

TITLE: Chronic cadmium exposure decreases the dependency of MCF7 breast cancer cells on ER α

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KEYWORDS: Breast cancer, cadmium, chronic exposure, estrogen receptor, ER α

A.

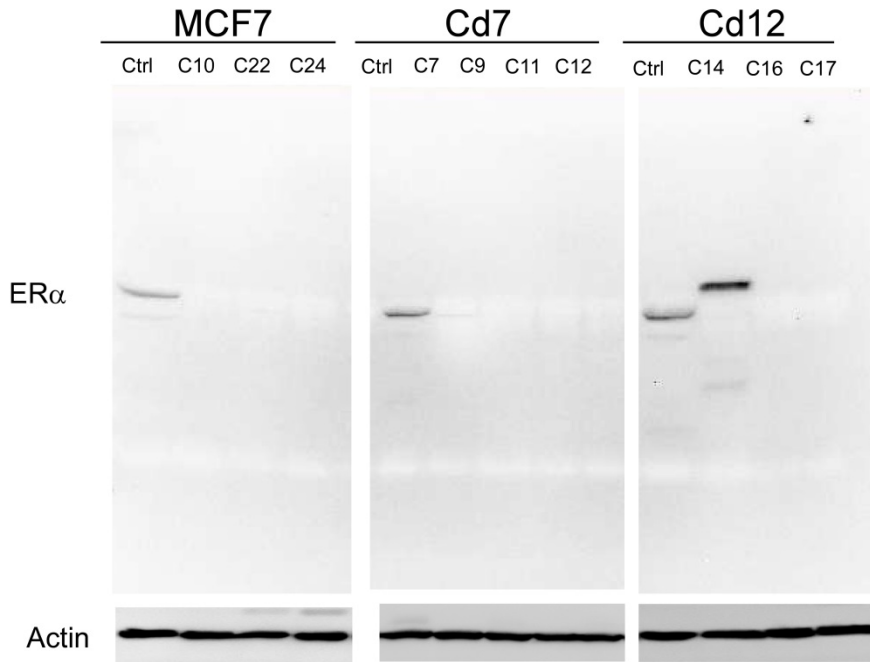
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Cd12-C16     GCCCCTGGGCGAGGTGTACCTGGACAGCAGCAAGCCCGCC-----GTGTACAACACTACCCCGAGGGCG
MCF7-C24     GCCCCAGGGCGCCGCCCTACCTGGACAAC-----TACCCCGAGGGCG
MCF7-C10     GCCCCTGGGCGAGGTGTACCTGGACAGCAGCAAGCCCGCCGTGTACAAGTGTACAACACTACCCCGAGGGCG
Cd12-C17     GCCCCTGGGCGAGGTGTACCTGGACAGCAGCAAGCCCAAC-----ATGGACATCTAGGAOTACCG--
Cd7-C7       GCCCCTGGGCGAGGTGTACCTGGACAGCAGCAAGCCCGCC-----GTGTACAACACTACCCCTAGG--
Cd7-C9       GCCCCTGGGCGAGGTGTACCTGGACAGCAGCAAGCCCGCC-----GTGTACAACACTACCCCTAGG--
Cd7-C11     GCCCCTGGGCGAGGTGTACCTGGACAGCAGCAAGCCCGCC-----G-----CCGAGGGGCG
MCF7-Ctrl    GCCCCTGGGCGAGGTGTACCTGGACAGCAGCAAGCCCGCC-----GTGTACAACACTACCCCGAGGGCG
Cd-Ctrl      GCCCCTGGGCGAGGTGTACCTGGACAGCAGCAAGCCCGCC-----GTGTACAACACTACCCCGAGGGCG

MCF7-C22      ATTTACAGGCTATTTACAGGCTTGGTATGGTCTGGATTTTGTCCCTCCCACTACCCCGAG--GGCGC
Cd12-C16     -----COGCCTACGAGTTTCAGCCGATCTG
MCF7-C24     -----COGCCTACGAGTTTCAC--GCCGC
MCF7-C10     -----GGACCCTCAGGCGCCGCTACCAACGCCCGCCGCTGCGAGTTCACC--GCCGC
Cd12-C17     -----CCACCTAGGAGTTTCAC--ACCGC
Cd7-C7       -----TAGTACTTCTAC--CCCGA
Cd7-C9       -----AAC--GCCGC
Cd7-C11     -----COGCCTACGAGTTTCAC--GCCGC
MCF7-Ctrl    -----COGCCTACGAGTTTCAC--GCCGC
Cd-Ctrl      -----COGCCTACGAGTTTCAC--GCCGC

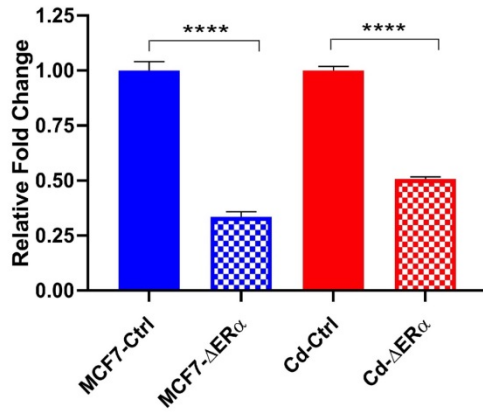
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B.

