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Supplemental information

Functional assessment of the cell-autonomous role of NADase CD38 in regulating $CD8^+$ T cell exhaustion

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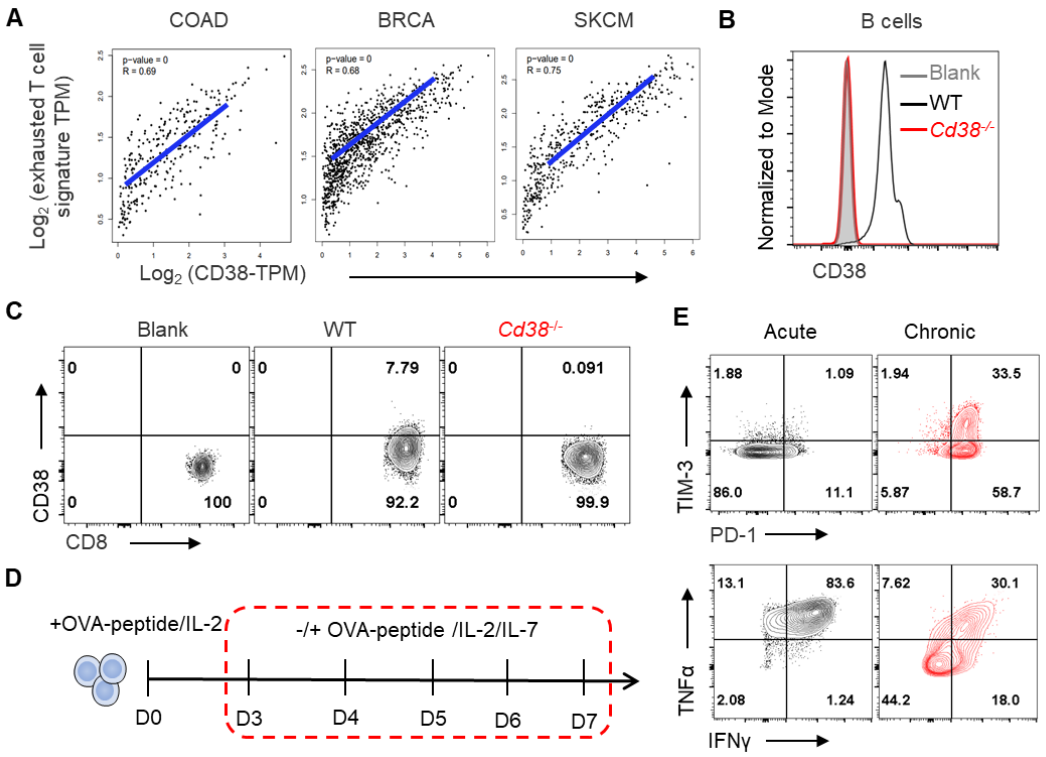

Figure S1. Flow cytometry analysis the expression of CD38 and antigen chronic stimulation model, Related to Figure 1. (A) The correlation between the expression of CD38 and exhausted T-cell signature genes in human colon adenocarcinoma (COAD), breast invasive carcinoma (BRCA), or skin cutaneous melanoma (SKCM) was performed using the GEPIA 2 online tool (Tang et al., 2019). TPM, transcripts per kilobase of exon model per million mapped reads. **(B)** Flow cytometry analysis of CD38 expression in WT or *Cd38*$^{-/-}$ mice B cells. **(C)** Representative FACS plots of CD38 expression in WT or *Cd38*$^{-/-}$ OT-1 cells at 4th day after activation. **(D)** Schematic representation of antigen persistent stimulation model. (E) Analysis of PD-1, TIM-3 expression and cytokines TNFα, IFNγ production of OT-1 cells after persistent antigen stimulation or not.

