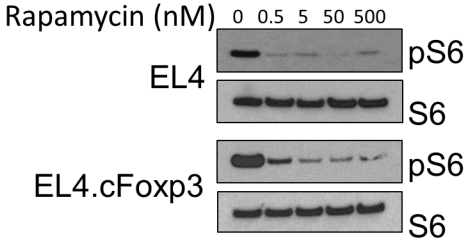
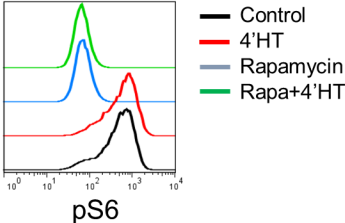


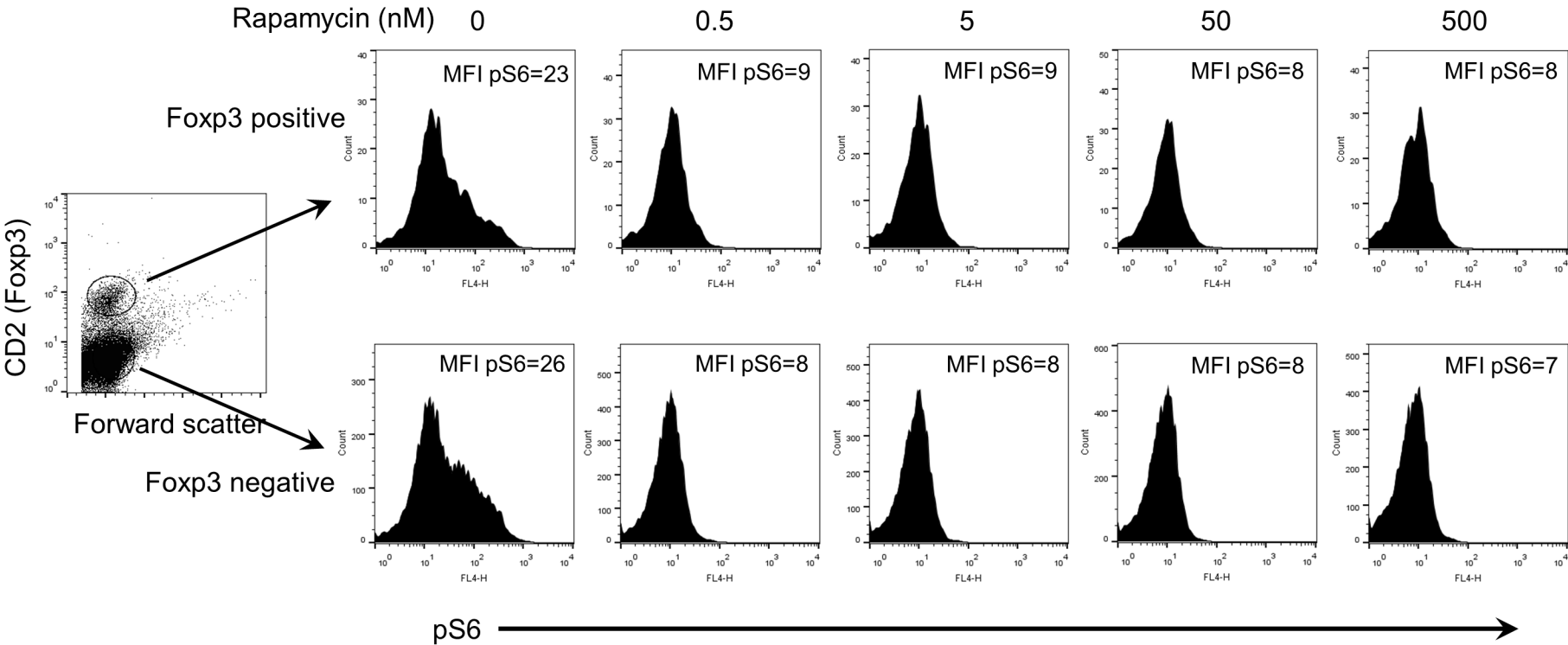
a.



b.



