Supplementary Materials

Supplementary Methods

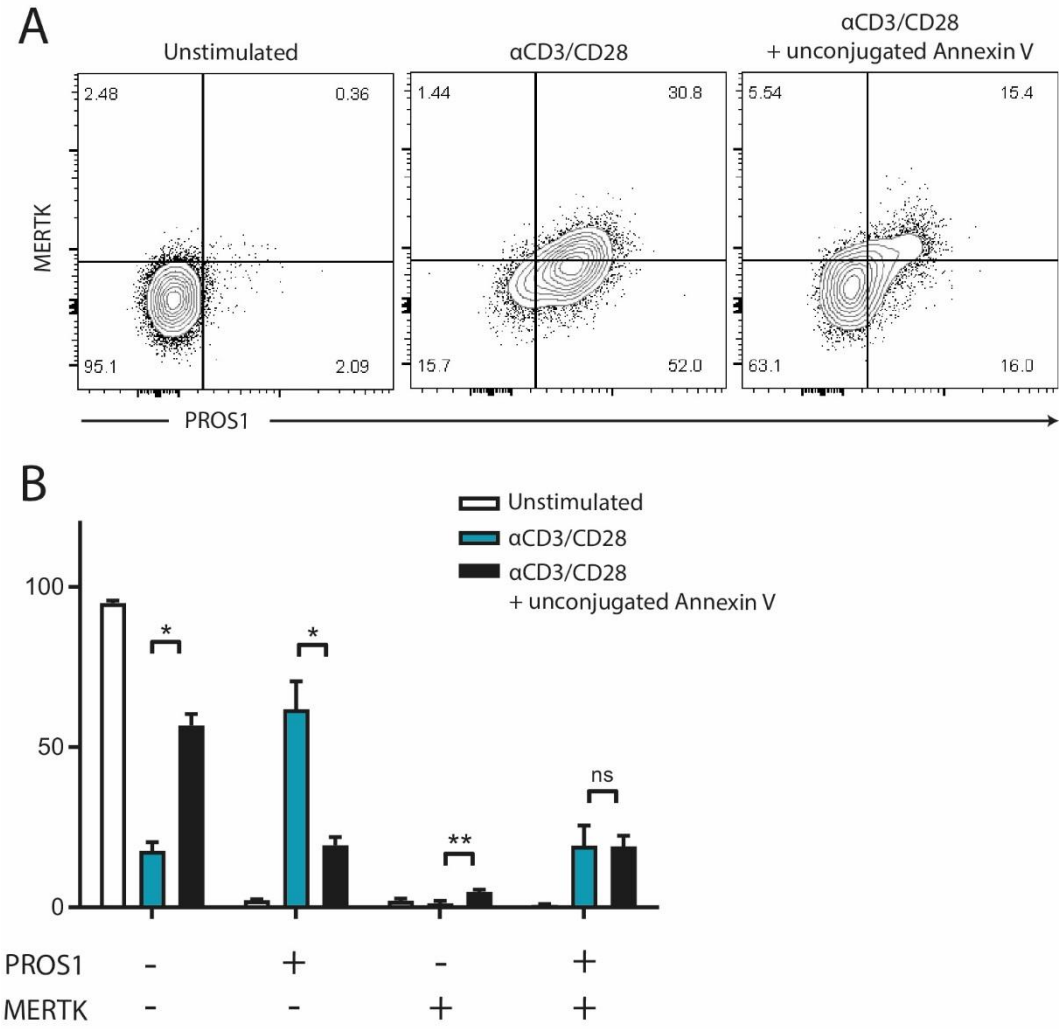
Phosphatidylserine measurements

For phosphatidylserine surface exposure measurements, negatively sorted human $CD8^+$ T cells were activated with αCD3/CD28 beads. $CD8^+$ T cells were cultured in X-VIVO 15 medium supplemented with 5% human serum and with or without 10 µM Q-VD-OPh (general caspase inhibitor, BD Biosciences). Samples were taken daily, stained with Annexin-V-FITC and propidium iodide in Annexin binding buffer according to manufacturer's instructions (Invitrogen), and analyzed by flow cytometry.

