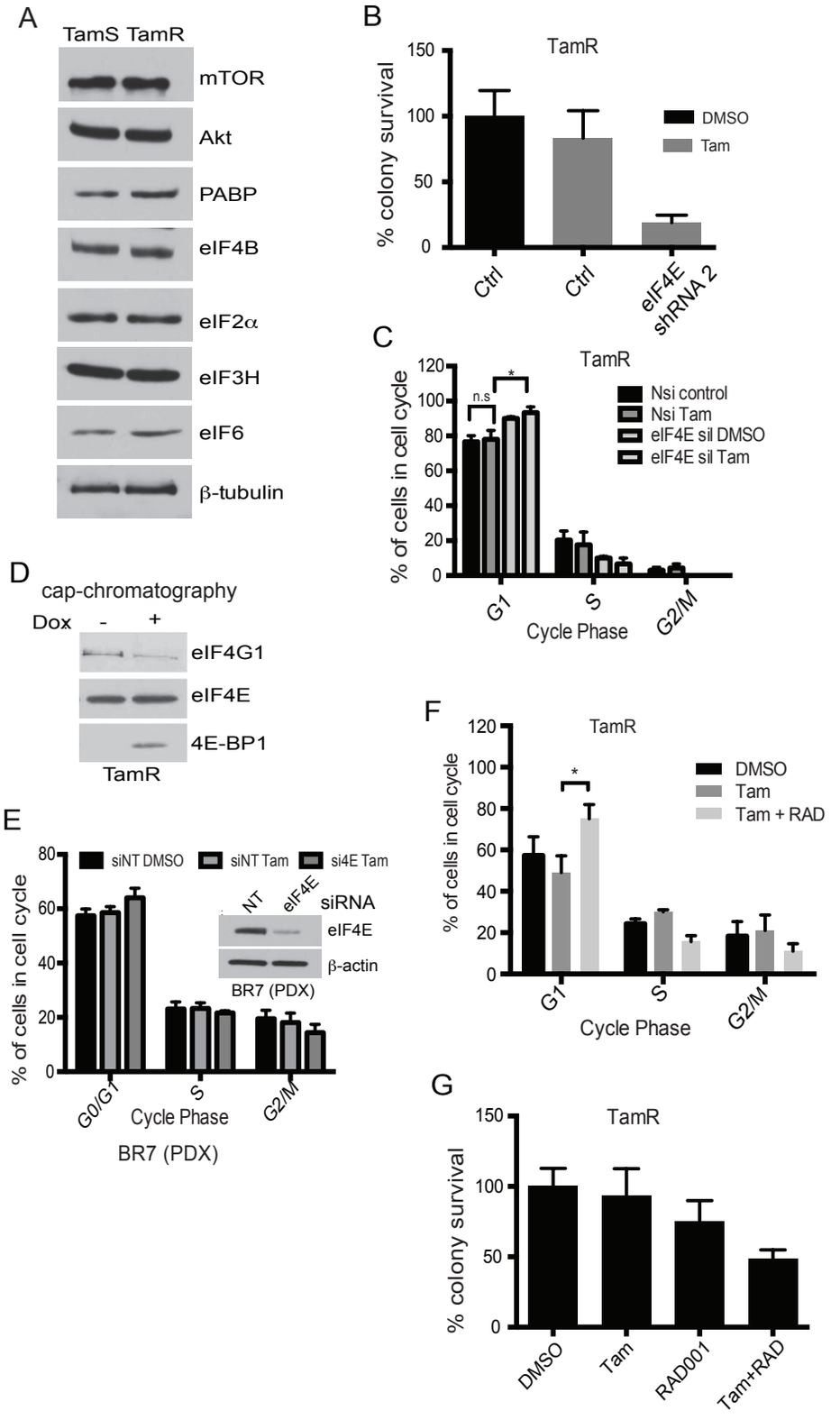
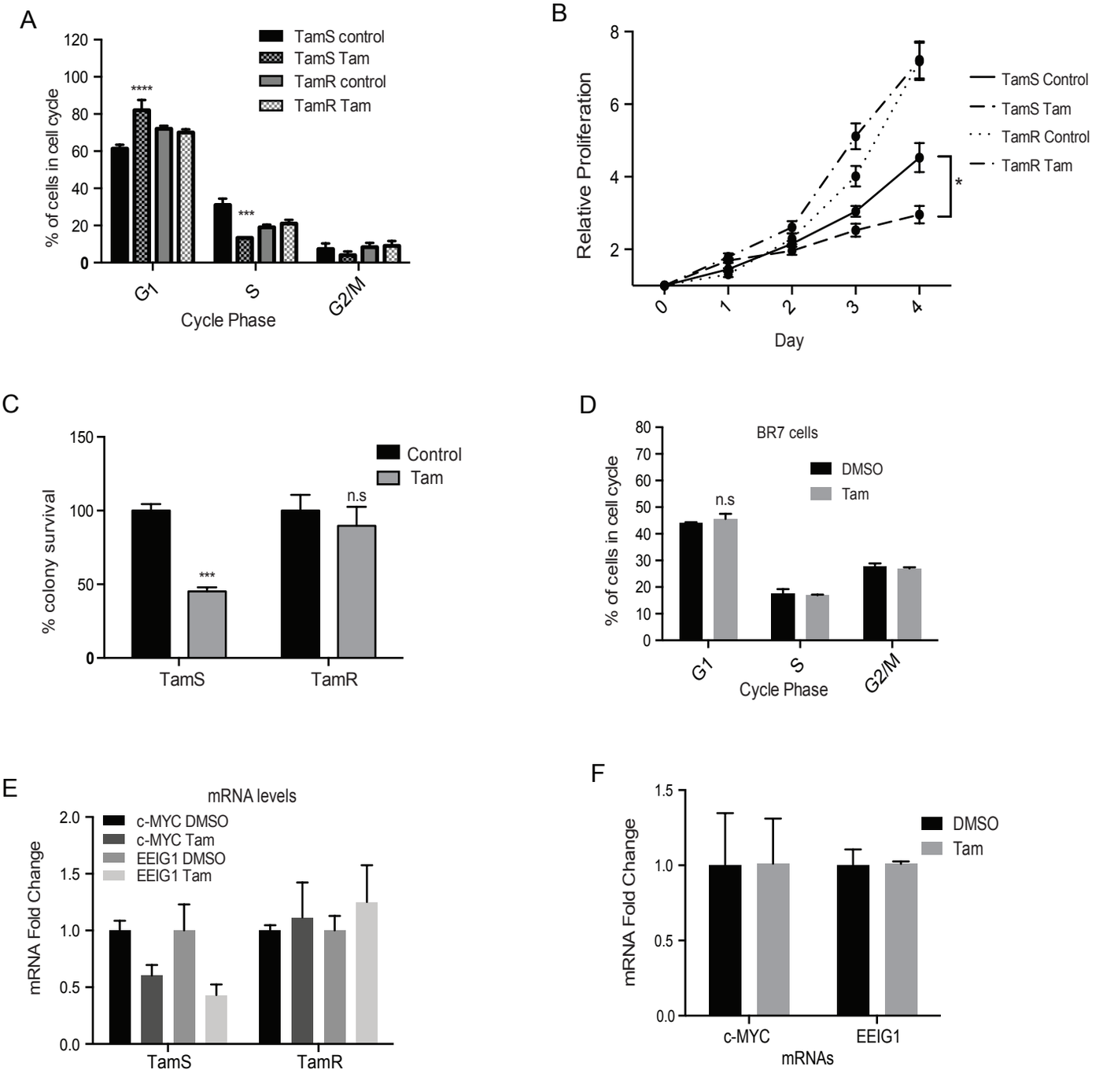
