Associazione Italiana per la Ricerca sul Cancro

Con la ricerca, contro il cancro.

AIRC

Paola Defilippi

Molecular Biotechnology Center (MBC), Torino

"The scaffold protein p140Cap as a molecular hub for limiting cancer progression: a paradigm in breast cancer and neuroblastoma." .

Firenze, January 21, 2020

Università di Torino

MBC

Molecular Biotechnology Center

www.metafight.eu

META FIGHT

Financial support from

AIRC

AICR

PRIN MIUR

EU FP7 program Metafight,
www.metafight.eu

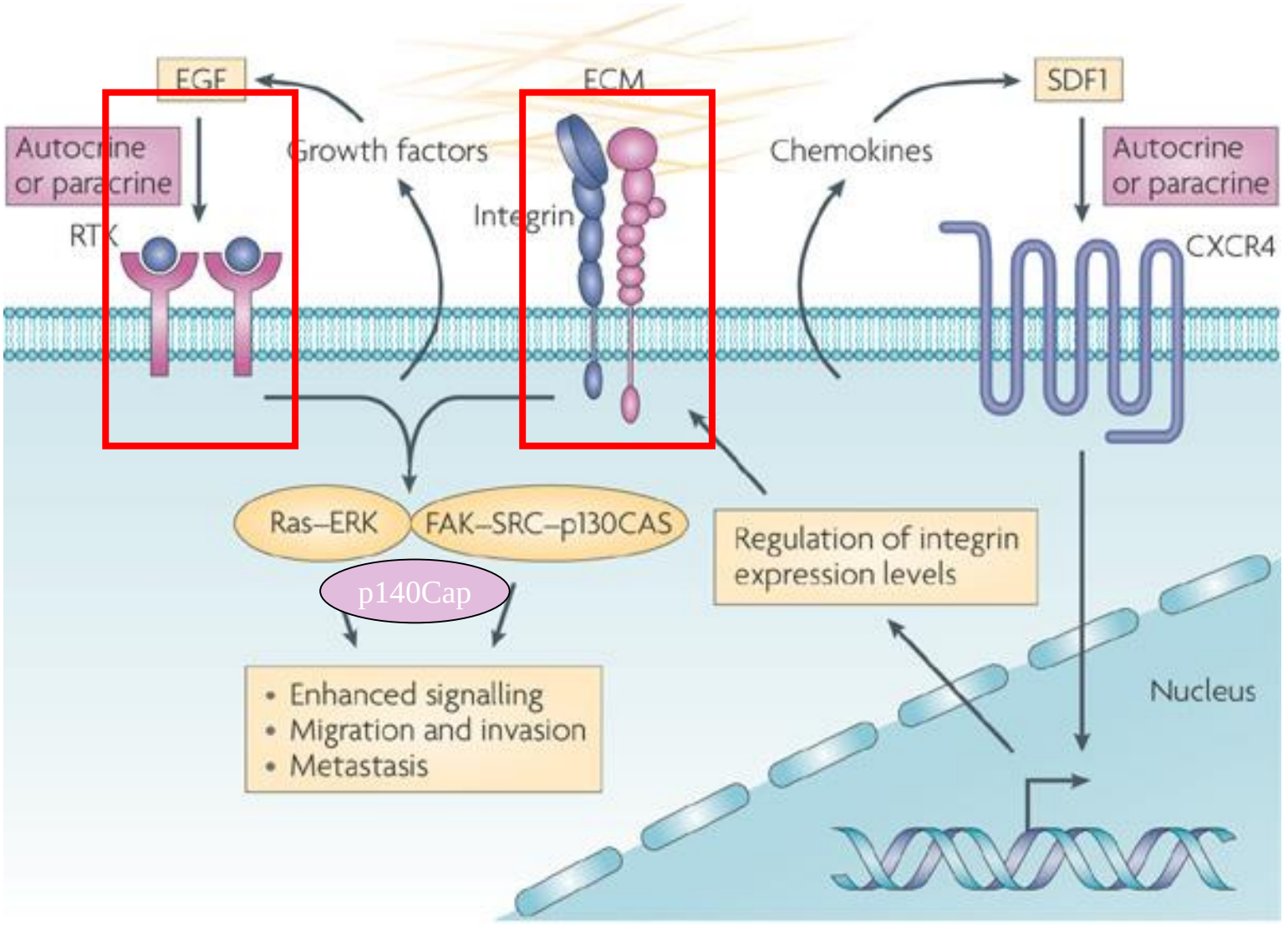
Regione Piemonte

Fondazione San Paolo

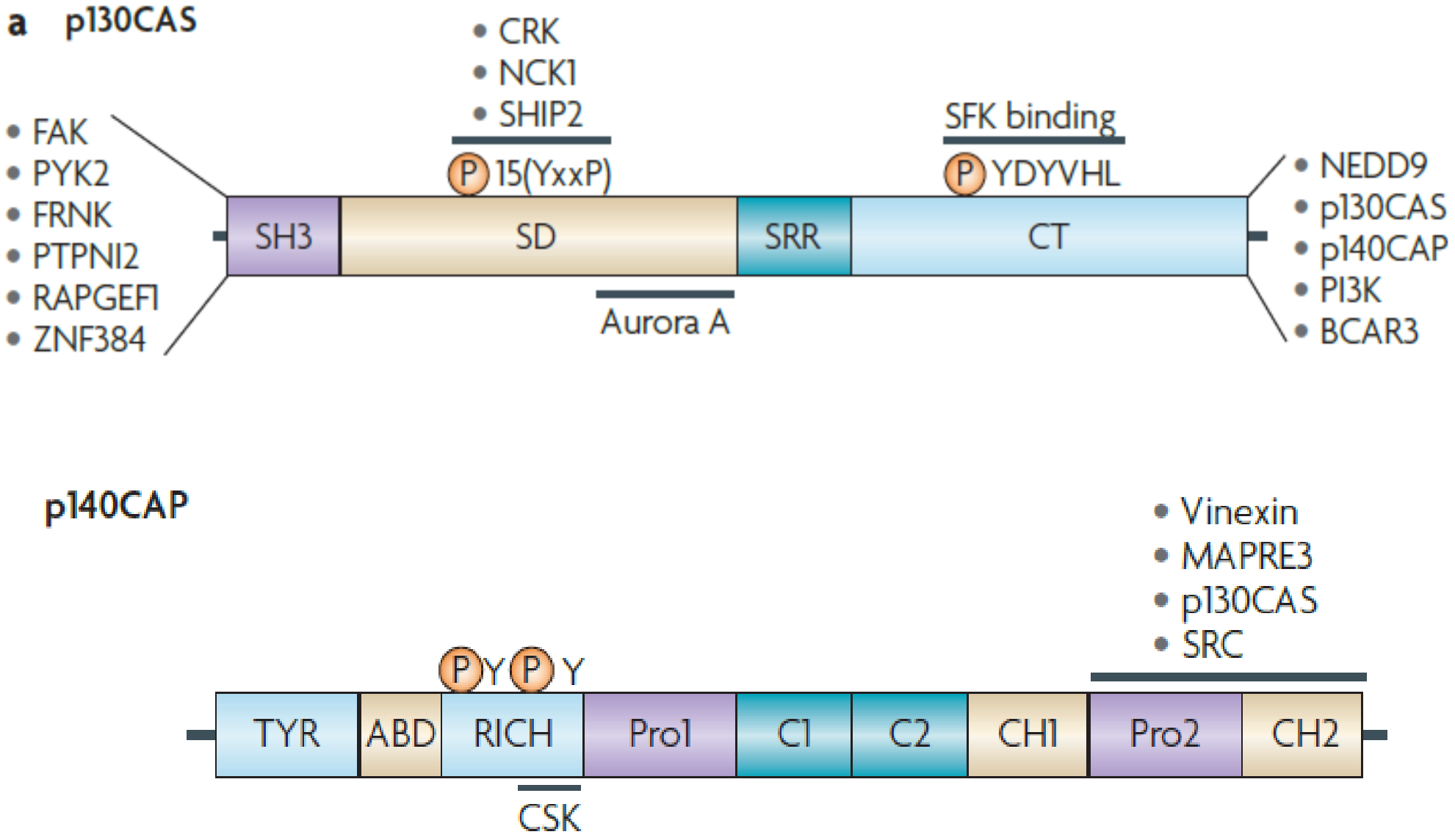
Fondazione CRT

Università di Torino
M B C
Molecular Biotechnology Center

Integrin signalling (in normal and cancer cells)

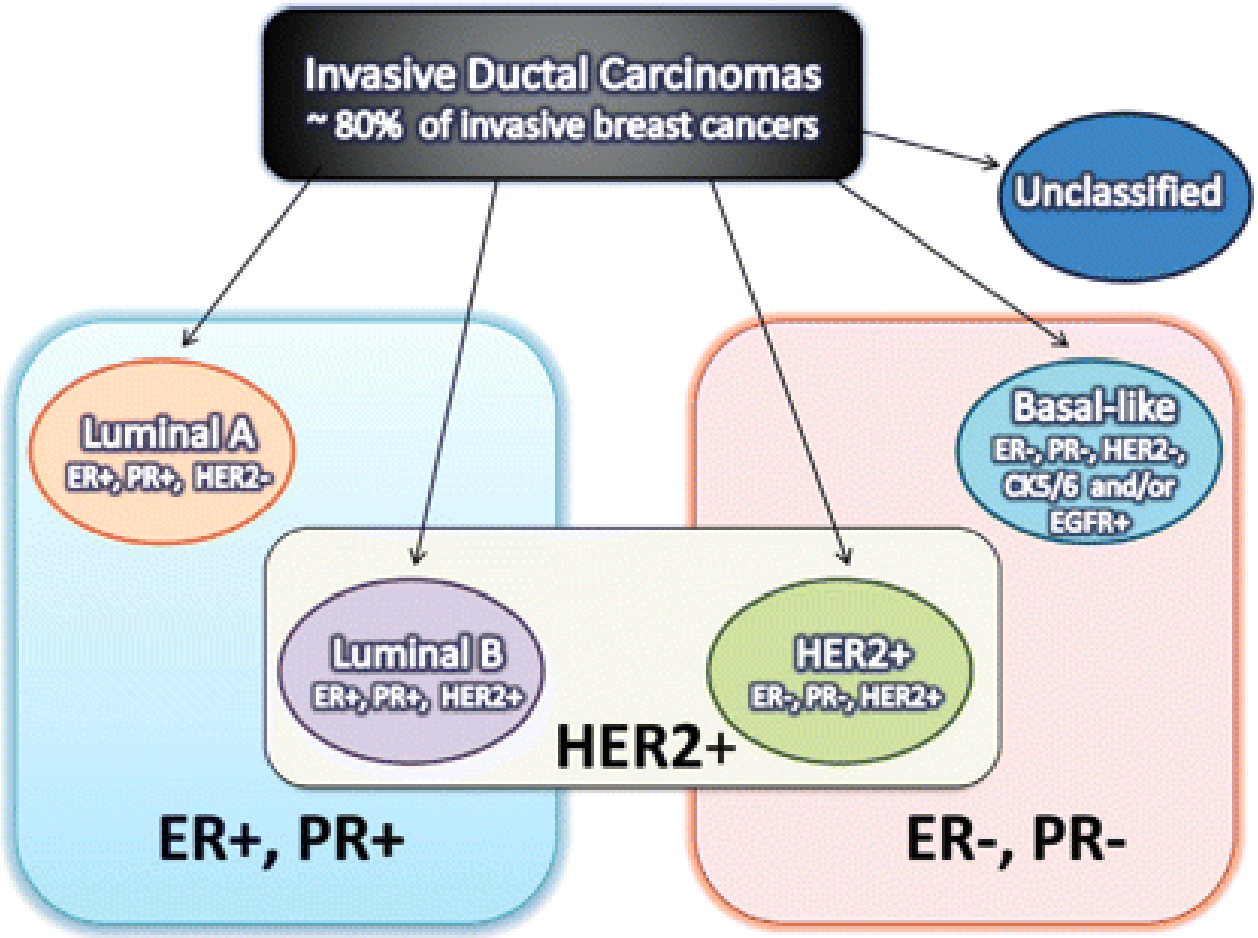


Desgrolliers and Cheresh , 2009



Cabodi et al, 2010, NATuRE REVIEwS | **CanCer**

Breast cancer subtypes



Sandhu et al., 2013

p130Cas and p140Cap scaffold proteins as opposite key players for transformation and cancer progression

cell proliferation
cell survival
EMT
ECM degradation
migration and invasion

p130Cas

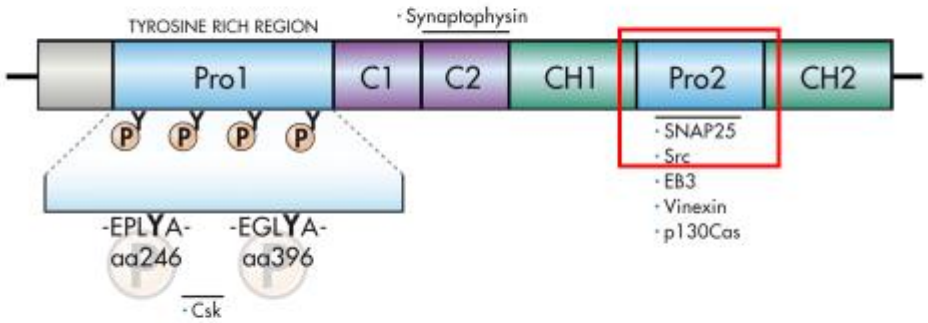
p140Cap/*SRCIN1*

cell proliferation
EMT
ECM degradation
migration and invasion

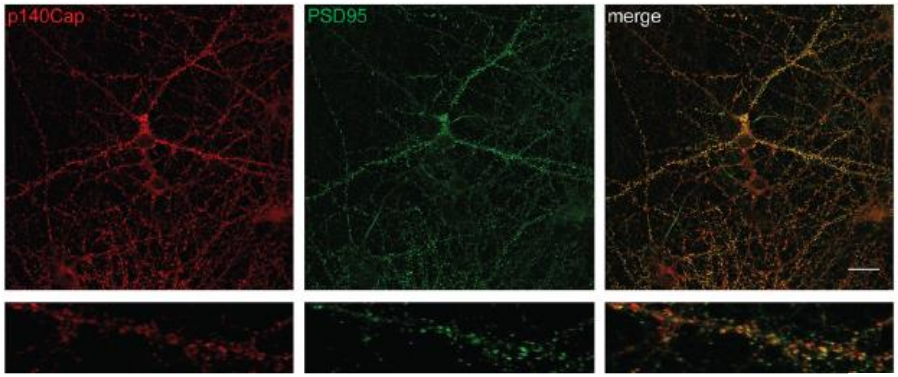
Cabodi et al., 2006, 2010 ERBB2 +
Tornillo et al., 2010 ERBB2+
Bisaro et al., 2012 Triple negative
Pincini et al., 2013 ERBB2+
Tornillo et al., 2014
Camacho et al., 2015
Rea et al., 2015 (ovarian cancer)
Bisaro et al.,2016 ERBB2+
Costamagna et al., 2019 ERBB2+

Di Stefano et al., 2007 (TNBC)
Damiano, Di Stefano et al., 2010 (Luminal A)
Damiano, Le Devedec, Di Stefano et al., 2012
Repetto et al., 2012
Sharma et al., 2013
Grasso et al., 2017 (HER2+)
Grasso, Cangelosi, Chapelle et al., 2019 (Neuroblastoma)
Chapelle, Sorokina, McLean et al., 2019 (HER2+)

The p140Cap adaptor

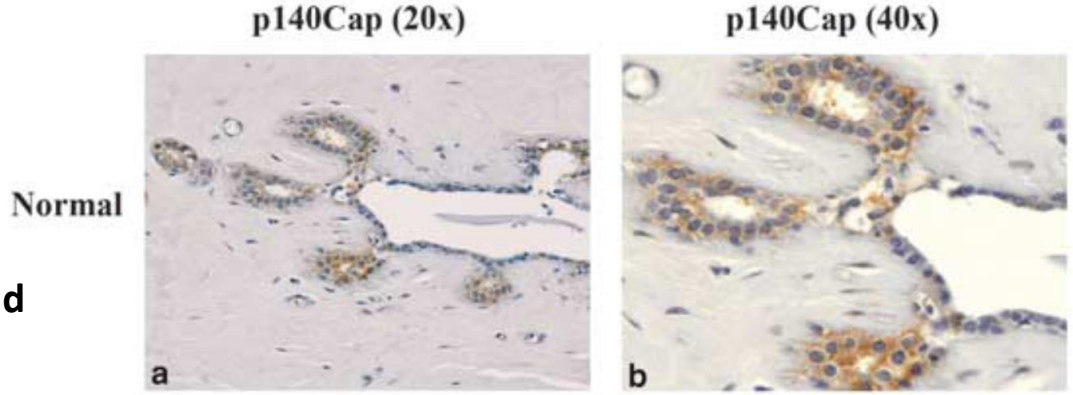


Primary neurons

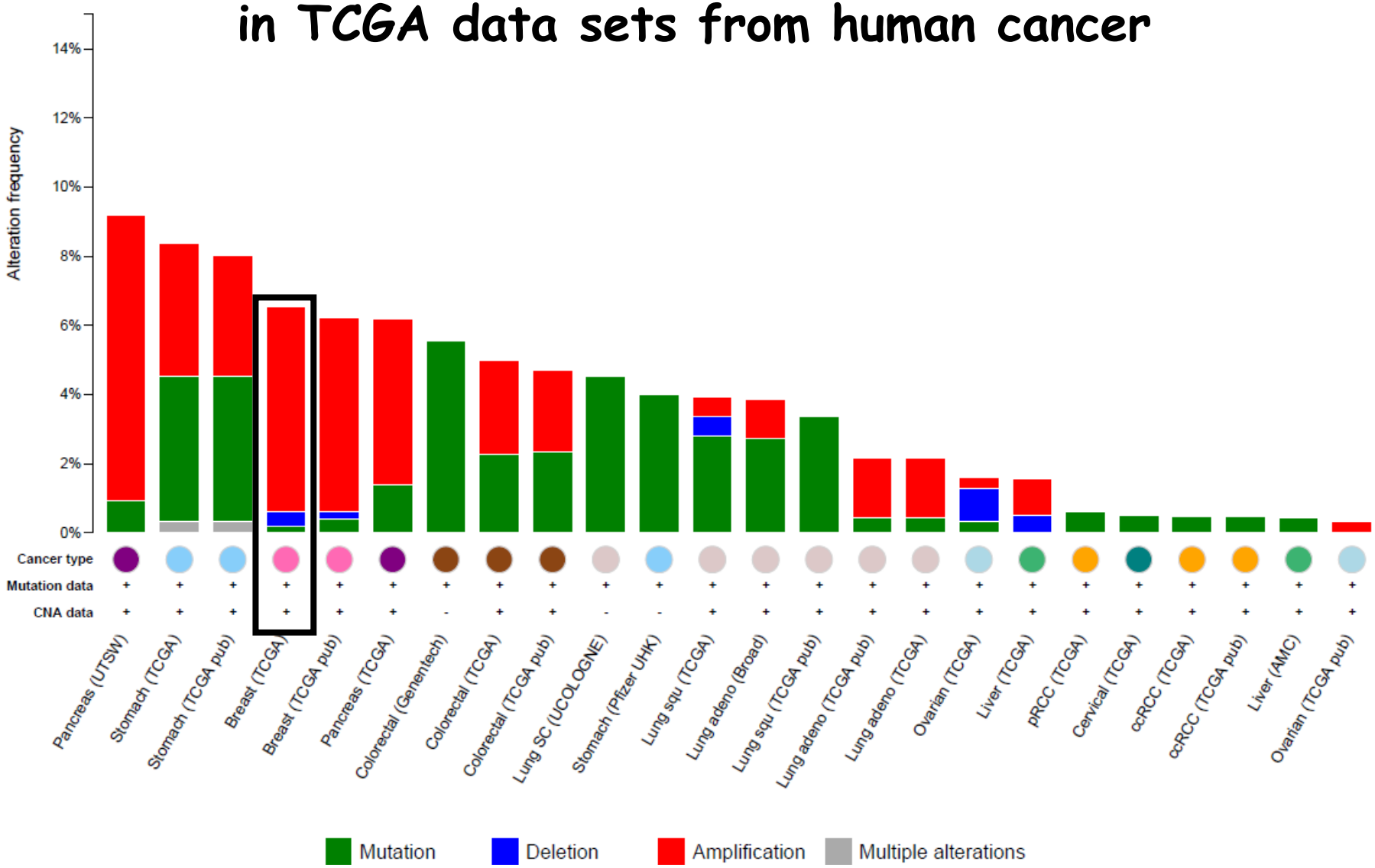


Jaworski et al. 2009
Repetto et al., 2014
Alfieri et al. 2017
Russo et al., 2017
Chapelle, Sorokina, McLean et al., 2019

Mammary gland



The *SRCIN1* gene, coding for p140Cap, is altered in TCGA data sets from human cancer



p140Cap as a breast tumor suppressor

The EMBO Journal (2007) 26, 2843–2855
www.embojournal.org

p140Cap protein suppresses tumour cell properties, regulating Csk and Src kinase activity

Oncogene (2010), 1–14

www.nature.com/onc

ORIGINAL ARTICLE

p140Cap dual regulation of E-cadherin/EGFR cross-talk and Ras signalling in tumour cell scatter and proliferation

L Damiano[1,5], P Di Stefano[1,5], MP Camacho Leal[1], M Barba[2], F Mainiero[2], S Cabodi[1], L Tordella[1], A Sapino[3], I Castellano[3], M Canel[4], M Frame[4], E Turco[1] and P Defilippi[1]

