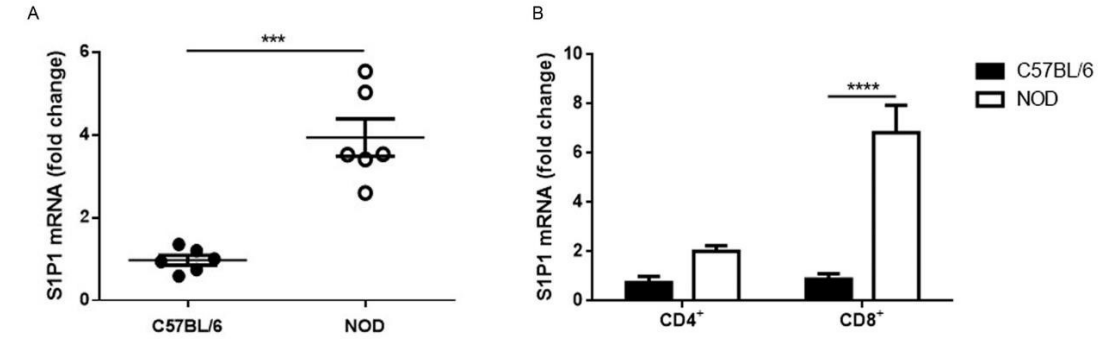

Figure S1. NOD thymocytes have increased expression of S1P1 mRNA. (A), the graph shows the mRNA expression of S1P1 in total thymocytes of C57BL/6 (black balls) and NOD (white balls) mice. Panel B depicts the mRNA expression of S1P1 in $CD4^+$ and $CD8^+$ SP thymocyte subpopulations in C57BL/6 (black bars) and NOD (white bars) mice. $CD4^+$ = $CD4^+CD8^-$ single-positive; $CD8^+$ = $CD4^-CD8^+$ single-positive. The relative quantitation was performed by the comparative *C*t method ($\Delta\Delta$Ct), using C57BL/6 as calibrator (fold change value = 1). Horizontal bars represent the mean ± SEM of six biological replicates for total thymocytes (A) and sixteen biological replicates for SP subpopulations (B). Results were analyzed by Student's t test for total thymus and 2 way ANOVA followed by Tukey post-test for sorted thymocyte subsets. Differences were considered statistically significant when *** $p < 0.001$; **** $p < 0.0001$.

