Supporting Information

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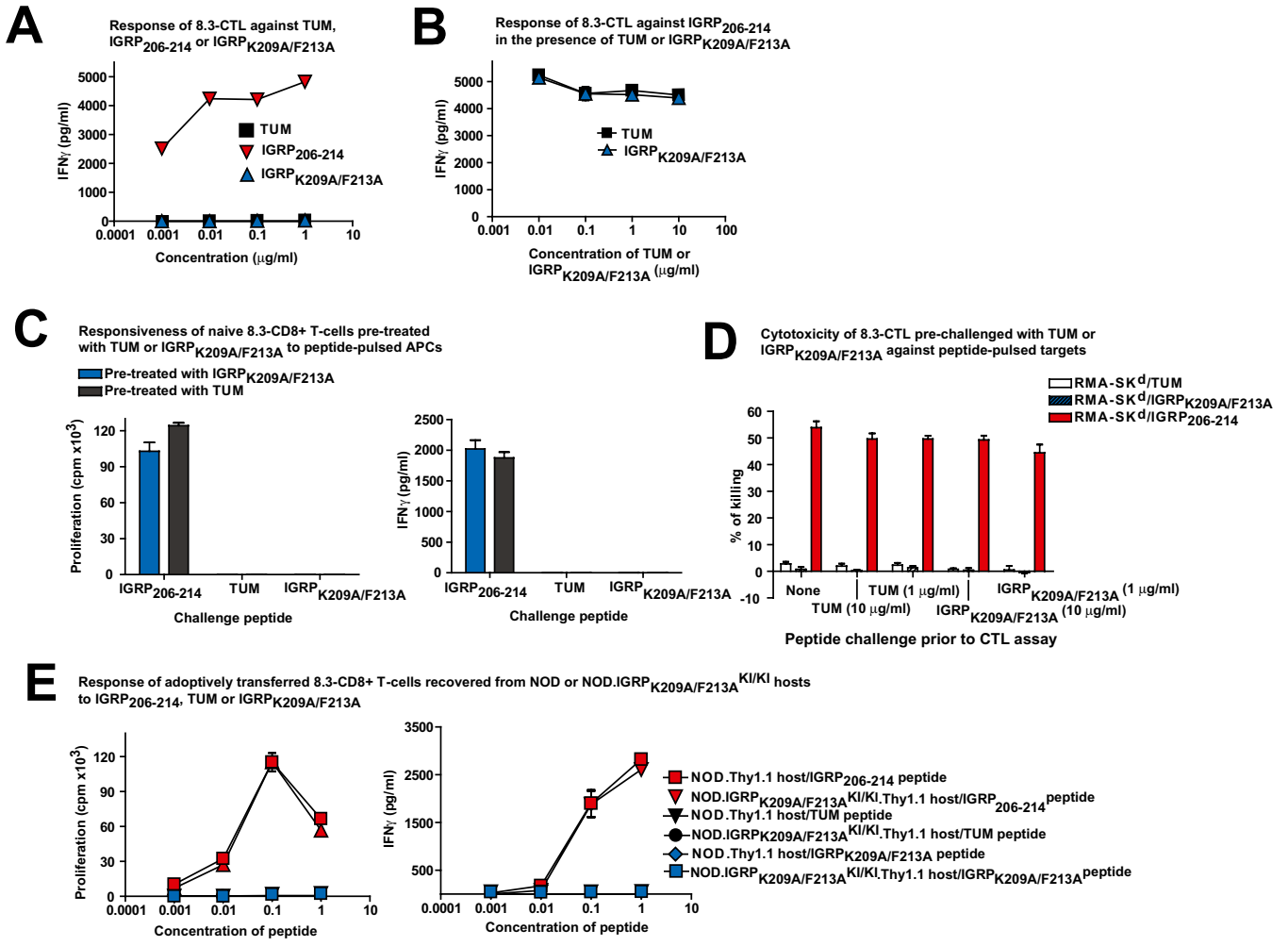

