5β-Dihydrotestosterone reveals a mutant androgen receptor vulnerability in prostate cancer

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Supplementary figure legends:

Fig. S1. AR dependence of 5β-DHT- and 3β-ecdiol-induced cell growth and abrogation by enzalutamide. (**A**) LNCaP proliferation with 10 nM T metabolites in csFBS medium. (**B**) AR-null PC-3 parental cells show no response to T, 5β-DHT, or 3β-ecdiol. (**C**) Enz (10 μM) abrogates 5β-DHT- and 3β-ecdiol-induced growth in C4-2 cells. Data are mean ± SEM ($n \geq 3$). $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$.

Fig. S2. 5β-DHT activates AR W742C and AR H875Y at high doses. PC-3 AR W742C and AR H875Y cells in FBS medium were treated with 100 nM or 1 μM T or 5β-DHT for 48 hours. (**A** and **B**) ARR3tk-eGFP/SV40-mCherry reporter fluorescence images in PC-3 AR W742C and AR H875Y cells (Ctrl, 100 nM T, 100 nM 5β-DHT). (**C** and **D**) GFP+/RFP+ ratios in PC-3 AR W742C and PC-3 AR H875Y over time with Ctrl, 100 nM or 1 μM T or 5β-DHT. Data are mean ± SEM ($n \geq 3$). $^{****}p < 0.0001$.

