



Next-generation cancer models – a human stem cell-based model for retinoblastoma

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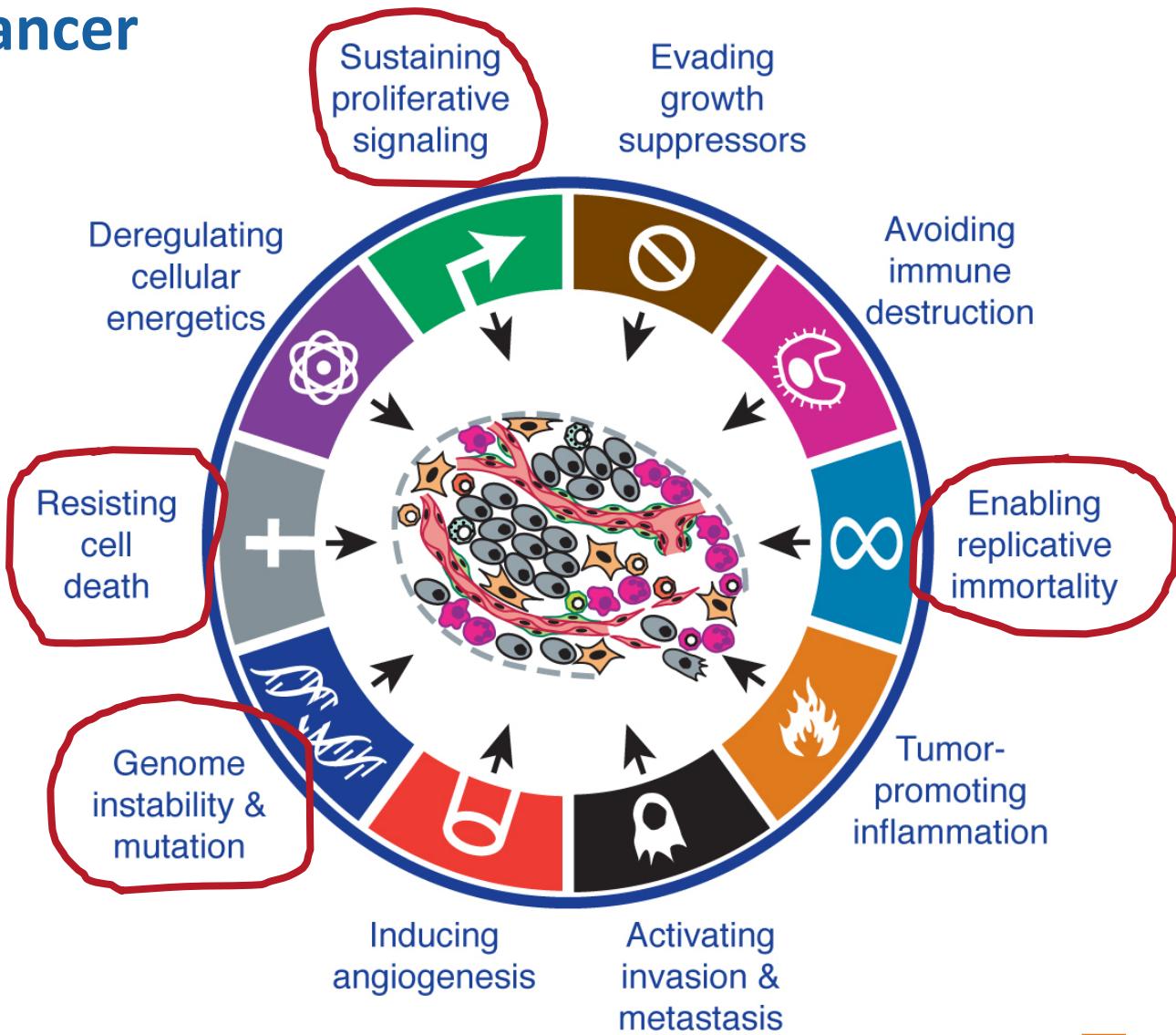


ECCO XL Braunschweig 28th September 2022



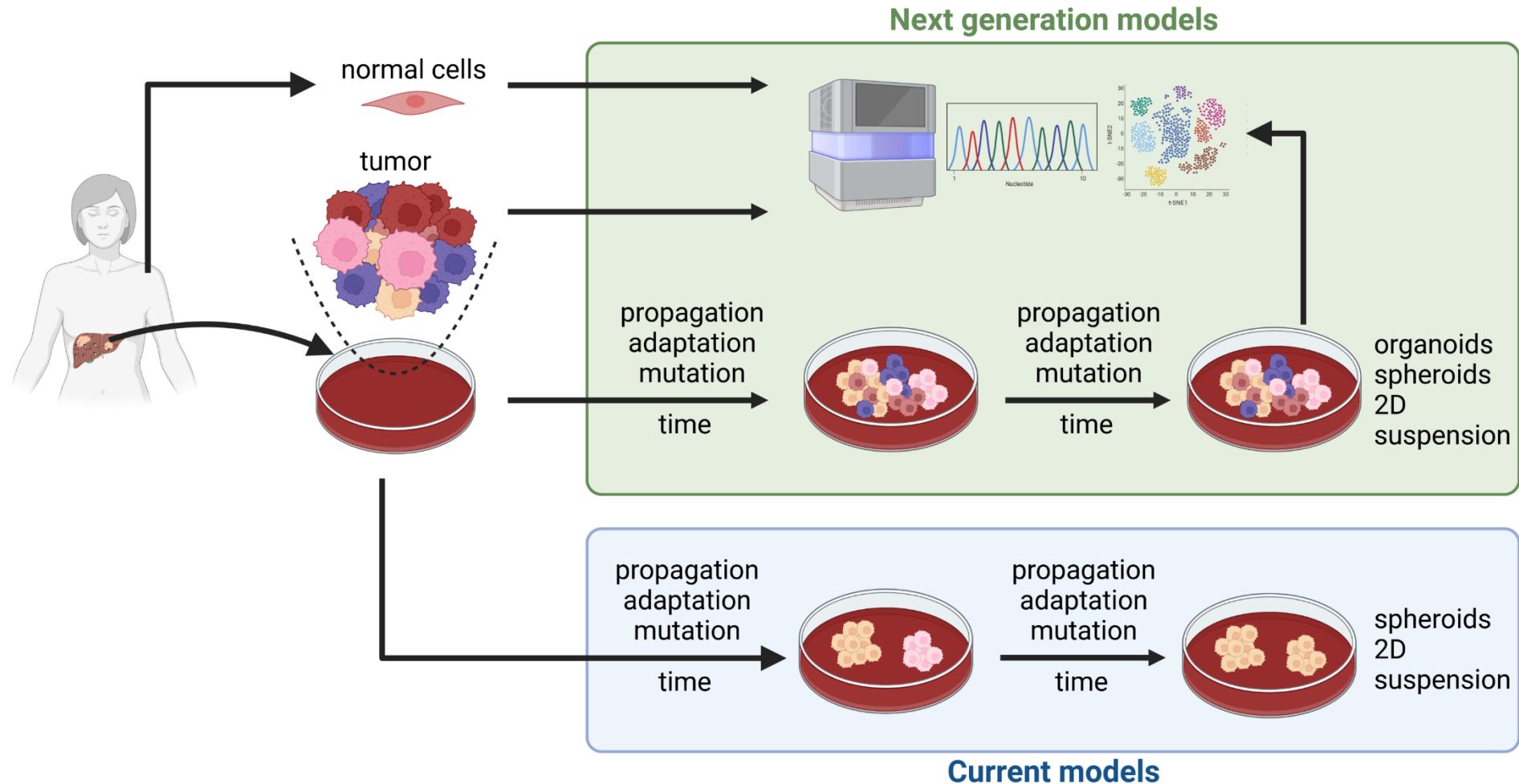
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Hallmarks of Cancer



