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Supplemental Data

Structurally Distinct Phosphatases CD45 and CD148 Both Regulate B Cell and Macrophage Immunoreceptor Signaling

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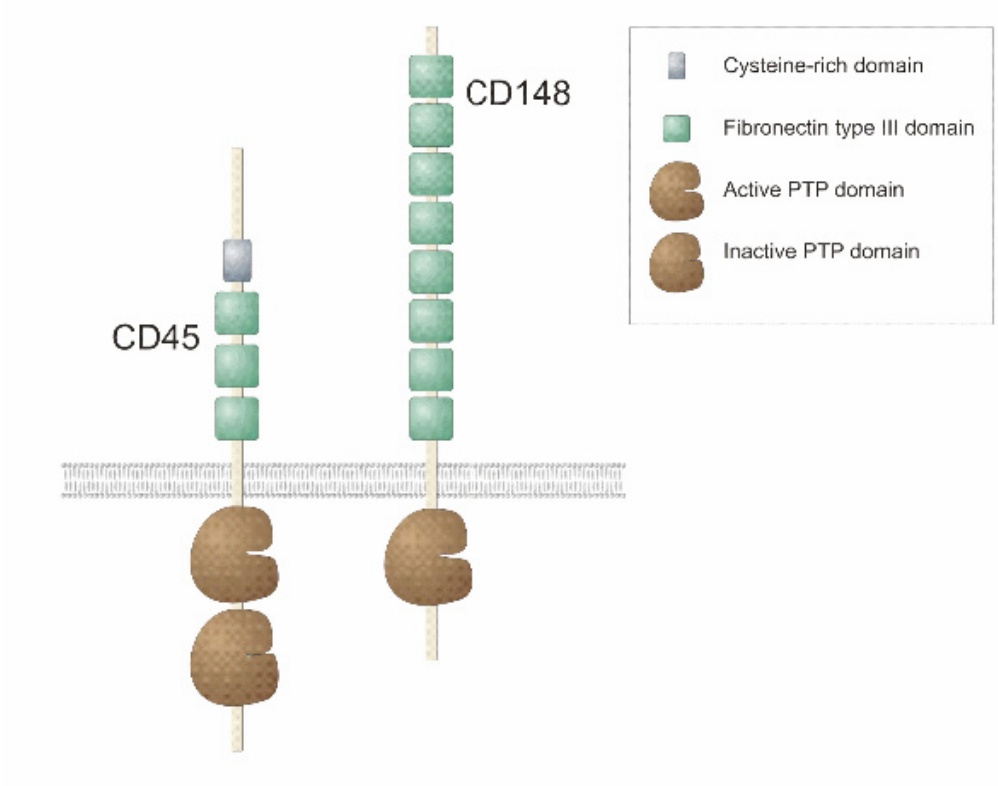
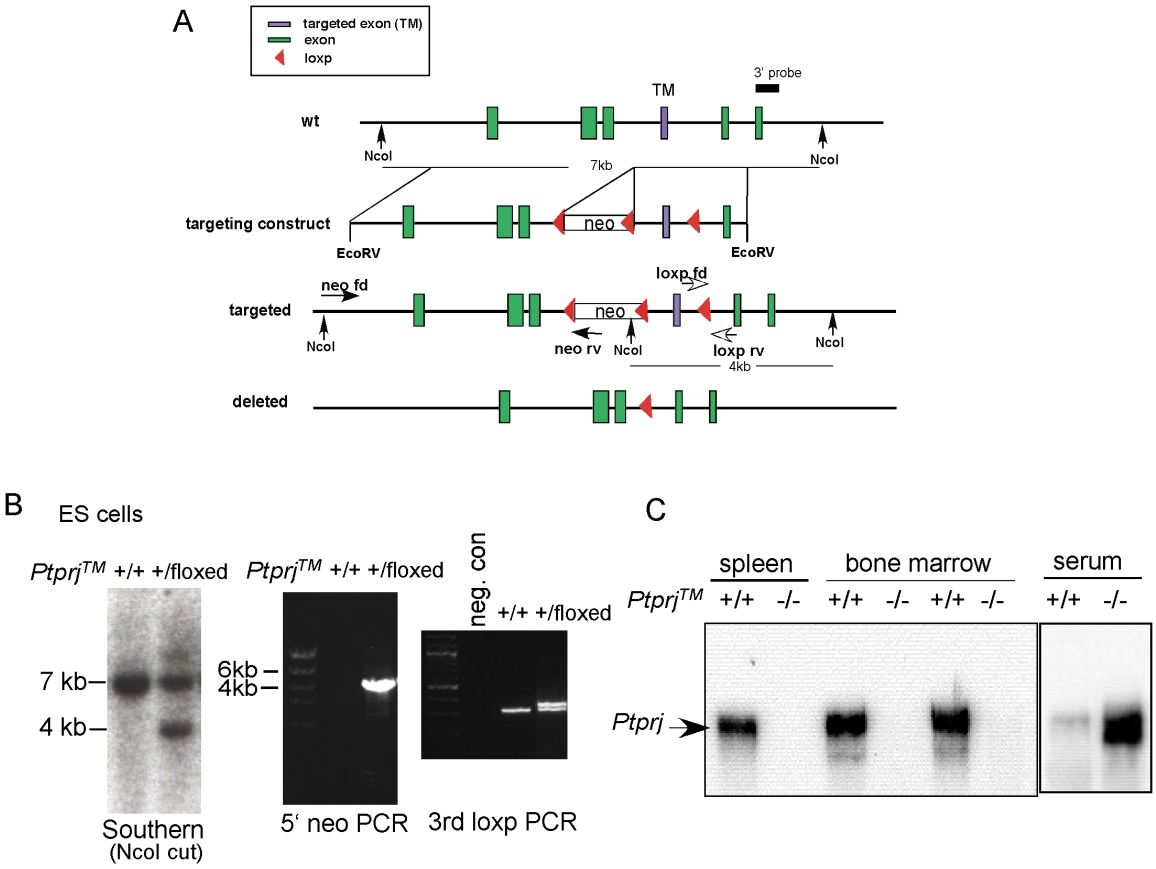


