Supplementary material

**Supplementary Table S1 - List of primers used for qPCR in this study**

Target gene symbol	Catalogue reference or sequences 5' – 3'	Supplier
ABCA8	QT00011095	Qiagen
ACTL8	QT00199934	Qiagen
ASF1B	QT00007224	Qiagen
B2M	QT00088935	Qiagen
BARHL1	QT00214599	Qiagen
BLM	QT00027671	Qiagen
BNIP3	QT00024178	Qiagen
BRCA1	QT00039305	Qiagen
BRCA2	QT00008449	Qiagen
CA9	F: GGAAGGCTCAGAGACTCA R: CTTAGCACTCAGCATCAC	Sigma-Aldrich
CDH1	QT00019418	Qiagen
E2F1	QT00016163	Qiagen
EXO1	QT00080717	Qiagen
FASN	QT00014588	Qiagen
FEN1	QT00064722	Qiagen
HK2	QT00013209	Qiagen
LOX	F: GTTCCAAGCTGGCTACTC R: GGGTTGTCGTCAGAGTAC	Sigma-Aldrich
LPIN1	QT00082873	
MCM6	QT00059570	Qiagen
MMP1	QT00014581	Qiagen
MMP14	F: CCCCGAAGCCTGGCTACA R: GCATCAGCTTTGCCTGTTACT	Sigma-Aldrich
PPARG	QT00029841	Qiagen
PTGS2	QT00040586	Qiagen
RAB6C	QT00219366	Qiagen
RAD51	QT00031493	Qiagen
RRM1	QT00066717	Qiagen
RRM2	QT00039480	Qiagen
SCD	QT00052381	Qiagen
SNA1L	F: GACCACTATGCCGCGCTCTT R: TCGCTGTAGTTAGGCTTCCGATT	Sigma-Aldrich
VEGFA	F: CTACCTCCACCATGCCAAGT R: CTCGATTGGATGGCAGTAGC	Sigma-Aldrich
VIM	QT00095795	Qiagen
WEE1	QT00038199	Qiagen
WSB1	QT00064127	Qiagen

Supplementary Table S2 – Antibodies used in this study

| Target	Manufacturer	Reference or Cataloge No.	Dilution	Origin	Expected band size (kDa)
β-Actin	Santa Cruz	sc-47778	1:2000	M	42
E2F1	Cell Signaling		1:1000		70
E-cadherin	Cell Signaling	3195	1:1000	R	135
HIF-1α	Becton Dickinson (BD) Biosciences	610958	1:500	M	120
HK2	Cell Signaling	2867	1:1000	R	102
p53 (DO-1)	Santa Cruz	sc-126	1:2000	M	53

