Molecular Medicine

Supplemental Data

Role of Glycine *N*-Methyltransferase in the Regulation of T-Cell Responses in Experimental Autoimmune Encephalomyelitis

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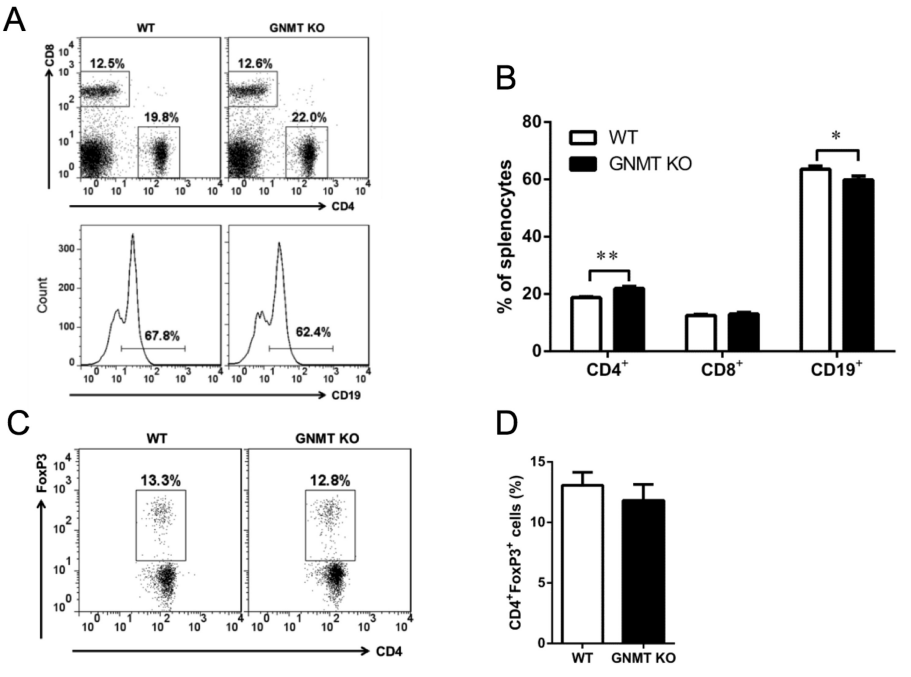
SUPPLEMENTARY MATERIALS AND METHODS

Flow Cytometric Analysis

Lymphocytes from spleens of 8- to 12-wk-old mice (n=6/group) were stained with fluorochrome-conjugated Abs specific for murine CD4 (RM4-5), CD62L (MEL-14), CD44 (IM7), Foxp3 (FJK-16s), CD8a (53-6.7) and CD69 (H1.2F3) which were purchased from eBioscience (San Diego, CA, USA). Fluorochrome-conjugated Ab to murine CD19 (1D3) was purchased from BD Biosciences (San Jose, CA, USA). Cells were analyzed using a FACSCalibur flow cytometer (BD Biosciences) and Flow Jo software (Tree Star, Ashland, OR, USA)



Supplementary Figure S1. Lymphoid development in *Gnmt*$^{-/-}$ mice. (A, B) The percentages of different lymphocyte populations in spleens were analyzed by flow cytometry. The percentages of CD4$^+$FoxP3$^+$, (C, D) and CD4$^+$CD69$^+$, CD8$^+$CD69$^+$ T cells (E, F) were analyzed by flow cytometry. Representative flow cytometric analysis of CD44 and CD62L in CD4$^+$ and CD8$^+$ T cells (G) and the histogram of percentage average of memory (CD44^{hi}CD62L^{hi}) or effector (CD44^{hi}CD62L^{lo}) T cells (H). All data are presented as mean ± SEM from six mice in each group. Statistics were calculated by the Student *t* test. *, p <0.05 and **, p <0.01.

