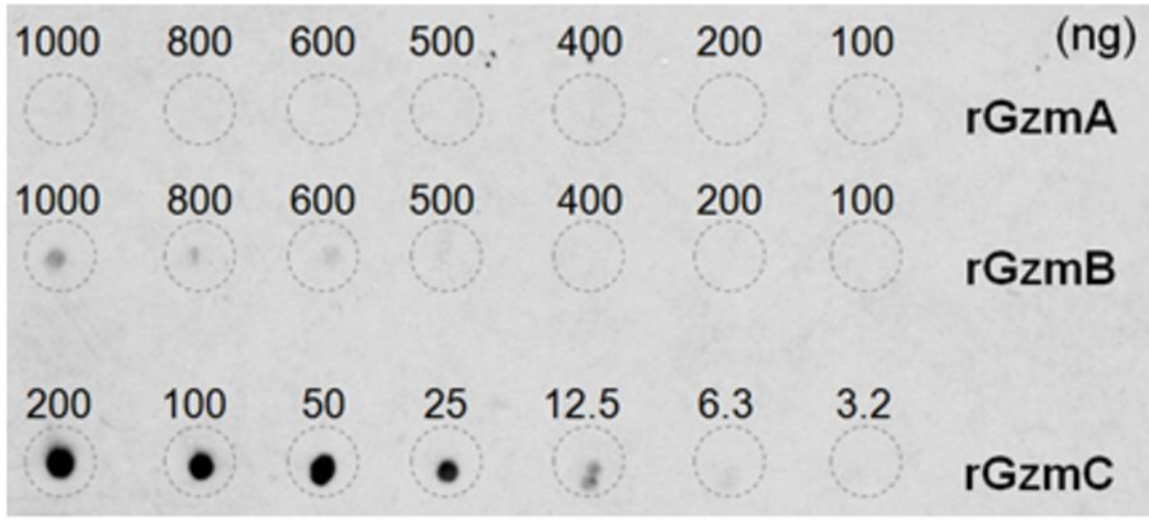
Supplemental Figure Legends

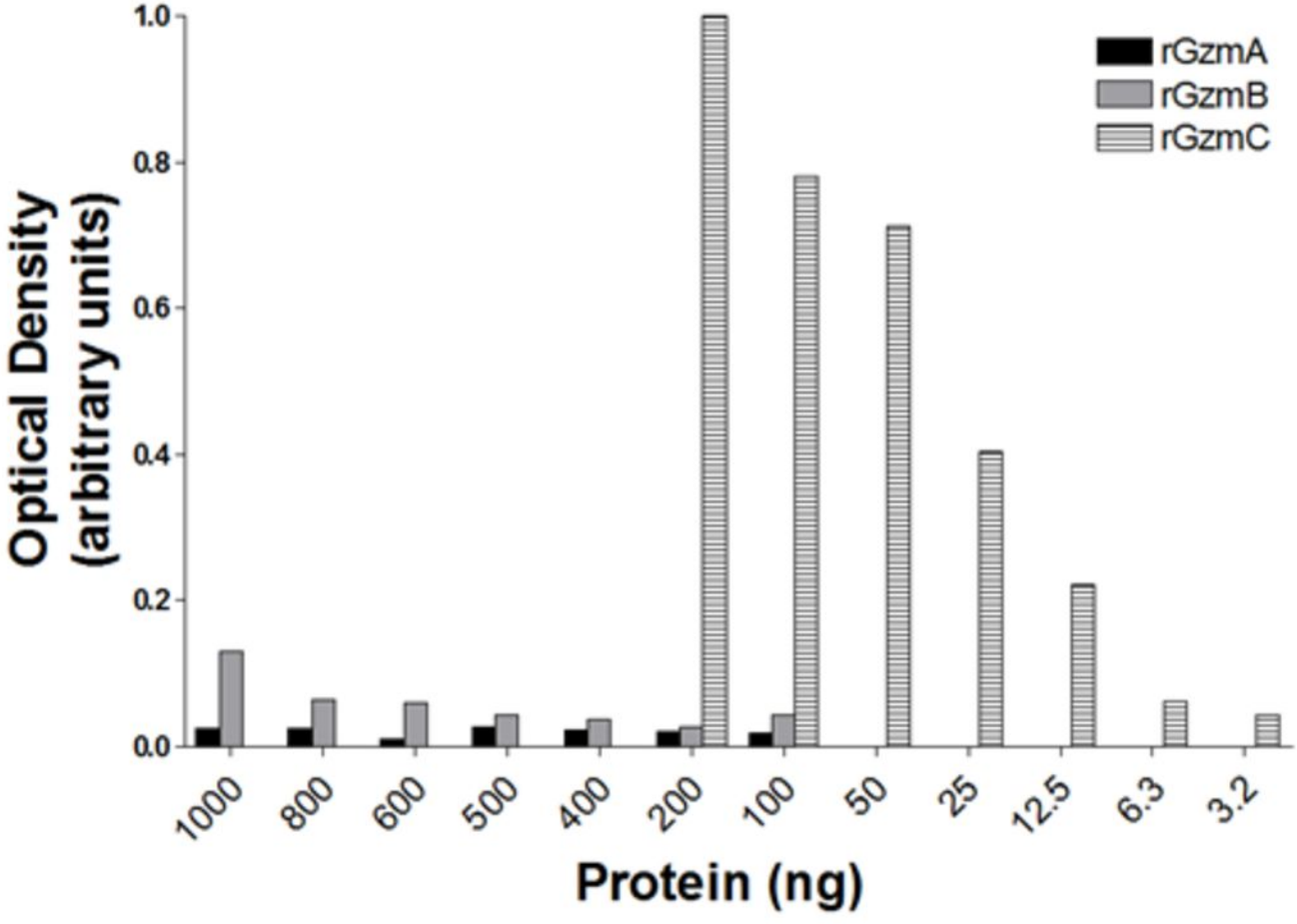
Supplemental Figure 1: SFC1D8 mAb has minimal cross-reactivity with native recombinant murine granzymes A and B. Various amounts of recombinant murine granzymes A, B, and C were spotted onto nitrocellulose membranes. Membranes were stained with Ponceau S to visualize spotted proteins. Nonspecific reactivity was blocked by incubation in Tris-buffered saline containing 0.1% Tween 20 (TBS-T) and 5% nonfat dried milk. Granzyme C mAb (SFC1D8) purified from hybridoma supernatants was used at 1:1000 dilution in TBS-T. Goat anti-Armenian hamster IgG-HRP conjugate (Santa Cruz Biotech) was used at 1:500 dilution in TBS-T to detect bound primary antibody. Reactive proteins were detected by incubation of washed filters in the enhanced chemiluminescence system (GE Healthcare Life Sciences) followed by exposure to autoradiographic film (A). Immunoblots were also quantified using ImageJ software (NIH) (B). Data shown are representative of two independent experiments.