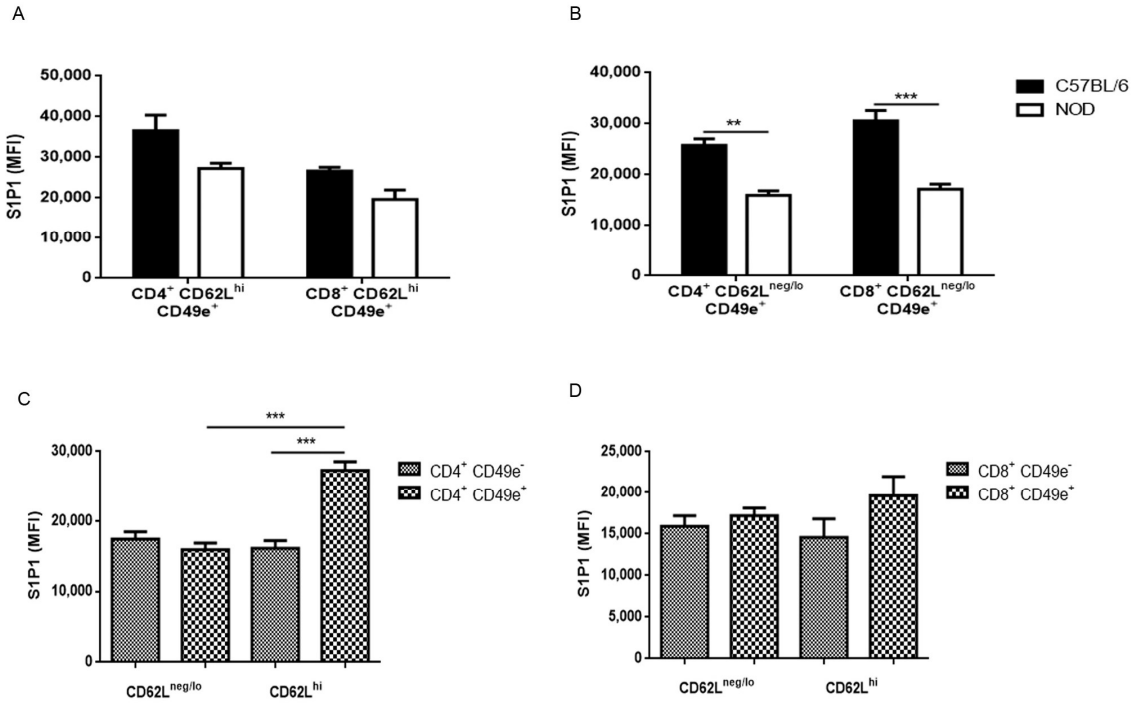

Figure S2. NOD $CD4^+CD49e^+$ SP cells increase S1P1 expression during differentiation. The panels show S1P1 expression in (A) $CD4^+$ and $CD8^+$ SP $CD62L^{neg/lo}CD49e^-$ cells, (B), SP $CD4^+$ and $CD8^+$ $CD62L^{neg/lo}CD49e^+$ cells, (C), NOD SP $CD4^+CD62L^{neg/lo}$ or $CD4^+CD62L^{hi}$, expressing or not CD49e and (D) NOD SP $CD8^+CD62L^{neg/lo}$ or $CD8^+CD62L^{hi}$ thymocytes expressing or not CD49e, as indicated, analyzed by flow cytometry. $CD4^+$ = $CD4^+CD8^-$ single-positive; $CD8^+$ = $CD4^-CD8^+$ single-positive; MFI = median fluorescence intensity. Results are expressed as mean ± SEM and were analyzed by 2way ANOVA followed by Tukey post-test or Student's t test. Differences were considered statistically significant when ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$, after evaluating 3 C57BL/6 and 4 NOD thymi.

