

cBio@MSKCC



Memorial Sloan-Kettering
Cancer Center

Correlating Protein Phosphorylation with Genomic Alterations in Cancer

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Memorial Sloan-Kettering Cancer Center

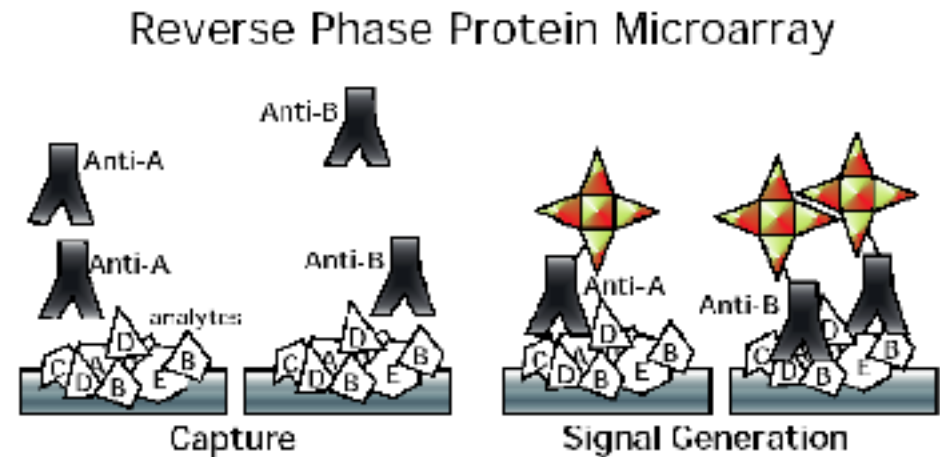
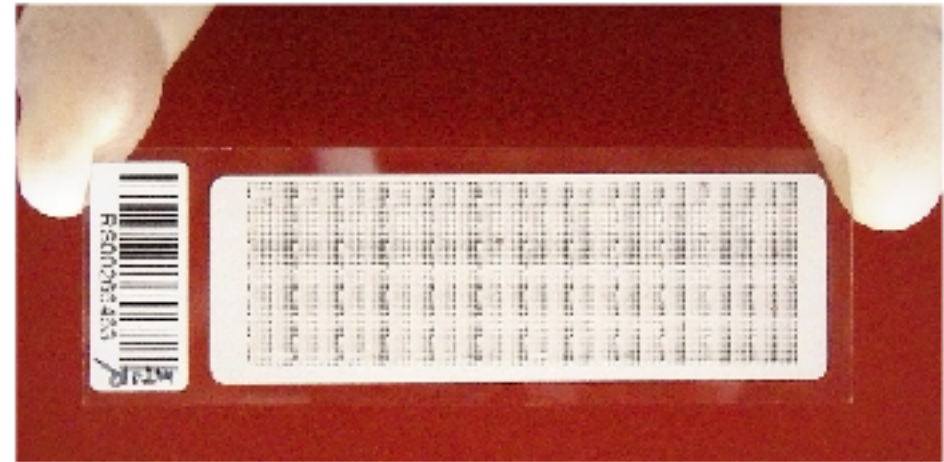
RPPA: Reverse phase protein arrays

Quantitative high throughput multiplexed inexpensive ELISA

Protein from cell lysates printed on slide: each slide developed with a single antibody

1000 samples per slide with 1000 controls

300 validated antibodies available



Gordon Mills, MD Anderson

Antibodies & TCGA data

All RPPA data were generated by Yiling Lu / Gordon Mills at MD Anderson

Tumor Type	Protein antibodies	Phospho-antibodies	Samples
Breast	122	43	410
Ovarian	122	43	412
GBM	142	49	215
Colorectal	142	49	463
Kidney	143	47	454
Endometrioid	143	47	200

Total samples: 2154

Antibody examples

Protein antibodies

- PTEN
- TP53
- ER
- AR
- AKT
- ATM
- b-Raf
- ERBB2
- ERBB3
- KRAS
- AMPK
- GSK-3 α/β
- and ~120 more

