Electronic Supplementary Information

Ligand design for specific MHC class I molecules on the cell surface

Xizheng Sun[a], Reika Tokunaga[a], Yoko Nagai[a], Ryo Miyahara[a], Akihiro Kishimura[a,b,c,d], Shigeru Kawakami[e], Yoshiki Katayama[a,b,c,d,f,g,*], Takeshi Mori[a,b,c,*]

[a]Graduate School of Systems Life Sciences, Kyushu University, 744 Motooka, Nishi-ku, Fukuoka 819-0395, Japan.

[b]Department of Applied Chemistry, Faculty of Engineering, Kyushu University, 744 Motooka, Nishi-ku, Fukuoka 819-0395, Japan.

[c]Center for Future Chemistry, Kyushu University, 744 Motooka, Nishi-ku, Fukuoka 819-0395, Japan.

[d]International Research Center for Molecular Systems, Kyushu University, 744 Motooka, Nishi-ku, Fukuoka 819-0395, Japan.

[e]Department of Pharmaceutical Informatics, Graduate School of Biomedical Sciences, 1-7-1 Sakamoto, Nagasaki 852-8588, Japan.

[f]Center for Advanced Medical Innovation, Kyushu University, 744 Motooka, Nishi-Ku, Fukuoka 819-0395, Japan.

[g]Department of Biomedical Engineering, Chung Yuan Christian University, 200 Chung Pei Rd., Chung Li, 32023 ROC, Taiwan.

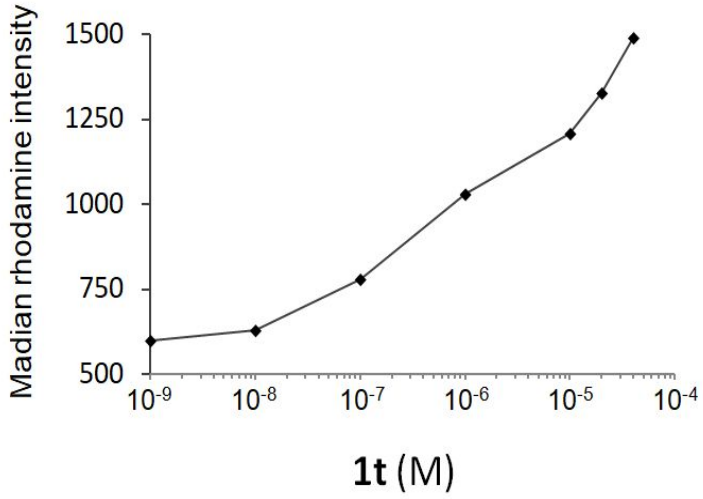


Figure S1. Effect of concentration of peptide **1t** on the binding with RMA cells. Cells were treated with **1t** in serum-containing medium for 1 h at 37 °C. After washing three times with serum-free medium, cells were detected by image-based cytometer. The fluorescence intensity was not saturated in the examined concentration range (up to 40 μM). Since we found that 0.8 μM of peptide provided enough fluorescence to be detectable by fluorescence microscopy, we chose this concentration in most of the experiments.

