








## CORRECTION

# Correction to: Potentiating CD8<sup>+</sup> T cell antitumor activity by inhibiting PCSK9 to promote LDLR-mediated TCR recycling and signaling

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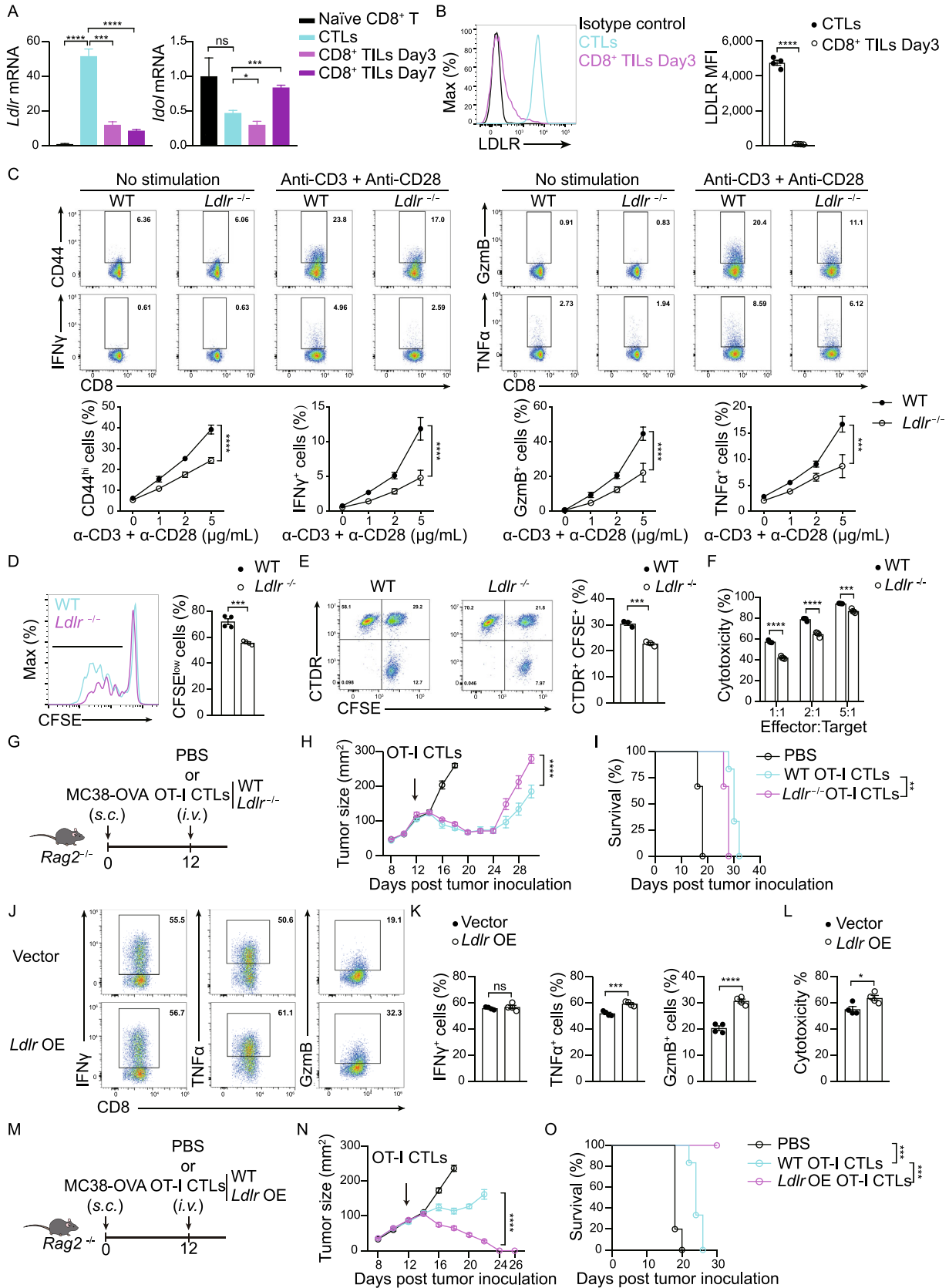
