Figure S1

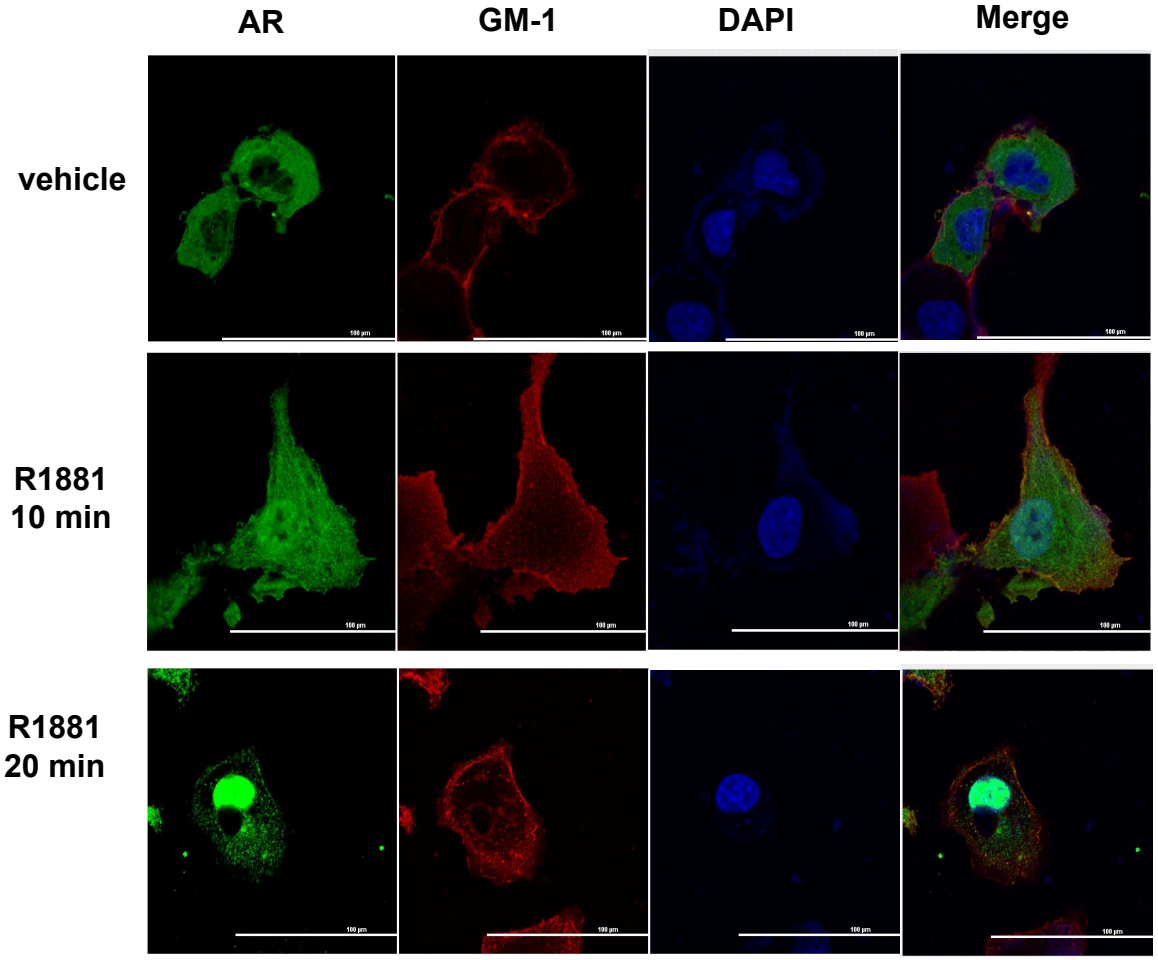


Figure S1. Intracellular localization of AR by immunofluorescence. COS-7 cells transfected with AR were cultured on chamber slides in phenol-red free DMEM containing 5% charcoal-stripped FBS and treated with 1 nM R1881 for the indicated times. Following fixation, the slides were stained for AR by an anti-AR (green), for membrane marker GM-1 by Cholera Toxin Subunit B (red), and for nuclei by DAPI (blue). Scale bar is 100 μm.

Figure S2

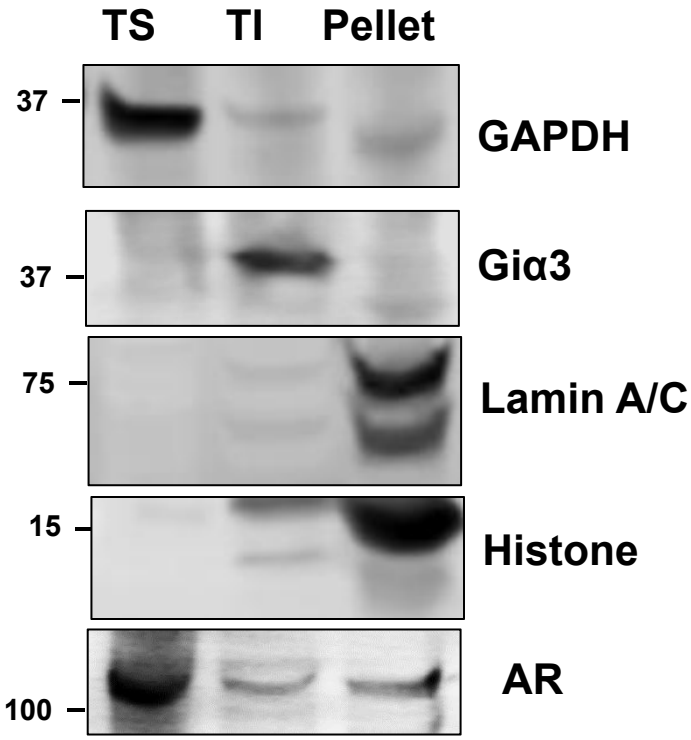


Figure S2. Assessment of the membrane fractionation assay. LNCaP cells cultured in an androgen-depleted condition were separated into Triton X-100-soluble (TS), Triton X-100-insoluble/octylglucoside-soluble (TI) and pellet (insoluable in both Triton X-100 and octylglucoside) fractions. Western blotting was performed to detect the distribution of GAPDH (cytoplasm marker), Giα3 (membrane marker), histone, and lamin A/C (nuclear markers). AR was detected in all three fractions.