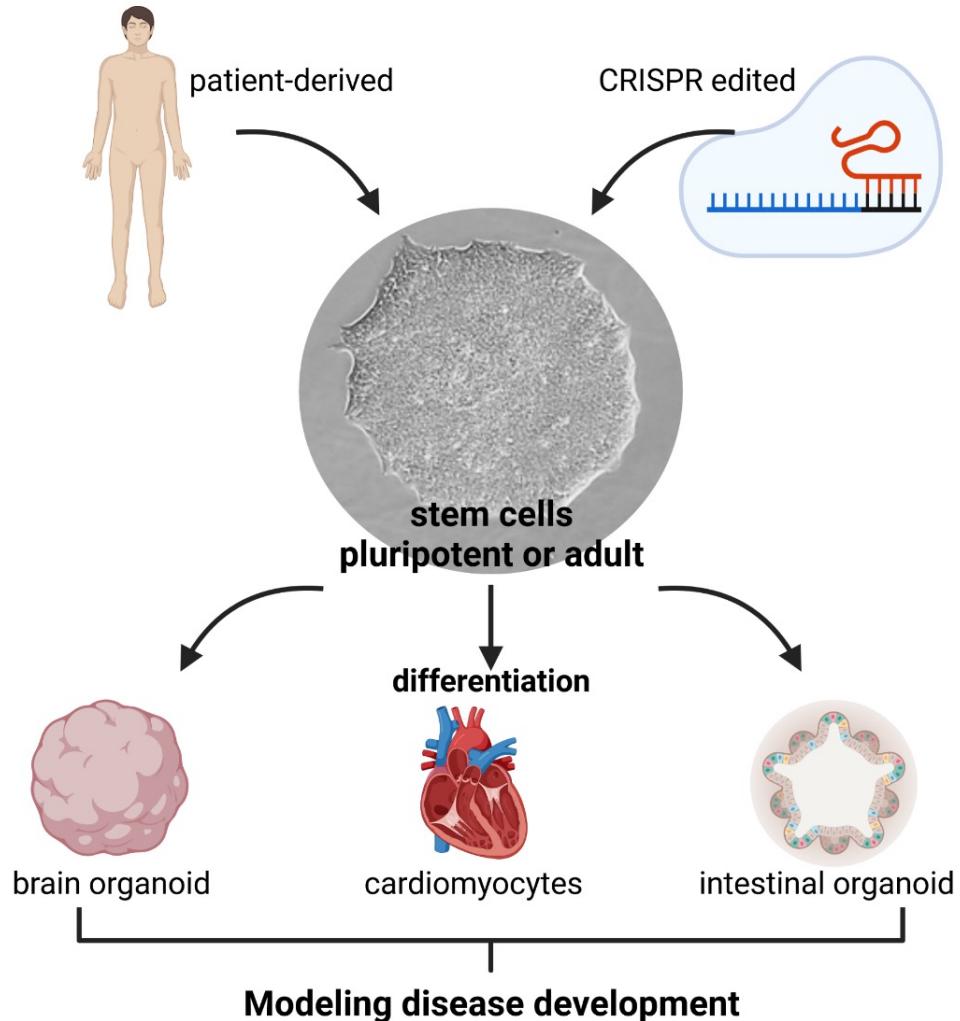
Hanahan and Weinberg, 2011

Next-generation cancer models – patient derived



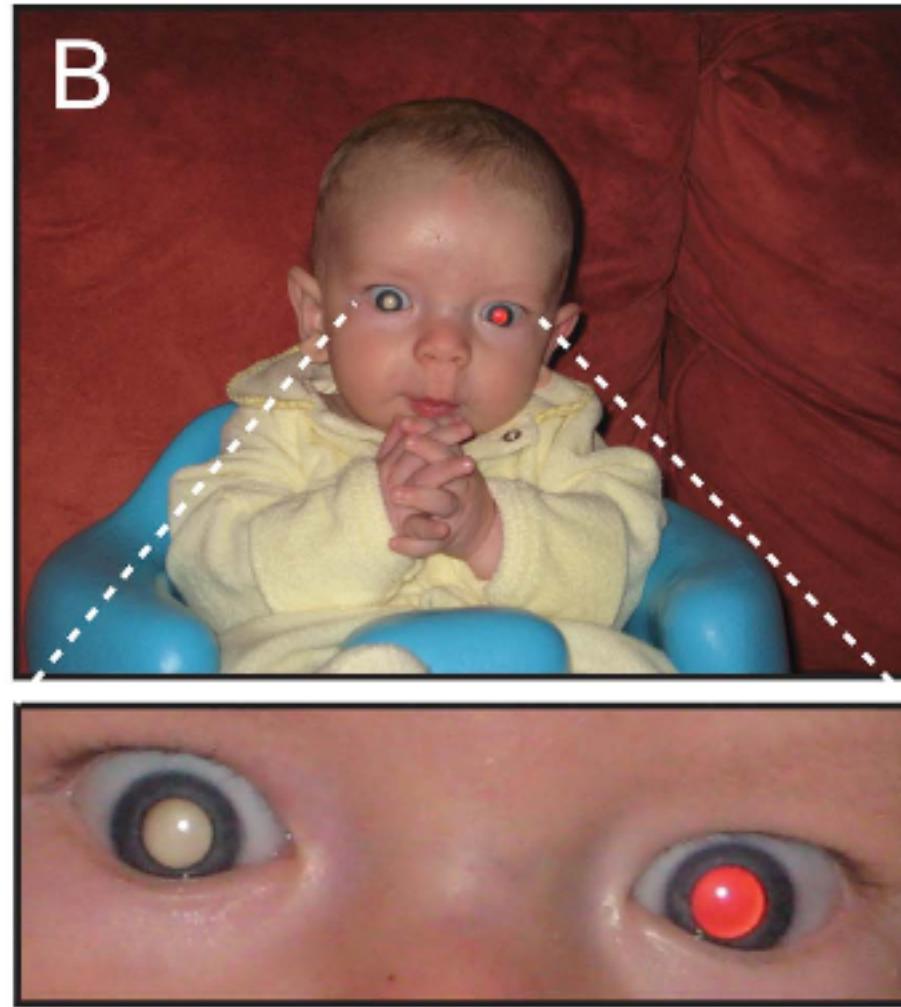
Next-generation cancer models – stem cell derived

- Tailored genetic modifications
- Same genetic background
- Derivation of target tissue
- Development instead of end-stage



