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Supporting Information

A Single Atom Change Facilitates the Membrane Transport of Green Fluorescent Proteins in Mammalian Cells

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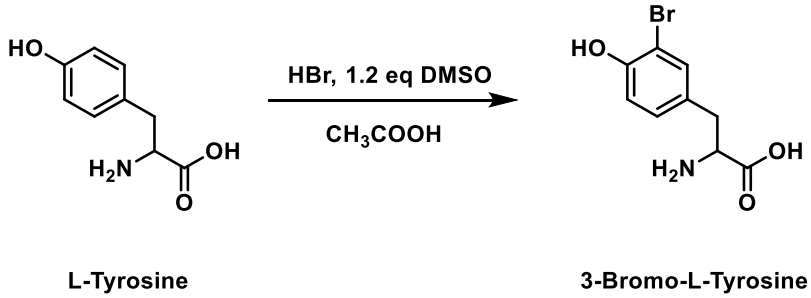
1. Experimental procedure

1.1. Synthesis of 3-bromo-L-tyrosine



L-Tyrosine **3-Bromo-L-Tyrosine**

Scheme **S1**. Synthesis of 3-bromo-L-tyrosine.[1]

L-Tyrosine (1.0 g, 5.5 mmol) was suspended in 10 mL AcOH, and 2.4 mL 48% HBr (21.6 mmol) was added. Then, 480 µL DMSO (6.6 mmol) was added, and the colorless suspension immediately turned yellow–orange. The suspension was warmed to 65 °C for 2 h, giving a clear light-yellow solution. It was then removed from the heat and the stirring was continued at 27 °C for 8 h. The clear yellow solution was then evaporated under reduced pressure to give a white solid, which was dissolved in 10 mL hot water, the pH was adjusted to 6 with solid $NaHCO_3$, and the product was crystallized at 4 °C. The crystalline suspension was filtered, the white crystals were washed with cold water and dried. A few more crystals were obtained from the filtrate upon standing. Yield: 935 mg (65.2%), ^{1}H NMR: (D_2O) δ 7.27 (d, 1H), 6.99 (dd, 1H), 6.82 (d, 1H,), 3.76 (dd, 1H), 3.01 (dd, 1H), 2.88 (dd, 1H); MS(ESI): 260.98.

1.2. Synthesis of ppTG21 peptide

A literature method was used with modifications.[2] The ppTG21 peptide was synthesized by standard Fmoc-based solid phase synthesis using microwave assisted automated peptide synthesizer Liberty 1 from CEM. The synthesis was performed on a 0.1 mmol scale using Rink Amide MBHA resin. The resin (270 mg) was swollen in 10 mL DMF for 1 h prior to the synthesis. For each coupling reaction, a 5-fold excess of reagents (2.5 mL of 0.2 M Fmoc protected amino acid, 1.0 mL of 0.5 M DIC and 0.5 mL of 1M HOBt solutions in NMP) were used. The side chain protecting groups used for His, Ser and Trp were Trityl (Trt), *ter*-Butyl (tBu) and *ter*-Butoxycarbonyl (Boc) respectively. All the coupling reactions except that of His were performed for 2 min with maximum temperature of 90 °C as has been reported earlier.[2] The His residues were coupled at a lower temperature of 50 °C for 6 min and the coupling was repeated twice with fresh reagents each time. Deprotection of the Fmoc group was achieved by 20% Piperidine in DMF (7 mL) for 30 sec followed by 3 min with a maximum temperature of 75 °C. The peptidyl resin was washed thoroughly with DCM (10 mL x 3) after completion of the synthesis. Cleavage of the peptide from the resin was performed manually by shaking at 27 °C for 2 h using the cleavage cocktail (8 mL) comprising of 92.5% TFA , 2.5% TIS , 2.5% DODT and 2.5% water. The resin was filtered and washed with 2 mL of fresh cleavage cocktail and the combined filtrate containing the cleaved peptide was collected. The peptide was precipitated from the cleavage

cocktail solution by adding ice-cold diethyl ether. The precipitate was collected by centrifugation, dried under reduced pressure, dissolved in 50% acetonitrile in water and lyophilized the crude and lyophilized peptide was purified reverse phase HPLC using Waters semipreparative HPLC system comprising of Waters 1525 binary pump and Waters 2489 UV/Visible detector. A semipreparative column from Phenomenax (Gemini Nx-C18; 5 µM; 110 Å 150 X 10 mm) was used with a flow rate of 1 mL/min. A linear gradient of 10% B to 90% B over 60 min was applied where solvent A was 0.1% TFA in water and solvent B was 0.1% TFA in acetonitrile. The purity of the peptide was determined to be 98% by analytical HPLC. The peptide was further confirmed by MALDI (Expected Mass – 2340.86 Observed Mass – 2341.0 $(M+H)^+$).

1.3. Plasmid construction, Protein Expression and Purification

Plasmid that can site specifically insert 3-iodo-L-tyrosine (3IYRS) in response to amber codon was generated from pEVOL-uaa (a gift from Prof. Peter G. Schultz, The Scripps Research Institute, Figure S1A). pEVOL plasmid contained one copy of $tRNA_{CUA}^{opt}$ and two copies of the evolved M. jannaschii tyrosyl-tRNA synthetase (aaRS) for L-(7-hydroxycoumarin-4-yl) ethylglycine, one of which was under an arabinose promoter and another under a constitutively activated glnS' promoter.[3,6] The second copy of aaRS including glnS' promoter was replaced with cassette having Emerald GFP under t5 promoter. The cassette was amplified with primers 11-12 (Table S2). Similarly, pEVOL-uaa vector backbone excluding the aaRS copy under glnS' promoter was amplified with primers 9 & 10 (Table S2). Both pEVOL vector backbone and EmGFP cassette (provided by Prof. Umesh Varshney, IISc) were ligated by using NEBuilder® HiFi DNA Assembly Cloning Kit (E5520S). The clones were confirmed by DNA sequencing.

Table S1: List of oligos used for this study.

S. No.	DNA Oligo name#	Sequence (5′-3′)
1	MjTyrRS32Alafp new*	TATAGGTTTTGAACCAAGTGG
2	MjYRS 32Tyr rpNO new	TAAGCAGATTTTTCATCTTT
3	mjYRS 65 Leu rp NO	GGCCAACAATATAATTATATCAAATCCAG
4	MjYRS70AlafpNew	GATTTAGCCGCCTATTTAAACCAG
5	MjYRS108F109Qfp new	TTTCAGCTTGATAAGGATTATACAC
6	MjYRS108109rpNO new	TTCACTTCCATAAACATATTTTG
7	mjYRS158ThrrpNO new	CGTATTAACCTGCATTATTGGATAGATAAC
8	mjYRS159Serfp new	TCTCATTATCTTGGCGTTGATGTTGCAGTTGG
9	EmGFPpromo-pEVOLrp	GGCCTCGTGATTTGATAATCTAACAAGGATTATG
10	EmGFP- pEVOLfp	TGGTGAGAATCTGCAGTTTCAAACGCTAAATTGC
11	pEVOL-EmGFPPromfp	ATTATCAAATCACGAGGCCCTTTCGTCTTC
12	pEVOL-EMGFPrp	TGAAACTGCAGATTCTCACCAATAAAAAACGCCC
13	EmGFPY39Amfp	GGGCAAGCTGACCCTGAAG
14	EmGFPY39AmrpNO	TAGGTGGCATCGCCCTC
15	EmGFP K214Am fp	CCAACGAGTAGCGCGATCAC
16	EmGFP K214Am rp NO	GGTCTTTGCTCAGGGCGG

#DNA Oligo =Primer; *DNA oligo used as common forward primer with Non-overlapping reverse primer to get mutations such as 32Tyr, 32Gly

The resultant plasmid subjected to site-directed mutagenesis based on known active site mutations reported (Table S1)[4] to achieve a synthetase that can charge 3-iodo-L-tyrosine. The site

directed mutagenesis was carried out with Q5® Site-Directed Mutagenesis Kit (E0554S from NEB), and primers 1-8 (Table S1) were used. The final clones were confirmed by sequencing the constructs (Figure S1B).

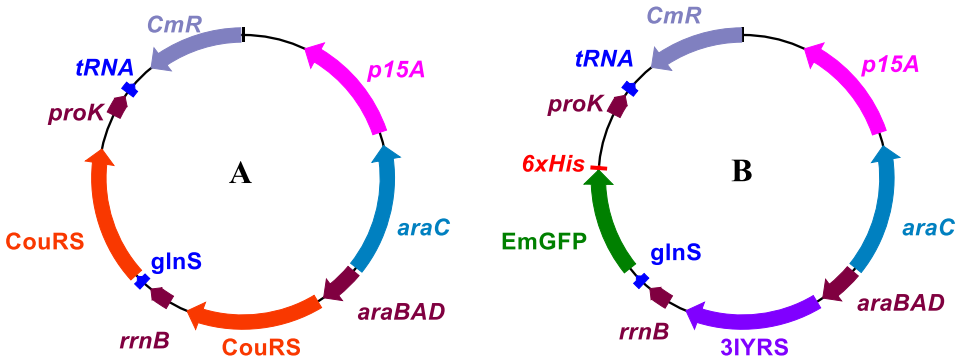
Table S2: List of mutations in the synthetases Cou-Etglycyl and 3IYRS.

Synthetase	Position									References
	32	65	67	70	108	109	158	159	162	
Tyrosyl (native)	Tyr	Leu	Ala	His	Phe	Gln	Asp	Ile	Leu	[5]
Coumaryl*	Glu	His	Gly	Gly	Tyr	His	Gly		Gly	[6,3]
3-iodo-tyrosyl$			Ala	Ala			Thr	Ser		[4]

*L-(7-hydroxycoumarin-4-yl) ethylglyl,
$Empty boxes in table indicates residues same as in wild type tyrosyl synthetase

Similarly, the introduction of Amber mutations in EmGFP at 39 and 214 positions were carried out by above mentioned procedure by using primers 13-16.

Figure S1: Plasmid map of pEVOL-uaa (A) and pEVOL-3IYRS-EmGFP (B)



pEVOL-3IYRS-EmGFP (WT), pEVOL-3IYRS-EmGFP-1TAG in combination with 3-Chloro, 3-Bromo, and 3-iodo-L-tyrosine) and pEVOL-3IYRS-EmGFP-2TAG with 3-iodo-L-tyrosine variants (listed in Table S3) were expressed and purified using E. *coli* strain *C321ΔA.exp* (Church Lab, Addgene plasmid # 49018).[7a]

Table S3: List of mutations in combination with 3-halo-L-tyrosines.

Sl. No	Plasmid	TAG position in EmGFP	3-Halo-L-tyrosine	Protein label
1	pEVOL-3IYRS-EmGFP	---	L-tyrosine*	WT
2	pEVOL-3IYRS-EmGFP	39	3-chloro-L-tyrosine	EmGFP-1TAG-3ClY
3	pEVOL-3IYRS-EmGFP	39	3-bromo-L-tyrosine	EmGFP-1TAG-3BrY
4	pEVOL-3IYRS-EmGFP	39	3-iodo-L-tyrosine	EmGFP-1TAG-3IY
5	pEVOL-3IYRS-EmGFP	39 & 214	3-iodo-L-tyrosine	EmGFP-2TAG-3IY

*endogenous L-tyrosine

E. coli C321.ΔA.exp cells were transformed with pEVOL-3IYRS-EmGFP or pEVOL-3IYRS-EmGFP1TAG or pEVOL-3IYRS-EmGFP-2TAG, individual colonies were inoculated in L.B broth supplemented with chloramphenicol (34 µg/mL) and grown at 37 °C for 12-14 h with a speed of 220 rpm in orbital shaking incubator. Saturated cultures were diluted to a 0.08 OD_{600} with fresh L.B

supplemented with chloramphenicol (34 µg/mL), IPTG (1.0 mM) and L-arabinose (0.2%). To express the variants having 3-iodo-L-tyrosine, 3-bromo-L-tyrosine and 3-chloro-L-tyrosine at 39-position of EmGFP-1TAG and only 3-iodo-L-tyrosine in 39- and 214-position of EmGFP-2TAG, the corresponding amino acids 3-iodo-L-tyrosine, 3-bromo-L-tyrosine and 3-chloro-L-tyrosine were supplemented in L.B to a final concentration of 1.0 mM allowed the cultures to grow for 24 h at 37 °C shaking with a speed of 220 rpm.[7b]

Cells were pelleted down and suspended in Lysis buffer and sonicated to lyse the cells. The cell debris was removed by centrifugation at 21,000 *g* for 1.0 h, further the lysates were subjected to ultracentrifugation at 1,00,000 *g* for additional 1.0 h. The clear supernatant was loaded into Ni-NTA Columns (HisTrap™ HP Columns form G.E. Healthcare were used with Bio-Rad Due flow FPLC system). The C-terminal 6xHis-tagged proteins were purified by Ni-NTA affinity chromatography protocol. Briefly, 40 mL lysate was loaded into Ni-NTA column and washed with 15 column volumes of wash buffer and eluted with elution buffer by concentration gradient of imidazole (50 mM to 400 mM in 100 mL elution volume). Imidazole was removed and proteins were concentrated by using Amicon Ultra centrifugal filters having 10 kDa MWCO. Finally, the proteins were stored in 50 mM sodium phosphate buffer with 10% glycerol at -80 °C. The purity of proteins was analyzed by 12% SDS-PAGE (Fig 1C and 4C). Bradford assays were performed to determine the protein concentrations by comparing with a set of bovine serum albumin (BSA) standards. The incorporation of unnatural amino acids (3-iodo-L-tyrosine, 3-bromo-L-tyrosine and 3-chloro-L-tyrosine) was confirmed by mass spectrometry (Table S4 and Figure S6-S10). All the proteins showed expected mass.

Table S4: Table showing the mass list of expected, observed mass of proteins.

Protein	Expected mass (Da)	Observed mass (Da)	Difference (Da)
WT	27943.52	27943.18 (Fig. S6)	-0.34
EmGFP-1TAG-3ClY	27979.02	27977.46 (Fig. S7)	-1.56
EmGFP-1TAG-3BrY	28023.42	28022.47 (Fig. S8)	-0.95
EmGFP-1TAG-3IY	28070.42	28069.32 (Fig. S9)	-1.1
EmGFP-2TAG-3IY	28231.31	28233.00 (Fig. S10)	+1.69

1.4. Mass spectrometry

Mass spectra were recorded using LC-ESI-QTOF (maXis Impact, Bruker Daltonics, Germany) mass spectrometer at the Proteomics facility, Molecular Biophysics Unit, Indian Institute of Science.

1.5. UV-Visible and Fluorescence Spectroscopy

The UV-Visible absorption spectra were recorded by using a Shimadzu UV-2600, UV-Vis spectrophotometer, and Fluorescence spectra were recorded by using FluoroMax-4, HORIBA JOBIN YVON, spectrofluorimeter. The UV-Vis and fluorescence studies were carried out in 50 mM sodium

phosphate buffer at a pH of 7.5 using the protein concentrations 25 µM and 5 µM, respectively. The spectra were plotted using OriginLab 8.0 and are normalized (Figure 2A).