Figure S3

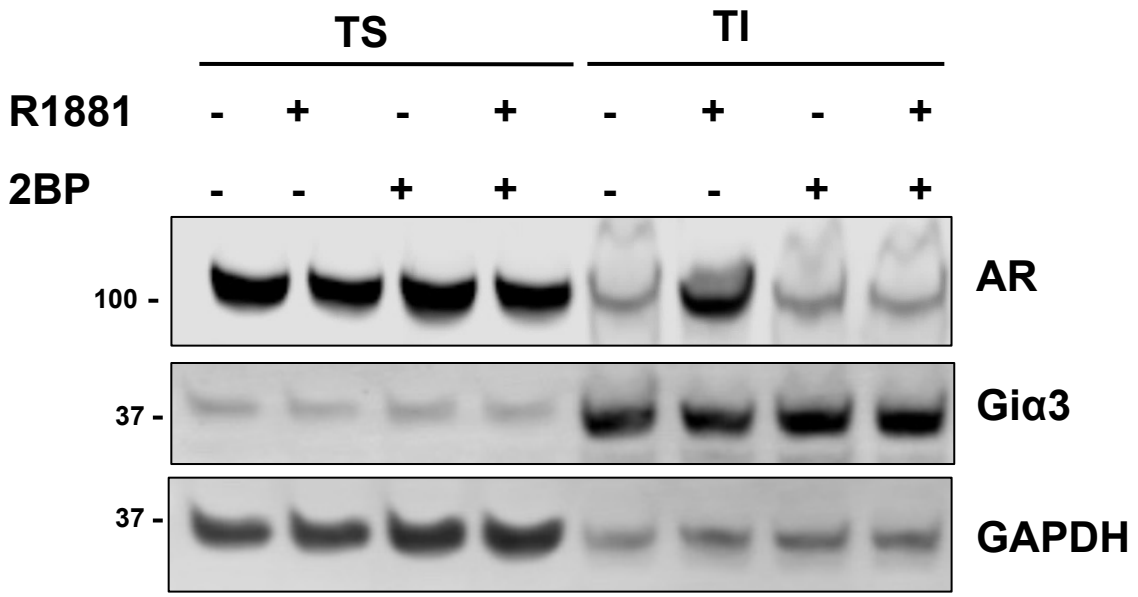


Figure S3. Androgen-induced AR membrane translocation is blocked by a palmitoylation inhibitor. LNCaP cells were pretreated with 10 μM 2-BP for 16 h, then stimulated with 1 nM R1881 for 20 min. TS/ TI fractions were extracted and analyzed by Western blots.

