Figure S1

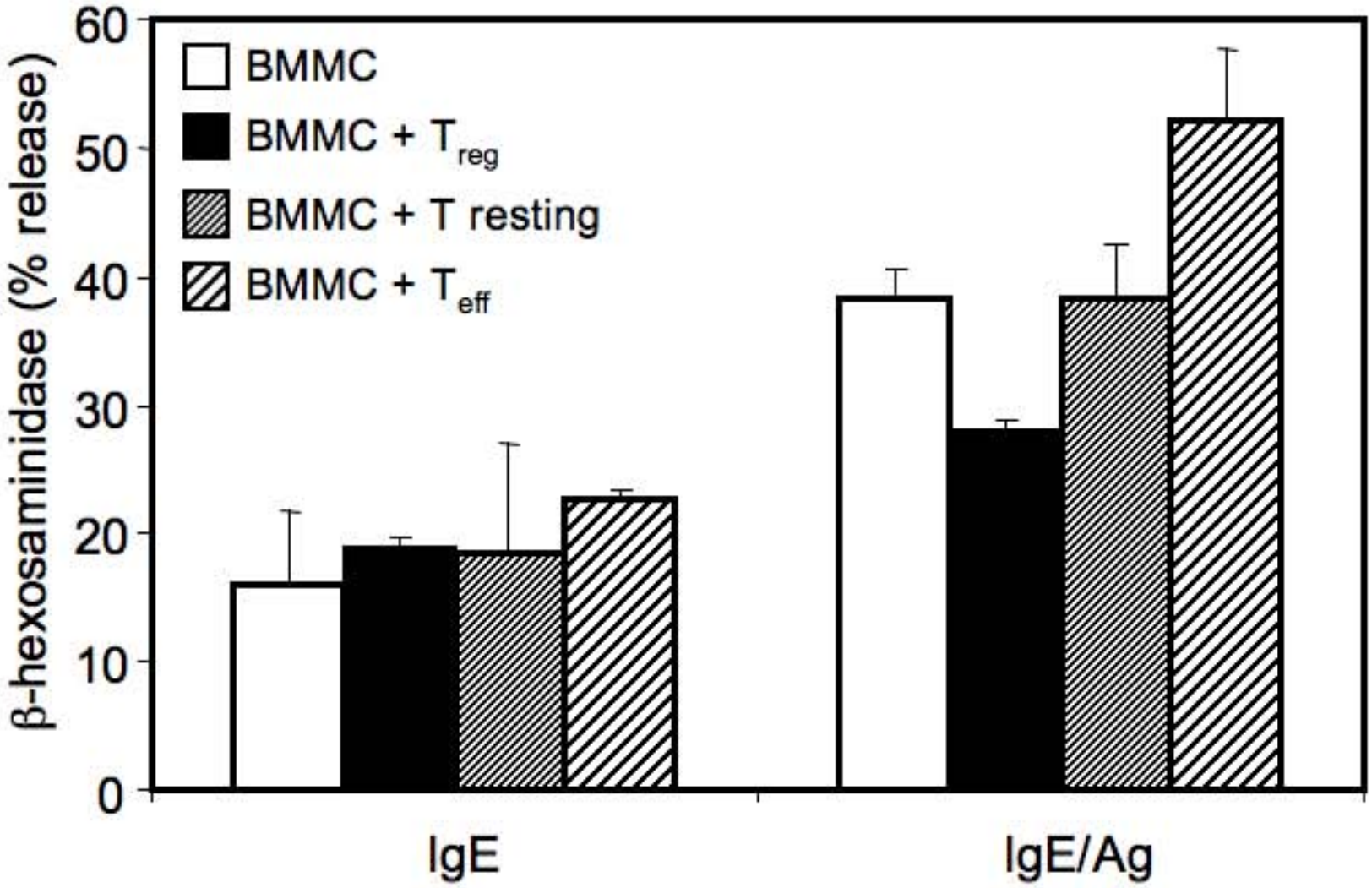

Figure S1. Impairment of IgE-mediated *in vitro* degranulation by BMMCs in the presence of Tregs. Primary cultures of BMMCs from Balb/c mice were sensitized with IgE anti-DNP (IgE) and challenged with Ag (IgE/Ag) in the absence or presence of equal amount of $CD4^+CD25^+$ T_{regs} (T_{reg}), CD4+CD25- T cells (T resting) or anti-CD3- plus anti-CD28-stimulated $CD4^+CD25^-$ effector T cells (Teff), were examined for release of β-hexosaminidase expressed as percentage of the cells' total mediator content obtained by lysis of cells with Triton X-100. Shown are the means ± SD of three independent experiments, each performed in duplicate.

