Expanded View Figures

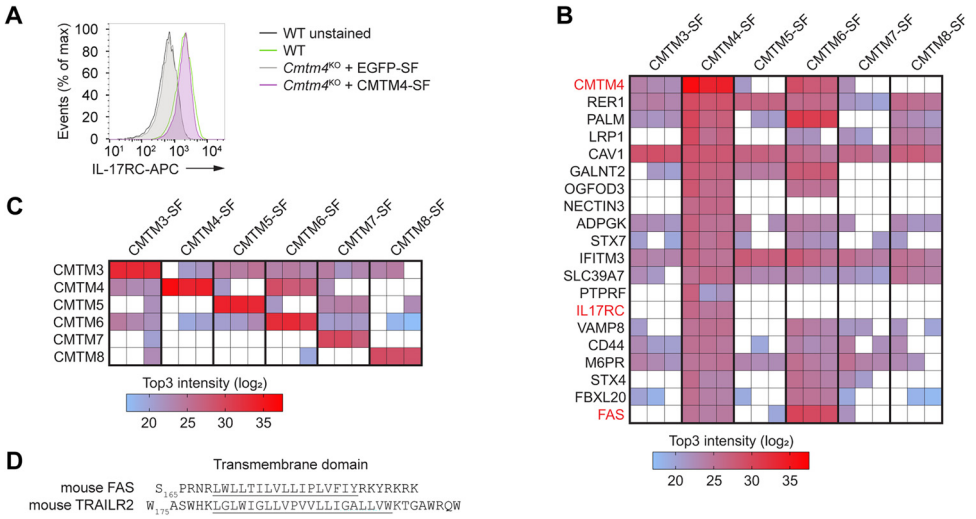

Figure EV1. Analysis of the CMTM family interactome.

(**A**) Flow cytometry analysis of surface IL-17RC expression in ST2 WT or *Cmtm4*^{KO} cells transduced with expression vectors encoding EGFP-SF or CMTM4-SF. (**B**) Mass spectrometry analysis of the indicated Strep-Flag (SF)-tagged CMTM family members isolated from mouse ST2 cells via tandem affinity purification. The heatmap displays the most abundant CMTM4-associated proteins that were not detected in EGFP-SF control samples, based on Top3 intensities from 3 independent experiments. (**C**) Heatmap showing Top3 intensity values for the associations between various CMTM family members. (**D**) Sequence comparison of murine FAS and TRAILR2 transmembrane (underlined) and juxtamembrane domains. Source data are available online for this figure.

