Supporting Information

miR-191 modulates B-cell development and targets transcription factors E2A, Foxp1, and Egr1

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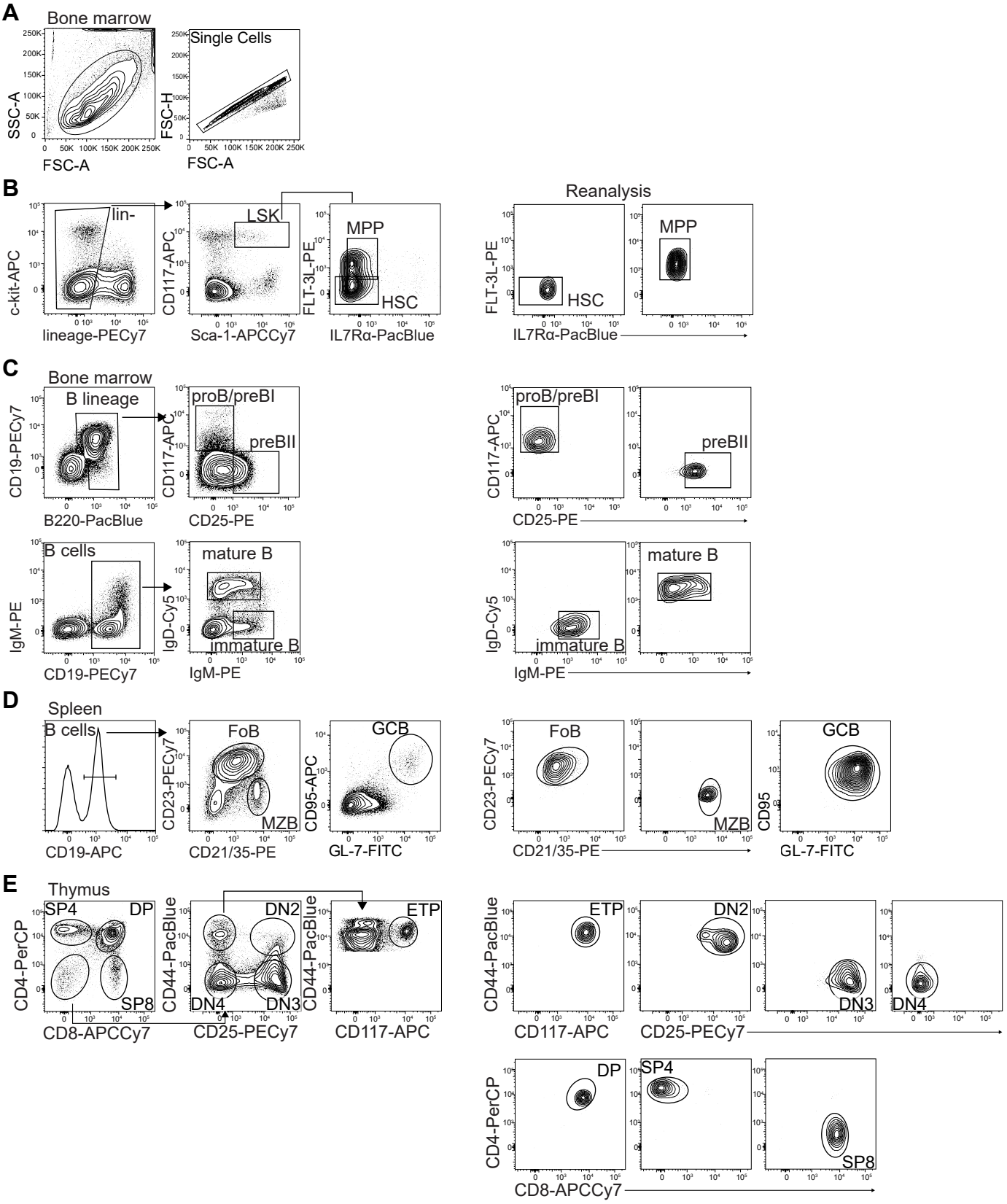

