Supporting Information

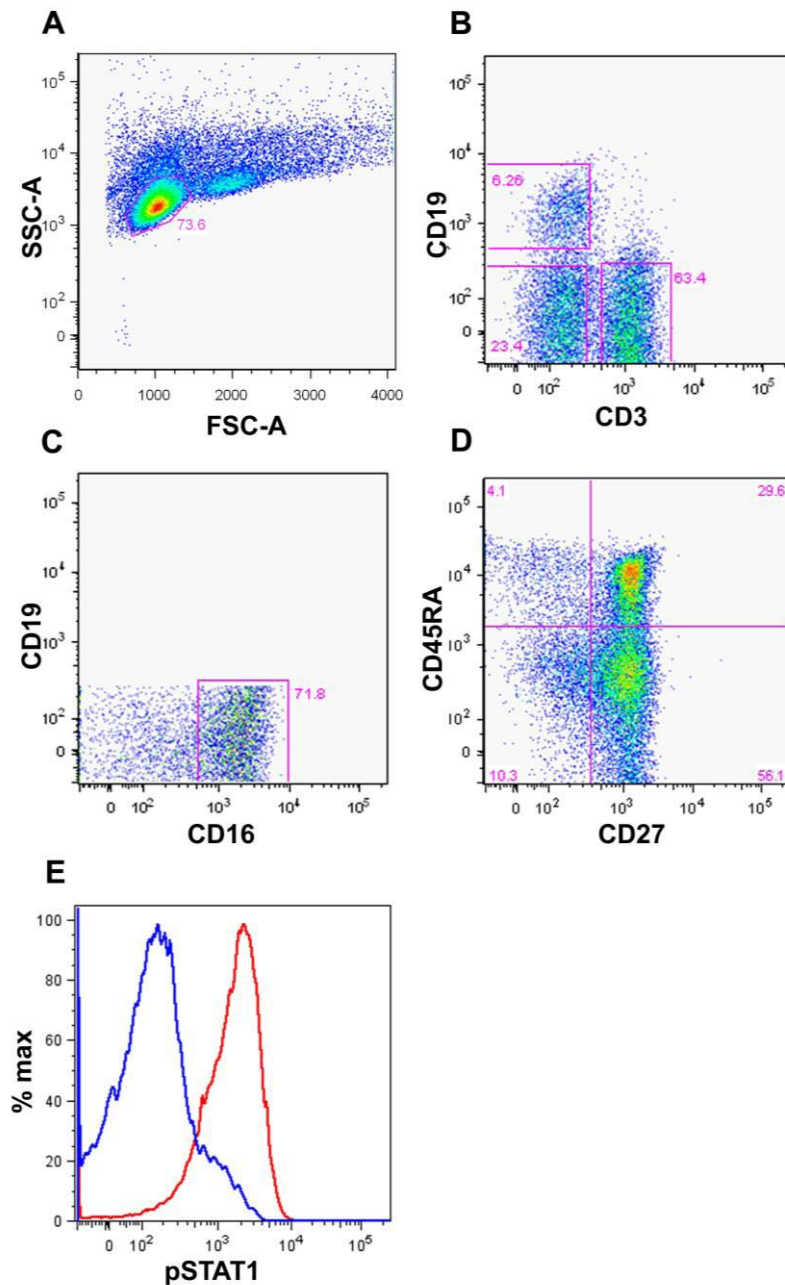## Critchley-Thorne et al. 10.1073/pnas.0901329106

