Dottorato in Genetica, Biologia Molecolare e Cellulare
Scuola di Dottorato in Scienze della Vita "Camillo Golgi" - Università degli Studi di Pavia

(XXIX Cycle)

Course: Frontiers in Cellular Biology

PhD student: **Santonastaso Alice**

In Vivo Imaging Reveals Extracellular Vesicle-Mediated Phenocopying of Metastatic Behavior

Authors

Anoek Zomer,[1] Carrie Maynard,[1] Frederik Johannes Verweij,[2] Alwin Kamermans,[1] Ronny Schäfer,[1] Evelyne Beerling,[1] Raymond Michel Schiffelers,[3] Elzo de Wit,[1] Jordi Berenguer,[4] Saskia Inge Johanna Ellenbroek,[1] Thomas Wurdinger,[4,5] Dirk Michiel Pegtel,[2] and Jacco van Rheenen[1,*]

Cell *161*, 1046–1057, May 21, 2015

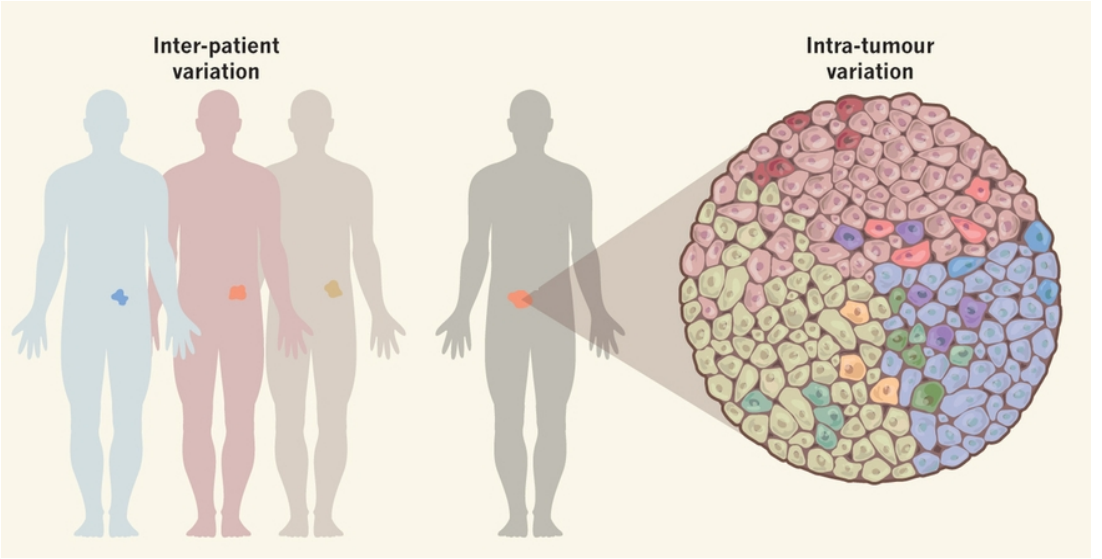
Cell

7th April 2016

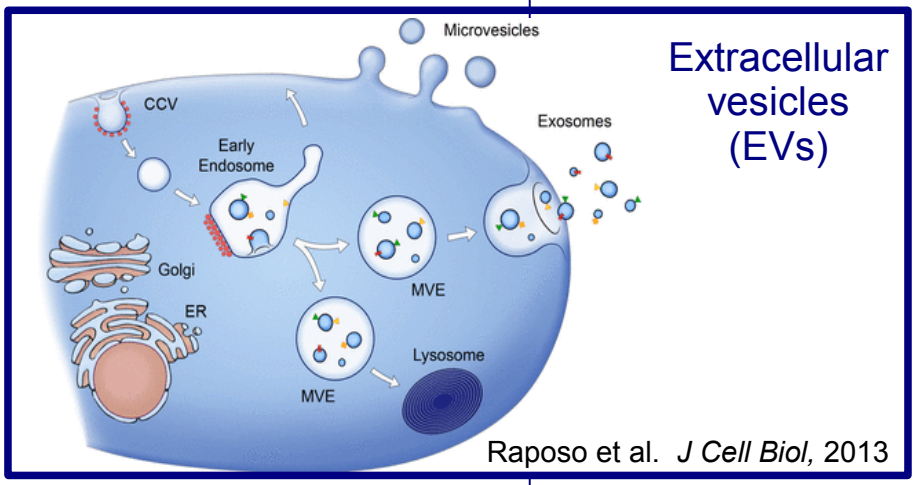
Tumor heterogeneity

Heterogeneous nature of tumors

Tumor cells genetic variability

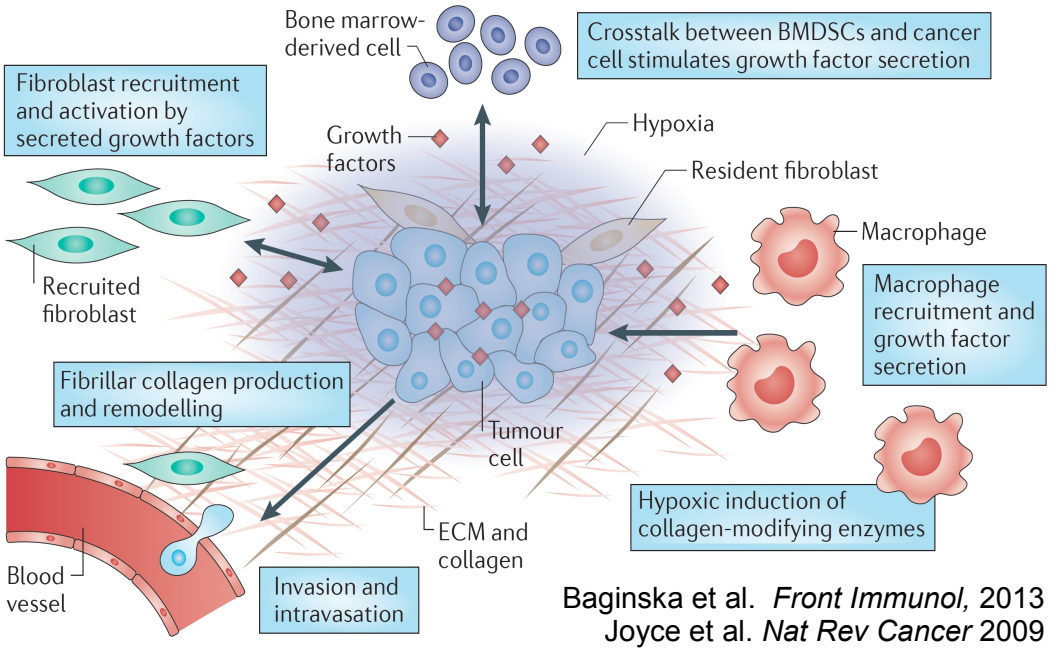


Divergence in tumor microenvironment



Extracellular vesicles (EVs)

Raposo et al. *J Cell Biol,* 2013

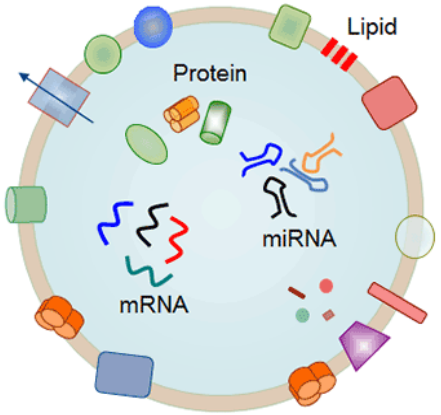


Baginska et al. *Front Immunol,* 2013
Joyce et al. *Nat Rev Cancer* 2009

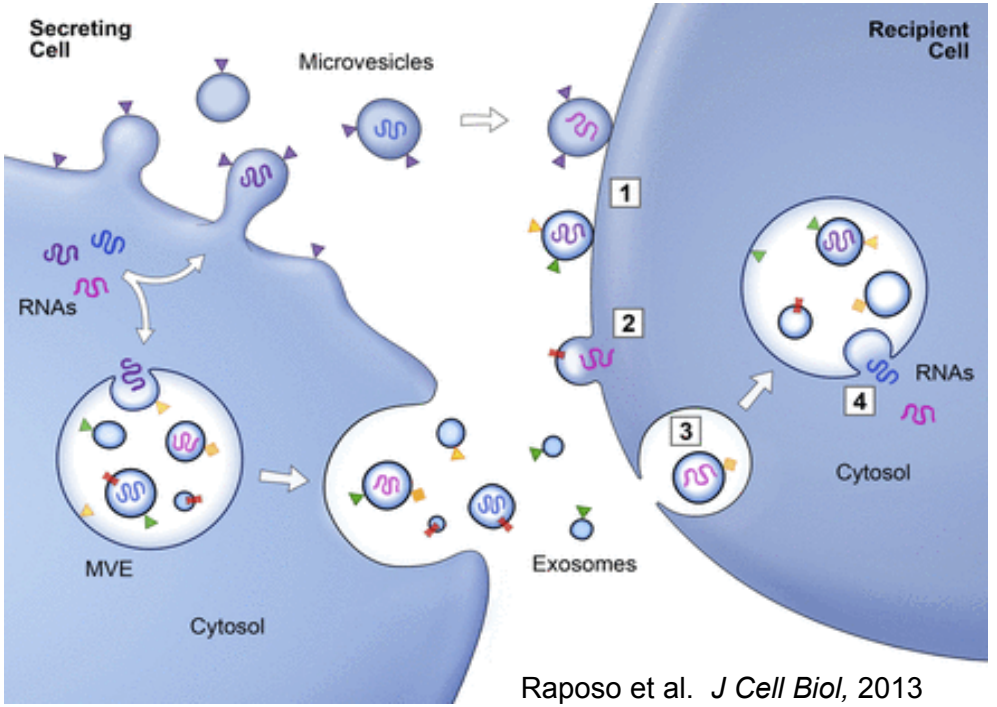
Extracellular vesicles (EVs)

EVs { Exosomes
Microvesicles

EV cargo



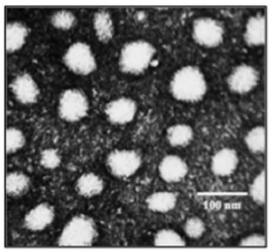
Important mode of intercellular communication



Raposo et al. *J Cell Biol*, 2013

Embryonic stem cell-derived microvesicles reprogram hematopoietic progenitors: evidence for horizontal transfer of mRNA and protein delivery

