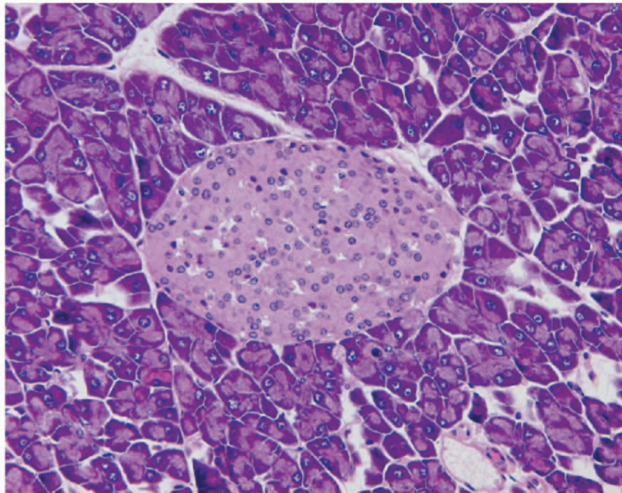
Supporting Information

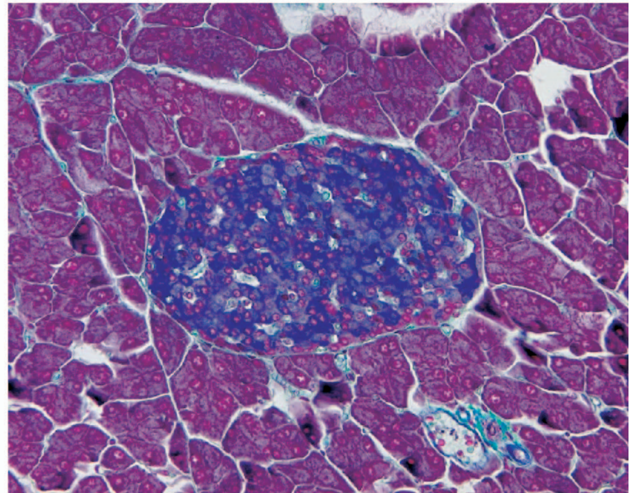
Parish et al. 10.1073/pnas.0810427106

Media Control

H&E

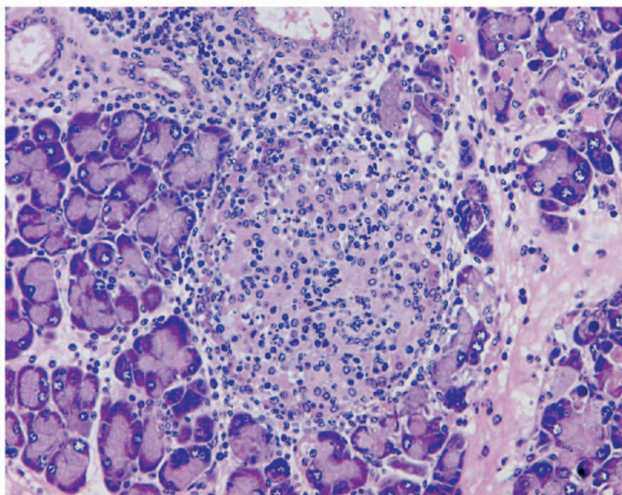


GAF



0.22 x 10^6 CTL

H&E



GAF

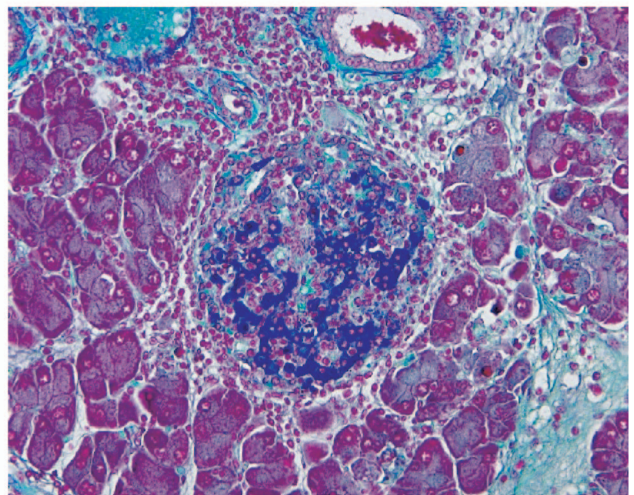


Fig. S1. Low CTL doses induce histologically detectable islet disruption. RIP-OVA^{lo} mice were given either media alone (*Upper*) or 220,000 CTLs (*Lower*). Four or 7 days later pancreases were harvested from each mouse, fixed, and sectioned. The tissue sections were stained with either hematoxylin and eosin (H&E) to visualize tissue architecture and infiltrates or Gomori's Aldehyde Fuchsin (GAF) to visualize islet cells (blue/dark purple). Representative data from 7 media- and 7 CTL-treated mice from 3 separate experiments are shown.

