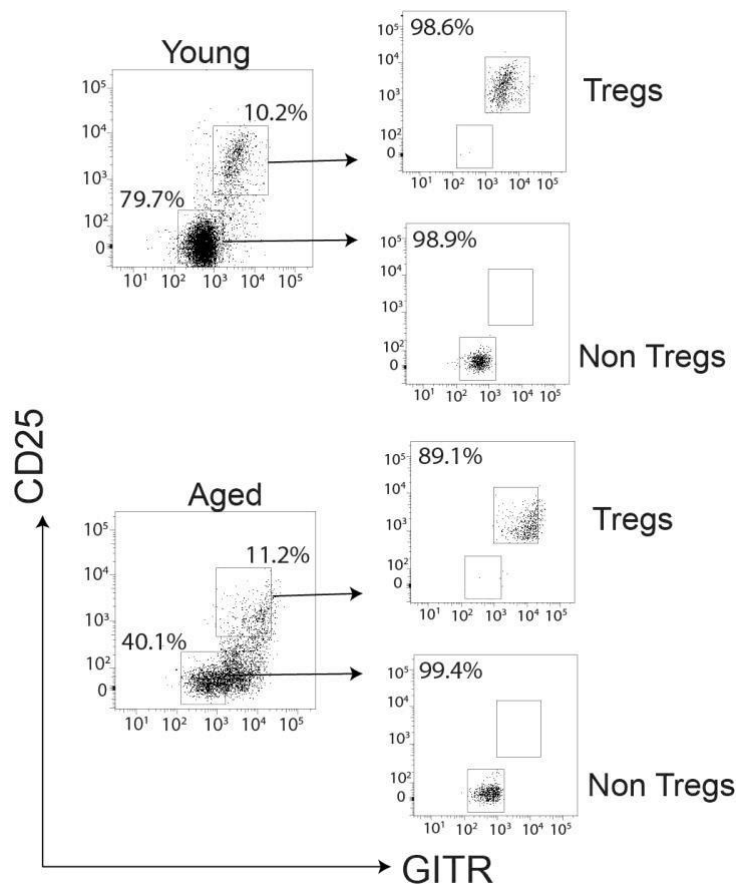
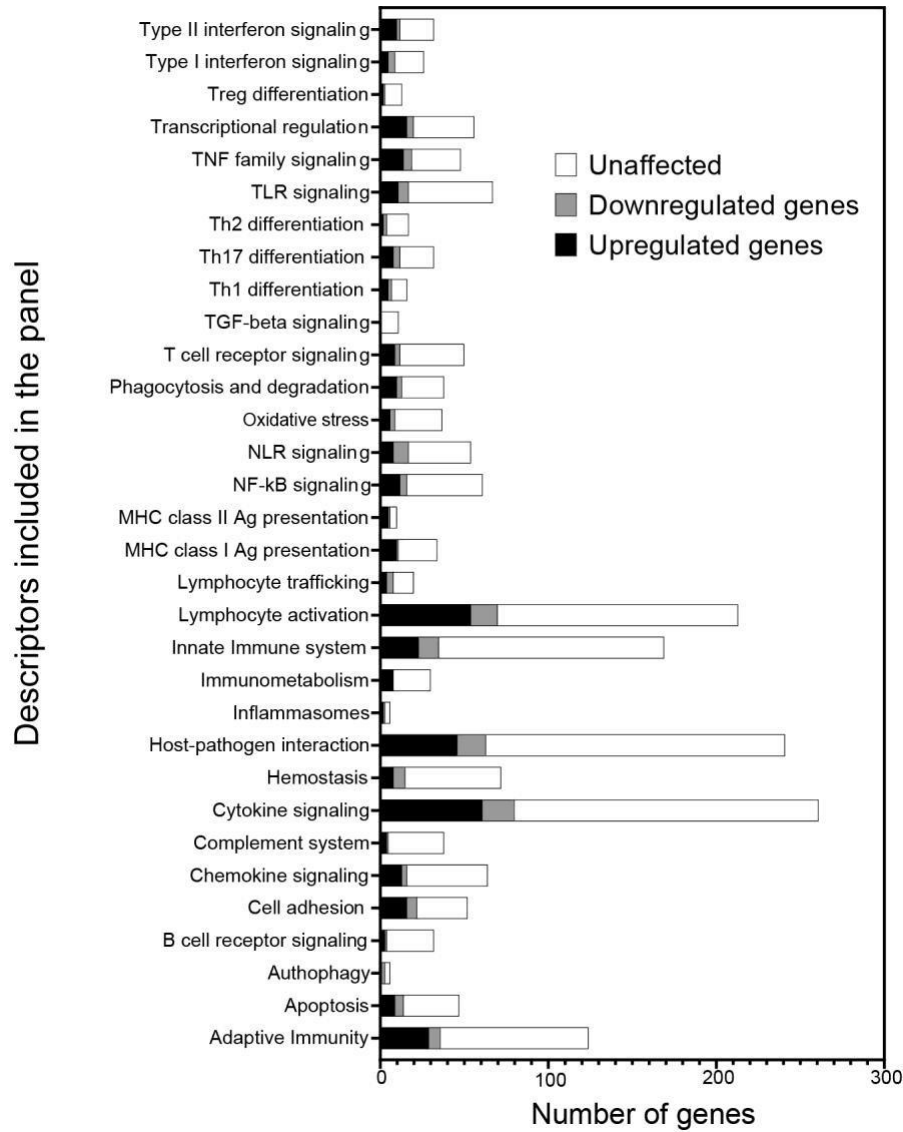


Supplemental Figure 1.



Supplementary Figure 1. Representative dot plots showing T regulatory cell sorting strategy. Single cell suspensions of a mixture of draining cervical lymph nodes (CLN) and spleen of young (2-3 months) and aged mice (22-24 months) (n=5-7 biological replicates/group; each replicate include the CLN and spleens from three mice) were obtained and fluorescently labelled with the corresponding antibodies. The following gating strategy was used: cells were identified by forward scatter area versus side scatter area; doublets were discriminated by forward scatter height versus forward scatter area (Singlets 1) followed by side scatter height versus side scatter area (Singlets 2); thereafter dead cells were excluded with DAPI and CD4⁺ cells were gated and then either T regulatory (Tregs, CD4⁺CD25⁺GITR⁺) and non-Tregs, CD4⁺CD25^{neg}GITR^{neg}) were selected for sorting. Frequency of cells is shown as percentage of CD4⁺ cells. The numbers above the squares indicate the purity of the sorted population before and after the cell sorting (Left or right panels, respectively). Neg = negative.

Supplemental Figure 2.



Supplemental Figure 2. Number of immunity-related genes modified in the mice lymphoid tissues by aging. Tregs from a mixture of mice CLN and spleen from either young or aged mice were sorted and then subjected to NanoString® analysis. The bars indicated the number of genes either up, down regulated or unmodified in the aged tissues from those included in the panel, as compared with young tissues.