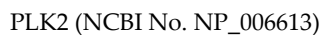


Figure S1: Peptide sequence of biotinylated lysine of PLK2 detected by mass spectrometry.



MELLRTITYQ	PAASTKMCEQ	ALGKGCAD	SKKRPPQPE	ESQPPQSQQ	VPPAAPHHH	60
HSHSGPEIS	RIIVDPTTG	RYCRGKVLG	GGFAKCYEM	DLTNNKVYA	KIIPHRSVAK	120
PHQREKIDKE	IELHRIHHK	HVVOFYHYFE	DKENIYILLE	YCSRRSMAHI	LKARKVLTEP	180
EVRYYLRLQIV	SGLKYLHEQE	ILHRDLKLGN	FFINAMELKL	VGDFGLAARL	EPLEHRRRTI	240
CGTPNLYSPE	VLNKGQGHGE	SDIHALGDM	FTYMLGRPF	ETNLKETYR	CIREARYTMP	300
LKLAPAKHL	IASMLSKNPE	DRPDLSDIIR	HDFFLQGFT	DLRSSCSCHT	VPDFHLSSPA	360
KNFFKAAAA	LFGGKKDKAR	YIDTHNRVSK	EDEDIYKLRL	DLKKTSTIQQ	PSKHRTDEEL	420
QPTTTTVARS	GTPAVENKQQ	IGDAIRMIVR	GTLGSCSSSS	ELEDSTMG	VADTVARVLR	480
GLENMPEAD	CIPKEQLSTS	FQWYTKWVDY	SNKYGFYQL	SDHTVGVLFN	NGAHMSLLPD	540
KYTVHHYASL	QQSCVFPA	APQFVLSQVT	VLKYFSHYME	ENLMDGGDLP	SVTDIRRPRL	600
YLLQWLKDK	ALMMLFNDGT	FQNVFYHDHT	KIICSQNEE	YLLTYINEDR	ISTFRLTTL	660
LMSGSSSELK	NRMEYFNML	LQRGN				

Figure S2: Induction of PLK2 and HSPB5 expression under ER stress by MG132 treatment. Undifferentiated L6 cells were treated with 5 μ M MG132. The induction of PLK2, HSPB5 and GAPDH protein expression was detected by western blotting.

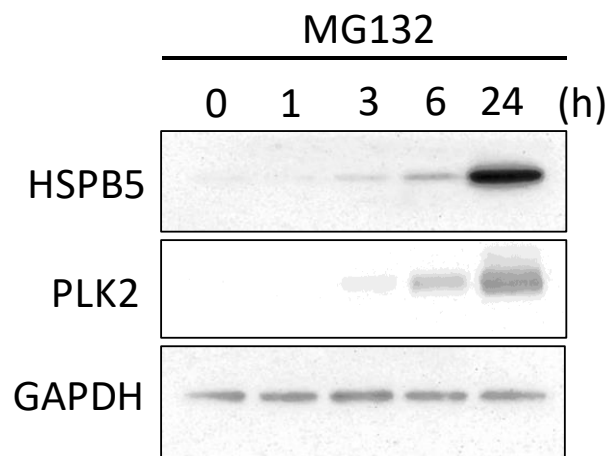


Figure S3: Induction of HSPB5 and PLK2 expression under ER stress by bortezomib treatment. Undifferentiated L6 cells were treated with 5 μ M MG132 or 1 μ M bortezomib (#A10160, Adooq Bioscience, CA, USA). The induction of HSPB5 and PLK2 protein expression was detected by western blotting. The graph shows the expression level of endogenous HSPB5 and PLK2. Data represent the mean \pm standard error ($n = 3$). Statistical analyses were performed using One-way ANOVA with Tukey test. $\ddagger p < 0.01$.

