Supplementary Table and Supplementary Figures

"Converting a Binding Protein into a Biosensing Conformational Switch Using Protein Fragment Exchange (FREX)" by Huimei Zheng, Jing Bi, Mira Krendel, and Stewart N. Loh

Table S1. Stability parameters of FN3 variants (pH 7.0, 20 °C) obtained by fitting denaturation data to the equation $\Delta G = \Delta G^{H2O} - m[\text{GdnHCl}]$. C_m is the midpoint of denaturation. Errors are standard deviations of triplicate experiments.

FN3 variant	ΔG^{H2O} (kcal mol^{-1})	m (kcal mol^{-1} M^{-1})	C_m (M)
$FN3^{BN}$	5.79 ±0.7	2.02 ±0.3	2.78 ±0.04
$FN3^{BN+V77A}$	4.18 ±0.5	2.29 ±0.6	1.96 ±0.3
$FN3^{BN+V77G}$	4.50 ±0.1	2.80 ±0.1	1.61 ±0.04
$FN3^{BN+I75V}$	4.10 ±0.3	1.47 ±0.1	2.77 ±0.01
$FN3^{BN+I75A}$	2.25 ±0.1	2.05 ±0.1	1.11 ±0.03

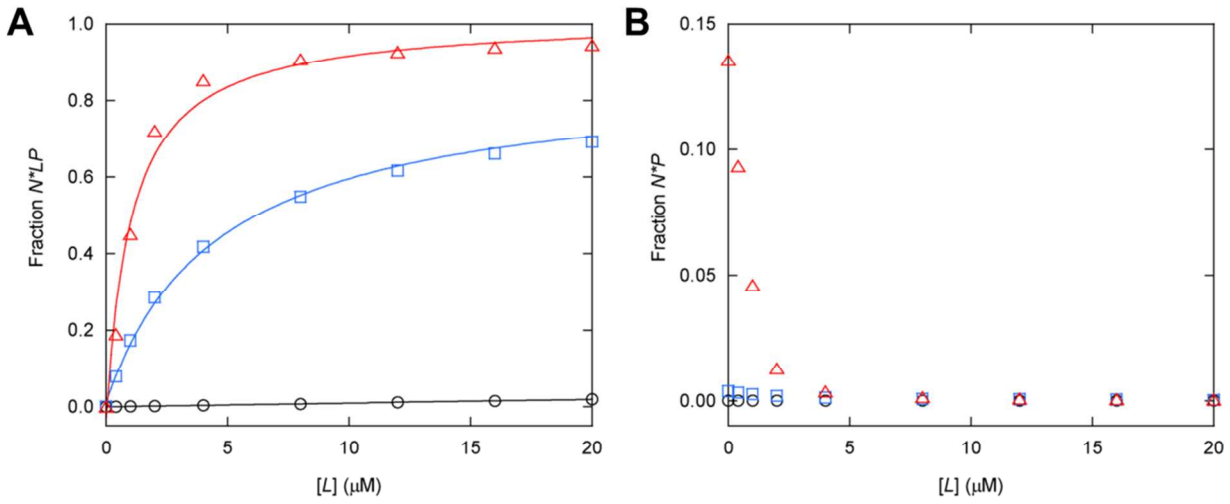

Figure S1. Simulations of Eqs. 1-3 (see text) showing fractions of *N*LP* **(A)** and *N*P* **(B)** as a function of ligand concentration. Data sets were generated using $K_{ex} = 10^5$ M^{-1}, $K_a = 10^8$ M^{-1}, and K_{unf} values of 5×10^{-5} (black circles), 0.02 (blue squares), and 10 (red diamonds). Concentrations of *N* and *P* were set to 2 μM each. Lines in panel A are best fits of the simulated data to the one-site binding equation; fitted $K_{a,app}$ values are 9.7×10^5 M^{-1} and 2.2×10^5 M^{-1} for the red and blue data sets, respectively. Simulations were performed using the Gepasi program [Mendes, P. (1997) Biochemistry by numbers: simulation of biochemical pathways with Gepasi 3. *Trends Biochem. Sci. 22*, 361-363].

