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Supplemental Information

Mitochondrial Oxidative Phosphorylation Regulates the Fate Decision between Pathogenic Th17 and Regulatory T Cells

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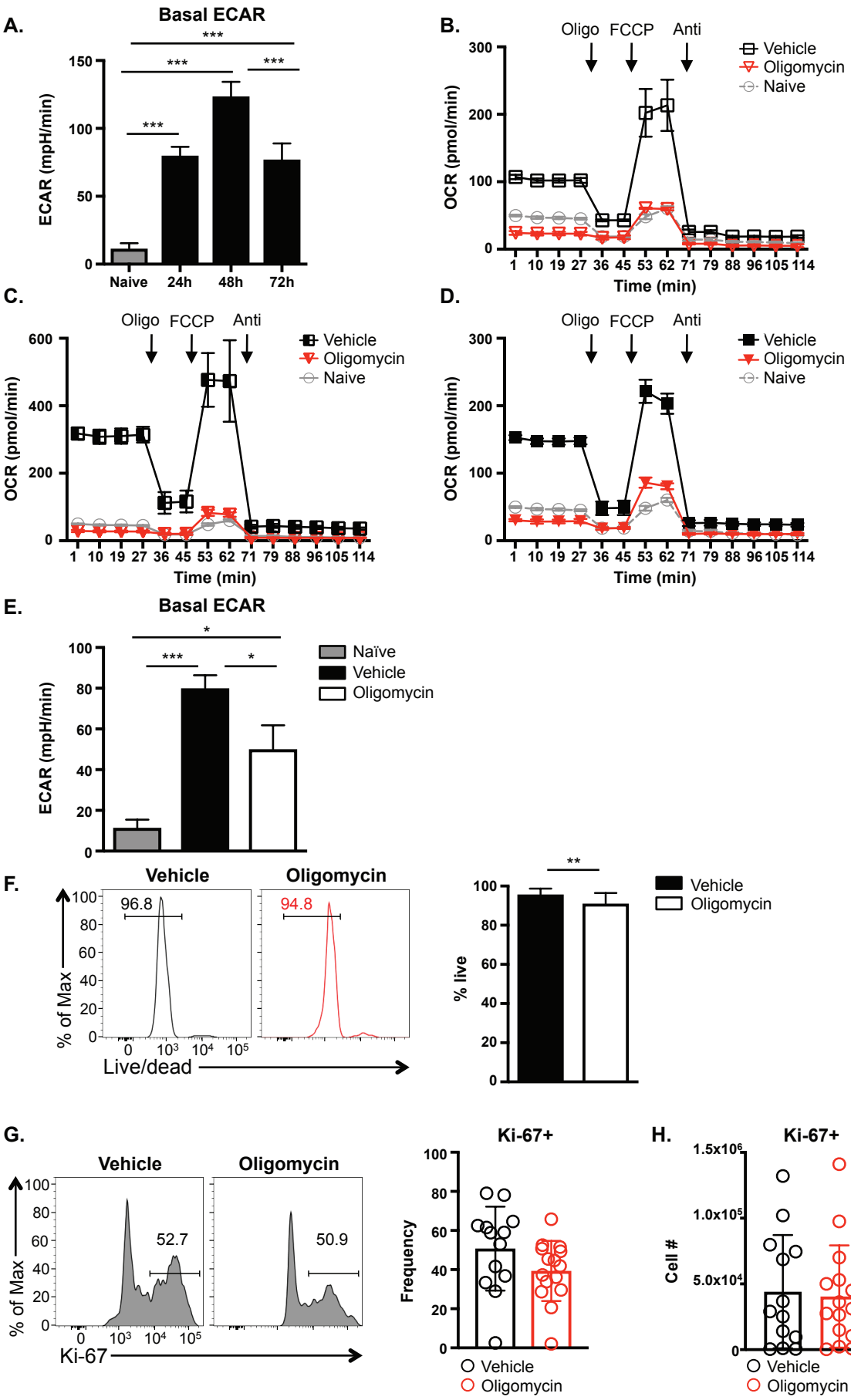
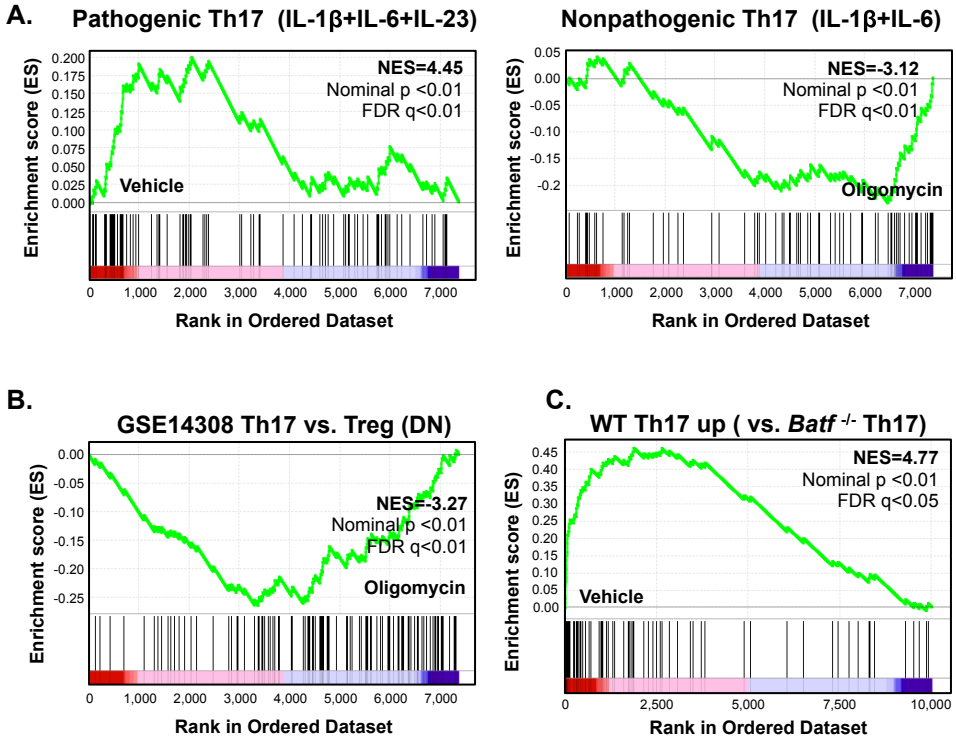
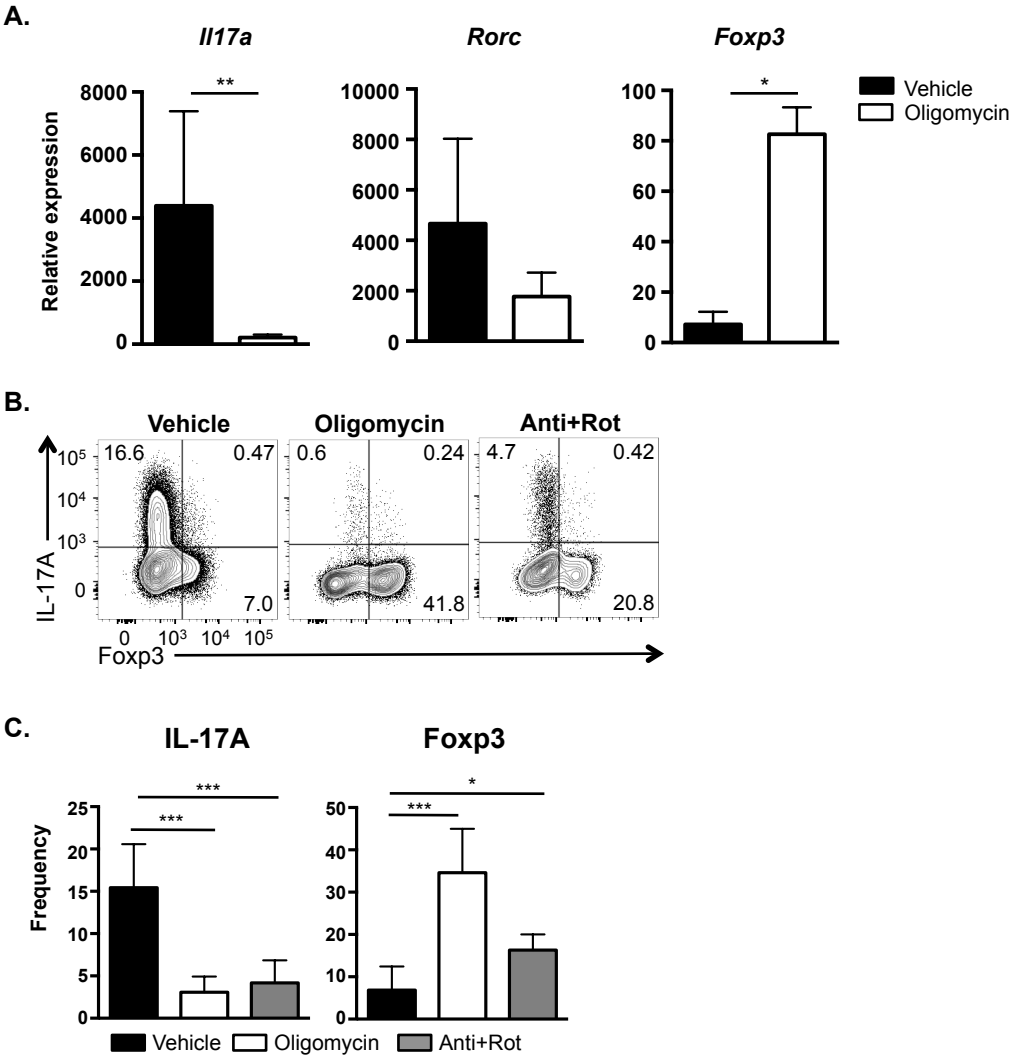

