Intravital imaging of tissue homeostasis and cancer

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Hubrecht Institute
Developmental Biology and Stem Cell Research

http://www.hubrecht.eu/onderzoekers/van-rheenen-group/

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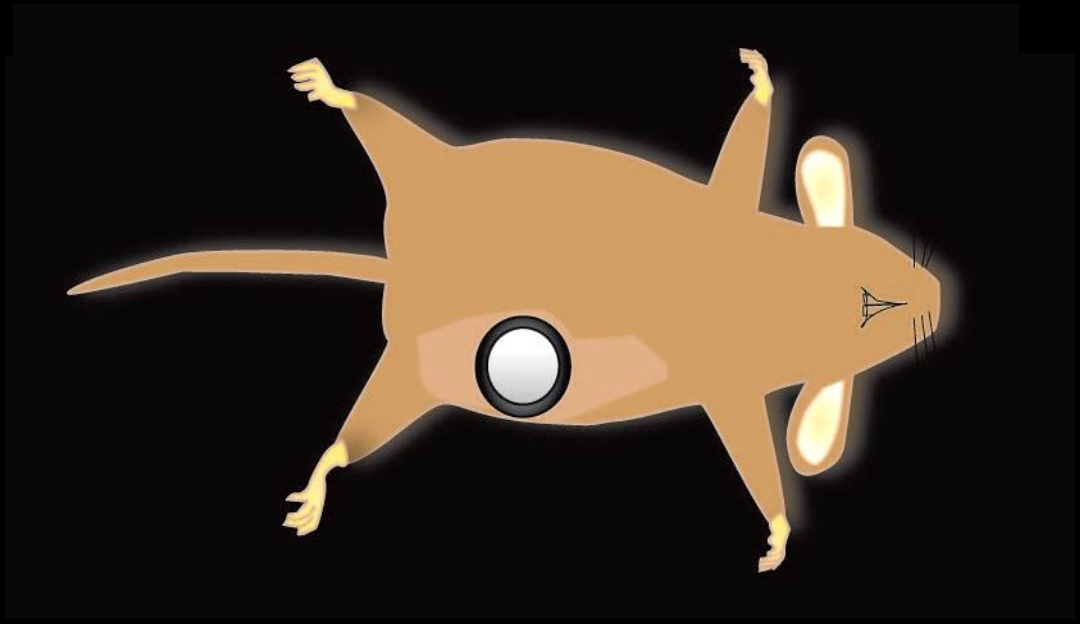
van Rheenen lab: intravital imaging

Imaging window: the next step in intravital imaging



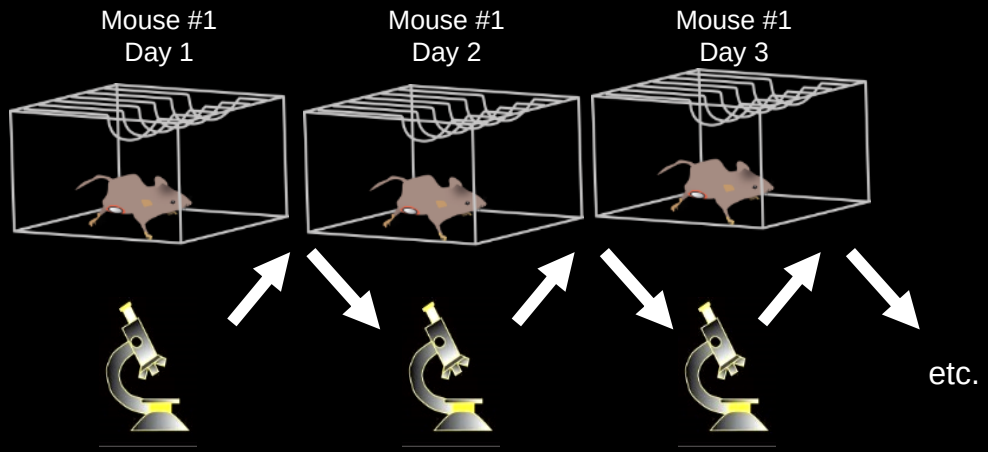
Mammary Imaging Window

Kedrin *et al*, Nat Meth, 2008

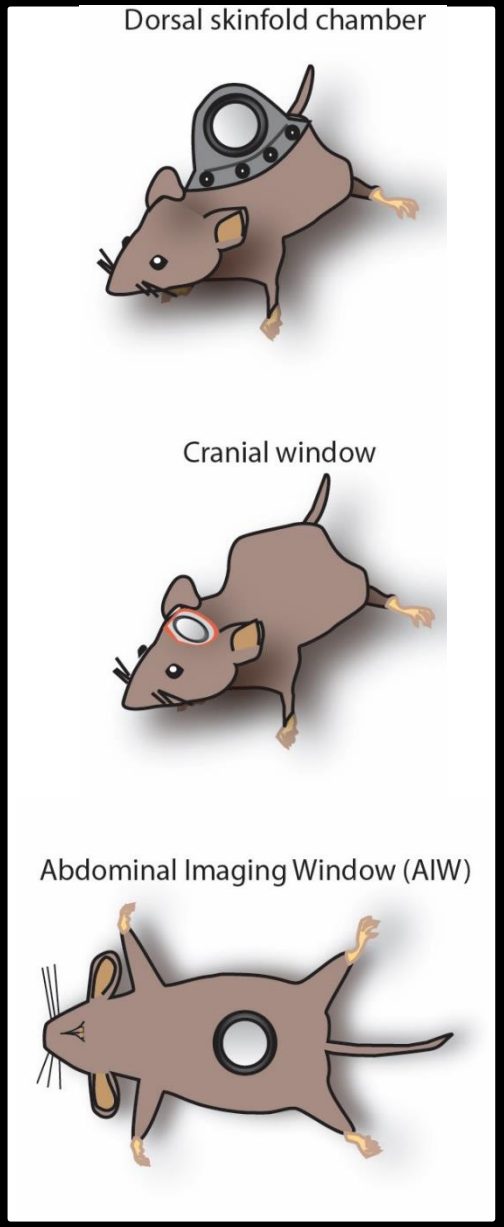
Gligorijvic *et al*, JVisExp, 2009

Ritsma *et al*, Nat Prot, 2013

- Window allows multiple imaging sessions

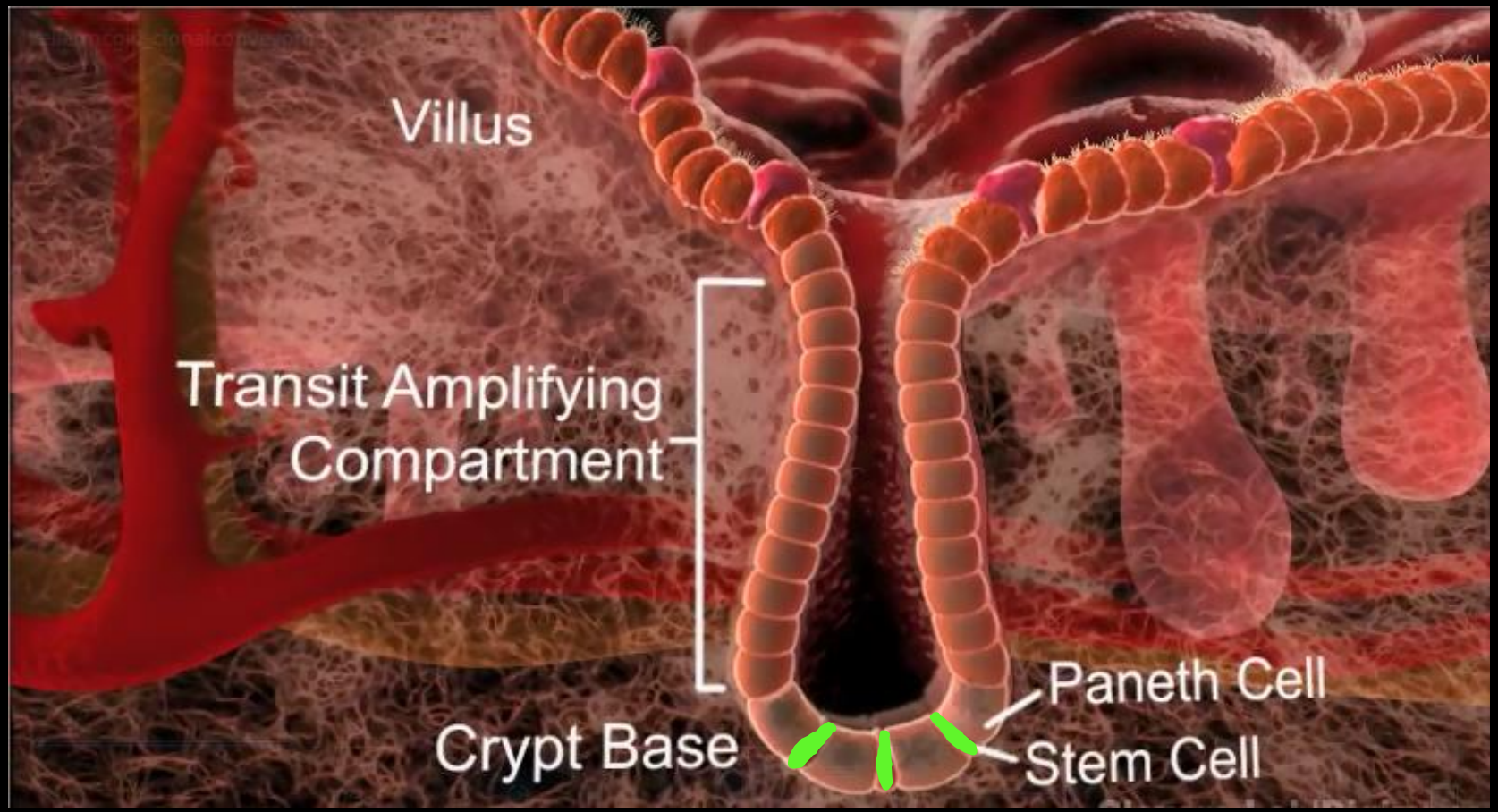


Imaging windows – long term IVM



Intestinal stem cell competition during homeostasis

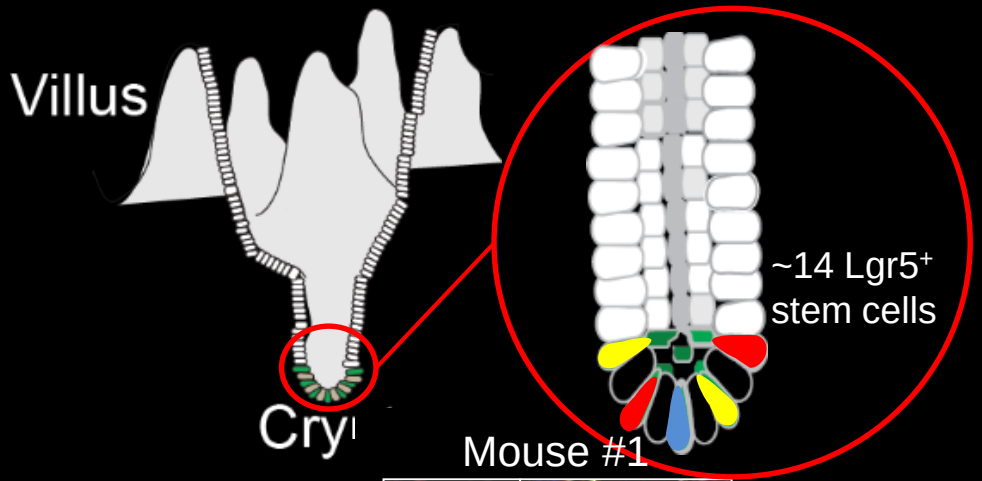
Intestinal stem cell homeostasis



- Rapidly self-renewing
- Intestinal lining refreshed every 2-4 days
- Bottom of crypt contains $Lgr5^{+}$ stem cells

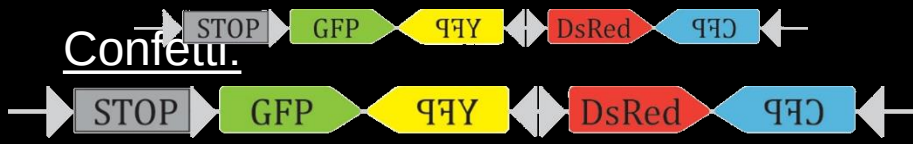
Clevers lab

Intestinal crypt homeostasis



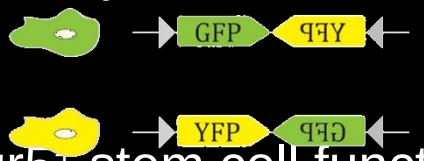
Lgr5: $Lgr5^{EGFP}$-IRES-$CreER^{T2}$

Confetti:

