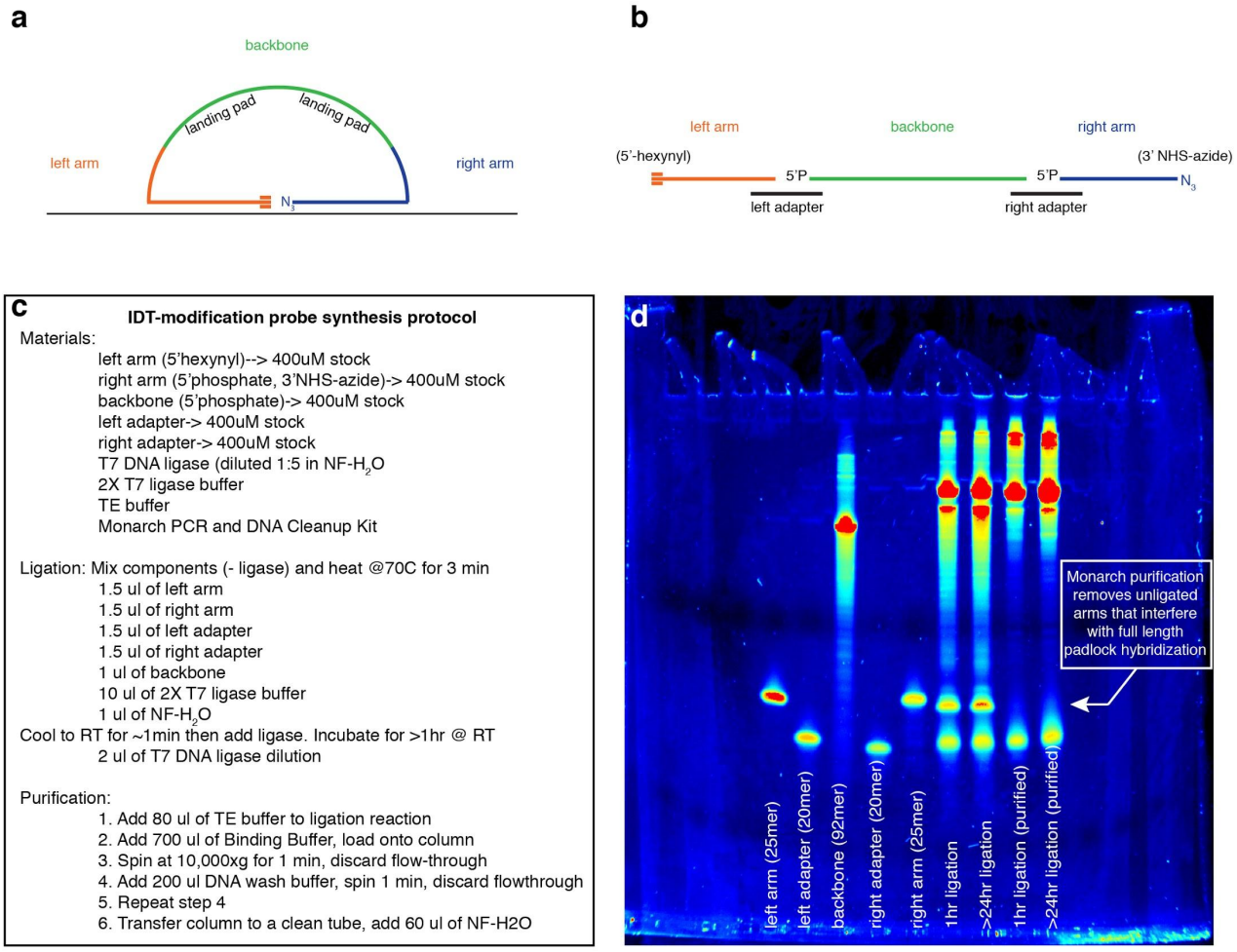
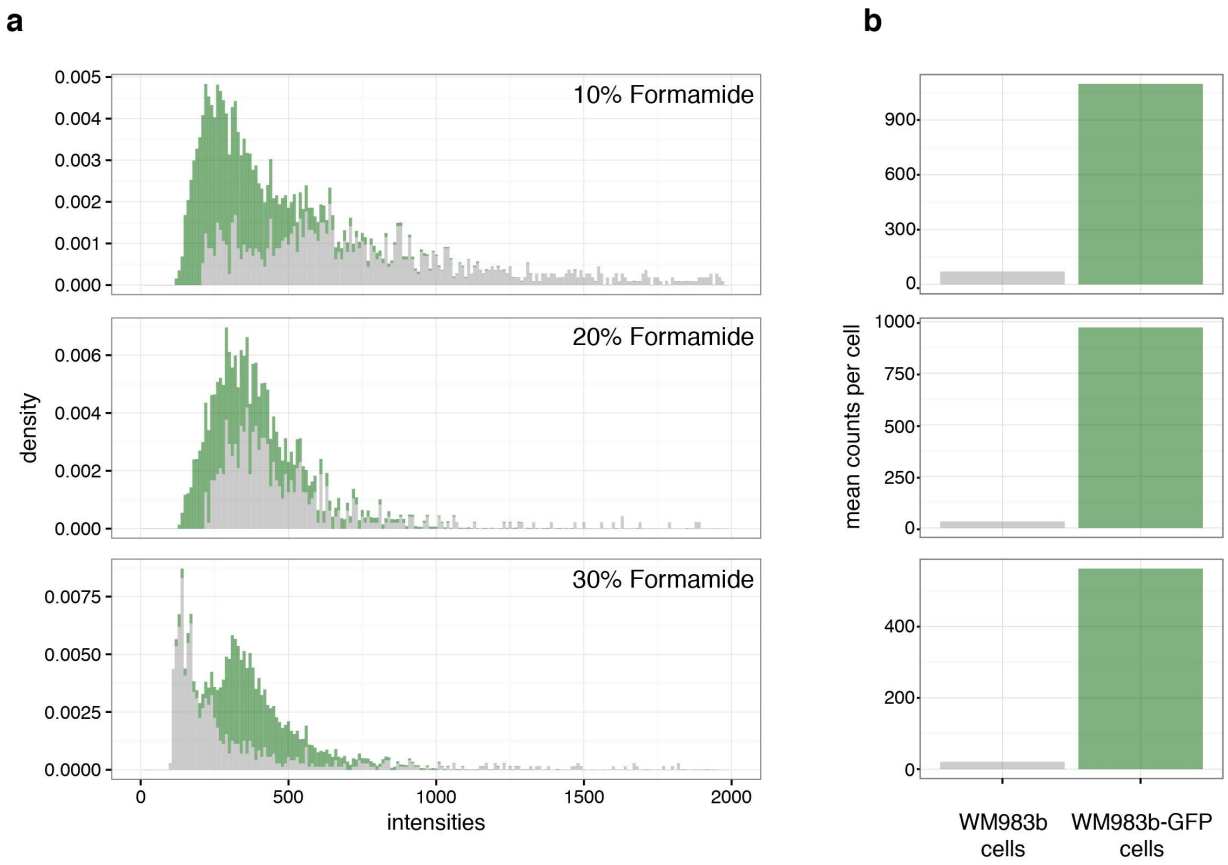
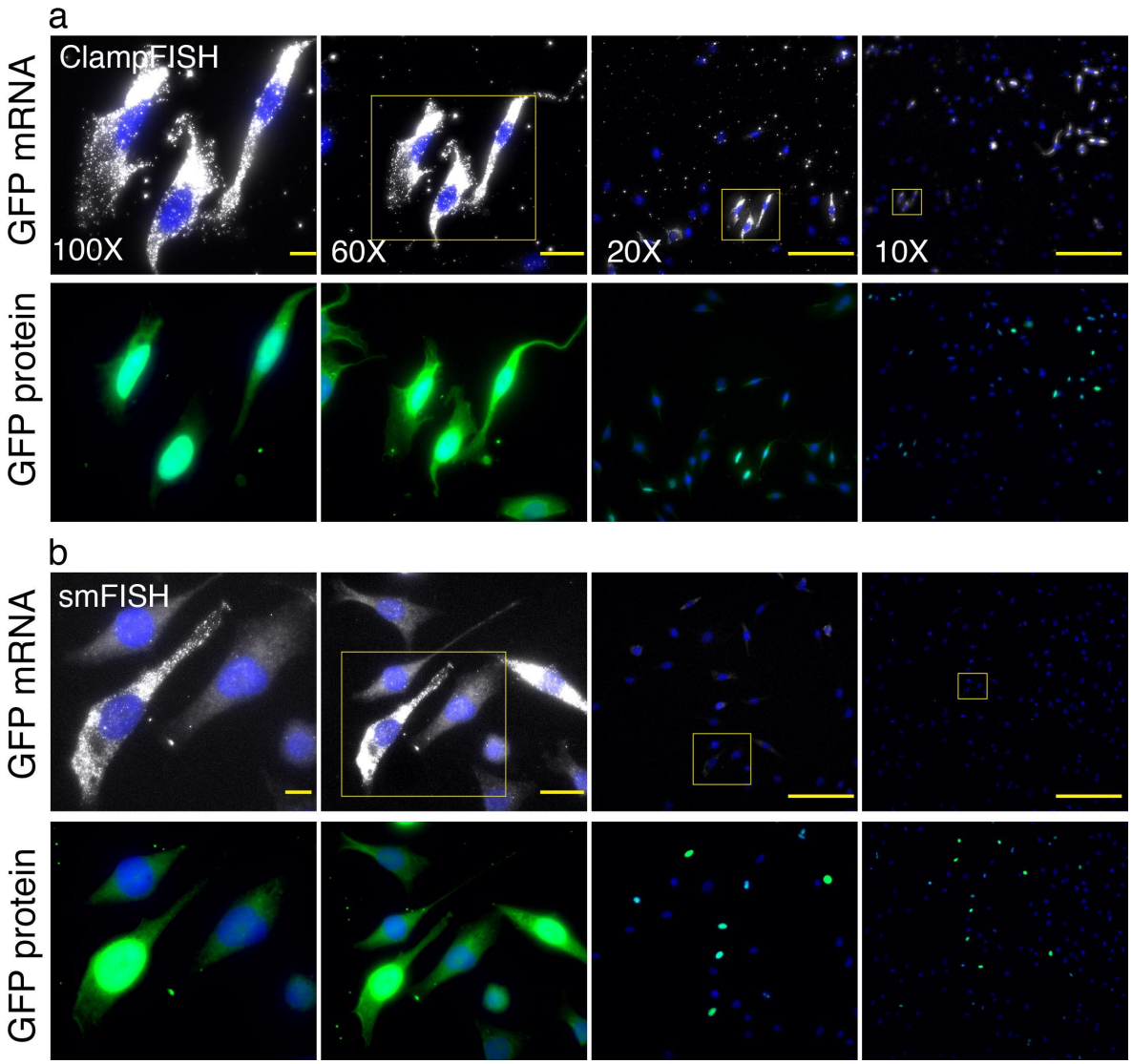
Supplementary Information



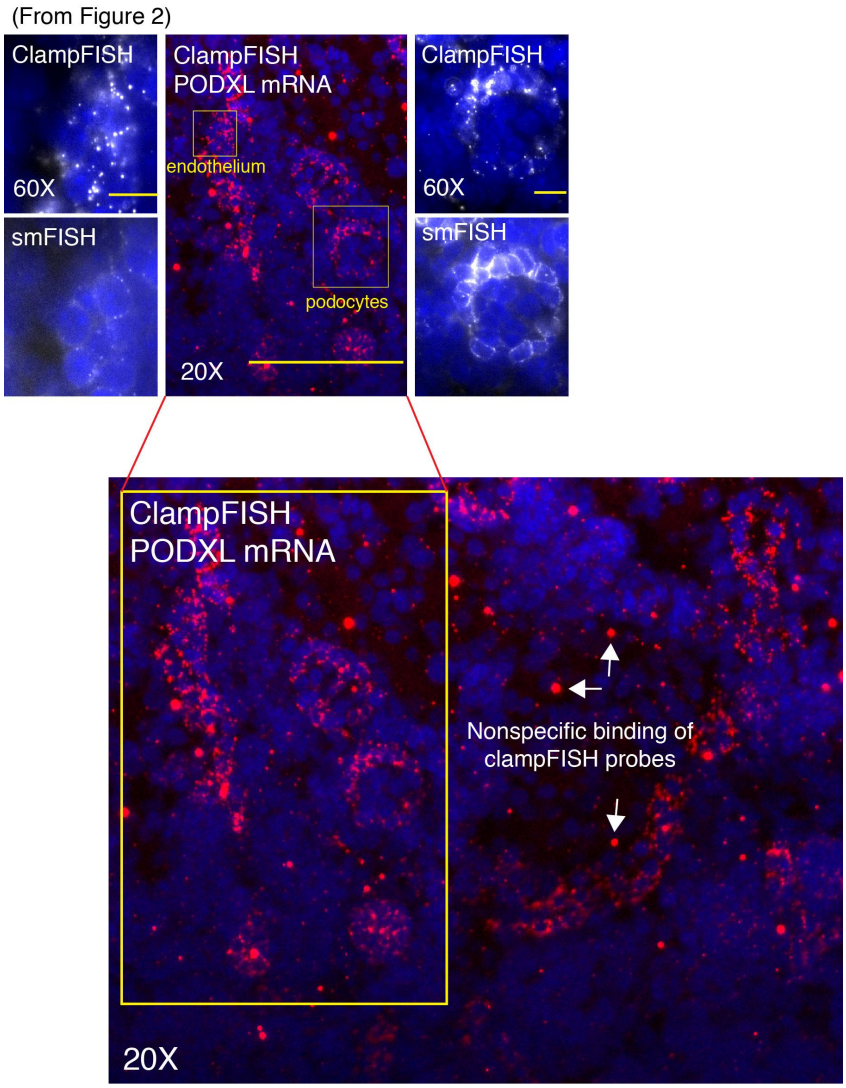
Supplementary Figure 1. Generation of ClampFISH probes. (a) Diagram of individual pieces of each clampFISH