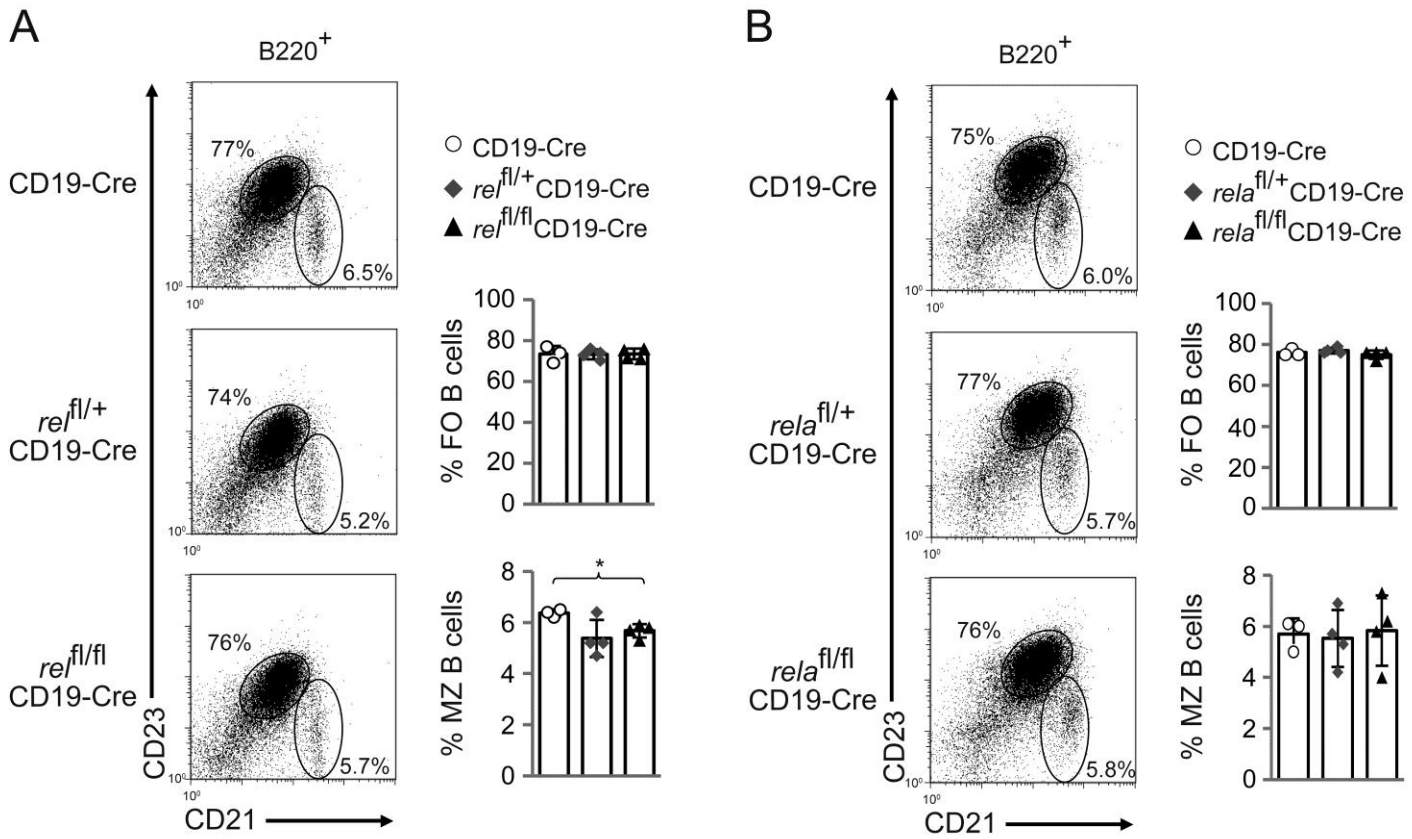
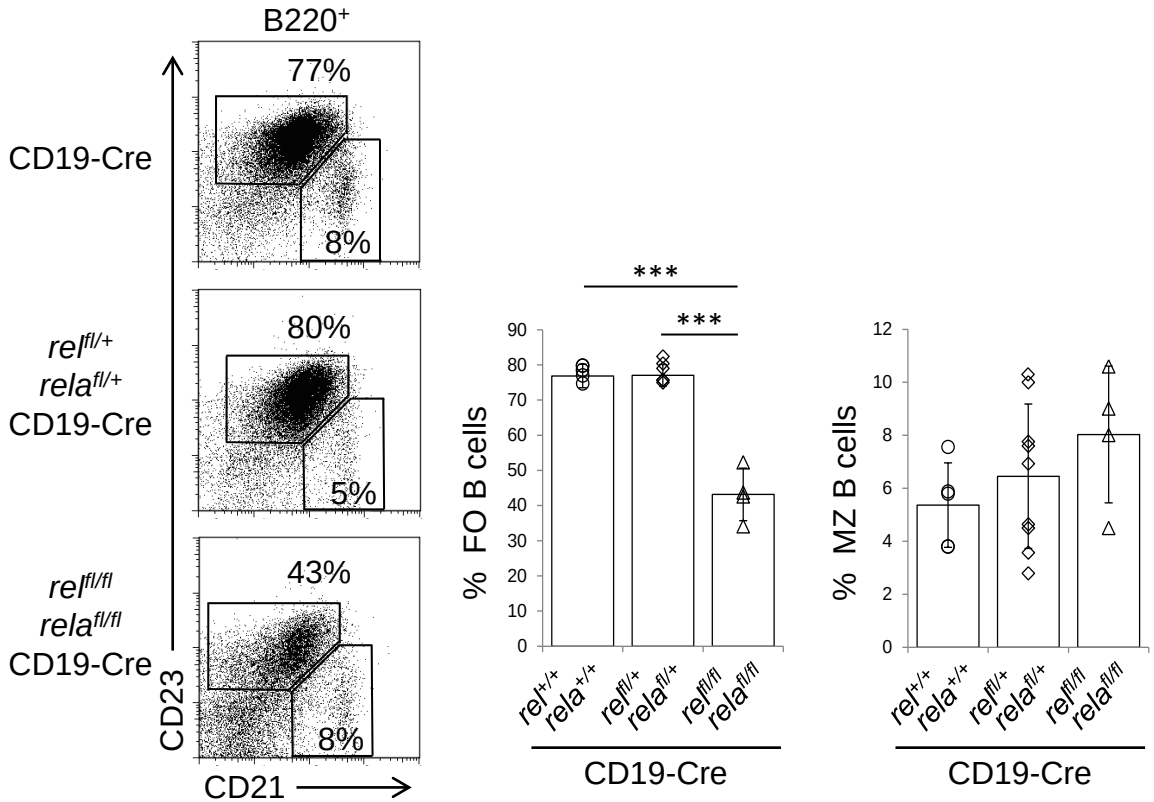
