Supplementary Figure Legends:

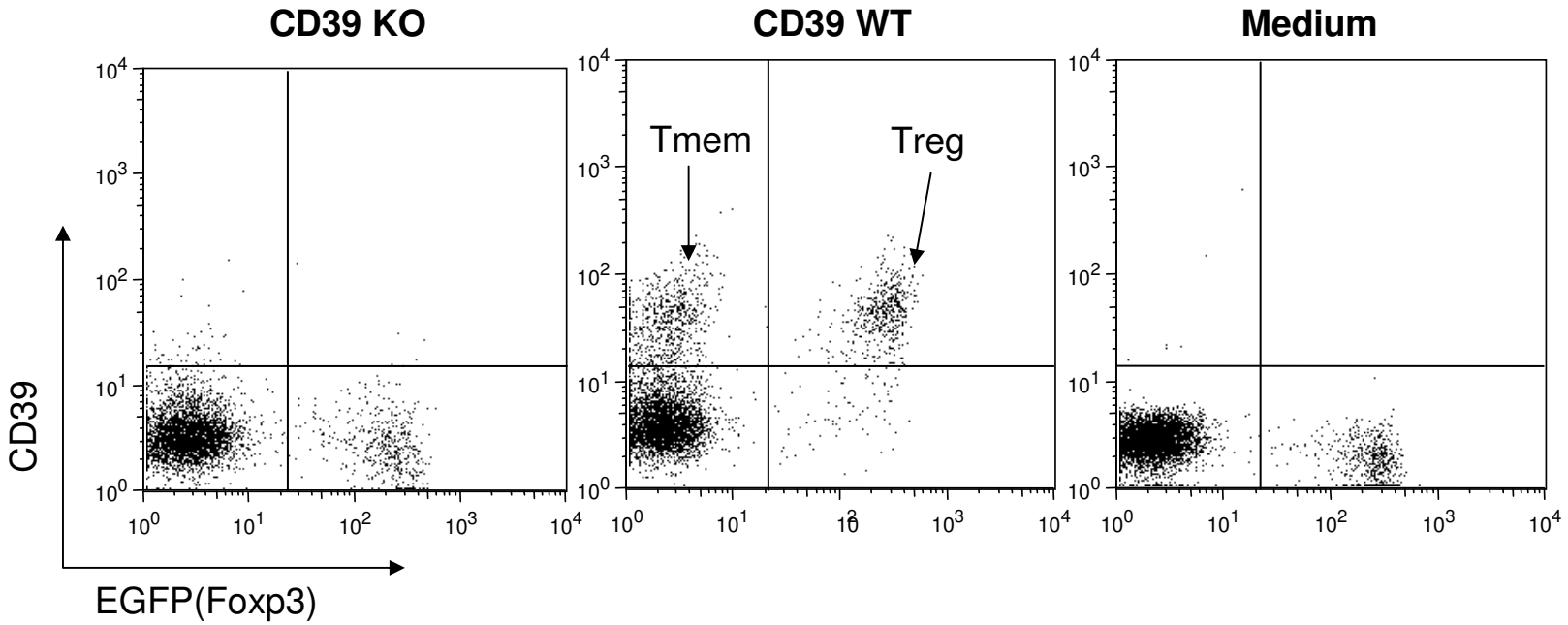
S1. Polyclonal anti-CD39 specifically recognizes mCD39. Splenocytes from CD39 KO mice crossed onto Foxp3GFP knockin background (left panel) or from wildtype Foxp3GFP knockin mice (middle panel) were stained with polyclonal anti-CD39. Only the wildtype cells give the typical $GFP^{+}CD39^{+}$ (Treg) and $GFP^{-}CD39^{+}$ (T_{mem}) staining pattern. Isotype control staining is shown in the right panel.

S2. Titration of anti-CD73 staining. Spleen cells isolated from 6-8 wk old Foxp3GFP knockin mice were stained with polyclonal anti-CD39 (1:200), and different concentrations of anti-CD73 (BD Biosciences, San Diego, CA) at 1:200, 1:400 and 1:2,000. Histograms of anti-CD73 staining (solid line) of gated CD4+ T cells were plotted against those of the isotype Ig control staining (shaded area).