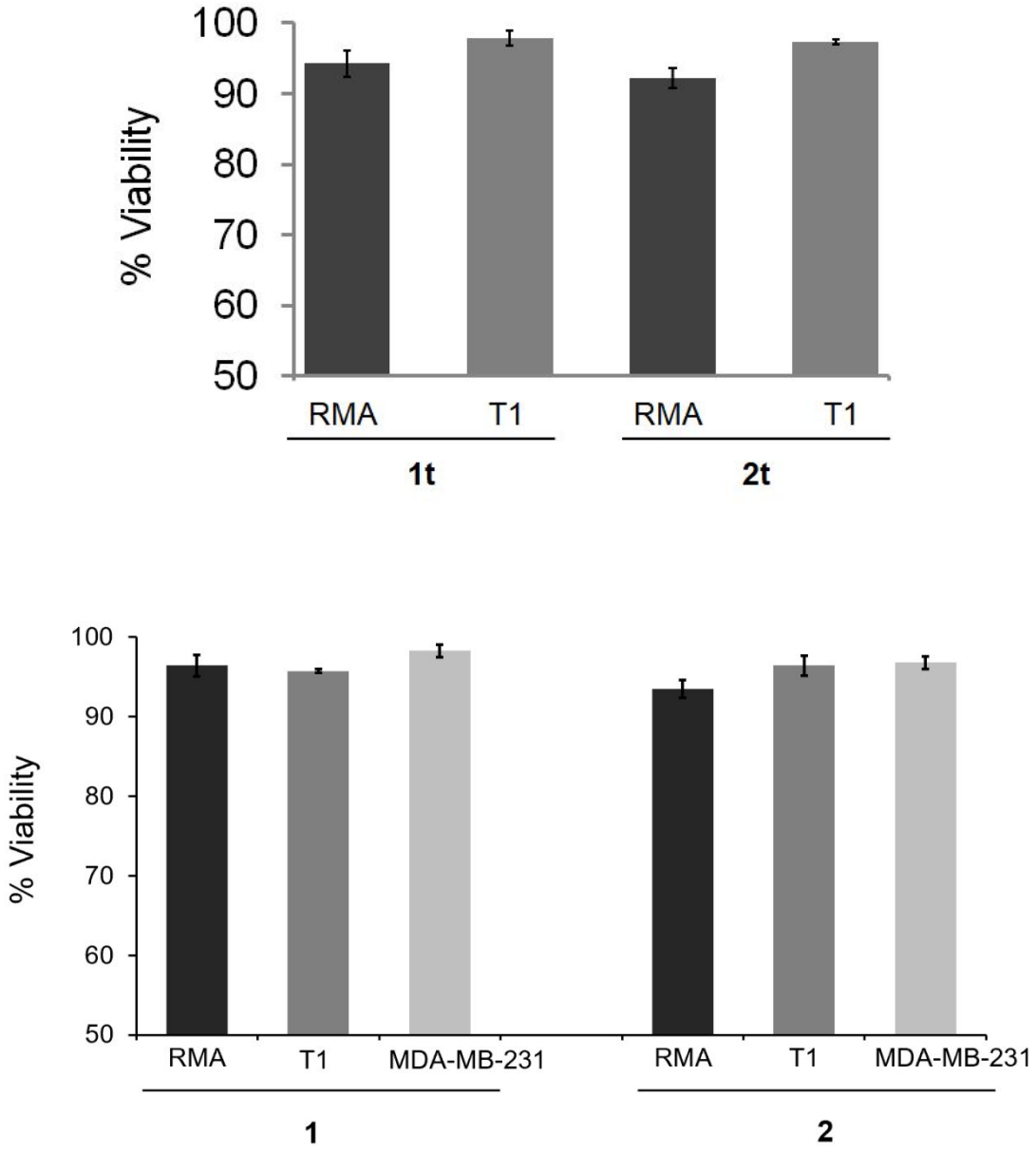


Figure S2. Viability of cells after treatment of **1t** or **2t** (0.8 μM) (above), and after treatment of **1** or **2** (80 μM) (below). Cells were treated with each peptide in serum-containing medium for 1 h at 37 °C. After washing three times with serum-free medium, dead cells were stained with propidium iodide (PI) solution (2 μg/ml) (Sigma, St. Louis, USA) for 15 min on ice. The dead cells were counted by image-based cytometer.