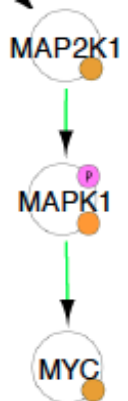
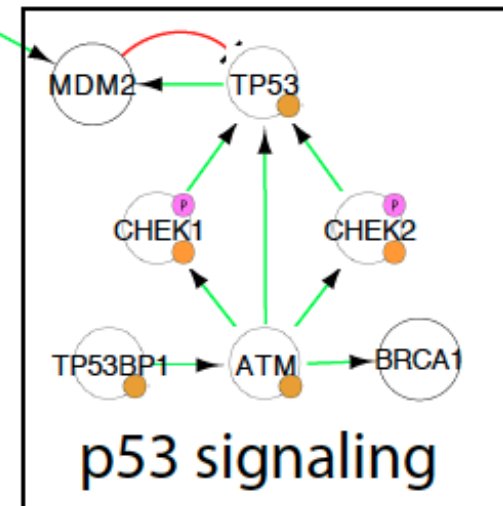
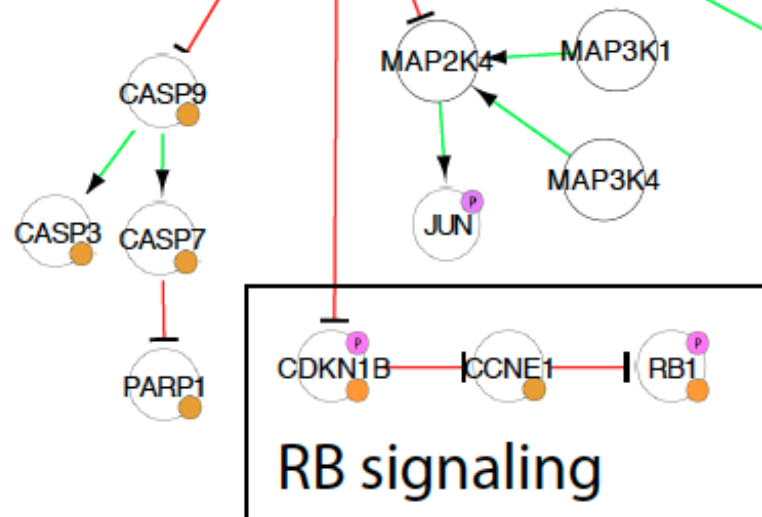
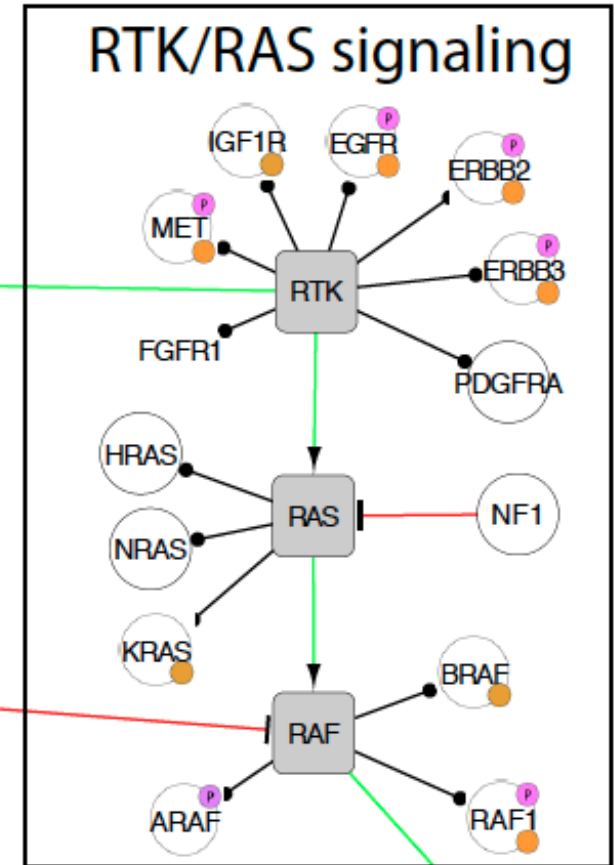
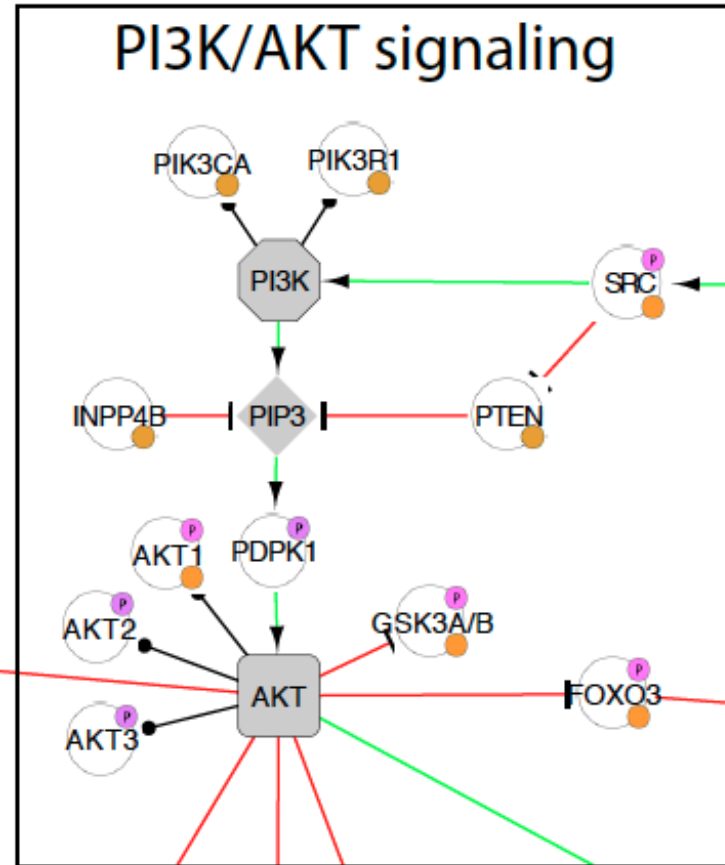
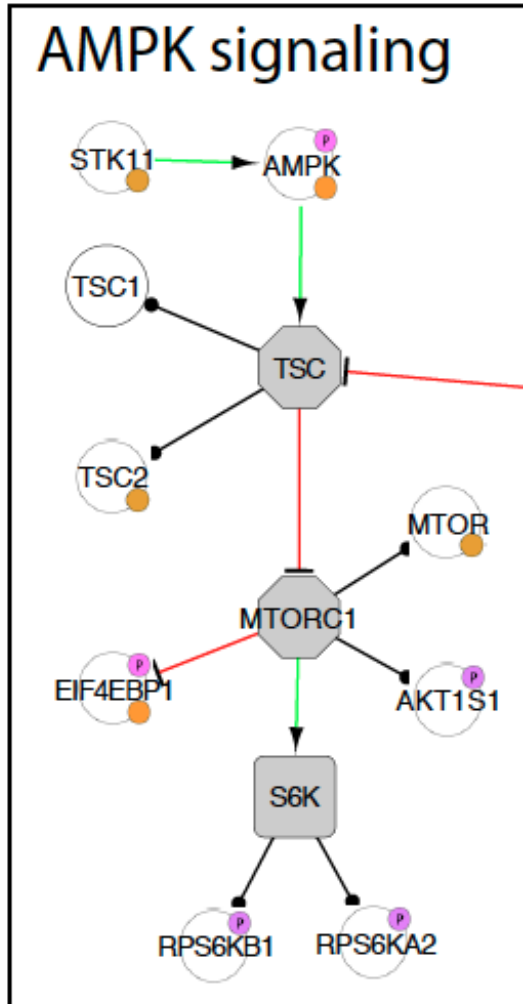
Phosphoprotein antibodies

- AKT (pT308 & pS473)
- EGFR (pY992, pY1068 & pY1173)
- ERBB2 (pY1248)
- ERBB3 (pY1298)
- MAPK (pT202/204)
- AMPK (pT172)
- MEK1 (pS217/212)
- mTOR (pY1235)
- PDK1 (pS241)
- RB (PS807/811)
- c-Raf (pS338)
- GSK-3 α/β (pS21/9)
- and ~40 more

