**Supplementary Table 1: Buffers and Aptamers**

| **Name** | **Buffer contents/RNA sequence** (5' → 3') |
|---|---|
| Buffer IC Intracellular buffer | 13.5 mM NaCl, 150 mM KCl, 20 mM HEPES, 0.22 mM $Na_2HPO_4$, 0.44 mM $KH_2PO_4$, 0.12 mM $MgCl_2$, 120 nM $CaCl_2$, 0.1 mM $MgSO_4$, pH 7.3 |
| Buffer IC+Mg | 13.5 mM NaCl, 150 mM KCl, 20 mM HEPES, 0.22 mM $Na_2HPO_4$, 0.44 mM $KH_2PO_4$, 5 mM $MgCl_2$, 120 nM $CaCl_2$, 0.1 mM $MgSO_4$, pH 7.3 |
| Buffer S selection buffer | 20 mM HEPES, 125 mM KCl, 5 mM $MgCl_2$, pH 7.4 |
| 98SPN1A | GAC GCA ACU GAA UGA AAU GGU GAA GGA CGG GUC CAG GUG UGG CUG CUU CGG CAG UGC AGC UUG UUG AGU AGA GUG UGA GCU CCG UAA CUA GUC GCG UC |
| 97SPN2A | GGG AUG UAA CUG AAU GAA AUG GUG AAG GAC GGG UCC AGU AGG CUG CUU CG GCA GCC UA CUU GUU GAG UAG AGU GUG AGC UCC GUA ACU AGU UAC AUC |
| Spinach2 tandem aptamer (2xSPN2A) | GGG AUG UAA CUG AAU GAA AUG GUG AAG GAC GGG UCC AGU AGG CGC UGA AUG AAA UGG UGA AGG ACG GGU CCA GUA GGC UGC UUC GGC AGC CUA CUU GUU GAG UAG AGU GUG AGC UCC GUA ACU AGC GCC UAC UUG UUG AGU AGA GUG UGA GCU CCG UAA CUA GUU ACA UC |
| MGA | GGA UCC CGA CUG GCG AGA GCC AGG UAA CGA AUG GAU CC |
| 4xMGA | GGA UCC CGA CUG GCG AGA GCC AGG UAA CGA AUG GAU CCU AAA AAC GGA UCC CGA CUG GCG AGA GCC AGG UAA CGA AUG GAU CCU AAA AAC GGA UCC CGA CUG GCG AGA GCC AGG UAA CGA AUG GAU CCU AAA AAC GGA UCC CGA CUG GCG AGA GCC AGG UAA CGA AUG GAU CC |
| 8xMGA | GGA UCC CGA CUG GCG AGA GCC AGG UAA CGA AUG GAU CCU AAA AAC GGA UCC CGA CUG GCG AGA GCC AGG UAA CGA AUG GAU CCU AAA AAC GGA UCC CGA CUG GCG AGA GCC AGG UAA CGA AUG GAU CCU AAA AAC GGA UCC CGA CUG GCG AGA GCC AGG UAA CGA AUG GAU CCU AAA AAC GGA UCC CGA CUG GCG AGA GCC AGG UAA CGA AUG GAU CCU AAA AAC GGA UCC CGA CUG GCG AGA GCC AGG UAA CGA AUG GAU CCU AAA AAC GGA UCC CGA CUG GCG AGA GCC AGG UAA CGA AUG GAU CC |

**Mammalian Expression Plasmids**

pDsRed-Express-C1 (Clontech #632430)

pJJR176: DsRed coding sequence replaced with spinach1 aptamer insert [tRNA(Lys).a98SPN1A.tRNA(Lys)] cloned into pDsRed-Express-C1 between the NheI and XmaI sites.

Spinach Insert:
GCCCGGATAGCTCAGTCGGTAGAGCAGCGGCCG**GACGCAACTGAATGAAATGGT**
**GAAGGACGGGTCCAGGTGTGGCTGCTTCGGCAGTGCAGCTTGTTGAGTAGAGTG**
**TGAGCTCCGTAACTAGTCGCGTC**CGGCCGCGGGTCCAGGGTTCAAGTCCCTGTT
CGGGCGCCATCTAGACGGACTTCGGTCCGCTTTTTTT

pTZ U6+ 19 plasmid (obtained from John J. Rossi, Department of Molecular Biology, Beckman Research Institute of the City of Hope, Duarte, California 91010, USA (1).