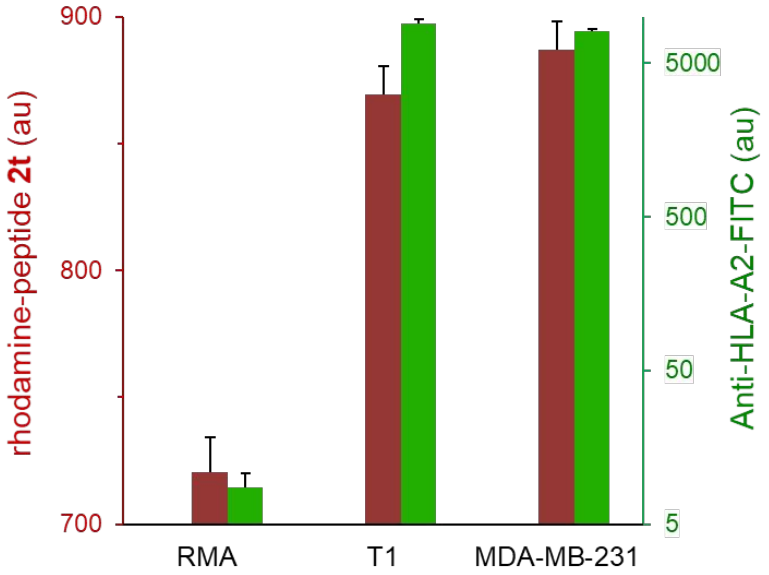


Figure S3. Correlation of bound ligand peptide **2t** and cell surface expression of HLA A02:01. To stain HLA A02:01, cells were treated with FITC-labeled anti-HLA-A2 mAb (8 μg/ml) for 30 min at 4°C. The fluorescence intensity of FITC was quantified by flow cytometer (Cytoflex). The binding of **2t** with HLA A02:01 on the cell surface was conducted as follows. Cells were treated with **2t** (0.8 μM) in serum-containing medium for 1 h at 37°C, then washed three times with serum-free medium. The fluorescence intensity was quantified by image-based cytometer.

