Electronic Supplementary Material (ESI) for Analyst.

Supplementary information

Genetically encoded biosensors for the detection of rapamycin: towards the screening of agonists and antagonists

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Plasmid Construction

DNA cloning was carried out according to standard protocols. The sequence of all plasmids was confirmed by DNA sequencing and amplified using the E. coli strain DH5α.

6His-NLS_N-VMA_N-FKBP12 (pKDH029): The synthetic FKBP12 encoding gene was inserted into the pKDH018 (*Anal. Chem.*2018, 90, 9779-9786) between Sal I and Xho I sites to create pKDH067.

Npu DnaE whole intein, (pJDH012): The whole Npu DnaE intein sequence was constructed by fusing the N- and C-fragment coding sequences of the Npu DnaE split-intein. The wI_N coding sequence was amplified by PCR using the total DNA from Nostoc punctiforme (strain ATCC 29133/PCC 73102) as a template using a pair of oligo-nucleotide primers (5'-GGT CGC CAT ATG TGT TTA AGC TAT-3' and 5'-CAA ATT GTC GAC CCG CAT-3', restriction sites are indicated by underlines). The wI_C coding sequence was obtained in similar way, using a pair of oligo-nucleotide primers (5'-TAA GTC GAC AAT TTG CCG AAT ATC AAA ATA GCC-3' and 5'-TCC CGT CTC GAG ATT AGA AGC TAT-3'). Resulting PCR products were digested with appropriate restriction enzymes (Nde I, Sal I and Xho I) and were placed in a pET28a vector between Nde I and Xho I sites to create pJDH012.

6His-NLS_N-Npu_N (pLMH006): The PCR amplified 6His-NLS_N gene (template: pKDH008, primer set: 5'-CGAAATTAATACGACTCACTATAGG-3' and 5'-CCTTGGCAAAAGCTTtAGCTTTCTTGG-3') were inserted into pET28a vector using Nco I and Hind III restriction sites to produce 6His-NLS_N. Then, Npu_N (123 aa) coding sequence (template: pJDH012) were inserted into pET28a using Hind III and Not I sites to create pLMH006 plasmid.

NLS-mCherry (pJHJ004): The synthetic DNA oligo encoding the NLS (KRPAATKKAGQAKKKKLD) was inserted into the pET28a vector between NdeI and EcoRI sites. Then the PCR amplified mCherry gene using primer set (5'-GAATTCATGGTGAGCAAGGGCG-3' and 5'-CTCGAGTTACTTGTACAGCTCGTCCA-3') was inserted to the resulting vector between EcoRI and XhoI sites to create pJHJ004 plasmid.

NES-Npu_C-NLS_C-mCherry-6His (pLMH007): The PCR amplified Npu_C gene (template: pJDH012, primer set: 5'- GCCATATGATCAAAATAGCCACAC-3' and 5'-GGATCCATTGAAACAATTAGAAGCTAT-3') were inserted into pET28a between Nde I and BamH I sites. Then, PCR amplified NLS_C-mCherry gene (template: pJHJ004, primer set: 5'-CATAACTGTTTCAACGGATCCGGCCAGG-3' and 5'-GGTGGTGCTCGAGTGACTTGTAC-3') were inserted into pET28a between BamH I and Xho I sites. The resulting construct was modified by inserting the cDNA encoding the NES (NELALKLAGLDINKT) between NcoI and NdeI sites to create pLMH007 plasmid, which encodes NES-Npu_C-NLS_C-mCherry-6His.

