Supporting Information

Supplemental Figures

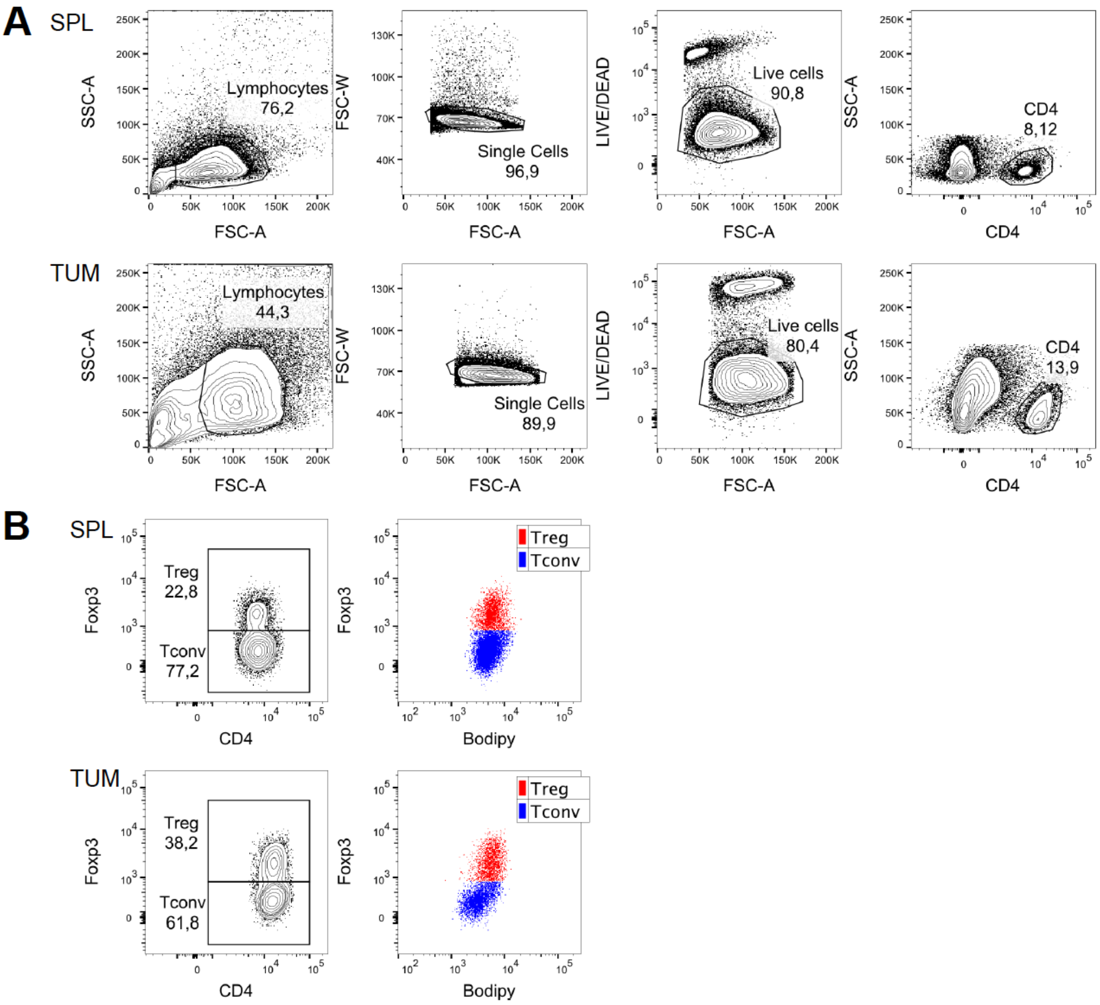


Figure S1. Gating strategy for Treg/Tconv identification in SPL and TUM by flow cytometry.

Flow cytometry analysis was performed on lymphocytes extracted from single spleens (SPL) and tumors (TUM) from C57BL/6 mice bearing MCA38 nodules. **(A)** Sequential hierarchical gating strategy for the selection of CD4 T cells. **(B)** In gated CD4 T cells, Tregs and Tconvs were selected according to Foxp3 intracellular expression. *Right*, Bodipy levels in overlaid Treg/Tconv subsets.

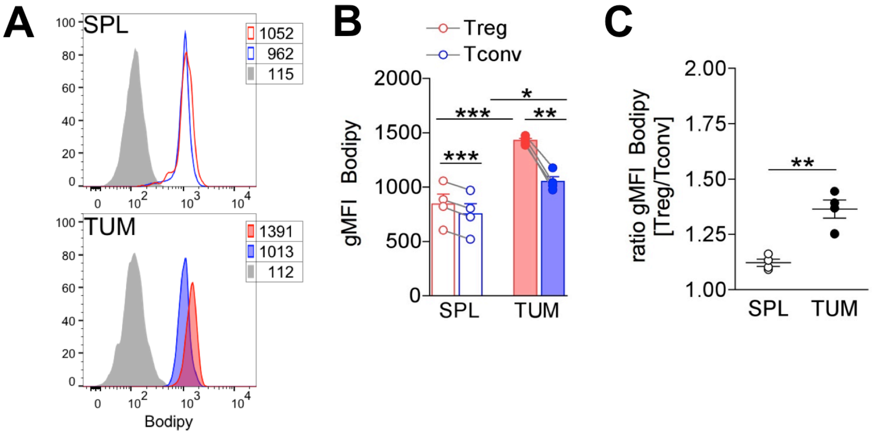


Figure S2. Tumor-infiltrating Tregs accumulate intracellular lipids also in another tumor model (B16F10).

10^5 B16F10 cells were injected s.c. into C57BL/6 mice, and flow cytometry analysis was performed on lymphocytes extracted from spleen (SPL) and tumor (TUM) after 14 days. **(A)** Representative histograms and **(B)** geometric mean fluorescence intensity (gMFI) analysis of Bodipy staining in gated CD25+Foxp3+ Tregs (red) and CD25-Foxp3- Tconvs (blue) in SPL (empty peaks and bars) and TUM (solid peaks and bars) from tumor-bearing mice. Peaks in grey represent the fluorescence-minus-one (FMO) controls. **(C)** Ratio between Bodipy gMFI in Tregs and Tconvs from SPL and TUM. Error bars show mean ± SEM, each dot corresponds to a single mouse. Data shown are from a representative out of two independent experiments. *p < 0.05, **p < 0.01, by Student t test, paired between Tregs and Tconvs in the same samples; unpaired, between populations of different samples.