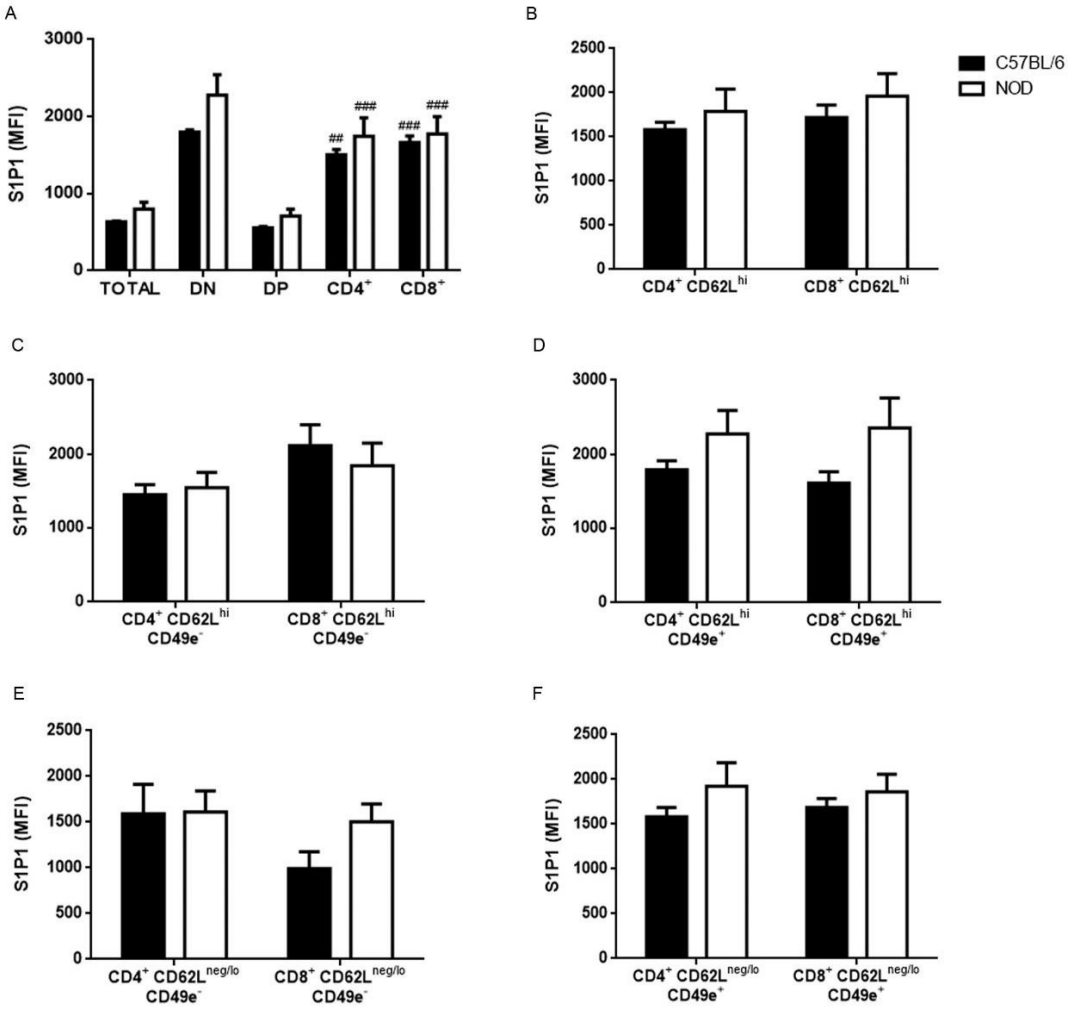

Figure S3. Younger NOD mice have no alterations in S1P1 expression. The graphs show S1P1 expression in C57BL/6 (black bars) and NOD (white bars) mice with 4 weeks of age, analyzed by flow cytometry. (A), S1P1 expression on CD4/CD8$^-$defined thymocyte subpopulations, (B), S1P1 expression in CD4$^+$CD62L^{hi} and CD8$^+$CD62L^{hi} subpopulations. (C), S1P1 expression in CD4$^+$CD62L^{hi}CD49e$^-$ and CD8$^+$CD62L^{hi}CD49e$^-$ cells and (D), S1P1 expression in CD4$^+$CD62L^{hi}CD49e$^+$ and CD8$^+$CD62L^{hi}CD49e$^+$ cells, (E), S1P1 expression in CD4$^+$CD62L$^{neg/lo}$CD49e$^-$ and CD8$^+$CD62L$^{neg/lo}$CD49e$^-$ cells and (F), S1P1 expression in CD4$^+$CD62L$^{neg/lo}$CD49e$^+$ and CD8$^+$CD62L$^{neg/lo}$CD49e$^+$ cells. Total = total thymocytes; DN = CD4$^-$CD8$^-$ double$^-$ negative; DP = CD4$^+$CD8$^+$ double-positive; CD4$^+$ = CD4$^+$CD8$^-$ single$^-$positive; CD8$^+$ = CD4$^-$CD8$^+$ single-positive; MFI = median fluorescence intensity. Results are expressed as mean ± SEM and were analyzed by 2way ANOVA followed by Tukey post-test. Differences were considered statistically significant when ## $p < 0.01$; ### $p < 0.001$. Hash marks represent statistical significance between DP and SP subpopulations in the same mouse strain, after evaluating 4 C57BL/6 and 4 NOD thymi.