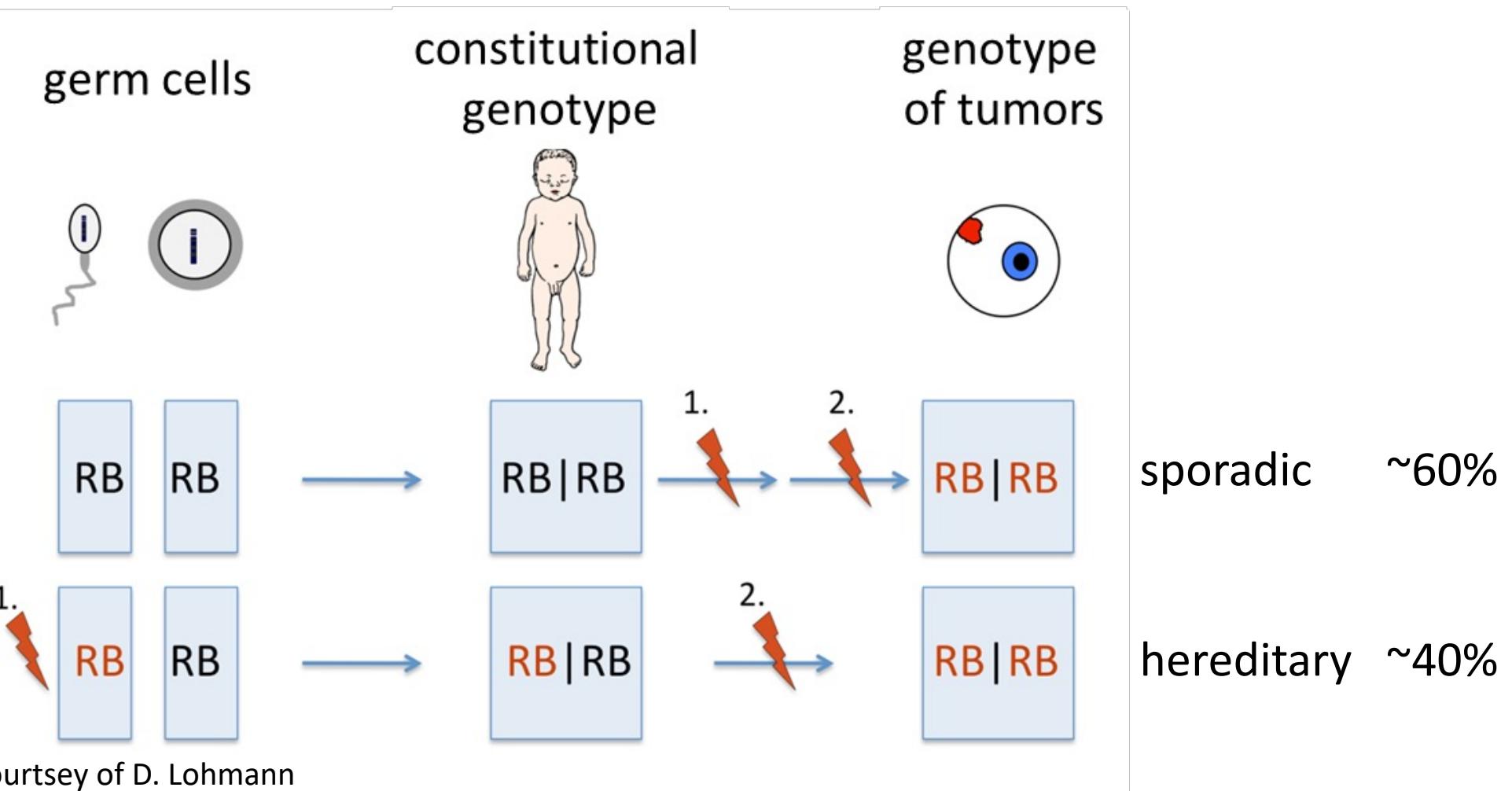
Retinoblastoma

Tumor of the retina
Children under the age of 5 years
Incidence ~1:20.000



Abdolvahabi et al, PlosOne 2013

Two-Hit-Hypothesis



AG Knudson, 1971: Mutation and Cancer: A statistical study on retinoblastoma

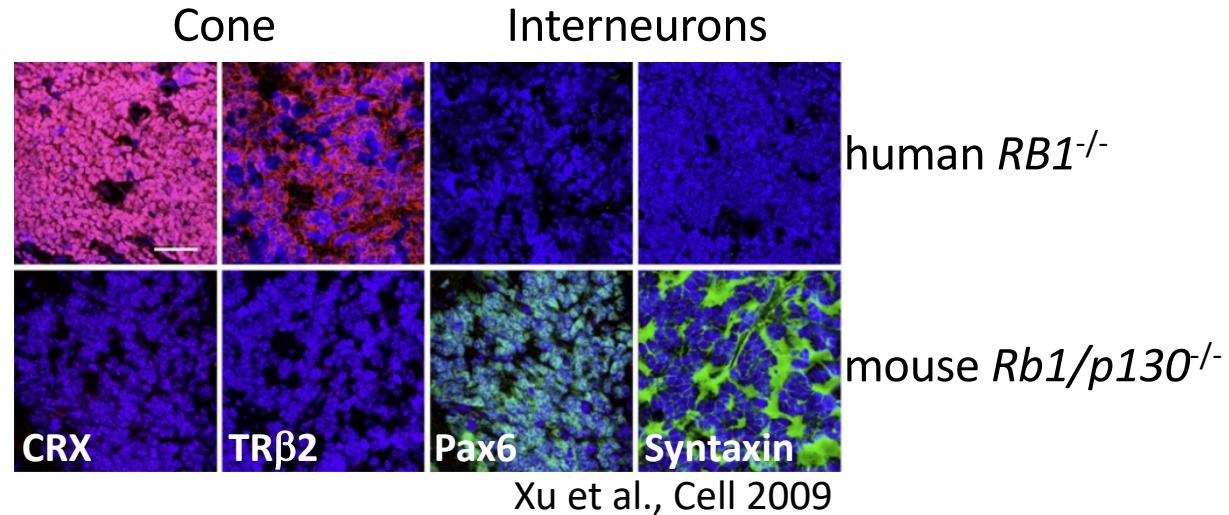
Modelling Retinoblastoma

The mouse is no good model for Rb

Knock-out mice do not develop Rb

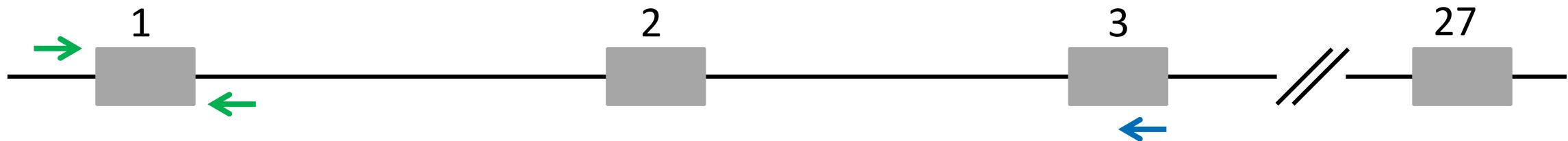
Heterozygote animals develop tumors in the brain

Cell-of-Origin differs between human and mice: photoreceptors vs interneurons



=> Differentiation of human pluripotent stem cells into retina

Knock-out of *RB1* in hESCs

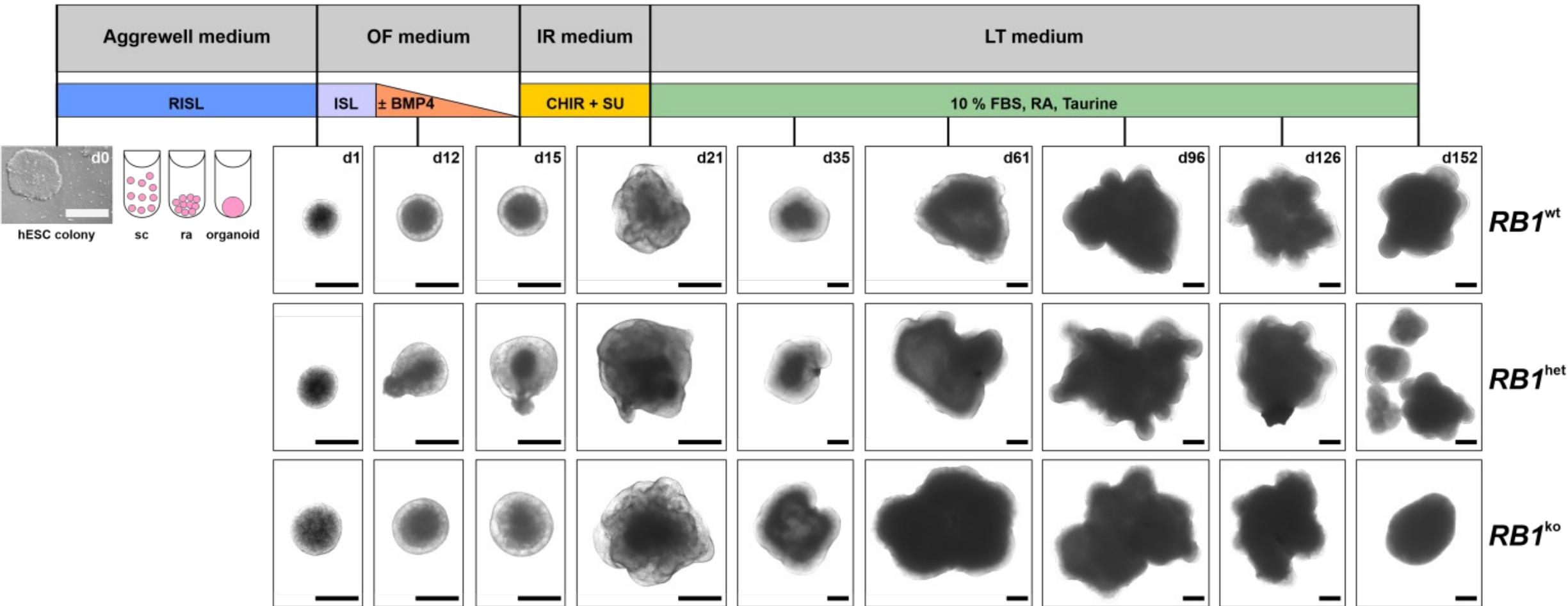


	RB1 Exon 3 Indel				RB1 Exon 1 Deletion			
hESC	H1		H9		H1		H9	
clones	G3	G4	G12LS	C7	E9	D6	8B3	8F2
	+/-	-/-	+/-	-/-	+/-	-/-	+/-	-/-
allele 1	wt	ins2_Δ7	wt	Δ7	wt	Δ400	wt	Δ417
allele 2	Δ7	ins2_Δ7	Δ7	Δ7	Δ365	insA_Δ416	Δ417	Δ417

