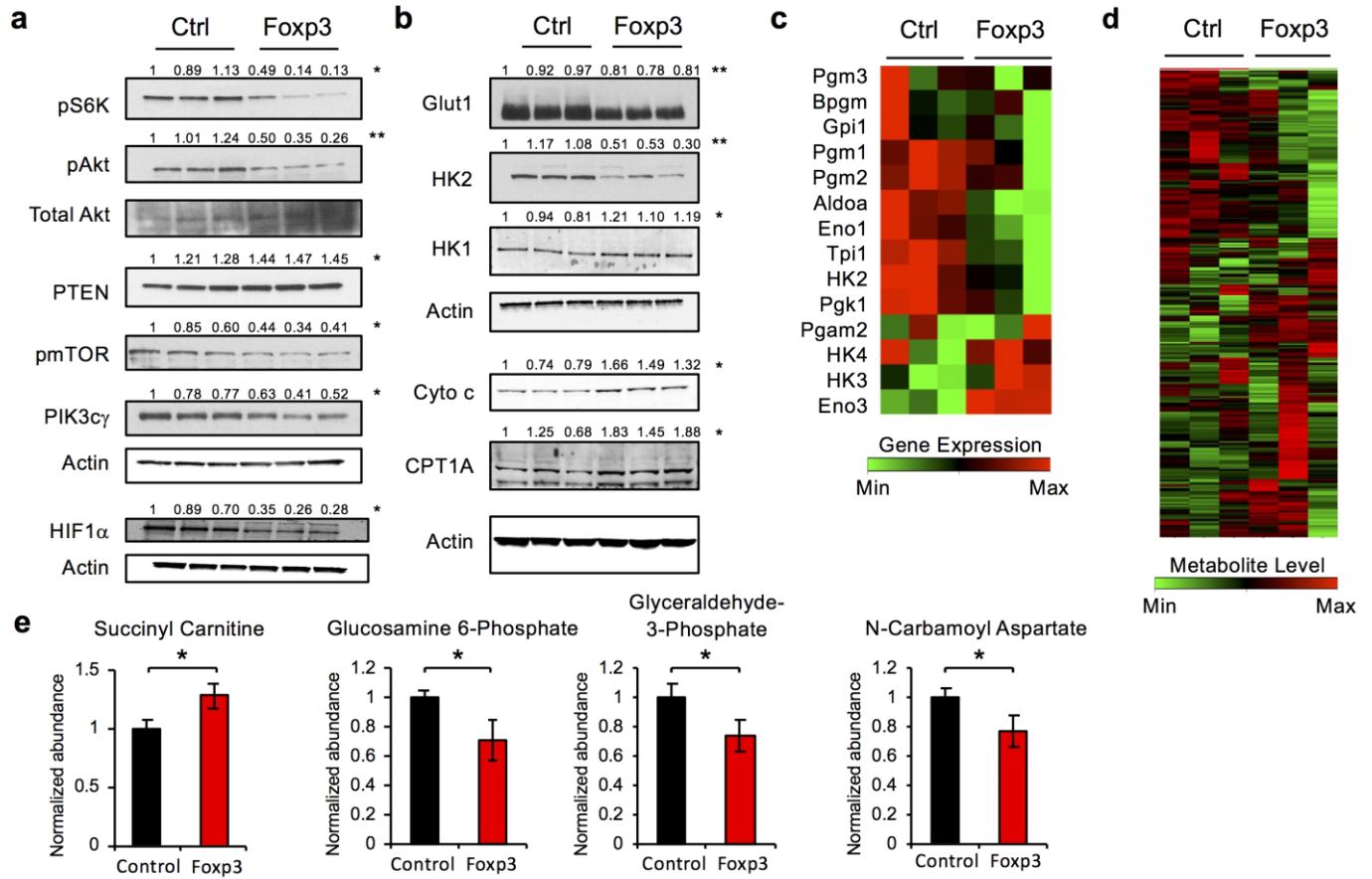


Supplementary Figure 1

T_{reg} cell metabolism is regulated by Foxp3 and inflammatory signals.

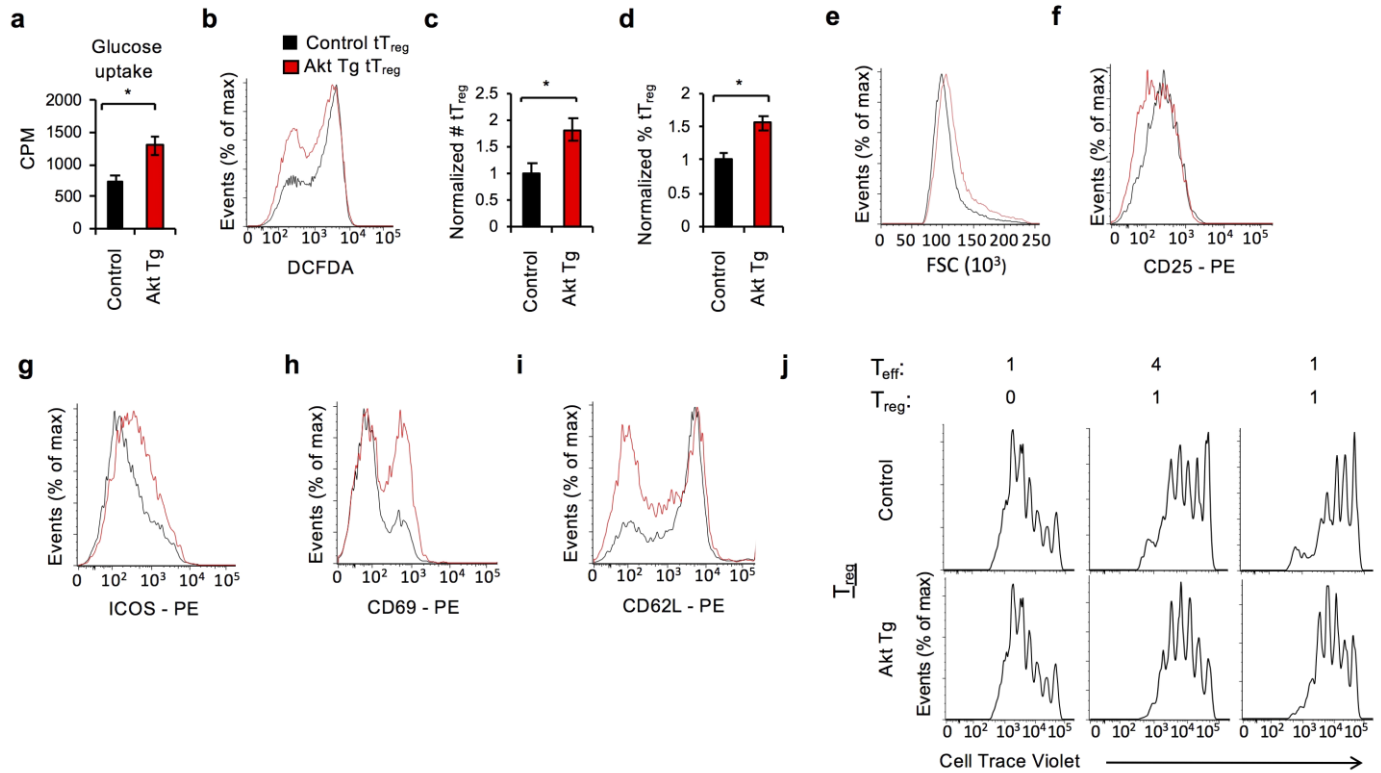
(a) CD4⁺Foxp3⁺ T cells and CD4⁺Foxp3⁻ T cells were analyzed by flow cytometry for Ki67 expression levels. **(b-c)** CD4⁺CD25⁻ T cells were isolated from the spleens of WT mice, polarized under T_{reg} skewing conditions for 5 days and treated with vehicle (H₂O) or 5 μg/mL Pam3CSK4 for the final 24 hrs. Cells were re-isolated by magnetic separation and analyzed for **(b)** forward scatter (FSC) and **(c)** glycolytic capacity using the Seahorse Extracellular Flux Analyzer. **(d)** Gene ontology analysis using PANTHER of pathways altered by Foxp3 deletion using gene expression data published by Williams and Rudensky (*Nat. Immunol* 8:277). **(e-f)** Primary murine CD4⁺CD25⁻ T cells were activated and transduced with control or Foxp3 expressing retrovirus and **(e)** analyzed by chromatin immunoprecipitation-sequencing showing Foxp3 associated sites in the *pyruvate dehydrogenase kinase 3 (PDK3)* and *PIK3cg* loci or **(f)** analyzed by QPCR for expression of *PDK3* mRNA. Data are representative of biological triplicate experiments **(a-b, e-f)**, two independent experiments **(c)**, or an analysis of previously published datasets with biological duplicates **(d)**. Means and standard deviations are shown, * p<0.05.



