Figure S1. Agarose gel electrophoresis images of reverse transcription-PCR products showing the time-course of induction of BLID mRNA in drug-treated (A) MCF-7 and (B) T47D cells. EtOH, ethanol; DXR, doxorubicin; DTX, docetaxel; 5-FU, 5-fluorouracil; BLID, BH-3 like motif containing inducer of cell death.

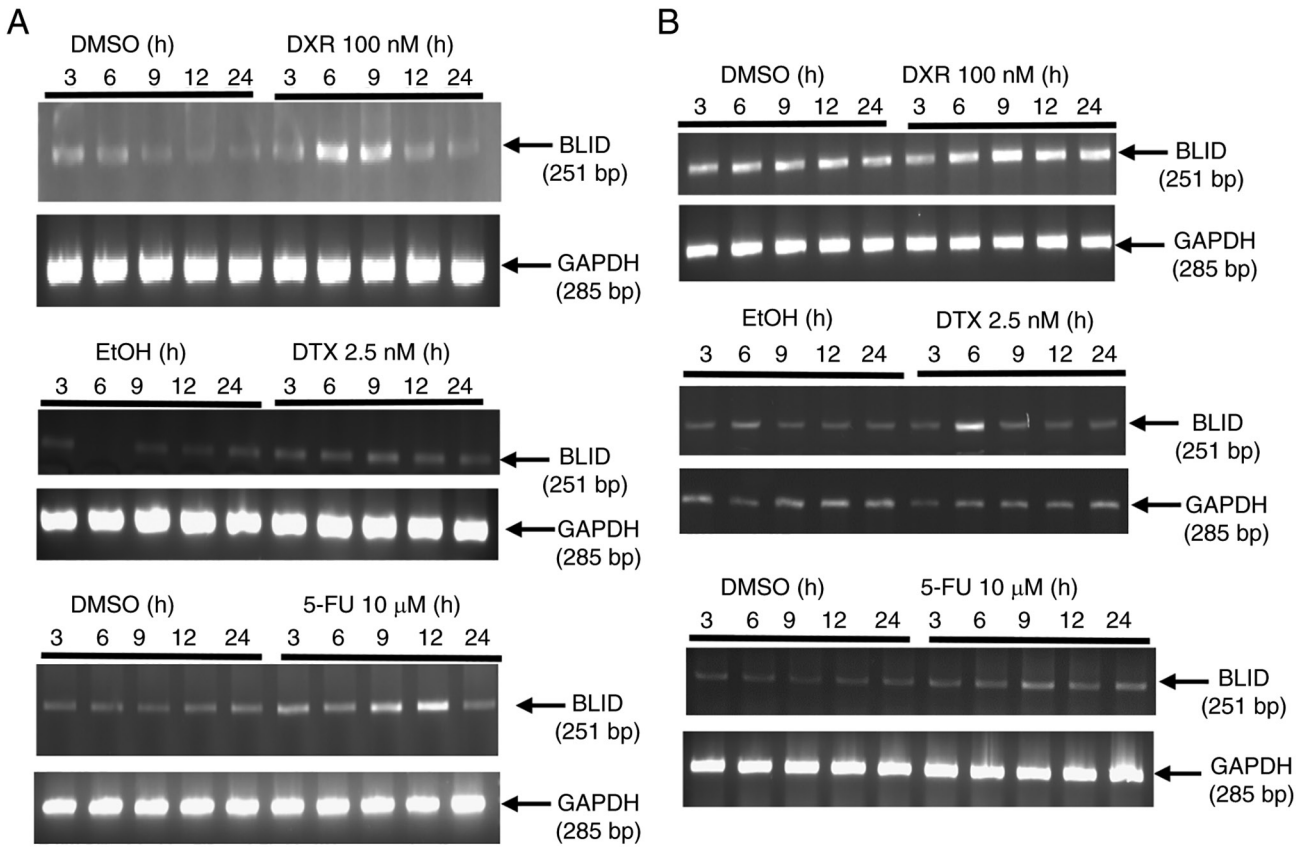


Figure S2. Dose-response experiments showing induction of BLID expression in MCF-7 cells treated with DXR or PTX. (A) Cells were treated with the indicated doses of DXR or PTX, and reverse transcription-PCR products were analyzed by agarose gel electrophoresis. (B) Reverse transcription-quantitative PCR analysis of BLID expression in the cells treated with various concentrations of DXR or PTX. The y-axis title of the right graph is identical to the y-axis title of the left graph. Data are presented as the mean ± SD normalized to GAPDH. DXR, doxorubicin; PTX, paclitaxel; BLID, BH-3 like motif containing inducer of cell death.

