

Expanded View Figures

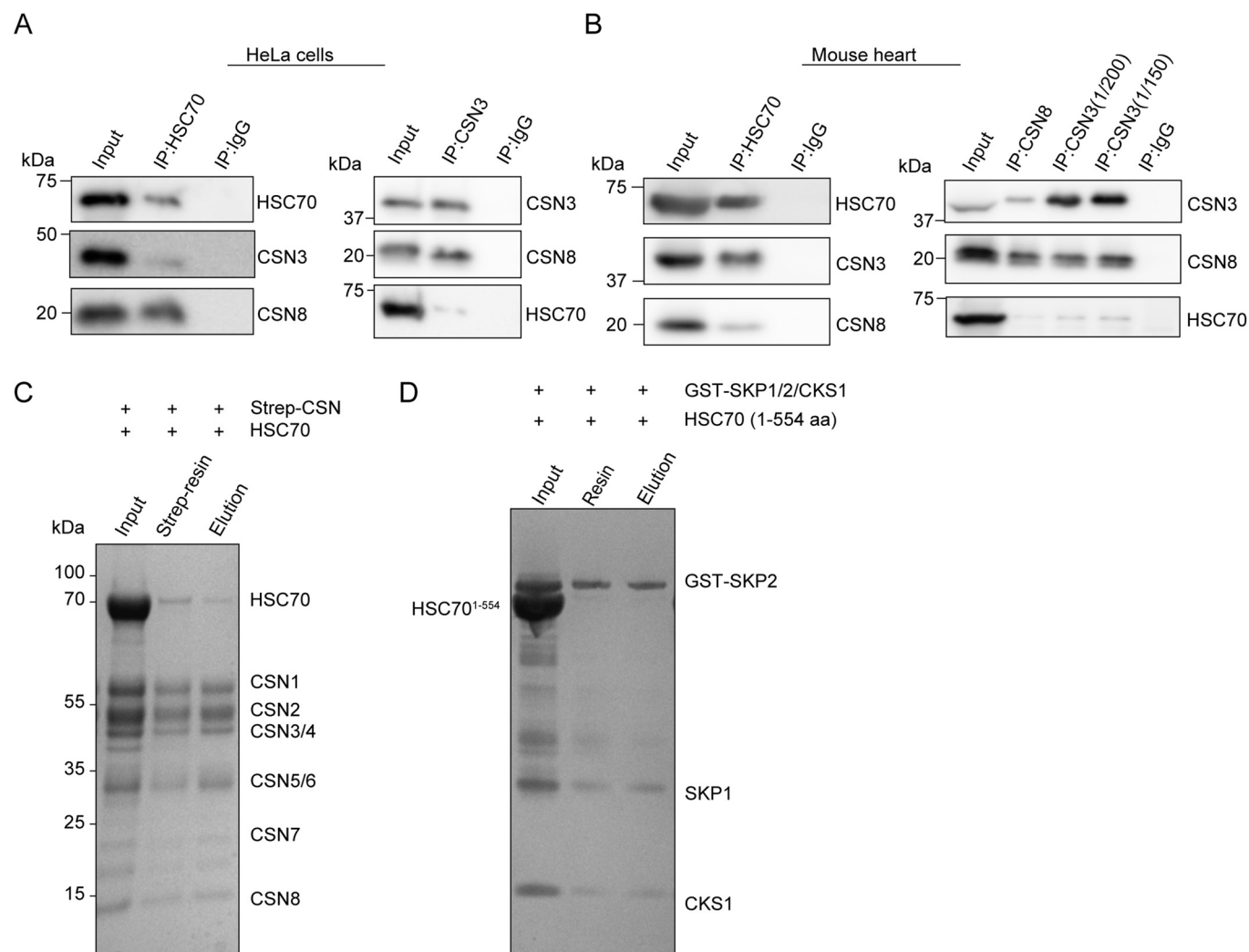


Figure EV1. HSC70 interacts with CSN in vivo and in vitro, but does not interact with SKP1/2/CKS mini complex.

(A) Co-immunoprecipitation in HeLa cells using HSC70 and CSN3 antibody, with rabbit-IgG serving as a negative control. (B) Co-immunoprecipitation in mouse heart tissue using HSC70, CSN8, and CSN3 antibody (1/150 or 1/200 dilution), with rabbit-IgG serving as a negative control. (C) In vitro pulldown assay. A mixture containing strep-tagged CSN and recombinant HSC70 was subjected to immunoprecipitation with Streptactin Sepharose. The result was visualized by Coomassie brilliant blue staining. (D) In vitro pulldown assay. A mixture containing GST-tagged SKP1/2/CKS1 and HSC70^{1-554aa} was subjected to immunoprecipitation with Glutathione Sepharose 4 Fast Flow. The results were visualized by Coomassie brilliant blue staining.