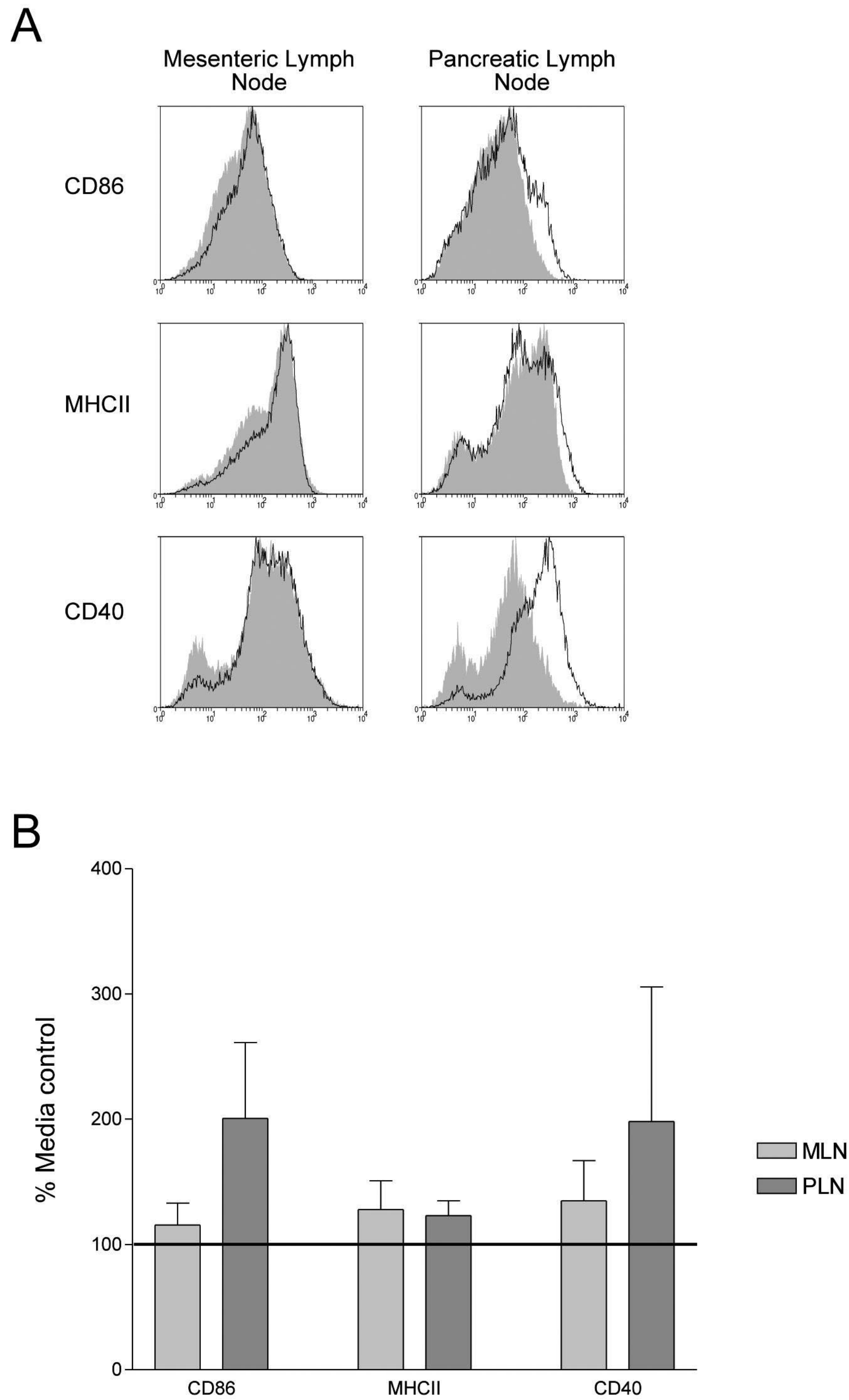


Fig. S2. CTL damage induces minimal DC maturation. The draining pancreatic or nondraining mesenteric lymph nodes were harvested from RIP-OVA^{lo} mice given either media or 220,000 CTLs i.v. after which the lymph node cells were enriched for dendritic cells and phenotyped by flow cytometry. (*A*) Plots show the staining of CD11c^{+} cells from media- (shaded histogram) or CTL-treated (open histogram) mice. Representative plots from 1 of 4 independent experiments are shown. (*B*) The data from *A* displayed as a bar graph with pooled data from the 4 experiments. Data were normalized before pooling by expressing each mean fluorescence index as a percentage of the media control value for that lymph node (represented as percentage of media control on the graph). None of the observed shifts were statistically significant. Error bars represent the standard deviation. MLN, mesenteric lymph node; PLN, pancreatic lymph node.

Fig. S3. OT-I cells make IFN-γ in response to OVA released by CTL-mediated tissue destruction. RIP-OVA^{hi} mice (R-OVA^{hi}), RIP-OVA^{lo} mice given 220,000 CTLs i.v. 4 days earlier (R-OVA^{lo}), or B6 mice given 20 million OVA-coated splenocytes (1) with 1 μg LPS i.v. (OVA-primed) were injected with 2 million CFSE-labeled OT-I cells (i.v.). Sixty hours later, cells from either the pancreatic lymph nodes (R-OVA^{hi} and R-OVA^{lo}) or the spleen (OVA-primed) were restimulated with 1 μg/mL OVA peptide (OVA257–264) in the presence of Brefeldin A and stained intracellularly for IFN-γ. IFN-γ vs. CFSE profiles are shown for each condition alongside the corresponding no peptide control. The proportions of divided (CFSE low) cells that were IFN-γ^+ are shown in each plot (*Inset Upper Left*). Representative profiles from 6 RIP-OVA^{hi}, 6 RIP-OVA^{lo}, and 6 OVA primed mice are shown.