A.
Basal ECAR
ECAR (mpH/min)
Naive
24h
48h
72h
B.
Oligo
FCCP
Anti
Vehicle
Oligomycin
Naive
OCR (pmol/min)
Time (min)
C.
D.
E.
Basal ECAR
Naïve
Vehicle
Oligomycin
F.
Vehicle
Oligomycin
96.8
94.8
% of Max
Live/dead
% live
G.
52.7
50.9
Ki-67
H.
Ki-67+
Frequency
Cell #

Supplementary Figure 1. Related to Figures 1 and 2. OXPHOS inhibition alters metabolic profiles of CD4 T cells during Th17 differentiation.
(A-E) The OCR and ECAR of CD4 T cells under Th17 differentiation conditions were measured at indicated time points. **(A)** Basal ECAR of CD4 T cells. **(B-D)** The OCR of CD4 T cells during Th17 development in the presence of vehicle or oligomycin were measured at **(B)** 24h, **(C)** 48h and **(D)** 72h. **(E)** The basal ECAR of CD4 T cells at 24h of Th17 culture with vehicle or oligomycin treatment (4-7 independent experiments). **(F)** The percent of live cells were determined by flow cytometry using live/dead exclusion dye after Th17 differentiation in the presence of vehicle or oligomycin for 3 days (18 independent experiments). **(G,H)** 2D2 CD4 T cells were differentiated under Th17 conditions in the presence of vehicle or oligomycin. After 5 days, cells were transferred into Rag1 deficient recipient mice. Proliferation of cells from cervical lymph nodes was analyzed using flow cytometry on day 16-19 (3 independent experiments, n=13-15). Graphs show the average ± S.D.; (A,E,G,H) One-way ANOVA, (F) unpaired t-test, *$p<0.05$, ***$p<0.001$.

