Appendix

Postnatal expression of CD38 in astrocytes regulates synapse formation and adult social memory

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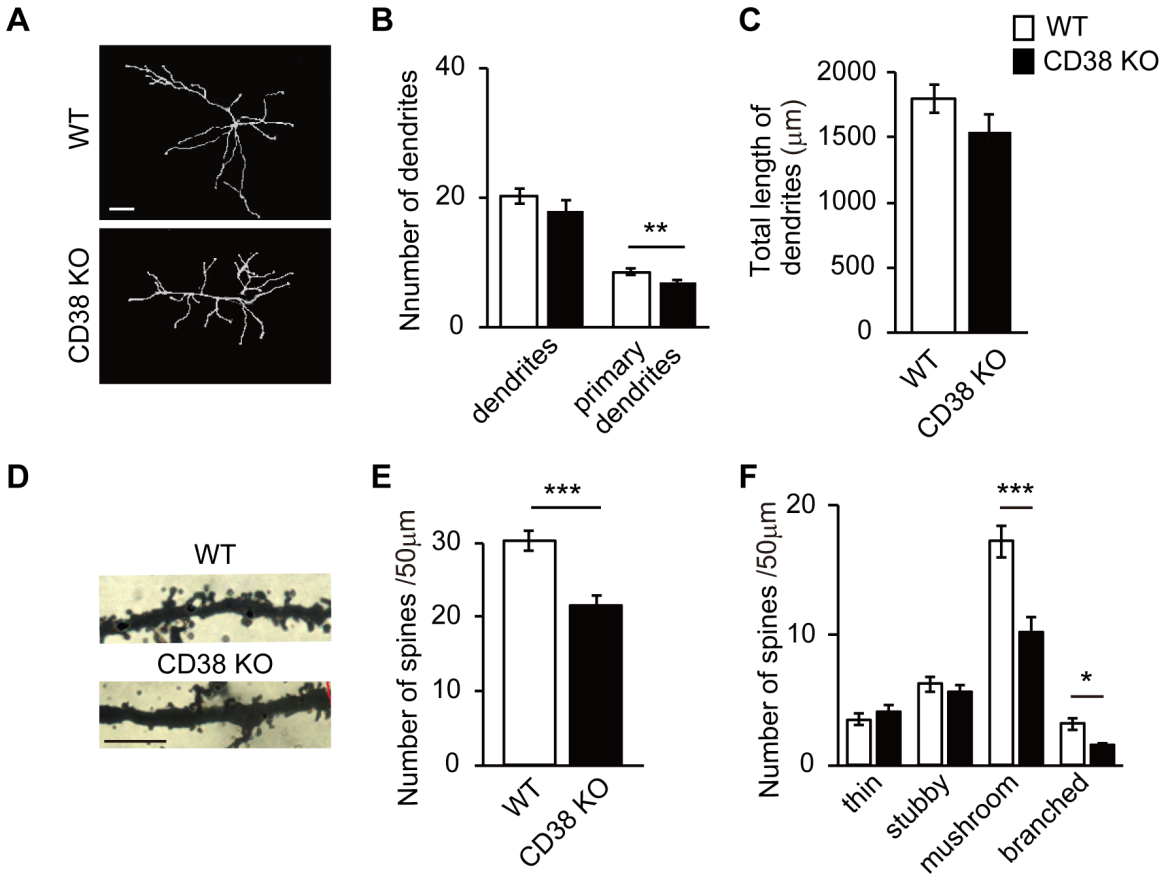


Appendix Figure S1. Morphology of dendrites and spines is altered in the mPFC pyramidal neurons in constitutive CD38 KO mice.

(A) Images of tracing of Golgi-stained pyramidal neurons in the mPFC of WT and CD38 KO mice. Scale bar, 20 μm.

(B, C) Quantification of the total number (B) or total length (C) of dendrites and primary dendrites in WT and CD38 KO mice. (n = 24 cells from four animals per genotype, two-way ANOVA followed by Bonferroni's multiple comparisons test).