J Ratajczak[1], K Miekus[1,2], M Kucia[1], J Zhang[1], R Reca[1], P Dvorak[3] and MZ Ratajczak[1] **Leukemia (2006) 20**, 847–856

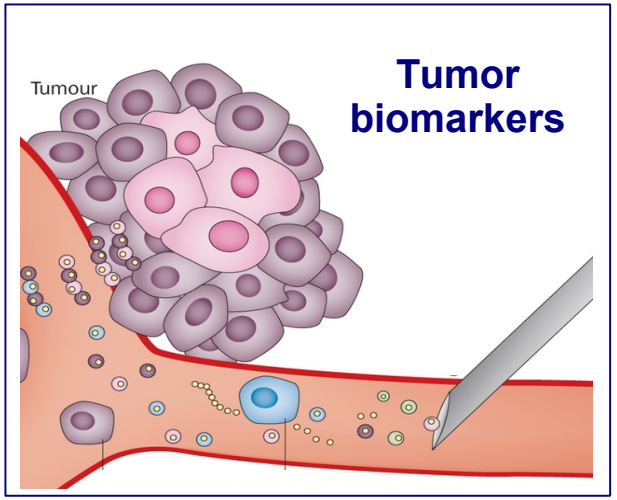


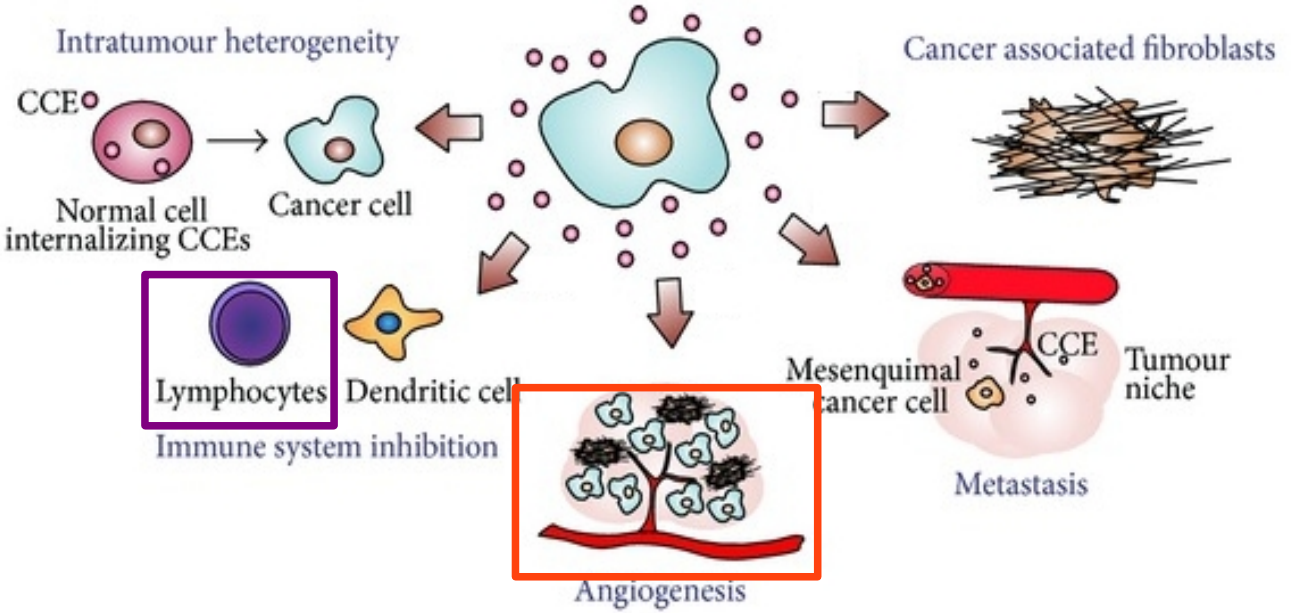
Exosomal Signaling during Hypoxia Mediates Microvascular Endothelial Cell Migration and Vasculogenesis

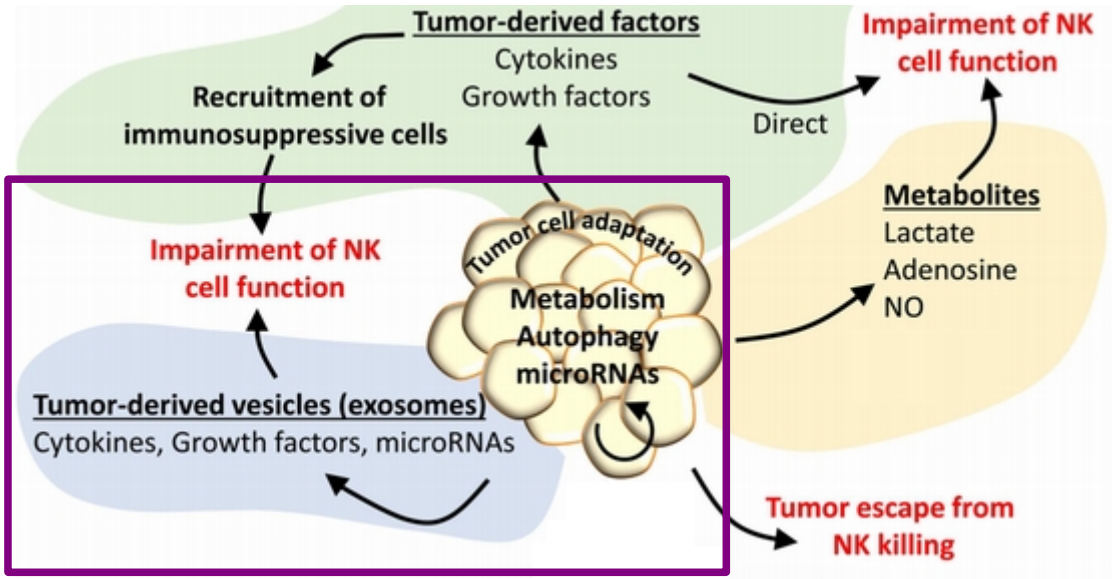
PLOS ONE

Carlos Salomon[1]*, Jennifer Ryan[1], Luis Sobrevia[1,2], Miharu Kobayashi[1], Keith Ashman[1], Murray Mitchell[1], Gregory E. Rice[1]

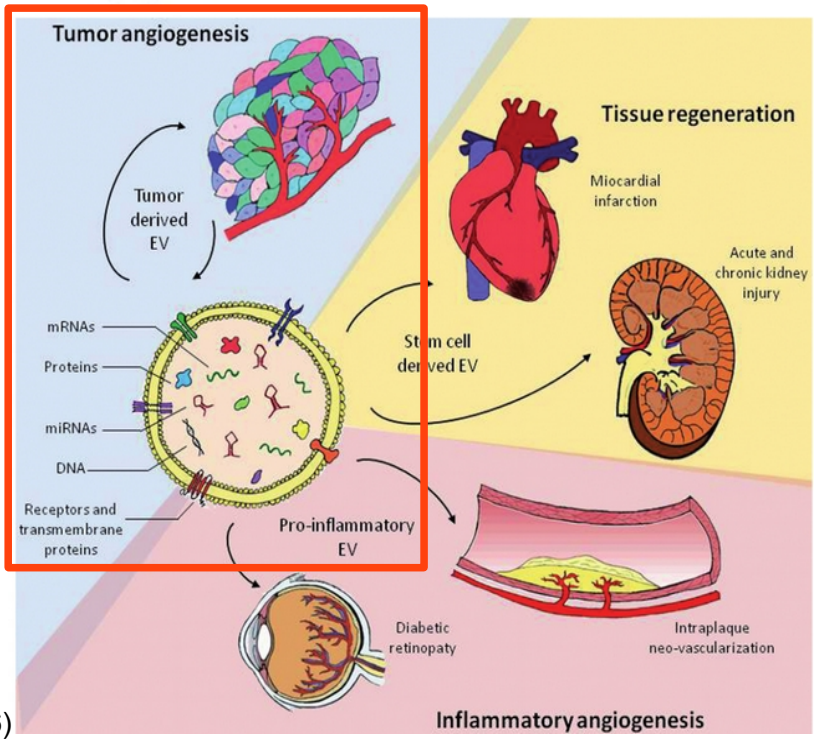
Tumor-derived EVs







Baginska et al. *Front Immunol,* 2013



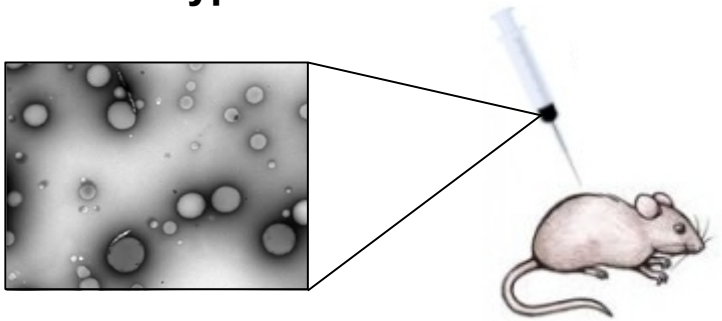
Gai et al. *Histol Histopathol* (2016)

Aim of the study

Challenges in the ***in vivo*** study of EV-mediated cell-cell communication:

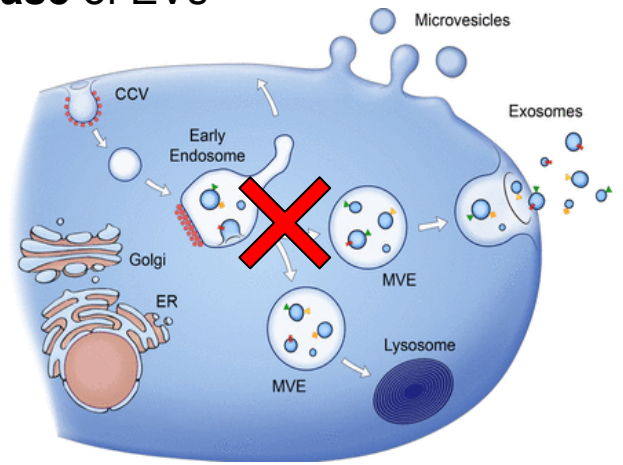
- Tumor cells are exposed to EVs released by **various cell types**

Isolation of concentrated EV preps
from cancer cell culture



- **Non-natural** location, concentration and continuous **release** of EVs

- **Inhibition of MVE pathways** → only partial reduction of EV production and alteration of EV-independent factors



