Supplemental Videos (Online Appendices 2-5). NIT-1 or NIT-4 cells were seeded in chambered coverglass (Nunc Lab-Tek II, Fisher Scientific) at 1×10^5 cells/chamber. After culturing for 24 hours, cells were washed once with phenol red-free DMEM culture medium, and stained with 25 nM of the mitochondrial membrane potential-sensitive dye, Tetramethyl Rhodamine Methyl Ester (TMRM, Invitrogen), for 30 minutes at 37°C. Mitochondrial membrane potential was used as an indicator of cell death as loss of membrane potential is a characteristic of necrotic and apoptotic cell death. The stained cells were refed with fresh medium containing 5 nM TMRM. Splenocytes from NOD-AI4a/b transgenic mice were harvested and activated. Jurkat cells were purchased from American Type Culture Collection and cultured in RPMI supplemented with 10% FBS, 1 mM sodium pyruvate, 10 mM HEPES, penicillin, and streptomycin. AI4 and Jurkat cells were stained with 1 µl/ml Picogreen (Invitrogen) for 15 minutes in phenol red-free medium, washed twice with fresh medium. T cells, either autoreactive AI4 or non-sepcific Jurkat, at an effector to target ratio (E:T) of 2.5:1 were added to the wells containing NIT-1 or NIT-4 cells in the chambered cover glass. The chambered cover glass was mounted in the live cell chamber unit of a Zeiss LSM 510 meta confocal microscope. The live cell chamber was maintained at 37°C and 5% CO_2 with moisture. Images were taken using Laser excitation wavelength 488 nm for Picogreen detection and 543 nm for the visualization of TMRM. Time laps series of images were taken at the intervals indicated in the upper right hand corner of the video image. The movie files were created using LSM software.

(A) AI4 T cells were added to the NIT-1 culture. The movie demonstrates that AI4 T cells [Green] migrated [8 mins (Top) and 48 mins (right)], adhered [10 mins (Top) and 52 min (right)], and dissipated mitochondrial membrane potential [78 mins] in NIT-1 cells over the course of the 80 min period. In additional experiments (not shown), the live cell studies have mirrored the results from Cell Mediated Lymphocytotoxicity assays. By microscopy we have observed that approximately 25% of the NIT-1 clusters are killed over 16 hour culture periods at an E:T ratio of 2.5:1.

(B) Control wells using human Jurkat cells cultured with NIT-1 cells for 194 min duration did not induce NIT-1 mitochondrial membrane potential change.

(C) AI4 T cells did not ablate mitochondrial membrane potential of NIT-4 cells during the culture period. Identical to the results of the CML data (Figure 3B).

(D) Control human Jurkat cells cultured with NIT-4 cells did not induce NIT-4 mitochondrial membrane potential change.

As the Picogreen dye is not specific to the T cells and does diffuse out of the T cells, both the NIT-1 and NIT4 clusters gain green fluorescence over the course of the incubation period, suggesting the take up of this dye. As the stacks of images represent only a single focal plane from a cluster of beta cells it is not possible to observe all of the T cells attached to a cluster.

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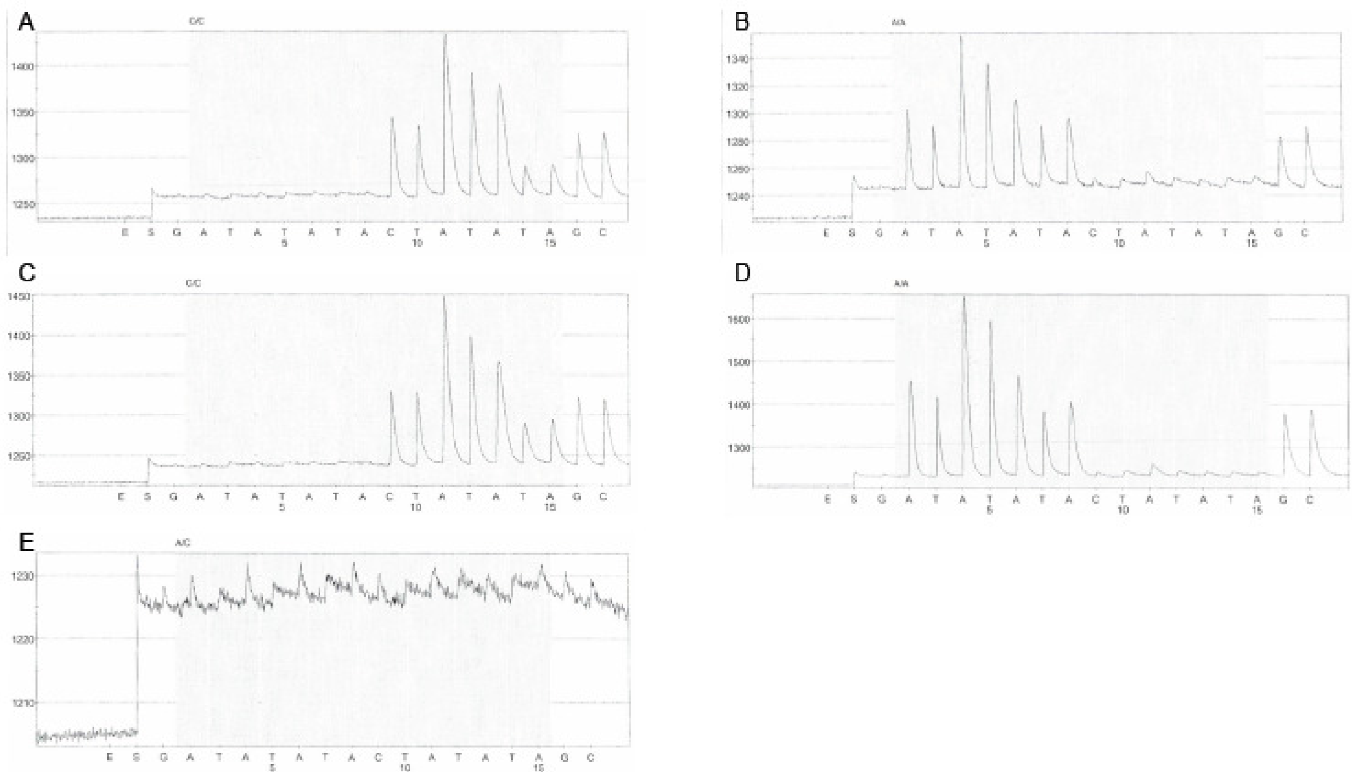
Supplemental Figure 1. Genotypes of *mt-Nd2* SNP in NIT-1 and NIT-4 were confirmed using Pyrosenquencing. (A) Pyrosequencing of NOD DNA as a positive control for *mt-Nd2*^{c}. (B) Genotype of ALR DNA as a positive control for *mt-Nd2*^{a}. (C) Genotype of NIT-1 is identical to NOD demonstrating the presence of *mt-Nd2*^{c}. (D) Genotype of NIT-4 is identical to ALR demonstrating the presence of *mt-Nd2*^{a}. (E) Water control was negative for DNA amplification.



