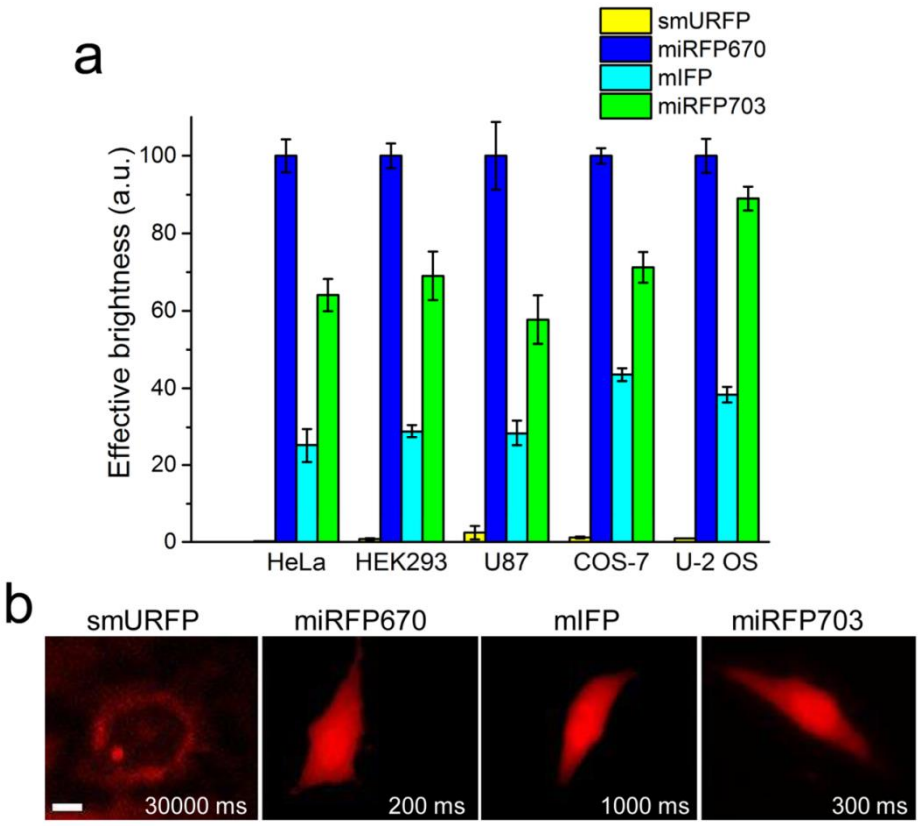


**Figure S1, related to Figure 2 and Table 1. Effective brightness of NIR FPs co-expressed with EGFP from two separate plasmids in different mammalian cells. (a)** NIR fluorescence intensity of cells transiently transfected with smURFP, miRFP670, mIFP and miRFP703 were analyzed using flow cytometry 48 h after co-transfection. Respective NIR FP was encoded by one plasmid, and EGFP was encoded by another plasmid. Mean NIR fluorescence intensity was normalized to mean fluorescence intensity of co-transfected from the other plasmid EGFP to account for cell transfection efficiency. NIR effective brightness of miRFP670 was assumed to be 100%. Error bars, s.e.m. (n=3; transfection experiments). **(b)** Representative fluorescence images of NIR FPs in live HeLa cells. Acquisition time for each image is indicated. Scale bar, 10 μm.

