Expanded View Figures

Figure EV1. miPEP31 does not affect the inhibitory function of T_{reg} cells.

A, B WT mice were treated with synthetic scPEP or miPEP31 at day 8 postimmunization every 2 days for three injections. FoxP3^{EGFP+} T_{reg} cells were sorted from splenocytes derived from mice treated with synthetic scPEP or miPEP31. The CellTrace™ Violet (CTV)-labeled memory T_{conv} cells (1×10^5) were cultured in 96-well plates for 72 h together with a decreasing ratio of sorted T_{reg} cells in the presence of anti-CD3 plus γ-irradiated antigen-presenting cells (1×10^5). The suppressive function of T_{reg} cells was determined by the proliferation of activated responder T cells (T_{resp}) on the basis of CTV dilution. Data in (B) are presented as mean ± SEM of four biological replicates, ns, not significant (1:2 or 1:4 or 1:8 or 1:16 miPEP31 vs. scPEP), nonparametric one-way ANOVA, Kruskal–Wallis test.

C–G Flow cytometric analysis of ICOS (C), LAG3 (D), GITR (E), and CTLA-4 (F) expression in T_{reg}-cell induction in the presence of 10 μM synthetic scPEP or miPEP31. Data in (G) are presented as mean ± SEM of six biological replicates. ns, not significant (scPEP vs. miPEP31), unpaired two-tailed Student's *t*-test.