1.6. Circular Dichroism

The proteins were diluted in 50 mM phosphate buffer (pH 7.5) to the final concentration of 10 µM. The CD spectra were recorded in the 190-230 nm range using a JASCO J-715 model spectropolarimeter and scanned 3 times with data patch of 0.5 nm bandwidth and 100 nm/sec speed. All the spectra are reported after a baseline correction with 50 mM phosphate buffer (pH 7.5).

1.7. Cell lines and culture condition

HepG2 hepatocellular carcinoma (HB8065) was obtained from ATCC, Washington DC, USA. The cells were grown in MEM supplemented with 2.2 g/L sodium bicarbonate, 10% supplemental FBS, penicillin G (100 unites/mL) streptomycin (100 mg/mL). The cells were cultured till 80-90% confluence and further sub-cultured in different sized cell culture dishes/plates depending on the type of experiment in a humidified incubator at 37 °C with 5% CO_2. For fluorescence reader assays, the cells were cultured on black walled 96-well plates with clear film bottom.

1.8. Cell viability assay with MTT

The viability of the cells was determined using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) colorimetric assay as described previously.[8] The cells were plated on 96-well microtiter cell culture plates at 20,000 cells per well for 24 h before they were incubated for 90 min with various concentrations of EmGFP variants. After the indicated treatment period, the cells were incubated for 3 h at 37 °C in a culture medium containing 10 µmol L−1 MTT in phosphate-buffered saline (PBS). The blue MTT formazan precipitate was then dissolved in 100 µL of DMSO, and the absorbance was measured at 570 nm with a multi-well plate reader. The cell viability was expressed as the percentage of the absorption values in the treated cells relative to the non-treated (control) cells. The data are presented as the means ± the standard error of the triplicated cultures.

1.9. Cell treatment conditions

For the low temperature studies, the cells were first incubated at 4 °C for 1 h and then incubated with EmGFP proteins for 1 h at 4 °C and cold PBS containing Heparin (1 mg/mL) and subsequently the cells were analysed by microplate reader (or) confocal imaging. For the ATP depletion studies, HepG2 cells were incubated with ATP depletion medium (glucose-free DMEM with 10 mM sodium azide and 6 mM 2-deoxy-D-glucose) containing 10% fetal calf serum for 1.5 h, followed by incubation with different EmGFP proteins1 h in serum-free ATP depletion medium. For the studies with endocytosis inhibitors, the HepG2 cells were first pre-treated with chlorpromazine (CPZ, C8138 Sigma, 10 µM/mL) or methyl-β-cyclodextrin-MβCD (C4555, Sigma, 10 mM) or genistein (GST, G6649

Sigma, 200 µM) in normal culture medium for 30 min at 37 °C. Subsequently, the cells were further incubated with EmGFP proteins for 90 min and were analyzed by microplate reader (or) confocal imaging.

1.10. Intracellular fluorescence intensity assay

After seeding the cells in to 96-well cell culture grade microtiter plates at 20,000 cells/well and culturing for 24 h, the cells were treated with proteins and inhibitors for various time points according to the experimental conditions, then washed 3 times with cold PBS containing Heparin (1 mg/mL). After washing, the MEM medium was added and the fluorescence intensity in the cells was measured by GloMax Microplate Reader (Promega).

1.11. Confocal microscopy

The cells were cultured on glass coverslips in 12-well culture plates (0.2 X 10^6 per well). They were allowed to adhere and proliferate for 24 h. Cells were then incubated with EmGFP proteins or inhibitors at different time points depending on the experimental condition. After the treatment, the cells were washed with cold PBS containing Heparin (1 mg/mL) and fixed with 4% PFA (paraformaldehyde) followed by wash with PBS buffer. Finally, the cover slips containing fixed cells were mounted on microscopic slides using fluoromount (F4680, Sigma). Images were captured with Zeiss LSM880 (Airyscan) and Leica TCS SP8 confocal microscopy and images were further analysed and extracted using Zen v2.3 (blue edition) and LAS X (Leica) software.

1.12. FACS Analysis

Cells were seeded in 6-well plates (1 × 10^5 cells/well) and allowed to adhere and proliferate for 24 h. Then, the cells were treated with EmGFP proteins or inhibitors at different time points depending on the experimental conditions. After the treatment, the cells were washed with PBS and treated with 0.5% trypsin/EDTA solution. The detached cells were pelleted down by centrifugation and cells were re-suspended in MEM. Then, the fluorescence intensity was measured using FACSCelesta flow cytometer (BD Biosciences, San Jose, CA) using CellQuest (BD Biosciences) acquisition software. Data analysis was performed with FSC Express 6.0 Flow Cytometry Analysis software.

2. Results

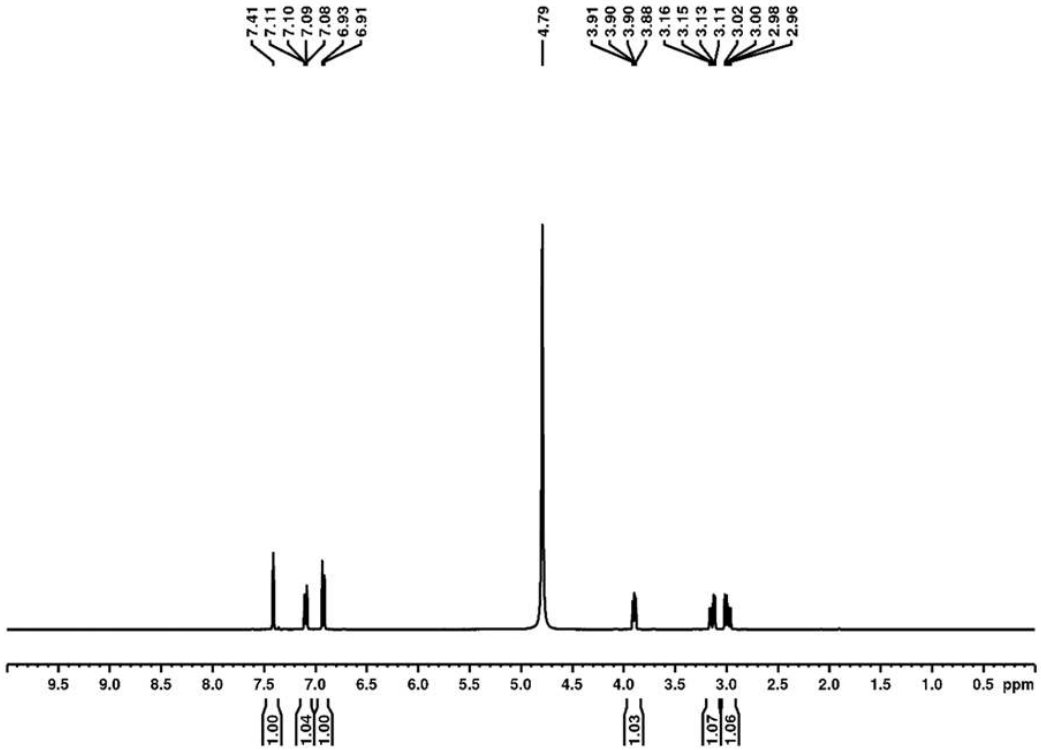


Figure S2: ^{1}H NMR spectrum (D_2O, 400 MHz) of 3-bromo- L-tyrosine.

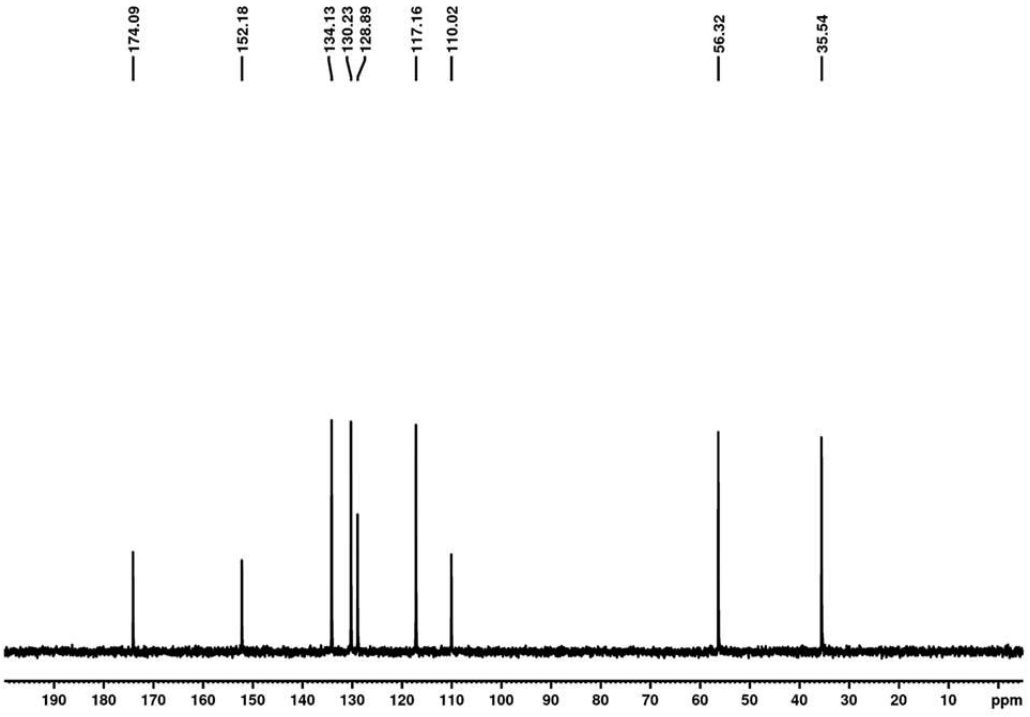


Figure S3: ^{13}C NMR spectrum (D_2O, 100.59 MHz) of 3-bromo-L-tyrosine.

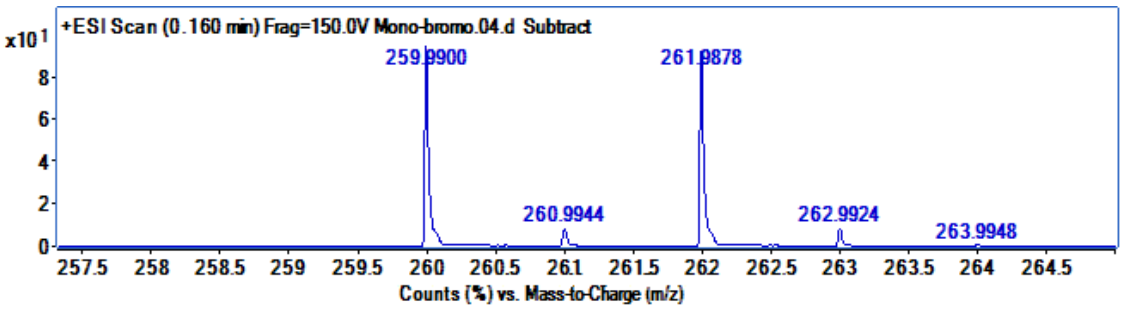
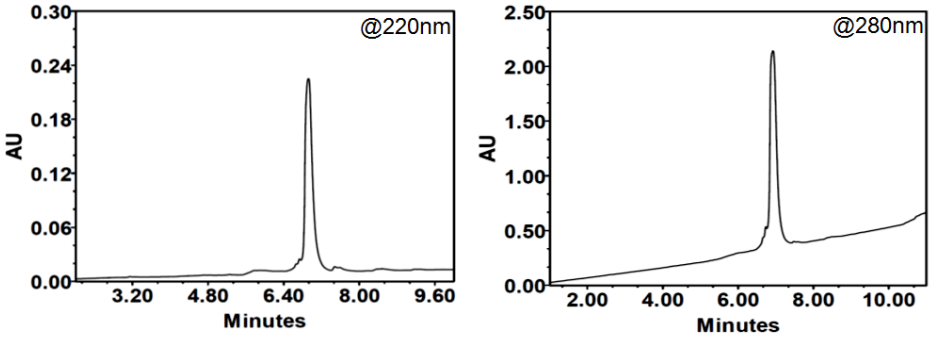


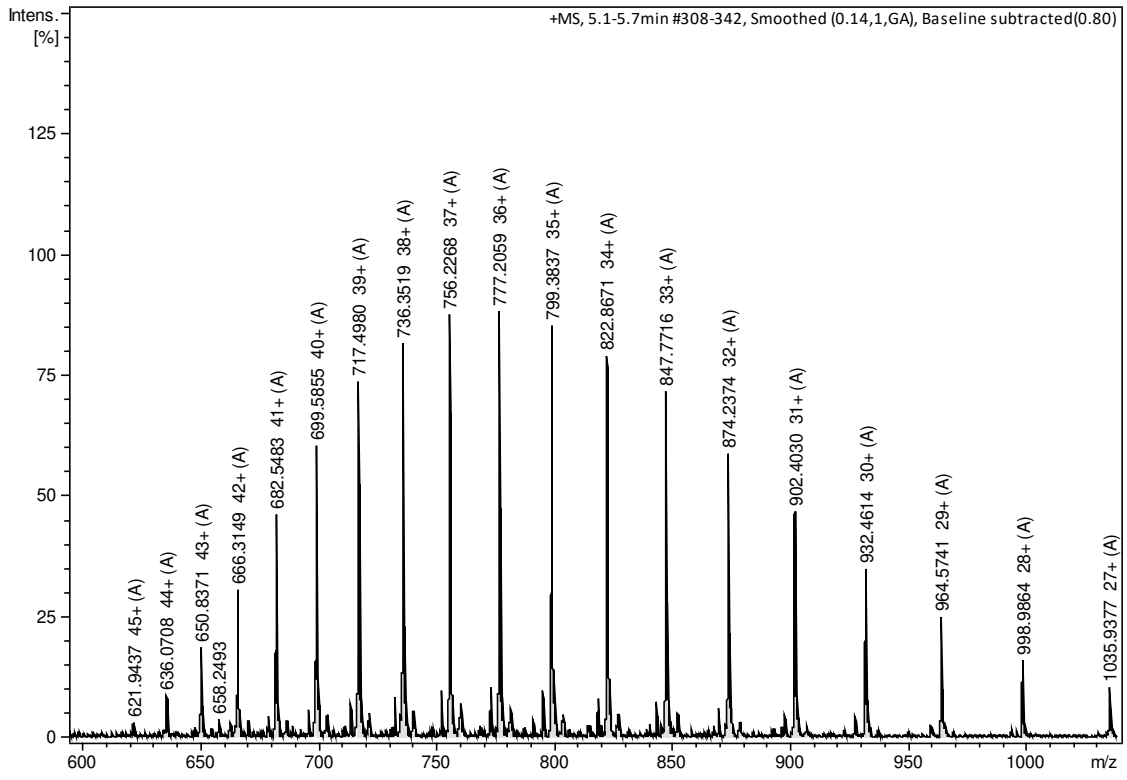
Figure S4. ESI- MS of 3-bromo-L-tyrosine.



(A) (B)

Figure S5: HPLC chromatogram of ppTG21 peptide at 220 nm (A) and 280 nm (B).