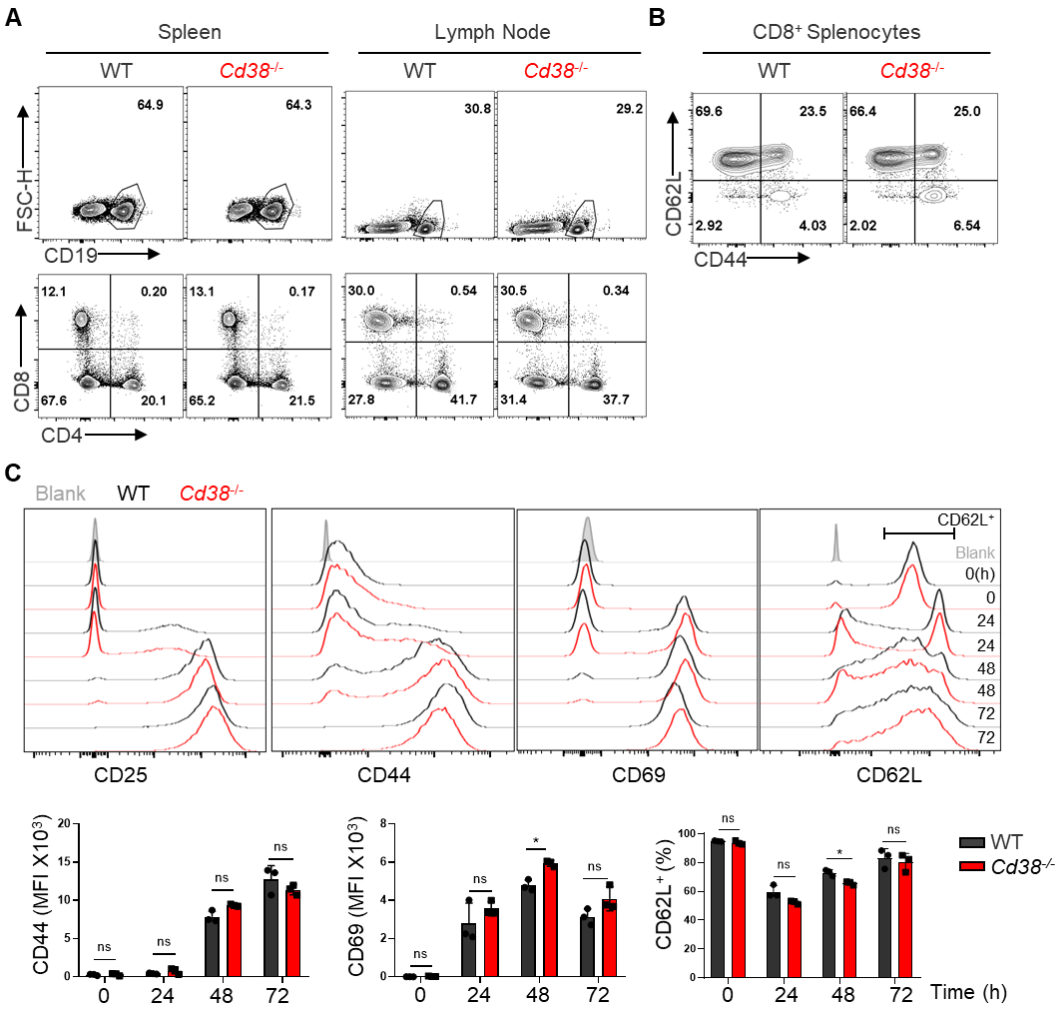

Figure S2. **The development and activation phenotype of *Cd38* knockout CD8$^+$ T cells, Related to Figure 2. (A)** Representative contour plots of CD4, CD8$^+$ or B cells in splenocytes or lymph node cells from 8-week-old mice of the indicated genotypes, respectively. **(B)** Representative contour plots of CD62L and CD44 expression in CD8$^+$ T cells from the spleen of WT or *Cd38*$^{-/-}$ mice. **(C)** Upper: The representative histogram of surface activation marker CD25, CD44, CD69 and CD62L expression in WT or *Cd38*$^{-/-}$ OT-1 cells during αCD3/CD28 activation. Down: statistical analysis of activation markers expression. Data are shown as Mean ± SD (n = 3), Student's t-test, *: $P < 0.05$; ns: not significant.