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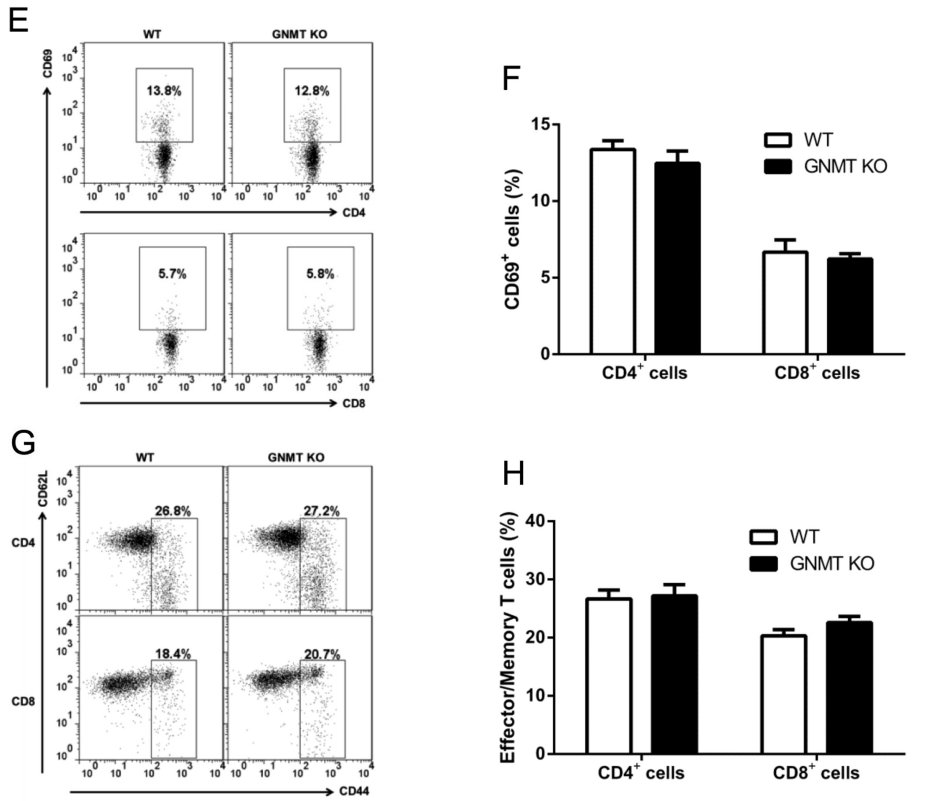
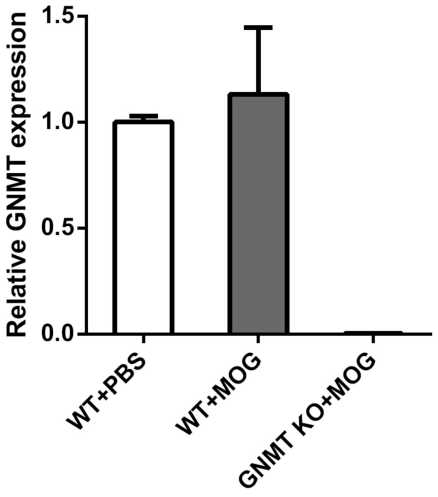
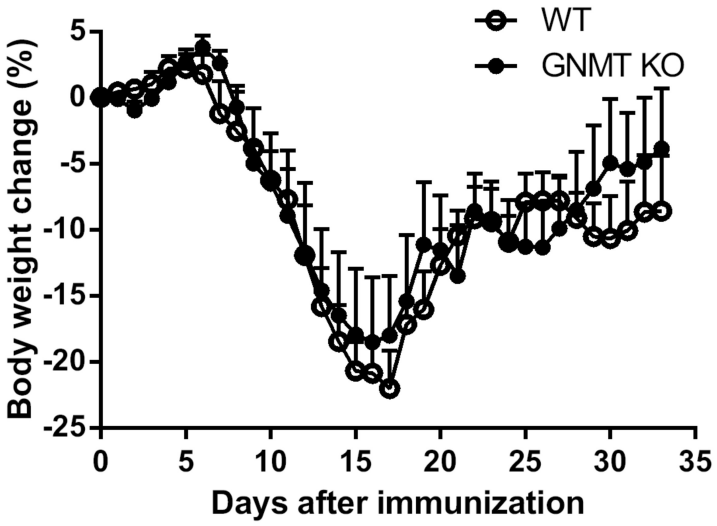


