

Supporting Information for:

Dynamic imaging of small molecule-induced protein-protein interactions in living cells with a fluorophore phase transition-based approach

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Table of Contents:

S. 1 Supplemental Figures

Figure S1. Amino acid sequence of HO-Tag3 and HO-Tag6.

Figure S2. Quantification of SPPIER signal.

Figure S3. SPPIER signal dependence on ligand concentration.

Figure S4. Kinetics of the SPPIER signal upon addition of rapamycin, followed by DMSO.

S. 2 Video legends

Figure S1. Amino acid sequence of HO-Tag3 and HO-Tag6.

HOTag3 (hexamer) GEIAKSLKEIAKSLKEIAWSLKEIAKSLKG

HOTag6 (tetramer) TLREIEELLRKIIEDSVRSVAELEDIEKWLKKI

Figure S2. Quantification of SPPIER signal related to time-lapse imaging data in Figures 1 and 2. (A) SPPIER signal formula. (B) SPPIER signal related to Fig. 1B. (C) SPPIER signal related to Fig. 1D. (D) SPPIER signal related to Fig. 2A. (E) SPPIER signal related to Fig. 2B. (F) SPPIER signal related to Fig. 2C.

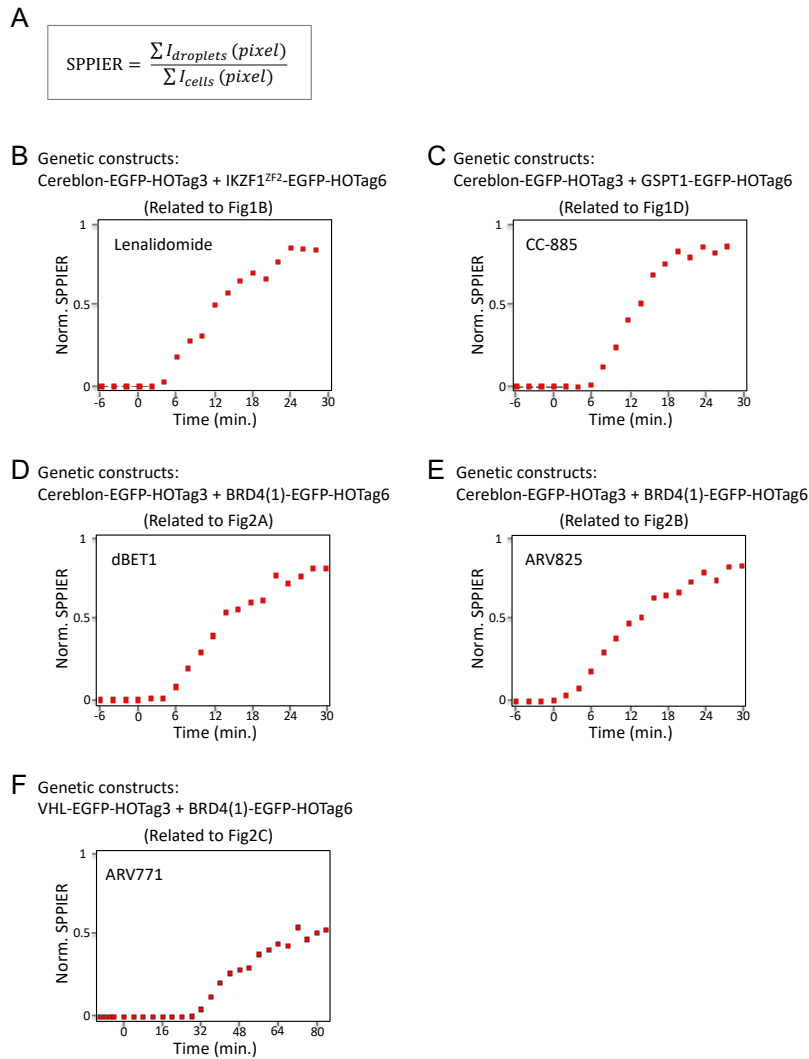


Figure S3. SPPIER signal dependence on ligand concentration. (A) Fluorescence images of transfected cells expressing the constructs incubated with various concentrations of ARV825. (B) Quantified SPPIER signal over time. (C) Quantified SPPIER signal (at 60 minutes) over concentration of ARV825. HEK293 cells transiently expressed cereblon-EGFP-HOTag3, BRD4(1)-HOTag6. Scale bar, 20 μm .

