

Figure 1- figure supplement 1

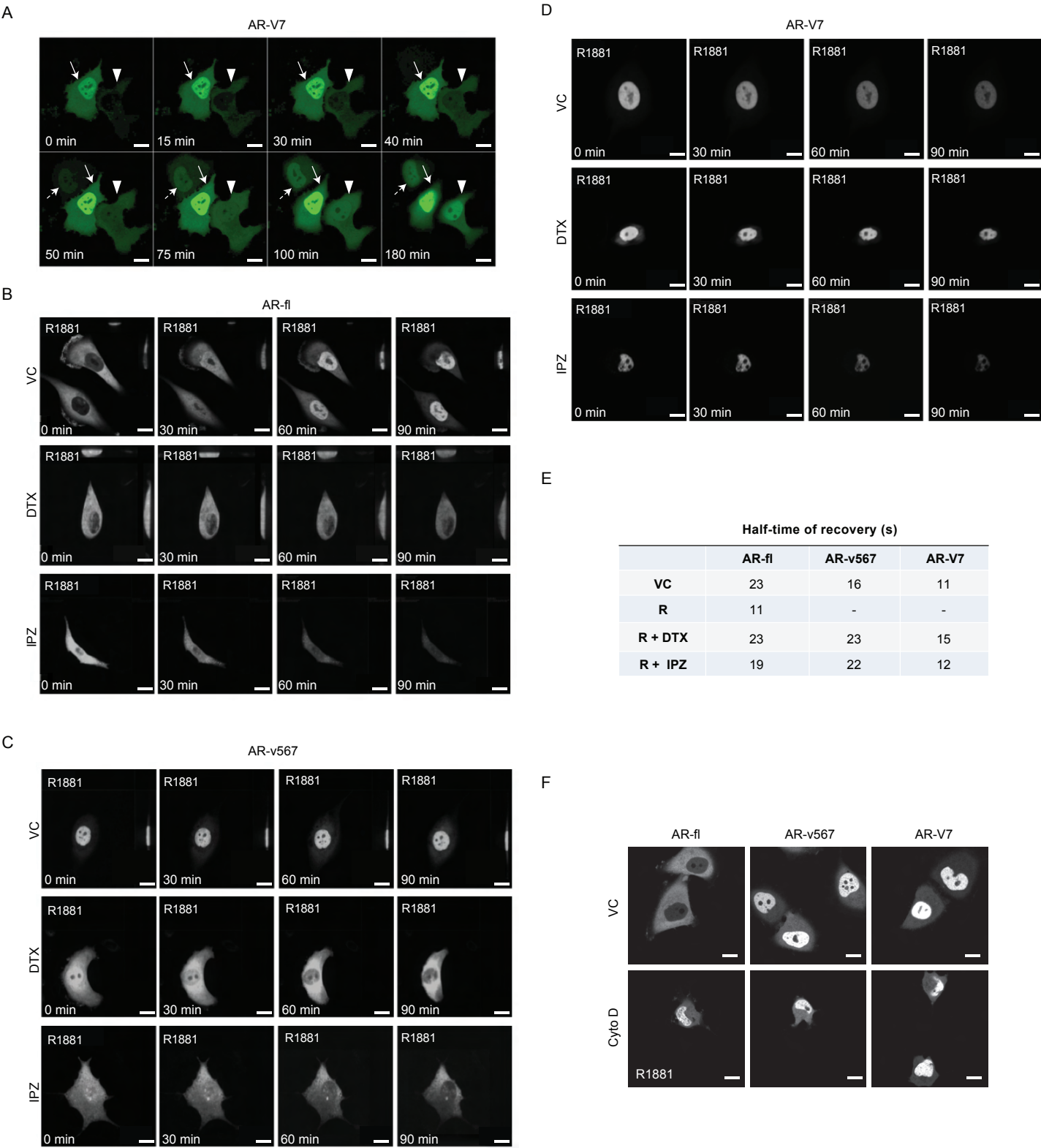


Figure 1- figure supplement 1. AR-V7 exhibits fast nuclear import kinetics independently of microtubules, actin or the importin- α/β pathway. **A.** Plasmid encoding GFP-tagged AR-V7 was microinjected into nuclei of PC3 cells and as soon as GFP was detected in the cytoplasm (~45 min post micro-injection) the kinetics of GFP-AR-V7 nuclear import were monitored by live-cell time-lapse confocal microscopy at 5 min intervals for a total of 180 min. Representative time lapse images are shown at the indicated time points. Solid arrow: cell with both cytoplasmic and nuclear AR-V7 at time 0; Arrowhead: cells with cytoplasmic only AR-V7 at time 0; Dashed Arrow: cell with primarily nuclear AR-V7 first detected at +50 min after the start of imaging. Enhanced AR-V7 nuclear translocation is observed over time for all cells. Notice that there are cells with already extensive nuclear accumulation of AR-V7 at 0 min, suggesting very fast nuclear import kinetics from the time of microinjection (~45 min). Scale bar, 10 μ m. **B-D.** Corresponds to Figure 1D with additional time points. Briefly, M12 prostate cancer cells stably expressing GFP-tagged **(B)** AR-fl, **(C)** AR-v567 or **(D)** AR-V7 were treated as indicated and subjected to live-cell time lapse imaging. R1881: synthetic androgen used to stimulate AR-fl nuclear translocation; DTX: docetaxel, MT-stabilizing drug; IPZ: importazole, importin- inhibitor. Representative images are shown. Scale bar 10 μ m. **E.** Table with T1/2 (half-time recovery) values for each variant (related to Fig. 1F). **F.** PC3 cells were treated with 1 μ g/ml cytochalasin D (Cyto D) or vehicle control (VC) for 1 hour at 37°C following plasmid micro-injection into the nuclei of PC3 cells. Cells were then treated with 10 nM R1881 for 4 hrs and subjected to point-scanning confocal microscopy. Representative images showing are shown. Scale bar 10 μ m. Experiments were repeated at least twice.