



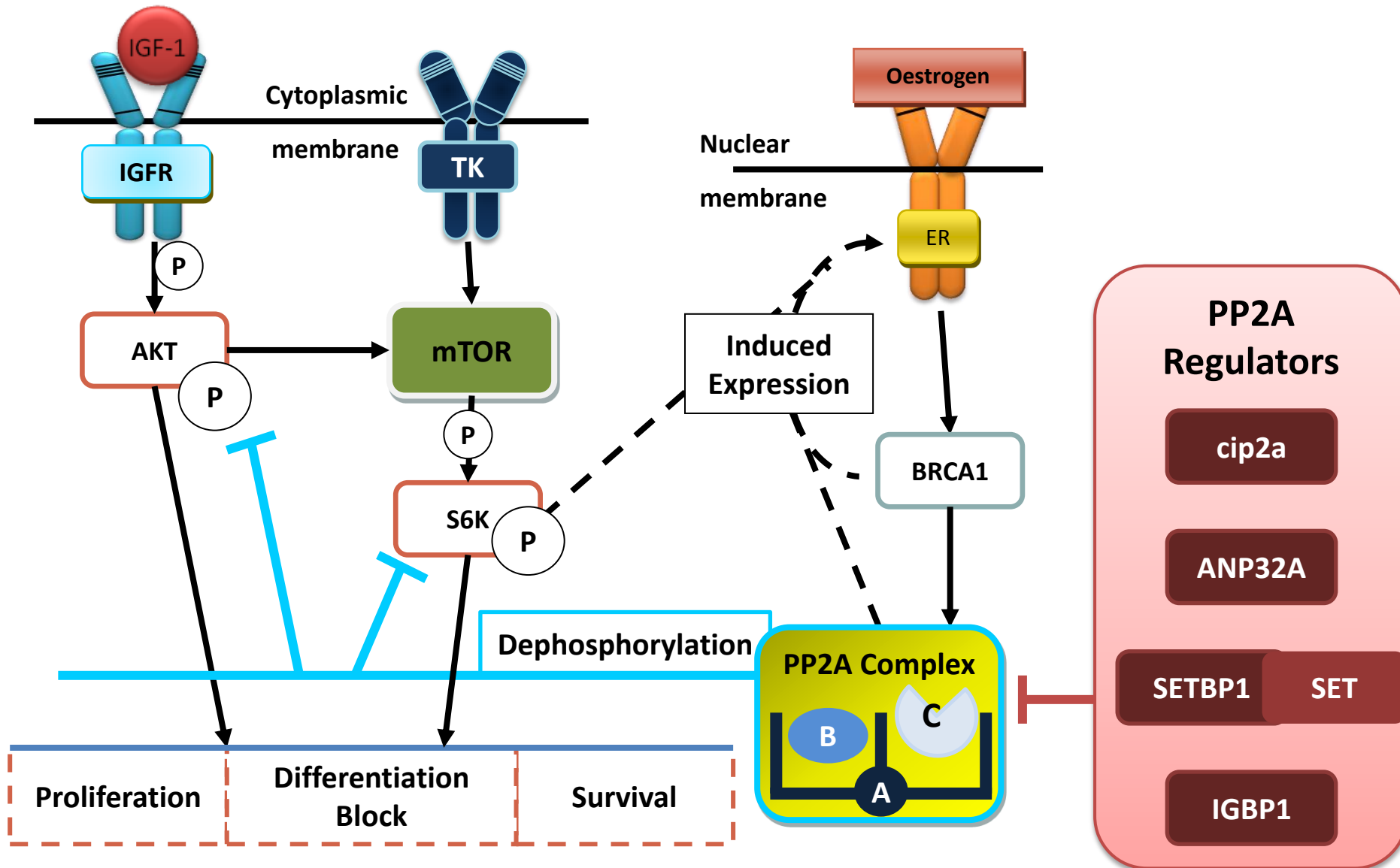
Department of Pathology and
The Department of Physiology and
Biochemistry
UNIVERSITY OF MALTA



Variation in the protein phosphatase 2A (PP2A) complex is a common event in breast cancer patients

*Shawn Baldacchino, Christian Saliba, Sharon Falzon, Keith Sacco, James DeGaetano, Gordon Caruana
Dingli, Joseph Debono, Anthony G. Fenech, Christian Scerri, Godfrey Grech*

Cellular mechanisms of PP2A



Downstream PP2A activity markers – S6K and AKT

- **p-AKT** positivity in **26%** of Triple Negative Breast Cancer (TNBC)

- **p-S6K** nuclear positivity in **45%** of Breast Cancer

Ref: Borg, N., Baldacchino, S., Saliba, C., Falzon, S., DeGaetano, J., Scerri, C., & Grech, G. (2014). Phospho-Akt expression is high in a subset of Triple Negative Breast Cancer Patients. *Xjenza*, 2(1).