M = mouse; R = rabbit
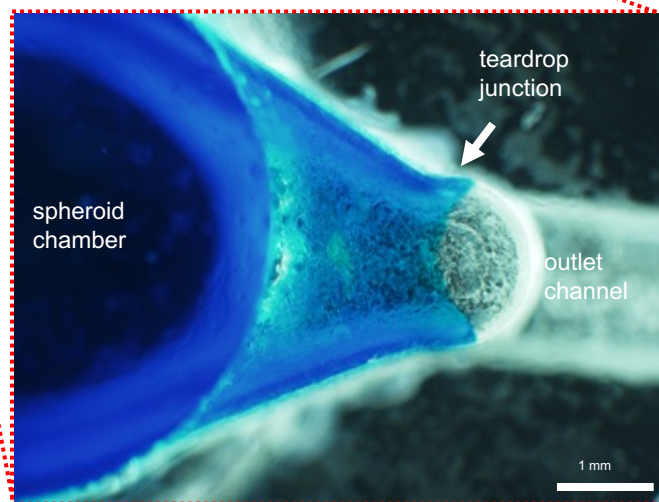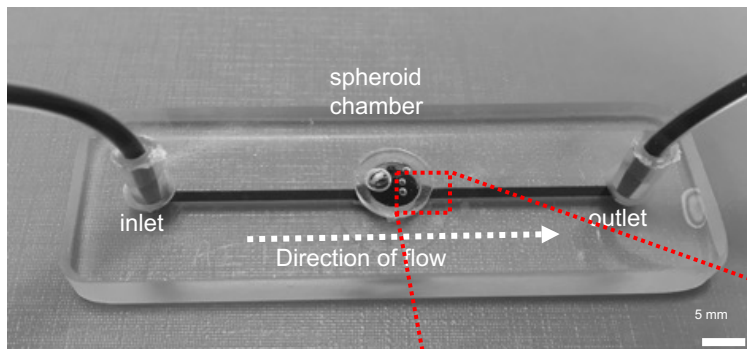
Supplementary Figure S1 – Detail of teardrop junction in spheroid-on-chip perfusion device
Photograph and microscope image detail of the spheroid-on-chip microfluidic perfusion device, highlighting the teardrop junction. Brilliant blue dye flowing through the chip demonstrates the location of the channel and shape of junction.