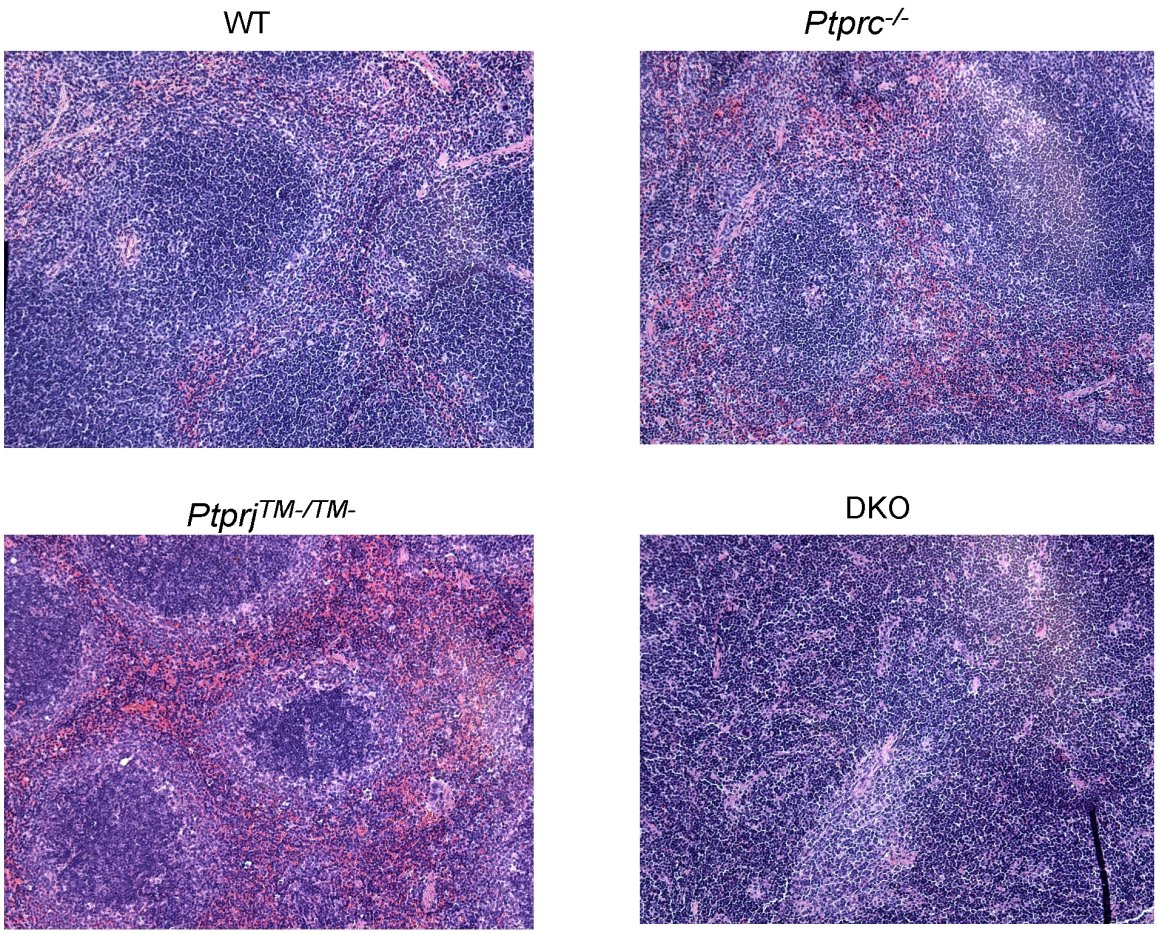
Supplementary figure 1. Comparison of the domain composition of CD45 and CD148.



