Survival A) CD3/CD28 Beads (1:1) >0.9999 100 %Live CD4+ cells tTreg IL-2 300IU/ml expansion 7AAD 60 IL-2 300IU/ml 40 tTregs TGF-β 5ng/ml in iTreg condition 20 10nM ATRA ٥ 110g Treginifiee 0 3 Annexin %FOXP3 **Proliferation** MFI of FOXP3 0.1294 0.4206 0.8413 Normalized To Mode 2.0 Normalized To Mode 100 2000 Proliferation index MFI of FOXP3 80 %FOXP3 60 -60 1000 40 40 500 20 0.0 Treginitreg 0 C ITTERS IN ITES 110g treg Treg Treginfre Cell Trace Violet FOXP3 B) Glycolytic Glucose Oligomycin Glycolysis 2DG capacity 250 250 0.3575 0.0751 250 200 ECAR (mpH/min) 200 200 150 uim/Hdm 100 mpH/min 150 100 100 50 50 50 0 0 Tregintreg Tregintreg Tregli Treglin Ó 10 30 40 60 70 80 90 20 50 Time (min) Oligomycin Rot/AA FCCP Basal OCR OCR/ECAR 500 400 0.2785 0.3673 2.0 OCR (pmol/min) 400 300 pmol 02/min 300 OCRIECAR 200 200 1. 100 100 0 Treghtreg Treghtreg

S2 Fig. Lack of effect of culturing tTregs in iTreg generating conditions

S2 Fig; Ex vivo Tregs were cultured in iTreg generating conditions (green) to compare with typical Treg activating conditions (red). (A) Cell survival, proliferation status and FOXP3 expression were analyzed at day 3 post activation. Data are shown in representative histograms and plotted with mean line and s.d. bar, respectively (n = 5). (B) Realtime measurements of extra cellular acidification rate (ECAR) and oxygen consumption rate (OCR) were assessed in same manner as figure 2. Representative data are from one of 3 independent experiments. Statistical analysis did not show any significance (Student's t-test).

0

10

20

30

40

Time (min)

50

60

70

80

90

Treglin

Treg