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Supplemental information

Overexpression of BIRC6 driven by EGF-JNK-HECTD1 signaling is a potential therapeutic target for triple-negative breast cancer

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Supplemental Data list:

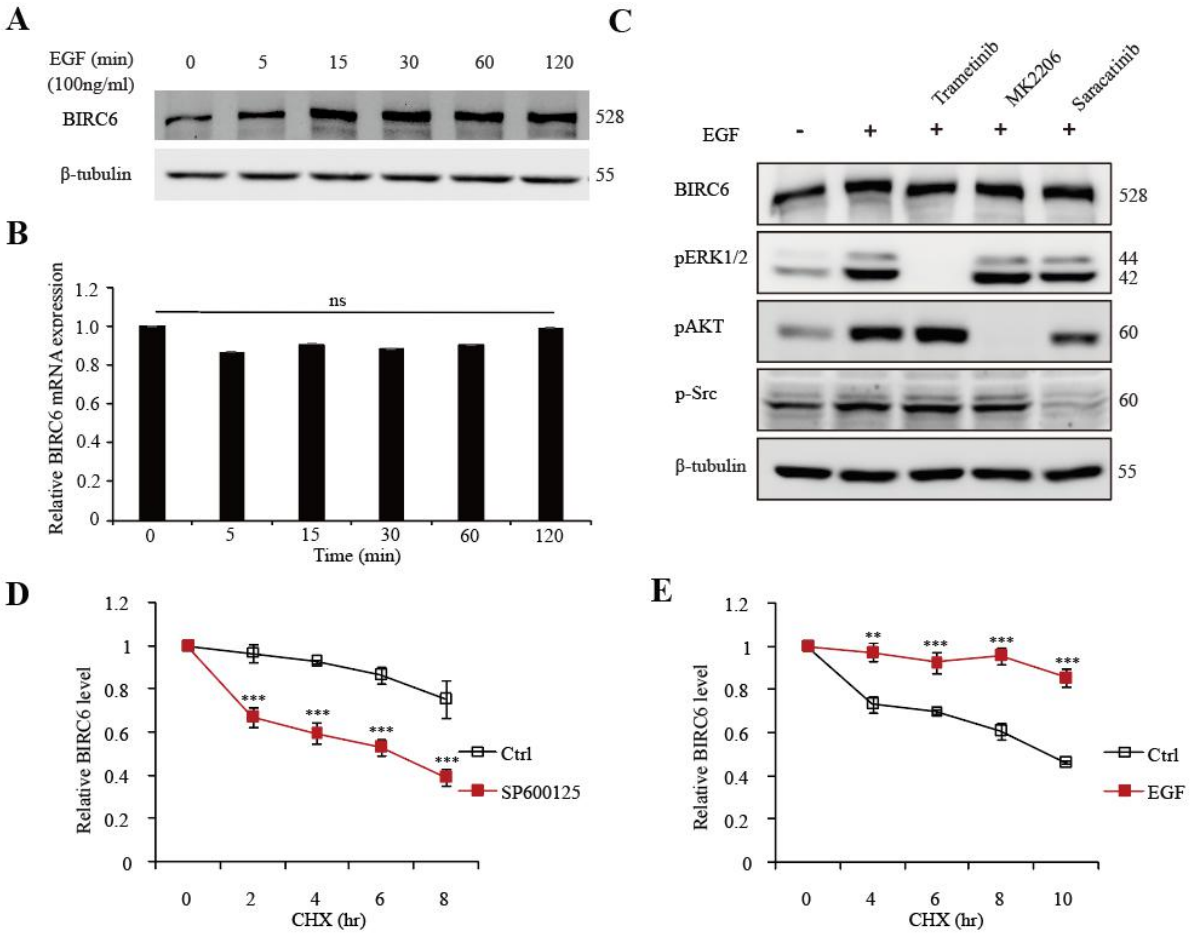

Fig. S1 (related to Fig. 2). The EGF-JNK Signaling Regulates BIRC6 Stability. (**A**) After serum starved for 48 hr, Hs578T cells were treated with EGF for the indicated times. Immunoblot analysis was performed to measure the protein levels of BIRC6. (**B**) After serum starvation, Hs578T cells were treated with EGF (100 ng/ml) for the indicated times. qRT-PCR analysis was performed to measure the mRNA levels of BIRC6. Data are means ± SD. (**C**) After serum starvation, Hs578T cells were treated with inhibitors to MEK (trametinib), AKT (MK2206), Src (saracatinib) for 1.5 hr and then treated with EGF (100 ng/ml) for 30 min. Equal amounts of cell lysates were immunoblotted with the indicated antibodies. (**D-E**) Quantification of BIRC6 protein levels in Figure 2E (D) and Figure 2F (E) from three independent experiments.

