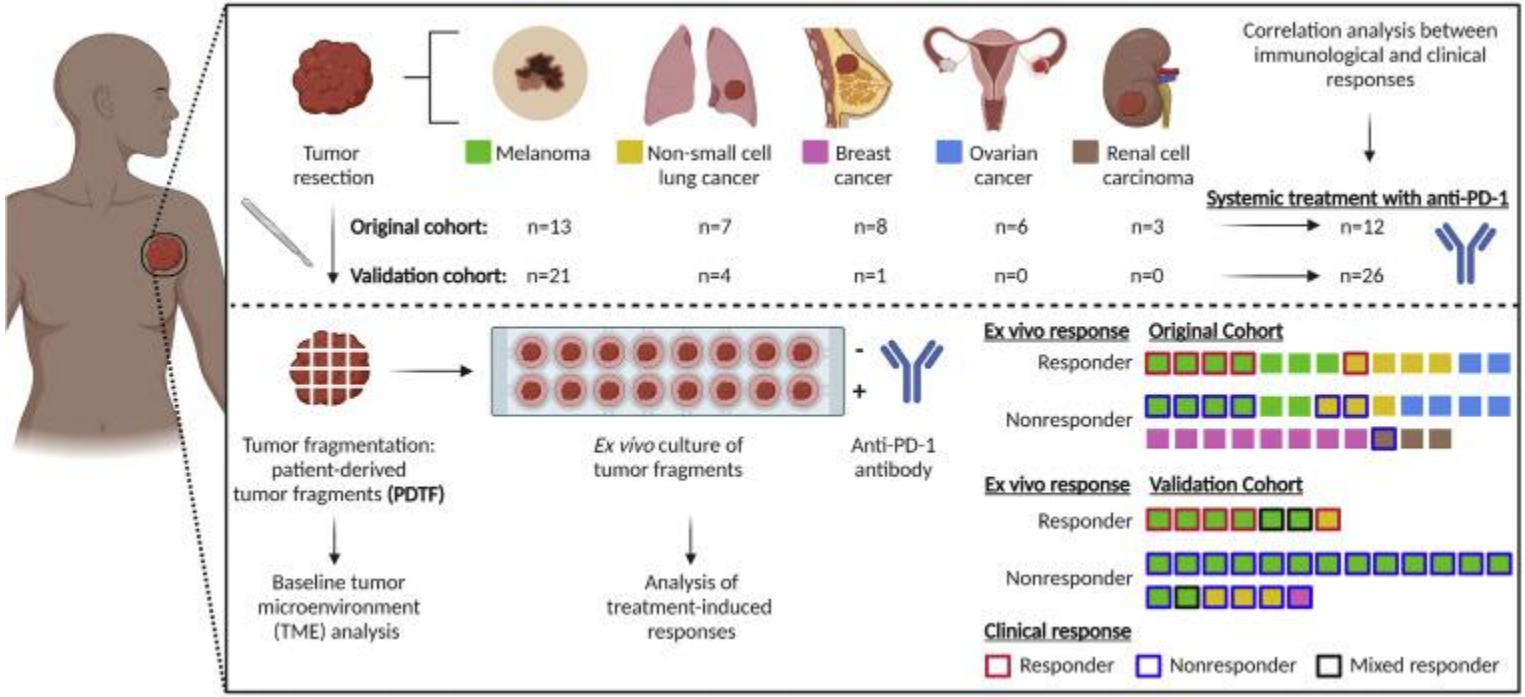
Devito, L. G. *et al*. Generation of TWO iPSC lines (CRICKi009-A; CRICKi010-A) from patients with type 1 von Hippel-Lindau (VHL) and histopathologically confirmed renal cell carcinoma (RCC). *Stem Cell Research* **81**, 103611 (2024). https://doi.org:10.1016/j.scr.2024.103611

4. How can we incorporate the immune system?



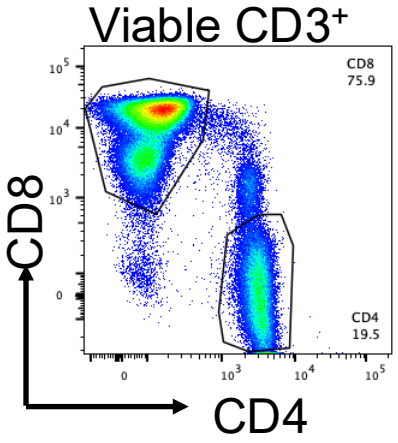
Polak, R., et al. *Nat Rev Cancer* **24**, 523-539 (2024).