To obtain the mammalian expression vectors for MGA and 4xMGA, pTZ U6+19 was digested with Sal1 and Xba1 restriction endonucleases to cut out the ribozymes in place and insert MGA and 4xMGA downstream of U6 promoter for their expression in mammalian cells.

**Yeast and plasmids**

*Saccharomyces cerevisiae* (BY4735, Genotype: MATα ade2Δ::hisG his3Δ200leu2Δ0 met15Δ0 trp1Δ63 ura3α0) were cultured in YPD medium. The yeast expression plasmid, pYES2, is a yeast 2 micron plasmid carrying a URA3 marker and a GAL1 promoter for galactose inducible gene expression in *S. cerevisiae*. The pYES2 was modified to express a reporter RNA consisting of a series of tandem aptamers (IMAGEtags) with specificity for PDC or it was modified to express the spinach aptamer under 5S promoter. Plasmids expressing multiaptamers and spinach aptamers were transformed into BY4735 using the Lazy Bones yeast transformation method by selection on SD-uracil plates (2).

**FRET Image Acquisition and Analysis**

For FRET analysis by sensitized emission, yeast cells were cultured in SD-uracil medium containing 2 % galactose for the induction period and incubated with the Cy3- and Cy5- modified ligands. Cells were washed once with TBS, resuspended in TBS and a 30 µL volume placed on a poly L-lysine coated cover glass or a poly d-lysine coated glass bottom culture dish (MatTek). The cells were observed using Leica SP5X laser scanning confocal microscope with a 63X objective and immersion oil. Cells were excited sequentially by a 550 nm white light laser (WLL, Leica) and donor emission was taken at 560-626 nm and FRET images were taken to measure sensitized emission using emission filters of 660-754 nm. In the second sequence cells were excited by 650 nm laser and acceptor emission was taken at or 660-754 nm.