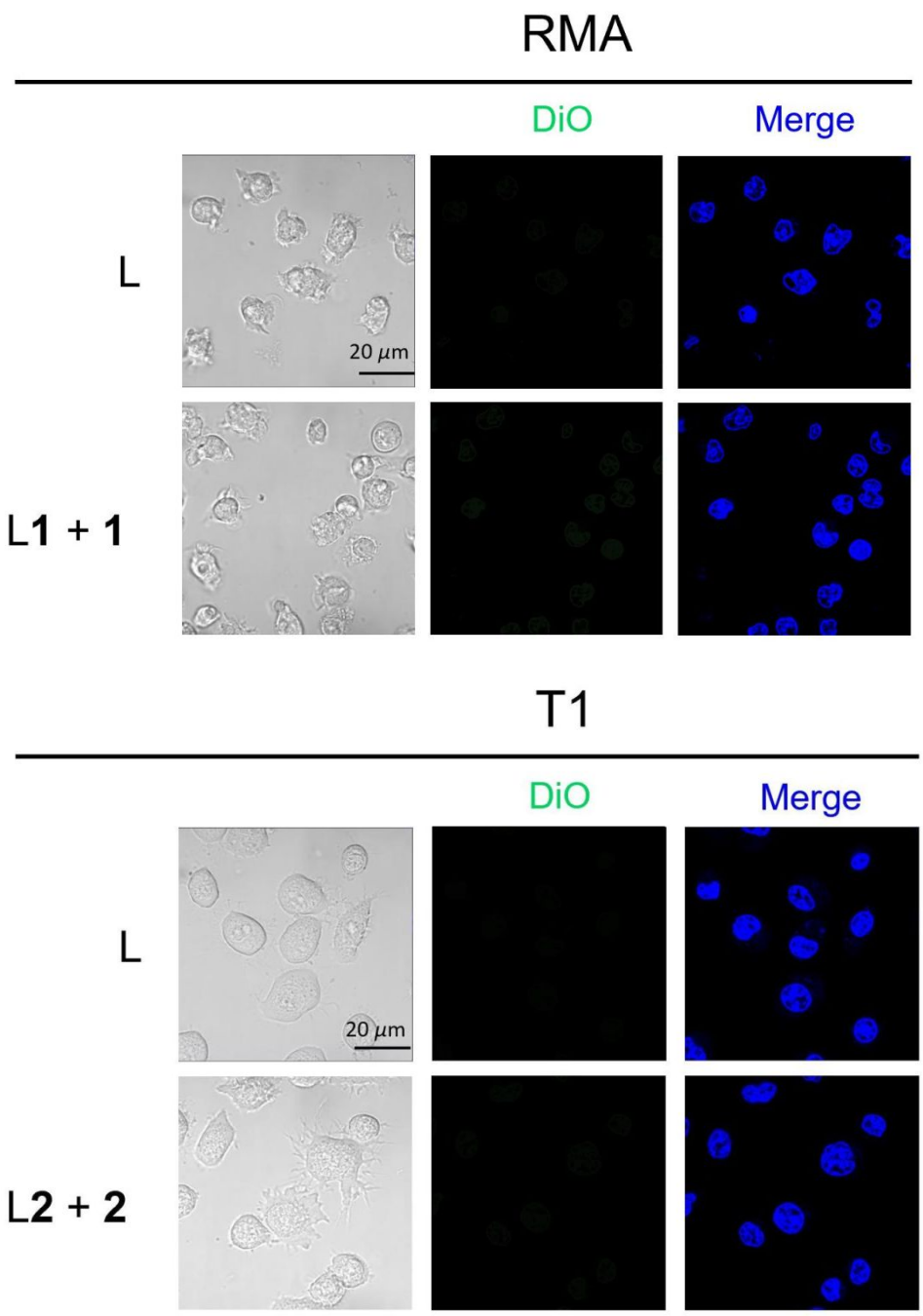


Figure S4. Evaluation of the specific binding of peptide-modified liposomes to RMA or T1 cells. Cells in group **L** were treated with 100 μM of liposomes (containing no ligand peptide); cells in group **L1** + **1** and **L 2**+ **2** were preincubated with no-labeled peptides **1** or **2** (100 μM) for1 hour before adding the **L1** or **L2** liposomes (100 μM, containing 3 μM of ligand peptides) respectively. After washing three times with serum-free medium, cells were observed by confocal laser scanning microscopy. Blue and green color represent the nucleus and DiO-labeled liposome, respectively.