Fig. S1. Gating of lymphocyte subsets and pSTAT1 measurement for Phosflow analysis. Gates were set on the lymphocytes on a FSC-A versus SSC-A plot (*A*). Within the lymphocyte gate, a further gate was set on the CD19+ CD3– cells to identify B cells, on the CD3+ CD19– cells to identify T cells, and on the CD3– CD19– cells (*B*). Within the CD3– CD19– gate, a further gate was set on the CD16+ cells to identify NK cells (*C*), as indicated in the plots. Within the T-cell gate, further gates were set on CD27+ CD45RA+ cells to identify naive T cells, CD27+ CD45RA– cells to identify memory T cells, and CD45RA+ CD27– cells to identify effector T cells (*D*), as indicated in the plots. Phosphorylated-STAT1 levels were measured in IFN-stimulated cells (*E*, *red line*) versus unstimulated cells (*E*, *blue line*).
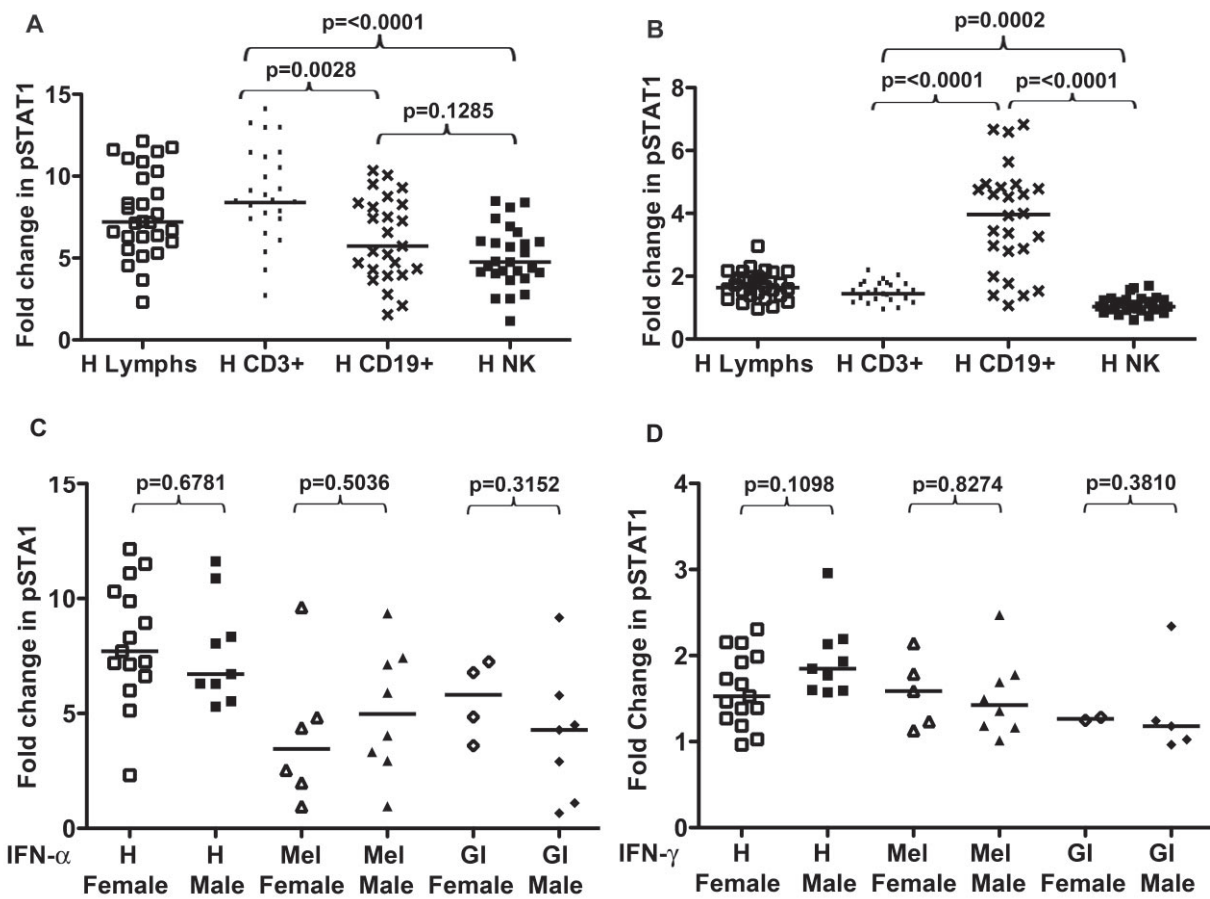
Fig. S2. Comparison of IFN-stimulated fold-change in pSTAT1 between lymphocyte subsets and in females versus males. PBMCs from healthy donors were stimulated with IFN-α or IFN-γ, or left unstimulated and pSTAT1 was measured in total healthy lymphocytes (open squares), T cells ('), B cells (x), and NK cells (filled squares) by Phosflow (*A* and *B*). The groups of healthy controls (H), melanoma patients (Mel), and GI cancer patients (GI) were divided into female and male (*C* and *D*) to determine whether the fold-change in pSTAT1 in response to IFN-α or IFN-γ was associated with gender. (*A* and *C*) IFN-α-stimulated cells; (*B* and *D*) IFN-γ-stimulated cells. The median is indicated by the bar in each data set. Two-sided Mann-Whitney tests were performed to determine whether there was a significant difference between fold-changes in IFN-induced pSTAT1 between lymphocyte populations and between females versus males. *P*-values from the comparisons are indicated on the plots. Because there was no significant difference in the IFN-stimulated pSTAT1 levels between males and females in the healthy controls or cancer patients, the breast cancer patients (all female) were compared to the mixed-gender group of healthy controls.

Fig. S3. IFN-α- and IFN-γ-stimulated fold-change in pSTAT1 in PBMC subsets from stage III breast cancer patients treated/untreated with neoadjuvant chemotherapy and healthy controls. PBMCs were stimulated with IFN-α, IFN-γ, or unstimulated and pSTAT1 was measured by Phosflow in healthy controls (H open squares), patients with stage III breast cancer that had not received systemic therapy (BC None filled squares) and patients with stage III breast cancer who had received neoadjuvant chemotherapy (BC Neoadj open circles). (*A*) IFN-α-stimulated T cells, (*B*) IFN-α-stimulated B cells, (*C*) IFN-α-stimulated NK cells, (*D*) IFN-γ-stimulated T cells, (*E*) IFN-γ-stimulated B cells, (*F*) IFN-γ-stimulated NK cells. The median is indicated by the bar in each data set. There were no significant differences between stage III breast cancer patients not treated or treated with neoadjuvant therapy (*P*-values for each comparison >0.1) (see Table S2).

Other Supporting Information Files