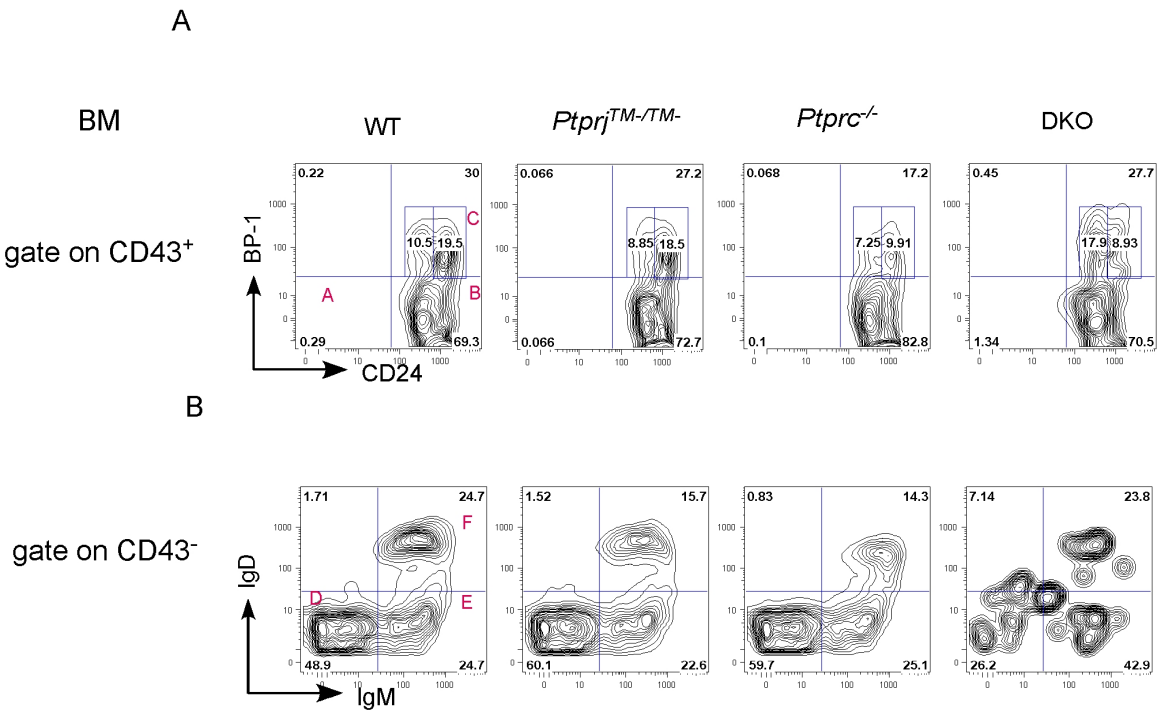
Supplementary figure 2. Generation of *Ptprj*$^{TM-/TM-}$ mice. (A). Schematics of part of the CD148 locus and strategy for conditional deletion. A loxP-flanked neomycin-resistance cassette and a third loxP site were introduced into both sides of exon 18 which encodes the transmembrane domain of CD148. Fragment sizes generated by digestion with NcoI are indicated, along with the 3' probe used for Southern blot analysis. **(B)** The targeting construct was transfected into the 129 strain embryonic stem (ES) cell line (Tsuda et al., 1997). ES cell clones were screened for homologous recombination. Southern blot and PCR analysis of DNA were prepared from the ES cell line (WB). The targeted allele gives rise to a 4 kb fragment in southern blot (left panel). PCR primers confirming 5' orientation of the construct and neomycin include neo-forward (neo fd), which is outside of 5' homologous arm, and neo-reverse (neo rv), which is within the neomycin cassette. The neo primer set amplifies a 4.3 kb product only