- Current techniques **lack of direct tracking** and do not discriminate cells that take up EVs

Aternative approach: **combination of high-resolution intravital imaging with a Cre recombinase-based method to study EV exchange between tumor cells**

→ Direct visualization of EV release by MDA-MB-231

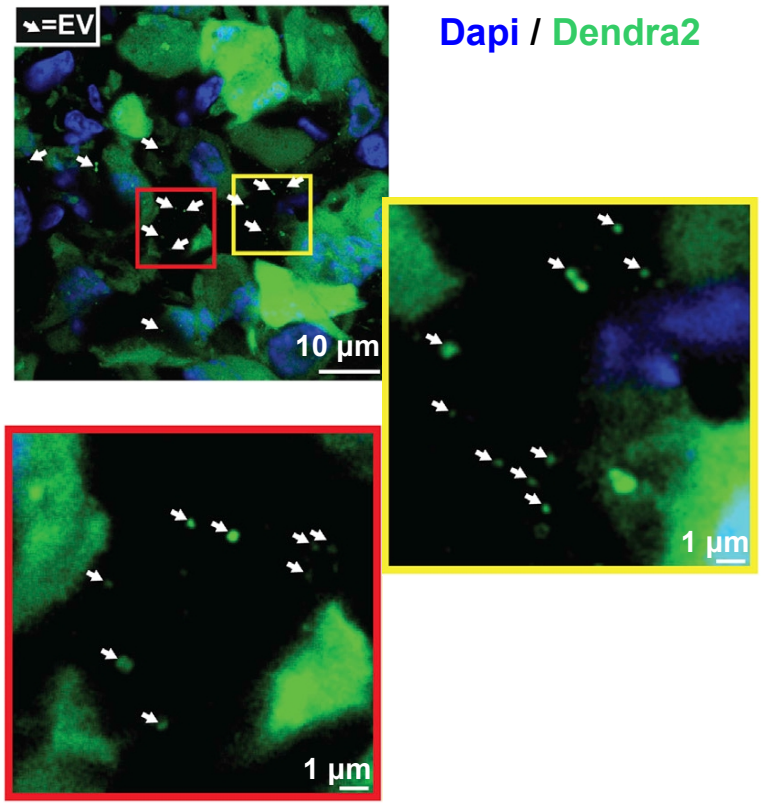
→ Observation of behavior of T47D cells that take up tumor-derived EVs

Evaluation of *in vivo* release of tumor EVs

Orthotopic transplantation of MDA-MB-231 cells

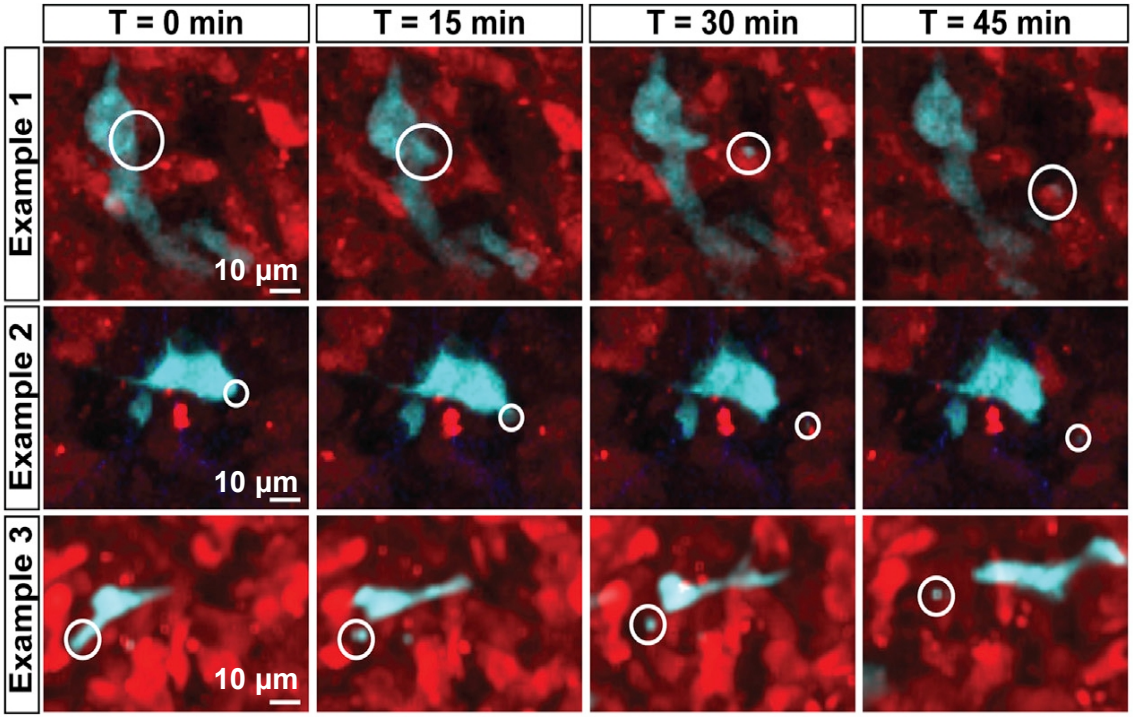
Confocal microscopy of tumor sections

Intravital imaging of mammary tumors (multiphoton microscope)



Dapi / **Dendra2**

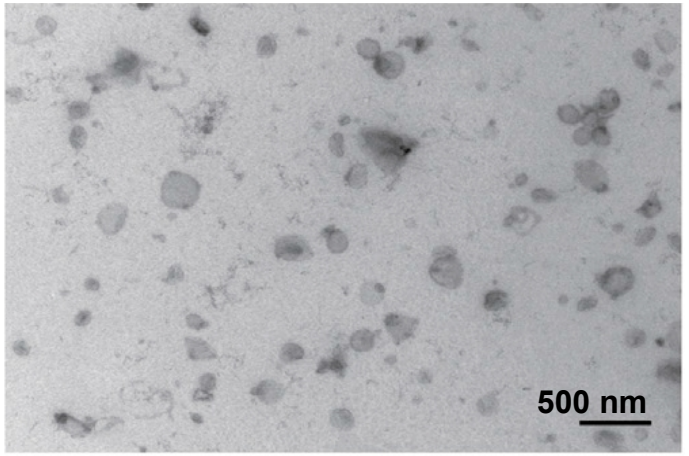
CFP-marked MDA-MB-231 / **DsRed-marked MDA-MB-231 cells**



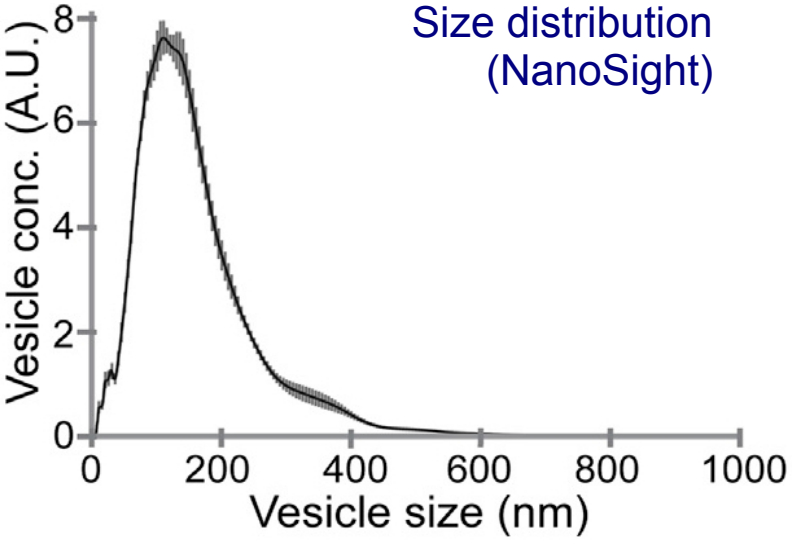
Characterization of MDA-Mb-231 tumor EVs

Isolation of EVs from MDA-MB-231 tumors

EM

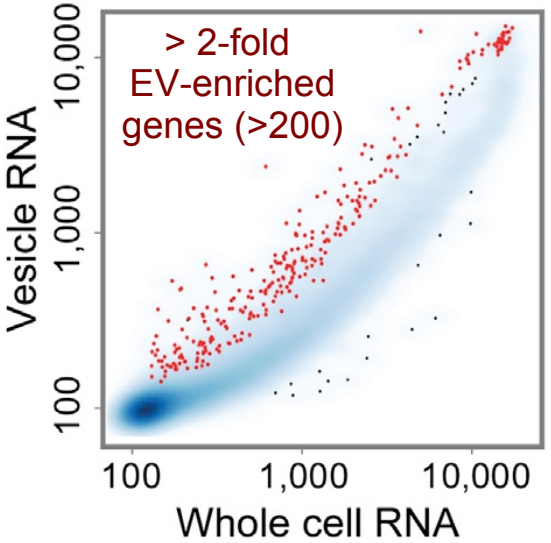


Size distribution (NanoSight)