Figure S4

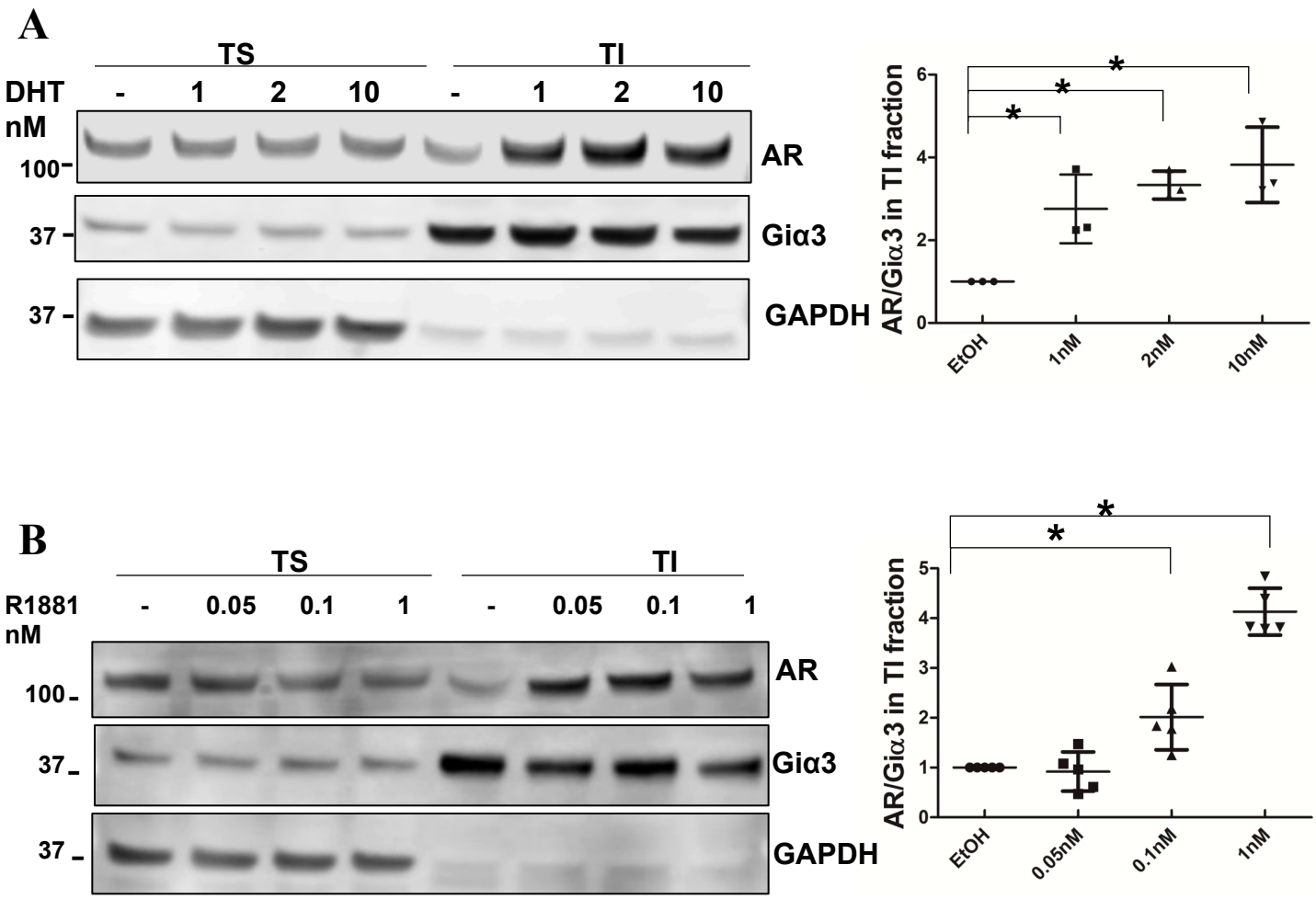


Figure S4. AR membrane transport induced by different concentrations of androgens. LNCaP cells were treated with DHT (1, 2, 10 nM, in panel A) or R1881 (50, 100 pM, 1 nM, in panel B) for 20 min. TS/TI fractions were extracted and analyzed by Western blots. Membrane AR levels were calculated as AR/Giα3 ratios and expressed relative to that of the leftmost group. The mean ± SEM from three experiments are plotted. **, P<0.05.*

Figure S5

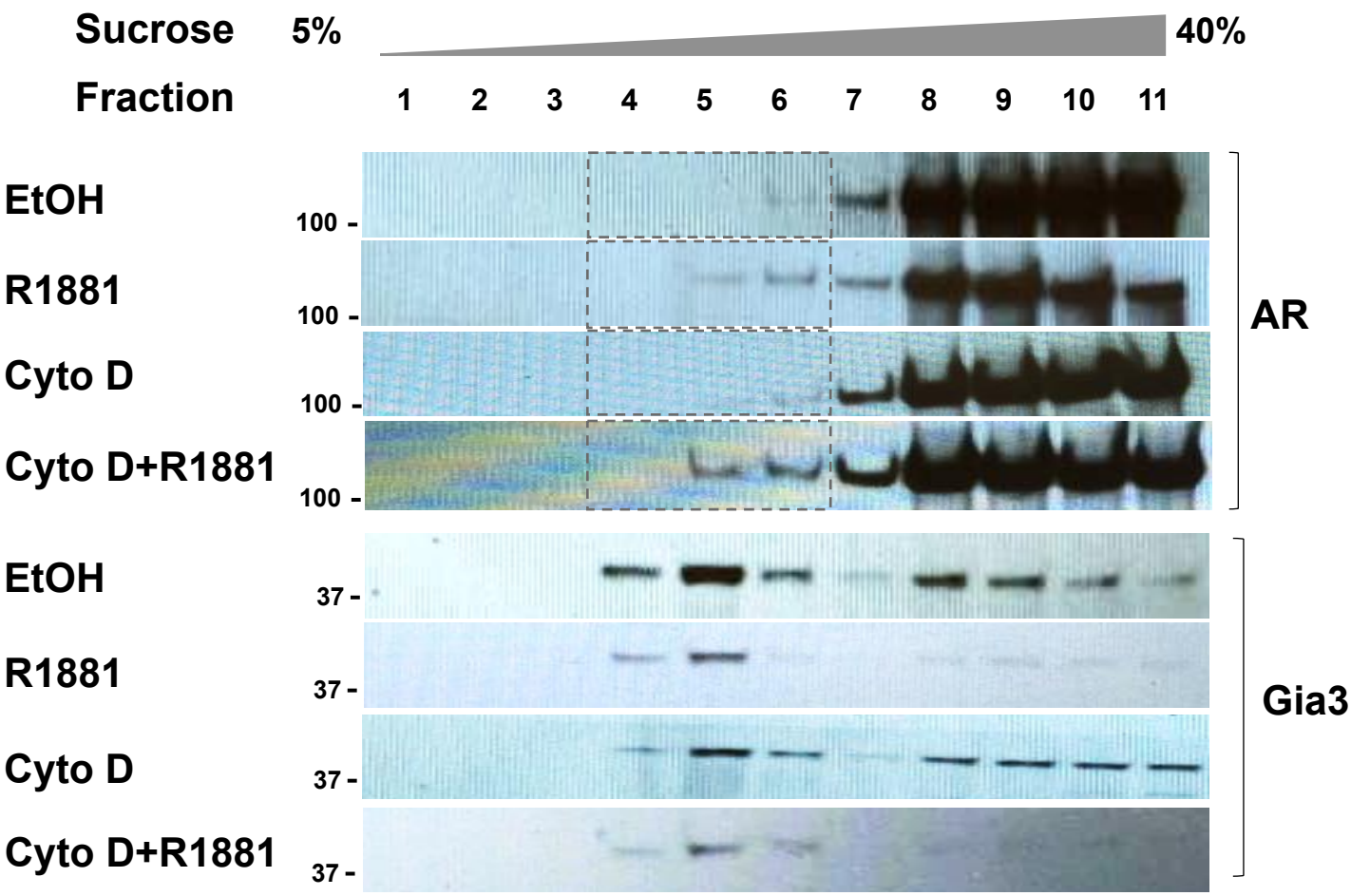


Figure S5. AR transport to the membrane is not affected by cytochalasin D. LNCaP cells were pretreated with EtOH or 0.5 mg/ml cytochalasin D overnight, followed by 1 nM R1881 for an additional 20 min. Cell lysates were subjected to sucrose gradient ultracentrifugation and analyzed by Western blots. Giα3 was used as the membrane marker.

