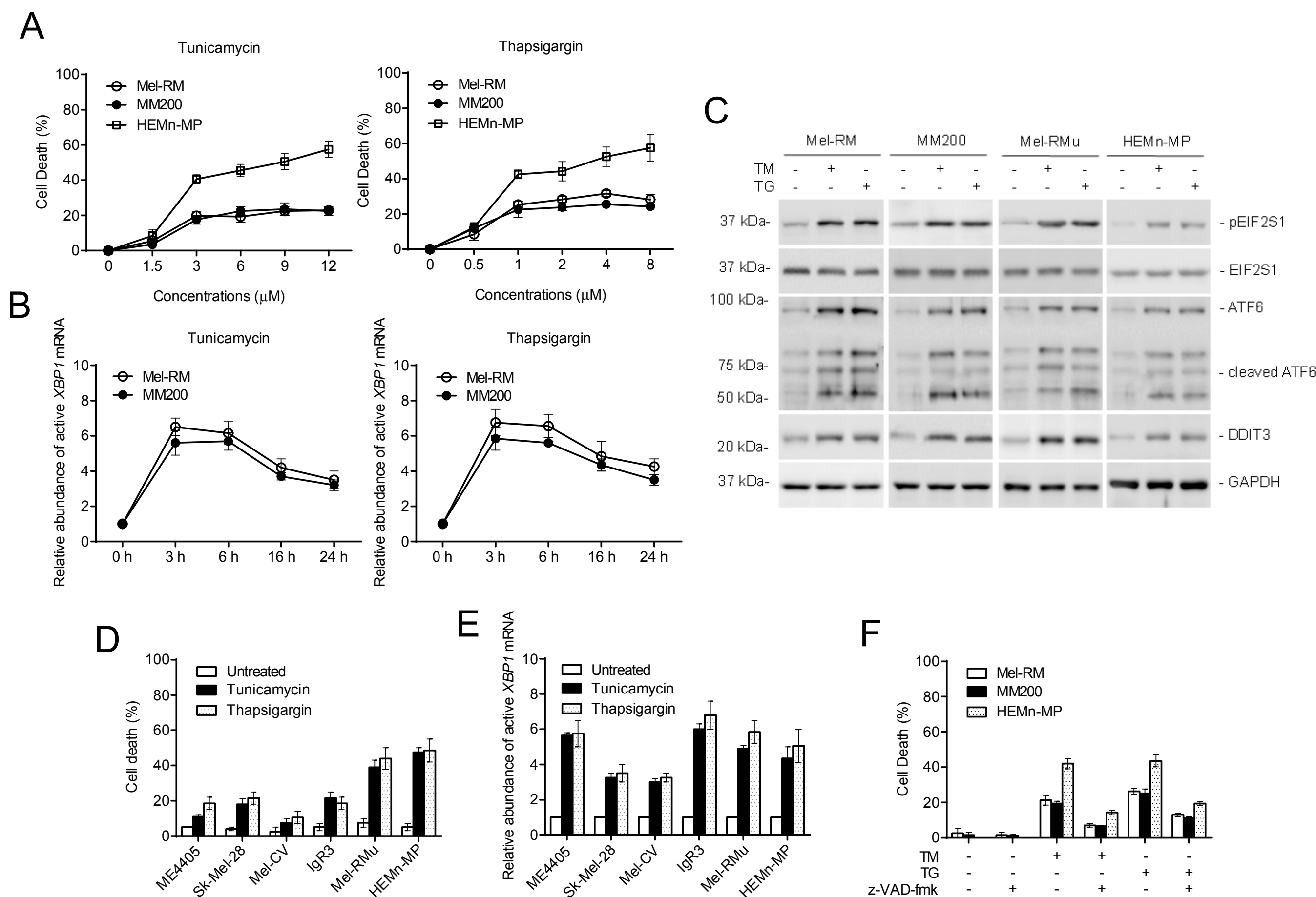

Figure S1. Mel-RM and MM200 cells are resistant to ER stress-induced apoptosis. (**A**) Mel-RM and MM200 melanoma cells and HEMn-MP melanocytes were treated with the indicated concentrations of tunicamycin (left panel) or thapsigargin (right panel) for 48 h. Apoptosis was quantitated by the ANXA5/annexin V and FITC-propidium iodide (PI) staining (n=3, mean ± SEM); (**B**) Mel-RM and MM200 cells were treated with tunicamycin (3 μM) (left panel) and thapsigargin (1 μM) (right panel) for the indicated periods. Total RNA was subjected to qPCR analysis of the expression of active *XBP1* mRNA. The relative abundance of the active *XBP1* mRNA before treatment was arbitrarily designated as 1 (n=3, mean ± SEM); (**C**) Whole cell lysates from Mel-RM, MM200 and Mel-RMu melanoma cells and HEMn-MP melanocytes with or without treatment with tunicamycin (TM) (3 μM) or thapsigargin (TG) (1 μM) were subjected to western blot analysis of pEIF2S1, EIF2S1, ATF6, DDIT3 and GAPDH (as a loading control) (n=3); (**D**) ME4405, Sk-Mel-28, Mel-CV, IgR3, and Mel-RMu melanoma cells and HEMn-MP melanocytes were treated with tunicamycin (3 μM) or thapsigargin (1 μM) for 48 h. Apoptosis was