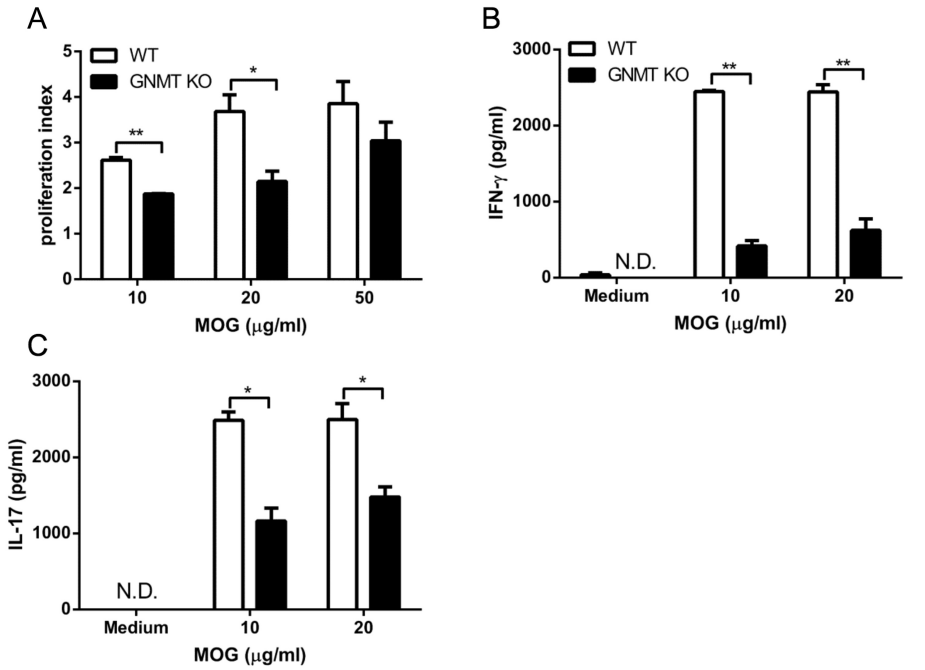
Supplementary Figure S1. ***Continued.***



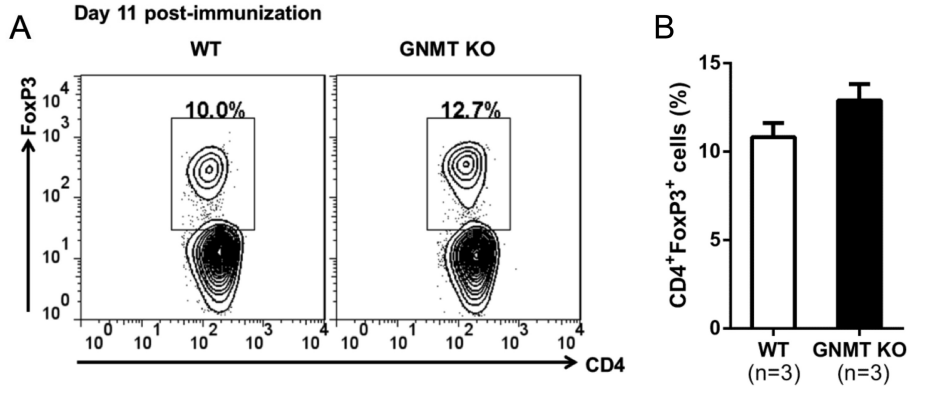
Supplementary Figure S3. GNMT gene expression of inflamed CNS was not affected by immunization of MOG_{35-55} peptide. RNA was extracted from spinal cord of PBS control, MOG_{35-55}-immunized wild-type, *Gnmt*$^{-/-}$ mice. The expressions of mRNA were analyzed by q-PCR. The mRNA level was normalized to the β-actin mRNA level and subsequently expressed as fold changes relative to wild-type (WT) mice. All data are presented as mean ± SEM from four mice in each group. Statistics were calculated by the Student *t* test.



