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Supplementary Materials for

The ion channel TRPM7 is required for B cell lymphopoiesis

Mithunah Krishnamoorthy, Fathima Hifza Mohamed Buhari, Tiantian Zhao, Patrick M. Brauer, Kyle Burrows, Eric Yixiao Cao, Vincent Moxley-Paquette, Arthur Mortha, Juan Carlos Zúñiga-Pflücker, Bebhinn Treanor*

*Corresponding author. Email: bebhinn.treanor@utoronto.ca

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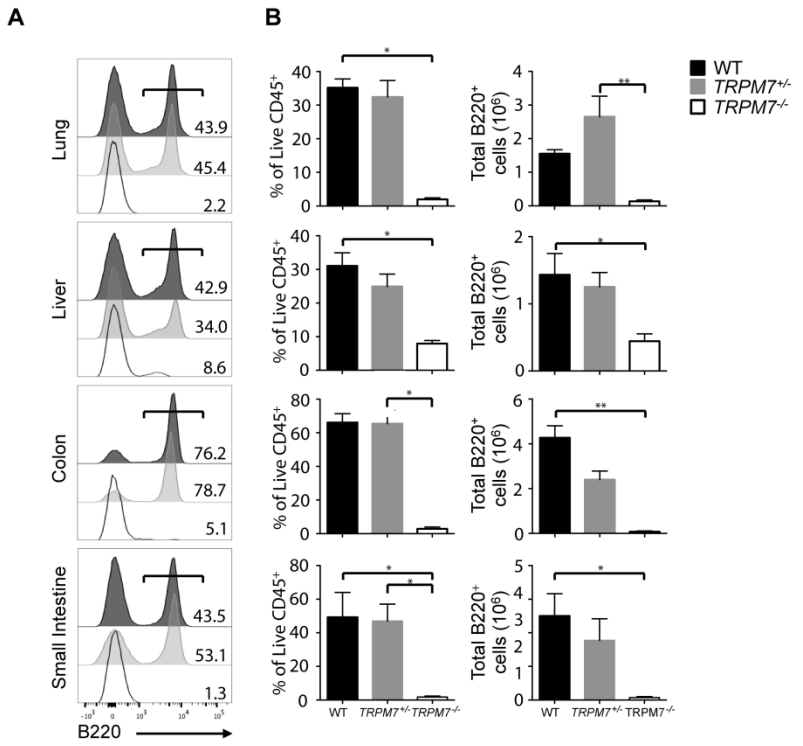

Fig. S1. TRPM7 is essential for B cell development in the gut, liver, and lung. (**A**) Flow cytometry analysis of B220 expression on gated viable, $CD45^+$ cells from the lung, liver, colon, and small intestine of WT, *TRPM7*$^{+/-}$, and *TRPM7*$^{-/-}$ mice. Histograms are representative of two independent experiments. (**B**) Quantification of $B220^+$ cells in peripheral tissues from the experiments represented in (A). Data are means ± SEM from 4 mice. Statistical significance was determined with Kruskal-Wallis test followed by Dunn's multiple comparisons test. **P* < 0.05, ***P* < 0.01.

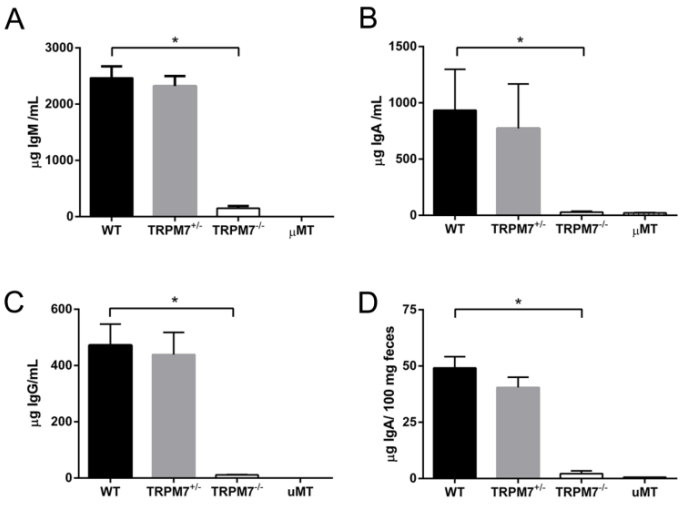

Fig. S2. Basal antibody concentrations are markedly reduced in *TRPM7*$^{-/-}$ mice. (**A** to **D**) Serum concentrations of IgM (A), IgA (B), and IgG (C), as well as fecal concentrations of IgA (D) in WT, *TRPM7*$^{+/-}$, *TRPM7*$^{-/-}$, and μMT mice were measured by ELISA. Data are means ± SEM from three mice of each genotype in 3 independent experiments. Statistical significance was determined with Kruskal-Wallis test followed by Dunn's multiple comparisons test. **P* < 0.05.