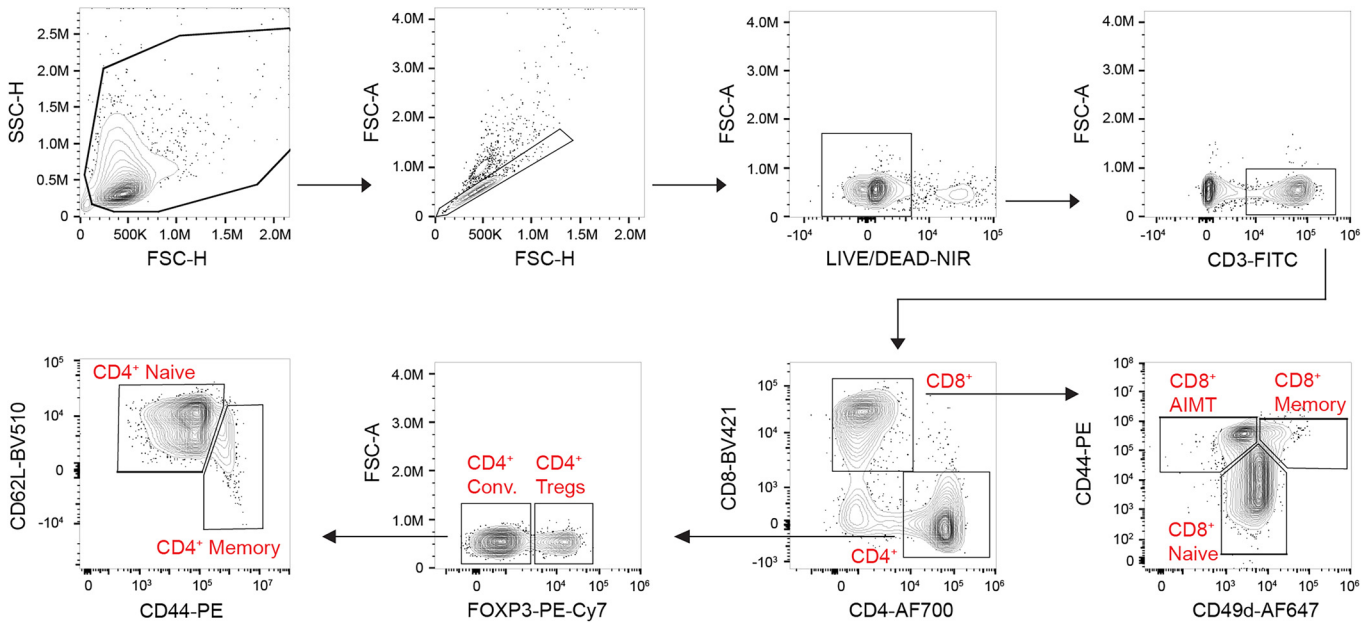

Figure EV2. Mouse T cells gating strategy.

The gating strategy used for analyzing T cells isolated from the spleen (Fig. 3) and peripheral lymph nodes (Fig. EV3).

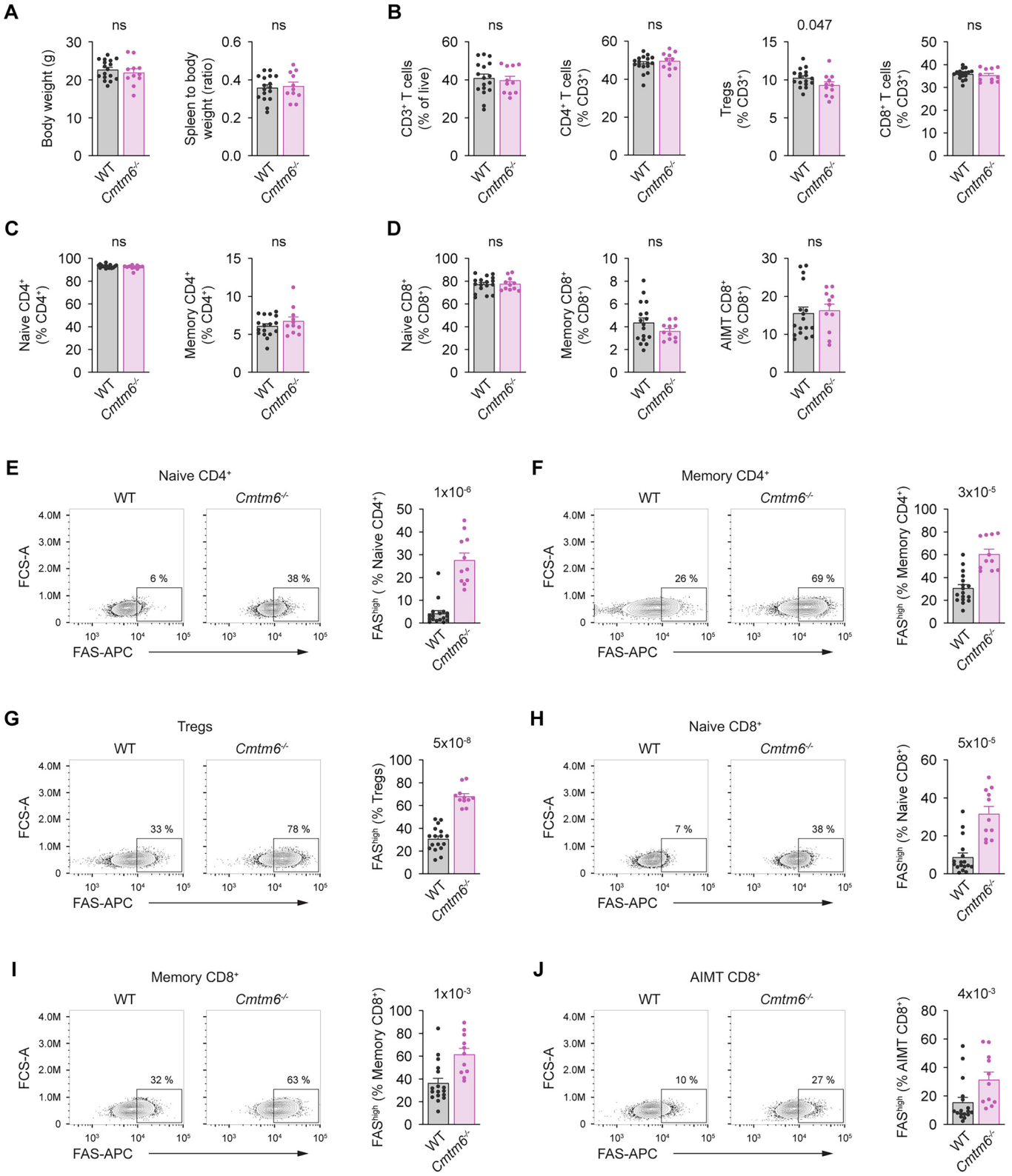
A
Body weight (g)
Spleen to body weight (ratio)
ns
WT
Cmtm6-/-
B
CD3+ T cells (% of live)
CD4+ T cells (% CD3+)
Tregs (% CD3+)
CD8+ T cells (% CD3+)
0.047
C
Naive CD4+ (% CD4+)
Memory CD4+ (% CD4+)
D
Naive CD8+ (% CD8+)
Memory CD8+ (% CD8+)
AIMT CD8+ (% CD8+)
E
Naive CD4+
6 %
38 %
FAS-APC
FCS-A
FAShigh (% Naive CD4+)
1x10-6
F
Memory CD4+
26 %
69 %
FAShigh (% Memory CD4+)
3x10-5
G
Tregs
33 %
78 %
FAShigh (% Tregs)
5x10-8
H
Naive CD8+
7 %
38 %
FAShigh (% Naive CD8+)
5x10-5
I
Memory CD8+
32 %
63 %
FAShigh (% Memory CD8+)
1x10-3
J
AIMT CD8+
10 %
27 %
FAShigh (% AIMT CD8+)
4x10-3