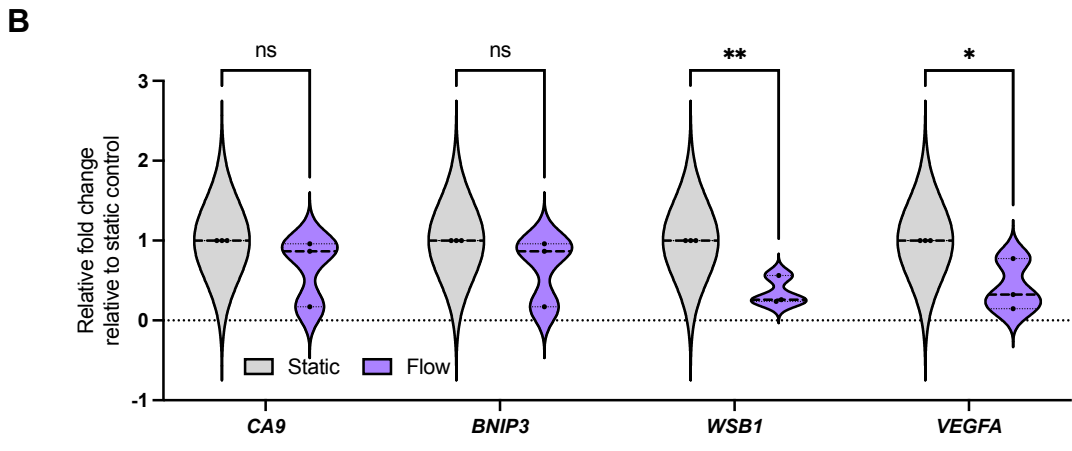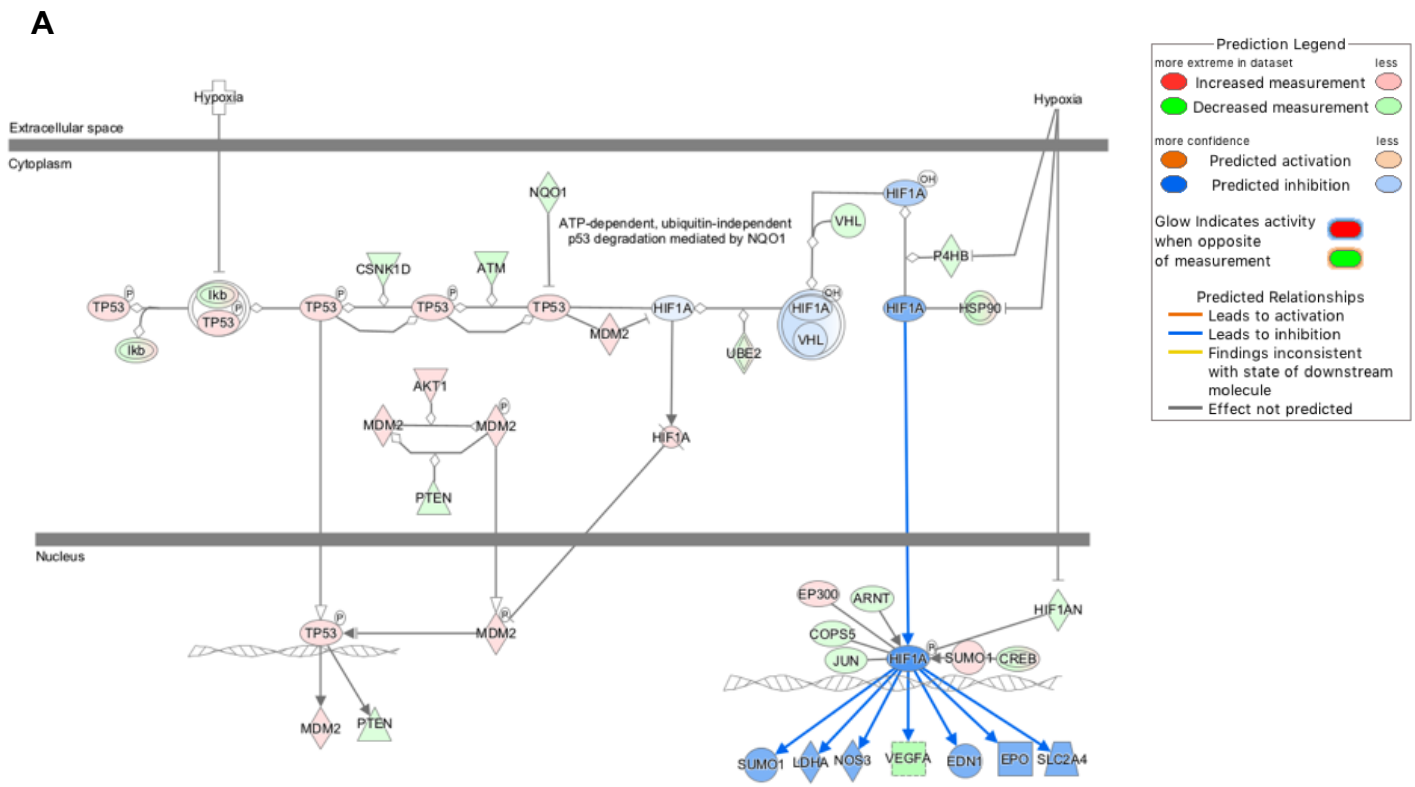
A
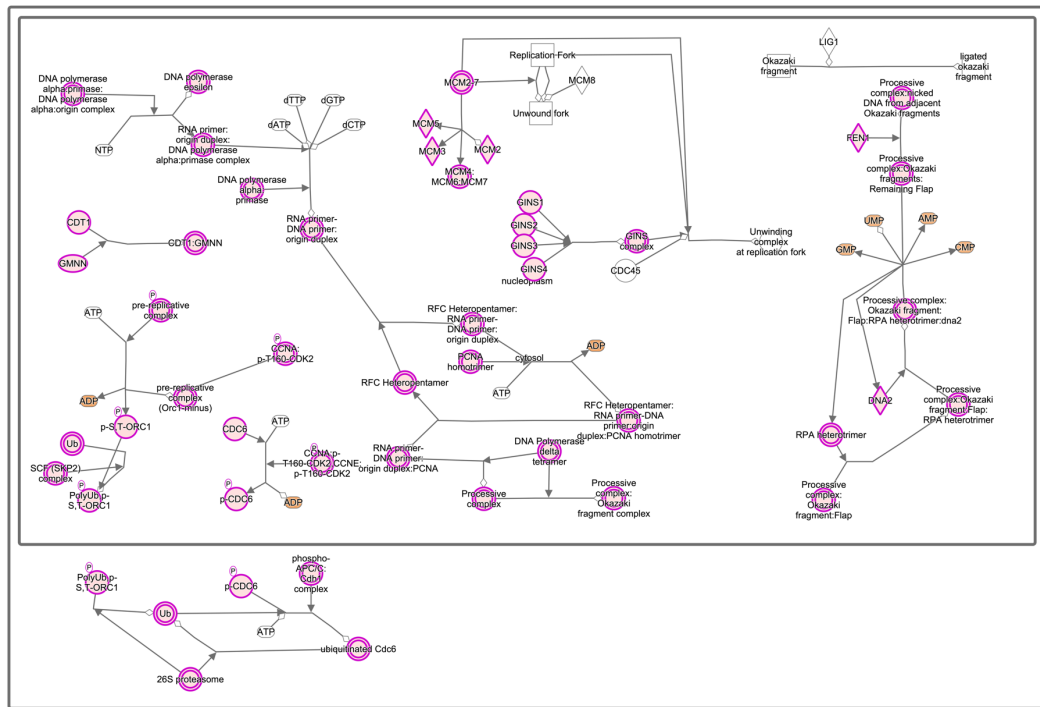
B
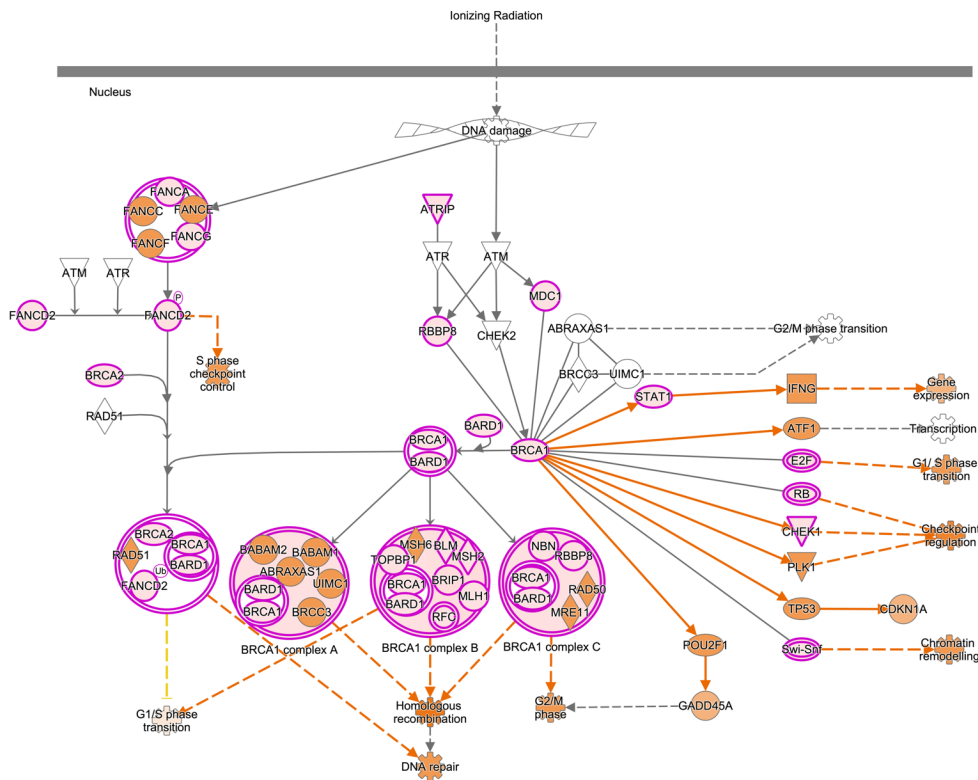
Supplementary Figure S2 - IFF-like perfusion/flow led to upregulation of DNA replication, cell cycle, and DNA repair pathways
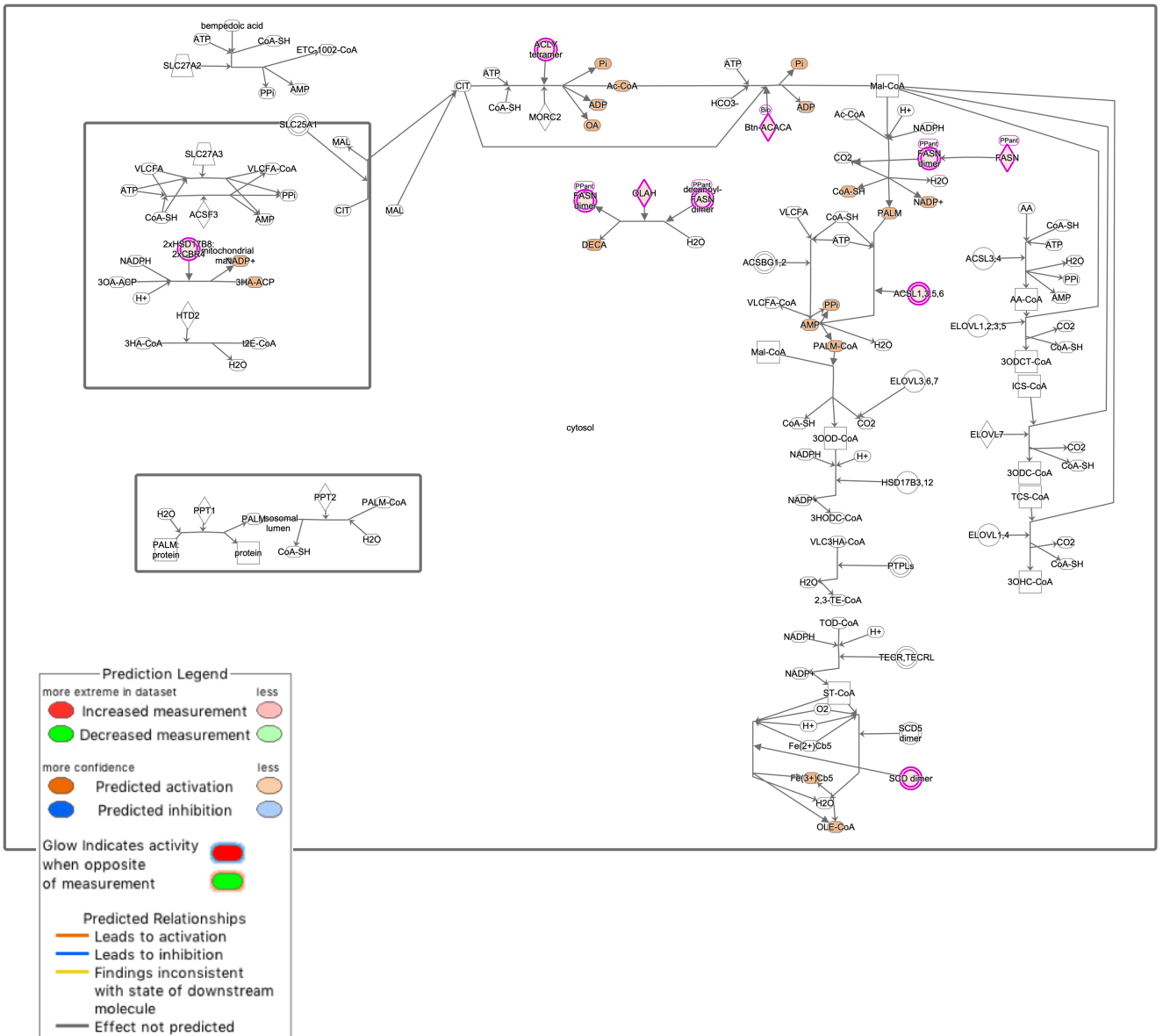Diagrams represent canonical pathway diagrams for DNA replication (A) and double strand break repair (B) generated using the Ingenuity Pathway Analysis platform.