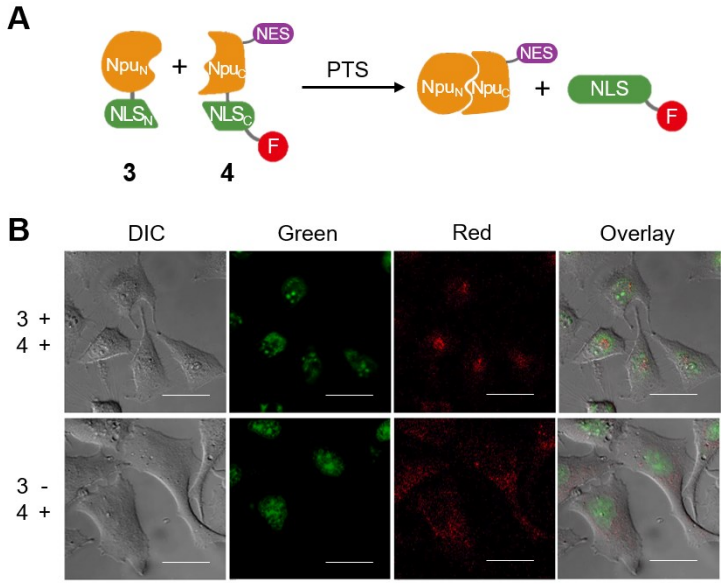

Fig. S1 Naturally occurring signal peptide reconstitution by Npu DnaE intein. (a) Schematic representation of trans-splicing. (b) The HeLa cells co-expressing protein 3 and 4 show the fluorescence signal travels to the nucleus (rows 1). The cells expressing only protein 4 do not respond to target stimulation (row 2). Scale bar=25 µm.

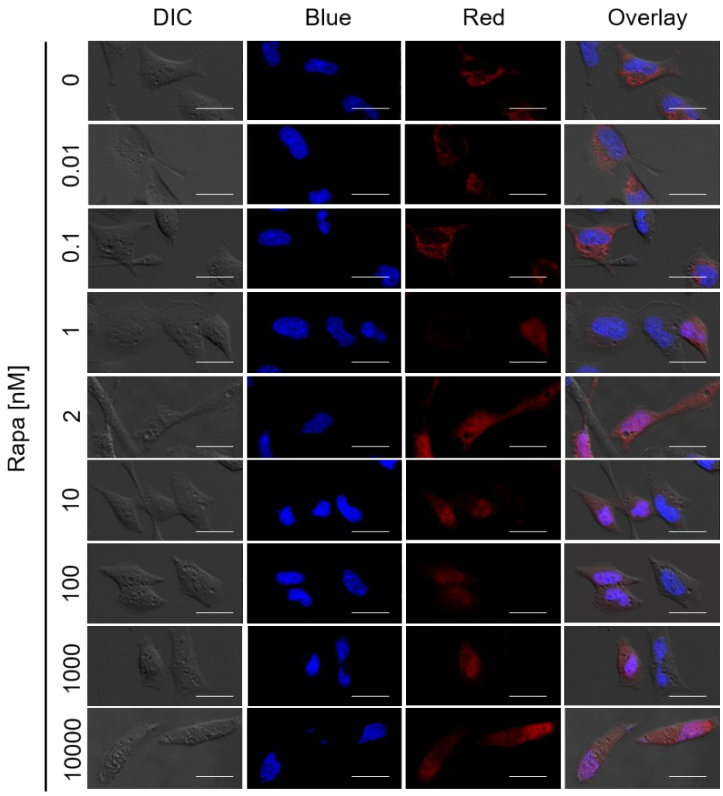

Fig. S2 Dependence of the relative change in fluorescence image on the Rapa concentration (Scale bar=25 µm).

