nature | methods

Imaging individual mRNA molecules using multiple singly labeled probes

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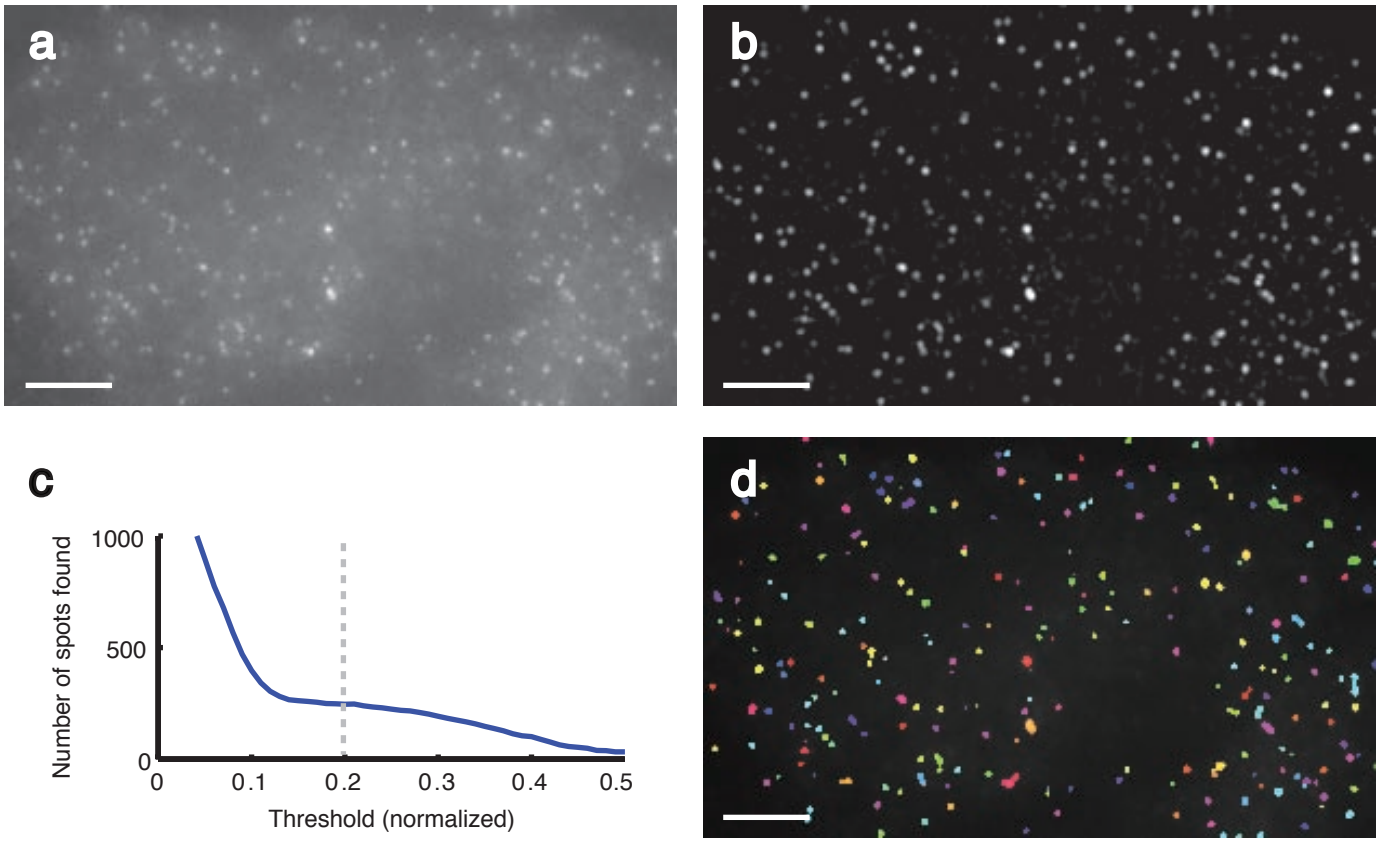
Supplementary Methods	

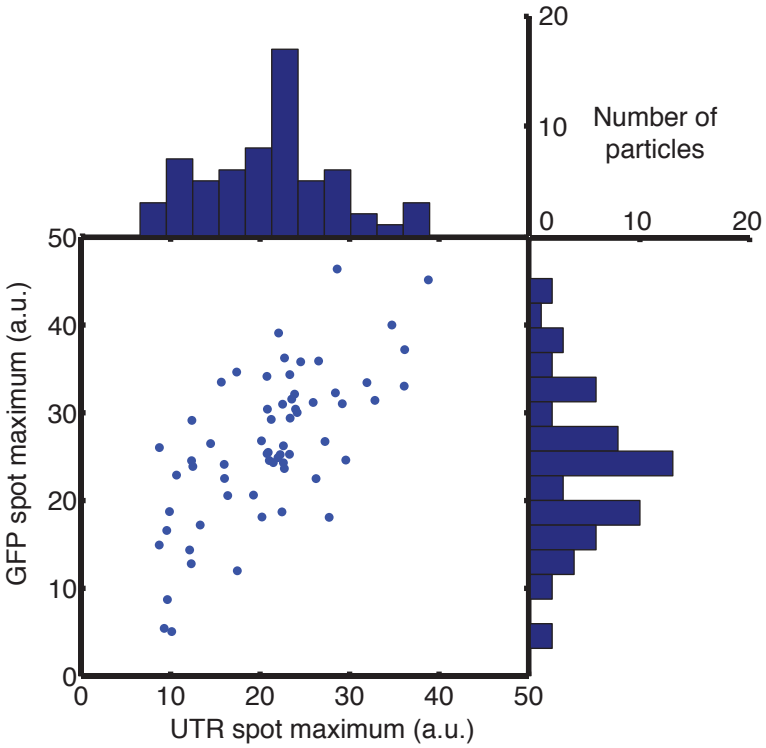
Note: Supplementary Software is available on the Nature Methods website.

Supplementary Figure 1



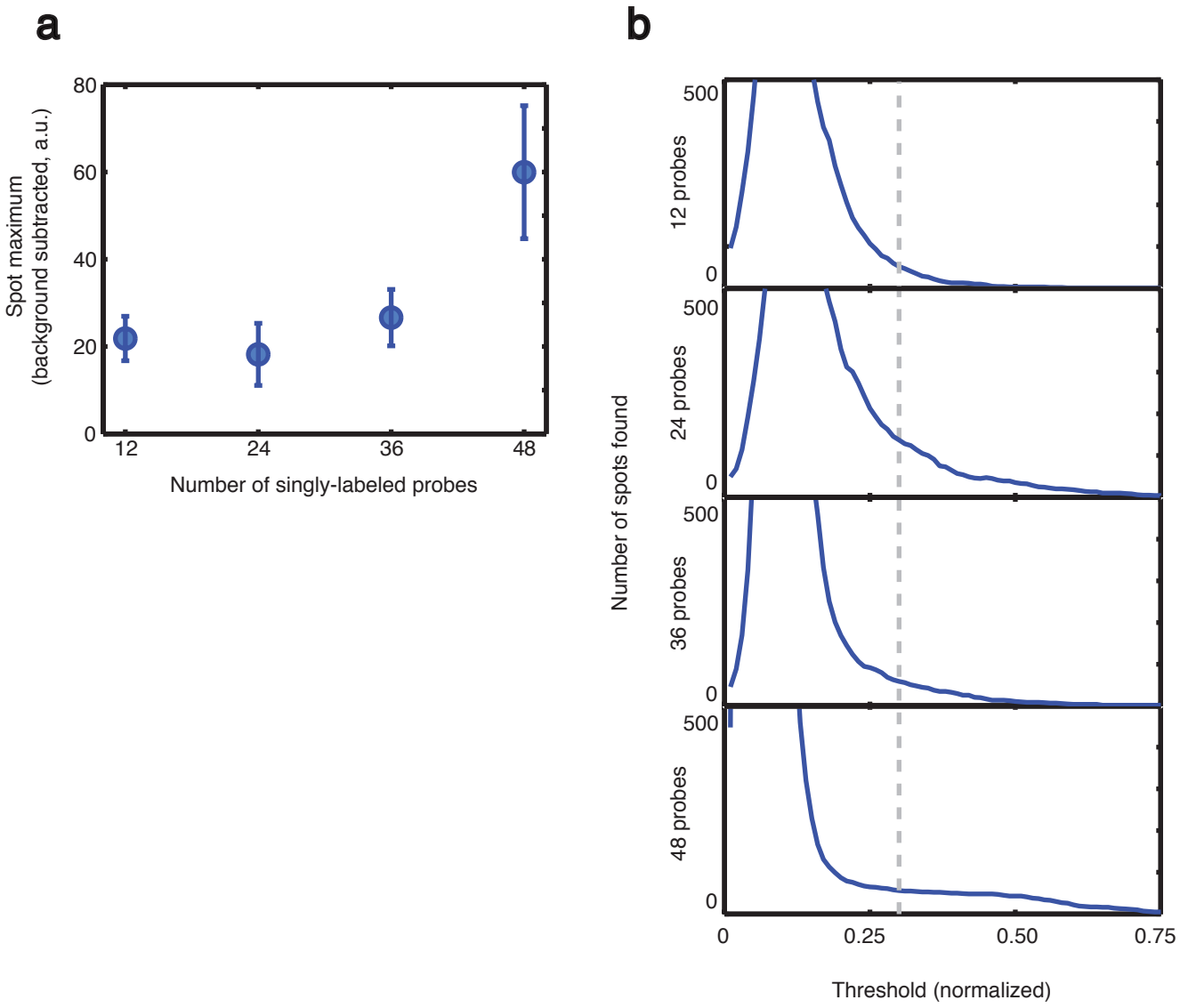
Supplementary Figure 1 | Computational identification of mRNA spots. (**a**) Raw image data (maximum intensity merge) obtained from imaging *FKBP5* mRNA particles in A549 cells induced with dexamethasone. (**b**) Image (maximum merge) obtained by running raw data through Laplacian of a Gaussian filter designed to enhance spots of the correct size and shape while removing the slowly varying background. (**c**) The number of spots (i.e., connected components) found upon thresholding the filtered image from (**b**) is plotted as a function of the threshold value, ranging from 0 to the maximum intensity of the filtered image (normalized to 1). The presence of a plateau indicates that there is a region over which the number of particles detected is fairly insensitive to the particular threshold chosen. The grey line represents the threshold used (within the plateau) for determining the actual number of mRNA in the image. (**d**) Image showing the results of using the threshold represented by the grey line in (**c**) on the filtered image in (**b**), with each distinct spot assigned a random color. The spots detected correspond very well with those identified by eye. All scale bars are 5 μm long.