Figure S6

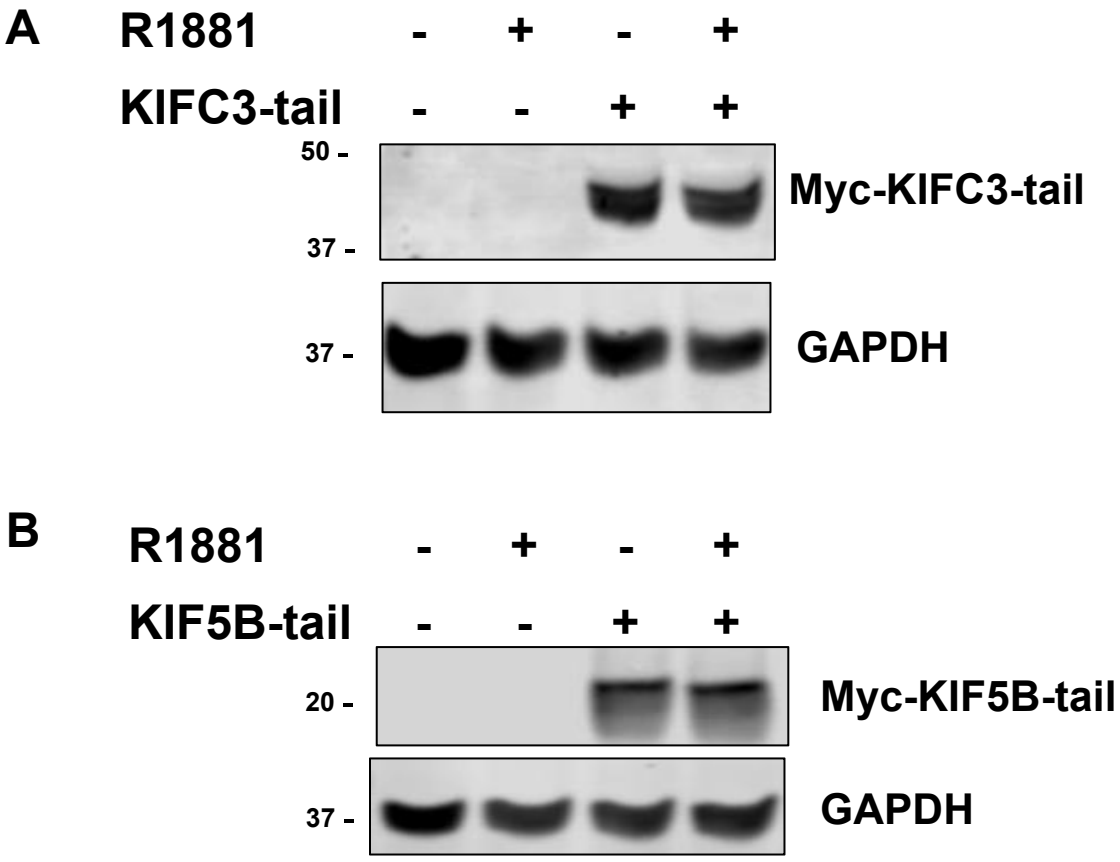


Figure S6. Expression of KIFC3-tail and KIF5B-tail in LNCaP. Cells were transiently transfected with KIFC3-tail or KIF5B-tail. Whole cell lysates were collected after 48 h and analyzed by Western blots to confirm the expression of KIFC3-tail and KIF5B-tail.