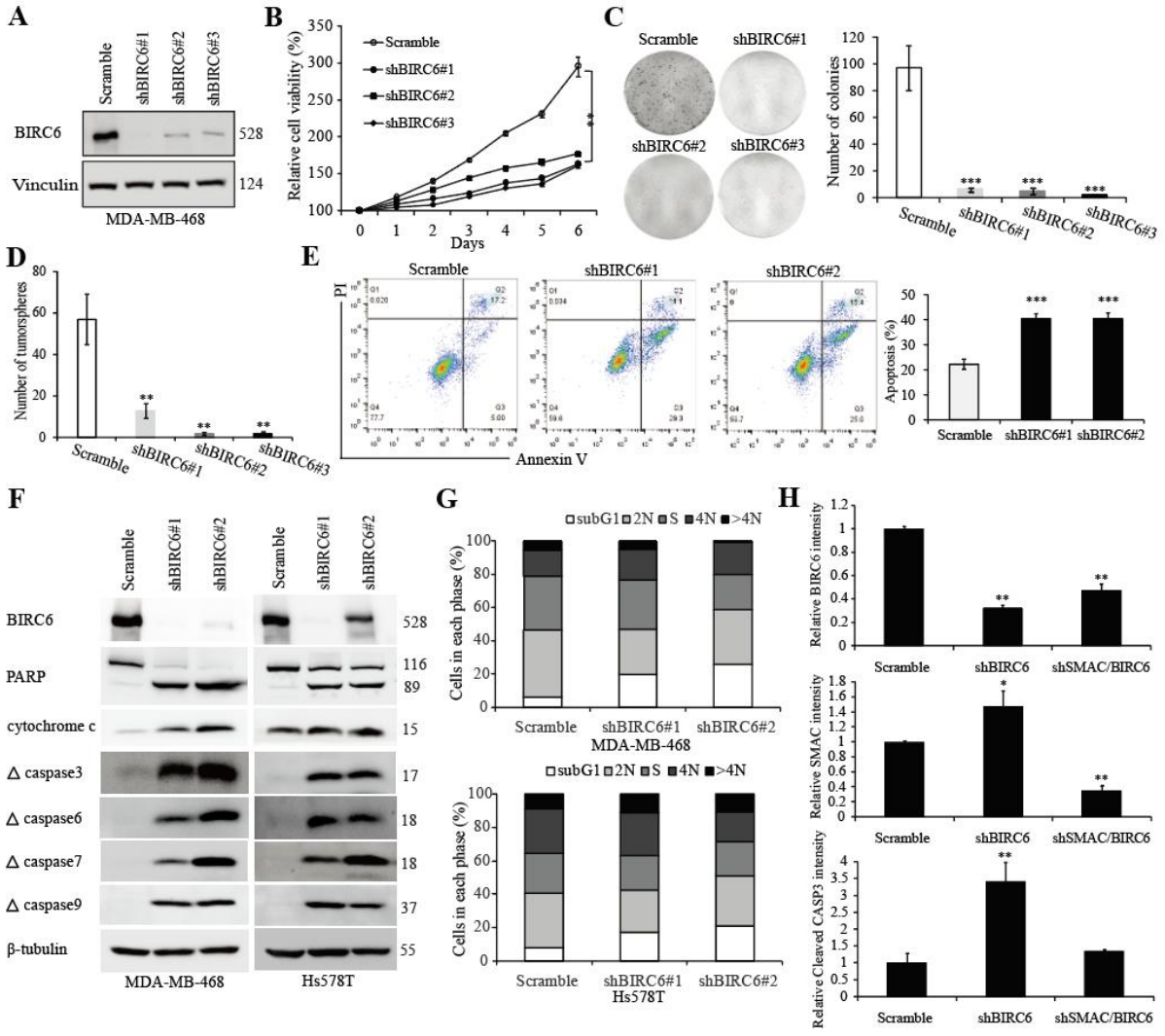

Fig. S2 (related to Fig. 4). BIRC6 Promotes TNBC Cell Growth and Tumorigenesis by Blocking SMAC-Mediated Apoptosis. Immunoblotting analysis (**A**), MTT assay showing growth (**B**), colony formation assay (**C**) and tumorsphere assay (**D**) were performed in MDA-MB-468 cells transduced with scramble or BIRC6 shRNA. (**E**) Representative plots of cell apoptosis analysis (left) and data quantification of the apoptotic cells (right) in MDA-MB-468 cells with or without BIRC6 knockdown. (**F**) Immunoblot analysis of the indicated proteins in MDA-MB-468 and Hs578T cells transduced with scramble or two independent BIRC6 shRNAs. (G) Cell cycle distribution in MDA-MB-468 (upper) and Hs578T (lower) cells was assessed by PI staining followed by flow cytometry analysis. (**H**) The immunoreactive areas in the IHC images were quantified using ImagePro Plus 6.0 software. Data are means ± SD. $^{**}P<0.01$, $^{***}P<0.001$.

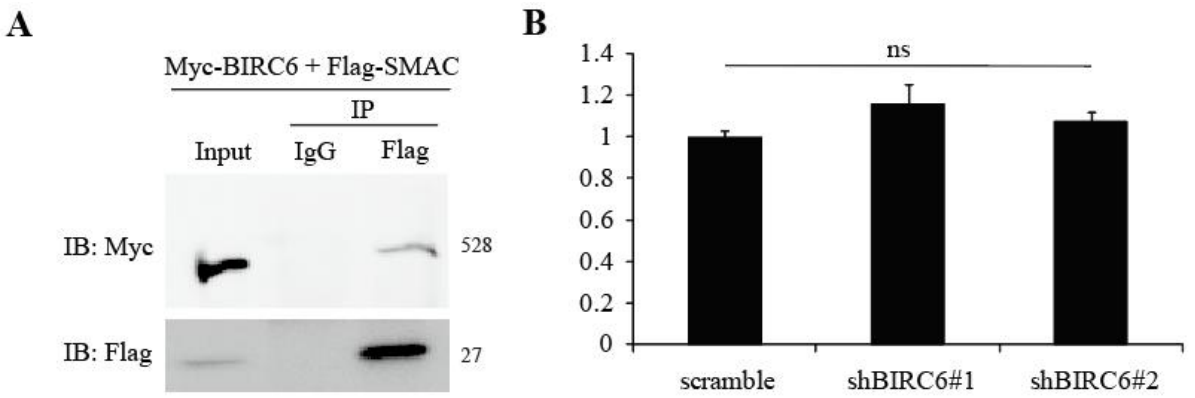

Fig. S3 (related to Fig. 5). BIRC6 Decreases SMAC Expression by Regulating Ubiquitination in TNBC. (**A**) After transiently transfected with myc-tagged BIRC6, Flag-tagged SMAC, 293T cells were immunoprecipitated with anti-Flag antibody then subjected to western blot analysis. (**B**) qRT-PCR analysis was performed to measure the mRNA levels of SMAC in Hs578T cells transduced with scramble or BIRC6 shRNA. Data are means ± SD.

