SUPPLEMENTAL MATERIAL

Thrombospondin-1 and Angiotensin II Inhibit Soluble Guanylyl Cyclase through an Increase in Intracellular Calcium Concentration

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Figure Legends

Fig. S1. Western blot of immunoprecipitated sGC. MCF7 cells were transiently transfected with myc-tagged sGCα and FLAG-tagged sGCβ, and trypsinized 12 hours post transfection. sGC was immunoprecipitated using beads conjugated to anti-FLAG antibody. The western blot was performed with mouse monoclonal anti-FLAG antibody and goat anti-mouse secondary antibody conjugated to IR-dye. Equal quantities of sGC were recovered under all conditions tested. (*Upper*) Each lane corresponds to one assay condition in Fig. 6C. (*Lower*) Representative samples used for kinetic measurements in Fig. 7.

Fig. S2. Dot-plot profiles of Jurkat T cells showing homogenous expression of CD47. *A.* Jurkat T cells only. *B.* Jurkat T cells stained with isotype control antibody. *C.* Jurkat T cells stained with monoclonal anti-CD47 antibody conjugated to FITC. 1 x 10^6 cells were used per assay condition.

Fig. S3. Dose dependence for E3CaG1. Shown is inhibition of sGC activity in Jurkat T cells as a function of E3CaG1 concentration (two independent experiments). Error bars represent the range in values. Jurkat T cells were incubated with E3CaG1 (0-220 nM) for 15 min prior to the addition of 10 μM DEA/NO. Cell pellets were lysed in lysis buffer and cGMP accumulation measured.

Fig. S4. Flow cytometry histograms of green fluorescence emission of Ca^{2+} binding dye Fluo-3 as a function of changing $[Ca^{2+}]_i$. *A.* Blocking of CD47 with anti-CD47 antibody (B6H12) abolishes E3CaG1 induced changes in $[Ca^{2+}]_i$, whereas antibodies to αV integrin (P2W7 and 272-17E6) have no effect. Jurkat T cells were loaded with 5 μM Fluo-3AM. Cells were then incubated with appropriate antibodies (B6H12, P2W7 or 272-17E6) for 20 minutes prior to the addition of E3CaG1 (22 nM). *B.* E3CaG1 (2.2-220 nM) causes increases in intracellular calcium. *C.* Pretreatment of Jurkat T cells with pertussis toxin (500 ng/ml) has no effect on E3CaG1 or Ang II induced changes in intracellular calcium. The Ang II receptor functions through G_q protein, which is pertussis toxin insensitive.

Fig. S5. Compounds YC-1 and BAY 41-2272 do not overcome direct Ca^{2+} inhibition of immunoprecipitated sGC. sGC immunoprecipitated from transiently-transfected MCF-7 cells was treated with 0 μM or 100 μM $CaCl_2$ prior to the addition of 10 μM YC-1 or 10 μM BAY 41-2272. This was followed immediately by the addition of 10 μM DEA/NO. Reactions were carried out at 37 °C for 5 min and cGMP accumulation was measured. Error bars represent the standard deviation from the mean of independent experiments (n = 5), and * denotes $p < 0.001$.

Movie M1. Calcium imaging in Jurkat cells. Shown is the full movie of Fig 2A (10 min elapsed time). The arrows indicate typical cells during calcium transients. There are 40 cells in the field of view.

