Functional and Biochemical Characterization of a T Cell Associated Anti-apoptotic Protein, GIMAP6

Running title: Anti-apoptosis Function of GIMAP6

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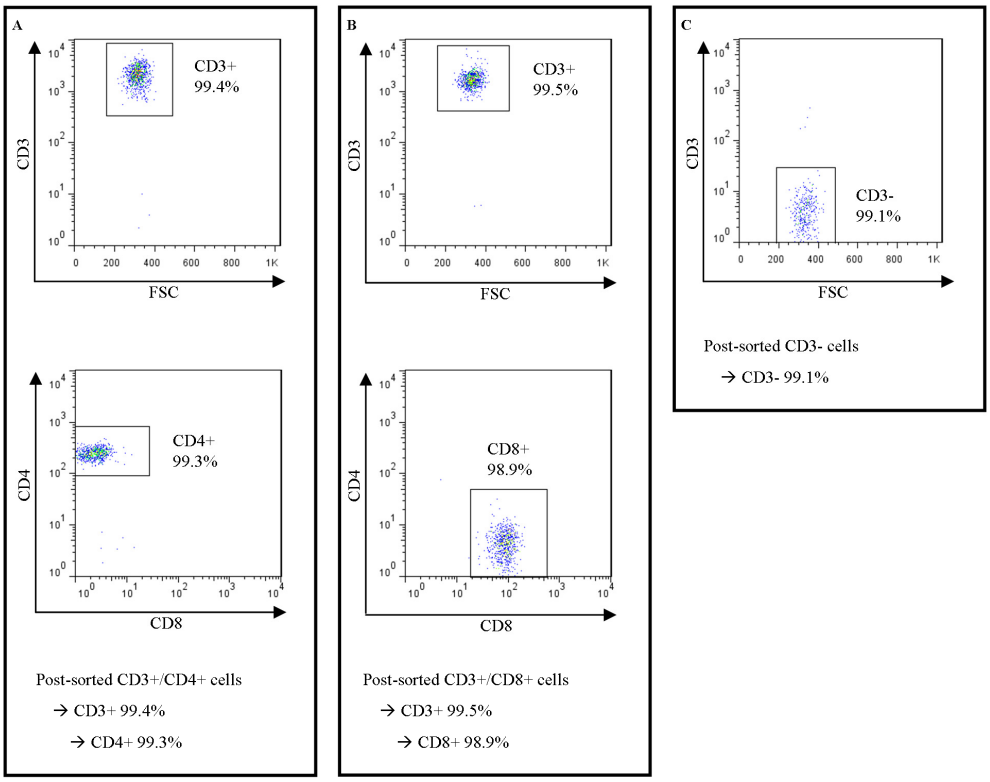

Fig. S1. The post-sort purity of three subsets of PBMC as revealed by the dot plot of flow cytometry. Three primary T cell subsets (A: CD3+/CD4+, B: CD3+/CD8+, and C: CD3–) from the PBMC of one healthy donor were isolated by flow cytometry using CD3-PE, CD4-FITC, and CD8-APC triple staining. Collected post-sort cells were re-flowed again to verify the purity.

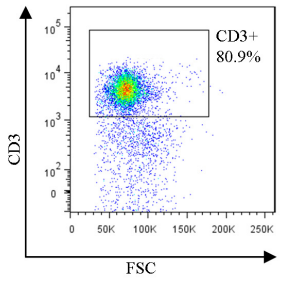

Fig. S2. The purity of enriched primary CD3+ T cells as revealed by dot plot of flow cytometry. After PBMC collection from the healthy donors, CD3+ T cells were further enriched using human CD3 T cell negative selection kit (Biolegend, #480022). The purity of CD3+ population was examined by flow cytometry with anti-CD3-PE (BD 555333) antibody.