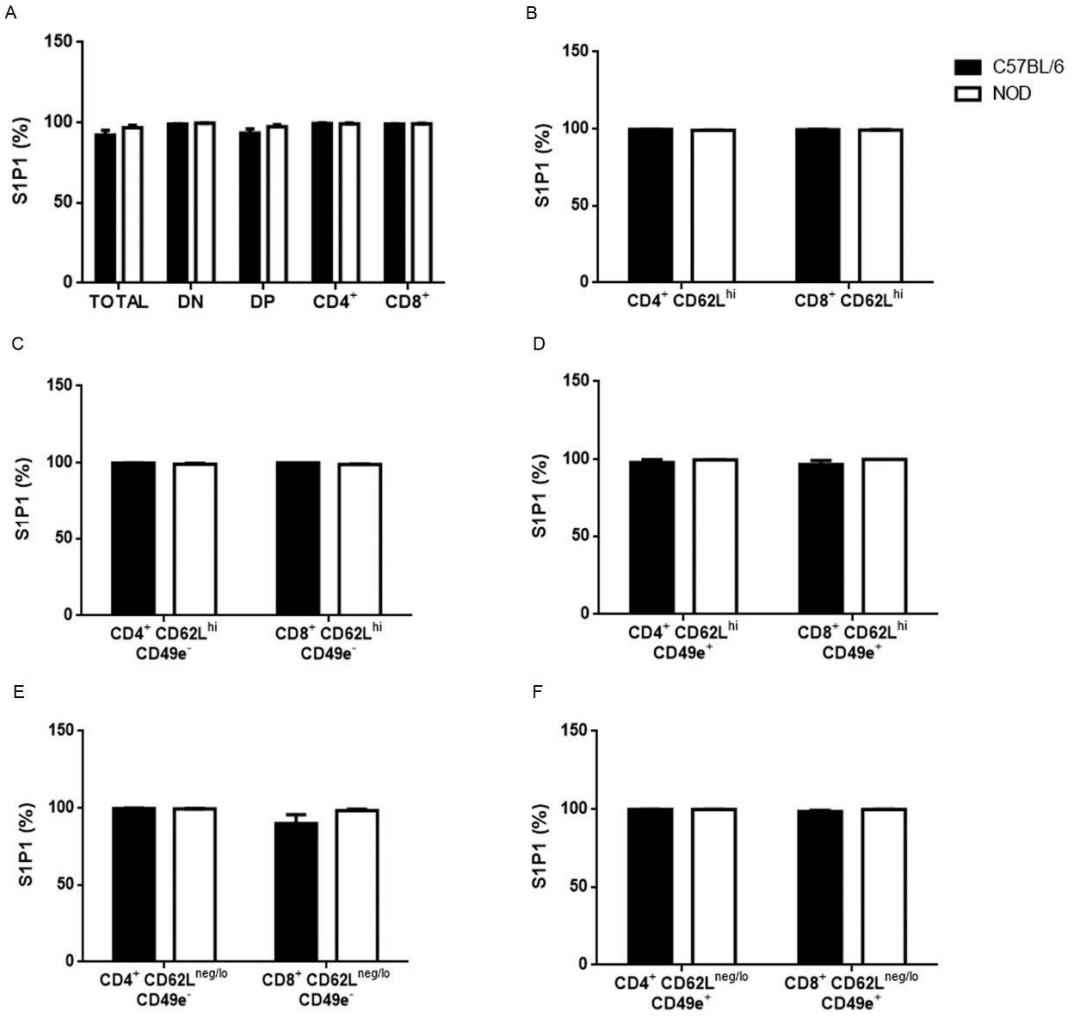

Figure S4. Younger NOD mice have no differences in percentage of cells expressing S1P1. The bars show the S1P1 expression on thymocyte subpopulations defined by CD4 and CD8 in C57BL/6 (black bars) and NOD (white bars) mice, analyzed by flow cytometry. (A), S1P1 in total thymocytes, as well as $CD4^{-}CD8^{-}$ double^{-}negative, $CD4^{+}CD8^{+}$ double-positive, $CD4^{+}$ and $CD8^{+}$ SP subpopulations, (B), S1P1 in mature $CD4^{+}CD62L^{hi}$ and $CD8^{+}CD62L^{hi}$ SP thymocytes, (C), S1P1 in mature $CD4^{+}CD62L^{hi}CD49e^{-}$ and $CD8^{+}CD62LhiCD49e^{-}$ cells, (D), S1P1 in mature $CD4^{+}CD62L^{hi}CD49e^{+}$ and $CD8^{+}CD62L^{hi}CD49e^{+}$ subpopulations, (E), S1P1 in $CD4^{+}CD62L^{neg/lo}CD49e^{-}$ and $CD8^{+}CD62L^{neg/lo}CD49e^{-}$ cells and (F), S1P1 in $CD4^{+}CD62L^{neg/lo}CD49e^{+}$ and $CD8^{+}CD62L^{neg/lo}CD49e^{+}$ subsets. Total = total thymocytes; DN = $CD4^{-}CD8^{-}$ double^{-}negative; DP = $CD4^{+}CD8^{+}$ double-positive; $CD4^{+}$. = $CD4^{+}CD8^{-}$ single-positive; $CD8^{+}$ = $CD4^{-}CD8^{+}$ single-positive; % = relative cell numbers. Results are expressed as mean ± SEM and were analyzed by 2way ANOVA followed by Tukey post-test. n = 4 C57BL/6; n = 4 NOD.