measured by the ANXA5 and FITC-PI staining (n=3, mean ± SEM); (**E**) ME4405, Sk-Mel-28, Mel-CV, IgR3, and Mel-RMu melanoma cells and HEMn-MP melanocytes were treated with tunicamycin (3 μM) or thapsigargin (1 μM) for 3 h. Total RNA was subjected to qPCR analysis for the expression of active *XBP1* mRNA. The relative abundance of the active *XBP1* mRNA before treatment was arbitrarily designated as 1 (n=3, mean ± SEM); (**F**) Mel-RM and MM200 melanoma cells and HEMn-MP melanocytes were pre-treated with or without z-VAD-fmk (20 μM) followed by treatment with TM (3 μM) or TG (1 μM) for 48 h. Apoptosis was measured by the ANXA5 and FITC-PI staining (n=3, mean ± SEM).

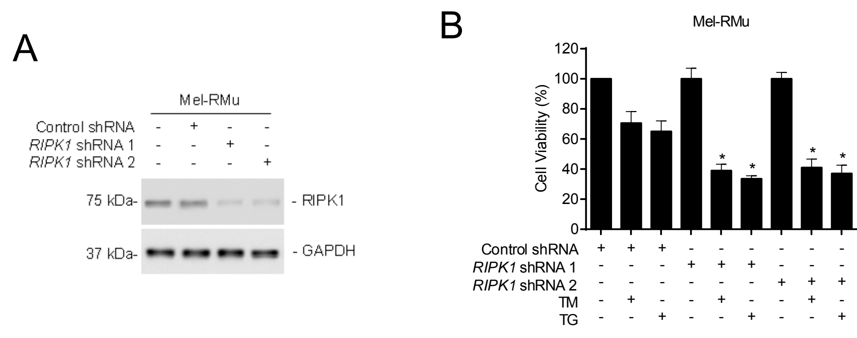

Figure S2. RIPK1 protects melanoma cells from killing by pharmacological ER stress. (**A**) Whole cell lysates from Mel-RMu transduced with the control or *RIPK1* shRNA were subjected to western blot analysis of RIPK1 and GAPDH (as a loading control) (n=3); (**B**) Mel-RMu cells transduced with the control or *RIPK1* shRNA were treated with tunicamycin (TM) (3 μM) or thapsigargin (TG) (1 μM) for 48 h. Cell viability was measured by CellTiter-Glo assays (n=3, mean ± SEM, *$P<0.05$, Student t test).

