Supplementary Table S2. Three-Day Five-Plate DMSO Validation								
		Max[a] controls All[b] AR-RFP in nucleoli			Min[c] controls All[b] AR-RFP in nucleoli			Plate performance statistics	
Day	Plate	Mean ($n=32$)	SD	CV	Mean ($n=32$)	SD	CV	Z'-factor	S:B ratio
1	1	4,988	480	9.6	459	44	9.7	0.65	10.9
	2	4,883	340	7.0	456	54	11.7	0.73	10.7
	3	4,373	311	7.1	419	35	8.4	0.74	10.4
	4	4,646	378	8.1	436	52	12.0	0.69	10.7
	5	4,539	307	6.8	409	34	8.3	0.75	11.1
2	1	4,949	551	11.1	537	45	8.3	0.59	9.2
	2	5,041	506	10.0	522	37	7.2	0.64	9.7
	3	4,874	390	8.0	539	55	10.2	0.69	9.0
	4	4,857	403	8.3	526	46	8.8	0.69	9.2
	5	4,940	471	9.5	508	29	5.8	0.66	9.7
3	1	5,476	447	8.2	529	40	7.5	0.71	10.4
	2	5,133	297	5.8	492	33	6.6	0.79	10.4
	3	4,990	292	5.9	477	34	7.2	0.78	10.5
	4	5,009	357	7.1	481	35	7.2	0.74	10.4
	5	2,910	196	6.7	281	20	7.0	0.75	10.4

The average inner intensity of AR-RFP (Ch3) within TIF2-GFP-positive nucleoli (Ch2) data presented was generated in three independent experiments of 5×384-well DMSO validation plates. These experiments were designed to mimic 3 days of automated screening operations in the AR-TIF2 PPIB HCS assay, save that DMSO was added to wells instead of compounds. The average inner intensity of AR-RFP Ch3 data of the maximum and minimum plate controls from each of the 15×384-well plates are presented.

[a]Max, maximum plate controls (20 nM DHT and 0.2% DMSO, $n=32$).
[b]All, average inner intensity AR-RFP within TIF2-GFP-positive nucleoli.
[c]Min, minimum plate controls (0.2% DMSO, $n=32$).
PPIB, protein–protein interaction biosensor.