Supplementary Figures

BBIT20 inhibits homologous DNA repair with disruption of the BRCA1-BARD1 interaction in breast and ovarian cancer

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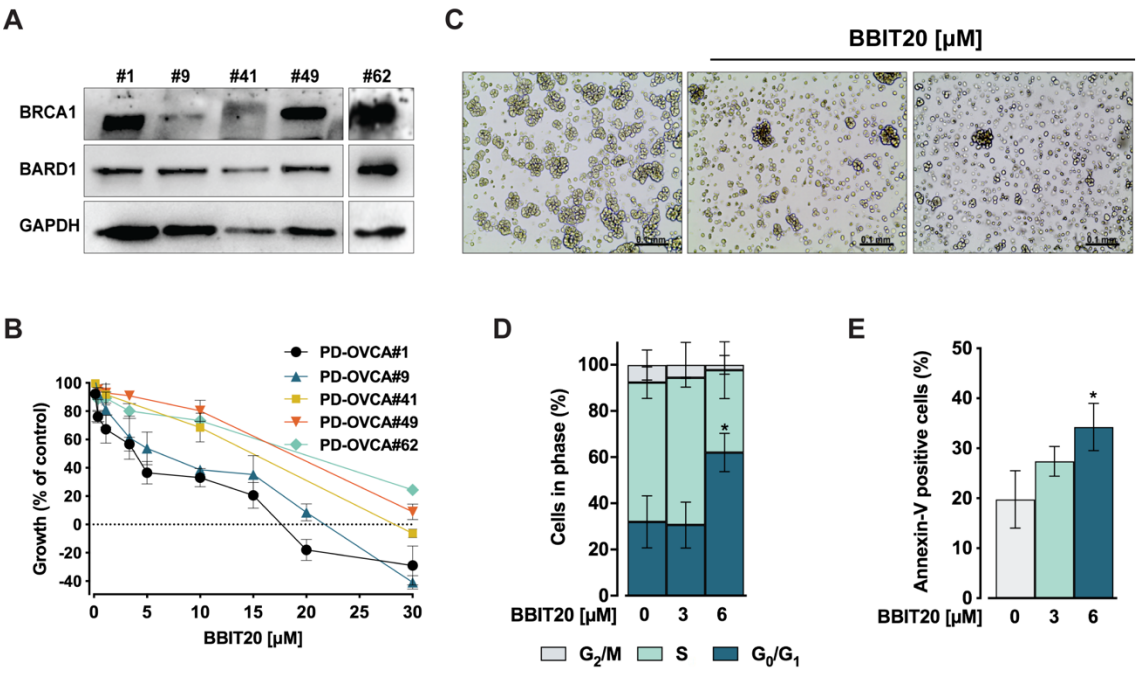
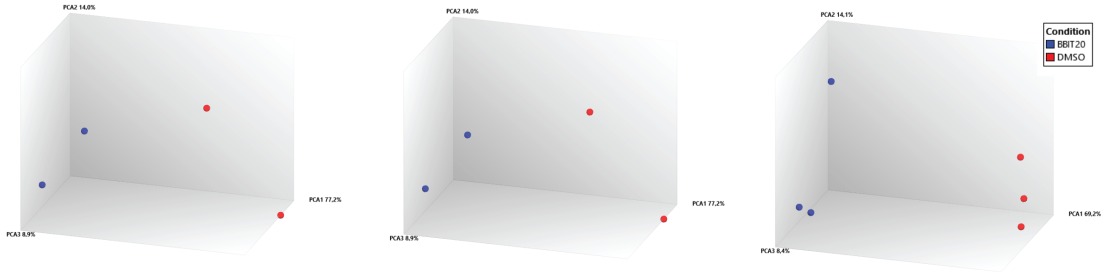
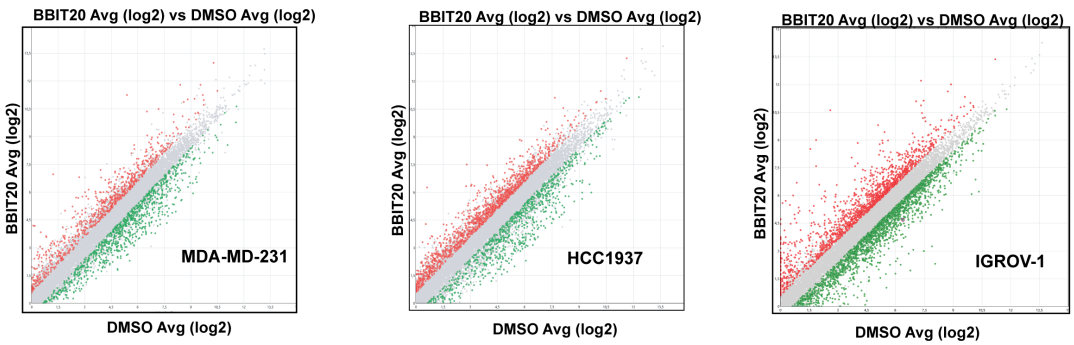