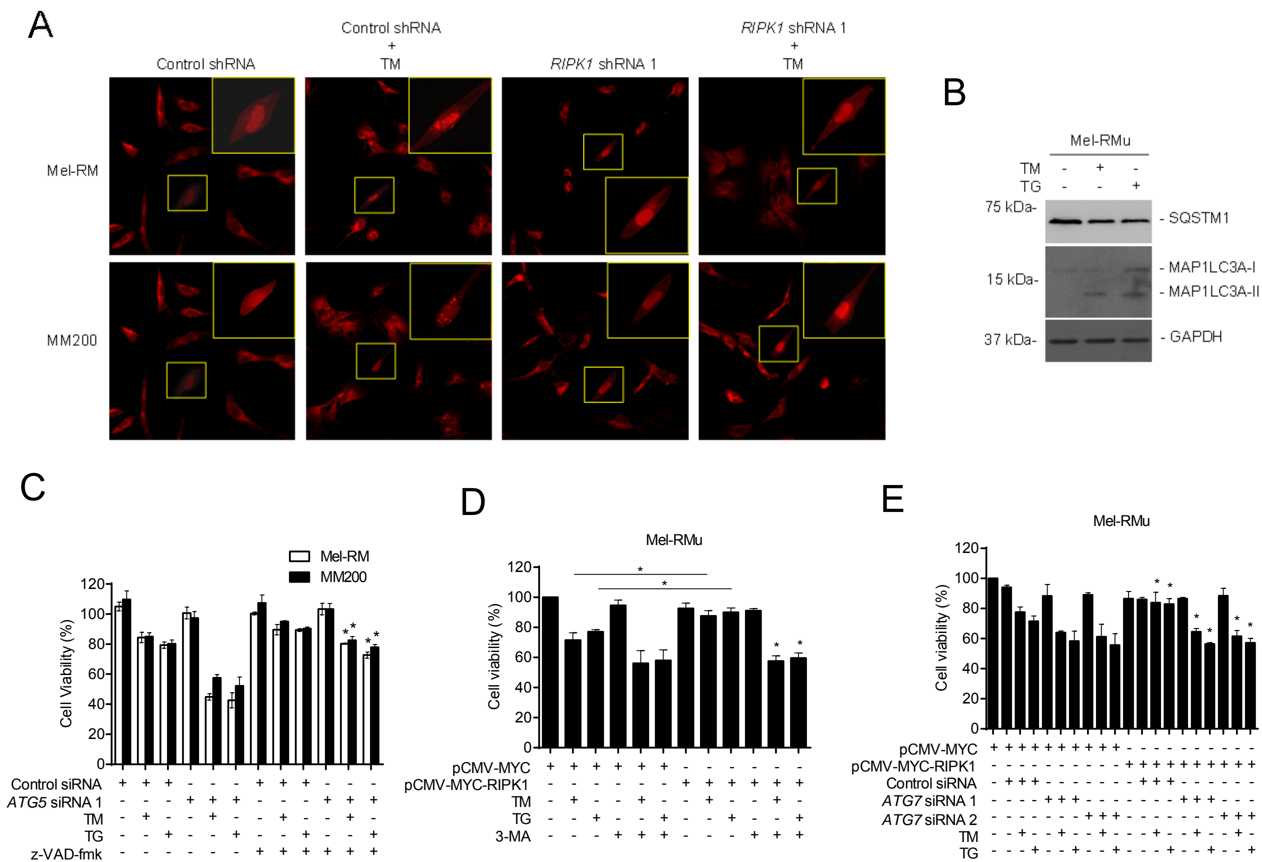

Figure S3. RIPK1 protects melanoma cells from ER stress-induced killing by activation of autophagy. (**A**) Mel-RM and MM200 cells transduced with the control or *RIPK1* shRNA 1 were transiently transfected with a mCherry-hLC3B-pcDNA3.1 construct. Forty-eight h later, cells were exposed to tunicamycin (TM) (3 μM) for a further 24 h. A representative cell was enlarged and shown at the top-right or bottom-right corner of each panel. Data shown are representative fluorescence confocal microscopy photographs; (**B**) Whole cell lysates from Mel-RMu cells with or without treatment with TM (3 μM) or thapsigargin (TG) (1 μM) for 16 h were subjected to western blot analysis of SQSTM1, MAP1LC3A, and GAPDH (as a loading control) (n=3); (**C**) Mel-RM and MM200 transfected with the control or *ATG5* siRNA 1 were pretreated with or without z-VAD-fmk (20 μM) followed by treatment with TM (3 μM) or TG (1 μM) for 48 h. Cell viability was measured by CellTiter-Glo assays (n=3, mean ± SEM, **P*<0.05, Student t test); (**D**) Mel-RMu cells stably transfected with the pCMV-MYC or pCMV-MYC-RIPK1 vector with or without pretreatment with 3-methyladenine (3-MA) (5 mM) were treated with TM (3 μM) or TG (1 μM) for 48 h. Cell viability