Fig. S3. AR specificity of supraphysiologic growth suppression. (**A** and **B**) C4-2 and LNCaP cells treated with inactive metabolites at 100 nM or 1 μM in FBS medium show no growth suppression. (**C**) AR-null PC-3 cells are unaffected by all compounds at high doses. Data are mean ± SEM ($n \geq 3$).

Fig. S4. Progesterone analogs cannot suppress growth at high doses. (**A**) Progesterone metabolic pathway schematic. (**B**) C4-2 growth in csFBS medium with progestins at 0.1–10 nM. (**C**) C4-2 growth in FBS medium with progestins at 100 nM or 1 μM: none suppresses proliferation. Data are mean ± SEM ($n \geq 3$). $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$.

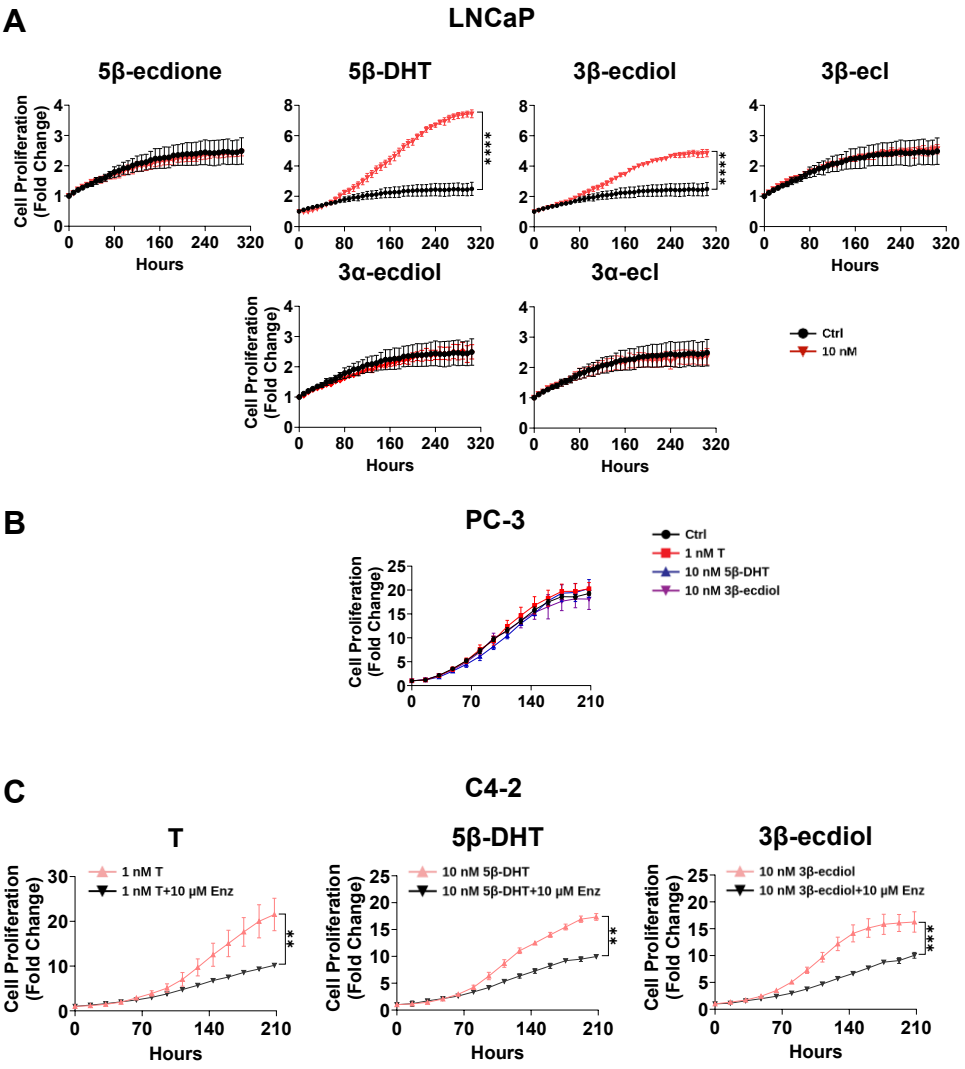
Fig. S5. High-dose 5β-DHT induces senescence-associated protein changes in PC-3 cells expressing mutant AR. Parental PC-3 cells and PC-3 cells stably expressing AR-W742C or AR-H875Y were cultured in FBS-containing medium and treated with vehicle control (Ctrl), 100 nM testosterone (T), or 100 nM 5β-DHT for 3 days. Representative cropped immunoblots for phospho-Rb (Ser807/811), SKP2, p27, p21, and β-actin are shown. For each protein, the three cell-line groups were cropped from non-adjacent regions of the same full-length blot and are