Fig. S1. Gating strategies for cell population identified by flow cytometry and Reanalysis of FACS sorted populations Representative contour plots reflecting gating strategies used to identify (A) single cells, (B) Lin-,CD117+,Sca-1+ (LSK), Lin-,CD117+,Sca-1+, FLT3L- Hematopoietic stem cells (HSC) and Lin-,CD117+,Sca-1+, FLT3L+ multipotent progenitor cells (MPP), (C) CD19+B220+CD117+ proB/preBI, CD19+B220+CD25+preBII, CD19+IgM+ imature and CD19+IgD+ mature B cells, (D) CD19+CD23+ Folicular (FoB), CD19+CD21/35+marginal zone (MZB) and CD19+CD95+GL-7+ germinal center (GC) B cells and (E) CD4-CD8-CD44+CD117+ early T cell progentitor (ETP), CD4-CD8-CD44+CD25+ double negative 2 (DN2), CD4-CD8-CD25+DN3, CD4-CD8-DN4, CD4+CD8+double positive (DP), CD4+ single positive (SP4) and CD8+ single positive (SP8) T cells from indicated organs. Hematopoietic progentitor cells were identified after excluding lineage positive cells (CD19, CD4, CD8, Gr-1, CD11b, CD11c, NK1.1) labelled with PE-Cy7 conjugated antibodies.

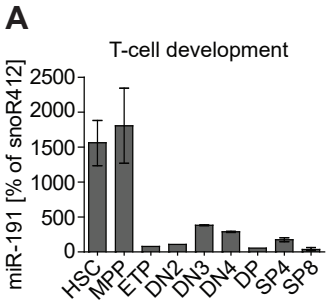

Fig. S2. Dynamic expression of miR-191 during T-cell development. (A) Analysis of expression of miR-191 in developing T cells by quantitative RT-PCR (qRT-PCR). Expression levels are relative to snoR412. Pooled data of four independent experiments are depicted. Data are shown as mean relative expression + SD.