Supplementary Figure 2



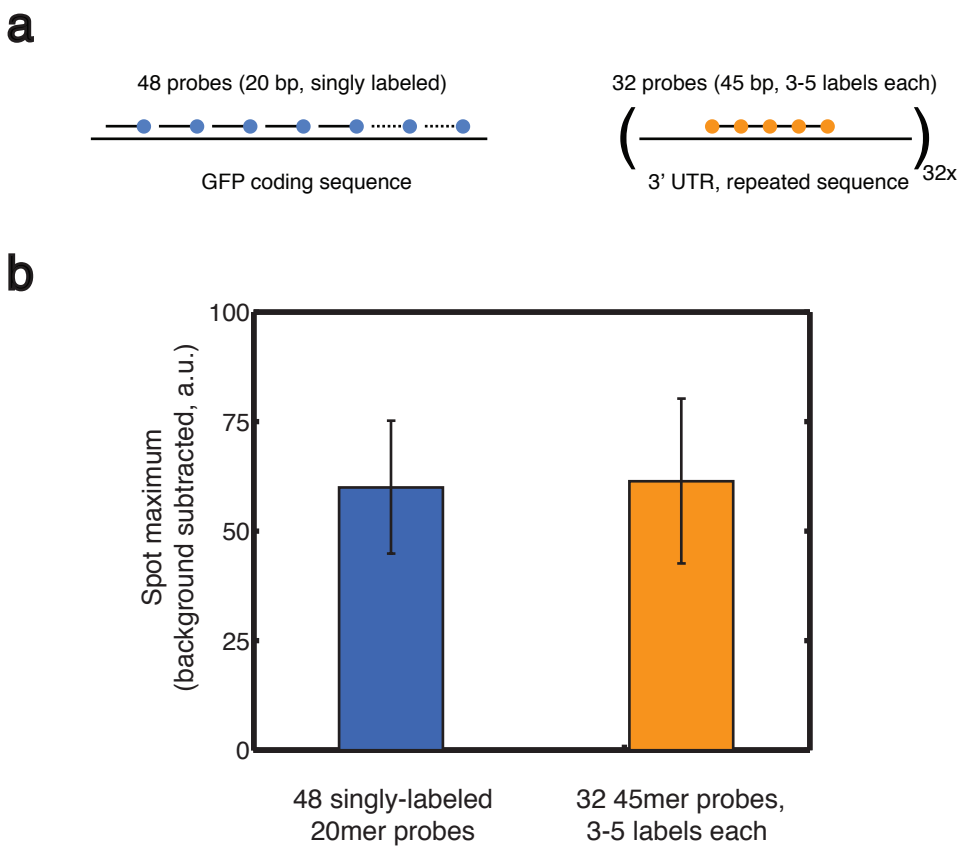
Supplementary Figure 2 | Intensity analysis of colocalized spots. Spot intensities corresponding to the GFP-targeted probes (Alexa 594 channel, y axis) and multimeric UTR-targeted probes (TMR channel, x axis) were computed by taking the maximum intensity in the computationally identified spot region and subtracting the mean intensity of an annular region surrounding the spot. Marginal histograms show the distributions of GFP spot intensities (right) and UTR spot intensities (top).

Supplementary Figure 3



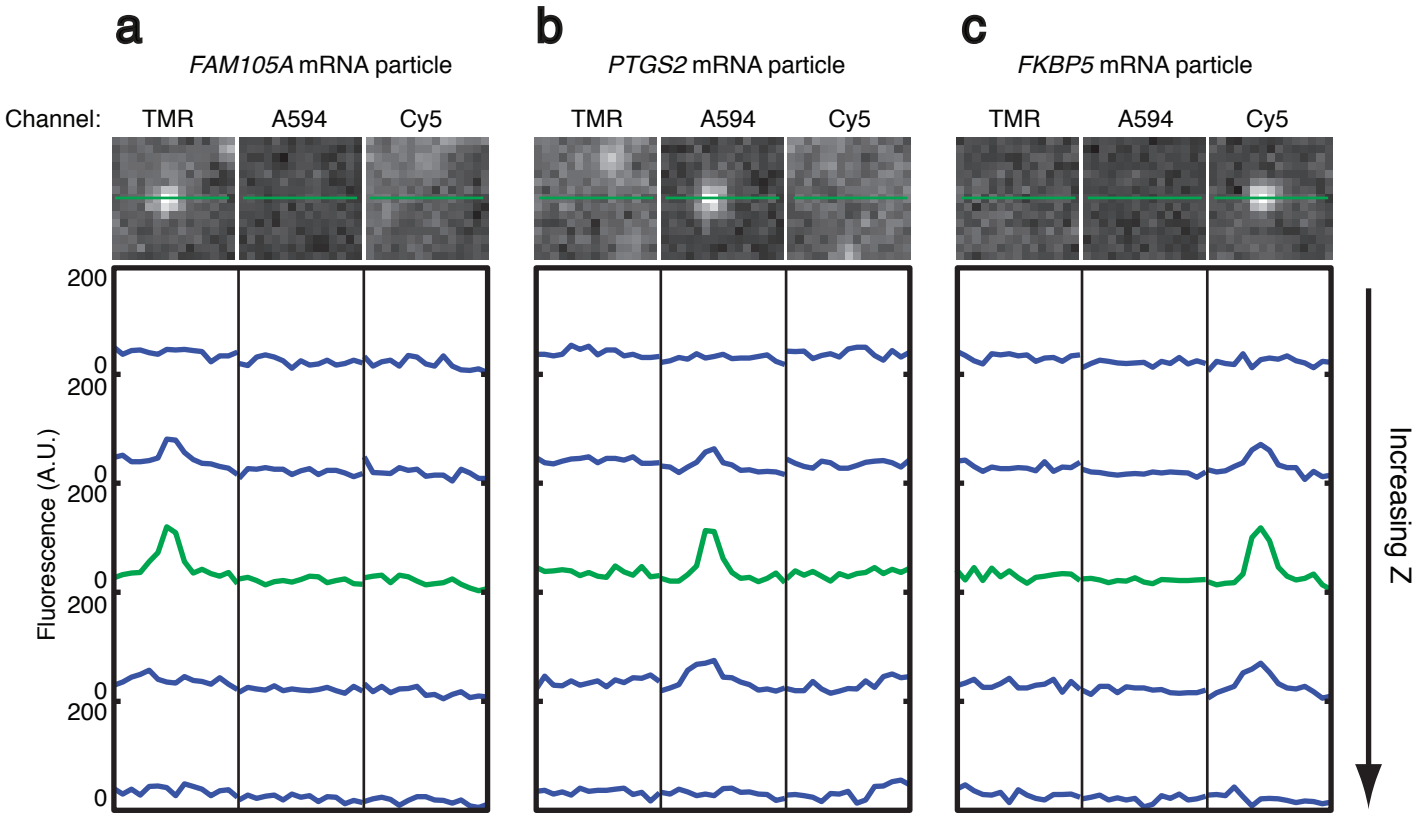
Supplementary Figure 3 | Sensitivity of method when using different numbers of probes. (**a**) Spot intensity (defined as maximum intensity within the spot minus the mean background taken in an annular region surrounding the spot) as a function of the number of probes chosen. Intensities for 12 and 24 probes are artifactual in that spots were not readily identifiable in those cases, so spots identified were biased towards being brighter. (**b**) The number of spots (i.e., connected components) found upon thresholding the filtered image plotted as a function of the threshold value, ranging from 0 to the maximum intensity of the filtered image (normalized to 1) for different numbers of probes. The grey bar indicates the threshold used for the analysis in (**a**).

Supplementary Figure 4



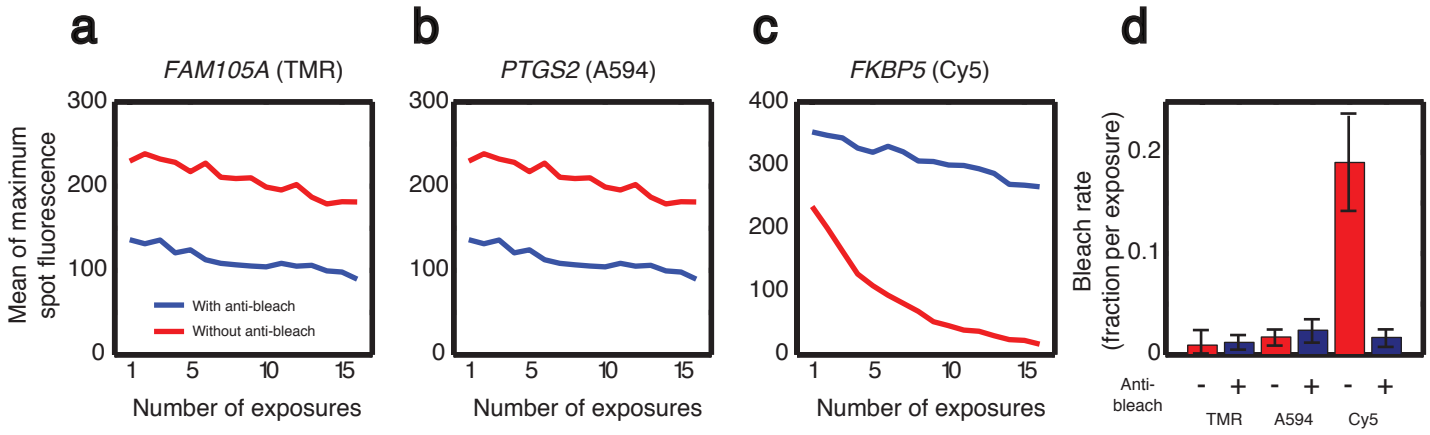
Supplementary Figure 4 | Comparison with the mRNA detection method of Femino et al. (*Science* 1998). (**a**) Schematic depicting the method described in this manuscript with 48 singly-labeled probes (left) and the method of Femino et al. in which each 45 bp probe contains five fluorophores each and is targeted to a sequence element that is repeated 32 times in the 3'UTR of the target mRNA expressed from a transgene in Chinese hamster ovary cells. (**b**) Comparison of spot intensities when using 48 singly labeled probes or using a 45 bp probe labeled with five fluorophores. Error bars represent one standard deviation.

Supplementary Figure 5



Supplementary Figure 5 | Examination of fluorescent spot bleedthrough. (**a**) Images of an *FAM105A* mRNA spot labeled with TMR as seen through the TMR, Alexa 594 and Cy5 filter channels. Linescans of fluorescent intensity corresponding to the line through the image are given below, with the different linescans corresponding to measurements taken at increasing z (0.25 μm spacing). The green linescan corresponds to the z-slice shown in the image itself. A similar analysis was performed for a *PTGS2* mRNA spot labeled with Alexa 594 (**b**) and an *FKBP5* mRNA particle labeled with Cy5 (**c**). All linescan intensity measurements had the camera background subtracted but range between 0 and 200 arbitrary fluorescence units.