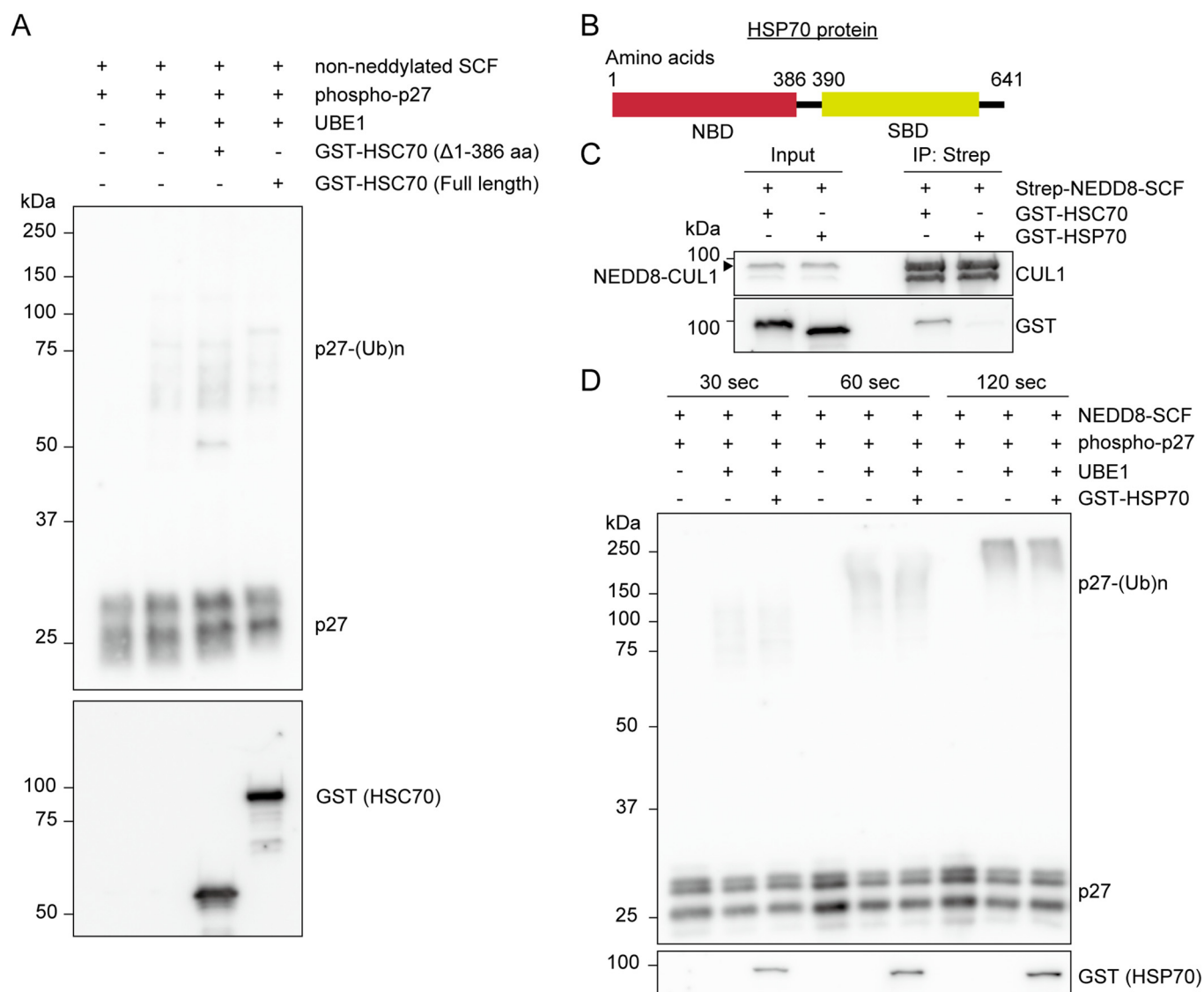