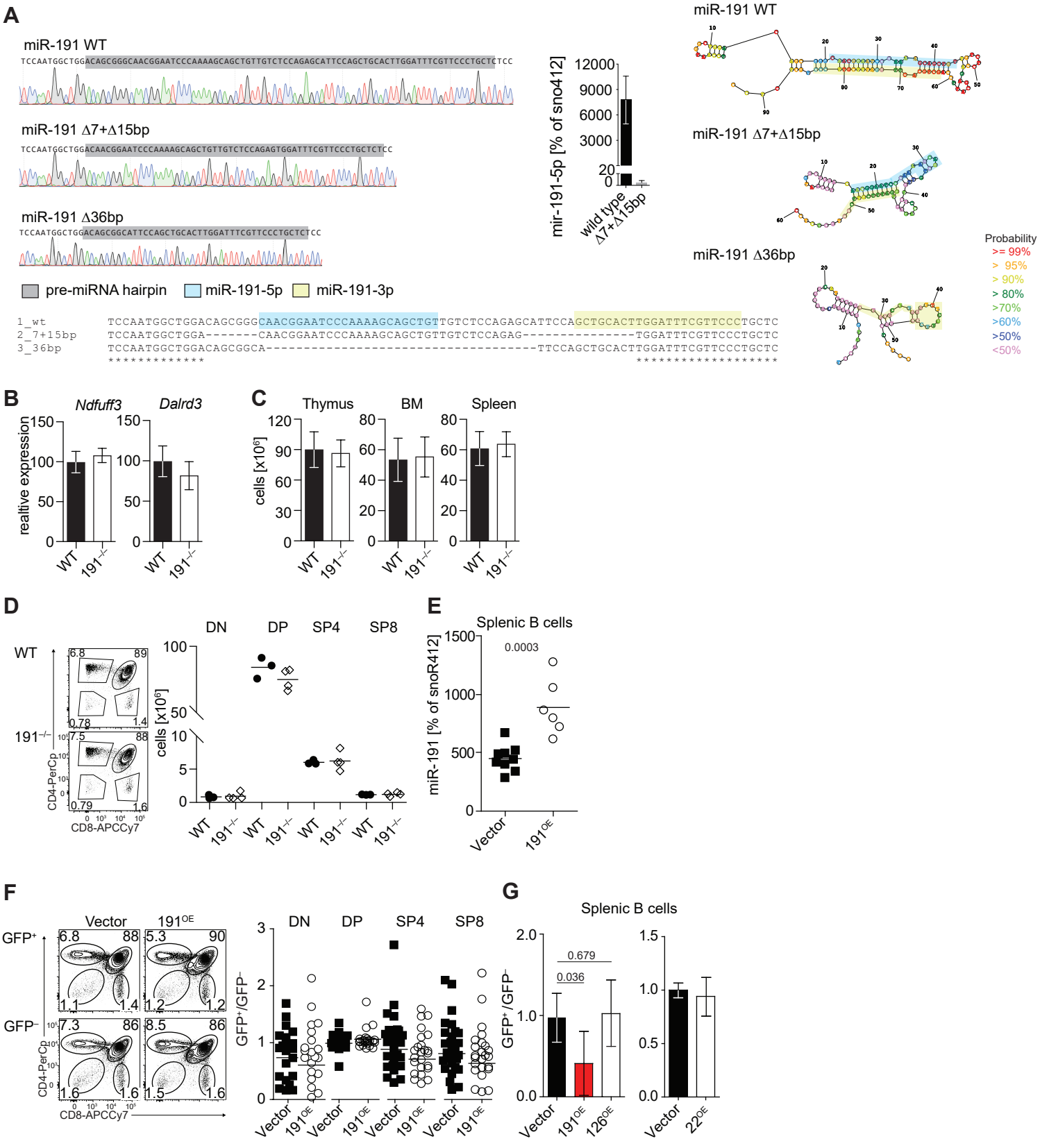

Fig. S3. Deletion of miR-191 by CRISPR/Cas9 is specific. Over-expression of miR-191 in B cells and 3T3 cells. The block in B-cell development is specific to miR-191. (A) Sanger-sequencing reads of 7+15bp and 36bp deletions in DNA isolated from CRISPR/Cas9 genetically modified mice, alignment to highlight deleted regions and expression of miR-191 in B cells from wild type and 7+15bp deleted mice by quantitative RT-PCR (qRT-PCR). Expression levels are relative to snoR412, (n=3, mean+SEM). Predictions of secondary structure were calculated using RNAStructure v6.0 (rna.urmc.rochester.edu) (B) Expression of genes surrounding the mmu-miR-191 encoding locus in WT and miR-191 deficient B cells, Expression relative to Hprt and normalized for WT expression (n=3, mean+SEM). (C) Cellularity of indicated organs from WT and 191–/– mice (n=5 mean + SEM). (D) Representative contour plots indicating development of T cells upon deletion of miR-191(191–/–) and in control mice (WT) (left) and quantification of two independent experiments with two mice per group shown as total number (right) (E) expression of miR-191 in total splenic B cells isolated from overexpression chimeras. Data are shown as mean relative expression + SD of one experiment with six to nine mice per group. (F) Representative contour plots indicating development of T cells upon overexpression of miR-191 (191OE) and in control mice (Vector) (left) and quantification of at least three independent experiments with six mice per group (right). In all charts each dot represents an individual mouse. (G) Development of B cells in chimeric mice upon overexpression of miR-191, miR-126, or miR-22, (n=5 mean + SEM). Indictated p-Values were determinded by unpaired *t*-tests. Values in contour plots indicate percentage of cells in gates closest to the numbers.