(A)



(B)

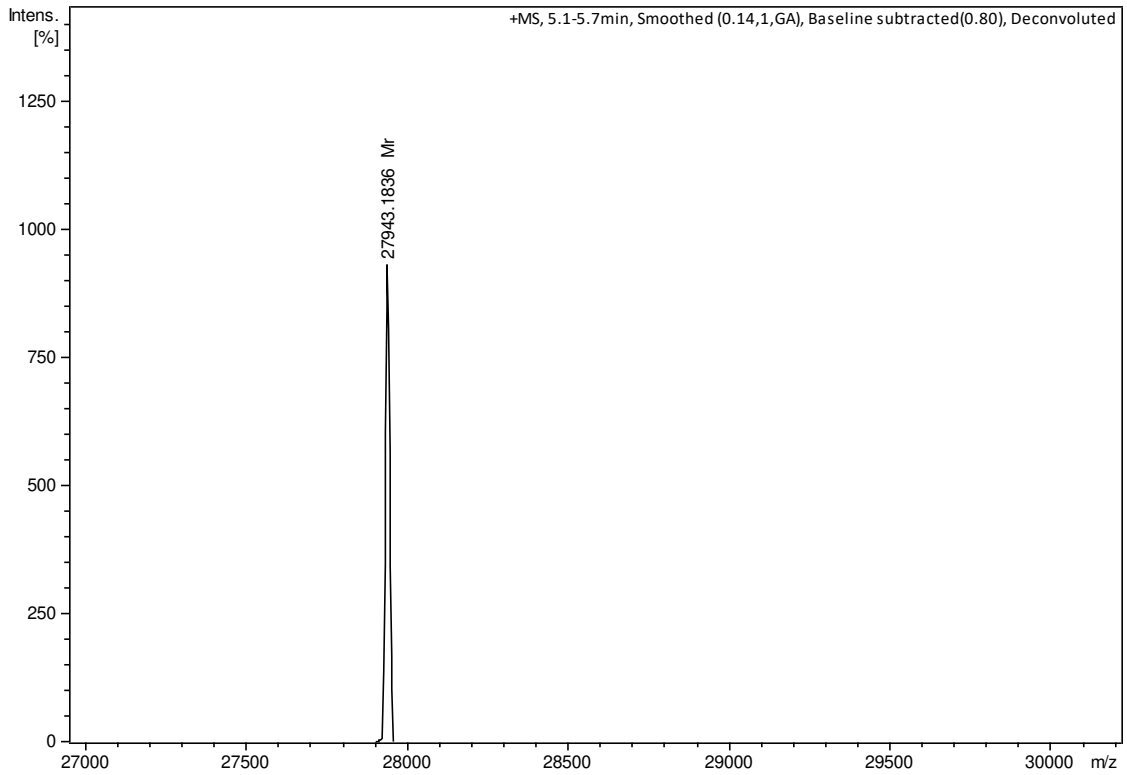
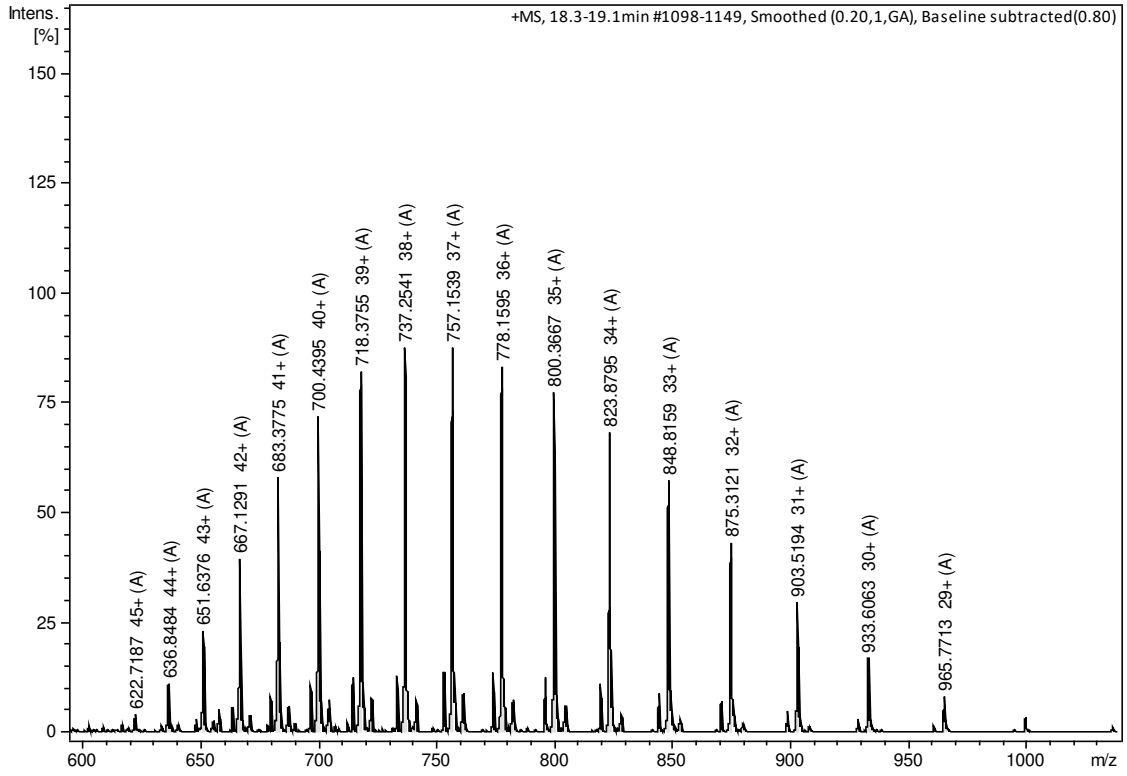


Figure S6. Mass Spectrum of **EmGFP-WT** showing the charged envelope (A) and deconvoluted spectrum (B).

(A)



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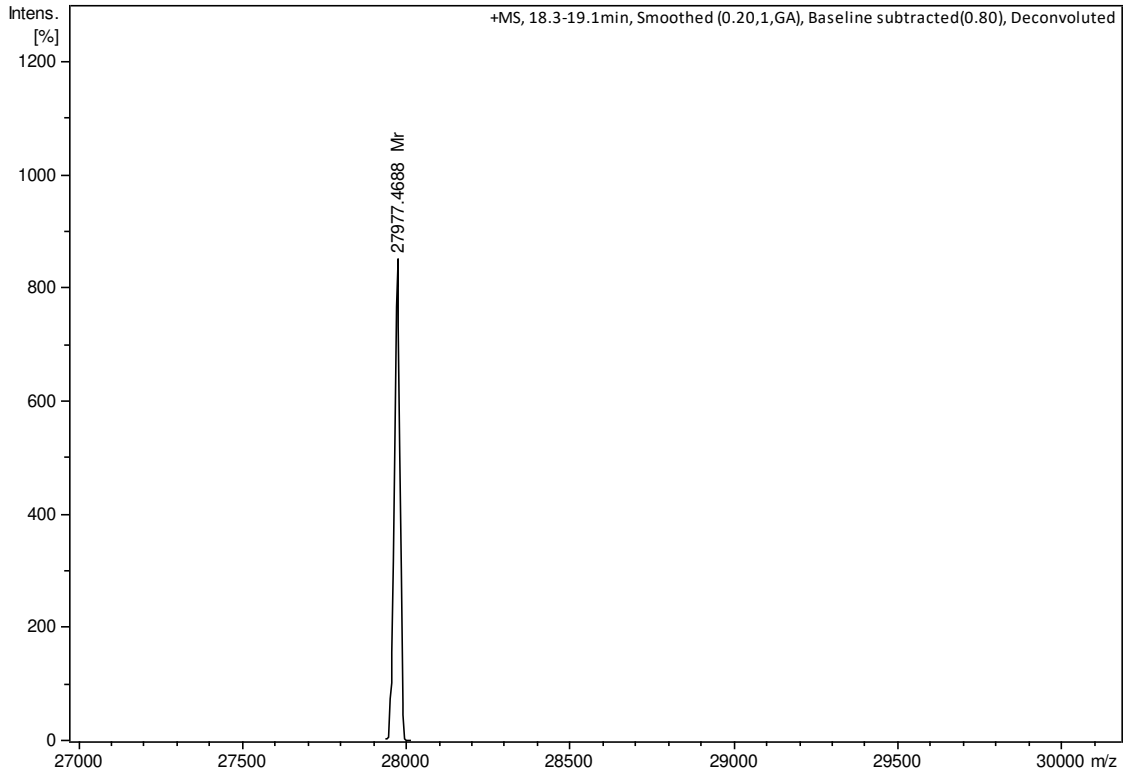
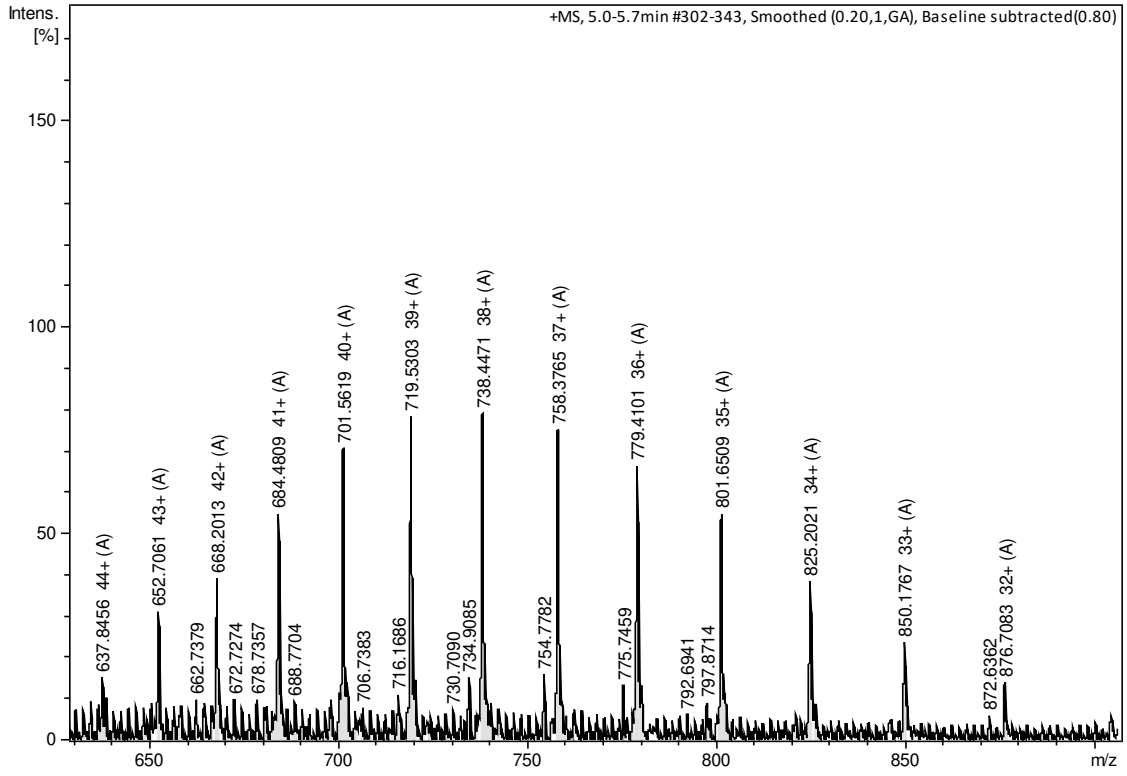


Figure S7. Mass Spectrum of **EmGFP-1TAG-3ClY** showing the multiply charged envelope (A) and deconvoluted spectrum (B).

(A)



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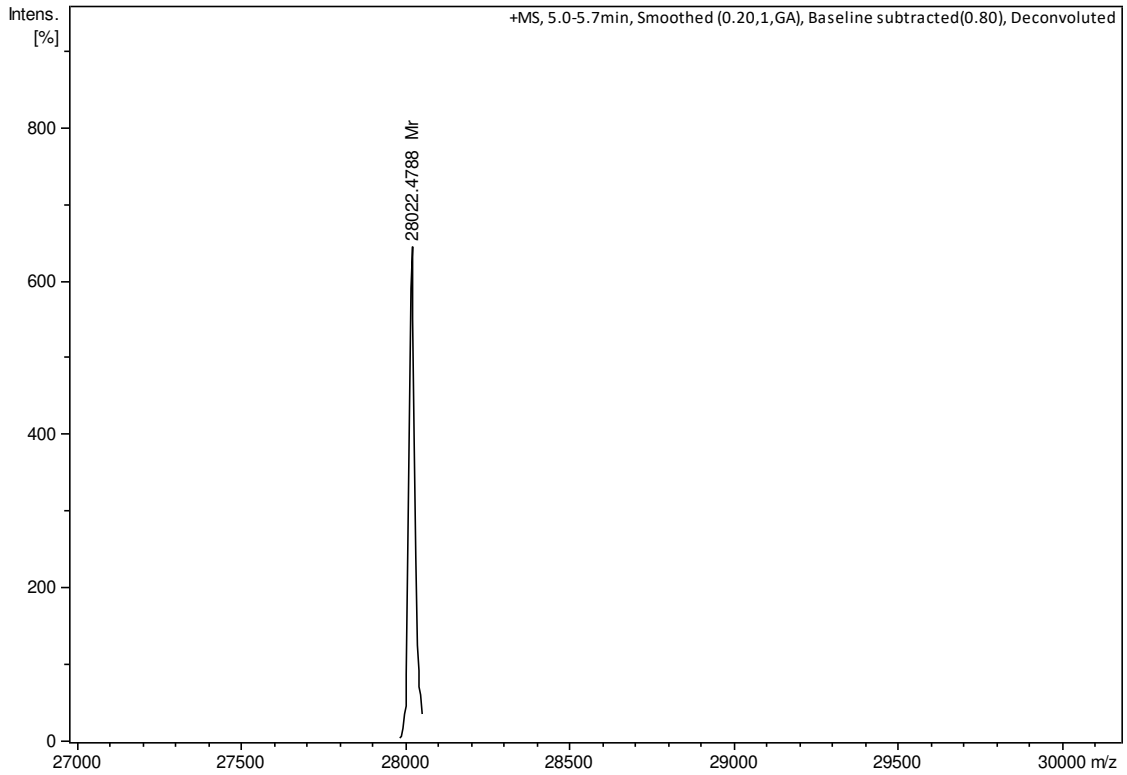
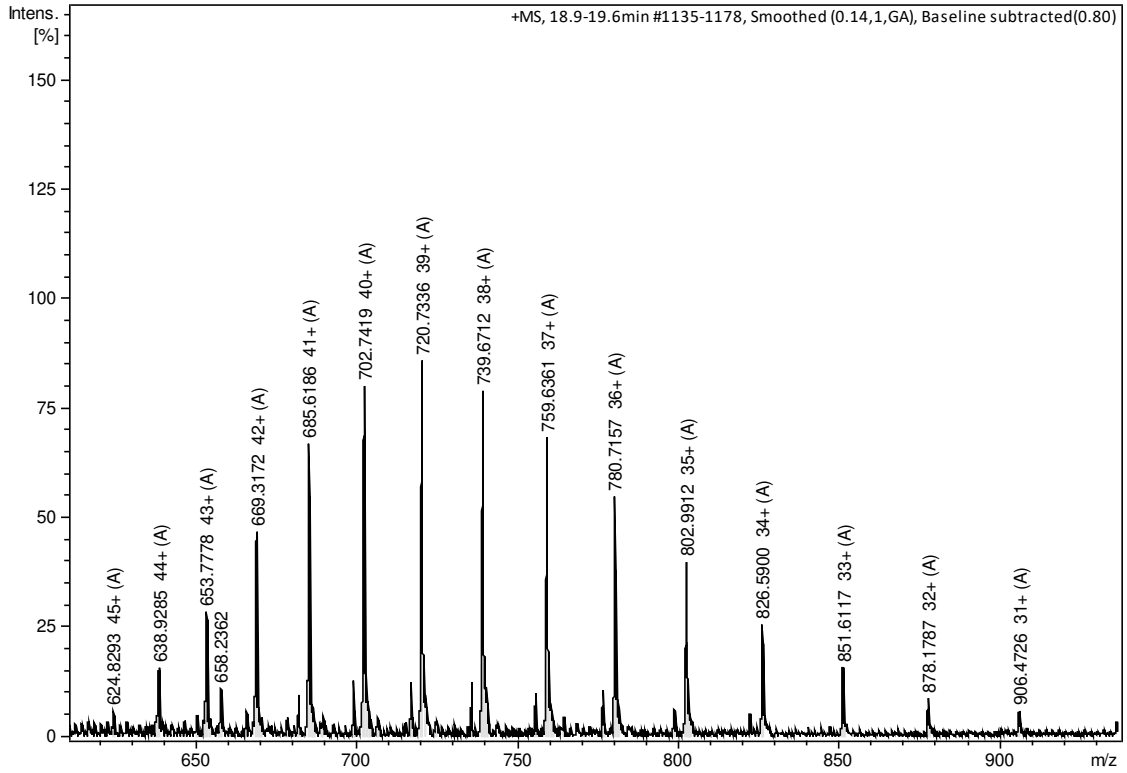