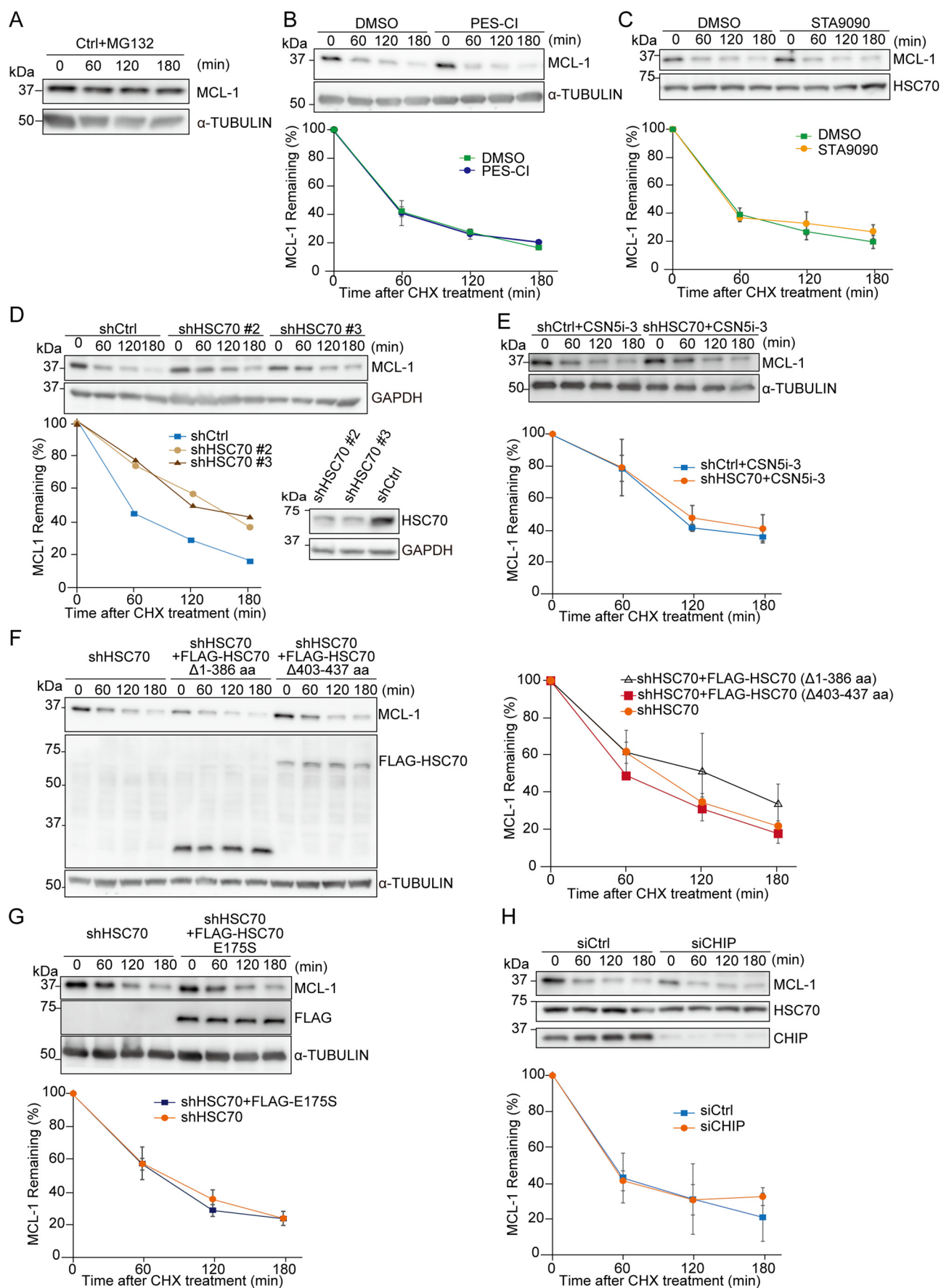


