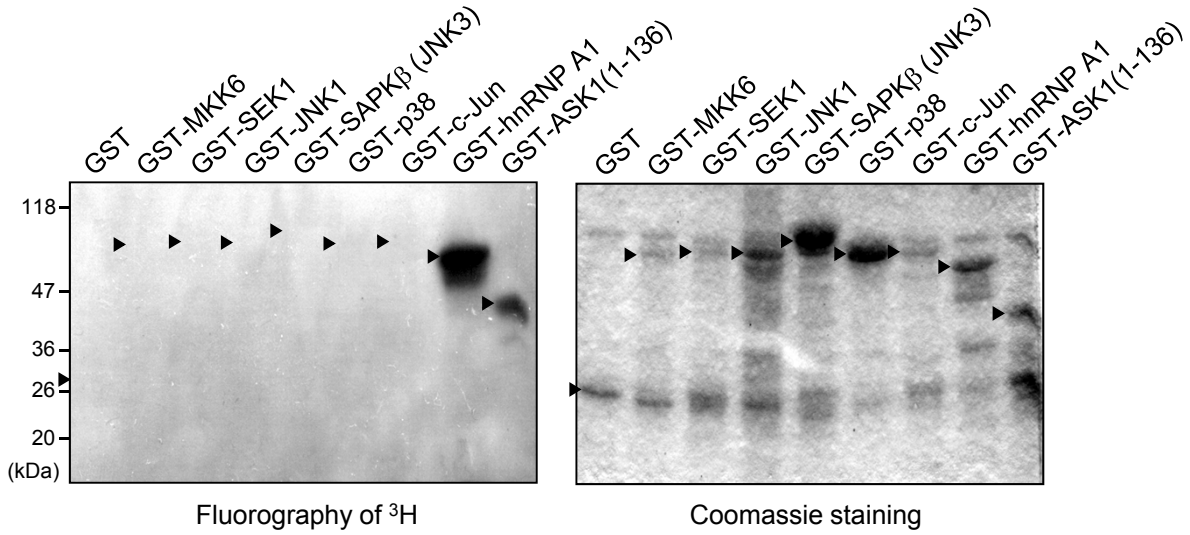
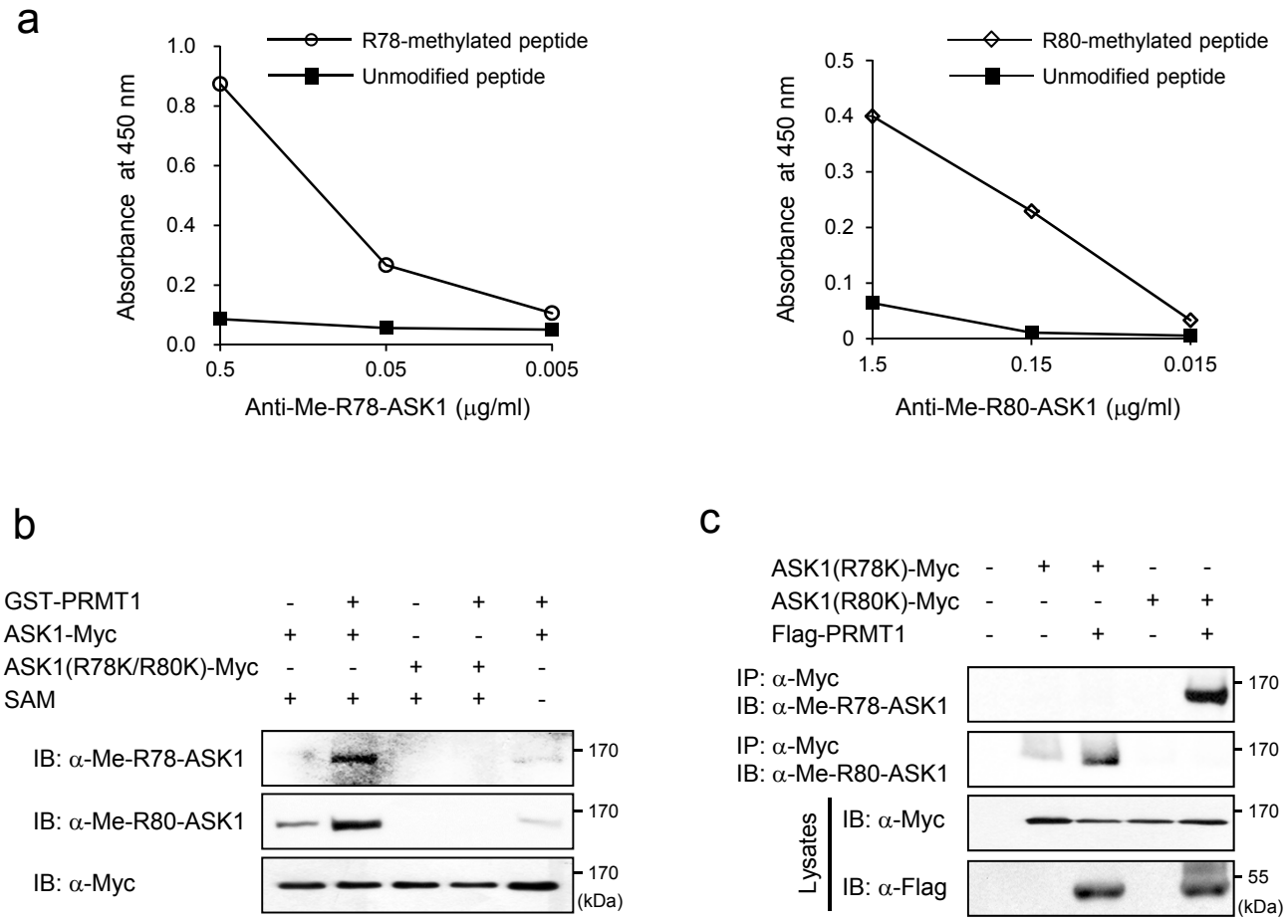
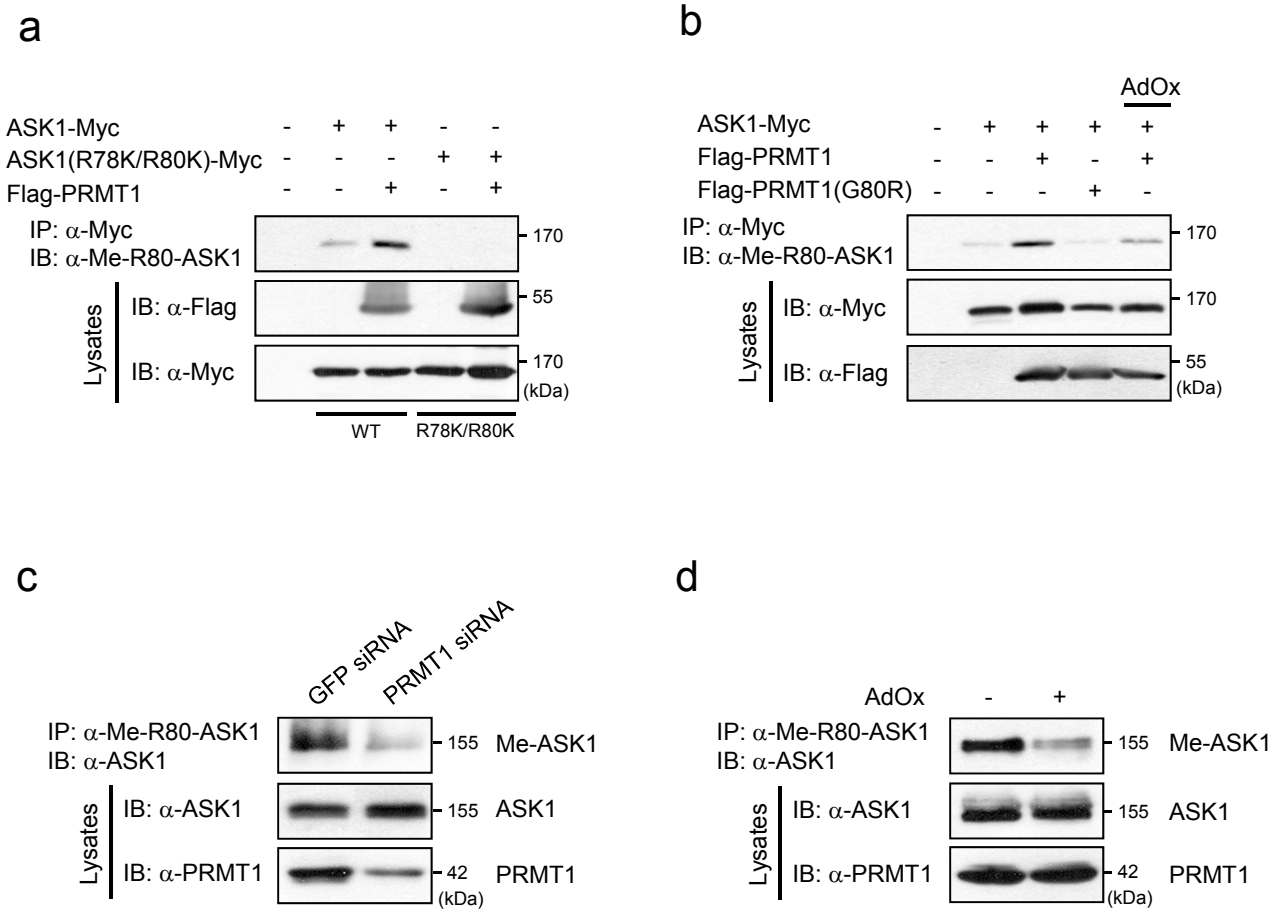