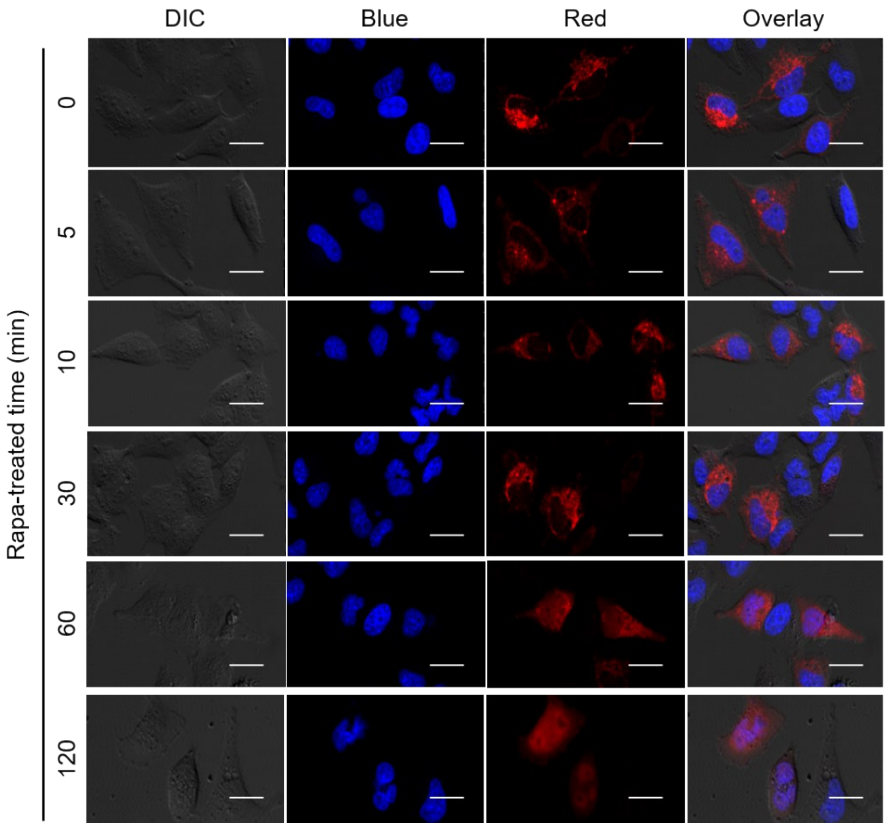

Fig. S3 Dependence of the relative change in fluorescence image on the Rapa-treated time (Scale bar=25 μm).

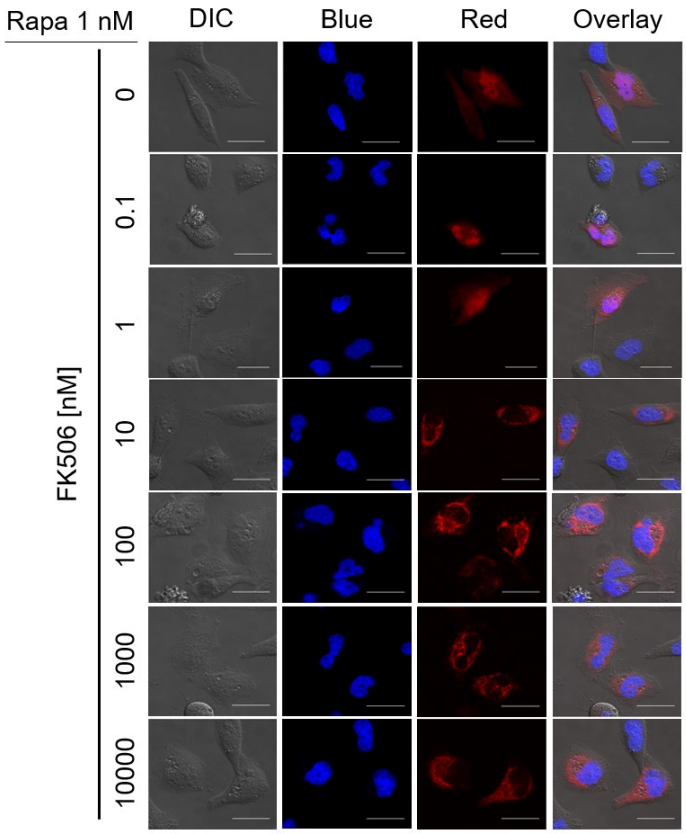

Fig. S4 Fluorescence image of competitive inhibition assay using FK506/Rapa (Scale bar=25 μm).