Supplementary Figure 6

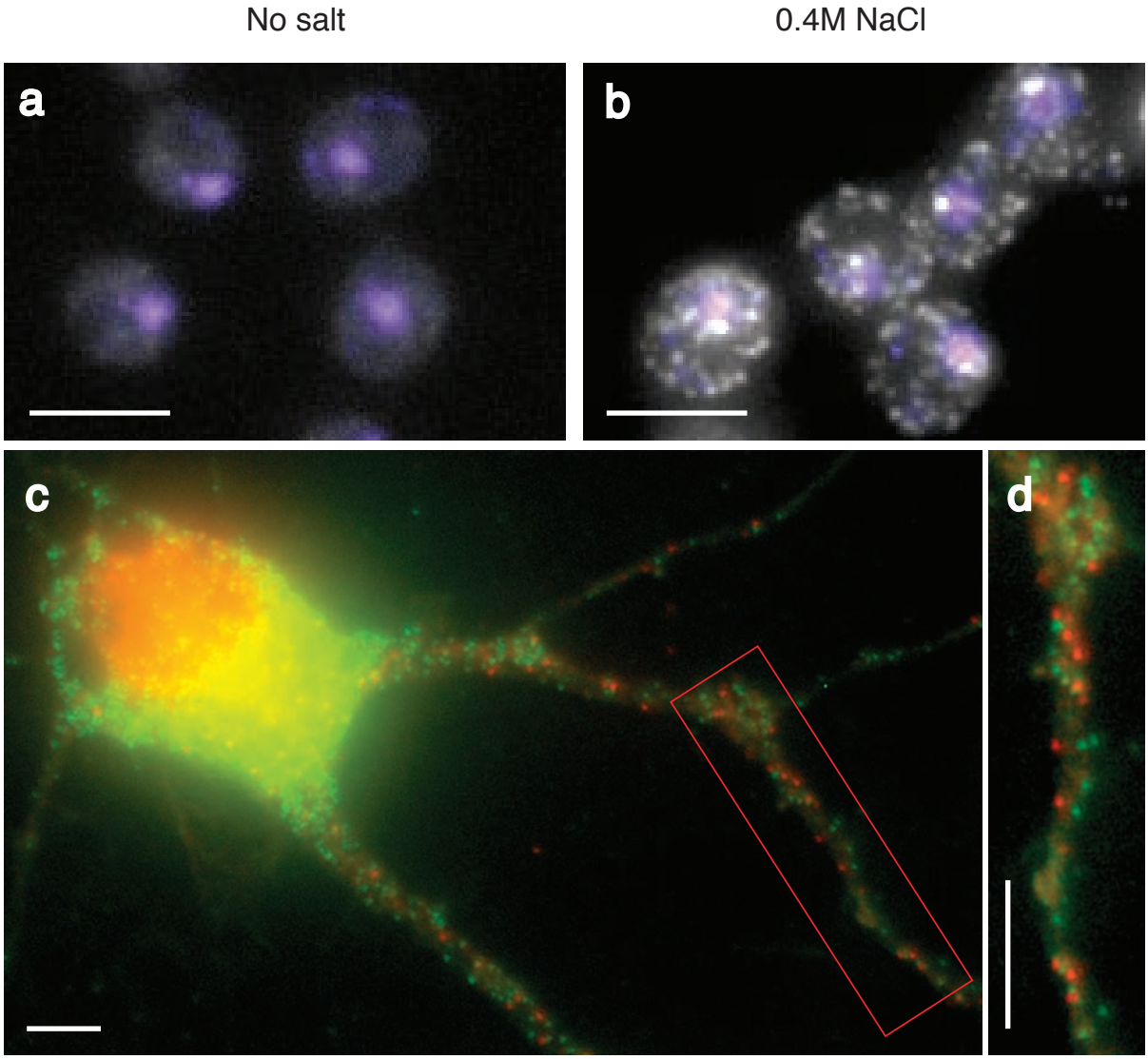


Supplementary Figure 6 | Demonstration that the oxygen-scavenger increases photostability of Cy5. (**a**) The mean of the maximum spot fluorescence for a number of *FAM105A* mRNAs labeled using TMR conjugated probes was plotted as a function of the number of 2 second exposures using a filter specific for TMR. Curves were generated for images taken both with (blue) and without (red) the oxygen scavenging system. A similar analysis was performed for *PTGS2* mRNAs labeled using Alexa 594 conjugated probes with 2 second exposures (**b**) and *FKBP5* mRNAs labeled using Cy5 conjugated probes with 2.5 second exposures (**c**). (**d**) The bleach rate per exposure (in units of fraction of fluorescence lost per exposure) for the TMR, Alexa 594 and Cy5 conjugates probes in (**a**-**c**) both with and without the oxygen-scavenging anti-bleach system. The bleach rate was calculated by fitting each individual particle's decay curve to an exponential and taking the mean of the fitted decay constants. The error bars correspond to one standard deviation. A minimum of 6 particles were chosen in each condition.

Figure overview: One technical challenge that arose when imaging multiple mRNAs simultaneously was fluorophore photolability, particularly in the case of Cy5. In order to image all of the mRNA molecules within a single cell, we acquire 10 to 30 "z-section" images for each visual field, utilizing a one-to-three second exposure for each image and a high numerical aperture objective. Only TMR and (to a lesser extent) Alexa 594 could withstand this intense and relatively prolonged exposure to light; Cy5, for instance, proved extremely photolabile under these conditions. To overcome this problem, we employed a special mounting medium in which fluorophores are much more photostable. This method was adapted from Yildiz et al.[1] with minor modifications. In this medium, a mixture of catalase, glucose oxidase, and glucose enzymatically removes molecular oxygen from the medium, thereby inhibiting oxygen-dependent, light-initiated pathways that destroy fluorophores. The use of these enzymes lead to a dramatic 10-fold enhancement of Cy5 photostability while not adversely affecting the imaging of TMR and Alexa 594, thus facilitating the acquisition of multiple z-sections when performing three color imaging.

1. Yildiz, A. et al. Myosin V walks hand-over-hand: single fluorophore imaging with 1.5-nm localization. *Science* **300**, 2061-2065 (2003).

Supplementary Figure 7



Supplementary Figure 7 | Imaging single mRNA molecules in yeast and neurons. (**a**) *STL1* mRNA particles in both unperturbed cells and (**b**) cells subjected to a 10 minute 0.4M NaCl salt shock, with nuclear DAPI counterstaining in purple. *STL1* is one among a number of yeast genes whose expression is significantly upregulated by the addition of salt to the growth medium[1]. (**c**) Expression of *Actb* (green) and *Mtap2* (red) mRNAs in rat hippocampus neurons in a dissociated neuron culture. (**d**) Enlarged and contrasted image of a segment of a dendrite enclosed by the red box in (**c**). Particle counts indicated that 15% of the 791 *Actb* mRNA molecules were located in dendrites, whereas 37% of the 140 *Mtap2* mRNA molecules were located in the dendrites, similar to previously reported distributions[2,3]. All scale bars are 5 μm long.

References:

1. Rep, M., Krantz, M., Thevelein, J.M. & Hohmann, S. The transcriptional response of Saccharomyces cerevisiae to osmotic shock. Hot1p and Msn2p/Msn4p are required for the induction of subsets of high osmolarity glycerol pathway-dependent genes. *J Biol Chem* **275**, 8290-8300 (2000).

2. Tiruchinapalli, D.M. et al. Activity-dependent trafficking and dynamic localization of zipcode binding protein 1 and beta-actin mRNA in dendrites and spines of hippocampal neurons. *J Neurosci* **23**, 3251-3261 (2003).

3. Blichenberg, A. et al. Identification of a cis-acting dendritic targeting element in MAP2 mRNAs. *J Neurosci* **19**, 8818-8829 (1999).

Supplementary Methods

Synthesis of the probes

The probes were chosen with the following criteria: 1) they should be roughly 17-22 bases long, 2) their GC content should be as close to 45% as possible and 3) there should at least three nucleotides of space between their target regions. To design large number of probes satisfying these conditions, we wrote a program using dynamic programming that optimizes the GC content of each probe subject to the constraints just outlined. This program is available for use on the internet at

http://www.singlemoleculefish.com

A listing of all the probes used in this study aligned with their target mRNA sequence is given at the end of this document (not all probes were designed using the algorithm just described). The oligonucleotides were ordered with 3'-amino modifications from BioSearch Technologies (Novato, California, United States) in plate format with the concentration of each oligonucleotide normalized to 100 μM in water. To couple the oligonucleotides to the appropriate fluorophore, 10 μl were taken from each oligonucleotide, pooled, precipitated and then resuspended in 0.1 M sodium bicarbonate (pH 8.5) with an excess of fluorophore. The fluorophores used were succinomydyl ester derivatives of tetramethylrhodamine (TMR), Alexa-594 (Invitrogen, Carlsbad, California, United States) and Cy5 (Amersham Bioscience, Pittsburgh, Pennsylvania, United States). The coupling proceeded overnight at 37˚C. The excess fluorophore was then removed by ethanol precipitation and then the uncoupled oligonucleotides were separated from the coupled oligonucleotides by reverse phase high pressure liquid chromatography. The coupled fractions were then air-dried in a Speedvac and resuspended in Tris-EDTA, pH 8.0. For each probe, the appropriate concentration for in situ hybridization was determined empirically and was typically around 5-50 ng per hybridization reaction consisting of 50 or 100 μl of hybridization solution.

For the experiments in which different numbers of probes targeting the GFP open reading frame were used, we coupled and purified the first 12, 24, 36 and 48 probes from the set described at the end of this document (*d2EGFP*).

We also generated a probe similar to those used in Femino et al.[1]. The probe sequence was

5′ CGGCRGGTAAGGGRTTCCATARAAACTCCTRAGGCCACGA 3′

where R represents locations where an amino-dT was introduced in place of a regular dT during synthesis; an additional amine group was added by synthesizing the oligo using a controlled pore glass column (Glen Research) that added this group to the 3' end. The probes were coupled to TMR and purified to obtain the most heavily labeled oligonucleotides. Estimates of labeling efficiency show that anywhere between 3 and 5 of the amine groups should be labeled. The construction of the mRNA UTR sequence that this gene targeted was outlined in Raj et al.[2] (designated the M1 multimer).

Mammalian Cell culture

A549 cells (American Type Culture Collection, Manassas, Virginia, United States) were grown on 2-well Lab-tek chambered coverglasses (Nalgene Nunc, Rochester, New York, United States) coated with 0.1% gelatin in DMEM supplemented with L-glutamine, penicillin/streptomycin and 10% Characterized Fetal Bovine Serum (HyClone, Thermo Fisher Scientific, Logan, Utah, United States). The cells were induced for 24 hours through the addition of medium containing 24 nM dexamethasone (Sigma, St. Louis, Missouri, United States). CHO cells were cultured on plain glass coverslips coated with gelatin and placed in culture dishes.

Hippocampus neurons isolated from prenatal Day 18 rats (Brainbits, Springfield, Illinois, United States) were cultured for two weeks on glass coverslips coated with poly-D-lysine and laminin using neurobasal medium (Invitrogen) supplemented with 2% B27 and 0.5% L-glutamine.