Figure S8. Mass Spectrum of **EmGFP-1TAG-3BrY** showing the multiply charged envelope (A) and deconvoluted spectrum (B).

(A)



(B)

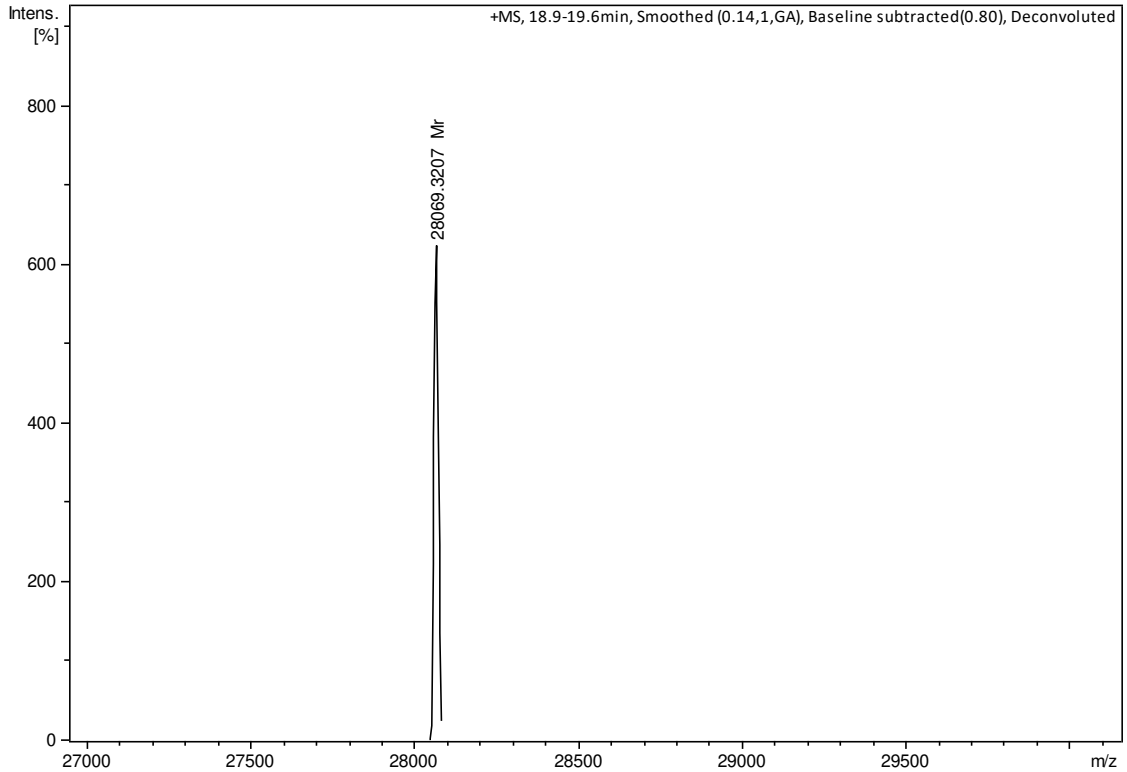
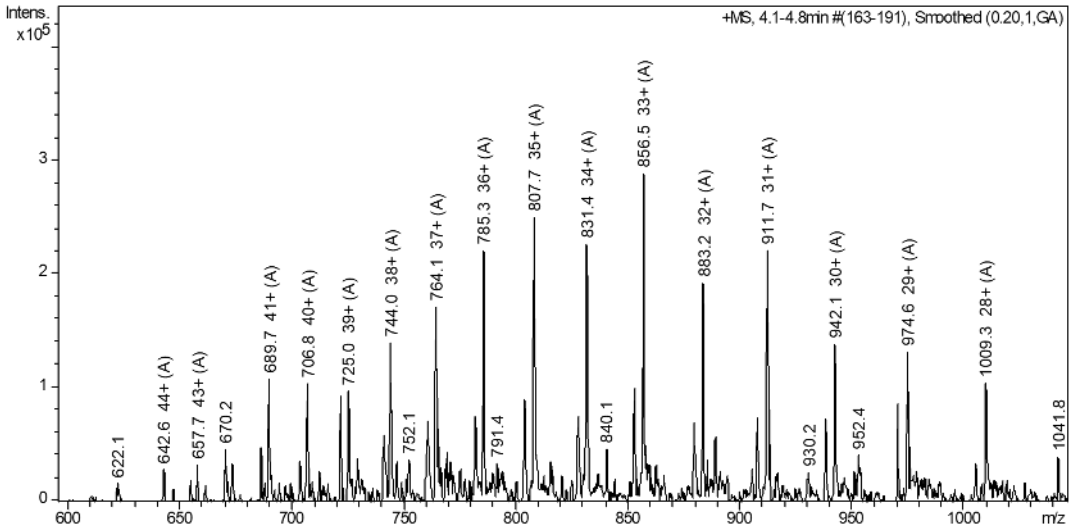


Figure S9. Mass Spectrum of **EmGFP-1TAG-3IY** showing charged envelope (A) and deconvoluted spectrum (B).

(A)



(B)

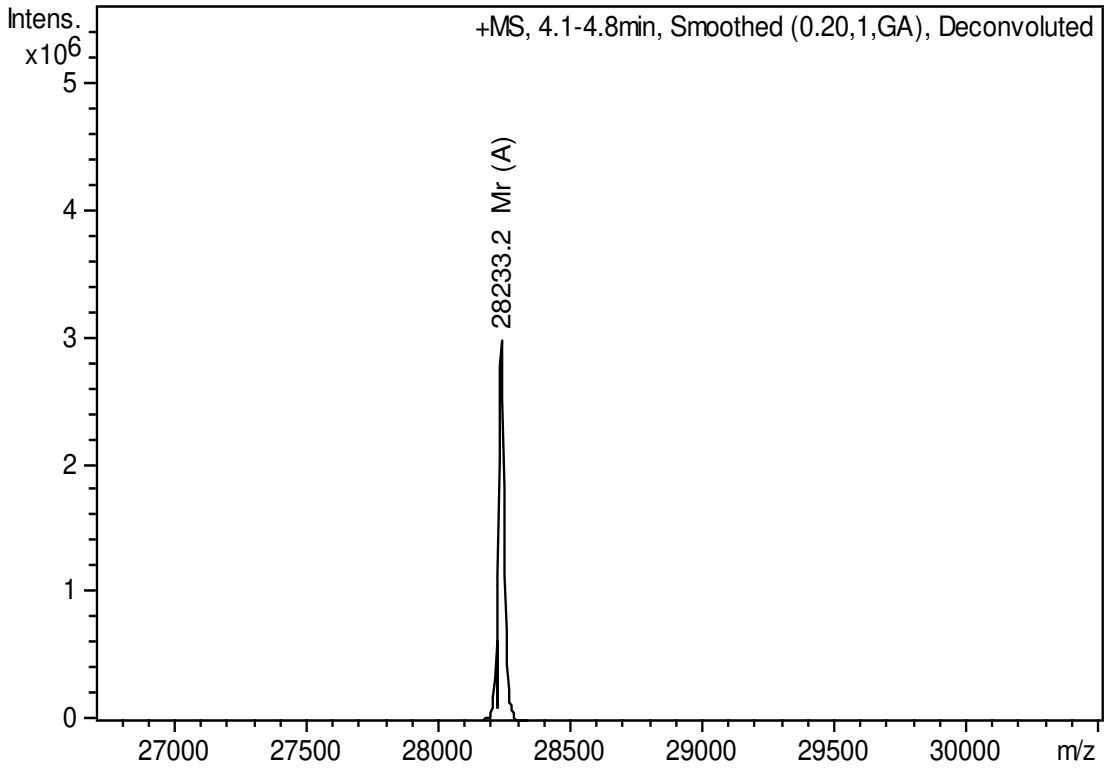


Figure S10: Mass Spectrum of **EmGFP-2TAG-3IY** showing charged envelope (A) and deconvoluted spectrum (B).

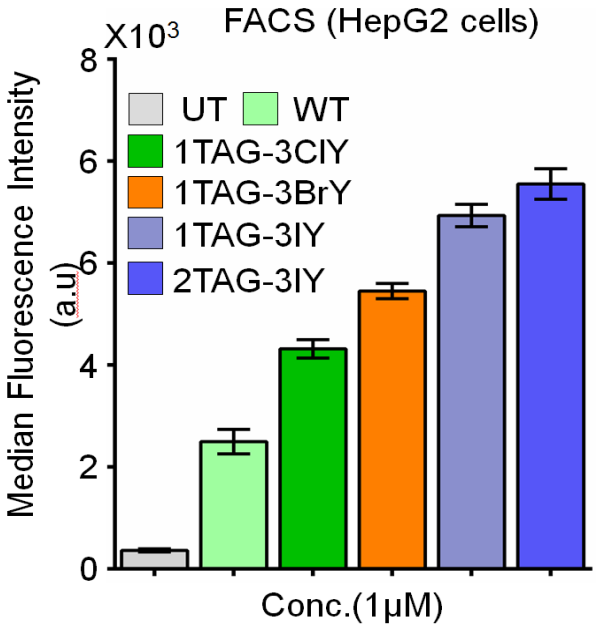
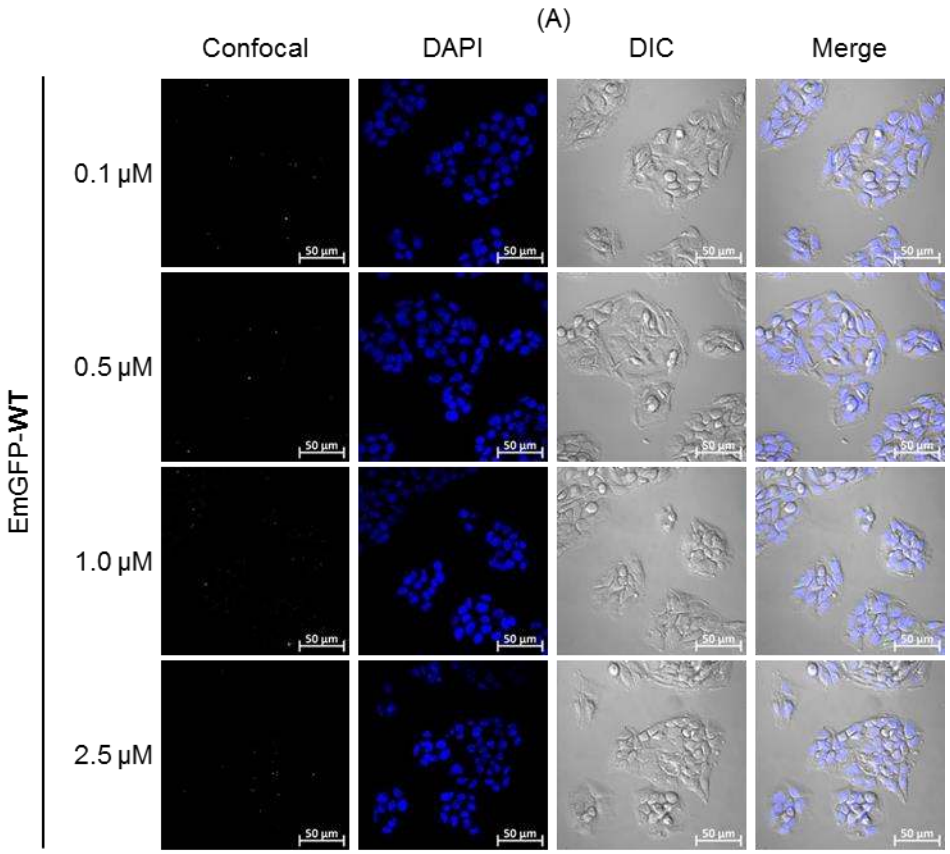
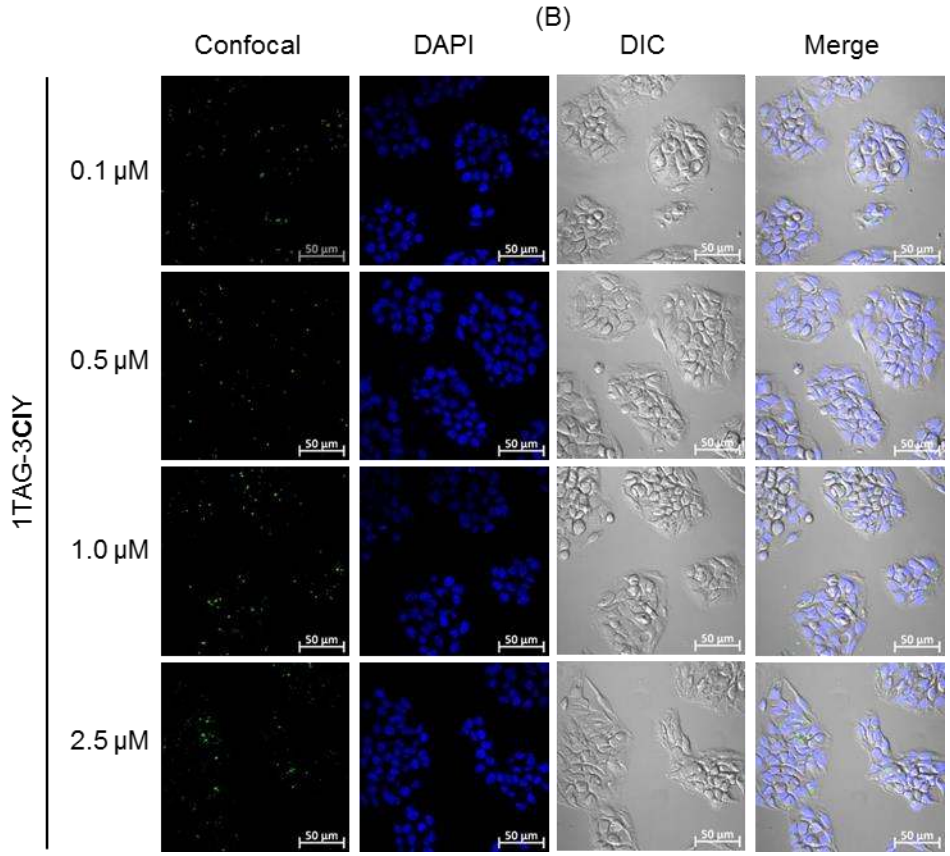


Figure S11: The fluorescence intensity measured by FACS after 90 min of treatment of HepG2 cells with 1 µM halo-EmGFP.