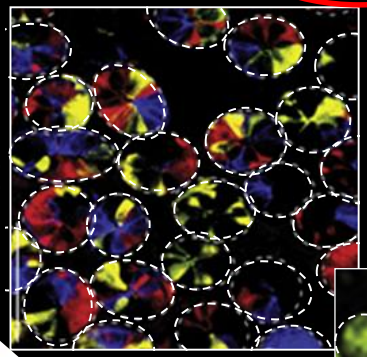


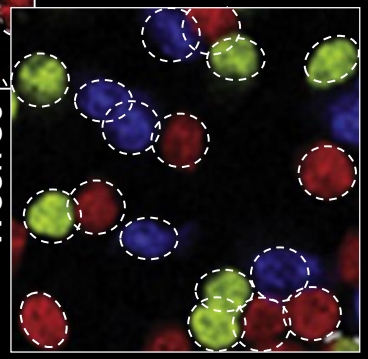
+ Tamoxifen =

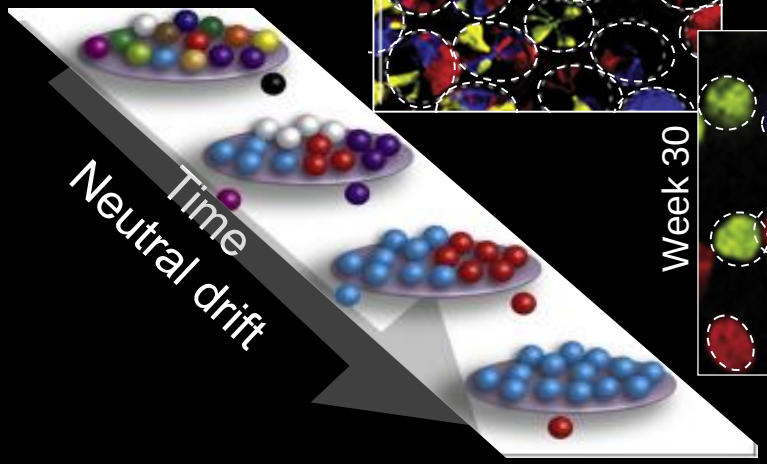
Stochastically induced color

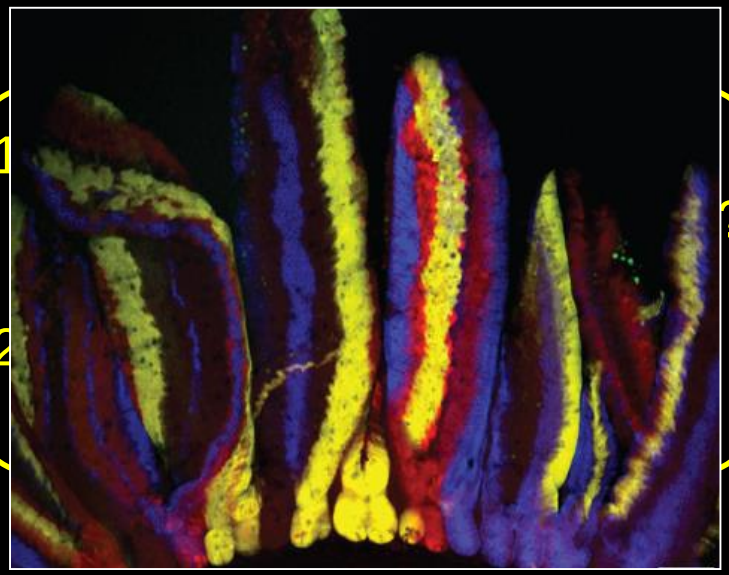


Only one $Lgr5^{+}$ stem cell functions as long-term stem cell







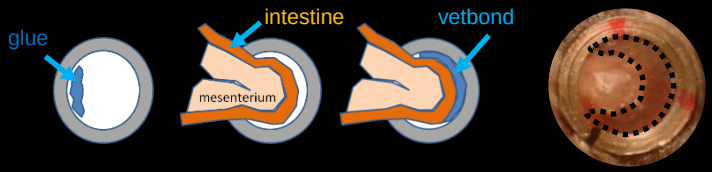


Neutral drift model: shown by the labs of Clevers and Winton
Snippert *et al.*, Cell 2010; Lopez-Garcia *et al.*, Science 2010

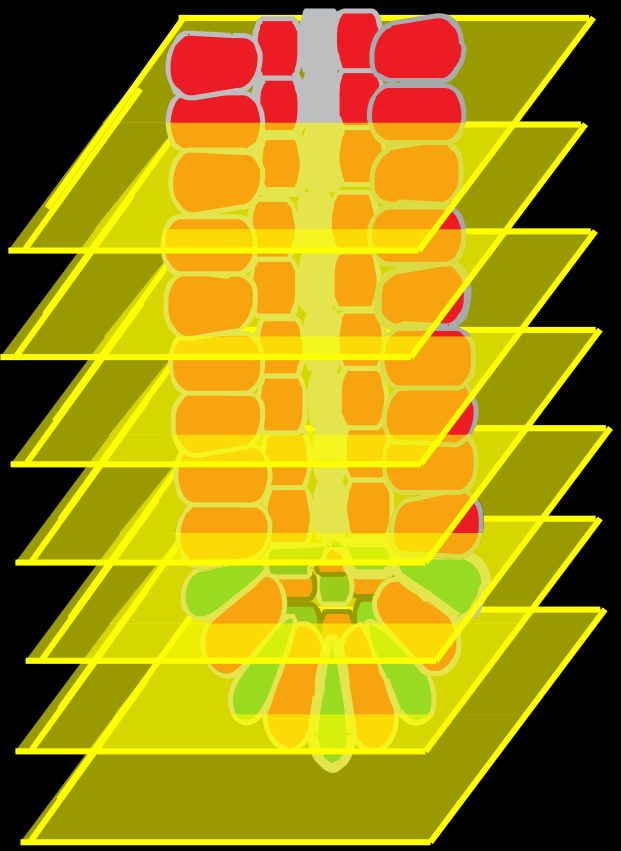
Do all cells participate in the competition?

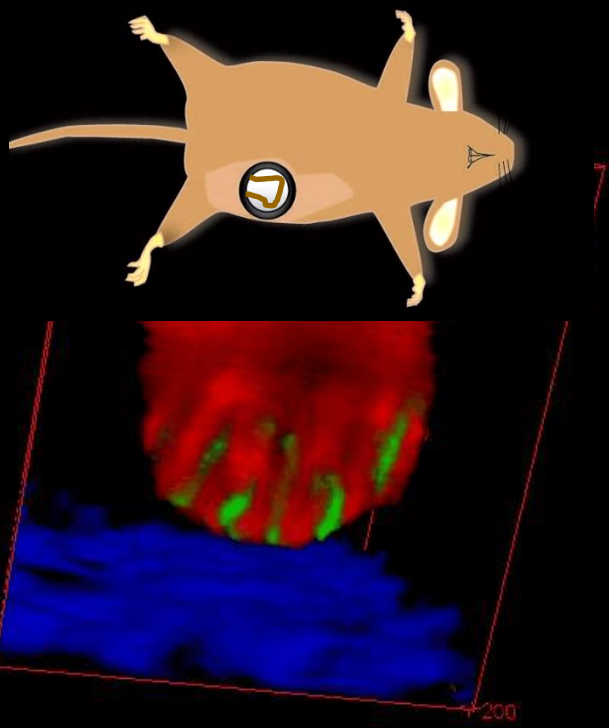
- ✓ Stem cell marker: Lgr5^{EGFP}-IRES-CreER^{T2}
- ✓ Lineage tracing: Confetti

- ✓ Imaging window: Abdominal imaging window



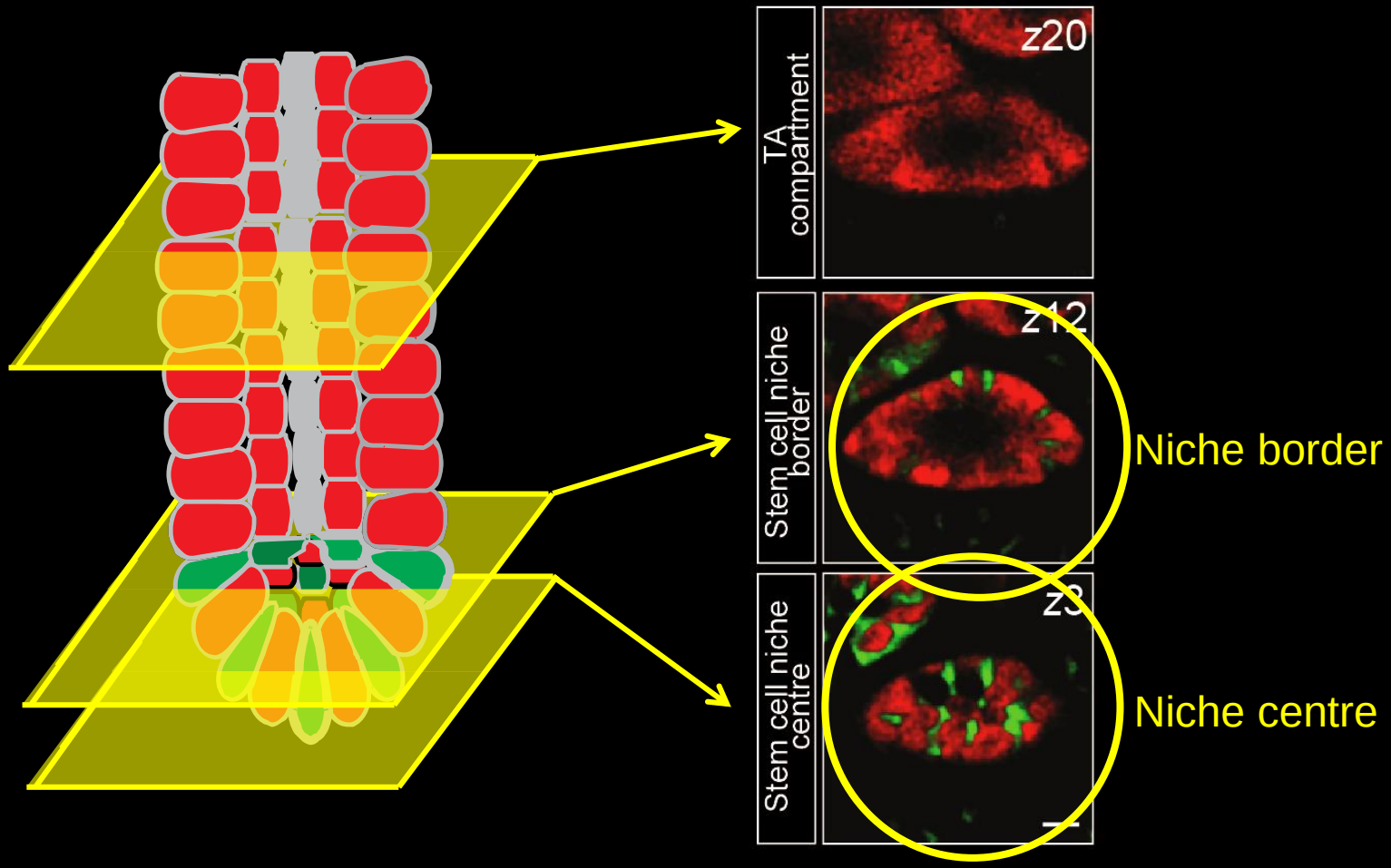
Reconstruction of a crypt





Laila Ritsma

Do all cells participate in the competition?

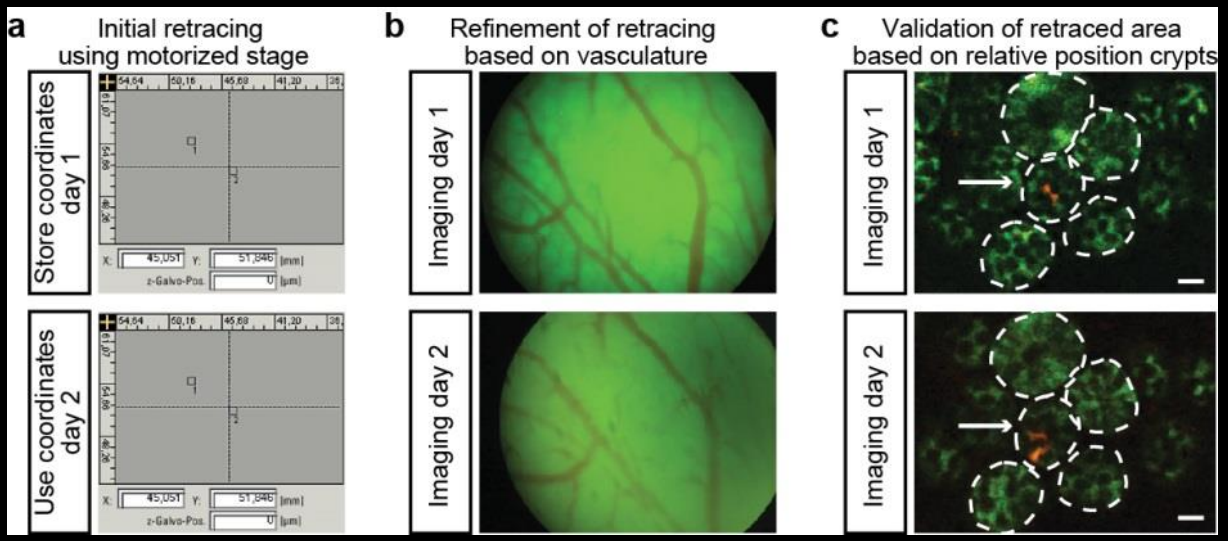


Can they both win the competition?

Equal competitional strength?