Pathway View

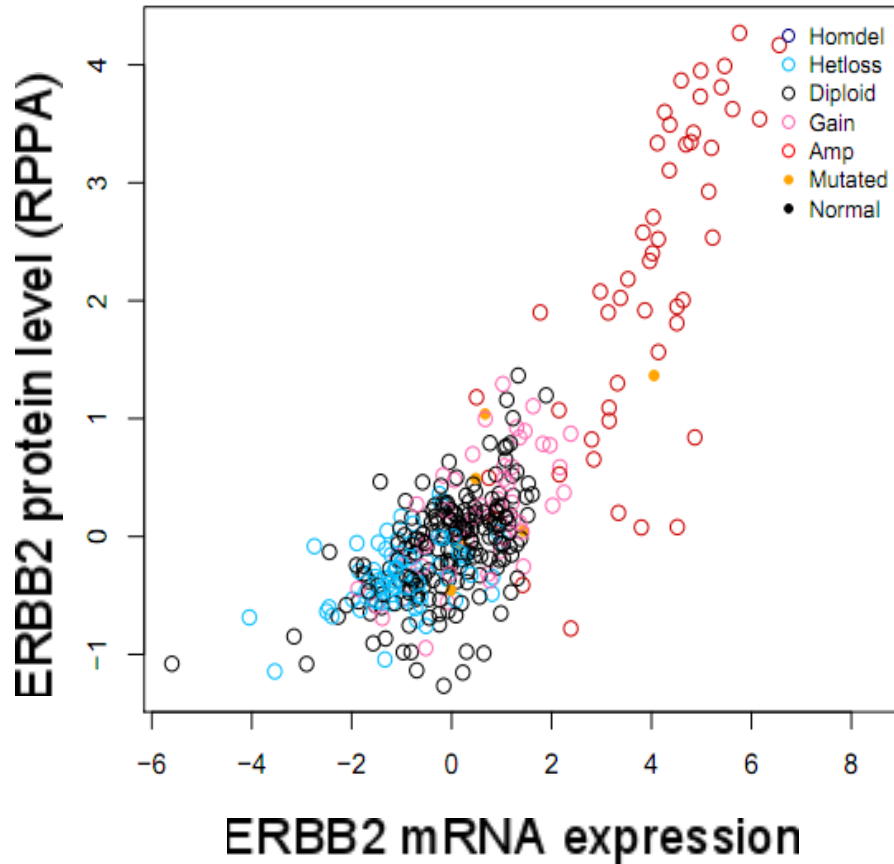
P Phospho-antibody

O Protein antibody

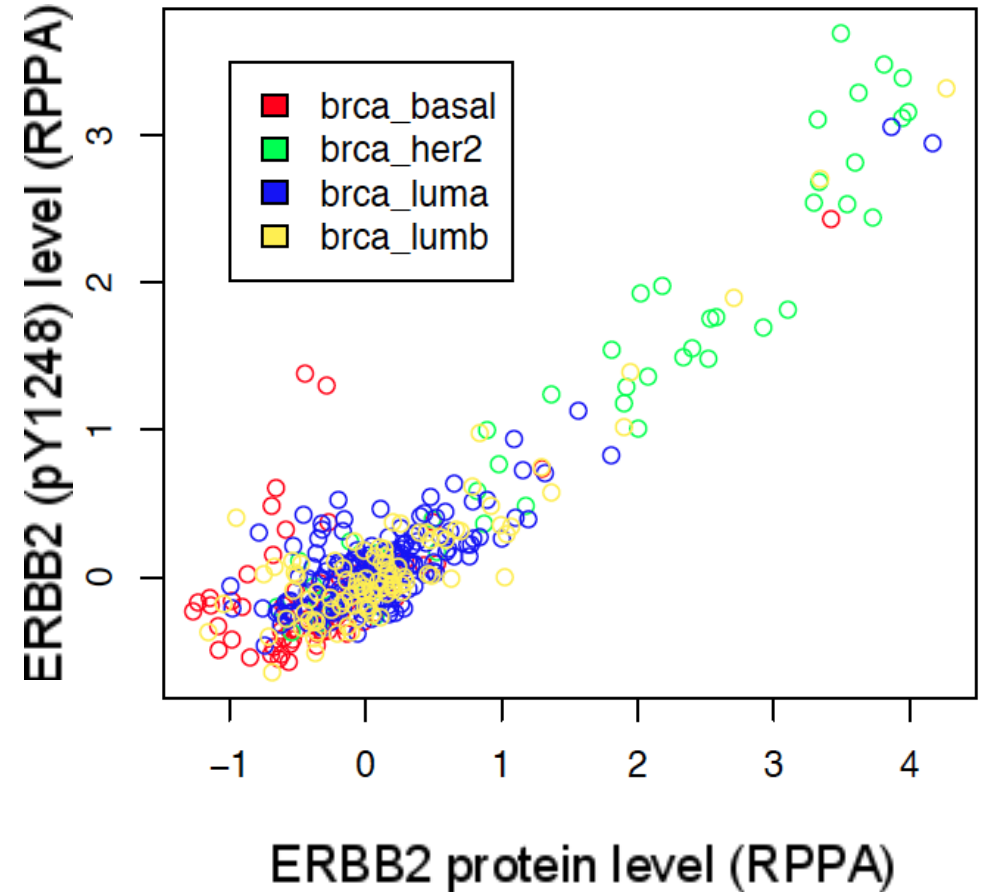


ERBB2 mRNA, protein level, and phosphorylation levels are well correlated in breast cancer

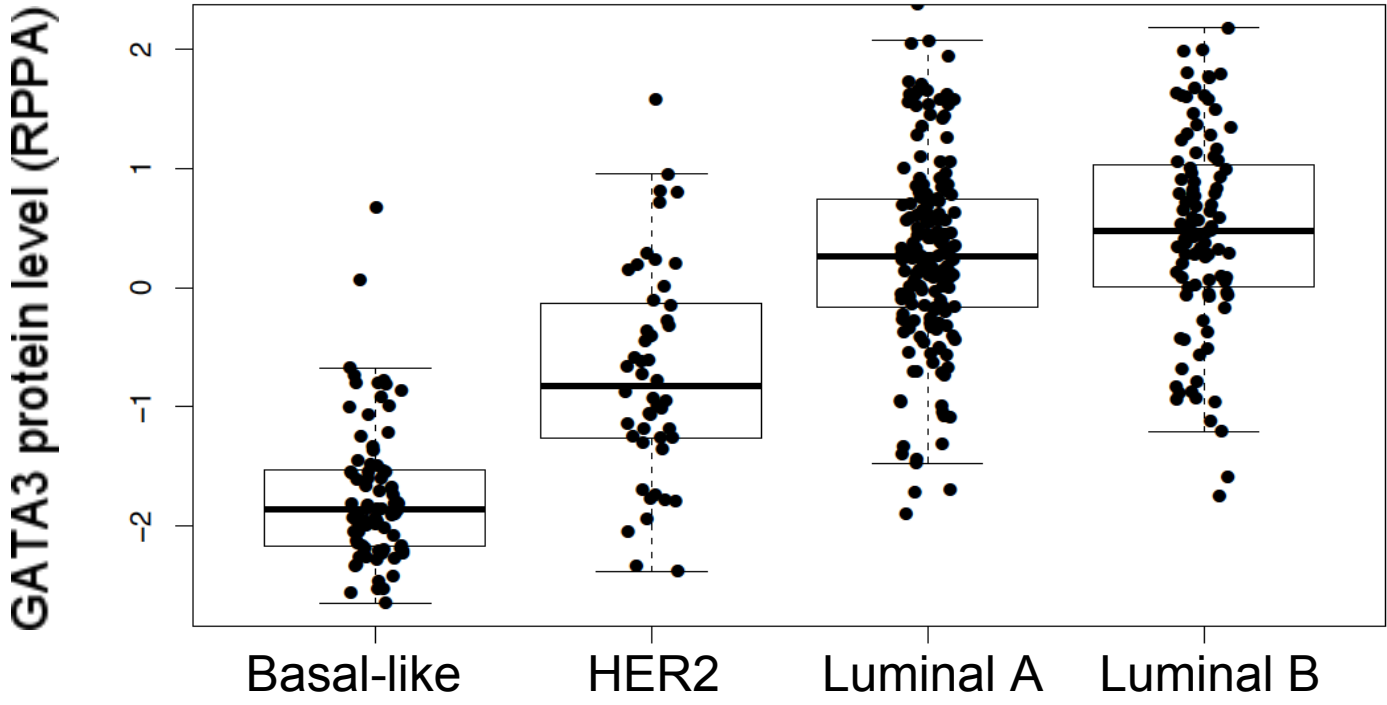
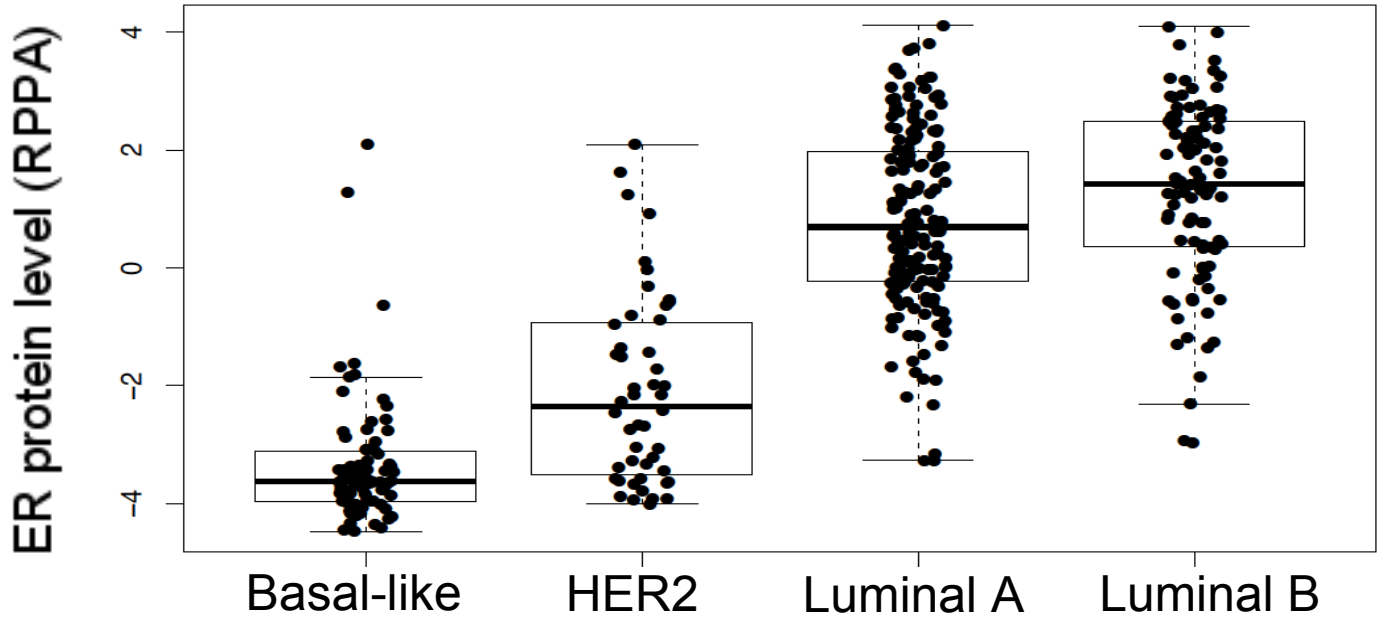
Protein level vs mRNA



