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Supplemental Information

Modeling of Cisplatin-Induced Signaling Dynamics in Triple-Negative Breast Cancer Cells Reveals Mediators of Sensitivity

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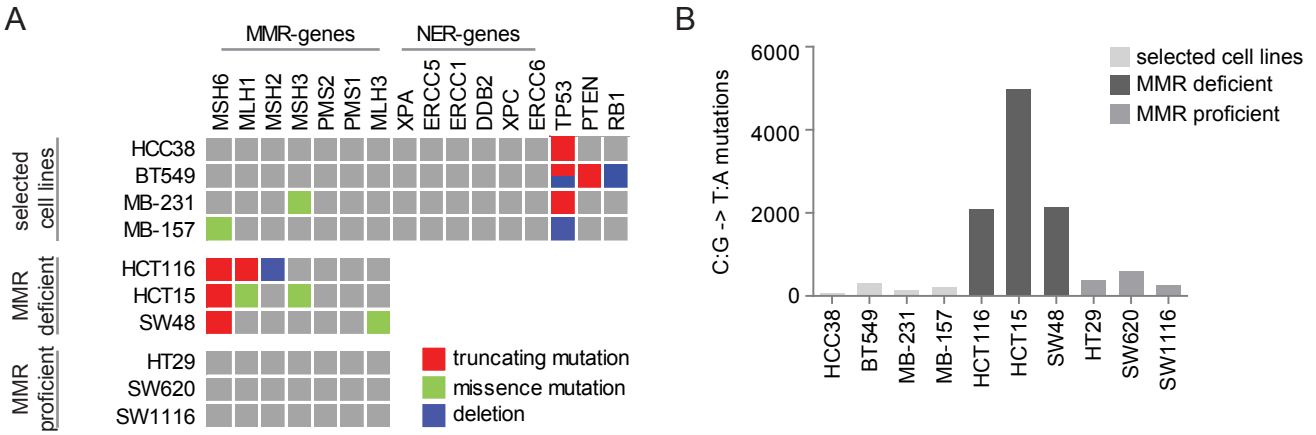

Figure S1: Selected TNBC cell lines are not deficient in MMR and NER. Related to Figure 1. (**A**) MMR and NER gene mutation frequency in the selected TNBC cell lines, MMR deficient cell lines and MMR proficient cell lines. (**B**) Number of CG-to-TA mutations in the same panel of cell lines as (A). Gene mutation status and CG-to-TA mutations were obtained from the COSMIC database.

