Electronic Supplementary Information (ESI)

Intramolecular catalytic hairpin assembly on DNA tetrahedron for mRNA imaging in living cells: improving reaction kinetics and signal stability

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Table S1. Sequences of oligonucleotides used in this work.

Oligo	Sequence (5'-3')
S1	ATCAC CCAAA CCCTC AATCT TTTAC ATTCC TAAGT CTGAA ACATT ACAGC TTGCT ACACG AGAAG AGCCG CCATA GTA
S2	TCAGC CAAGC ATACT AACTA TTTTA TCACC AGGCA GTTGA CAGTG TAGCA AGCTG TAATA GATGC GAGGG TCCAA TAC
S3	TCAAC TGCCT GGTGA TAAAA CGACA CTACG TGGGA ATCTA CTATG GCGGC TCTTC
S4	TTCAG ACTTA GGAAT GTGCT TCCCA CGTAG TGTCG TTTGT ATTGG ACCCT CGCAT
H1	AGATT GAGGG TTTGG GTGAT TTTCA GTTAC ATTCT CCCAG TTGAT TCCA(-Cy3)T GTGTA GAAAT CAACT GGGAG AA
H2	TAGTT AGTAT GCTTG GCTGA TTTAG TTGAT TTCTA CACAT(-Cy5) GGAAT CAACT GGGAG AACCA TGTGT AGA
MnSOD DNA target	AATCA ACTGG GAGAA TGTAA CTG
MnSOD RNA target	AAUCA ACUGG GAGAA UGUAA CUG
random DNA	ATTAG CGATG TCTAT TAGTC GC
miRNA 21	TAGCT TATCA GACTG ATGTT GA
miRNA 221	CCTGA AATCT ACATT GTATG CCAGG TTGGT
miRNA 205	ACCAG ATTTC AGTGG AGTGA AGTTC AGG
c-myc mRNA	TTGGT GAAGC TAACG TTGAG G

Considering easy synthesis of DNA, corresponding DNA sequences were used instead of RNA targets in vitro experiments, because of their same base-pairing recognitions and similar fluorescence response from the intra-CHA system.

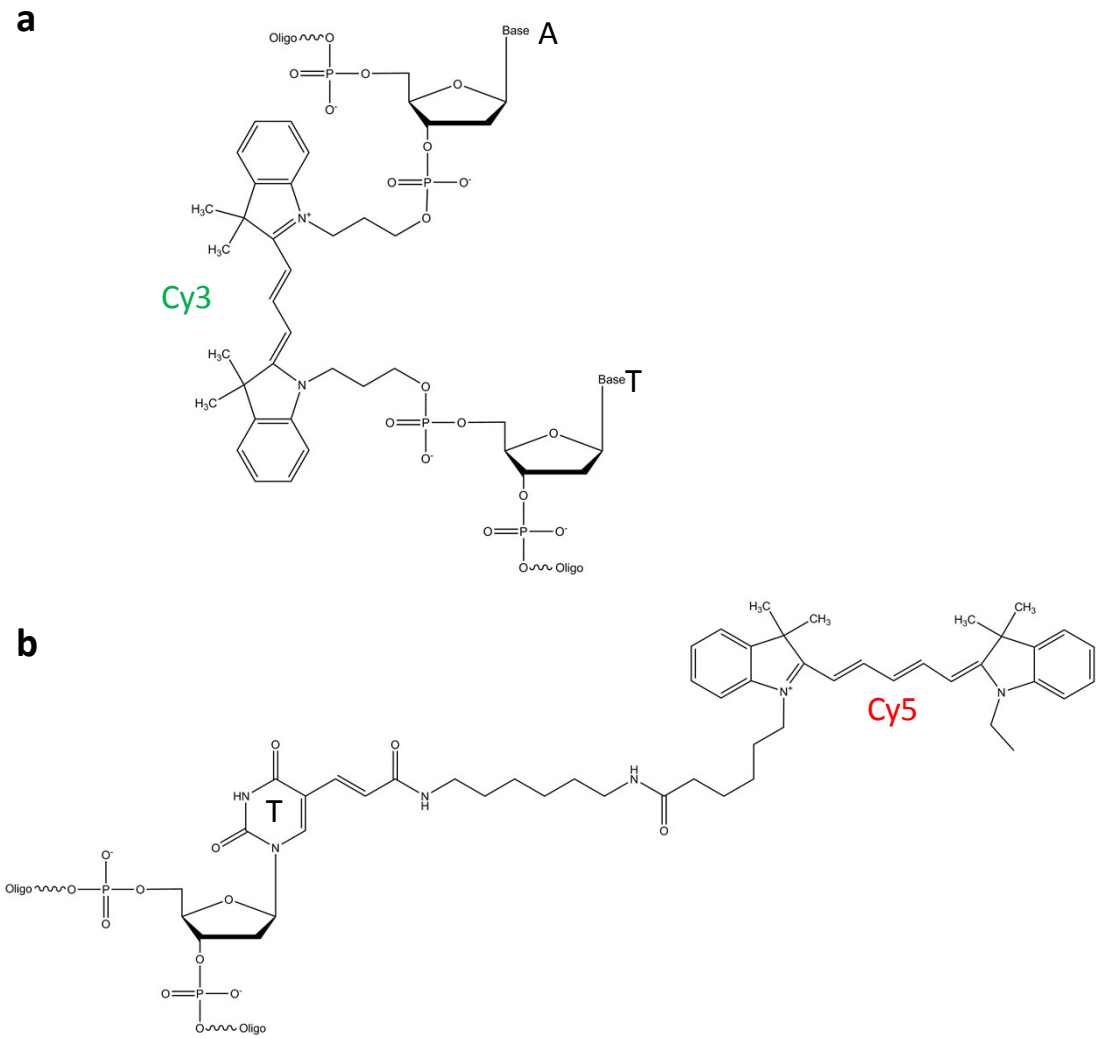

Fig. S1. Nucleotides modified withfluorophores Cy3 (a) and Cy5 (b), and their molecular structures. Cy3 is modified on the backbone between A and T, (b) Cy5 is modified on T.