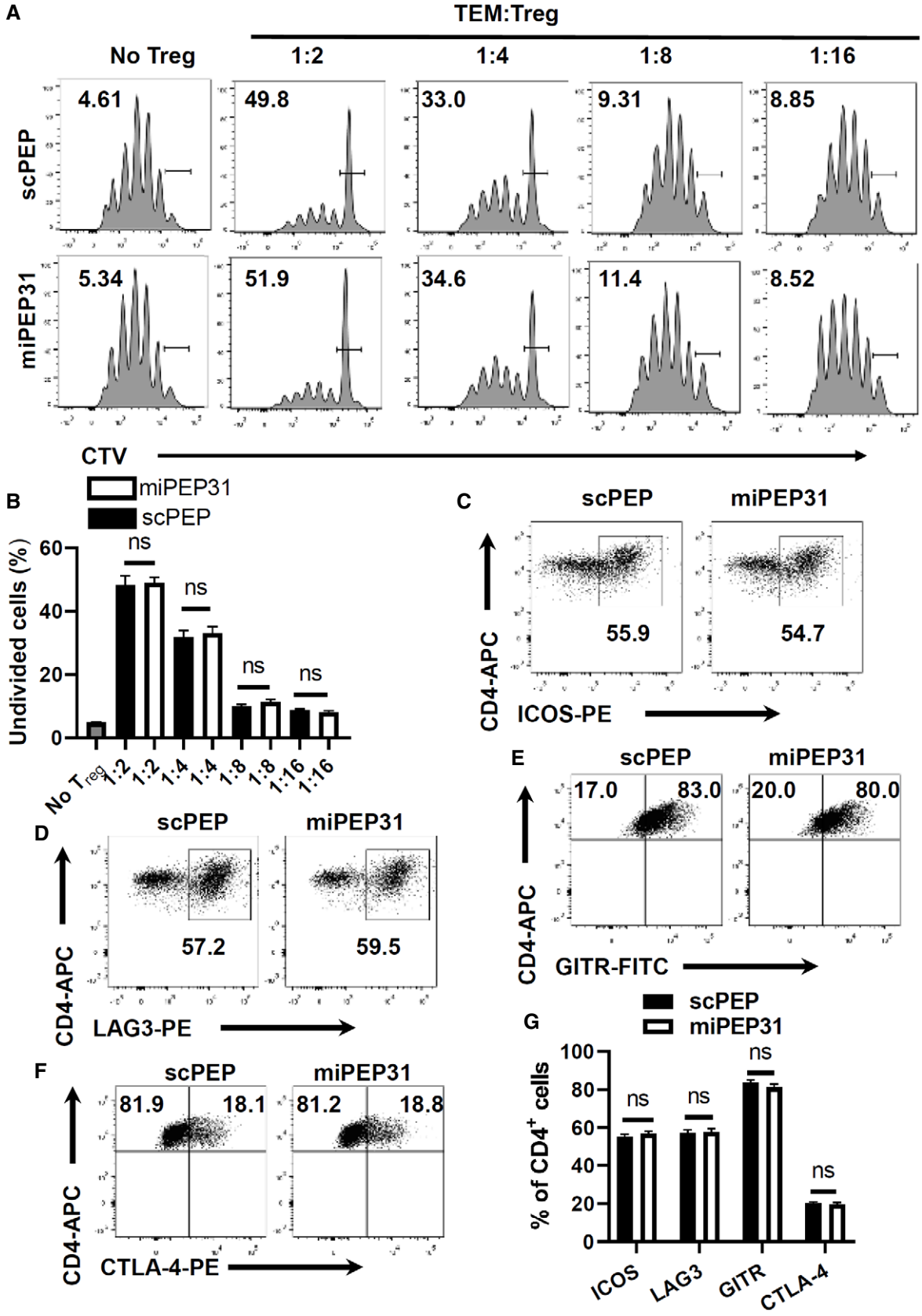


Figure EV1.

Figure EV2. The role of miPEP31 in the induction of immune cells in EAE mice.

A–E EAE was induced by MOG_{35-55}. scPEP or miPEP31 (50 μg) was injected intravenously every 2 days starting from day 8 postimmunization. Splenocytes from scPEP- or miPEP31-treated EAE mice were isolated on day 15 postimmunization and analyzed by flow cytometry. (A) B220 for total B lymphocytes. (B) CD3 for total T lymphocytes. (C) CD4 and CD8 for T-helper cells and cytotoxic T cells (gate on $CD3^+$ T cells). (D) CD11b for macrophages. (E) CD11c for dendritic cells. Data in (A–E) are presented as mean ± SEM of five biological replicates. ns, not significant, unpaired two-tailed Student's *t*-test.

F–H EAE was induced by MOG_{35-55} in $FoxP3^{EGFP}$ mice. scPEP or miPEP31 (50 μg) was injected intravenously every 2 days starting from day 8 postimmunization. Splenocytes of scPEP- or miPEP31-treated EAE mice were isolated on day 15 postimmunization. (F,H) Flow cytometric analysis of $FoxP3^{EGFP+}$ cells in $CD4^+$ T cells. (G) Flow cytometric analysis of IFN-γ^+ cells and IL-17^+ cells in $CD4^+$ T cells. Data in (H) are presented as mean ± SEM of five biological replicates. $^{**}P < 0.01$, unpaired two-tailed Student's *t*-test.

I Naive $CD4^+$ T cells were sorted from C57BL/6 mice and cultured in Th1, Th2, and Th17 polarizing medium for 3 days with 10 μM synthetic scPEP or miPEP31. The differentiation of Th1, Th2, and Th17 was analyzed by flow cytometry.