Oncogene (2011) 1–10

www.nature.com/onc

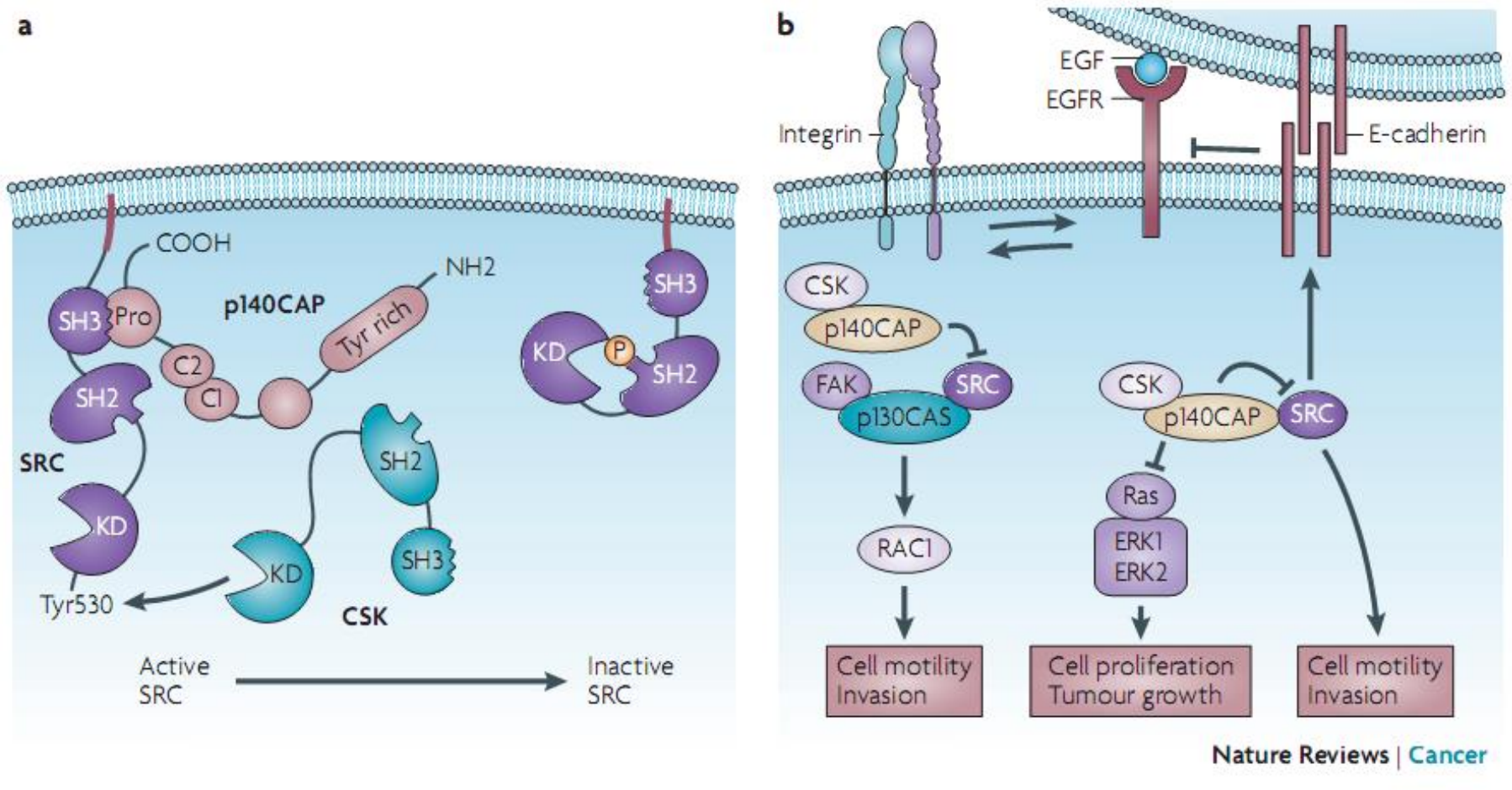
ORIGINAL ARTICLE

p140Cap suppresses the invasive properties of highly metastatic MTLn3-EGFR cells via impaired cortactin phosphorylation

L Damiano[1,5], SE Le Dévédec[2,5], P Di Stefano[1,5], D Repetto[1], R Lalai[2], H Truong[2], JL Xiong[2], EH Danen[2], K Yan[3], FJ Verbeek[3], F Attanasio[4], R Buccione[4], B van de Water[2] and P Defilippi[1]

I

In the ER+ breast tumors, the direct binding of p140Cap with Src and Csk allows the regulation of Src activity, and of E-cadherin-dependent EGFR activity



Di Stefano et al., 2007; Damiano, Di Stefano et al., 2010

Gain of function of p140Cap in highly metastatic breast cancer cells inhibits *in vivo* metastasis formation

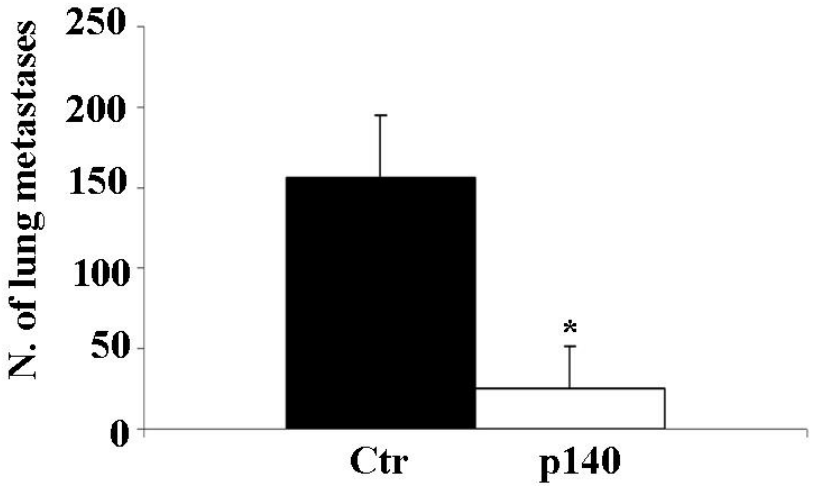
Oncogene (2011) 1–10

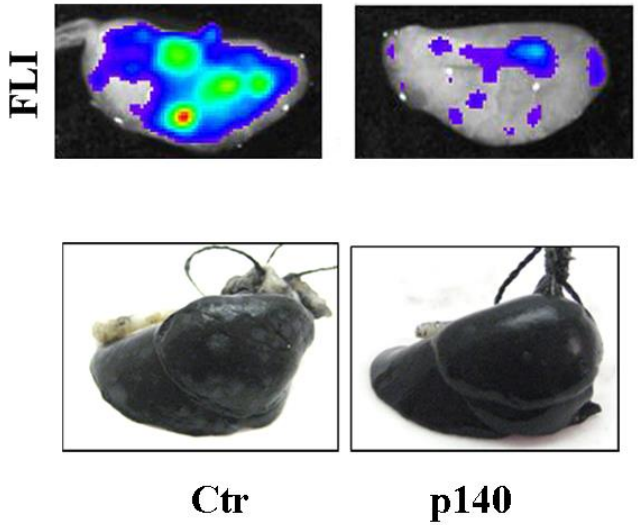
www.nature.com/onc

ORIGINAL ARTICLE

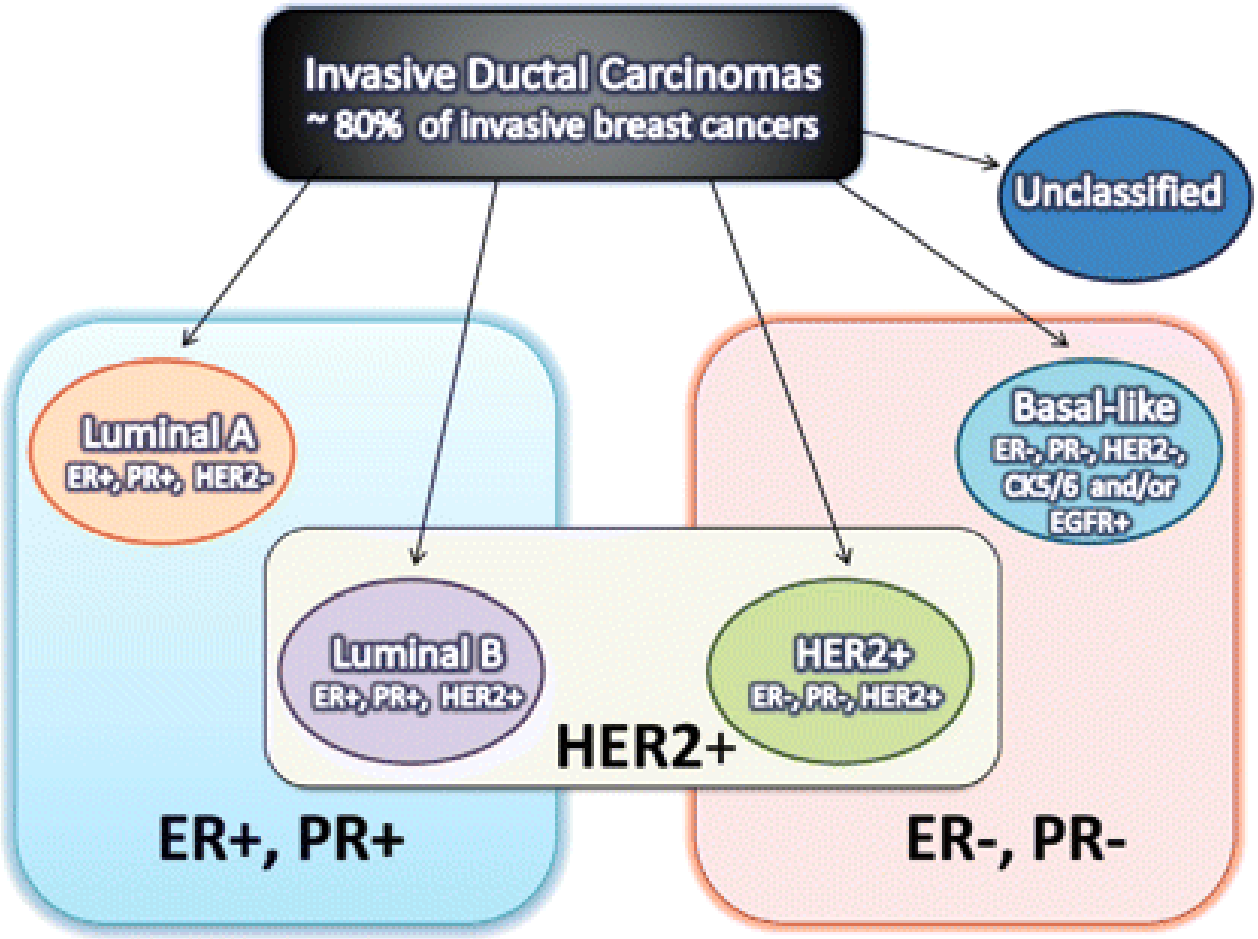
p140Cap suppresses the invasive properties of highly metastatic MTLn3-EGFR cells via impaired cortactin phosphorylation

L Damiano[1,5], SE Le Dévédec[2,5], P Di Stefano[1,5], D Repetto[1], R Lalai[2], H Truong[2], JL Xiong[2], EH Danen[2], K Yan[3], FJ Verbeek[3], F Attanasio[4], R Buccione[4], B van de Water[2] and P Defilippi[1]



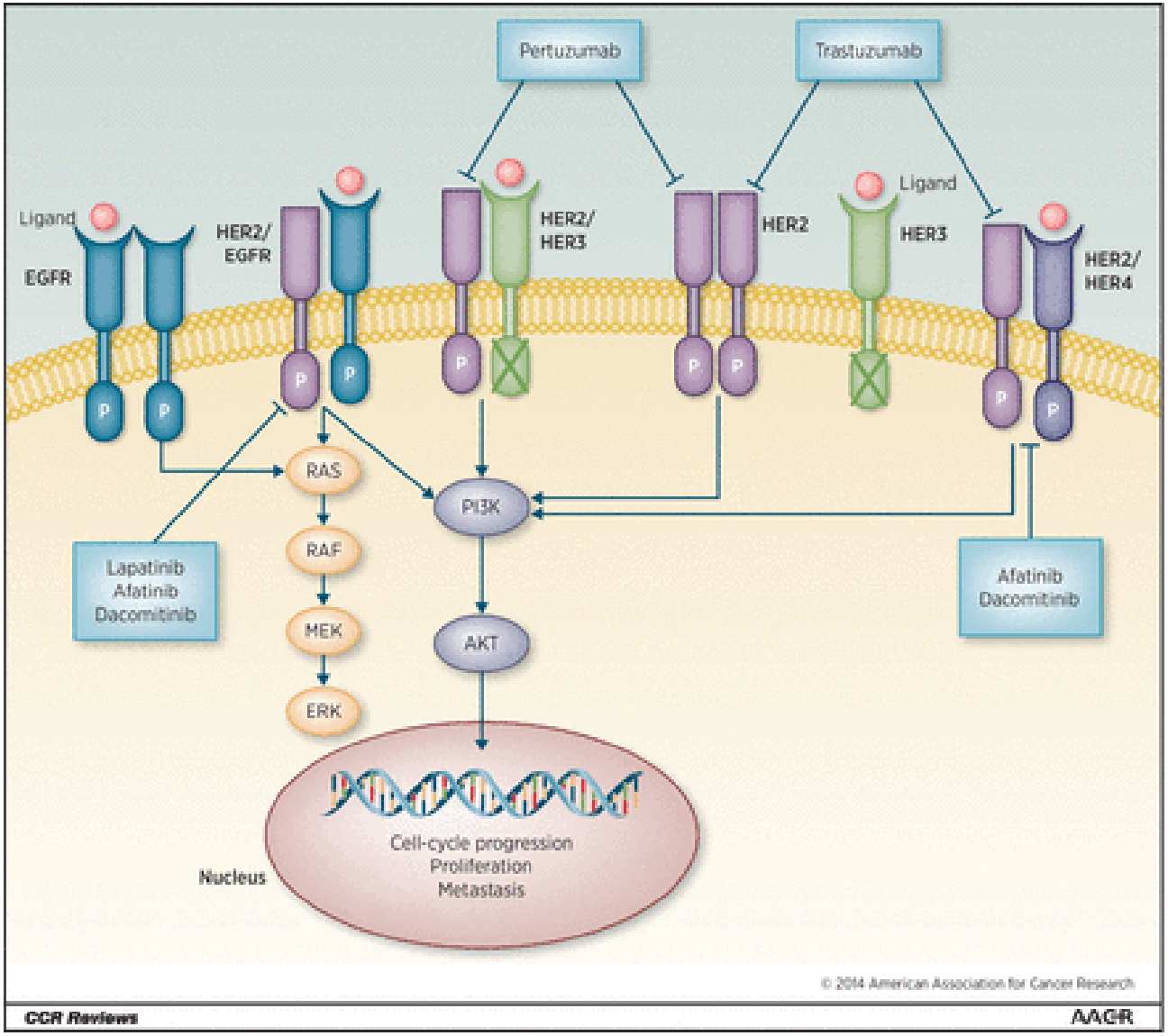


p140Cap in HER2+ breast tumors

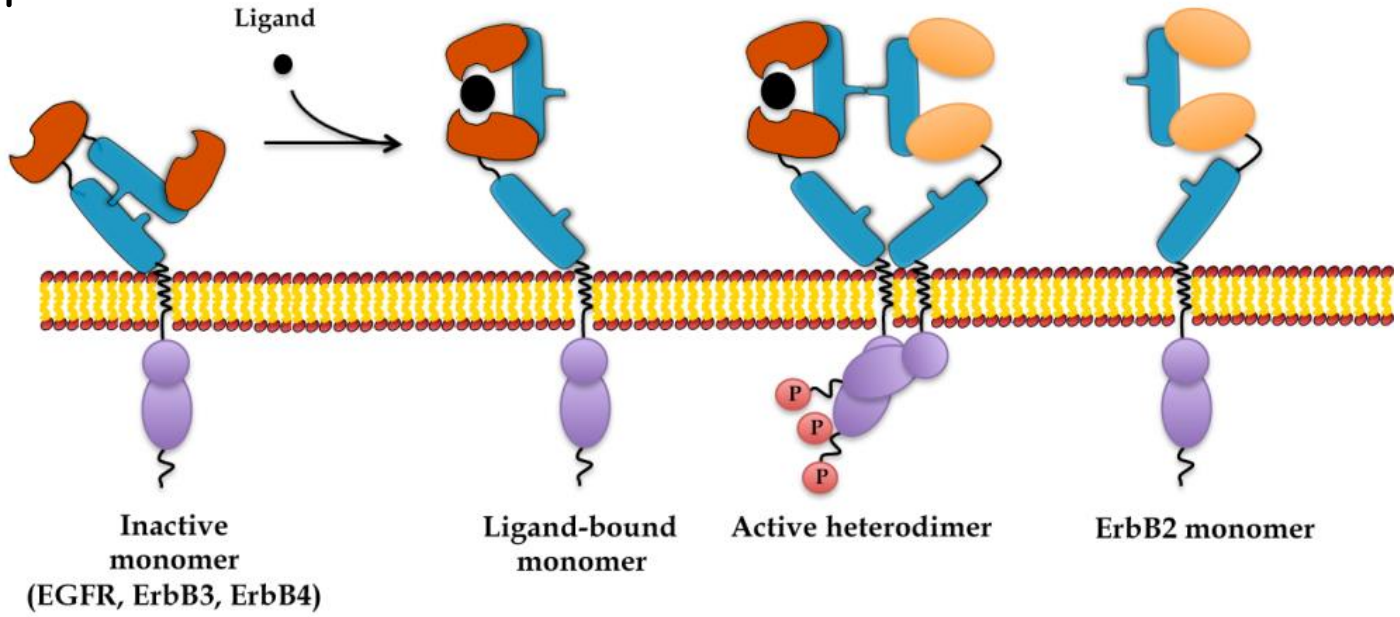


Sandhu et al., 2013

HER2 oncogene Receptor Tyrosine Kinase family



In the ligand-free state, HER1, HER3, and HER4 have a closed conformation. Binding of ligand, involving subdomains I and III, creates an extended conformation, allowing for receptor homo- and heterodimerization. Receptor dimerization leads to C-terminal tyrosine phosphorylation, creating phosphotyrosine binding sites for binding of adaptors, signaling molecules and regulatory proteins.

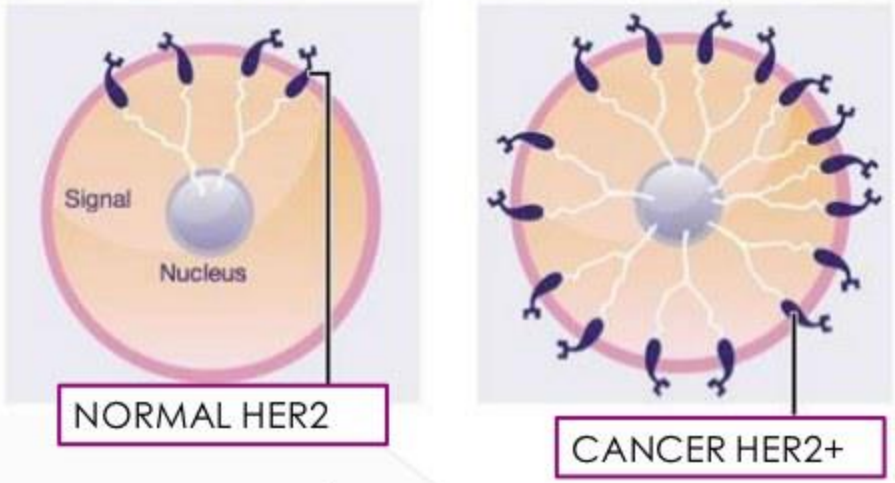


HER2 is unique in that it is fixed in the active conformation ready to interact with other HER receptors

Normal vs. Cancerous HER2+

Yes, normal cells have HER2

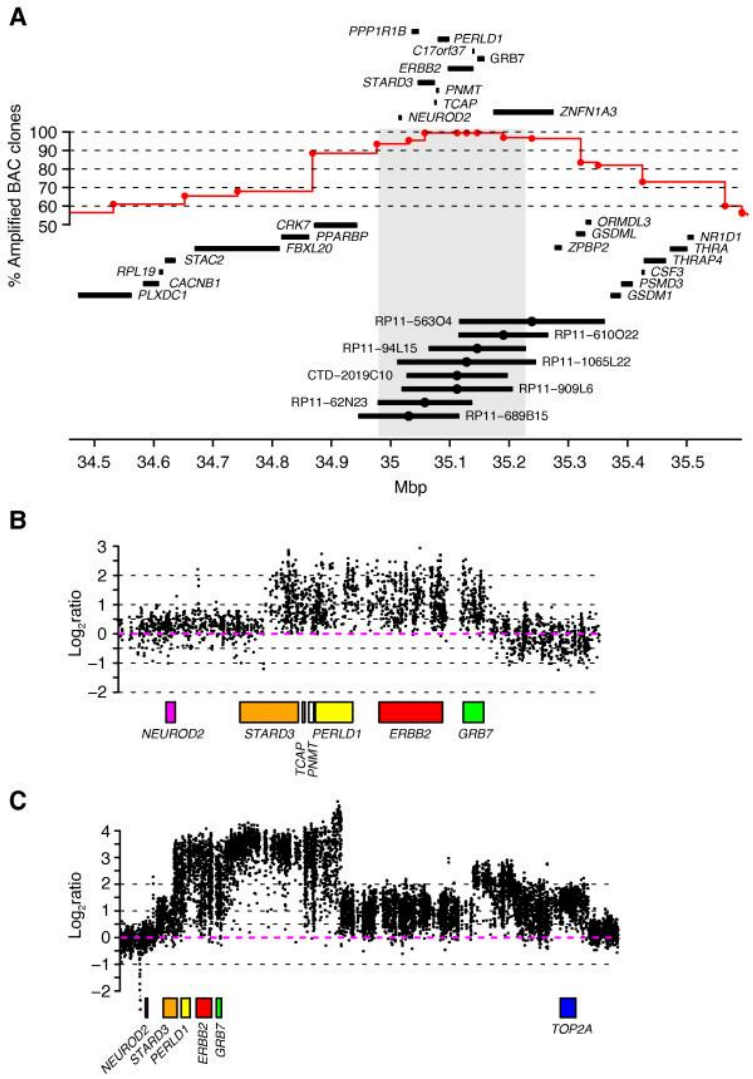
The difference:



1.) receptor overexpression
2.) dysregulation of receptor activation

http://www.herceptin.com/metastatic/what-is/how-does-it-work.jsp

HER2 amplicon is localised on chr17



nature COMMUNICATIONS

ARTICLE

Received 5 May 2016 | Accepted 27 Jan 2017 | Published 16 Mar 2017

DOI: 10.1038/ncomms14797 OPEN

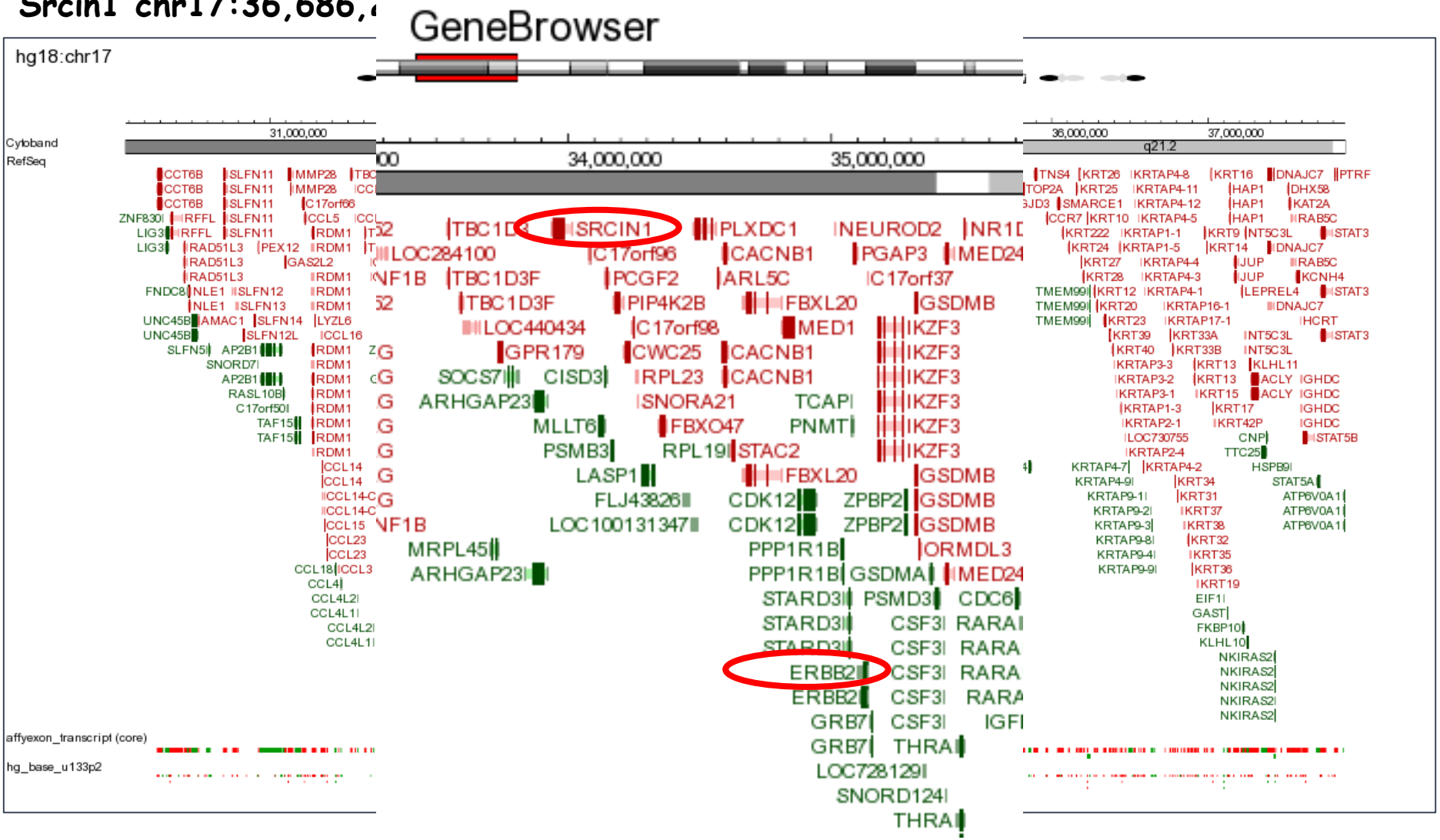
The scaffold protein p140Cap limits ERBB2-mediated breast cancer progression interfering with Rac GTPase-controlled circuitries

Silvia Grasso[1], Jennifer Chapelle[1], Vincenzo Salemme[1], Simona Aramu[1], Isabella Russo[1], Nicoletta Vitale[1], Ludovica Verdun di Cantogno[2], Katiuscia Dallaglio[3], Isabella Castellano[2], Augusto Amici[4], Giorgia Centonze[1], Nanaocha Sharma[1], Serena Lunardi[1], Sara Cabodi[1], Federica Cavallo[1], Alessia Lamolinara[5], Lorenzo Stramucci[5], Enrico Moiso[1], Paolo Provero[1], Adriana Albini[6], Anna Sapino[2], Johan Staaf[7], Pier Paolo Di Fiore[8,9,10], Giovanni Bertalot[8], Salvatore Pece[8,10], Daniela Tosoni[8], Stefano Confalonieri[8,9], Manuela Iezzi[5], Paola Di Stefano[1], Emilia Turco[1,*] & Paola Defilippi[1,*]

p140Cap gene sits near the HER2 amplicon

Srcin1 (the human p140Cap gene) is localised on chr17, close to the HER2 amplicon

Srcin1 chr17:36,686,[illegible]



Alteration in the copy of *Srcin1* gene is specific for nearly 50% of the HER2+ patients

Staaf et al. Breast Cancer Research 2010, 12:R25
http://breast-cancer-research.com/content/12/3/R25

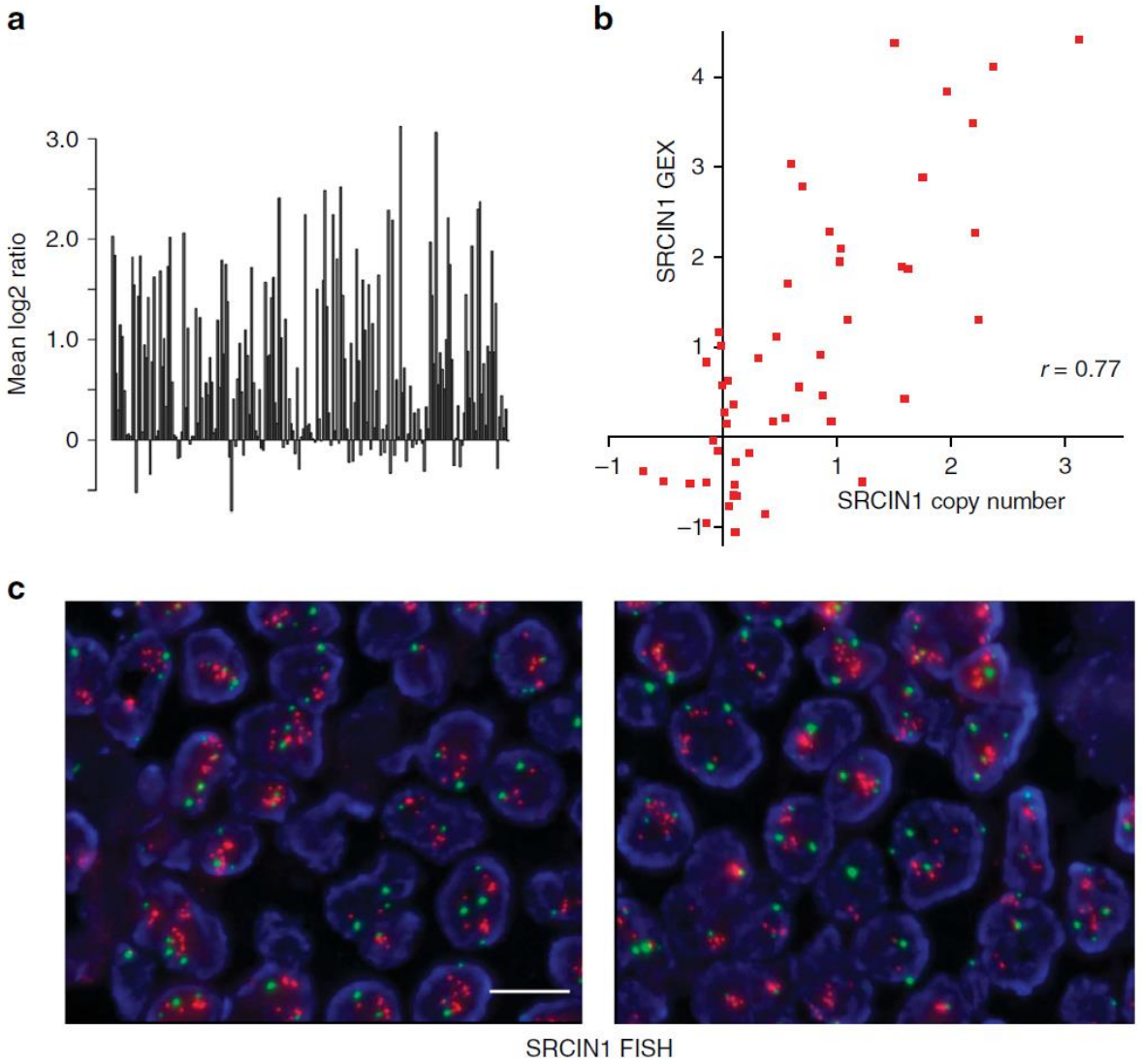
Breast Cancer RESEARCH

RESEARCH ARTICLE Open Access

High-resolution genomic and expression analyses of copy number alterations in HER2-amplified breast cancer

Johan Staaf*[1,2], Göran Jönsson[1,2], Markus Ringnér[1,2], Johan Vallon-Christersson[1,2], Dorthe Grabau[3], Adalgeir Arason[4], Haukur Gunnarsson[4], Bjarni A Agnarsson[4,5], Per-Olof Malmström[1], Oskar Th Johannsson[5,6], Niklas Loman[1], Rosa B Barkardottir[4,5] and Åke Borg[1,2,7]

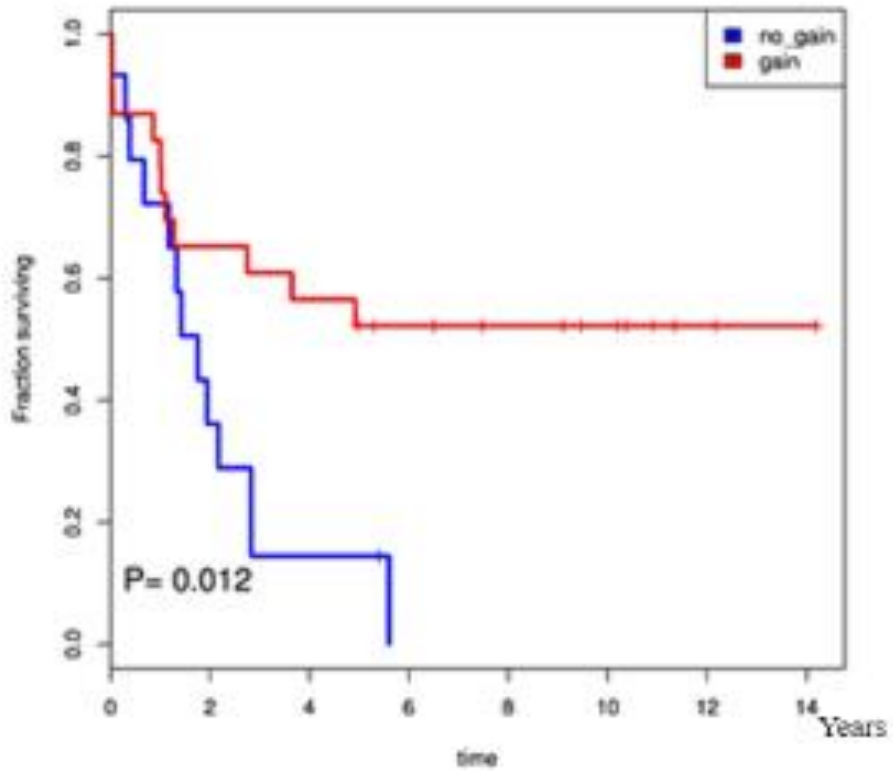
SRCIN1 copy number in 200 HER2+ samples
61,5% gain and 9% loss



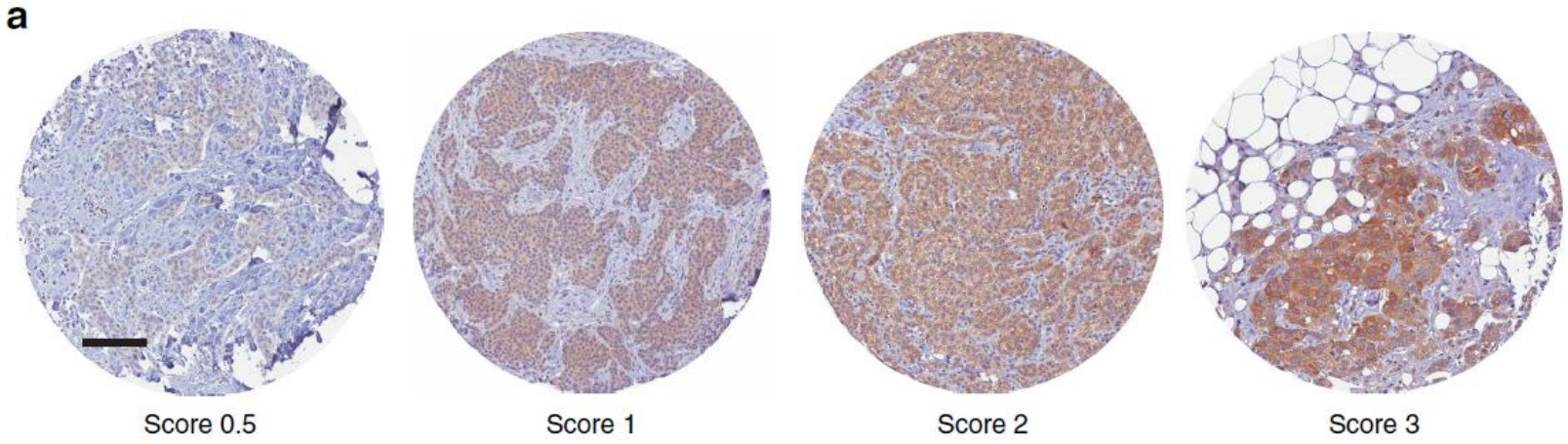
Staaf' and Sapino' lab

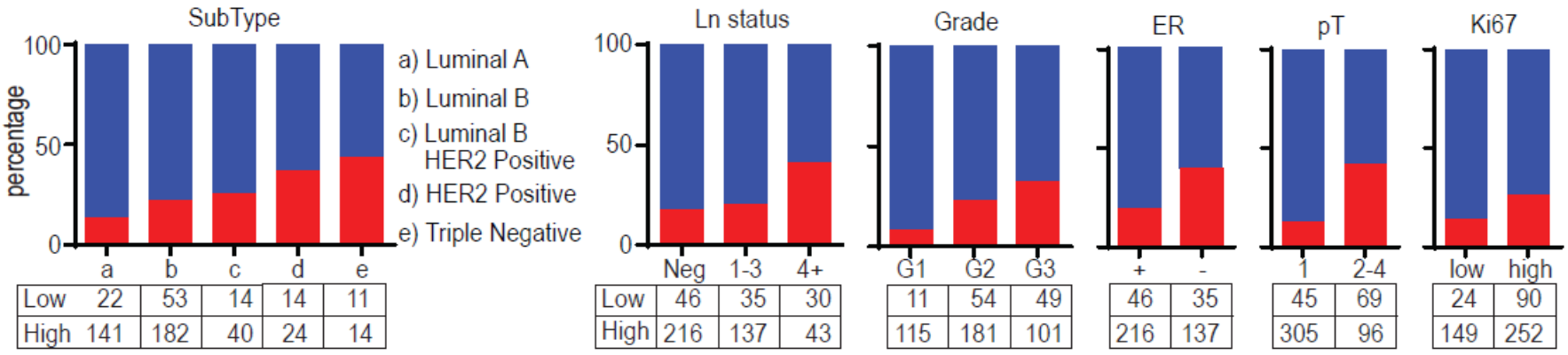
SRCIN1 amplification together with HER2 improves the survival of HER2+ patients

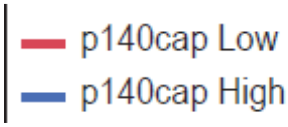
SRCIN1 no gain/gain



p140Cap expression in 622 consecutive breast tumor samples associates with good prognosis marker

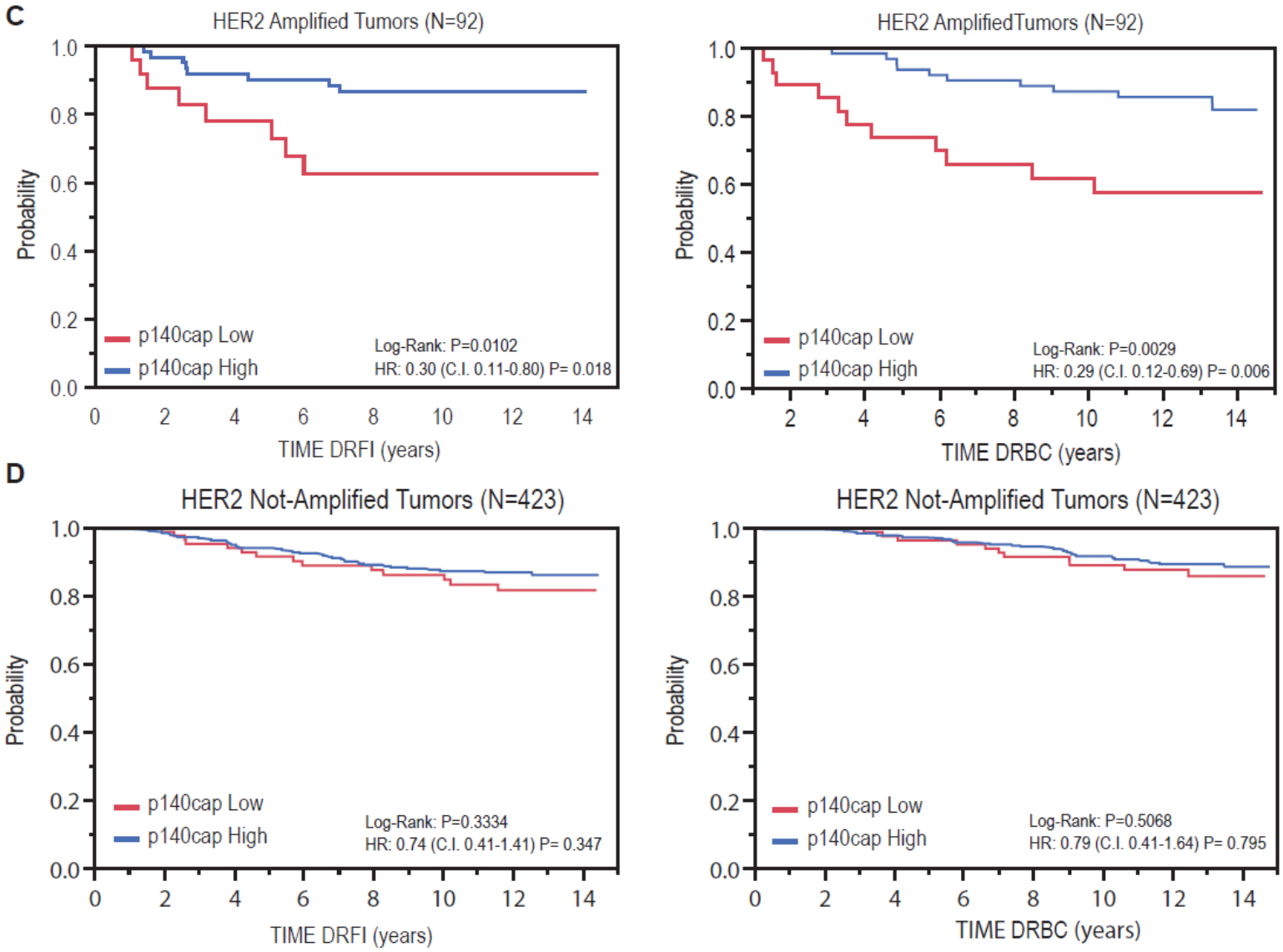






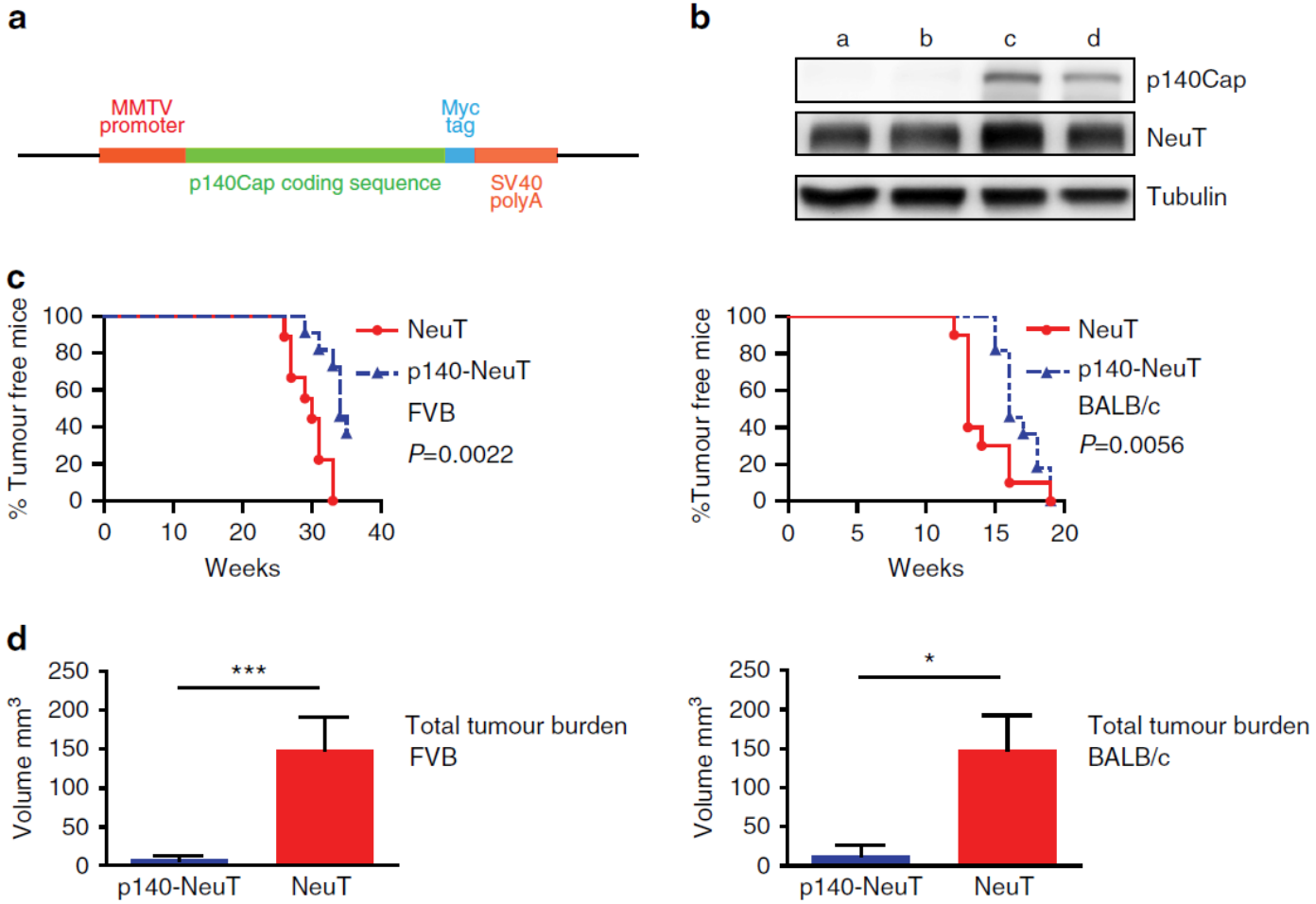
(European Institute of Oncology, Milano, Italy,from 2000)