Supplementary Figure 2

Foxp3 expression in non-T cell lineage inhibits anabolic growth signaling and gene expression.

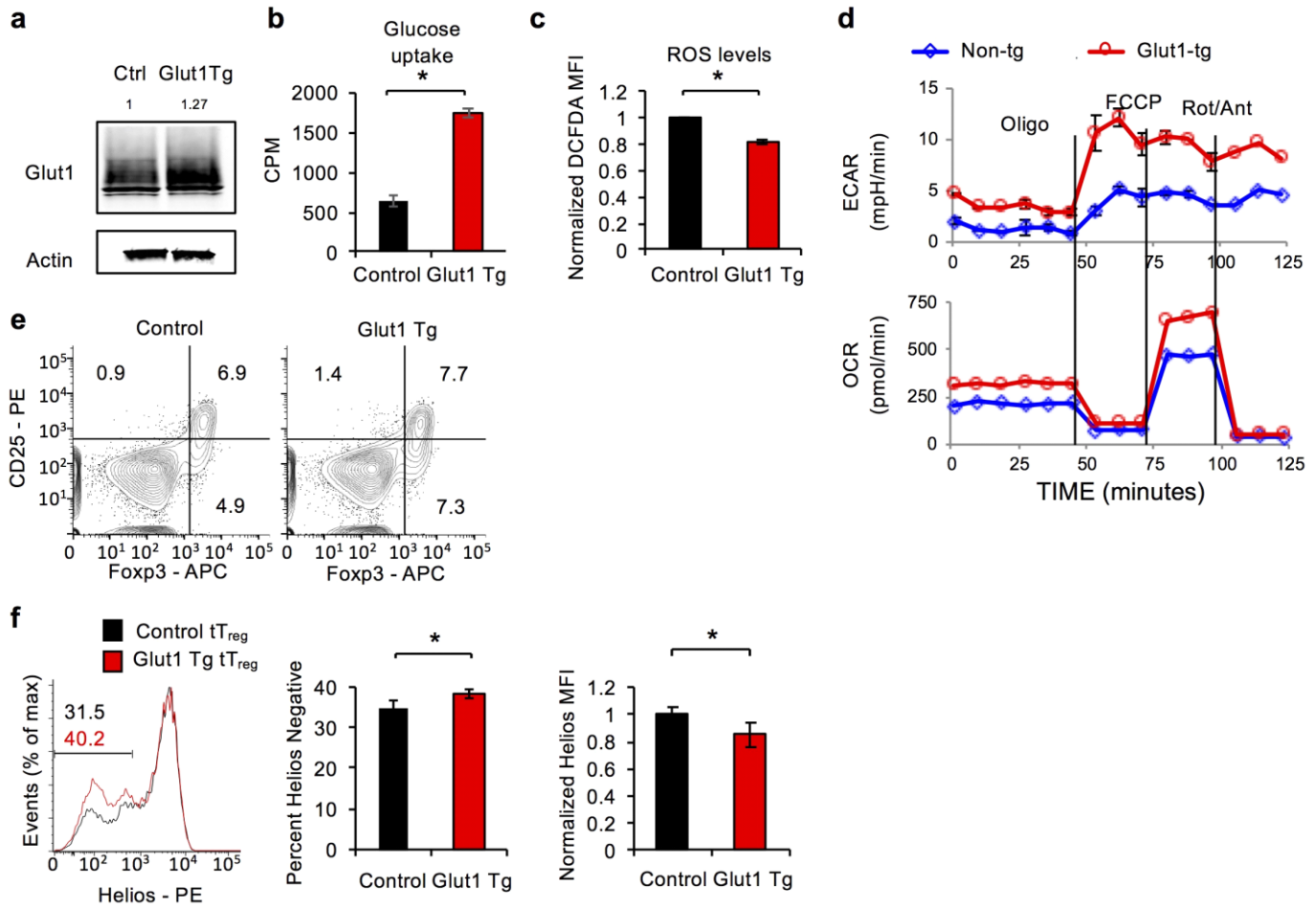
a-e. Three individual clones of control and Foxp3-ER expressing FL5.12 cells were treated with 4OHT to activate Foxp3 and examined for **(a-b)** the expression of metabolic and related proteins, **(c)** select glycolytic gene expression by QPCR or **(d-e)** were extracted and analyzed using high-resolution LC-QE-MS. **(d)** A heat map with relative levels of metabolites using unsupervised hierarchical clustering or **(e)** select metabolite levels are shown. Data are representative of three independent experiments **(a-b)** or an analysis of three independent clones **(c-e)**. **(a-b)** Gel bands are quantified, * $p < 0.05$, ** $p < 0.005$.



Supplementary Figure 3

Constitutive Akt expression increases the number and frequency of T_{reg} cells but diminishes suppressive function.

