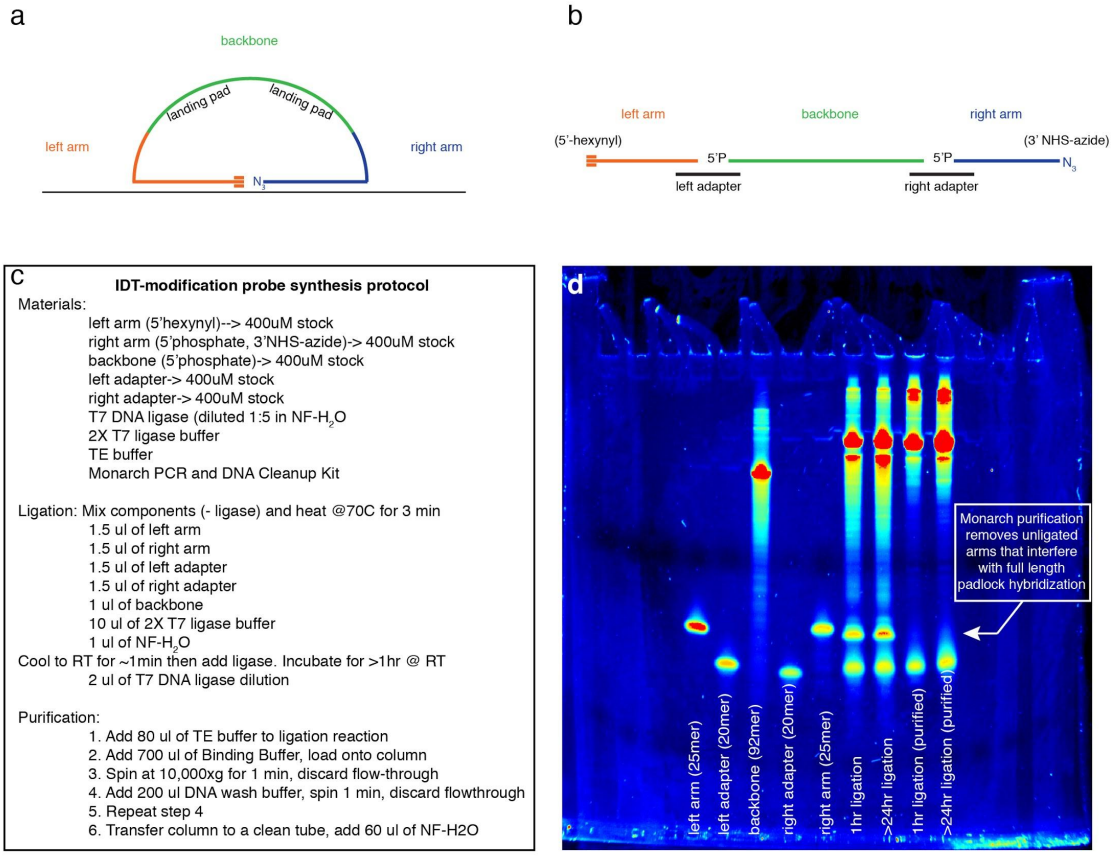
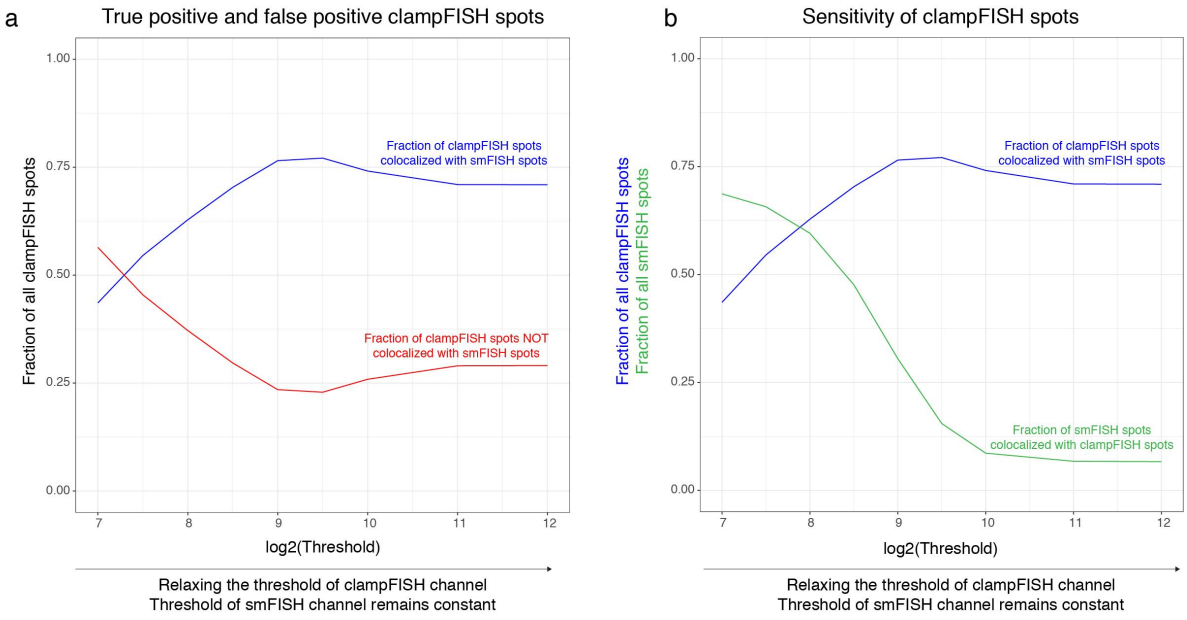
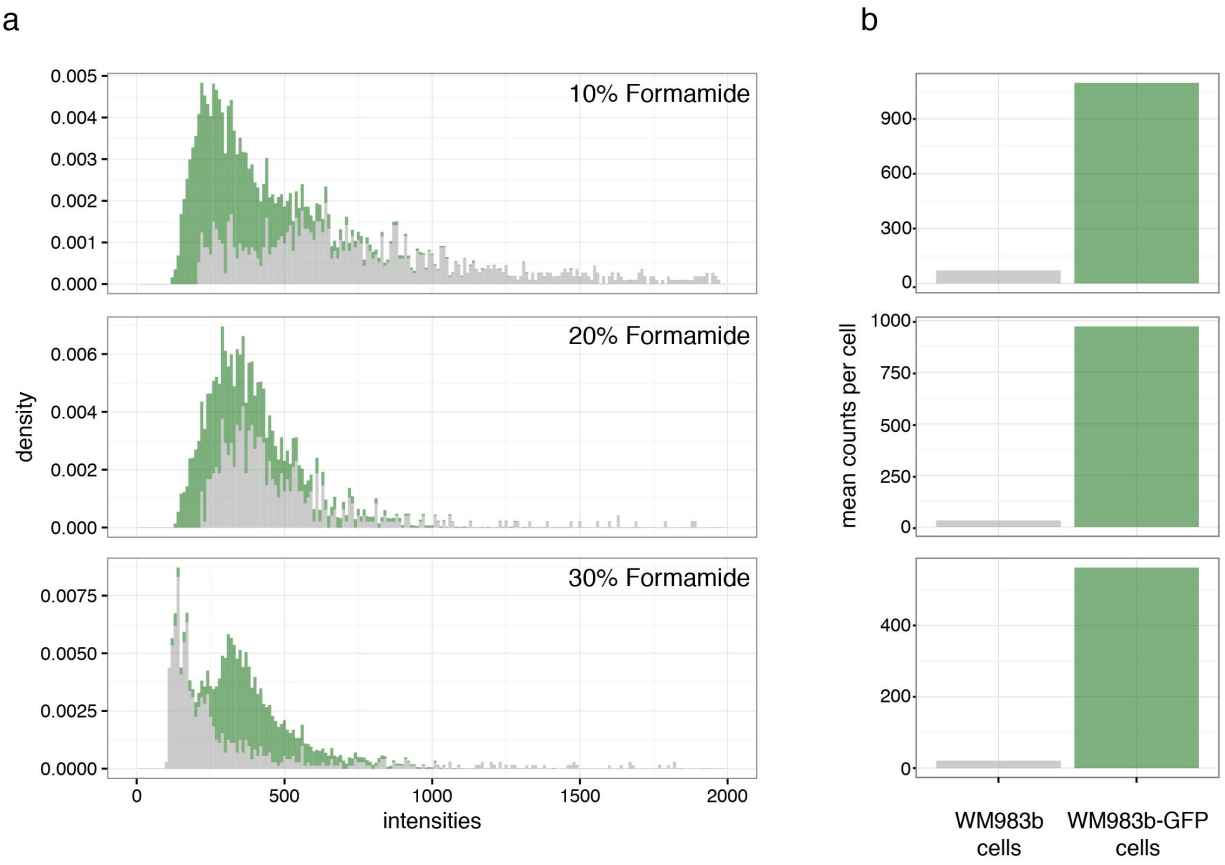
Supplementary information



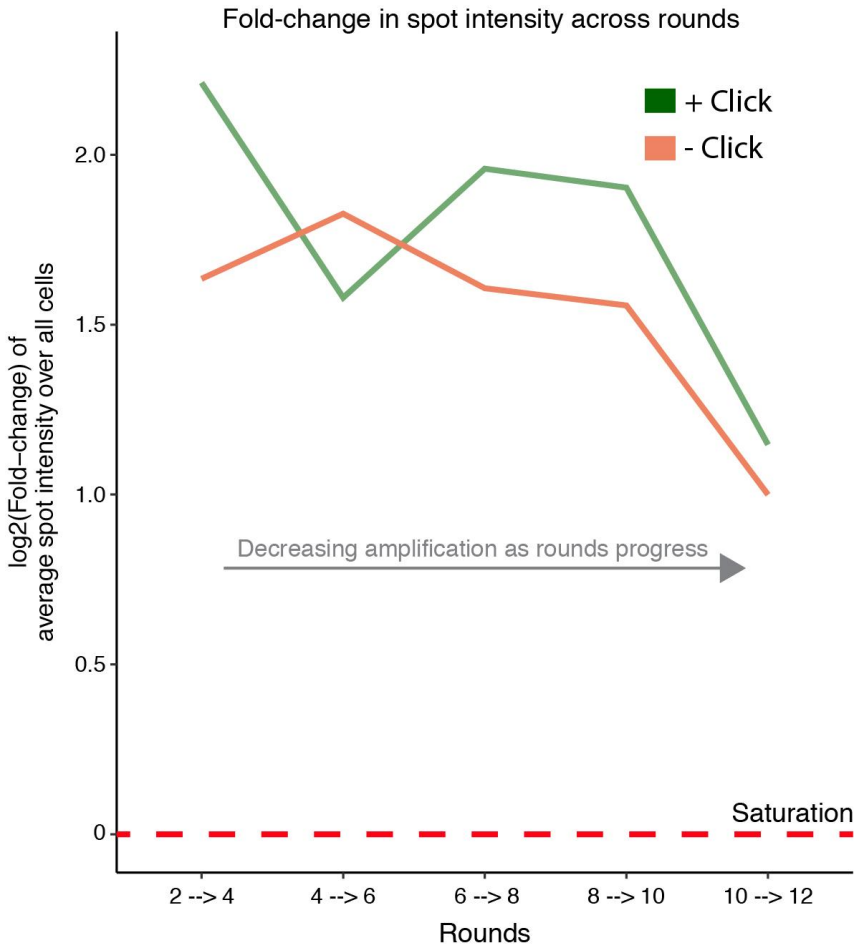
Supplementary Figure 1. Generation of ClampFISH probes. (a) Diagram of individual pieces of each clampFISH probe. (b) Linear diagram of clampFISH probe ligation scheme. (c) ClampFISH probe ligation protocol. (d) 15% TBE-UREA gel showing separation of individual pieces, ligation product, and product after purification.