separated by white space. β-actin served as the loading control. Membranes were cut into strips according to molecular-weight markers prior to antibody hybridization; full-length membrane images are therefore not available. Uncropped images of all blots, including all replicates, are shown in Supplementary Fig. S7.

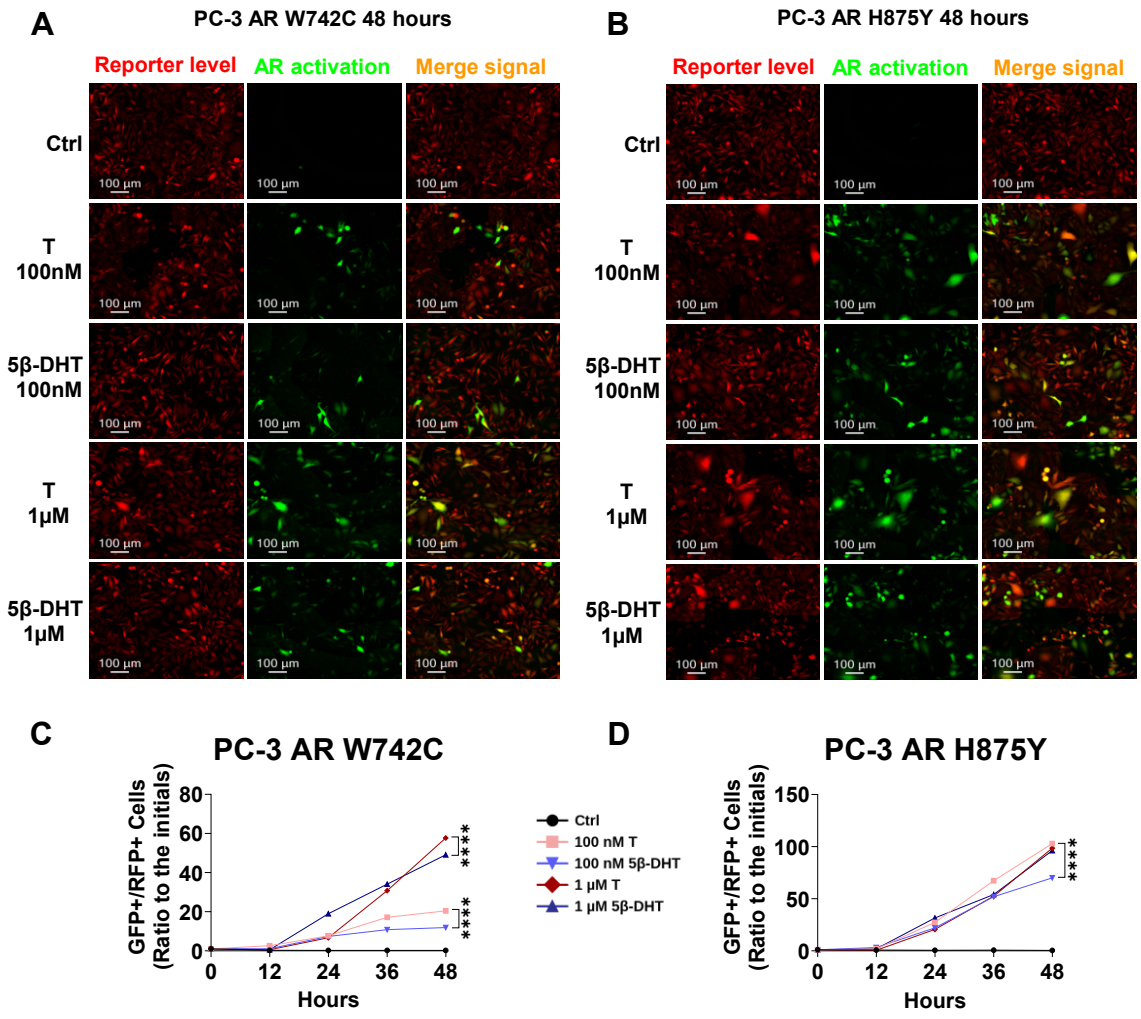
Supplementary Fig. S6. Uncropped immunoblot images corresponding to Fig. 5D.
(**A, B**) Uncropped immunoblot images from three biological replicates. C4-2 cells were treated with vehicle control (Ctrl), 100 nM testosterone (T), or 100 nM 5β-DHT for 1 or 3 days. Blots were probed for phospho-Rb (Ser807/811), p57, SKP2, p27, p21, and β-actin, as indicated. (**A**) Immunoblots from the first and second biological replicates; red boxes indicate the regions presented in **Fig. 5D**. (**B**) Immunoblots from the third biological replicate. The uncropped images of all available membrane sections are shown. Separate membrane sections or blot images are separated by gray or white space. Molecular masses are indicated in kilodaltons (kDa), and β-actin served as the loading control.

Supplementary Fig. S7. Uncropped immunoblot images corresponding to Supplementary Fig. S5.
(**A–C**) Uncropped immunoblot images from three biological replicates. Parental PC-3, PC-3 AR-W742C, and PC-3 AR-H875Y cells were treated with vehicle control (Ctrl), 100 nM testosterone (T), or 100 nM 5β-DHT for 3 days. Blots were probed for phospho-Rb (Ser807/811), SKP2, p27, p21, and β-actin, as indicated. (**A–C**) Immunoblots from the first, second, and third biological replicates, respectively. Red boxes in (**A**) indicate the regions presented in **Supplementary Fig. S5**. The uncropped images of all available membrane sections are shown. Separate membrane sections or blot images are separated by gray or white space. Molecular masses are indicated in kilodaltons (kDa), and β-actin served as the loading control.