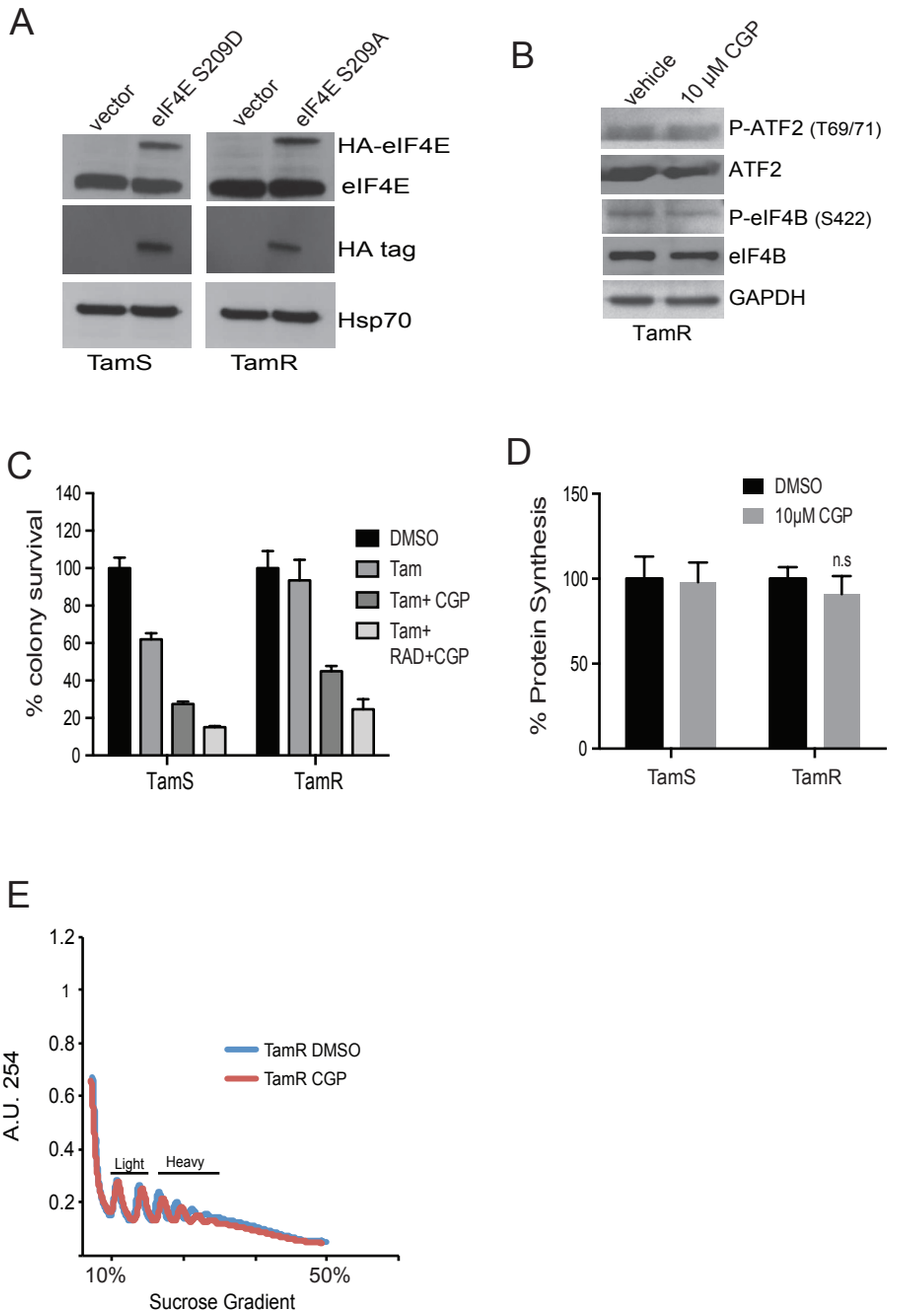
Geter et. al., Supplemental Figure 2. Related to Figures 2 & 3.