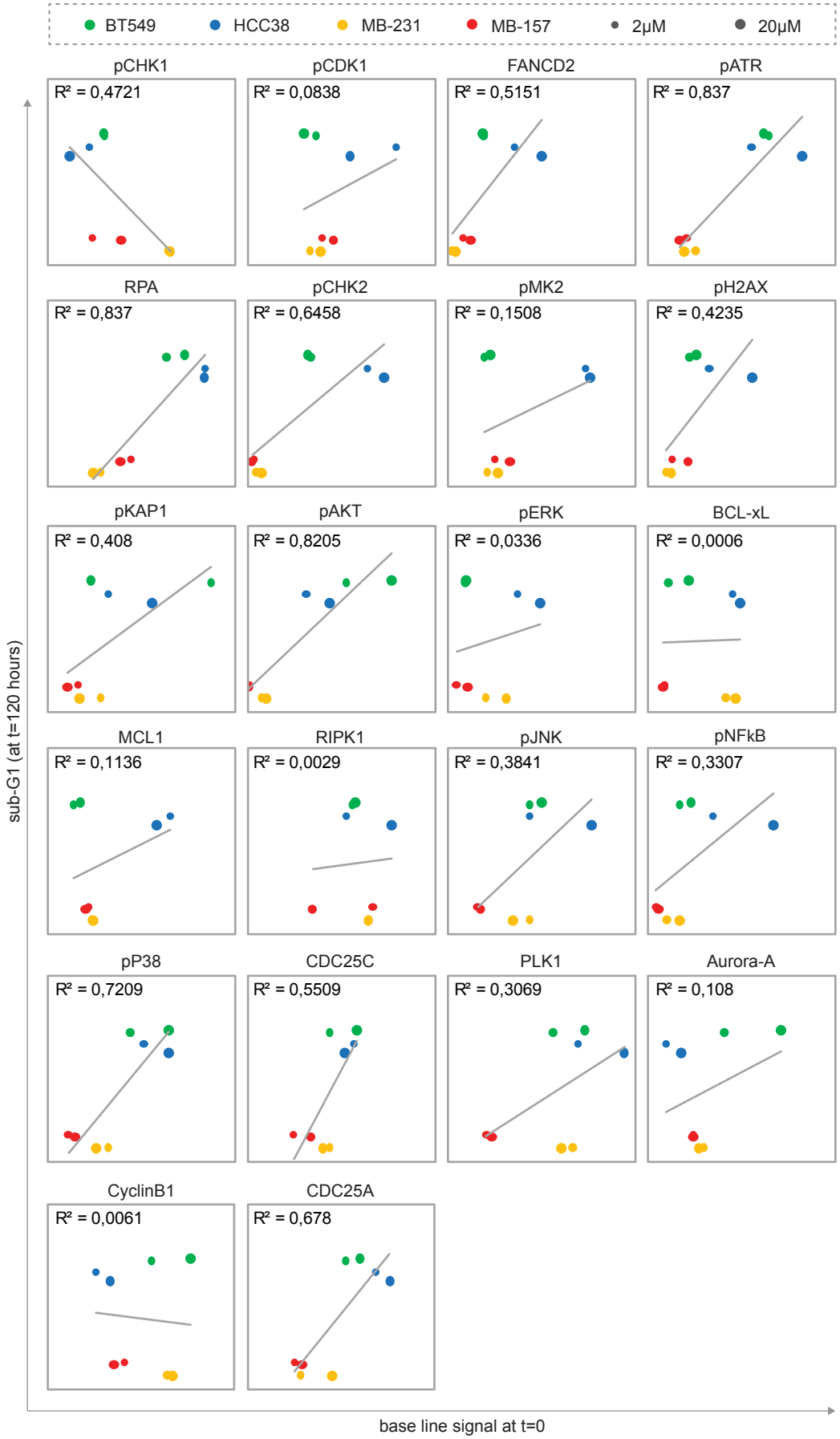

Figure S2: Correlation between baseline signal measurements and sub-G1. Related to Figure 2. Baseline signal values and sub-G1 measurements at t=120 hours after cisplatin treatment are presented as scatterplots. The linear correlation (R2) between signals and sub-G1 was calculated for all four cell lines treated with 2 and 20 µM cisplatin.