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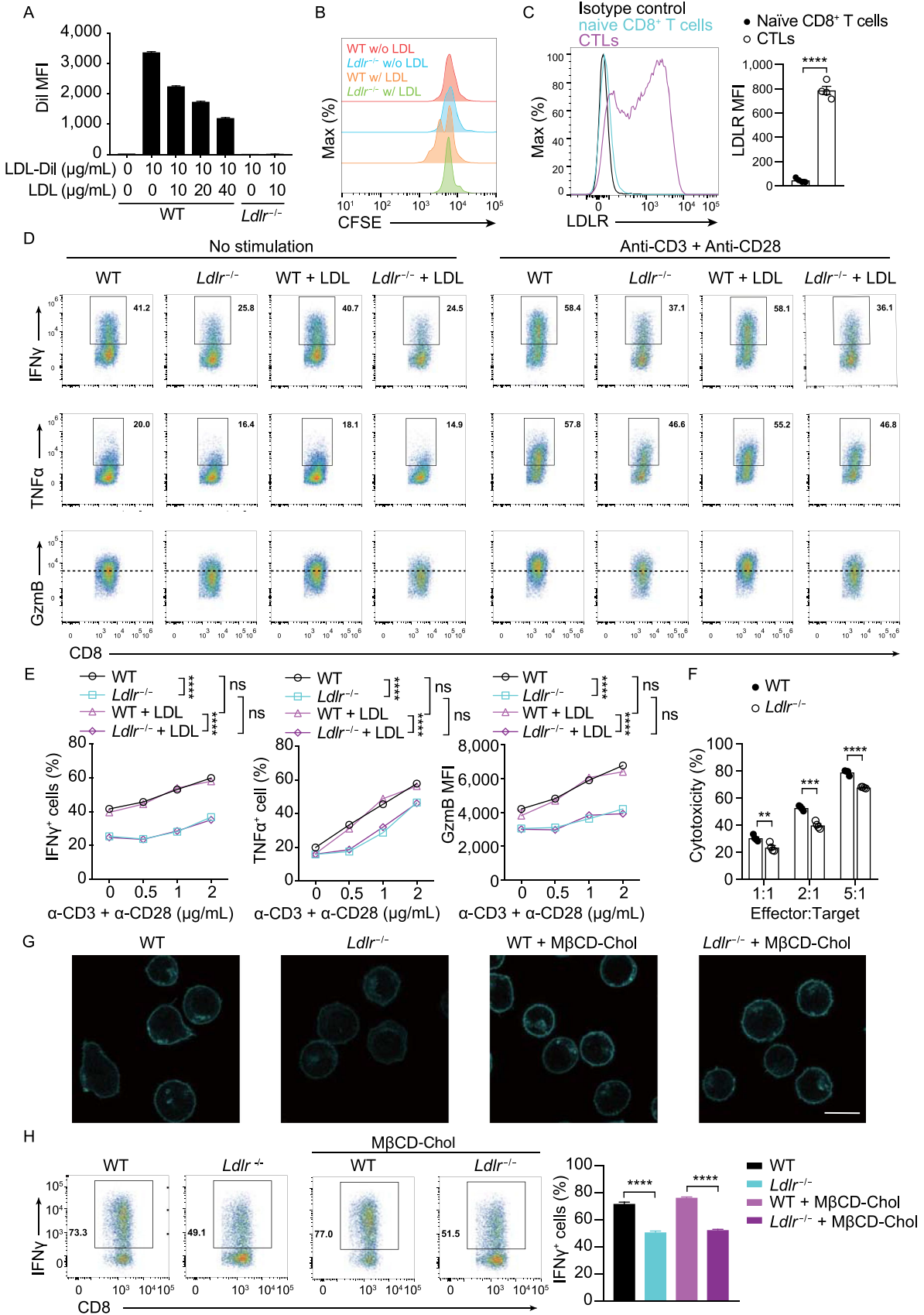
PROTEIN CELL

[HTTPS://DOI.ORG/10.1007/S13238-021-00821-2](https://doi.org/10.1007/s13238-021-00821-2)