Figure S2

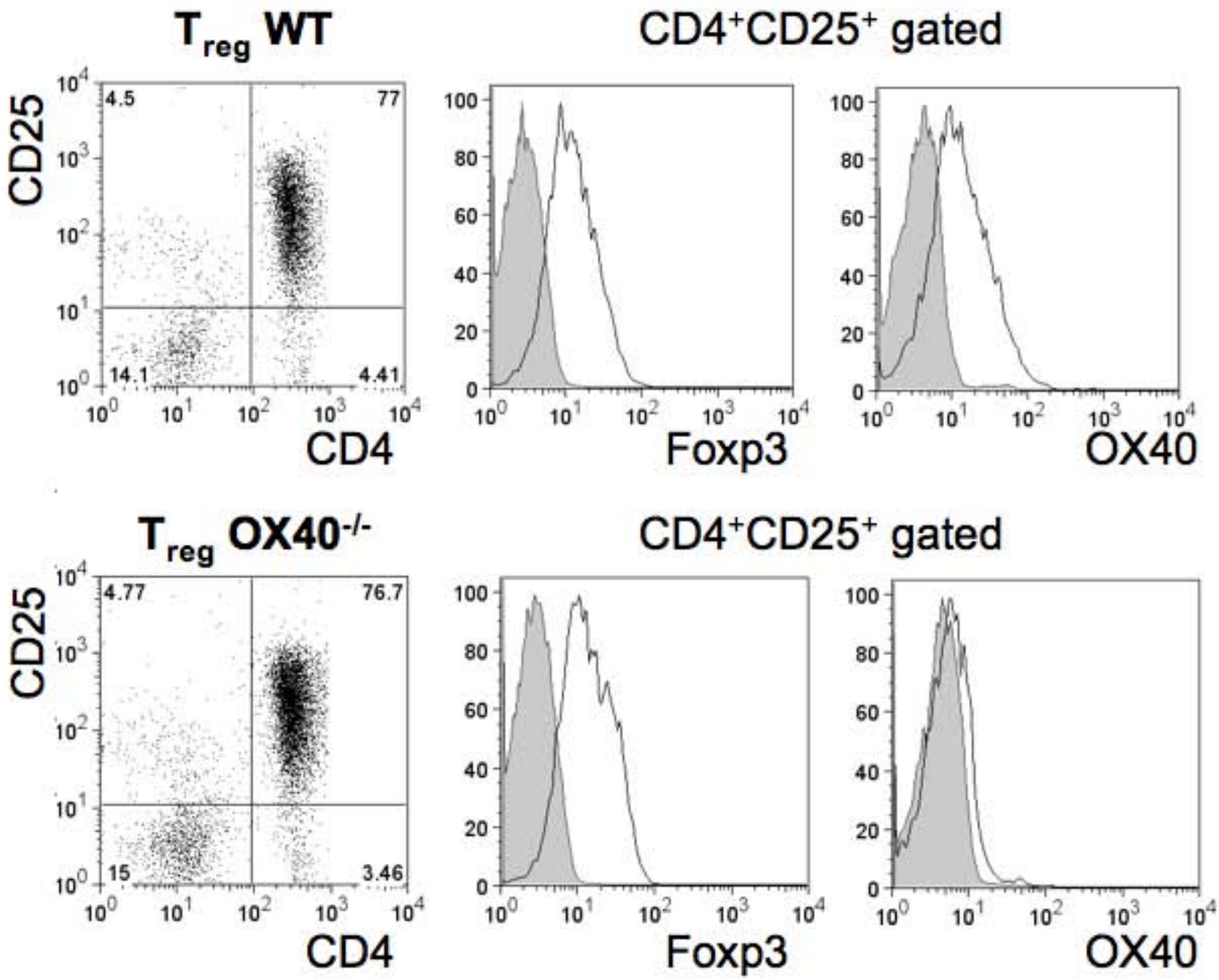

Figure S2. CD4$^+$CD25$^+$ T$_{regs}$ purification and OX40 expression. Flow cytometry showed that the separate fractions were more than 80% pure, CD4$^+$CD25$^+$ were mainly Foxp3 positive and wild type CD4$^+$CD25$^+$ were OX40 positive in contrast to CD4$^+$CD25$^+$ from *OX40*$^{-/-}$ mice. Shaded areas show staining with isotype-matched control Ab.