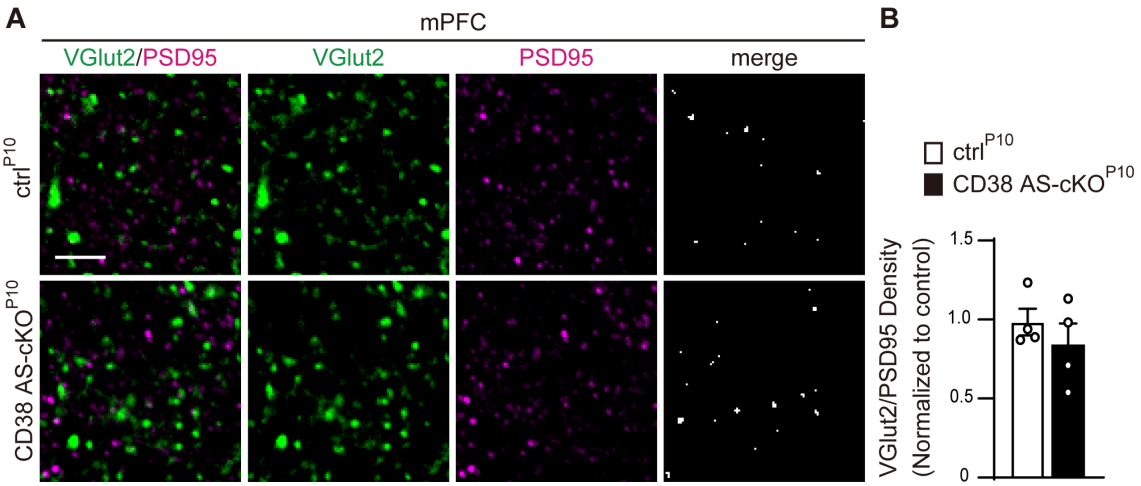
(D) Images of Golgi-stained pyramidal neurons in the mPFC of WT and CD38 KO mice. Scale bar, 5 μm.

(E) Quantification of the total number of spines on apical dendrites of WT and CD38 KO mice. (n = 16 dendrites from four animals per genotype, Student's unpaired t-test).

(F) The percentage of branched, mushroom, stubby and thin-type spines in cortical apical dendritic spines. (n = 16 dendrites from four animals per genotype, two-way ANOVA followed by

Bonferroni's multiple comparisons test).

Data information: $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ vs WT. Data represent means ± SEM.

