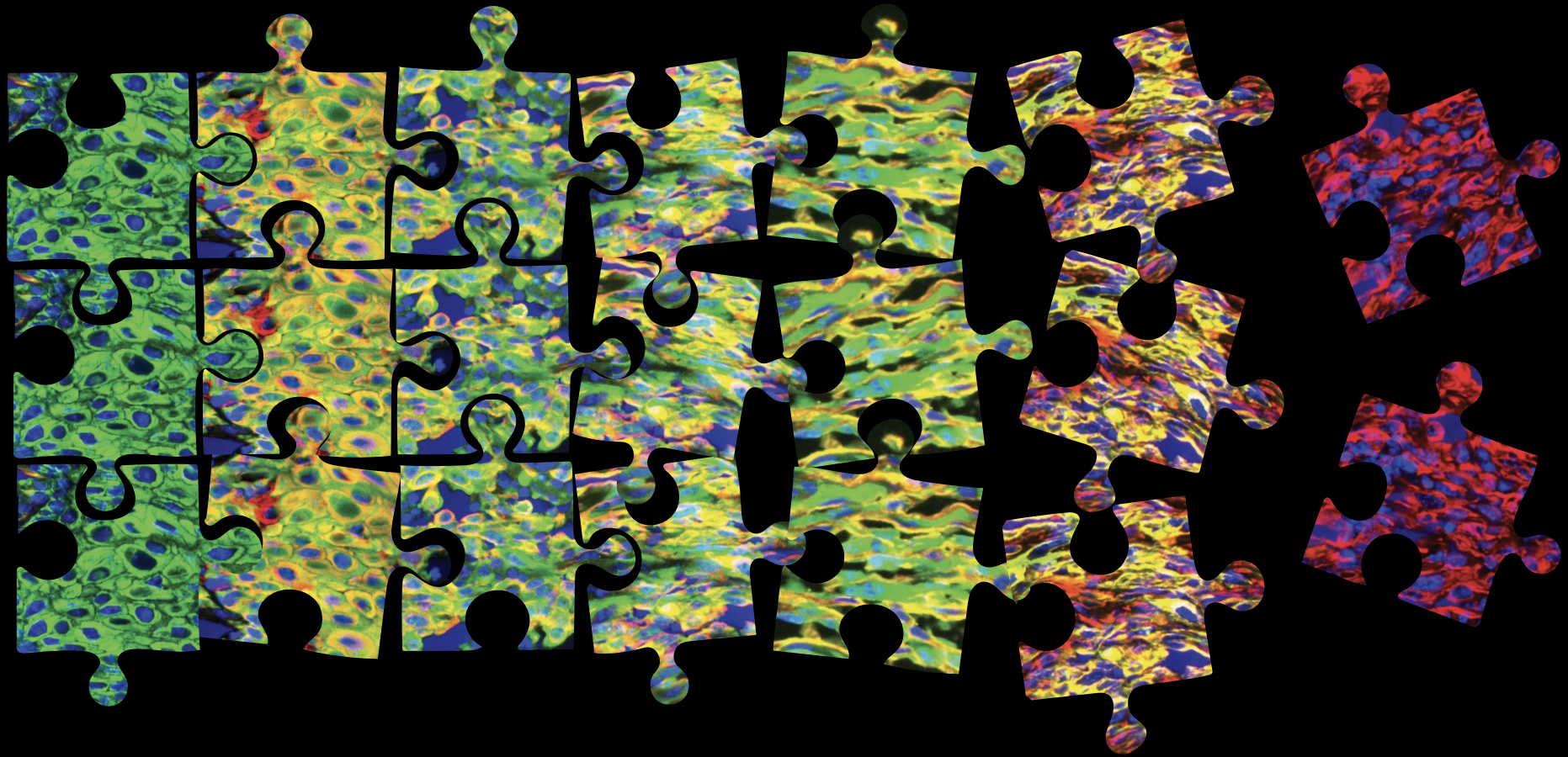
Tumor transition states occuring during EMT



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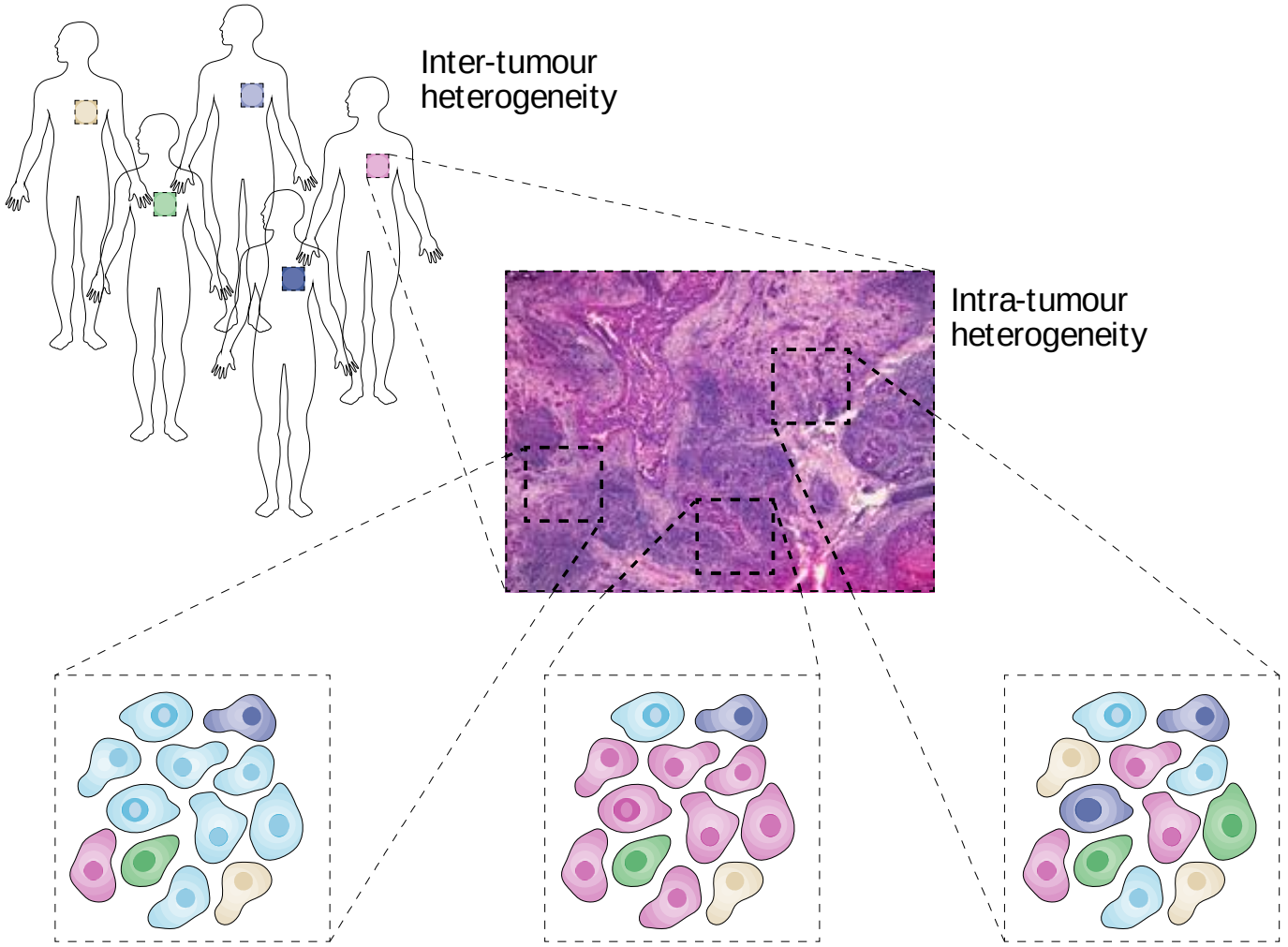
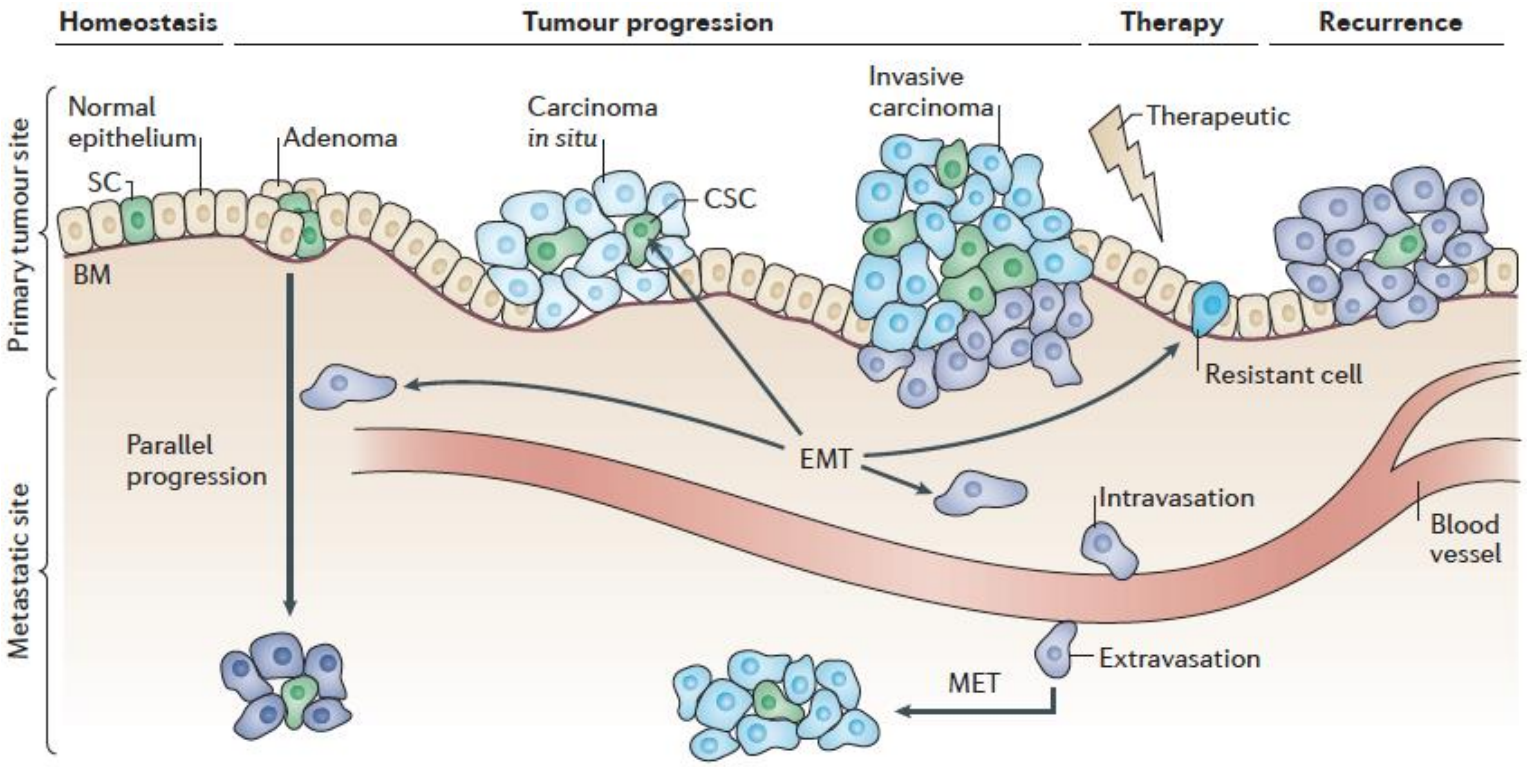
Dermatology Department, CHU Brugmann

Tumor heterogeneity



Marusyk A et al. Nat Rev Cancer 2012

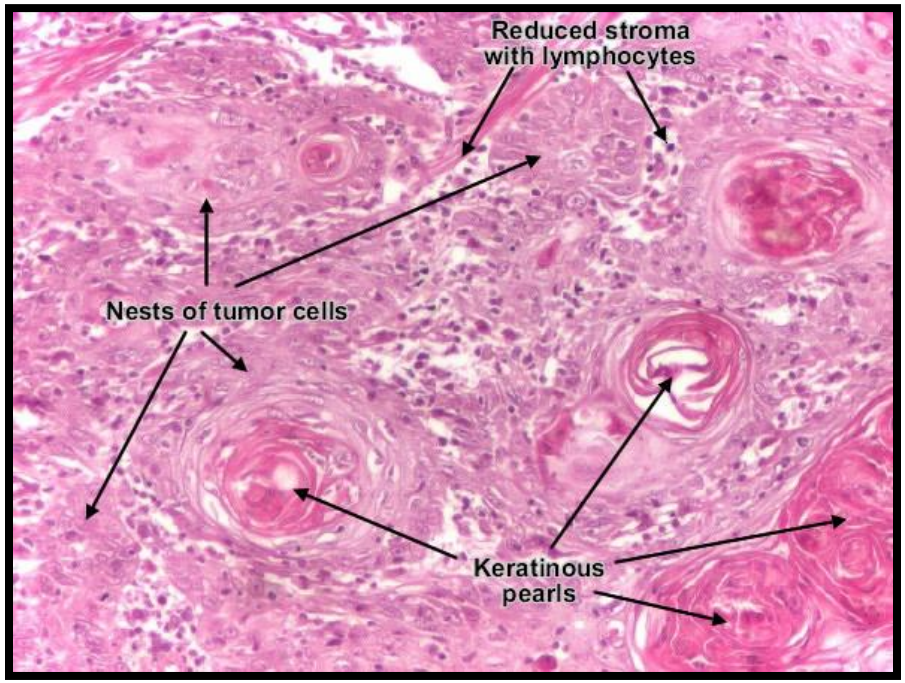
Implication of EMT for tumor heterogeneity and metastasis



De Craene & Berx, Nat Rev Cancer 2013

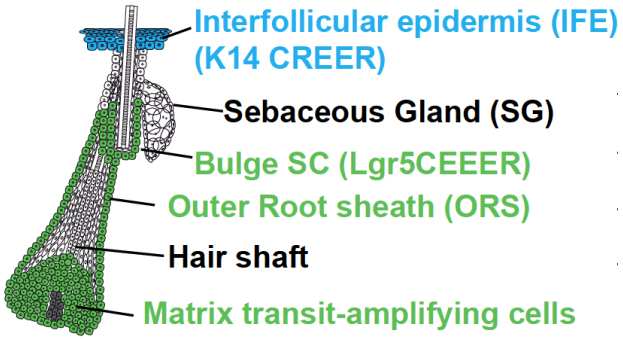
Squamous cell carcinoma

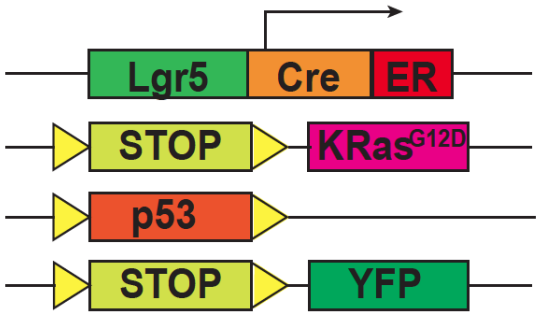
- 2nd most frequent cancer
- 500.000 patients /year in US
- Surgical excision
- 5% with metastasis leading to poor survival prognosis

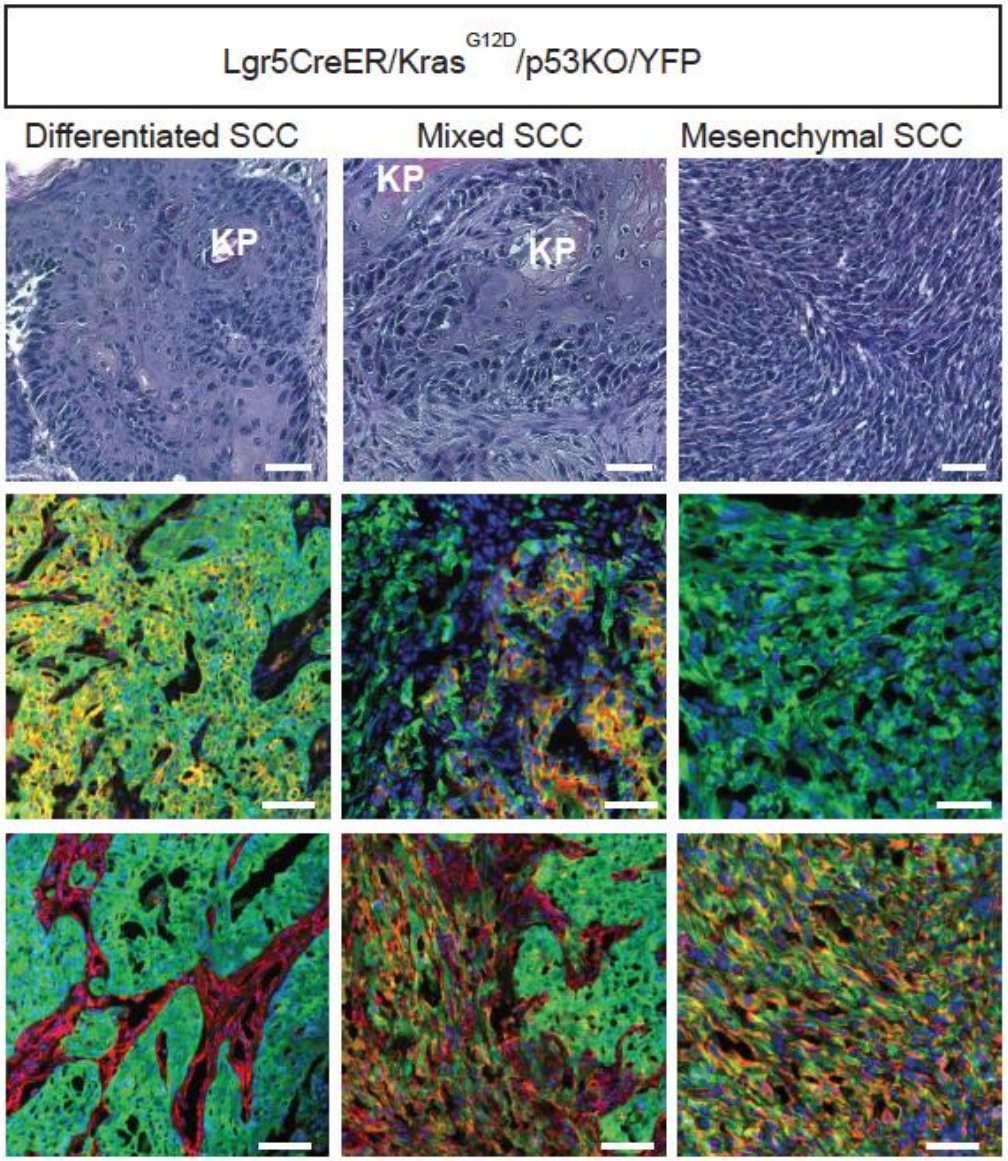


Mutations of the **Ras** pathway in mice and human SCC
⇒Hras (Kamino et al., 1991, Corominas et al., 1989, Nassar et al. Nature Medicine 2015)
⇒Kras (Spencer et al., 1995; Sutter et al., 1993; van der Schroeff et al., 1990, Nassar et al. Nature Medicine 2015)
⇒RRas2 (Nassar et al. Nature Medicine 2015)