Supplementary Figure S3 - Hypoxia signalling is downregulated in the better perfused spheroid-on-chip model

(A) Canonical pathway diagram for low oxygen/hypoxia signalling generated using the Ingenuity Pathway Analysis platform.

(B) A panel of hypoxia-regulated genes was validated through qPCR as described before for 72 hours post flow, representing n=3 independent experiments. Unpaired student's t-test was performed to test for statistical significance between samples. ns = nonsignificant; * p<0.05; ** p<0.01

Supplementary Figure S4 - Gene expression changes mediated by IFF-like perfusion/flow differ depending on environmental oxygen tensions

Diagram represents canonical pathway diagrams for Phospholipid metabolism signalling pathways were enriched in upregulated DEGS after flow generated using the Ingenuity Pathway Analysis platform.
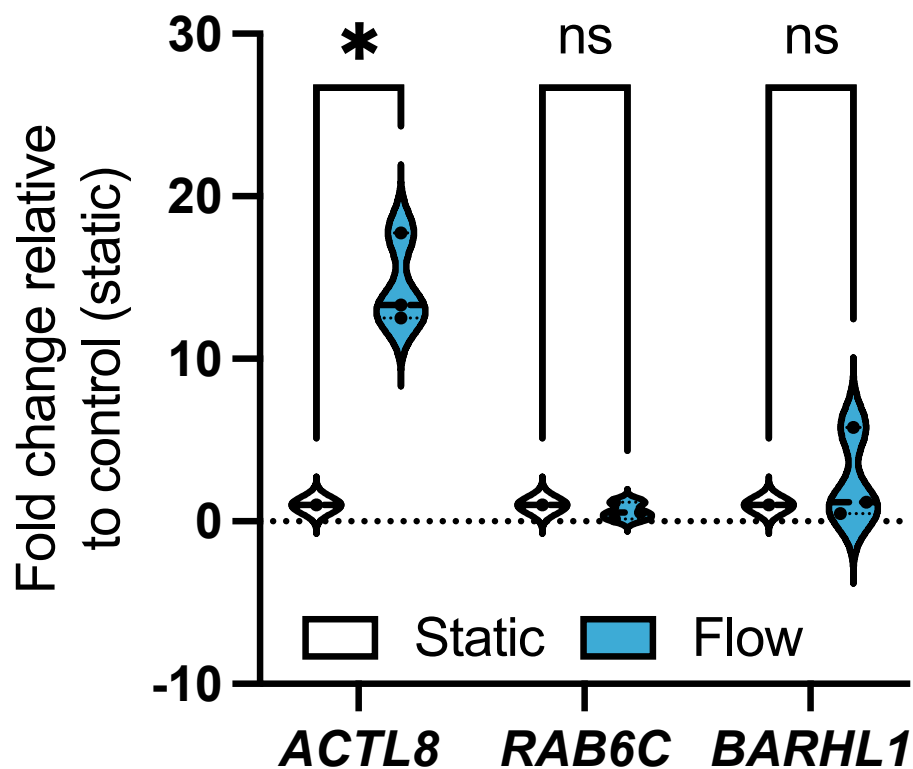
Supplementary Figure S5 – Identification of potential IFF-associated clinically relevant biomarkers – validation of candidate panel at 72 hours
A panel of genes uniquely linked with either static or flow conditions were selected from the normoxic (20% O_2) RNA-sequencing dataset (as determined in Figure 2). MCF7 spheroids were formed as before and exposed to static or flow conditions for 72 hours in the presence of Matrigel in normoxic conditions (20% O_2), and gene expression for the gene panel validated through qPCR as described before, with plots (B) representing n=3 independent experiments.

Supplementary Table S3 - Patient demographics of breast invasive carcinoma TCGA pan cancer Atlas dataset

Age median (range)	58 (26 - 90)	
Sex n (%)	*Female*	1072 (99)
	Male	12 (1)
Disease Stage * n (%)	I	189 (17)
	II	613 (57)
	III	244 (22)
	IV	19 (2)
	Unknown	19 (2)
Molecular Subtype n (%)	Basal	171 (16)
	Her2	78 (7)
	Luminal A	499 (46)
	Luminal B	197 (18)
	'normal'	36 (3)
	Unknown	103 (10)

