

Supporting information Fig. 2. (*A*) We isolated CD8⁺ T cells from splenocytes of C57BL/6 mice to >95% purity (*right panel*), treated them with GSI *in vitro*, washed them twice with PBS and mixed them with CD8-depleted antigen presenting cells (APCs) that we purified to >99% (*left panel*). The purity of isolated cells was confirmed in each experiment. (*B*) N1ICD expression was determined in total lysates from CD8⁺ T cells from Notch1 AS mice (*upper panels*) either left unstimulated, or stimulated with anti-CD3ε plus anti-CD28 for 2 days, or from unstimulated or stimulated splenocytes from *in vivo* GSI-treated mice (*lower panels*). (*C*) ELISA was used to assess IFN-γ levels in the supernatants from similarly-prepared cells, stimulated for 1-3 days. Results in each panel are representative (*B*) or the mean \pm s.d (*C*) of at least two separate experiments.