Figure S7

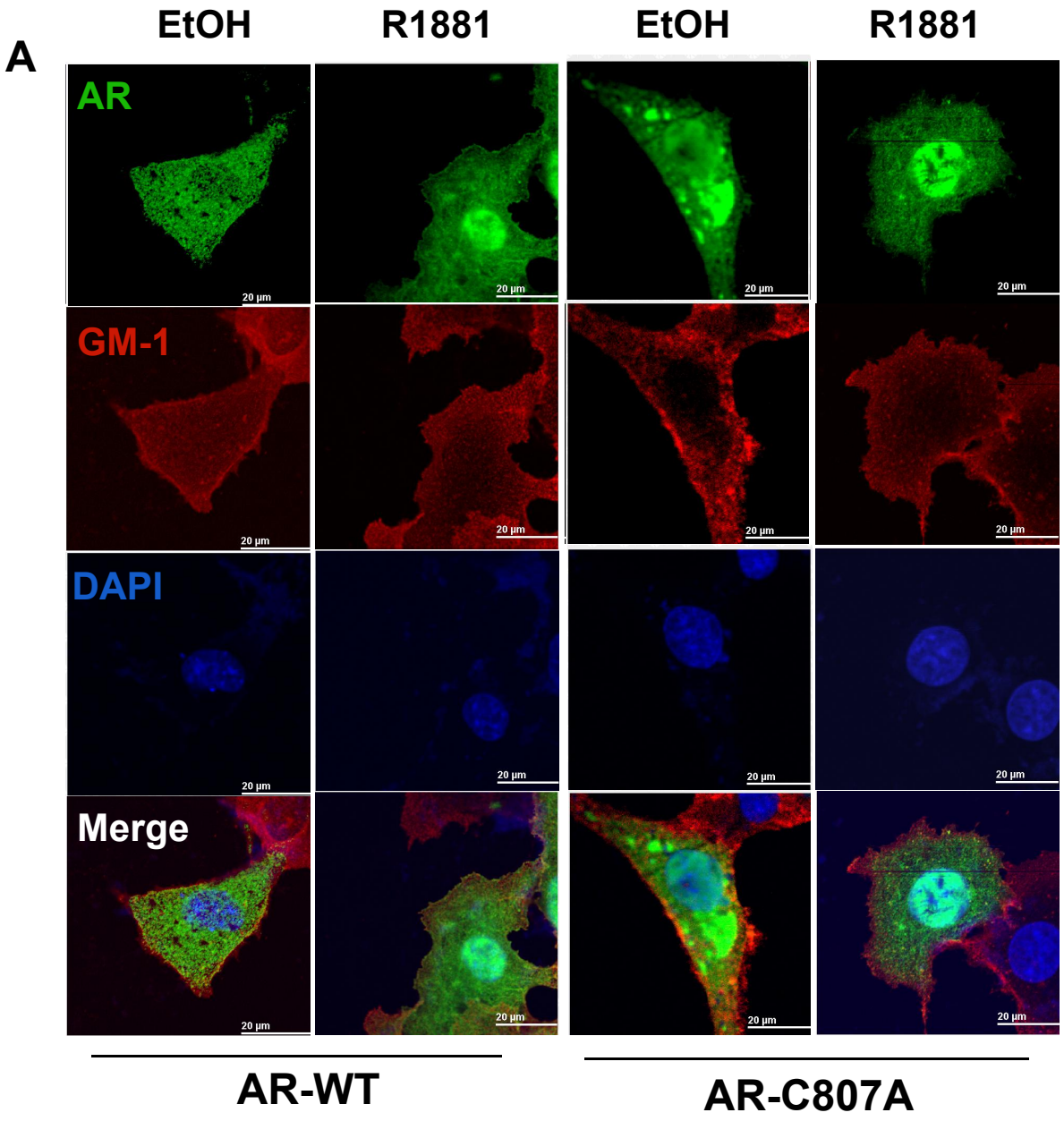
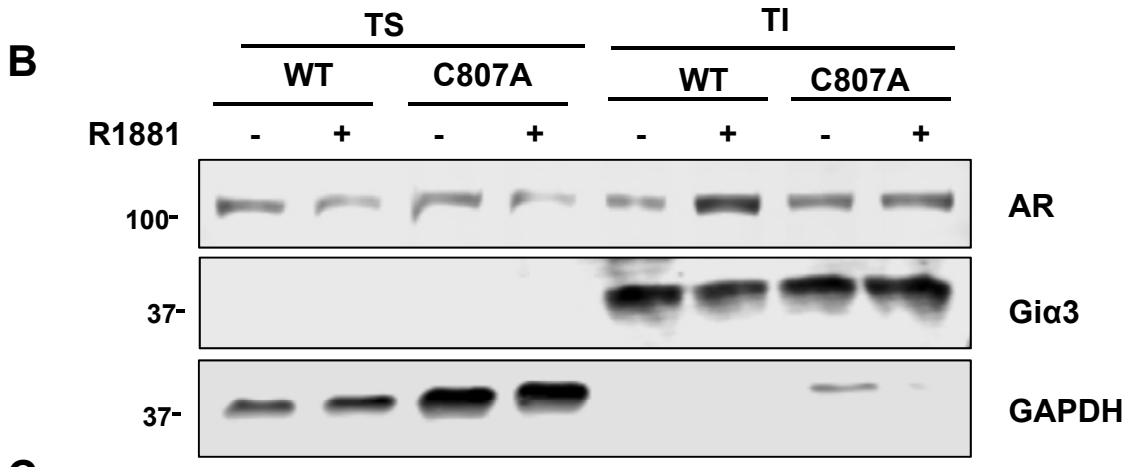
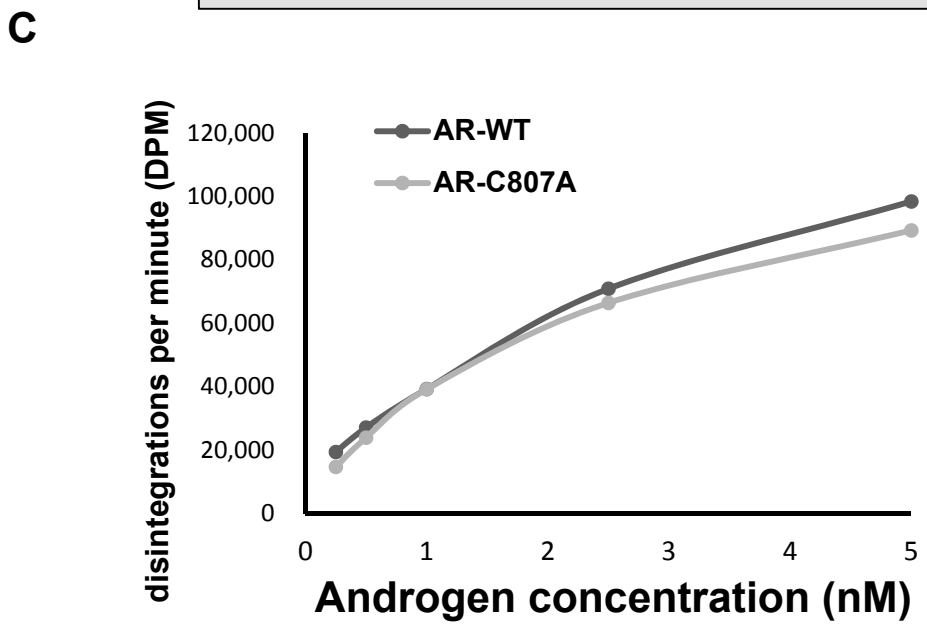