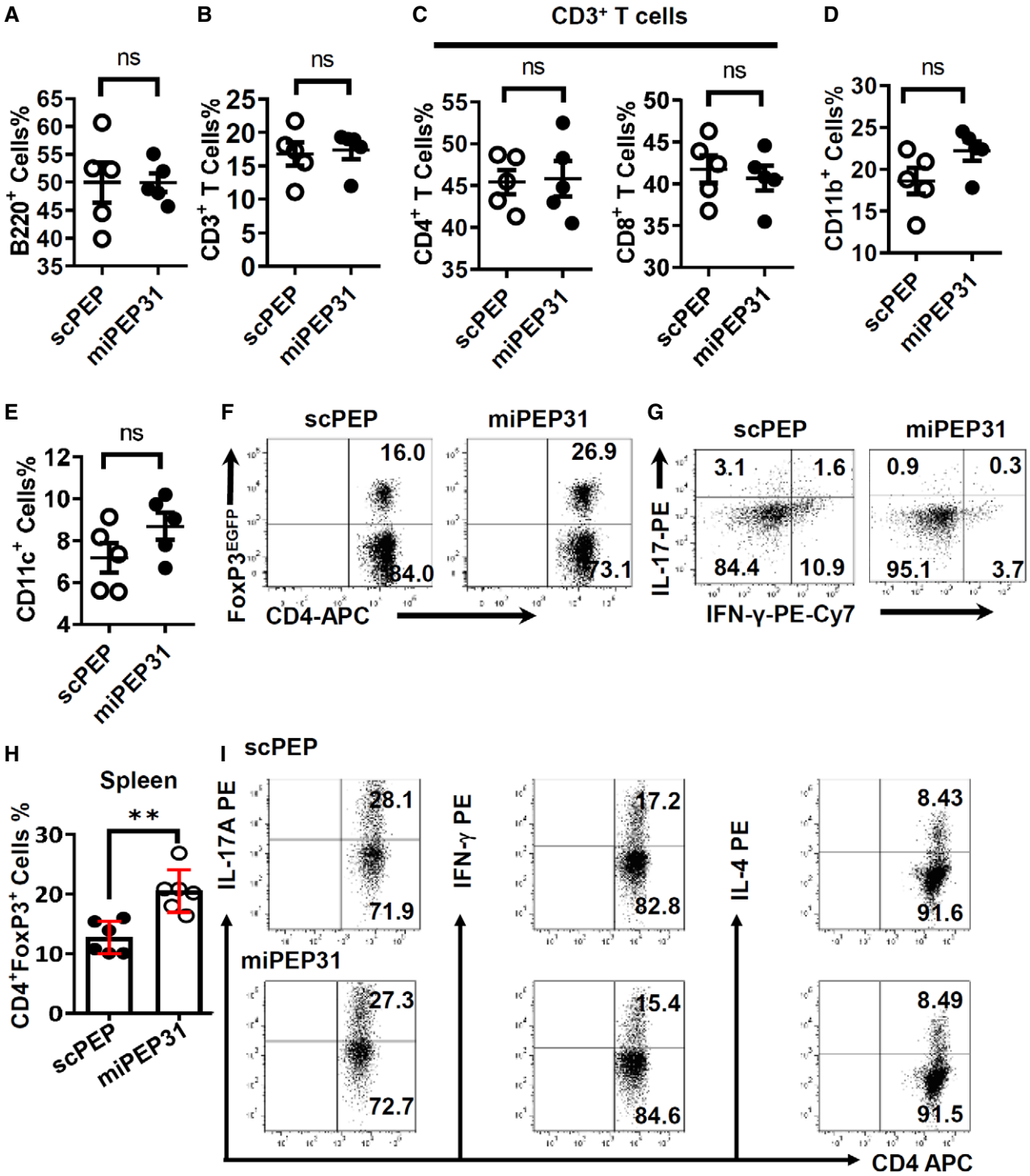


Figure EV2.