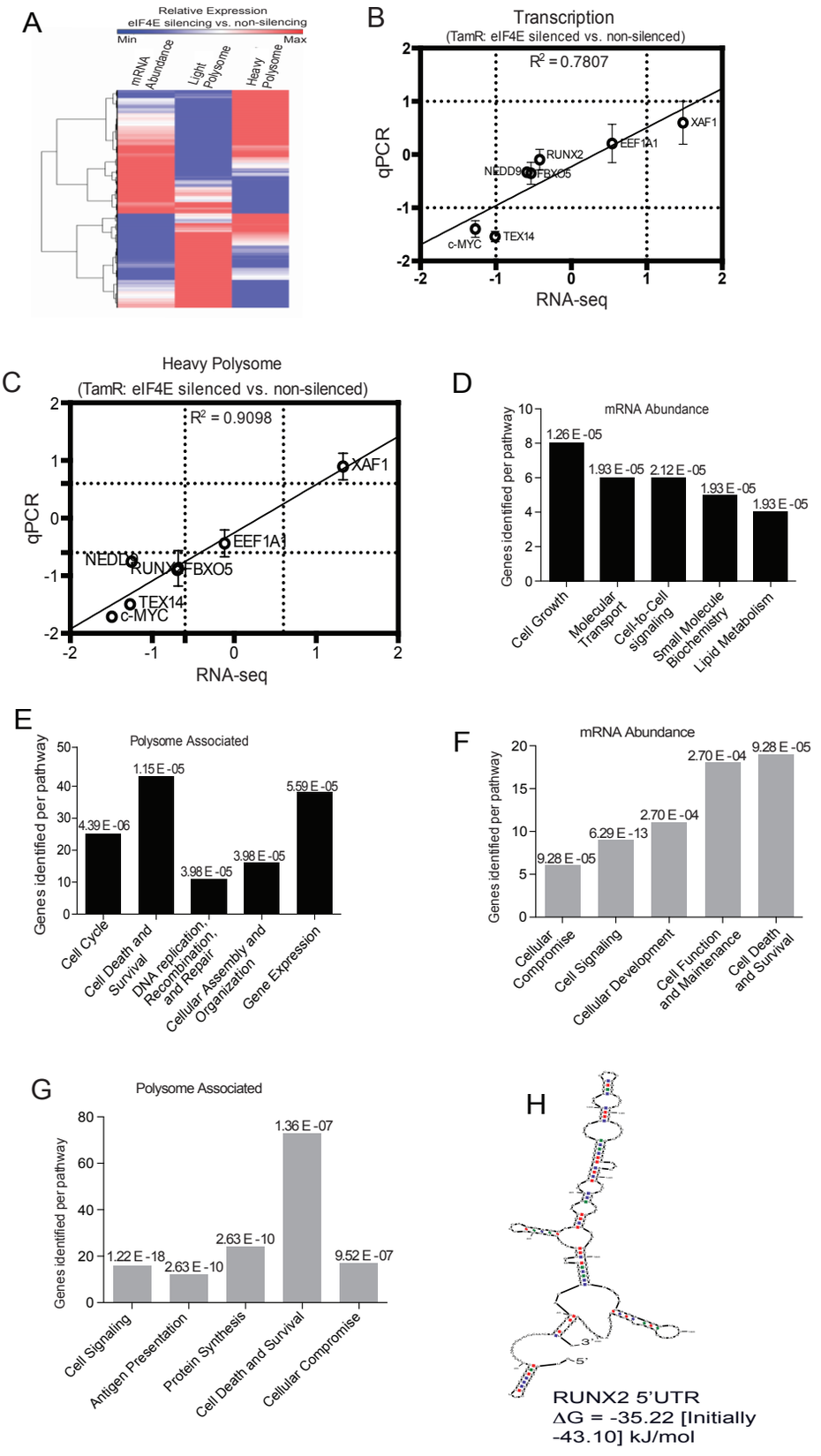
Geter et. al., Supplemental Figure 1. Related to Figures 1 & 2