Cells attached to chambered coverglass or plain coverslips were fixed by first removing the medium, washing them with PBS buffer (100 mM Na_2HPO_4, 20 mM KH_2PO_4, 137 mM NaCl, 27 mM KCl, pH 7.4), and then incubating them in a solution of PBS with 3.7% formaldehyde for 10 minutes. After the formaldehyde fixation, the cells were washed twice with PBS, permeabilized in 70% ethanol for at least two hours, and then used immediately or stored at 4˚C.

Yeast culture

The yeast strain we utilized was BY4741; Mat a; his3D1; leu2D0; met15D0; ura3D0; YER118c::kanMX4. This strain was grown to an optical density of 0.56 in a 50 mL volume of complete supplemental media without histidine or uracil. The cells were then shocked osmotically by replacing the media with medium containing 0.4M NaCl for 10 minutes. Both shocked and unshocked cells were fixed by adding 5 ml of 37% formaldehyde directly to the medium for 45 minutes. The rest of the fixation and spheroplasting followed the procedure outlined in Long et al.[3] with the following important exception: after spheroplasting, the cells were incubated in concanavalain A (0.1mg/mL, Sigma) for approximately 2 hours before letting them settle onto chambered coverglasses coated overnight with concanavalin A. This increased the efficiency of attachment of the yeast as compared to the more traditional approach of coating the slides with poly-L-lysine.

Worm culture and embryo harvest

The N2 strain of *Caenorhabditis elegans* was grown on plates seeded with OP50 *E. coli* in the standard manner. To extract the embryos from gravid adult hermaphrodites, the worms were washed off the plates in M9 buffer, spun down, and resuspended in a bleaching solution and vigorously shaken for around 5 minutes, at which point the worm bodies themselves are dissolved and only the embryos with their tough chitinous eggshell remain. These embryos were then washed twice with distilled water and then incubated in 1 ml of PBS with 3.7% formaldehyde for 15 minutes. The embryos were then flash frozen by submersion of the tube into liquid nitrogen for 1 minute to crack the eggshells, after which the samples were thawed and incubated for an additional 20 minutes on ice. After that, the embryos were washed twice in PBS and then permeabilized in 70% ethanol for at least 24 hours at 4˚C.

For hybridization with the L1 worm larvae, worms were grown in a mixed stage population as above, washed in PBS, and then resuspended in 1mL of PBS, 3.7% formaldehyde for 45 minutes. After the fixation, the worms were washed twice in PBS and then permeabilized in 70% ethanol for at least 24 hours at 4˚C.

Fly culture and wing imaginal disc harvest

The y cn bw sp strain of *Drosophila melanogaster* was grown according to standard methods. Third instar larvae were isolated and wing discs were manually located and dissected away from the rest of the organism in PBS. The wing discs were placed onto a chambered coverglass and fixed by adding 1 mL of PBS with 3.7% formaldehyde to the chamber for 45 minutes; the fixative was removed by two washes in PBS followed by permeabilization in 70% ethanol for 24 hours at 4˚C.

Fluorescence *in situ* hybridization and imaging

Cells adhered to plain or chambered coverglasses were first rehydrated in a solution of 10% formamide and 2x SSC for 5 minutes. Thereafter, hybridization reactions were performed in 50 µl of hybridization solution containing 10% dextran sulfate, 2 mM vanadyl-ribonucleoside complex, 0.02% RNAse-free BSA, 50 µg E.coli tRNA, 2x SSC, 10% formamide and an empirically determined amount of probe cocktail (typically around 5-50 ng) for 3-24 hours at 37˚C (Singer lab protocol). For the cells attached to chambered coverglass, a coverslip was placed over the hybridization solution both to spread the small volume over the entire surface of the chamber and to prevent evaporation during the overnight incubation. The hybridization reactions with cells grown on plain coverslips were performed by placing them over a drop of hybridization solution on a flat piece of parafilm.

After hybridization the cells were washed twice for 30 minutes at 30˚C using a wash buffer (usually 10%, 15% or 20% formamide and 2xSSC). Nuclear staining was performed by adding DAPI (Invitrogen) to the wash solution during the second wash. For TMR and Alexa-594 labels the imaging was performed using either PBS or 2xSSC as mounting medium. When the Cy5 dye was used as the label, or when we employed long exposure times for imaging with other fluorophores, we used freshly prepared oxygen depleted mounting media. We prepared 100 µl aliquots of this medium just before use by adding 1µl of 3.7mg/mL glucose oxidase and 1µl of catalase suspension (both from Sigma, St.Louis, Missouri, United States) to 10 mM Tris HCl pH 8.0, 2xSSC, and 0.4% glucose, a modification of the system developed by Yildiz et al.[4]. The plain coverslips were mounted on glass slides and sealed with clear nail-polish and chambered coverglasses were covered with a coverslip to prevent evaporation and any influx of oxygen during imaging.

The C. elegans embryos and worms were hybridized in solution. For hybridization, the samples were first spun down using a swinging bucket centrifuge, after which the 70% ethanol was aspirated and the samples were resuspended in a solution of 10% formamide and 2xSSC for 5 minutes. The samples were then spun down again and the supernatant was aspirated. Then 100µl of hybridization solution containing 10% formamide was added to the sample and incubated overnight at 30˚C. The next day, the samples were washed twice by adding 1 ml of wash solution consisting of 10% formamide and 2xSSC, spinning and aspirating the supernatant, and then adding

another 1 ml of wash solution and incubating the samples for 30 minutes at 30˚C. These two washes were followed by another wash and incubation in the same solution supplemented with 5 µg/ml DAPI. Finally the samples were allowed to settle to the bottom of a chambered coverglass and imaged.

For the fly wing discs, immunofluorescence was incorporated into the FISH protocol in the following manner. During the overnight hybridization, the 55 µl of hybridization solution (with 10% formamide) was supplemented with 1 µl of mouse anti-Engrailed antibody (4D9 anti-engrailed/invected-s, Developmental Studies Hybridoma Bank at the University of Iowa) in addition to the probe mixture for *dpp* mRNA detection. In the morning, the samples were washed 2x in 10% formamide, 2xSSC for 30 minutes at 30C. Afterwards, another 55 µl of hybridization solution with 10% formamide supplemented with 1 µl of Alexa-488 goat anti-mouse secondary antibody (Invitrogen) was added to the sample and covered with a cover slip. The secondary labeling proceeded for 1 hour after which the sample was washed twice with 10% formamide, 2xSSC for 30 minutes at 30˚C. Finally, the samples were kept in 2xSSC for imaging.

The images for the colocalization analysis and the images for the neurons were taken with a Zeiss Axiovert 200M inverted fluorescence microscope (Zeiss, Oberkochen, Germany) equipped with a 100x oil-immersion objective and a CoolSNAP HQ camera (Photometrics, Pleasanton, California, United States) using Openlab acquisition software (Improvision, Sheffield, United Kingdom). All other images were taken with a Nikon TE2000 inverted fluorescence microscope equipped with a 100x oil-immersion objective and a Princeton Instruments camera using MetaMorph software (Molecular Devices, Downington, Pennsylvania, United States). Custom narrow band filters to discriminate between TMR and Alexa 594 were obtained from Omega Optical (Brattleboro, Vermont, United States) and broad band filters for TMR, Alexa 594 and Cy5 was obtained from Chroma (Rockingham, Vermont, United States). Typical exposure times used were 1 second for the broad band filters for TMR and Alexa 594 and 3 seconds for the narrow band filters and for the Cy5 filter. Stacks of images were taken automatically with 0.25 microns between the *z*-slices.

Measurements of bleaching rate

A549 cells were grown in 2 chambers of chambered coverglass and induced overnight with medium containing 24nM dexamethasone. After we performed fixation, hybridization and washing as outlined above, we added 2xSSC to one of the chambers and added the anti-bleach solution described above to the other chamber. To measure the rate of photobleaching, we repeatedly imaged spots using 2.5 second exposures for Cy5 and 2 second exposures for TMR and Alexa 594. We then computationally identified spots as described below and measured their maximum intensity as a function of the number of exposures taken. We then computed the average photobleach rate for each spot individually by fitting the intensity profile to an exponential; the average of this value was taken to be the rate of photobleaching.

Measurements of spot intensities using different numbers of probes

A549 cells were grown in 2 chambers of chambered coverglass and induced overnight with medium containing 24nM dexamethasone. After we performed fixation, hybridization and washing as outlined above, we added 2xSSC to one of the chambers

and added the anti-bleach solution described above to the other chamber. To measure the rate of photobleaching, we repeatedly imaged spots using 2.5 second exposures for Cy5 and 2 second exposures for TMR and Alexa 594. We then computationally identified spots as described below and measured their maximum intensity as a function of the number of exposures taken. We then computed the average photobleach rate for each spot individually by fitting the intensity profile to an exponential; the average of this value was taken to be the rate of photobleaching.

Image analysis and particle identification

In order to identify and locate particles in a semi-automated way, images were processed using custom software written in MATLAB (The Mathworks, Natick, Massachusetts, United States).. The first step was to apply a linear filter roughly corresponding to a Laplacian convolved with a Gaussian to remove the non-uniform background while enhancing particles. The optimal bandwidth of the filter (corresponding to the width of the Gaussian) depends on the size of the observed particle and was empirically adjusted to maximize the signal to background of the particles. While this filter enhances particulate signal, noise still appears in the filtered image, necessitating the use of a threshold. The choice of threshold is an important step in accurate determination of mRNA particle number. To perform this in a principled manner, we counted the number of spots for all possible thresholds, where a spot was defined as a connected component in three dimensions. Upon plotting the number of spots detected as a function of the threshold, we typically observed a plateau corresponding to a range of thresholds over which the number of mRNAs detected did not vary. The existence of this plateau indicates that the particle signals are easily detected above background. Moreover, thresholds chosen in this region yielded spot detection that matched very nicely with spots identified by eye. In some cases (typically those with very high background), a plateau did not appear but was rather replaced by a "kink" in the graph. Choosing a threshold at the location of the kink yielded mRNA spot detection that corresponded closely with manual identification. Overall, it was difficult to computationally identify the region where the plateau was without some human guidance, so our method is only semi-automated, but with graphical aides, hundreds of thresholds can be chosen an hour due to the robust appearance of the plateaus. Generally, our algorithm was run on a cell-by-cell basis, thus removing potential differences in signal to background level in different cells in the same field.

For the colocalization analysis, we looked for thresholded particle voxels that overlapped in the two channels; such particles were considered to be colocalized. Software, including all the custom filters used, is available upon request.

For particle intensity measurements, we would first identify the particles as described and take the maximum intensity within the connected component that defines the spot. From this value, we subtracted the local background by taking the mean intensity of an annular region surrounding the spot. We used maximum intensities rather than mean intensities because the use of mean or total intensities is heavily dependent on the size of the spot, which is not a mathematically well-defined quantity and can lead to artifacts in the analysis.

