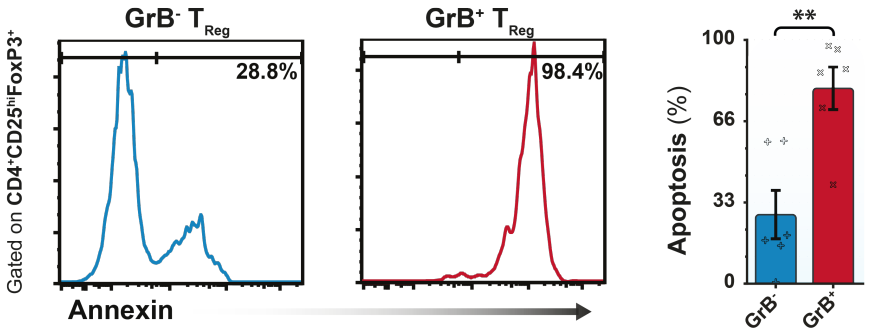
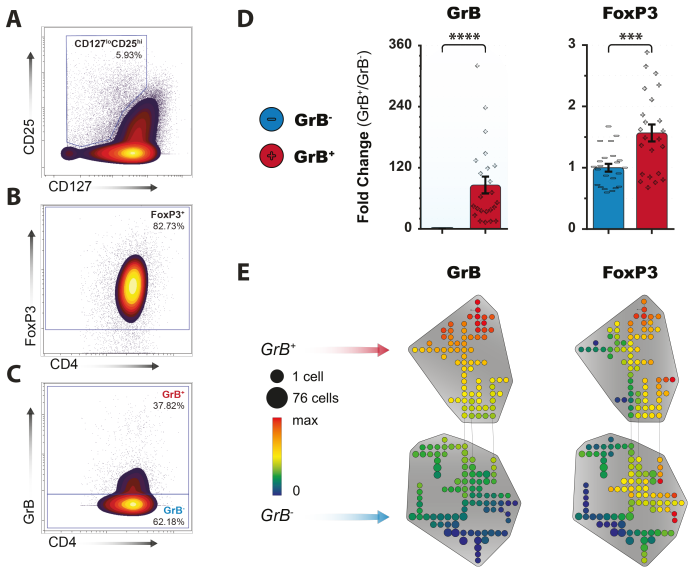
Supplemental Figures, Videos, and Legends



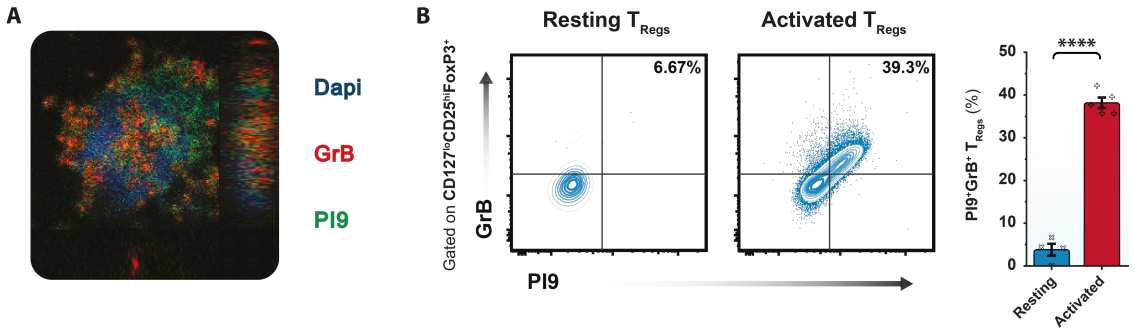
Supplemental Figure 1. *Increased apoptosis among GrB-expressing T_{Regs} in the peripheral blood of renal allograft recipients undergoing cellular rejection.* The GrB^+ T_{Regs} of patients undergoing cellular rejection (as proven by a concurrent biopsy) showcase greater staining of Annexin V, an apoptotic marker. Representative flow cytometry histograms and a corresponding descriptive bar chart show increased Annexin expression in GrB^+ T_{Regs} compared to GrB^- T_{Regs} (n=5/group, $^{**}p<0.01$).

CR, cellular rejector; FoxP3, forkhead box P3; GrB, granzyme B; NR, non-rejector; T_{Reg}, regulatory T cell.



