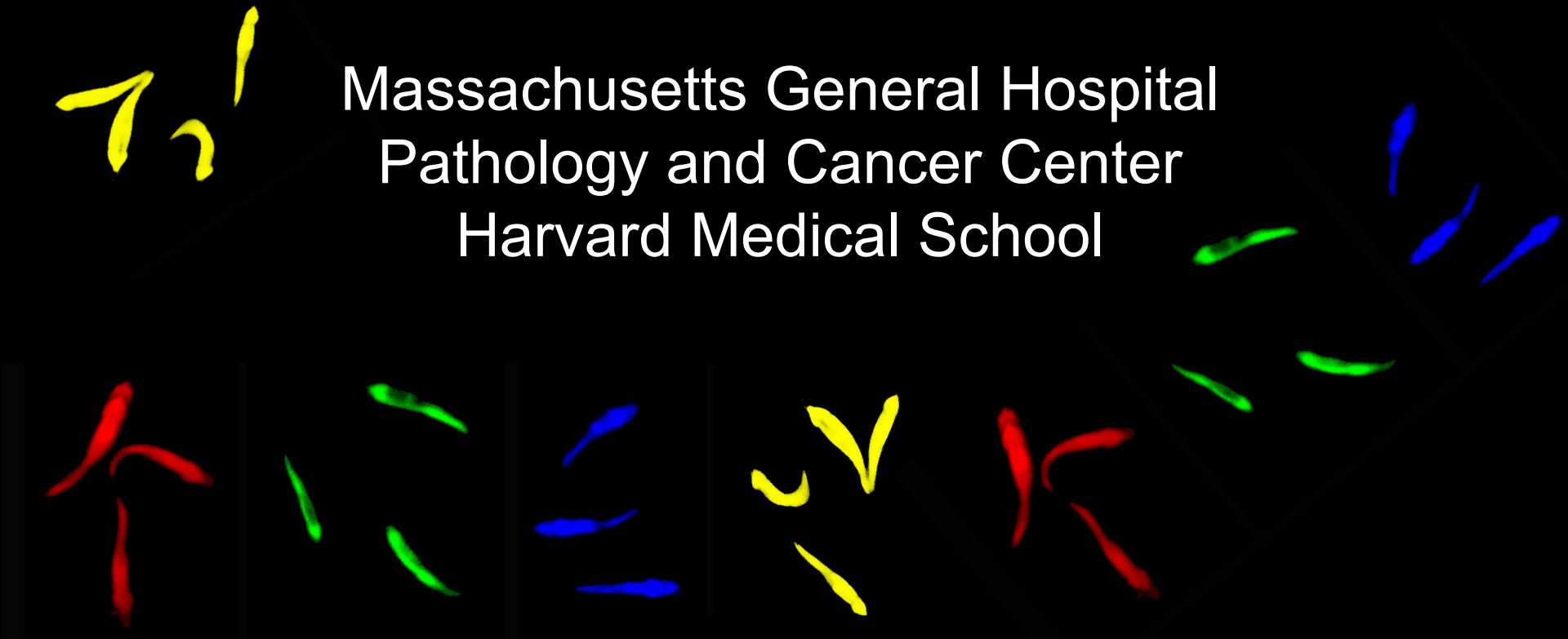


Preclinical Modeling Using Zebrafish

David M. Langenau, Ph.D.

Massachusetts General Hospital
Pathology and Cancer Center
Harvard Medical School

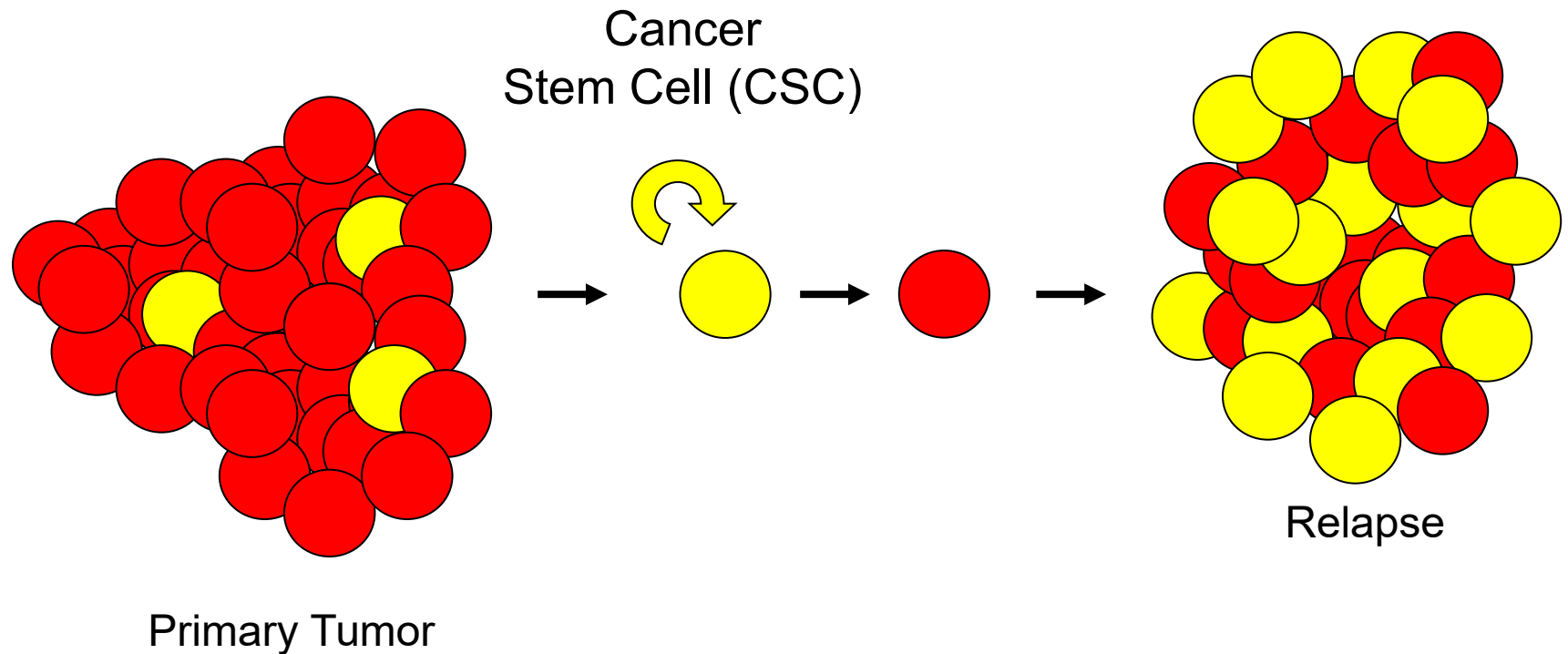


Disclosures

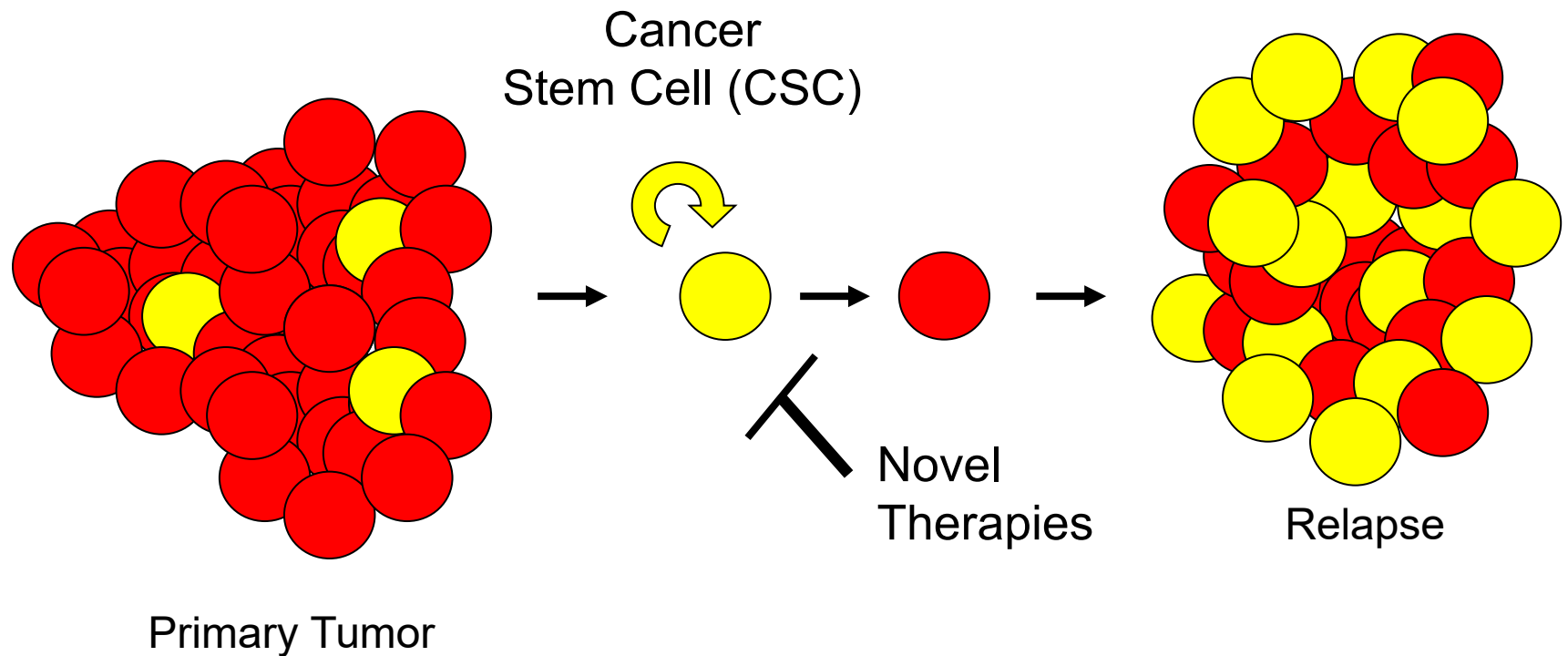
Prior/current sponsored research support from Merck, Novartis, and NextCure.

Patents awarded/filed on many of the technologies described.

Langenau lab: Relapse and progression in pediatric cancer

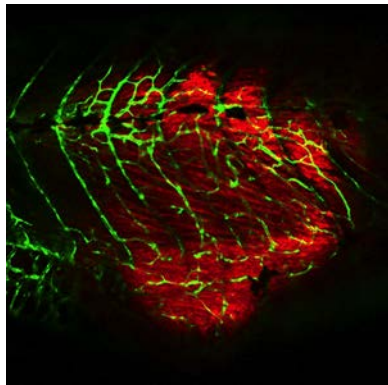
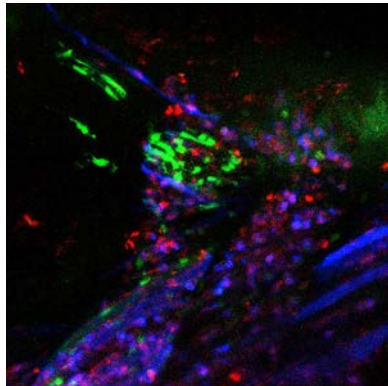


Langenau lab: Relapse and progression in pediatric cancer

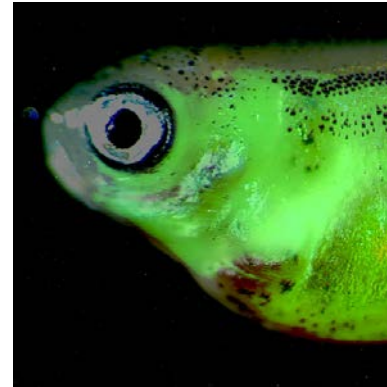
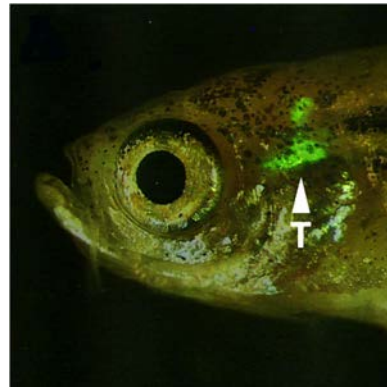
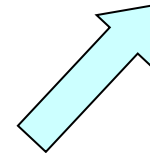
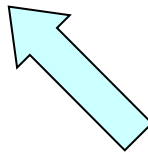


Langenau lab: Relapse and progression in pediatric cancer

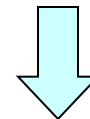
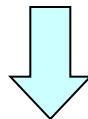
In vivo
Imaging



Relapse and Cell
Transplantation



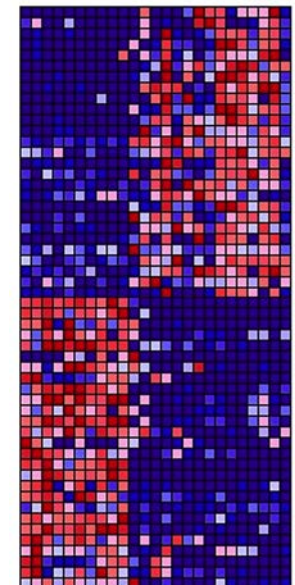
Zebrafish Models of Leukemia and Sarcoma



- Transgenesis
- Loss-of-function

Chemical Genetics

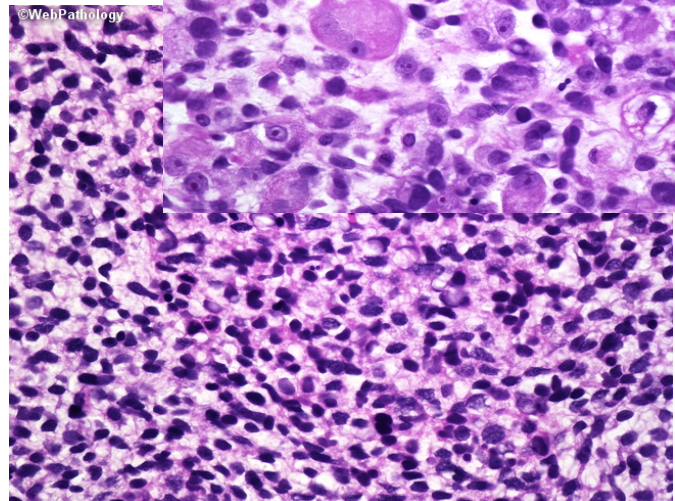
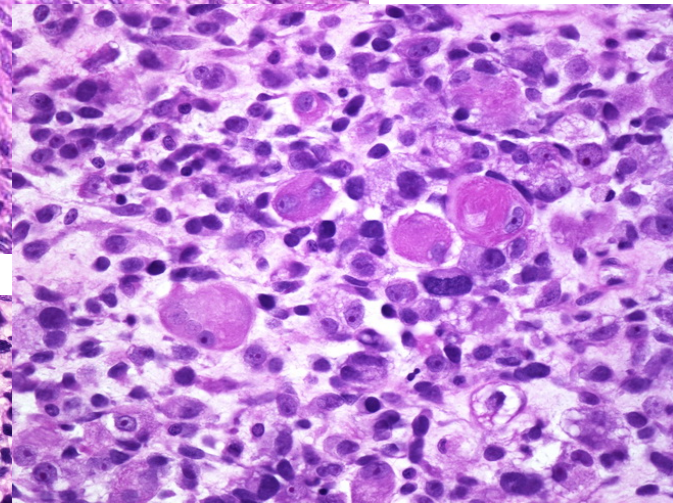
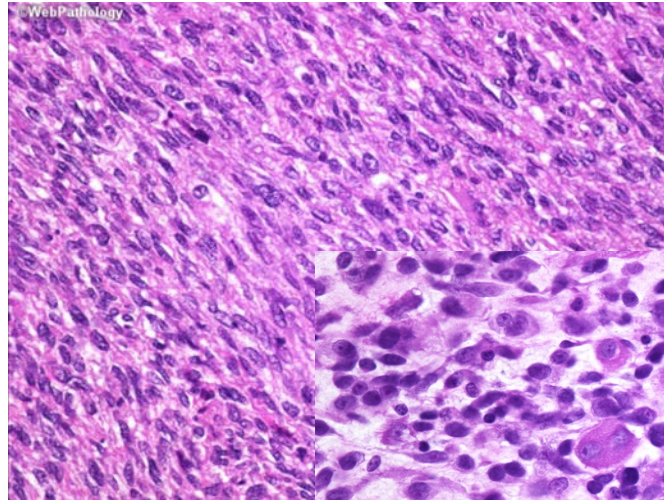
Conserved
Genetic
Programs



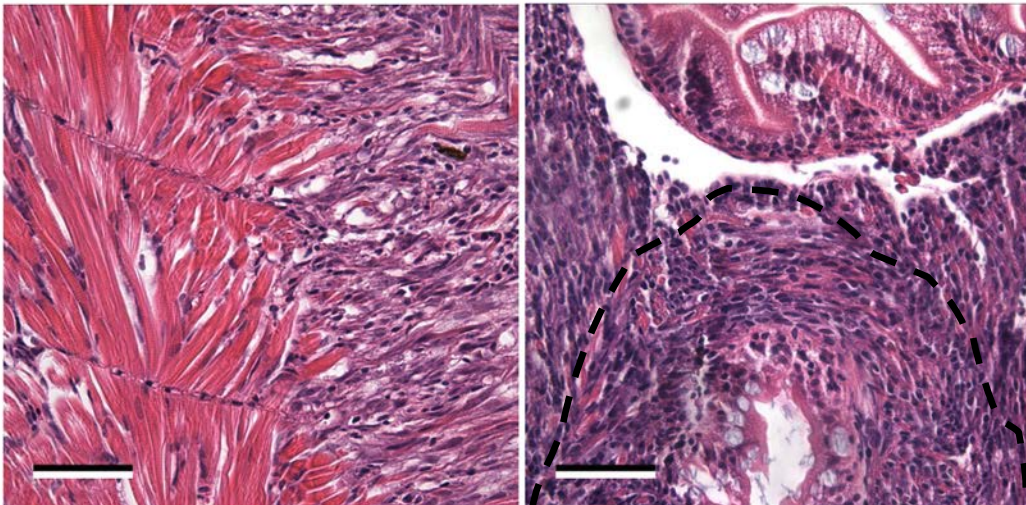
MYF5
MYF6
GAS2
PTN
PLA31
FBN2
ITM2A
HMG22
COL9A3
PTN
EGFR
FZD4
HOXC6
PLK2
SNCAIP
FABP5
GULP1
FZD10
PTN
CCND1
FZD7
PRAME
NFXN2
IGF2BP2
HEY1
HMG1
MFO2
CNF1
PIPOX
BSGAT1
DCX
ASS1
STMN2
TOX3
GHEM1
NELL1
ABAT
DCX
ABAT
ELOVL2
PCP4
PDM1H
OLIG2
CELA2A
IPAK3
ARHGAP25
NEUROD2
NRN1
WSCD1
FGFR2
BMP5
GHEM1
SGMS1
NRCAM

Rhabdomyosarcoma

- A common pediatric malignancy of muscle
- Two major subtypes:
 - Embryonal (FN)
 - Alveolar (FP) –
PAX/FOXO gene fusion
- ~80% of relapse patients will succumb to disease
- Relapse is driven by cancer stem cells (FN-CSCs)