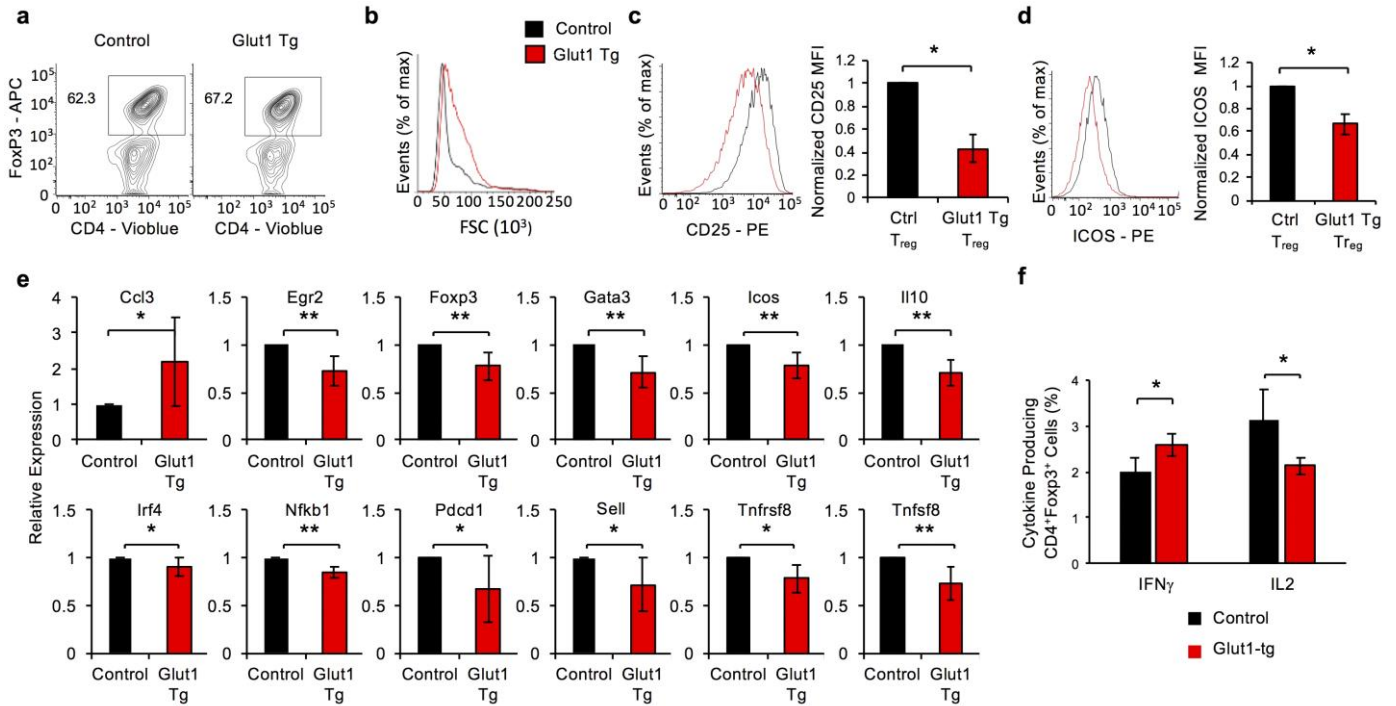
a-b. CD4⁺CD25⁻ T cells were isolated from the spleens of control and mAkt-Tg mice and polarized under T_{reg} skewing conditions. Cells were examined for **(a)** glucose uptake and **(b)** ROS levels as measured by DCFDA. **c-i.** Foxp3⁺ tTreg from the spleen of control and mAkt-Tg mice were examined for **(c)** tTreg number, **(d)** percentage and **(e)** cell size determined by forward scatter and were measured by flow cytometry. **(f)** CD25, **(g)** ICOS, **(h)** CD69 and **(i)** CD62L protein expression in CD4⁺Foxp3⁺ control and mAkt-Tg cells were measured by flow cytometry. **(j)** CD4⁺CD25⁻ T cells were isolated from the spleens of control and mAkt-Tg mice and were polarized under T_{reg} skewing conditions to measure inhibition of effector T cell (T_{eff}) proliferation in an *in vitro* suppression assay. Data are representative of two independent experiments **(a, b)**, three independent experiments **(c, d, f-i)**, four independent experiments **(e)**, or two experiments **(f, j)**. Means and standard deviations are shown, * p<0.05.



Supplementary Figure 4

Transgenic expression of Glut1 results in altered metabolic and immune phenotypes in T_{reg} cells.

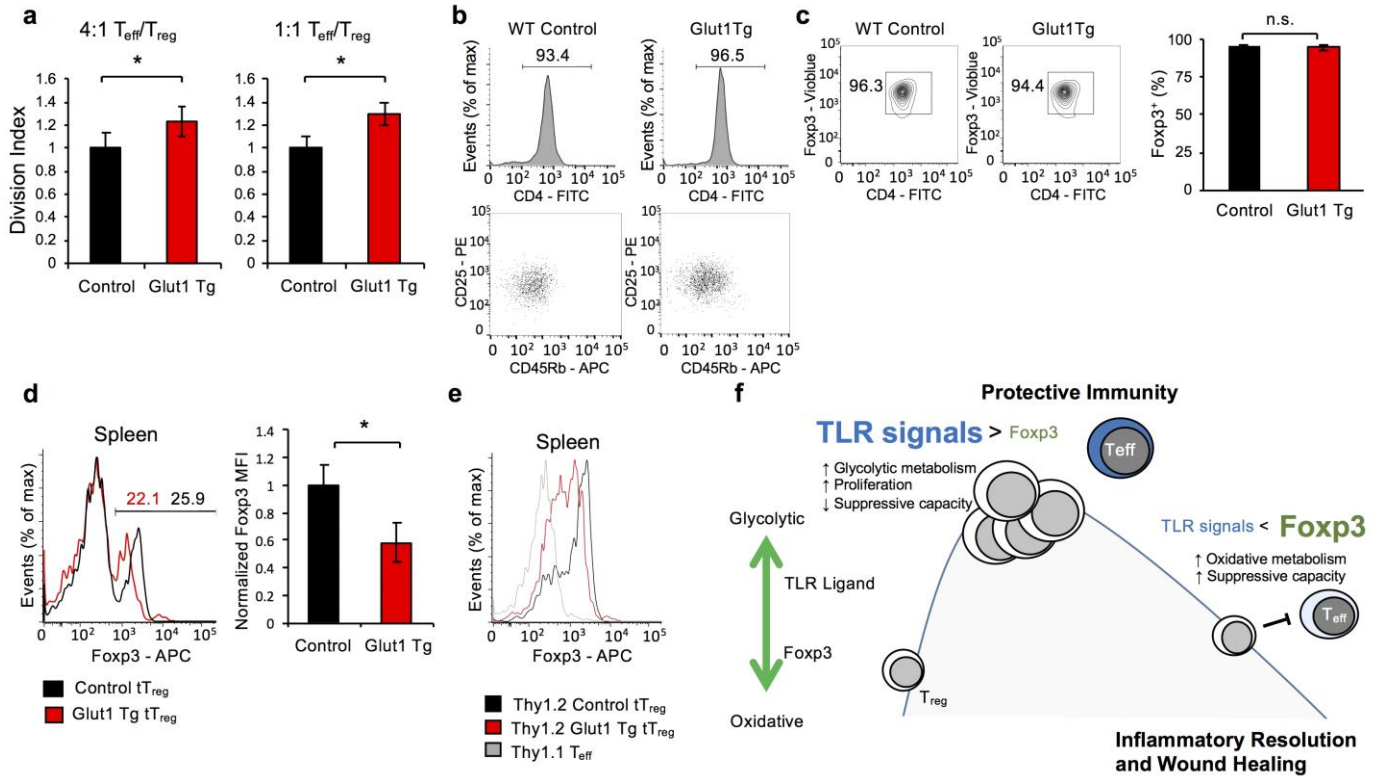
a-d. CD4⁺CD25⁺ T cells were isolated from the spleens of control and Glut1-Tg mice and polarized under T_{reg} skewing conditions. Cells were examined for **(a)** Glut1 expression levels by immunoblot, **(b)** glucose uptake, **(c)** ROS production as measured by DCFDA and **(d)** ECAR and OCR levels were measured using the Seahorse Extracellular Flux Analyzer before and after the addition of the specified inhibitors. **e-f.** CD4⁺Foxp3⁺ T cells from the spleens of control and Glut1-Tg mice were examined for expression of **(e)** CD25 and **(f)** Helios proteins. Data are representative of three independent experiments (**a-b, d, e**) or compiled data from three independent experiments (**c, f**). Means and standard deviations are shown, * p<0.05.