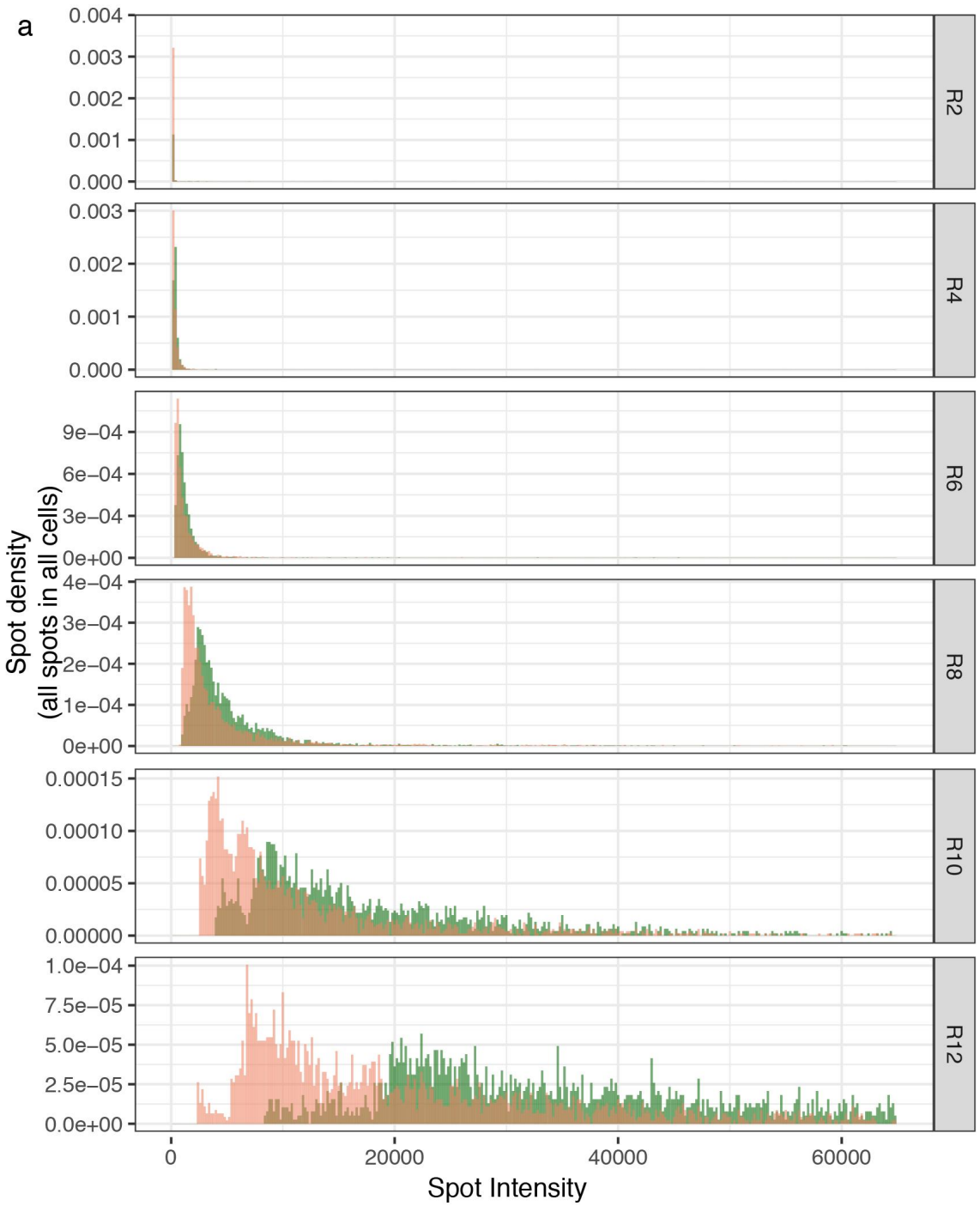
Supplementary Figure 2. (a) Fraction of clampFISH spots that colocalize with single molecule RNA FISHspots (blue) and spots that do not colocalize with single molecule RNA FISH spots (red) as the threshold for spot calling is relaxed. **(b)** (blue) Fraction of all clampFISH spots that colocalize with single molecule RNA FISH spots as the threshold is relaxed, (green) fraction of all single molecule RNA FISH spots that colocalize with clampFISH spots. Note: Single-molecule spot localization is difficult at very high fluorescence levels, making stringent spot localization difficult for bright clampFISH spots. This may contribute to an apparent (false) loss of colocalization with single molecule RNA FISH spots, whose location is easier to computationally estimate.