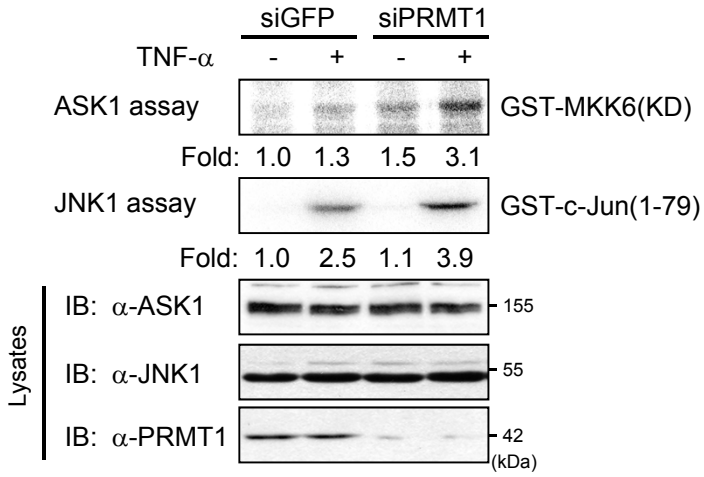
Supplementary Figure 1 PRMT1 does not methylate MKK6, SEK1, JNK1, SAPKβ (JNK3), p38, or c-Jun in vitro. GST-PRMT1 expressed in and purified from *Escherichia coli* was incubated with the indicated proteins in the presence of S-adenosyl-L-(methyl-^{3}H)-methionine. The reaction mixtures were then subjected to SDS-PAGE followed by fluorography (left) and Coomassie blue staining (right). The arrowheads indicate the positions of the tested proteins. hnRNP A1 was used as a positive control.