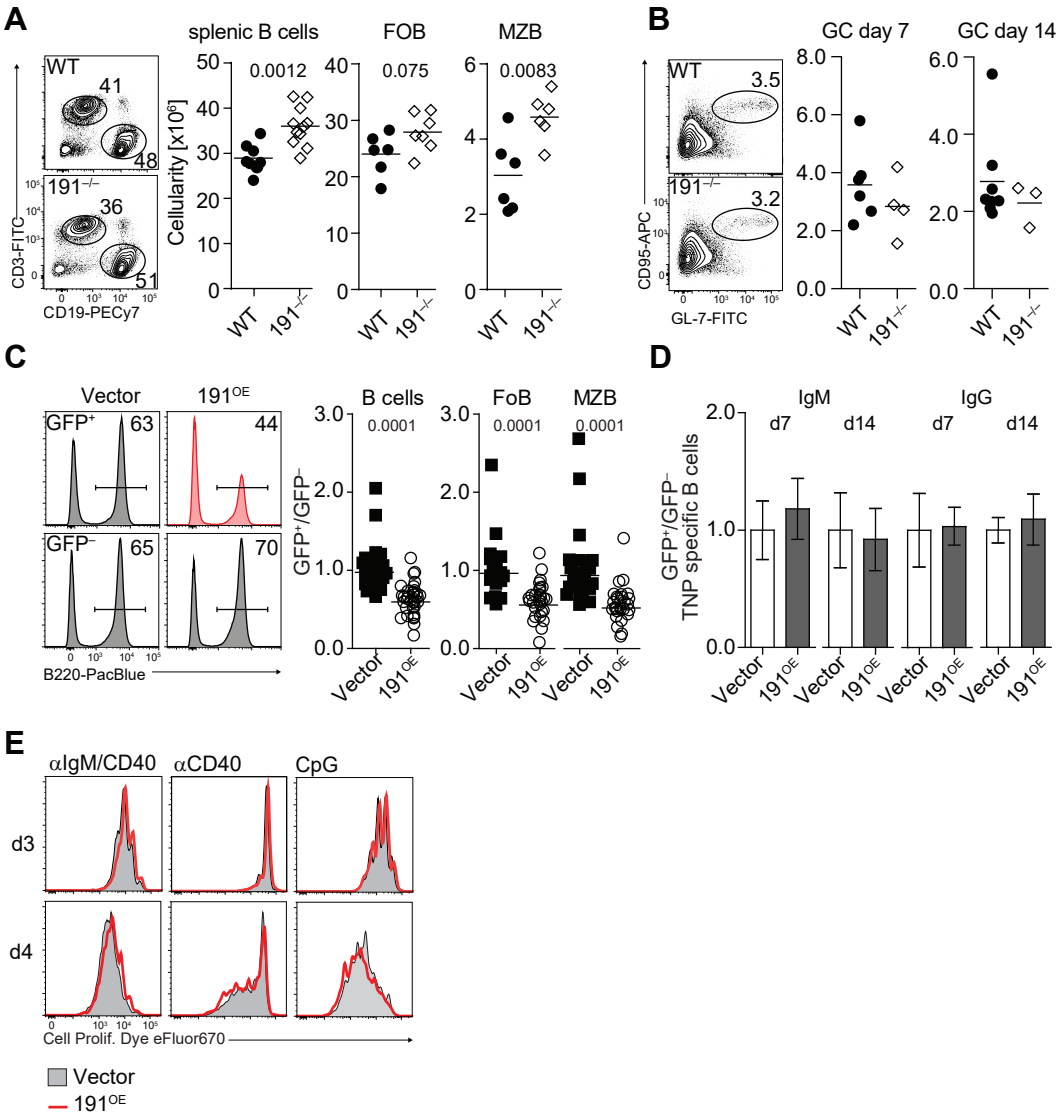

Fig. S4. Over-expression but not loss of miR-191 affect B cells in the periphery. (A) Representative contour plots of splenic B and T cells (left) and quantification of total splenic B cells and follicular (FoB - CD19+CD23hiCD21/35lo) and marginal zone (MZB - CD19+CD23loCD21/35hi) B cells in miR-191KO (open diamonds) and control littermates (WT, black circles). (B) miR-191 deficient (191–/–) and littermates (WT) mice were i.v. immunized with SRBC. 7 and 14 days post immunization GC B cells were analyzed by flow cytometry. Representative contour plots (left) and quantification (right). (C) Representative histograms of splenic B cells (left) and quantification of total B cells and follicular (FoB) and marginal zone (MZB) B cells in miR-191 chimeras given as ratio of GFP+/GFP– (right). (D) Chimeric mice overexpressing miR-191 (191OE) or control mice (Vector) were immunized with TNP-KLH and ratios of antigen-specific IgM+ and IgG+ B cells were determined by ELISPOT after 7 and 14 days (n=6, +SEM). (E) Proliferation of follicular B cells sorted from chimeric mice stimulated with αIgM, αIgM and αCD40 or CpG for 3 and 4 days assessed by Cell Proliferation Dye eFluor670. Numbers next to the gates indicate percentages. Numbers above the plots indicate p values. Each dot represents an individual mouse. Statistical analysis was performed using unpaired Student's t test and p-Values are indicated in plots.