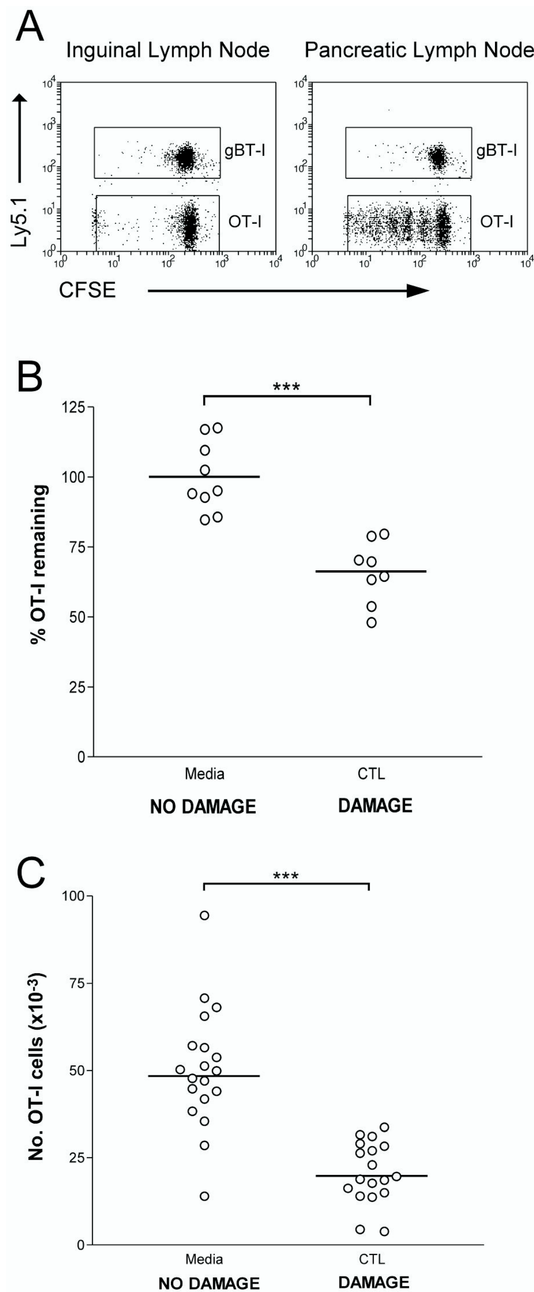


Fig. S4. OT-I deletion is antigen specific and is augmented by reducing initial naive T cell numbers. (*A*) RIP-OVA^{lo} mice were given 220,000 CTLs i.v. and 4 days later were injected i.v. with 2 million CFSE-labeled Ly5.2$^+$ OT-I cells and 2 million CFSE-labeled Ly5.1$^+$ gBT-I cells. Proliferation was examined by flow cytometry of draining pancreatic and nondraining inguinal lymph node cells 60 h later. Representative data from 3 independent experiments are shown. (*B*) RIP-OVA^{lo} mice were given either media or 220,000 Ly5.2$^+$ CTLs i.v. and 4 days later were injected i.v. with 5 million Ly5.1$^+$ naive OT-I cells and 5 million Ly5.1$^+$ naive gBT-I cells. Four weeks after naive T cell injection, the number of Ly5.1$^+$ Vβ8.1/8.2$^-$ (OT-I) cells and Ly5.1$^+$ Vβ8.1/8.2$^+$ (gBT-I) cells remaining in the spleen and lymph nodes of the mice was determined by flow cytometry. *y*-axis values display the number of remaining OT-I cells divided by the number of gBT-I cells and multiplied by 100. Thus, the number of OT-I cells remaining is expressed as a percentage of the gBT-I cells remaining and overall this gives the survival of OT-I cells relative to gBT-I cells. To pool data from 2 independent experiments, final values were normalized such that the media control group's average was 100%. Circles represent individual mice and the bar represents the average. ***, $P < 0.001$. Pooled data from 2 independent experiments are shown. (*C*) RIP-OVA^{lo} mice were given either media (NO DAMAGE) or 220,000 Ly5.1$^+$ CTL (DAMAGE) i.v. followed by 1 million Ly5.2$^+$ naive OT-I cells i.v. 4 days later. Four weeks after naive OT-I injection, the number of Ly5.1$^-$ OVA-tetramer$^+$ cells (derived from the original naive Ly5.2$^+$ OT-I cells) remaining in the spleen and lymph nodes of the mice was determined by flow cytometry. Pooled data are shown from 2 independent experiments.