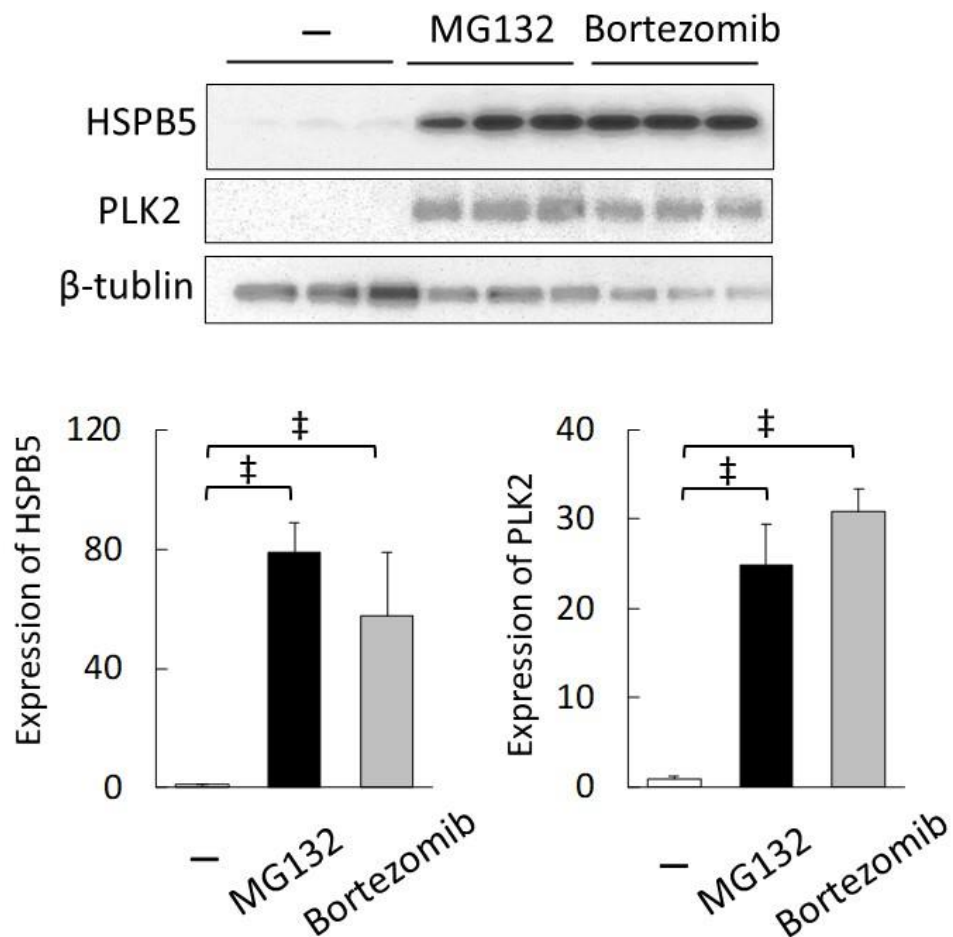


Figure S4: HSPB5 co-localizes with the ER marker, protein disulfide isomerase (PDI), by MG132 treatment. After 5 μ M MG132 treatment (24 h), FLAG-tagged HSPB5 and endogenous PDI in undifferentiated L6 cells were immunofluorescently stained with anti-tag (#1E6, Fujifilm Wako) and anti-PDI (#3501, Cell Signaling Technology) antibodies. Nuclei were fluorescently stained with DAPI solution. Yellow color in Merge image indicates co-localization of HSPB5(WT) and PDI.

MG132 treatment

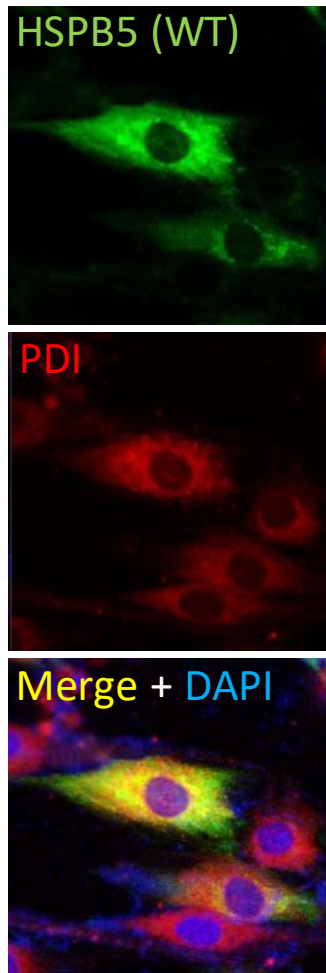


Figure S5. PLK2 is not involved in the activation of p38 and MK2.

After MG132 treatment (24 h), phosphorylation of MK2 (pThe334) and p38 (pThr180/pTyr182) was detected by western blotting. The graph shows the phosphorylation level of endogenous MK2 and p38. Data represent the mean \pm standard error ($n = 3$). Statistical analyses were performed using One-way ANOVA with t -test. n.s. means not significant.