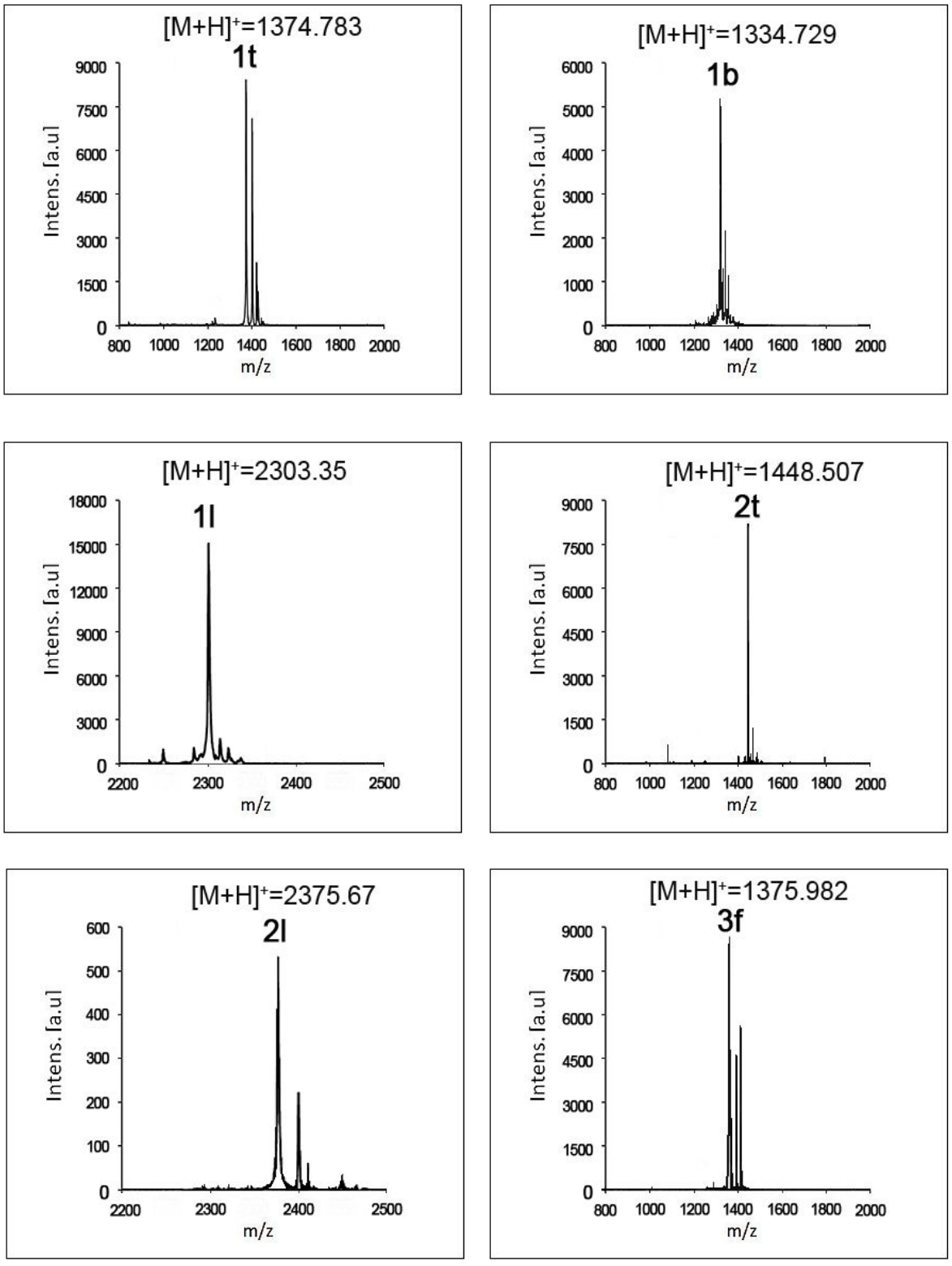
