

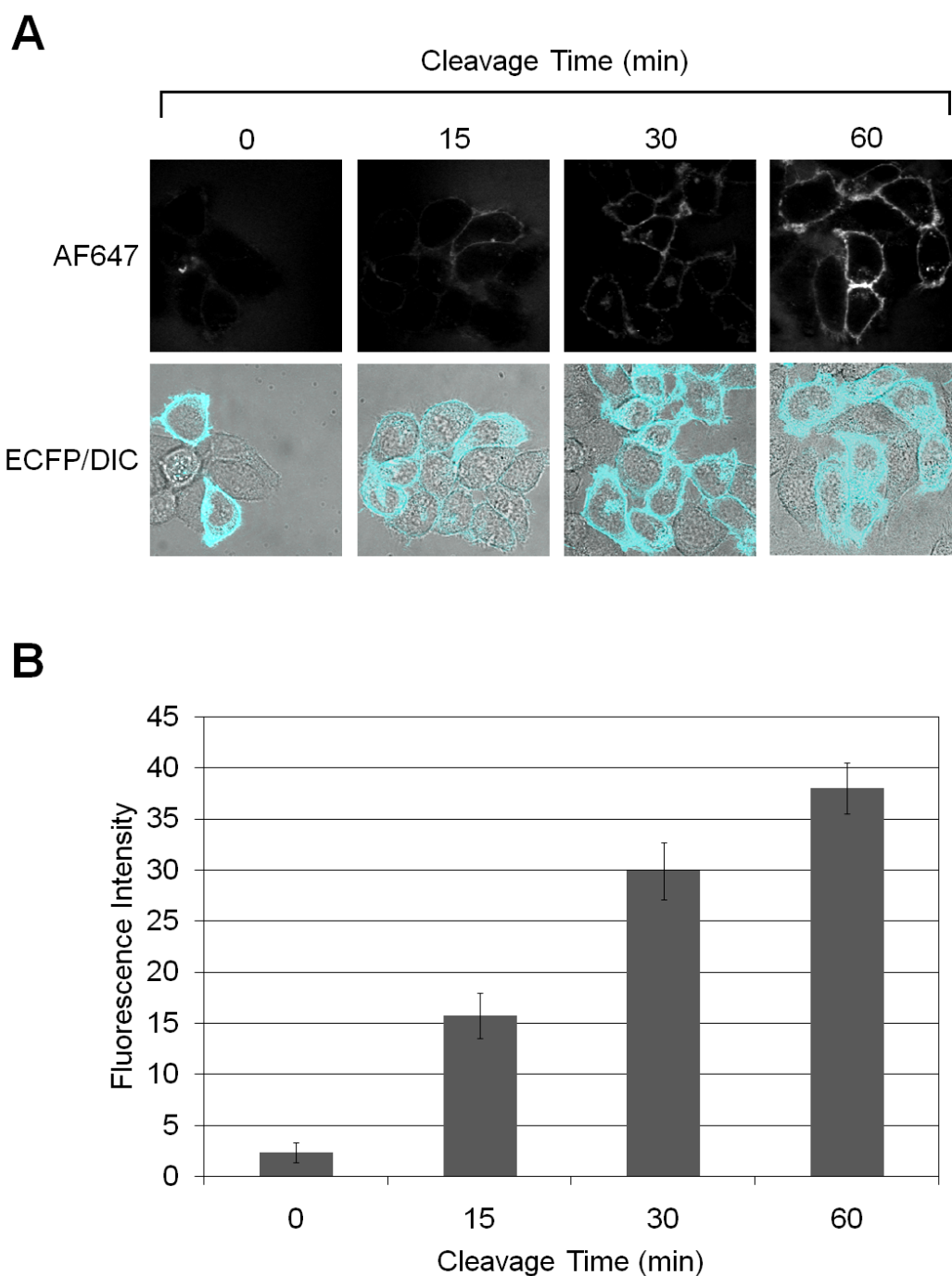
**Supplementary Information for**  
**Expansion of the sortase-mediated labeling method for site-specific**  
**N-terminal labeling of cell surface proteins on living cells**