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1116 Supplementary Figure 1

1117 **a.** Western blot for phospho-ribosomal protein S6 and non phosphorylated S6 in EL4 and
1118 EL4.cFoxp3 treated for 24 hours with the indicated concentrations of rapamycin. Results
1119 representative of two experiments.

1120 **b.** Flow cytometric measurement of phospho ribosomal protein S6 in EL4.cFoxp3 cells
1121 treated for 24 hours with ⁴HT, rapamycin or a combination of both. Results representative of
1122 two experiments.

1123 **c.** Flow cytometric measurement of nTreg and Tconv sensitivity to rapamycin at different
1124 concentrations. CD4⁺ T cells from C57Bl/6.foxp3-hCD2 knockin mice were cultured
1125 overnight with anti-CD3/CD28 beads plus IL2 and IL7 along with the indicated
1126 concentrations of rapamycin. Cells were stained for human CD2 and, following
1127 permeabilisation, phospho-S6 ribosomal protein. Results representative of two experiments.

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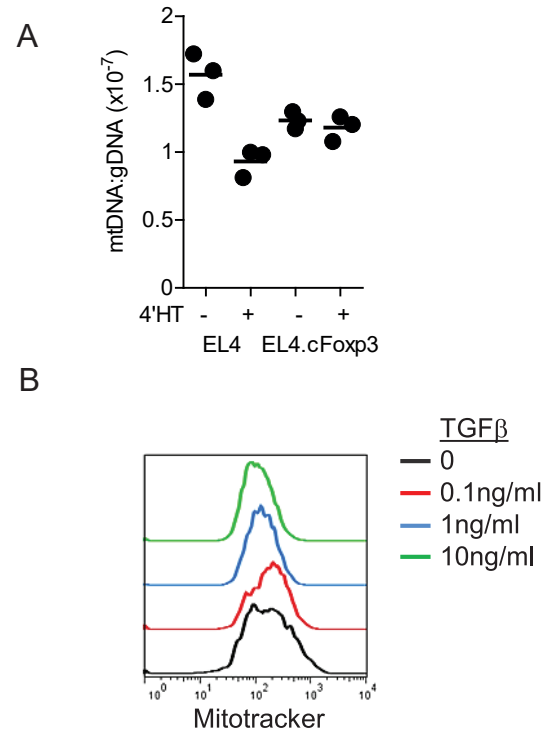
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Howie et al Supplementary Figure 2



1141 **Supplementary Figure 2.**

1142 **a.** Mitochondrial DNA (mtDNA) to genomic DNA (gDNA) ratios in EL4 T cells versus EL4
1143 T cells expressing cFoxp3. A representative mtDNA gene; cytochrome *c* oxidase subunit I
1144 (CO1) and a representative gDNA gene NDUFv1 were measured using quantitative PCR.
1145 Data representative of two separate experiments.

1146 **b.** Quantification of mitochondrial mass in iTreg cultured RAG^{-/-}Marilyn.Foxp3hCD2 T cells
1147 using mitotracker-deep red FM staining. T Cells were cultured with dendritic cells, peptide
1148 and titrated doses of TGFβ as indicated. Results representative of three separate experiments.