Figure S7 Cont'd





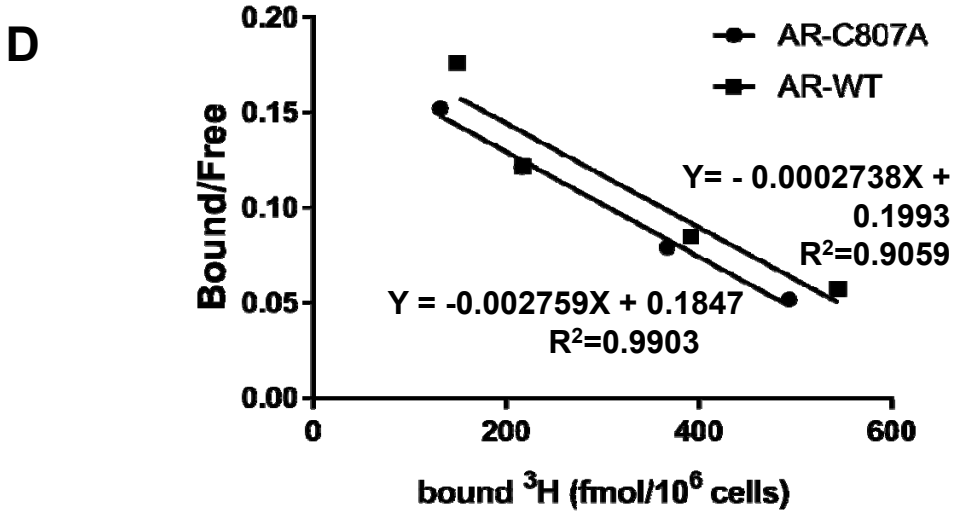


Figure S7 Cont'd

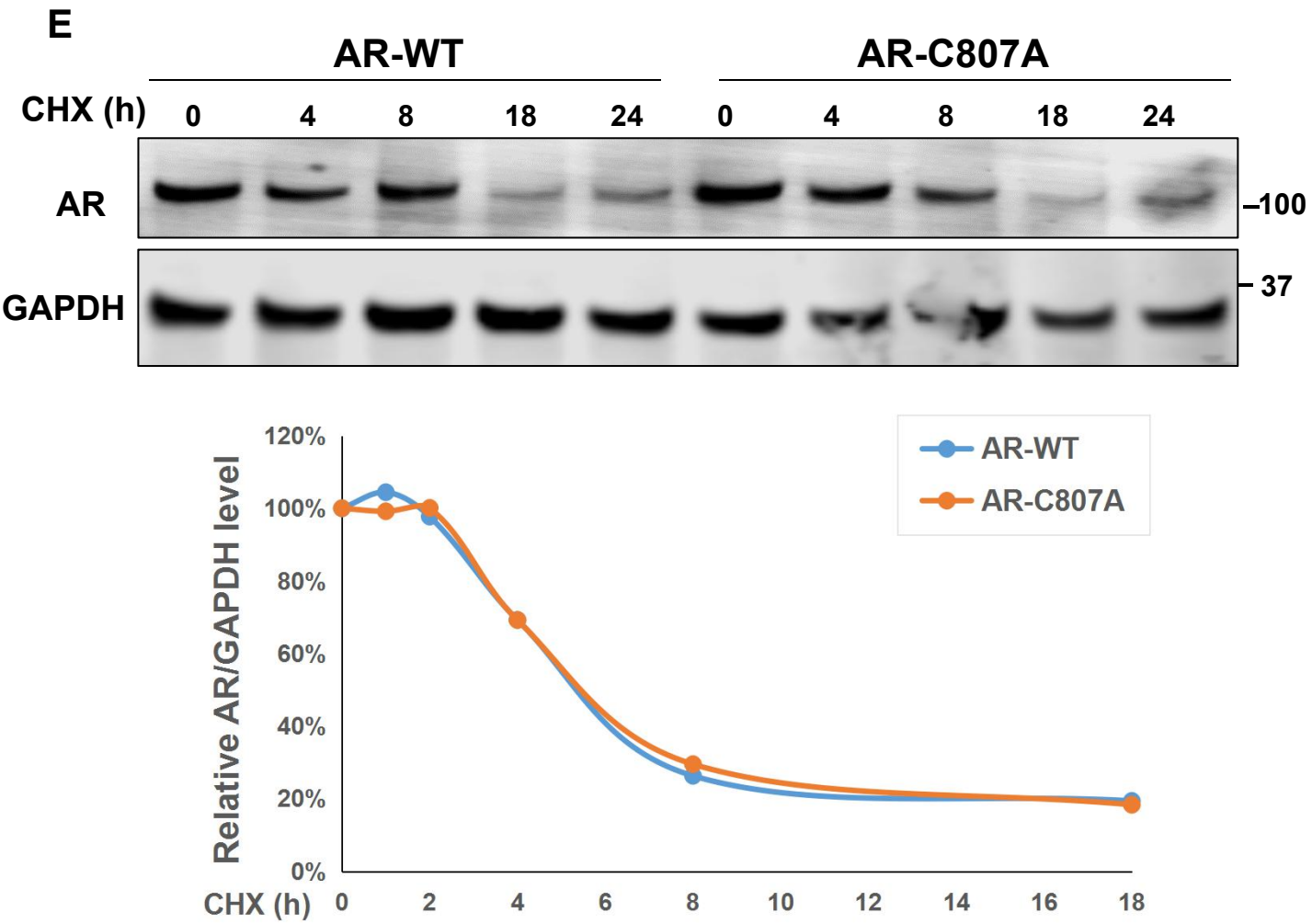


Figure S7. Characterization of the AR-C807A mutant. A, intracellular localization of AR-WT/AR-C807A by immunofluorescence. COS-7 cells were transiently transfected with AR-WT or AR-C807A, cultured in phenol-red free DMEM with 5% cs-FBS for 48 h, and stimulated with 1 nM R1881 for 20 min. Following fixation, cells were stained for AR by an anti-AR (green). B, the localization of AR-WT/AR-C807A at plasma membrane was detected by the TS/TI fractionation assay. C, androgen binding activities of AR-WT and AR-C807A. D, Scatchard plots for AR-WT/AR-C807A ligand binding. The calculated dissociation constant (Kd) is 3.652 pM for AR-WT, and is 3.625 pM for AR-C807A. E, stability of AR-WT and AR-C807A. COS-7 cells were transfected with AR-WT or AR-C807A for 48h, and treated with 20 μg/ml CHX for different times. AR protein in whole cell lysates were analyzed by Western blotting.