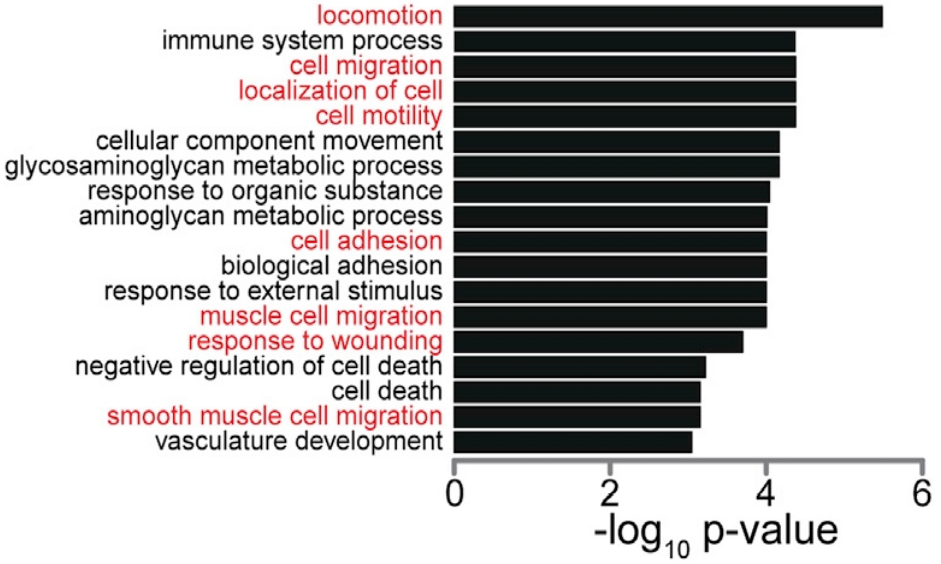


Isolation of mRNA from EVs and cells of MDA-MB-231 tumors

Gene expression array



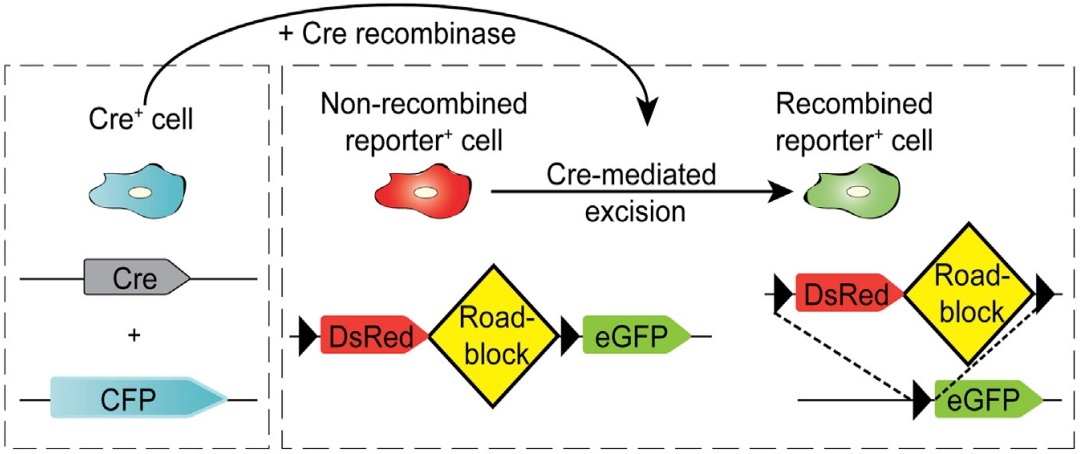
Gene ontology analysis

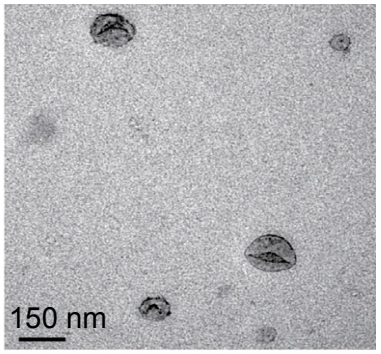


Cre-LoxP system to report EV transfer

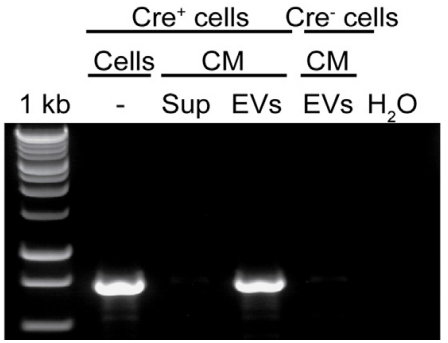
The Cre-LoxP system:

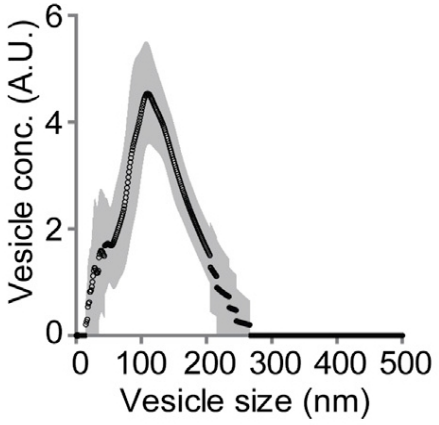
Detection is restricted to uptake of EVs from Cre⁺ cells



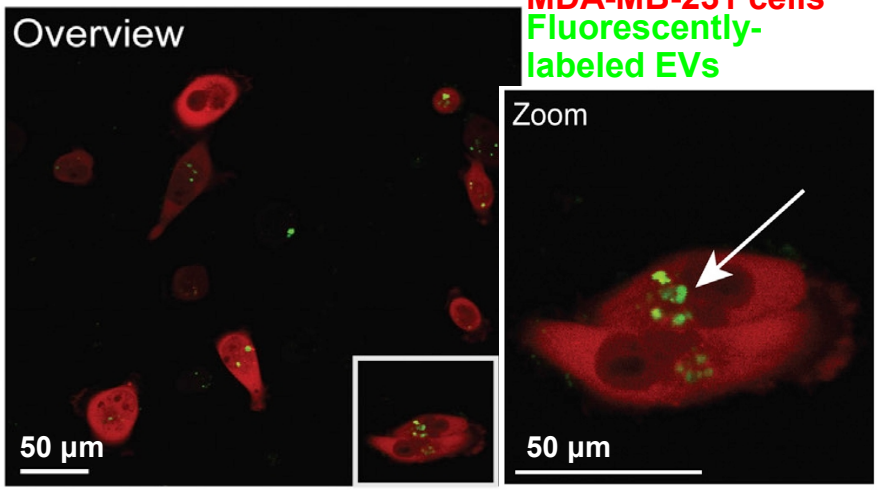


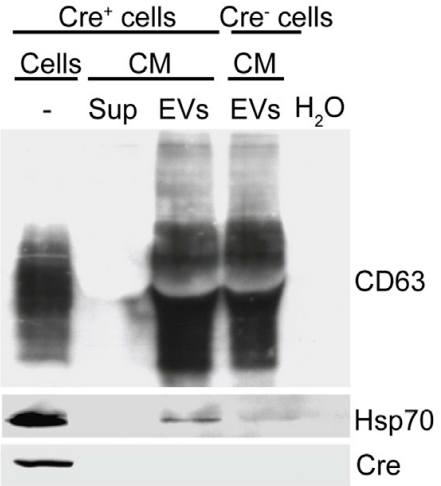
Generation of
MDA-MB-231 cells expressing
Cre and CFP



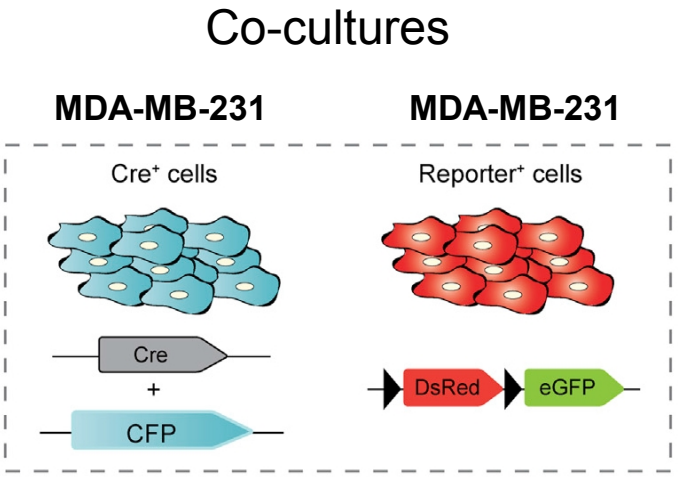


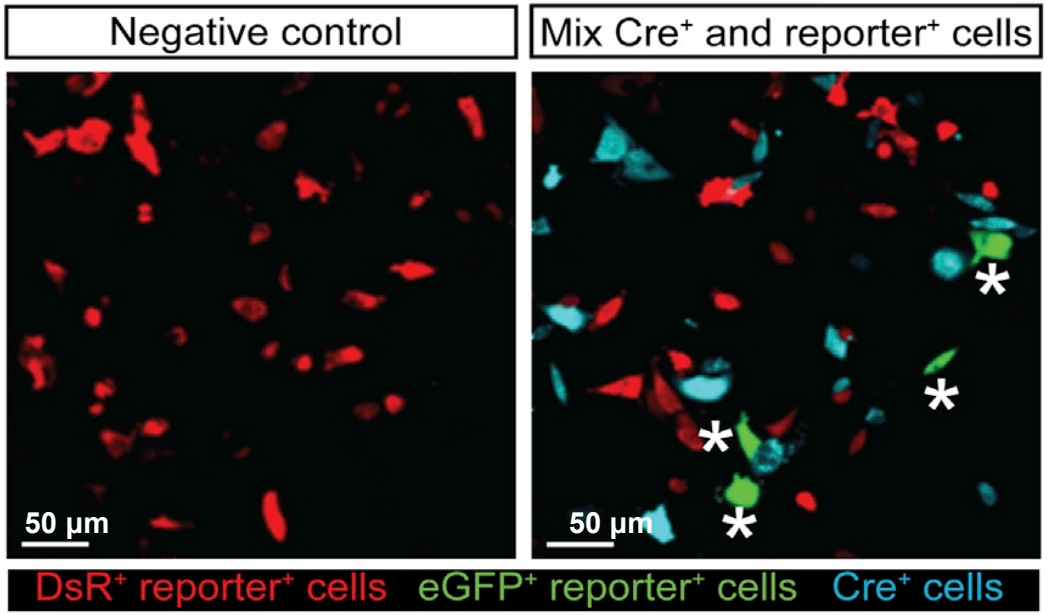
3h after EV addition to cells

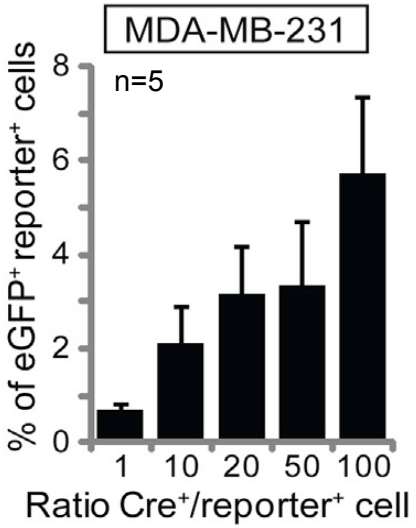


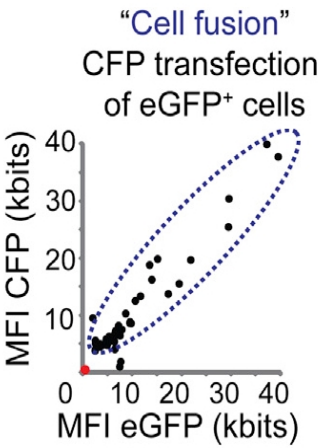


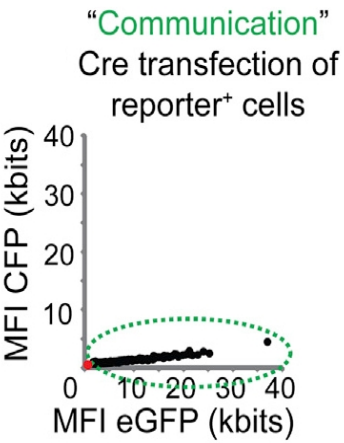
In vitro tumor EV transfer using Cre-LoxP

