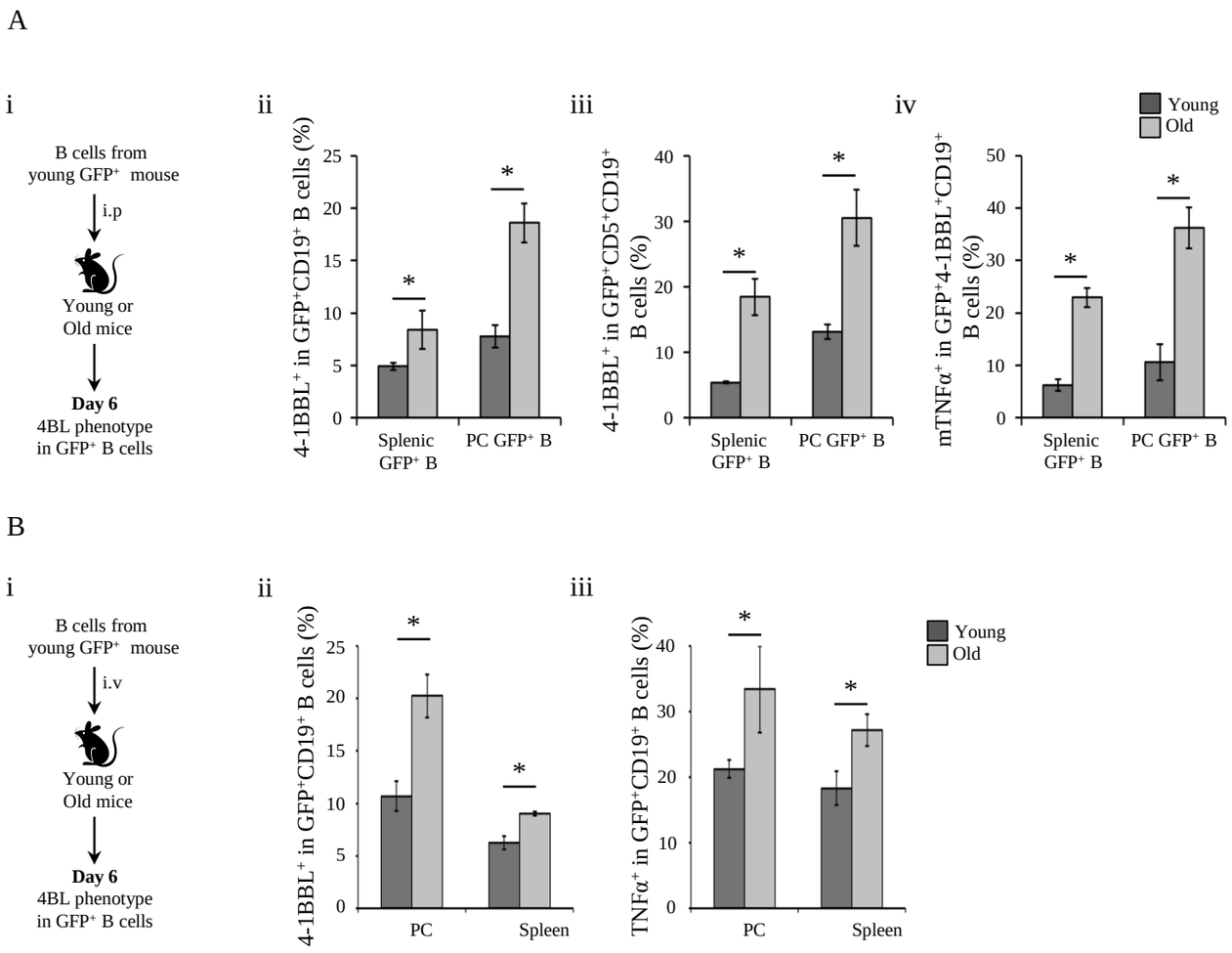
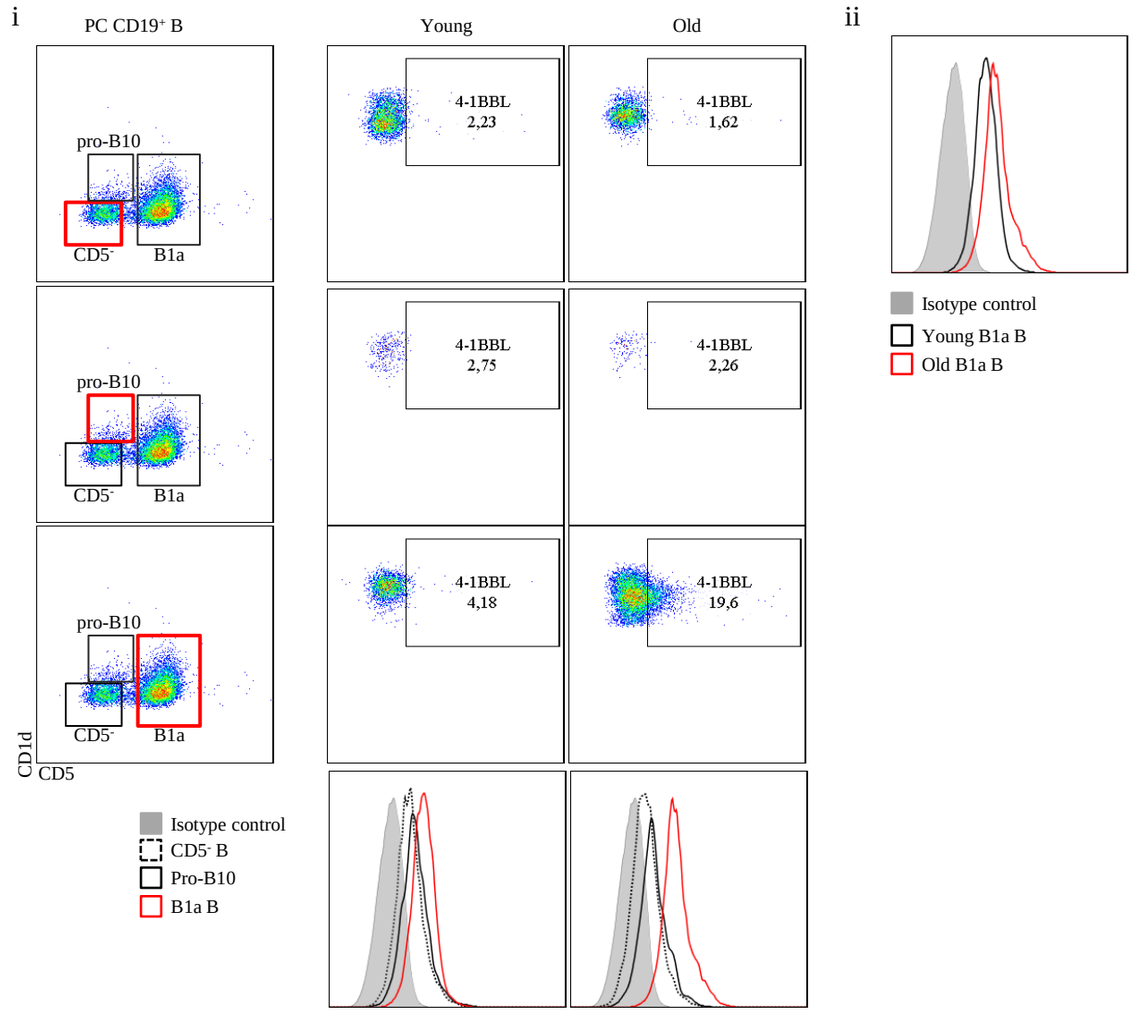
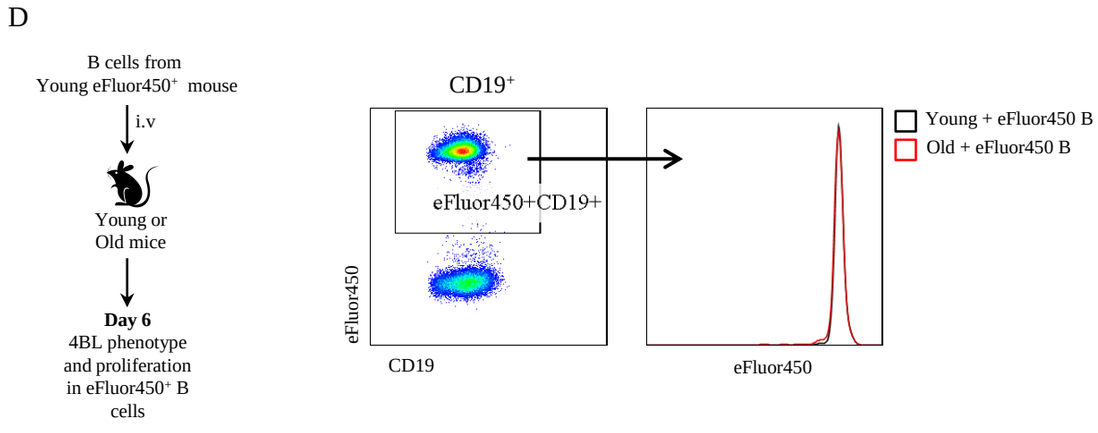
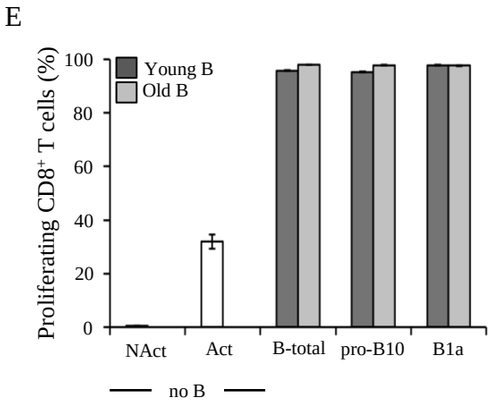
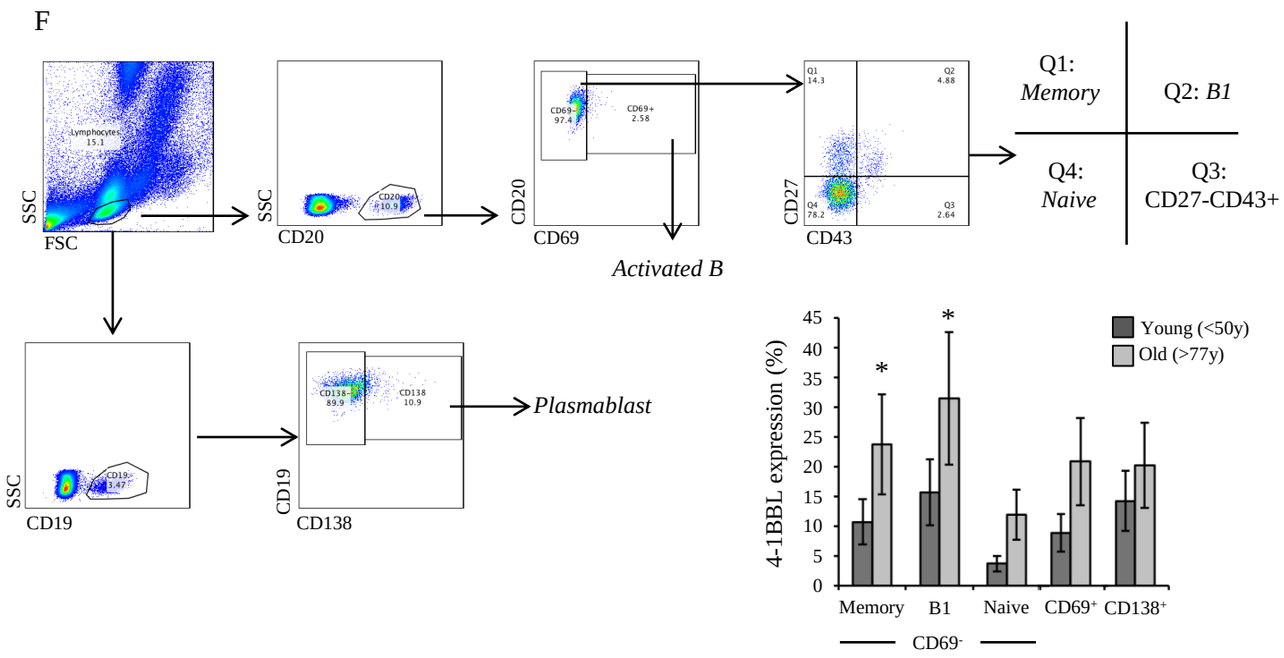
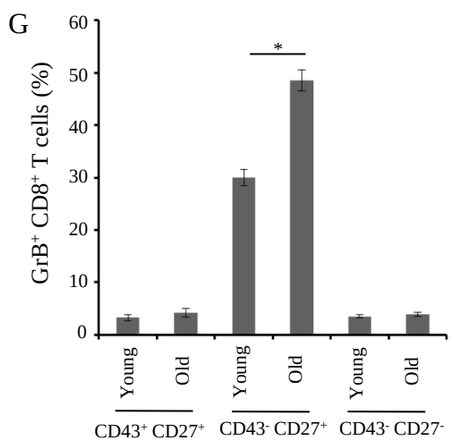


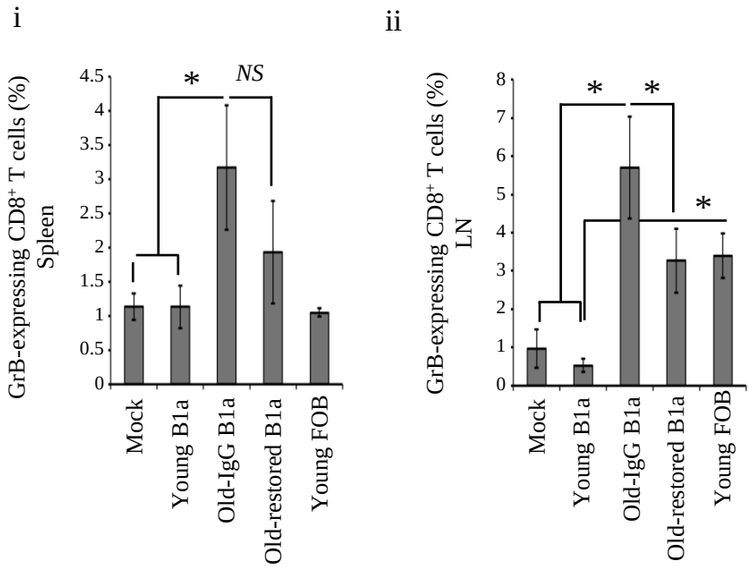




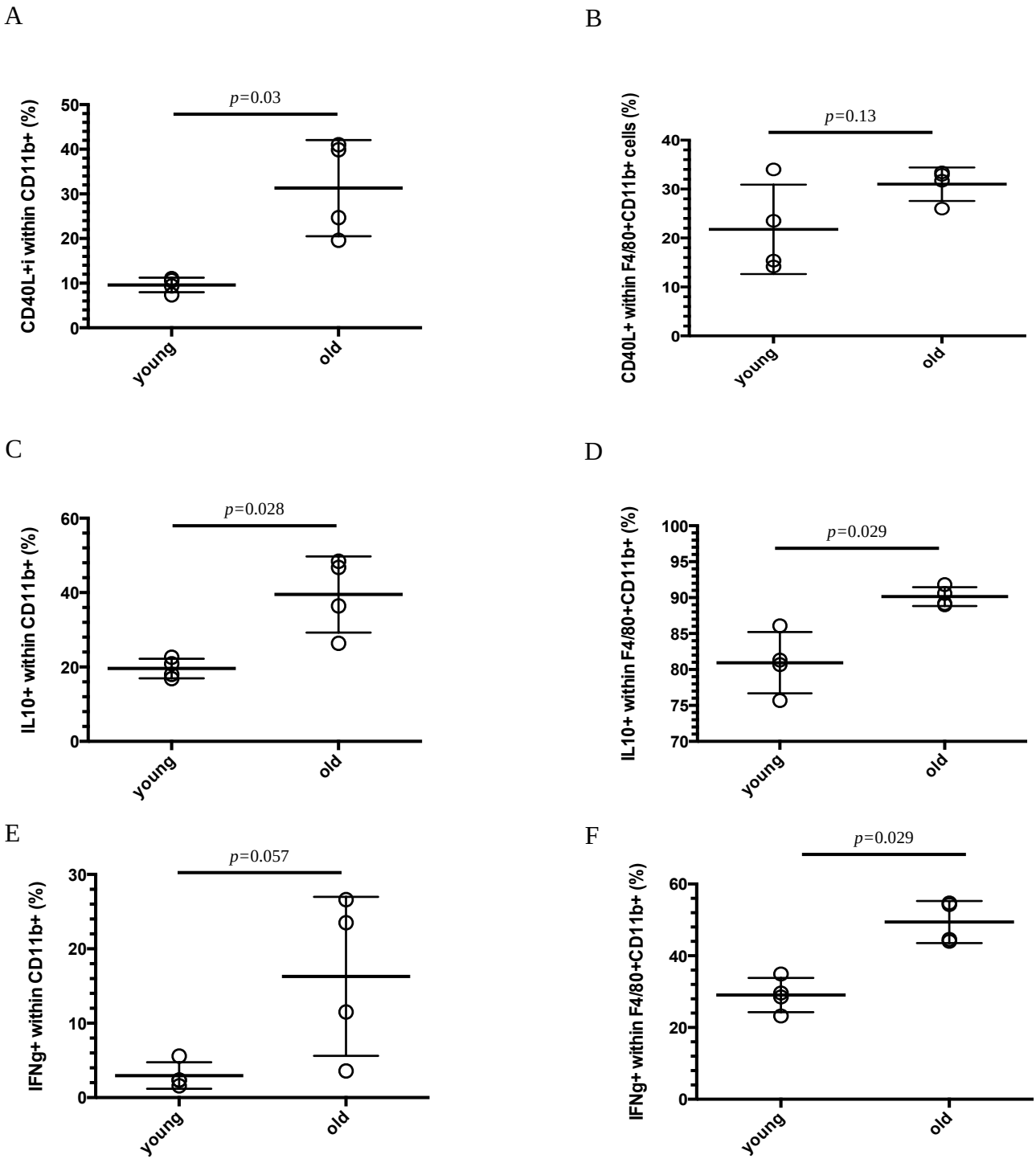




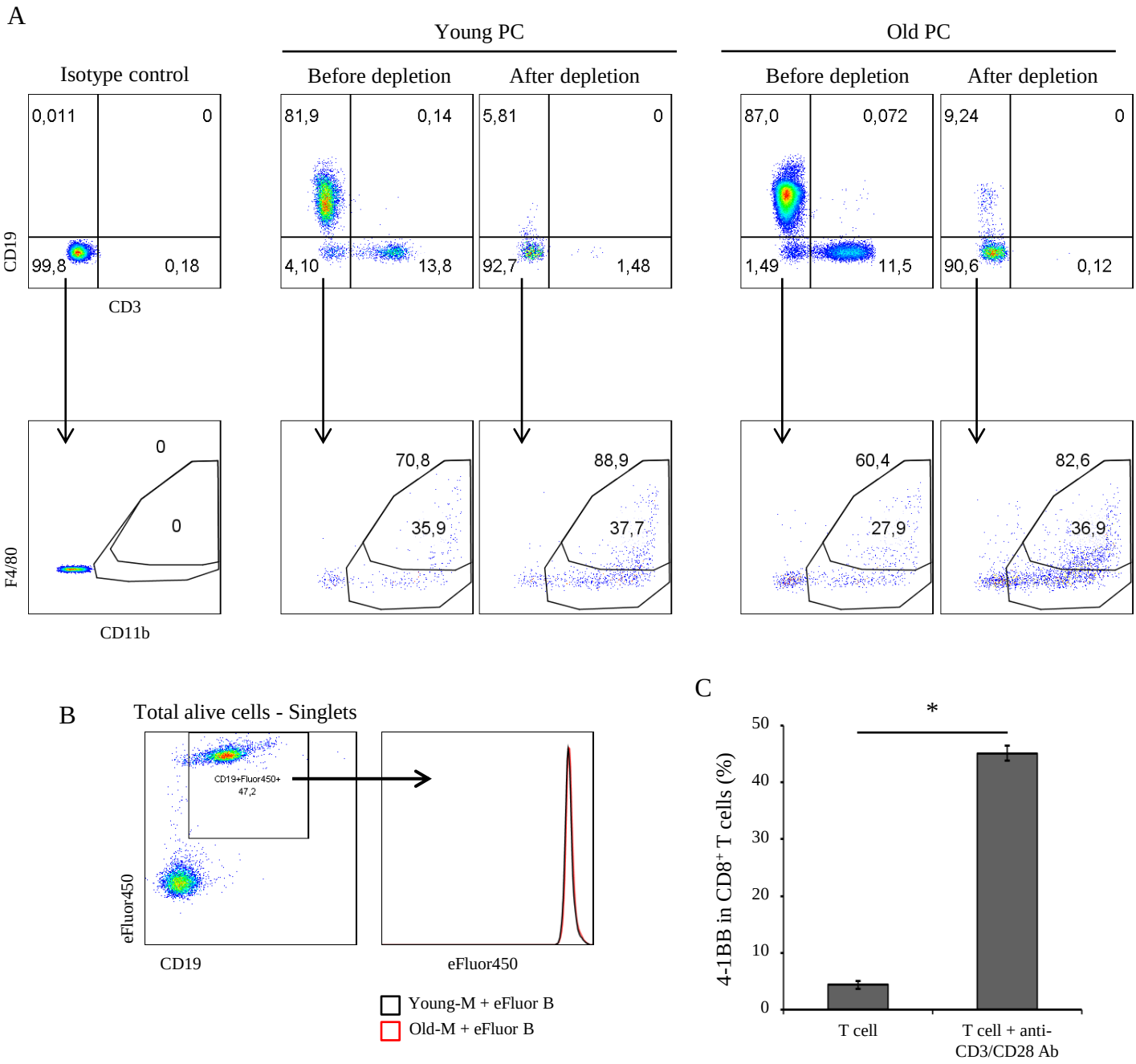




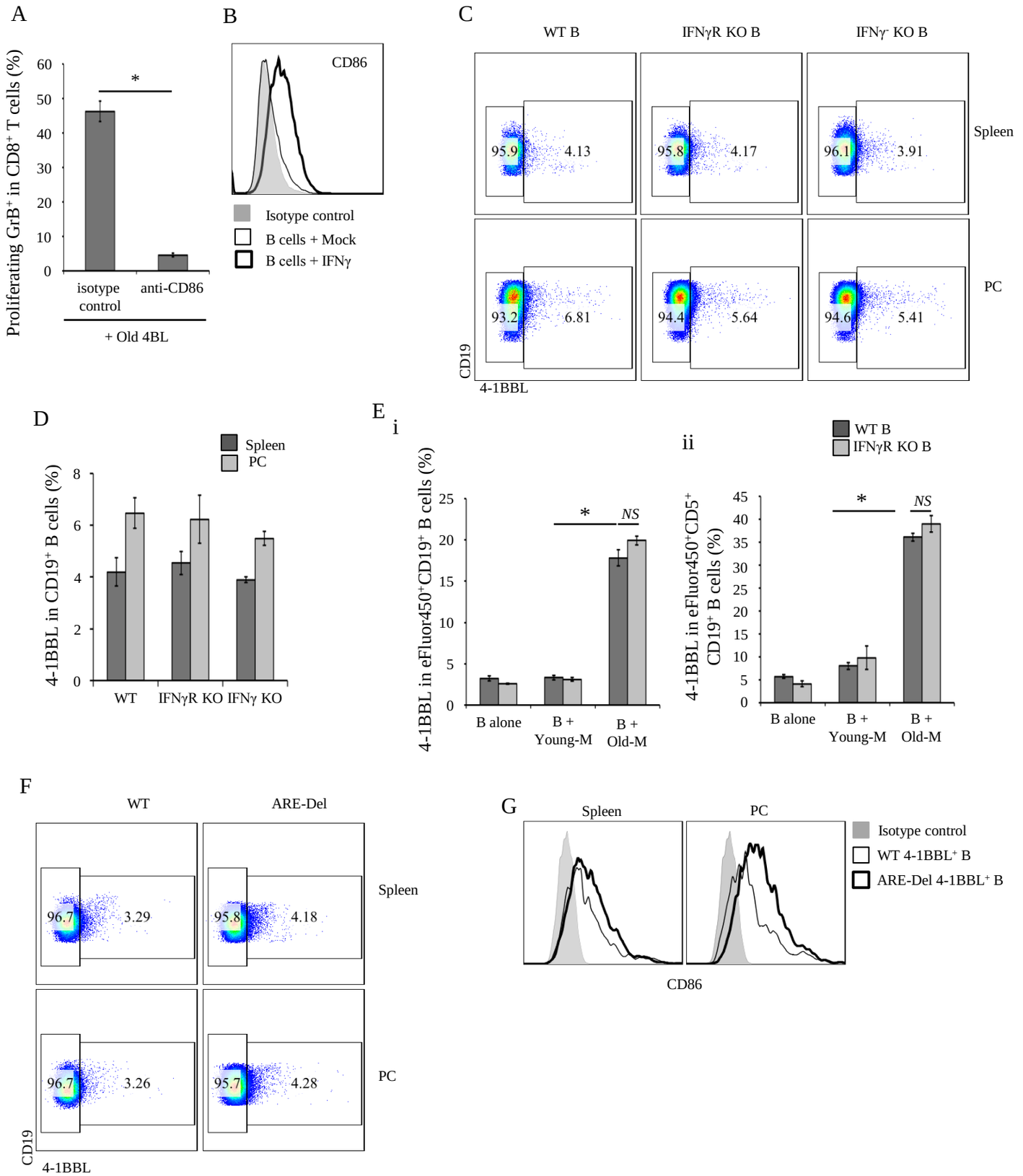
S Fig1. In vivo 4BL cells induction. (A) The 4BL cell conversion of splenic and peritoneal cavity (PC) B cells of young GFP$^+$ mice upon their i.p. (A) or i.v. (B) injection