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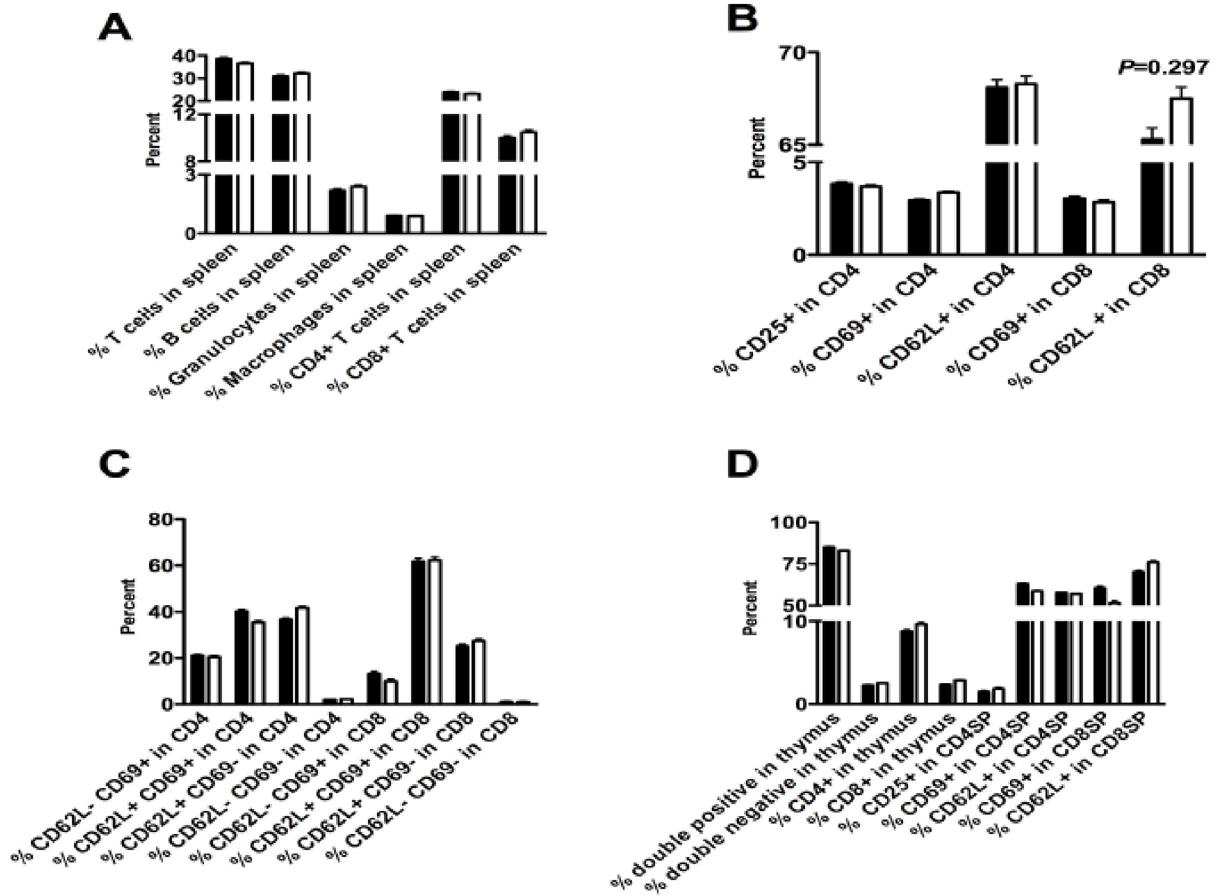
Supplemental Figure 2. Profiles of spleen and thymus comparing NOD and NOD.mt^{ALR} mice by flow cytometry. **(A)** Percentages of B cells, T cells (CD3^{+}), granulocytes, macrophages, CD4^{+} and CD8^{+} T cells in spleen. **(B)** Percentage of activation markers CD25 and CD69 on CD4^{+} and CD8^{+} spleen cells. **(C)** Percentage of activation markers CD69 and CD62L on CD4^{+} and CD8^{+} spleen cells. **(D)** Percentage of CD4^{+} CD8^{+} (double positive), CD4^{-} CD8^{-} (double negative), CD4 single positive, CD8 single positive in thymus. Also shown are the percentages of activation markers CD69 and CD62L on CD4 single positive (CD4SP) and CD8 single positive (CD8SP) thymocytes. N=5 for all measures.



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