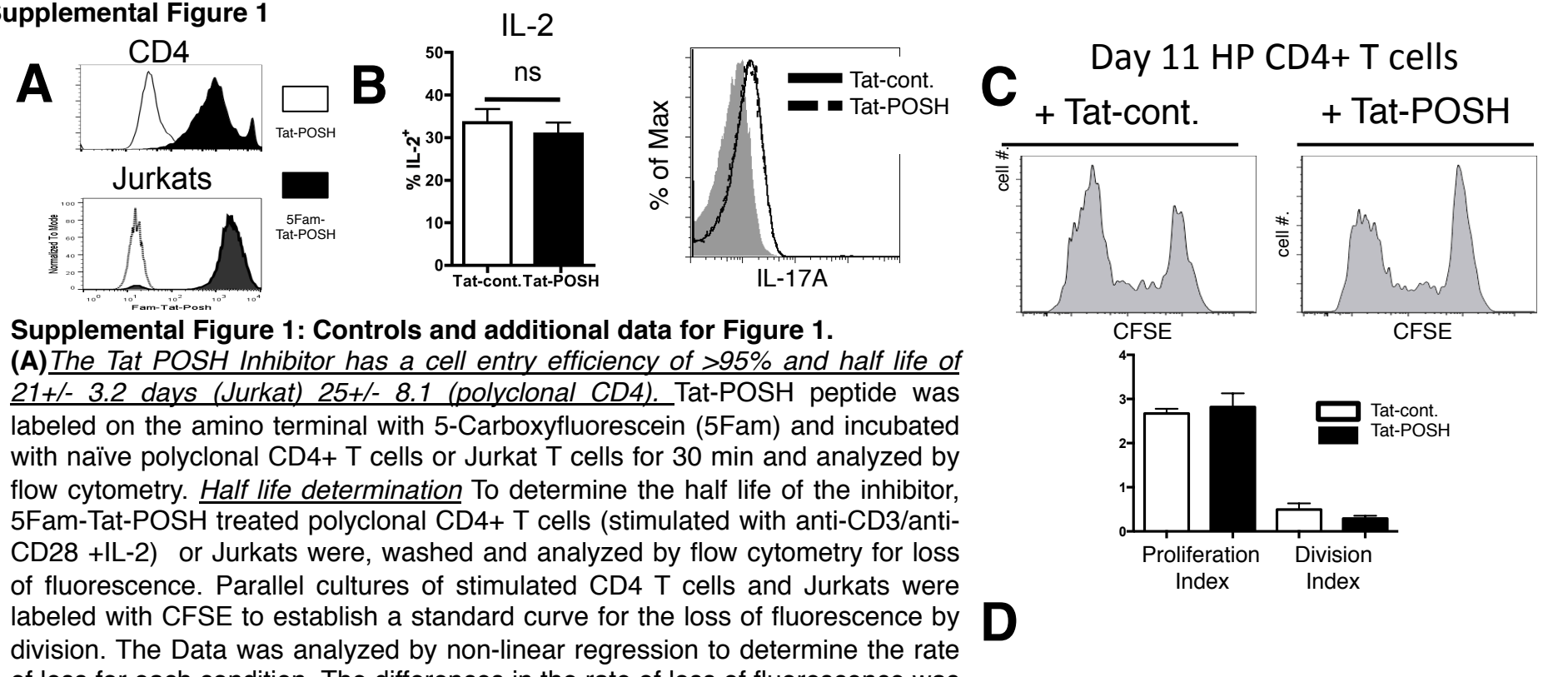