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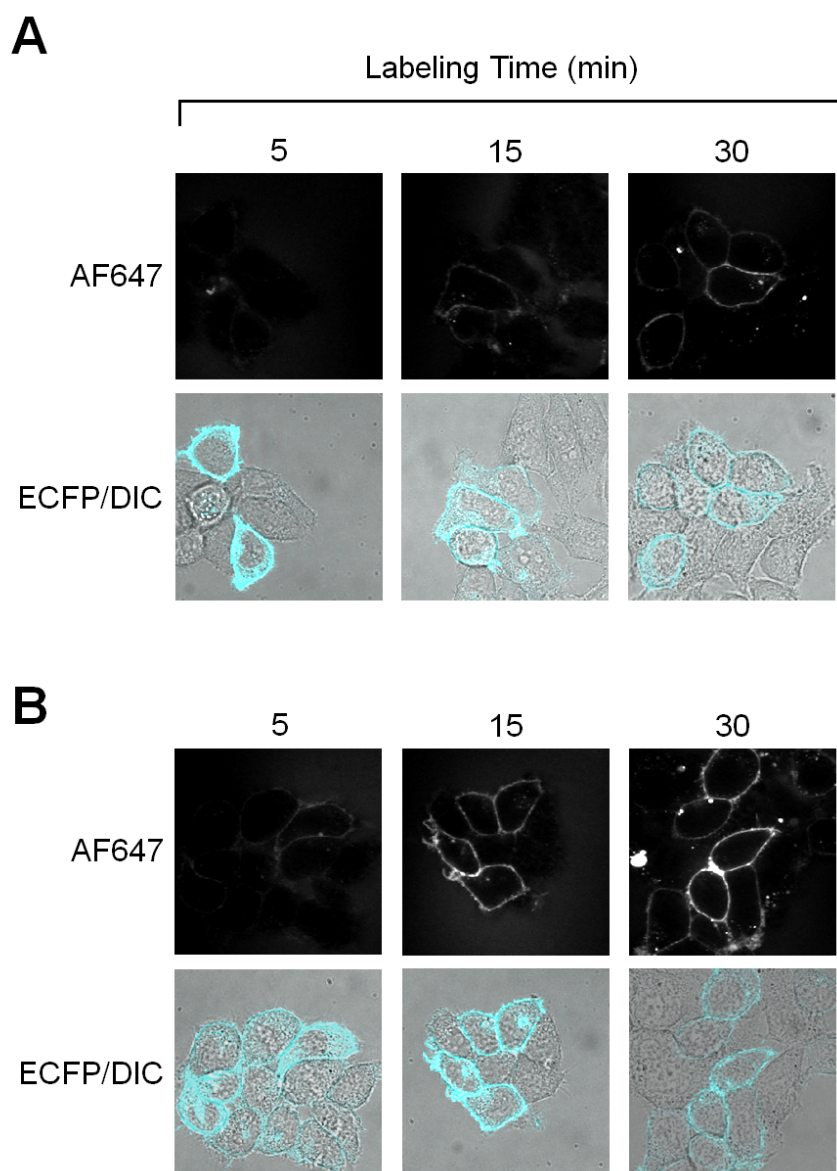
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**Fig. S1** Labeling of LPETG<sub>5</sub>-ECFP-TM with AF647-LPETGG with various cleavage times. HEK 293T cells transiently expressing LPETG<sub>5</sub>-ECFP-TM were preincubated with SrtA (30  $\mu$ M) and triglycine (1 mM) at 37°C for 15-60 min, and then labeled with SrtA (30  $\mu$ M) and AF647-LPETGG (10  $\mu$ M) at 37°C for 5 min. (A) Laser scanning confocal images of the cells. (B) Alexa Fluor 647-derived fluorescence signals on the surfaces of transfected cells obtained from confocal images (N = 10).



**Fig. S2** Labeling of LPETG<sub>5</sub>-ECFP-TM with AF647-LPETGG without the cleavage reaction. (A) Without the preincubation, HEK 293T cells transiently expressing LPETG<sub>5</sub>-ECFP-TM were labeled with SrtA (30 μM) and AF647-LPETGG (10 μM) at 37°C for 5-30 min. (B) Following preincubation with SrtA (30 μM) and triglycine (1 mM) at 37°C for 15 min, HEK 293T cells transiently expressing LPETG<sub>5</sub>-ECFP-TM were labeled with SrtA (30 μM) and AF647-LPETGG (10 μM) at 37°C for 5-30 min.