*Neoplasm Disease Stage American Joint Committee on Cancer Code
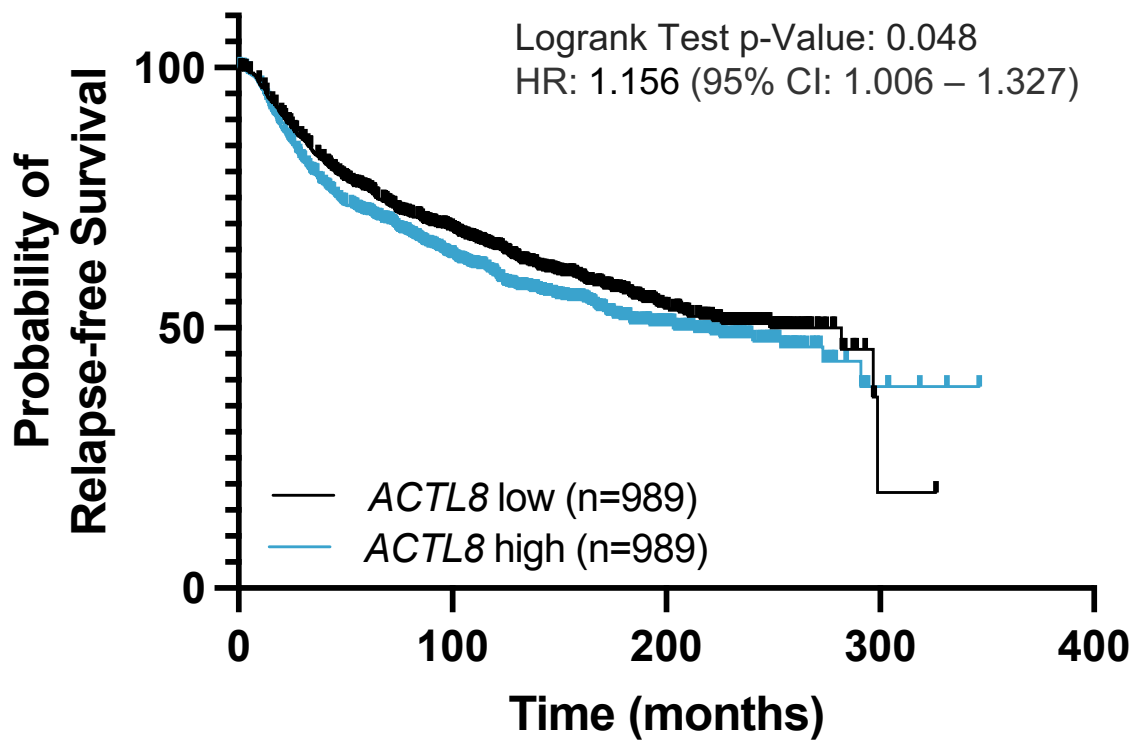
Supplementary Figure S6 – Prognostic value for *ACTL8* expression in breast cancer patient datasets (Metabric Dataset)
Breast invasive carcinoma patient samples was determined using the Metabric RNA-seq dataset (n=1978). Relapse-free Survival plot compare high and low *ACTL8* expression, determined by median *ACTL8* expression.
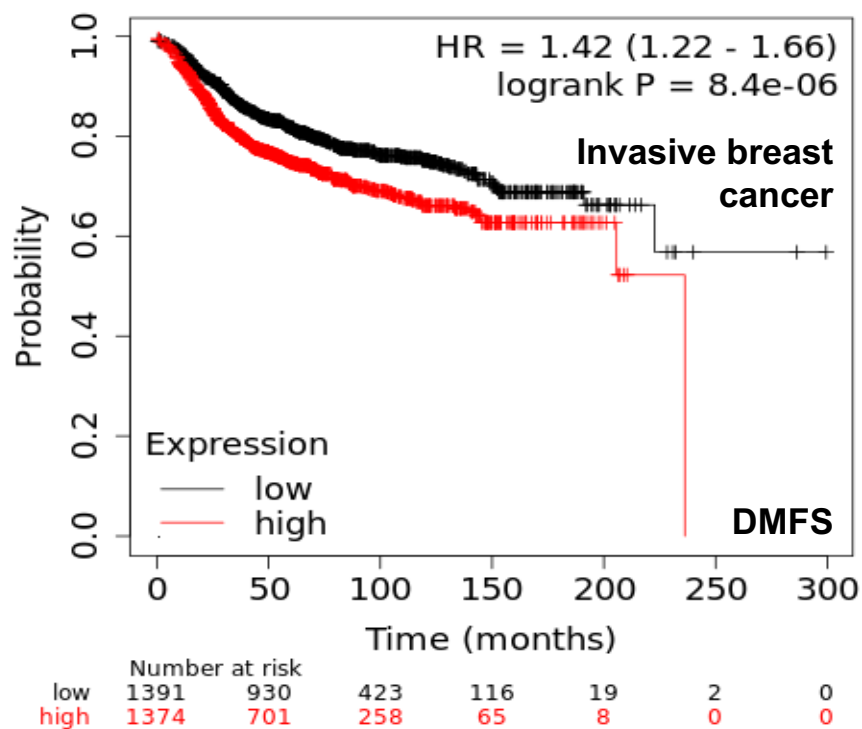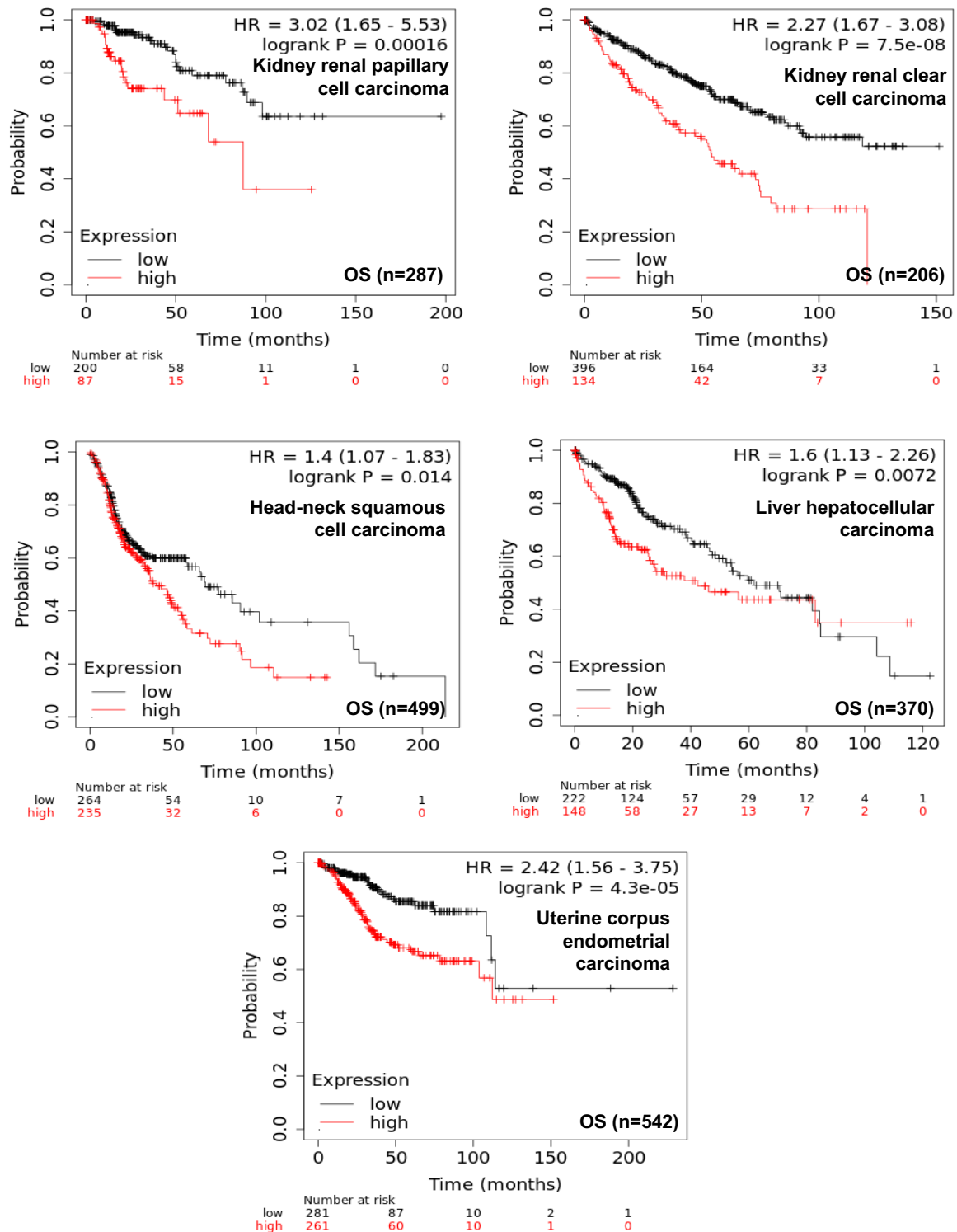
**Supplementary Figure S7 – Distant Metastasis-Free Survival prognostic value for *ACTL8*
expression in breast cancer patient datasets**