Patients with high expression of p140Cap display a lower risk of developing distant metastases and of death from breast cancer

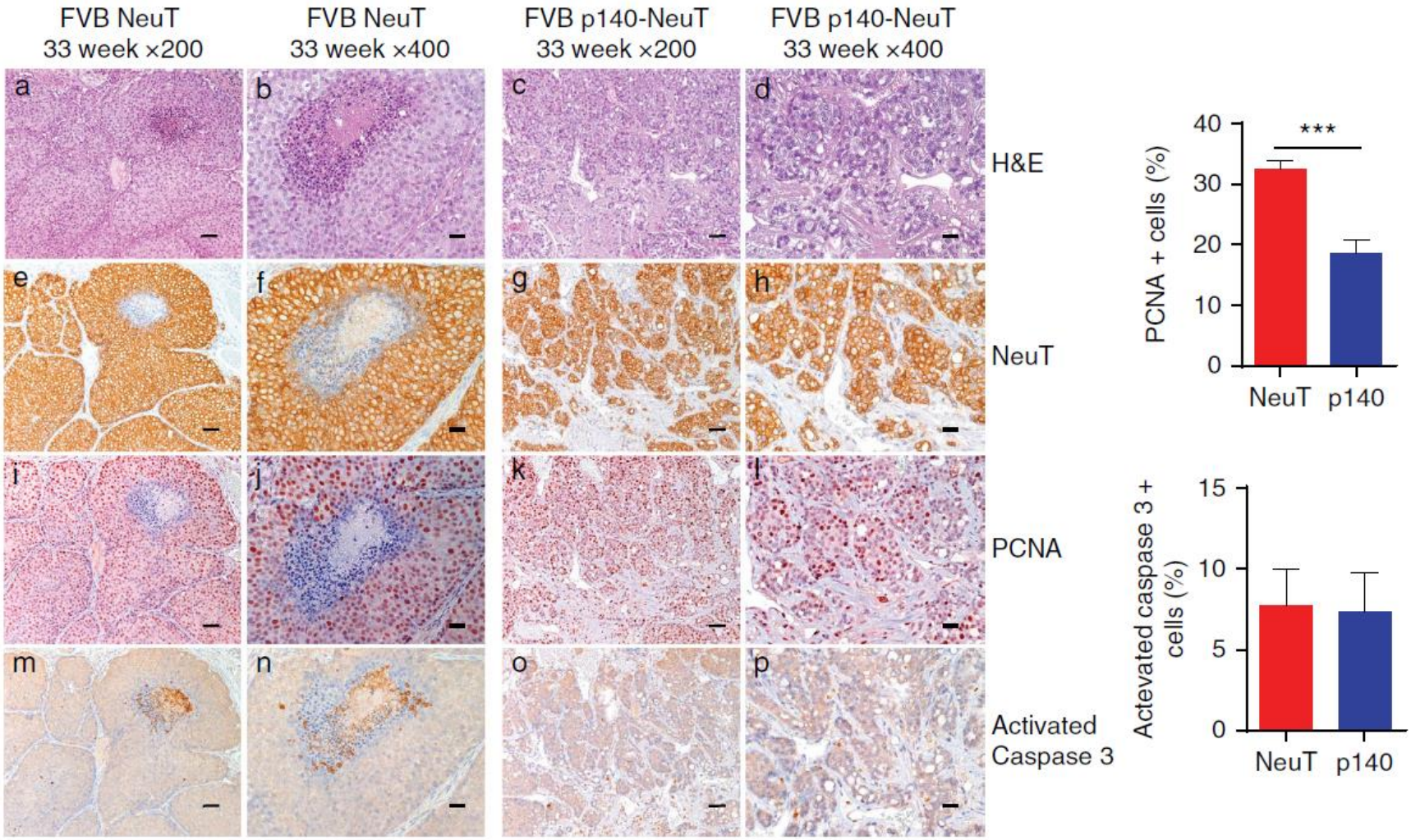


p140Cap protein over-expression limits tumorigenicity in the NeuT mice

Generation of p140-NeuT double Tg mice



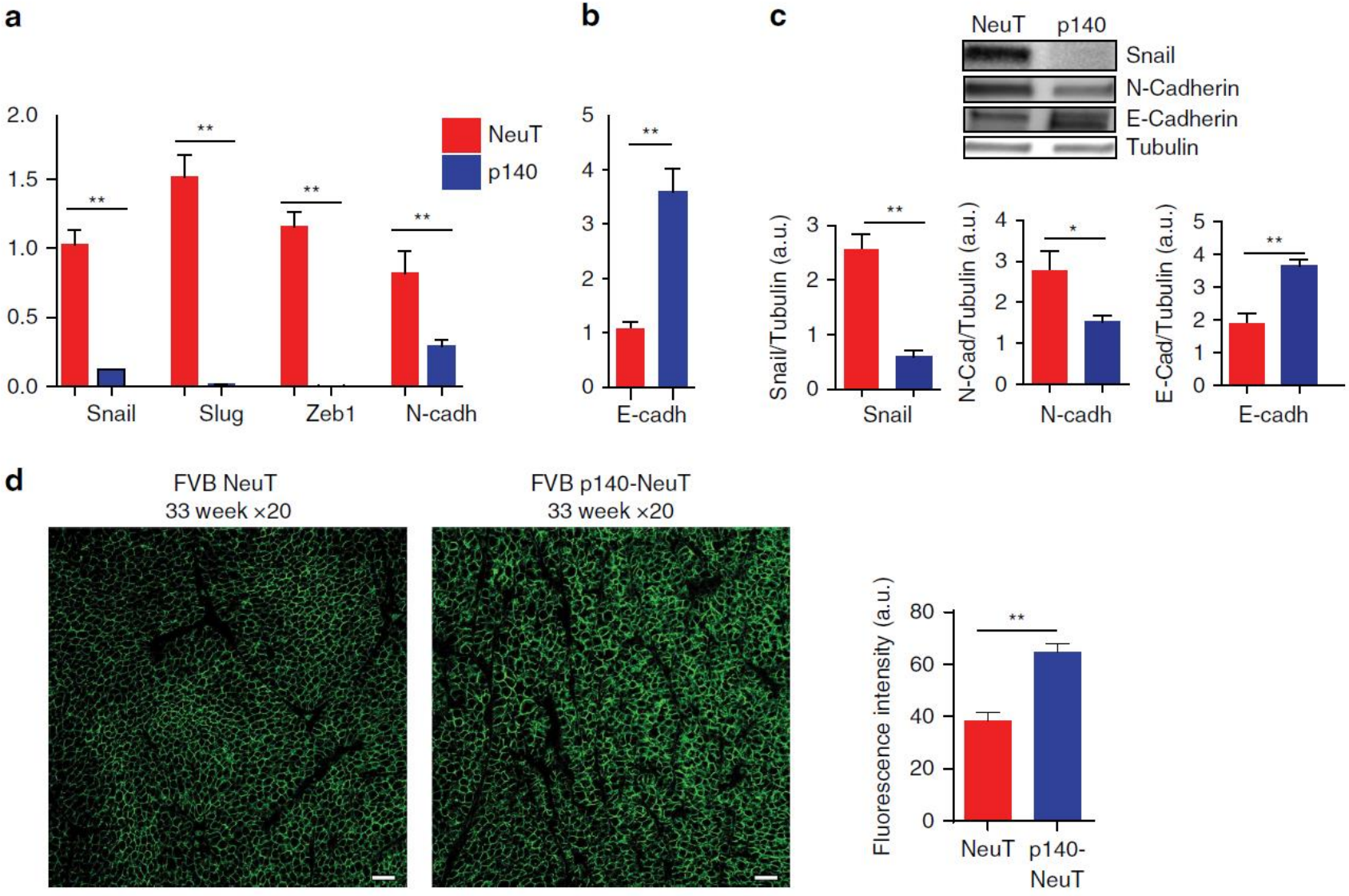
Morphological differences in the appearance of the two types of tumours



Large solid nodules

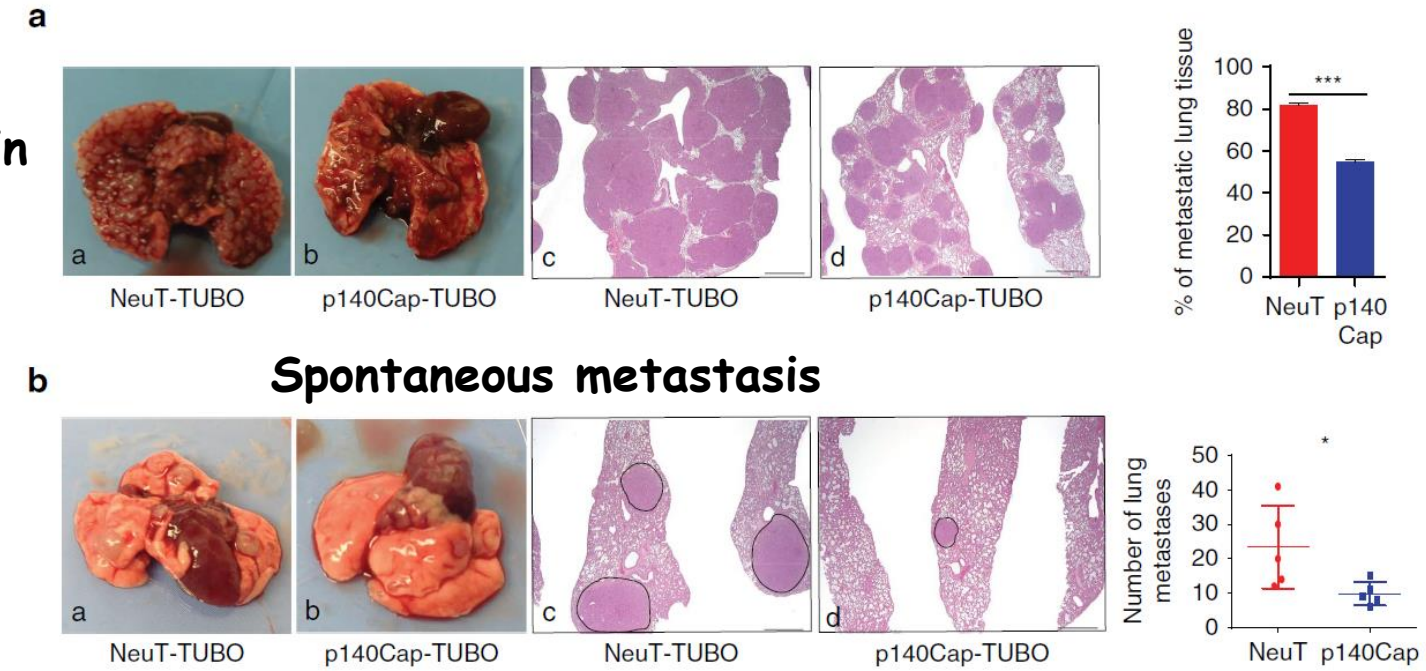
Smaller nodules and sheets of cells separated by more abundant stroma

p140Cap limits EMT in the NeuT cells

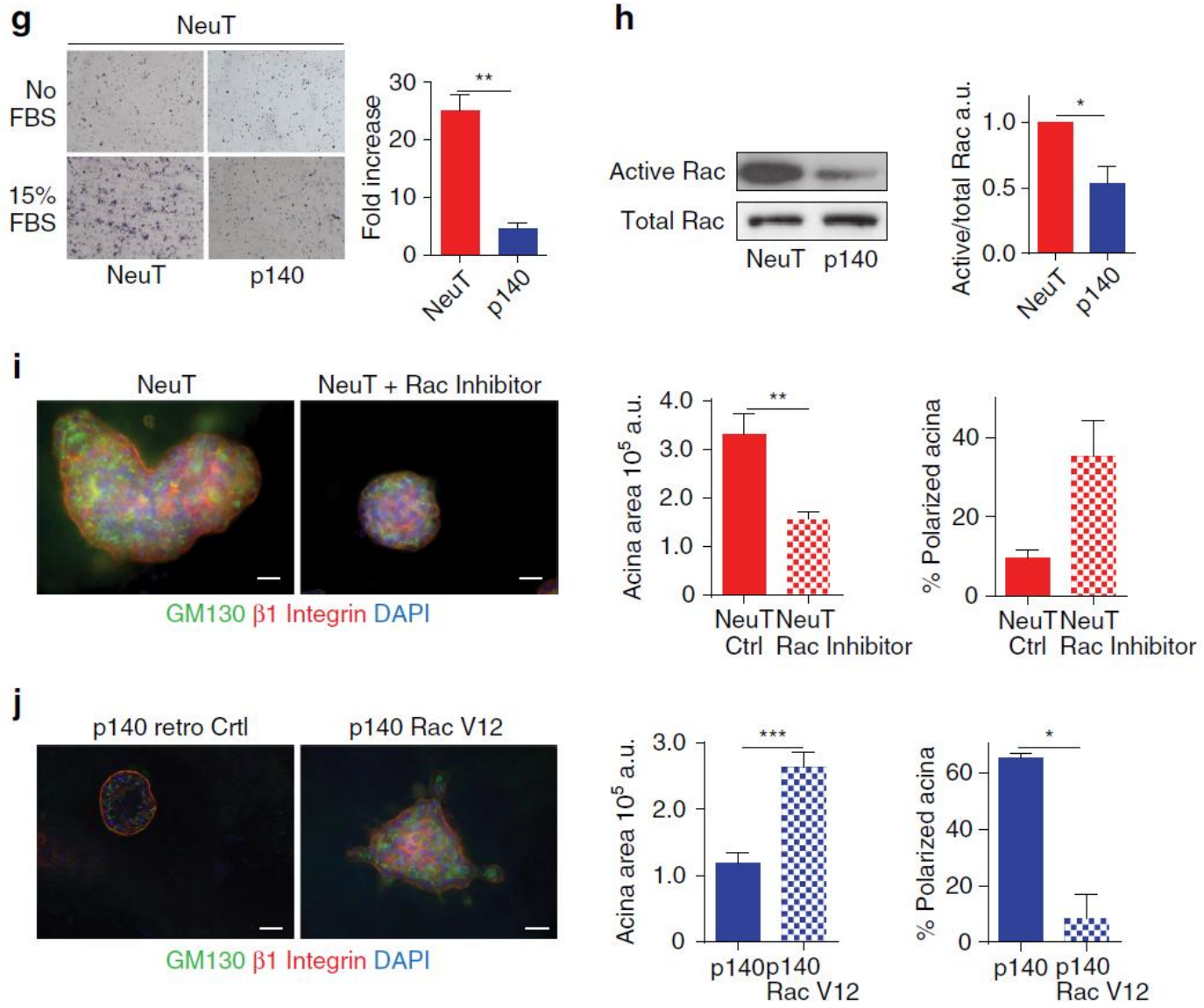


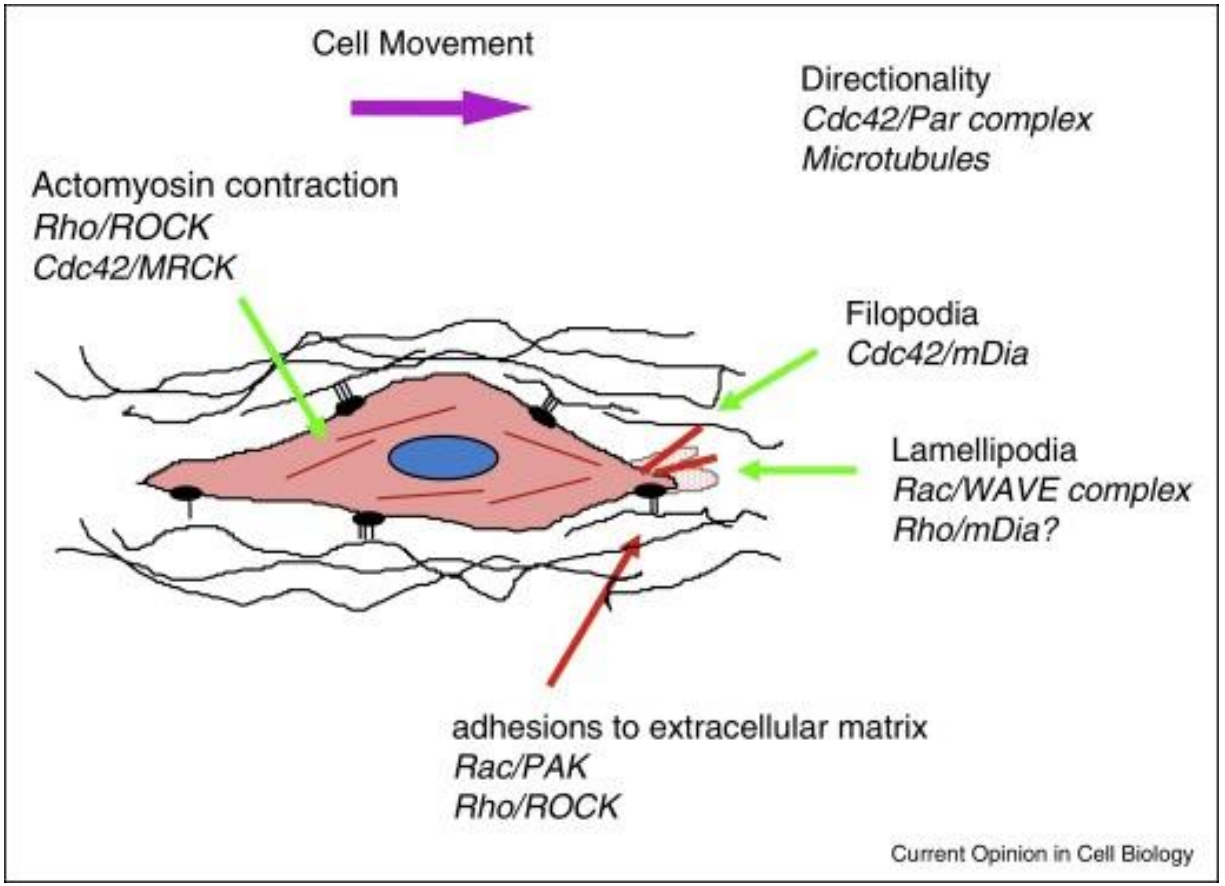
p140Cap limits metastasis in NeuT expressing cells