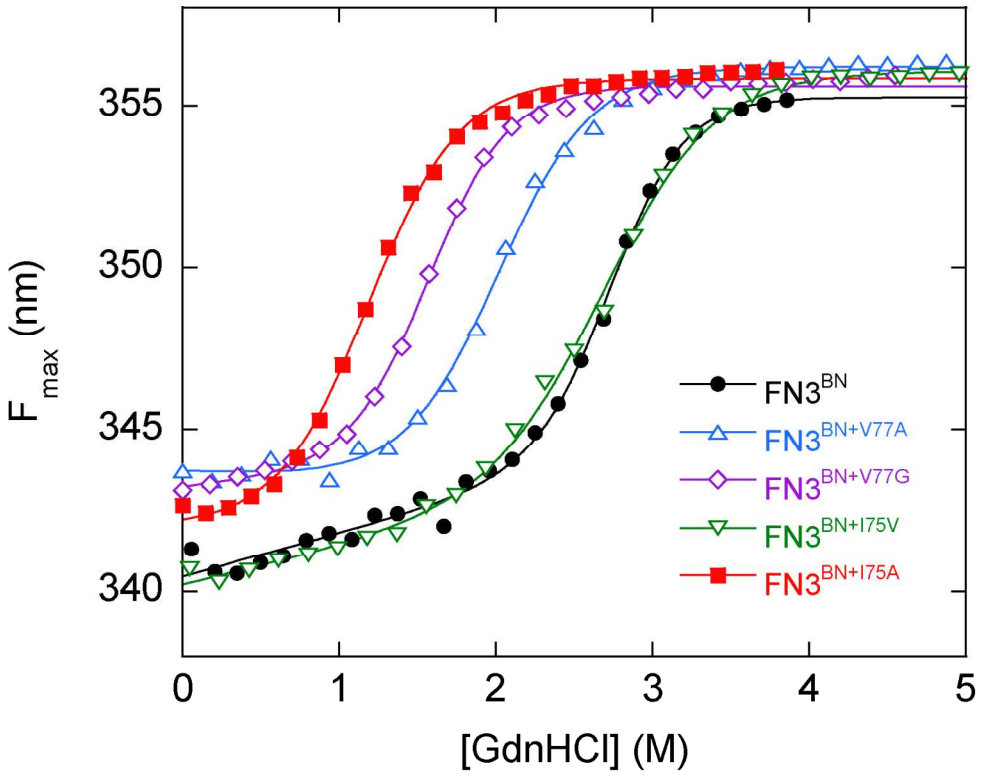

Figure S2. Guanidine hydrochloride (GdnHCl)-induced denaturation of FN3 variants monitored by Trp fluorescence. For each emission spectrum (350 – 450 nm), the wavelength of maximum fluorescence (F_{max}) was determined using the peak analysis package of the Igor Pro program (WaveMetrics). These data were then fit to the two-state linear extrapolation equation to generate the lines above. Samples were prepared by mixing a solution of protein (5 – 10 μM) in 20 mM sodium phosphate (pH 7.0) with an identical solution of protein in the same buffer plus 5 – 7 M GdnHCl, using a Hamilton Microlab 540B dispenser. Samples were equilibrated for 3 h at 20 °C. Data were collected on a Horiba FluoroMax-4 fluorometer with an excitation wavelength of 280 nm. Final denaturant concentrations were determined by index of refraction.