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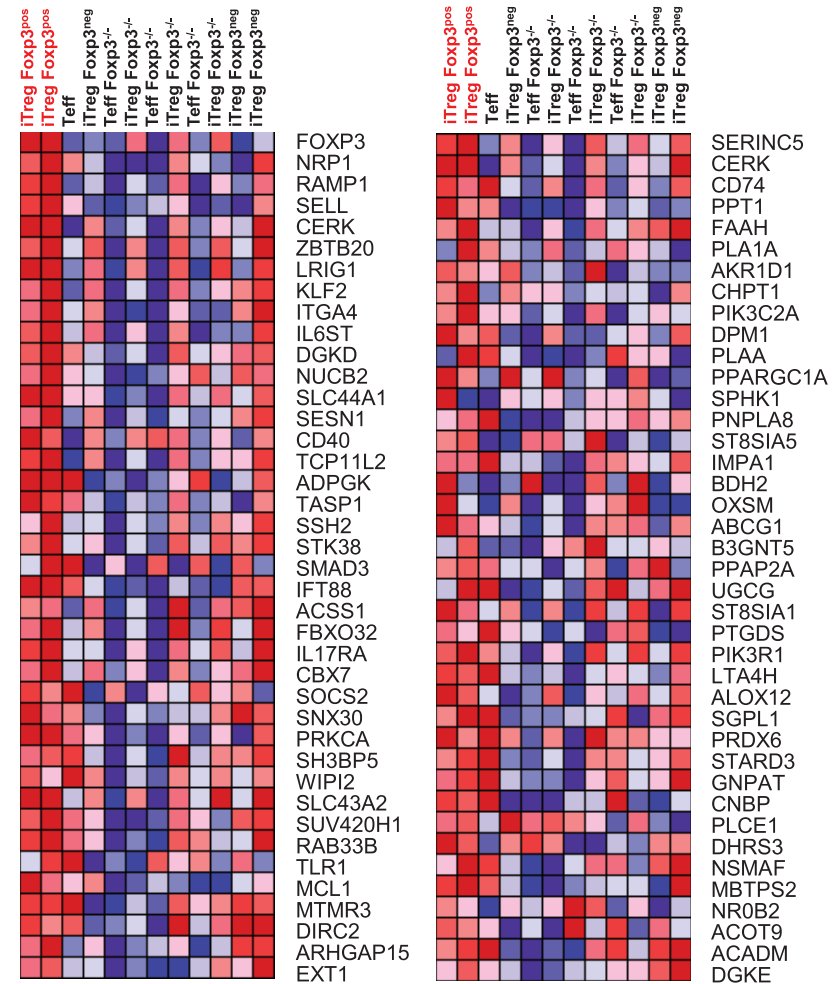
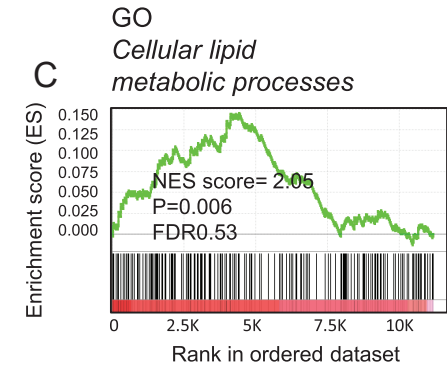
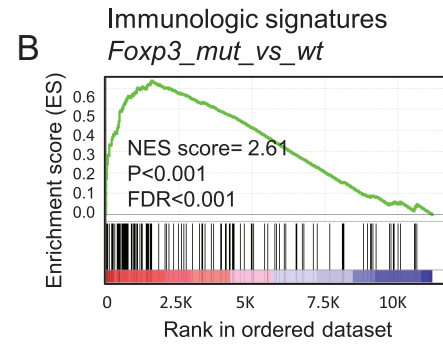
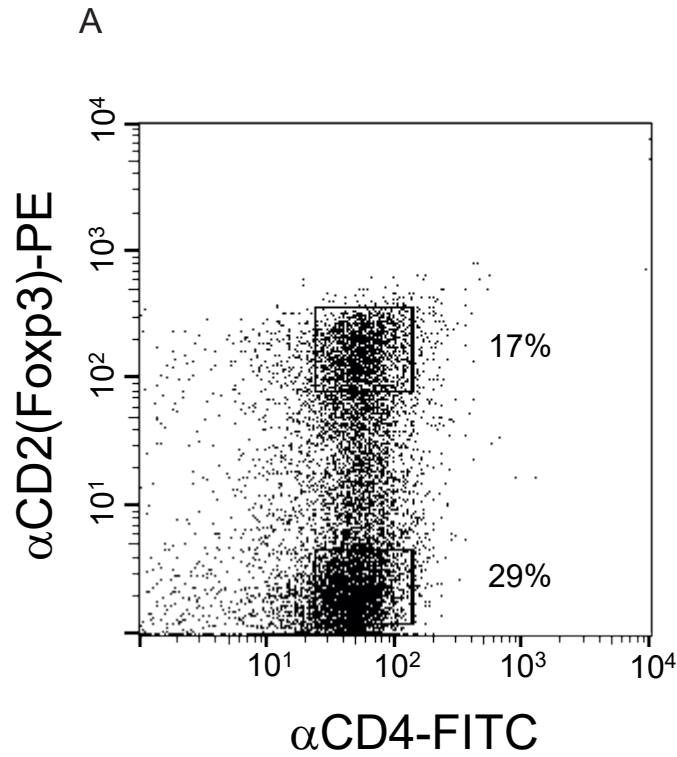
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1166 **Supplementary Figure 3.**