Fig. S5. Surviving OT-I cells are antigen inexperienced. When naive OT-I cells are transferred into RIP-OVA^{lo} mice they remain phenotypically naive and CD44 low (2). For the experiment shown in Fig. S4C, CFSE-labeled OT-I cells were also analyzed for (*A*) CD44 expression vs. CFSE dilution or (*B*) undivided cells were examined for CD44 expression (shaded histogram represents media treated, and solid line represents CTL treated). As a positive control for high expression of CD44, OT-I cells from an OVA primed mouse (dotted line) are also shown. The bulk of cells in CTL-treated mice are not only undivided, but also the undivided cells display the same CD44 levels as cells from media-treated mice and hence are antigen inexperienced. Representative data from 2 independent experiments are shown.

Fig. S6. Surviving OT-I cells are not anergic. RIP-OVA[lo] mice were given either media or 220,000 Ly5.2$^+$ CTLs i.v. and 4 days later were injected i.v. with 5 million Ly5.1$^+$ naive OT-I cells. Four weeks later, CD8$^+$ cells were enriched from the spleen and lymph nodes, CFSE labeled, serially diluted, and incubated with OVA257–264-peptide pulsed B6 spleen cells in a 96-well plate. Sixty hours later the CFSE dilution of the CD8$^+$ Ly5.1$^+$ cells was examined by flow cytometry. (*A*) CFSE profiles of cells from media- and CTL-treated mice. (*B*) The number of proliferating cells recovered after 60 h vs. the number of input CD8$^+$Ly5.1$^+$ cells. Open circles, media control mice; solid triangles, CTL-treated mice. Representative (*A*) or pooled (*B*) data from 2 independent experiments are shown.

1. Carbone FR, Bevan MJ (1990) Class I-restricted processing and presentation of exogenous cell-associated antigen in vivo. *J Exp Med* 171:377–387.
2. Feng H, Zeng Y, Graner MW, Katsanis E (2002) Stressed apoptotic tumor cells stimulate dendritic cells and induce specific cytotoxic T cells. *Blood* 100:4108–4115.