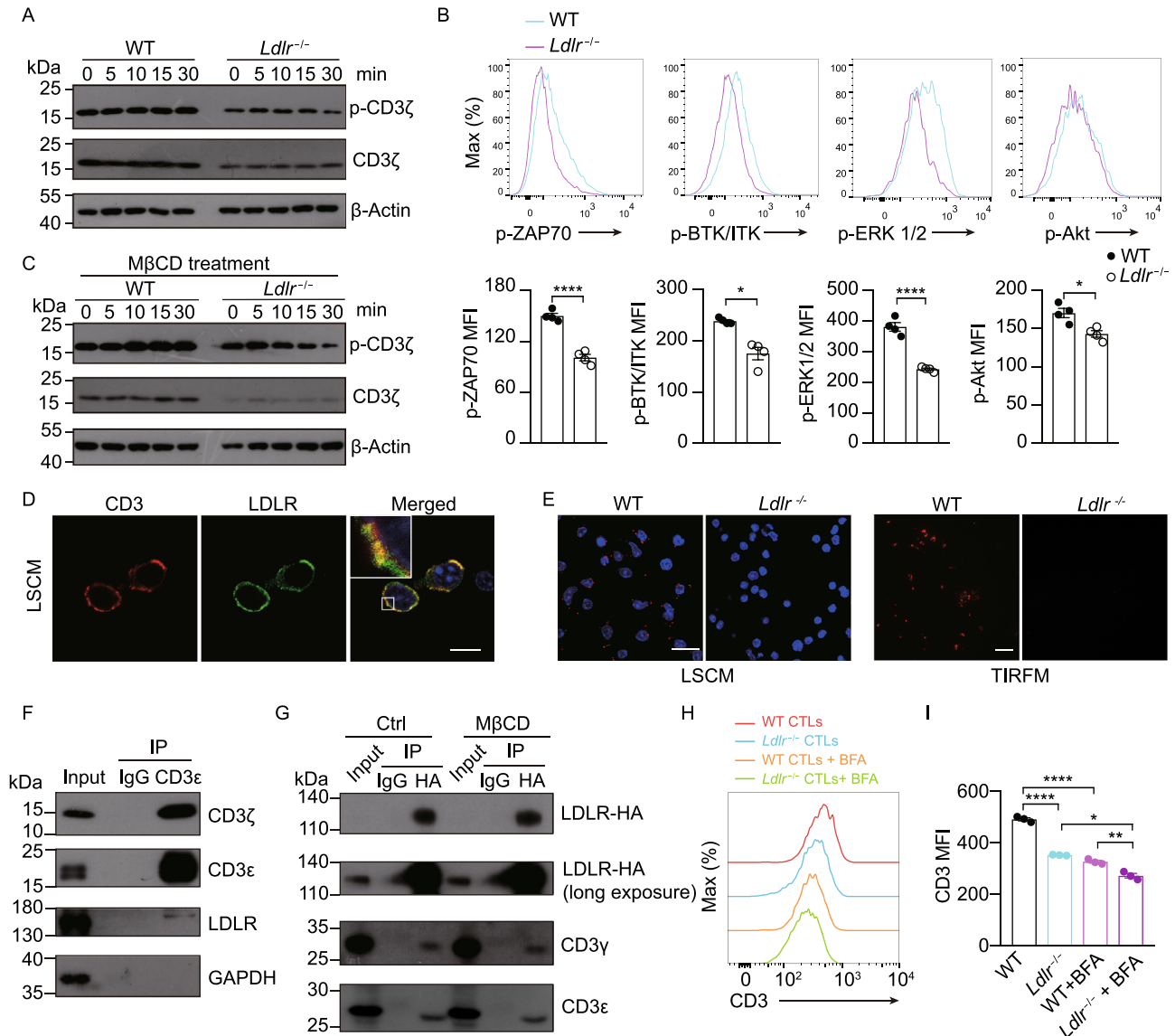
In the original publication Figs. 1, 2 and 3 are incorrectly published, the correct Figs. 1, 2 and 3 are provided in this correction. The original article has been corrected.