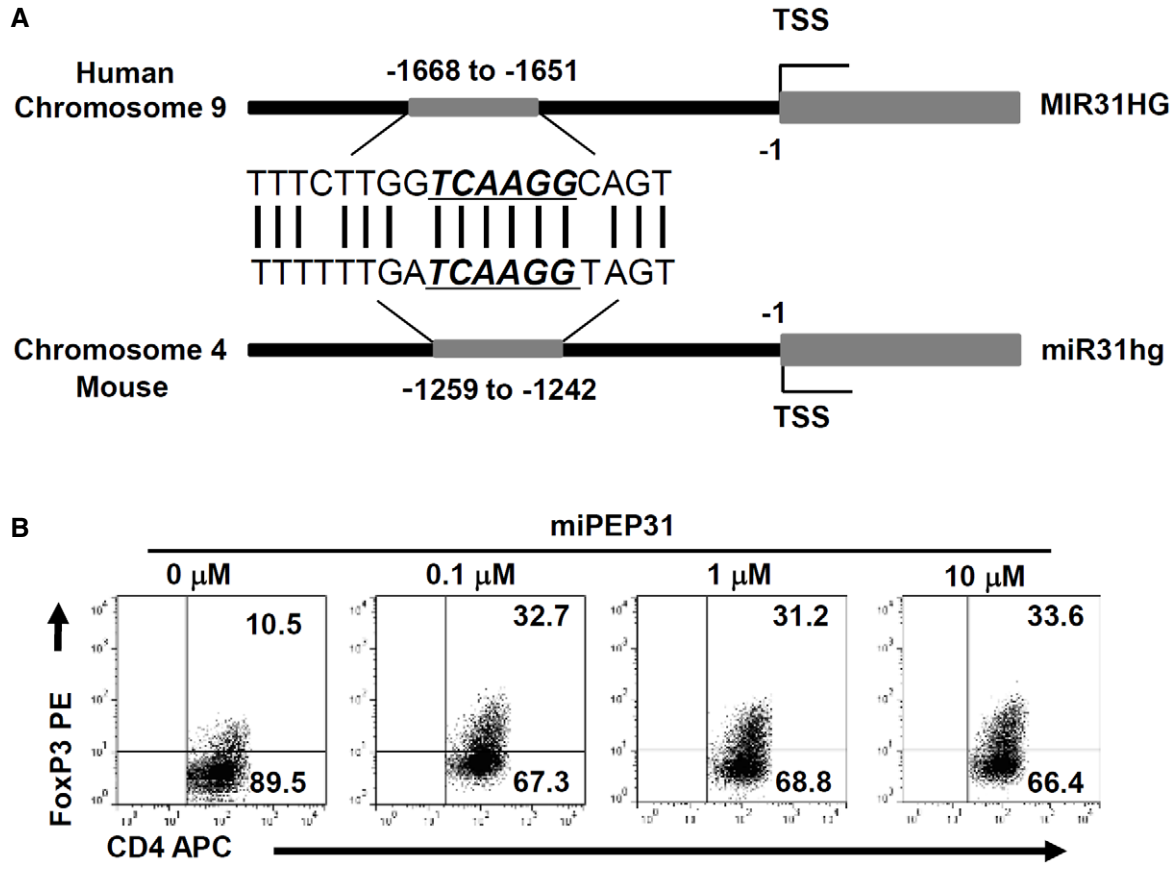


Figure EV3. miPEP31 promotes the differentiation of human T_{reg} cells.

A Homologous alignment of miPEP31-binding site in human and mouse miR-31 host gene promoter.

B Flow cytometric analysis of human T_{reg}-cell induction from $CD4^+$ T cells isolated from peripheral blood mononuclear cells in the presence of different doses of miPEP31. Shown in (B) is representative of three independent experiments.

Figure EV4. Existence of the homologous peptide of miPEP31 in human HCT 116 cells.

A miR-31 host gene in *Mus musculus*, *Rattus norvegicus*, *Homo sapiens*, and *Macaca mulatta*. The coding sequence of miPEP31 located on Exon 2 (106 bp, in blue) and Exon 3 (857 bp, in green) of mouse miR-31 host gene. The coding sequence of miPEP31 (under the green line) on Exon 3 is conserved across the four species.

B Workflow of mass spectrometry analysis to detect small peptides in HCT 116 cells. Protein lysates of HCT 116 cells were subjected to anti-miPEP31 immunoprecipitation. Purified lysates were subject to mass spectrometry analysis.

C Representative secondary mass spectrometry analysis of fragment. Identified fragment ions (632.85 Da, charge = 4) are indicated below the peptide sequence. Shown in (C) is representative of three independent experiments.