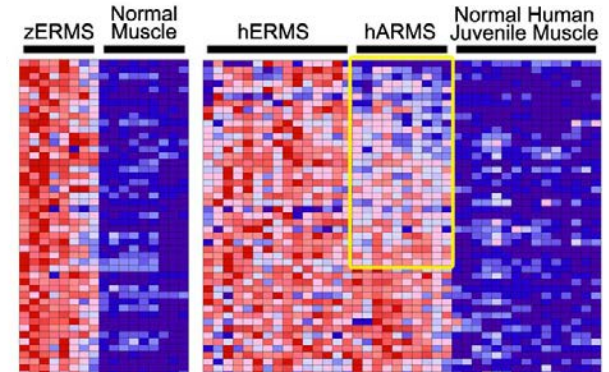


Zebrafish ERMS is Similar to Human Disease

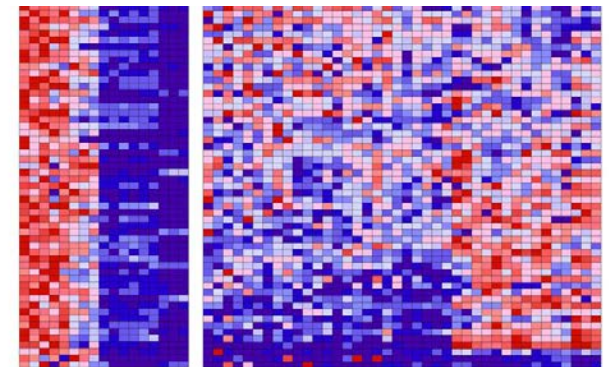


Skeletal Muscle

Intestine



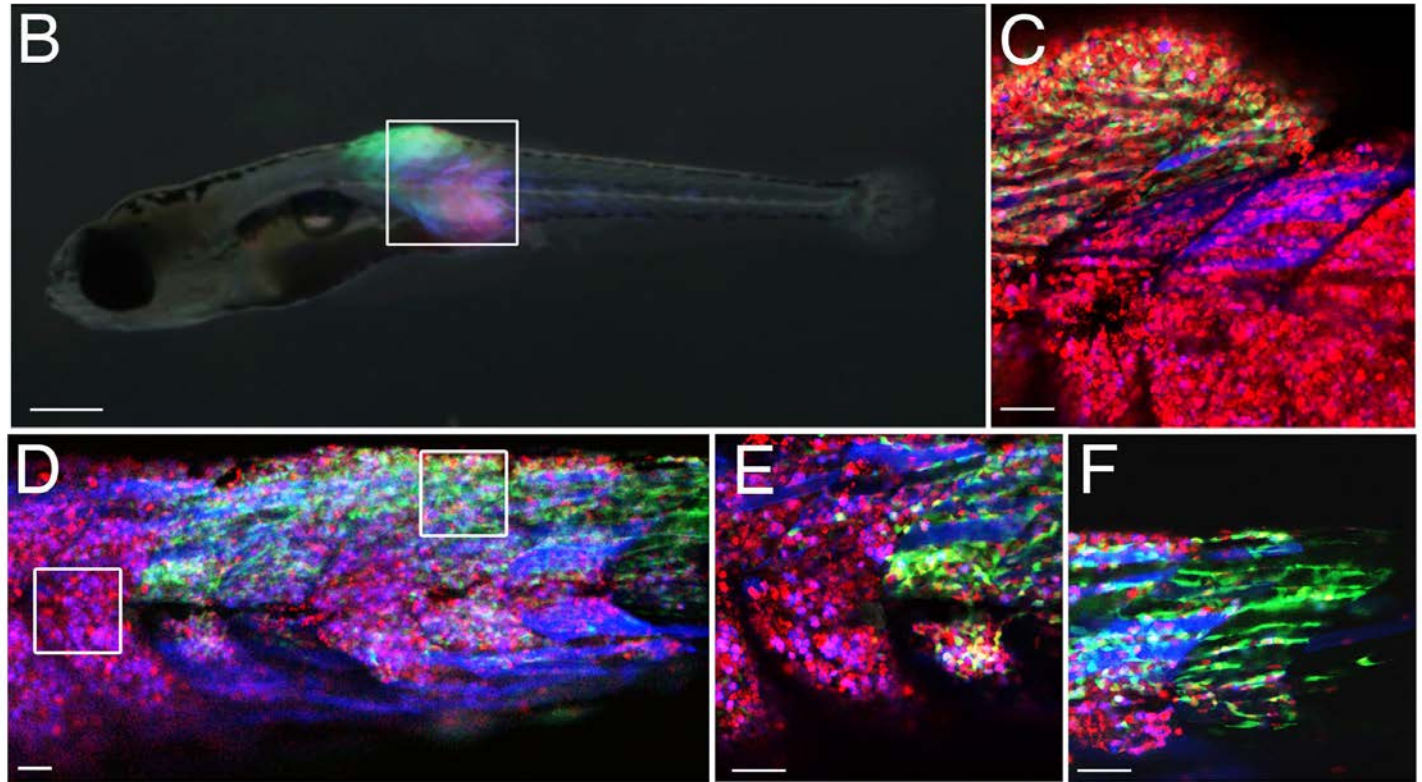
RAS is the dominant oncogenic pathway in 90% of human ERMS



Langenau et al., G&D 2007

Transgenic zebrafish models of Embryonal Rhabdomyosarcoma (ERMS)

rag2-kRASG12D
+ *myog-H2b-RFP*
+ *myl2-lyn-cyan*
(*myf5-GFP*)²



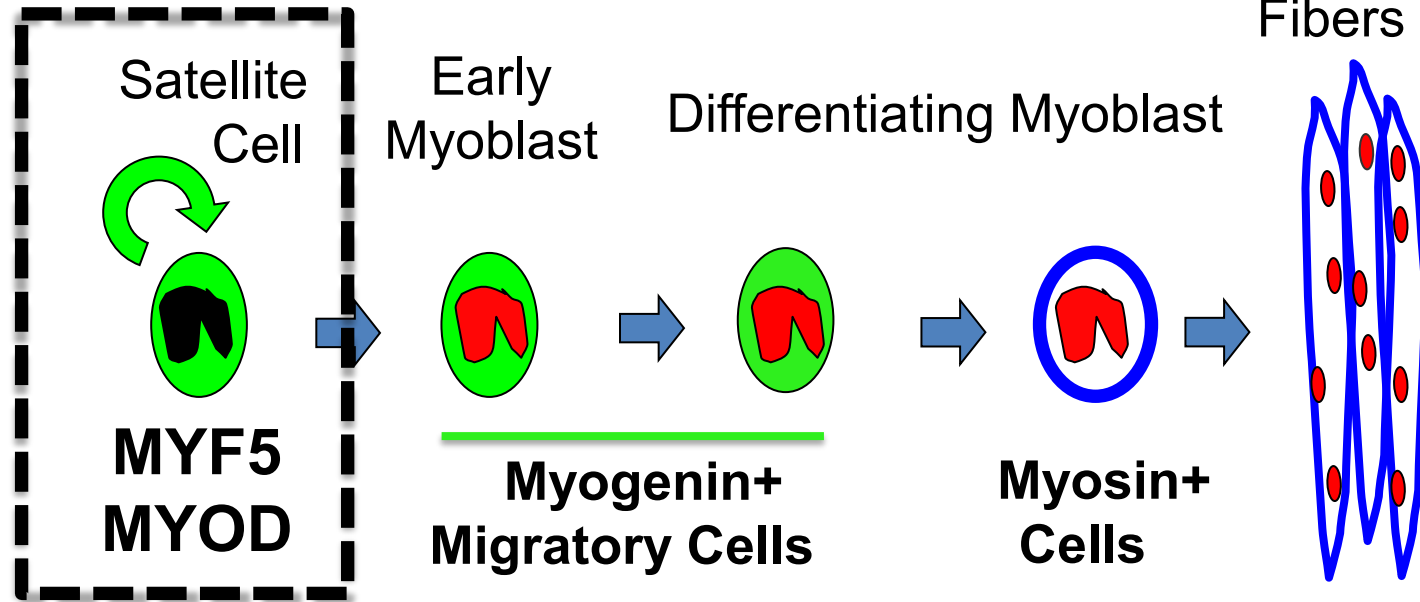
Imaging tumor heterogeneity.

myf5-GFP/differentiation-negative CSC.

Cancer stem cell pathways in ERMS

Cancer stem cells (CSCs)

Differentiated
Fibers



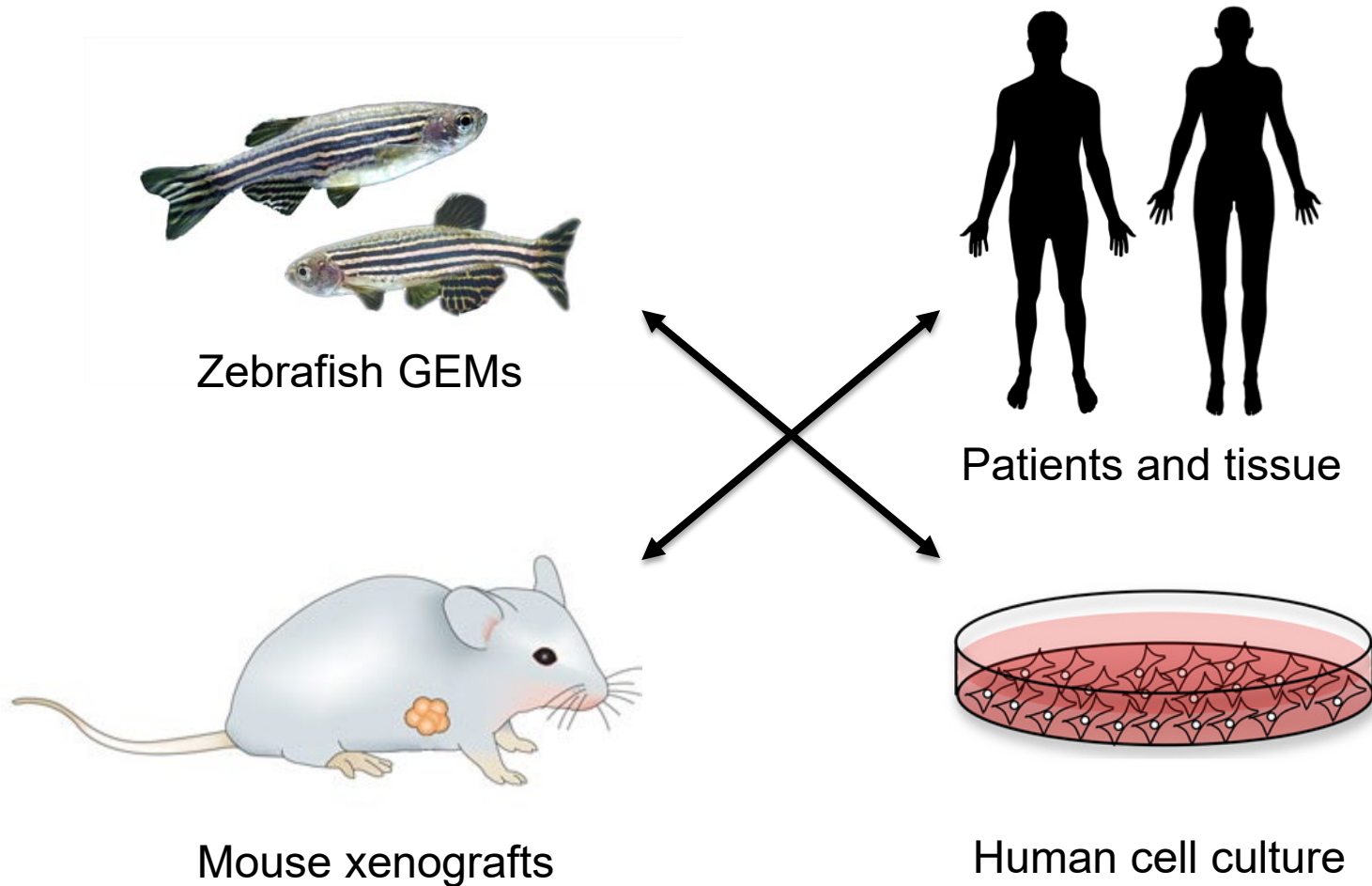
Notch1

WNT/ β -catenin

Vangl2/PCP

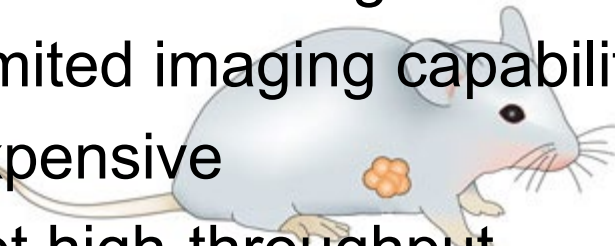
Ignatius et al., Cancer Cell 2012
Chen et al., PNAS 2014
Tenente et al., Elife 2017
Ignatius et al., Cell Reports 2017
Hayes et al., Cell Stem Cell 2018

Iterative modeling to discover mechanisms of human cancer



Iterative modeling to discover mechanisms of human cancer

- ❖ Pre-clinical drug studies
- ❖ Limited imaging capability
- ❖ Expensive
- ❖ Not high-throughput



Mouse xenografts

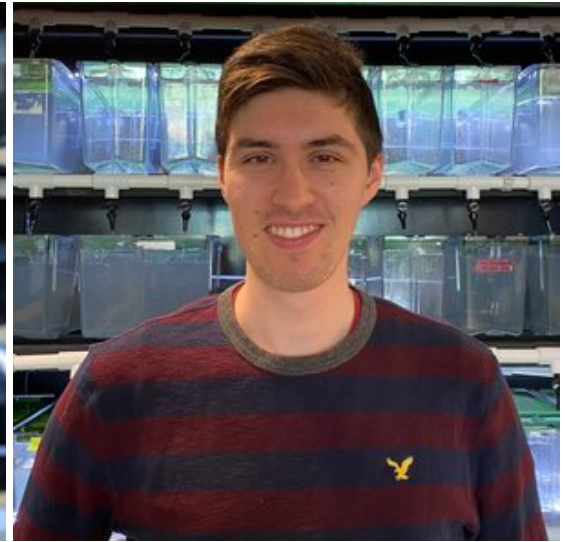
Single cell imaging of human cancer xenografts and therapy responses in immunodeficient zebrafish



Chuan Yan



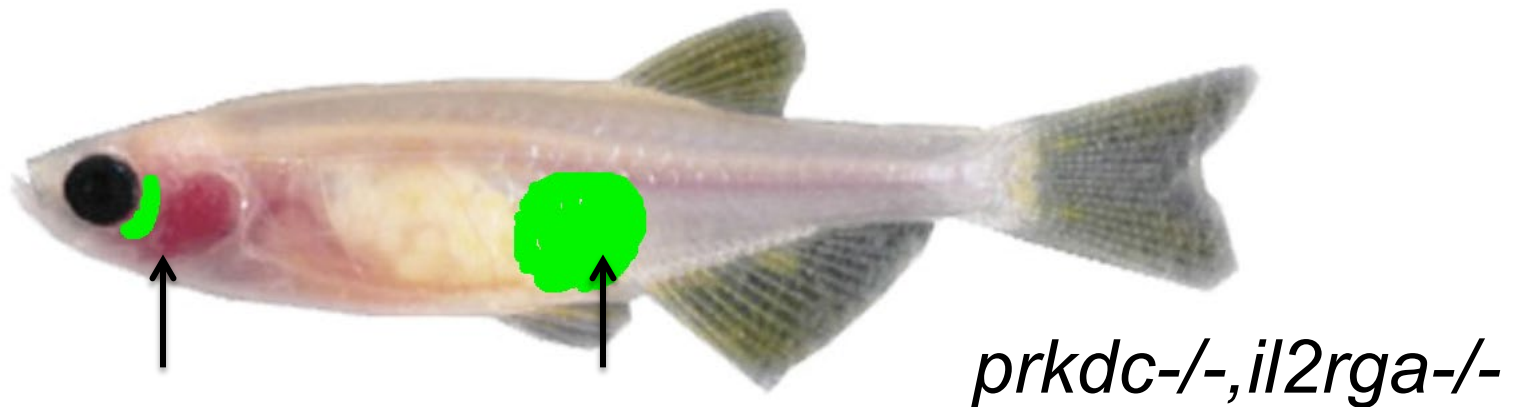
Qiqi Yang



Eric Alpert

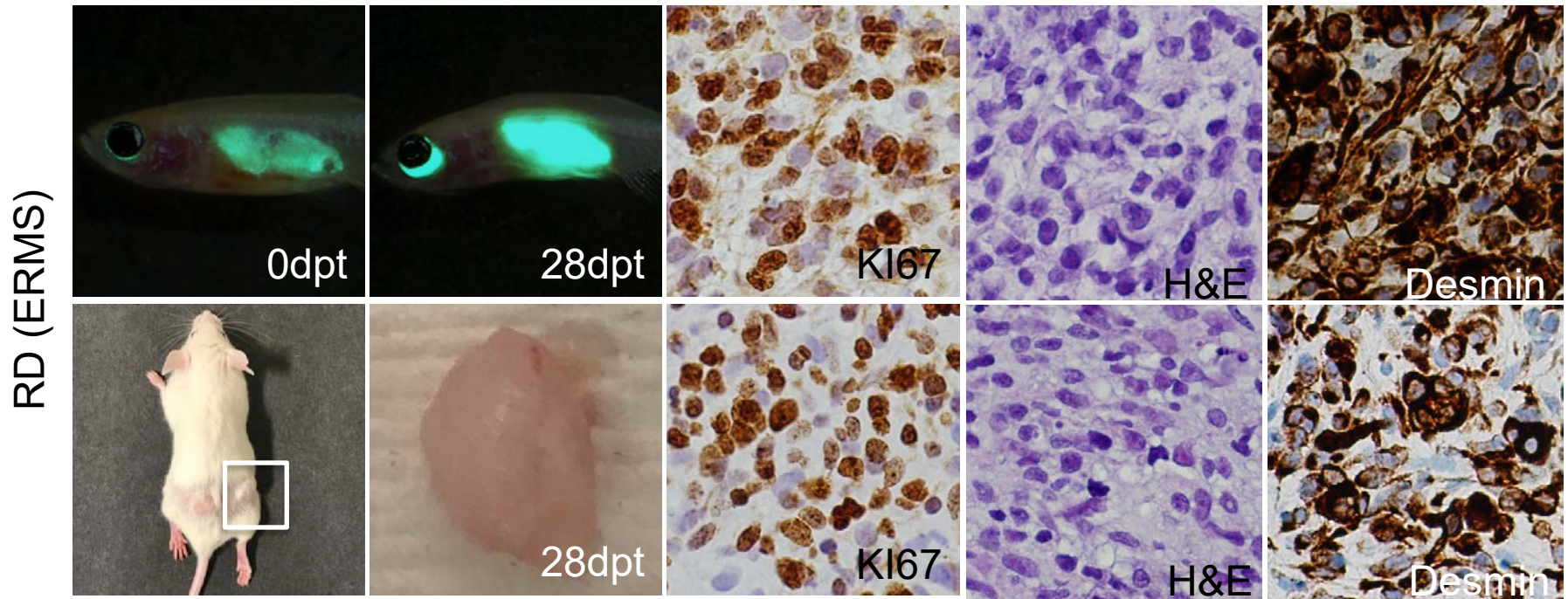
Yan et al., Cell 2019
Yan et al., Nat. Proc. 2020
Yan, Yang, et al., unpublished
Zhang, Yan, Millar, unpublished

