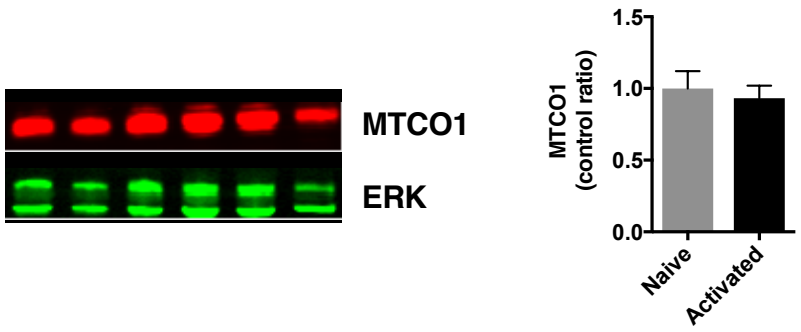
SUPPLEMENTAL FIGURES

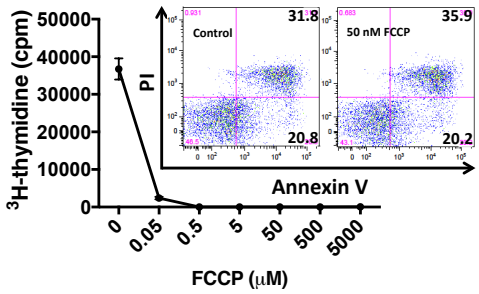
A



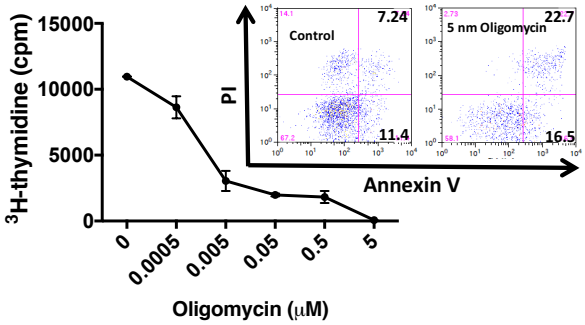
B

Accession	Description	Gene ID	Ratio: (Stimulated) / (Control)	Control SE [%]	Stimulated SE [%]	Labeled Peptides
P00405	Cytochrome c oxidase subunit 2 OS=Mus musculus GN=Mtco2 PE=1 SV=1	COX2	0.906	11.01	36.53	6
P19783	Cytochrome c oxidase subunit 4 isoform 1, mitochondrial OS=Mus musculus GN=Cox4i1 PE=1 SV=2	Cox4i1	0.982	8.84	32.87	10
