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Supplemental information

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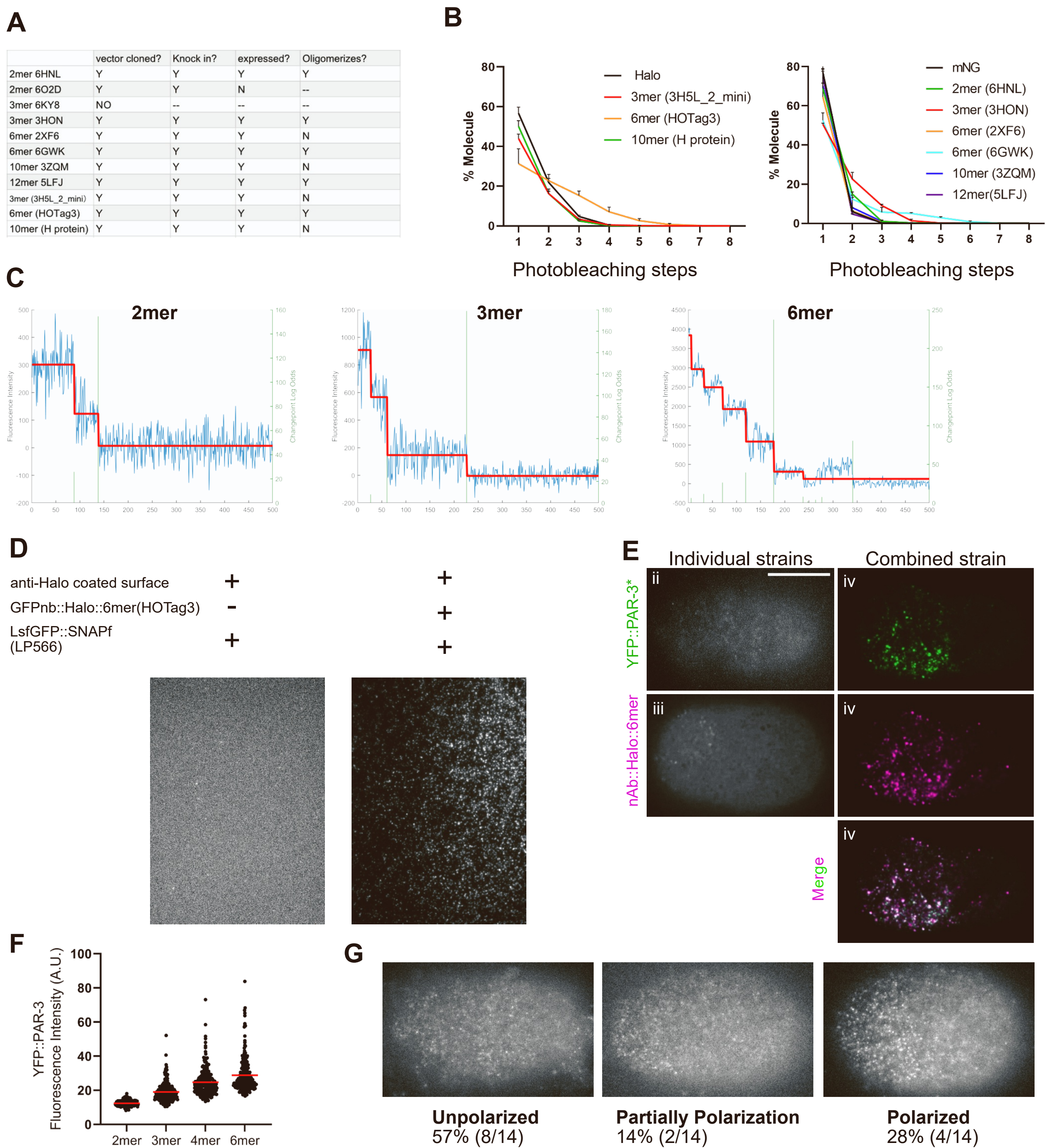