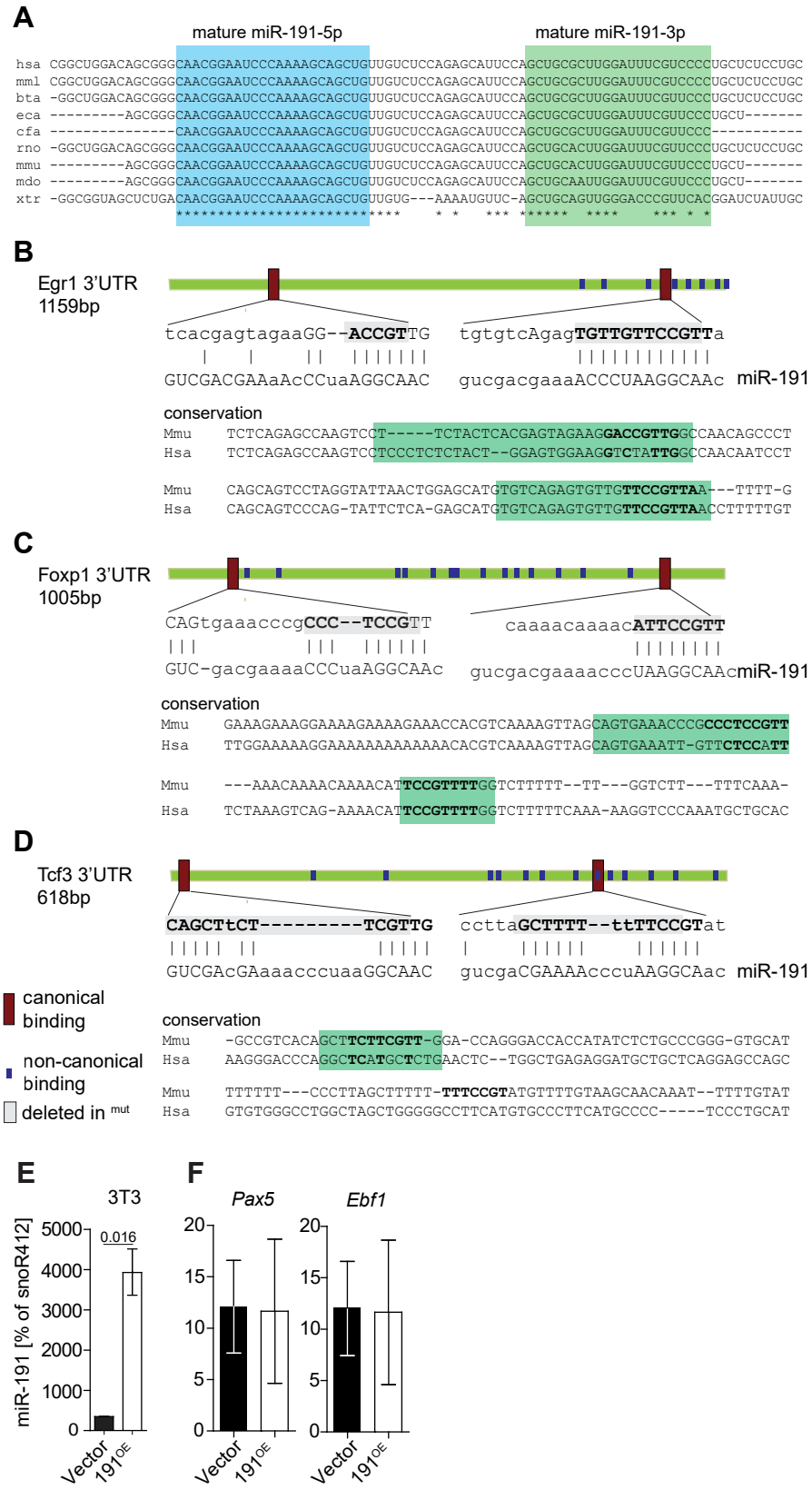

Fig. S5 Conservation of miR-191 and its putative binding sites in 3' untranslated regions (UTR) of Egr1, Foxp1 and E2A. (A) Alignment of miR-191 stem loop sequences from multiple species. Sequences of miR-191-5p (blue) and miR-191-3p (green) are highlighted. Conservation among species is indicated with an asterisk. hsa=human, mml= Rhesus macaque, bta=cattle, eca=horse, cfa=dog, rno=rat, mmu=mouse, mdo=opossum, xtr=frog. (B-D) Putative binding sites of miR-191 in 3'UTR of Egr1 (B), Foxp1 (C) and Tcf3 (D). Red boxes indicate canonical binding sites also depicted below. Sequences highlighted in gray are deleted in mutated UTRs used for luciferase assays. Dark blue boxes indicate multiple poly-T fragments which may serve as non-canonical binding sites of miR-191. Alignment of respective UTRs from human and mouse. Canonical binding sites are highlighted in green. (E) expression of miR-191 in 3T3 lines after transduction with miR-191 overexpression vector or control vector. (n=3 +SD) (F) Expression of transcription factors