(A)
Confocal
DAPI
DIC
Merge
EmGFP-WT
0.1 μM
0.5 μM
1.0 μM
2.5 μM
50 μm


(B)
Confocal
DAPI
DIC
Merge
1TAG-3CIY
0.1 μM
0.5 μM
1.0 μM
2.5 μM
50 μm

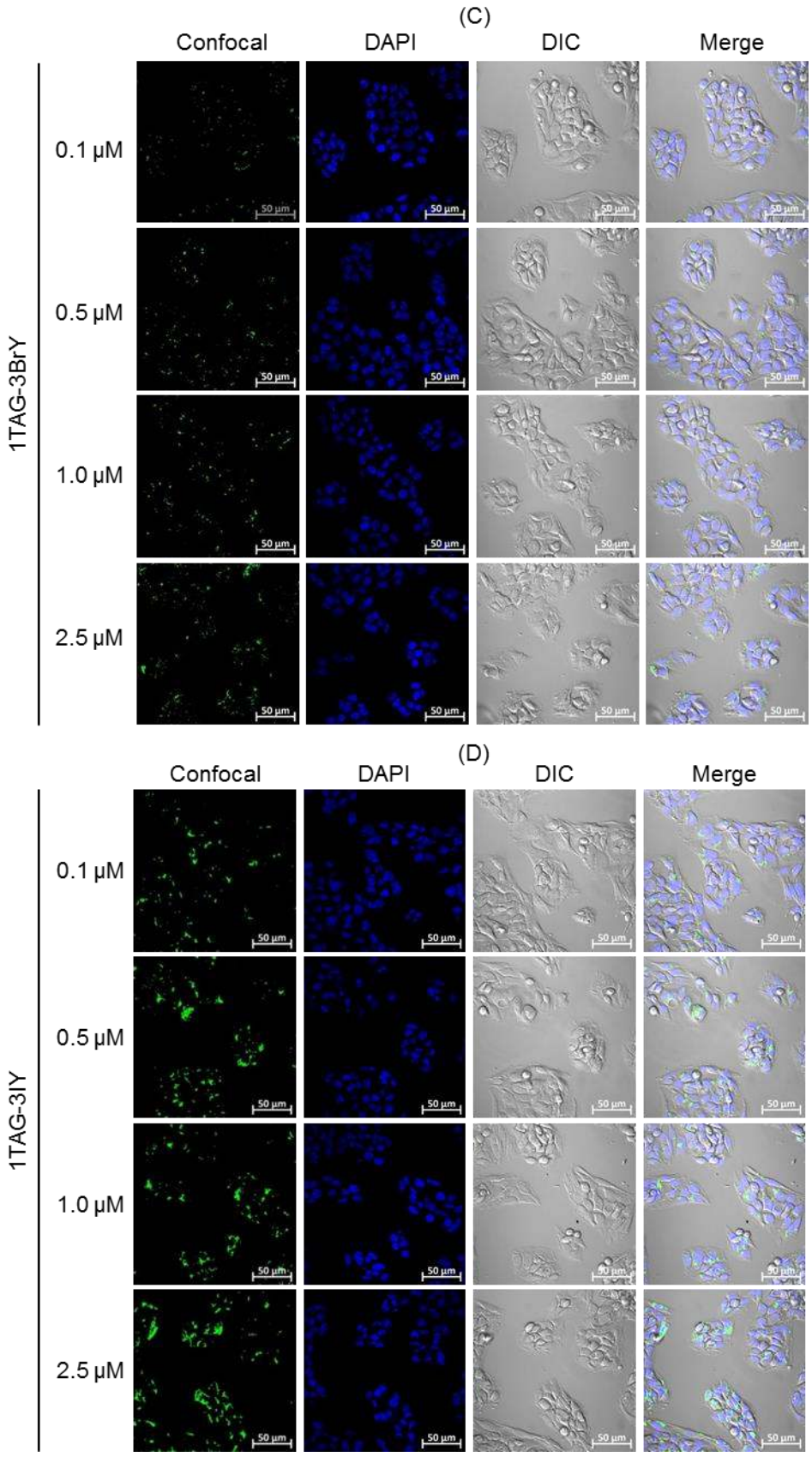


Figure S12: Confocal images of HepG2 cells treated with various concentrations of halo-EmGFP (1TAG) for 90 min.

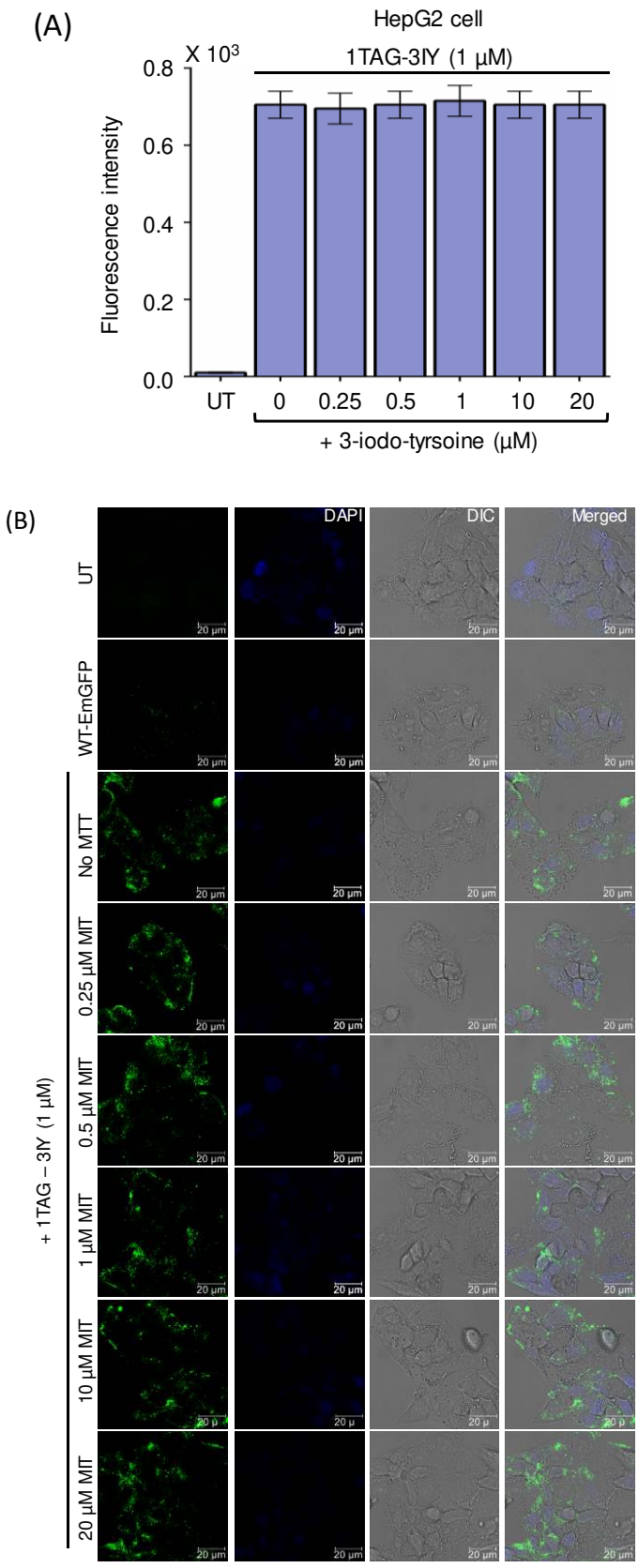
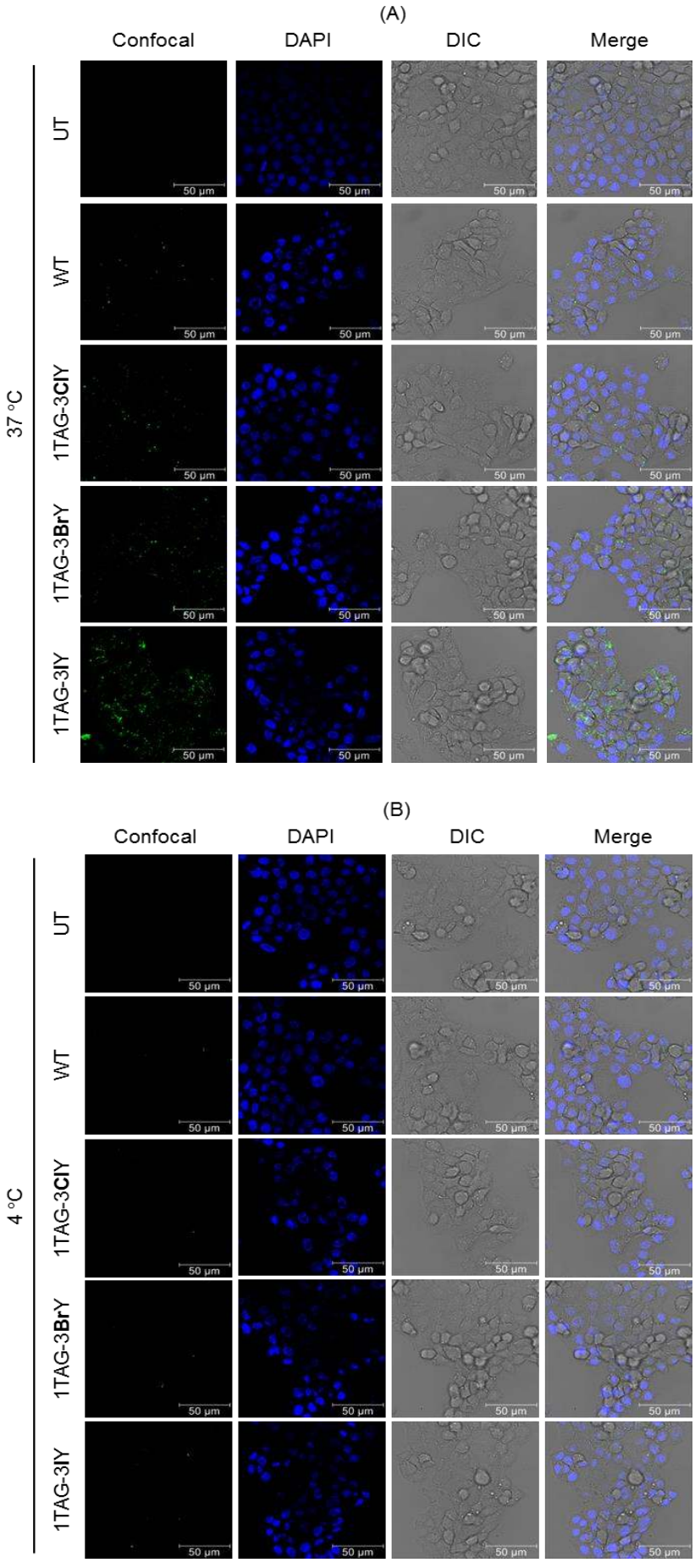
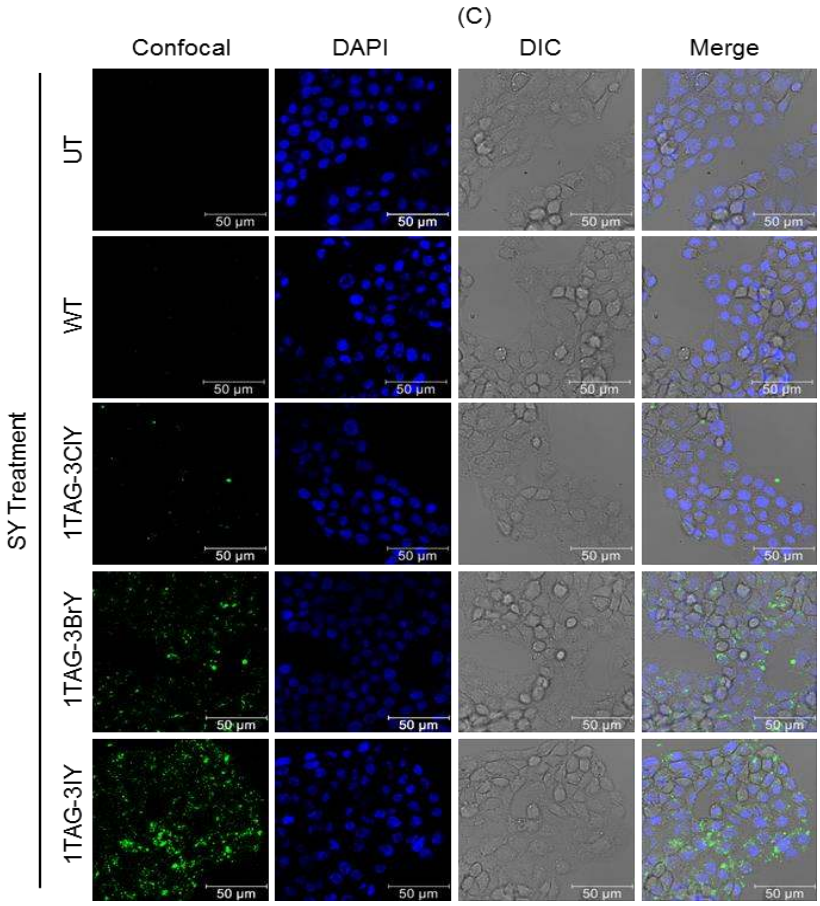
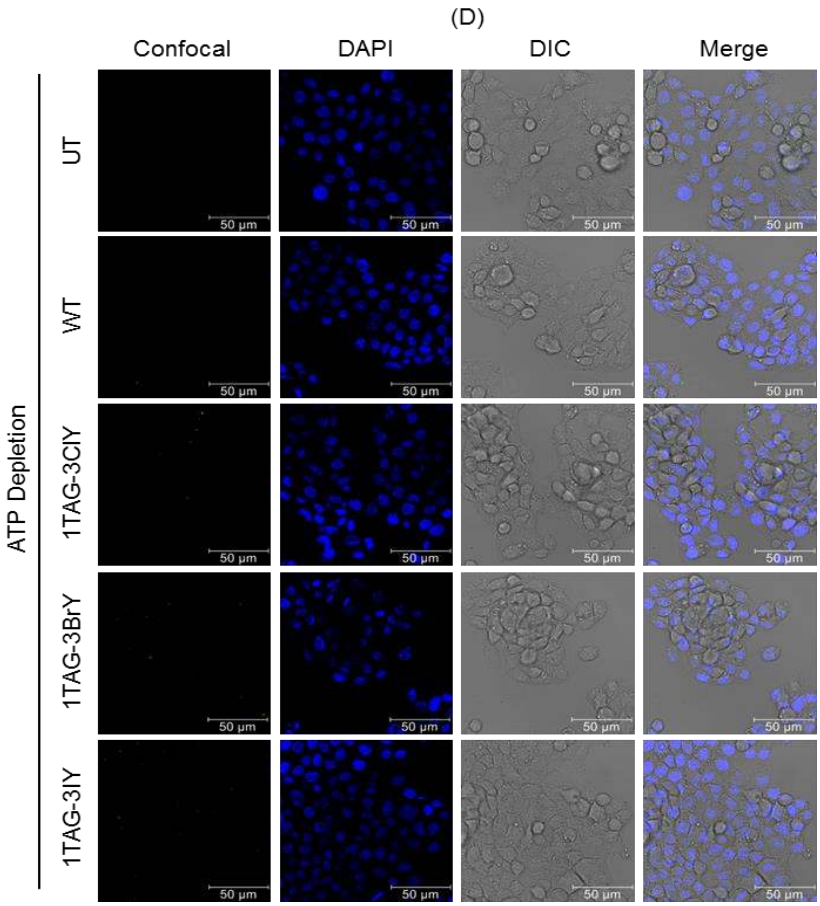
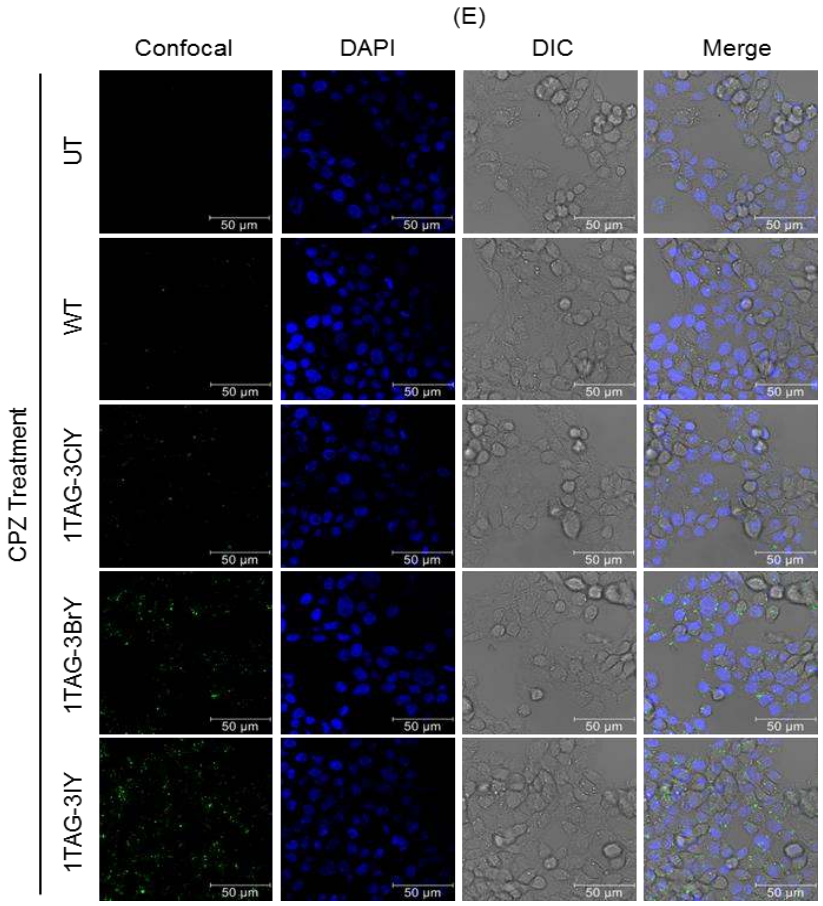