Genetic lineage tracing in mouse model of skin SCC that undergo spontaneous EMT

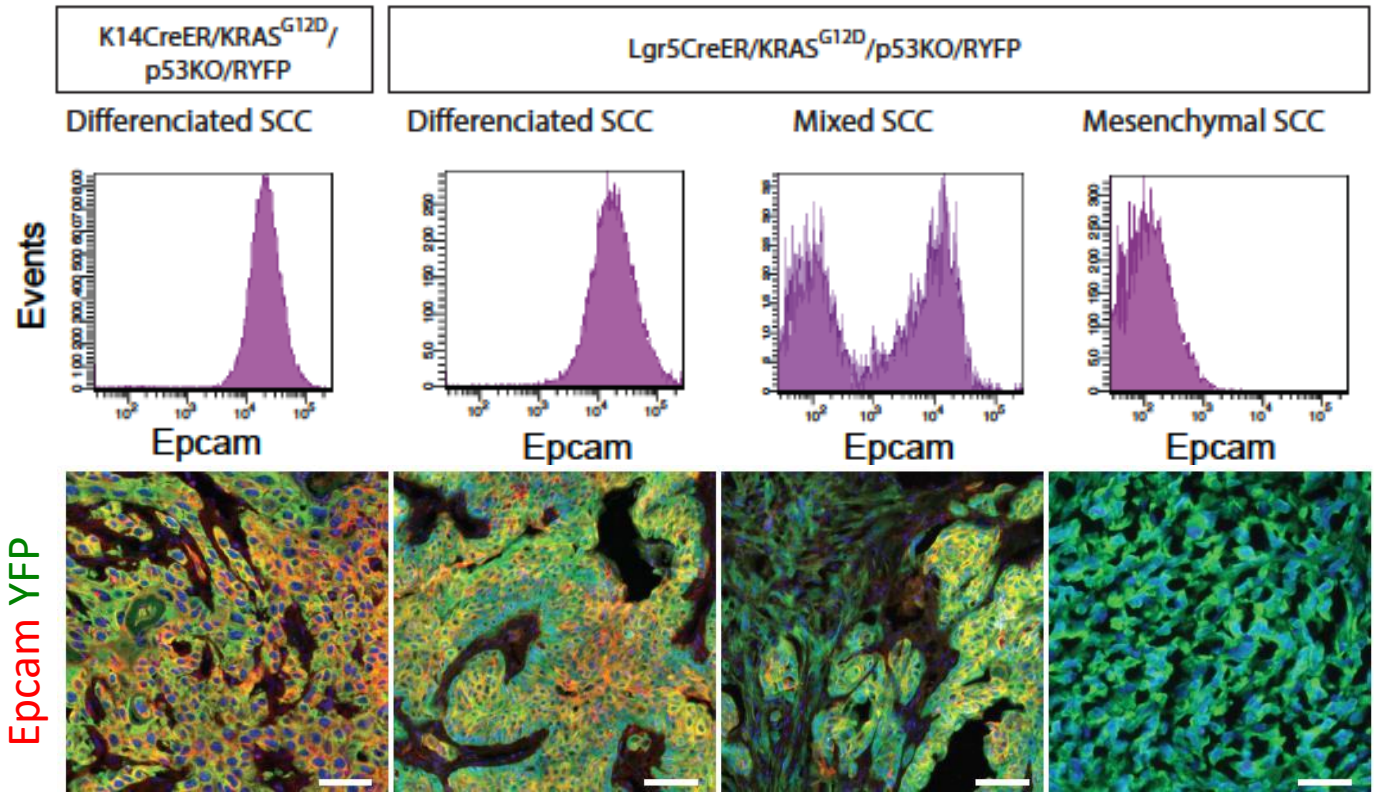


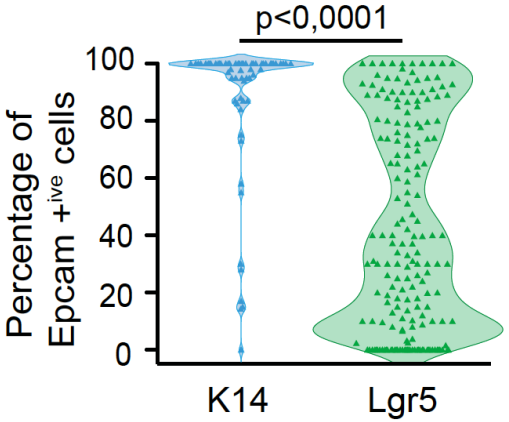


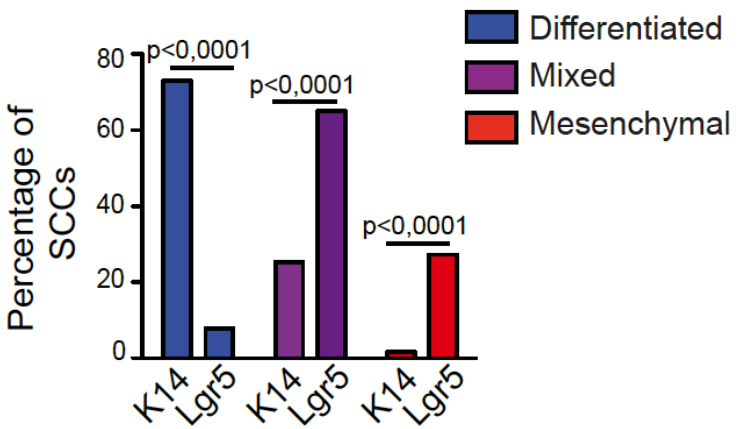


Latil et al, Cell Stem Cell 2017

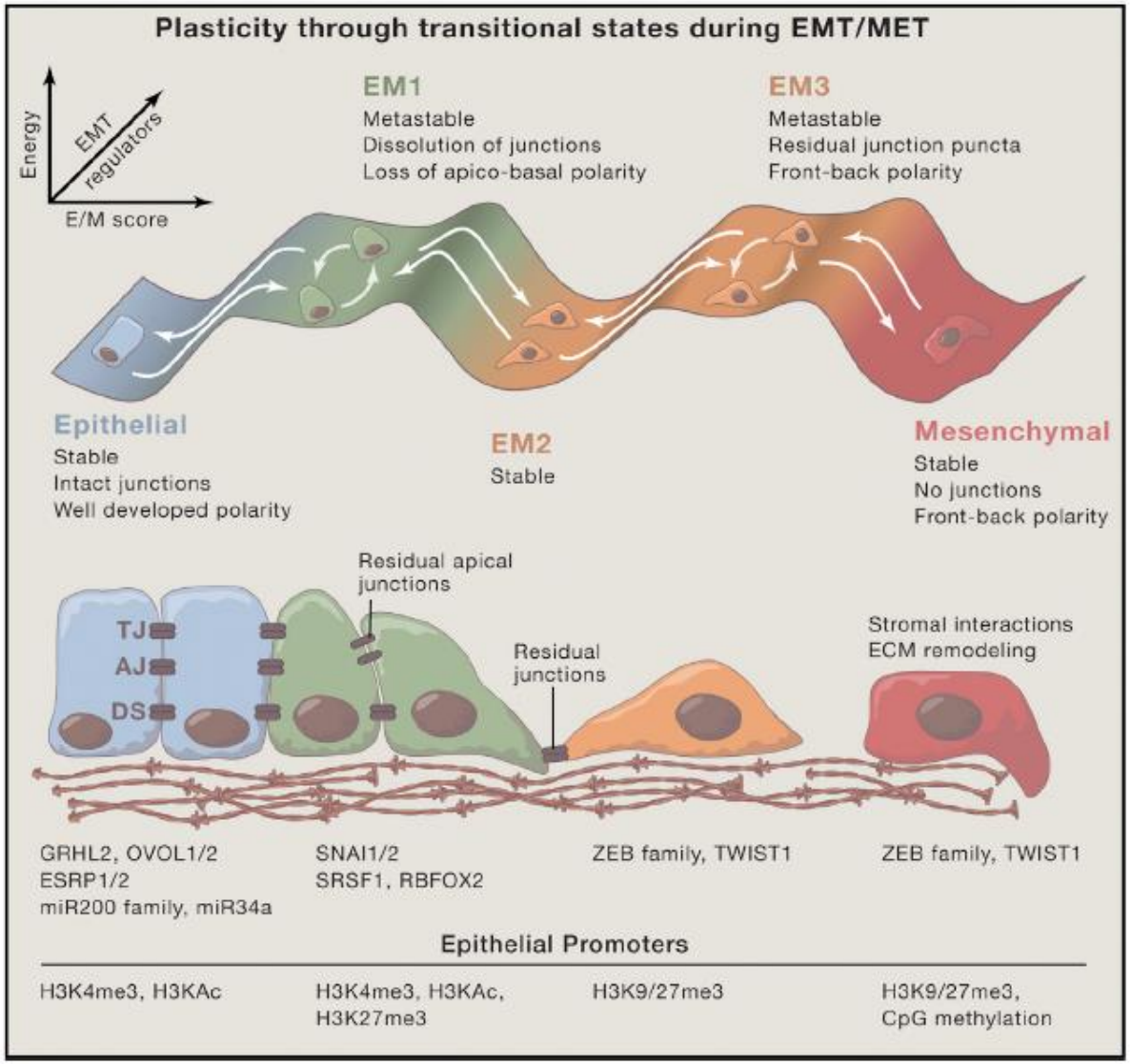
Loss of Epcam expression during EMT





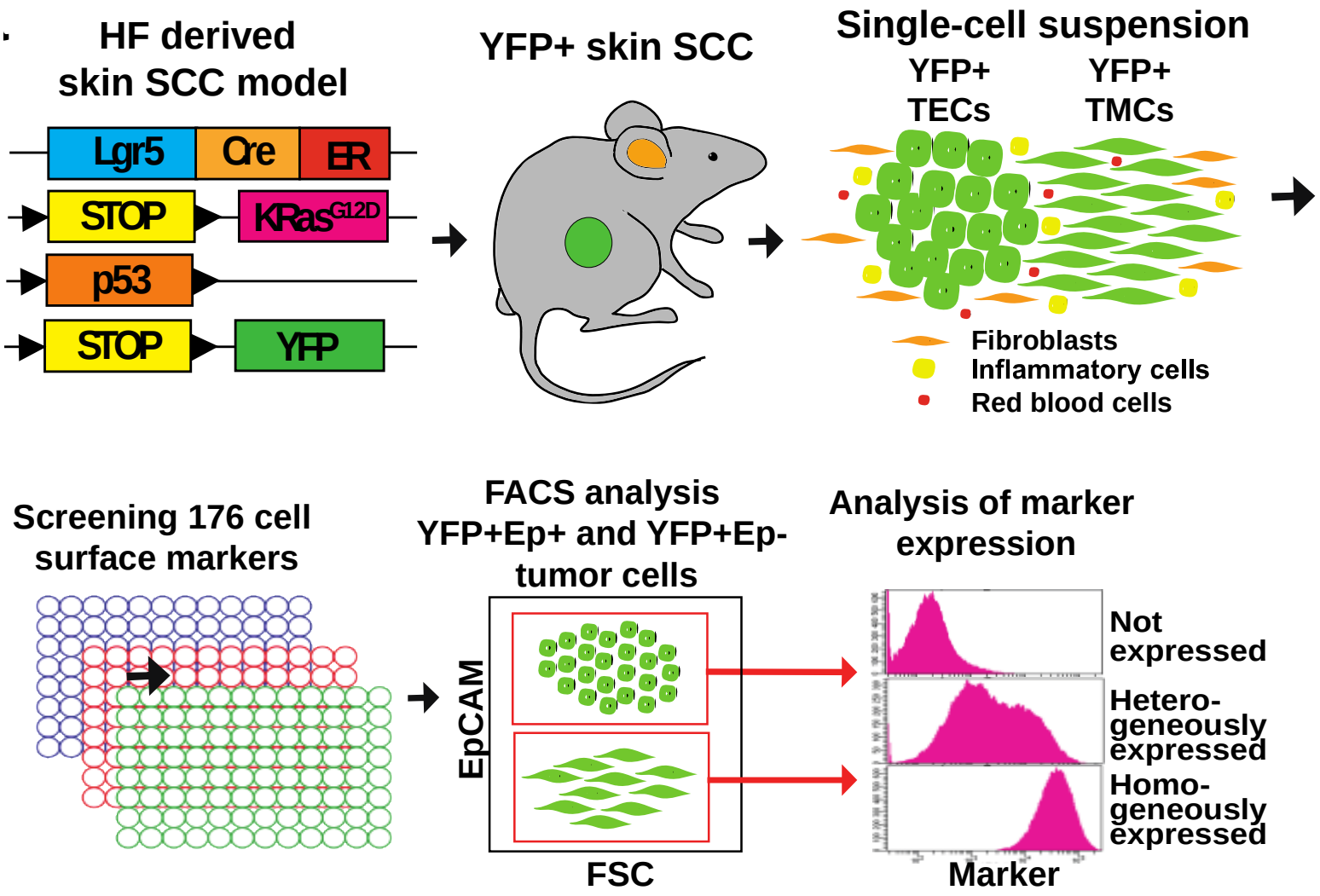


Does EMT occur through distinct transitional states ?