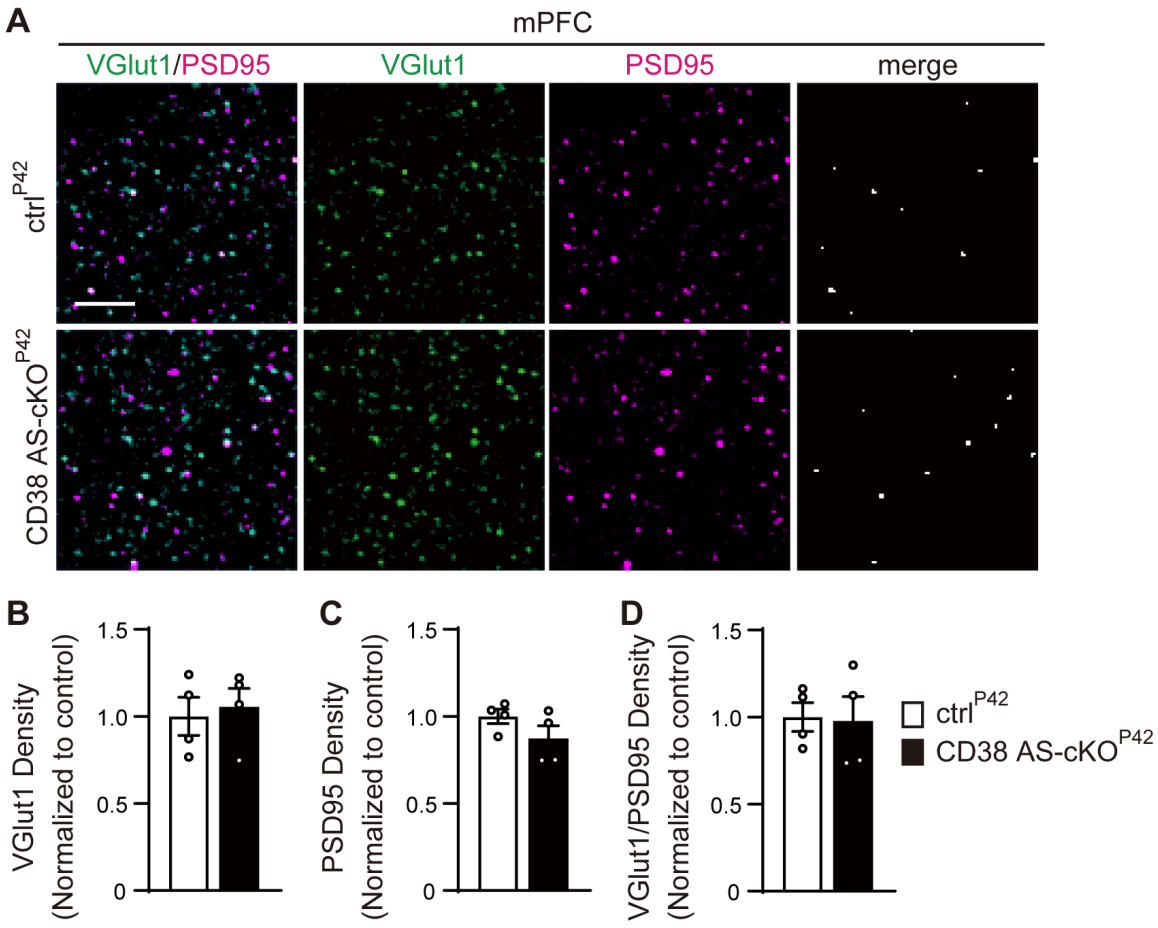


Appendix Figure S2. Number of VGlut2+ excitatory synapses are not changed in the mPFC of CD38 AS-cKO^{P10} mice.

(A) Immunohistochemistry for VGlut2 (green) and PSD95 (magenta) in the mPFC of ctrl^{P10} and CD38 AS-cKO^{P10} mice at P70. The VGlut2 and PSD95 channels are separated in the middle panels. Right panels are colocalized VGlut2 and PSD95 puncta. Scale bar, 10 μm.

(B) Quantification of VGlut2+ synapse densities in the mPFC in ctrl^{P10} and CD38 AS-cKO^{P10} mice. (n = 4 animals per genotype, Student's unpaired t-test).

Data information: Data represent means ± SEM.