Figure S3

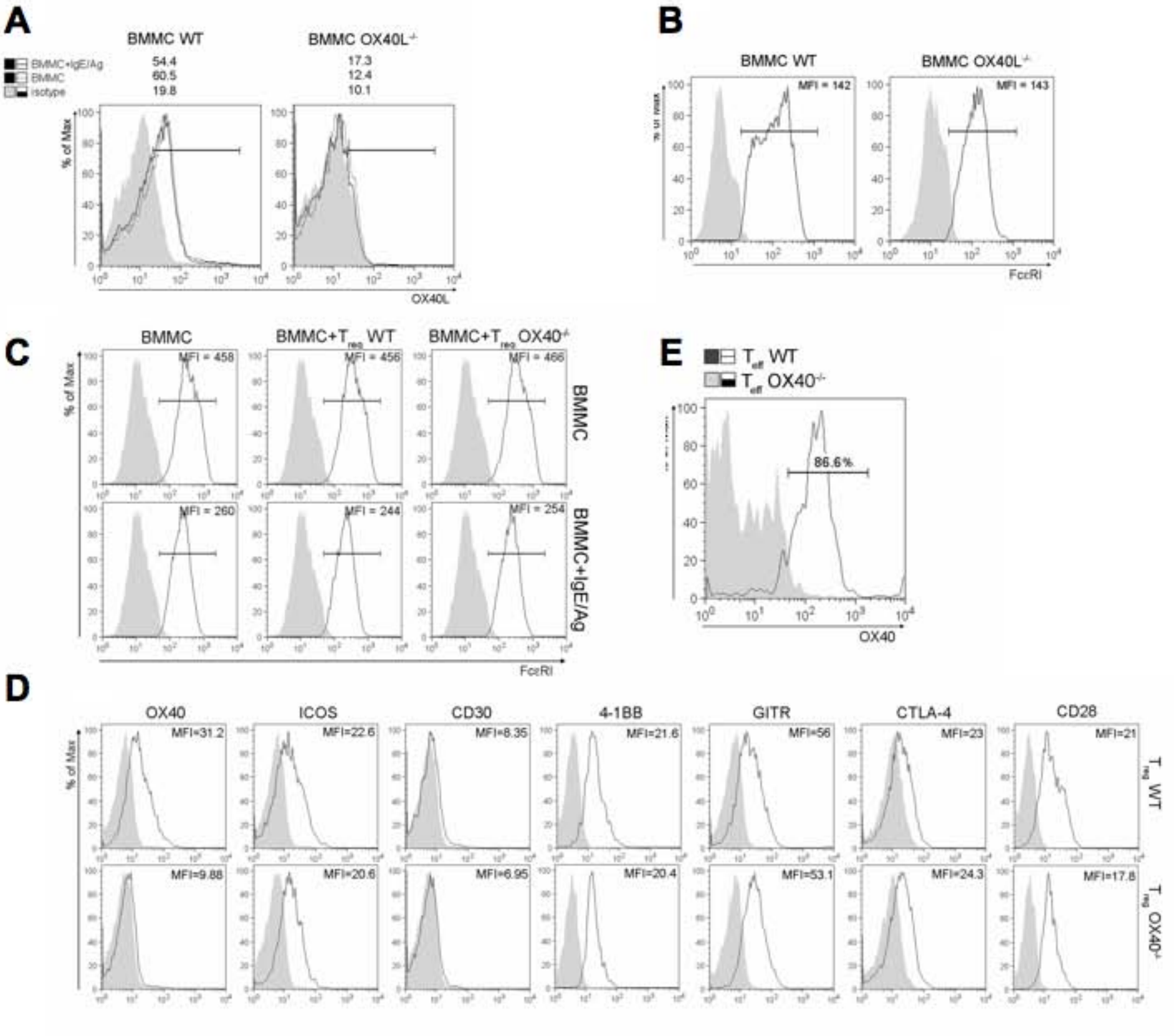

Figure S3. Surface phenotype of BMMC, T_{reg} and T_{eff}. (A) OX40L expression on WT or *OX40L*$^{-/-}$ BMMCs unstimulated (dotted lines) or stimulated with IgE and Ag (bold lines) was determined by flow cytometry. Percentages of positive cells are reported for each condition. (B) FcεRI expression on unstimulated WT or *OX40L*$^{-/-}$ BMMCs. Mean fluorescence intensity (MFI) of positive cells is shown on each histogram. (C) FcεRI expression on unstimulated or stimulated WT BMMC after 30 min culture in absence or in presence of T_{regs} from WT or OX40$^{-/-}$ mice. Mean fluorescence intensity (MFI) of positive cells is reported. Shaded areas show staining with isotype-matched control Ab. (D) Surface phenotype of WT or OX40$^{-/-}$ T_{regs}. The expression of each marker on gated CD4^{+} Foxp3^{+} splenic T_{regs} (bold lines) is overlayed to matched isotype control Ab (shaded areas). Mean fluorescence intensity (MFI) is shown on each histogram. (E) Percentage of OX40 expression by WT (bold line) or *OX40*$^{-/-}$ (shaded area) T_{eff} after *in vitro* activation.