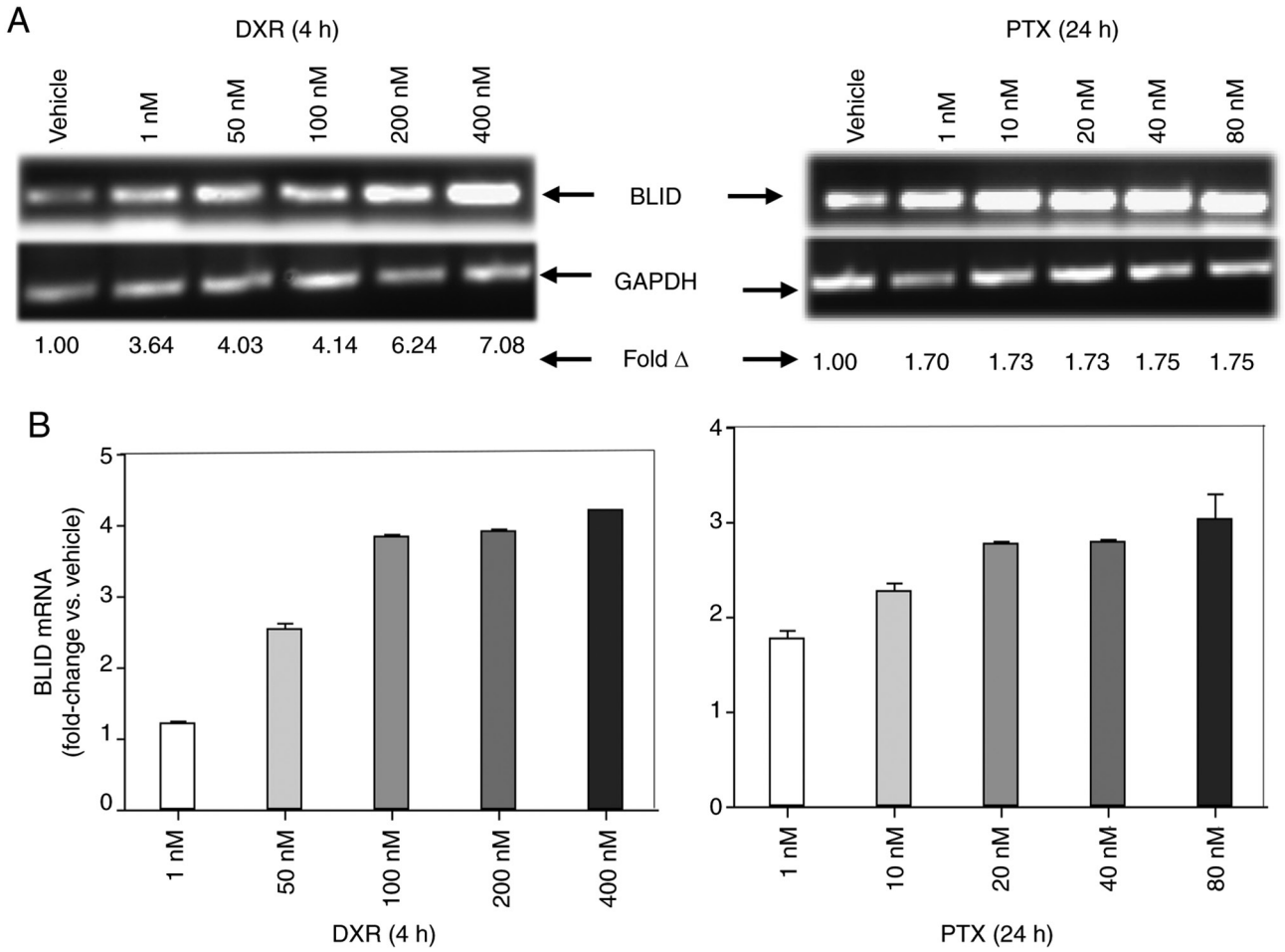
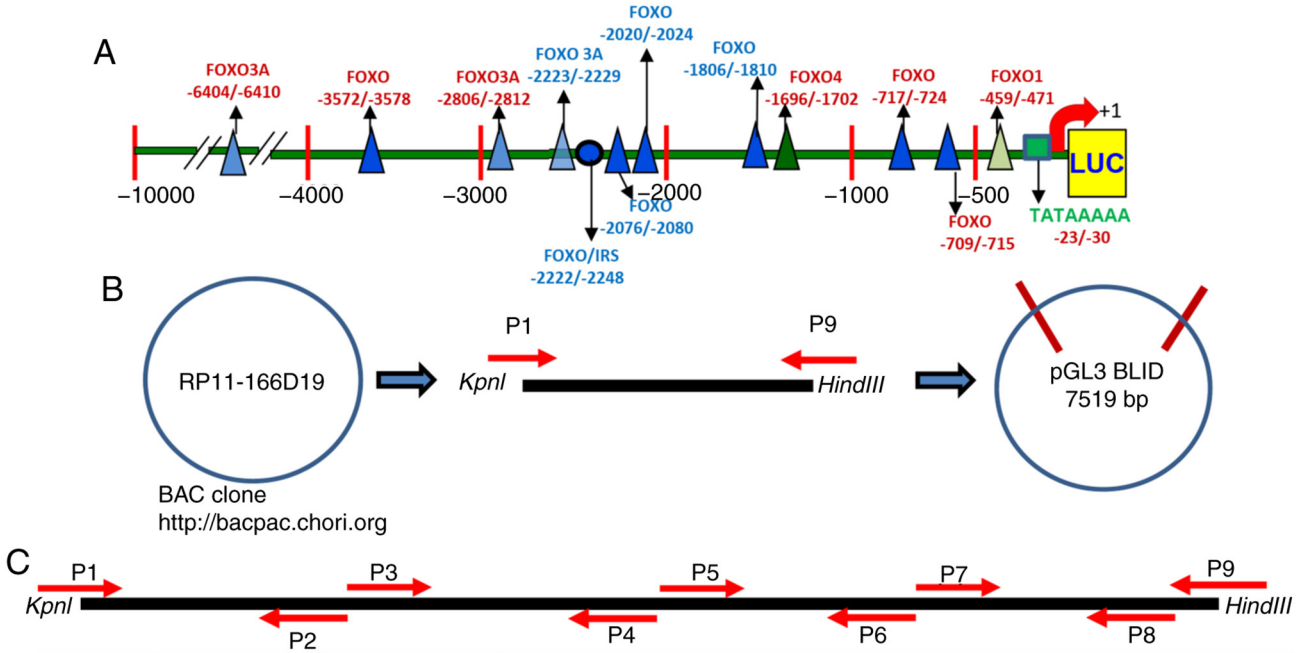