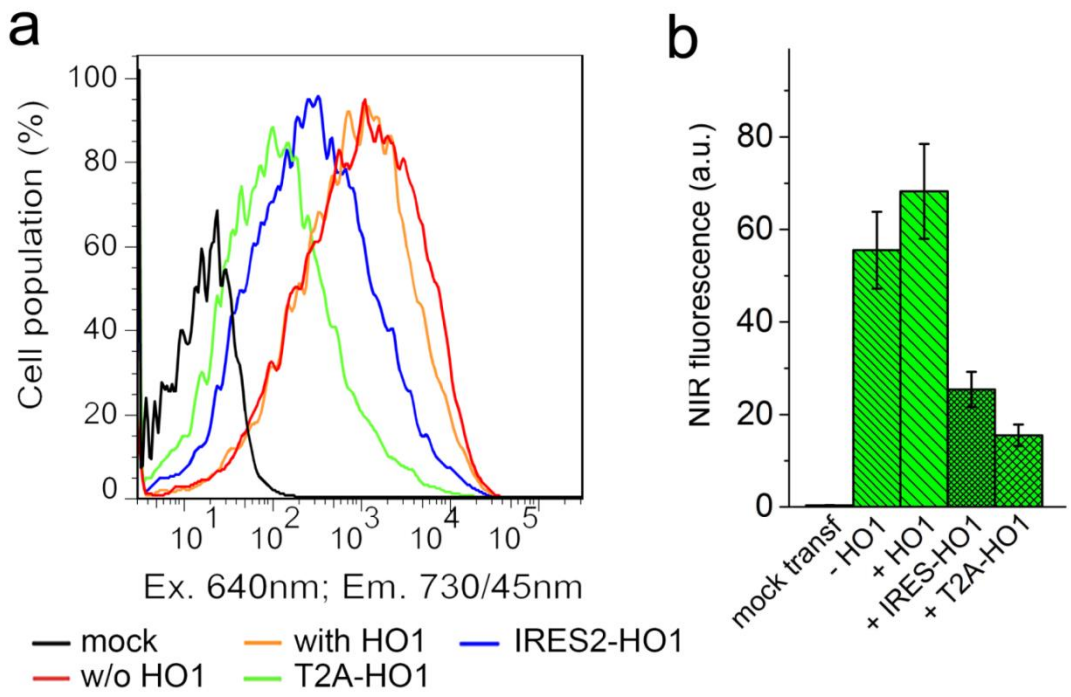


**Figure S2, related to Figure 3. Co-expression of miRFP703 with HO1. (a)** Fluorescence intensity distribution of live HeLa cells, co-expressing miRFP703 with HO1 either from the two separate plasmids (miRFP703 was encoded by one plasmid, and HO1 was encoded by another plasmid) or from the same plasmid encoded via either IRES element (miRFP703-IRES2-HO1) or T2A peptide (miRFP703-T2A-HO1) was analyzed using flow cytometry 48 h after transfection. **(b)** Quantification of the data represented in (a). Mean NIR fluorescence intensity was normalized to mean fluorescence intensity of co-transfected EGFP to account for transfection efficiency and to mean fluorescence intensity of mock transfected cells in the 730/45 nm channel (to account for cellular autofluorescence). EGFP was co-expressed from the third plasmid when miRFP703 and HO1 were co-expressed from two separate plasmids or from the second plasmid when miRFP703 and HO1 were co-expressed from the same plasmid via either IRES2 or T2A. Error bars, s.e.m. (n = 3; transfection experiments).

**Table S1, related to Table 1. Amount of NIR FP holoform in mammalian cell lines.**

| NIR FP | Holoform amount of NIR FP in live cells relative to that of miRFP670: co-expression with EGFP downstream of T2A from the same plasmid (co-expression with EGFP from two separate plasmids), % | | | | |
|---|---|---|---|---|---|
| | **HeLa** | **HEK293** | **U87** | **COS-7** | **U-2 OS** |
| smURFP | 0.7 (0.1) | 3 (0.3) | 0.4 (0.9) | 0.6 (0.4) | 0.2 (0.3) |
| miRFP670 | 100 (100) | 100 (100) | 100 (100) | 100 (100) | 100 (100) |
| mIFP | 20 (44) | 22 (51) | 29 (49) | 25 (78) | 26 (68) |
| miRFP703 | 79 (98) | 81 (107) | 88 (88) | 107 (113) | 96 (136) |

**Table S2, related to Key Resource Table and Design of plasmids section of STAR Methods. DNA oligonucleotides utilized for design and cloning of plasmids.**

| Oligonucleotide name | Direction | Sequence (5'-3') |
|---|---|---|
| MCS_HO1_sense | forward | ttaagataaagcttataggtaccgagggatccacagcgatcgcgtggaattc tcagcggccgcatactcgagatagg |
| MCS_HO1_asense | reverse | cgcgcctatctcgagtatgcggccgctgagaattccacgcgatcgctgtgg atccctcggtacctataagctttatc |
| EGFP_HindIII_fw | forward | ataaagcttaccatggtgagcaagggcgagg |
| EGFP_XhoI_rv | reverse | tatctcgagccttgtacagctcgtccatgccg |
| HO1_AgeI_del_fw | forward | cagccacaaagttcaagcagctctatcggtccaggatgaacagcctg |
| HO1_AgeI_del_rv | reverse | caggctgttcatcctggaccgatagagctgcttgaactttgtggctg |
| HO1_AgeI_fw | forward | ataaccggtgaaagaccacagccagactctatgc |
| HO1_NotI_rv | reverse | atagcggccgctcacatagcatagagccccactgc |
| EGFP_SacI_fw | forward | atgagctcaccatggtgagcaagggcgagg |
| HO1_XmaI_rv | reverse | atcccgggtcacatagcatagagccccactgc |
| HO1_SpeI_add_fw | forward | cgaggagaatcctggcccaactagtatggaaagaccacagccagactc |
| HO1_SpeI_add_rv | reverse | gagtctggctgtggtctttccatactagttgggccaggattctcctcg |
| miRFP_SpeI_fw | forward | atactagtatggtagcaggtcatgcctctgg |
| miRFP_XmaI_rv | reverse | atacccgggtcagctctcaagcgcggtgatcc |
| mIFP_SpeI_fw | forward | atactagtatgtcggtaccgctgactacctc |
| mIFP_XmaI_rv | reverse | atacccgggtcatttggactgagactgtgcaaagctc |
| smURFP_SpeI_fw | forward | atactagtatggctaagacttccgaacagagg |
| smURFP_XmaI_rv | reverse | atcccgggtcagctcatagccttaataatgtaatcaaagtag |
| HO1_H25A_fw | forward | caaggaggccaccaaggaagtggccacccaggccgagaacgc |
| HO1_H25A_rv | reverse | gcgttctcggcctgggtggccacttccttggtggcctccttg |
| HO1_D140F_fw | forward | ctacacccgctacctgggcttcctgtcaggggtcaagttctg |
| HO1_D140F_rv | reverse | cagaacttgacccccctgacaggaagcccaggtagcgggtgtag |