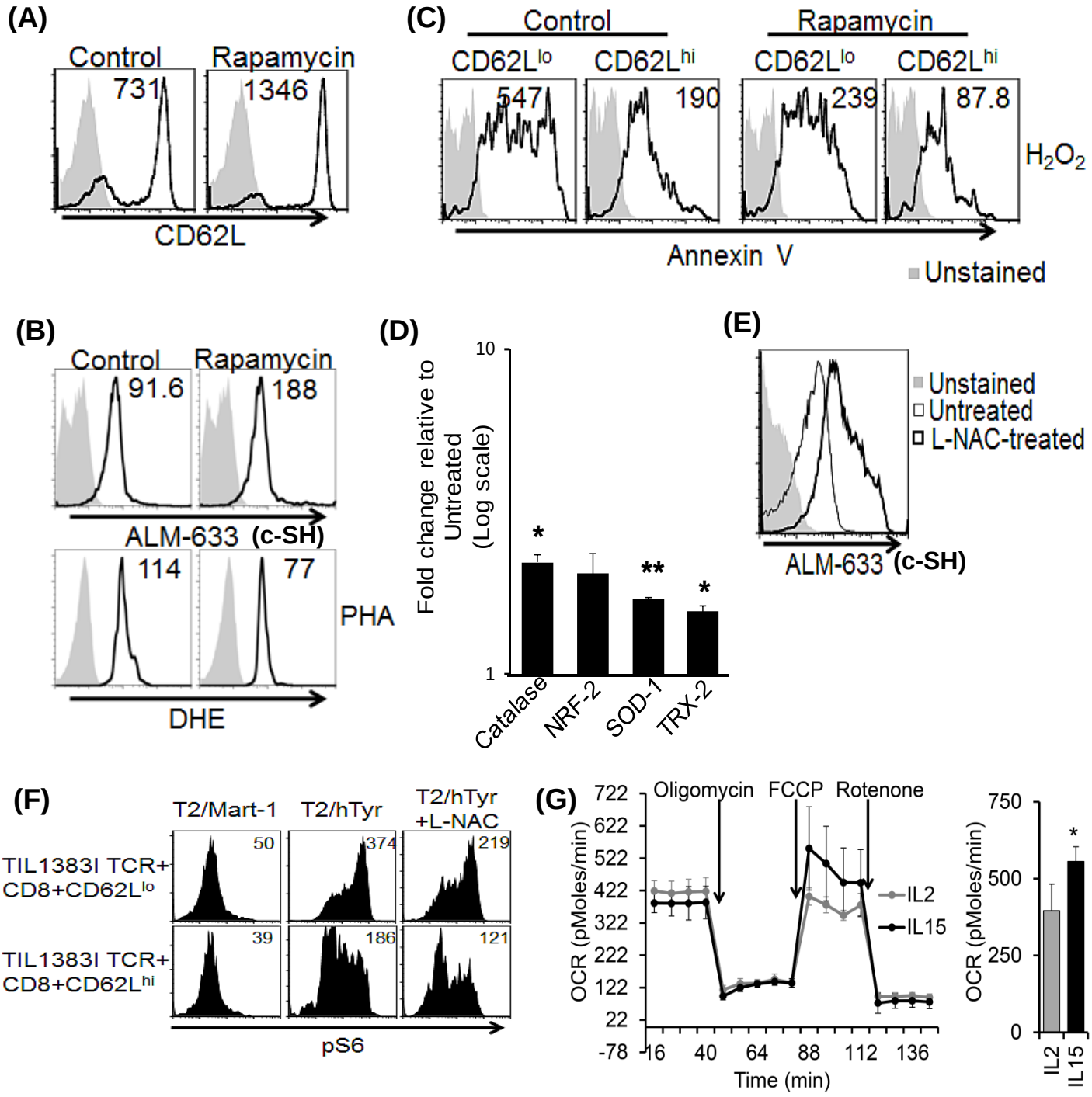
Figure S3. Rapamycin-preconditioned $CD8^+$ T cells upregulate the levels of antioxidant molecules. PBMCs were cultured with or without rapamycin for 5 days. (**A**) Cells were gated on CD8 and analyzed for the expression of CD62L. (**B**) *Upper panel:* Cells were gated on CD8 and analyzed for the expression of cell surface thiols (c-SH) using ALM-633. *Lower panel:* Cells were left untreated or re-stimulated for 4h with PHA. Cells were gated on CD8 and flow cytometric analysis of DHE (superoxide) was performed. Experiments were repeated thrice with similar results. (**C**) Cells were left untreated or treated with H_2O_2 for 4 h in the presence or absence of rapamycin. Annexin V levels were then determined in $CD62L^{lo}$ and $CD62L^{hi}$ $CD8^+$ T cells. Result from one of the two experiments with similar results is shown. (**D**) Purified $CD8^+$ T cells were analyzed for mRNA of key antioxidant molecules (as indicated) by real-time PCR. Results are representative from one of the two similar experiments. (**E**) The c-SH levels on $CD8^+$ T cells, either untreated or pretreated with 5 mM L-NAC for 30 minutes, was determined using ALM-633 staining. (**F**) For antigen-specific pS6 expression, TIL1383I TCR-transduced $CD8^+$ T cells were co-cultured for 4 h with T2 cells that were pulsed with cognate tyrosinase or control MART-1 peptides and stained with anti-CD8, CD34, CD62L and phosphoS6 (pS6) antibodies. pS6 was evaluated on $CD8^+CD34^+CD62L^{lo}$ and $CD8^+CD34^+CD62L^{hi}$ fractions by intracellular staining. Results are representative from one of three similar experiments. (**G**) Human TIL1383I TCR were cultured in IL2 or IL15 and analyzed for extracellular acidification rate using seahorse XF24 analyzer. Experiments were repeated thrice with similar results. ** p <0.005, * p <0.05.


(A)
Control
Rapamycin
731
1346
CD62L
(C)
Control
Rapamycin
CD62L^lo
CD62L^hi
CD62L^lo
CD62L^hi
547
190
239
87.8
H2O2
Annexin V
Unstained
(B)
Control
Rapamycin
91.6
188
ALM-633 (c-SH)
114
77
PHA
DHE
(D)
Fold change relative to Untreated (Log scale)
10
1
*
**
*
Catalase
NRF-2
SOD-1
TRX-2
(E)
Unstained
Untreated
L-NAC-treated
ALM-633 (c-SH)
(F)
T2/Mart-1
T2/hTyr
T2/hTyr +L-NAC
TIL1383I TCR+ CD8+CD62L^lo
TIL1383I TCR+ CD8+CD62L^hi
50
374
219
39
186
121
pS6
(G)
Oligomycin
FCCP
Rotenone
OCR (pMoles/min)
722
622
522
422
322
222
122
22
-78
16
40
64
88
112
136
Time (min)
IL2
IL15
750
500
250
0
*
IL2
IL15