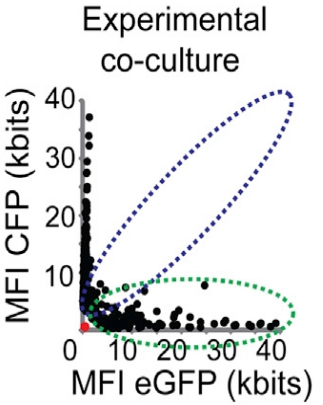




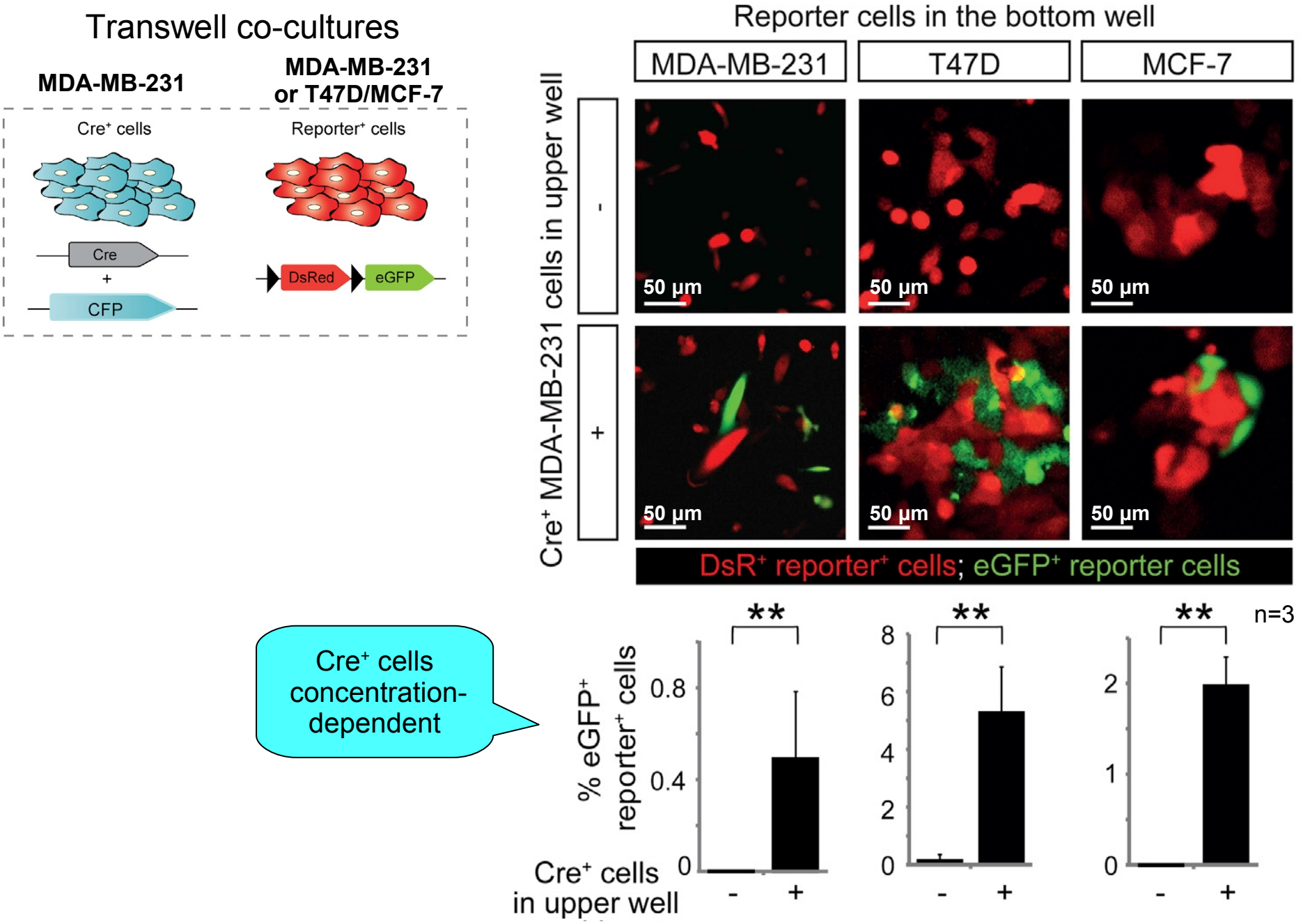








In vitro tumor EV transfer using Cre-LoxP



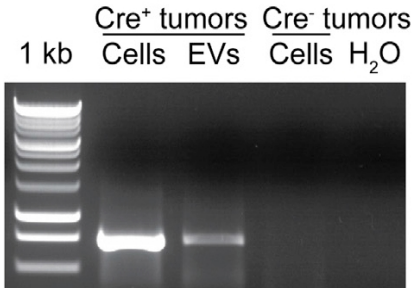
In vivo tumor EV transfer using Cre-LoxP

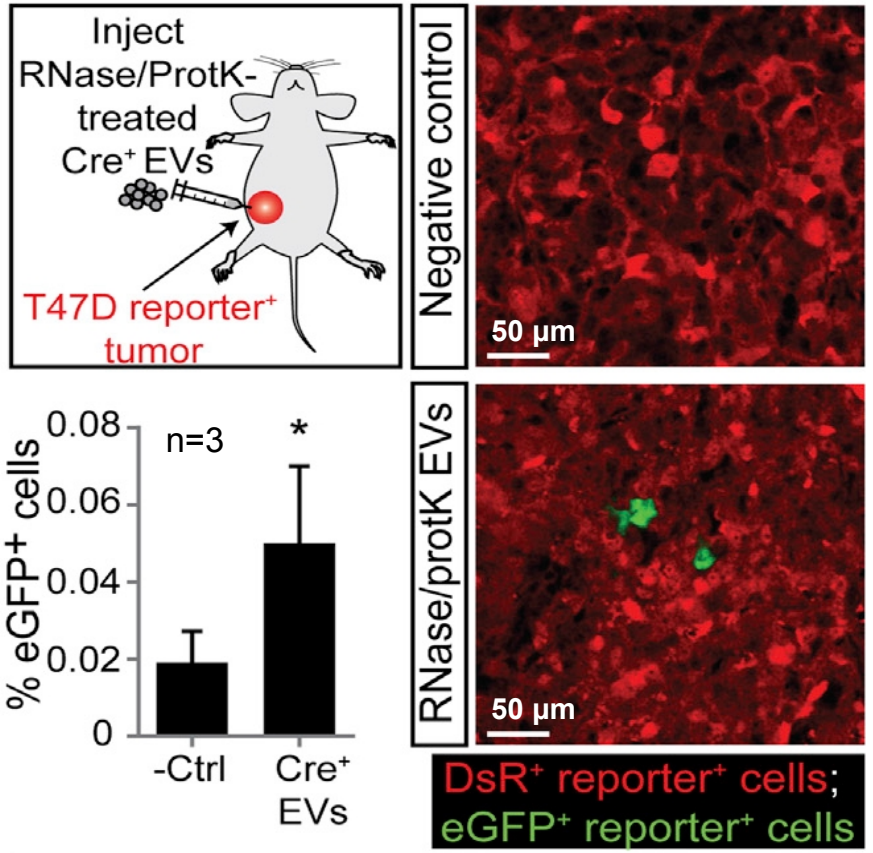
Human mammary xenograft and melanoma allograft tumor models

