

Supplemental material

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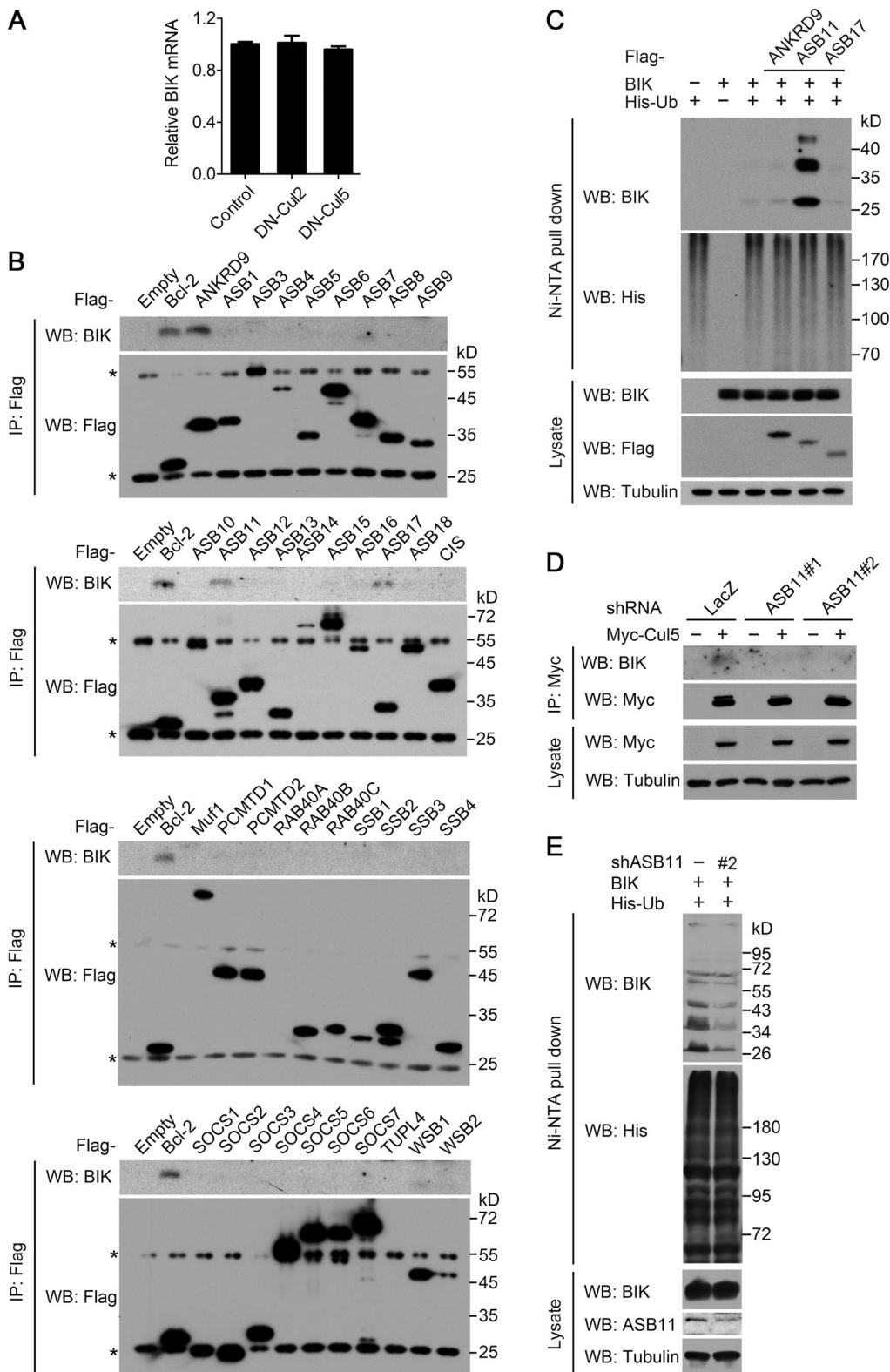