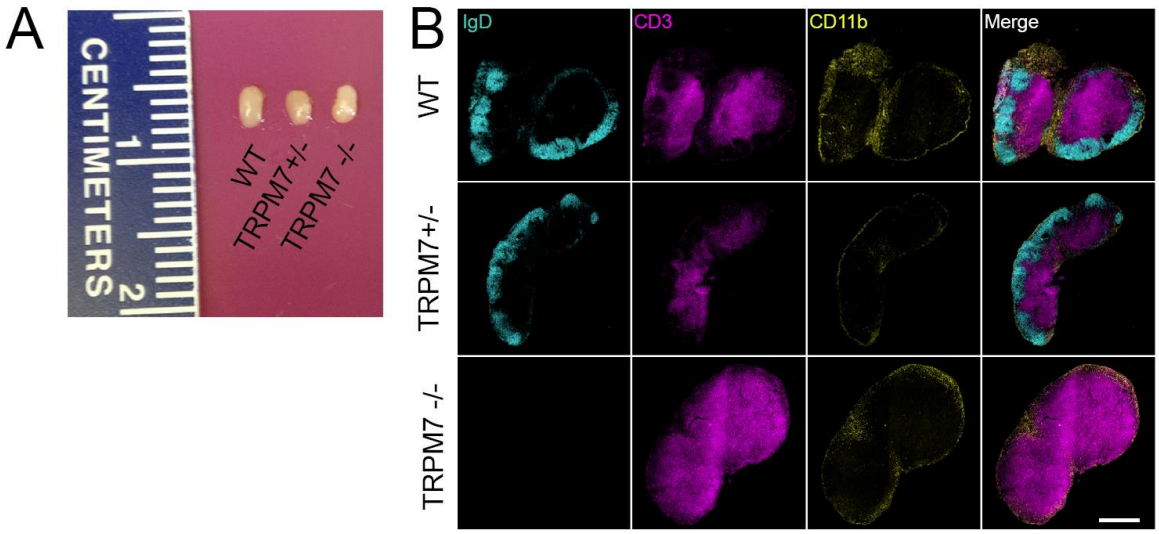

Fig. S3. Architecture of lymph nodes in *TRPM7*$^{-/-}$ mice. (**A**) Comparison of lymph node size from WT, *TRPM7*$^{+/-}$, and *TRPM7*$^{-/-}$ mice. Images are representative of 3 independent experiments. (**B**) Epifluorescence microscopy analysis of lymph node morphology in WT, *TRPM7*$^{+/-}$, and *TRPM7*$^{-/-}$ mice. Tissue sections were stained with antibodies against IgD (cyan), CD3 (magenta), and CD11b (yellow). Images are representative of at least three mice of each genotype. Scale bar, 0.5 mm.

