

## Supplemental Table Legends

**Supplementary Table 1** Expressed genes common to iT<sub>reg</sub> (n=5 independent replicates) and Freshly isolated nT<sub>reg</sub> (n=4) cell preparations as compared to CD4<sup>+</sup>EGFP<sup>-</sup> T<sub>conv</sub> cells (n=3) of *Foxp3*<sup>EGFP</sup> mice. Common genes were selected at 2 fold change cut-off. Results represent means  $\pm$  S.E.M., with each replicate derived from pooled cells from several mice (4-10). Probe comparisons were analyzed by the Student unpaired two-tailed T test with p values <0.05 considered significant.

**Supplementary Table 2** Expressed genes common to iT<sub>reg</sub> (n=5 independent replicates) and nT<sub>reg</sub> cells that have been activated *in vitro* with anti-CD3 mAb + IL-2 (A-nT<sub>reg</sub> cells; n=3) as compared to in vitro activated CD4<sup>+</sup>EGFP<sup>-</sup> T<sub>conv</sub> (A-T<sub>conv</sub>) cells, all originally derived from *Foxp3*<sup>EGFP</sup> mice (n=4). Common genes were selected at 2 fold change cut-off. Results represent means of independent replicates (n=5 for iT<sub>reg</sub> and 4 for nT<sub>reg</sub>)  $\pm$  S.E.M., with p<0.05 by Student unpaired two tailed T test.

**Supplementary Table 3** Differential gene expression between iT<sub>reg</sub> and freshly isolated nT<sub>reg</sub> cell preparations (n=5 and 4, respectively). Results were analyzed at a 2 fold change cut off, and represent means ( $\pm$  S.E.M) that scored at p<0.05 by Student's unpaired two-tailed T test.

**Supplementary Table 4** Differential gene expression between iT<sub>reg</sub> and activated nT<sub>reg</sub> cell preparations (n=5 and 3, respectively). Results were analyzed at a 2 fold change cut off, and represent means ( $\pm$  S.E.M) that scored at p<0.05 by Student's unpaired two-tailed T test.

**Supplementary Table 5** Differential gene expression between successfully derived iT<sub>reg</sub> cells and CD4<sup>+</sup>EGFP<sup>-</sup> cells from the same cultures that failed to convert into iT<sub>reg</sub> cells following anti-CD3 mAb+TGF- $\beta$  treatment (n=5 and 3, respectively). Results were analyzed at a 2 fold change

cut off, and represent means ( $\pm$  S.E.M) that scored at  $p < 0.05$  by Student's unpaired two-tailed T test.

**Supplementary Table 6** Differential gene expression between  $iT_{reg}$  and  $\Delta iT_{reg}$  cell preparations (n=5 and 3, respectively). Results were analyzed at a 2 fold change cut off, and represent means ( $\pm$  S.E.M) that scored at  $p < 0.05$  by Student's unpaired two-tailed T test. 2 genes were found selectively enriched in each population: *Ddx3y* and *Jarid1d* in  $iT_{reg}$  cells and *2310047K21Rik* and *Foxp3* in  $\Delta iT_{reg}$  cells. Both *Ddx3y* and *Jarid1d* are found on the Y chromosome. Their enrichment in  $iT_{reg}$  cells reflects a sex bias in the origin of the analyzed samples in that a majority of the  $iT_{reg}$  cell populations (3/5) were from *Foxp3<sup>EGFP</sup>* males (3/5) whereas all the  $\Delta iT_{reg}$  cells were from heterozygous *Foxp3<sup>ΔEGFP+/+</sup>* females. The latter choice was necessitated by the need to avoid the problem of intense T cell activation at baseline associated with sick *Foxp3<sup>ΔEGFP</sup>* males, which interferes with the derivation of  $\Delta iT_{reg}$  cells. The identification of *Foxp3* as the more enriched of the 2 genes found overexpressed in  $\Delta iT_{reg}$  cells reflects a technical anomaly, namely that the gene array probe for *Foxp3* lies distal to the poly-A start site introduced as part of the bicistronic EGFP cassette in the *Foxp3<sup>EGFP</sup>* allele. This results in a false negative signal for *Foxp3* in *Foxp3<sup>EGFP</sup>* T cells.

**Supplementary Table 7** Differential gene expression between  $iT_{reg}$  and  $\Delta iT_{reg}$  cells that have been rested in culture for 7 days with IL-2 (n=5 and 3, respectively). Results were analyzed at a 2 fold change cut off, and represent means ( $\pm$  S.E.M) that scored at  $p < 0.05$  by Student's unpaired two-tailed T test.