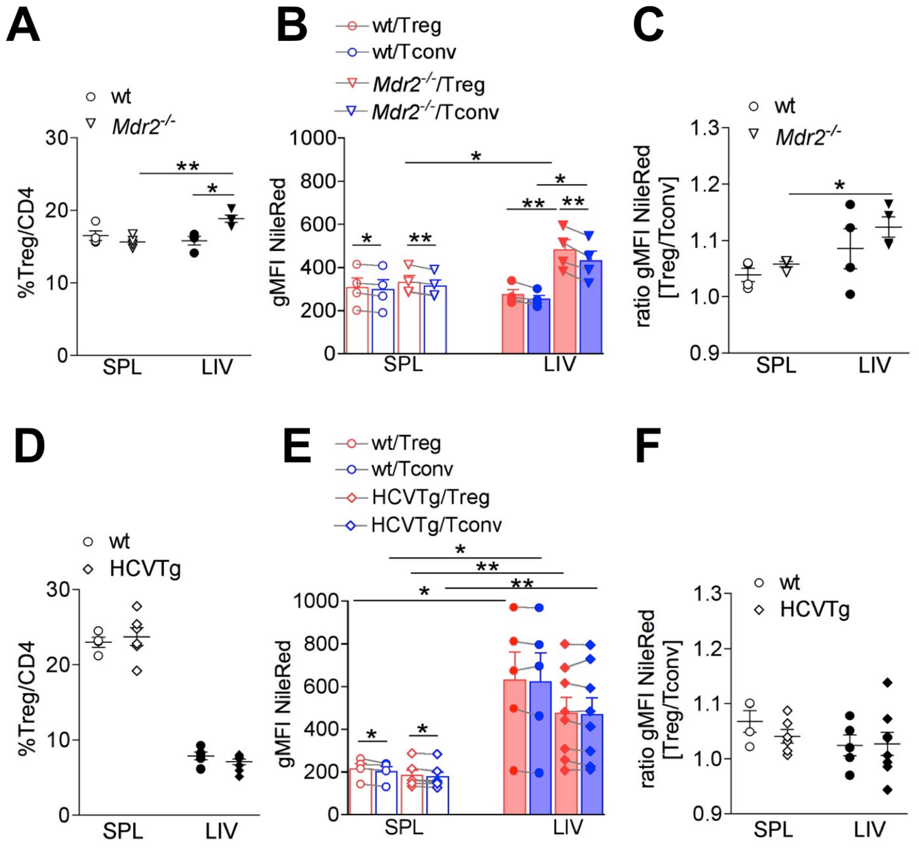


Figure S3. High intracellular lipid accumulation occurs in expanding Tregs also in the inflamed hepatic microenvironment.

(A-C) Analysis of CD25+Foxp3+ Tregs and CD25-Foxp3- Tconvs from spleen (SPL) and liver (LIV) of wild type (wt) and *Mdr2*$^{-/-}$ mice. **(A)** Frequency of Tregs in gated CD4+ lymphocytes from the indicated samples. **(B)** Geometric mean fluorescence intensity (gMFI) of Nile Red staining in Tregs (red) and Tconvs (blue) from spleen (empty bars) and liver (filled bars) of wt and *Mdr2*$^{-/-}$ mice. **(C)** Ratio between Nile Red gMFI in Tregs and Tconvs in spleen and liver of wt and *Mdr2*$^{-/-}$ mice.

(D-F) Analysis of CD25+Foxp3+ Tregs and CD25-Foxp3- Tconvs from spleen (SPL) and liver (LIV) of wild type (wt) and HCV transgenic (HCVTg) mice. **(D)** Frequency of Tregs in gated CD4+ lymphocytes from the indicated samples. **(E)** gMFI of Nile Red staining in Tregs (red) and Tconvs (blue) from spleen (empty bars) and liver (filled bars) of wt and HCVTg mice. **(F)** Ratio between Nile Red gMFI of Tregs and Tconvs in spleen and liver of wt and HCVTg mice.

Error bars show mean ± SEM, $*p < 0.05$, $**p < 0.01$, by Student t test, paired between Tregs and Tconvs in the same sample; unpaired, between populations of different samples. Each dot corresponds to a single mouse; data shown are from a representative out of two independent experiments.