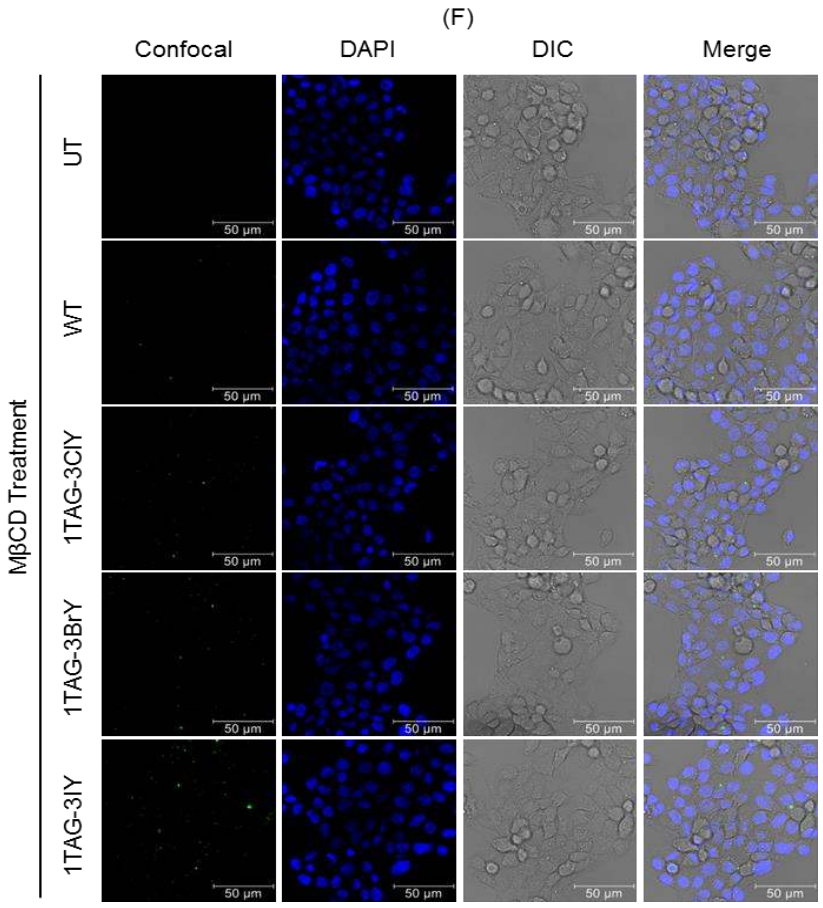
Figure S13: The fluorescence measured by a plate reader after 90 min treatment of HepG2 cells with 1 µM of 1TAG-3IY EmGFP at 37 °C after co-treatment with 3-iodo-L-tyrosine (MIT) (A) and the corresponding confocal images (B).


(A)
Confocal
DAPI
DIC
Merge
37 °C
UT
WT
1TAG-3ClY
1TAG-3BrY
1TAG-3IY
50 μm
(B)
Confocal
DAPI
DIC
Merge
4 °C
UT
WT
1TAG-3ClY
1TAG-3BrY
1TAG-3IY
50 μm


(C)
Confocal
DAPI
DIC
Merge
SY Treatment
UT
WT
1TAG-3ClY
1TAG-3BrY
1TAG-3IY
50 µm


(D)
Confocal
DAPI
DIC
Merge
ATP Depletion
UT
WT
1TAG-3ClY
1TAG-3BrY
1TAG-3IY
50 µm


(E)
Confocal
DAPI
DIC
Merge
CPZ Treatment
UT
WT
1TAG-3ClY
1TAG-3BrY
1TAG-3IY
50 µm


(F)
Confocal
DAPI
DIC
Merge
MβCD Treatment
UT
WT
1TAG-3ClY
1TAG-3BrY
1TAG-3IY
50 µm

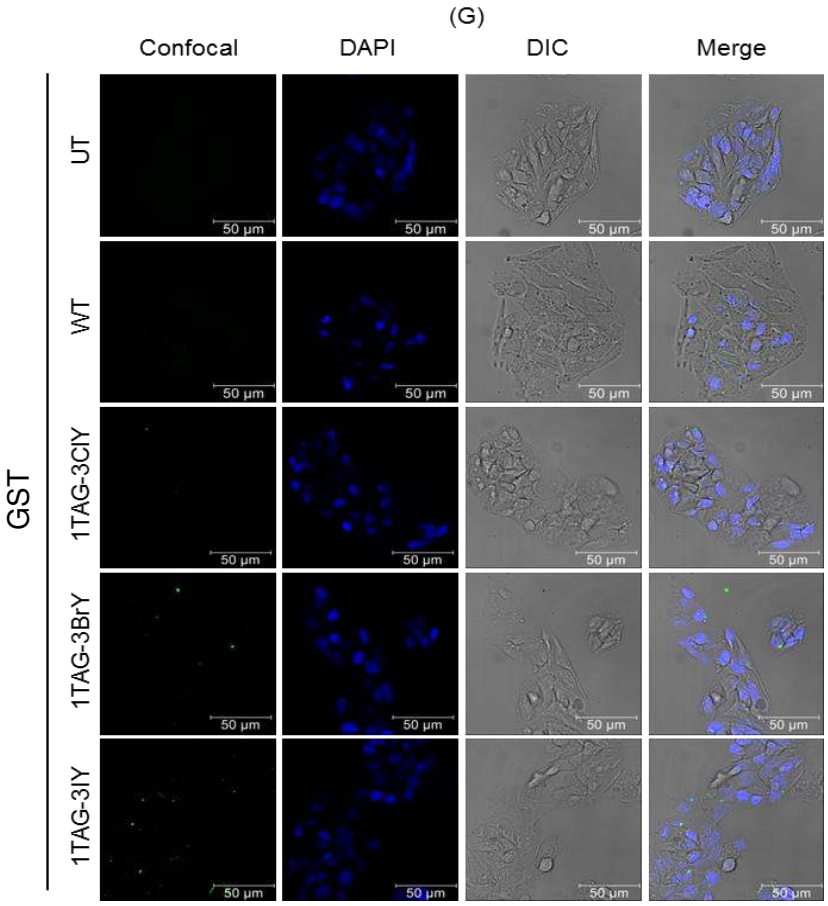


Figure S14: Confocal images of internalization and distribution of halo-EmGFP (1 µM) having 3-IY in positions 39 (1TAG) at 37 °C (A) (or) 4 °C (or) under ATP depletion (or) after pre-treatment with MCT8 inhibitor or endocytosis inhibitors in HepG2 cells.

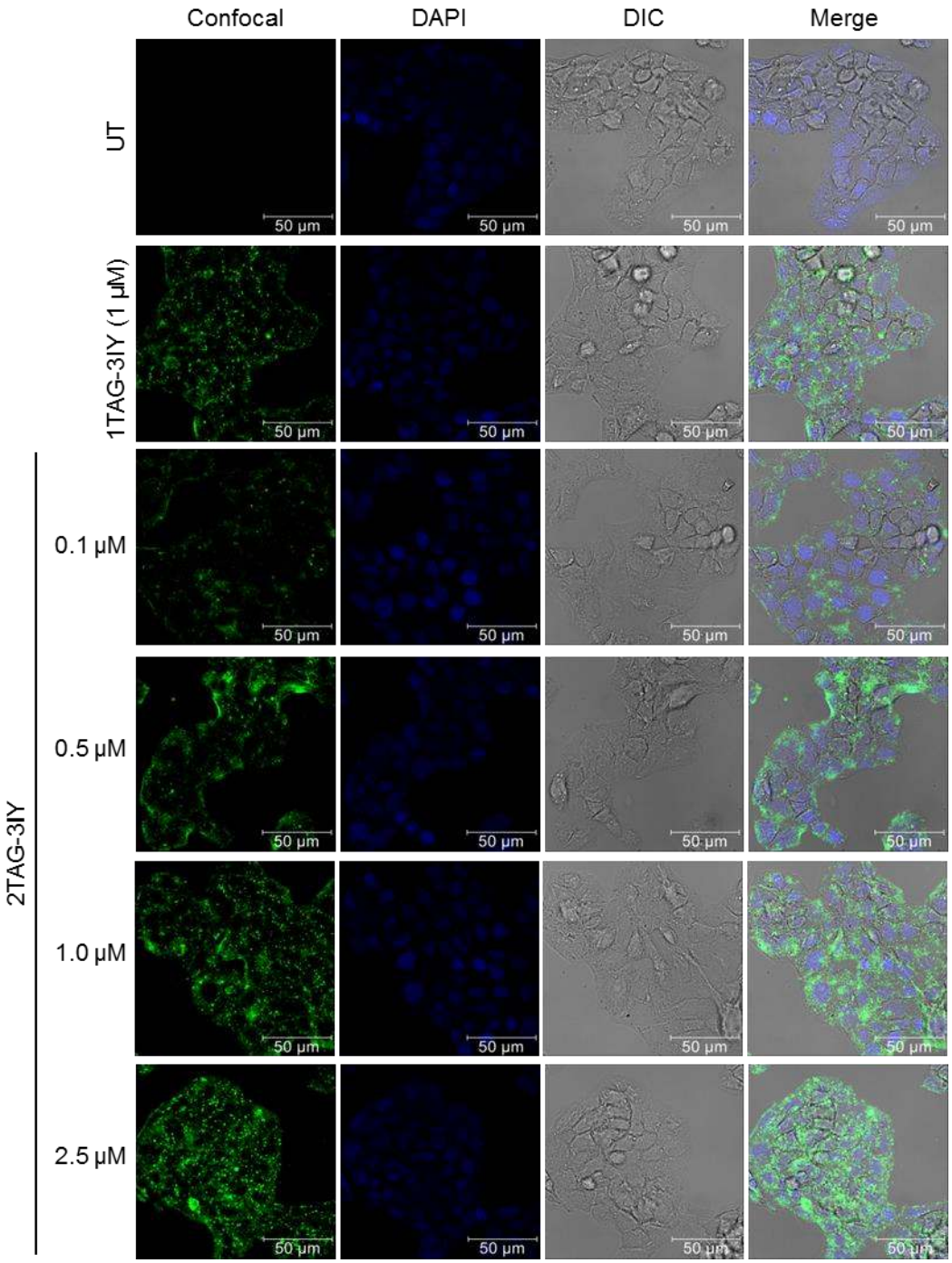
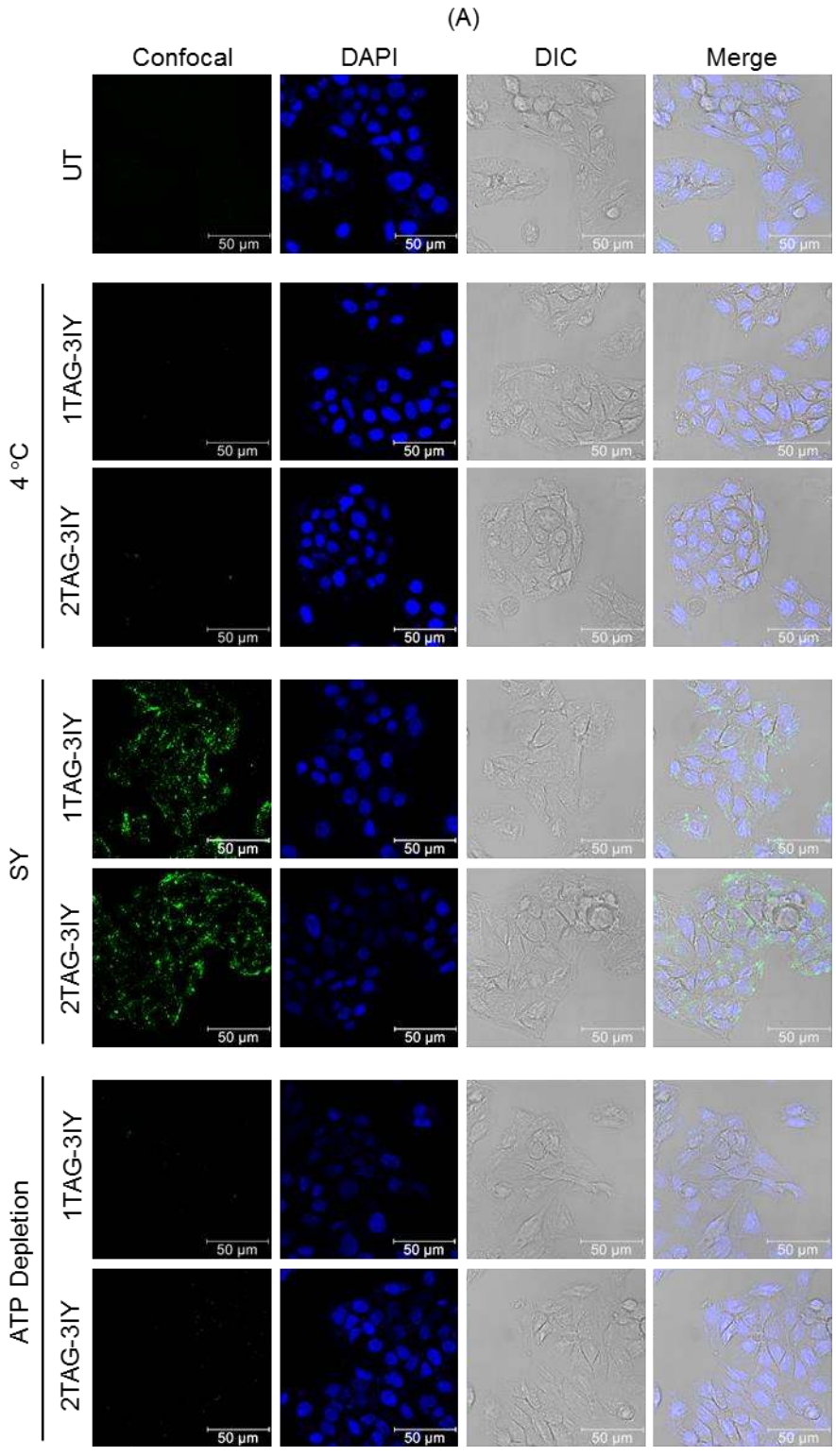