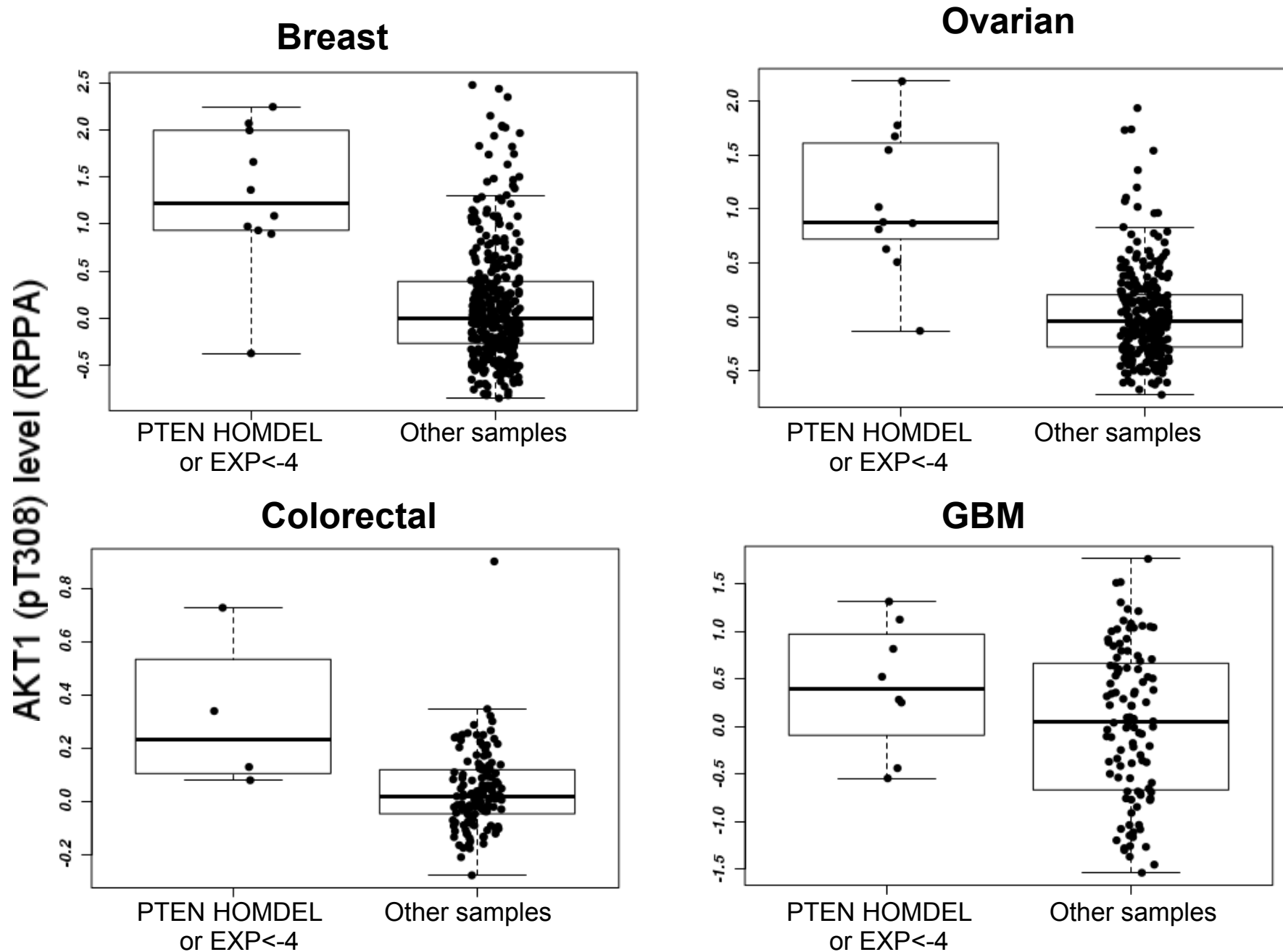
Phosphorylation vs protein level



ER and GATA3 protein levels differ in breast cancer subtypes



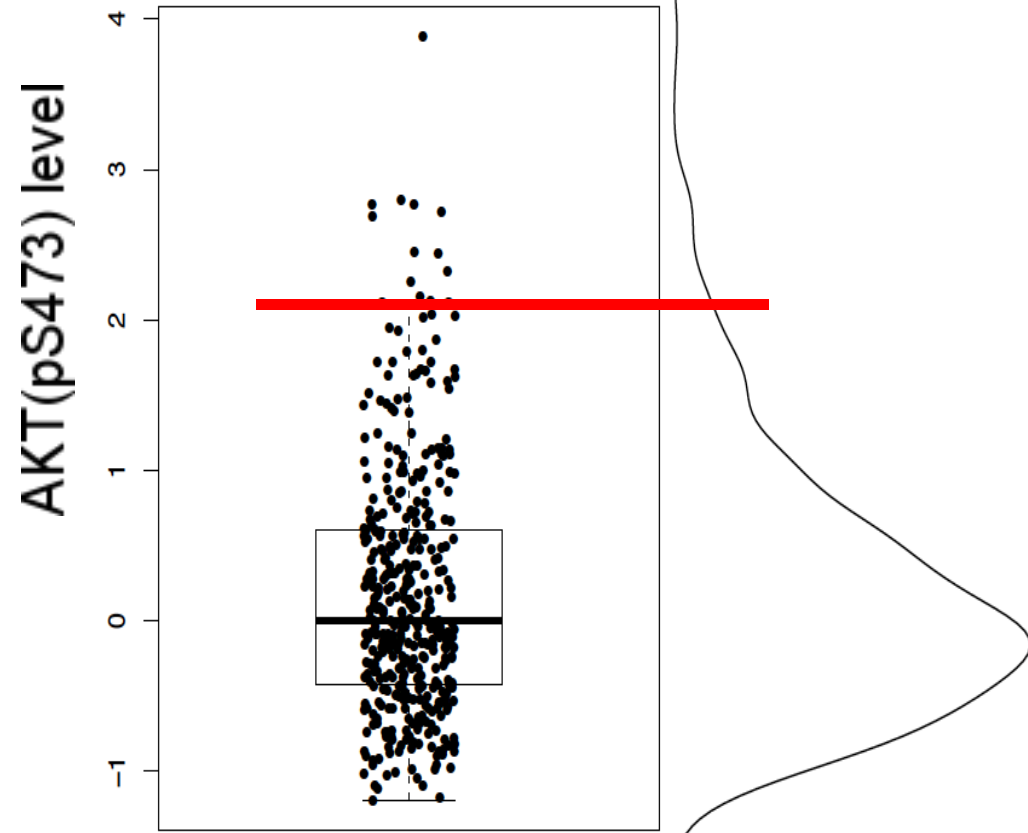
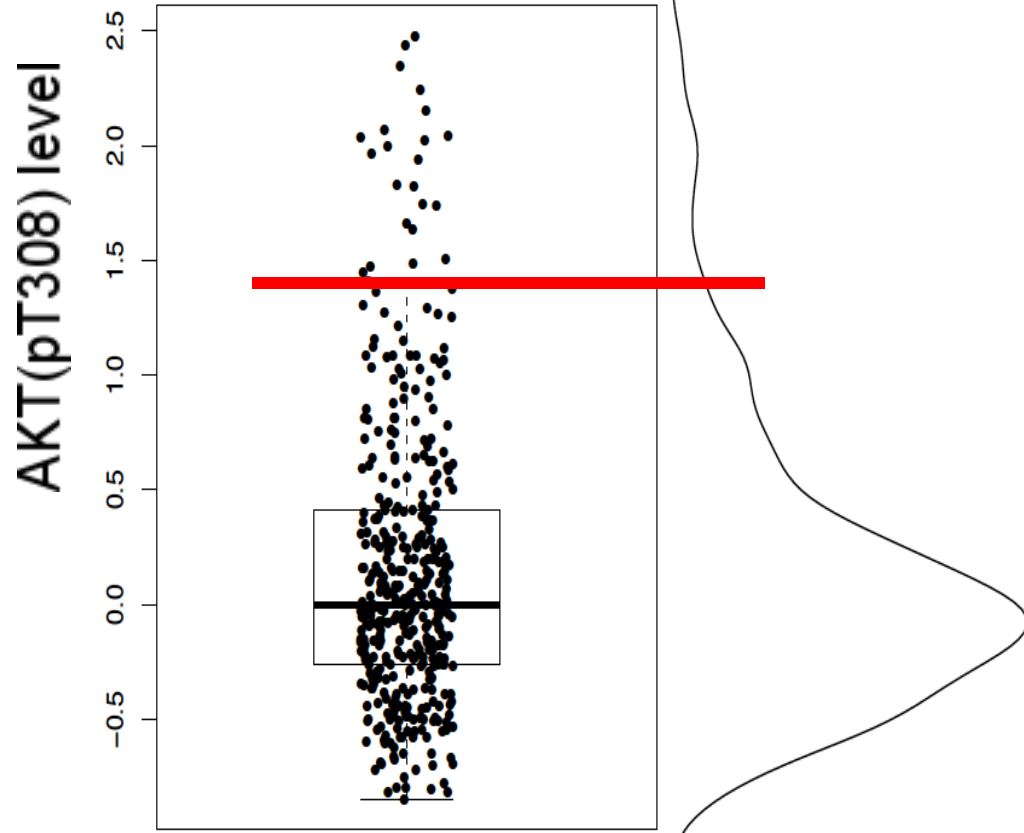
***PTEN* deletion and under-expression are correlated with elevated phospho-AKT**



Case study: *AKT phosphorylation in breast cancer*

- AKT activation/phosphorylation has been found in many cancer types