	vector cloned?	Knock in?	expressed?	Oligomerizes?
2mer 6HNL	Y	Y	Y	Y
2mer 6O2D	Y	Y	N	--
3mer 6KY8	NO	--	--	--
3mer 3HON	Y	Y	Y	Y
6mer 2XF6	Y	Y	Y	N
6mer 6GWK	Y	Y	Y	Y
10mer 3ZQM	Y	Y	Y	N
12mer 5LFJ	Y	Y	Y	Y
3mer (3H5L_2_mini)	Y	Y	Y	N
6mer (HOTag3)	Y	Y	Y	Y
10mer (H protein)	Y	Y	Y	N

Figure S1. Testing and Optimization of Extra Oligomerization Domains, Related to Figure 1

(A) Summary of the potential EOD domains tested. (B) The validation of EOD oligomer sizes using sc-SiMpull followed by photobleaching step counting. (3 replicates for each strain). EODs attached to Halo and tested by photobleaching Halo (left panel) and EODs attached to mNG and tested by photobleaching mNG (right panel). Please note that under this methodology, the photobleaching steps are predicted to be smaller than the real number of molecules in an oligomer due to the non-perfect maturation rate (50-80%) of fluorescent proteins and simultaneous bleaching of multiple fluorophores. (C) Examples of photobleaching traces of 2mer, 3mer and 6mer bleaching in 2 steps, 3 steps and 6 steps respectively. (D) sc-SiMpull assay testing the binding between GFP nanobody and GFP. The device surface was functionalized with anti-Halo antibodies. First, a GFPnb::Halo::6mer embryo was lysed to capture the EOD construct by means of the Halo – anti-Halo interaction. Then, a second embryo expressing sfGFP::SNAPf embryo was lysed to test whether the GFP nanobody, present in the EOD construct, could capture sfGFP. TIRF imaging for GFP signal. (E) Detailed illustration of the rescue experiment result, hexamer(HOTag3) was shown as an example. Left panels: (Top) TIRF imaging of embryo from YFP::PAR-3* monomeric mutant strain after polarization stage, corresponding to ii in panel figure 1A. (Bottom) TIRF imaging of embryos from GFPnanobody::HaloTag::6mer(HOTag3) construct strain after polarization stage, corresponding to iii in panel figure 1A. Right panels: TIFR imaging of polarized zygote from YFP::PAR-3;nAb::HaloTag::6mer(HOTag3) strain, corresponding to iv in panel figure 1A (Top) YFP::PAR-3 channel. (Middle) nb::Halo::6mer(HOTag3) channel. (Bottom) composite image with both YFP and Halo signal. Scale bar: 10 µm. (F) YFP fluorescence intensity of the foci on the cortex of YFP::PAR-3*;nAB::BFP::EOD embryos dissected from mlc-4 RNAi treated worms. (G) The degree of polarity defect in the 2mer strain and the fraction of each category.