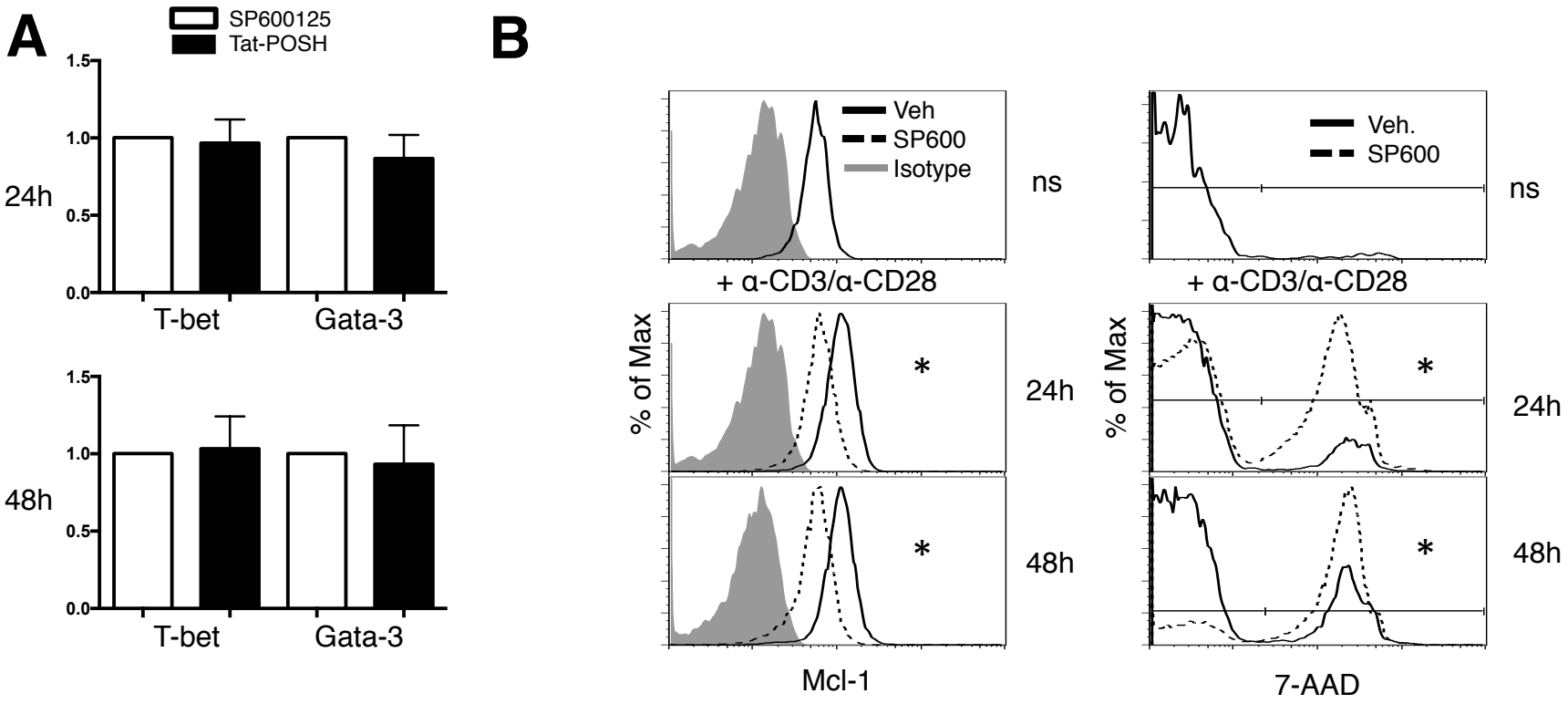
Supplemental Figure 1



