INSTITUTE FOR RESEARCH IN BIOMEDICINE

IRB BARCELONA

Mechanisms of lymphatic metastases in squamous cell carcinoma of the oral cavity

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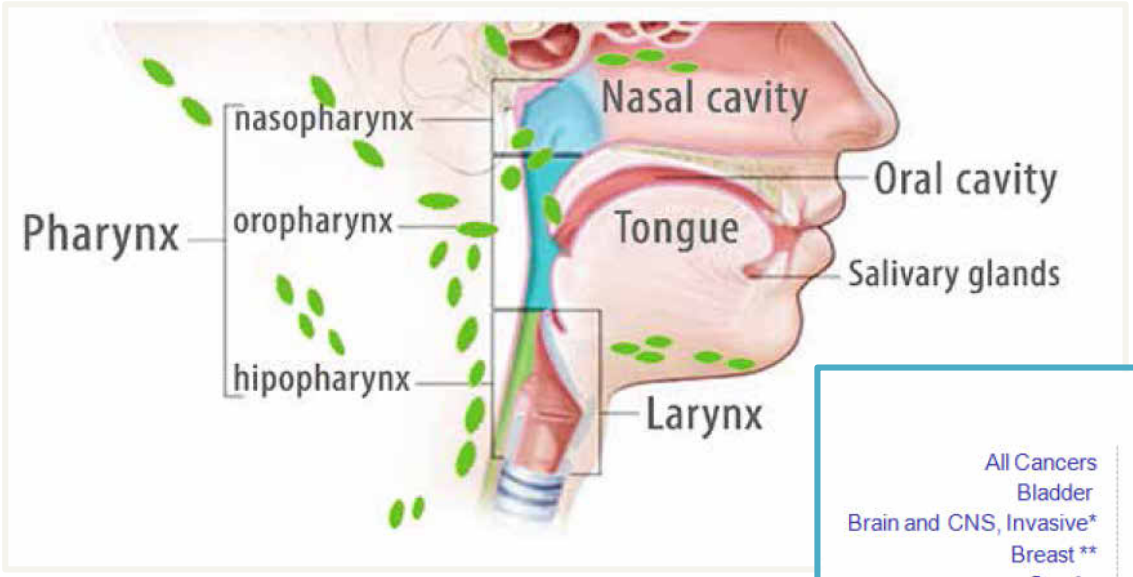
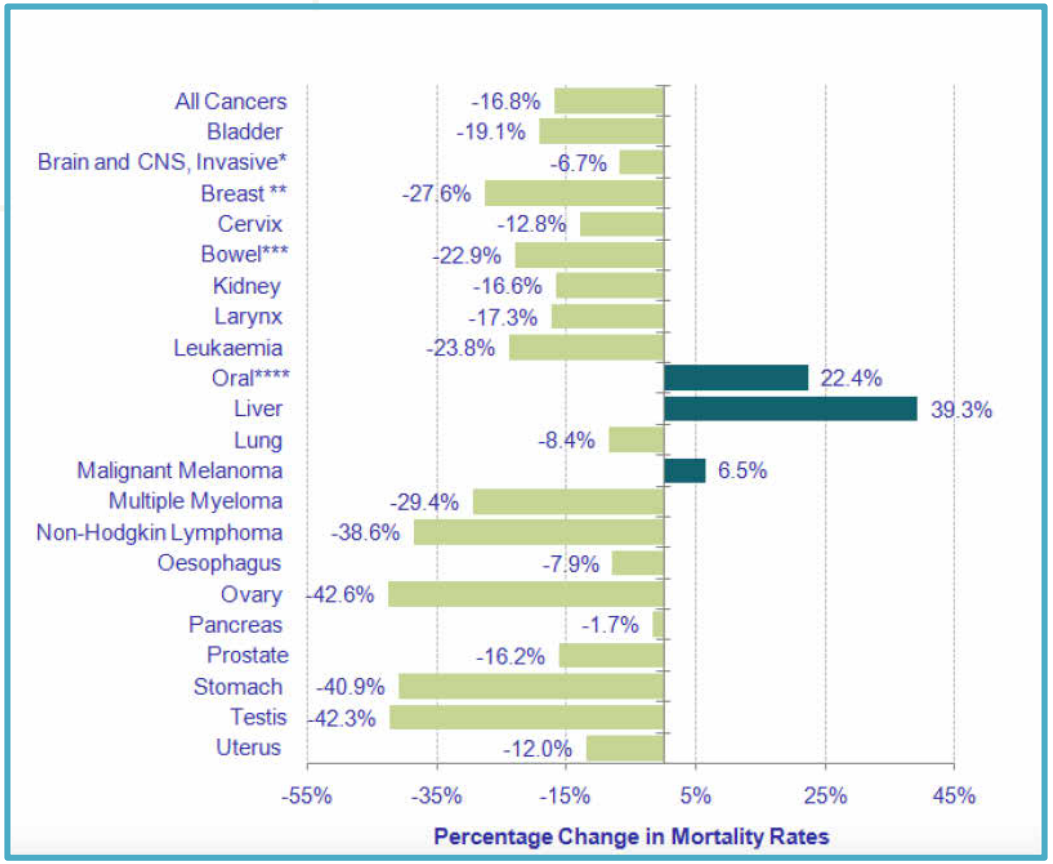
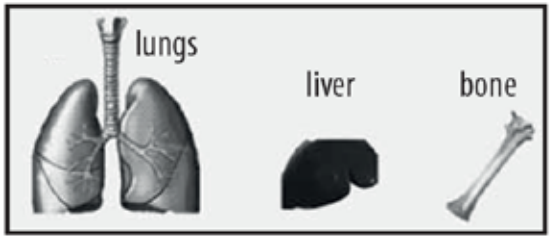
Head and Neck Squamous Cell Carcinoma (HNSCC)





Local Metastasis:
Nodal spread

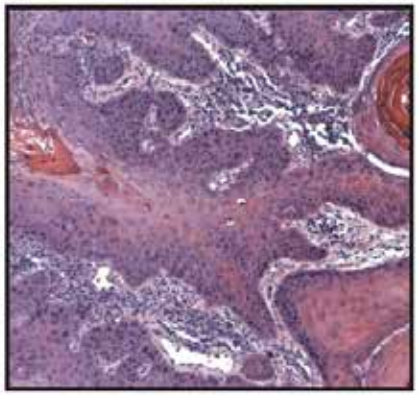
Distant Metastasis:



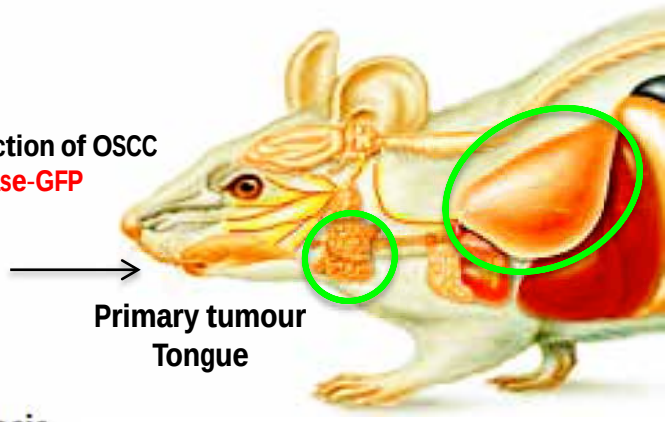
70-85% 10-30% 15-35%

Orthotopic model of human oral SCC using primary samples

SCC-human biopsye



Sublingual injection of OSCC
*p*Luciferase-GFP

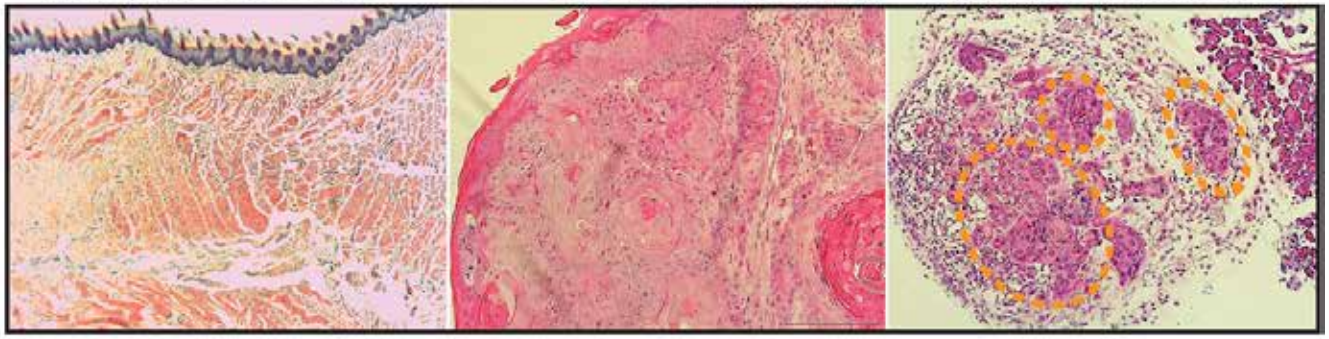


Primary tumour
Tongue

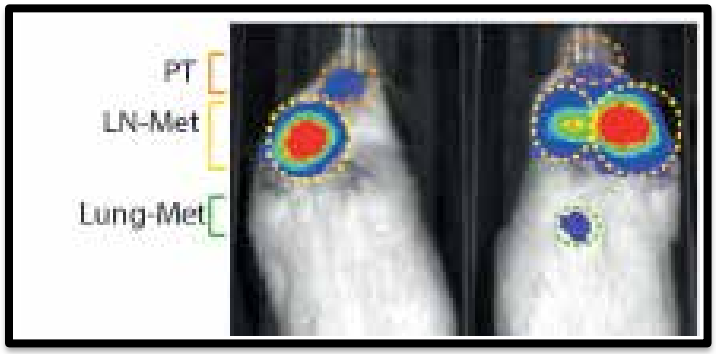
Local Met
Lymph nodes

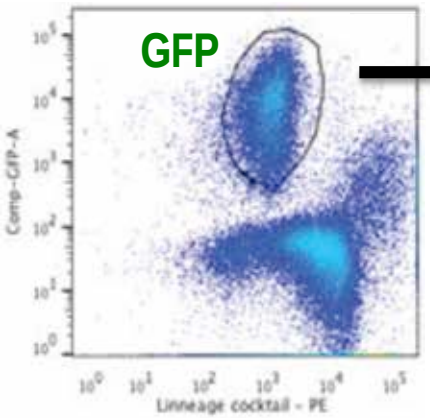
Distant Met

Normal mouse tongue | Orthotopic OSCC transplant | Lymph node Metastasis



***In vivo* tumour growing monitorization**





OSCC isolation/purification

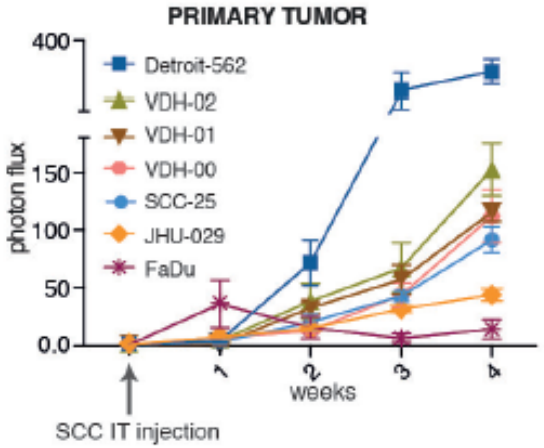
PDTs and established cell lines with varying degrees of metastatic potential