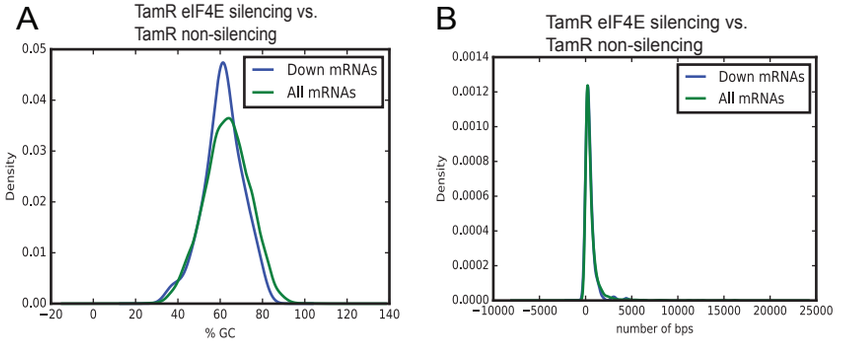
Geter et. al., Supplemental Figure 3. Related to Figures 2 & 3

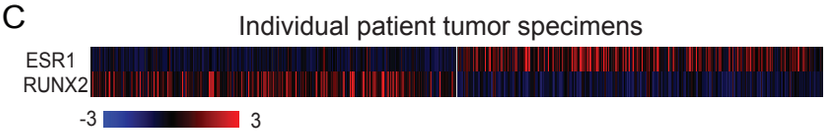


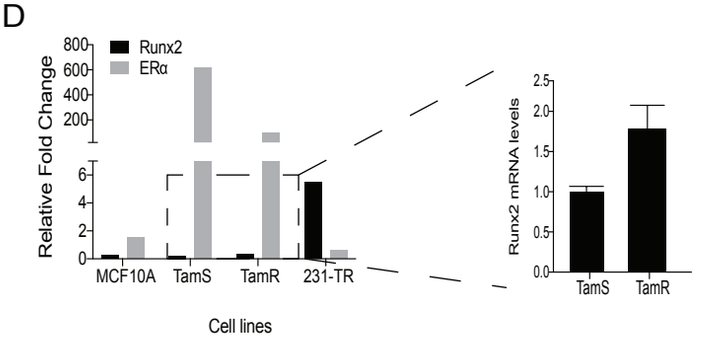
Geter et. al., Supplemental Figure 4. Related to Figures 6 and 7



Geter et. al., Supplemental Figure 5. Related to Figures 6 and 7







SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure S1. Related to Figures 1 & 2. Tamoxifen resistant breast cancer cells display ER independent survival when exposed to tamoxifen. (*A*) Cell cycle analysis performed on the TamS and TamR cells treated with either DMSO control or 1 μM 4-OH Tam for 72 h in 1% CS-FBS. Cells were fixed in 70% ethanol overnight. Cells were subjected to RNaseA treatment and stained with propidium iodide (PI). Data was collected using FACScalibur and analyzed with FloJo 10.0 software. Data from 3 independent experiments are shown. SEMs are shown. ** *P<0.01* and *** *P <0.001* by two-way ANOVA. (*B*) Cell proliferation as assayed by MTT assay initiated 24 h after plating (day 0). TamS and TamR cells were treated with either DMSO or 1 μM 4-OH Tam and initiated on day 0. Results are representative of 3 independent experiments and presented as relative proliferation in which all samples are normalized to Day 0. Standard deviations are shown. **P<0.05* by t-test. (*C*) Colony survival assay performed by low density seeding (1000 cells) of TamS and TamR cells. Cells were treated with either DMSO or 1 μM 4-HT 24 h after plating. Colonies scored after 10 days counting only ≥50 cells/colony. Results are representative of 3 independent experiments. SEMs are shown. ***P< 0.001 by two-way ANOVA. (*D*) Cell cycle analysis performed as described in (*A*) on BR7 (PDX) cells treated with either DMSO control or 1 μM 4-OH Tam; n.s.; not significant. (*E*) Expression of c-Myc and EEIG1 mRNA in MCF7/TamS and MCF7/TamR cells following treatment with either DMSO or Tam for 24 h. Equal amounts of RNA were reverse transcribed and quantified through real-time PCR. RNA levels normalized to GAPDH using the $^{-\Delta\Delta}$Ct method. Error bars represent SEM. (*F*) Experiment was performed as in (*E*) on BR7 (PDX) cells following treatment with either DMSO or Tam for 24 h. RNA levels normalized to GAPDH using the $^{-\Delta\Delta}$Ct method. Error bars represent SEM.