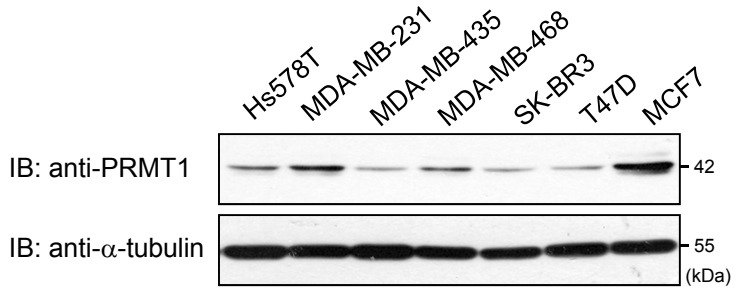
Supplementary Figure 2 Confirmation of the specificity of antibodies to Arg^{78}-methylated ASK1 or to Arg^{80}-methylated ASK1. (a) Enzyme-linked immunosorbent assay with affinity-purified antibodies to Arg^{78}-methylated ASK1 (anti-Me-R78-ASK1) or to Arg^{80}-methylated ASK1 (anti-Me-R80-ASK1). Various concentrations of the antibodies were incubated with the corresponding methylated peptide antigen or the unmodified peptide at a concentration of 1 μg/ml. (b) 293T cells were transfected for 48 h with a plasmid vector for ASK1-Myc or ASK1 (R78K/R80K)-Myc, after which cell lysates were subjected to immunoprecpitation with anti-Myc antibody. The resulting immunoprecipitates were incubated with recombinant GST-PRMT1 in the absence or presence of unlabeled S-adenosyl-L-methionine (cold-SAM), and the reaction mixtures were then subjected to immunoblot analysis with the indicated antibodies. (c) 293T cells were transfected for 48 h with the indicated combinations of expression vectors for ASK1(R78K)-Myc, ASK1(R80K)-Myc, and Flag-PRMT1, after which cell lysates were subjected to immunoprecipitation with anti-Myc antibody. The resulting precipitates were subjected to immunoblot analysis with antibodies to Arg^{78}-methylated ASK1 or to Arg^{80}-methylated ASK1. Cell lysates were also examined directly by immunoblot analysis with antibodies to Myc or to Flag.