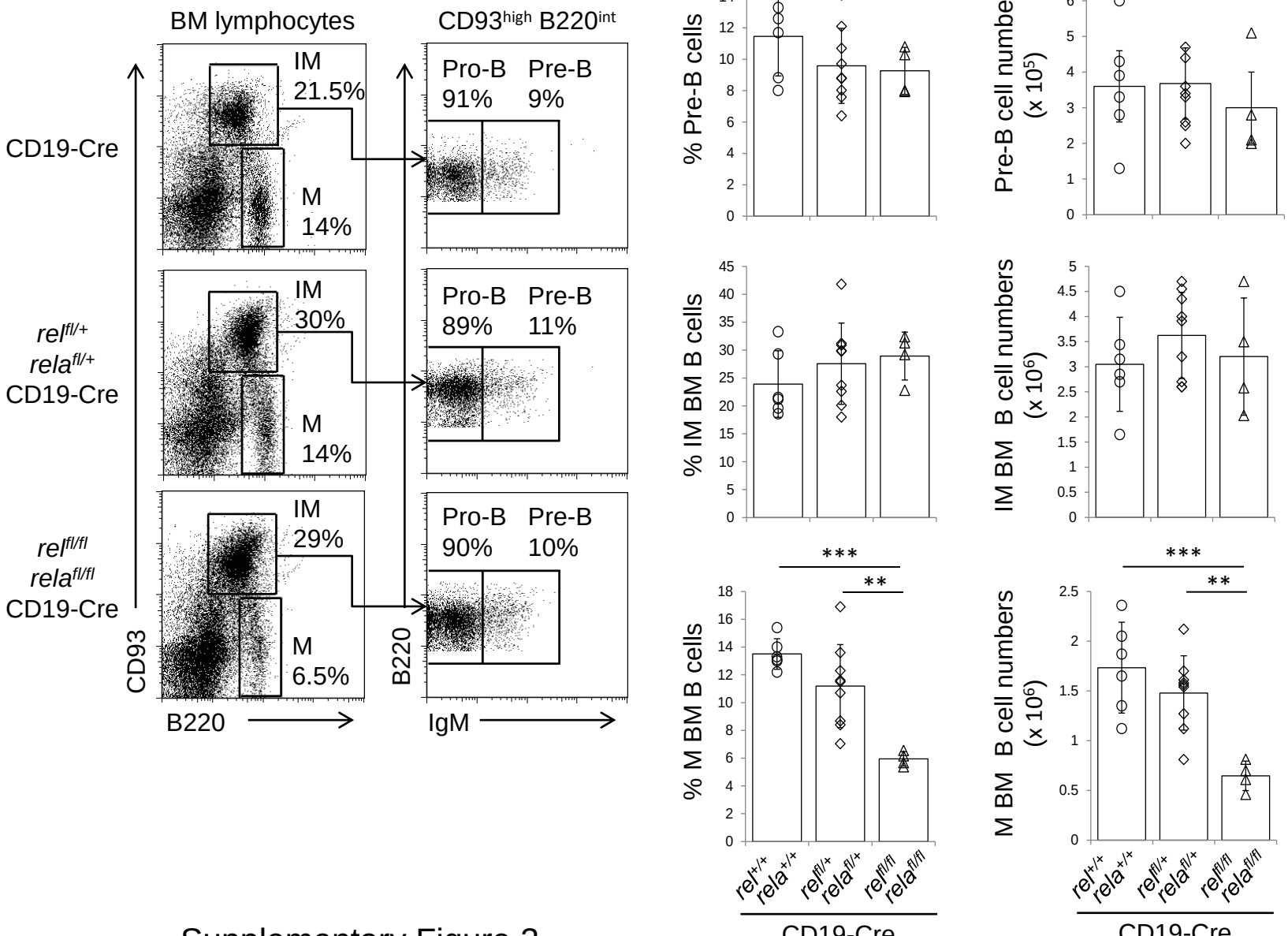