**Figure S1 Yeast imaging with DFHBI**

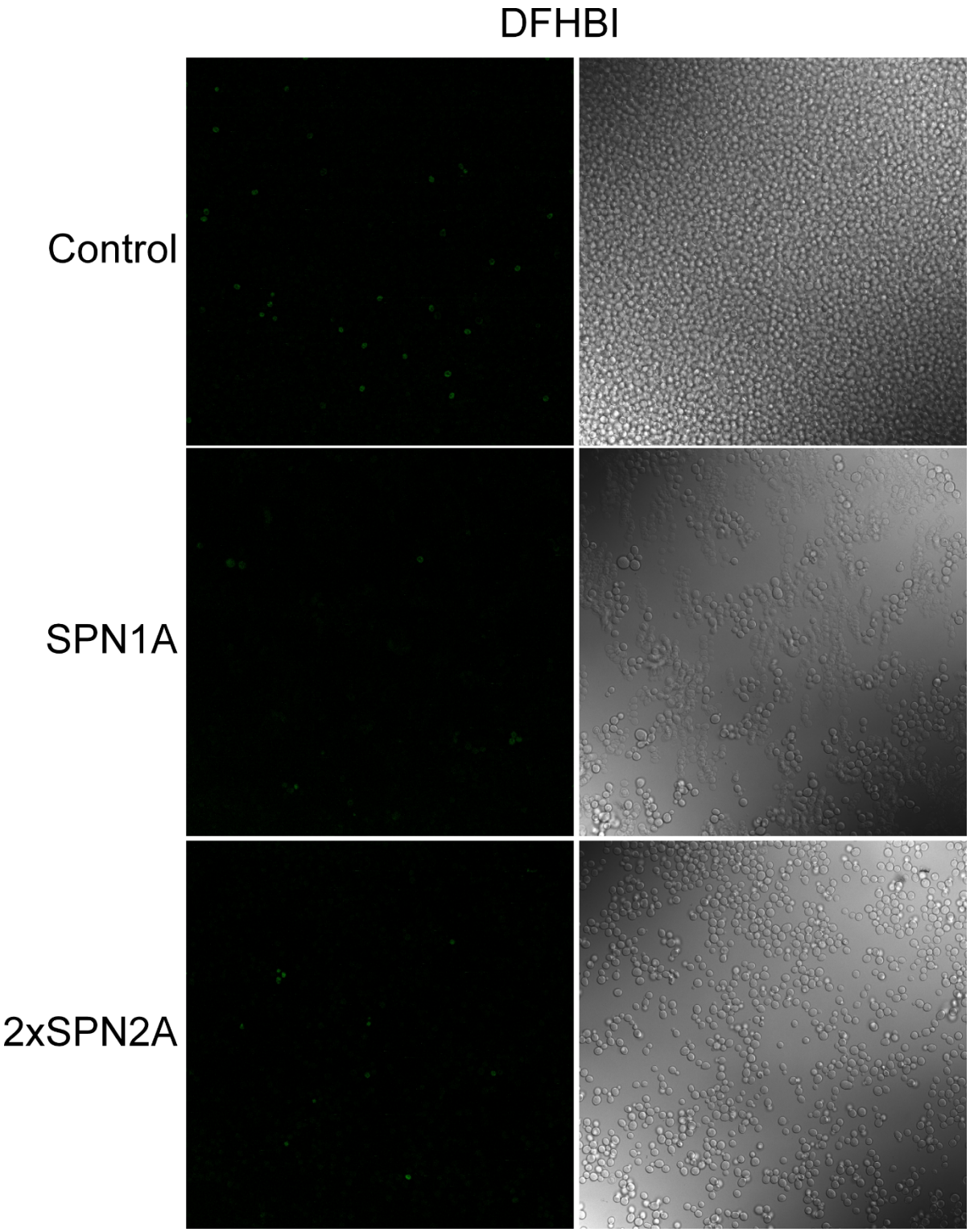


Fig. S1. The full color versions of the microscope fields shown in Fig. 5 for DFHBI.