Figure S1. ASB11 targets BIK to the Cul5 complex for ubiquitination. **(A)** RT-qPCR analysis of *BIK* mRNA expression in 293T cells transiently transfected with the indicated Cullin DN mutants. **(B)** The indicated substrate adaptors of Cul5 ubiquitin ligase were expressed in 293T cells, and their interaction with endogenous BIK was assayed by immunoprecipitation (IP). Bcl-2 was used as a positive control. The positions of immunoglobulin heavy and light chains are denoted with asterisks. **(C)** Analysis of BIK ubiquitination in 293T cells transfected with the indicated constructs. The ubiquitinated proteins were pulled down under denaturing conditions by Ni-NTA agarose and analyzed by Western blot (WB). **(D)** Immunoprecipitation analysis of BIK interaction with Cul5 in 293T derivatives as in Fig. 2 D. **(E)** Analysis of BIK ubiquitination in H1299 stable line carrying control or ASB11 shRNA and transfected with the indicated constructs. The ubiquitinated proteins were pulled down under denaturing conditions by Ni-NTA agarose and analyzed by Western blot.

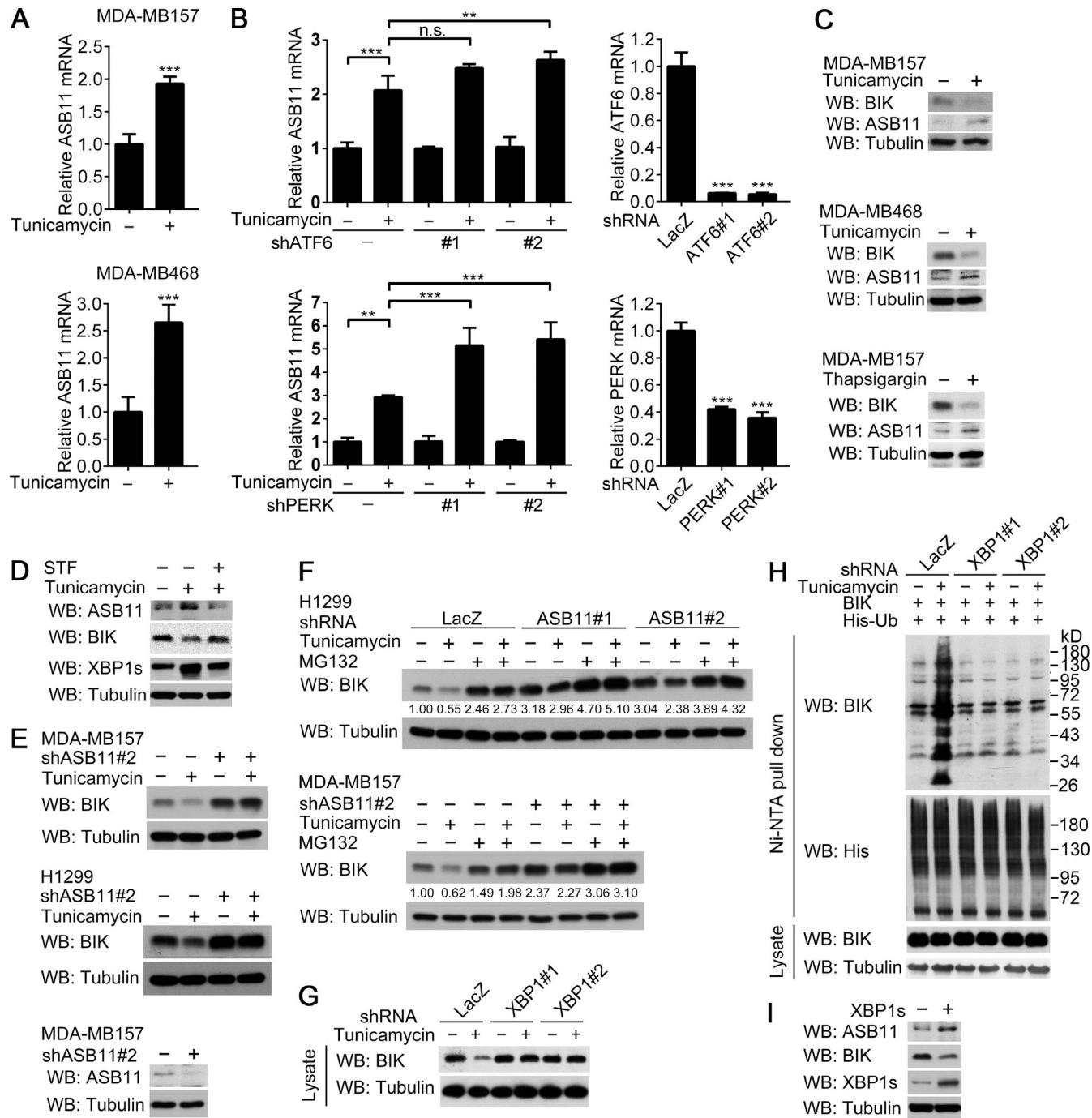


Figure S2. ASB11 and XBP1 mediate ER stress–induced BIK degradation. **(A)** RT-qPCR analysis of ASB11 mRNA in the indicated cells treated with 10 µg/ml tunicamycin for 8 h. Data are mean ± SD; ***P < 0.001 by t test; n = 3. **(B)** RT-qPCR analysis of ASB11 mRNA in 293T cells stably expressing the indicated shRNAs and treated with 10 µg/ml tunicamycin for 16 h. The