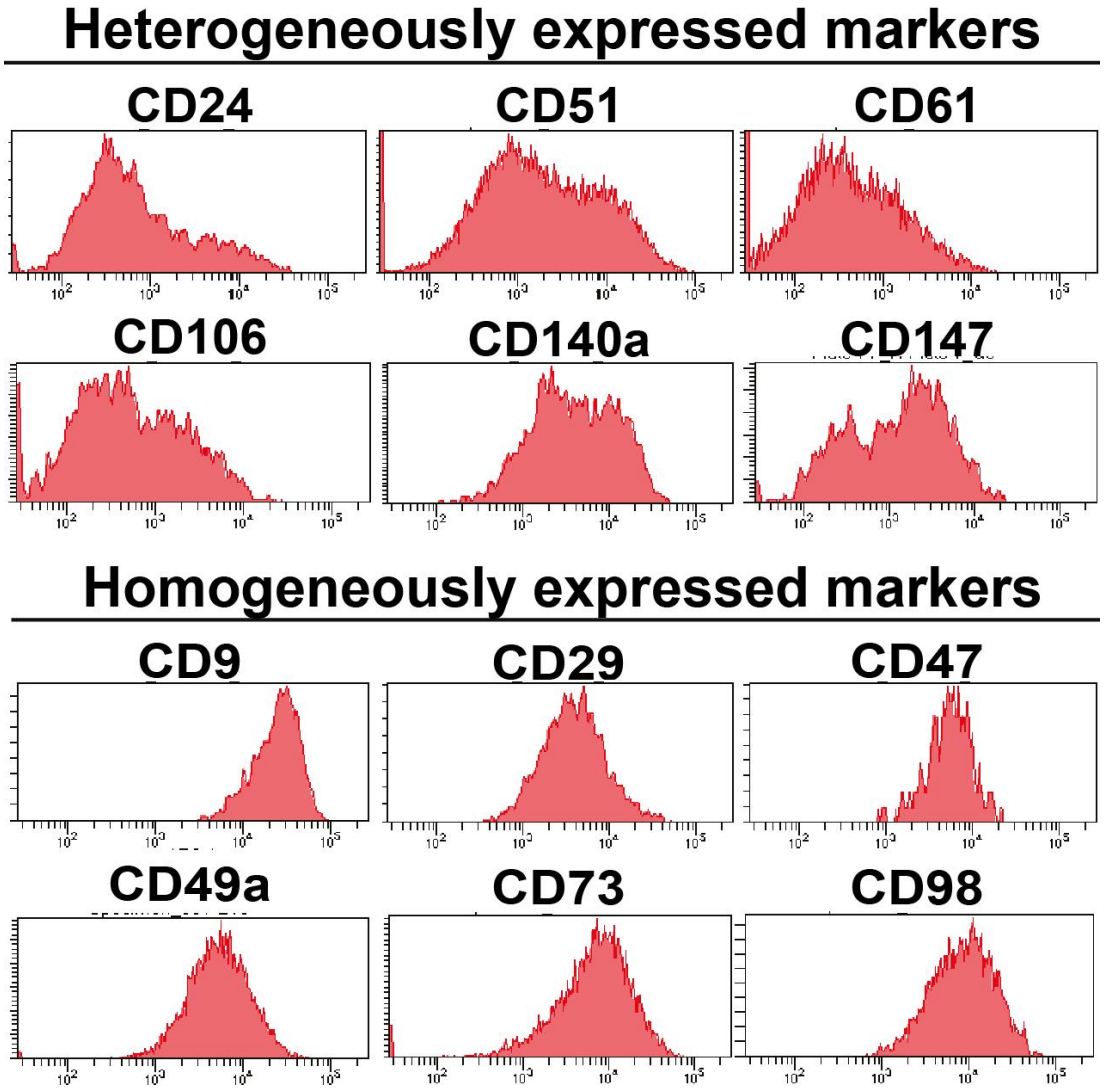


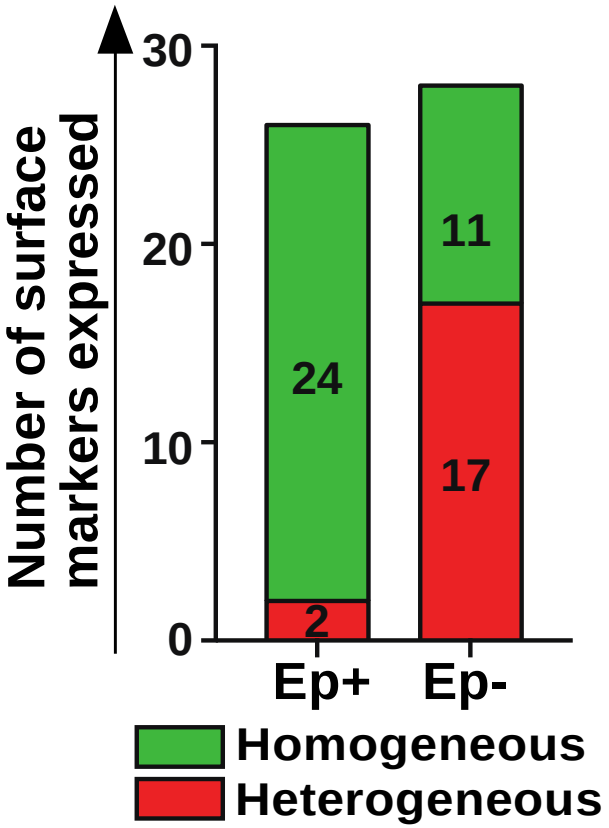
Nieto et al. Cell 2016

Identification of the tumor transition states occuring during EMT in vivo

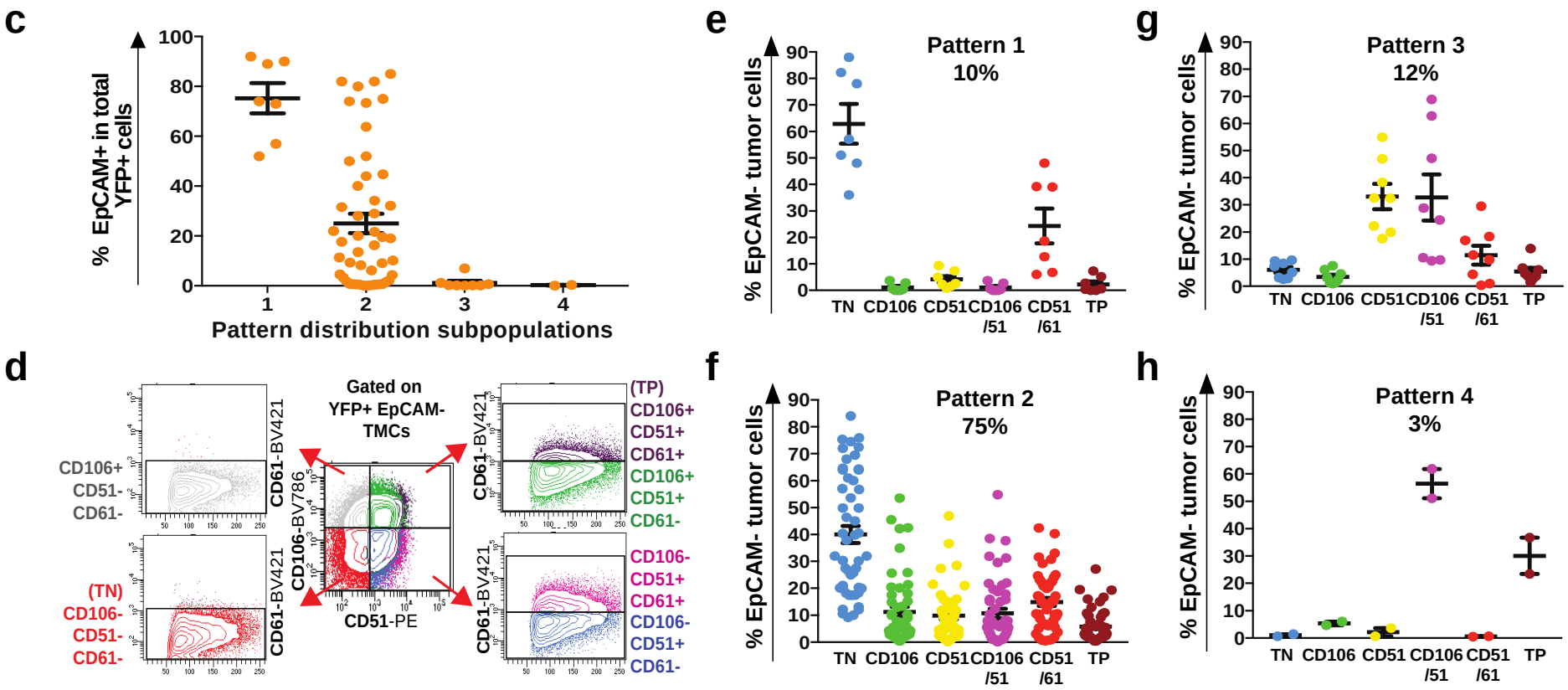


Identification of cell surface markers heterogenously expressed during EMT in vivo

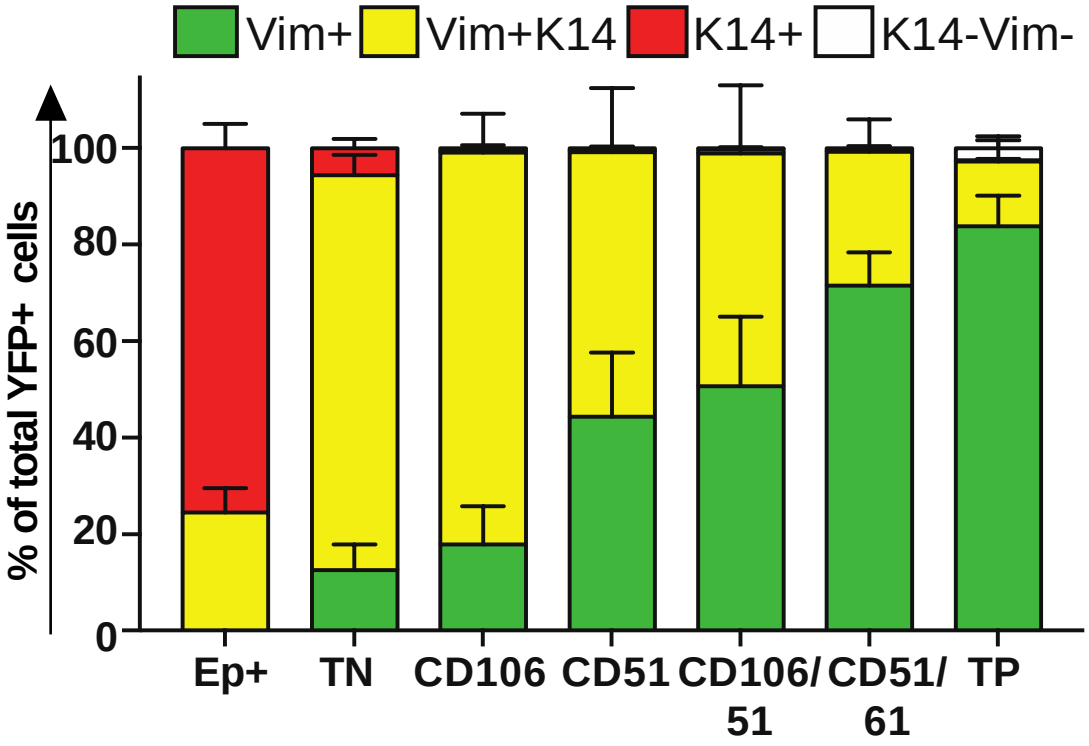


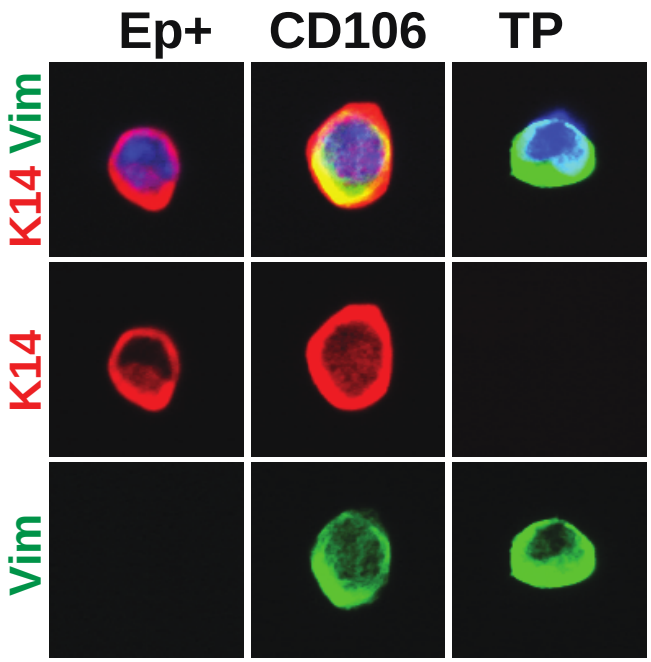


Identification of the tumor transition states occuring during EMT in vivo

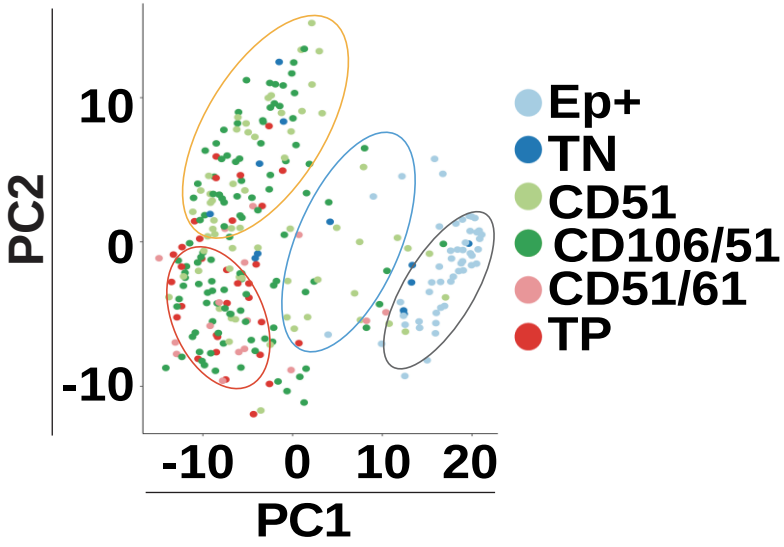


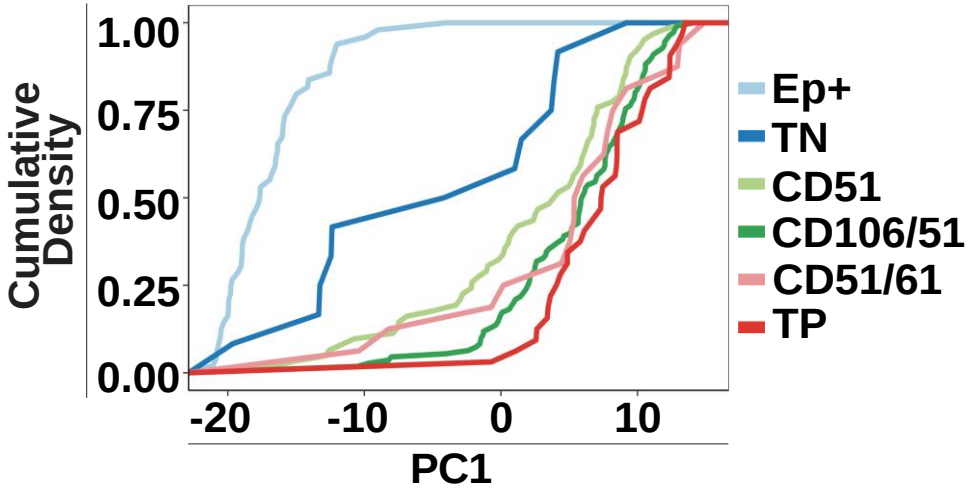
Uncovering the order of transition during EMT