- Phospho-AKT has diverse targets regulating proliferation, invasion and apoptosis
- It contributes to breast cancer progression
- It confers resistance to conventional therapies
- Targeted AKT inhibition could be beneficial to some patients

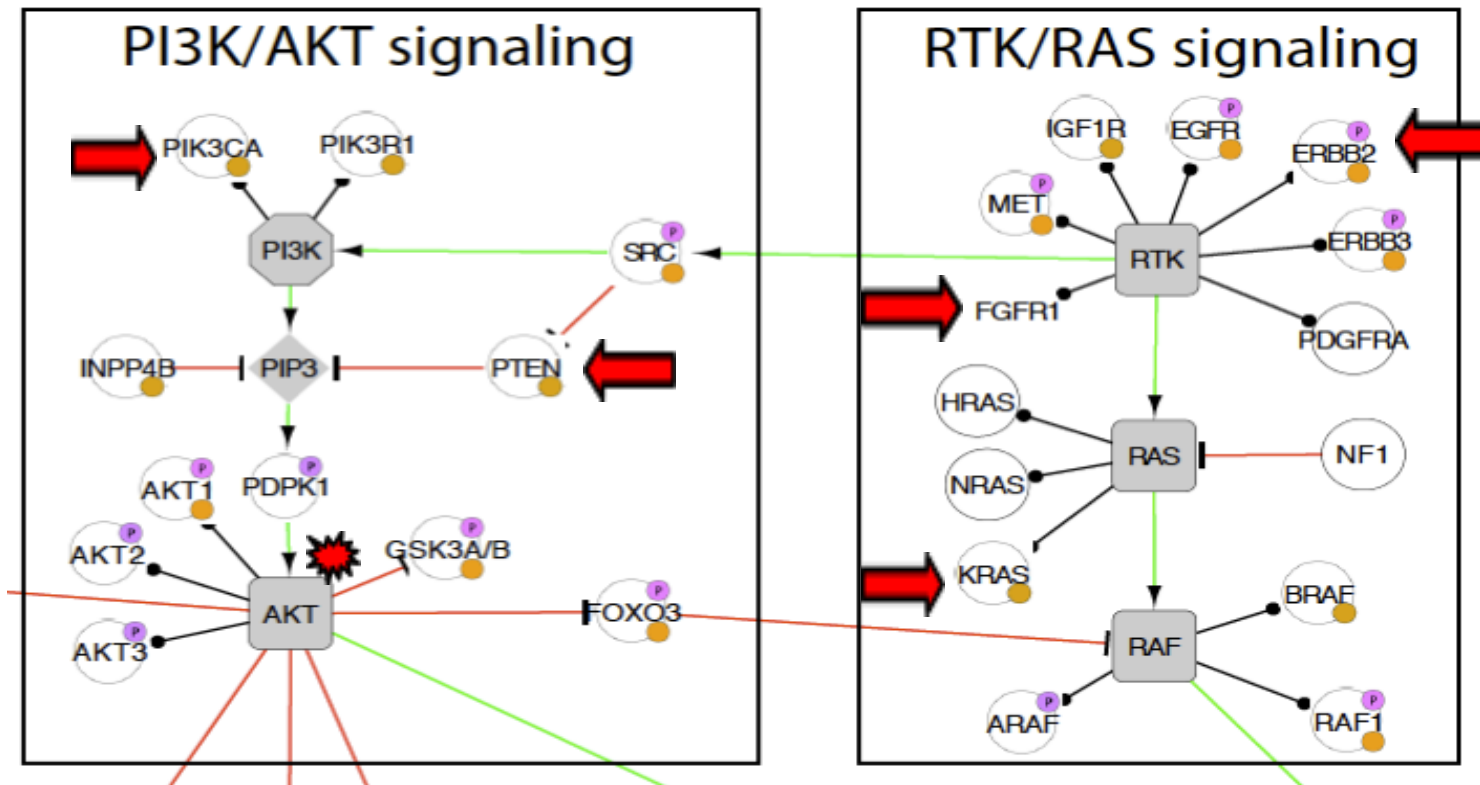
AKT phosphorylation in TCGA breast cancer samples



Question:

What causes AKT activation in breast tumors?

