Supplemental data

Supplemental Figure 1

Shown is the incidence, after birth, of severe EAE-like disease observed in IgH^{MOG} x TCR^{MOG} double-transgenic “OSE” mice housed under conventional conditions (n = 9). Single transgenic littermates (IgH^{MOG} : n = 7; TCR^{MOG} : n = 3) mice remained free of clinical signs during the observation period. For comparison, the disease kinetics of OSE mice housed under SPF conditions (Figure 1) is added (grey line). The difference in the disease kinetics between SPF and non-SPF grown mice is statistically not significant.

Supplemental Figure 2

(A) Onset of spontaneous EAE in OSE mice is associated with weight loss. The body weight (blue) and clinical score (red) of two individual animals over time is on display. Note the significant drop of body weight at the onset of severe EAE.

(B) Shown is the mean severity score of EAE-like disease in OSE mice housed either under SPF (n = 5) or under conventional (non SPF; n = 4) conditions after the onset of clear signs of disease, i.e. after the first detection of a clinical score of ≥ 1. Error bars represent the SEM.

Supplemental Figure 3

The entire CNS of a healthy OSE mouse (7.5 weeks old) was analyzed after histological staining. Blue areas indicate inflammation; demyelinating lesions were not observed.

Supplemental Figure 4

Shown is the spinal cord (left column) and optic nerves (right column) of a diseased OSE mouse grown under SPF conditions. Upper panels are stained with hematoxylin/eosin, middle panels with luxol-fast blue and lower panels with Bielschowsky silver impregnation.

Supplemental Figure 5

(A) Proliferation of splenocytes from TCR^{MOG}, IgH^{MOG} and C57BL/6 wild-type mice combined at equal cell numbers as indicated (total of 2×10^5 cells per well).

(B) Proliferation of non-separated splenocytes of a OSE mouse and that of its purified and re-combined transgenic T and B cells (purity of transgenic cells >90%; total of 2×10^5 cells per well).

Error bars indicate the standard error.

Supplemental Figure 6

OSE transgenic T cells (upper graph) and B cells (lower graph) stimulated with rMOG express the activation markers CD25 (IL-2R) and CD86, respectively.

black line: parallel cultures that were not activated with rMOG.