Tail vein



p140Cap attenuates NeuT-driven migratory ability and Rac activation

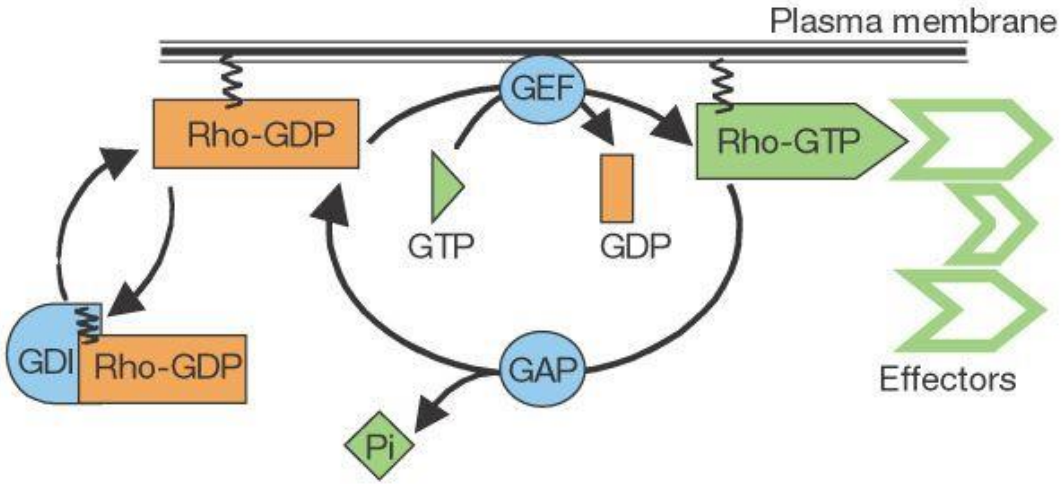




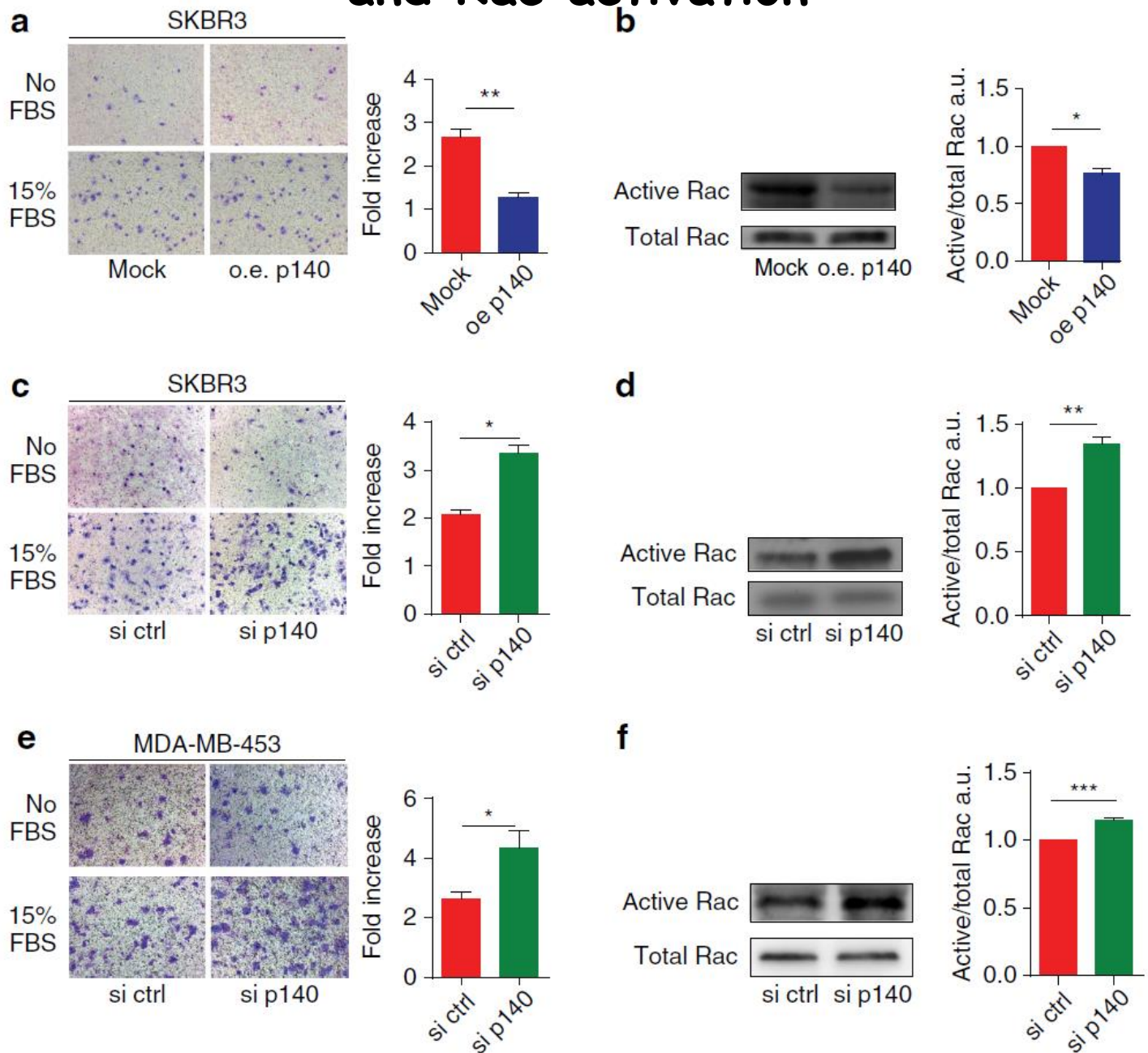
Rho

Rac

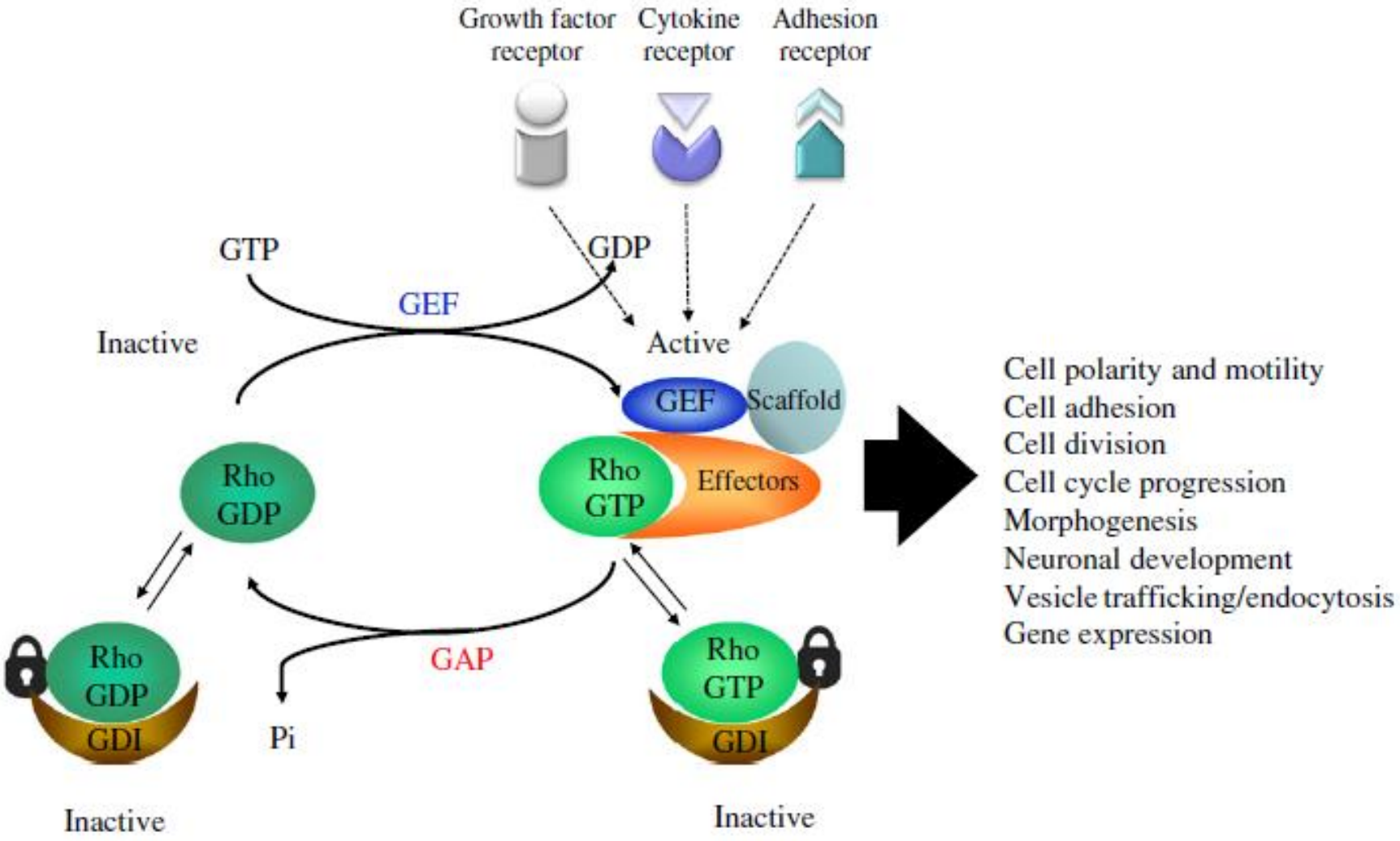
Cdc42



p140Cap attenuates ERBB2-driven migratory ability and Rac activation



Rho family dependent circuitries

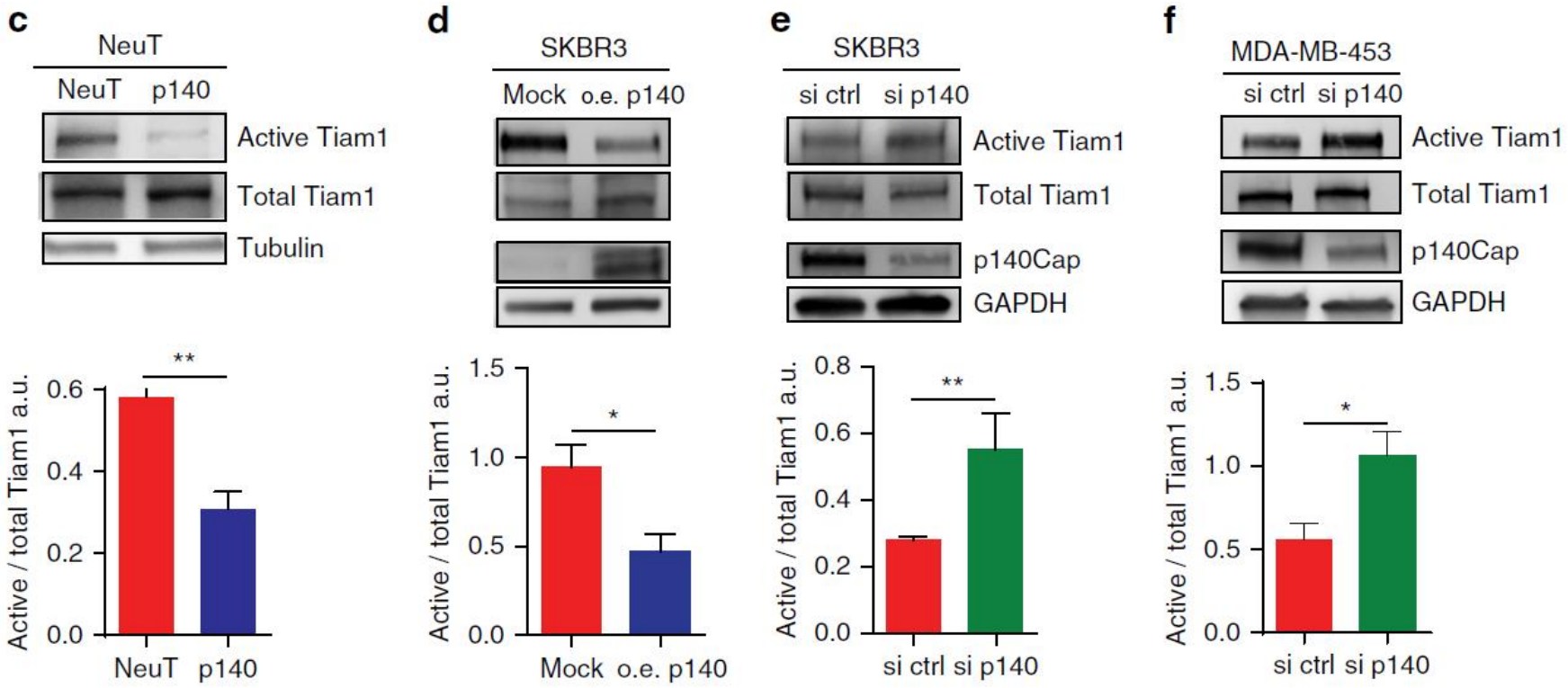


Boissier et al., 2014

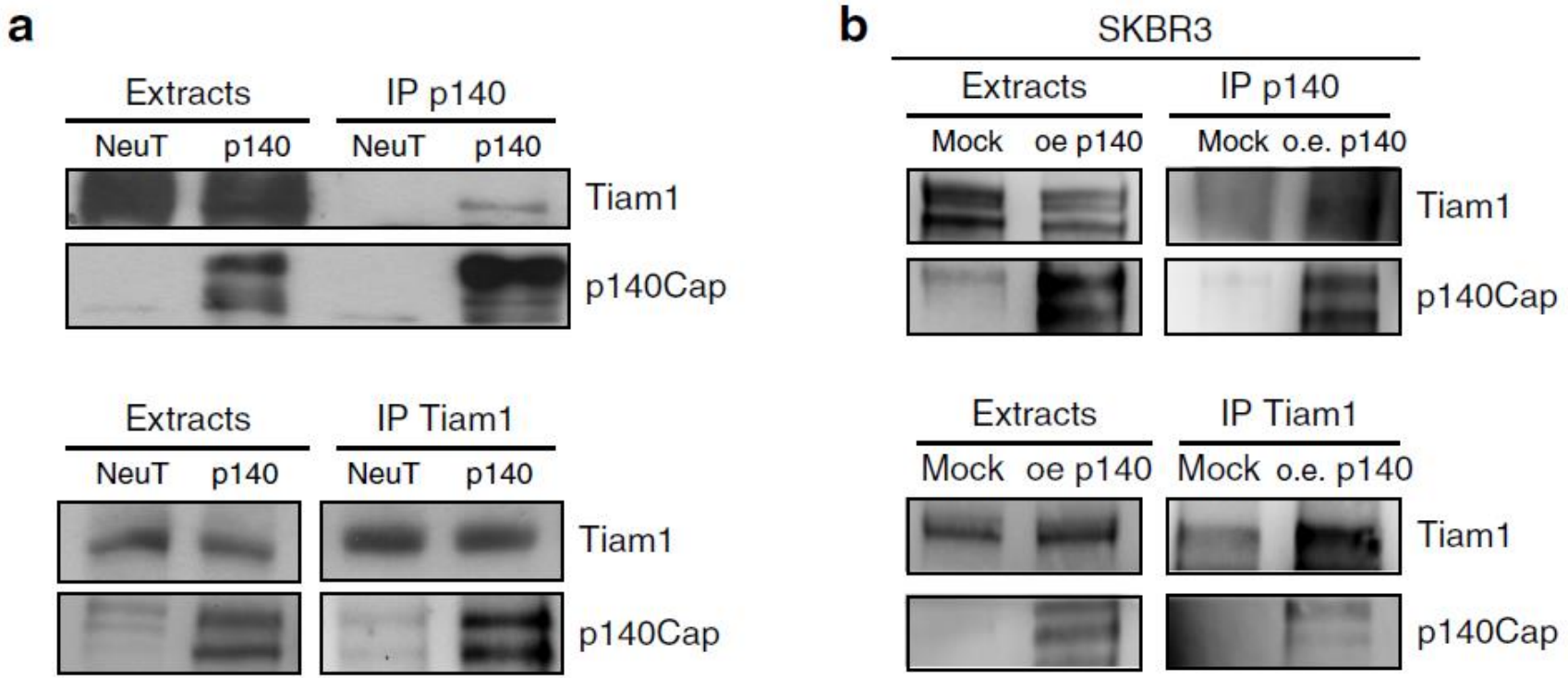
p140Cap limits the activation of the Rac1 GEF Tiam1

Expression

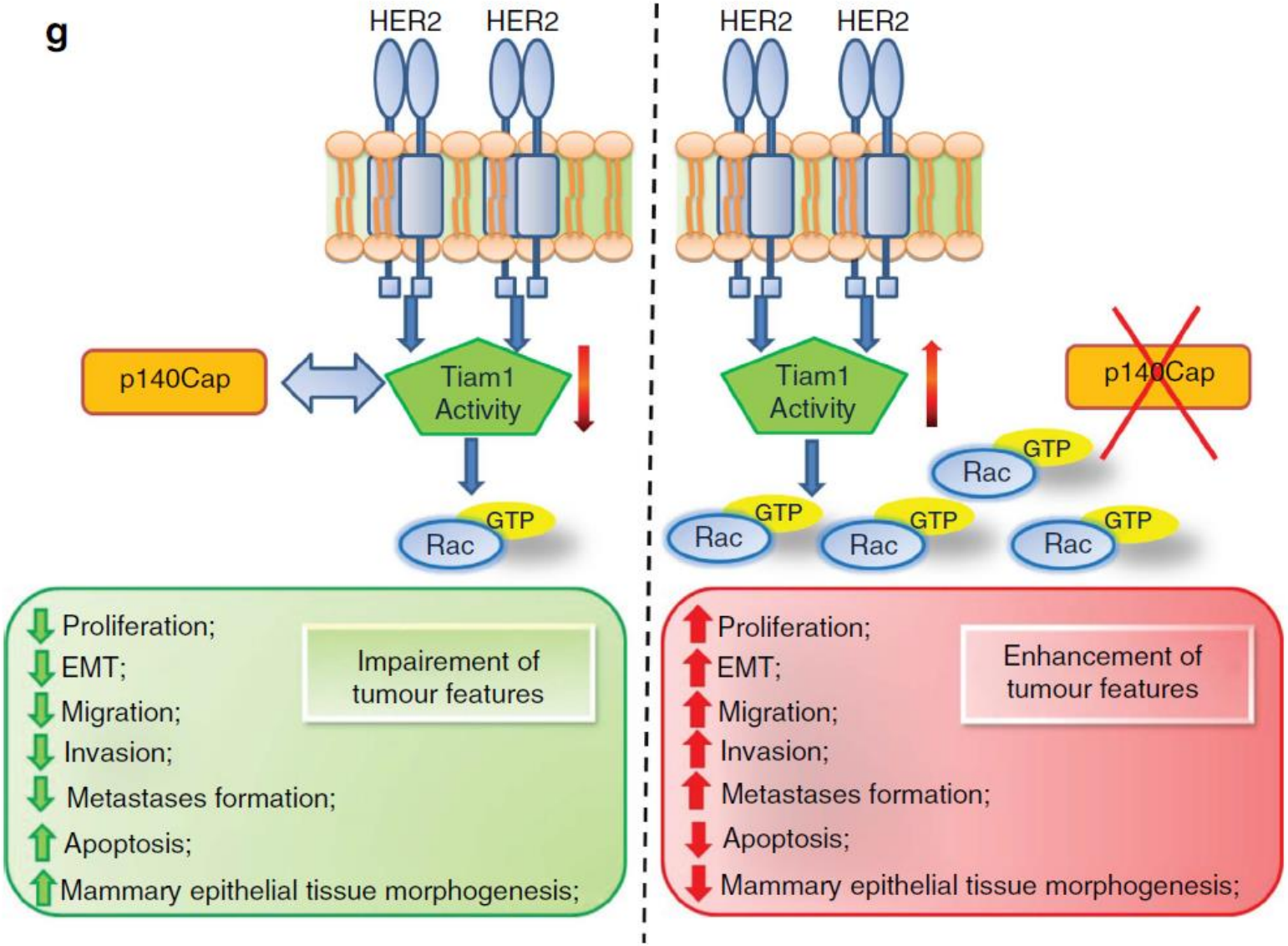
Silencing



p140Cap/Tiam1 association may impact on the activity of Tiam1 negatively regulating Rac1 circuitries in HER2 tumors



Conclusion I



Cell Death & Differentiation
https://doi.org/10.1038/s41418-019-0386-6

Cell Death & Differentiation

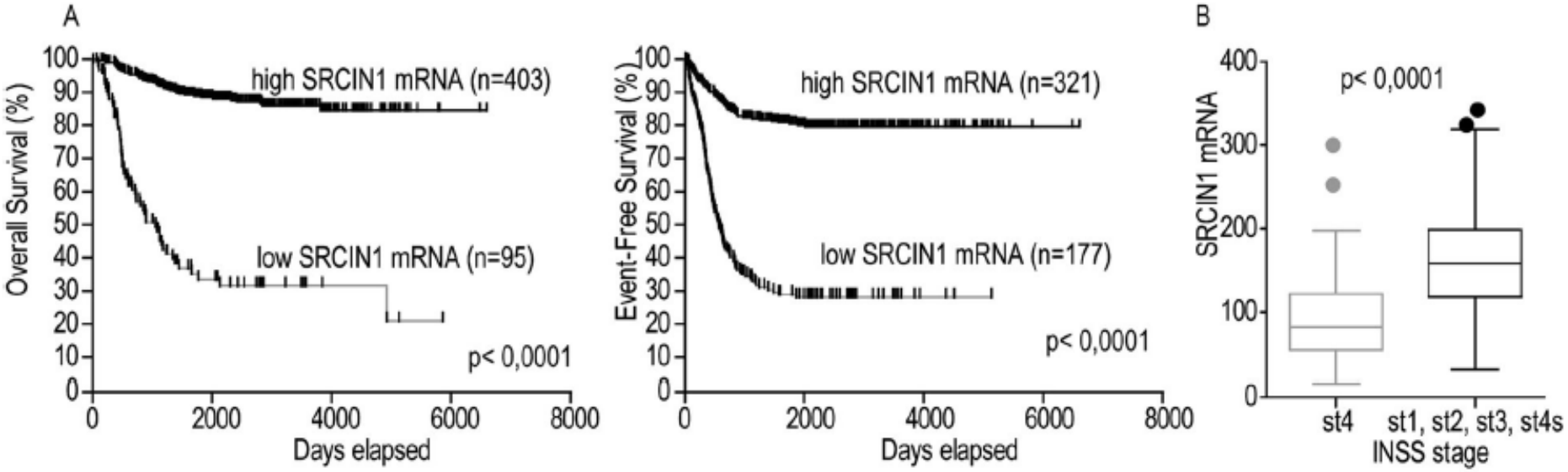
ARTICLE

The SRCIN1/p140Cap adaptor protein negatively regulates the aggressiveness of neuroblastoma