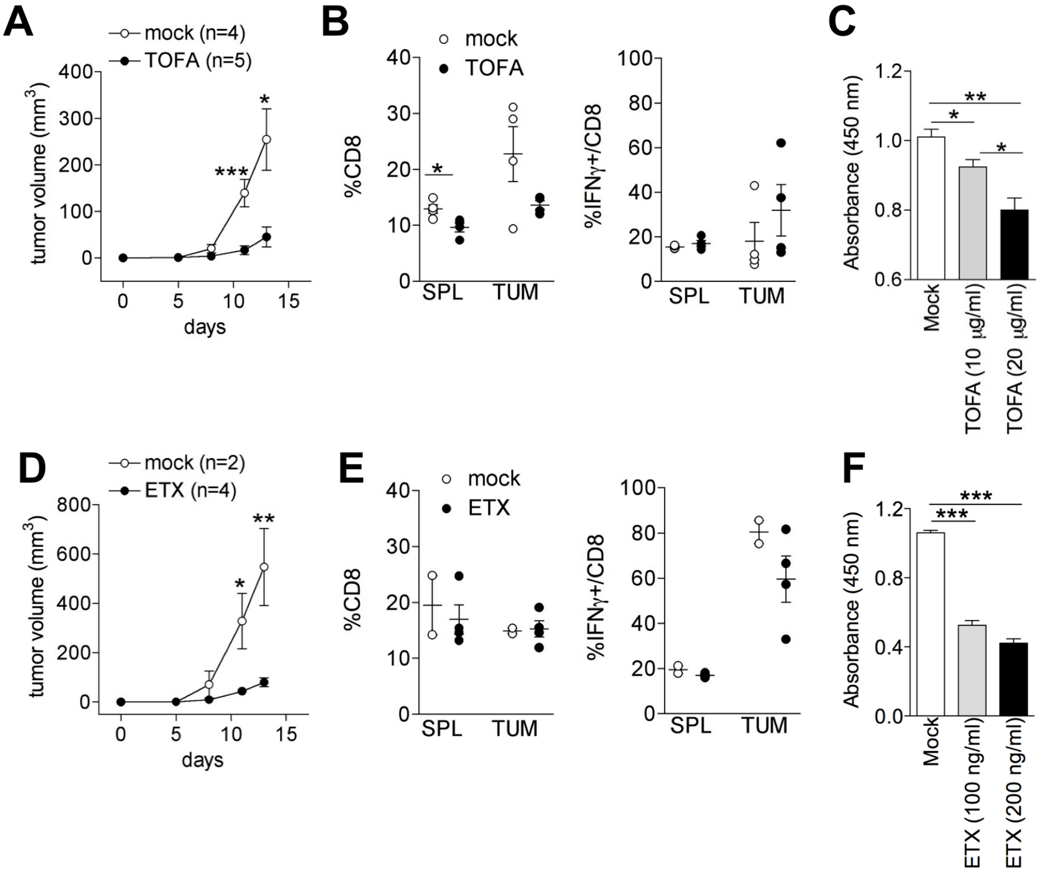


Figure S4. TOFA and Etx treatments mostly target tumor cells.

(A, D) Tumor-bearing mice were treated with repeated intra-tumor (i.t.) TOFA **(A)** or Etx **(D)** injections at days 5, 8, 11, 13 after MCA38 s.c. transplantation, and tumor growth was monitored. **(B, E)** At day 14, spleen (SPL) and tumors (TUM) were collected and tumor-infiltrating CD8 T cells characterization was performed, in terms of frequency (left panels) and IFNγ production (right panels). Data are shown as mean ± SEM, 2-5 mice were included in each experimental group; *p < 0.05, **p < 0.01, ***p < 0.005, by Student t test, unpaired.

(C, F) MCA38 cells were cultured in vitro with the indicated TOFA **(C)** or Etx **(F)** concentrations, and XTT cytotoxicity assay was performed after 24 hours. Error bars show mean ± SEM, data shown are representative of one out of two independent experiments. *p < 0.05, **p < 0.01, ***p < 0.005, by Student t test, unpaired.

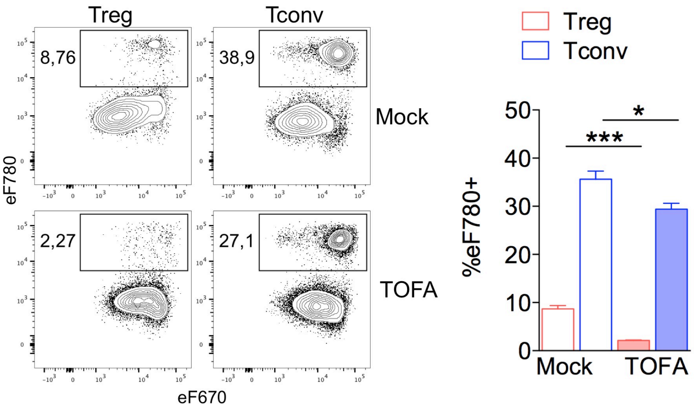


Figure S5. TOFA exposure increases Tconv and Treg survival in vitro.

Magnetically purified Tregs and Tconvs were labelled with eFluor670 and co-cultured at 1:1 ratio with feeder cells, anti-CD3 and IL-2, with or without TOFA (5 µg/ml). After 4 days, cells were stained with the viability dye eFluor780, Bodipy, CD4 and Foxp3, and flow cytometry analysis was performed in gated Tregs (CD4+Foxp3+, red) or Tconvs (CD4+Foxp3-, blue). Numbers indicate the frequency of dead cells, evaluated as the percentage of cells labelled with the viability dye eFluor780 in each gate.

Each condition was tested in triplicates; data shown are from a representative out of two independent experiments. Error bars show mean ± SEM, $*p < 0.05$, $***p < 0.005$, by Student t test, unpaired.