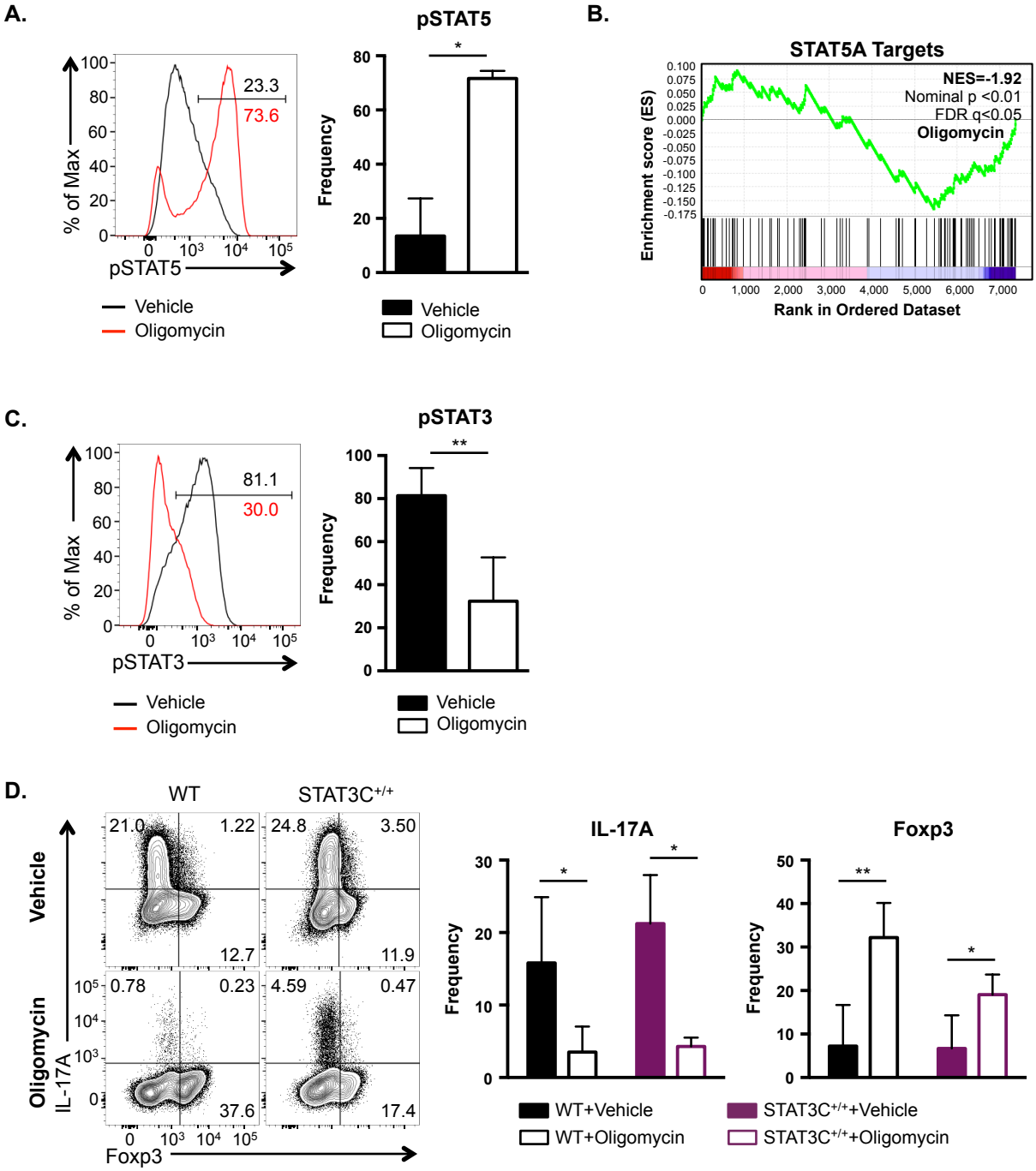

A.
Pathogenic Th17 (IL-1β+IL-6+IL-23)
Enrichment score (ES)
NES=4.45
Nominal p <0.01
FDR q<0.01
Vehicle
Rank in Ordered Dataset
Nonpathogenic Th17 (IL-1β+IL-6)
NES=-3.12
Nominal p <0.01
FDR q<0.01
Oligomycin
Rank in Ordered Dataset
B.
GSE14308 Th17 vs. Treg (DN)
NES=-3.27
Nominal p <0.01
FDR q<0.01
Oligomycin
Rank in Ordered Dataset
C.
WT Th17 up (vs. Batf -/- Th17)
NES=4.77
Nominal p <0.01
FDR q<0.05
Vehicle
Rank in Ordered Dataset

Supplementary Figure 2. Related to Figures 2 and 3. Mitochondrial OXPHOS is necessary for expression of both pathogenic and BATF-dependent Th17 gene signatures.
GSEA plots demonstrate enrichment of RNA sequencing data sets from vehicle treated and oligomycin treated CD4 T cells with previously published data sets. **(A)** Enrichment with pathogenic vs. non-pathogenic Th17 gene signature. **(B)** Enrichment with Th17 vs. Treg signature. **(C)** Enrichment with WT vs. BATF deficient Th17 gene set. NES=Normalized Enrichment Score, FDR= False Detection Rate.