Figure S15: Confocal images of HepG2 cells treated with various concentrations of halo-EmGFP (2TAG) for 90 min.


(A)
Confocal
DAPI
DIC
Merge
UT
4 °C
1TAG-3IY
2TAG-3IY
SY
1TAG-3IY
2TAG-3IY
ATP Depletion
1TAG-3IY
2TAG-3IY
50 μm

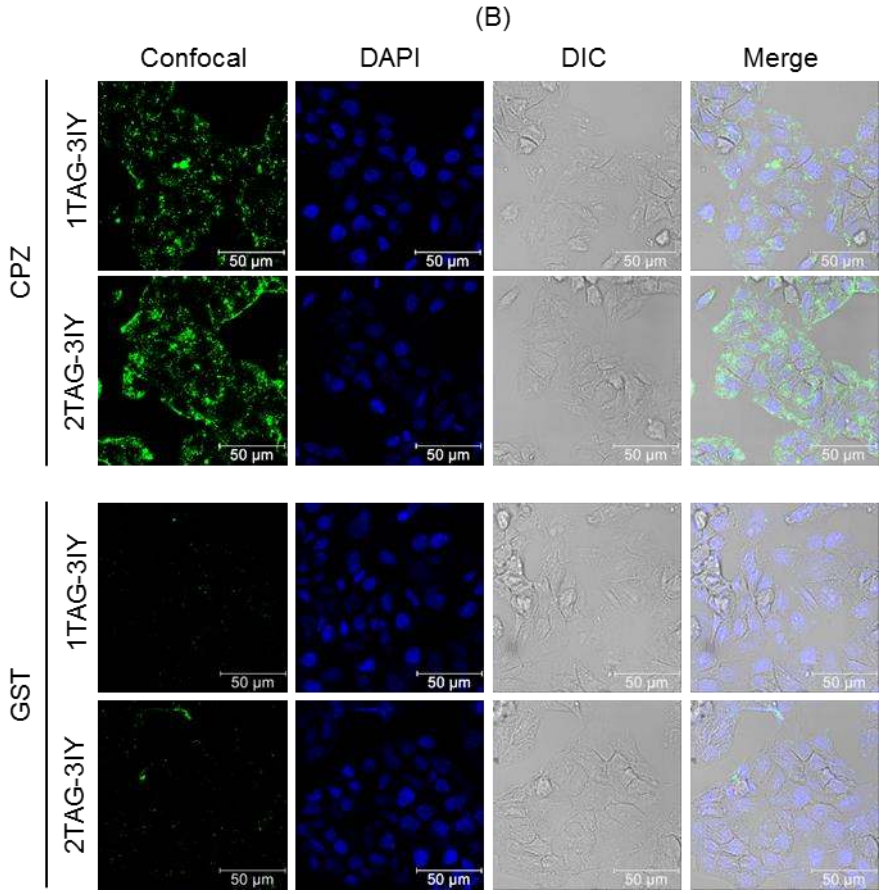
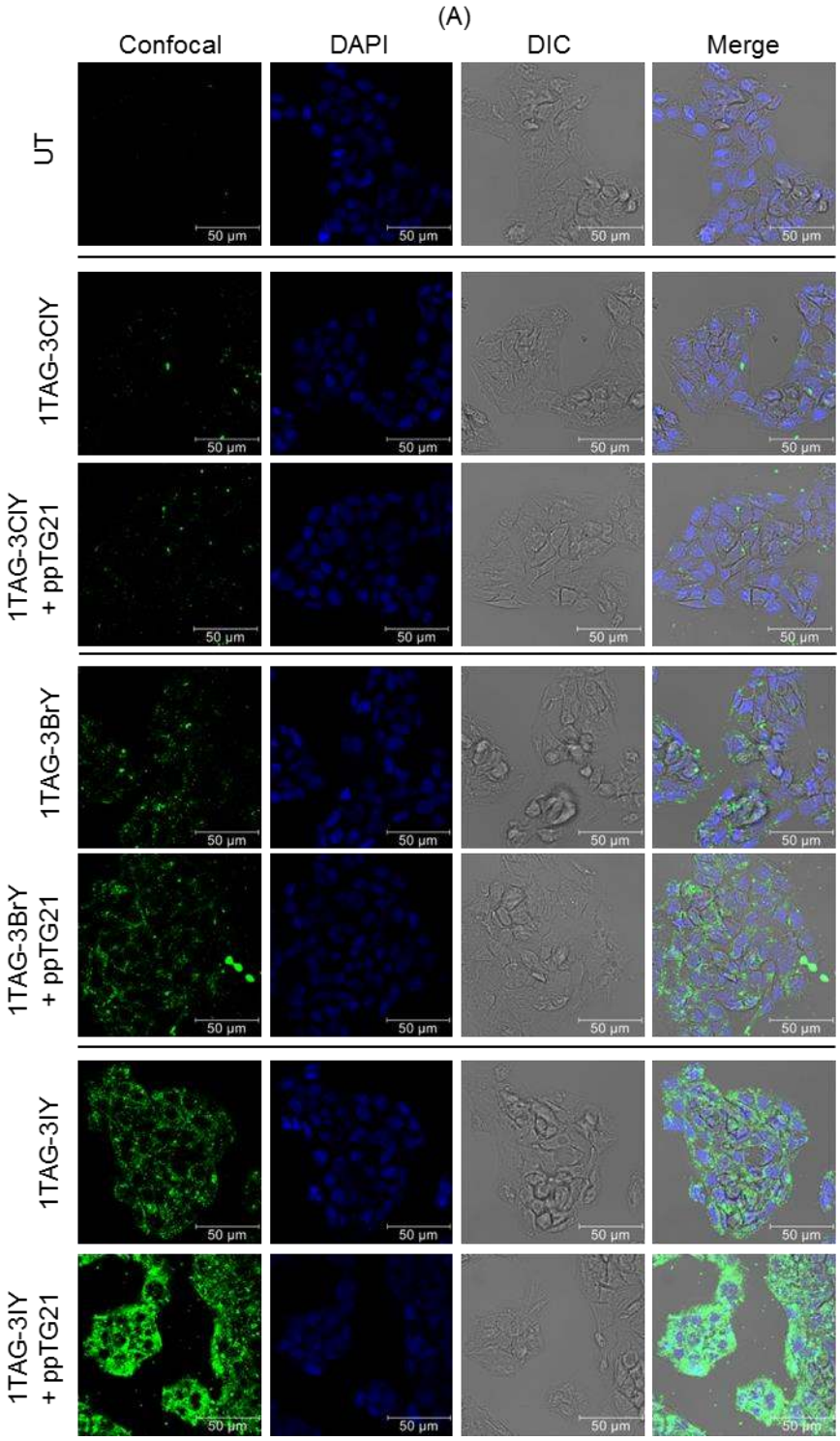


Figure S16: Confocal images of internalization and distribution of halo-EmGFP-2TAG (1 µM) at 37 °C (or) 4 °C (or) under ATP depletion (or) after pre-treatment with MCT8 inhibitor or endocytosis inhibitors in HepG2 cells (A & B).


(A)
Confocal
DAPI
DIC
Merge
UT
1TAG-3ClY
1TAG-3ClY + ppTG21
1TAG-3BrY
1TAG-3BrY + ppTG21
1TAG-3IY
1TAG-3IY + ppTG21
50 µm

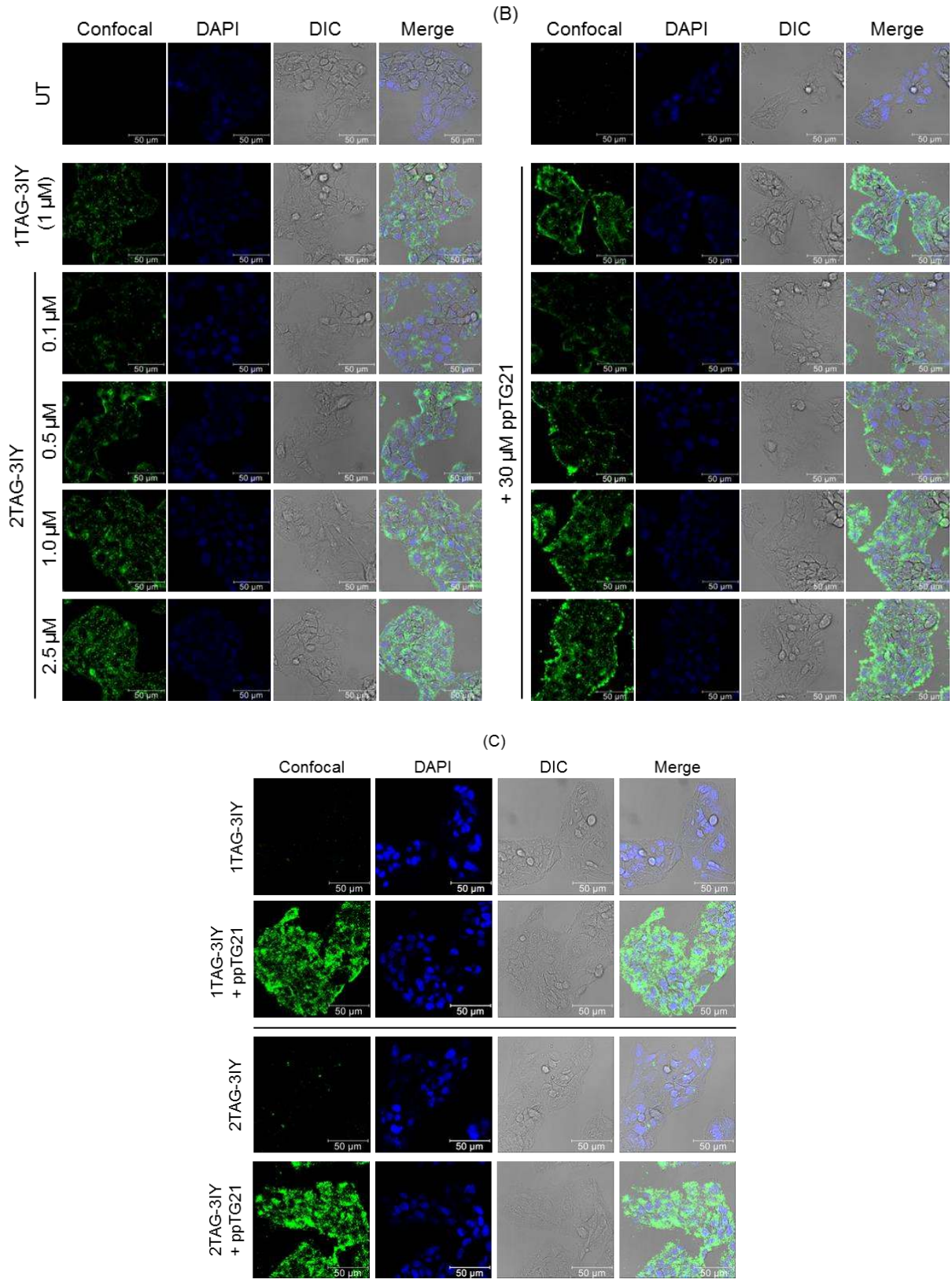


Figure S17: Confocal images of HepG2 cells after 90 min (A & B) or 24 h (C) of co-treatment with 1 µM halo-EmGFP and 30 µM ppTG21.

