Pichon et al., http://www.jcb.org/cgi/content/full/jcb.201605024/DC1

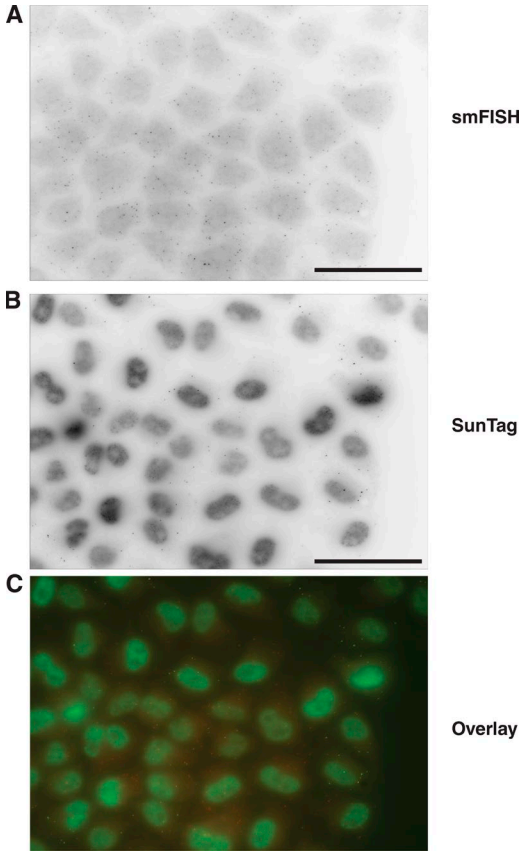


Figure S1. **Colocalization of the bright protein foci labeled by the scFv-sfGFP with the SunTagx56-Ki67 mRNAs.** Panels represent microscopy images of cells stably expressing the scFv-sfGFP and the SunTagx56-Ki67 mRNA and labeled with smFISH probes recognizing the SunTag-Hygro (B) sequences. (C) Color overlay of the smFISH image (red, A) and the SunTag signal (green, B). Bars, 45 µm. Signal quality can be appreciated by zooming in the images.