knockdown efficiencies of ATF6 and PERK shRNAs are shown on the right. Data are mean ± SD; **P < 0.01, ***P < 0.001 by one-way ANOVA with Tukey's post hoc test (left panel) or t test (right panels); n = 3. **(C)** Western blot (WB) analysis of ASB11 and BIK expression levels in the indicated cells treated with 10 µg/ml tunicamycin or 500 nM thapsigargin for 8 h. **(D)** Western blot analysis of ASB11 and BIK expression in 293T cells pretreated with IRE1α inhibitor and then treated with 10 µg/ml tunicamycin for 16 h. **(E)** Western blot analysis of BIK and ASB11 expression levels in the indicated stable cell lines treated with 10 µg/ml tunicamycin for 8 h. The knockdown efficiencies of ASB11 shRNAs are shown in the bottom panel and in Fig. 2 D. **(F)** Western blot analysis of BIK expression in the indicated stable cell lines cotreated with tunicamycin and MG132 for 8 h. The relative amounts of BIK are indicated by assigning the value of the first lane as 1. **(G)** Western blot analysis of BIK expression in 293T derivatives as in Fig. 3 C treated with or without tunicamycin for 16 h. **(H)** Immunoprecipitation analysis of BIK ubiquitination in 293T derivatives as in Fig. 3 C transfected with BIK and His-ubiquitin and treated with tunicamycin for 16 h. The ubiquitinated proteins were pulled down under denaturing conditions by Ni-NTA agarose and analyzed by Western blot. **(I)** Western blot analysis of ASB11 and BIK expression in 293T cells transfected with XBP1s.