Intravital imaging of intestinal stem cells: re-tracing

- ✓ Retracing imaging area:

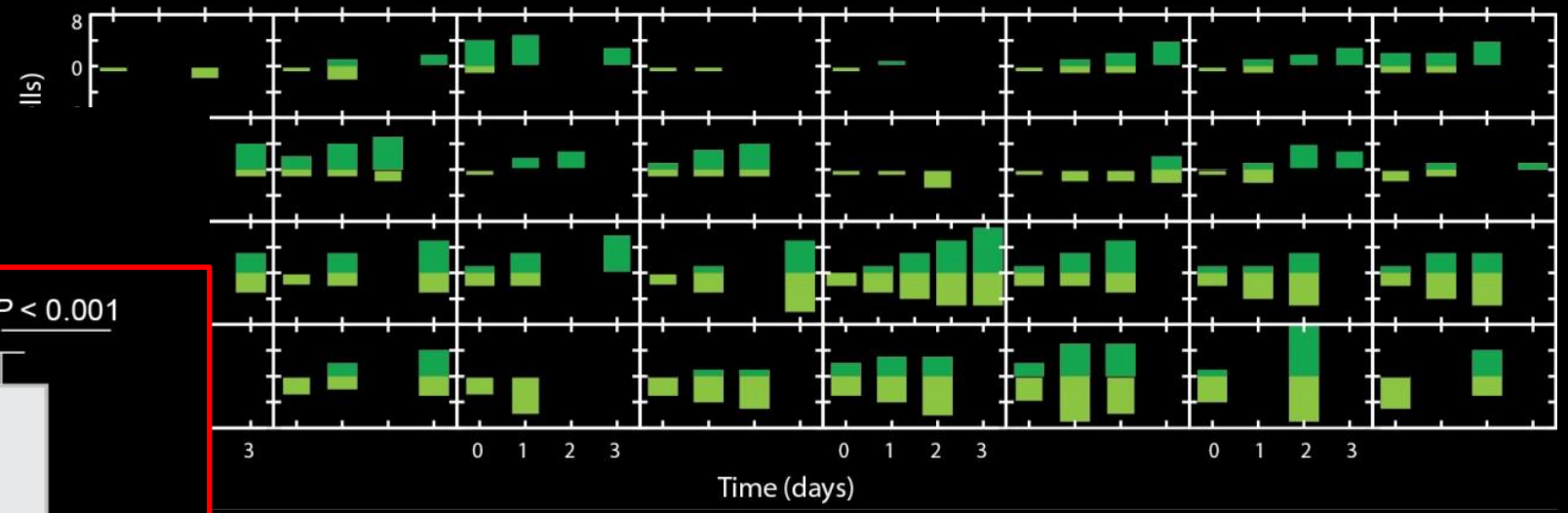


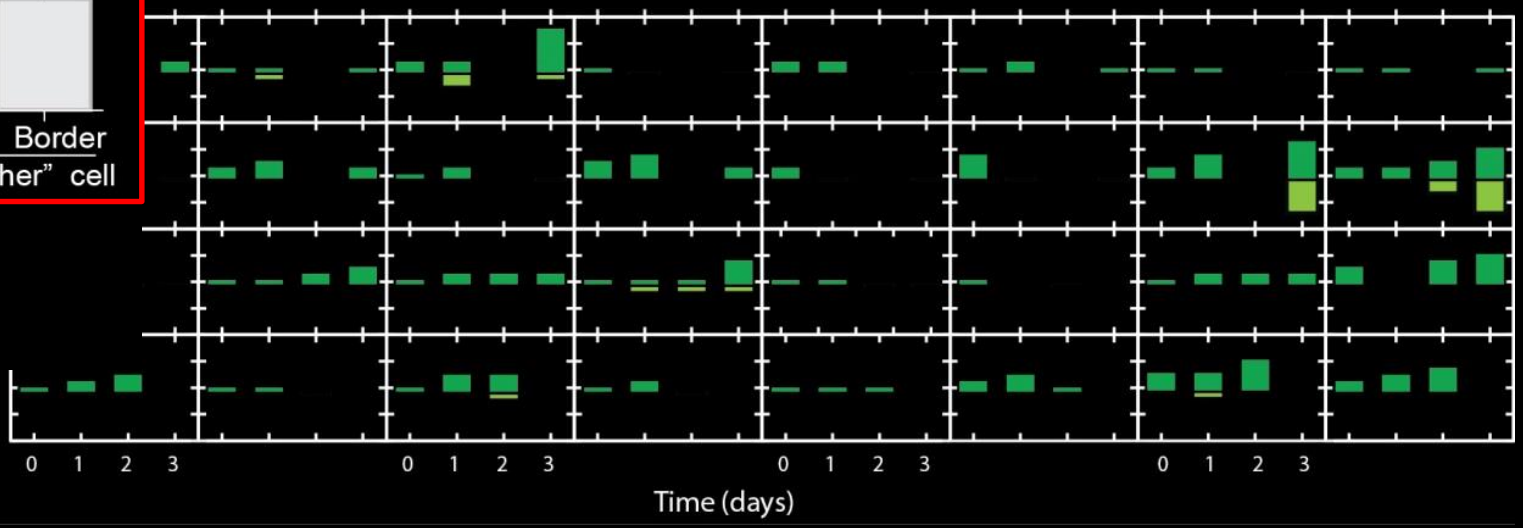
Ritsma*, Ellenbroek* *et. al*., Nature, 2014

Central and border stem cells participate in the competition

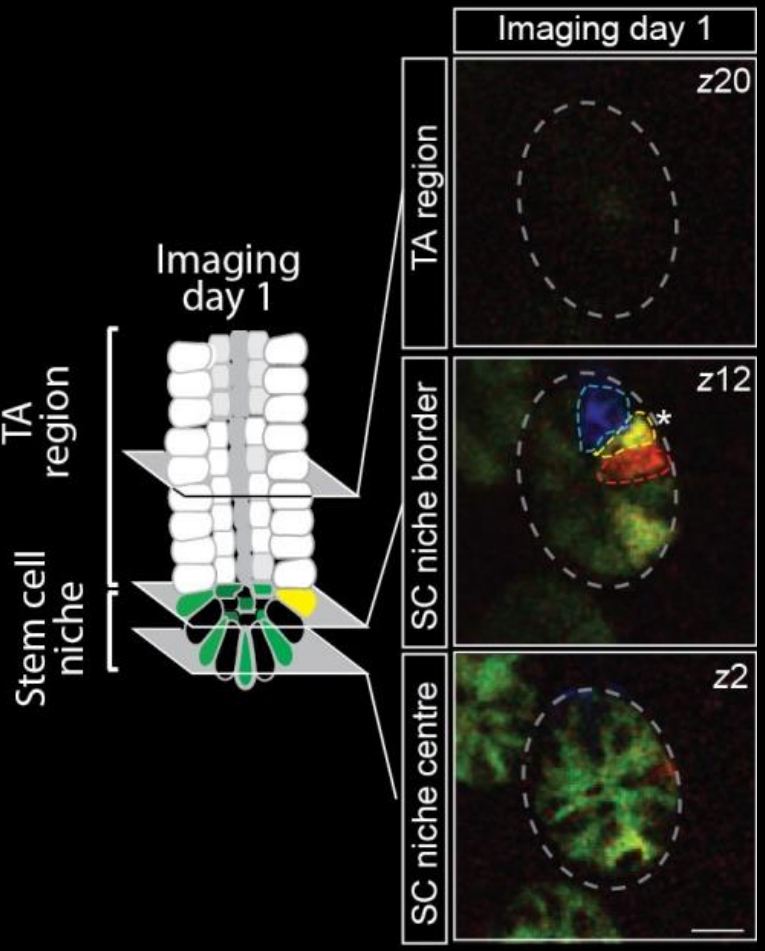
Start @ centre

Start @ border





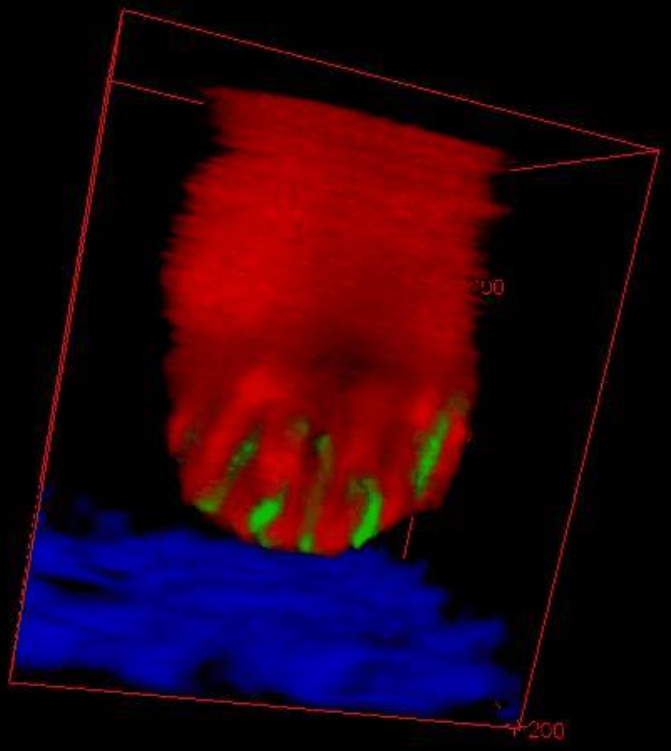
SCs can be expelled from the SC niche by passive displacement



Competition for space:
Repulsion from niche due to division of neighbouring stem cells

Ritsma*, Ellenbroek* *et al.*, Nature 2014

Conclusions IVM analysis stem cell homeostasis in SI



- There are ~14 stem cells, but only one of them wins the competition and is therefore a functional (long-term) stem cell
- Microenvironment determines stemness

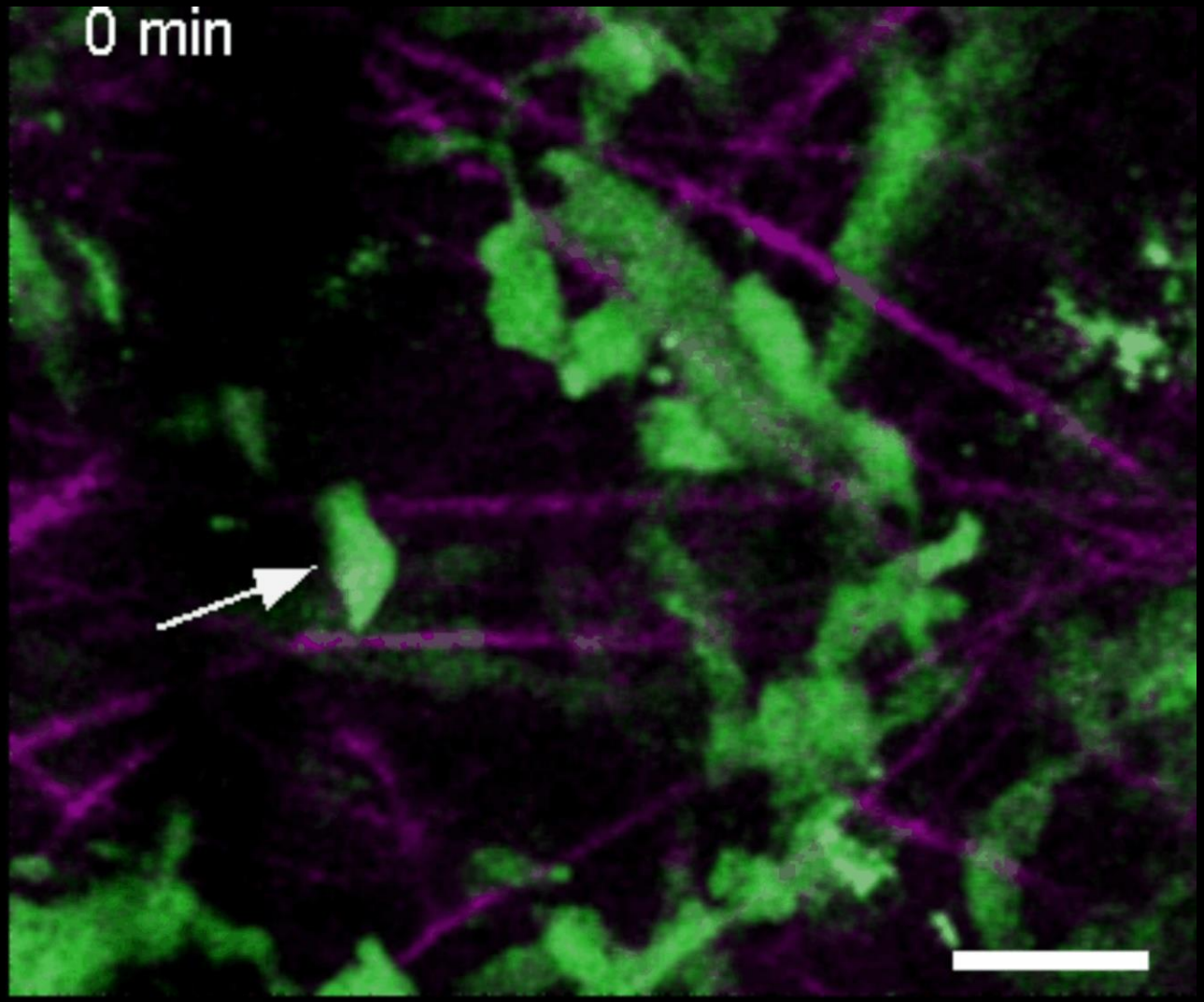
Vermeulen & Snippert, Nat Rev Cancer 2014

- Stem cells at the center of the niche have an advantage over stem cells at the border
- Position determines probability of ISC functionality
- Through transfer between centre and border region all Lgr5 stem cells can act as long-term stem cells

Transfer of extracellular vesicles between tumor cells

Tumor cells release extracellular vesicles (EVs)

Anoek Zomer



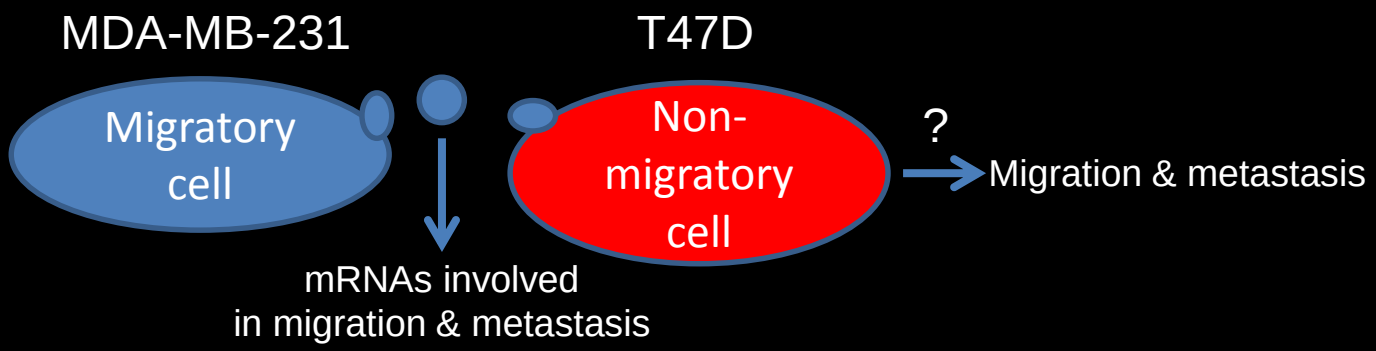
Green: Dendra2 mammary tumor cells

Purple: SHG (Type I Collagen)