RT-PCR analysis of *FKBP5* induction

A549 cells were grown in 8 chambers of chambered coverglass, of which 4 were induced with medium containing 24nM dexamethasone while the 4 others were grown in medium without dexamethasone. After 8 hours of induction, one of the chambers each from both the induced and uninduced batches was fixed as outlined above while the cells in the remaining 3 chambers were harvested for RT-PCR using the RNeasy Mini Kit (Qiagen, Valencia, California, United States). The RNA was extracted by removing the medium and washing once with PBS, after which 300µl of the cell lysis buffer was added directly to each chamber and mixed by pipetting as per the protocol accompanying the kit, after which the protocol was followed to completion, resulting in three independent RNA isolations each for both the induced and uninduced cells.

The RT-PCRs were performed using the one-step QuantiFast SYBR Green RT-PCR kit (Qiagen) using 5 µl of a 1:10 dilution of template RNA in a 25µl reaction with the following primers (5' to 3'):

FKBP5:	ATTGTCAAAAGAGTGGGGAATG	(forward)
	GCCAAGACTAAAGACAAATGG	(reverse)
PTGS2:	GTCAAAACCGAGGTGTATGT	(forward)
	ATAATTGCATTTCGAAGGAA	(reverse)
FAM105A:	GGACATTGTTAAGCTTCCTG	(forward)
	TTCAGTCCACTGTGTTTTCA	(reverse)

RT-PCRs on each of the three independent isolations were done in triplicate, resulting in 9 measurements for both the uninduced and induced samples. A standard curve was generated by performing RT-PCR on 5µl of 1:1, 1:10, 1:100 and 1:1000 dilutions of one of the induced samples. The variability in the threshold cycles for the uninduced and induced samples was used to compute the error for the RT-PCR fold induction as follows: the standard error was computed for both uninduced and induced threshold cycles and error bars were determined by computing the fold induction for the mean threshold cycle with the standard errors added and subtracted in such a manner as to maximize the error.

In parallel, we performed in situ hybridization on the fixed sample and obtained stacks of fluorescence images for 53 uninduced and 57 induced cells. These images were then analyzed as above to obtain the number of *FKBP5*, *PTGS2* and *FAM105A* mRNAs per cell. Errors in the fold induction as determined by in situ hybridization were obtained by bootstrapping 1000 averages of both the induced and uninduced populations and then finding the standard deviation of the fold induction of the bootstrapped means.

References

1. Femino, A.M., Fay, F.S., Fogarty, K. & Singer, R.H. Visualization of single RNA transcripts in situ. *Science* **280**, 585-590 (1998).
2. Raj, A., Peskin, C.S., Tranchina, D., Vargas, D.Y. & Tyagi, S. Stochastic mRNA synthesis in mammalian cells. *PLoS Biol* **4**, e309 (2006).
3. Long, R.M., Elliott, D.J., Stutz, F., Rosbash, M., Singer, R.H. Spatial consequences of defective processing of specific yeast mRNAs revealed by fluorescent in situ hybridization. *RNA* 1(10), 1071-1078 (1995)
4. Yildiz, A. et al. Myosin V walks hand-over-hand: single fluorophore imaging with 1.5-nm localization. *Science* **300**, 2061-2065 (2003).

Probe sequences used in this study:

Probes for the repeated 3' UTR multimer sequence (4 oligos):

```
TCGACGCGGAGACCACGCTC-GGCTTGTCTTTCGCGCGCAA-TGCGACGCACGCGGATAGTT-AGCTG
AGCTGCGCCTCTGGTGCGAG CCGAACAGAAAGCGCGCGTT ACGCTGCGTGCGCCTATCAA TCGAC

CGGCGACGAGGCACC
GCCGCTGCTCCGTGG
```

Actb, accession #BC063166 (48 oligos):

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           ATGGATGACGATATCGCTGCGCTCGTCGTCGACAACGGCTCCGGCATGTGCAAGGCCGGCTTCGC
                                      CAGCTGTTGCCGAGGCCGTA   GTTCCGGCCGAAGCG

GGGCGACGATGCTCCCCGGGCCGTCTTCCCCTCCATCGTGGGCCGCCCTAGGCACCAGGGTGTGATGGTGGGTAT
CCCGC    TACGAGGGGCCCGGCAGAAG    AGGTAGCACCCGGCGGGATC    GGTCCCACACTACCACCCAT

GGGTCAGAAGGACTCCTACGTGGGCGACGAGGCCCAGAGCAAGAGAGGCATCCTGACCCTGAAGTACCCCATTGA
  CAGTCTTCCTGAGGATGCAC    CTGCTCCGGGTCTCGTTCTC    GTAGGACTGGGACTTCATGG    AACT

ACACGGCATTGTCACCAACTGGGACGATATGGAGAAGATTTGGCACCACACTTTCTACAATGAGCTGCGTGTGGC
TGTGCCGTAACAGTGG    ACCCTGCTATACCTCTTCTA    CGTGGTGTGAAAGATGTTAC    ACGCACACCG

CCCTGAGGAGCACCCTGTGCTGCTCACCGAGGCCCCTCTGAACCCTAAGGCCAACCGTGAAAAGATGACCCAGAT
GGGACTCCTC    GGACACGACGAGTGGCTCCG    AGACTTGGGATTCCGGTTGG    TTTTCTACTGGGTCTA

CATGTTTGAGACCTTCAACACCCCAGCCATGTACGTAGCCATCCAGGCTGTGTTGTCCCTGTATGCCTCTGGTCG
GTAC    CTCTGGAAGTTGTGGGGTCG    CATGCATCGGTAGGTCCGAC    ACAGGGACATACGGAGACCA

TACCACTGGCATTGTGATGGACTCCGGAGACGGGGTCACCCACACTGTGCCCATCTATGAGGGTTACGCGCTCCC
 TGGTGACCGTAACACTACCT    GCCTCTGCCCCAGTGGGTGT    ACGGGTAGATACTCCCAATG    GAGGG

TCATGCCATCCTGCGTCTGGACCTGGCTGGCCGGGACCTGACAGACTACCTCATGAAGATCCTGACCGAGCGTGG
AGTACGGTAGGACGC    CCTGGACCGACCGGCCCTGG    GTCTGATGGAGTACTTCTAG    TGGCTCGCACC

CTACAGCTTCACCACCACAGCTGAGAGGGAAATCGTGCGTGACATTAAAGAGAAGCTGTGCTATGTTGCCCTAGA
GATGTCGAA    GTGGTGTCGACTCTCCCTTT    ACGCACTGTAATTTCTCTTC    ACGATACAACGGGATCT

CTTCGAGCAAGAGATGGCCACTGCCGCATCCTCTTCCTCCCTGGAGAAGAGCTATGAGCTGCCTGACGGTCAGGT
GAA    CGTTCTCTACCGGTGACGGC    GGAGAAGGAGGGACCTCTTC    ATACTCGACGGACTGCCAGT

CATCACTATCGGCAATGAGCGGTTCCGATGCCCCGAGGCTCTCTTCCAGCCTTCCTTCCTGGGTATGGAATCCTG
GTAGTGATAGCCGTTACTCG    AGGCTACGGGGCTCCGAGAG    GTCGGAAGGAAGGACCCATA    TAGGAC

TGGCATCCATGAAACTACATTCAATTCCATCATGAAGTGTGACGTTGACATCCGTAAAGACCTCTATGCCAACAC
ACCGTAGGTACTTT    GTAAGTTAAGGTAGTACTTC    CTGCAACTGTAGGCATTTCT    GATACGGTTGTG

AGTGCTGTCTGGTGGCACCACCATGTACCCAGGCATTGCTGACAGGATGCAGAAGGAGATTACTGCCCTGGCTCC
TCACGACA    CACCGTGGTGGTACATGGGT    TAACGACTGTCCTACGTCTT    CTAATGACGGGACCGAGG

TAGCACCATGAAGATCAAGATCATTGCTCCTCCTGAGCGCAAGTACTCTGTGTGGATTGGTGGCTCTATCCTGGC
AT    GGTACTTCTAGTTCTAGTAA    GGAGGACTCGCGTTCATGAG    CACCTAACCACCGAGATAGG    G

CTCACTGTCCACCTTCCAGCAGATGTGGATCAGCAAGCAGGAGTACGATGAGTCCGGCCCCTCCATCGTGCACCG
GAGTGACAGGTGGAAGGTC    TACACCTAGTCGTTCGTCCT    GCTACTCAGGCCGGGGAGGT    ACGTGGC
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CAAATGCTTCTAG
GTTTACGAAGATC
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d2EGFP (48 oligos):

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          GCGGATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAGCTGGACG
             CTACCACTCGTTCCCGC   TCGACAAGTGGCCCCAC   GGGTAGGACCAGCTCGA   GC

GCGACGTAAACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCCTGA
CGCTGCATTTGCCGG   TCAAGTCGCACAGGCCG   CCGCTCCCGCTACGGTG   GCCGTTCGACTGGGACT

AGTTCATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCACCCTCGTGACCACCCTGACCTACGGCGTGCAGT
   AGTAGACGTGGTGGCCG   GACGGGCACGGGACCGG   GGAGCACTGGTGGGACT   TGCCGCACGTCA

GCTTCAGCCGCTACCCCGACCACATGAAGCAGCACGACTTCTTCAAGTCCGCCATGCCCGAAGGCTACGTCCAGG
CGAAG   GCGATGGGGCTGGTGTA   CGTCGTGCTGAAGAAGT   GGCGGTACGGGCTTCCG   CAGGTCC

AGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGG
TCGCGTGGTA   GAAGTTCCTGCTGCCGT   TGTTCTGGGCGCGGCTC   TTCAAGCTCCCGCTGTG   CC

TGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTACAACT
ACTTGGCGTAGCTCG   TCCCGTAGCTGAAGTTC   CTGCCGTTGTAGGACCC   GTTCGACCTCATGTTGA

ACAACAGCCACAACGTCTATATCATGGCCGACAAGCAGAAGAACGGCATCAAGGTGAACTTCAAGATCCGCCACA
   TGTCGGTGTTGCAGATA   TACCGGCTGTTCGTCTT   GCCGTAGTTCCACTTGA   TCTAGGCGGTGT

ACATCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAGCAGAACACCCCCATCGGCGACGGCCCCGTGCTGC
TGTAG   CTGCCGTCGCACGTCGA   GCTGGTGATGGTCGTCT   GGGGGTAGCCGCTGCCG   CACGACG

TGCCCGACAACCACTACCTGAGCACCCAGTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCC
ACGGGCTGTT   GATGGACTCGTGGGTCA   GGGACTCGTTTCTGGGG   CTCTTCGCGCTAGTGTA   GG

TGCTGGAGTTCGTGACCGCCGCCGGGATCACTCTCGGCATGGACGAGCTGTACAAGAAGCTTAGCCATGGCTTCC
ACGACCTCAAGCACT   GGCGGCCCTAGTGAGAG   TACCTGCTCGACATGTT   CGAATCGGTACCGAAGG