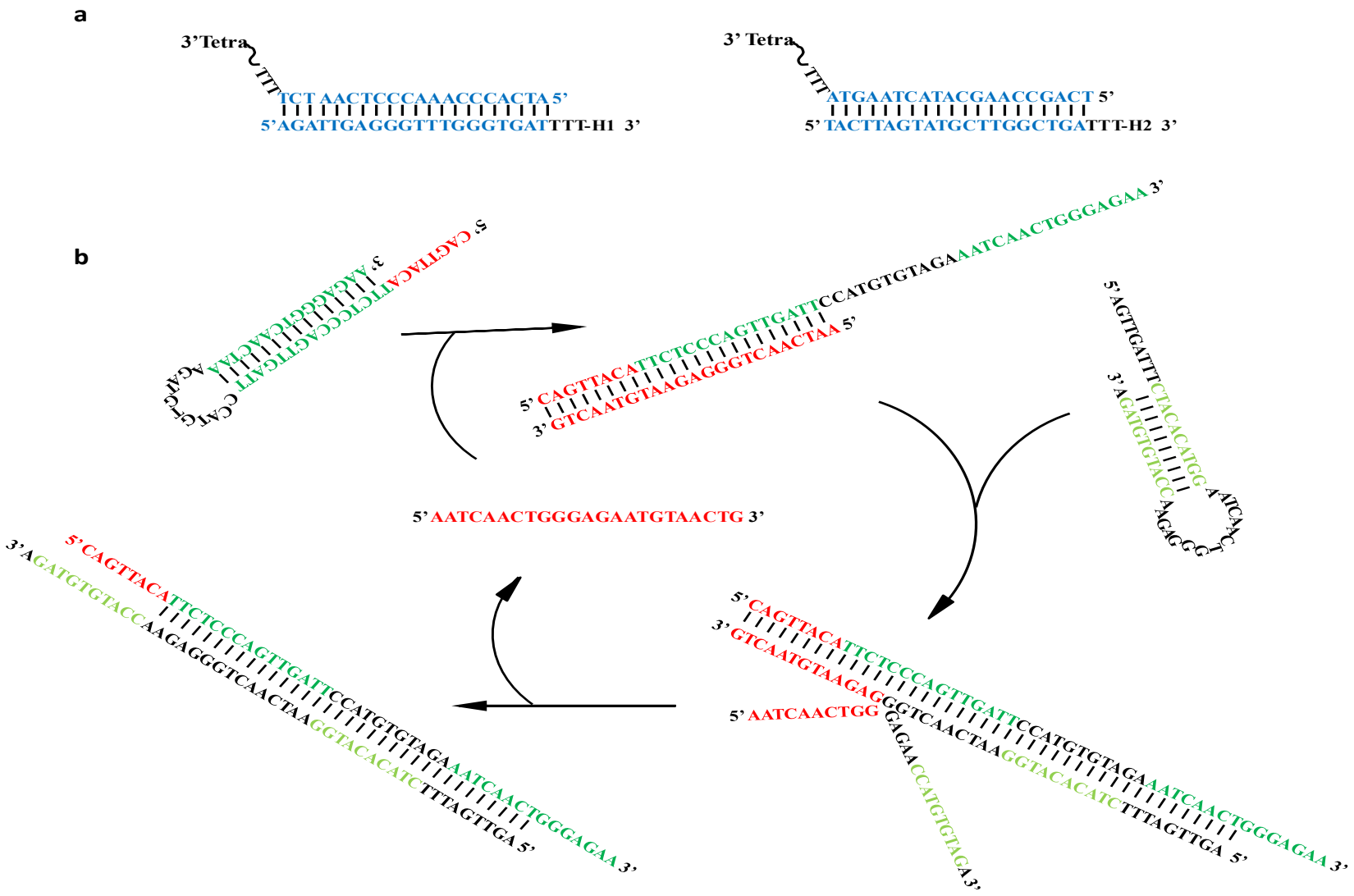

Fig. S2. (a) The contact structures of H1 and H2 with the tetrahedron (Tetra); (b) CHA reaction scheme with detailed sequence information.

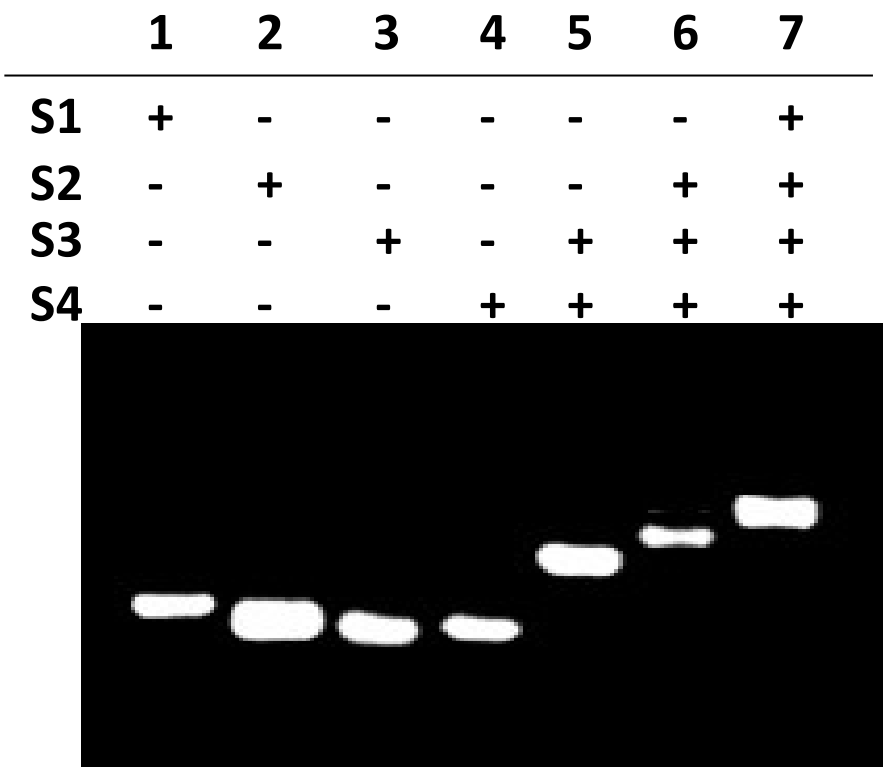

Fig.S3. Agarose electrophoresis characterization of the construction of the DNA tetrahedron. Lane 1: S1; Lane 2: S2; Lane 3: S3; Lane 4: S4; Lane 5: S3+S4; Lane 6: S2+S3+S4; Lane 7: S1+S2+S3+S4 (DNA tetrahedron).

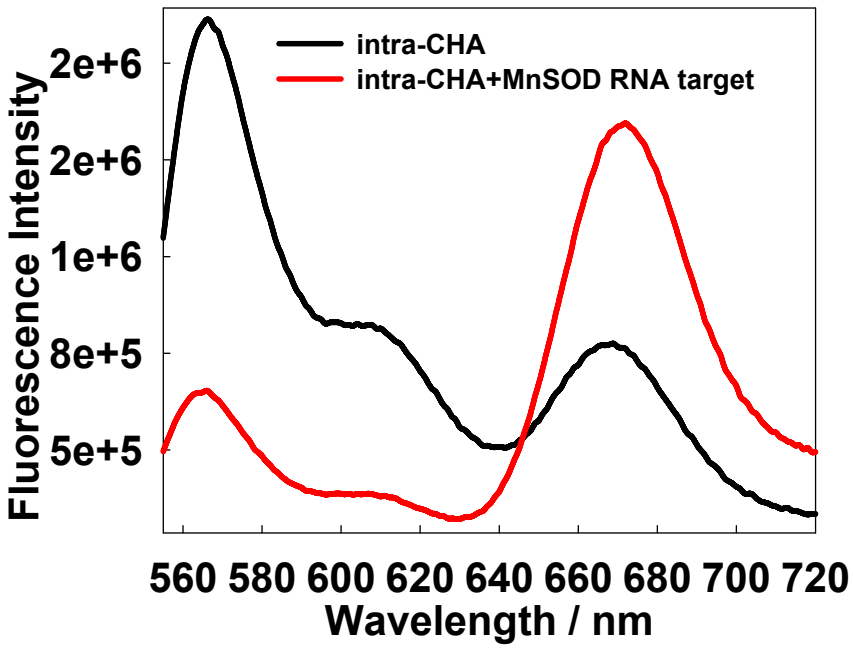

Fig. S4. Fluorescence spectra of the intra-CHA system in the absence (black curve) and presence (red curve) of the MnSOD target of mRNA sequence.