was measured by CellTiter-Glo (n=3, mean ± SEM, **P*<0.05, Student t test); (**E**) Mel-RMu cells stably transfected with the pCMV-MYC or pCMV-MYC-RIPK1 vector were transfected with the control or *ATG7* siRNA followed by treatment with TM (3 μM) or TG (1 μM) for 48 h. Cell viability was measured by CellTiter-Glo (n=3, mean ± SEM, **P*<0.05, Student t test).

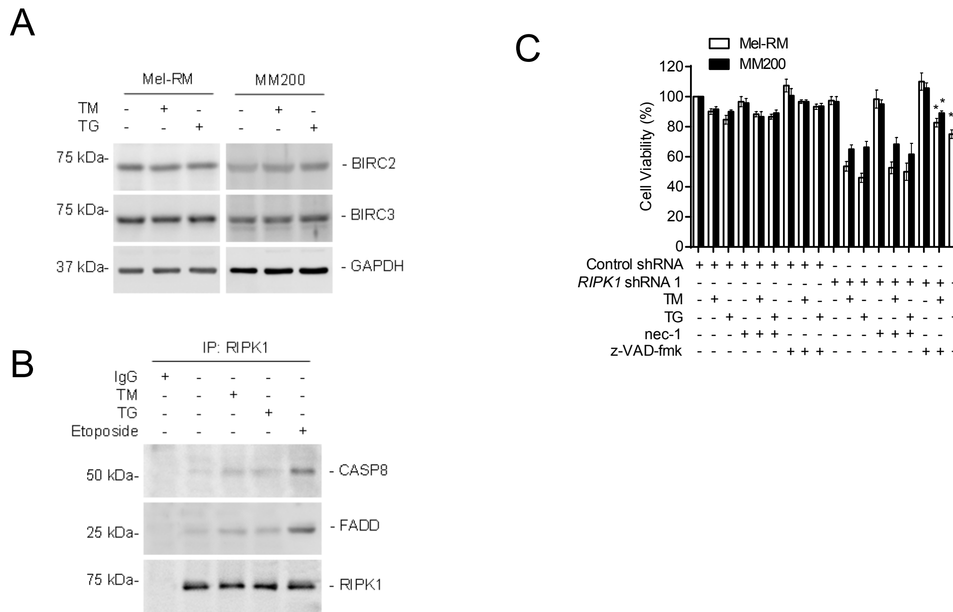

Figure S4. RIPK1-mediated activation of MAPK8/9 contributes to induction of autophagy by ER stress in melanoma cells. (**A**) Whole cell lysates from Mel-RM and MM200 cells treated with tunicamycin (TM) (3 µM) or thapsigargin (TG) (1 µM) for 16 h were subjected to western blot analysis of BIRC2, BIRC3 and GAPDH (as loading control) (n=3); (**B**) Whole cell lysates from Mel-RM cells treated with TM (3 µM) or TG (1 µM) for 16 h or etoposide (40 µM) for one h were immunoprecipitated by RIPK1 antibody. The resulting precipitates were subjected to western blot analysis of CASP8, FADD and RIPK1 (n=3); (**C**) Mel-RM and MM200 cells were pretreated with or without necrostatin-1 (nec-1) (30 µM) or z-VAD-fmk (20 µM) followed by treatment with TM (3 µM) or TG (1 µM) for 48 h. Cell viability was measured by CellTiter-Glo (n=3, mean ± SEM, **P*<0.05, Student t test).

