Supplementary Information

Live cell monitoring of separase activity, a key enzymatic reaction for chromosome segregation, with chimeric FRET-based molecular sensor upon cell cycle progression

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Supplementary Figure 1

Amino acid sequences for NLS based GFP marked with red for NLS, blue for separase recognition sequence, green for dye attached cysteine. In case of WNLS, GFP with simply removed GPKKKRKV

MASMTGGQQMGR **GPKKKRKV** MSKGEELFTG VVPILVELDG DVNGHKFSVS GEGEGDATYG KLTLKFISTT GKLPVPWPTL VTTLTYGVQC FSRYPDHMKR HDFFKSAMPE GYVQERTISF KDDGNYKTRA EVKFEGDTLV NRIELKGIDF KEDGNILGHK LEYNYNSHNV YTTADKQKNG IKANFKTRHN IEDGSVQLAD HYQQNTPIGD GPVLLPDNHY LSTQSALLKD PNEKRDHMVL LEFVTAAGSGSSG **DREIMRE**GTC ELYK GG HHHHHH

Supplementary Figure 2

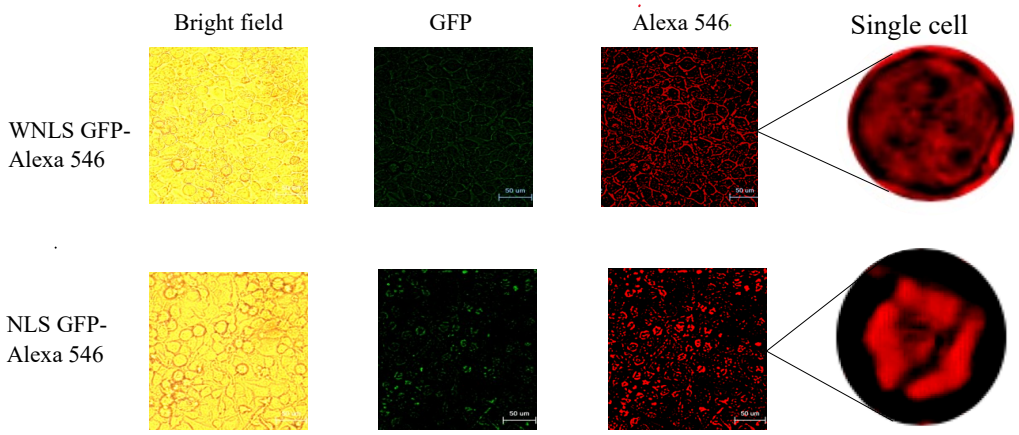

Figure S2: General fluorescence microscopic observation to compare two types of molecular sensors localizations in cells. It appeared that not localized type of molecular sensor (WNLS based) stays at endosome surrounding the nucleus to disperse into the cytosol. On the other hand, nucleus localized type of molecular sensor (NLS based) appeared to be accumulated inside nucleus.

Supplementary Figure 3

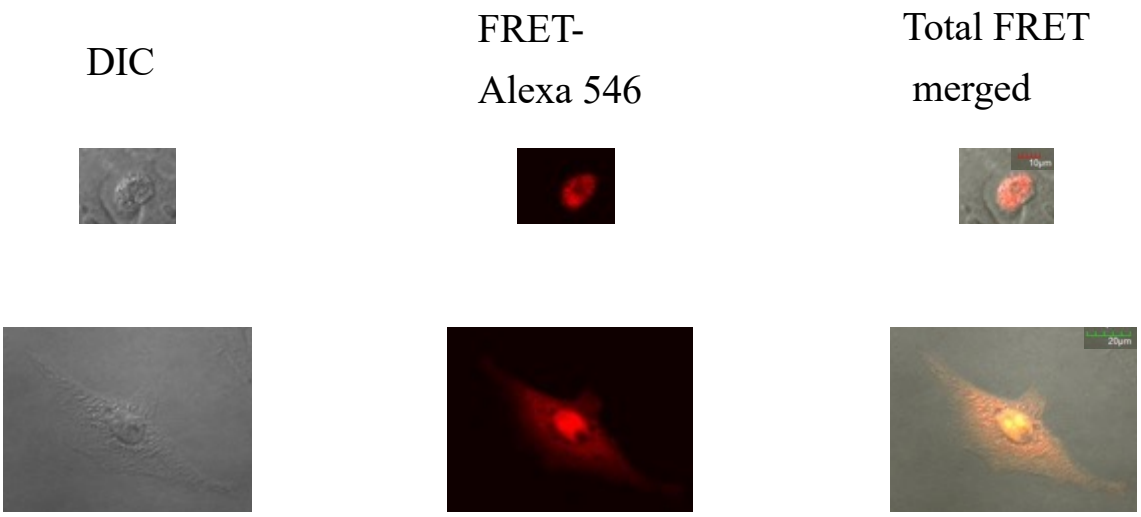

Figure S3: Identification of different cell states upon NLS based molecular sensor uptake using fluorescence microscopic observations in detail. Although ~~f~~orms of introduced cells were varied as a spherical shape or spread one, they still exhibited identical localization patterns for molecular sensors.