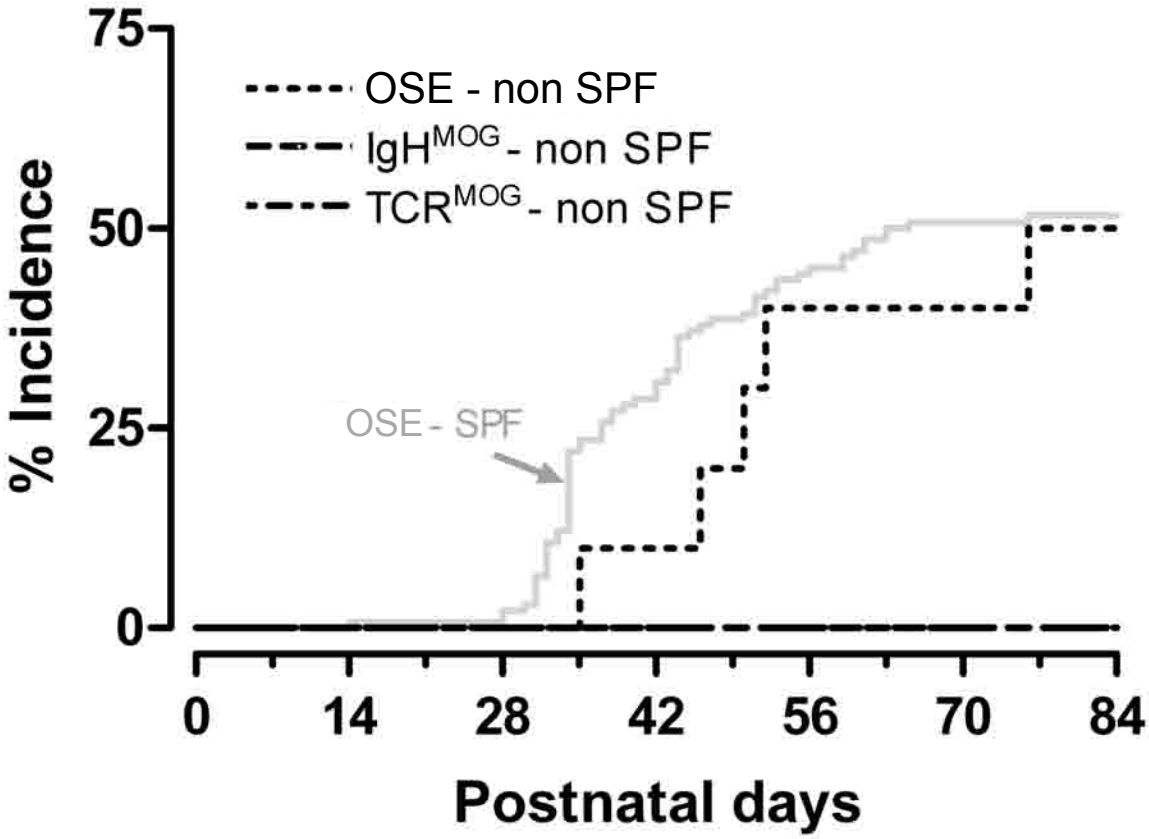
Supplemental Figure 7

MOG-specific antibody levels were determined in the serum obtained from two individual OSE (A + B), IgH^{MOG} (C) and TCR^{MOG} mice by ELISA. The double-transgenic mice shown in A and B developed clinical symptoms as indicated in the lower panels; in contrast the single-transgenic animals shown in C and D did not develop any clinical signs. Serum was drawn from all animals at two indicated occasions. Note presence of high anti-MOG IgG1 antibody levels in the serum of OSE mice that remained similar before and after the onset of clinical disease.