Growing human tumors into immune-deficient zebrafish



- ✓ Single cell imaging of cancer
- ✓ Grow fish at 37°C
- ✓ Oral dosing of drugs

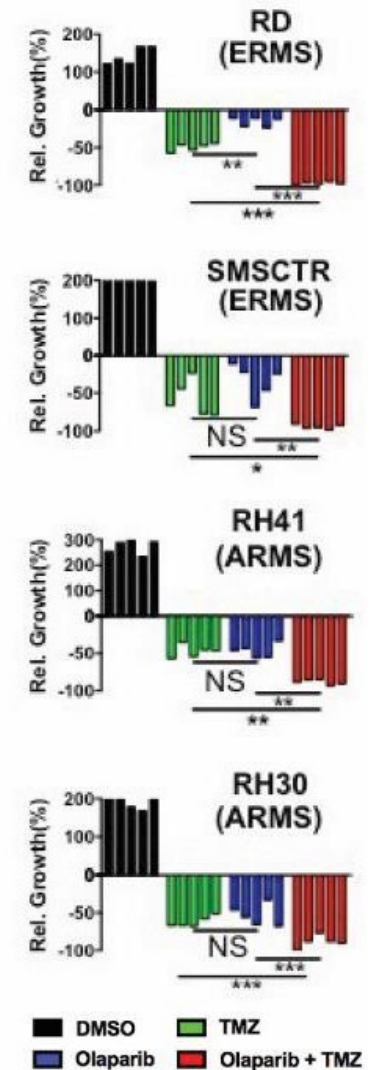
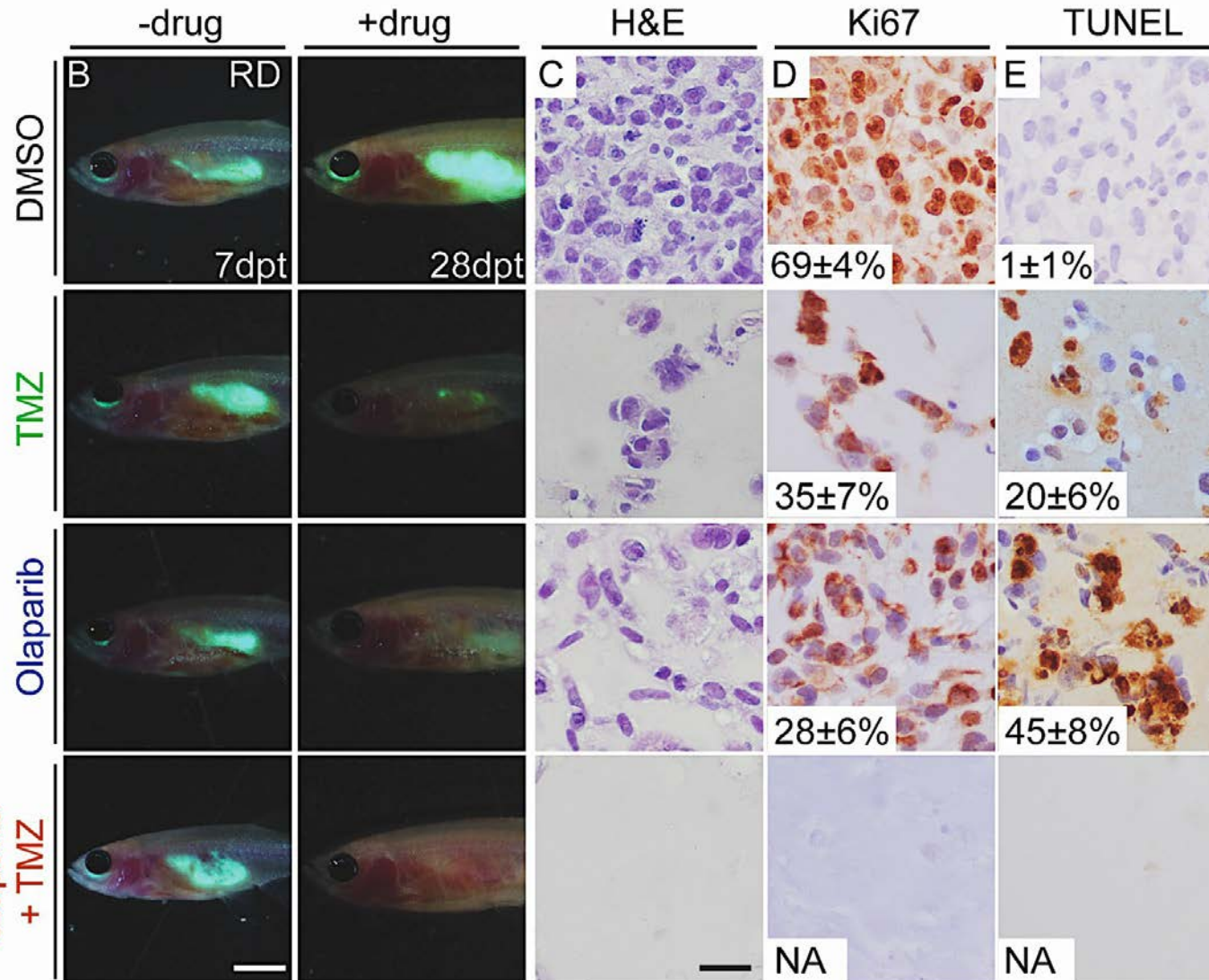
Human tumors grow the same in zebrafish and mice



Engraftment of sarcomas, TN breast cancer, melanoma, renal cell carcinoma, CML, glioblastoma, MPNSTs, **PDXs**

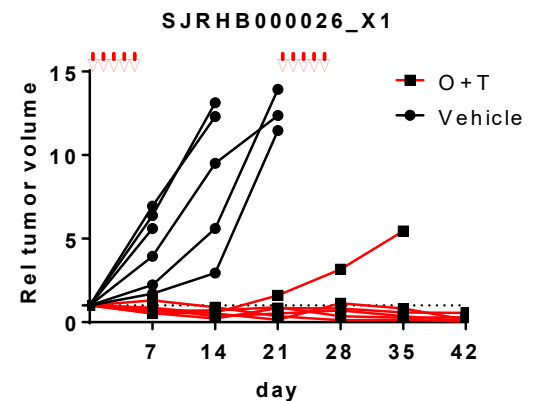
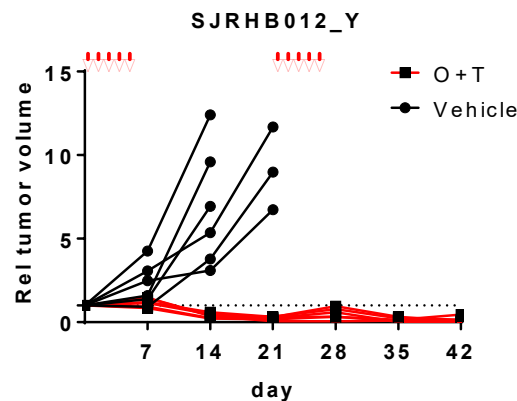
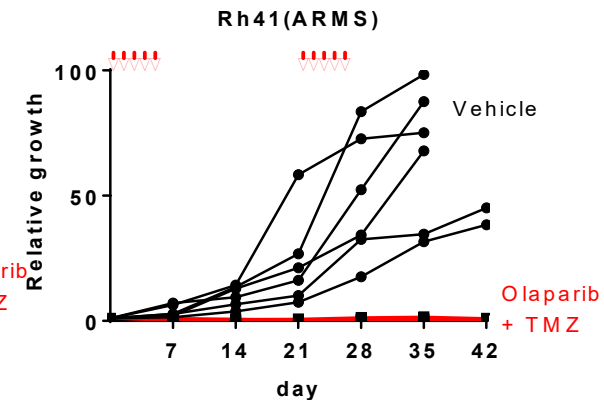
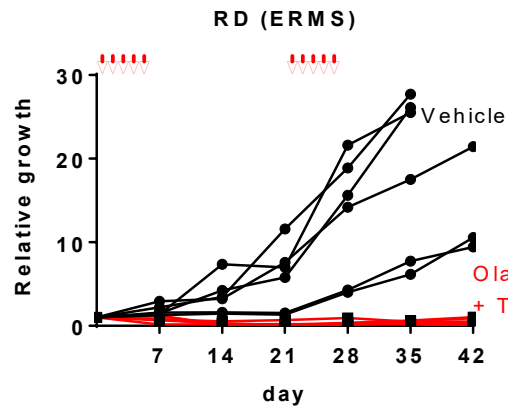
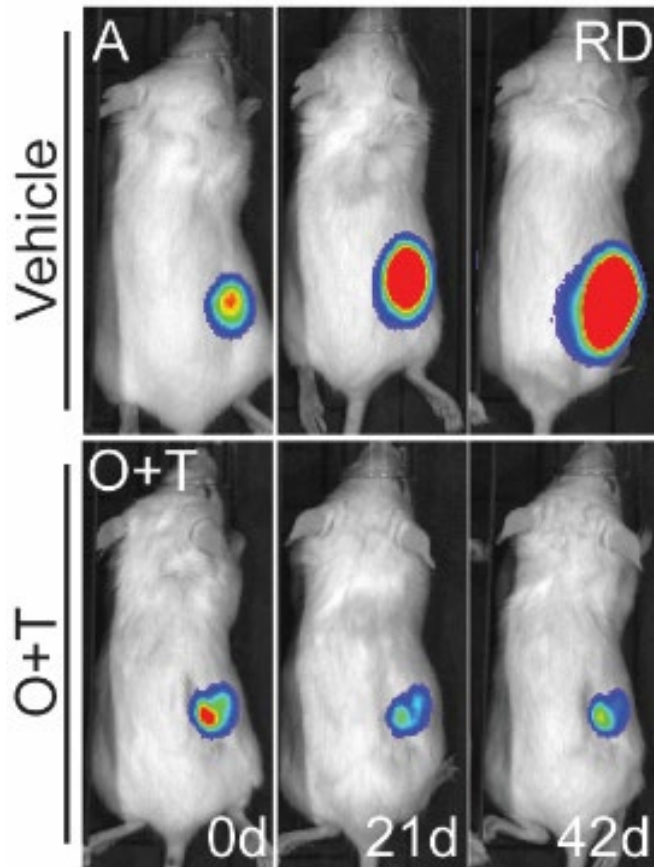
New combination therapy for muscle cancer

histological analysis

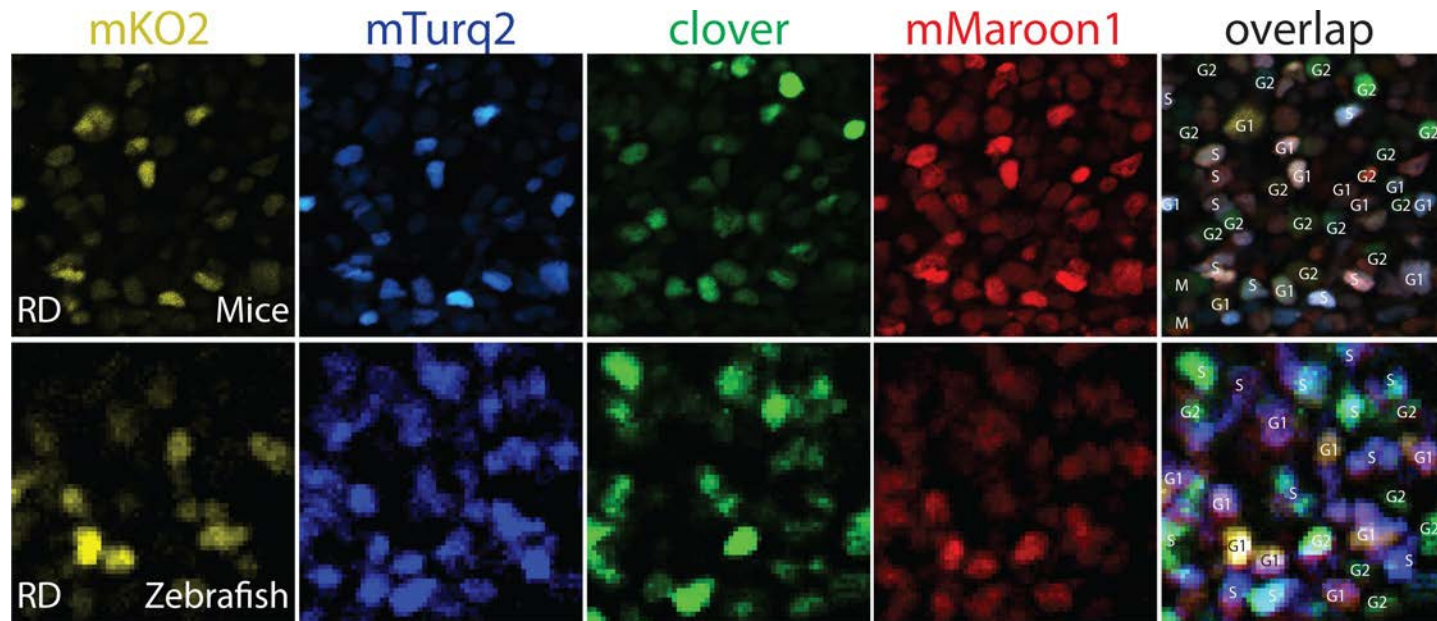
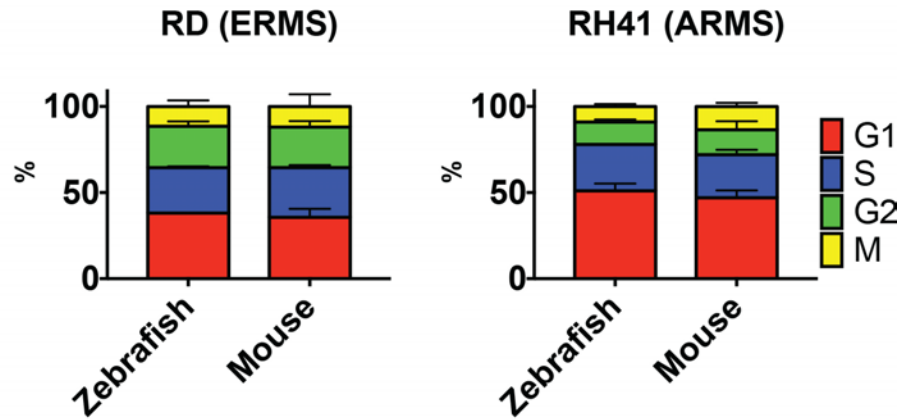
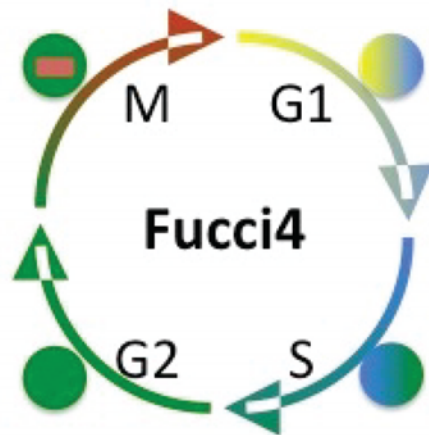


Validating combination therapy using patient-derived xenograft ERMS models

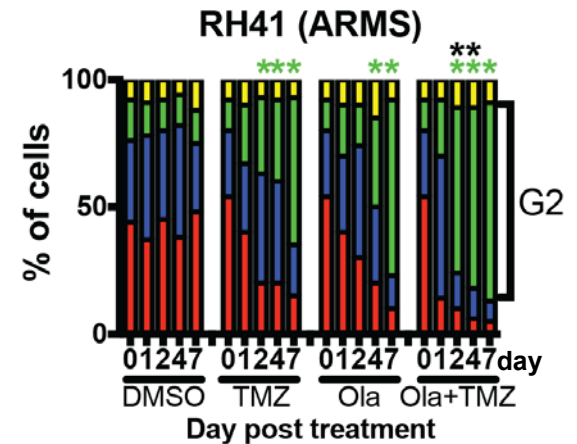
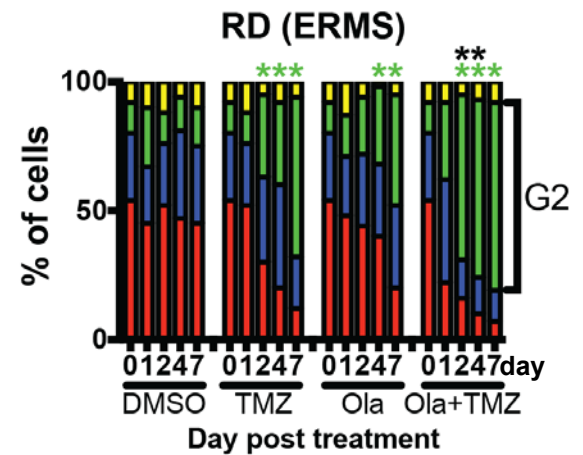
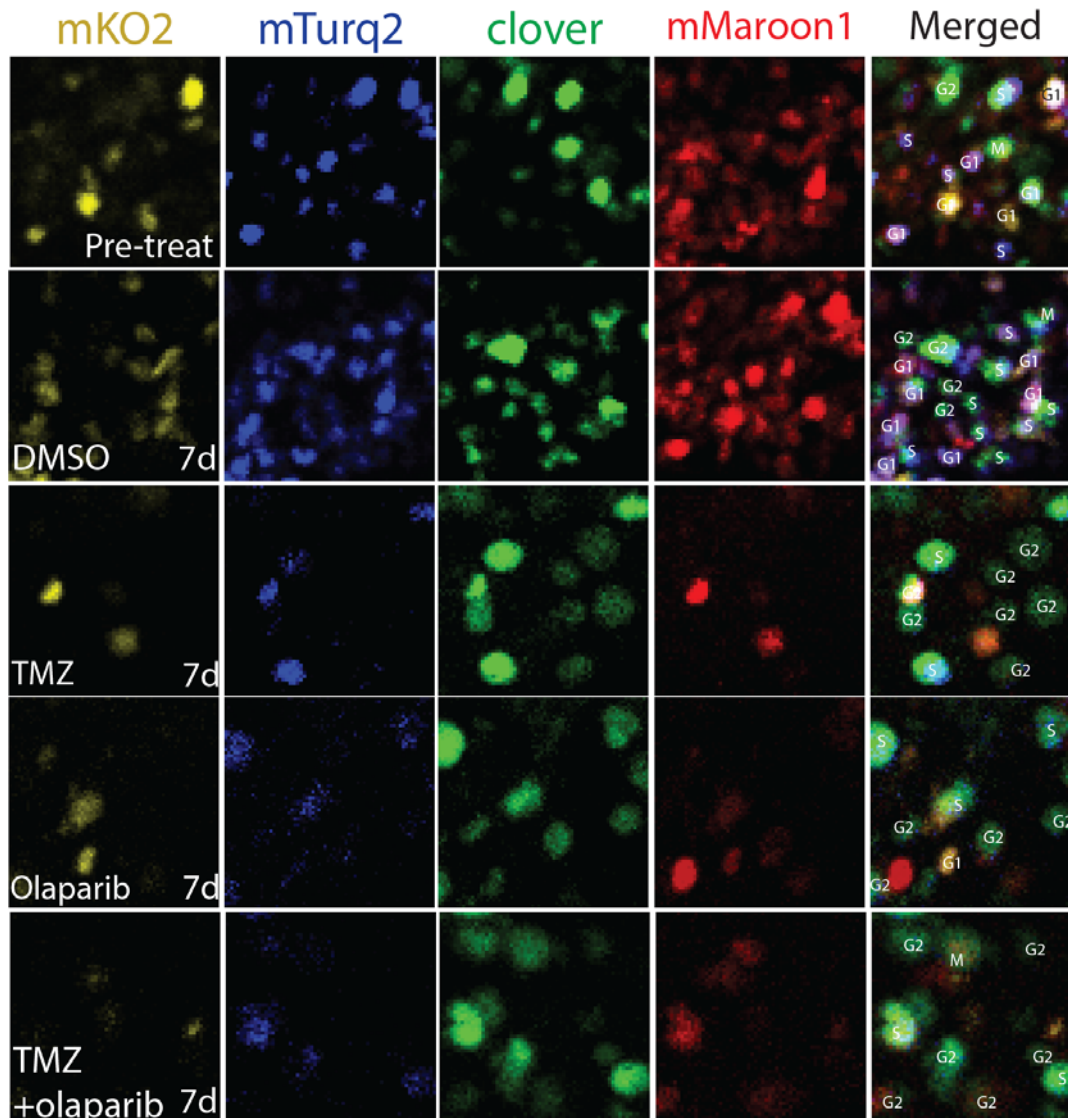
Whole animal