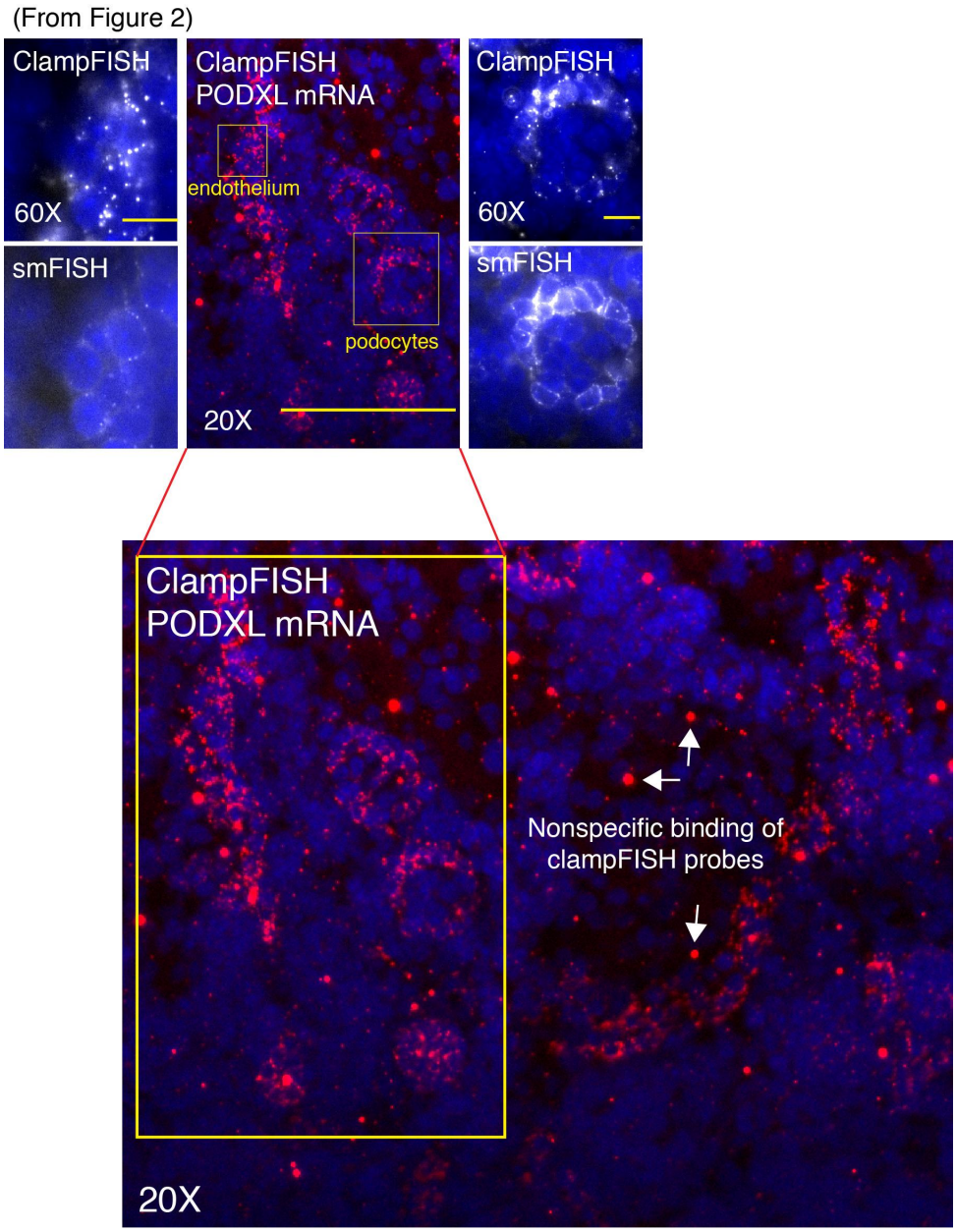
Supplementary Figure 3. Optimizing [Formamide] in hybridization buffer. (a) Density plots showing single spot intensity distributions across 3 different formamide concentrations in hybridization buffer. WM983b-GFP cells were probed for GFP mRNA using clampFISH probes out to round 2 (green) and WM983b cells were also stained for GFP mRNA using GFP targeting clampFISH probes (gray). (b) Mean spot counts per cell were assessed in WM983b cells (gray) and WM983b-GFP cells (green) after 2 rounds of amplification.



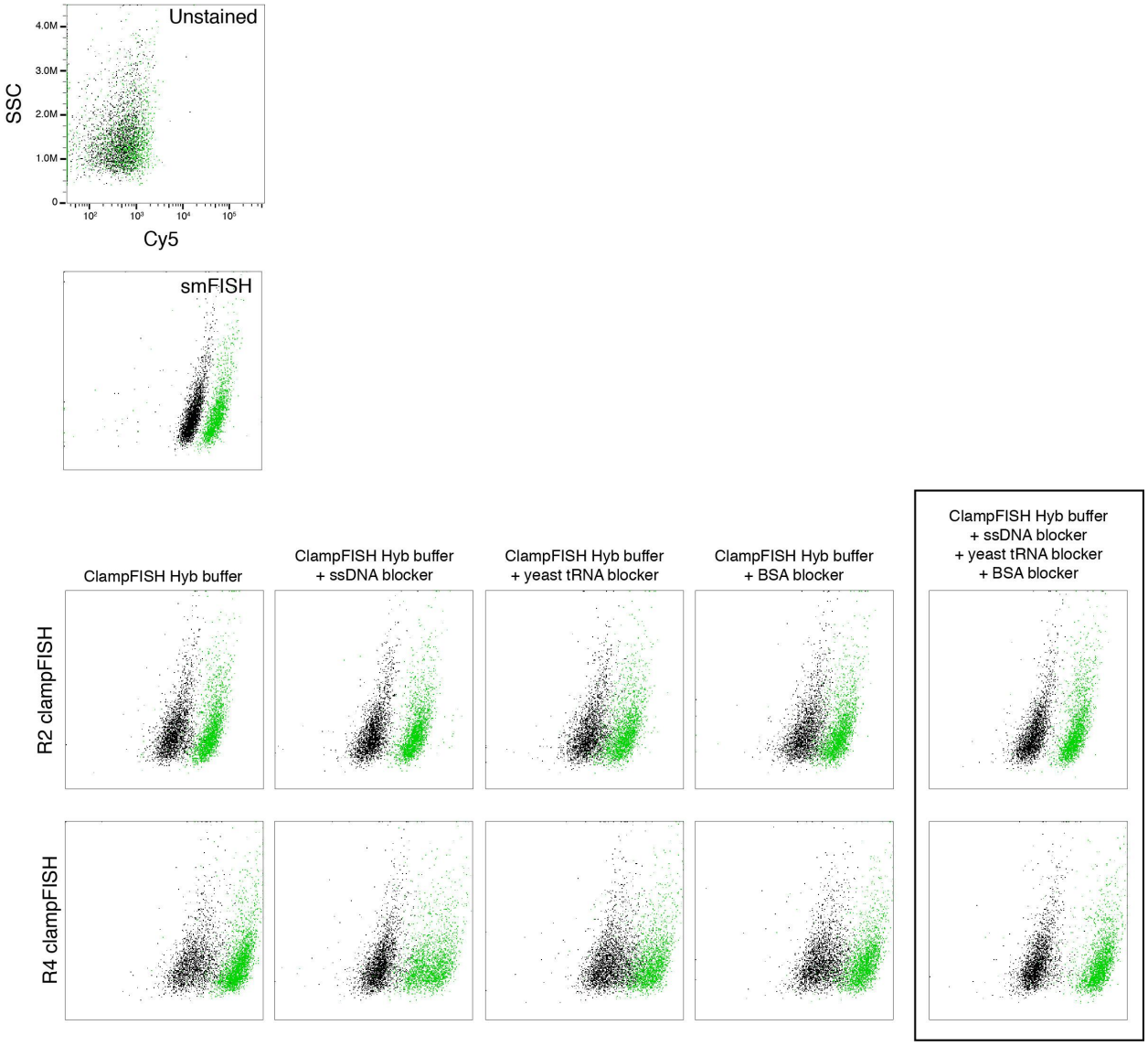
Supplementary Figure 4. Saturation curve showing the fold-change in amplification as the number of rounds of clampFISH amplification progress.