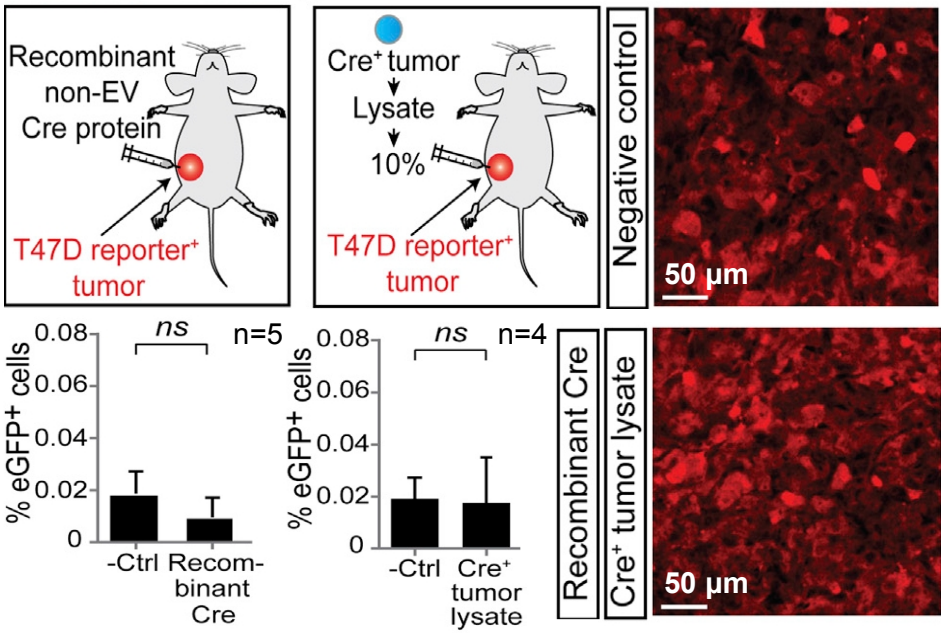
Isolation of EVs from Cre+ tumors

↓

Intratumoral injection

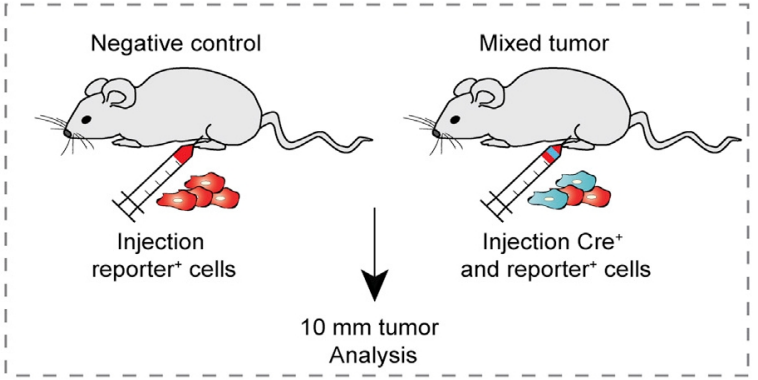


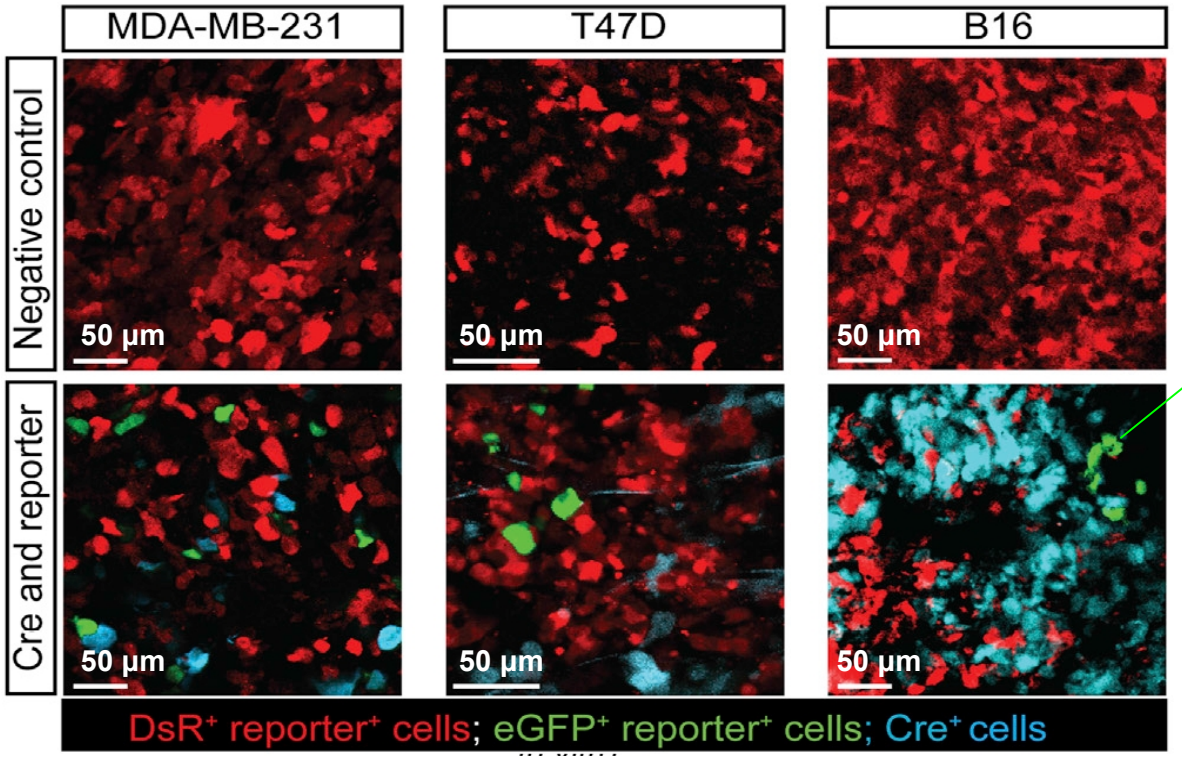




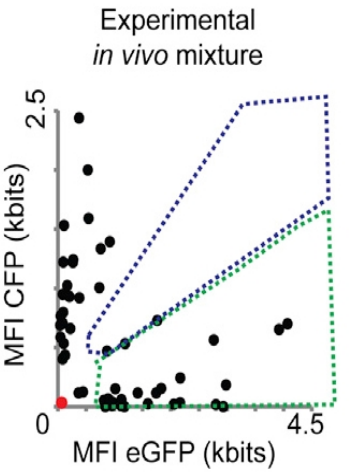
In vivo tumor EV transfer using Cre-LoxP

Human mammary xenograft and melanoma allograft tumor models





Negative for CFP



EV exchange between tumor and non-tumor cells

(n=3 mice)

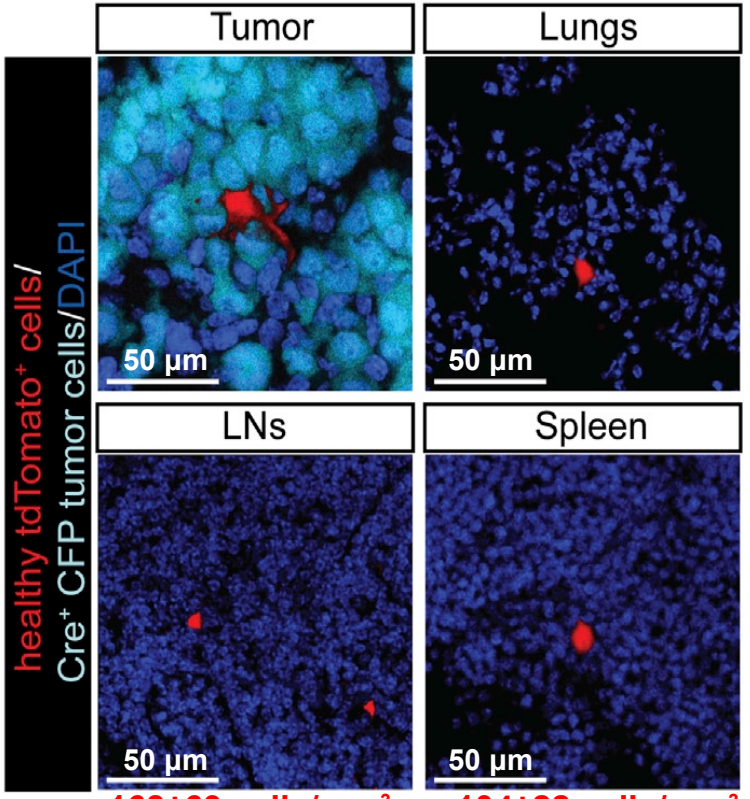
CFP⁺ Cre⁺ B16 melanoma cells

R26-LSL-tdTomato B6 mice

Tumor growth *~2 weeks*

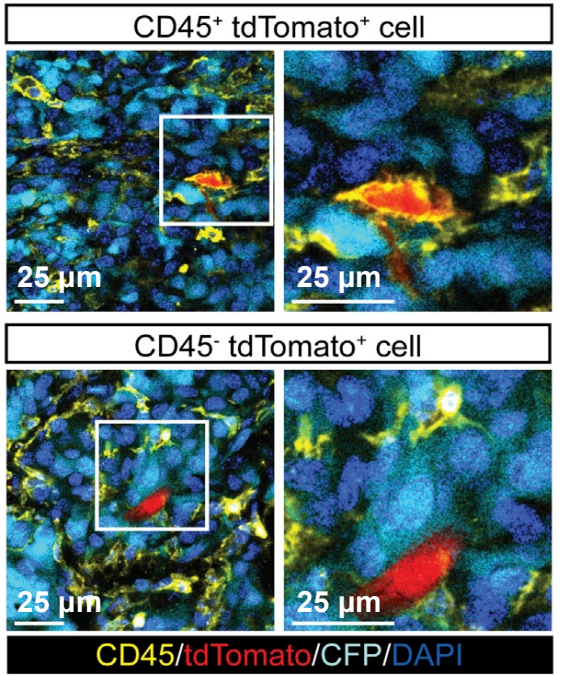
Check for tdTomato⁺ non-tumor cell types

374±76 cells/mm³ | 69±17 cells/mm³

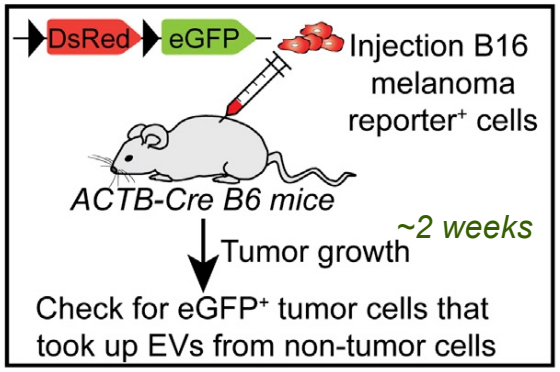


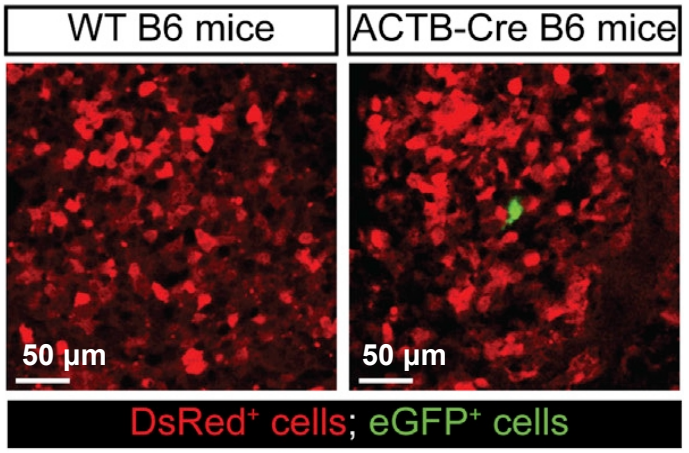
168±69 cells/mm³ | 104±22 cells/mm³

Immune and non-immune cells (CD45)



(n=3 mice)