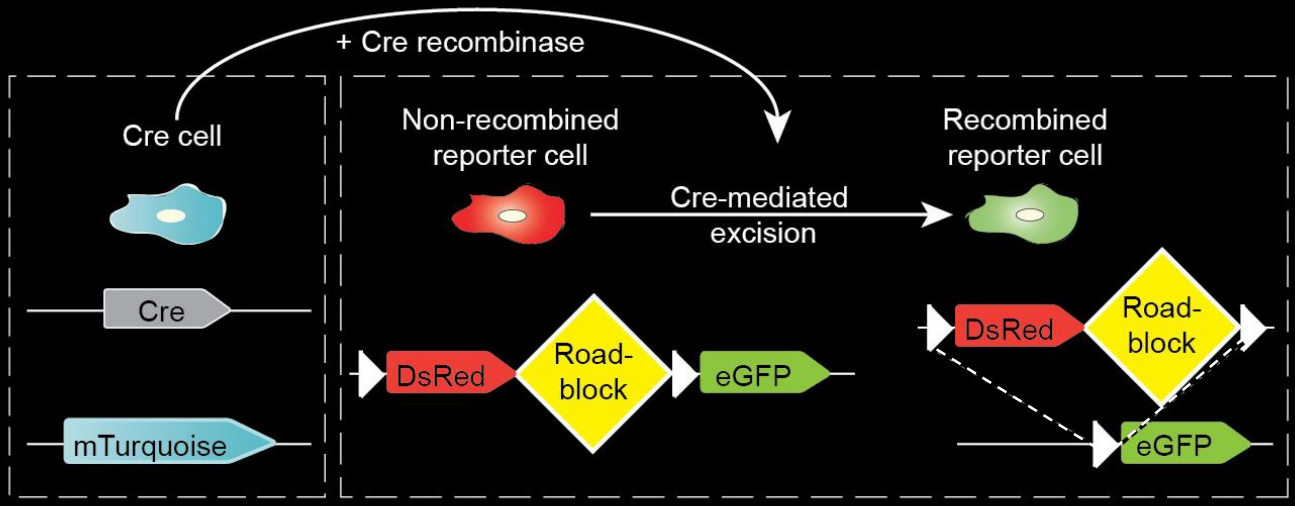
Do the extracellular vesicles (EVs) have a function in the observed migration?

Total time movie: 3 hrs

Labeling cells that have taken up EVs using a Cre-LoxP method



How to identify those cells that have taken up EVs to study their behavior?