a

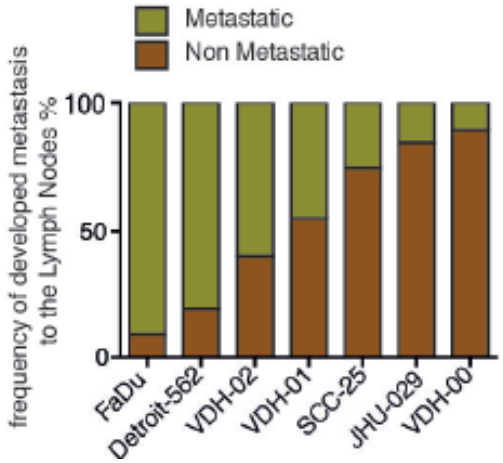
SCC cell line	% Developed Primary tumors	% Developed Metastasis (LN)	Time until develop Metastasis	Site of Metastasis
FaDu	100	91	1 week	Lymph node, Lung*
Detroit-562	100	81	1 week	Lymph node
SCC-25	100	25	1,5 weeks	Lymph node
JHU-029	100	15	1,5 weeks	Lymph node
VDH-00	100	10	>2,5 weeks	Lymph node
VDH-01	100	45	>2,5 weeks	Lymph node
VDH-02	100	60	1,5 weeks	Lymph node

(* % of developed metastasis to the lung in FaDu SCC cell line is 45%)

b



c

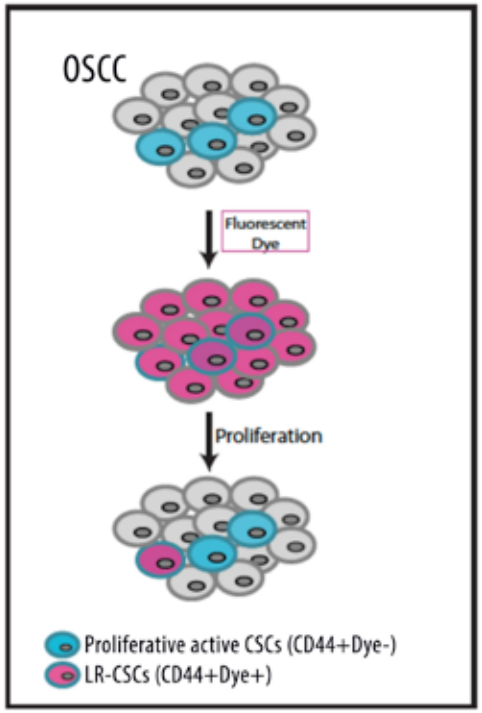


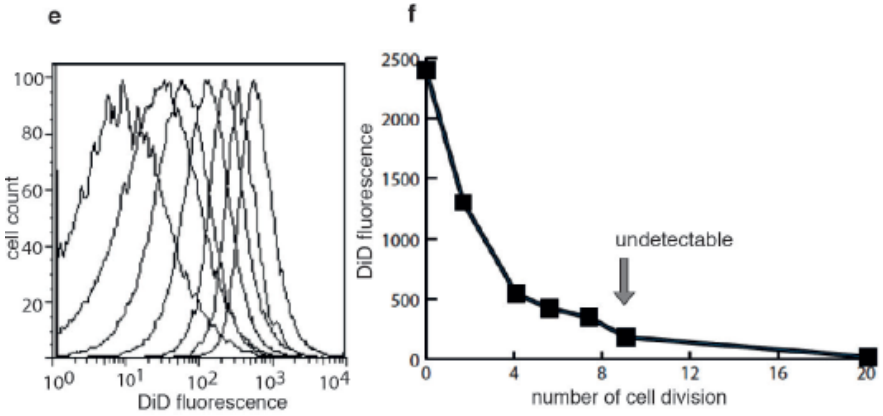
Is there cancer stem cell heterogeneity *in vivo* in human SCC?

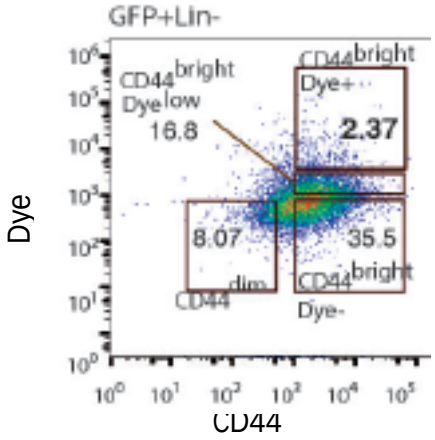
Quiescent/Slow-cycling cell populations in cancer

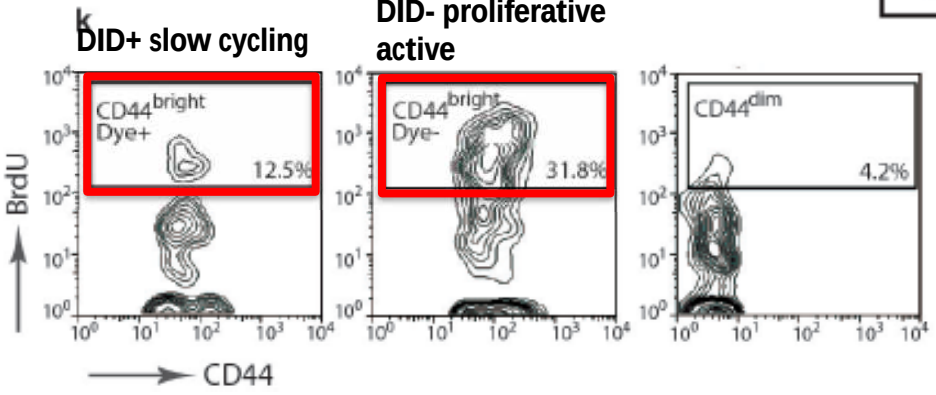
- *Source* of tumor-initiating potential
- *Sustain* tumorigenesis
- *Resistant* to radio-chemotherapy

Lipophilic tracers
Long-chain carbocyanines

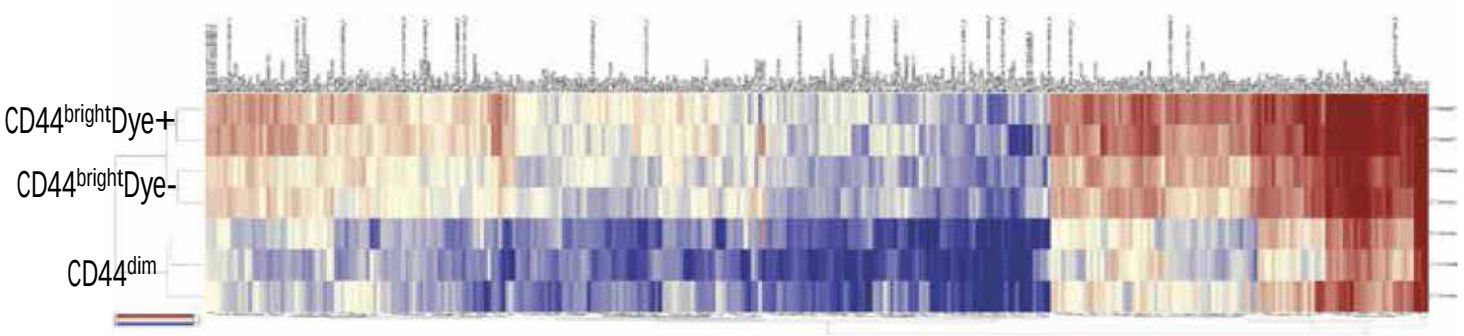






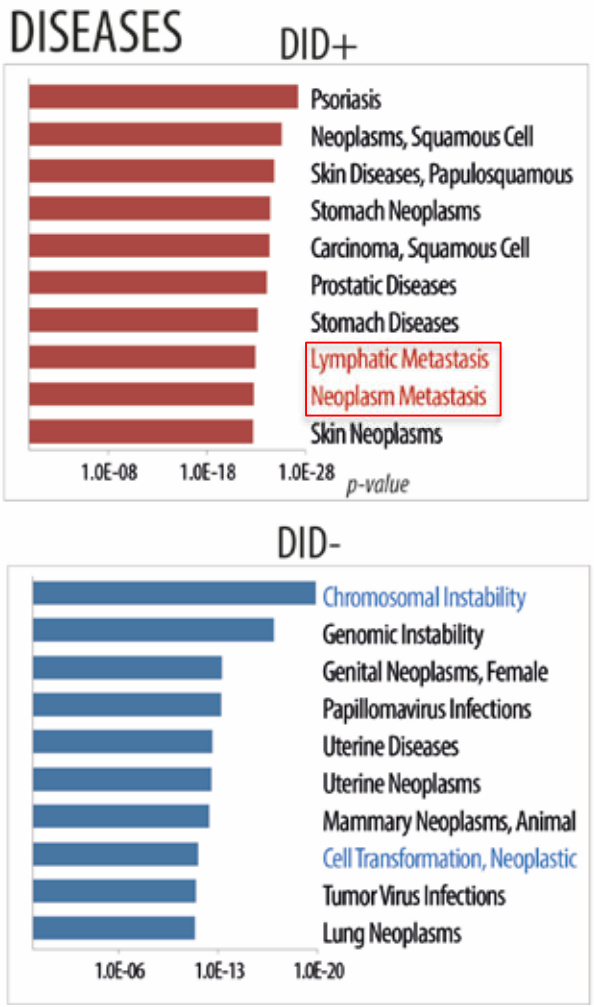


Molecular characterization of tumor LRCs and actively proliferating CD44^{bright} cells *in vivo*