probe. (b) Linear diagram of clampFISH probe ligations cheme. (c) ClampFISH probe ligation protocol. (d) 15% TBE-UREA gel showing separation of individual pieces, ligation product, and product after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



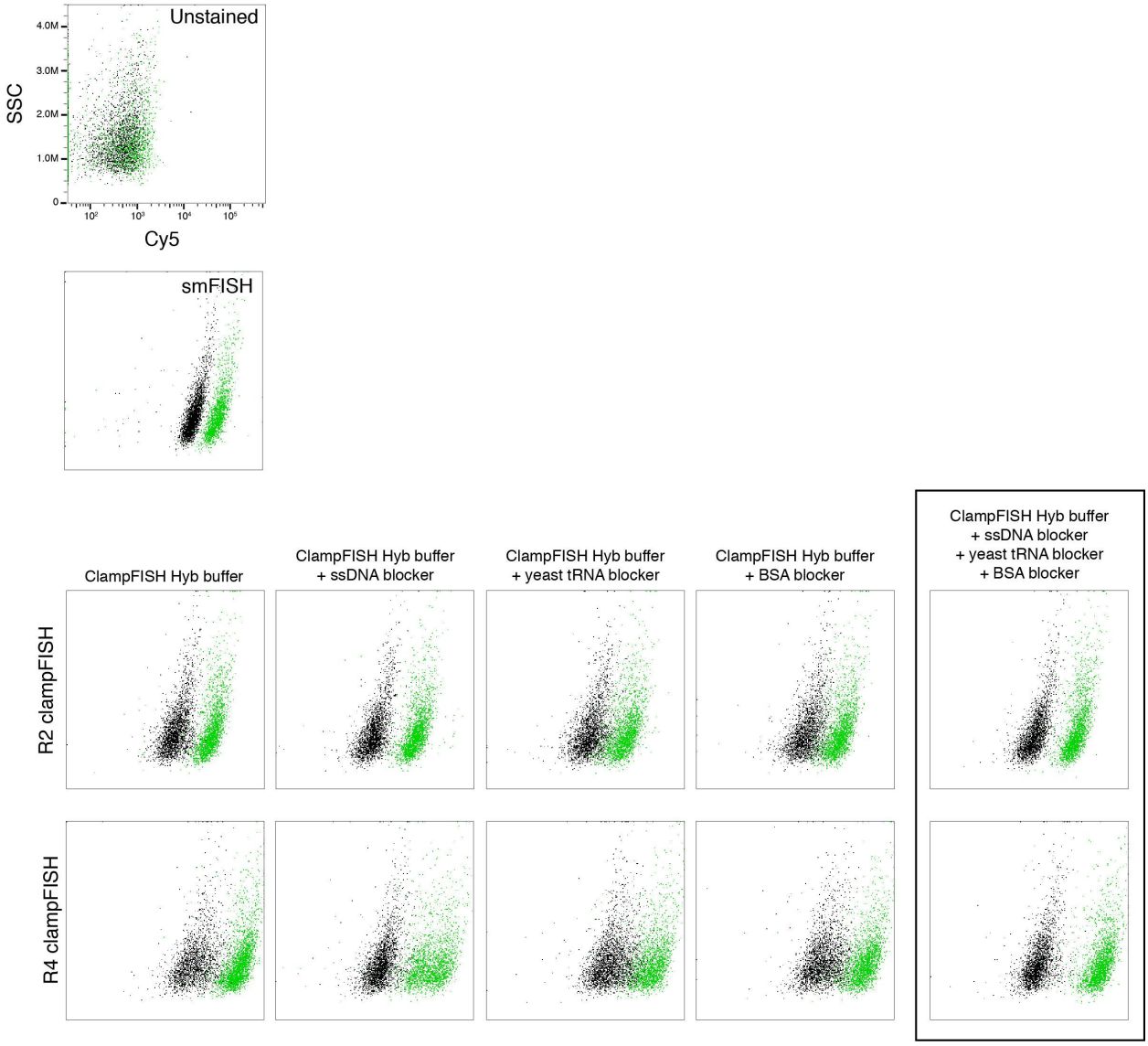