in the targeted allele, but no product in the wt allele (middle panel). LoxP primers include loxP-fd and loxP-rv that generate a 344 bp product in the targeted and flox alleles, but a 310 bp product in the wt allele and no product in deleted allele (right panel). Correctly targeted ES cell lines were used to generate chimeric mice. The floxed neo cassette was removed by breeding the targeted mice with X-linked CMV-Cre transgenic female mice (Jackson Lab). From this cross, we isolated heterozygous mice in which the transmembrane loxP site had undergone recombination, leaving only a single loxP site and resulting in inactivation of the CD148 locus. We then screened for mice that lost the CMV-Cre transgene. The CMV-Cre negative *Ptprj* $^{TM-/TM-}$ mice were selected for further breeding **(C).** Western blot analyses of splenocytes, bone marrow cells and serum from mice of indicated genotypes with mAb to murine CD148.

Spleen 10X

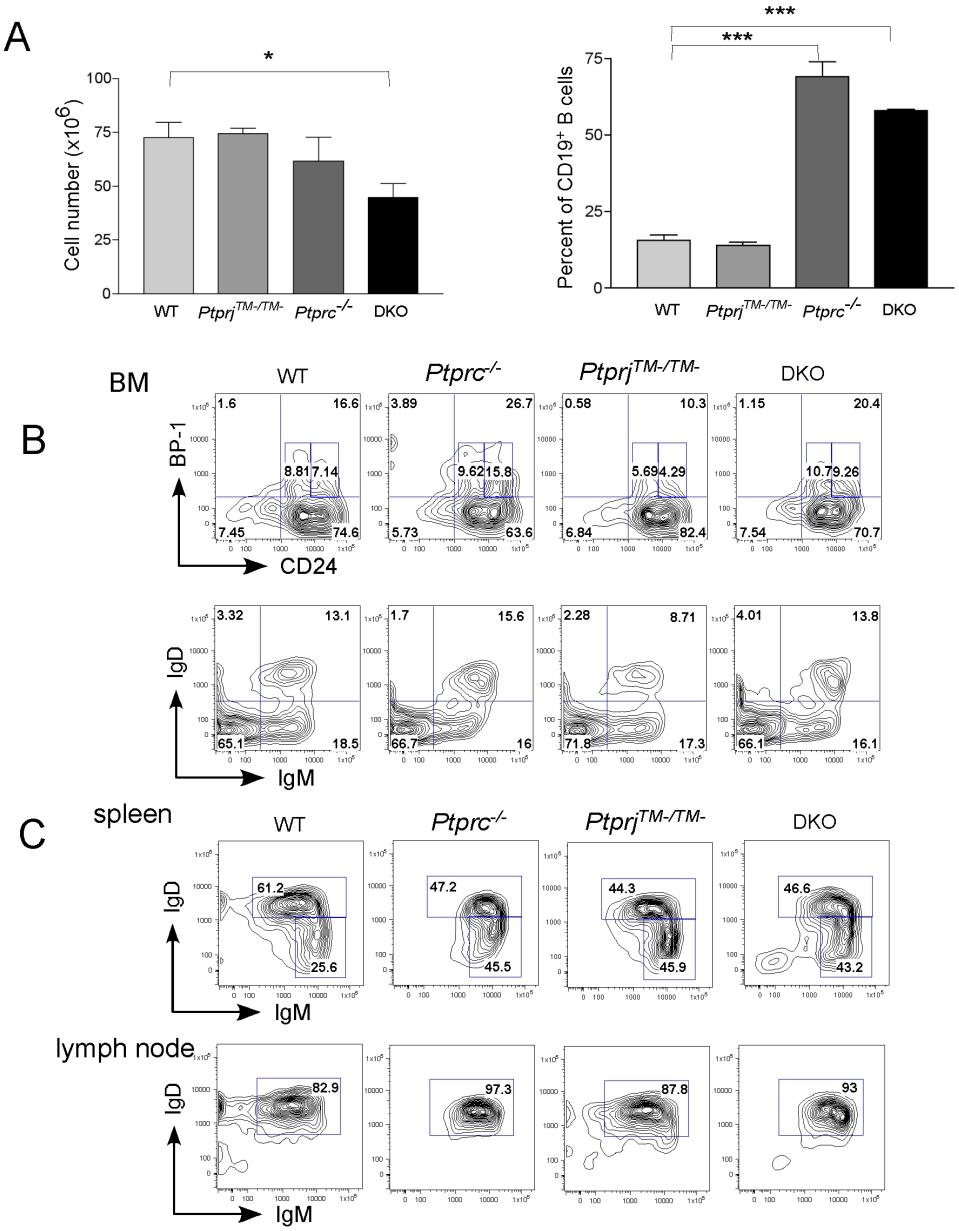


Supplementary figure 3. Distorted splenic architecture of DKO spleen. Splenic sections from mice of the indicated genotypes stained with H&E.