A.
Il17a
Rorc
Foxp3
Relative expression
8000
6000
4000
2000
0
10000
8000
6000
4000
2000
0
100
80
60
40
20
0
**
*
Vehicle
Oligomycin
B.
Vehicle
Oligomycin
Anti+Rot
16.6
0.47
7.0
0.6
0.24
41.8
4.7
0.42
20.8
IL-17A
Foxp3
C.
IL-17A
Foxp3
Frequency
***
***
*
Vehicle
Oligomycin
Anti+Rot

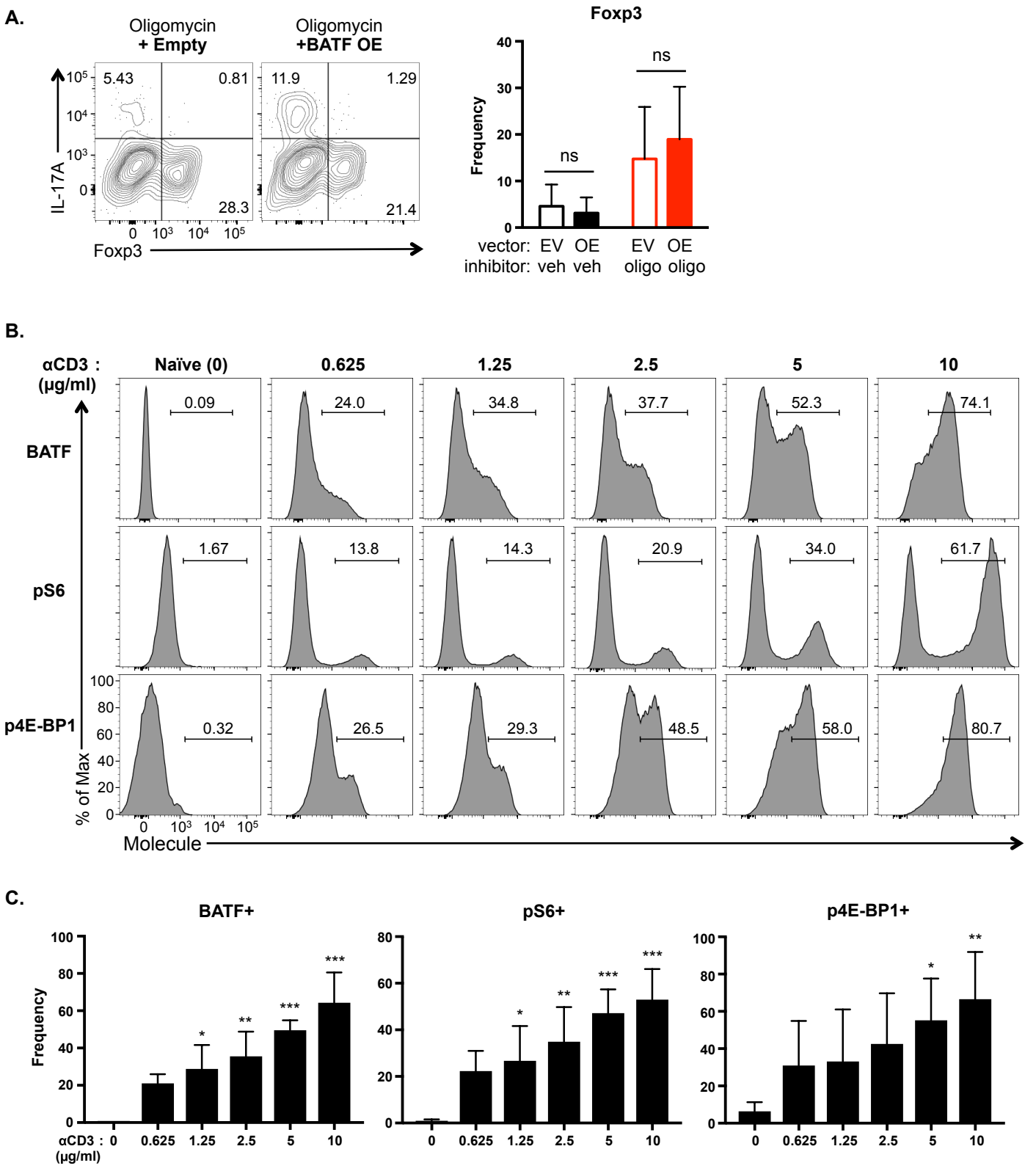
Supplementary Figure 3. Related to Figure 2. Mitochondrial OXPHOS regulates the balance between Th17 and Treg.