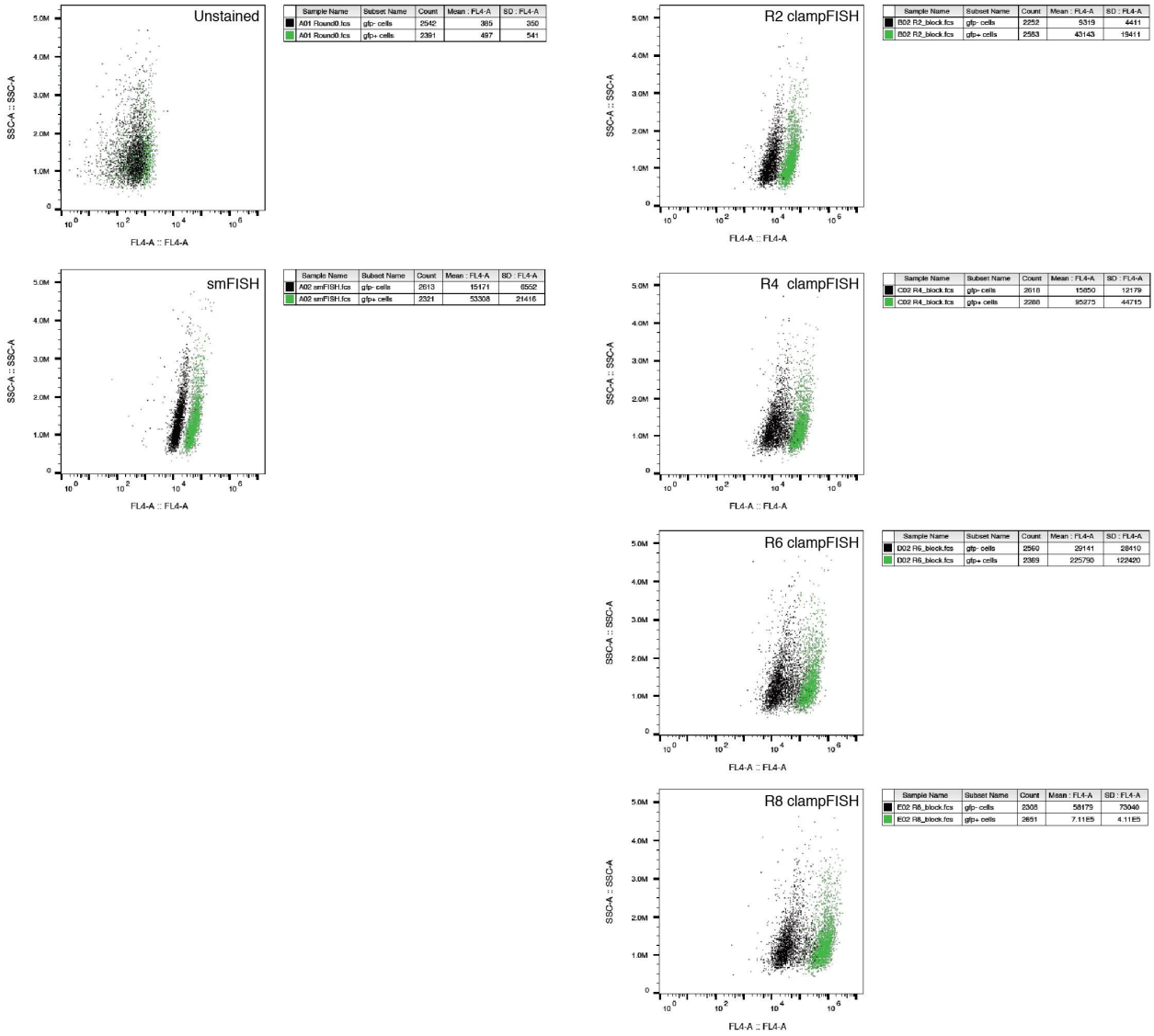
Supplementary Figure 5. Density of the intensity of all spots detected at each round in click vs. no click samples.