Supplementary Fig. S1



Supplementary Fig. S2



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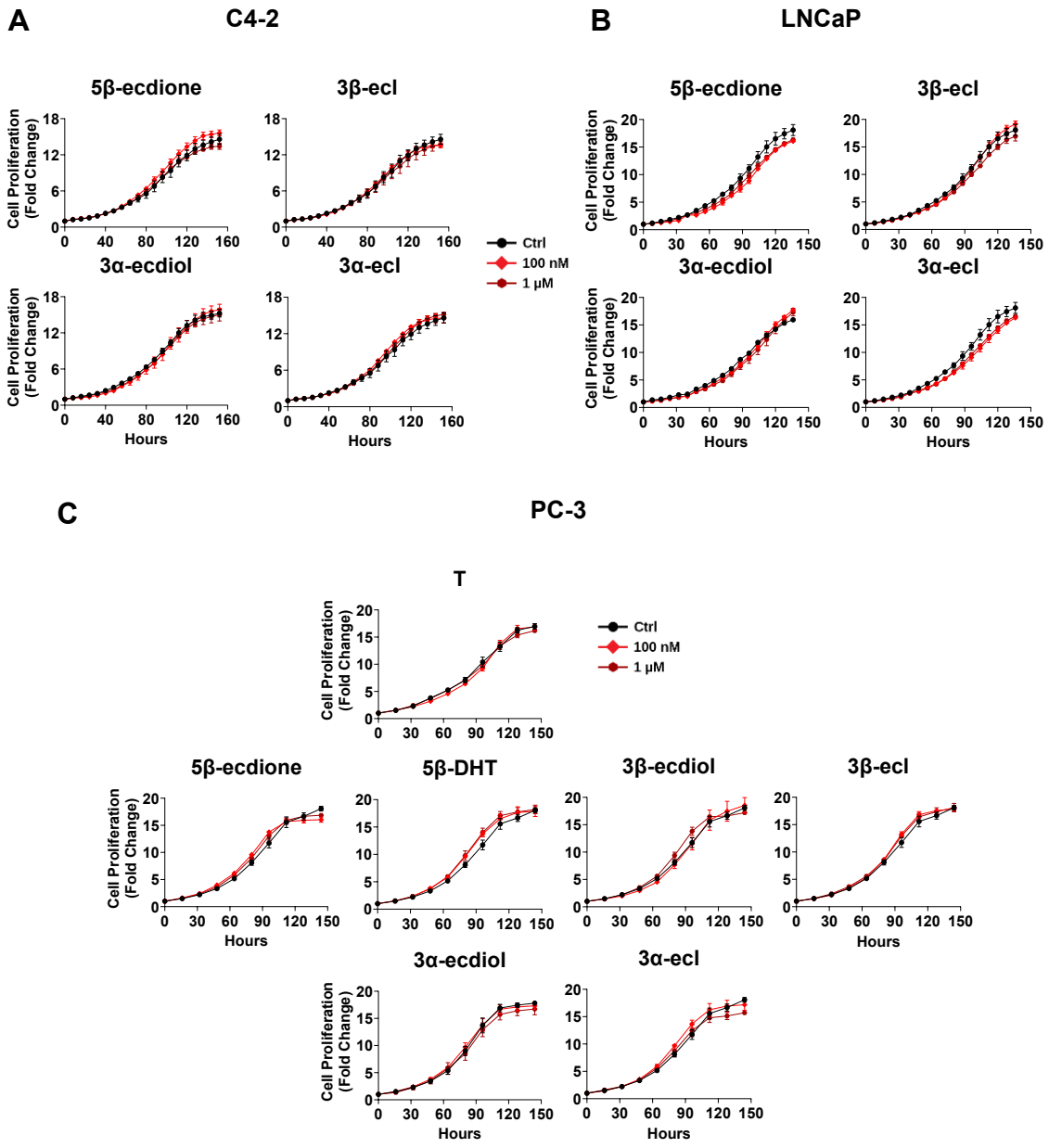