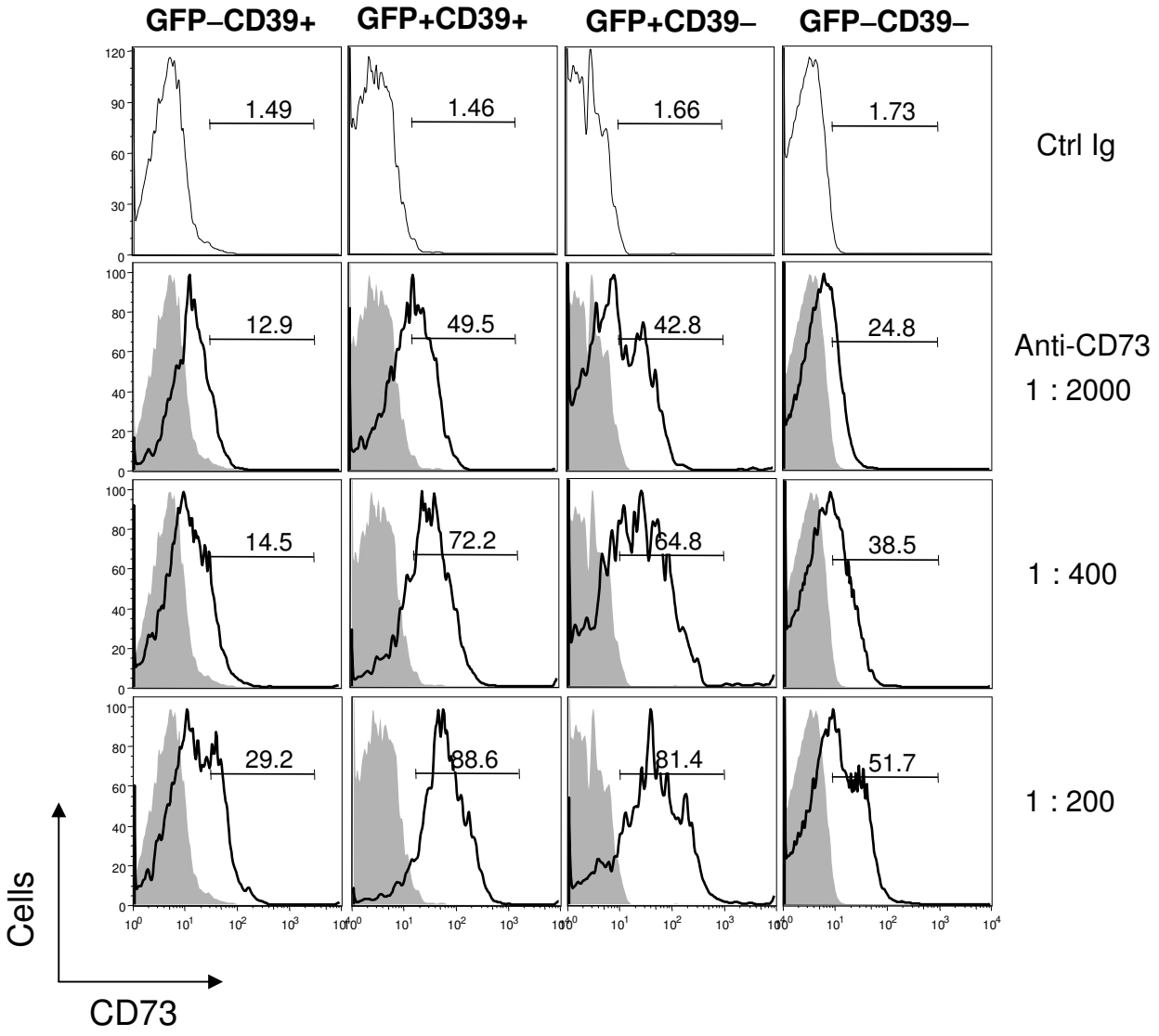
S3. $Foxp3^{-}CD39^{+}$ cells do not suppress. FACS-sorted $GFP^{-}CD39^{+}$, $GFP^{+}CD39^{+}$ or $GFP^{+}CD39^{-}$ testing suppressor cells were mixed with $GFP^{-}CD39^{-}$ effector cells at 0.5:1 (gray bar) or 1:1 (black bar), and stimulated with soluble anti-CD3 (2 μg/mL) in the presence of mitomycin C treated CD4-depleted syngeneic splenocytes for 3 days. Cytokine levels were determined by ELISA.