Supplemental Figure 2: Extended MLR Time course. Splenocytes from wild-type or granzyme B-/- cre-deficient mice were cultured with irradiated Balb/c splenocytes (2000 cGy) in K10 medium supplemented with IL-2 (50 U/ml). MLR cultures were harvested for flow cytometric analysis after 5-8 days. T cell expression of granzymes B and C are shown. The percent CD4+ T cells positive for granzymes B and C are summarized in A, and the percent CD8+ T cells positive for granzymes B and C are summarized in B. Summary results (mean + SD) represent data pooled from three independent experiments.

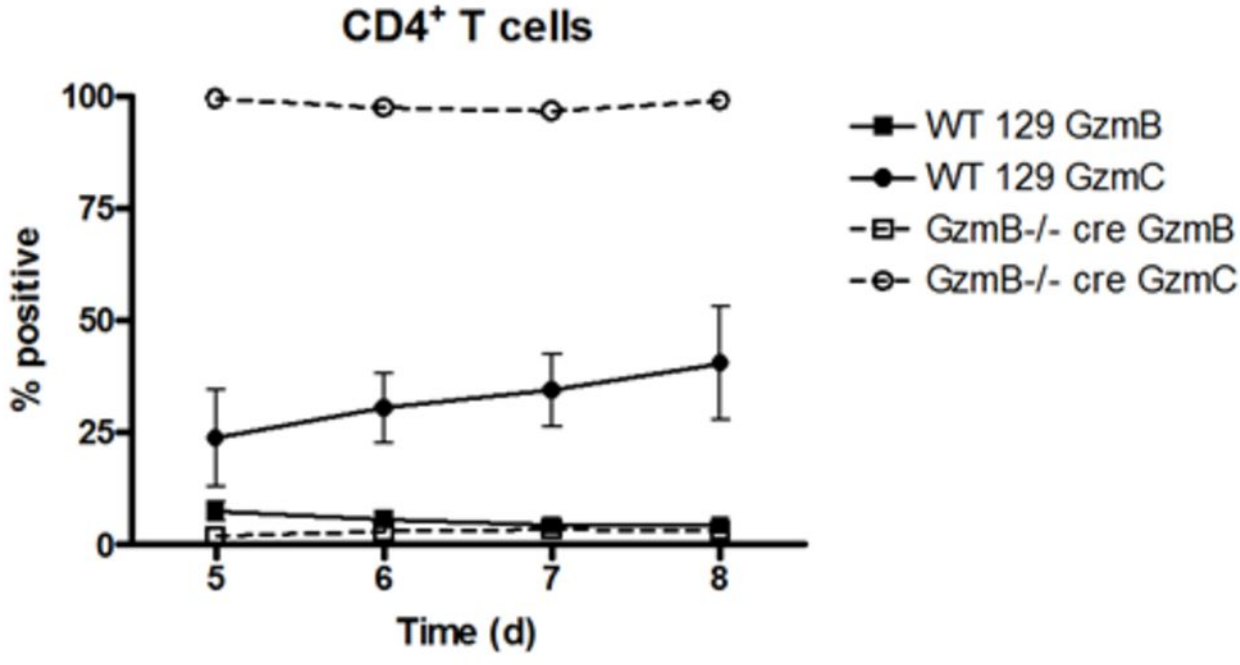
A


1000 800 600 500 400 200 100 (ng)
rGzmA
1000 800 600 500 400 200 100
rGzmB
200 100 50 25 12.5 6.3 3.2
rGzmC

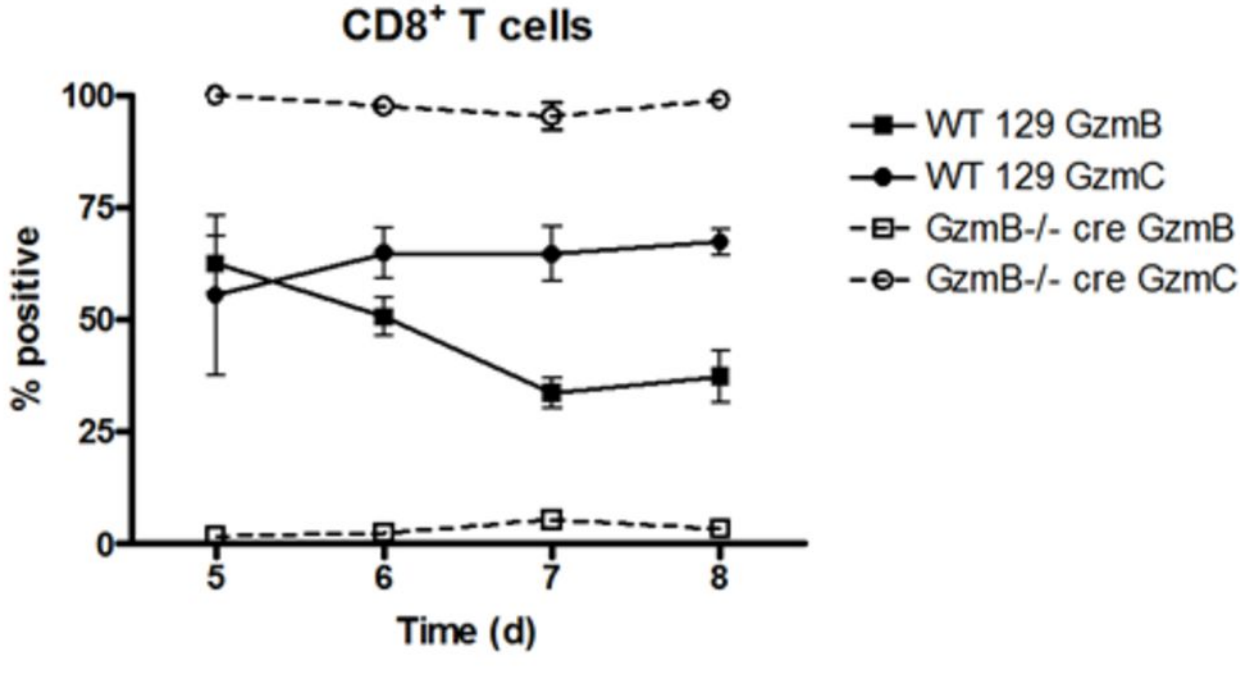
B


rGzmA
rGzmB
rGzmC
Optical Density (arbitrary units)
1.0
0.8
0.6
0.4
0.2
0.0
1000 800 600 500 400 200 100 50 25 12.5 6.3 3.2
Protein (ng)

A


CD4+ T cells
100
75
50
25
0
% positive
5
6
7
8
Time (d)
WT 129 GzmB
WT 129 GzmC
GzmB-/- cre GzmB
GzmB-/- cre GzmC

B


CD8+ T cells
100
75
50
25
0
% positive
5
6
7
8
Time (d)
WT 129 GzmB
WT 129 GzmC
GzmB-/- cre GzmB
GzmB-/- cre GzmC