Supplementary Figure 2. Optimizing [Formamide] in hybridization buffer. (a) Density plots showing single spot intensity distributions across 3 different formamide concentrations in hybridization buffer. WM983b-GFP cells were probed for GFP mRNA using clampFISH probes to round 2 (green) and WM983b cells were also stained for GFP mRNA using GFP targetting 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.



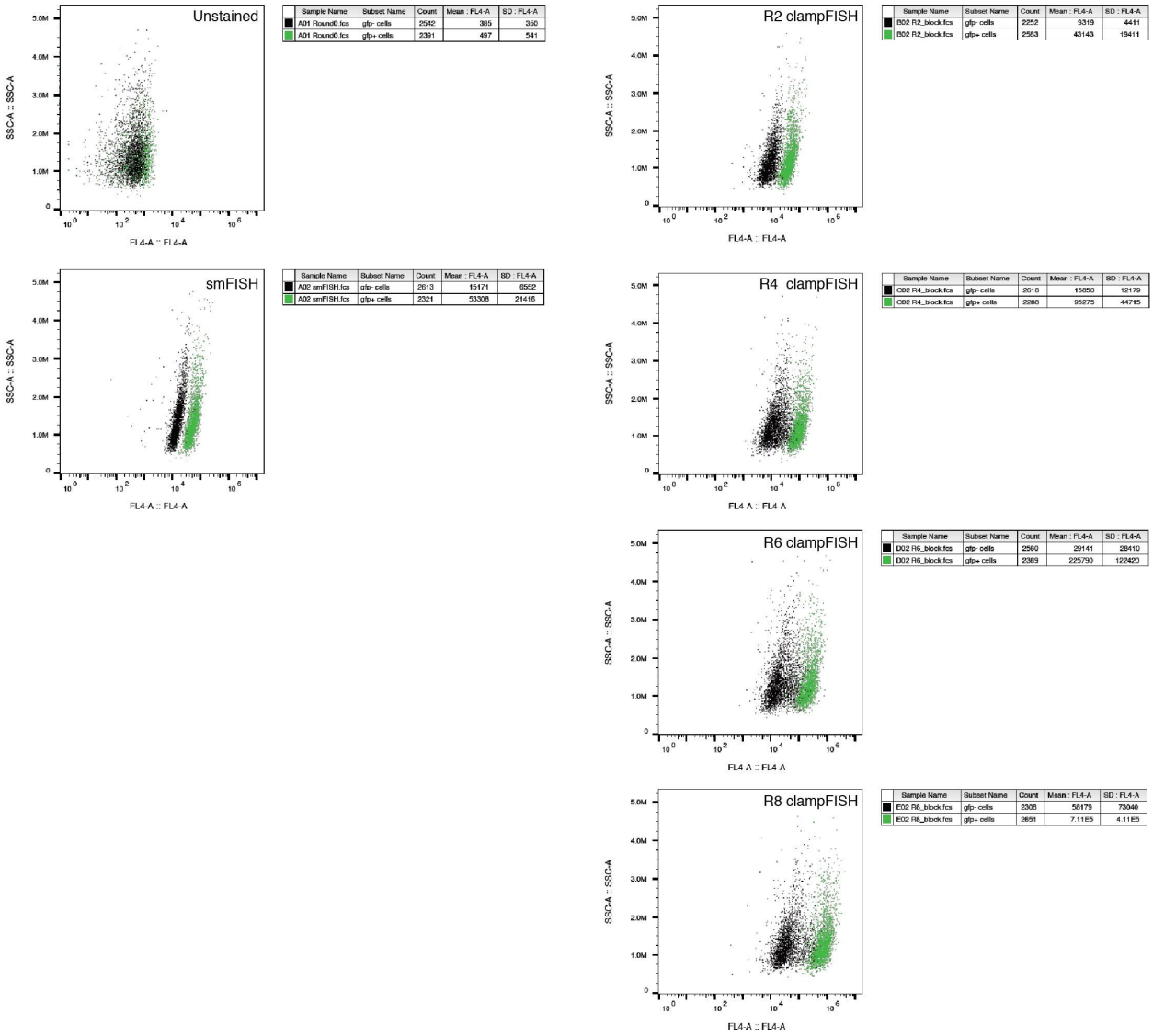
Supplementary Figure 3. Colocalization of GFP mRNA signal with GFP protein. (a) ClampFISH images as shown in Figure 2 of main text with a panel showing which cells