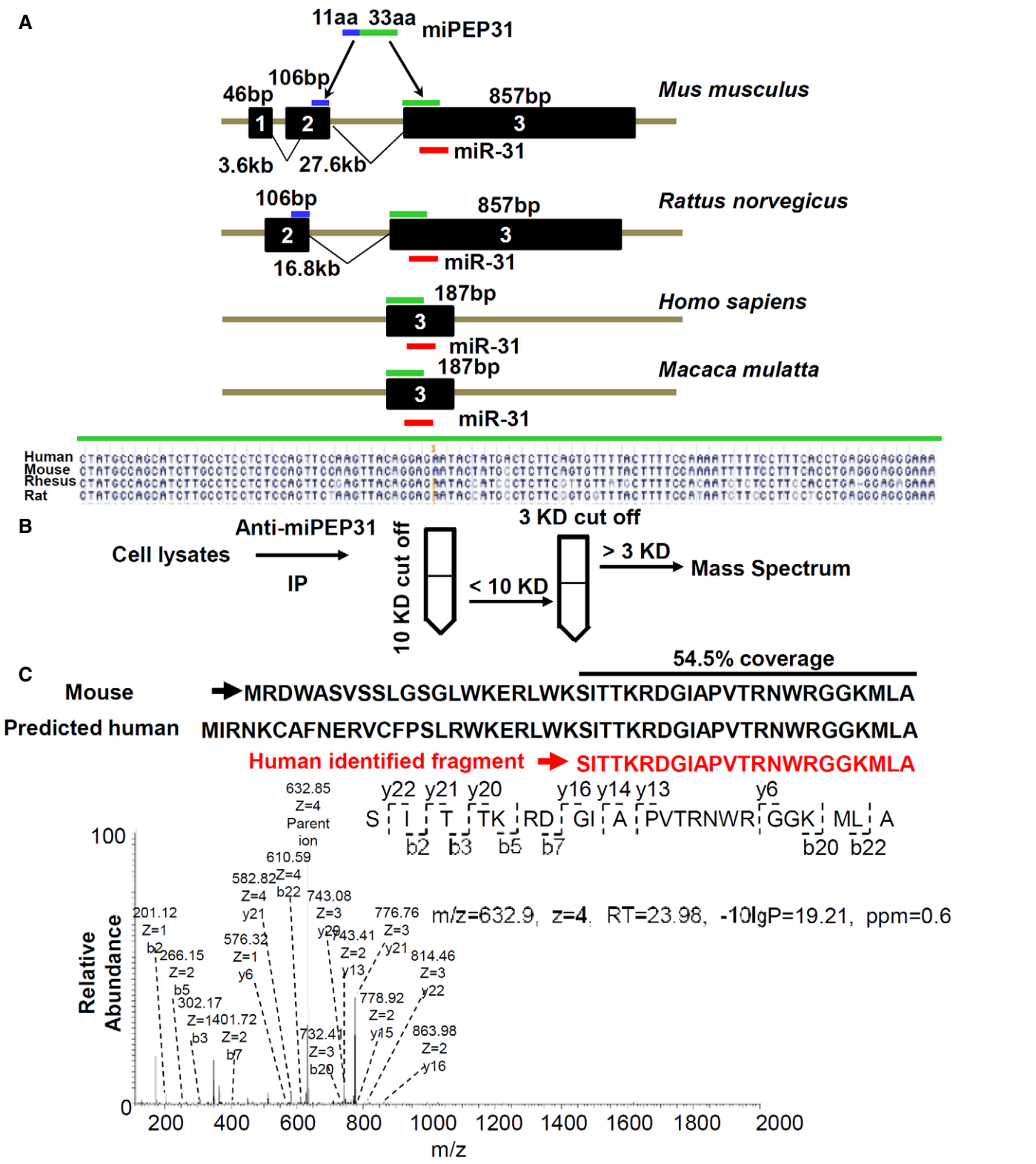


Figure EV4.

Figure EV5. Secondary structures of scPEP and miPEP31.

A, B miPEP31 or scPEP with the concentration of 100 μg/ml in phosphate buffer (A) or phosphate buffer with 50% TFE (B) was analyzed by circular dichroism. The ratio between molar ellipticity at 222 nm and 207 nm was used to confirm an α-helical structure of peptides. The arrows indicate the α-helical structure of miPEP31 and scPEP.

C Secondary structures of miPEP31 and scPEP.

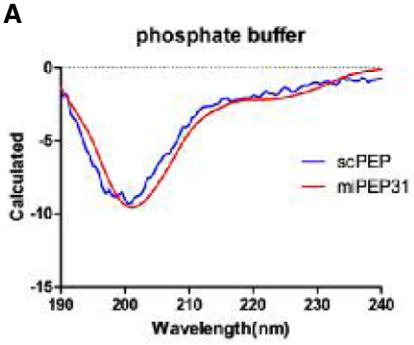
D The secondary structure of miPEP31 was predicted by I-TASSER. H, S, and C stands for helix, stand, or coil tend of different residues.