Fig. S1

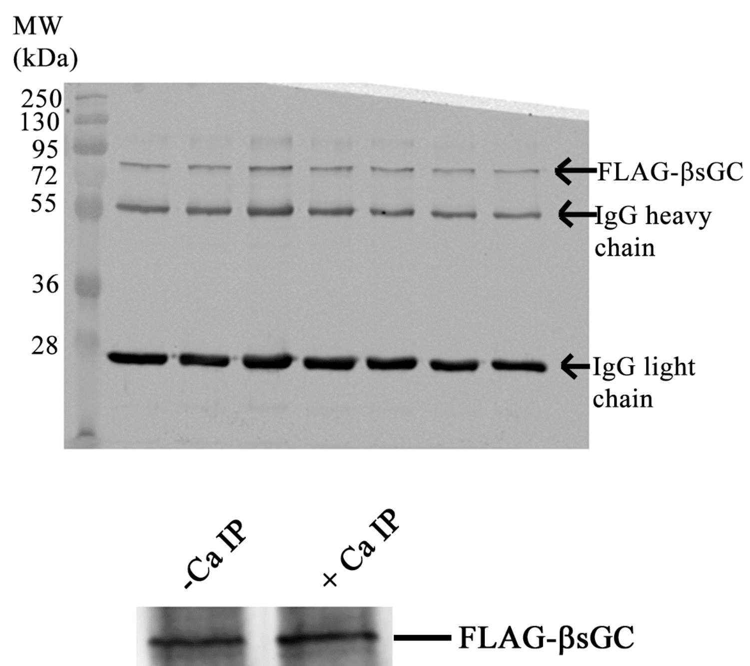

Fig. S2

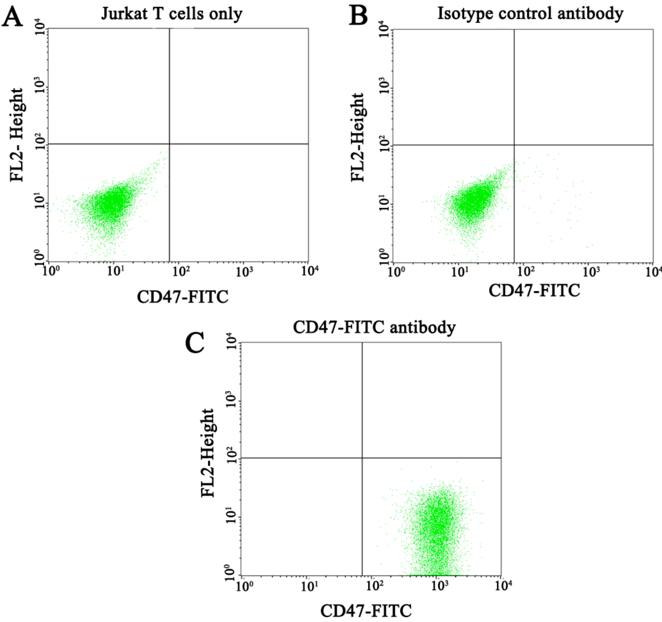

Fig. S3

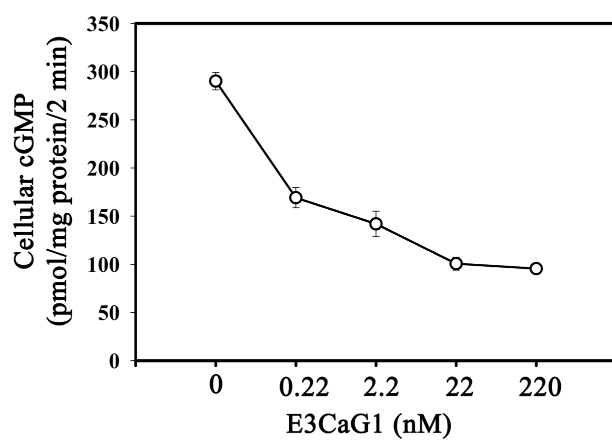

Fig. S4

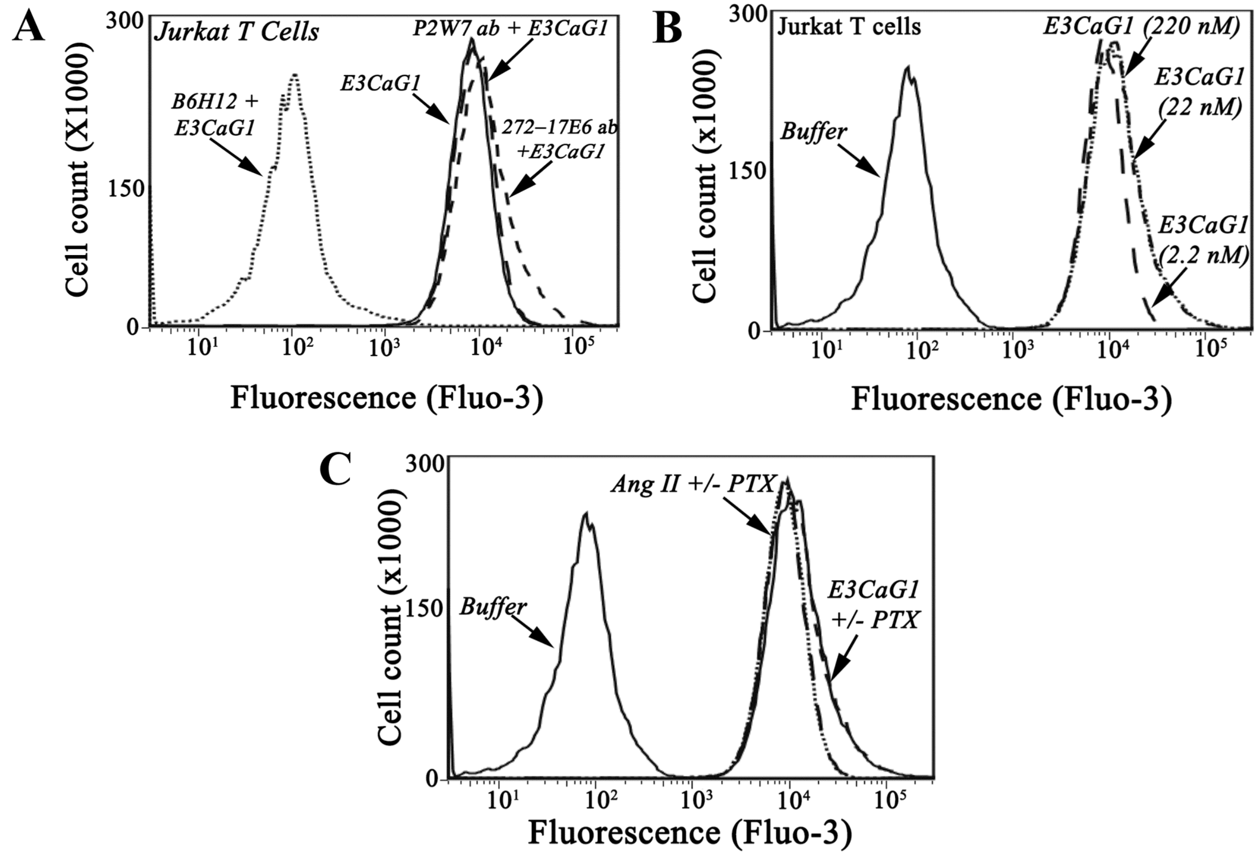

Fig. S5