Imaging cell cycle kinetics of RMS *in vivo* at single cell resolution

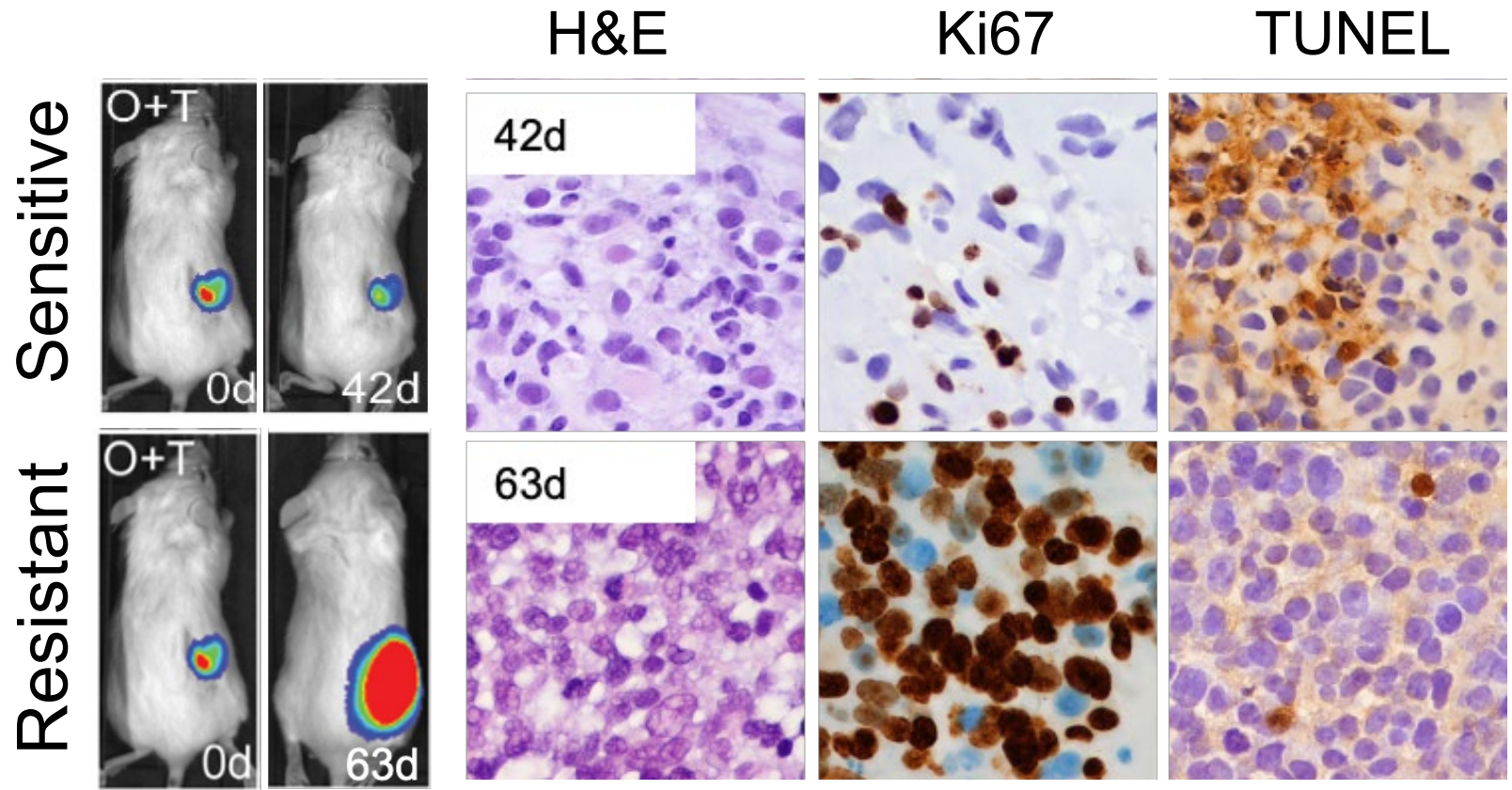


Assessing therapy responses of RMS *in vivo* and at single cell resolution

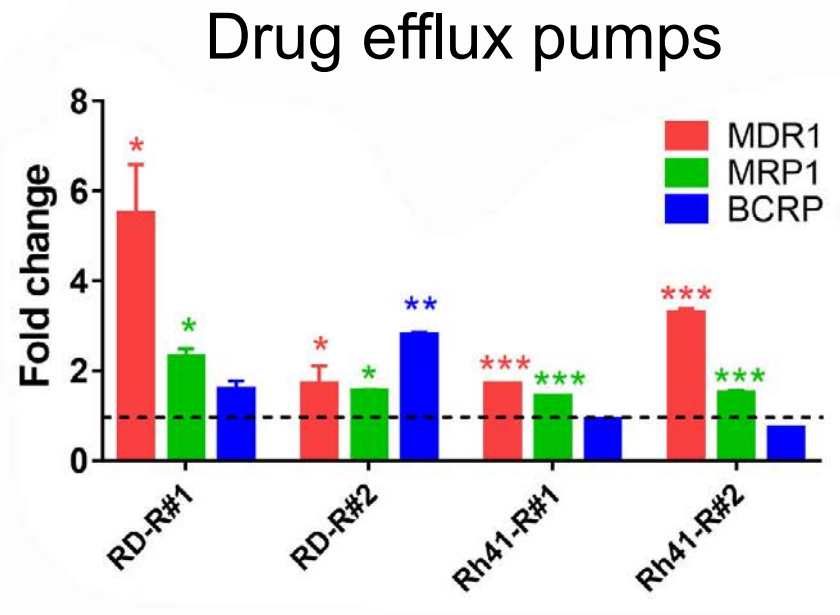
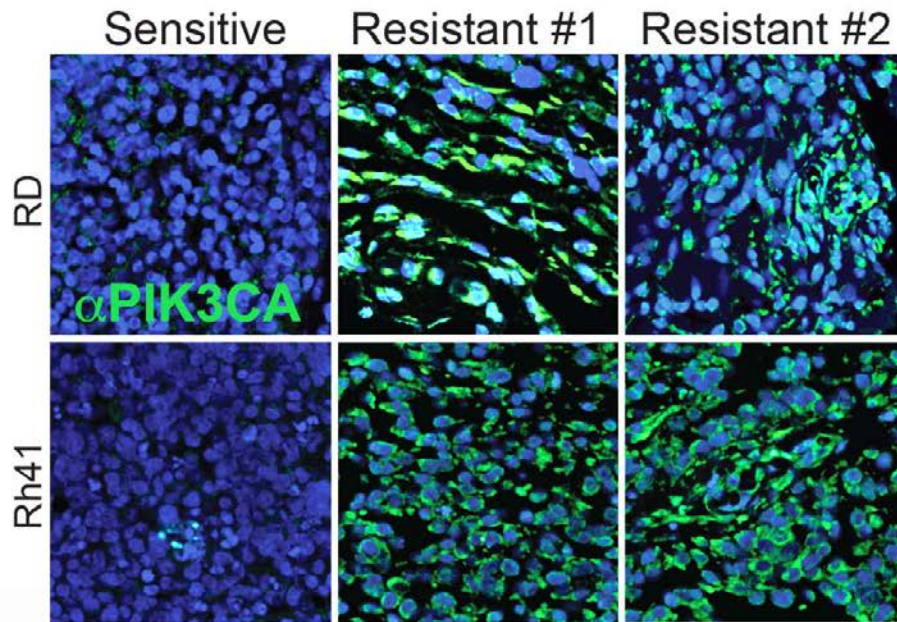


Legend: G1 (red), S (blue), G2 (green), M (yellow)

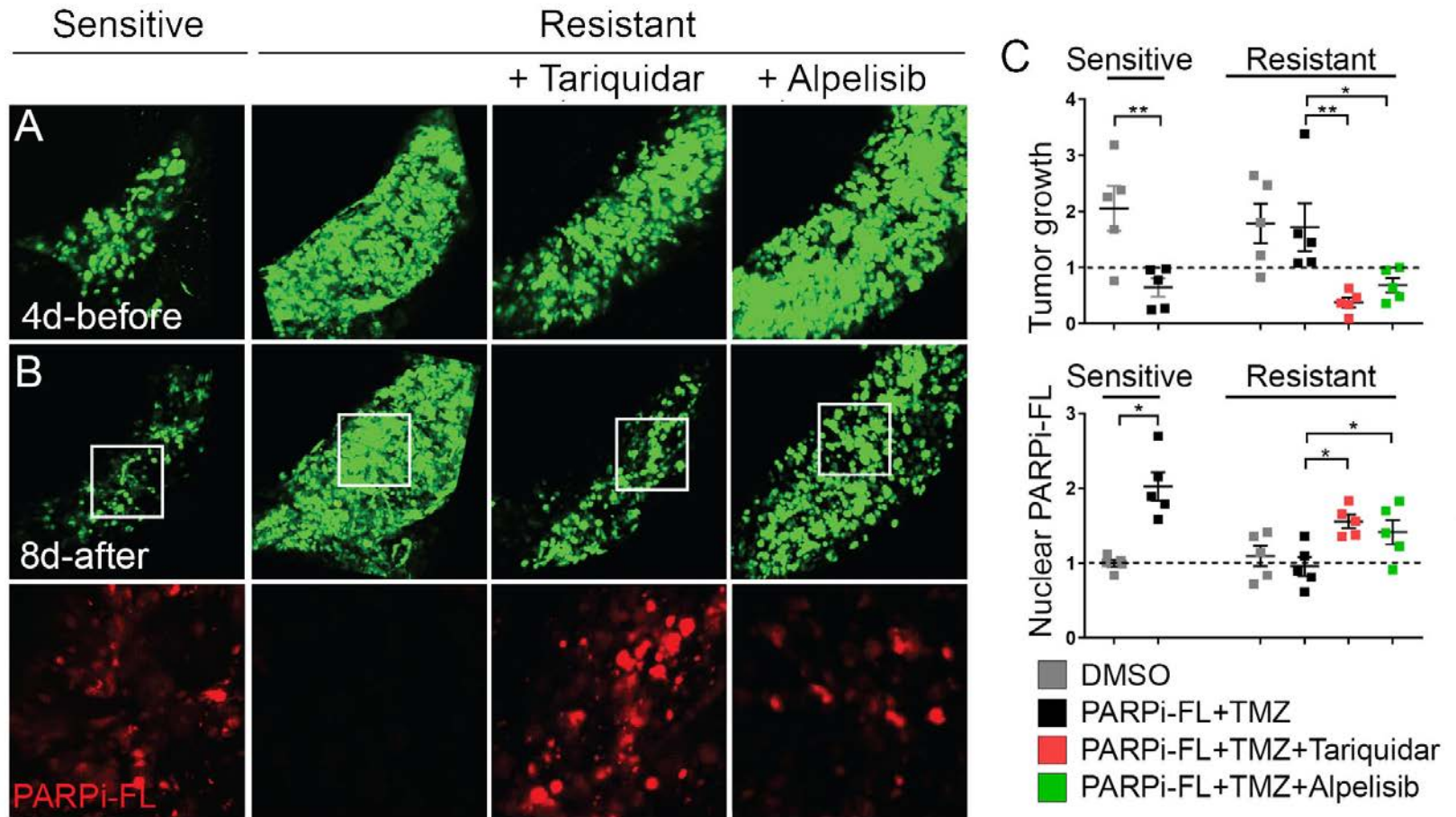
A subset of RMS develop resistance to Olaparib + TMZ



Resistant RMS upregulate the PI3K/AKT/ABC transporter pathway

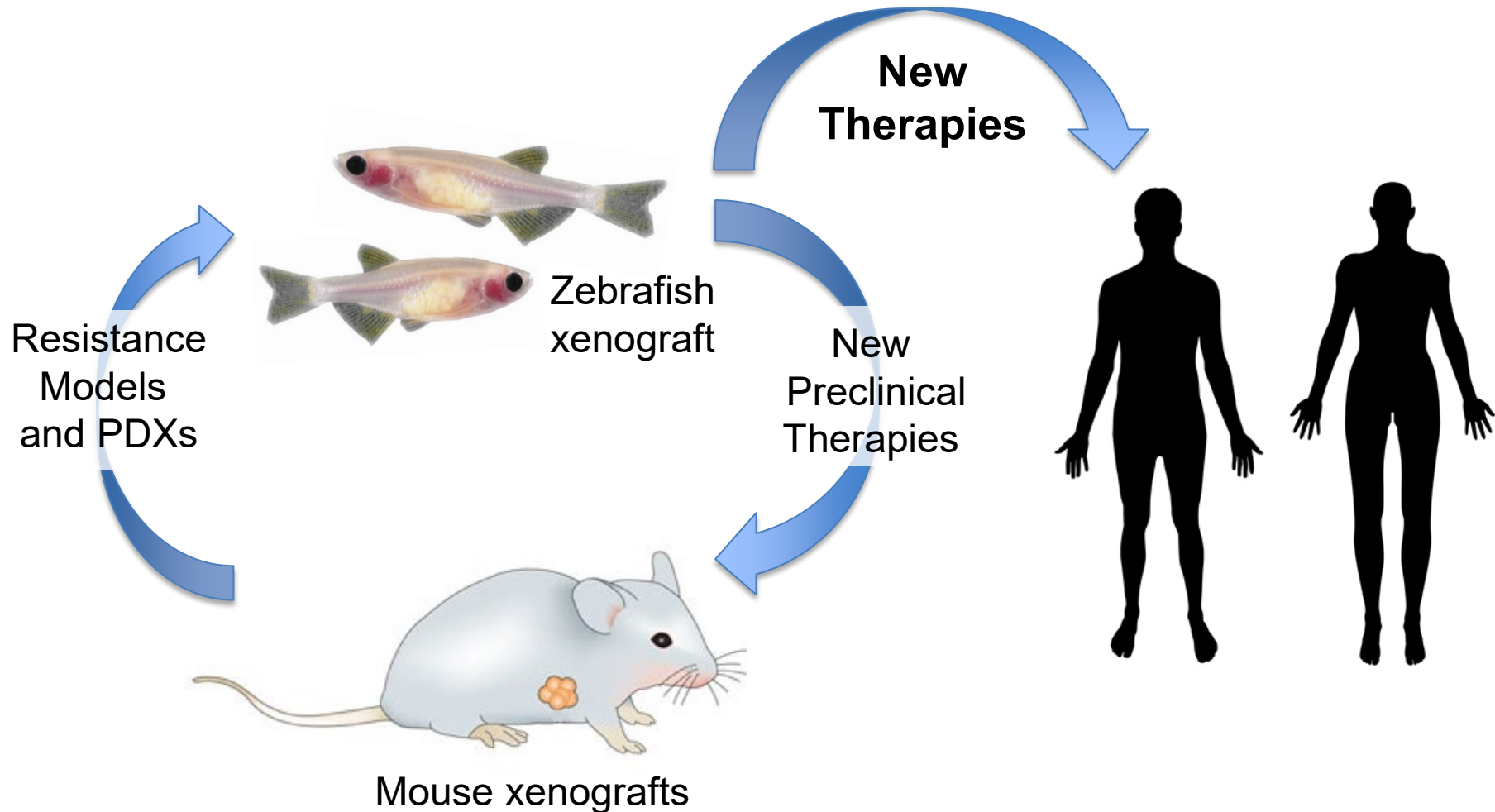


PIK3CA and ABC transport inhibitors



N=166 engrafted tumors, >20,000 analyzed cells

A new xenograft pipeline for identifying combination therapies



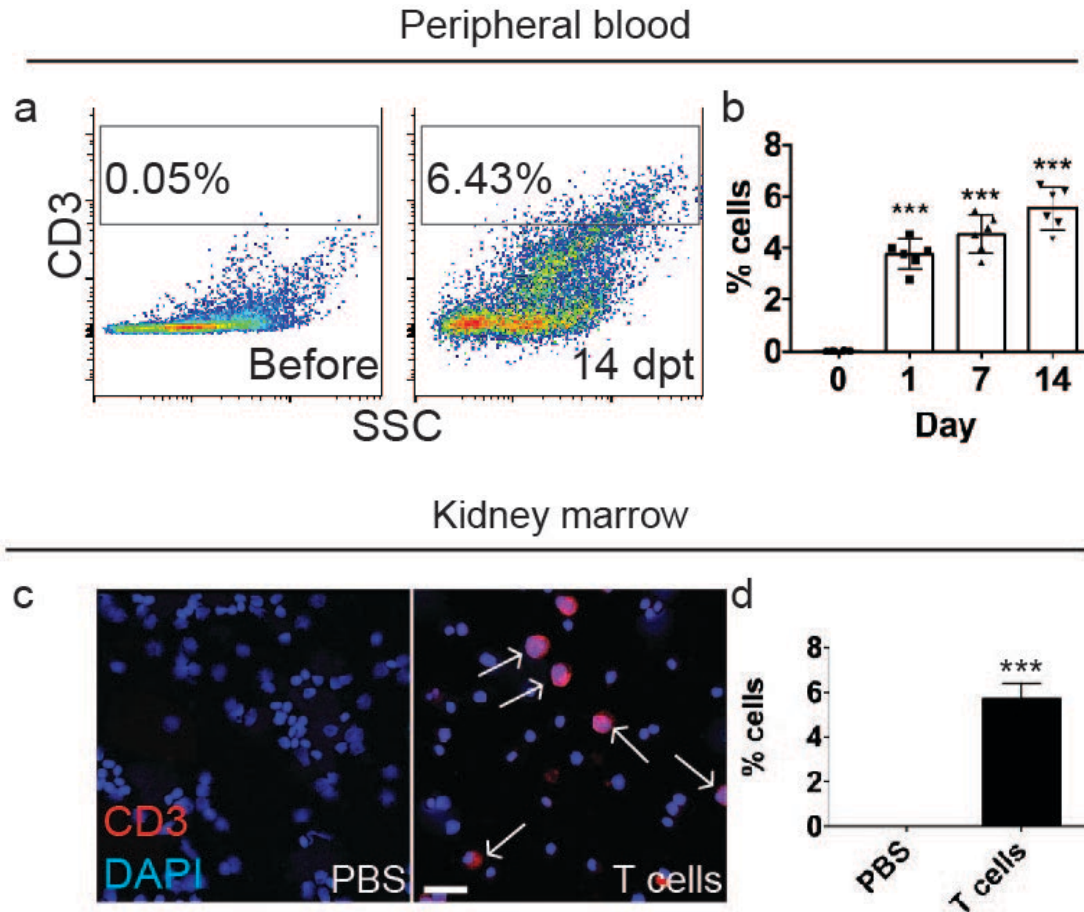
Better immune deficient zebrafish models for engrafting human cancer