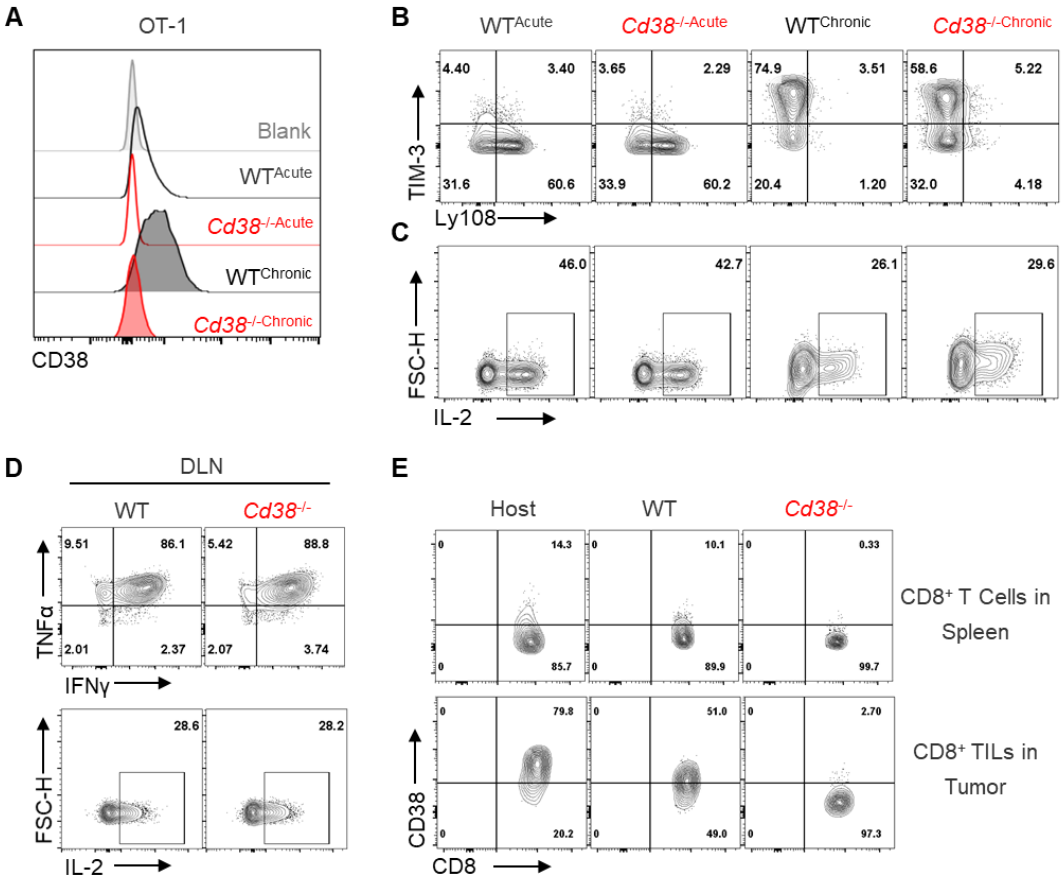

Figure S3. Specific flow cytometry information, Related to Figure 3 and 4. (A) The representative histogram of CD38 expression in indicated cells. **(B)** Representative contour plots of Ly108 and TIM-3 expression in WT or *Cd38*$^{-/-}$ OT-1 cells after persistent antigen stimulation or not. **(C)** Representative contour plots of IL-2 expression of indicated cells after treatment. **(D)** Representative contour plots of indicated OT-1 cells from recipient mice DLN producing cytokines TNFα, IFNγ, IL-2 after stimulation with PMA and Ionomycin. (**E**) The representative contour plots of CD38 expression in tumor infiltrated host, adoptive transferred WT or CD38 knockout CD8+ T cells.