**Figure S2. Yeast imaging with PFP-DFHBI**

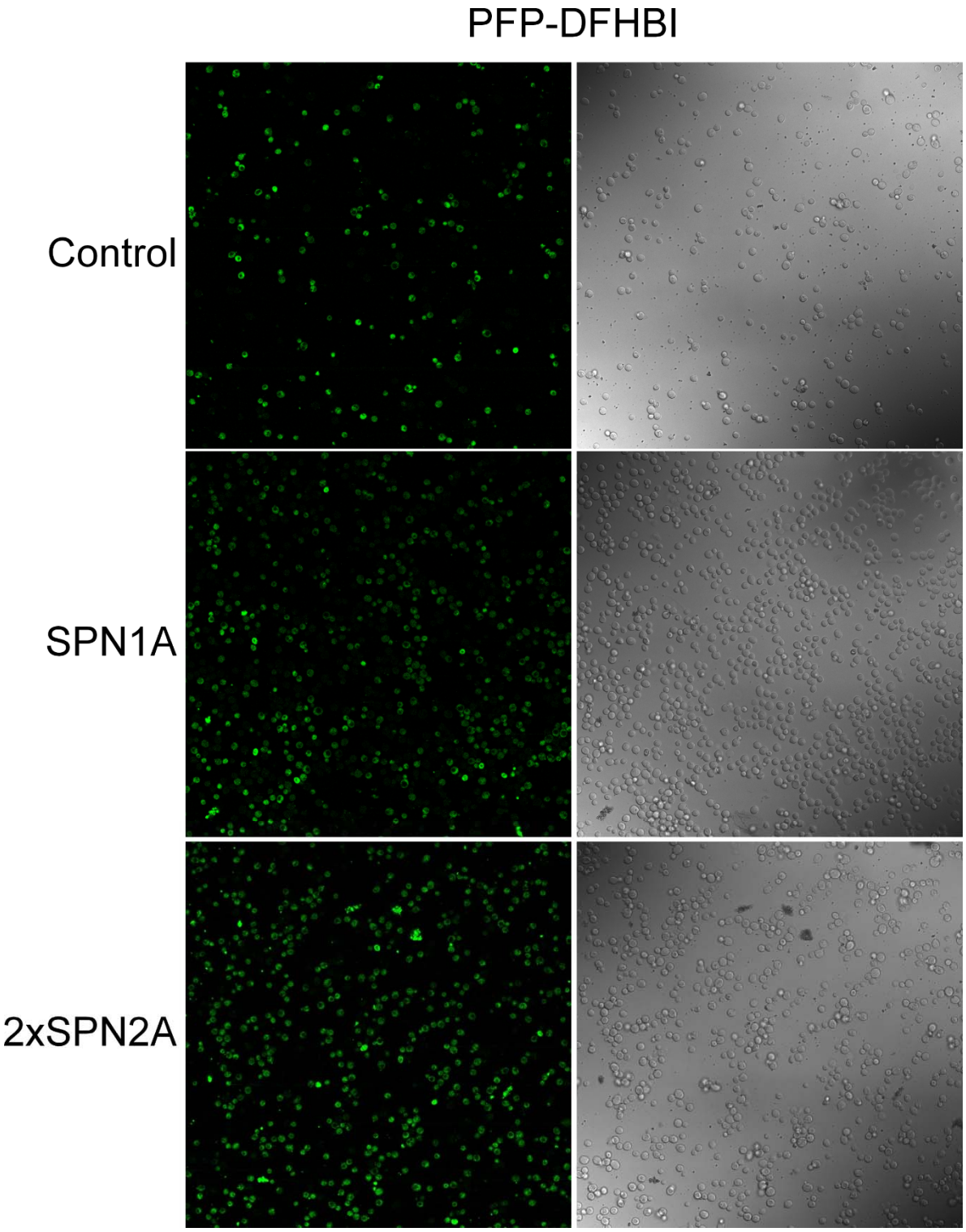


Fig. S2. The full color versions of the microscope fields shown in Fig. 5 for PFP-DFHBI.