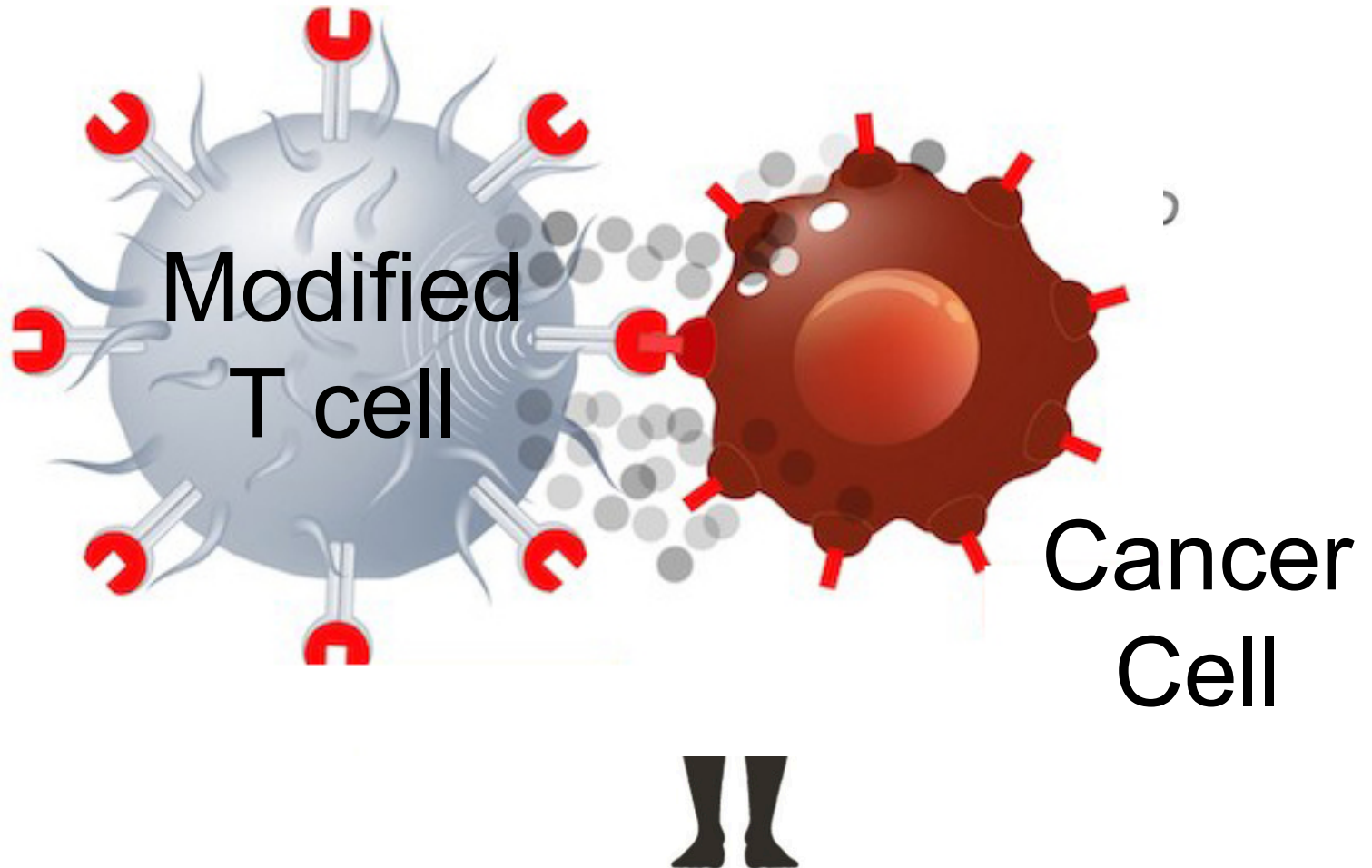
rag2 Δ/Δ , il2rga $^{-/-}$

- ✓ Fully ablate mature T, B, and NK cells
- ✓ Enhanced engraftment at 37°C
- ✓ Radiation competent

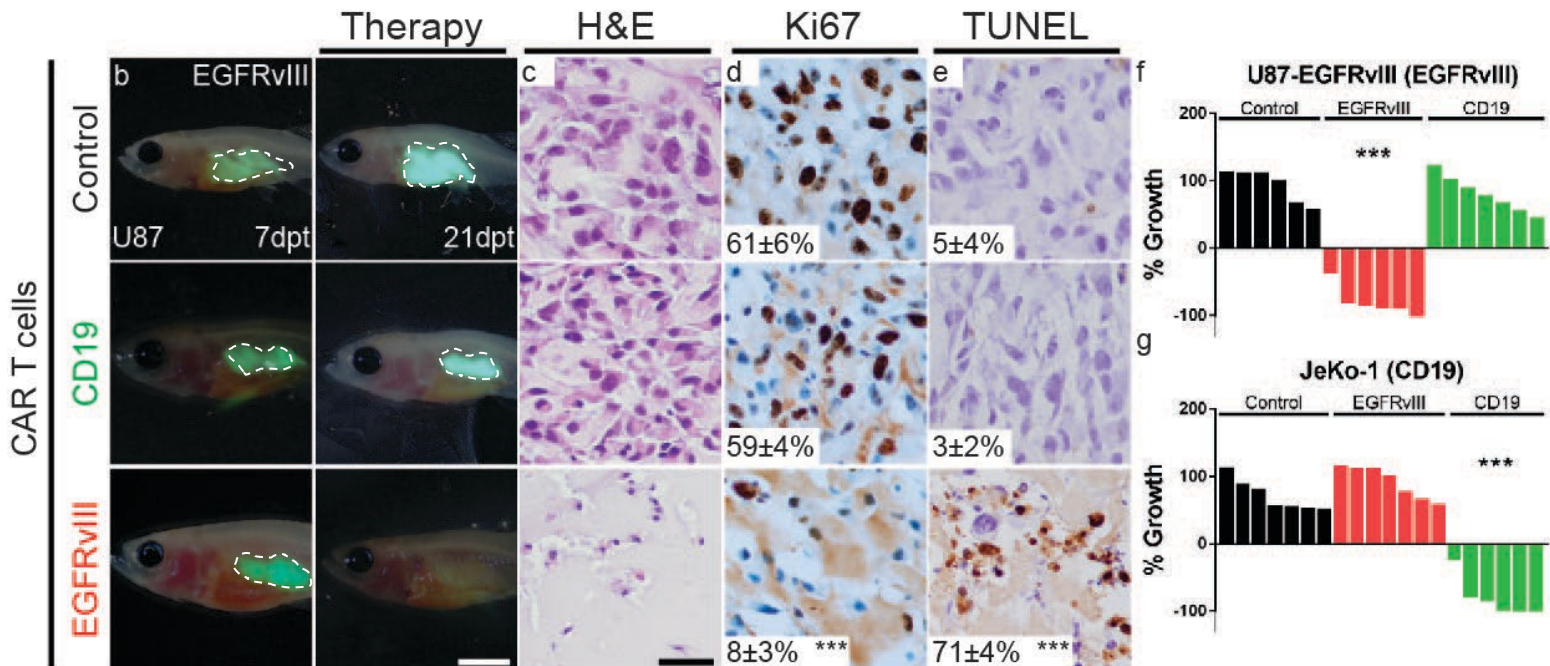
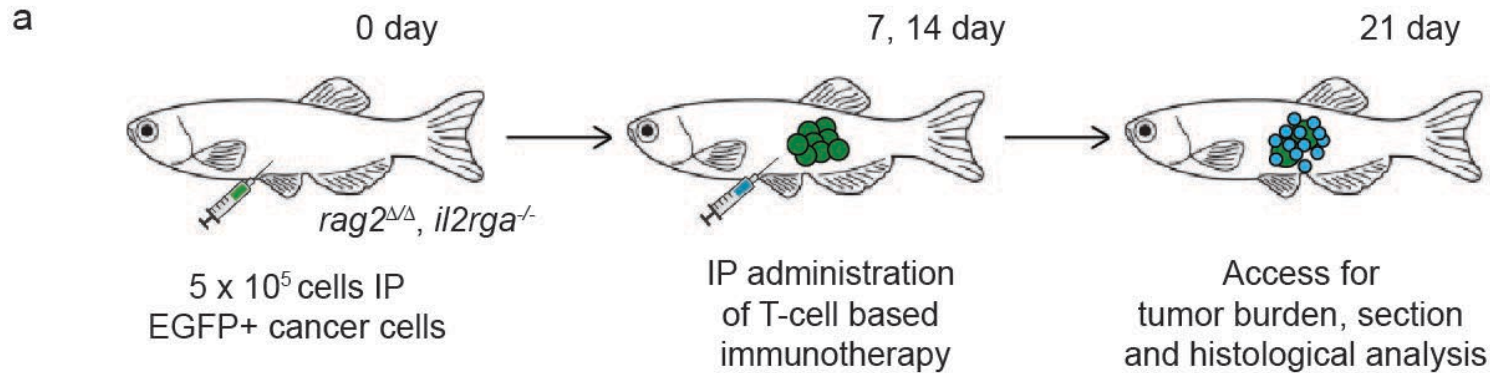
rag2 Δ/Δ , *il2rga* $^{-/-}$ zebrafish engraft human T cells



Chimeric Antigen Receptor T Cells

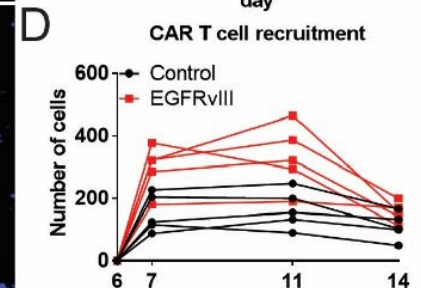
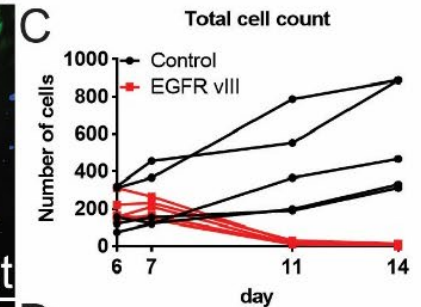
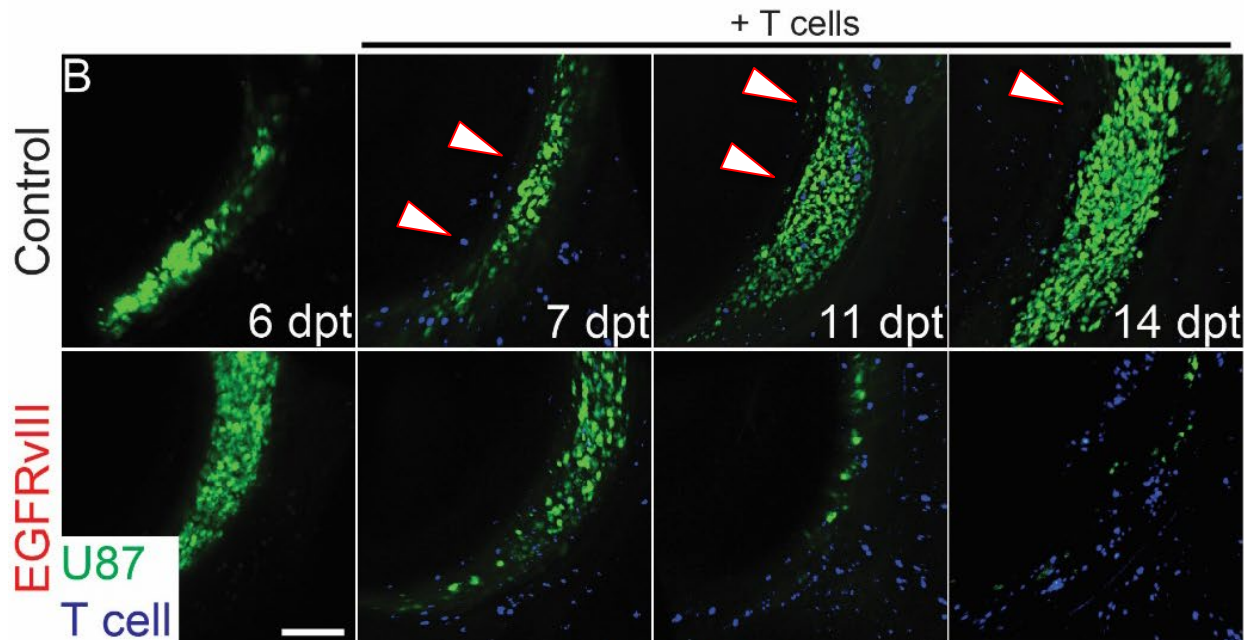
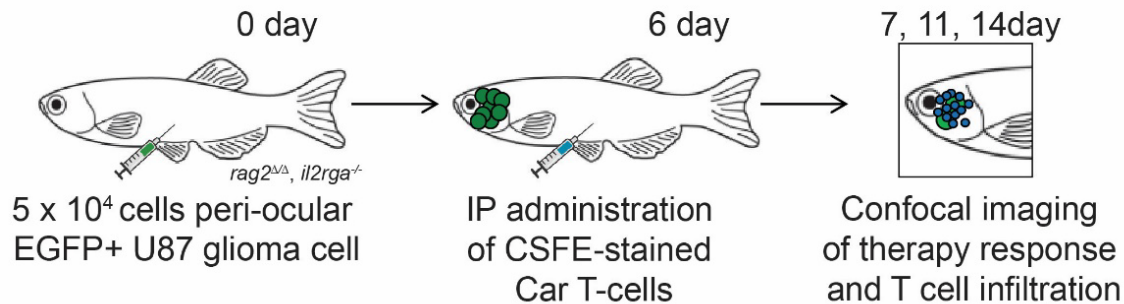


Assessing CAR-T cell function *in vivo*

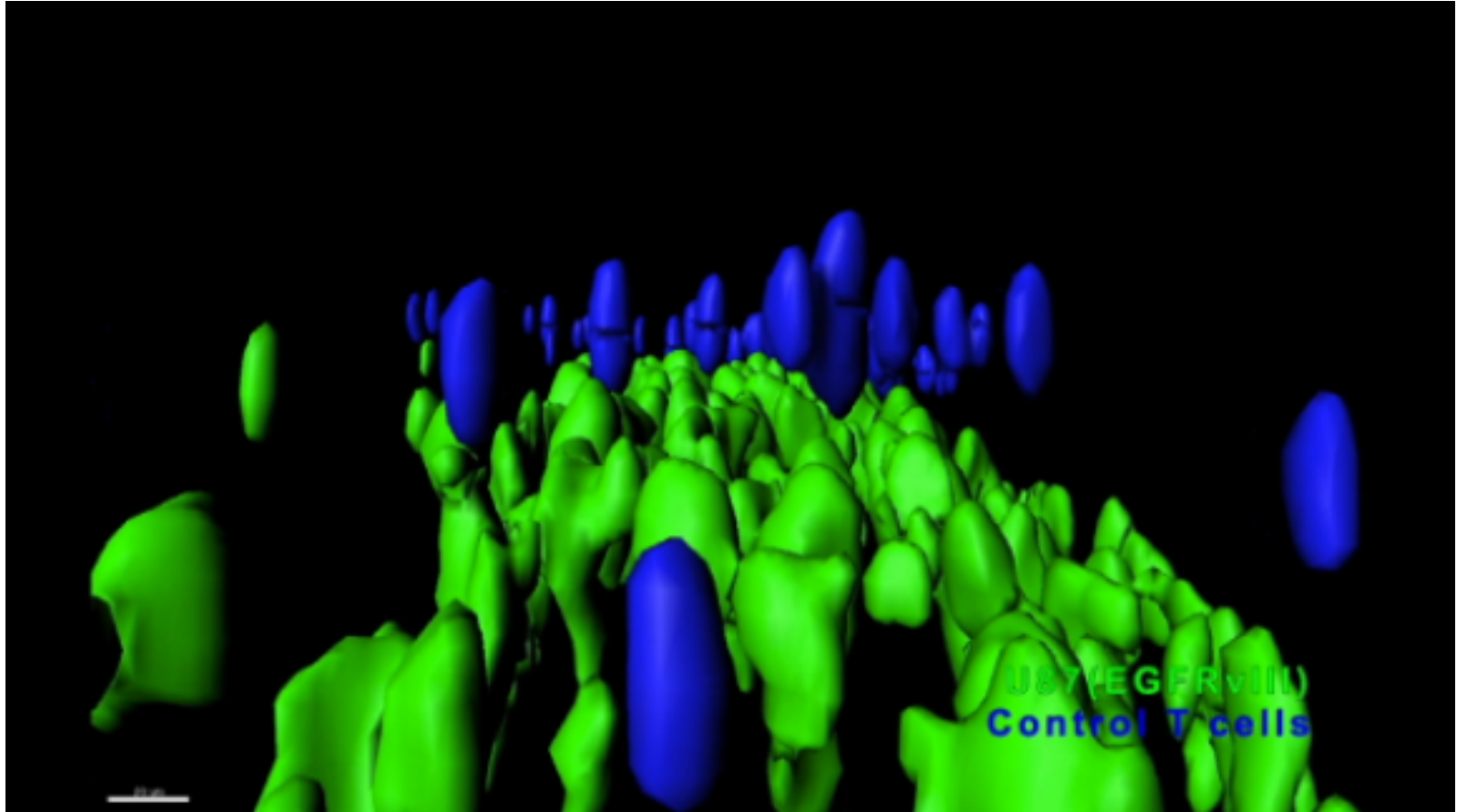


Single cell imaging of CAR-T cell function *in vivo*

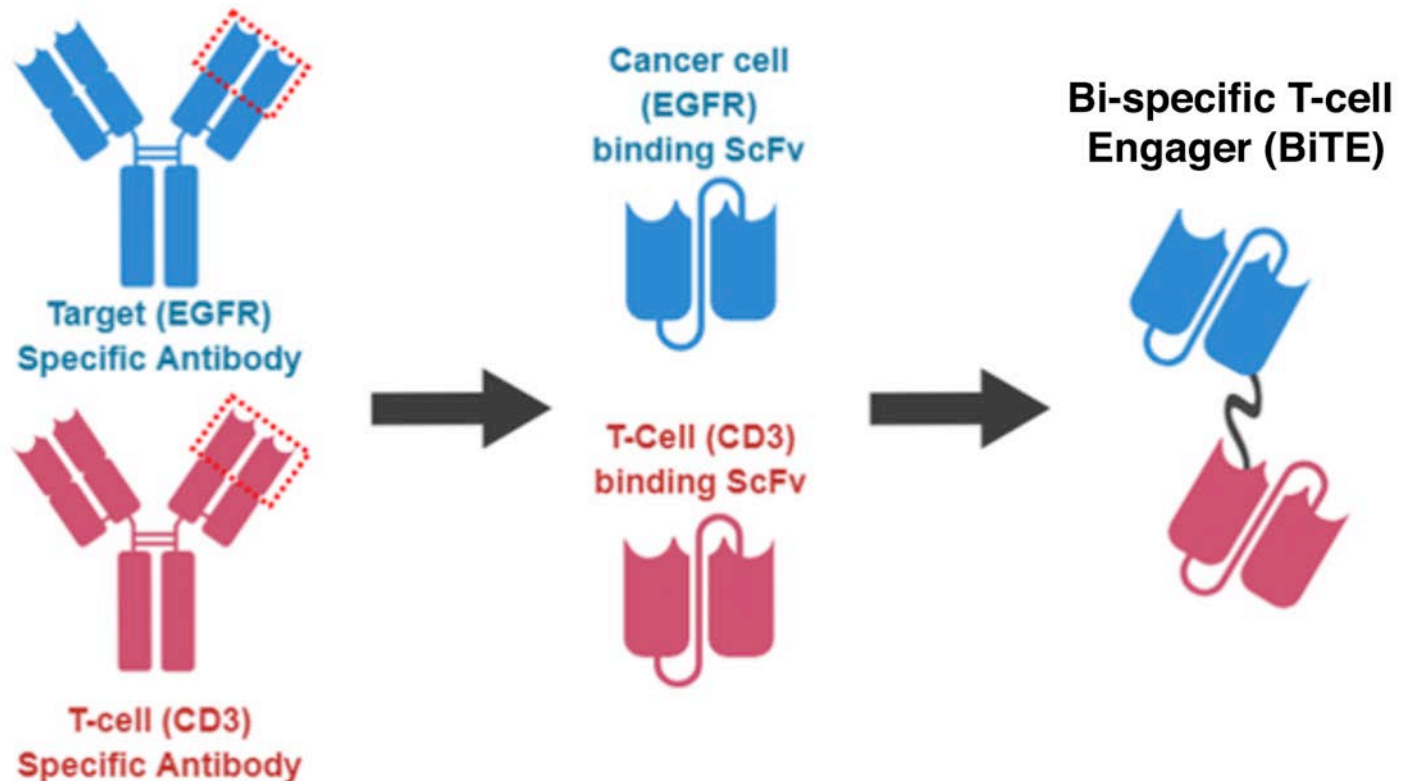
A



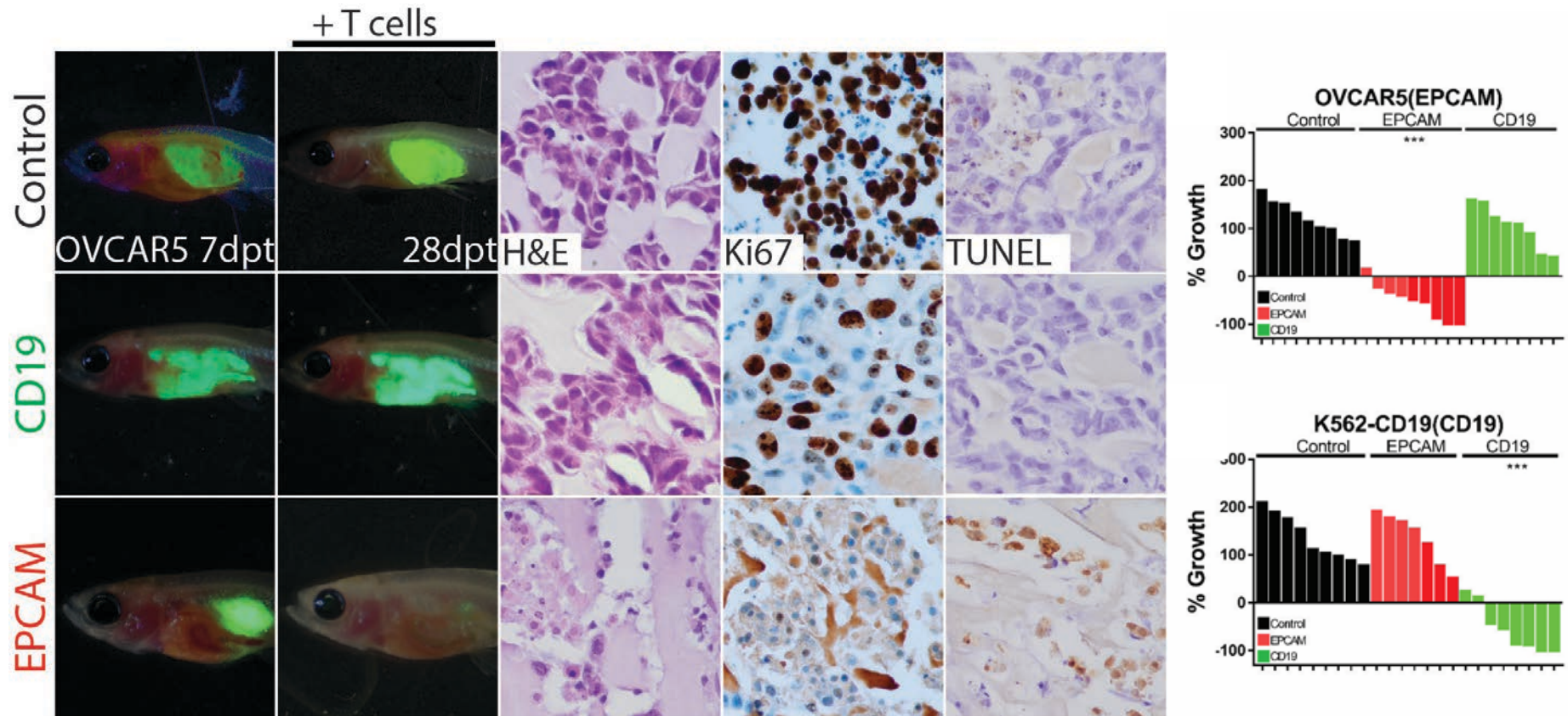
Single cell imaging of CAR-T cell function *in vivo*



Bi-specific T-cell Engager (BiTE)

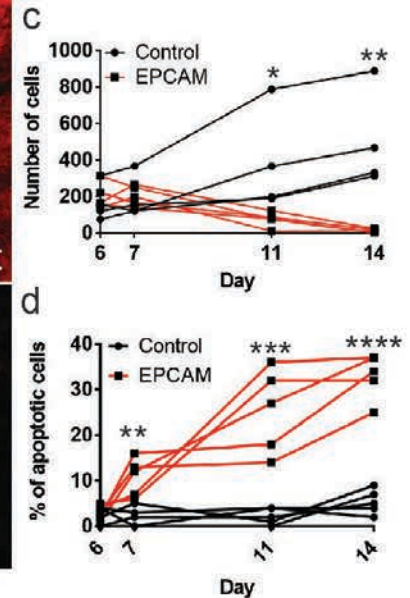
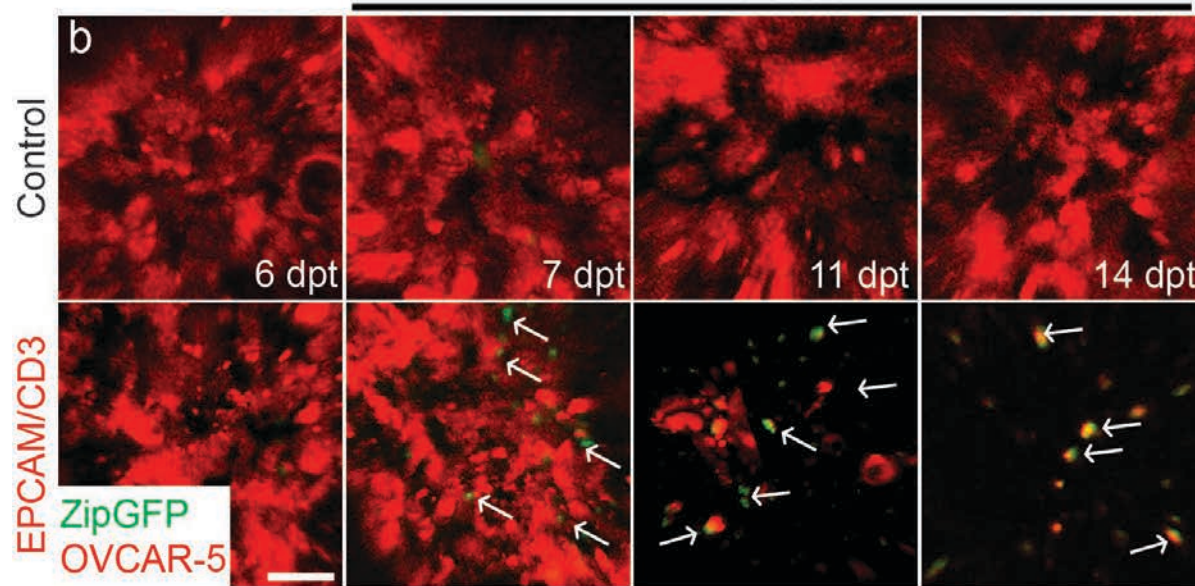
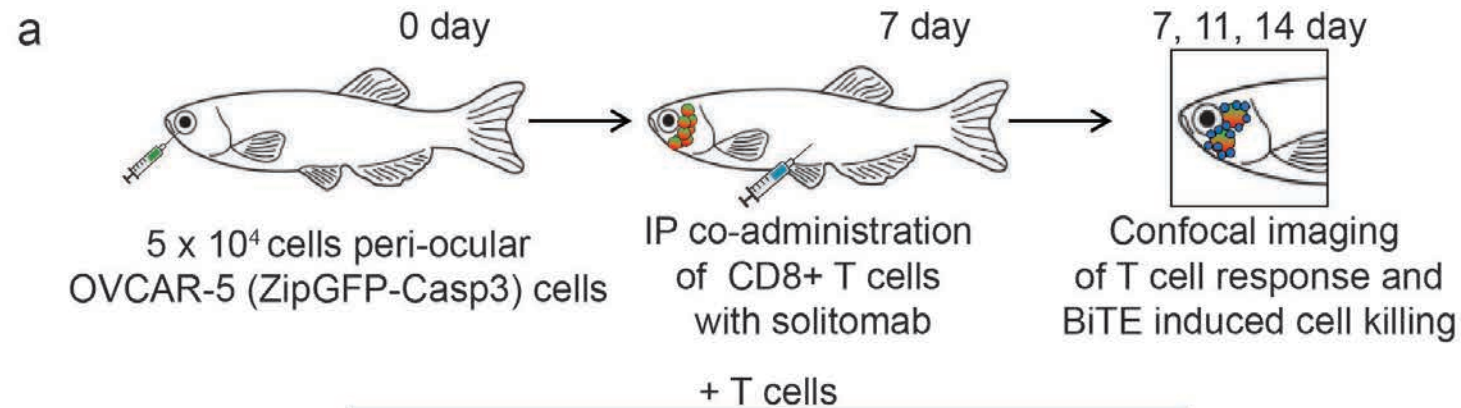


Assessing BiTE cell function *in vivo*

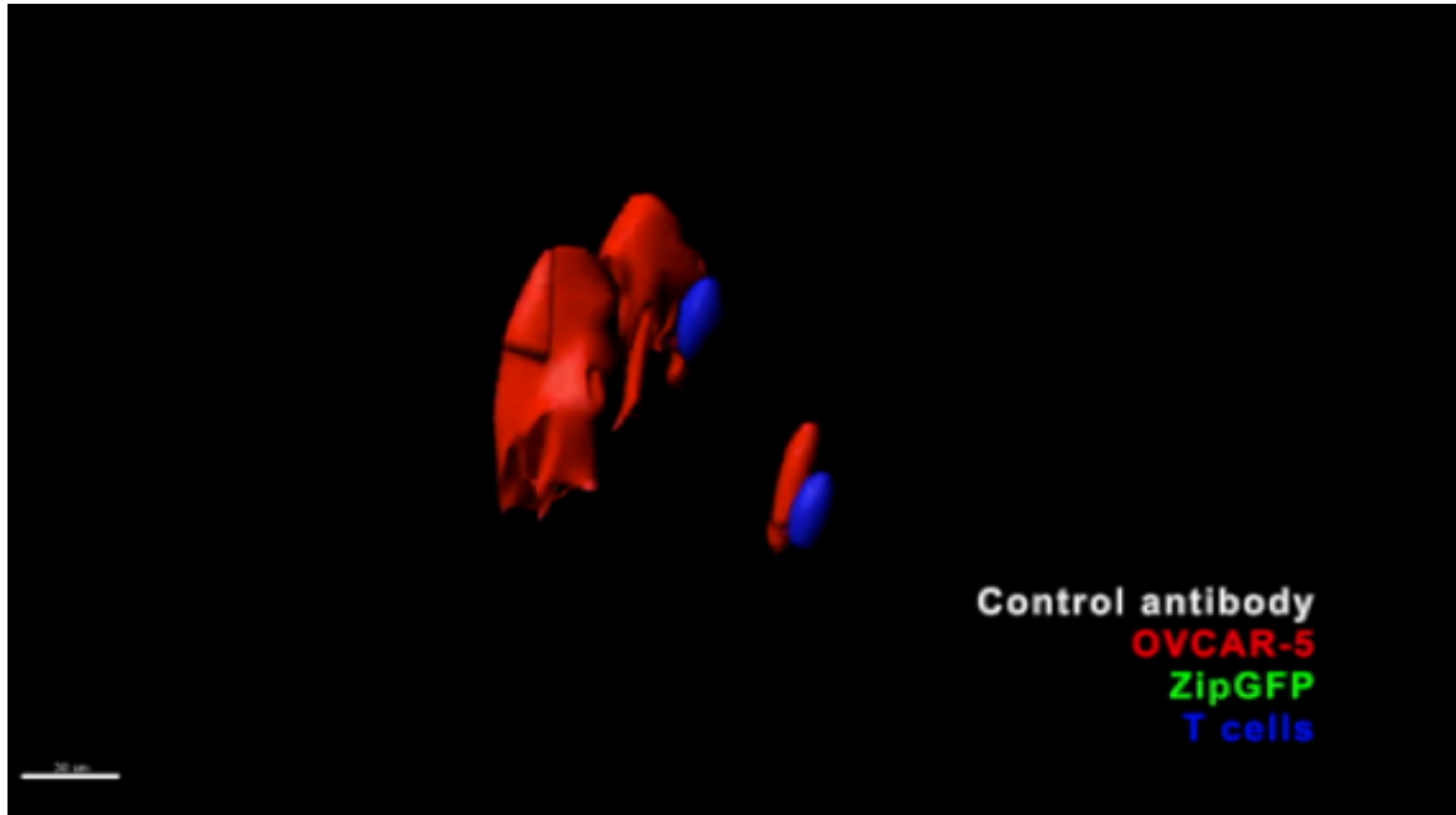


Specificity of killing and single cell imaging

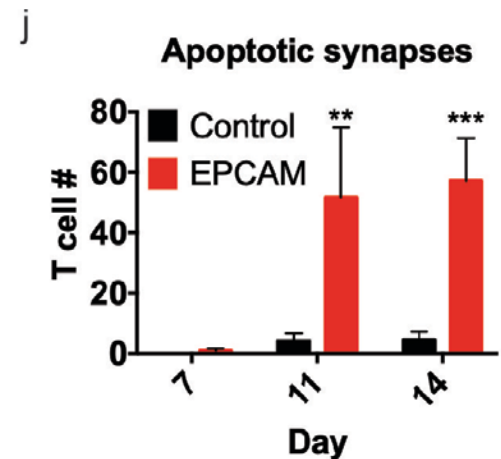
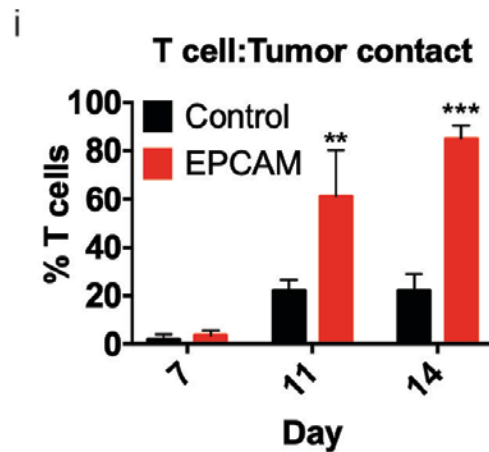
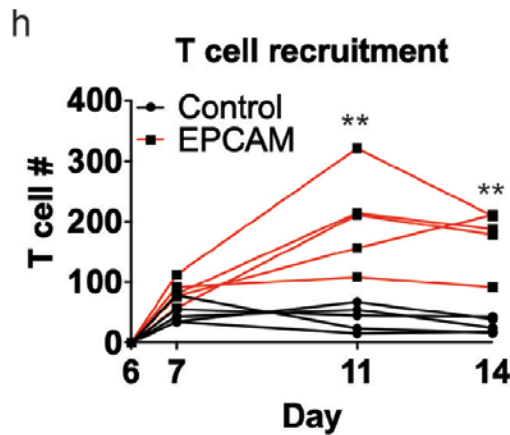
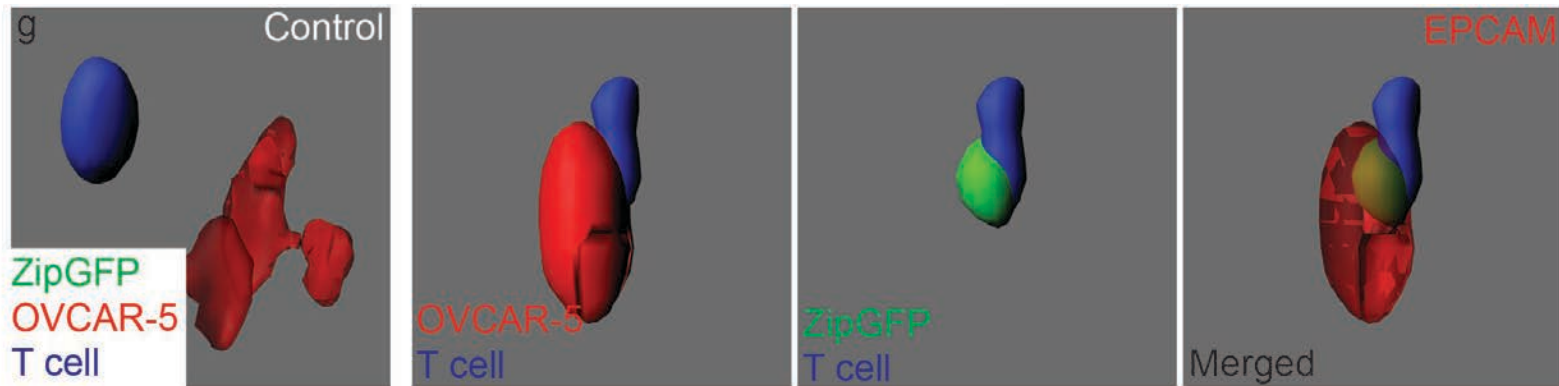
Assessing BiTE cell function *in vivo*