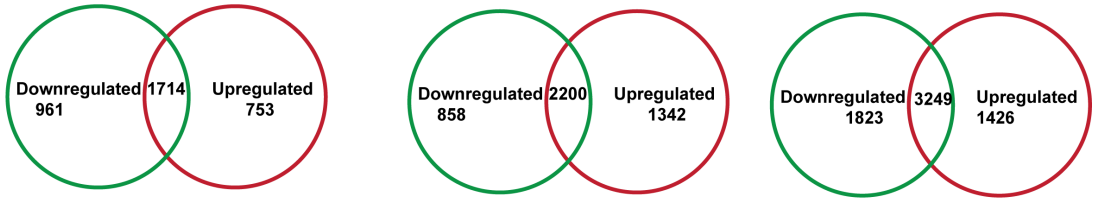
Figure S1. BRCA1 and BARD1 protein expression levels and BBIT20 growth inhibitory activity, in PD-OVCA cells. **(A)** PD-OVCA cells expressing mutBRCA1 (#1 and #9) or wtBRCA1 (#41, #49 and #62). **(B)** Dose-response curves for the growth inhibitory effect of BBIT20 on patient-derived ovarian cancer (PD-OVCA) cells after 48 h treatment; growth obtained with control was set as 100%; data are mean ± SD, n=4 independent experiments. **(C)** Representative images of BBIT20 effect on PD-OVCA#1 morphology after 48 h treatment (scale bar=0.1 mm; 400× amplification). (**D**, **E**) Effect of 3 and 6 µM BBIT20 on cell cycle progression (D) and apoptosis (**E**) in PD-OVCA#1, after 48 h treatment; data are mean ± SD, n=4-5 independent experiments; values significantly different from DMSO: **P*<0.05 (two-way ANOVA followed by Dunnett's test).

A


PCA2 14,0%
PCA1 77,2%
PCA3 8,9%
PCA2 14,0%
PCA1 77,2%
PCA3 8,9%
PCA2 14,1%
PCA1 69,2%
PCA3 8,4%
Condition
BBIT20
DMSO

B


BBIT20 Avg (log2) vs DMSO Avg (log2)
BBIT20 Avg (log2)
DMSO Avg (log2)
MDA-MD-231
BBIT20 Avg (log2) vs DMSO Avg (log2)
BBIT20 Avg (log2)
DMSO Avg (log2)
HCC1937
BBIT20 Avg (log2) vs DMSO Avg (log2)
BBIT20 Avg (log2)
DMSO Avg (log2)
IGROV-1


Downregulated 961
1714
Upregulated 753
Downregulated 858
2200
Upregulated 1342
Downregulated 1823
3249
Upregulated 1426