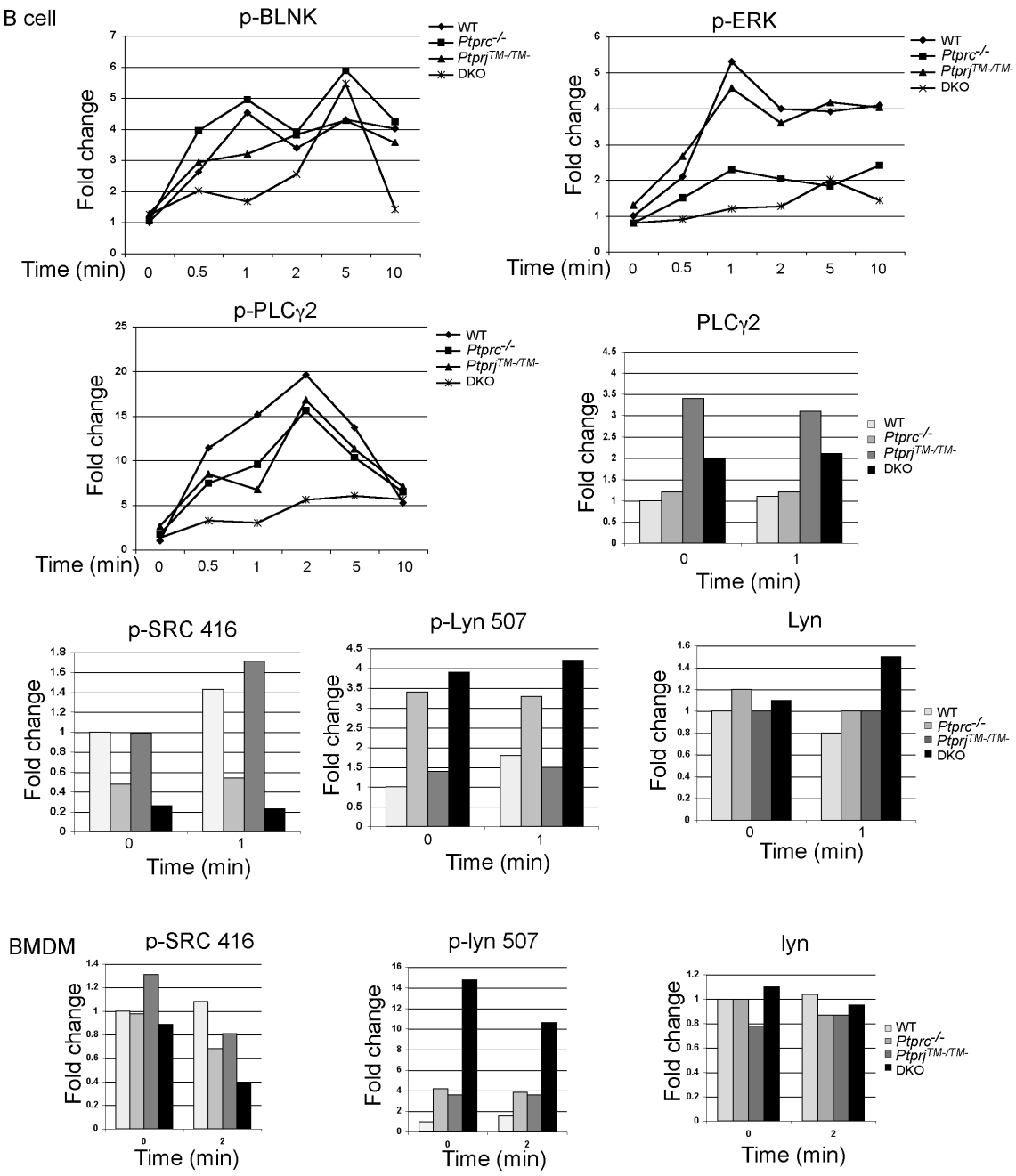
Supplementary figure 4. B cell developmental block in CD45/CD148 DKO mice in the B6 background. (A) Representative FACS analysis of bone marrow cells from 4-6 week-old mice of the indicated genotypes stained with mAb to CD19 and CD43. $CD19^+$ $CD43^+$ BM B cells were further identified as fraction B ($CD43^+$ $CD24^+$, $BP1^-$), fraction C ($CD43^+$ $CD24^+$, $BP1^+$). Fraction A ($CD19^-B220^+$) could not be analyzed due to the lack of CD45 (i.e. B220) expression in CD45 KO and DKO mice. **(B)** CD19+ CD43- BM B cells were further separated as fraction D ($CD43^-$ IgM^- IgD^-), fraction E ($CD43^-$ IgM^+ IgD^-), fraction F ($CD43^-$ IgM^+ IgD^+). Percentage of each B cell subset is shown.