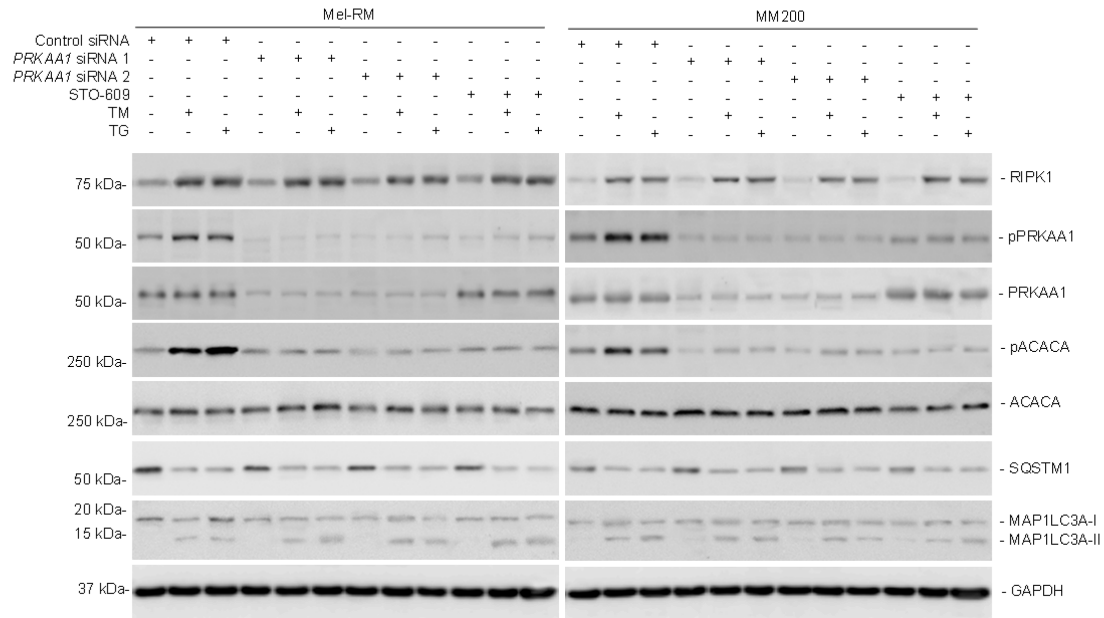

Figure S5. The Calcium-CAMKK2-AMPK pathway does not play a major role in pharmacological ER stress upregulation of RIPK1 in melanoma cells. Mel-RM and MM200 cells were transfected with the control or *PRKAA1* siRNA. Whole cell lysates from transfected cells treated with tunicamycin (TM) (3 μM) or thapsigargin (TG) (1 μM) for 16 h and whole cell lysates from untransfected cells treated with STO-609 (1 μg/ml) and TM (3 μM) or TG (1 μM) were subjected to western blot analysis of RIPK1, pPRKAA1, PRKAA1, pACACA, ACACA, SQSTM1, MAP1LC3A, and GAPDH (as a loading control) (n=3).