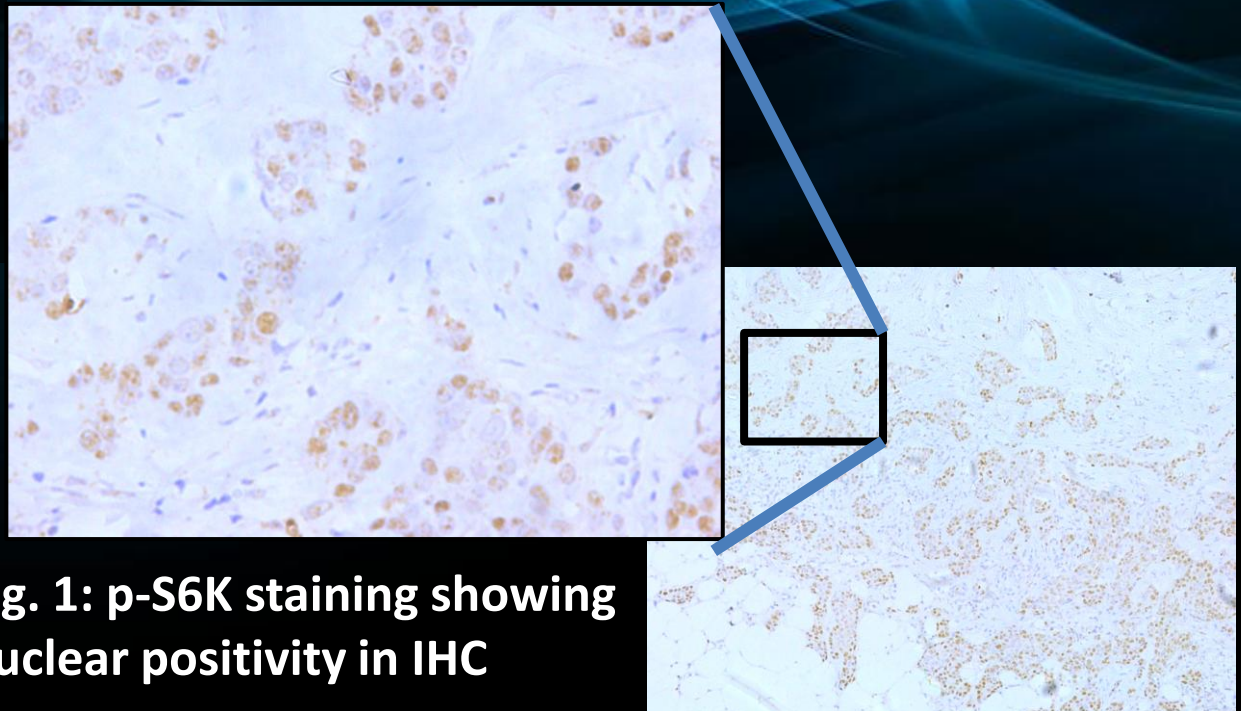


Fig. 1: p-S6K staining showing nuclear positivity in IHC

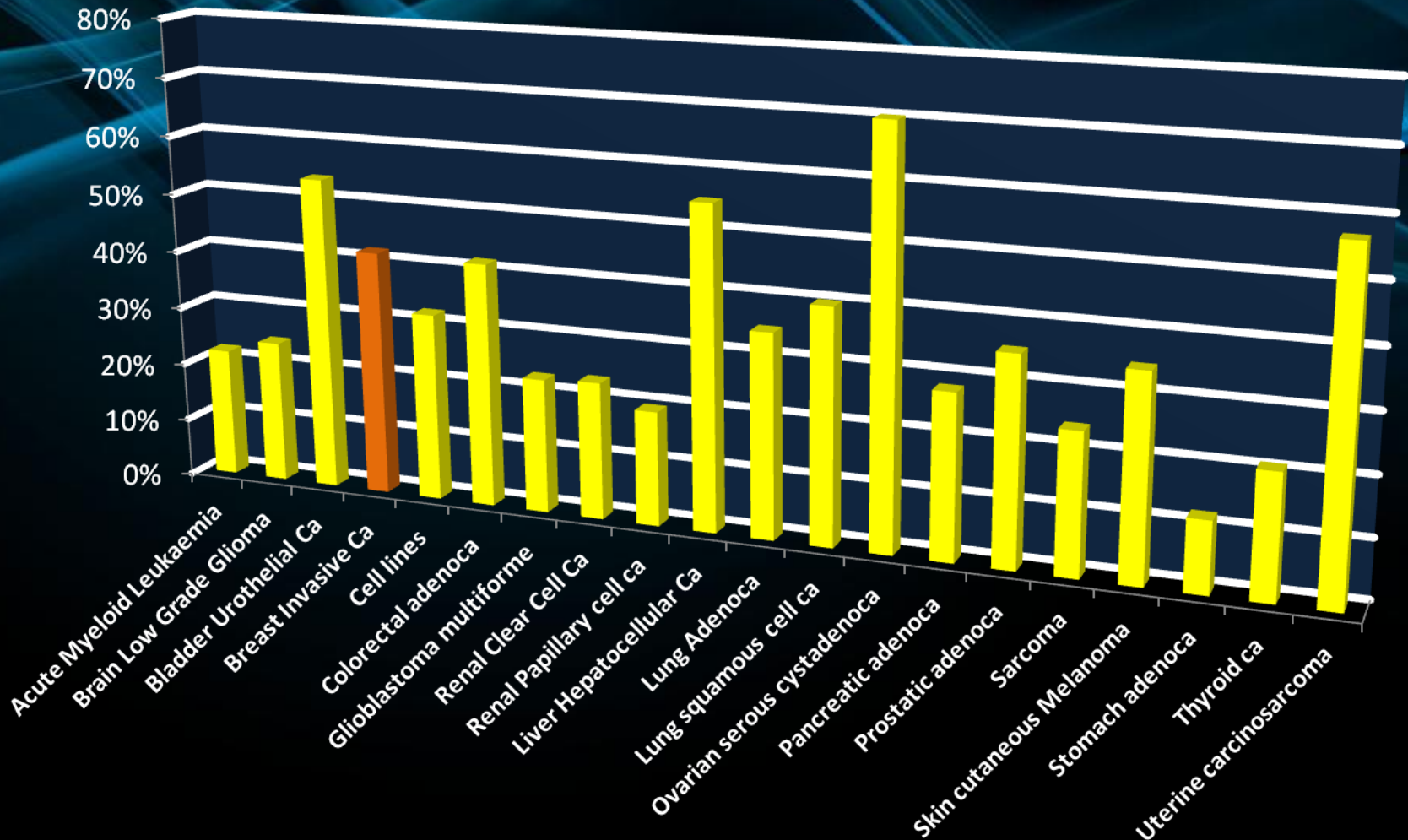
PP2A deregulation across all tumors