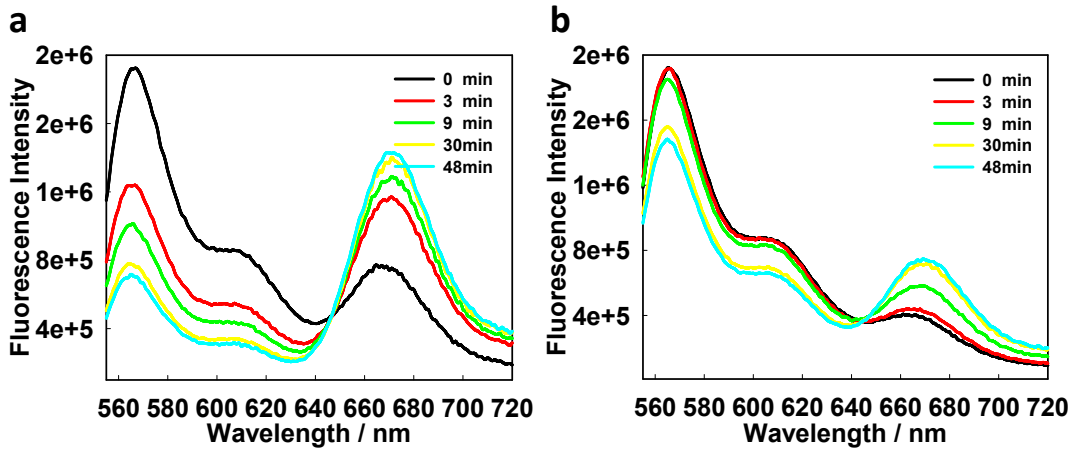

Fig.S5. Fluorescence spectra of (a) intra-CHA system and (b) free-CHA system at different reaction time points. The target concentration was 25 nM.

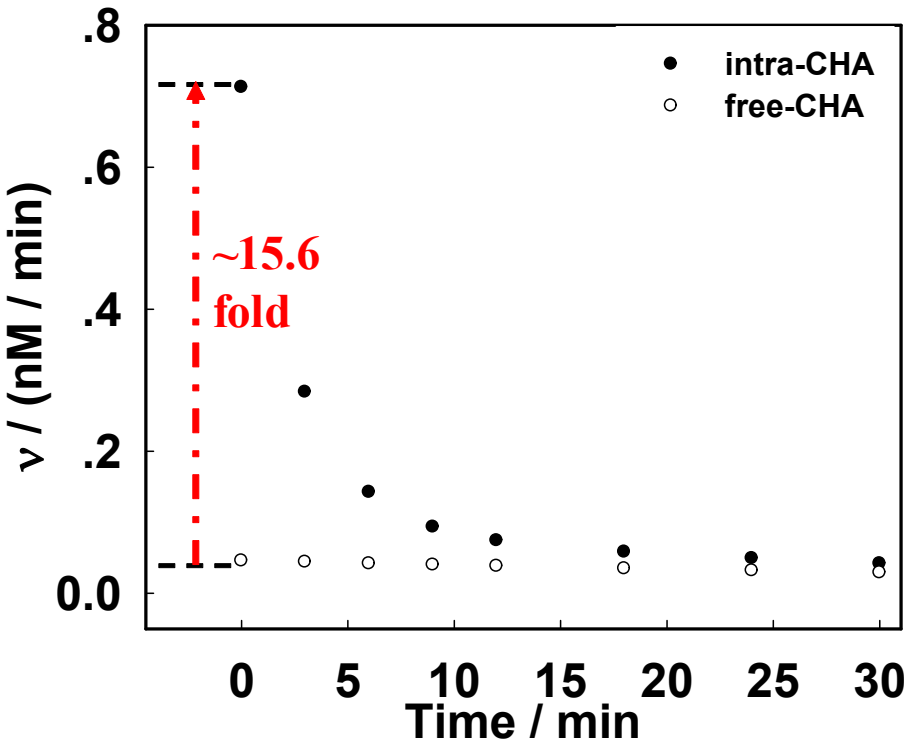

Fig. S6. Quantitativeanalysis of the reaction rates of intra-CHA and free-CHA system, vs reaction time.

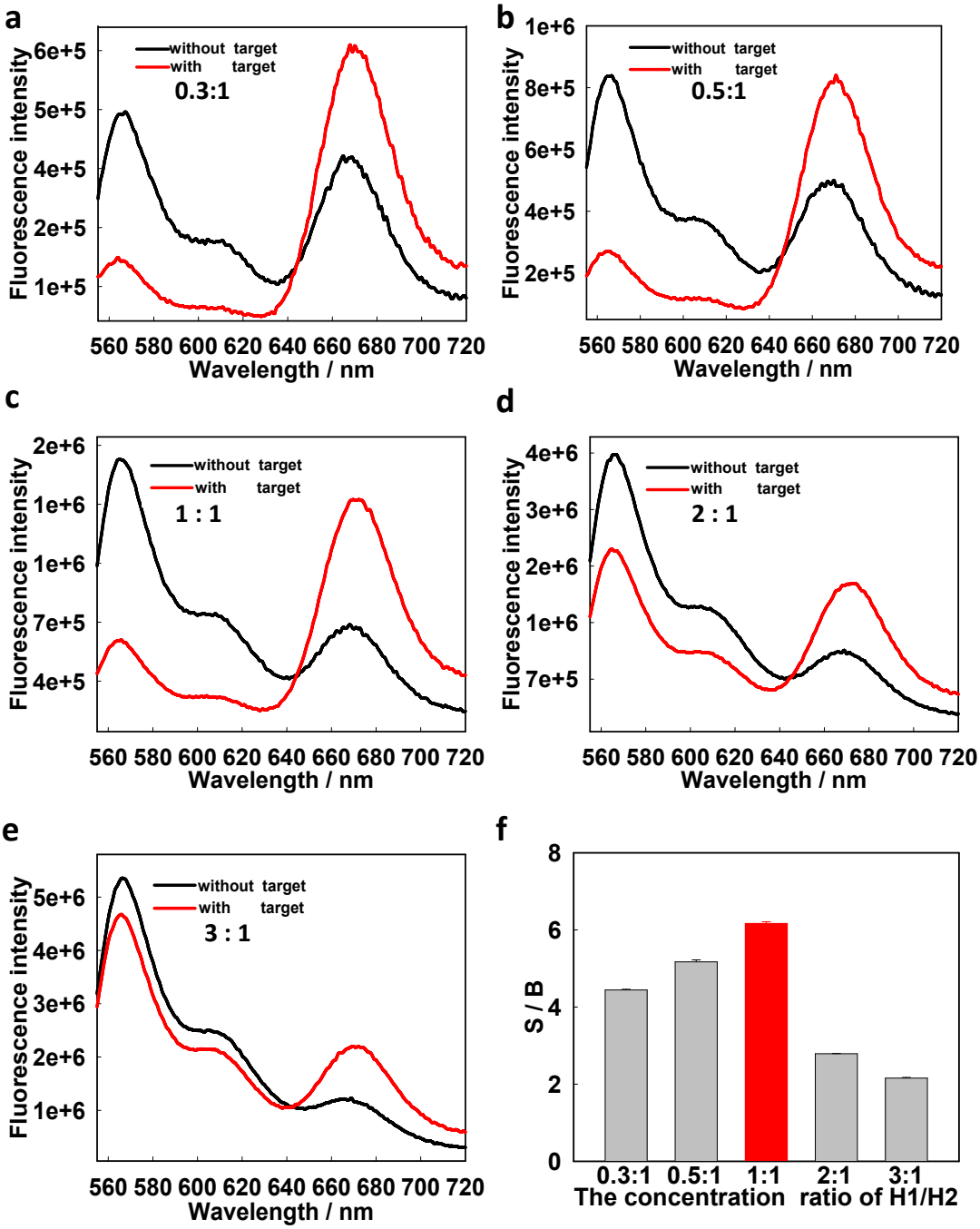

Fig. S7. (a-e) Fluorescence spectra of intra-CHA in different ratio of H1 and H2 (0.3:1, 0.5:1, 1:1, 2:1, 3:1); (f) Signal-to-background (S/B) of intra-CHA in different ratio of H1 and H2. The concentration of H2 was fixed at 50 nM, the reaction time was 3 h.