KMplotter was used to analyse *ACTL8* expression levels in gene chip array-based patient
data and correlate it to distant metastases-free survival (DMFS) *n* =2765. Patients were split
by *ACTL8* median expression, and no other patient selection thresholds were applied.
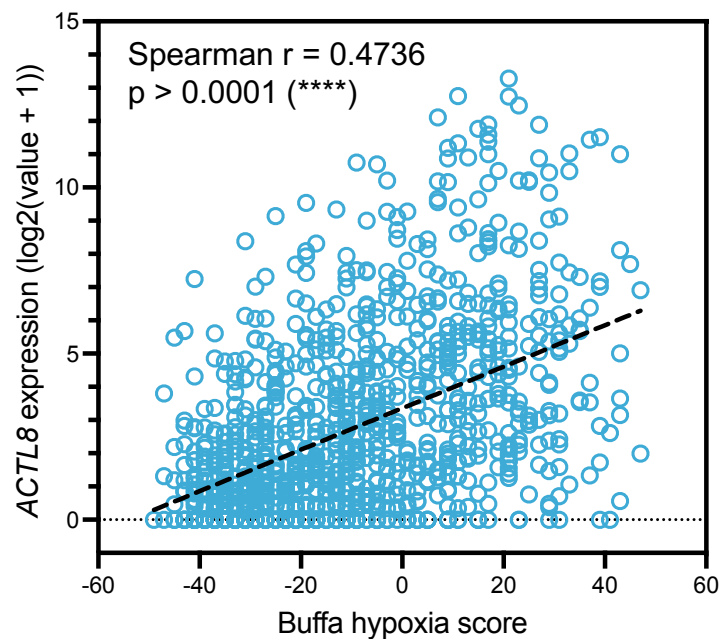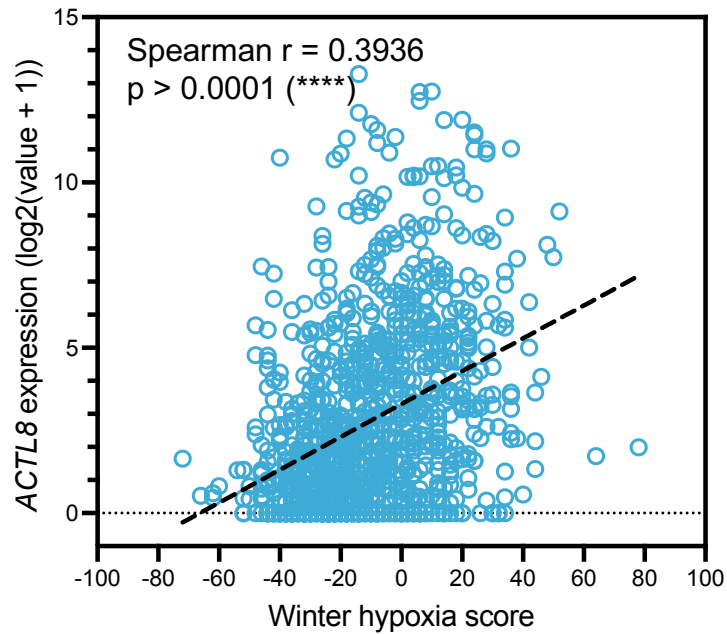
Supplementary Figure S8 – *ACTL8* expression in relation to hypoxia scores
ACTL8 expression in patients (*n* = 994) from the TCGA PanCancer Atlas Breast Invasive Carcinoma study is plotted on the y-axis against the Winter and Buffa hypoxia score. The hypoxia score is representative of a hypoxia metagene signature. The expression data was log2 transformed.