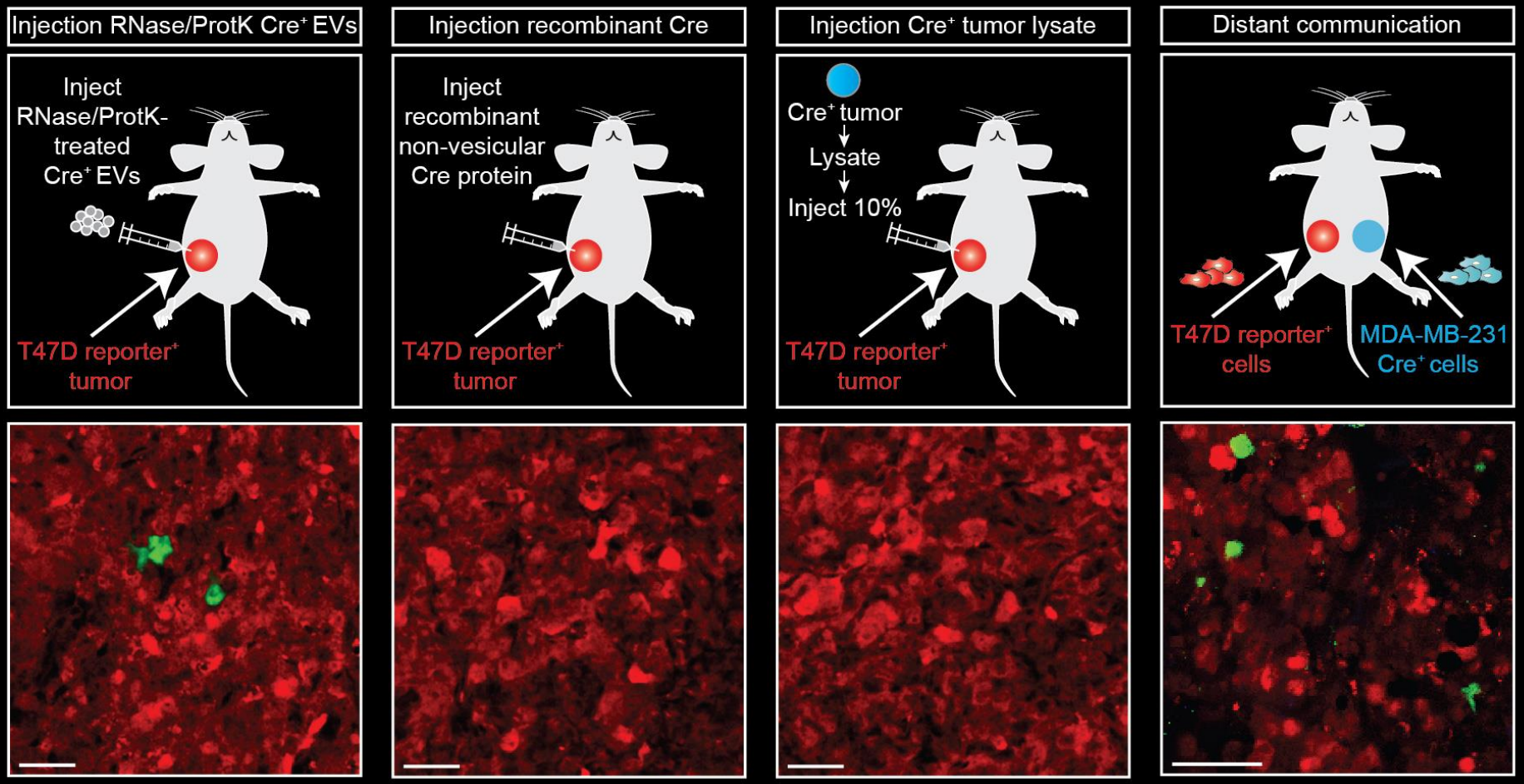


DsR^{+} reporter cells: no vesicle uptake

eGFP^{+} reporter cells: vesicle uptake

Zomer et al, Cell, 2015

Tumor cells exchange EV-mRNA locally and at large distances

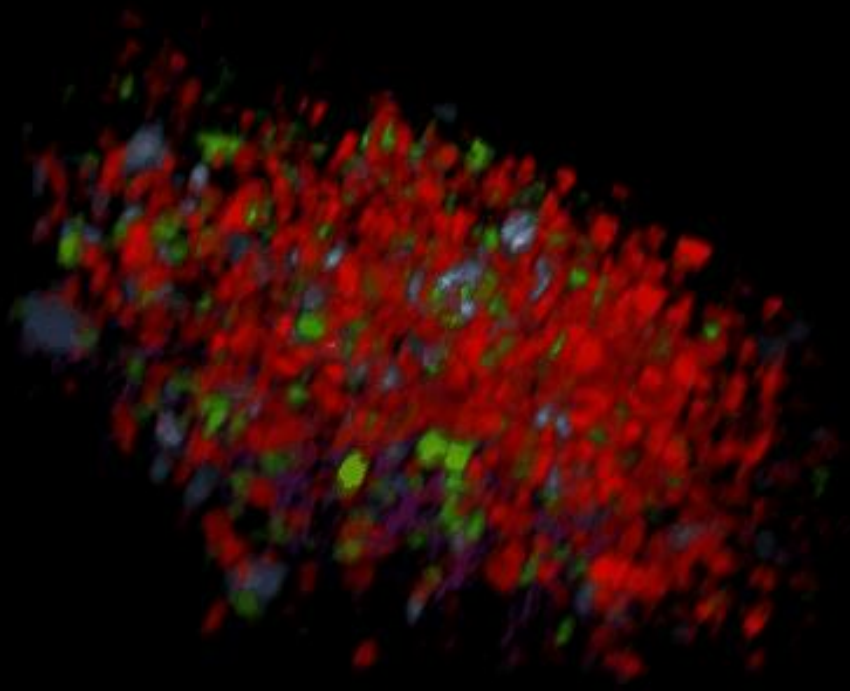


Cre+ cells; DsRed+ reporter cells; eGFP+ reporter cells

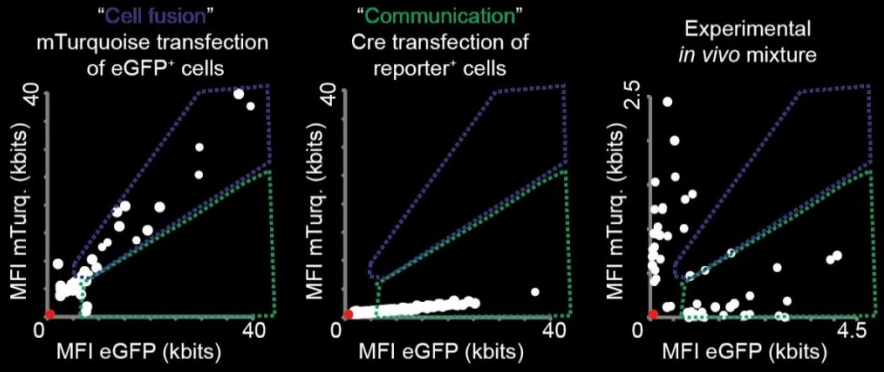
Anoek Zomer

Zomer *et al.*, Cell, 2015

Tumor cells exchange EV-mRNA locally and at large distances



Cre$^+$ cells; DsRed$^+$ reporter cells; eGFP$^+$ reporter cells

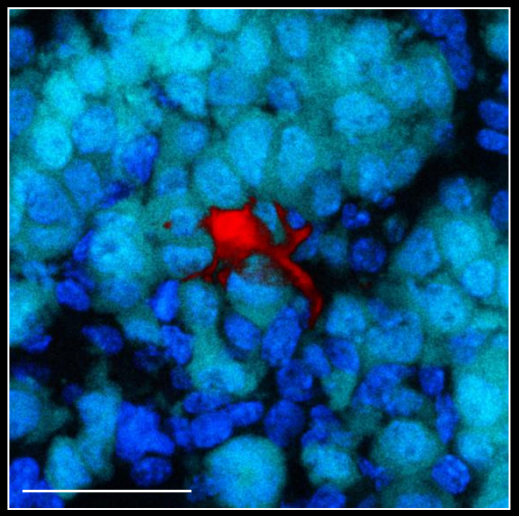


Anoek Zomer

Zomer *et al.*, Cell, 2015

Tumor cells exchange EV-mRNA to cells throughout the body

Tumor

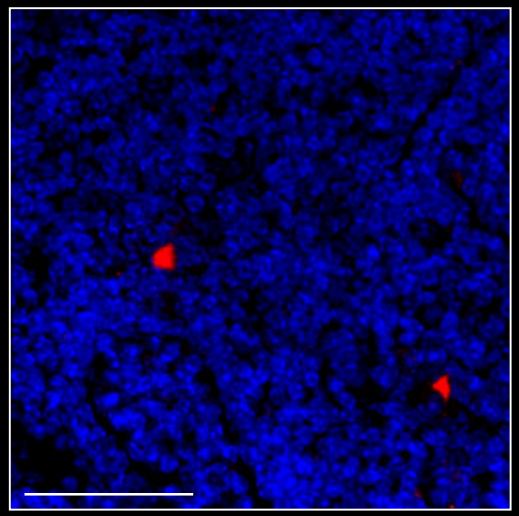


Cre+ melanoma tumor cells

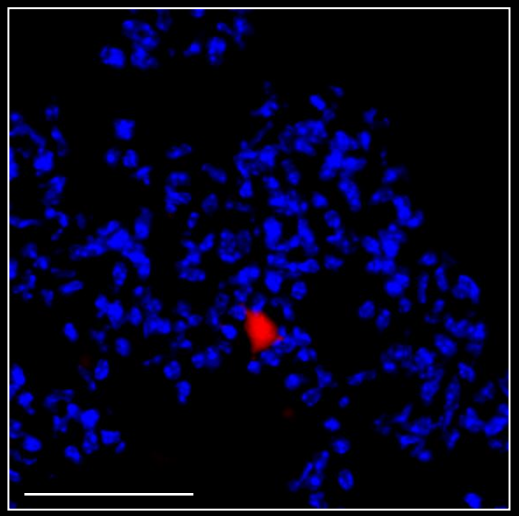
Non-tumor reporter cells

Dapi

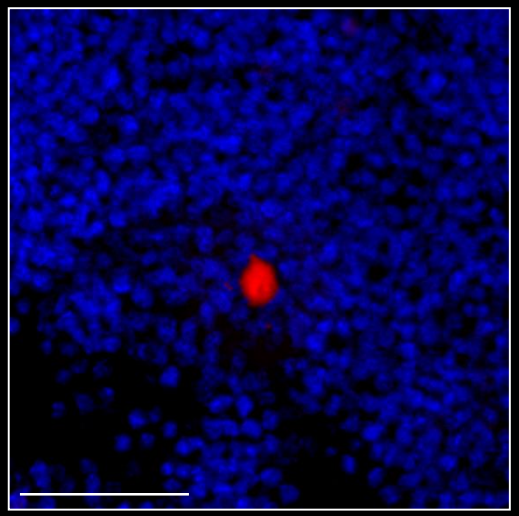
Lymph node



Lungs



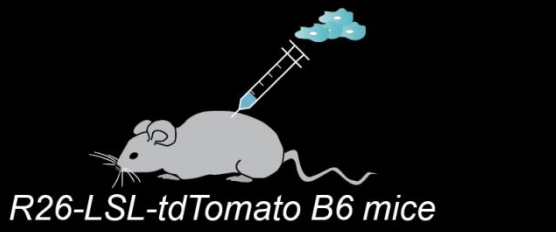
Spleen



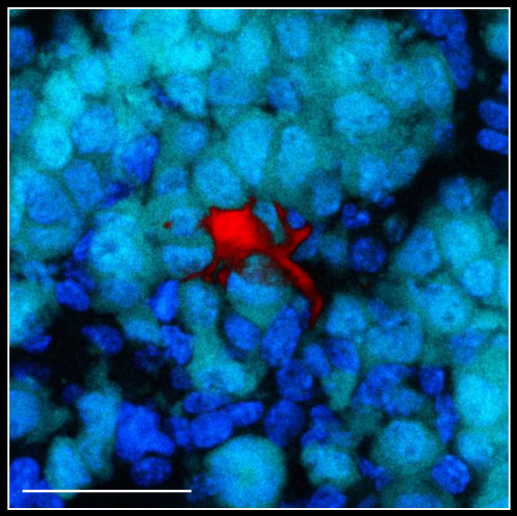
With the help of Carrie Maynard

Zomer *et al.*, Cell, 2015

Tumor cells exchange EV-mRNA to cells throughout the body



Tumor



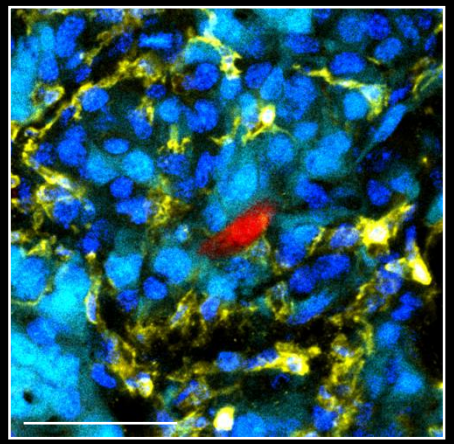
Cre+ melanoma tumor cells

Non-tumor reporter cells

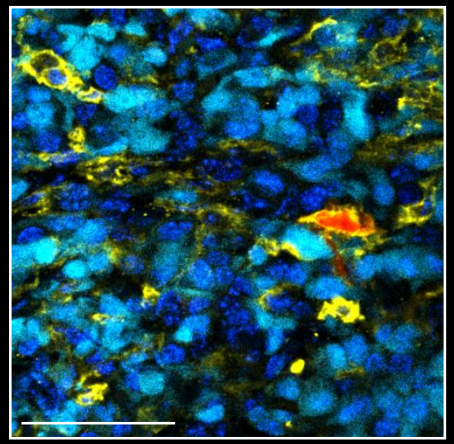
Dapi

CD45 immune cell staining

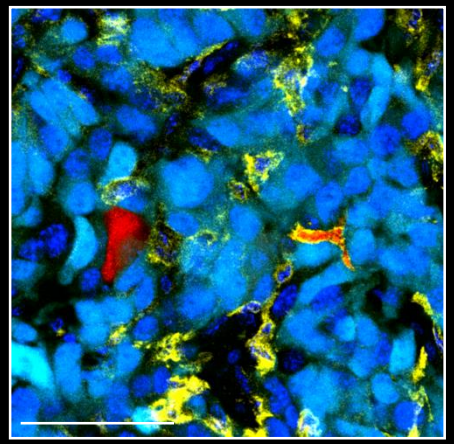
CD45- reporter cell



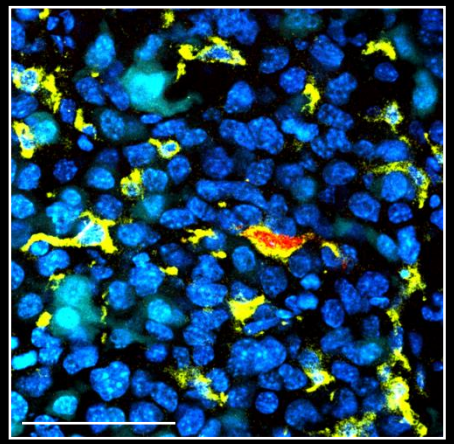
CD45+ reporter cell



F4/80 macrophage staining



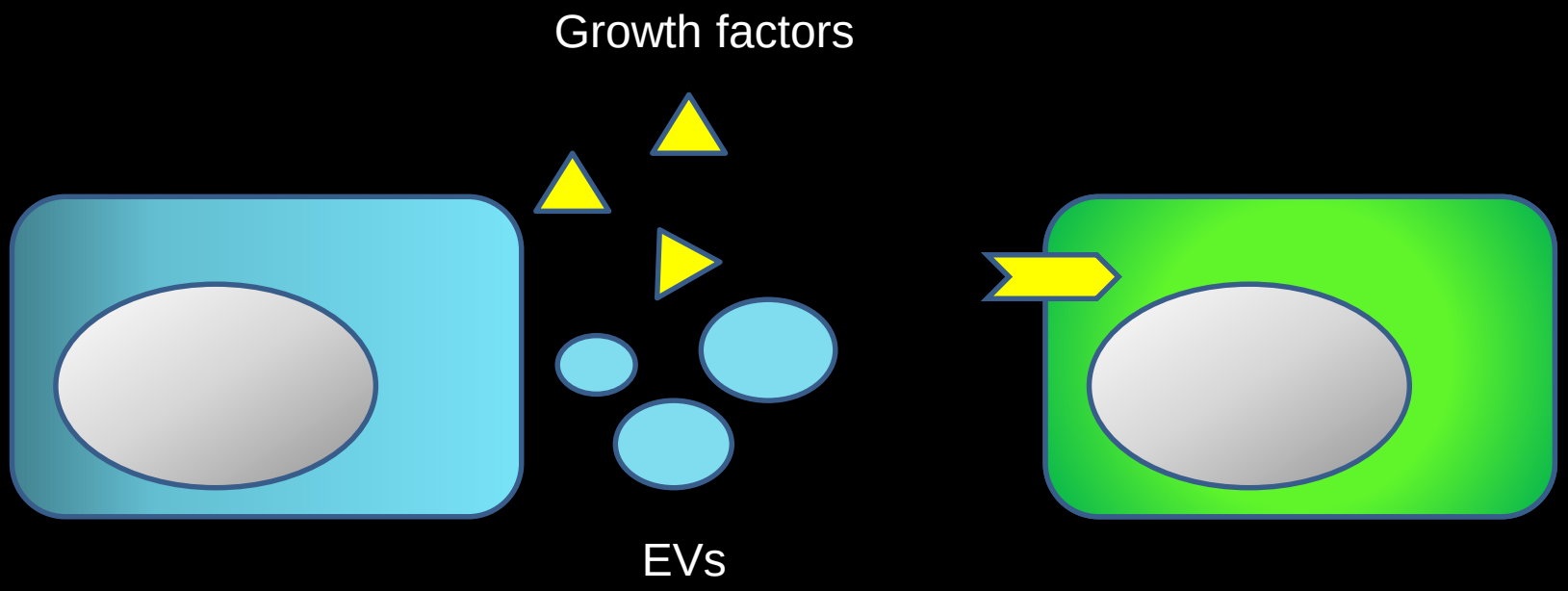
Gr1 neutrophil staining



With the help of Carrie Maynard

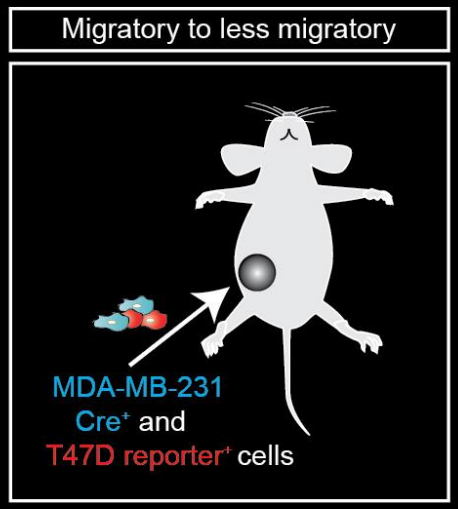
Zomer *et al.*, Cell, 2015

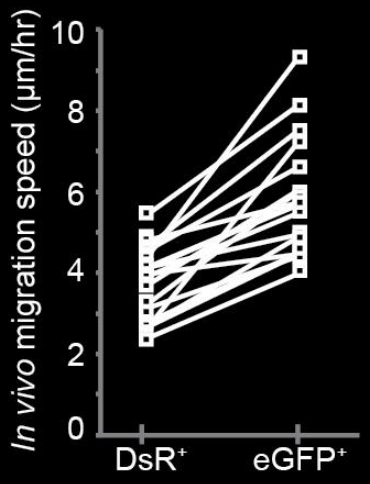
Intercellular communication through extracellular vesicles

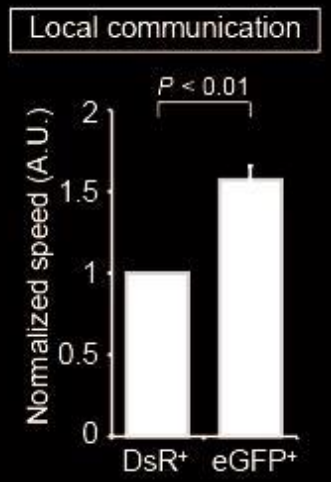


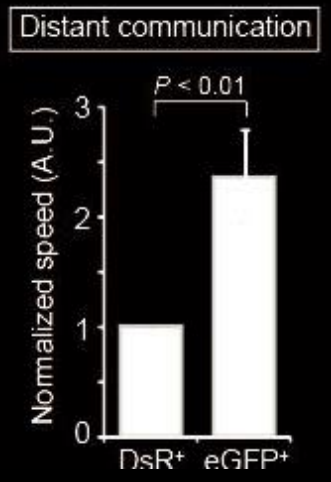
As with growth factors, EV-mediated communication may have various effects

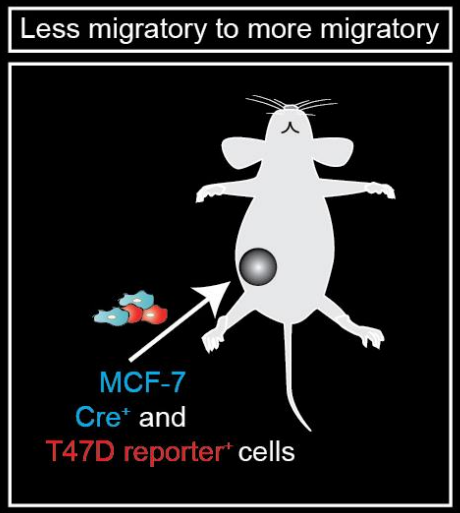
Is transfer of EVs linked to migratory behavior of tumor cells?

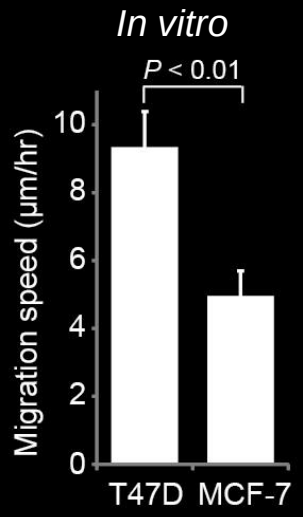


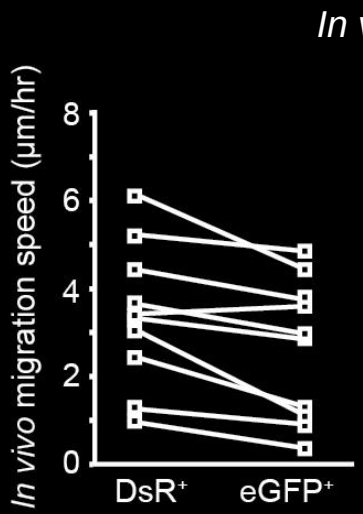


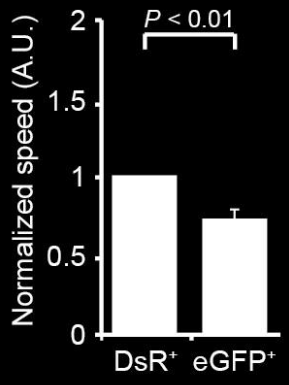






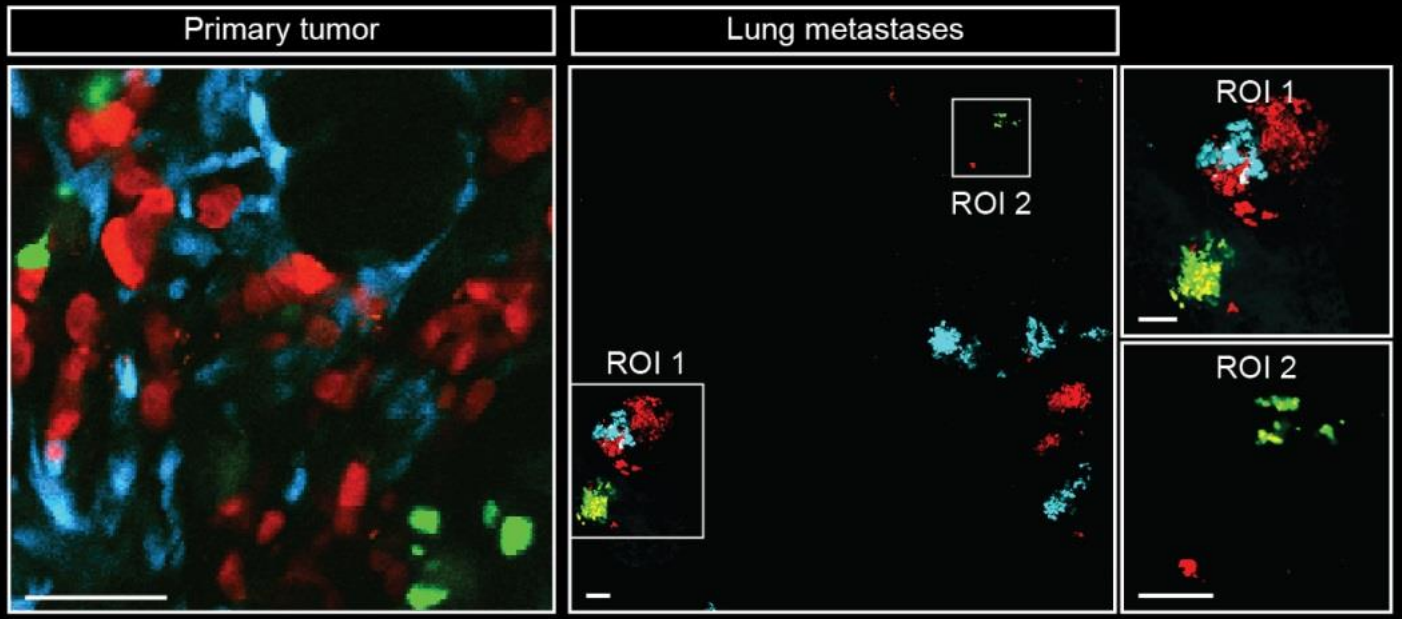






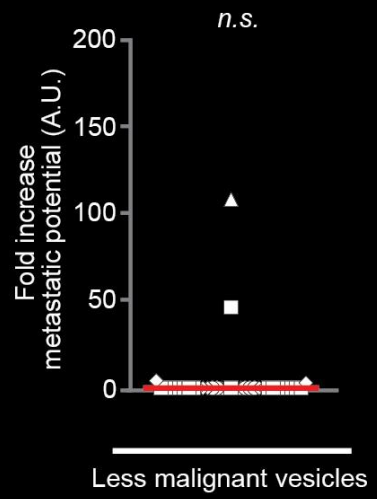
Zomer *et al.*, Cell, 2015

Is transfer of EVs linked to metastatic behavior of tumor cells?