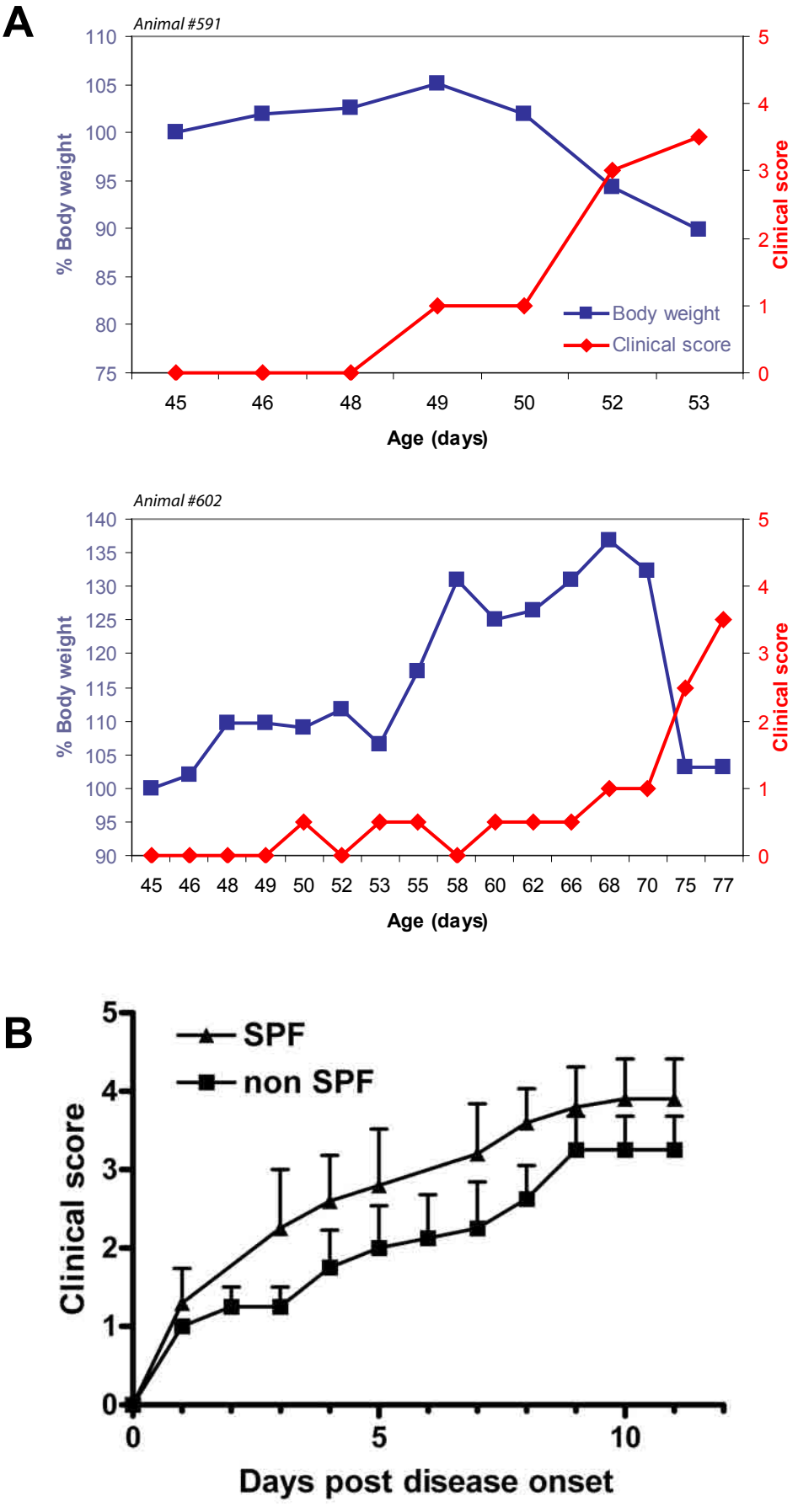
Supplemental Figure 8

The frequency of regulatory T cells is low in OSE mice and remains unaltered after the onset of disease. (A) Gated $CD4^+$ T cells from the spleen of healthy and sick OSE mice (each n = 3) were stained with Foxp3 and V alpha 3.2 antibodies. The numbers indicate the relative cell frequencies in each quadrant. Note the overall low frequency of $CD4^+/Foxp3^+$ T cells in OSE mice. (B) Real-time PCR was used to assess the relative expression of the Foxp3 gene among splenocytes from age-matched (4-8 week old) healthy (n = 9) and sick (n = 9) OSE, healthy TCR^{MOG} (n = 7), and healthy IgH^{MOG} (n = 8) mice. Foxp3 gene expression was measured in triplicates and normalized to GAPDH transcripts. Error bars indicate the SEM.

Supplemental Figure 1:



Supplemental Figure 2:



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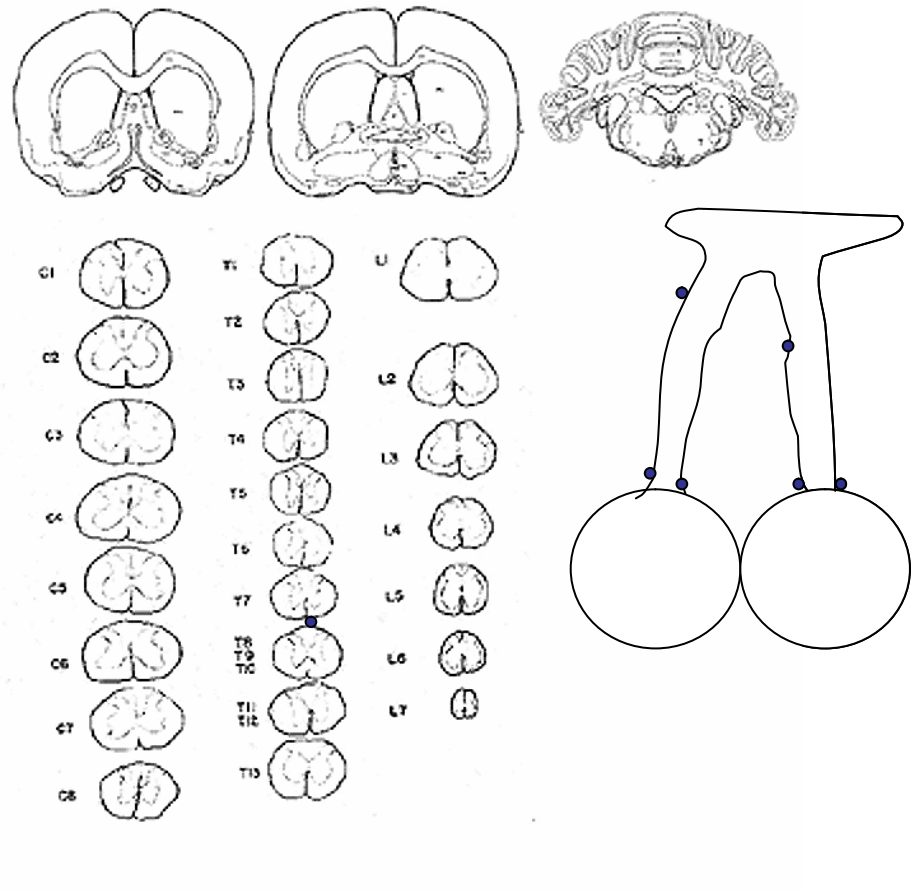