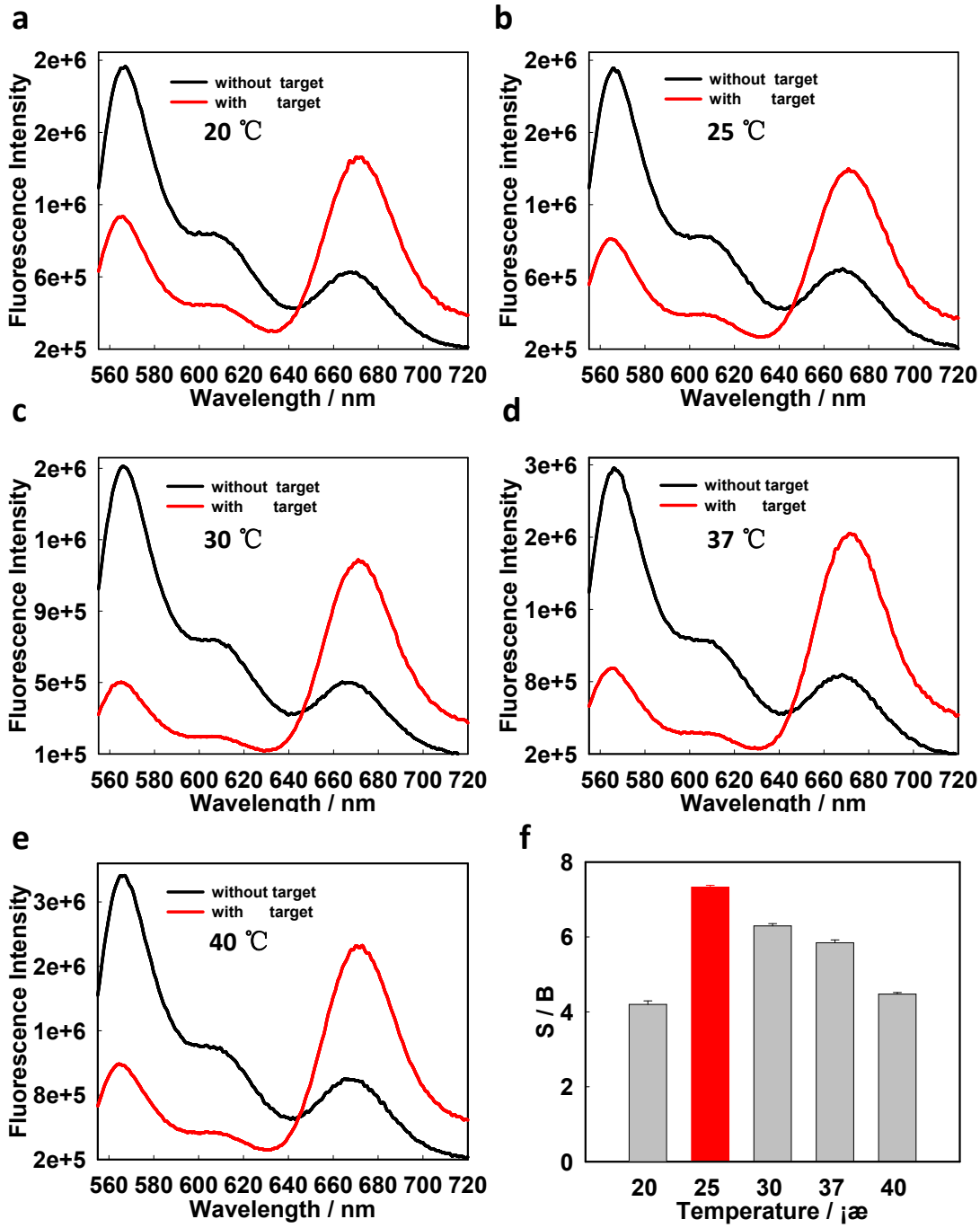

Fig. S8. (a-e) Fluorescence spectra of intra-CHA in different temperature (20, 25, 30, 37, 40 °C); (f) Signal-to-background (S/B) of intra-CHA in different temperature. The concentrations of amplifiers and targets were 50 nM and 25 nM, respectively. The reaction time was 3 h.

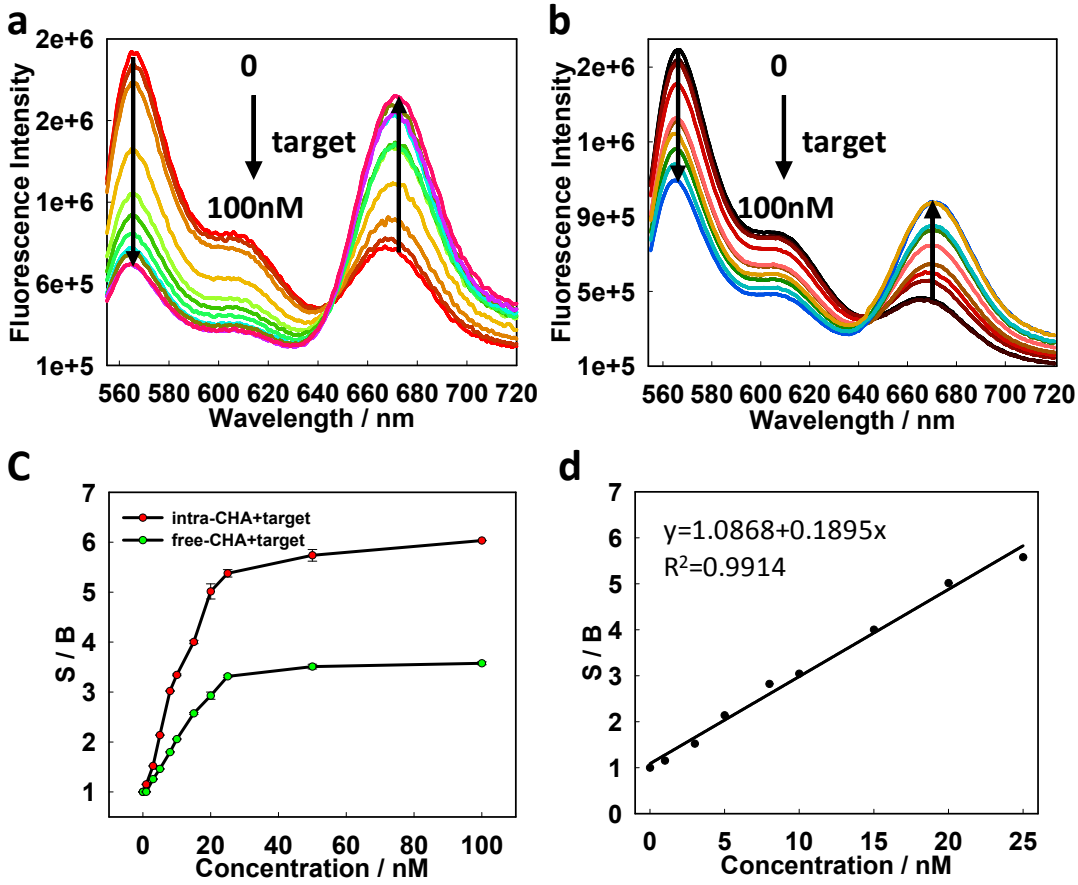

Fig.S9. Fluorescence spectra of (a) intra-CHA systemand (b) free-CHA system in the presence of MnSOD mRNA of different concentration.(c) Signal-to-background (S/B) of intra-CHA system (up) and free-CHA system (down). The concentrations of amplifiers were 50nM. The reaction time was 3 h. (d) Linear curve of intra-CHA system. The linear range is from1 to 25 nM.