Figure S3. Luciferase reporter design. (A) Schematic map of the BLID promoter showing binding locations of the FOXO proteins. The core promoter region of the BLID gene was predicted within the 2.5 kb sequence upstream from the translation start site (Promoter Inspector and Gene2Promoter; Genomatix platform). The websites interrogated for identification of transcription factor binding sites on the putative BLID promoter were: http://www.genomatix.de and https://gene-regulation.com/pub/databases.html (Transfac 7.0). (B) Strategy for constructing the BLID luciferase reporter plasmid (pGL3 BLID). The primers P1 (5'-CTAGGGTACCCCAACATGGTAAAACCTTGT-3'; restriction site underlined) and P9 (5'-CTAGAAGCTTAAAATTTAACTTCCATT-3'; restriction site underlined) were used to amplify 2,444 bp of the BLID promoter. PCR was performed using Takara LA Taq (code no. RR002A; Takara Bio, Inc.), a Taq polymerase with proofreading activity, and the BAC clone RP11-166D19 as template. The source URL for the BAC clone is shown in this panel. The PCR conditions were: 94˚C for 1 min, followed by 30 cycles of 98˚C for 10 sec and 68˚C for 30 sec, and final extension at 72˚C for 10 min. The PCR product was extracted using the QIAquick gel extraction kit (Qiagen, Inc.) and digested with the corresponding enzymes. The product was ligated upstream of the firefly luciferase gene into the pGL3-Enhancer Vector (Promega Technical Manual: pGL3-enhancer cat. no. E1771; Promega Corporation). (C) Locations and sequences of primers, and the restriction enzymes used to verify the BLID promoter sequence (sequences in bold are the restriction enzyme recognition sites). IRS, insulin responsive sequence; LUC, location of the luciferase reporter; BAC, bacterial artificial chromosome; BLID, BH-3 like motif containing inducer of cell death.



Primer ID	Working ID	Primer Orientation	Sequence
P1	GKF1-Lu	Forward	5'-CTAG**GGTACC**CCAACATGGTAAAACCTTGT-3'-(**-2444/-2423)**
P2	GK1R	Reverse	5'-GGCAAAGACCCAAAGATGTATAAG-3'-(**-2151/-2128)**
P3	GK2F	Forward	5'-CCACCTTGTATGGTGCCTGGTATA-3'– **-2129/-2104)**
P4	GK2R	Reverse	5'- GGGGGATTAATGAGGGAAGGAATA- 3' **–(-1755/-1741)**
P5	GK3F	Forward	5'- ACCAGACTTTGCTCTCAGAGTAGG- 3' **– (-1385/-1362)**
P6	GK3R	Reverse	5'- TCTTACTTAGCAGCAGCAGAGAGG- 3' **-(- 999/-976)**
P7	GK4F	Forward	5'- CCTCTCTGCTGCTGCTAAGTAAGA - 3' **-(-999/- 976)**
P8	GK4R	Reverse	5'- CCAGCTCTCTTGTCTGGTGCTTAT - 3' **- (-550/-526)**
P9	R2R	Reverse	5'- CTAG**AAGCTT**AAAATTTAACTTCCATT-3'

Figure S4. Time-course analysis of drug-induced BLID luciferase reporter activity in MCF-7 cells. The y-axis title of the right graph is identical to the y-axis title of the left graph. Data are presented as the mean ± SD. pGL3-BLID, BLID luciferase reporter; pGL3 Basic, pGL3-Enhancer; DXR, doxorubicin; PTX, paclitaxel; BLID, BH-3 like motif containing inducer of cell death.

