## Supplementary Methods:

The following SNPs were utilized in the authentication of cell lines:

rs10018359, rs10050093, rs10108245, rs10410468, rs10461909, rs10740186, rs10755578, rs10828176, rs10834627, rs10885378, rs10977980, rs11083145, rs11100847, rs11180435, rs11638893, rs11746396, rs12486048, rs12537, rs13032222, rs1325922, rs1425916, rs1529192, rs1635191, rs16888998, rs16928965, rs16999576, rs1709795, rs17174920, rs1726254, rs1784232, rs1912640, rs1934395, rs1956898, rs2069492, rs2172614, rs2280916, rs2355988, rs2391691, rs2563263, rs2590442, rs2714679, rs2848745, rs2927899, rs2928432, rs2999156, rs3125842, rs3739422, rs3783412, rs3817731, rs387232, rs3923878, rs3952312, rs4323418, rs4342761, rs4355349, rs4355471, rs4421732, rs4614318, rs4669243, rs4695891, rs4715321, rs4825186, rs4828565, rs4862450, rs4903262, rs4917823, rs4947934, rs522171, rs564597, rs5749504, rs5749505, rs5908093, rs6075993, rs6080649, rs6135489, rs6532589, rs6682171, rs689095, rs7052801, rs706691, rs7083994, rs7123344, rs7528533, rs7847847, rs7971921, rs8087158, rs8095861, rs828566, rs919306, rs9297351, rs9367063, rs937831, rs9471874, rs9535482, rs9552910, rs9817493

Western blot and ELISA assays were utilized to interrogate signalling pathway alterations in the setting of PPM1H knockdown (Supplementary Fig. S2). The antibodies used for Western blot were as follows: phos-ERK1/2 (Thr202/Tyr204), Cell Signaling, #4370; total ERK1/2, Cell Signaling, #4695; phos-AKT (Ser473), Cell Signaling, #4060; total AKT, Cell Signaling, #4685; phos-Her3 (Tyr1289), Cell Signaling, #4791; total Her3, Santa Cruz Biotechnology, SC-285. Phospho and total AKT ELISA was

performed with kits #7160 and # 7170 from Cell Signaling, according to manufacturer's instructions.

## Supplementary Figure Legends

**Supplementary Figure S1. Additional data on PPM1H knockdown in 2D and 3D culture, expanding upon Figure 2.** Proliferation of BT474M1 cells (A) or SKBR3 cells (B) was measured after 7 days of treatment with trastuzumab (8 days of knockdown). Cell number was measured via CellTiter-Glo (cellular ATP measurement). Data are represented as mean ± SEM. (C) Percent knockdown by qRT-PCR of each gene in each cell line after 8 days. (D) PPM1H knockdown with each of 4 individual oligonucleotides and the pool of 4. (E) In parallel with the data shown in Fig. 2C, we included a dox inducible shLacZ cell line as a negative control and saw no difference in the effect of trastuzumab with and without dox administration. (F) Western blot showing PPM1H knockdown with dox administration in the 3D culture model. (G) Phase contrast photographs of 3D culture acini