Tumor LRCs are uniquely defined by a signature indicative of metastatic process

CD44^{bright} Dye+ LR-CSC signature



Metastatic Process

DID+

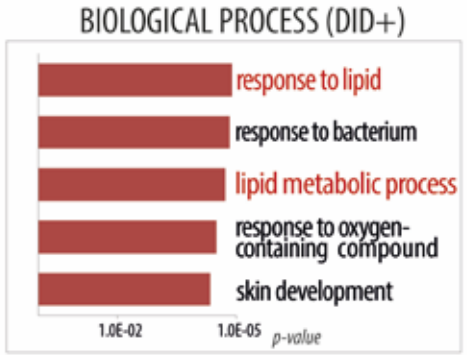
LYMPHATIC METASTASIS	ABCA4	ELN	NOD2
	ACPP	ENO2	NOTCH2NL
	ACSBG1	EPB41L1	NR1D1
	ALDH1A1	ERBB3	OCLN
	AQP3	FBXO32	POSTN
	CD36	FUT3	PTGES
	CFLAR	GABARAPL1	RAPH1
	CHI3L1	ID2	S100A8
	CLDN1	IGFBP3	S100A9
	COL1A1	IL1RN	SEC14L2
	COL1A2	KLK6	SERPINA9
	CST6	KLK7	SLC28A3
	CYP3A5	KRT7	SORT1
	CYP4F3	MUCL1	SOX9
	DUSP16	MYEOV	SPARC
	EGR2	NET1	TACSTD2
			TNFSF10
NEOPLASM METASTASIS	ABCA1	LPHN2	SLC28A3
	ABCA4	ELN	TMPRSS4
	ACPP	ID2	SLC10A1
	ACSBG1	SLC39A2	KRT23
	CD36	SPARC	IGFBP3
	CYP4F3	ERBB3	KRT7
	PNLIPRP3	S100A9	EPB41L1
	EGR2	NET1	COL1A1
	SEC14L2	MYEOV	AQP3
	SASH1	ADAM12	GABARAPL1
	CHI3L1	NOTCH2NL	MLLT4
	CLDN1	ACER2	DUSP16
	CRYAB	PTGES	OCLN
	SERPIND1	IL1RN	PCSK5
	S100A8	NR1D1	SLC15A1
	CST6	KLK5	CAPN3
	CYP3A5	CARD14	ENO2
	FBXO32	KLK7	SERPINA9
	PADI1	COL1A2	IRF5
	TACSTD2	ALDH1A1	S100A7
	TINAGL1	MUCL1	POSTN
	FAM83A	SOX9	SORT1
	LAMA4	COL6A3	RAPH1
	HSD17B2	CFLAR	NOD2
	TNFSF10	FUT3	PIK3R3
			KLK6

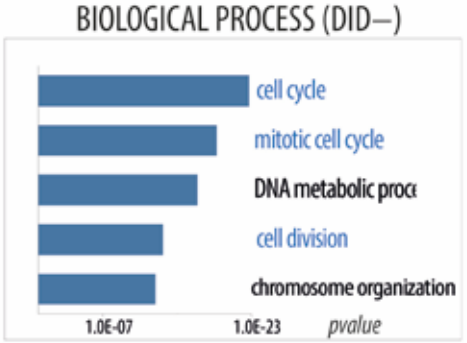
Neoplasm

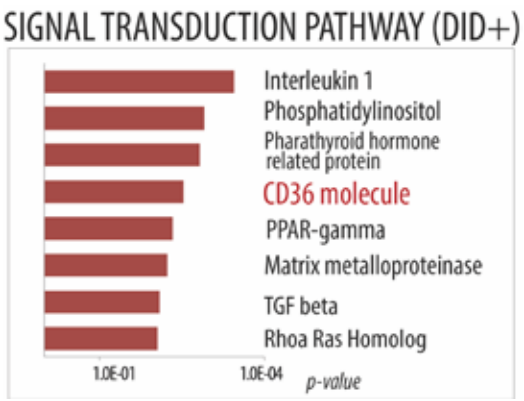
DID-

CELL TRANSFORMATION, NEOPLASTIC	DLGAP5	MCM3
	LFNG	HJURP
	FANCD2	DEPDC1
	NREP	CLSPN
	KIF2C	ITGA6
	CCNB1	VAV2
	CCL5	CDCA8
	STIL	MLH1
	STMN1	BLM
	PDGFA	TGFBR2
	CCNA2	HIST1HA
	PCNA	GMNN
	UBE2T	TOP2A
	PRR11	BUB1
	PEG10	HSPD1
	TACC3	UHRF1
	PLK1	CSPG4
	RFC4	PDGFC
	CENPA	ZWINT
	PARP1	KIF20A
	MKI67	DKK3
	NUSAP1	CDCA5
	CAV1	AURKA
	AURKB	PHLDB1
	CDK1	IGF2
	SLC7A1	CDKN3
	EXO1	CEP55
	MYLK	HIST1H8
	FST	TYMS

CD44^{bright} Dye+ LR- CSC signature

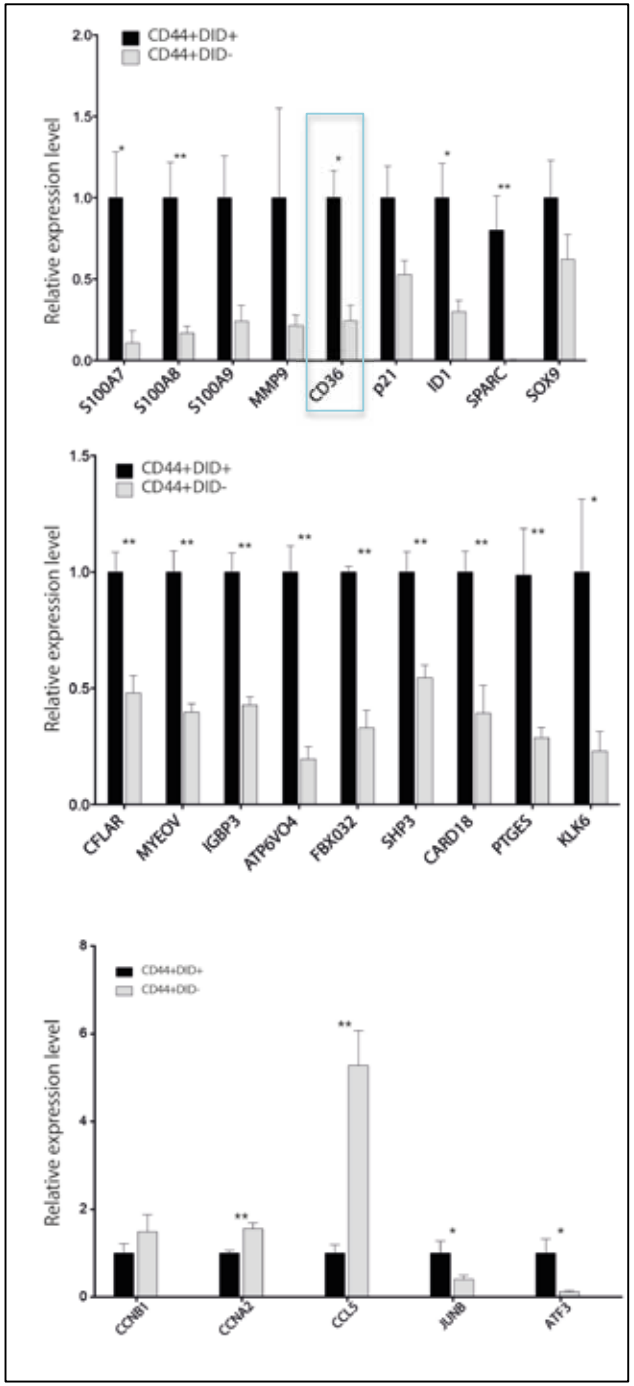






Lipid Metabolic Process DID+

Fatty Acid uptake	CD36 ABCA1 SLC10A1
Lipid Catabolism Fatty Acid beta oxidation Fatty Acid alpha oxidation	ACSL1 ACSBG1 PPAR-alpha PNLIPRP3 LIPH PLA2G4E PNPLA2 (ATGL) HSD17B2 FA2H
Lipid Biosinthesis	ACSL1 ACSBG1 FA2H HSD17B2 CYP4F3
Triglyceride Sinthesis/Lipid Storage	DGAT2 SEC14L2

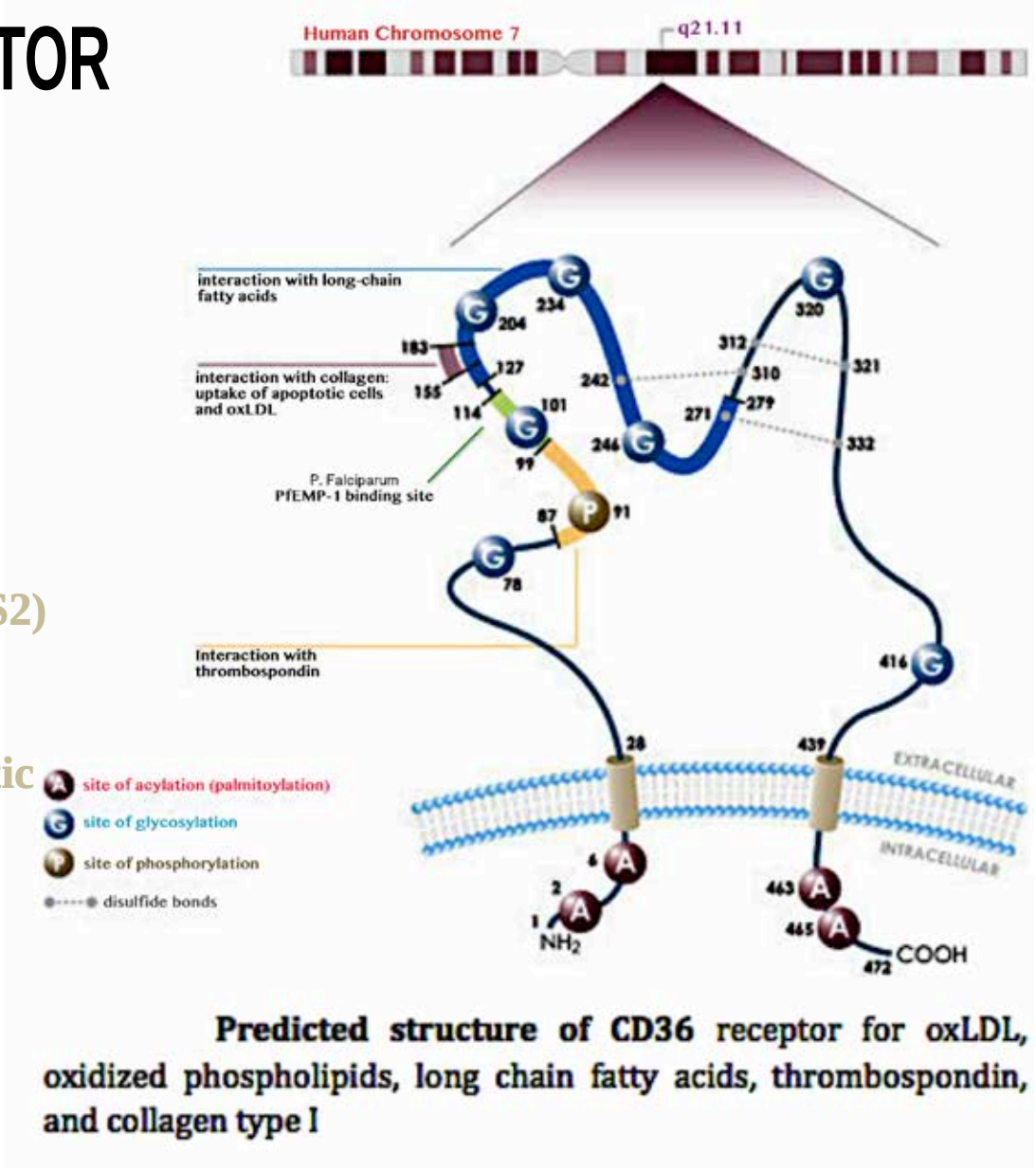


CD36 SCAVENGER RECEPTOR

(FAT/SCARB3/PASIV/GPIV)