Supplementary Figure 3 Detection of PRMT1-mediated methylation of ASK1 at Arg^{80} with antibodies specific for Arg^{80}-methylated ASK1. (a, b) 293T cells were transfected for 48 h with the indicated combinations of expression vectors encoding ASK1-Myc, ASK1(R78K/R80K)-Myc, and Flag-PRMT1 (a) or ASK1-Myc, Flag-PRMT1, and Flag-PRMT1(G80R) (b). In (b), the transfected cells were then incubated for 12 h with DMSO (0.1%) or AdOx (10 μM). In both (a) and (b), cell lysates were subjected to immunoprecipitation with anti-Myc antibody, and the resulting precipitates were immunoblotted with antibodies specific for Arg^{80}-methylated ASK1 (α-Me-R80-ASK1). Cell lysates were also examined directly by immunoblot analysis with antibodies to Myc or to Flag. (c, d) MCF7 cells were transfected for 48 h with control (GFP) or PRMT1 siRNAs (c) or were incubated for 12 h with either DMSO (0.1%) or AdOx (10 μM) (d). The cells were then lysed and subjected to immunoprecipitation with antibodies to Arg^{80}-methylated ASK1, and the resulting precipitates were subjected to immunoblot analysis with anti-ASK1 antibody. Cell lysates were also examined directly by immunoblot analysis with antibodies to ASK1 or to PRMT1.

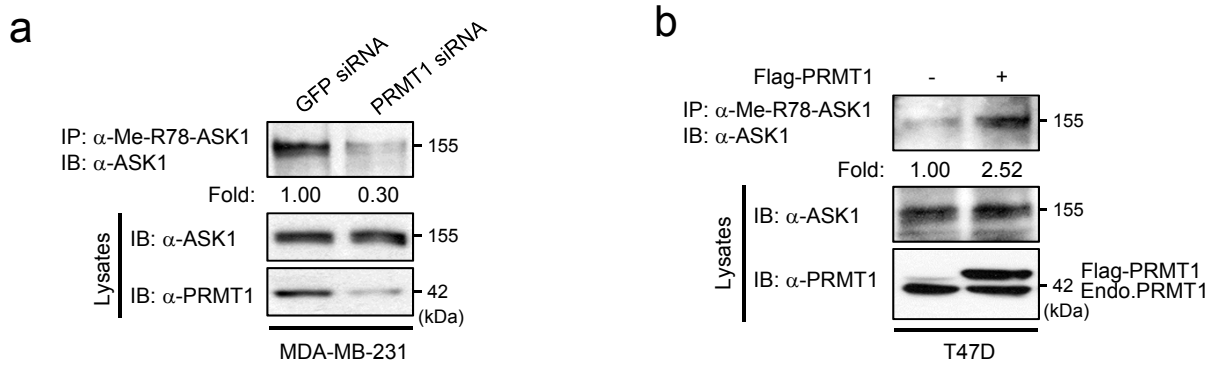


Supplementary Figure 4 Depletion of PRMT1 by RNAi potentiates TNF-α-induced stimulation of ASK1 and JNK1 in HeLa cells. HeLa cells stably expressing GFP (control) or PRMT1 siRNAs were left untreated or treated with 20 nM TNF-α for 20 min, lysed, and subjected to immunoprecipitation with antibodies to ASK1 or to JNK1. The resulting precipitates were subjected to immune complex kinase assays of ASK1 or JNK1 activity, respectively, and cell lysates were also examined directly by immunoblot analysis with antibodies to ASK1, JNK1, or to PRMT1.