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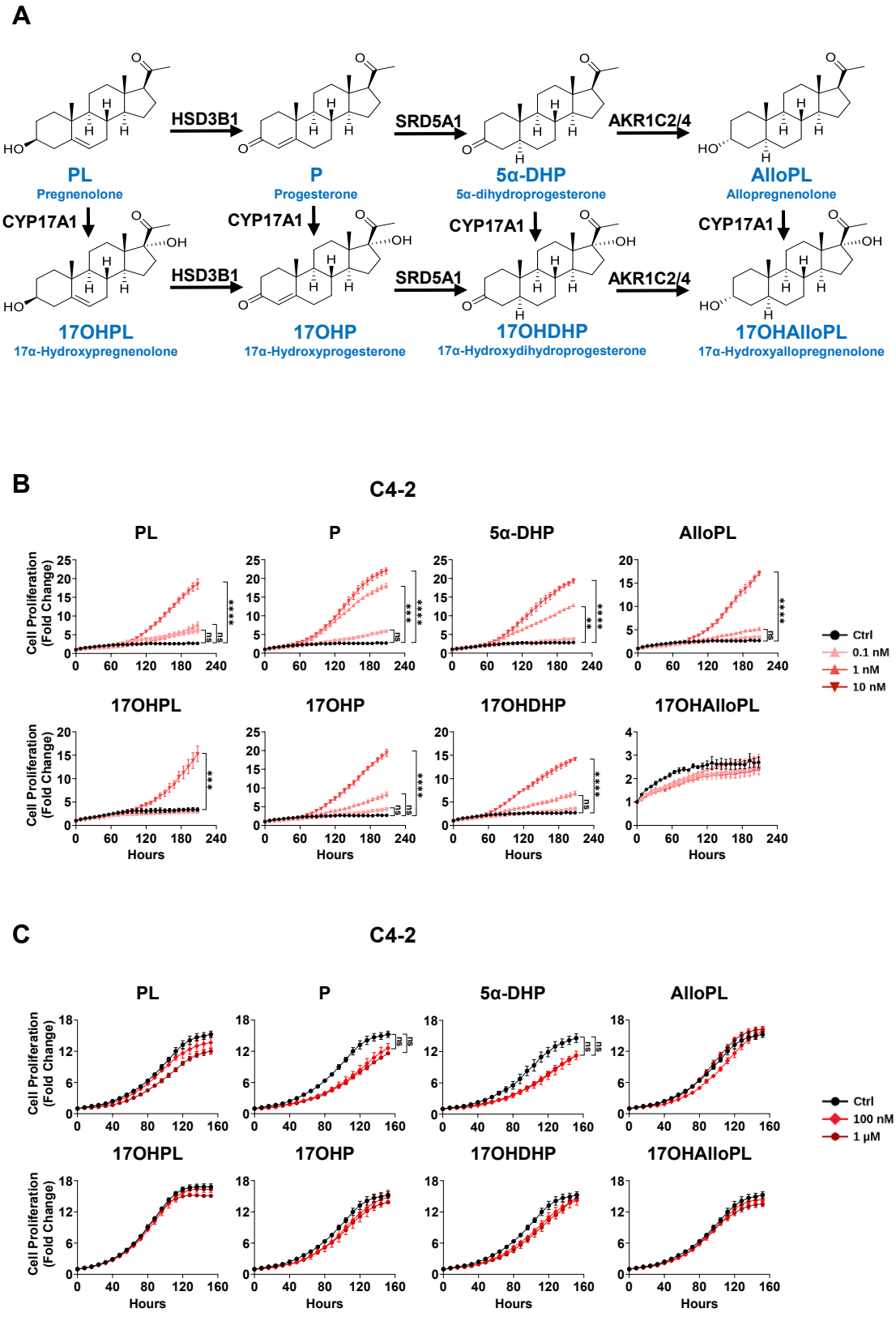