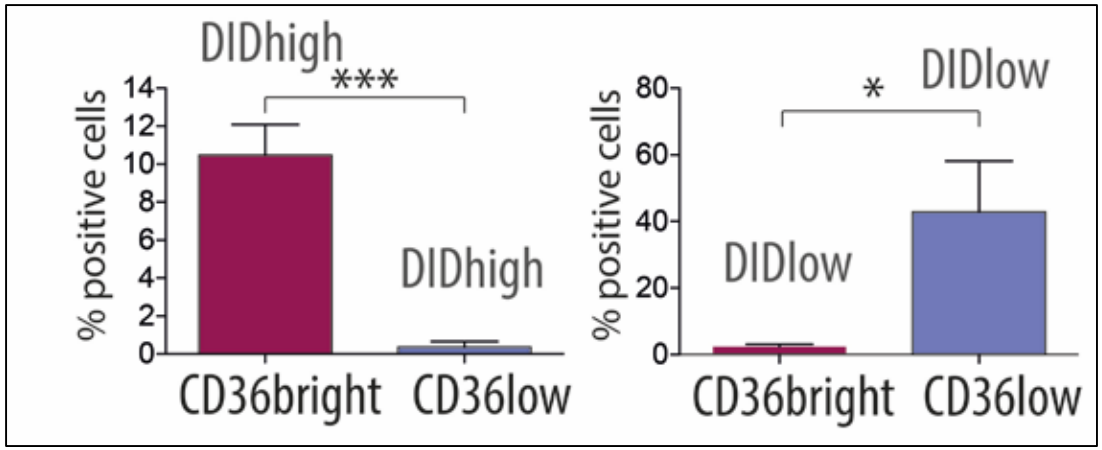
LIGANDS:

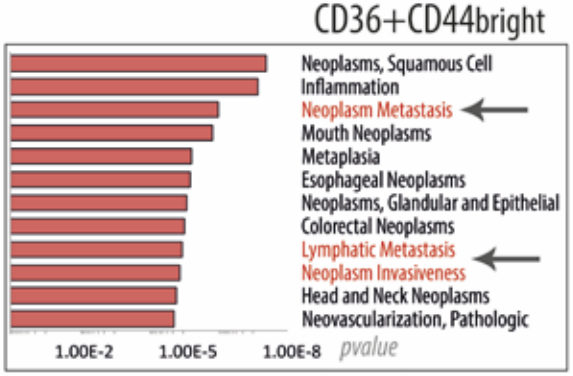
Long chain fatty acids
oxLDL
Anionic phospholipids
Thrombospondins (THBS1 and THBS2)
Collagen type I and IV
CoenzimeQ (CoQ)
Phagocytose and clear damage, apoptotic cells, debris and ROS
Signal transduction

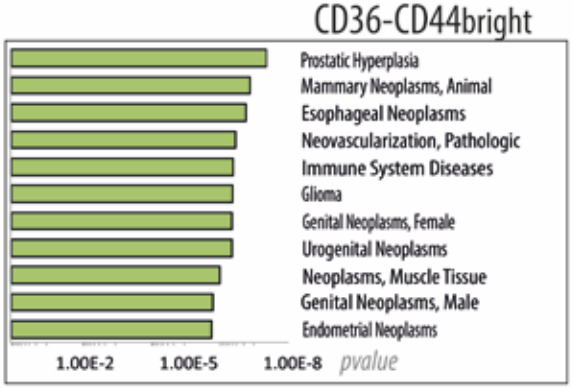


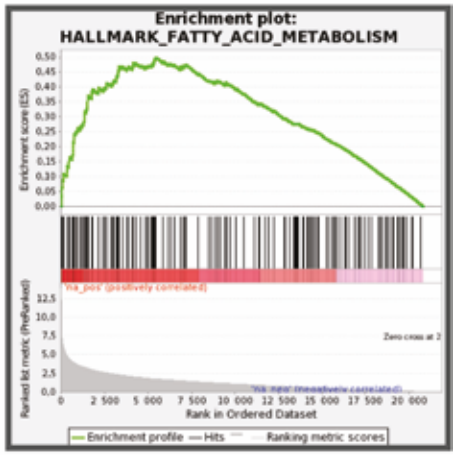
Predicted structure of CD36 receptor for oxLDL, oxidized phospholipids, long chain fatty acids, thrombospondin, and collagen type I

Long-term tumour LRCs correspond to the CD36+/CD44bright population and are enriched in lipid metabolism









Fatty-acid metabolism enrichment

commom Dye+/CD36+

LIPID METABOLISM

ACSL1	acyl-CoA synthetase long-chain family member 1
CD36	CD36 molecule (thrombospondin receptor)
DGAT2	diacylglycerol O-acyltransferase 2
PPM1L	protein phosphatase, Mg2+/Mn2+ dependent, 1L

CANCER INVASION AND METASTASIS

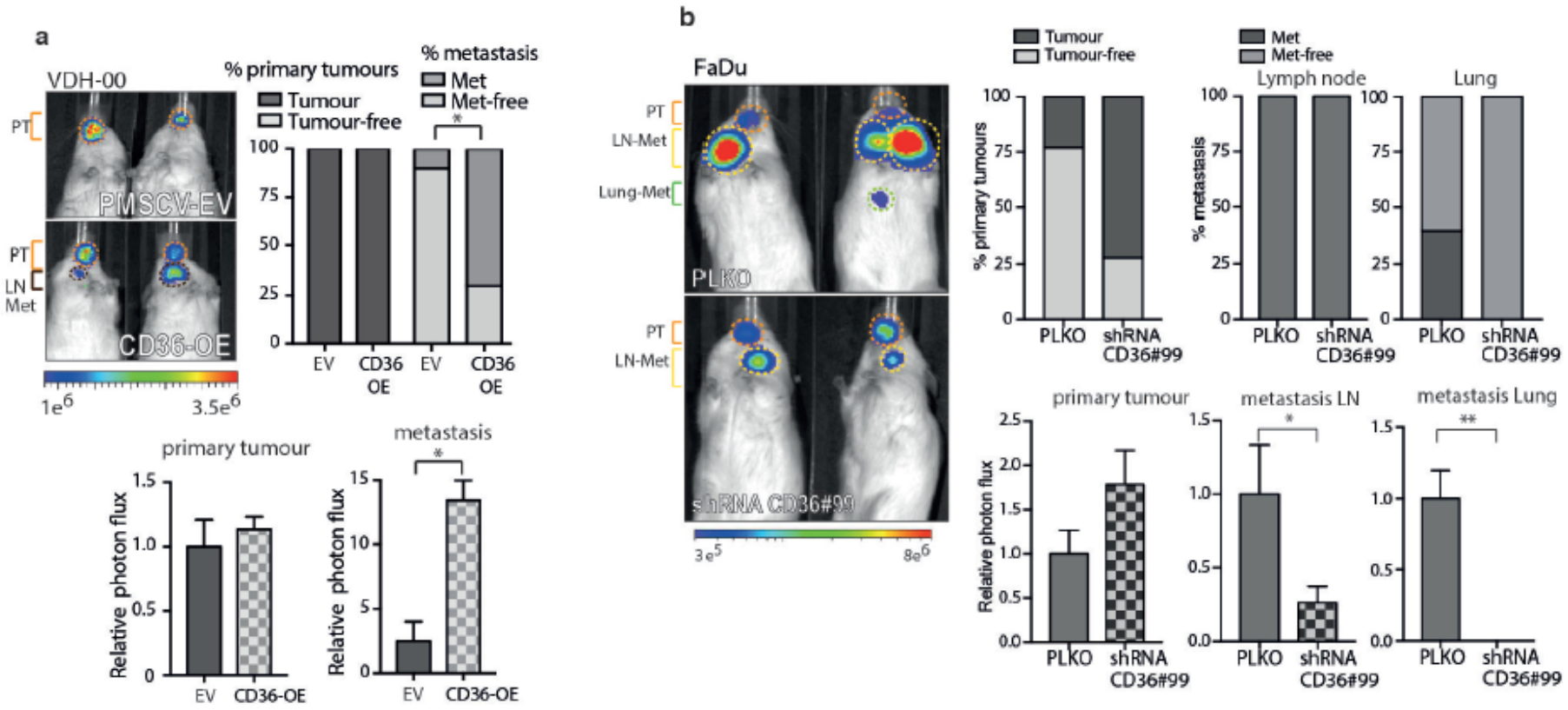
S100A7	S100 calcium binding protein A7
KLK7	kallikrein-related peptidase 7
MUC15	mucin 15, cell surface associated

TRANSPORT AND METABOLISM OF NUCLEOSIDE DRUGS

SLC28A3	solute carrier family 28 (concentrative nucleoside transporter), member3

What happens if we modulate the expression of CD36 in oral SCC tumors?