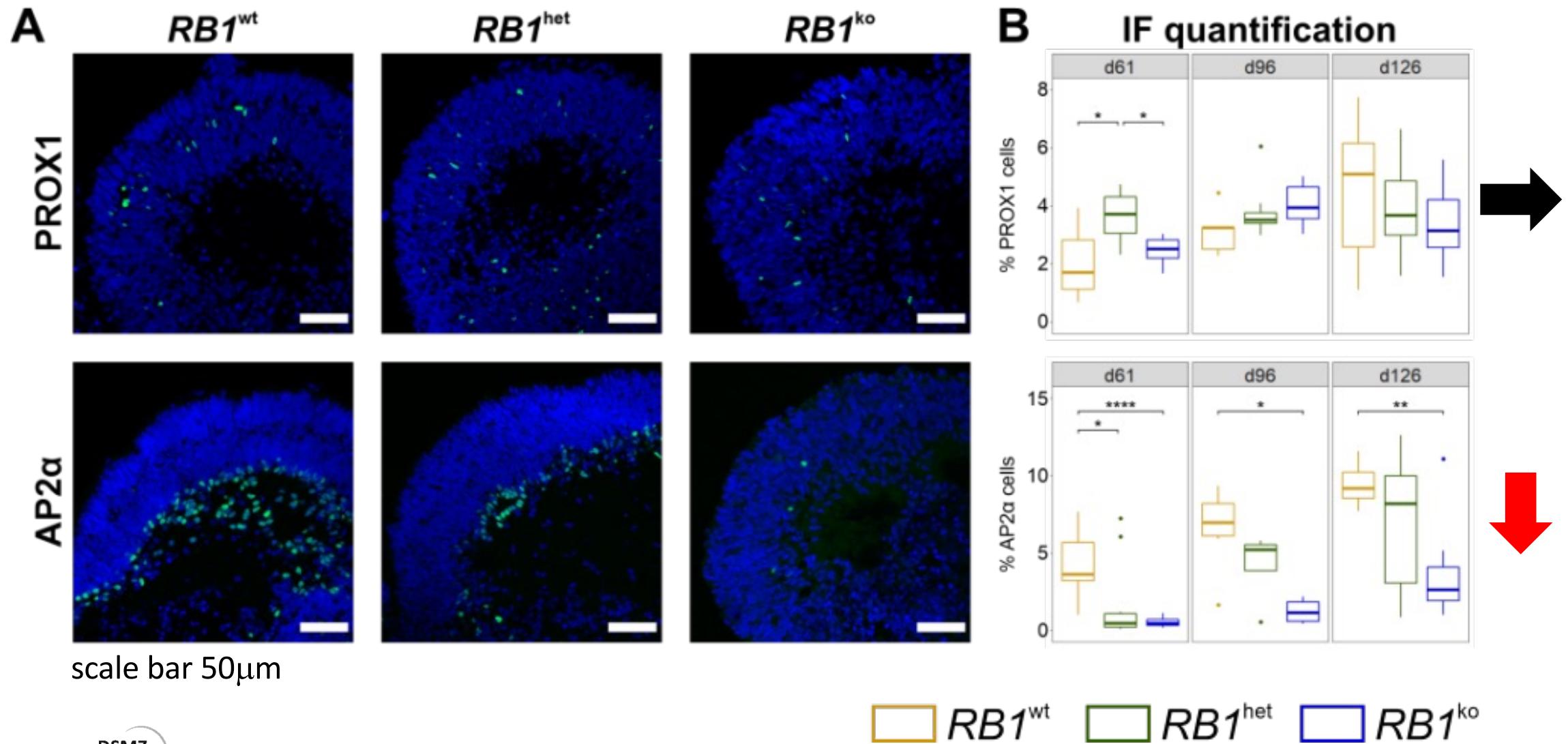
Steenpass 2017; Schipper et al., 2018; Menges et al., 2019; Döpper et al., 2020; all StemCellRes

Differentiation towards neural retina

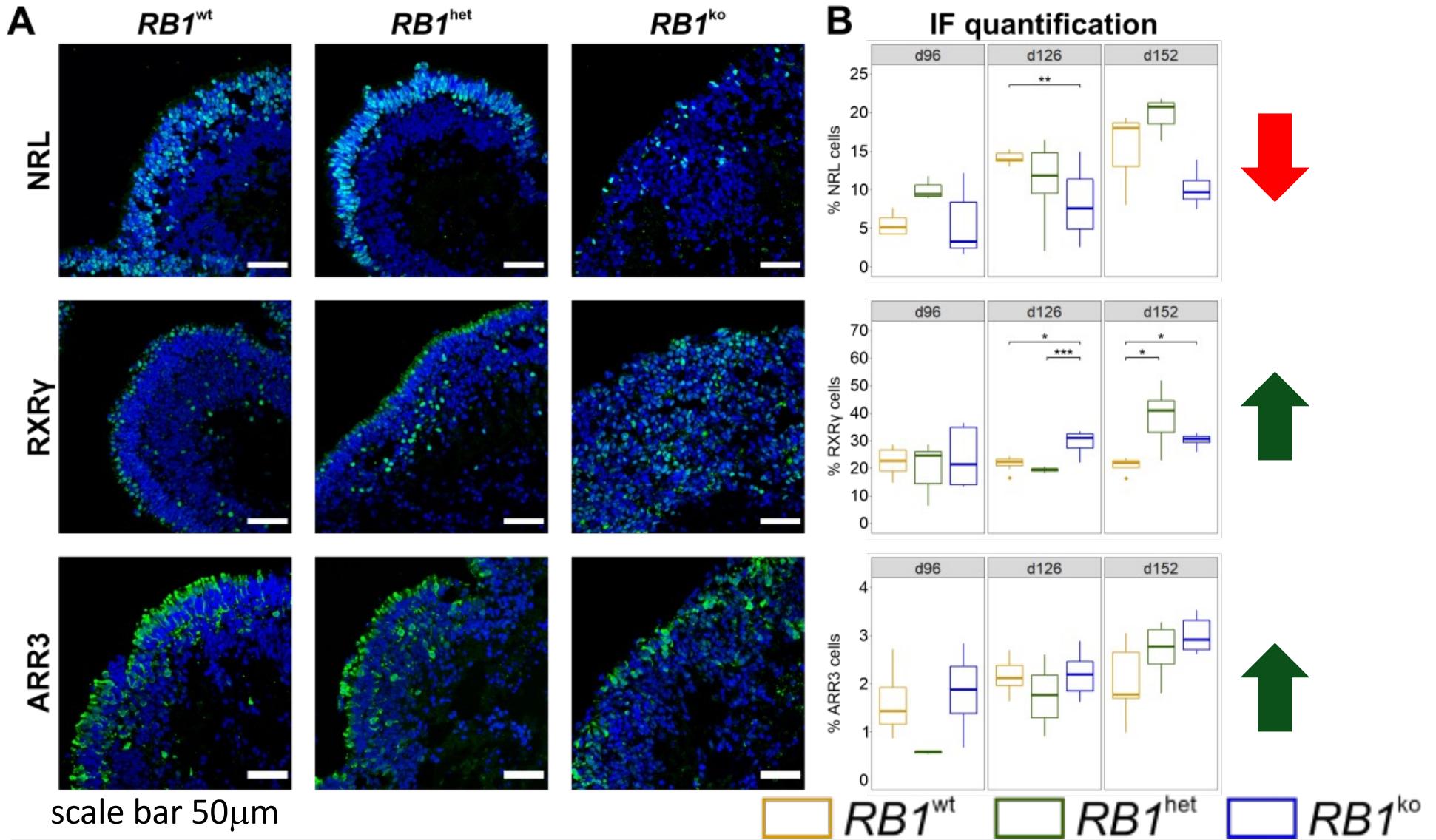
modified after Kuwahara et al., NatCommun, 2015