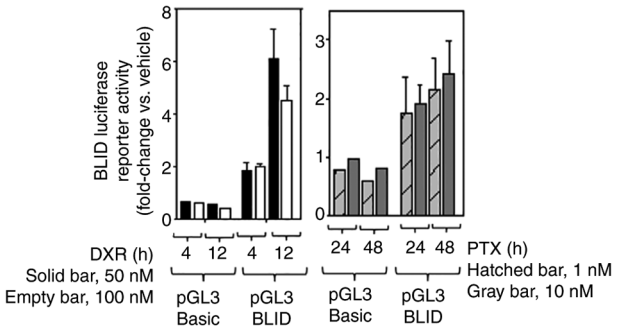


Figure S5. Dose-response experiments showing drug-induced BLID luciferase reporter activity in (A) MCF-7 and (B) T47D cells. All y-axes denote BLID luciferase reporter activity (fold change vs. vehicle). Data are presented as the mean ± SD of two to three replicates from one of the two independent experiments. *P<0.05 (drug vs. vehicle treatment at the time corresponding to the corresponding experimental treatment time period). DXR, doxorubicin; DTX, docetaxel; Cis, cisplatin; BLID, BH-3 like motif containing inducer of cell death.

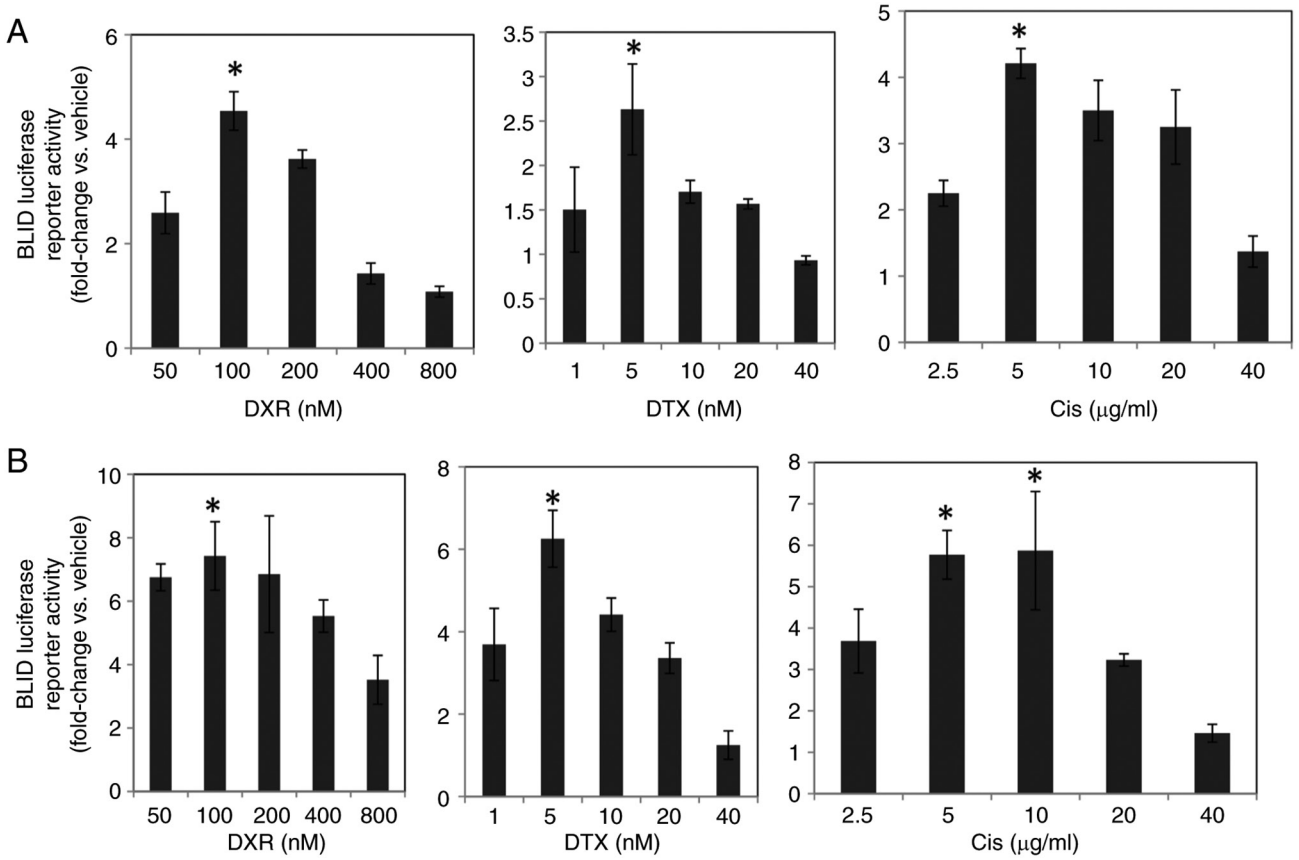


Figure S6. BLID expression is decreased in breast cancer cells following FOXO3a knockdown. (A) BLID and FOXO3a expression was analyzed in MCF-7 cells by sequential immunoblotting with anti-FOXO3a (1:1,000 dilution), anti-BLID (1:10,000 dilution) and anti-α-Tubulin (1:1,000 dilution) antibodies. (B) Quantification data showing percent inhibition of BLID and FOXO3a expression in FOXO3a siRNA-treated MCF-7 cells (left panel) and T47D cells (right panel). The y-axis title of the right graph is identical to the y-axis title of the left graph. (C) Inhibition of drug-induced BLID luciferase reporter activity in MCF-7 cells treated with FOXO3a siRNA. Cells were treated with 100 nM siRNA for 24 h and then cotransfected with pGL3-BLID luciferase reporter and pRL-TK vector (internal transfection control) for 48 h. This was followed by treatment with DXR or DMSO (vehicle) and the dual-luciferase reporter assay was performed. The experiment was repeated two to three times. Data are presented as the mean ± SD from a representative experiment. siRNA, small interfering RNA; Scr, scrambled control siRNA; DXR, doxorubicin; BLID, BH-3 like motif containing inducer of cell death.