Targeting fatty acid receptor CD36 severely affects metastasis initiation and progression but not primary tumor growth



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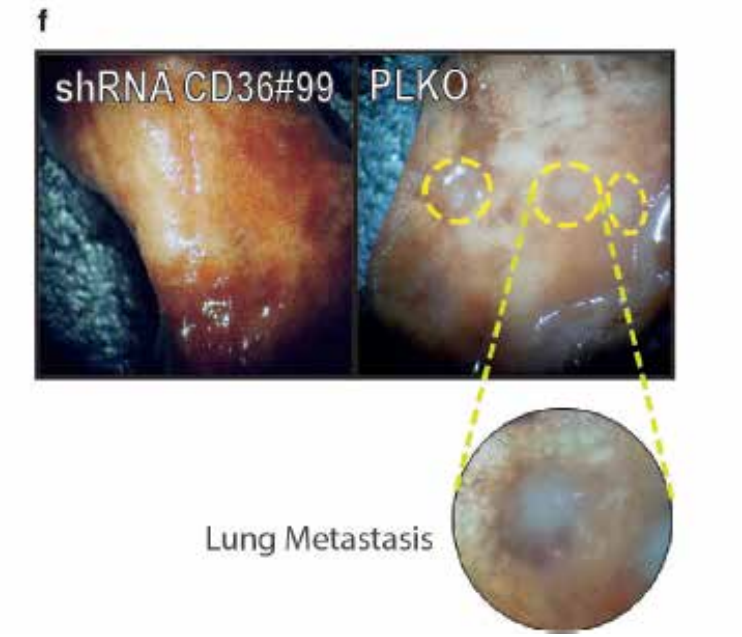
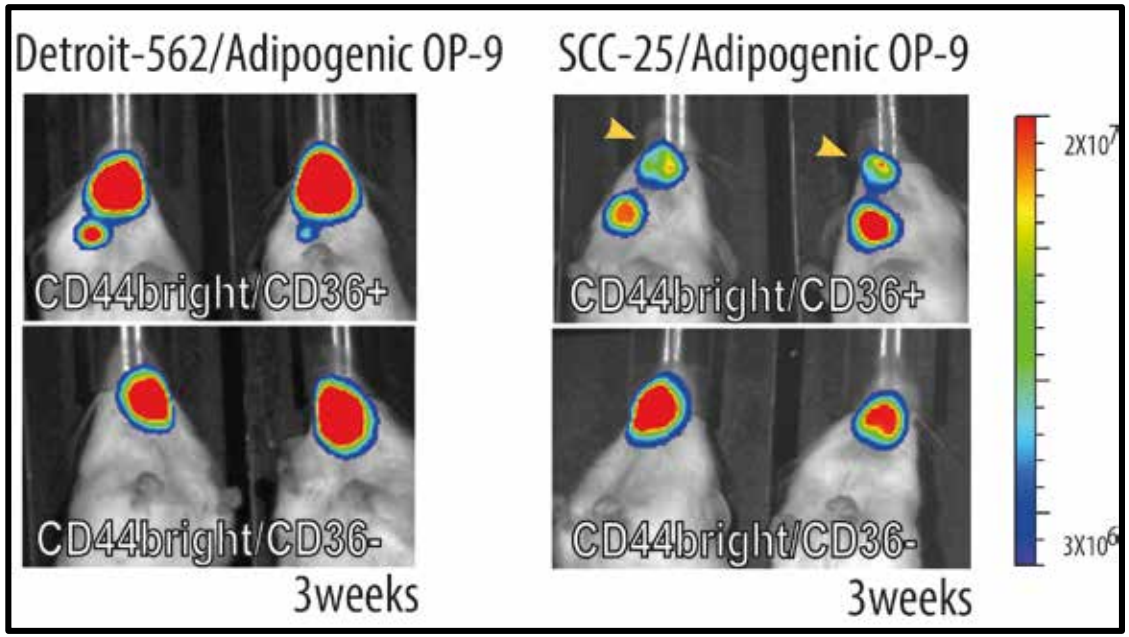
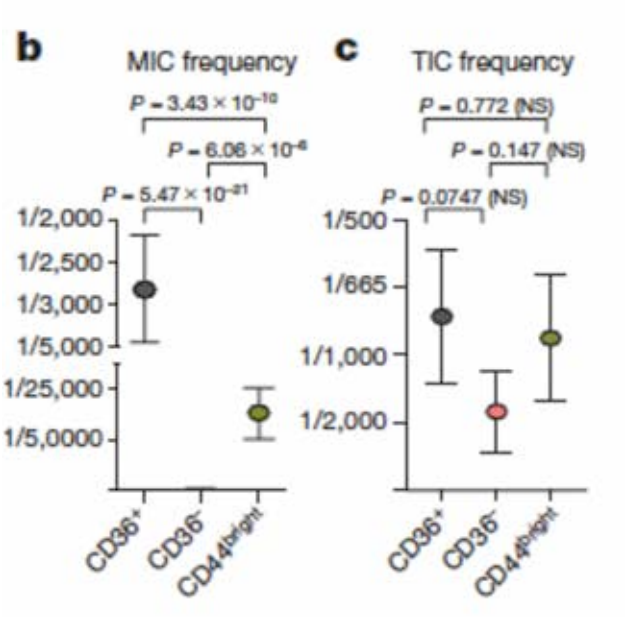


Have CD36 positive cells a role in initiating and/or promoting OSCC metastasis?

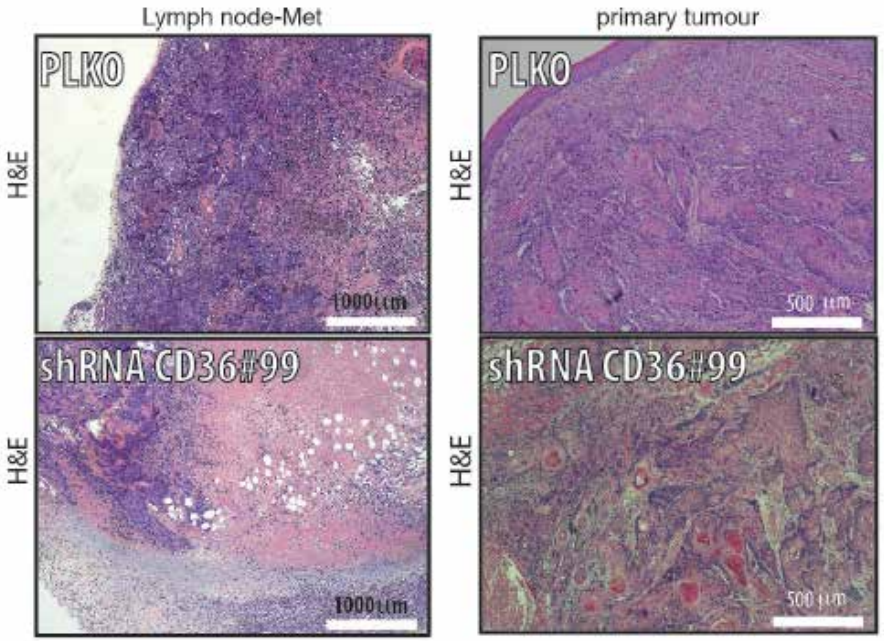
CD36 positive cells initiate and promote OSCC metastasis



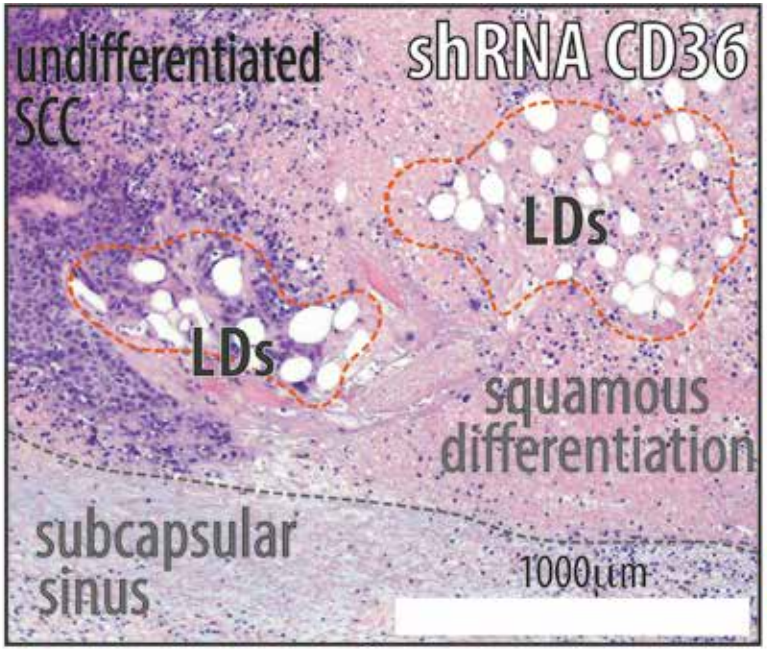


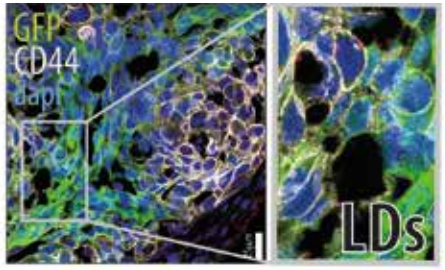
CD36 positive cells require fatty acid internalization to promote metastasis

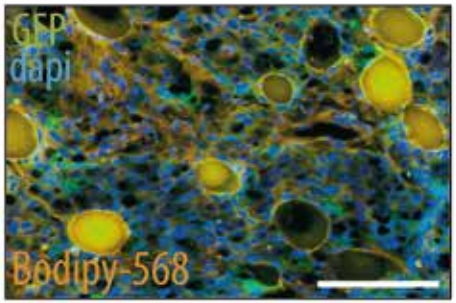
CD36 positive cells require fatty acid internalization to promote metastasis