**Figure S3. Yeast imaging with PFP-DFHBI**

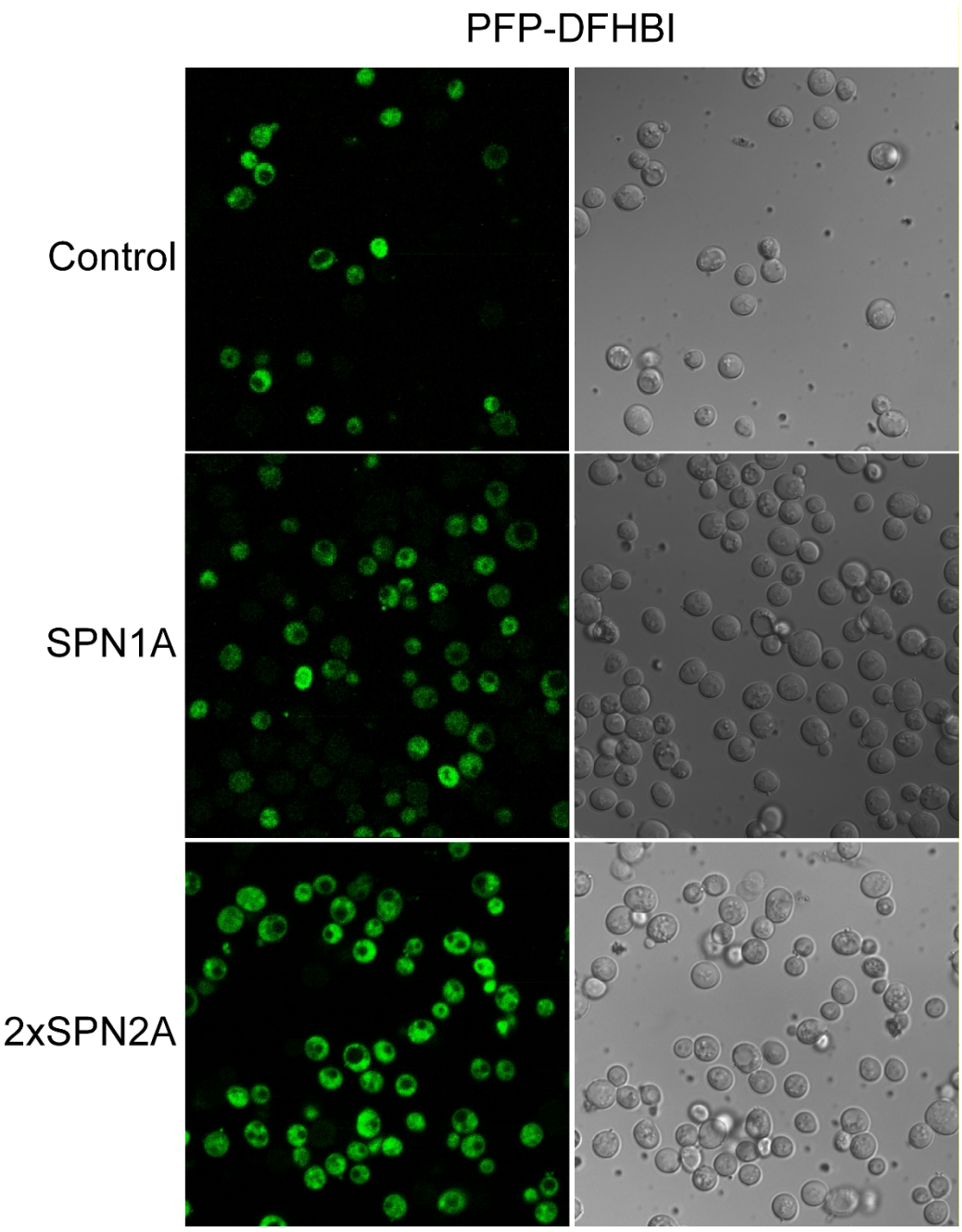


Fig. S3. Higher resolution images from the experiment shown in Fig. 5

**Figure S4. ROIs Chosen for Analysis**

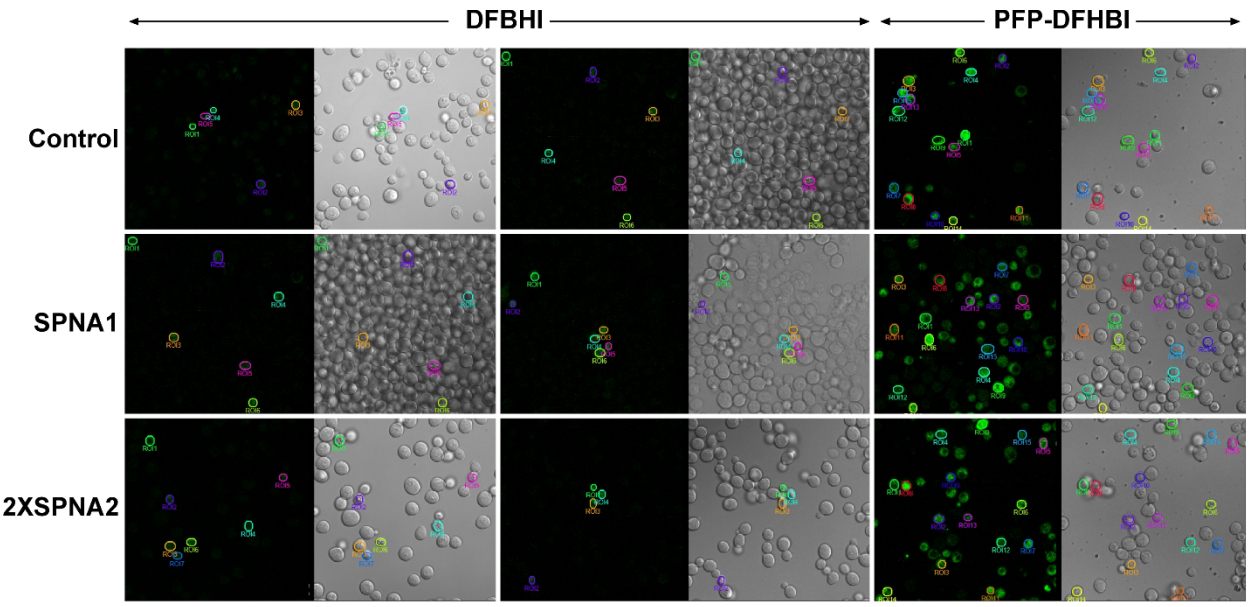


Fig. S4. Examples of ROIs from which intensities were collected to provide the quantitative data in Fig. 5B. Colored circles identify the circumference within which the fluorescence intensity was measured.

