Foley *et al*. Supplemental data

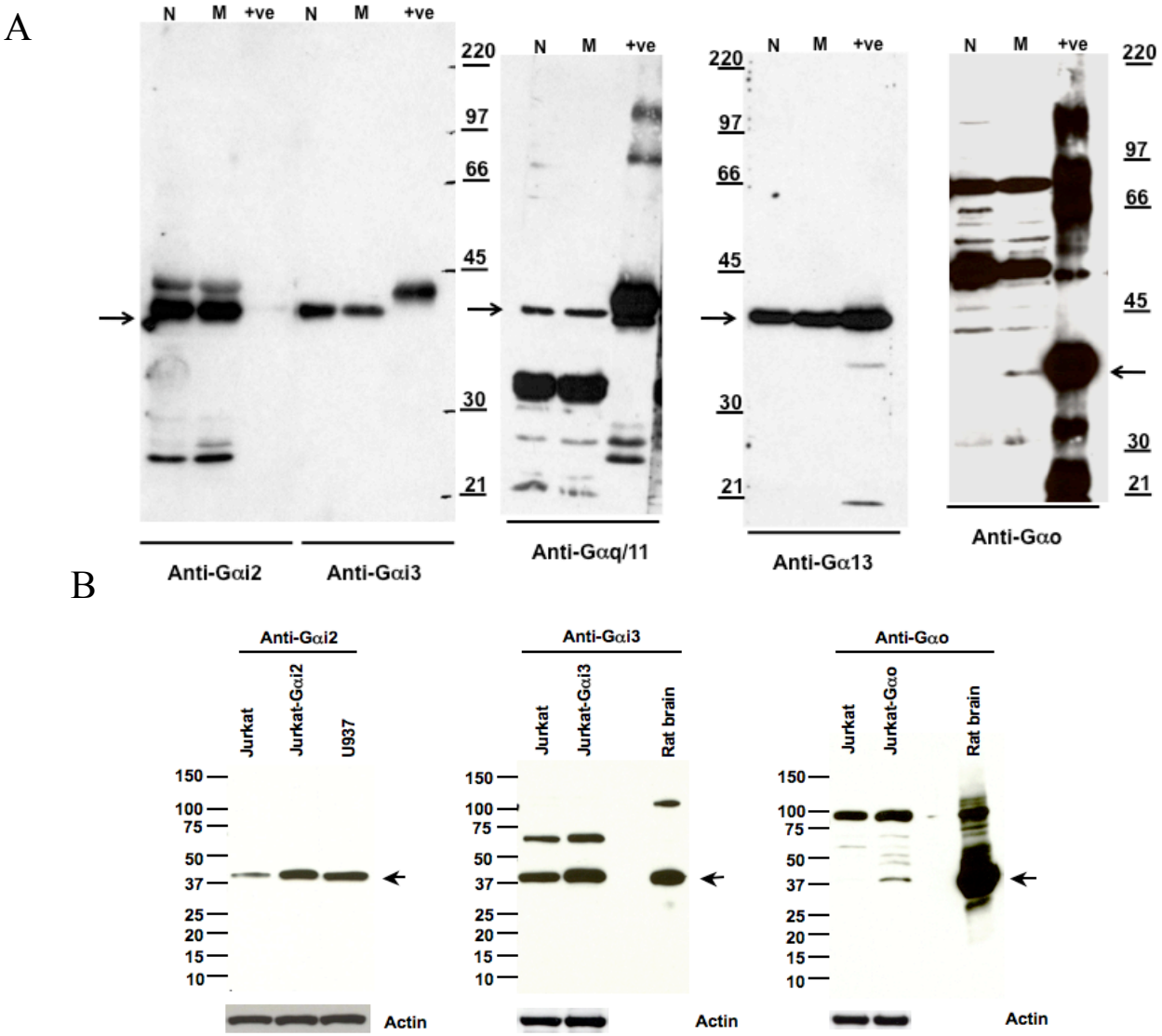

Figure S1. Western blots of Gα proteins in naïve (N) and memory (M) subsets of human $CD4^+$ T cells and in Jurkat cells transfected to over-express Gα proteins. *A,* Naïve and memory subsets of human $CD4^+$ T cells were isolated from peripheral blood and analyzed by Western blotting for the presence of the indicated Gα proteins as described in Experimental Procedures and in the legend for Fig. 1. Recombinant proteins (100 ng) were used as positive controls (+ve) for all antibodies except anti-$G\alpha_{13}$, for which mouse brain (25 μg protein) was used. The anti-Gα protein antibodies used are noted below the lanes. The left-most blot was split for probing. *B,* Jurkat cells, transfected with control plasmid or plasmid encoding Gα proteins as noted were analyzed by Western blotting 24 hours post-transfection. Extracts of U937 cells or rat brain were used as positive controls. The anti-Gα protein antibodies used are noted above the lanes. Probing for actin was done to demonstrate equal loading. Lanes contained 14 μg protein, except for rat brain, where 0.5 μg was used due to the high amounts of Gα proteins. For both *A* and *B* molecular mass markers (kD) are shown beside the blots. Arrows indicate the Gα proteins of interest. Data shown are representative of three experiments.