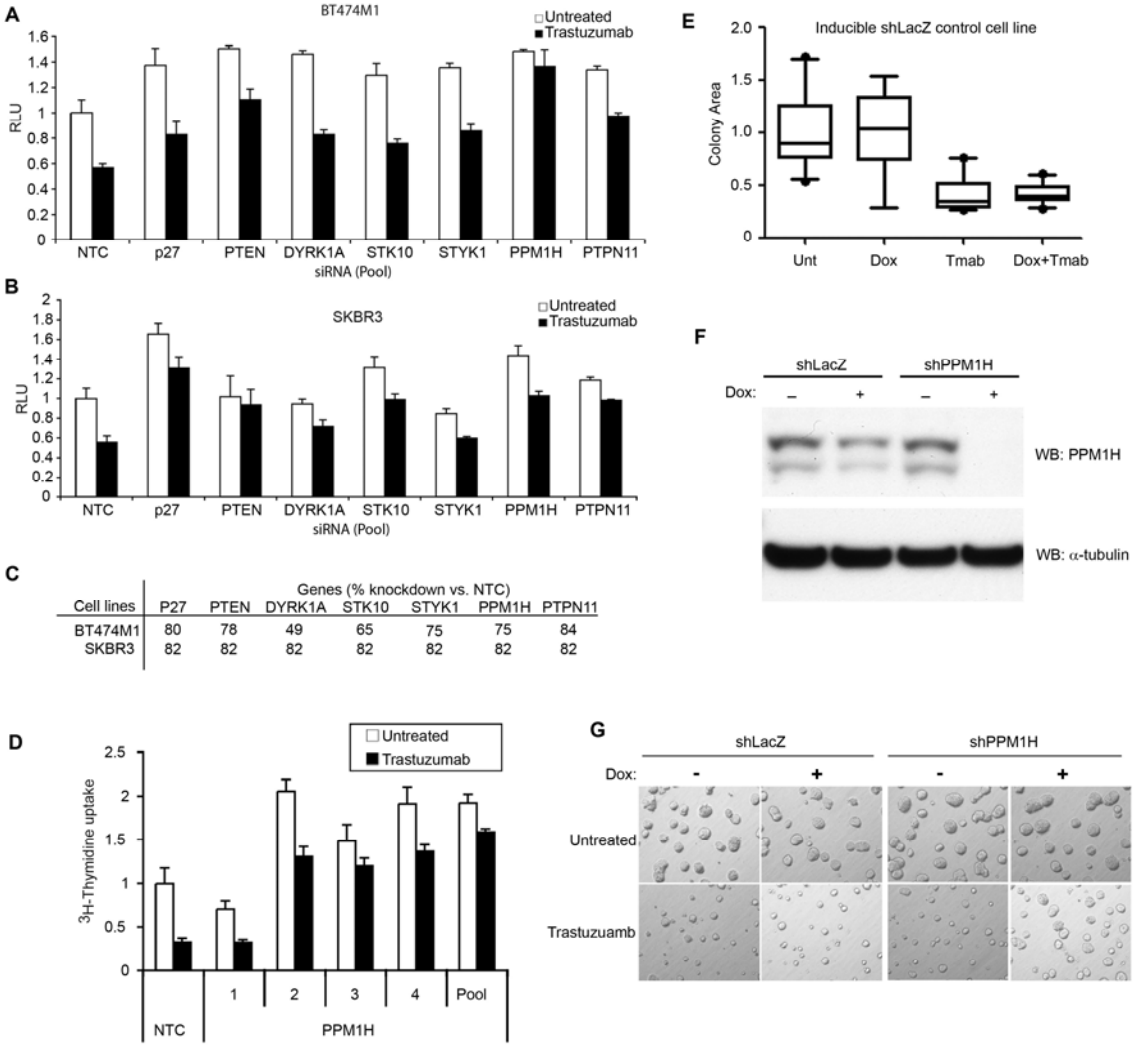
**Supplementary Figure S2. Assessment of PPM1H effect on downstream signaling, expanding upon Figure 3.** (A) Western blot of pAKT and pERK with various trastuzumab treatment times after transfection with NTC siRNA or PPM1H siRNA. (B) ELISA assay for pAKT with various trastuzumab treatment times after transfection with NTC siRNA, PTEN siRNA or PPM1H siRNA. (C) ELISA assay for phospho-HER2 and phospho-HER3 with various trastuzumab treatment times after transfection with NTC siRNA or PPM1H siRNA. (D) Percent of nuclei with positive staining for p27 after transfection with NTC siRNA or PPM1H siRNA. (E) Percent of nuclei with positive staining for SKP2 after transfection with NTC siRNA or PPM1H siRNA. Error bars represent SEM.

**Supplementary Figure S3. PPM1H enzyme activity, expanding upon Figure 4.** (A) Cells were fractionated into nuclear and cytoplasmic compartments followed by PPM1H Western blot. C, cytoplasm; N, nucleus; T, Total. A non-specific band that does not disappear with PPM1H knockdown is noted. (B) Recombinant Flag-PPM1H and flag-PPM1H-H153L were tested for activity using a synthetic phosphatase assay kit (EnzChek, Molecular Probes). (C) Recombinant GST-PPM1H and GST-PPM1J were tested for activity using the EnzChek phosphatase assay kit. (D) Recombinant HA-SKP2 was incubated with AKT in a kinase reaction with $\gamma$-$^{32}$P ATP which results in radiolabeling of SKP2. The $^{32}$P-SKP2 was then incubated with recombinant flag-PTEN as a negative control or recombinant flag-PPM1H. $\lambda$-phosphatase was added in addition to flag-PPM1H as a positive control, and to rule out the presence of phosphatase inhibitors in the flag-PPM1H preparation. The addition of $\lambda$-phosphatase removed all detectable $^{32}$P from SKP2. In contrast, neither PTEN nor PPM1H exhibited any evidence of phosphatase activity on SKP2.