A

Materials	Size (nm)	PI (-)	Zeta potental (mv)
pCLNs	100.2 ± 11.2	0.26 ± 0.01	40.6 ± 4.41
pCLNs/siRNA	155.3 ± 1.6	0.24 ± 0.01	18.9 ± 1.31

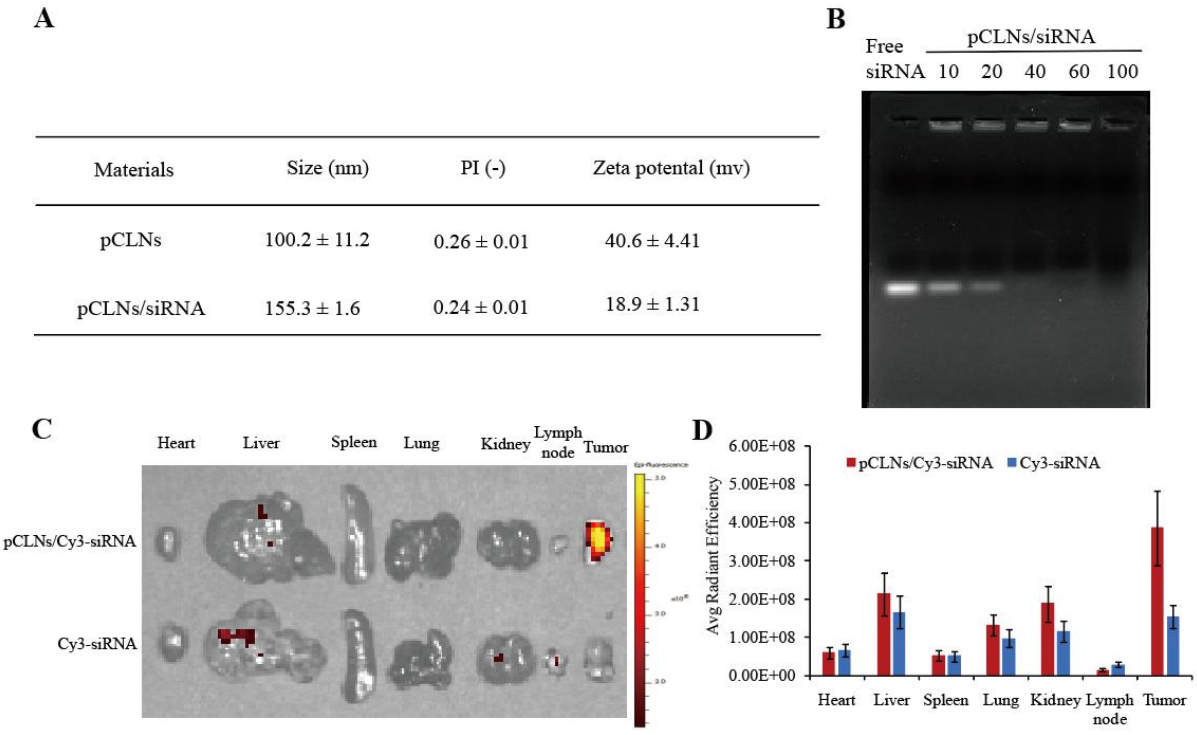

Fig. S4 (related to Fig. 6). pCLNs Can Efficiently Deliver the BIRC6-siRNA into TNBC Cells *in vitro* and *in vivo*. (**A**) Sizes and zeta potentials of pCLNs and pCLNs/siRNA. Data are means ± SD (n = 3). PI: polydispersity index. (**B**) Gel retardation assay was performed to test the binding ability of pCLNs to siRNA at different mass ratios. (C) Ex vivo images of major organs at 36 h post i.v.

injection with pCLNs/Cy3-siRNA or Cy3-siRNA. (D) The average fluorescence intensity of major organs after mice injected with pCLNs/Cy3-siRNA or Cy3-siRNA for 36h (n = 3).