Naïve CD4 T cells were activated under Th17 conditions for 72h in the presence of vehicle or indicated OXPHOS inhibitor. **(A)** The relative expression of indicated genes were measured by realtime PCR (3 independent experiments). **(B,C)** The frequency of IL-17A+ and Foxp3+ CD4 T cells was assessed by flow cytometry (10 independent experiments for vehicle and oligomycin, 3 independent experiments for antimycin + rotenone). Graphs show the average ± S.D.; (A) Ratio paired t-test, (C) One-way ANOVA *$p<0.05$, **$p<0.01$, ***$p<0.001$.


A.
pSTAT5
% of Max
pSTAT5
23.3
73.6
Vehicle
Oligomycin
Frequency
*
Vehicle
Oligomycin
B.
STAT5A Targets
NES=-1.92
Nominal p <0.01
FDR q<0.05
Oligomycin
Enrichment score (ES)
Rank in Ordered Dataset
C.
pSTAT3
% of Max
pSTAT3
81.1
30.0
Vehicle
Oligomycin
Frequency
**
Vehicle
Oligomycin
D.
WT
STAT3C+/+
Vehicle
Oligomycin
IL-17A
Foxp3
21.0
1.22
12.7
24.8
3.50
11.9
0.78
0.23
37.6
4.59
0.47
17.4
IL-17A
Foxp3
Frequency
WT+Vehicle
WT+Oligomycin
STAT3C+/++Vehicle
STAT3C+/++Oligomycin

Supplementary Figure 4. Related to Figure 2. Mitochondrial OXPHOS skews STAT5/STAT3 signaling.

(A) Naïve CD4 T cells were cultured under Th17 conditions in the presence of vehicle or oligomycin for 60h. Phosphorylation of STAT5 was measured by flow cytometry (2-3 independent experiments). **(B)** GSEA plots demonstrate enrichment of RNA sequencing data sets from vehicle treated and oligomycin treated CD4 T cells with previously published STAT5A target gene set. **(C)** Naïve CD4 T cells were cultured under Th17 conditions in the presence of vehicle or oligomycin for 24h. Phosphorylation of STAT3 was measured by flow cytometry (2-3 independent experiments). **(D)** Naïve CD4 T cells from WT or STAT3C$^{+/+}$ mice were activated under Th17 conditions in the presence of vehicle or oligomycin for 72h. Representative plots are gated on live CD4 T cells and show the frequency of IL-17A^{+} and Foxp3^{+} (3 independent experiments). Graphs show the average ± S.D.; (A,C) unpaired t-test and (D) One-way ANOVA *$p<0.05$, **$p<0.01$.


A.
Oligomycin
+ Empty
Oligomycin
+BATF OE
5.43
0.81
11.9
1.29
28.3
21.4
IL-17A
Foxp3
Foxp3
Frequency
ns
ns
vector: EV OE EV OE
inhibitor: veh veh oligo oligo
B.
αCD3 :
(μg/ml)
Naïve (0)
0.625
1.25
2.5
5
10
BATF
pS6
p4E-BP1
0.09
24.0
34.8
37.7
52.3
74.1
1.67
13.8
14.3
20.9
34.0
61.7
0.32
26.5
29.3
48.5
58.0
80.7
% of Max
Molecule
C.
BATF+
pS6+
p4E-BP1+
Frequency
αCD3 : 0 0.625 1.25 2.5 5 10
(μg/ml)

Supplementary Figure 5. Related to Figures 3, 4, and 5. TCR signal strength regulates BATF and mTOR activation but BATF overexpression does not suppress Foxp3^{+} Treg generation in oligomycin treated Th17 cultures.

(A) Activated CD4 T cells were retrovirally transduced with EGFR-empty vector (EV) or EGFR-BATF overexpression vector (OE) at 24h. The expression of Foxp3^{+} and IL-17A^{+} CD4 T cells (gated on hNGFR^{+}) were analyzed on day 3-4 using flow cytometry (4 independent experiments). **(B,C)** Naïve CD4 T cells were activated with varying concentrations of plate-bound anti-CD3ε and 1μg/ml of anti-CD28 under Th17 conditions for 24h. Expression of BATF and phosphorylation of S6 (pS6) or 4E-BP1 (p4E-BP1) were measured by flow cytometry (3-4 independent experiments). Graphs show the average ± S.D.; One-way ANOVA ns=not significant, *$p<0.05$, **$p<0.01$, ***$p<0.001$.