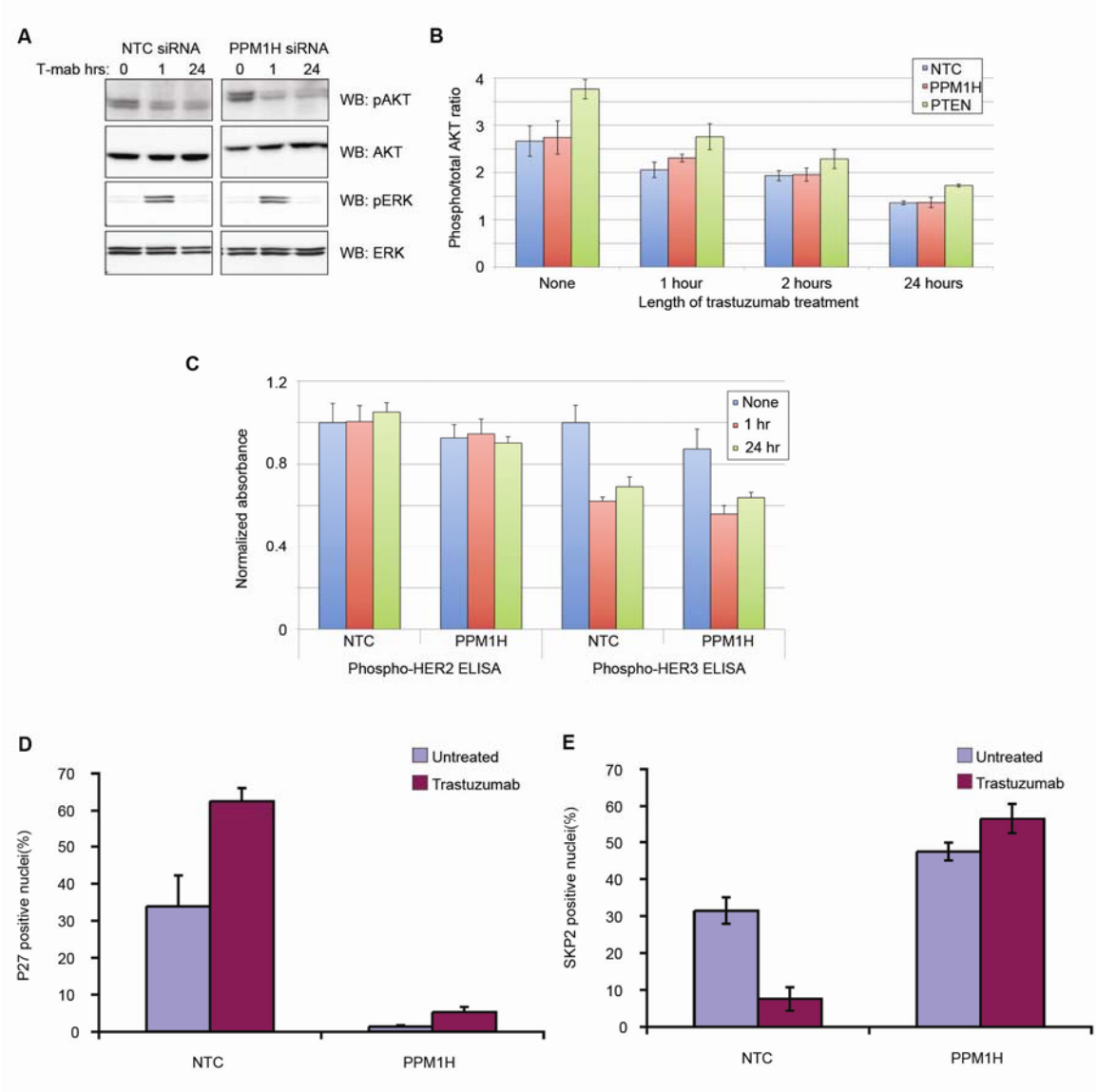
**Supplementary Figure S4. PPM1H expression in breast tissues, expanding upon Figure 5.** *PPM1H in situ* hybridization was performed as noted in Fig. 5. In addition to analysis of the overall population, *PPM1H* status in relation to clinical outcome was examined based on ER status: (A) ER-negative patients, (B) ER-positive patients. While neither analysis achieved statistical significance, the trend toward poor clinical outcome with low PPM1H expression was observed only in the ER-negative population (HR 1.8, p = 0.15, 95%CI 0.87-3.7). (C) PTEN IHC was performed on the Ventana Discovery platform using a tissue microarray representing the same samples included in the PPM1H analysis. The IHC was scored by comparing staining in tumour cells to staining in normal tissue elements. Samples with decreased staining in tumour were considered to have decreased PTEN expression (33%). In a Kaplan-Meier analysis, there was no statistically significant difference in outcome based on PTEN status. (D) PIK3CA

