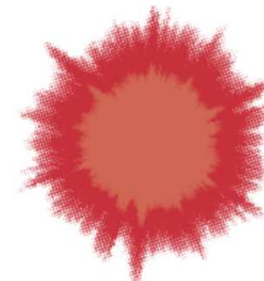


EMT during cancer initiation and progression

Lab: prof. Geert Berx

Dr. Bram De Craene

American Biotechnology and Life-Science Trade Mission
October 21 2104



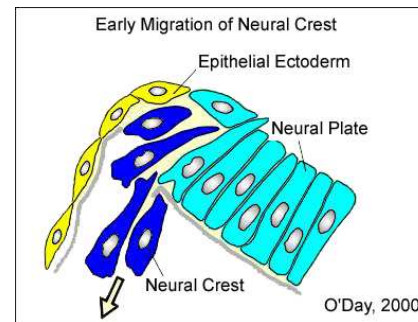
IRC
A VIB-UGENT DEPARTMENT

Epithelial to Mesenchymal Transition (EMT)

EMT is a morphogenetic process in which cells lose their epithelial characteristics and gain mesenchymal properties

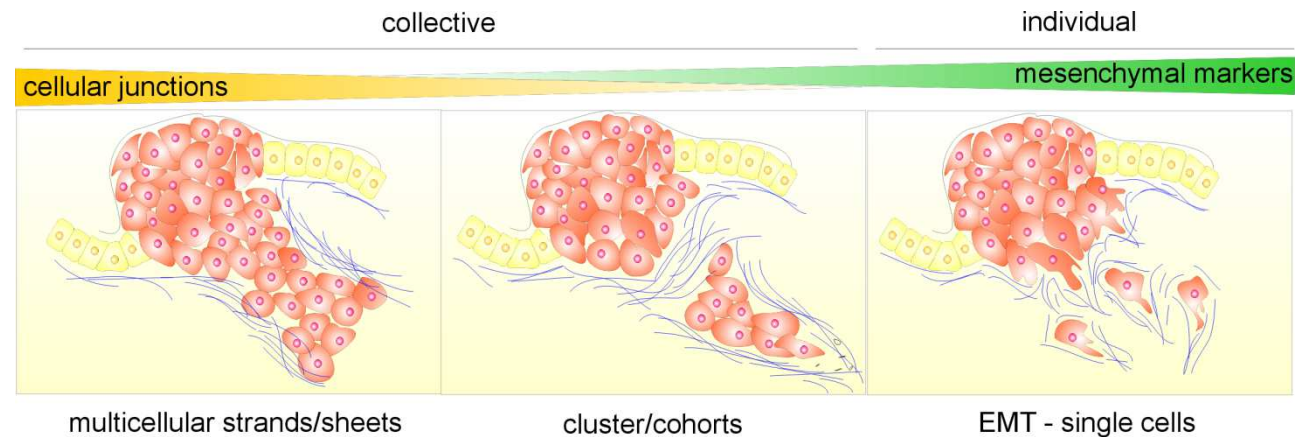
Normal regulation during development/adulthood

- Neural crest
- Mesoderm
- Palate
- Cartilage
- Wound healing

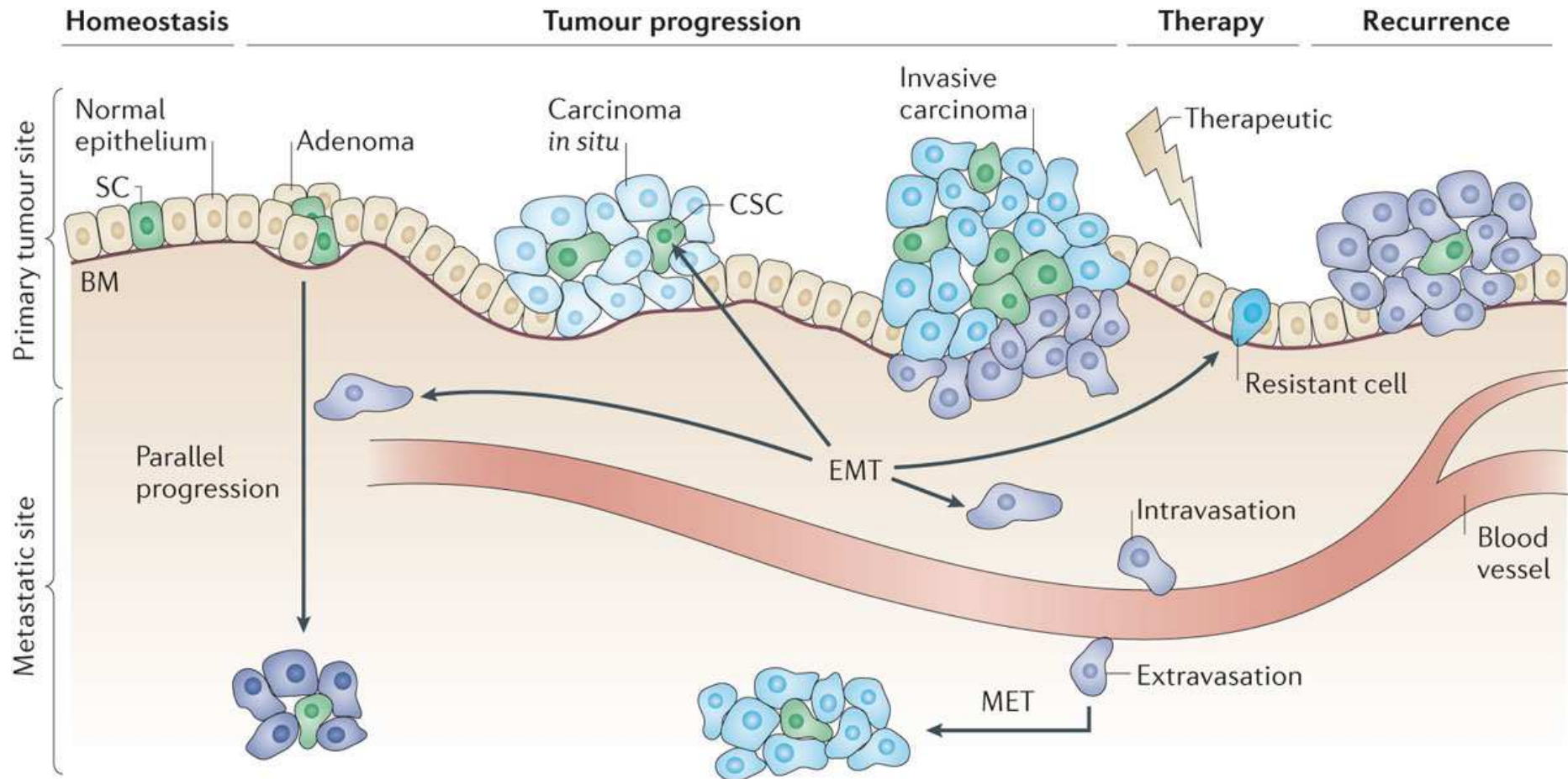


Aberrant regulation late in development or adult:

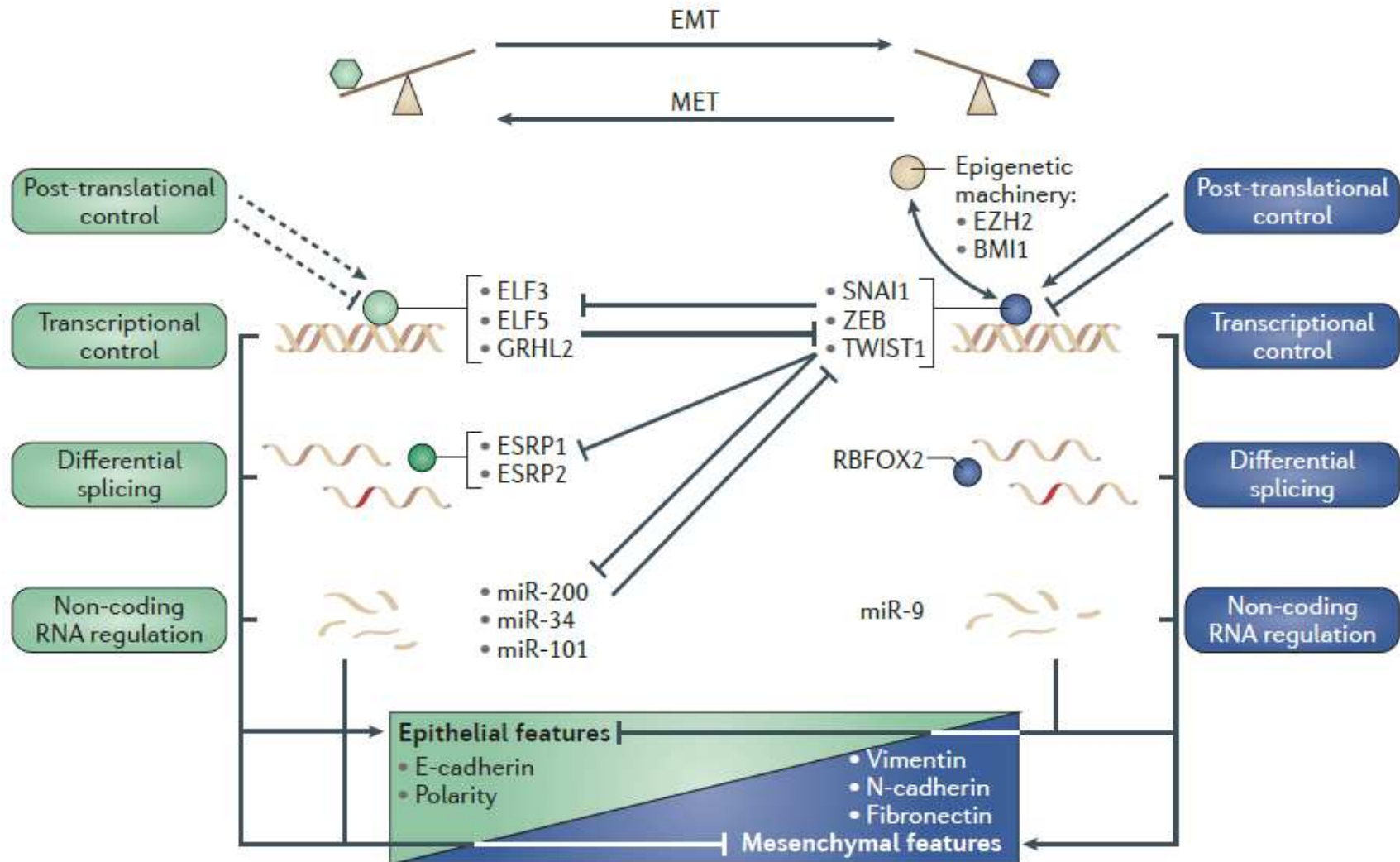
- Tumour malignancy, invasive tumour cells
- Fibrosis
- Drug resistance
- Leukemias