Supplementary Figure 5

Transgenic Glut1 expression alters the immunological phenotype of T_{reg} cells.

a-f. CD4⁺CD25⁻ T cells were isolated from the spleens of control and Glut1-Tg mice and polarized under T_{reg} skewing conditions. Skewed cells were analyzed for **(a)** Foxp3 expression by flow cytometry, **(b)** cell size by FSC analysis, **(c)** CD25 and **(d)** ICOS protein expression by flow cytometry. **(e)** RNA expression levels of a panel of immunosuppressive related genes by QPCR and **(f)** percentage of IFN γ and IL-2 producing CD4⁺Foxp3⁺ cells by flow cytometry are shown. Data are representative of three independent experiments **(a-d)** or the average of six biological replicates **(e)** or four biological replicates **(f)**. Means and standard deviations are shown, * p<0.05.



Supplementary Figure 6

Transgenic expression of Glut1 diminishes the suppressive ability of T_{reg} cells *in vitro* and *in vivo*.

a. $CD4^+CD25^-$ T cells were isolated from the spleens of control and Glut1-Tg mice and polarized under T_{reg} skewing conditions. Control and Glut1-Tg T_{reg} were functionally examined in an *in vitro* suppression assay to measure inhibition of effector T cells (T_{eff}) proliferation and the T_{eff} division index was calculated by Flowjo flow cytometry analysis software. **b-d.** $RAG1^{-/-}$ mice were injected with naïve effector ($CD4^+CD25^+CD45RB^{hi}$) T cells to induce colitis. After weight loss indicated active disease was apparent, control or Glut1-Tg $CD4^+CD25^+CD45RB^{lo}$ T_{reg} were sorted and analyzed by flow cytometry to assess sorted T_{reg} . **(b)** The expression of CD25 and CD45Rb and **(c)** Fopx3 protein of sorted rescue T_{reg} are shown. **(d)** At the termination of the experiment Fopx3 levels were assessed on $CD4^+$ gated T cells in the spleens of recipient animals. **(e)** Thy1.1 naïve effector ($CD4^+CD25^+CD45RB^{hi}$) T cells were adoptively transferred into $RAG1^{-/-}$ mice to initiate IBD. Thy1.2 control or Glut1-tg tT_{reg} ($CD4^+CD25^+CD45RB^{lo}$) T cells were sorted and injected after disease was apparent Fopx3 levels were then assessed by flow cytometry on adoptively transferred Thy1.1 effectors and Thy1.2 $CD4^+$ control and Glut1-tg T_{reg} from mesenteric lymph nodes and spleens. Data are the result of three independent experiments **(a)**, representative of three independent experiments **(b)** or is representative of two independent experiments with at least 5 mice per group **(c, d)**. Means and standard deviations are shown, * $p < 0.05$. **(f)** Model of our findings. Our findings show that T_{reg} are metabolically heterogeneous and depend on activating and inflammatory signals as well as Fopx3 itself to coordinate metabolism. In the presence of inflammatory stimuli, such as TLR ligands, T_{reg} increase mTORC1 signaling, Glut1, and glycolysis, which results in increased cell growth and proliferation. Suppressive capacity, however, can be impaired. As inflammatory signals are reduced, Fopx3 can tilt the balance away from mTORC1 signaling to favor oxidative metabolism that lowers proliferative ability but enhances suppression to promote inflammatory resolution. Metabolic transitions are critical in this process as increased Glut1 expression is sufficient to promote T_{reg} growth while reducing suppression and stability.

Supplementary Table 1. Genes in Primary Metabolic Process with altered expression upon Foxp3 deletion. Microarray data from Foxp3 gene targeted T_{reg} was analyzed using Geo Dataset browser of GDS2525 (p<0.1) and genes with 1.5-fold change from control were analyzed using PANTHER gene ontology analysis classification system (pantherdb.org). Genes in Primary Metabolic Processes (GO:0044238; as shown in Supplementary Fig. 1d) are indicated.