Silvia Grasso[1] · Davide Cangelosi[2] · Jennifer Chapelle[1] · Melissa Alzona[1] · Giorgia Centonze[1] · Alessia Lamolinara[3] · Vincenzo Salemme[1] · Costanza Angelini[1] · Alessandro Morellato[1] · Andrea Saglietto[4] · Federico Tommaso Bianchi[1,5] · Sara Cabodi[1] · Iris Chiara Salaroglio[1,6] · Federica Fusella[1] · Marzia Ognibene[7] · Manuela Iezzi[3] · Annalisa Pezzolo[7] · Valeria Poli[1] · Ferdinando Di Cunto[5] · Alessandra Eva[2] · Chiara Riganti[6] · Luigi Varesio[2] · Emilia Turco[1] · Paola Defilippi[1]

Received: 14 September 2018 / Revised: 21 May 2019 / Accepted: 21 June 2019

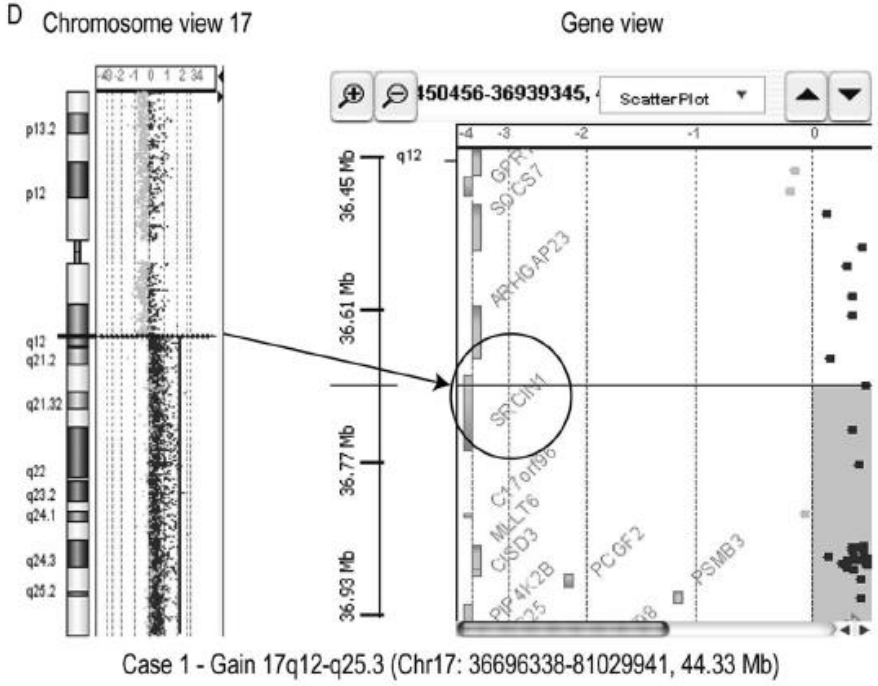
SRCIN1 mRNA is a prognostic risk factor for NB



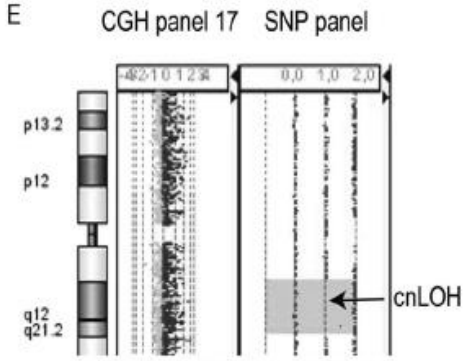
C

Covariate	Multivariate cox analysis (OS)				Multivariate cox analysis (EFS)			
	coefficient	HR	95% CI	P-value	coefficient	HR	95% CI	P-value
SRCIN1 (Low vs High)	-1.07	0.34	(0.2,0.5)	1.20E -05	-1.3	0.3	(0.1,0.3)	2.30E -11
Age group (<=12 months vs > 12 months	0.9	2.5	(1.3,4.7)	4.00E -03	-	-	-	
INSS stage (1, 2, 3, 4s vs 4	1.3	3.7	(1.5,5.5)	3.10E -07	-	-	-	
MYC status (normal vs amplified	0.7	2.2	(1.3,3.5)	1.00E -03	0.1	1.1	(0.8,1.6)	1.00E -04

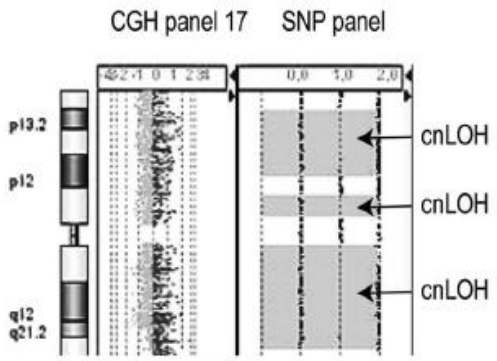
SRCIN1 mRNA is **a risk factor independent** from the other known risk factors, such as MYCN oncogene amplification, INSS stage and age at diagnosis, so far the strongest indicators of aggressive tumor behavior in NB patients.



Case 1 - Gain 17q12-q25.3 (Chr17: 36696338-81029941, 44.33 Mb)



Case 8 - cn(LOH) 17q11.2-q21.2
(Chr17: 31571877-40588363, 9.01 Mb)



Case 6 - cn(LOH) 17q11.1-q21.31
(Chr17: 25569094-42949451, 17.38 Mb)

- The *SRCIN1* gene is located on chromosome 17q12, a region frequently altered in NB, and associated with poor prognosis.

- The 17q gain, the most frequent chromosome imbalance occurring in 50–70% of all high stage
- NB, associates with poor prognosis as an independent
- indicator of adverse outcome.

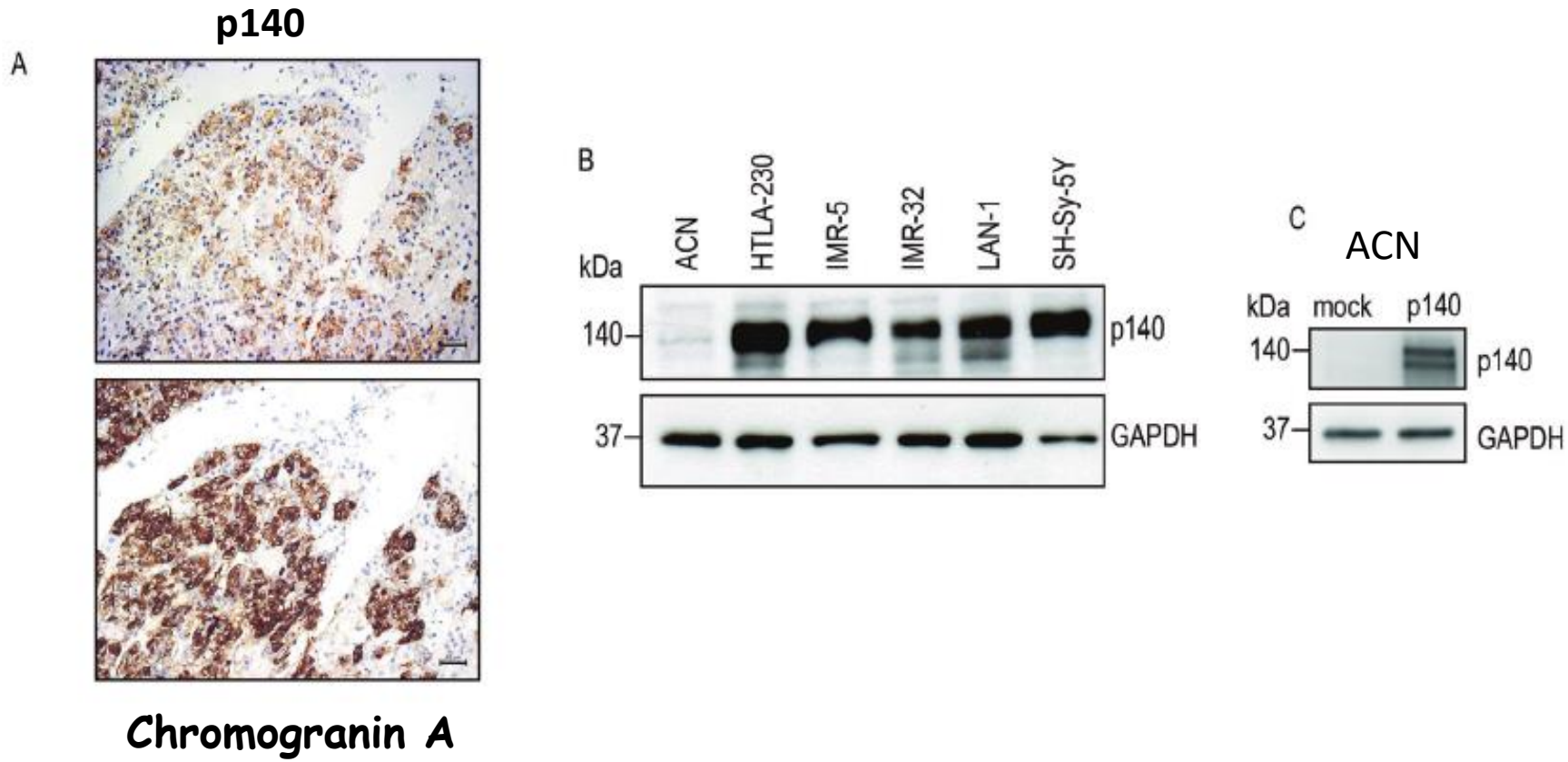
- The status of the *SRCIN1* gene in NB was assessed by high-resolution oligonucleotide a-CGH and SNP-array on 225 NB primary tumors of all stages with 17q gain.

Table 1 *SRCIN1* loss/cn-LOH or disruption in the breakpoint on 17 NB patients

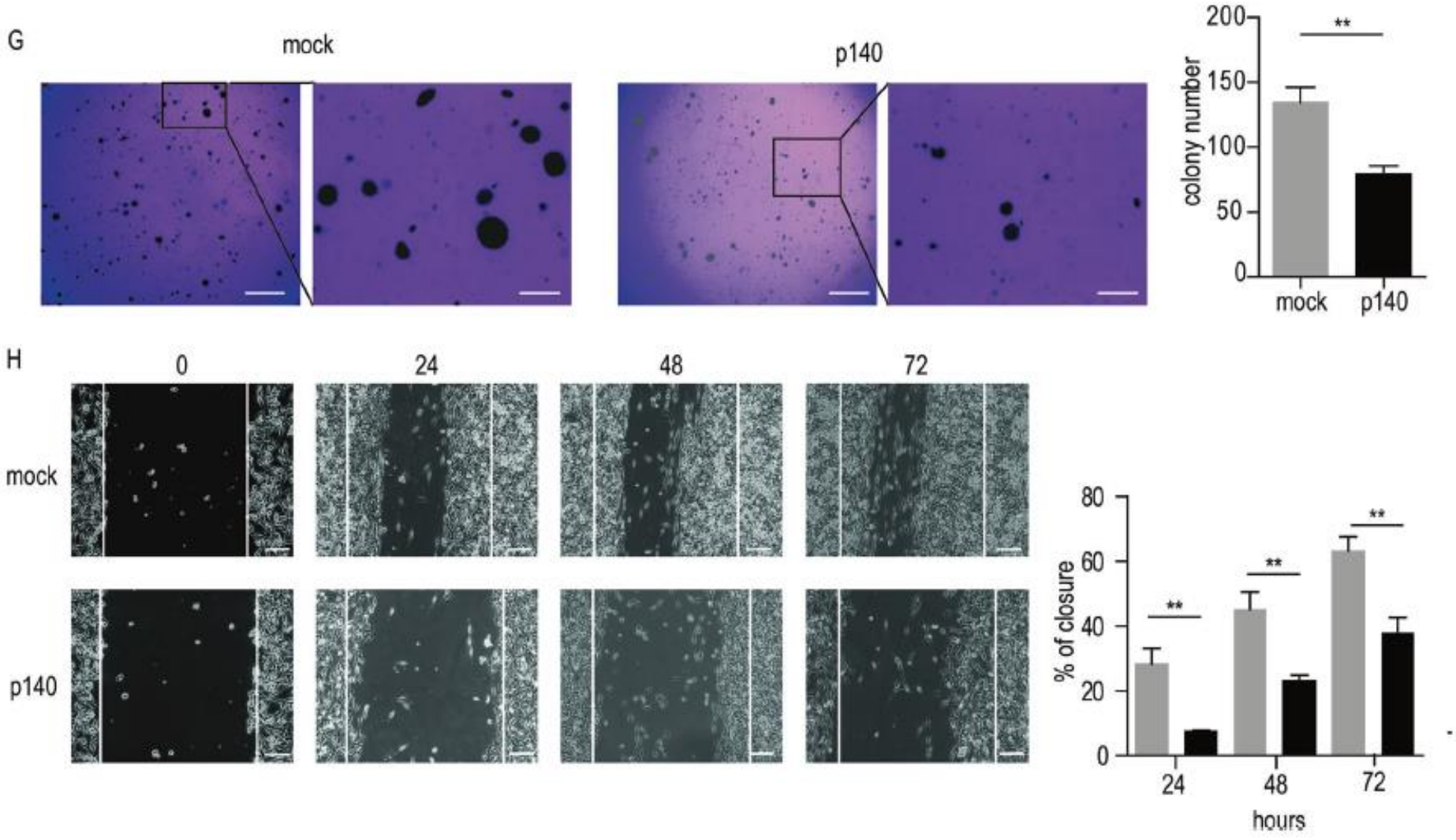
NB patients	*SRCIN1* gene status	Chromosomal coordinates
Case 1	disrupted in the breakpoint	Chr17: 36696338-81029941 Cytoband: 17q12-q25.3 Size: 44.33 Mb
Case 2	disrupted in the breakpoint	Chr17: 36694901-80943345 Cytoband: 17q12-q25.3 Size: 44.24 Mb
Case 3	loss	Chr17: 25311574-36777884 Cytoband: 17q11.1-q12 Size: 11.46 Mb
Case 4	disrupted in the breakpoint	Chr17: 36696338-80969424 Cytoband: 17q12-q25.3 Size: 44.27 Mb
Case 5	disrupted in the breakpoint	Chr17: 36696279-81029941 Cytoband: 17q12-q25.3 Size: 44.33 Mb
Case 6	copy neutral LOH	Chr17: 25569094-42949451 Cytoband: 17q11.1-q21.31 Size: 17.38 Mb
Case 7	copy neutral LOH	Chr17: 29149425-45297941 Cytoband: 17q11.1-q21.31 Size: 16.14 Mb
Case 8	copy neutral LOH	Chr17: 31571877-40588363 Cytoband: 17q11.2-q21.2 Size: 9.01 Mb
Case 9	loss	Chr17: 25278114-37876263 Cytoband: 17q11.1-q12 Size: 12.59 Mb
Case 10	loss	Chr17: 25278114-68301170 Cytoband: 17q11.1-q24.3 Size: 43.02 Mb
Case 11	disrupted in the breakpoint	Chr17: 36696279-81029941 Cytoband: 17q12-q25.3 Size: 44.33 Mb
Case 12	disrupted in the breakpoint	Chr17: 36696338-81029941 Cytoband: 17q12-q25.3 Size: 44.33 Mb
Case 13	disrupted in the breakpoint	Chr17: 36740844-80943189 Cytoband: 17q12-q25.3 Size: 44.20 Mb
Case 14	disrupted in the breakpoint	Chr17: 36740903-80993001 Cytoband: 17q12-q25.3 Size: 44.25 Mb
Case 15	loss	Chr17: 25278114-81029941 Cytoband: 17q11.1-q25.3 Size: 55.75 Mb
Case 16	disrupted in the breakpoint	Chr17: 36672992-77470237 Cytoband: 17q12-q25.3 Size: 40.79 Mb
Case 17	disrupted in the breakpoint	Chr17: 36694044-81099040 Cytoband: 17q12-q25.3 Size: 44.40 Mb

- in 4 patients *SRCIN1* was hemizygously deleted
- in 3 patients was subjected to copy-neutral Loss Of Heterozigosity (cn-LOH)
- in 10 patients was disrupted because located at breakpoint of 17q segment involved in generation of 17q gain

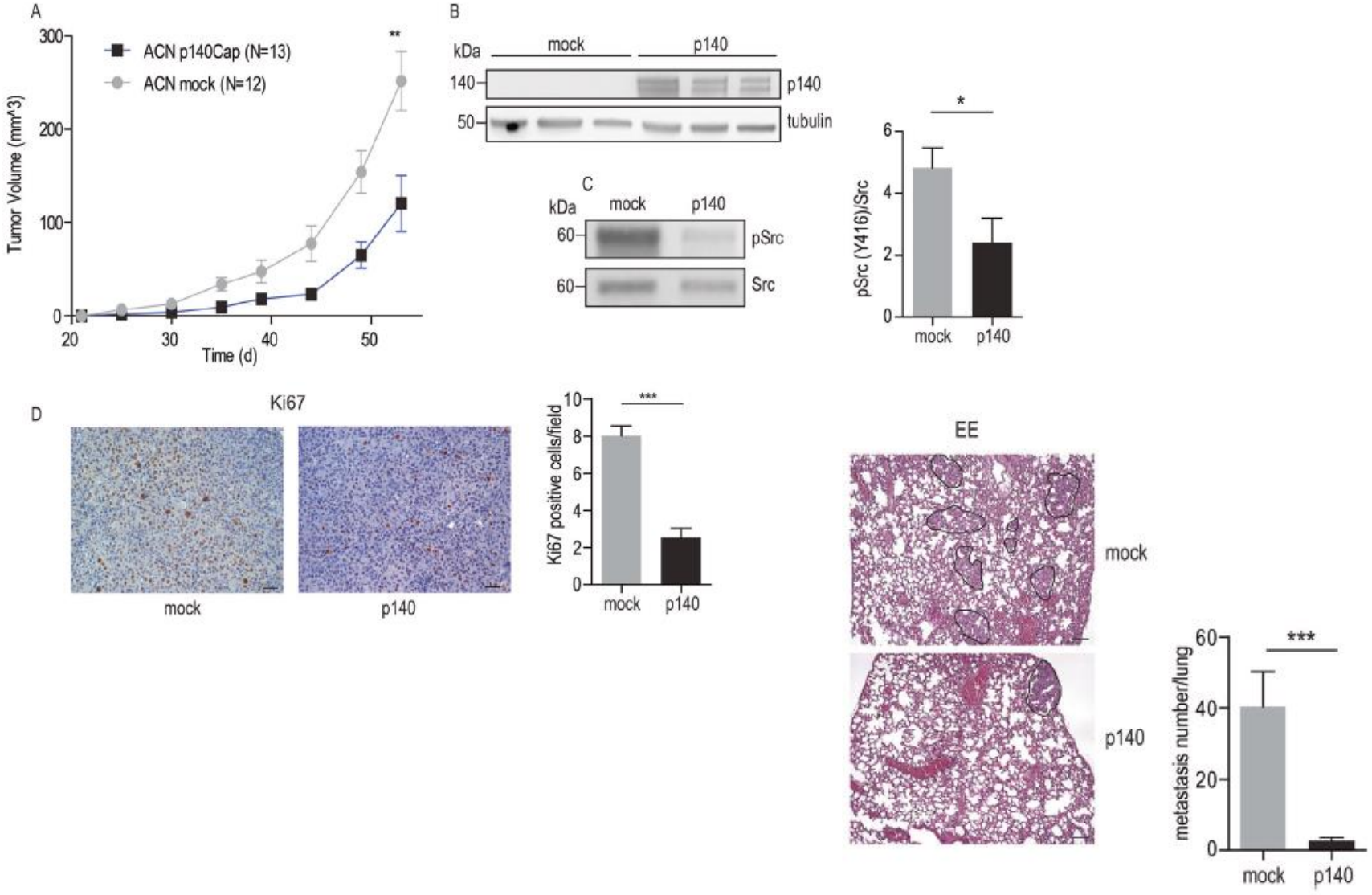
p140Cap is expressed in human neonatal adrenal gland and in NB cell lines