Fig. S1. The $IGRP_{K209A/F213A}$ epitope is not recognized by, and does not alter the functional responsiveness of $IGRP_{206–214}$-reactive 8.3-$CD8^+$ T cells either in vitro or in vivo. (*A*) Differentiated 8.3-$CD8^+$ T cells secrete IFNγ in response to splenocytes pulsed with $IGRP_{206–214}$ but not $IGRP_{K209A/F213A}$. IFNγ content in the supernatants was measured at 24 h of culture. (*B*) $IGRPK_{209A/F213A}$ does not inhibit the responsiveness of differentiated 8.3-$CD8^+$ T cells to $IGRP_{206–214}$. Differentiated 8.3-$CD8^+$ T cells were cultured with $IGRP_{206–214}$-pulsed (0.1 μg/mL) splenocytes in the presence of various concentrations of TUM or $IGRP_{K209A/F213A}$ for 24 h and the supernatants collected to measure the IFNγ concentration. (*C*) Pretreatment of naive 8.3-$CD8^+$ T cells with $IGRP_{K209A/F213A}$ (or TUM) peptide does not alter their subsequent responsiveness to $IGRP_{206–214}$. Naive splenic 8.3-$CD8^+$ T cells were preincubated with 10 μg/mL $IGRP_{K209A/F213A}$ or TUM for 2 days. $CD8^+$ T cells were then purified and tested for their ability to proliferate (*Left*) and secrete IFNγ (*Right*) in response to bone marrow-derived dendritic cells pulsed with 0.1 μg/mL $IGRP_{206–214}$, TUM, or $IGRP_{K209A/F213A}$. (*D*) Differentiated 8.3-$CD8^+$ T cells cannot kill targets pulsed with $IGRP_{K209A/F213A}$, and preincubation of 8.3-$CD8^+$ CTL with $IGRP_{K209A/F213A}$ does not inhibit their cytotoxic activity against RMA-SK^d targets pulsed with $IGRP_{206–214}$. The 8.3-$CD8^+$ CTLs were preincubated with bone marrow-derived dendritic cells pulsed with 1 or 10 μg/mL of either TUM or $IGRP_{K209A/F213A}$ for 24 h, purified away from DCs using mAb-coated magnetic beads, and used as effectors in a standard ^{51}Cr-release assay using RMA-SK^d cells pulsed with 10 μg/mL of $IGRP_{206–214}$, TUM or $IGRP_{K209A/F213A}$ at an 8:1 effector:target ratio in triplicate wells. Percentage of killing was calculated as (^{51}Cr in test well − spontaneous release)/(maximum release − spontaneous release) × 100. (*E*) Encounter of $IGRP_{K209A/F213A}$ by 8.3-$CD8^+$ T cells in vivo does not impair their functional responsiveness to $IGRP_{206–214}$ ex vivo. Ten million 8.3-$CD8^+$ T cells ($Thy1.2^+$) were adoptively transferred into NOD.Thy1.1 or $NOD.IGRP_{K209A/F213A}^{KI/KI}$.Thy1.1 hosts (8- to 12-weeks old). Seven days posttransfer, $Thy1.2^+$ 8.3-$CD8^+$ T cells were isolated from the spleen and lymph nodes of the hosts using antibody-coated magnetic beads and tested for proliferation and IFNγ against irradiated NOD splenocytes pulsed with TUM, $IGRP_{206–214}$, or $IGRP_{K209A/F213A}$. Data correspond to mean ± SE of triplicate cultures.