are GFP positive (green). (b) smFISH images as shown in Figure 2 of main text with a panel showing which cells are GFP positive (green).



Supplementary Figure 4. Enlarged center panel from Figure 2 showing nonspecific binding of clampFISH probes on mouse kidney tissues.



Supplementary Figure 5. 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 6. Biological replicate of flow cytometry clampFISH from Figure 2C.

a **Expansion clampFISH workflow:**

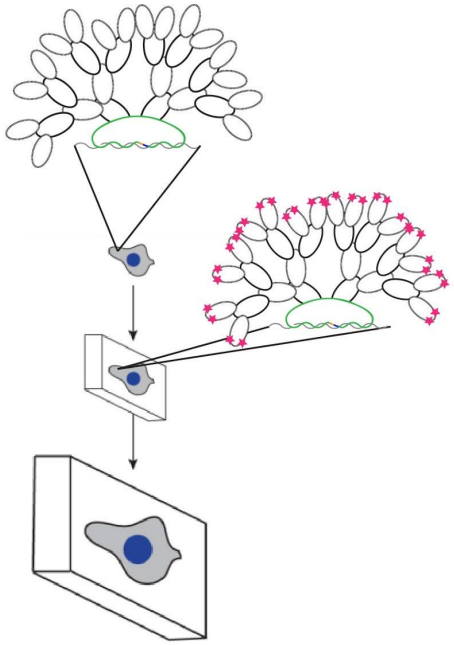
1. Perform 6 rounds of clampFISH using probes with no internal fluorophores on the target of interest.

