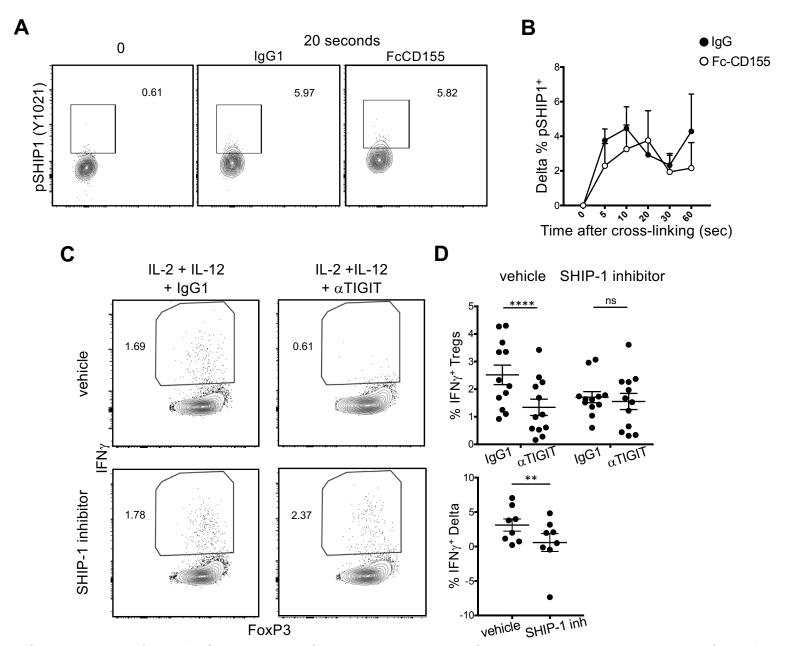


Supplementary figure 1. Kinetic and correlation with suppressor activity of the effect of TIGIT stimulation on IFN $\gamma$  production. (A) Tregs were stimulated for the indicated time points with  $\alpha$ CD3+ $\alpha$ CD28, IL-2+IL-12 with or TIGIT stimulation through FcCD155. Quantification of the frequency of IFN $\gamma$ <sup>+</sup> Tregs over time from 2 donors. Tregs were pre-activated for 4 days with IL-2 and IL-12 in the presence of IgG1 control (B) or Fc-CD155 stimulation (C) and co-cultured with Teff in suppression assays. Correlation between suppression of Teff proliferation and production of IFN $\gamma$  by Tregs. Each dot represents a Treg:Teff ratio.

C



Supplementary figure 2. Suppression of IFN $\gamma$  by agonistic  $\alpha$ TIGIT stimulation is dependent on SHIP-1 function. (A, B) Tregs were stimulated for the indicated times with cross  $\alpha$ CD3+ $\alpha$ CD28 as well as Fc-CD155 or isotype control. One representative staining for pSHIP1 (Y1021, A) and quantification of the delta between the indicated time points and the 0 condition for 5 donors (B, paired t-test with Bonferroni's correction for multiple comparisons highlighted no significant differences between the IgG1 and FcCD155 conditions). (C, D) Tregs were stimulated for 4 days with  $\alpha$ CD3+ $\alpha$ CD28, IL-2 or IL-2+IL-12 with or without  $\alpha$ TIGIT and in the presence of a SHIP-1 inhibitor (3AC 1 $\mu$ M) or vehicle (EtOH). 15 donors were tested and 3 were removed because no TIGIT effect in the *vehicle* condition was observed. Representative staining (C) and quantifications