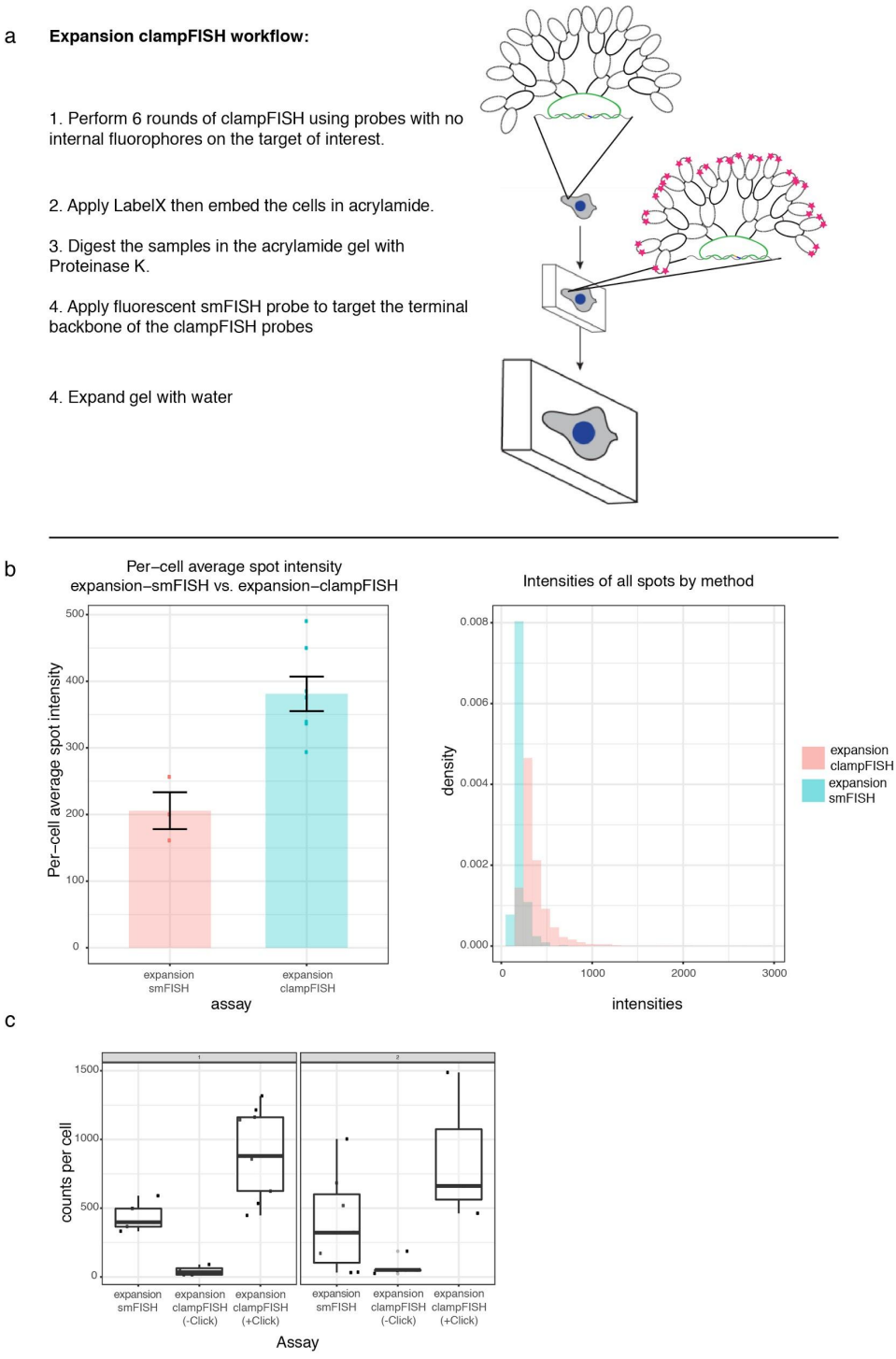
Supplementary Figure 6. Enlarged center panel from Figure 2 showing nonspecific binding of clampFISH probes on mouse kidney tissues.



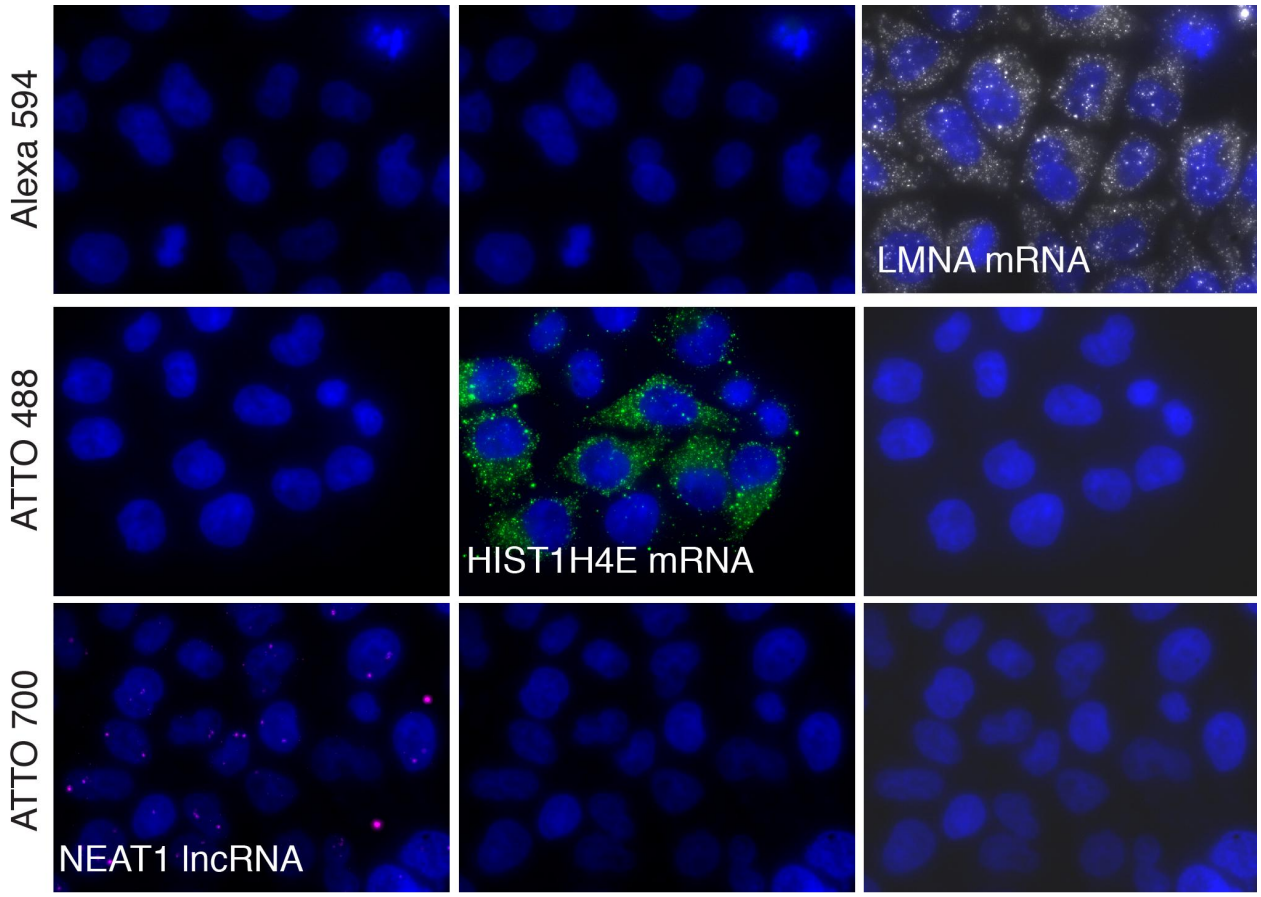
Supplementary Figure 7. Addition of blocking reagents to hybridization buffer for flow cytometry. MDA-MB 231 cells expressing GFP were mixed with MDA-MB 