Supplementary Information (SI) for Biomaterials Science.
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Supporting Information

Cholesterol- and ssDNA-binding fusion protein-mediated DNA tethering on plasma membrane

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S1. Modified Rep–ALOD4 construction

The hexapeptide sequence of ALOD4 (GTTLYP; amino acid 98–103) was substituted with AAAAAA by point mutagenesis to obtain the modified Rep–ALOD4. Briefly, the DNA sequence encoding GTTLYP in pET–Rep–$(GGGS)_2$–Rep–ALOD4 was substituted with AAAAA using inverse PCR-based site-directed mutagenesis. pET–Rep–$(GGGS)_2$–Rep–ALOD4 was amplified using KOD-plus-Neo polymerase (TOYOBO, Tokyo, Japan) and mutation primers. *E. coli* KRX strain was transformed with the constructed plasmids to obtain pET–Rep–$(GGGS)_2$–modified Rep–ALOD4, which encodes the modified Rep–ALOD4. DNA was sequenced to verify the correct sequences and corresponding amino acid sequences. The modified Rep–ALOD4 was expressed and purified using the procedure described in **2.3. Protein Expression and Purification**.

S2. Size exclusion chromatography (SEC) analysis

SEC was performed using an AKTA Explorer 10S (Cytiva, MA, USA) equipped with a combination of TSKgel G3000SW_{XL} (Tosoh). The system was eluted with 0.2 M phosphate buffer at pH 7.5 at 0.5 mL/min at 30 ºC. A calibration curve for the protein molecular weight was prepared using a molecular weight marker kit (MW-GF-200; Sigma-Aldrich).

S3. Association constant of Rep–ALOD4 with membrane cholesterol

From QCM analysis, the adsorption amount (Δm) of Rep–ALOD4 with various concentrations to the

liposome-modified SiO_2 sensor chip (DOPC:cholesterol = 7:3) was calculated. Δm and Rep–ALOD4 concentration (C_0) were plotted (**Figure S3b**). Then, $C_0/\Delta m$ and C_0 were plotted according to Langmuir's adsorption isotherm (**Figure S3c**):

$$\frac{C_0}{\Delta m} = \frac{1}{\Delta m_{max}}[C_0] + \frac{1}{\Delta m_{max} K_a}$$

where C_0 = the concentration of protein, Δm_{max} = the maximum adsorption amount, and K_a = apparent association constant of protein. K_a was calculated from the slope and intercept of $C_0/\Delta m$-C_0 plot (**Fig. S3c**).

S4. Interaction between S-FITC-RepALOD4 and serum proteins

S-FITC and S-FITC-Rep-ALOD4 (10 μM) were dissolved in DMEM medium containing 10% FBS and incubated at 37°C for 30, 60, and 90 min. For S-FITC, the solutions were measured by 3.5% agarose gel (MetaPhor Agarose, Lonza) electrophoresis. The agarose gel was irradiated with UV lamp at 365 nm. For S-FITC-Rep-ALOD4, the solutions were measured by SDS-PAGE, and the acrylamide gel was stained with CBB. Band ratio was calculated from using the ratio of the band at without to the band at each time.

(a) Rep–ALOD4

MHHHHHHAMGEFMPSKKNGRSGPQPHKRWVFTLNNPSEDERKKIRDLPISLFDYFIVGEE
GNEEGRTPHLQGFANFVKKQTFNKVKWYLGARCHIEKAKGTDQQNKEYCSKEGNLLMEC
GAPRSQGQRGSGGGGSGGGGSRSSAKMTLDHYGAYVAQFDVSWDEFTFDQNGKEVLT
HKTWEGSGKDKTAHYSTVIPLPPNSKNIKIVAREATGLAWEWWRTIINEQNVPLTNEIKVSI
GGTTLYPTATISH

(b) Modified Rep–ALOD4

MHHHHHHAMGEFMPSKKNGRSGPQPHKRWVFTLNNPSEDERKKIRDLPISLFDYFIVGEE
GNEEGRTPHLQGFANFVKKQTFNKVKWYLGARCHIEKAKGTDQQNKEYCSKEGNLLMEC
GAPRSQGQRGSGGGGSGGGGSRSSAKMTLDHYGAYVAQFDVSWDEFTFDQNGKEVLT
HKTWEGSGKDKTAHYSTVIPLPPNSKNIKIVAREATGLAWEWWRTIINEQNVPLTNEIKVSI
GAAAAAATATISH

(c) Venus–Rep

MHHHHHHAMGEFMSKGEELFTGVVPILVELDGDVNGHKFSVSGEGEGDATYGKLTLKLIC
TGKLPVPWPTLVTTLGYGLQCFARYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKT
RAEVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNYNSHNVYITADKQKNGIKANFKIRHNI
EDGGVQLADHYQQNTPIGDGPVLLPDNHYLSYQSALSKDPNEKRDHMVLLEFVTAAGGT
EFMPSKKNGRSGPQPHKRWVFTLNNPSEDERKKIRDLPISLFDYFIVGEEGNEEGRTPHL
QGFANFVKKQTFNKVKWYLGARCHIEKAKGTDQQNKEYCSKEGNLLMECGAPRSQGQR
VD

Fig. S1 (a–c) The amino acid sequences of Rep–ALOD4 (a), modified Rep–ALOD4 (b), and His_6–Venus–Rep (c). Highlighted sequences for Rep–ALOD4 and modified Rep–ALOD4 represent the mutated position.