Figure EV2. HSC70 does not enhance the ubiquitination activity of non-neddylated SCF, and HSP70 does not enhance NEDD8-SCF ubiquitination activity.

(A) Non-neddylated SCF mediated in vitro ubiquitination of p-p27 assayed in the absence or presence of indicated recombinant proteins. Unmodified and polyubiquitinated p-p27 were detected by immunoblotting with anti-p27 antibody. (B) Schematic diagram of HSP70 protein structure. (C) In vitro pulldown assay. A mixture containing GST-tagged HSC70 or HSP70, and Strep-tagged NEDD8-SCF were subjected to immunoprecipitation with Streptactin Sepharose. (D) NEDD8-SCF mediated in vitro ubiquitination of p-p27 assayed in the absence or presence of GST-HSP70.



◀ **Figure EV3. Cycloheximide (CHX) chase assay in various conditions.**

(A) CHX chase assay of endogenous MCL-1 in the presence of proteasome inhibitor MG132. (B) CHX chase assay of endogenous MCL1 with pharmacological inhibition with PES-Cl. The graphs show the mean $\pm$ s.e.m. ($n = 3$, respectively; Welch's t test). No significant difference was observed at each time point. (C) CHX chase assay of endogenous MCL1 with pharmacological inhibition with STA9090. The graphs show the mean $\pm$ s.e.m. ($n = 3$, respectively; Welch's t test). No significant difference was observed at each time point. (D) CHX chase assay of endogenous MCL-1 in other *HSC70* knockdown cell lines (shHSC70 clones #2 and 3). The lower right panel shows the extent of *HSC70* knockdown in these cell lines by immunoblotting. (E) CHX chase assay of endogenous MCL1 in *HSC70* knockdown cells with pharmacological inhibition with CSN5i-3. The graphs show the mean $\pm$ s.e.m. ($n = 3$, respectively; Welch's t test). No significant difference was observed at each time point. (F) CHX chase assay of endogenous MCL-1 with or without transient FLAG-HSC70 mutant expression. The graphs show the mean $\pm$ s.e.m. ($\Delta 1-386$ aa or $\Delta 403-437$ aa; $n = 3$, respectively; Welch's t test). No significant difference was observed at each time point. (G) CHX chase assay of endogenous MCL-1 with or without transient FLAG-HSC70 E175S mutant expression. The graphs show the mean $\pm$ s.e.m. ($n = 3$, respectively; Welch's t test). No significant difference was observed at each time point. (H) CHX chase assay of endogenous MCL1 with or without *CHIP* knockdown. The graphs show the mean $\pm$ s.e.m. ($n = 3$, respectively; Welch's t test). No significant difference was observed at each time point. (B–H) Each protein level was densitometrically quantified (normalized to 0 min) and shown in the graph below.