Supplementary Figure S2. The average percentage of body weight change in MOG_{35-55}-immunized wild-type and *Gnmt*$^{-/-}$ mice. Eight- to 12-wk-old female wild-type, *Gnmt*$^{-/-}$ mice were induced EAE and recorded for body weight. Pooled data of two independent experiments including 7–12 mice per group are presented. Data are presented as mean of body weight change ± SEM. Statistics were calculated by the Student *t* test.



Supplementary Figure S4. GNMT deficiency suppressed MOG-specific T cell responses in peripheral lymphoid tissue. (A) The recovered lymphocytes from 23-d post-immunized mice were restimulated with various concentrations of $MOG_{35–55}$ peptide for 48 h. The proliferative response was determined by (^{3}H)thymidine incorporation as described in the Materials and Methods. Data is presented as mean ± SEM from three mice in each group. (B and C) Lymphocytes were recovered from 23-d post-immunized mice and restimulated with 10 and 20 μg/mL $MOG_{35–55}$ peptide for 48 h. The supernatants were harvested and measured for IFN-γ and IL-17A concentrations by ELISA. Data is presented as mean ± SD from three mice in each group. Statistics were calculated by the Student *t* test. *, p <0.05 and **, p <0.01. N.D., Non-determined.



Supplementary Figure S5. The effect of GNMT deficiency in the induction of Treg cells during EAE development. Lymphocytes were recovered from draining LNs of 11-d post-immunized mice. The percentages of $CD4^+$ $FoxP3^+$ T cells were analyzed by flow cytometry. Representative flow cytometric analysis (A) and the average percentages of $CD4^+$ $FoxP3^+$ T cells (B) in MOG_{35-55}-immunized wild-type and *Gnmt*$^{-/-}$ mice. All data are presented as mean ± SEM. Statistics were calculated by the Student *t* test.

Supplementary Table S1. Sequences of primers for real-time PCR.

Primer name	Sequence
IFN-γ (F)	5′-AAAAACCTGGATCGGAACCAA-3′
IFN-γ (R)	5′-CGGGTCAACTTCACATTCAAAG-3′
IL-17A (F)	5′-AGAAGGCCCTCAGACTAC-3′
IL-17A (R)	5′-CAGGATCTCTTGCTGGATG-3′
IL-6 (F)	5′-TTCCATCCAGTTGCCTTCTTG-3′
IL-6 (R)	5′-TTGGGAGTGGTATCCTCTGTGA-3′
TNF-α (F)	5′-CTGGAAATAGCTCCCAGAA-3′
TNF-α (R)	5′-CATTTGGGAACTTCTCATCC-3′
IL-10 (F)	5′-AACTGCACCCACTTCCCAGTC-3′
IL-10 (R)	5′-CATTAAGGAGTCGGTTAGCAG-3′
IL-2 (F)	5′-CCTGAGCAGGATGGAGAATTACA-3′
IL-2 (R)	5′-TCCAGAACATGCCGCAGAG-3′
CD25 (F)	5′-CCAGGACGTGGACTTAGATGCT-3′
CD25 (R)	5′-GCATCAGATTAGTGCAGGCTAAATT-3′
GNMT (F)	5′-GTTGACGCTGGACAAAGA-3′
GNMT (R)	5′-AGCCTGTGCTGAGGATA-3′
β-actin (F)	5′-TGTCCACCTTCCAGCAGATGT-3′
β-actin (R)	5′-AGCTCAGTAACAGTCCGCCTAGA-3′