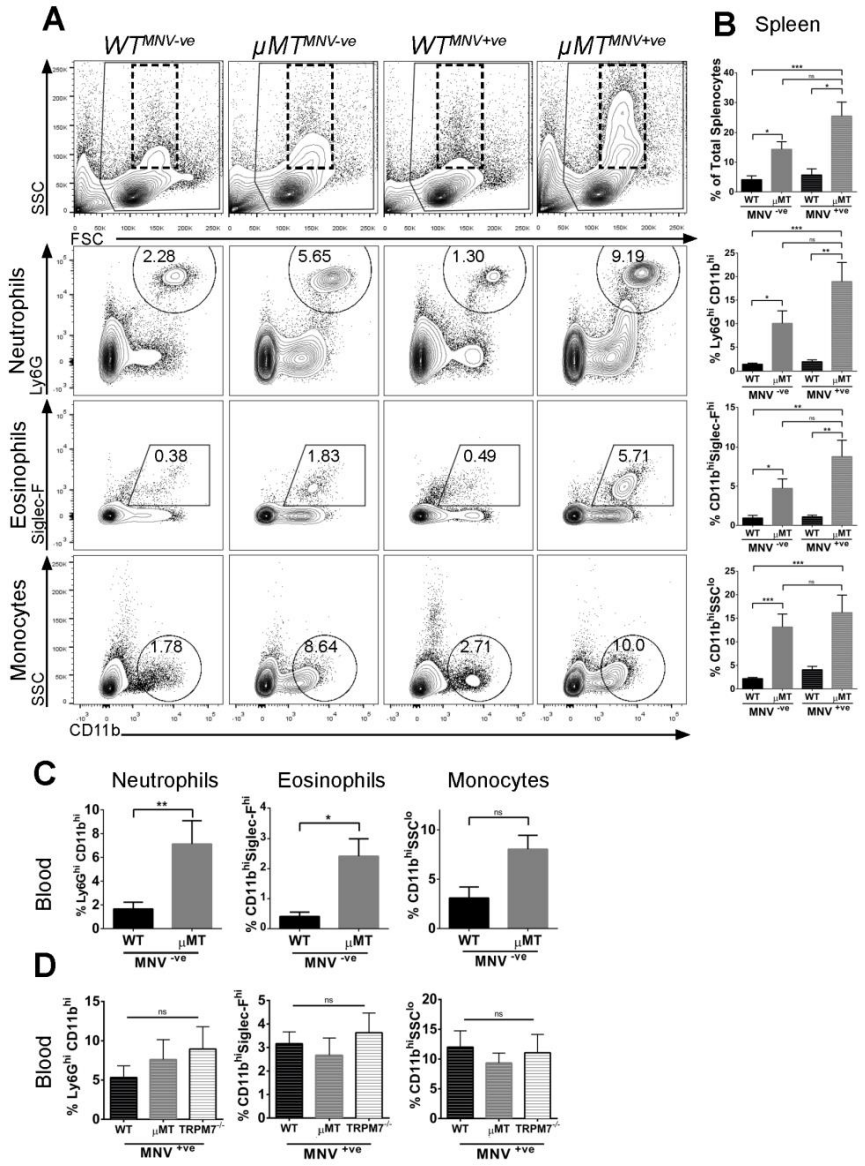

Fig. S4. The myeloid population is expanded in μMT mice. (**A** to **D**) Flow cytometry analysis of myeloid cell populations in the spleens of WT and μMT mice that were or were not infected with murine norovirus (MNV). (A) The gating strategy used to detect total granulocytes ($FSC^{hi}SSC^{hi}$), neutrophils ($CD11b^{hi}Ly6G^{hi}$), eosinophils (Siglec-$f^{hi}CD11b^{hi}$), and monocytes ($CD11b^{hi}SSC^{lo}$) from live singlet cells. (B) Percentages of the indicated cell types in the spleens of the indicated mice. Data are means ± SEM from 3 mice of each genotype. (C and D) Percentages of the indicated myeloid populations in the blood of uninfected (C) and MNV-infected (D) WT,μMT and *TRPM7*$^{-/-}$ (D only) mice. Data are means ± SEM from 4 mice of each genotype. Statistical significance was determined with Kruskal-Wallis test followed by Dunn's multiple comparisons test for (B) and (D) and the Mann-Whitney test for (C). **P* < 0.05, ***P* < 0.01, ****P* < 0.001.

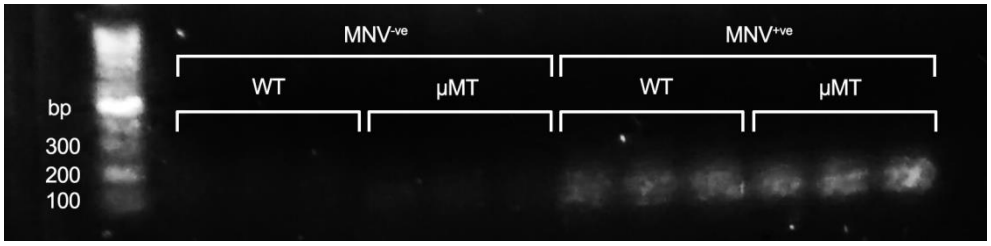

Fig. S5. Confirmation of MNV infection. MNV infection status was checked for 3 mice of each condition (WT MNV^{+ve}; µMT MNV^{+ve}; WT MNV^{-ve}; µMT MNV^{-ve}). MNV mRNA was extracted from fecal samples collected from each mouse and converted into cDNA. PCR was used to amplify a ~97bp region of the viral genome.