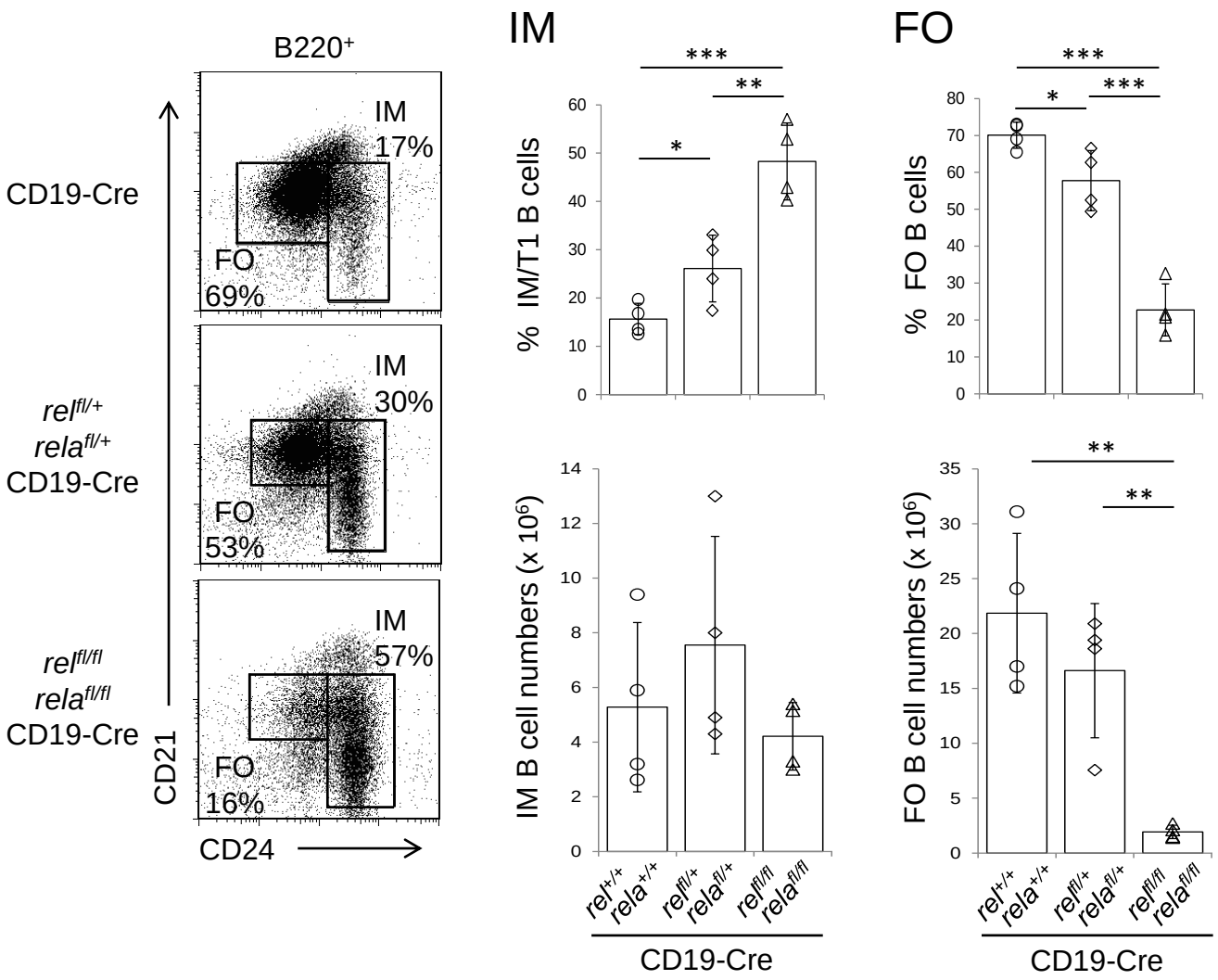
Supplementary Figure 1



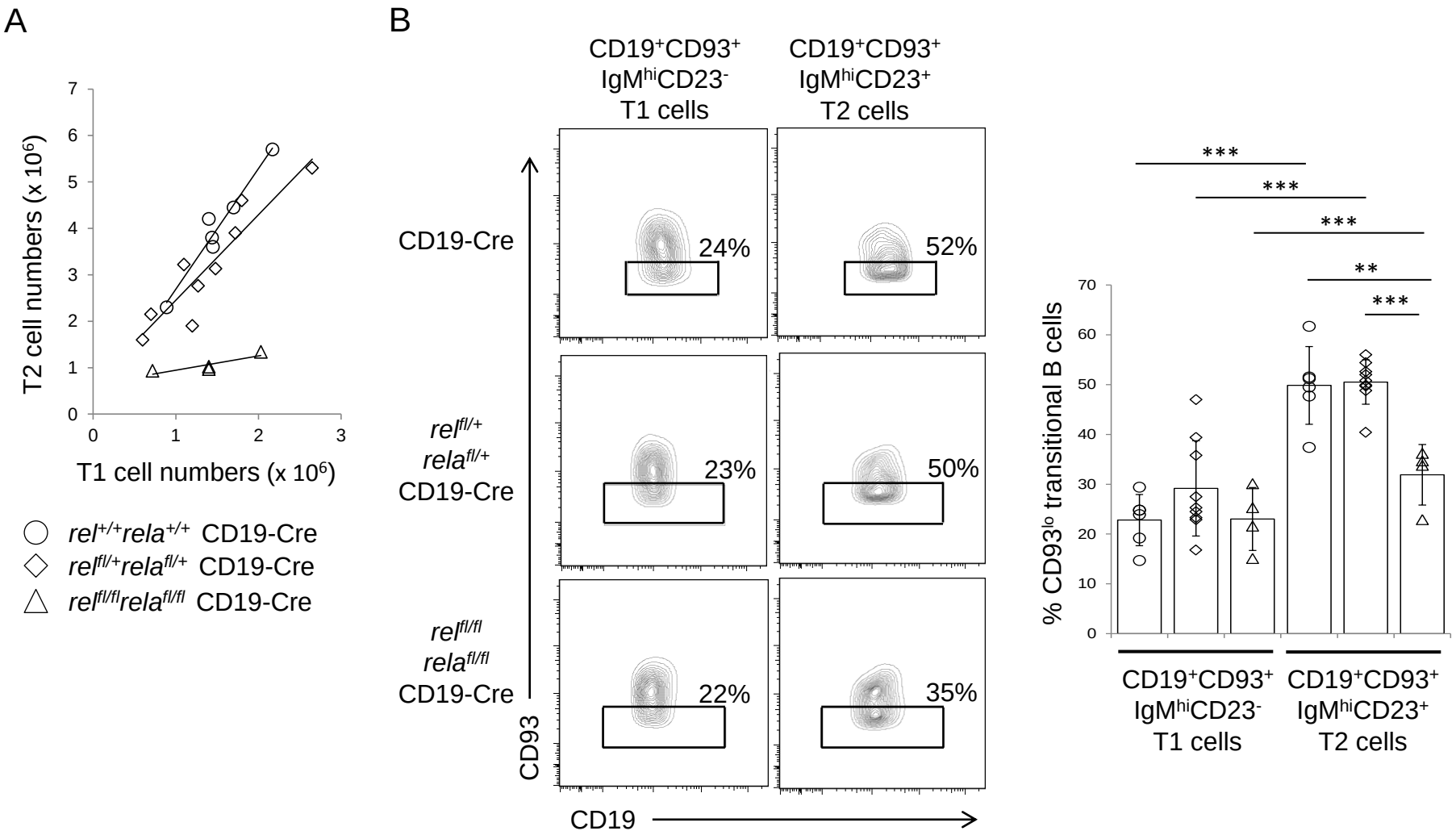
Supplementary Figure 2



Supplementary Figure 3



Supplementary Figure 4



Supplementary Figure 5

Supplementary Fig. 1. Mice deficient for c-REL or RELA in B-cells show normal development of splenic B-cell subsets. (**A,B**) CD23 and CD21 expression by splenic B-cells from *rel*$^{fl/fl}$CD19-Cre or *rela*$^{fl/fl}$CD19-Cre and heterozygous and CD19-Cre control mice were analyzed by flow cytometry. (*left*) Numbers beside gates indicate the percentage of CD23^{+}CD21^{low} follicular (FO) or CD23^{-}CD21^{hi} marginal zone (MZ) B-cells. (*right*) Summary of the frequencies of follicular and MZ B-cells. Data are cumulative from independent experiments (n=3-5 per group), with each symbol representing a mouse. Data are shown as mean ± standard deviation. Statistical significance was determined by Student's *t* test (*, $P<0.05$).

Supplementary Fig. 2. Mice deficient for both c-REL and RELA in B-cells show a reduction in the fraction of FO B-cells. CD21 and CD23 expression of splenic B-cells from mice of the indicated genotypes were analyzed by flow cytometry. (*left*) Numbers beside gates indicates the percentage of follicular (CD23^{+}CD21^{int}) and MZ (CD21^{hi}CD23^{-}). (*right*) Summary of the frequencies of follicular and MZ B-cells. Data are