mixed background

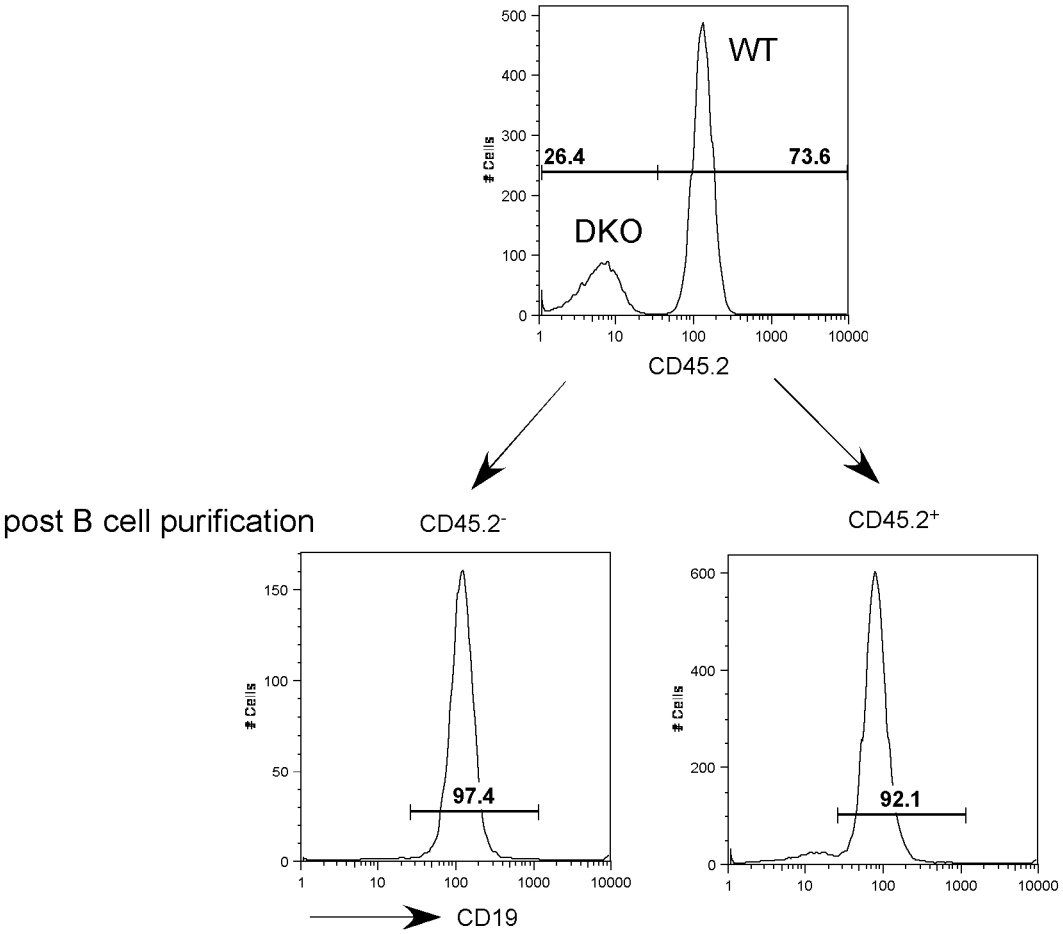


Supplementary figure 5. B cell phenotypes in mixed background DKO mice. **(A)** Total cell numbers (left panel) and percentage of $CD19^+$ B cells (right panel) in the spleen from mice of the indicated genotypes are shown in bar graph. Graphs show mean±SEM, n=5. * indicates $p<0.05$, *** indicates $p<0.0001$. **(B)** Representative FACS analysis of bone marrow cells from 8-10 week-old mice of the indicated genotypes stained with mAb to CD19, CD43. $CD19^+$ $CD43^+$ BM B cells were further identified as fraction B ($CD43^+$ $CD24^+$, $BP1^-$) and fraction C ($CD43^+$ $CD24^+$, $BP1^+$). Fraction A was not analyzed (upper panel). $CD19^+$ $CD43^-$ BM B cells were further identified as fraction D ($CD43^-$ IgM^- IgD^-), fraction E ($CD43^-$ IgM^+ IgD^-), fraction F ($CD43^-$ IgM^+ IgD^+) (lower panel). Percentage of each B cell subset is shown. **(C)** Representative FACS analysis of cells from

spleen (upper panel) or lymph node (lower panel) stained with mAb to CD19, IgM and IgD. Percentages of T1 (IgM^{hi} IgD^{lo}) cells and T2 (IgM^{hi} IgD^{hi}) plus follicular mature (FM) (IgM^{lo} IgD^{hi}) B cells in $CD19^{+}$ gate are shown.