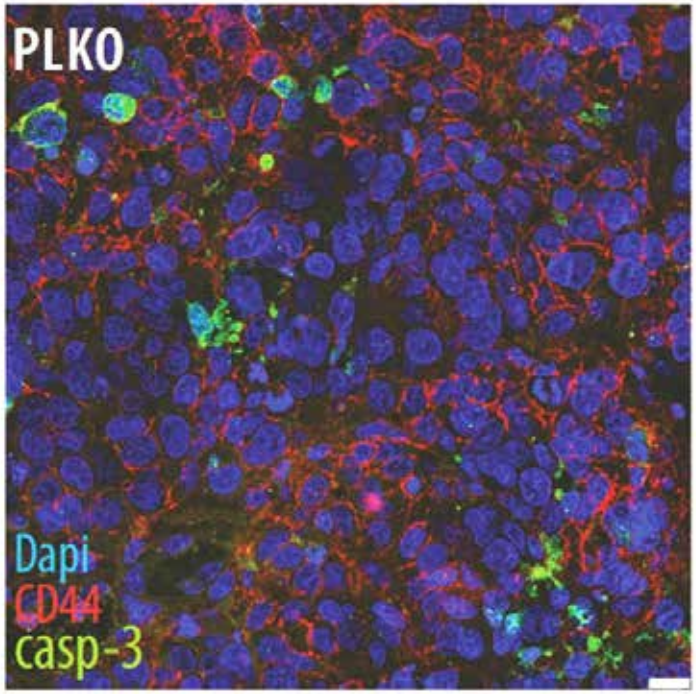


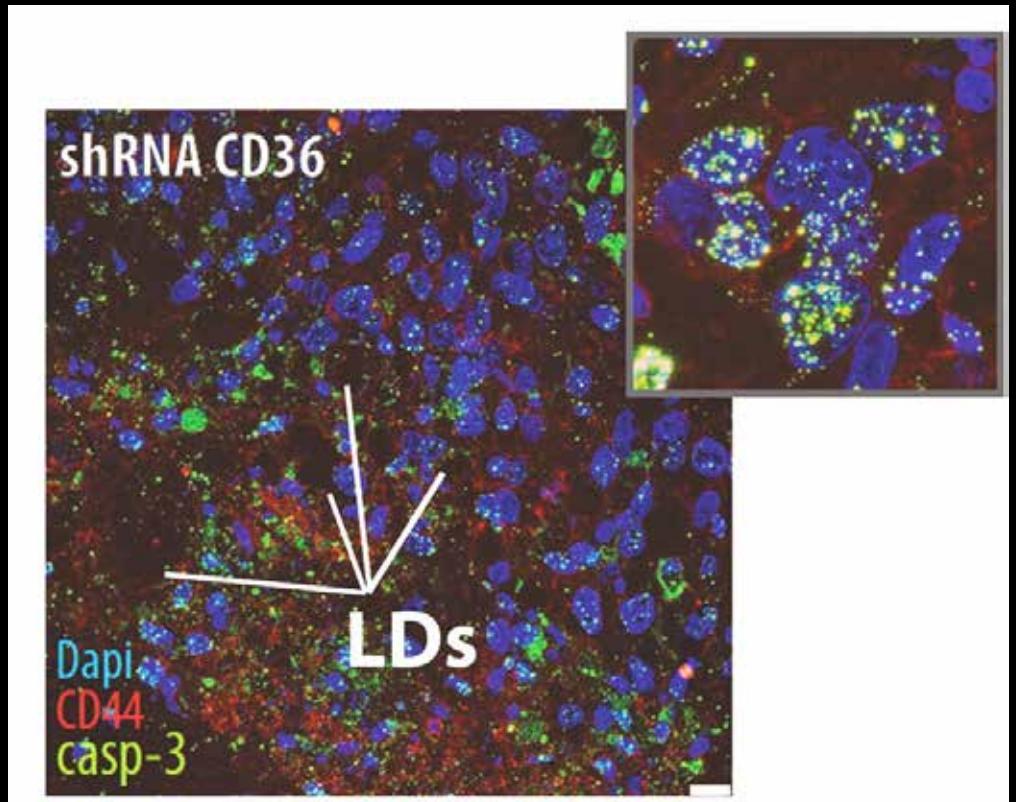
Depletion of CD36 results in the accumulation of large lipid droplets



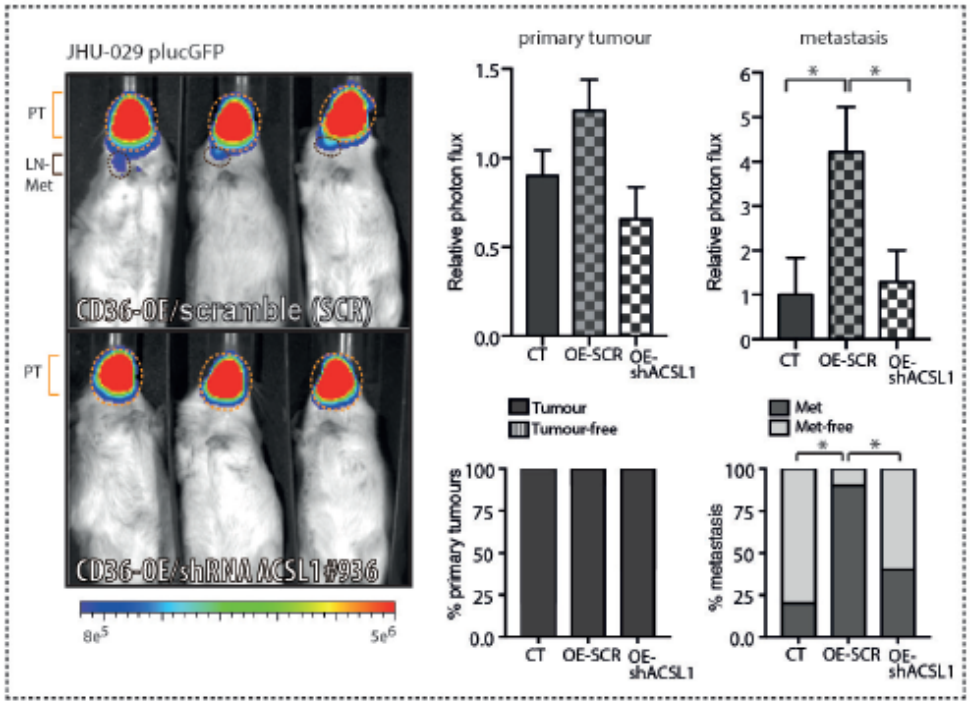





PLKO
Dapi
CD44
casp-3


shRNA CD36
LDs
Dapi
CD44
casp-3

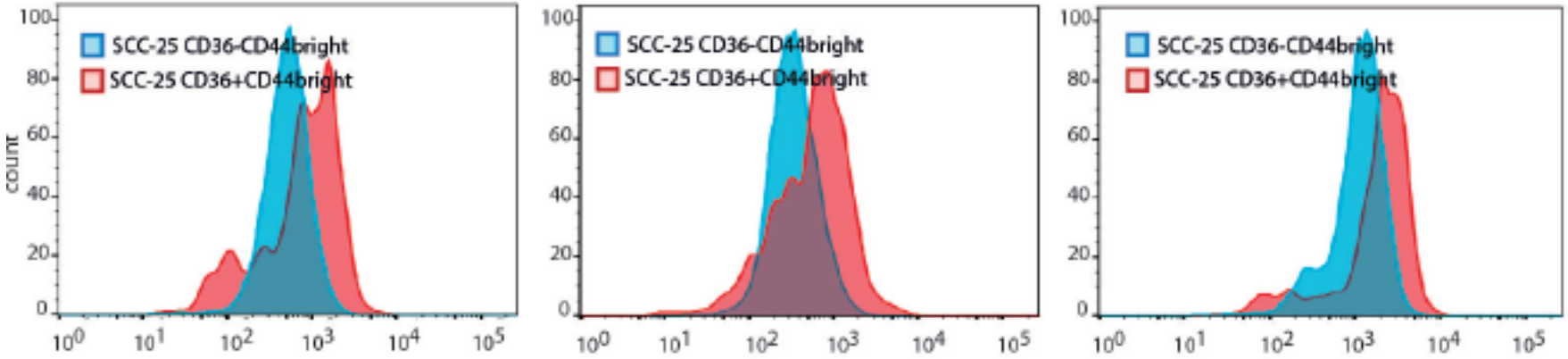
h



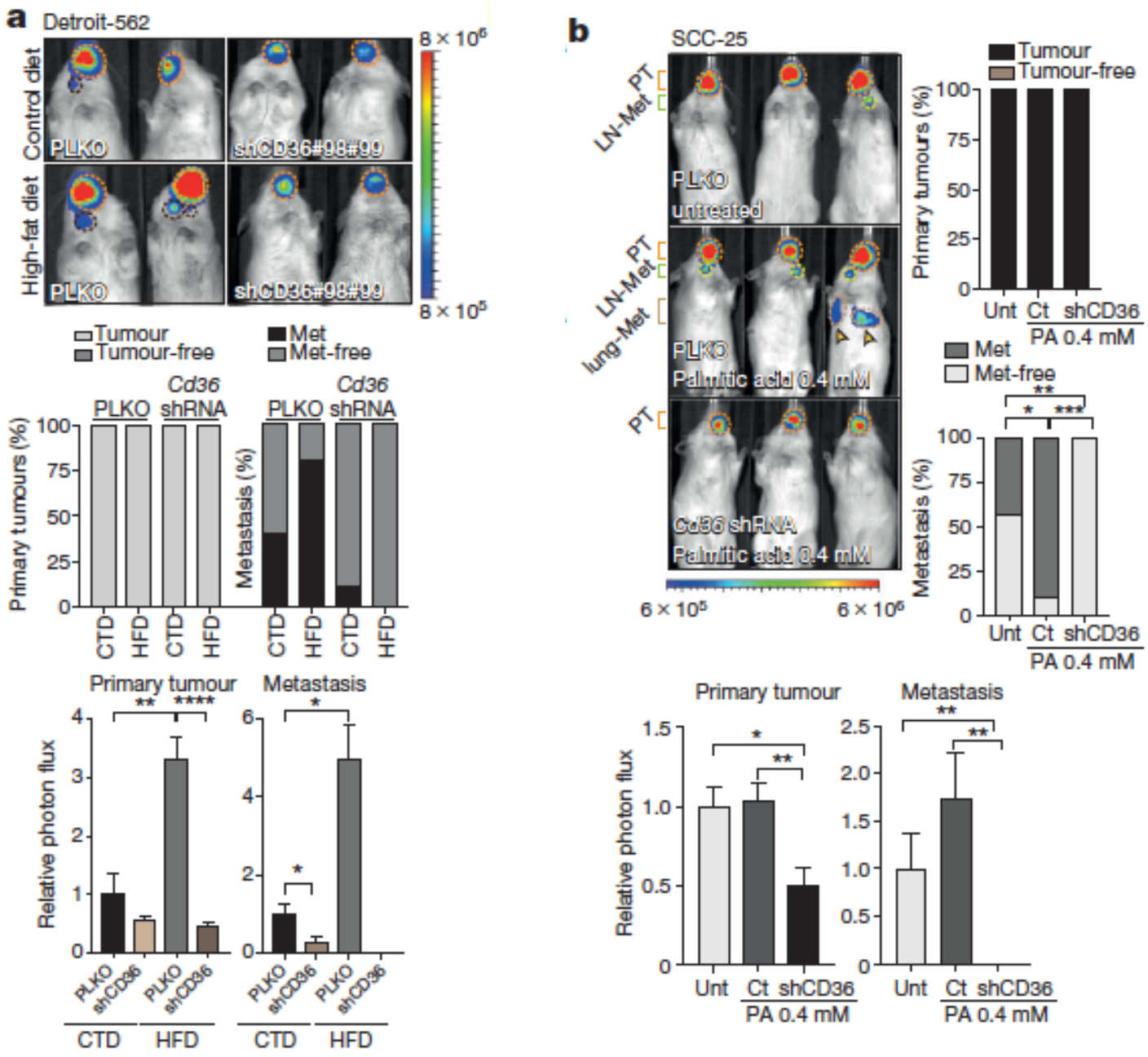
CD36 is promoting metastasis through ACSL-1 enzyme