cumulative from independent experiments (n=4-9 per group), with each symbol representing a mouse. Data are shown as mean ± standard deviation. Statistical significance was determined by Student's *t* test (***, $P<0.001$).

Supplementary Fig. 3. Combined c-REL and RELA deficiency does not impair B-cell maturation in the bone marrow. B220, CD93 and IgM expression of bone marrow (BM) cells from mice of the indicated genotypes were analyzed by flow cytometry. (*left*) Numbers beside gates indicates the percentage of immature B-cells (IM; B220^{lo}CD93^{hi}), mature B-cells (M; B220^{+}CD93^{lo}), pro-B (B220^{lo}CD93^{hi}IgM^{-}) and pre-B (B220^{lo}CD93^{hi}IgM^{+}) cells. (*right*) Summary of the frequencies of pre-B, immature and mature BM B-cells. Data are cumulative from independent experiments (n=4-9 per group), with each symbol representing a mouse. Data are shown as mean ± standard deviation. Statistical significance was determined by Student's *t* test (**, $P<0.01$; ***, $P<0.001$).

Supplementary Fig. 4. Most peripheral B-cells in c-REL/RELA-deficient mice express high levels of CD24. B220, CD24 and CD21 expression of splenic B-cells from mice of the indicated genotypes were analyzed by flow cytometry. (*left*) Numbers beside gates indicate the percentage of immature B-cells (IM; $B220^{+}CD24^{hi}CD21^{lo}$) and FO B-cells ($B220^{+}CD24^{lo}CD21^{int}$). (MZ B-cells are found among $B220^{+}CD24^{int}CD21^{hi}$ B-cells.) (*right*) Summary of the frequencies of immature and mature B-cells. Data are cumulative from independent experiments (n=4 per group), with each symbol representing a mouse. Data are shown as mean ± standard deviation. Statistical significance was determined by Student's *t* test (*, $P<0.05$; **, $P<0.01$; ***, $P<0.001$).

Supplementary Fig. 5. Further characterization of the block in the T1 to T2 transition in *rel*$^{fl/fl}$*rela*$^{fl/fl}$CD19-Cre mice. (**A**) T2 cellularity as a function of T1 cell numbers. Data are from independent experiments (n=4-9 per group), with each symbol representing a mouse. Lines represent the best-fitting linear function, forced to go through the origin. (**B**) Determination of the percentage of $CD93^{lo}$ cells within T1 and T2 populations of the indicated genotypes. (*left*) Numbers beside gates indicates the percentage of $CD93^{lo}$ cells among $CD19^{+}CD93^{+}IgM^{hi}CD23^{-}$ T1 and $CD19^{+}CD93^{+}IgM^{hi}CD23^{+}$ T2 cells. (*right*) Summary of the frequencies of $CD93^{lo}$ transitional B-cells. Data are cumulative from independent experiments (n=4-9 per group), with each symbol representing a mouse. Data are shown as mean ± standard deviation. Statistical significance was determined by Student's *t* test (**, $P<0.01$; ***, $P<0.001$).