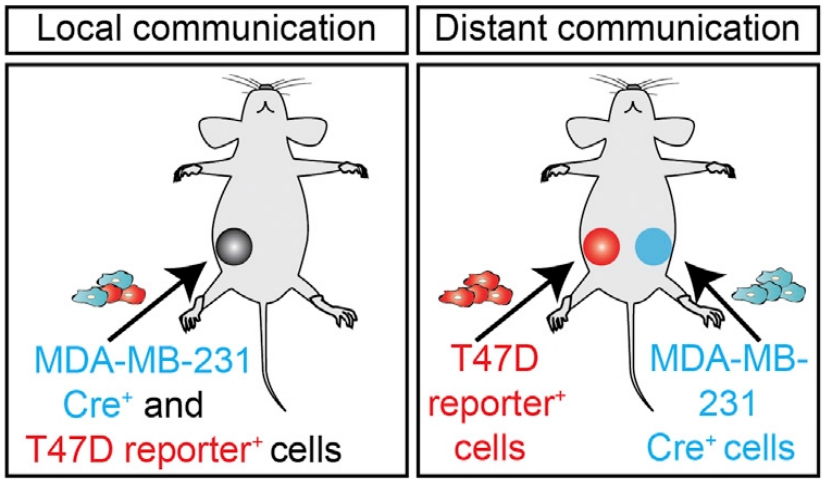


2 ± 0.7 cells/mm³

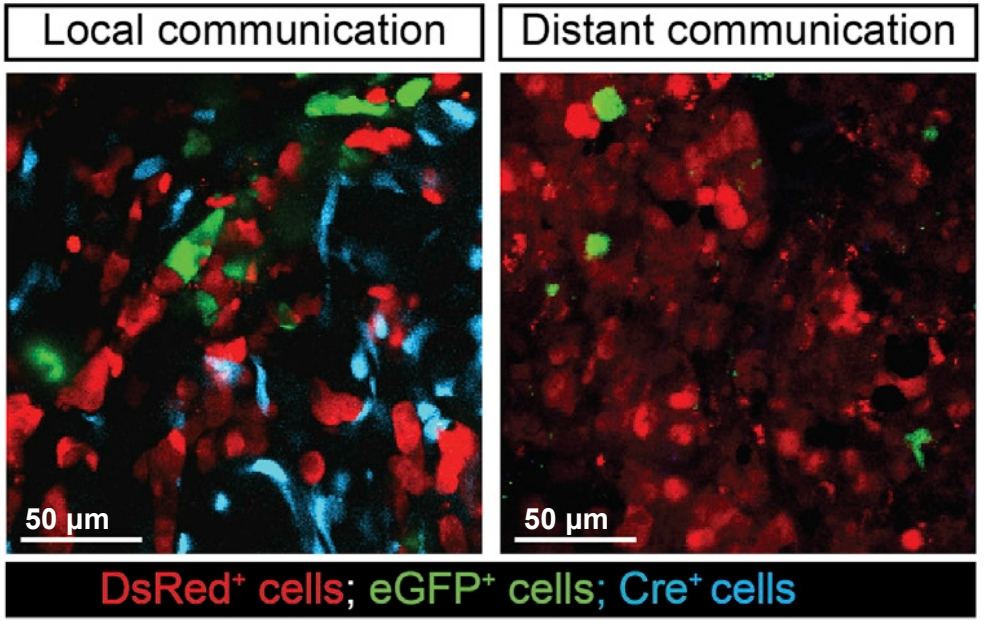
Local and systemic tumor EV transfer

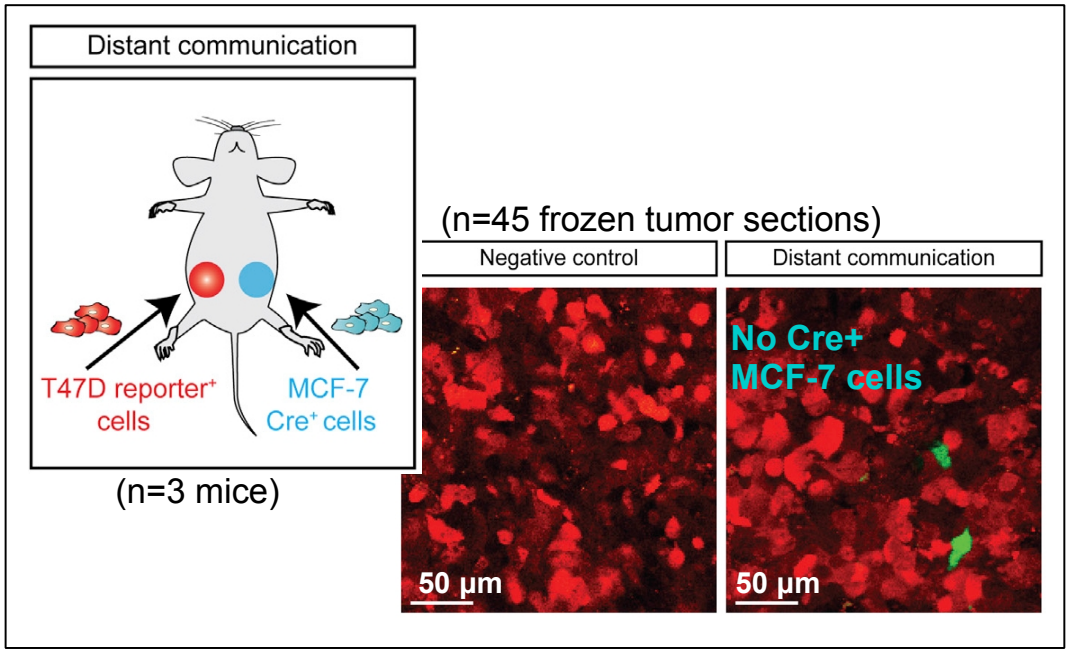
Testing the possibility of systemic transfer of Cre activity

(n=3 mice) (n=3 mice)

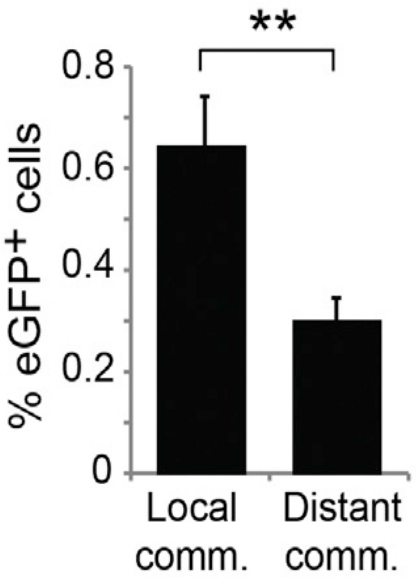


(n=26 frozen tumor sections)



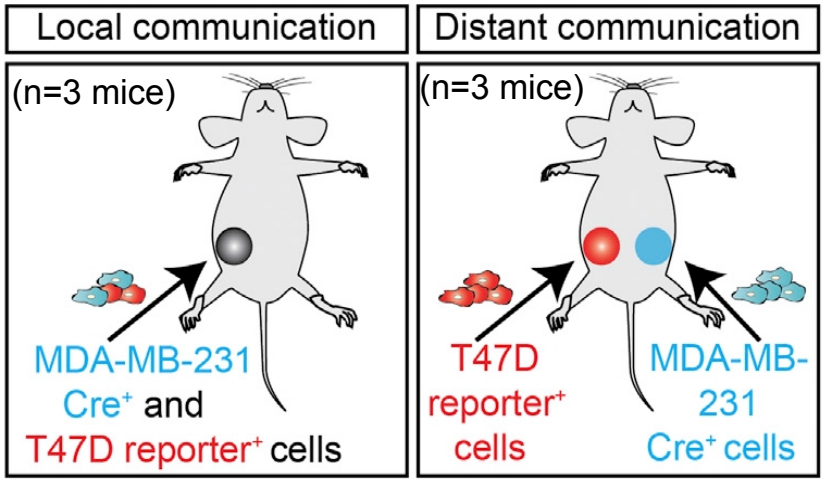


A slight *reseeding effect* was observed (**23 ± 10 cells/mm³**)

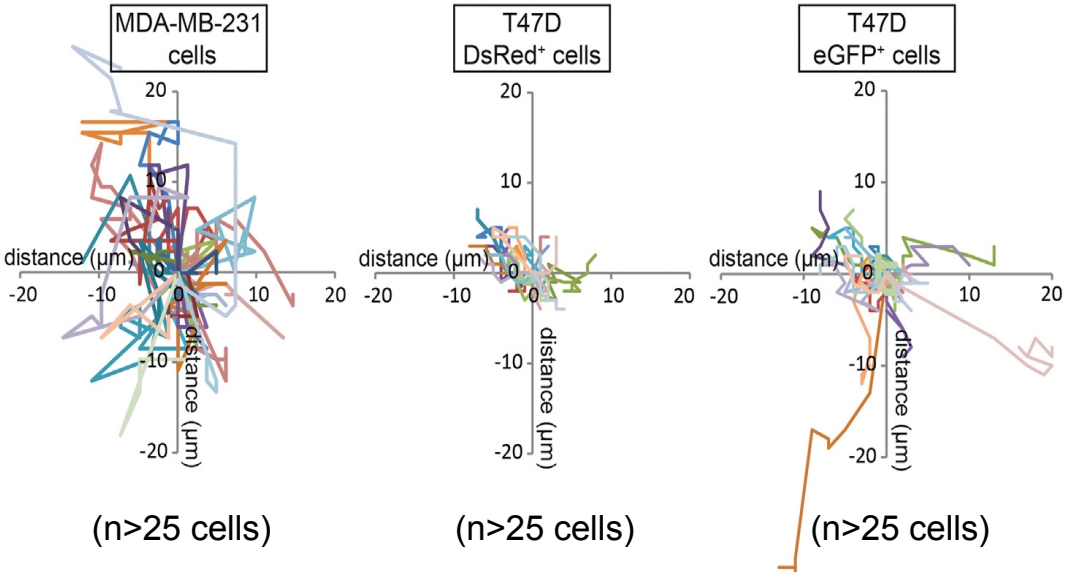


Migration of T47D cells upon malignant tumor EV uptake

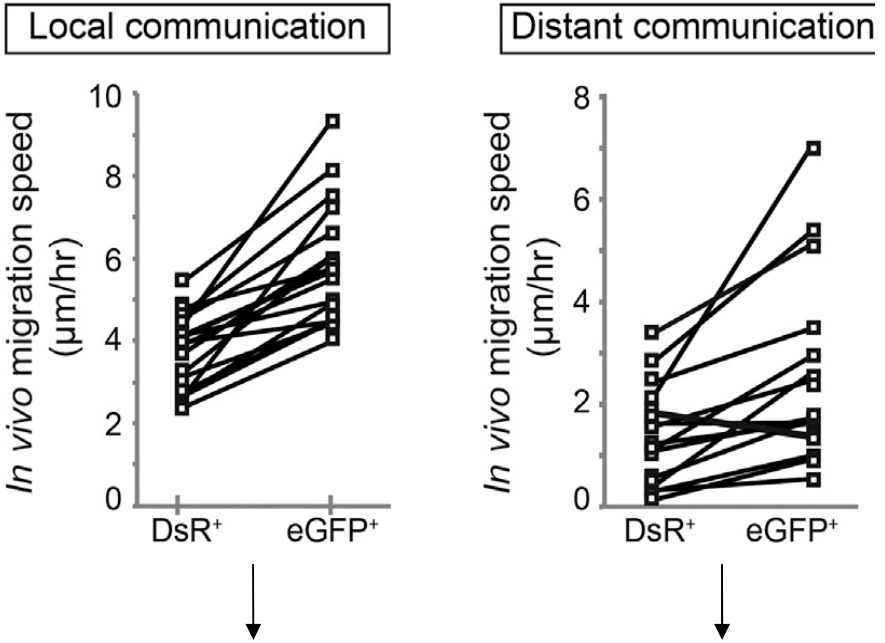
Testing the possibility of alteration of T47D recipient cells migratory behavior



