

Supplemental Figure 1. S-CM inhibition of CD4⁺ T-cell responses. (A) PHA-stimulated T-cells cultured in media alone or the presence of a representative normal or Crohn's S-CM were analyzed on day 4 for proliferation by flow cytometry for CFSE dilution. The percent of cells that proliferated is indicated in the top left corner of the plots. (B) Percent inhibition of proliferation for PHA-stimulated T-cells cultured in normal S-CM ($n=7$) or Crohn's S-CM ($n=6$). (C) IFN- γ release by PHA-stimulated T-cells cultured for 4 days in the presence of media, normal S-CM ($n=2$) or Crohn's S-CM ($n=2$). (D) Western blot analysis of T-bet protein expression in PHA-stimulated T-cells cultured 24 hr in the presence of a representative normal S-CM or Crohn's S-CM ($n=2$ each). For B and C, error bars indicate the standard error of the mean. * $p \leq 0.05$, ** $p \leq 0.01$.

Supplemental Figure 2. Stromal factors inhibit pre-activated T-cell proliferation and IFN- γ release. T-cells were cultured in media plus IL-2 \pm CD3/CD28

or PHA. After 3 days, the cells were harvested, CFSE stained and re-cultured for an additional 4 days with IL-2 in media, normal S-CM, or Crohn's S-CM. **(A)** Representative T-cell proliferation for days 3-7. The percent of cells that proliferated is indicated in the top left corner of the plots. **(B)** Percent inhibition of T-cell proliferation induced by normal or Crohn's S-CM compared to media alone (mean \pm SEM; $n=3$). **(C)** Average percent inhibition of T-cell IFN- γ release by normal or Crohn's S-CM compared to media (mean \pm SEM; $n=3$).

Supplemental Figure 3. Stromal factors inhibit CD3/CD28 reactivated T-cell proliferation and IFN- γ release. T-cells were cultured in media plus IL-2 \pm CD3/CD28 or PHA. After 3 days, the cells were harvested, CFSE stained and re-cultured for an additional 4 days with CD3 restimulation in the presence of media, normal S-CM, or Crohn's S-CM. **(A)** Representative T-cell proliferation for days 3-7. The percent of cells that proliferated is indicated in the top left corner of the plots. **(B)** Percent inhibition of T-cell proliferation induced by normal or Crohn's S-CM compared to media alone (mean \pm SEM; $n=3$). **(C)** Average percent inhibition of T-cell IFN- γ release by normal or Crohn's S-CM compared to media (mean \pm SEM; $n=3$).

Supplemental Figure 4. Neutralization of TGF- β reverses normal S-CM-mediated down-regulation of CD4⁺ T-cell responses and TGF- β down-regulates CD4⁺ T-cell responses. **(A)** Proliferation of PHA-stimulated T-cells cultured for 4 days in the presence of media or a representative normal S-CM (250 μ g/mL) \pm anti-TGF- β

neutralizing antibody ($n=2$). **(B)** IFN- γ release by PHA-stimulated T-cells cultured for 4 days in the presence of media or normal S-CM \pm anti-TGF- β antibody ($n=2$). **(C)** Proliferation of PHA-stimulated T-cells cultured in the presence of rhTGF- β alone for 4 days in a representative experiment ($n=2$). **(D)** PHA-stimulated IFN- γ release by T-cells cultured in the presence rhTGF- β $n=2$). Error bars indicate standard error of the mean.

Supplemental Figure 5. Irrelevant control antibodies and neutralization of cytokines have no effect on T-cell proliferation and IFN- γ release. **(A, B, C)** T-cell proliferation and IFN- γ release were not affected by treatment with media pre-incubated with α -TGF- β or irrelevant isotype control ($n=3$). **(D, E, F)** T-cell proliferation and IFN- γ release also were not affected by treatment with media pre-incubated with α -IL-6, α -IL-1 β , or α -IL-6 plus α -IL-1 β antibodies ($n=3$).