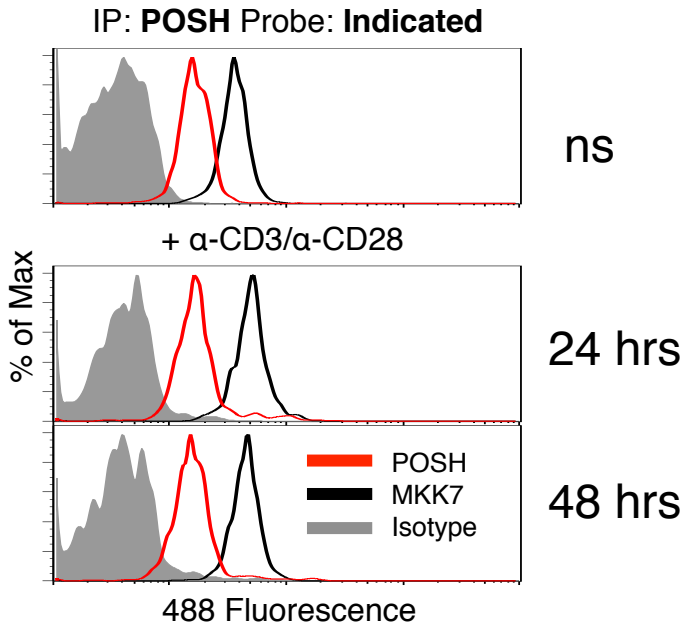
Supplemental Figure 1: Controls and additional data for Figure 1.
(A) *The Tat POSH Inhibitor has a cell entry efficiency of >95% and half life of 21+/- 3.2 days (Jurkat) 25+/- 8.1 (polyclonal CD4).* Tat-POSH peptide was labeled on the amino terminal with 5-Carboxyfluorescein (5Fam) and incubated with naïve polyclonal CD4+ T cells or Jurkat T cells for 30 min and analyzed by flow cytometry. *Half life determination* To determine the half life of the inhibitor, 5Fam-Tat-POSH treated polyclonal CD4+ T cells (stimulated with anti-CD3/anti-CD28 +IL-2) or Jurkats were, washed and analyzed by flow cytometry for loss of fluorescence. Parallel cultures of stimulated CD4 T cells and Jurkats were labeled with CFSE to establish a standard curve for the loss of fluorescence by division. The Data was analyzed by non-linear regression to determine the rate of loss for each condition. The differences in the rate of loss of fluorescence was used to calculate the half life of Tat-POSH. **(B)** *The POSH/JNK axis does not regulate IL-2 and IL-17 production in CD4+ T cells.* Naïve CD4+ T cells were stimulated in the continuous presence of Tat-cont. or Tat-POSH for 4 days. Cells were washed and re-stimulated with PMA/ionomycin plus Brefeldin A for 5 hours. IL-2 **(left)** and IL-17 **(right)** were measured by ICCS. Grey histogram is isotype control. **(C-D)** *In vivo proliferation of Tat-POSH treated CD4+ T cells are similar to control treated cells.* CD4+ T cells were purified incubated for 30 minutes in the presence Tat-POSH or Tat-cont. T cells were either unstimulated **(C)** or stimulation with CD3/CD28. 1x 10^6 cells / condition were injected into congenically marked hosts and harvested at the times indicated. Graphs **(B, C)** show mean plus and minus SD and facs plot **(A-D)** are representative for n>3 independent experiments. Only the differences shown in **(A)** are significant as analyzed by Prism6 by Graph pad. **(C)** Proliferation and Division index were calculated using FlowJo9.9, Treestar.

