Online Figure Legends

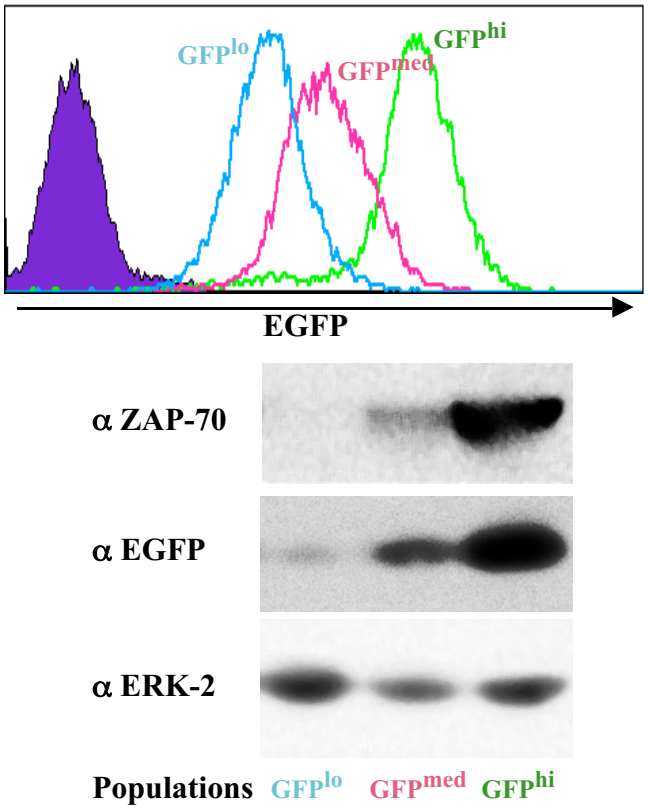
Online Figure 1. **Coordinated expression of ZAP-70 and eGFP in T cells transduced with the pT-ZAP lentiviral vector**. ZAP-70-deficient Jurkat T cells (clone p116) were transduced with the pT-ZAP vector. (A) Following transduction, cells were sorted on the basis of EGFP expression, resulting in the maintenance of populations with distinct EGFP levels. These populations are designated as GFP^{lo}, GFP^{med}, and GFP^{hi}, and their relative fluorescence is shown. The basal fluorescence of untransduced cells is shown in the filled histogram. (B) ZAP-70 and eGFP levels were monitored by western blotting of whole cell lysates from the three populations with monoclonal α-ZAP-70 and polyclonal α-EGFP antibodies, respectively. Protein loading was verified by immunoblotting with an α-Erk-2 mAb.

Online Figure 2. **Thymocyte and splenocyte profiles in ZAP-$70^{-/-}$ mice following in situ injection of an EGFP-expressing lentiviral vector.** ZAP-$70^{-/-}$ mice (14-17 days of age) were intrathymically injected with an eGFP-expressing lentiviral vector ($2x10^7$ TU in a total volume of 20 μls). Thymi and spleens were harvested from euthanized animals 14 weeks later. The percentages of double negative, double positive and CD4+ and CD8+ single positive thymocytes in 1 of 3 mice are shown. The percentages of CD3+ T cells and CD19+ B cells in spleens isolated from 1 of 3 mice are shown.