1167 **Comparison of Foxp3⁺ and Foxp3⁻ cells polarised under iTreg conditions.**

1168 **a.** Flow cytometric analysis of CD4⁺ T cells from a seven day iTreg induction culture (see
1169 materials and methods). Cells were stained for CD4 and human CD2. Rectangular gates
1170 indicate populations of CD2 (Foxp3) high and low cells selected for sorting for downstream
1171 analysis.

1172 **b.** Gene set enrichment analysis of microarray data from flow cytometry-purified
1173 Marilyn.foxp3-hCD2 knock-in Foxp3⁺ iTreg compared with Foxp3⁻ Marilyn T cells grown
1174 under the same conditions or Marilyn.foxp3^{-/-} T cells grown under the same conditions.
1175 Comparison of all datasets against immunologic signatures. Top panel; gene set enrichment
1176 analysis showing the influence of Foxp3 expression on the overall gene expression profile of
1177 CD4 T cells. Bottom panel; Heat map of the top 40 leading edge genes influenced positively
1178 by Foxp3 compared across all array datasets analysed.

1179 **c.** Comparison of all datasets, as in (a) against Gene Ontology biochemical process
1180 signatures. Top panel; gene set enrichment analysis showing the influence of Foxp3
1181 expression on the overall gene expression profile of CD4 T cells. Bottom panel; Heat map of
1182 the top 40 leading edge genes influenced positively by Foxp3 compared across all array
1183 datasets analysed.