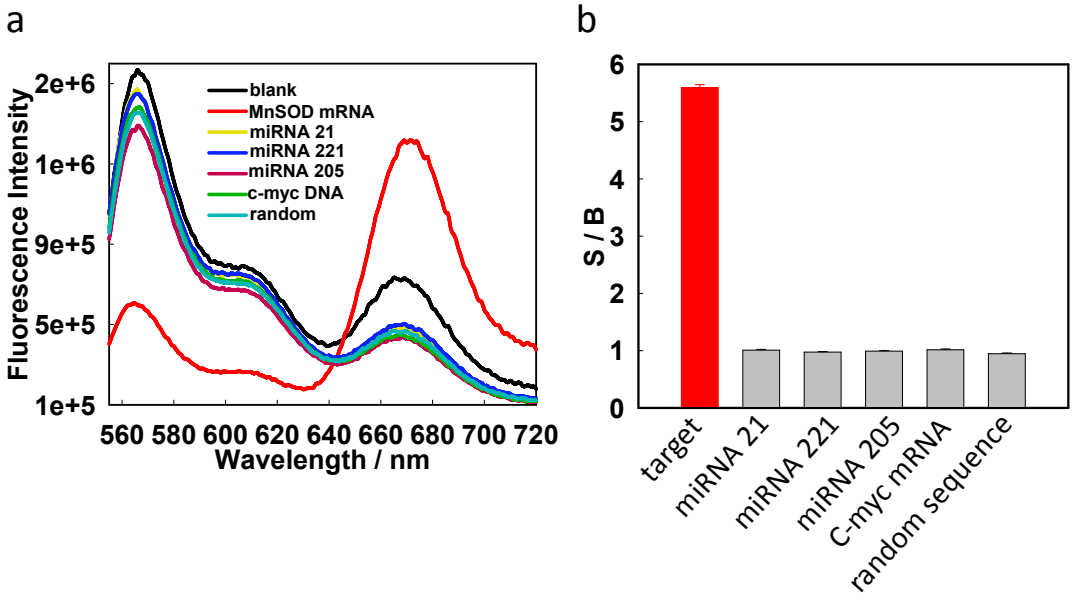

Fig. S10. (a) Fluorescence spectra and (b) Signal-to-background (S/B)of intra-CHA system towards different oligonucleotides. The concentration of amplifiers and oligonucleotides were 50 nM and 25 nM, respectively. The reaction time was 3 h.

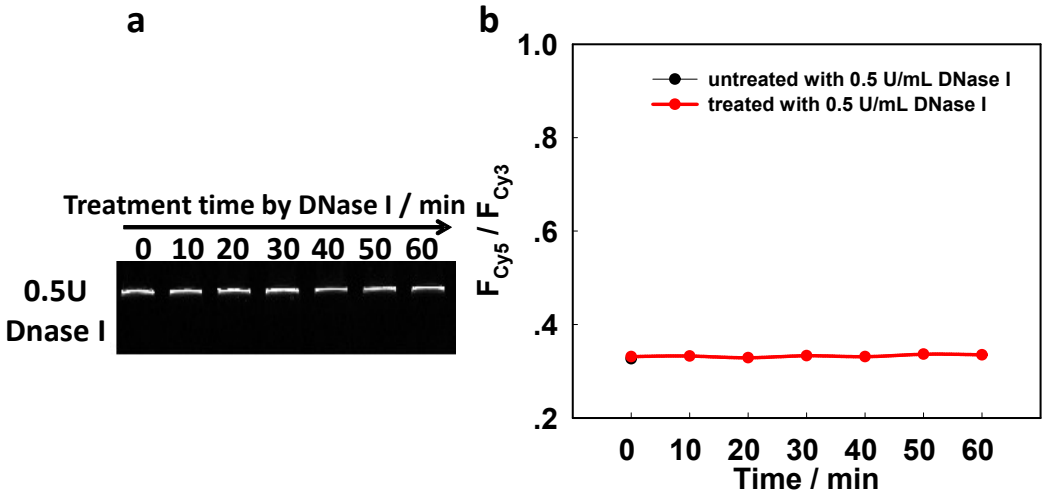

Fig. S11. Ability of intra-CHA amplifiers to avoid false-positive signals. (a) Gel characterization for the degradation of intra-CHA amplifiers by 0.5 U/mL DNase I. (b) FRET signal (F_{Cy5}/F_{Cy3}) as a function of time treated with 0.5 U/mL DNase I.

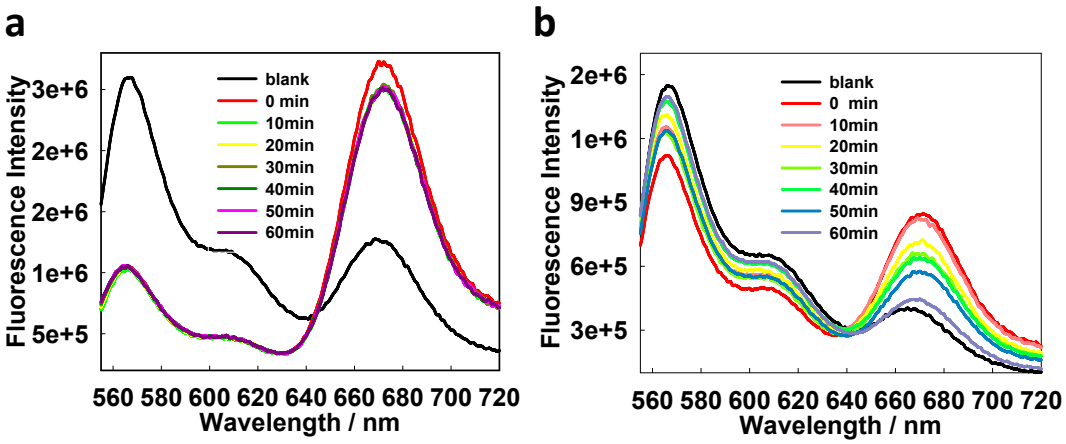

Fig. S12. Fluorescence spectra of (a) intra-CHA and (b) free-CHA products with different treatment time by DNase I.