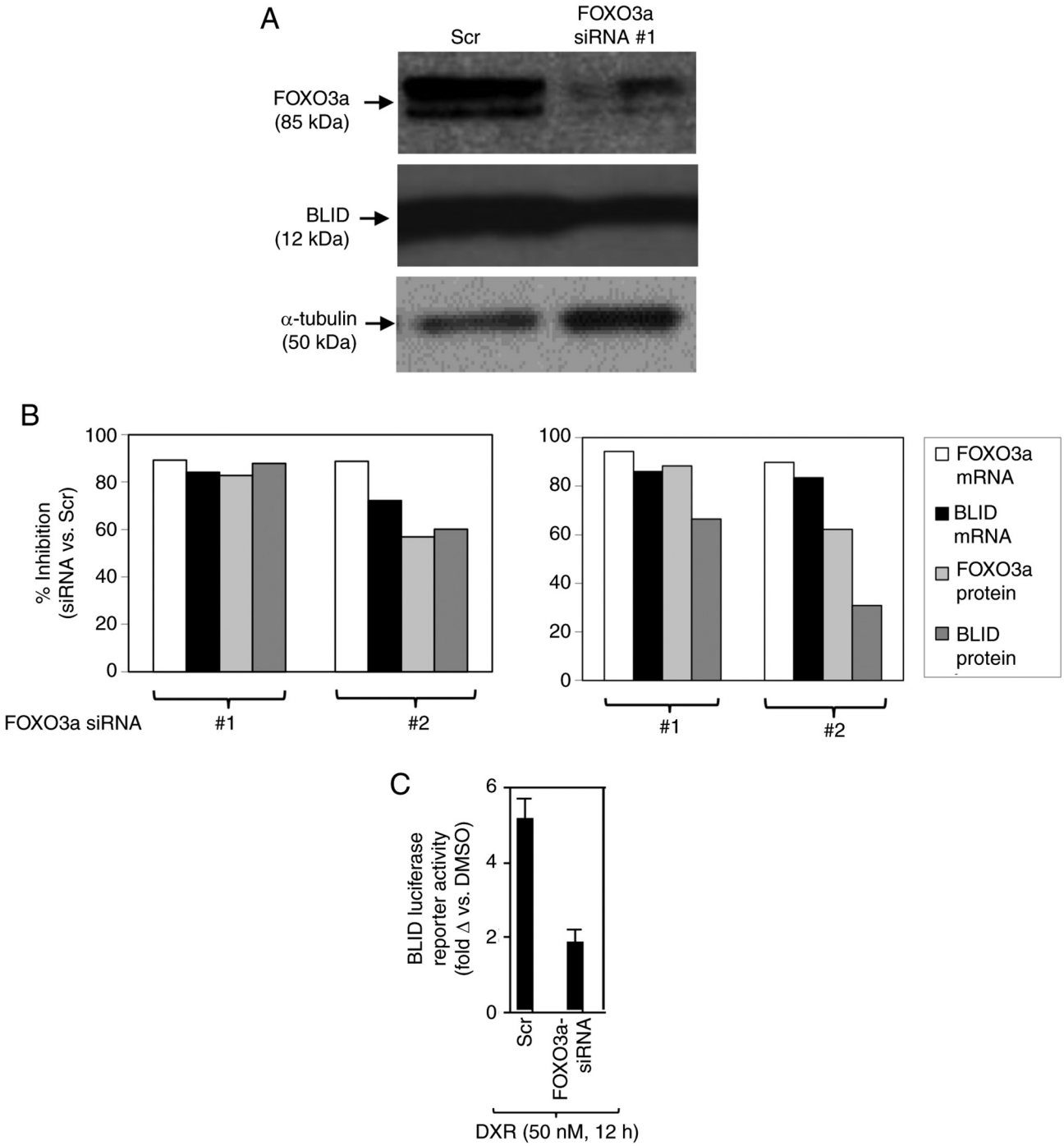


Figure S7. Representative network inferred using the Ingenuity Pathway Analysis tool showing transcriptome changes in MCF-7 cells stably transduced with BH-3 like motif containing inducer of cell death shRNA47 (sh47) vs. scramble control shRNA. The MCF-7 network 2 signals shown are known to be primarily involved in anti-microbial response, cancer and the inflammatory response. shRNA, short hairpin RNA.