FATTY ACID BETA-OXIDATION

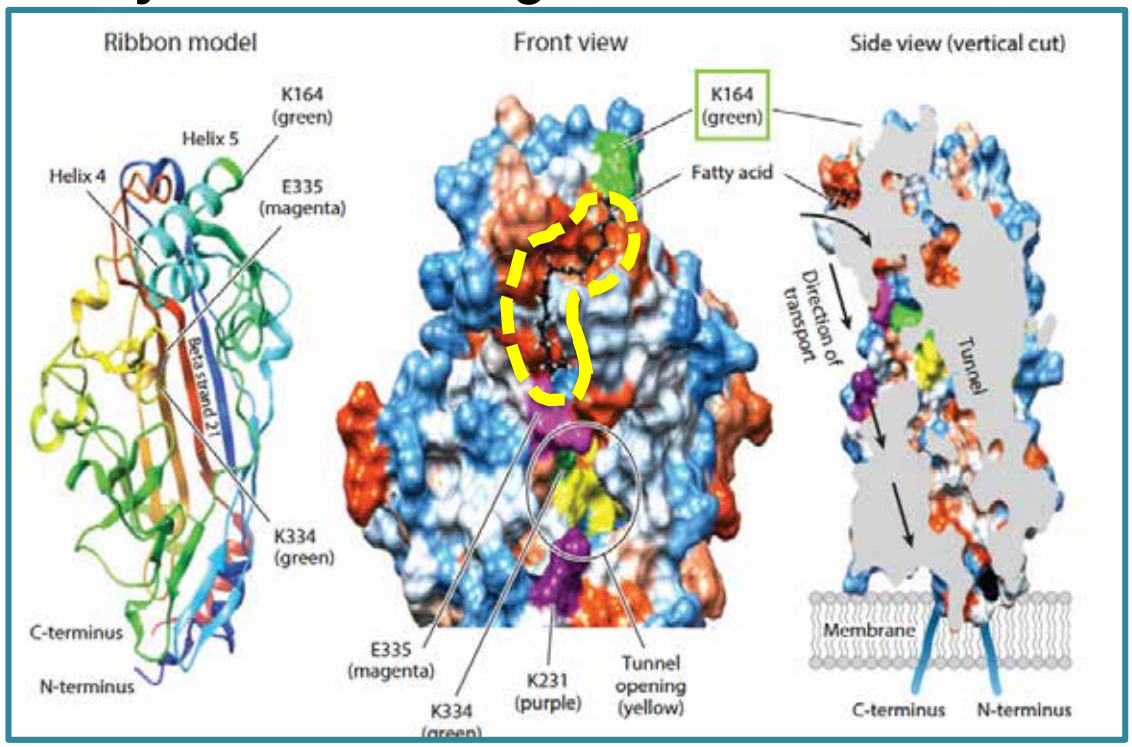
e



High fat diet boosts LN metastasis in a CD36-dependent manner

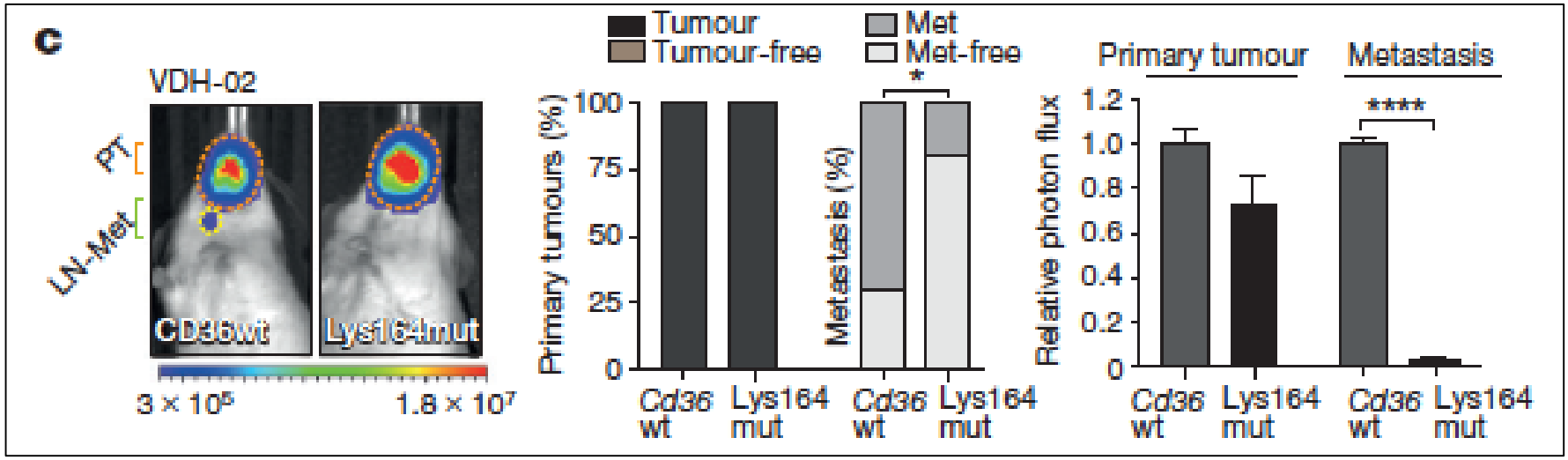


Fatty Acid binding site

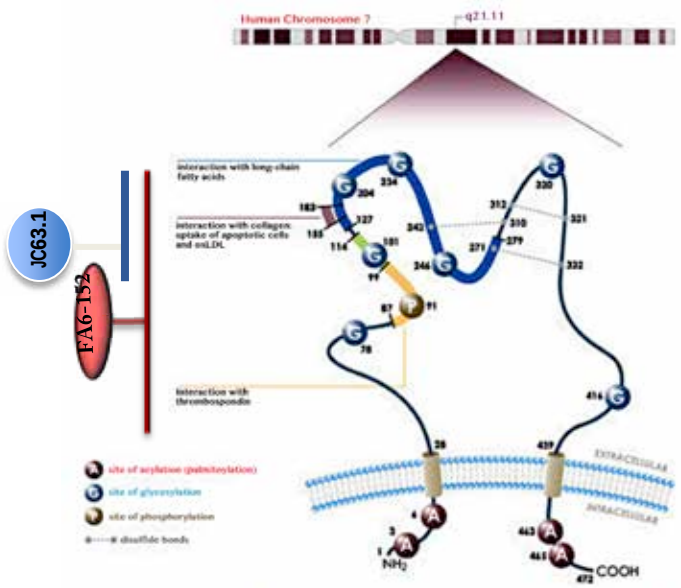


Lys164Ala

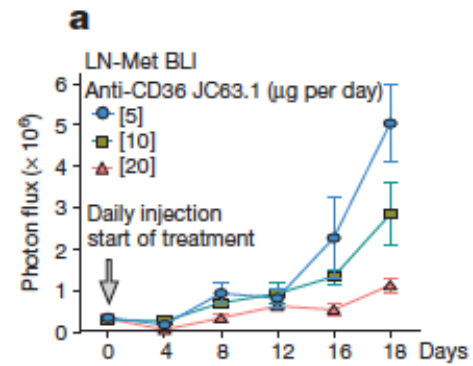
Structure-Function of CD36 and Importance of Fatty Acid Signal Transduction in Fat Metabolism. Annu Rev Nutr. 2014;34:281-303.

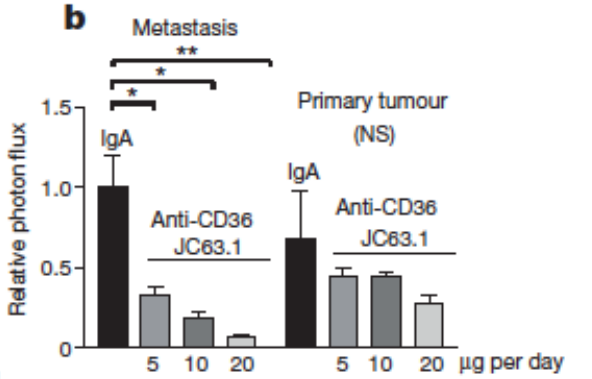


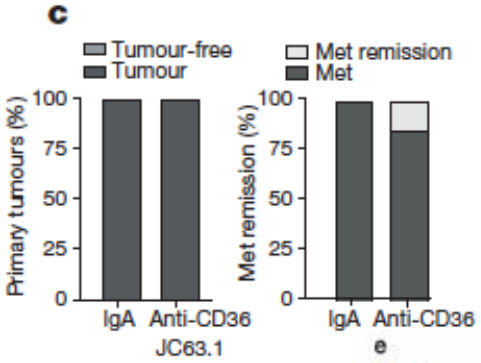
Targeting therapy against CD36+ cells

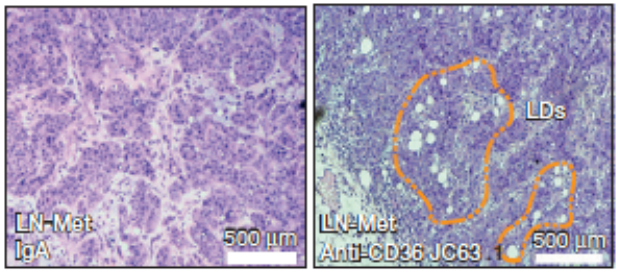


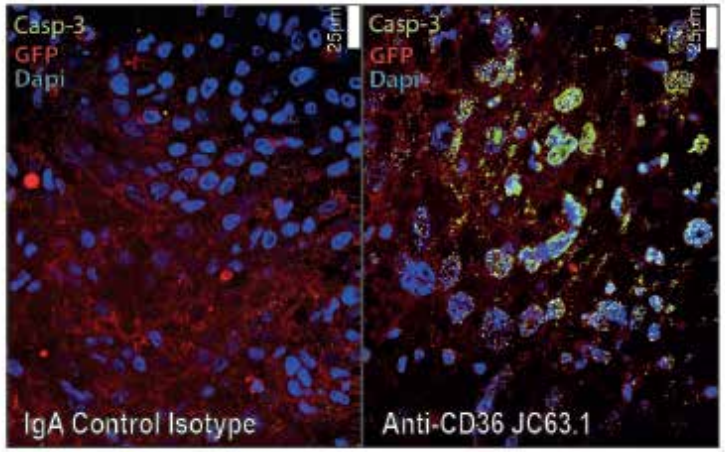
Predicted structure of CD36 receptor for oxLDL, oxidized phospholipids, long chain fatty acids, thrombospondin, and collagen type I