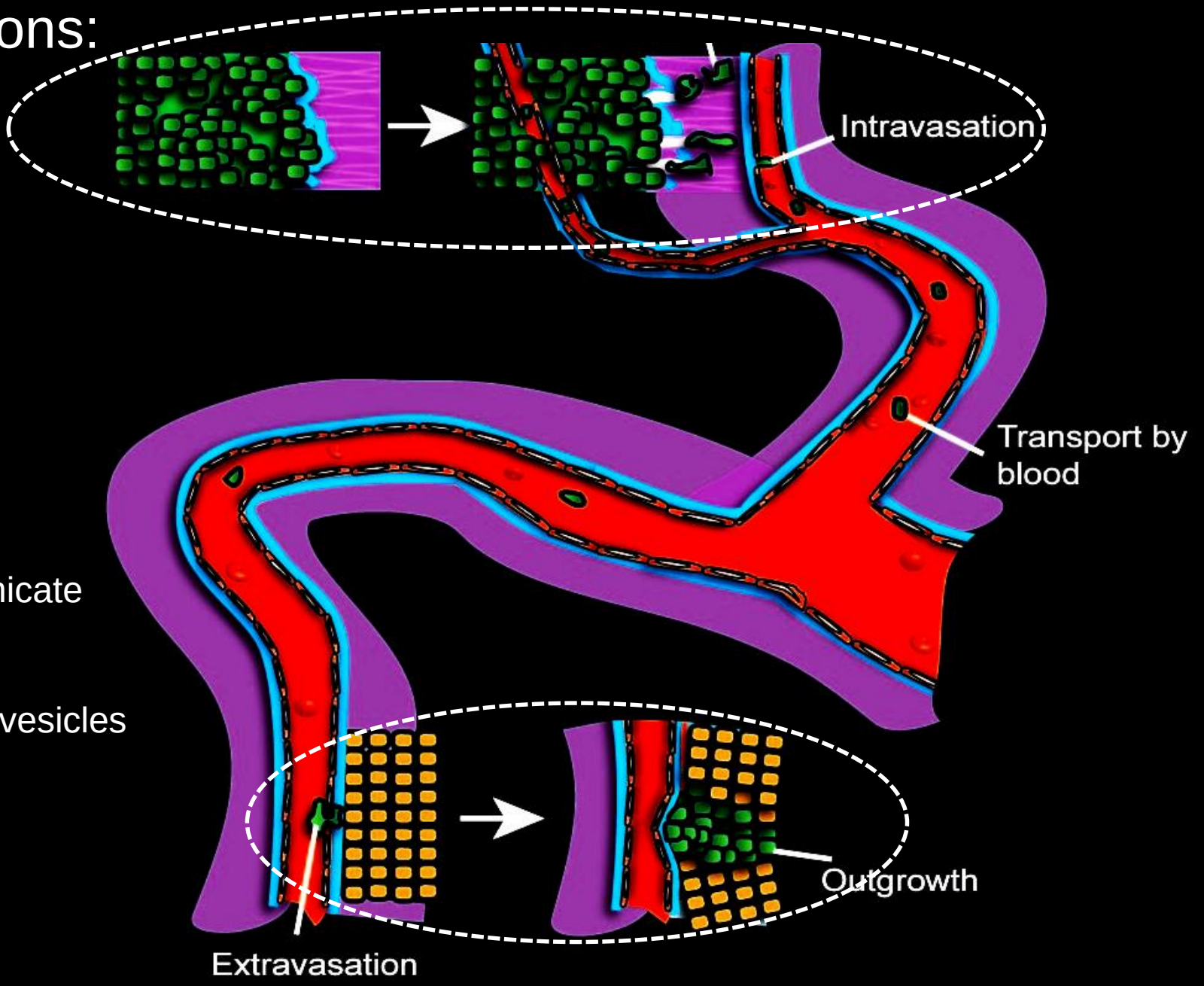
No increased metastatic potential

$$\frac{\text{Metastases Green/Red}}{\text{Primary tumor Green/Red}} = 1$$



Zomer *et al.*, Cell, 2015

Conclusions:



Cells communicate locally and systemically through microvesicles

Genetic dissection of colorectal cancer progression by orthotopic transplantation of engineered cancer organoids

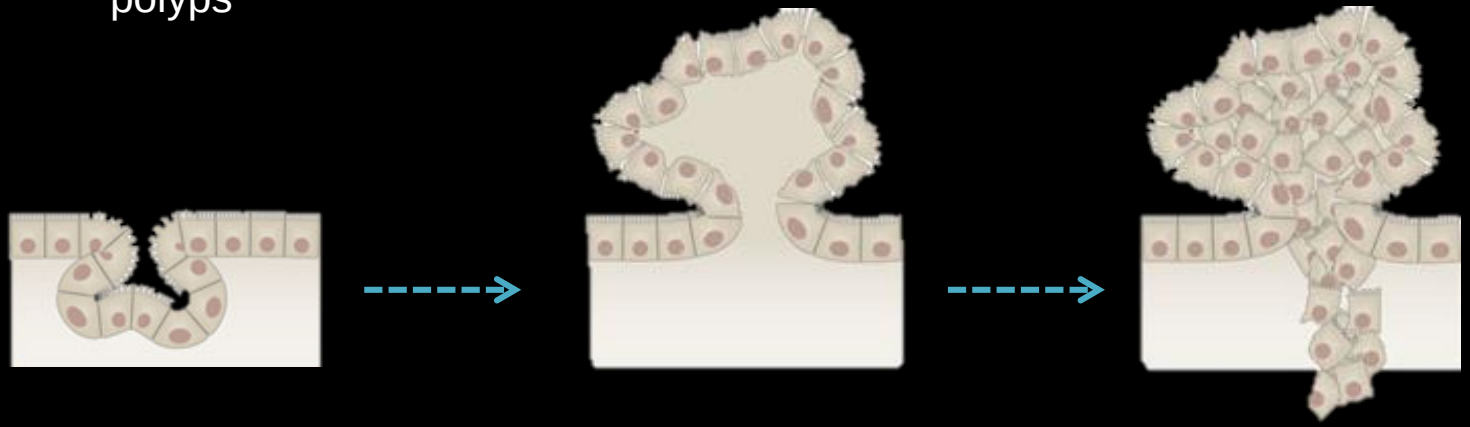
Study the genetics of human colorectal cancer

Colorectal cancer adenoma-carcinoma sequence *(*Fearon and Vogelstein*, Cell 1990)*

ACF/adenomatous polyps

Intermediate adenoma

Carcinoma/ metastatic carcinoma



Mutations in Wnt pathway

Mutation in EGFR pathway

Mutations in P53, BMP, TGFβ pathway + other chromosomal aberration

What is the contribution of the different mutations to the different steps of CRC progression?

Current tools to study CRC cancer

Genetic mouse models

Mice die before developing metastases

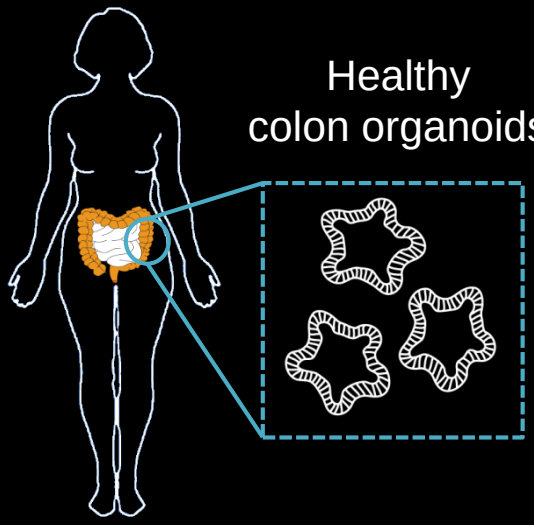
Most tumors are found in the small intestine

Injection of cell lines

No progression since it is an end-stage tumor

Do not represent tumor heterogeneity

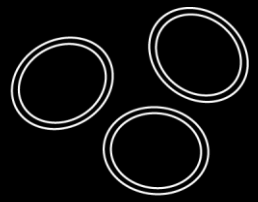
Model the adenoma-carcinoma sequence *in vitro*



Healthy colon organoids

In vitro engineering with CRISPR/Cas9

To introduce mutations in CRC driver genes (*KRAS*, *APC*, *P53*, *SMAD4*)



Tumor organoids

Jarno Drost

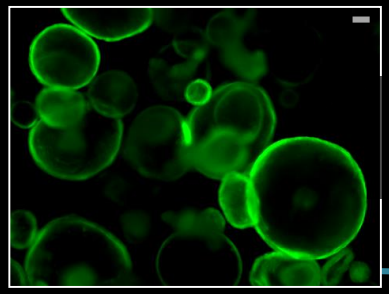
Hans Clevers

Can we make use of this system *in vivo*?

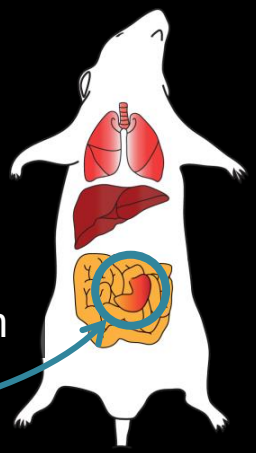
Drost *et al.*, Nature 2015

An organoid-based tumor model to study colorectal cancer *in vivo*

Murine
intestinal carcinoma organoids
$APC^{fl/fl}$, $KRAS^{G12D/+}$, $p53^{fl/R172H}$

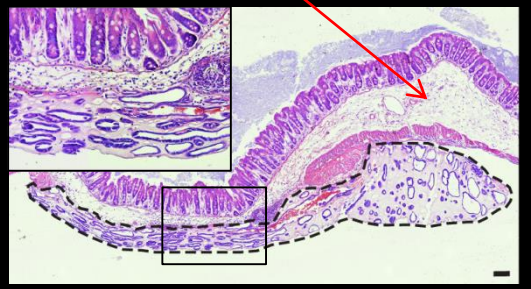


Orthotopic
transplantation



Proximal colon
(caecal wall)

submucosa



Fumagalli *et al*., PNAS, 2017

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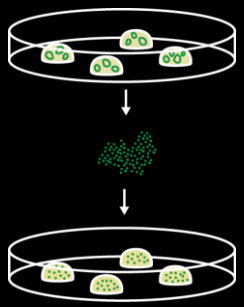

Orthotopic
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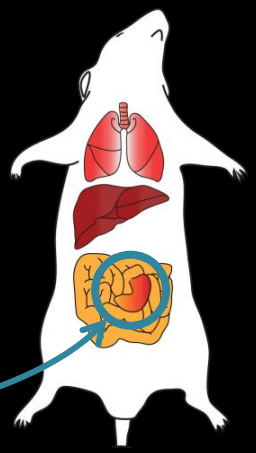
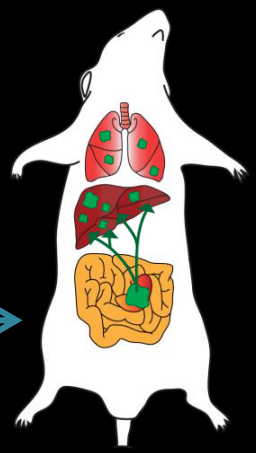
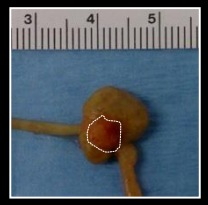
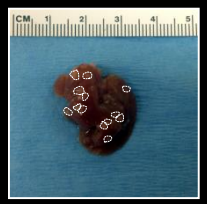
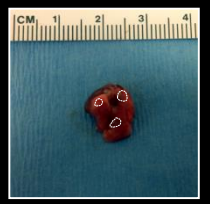