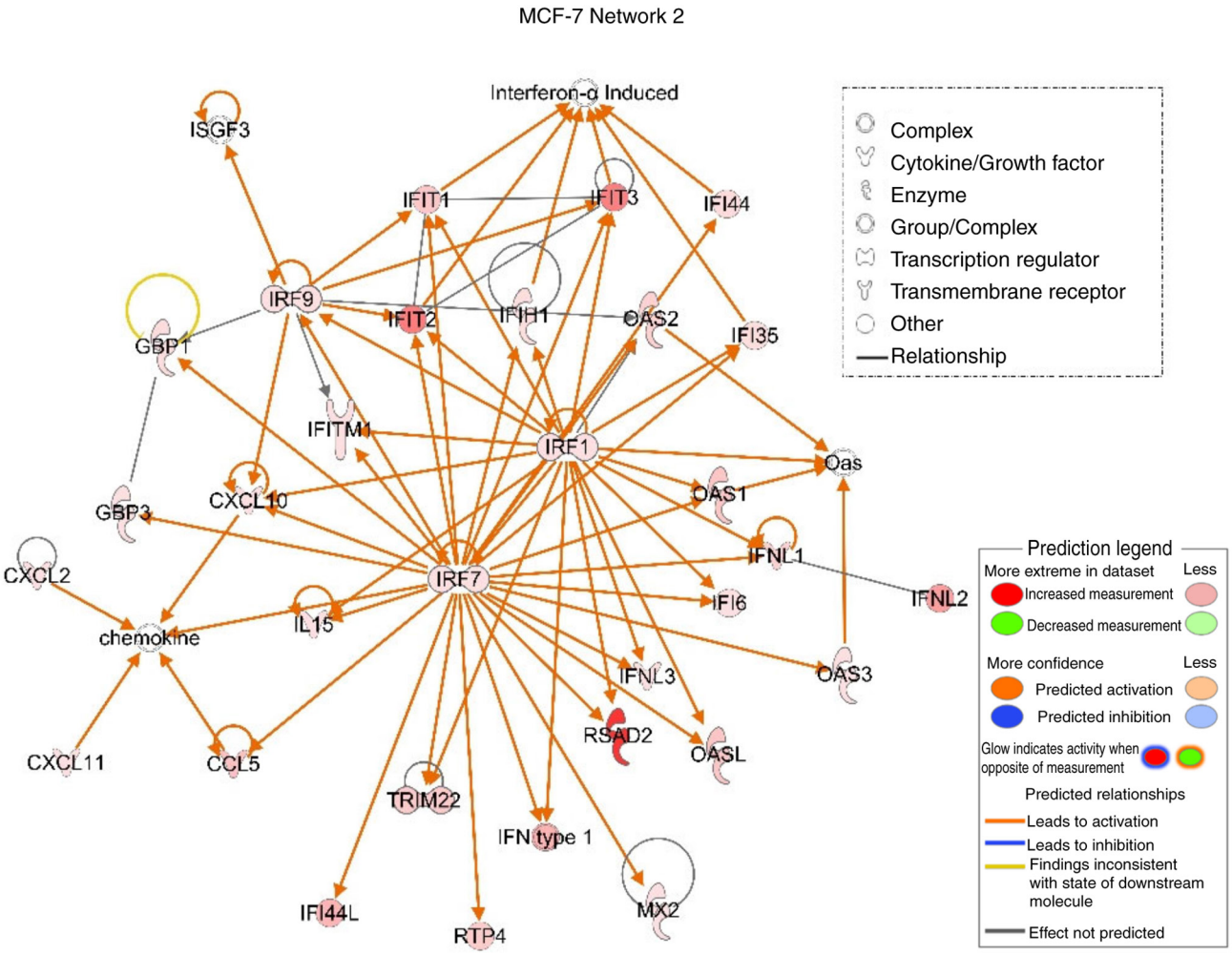


Figure S8. Representative network inferred using the Ingenuity Pathway Analysis tool showing proteome changes in MDA-MB-231 cells stably transduced with BH-3 like motif containing inducer of cell death shRNA47 (sh47) vs. scrambled control shRNA. The MDA-MB-231 Network 1 targets shown are known to be associated with cancer, free radical scavenging and hematological disease. shRNA, short hairpin RNA.