Figure EV3. CMTM6 suppresses FAS expression in T cells isolated from mouse lymph nodes.

(**A**) Body weight and spleen-to-body weight ratio in 8-12-week-old WT and *Cmtm6*$^{-/-}$ mice. (**B**) Flow cytometry analysis of T cells isolated from peripheral lymph nodes of 8-12-week-old WT and *Cmtm6*$^{-/-}$ mice. T cells (gated as CD3^{+}) were subdivided into conventional CD4^{+} (CD4^{+}, FOXP3^{-}), Tregs (CD4^{+}, FOXP3^{+}), and conventional CD8^{+} (CD8^{+}). (**C**) Conventional CD4^{+} T cells were further gated as naïve (CD44^{-}, CD62L^{+}) and memory (CD44^{+}, CD62L^{-}). (**D**) Conventional CD8^{+} T cells were gated as naïve (CD44^{-}), memory (CD44^{+}, CD49d^{+}), and antigen-inexperienced memory T (AIMT) (CD44^{+}, CD49d^{-}) cells. (**E–J**) FAS expression in the indicated subsets of T cells isolated from peripheral lymph nodes. Data are presented as mean + SEM, $n = 17$ WT and 11 *Cmtm6*$^{-/-}$ mice. Two-tailed Mann-Whitney test. ns not significant. Source data are available online for this figure.