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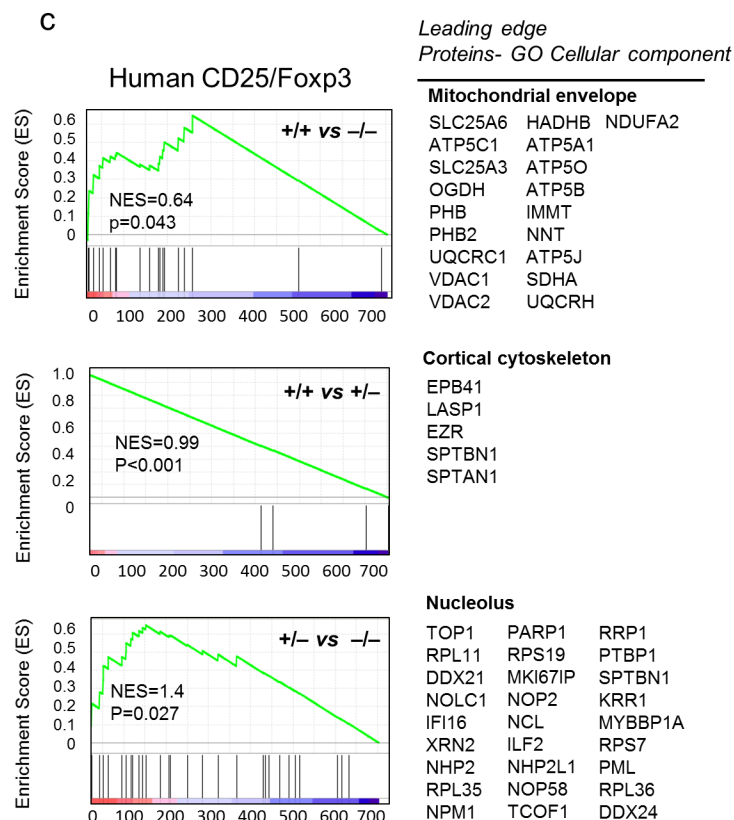
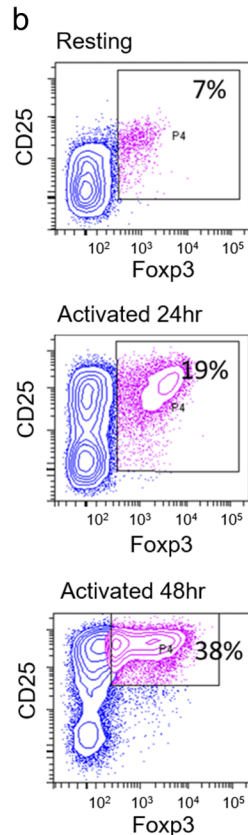
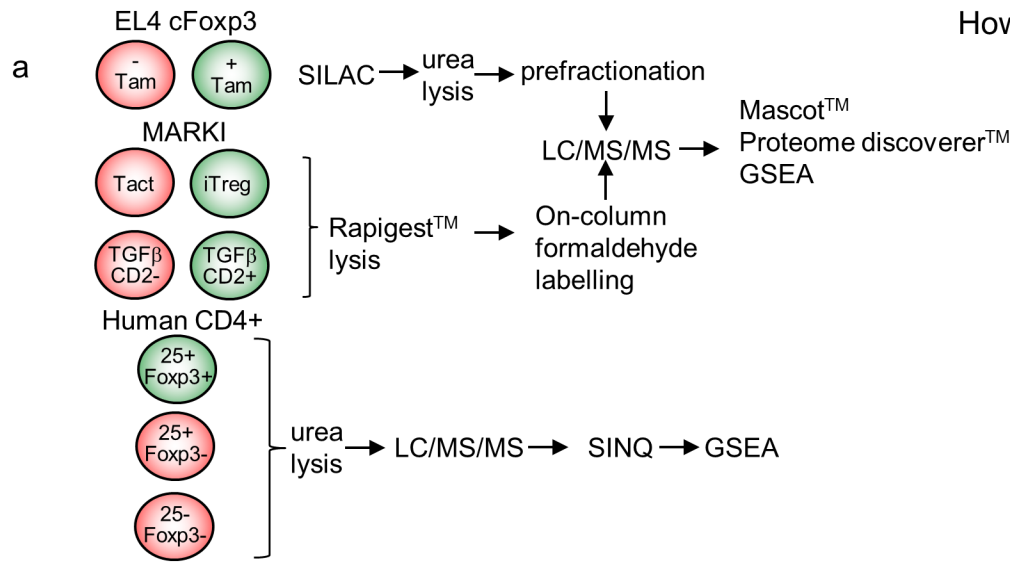
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1191 **Supplementary Figure 4.**

1192 **a.** Quantitative mass spectrometry strategy for identification of Foxp3 and TGF β controlled
1193 proteomes. Overall strategy of cell fractionation, labelling, lysis and proteomic quantitation
1194 of mouse and human Treg proteomes. EL4.cFoxp3, EL4 T cell line transfected with GFP-
1195 Foxp3-ERT fusion protein “cFoxp3”. RAG^{-/-}Marilyn.Foxp3^{hCD2}, human CD2 Foxp3-reporter
1196 mouse TCR transgenic for anti HY TCR. SILAC, stable isotope labelling of cells in culture.
1197 SINQ, spectral index quantification. GSEA, gene set enrichment analysis.