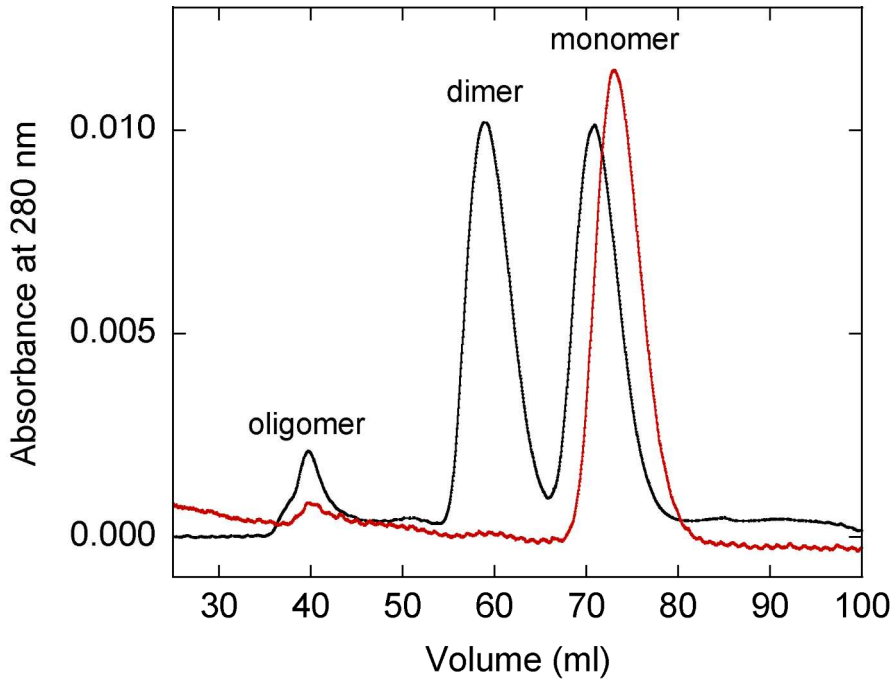

Figure S3. Size exclusion chromatograms of WT FN3-HA4 (black) and the GSSV → YGGG mutant used in the present study (red). Protein concentrations are ~30 μM. Data were collected using a Superdex-75 column (GE Healthcare).

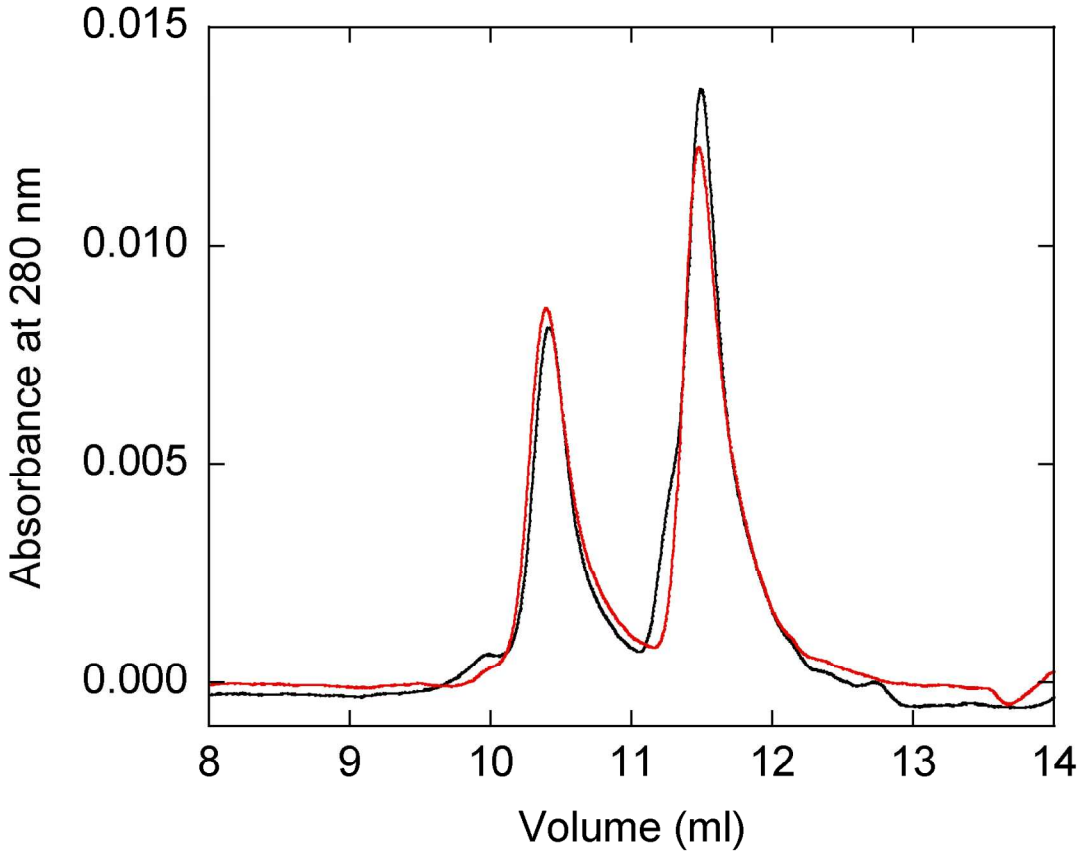

Figure S4. Size exclusion chromatograms of ($FN3^{BN}$+P48+SH2) (black) and ($FN3^{BN+I75V}$+P48+SH2) (red). Protein concentrations and experimental conditions are identical to those of Fig. 2 of the text.