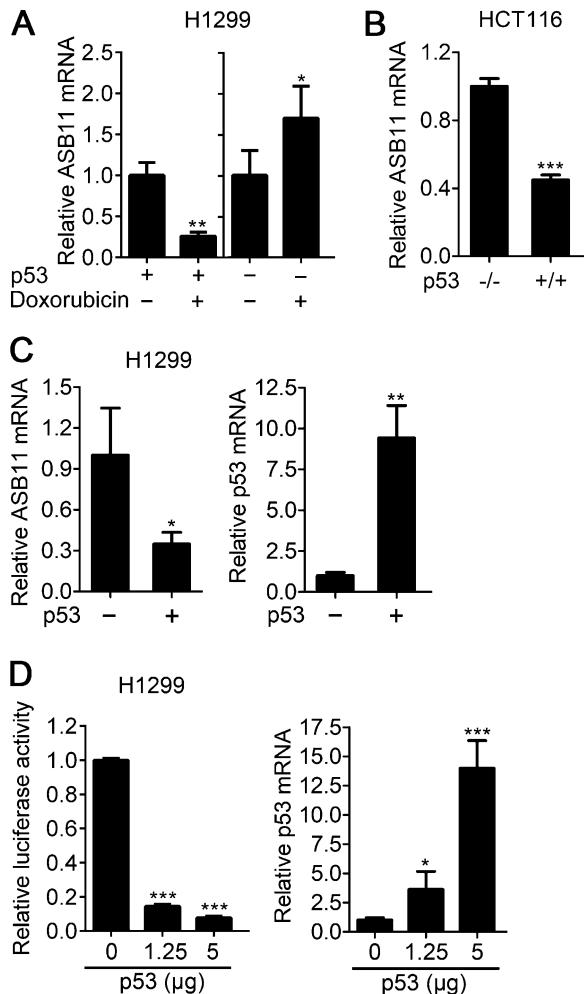


Figure S3. DNA damage acts through p53 to down-regulate ASB11. **(A)** RT-qPCR analysis of ASB11 mRNA expression in H1299 cells or p53-transfected H1299 cells treated with 3 μg/ml doxorubicin for 24 h. **(B and C)** RT-qPCR analysis of ASB11 mRNA expression in HCT116 p53^{-/-} and p53^{+/+} cells (B) or in H1299 cells transfected with control vector or p53 (C). **(D)** Luciferase assay for ASB11 promoter activity in H1299 cells transfected with the indicated amounts of p53 and the 2-kb reporter construct (see Fig. 3 E). The expression of p53 is shown on the right for C and D. Data in all panels are mean ± SD; *P < 0.05, **P < 0.01, ***P < 0.001 by t test; n = 3.