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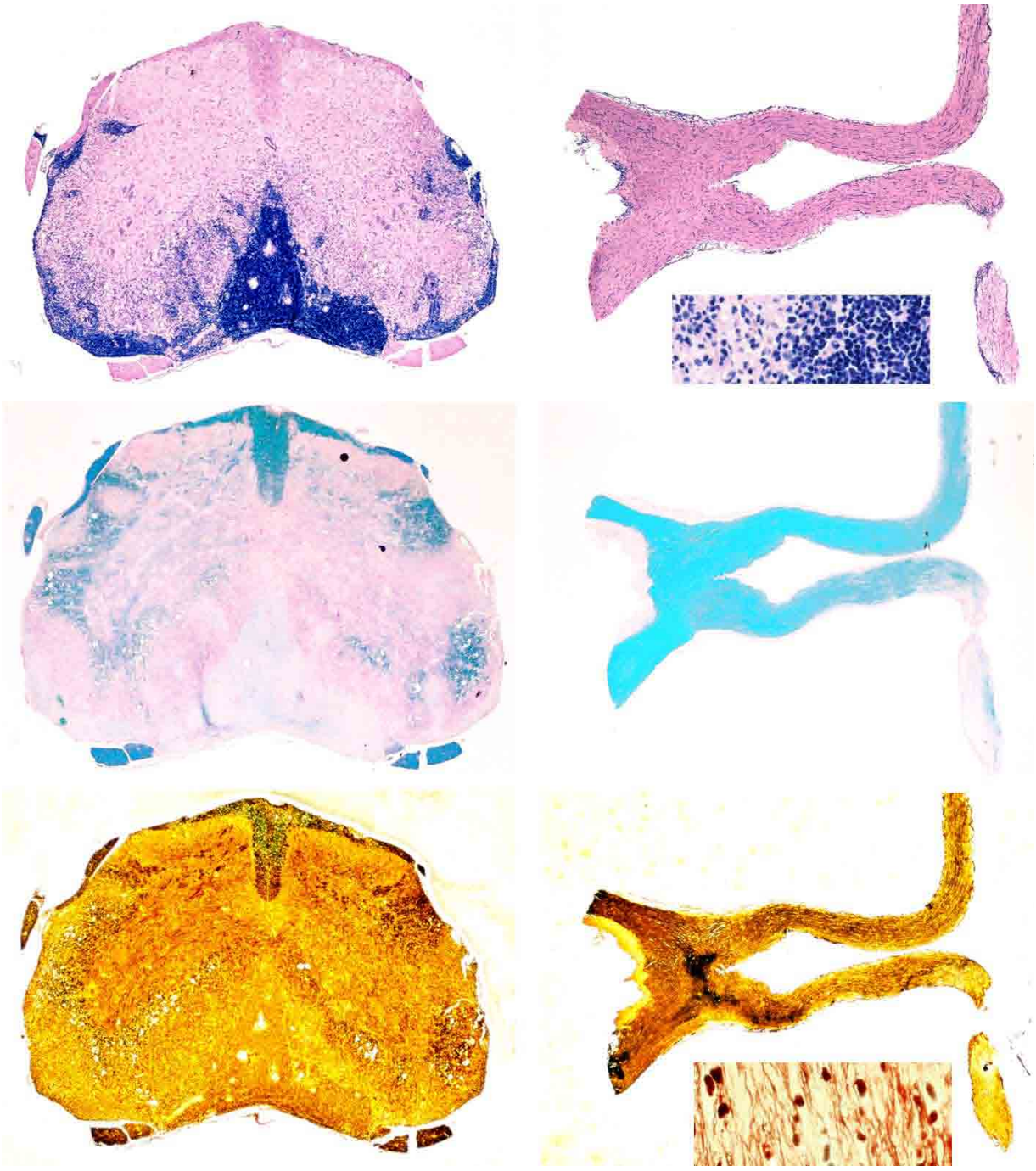