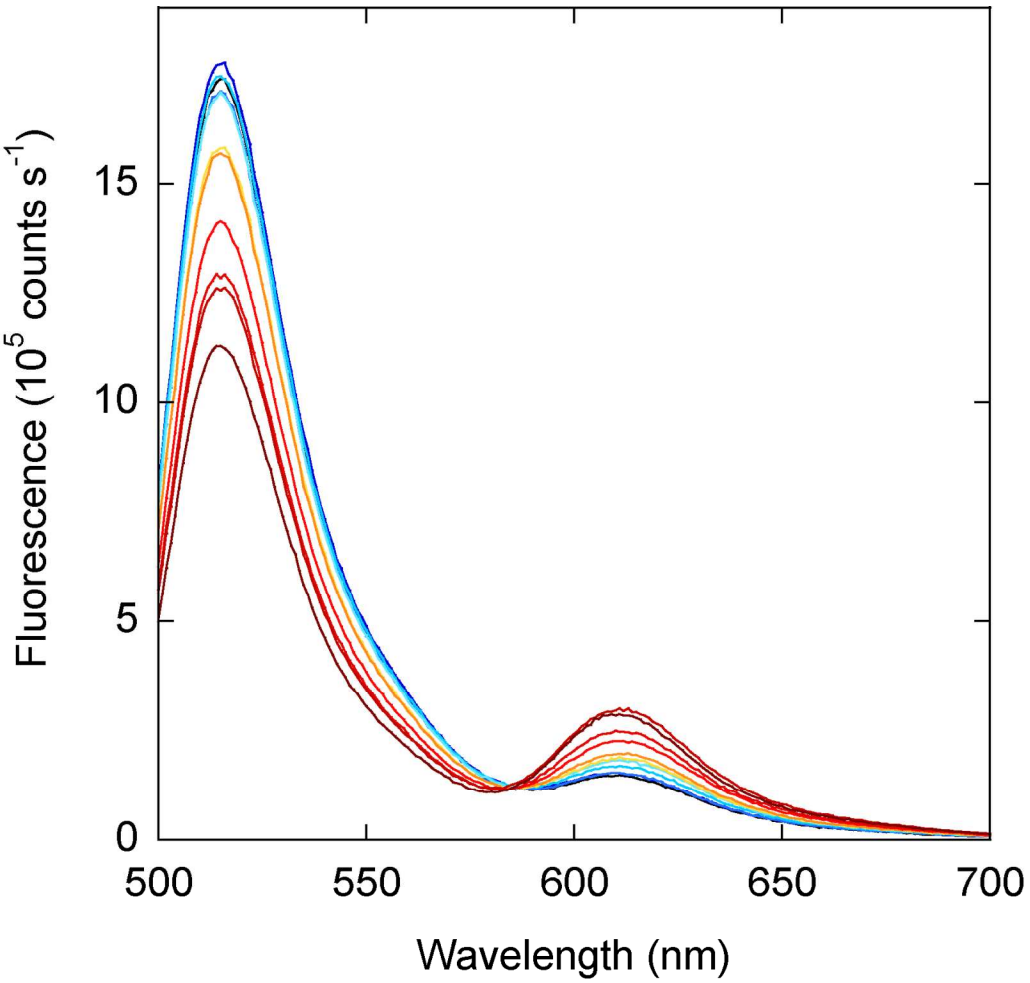

Figure S5. Unprocessed fluorescence spectra of $FN3^{BN+I75A}$ (labeled with donor at the N-terminus) plus acceptor-labeled P60 are overlaid to show ratiometric changes in fluorescence intensity as a function of increasing SH2 concentration, from zero (black) to 50 μM (dark red) SH2.