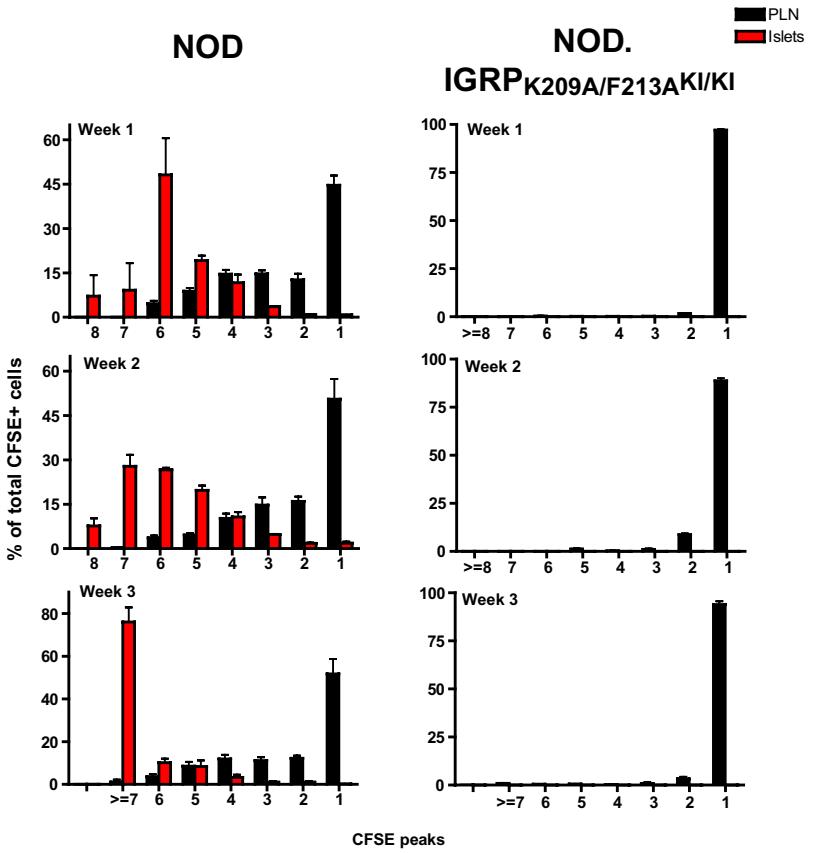

Fig. S2. Recruitment of naive 8.3-CD8$^+$ T cells into the pancreatic islets of NOD mice is preceded by antigen-induced proliferation in the PLN. Recruitment and proliferation of adoptively transferred naive 8.3-CD8$^+$ T cells (10^7) from 8.3-NOD.Thy1.1 donor mice in the lymphoid organs and islets of insulitic NOD and NOD.IGRP$_{K209A/F213A}^{KI/KI}$ hosts 1, 2, and 3 weeks after transfer. Histograms correspond to percentages of cells within each CFSE peak (i.e., from Fig. 2*C*) (mean ± SEM). Data correspond to three to six experiments for each time point and host type.

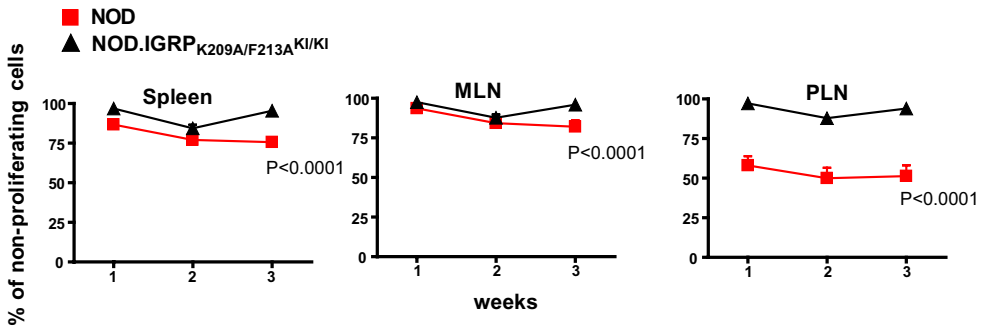

Fig. S3. Naive 8.3-CD8$^+$ T cells fail to proliferate in the PLN of NOD.IGRP$^{KI/KI}$ hosts. Proliferation of adoptively transferred naive 8.3-CD8$^+$ T cells (10^7) from 8.3-NOD.Thy1.1 donor mice in the lymphoid organs of insulitic NOD and NOD.IGRP$_{K209A/F213A}^{KI/KI}$ hosts 1, 2, and 3 weeks after transfer. Histograms correspond to percentages of nonproliferating CFSE$^+$ cells (mean ± SEM). Data correspond to three to six experiments for each time point and host type. *P* values shown were obtained with two-way ANOVA. Values corresponding to the 1-week and 3-week time points in the spleen and MLNs were also statistically different as measured with Mann-Whitney *U*-test (spleen: $P = 0.0011$ and 0.0029, respectively; and MLN: $P = 0.002$ and 0.0343, respectively).