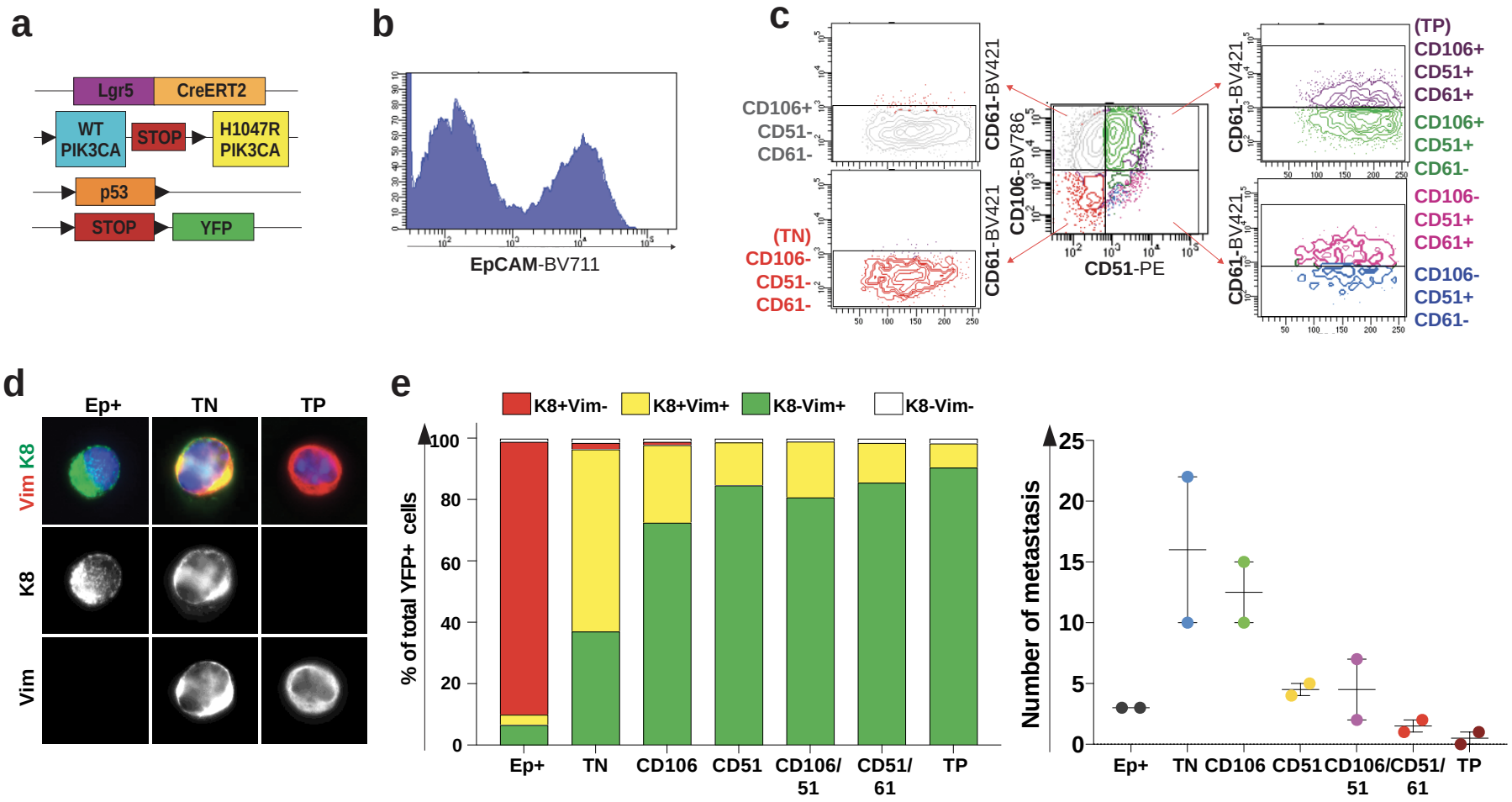


EMT transition states revealed by tumor by single cell RNA-seq

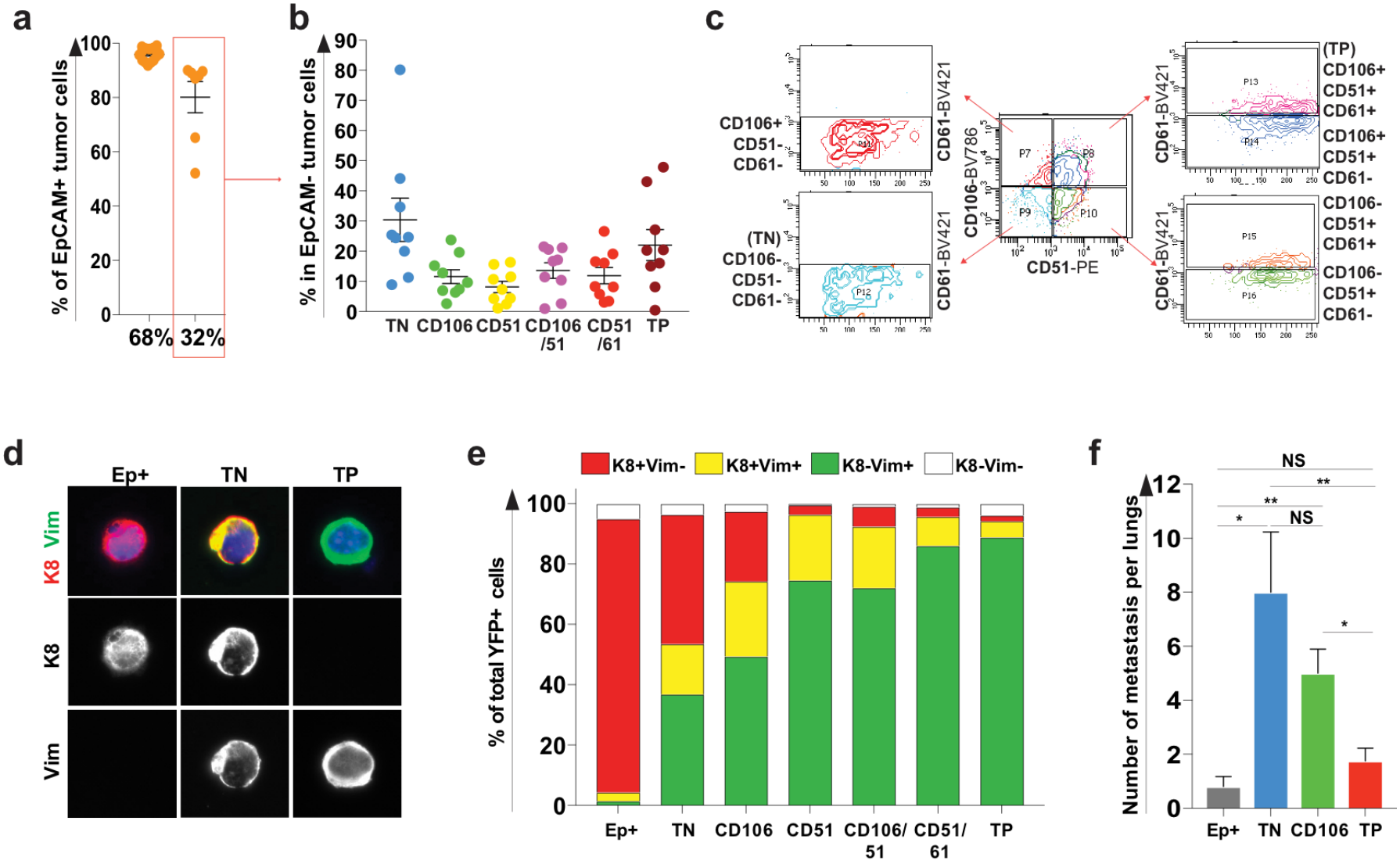




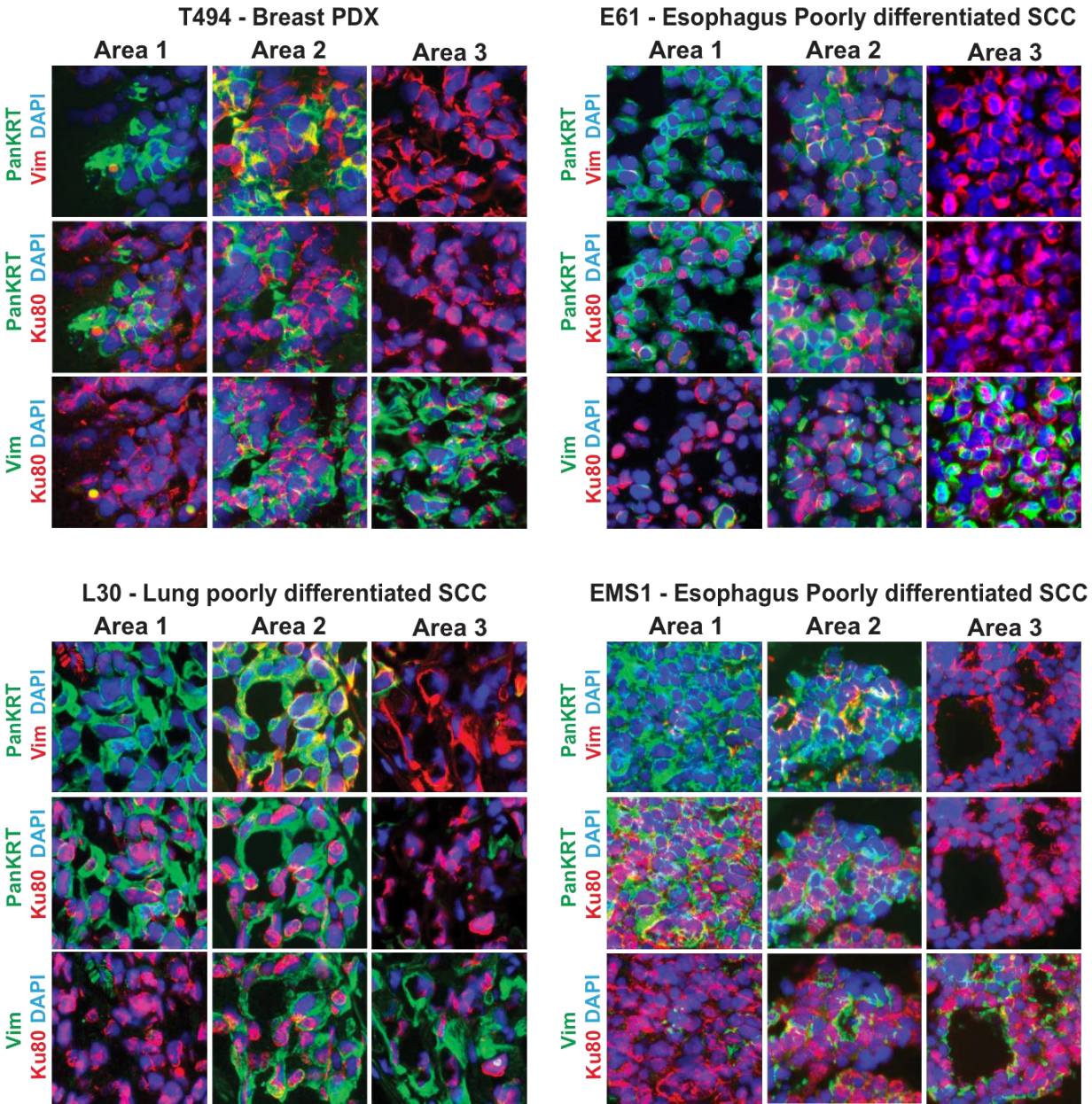
Metaplastic mammary tumors progress through the same EMT transition states



EMT transition states in MMTV-PyMT mammary tumors



EMT transition states in human cancer



EMT transition states present similar TPC capacity but exhibit different plasticity

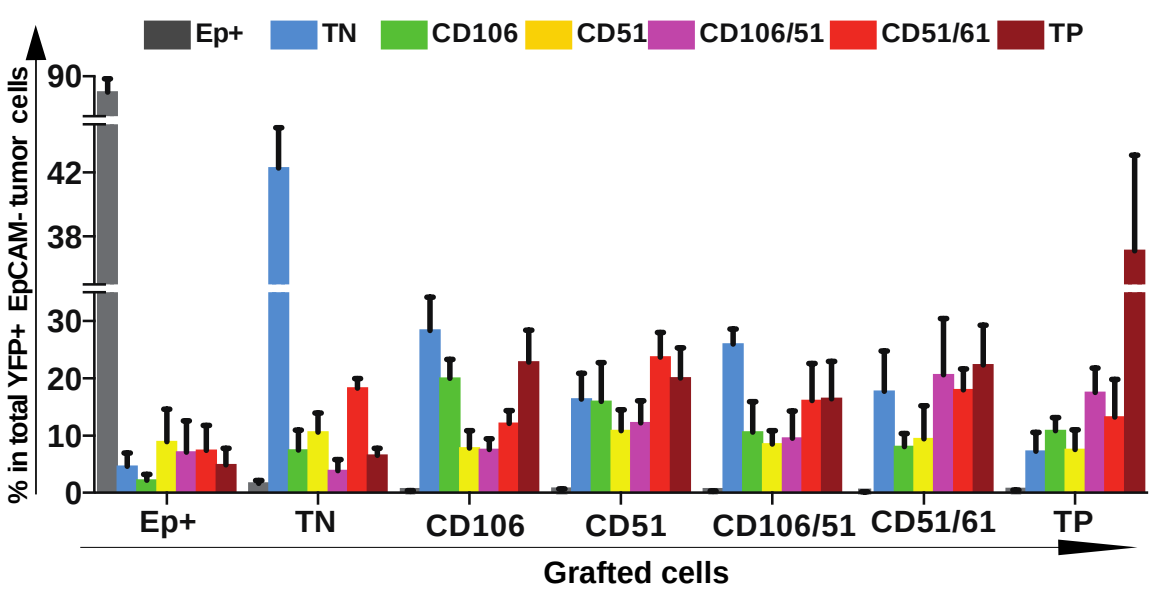
Number of grafted cells	Ep+	Ep-	TN	CD106	CD51	CD106/51	CD51/61	TP
1000	7/9 (n=3)	10/12 (n=3)	9/12 (n=2)	15/17 (n=2)	12/15 (n=3)	6/6 (n=2)	14/18 (n=3)	10/12 (n=2)
100	1/9 (n=3)	15/18 (n=4)	23/24 (n=4)	17/24 (n=4)	3/3 (n=3)	13/18 (n=3)	19/24 (n=3)	13/17 (n=4)
10	1/9 (n=3)	18/24 (n=3)	7/30 (n=4)	21/46 (n=4)	14/19 (n=3)	6/18 (n=3)	14/30 (n=3)	12/21 (n=3)
TPC frequency	**1/614** (1/1266-1/297)	**1/93** (1/159-1/54)	**1/146** (1/246-1/86)	**1/99** (1/156-1/63)	**1/130** (1/246-1/68)	**1/59** (1/99-1/35)	**1/168** (1/285-1/99)	**1/124** (1/226-1/69)

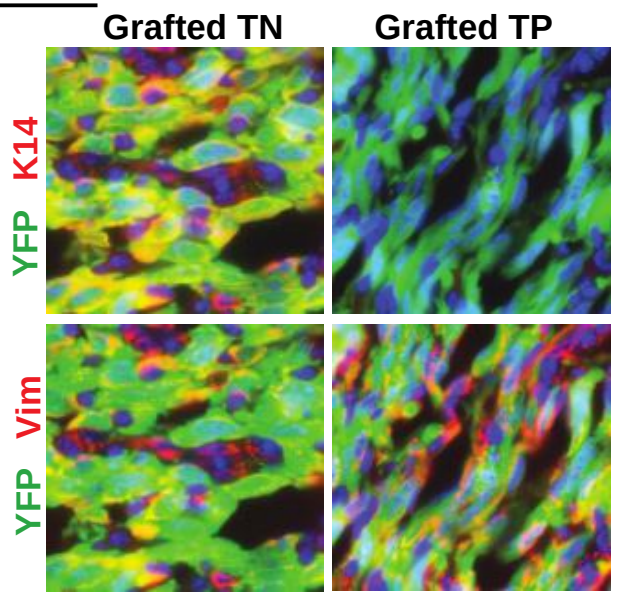
p= 0.004-3.35e-08

p=0.0049

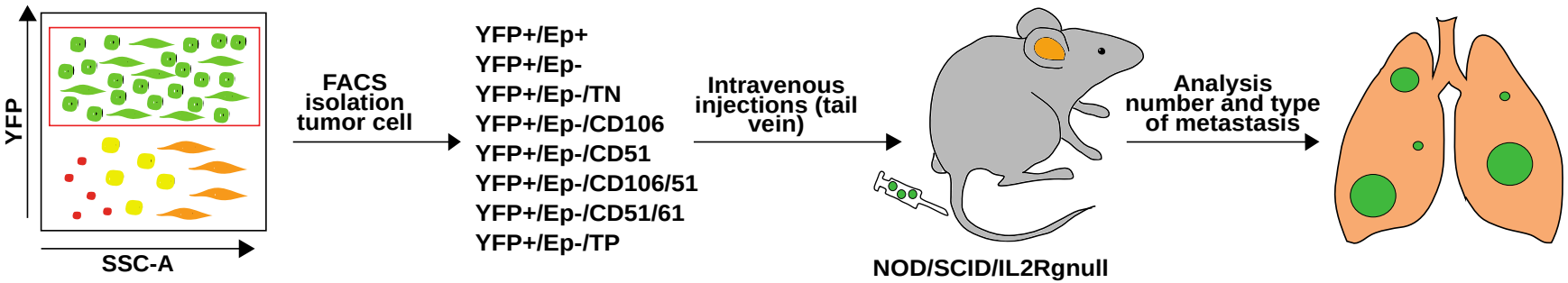
p=0.001

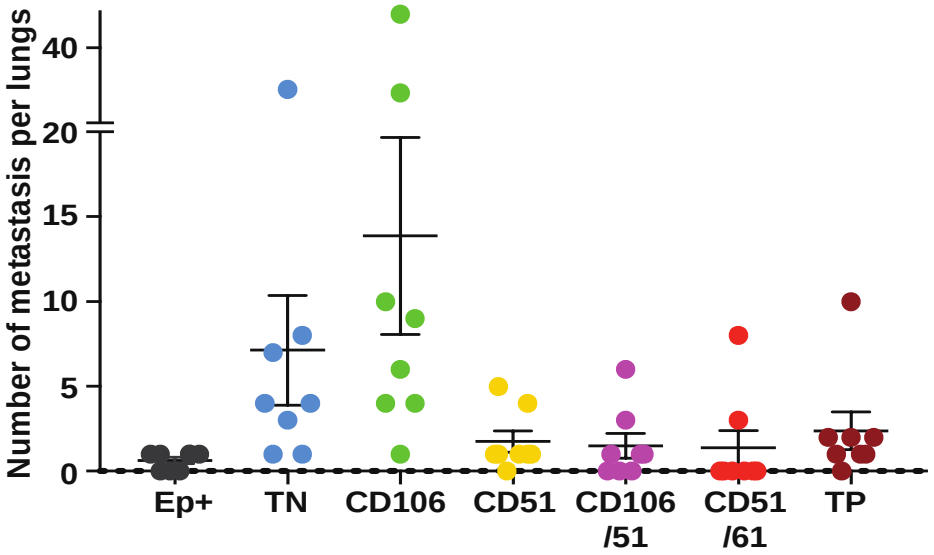
c

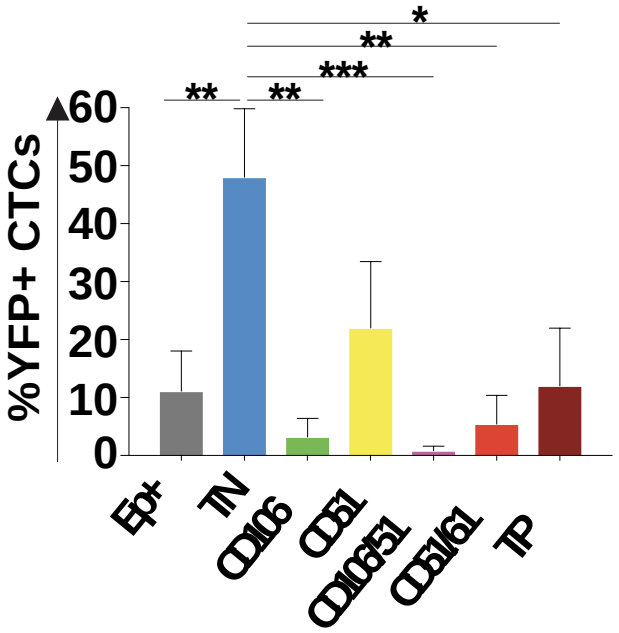




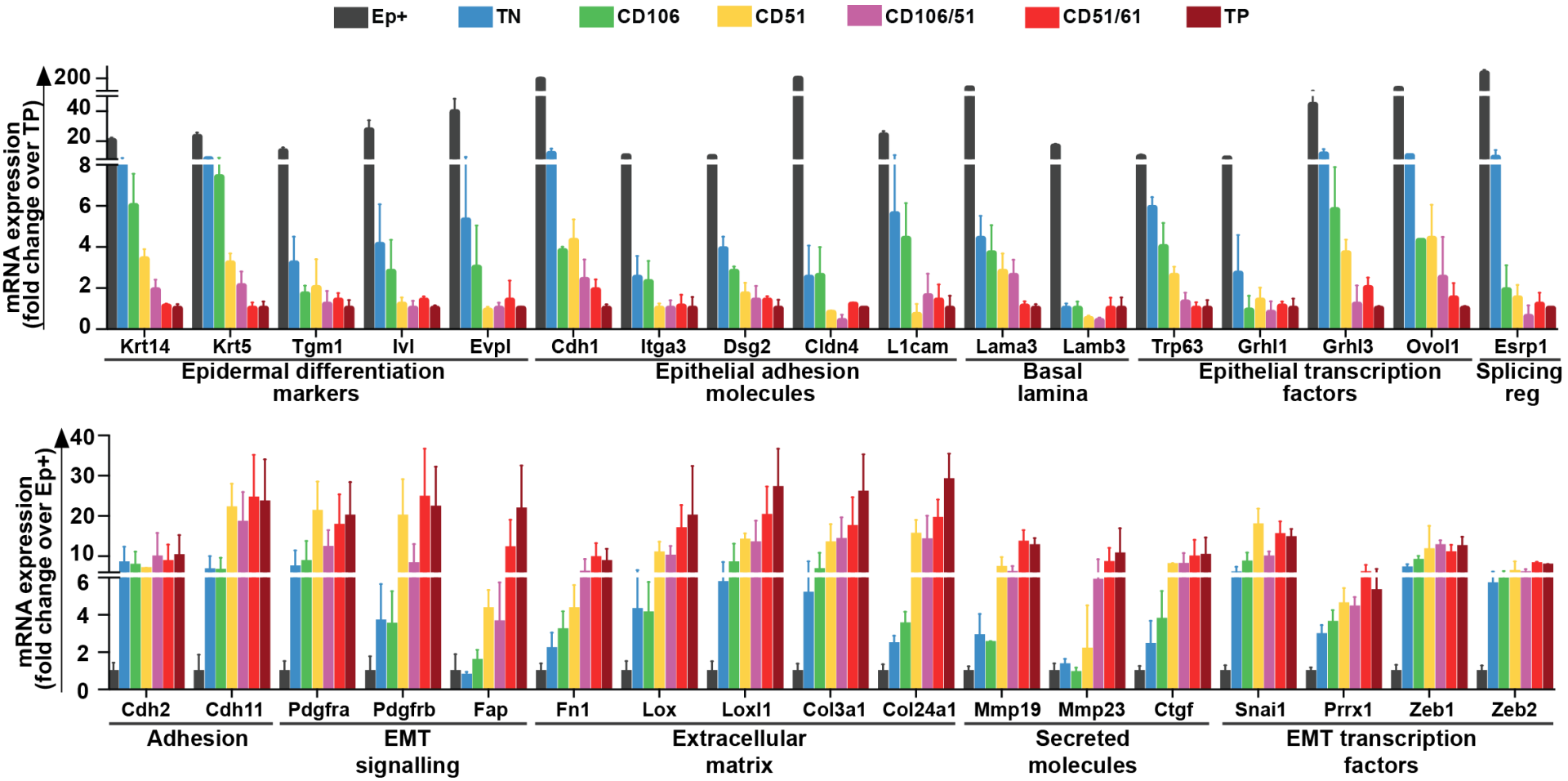
Different EMT transition states present different metastatic potential