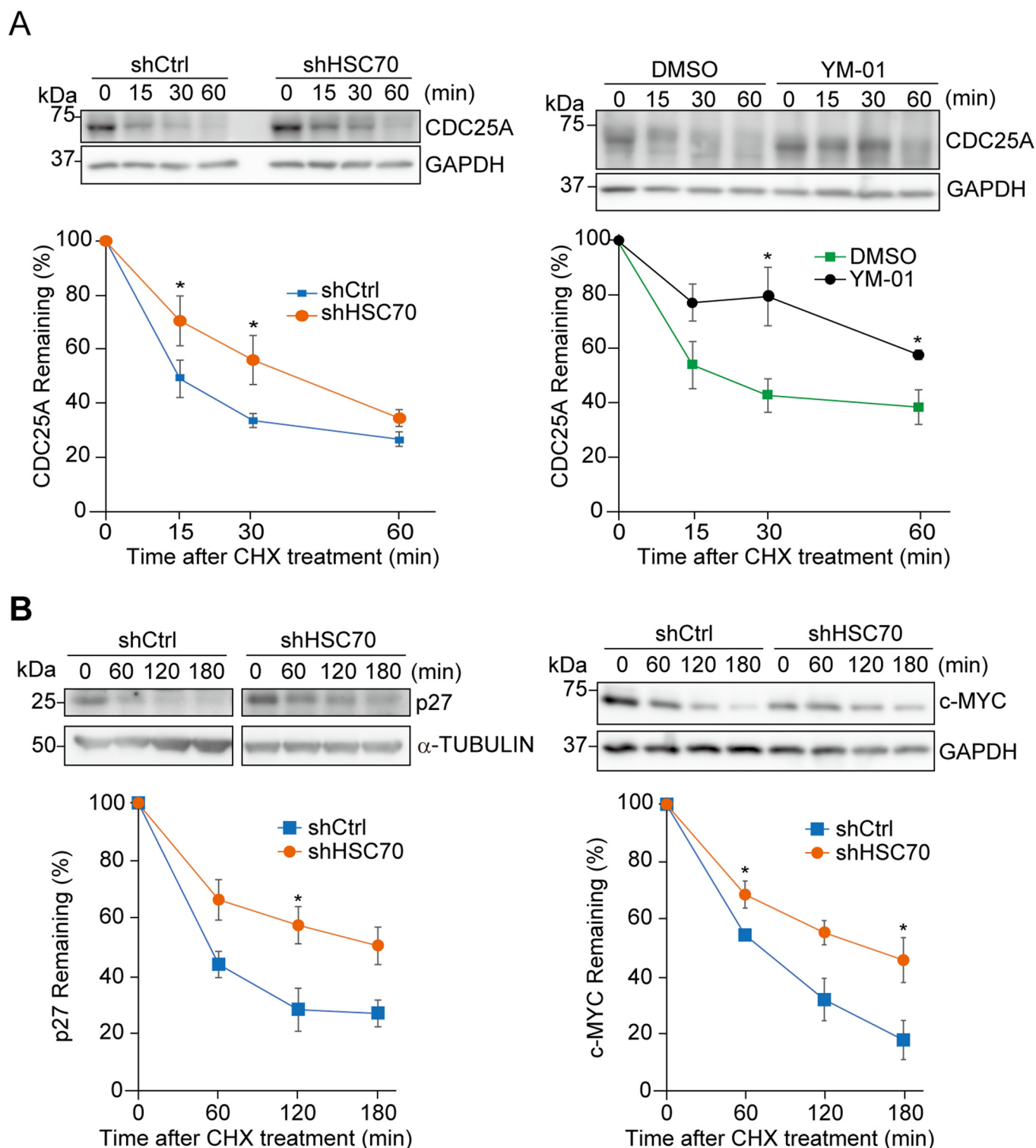


Figure EV4. Pharmacological or genetic inhibition of HSC70 prolonged the half-life of other SCF substrates.

(A) CHX chase assay of endogenous CDC25A with or without *HSC70* knockdown, or pharmacological inhibition with YM-01. The bottom graphs show the mean $\pm$ s.e.m. * $P < 0.05$ ($n = 3$ independent experiments; Welch's t test). Compared with shCtrl cells or DMSO treatment, a significant difference was observed at 15 min ($P = 0.025$) and 30 min ($P = 0.045$) in shHSC70 cells and at 30 min ($P = 0.042$) and 60 min ($P = 0.011$) in YM-01 treated cells. (B) CHX chase assay of endogenous p27 or c-MYC with or without *HSC70* knockdown. The bottom graphs show the mean $\pm$ s.e.m. * $P < 0.05$ ($n = 3$ independent experiments, respectively; Welch's t test). For p27 protein, a significant difference was observed at 120 min ($P = 0.032$). For c-MYC protein, significant difference was observed at 60 min ($P = 0.033$) and 180 min ($P = 0.045$). (A, B) Each protein level was densitometrically quantified (normalized to 0 min) and shown in the graph below.

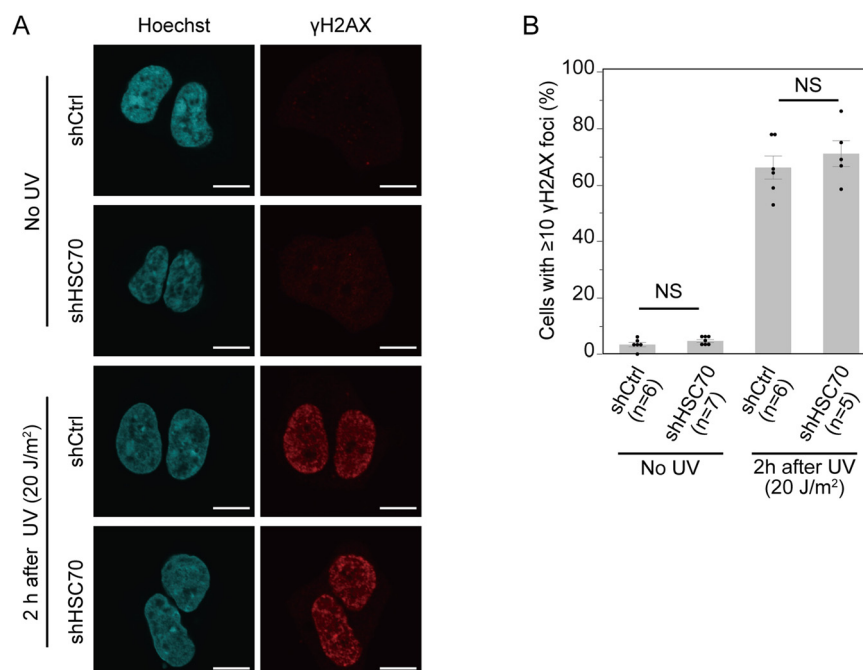


Figure EV5. Ultraviolet-induced DNA damage in HSC70 knockdown cells.

(A) Immunostaining of control or HSC70 knockdown HEK293T cells with or without low-dose UV irradiation (20 J/m²) using γH2AX antibody and Hoechst 33342. Scale bars, 10 μm. (B) Quantification of cells with γH2AX positive foci with or without UV irradiation ($n = 6$ independent experiments for shCtrl cells with or without UV, respectively; $n = 7$ or 5 independent experiments for shHSC70 cells with or without UV, respectively; Welch's t test). The graphs show the mean $\pm$ s.e.m. NS not significant.