231 cells not expressing GFP at 50%. The mixed cell population was subsequently stained with clampFISH probes targeting GFP mRNA and the separation was assessed with the addition of different blocking reagents in the hybridization buffer.



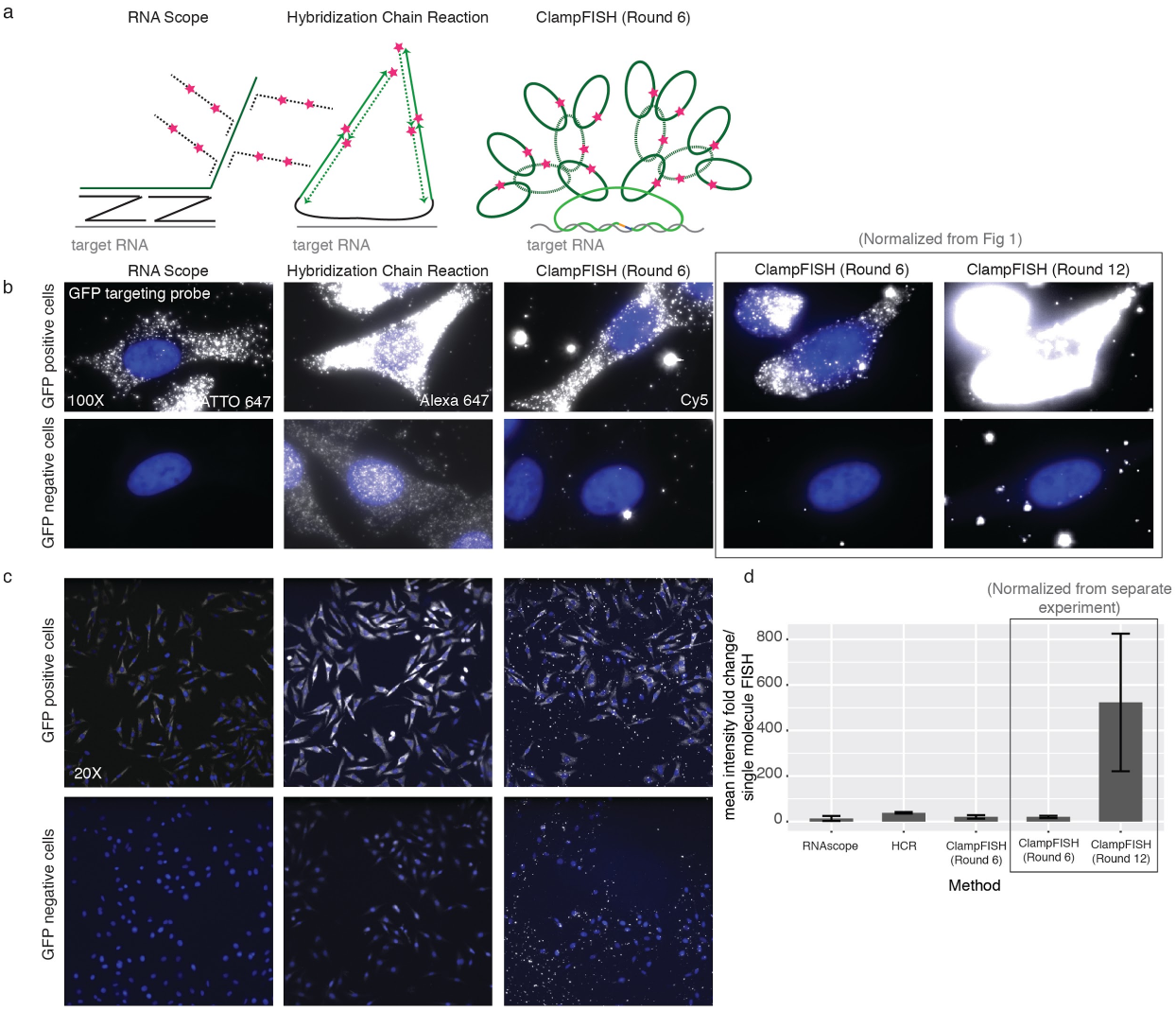
Supplementary Figure 8. Biological replicate of flow cytometry clampFISH from Figure 2C.