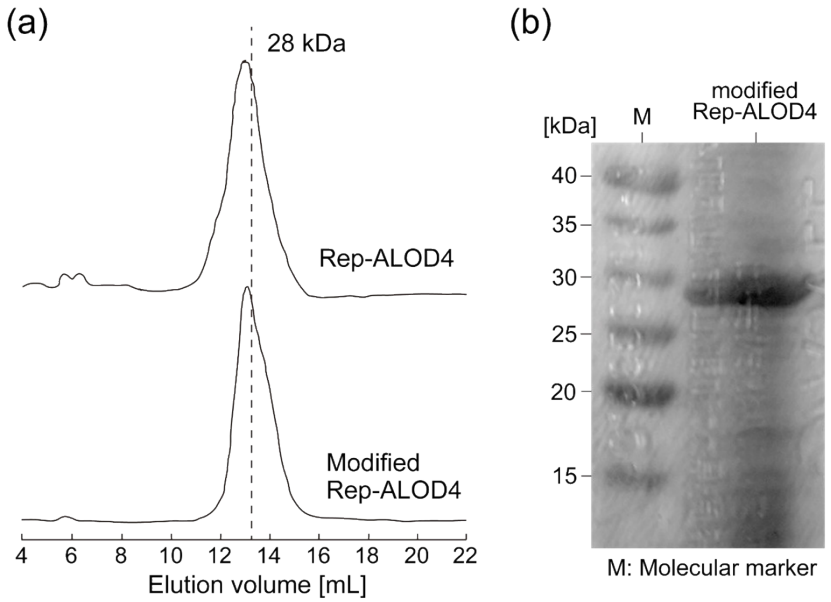

Fig. S2 (a) SEC charts of Rep–ALOD4 and modified Rep–ALOD4. (b) SDS–PAGE patterns of modified Rep–ALOD4.

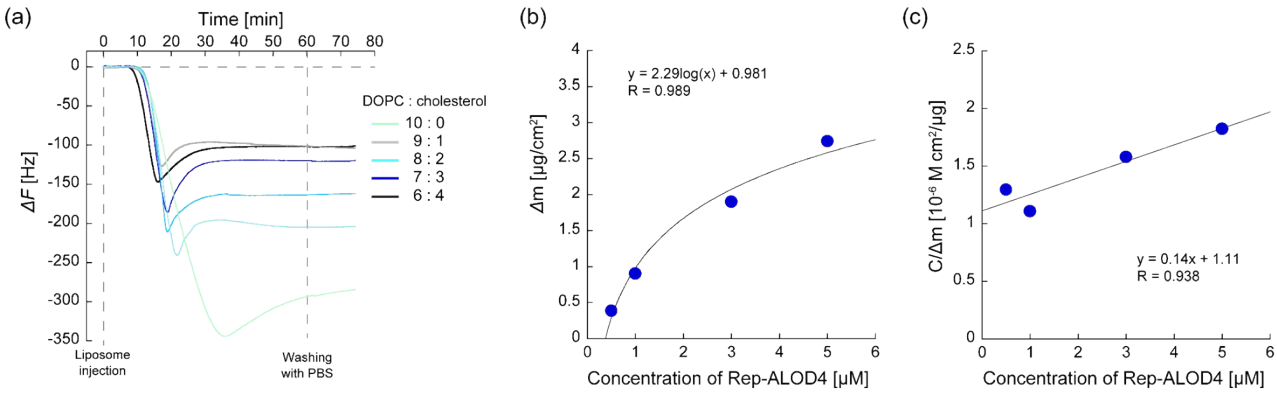

Fig. S3 (a) Representative QCM profiles with liposomes to the sensor chip in various ratios of DOPC:cholesterol (10:1, 9:1, 8:2, 7:3, and 6:4). (b) The plot of mass change of adsorbed Rep–ALOD4 as a function of Rep–ALOD4 concentration. (c) A linear representation of the Langmuir isotherm for Rep–ALOD4 adsorption to cholesterol.