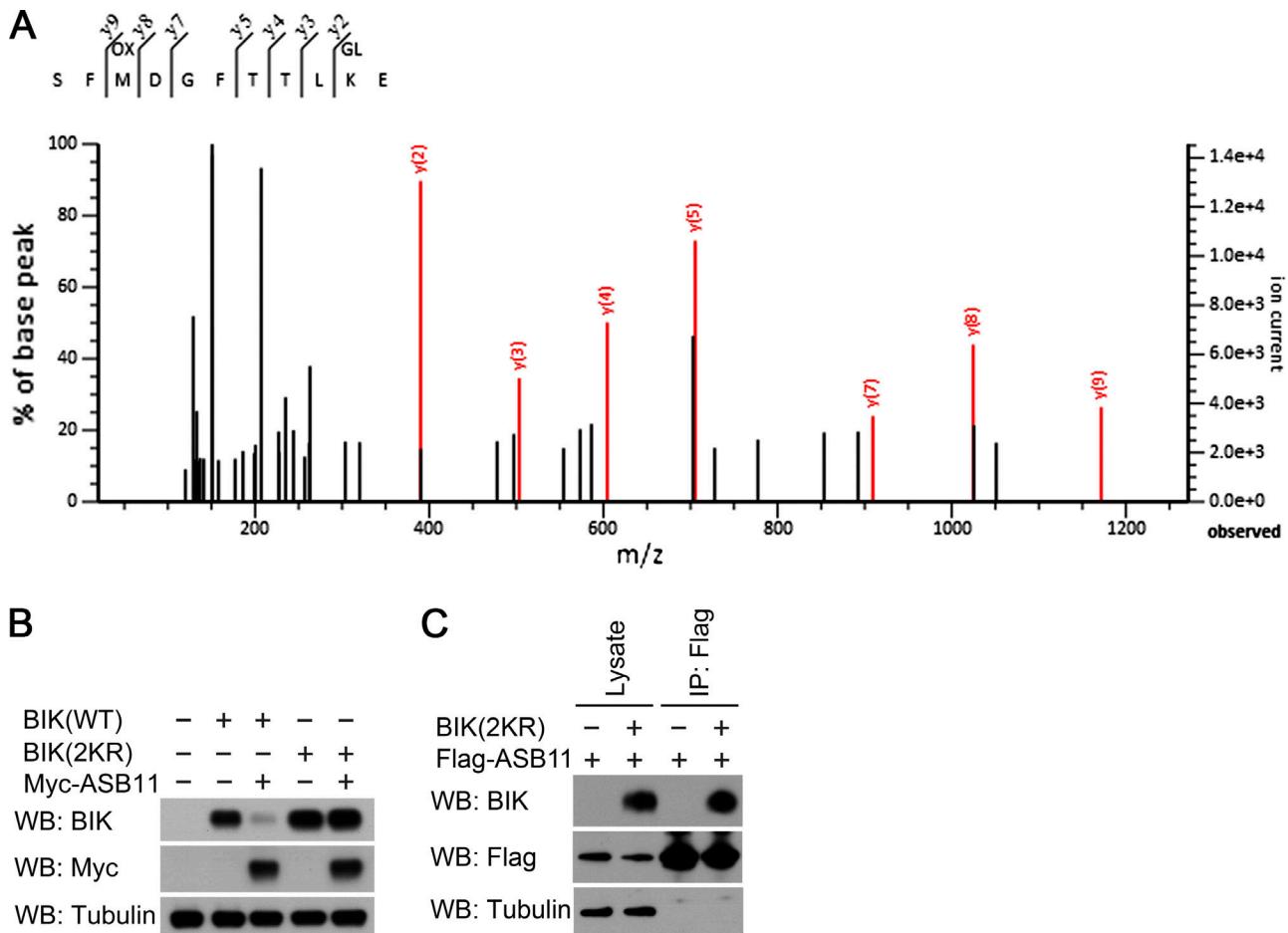


Figure S4. BIK(2KR) mutant is resistant to ASB11-mediated ubiquitination and degradation. **(A)** Tandem mass spectrometry of a peptide (corresponding to amino acids 106–116) derived from ubiquitinated BIK showing ubiquitin conjugation at the K115 residue. Amino acid residues marked by OX and GL denote the existence of oxidation and GlyGly modifications, respectively. **(B)** Western blot (WB) analysis of BIK levels in 293T cells transfected with the indicated constructs. **(C)** Immunoprecipitation (IP) analysis of BIK(2KR) binding to Flag-ASB11 in 293T cells transfected with the indicated constructs.