C

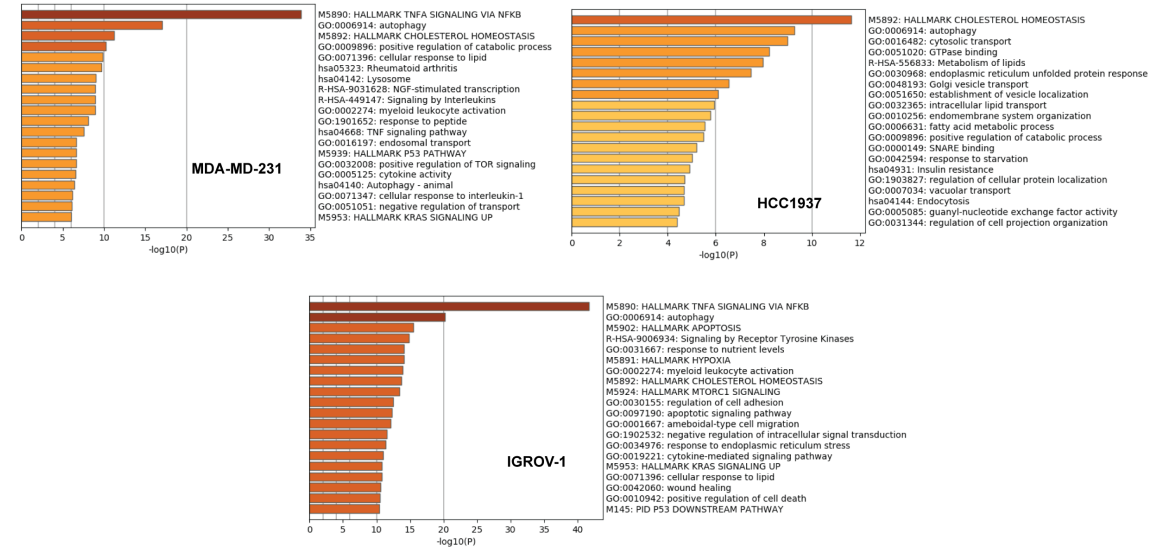

M5890: HALLMARK TNFA SIGNALING VIA NFKB
GO:0006914: autophagy
M5892: HALLMARK CHOLESTEROL HOMEOSTASIS
GO:0009896: positive regulation of catabolic process
GO:0071396: cellular response to lipid
hsa05323: Rheumatoid arthritis
hsa04142: Lysosome
R-HSA-9031628: NGF-stimulated transcription
R-HSA-449147: Signaling by Interleukins
GO:0002274: myeloid leukocyte activation
GO:1901652: response to peptide
hsa04668: TNF signaling pathway
GO:0016197: endosomal transport
M5939: HALLMARK P53 PATHWAY
GO:0032008: positive regulation of TOR signaling
GO:0005125: cytokine activity
hsa04140: Autophagy - animal
GO:0071347: cellular response to interleukin-1
GO:0051051: negative regulation of transport
M5953: HALLMARK KRAS SIGNALING UP
MDA-MD-231
-log10(P)
M5892: HALLMARK CHOLESTEROL HOMEOSTASIS
GO:0006914: autophagy
GO:0016482: cytosolic transport
GO:0051020: GTPase binding
R-HSA-556833: Metabolism of lipids
GO:0030968: endoplasmic reticulum unfolded protein response
GO:0048193: Golgi vesicle transport
GO:0051650: establishment of vesicle localization
GO:0032365: intracellular lipid transport
GO:0010256: endomembrane system organization
GO:0006631: fatty acid metabolic process
GO:0009896: positive regulation of catabolic process
GO:0000149: SNARE binding
GO:0042594: response to starvation
hsa04931: Insulin resistance
GO:1903827: regulation of cellular protein localization
GO:0007034: vacuolar transport
hsa04144: Endocytosis
GO:0005085: guanyl-nucleotide exchange factor activity
GO:0031344: regulation of cell projection organization
HCC1937
-log10(P)
M5890: HALLMARK TNFA SIGNALING VIA NFKB
GO:0006914: autophagy
M5902: HALLMARK APOPTOSIS
R-HSA-9006934: Signaling by Receptor Tyrosine Kinases
GO:0031667: response to nutrient levels
M5891: HALLMARK HYPOXIA
GO:0002274: myeloid leukocyte activation
M5892: HALLMARK CHOLESTEROL HOMEOSTASIS
M5924: HALLMARK MTORC1 SIGNALING
GO:0030155: regulation of cell adhesion
GO:0097190: apoptotic signaling pathway
GO:0001667: ameboidal-type cell migration
GO:1902532: negative regulation of intracellular signal transduction
GO:0034976: response to endoplasmic reticulum stress
GO:0019221: cytokine-mediated signaling pathway
M5953: HALLMARK KRAS SIGNALING UP
GO:0071396: cellular response to lipid
GO:0042060: wound healing
GO:0010942: positive regulation of cell death
M145: PID P53 DOWNSTREAM PATHWAY
IGROV-1
-log10(P)