Cell tracking through intravital imaging

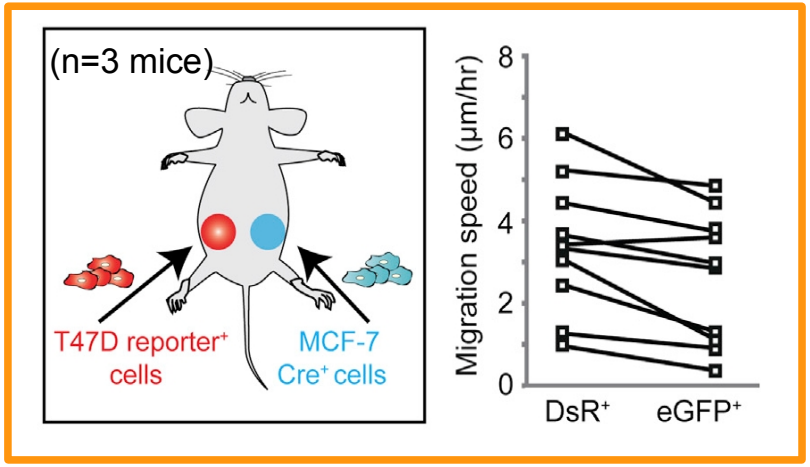


(n>25 cells) (n>25 cells) (n>25 cells)



In all 18 imaging fields, **eGFP+** migrate faster than **DsR+**

In 15/17 imaging fields, **eGFP+** migrate faster than **DsR+**

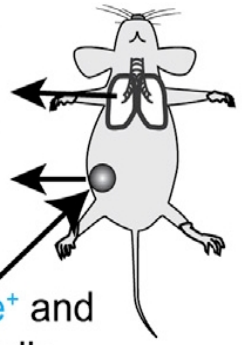


Metastatic potential of T47D cells upon malignant tumor EV uptake

Analysis on > 1000 lung metastasis sections

ratio of eGFP⁺ /DsRed⁺ metastases

ratio of eGFP⁺ /DsRed⁺ tumor cells

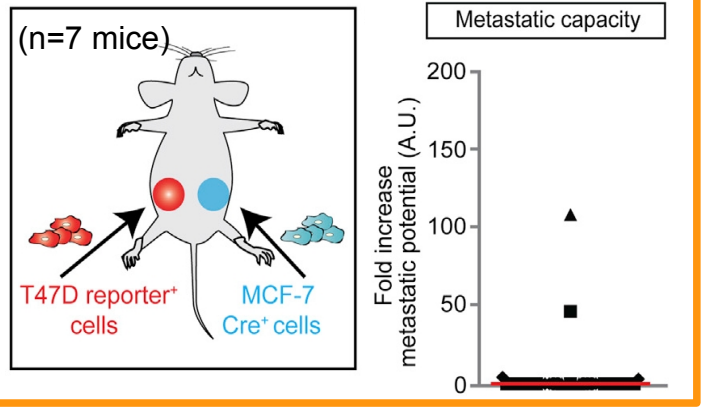


MDA-MB-231 Cre⁺ and T47D reporter⁺ cells

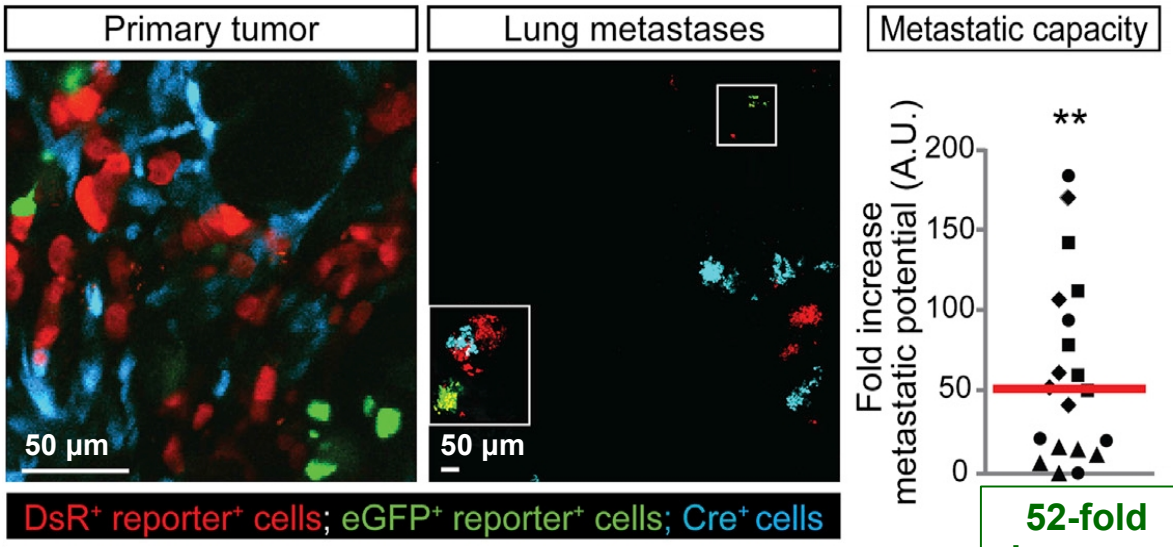
(n=4+4 mice)

Increased metastatic potential if:

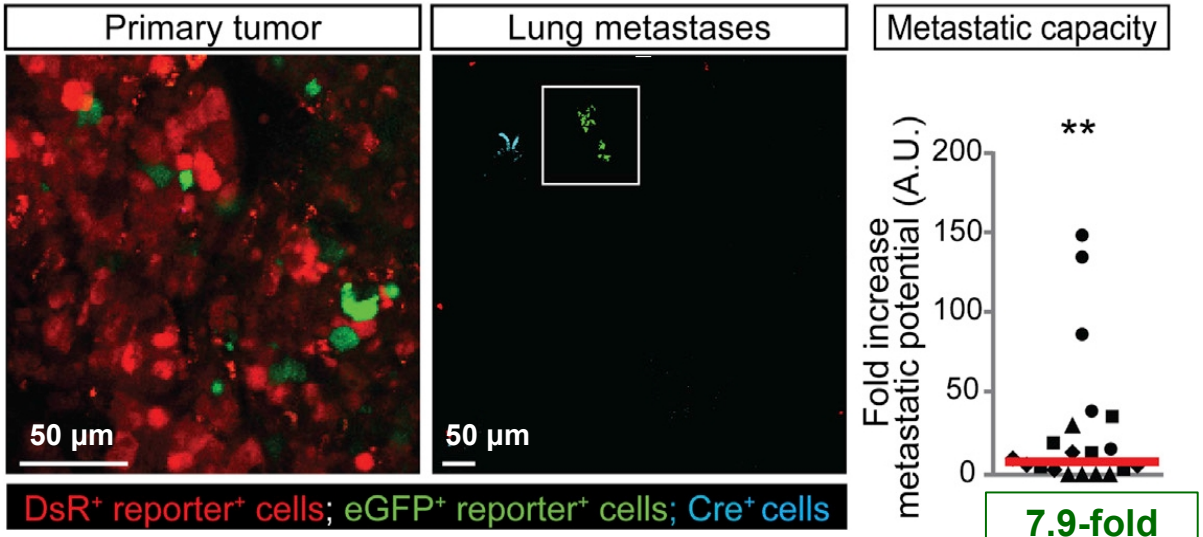
$$\frac{\text{ratio of eGFP}^+ /\text{DsRed}^+ \text{ metastases}}{\text{ratio of eGFP}^+ /\text{DsRed}^+ \text{ tumor cells}} > 1$$



Local communication



Distant communication



Conclusions

1) Tumor cells (MDA-MB-231 cells) release a **heterogeneous population** of EVs.

- enrichment in genes involved in migration and metastasis

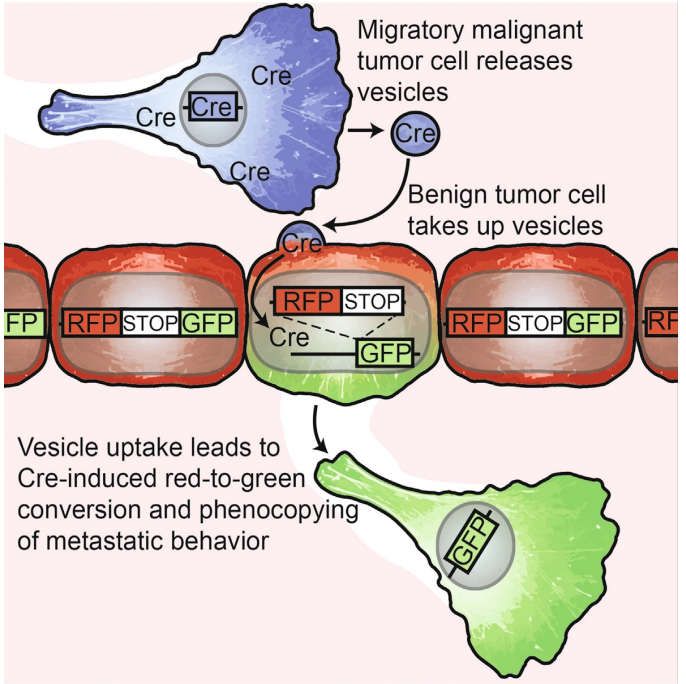
2) Tumor cells with high metastatic potential can **transfer functional biomolecules** to less malignant cells and to non-tumor cells through EVs.

- both *in vitro* and *in vivo* demonstration
- both local and systemic transfer

3) Malignant cells-derived Evs can **enhance** the **migratory behavior** and **metastatic potential** of tumor cells, possibly through transfer of mRNA molecules.

***In vivo* and *in vitro* visualization of EV transfer from a defined cell population to detectable recipient cells**

Need for evidence on the exact funcional biomolecules that are transferred



Thank you for your attention!