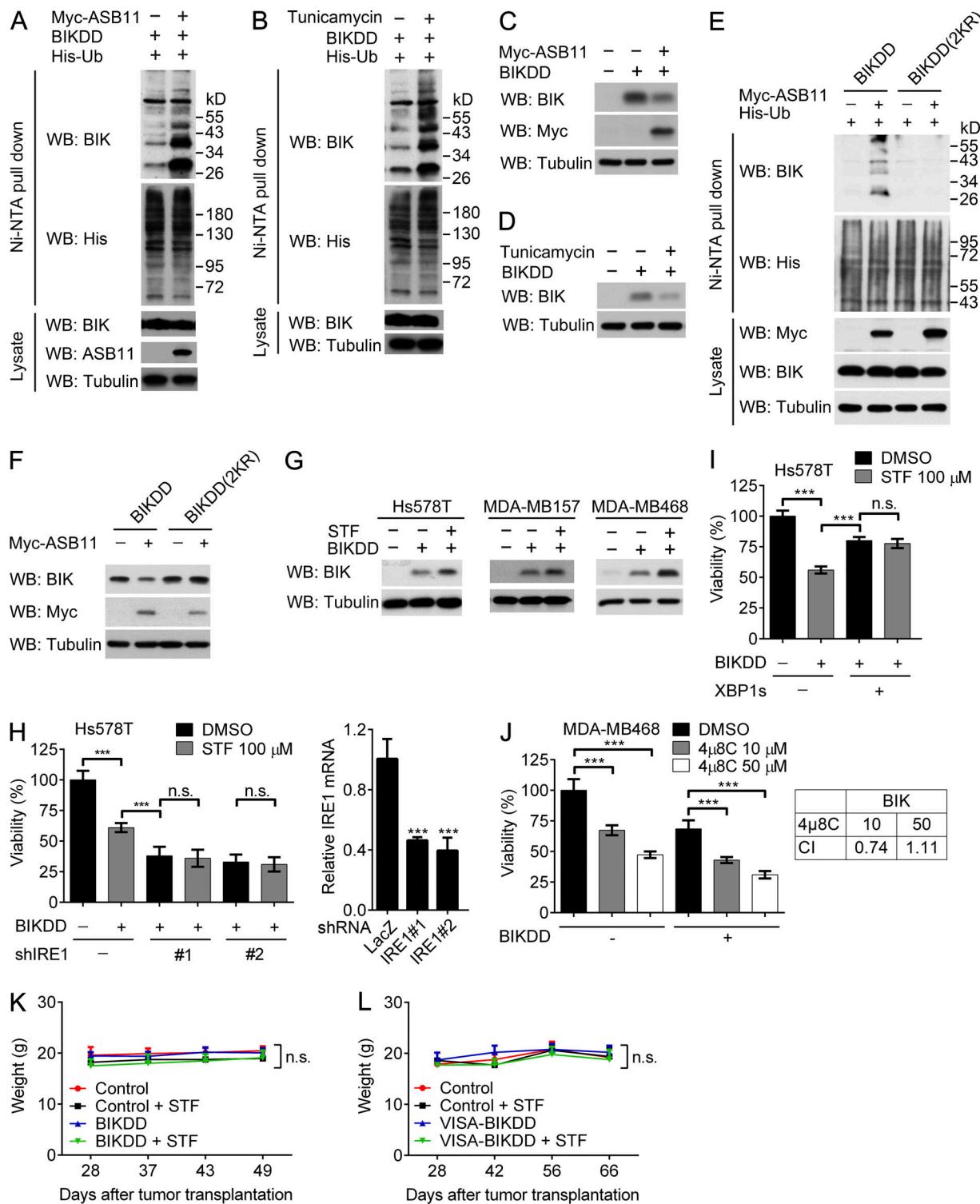


Figure S5. BIKDD is a target of ASB11, and its stability and tumor-killing effect are enhanced by IRE1 α inhibitor. **(A, B, and E)** Analysis of BIKDD or BIKDD(2KR) ubiquitination in 293T cells transfected with the indicated constructs and/or treated with tunicamycin for 16 h. **(C and D)** Western blot (WB) analysis of BIKDD levels in 293T cells transfected with the indicated constructs and/or treated with tunicamycin for 16 h. **(F)** Western blot analysis of BIKDD and BIKDD(2KR) levels in 293T cells transfected with or without ASB11. **(G)** Western blot analysis of BIKDD levels in the indicated TNBC cells transfected with BIKDD and treated with 100 μ M STF-083010 for 12 h. **(H)** MTT assay for the viability of Hs578T cells stably expressing IRE1 α shRNAs, transfected with BIKDD and treated with 100 μ M STF-083010 for 48 h. The knockdown efficiencies are shown on the right. **(I)** MTT assay for the viability of Hs578T cells transiently transfected with XBP1s and BIKDD and treated with 100 μ M STF-083010 for 48 h. **(J)** MTT assay for the viability of MDA-MB468 cells transfected with BIKDD and treated with the indicated concentrations of 4 μ 8C for 48 h. The CI values are indicated. **(K and L)** The body weight of mice treated with BIKDD and/or STF-083010 as shown in Fig. 8, D and E, respectively, was measured at the indicated time points and plotted. Data in H–J are mean \pm SD; ***P < 0.001 by one-way ANOVA with Tukey's post hoc test; n = 3. Data in K and L are mean \pm SD; n = 5. n.s., not significant by t test.

Provided online are three tables in Excel. Table S1 contains information for antibodies used in this study; Table S2 contains primers for PCR, qPCR, and cloning; and Table S3 contains targeting sequences of shRNAs.