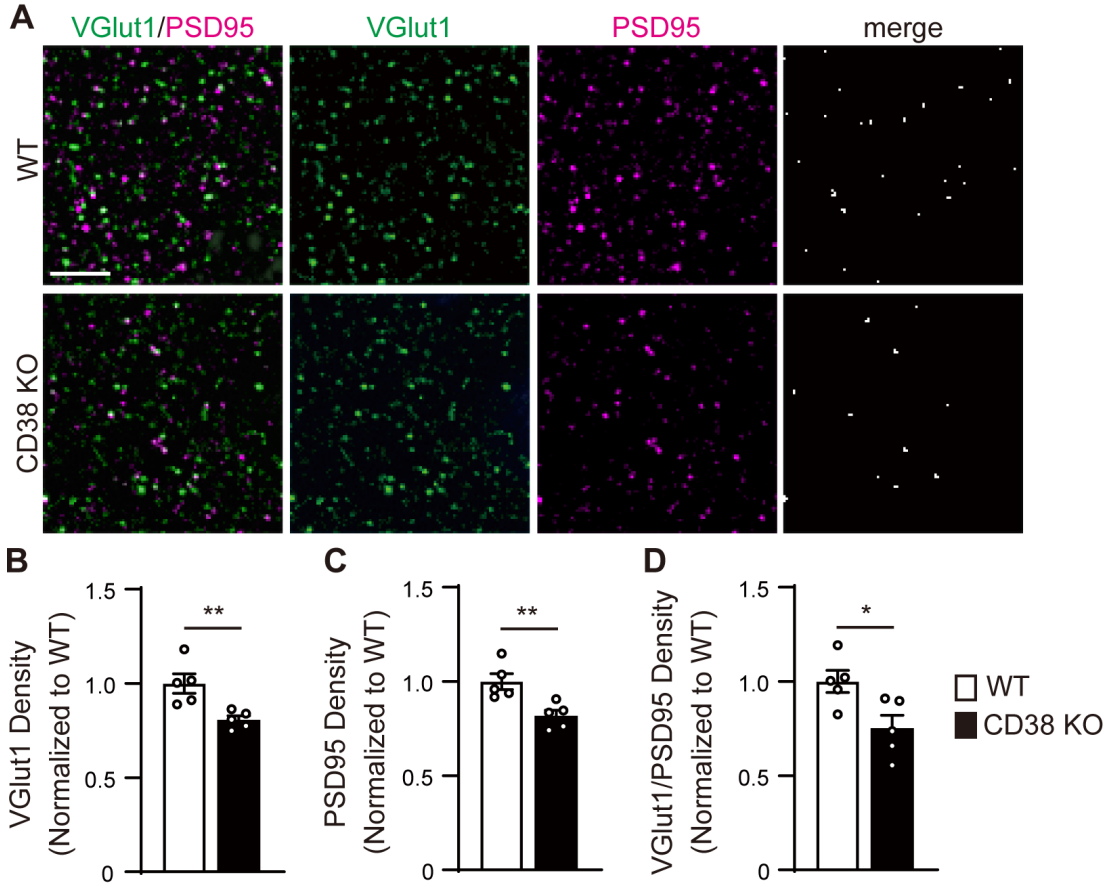


Appendix Figure S3. Number of excitatory synapses are not changed in the mPFC cortical pyramidal neurons in CD38 AS-cKO^{P42} mice.

(A) Immunohistochemistry for VGlut1 (green) and PSD95 (magenta) in the mPFC of ctrl^{P42} and CD38 AS-cKO^{P42} mice at P70. The VGlut1 and PSD95 channels are separated in the middle panels. Right panels are colocalized VGlut1 and PSD95 puncta. Scale bar, 10 μm.

(B–D) Quantification of VGlut1, PSD95 and synapse densities in the mPFC in ctrl^{P42} and CD38 AS-cKO^{P42} mice. (n = 4 animals per genotype, Student's unpaired t-test).

Data information: Data represent means ± SEM.

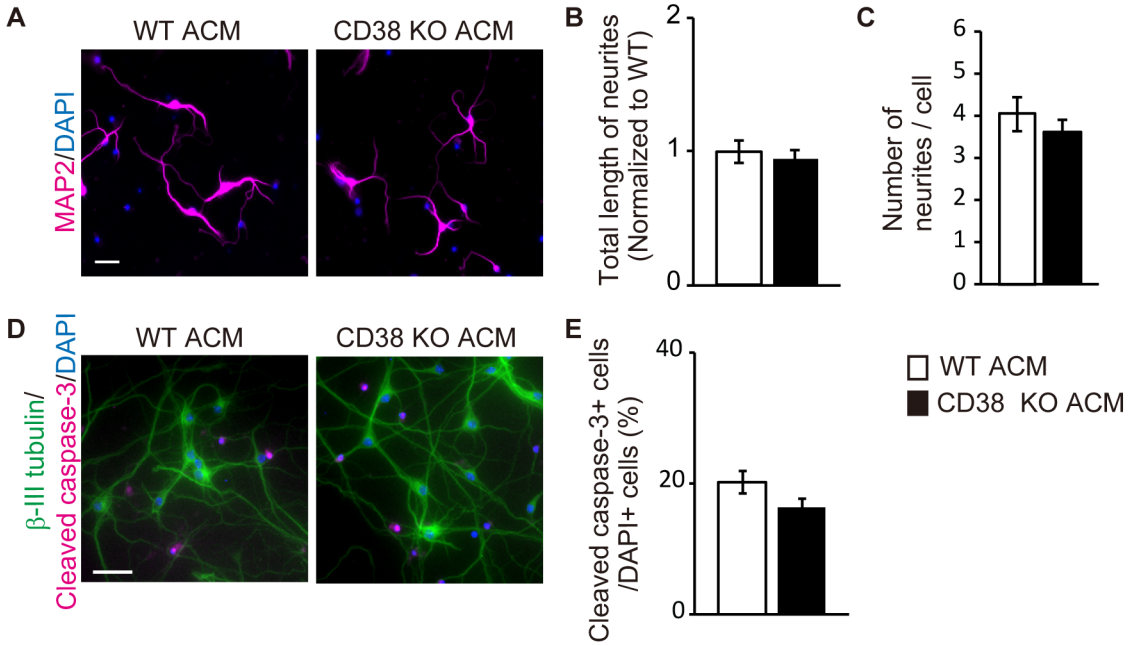


Appendix Figure S4. Excitatory synapses are reduced in the mPFC cortical pyramidal neurons in constitutive CD38 KO mice.

(A) Immunohistochemistry for VGlut1 (green) and PSD95 (magenta) in the mPFC of WT and CD38 KO mice at P70. The VGlut1 and PSD95 channels are separated in the middle panels. Right panels are colocalized VGlut1 and PSD95 puncta. Scale bar, 10 µm.