CGCCGGAGGTGGAGGAGCAGGATGATGGCACGCTGCCCATGTCTTGTGCCCAGGAGAGCGGGATGGACCGTCACC
   GCCTCCACCTCCTCGTC   CTACCGTGCGACGGGTA   AACACGGGTCCTCTCGC   ACCTGGCAGTGG

CTGCAGCCTGTGCTTCTGCTAGGATCAATGTGTAGGAATTCGTGACATGATAAGATACATTGATGAGTTTGGACA
GACGT   ACACGAAGACGATCCTA TTACACATCCTTAAGCA   TACTATTCTATGTAACT   CAAACCTGT

AACCACAACTAGAATGCAGTGAAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTG
TTGGTGTT   CTTACGTCACTTTTTTT   AAATAAACACTTTAAAC   ACGATAACGAAATAAAC
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dpp, accession # NM_164485 (48 oligos):

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          ATGCGCGCATGGCTTCTACTCCTCGCAGTGCTGGCGACTTTTCAAACGATTGTTCGAGTTGCTAG
            CGCGCGTACCGAAGATGAGG      CACGACCGCTGAAAAGTTTG      AGCTCAACGATC

CACCGAGGATATATCCCAGAGATTCATCGCCGCCATAGCGCCCGTTGCCGCTCATATTCCGCTGGCATCAGCATC
GTGGCTCC                 GTAGCGGCGGTATCGCGGGC                GGCGACCGTAGTCGTAG

AGGATCAGGATCAGGACGATCTGGATCTAGATCGGTAGGAGCCTCGACCAGCACAGCATTAGCAAAAGCATTTAA
TCC                               GCCATCCTCGGAGCTGGTCG

TCCATTCAGCGAGCCCGCCTCGTTCAGTGATAGTGATAAAAGCCATCGGAGTAAAACAAACAAAAAACCTAGCAA
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      GTCGCTCGGGCGGAGCAAGT      TCACTATTTTCGGTAGCCTC

AAGTGACGCGAACCGACAGTTCAACGAAGTGCATAAGCCAAGAACAGACCAATTAGAAAATTCCAAAAATAAGTC
      GCGCTTGGCTGTCAAGTTGC

TAAACAATTAGTTAATAAACCCAACCACAACAAAATGGCTGTCAAGGAGCAGAGGAGCCACCACAAGAAGAGCCA
                                            GTTCCTCGTCTCCTCGGTGG       CTCGGT

CCACCATCGCAGCCACCAGCCAAAGCAGGCCAGTGCATCCACAGAATCTCATCAATCCTCGTCGATTGAATCAAT
GGTGGTAGCGTCGG           CGTCCGGTCACGTAGGTGTC

CTTCGTGGAGGAGCCGACGCTGGTGCTCGACCGCGAGGTGGCCTCCATCAACGTGCCCGCCAACGCCAAGGCCAT
   GCACCTCCTCGGCTGCGACC                                 GCACGGGCGGTTGCGGTTCC

CATCGCCGAGCAGGGCCCGTCCACCTACAGCAAGGAGGCGCTCATCAAGGACAAGCTGAAGCCAGACCCCTCCAC
     GGCTCGTCCCGGGCAGGTGG                                  TCGACTTCGGTCTGGGGAGG

TCTAGTCGAGATCGAGAAGAGCCTGCTCTCGCTGTTCAACATGAAGCGGCCGCCCAAGATCGACCGCTCCAAGAT
             CTCTTCTCGGACGAGAGCGA                  GGCGGGTTCTAGCTGGCGAG

CATCATCCCCGAGCCGATGAAGAAGCTCTACGCCGAGATCATGGGCCACGAGCTCGACTCGGTCAACATCCCCAA
  AGTAGGGGCTCGGCTACTTC          CGGCTCTAGTACCCGGTGCT                GTAGGGGTT

GCCGGGTCTGCTGACCAAGTCGGCCAACACAGTGCGAAGTTTTACACACAAAGATAGTAAAATCGACGATCGATT
CGGCCCAGACG      TTCAGCCGGTTGTGTCACGC

TCCGCACCACCACCGGTTTCGGCTGCACTTCGACGTGAAGAGCATTCCCGCCGACGAGAAGCTGAAGGCGGCGGA
      GGTGGTGGCCAAAGCCGACG                                 GCTCTTCGACTTCCGCCGCC

GCTGCAGCTGACCCGGGACGCACTCAGTCAACAGGTGGTGGCCAGCAGATCGTCGGCGAATCGGACGCGCTACCA
    GTCGACTGGGCCCTGCGTGA             CCACCGGTCGTCTAGCAGCC     GCCTGCGCGATGGT

GGTGCTTGTCTACGACATCACGCGCGTCGGGGTGCGTGGTCAGCGGGAGCCGAGCTATCTGCTGTTGGACACCAA
CCACGA             GTGCGCGCAGCCCCACGCAC                                CCTGTGGTT

GACGGTCCGGCTTAACAGCACGGACACGGTGAGCCTCGATGTCCAGCCGGCCGTGGACCGGTGGCTGGCGAGTCC
CTGCCAGGCCG       CGTGCCTGTGCCACTCGGAG             CGGCACCTGGCCACCGACCG

GCAGCGCAACTACGGACTGCTGGTGGAGGTGCGGACGGTCCGCTCCCTGAAGCCGGCCCCACACCACCATGTACG
  TCGCGTTGATGCCTGACGAC      CCACGCCTGCCAGGCGAGGG             GTGTGGTGGTACATGC

CCTGCGCCGCAGCGCGGACGAGGCGCACGAGCGGTGGCAGCACAAGCAGCCGCTCCTGTTCACCTACACGGACGA
GGAC      GTCGCGCCTGCTCCGCGTGC       CCGTCGTGTTCGTCGGCGAG        GGATGTGCCTGCT

CGGGCGGCACAAGGCGCGCTCCATTCGGGACGTGTCTGGCGGAGAGGGCGGTGGCAAGGGCGGCCGGAACAAGCG
GCCCGCC                                GACCGCCTCTCCCGCCACCG                   C

GCAGCCGAGACGGCCTACGAGGCGCAAGAACCACGACGACACCTGCCGGCGGCACTCGCTGTACGTGGACTTCTC
CGTCGGCTCTGCCGGATGC                       GTGGACGGCCGCCGTGAGCG              GAG

GGACGTGGGCTGGGACGACTGGATTGTGGCGCCTCTGGGCTACGATGCATATTACTGCCACGGGAAGTGCCCCTT
CCTGCACCCGACCCTGC        ACACCGCGGAGACCCGATGC                          CACGGGGAA

CCCGCTGGCCGACCACTTTAACTCGACCAATCACGCCGTGGTGCAGACCCTGGTCAACAATATGAATCCCGGCAA
GGGCGACCGGC                     AGTGCGGCACCACGTCTGGG                   GGGCCGTT

GGTGCCGAAGGCGTGCTGCGTGCCCACGCAACTGGACAGCGTGGCCATGCTCTATCTCAACGACCAAAGTACGGT
CCACGGCTTCCG      CGCACGGGTGCGTTGACCTG                          GCTGGTTTCATGCCA

GGTGCTGAAGAACTACCAGGAGATGACCGTGGTGGGCTGTGGCTGTCGATAG
CCACG                   ACTGGCACCACCCGACACCG
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elt-2, accession # NM_077354 (48 oligos):

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                                        GTTGCCGACCCGGCTTTACC     TAGAGTTGGTTACCC

AGGTCTGCGCCTACCAACTCAGAACATGGATCCACCAGAGCAAAATAATGAGTCACAATTGAGTGAACTACCGAG
TCCAG     GGATGGTTGAGTCTTGTACC     TGGTCTCGTTTTATTACTCA     TAACTCACTTGATGGCTC

AATGAAAATTGATAATGATTACGCATCTCCAATTGAACGGCAAAGTGTTATCACAAGTGGCACAAATAACTATGA
TT                                TTAACTTGCCGTTTCACAAT     TTCACCGTGTTTATTGATAC

GCCGAAAGTGGAAACTGTTACATCATTTTTCCATACTGGCATAGACTACTCAAACTTTGGAATGTTGGACCAAAC
   CTTTCACCTTTGACAATGTA     AAAGGTATGACCGTATCTGA     TTTGAAACCTTACAACCTGG

TACCATGCAACCGTTTTATCCTCTTTACAGTGGAATTCCCGTAAACACTCTTGGAACTTTTTCGGGATATACAAA
ATGGTACGTTGGCAAAATAG     AATGTCACCTTAAGGGCATT     AGAACCTTGAAAAAGCCCTA     TTT

CTCCATATACGACAAACCCTCTCTGTACGACCCCAGTATTCCTACCATTAACATCCCTTCTACTTATCCAACTGT
GAGGTATATGCTGTTTG     AGACATGCTGGGGTCATAAG     GTAATTGTAGGGAAGATGAA     TTGACA

GGCTCCAACTTACGAATGCGTCAAATGCTCACAAAGTTGTGGGGCCGGGATGAAGGCAGTAAACGGAGGAATGAT
CCGAGGTTGAATGC     GCAGTTTACGAGTGTTTCAA     CCGGCCCTACTTCCGTCATT     TCCTTACTA

GTGCGTCAACTGTTCAACACCAAAAACCACGTATTCTCCTCCAGTCGCGTATAGCACTTCTTTGGGACAACCCCC
CACGCAGTTGA     TTGTGGTTTTTGGTGCATAA     AGGTCAGCGCATATCGTGAA     CCCTGTTGGGGG

GATTCTGGAAATACCTTCAGAGCAGCCAACTGCTAAAATTGCCAAGCAATCCTCTAAAAAGTCAAGTAGCTCAAA
CTAAGACC     TGGAAGTCTCGTCGGTTGAC     TTAACGGTTCGTTAGGAGAT     CAGTTCATCGAGTTT

TAGGGGGTCAAACGGATCTGCGTCCCGTCGGCAGGGACTTGTGTGCTCCAATTGCAATGGTACCAACACAACTCT
ATCCC     TTTGCCTAGACGCAGGGCAG     CCCTGAACACACGAGGTTAA     ACCATGGTTGTGTTGAGA

CTGGAGAAGAAATGCTGAAGGAGATCCGGTCTGCAATGCTTGCGGGCTTTACTTCAAACTCCATCACATCCCTCG
GA     TTCTTTACGACTTCCTCTAG     GACGTTACGAACGCCCGAAA     GTTTGAGGTAGTGTAGGGAG

GCCGACCTCAATGAAGAAAGAAGGTGCTTTACAGACAAGAAAGAGAAAATCAAAAAGCGGAGACTCTTCCACACC
   CTGGAGTTACTTCTTTCTTC                                  TTTTTCGCCTCTGAGAAGGT

ATCAACGTCACGGGCCCGAGAAAGGAAGTTTGAGAGAGCCTCTTCTTCGACCGAAAAGGCTCAAAGGTCATCTAA
TAGTTGCAGTGCCCGGGCTC     CTTCAAACTCTCTCGGAGAA     CTGGCTTTTCCGAGTTTCCA     ATT

