Development of split SNAP-tag protein complementation assay for visualization of protein-protein interaction in living cells

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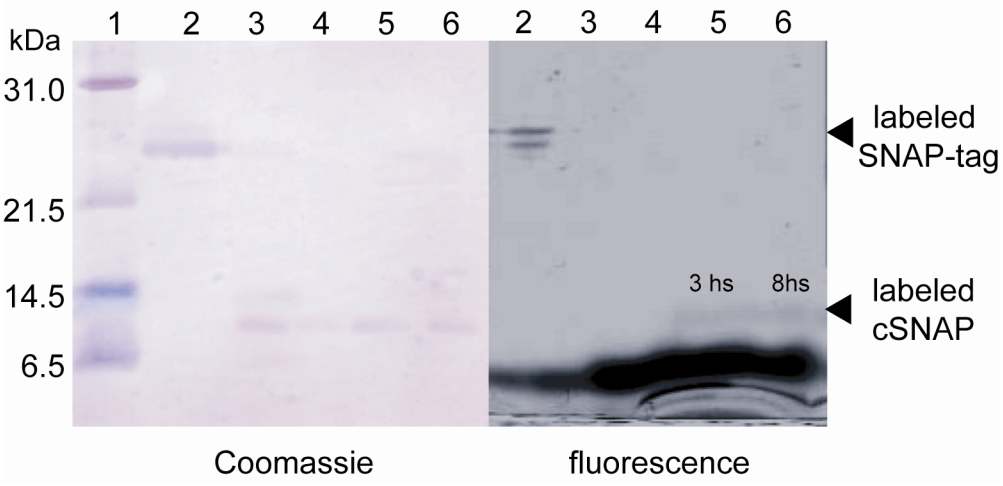
Supplementary information

Table S1

Figure S1-S4

Table S1. Synthetic nucleotides for PCR and insertion

KpnI-NheI-nSNAP	GCTAGGTACCGCTAGCCATGGACAAAGACTGCGAA
nSNAP-HindIII-EcoRI	CCCGAATTCAAAGCTTCTGCTGGAACACTGGGTGGT
KpnI-ATG-cSNAP	GCTAGGTACCATGGTCGACGAGAGCTTTACCCGCCAGGT
cSNAP-HindIII-EcoRI	GCCGAATTCAAGCTTTCACTCGAGGGATCCTGGC
FKBP12 (s)	GCTAGCCTCGAGATGGGAGTGCAGGTGGAAACCAT
FKBP12(as)	GAATTCTTAGGTACCTTCCAGTTTTAGAAGCTCCACATCG
FKBP12 F36M(s)	GAAGATGGAAAGAAAATGGATTCCTCCCGGGACAG
FKBP12 F36MA(as)	CTGTCCCGGGAGGAATCCATTTTCTTTCCATCTTC
Mem(s)	CTAGCCATGCTGTGCTGTATGAGAAGAACCAAACAGGTTGAAAAG AATGATGAGGACCAAAAGATCC
Mem(as)	TCGAGGATCTTTTGGTCCTCATCATTCTTTTCAACCTGTTTGGTTCT TCTCATACAGCACAGCATGG
NLS(s)	CTAGCCATGCCTAAGAAGAAGAGAAAGGTGAAGCTTC
NLS(as)	TCGAGAAGCTTCACCTTTCTCTTCTTCTTAGGCATGG



Figuere S1. Split SNAP-tag reconstitution *in vitro*. Purified split SNAP-tag proteins were mixed without freeze-thawing. Before addition of SNAP-Cell Oregon Green, mixed proteins were incubated for 3hr or 8hr. After addition of SNAP-Cell Oregon Green, proteins were incubated for 40 min and analyzed using a fluorescence scanner. Lane 1: molecular weight protein marker. Lane 2: SNAP-tag (21.3 kDa). Lane 3; nSNAP (11.1 kDa). Lane 4: cSNAP (11.8 kDa). Lane 5: nSNAP and cSNAP incubated 3hr. Lane 6: nSNAP and cSNAP incubated 8hr