The Cancer Genome Atlas




Understanding genomics
to improve cancer care

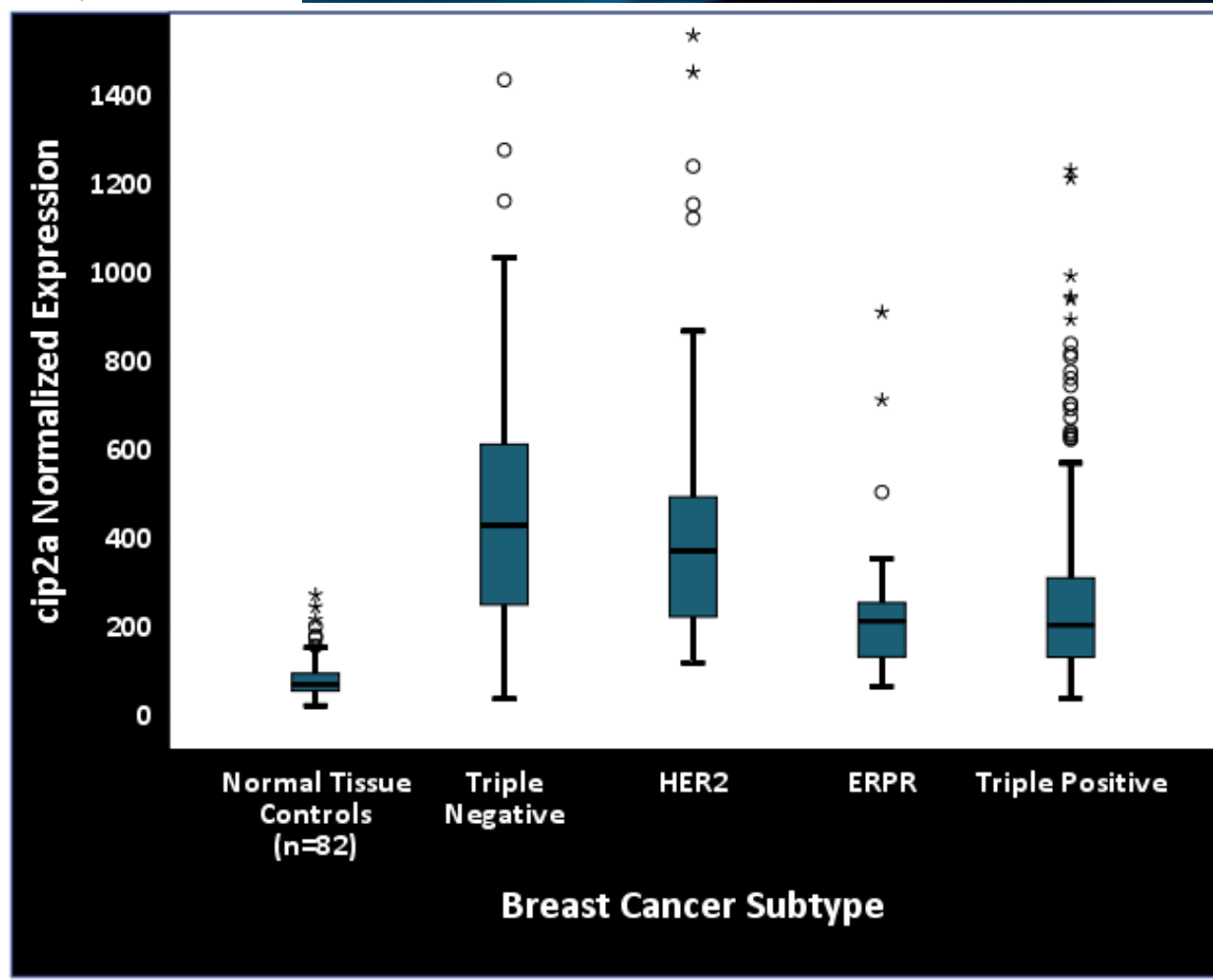
Percentage of potential cases with deregulated PP2A



