Supplementary Figures

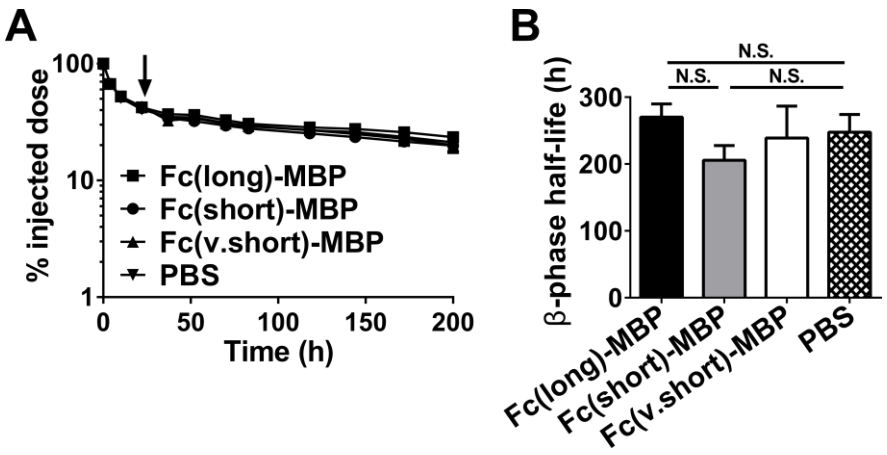

Figure S1. Fc-MBP fusions do not affect the clearance rate of mouse IgG1. B10.PL mice (n = 4-5 mice/group) were injected with ^{125}I-labeled mouse IgG1. 24 hours later (indicated by arrow in panel A), the mice were injected with DPBS or 1 μg Fc-MBP fusion. (A) Remaining radioactivity levels in blood samples. (B) β-phase half-lives of mouse IgG1, calculated for fitted data. Error bars indicate SEM. N.S., no significant difference ($p > 0.05$; two-tailed Student's *t*-test).

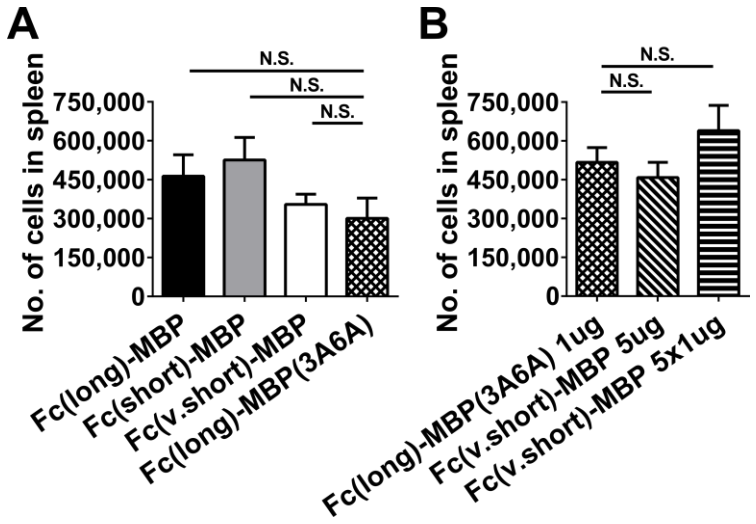

Figure S2. Prophylactic tolerance induction does not result in increased numbers of $CD4^+Foxp3^+$ T cells. B10.PL mice were treated as in Fig. 3. (A, B) Numbers of $CD4^+Foxp3^+$ T cells in the spleens were determined using flow cytometry ten days following immunization of mice with MBP1-9. Data are derived from 4-7 mice per treatment group. Error bars indicate SEM. N.S., no significant difference ($p > 0.05$; two-tailed Student's *t*-test).