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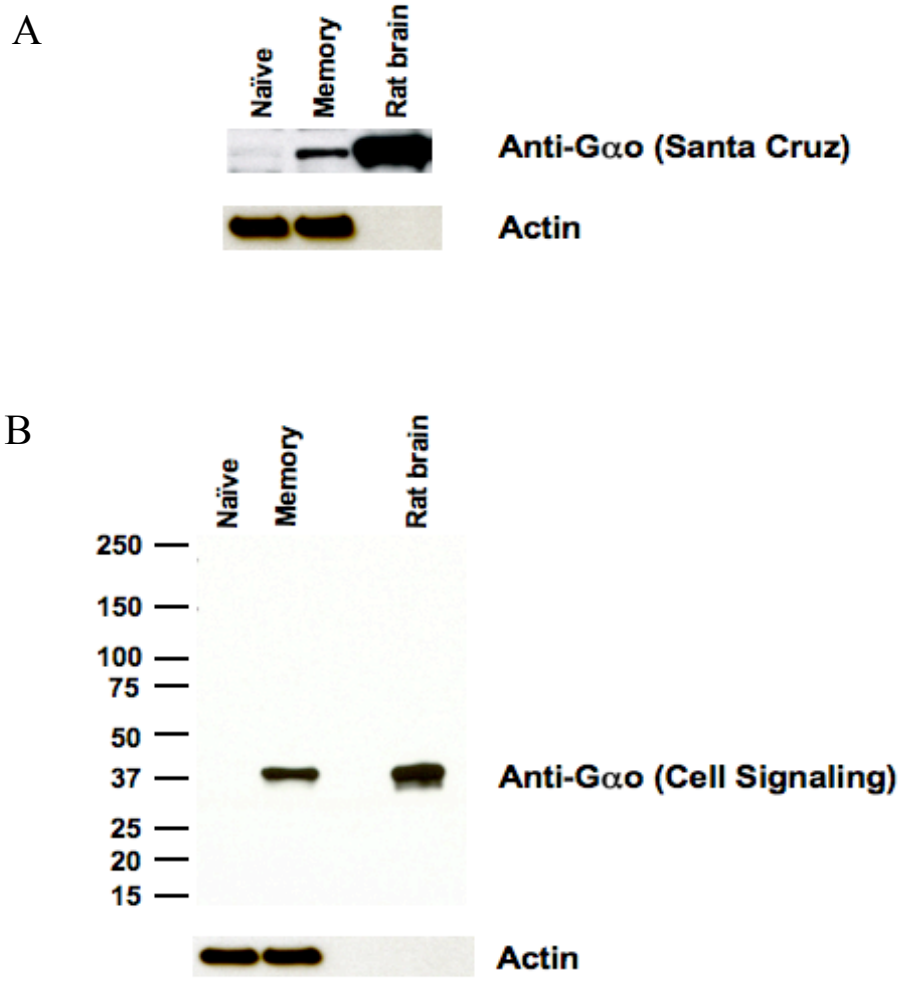

Figure S2. Comparison of $G\alpha_o$-specific antibodies in Western blotting. Human naïve and memory $CD4^+$ T cells were sorted from peripheral blood and processed for Western blotting as described in Experimental Procedures. Samples were analyzed with either the Santa Cruz anti-$G\alpha_o$ antibody, *A,* or the Cell Signaling anti-$G\alpha_o$ antibody, *B*, and as a loading control, with an anti-actin antibody. Lanes contained 14 μg protein, except for rat brain, where 0.5 μg was used due to the high amounts of Gα proteins. Molecular mass markers (kD) are shown on the left of the blot in *B*. Data are representative of two experiments.