CCGGCGTGCGGGAAGTGCAAAAGCAGACCGAGAACTGAGCACTGCTGCCGTCGCAGCTGCGACTGCCACATATGT
GGCCGCACGCCCTTCAC     TCGTCTGGCTCTTGACTCGT                                  TATACA

GTCACATGCCGACTTGTATCCCGTTTCCTCAGCTGCCGTCACCTTGCCAGATCAAACGTACAGTAATTACTATCA
CAGTGTACGGCTGA     AGGGCAAAGGAGTCGACGGC     GAACGGTCTAGTTTGCATGT

ATGGAACACTGCCGCTACAGCTGGGTTGATGATGGTTCCAAACGATCAAAACTACGTGTATGCAGCAACAAACTA
               ATGTCGACCCAACTACTACC                                  TCGTTGTTTGAT

CCAGACTGGCCTAAGACCTGCCGATAACATCCAAGTTCATGTGATGCCAGTTCAGGATGATGAAACCAAAGCTGC
GGTCTGAC     TTCTGGACGGCTATTGTAGG     AGTACACTACGGTCAAGTCC     ACTTTGGTTTCGACG

GGCTCGCGATTTGGAAGCGGTCGACGGAGATTCTTAA
CCGAG     AAACCTTCGCCAGCTGCCTC
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FAM105A, accession # NM_019018.1 (53 oligos):

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TTCCTCGGGGTGCGCCC CCCTCGCCCTCGCCAGA CGAGGGCGGCGTCC

AAGTGACCAAGTTCACTCCTGGATGCTAGCTACAAGCCAAGCCTTAGACACTGTCTGGAGAATGGCAAAAGGCTT
TTC GGTTCAAGTGAGGACCT ATCGATGTTCGGTTCGG CTGTGACAGACCTCTTA TTTTCCGAA

TGTGATGTTGGCAGTTTCATTTCTGGTGGCTGCCATCTGCTACTTCCGGAGGCTACATTTATATTCAGGGCACAA
ACACTACA GTCAAAGTAAAGACCAC CGGTAGACGATGAAGGC CGATGTAAATATAAGTC TGTT

GCTGAAATGGTGGATTGGATATCTGCAGAGAAAATTCAAAAGGAACCTCAGTGTGGAGGCAGAGGTTGATTTACT
CGACTTTACCACC CCTATAGACGTCTCTTT GTTTTCCTTGGAGTCAC TCCGTCTCCAACTAAAT

CAGTTATTGTGCAAGAGAATGGAAAGGAGAGACACCCCGTAACAAGCTGATGAGGAAGGCTTATGAGGAGCTATT
TCAATAACACGTTCTCT CTTTCCTCTCTGTGGGG TGTTCGACTACTCCTTC ATACTCCTCGATAA

TTGGCGGCATCACATTAAATGTGTTCGACAAGTAAGGAGAGATAACTATGATGCTCTCAGATCAGTGTTATTTCA
AAC CGTAGTGTAATTTACAC CTGTTCATTCCTCTCTA ATACTACGAGAGTCTAG CAATAAAGT

GATATTCAGCCAGGGCATCTCTTTTCCATCATGGATGAAAGAAAAGGACATTGTTAAGCTTCCTGAAAAACTGCT
CTATAAGT TCCCGTAGAGAAAAGGT ACCTACTTTCTTTTCCT ACAATTCGAAGGACTTT ACGA

GTTTTCACAAGGTTGTAATTGGATTCAGCAGTACAGTTTTGGTCCTGAGAAGTATACAGGCTCGAATGTGTTTGG
CAAAAGTGTTCCA TTAACCTAAGTCGTCAT AAAACCAGGACTCTTCA GTCCGAGCTTACACAAA

AAAACTACGGAAATATGTGGAATTATTGAAAACACAGTGGACTGAATTTAATGGCATTAGAGATTATCACAAGAG
TTTGATGCCTTTATACA TAATAACTTTTGTGTCA GACTTAAATTACCGTAA CTAATAGTGTTCTC

AGGAAGTATGTGCAACACCCTTTTTTCAGATGCCATTCTGGAATATAAACTTTATGAAGCTTTAAAGTTCATCAT
TCC ATACACGTTGTGGGAAA GTCTACGGTAAGACCTT TTTGAAATACTTCGAAA CAAGTAGTA

GCTGTATCAAGTCACTGAAGTTTATGAACAAATGAAGACTAAAAAGGTCATTCCCAGTCTTTTTAGACTCCTGTT
CGACATAG AGTGACTTCAAATACTT TACTTCTGATTTTTCCA AGGGTCAGAAAAATCTG ACAA

TTCCAGGGAGACATCCTCTGATCCTTTGAGCTTCATGATGAATCACCTGAATTCTGTAGGCGACACATGTGGACT
AAGGTCCCTCTGT AGACTAGGAAACTCGAA CTACTTAGTGGACTTAA ATCCGCTGTGTACACCT

AGAGCAGATTGATATGTTTATACTTGGATACTCCCTTGAAGTAAAGATAAAAGTGTTCAGACTGTTCAAGTTTAA
CTCGTCTAACTATACAA TGAACCTATGAGGGAAC ATTTCTATTTTCACAAG GACAAGTTCAAATT

CTCCAGAGACTTTGAAGTCTGCTACCCAGAGGAGCCTCTCAGGGACTGGCCGGAGATCTCCCTGCTGACCGAGAA
GAG TCTGAAACTTCAGACGA GTCTCCTCGGAGAGTCC ACCGGCCTCTAGAGGGA CTGGCTCTT

CGACCGCCACTACCACATTCCAGTCTTTTAA
GCTGGCGG TGGTGTAAGGTCAGAAA

FKBP5, accession # NM_004117.2 (63 oligos):

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AGAGGATATTACCTCCAAAAAAGACAGGGGAGTATTAAAGATTGTCAAAAGAGTGGGGAATGGTGAGGAAACGCC
TCCTTTGCGG

GATGATTGGAGACAAAGTTTATGTCCATTACAAAGGAAAATTGTCAAATGGAAAGAAGTTTGATTCCAGTCATGA
CTACTAA CTGTTTCAAATACAGGT GTTTCCTTTTAACAGTT CTTTCTTCAAACTAAGG GTACT

TAGAAATGAACCATTTGTCTTTAGTCTTGGCAAAGGCCAAGTCATCAAGGCATGGGACATTGGGGTGGCTACCAT
ATCTTTACTTGG ACAGAAATCAGAACCGT CGGTTCAGTAGTTCCGT CTGTAACCCCACCGATG

GAAGAAAGGAGAGATATGCCATTTACTGTGCAAACCAGAATATGCATATGGCTCGGCTGGCAGTCTCCCTAAAAT

CTTCTTTCCTCTCTATA TAAATGACACGTTTGGT ATACGTATACCGAGCCG GTCAGAGGGATTTTA

TCCCTCGAATGCAACTCTCTTTTTTGAGATTGAGCTCCTTGATTTCAAAGGAGAGGATTTATTTGAAGATGGAGG
AG GCTTACGTTGAGAGAAA CTCTAACTCGAGGAACT GTTTCCTCTCCTAAATA TTCTACCTCC

CATTATCCGGAGAACCAAACGGAAAGGAGAGGGATATTCAAATCCAAACGAAGGAGCAACAGTAGAAATCCACCT
GTAATAG TCTTGGTTTGCCTTTCC CCCTATAAGTTTAGGTT TTCCTCGTTGTCATCTT GTGGA

GGAAGGCCGCTGTGGTGGAAGGATGTTTGACTGCAGAGATGTGGCATTCACTGTGGGCGAAGGAGAAGACCACGA
CCTTCCGGCGAC ACCTTCCTACAAACTGA CTCTACACCGTAAGTGA CCGCTTCCTCTTCTGGT

CATTCCAATTGGAATTGACAAAGCTCTGGAGAAAATGCAGCGGGAAGAACAATGTATTTTATATCTTGGACCAAG
GTAAGGTTAACCTTAAC TTCGAGACCTCTTTTAC GCCCTTCTTGTTACATA TATAGAACCTGGTTC

ATATGGTTTTGGAGAGGCAGGGAAGCCTAAATTTGGCATTGAACCTAATGCTGAGCTTATATATGAAGTTACACT
TA CAAAACCTCTCCGTCCC GGATTTAAACCGTAACT ATTACGACTCGAATATA TTCAATGTGA

TAAGAGCTTCGAAAAGGCCAAAGAATCCTGGGAGATGGATACCAAAGAAAAATTGGAGCAGGCTGCCATTGTCAA
ATTCTCG CTTTTCCGGTTTCTTAG CCTCTACCTATGGTTTC TTAACCTCGTCCGACGG CAGTT

AGAGAAGGGAACCGTATACTTCAAGGGAGGCAAATACATGCAGGCGGTGATTCAGTATGGGAAGATAGTGTCCTG
TCTCTTCCCTTG TATGAAGTTCCCTCCGT TGTACGTCCGCCACTAA ATACCCTTCTATCACAG

GTTAGAGATGGAATATGGTTTATCAGAAAAGGAATCGAAAGCTTCTGAATCATTTCTCCTTGCTGCCTTTCTGAA
CAATCTCTACCTTATAC ATAGTCTTTTCCTTAGC CGAAGACTTAGTAAAGA ACGACGGAAAGACTT

CCTGGCCATGTGCTACCTGAAGCTTAGAGAATACACCAAAGCTGTTGAATGCTGTGACAAGGCCCTTGGACTGGA
GG GGTACACGATGGACTTC TCTCTTATGTGGTTTCG ACTTACGACACTGTTCC AACCTGACCT

CAGTGCCAATGAGAAAGGCTTGTATAGGAGGGGTGAAGCCCAGCTGCTCATGAACGAGTTTGAGTCAGCCAAGGG
GTCACGG CTCTTTCCGAACATATC CCCACTTCGGGTCGACG ACTTGCTCAAACTCAGT TTCCC

TGACTTTGAGAAAGTGCTGGAAGTAAACCCCCAGAATAAGGCTGCAAGACTGCAGATCTCCATGTGCCAGAAAAA
ACTGAAACTCTT CGACCTTCATTTGGGGG TATTCCGACGTTCTGAC TAGAGGTACACGGTCTT

GGCCAAGGAGCACAACGAGCGGGACCGCAGGATATACGCCAACATGTTCAAGAAGTTTGCAGAGCAGGATGCCAA
CCGGTTCCTCGTGTTGC CCCTGGCGTCCTATATG TTGTACAAGTTCTTCAA TCTCGTCCTACGGTT

GGAAGAGGCCAATAAAGCAATGGGCAAGAAGACTTCAGAAGGGGTCACTAATGAAAAAGGAACAGACAGTCAAGC
CC TCCGGTTATTTCGTTAC TTCTTCTGAAGTCTTCC GTGATTACTTTTTCCTT TGTCAGTTCG