Table S1. Conditional deletion of *TRPM7* in B cells does not alter embryonic survival. Mendelian inheritance tabulations for the indicated breeding schemes showing expected and actual genotypes. Numbers in parentheses indicate the numbers of weaned pups for each genotype. Genotype effect on survival was assessed using the Chi-squared test to compare between theoretical (expected) and actual frequencies of each genotype produced from each breeding cross. ($TRPM7^{fl/fl}CD79a^{+/+}$ X $TRPM7^{+/+}CD79a^{cre/+}$:X^2=0.0007, $TRPM7^{fl/fl}CD79a^{+/+}$ X $TRPM7^{fl/fl}CD79a^{cre/+}$: X^2=0.04, $TRPM7^{fl/fl}CD79a^{cre/+}$ X $TRPM7^{fl/+}CD79a^{cre/+}$:$X^2$=1.72).

Breeding Scheme	Genotype	Expected	Actual
$TRPM7^{fl/fl}CD79a^{+/+}$ X $TRPM7^{+/+}CD79a^{cre/+}$	$TRPM7^{fl/+}CD79a^{+/+}$	50%	46.9 % (53)
	$TRPM7^{fl/+}CD79a^{cre/+}$	50%	53.1 % (55)
$TRPM7^{fl/fl}CD79a^{+/+}$ X $TRPM7^{fl/fl}CD79a^{cre/+}$	$TRPM7^{fl/fl}CD79a^{+/+}$	50%	54.9 (84)
	$TRPM7^{fl/fl}CD79a^{cre/+}$	50%	45.1% (69)
$TRPM7^{fl/fl}CD79a^{cre/+}$ X $TRPM7^{fl/+}CD79a^{cre/+}$	$TRPM7^{fl/+}CD79a^{+/+}$	25%	26.4% (52)
	$TRPM7^{fl/fl}CD79a^{+/+}$	25%	27.9 % (55)
	$TRPM7^{fl/+}CD79a^{cre/+}$	25%	21.8 % (43)
	$TRPM7^{fl/fl}CD79a^{cre/+}$	25%	23.9 % (47)

Table S2. The proportion of T cells is increased in the peripheral lymphoid tissues of *TRPM7*$^{-/-}$ mice. Quantification of the percentages of T cells in the indicated tissues. Data are means ± SEM and are representative of at least three mice of each genotype. Comparisons to the tissues from WT mice were made using the Kruskall-Wallis test followed by Dunn's multiple comparisons test (spleen, lymph node, and blood) or Mann-Whitney test (Peyer's patch). *$P < 0.05$.

Tissue	WT	*TRPM7*$^{+/-}$	*TRPM7*$^{-/-}$
Spleen	43.10± 2.60	50.88 ± 6.92	59.43 ± 4.503 *
Lymph Node	71.25 ± 2.89	77.05 ± 3.59	89.92 ± 3.691 *
Blood	52.17 ± 5.38	50.73 ± 8.35	79.67 ± 1.48*
Peyer's Patch	20.73 ± 3.634	15.14 ± 3.677	N/A

Table S3. The number of T cells is decreased in the spleen of *TRPM7*$^{-/-}$ mice. Quantification of the absolute numbers of T cells in the indicated tissues. Data are means ± SEM and are representative of at least three mice of each genotype. Comparisons to the tissues from WT mice were made using the Kruskall-Wallis test followed by Dunn's multiple comparisons test (spleen, and lymph node) or Mann-Whitney test (Peyer's patch) *$P < 0.05$. N/A; not applicable.

Tissue	**WT**	***TRPM7*$^{+/-}$**	***TRPM7*$^{-/-}$**
Spleen	$1.83x10^7 \pm 3.80\ x10^6$	$2.76x10^7 \pm 1.09x10^7$	$4.58x10^6 \pm 1.64x10^6$ *
Lymph Node	$4.67x10^5 \pm 7.26\ x10^4$	$7.83x10^5 \pm 5.60\ x10^4$	$6.23x10^5 \pm 2.02x10^5$
Peyer's Patch	$5.69x10^4 \pm 8.49\ x10^3$	$7.79x10^4 \pm 1.91\ x10^4$	N/A