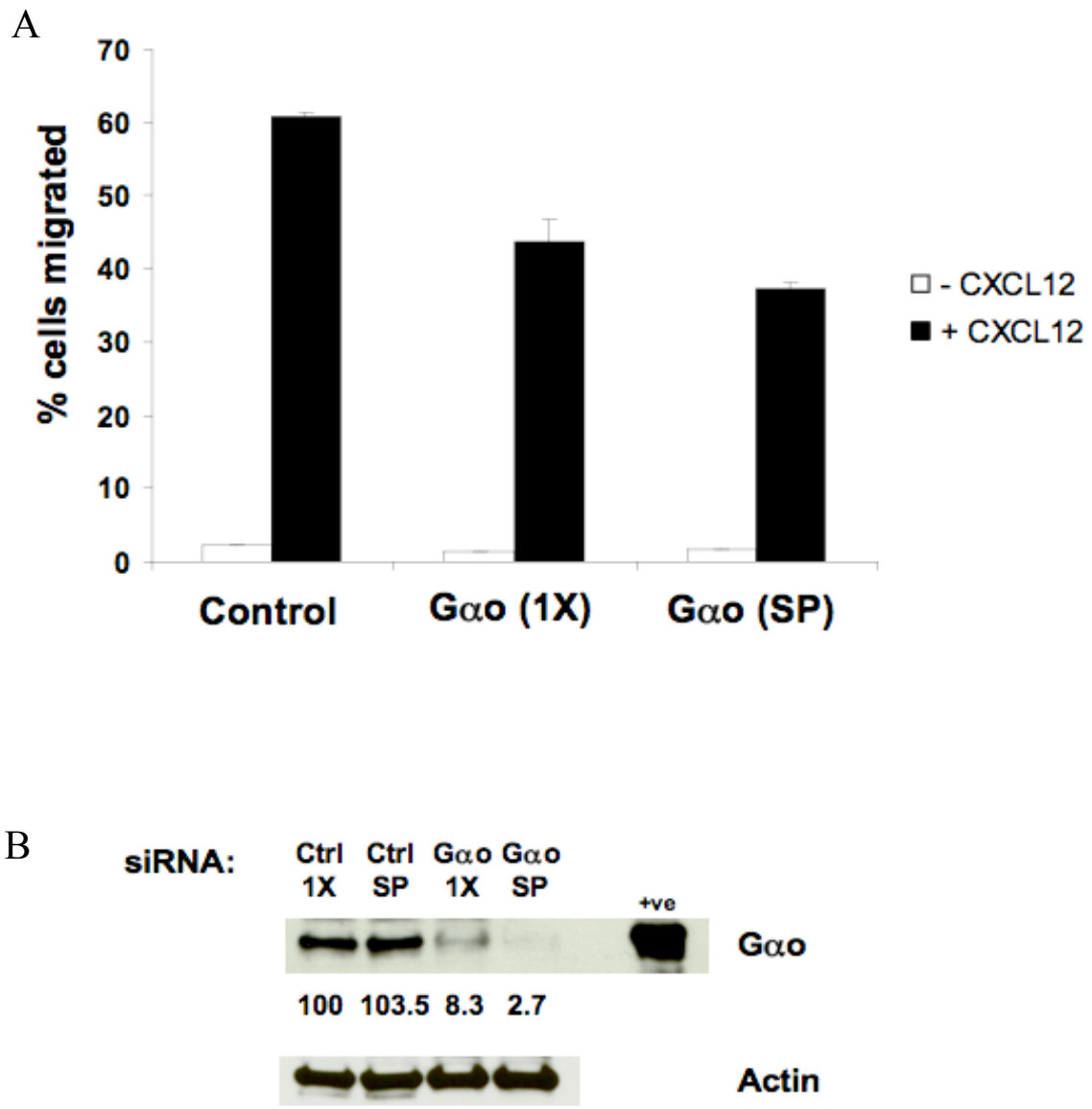

Figure S3. Comparison of the effectiveness of SmartPool siRNA and a single, independent siRNA on knockdown of $G\alpha_o$ and inhibition of CXCL12-dependent chemotaxis of Jurkat cells. Jurkat cells were transfected with either the SmartPool combination of $G\alpha_o$-specific siRNA (SP), a single, independent siRNA (1X), or the appropriate control, as described in Experimental Procedures. After 72 hours, cells were harvested and subjected to chemotaxis assay, *A,* and Western blotting, *B*. Lanes in *B* contained 14 μg protein, except for rat brain (+ve), where 0.5 μg was used due to the high amounts of $G\alpha_o$ protein. Bands were analyzed by densitometry and the quantitative data, normalized to actin and then to Ctrl 1X, which was set to 100, are shown below the blot. Data are representative of two experiments.