**Figure S5. Signal acquisition from cells expressing control RNA**

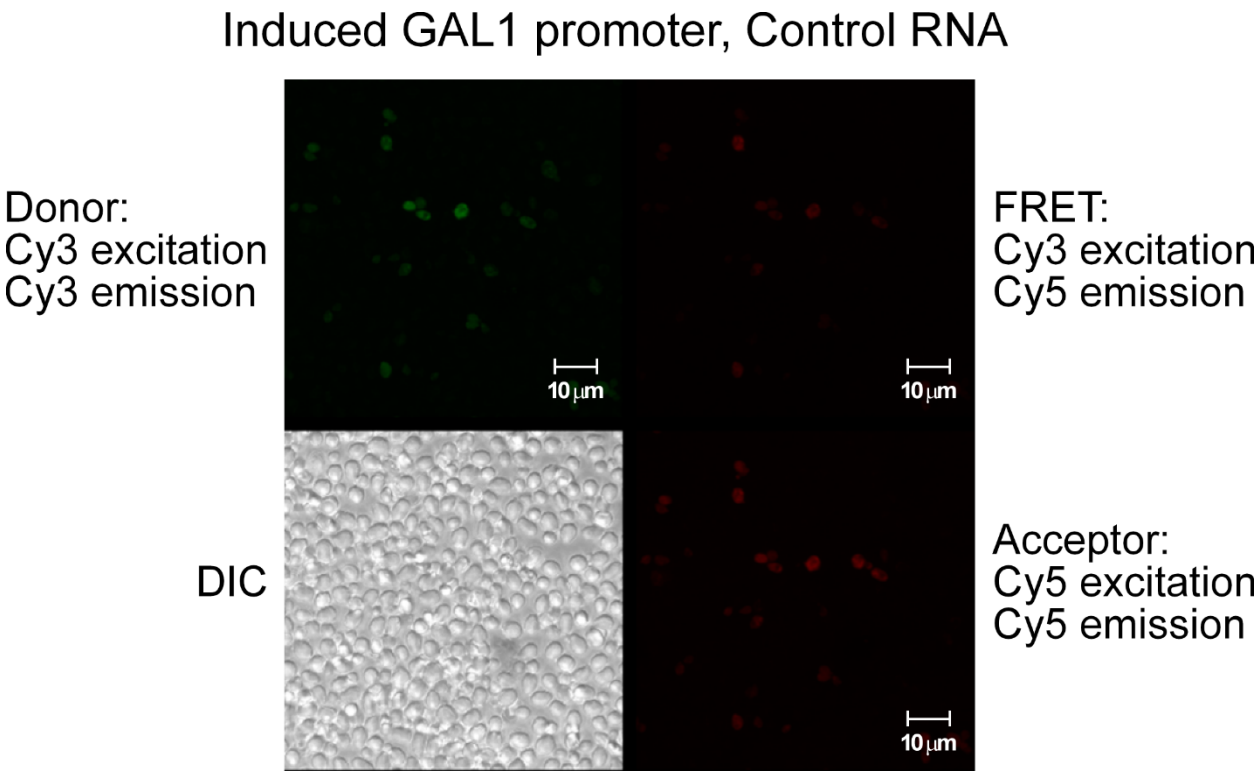


Fig. S5. Yeast cells expressing control RNA from a plasmid template were incubated with both PDC-Cy3 (Donor) and PDC-Cy5 (Acceptor). Cells were excited sequentially at 550nm with a white light laser and emissions were recorded for Cy3, Cy5 (FRET) channels. In the next sequence cells were excited at 650nm and emissions were recorded for Cy5 channel. The DIC image shows the cells in the field of view. Images were collected by z stacking and maximum projection of the images are shown.