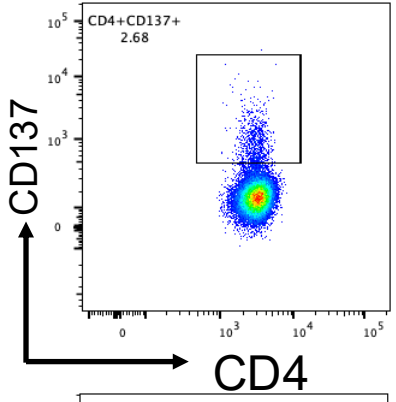
Fragment cultures represent TIME heterogeneity and predictive responses to IO

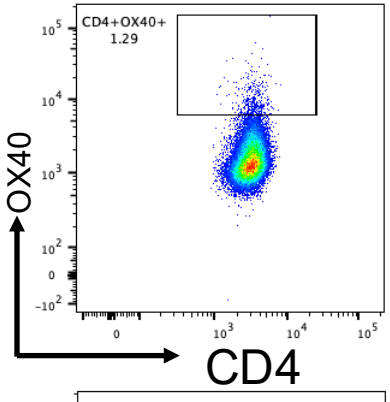


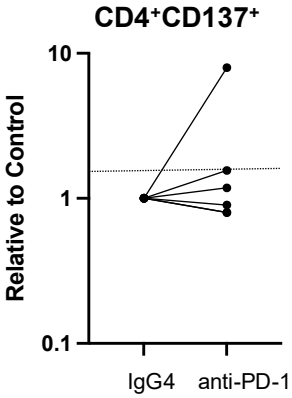
Voabil P, et al. Nature Medicine. 2021.
Cimen Bozkus C, Bhardwaj N. Cancer Cell. 2021.

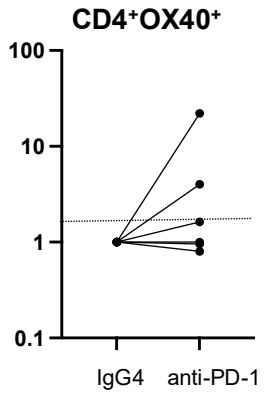
Flow assay to identify activated T cells post nivolumab treatment