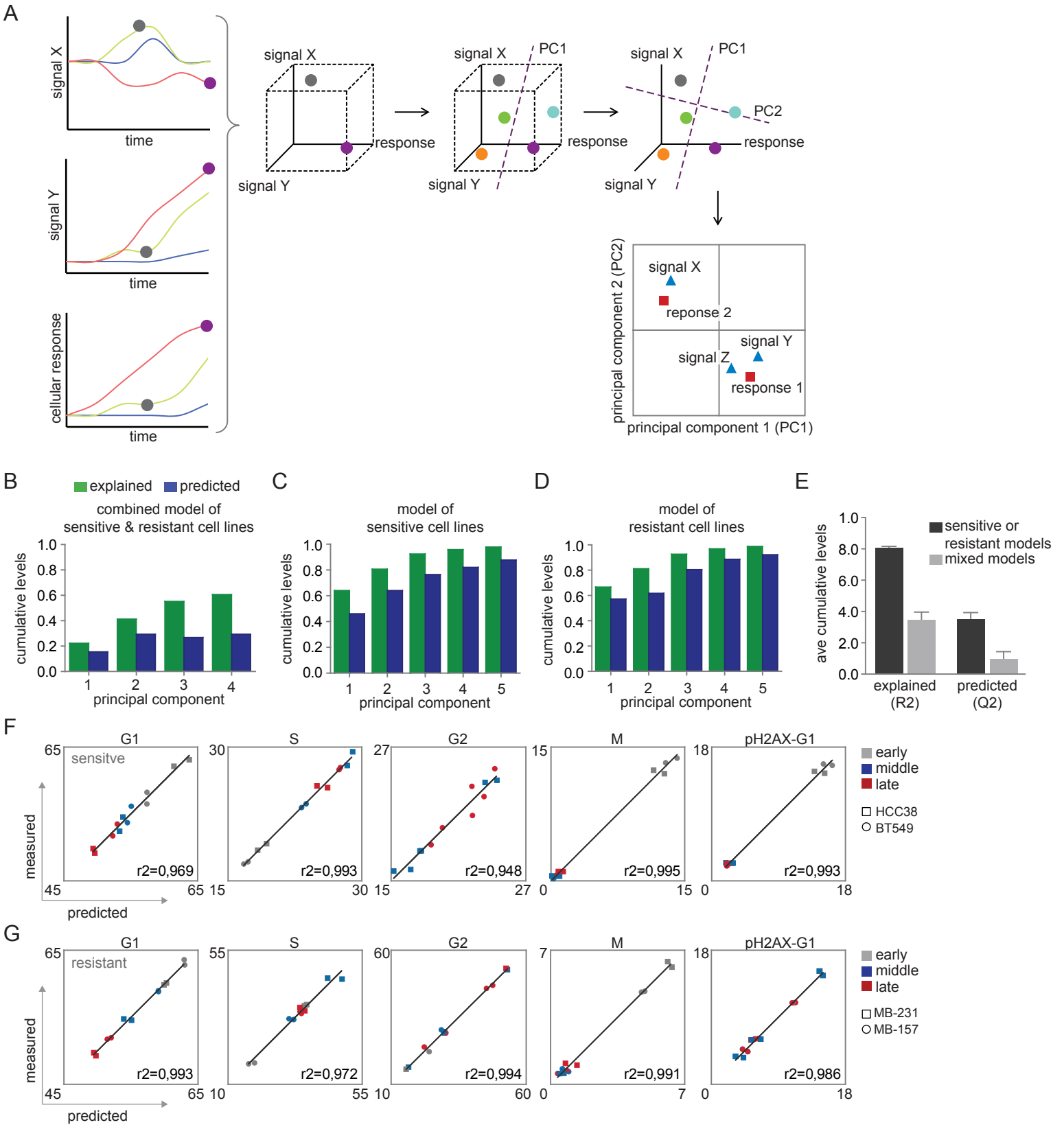

Figure S3: Basic explanation and PLSR modeling of cisplatin-sensitive and -resistant TNBC cell lines. Related to Figure 3. (**A**) Left panel: In this simplified example, 2 signaling components and 1 response marker make up the "signaling space". These can separately be plotted as time-course plots (as tradi-tionally done and shown left panels) or can be plotted in multidimensional "data space" (top middle panels). Further reduction of the data space can be achieved by identification of principle components (PCs), which are defined as latent axes that maximally capture the variance in the dataset. When signaling vectors are regressed against response vectors, the PC-space can be used to identify co-variation between molecular signals and corresponding cellular responses (shown in bottom right panel). Our data space was comprised of 6,336 signaling vectors (6 metrics * 22 signals * 3 timeframes * 4 cell lines * 2 duplicates * 2 concentrations) and 288 response vectors (6 responses * 3 timeframes * 4 cell lines * 2 duplicates * 2 concentrations). (**B-D**) Explained variation (R2), and predicted variation (Q2) for PLS models built with increasing numbers of principal components. (B) Model built with combined data of cisplatin-sensitive and -resistant cell lines. (C) Model built with data from cisplatin-sensitive cell lines. (D) Model built with data from cisplatin-resistant cell lines. (**E**) Average (ave) cumulative explained variation (R2) and predicted variation (Q2) by combined models for cisplatin-sensitive and cisplatin-resistant models ('sensitive or resistant models') versus cumulative explained variation (R2) and predicted variation (Q2) upon modeling of random pairs of cell lines ('mixed models'). Error bars show the standard deviation of R2 and Q2 values for models built using sensitive/resistant or mixed pairs of cells. (**F, G**) Correlation between measured signals (y-axis) and cross-validated predictions (x-axis) by the PLS models. Panel (F) indicates correlation based on model of cisplatin-sensitive cell lines. Panel (G) indicates correlation based on model of cisplatin-resistant cell lines.