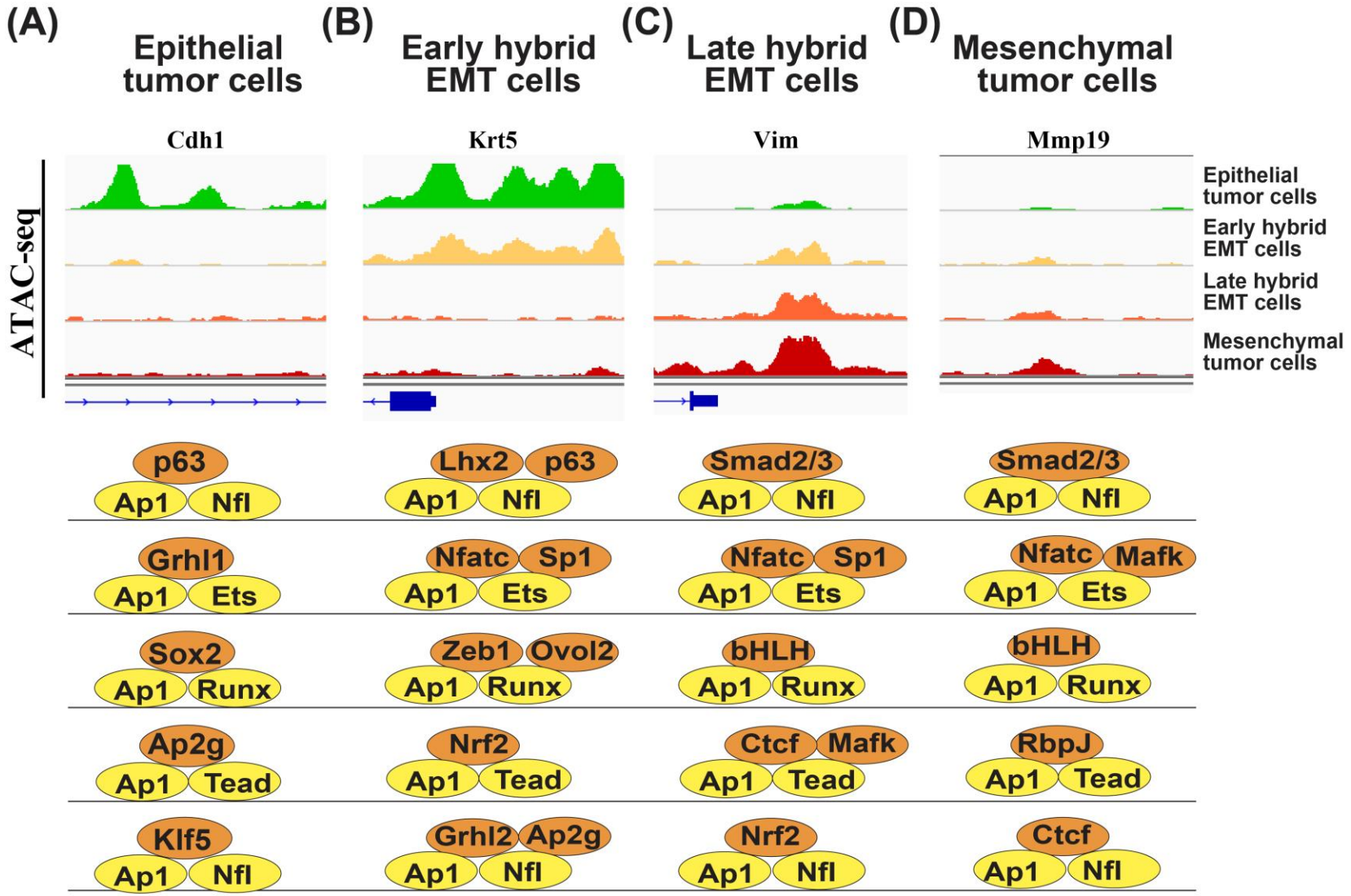




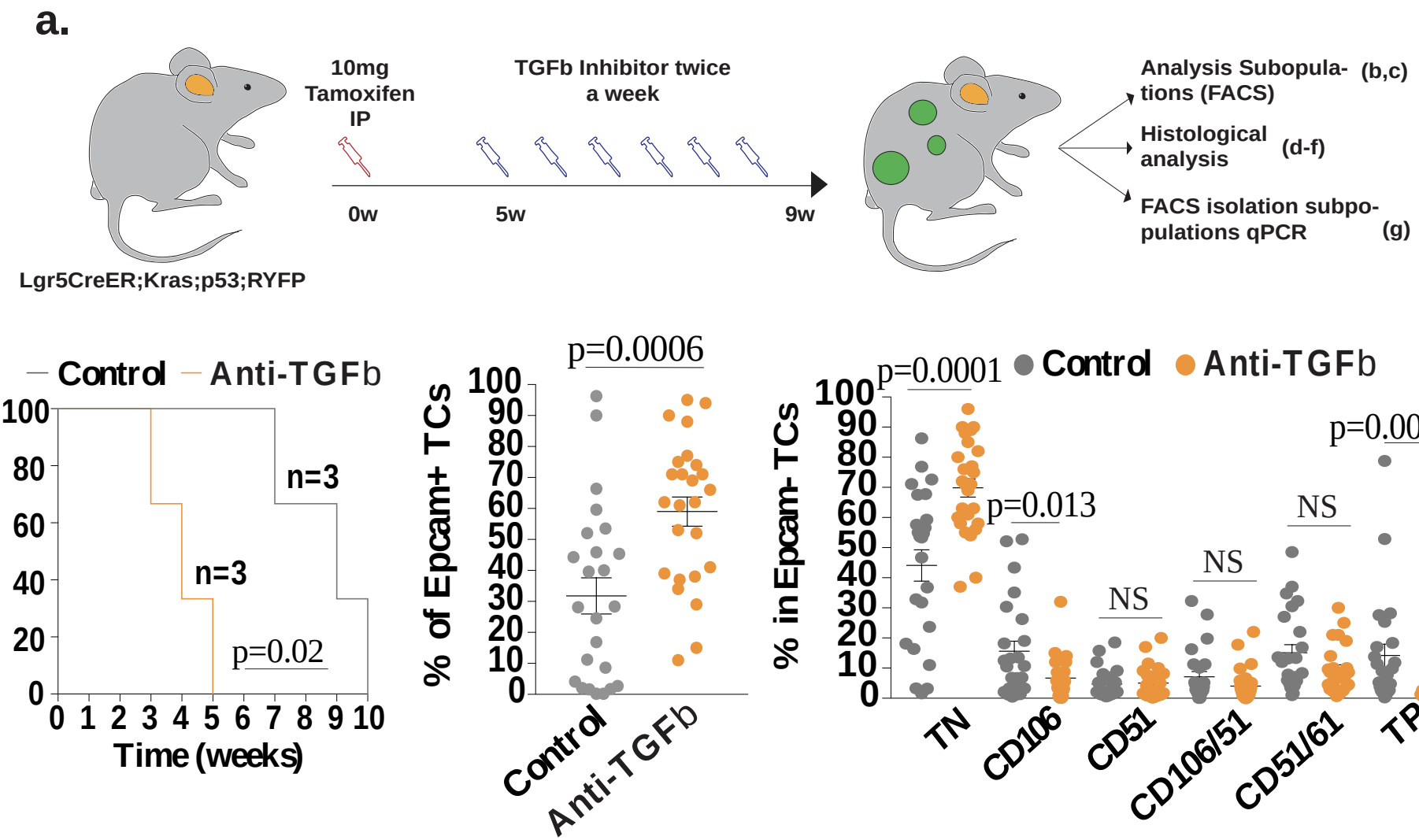
Transcriptional landscape of EMT transition states



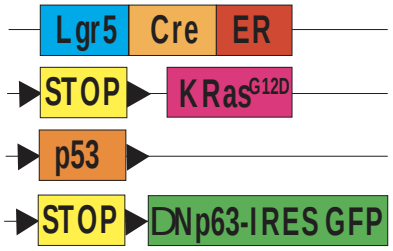
Uncovering transcription factors operating during EMT at each transition states



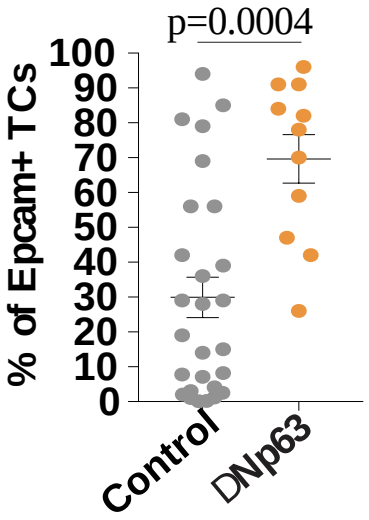
TGFb/Smad2 inhibition accelerates tumorigenesis and blocks EMT progression

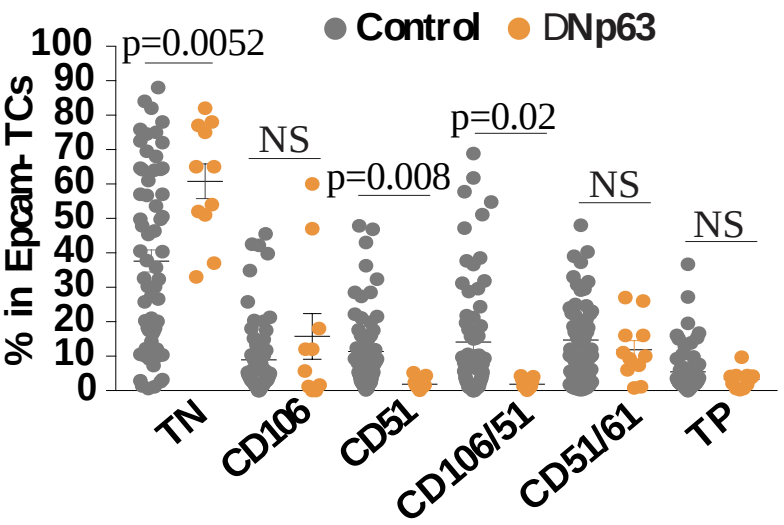


p63 overexpression blocks late steps of EMT progression

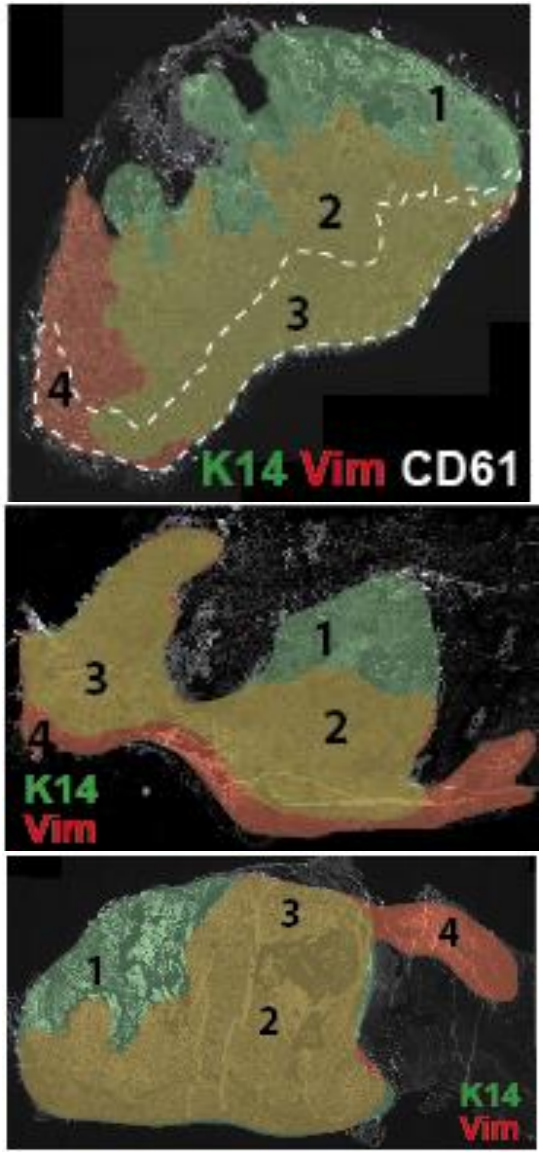


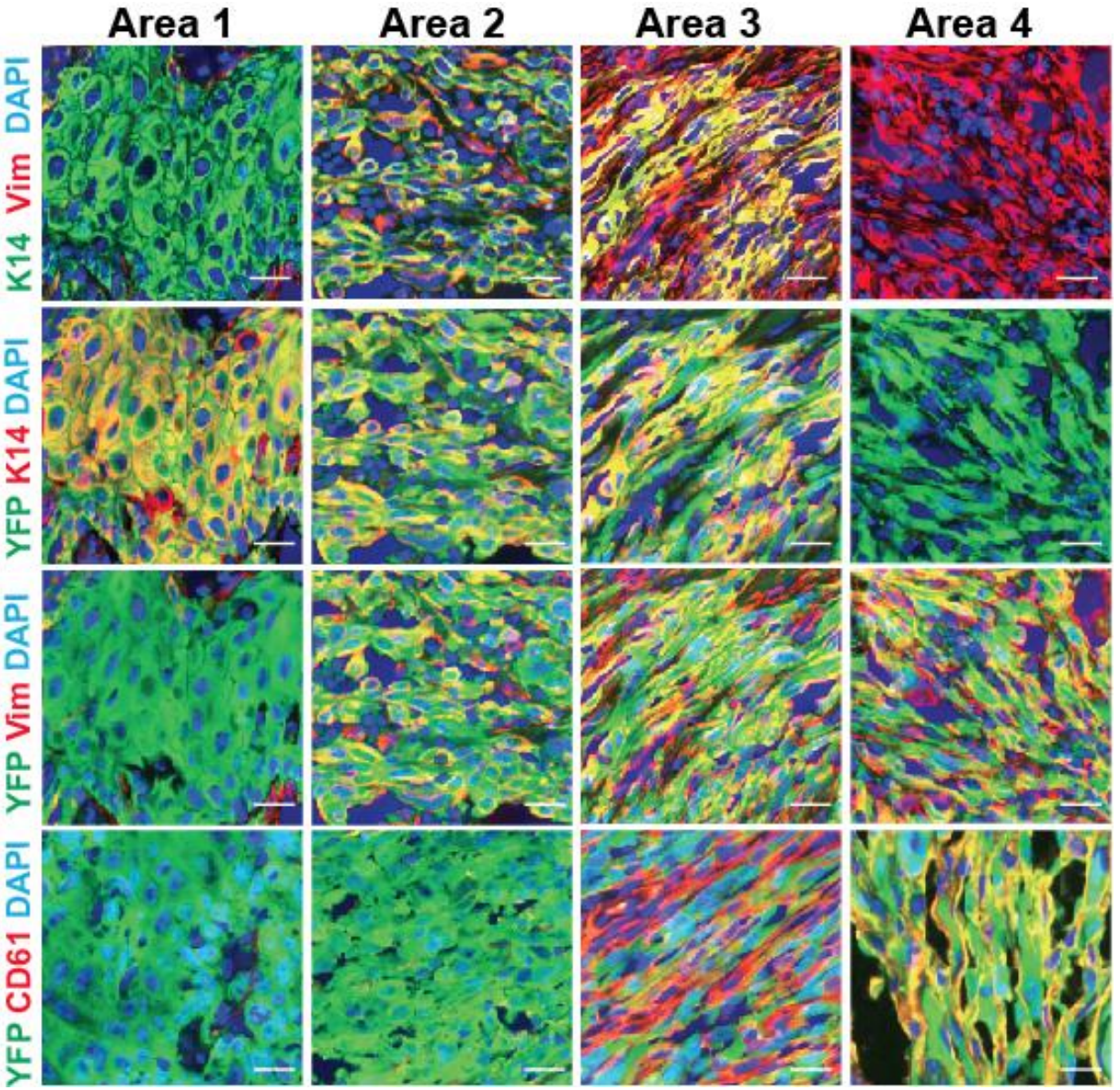
Lgr5/Kras/p53/
DNp63 IRES GFP



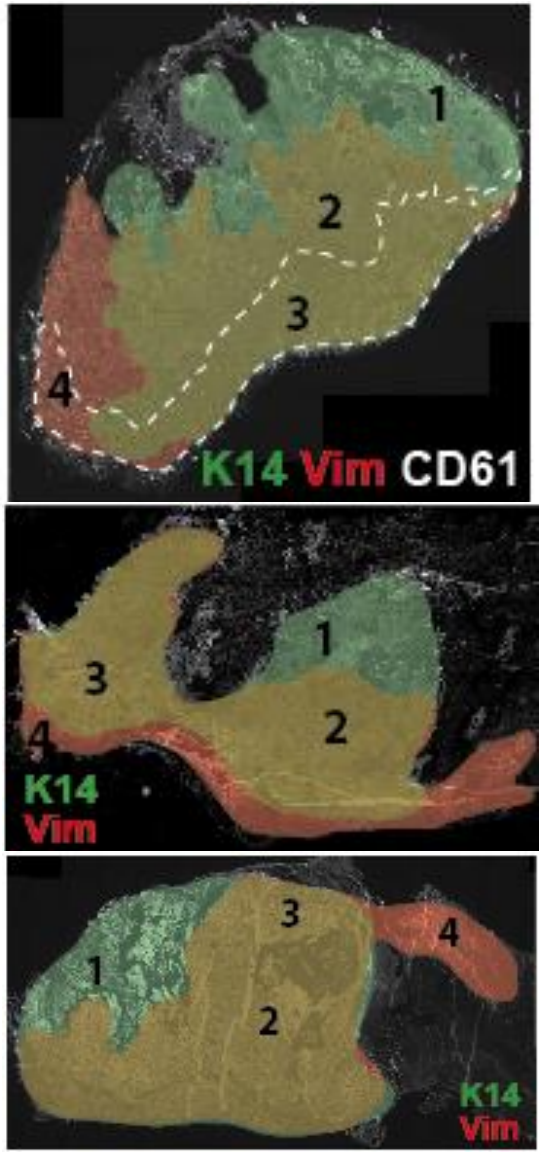


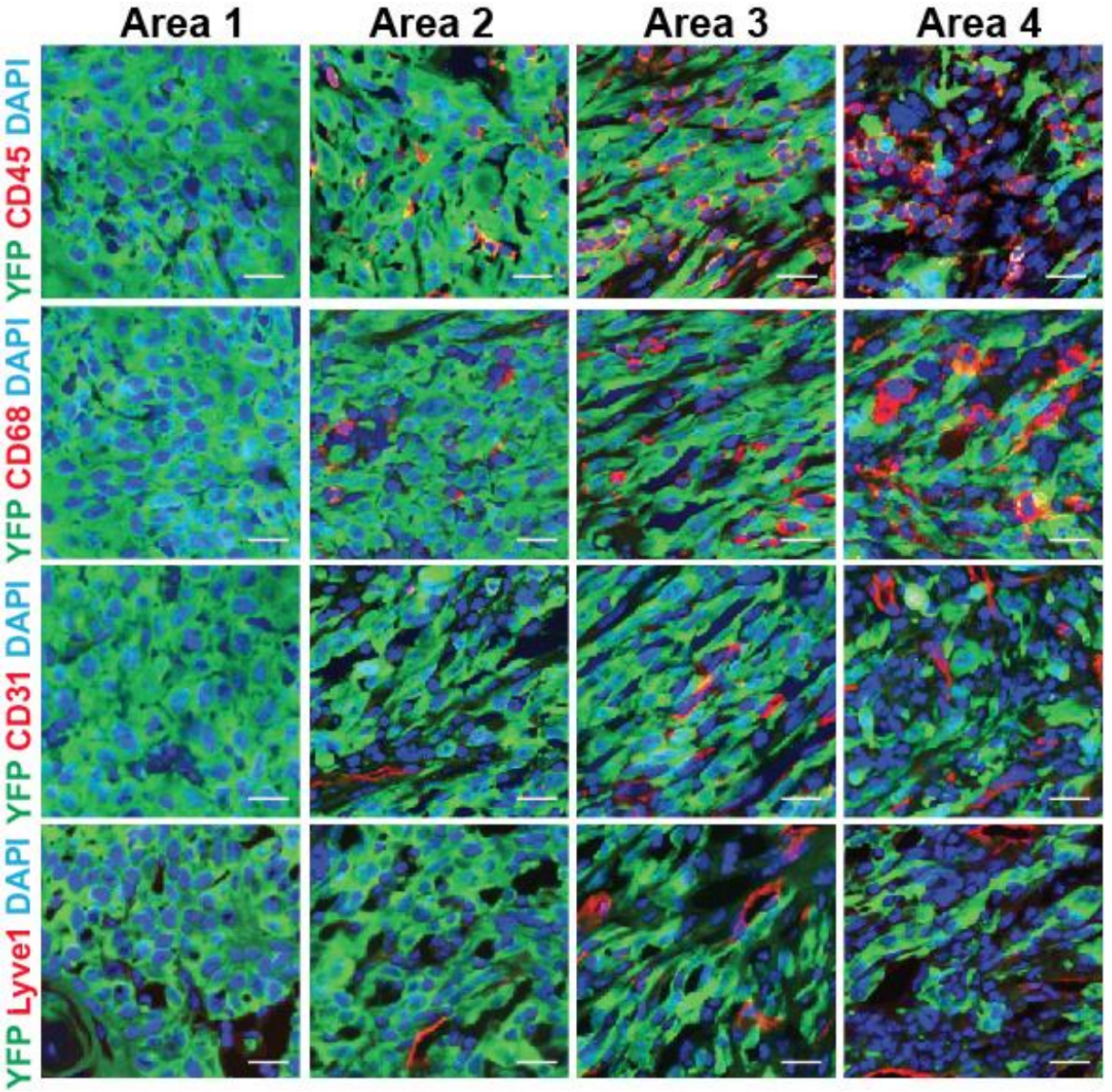
Different EMT transition states are localized in different niches