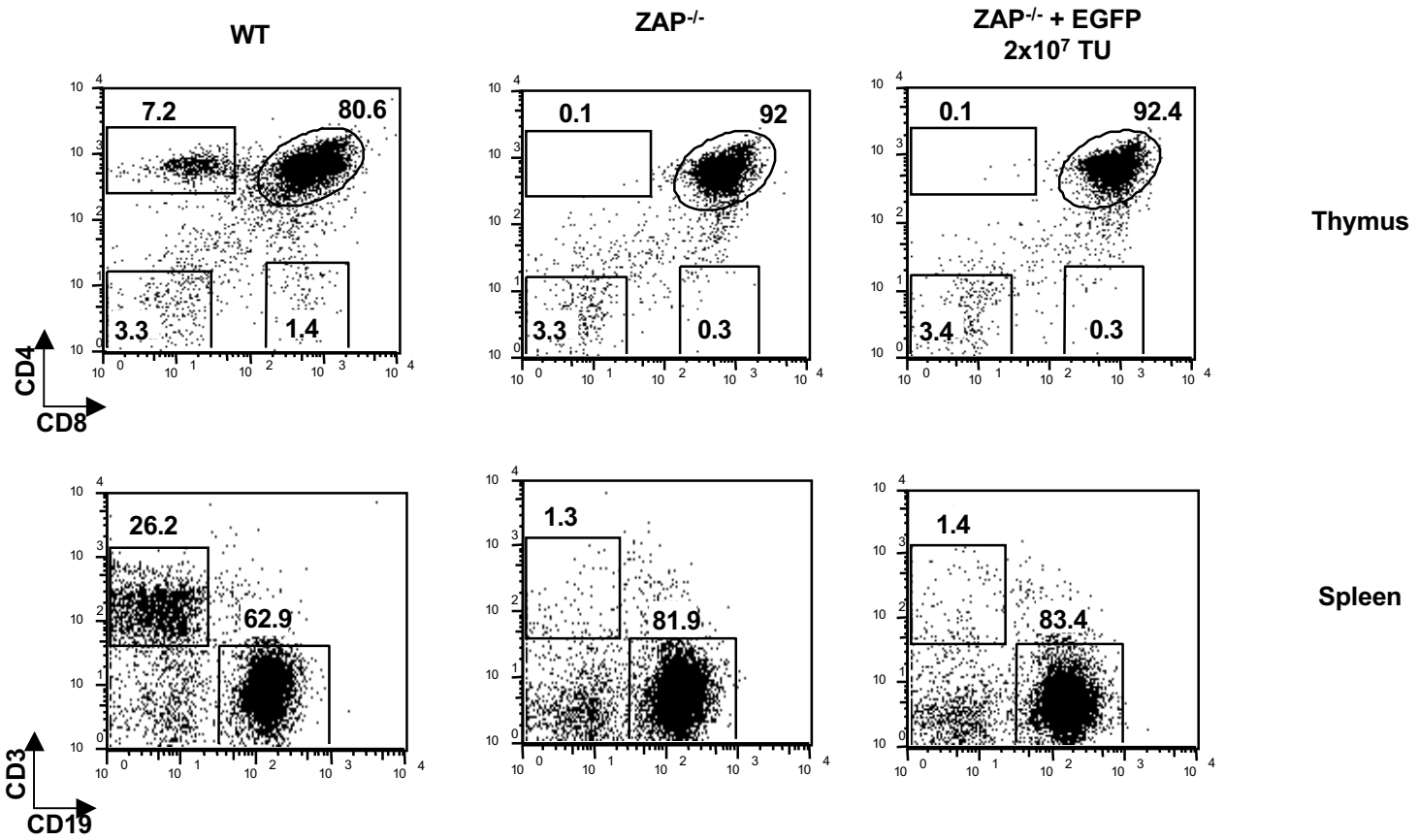
Online Figure 3. **Thymocyte profiles in ZAP-$70^{-/-}$ mice 72 hours post in situ injection of EGFP- and ZAP-70-EGFP expressing lentiviral vectors.** Virions harboring a ZAP-70-expressing lentiviral vector (pT-ZAP) or a control EGFP-expressing lentiviral vector were injected intrathymically into ZAP-$70^{-/-}$ mice and thymocytes were harvested from euthanized animals 3 days later. The percentages of double negative, double positive and CD4+ and CD8+ single positive thymocytes in WT (C57/Bl6) and ZAP-$70^{-/-}$ mice are indicated in the top left dot plots. Representative thymi from 1 of 3 animals injected with each type of virions ($1x10^7$ transducing units, in a total volume of 20 μls) are shown 3 days following injection. The percentages of cells in each thymocyte population are noted. The percentages of eGFP+ cells within each of these subsets were assessed and are presented as SSC/EGFP dot plots.

Online Figure 4. **Thymocyte profile of a ZAP-70$^{-/-}$ mouse 1 year following in situ injection of a ZAP-70-EGFP expressing lentiviral vector.** A ZAP-70$^{-/-}$ mouse, IT-injected with pT-ZAP lentiviral virions, was maintained in pathogen-free conditions, for one year post-treatment. The animal was then euthanized and the distribution of thymocyte populations and eGFP+ cells was analyzed. The percentages of eGFP+ cells within each thymocyte population are indicated and conversely, the relative distribution of thymocyte populations within the eGFP- and eGFP+ subsets are shown.