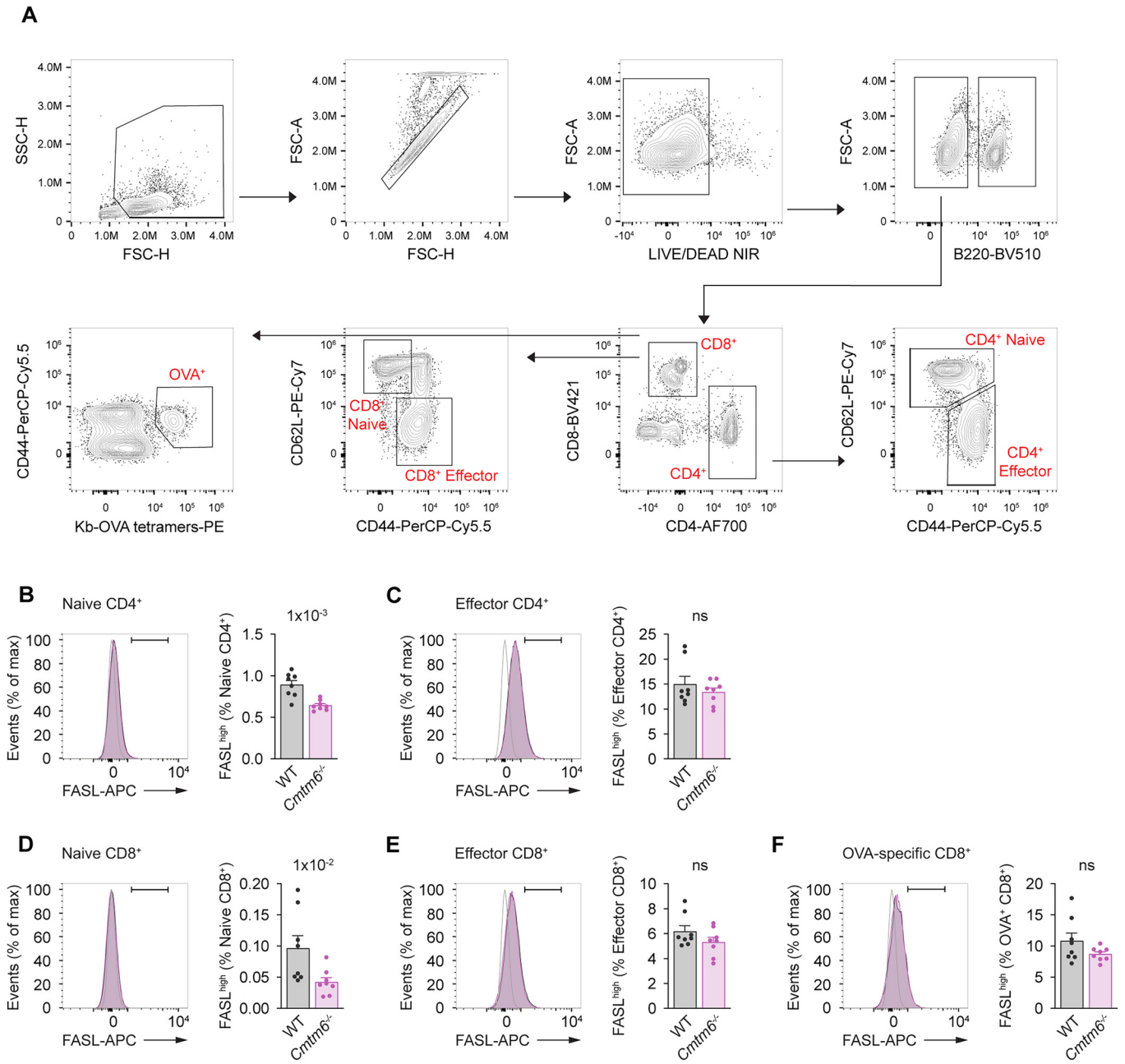

Figure EV4. Role of CMTM6 in infection with *L. Monocytogenes*.

(**A**) The gating strategy used for the analysis of T cells isolated from the spleens of mice 10 days post-infection with *L. Monocytogenes* (Figs. 4 and EV4). (**B–F**) Surface FASL levels in the indicated subsets of splenic T cells. Data are presented as mean + SEM from two independent experiments. $n = 8$ mice per group. Two-tailed Mann-Whitney statistical test. ns not significant. Source data are available online for this figure.