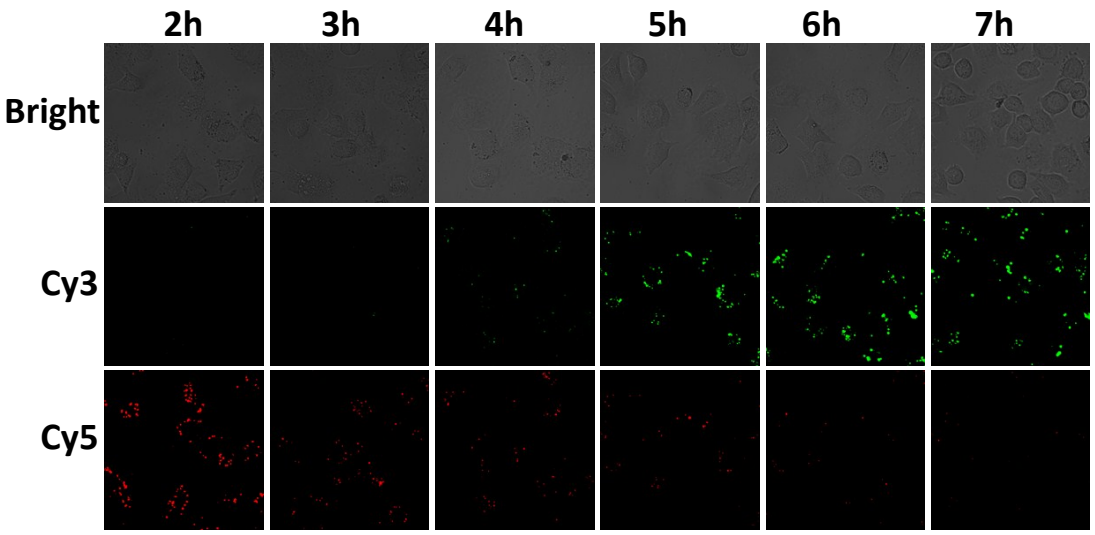

Fig. S13. Confocal fluorescence images for Cy3 and Cy5 in MDA-MB-231 cells transfected with free-CHA products, with increasing the incubation time. Excitation wavelength for imaging was set at 560 nm.

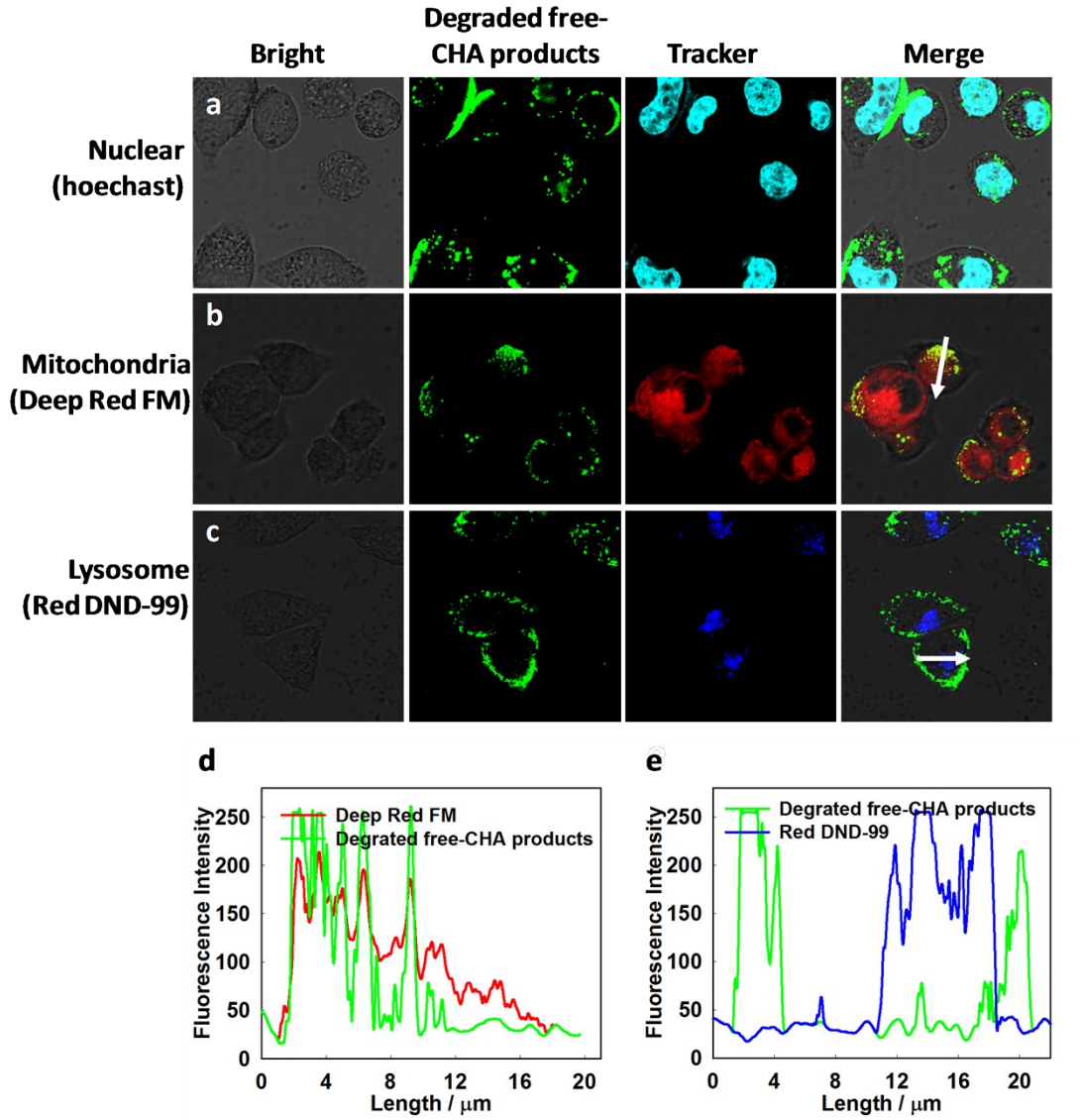

Fig. S14. Fluorescent location images of degraded free-CHA products and trackers of (a) nuclear, (b) mitochondria and (c) lysosome in MDA-MB-231 cells. (d) Intensity profile of the linear region of interest across MDA-MB-231 cells of degraded free-CHA products with Mito Tracker Deep Red FM. (e) Intensity profile of the linear region of interest across MDA-MB-231 cells of degraded free-CHA products with Lyso Tracker Red DND-99.The involved length in d and e is the area indicated by the arrow in b and c, respectively.

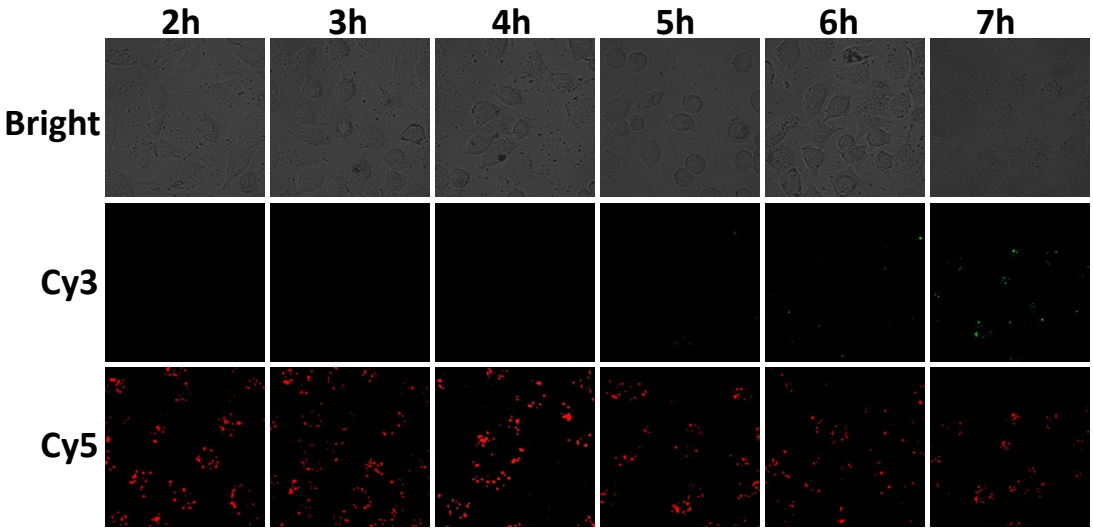

Fig. S15. Confocal fluorescence images for Cy3 and Cy5 in MDA-MB-231 cells transfected with intra-CHA products, with increasing the incubation time. Excitation wavelength for imaging was set at 560 nm.

