Supplemental Text

Results

We co-administered NaHS (500 nM) and a range of concentrations of the K+ATP channel blocker glibenclamide while measuring TCR-driven proliferation (SI Fig. 1A). Unexpectedly, glibenclamide stimulated T cell proliferation in the absence of NaHS nearly 50% starting at the lowest dose (100 nM). Furthermore, Glibenclamide was unable to inhibit the stimulatory actions of NaHS at physiologically relevant concentrations[37] of 100 nM and 1 µM. Due to the lack of effect of glibenclamide on NaHS stimulated proliferation, we concluded that the K+ATP channel does not play a role in the T cell effects of H_2S.

Phosphodiesterases (PDEs) are another recently reported target of H_2S signaling[27]. H_2S was reported to inhibit the degradation of cGMP in smooth muscle cells at 10nM-50µM using semi-purified cGMP- and cAMP -specific isoforms. To address the effect of H_2S on PDE activity in T cells, we used an NO donor to increase cGMP levels in Jurkat T cells with and without NaHS present (SI Fig. 1B). After 4 hours of treatment, there was no difference in the levels of cGMP in the NO or NO-NaHS co-treated cells. We conclude that H_2S does not affect cGMP-sensitive PDEs and this is not the source of H_2S-driven responses in T cells.

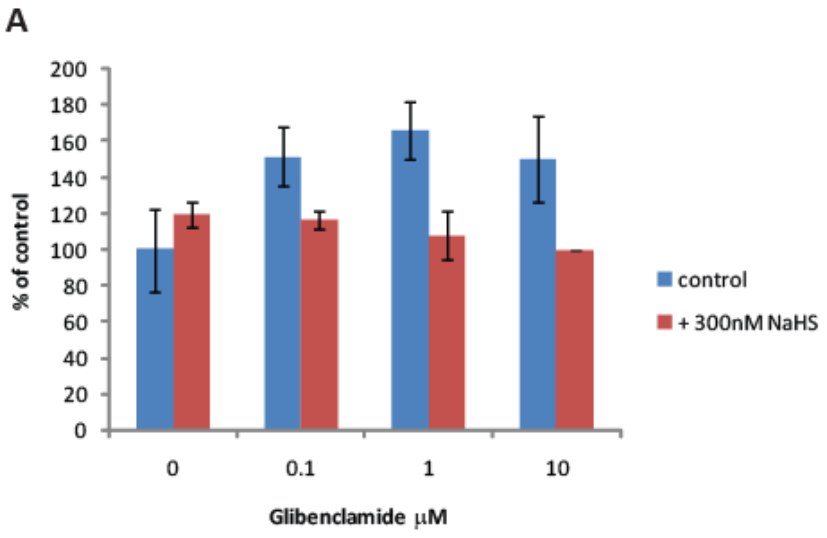
Next we investigated the source of the H_2S-dependent stimulation of IL-2 expression to assess whether IL-2 promoter binding transcription factors are a target of H_2S including the previously mentioned Akt MAPK target, NFκB. We transfected luciferase constructs of NFAT, NFκB, STAT5, CRE, and AP-1 into Jurkat T cells and measured their TCR-stimulated activities in the presence of H_2S (SI Fig. 1C). While there were no significant effects of H_2S on the transcription factor activities that were tested, NFκB showed a significant dose-dependent decrease in activity starting with 18% at 10uM NaHS. However, these concentrations are much higher than the effective concentrations cited earlier in this work, and while it may be interesting data for the pharmacological application of H_2S, we conclude that its effect on NFκB does not contribute to the mechanism of H_2S-dependent T cell proliferation.

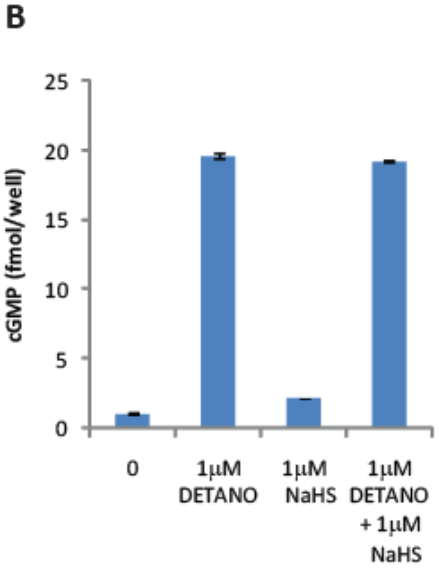
SI Fig. 1 (A) Mouse splenocytes were activated with plate-bound anti-CD3/CD28 antibodies in the presence of glibenclamide or vehicle and proliferation was assessed either in a 1% O_2 atmosphere via an MTS assay at 72 hours post activation. (B) Jurkat T cells (500,000 cells) were stimulated with DETANO (1μM) or DETANO with NaHS (300nM) for 4 hours in a 1% O_2 atmosphere, after which cGMP was measured. (C) Jurkat T cells were transfected with a luciferase reporter construct bearing the IL-2 promoter binding elements for NFκB as well as a constitutively expressed Renilla luciferase reporter construct. Cells were activated by plate bound anti-CD3 and anti CD28 antibodies in the presence of NaHS in a 1% O_2 atmosphere for 6 hours, after which the ratio of NFκB to Renilla luciferase activity was measured. Data are normalized to non-activated control for each treatment, n=3, error bars indicate standard deviation, * denotes $P < 0.05$.

SI Fig. 2 (A) Mouse CD3+ cells were labeled with CFSE and activated with plate-bound anti-CD3/CD28 antibodies in the presence of 100 nM Na_2S or vehicle and proliferation was assessed in a 1% O_2 atmosphere by flow cytometry at 72 hours post activation. (A) shows proliferation of CD4+ and CD8+ populations. (B) shows proliferation analysis of gated CD8+ cells.

Supplemetary Information

Figure 1





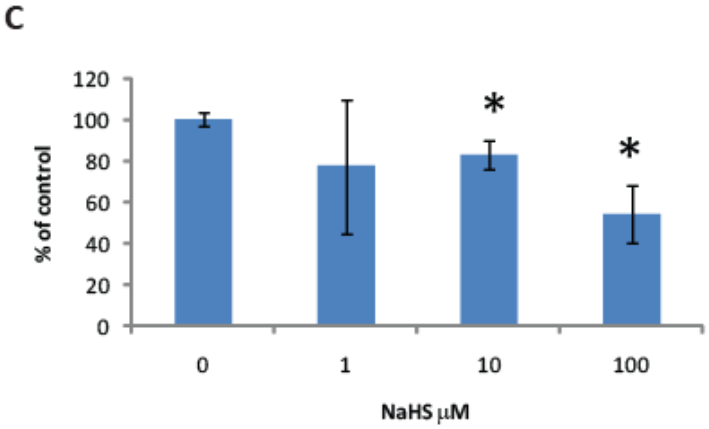
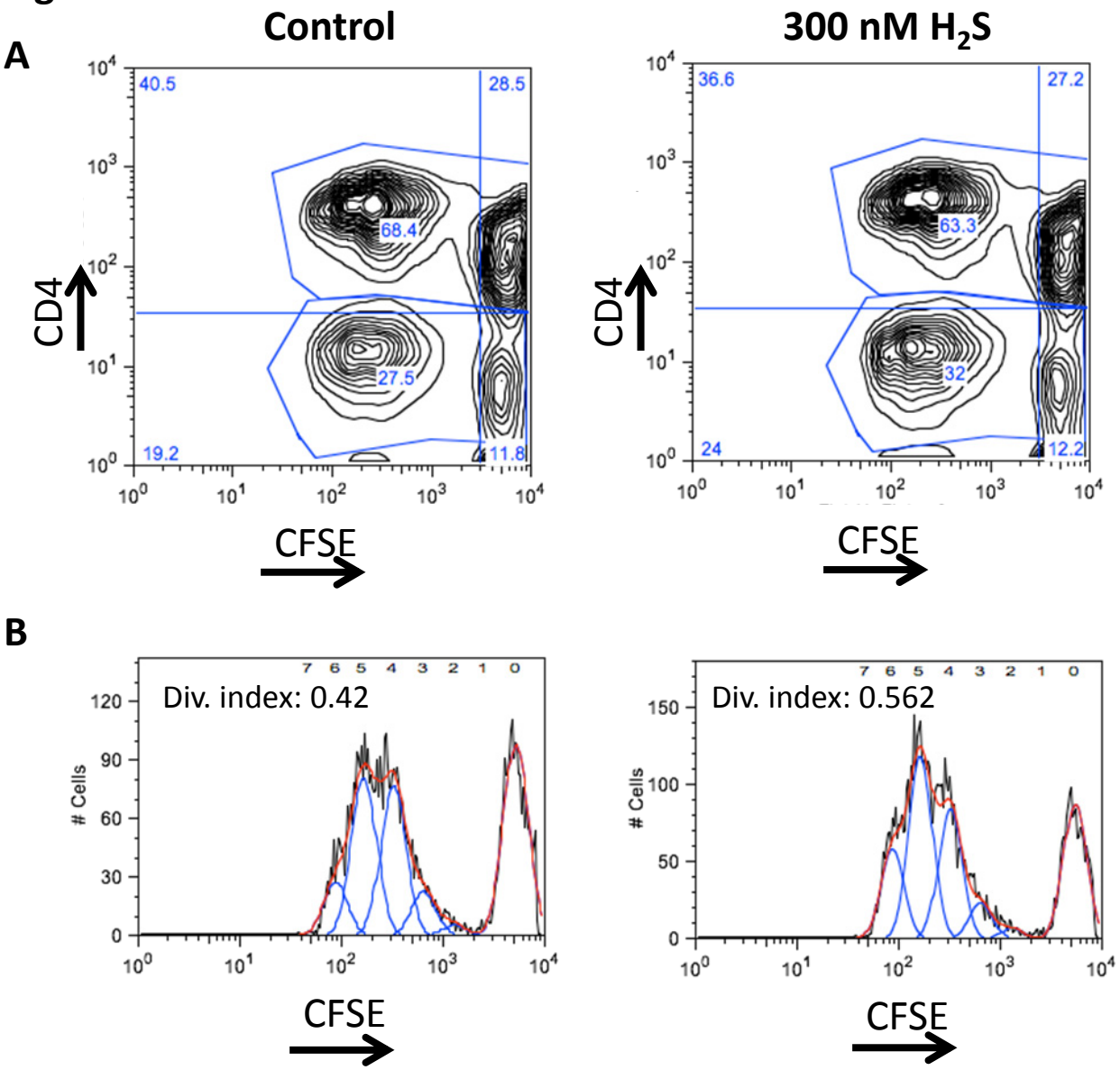


Figure 2


A
Control
300 nM H_2S
CD4
CFSE
40.5
28.5
68.4
27.5
19.2
11.8
36.6
27.2
63.3
32
24
12.2
B
Div. index: 0.42
Div. index: 0.562
Cells
7 6 5 4 3 2 1 0