Supplemental Figure S2. Related to Figures 2 & 3. Selective therapeutic inhibition of mTORC1 can restore tamoxifen sensitivity to ER^+ breast cancer cells. (*A*) Immunoblot of equal amounts of protein from TamS and TamR cells during exponential growth. Cells were lysed in NP-40 buffer and probed for key translation factors. β-tubulin (loading control). (*B*) Colony survival assay was performed by low density seeding (1000 cells) of stably transduced TamR cells (sh-control or sh-eIF4E-2). Cells were treated with either DMSO or 1 μM 4-OH Tam 24 h after plating. Dox (1 μg/mL) was administered 24 h after plating and removed after 72 h. Colonies scored after 10 days counting only ≥50 cells/colony. SEM is shown. (*C*) Cell cycle analysis performed on TamR cells following eIF4E silencing (sh-eIF4E-2). Cells were treated with either DMSO control or 1 μM 4-OH Tam for 72 h in 1% CS-FBS. Dox (1 μg/mL) was administered to cells for 72 h. Cells were fixed in 70% ethanol overnight and subjected to RNaseA treatment. Cells were stained with Hoechst 33342. Data was collected using LSRII UV and analyzed with FloJo software. Data from 3 independent experiments are shown. SEM is shown. * *P<0.05* by two-way ANOVA. (*D*) Overexpressing 4E-BP1 in breast cancer cells increases its association with eIF4E. Cap chromatography performed on TamR cells with or without 4E-BP1 cDNA overexpression. Cells were lysed in NP-40 buffer and pulldown was performed for 1 h at 4ºC. Proteins were eluted and resolved via SDS-PAGE. Membrane was probed for eIF4E, eIF4GI and 4E-BP1. Representative blot shown. (*E*) Cell cycle analysis performed on the BR7 (PDX) cells treated with either DMSO control or 1μM 4-OH Tam for 72 h in 1% CS-FBS with or without eIF4E silencing. Cells were fixed in 70% ethanol overnight. Cells were subjected to RNaseA treatment and stained with propidium iodide (PI). Data was collected using FACScalibur and analyzed with FloJo 10.0 software. BR7 cells were transfected with 25 nM of either non-targeting (NT) or eIF4E specific siRNA for 72 h. Cells were lysed in NP-40 buffer and equal amounts of protein were resolved via SDS-PAGE and transferred to a PVDF membrane. Membrane was probed for eIF4E. β-actin (loading control). (*F*) Cell cycle analysis performed on TamR cells treated with

DMSO control, 1 μM 4-OH Tam, or 4-OH Tam and RAD001 (20 nM) for 72 h in 1% CS-FBS. Cells were fixed in 70% ethanol overnight. Cells were subjected to RNaseA treatment and stained with propidium iodide (PI). Data was collected using FACScalibur and analyzed with FloJo 10.0 software. Data from 3 independent experiments are shown. SEM is shown. * *P<0.05* by two-way ANOVA. (*G*) Colony survival assay was performed by low density seeding (1000 cells) of TamS and TamR cells. Cells were treated with DMSO, 1 μM 4-OH Tam, 20 nM RAD001, or combination therapy 24 h after plating. Treatment was changed every 72 h. Colonies scored after 10 days counting only ≥50 cells/colony. Results are representative of 3 independent experiments. SEM is shown.