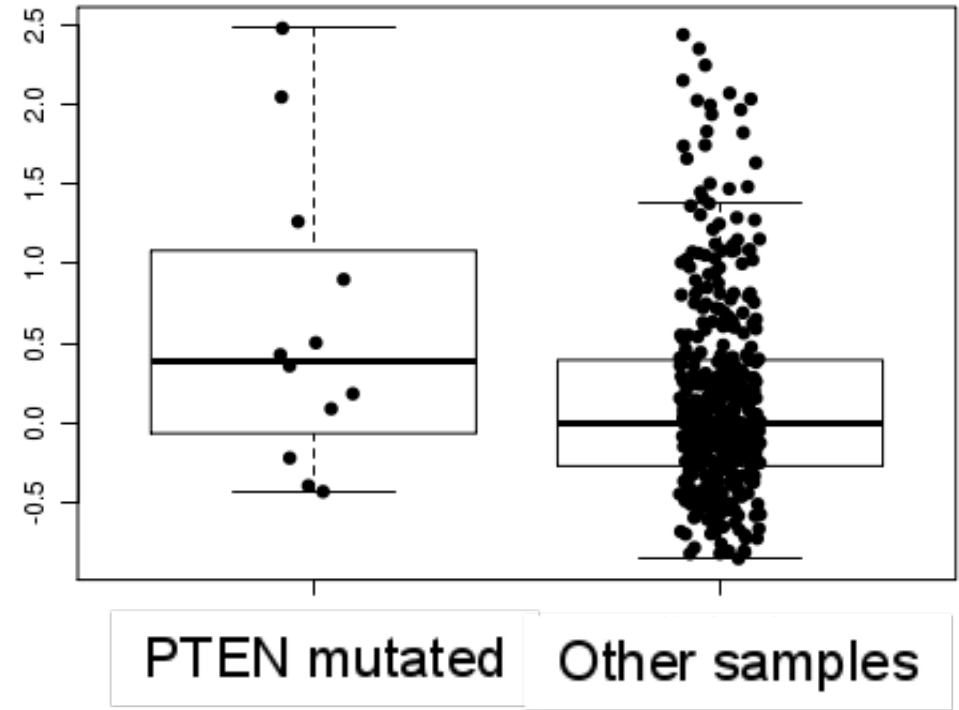
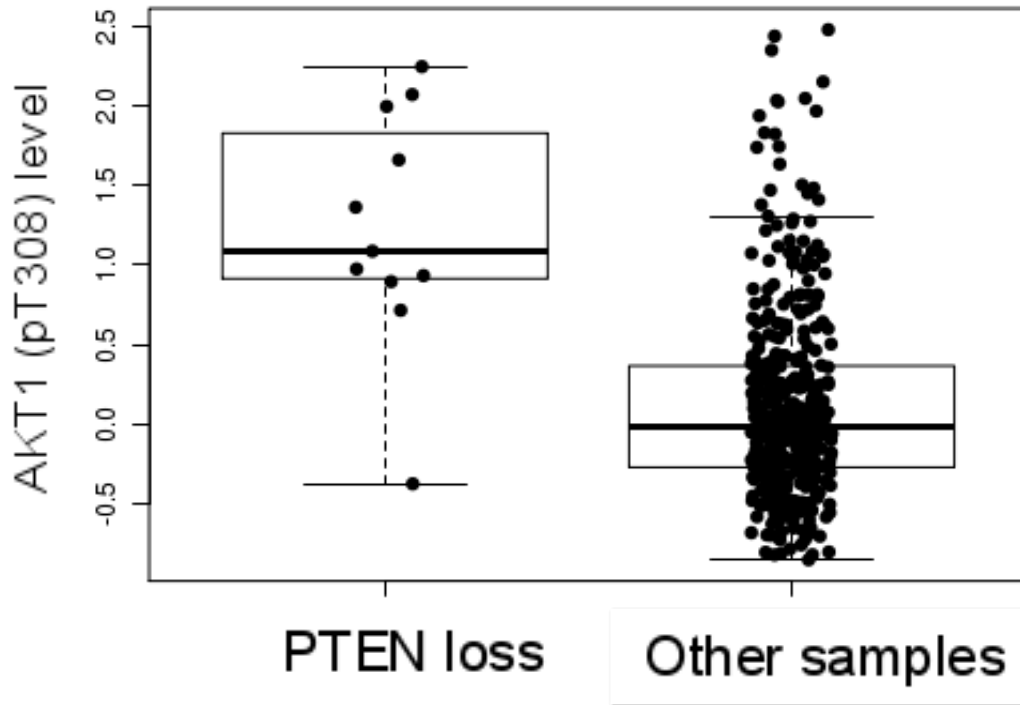
AKT activation: Some of the usual suspects



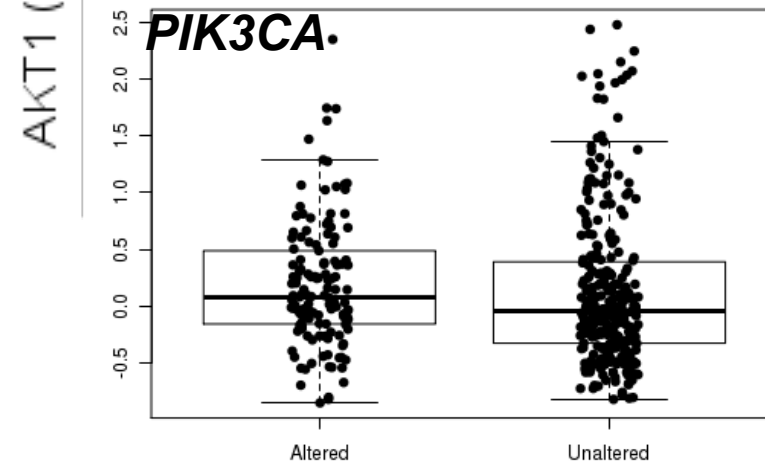
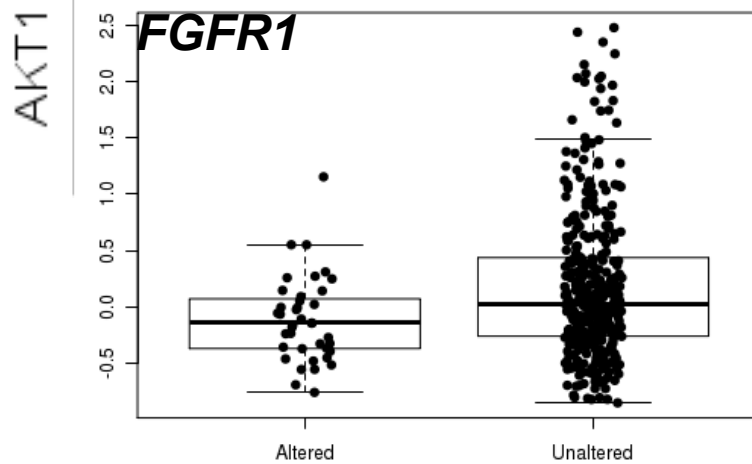
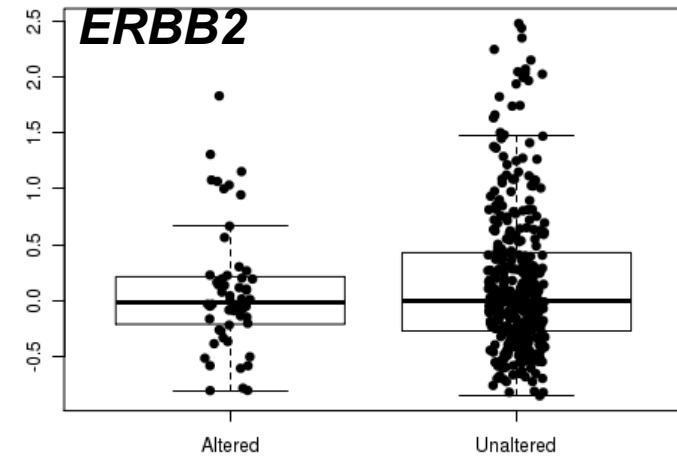
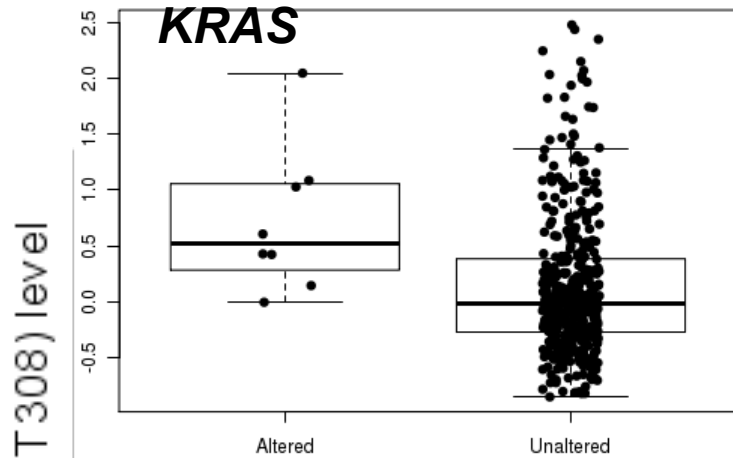
Loss of PTEN but not mutation is strongly associated with pAKT