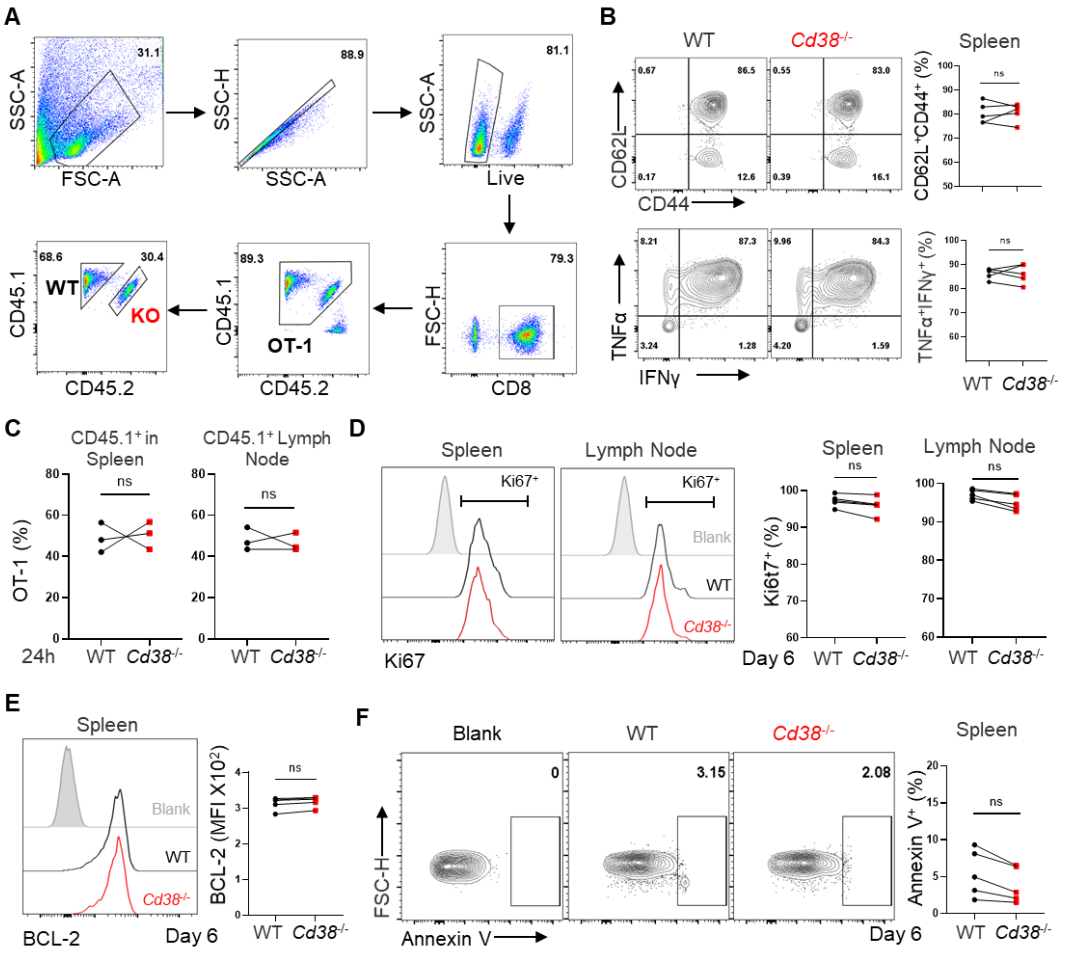

Figure S4. The deficiency of CD38 did not impact the migration and apoptosis of OT-1 cells in vivo, Related to Figure 5. (A) Representative FACS plots of gating scheme for WT and CD38 knockout OT-1 cells. **(B)** Left: representative contour plots of CD62L, CD44 expression, or TNFα, IFNγ production in two genotypes OT-1 cells from recipient mice spleen. Right: summary of the frequencies of $CD62L^{+}CD44^{+}$ or $TNF\alpha^{+}IFN\gamma^{+}$ OT-1 cells in the spleen. Above all error bars in this figure indicate Mean ± SD (n = 5), Student's t-test, ns: not significant. **(C)** Naïve WT or Cd38-/- OT-1 cells were mixed at a 1:1 ratio, and then were co-adoptive transferred in recipient mice. Analysis percentage of indicated OT-1 cells in total transferred OT-1 cells of recipient mice