1198 **b.** Human peripheral blood regulatory CD4⁺ T cells before and after 24hr and 48 hr activation
1199 with anti-TCR stained for Foxp3 and CD25, representative of three experiments. The extreme
1200 top and bottom 15% of cells from resting and 24hr activated cultures were gated for cell
1201 sorting.

1202 **c.** Gene set enrichment analysis results of the indicated T cell subset comparisons.
1203 CD25⁺Foxp3⁺ (+/+), CD25⁺Foxp3⁻ (+/-), CD25⁻Foxp3⁻ (-/-). Representative of three pooled
1204 patient samples.

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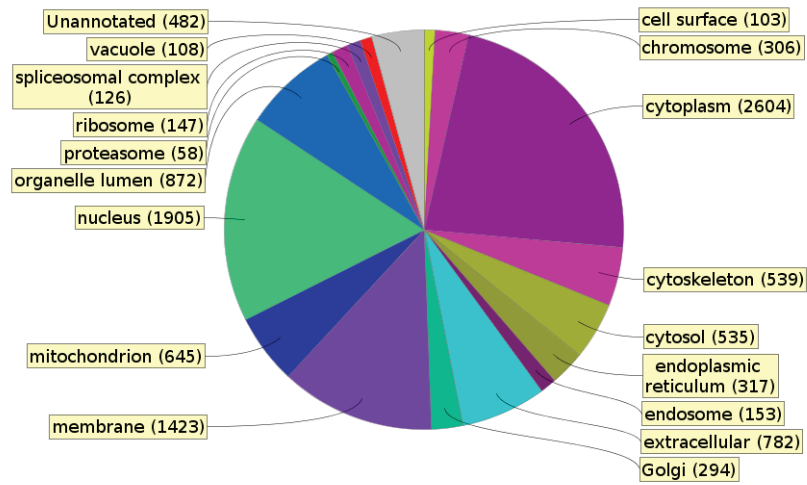
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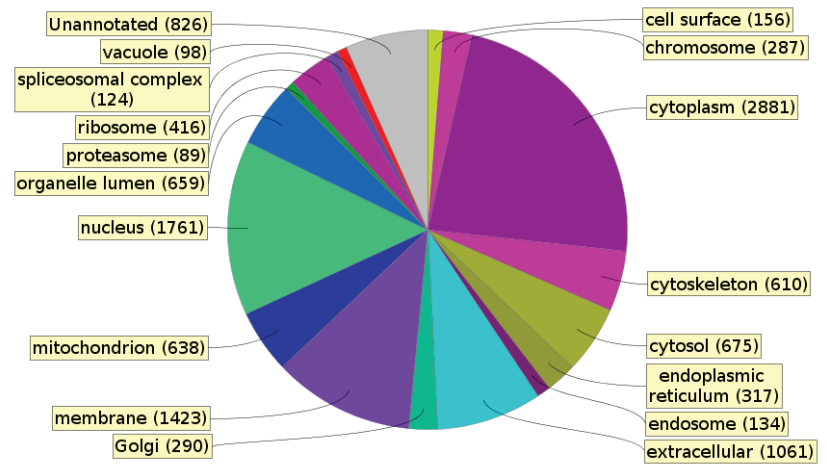
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iTreg Fxp3^{pos} & Fxp3^{neg}
Rapigest™



EL4 +/- 4'HT
8M Urea



1216 Supplementary Figure 5

1217 Comparison of the number of proteins from multiple subcellular compartments extracted
1218 from proteomic experiments using RAG^{-/-}Marilyn.Foxp3hCD2 knock-in T cells with
1219 RapigestTM surfactant and EL4cFoxp3 cells with 8M urea. Data calculated using
1220 ProteinCenterTM software.

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