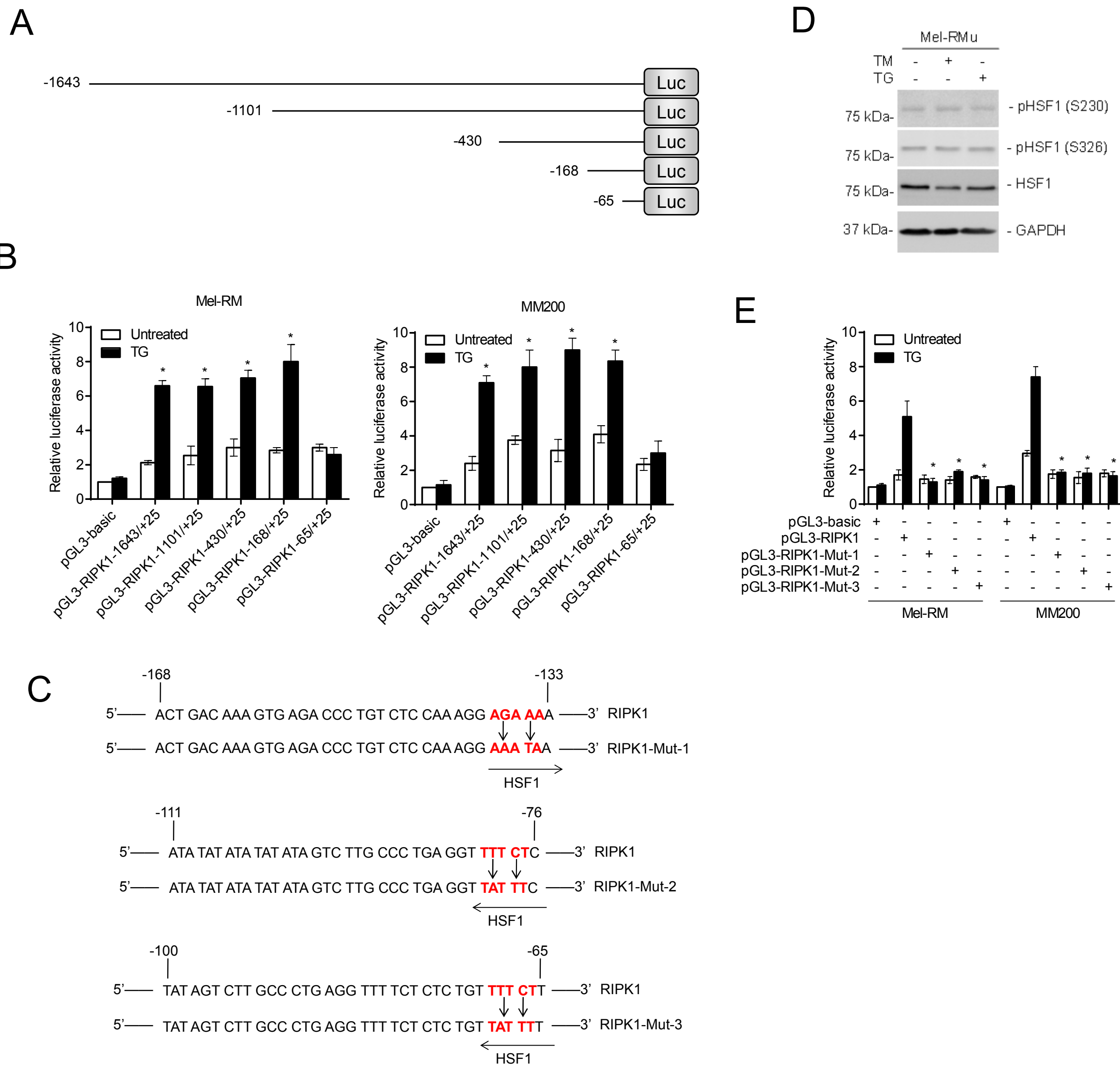

Figure S6. HSF1 (heat shock transcription factor 1) is responsible for transcriptional upregulation of *RIPK1* in melanoma cells upon pharmacological ER stress. (**A**) A schematic illustration of construction of the luciferase reporter constructs; (**B**) Mel-RM (left panel) and MM200 (right panel) cells transfected with indicated pGL3-basic based reporter constructs were treated with thapsigargin (TG) (1 μM) for 16 h followed by measurement of the luciferase activity (n=3, mean ± SEM, **P*<0.05, Student t test); (**C**) A schematic illustration of a potential HSF1 binding motif located within the -168 to -133 region (top panel), the -111 to -76 region (middle panel) and the -100 to -65 region (bottom panel) of the *RIPK1* promoter. Experimental mutagenesis of the region is also depicted; (**D**) Whole cell lysates of Mel-RMu cells treated with tunicamycin (TM) (3 μM) or TG (1 μM) for 16 h were subjected to western blot analysis of pHSF1 (Ser230), pHSF1 (Ser326), HSF1 and GAPDH (as loading control) (n=3); (**E**) Mel-RM and MM200 cells transfected with indicated pGL3-basic based reporter constructs were treated with TG (1 μM) for 16 h followed by measurement of the luciferase activity (n=3, mean ± SEM, **P*<0.05, Student t test) .