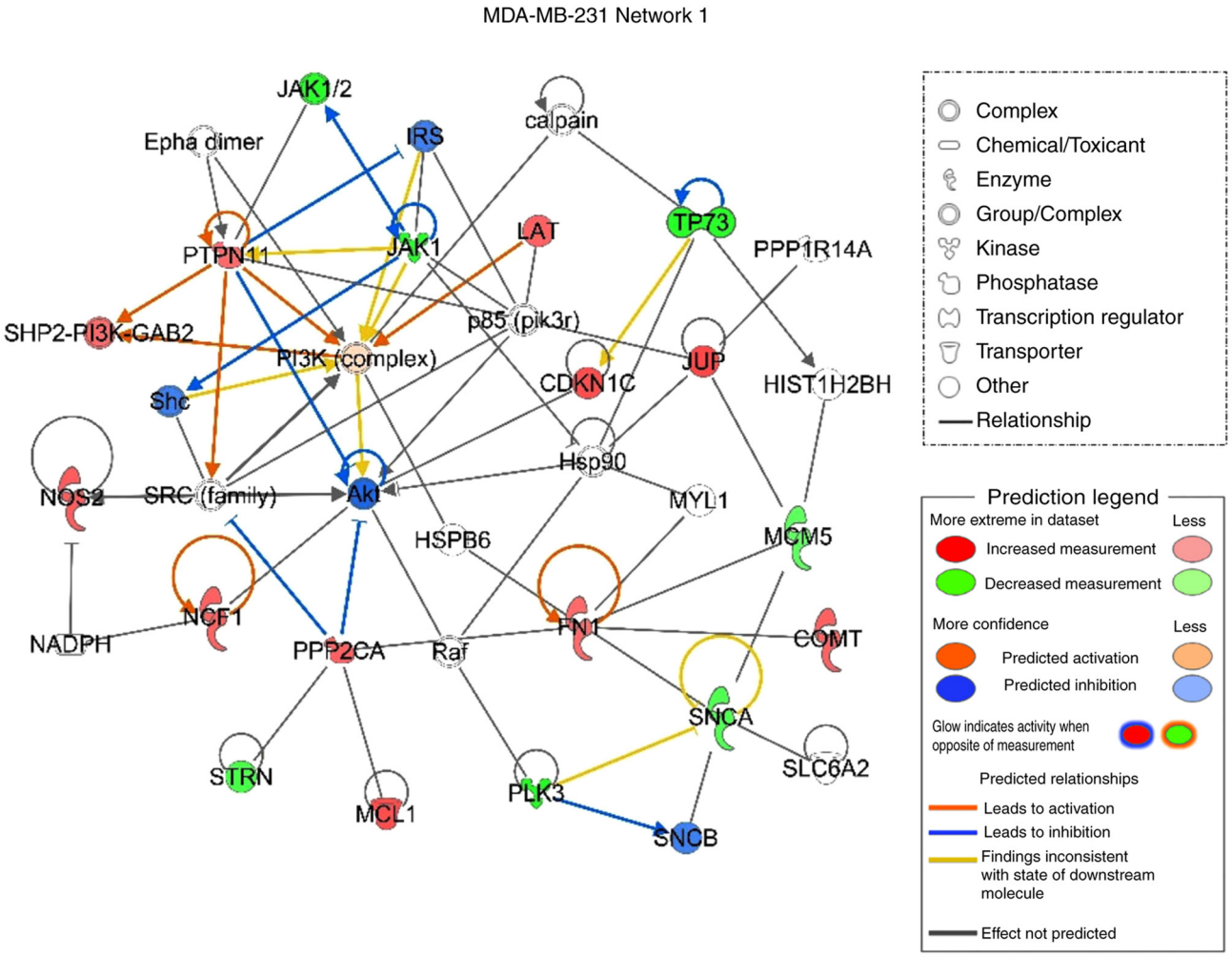


Figure S9. Validation of expression levels of a subset of genes by (A) reverse transcription-quantitative PCR or (B) western blotting following BLID knockdown in LCC9 cells. (A) The y-axis title of the right graph is identical to the y-axis title of the left graph. **P<0.01 (BLID shRNA vs. the corresponding Scr group), n=3. shRNA, short hairpin RNA; Scr, scrambled control shRNA; Ctl, control; BLID, BH-3 like motif containing inducer of cell death.

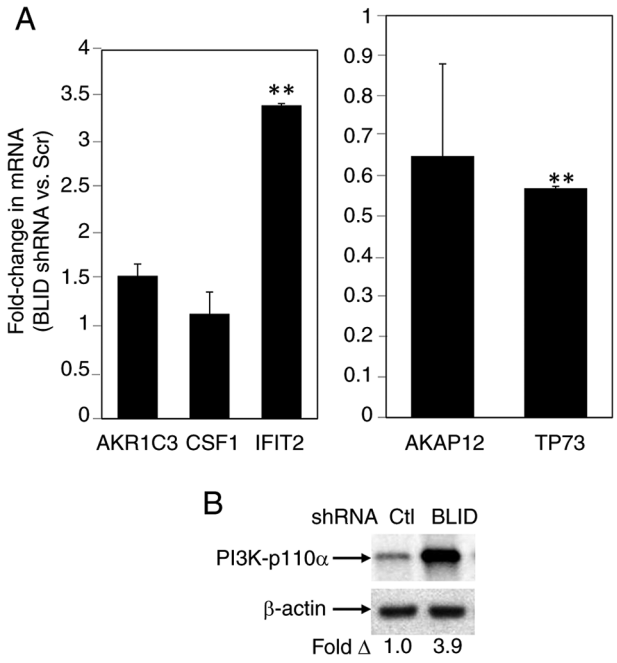


Figure S10. STRING protein-protein interaction network of BLID-interacting proteins. BLID STRING (version 11.5; August 2022) data can be found at BLID protein (human)-STRING interaction network (string-db.org). STRING, Search Tool for the Retrieval of Interacting Genes/Proteins; BLID, BH-3 like motif containing inducer of cell death.