**Figure S6. Signal acquisition from cells with uninduced 6xPDC IMAGEtag plasmid**

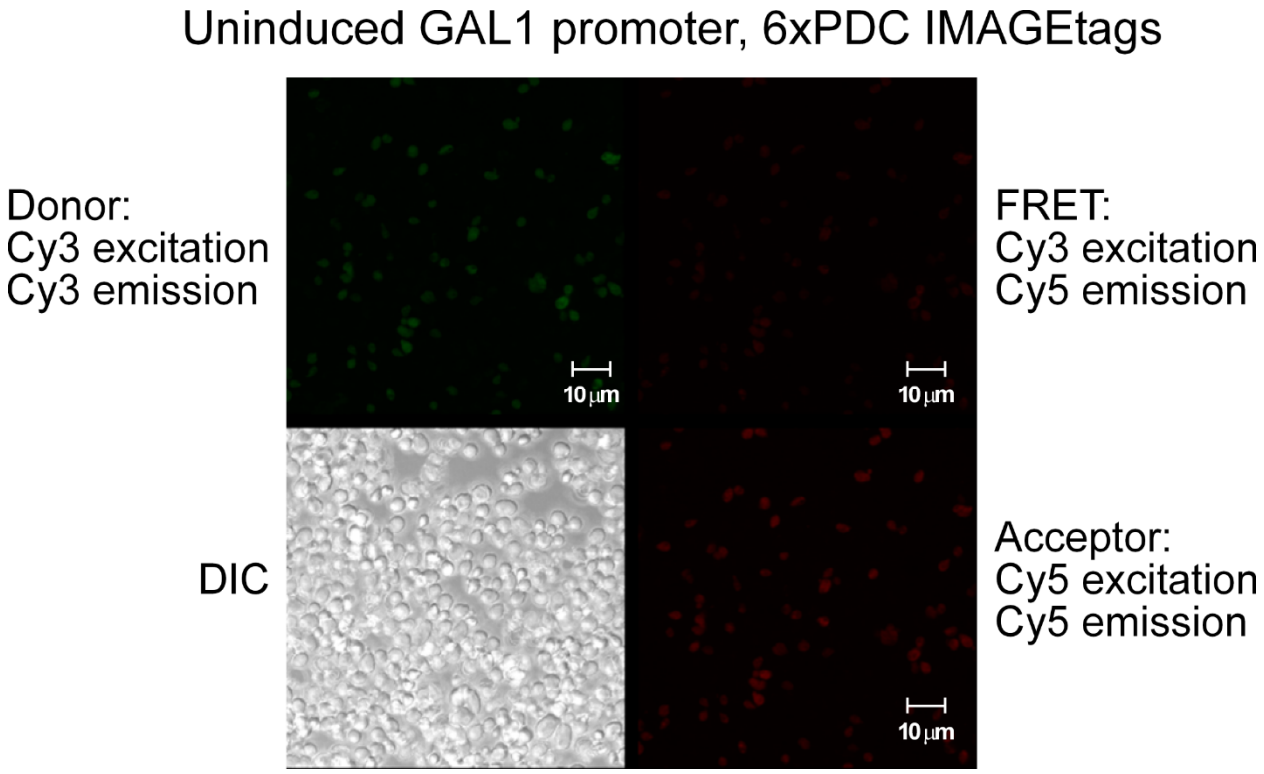


Fig. S6. Yeast cells harboring 6xPDC IMAGEtag plasmid were not induced by galactose and incubated with both PDC-Cy3 (Donor) and PDC-Cy5 (Acceptor). Cells were excited sequentially at 550nm with a white light laser and emissions were recorded for Cy3, Cy5 (FRET) channels. In the next sequence cells were excited at 650nm and emissions were recorded for Cy5 channel. The DIC image shows the cells in the field of view. Images were collected by z stacking and maximum projection of the images are shown.