Slc25a44	Solute carrier family 25 member 44;Slc25a44;ortholog
Pten	Phosphatidylinositol 3,4,5-trisphosphate 3-phosphatase and dual-specificity protein phosphatase PTEN;Pten;ortholog
Gfpt1	Glutamine--fructose-6-phosphate aminotransferase [isomerizing] 1;Gfpt1;ortholog
Ucp2	Mitochondrial uncoupling protein 2;Ucp2;ortholog
Pfkfb1	6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 1;Pfkfb1;ortholog
Slc35c1	GDP-fucose transporter 1;Slc35c1;ortholog
Eno2	Gamma-enolase;Eno2;ortholog
Tkt	Transketolase;Tkt;ortholog
Gapdh	Glyceraldehyde-3-phosphate dehydrogenase;Gapdh;ortholog
Prps1	Ribose-phosphate pyrophosphokinase 1;Prps1;ortholog
Atp5e	ATP synthase subunit epsilon, mitochondrial;Atp5e;ortholog
Slc35e1	Solute carrier family 35 member E1;Slc35e1;ortholog
Ahr	Aryl hydrocarbon receptor;Ahr;ortholog
Acox1	Acyl-coenzyme A oxidase-like protein;Acox1;ortholog
Foxp3	Forkhead box protein P3;Foxp3;ortholog
Cpt1a	Carnitine O-palmitoyltransferase 1, liver isoform;Cpt1a;ortholog
Slc25a29	Mitochondrial carnitine/acylcarnitine carrier protein CACL;Slc25a29;ortholog
Faah	Fatty-acid amide hydrolase 1;Faah;ortholog
Rraga	Ras-related GTP-binding protein A;Rraga;ortholog
Acat2	Acetyl-CoA acetyltransferase, cytosolic;Acat2;ortholog
Pfkfb3	6-phosphofructokinase type C;Pfkfb3;ortholog
Acss1	Acetyl-coenzyme A synthetase 2-like, mitochondrial;Acss1;ortholog
Txnrd2	Thioredoxin reductase 2, mitochondrial;Txnrd2;ortholog
Mgll	Monoglyceride lipase;Mgll;ortholog
Acss2	Acetyl-coenzyme A synthetase, cytoplasmic;Acss2;ortholog
Ogdh	2-oxoglutarate dehydrogenase, mitochondrial;Ogdh;ortholog
Slc7a5	Large neutral amino acids transporter small subunit 1;Slc7a5;ortholog
Gpd2	Glycerol-3-phosphate dehydrogenase, mitochondrial;Gpd2;ortholog
Slc27a4	Long-chain fatty acid transport protein 4;Slc27a4;ortholog
Pik3c2a	Phosphatidylinositol 4-phosphate 3-kinase C2 domain-containing subunit alpha;Pik3c2a;ortholog
Slc25a36	Solute carrier family 25 member 36;Slc25a36;ortholog
Elovl1	Elongation of very long chain fatty acids protein 1;Elovl1;ortholog

Pfkl	6-phosphofructokinase, liver type;Pfkl;ortholog
Txnrd1	Thioredoxin reductase 1, cytoplasmic;Txnrd1;ortholog
Ggt1	Gamma-glutamyltranspeptidase 1;Ggt1;ortholog
Xdh	Xanthine dehydrogenase/oxidase;Xdh;ortholog
Slc45a4	Solute carrier family 45 member 4;Slc45a4;ortholog
Pprc1	Peroxisome proliferator-activated receptor gamma coactivator-related protein 1;Pprc1;ortholog
Alox8	Arachidonate 8S-lipoxygenase;Alox8;ortholog
Gsk3b	Glycogen synthase kinase-3 beta;Gsk3b;ortholog
Slc25a30	Kidney mitochondrial carrier protein 1;Slc25a30;ortholog
Odc1	Ornithine decarboxylase;Odc1;ortholog
Tpi1	Triosephosphate isomerase;Tpi1;ortholog
Gclc	Glutamate--cysteine ligase catalytic subunit;Gclc;ortholog

Supplementary Table 2. Overlapping genes with Foxp3 deletion or overexpression. Microarray data from Foxp3 gene targeted T_{reg} was analyzed using Geo Dataset browser of GDS2525 (p<0.1) and genes with 1.5-fold change from control were compared to genes altered by retroviral Foxp3 overexpression (ArrayExpress E-MTAB-4561; p<0.05). Overlapping genes that increased or decreased with modulation of Foxp3 expression are shown.

Genes Lower with FoxP3 Deletion and Increased with FoxP3 Expression	Genes Increased with FoxP3 Deletion and Decreased with FoxP3 Expression
Arl5a	2610018G03Rik
Arrdc4	Acpp
Atp6v0d2	Ar
Ccrl2	Arhgap26
Ccs	Auh
Cd72	B4galnt1
Cd81	Bcl2
Crem	Bdh1
Crim1	Bhlhe40
Ctla4	Btg2
Cxcl2	Cd160
Cyfp1	Cd96
Dusp4	Cdyl2
Ebi3	Dap
Entpd1	Dusp6
Epcam	Ehd3
Foxp3	Gab3
Gem	Ggt1
Glrx	Golm1
Gpr83	Gpr171
Hgfac	Gramd1a
Hspbap1	Grap2
Icos	Higd2a
Ighm	Il1rl2
Ikzf2	Kcnk5
Il10	Klrb1f
Il1r2	Ldlr
Il1rl1	Lgals3
Itgae	Lgals3bp
Itgav	Lmo4
Itgb8	Mfsd6
Klrg1	Mgll
Mab21l1	Ms4a6d
Micall1	Nebi

Naip5	Nqo2
Neb	Pde3b
Nrn1	Pde7a
Nucb2	Phf6
Odc1	Pim2
Pde2a	Pltp
Pdzk1ip1	Rab4b
Phlpp1	Rnf19a
Plxnc1	Runx2
Plxnd1	Satb1
Prdm1	Sco1
Prdx4	Sema4a
Ptprj	Slco3a1
Rab6b	Snai3
Rrad	Sorl1
Rxra	Ssrp1
Samsn1	Stat4
Slc14a1	Stk39
Slc45a4	Tab2
Snx9	Tcf7
Tcn2	Themis
Tjp3	Tnfsf8
Tlr1	Tomm22
Tns1	Topors
Tspan17	Trim36
Tspan32	Xdh
Vamp5	
Vav2	
Zbtb10	
Zfp612	

Supplementary Table 3. Gene ontology analysis of pathways altered based on shared gene expression changes with modulation of Foxp3 expression. Genes identified in Supplementary Table 2 as overlapping between Foxp3 gene deletion or overexpression were analyzed by DAVID Gene Functional Classification Tool and sorted by fold-enrichment for the pathway.

Pathway	Fold-Enrichment	Genes
GO:0006687~glycosphingolipid metabolic process	23.32036613	ITGB8, CREM, B4GALNT1
GO:0046632~alpha-beta T cell differentiation	23.32036613	SATB1, BCL2, FOXP3
GO:0006664~glycolipid metabolic process 3	20.14031621	ITGB8, CREM, B4GALNT1
GO:0046631~alpha-beta T cell activation	19.26465028	SATB1, BCL2, FOXP3
GO:0007160~cell-matrix adhesion	8.86173913	ITGB8, BCL2, RAB4B
GO:0050864~regulation of B cell activation	8.056126482	CD81, FOXP3, IL10
GO:0030217~T cell differentiation	7.773455378	SATB1, THEMIS, BCL2, FOXP3
GO:0031589~cell-substrate adhesion	7.773455378	ITGB8, BCL2, RAB4B
GO:0006665~sphingolipid metabolic process	7.146563815	ITGB8, CREM, B4GALNT1
GO:0045454~cell redox homeostasis	7.146563815	PRDX4, SCO1, GLRX
GO:0006643~membrane lipid metabolic process	6.923233696	ITGB8, CREM, B4GALNT1
GO:0007229~integrin-mediated signaling pathway	5.830091533	ITGB8, ITGAE, ITGAV
GO:0031401~positive regulation of protein modification process	5.680602007	BCL2, CD81, FOXP3
GO:0002521~leukocyte differentiation	5.200551133	SATB1, THEMIS, GAB3, BCL2, FOXP3
GO:0030098~lymphocyte differentiation	5.182303585	SATB1, THEMIS, BCL2, FOXP3
GO:0006470~protein amino acid dephosphorylation	5.182303585	PTPRJ, DUSP4, BCL2, DUSP6
GO:0042110~T cell activation	5.092953523	SATB1, THEMIS, BCL2, FOXP3
GO:0001763~morphogenesis of a branching structure	4.72626087	AR, BCL2, PLXND1, IL10
GO:0010876~lipid localization	4.61548913	LDLR, SORL1, PLTP, B4GALNT1
GO:0016311~dephosphorylation	4.189947579	PTPRJ, DUSP4, BCL2, DUSP6