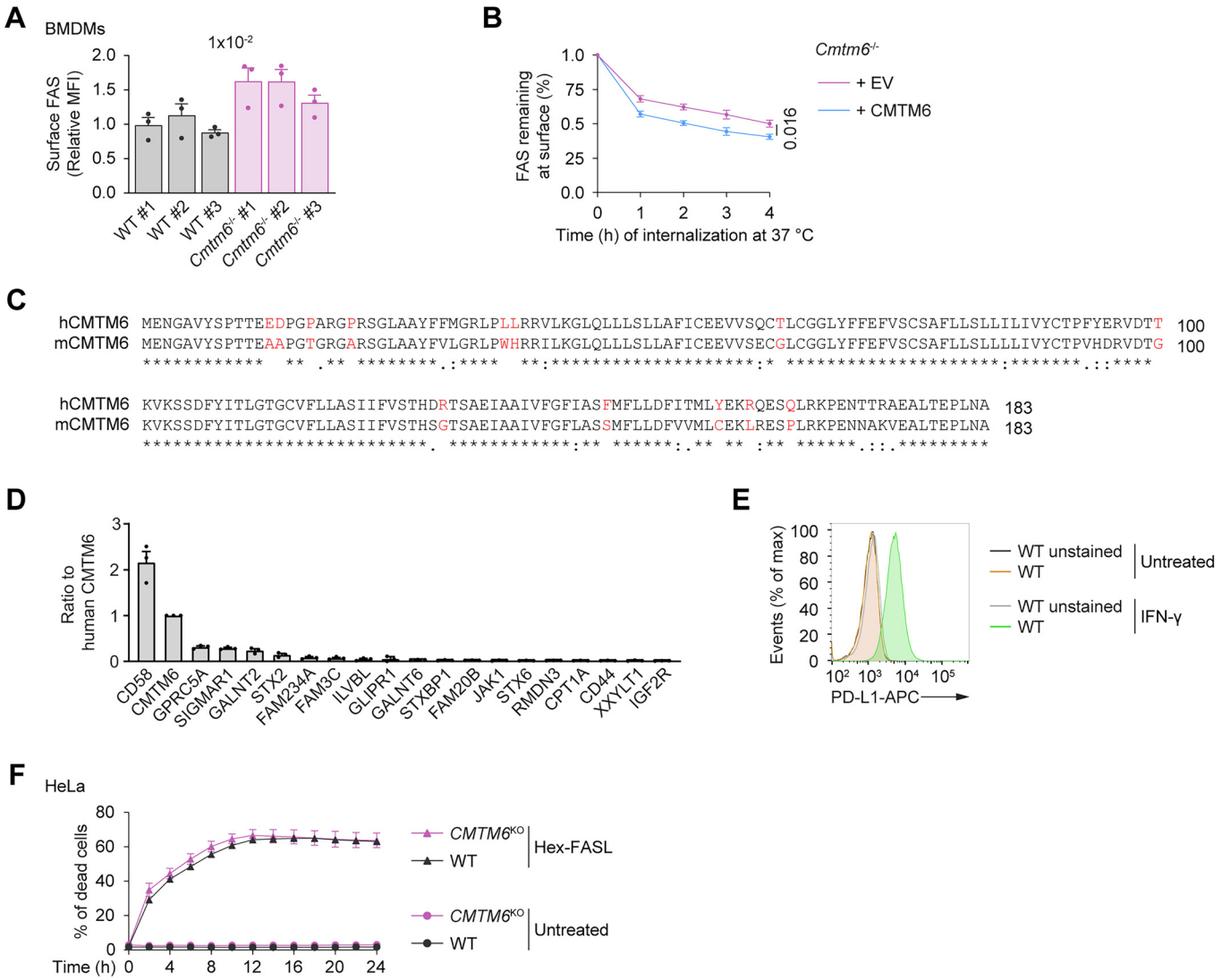

Figure EV5. CMTM6 regulates FAS surface expression and activity in mice, but not humans.

(**A**) Flow cytometry analysis of surface FAS expression in bone BMDMs isolated from 10-week-old WT and *Cmtm6*$^{-/-}$ mice, $n = 3$ per group. Cells from each mouse were analyzed in three separate experiments. (**B**) FAS internalization assay in *Cmtm6*$^{-/-}$ MEFs (clones #1 and #2) reconstituted with murine CMTM6 or empty vector (EV). Cells were labeled on ice with unconjugated FAS antibody and subsequently incubated for the indicated time at 37 °C. The remaining surface FAS was detected using a fluorescently labeled secondary antibody and analyzed by flow cytometry. (**C**) Comparison between murine and human CMTM6 sequences. (**D**) Mass spectrometry analysis of the SF-tagged human CMTM6 isolated from unstimulated HeLa cells via tandem affinity purification. The 20 most abundant CMTM6 interactors not detected in control EGFP-SF samples are shown, based on Top3 intensities from 3 independent experiments. (**E**) Flow cytometry analysis of surface PD-L1 expression in WT HeLa cells stimulated or not with human IFN-γ (100 ng/ml). (**F**) Cell death induction in WT or CMTM6-deficient HeLa cells stimulated or not with Hex-FASL (500 ng/ml). Cell death was monitored every 2 h using Incucyte. Data are presented as mean + SEM (**A**) or mean ± SEM (**B, F**). Data are representative of two (**F**), three (**A**, **B**, **D**), or five (**E**) independent experiments using two different knockout clones (**B**, **F**). One-way ANOVA (**A**), unpaired two-tailed t-test (**B**). ns not significant, MFI median fluorescent intensity. Source data are available online for this figure.