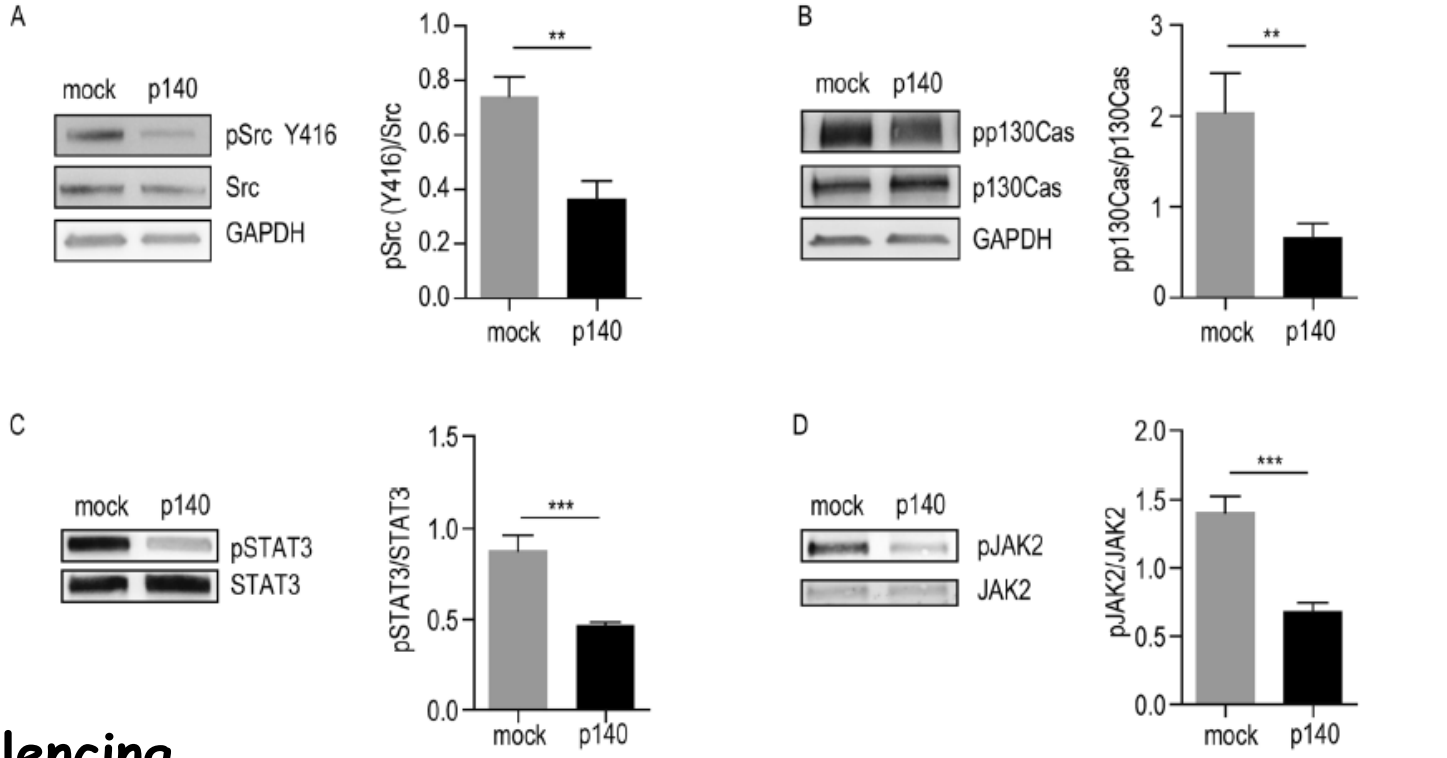
p140Cap negatively affects tumorigenic features



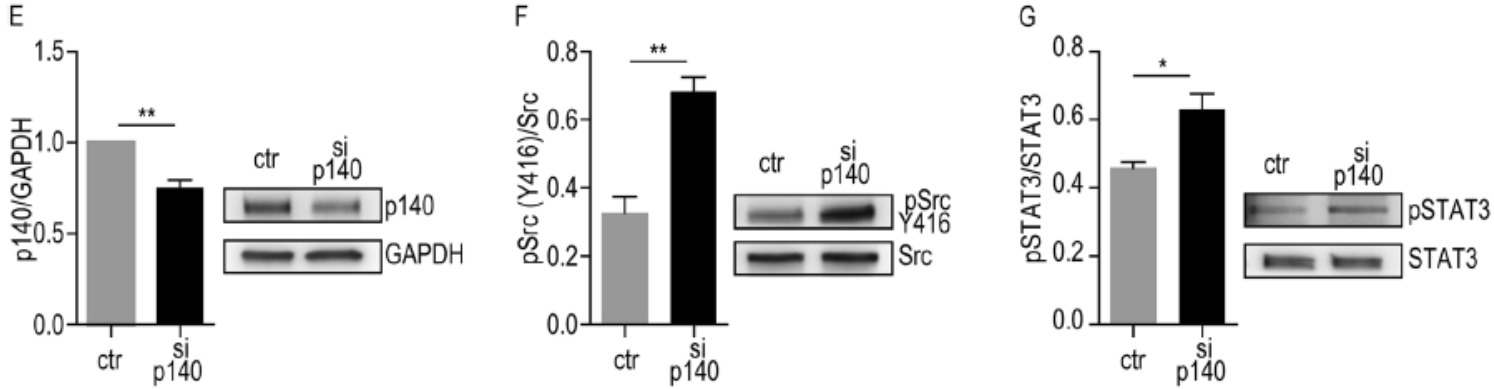
p140Cap dampens in vivo tumor growth and spontaneous metastasis formation



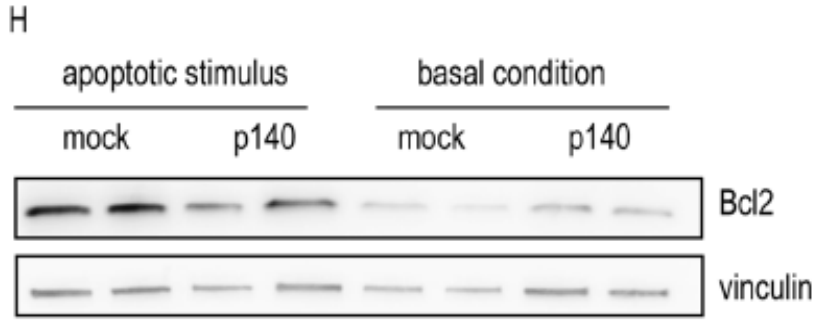
p140Cap impairs the Src/p130Cas and the STAT3/ Jak2 signaling pathways

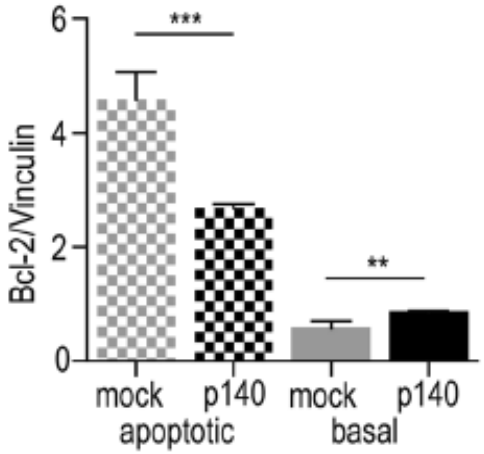


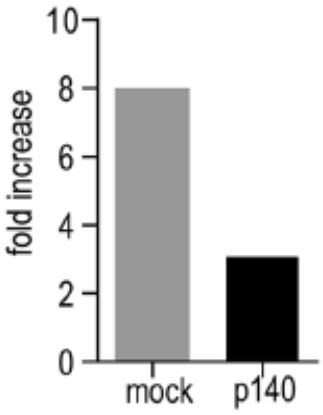
p140Cap silencing

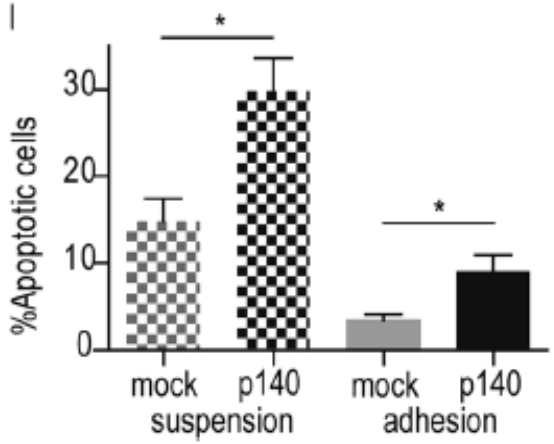


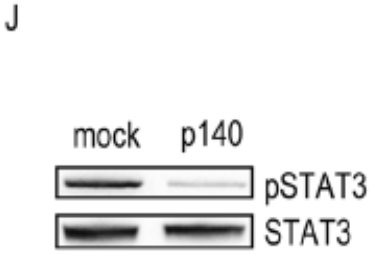
p140Cap limits the ability of NB cells to induce survival pathways upon anoikis

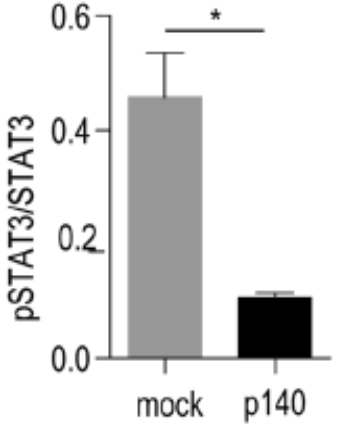




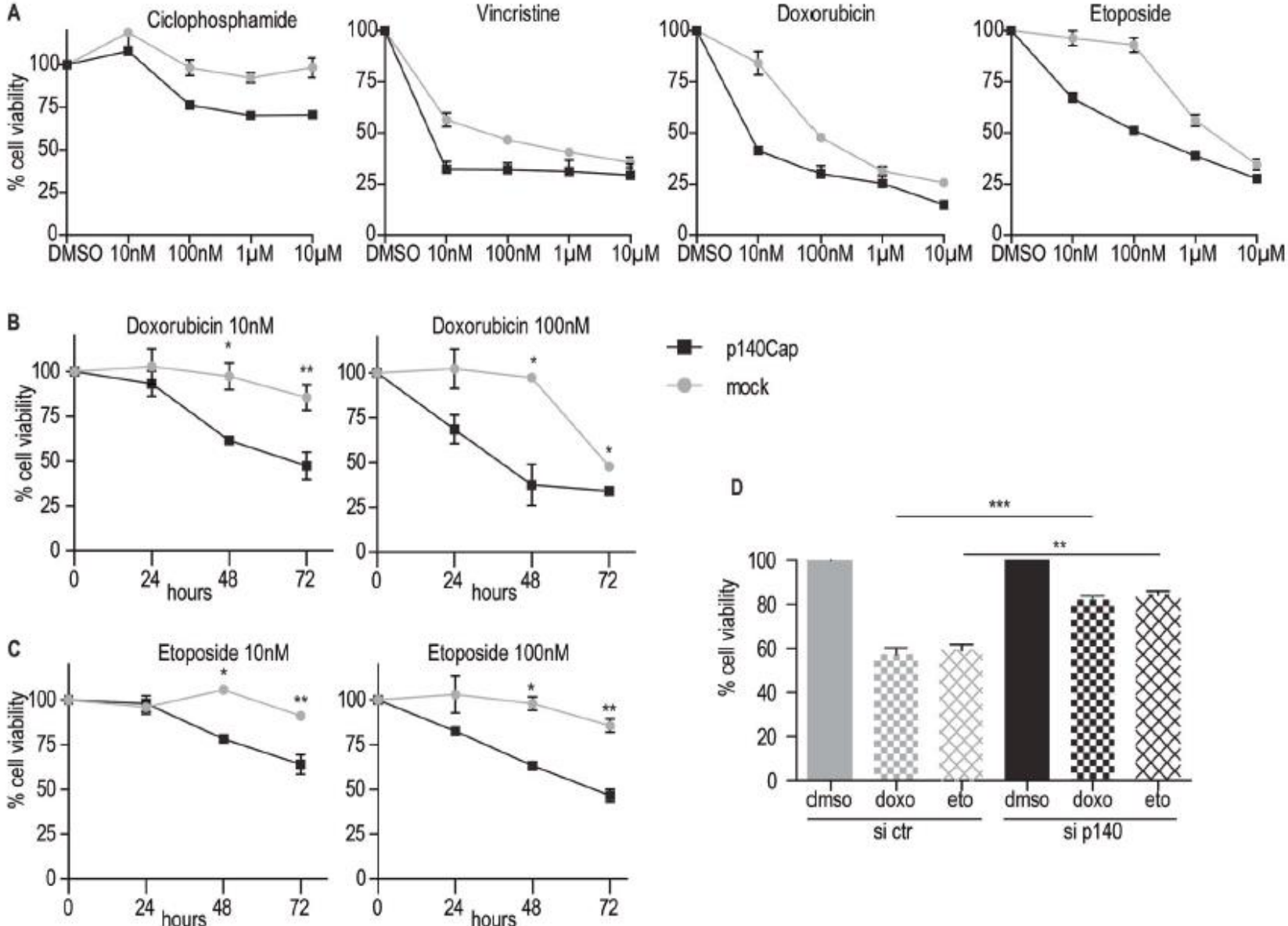




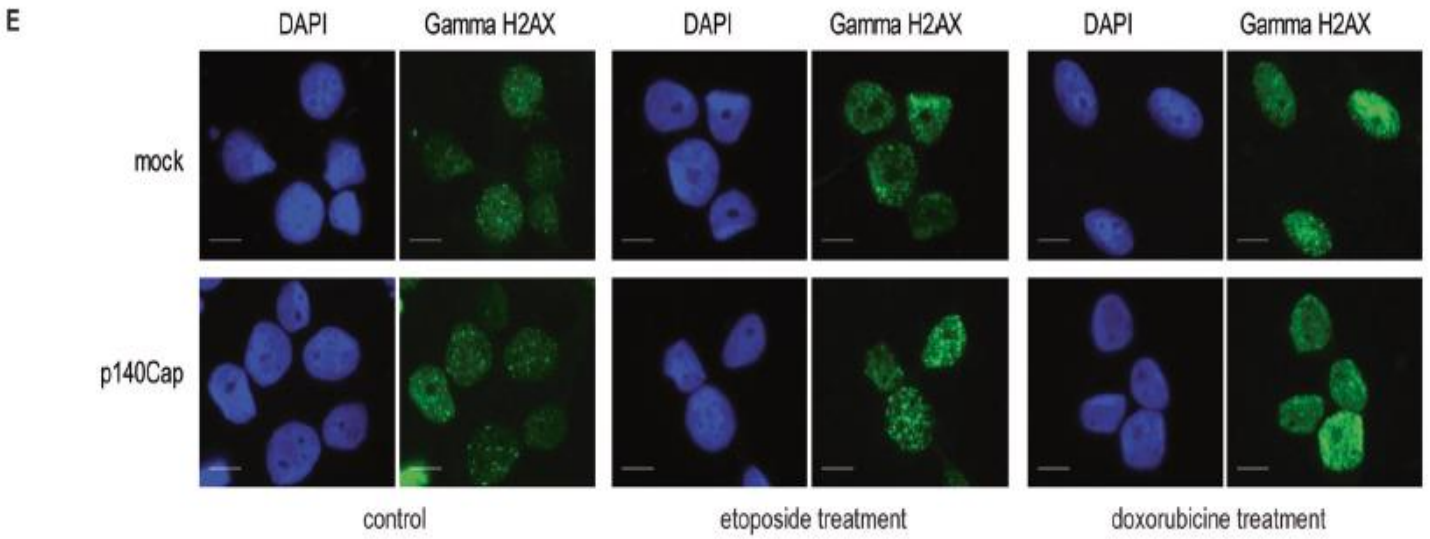




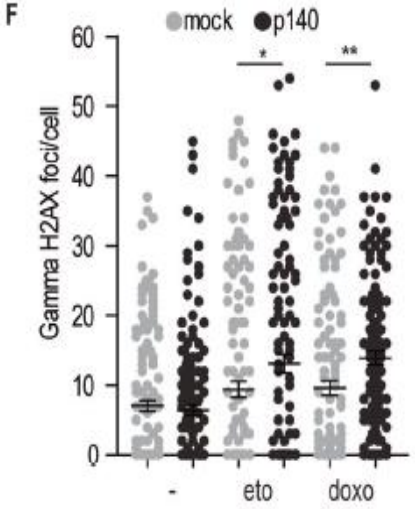
p140Cap increases NB cell sensitivity to chemotherapeutic treatment with a significant decrease in cell viability

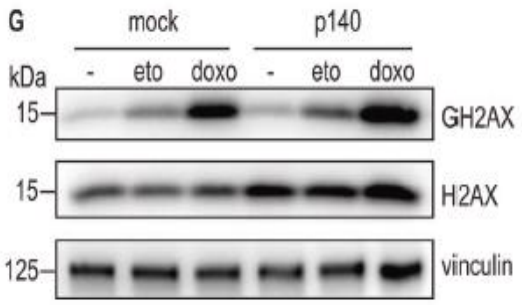


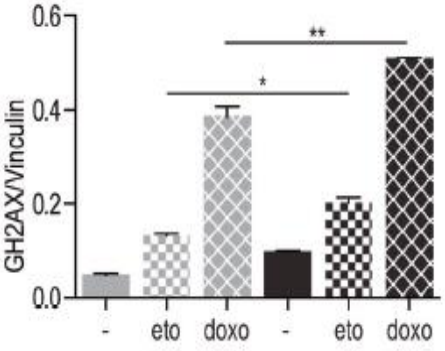
p140 cells display an increased sensitivity to drug-dependent DNA damage.



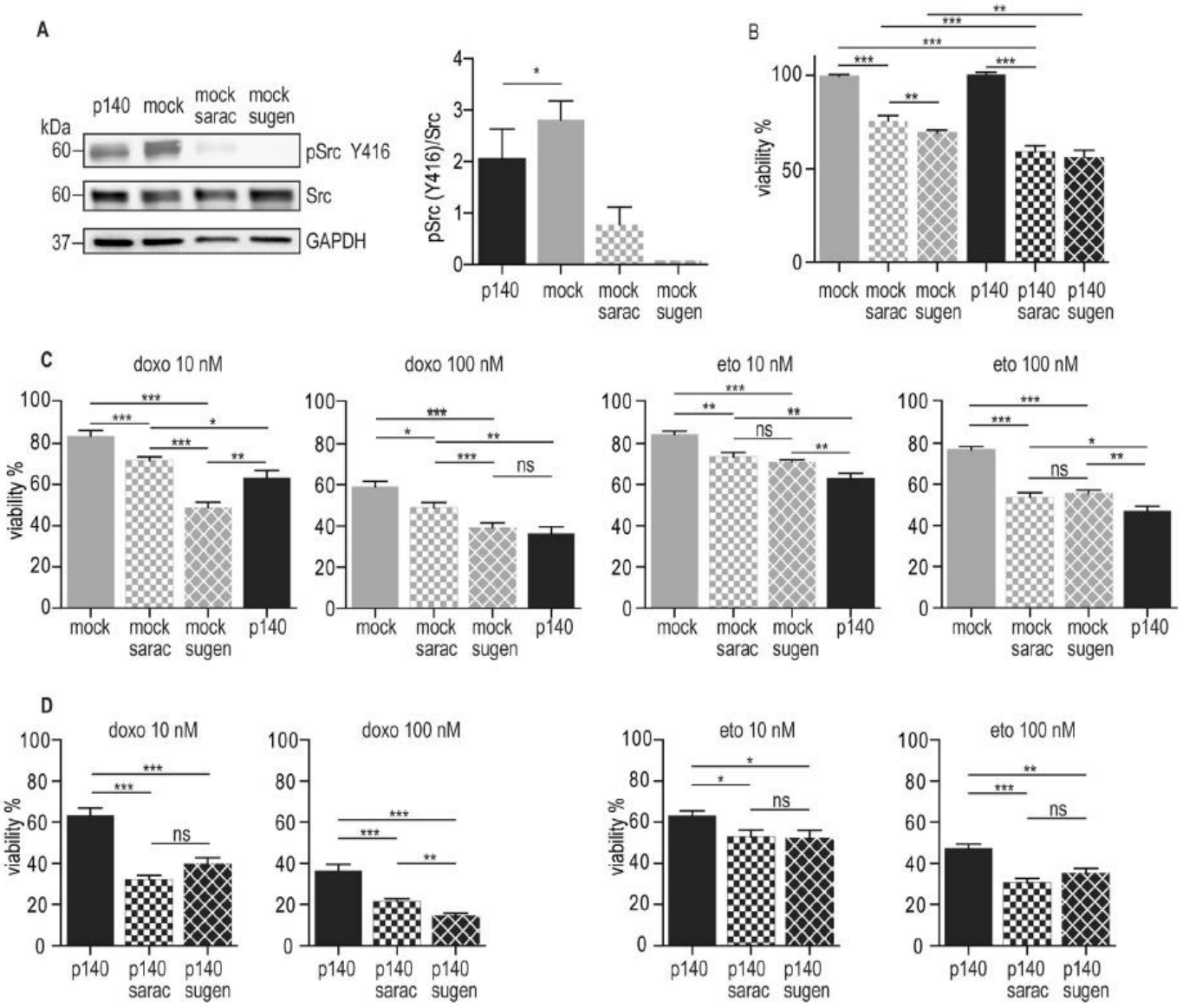
Etoposide and doxorubicin prevent ligation of the DNA strands, stopping the process of replication.







p140Cap sensitizes cells to combined treatment with chemotherapy and Src kinase inhibitors



The combination of Src inhibitors with doxorubicin or etoposide, sensitize mock cells, reducing cell viability to that of p140 cells treated with chemotherapy alone

The combined treatment was synergistic both in mock and p140 cells, because the Combination Index (CI) values computed for the different combinations of drugs were <1 in all the experimental settings

treatment	Cell line	CI	DRI50	r	Cell line	CI	DRI50	r
Doxo, Sug	mock	0.08838	Doxo -12.39; Sug -130.442	0.97494	p140	0.17652	Doxo -5.814; Sug -221.354	0.93442
Doxo, Sara	mock	0.1775	Doxo -9.021; Sara -14.904	0.98575	p140	0.3368	Doxo -3.219; Sara -38.115	0.95530
Eto, Sug	mock	0.277	Eto -5.606; Sug -10.041	0.99194	p140	0.07017	Eto -18.881; Sug -58.115	0.96845
Eto, Sara	mock	0.08899	Eto -28.921; Sara -18.377	0.97889	p140	0.07185	Eto -26.208; Sara -29.676	0.92729

Conclusion II

Oncosuppressive function of *SRCIN1*/p140Cap in NB tumors.

- *SRCIN1* gene expression correlates with good outcomes in NB, likely due to the ability of the p140Cap protein to negatively regulate molecular pathways exploited for tumor progression.

- High levels of *SRCIN1* mRNA are clinically relevant in NB patients, positively correlating with good prognosis and high survival rate, both OS and EFS, meaning that SRCIN1 expression correlates with decreased metastatic recurrences in NB patients.

- *SRCIN1* expression is a risk factor independent from the other known risk factors, such as MYCN oncogene amplification, INSS stage and age at diagnosis, recognized as the strongest indicators of aggressive tumor behavior in NB patients.

- Thus, *SRCIN1* could provide a useful, additional marker for better stratifying NB patient cohorts.