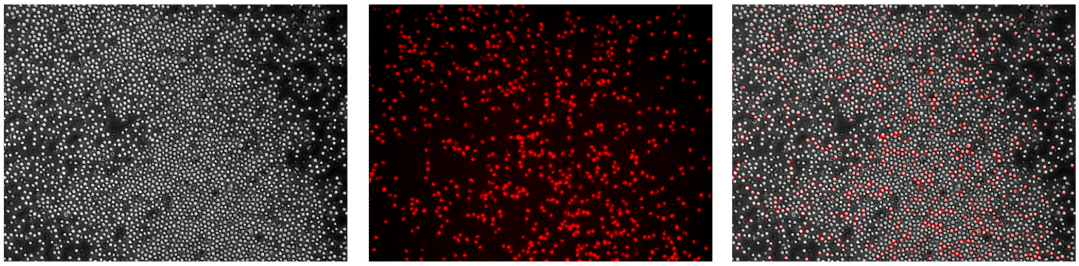

Fig. S3. Transfection efficiency of siRNA as revealed by fluorescence microscopy. (A) The transfection efficiency was monitored by transfecting Cy3-labeled negative control siRNA (AM4621). After transfection for 48 hours, transfection efficiency was checked on fluorescence microscopy with an average of 20.65±6.68%.

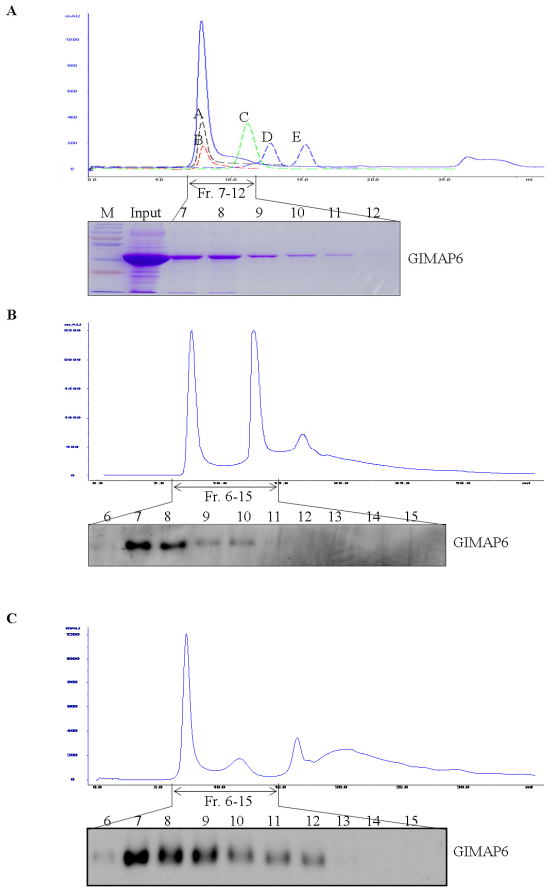

Fig. S4. Elution profile of recombinant and mammalian GIMAP6 using gel filtration chromatography. (A) The elution profile of recombinant GIMAP6. Five standard proteins (GE, a high molecular weight calibration kit, A: Blue dextran 2000 kDa, B: Thyroglobulin 669 kDa, C: Catalase 250 kDa, D: Ovalbumin 48.1 kDa, E: Ribonuclease A 15.2 kDa) are shown as the dashed line; these were used to calibrate the column (Superose 12 10/300 GL prepacked column, GE Healthcare). The elution profile of GIMAP6 is presented as a solid blue line and this is superimposed on the above-mentioned standards. The eluates were collected as 1 ml fractions using an elution velocity of 0.5 ml/min. Protein signal, which could be identified as GIMAP6, was detected in fractions 7 to 12 by measuring the absorbance in the near UV (280 nm). Fractions 7 to 12 of the size exclusion chromatography were analyzed by SDS-PAGE, and the proteins were then visualized by staining with Coomassie brilliant blue R250. “M” indicates the protein markers, and “Input” indicates the sample before gel filtration. Total cell lysates of Jurkat T-lymphocytes (B) or 293T cells stably

transfected with GIMAP6 (C) were also analyzed by gel filtration chromatography. Immunoblot analysis revealed that GIMAP6 was also eluted in fractions 7 to 12, when either Jurkat T-lymphocyte or 293T stable transfectant total cell lysates was used as input.