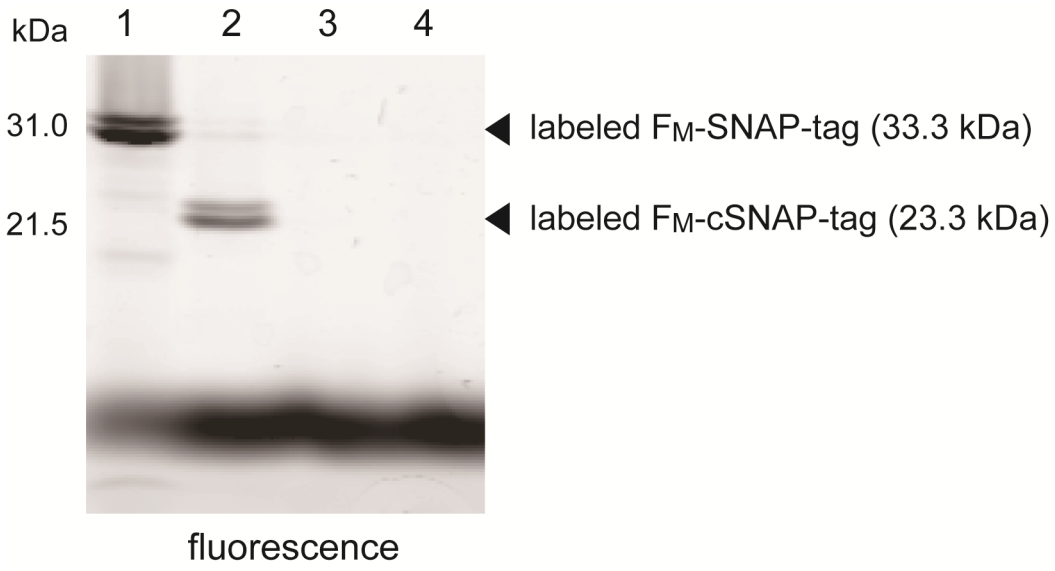


Figure S2. F_M interaction dependent split SNAP-tag reconstitution *in vitro*. Cells, expressed F_M-nSNAP and/or F_M-cSNAP, were lysed and added SNAP-Cell Oregon Green for 30 min. After separation by SDS-PAGE, protein labeling was analyzed using a fluorescence scanner. Lane 1: F_M-SNAP-tag. Lane 2: F_M-nSNAP and F_M-cSNAP. Lane 3; F_M-nSNAP. Lane 4: F_M-cSNAP.

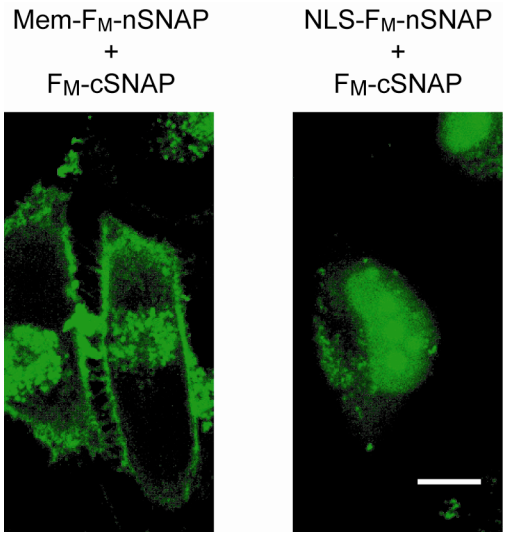


Figure S3. Replaced the split SNAP-tag fragments of Figure 4. HeLa cells were expressed F_M-cSNAP and Mem-F_M-nSNAP or NLS-F_M-nSNAP.

Scale bar= 20 μ m

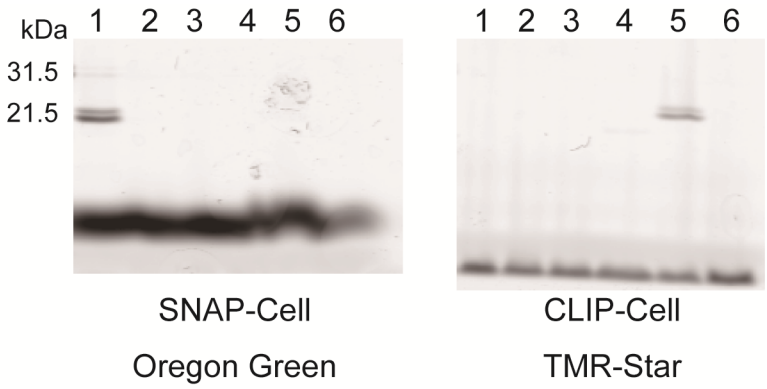


Figure S4. Substrate specificity of split CLIP-tag reconstitution with nSNAP

The cell lysates, derived from cells expressed F_M fused split SNAP- and/or CLIP-tag fragments, were incubated with SNAP-Cell Oregon Green or CLIP-Cell TMR-Star. Lane 1: F_M-nSNAP and F_M-cSNAP . Lane 2: F_M-nSNAP. Lane 3; F_M-cSNAP. Lane 4: Cell lysate without transfection. Lane 5: F_M-nSNAP and F_M-cCLIP. Lane 6; F_M-cCLIP.