## Supplementary Methods

### Construction of expression plasmids

*Construction of expression plasmids for LPETG<sub>5</sub>-ECFP-TM.* A gene fragment encoding LPETG<sub>5</sub>-ECFP was amplified by PCR from ECFP-C1 (Clontech) using the following primers: forward primer, 5'-GCC GAA TTC GAC AGA TCT CTG CCG GAA ACT GGT GGC GGT GGC GGT TCT GGA GTG AGC AAG GGC GAG GAG CTG TTC-3'; reverse primer, 5'-CGT GGA TCC GAA CTG CAG GCC CTT GTA CAG CTC GTC CAT GCC GAG AGT GAT CCC GGC-3'. The resultant gene fragment was subcloned into pBlueScript II SK(-) (Stratagene) with EcoRI/BamHI double digestion and sequenced with a CEQ8000 (Beckman Coulter). The gene fragment was subcloned into the expression vector pDisplay Vector (Invitrogen) with BglIII/PstI double digestion. An *E. coli* strain, XL-10 Gold (Stratagene), was used as the bacterial host for construction of the plasmid. All cloning enzymes were obtained from Takara Biomedical and used according to the manufacturer's instructions.

*Construction of expression plasmids for LPETA<sub>5</sub>-ECFP-TM.* Except for the forward primer sequence, the expression plasmids for LPETA<sub>5</sub>-ECFP-TM were constructed using the same procedure as that described above for LPETG<sub>5</sub>-ECFP-TM. The forward primer was replaced with 5'-GCC GAA TTC GAC AGA TCT CTG CCG GAA ACT GCC GCT GCC GCC GCC TCT GGA GTG AGC AAG GGC GAG GAG CTG TTC-3'.

### Expression and purification of His<sub>6</sub>-SrtA

In all experiments, SrtA was used as a His-tag fusion (His<sub>6</sub>-SrtA). Overproduction and purification of His<sub>6</sub>-SrtA were performed as described previously.<sup>1</sup>

### Preparation of AF647-LPETGG and biotin-LPETGG

*Peptide synthesis.* The peptides H-LPETGG-NH<sub>2</sub> and H-YGLPETGG-NH<sub>2</sub> were manually synthesized on Rink Amide Resin (Watanabe Chemical Industries) by standard Fmoc-based solid-phase peptide synthesis protocols. Fmoc-Tyr(tBu)-OH, Fmoc-Leu-OH, Fmoc-Pro-OH, Fmoc-Glu(OtBu)-OH, Fmoc-Thr(tBu)-OH and Fmoc-Gly-OH (Watanabe Chemical Industries) were used as building blocks. Fmoc deprotection was carried out with 20% piperidine in DMF, and the coupling reactions were carried out in DMF with 4 equiv of Fmoc-amino acid, 4 equiv of *N,N*-diisopropylcarbodiimide and 4 equiv of 1-hydroxybenzotriazole. Following chain assembly, peptide cleavage from the resin and side-chain deprotection were performed by treatment with TFA containing 2.5% triisopropylsilane and 2.5% H<sub>2</sub>O for 3 h at room temperature. The crude peptide products were precipitated by Et<sub>2</sub>O and purified by reversed-phase HPLC using a semipreparative YMC-Pack ODS-A column with a linear gradient of 0.1% aqueous TFA and CH<sub>3</sub>CN containing 0.1% TFA. The peptides were identified by MALDI-TOF-MS (matrix: CHCA). *m/z* for H-LPETGG-NH<sub>2</sub>: calcd for [M+H]<sup>+</sup> 572.30,

obsd 572.86;  $m/z$  for H-YGLPETGG-NH<sub>2</sub>: calcd for [M+H]<sup>+</sup> 792.39, obsd 792.96.

*Synthesis of AF647-LPETGG and biotin-LPETGG.* The purified peptides H-LPETGG-NH<sub>2</sub> and H-YGLPETGG-NH<sub>2</sub> were reacted with 0.5 equiv of Alexa Fluor 647 carboxylic acid, succinimidyl ester (Molecular Probes) and 0.5 equiv of NHS-PEO<sub>4</sub>-Biotin (Pierce), respectively, in anhydrous DMSO with 4 equiv of *N,N*-diisopropylethylamine for 3 h, and purified by reversed-phase HPLC. The peptides were identified by MALDI-TOF MS (matrix: CHCA).  $m/z$  for AF647-LPETGG: calcd for M<sup>-</sup> 1411, obsd 1411.18;  $m/z$  for biotin-LPETGG: calcd for M<sup>-</sup> 1264.60, obsd 1264.32.