◀ **Figure 1. LDLR deficiency hinders the antitumor activity of CD8<sup>+</sup> T cells.** (A) Transcriptional level of genes involved in cholesterol transport in naïve CD8<sup>+</sup> T cells, CTLs and CD8<sup>+</sup> TILs (isolated at Day3 or Day7 post CTLs adoptive transfer), ( $n = 4$ ). (B) LDLR expression level on CTLs and CD8<sup>+</sup> TILs (isolated at Day3 post CTLs adoptive transfer), ( $n = 4$ ). (C) Activation and cytokine/granule productions of WT and *Ldlr*<sup>-/-</sup> CD8<sup>+</sup> T cells. Naïve CD8<sup>+</sup> T cells were isolated from the spleen and stimulated with anti-CD3 and anti-CD28 antibodies for 24 h at indicated concentrations. Data were analyzed by two-way ANOVA ( $n = 4$ ). (D) CD8<sup>+</sup> T cell proliferation was measured by CFSE dilution assay. CD8<sup>+</sup> T cells were isolated from the spleen and stimulated with 1 µg/mL plate-coated anti-CD3 and anti-CD28 antibodies for 72 h, ( $n = 4$ ). (E) Immunological synapse formation of WT and *Ldlr*<sup>-/-</sup> CTLs. CFSE-labeled CTLs and CellTracker Deep Red (CTDR)-labeled OVA-pulsed EL4 cells were cocultured for 30 min, ( $n = 3$ ). (F) Cytotoxicity of WT and *Ldlr*<sup>-/-</sup> CTLs. Splenocytes from WT and *Ldlr*<sup>-/-</sup> OT-I mice were stimulated with OVA<sub>257-264</sub> and IL-2 to generate mature CTLs. CTLs were incubated with OVA-pulsed CTDR-labeled EL4 cells and CFSE-labeled non-pulsed EL4 cells for 4 h. The ratio of OVA-pulsed and non-pulsed EL4 cells was calculated to determine the cytotoxicity of CTLs, ( $n = 4$ ). (G) Illustration of adoptive transfer of PBS, WT or *Ldlr*<sup>-/-</sup> CTLs to MC38-OVA tumor-bearing *Rag2*<sup>-/-</sup> mice. (H and I) Tumor growth (H) and survival (I) of MC38-OVA tumor-bearing *Rag2*<sup>-/-</sup> mice with CTL transfer as shown in (G), ( $n = 6$ ). (J and K) Cytokine and granule productions of control and *Ldlr* OE CTLs. *Ldlr* was overexpressed in CTLs with retrovirus infection. The sorted cells were stimulated with 1 µg/mL plate-coated anti-CD3 and anti-CD28 antibodies for 4 h, ( $n = 4$ ). (L) Cytotoxicity of control and *Ldlr* OE CTLs. CTLs were incubated with OVA-pulsed EL4 cells and non-pulsed EL4 cells for 4 h, ( $n = 4$ ). (M) Illustration of adoptive transfer of PBS, WT or *Ldlr* OE CTLs to MC38-OVA tumor-bearing *Rag2*<sup>-/-</sup> mice. (N and O) Tumor growth (N) and survival (O) of MC38-OVA tumor-bearing *Rag2*<sup>-/-</sup> mice with CTL transfer as shown in (M), ( $n = 5-6$ ). Data were analyzed by *t* test (A, B, D, E, F, K and L) or two-way ANOVA (C, H, I, N and O). \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ ; \*\*\*\* $P < 0.0001$ . Error bars denote for the s.e.m.