spleen after 24 h. **(D)** Flow cytometry analysis and summary of $Ki67^{+}$ WT or *Cd38*$^{-/-}$ OT-1 cells in the spleen and lymph node at day 6 post OVA peptide-stimulation as Fig.5f. **(E)** Histogram and summary of BCL-2 expression in the recipient mice spleen OT-1 cells. **(F)** Representative contour plots and summary of Annexin V^{+} WT or *Cd38*$^{-/-}$ OT-1 cells in the spleen at day 6 post activation by OVA peptide.

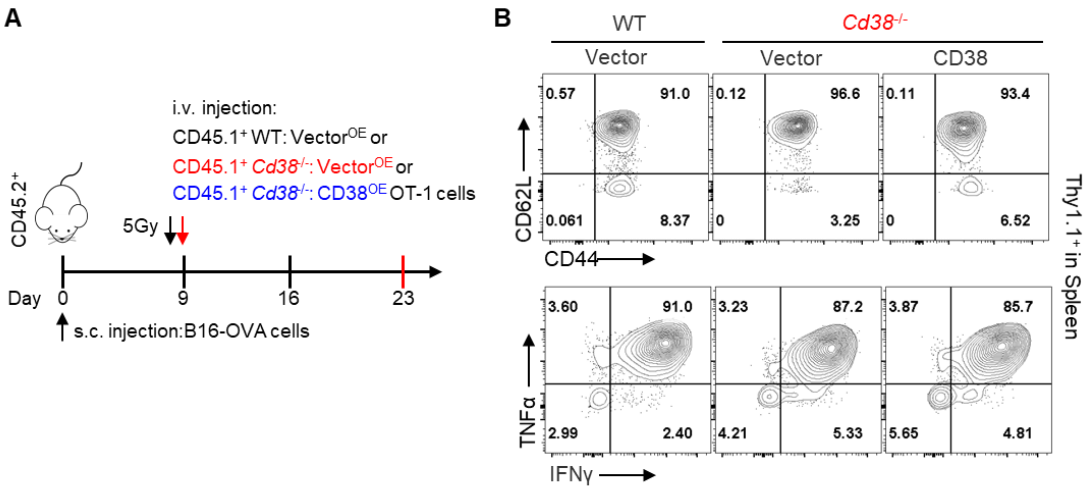

Figure S5. The phenotype of CD38 overexpressed OT-1 cells in the spleen, Related to Figure 6. (A) Schematic representation of adoptive transfer indicated OT-1 cells with CD38 overexpression or not in the tumor model. **(B)** Representative contour plots of CD62L, CD44 expression, or TNFα, IFNγ production of three Thy1.1$^+$ groups in recipient mice spleen.