P12787	Cytochrome c oxidase subunit 5A, mitochondrial OS=Mus musculus GN=Cox5a PE=1 SV=2	Cox5a	0.946	18.15	10.72	6
P19536	Cytochrome c oxidase subunit 5B, mitochondrial OS=Mus musculus GN=Cox5b PE=1 SV=1	Cox5b	1.028	7.49	17.92	8
P43024	Cytochrome c oxidase subunit 6A1, mitochondrial OS=Mus musculus GN=Cox6a1 PE=1 SV=2	Cox6a1	0.947	7.86	19.23	2
P56391	Cytochrome c oxidase subunit 6B1 OS=Mus musculus GN=Cox6b1 PE=1 SV=2	Cox6b1	0.836	1.78	17.1	7
Q9CPQ1	Cytochrome c oxidase subunit 6C OS=Mus musculus GN=Cox6c PE=1 SV=3	Cox6c	0.986	10.42	38.83	3
P48771	Cytochrome c oxidase subunit 7A2, mitochondrial OS=Mus musculus GN=Cox7a2 PE=1 SV=2	Cox7a2	0.964	13.43	33.16	3
P17665	Cytochrome c oxidase subunit 7C, mitochondrial OS=Mus musculus GN=Cox7c PE=1 SV=1	Cox7c	1.037	5.55	27.92	2

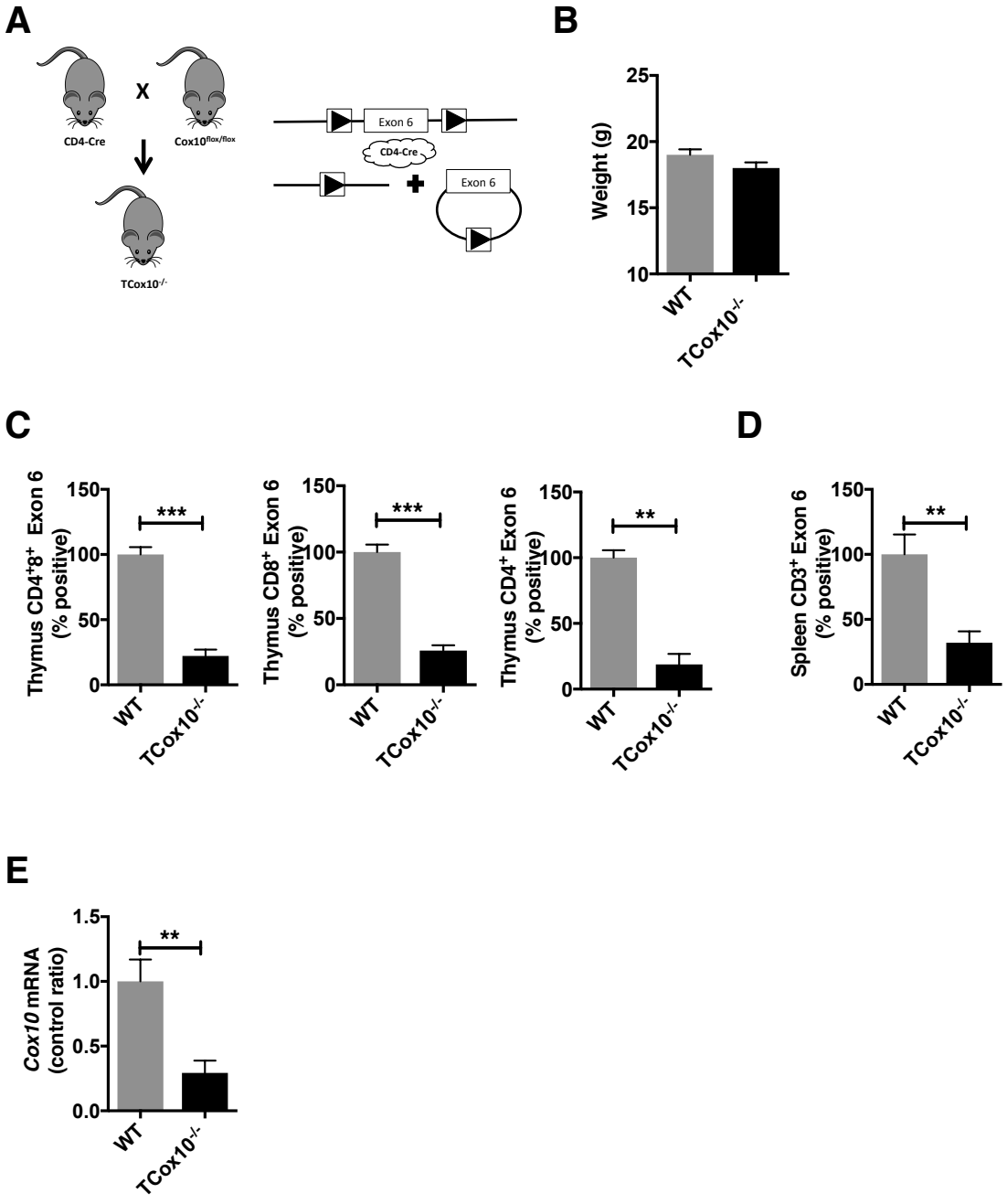
C



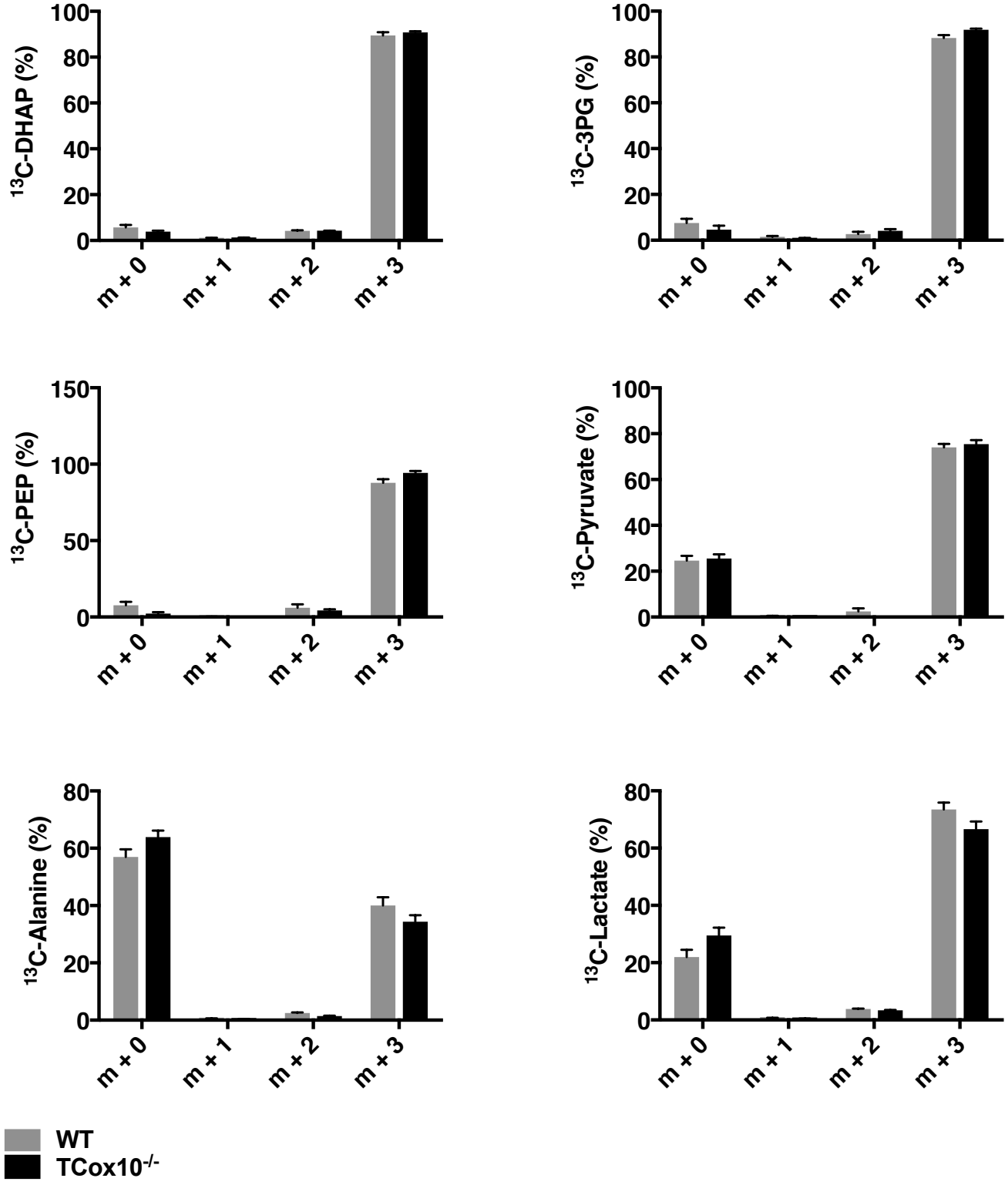
D



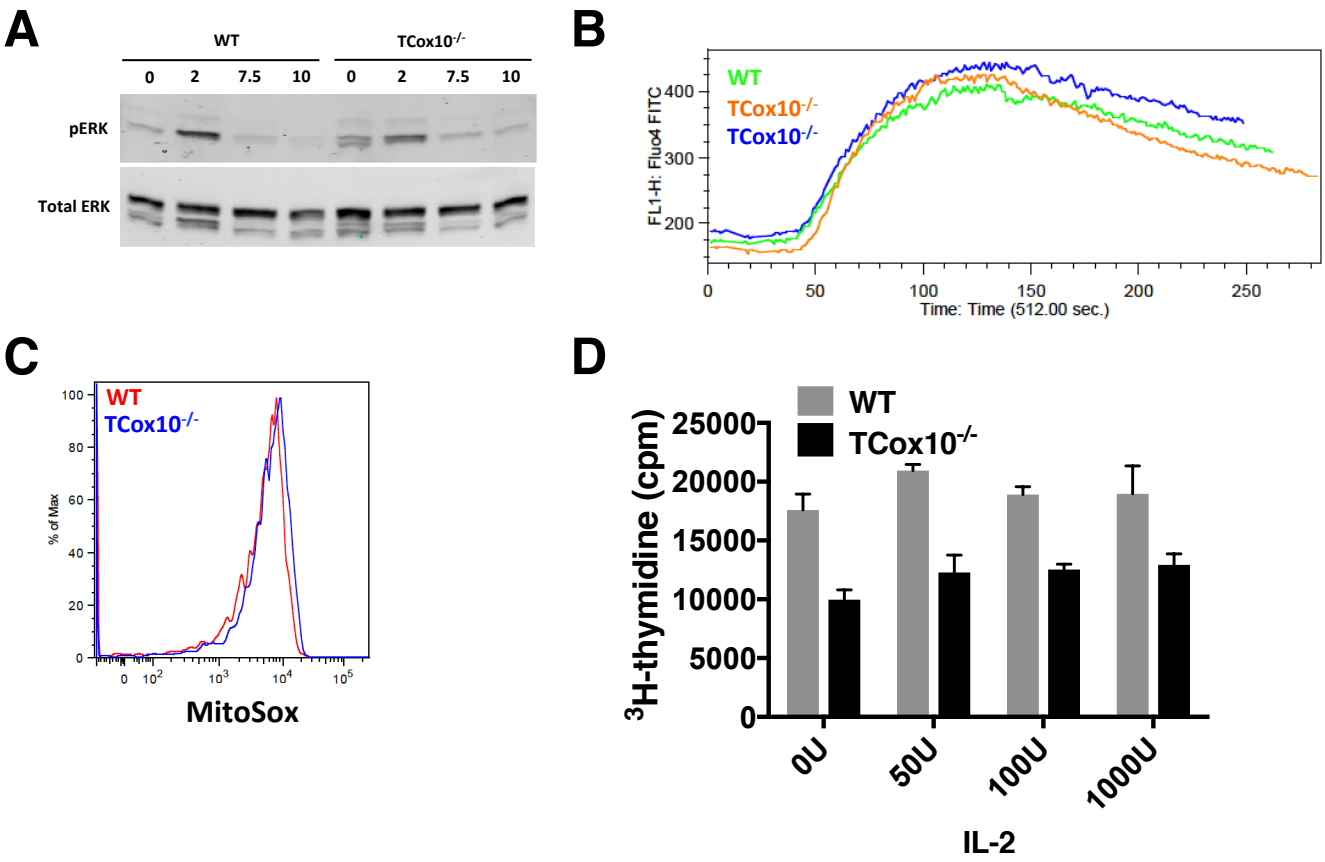
Supplemental Figure 1: Mitochondrial proteins and cell proliferation. Related to Figure 2. Mouse splenic T-cells were stimulated for 24 hours with anti-CD3/CD28. (A) Immunblot for MTCO1 (complex IV) (N = 3 / condition). (B) Proteomic analysis of COX subunits (N = 3 / condition). (C) T-cell proliferation in the presence of a mitochondrial uncoupler, FCCP (N = 3 / condition). (D) T-cell proliferation in the presence of ATP synthase inhibitor, oligomycin (N = 3 / condition). MTCO1 = Mitochondrially Encoded Cytochrome C Oxidase I. Experiments were performed 3 or more times with representative data shown. FCCP = Carbonyl cyanide 4-(trifluoromethoxy)phenylhydrazone. *P < 0.05.



