Supporting Information

Sharma et al. 10.1073/pnas.1019711108

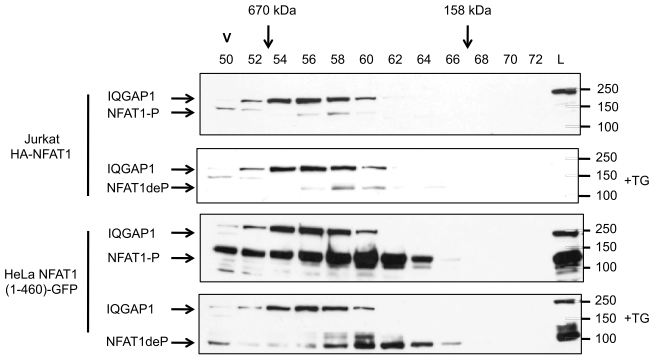

Fig. S1. Migration of nuclear factor of activated T cells (NFAT1) and NFAT1-GFP complexes upon size-exclusion chromatography of cytoplasmic extracts from untreated and thapsigargin-treated cells. Jurkat cells stably expressing HA-NFAT1 (*Upper*) and HeLa cells stably expressing NFAT1(1-460)-GFP (*Lower*) were left unstimulated or stimulated with 1 μM thapsigargin for 10 min. Hypotonic lysates were prepared and fractionated on a Superdex 200 size-exclusion column. Individual fractions were analyzed by 10% one-dimensional SDS-PAGE and Western blotting with anti-HA and anti-IQ motif-containing, GTPase-activating protein (IQGAP1). Column void volume (**V**) is indicated. **L** denotes 10% of the input loaded on the column. Note that full-length HA-NFAT1 from Jurkat lysates migrates at a slightly higher apparent molecular weight compared to NFAT1(1-460)-GFP from HeLa cell lysates, potentially because the region of NFAT1 C-terminal to residue 460 recruits additional components to the complex. The decreased amount of NFAT1-GFP in cytoplasmic extracts of stimulated HeLa cells likely reflects nuclear translocation of a substantial proportion of the protein. For a detailed discussion, see text.

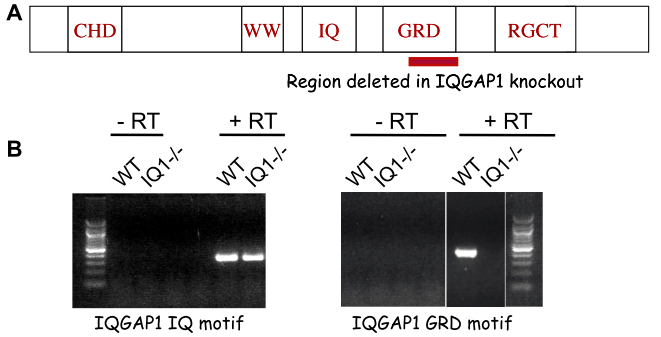

Fig. S2. *Iqgap1* mutant (IQ-/-) $CD8^+$ T cells still express *Iqgap1* mRNA. (*A*) Diagram of IQ motif containing GTPase activating protein 1 (IQGAP1) showing protein domains (1, 2). (*B*) Total RNA was isolated from WT or IQGAP1-/- $CD8^+$ T cells differentiated for 6 d in the presence of Interleukin-2 (100 units per milliliter) and subjected to RT-PCR for sequences corresponding to the IQ and GAP-related domain (GRD) motifs of IQGAP1. *Iqgap1* mRNA transcripts from IQGAP1-/- cells lack sequences corresponding to the N-terminal part of the GRD region of IQGAP1, but contain sequences from the IQ domain. Essentially identical data were also obtained using primers to different regions of IQGAP1. Note that IQGAP1-/- mice bear a deletion of exon 27 of IQGAP1, which encodes the C-terminal region of the GRD motif; this deletion is predicted to yield an in-frame transcript that could potentially result in expression of a truncated but partially functional protein.

1 Briggs MW, Sacks DB (2003) IQGAP proteins are integral components of cytoskeletal regulation. *EMBO Rep* 4:571–574.
2 White CD, Brown MD, Sacks DB (2009) IQGAPs in cancer: A family of scaffold proteins underlying tumorigenesis. *FEBS Lett* 583:1817–1824.