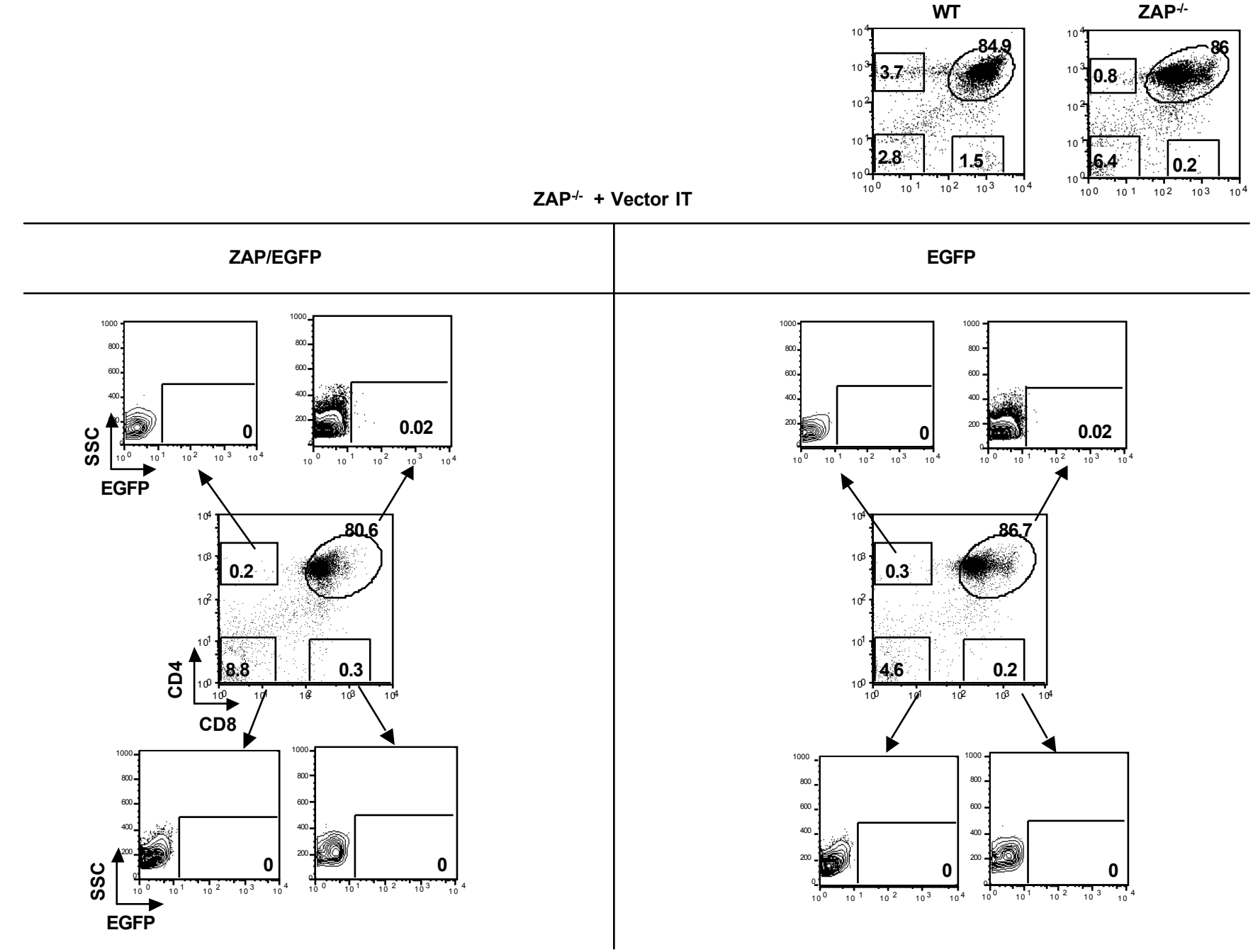
Online figure 1



Online figure 2



Online figure 3



Online figure 4