S4. Additional real-time PCR comparison between Foxp3^{-}CD39^{-} and Foxp3^{-}CD39^{+} cells. FACS-sorted naïve (GFP^{-}CD39^{-}) and memory (GFP^{-}CD39^{+}) T cells were activated by anti-CD3 and anti-CD28. At 48 hrs, aliquots of activated naïve (GFP^{-}CD39^{-}, empty circle) and memory (GFP^{-}CD39^{+}, filled circle) T cells were analyzed by real-time PCR for cytokine messages. Data represent two independent experiments.

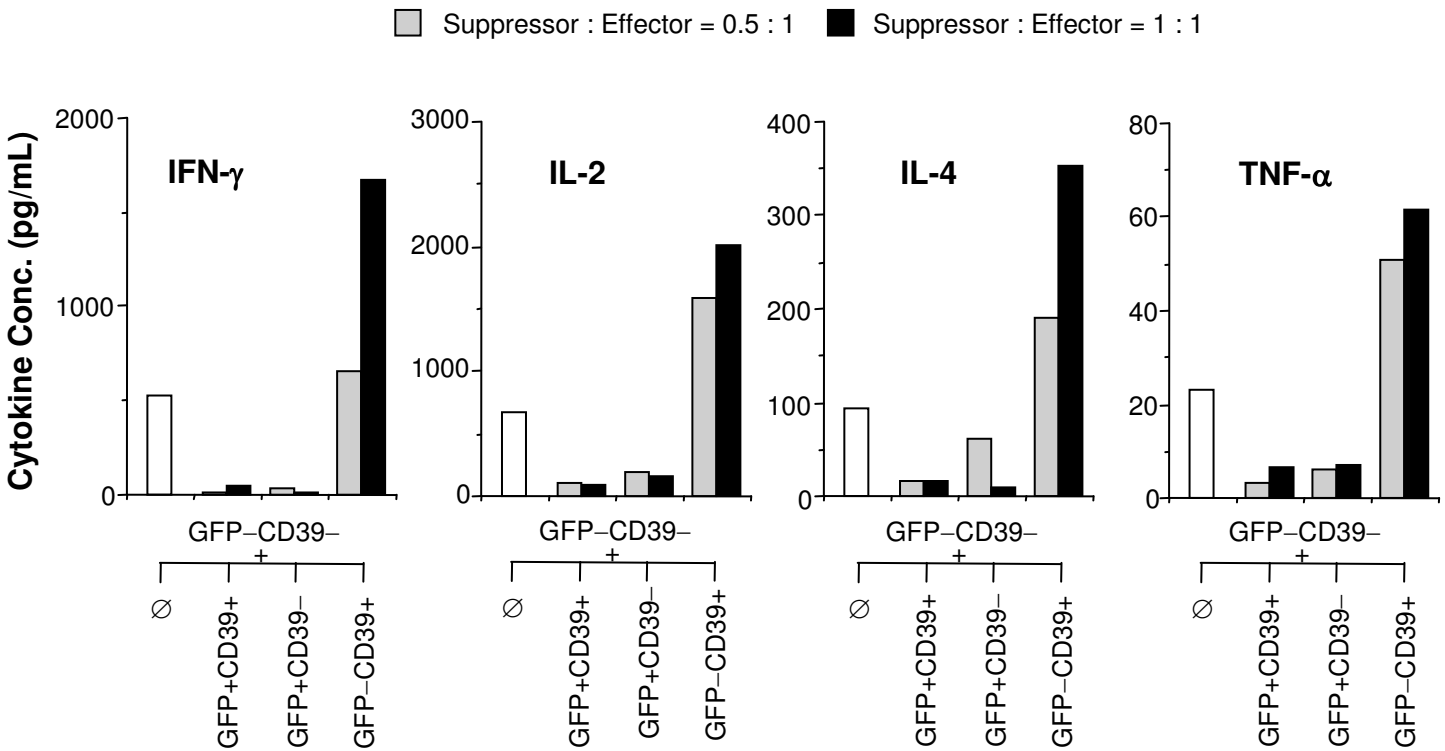
S5. Foxp3^{-}CD39^{+} cells produce multiple cytokines upon activation. FACS-sorted naïve (GFP^{-}CD39^{-}) and memory (GFP^{-}CD39^{+}) T cells were activated by anti-CD3 and anti-CD28. At 72 hrs, supernatants were collected and cytokine levels were determined by ELISA. Note that *y* axes for cytokines IFN-γ, IL-4, IL-10 and IL-17A are in log scale. Data represent two independent experiments.