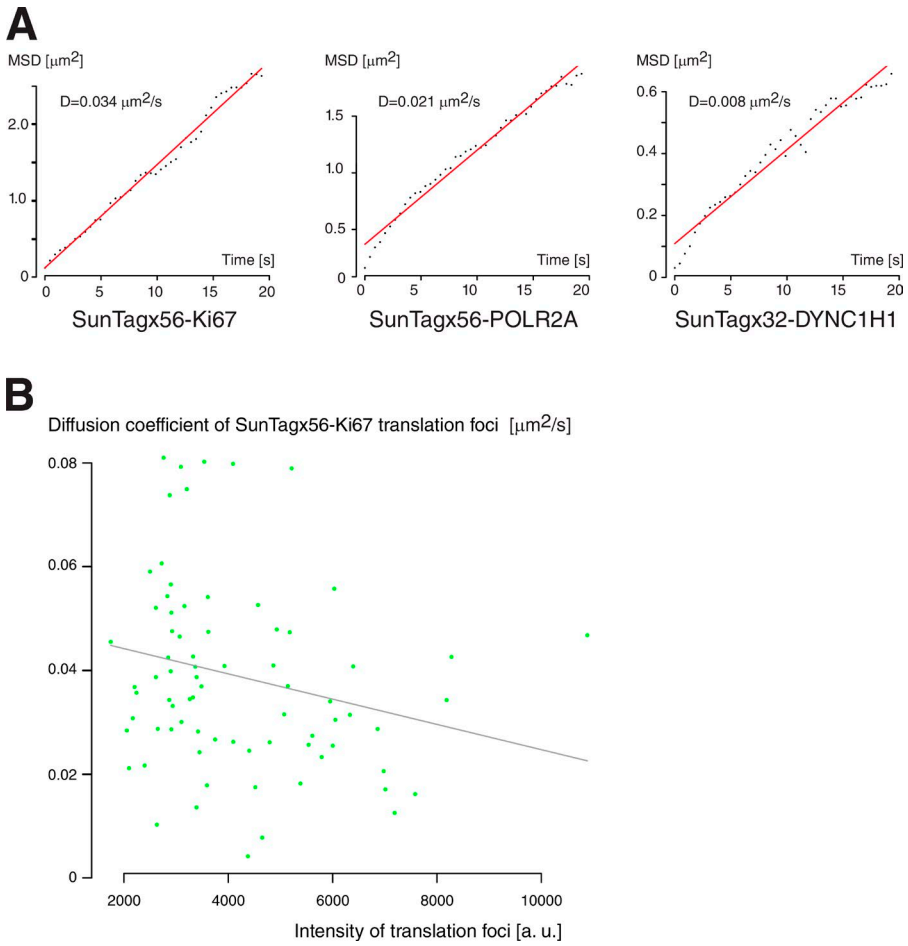


Figure S2. **Diffusion of polysomes and correlation with the number of nascent proteins.** (A) Graph depicts a linear fit to MSD for the three polysomes analyzed (Ki67, POLR2A, and DYNC1H1). Only the first 20 s of the curves are taken into account. (B) Graph depicts the diffusion coefficient of individual DYNC1H1 polysomes as a function of their brightness. The diffusion coefficient of individual polysome is obtained from the variance of the single-step jumps of this particle over time.

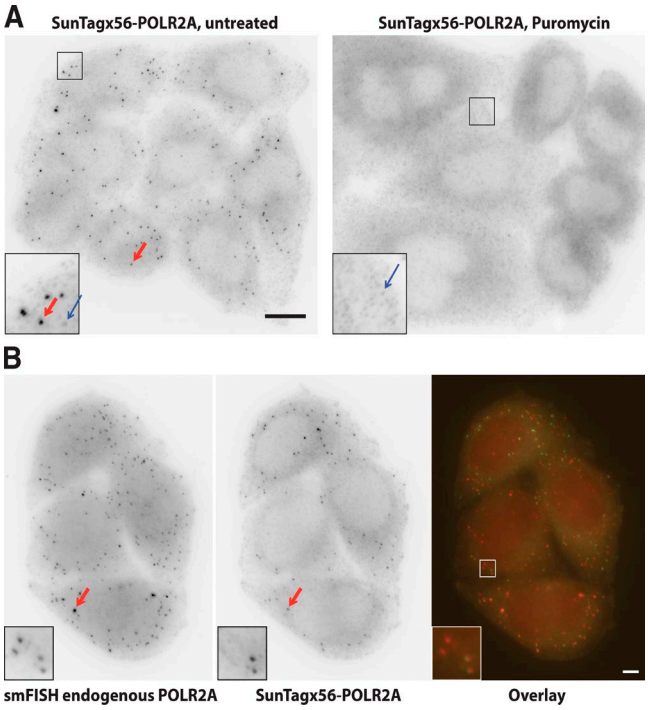


Figure S3. **Characterization of the SunTagx56-POLR2A polysomes.** (A) Effect of puromycin on the brighter protein foci labeled by the scFv-sfGFP. Panels represent microscopy images of cells stably expressing the scFv-sfGFP containing the SunTagx56-POLR2A allele, untreated (left panel) or treated with puromycin (right panel). The blue arrows indicate a spot corresponding to a single molecule of SunTagx56-Ki67 protein, whereas the red arrows point to a brighter protein foci. Inset: a zoom of the boxed area (12 × 12 µm). Bar, 12 µm. (B) Colocalization of the bright protein foci labeled by the scFv-sfGFP with endogenous POLR2A mRNAs. Panels represent microscopy images of cells stably expressing the scFv-sfGFP containing the SunTagx56-POLR2A allele and labeled with smFISH probes recognizing the POLR2A sequences. Note that these probes recognize both tagged and untagged mRNA, as cells are heterozygous and thus yield less colocalization than in Fig. 3 B, in which only the tagged mRNA are labeled. (right) Color overlay of the smFISH image (red, left panel) and the SunTag signal (green, middle panel). The red arrows point to a brighter protein foci that colocalizes with the mRNA. (inset) A zoom of the boxed area (4 × 4 µm). Bar, 4 µm.