Figure S8

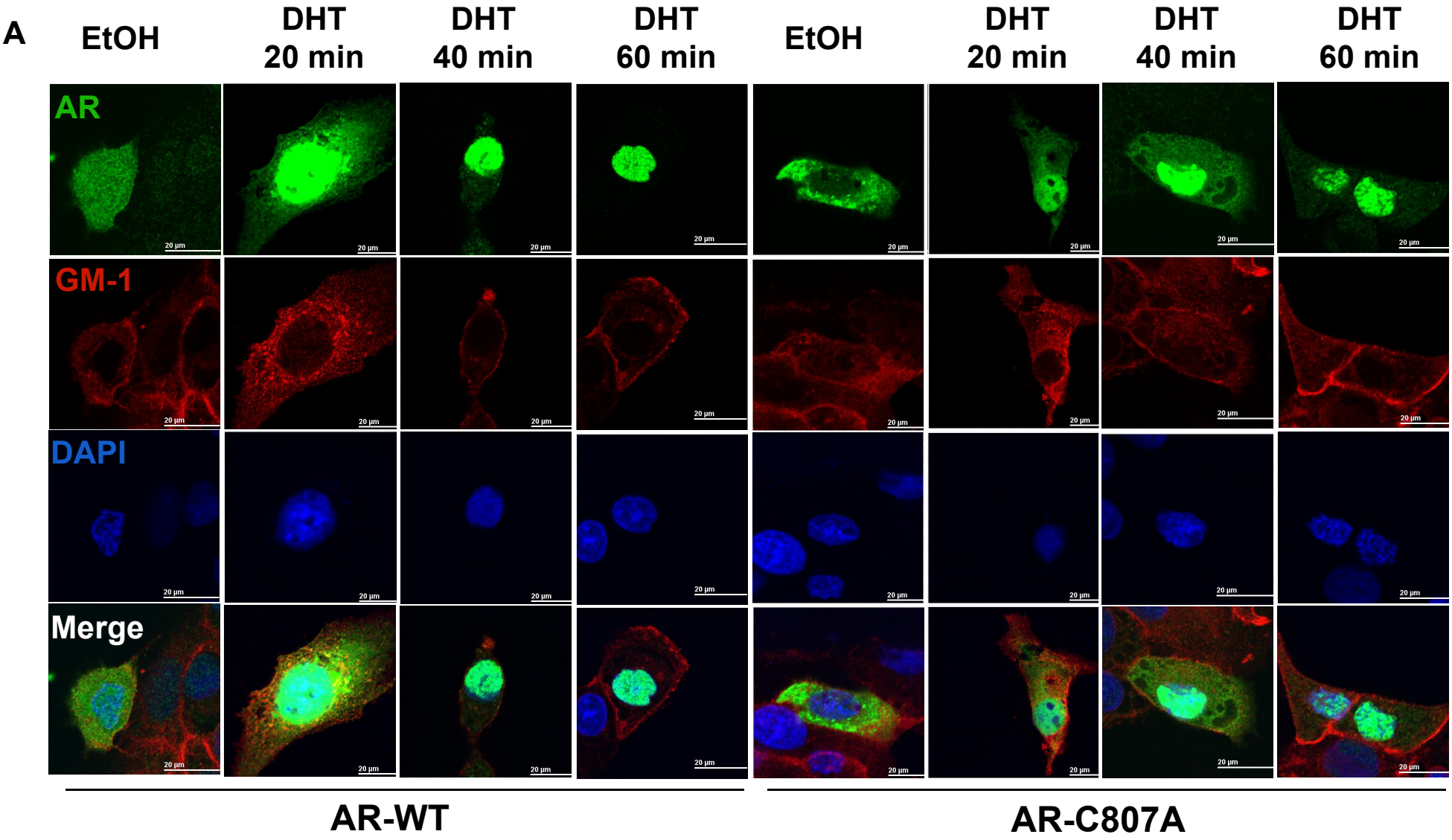

A
EtOH
DHT 20 min
DHT 40 min
DHT 60 min
EtOH
DHT 20 min
DHT 40 min
DHT 60 min
AR
GM-1
DAPI
Merge
20 μm
AR-WT
AR-C807A

Figure S8 Cont'd

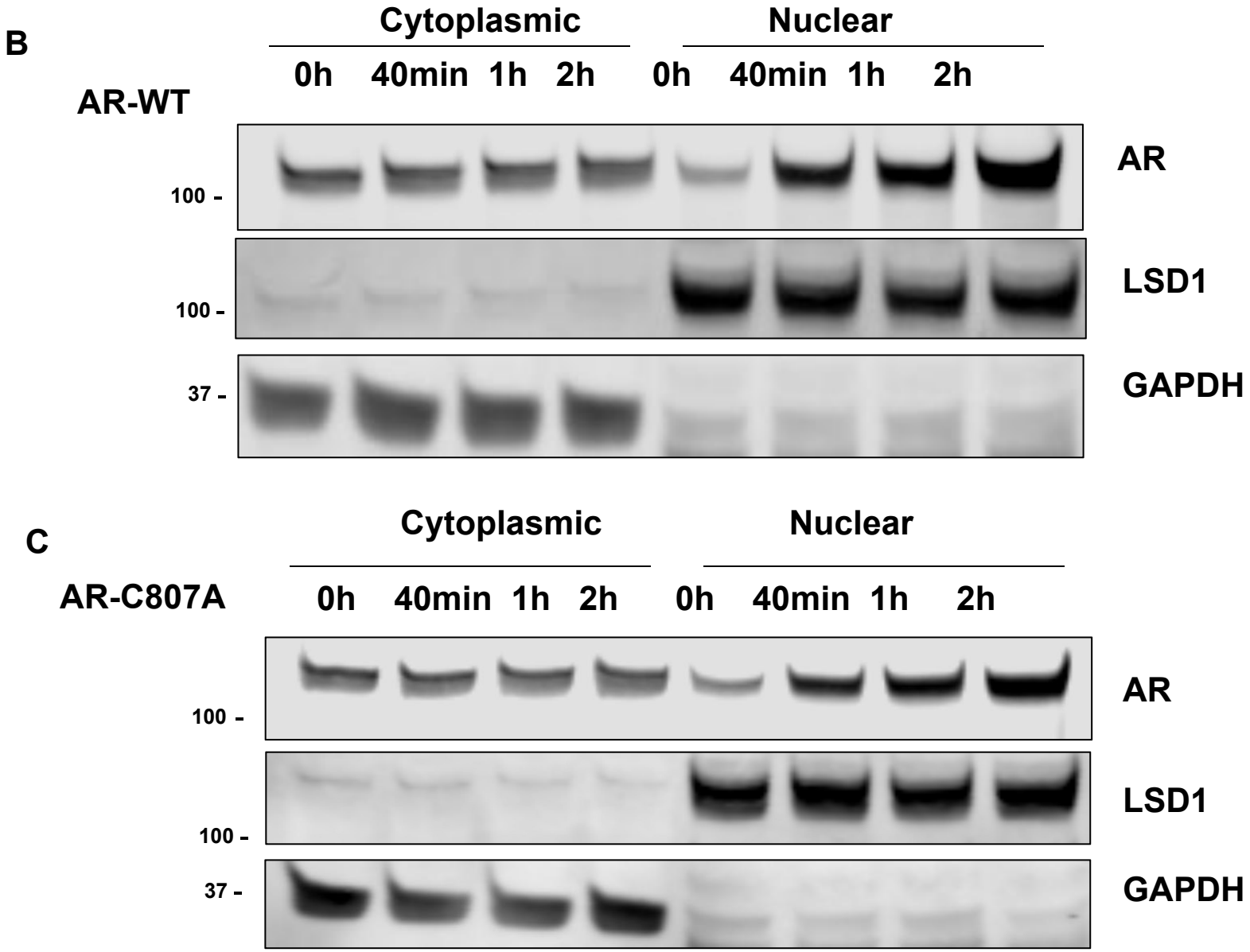


Figure S8. Nuclear import of AR-WT and AR-C807A. A, immunofluorescence assay. Transfected COS-7 cells were treated with 1 nM R1881 for the indicated time points and stained for AR, GM-1, and nuclei. Scale bar is 20 μm. B and C, subcellular fractionation assay. COS-7 cells was treated with 1 nM R1881 for different time points (0, 40 min, 1 h, 2 h), lysed for cytoplasm/nuclear fractionation, and analyzed by Western blots. LSD1 was used as the nuclear marker and GAPDH as the cytoplasmic marker.

Figure S9

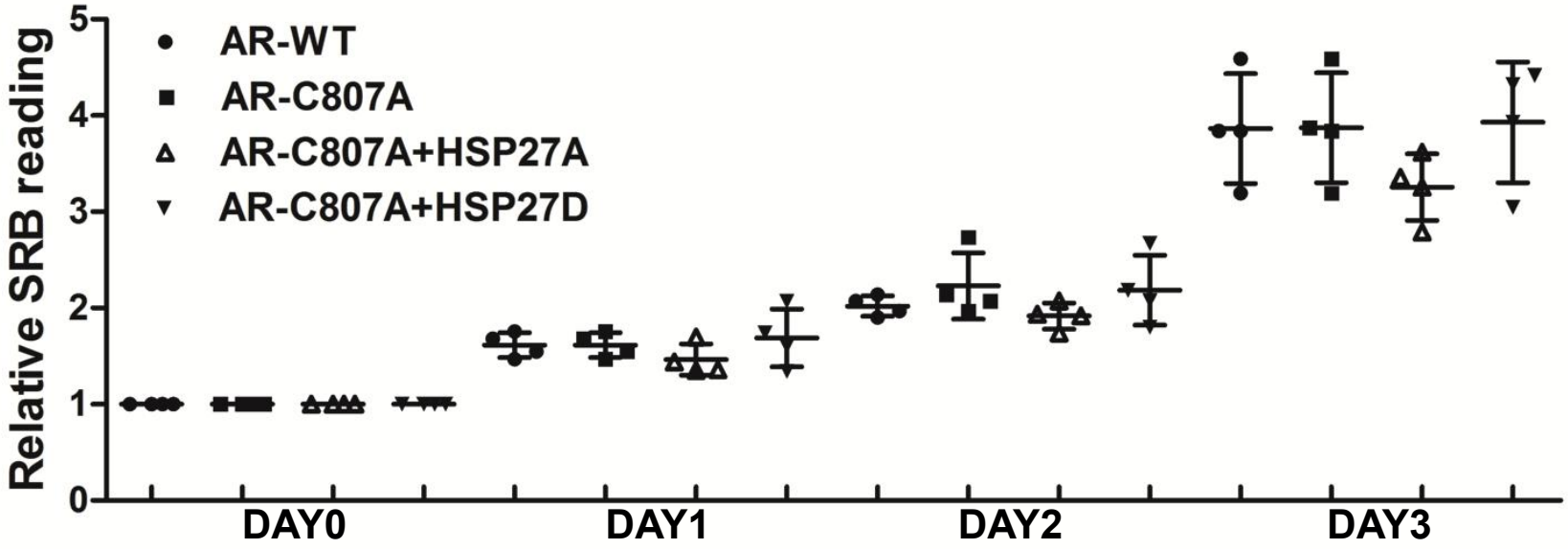


Figure S9. Cell growth assay in DU145 cells without androgen stimulation. Cells were co-transfected with AR-WT/AR-C807A and vector/HSP27A/HSP27D, cultured in RPMI 1640 supplemented with 10% cs-FBS for 1 to 3 days, and the SRB assay was performed. The data were expressed as folds of the day 0 readings and plotted as mean ± SD from three experiments.