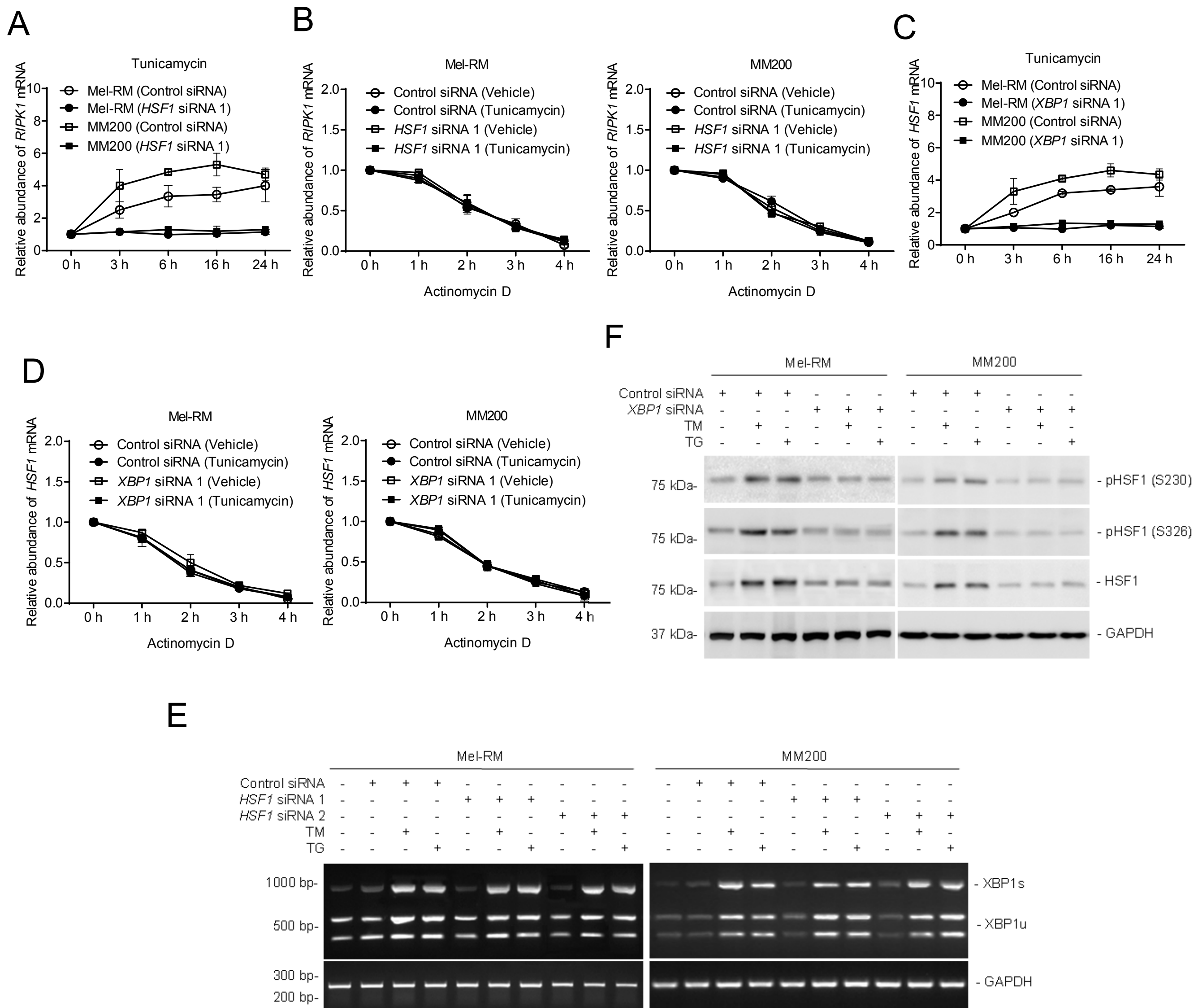

Figure S7. Heat shock factor protein 1 (HSF1) is transcriptionally upregulated downstream of XBP1 in melanoma cells under pharmacological ER stress. (**A and C**) Mel-RM and MM200 cells transfected with the control siRNA, *HSF1* siRNA 1 (A), or *XBP1* siRNA 1 (C) were treated with tunicamycin (3 μM) for the indicated period. Total RNA was subjected to qPCR analysis of the expression of *RIPK1* (A) or *HSF1* (C) mRNA. The relative abundance of *RIPK1* (A) or *HSF1* (C) mRNA before treatment was arbitrarily designated as 1 (n=3, mean ± SEM); (**B and D**) Mel-RM (left panel) and MM200 (right panel) cells transfected with the control siRNA, *HSF1* siRNA 1 (B) or *XBP1* siRNA 1 (D) were treated with tunicamycin (3 μM) for 16 h followed by treatment with actinomycin D (100 ng/ml) for the indicated period. Total RNA was subjected to qPCR analysis for the expression of *RIPK1* (B) or *HSF1* (D) mRNA. The relative abundance of the *RIPK1* (B) or *HSF1* (D) mRNA without actinomycin D treatment was arbitrarily designated