E The structure model of miPEP31 predicted by I-TASSER.

F $CD4^+$ T cells were activated with anti-CD3/28 and treated with 10 μM FAM-scPEP for 24 h, the fluorescein was detected by flow cytometry.

G, H C57BL/6 mice were injected intravenously with 100 μg FAM-labeled scPEP. Splenocytes were isolated and stained for CD4 and DAPI. Imaging flow cytometry analysis of FAM-scPEP in $CD4^+$ T cells (G) and non-CD4 cells (H). Shown in (G, H) are representative of two independent experiments, scale bar 10 μm.

A



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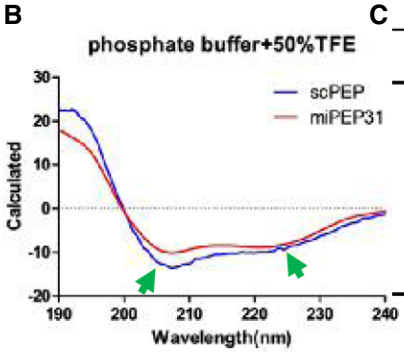

C

	scPEP	miPEP31
Helix	50.5	59.2
Beta	6.3	9.4
Turn	13.5	4.9
Random	29.6	26.4

D

Predicted Secondary Structure

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Sequence    MRDWASVSSLGSGLWKERLWKSITTKRDGIAPVTRNWRGGKMLA
Prediction  CCHHHHHHHHHHHHHHHHHHHHHCCCCCCCCCCCCCCCCCCCCC
Conf.Score  930365542200278999988721044664433135556602 59
            H:Helix; S:Strand; C:Coil
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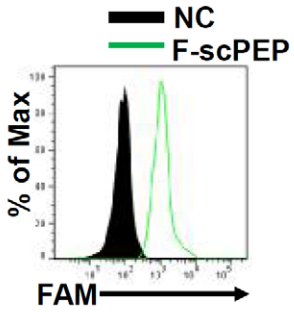
E

F



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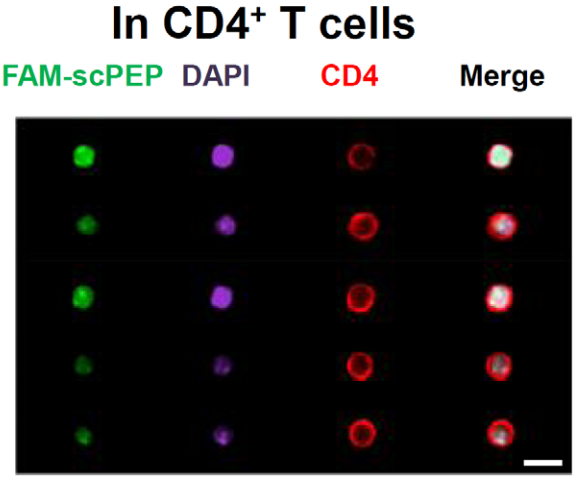

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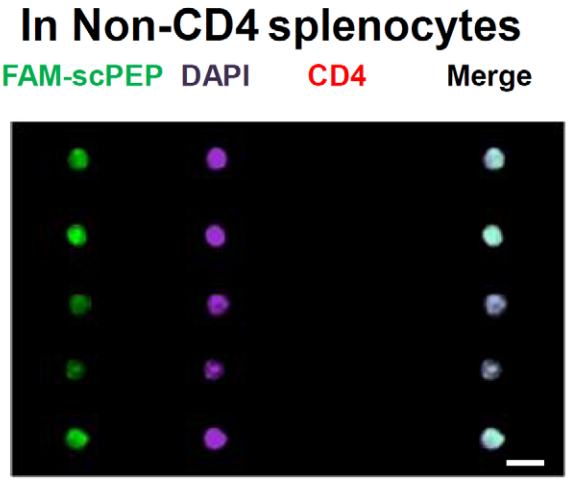

Figure EV5.