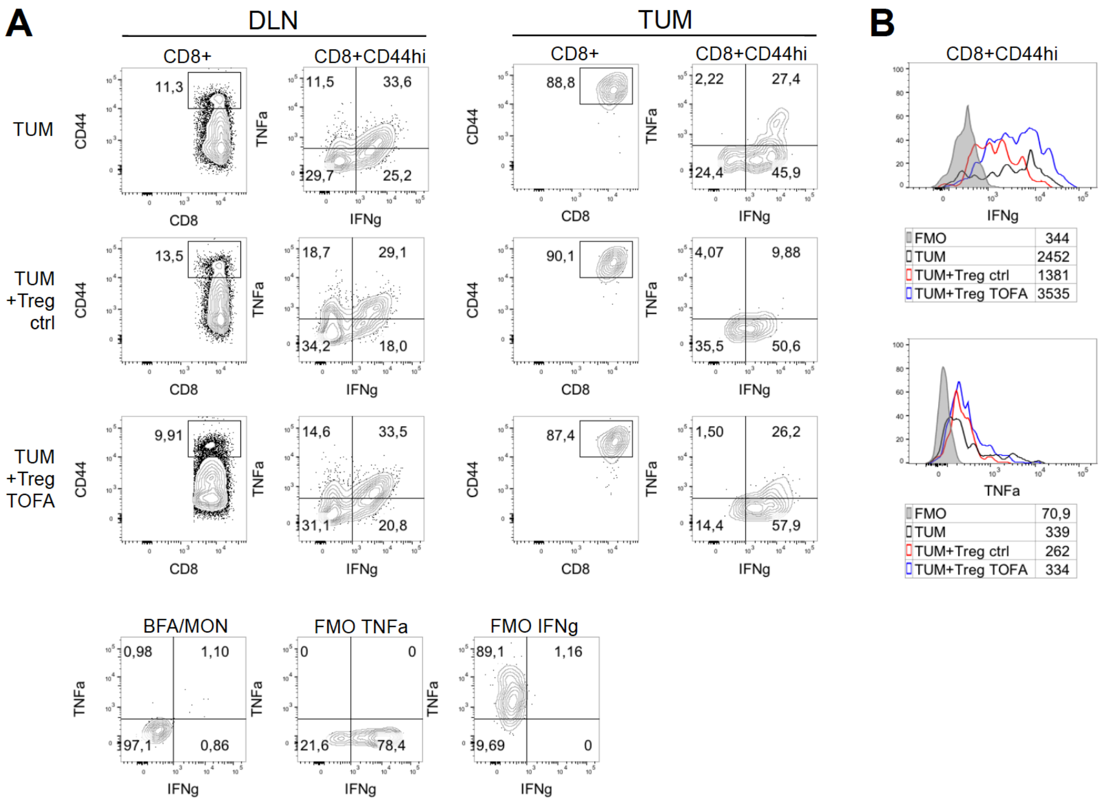
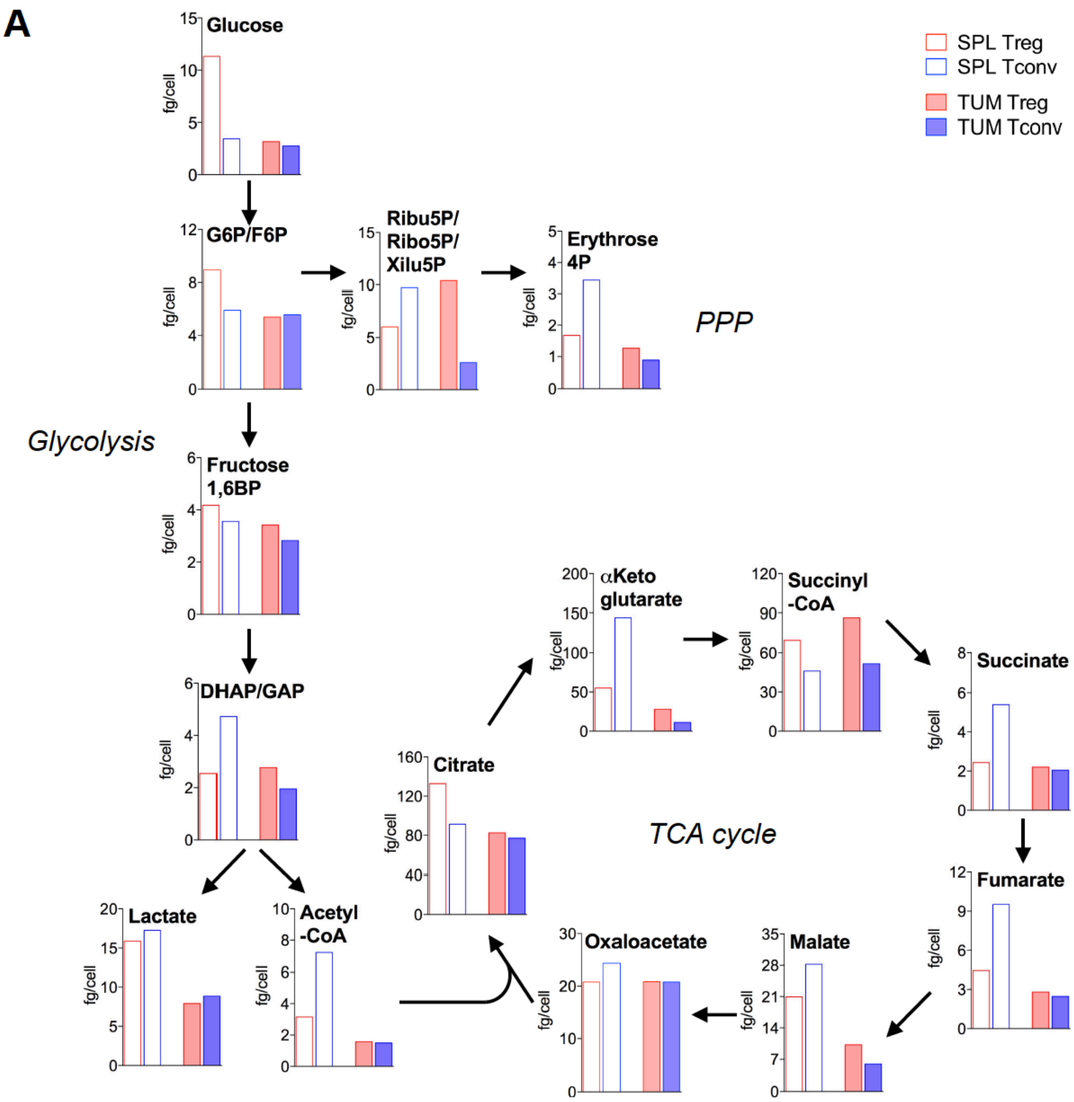


Figure S6. TOFA-treated Tregs may display lower suppressive activity against anti-tumor concomitant immunity in vivo.

Tregs were isolated through immunomagnetic separation from the spleens of naïve mice, cultured in vitro 40 hours with aCD3/IL-2, in the presence of TOFA or DMSO (ctrl). Then, Tregs were co-injected at 1:1 ratio with 10^5 MCA38 tumor cells, subcutaneously in the left flank of mice already bearing palpable MCA38-tumors on the right flank (10 days after the primary tumor inoculation); as control, tumor cells only (TUM) were injected. After further 13 days, flow cytometry analysis was performed in lymphocytes extracted from secondary tumor-draining lymph nodes (DLN) and the growing secondary tumors (2 in the group TUM, 2 in the group TUM+Treg TOFA, and only 1 in the TUM+Treg ctrl). **(A)** Contour plots showing the frequency of CD44hi in gated CD8 T cells, and of TNFα+/IFNγ+ in gated CD8+CD44hi T cells, in DLN and TUM in each experimental group. Samples in the bottom line show the staining controls on CD8+CD44hi splenocytes; FMO, fluorescence-minus-one; BFA/MON, cultured with BFA/MON only in the absence of PMA/ionomycin. **(B)** Histogram overlay of IFNγ and TNFα in TUM-infiltrating gated CD8+CD44hi in the indicated groups; numbers indicate the geometric mean fluorescence intensity (gMFI).


