Supplemental Figures

**Supplemental Figure 1.** CHO cell transfectants expressing human CD96 were incubated with biotinylated CD155-Fc and titrated amounts of D265A m2a anti-CD96 Ab clones or an isotype control (IC). Biotinylated CD155-Fc binding was detected using fluorescently-labelled streptavidin and flow cytometry. Data are shown as the percentage of inhibition of CD155-CD96 interaction. Dose response curves were obtained from GraphPad Prism software using a non-linear regression fit with a variable slope model.
Supplemental Figure 2. The expression of CD96 and CD155 on resting and activated PBMCs from HD was analyzed by flow cytometry. (A) Frequency of CD96+ cells among resting naive (CCR7hi CD45RAhi), effector memory (TEM; CCR7lo CD45RAlo), central memory (TCM; CCR7hi CD45RAlo) and terminally-differentiated memory (TEMRA; CCR7lo CD45RAhi) CD4+ and CD8+ T cells. Data show the mean ± SEM, with each symbol representing an individual HD. (B) Data show representative examples of CD96 expression on T-cell subsets, prior to and 1 and 3 days after stimulation with soluble OKT3. (C and D) Data show representative examples of CD155 expression (black lines) or the corresponding isotype control (shaded histograms) on myeloid and lymphoid subsets prior to OKT3 stimulation and (D) of CD155 expression on T-cell subsets prior to and 1 and 3 days after soluble OKT3 stimulation. (B-D) Data are from one HD representative of 4, analyzed in n=2 independent experiments.
Supplemental Figure 3. The expression of FcγRI, FcγRIIA/B and FcγRIIIA on monocytes, B cells, NK cells, and T-cell subsets was analyzed by flow cytometry on PBMCs from HD, either (A) unstimulated or (B) stimulated for 3 days with soluble OKT3. Data show examples of expression for one HD representative of 4 for monocytes, B cells and NK cells, and representative of 6 for T-cell subsets, from n=3 independent experiments.
Supplemental Figure 4. T-cell subsets were purified from PBMCs from HD by immunomagnetic selection. (A) CFSE-labelled CD4+ or CD8+ T cells were stimulated with plate-bound OKT3 and plate-bound anti-CD96 huG1 mAb (19-134) or a matching isotype control (IC) for 4 days and the frequency of dividing cells was determined by flow cytometry. (B) CD4+ and CD8+ T cells were stimulated for 6 hours with plate-bound OKT3 and 19-134 huG1 mAb and IL-2 production was quantified by ELISA. Each data point represents the mean of triplicate wells for an individual donor.
Supplemental Figure 5. Purified CD3+ T cells were stimulated for 6 hours with plate-bound OKT3 and 19-134 huG1 mAb clone or an isotype control, RNA was isolated and samples were submitted to RNA-Seq analysis. Data show upstream regulators of genes that are predicted to be (A) activated or (B) inhibited by anti-CD96 mAb, as identified by IPA: p values (crosses; right axis) and z-scores (line; left axis). Regulators with z-scores $\geq 2$ are predicted to be activated; regulators with z-scores $\leq -2$ are predicted to be inhibited.