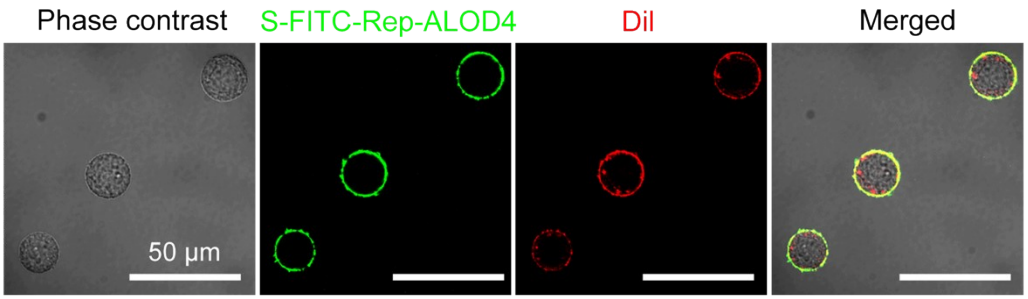

Fig. S4 CLSM images of DiI-stained HeLa cells treated with 3 μM S-FITC–Rep–ALOD4 after 10 min of incubation. Scale bars: 50 μm

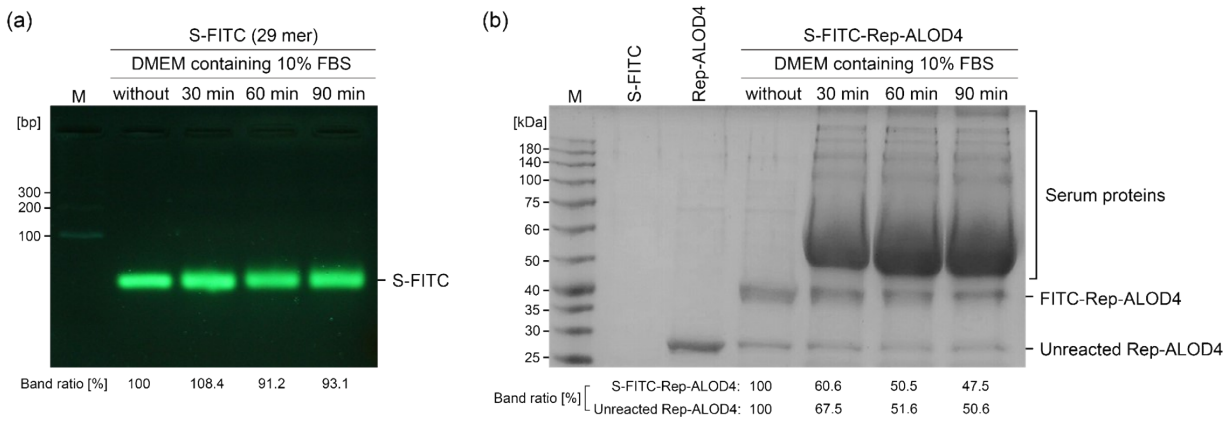

Fig. S5 (a) Agarose gel analysis of S-FITC (10 μM) incubated with 10% FBS for 30, 60, and 90 min. The band was irradiated with UV lamp at 365 nm. **(b)** SDS–PAGE analysis of S-FITC-Rep–ALOD4 (10 μM) incubated with 10% FBS for 30, 60, and 90 min. Band ratio was calculated from using the ratio of the band at without to the band at each time.

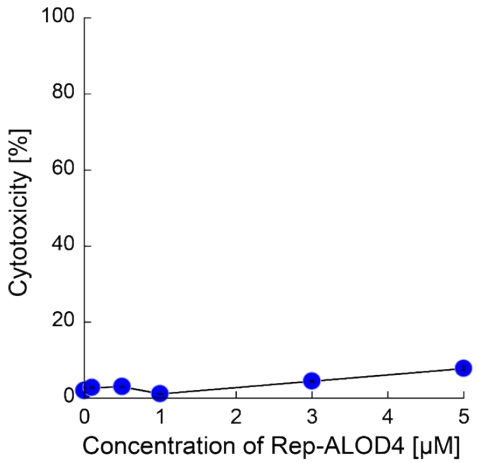

Fig. S6 Concentration-dependent cytotoxicity of Rep–ALOD4 to HeLa cells for 24 h of incubation. Data expressed as mean ± SD (n=3).

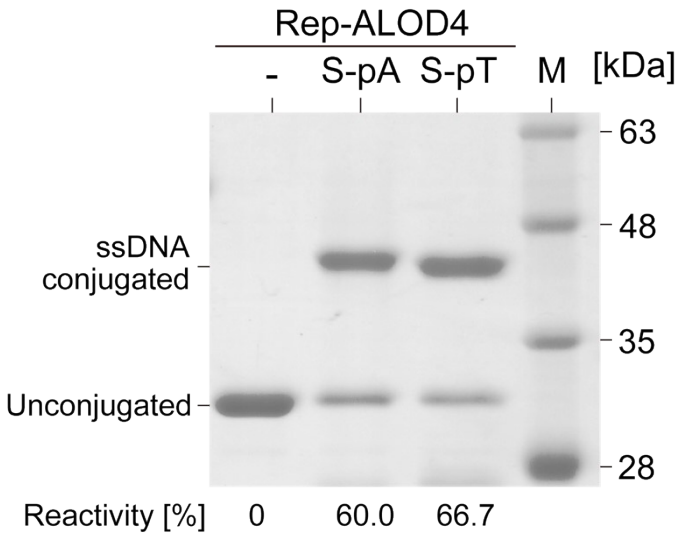

Fig. S7 SDS–PAGE patterns of Rep–ALOD4 and S-pA- and S-pT-reacted Rep–ALOD4.