Supplementary Figure S9 – Overall Survival prognostic value for *ACTL8* expression in other tumour types (pan-cancer analysis)
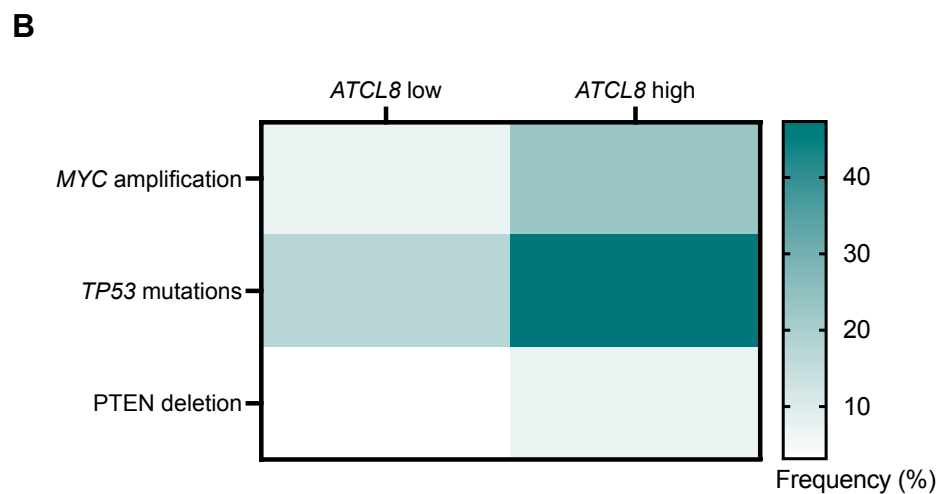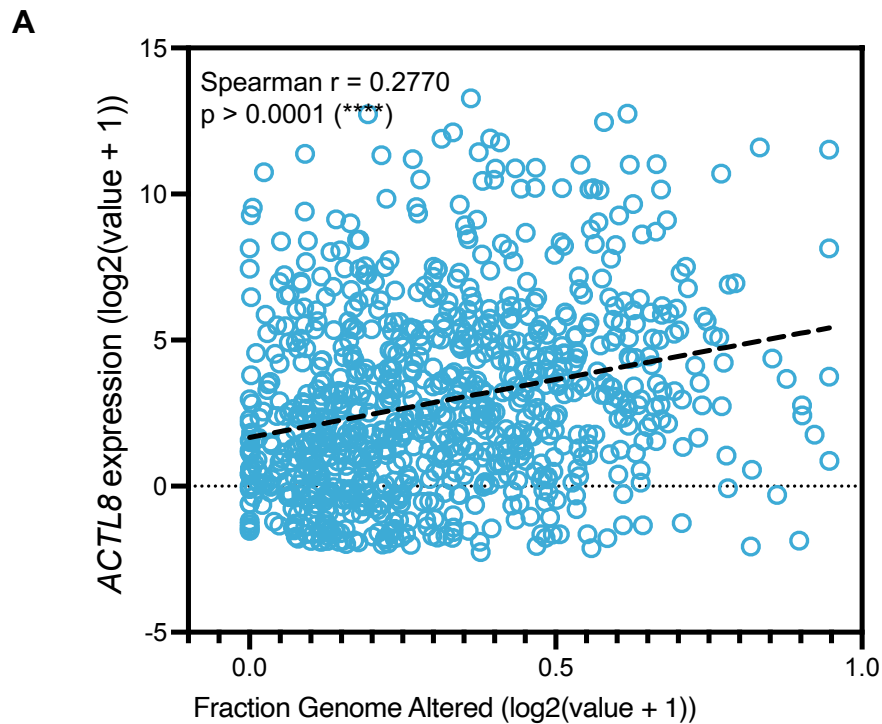KMplotter was used to analyse *ACTL8* expression levels in gene chip array-based patient data and correlate it to Overall Survival. Patients were split by *ACTL8* median expression, and no other patient selection thresholds were applied.
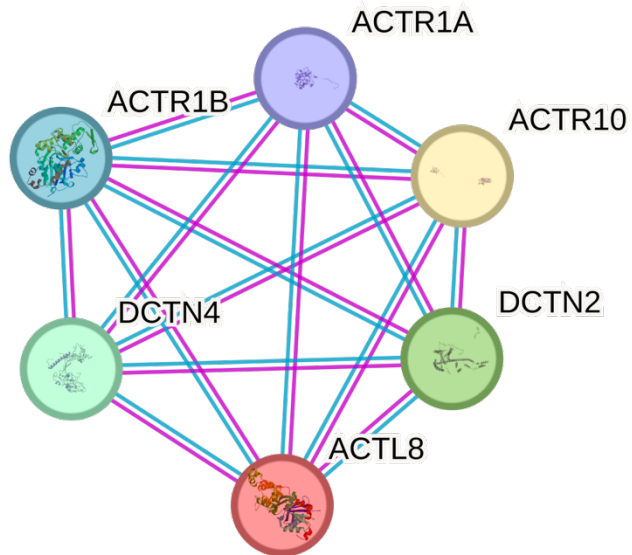
Supplementary Figure S10 – STRING PPI (protein-protein interaction) analysis network for ACTL8