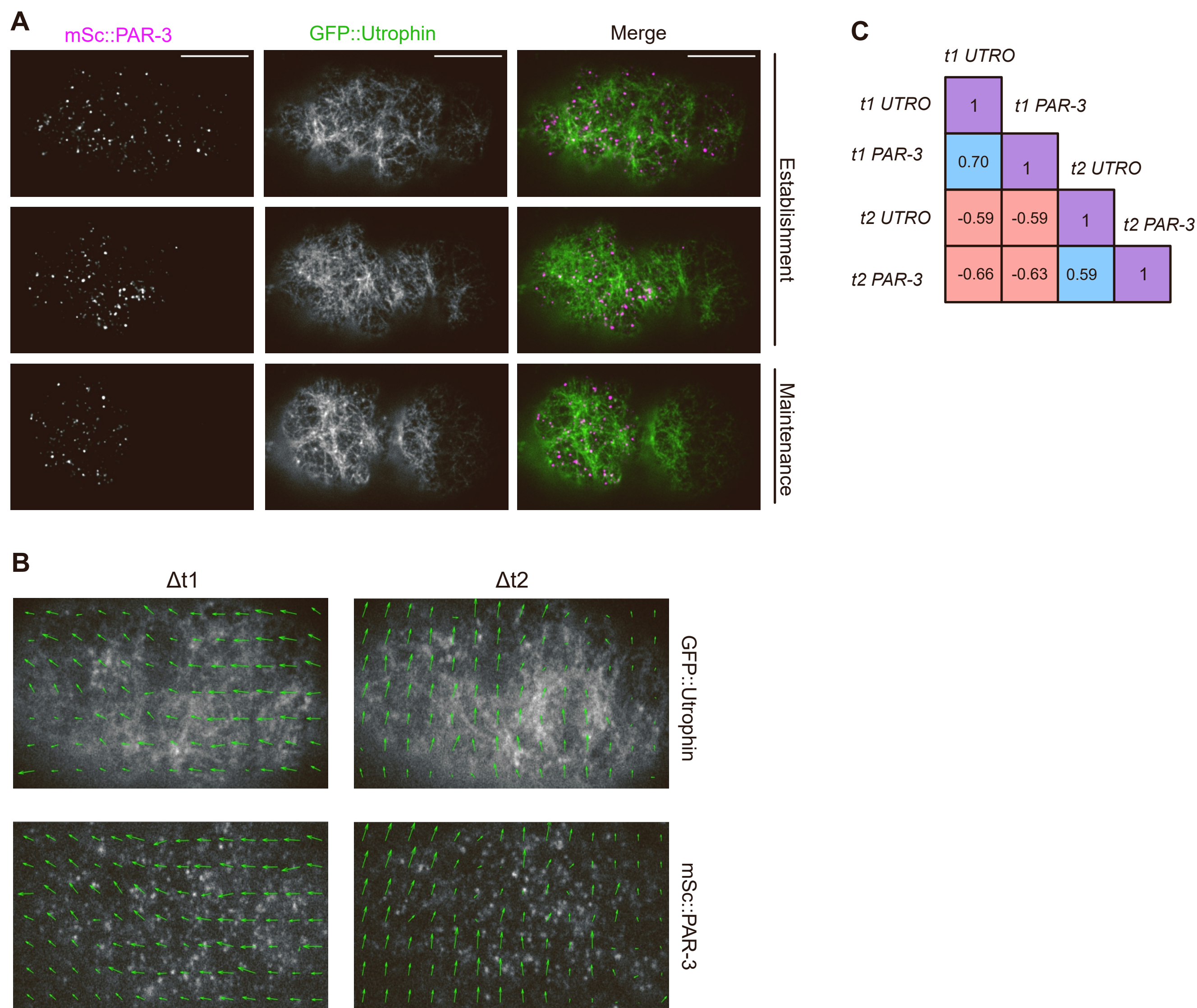

Figure S2. Correlation between PAR-3 and Actin Movement, Related to Figure 3

(A) TIRF imaging of mSc::PAR-3;GFP::Utrophin zygote. Anterior to the left. Scale bar = 10µm. (B) The vector field generated by PIVlab at the polarization stage. (C) Pearson's correlation of the vector fields in (B). Blue boxes represent experiments where the vector field of actin and PAR-3 from the same time point is compared, red boxes represent negative controls where unrelated images are compared, and purple boxes serve as positive controls where the same image is compared to itself.