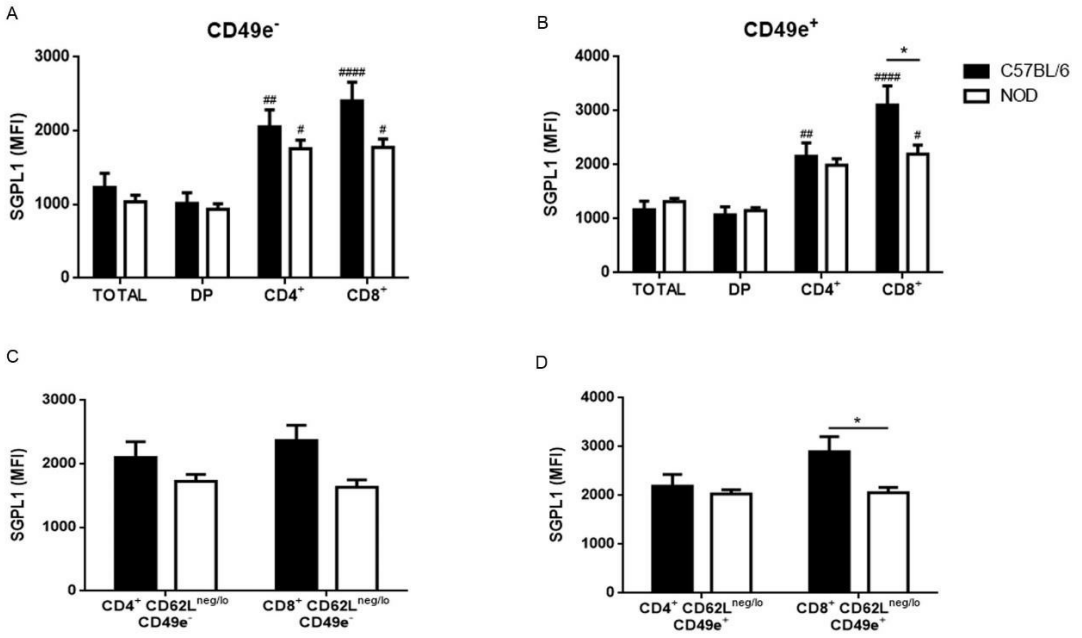

Figure S5. NOD CD49e$^+$ and CD49e$^-$ SP thymocytes present lesser SGPL1 expression. The bars show the SGPL1 expression on thymocyte subpopulations defined by CD4 and CD8 in C57BL/6 (black bars) and NOD (white bars) mice, analyzed by flow cytometry. (A), CD49e$^+$ total thymocytes and DP, CD4$^+$ and CD8$^+$ SP subpopulations and (B), CD49e$^-$ total thymocytes and DP, CD4$^+$ and CD8$^+$ SP subpopulations, (C), CD4$^+$ and CD8$^+$ SP CD62L$^{neg/lo}$CD49e$^-$ cells and (D), SP CD4$^+$ and CD8$^+$ CD62L$^{neg/lo}$CD49e$^+$ cells. Total = total thymocytes; DN = CD4$^-$CD8$^-$ double$^-$negative; DP = CD4$^+$CD8$^+$ double-positive; CD4$^+$ = CD4$^+$CD8$^-$ single-positive; CD8$^+$ = CD4$^-$CD8$^+$ single-positive; MFI = median fluorescence intensity. Results are expressed as mean ± SEM and were analyzed by 2way ANOVA followed by Tukey post-test. Differences were considered statistically significant when * or # $p < 0.05$; ## $p < 0.01$; #### $p < 0.0001$. Asterisks represent statistical significance between C57BL/6 and NOD subpopulations; hash marks represent statistical significance between DP and SP subpopulations in the same mouse strain. $n = 8$ C57BL/6; $n = 8$ NOD.