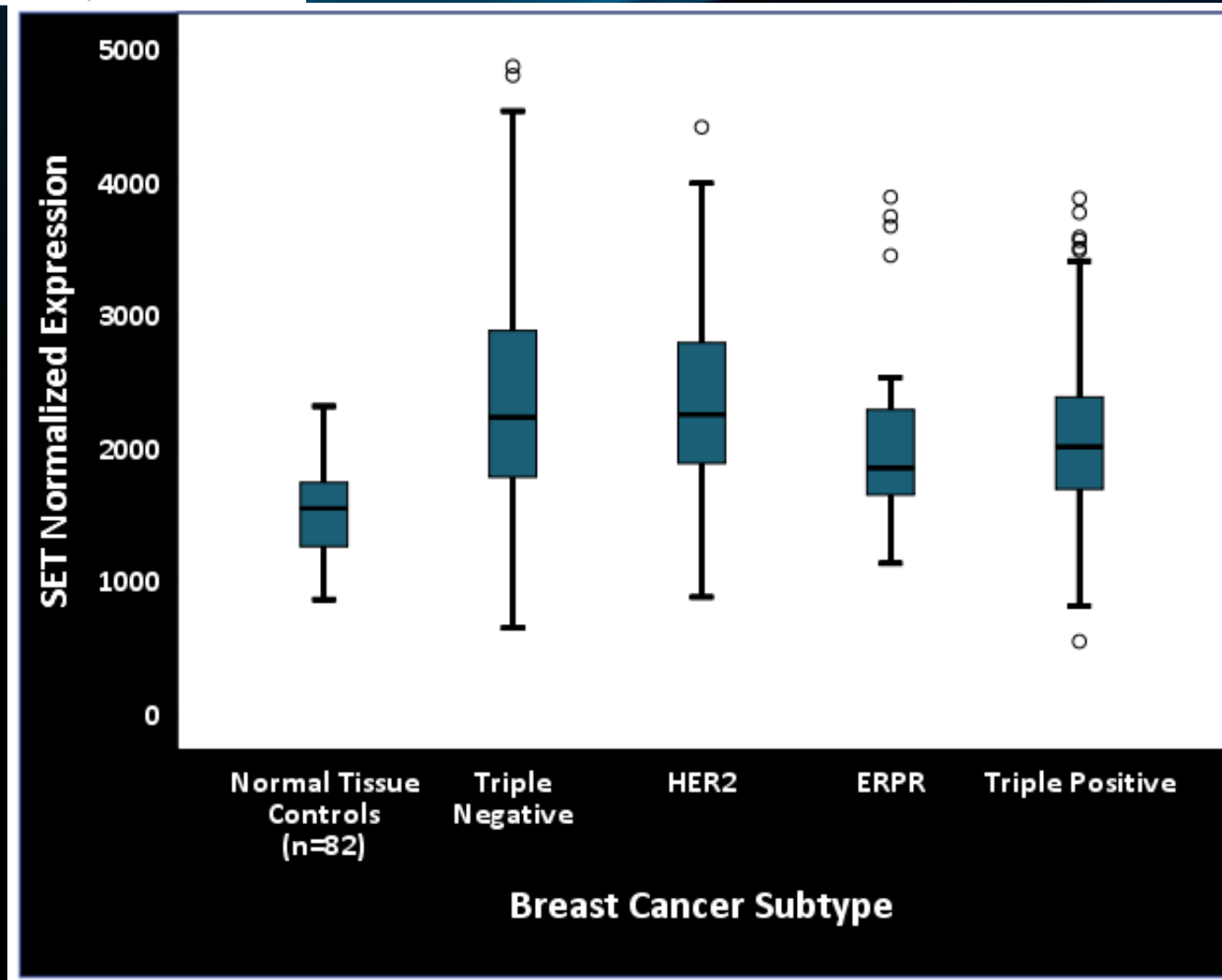
PP2A deregulation across breast tumor subtypes

The Cancer Genome Atlas 	Triple Negative (N=88)	HER2 Positive (N=80)	ERPR Positive (N=267)	Triple Positive (N=296)	Total (N=731)
PPP2CA	16%	13%	2%	1%	5%
PPP2CB	24%	24%	17%	15%	18%
PPP2R2A	18%	28%	15%	12%	15%
CIP2A	17%	11%	4%	5%	7%
SETBP1	0%	0%	6%	6%	5%
SET	20%	15%	3%	5%	6%
IGBP1	0%	3%	5%	5%	4%
ANP32A	8%	9%	4%	3%	5%
Total PP2A deregulation	60%	65%	38%	35%	42%