hotspot mutations (H1047, E545, E542) were assessed by Sequenom mass spectrometry. 21% of samples were mutant. Kaplan-Meier analysis reveals no significant difference in outcome based on PIK3CA mutation status. (E) Image of *in situ* hybridization and H&E of one sample with increased PPM1H expression in DCIS, but low expression in invasive carcinoma. Scale bar = 50 μm. (F) PPM1H expression in breast tissue samples including normal, benign disease, invasive cancer and metastasis. Expression data were derived from Affymetrix HGU-133A and HGU-133B arrays and were normalized using the MAS 5.0 algorithm. Error bars represent standard deviation. (G) *PPM1H* expression as determined by qRT-PCR in BT474 cells transfected with non-targeting control (NTC), HER2 or HER3 siRNA. The data are normalized to *PPM1H* expression in NTC (set at 1.0). (H) *PPM1H* expression in several examples of normal tissue and cancer tissue. Expression data were derived from Affymetrix HGU-133A and HGU-133B arrays and were normalized using the MAS 5.0 algorithm. Error bars represent standard deviation.

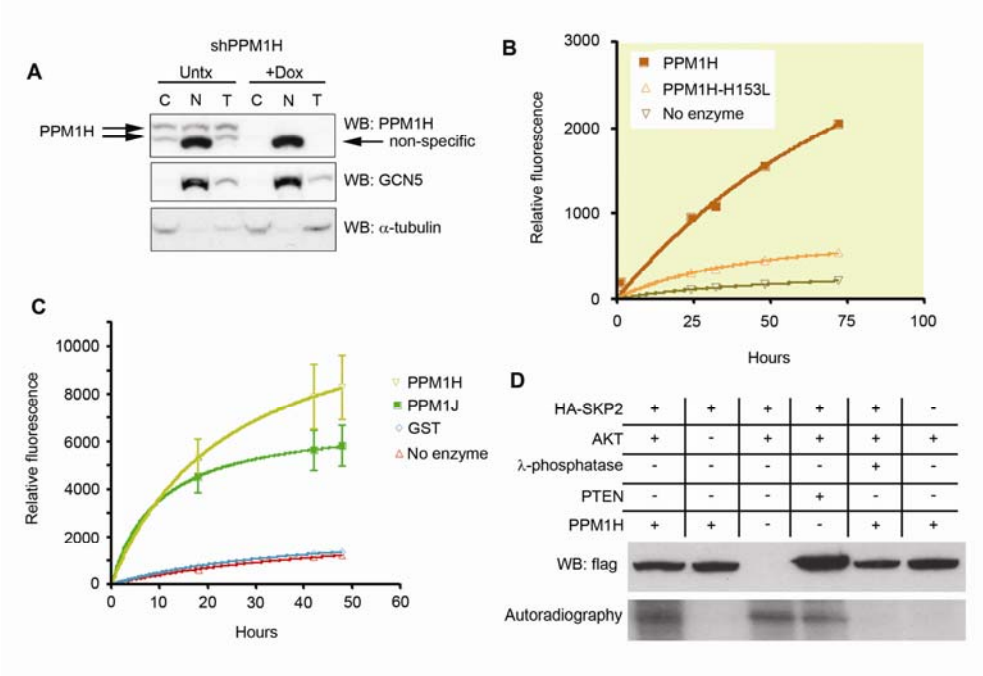
Supplementary Fig S1



Supplementary Fig S2



Supplementary Fig S3



Supplementary Fig S4