Role of EMT during cancer progression

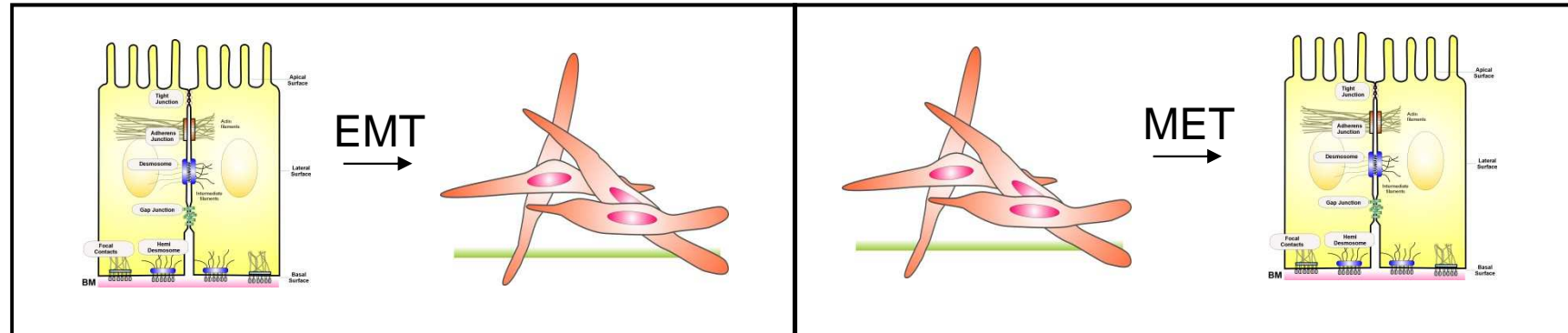


Regulatory levels of EMT



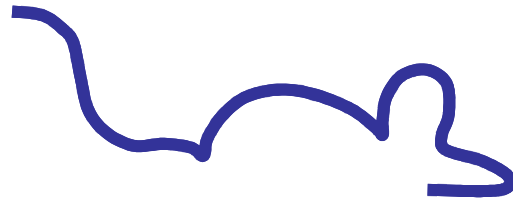
Study of molecular mechanisms of EMT driven cancer progression

In vitro



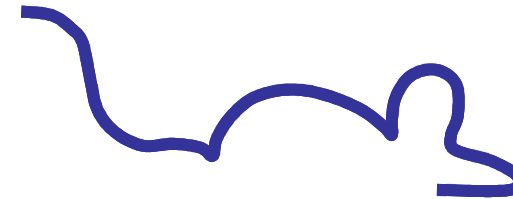
In vivo

EMT and skin cancer



K14-EMT mice

EMT and homeostasis/disease



ROSA-EMT mice

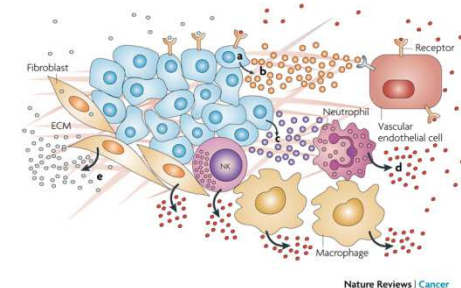
Microenvironment

Tool development

Cp-values	gen	cancer Inducte			EMT Inducte			p-values
		stai 1	stai 2	stai 3	stai 1	stai 2	stai 3	
31.19	Cx2c							0.015142
34.93	Cd34-Pr01							0.025228
36.99	Et1br							0.025993
38.04	Stat1							0.098819
37.35	H1n							0.000796
38.24	Fluc							0.043569
31.15	Mbl1							0.027599
31.64	Bcl2							0.056484

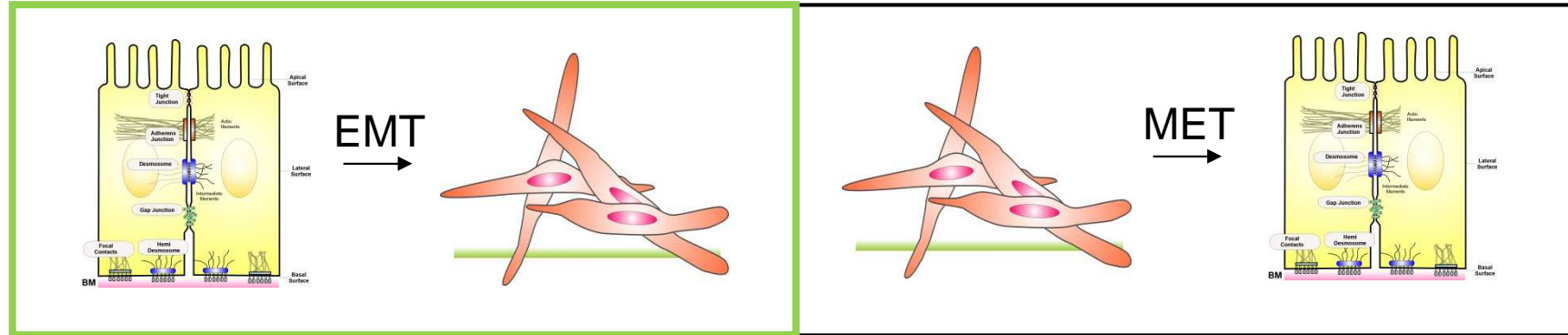


Study of EMT – tumour stroma interface



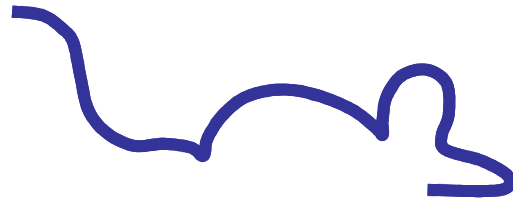
Study of molecular mechanisms of EMT driven cancer progression

In vitro



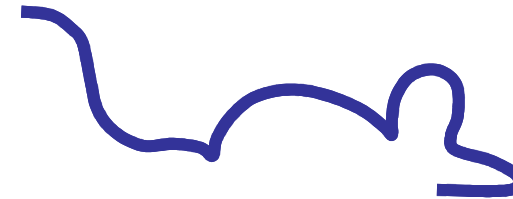
In vivo

EMT and skin cancer



K14-EMT mice

EMT and homeostasis/disease



ROSA-EMT mice

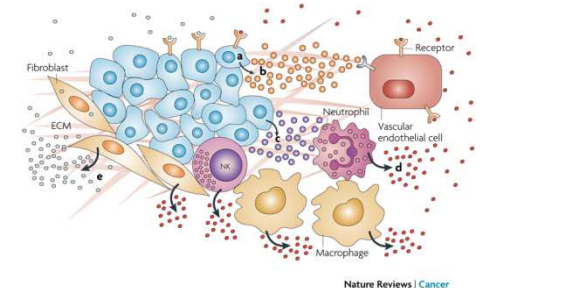
Microenvironment

Tool development

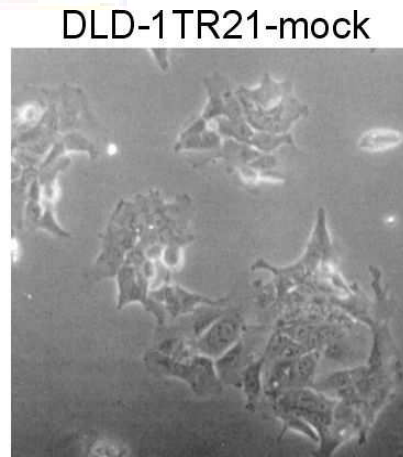
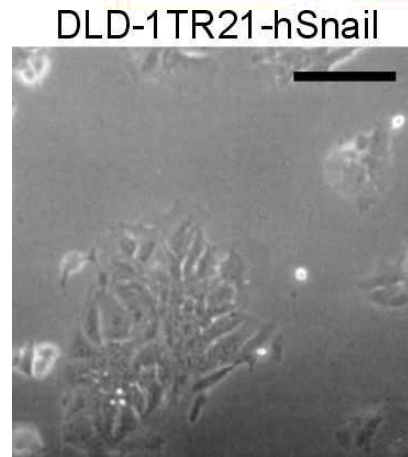
Cp-values	gen	cancer inducible			EMT inducible			p-values
		stad 1	stad 2	stad 3	stad 1	stad 2	stad 3	
31.19	CcG							0.015142
34.93	Cd38 (Pdgfr)							0.025228
36.99	Et1br							0.025993
38.04	Stat1							0.008819
37.35	Itin							0.000798
38.24	Itic							0.043569
31.15	Mbl1							0.027599
31.64	Itat							0.004884