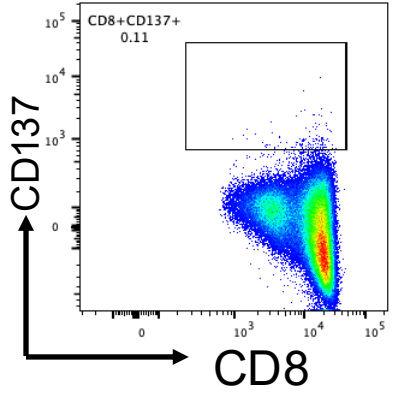


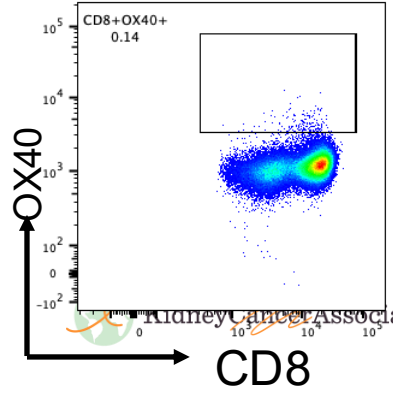


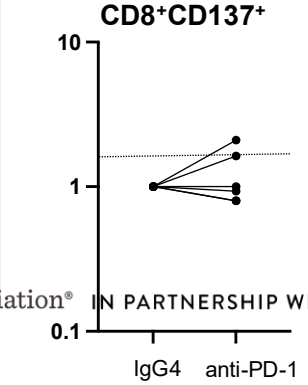


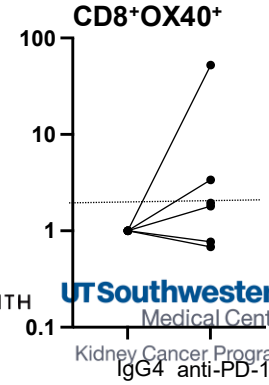












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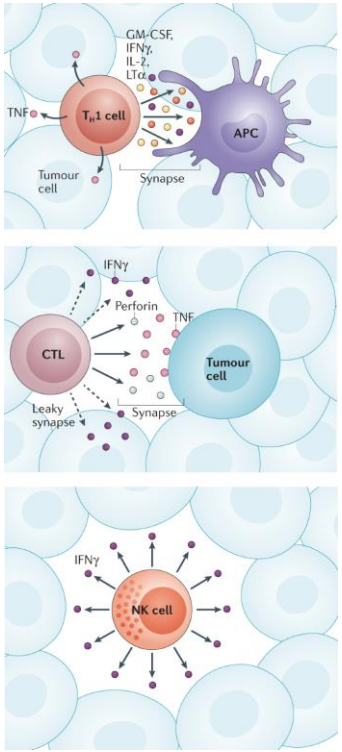
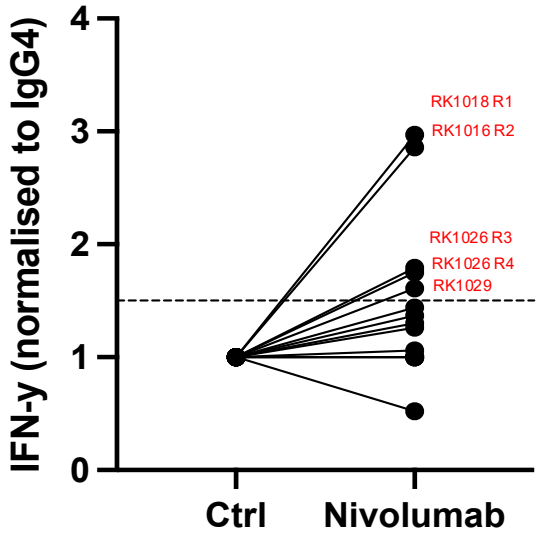
IFN-γ secretion from activated cells is an alternative method to quantify response





Different regions from the same patient show concordant responses

Case number	Region	Response (IFN-γ)	Response (CD4)	Response (CD8)
RK1015	R10	NR		
RK1015	R12	NR	NR	NR
RK1016	R2	R		
RK1018	R1	R		
RK1025	R1+2	NR		
RK1026	R3	R		
RK1026	R4	R	R	R
RK1027	R4	NR		
RK1027	R13	NR	NR	NR
RK1028	R9	NR	NR	(N)R
RK1028	R2	NR		
RK1029	Swiss cheese	R		
RK1030	R6	NR		
RK1031	R5	NR	R	R
RK1034	Central	NR		
RK1034	North	NR	NR	NR

NR, non-responder; R, responder

Conclusions

- PDO can maintain regenerative and differentiation capacity over culture
- Culture conditions should allow tumor cells to outcompete other cell types
- PDO can be established from different subclones of the primary tumor and represent these regions
- Genetic engeneering in PDO can be used to functionally study tumorigenesis and evolution in vitro
- Incorporating the immune system is esential for predicting therapy outcomes for RCC

Thank you

Fendler Lab @Charite

Emily Riemer
Patricia Tscheak
Bettina Ergün
Siegrun Blauhut
Lucas Raue
Cora Nordbrock
Kira Furlano
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Oncology
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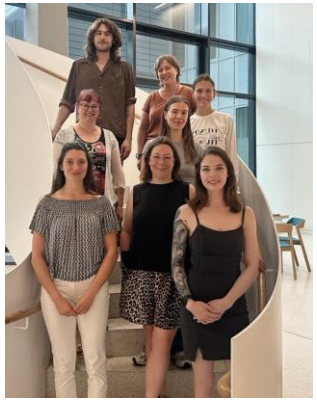
Lars Bullinger

Pathology
Franz Dressler

Physiology
Bianca Nitzsche



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