A
SPL Treg
SPL Tconv
TUM Treg
TUM Tconv
Glycolysis
PPP
TCA cycle
Glucose
G6P/F6P
Ribu5P/ Ribo5P/ Xilu5P
Erythrose 4P
Fructose 1,6BP
DHAP/GAP
Lactate
Acetyl -CoA
Citrate
αKeto glutarate
Succinyl -CoA
Succinate
Fumarate
Malate
Oxaloacetate
fg/cell

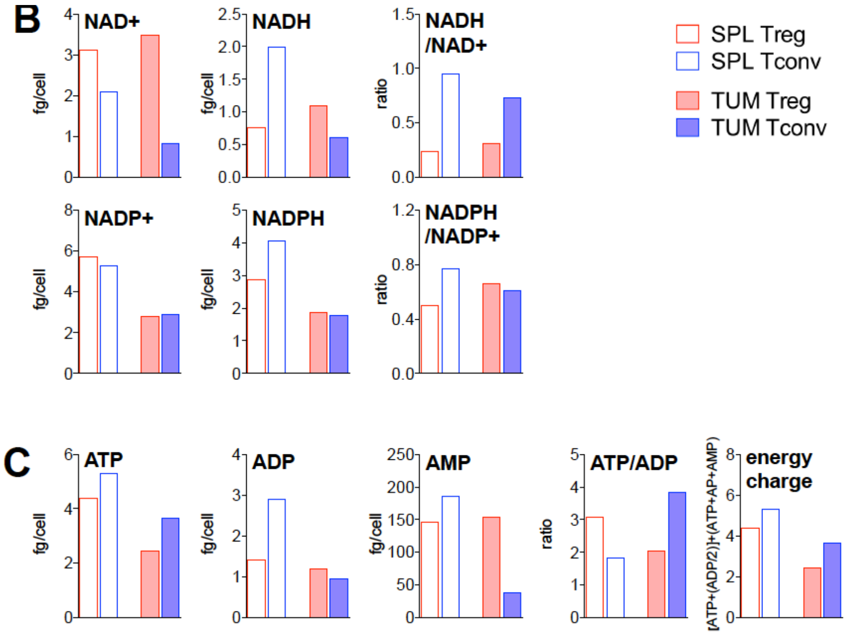


Figure S7. Metabolomic profile of Tregs and Tconvs freshly extracted from tumor and spleens.

CD4+CD25+ Tregs and CD4+CD25- Tconvs were magnetically purified from pooled spleens and tumors of tumor-bearing mice, and metabolomic profiling was performed directly ex vivo. **(A)** Diagram showing the levels of each metabolite in the context of glycolysis, pentose-phosphate, and TCA cycle pathways. **(B-C)** Levels of analytes and parameters, indicating the redox and energy status in each condition.

Table S1

List of the 216 genes found upregulated >2 fold only in Treg CT OX40+ versus Treg PB OX40-, and not in all the other cell subsets analyzed (Treg CT OX40-, Treg PB OX40+ and all Tconvs counterparts).

	RefSeq Gene	**Gene Symbol**	**Gene description**
1	Hs.413494		
2	Hs.534439		
3	Hs.554324		
4	Hs.564874		
5	Hs.571502		
6	NM_000093.2	COL5A1	collagen type V alpha 1 chain
7	NM_000130.2	F5	coagulation factor V (proaccelerin, labile factor)
8	NM_000169.1	GLA	galactosidase, alpha
9	NM_000211.1	ITGB2	integrin, beta 2
10	NM_000259.1	MYO5A	myosin VA (heavy chain 12, myoxin)
11	NM_000310.2	PPT1	palmitoyl-protein thioesterase 1
12	NM_000365.4	TPI1	triosephosphate isomerase 1
13	NM_000474.3	TWIST1	twist family bHLH transcription factor 1
14	NM_000521.2	HEXB	hexosaminidase B
15	NM_000595.2	LTA	lymphotoxin alpha (TNF superfamily, member 1)
16	NM_000616.2	CD4	CD4 molecule
17	NM_000675.3	ADORA2A	adenosine A2a receptor
18	NM_000877.2	IL1R1	interleukin 1 receptor, type I
19	NM_001001870.1	C17orf91	chromosome 17 open reading frame 91
20	NM_001017980.1	LOC203547	hypothetical protein LOC203547
21	NM_001024912.1	CEACAM1	carcinoembryonic antigen-related cell adhesion molecule 1 (biliary glycoprotein)
22	NM_001033046.1	C17orf62	chromosome 17 open reading frame 62
23	NM_001039933.1	CD79B	CD79b molecule
24	NM_001040168.1	LFNG	LFNG O-fucosylpeptide 3-beta-N-acetylglucosaminyltransferase
25	NM_001066.2	TNFRSF1B	tumor necrosis factor receptor superfamily member 1B (TNFR2)
26	NM_001130677.1	C17ORF96	chromosome 17 open reading frame 96
27	NM_001183.3	ATP6AP1	ATPase, H+ transporting, lysosomal accessory protein 1
28	NM_001252.2	TNFSF7	tumor necrosis factor (ligand) superfamily, member 7 (CD70)
29	NM_001288.4	CLIC1	chloride intracellular channel 1
30	NM_001386.4	DPYSL2	dihydropyrimidinase-like 2
31	NM_001398.2	ECH1	enoyl-CoA hydratase 1, peroxisomal
32	NM_001418.3	EIF4G2	eukaryotic translation initiation factor 4 gamma, 2
33	NM_001428.2	ENO1	enolase 1, (alpha)
34	NM_001559.2	IL12RB2	interleukin 12 receptor subunit beta 2
35	NM_001611.2	ACP5	acid phosphatase 5, tartrate resistant
36	NM_001613.1	ACTA2	actin, alpha 2, smooth muscle, aorta
37	NM_001615.3	ACTG2	actin, gamma 2, smooth muscle, enteric
38	NM_001618.2	PARP1	poly (ADP-ribose) polymerase family, member 1
39	NM_001659.1	ARF3	ADP-ribosylation factor 3
40	NM_001693.2	ATP6V1B2	ATPase, H+ transporting, lysosomal 56/58kDa, V1 subunit B, isoform 2
41	NM_001759.2	CCND2	cyclin D2
42	NM_001767.2	CD2	CD2 molecule
43	NM_001776.3	ENTPD1	ectonucleoside triphosphate diphosphohydrolase 1 (CD39)
44	NM_001779.1	CD58	CD58 molecule
45	NM_001780.3	CD63	CD63 antigen (melanoma 1 antigen)