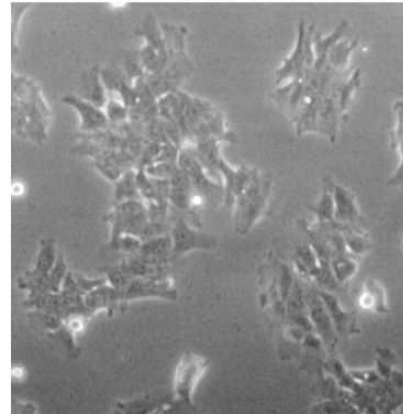
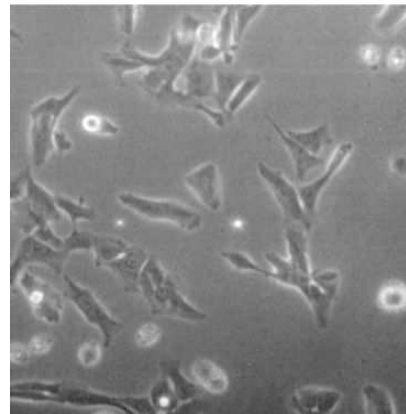
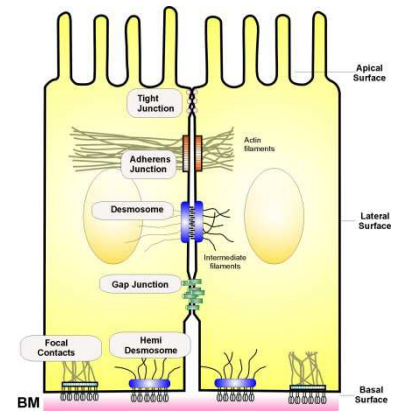
Study of EMT – tumour stroma interface



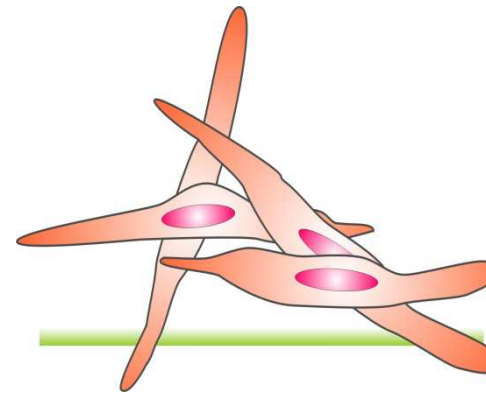
In vitro: Colorectal cancer cells with inducible expression of hSnail or hSlug



-Dox



+Dox

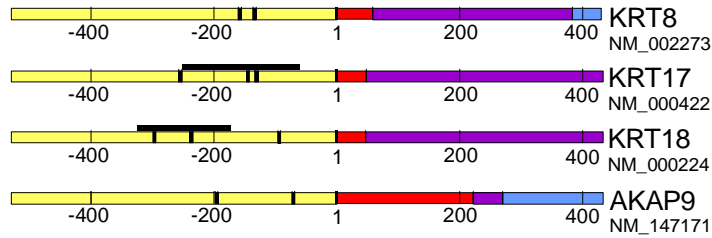


De Craene *et al.*, 2005

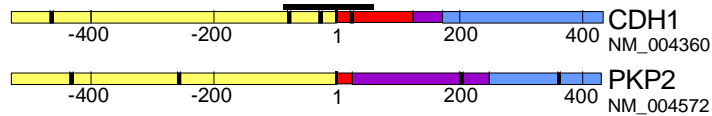
Identification of hSnail target genes by ChIP analysis

Epithelial differentiation

Cytoskeletal organization

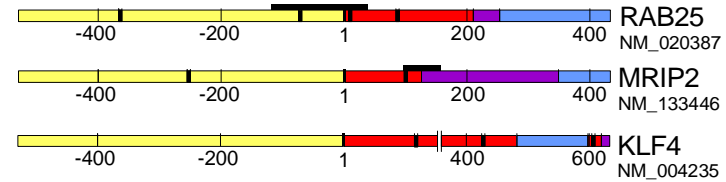


Cell adhesion

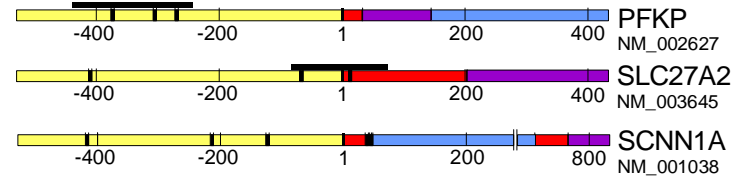


■ promoter region
■ 5' UTR
■ ORF

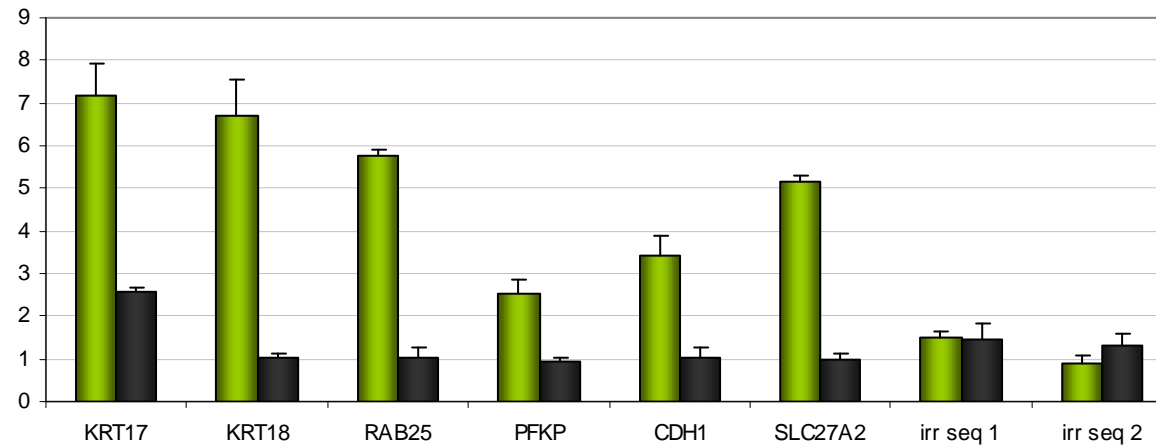
Signaling



Metabolism



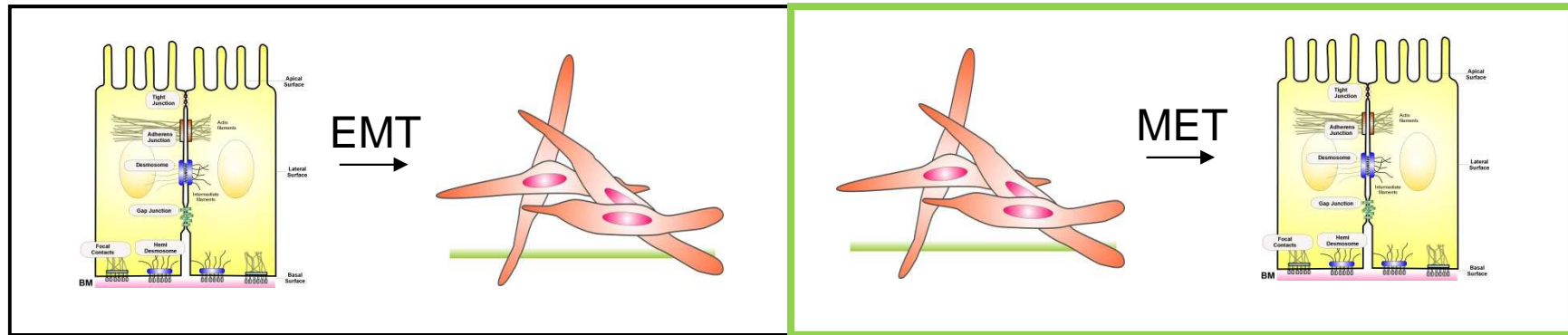
■ E-box CACCTG or CAGGTG
— amplicon in ChIP analysis



■ DLD-1TR21-EGFP-hSnail ■ DLD-1TR21-EGFP

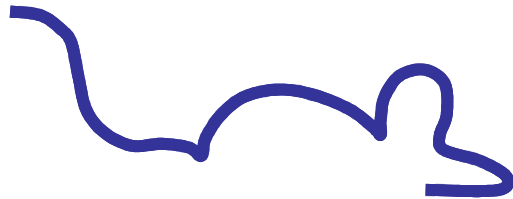
Study of molecular mechanisms of EMT driven cancer progression

In vitro



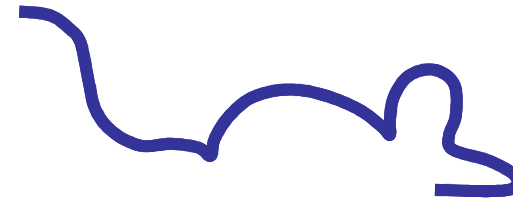
In vivo

EMT and skin cancer



K14-EMT mice

EMT and homeostasis/disease



ROSA-EMT mice

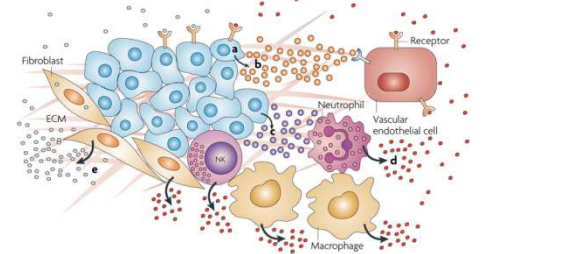
Microenvironment

Tool development

Cp-values	gen	TGFβ Inductia			TGFβ Inhibitor			p-values
		stad 1	stad 2	stad 3	stad 1	stad 2	stad 3	
31.19	CcG							0.015142
24.93	Col5A1-Prom1							0.025228
26.99	β1-Int							0.025993
26.04	Shaf1							0.008819
27.25	Itih1							0.000796
33.24	Itih5							0.043569
31.15	Mbl1							0.027599
31.64	Itih4							0.005484