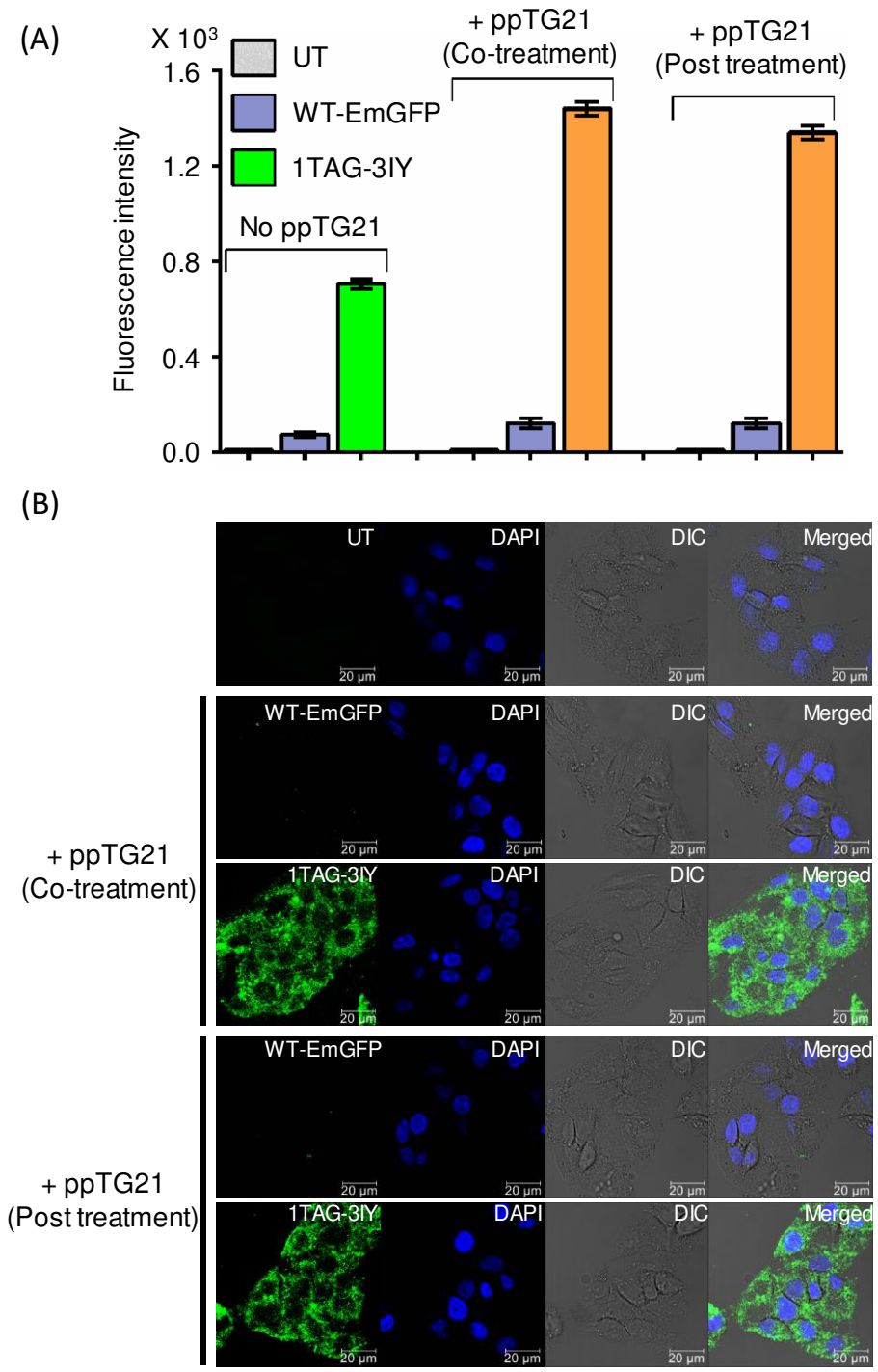


Figure S18: (A) The fluorescence measured by a plate reader after 60 min co-treatment of HepG2 cells with 1 µM of 1TAG-3IY EmGFP and 30 µM of ppTG21 at 37 °C. For the post-treatment of ppTG21, the cells were washed thoroughly with PBS buffer after 60 min and then treated with 30 µM ppTG21 for 30 min. (B) The confocal images obtained after 60 min co-treatment of HepG2 cells with 1 µM of 1TAG-3IY EmGFP and 30 µM of ppTG21 at 37 °C. For the post-treatment of ppTG21, the cells were washed thoroughly with PBS buffer after 60 min and then treated with 30 µM ppTG21 for 30 min. In all experiments, the cells were washed with PBS buffer containing heparin (1 mg/mL) to remove surface-bound proteins.

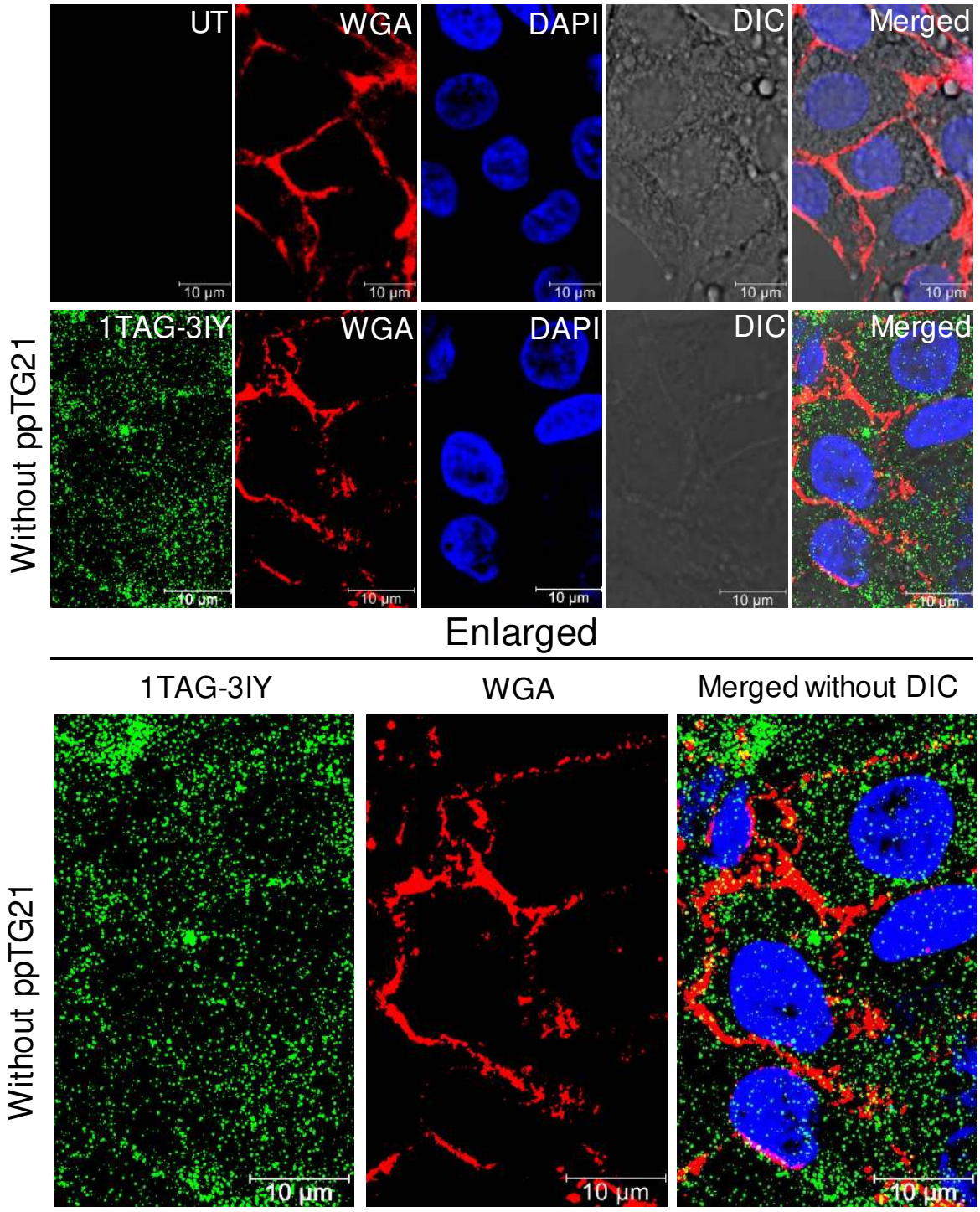


Figure S19: Confocal images of HepG2 cells after treatment with the plasma membrane tracker (wheat germ agglutinin, WGA, 5 µg/mL, 10 min incubation time) or 1 µM 1TAG-3IY-EmGFP for 60 min without ppTG21. In all experiments, the cells were washed with PBS buffer containing heparin (1 mg/mL) to remove surface-bound proteins.

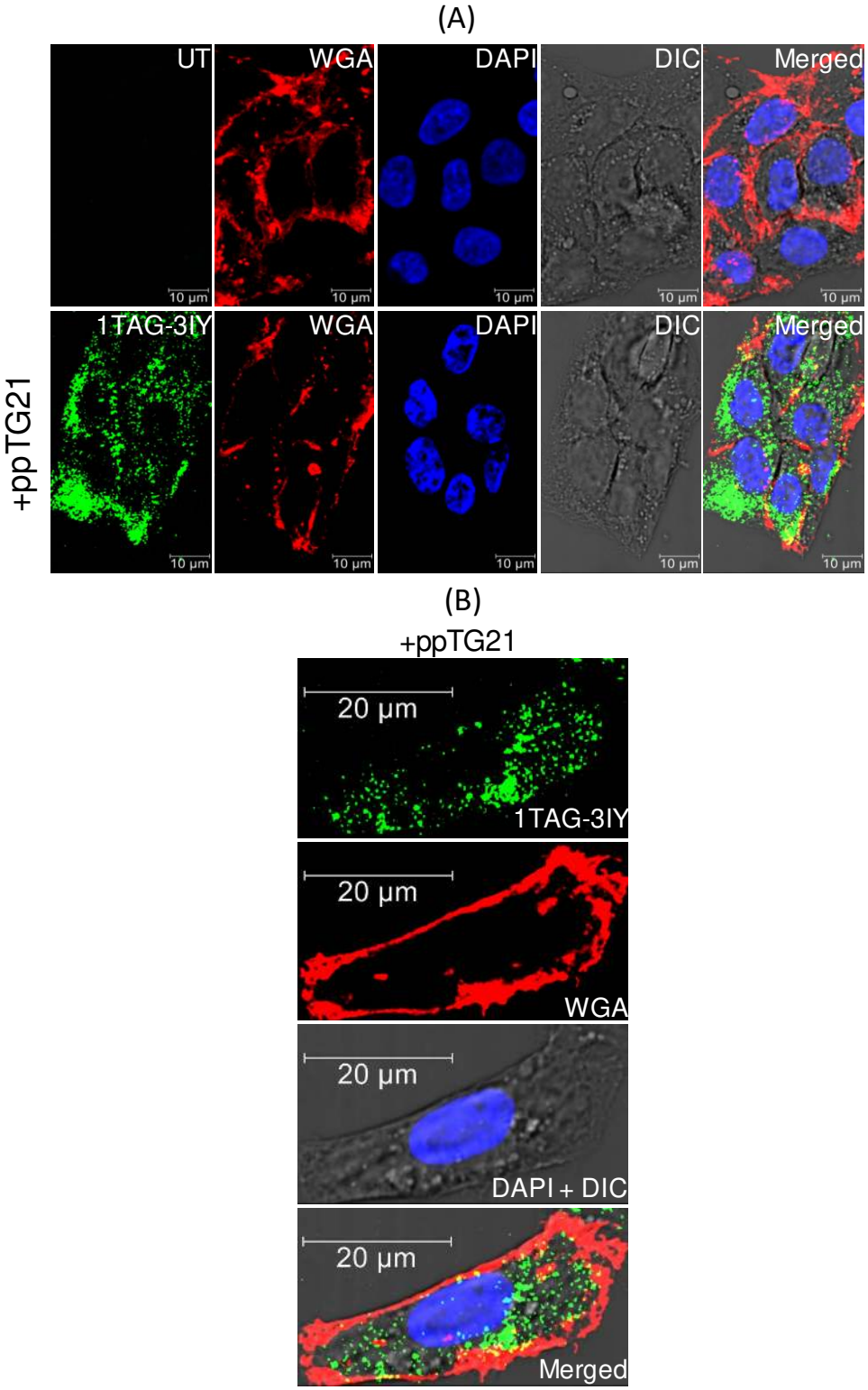


Figure S20: Confocal images of HepG2 cells after treatment with 1 µM 1TAG-3IY-EmGFP for 60 min, followed by washing with PBS buffer and then treatment with 30 µM ppTG21 for 30 min. For staining the plasma membrane and nucleus, wheat germ agglutinin (WGA, 5 µg/mL, 10 min incubation time) and DAPI (300 nM, 10 min incubation time), were used respectively. In all experiments, the cells were washed with PBS buffer containing heparin (1 mg/mL) to remove surface-bound proteins.

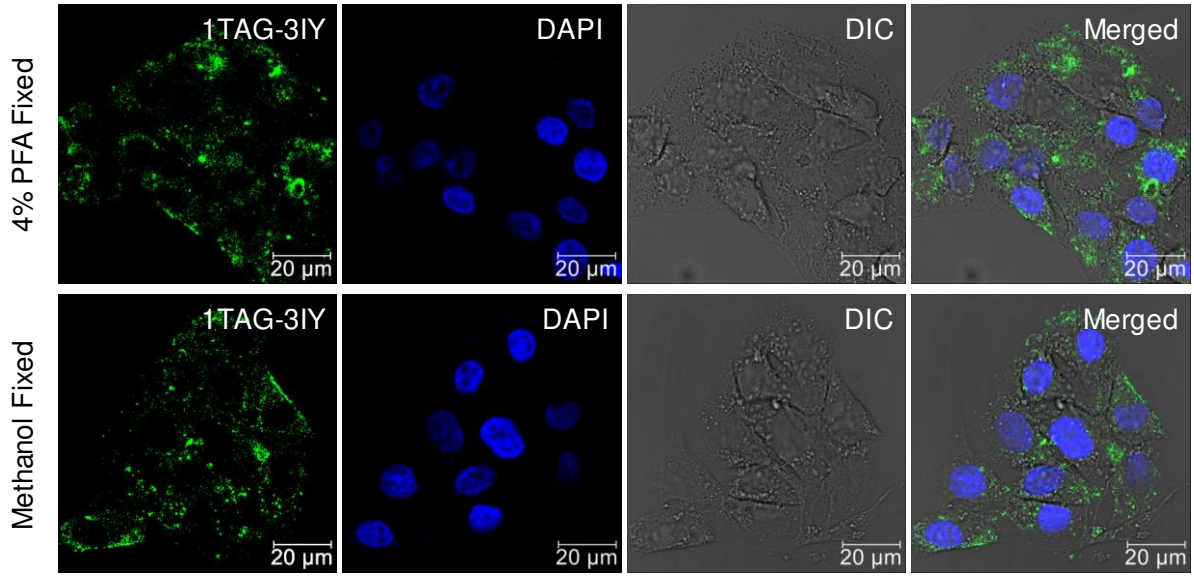


Figure S21: Confocal images of HepG2 cells after 90 min of treatment with 1 µM 1TAG-3IY-EmGFP. Fixing the cells with two different methods gave identical results.

3. References

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