Future directions

frontiers in Molecular Neuroscience

ORIGINAL RESEARCH
published: 30 June 2017
doi: 10.3389/fnmol.2017.00212

Synaptic Interactome Mining Reveals p140Cap as a New Hub for PSD Proteins Involved in Psychiatric and Neurological Disorders

Annalisa Alfieri[1†], Oksana Sorokina[2†], Annie Adrait[3,4,5], Costanza Angelini[1], Isabella Russo[1], Alessandro Morellato[1], Michela Matteoli[6,7], Elisabetta Menna[6,7], Elisabetta Boeri Erba[8,9,10], Colin McLean[2], J. Douglas Armstrong[2], Ugo Ala[1,11], Joseph D. Buxbaum[12,13,14,15,16,17], Alfredo Brusco[18,19], Yohann Couté[3,4,5], Silvia De Rubeis[12,13], Emilia Turco[1] and Paola Defilippi[1*]*

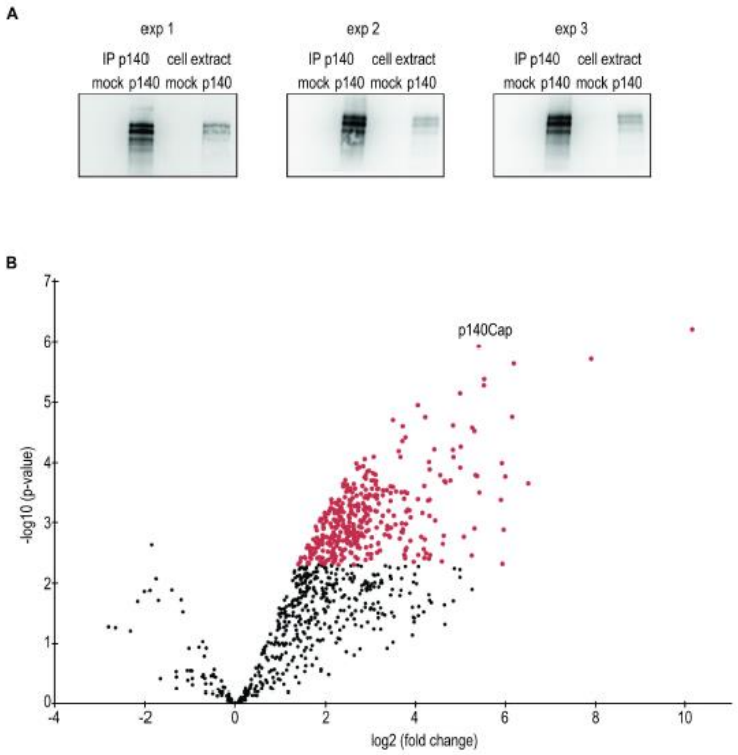
frontiers in Cell and Developmental Biology

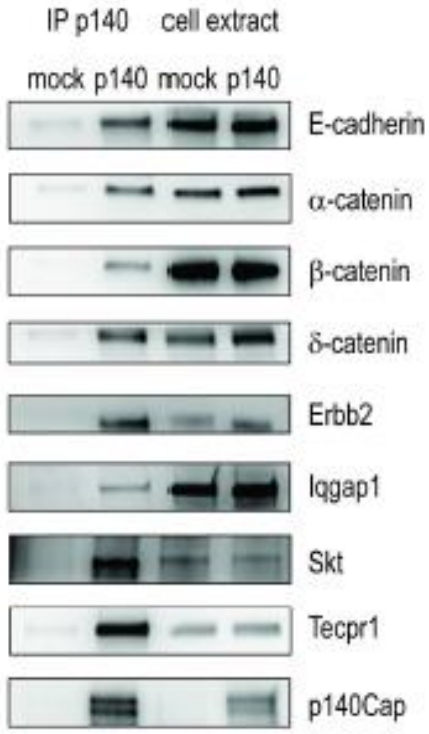
ORIGINAL RESEARCH
published: xx September 2019
doi: 10.3389/fcell.2019.00222

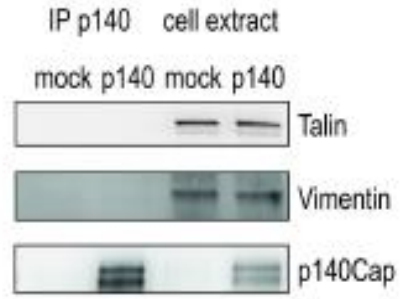
Dissecting the Shared and Context-Dependent Pathways Mediated by the p140Cap Adaptor Protein in Cancer and in Neurons

Jennifer Chapelle[1†], Oksana Sorokina[2†], Colin McLean[2†], Vincenzo Salemme[1], Annalisa Alfieri[1], Costanza Angelini[1], Alessandro Morellato[1], Annie Adrait[3], Elisabetta Menna[4,5], Michela Matteoli[4,5], Yohann Coute[3], Ugo Ala[6], Emilia Turco[1], Paola Defilippi[1*] and J. Douglas Armstrong[2*]*

To gain insight on p140Cap interacting proteins in breast cancer and the underlying molecular complexes:
p140Cap interactome from ERBB2-positive breast cancer cells







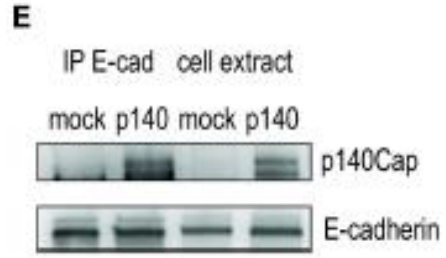
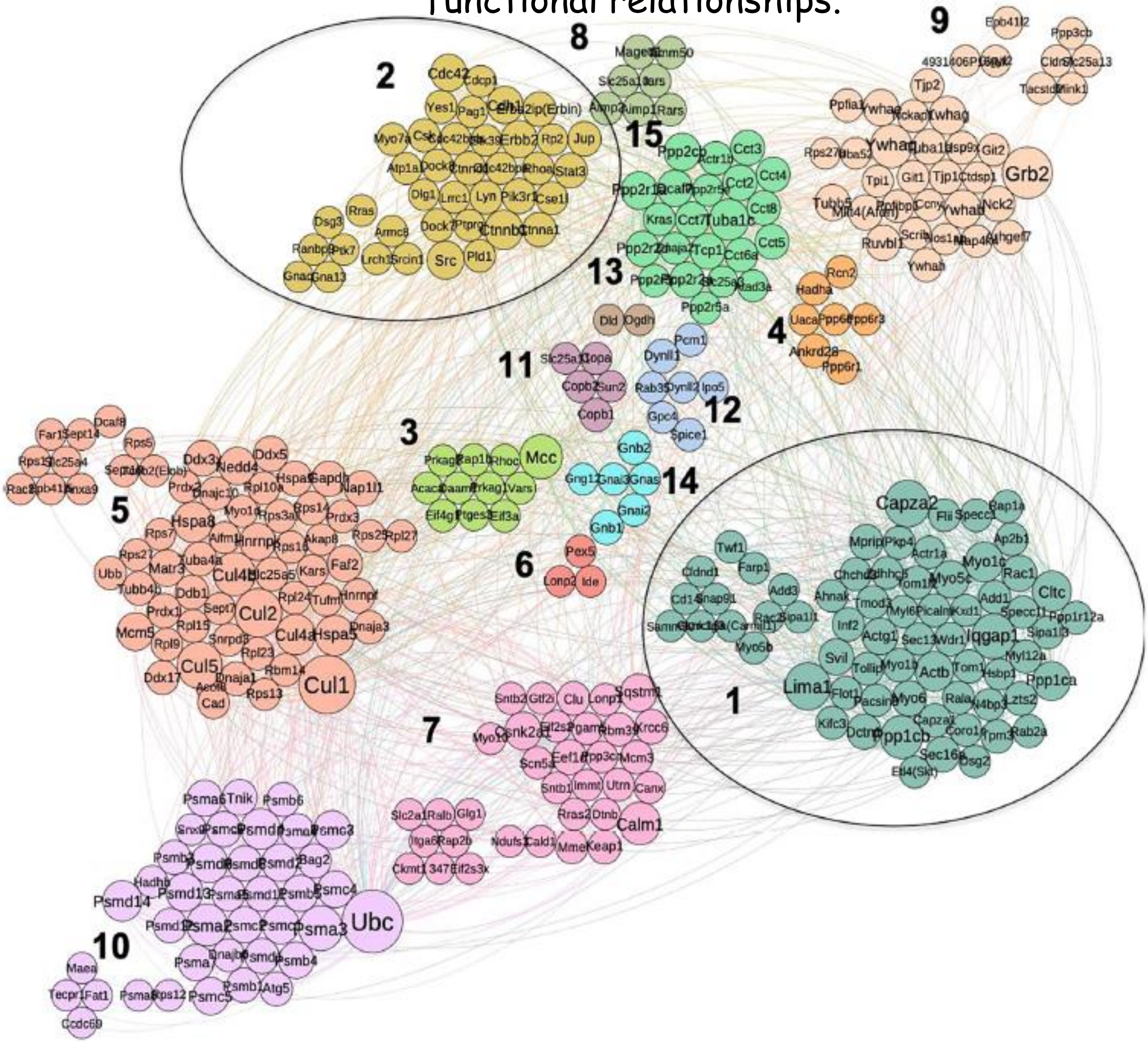


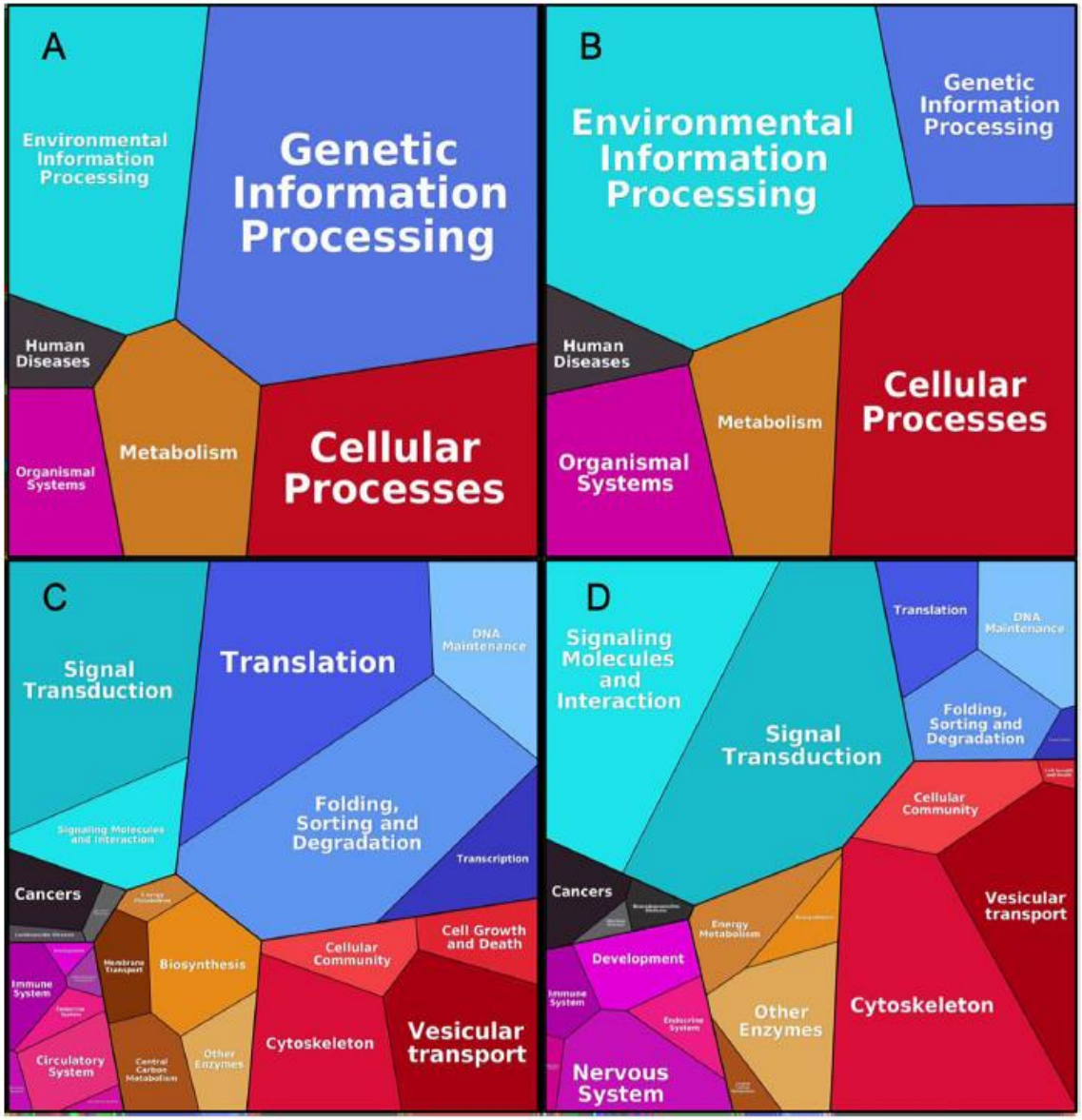
TABLE 1 | Top enrichment terms for cancer P140Cap interactome.

Annotation type	Annotation terms	*P*.adjust
GO CC	Cell-substrate junction	4.96E-39
	Focal adhesion	8.47E-39
	Proteosome complex	1.32E-27
	Endopeptidase complex	1.32E-27
	Extrinsic component of plasma membrane	1.20E-12
GO BP	Wnt signaling pathway, planar cell polarity pathway	2.95E-33
	Positive regulation of ubiquitin-protein ligase activity involved in regulation of mitotic cell cycle transition	2.83E-30
	Regulation of mRNA stability	1.50E-23
	TNF- regulated signaling pathway	4.21E-19
	Positive regulation of cellular catabolic process	3.05E-16
GO MF	Cadherin binding involved in cell-cell adhesion	2.31E-25
	Threonine-type endopeptidase activity	3.16E-13
	GTP binding	1.02E-10
Reactome	Vif-mediated degradation of APOBEC3G	8.20E-32
	Regulation of activated PAK-2p34 by proteasome mediated degradation	1.14E-30
	Regulation of apoptosis	1.29E-30
	Ubiquitin-dependent degradation of Cyclin D1	1.29E-30
	Stabilization of p53	4.05E-30
	G1/S DNA damage checkpoints	2.09E-28

Within the interactome, we identified 15 communities (clusters) with topology-functional relationships.



Comparing the breast cancer to the synaptic interactome



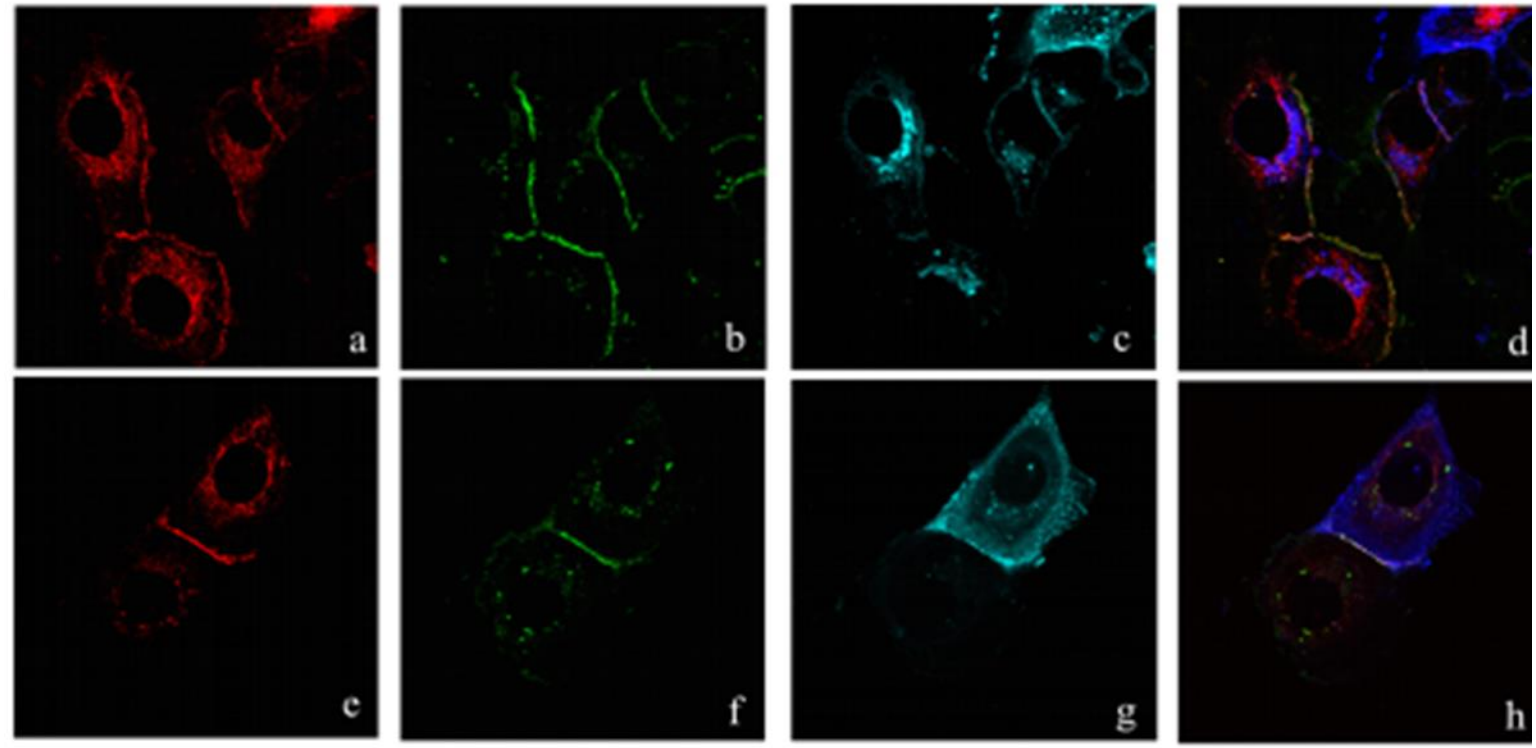
39 overlapping proteins

Cell adhesion and remodeling of actin cytoskeleton clearly emerge as common terms in the shared subset.

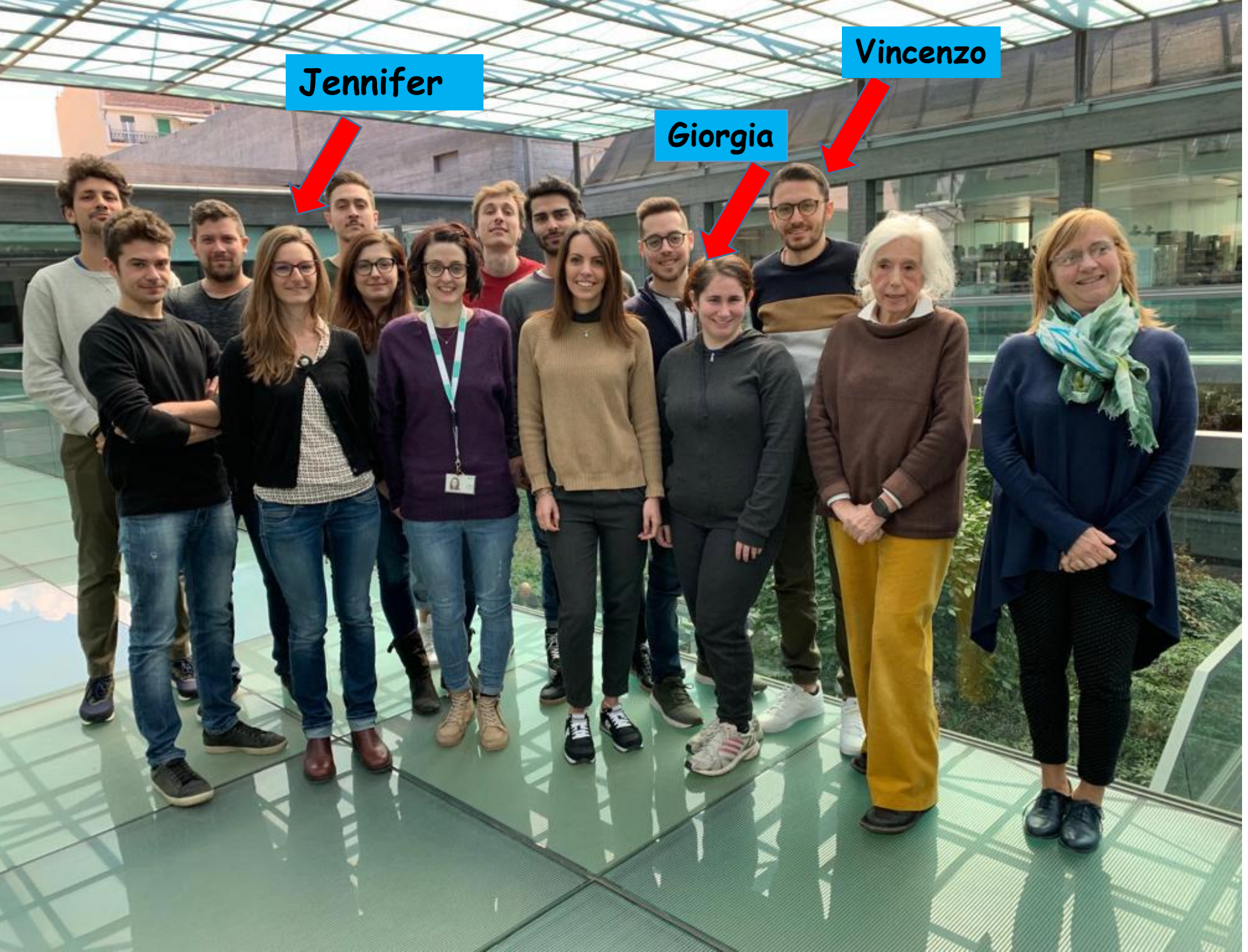
The functional signature of the two interactomes is primarily determined by organ/tissue and functional specificity.

The overlap provides a list of shared functional terms, which might be linked to both cancer and neurological functions.

p140Cap localizes at the cell membrane and forms a macromolecular complex with E-Cadherin and EGFR



Damiano, Di Stefano et al., 2010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