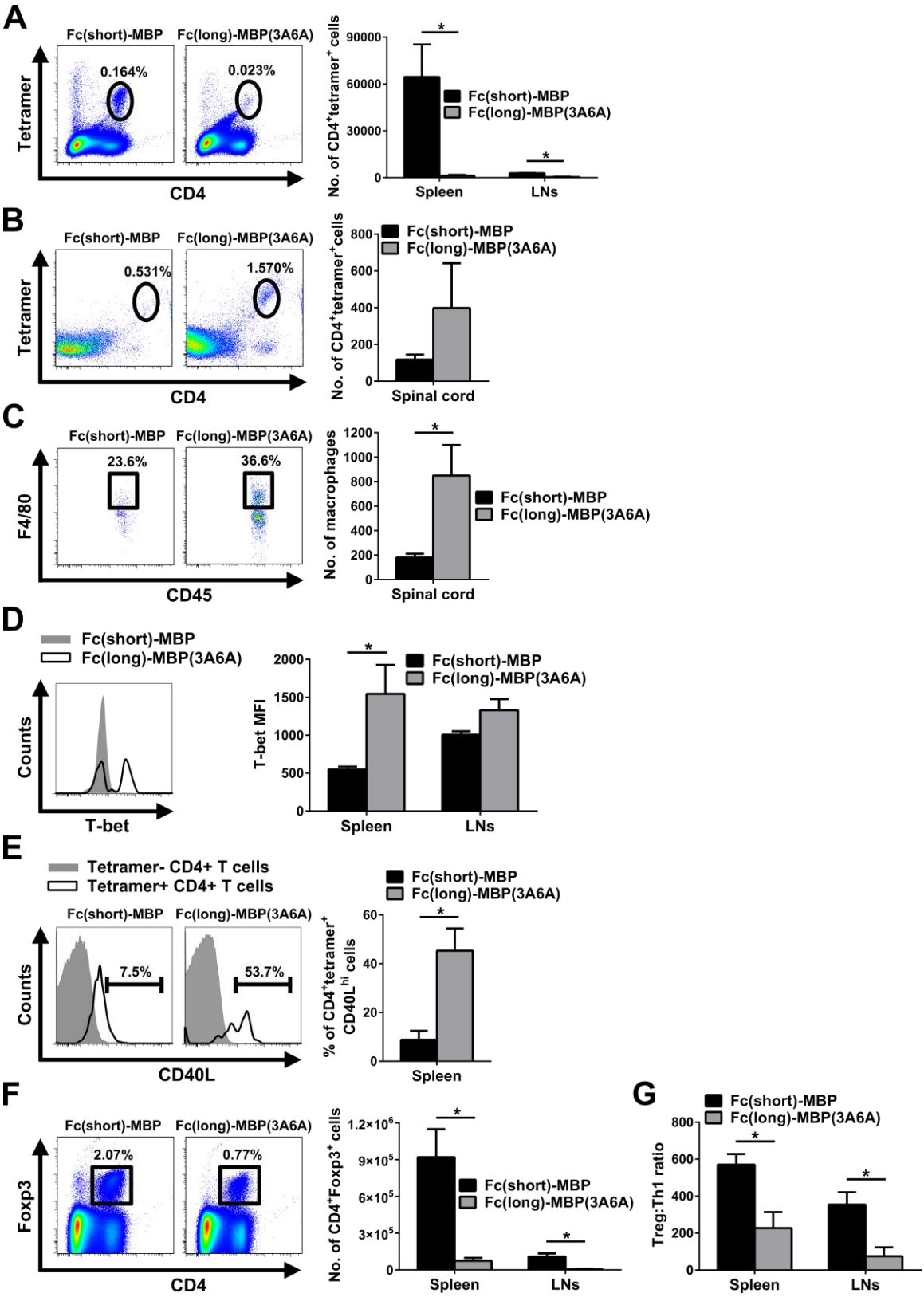
A
Fc(short)-MBP
Fc(long)-MBP(3A6A)
0.164%
0.023%
Tetramer
CD4
No. of CD4+tetramer+ cells
90000
60000
30000
0
Fc(short)-MBP
Fc(long)-MBP(3A6A)
*
*
Spleen
LNs
B
Fc(short)-MBP
Fc(long)-MBP(3A6A)
0.531%
1.570%
Tetramer
CD4
No. of CD4+tetramer+ cells
800
600
400
200
0
Fc(short)-MBP
Fc(long)-MBP(3A6A)
Spinal cord
C
Fc(short)-MBP
Fc(long)-MBP(3A6A)
23.6%
36.6%
F4/80
CD45
No. of macrophages
1200
1000
800
600
400
200
0
*
Fc(short)-MBP
Fc(long)-MBP(3A6A)
Spinal cord
D
Fc(short)-MBP
Fc(long)-MBP(3A6A)
Counts
T-bet
T-bet MFI
2000
1500
1000
500
0
*
Fc(short)-MBP
Fc(long)-MBP(3A6A)
Spleen
LNs
E
Tetramer- CD4+ T cells
Tetramer+ CD4+ T cells
Fc(short)-MBP
Fc(long)-MBP(3A6A)
7.5%
53.7%
Counts
CD40L
% of CD4+tetramer+ CD40Lhi cells
60
40
20
0
*
Fc(short)-MBP
Fc(long)-MBP(3A6A)
Spleen
F
Fc(short)-MBP
Fc(long)-MBP(3A6A)
2.07%
0.77%
Foxp3
CD4
No. of CD4+Foxp3+ cells
1.2×10^6
9×10^5
6×10^5
3×10^5
0
*
Fc(short)-MBP
Fc(long)-MBP(3A6A)
*
Spleen
LNs
G
Fc(short)-MBP
Fc(long)-MBP(3A6A)
Treg:Th1 ratio
800
600
400
200
0
*
*
Spleen
LNs

Figure S3. Tolerance induction during ongoing EAE results in increased numbers of peripheral antigen-specific $CD4^+$ T cells with downregulated T-bet and CD40L levels combined with reduced inflammatory infiltrates in the CNS. B10.PL mice were immunized and treated with Fc(short)-MBP or Fc(long)-MBP(3A6A) as in Fig. 5. Six days following treatment, mice were sacrificed and tissues isolated for flow cytometry analyses to determine: (A) % (in spleens) and total numbers (in spleens, LNs) of $CD4^+$tetramer$^+$ T cells; (B, C) % and total numbers of mononuclear infiltrates in the spinal cords that are $CD4^+$tetramer$^+$ T cells (B) or F4/80$^+$CD45^{hi} macrophages (C); (D) MFI levels for T-bet amongst $CD4^+$tetramer$^+$ T cells in spleens and LNs; (E) % $CD4^+$tetramer$^+$ CD40L^{hi} T cells in spleens; (F) % (in spleens) and total numbers (in spleens, LNs) of $CD4^+Foxp3^+$ T cells; (G) Treg ($CD4^+Foxp3^+$ T cells):Th1 ($CD4^+$tetramer$^+$T-bet$^+$ T cells) ratios in spleens and LNs. For A-F, data for one representative mouse from each treatment group is presented in the left panels. For A-C, F, populations of interest are indicated in dot plots by solid circles or boxes. Percentages (± SEM) of $CD4^+$ T cells for mice treated with the Fc-MBP fusions were: Fc(short)-MBP, 9.5 ± 0.8 (spleens) and 28.8 ± 0.8 (LNs); Fc(long)-MBP(3A6A), 11.5 ± 0.6 (spleens) and 19.7 ± 2.7 (LNs). Data are derived from 3-4 mice/group (A-C, E-G) or 7 mice/group (D). Error bars indicate SEM and significant differences ($p < 0.05$; two-tailed Student's *t*-test) are indicated by ⋆.