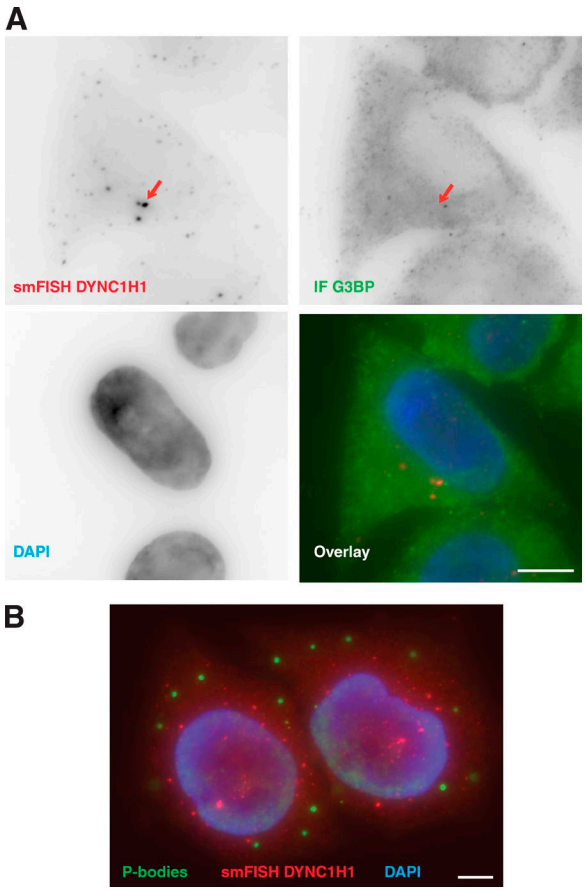


Figure S4. **Characterization of DYNC1H1 RNA blobs.** (A) Simultaneous localization of the stress granule marker G3BP and DYNC1H1 RNA blobs in HeLa cells. Image depicts fluorescent microscopy signals of cells stained for the stress granule marker G3BP (green), DYNC1H1 mRNA (red), and DAPI (blue). Red arrow, RNA blob. Bar, 7 µm. (B) Simultaneous localization of P-bodies and DYNC1H1 RNA blobs in HeLa cells expressing the DYNC1H1 BAC. Image depicts fluorescent microscopy signals of cells stained for the P-body marker GE-1/helds (green), DYNC1H1 mRNA (red), and DAPI (blue). Bar, 7 µm.

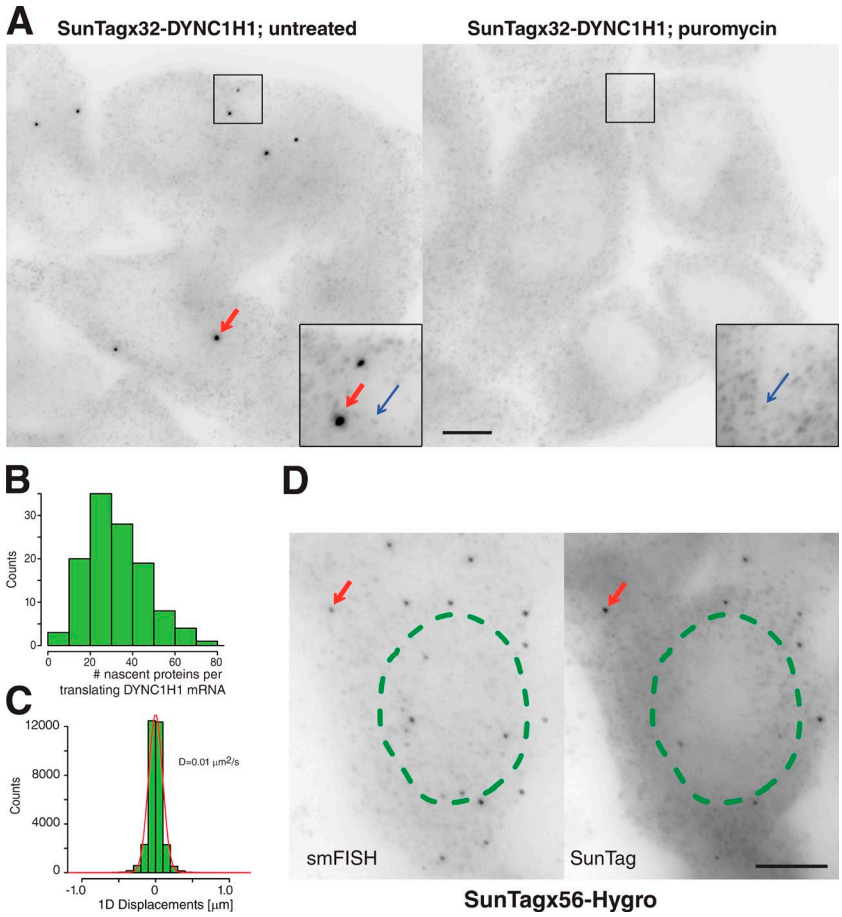
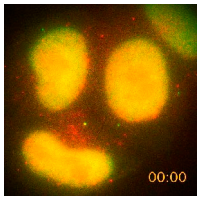
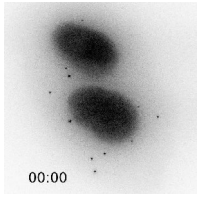