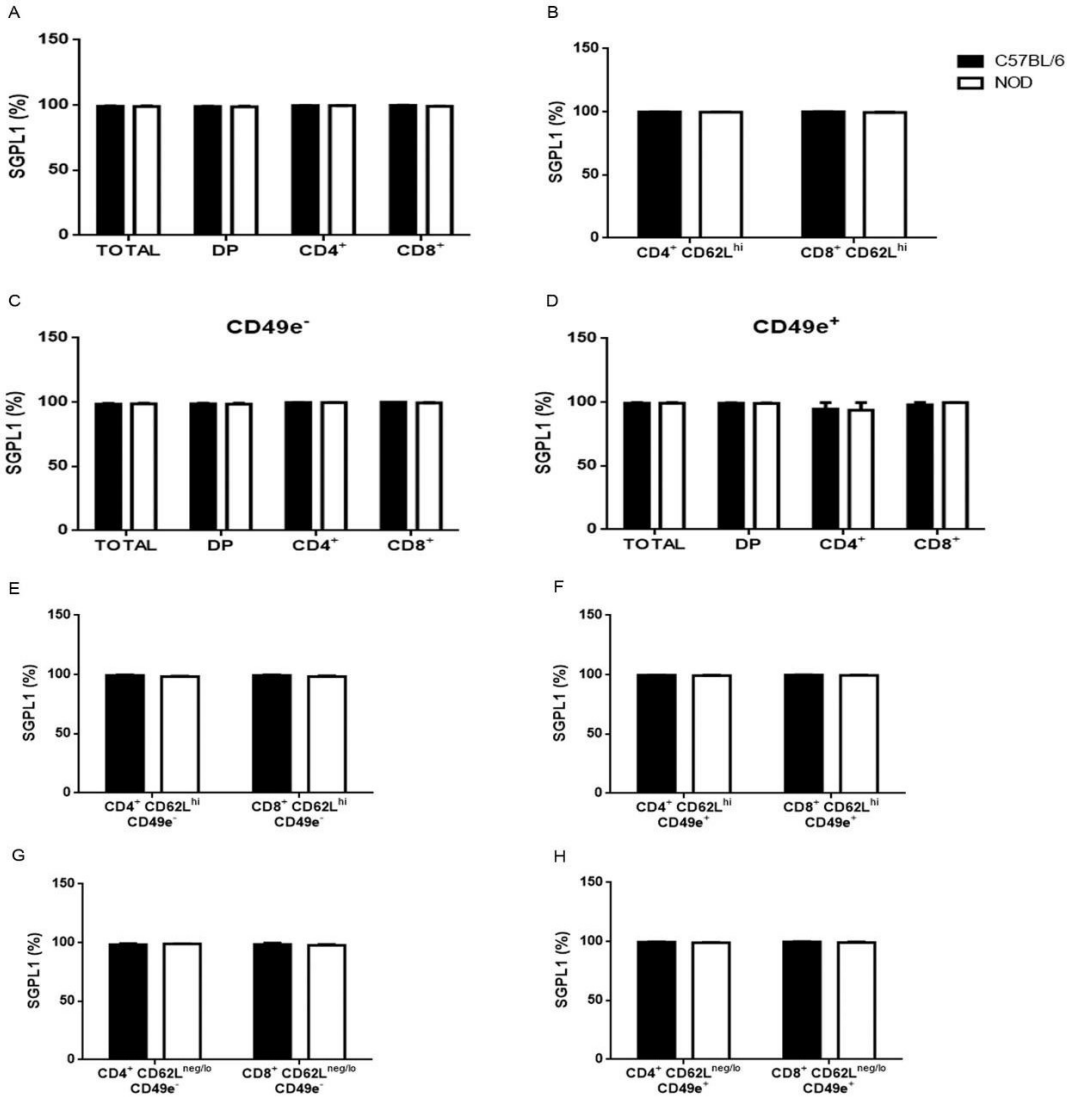

Figure S6. NOD mice have similar frequency of cell expressing SGPL1. The bars show the SGPL1 expression on thymocyte subpopulations defined by CD4 and CD8 in C57BL/6 (black bars) and NOD (white bars) mice, analyzed by flow cytometry. (A), SGPL1 in total thymocytes, as well as $CD4^+CD8^+$ double-positive, $CD4^+$ and $CD8^+$ SP subpopulations, (B), SGPL1 in mature $CD4^+CD62L^{hi}$ and $CD8^+CD62L^{hi}$ SP thymocyte subpopulations, (C), SGPL1 in $CD49e^-$ total thymocytes and DP, $CD4^+$ and $CD8^+$ SP subpopulations, (D), SGPL1 in $CD49e^+$ total thymocytes and DP, $CD4^+$ and $CD8^+$ SP subpopulations, (E), SGPL1 in $CD4^+$ and $CD8^+$ SP $CD62L^{hi}CD49e^-$ cells, (F), SGPL1 in SP $CD4^+$ and $CD8^+$ $CD62L^{hi}CD49e^+$ cells, (G), SGPL1 in $CD4^+$ and $CD8^+$ SP $CD62L^{neg/lo}CD49e^-$ cells and (H), SGPL1 in SP $CD4^+$ and $CD8^+$ $CD62L^{neg/lo}CD49e^+$ cells. Total = total thymocytes; DN = $CD4^-CD8^-$ double$^-$negative; DP = $CD4^+CD8^+$ double-positive; $CD4^+$ = $CD4^+CD8^-$ single-positive; $CD8^+$ = $CD4^-CD8^+$ single-positive; % = relative cell numbers. Results are expressed as mean ± SEM and were analyzed by 2way ANOVA followed by Tukey post-test. n = 8 C57BL/6; n = 8 NOD.