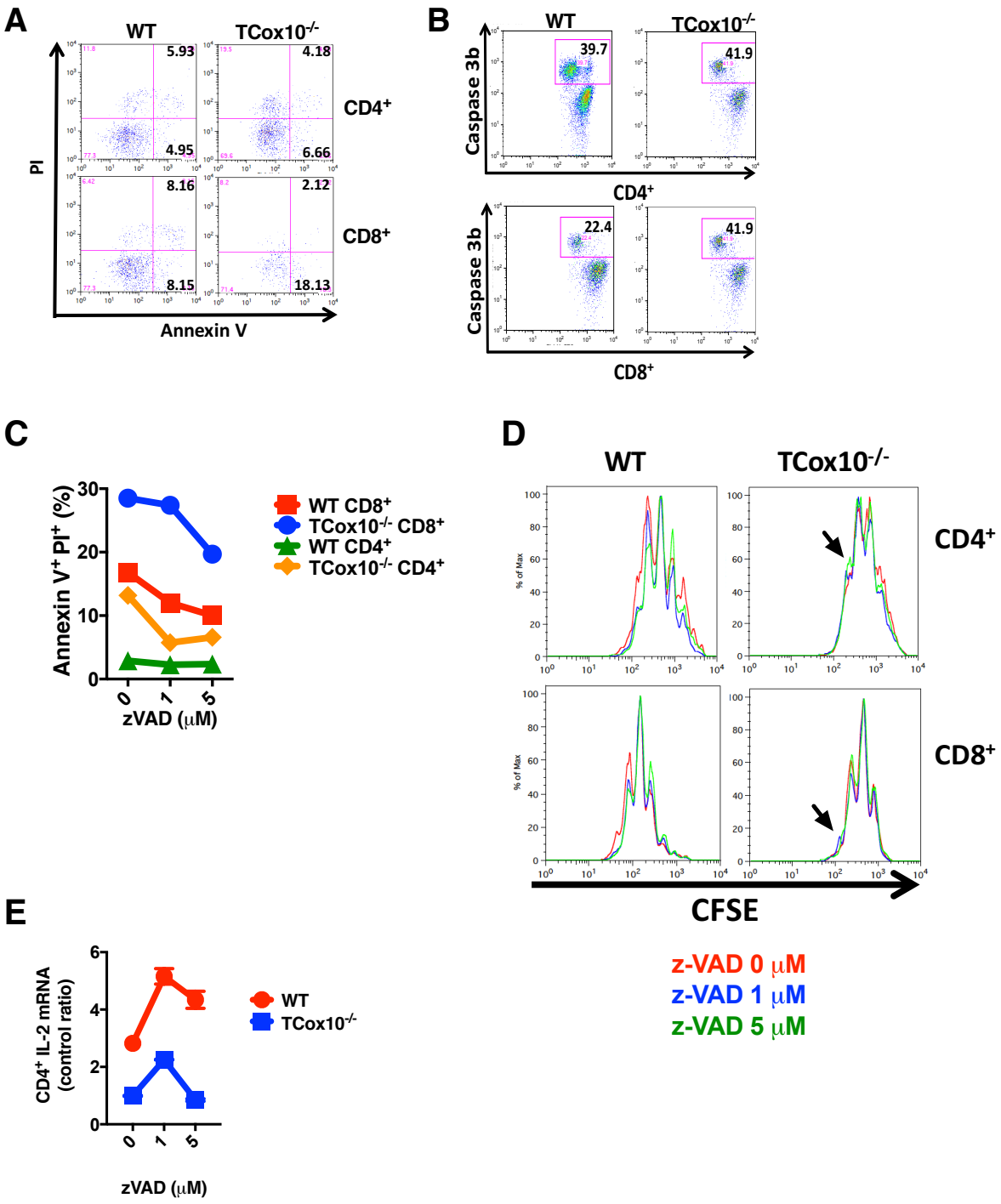
Supplemental Figure 2: Model of T-cell COX deficiency TCox10$^{-/-}$. Related to Figure 3. (A) Breeding strategy. (B) Mouse weight (N = 3 / condition). (C) Thymic *Cox10* exon 6 deletion status by PCR using targeted primers (N = 3 / condition). (D) Splenic *Cox10* exon 6 deletion status by PCR (N = 4 / condition). (E) Cox10 mRNA by PCR (N = 4 - 5 / condition). Experiments were performed 3 or more times with representative data shown. *P < 0.05., ** P < 0.01, *** P < 0.001.