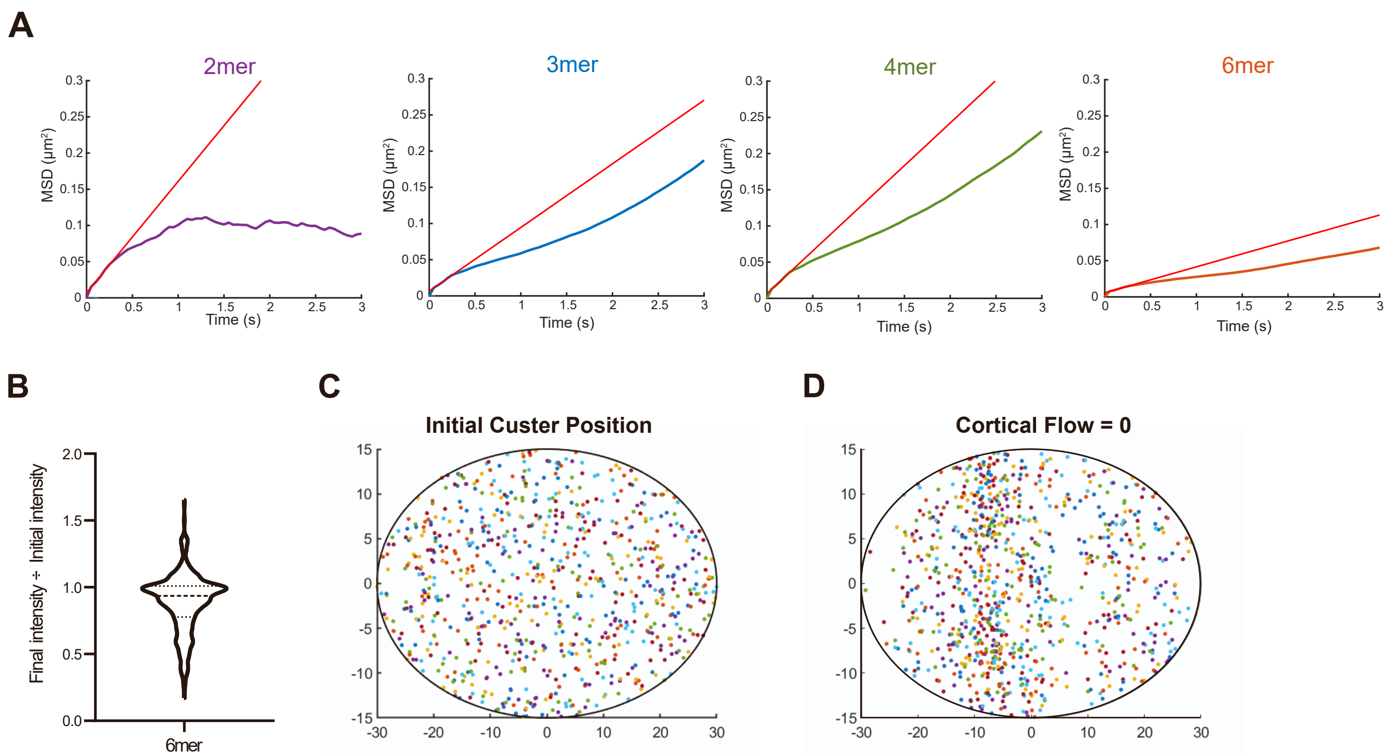

Figure S3. Additional information for simulating PAR-3 polarization, Related to Figure 5

(A) Curve fits used to determine the diffusion coefficient for engineered PAR-3 oligomers. (B) Ratio of final:initial cluster intensity for 6mers under our imaging conditions. n=4,309 particles. (C) An example of the random initial cluster positions used for simulations. (D) PAR-3 cluster segregation simulated with cortical flow = 0 globally. Positive feedback leads to transient enrichment of particles in random locations but does not result in polarity.