GO:0001655~urogenital system development	4.046456224	ODC1, AR, RXRA, BCL2
GO:0048732~gland development	3.748620614	XDH, AR, RXRA, BCL2, PLXND1
GO:0032535~regulation of cellular component size	3.669457197	NEB, BCL2, CD81, CYFIP1
GO:0001775~cell activation	3.60233298	SATB1, THEMIS, BCL2, TLR1, ENTPD1, FOXP3
GO:0007264~small GTPase mediated signal transduction	3.434782609	ARL5A, RAB4B, RRAD, RAB6B, GEM, VAV2
GO:0045321~leukocyte activation	3.372046853	SATB1, THEMIS, BCL2, TLR1, FOXP3
GO:0006955~immune response	3.135788794	MFSD6, IL1RL1, THEMIS, IL1RL2, CXCL2, TLR1, CTLA4, FOXP3, IL10, TNFSF8
GO:0008284~positive regulation of cell proliferation	3.12033068	ODC1, BCL2, CD81, MAB21L1, FOXP3, RUNX2
GO:0048729~tissue morphogenesis	3.102849836	AR, LMO4, RXRA, BCL2, PLXND1
GO:0030097~hemopoiesis	2.942144466	SATB1, THEMIS, GAB3, BCL2, FOXP3
GO:0043085~positive regulation of catalytic activity	2.829418624	BCL2, CD81, TLR1, CCS, VAV2
GO:0042127~regulation of cell proliferation	2.745272345	ODC1, TCF7, AR, BCL2, CD81, TOPORS, MAB21L1, FOXP3, RUNX2, IL10
GO:0010604~positive regulation of macromolecule metabolic process	2.566591112	AR, IKZF2, RXRA, BCL2, CD81, TLR1, TOPORS, PRDM1, FOXP3, RUNX2, IL10
GO:0042592~homeostatic process	2.276131626	XDH, LDLR, BCL2, NUCB2, PRDX4, PDE3B, FOXP3, SCO1, GLRX
GO:0010557~positive regulation of macromolecule biosynthetic process	2.229368335	AR, IKZF2, RXRA, TLR1, TOPORS, FOXP3, RUNX2, IL10
GO:0006915~apoptosis	2.223375409	PHLPP1, 2610018G03RIK, BCL2, NAIP5, DAP, TOPORS, PIM2
GO:0012501~programmed cell death	2.185770751	PHLPP1, 2610018G03RIK, BCL2, NAIP5, DAP, TOPORS, PIM2
GO:0009891~positive regulation of biosynthetic process	2.121302006	AR, IKZF2, RXRA, TLR1, TOPORS, FOXP3, RUNX2, IL10

Supplementary Table 4. Glucose metabolism QPCR Array Ct values for Foxp3 expressing cells. Control and Foxp3-ER expressing FL5.12 cells were treated with 4-OHT and metabolic gene expression was determined by QPCR array. Ct values for each cell line are provided.

Gene Symbol	Control line 1	Control line 2	Control line 3	FoxP3 line 1	FoxP3 line 2	FoxP3 line 3
Acly	24.16	25.54	23.55	24.43	24.28	23.54
Aco1	35	35	35	35	35	35
Aco2	27.25	28.08	26.25	26.99	27.06	25.83
Agl	26.69	27.45	25.47	25.47	25.69	24.95
Aldoa	21.05	22.1	19.83	20.65	21.04	20.01
Aldob	35	35	35	35	35	35
Aldoc	35	34.16	33.46	33.89	33.04	32.53
Bpgm	29.18	30.16	27.69	28.32	28.17	27.47
Cs	29.66	30.56	27.92	28.31	28.38	27.64
Dlat	26.84	27.51	25.32	26	26.06	25.23
Dld	24.53	25.48	23.39	24.03	23.98	23.12
Dlst	25.02	25.67	23.59	23.85	24.08	23.63
Eno1	21.3	22.26	19.97	20.92	21.22	20.44
Eno2	35	35	35	35	35	35
Eno3	27.23	28.4	25.68	26.04	25.36	24.27
Fbp1	35	35	35	35	35	35
Fbp2	35	35	35	35	35	35
Fh1	24.53	25.48	23.92	24.38	24.42	23.76
G6pc	35	35	35	35	35	35
G6pc3	26.19	27.09	25.22	25.18	25.14	24.19
G6pdx	25.4	26.43	23.89	24.13	24.08	23.23
Galm	35	35	35	35	35	35
Gapdhs	35	35	35	35	35	35
Gbe1	29.1	30.24	28.13	28.44	28.6	28.23
Gck	35	31.9	31.17	34.62	31.42	28.62
Gpi1	23.38	24.38	22.08	22.53	22.64	21.72
Gsk3a	26	27.02	24.26	25.08	24.88	23.93
Gsk3b	26.53	27.59	25.52	25.89	25.66	24.92
Gys1	26.9	28.04	25.69	26.02	26.22	25.36
Gys2	35	35	35	35	35	35
H6pd	28.45	29.11	26.61	27.15	27.28	26.14
Hk2	26.52	27.31	25.21	26.06	26.12	25.81
Hk3	31.48	32.01	28.88	29.33	29.08	28.25
ldh1	26.9	27.92	25.66	26.1	26.02	25.44