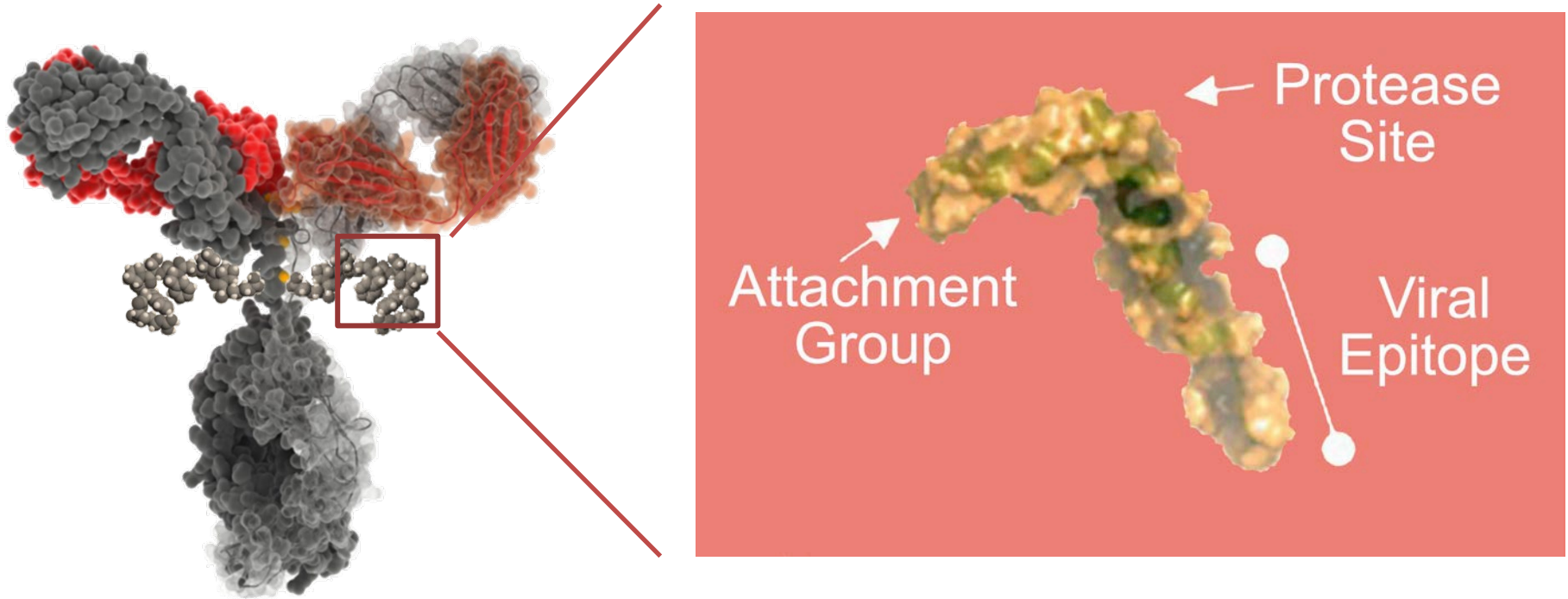
Assessing BiTE cell function *in vivo*



Assessing BiTE cell function *in vivo*



Antibody peptide epitope conjugates



Mark Cobbold

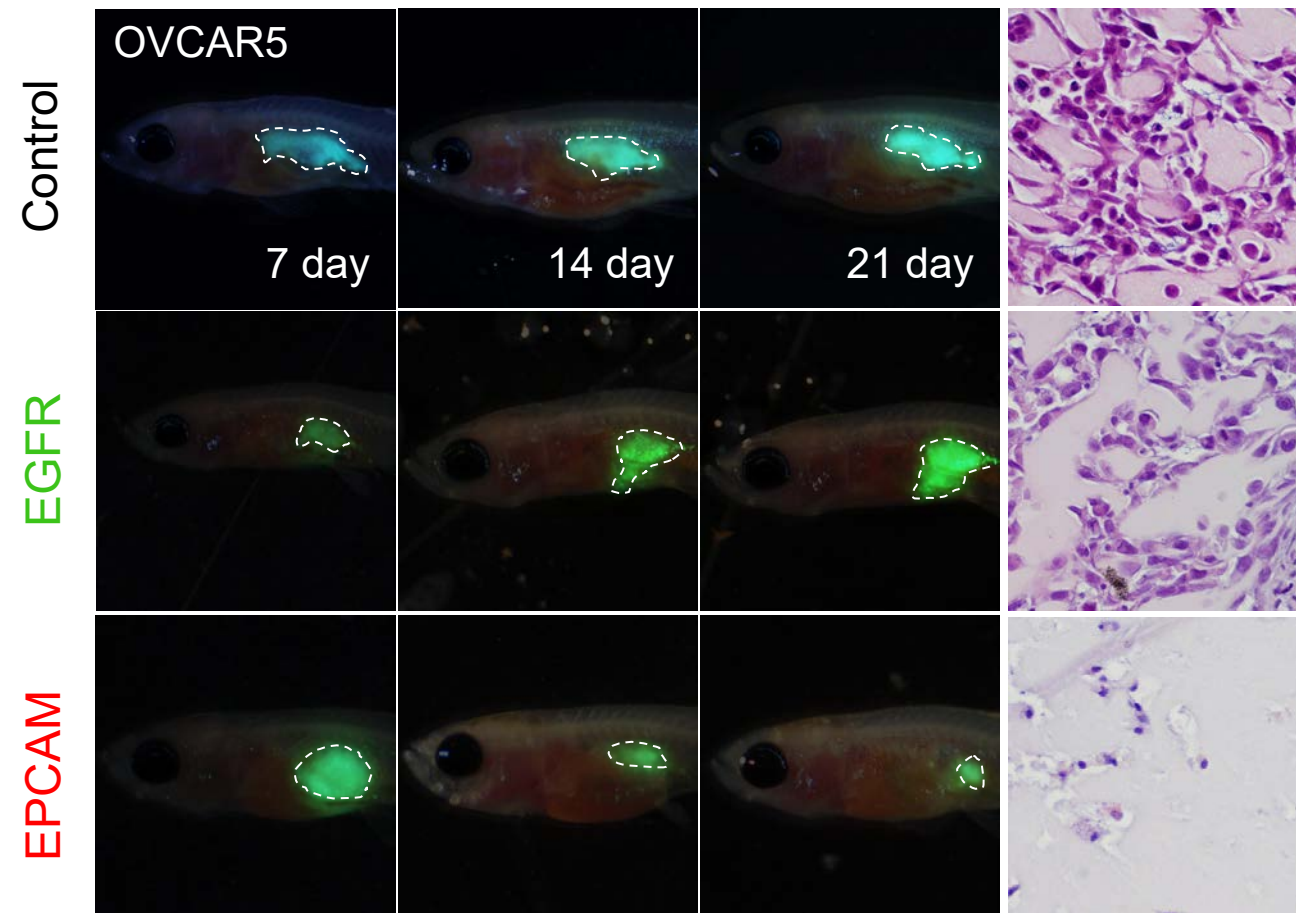
Songfa Zhang

David Millar

Chuan Yan

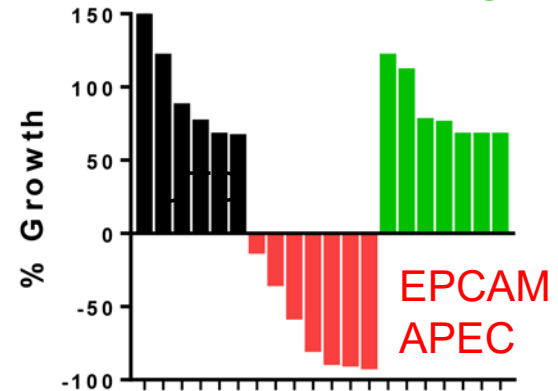
Antibody peptide epitope conjugates (APECs)

+ APEC and CMV T cells



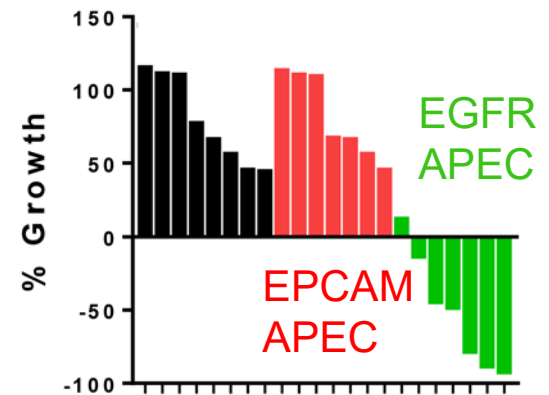
OVCAR5

EGFR
APEC



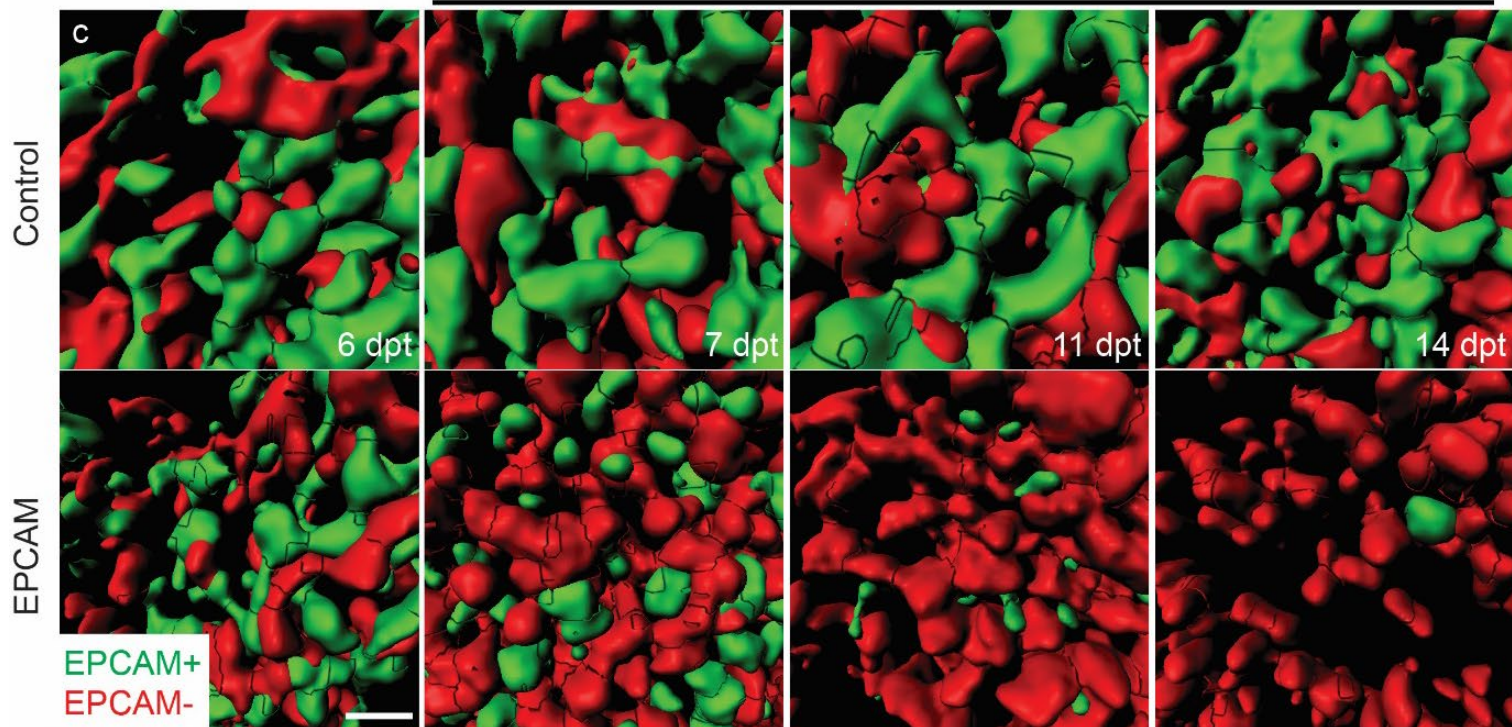
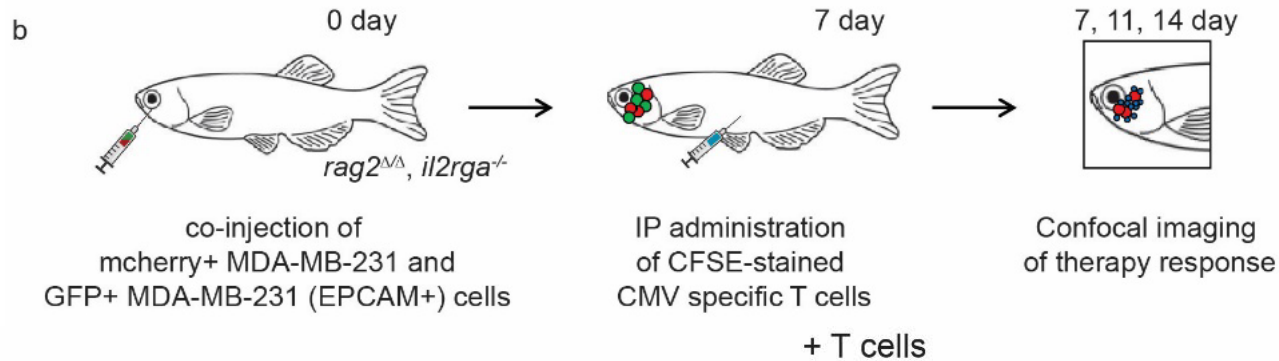
MDA-MB231

EGFR
APEC

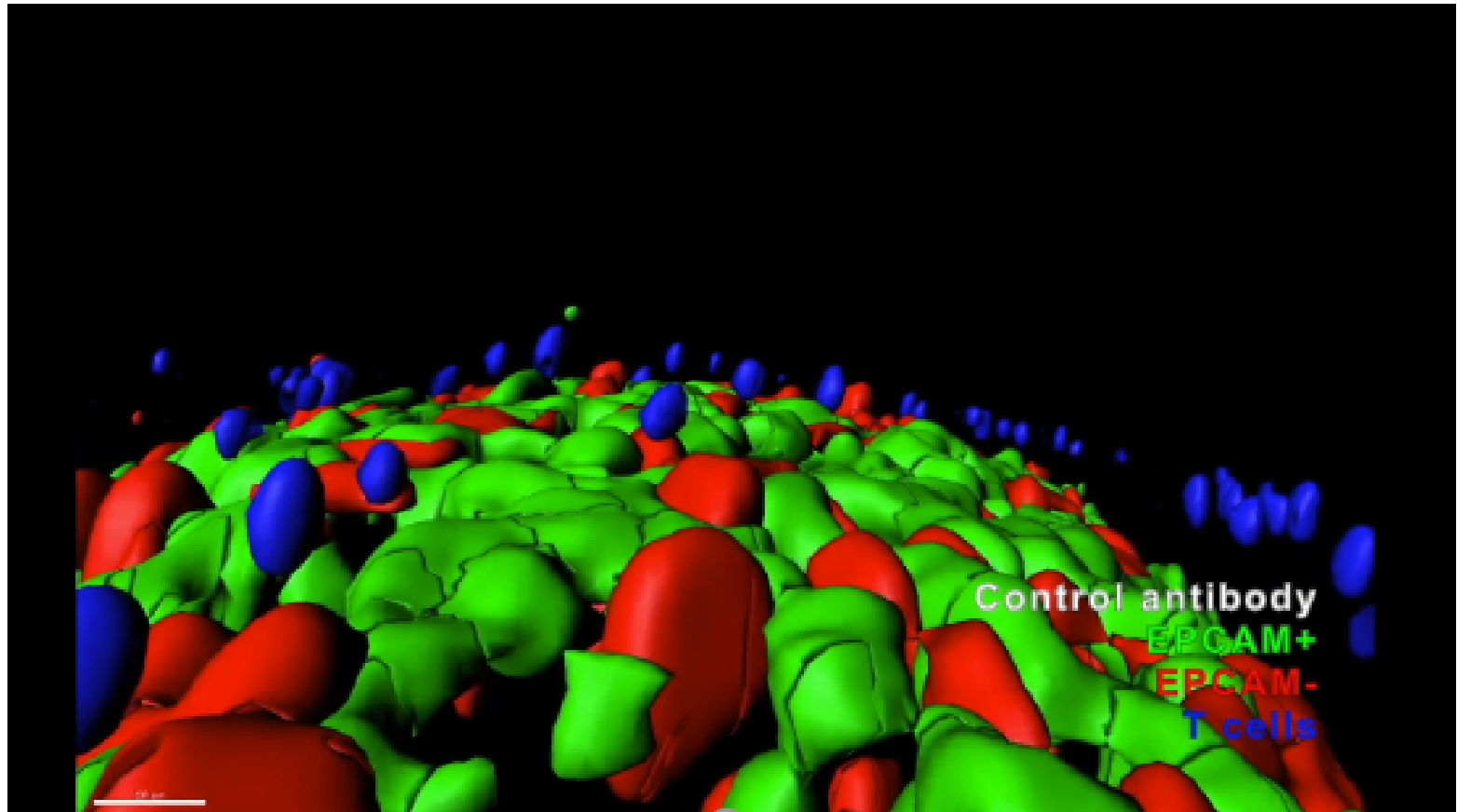


p<0.01

Specificity of APEC killing *in vivo*



Specificity of APEC killing *in vivo*



No collateral cell killing in Breast Cancer Models!

Real-time visualization of APEC-mediated T cell killing *in vivo*

Overlap

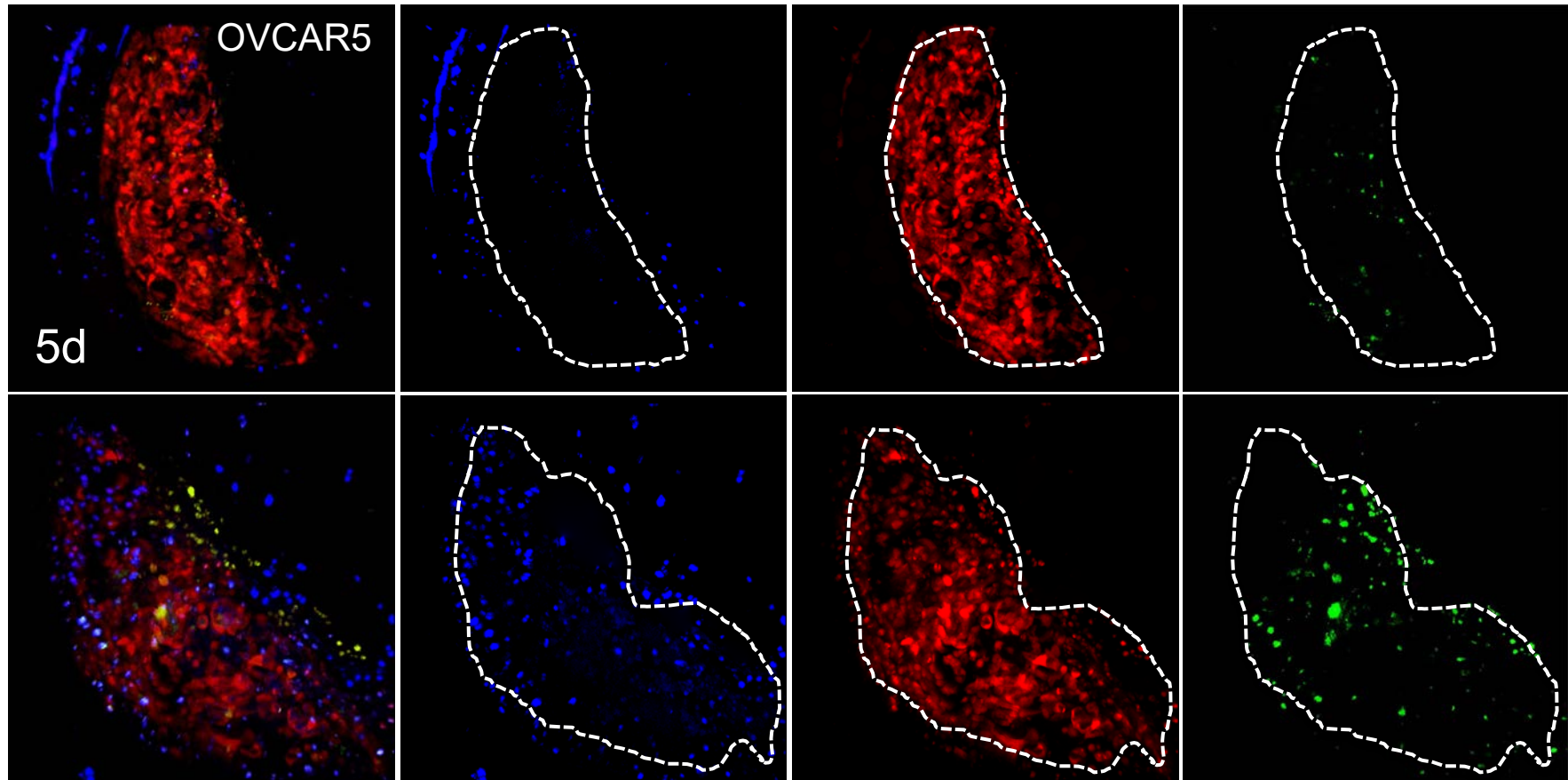
T-cells

Tumor

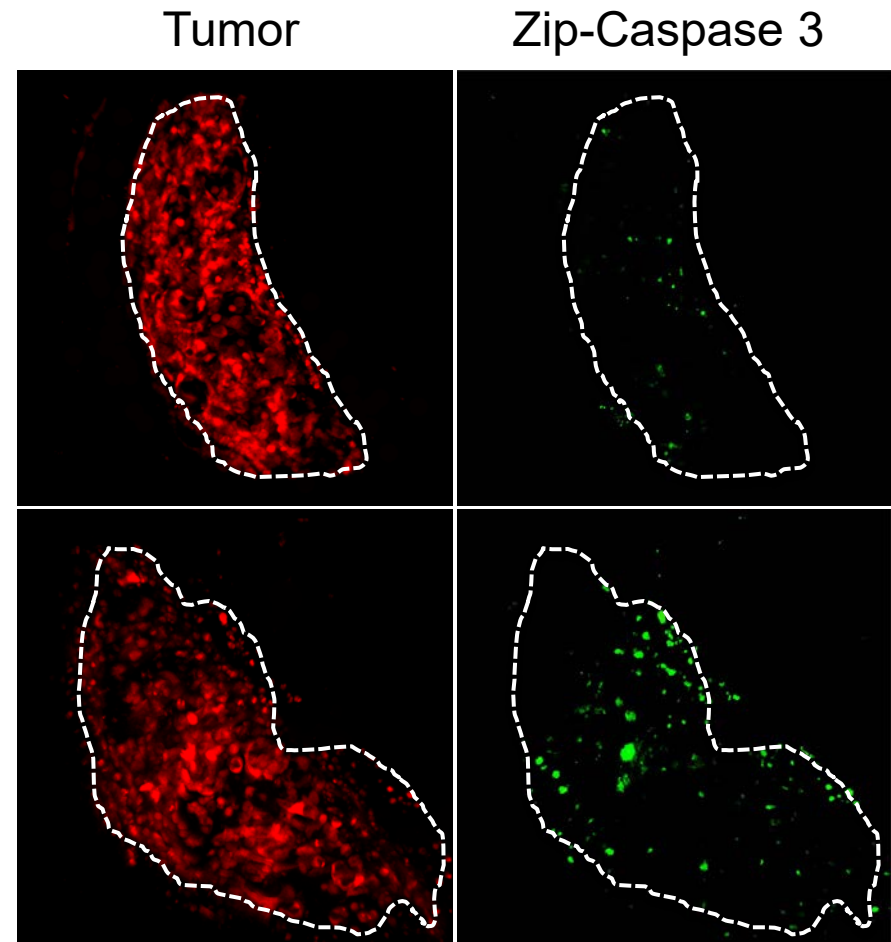
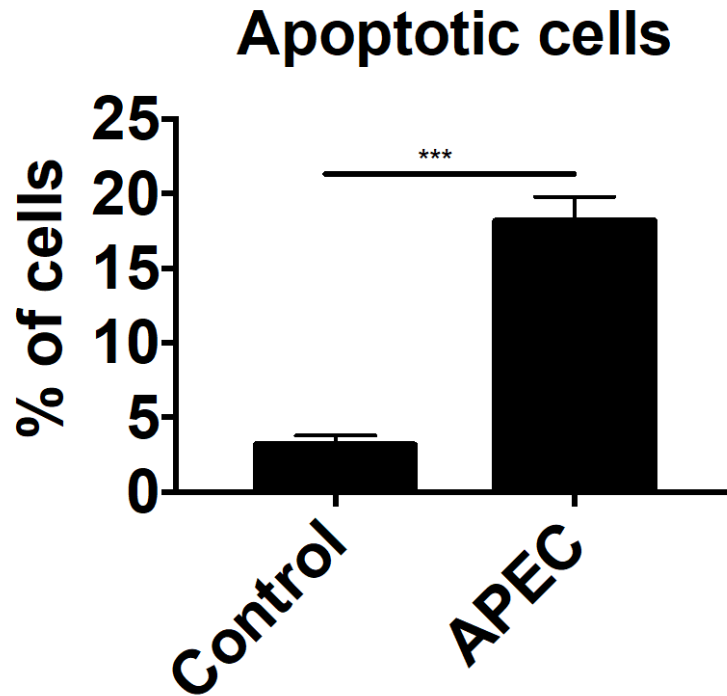
Zip-Caspase 3