***PTEN* deletions & loss of expr.** ***PTEN* mutations** (mostly truncating)



PIK3CA mutations and RTK amplifications are not associated with elevated pAKT



What other genomic events can explain AKT phosphorylation in breast cancer?

A more systematic approach:

Enrichment test of all GISTIC ROIs and frequently mutated genes

Genomic events correlated with pAKT

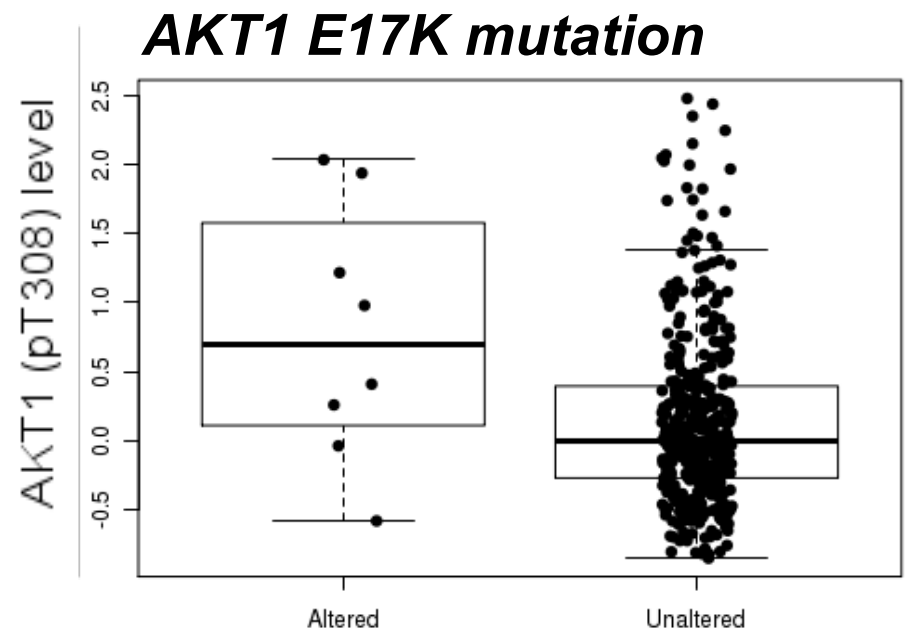
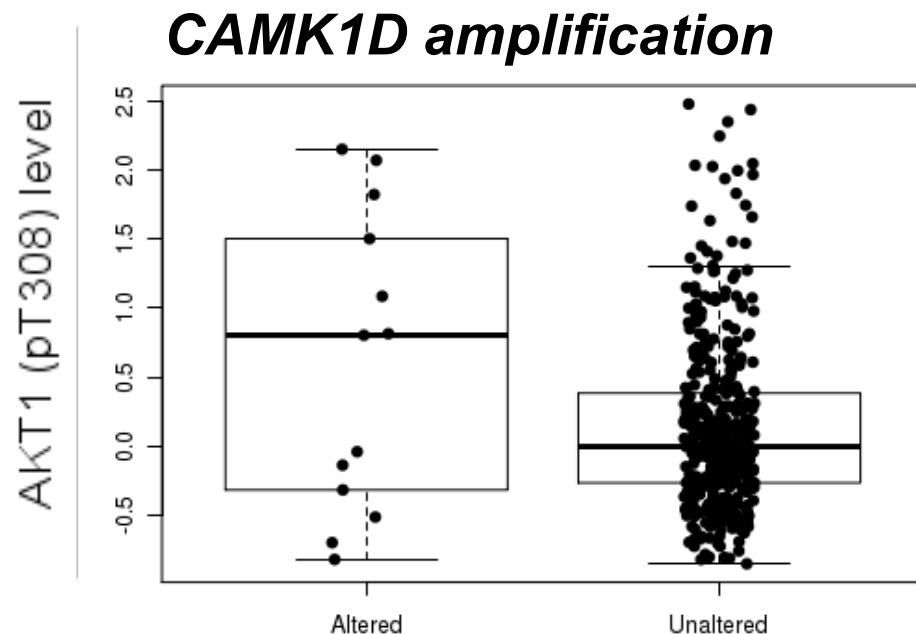
<i>COPY NUMBER</i>	genes	multivariate	p-value	Enriched Set	AKT_HI	AKT_LOW
chr10:12118816-12131522	CAMK1D + 7 others	0.0011	0.0013	AKT_HI	7	4
chr8:37835997-37863117	EIF4EBP1	0.0040	0.0050	AKT_LOW	3	47
chr10:89611041-89720627	PTEN	0.0128	0.0140	AKT_HI	4	2
chr20:51775900-51788738	ZNF217, SUMO1P1, BCAS1	0.0301	0.0350	AKT_LOW	1	23
chr11:69185447-69190570	CCND1	0.0242	0.0388	AKT_LOW	6	52
<i>MUTATIONS</i>		multivariate	p-value	Enriched Set	AKT_HI	AKT_LOW
RYR2		0.0057	0.0072	AKT_HI	8	9
ABCA13		0.0073	0.0086	AKT_HI	6	5
MUC16		0.0102	0.0144	AKT_HI	10	16
RYR3		0.0217	0.0263	AKT_HI	5	5
AKT1		0.0397	0.0478	AKT_HI	4	4

Enrichment test of all GISTIC ROIs and frequently mutated genes

Genomic events correlated with pAKT

COPY NUMBER	genes	multivariate	p-value	Enriched Set	AKT_HI	AKT_LOW
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chr20:51775900-51788738	ZNF217, SUMO1P1, BCAS1	0.0301	0.0350	AKT_LOW	1	23
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Enrichment test of all GISTIC ROIs and frequently mutated genes



Summary and future work

Very good correlation between genomic and proteomic data on the level of individual genes and proteins


Downstream effects are harder to link to genomic events:

We can explain some but not all cases of AKT phosphorylation
Analysis of genomic event combinations may help

Systematic analysis of all antibodies needed

Correlations between protein data may help elucidate active signaling pathways

RPPA data in the cBio GDAC Portal



Memorial Sloan-Kettering Cancer Center

cBio Cancer Genomics Portal

Visualize, analyze, discover.

HOME NEWS FAQ DATA SETS ABOUT WEB API R/MATLAB NETWORKS

Gene Set / Pathway is altered in 15.4% of all cases.