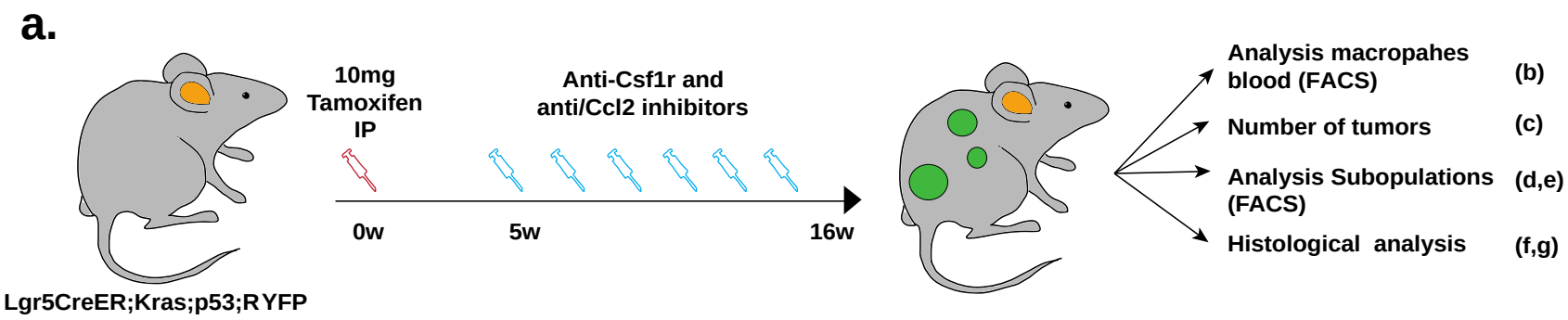


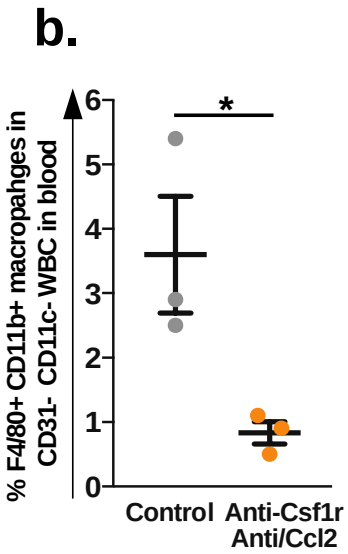
Different EMT niches are associated with different immune and vascular infiltration

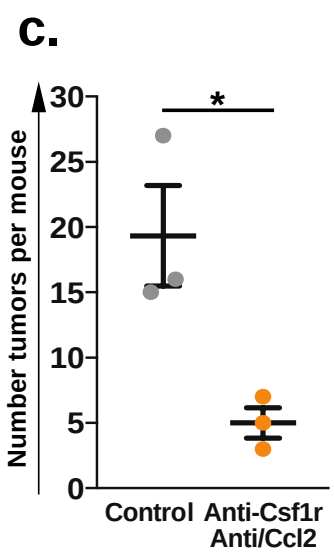


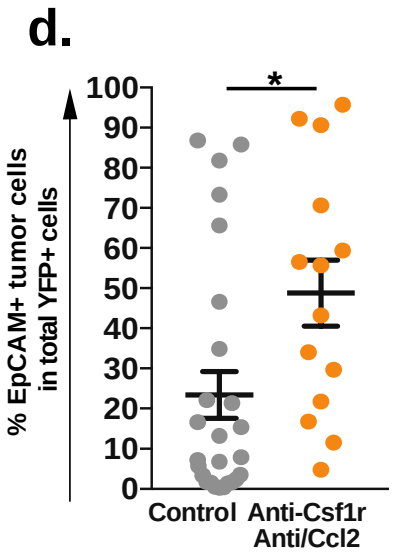


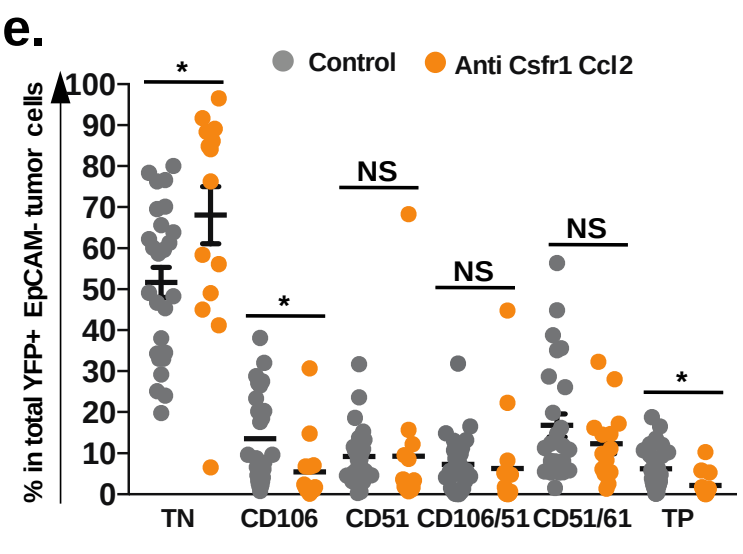
Macrophages regulate EMT transition states



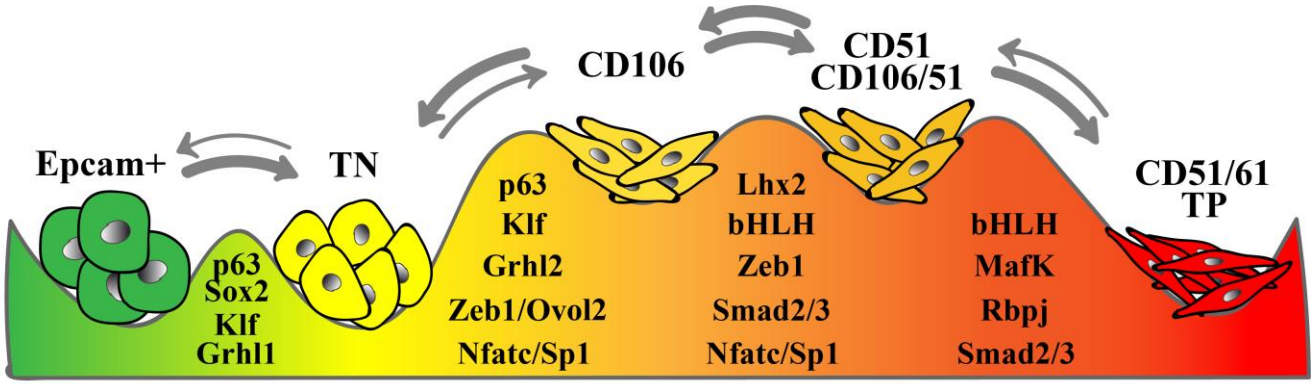








Transition through the different EMT states

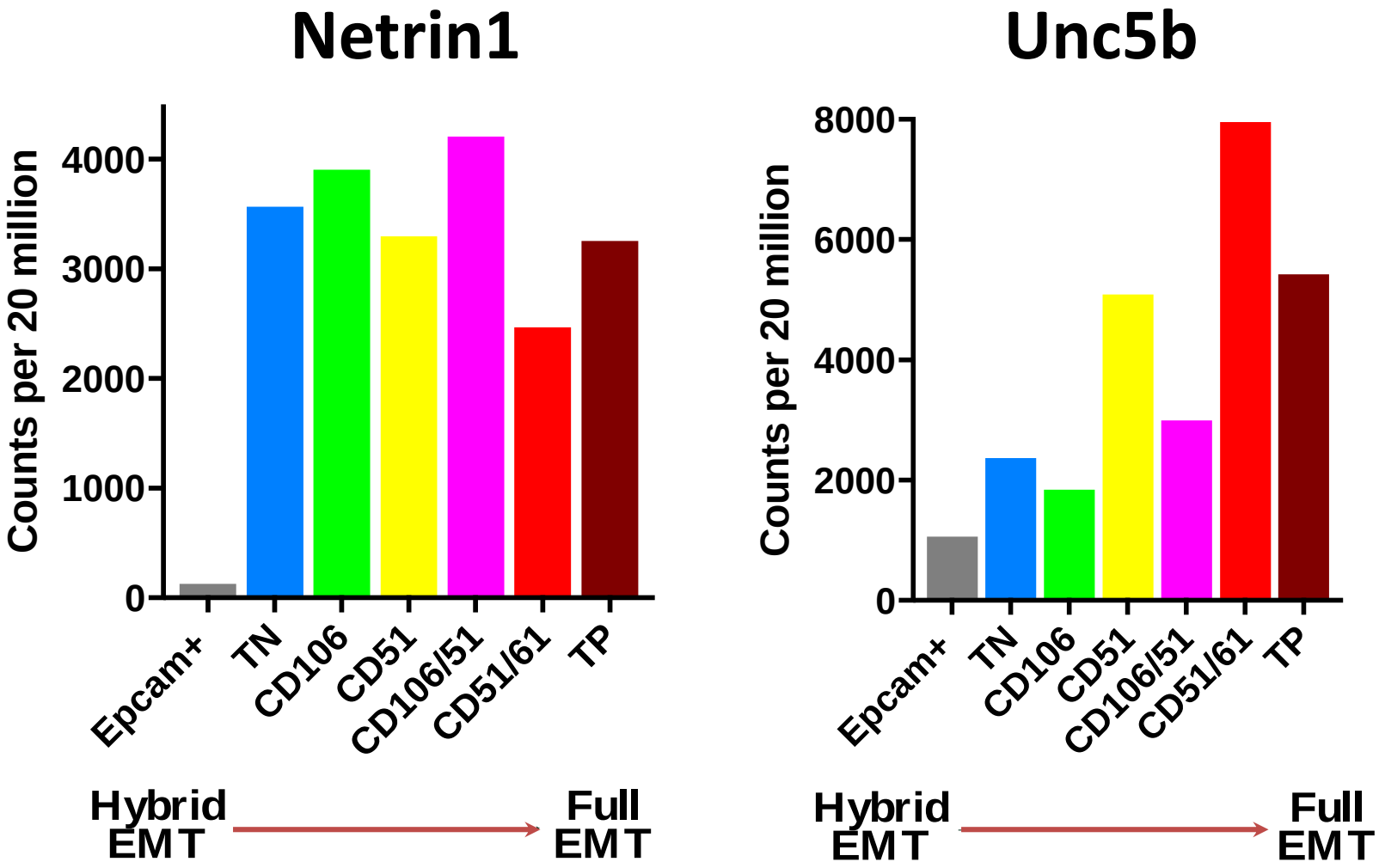


	Epithelial Tumor cells	Early Hybrid EMT state	Hybrid EMT state	Late Hybrid EMT state	Mesenchymal Tumor cells
Proliferation	++++	++	++	++	+
Invasion	+	++	+++	++++	+++++
Plasticity	+	++	+++	++++	++
Stemness	+	+++	+++	+++	+++
Metastasis	+	++++	++++	++	+

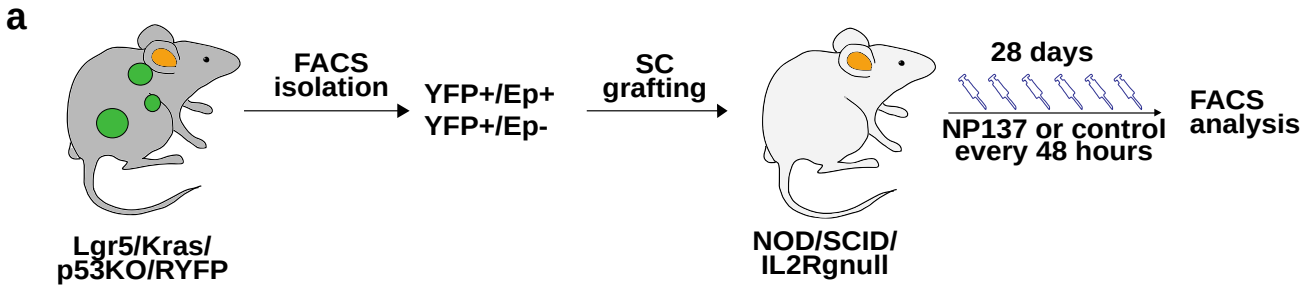
- EMT occurs through distinct transition states
- Early hybrid EMT exhibit the highest metastatic capacity
- Different transition states are associated with different microenvironment
- Depletion of macrophages prevents progression towards complete EMT

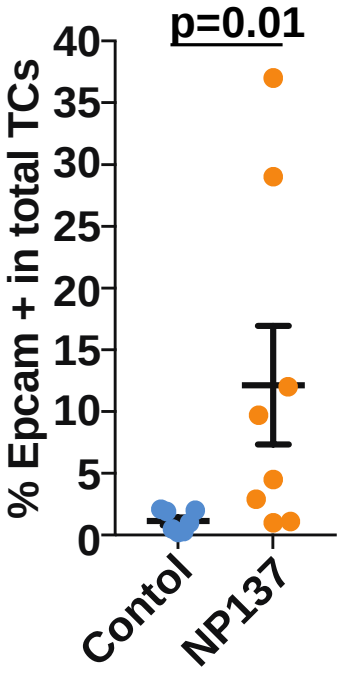
Pastushenko et al. Nature 2018

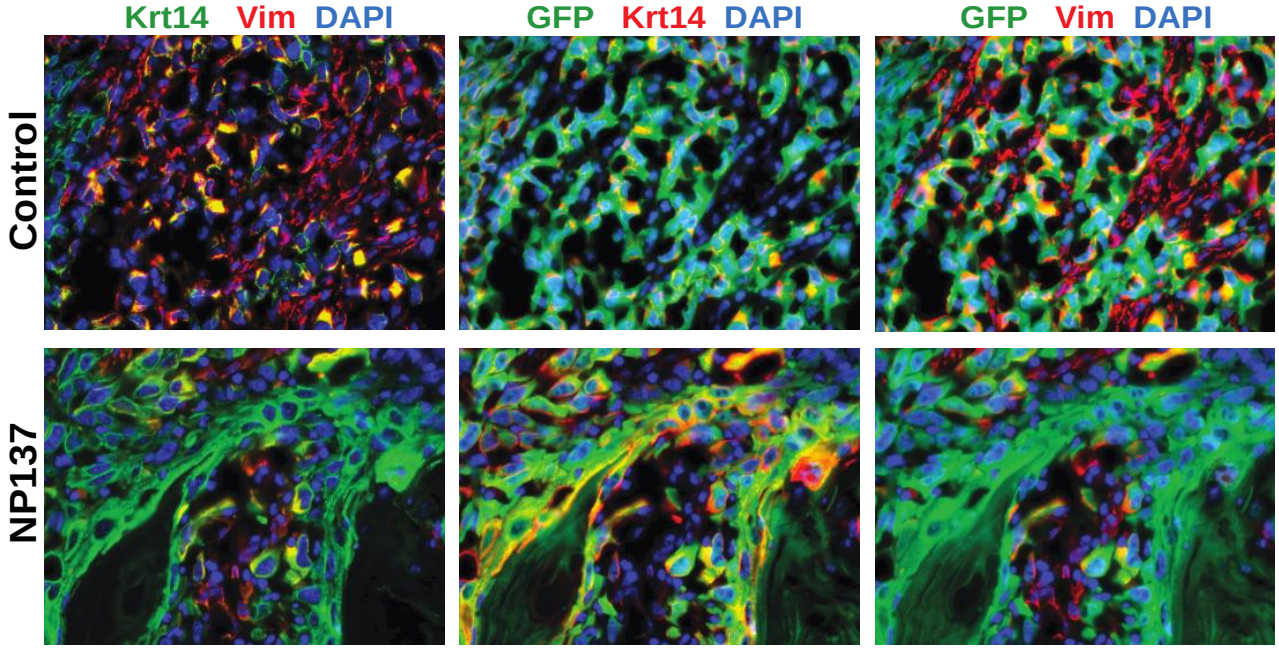
Role of Netrin-1 in EMT



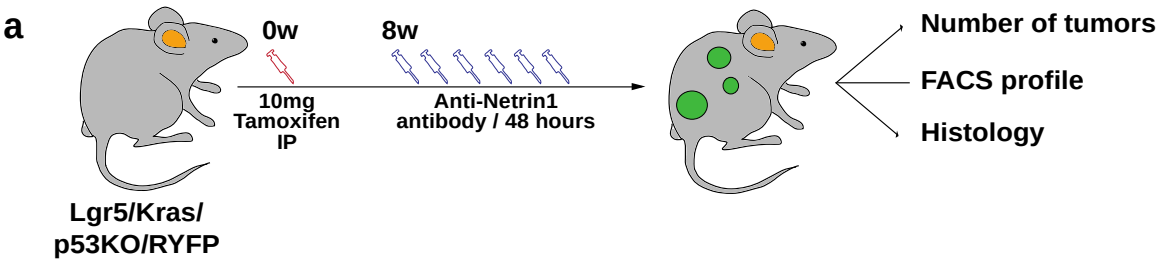
Netrin-1 inhibition reverts EMT upon subcutaneous grafting of Epcam- tumor cells

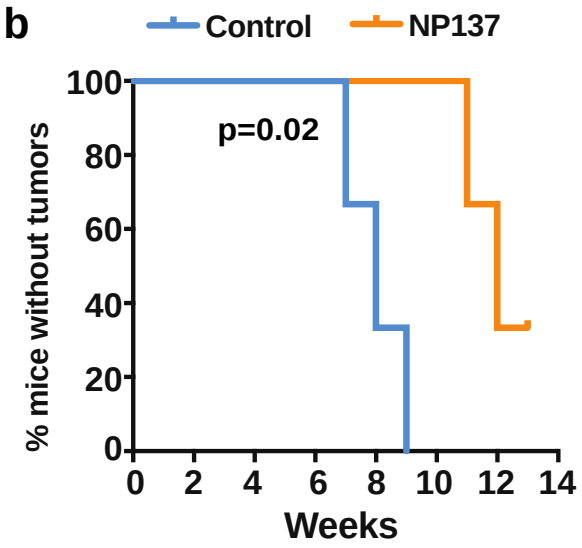


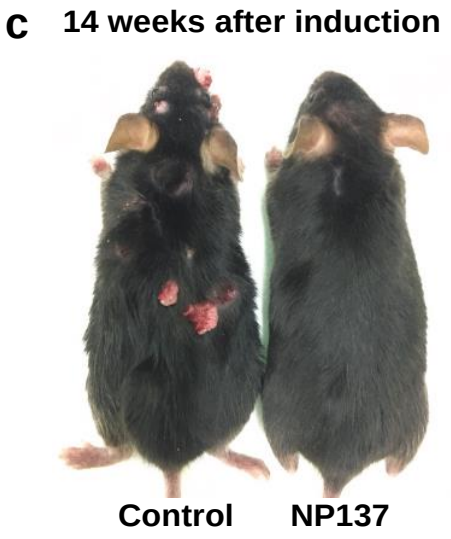


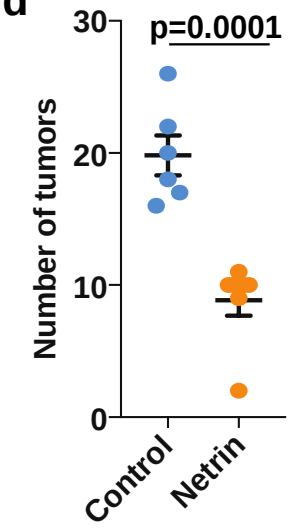


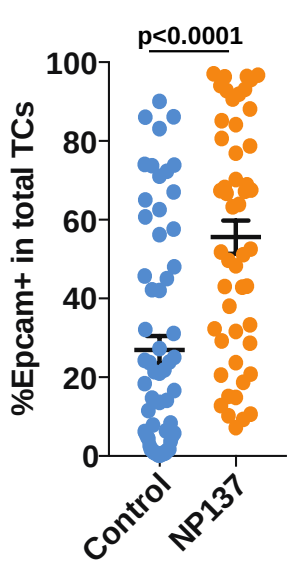
Netrin-1 inhibition reduces tumorigenesis and prevents EMT in primary skin SCC



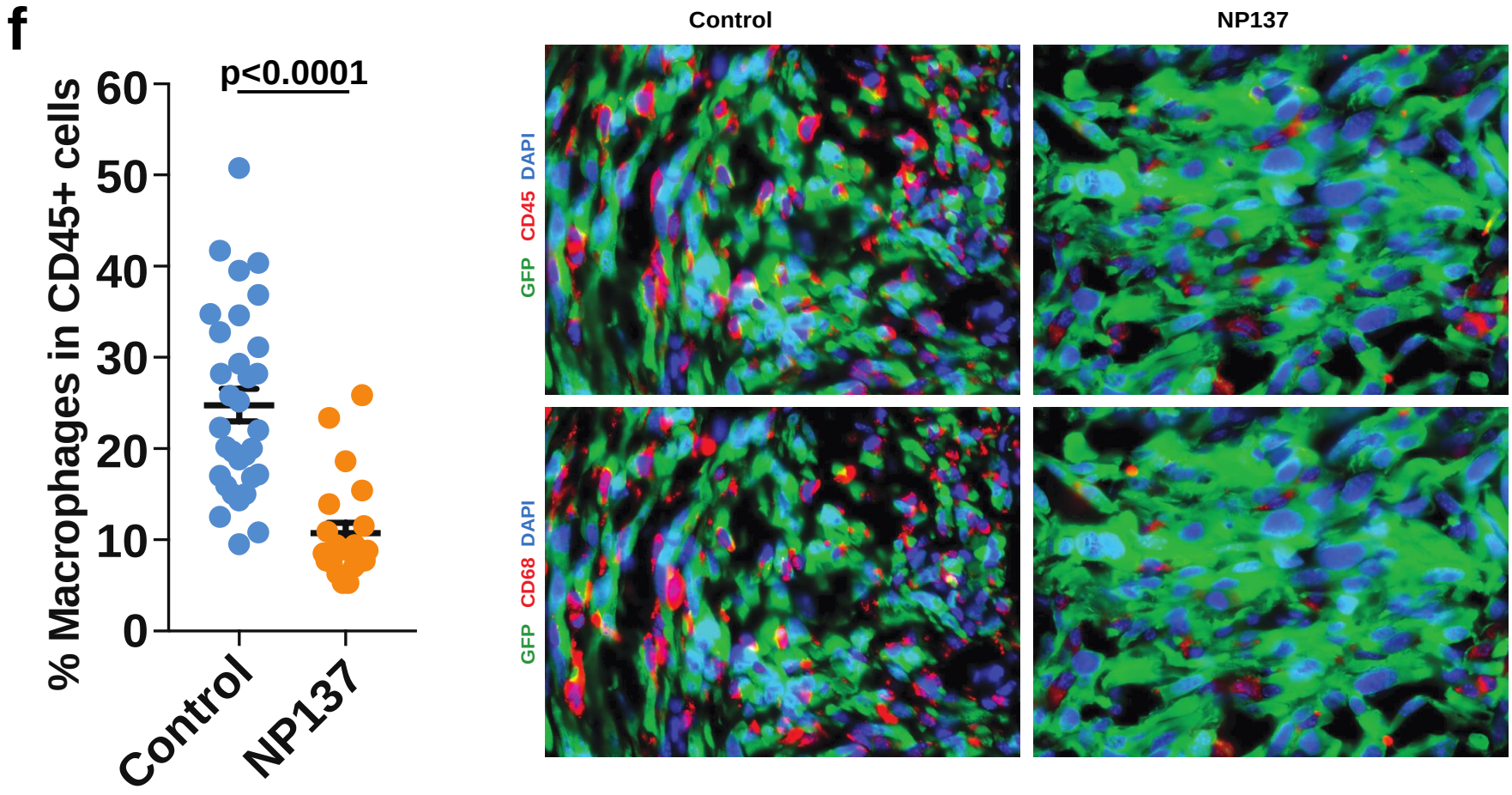




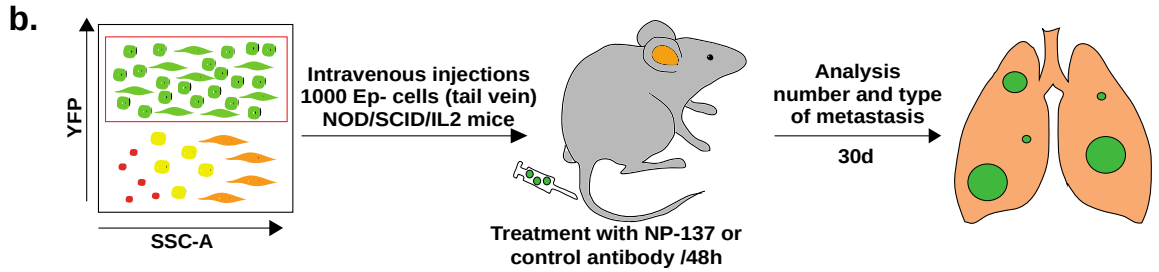


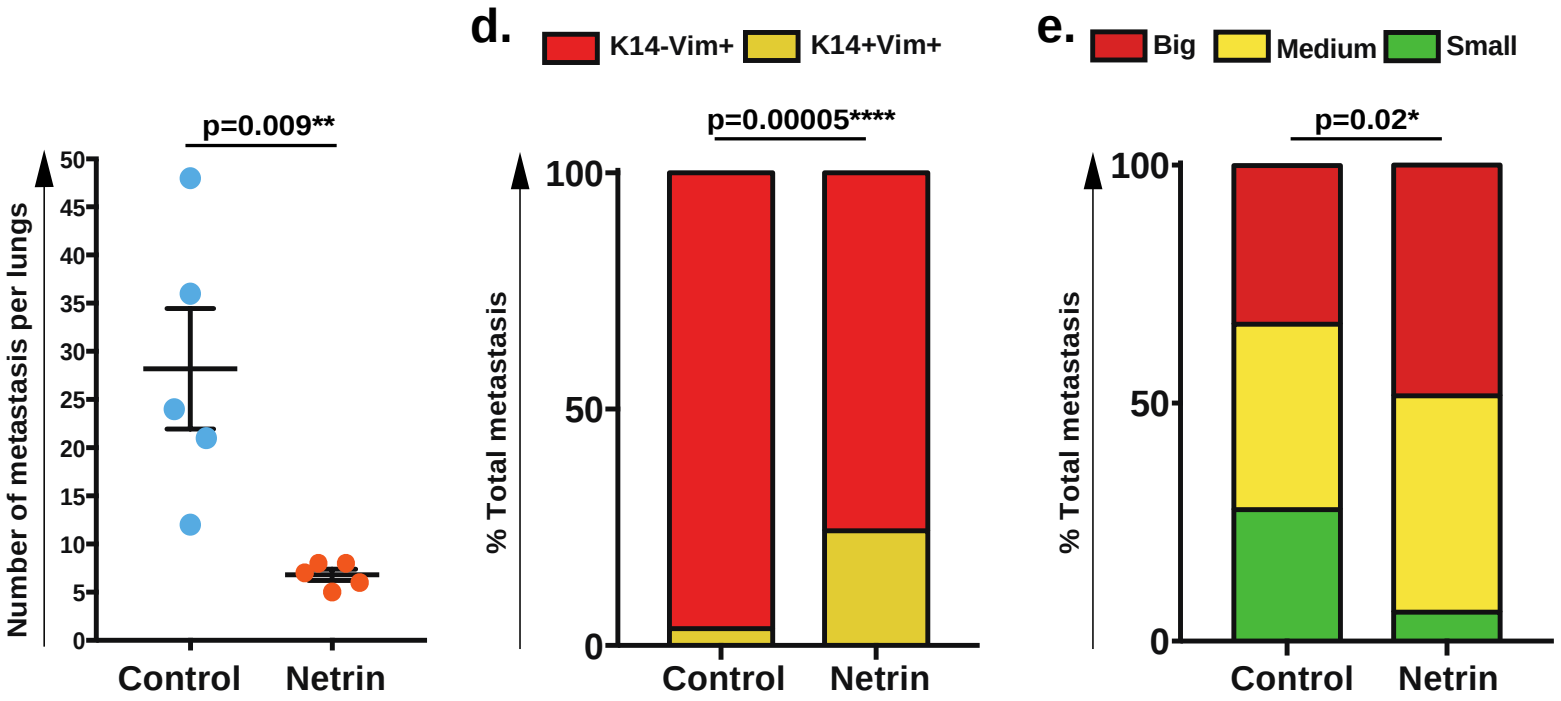


Netrin-1 inhibition reduces macrophage infiltration in primary skin SCC

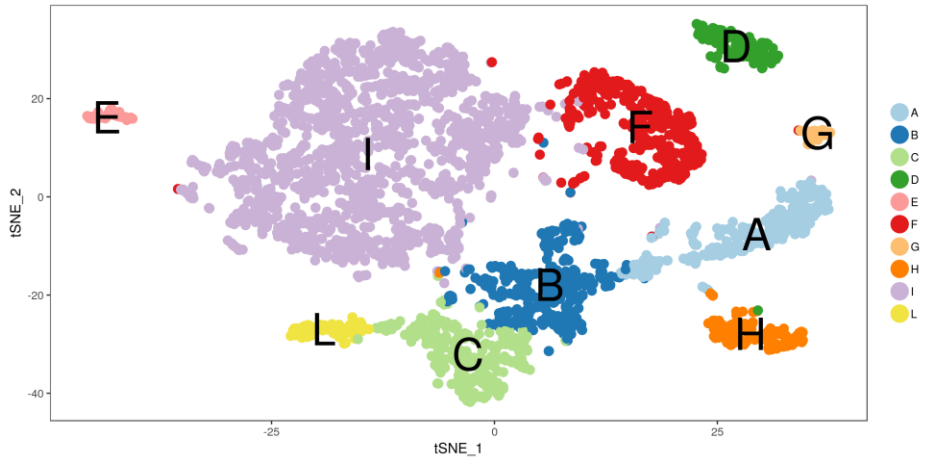


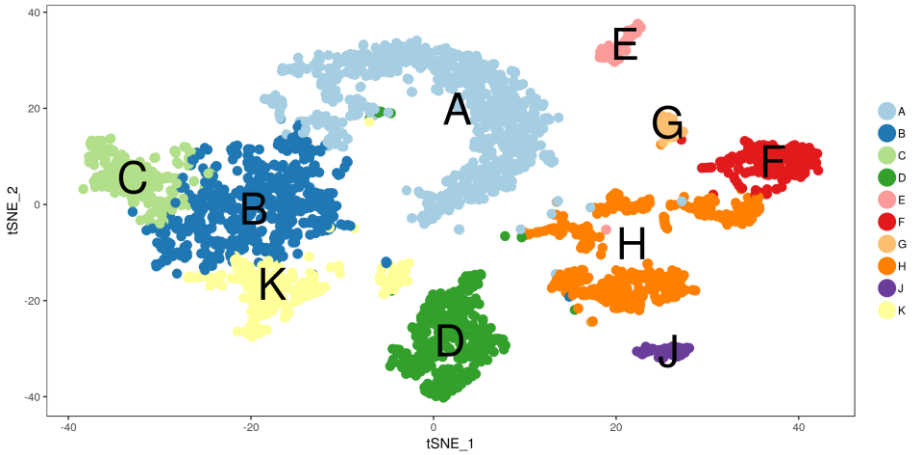
Netrin-1 inhibition prevents lung colonization upon intravenous injection of Epcam- TCs

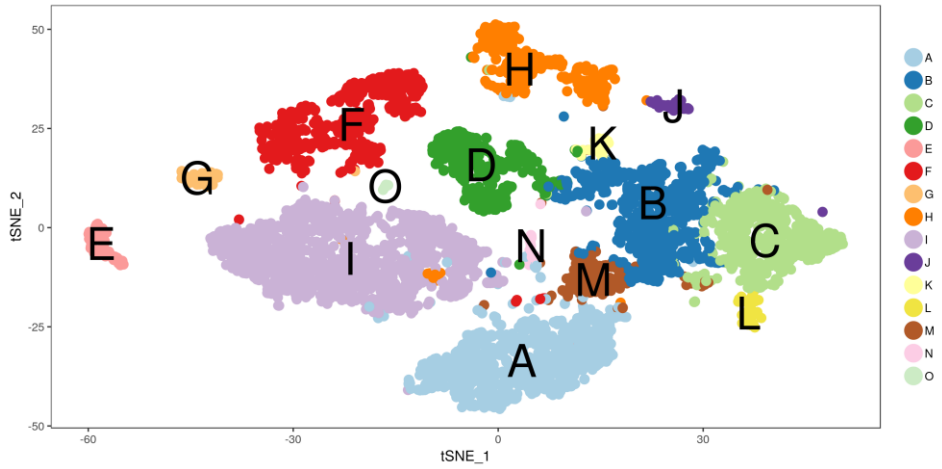




Regulation of EMT and metastasis through modulation of the microenvironment







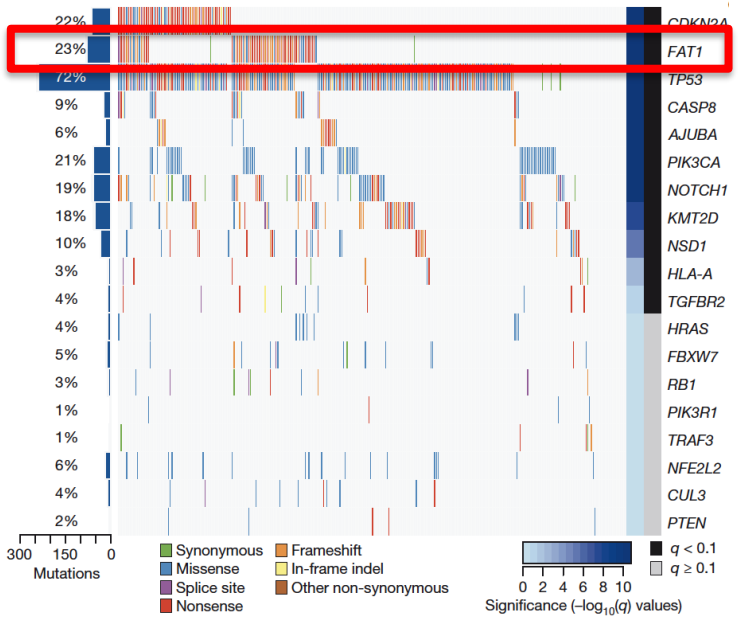
A	Neutrophils	I	Cancer (VCAM1)
B	DC/Macrophages	J	Melanocytes
C	M2 Macrophages	K	M1 Macrophages
D	T-Cells	L	Osteoclast markers
E	Endothelial	M	Cluster 9 macrophages (total)
F	Fibroblasts	N	NK
G	Fibroblasts (special)	O	Cluster 16 (total)
H	Cancer (EPCAM +)		

Pastushenko & Lengrand, unpublished data

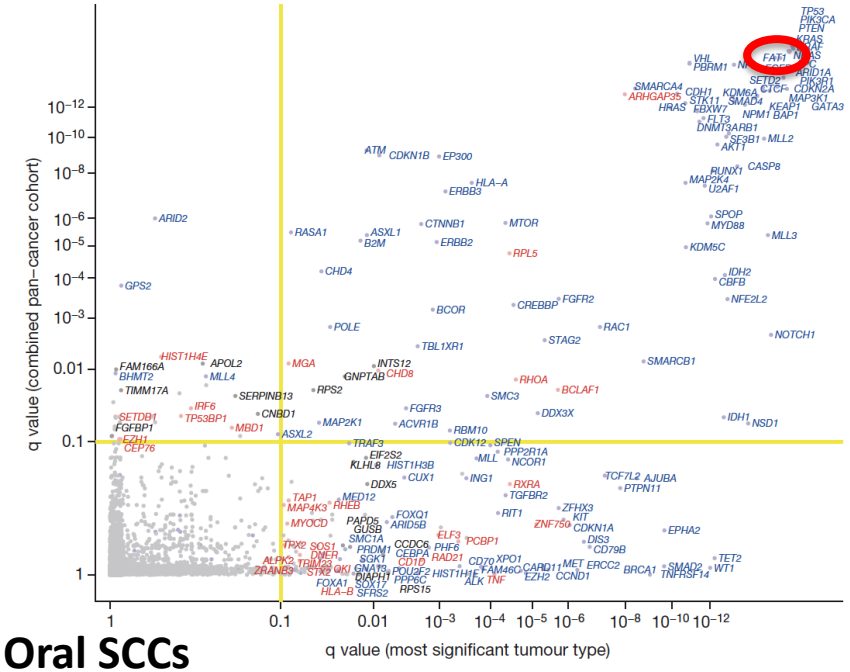
Can genetic hints stabilize specific EMT state?