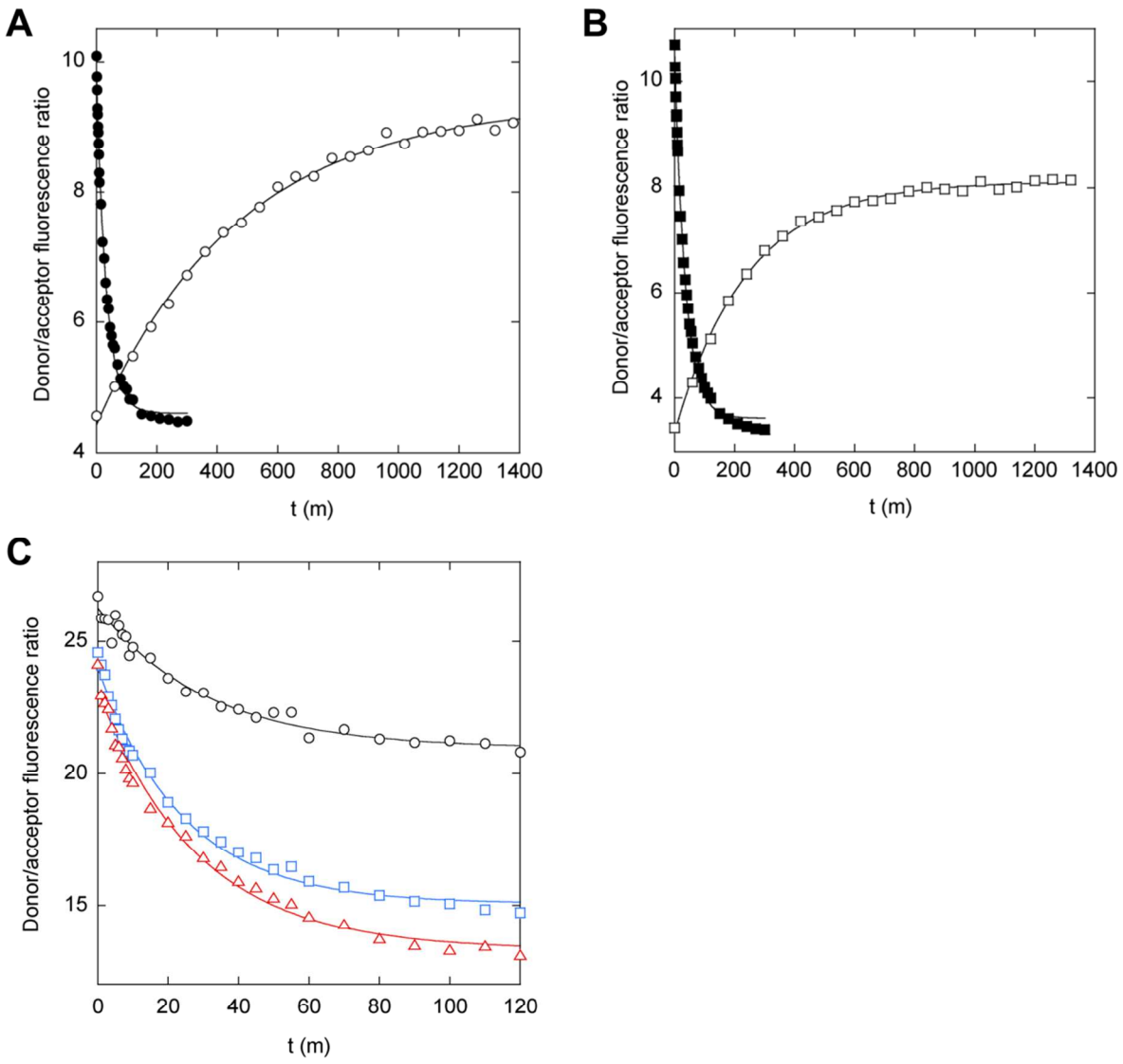

Figure S6. Switching kinetics monitored by FRET. **(A)** and **(B)** show on-rates (closed symbols) and off-rates (open symbols) of the P48 and P60 sensors, respectively. $FN3^{BN+I75A}$ labeled with Alexa488 at the N-terminus (2 μM) was pre-mixed with Alexa495-labeled P48 or P60 (2 μM). SH2 (50 μM) was then added and fluorescence emission spectra were recorded as described in the text. Dissociation measurements were performed by adding a 10-fold excess of unlabeled $FN3^{BN+I75A}$ (20 μM) to the above ternary complexes. **(C)** The rate of ternary complex formation

does not depend on SH2 concentration. Experimental conditions are identical to those in panel A except protein concentrations are 0.5 μM $FN3^{BN+I75A}$, 0.5 μM P48, and 10 μM SH2 (black circles), 20 μM SH2 (blue squares), or 40 μM SH2 (red triangles). Lines are best fits of the data to single-exponential functions. Fitted k_{on} values are (from lowest to highest SH2 concentration and in units of 10^{-4} s^{-1}): 5.45, 6.84, and 5.86.