2. Apply LabelX then embed the cells in acrylamide.

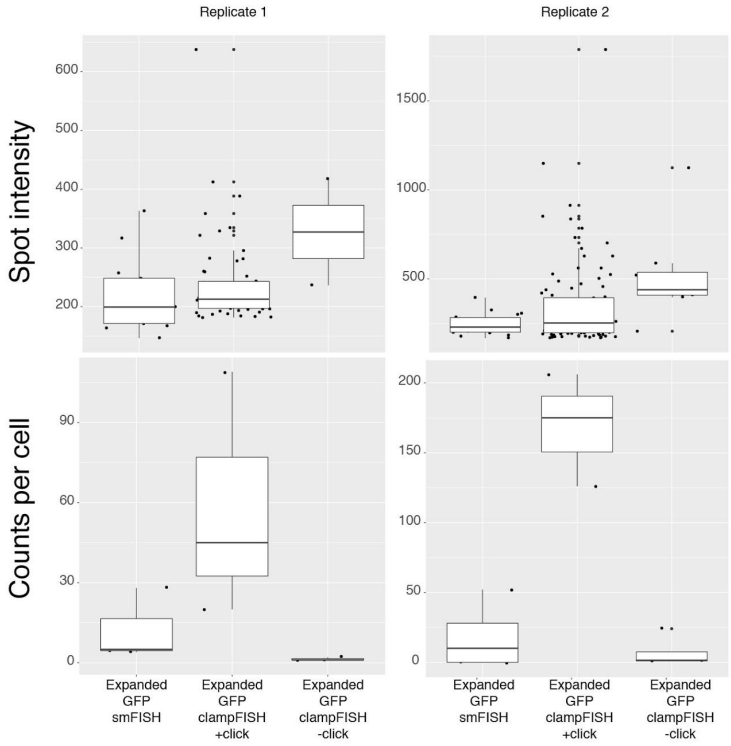
3. Digest the samples in the acrylamide gel with Proteinase K.

4. Apply fluorescent smFISH probe to target the terminal backbone of the clampFISH probes

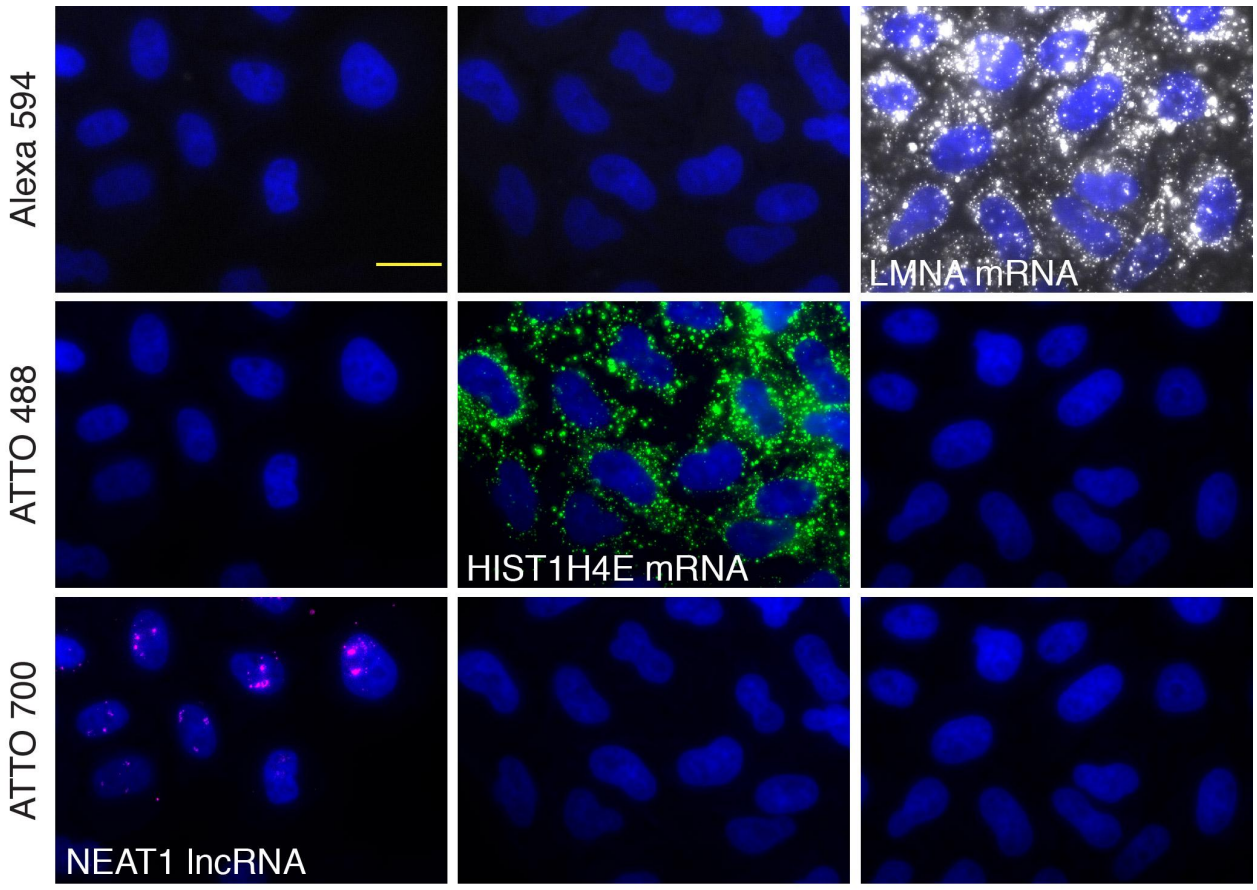
4. Expand gel with water



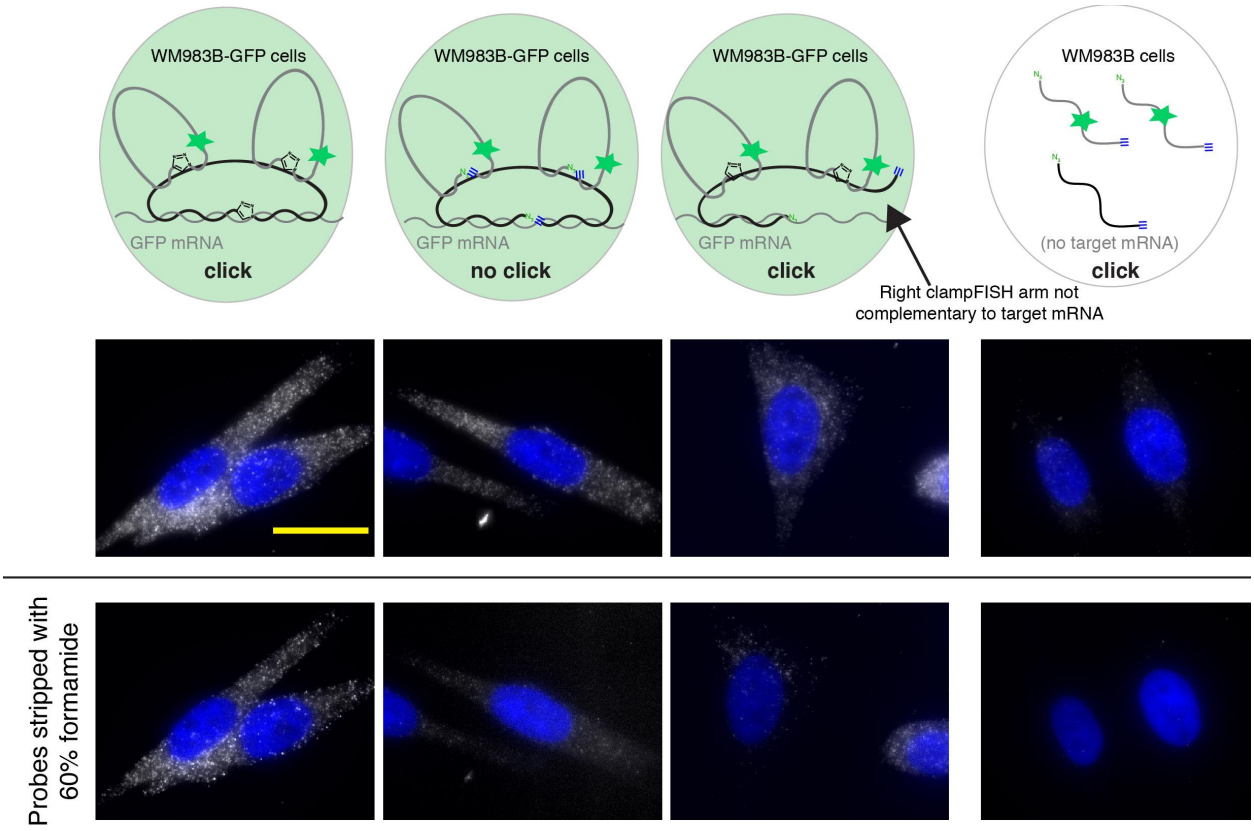
b



Supplementary Figure 7. (a) Expansion clampFISH workflow. (b) Expansion clampFISH samples were assessed for spot intensity and mRNA counts per cell with smFISH and clampFISH on WM983bGFP cells.



Supplementary Figure 8. 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 9. ClampFISH probes require adjacent hybridization of probe arms and click reaction to survive harsh conditions. 20 um scale bar