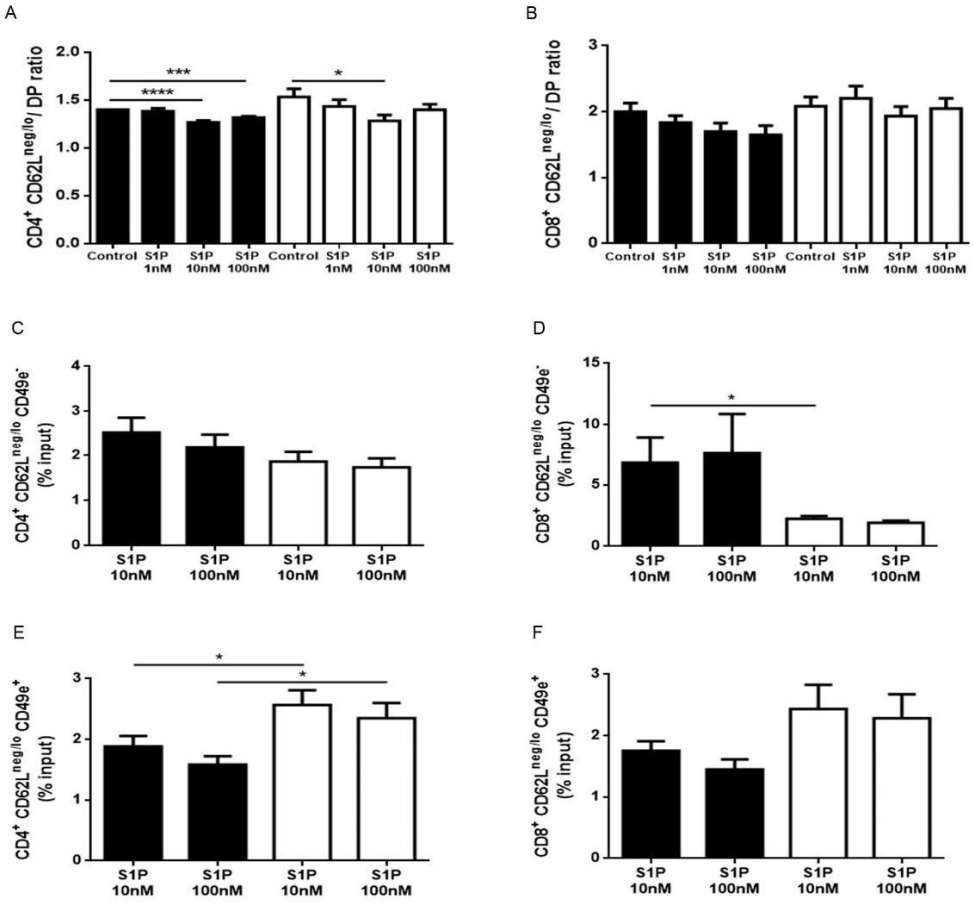

Figure S7. CD62L$^{neg/lo}$ thymocyte subpopulations have no chemotactic response under S1P stimulation. The panels show (A), SP CD4^{+}CD62L$^{neg/lo}$, (B), SP CD8^{+}CD62L$^{neg/lo}$, (C), CD4^{+}CD62L$^{neg/lo}$CD49e^{-}, (D), CD8^{+}CD62L$^{neg/lo}$CD49e^{-}, (E), CD4^{+}CD62L$^{neg/lo}$CD49e^{+} and (F), CD8^{+}CD62L$^{neg/lo}$CD49e^{+} cells migration towards 1 nM, 10 nM or 100 nM of S1P, in C57BL/6 (black bars) and NOD (white bars) mice. CD4^{+} = CD4^{+}CD8^{-} single-positive; CD8^{+} = CD4^{-}CD8^{+} single-positive; MFI = median fluorescence intensity. Results are shown as the ratio of the percentages of input (single-positive to double-positive thymocytes) or percentages of input, and are expressed as mean ± SEM, being analyzed by 2way ANOVA followed by Tukey post-test or Student's t test. Differences were considered statistically significant when * $p < 0.05$; *** $p < 0.001$; **** $p < 0.0001$, after evaluating 6 C57BL/6 versus 6 NOD animals.