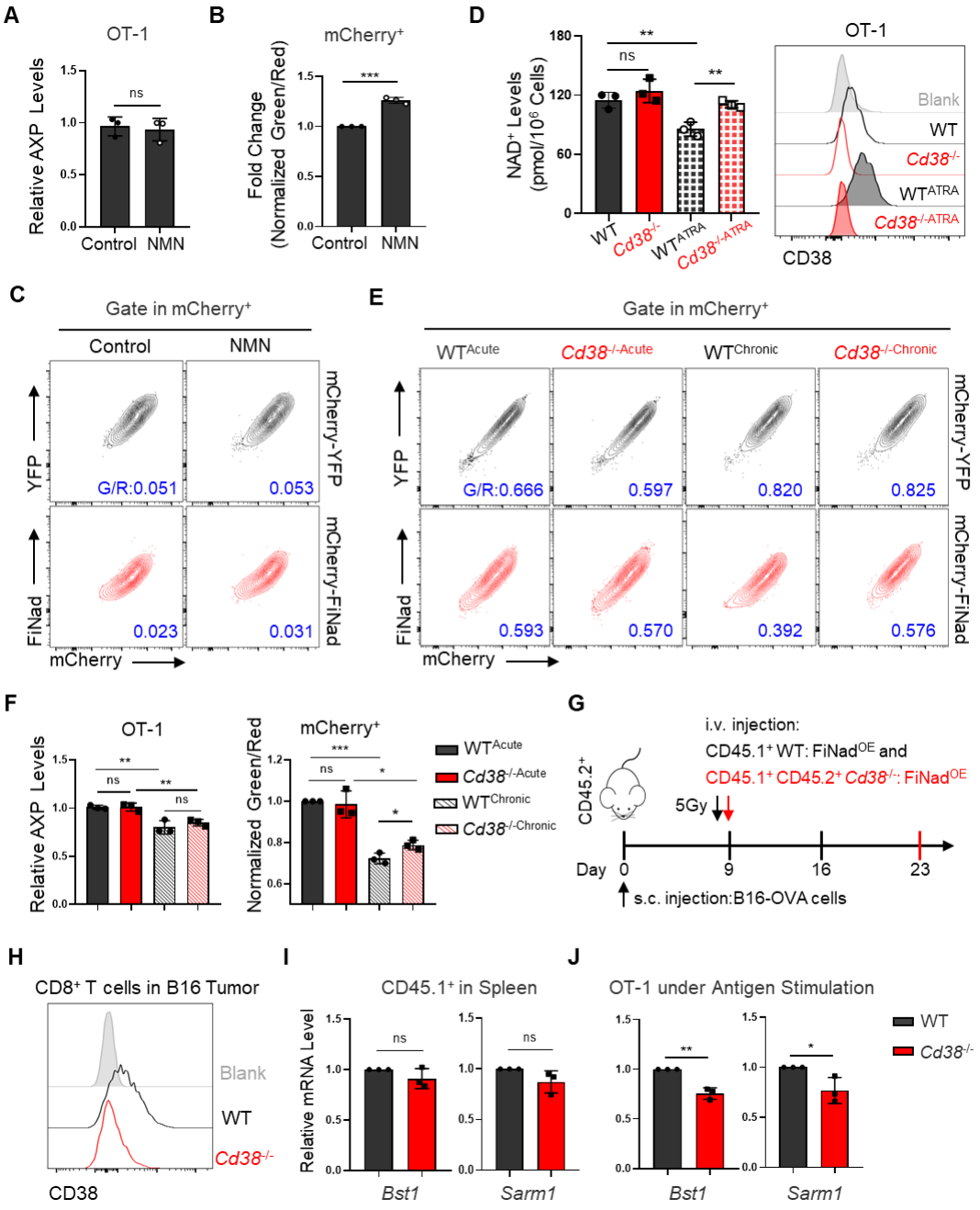

Figure S6. CD38 played a crucial role in regulating NAD+ levels in vitro, Related to Figure 7. **(A)** WT OT-1 cells were treated with 0.1mM NMN for 48 h or not. The relative AXP (ATP + ADP) levels were measured by kit as the method. Data are shown as Mean ± SD (n = 3), Student's t-test, ns, not significant. **(B)** The mCherry-YFP or mCherry-FiNad overexpressed-WT OT-1 cells were treated with 0.1mM NMN for 48 h or not. Comparing the fold change of normalized mCherry-FiNad (green to red) ratios in two groups. Data are shown as Mean ± SD (n = 3), Student's t-test, ***: $P < 0.001$. **(C)** Representative FACS plots of mCherry-YFP and mCherry-FiNad fluorescence intensity in WT OT-1 cells under NMN treatment or not. **(D)** Left: Activated WT or *Cd38*$^{-/-}$ OT-1 cells treated with 500 nM ATRA for 72h, the equal number of indicated alive cells were collected