into congenic young or old C57BL/6 mice. Shown, a representative result (mean ± SEM) of 3 independent experiments (n ≥ 4 mice/group/experiment). (C) Representative plot showing the expression of 4-BBL and TNFα in PC B-cell subsets (CD5$^-$, pro-B10, and B1a B cells) of young and old mice (i). Compared to young mice, B1a cells of old mice express higher levels of mTNFα (ii). Shown, a representative result (mean ± SEM) of 4 independent experiments (n ≥ 4mice/group/experiment). (D) B cells do not proliferate in the PC of young (black line) or old (red line) mice upon their injection. Shown is a representative result (mean ± SEM) of 2 independent experiments (n ≥ 4mice/group/experiment). (E) Sort-purified pro-B10 and B1a cells, as well as unsorted total B cells, from PC of old (light grey) and young (dark) mice exhibit a comparable ability to induce proliferation of CD8$^+$ T cells stimulated with anti-CD3 Ab. Shown, a representative result (mean ± SEM) of 3 independent experiments performed in triplicates. (F) 4-1BBL is highly expressed in memory and B1 cells of elderly humans. Shown is a representative result of 11 young (41 ± 7.2 years old) and 19 elderly donors (79 ± 6.45 years old), where we compared expression of 4-BBL in memory, naïve, activated CD69$^+$B cells, plasmablasts, and B1 cells. (G) Elderly human memory CD27$^+$ B-cells strongly induce GrB$^+$CD8$^+$ T cells in vitro, while CD43$^+$B cells and CD27$^-$ B cells failed to do so. Shown is a representative result (mean ± SEM) of 3 independent experiments (n ≥ 2 human cells/group/experiment). (H) Adoptively transferred sB1a cells of old mice, but not FOB or young mouse sB1a cells, increase frequency of GrB$^+$CD8$^+$ T cells in B-cell deficient J_HT mice with B16 melanoma. Shown