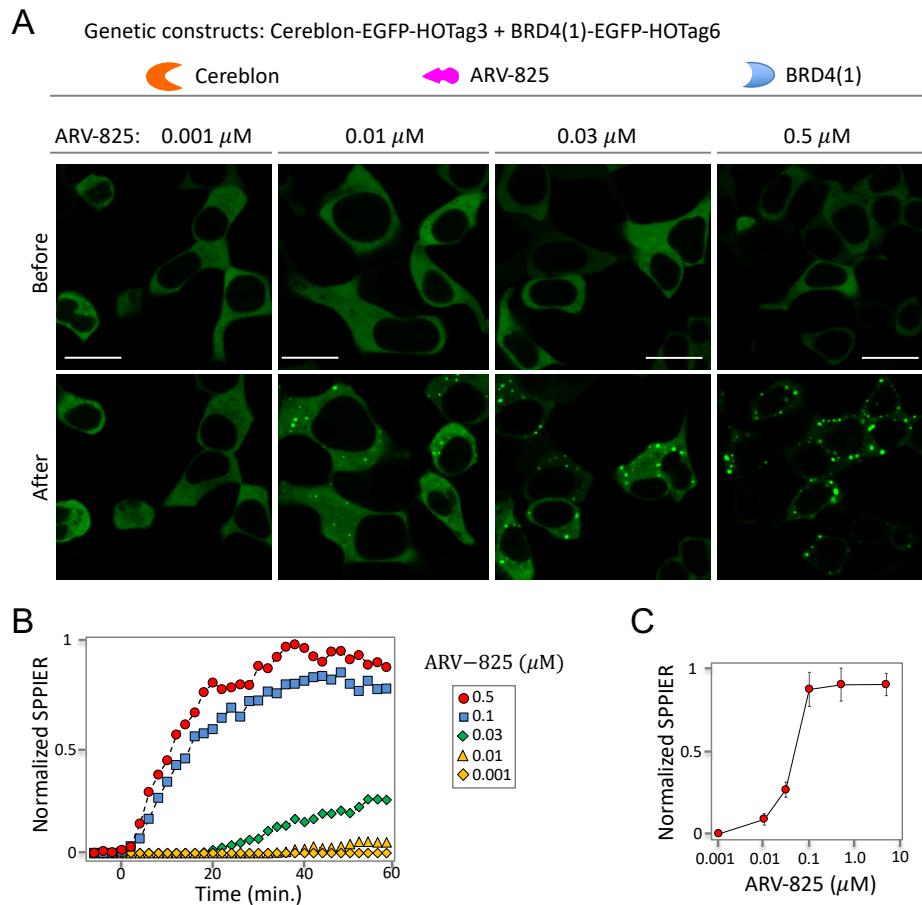
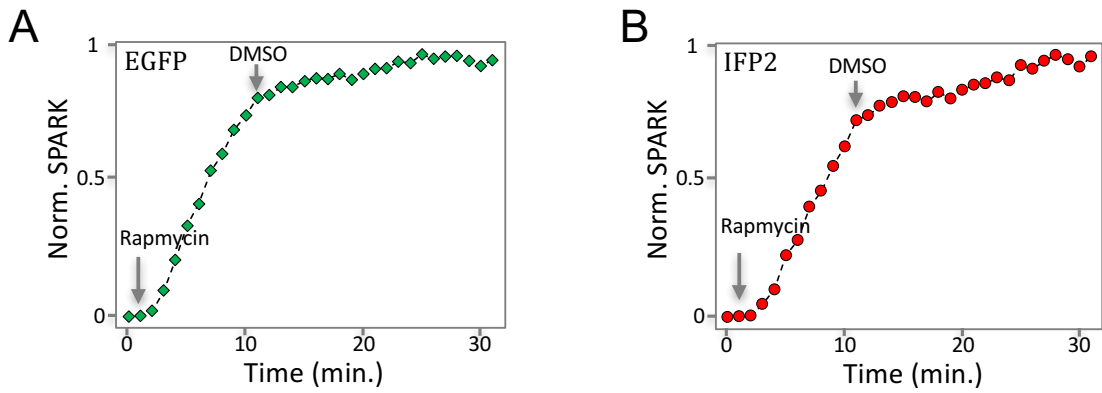


Figure S4. Kinetics of the green (A) and infrared (B) fluorescent SPPIER signal upon addition of rapamycin (0.1 μ M) followed by DMSO. HEK293 cells transiently expressed FKBP12-EGFP-HOTag3, Frb-p53^{TAD}-HOTag6, and HDM2^{p53BD}-IFP2. IFP2 is an improved infrared fluorescent protein (IFP).



Supplementary Video

Video 1. Time-lapse confocal imaging of lenalidomide (1 μ M)-induced interaction between CRBN and IKZF1. HEK293 cells were transiently transfected with CRBN-EGFP-HOTag3 and IKZF1^{ZF2}-EGFP-HOTag6.

Video 2. Time-lapse confocal imaging of CC-885 (1 μ M)-induced interaction between CRBN and GSPT1. HEK293 cells were transiently transfected with CRBN-EGFP-HOTag3 and GSPT1-EGFP-HOTag6.

Video 3. Disassembly of ARV-825 induced GFP droplet upon ARV-825 wash. HEK293 cells were transiently transfected with CRBN-EGFP-HOTag3 and BRD4(1)-EGFP-HOTag6. Cells were pre-incubated with 100 nM ARV-825. Left, ARV-825 was washed. Right, ARV-825 was not washed.

Video 4. Time-lapse confocal imaging of cells upon addition of rapamycin (0.1 μ M) and nutlin-3a (10 μ M). HEK293 cells transiently expressed FKBP12-EGFP-HOTag3, Frb-p53^{TAD}-HOTag6, and HDM2^{p53BD}-IFP2. IFP2 is an improved infrared fluorescent protein (IFP).

Video 5. Time-lapse confocal imaging of the zoom-in area in video 23 (boxed area shown in Figure 4B).