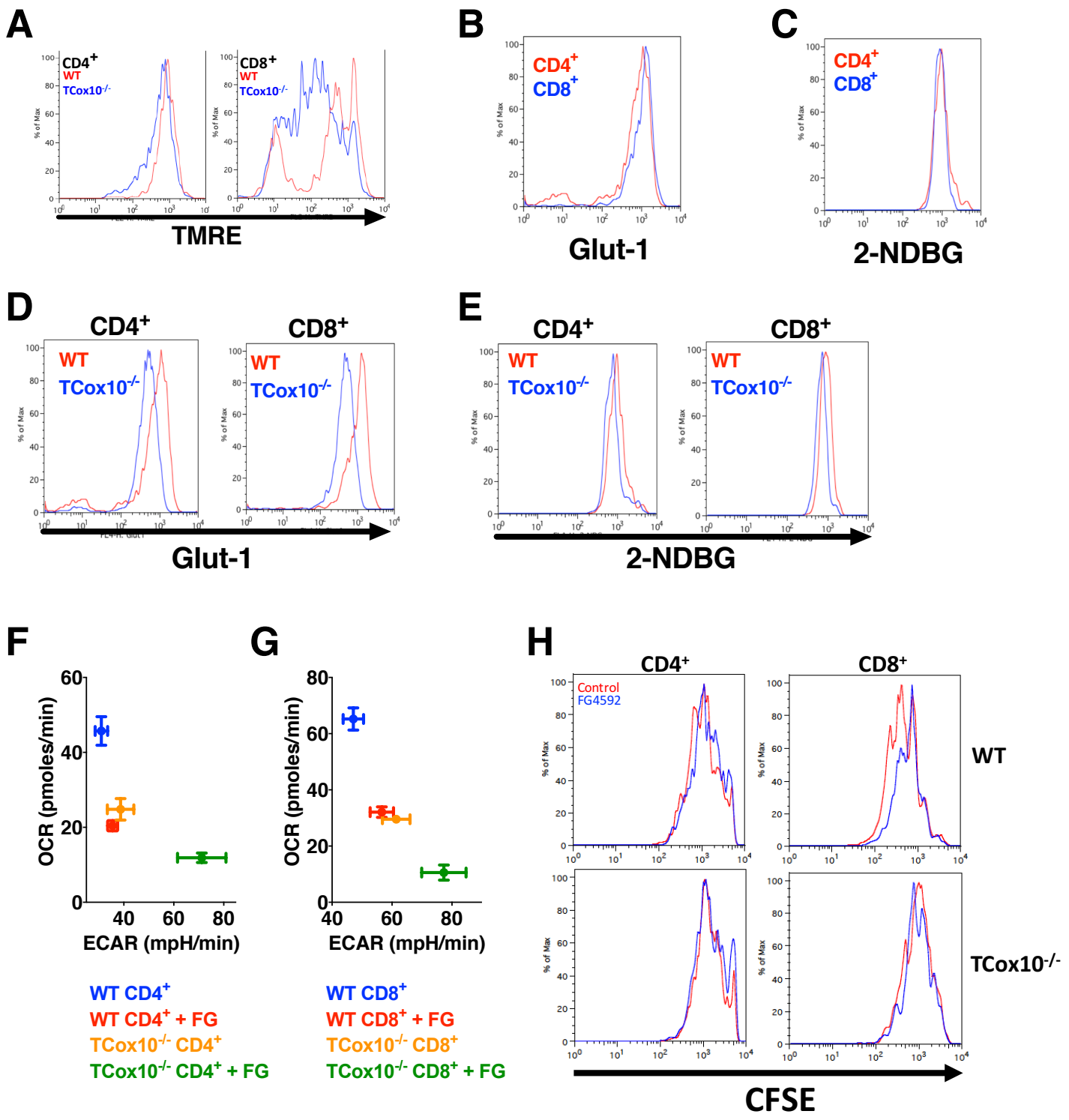
Supplemental Figure 3: Stable isotopic tracing of glycolytic intermediates. Related to Figure 3. WT and TCox10$^{-/-}$ T-cells were stimulated for 24 hours with anti-CD3/CD28. Glucose in the media was replaced with 11 mM [U ^{13}C] glucose (N = 3 / condition).



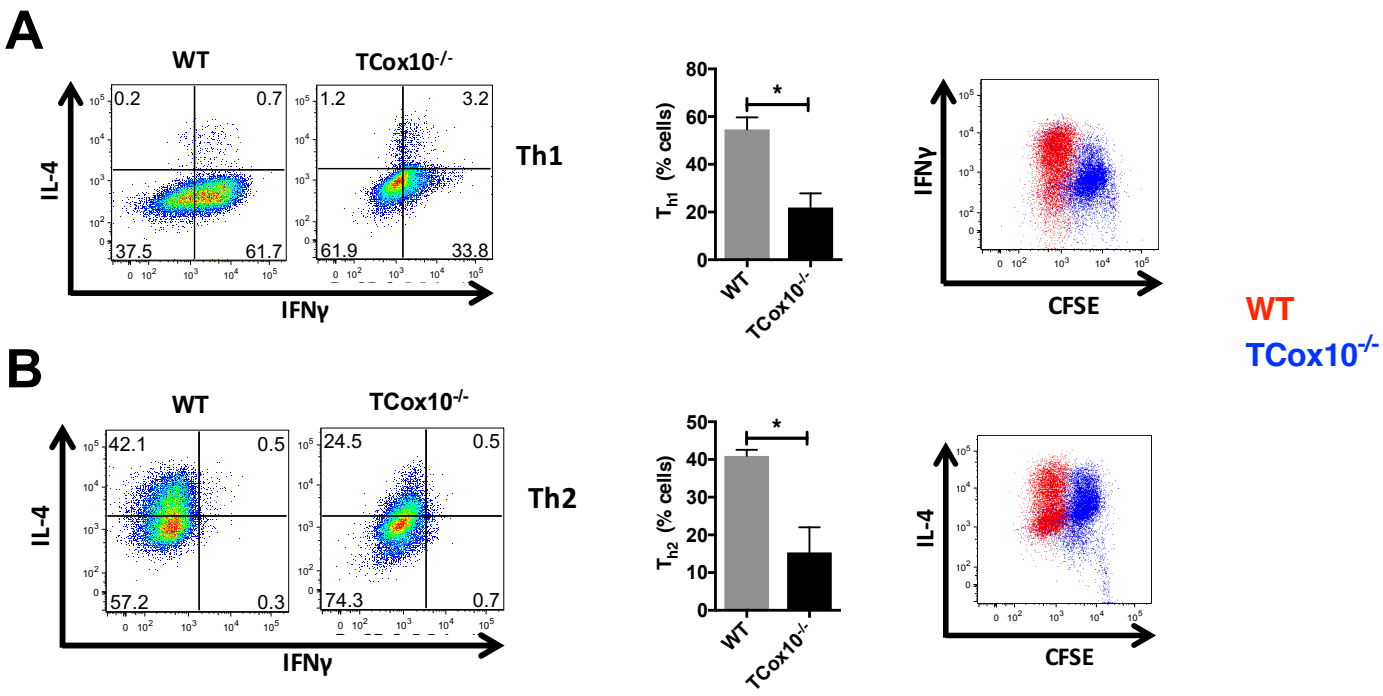
Supplemental Figure 4: TCox10$^{-/-}$ T-cell activation parameters. Related to Figure 4. Splenic T-cells were stimulated with anti-CD3/CD28 and were examined at defined timepoints. (A) ERK phosphorylation at 0, 2, 7.5 and 10 minutes. (B) Calcium flux. (C) ROS production at 24 hours (N = 3 / condition). (D) T-cell proliferation with IL-2 rescue (N = 3 / condition). Experiments were performed 3 or more times with representative data shown.



Supplemental Figure 5: Cell death in CD4$^+$ and CD8$^+$ TCox10$^{-/-}$ T-cells. Related to Figure 5. WT and TCox10$^{-/-}$ splenic T-cells were stimulated *in vitro* with anti-CD3/28 for 24 