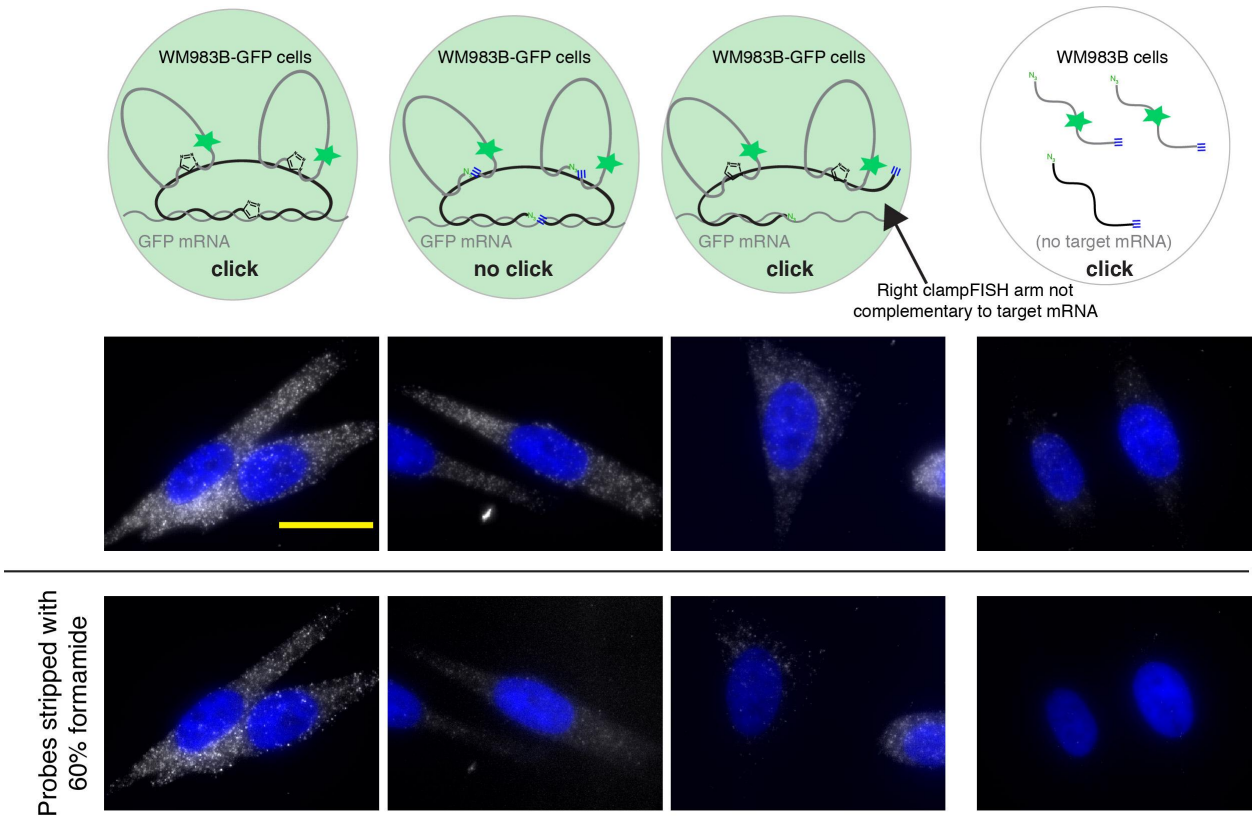
Supplementary Figure 9. (a) Expansion clampFISH workflow. (b) (left) Expansion clampFISH samples were assessed for spot intensity and compared to Expansion smFISH samples targeting the same RNA (GFP). (right) Density plots showing single spot intensity distributions across all cells using expansion clampFISH vs. expansion smFISH. (c) Mean counts per cell for each experimental condition. Left represents replicate 1 and right represents replicate 2. Note that the fully expanded cells are larger than the imaging field, and the values reported are from partial cells in all experimental conditions.



Supplementary Figure 10. Multiplexing ClampFISH to Round 7 bleedthrough. Cells were stained with clampFISH probes to round 7 individually and assessed using the same exposure times from the multiplexing experiment in Figure 3 for bleedthrough.



Supplementary Figure 11. Alternative amplification methods. (a) Comparison of 3 nucleic acid based amplification schemes. Pink star represents fluor. (b) Fluorescent micrographs of GFP targeting probes using each amplification method using 100X magnification. Top images are from WM983b-GFP cells and bottom images are from WM983b cells. We fixed contrast parameters independently for each method and used those for all samples of a given method; however, contrast parameters varied between methods. Images of rounds 6 and 12 of clampFISH are from Figure 1 using a lower exposure time and have been normalized to round 6 clampFISH from the current experiment. (c) Fluorescent micrographs acquired using 20X magnification on GFP positive cells (top) and GFP negative cells (bottom). (d) Mean Fold Change Intensities of each individual method over single molecule RNA FISH signal intensities using the same fluorophore. Normalized from experiments taken to 12 rounds of amplification, including the experiment shown in Fig 1. Round 6 and round 12 converted intensities are normalized to clampFISH mean round 6 clampFISH intensity from this experiment to account for differences in exposure time (error bars show standard error of the mean over 2 biological replicates). ClampFISH leads to much higher signal intensity than the maximum signal intensity from the other methods. Hybridization chain reaction showed some background in the negative controls.



Supplementary Figure 12. ClampFISH probes require adjacent hybridization of probe arms and click reaction to survive harsh conditions. 20 μm scale bar