**Figure S7. Signal acquisition from cells expressing 6x PDC IMAGEtag RNA**

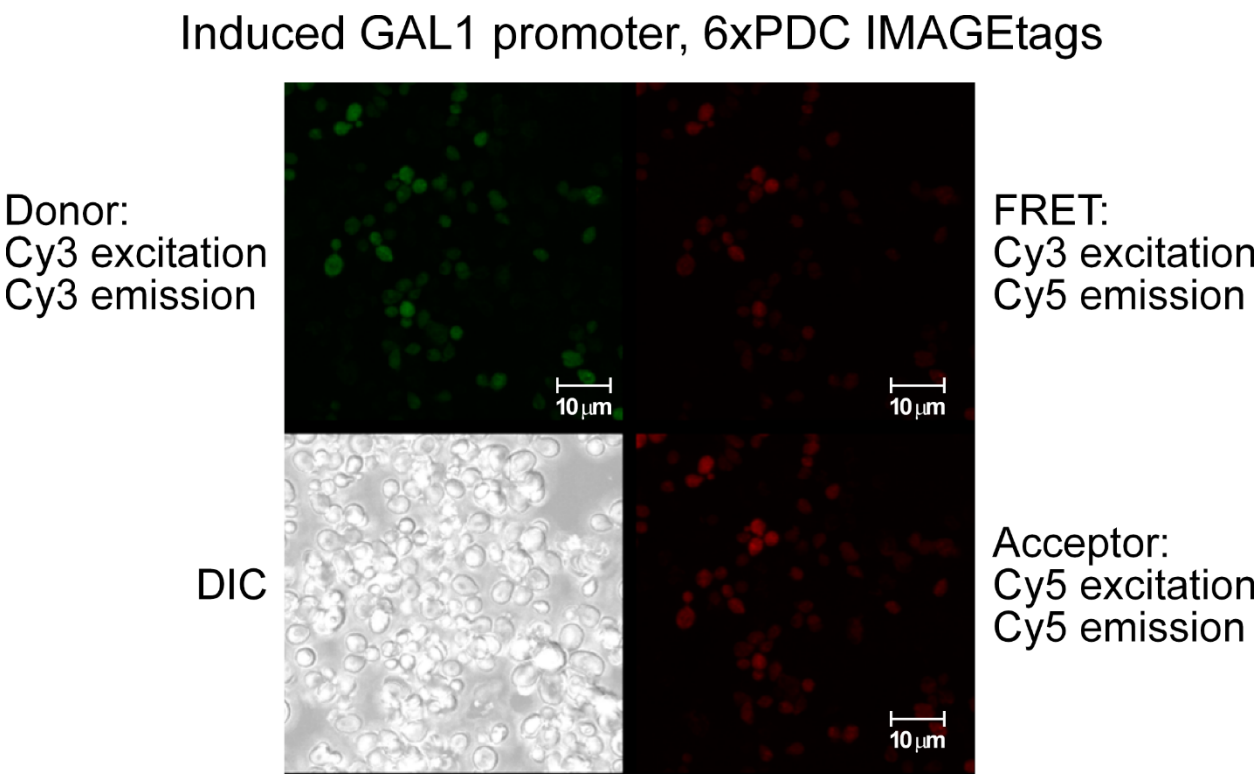


Fig. S7. Yeast cells expressing 6x PDC IMAGEtag RNA from a plasmid template were incubated with both PDC-Cy3 (Donor) and PDC-Cy5 (Acceptor). Cells were excited sequentially at 550nm with a white light laser and emissions were recorded for Cy3, Cy5 (FRET) channels. In the next sequence cells were excited at 650nm and emissions were recorded for Cy5 channel. The DIC image shows the cells in the field of view. Images were collected by z stacking and maximum projection of the images are shown.

## References


1. Bertrand, E., Castanotto, D., Zhou, C., Carbonnelle, C., Lee, N. S., Good, P., Chatterjee, S., Grange, T., Pictet, R., Kohn, D., Engelke, D., and Rossi, J. J. (1997) *RNA* **3**, 75-88
2. Amberg, D. C., Burke, D. J., and Strathern, J. N. (2005) *Methods in yeast genetics*,