hours. (A) Apoptosis markers Annexin V and Propidium Iodide (PI) (N = 3 / condition). (B) Activated caspase 3 by flow cytometry (N = 3 / condition). (C) Cell death following treatment with zVAD-FMK. (D) Cell proliferation by CFSE during zVAD-FMK treatment. (E) IL-2 production following treatment with zVAD-FMK (N = 3 / condition). Experiments were performed 3 or more times with representative data shown.



Supplemental Figure 6: Bioenergetics of CD4$^+$ and CD8$^+$ TCox10$^{-/-}$ T-cells. Related to Figure 5. WT and Tcox10-/- splenic T-cells were stimulated *in vitro* with anti-CD3/28. (A) Mitochondrial membrane potential Ψ_m at 24 hours (N = 3 / condition). (B) Glut-1 expression on WT T-cells at 24 hours (N = 3 / condition). (C) 2-NBDG transport by WT T-cells at 24 hours (N = 3 / condition). (D) Glut-1 expression on TCox10$^{-/-}$ T-cells at 24 hours (N = 3 / condition). (E) 2-NDBG transport by Tcox10$^{-/-}$ T-cells at 24 hours (N = 3 / condition). (F) CD4$^+$ T-cell metabotype by extracellular flux analysis at 24 hours (N = 3 - 5 / condition). (G) CD8$^+$ T-cell metabotype by extracellular flux analysis at 24 hours (N = 3 - 5 / condition). (H) T-cell proliferation during treatment with FG4592 for 3 days (N = 3 / condition). 2-NBDG = 2-(*N*-(7-Nitrobenz-2-oxa-1,3-diazol-4-yl)Amino)-2-Deoxyglucose; 2-DG = 2-deoxyglucose. Experiments were performed 3 or more times with representative data shown.



Supplemental Figure 7: *In vitro* helper T-cell differentiation. Related to Figure 5. Sorted naïve CD4 T cells ($CD4^{+}CD62L^{high}CD44^{low}CD25^{neg}$) were differentiated under T_{h1} and T_{h2} conditions in the presence of T-depleted splenocytes as APCs for 3 days. Cells were re-stimulated with PMA + ionomycin and Golgi stop for 4 h and stained for intracellular IFN-γ and IL-4. (A) Th1 cells (N = 3 / condition); (B) Th2 cells (N = 3 / condition). Experiments were performed 3 or more times with representative data shown. * $P < 0.05$