The interactome was produced using the online STRING database (Szklarczyk et al., 2015). Colour of the connectors depends on the type of evidence for the interaction: blue: from curated databases; pink: experimentally determined.

Key: ACTL8 (Actin like 8); DCTN2 (Dynactin subunit 2); DCTN4 (Dynactin subunit 4); ACTR1A (Alpha-centractin); ACTR1B (Beta-centractin); ACTR10 (Actin related protein 10)
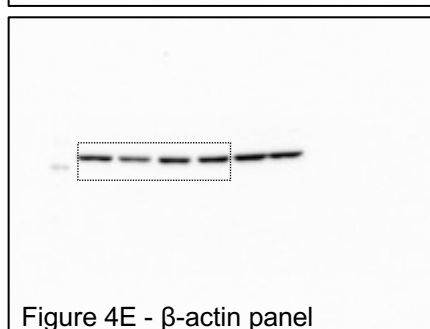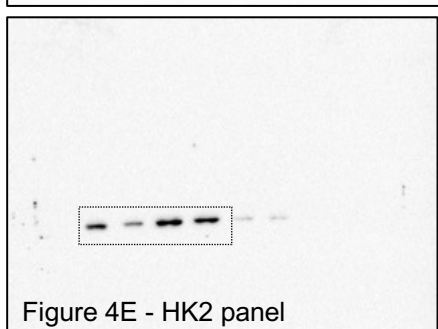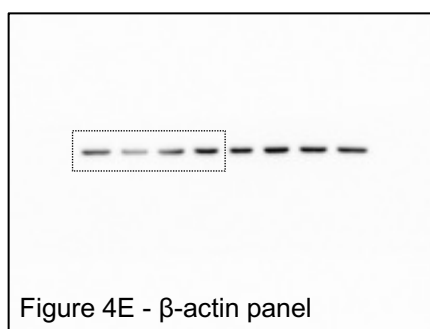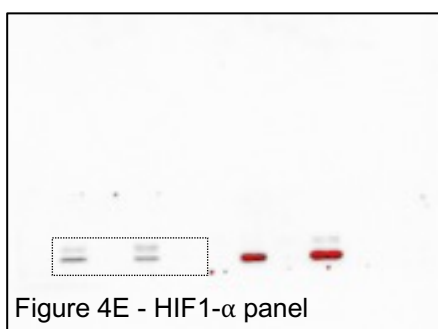
A

Spearman r = 0.2770
p > 0.0001 (****)

ACTL8 expression (log2(value + 1))

Fraction Genome Altered (log2(value + 1))

B

ATCL8 low   *ATCL8* high

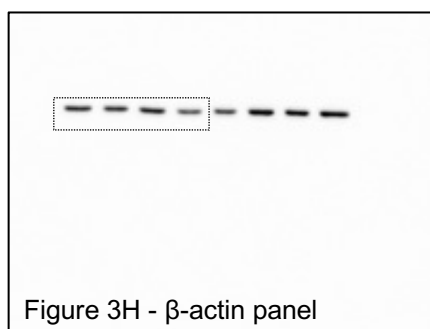*MYC* amplification

TP53 mutations
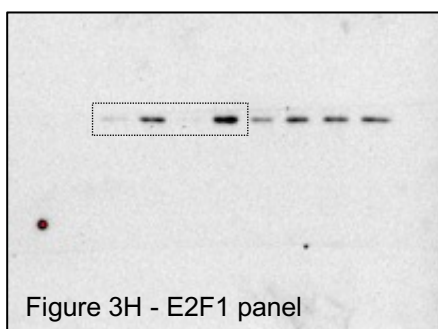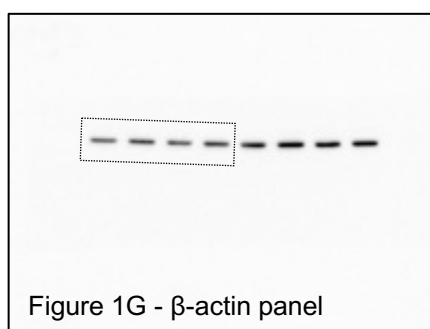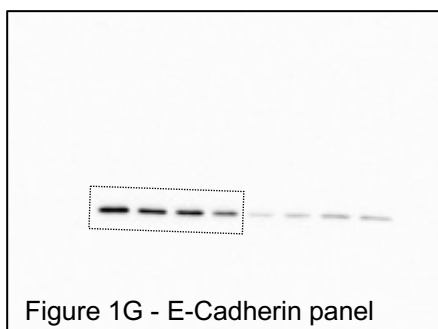
PTEN deletion

Frequency (%)

Supplementary Figure S11 – Analyses of relationship between ACTL8 mRNA expression and genomic instability markers

ACTL8 expression in patients (*n* = 994) from the TCGA PanCancer Atlas Breast Invasive Carcinoma study were analysed for links with genomic instability markers.

A - Correlation analysis of *ACTL8* mRNA levels vs Genomic instability marker 'Fraction Altered Genome'.

B - Frequency of genomic alterations linked with increased genomic instability (*MYC* amplification, *TP53* mutations, and *PTEN* deletions) comparing patient samples with low of high *ACTL8* mRNA expression.

Figure 1G - E-Cadherin panel

Figure 1G - β-actin panel

Figure 3H - E2F1 panel

Figure 3H - β-actin panel

Figure 3H – p53 panel

Figure 3H - β-actin panel

Figure 4E - HIF1-α panel

Figure 4E - β-actin panel

Figure 4E - HK2 panel

Figure 4E - β-actin panel
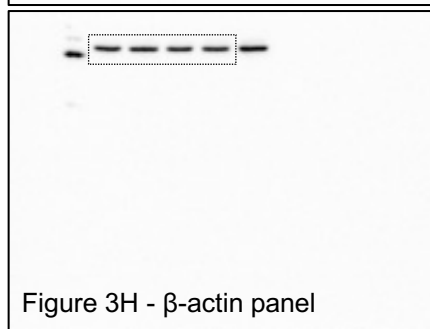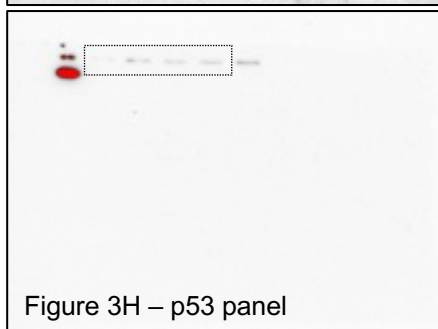
Supplementary Figure S12 – Raw blots from immunoblotting panels in manuscript