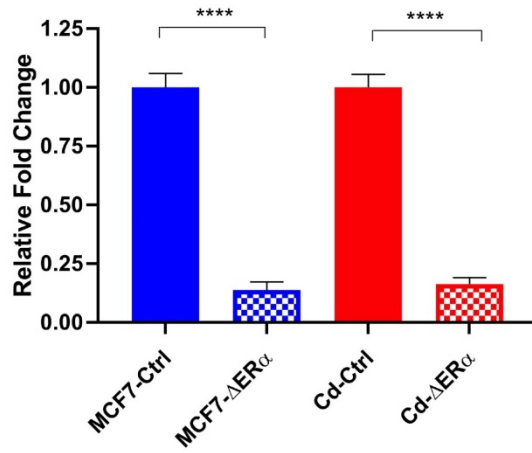


Supplementary Figure S1. Generation of ER α -KO clones using the CRISPR/Cas9 gene-editing system. (A) DNA sequence alignment showing a region that flanks the CRISPR target sequence showing the presence of insertion or deletion mutations (shown in red letters) in single cell-derived clones after transfection with plasmids containing sgRNA oligos and Cas9 enzyme. (B) The presence or absence of ER α in control or CRISPR-transfected clones was verified using western blot analysis with actin as the loading control.

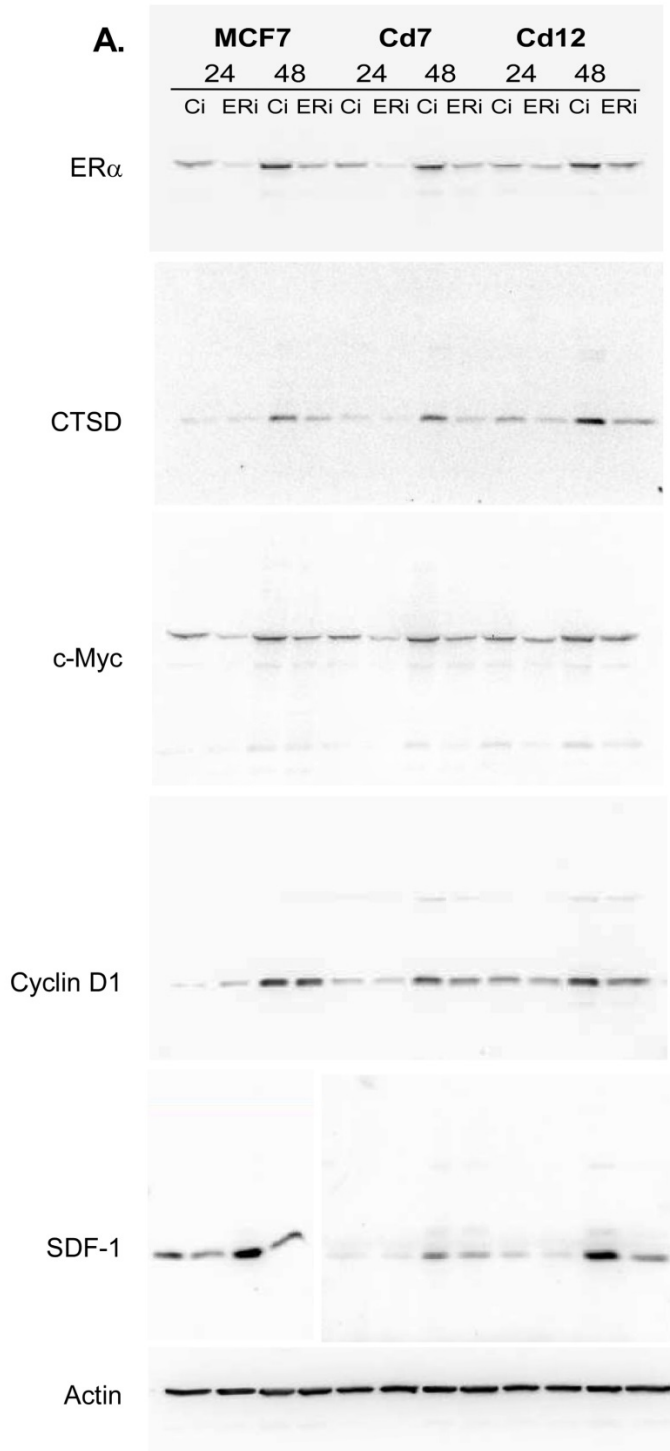
A.



B.