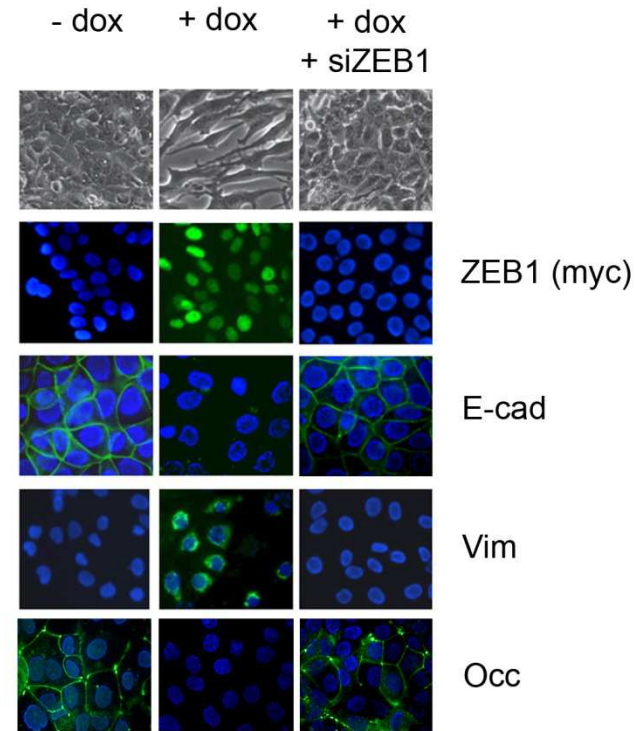
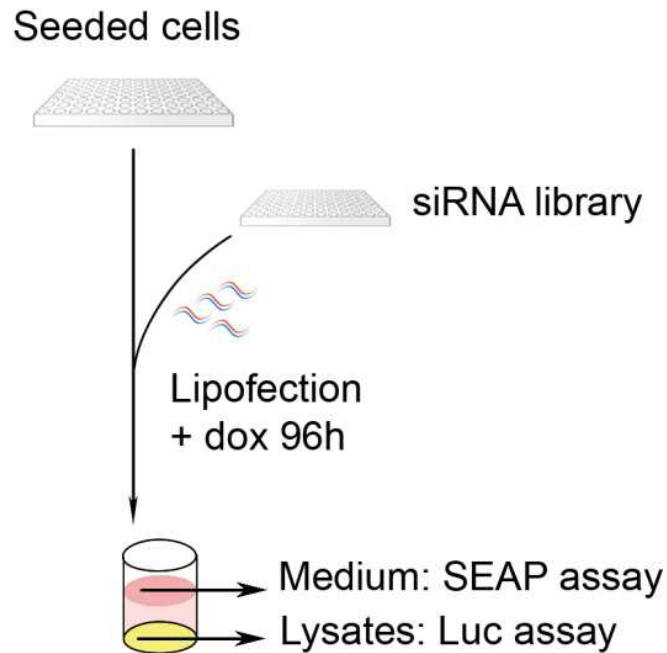


Study of EMT – tumour stroma interface

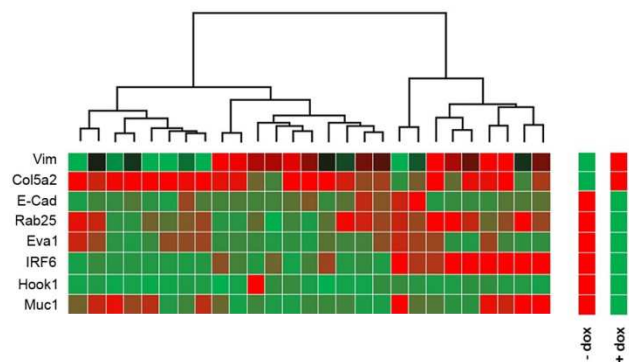


Construction of *in vitro* screening system for functional EMT interactors

EMT sensor based on luciferase reporter gene driven by the vimentin promoter



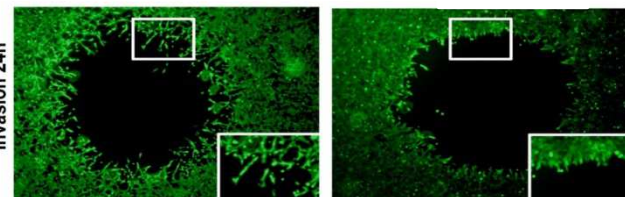
qPCR analysis



migration assay

shcon

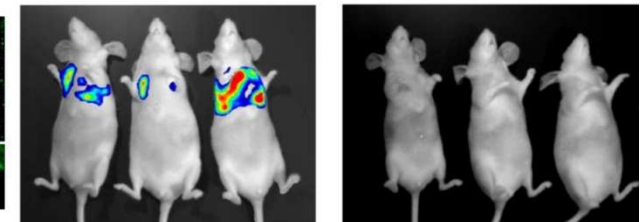
shTG



experimental metastasis

shcon

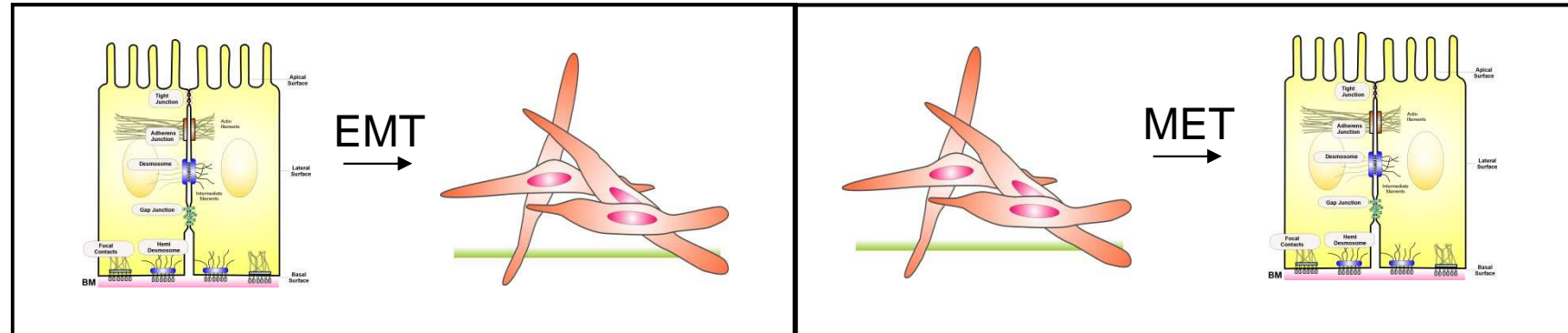
shTG



Gheldof *et al.*, in preparation

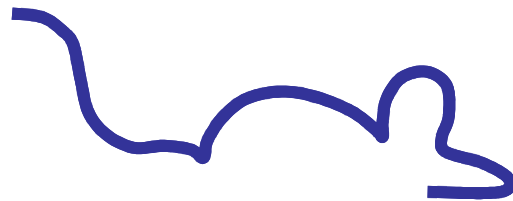
Study of molecular mechanisms of EMT driven cancer progression

In vitro



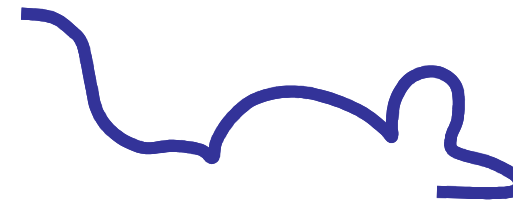
In vivo

EMT and skin cancer



K14-EMT mice

EMT and homeostasis/disease



ROSA-EMT mice

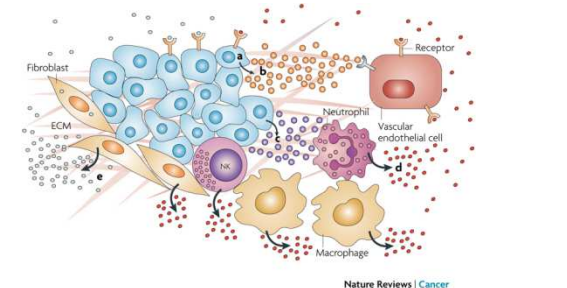
Microenvironment

Tool development

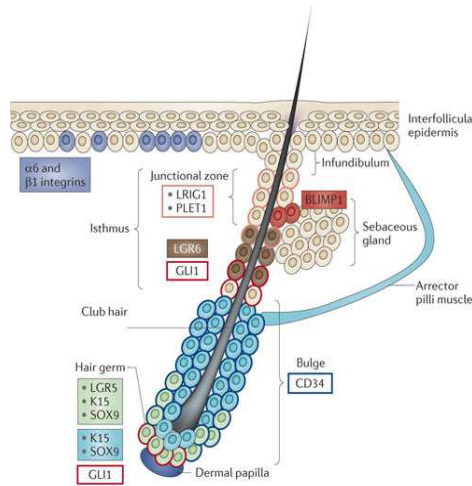
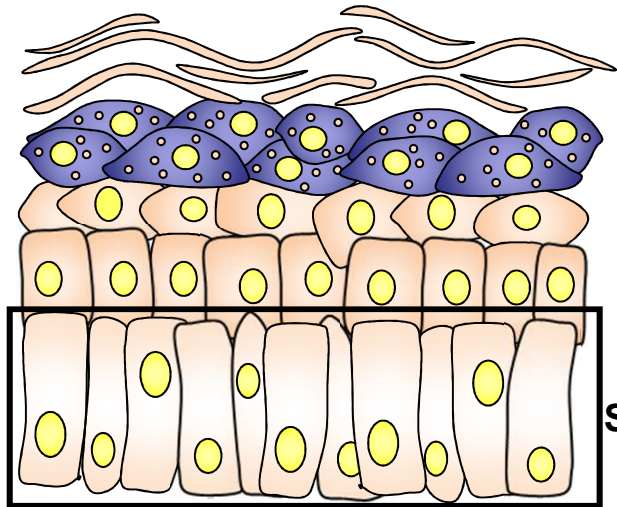
Cp-values	gen	cancer inducible			EMT inducible			p-values
		stad 1	stad 2	stad 3	stad 1	stad 2	stad 3	
31.19	CcG							0.015142
34.93	Cd34-PRO1							0.025228
36.99	Et1ba							0.025993
38.04	Shaf1							0.008819
37.35	H1m							0.000798
38.24	Foxc							0.043569
31.16	Mbl1							0.027599
31.64	Bfat							0.005484