46	NM_001814.2	CTSC	cathepsin C
47	NM_001852.3	COL9A2	collagen, type IX, alpha 2
48	NM_001903.2	CTNNA1	catenin (cadherin-associated protein), alpha 1, 102kDa
49	NM_001979.4	EPHX2	epoxide hydrolase 2, cytoplasmic
50	NM_002046.2	GAPDH	glyceraldehyde-3-phosphate dehydrogenase
51	NM_002064.1	GLRX	glutaredoxin (thioltransferase)
52	NM_002076.2	GNS	glucosamine (N-acetyl)-6-sulfatase (Sanfilippo disease IIID)
53	NM_002080.2	GOT2	glutamic-oxaloacetic transaminase 2
54	NM_002125.3	HLA-DRB5	major histocompatibility complex, class II, DR beta 5
55	NM_002167.2	ID3	inhibitor of DNA binding 3, dominant negative helix-loop-helix protein
56	NM_002194.2	INPP1	inositol polyphosphate-1-phosphatase
57	NM_002284.3	KRT86	keratin 86, type II
58	NM_002298.2	LCP1	lymphocyte cytosolic protein 1 (L-plastin)
59	NM_002305.2	LGALS1	lectin, galactoside-binding, soluble, 1 (galectin 1)
60	NM_002351.1	SH2D1A	SH2 domain protein 1A, Duncan's disease (lymphoproliferative syndrome)
61	NM_002413.3	MGST2	microsomal glutathione S-transferase 2
62	NM_002460.1	IRF4	interferon regulatory factor 4
63	NM_002475.2	MLC1SA	myosin light chain 1 slow a
64	NM_002525.1	NRD1	nardilysin (N-arginine dibasic convertase)
65	NM_002654.3	PKM2	pyruvate kinase, muscle
66	NM_002674.2	PMCH	pro-melanin concentrating hormone
67	NM_002708.3	PPP1CA	protein phosphatase 1, catalytic subunit, alpha isozyme
68	NM_002748.2	MAPK6	mitogen-activated protein kinase 6
69	NM_002790.2	PSMA5	proteasome (prosome, macropain) subunit, alpha type, 5
70	NM_002818.2	PSME2	proteasome activator subunit 2
71	NM_002872.3	RAC2	ras-related C3 botulinum toxin substrate 2 (rho family, small GTP binding protein Rac2)
72	NM_002922.3	RGS1	regulator of G-protein signaling 1
73	NM_002999.2	SDC4	syndecan 4
74	NM_003234.1	TFRC	transferrin receptor (p90, CD71)
75	NM_003355.2	UCP2	uncoupling protein 2 (mitochondrial, proton carrier)
76	NM_003365.2	UQCRC1	ubiquinol-cytochrome c reductase core protein I
77	NM_003650.2	CST7	cystatin F (leukocystatin)
78	NM_003656.3	CAMK1	Calcium/calmodulin-dependent protein kinase I
79	NM_003689.2	AKR7A2	aldo-keto reductase family 7, member A2 (aflatoxin aldehyde reductase)
80	NM_003745.1	SOCS1	suppressor of cytokine signaling 1
81	NM_003746.1	DNCL1	dynein, cytoplasmic, light polypeptide 1
82	NM_003805.3	CRADD	CASP2 and RIPK1 domain containing adaptor with death domain
83	NM_003855.2	IL18R1	interleukin 18 receptor 1
84	NM_003884.4	KAT2B	K(lysine) acetyltransferase 2B
85	NM_003975.2	SH2D2A	SH2 domain protein 2A
86	NM_003978.2	PSTPIP1	proline-serine-threonine phosphatase interacting protein 1
87	NM_004036.2	ADCY3	adenylate cyclase 3
88	NM_004040.2	RHOB	ras homolog family member B
89	NM_004120.3	GBP2	guanylate binding protein 2, interferon-inducible
90	NM_004124.2	GMFB	glia maturation factor, beta
91	NM_004225.1	MFHAS1	malignant fibrous histiocytoma amplified sequence 1
92	NM_004251.3	RAB9A	RAB9A, member RAS oncogene family
93	NM_004355.2	CD74	CD74 molecule, major histocompatibility complex, class II invariant chain
94	NM_004364.2	CEBPA	CCAAT/enhancer binding protein (C/EBP), alpha
95	NM_004642.2	CDK2AP1	cyclin-dependent kinase 2 associated protein 1
96	NM_004910.1	PITPNM1	phosphatidylinositol transfer protein, membrane-associated