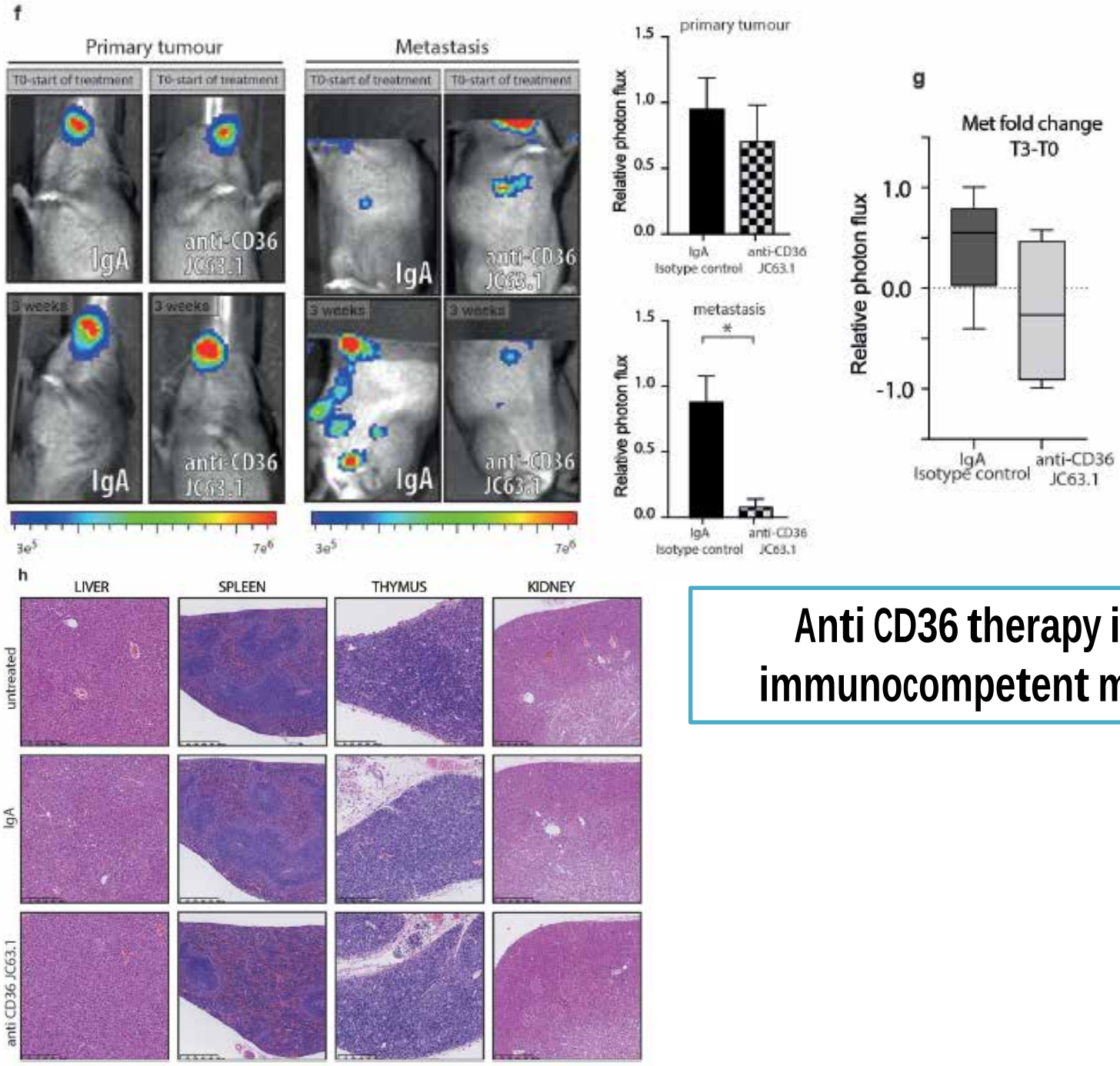








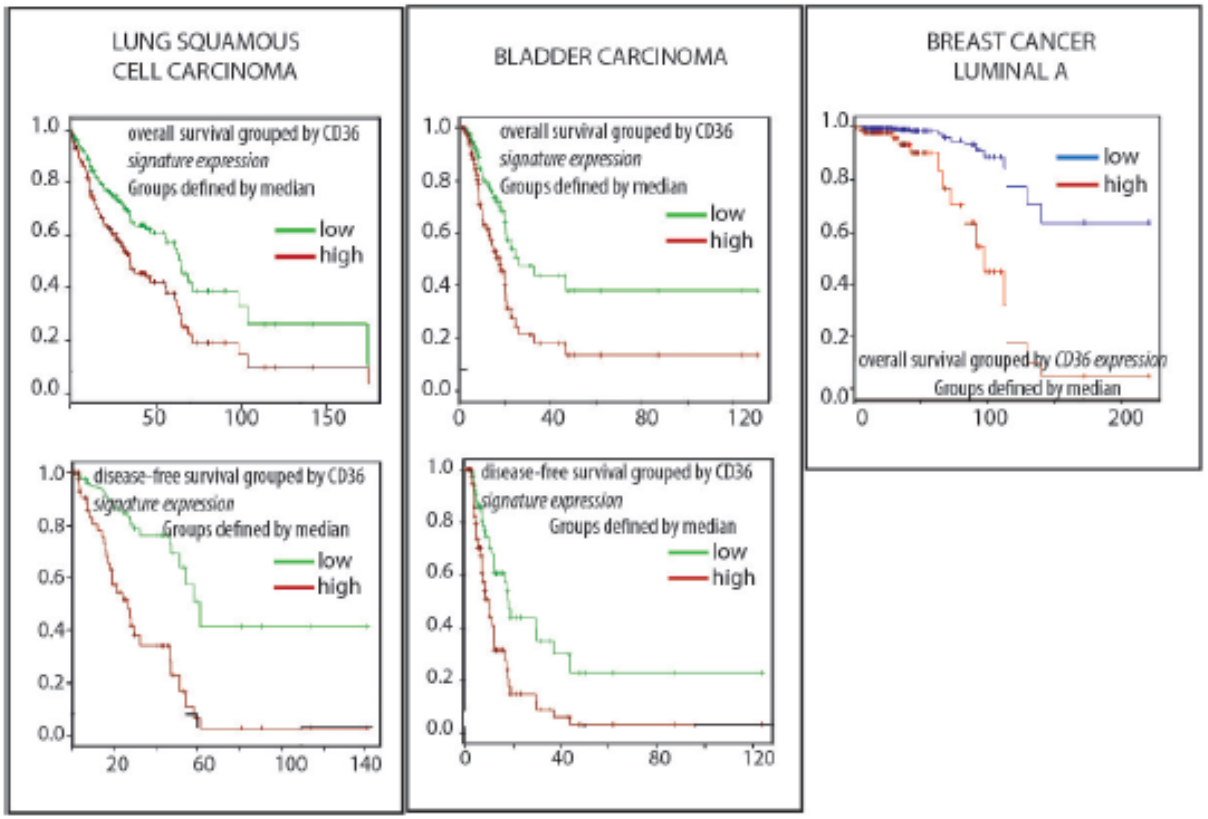


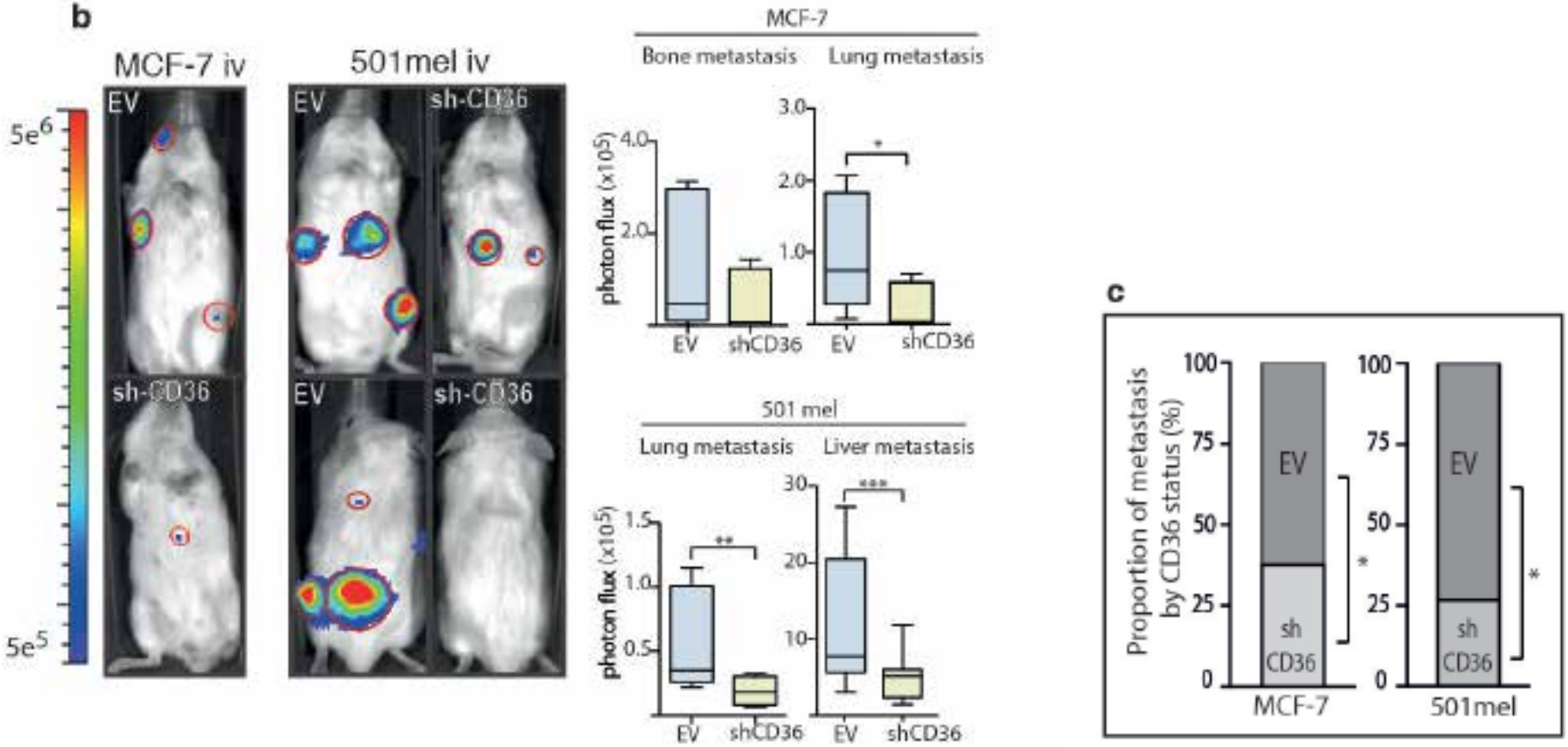


Anti CD36 therapy in immunocompetent mice

Is CD36 relevant in other types of tumours?

a


LUNG SQUAMOUS CELL CARCINOMA
overall survival grouped by CD36 signature expression
Groups defined by median
low
high
disease-free survival grouped by CD36 signature expression
Groups defined by median
low
high
BLADDER CARCINOMA
overall survival grouped by CD36 signature expression
Groups defined by median
low
high
disease-free survival grouped by CD36 signature expression
Groups defined by median
low
high
BREAST CANCER LUMINAL A
low
high
overall survival grouped by CD36 expression
Groups defined by median


b
MCF-7 iv
501mel iv
EV
sh-CD36
EV
sh-CD36
EV
sh-CD36
5e6
5e5
MCF-7
Bone metastasis
Lung metastasis
photon flux (x10⁵)
4.0
2.0
3.0
2.0
1.0
*
EV
shCD36
EV
shCD36
501 mel
Lung metastasis
Liver metastasis
photon flux (x10⁵)
1.5
1.0
0.5
30
20
10
**
***
EV
shCD36
EV
shCD36
c
Proportion of metastasis by CD36 status (%)
100
75
50
25
0
EV
sh CD36
*
100
75
50
25
0
EV
sh CD36
*
MCF-7
501mel

Stem cells and cancer lab

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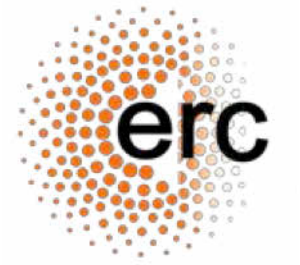

THANKS!