AATGGAAGAAGAGAAACCTGAGGGCCACGTATGA
TTACCTT CTCTTTGGACTCCCGGT

Mtap2, accession # NM_013066 (72 oligos):

ATGGCTGACGAGAGGAAAGACGAAGGAAAGGCACCACACTGGACATCAGCCTCACTCACAGAGGC
CTGCTTCCTTTCCGTGGTGT CGGAGTGAGTGTCTCCG

AGCTGCACACCCCCACTCGCCAGAGATGAAGGACCAGGGTGGCTCAGGGGAAGGGCTGAGCCGCAGCGCCAATGG
TCG GTGAGCGGTCTCTACTTCCT AGTCCCCTTCCCGACTCGGC CC

ATTTCCATACAGAGAGGAGGAGGAAGGCGCCTTTGGGGAGCACGGGTCACAGGGCACCTATTCAGATACCAAAGA
TAAAGGTATGTCTCTCCT CGGAAACCCCTCGTGCCCAG ATAAGTCTATGGTTTCT

GAACGGGATCAACGGAGAGCTGACCTCAGCTGACAGAGAAACAGCAGAGGAAGTGTCTGCAAGGATAGTTCAAGT
CTT CCTCTCGACTGGAGTCGACT CGTCTCCTTCACAGACGTTC CA

AGTCACAGCTGAAGCTGTAGCAGTCCTGAAAGGTGAACAAGAGAAGGAGGCCCAACACAAGGATCAGCCTGCAGC
TCAGTGTCGACTTCGACA TTTCCACTTGTTCTCTTCCT TTCCTAGTCGGACGTCG

TCTGCCTTTAGCAGCTGAAGAAACAGTTAATCTGCCACCTTCCCCACCACCATCGCCAGCATCAGAACAAACAGC
AGA CGACTTCTTTGTCAATTAGA GGTGGTGGTAGCGGTCGTAG CG

TGCACTGGAAGAAGCCTCGAAGATGGAATTCCCTGAGCAGCAGAAATTGCCTTCCTCATTCGCTGAGCCTTTAGA
ACGTGACCTTCTTCGGAG AAGGGACTCGTCGTCTTTAA AAGCGACTCGGAAATCT

CAAGGAGGAAACGGAGTTTAAGATGCAAAGTAAGCCTGGTGAAGACTTTGAACATGCTGCCTTAGTTCCTCAGCC
GTT CTCAAATTCTACGTTTCATT CTGAAACTTGTACGACGGAA GG

GGACACAAGTAAAACTCCCCAGGATAAAAAGGATCCCCAAGACATGGAAGGAGAAAAGTCGCCTGCCAGTCCATT
CCTGTGTTCATTTTGAGG TTCCTAGGGGTTCTGTACCT AGCGGACGGTCAGGTAA

TGCGCAGACTTTCGGTACCAACCTGGAAGACATAAAACAGATCACAGAACCAAGCATAACAGTACCTAGCATTGG
ACG CCATGGTTGGACCTTCTGTA TGTCTTGGTTCGTATTGTCA CC

CCTCTCCGCAGAGCCCCTAGCTCCAAAAGATCAGAAAGACTGGTTCATCGAAATGCCCGTGGAATCAAAGAAGGA
GGAGAGGCGTCTCGGGGA CTAGTCTTTCTGACCAAGTA CACCTTAGTTTCTTCCT

TGAATGGGGTTTAGCTGCCCCAATATCTCCTGGCCCCTTGACACCCATGAGGGAAAAAGATGTGCTGGAGGATAT
ACT CGACGGGGTTATAGAGGACC GGGTACTCCCTTTTTCTACA TA

CCCAAGATGGGAAGGAAAGCAGTTTGACTCTCCCATGCCTAGCCCCTTCCACAGTGGAAGTTTCACTCTTCCCTT
GGGTTCTACCCTTCCTTT AGAGGGTACGGATCGGGGAA TCAAAGTGAGAAGGGAA

AGATACTGTGAAAGATGAGAGAGTCACAGAAGGGTCACAACCCTTTGCCCCTGTCTTCTTCCAATCAGATGACAA
TCT CTACTCTCTCAGTGTCTTCC AAACGGGGACAGAAGAAGGT TT

AATGTCTCTGCAGGACACCAGTGGTTCAGCTACTTCCAAAGAGAGTTCTAAAGATGAGGAGCCACAGAAAGATAA
TTACAGAGACGTCCTGTG CGATGAAGGTTTCTCTCAAG CTCGGTGTCTTTCTATT

AGCAGACAAAGTGGCAGATGTTCCTGTCTCAGAAGCTACCACTGTACTGGGAGATGTTCACAGTCCAGCTGTGGA
TCG CGTCTACAAGGACAGAGTCT CATGACCCTCTACAAGTGTC CT

AGGCTTTGTCGGGGAGAACATTTCAGGAGAAGAAAAGGGTACCACAGATCAAGAGAAAAAAGAGACTTCGACACC
TCCGAAACAGCCCCTCTT CTTCTTTTCCCATGGTGTCT TTTCTCTGAAGCTGTGG

CAGTGTACAGGAACCTACACTCACTGAAACTGAACCACAGACAAAGCTTGAAGAGACATCAAAGGTTTCCATCGA
GTC GGATGTGAGTGACTTTGACT TTCGAACTTCTCTGTAGTTT CT

AGAAACTGTGGCAAAAGAAGAGGAATCCTTGAAATTAAAAGATGATAAAGCAGGTGTAATTCAGACTTCCACCGA
TCTTTGACACCGTTTTCT AACTTTAATTTTCTACTATT TAAGTCTGAAGGTGGCT

GCATTCTTTCTCCAAAGAAGACCAGAAAGGCGAAGAACAGACAATCGAAGCATTAAAACAAGACTCCTTTCCTAT
CGT TTTCTTCTGGTCTTTCCGCT

AAGTCTAGAACAGGCAGTTACAGATGCAGCCATGGCCACCAAGACCTTGGAAAAGGTTACGTCTGAGCCAGAGGC

AGTAAGTGAAAAGAGAGAAATCCAGGGACTTTTTGAAGAGGATATAGCTGACAAGAGTAAGCTCGAAGGCGCTGG
CGAGCTTCCGCGACC

GTCTGCAACAGTAGCCGAGGTTGAGATGCCATTTTATGAAGATAAATCAGGGATGTCCAAGTACTTTGAAACATC
CAGAC

TGCATTGAAAGAAGATGTGACCAGAAGCACTGGGTTGGGCAGTGATTACTACGAGCTGAGTGACTCAAGAGGAAA
GGTCTTCGTGACCCAACCCG

TGCCCAGGAATCTCTTGATACTGTATCTCCCAAGAACCAACAAGATGAAAAGGAACTTCTGGCAAAAGCTTCCCA
AGGGT

GCCTAGTCCTCCAGCACACGAAGCAGGGTACAGCACTCTTGCCCAGAGTTATACATCTGATCATCCGTCCGAGTT

CGGATCAGGAGGTCG

ACCTGAAGAACCAAGTTCTCCTCAAGAAAGAATGTTCACTATTGACCCCAAAGTTTATGGGGAGAAAAGGGACCT

TCATAGTAAGAACAAAGATGATCTGACACTTAGTCGAAGCTTGGGGCTGGGCGGAAGGTCTGCAATAGAACAGAG
GCTTCGAACCCCGACCCGCC

AAGCATGTCCATTAACTTGCCTATGTCTTGCCTTGATTCTATTGCCCTTGGGTTTAACTTTGGCCGGGGCCATGA
GAAACCGGCCCCGGTACT

TCTTTCCCCTCTGGCTTCTGATATTCTAACCAACACTAGCGGAACGATGGATGAAGGAGATGATTACCTGCCCCC
AG GGACGGGGG

CACCACACCTGCAGTGGAGAAGATTCCTTGCTTTCCAATAGAGAGCAAAGAGGAAGAAGATAAGACAGAGCAAGC
GTGGTGTGGAC

AAAAGTGACTGGAGGGCAAACTACCCAAGTTGAAACATCCTCCGAGTCACCCTTCCCAGCCAAAGAATATTACAA
GGCTCAGTGGGAAGGGTCGG

AAATGGCACTGTCATGGCCCCTGACCTGCCTGAGATGCTAGATCTAGCAGGGACCAGGTCCAGATTAGCTTCTGT
CAGTACCGGGGACTGGACGG GATCGTCCCTGGTCCAGGTC

GAGTGCAGATGCTGAGGTTGCCAGGAGGAAATCAGTCCCATCGGAGGCTGTGGTTGCAGAGAGCAGTACTGGTTT
GTCAGGGTAGCCTCCGACAC

GCCACCTGTTGCTGATGACAGCCAACCCGTAAAACCAGACAGTCAACTTGAAGACATGGGGTACTGTGTGTTCAA

CAAGTACACAGTCCCTCTCCCATCGCCAGTTCAAGACAGTGAGAATTTGTCAGGAGAGAGTGGTTCGTTTTATGA
GGGAGAGGGTAGCGGTCAAG

AGGAACCGATGACAAAGTCCGTAGAGATTTGGCCACTGACCTTTCACTAATTGAGGTAAAACTTGCAGCTGCTGG

AAGAGTCAAAGATGAATTCACTGCTGAGAAAGAGGCATCTCCACCCTCTTCTGCTGACAAATCAGGACTGAGTAG

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CAGAG

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GAGG

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PTGS2, accession # NM_000963 (48 oligos):

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         ACCTCTTTTGACGAGTTGTG                                AAGACTTTGGGTGAGGTTT

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G               TGAATGGGTGAAGTTCCCTA                          AGGGAAGGAAGCTTTACG

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TTGAGACCG            TTGACGCGGAAAAGTTCCTA                          ACTACCTCTCTA

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CATAGGAG           TCTATGAGTCCGTCTCTACT

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                  ACCAGACCACGGACCAGACT                AGACCGACGCCCTTGTGTTG
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                                  AAGTACTGGTCTTTATGTTG

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        ACGACCTTGTACCTTAATGG      ACAACTTAGTAAGTGGTCCG

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STL1, accession # NP_010825 (48 oligos):

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CAACT             AATGACCATTTGTCAAGTTG                          TTACCGCTAGTACT

CAGACACGCAACTGTAGTGCAGGGCGCTACAACCTCCTGTTATGAATTAGGTTGTTTCGCAGGTTCTCTATTCGT
GTCTGT                                          AATCCAACAAAGCGTCCAAG     AGCA

TATGTTCTGCGGTGAAAGAATTGGTAGAAAACCATTAATCCTGATGGGTTCCGTAATAACCATCATTGGTGCCGT
ATACAAGACGCCACTT                     ATTAGGACTACCCAAGGCAT

TATTTCTACATGCGCATTTCGTGGTTACTGGGCATTAGGCCAGTTTATCATCGGAAGAGTCGTCACCGGTGTTGG
   AAGATGTACGCGTAAAGCAC             ATCCGGTCAAATAGTAGCCT
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