Study of EMT – tumour stroma interface

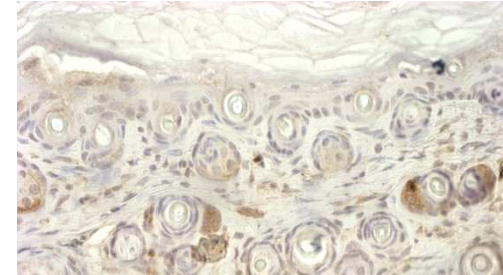


Mouse model: K14-driven overexpression of Snail: K14-Snail mice

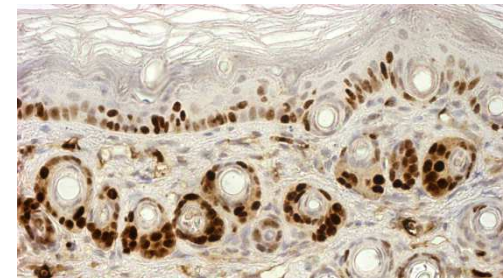


Arwert et al., 2012

HA-tag (Snail)



WT

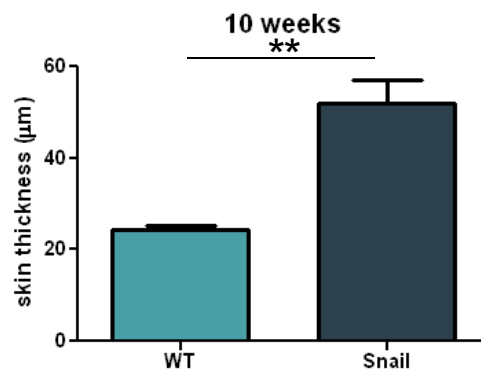


Snail

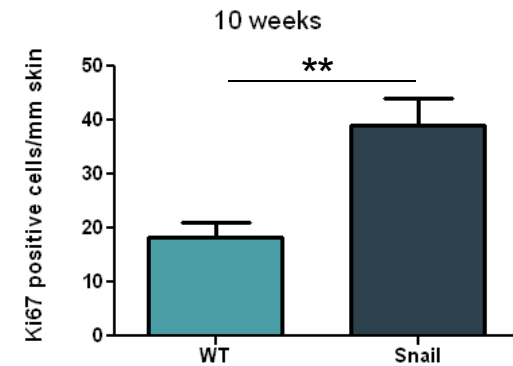
K14 → Snail (HA-tag)



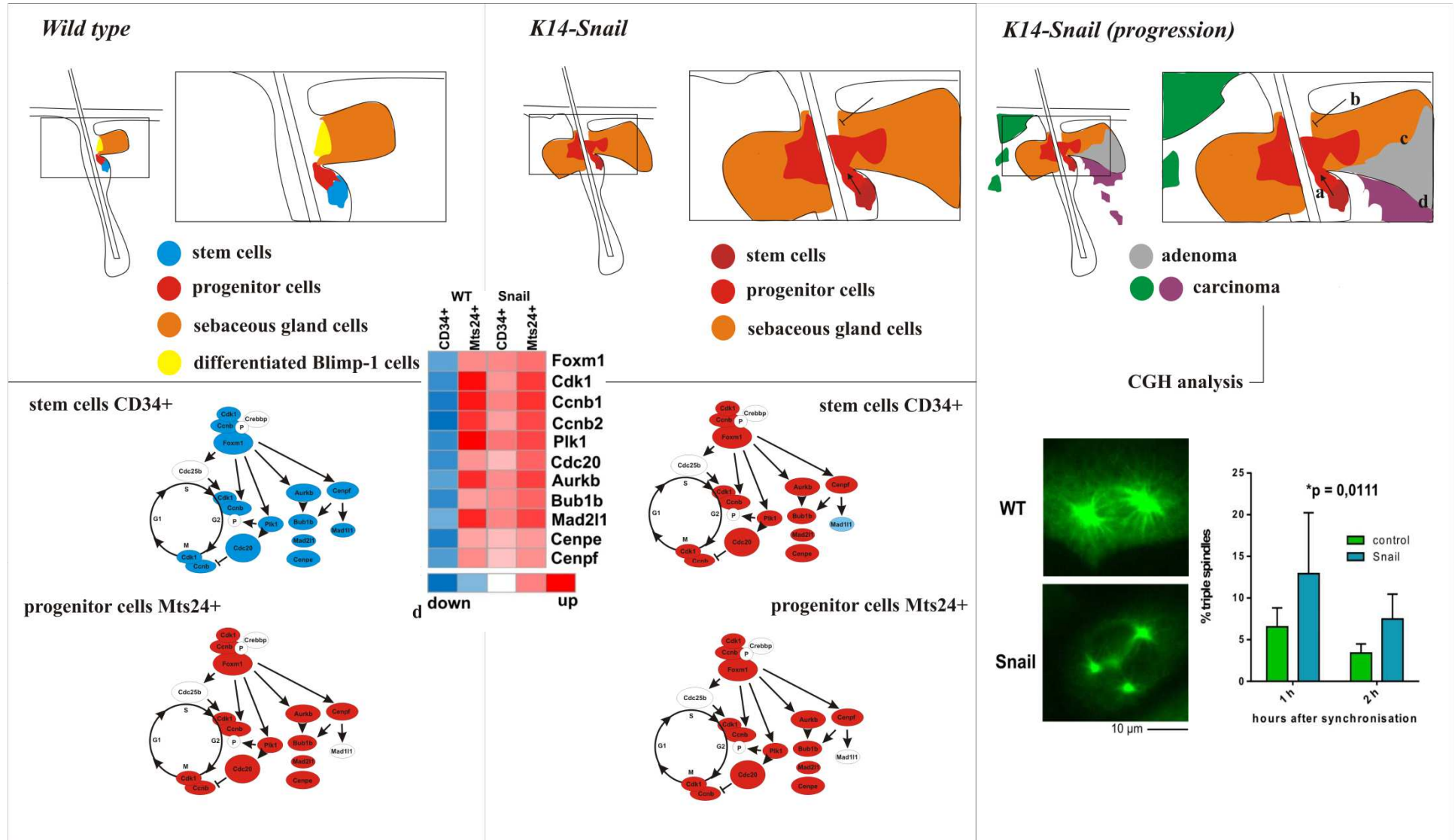
Increased epidermal thickness in K14-Snail mice



Increased amount of Ki67 pos. cells in the epidermis of Snail mice

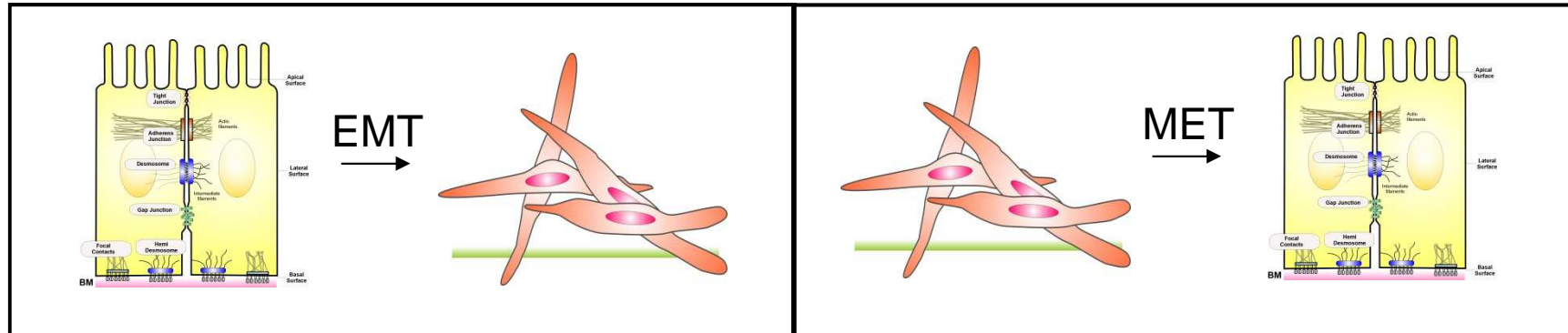


Snail induces FoxM1, a central regulator of the mitotic program and chromosome stability



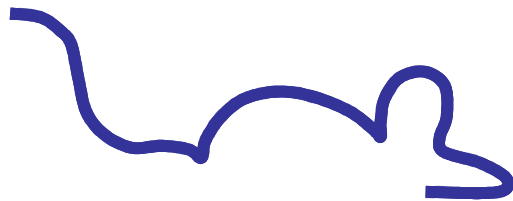
Study of molecular mechanisms of EMT driven cancer progression