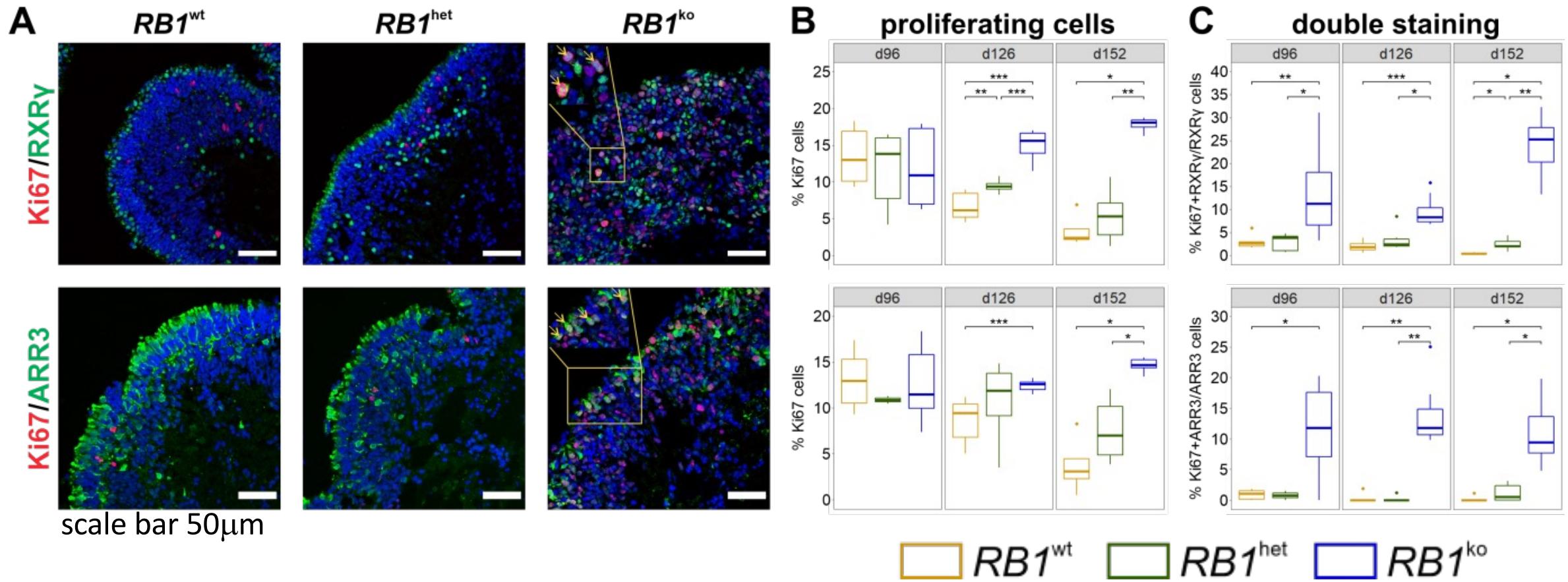
Horizontal and amacrine interneurons, day 126