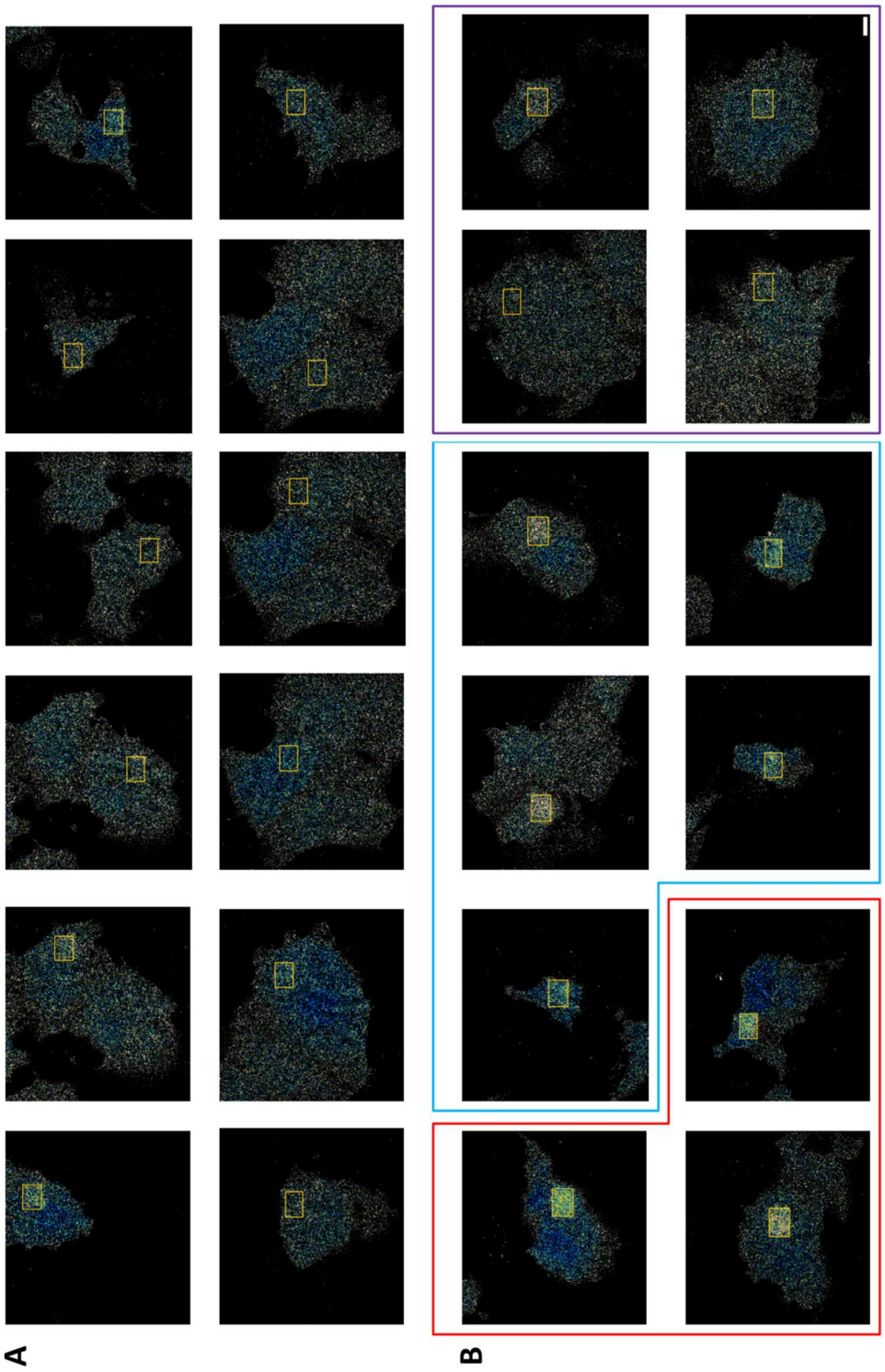

Figure S7. FRET images of Cos7 cells transfected with: **(A)** EGFP-FN3$^{BN+I75A}$ and mCherry-P48, and **(B)** EGFP-FN3$^{BN+I75A}$, mCherry-P48, and SH2. Boxes indicate areas in the cell that

were photobleached. Colored borders around images in panel B denote cells in which the ratio of FRET efficiency inside versus outside the box is >3 (red), 2 – 3 (blue), and <2 (purple).