(B–D) Quantification of VGlut1, PSD95 and synapse densities in the mPFC in WT and CD38 KO mice. (n = 5 animals per genotype, Student's unpaired t-test).

Data information: $^{*}p < 0.05$, $^{**}p < 0.01$. Data represent means ± SEM.



Appendix Figure S5. CD38 KO ACM does not affect neurite outgrowth and cell viability of cortical neurons.

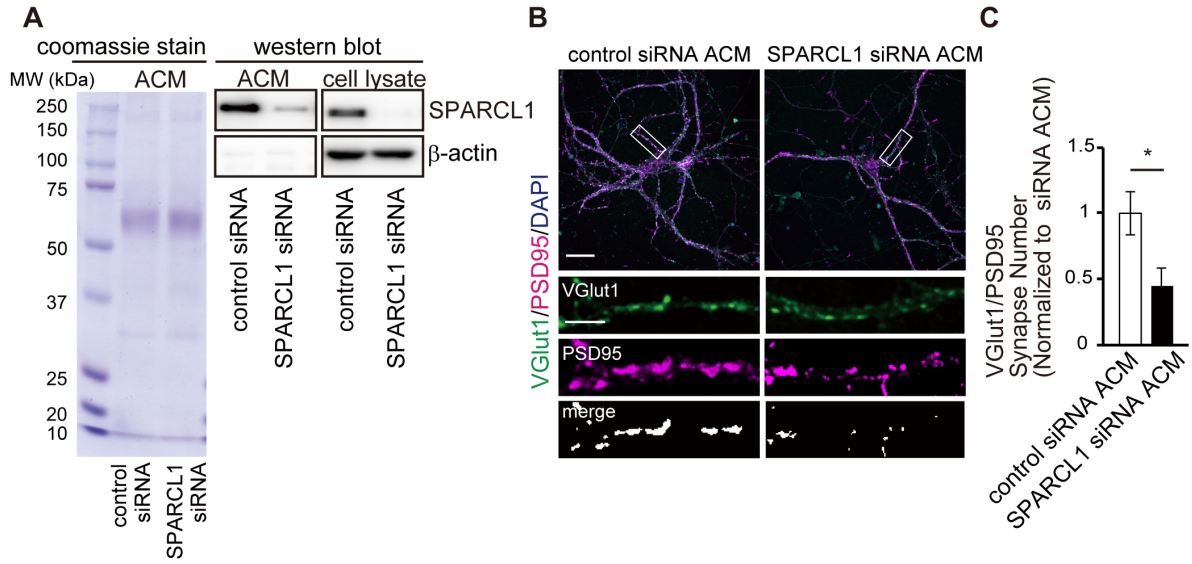
(A) Representative images of cortical neurons cultured in ACM of WT or CD38 KO mice at 6 DIV. Cells were stained with antibody against MAP2 (magenta). Nuclei were counterstained with DAPI. Scale bar, 50 µm.

(B, C) Quantification of the total length of neurites and number of neurites per cell. (n = 40 cells per genotype from three independent culture, Student's unpaired t-test).

(D) Representative images of cortical neurons cultured in ACM of WT or CD38 KO mice at 6 DIV. Cells are stained with antibody against β-III tubulin (green) and Cleaved caspase-3 (magenta). Nuclei were counterstained with DAPI. Scale bar, 50 µm.

(E) Quantification of cleaved caspase-3 positive cells out of DAPI positive cells. (n = 40, Student's unpaired t-test).

Data information: Data represent means ± SEM.



Appendix Figure S6. ACM of SPARCL1-knockdown astrocytes reduces synapse formation of cortical neurons.

(A) Coomassie stain shows the total protein composition of ACM of control siRNA or SPARCL1 siRNA-transfected astrocytes. Representative western blot images of SPARCL1 and β-actin in ACM and cell lysate of astrocytes transfected with control or SPARCL1 siRNA.

(B) Representative images of cortical neurons cultured for 14 DIV in ACM of control or SPARCL1 siRNA-transfected astrocytes. Insets show individual channels for VGlut1 (green) and PSD95 (magenta) staining, as well as the merged image. Nuclei were counterstained with DAPI. Scale bar, 20 μm (main image) and 10 μm (inset).

(C) Quantification of synapse number in cortical neurons cultured in ACM of control or SPARCL1 siRNA-transfected astrocytes. (n = 20 cells per condition from three independent culture, Student's unpaired t-test).

Data information: $^{*}p < 0.05$. Data represent means ± SEM.