Supplemental Figure S3. Related to Figures 2 & 3. eIF4E S209 phosphorylation promotes drug resistance via selective mRNA translation. (*A*) Immunoblot analysis of equal amounts of protein from TamS and TamR cells transfected with eIF4E phospho-mutants S209A or S209D, respectively or a control vector. Hsp70 (loading control). (*B*) TamR cells were untreated or treated with 10 μM CGP57380 for 6 h and equal protein amounts immunoblotted as shown. (*C*) Colony survival assay was performed by low density seeding (1000 cells) of TamS and TamR cells. Cells were treated with DMSO, 1 μM 4-OH Tam, 4-OH Tam and CGP 57380 (10 μM), or 1 μM 4-OH Tam, CGP 57380, and 20 nM RAD001 therapies 24 h after plating. Treatment was changed every 72 h. Colonies scored after 10 days counting only ≥50 cells/colony. Results are representative of 3 independent experiments. SEM is shown. (*D*) The overall protein synthesis activity of the TamS and TamR cells was measured by [^{35}S]-methionine labeling for 30 min, following treatment with either DMSO or 10 μM CGP 57380 for 24 h. Results from 3 independent experiments. Error bars represent SEM. n.s., not significant. (*E*) Polysomal profiling was performed on TamR cells treated with either DMSO or 10 μM CGP57380 for 24 h. Equal amounts of RNA were resolved by sucrose gradient centrifugation and ribosome profiles

monitored by UV absorbance 254 nm. Results are representative images of two independent experiments.

Supplemental Figure S4. Related to Figures 6 & 7. Genomic and pathway analysis of genes significantly altered in resistant cells with eIF4E silencing. (*A*) Heatmap (generated using GENE-E software) of genes altered in total abundance or translation (light and heavy polysome) with eIF4E silencing from TamR cells. Red indicates an increase and blue a decrease in expression. (*B*) RNA-seq was validated using RT-qPCR analysis. Plot shows the correlation of gene expression between the RNA-seq and qPCR analysis (qPCR values are a mean of $\log_2$ fold change +/- SD) from the total extracted mRNA. Pearson's correlation (R^2) is shown on each graph. Dotted lines represent significance cutoffs. (*C*) RNA-seq was validated using RT-qPCR analysis. Plot shows the correlation of gene expression between the RNA-seq and qPCR analysis (qPCR values are a mean of $\log_2$ fold change +/- SD) of mRNAs extracted from heavy (≥4 ribosomes) polysome fractions. Pearson's correlation (R^2) is shown on each graph. Dotted lines represent significance cutoffs. (*D*) Graph representing the top downregulated biological pathways from the total mRNA fraction with eIF4E silencing in TamR cells. Numbers above bars represent P-values. (*E*) Graph representing the top downregulated biological pathways from the heavy polysome fraction with eIF4E silencing in TamR cells. Numbers above bars represent *P*-values. (*F*) Graph representing the top upregulated biological pathways from the total mRNA fraction with eIF4E silencing in TamR cells. Numbers above bars represent *P*-values. (*G*) Graph representing the top upregulated biological pathways from the heavy polysome fraction with eIF4E silencing in TamR cells. Numbers above bars represent *P*-values. Pathways were identified using Ingenuity Pathway Analysis (IPA) software (*D*-*E*). (*H*) Predicted structure of *Runx2* 5'UTR using mfold web server. Computational folding was done under standard thermodynamic conditions. Analysis was repeated using both RNAstructure and IDT UNAFold

folding programs. Similar structural and thermodynamic results were returned. The ∆G value represents that predicted by mfold.

Supplemental Figure S5. Related to Figures 6 & 7. Genome-wide analysis of 5'UTRs of genes selectively downregulated with eIF4E silencing. (*A*) Histogram represents a genome-wide analysis of the GC percentage (normalized to 5'UTR length) of the 5'UTRs from all mRNAs or downregulated mRNAs extracted from the heavy polysome fraction. mRNAs were extracted from TamR sh-control and sh-eIF4E cells. (*B*) Histogram represents a genome wide analysis of the length of the 5'UTRs from all mRNAs or downregulated mRNAs extracted from the heavy polysome fraction. mRNAs were extracted from TamR sh-control and sh-eIF4E cells. Histograms generated using R studio software. (*C*) Heatmap generated from TCGA analysis of *Runx2* and ER (*ESR1*) mRNA expression from patients (n=594) diagnosed with ER^+ breast cancer. (*D*) qRT-PCR analysis of *Runx2* and ER (*ESR1*) mRNA in breast cancer cell lines (left panel). Zoomed comparison of *Runx2* mRNA levels in TamS and TamR cells (right panel).