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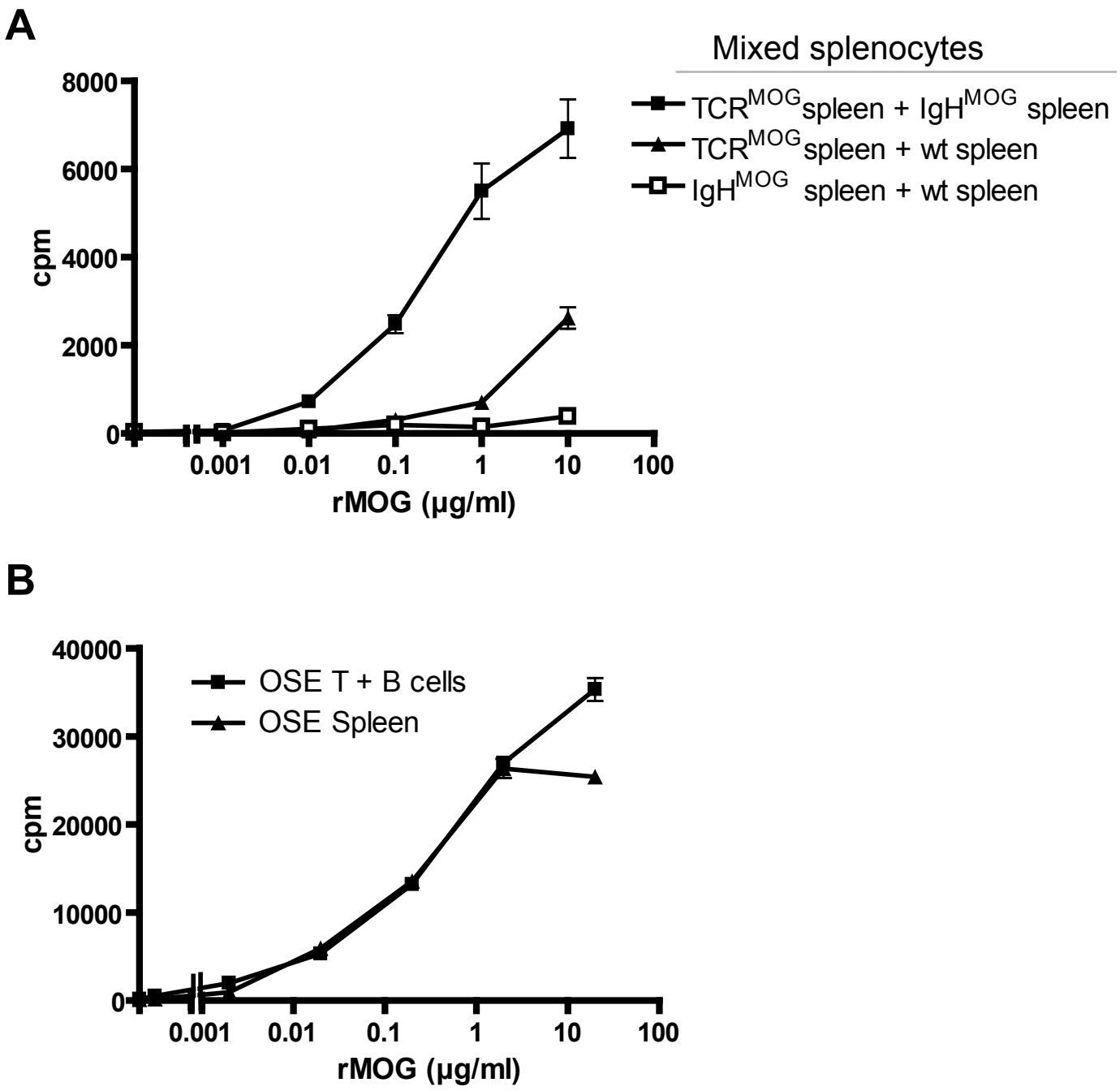
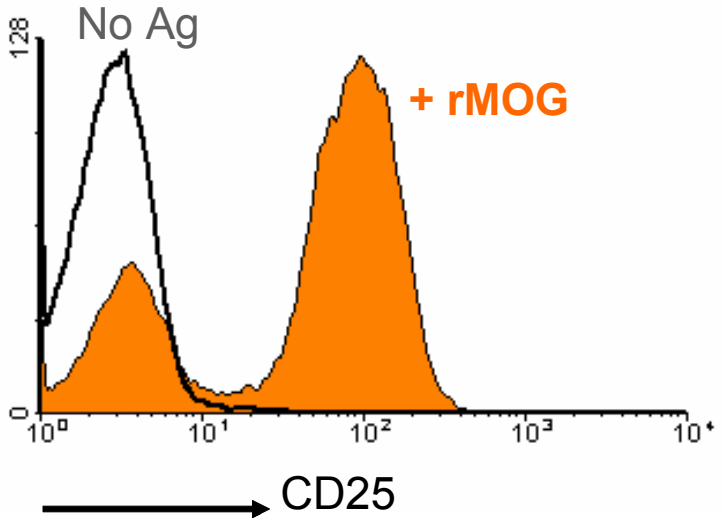
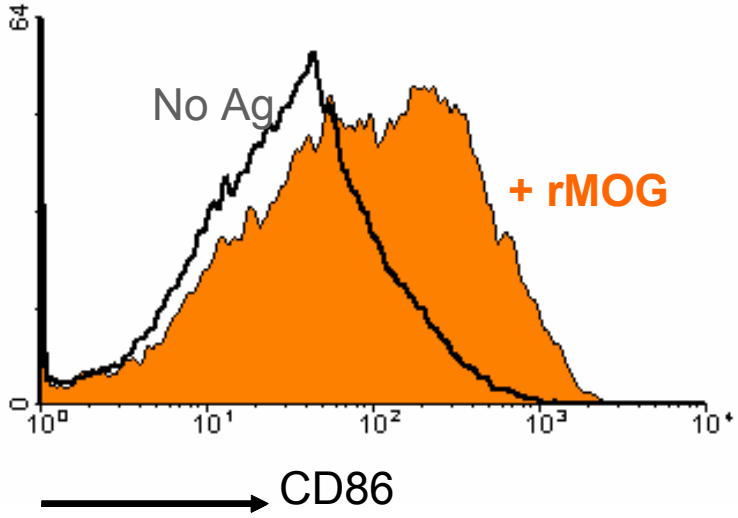