◀ **Figure 2. The regulation of LDLR on CD8<sup>+</sup> T cell effector function is not fully dependent on LDL/cholesterol.** (A) LDL uptake of activated WT and *Ldlr*<sup>-/-</sup> CD8<sup>+</sup> T cells. CD8<sup>+</sup> T cells were treated with LDL and LDL-Dil at indicated concentrations. The uptake of LDL-Dil was analyzed by flow cytometry. (B) Proliferation of WT and *Ldlr*<sup>-/-</sup> CD8<sup>+</sup> T cells was measured by CFSE dilution. Cells were cultured in medium containing lipoprotein-deficient serum (LPDS) with or without the addition of LDL. (C) LDLR expression of naïve CD8<sup>+</sup> T cells and CTLs was analyzed by flow cytometry, (*n* = 4). (D and E) Cytokine/granule productions of WT and *Ldlr*<sup>-/-</sup> CTLs. CTLs were generated from the splenocytes of WT and *Ldlr*<sup>-/-</sup> mice and pretreated in the medium containing LPDS for 4 h, with or without the presence of LDL. The cells were then stimulated with anti-CD3 and anti-CD28 antibodies for 4 h at indicated concentrations in corresponding medium, (*n* = 4). (F) Cytotoxicity of WT and *Ldlr*<sup>-/-</sup> CTLs. CTLs were pretreated in the medium containing LPDS for 12 h and cocultured with EL4 cells to determine the cytotoxicity, (*n* = 4). (G) Filipin III staining to analyze cellular cholesterol distribution in untreated or M $\beta$ CD-coated cholesterol treated WT and *Ldlr*<sup>-/-</sup> CTLs. Scale bar, 10  $\mu$ m. (H) IFN $\gamma$  production of WT and *Ldlr*<sup>-/-</sup> CTLs. Mature CTLs were generated from the splenocytes of WT and *Ldlr*<sup>-/-</sup> mice and treated with M $\beta$ CD-coated cholesterol or not. The cells were then stimulated with 1  $\mu$ g/mL plate-coated anti-CD3 and anti-CD28 antibodies for 4 h, (*n* = 4). Data were analyzed by *t* test (C, F and H) or two-way ANOVA (E). ns, no significance; \*\**P* < 0.01; \*\*\**P* < 0.001; \*\*\*\**P* < 0.0001. Error bars denote for the s.e.m.



**Figure 3. LDLR interacts with TCR and regulates TCR signaling in CD8<sup>+</sup> T cells.** (A) Immunoblotting to detect the phosphorylation of CD3 $\zeta$  of WT and *Ldlr*<sup>-/-</sup> CTLs. CTLs were stimulated with 1  $\mu$ g/mL anti-CD3, anti-CD28, anti-Armenian hamster IgG and anti-Syrian hamster IgG for indicated times. (B) Phosphorylation of ZAP70, BTK/ITK, ERK1/2 and Akt of WT and *Ldlr*<sup>-/-</sup> CTLs. CTLs were stimulated as in (A) for 10 min. Data were analyzed by *t* test (*n* = 4). (C) Immunoblotting to detect the phosphorylation of CD3 $\zeta$  of M $\beta$ CD-treated WT and *Ldlr*<sup>-/-</sup> CTLs. CTLs were stimulated as in (A). (D) Fluorescence staining of CD3 and LDLR in CTLs. DAPI was shown in blue. Scale bar, 10  $\mu$ m. LCSM, laser confocal scanning microscopy. (E) Proximity Ligation Assay (PLA) analysis of CD3 and LDLR interaction in WT and *Ldlr*<sup>-/-</sup> CTLs. Confocal images (left panel, scale bar, 20  $\mu$ m) and TIRFM images (right panel, scale bar, 10  $\mu$ m) were shown. Red, PLA signals; Blue, DAPI. TIRFM, total internal reflection fluorescence microscopy. (F) CD3 $\epsilon$  was immunoprecipitated (IP) in CTLs and its interaction with LDLR was analyzed by immunoblotting. (G) HA-tagged LDLR was overexpressed in EL4 cells. The EL4 cells were treated with M $\beta$ CD or not and then HA-tagged LDLR was immunoprecipitated with anti-HA antibody. The interaction between LDLR and CD3 was analyzed by immunoblotting. (H and I) WT and *Ldlr*<sup>-/-</sup> CTLs were treated with BFA (5  $\mu$ g/mL) or not for 2 h. CD3 expression was analyzed by flow cytometry. Data were analyzed by *t* test (*n* = 3). \**P* < 0.05; \*\**P* < 0.01; \*\*\*\**P* < 0.0001. Error bars denote for the s.e.m.

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