Figure S4

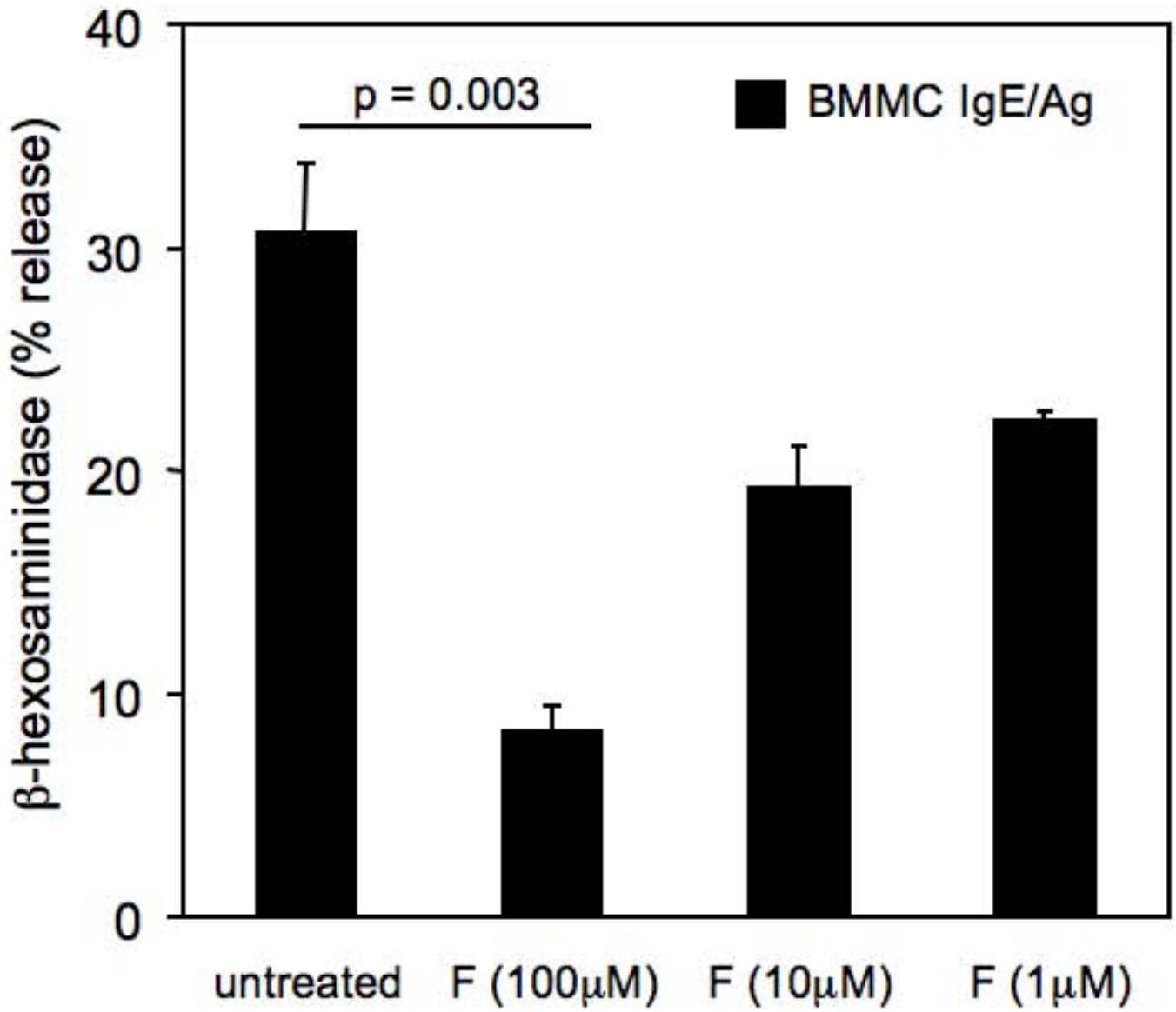

Figure S4. Inhibitory effect of cAMP-elevating agent forskolin on β-hexosaminidase release. Anti-DNP IgE preloaded BMMCs were untreated or pretreated with the indicated concentrations of forskolin (F) for 1 hour before challenge with DNP-HSA and the effect of forskolin on Ag-induced degranulation of BMMCs was measured. β-hexosaminidase release (mean ± SD) from two independent experiments is shown, each performed in duplicate.