Photoreceptors, day 152

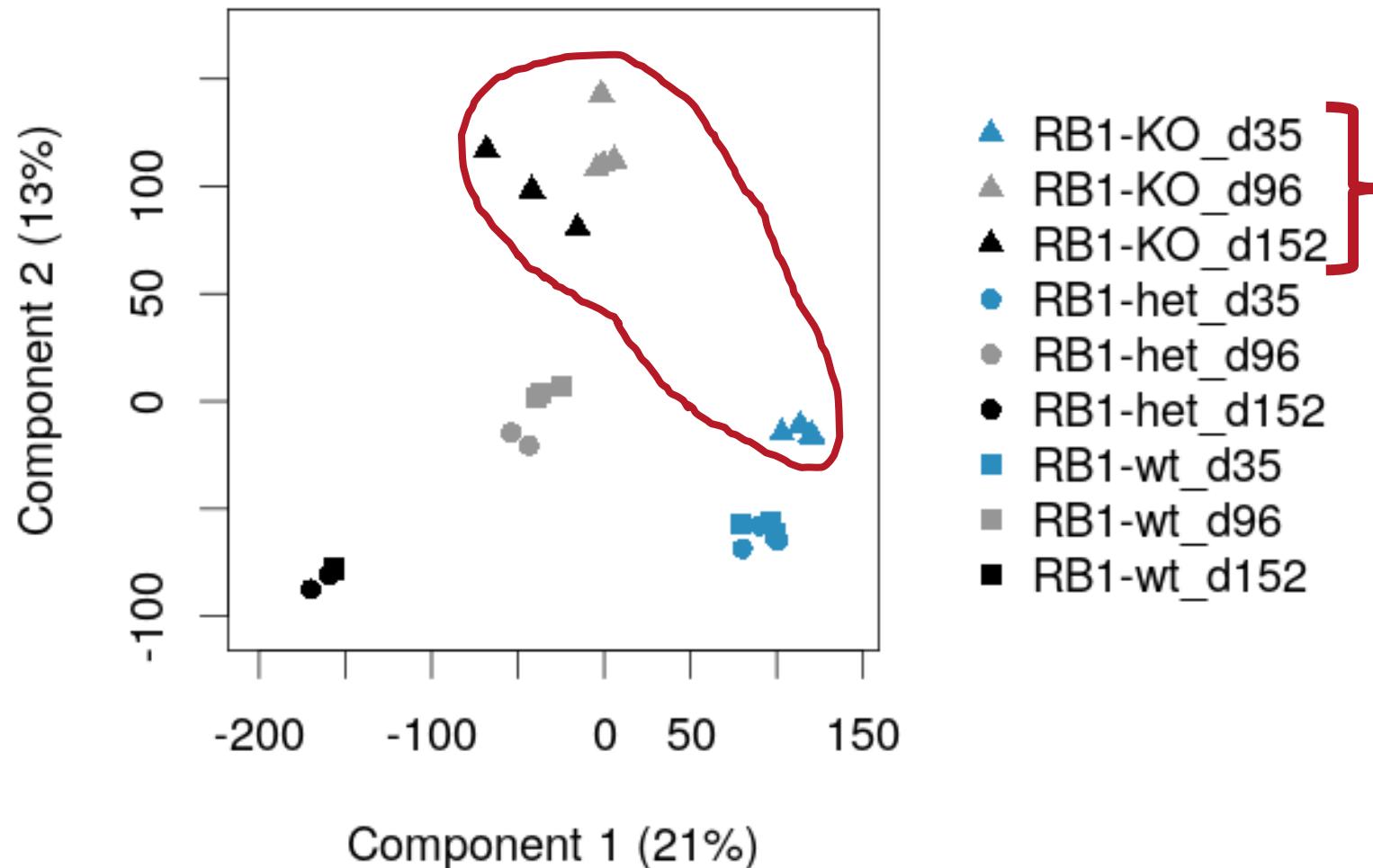


Proliferating cone photoreceptors, day 152

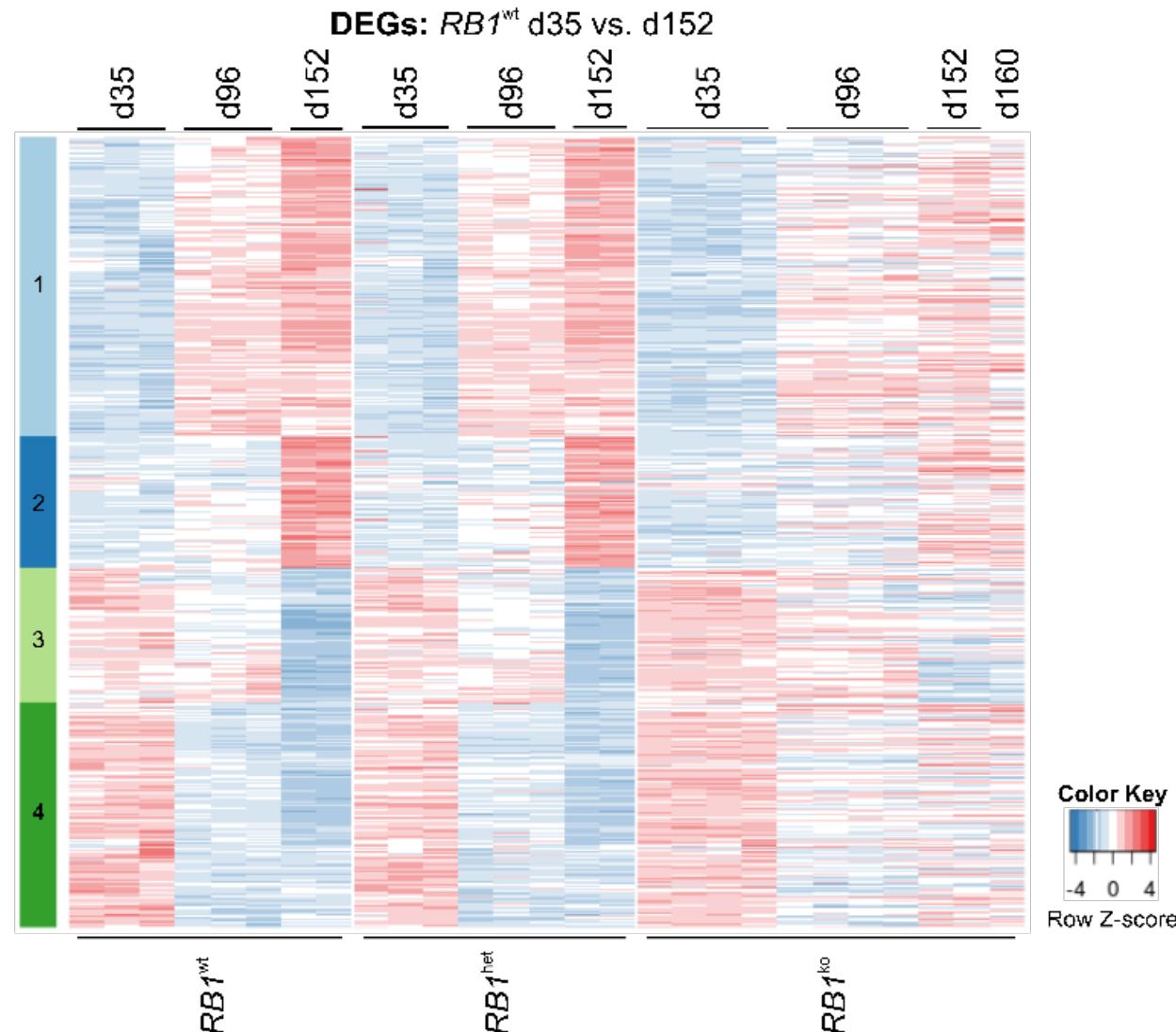


RNA-seq

PCA

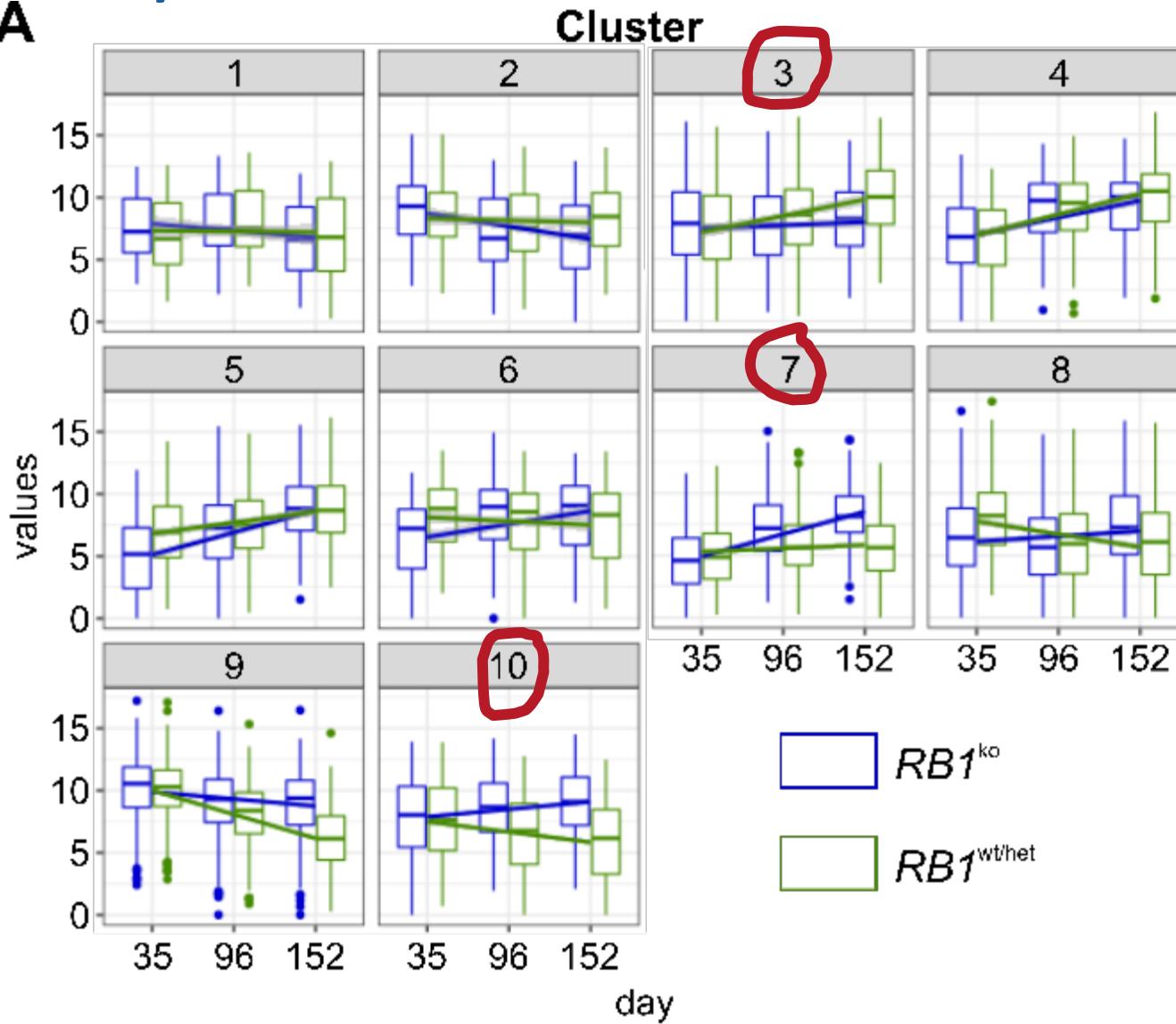


$RB1^{wt}$ and $RB1^{het}$ develop similar

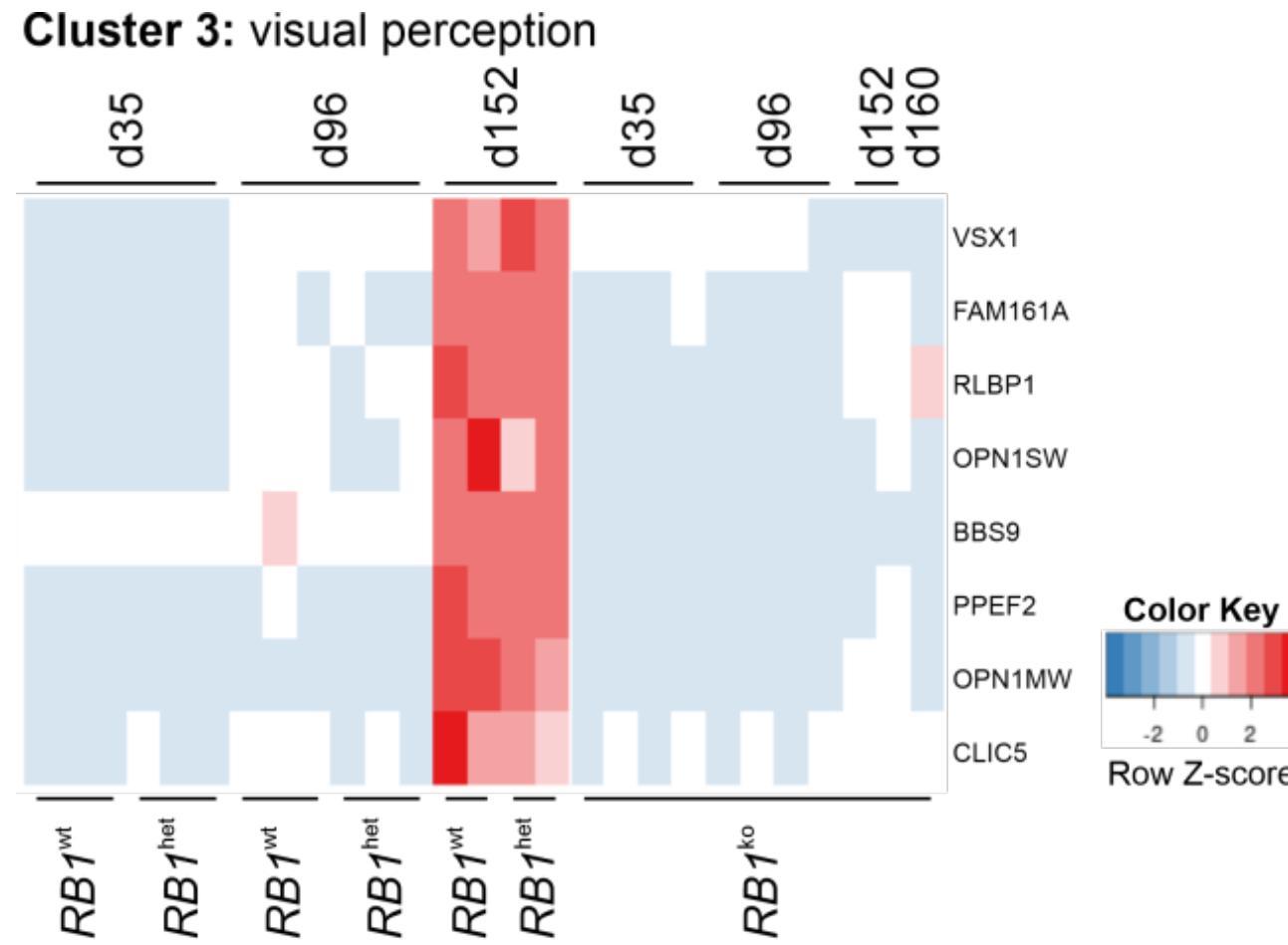


Comparison $RB1^{wt}/RB1^{het}$ vs $RB1^{ko}$

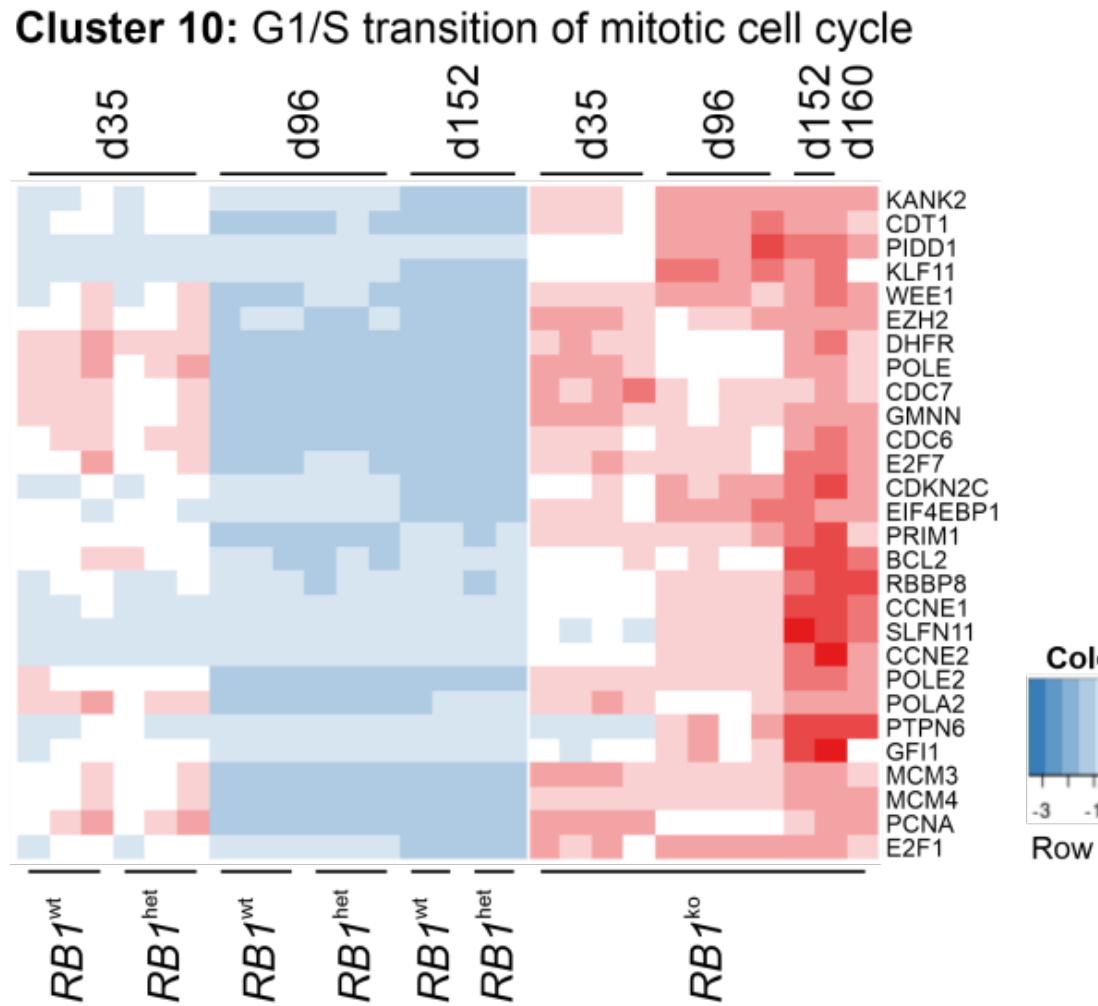
A



Lack of retinal differentiation

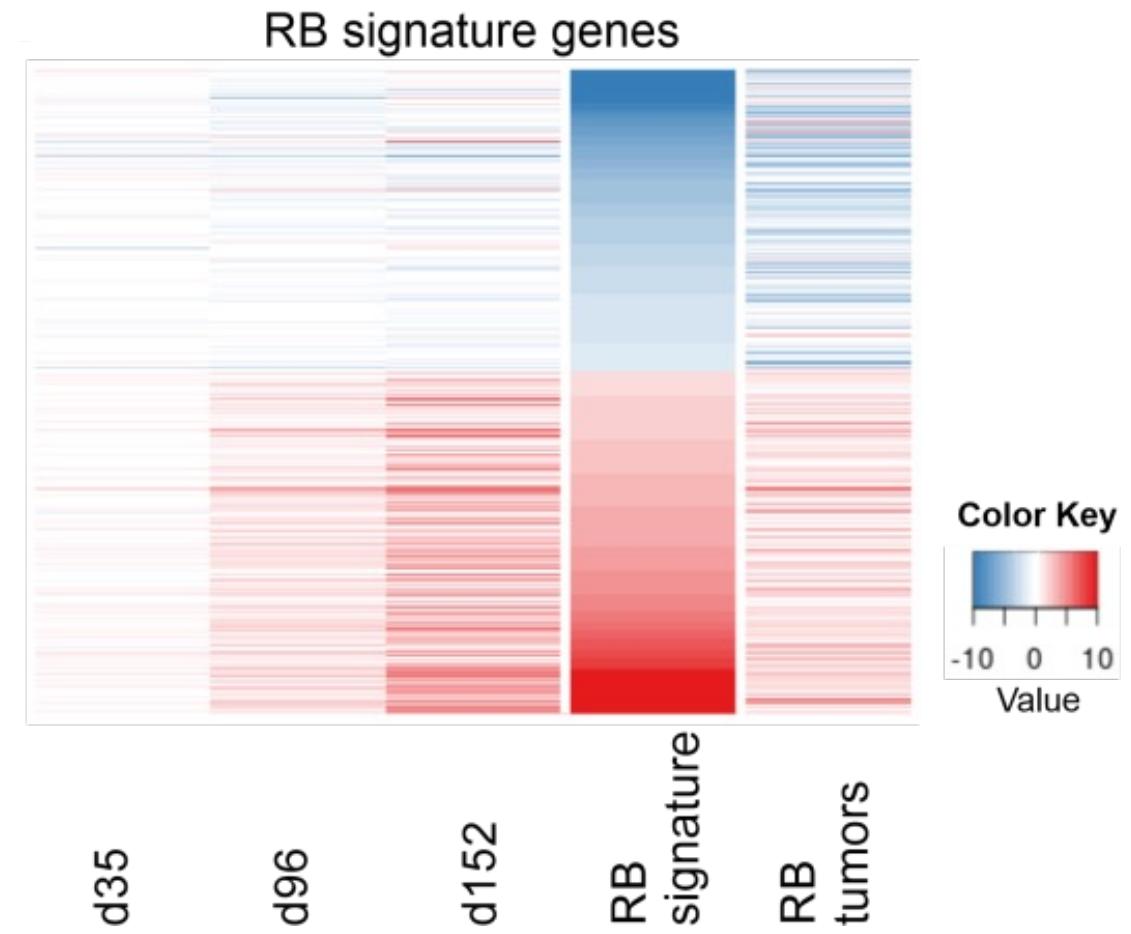


Persistent proliferation



RB1^{ko} develop Rb expression signature

Age@dx	Laterality	1 st mt	2 nd mt	Protein
125	unilateral	c.958C>T	LOH	p.(Arg320*)
62	unilateral	c.958C>T	LOH	p.(Arg320*)
320	unilateral	c.1072C>T	LOH	p.(Arg358*)
96	unilateral	c.1363C>T	LOH	p.(Arg455*)
46	unilateral	c.1390-1G>A	LOH	skipping exon 15/ p.(Glu464Lysfs*4)



Kapatai et al., BrJ Cancer, 2013
Ganguly & Shields, MolVis, 2010



Summary

Rb tumor	<i>RB1</i> ^{ko} organoids
semi-solid, soft tumor	loss of structure
photoreceptor markers	Increase in cones, loss of rods
L/M cones sensitive for <i>RB1</i> loss	proliferating L/M and S cones
No interneuron marker expression	loss of amacrine and bipolar interneurons
	Rb expression signature