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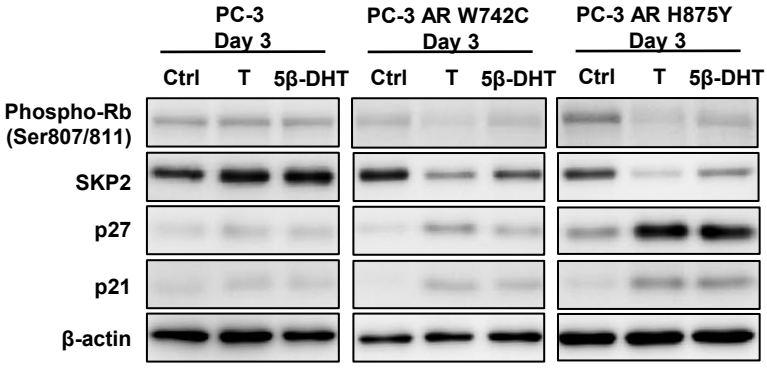
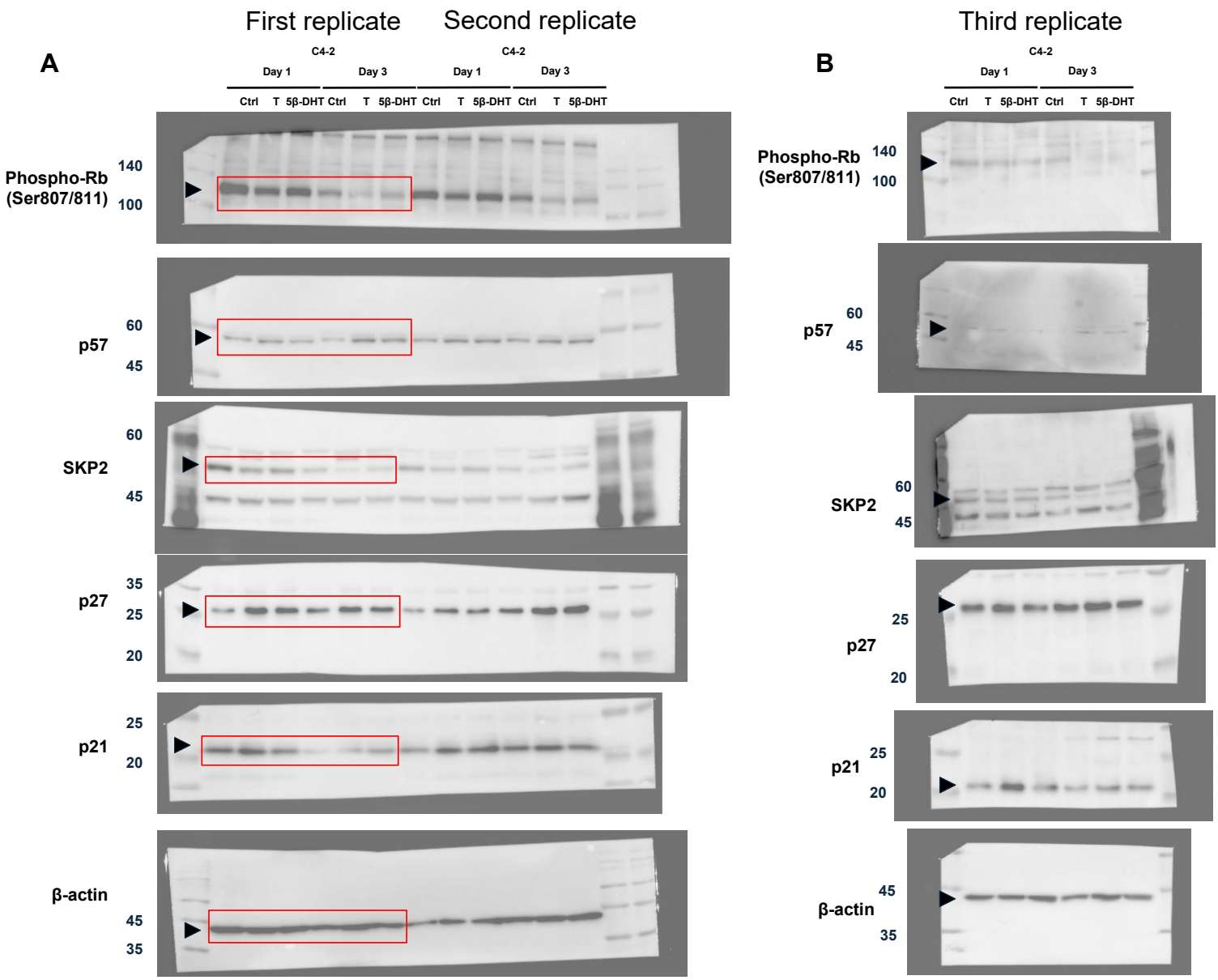


Supplementary Fig. S6



Supplementary Fig. S7