In vitro



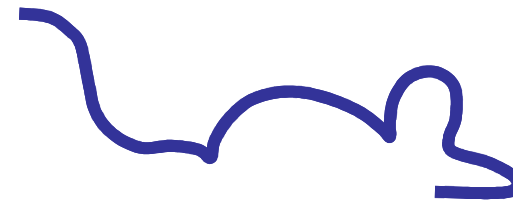
In vivo

EMT and skin cancer



K14-EMT mice

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ROSA-EMT mice

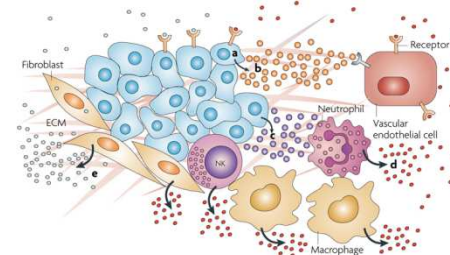
Microenvironment

Tool development

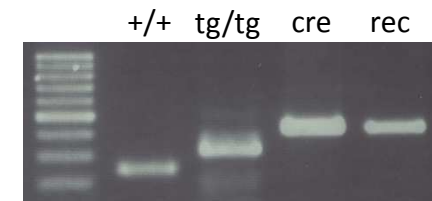
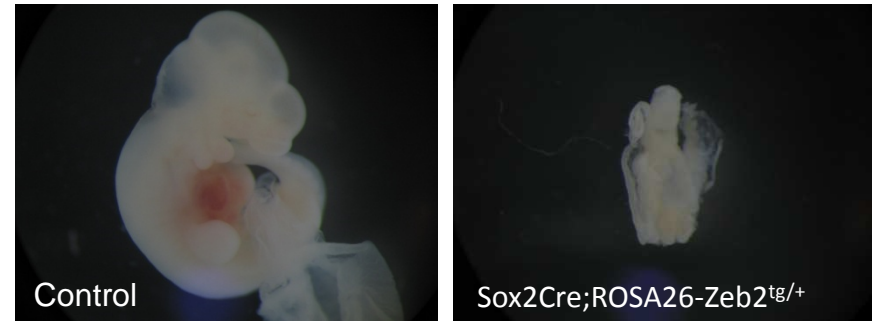
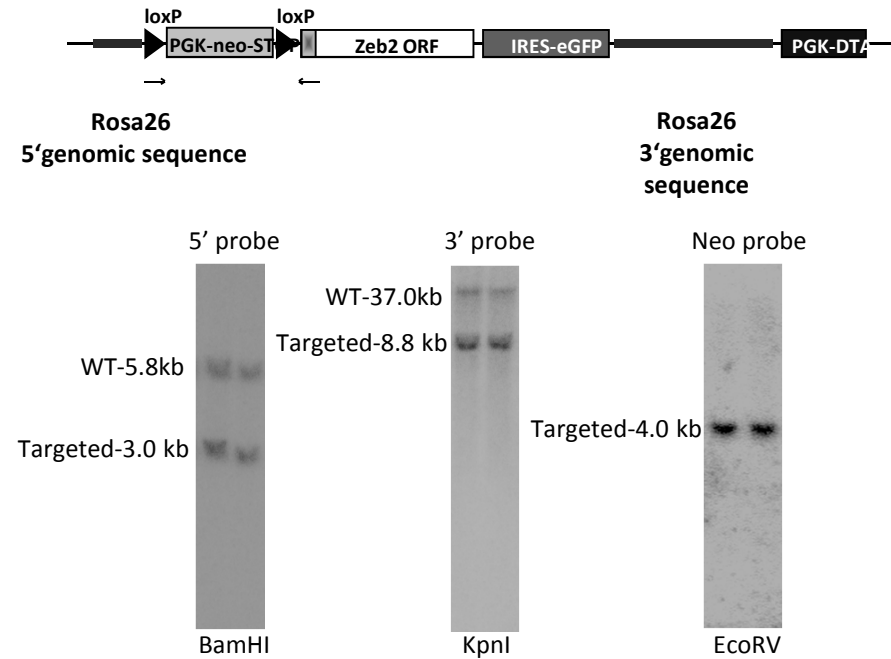
Cp-values	gen	cancer Inducte			EMT Inducte			p-values
		stad 1	stad 2	stad 3	stad 1	stad 2	stad 3	
31.19	CcG							0.015142
34.95	Col5A1-PRO1							0.020528
36.99	Elk3							0.020903
38.04	Shaf1							0.008819
37.35	Itih1							0.000796
38.24	Fluc							0.040368
31.16	Mbl1							0.027599
31.64	Blat							0.004884



Study of EMT – tumour stroma interface

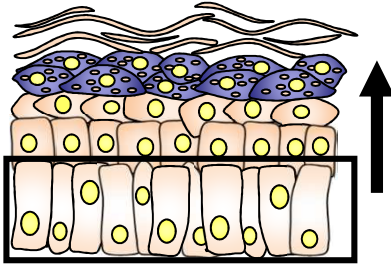


Generation of a conditional transgenic mouse model for Zeb2



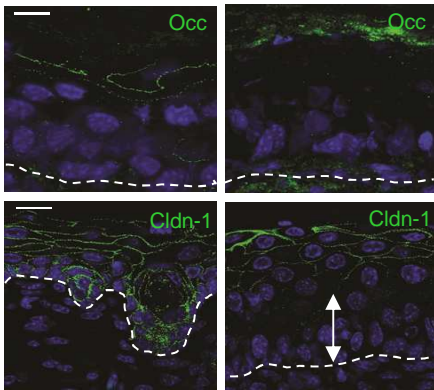
Crucial role of the EMT transcription factor Zeb2 in homeostasis and disease

skin/epidermis

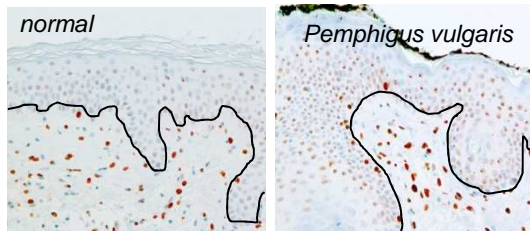


K14/K5 Cre driven expression

ROSA26-Zeb2^{tg/+} K5Cre;ROSA26-Zeb2^{tg/+}



Expression in human Pemphigus vulgaris



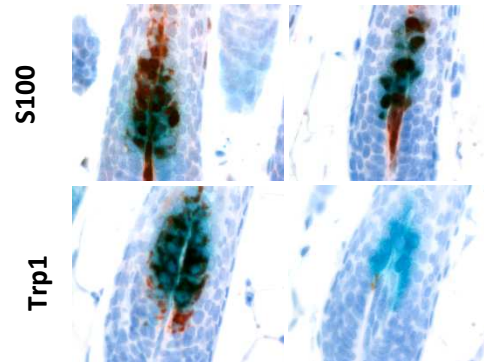
Tatari *et al.*, 2014

melanocyte

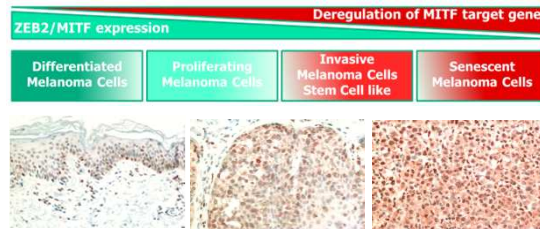


Tyr Cre driven deletion

ZEB2^{MC-WT} ZEB2^{MC-KO}

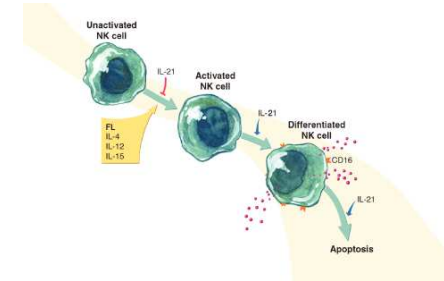


Role in melanoma

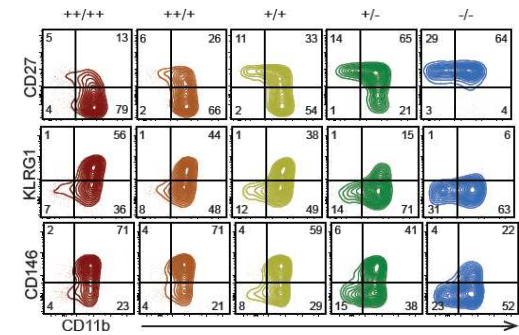


Denecker *et al.*, 2014, Vandamme *et al.*, in prep

immune cells



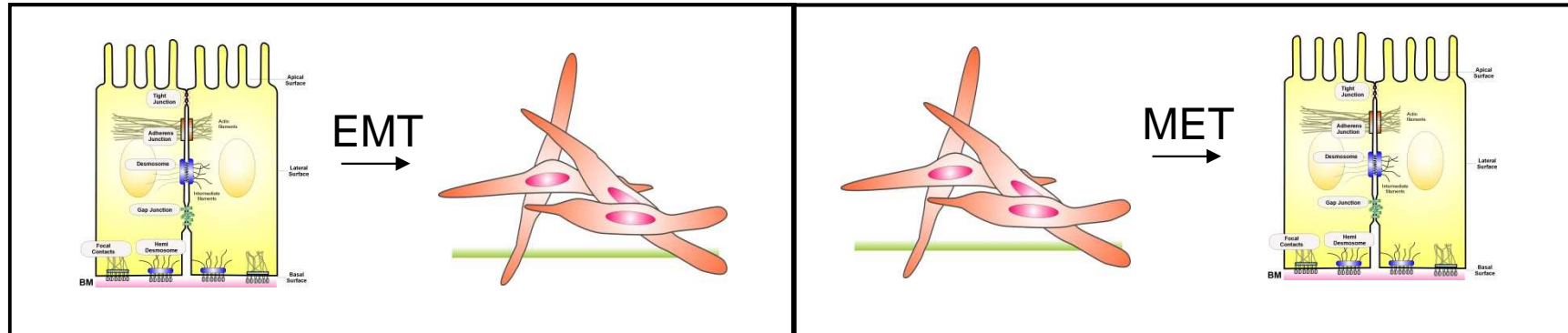
Cre driven expression or deletion



Van Helden *et al.*, submitted

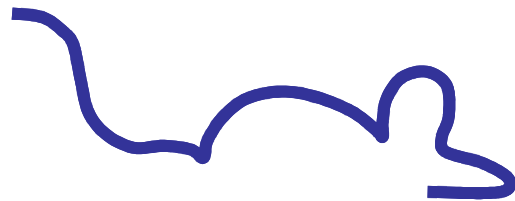
Study of molecular mechanisms of EMT driven cancer progression

In vitro



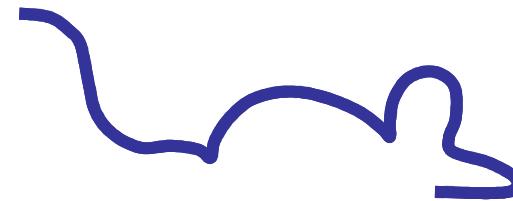
In vivo

EMT and skin cancer



K14-EMT mice

EMT and skin homeostasis/disease



ROSA-EMT mice

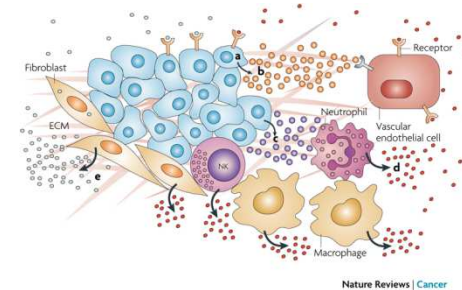
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Tool development