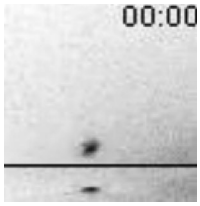
Figure S5. **Characterization of DYNC1H1 polysomes.** (A) Effect of puromycin on the brighter protein foci labeled by the scFv-sfGFP. Panels represent microscopy images of cells stably expressing the scFv-sfGFP and containing the SunTagx32-DYNC1H1 allele, untreated (left) or treated with puromycin (right). The blue arrows indicate a spot corresponding to a single molecule of SunTagx32-DYNC1H1 protein, whereas the red arrows point to a brighter protein foci. Inset: a zoom of the boxed area (8 × 8 µm). Bar, 8 µm. (B) Single-molecule polysome profile of DYNC1H1 mRNAs. Panels represent histograms of the number of nascent protein per translated DYNC1H1 mRNA (n = 120 mRNAs). (C) Quantification of the diffusion rates of SunTagx32-DYNC1H1 polysomes. The graph is a histogram of 1D displacements measured between two consecutive video frames (176 particles total). (D) Colocalization of the bright protein foci labeled by the scFv-sfGFP with SunTagx56-Hygro mRNAs. Panels represent microscopy images of cells stably expressing the scFv-sfGFP and the SunTagx56-Hygro reporter, labeled with smFISH probes recognizing the SunTagx56-hygro sequences. This reporter is identical to the SunTagx56-Ki67, except that it lacks the Ki67 coding sequence. The green dashed outlines represent the position of the nucleus. The red arrows point to a bright protein foci that colocalize with the mRNA. Bar, 5 µm.



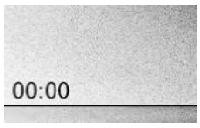
Video 1. **Fast imaging of the SunTagx56-Ki67 reporter (2.2 stacks/s; each Z-stack with 10 slices 0.6 µm apart), accelerated four times.** HeLa cells stably expressing SunTagx56-Ki67, scFv-sfGFP (green), and nls-MCP-TagRFPt (red) were imaged in two colors by epifluorescence microscopy. Images are maximal intensity projection along Z and corrected for photobleaching using histogram matching. Time is indicated as minutes:seconds.



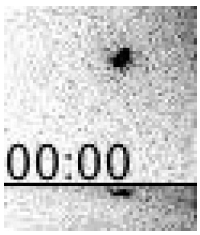
Video 2. **Slow imaging of the SunTagx56-Ki67 reporter (1 stack per 10 s), accelerated 160 times.** HeLa cells stably expressing the SunTagx56-Ki67 reporter and scFv-sfGFP were imaged in a single color by epifluorescence microscopy. The video corresponds to the SunTag images of Fig. 2 A. Bar, 5 µm.



