SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure S1. ICI-mediated receptor turnover in the presence of CHX. C4-12 ERα stable cells pretreated with CHX for 30 min prior to ICI (100nM) treatment for the indicated times. Experiments were performed in duplicate and repeated twice using two ERα and three ERα-AA clones. GAPDH was used as SDS-PAGE loading control. The band density of exposed film was evaluated with ImageJ software. Relative ERα levels are shown in the corresponding graph as the mean ± SE. ** $p<0.01$.

Supplemental Figure S2. Basal and ligand-induced ubiquitination of ERαK302R/K303R. HeLa cells were transfected with 250ng ERα-RR, along with 1μg HA-ubiquitin using LipofectAMINE/PLUS. Transfected cells were pretreated with vehicle (DMSO) or MG132 (25μM) for 1 h, followed by DMSO, E2 (10nM) or ICI (100nM) for 4 h. ERα was then immunoprecipitated with anti-ERα antibody. Precipitated proteins were resolved by SDS-PAGE and Western blot performed with an HA antibody. Levels of immunoprecipitated ERα were also determined by probing with an anti-ERα antibody (*lower panel*).

Supplemental Figure S3. Nuclear localization of wtERα and ERα-AA. C4-12 cells were pretreated with vehicle (DMSO) or E2 (10nM) for 20 minutes. Whole cell fractions (W) or nuclear extracts fractions (N) were isolated and Western blot was performed for ERα. GAPDH was used as an SDS-PAGE loading control.

Suppl. Fig. S1

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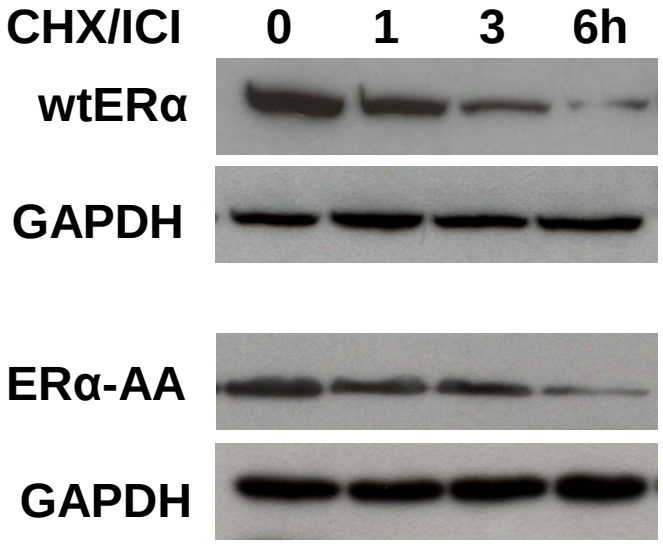

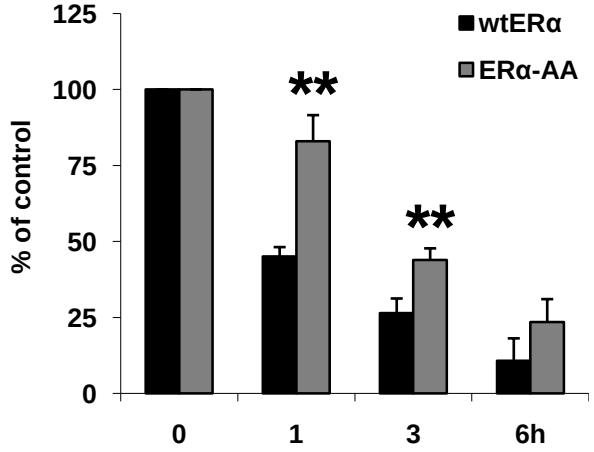

Suppl. Fig. S2

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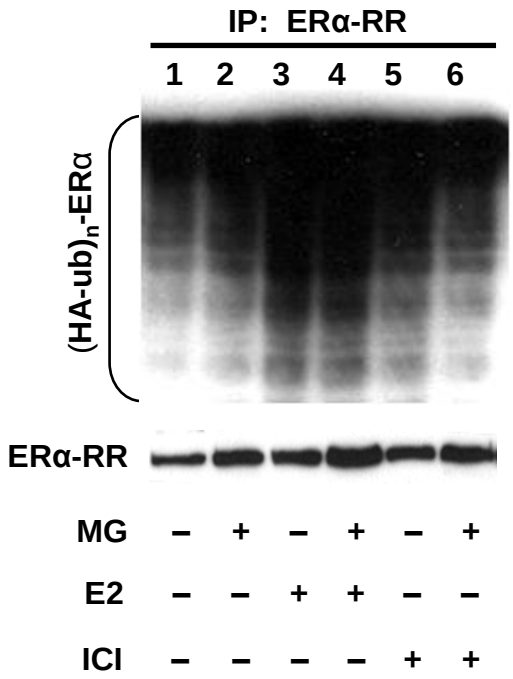

Suppl. Fig. S3

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