			1
97	NM_004926.2	ZFP36L1	zinc finger protein 36, C3H type-like 1
98	NM_005063.4	SCD	stearoyl-CoA desaturase (delta-9-desaturase)
99	NM_005177.2	ATP6V0A1	ATPase, H+ transporting, lysosomal V0 subunit a isoform 1
100	NM_005348.2	HSP90AA1	heat shock protein 90kDa alpha (cytosolic), class A member 1
101	NM_005384.2	NFIL3	nuclear factor, interleukin 3 regulated
102	NM_005415.3	SLC20A1	solute carrier family 20 (phosphate transporter), member 1
103	NM_005436.2	CCDC6	coiled-coil domain containing 6
104	NM_005532.3	IFI27	interferon, alpha-inducible protein 27
105	NM_005533.2	IFI35	interferon-induced protein 35
106	NM_005755.2	EBI3	Epstein-Barr virus induced 3
107	NM_005937.2	MLLT6	myeloid/lymphoid or mixed-lineage leukemia
108	NM_005966.3	NAB1	NGFI-A binding protein 1
109	NM_006007.1	ZFAND5	zinc finger, AN1-type domain 5
110	NM_006110.1	CD2BP2	CD2 (cytoplasmic tail) binding protein 2
111	NM_006120.2	HLA-DMA	major histocompatibility complex, class II, DM alpha
112	NM_006137.6	CD7	CD7 molecule
113	NM_006407.3	ARL6IP5	ADP ribosylation factor like GTPase 6 interacting protein 5
114	NM_006556.2	PMVK	phosphomevalonate kinase
115	NM_006564.1	CXCR6	chemokine (C-X-C motif) receptor 6
116	NM_006759.3	UGP2	UDP-glucose pyrophosphorylase 2
117	NM_006824.1	EBNA1BP2	EBNA1 binding protein 2
118	NM_006869.1	CENTA1	centaurin, alpha 1
119	NM_007063.2	TBC1D8	TBC1 domain family, member 8 (with GRAM domain)
120	NM_007200.3	AKAP13	kinase (PRKA) anchor protein 13
121	NM_007254.2	PNKP	polynucleotide kinase 3'-phosphatase
122	NM_012428.1	SDFR1	stromal cell derived factor receptor 1
123	NM_014038.1	BZW2	basic leucine zipper and W2 domains 2
124	NM_014061.3	MAGEH1	melanoma antigen family H, 1
125	NM_014142.2	NUDT5	nudix hydrolase 5
126	NM_014241.2	PTPLA	protein tyrosine phosphatase-like (proline instead of catalytic arginine), member a
127	NM_014380.1	NGFRAP1	nerve growth factor receptor (TNFRSF16) associated protein 1
128	NM_014383.1	ZBTB32	zinc finger and BTB domain containing 32
129	NM_014508.2	APOBEC3C	apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like 3C
130	NM_014583.2	LMCD1	LIM and cysteine-rich domains 1
131	NM_014661.3	FAM53B	family with sequence similarity 53 member B
132	NM_014832.1	TBC1D4	TBC1 domain family, member 4
133	NM_015124.2	GRAMD4	GRAM domain containing 4
134	NM_015150.1	RFTN1	raftlin, lipid raft linker 1
135	NM_015187.1	KIAA0746	KIAA0746 protein
136	NM_015404.1	DFNB31	deafness, autosomal recessive 31
137	NM_015528.1	RNF167	ring finger protein 167
138	NM_015559.1	SETBP1	SET binding protein 1
139	NM_015994.2	ATP6V1D	ATPase, H+ transporting, lysosomal 34kDa, V1 subunit D
140	NM_016053.1	CCDC53	coiled-coil domain containing 53
141	NM_016086.2	STYXL1	serine/threonine/tyrosine interacting-like 1
142	NM_016459.3	MZB1	marginal zone B and B1 cell-specific protein
143	NM_016497.2	MRPL51	mitochondrial ribosomal protein L51
144	NM_016633.2	AHSP	alpha hemoglobin stabilizing protein
145	NM_017515.3	SLC35F2	solute carrier family 35, member F2
146	NM_017654.2	SAMD9	sterile alpha motif domain containing 9
147	NM_017664.2	ANKRD10	ankyrin repeat domain 10
148	NM_017773.2	LAX1	lymphocyte transmembrane adaptor 1

149	NM_017775.2	TTC19	tetratricopeptide repeat domain 19
150	NM_017791.1	FLVCR2	feline leukemia virus subgroup C cellular receptor family, member 2
151	NM_018064.2	AKIRIN2	akirin 2
152	NM_018129.1	PNPO	pyridoxamine 5'-phosphate oxidase
153	NM_018407.4	LAPTM4B	lysosomal protein transmembrane 4 beta
154	NM_018590.3	CSGALNACT2	chondroitin sulfate N-acetylgalactosaminyltransferase 2
155	NM_018950.1	HLA-F	major histocompatibility complex, class I, F
156	NM_019026.2	TMCO1	transmembrane and coiled-coil domains 1
157	NM_019043.3	APBB1IP	amyloid beta (A4) precursor protein-binding, family B, member 1 interacting protein
158	NM_020441.2	CORO1B	coronin, actin binding protein, 1B
159	NM_020531.2	APMAP	adipocyte plasma membrane associated protein
160	NM_021127.1	PMAIP1	phorbol-12-myristate-13-acetate-induced protein 1
161	NM_021270.2	LAIR2	leukocyte-associated immunoglobulin-like receptor 2
162	NM_021983.4	HLA-DRB4	major histocompatibility complex, class II, DR beta 4
163	NM_022153.1	C10ORF54	chromosome 10 open reading frame 54
164	NM_022476.2	AKTIP	AKT interacting protein
165	NM_022748.9	TNS3	tensin 3
166	NM_023927.1	GRAMD3	GRAM domain containing 3
167	NM_023933.1	FAM173A	family with sequence similarity 173, member A
168	NM_024092.1	TMEM109	transmembrane protein 109
169	NM_024298.2	MBOAT7	membrane bound O-acyltransferase domain containing 7
170	NM_024508.3	ZBED2	zinc finger, BED-type containing 2
171	NM_024928.3	OBFC1	oligonucleotide/oligosaccharide-binding fold containing 1
172	NM_025216.2	WNT10A	wingless-type MMTV integration site family member 10A
173	NM_025250.2	TTYH3	tweety family member 3
174	NM_031243.1	HNRPA2B1	heterogeneous nuclear ribonucleoprotein A2/B1
175	NM_032199.1	ARID5B	AT rich interactive domain 5B (MRF1-like)
176	NM_032704.3	TUBA1C	tubulin, alpha 1c
177	NM_032802.2	SPPL2A	signal peptide peptidase-like 2A
178	NM_033255.2	EPSTI1	epithelial stromal interaction 1 (breast)
179	NM_052941.2	GBP4	guanylate binding protein 4
180	NM_053041.1	COMMD7	COMM domain containing 7
181	NM_054025.1	B3GAT1	beta-1,3-glucuronyltransferase 1 (glucuronosyltransferase P)
182	NM_080816.2	SIRPG	signal-regulatory protein gamma
183	NM_138371.1	PCED1B	PC-esterase domain containing 1B
184	NM_138578.1	BCL2L1	BCL2-like 1
185	NM_138801.1	GALM	galactose mutarotase (aldose 1-epimerase)
186	NM_138821.1	PAM	peptidylglycine alpha-amidating monooxygenase
187	NM_139266.1	STAT1	signal transducer and activator of transcription 1, 91kDa
188	NM_144641.1	PPM1M	protein phosphatase 1M (PP2C domain containing)
189	NM_145169.1	SFT2D1	SFT2 domain containing 1
190	NM_152405.1	JMY	junction-mediating and regulatory protein
191	NM_152833.2	C9ORF69	chromosome 9 open reading frame 69
192	NM_153201.1	HSPA8	heat shock 70kDa protein 8
193	NM_153214.1	FBLN7	fibulin 7
194	NM_153332.3	ERI1	exoribonuclease 1
195	NM_153609.2	TMPRSS6	transmembrane protease, serine 6
196	NM_173343.1	IL1R2	interleukin 1 receptor, type II
197	NM_173798.2	ZCCHC12	zinc finger, CCHC domain containing 12
198	NM_173799.2	TIGIT	T cell immunoreceptor with Ig and ITIM domains
199	NM_173815.3	FLJ37464	hypothetical protein FLJ37464
200	NM_175852.3	TXLNA	taxilin alpha
201	NM_178562.2	TSPAN33	tetraspanin 33
202	NM_178834.2	LAYN	layilin