Idh2	25.1	26.09	24.04	24.39	24.31	23.72
Idh3a	24.44	25.26	22.77	23.59	23.52	22.56
Idh3b	24.87	25.5	23.25	23.97	24.09	23.27
Idh3g	23.85	24.8	22.7	23.18	23.22	22.4
Mdh1	25.54	26.22	24.49	25.16	25.21	24.12
Mdh1b	35	35	35	35	35	35
Mdh2	23.59	24.1	22.55	23	23.12	22.33
Ogdh	25.28	26.34	23.88	24.64	24.62	23.44
Pck1	35	35	35	35	35	35
Pck2	26.11	27.04	24.75	25.24	25.65	24.82
Pcx	28.19	28.86	26.27	26.67	27.29	26
Pdha1	25.54	26.33	24.01	24.52	24.62	23.85
Pdhb	25.57	26.68	24.59	25.07	25.01	24.25
Pdk1	27.21	28.04	25.76	26.92	26.81	26.64
Pdk2	35	35	35	35	35	35
Pdk3	25.11	25.81	24.26	24.81	25.05	23.95
Pdk4	35	35	35	35	35	35
Pdp2	28.08	28.63	26.7	27.11	27.49	26.65
Pdpr	29.29	29.94	27.6	28.08	28.28	26.98
Pfkl	24.38	25.41	22.81	23.75	24.08	23.25
Pgam2	31.13	32.1	30.05	29.95	29.94	28.81
Pgk1	22.41	23.25	21.17	21.82	22.11	21.66
Pgk2	35	35	35	35	35	35
Pgm1	25.58	26.58	24.61	24.92	25.02	24.15
Pgm2	26.08	27.05	25.07	25.52	25.45	25.08
Pgm3	28.45	29.61	27.28	27.72	27.85	26.54
Phka1	35	35	35	35	35	35
Phkb	27.26	28.46	26.05	26.38	26.17	25.26
Phkg1	34.73	35	33.48	34.3	34.18	32.66
Phkg2	27.66	28.71	26.44	26.83	26.88	25.51
Pklr	35	35	35	35	35	35
Prps1	24.71	25.81	23.58	23.86	23.73	23.05
Prps1l1	35	35	35	35	35	35
Prps2	26.28	27.23	25.02	25.27	25.23	24.57
Pygl	35	35	35	33.65	35	35
Pygm	34.02	35	33.68	33.78	34.12	33.63
Rbks	28	29	27.44	27.66	27.9	26.94
Rpe	26.07	26.56	24.7	25.23	25.37	24.29
Rpia	24.54	25.49	23.64	24.31	24.17	23.54
Sdha	24.33	25.35	23.13	23.65	23.64	22.9
Sdhb	25.36	26.38	24.5	25.25	25.15	24.37

Sdhc	24.19	25.39	23.26	23.82	23.67	22.9
Sdhd	28.41	29.62	27.49	27.86	27.85	27.17
Sucla2	26.05	27.02	25.01	25.53	25.63	24.71
Suclg1	28.42	29.63	26.53	27.26	27.09	26.77
Suclg2	25.36	26.35	24.22	24.9	24.83	24.21
Taldo1	25.74	26.71	24.73	27.4	25.07	24.31
Tkt	22.23	23.37	21.16	21.56	21.5	20.69
Tpi1	22.8	23.69	21.64	22.47	22.53	22.04
Ugp2	26.77	27.76	25.46	25.85	25.94	24.67
Actb	19.7	20.95	18.45	18.68	18.58	17.31
B2m	23.53	24.64	22.97	23.1	23.02	21.41
Gapdh	21.21	22.61	19.85	20.62	20.94	19.65
Gusb	26.02	26.83	25.07	25.33	25.27	24.35
Hsp90ab1	21.16	22.13	19.81	20.28	20.39	19.57

Supplementary Table 6. Gene ontology analysis of pathways altered based on shared gene expression changes between age-matched Glut1 and control T_{reg} cohorts. Genes identified in RNAseq (Geo accession GSE84919) as $p < 0.05$, \log_2 0.3-fold different between normalized cohorts of control and Glut1-tg T_{reg} were analyzed by DAVID Gene Functional Classification Tool. Pathways were sorted by fold-enrichment as increased (positive Fold-Enrichment) or decreased (negative Fold-Enrichment) in Glut1-tg T_{reg}.

Pathway	Fold-Enrichment	Genes
GO:0033138~positive regulation of peptidyl-serine phosphorylation	18.97765	OSM, LIF, IFNG
GO:0002763~positive regulation of myeloid leukocyte differentiation	8.758917	LIF, TNFSF11, ID2
GO:0007259~JAK-STAT cascade	5.422187	OSM, LIF, IFNG, SOCS1
GO:0008213~protein amino acid alkylation	4.413408	PRMT1, PRMT7, GSPT1, PRMT5, EZH2
GO:0006730~one-carbon metabolic process	3.892852	MTHFD1, MTHFD2, PRMT1, SHMT2, AHCY, PRMT7, GSPT1, GM9826, PRMT5, EZH2, DNMT1, FPGS
GO:0016570~histone modification	3.300462	KAT2A, HDAC2, PRMT7, EZH2, RUVBL2, MBD3, RUVBL1, RBM14
GO:0043414~biopolymer methylation	3.207491	PRMT1, PRMT7, GSPT1, PRMT5, EZH2, DNMT1
GO:0016569~covalent chromatin modification	3.162942	KAT2A, HDAC2, PRMT7, EZH2, RUVBL2, MBD3, RUVBL1, RBM14
GO:0006325~chromatin organization	2.77134	HIST1H2AG, EZH2, CBX5, SET, PRMT7, PRMT5, HIST1H2BJ, HIST1H4F, H2AFX, HIST1H4C, HIST1H4D, HIST1H4I, ASF1B, ASF1A, HIST1H4H, KAT2A, HIST1H2BB, HIST1H1E, MBD3, HDAC2, NPTXR, HIST1H3A, HIST1H2AI, DNMT1, HIST1H3C, RUVBL2, HIST1H3E, RUVBL1, RBM14
GO:0051276~chromosome organization	2.724515	RAD51C, HIST1H2AG, EZH2, CBX5, NCAPH, SET, PRMT7, PRMT5, HIST1H2BJ, HIST1H4F, HIST1H4C, H2AFX, HIST1H4D, HIST1H4I, ASF1B, TOP2A, ASF1A,