***RB1*^{ko} organoids are a valid human Rb model**

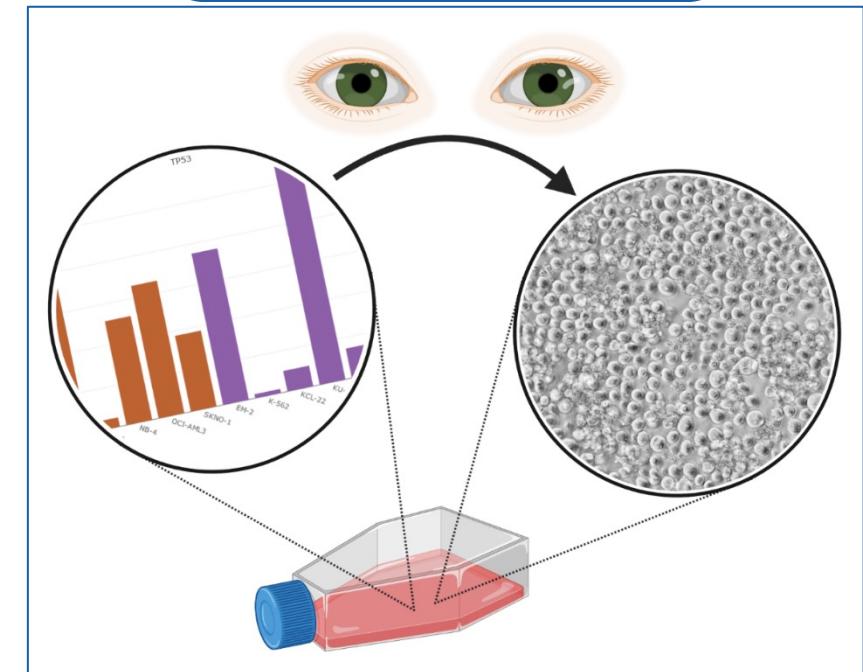
Digital collection – DSMZCellDive

Integration of

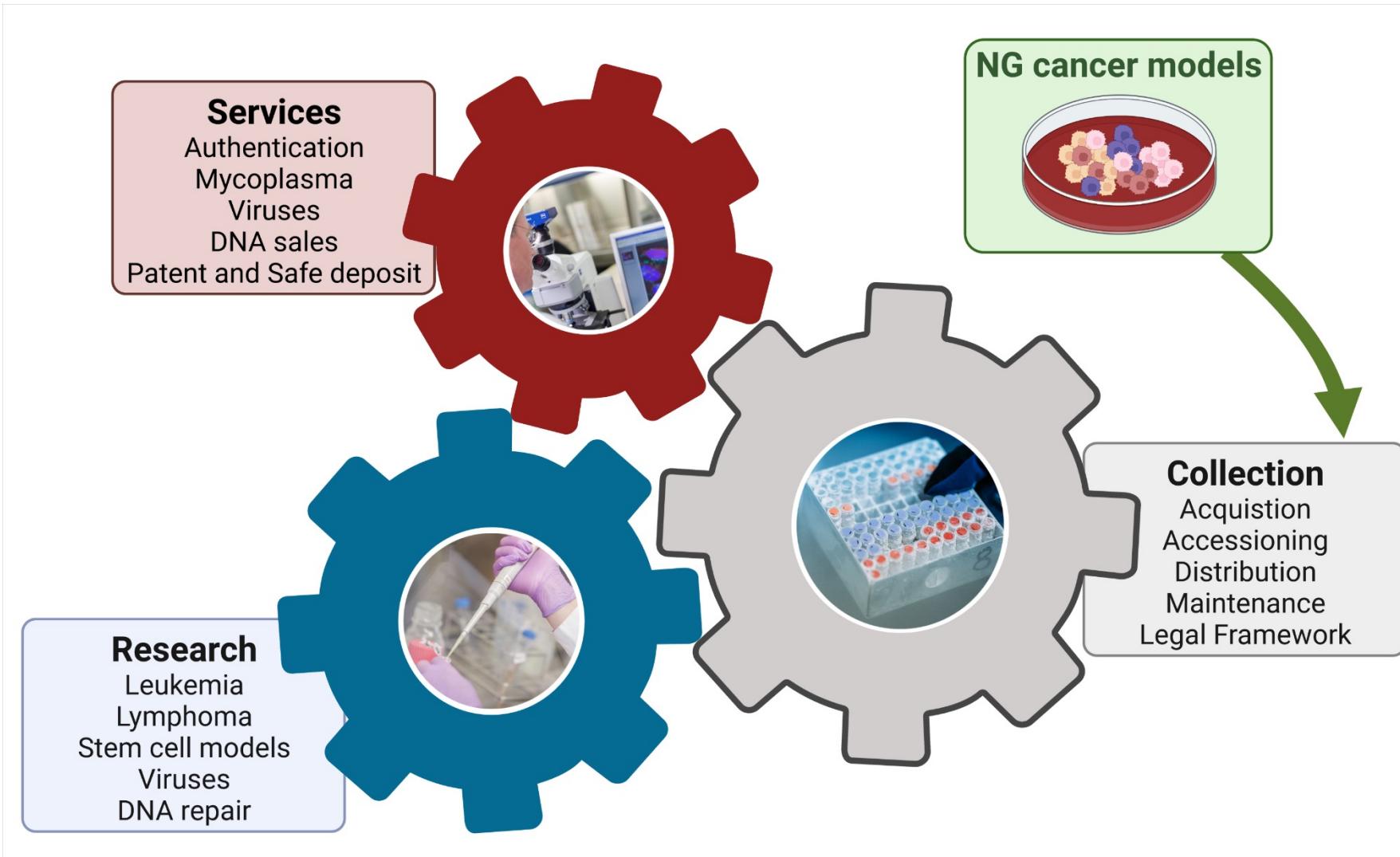
- RNA-seq data of LL100 panel
- STR profile browser and STR profile search
- COI DNA barcoding



**What you see is
what you get!**



Department of Human and Animal Cell Lines



Acknowledgment



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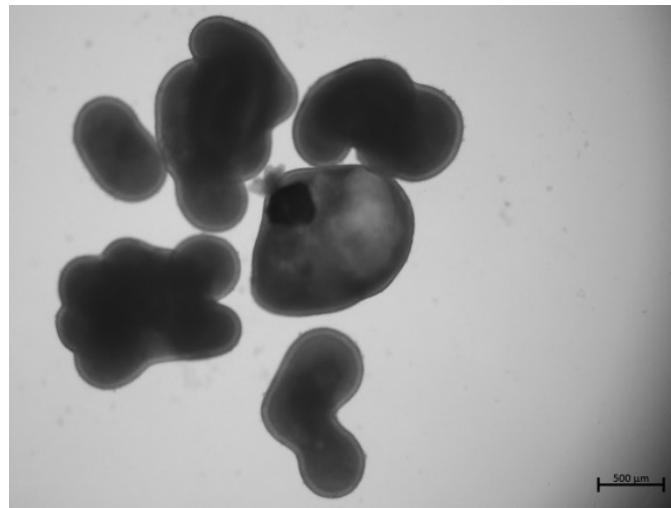


Differentiation towards neural retina

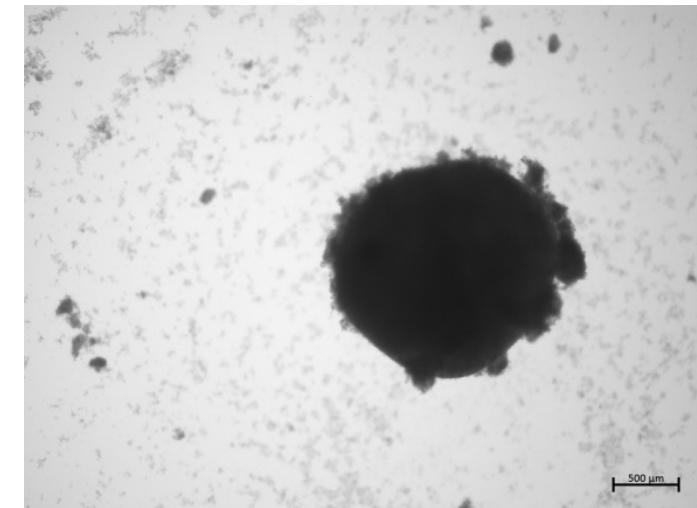
d152



RB1^{+/+}



RB1^{+/-}



RB1^{-/-}

Upregulation of ECM associated genes

Cluster 7: extracellular matrix organization