Pax5, Ebf1 and in proB/preBI cells sorted from control (Vector) and miR-191 overexpressing (191OE) chimeric mice. Data are shown as mean relative expression + SD of one experiment with three mice per group.

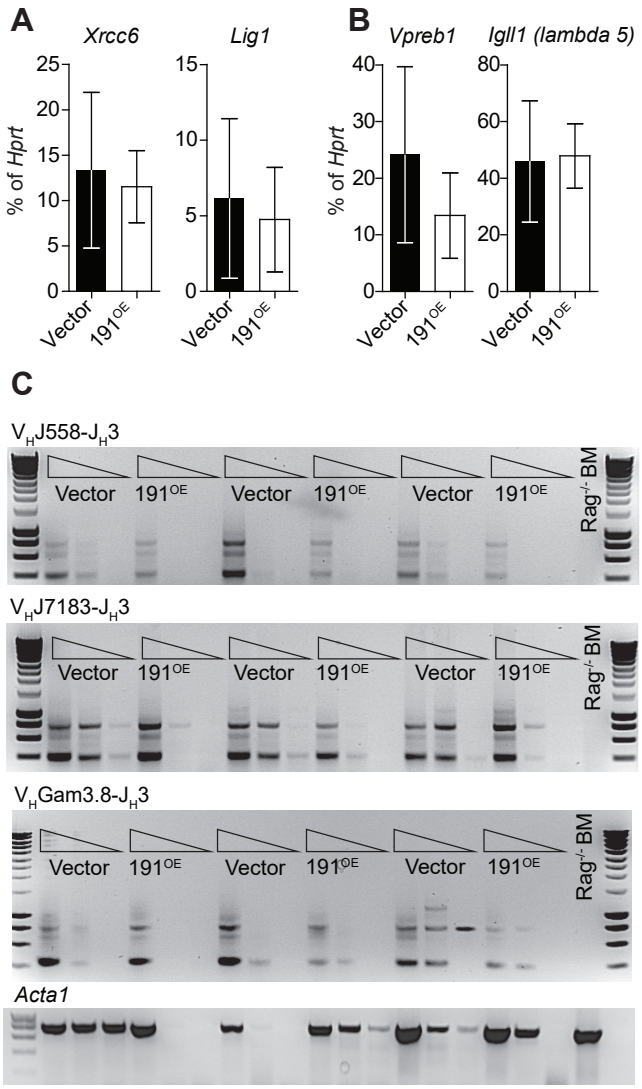

Fig. S6 . Expression of rearrangement genes, transcription factors and V(D)J recombination in early B cell progenitors. Expression of (A) preBCR associated genes *Vpreb1, Igll1* and (B) rearrangement genes *Xrcc6, Lig1* in proB/preBI cells sorted from control (Vector) and miR-191 overexpressing (191OE) chimeric mice. Data are shown as mean relative expression + SD of one experiment with three mice per group. (C) Semi-quantitative PCR analysis of genomic DNA for VHJ558, VHGam3.8 or VHJ71833 to JH3 rearrangements in proB/preBI cells sorted from control (Vector) and miR-191 overexpressing (191OE) chimeric mice. Expression of recombination-independent *Acta1* serves as loading control. PCR was carried out on the DNA content of 20,000, 4,000 and 800 cells. Each dilution series resembles DNA extracted from individual mice