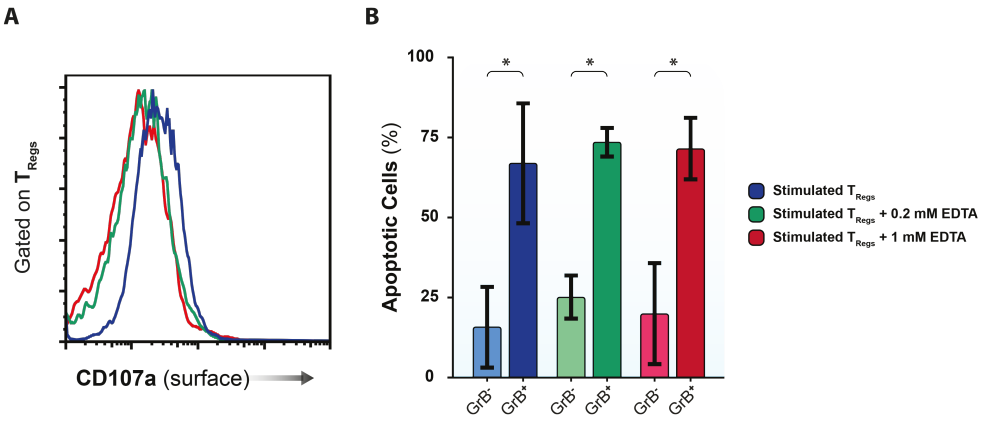
Supplemental Figure 2. *Increased expression of FoxP3 among the GrB$^+$ T_{Regs} compared to the GrB$^-$ T_{Regs}.* (A, B) CyTOF gating strategy used to identify the CD4$^+$CD127^{lo}CD25^{hi}FoxP3$^+$ cell population from the PBMCs of renal transplant patients, and to study the (C) GrB$^+$ and GrB$^-$ subsets. (D) Bar graphs show the fold change difference between the GrB subsets, based on their mean fluorescence intensity for the displayed markers. The primary GrB graph verifies that the chosen subsets are indeed GrB$^-$ and GrB$^+$ cells, while the other graph shows that among GrB$^+$ T_{Regs} there is increased expression of FoxP3 (1.57 ± 0.14, ***p<0.001). (E) Representative SPADE trees reiterating the increased expression of FoxP3 among the GrB$^+$ T_{Regs} compared to the GrB$^-$ counterpart. Each node represents a cell population, while the size of a node within the SPADE tree corresponds to the number of cells per population, and the node color indicates the signal intensity of the respective markers.

FoxP3, forkhead box P3; GrB, granzyme B.



Supplemental Figure 3. *Colocalization and concomitant upregulation of GrB and PI9 upon T_{Reg} stimulation.* Human T_{Regs} ($CD4^{+}CD25^{hi}$) were isolated from healthy individuals using magnetic beads and stimulated in vitro with anti-CD3/ CD28 and IL-2 for 72 hours. (A) Representative confocal microscopy image of an activated T_{Reg} showing nuclear DAPI staining, cytosolic and nuclear GrB staining, and colocalized PI9 staining. (B) Representative flow cytometry figures and bar chart outlining the concurrent increase in PI9 and GrB expression upon 72 hours of T_{Reg} stimulation (3.81 ± 1.39% vs. 38.18 ± 1.24%, respectively $PI9^{+}GrB^{+}$ resting vs. stimulated T_{Regs}, 2 separate experiments, ****p<0.0001).

DAPI, 4',6-diamino-2-phenylindole; GrB, granzyme B; PI9, proteinase inhibitor 9; T_{Reg}, regulatory T cell.



Supplemental Figure 4. *GrB originates from intracellular granular leakage.* To establish whether the GrB comes from T_{Reg} degranulation or intracellular granule leakage we treated cells with different concentrations of EDTA. Degranulation is a calcium dependent phenomenon and adding EDTA, which is a calcium chelator, would block degranulation. We used CD107 as a readout to verify whether EDTA blocked degranulation. CD107a or LAMP1 is a protein present on the granule membrane and is also present on the cell surface upon degranulation, as the granule membrane fuses with the cell membrane. (A) Representative figure of flow cytometry analysis of EDTA-treated cells stained with CD107a (surface staining). EDTA treated T_{Regs} expressed less CD107a on their surface compared to the control. (B) The bar graph shows the difference in the percentage of apoptosis between GrB^+ and GrB^- T_{Regs} in the EDTA groups vs. the control. Even after blocking degranulation, there are no differences in apoptosis, and GrB^+ T_{Regs} are characterized by much more apoptosis than the GrB^- T_{Regs}. This is an indicator that the apoptosis-inducing GrB in T_{Regs} originates from intracellular granule leakage. (Data represents one of two separate experiments, $^{**}p<0.01$).

EDTA, ethylenediaminetetraacetic acid; GrB, granzyme B; T_{Reg}, regulatory T cell.

Supplemental Video 1. Representative video of 3D microscopy of GrB leaking outside the granule and localizing within the nucleus after 36 hours of stimulation.

GrB, granzyme B.

Supplemental Video 2. Representative video of 3D microscopy of GrA leaking outside the granule and localizing within the cytoplasm after 36 hours of stimulation.

GrA, granzyme A.