6-8
wks



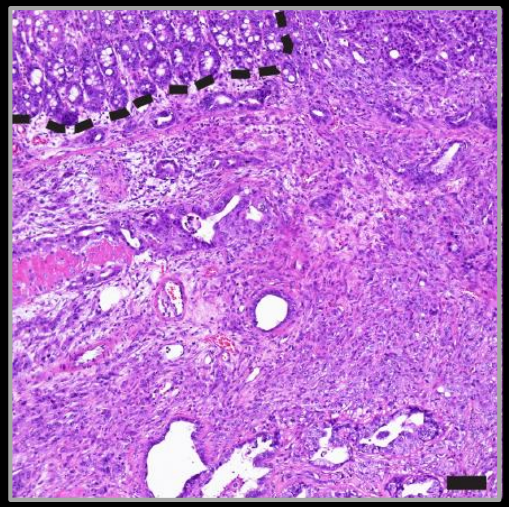




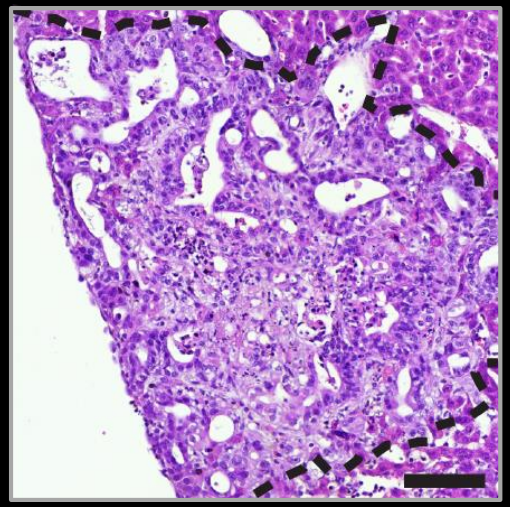


Spontaneous metastasis

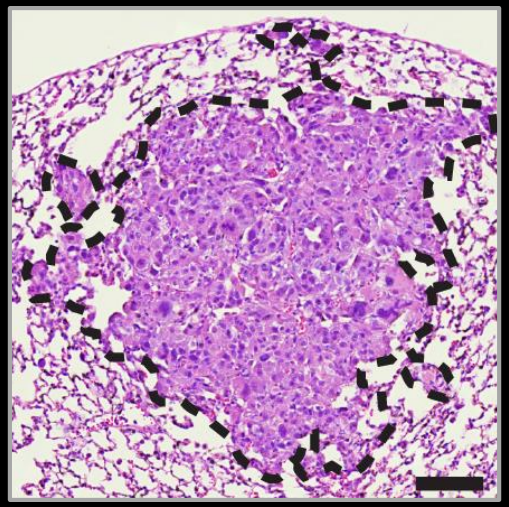
Primary tumor



Liver

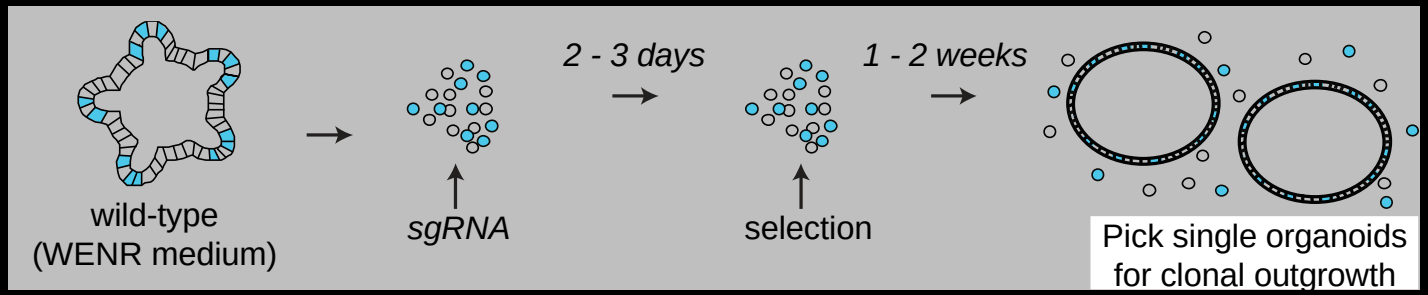


Lungs



Fumagalli *et al.*, PNAS, 2017

Generation of engineered human tumor-organoids



WNT/Rspondin
EGF
Nutlin-3
Noggin

Genotype					Selection
	KRAS^{G12D}	P53^{KO}	SMAD4^{KO}	Triple$^{APC WT}$	WNT + R-spondin / ~~EGF~~ / ~~Noggin~~
APC^{KO}		P53^{KO}	SMAD4^{KO}	Triple$^{KRAS WT}$	~~WNT + R-spondin~~ / EGF / ~~Noggin~~
APC^{KO}	KRAS^{G12D}		SMAD4^{KO}	Triple$^{P53 WT}$	~~WNT + R-spondin~~ / ~~EGF~~ / ~~Noggin~~
APC^{KO}	KRAS^{G12D}	P53^{KO}		Triple$^{SMAD4 WT}$	~~WNT + R-spondin~~ / ~~EGF~~ / Noggin
APC^{KO}	KRAS^{G12D}	P53^{KO}	SMAD4^{KO}	Quadruple	~~WNT + R-spondin~~ / ~~EGF~~ / ~~Noggin~~

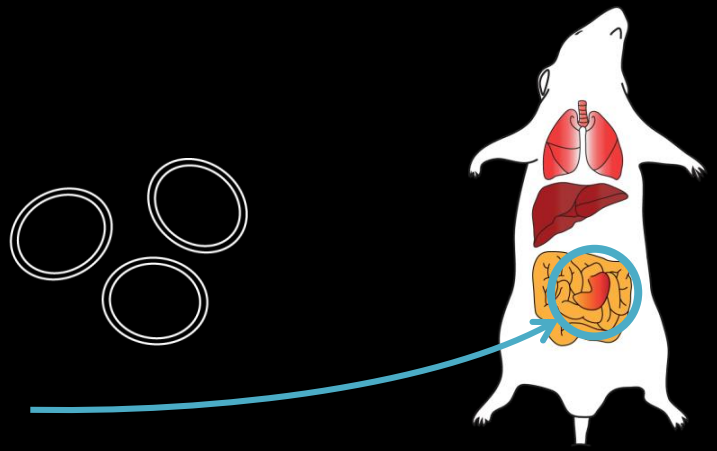
Drost *et al.*, Nature 2015

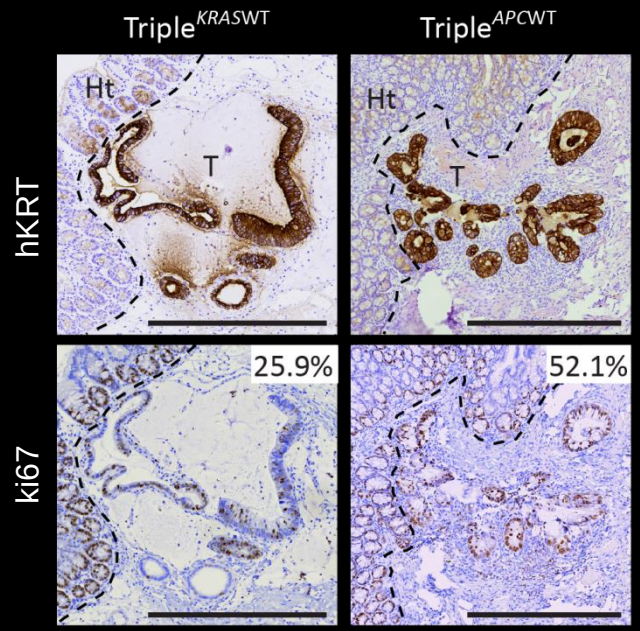

Jarno Drost

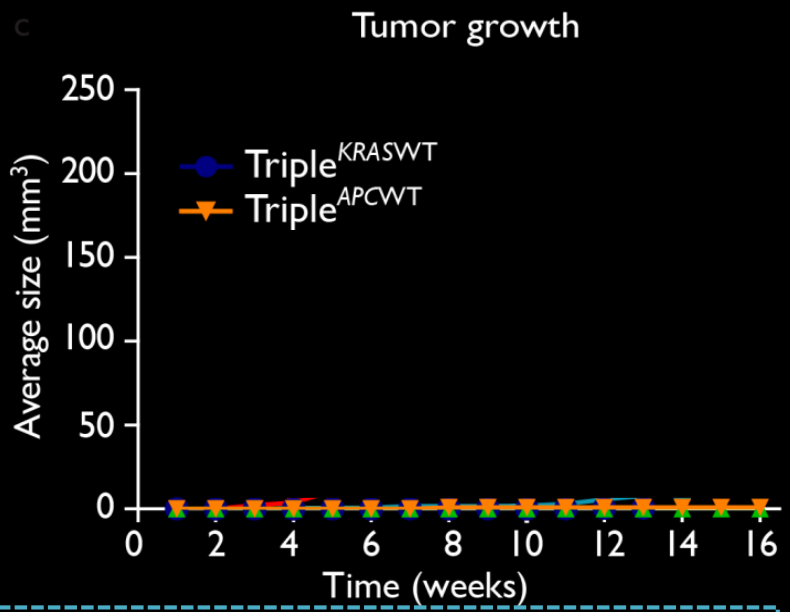

Hans Clevers

Orthotopic transplantation of human CRC organoids

Tumor organoids	Mutations
Triple^{KRASWT}	$APC^{KO}/P53^{KO}/SMAD4^{KO}$
Triple^{APCWT}	$KRAS^{G12D}/P53^{KO}/SMAD4^{KO}$
Triple^{P53WT}	$APC^{KO}/KRAS^{G12D}/SMAD4^{KO}$
Triple^{SMAD4WT}	$APC^{KO}/KRAS^{G12D}/P53^{KO}$
Quadruple	$APC^{KO}/KRAS^{G12D}/P53^{KO}/SMAD4^{KO}$



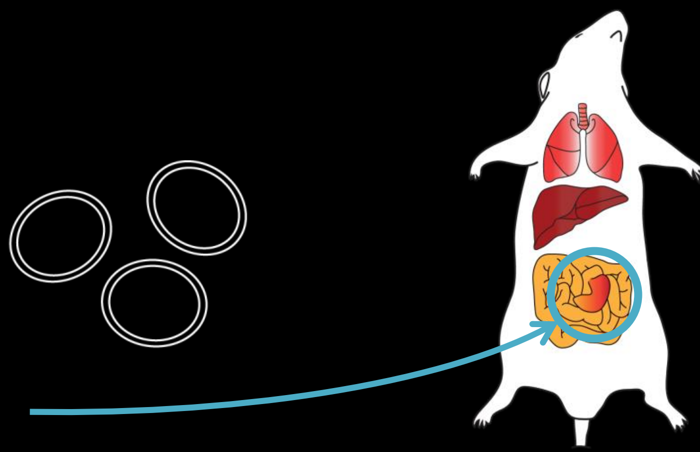


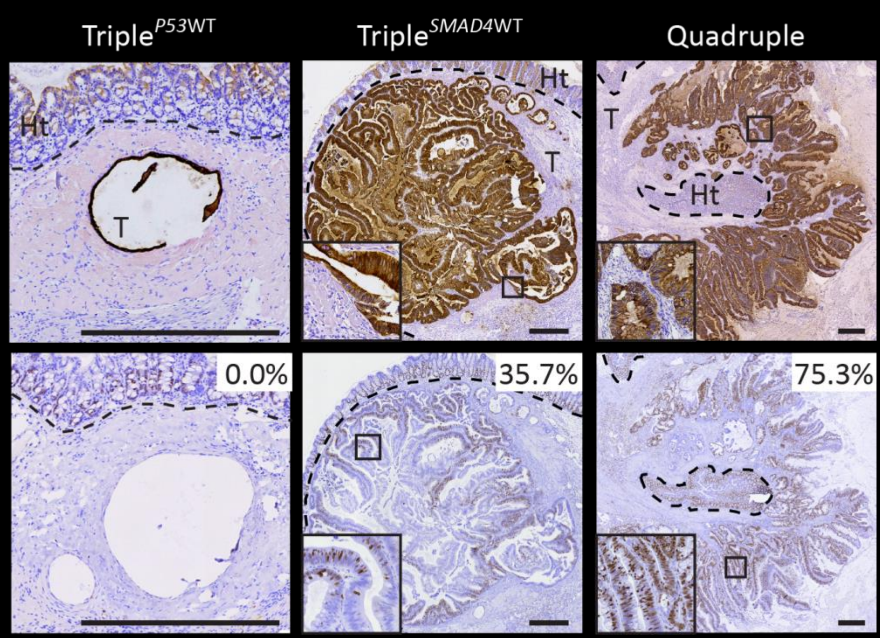


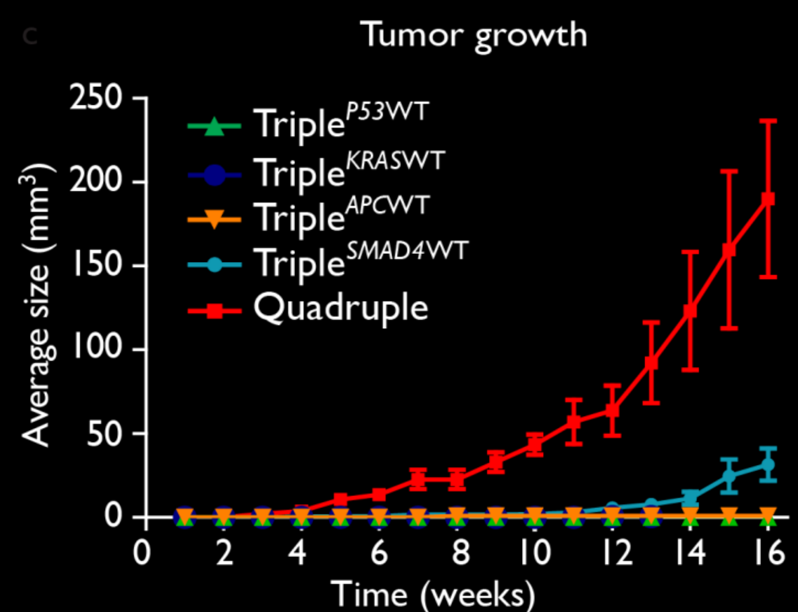
Absence of the driving *APC* or *KRAS* mutation: tumor cells lack proliferation-inducing signals