Figure S5

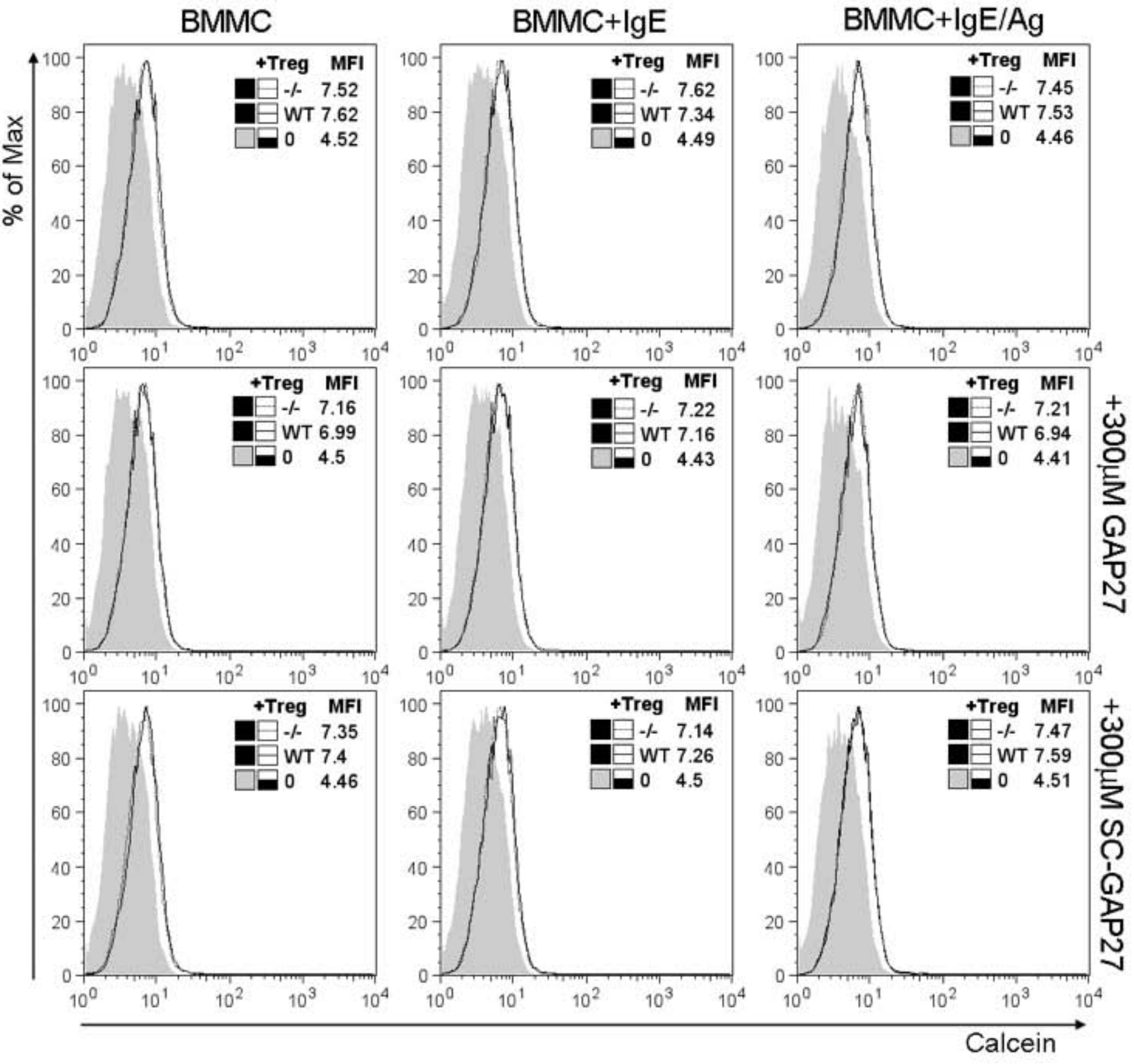

Figure S5. Calcein transfer from T_{reg} to BMMC.

BMMC were sensitized (BMMC+IgE) or not (BMMC) with anti-DNP IgE and stimulated for 30 min with DNP (BMMC+IgE/Ag) in absence (0 T_{regs}, shaded areas) or in presence of WT (bold lines) or *OX40*$^{-/-}$ (dotted lines) T_{regs} previously loaded with 1 µM calcein. Co-culture was performed in absence or in presence of 300 µM of the gap-junctions mimetic peptide GAP27 or of a scrambled control peptide (SC-GAP27), consisting of the same aminoacids in a different order. In the gated CD4^{-} cells, the mean fluorescence intensity (MFI) of calcein transferred to BMMC is reported.