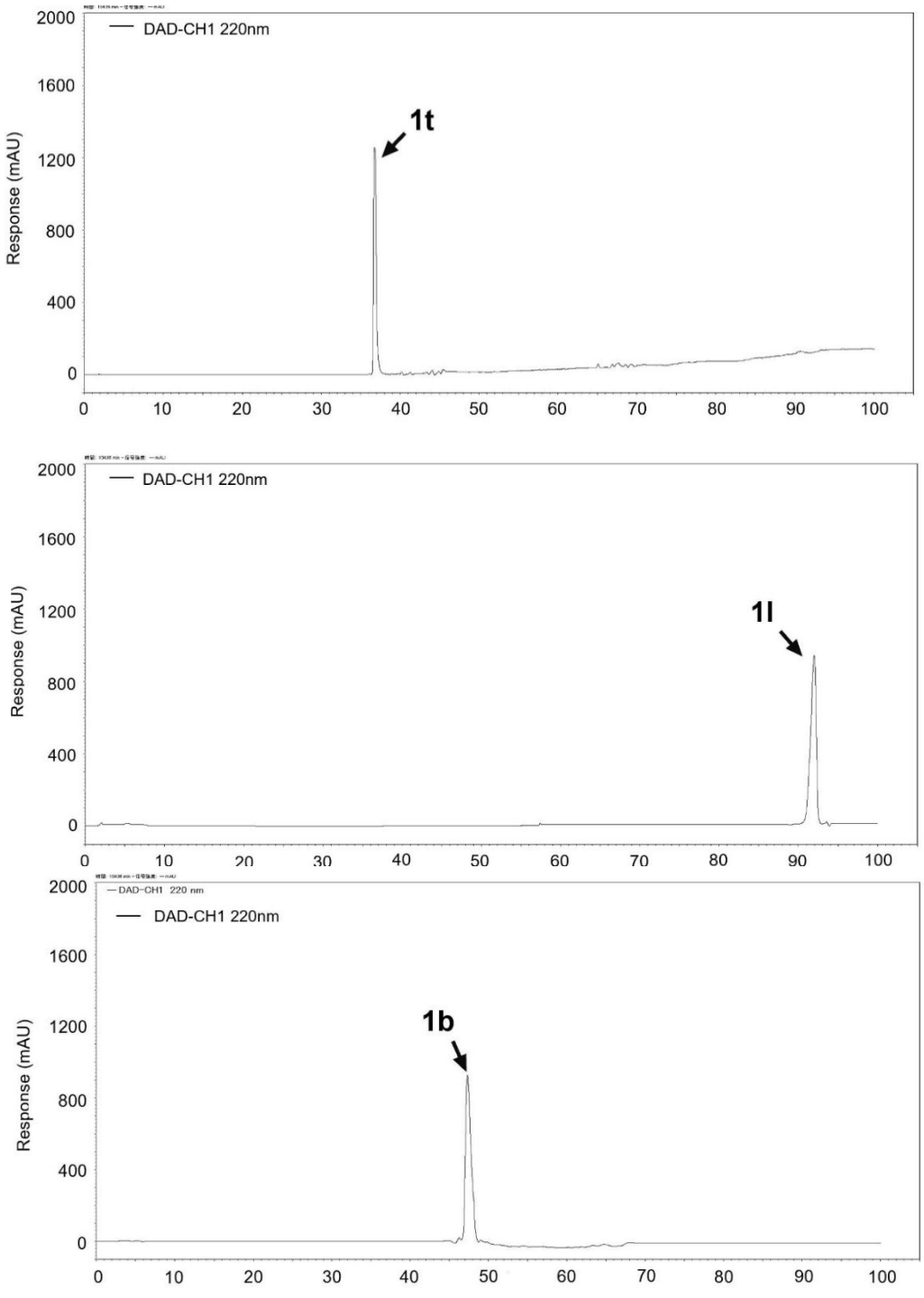