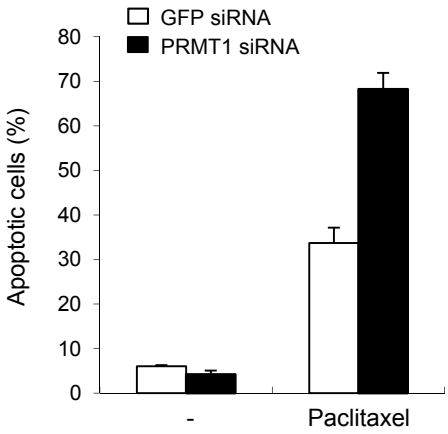


Supplementary Figure 5 Abundance of PRMT1 in various breast cancer cell lines.

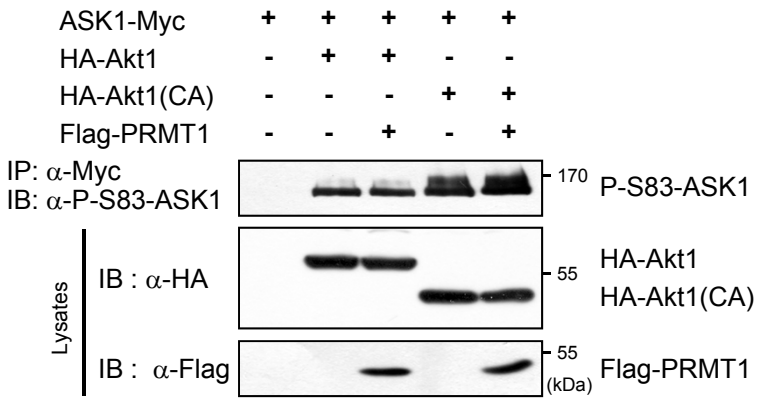
Lysates of the indicated breast cancer cell lines were subjected to immunoblot analysis with antibodies to PRMT1 or to α-tubulin (loading control).



Supplementary Figure 6 Effect of PRMT1 on Arg^{78} methylation of endogeneous ASK1 in MDA-MB-231 or T47D breast cancer cells. MDA-MB-231 cells were transfected for 48 h with control (GFP) or PRMT1 siRNAs (a) or T47D cells were transfected for 48 h with the expression vector encoding Flag-PRMT1, as indicated (b). The cells were then lysed and subjected to immunoprecipitation with antibodies to Arg^{78}-methylated ASK1, and the resulting precipitates were subjected to immunoblot analysis with anti-ASK1 antibody. Cell lysates were also examined directly by immunoblot analysis with antibodies to ASK1 or to PRMT1. Endo.PRMT1 indicates endogeneous PRMT1.



Supplementary Figure 7 Depletion of PRMT1 by RNAi enhances paclitaxel-induced apoptosis in MCF7 breast cancer cells. MCF7 cells transfected for 48 h with GFP or PRMT1 siRNAs were incubated with either DMSO (0.1%) or paclitaxel (1 μg/ml) for 48 h, fixed, and stained with 4',6'-diamidino-2-phenylindole dihydrochloride (DAPI). DAPI-stained nuclei were examined for apoptotic morphology by fluorescence microscopy, and the percentages of apoptotic cells are presented as means ± SD from three independent experiments.



Supplementary Figure 8 PRMT1 does not affect Akt1-mediated phosphorylation of ASK1 at Ser^{83}. 293T cells were transfected for 48 h with the indicated combinations of expression vectors for ASK1-Myc, HA-Akt1, HA-Akt1(CA) (a constitutively activated form of Akt1), and Flag-PRMT1. Cell lysates were subjected to immunoprecipitation with anti-Myc antibody, and the resulting precipitates were subjected to immunoblot analysis with antibodies to the Ser83-phosphorylated form of ASK1 (α-P-S83-ASK1). Cell lysates were also examined directly by immunoblot analysis with antibodies to HA or to Flag.