203	NM_181652.1	PRDX5	peroxiredoxin 5
204	NM_183079.1	PRNP	prion protein (p27-30)
205	NM_194071.2	CREB3L2	cAMP responsive element binding protein 3-like 2
206	NM_198828.1	LOC375449	similar to microtubule associated testis specific serine/threonine protein kinase
207	NR_001298.1	HLA-DRB6	major histocompatibility complex, class II, DR beta 6 (pseudogene)
208	NR_002733.1	DGCR5	DiGeorge syndrome critical region gene 5 (non-protein coding)
209	NR_004845.1	LOC644936	actin, beta pseudogene
210	NR_024392.1	HSPBL2	heat shock 27kDa protein-like 2 pseudogene
211	XM_925989.1	LOC642489	similar to FK506-binding protein 1A
212	XM_926168.1	LOC643025	similar to Probable phosphoglycerate mutase 4
213	XM_929420.1	LOC653377	similar to family with sequence similarity 36, member A
214	XM_939115.1	MGC4677	hypothetical protein MGC4677
215	XM_939906.1	LOC650815	similar to T-cell receptor alpha chain V region CTL-L17 precursor
216	XR_016557.2	LOC391075	misc_RNA

Table S2
List of the pathways significantly ($p<0.05$) enriched among the 216 genes upregulated in Treg CT OX40+ (www.reactome.org).

Pathway name	**Entities found**	**Entities total**	**Entities ratio**	**Entities pValue**
Interleukin-4 and 13 signaling	18	212	0.16	2.08E-7
Cytokine Signaling in Immune System	44	1006	0.075	2.45E-7
Signaling by Interleukins	31	688	0.051	1.01E-5
Fibronectin matrix formation	3	7	0.001	3.58E-4
Interferon Signaling	15	292	0.022	5.89E-4
Immune System	71	2616	0.195	9.66E-4
Transferrin endocytosis and recycling	5	39	0.003	1.01E-3
Neutrophil degranulation	20	480	0.036	1.05E-3
Glycolysis	*6*	*63*	*0.005*	*1.48E-3*
MHC class II antigen presentation	9	142	0.011	1.86E-3
Insulin receptor recycling	4	28	0.002	2.2E-3
Formation of the cornified envelope	8	130	0.01	3.9E-3
Glucose metabolism	8	131	0.01	4.08E-3
RHO GTPases activate IQGAPs	4	35	0.003	4.85E-3
Iron uptake and transport	6	83	0.006	5.64E-3
Activation of NOXA and translocation to mitochondria	2	6	0	6.08E-3
Interferon alpha/beta signaling	8	141	0.011	6.26E-3
BH3-only proteins associate with and inactivate anti-apoptotic BCL-2 members	2	8	0.001	1.05E-2
Cell surface interactions at the vascular wall	11	256	0.019	1.11E-2
Semaphorin interactions	5	73	0.005	1.38E-2
Intrinsic pathways for apoptosis	4	49	0.004	1.52E-2
Nef Mediated CD4 Down-regulation	2	10	0.001	1.61E-2
Aflatoxin activation and detoxification	4	50	0.004	1.62E-2
Sema4D induced cell migration and growth-cone collapse	3	28	0.002	1.71E-2
Regulation of IFNA signaling	3	28	0.002	1.71E-2
The role of Nef in HIV-1 replication and disease pathogenesis	3	29	0.002	1.87E-2
TNFa binds their physiological receptors	3	30	0.002	2.05E-2
Inteferon gamma signaling	8	186	0.013	2.11E-2
The role of GTSE1 in G2/M progression after G2 checkpoint	5	82	0.006	2.16E-2
AUF (hnRNP D0) binds and destabilizes mRNA	4	56	0.004	2.34E-2
Sema4D in semaphorin signaling	3	33	0.002	2.61E-2
Interleukin-12 family signaling	2	13	0.001	2.62E-2
Translocation of GLUT4 to the plasma membrane	5	87	0.006	2.69E-2
Activation of BH3-only proteins	3	36	0.003	3.26E-2
Regulation of IFNG signaling	2	16	0.001	3.82E-2
COPI-mediated anterograde transport	5	100	0.007	4.45E-2
Activation of gene expression by SREBF (SREBP)	*4*	*70*	*0.005*	*4.66E-2*
Translocation of ZAP-70 to Immunological synapse	3	42	0.003	4.76E-2