as 1 (n=3, mean ± SEM); (**E**) Mel-RM and MM200 cells transfected with the control or *HSF1* siRNA followed by treatment with tunicamycin (TM) (3 μM) or thapsigargin (TG) (1 μM) for 16 h were subjected to RT-PCR for the analysis of *XBP1* mRNA. *GAPDH* was used as a loading control (n=3); (**F**) Whole cell lysates from Mel-RM and MM200 transfected with the control or *XBP1* siRNA and treated with tunicamycin (TM) (3 μM) or thapsigargin (1 μM) were subjected to western blot analysis of pHSF1 (Ser230), pHSF1 (Ser326), HSF1 and GAPDH (as loading control) (n=3).

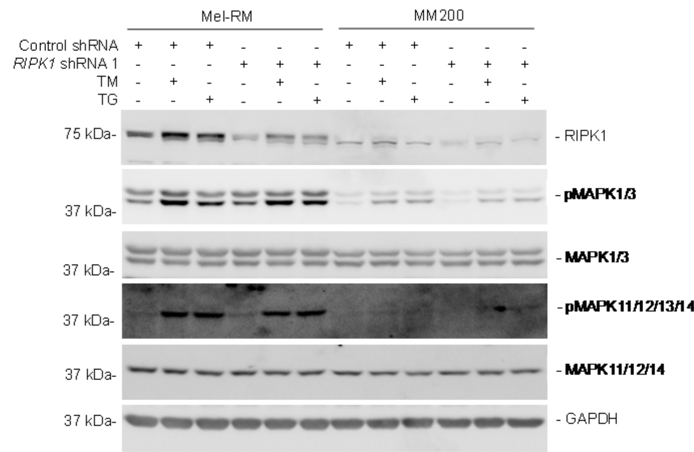

Figure S8. Activation of MAPK1/3 and MAPK11/12/13/14 was not affected by RIPK1 knockdown in melanoma cells under ER stress. Mel-RM and MM200 cells stably transduced with the control or *RIPK1* shRNA1 were treated with or without tunicamycin (TM) (3 μM) or thapsigargin (TG) (1 μM) for 16 h. Whole cell lysates were subjected to western blot analysis of RIPK1, pMAPK1/3, MAPK1/3, pMAPK11/12/13/14, MAPK11/12/14, and GAPDH (as a loading control) (n=3).