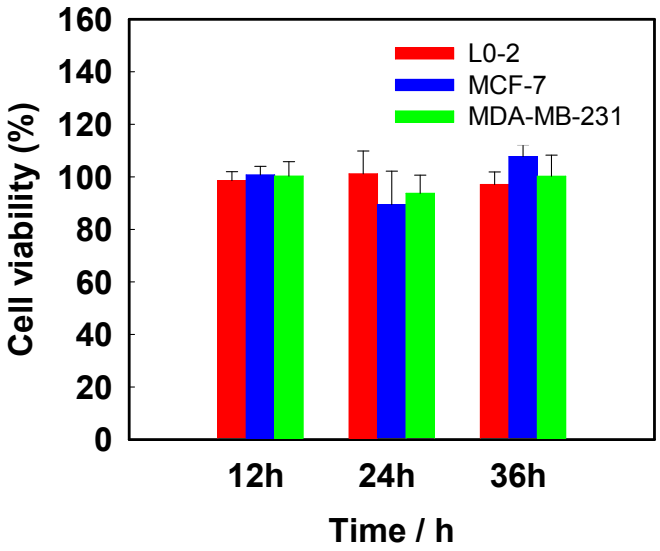

Fig. S16. Cell viability assay by MTT：L0-2, MCF-7 and MDA-MB-231 cells treated with intra-CHA amplifiers (250 nM) for 12, 24, and 36 h at 37 °C.

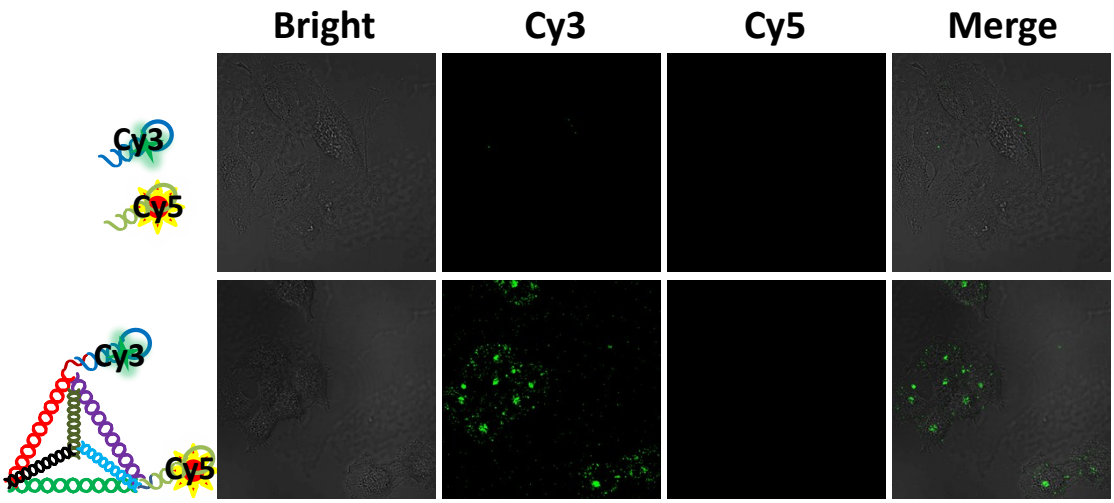

Fig. S17. Confocal fluorescence images for L0-2 cells after incubation with free-CHA or intra-CHA amplifiers for 4 h. The excitation wavelength was set at 560 nm.

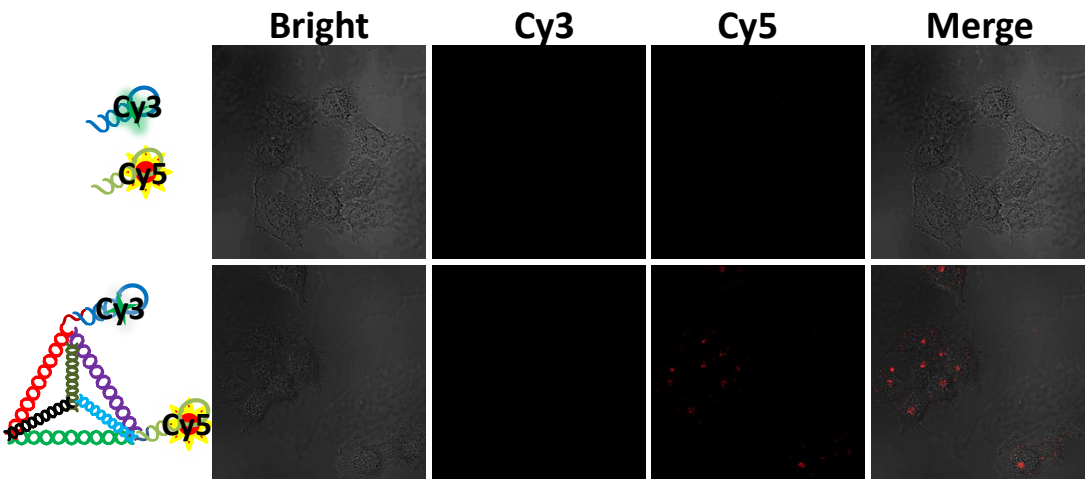

Fig. S18. Confocal fluorescence images for L0-2 cells after incubation with free-CHA or intra-CHA amplifiers for 4 h. The excitation wavelength was set at 640 nm.