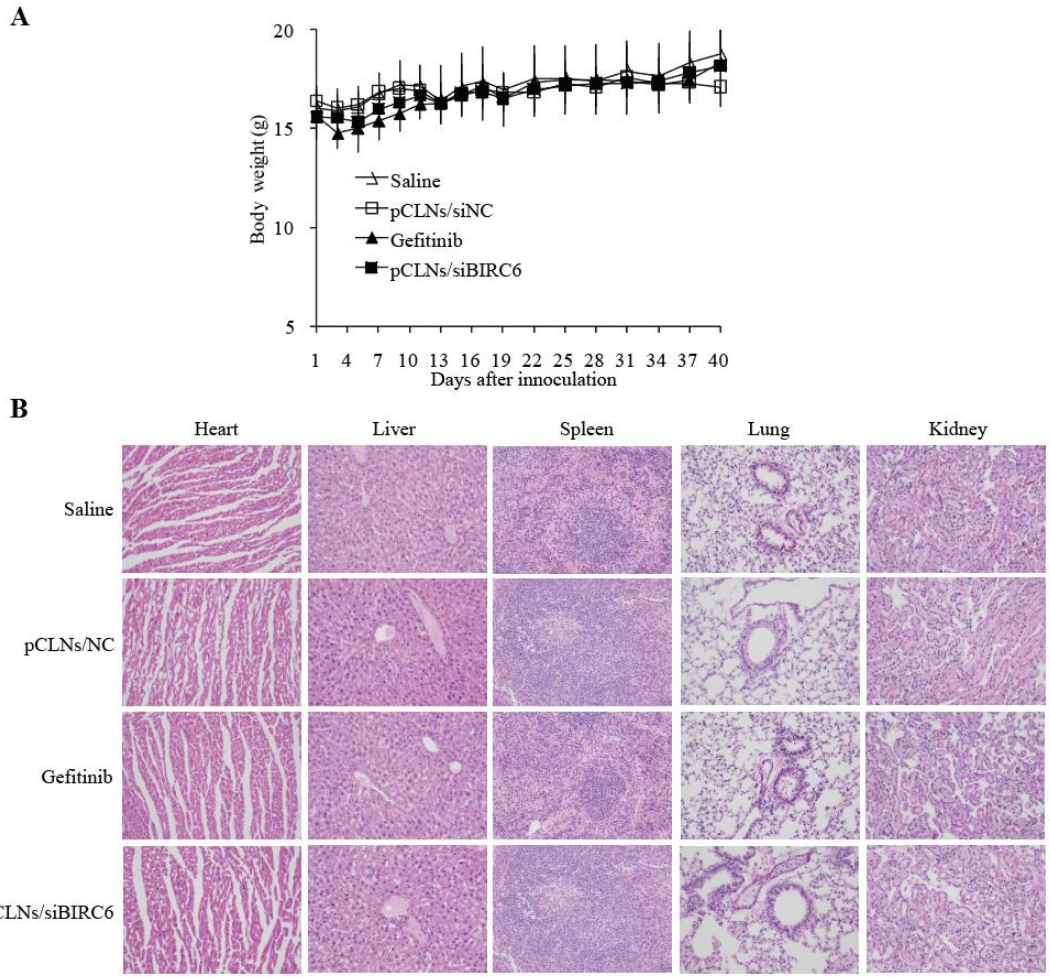

Fig. S5 (related to Fig. 7). pCLNs/siBIRC6 Complex is safe for TNBC therapy. (**A**) Body weight in MDA-MB-468 xenograft-bearing mice receiving various treatments. (**B**) Representative images of H&E staining of major organs in MDA-MB-468 xenograft-bearing mice receiving various treatments.

Table S1 The oligonucleotide sequences for shRNA interference.

Gene	Sequence	Index
BIRC6 #1	GCTGTAGTGAATGGTGCAAAT	TRCN0000004159
BIRC6 #2	GCGTTCTTTCTACAGTGCATT	TRCN0000004158
BIRC6 #3	AAACTACCTTAGGAAAGAATG	http://jura.wi.mit.edu/bioc/siRNAext/
HECTD1 #1	TCTCTCTTTGTTTGGCATATA	TRCN0000004087
HECTD1 #2	GCCACGAACAACATGAATCTA	TRCN0000004084

SMAC #1	CCGACAATATACAAGTTTACT	TRCN0000004513
SMAC #2	GCGTTGATTGAAGCTATTACT	TRCN0000004512
CASP9	CAGCTTCCAGATTGACGACAA	TRCN0000318563
HtrA2	GATCACATCCGGCATTGTTAG	TRCN0000344116
TP53	CGGCGCACAGAGGAAGAGAAT	TRCN0000003753
EGFR #1	GCTGCTCTGAAATCTCCTTTA	TRCN0000121067
EGFR #2	GAGAATGTGGAATACCTAAGG	TRCN000010329
Scramble	CCTAAGGTTAAGTCGCCCTCG	Addgene #1864