Supplemental Figure 6:

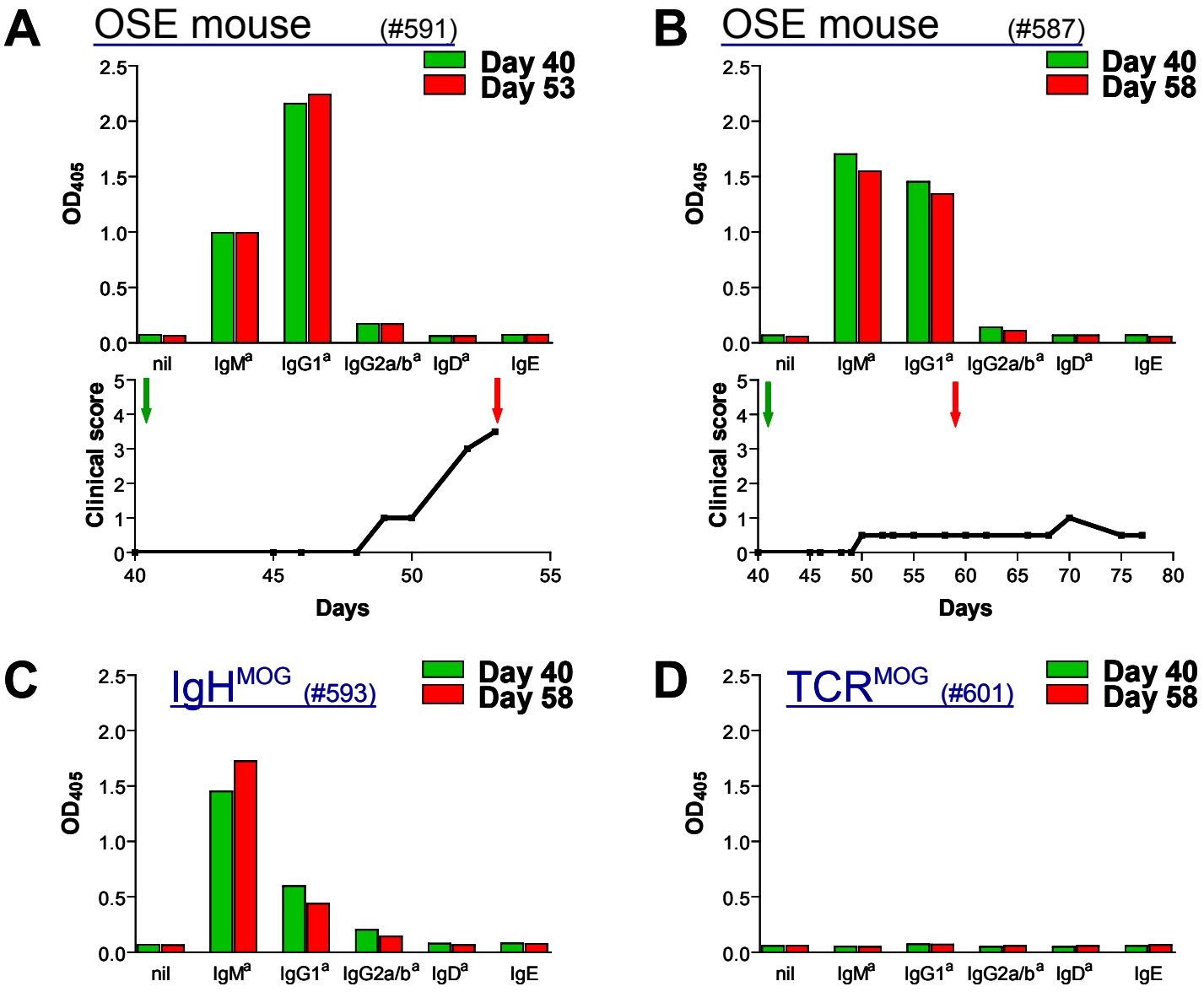
Live CD4+/CD3+
T cell gate



Live CD19+
B cell gate



Supplemental Figure 7:



Supplemental Figure 8: