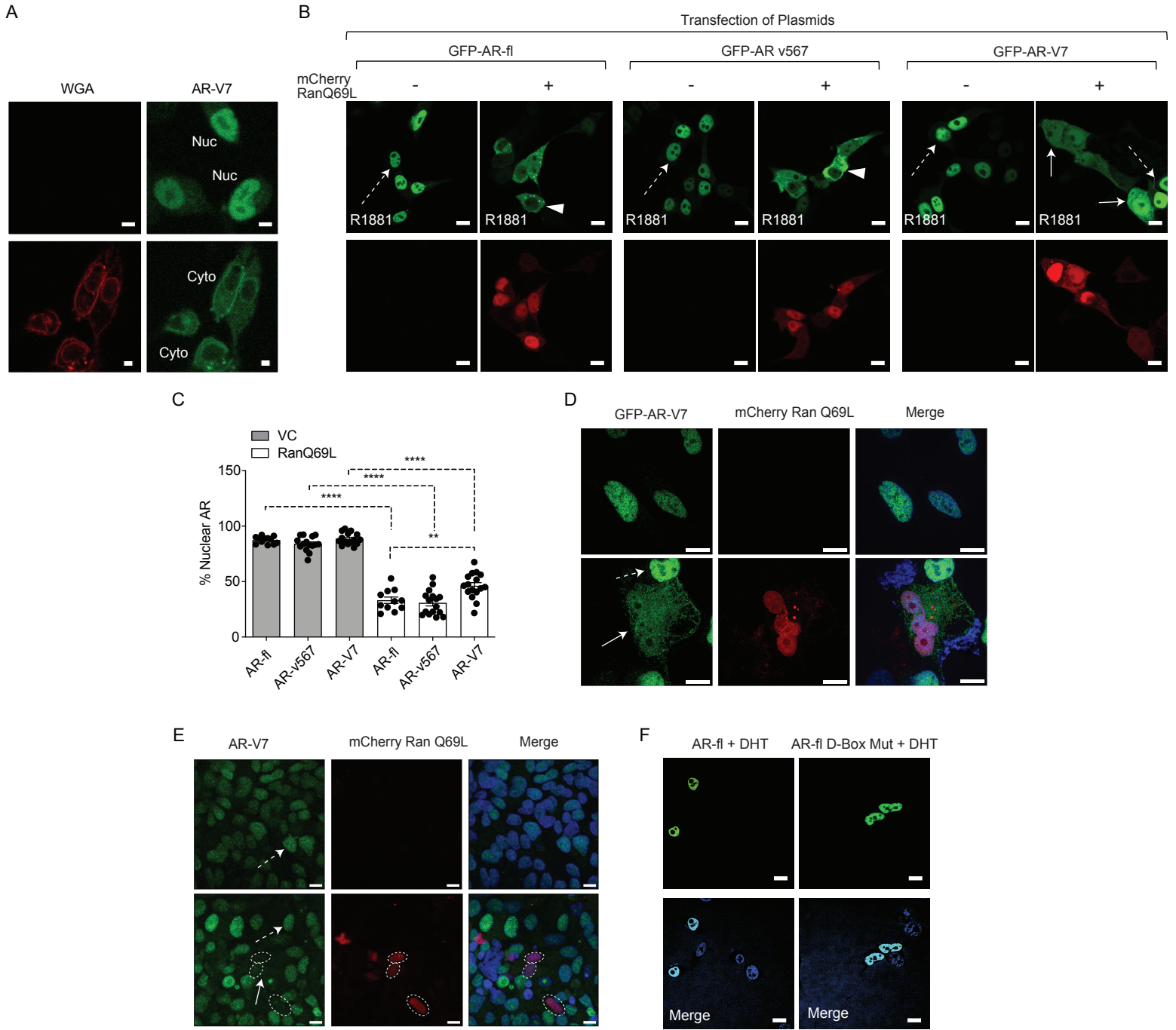


Figure 2- figure supplement 1



**Figure 2- figure supplement 1. AR-V7 nuclear import requires active transport via the nuclear pore complex, is dependent on Ran-GTP activity and is impaired upon mutation of the dimerization box domain (D-Box).** **A.** Wheat germ agglutinin (WGA) blocks AR-V7 nuclear import: we incubated cells with WGA, an inhibitor of nucleoporin-mediated nuclear transport and monitored GFP-AR-V7 localization by live cell imaging. WGA kept AR-V7 in the cytoplasm in the presence of doxycycline suggesting that AR-V7 nuclear import requires active transport via the NPC. Representative confocal microscopy images were shown. Red: WGA labeling of membranes; Green: GFP-AR-V7; Nuc: nuclear AR-V; Cyto: cytoplasmic AR-V7. Scale bars represent 10  $\mu$ m. **B.** (related to Fig. 2B) HEK293T cells were transfected with plasmids encoding GFP-tagged AR-fl, AR-v567 and AR-V7 in the presence of the catalytic mutant Ran-GTP (mCherry-tagged RanQ69L). Nuclear accumulation of each AR variant was calculated. Representative confocal microscopy images (63x magnification) for each condition are shown. Solid arrow: cell with both cytoplasmic and nuclear proteins; arrowheads: cells with cytoplasmic protein; dashed arrow: cell with nuclear protein. Scale bars represent 10  $\mu$ m. **C.** Graphic display of % Nuclear AR across 30 individual cells per condition. Data represent Mean  $\pm$  SEM with  $n > 10$ , p-value (\*\* $p < 0.01$ , \*\*\*\* $p < 0.0001$ ) was obtained using unpaired two-tailed t-test. **D.** C4-2 cells with endogenous AR-fl stably expressing inducible GFP-AR-V7 were transfected with the catalytic mutant mCherry-tagged RanQ69L. Representative confocal microscopy images are shown. Cytoplasmic enrichment for AR-V7 is shown when the mutant Ran is co-expressed in the same cell. **E.** 22RV1 cells with endogenous AR-fl and AR-V7 were transfected with the catalytic mutant mCherry-tagged RanQ69L. AR-V7 was detected by immunostaining with the RevMab antibody. Downregulation of endogenous AR-V7 protein was observed in cells co-expressing RanQ69L. Solid arrow: cell with cytoplasmic and nuclear AR-V7; dashed arrow: non-transfected cells with endogenous nuclear AR-V7. Dotted ellipses: cells transfected with mutant Ran where AR-V7 appears absent or downregulated. Scale bar, 10  $\mu$ m. **F.** PC3 cells were transfected with GFP-AR-fl or GFP-AR-fl-D-box mutant (A596T, S597T) and they were treated for 4 hr with 10 nM DHT. There was no effect of the D-box mutations on AR-fl nuclear import. Experiments were repeated at least twice.