Figure S6

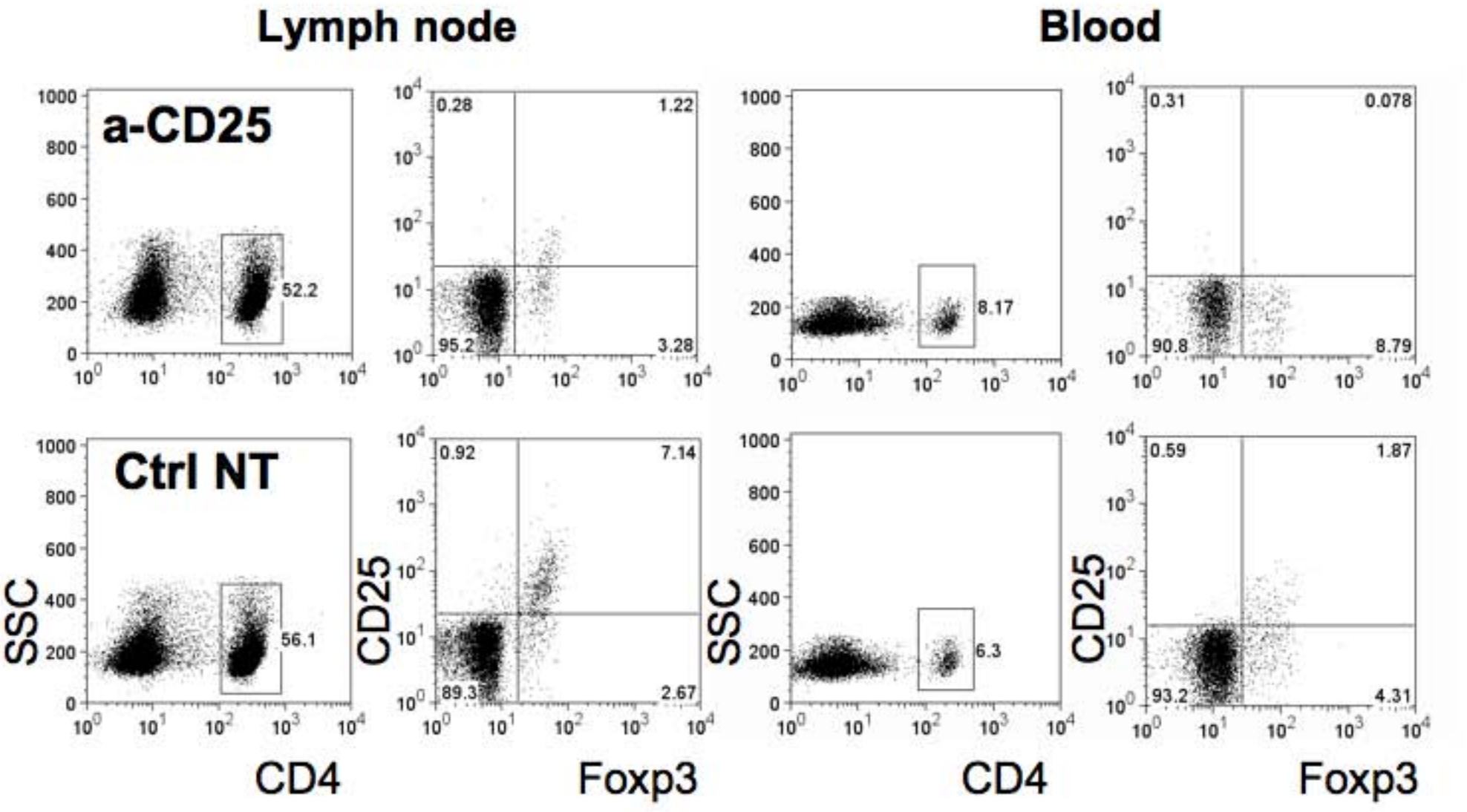

Figure S6. T_{reg} depletion induced by anti-CD25 Ab administration. Seven days after anti-CD25 mAb injection (clone PC61) blood and lymph nodes were collected and cells were stained for CD25 (clone 7D4) and Foxp3 to check T_{regs} depletion.