Fat1 mutations in human cancers

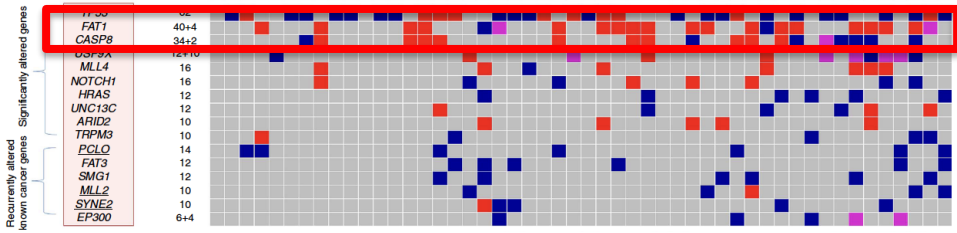
Head and neck SCCs



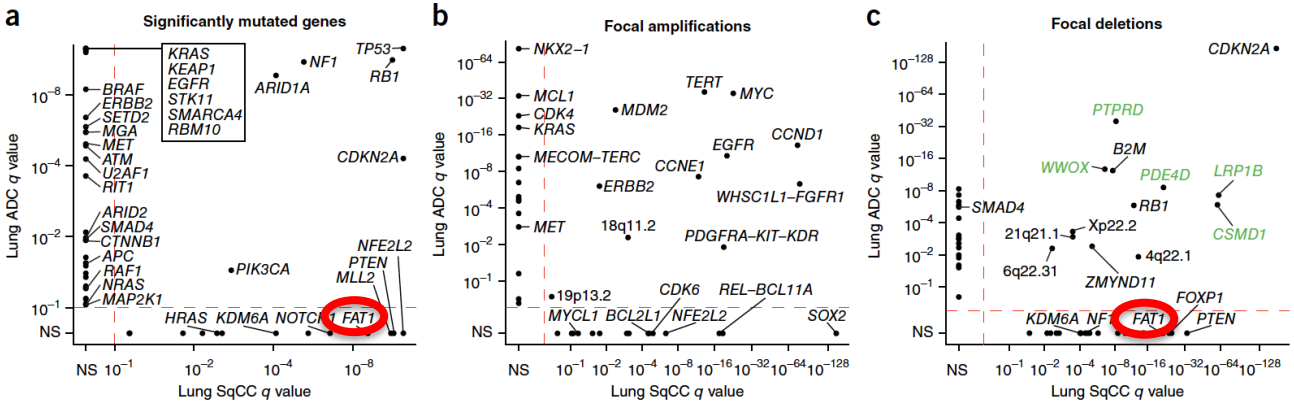
Pan Cancer



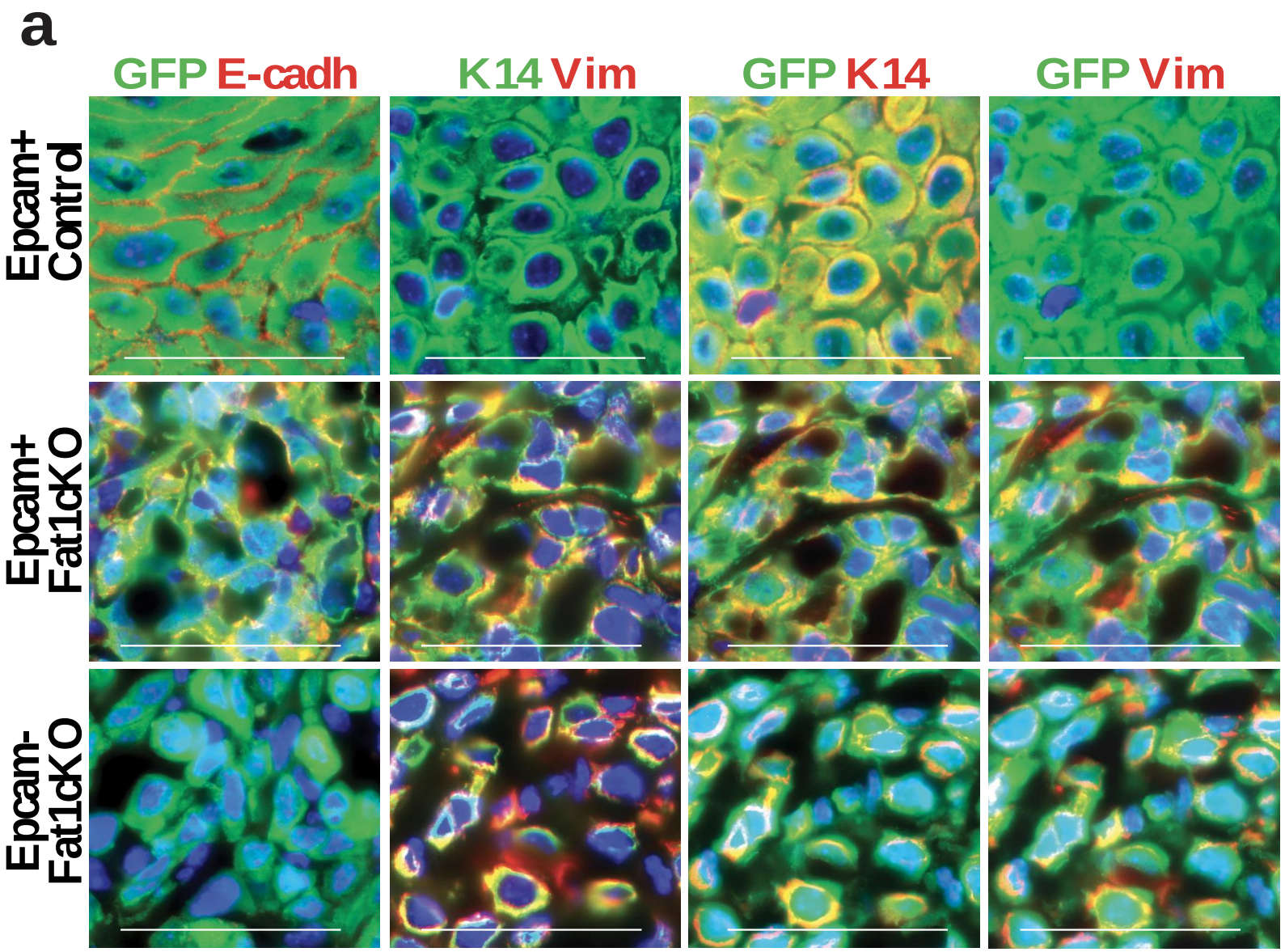
Oral SCCs



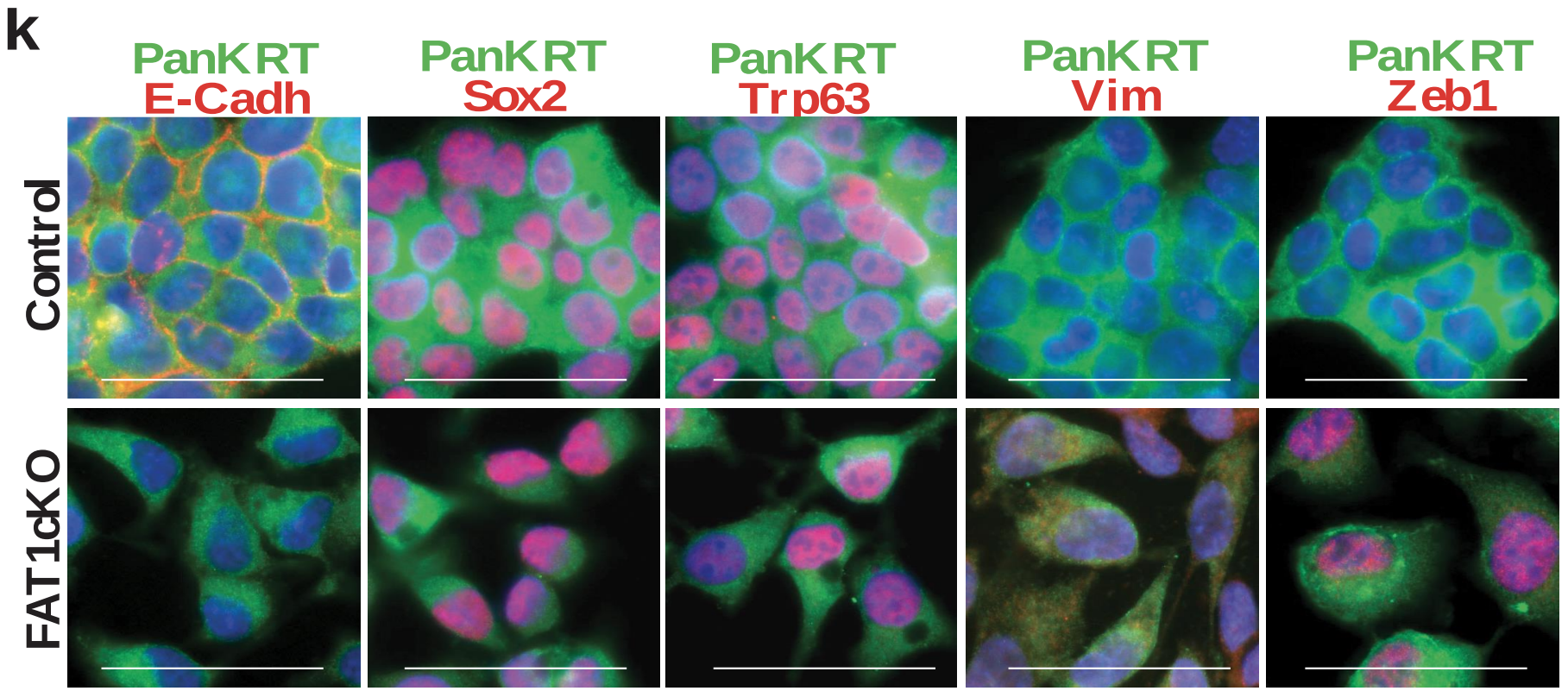
Lung SCCs



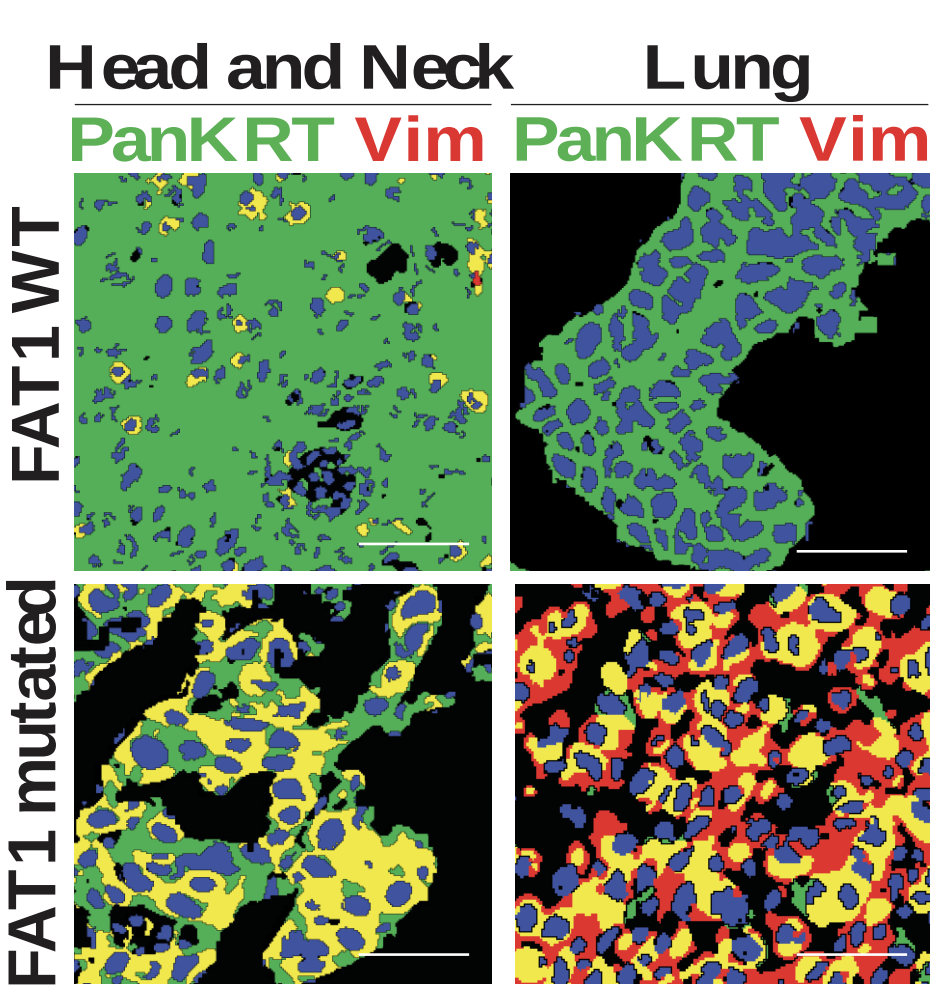
Fat1 deletion promotes hybrid EMT in SCC

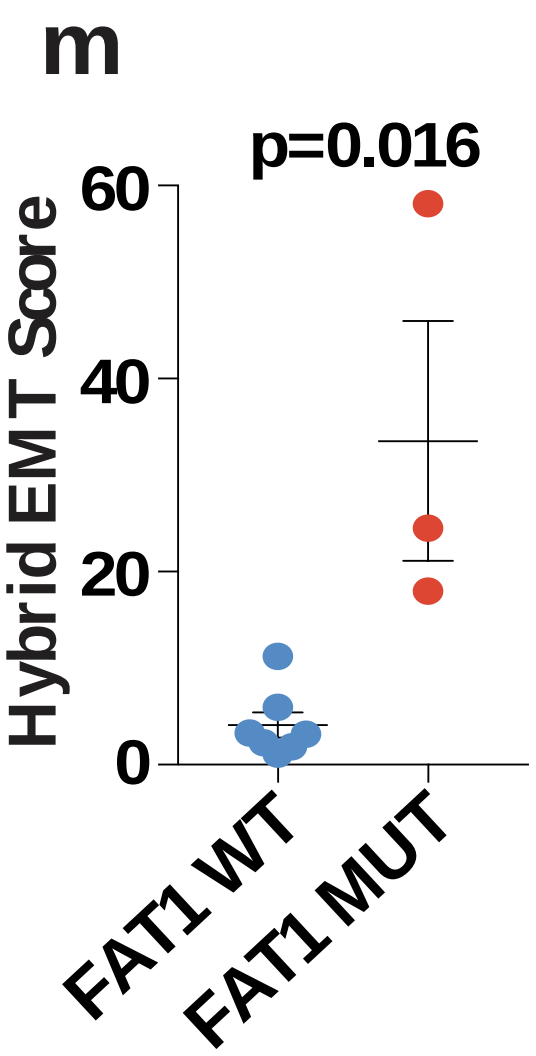


Deletion of Fat1 promotes hybrid EMT state in human SCCs

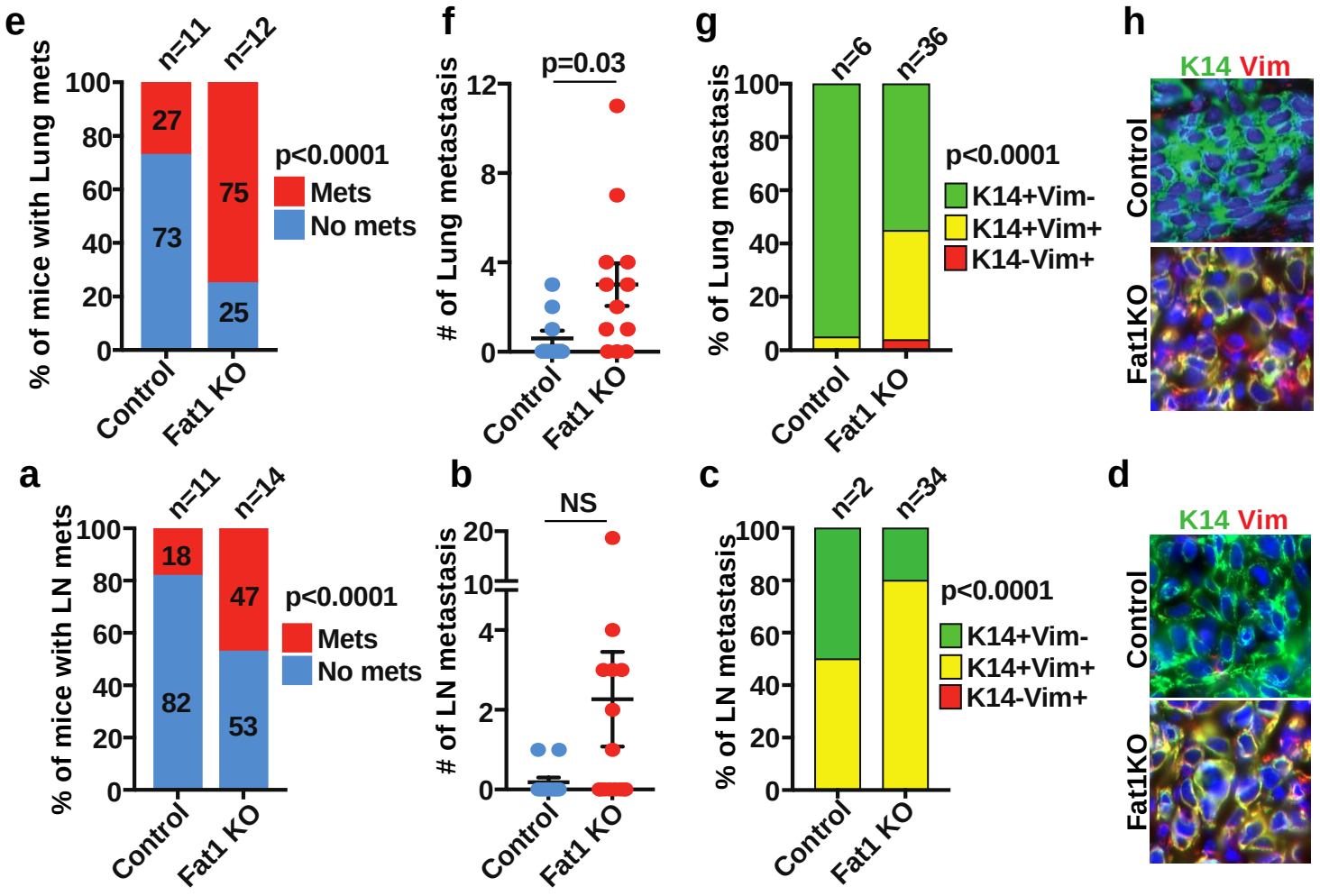


Somatic mutations in Fat1 is associated with hybrid EMT state in human cancers





Fat1 deletion increases lymph node and lung metastasis



Pastushenko et al, under review

- **EMT occurs through distinct transition states**
- **Early hybrid EMT exhibit the highest metastatic capacity**
- **Different transition states are associated with different microenvironment**
- **Depletion of macrophages prevents progression towards complete EMT**

- **Role of microenvironment in the modulation of EMT can be explored as therapeutic opportunity**
- **Specific mutations can stabilize highly metastatic hybrid EMT state and should be be explored as predictive factor of poor outcome**

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