Supplemental Figure 2

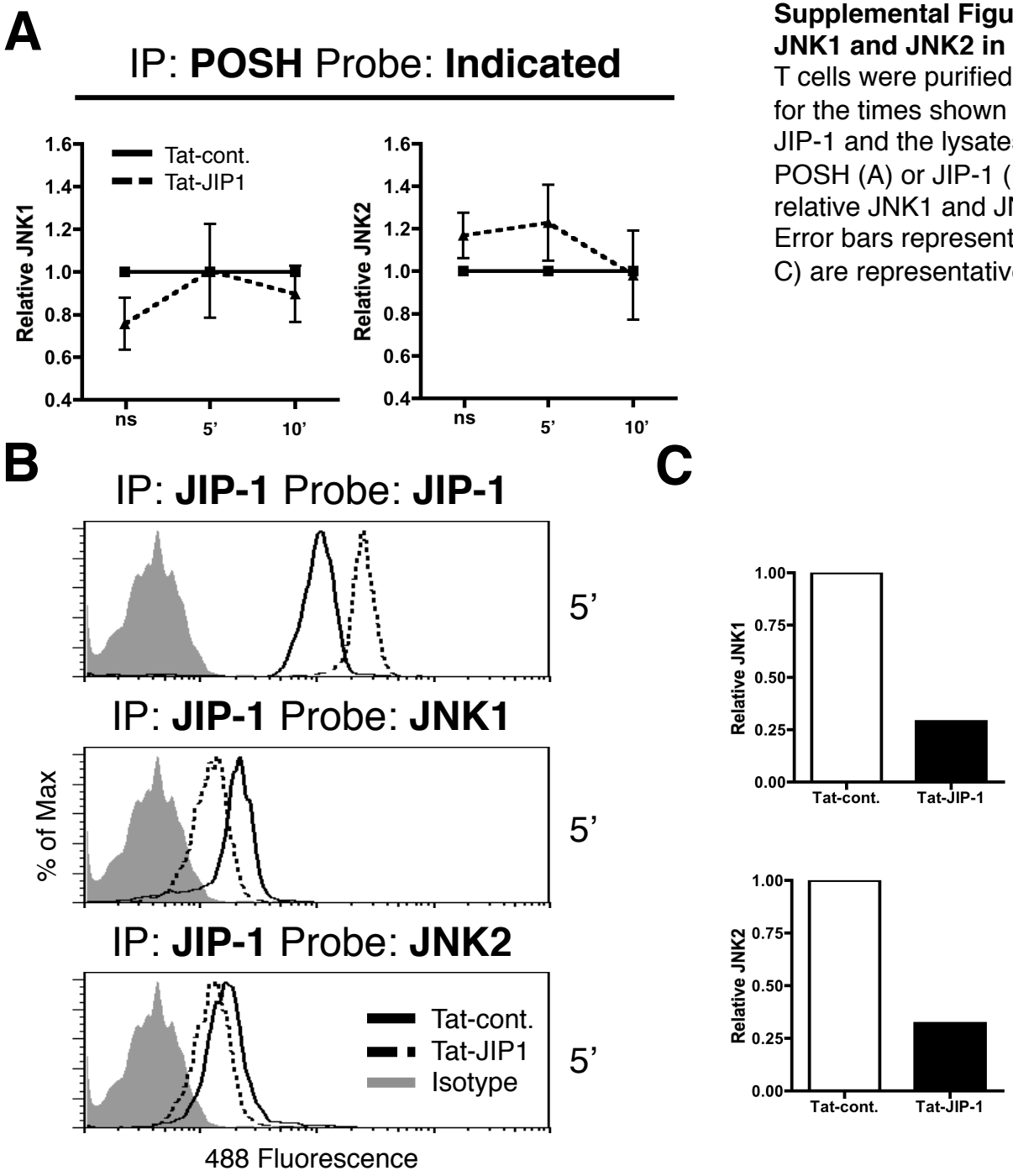


Supplemental Figure 2: Tat-POSH data replicated JNK kinase inhibitor controls. Naïve CD4+ T cells were stimulated with anti-CD3 and CD28 for the times shown in the presence of **(A)** SP600125 or Tat-POSH and **(B)** SP600125 or vehicle only control (Veh). **(A)** *Inhibition of T-bet and Gata-3 with Tat-POSH or the kinase activity of JNK* . Cells were stained by IC staining for T-bet or Gata-3 at the time shown. Data is normalized to inhibition by SP600125. Negative controls were used as described in Figure 3. Graph represents normalized data with +/- SD, n=3. **(B)** *Inhibition of the kinase activity of JNK leads to decreased induction of Mcl-1 and increased apoptosis.* Mcl-1 expression **(left)** was assessed by ICCS. **(right)** Apoptosis was determined by the percentage of cells positive for 7-AAD. All data is representative for n>3 independent experiments. * = $p < 0.05$

Supplemental Figure 3 The POSH complex contains MKK7



Supplemental Figure 3: The POSH complex contains MKK7.
CD4+ T cells were purified and stimulated with α-CD3 and α-CD28 for the times shown and the lysates were subjected to IP-FCM with α-POSH coated CML beads. POSH (red) represents loading control, isotype is shown as negative staining control. The histograms are representative for n>4 independent experiments.



Supplemental Figure 4. Tat-JIP un-couples JIP-1 from JNK1 and JNK2 in CD4⁺ T cells but not from POSH. CD4+ T cells were purified and stimulated with α-CD3 and α-CD28 for the times shown in the presence of Tat-control or Tat-JIP-1 and the lysates were subjected to IP-FCM with anti-POSH (A) or JIP-1 (B, C) coated CML beads. Graph depict relative JNK1 and JNK2 levels normalized to Tat-control. Error bars represent SD n=3 independent experiments. (B, C) are representative of n>3 independent experiments.