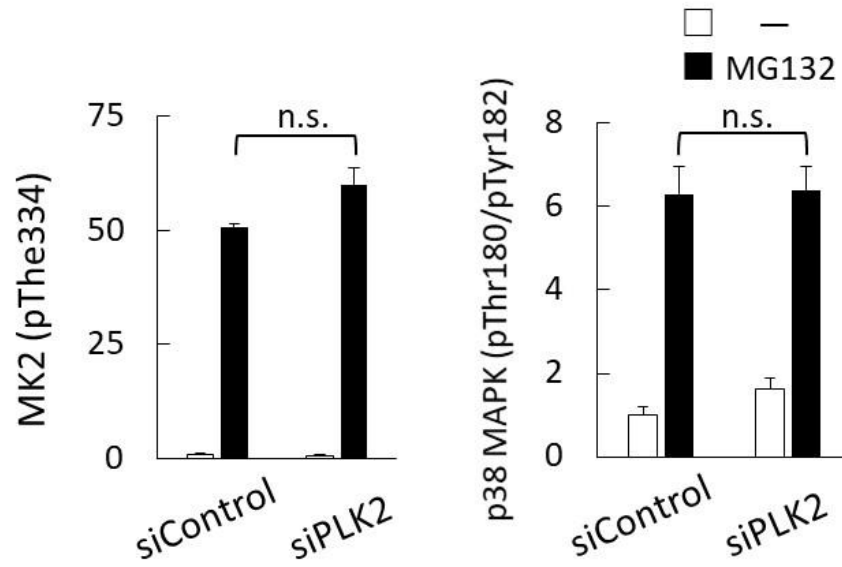


Figure S6: MK2 does not phosphorylate serine 19 of HSPB5.

After 5 μ M MG132 treatment (24 h) with various concentrations of MK2 inhibitors, phosphorylation of HSPB5 (pSer19) was detected by western blotting.

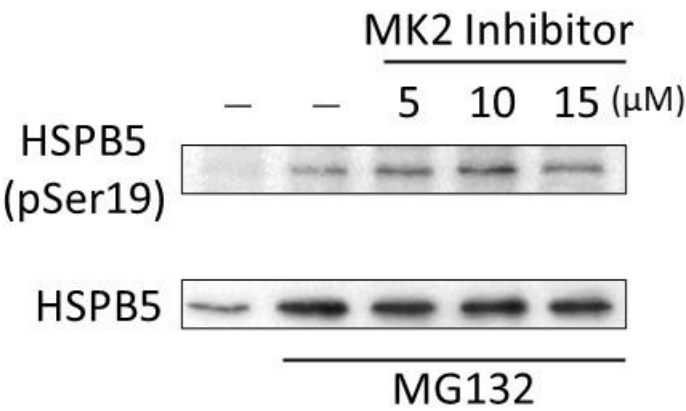


Figure S7: Comparison of the inhibitory effect of HSPB5 phosphorylation on ER stress-induced caspase-3 activity.

(a) Detection of cleaved caspase-3 positive cells in L6 cells expressing recombinant protein. Undifferentiated L6 cells were transfected with expression plasmids by lipofection. HSPB5 (3A) is a recombinant protein with triple point mutations in S19A, S45A, and S59A. Control cells were transfected with empty expression vectors. After 48 h of lipofection, undifferentiated L6 cells were treated with 5 μ M MG132 for 16 h. FLAG-tagged HSPB5 were immunofluorescently stained with anti-tag antibodies. Caspase-3 activity was detected using a fluorescently labeled antibody specific for cleaved caspase-3 Asp175 (#9661, Cell Signaling Technology). Fluorescent stained images of cells were observed using a confocal laser scanning microscope (LSM700, Zeiss Japan). Arrows in the column of cleaved caspase-3 indicate cells expressing recombinant HSPB5. White-framed arrows indicate cleaved caspase-3 positive cells. Blue arrows indicate non-positive cells. Scale bar indicates 20 μ m. **(b)** The graph shows the percentage of cleaved caspase-3 positive cells divided by the total number of cells (control) or the percentage of cleaved caspase-3 positive cells expressing HSPB5 divided by the total number of HSPB5 expressing cells (HSPB5). The mean of three independent experiments is shown. Statistical analyses were performed using One-way ANOVA with Tukey test. $\ddagger p < 0.01$; n.s. means not significant.

