



**Supplementary Fig. S2.** mIA nuclear spot analysis pipeline. HeLa cells containing a multicopy prolactin promoter element integration and stably expressing GFP-tagged estrogen receptor were treated with 10 nM 17-estradiol, 10 nM tamoxifen, or DMSO for 24 h. A total of 120 wells were used for each treatment condition. Metrics examined were (A) percent cells with spot following estradiol treatment, (B) percent cells with spot following tamoxifen treatment, (C) spot loading index, (D) spot total GFP signal, and (E) spot size. C through E were constructed using only estradiol- and tamoxifen-treated wells.