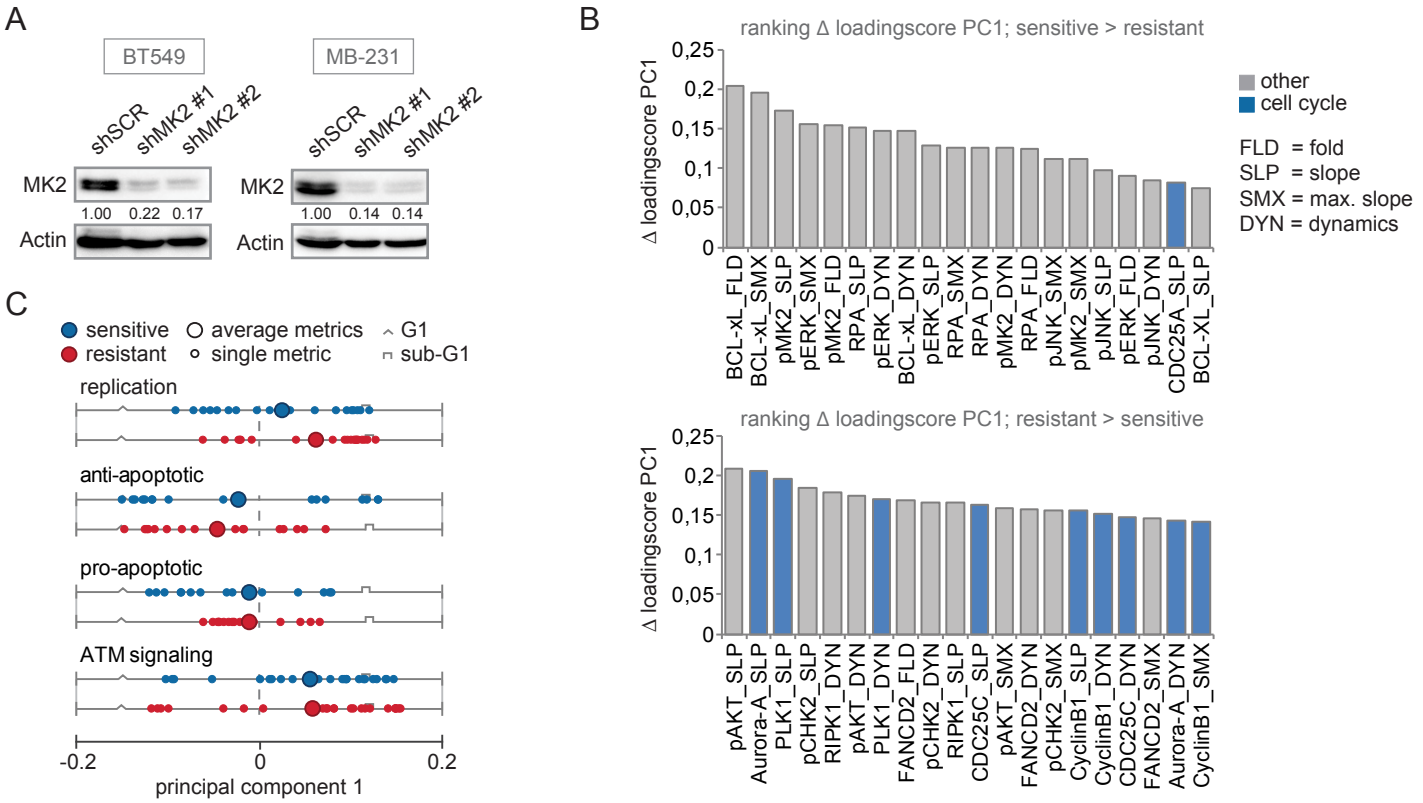

Figure S4: MK2-depletion and differentially relevant metrics for PC1. Related to Figure 3. (**A**) Knockdown efficiencies of BT549 and MDA-MB-231 cells transduced with shRNAs targeting MK2. Lysates were made 5 days after transduction. (**B**) Differential PC1 loading scores. PC1 loading scores were calculated for all signal-metrics combinations. Plotted in the top graph are the PC1-scores of the sensitive model subtracted by those of the resistant model. PC1-scores with the biggest positive difference between the sensitive and resistant model are shown. In the lower panel PC1-scores of the sensitive model were subtracted from the resistant model. PC1-scores with the biggest positive difference between the resistant and the sensitive model are plotted. (**C**) PC1 loading scores of the dynamical signaling metrics (FLD, DYN, SMX, SLP) and their average are plotted. Signals were grouped by signaling class.

