Supplementary Fig. S4. Concentration-dependent confirmation of disruptor actives identified in the AR-TIF2 protein–protein interaction biosensor (PPIB) LOPAC screens. **(A)** Concentration-dependent disruption of preexisting DHT-induced AR-TIF2 PPI complexes—compounds with incomplete curves. U-2 OS cells were coinfected with the AR-RFP and TIF2-GFP rAV biosensors, 2,500 cells were seeded into the wells of 384-well assay plates, cultured overnight at 37°C, 5% CO_2, and 95% humidity, and then exposed to 20 nM DHT for 30 min. Cells were then treated with compounds at the indicated concentrations for 3 h, fixed and stained with Hoechst, 20× images in three fluorescent channels were acquired on the IXU automated imaging platform, and the AR-TIF2 PPIs were quantified using the TE image analysis module as described previously. The mean ± SD ($n=3$) percent disruption of preformed AR-TIF2 PPI complexes in cells exposed to the indicated concentrations of 17-α-H-PG (○), nilutamide (▼), spironlactone (◆), budesonide (▲), cotexelone (△), or estrone (▼) are presented. Representative experimental data from one of the three independent experiments are shown. **(B)** Images of AR-RFP phenotypes in cells exposed to compounds that disrupt preexisting DHT-induced AR-TIF2 PPI complexes. U-2 OS cells were coinfected with the AR-RFP and TIF2-GFP rAV biosensors, 2,500 cells were seeded into the wells of 384-well assay plates, cultured overnight at 37°C, 5% CO_2, and 95% humidity, and then exposed to 20 nM DHT for 30 min. Cells were then treated with compounds for 3 h, fixed and stained with Hoechst, 20× images in three fluorescent channels were acquired on the IXU automated imaging platform. Grayscale images of the AR-RFP distribution phenotype of DHT treated cells exposed to 10 μM mifepristone, 10 μM 17-α-H-PG, 10 μM nilutamide, 10 μM spironlactone, 50 μM gugglesterone, 10 μM cyproterone, 50 μM 2-MOED, 50 μM budesonide, 50 μM cotexelone, or 50 μM estrone are presented. Grayscale images of the AR-RFP distribution phenotype of the 0.5% DMSO and 20 nM DHT controls are also presented. Representative images from one of the three independent experiments are shown.