cip2a expression

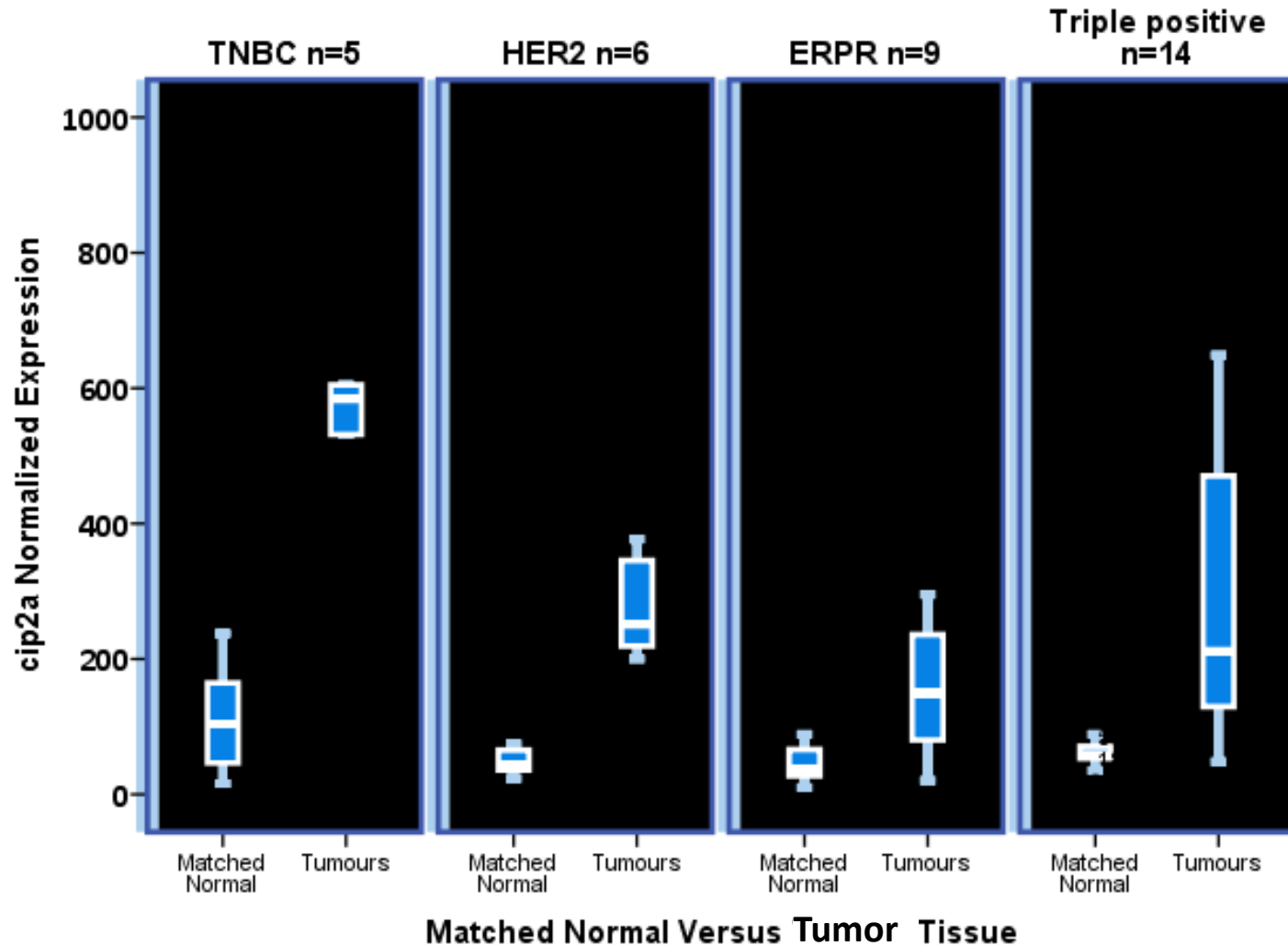


SET expression



cip2a expression – Normal to Tumor Matched Analysis

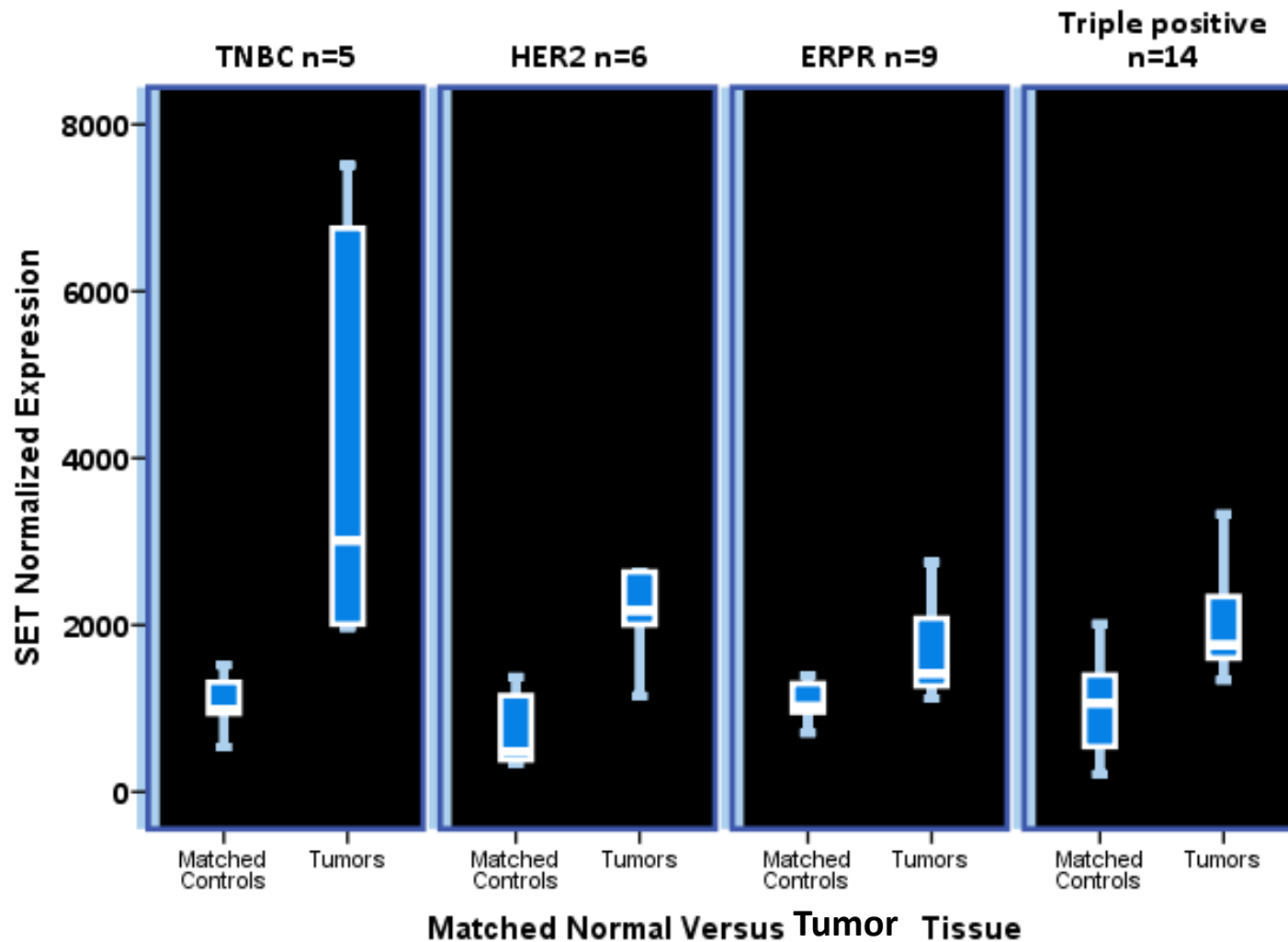
Breast Cancer Subtype



SET expression

Normal to tumor Matched Analysis

Breast Cancer Subtype



Multiplex expression assay (40-gene panel)

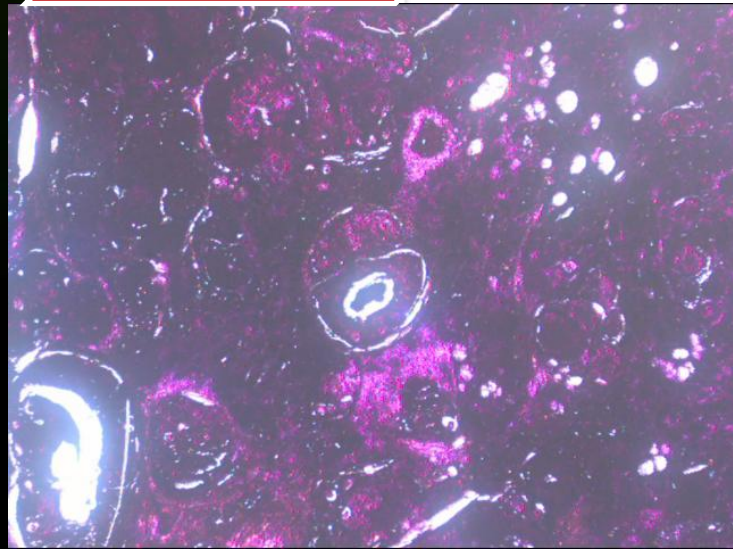
Laser
microdissection

Sample
preparation

Target
hybridization

Signal
amplification

Detection

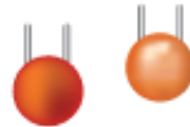


Lyse sample
and go

Lysate
(with target RNAs)



Luminex® beads
with Capture Probes



Incubate beads
with sample and
probes

Capture
Extenders (CE's)

Label
Extenders (LE's)

Blocking
Probes (BP's)

Lysate
(with target RNAs)