Cp-values	gen	cancer Inductie			EMT Inductie			p-values
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31.19	Cx2c							0.015142
34.93	Cd34-Pr01							0.025228
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37.25	Itin							0.000798
38.24	Fluc							0.043568
31.15	Mbl1							0.027599
31.64	Blat							0.004884

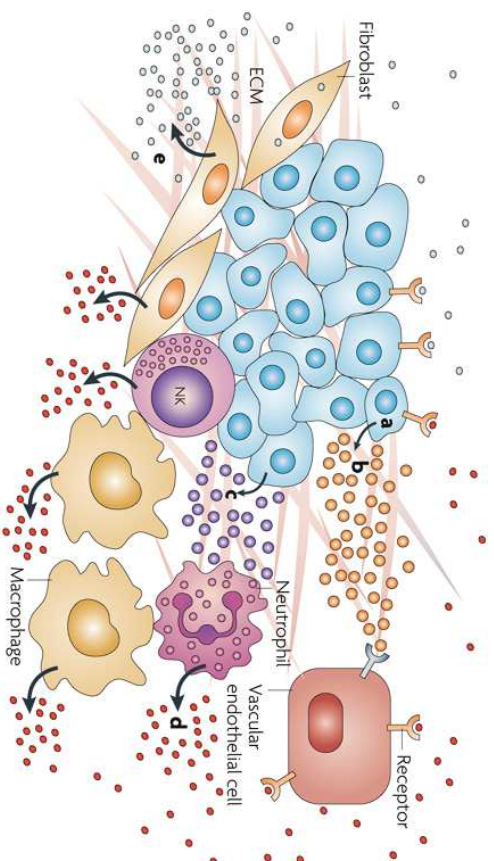


Study of EMT – tumour stroma interface

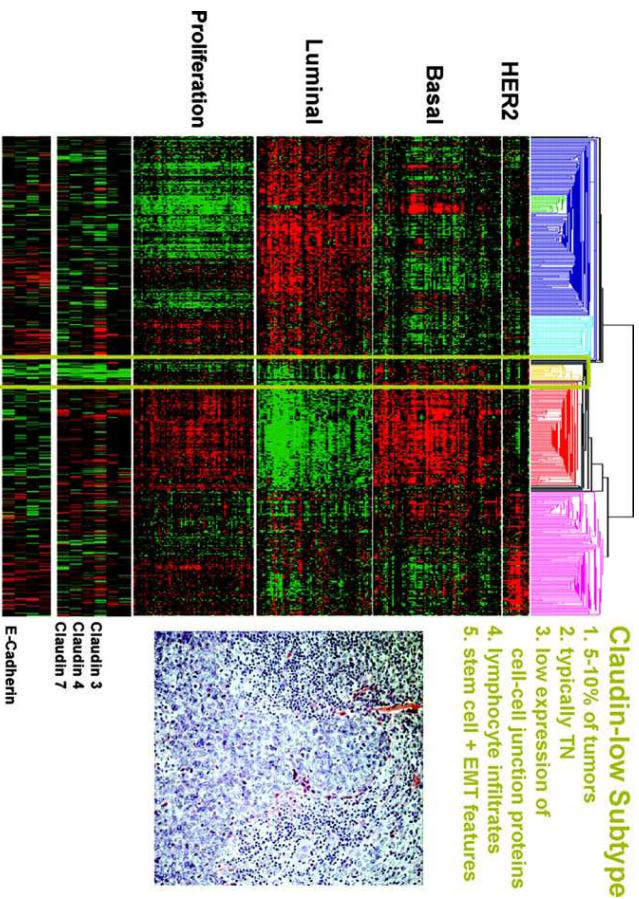


Tumour, microenvironment & tumour associated cells

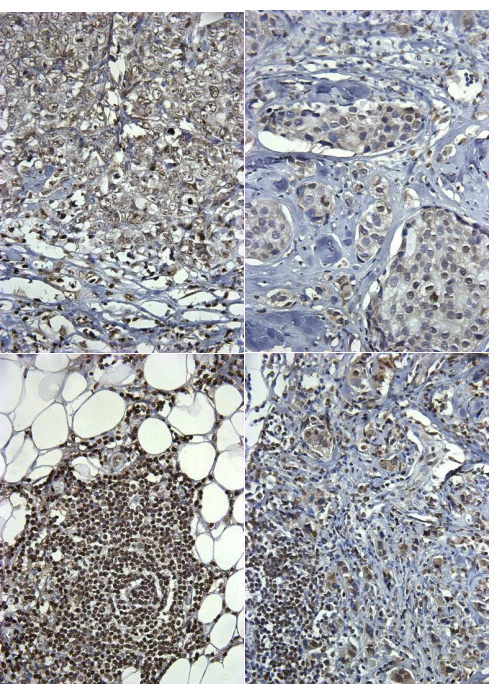
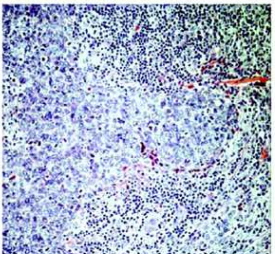
Contribution of Zeb2 in tumour cells?



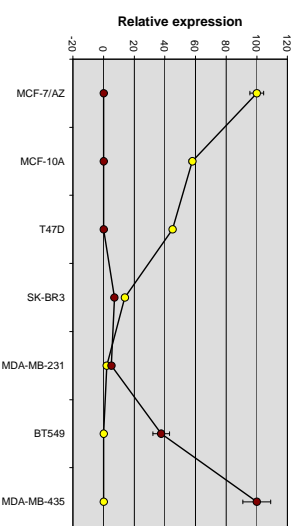
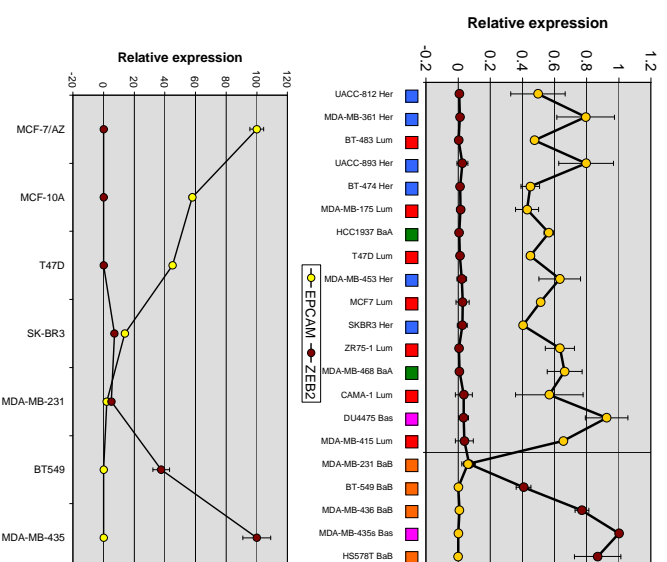
Nature Reviews | Cancer



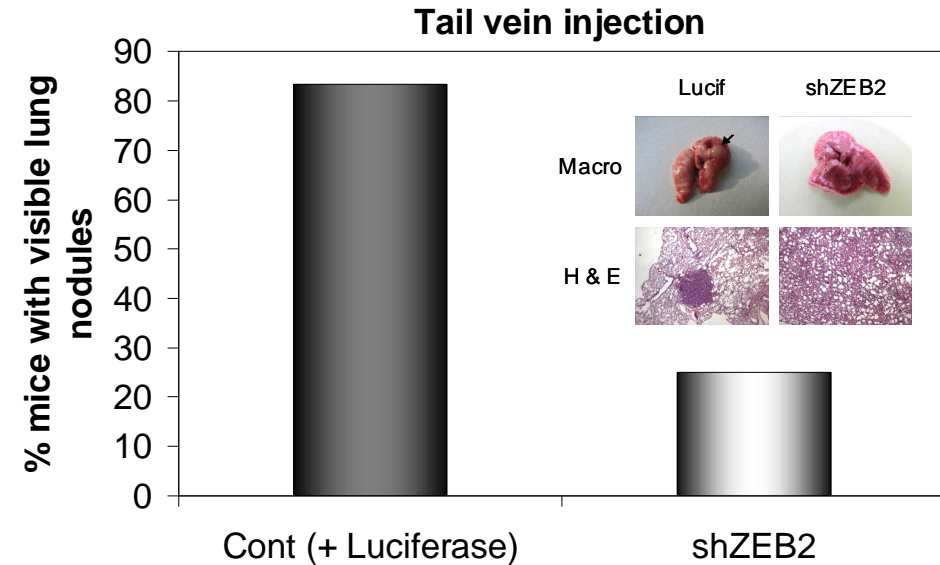
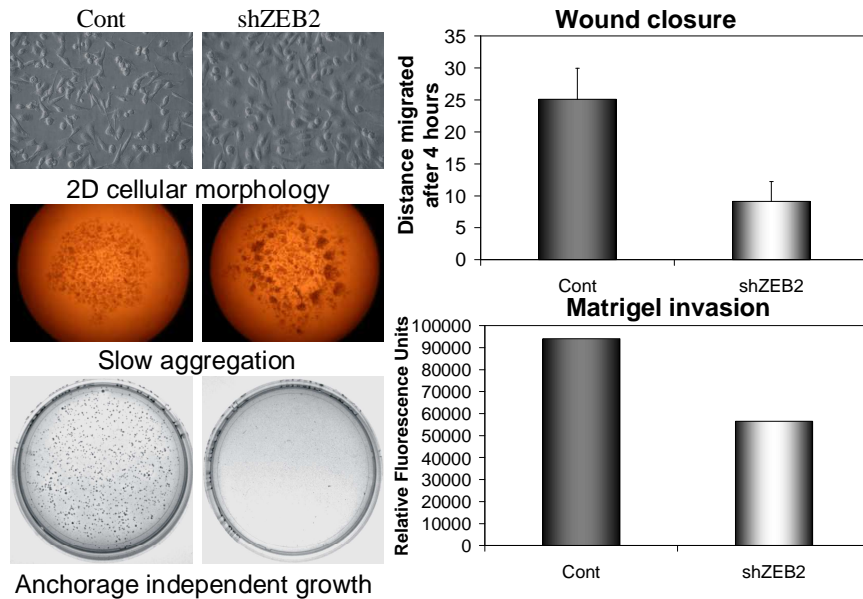
- Claudin-low Subtype**
1. 5-10% of tumors
 2. typically TN
 3. low expression of cell-cell junction proteins
 4. lymphocyte infiltrates
 5. stem cell + EMT features