### **Cell culture and plasmid transfection**

HEK 293T cells were cultured in Dulbecco's modified Eagle's medium (DMEM; Sigma) containing 10% fetal bovine serum at 37°C under 6% CO<sub>2</sub>. Plasmid transfection was performed using Lipofectamine reagent (Invitrogen) and PLUS reagent (Invitrogen) according to the manufacturer's instructions.

### **Immunofluorescence staining for evaluating the cleavage efficiency of the LPETG5 tags in LPETG5-ECFP-TM on cell surfaces**

*Preparation of an Alexa Fluor 647-labeled anti-HA antibody.* A rabbit anti-HA antibody (Bethyl Laboratories) was labeled with Alexa Fluor 647 using an Alexa Fluor 647 Monoclonal Antibody Labeling Kit (Molecular Probes) according to the manufacturer's instructions. Labeling with 3.7 moles of Alexa Fluor 647 per mole of antibody was accomplished.

*Immunofluorescence staining.* HEK 293T cells were transfected with the LPETG<sub>5</sub>-ECFP-TM plasmid as described above. Approximately 18-24 h after transfection, the cleavage reaction was performed by incubating the cells in serum-containing DMEM with 30 μM His<sub>6</sub>-SrtA and 1 mM triglycine (Sigma) for 15-60 min at 37°C. The cells were washed with phosphate-buffered saline (PBS) and incubated with 1.9 μg/ml Alexa Fluor 647-labeled anti-HA antibody at room temperature for 20 min. After three washes with PBS, the cells were observed in PBS using an LSM510 confocal laser-scanning microscope (Carl Zeiss MicroImaging) with a 63× oil-immersion objective. The 458-nm line of an argon laser and 633-nm line of a helium-neon laser were used for collecting the fluorescence images of ECFP and Alexa Fluor 647, respectively.

### **Labeling of LPETG5-ECFP-TM with AF647-LPETGG on cell surfaces**

HEK 293T cells were transfected with the LPETG<sub>5</sub>-ECFP-TM plasmid as described above. After 18-24 h of incubation at 37°C, the cleavage reaction was performed by incubating the cells in serum-containing DMEM with 30 μM His<sub>6</sub>-SrtA and 1 mM triglycine (Sigma) for 15-60 min at 37°C. The cells were washed three times with PBS, and the labeling reaction was

performed by incubating the cells in serum-containing DMEM with 30  $\mu\text{M}$  His<sub>6</sub>-SrtA and 10  $\mu\text{M}$  AF647-LPETGG for 5-30 min at 37°C. After five washes with PBS, the cells were observed using a confocal laser-scanning microscope as described above. Alexa Fluor 647-derived fluorescence signals on the surfaces of transfected cells were collected from the intensity profiles of the confocal images (N = 10).

#### **Labeling of LPETG<sub>5</sub>-ECFP-TM with biotin-LPETGG on cell surfaces**

HEK 293T cells were transfected with the LPETG<sub>5</sub>-ECFP-TM plasmid as described above. After 24 h of incubation at 37°C, the cleavage reaction was performed by incubating the cells in serum-containing DMEM with 30  $\mu\text{M}$  His<sub>6</sub>-SrtA and 1 mM triglycine (Sigma) for 120 min at 37°C. The cells were washed three times with PBS, and the labeling reaction was performed by incubating the cells in serum-containing DMEM with 15  $\mu\text{M}$  His<sub>6</sub>-SrtA and 10  $\mu\text{M}$  biotin-LPETGG for 5 min at 37°C. After three washes with PBS, the cells were lysed in 10 mM HEPES (pH 7.4), 150 mM NaCl, 0.5 mM EDTA, 0.5 mM EGTA, 1% Triton X-100, 1 mM Na<sub>3</sub>VO<sub>4</sub>, 1 mM NaF and 1 mM PMSF. The protein samples were fractionated by 12.5% SDS-PAGE and electrotransferred to PVDF membranes. The membranes were blocked with 5% bovine serum albumin in Tris-buffered saline containing Tween-20 (TBST) for 1 h at room temperature. Thereafter, the membranes were washed three times with TBST, and incubated with streptavidin-HRP (Invitrogen), or a rabbit anti-c-Myc antibody (Bethyl Laboratories) followed by a goat HRP-conjugated anti-rabbit antibody (Biosource). After three washes with TBST, blots were detected using Chemi-Lumi One L (Nacalai Tesque).

#### **References**

1. T. Tanaka, T. Yamamoto, S. Tsukiji and T. Nagamune, *ChemBioChem*, 2008, **9**, 802-807.