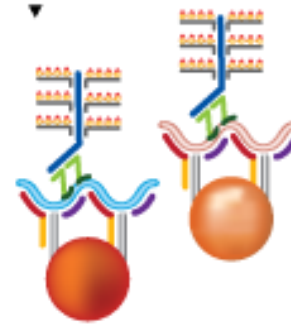
Pre-Amplifier

Amplifier

Label Probe

SAPE

Hybridizations
and Wash Steps



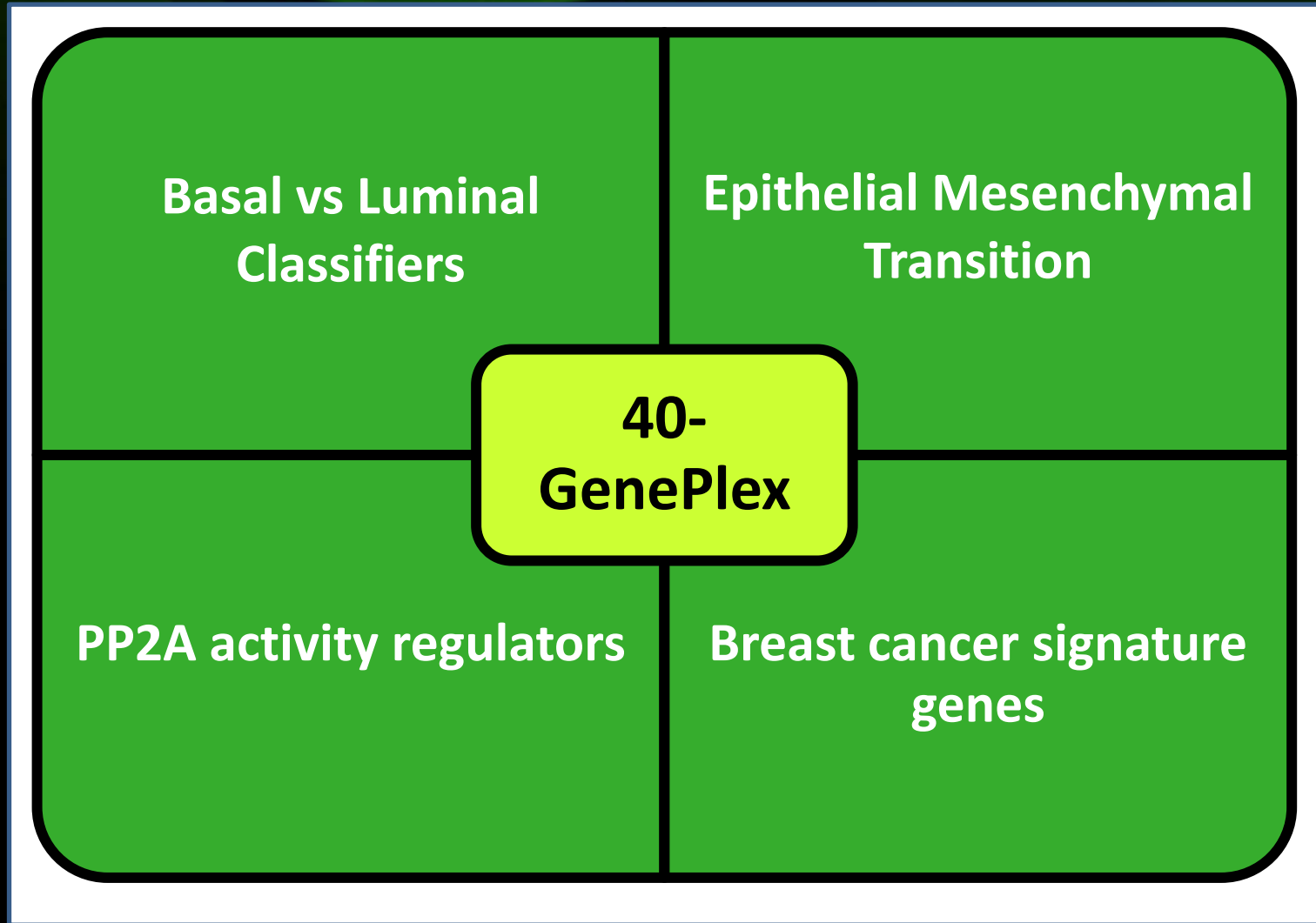
Streptavidin
phycoerythrin (SAPE)
(QuantiGene® Plex)