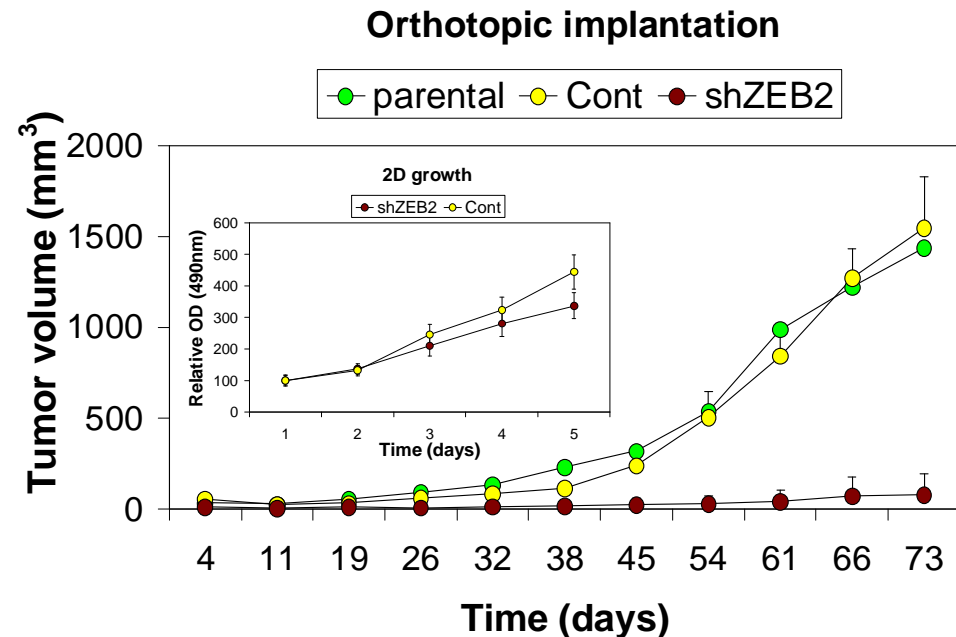
ZEB2 expression in breast tumour samples



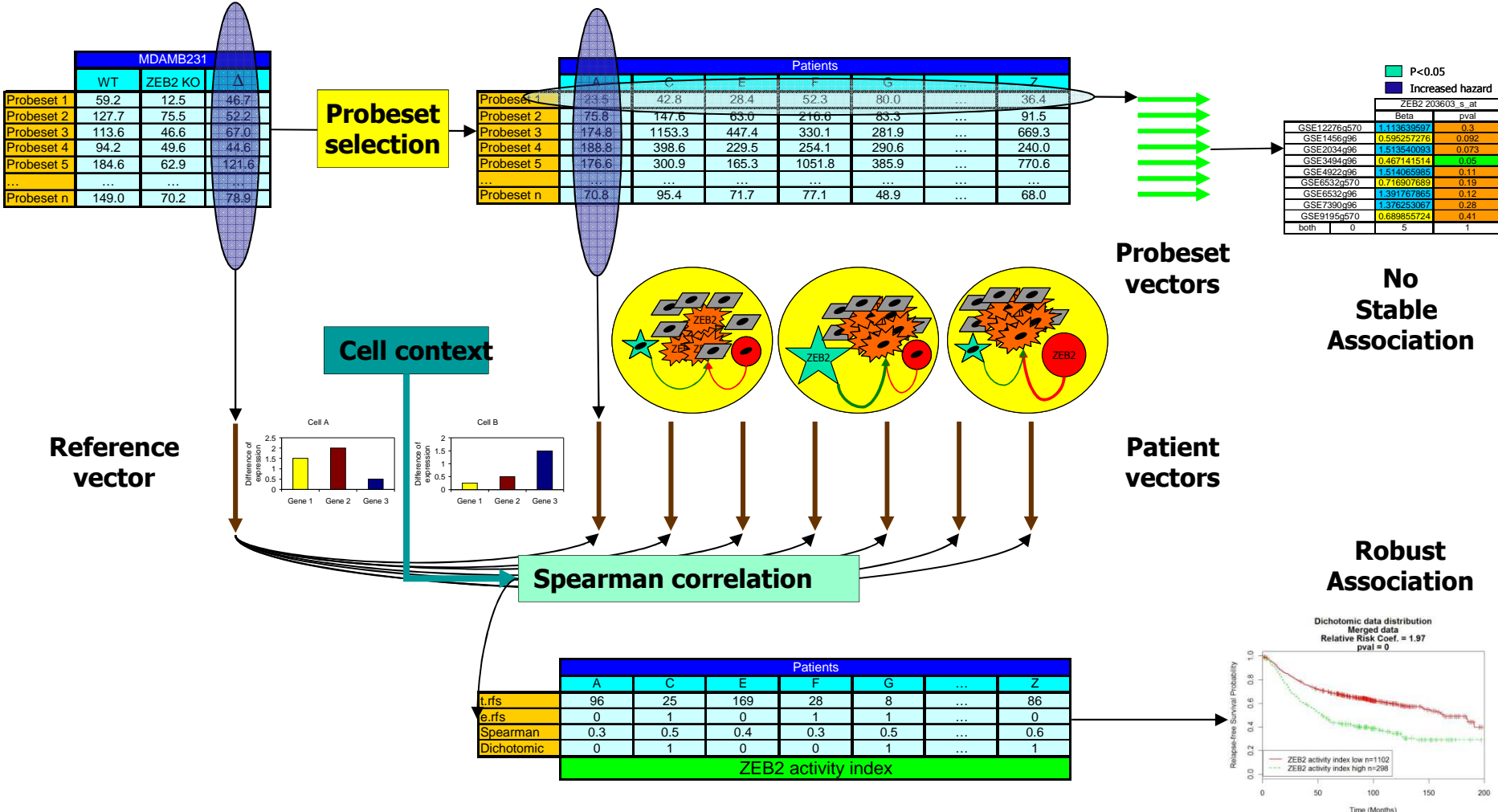
Impact of Zeb2 knock-down in basal breast cancer cells



- Increased epithelial morphology
- Increased aggregation
- Reduced 2D and 3D migration
- Impaired lung colonization
- Minor effect on 2D growth
- Impaired anchorage independent growth
- Impaired in vivo growth.



Breast cancer microarray: Zeb2 activity index building



- ZEB2 target genes → ZEB2 activity index.
- Which tumour behaves as if ZEB2 is knocked-down in MDAMB231 cells?

Zeb2 transcription factor activity: survival analysis

Full list n=36

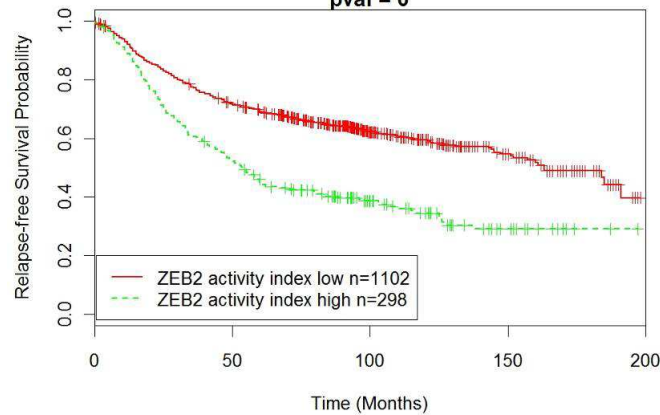
List optimization
Leave one out

Optimal list n=16

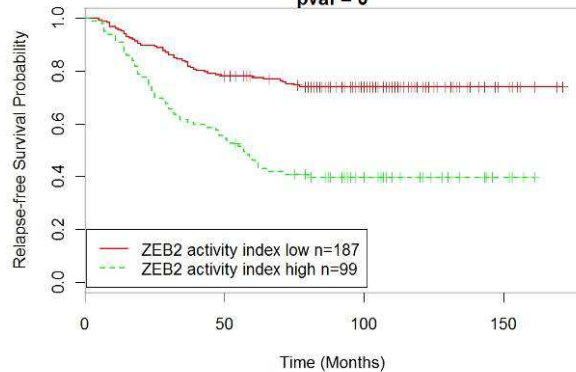
Raw ZEB2 activity index

	Studies	Pooled data (n=1400)	
	Count (tot=9) p<0.05 Rel Risk>1	Rel. Risk	p values
ZEB2	0	1.002	1.50E-01
Full List n=36	6	24.5	5.30E-11
Selected List n=16	6	5.3	6.90E-12

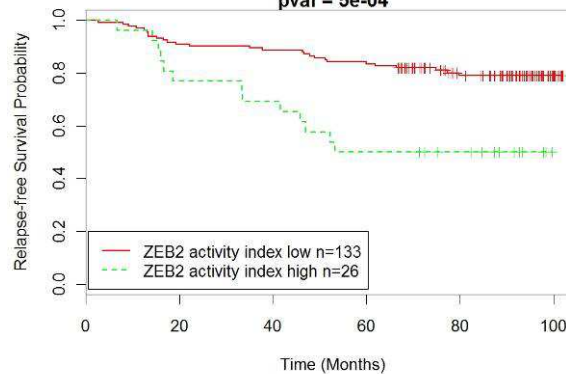
Dichotomic data distribution
Merged data
Relative Risk Coef. = 1.97
pval = 0



Dichotomic data distribution
GSE2034
Relative Risk Coef. = 2.96
pval = 0



Dichotomic data distribution
GSE1456
Relative Risk Coef. = 3.05
pval = 5e-04



Raspe et al., submitted

Metagene expression signature for prognosis of breast cancer patients.
Priority date 2010.10.29. Published: WO2012/056047 A1 (05.12.2013)

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PhD student position available !!!!
Postdoc position available !!!!