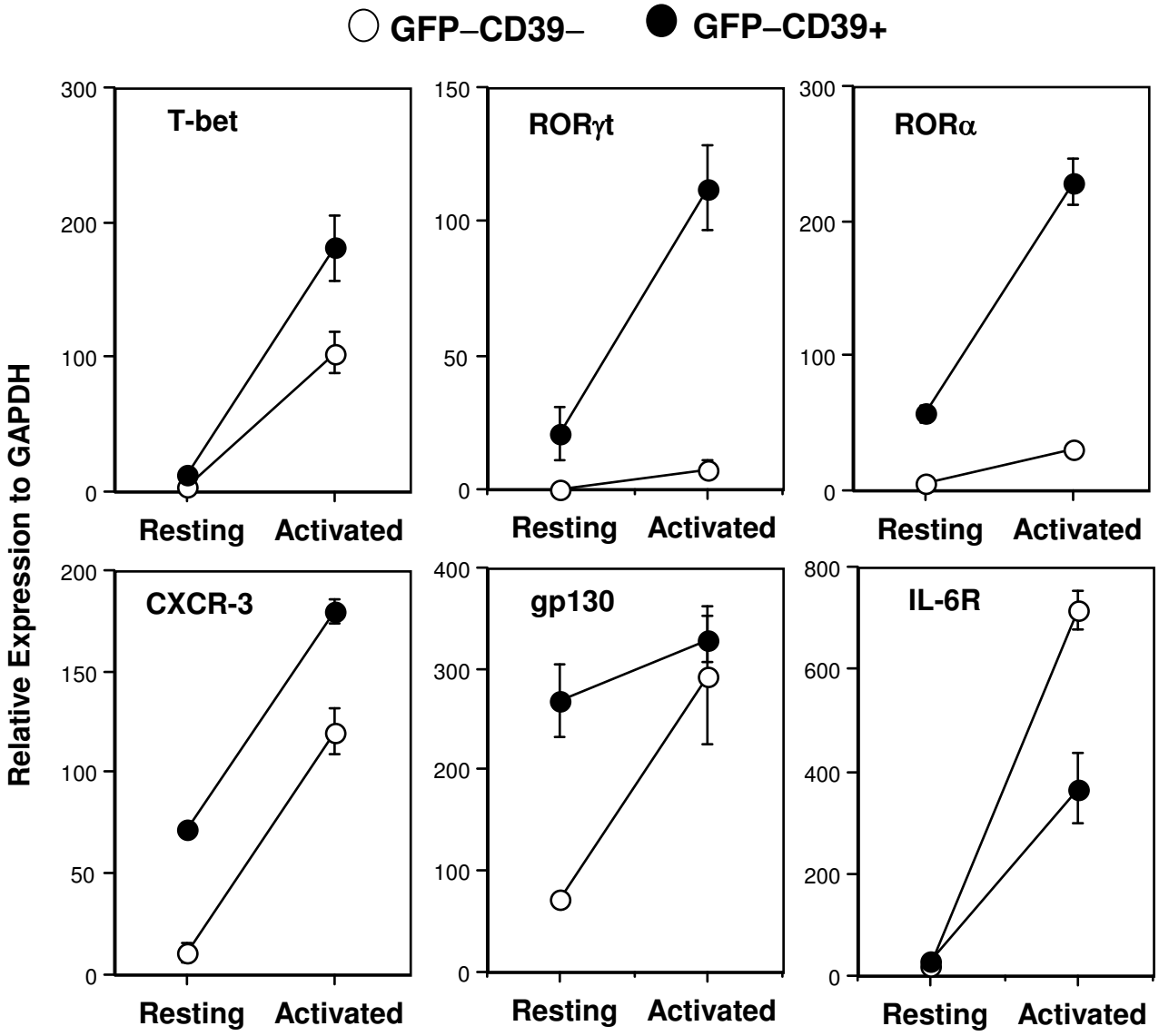
Supplementary Figure S1



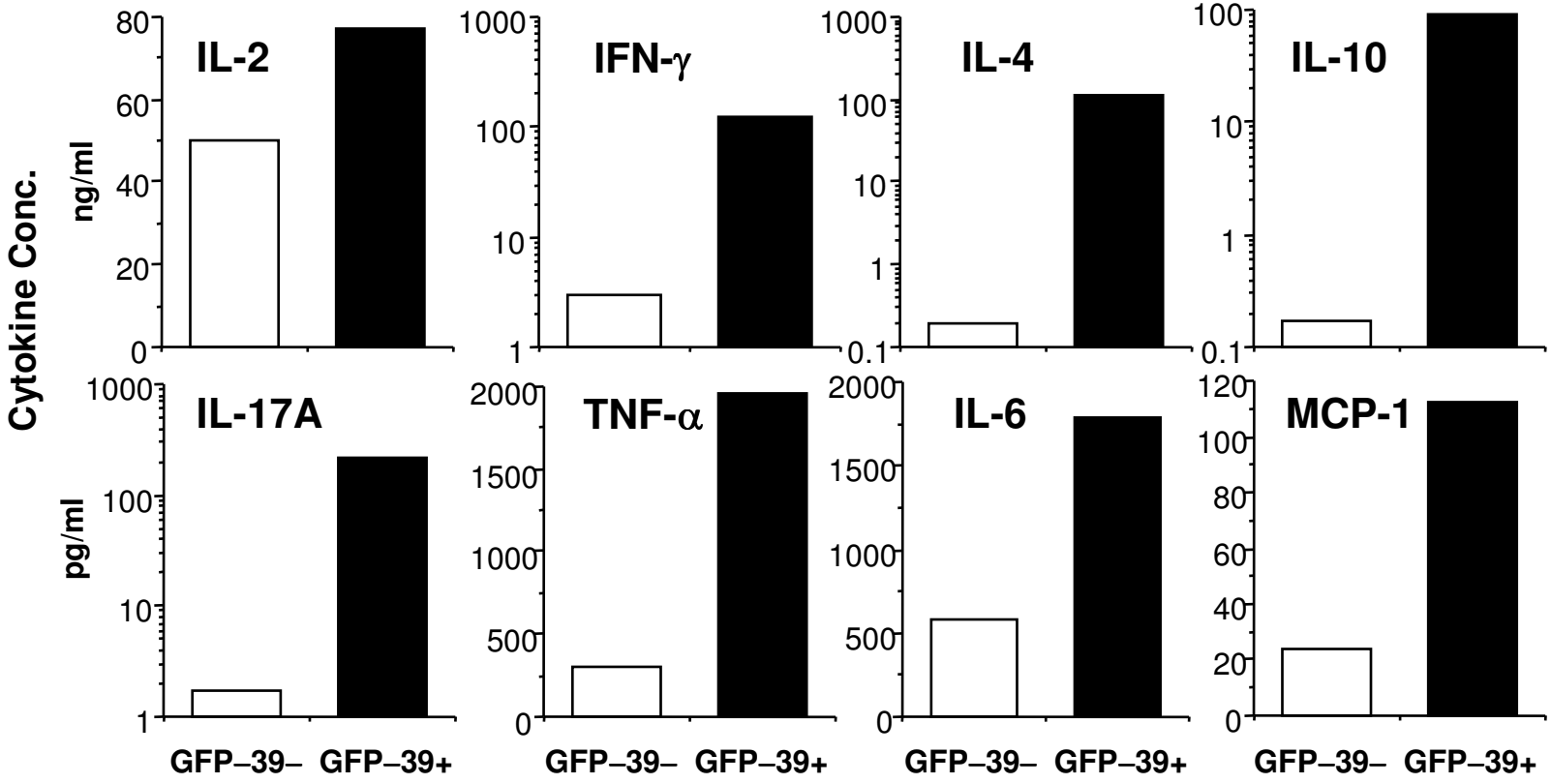
Supplementary Figure S2



Supplementary Figure S3



Supplementary Figure S4



Supplementary Figure S5