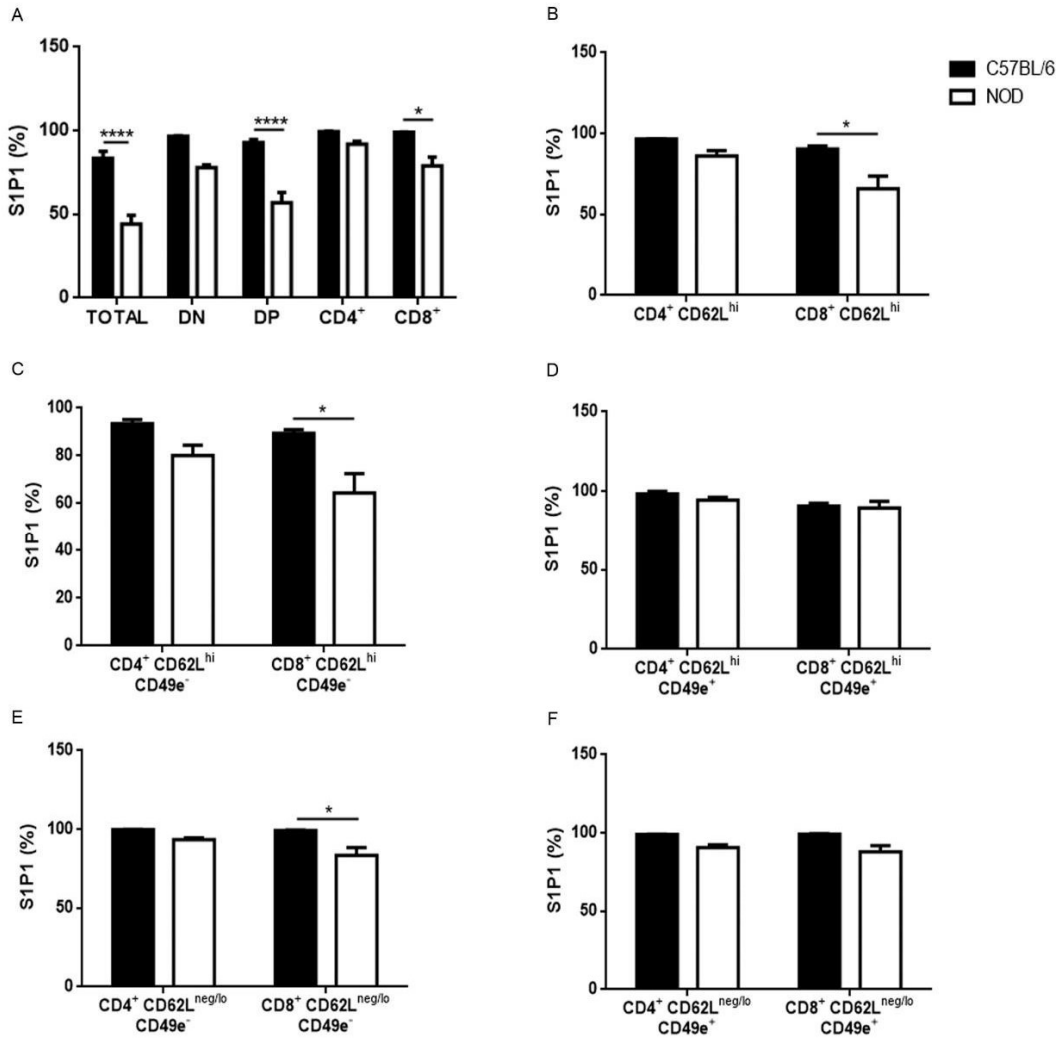

Figure S8. NOD mice have decreased percentage of $CD8^+CD49e^-$ cells expressing S1P1. The graphs show the S1P1 expression on thymocyte subpopulations defined by CD4 and CD8 in C57BL/6 (black bars) and NOD (white bars) mice, analyzed by flow cytometry. (A), S1P1 in total thymocytes, as well as $CD4^-CD8^-$ double$^-$negative, $CD4^+CD8^+$ double-positive, $CD4^+$ and $CD8^+$ SP subpopulations, (B), S1P1 in mature $CD4^+CD62L^{hi}$ and $CD8^+CD62L^{hi}$ SP thymocyte subpopulations, (C), S1P1 in mature $CD4^+CD62L^{hi}CD49e^-$ and $CD8^+CD62LhiCD49e^-$ cells, (D), S1P1 in mature $CD4^+CD62L^{hi}CD49e^+$ and $CD8^+CD62L^{hi}CD49e^+$ subpopulations, (E), S1P1 in $CD4^+CD62L^{neg/lo}CD49e^-$ and $CD8^+CD62L^{neg/lo}CD49e^-$ cells and (F), S1P1 in $CD4^+CD62L^{neg/lo}CD49e^+$ and $CD8^+CD62L^{neg/lo}CD49e^+$ subsets. Total = total thymocytes; DN = $CD4^-CD8^-$ double$^-$ negative; DP = $CD4^+CD8^+$ double-positive; $CD4^+$ = $CD4^+CD8^-$ single-positive; $CD8^+$ = $CD4^-CD8^+$ single-positive; % = relative cell numbers. Results are expressed as mean ± SEM and were analyzed by 2way ANOVA followed by Tukey post-test. Differences were considered statistically significant when * $p < 0.05$; **** $p < 0.0001$. $n = 3$ C57BL/6; $n = 4$ NOD.

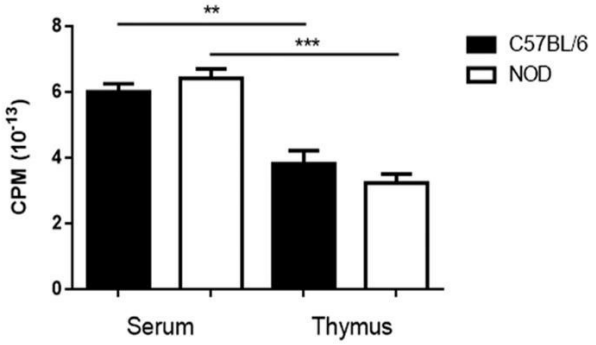

Figure S9. NOD mice have normal S1P gradient between thymus and serum. S1P was measured in thymi and sera of C57BL/6 (black bars) and NOD (white bars) by thin-layer chromatography (TLC). Four C57BL/6 and 4 NOD mice were used for these experiments. Results are expressed as mean ± SEM and were analyzed by 2 way ANOVA followed by Tukey post-test. Differences were considered statistically significant when ** $p < 0.01$; *** $p<0.001$. CPM = counts per minute.

Table S1. Primers and standard curve parameters for gene amplification in thymi from C57BL/6 and NOD mice.

Mouse gene target	Primer sequences (5'-3')	Slope	Intercept	Coefficient of linearity (r^2)	Amplification efficiency (%)
S1P1	FW - GTGTAGACCCAGAGTCCTGCG	-3,06	15,9	0,99	112,4
	RV - AGCTTTTCCTTGGCTGGAGAG				
HPRT	FW - TCCCAGCGTCGTGATTAGCGATG	-2,89	13,96	0,98	121,8
	RV - GGCCACAATGTGATGGCCTCCC				
GAPDH	FW - CCACTCACGGCAAATTCAACGGC	-3,16	9,62	0,98	107,2
	RV - CCACCCTTCAAGTGGGCCCCG				