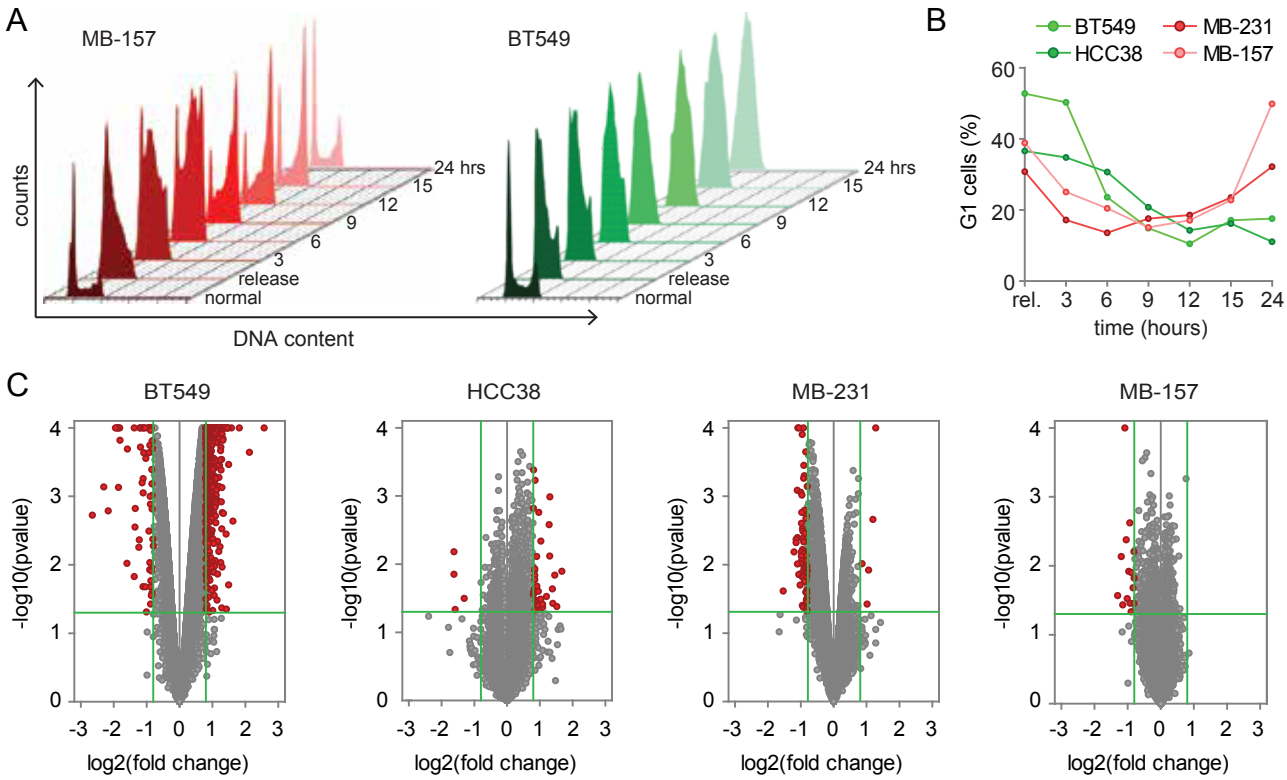

Figure S5: Cell cycle progression and differential gene expression upon cisplatin treatment. Related to Figure 4. (**A, B**) After cells were synchronized in early S-phase with thymidine, cells were released and treated with 2 µM cisplatin. Cells were fixed at indicated time points and stained with propidium iodide. Experiments of all four cell lines, cisplatin-resistant (red) and cisplatin-sensitive (green) were performed in duplicate. (A) Representative examples of cell cycle profiles of MDA-MB-157 (red) and BT549 (green). (B) Quantification of G1-cells. Averages from two biological experiments are shown. (**C**) Differential expressed genes (DEGs) following 2 µM cisplatin treatment for 72 hours versus untreated cells. Genes that meet the cut-off of ≥ 1.75-fold change and a p-value ≤ 0.05 are colored red. B score is the log of the odds of differential expression.