Control

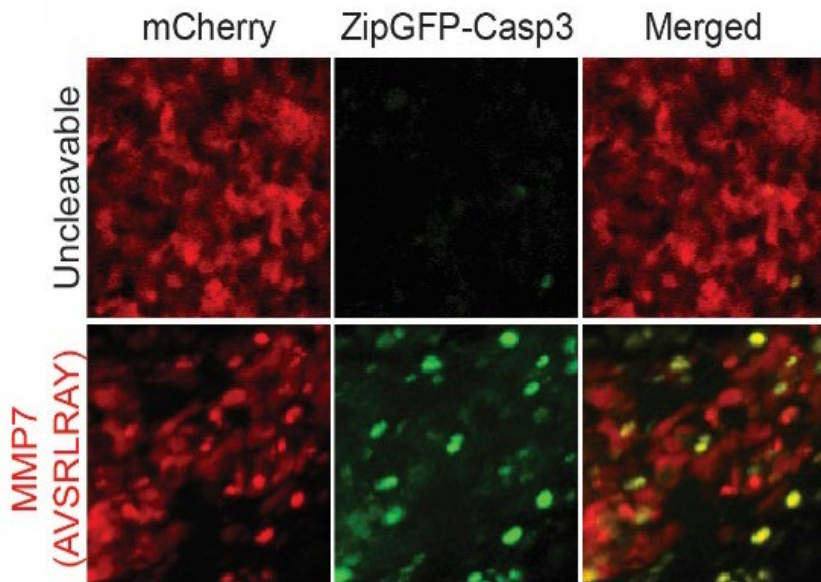
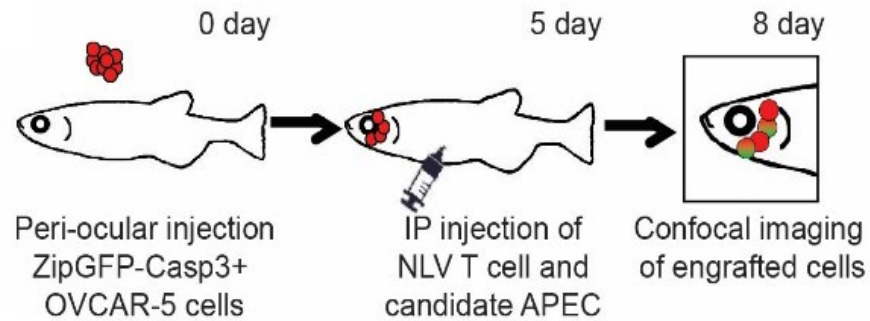
EPCAM+ APEC



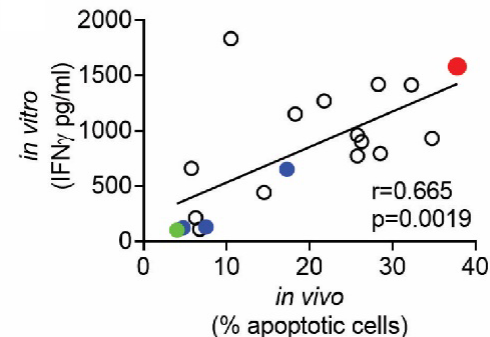
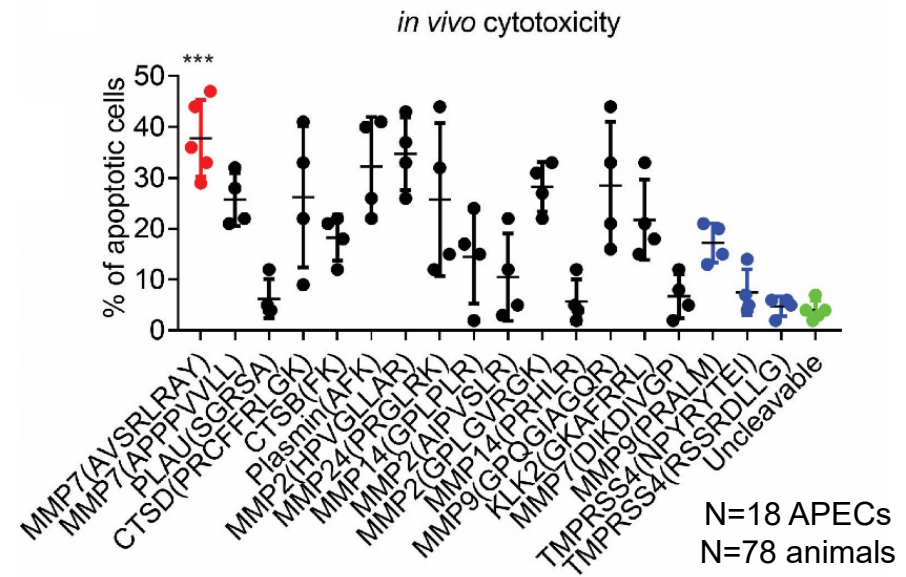
Real-time visualization of APEC-mediated T cell killing *in vivo*



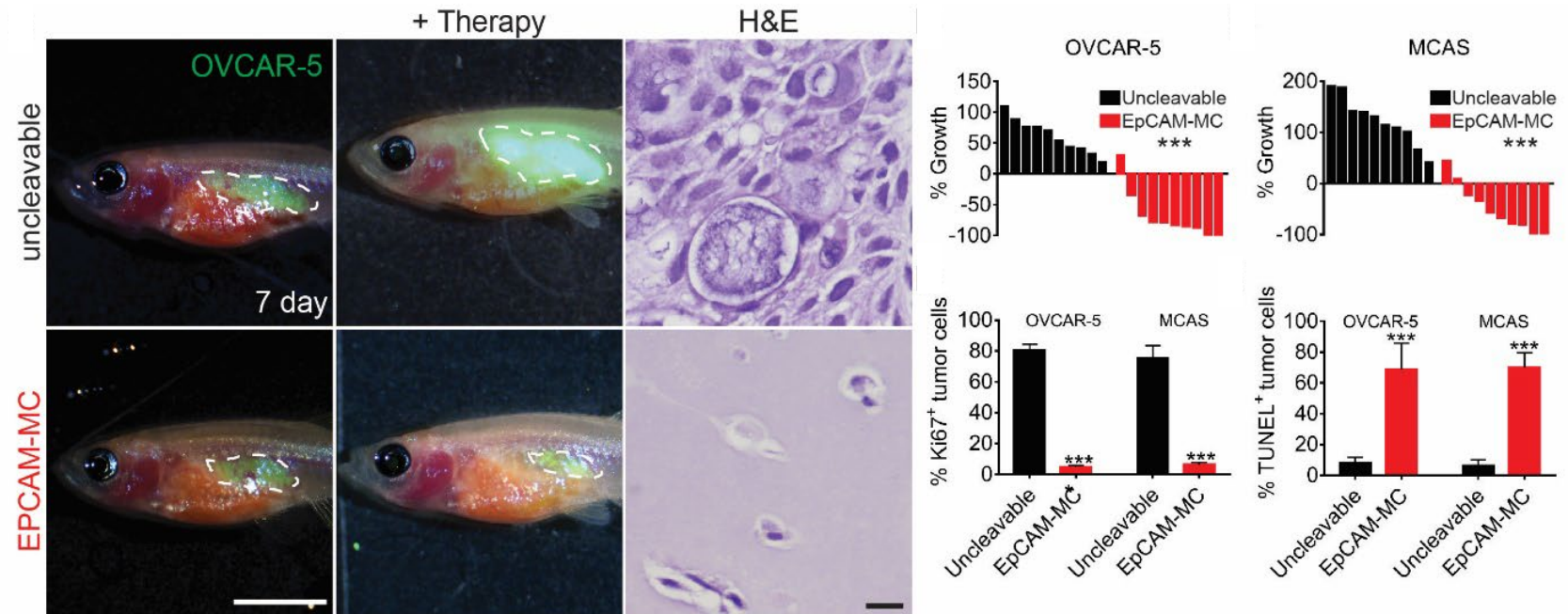
In vivo screening of best APEC killers



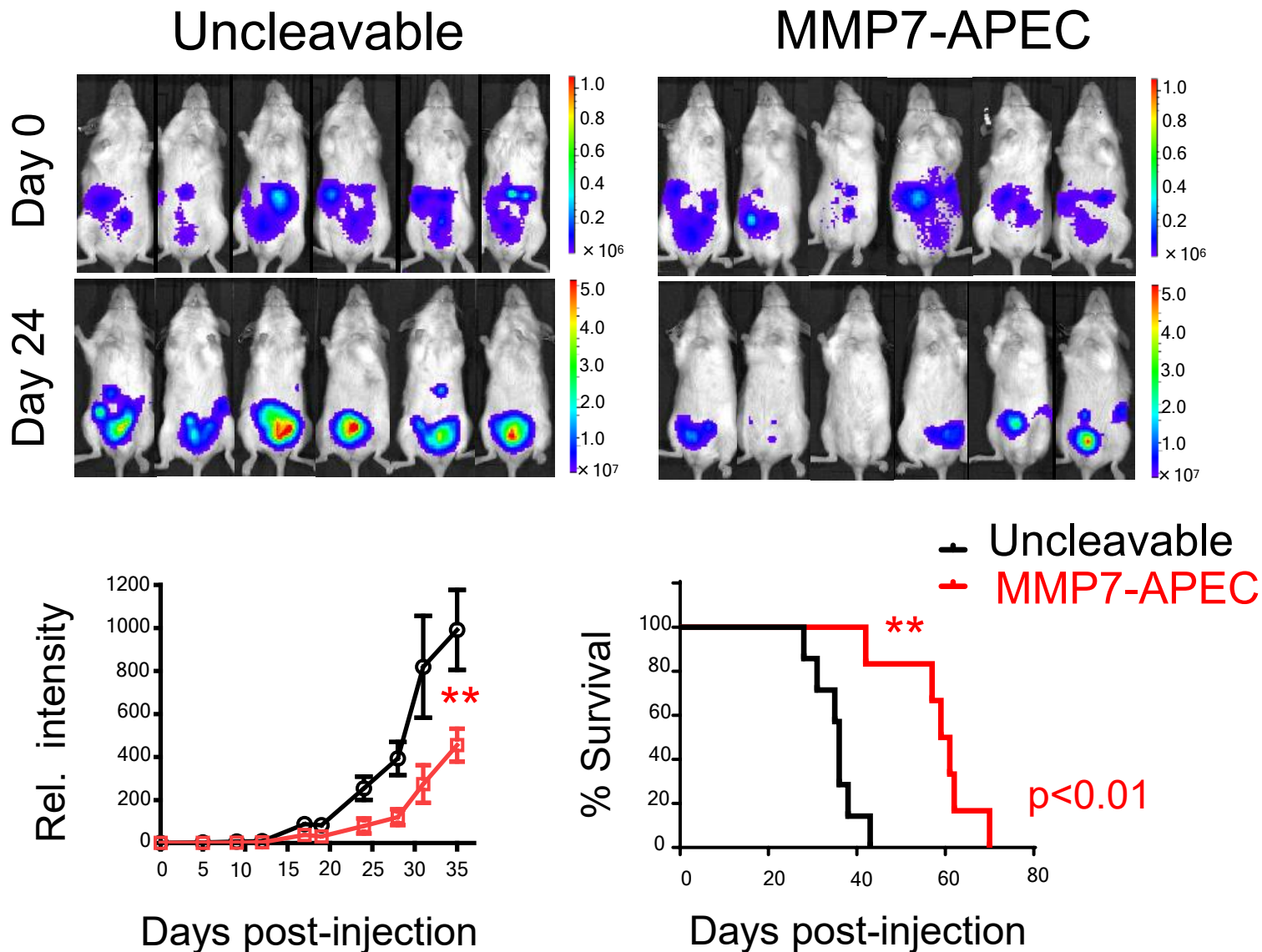
MMP7-APEC kills best *in vivo*.



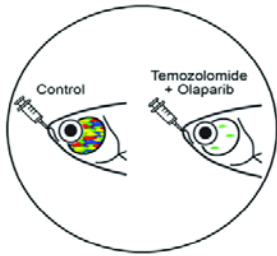
In vivo screening of best APEC killers



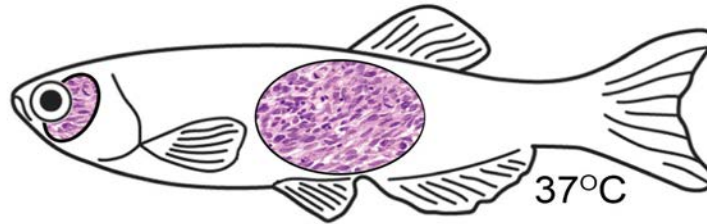
In vivo screening of best APEC killers



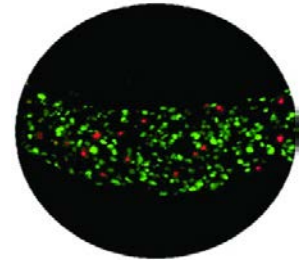
Zebrafish Xenograft Studies



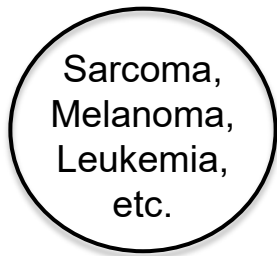
Drug
discovery



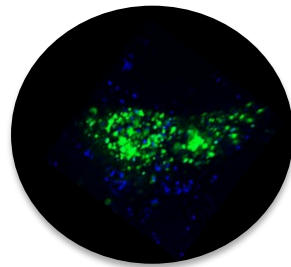
prkdc^{-/-}, *il2rga*^{-/-} (Cell, 2019)
rag2^{Δ/Δ}, *il2rga*^{-/-} (unpublished)



Single cell
imaging



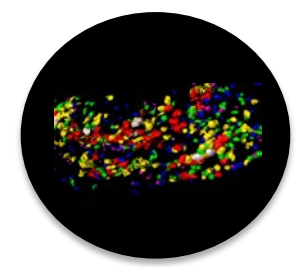
Robust
engraftment



Immunotherapy



Pharmacokinetics



Therapy
responses

Future Xenograft Applications for Immune Deficient Zebrafish

Humanizing zebrafish by engraftment of human PBMCs and CD34+ blood

Engraftment of ES and iPSCs

Chimeric/functional assays (muscle)

Non-invasive imaging using second and third harmonics and dyes

Langenaulab.com

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R01CA226926
R24OD016761



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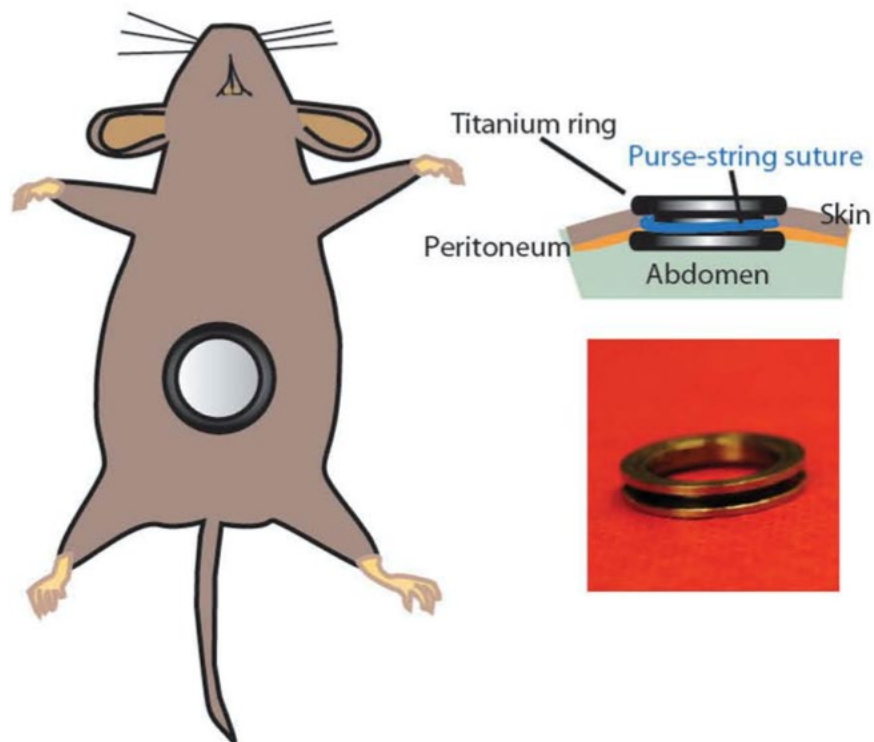
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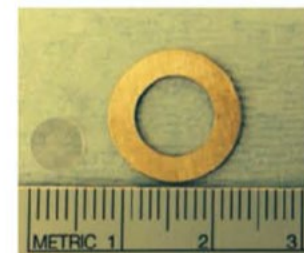
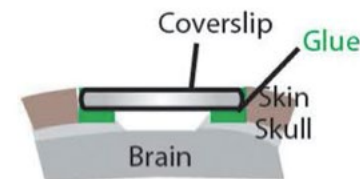
Mouse Xenograft Studies



High cost
Limited imaging
Low throughput

Longer time analysis
PK/PD established

Approved model for IND filings



Resource building: Critical Need for New Lines

Transgenics to eliminate macrophage function

Expression of human cytokines

Growth of AML, myeloid cells, and blood

Better “genotype-less” models

Only 1/8 of fish are currently used!

Scaling the husbandry to large BL2 facilities

Resource building: Education and Dissemination

Publish protocols (Yan et al, Nature Protocols, 2020)

Present on work widely

(ZDMS, Industry meetings, JAX seminar, etc.)

Efficient distribution of current/unpublished lines:

Over 75+ labs, Universal MTA, local distribution,
long-term plan to distribute through ZIRC.

User group webinars and website videos

Continue to credential the zebrafish models by
comparison to NSG mice.

Hurdles for Adoption

- ✓ Few published *xenograft* studies to date
- ✓ PK/PD studies required
- ✓ Optimized husbandry approaches and building of BL2 zebrafish facilities
- ✗ Innovation requires more users (i.e. Mouse)
- ✗ Perceived competition across species models, including at the NIH level (Mammalian Models of Cancer Grants)