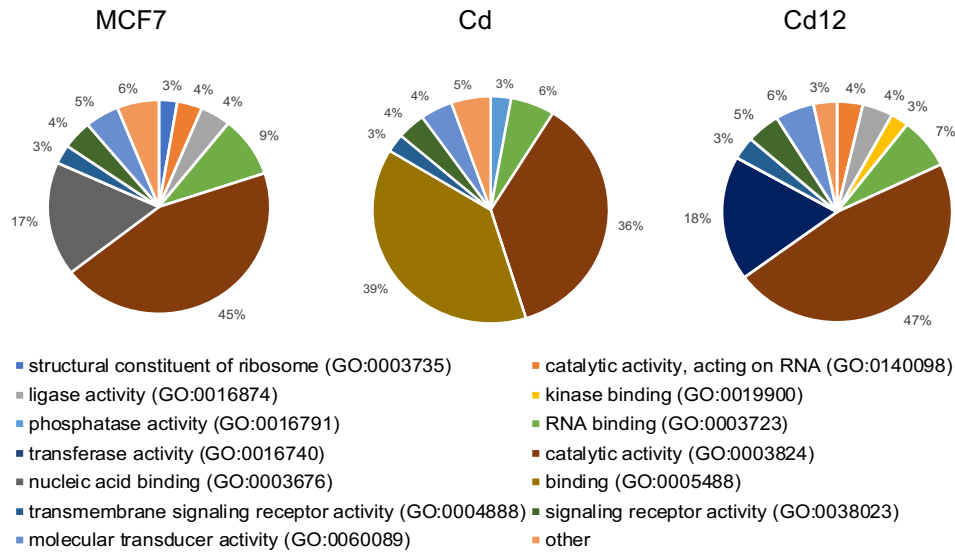
Supplementary Figure S2. Normalized invasion and colony formation abilities of MCF7- Δ ER α and Cd- Δ ER α clones. Bar graphs of the invasiveness (A) and tumorigenicity (B) of the MCF7- Δ ER α and Cd- Δ ER α clones shown as relative fold change to normalize for baseline differences existing between MCF7 and Cd cells (**** $p < 0.0001$).



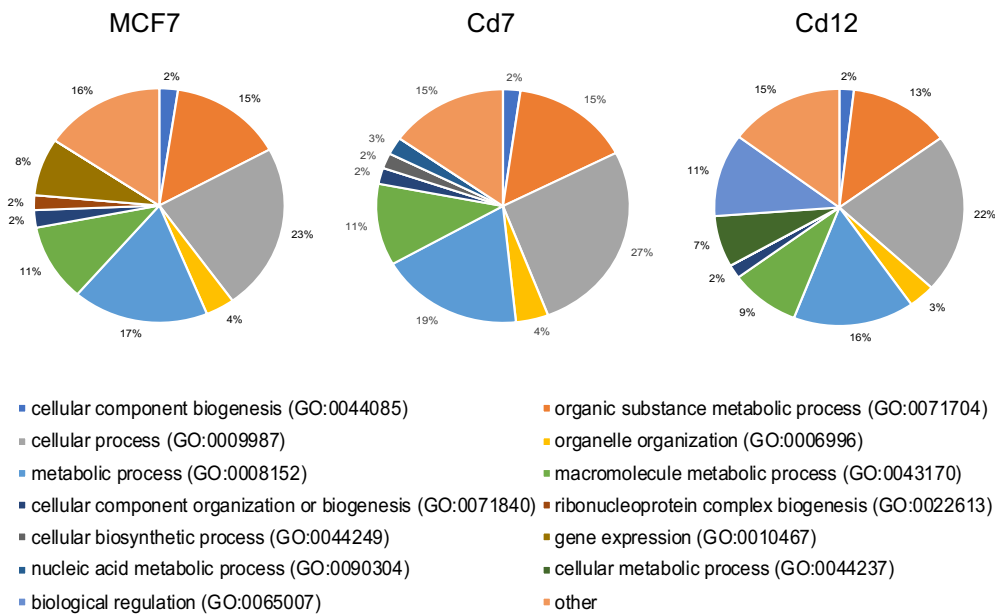
Supplementary Figure S3. Full-length of Western Blot Images for Figure 4C.

MCF7, Cd7, and Cd12 cells were transfected with si-ER α (ERi) or si-control (Ci) and cell lysates were collected from MCF7, Cd7, and Cd12 cells 24 or 48 hours after ER α knockdown for protein expression analysis using western blots with actin as the loading control. #SDF-1 blot is composed of two blots—one with MCF7 cells and the other with Cd7 and Cd12 cells, each transfected with either si-ER α or si-control.

A. GO Molecular Function



B. GO Biological Process



Supplementary Figure S4. Enrichment of GO terms in MCF7, Cd7, and Cd12 cells after ER α knockdown. Pie charts showing the over-represented (A) Molecular Function and (B) Biological Process using PANTHER GO-Slim in parental and cadmium-adapted MCF7 cells following exposure to ICI. Annotations where the corresponding number of DE genes was less than 1% were categorized as “other.”