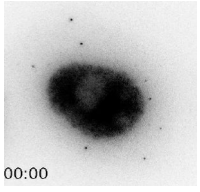
Video 3. **Slow imaging of the SunTagx56-Ki67 reporter (1 stack per 10 s), accelerated 160 times.** HeLa cells stably expressing the SunTagx56-Ki67 reporter and scFv-sfGFP were imaged in a single color by epifluorescence microscopy. The video is a zoom of Video 1 (frames 4–92) and corresponds to the violet trace of Fig. 3 B. The top panel is a maximal intensity projection along z (providing a top view of the cell), and the bottom panel is a maximal intensity projection along y (providing a side view of the cell). Bar, 2 µm.



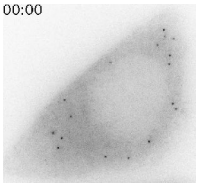
Video 4. **Slow imaging of the SunTagx56-Ki67 reporter (1 stack per 10 s), accelerated 80 times.** HeLa cells stably expressing the SunTagx56-Ki67 reporter and scFv-sfGFP were imaged in a single color by epifluorescence microscopy. The video is a zoom of Video 1 and corresponds to the green trace of Fig. 3 B. The top panel is a maximal intensity projection along z (providing a top view of the cell), and the bottom panel is a maximal intensity projection along y (providing a side view of the cell). Bar, 2 µm.



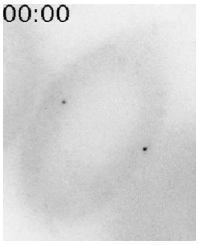
Video 5. **Slow imaging of the SunTagx56-Ki67 reporter (1 stack per 10 s), accelerated 80 times.** HeLa cells stably expressing the SunTagx56-Ki67 reporter and scFv-sfGFP were imaged in a single color by epifluorescence microscopy. The video is a zoom of Video 1 and corresponds to the end of the brown trace of Fig. 3 B. The top panel is a maximal intensity projection along z (providing a top view of the cell), and the bottom panel is a maximal intensity projection along y (providing a side view of the cell). Bar, 2 µm.



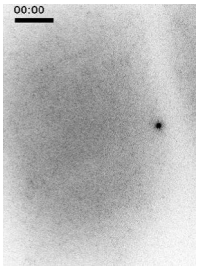
Video 6. **Fast imaging of the SunTagx56-Ki67 reporter (2.2 stacks/s), accelerated four times.** HeLa cells stably expressing the SunTagx56-Ki67 reporter and scFv-sfGFP were imaged in a single color by epifluorescence microscopy. The video corresponds to the SunTag images of Fig. 4 A. Bar, 5 µm.



Video 7. **Fast imaging of the SunTagx56-POLR2A gene (2.2 stacks/s), accelerated four times.** HeLa cells stably expressing the SunTagx56-POLR2A allele and the scFv-sfGFP were imaged in a single color by epifluorescence microscopy. The video corresponds to the SunTag images of Fig. 5 E. Bar, 5 µm.



Video 8. **Fast imaging of the SunTagx32-DYNC1H1 gene (2.2 stacks/s), accelerated four times.** HeLa cells stably expressing the SunTagx32-DYNC1H1 allele and the scFv-sfGFP were imaged in a single color by epifluorescence microscopy. The video corresponds to the SunTag images of Fig. 7 A. Bar, 5 µm.



Video 9. **Fast imaging of the SunTagx32-DYNC1H1 gene (2.2 stacks/s), accelerated four times.** Legend as for Video 8, except that the cells were treated with cytochalasin D (5 µM for 1 h). Bar, 5 µm.

Provided online is Table S1, providing the sequence of the oligo probes used for the smFISH experiments, as well as the sequence of the SunTagx32, SunTagx56, and MS2x132 tags. For the smFISH oligonucleotide probes, the pool of unlabeled oligonucleotide is hybridized with the fluorescent FLAP, and the resulting hybrid is used as a probe instead of a traditional fluorescent oligonucleotide.