Breast invasive carcinoma/All Complete Tumors: (494)/User-defined List/1 gene

Modify Query

Summary Network Plots Mutation Details **RPPA Data** Event Map Data Download Bookmark/Email

Antibody Type: phosphorylation Search:

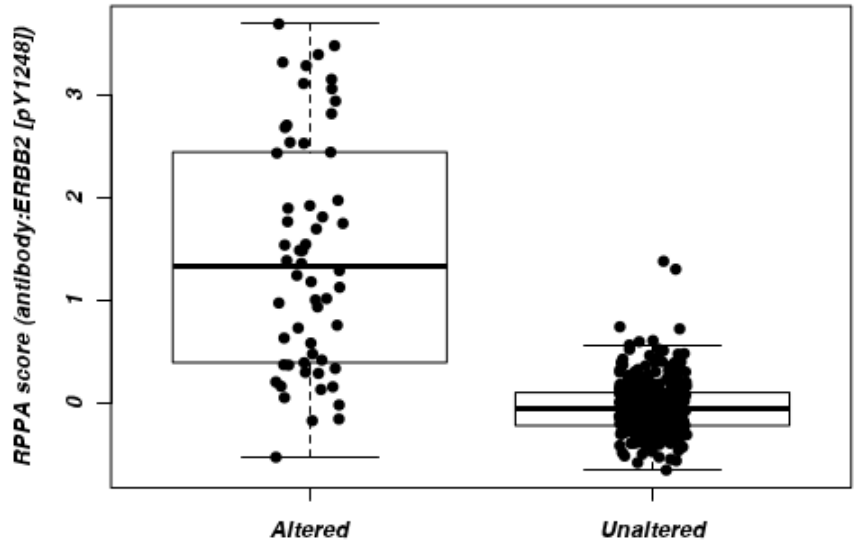
Target		Ave. Abundance		p-value	Plot
Protein	Residue	Unaltered	Altered		
ERBB2	pY1248	-0.03	1.44	5.03e-14 ▲	+
ESR1	pS118	0.21	-0.13	4.17e-9 ▼	+
EGFR	pY1068	-0.01	1.12	1.23e-8 ▲	+
ERBB3	pY1298	0.01	0.25	3.10e-5 ▲	+
SRC	pY416	-0.05	0.12	0.006 ▲	+
WWTR1	pS89	0.07	-0.01	0.014 ▼	+
RELA	pS536	-0.07	0.13	0.017 ▲	+
SHC1	pY317	-0.07	0.14	0.037 ▲	+
CDKN1B	pT198	0.02	0.10	0.039 ▲	+
ACACA/ACACB	pS79	0.02	0.29	0.042 ▲	+
EGFR	pY992	-0.04	0.13	0.042 ▲	+
AKT1/AKT2/AKT3	pS473	0.23	0.07	0.074 ▼	+

<http://cbio.mskcc.org/gdac-portal/>

RPPA data in the cBio GDAC Portal

Target		Ave. Abundance		p-value	Plot
Protein	Residue	Unaltered	Altered		
ERBB2	pY1248	-0.03	1.44	5.03e-14 ▲	⊖

Boxplots of RPPA data (antibody:ERBB2 [pY1248]) for altered and unaltered cases [PDF]



Query: ERBB2 (p-value: 5.03e-14)

ESR1	pS118	0.21	-0.13	4.17e-9 ▼	⊕
EGFR	pY1068	-0.01	1.12	1.23e-8 ▲	⊕
ERBB3	pY1298	0.01	0.25	3.10e-5 ▲	⊕
SRC	pY416	-0.05	0.12	0.006 ▲	⊕
WWTR1	pS89	0.07	-0.01	0.014 ▼	⊕
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Acknowledgements

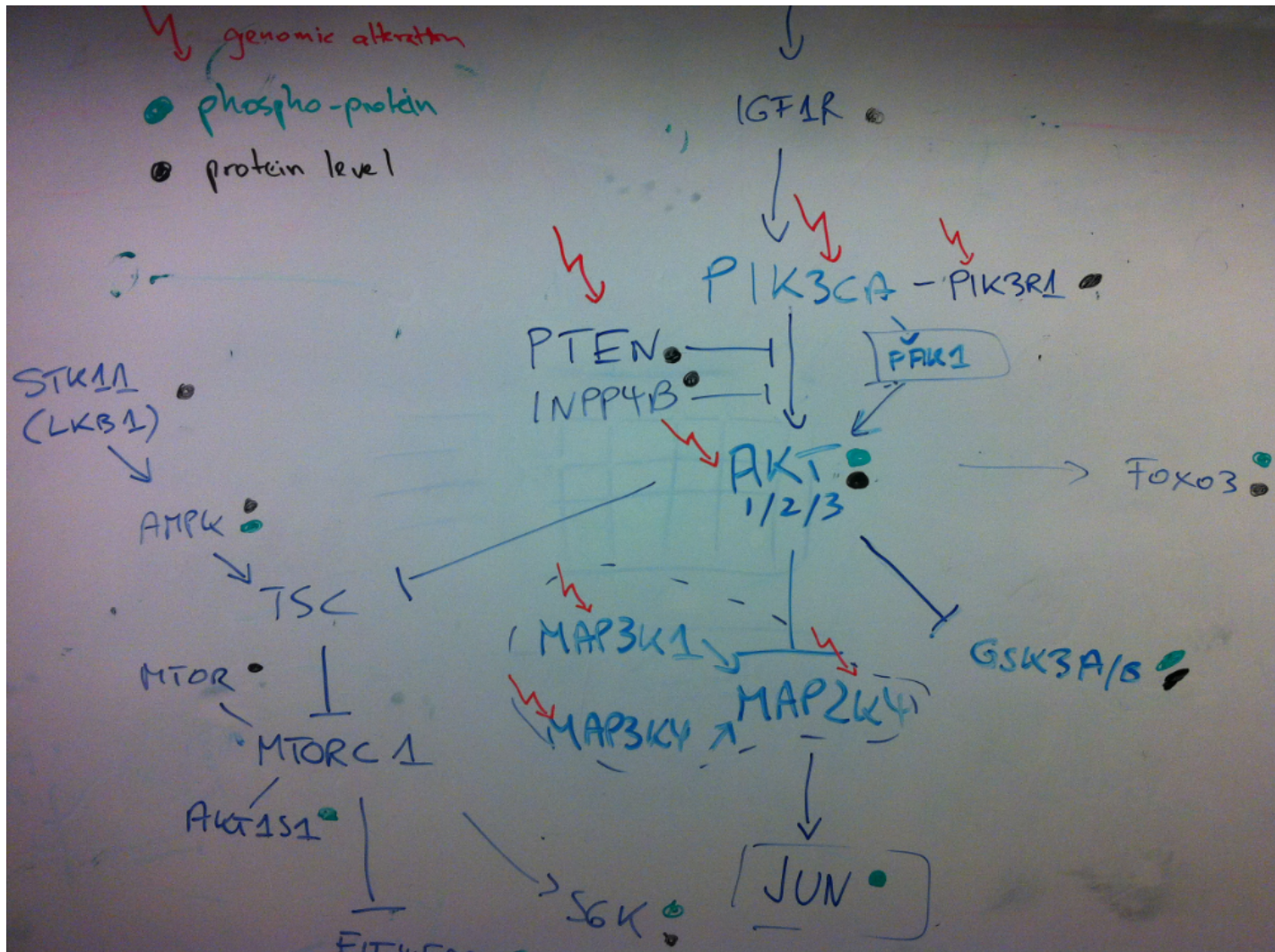
MSKCC

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Chris Sander

MD Anderson

Gordon Mills
Yiling Lu and the RPPA team





Thank you!

Poster #113