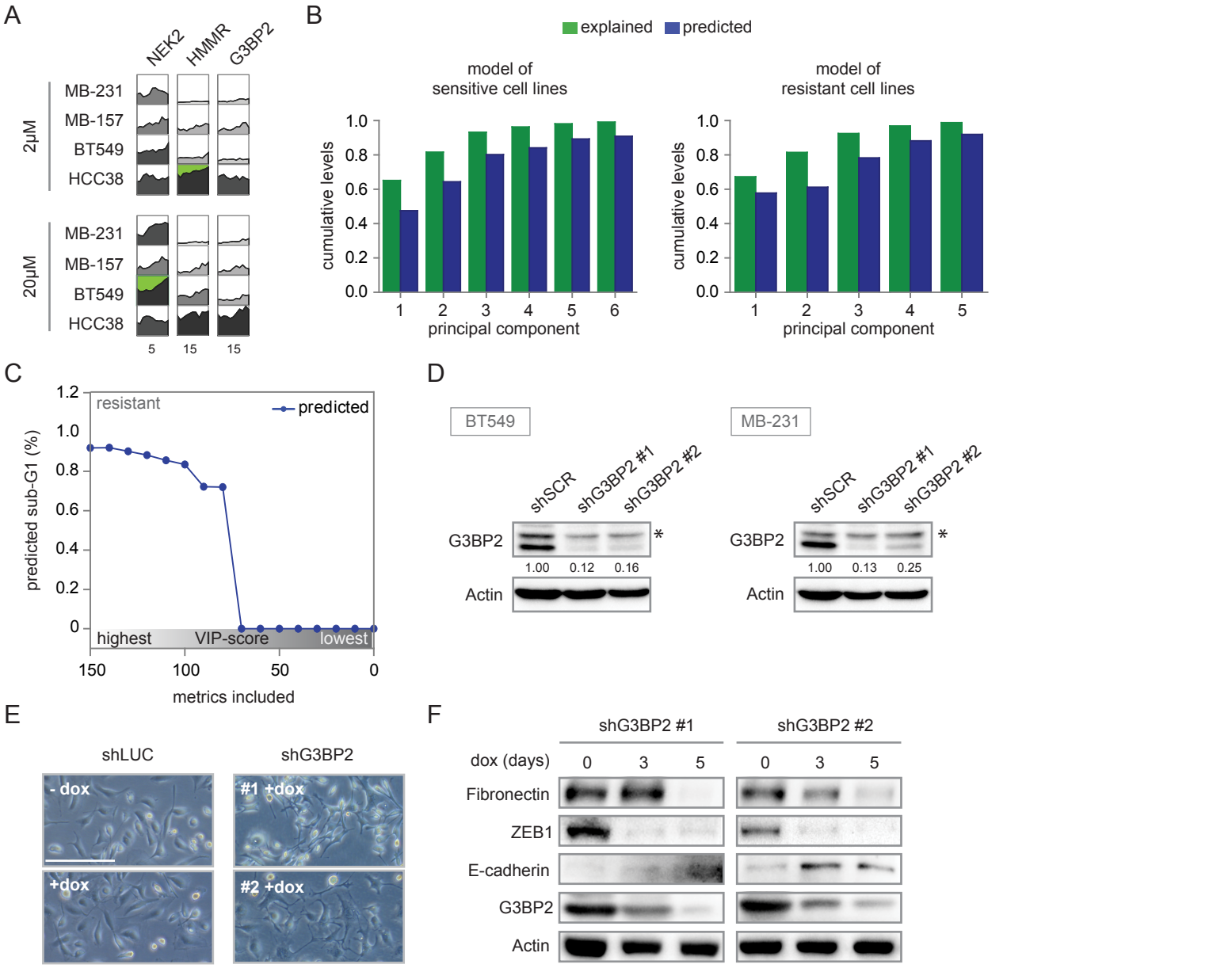

Figure S6: Dynamics of NEK2, HMMR and G3BP2 abundance upon cisplatin treatment, and EMT features upon G3BP2 depletion. Related to Figure 5. (**A**) Protein abundance levels of NEK2, HMMR and G3BP2 were measured. Signal intensity was quantified following 2 or 20 µM cisplatin treatment, normalized to actin and plotted as fold change compared to the lowest measurement. Each box represents an 11-point time course of biological duplicate experiments. Plots are colored by response profile, with 'early sustained increases' colored in green, 'late sustained increases' colored in red, and 'decreases' colored in blue. Responses that were not significantly changed by treatment were shaded grey to black with darkness reflecting response strength. Numbers below each plot report the maximum fold change on the y-axis. (**B**) PLS regression modeling using expanded models, which incorporate expression levels of NEK2, HMMR and G3BP2. (**C**) Breakpoint analysis of the model of cisplatin-resistant cell lines. Metrics were sequentially eliminated from the model of cisplatin-resistant cell lines, from lowest to highest VIP-score. (**D**) BT549 and MDA-MB-231 cells with shRNAs for luciferase or G3BP2 were lysed and immunoblotted for G3BP2 and Actin. Non-specific bands are indicated with *. Densitometry of G3BP2 bands were corrected for local background and intensity of Actin bands to estimate the indicated knockdown efficiencies. (**E**) 5 days after doxycycline-induced expression of shRNAs targeting luciferase or G3BP2, the morphology of MDA-MB-231 cells was recorded using bright light. Scale bar represents 100 µM. (**F**) Protein expression levels of EMT factors. MDA-MB-231 cells expressing doxycycline-inducible shRNAs against G3BP2 were lysed at indicated time points after doxycycline addition. Protein expression levels were detected by Western blot.