Gendelman et al., Supplemental Figure Legends

Figure S1. A. Schematic of the experimental framework. B. Treatment of cells with MEK inhibitor leads to G1 arrest. MDA-MB-231 cells were untreated or treated with U0126 for 24h and 48h and cell cycle was cycle distribution was assessed by FACS.

Figure S2. Predicted phenotypic value and assessment of predictive power of the model. A. Plot of simulated data for phenotypes (percentage of MDA-MB231 cells in G1 phase) vs. observed values. Dotted line represents the line of unity (y=x) and each circle represents an experimental condition in the training data. The correlation coefficient (r) is printed above the graph. **B.** Plot of simulated data for a transcript (IER2, or 202081_at) vs. observed values. Dotted line represents the line of unity (y=x) and each circle represents an experimental condition in the training data. The correlation coefficient (r) is printed above the graph. **c.** Plot of distribution of Pearson correlation coefficients for all the genes in the model.

Figure S3. Target knock-down. A. Cells were treated with siRNA pools against indicated genes for 24 hours RNA levels were assayed by TaqMan. All experiments were done in triplicate and presented as % NC-siRNA treatment. **B.** Cells were treated as in A and protein lysates were collected 72h post-treatment and assayed with available antibodies by SDS-PAGE/Western Blot.

Figure S4. Treatment with individual siRNAs against TRIB1 leads to G1-S arrest. Cells were treated with 3 individual siRNA duplexes against TRIB1 and cell cycle distribution was assessed by FACS 72 hours post-transfection.The siRNAs were synthesized by Dharmacon, the sense sequences are as follows:TRIB1-07: GCA AGG UGU UUC CCA UUA AUU; TRIB1-10: GAA CCC AGC UUA GAC UAG AUU; TRIB1-02M GGA AGA GGC UGC GGG AAG AUU; si-pool is a mixture of these three siRNAs. NC: ON-TARGET plus non-targeting pool (Dharmacon, D-001810). For more efficient knockdown of TRIB1 the transfection was repeated twice (on two consecutive days), each time using 50nM siRNA and 1.75ul of RNAiMax (Invitrogen).

Figure S5. RNA levels of cell cycle genes. MDA-MB-231 cells were grown in triplicate in 10%FBS, treated with designated siRNA pools and synchronized in G1 with mimosine. Cells were released from G1-block and RNA expression was measured with custom built TaqMan Low Density Array (TLDA). Data were analyzed with Cluster and plotted in TreeView, shown as heat map of expression relative to NC.

Figure S6. TRIB1 knockdown results in inhibition of NFκB-responsive promoter activity: comparison of individual siRNA duplexes. The cells were transfected with three individual duplexes of siRNAs constituting the pool as described in Figure 3C, after that they were transfected with NFkB-Luciferase reporter vector and SEAP control vector, and treated with TNFα or vehicle control for 24h. Cells were assayed for Luciferase activity, SEAP activity was used for transfection efficiency normalization. Based on the NFkB-effect, the siRNA-07 was selected for constructing a vector with siRNA-resistant TRIB1 (see Figure S7). (A). NFkB promoter is activated in response to

TNF. This response is inhibited by TRIB1-siRNAs (as compared to NC-siRNA). The data are expressed relatively to NC-siRNA, which was taken for one. Bars are the mean of three independent experiments +/- sd; (B). The degree of TRIB1 expression knockdown by the corresponding siRNA duplexes. The cells were transfected as in panel A, RNA was harvested at 24h post siRNA transfection and used for QPCR analysis to detect the levels of TRIB1. The data are expressed relatively to NC-siRNA, which was taken for 100%. Bars are the mean of three independent experiments +/- sd.

Figure S7. TRIB1 resistant to siRNA (TRIB1-siR) rescues the phenotype induced by TRIB1-siRNA. To create siRNA-resistant TRIB1, we introduced 5 silent mutations in the region of TRIB1 which was a target of siRNA duplex #07. TRIB1-GFP or TRIB1-siR-GFP was expressed in MDAMB231 cells, and the cell lines stably expressing these constructs were selected. MDAMB231 cells carrying TRIB1-GFP or TRIB1-siR-GFP were transiently transfected either with negative control (NC) or with TRIB1-07 siRNA. (A). NFkB promoter is activated in response to TNF. This response is inhibited by TRIB1-siRNA (as compared to NC-siRNA) in cells expressing TRIB1, but not in cells expressing siRNA-resistant TRIB1. The data are expressed relatively to NC-siRNA, which was taken for one and designated as a dashed line. Bars are the mean of three independent experiments +/- sd; (B). Both TRIB1 and GFP were significantly downregulated by TRIB1-siRNA in the cells expressing TRIB1, but not in the cells expressing TRIB1-resistant construct. The cells were transfected as in panel A, RNA was harvested at 24h post siRNA transfection and used for QPCR analysis to detect the levels of TRIB1 and GFP (The construct was TRIB1-GFP fusion). The data are expressed relatively to NC-siRNA, which was taken for one and designated as a dashed line. Bars are the mean of three independent experiments +/- sd.

Figure S8. Downregulation of NFkB target genes after treatment with TRIB1 siRNA. Cells were treated with TRIB1 or NC siRNA. RNA was collected following TNFα treatment and assayed using QPCR. Data represent three independent experiments.

Figure S9. TRIB1 knock down leads to inhibition of NFκB pathway: A. TRIB1 downregulation by siRNA results in inhibition of p105 processing and p50 nuclear translocation but has no effect on RelA phosphorylation (Ser536). Cells were treated with TRIB1 siRNA and exposed to TNFα for indicated times. Nuclear and cytoplasmic extracts were analyzed by SDS-PAGE. B. TRIB1 downregulation results in inhibition of IKKα/β phosphorylation and in inhibition of p52 generation. Total cell extract was analyzed at 72h post TRIB1 siRNA transfection.

Figure S10. TRIB1 siRNA promotes TRAIL-induced apoptosis through activation of caspase-8 cleavage. Cells were treated with TRIB1 or NC siRNA and exposed to TRAIL at two concentrations. Levels of Caspase-8 and its cleavage products were assayed by Western Blot.

Figure S11. TRIB1 knockdown results in decrease of YY1gene expression. The cells were transfected with single TRIB1 siRNA-07 or with the pool of three siRNAs as

described above in Supplementary Figure. mRNA expression of YY1 was assessed by QPCR. The data are expressed as mean from three independent experiments +/- standard deviation (sd). YY1 expression decreased moderately (1.6 fold) but statistically significant (* $p < 0.01$, t-test) as compared to negative control siRNA.

Figure S12. Kaplan-Meier plots and log-rank p-values comparing overall survival distributions (**A**) and Breast Cancer Specific Survival distributions (**C**) between individuals with *TRIB1* expression levels in the upper (66.66%) versus lower (33.33%) tertiles. **(B, D)** As in (A, D), but for *IL8* expression.

Figure S13. Hazard Ratios for BCCS distribution.

Figure S14. Hazard Ratios for OS distribution.