Supplementary figure 6: Quantification for westerns in Figure 4 and 7. Kodak ID image analysis software 3.5 was used to quantify expression levels. The expression levels of indicated proteins were determined by normalizing to ERK (fig.4) or tubulin (fig.7) expression. Fold changes were normalized to the protein level from WT cells at T=0, which was set at 1.



Supplementary Figure 7: B cell purity post MACS beads purification.
Lethally irradiated recipient mice were reconstituted with BM from CD45/CD148 DKO and WT control mice. The donor origins of reconstituted B cells were identified using CD45.2 as a congenic marker: WT B cells were $CD45.2^+$ and DKO B cells were $CD45.2^-$. Lymph node B cells were purified and the purity (indicated as $CD19^+$ cells) for $CD45.2^+$ and $CD45.2^-$ cells was shown in histogram.
The purified B cells were then used for detecting intracellular free Ca^{2+} levels after addition of IgM $F(ab')_2$ (5μg/ml) (**see Figure 5**).

Thymus genotype		Total cell (10^6)	DN cells (%)	DN cells (10^6)	DP cells (%)	DP cells (10^6)	$CD4^+$ cells (%)	$CD4^+$ cells (10^6)	$CD8^+$ cells (%)	$CD8^+$ cells (10^6)
Wild type	N=3	61.8±8.4	4.0±0.7	2.5±0.7	84.4±2.3	52.1±6.9	8.2±0.9	5.1±1.0	3.0±0.5	1.8±0.3
***Ptprj*$^{TM/TM-}$**	N=3	70.4±6.6	3.8±0.6	2.7±0.3	82.0±3.8	58.2±7.6	9.5±1.8	6.5±0.7	4.0±0.8	2.7±0.3

Peritoneal lavage genotype		Total cell (10^6)	B cells (%)	B cells (10^6)	B1a cells (%)	B1a cells (10^6)	B1b cells (%)	B1bcells (10^6)	B2 cells (%)	B2 cells (10^6)
Wild type	N=3	4.2±0.6	33.2±5.6	1.1±0.1	31.5±1.2	1.3±0.3	0.3±0.09	8.2±0.9	30.2±3.0	0.4±0.12
***Ptprj*$^{TM/TM-}$**	N=3	4.3±1.0	35.5±9.9	1.4±0.4	20.4±4.0	1.4±0.4	0.2±0.04	9.5±1.8	53.8±8.9	0.84±0.4

Spleen genotype		Total cell (10^6)	B cells (%)	B cells (10^6)	T1 cells (%)	T1 cells (10^6)	T2+FMcells (%)	T2+FMcells (10^6)	MZ cells (%)	MZ cells (10^6)
Wild type	N=3	84.5±12.4	53.0±1.6	44.7±6.7	5.7±0.4	1.3±0.3	68.9±0. 9	30.9±4.8	8.2±0.9	2.4±1.2
***Ptprj*$^{TM/TM-}$**	N=3	111.9±10.3	51.1±5.8	56.5±5.7	10.7±1.5	1.4±0.4	54.0±3.7	30.5±3.6	21.3±4.0	8.8±1.4

Supplementary table 1: Statistical characterization of T and B cell populations in *Ptprj*$^{TM-/TM-}$ mice.

Supplementary Reference:

Tsuda, H., Maynard-Currie, C.E., Reid, L.H., Yoshida, T., Edamura, K., Maeda, N., Smithies, O., and Jakobovits, A. (1997). Inactivation of the mouse HPRT locus by a 203-bp retroposon insertion and a 55-kb gene-targeted deletion: establishment of new HPRT-deficient mouse embryonic stem cell lines. Genomics *42*, 413-421.