Figure S5. Molecular weight of the ligand peptides. The obtained peptides were identified by a matrix-assisted laser desorption ionization time-of-flight mass spectrometer (Bruker Daltonics, Billerica, MA, USA).


DAD-CH1 220nm
1t
Response (mAU)
2000
1600
1200
800
400
0
0 10 20 30 40 50 60 70 80 90 100
DAD-CH1 220nm
1l
Response (mAU)
DAD-CH1 220nm
1b
Response (mAU)
Retention time (min)

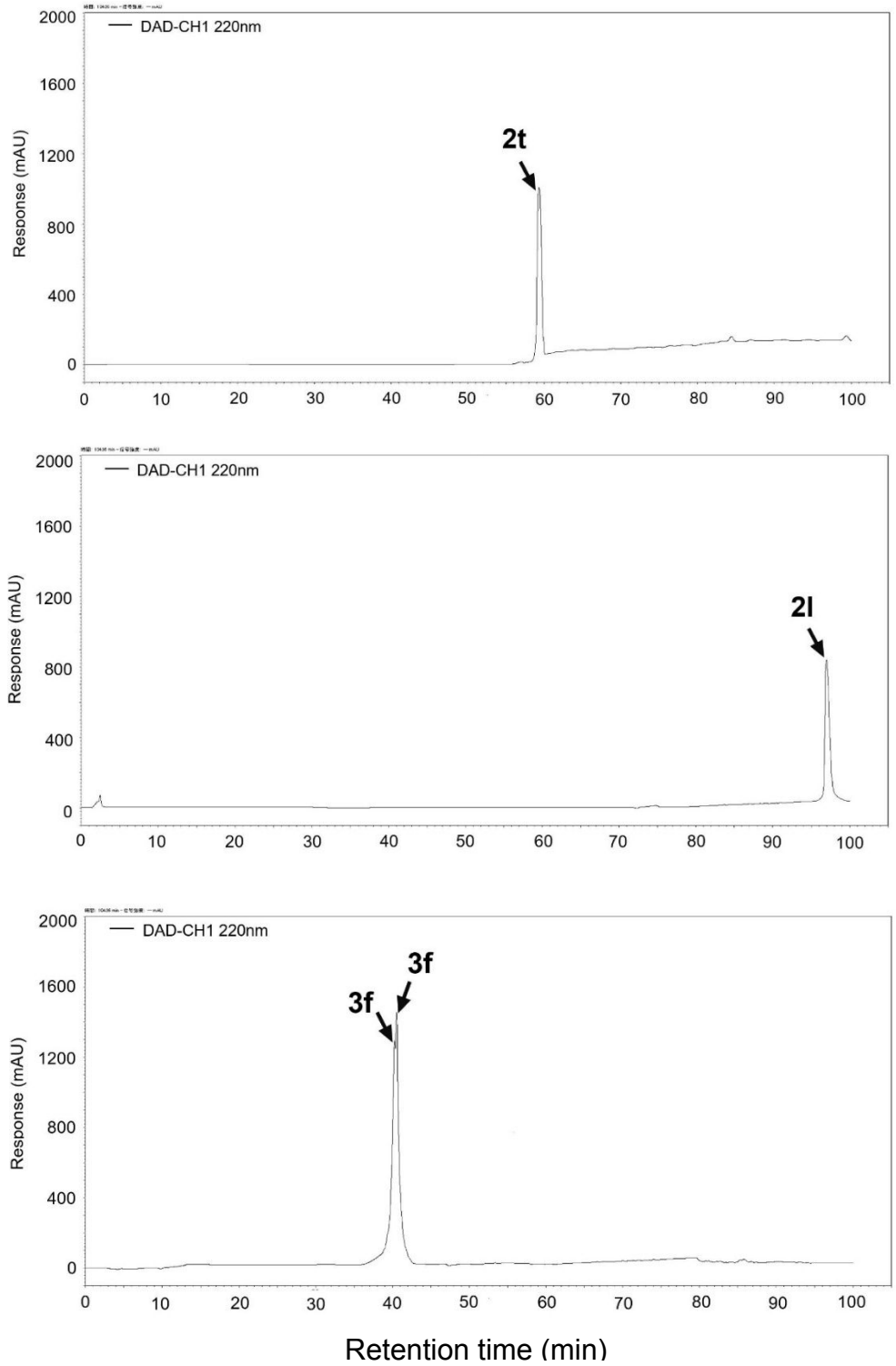


Figure S6. Chromatogams of the purified ligand peptides. The purified peptides were analized by reversed-phase HPLC (Hitachi La Chrom Elite, Tokyo, Japan) on a Protein-R column (Nacalai Tesque,

Kyoto, Japan) using a mobile phase consisting of acetonitrile/0.1% TFA (0% to 100% v/v) (for **1t**, **2t** and **3f**) or methanol/0.1% TFA (0% to 100% v/v) (for **1b**, **1l** and **2l**).

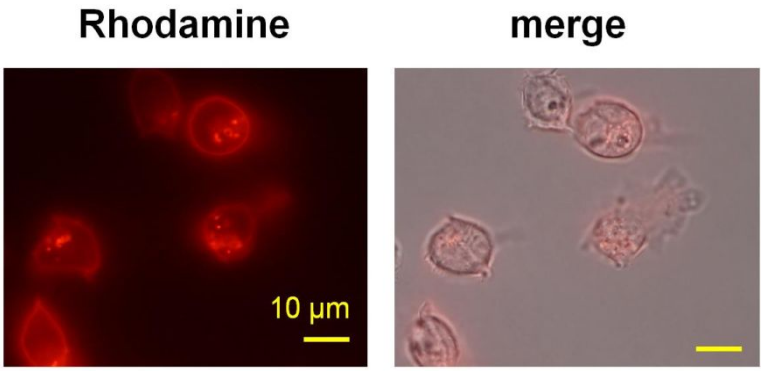


Figure S7. Fluorescence microscope images of RMA cells treated with TAMRA-labeled peptide **1t** (0.8 µM) in serum-containing medium for 3 h at 37 °C. After washing three times with serum-free medium, cells were observed by fluorescence microscopy.