Figure S2. Transcriptome analysis reveals differentially expressed genes in BBIT20-treated TNBC and OC cells. **(A)** Principal component analysis (PCA) of the gene expression counts (log2 normalized Trimmed Mean of M-values TMM) method counts) showing the first versus the second principal component (PC). Samples corresponding to the two conditions, BBIT20 or DMSO, are highlighted in blue and red colors, respectively. **(B)** Volcano plots of differentially expressed genes after 48 h treatment with 12 μM BBIT20 versus untreated cells. The differentially expressed genes are highlighted with red (upregulated genes) or green (downregulated genes) dots. **(C)** Effect of 12 μM BBIT20 in the transcriptome of cancer cells after 48h treatment; Metascape was used to perform gene set enrichment analysis of significantly up-regulated genes in cancer cells.

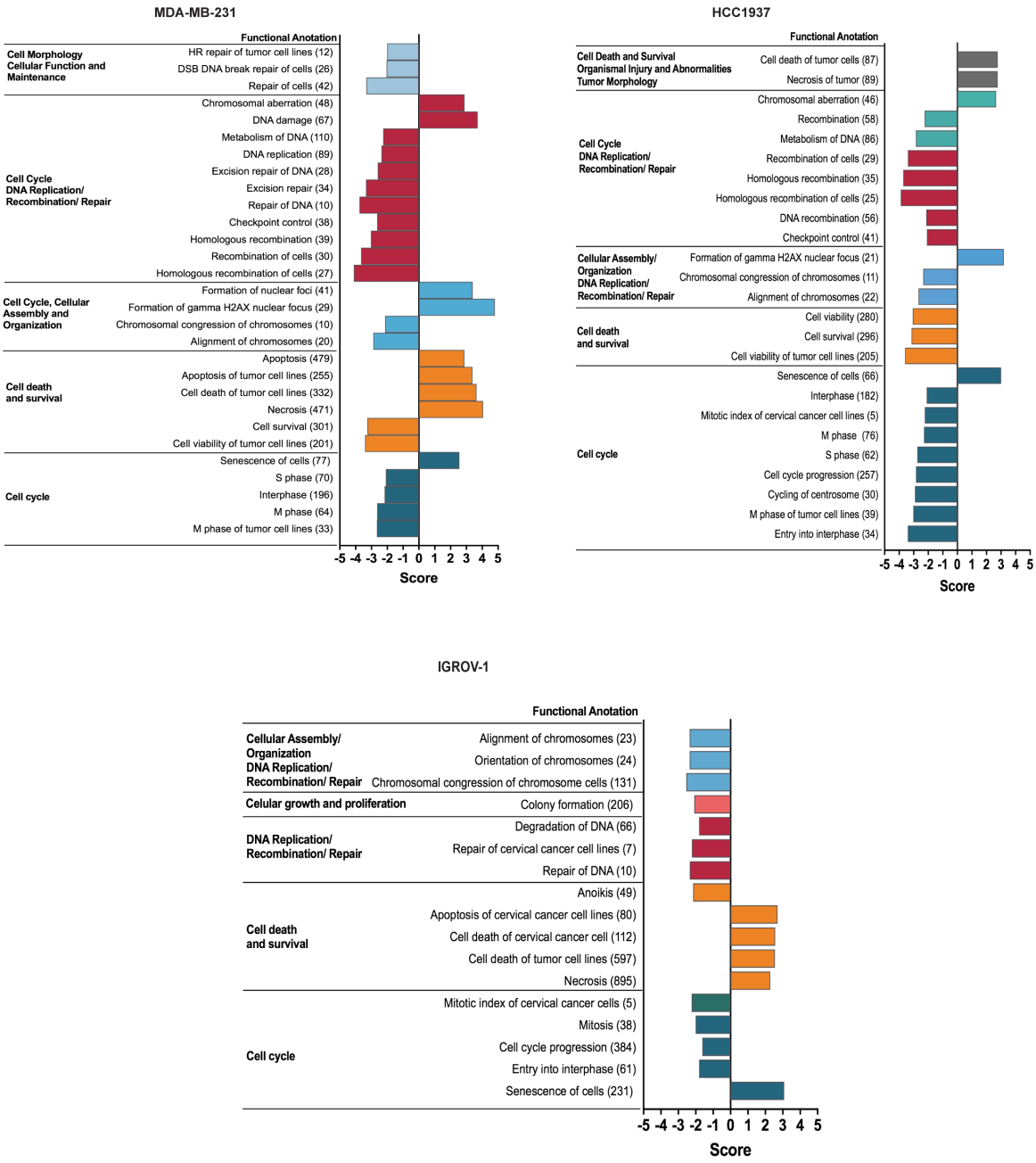