is the mean (%) ± SEM of GrB in CD8$^+$T cells in spleens (i) and LN (ii) at day 20 post tumor challenge. The experiment was reproduced twice with 4-5 mice/group. P-value significance is shown as *p<0.05, **p<0.01, ***p<0.005, and NS = not statistically significant.



S Fig2. PC myeloid cells and macrophages are dysregulated in old mice, as shown by enrichment for CD40L, IL10, and IFNγ –expressing $CD11b^+$ PC myeloid cells (A,C,E) and $CD11b^+F4/80^+$macrophages (B, D, F) of young and old mice (n=4 mice/group).



S Fig3. Aging myeloid cells induce 4BL cells (A,B**).** CD11b$^+$ myeloid cells isolated from PC of young and old mice (depleted of B cells, T cells, and NK cells with CD19-PE+CD3-PE + anti-PE MicroBeads and DX5-PE + anti-PE MicroBeads, which yielded >80% enrichment in CD11b$^+$ cells, (A) were cultured with eFluor® 450-labeled B cells (from naïve young mice) at 1:1 ratio (B) for 24 h. As shown in a representative experiment performed ≥ 4 times (B), B cells did not proliferate. (C) **Murine CD8$^+$ T cells express 4-1BB upon CD3/CD28 activation**. Murine CD8$^+$ T cells were activated with 1.5 μg/ml of soluble anti-CD3 Ab + 1.5 μg/ml of soluble anti-CD28 for 24 h. Shown, a representative result (mean ± SEM) of 2 independent experiments performed in triplicates. P-value significance is shown as *p<0.05.



S Fig4. Importance of IFNγ provided by myeloid cells in induction of 4BL cells. (A) 4BL cells use CD86 in induction of GrB^+CD8^+ T cells, as it was lost in the presence of an anti-CD86 blocking Ab. (B) Young mouse splenic B cells up-regulate CD86 upon treatment with IFNγ (100 U/ml, 24 h). (C, D) Expression of 4-BBL in splenic and PC B cells is not affected by the loss of IFNγ signaling, such as in IFNγR1 KO or IFNγ KO mice. (E) Despite the loss of IFNγR (light grey), B cells up-regulate 4-1BBL in response to aging myeloid cells of mice (Old-M vs Young-M) as well as WT B cells (dark bars). (F) Compared to WT mice, B cells from young ARE-Del mice, which constitutively produce IFNγ, express comparable levels of 4-1BBL in splenic and PC B cells. (G) However, only ARE-Del mouse B cells express higher levels of CD86. Shown is a representative result (mean ± SEM) of 3 independent experiments ($n \geq 3$ mice/group) performed in triplicates. P-value significance is shown as *$p<0.05$ and NS = not statistically significant.