Figure S4

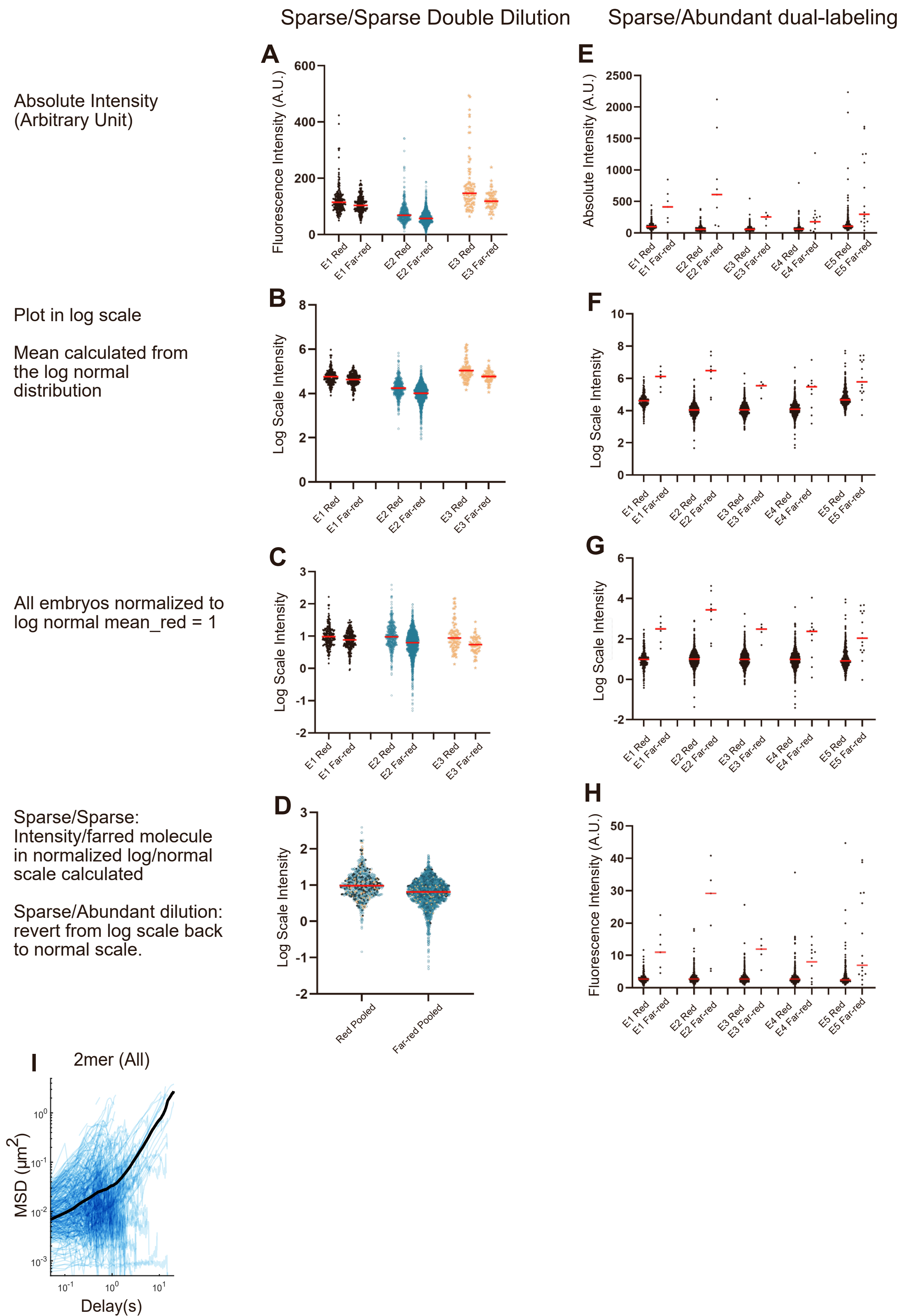

Figure S4. Calibration of single dye molecule fluorescence intensity, Related to Figure 6

(A) The global intensity of red and farred signals in double-dilution experiments. Each dot represents a single foci detected. Red lines indicate the means. (B) The log scale intensity of (A). (C) The datasets in (B) are normalized to 1. (D) All 3 datasets from the same color channel are pooled together for calculating the single red/single far-red ratio. (E) The global intensity of red and the farred channel intensity of particles of interest in sparse/abundant dual-labeling experiments. Each dot represents a single foci detected. Red lines indicate the means. (F) The log scale intensity of (E). (G) The datasets in (F) are normalized to 1. (H) The normalized log scale intensity is converted back to normal scale, ready for converting into cluster size. (I) log/log scale MSD curves for all 2mers, each curve describing the motion of a single PAR-3 cluster. Data was acquired and pooled from 3 embryos.