and detecting the NAD^+ levels by assay kit. Right: The representative histogram of CD38 expression in indicated cells. **(E)** Representative FACS plots of mCherry-YFP and mCherry-FiNad fluorescence intensity in WT or *Cd38*$^{-/-}$ OT-1 cells under antigen chronic stimulation or not. **(F)** Left: Summary of the relative AXP (ATP + ADP) levels of WT or *Cd38*$^{-/-}$ OT-1 cells under antigen chronic stimulation or not. Right: Summary of the relative of normalized mCherry-FiNad (green to red) ratios in WT or CD38 knockout OT-1 cells under persistent antigen stimulation or not. Data are shown as Mean ± SD (n = 3), Student's t-test, ***: $P < 0.001$; **: $P < 0.01$; *: $P < 0.05$; ns: not significant. **(G)** Schematic representation of co-adoptive transfer mCherry-FiNad expressed-WT or CD38 knockout OT-1 cells strategy. **(H)** The B16-F10 tumors were implanted in WT or *Cd38*$^{-/-}$ C57BL/6 female mice, after 15 days, flow cytometry analyzed the CD38 expression of tumor-infiltrating $CD8^+$ T cells. **(I)** qPCR experiment showed the relative mRNA levels of Bst1 and Sarm1 in WT or *Cd38*$^{-/-}$ OT-1 cells from recipient mice spleen. Data are shown as Mean ± SD (n = 3), Student's t-test, ns: not significant. **(J)** The transcript levels of Bst1 and Sarm1 of indicated OT-1 cells after persistent antigen stimulation.

Table S1. **Oligonucleotides sequence used in this paper, Related to STAR Methods.**

sgRNA sequence to target *Cd38* (5'-3')		
sgRNA-*Cd38*-1	AAGCTTCTCAGTCTAGACTCTGG	
sgRNA-*Cd38*-2	CTGATTCTCAACCCGAACTAGGG	
sgRNA-*Cd38*-3	CAGTGACCTTAGTGACAAACTGG	
sgRNA-*Cd38*-4	AGGGGAAACATCACCATAAGAGG	
Primers used in plasmids construction	PF(5'-3')	PR(5'-3')
CD38 (mouse)	CCGCTCGAGATGGCTAACTATGAATTTAGC	CCCAAGCTTCGTATTAAGTCTACACGATGG
mCherry-YFP	CGGAATTCGCCACCATGGTGAGCAAGGGCGAGG	CCCAAGCTTTTAGTTGTACTCCAGCTTGTGC
mCherry-FiNad	CGGAATTCGCCACCATGGTGAGCAAGGGCGAGG	CCCAAGCTTTTAGCCCATCATCTCCTCCCGCC
Primers used in qPCR		
Cd38 (mouse)	TCTCTAGGAAAGCCCAGATCG	AGAAAAGTGCTTCGTGGTAGG
B2m (mouse)	AGACTGATACATACGCCTGCAG	GCAGGTTCAAATG AATCTTCAG
Bst1 (mouse)	AGGGACAAGTCACTGTTCTGG	AACTTTGCCATACAGCACGTC
Sarm1 (mouse)	TTCCTTGGCTCCAGAAATGCT	GACCCTGAGTTCCTCCGGTA
Slc12a8 (mouse)	GGGGAGTCATTCTTCACTGTG	GTCAGCAGGGTCTCTCAGGT
Nampt (mouse)	GCAGAAGCCGAGTTCAACATC	TTTTCACGGCATTCAAAGTAGGA