Figure S3. Top enriched biological pathways grouped by broad categories based on Ingenuity Pathway Analysis (IPA). Analysis performed starting from the dataset of differentially expressed genes (DEGs) from BBIT20-treated cells at MDA-MB-231, HCC1937 and IGROV-1 cancer cells. The number of features for each functional annotation is given in parenthesis. The score combines the *log10 p-value* and predicted pathway activation or repression status of the

corresponding pathway/process, respectively for positive and negative score. The different colors correspond to the different functional annotation categories.

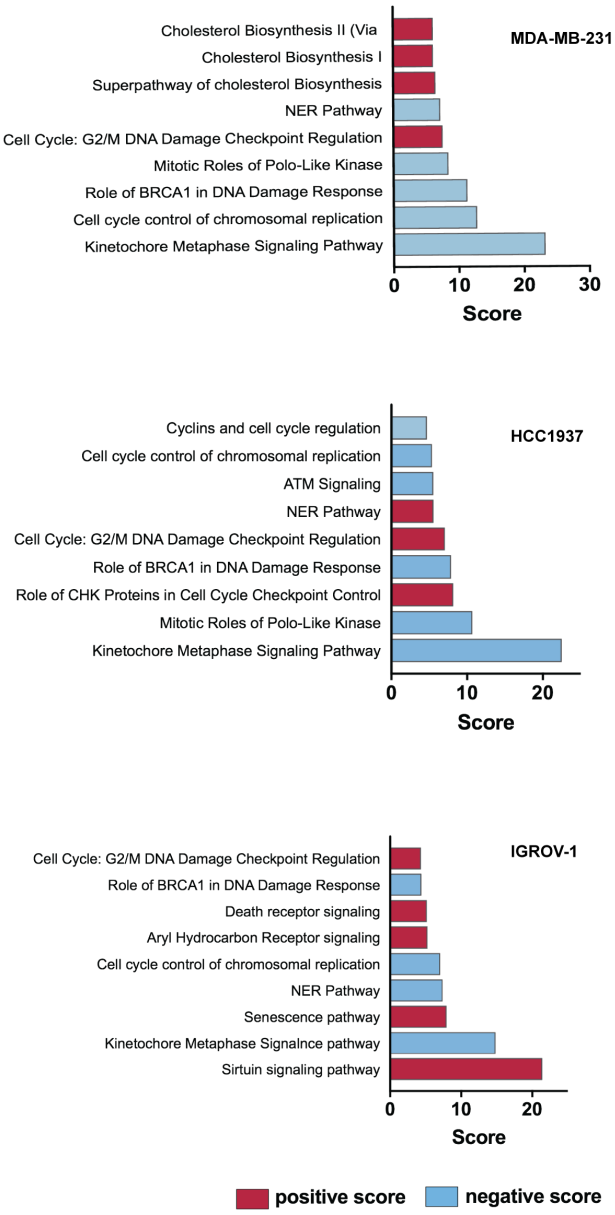


Figure S4. Top enriched canonical pathways based on Ingenuity Pathway Analysis (IPA). Analysis performed starting from the dataset of differentially expressed genes (DEGs) from BBIT20-treated cells at MDA-MB-231, HCC1937 and IGROV-1 cancer cells. The score combines the *log10 p-value* and predicted pathway activation or repression status of the corresponding pathway/process, respectively for positive and negative score. The different colors correspond to the different functional annotation categories.