		BUB3, HIST1H4H, KAT2A, HIST1H2BB, HIST1H1E, MBD3, RAD54L, MIS12, HDAC2, NPTXR, HIST1H3A, HIST1H2AI, HIST1H3C, DNMT1, RUVBL2, RUVBL1, HIST1H3E, RBM14
GO:0033554~cellular response to stress	2.442668	KIF22, APEX2, MRE11A, ROMO1, HMOX1, IFNG, H2AFX, POLQ, ASF1A, EIF2B5, RAD51AP1, NUDT1, LIG1, EME1, GTF2H4, BRIP1, RAD54L, EEPD1, RAD51, NUPR1, TIMELESS, TDP1, POLD1, RUVBL2, RAD54B, CHAF1A
GO:0016568~chromatin modification	2.090758	KAT2A, HDAC2, NPTXR, PRMT7, PRMT5, EZH2, DNMT1, RUVBL2, MBD3, RUVBL1, RBM14, ASF1B, ASF1A
GO:0006955~immune response	-2.22662	IL18R1, IL1R1, H2-Q5, HC, SLA2, TLR1, GBP9, CD1D1, FOXP3, H2-Q6, TLR6, TRAT1, CD1D2, H60B, CCR7, CBLB, H2-EB2, BNIP3L, GBP10, OAS1B, BCL6, H2-T24, GBP4, CD27
GO:0031327~negative regulation of cellular biosynthetic process	-2.22685	SATB1, SLA2, HR, SMAD3, TTF1, MAF1, FOXP3, ZFP128, NRIP1, CITED2, EPC1, CIR1, RNF2, TIA1, TRPS1, TGIF1, ZFP281, PER1, BCL6, EIF2AK3, NR1H3
GO:0002684~positive regulation of immune system process	-2.43481	GM614, CBLB, HC, BCL6, CD1D1, H2-Q6, FOXP3, GM5511, TRAT1, CD27, CLEC2I, CD1D2
GO:0009725~response to hormone stimulus	-2.48713	IRS2, FOXO1, PDE3B, JAK2, LPIN1, PIK3R1, HMGB1-PS5, ACVR1C, NR1H3
GO:0051249~regulation of lymphocyte activation	-2.53318	GM614, CBLB, BCL6, CD1D1, FOXP3, GM5511, CD27, CLEC2I, CD1D2
GO:0045087~innate immune response	-2.55686	IL18R1, IL1R1, HC, TLR1, TLR6, CD1D1, CD1D2
GO:0050865~regulation of cell activation	-2.63061	GM614, CBLB, BCL6, RORA, CD1D1, FOXP3, GM5511, CD27, CLEC2I, CD1D2
GO:0002694~regulation of leukocyte activation	-2.66478	GM614, CBLB, BCL6, RORA, CD1D1, FOXP3, GM5511, CD27, CLEC2I, CD1D2

GO:0045321~leukocyte activation	-2.70669	SATB1, SLA2, TLR1, JAG2, SMAD3, FOXP3, CD1D1, CD1D2, H60B, CBLB, CXCR4, BCL6, PIK3R1, CD27
GO:0009719~response to endogenous stimulus	-2.72593	GNAL, IRS2, FOXO1, PDE3B, JAK2, LPIN1, PIK3R1, DDIT3, HMGB1-PS5, ACVR1C, NR1H3
GO:0044092~negative regulation of molecular function	-2.76347	ADRB2, SPRY1, S1PR1, CDKN1B, ZFYVE28, JAK2, FOXP3, DDIT3
GO:0051094~positive regulation of developmental process	-2.76993	GM614, FGFR1, EPC1, ADRB2, ETS1, PLXNB1, BTG1, TGIF1, CD1D1, FOXP3, GM5511, CD27, HMGB1-PS5, CD1D2
GO:0046649~lymphocyte activation	-2.86475	H60B, SATB1, CBLB, CXCR4, SLA2, JAG2, SMAD3, BCL6, CD1D1, FOXP3, PIK3R1, CD27, CD1D2
GO:0045597~positive regulation of cell differentiation	-2.86612	GM614, EPC1, ETS1, PLXNB1, BTG1, TGIF1, CD1D1, FOXP3, GM5511, CD27, HMGB1-PS5, CD1D2
GO:0001817~regulation of cytokine production	-2.95234	TIA1, TLR1, BCL6, TLR6, CD1D1, H2-Q6, FOXP3, CD27, CLEC2I, CD1D2
GO:0042110~T cell activation	-3.14464	SATB1, CBLB, CXCR4, SLA2, JAG2, SMAD3, CD1D1, FOXP3, CD1D2
GO:0050863~regulation of T cell activation	-3.37758	GM614, CBLB, BCL6, CD1D1, FOXP3, GM5511, CD27, CLEC2I, CD1D2
GO:0032318~regulation of Ras GTPase activity	-3.4198	SPRY1, S1PR1, ASAP1, BCL6, RICTOR, ARAP2
GO:0043087~regulation of GTPase activity	-3.43206	SPRY1, S1PR1, RASGRP3, ASAP1, BCL6, RICTOR, ARAP2
GO:0051098~regulation of binding	-3.54646	ADRB2, SMAD3, JAK2, FOXP3, EIF2AK3, DDIT3, JMY
GO:0043434~response to peptide hormone stimulus	-3.83977	IRS2, FOXO1, PDE3B, JAK2, LPIN1, PIK3R1, ACVR1C, NR1H3
GO:0045619~regulation of lymphocyte differentiation	-4.71696	GM614, BCL6, CD1D1, FOXP3, GM5511, CD27, CD1D2
GO:0030335~positive regulation of cell migration	-4.92944	IRS2, S1PR1, PIK3R1, HMGB1-PS5
GO:0042035~regulation of cytokine biosynthetic process	-4.98721	TIA1, TLR1, TLR6, CD1D1, FOXP3, CD27, CLEC2I, CD1D2

GO:0032868~response to insulin stimulus	-5.40985	IRS2, FOXO1, PDE3B, LPIN1, PIK3R1, ACVR1C, NR1H3
GO:0032870~cellular response to hormone stimulus	-5.50312	IRS2, FOXO1, PDE3B, JAK2, LPIN1, PIK3R1, NR1H3
GO:0045580~regulation of T cell differentiation	-5.69966	GM614, BCL6, CD1D1, FOXP3, GM5511, CD27, CD1D2
GO:0032649~regulation of interferon-gamma production	-5.69966	CD1D1, H2-Q6, FOXP3, CD27, CD1D2
GO:0051100~negative regulation of binding	-6.07964	ADRB2, JAK2, FOXP3, DDIT3
GO:0032869~cellular response to insulin stimulus	-6.36242	IRS2, FOXO1, PDE3B, LPIN1, PIK3R1, NR1H3
GO:0008286~insulin receptor signaling pathway	-6.5139	IRS2, FOXO1, PIK3R1, NR1H3
GO:0046634~regulation of alpha-beta T cell activation	-6.70549	GM614, CBLB, BCL6, CD1D1, FOXP3, CD1D2
GO:0045621~positive regulation of lymphocyte differentiation	-6.70549	GM614, CD1D1, FOXP3, GM5511, CD27, CD1D2
GO:0045582~positive regulation of T cell differentiation	-7.12458	GM614, CD1D1, FOXP3, GM5511, CD27, CD1D2
GO:0046637~regulation of alpha-beta T cell differentiation	-8.29042	GM614, BCL6, CD1D1, FOXP3, CD1D2
GO:0045076~regulation of interleukin-2 biosynthetic process	-9.77085	CD1D1, FOXP3, CLEC2I, CD1D2
GO:0002711~positive regulation of T cell mediated immunity	-10.5225	CD1D1, H2-Q6, FOXP3, CD1D2
GO:0043370~regulation of CD4-positive, alpha beta T cell differentiation	-12.4356	GM614, BCL6, FOXP3
GO:0045072~regulation of interferon-gamma biosynthetic process	-12.4356	CD1D1, FOXP3, CD27, CD1D2