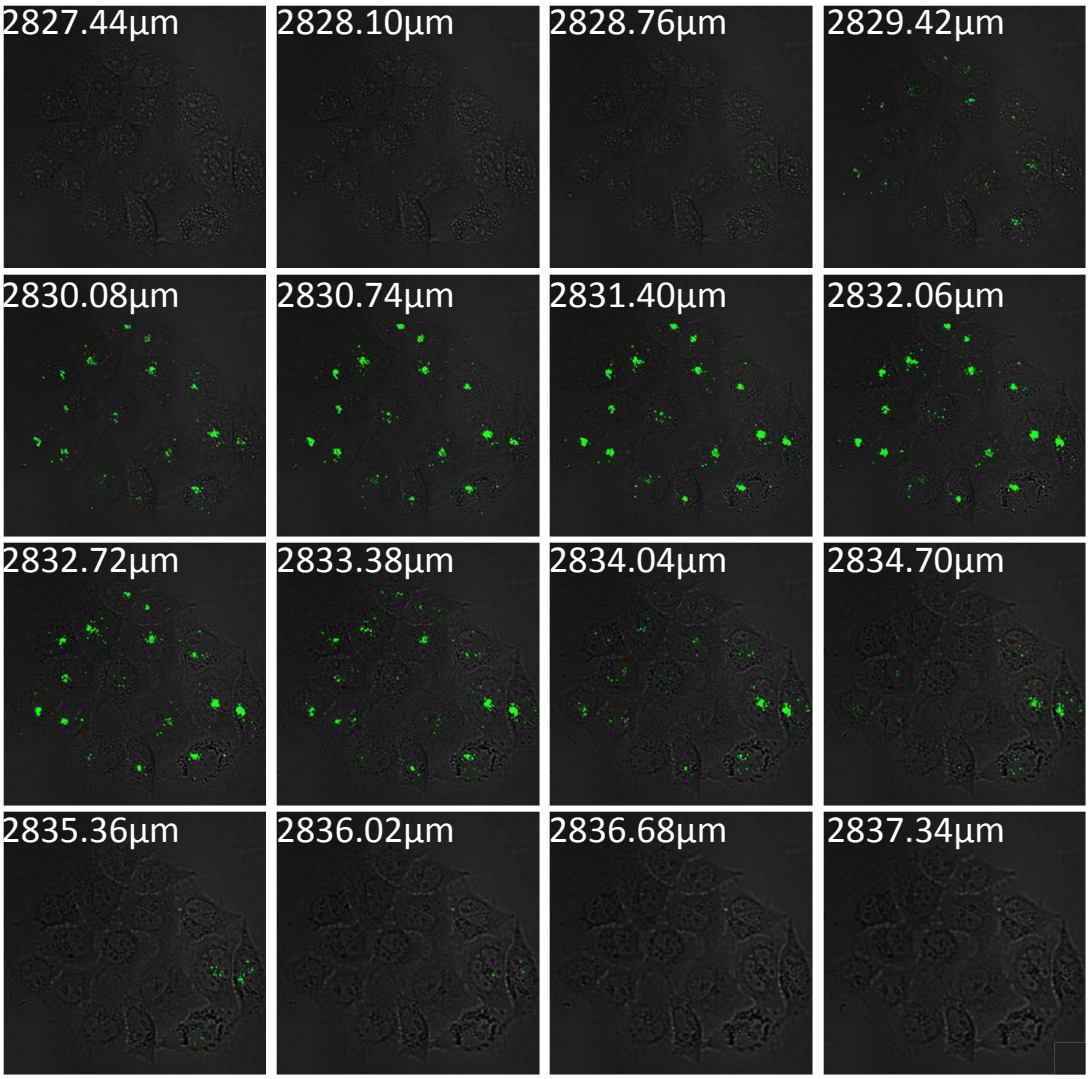

Fig. S19. The z-stack images of L0-2 cells after incubation with 250 nM intra-CHA amplifiers for 4 h. The excitation wavelength was set at 560 nm.

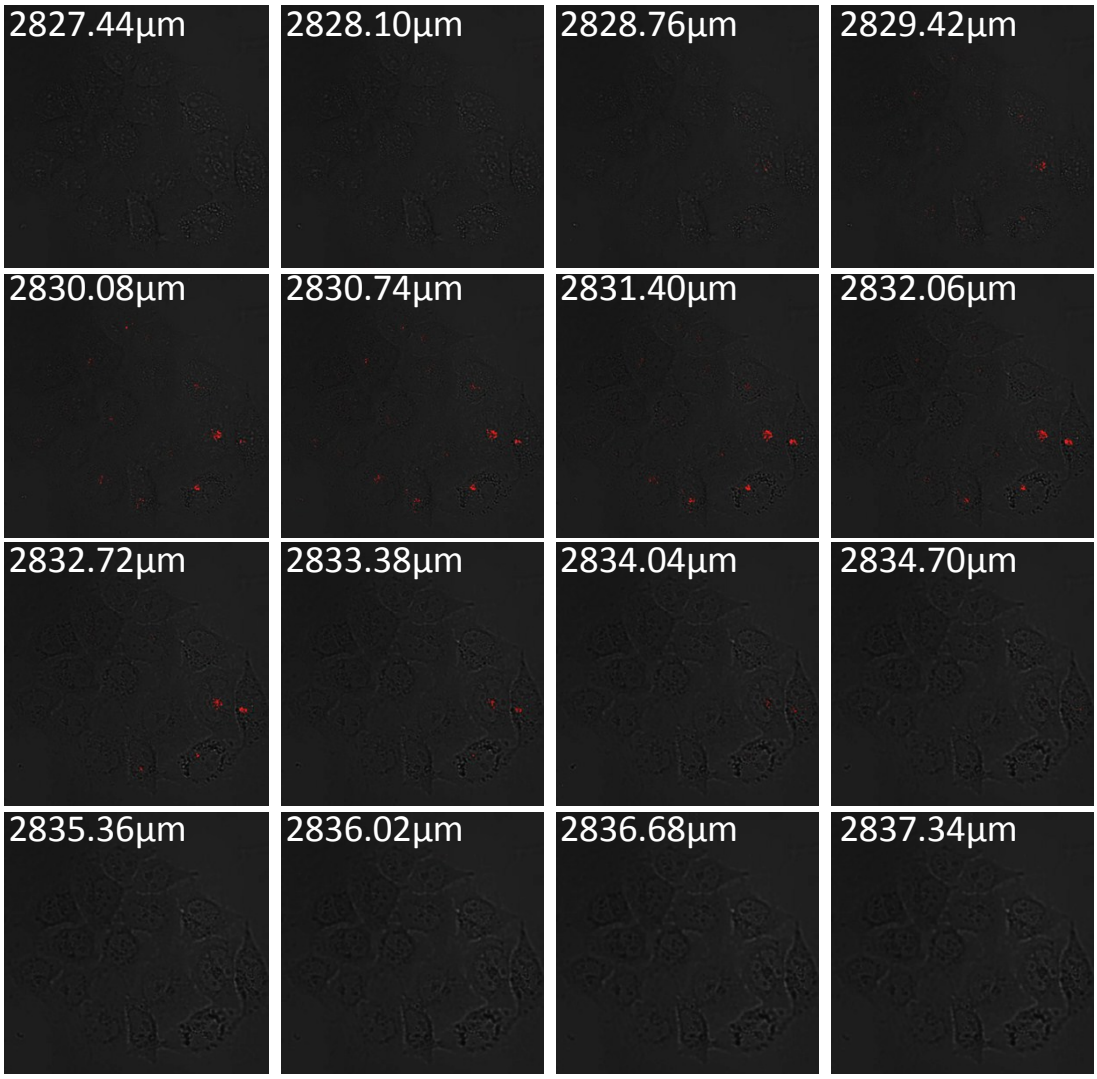

Fig. S20. The z-stack images of L0-2 cells after incubation with 250 nM intra-CHA amplifiers for 4 h. The excitation wavelength was set at 640 nm.

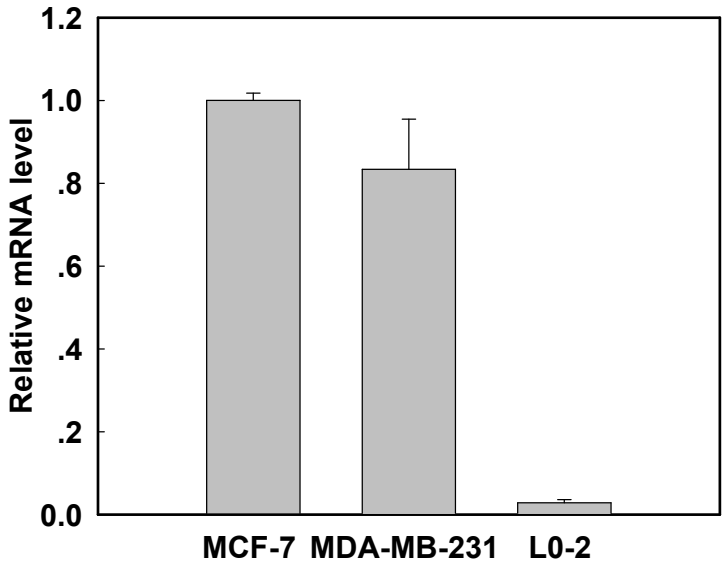

Fig. S21. Reverse transcriptase quantitative PCR analysis of MnSOD mRNA expression in MCF-7, MDA-MB-231 and L0-2 cells. Actin mRNA was also measured as an internal reference, which is usually used in relative quantification of RNA. The level of MnSOD mRNA in each cell was normalized to actin mRNA.