Read signal using
a Luminex®
instrument

3 to 80 RNA targets
per well



40-gene panel

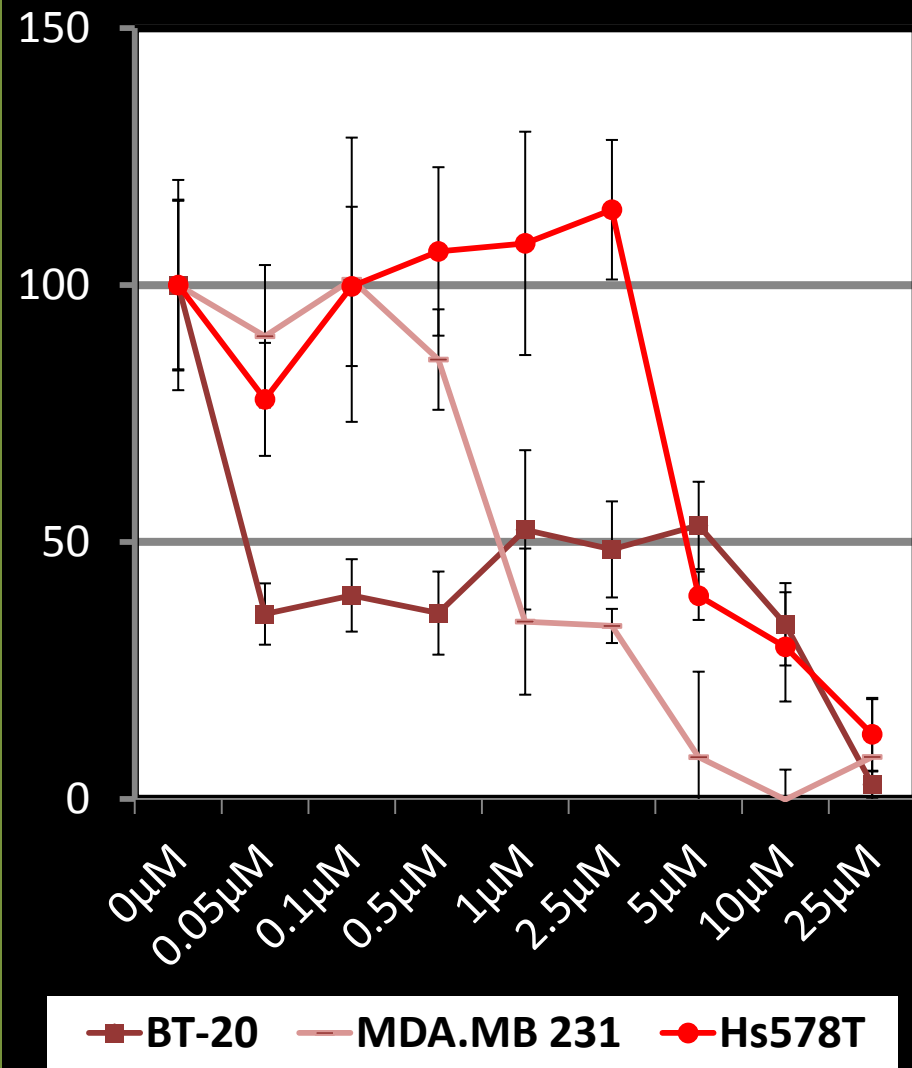


Molecular Classification and Multiplex Expression Signature

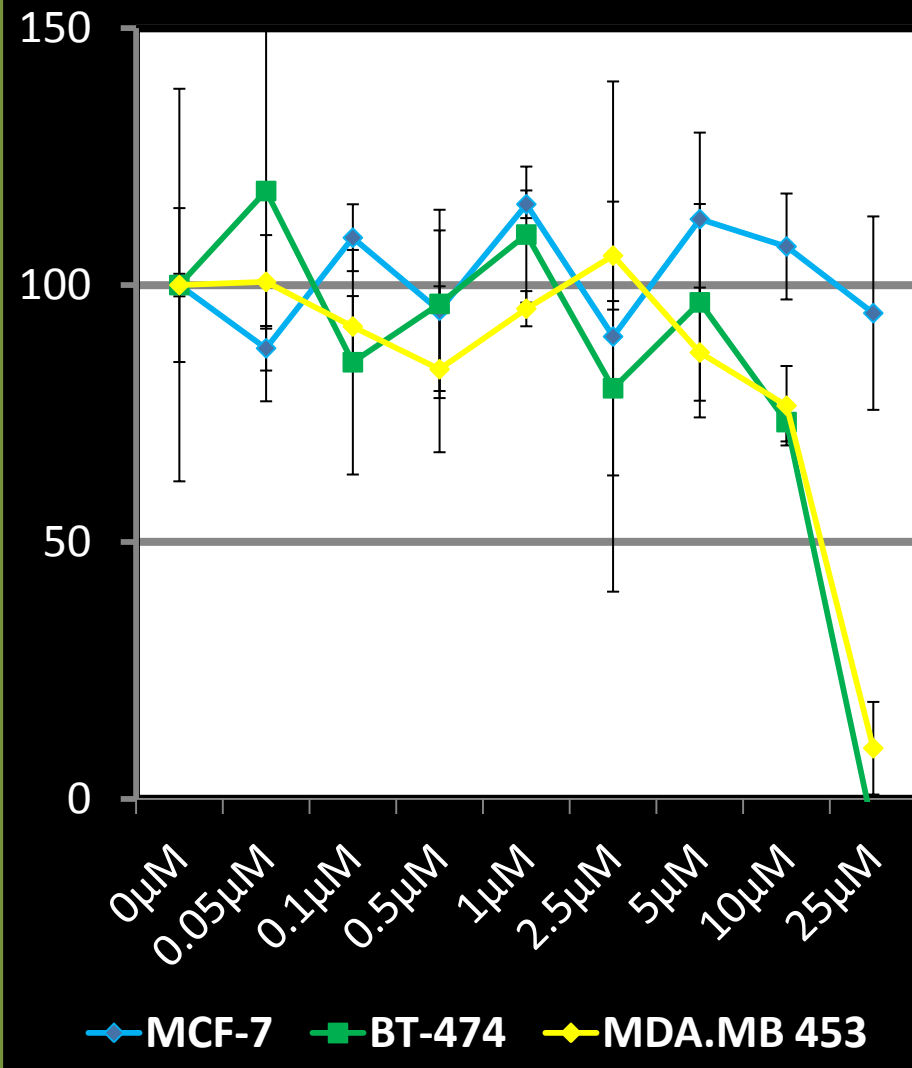
Case	Subtype	EMT	PP2A deregulation	mTOR targets	
				pS6K	HIF1 α
1	HER2-enriched	Mesenchymal	PPP2R2A - DOWN	+	+
2	HER2-enriched	Equivocal	NO	+	+
3	HER2-enriched	Mesenchymal	NO	--	--
4	HER2-enriched	Epithelial	SET - UP; ANP32A - UP	+	+
5	Luminal	Equivocal	cip2a - UP; ANP32A - UP	--	--
6	Luminal	Epithelial	PPP2R2A - DOWN	--	--
7	Basal	Epithelial	cip2a - UP; SET - UP	--	--
8	Basal	Mesenchymal	cip2a - UP	--	+
9	Basal	Epithelial	cip2a - UP	--	--
10	Equivocal	Mesenchymal	Undetermined	+	--

Sensitivity to FTY720 (Breast Cancer Cell lines)

FTY720 sensitivity assays - TNBC Cell lines



FTY720 sensitivity assays - Receptor Positive Cell lines



Summary of Results

- p-S6K and p-AKT (active proliferation) – 45% and 26%
- PP2A deregulation in **42%** of breast cancer
- PP2A deregulation is more common in TNBC especially through cip2a over-expression (17% of TCGA patients)
- Cip2a is upregulated in the basal subtype.

- Differential expression of TFF3, FN1, TWIST1 and CA12 between normal vs tumor matched samples is significant for classification of breast tumors.
- Normal vs Tumor matched data may identify biomarkers

Conclusions

- PP2A is commonly deregulated in breast cancer
- The 40-plex classifies breast cancer and identifies PP2A deregulation
- TNBC are sensitive to PP2A activation correlating with PP2A deregulation.
- Current analyses aim to classify tumors using expression signatures for PP2A-targeted therapy.

Collaborators



Funded by the
ImagenX Project



In Collaboration with
the
University of Leeds