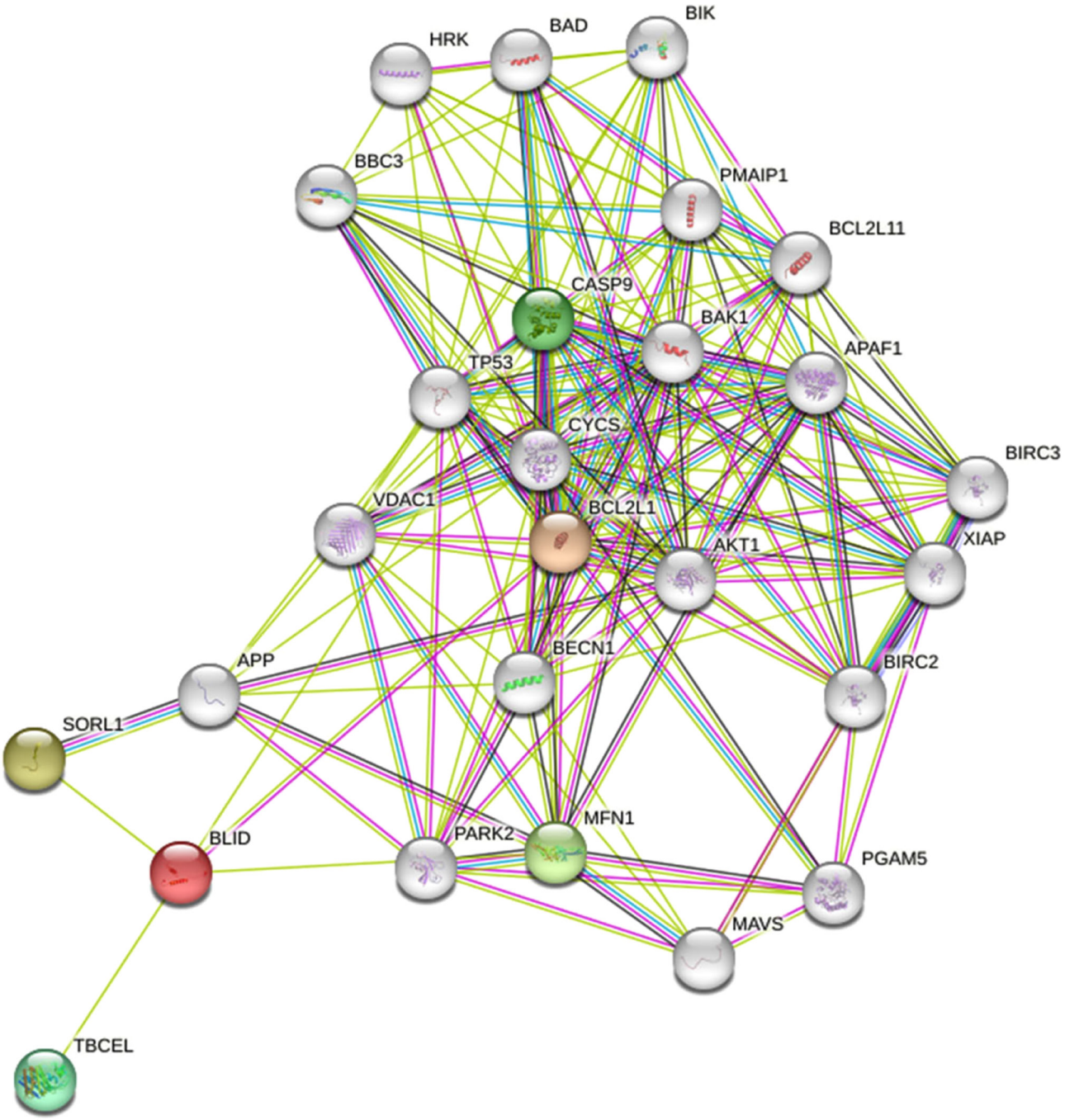


Figure S11. Analysis of BLID expression in the commercially purchased pre-made multi-tissue northern blots (Clontech; Takara Bio USA, Inc.). The commercial blots were probed with a 32P-radiolabeled BLID cDNA probe as reported previously (25). BLID, BH-3 like motif containing inducer of cell death; PBL, peripheral blood leukocytes.

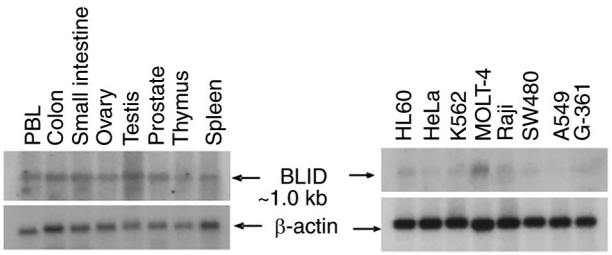


Figure S12. Association between BLID expression and melanoma response to immune checkpoint therapy examined using the ROC plotter tool. The experimental parameters were set as follows: Response based on calculated response, pretreatment sample acquisition, tissue of origin melanoma and treatment as any immune checkpoint inhibitor therapy. AUC, 0.59; ROC P=1.8x10^{-3}. ROC, receiver operating characteristic; AUC, area under the curve; TPR, true positive rate; TNR, true negative rate; BLID, BH-3 like motif containing inducer of cell death.