Orthotopic transplantation of human CRC organoids

Tumor organoids	Mutations
Triple^{KRASWT}	$APC^{KO}/P53^{KO}/SMAD4^{KO}$
Triple^{APCWT}	$KRAS^{G12D}/P53^{KO}/SMAD4^{KO}$
Triple^{P53WT}	$APC^{KO}/KRAS^{G12D}/SMAD4^{KO}$
Triple^{SMAD4WT}	$APC^{KO}/KRAS^{G12D}/P53^{KO}$
Quadruple	$APC^{KO}/KRAS^{G12D}/P53^{KO}/SMAD4^{KO}$



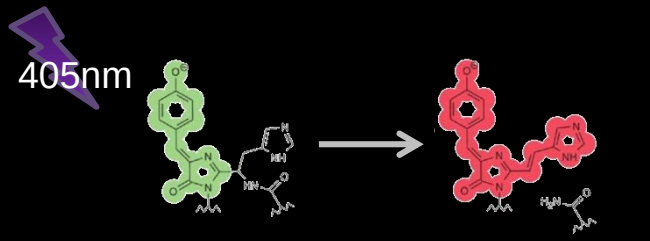




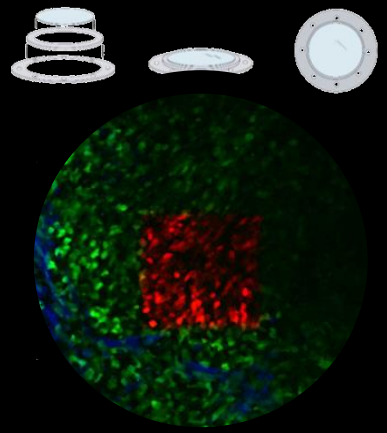
P53 loss is essential for proliferation of cells that possess the other oncogenic alterations

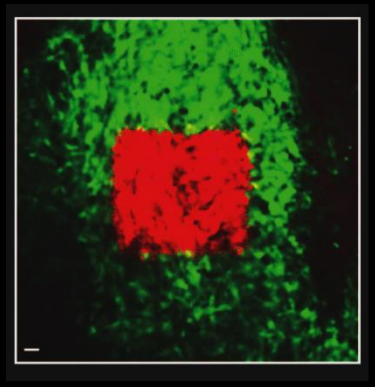
All four mutations are required for efficient growth of the primary tumor

Photomarking enables the visualization of motility within the tumor mass



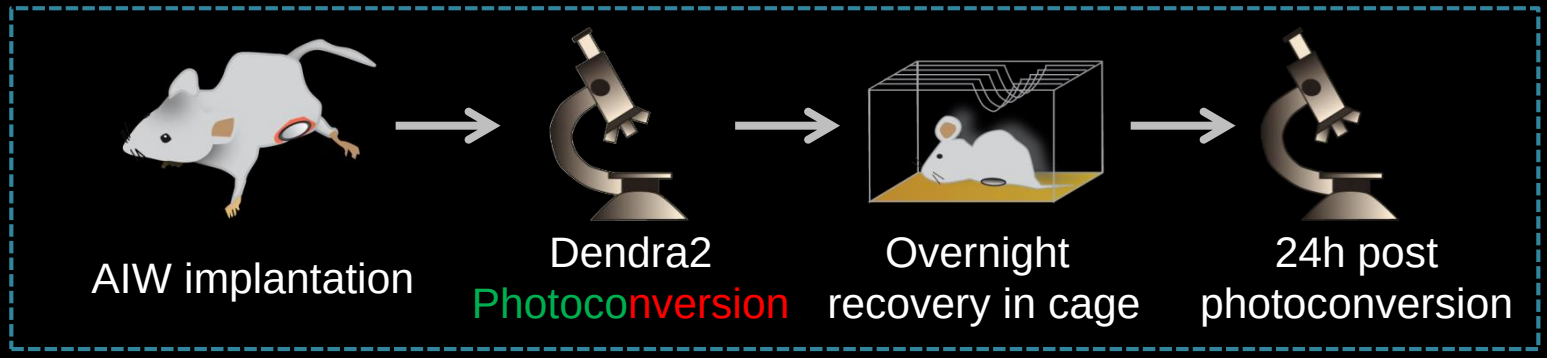
Kedrin *et al.,* Nature Methods 2008
Gligorijvic *et al*., JVisExp 2009

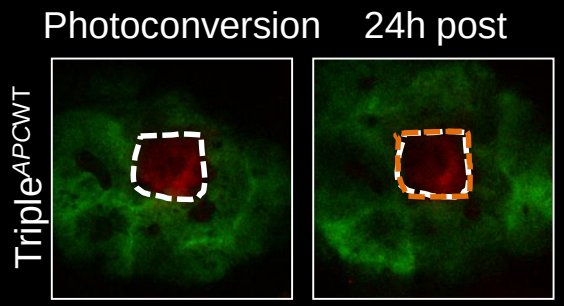


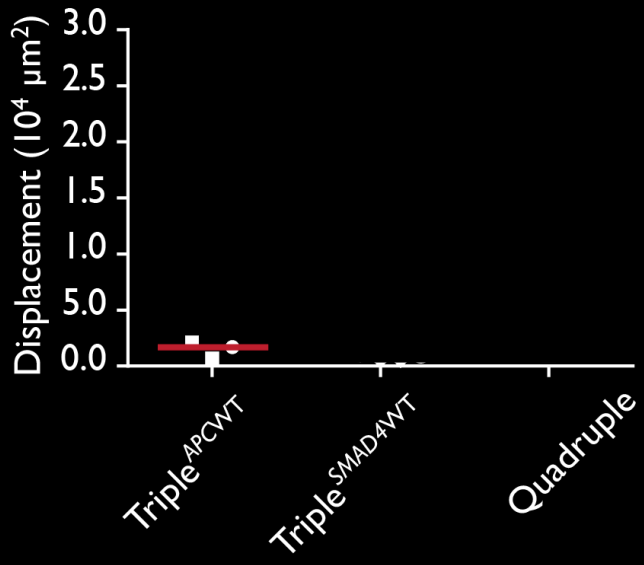


Beerling *et al.,* JCS 2011

Evaluate the role of CRC driver mutations in tumor cell migration





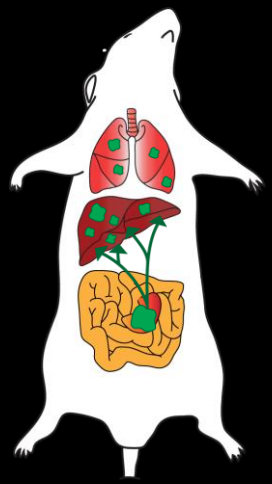


All four mutations are required for efficient migration

Fumagalli *et al.*, PNAS, 2017

Evaluate the role of CRC driver mutations in metastasis formation

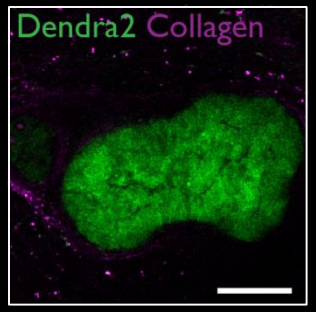
Spontaneous metastasis



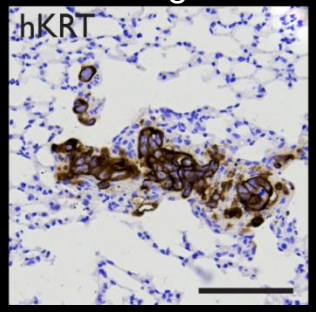
Triple^{P53WT}
Triple^{KRASWT}
Triple^{APCWT}
Triple^{SMAD4WT}
Quadruple

Quadruple

Liver



Lungs

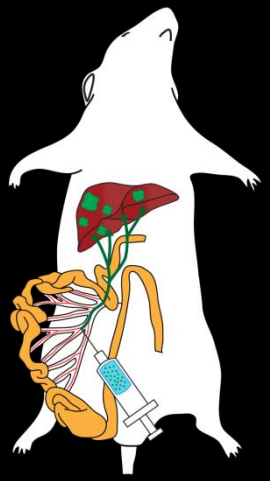


All four mutations are required for efficient metastasis

Fumagalli *et al.*, PNAS, 2017

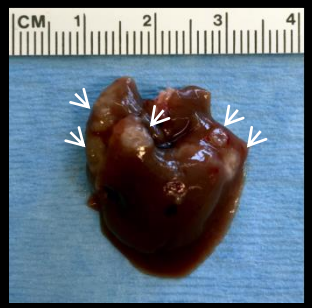
Evaluate the role of CRC driver mutations in metastasis formation

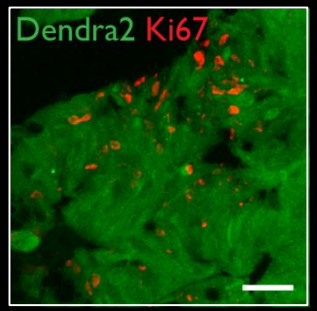
Metastatic colonisation



Triple^{P53WT}
Triple^{KRASWT}
Triple^{APCWT}
Triple^{SMAD4WT}
Quadruple

Quadruple

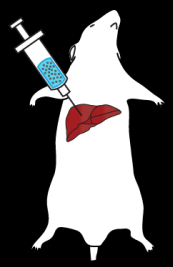




All four mutations are required for efficient metastatic outgrowth

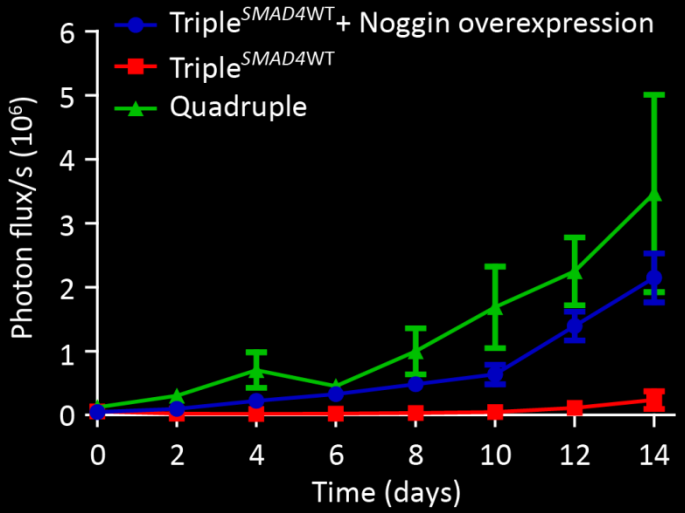
Fumagalli et al, PNAS, 2017

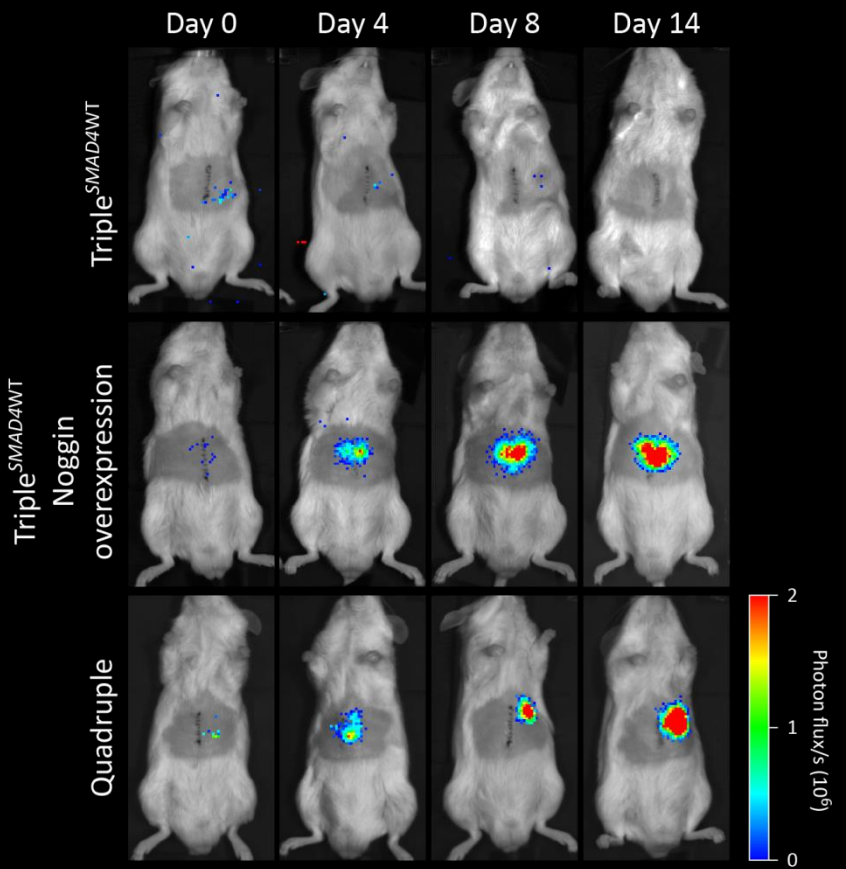
Reconstitution of Noggin enables metastasis formation



Injection of single cells

1. Quadruple
2. Triple^{SMAD4WT}
3. Triple^{SMAD4WT}+Noggin overexpression





Fumagalli *et al.*, PNAS, 2017

Conclusions

We developed a new *in vivo* strategy based on orthothopic transplantation of tumor organoids

- ✓ in their native environment
- ✓ allows visualization of CRC progression

We used this approach to dissect the adenoma-carcinoma sequence of human CRC *in vivo*

Mitotic errors are responsible for the acquisition of new mutations → Loss of *P53*

(Drost *et al.,* Nature 2015)

- ✓ We defined the gate keepers of tumor progression ➔ *P53* loss is crucial

➔ Metastasis occurs upon mutations in *APC*, *KRAS*, *P53* and *SMAD4*

- ✓ The ability to metastasize is the direct consequence of the loss of dependency on specific niche signals

Acknowledgements

Van Rheenen lab

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Fred Verweij
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Tom Wurdinger

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NWO

KWF KANKER BESTRIJDING

AICR
Cancer knows no boundaries.
Fortunately, neither do we.