Supplementary Table S1

Target	**Sequence 5' to 3'**
Human 18S forward	GTAACCCGTTGAACCCCATT
Human 18S reverse	CCATCCAATCGGTAGTAGCG
Human PROS1 forward	TGCTGGCGTGTCTCCTCCTA
Human PROS1 reverse	CAGTTCTTCGATGCATTCTCTTTCA
Human MERTK forward	AATTACAGATCCGCAGCCCC
Human MERTK reverse	TCAGTGATAGCTCTACGCCAG
Human MERTK siRNA 1 sense	GAACUUACCUUACAUAGCU
Human MERTK siRNA 1 antisense	AGCUAUGUAAGGUAAGUUC
Human MERTK siRNA 2 sense	CAGUAGCCGUGUUAACGAA
Human MERTK siRNA 2 antisense	UUCGUUAACACGGCUACUG
Human MERTK siRNA 3 sense	GGAUGAAGCCUCCGACUUAA
Human MERTK siRNA 3 antisense	UUAGUCGGAGGUUCAUCC

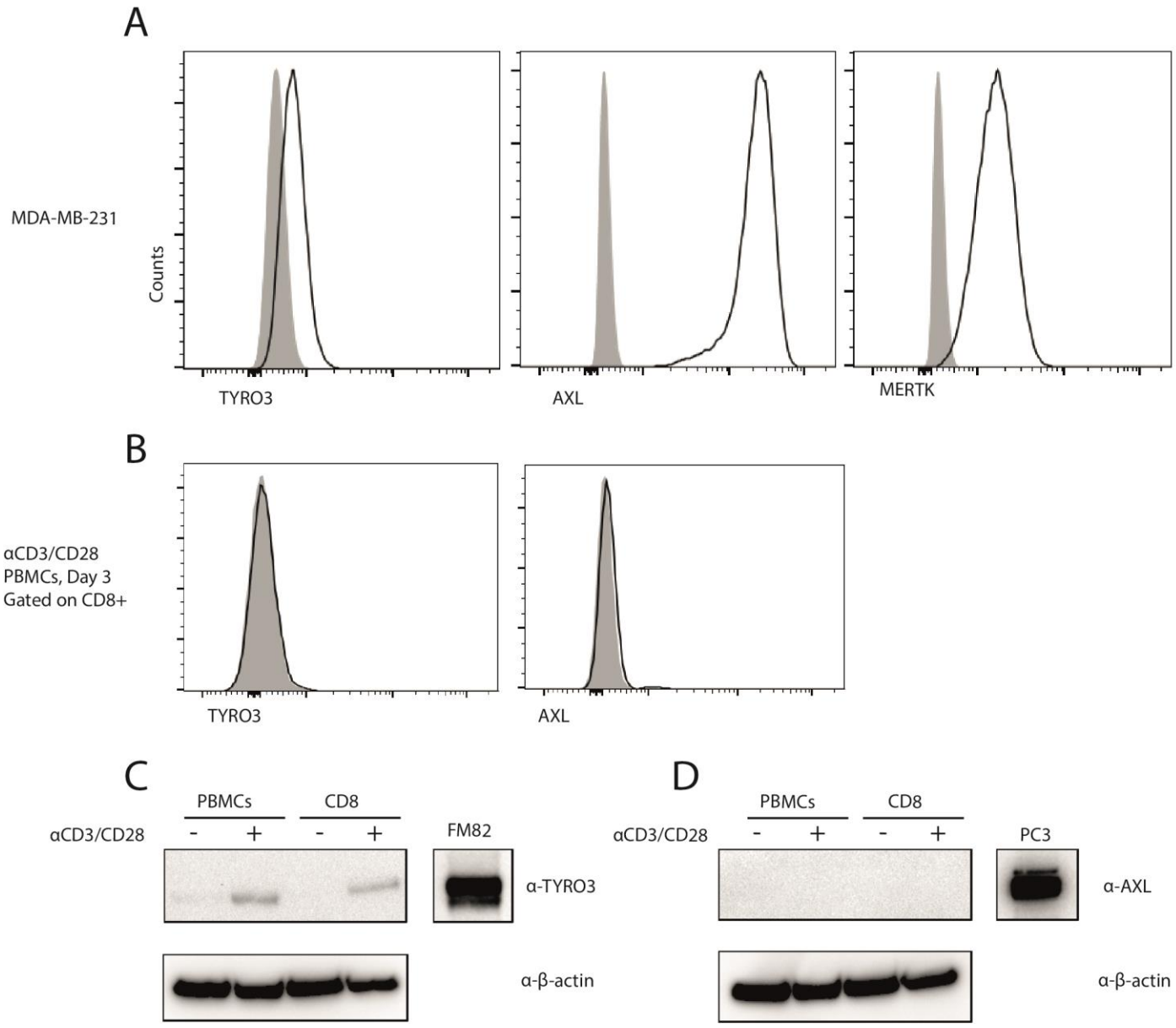
Figure S1



Supplementary Figure S1

PROS1 and MERTK co-expression on 3-day activated CD8$^+$ T cells. (A) Representative dot plots of PROS1 and MERTK co-staining on CD8+ T cells, activated for 3 days with αCD3/CD28 in the presence or absence of 5 µg/ml unconjugated Annexin V. (B) Percentage of PROS1 or MERTK negative, single-positive or double-positive CD8$^+$ T cells (n=4). Data are plotted as mean ± SEM and statistical significance was determined with two-way ANOVA with Bonferroni's multiple comparisons tests (B). *p<0.05, **p<0.01.

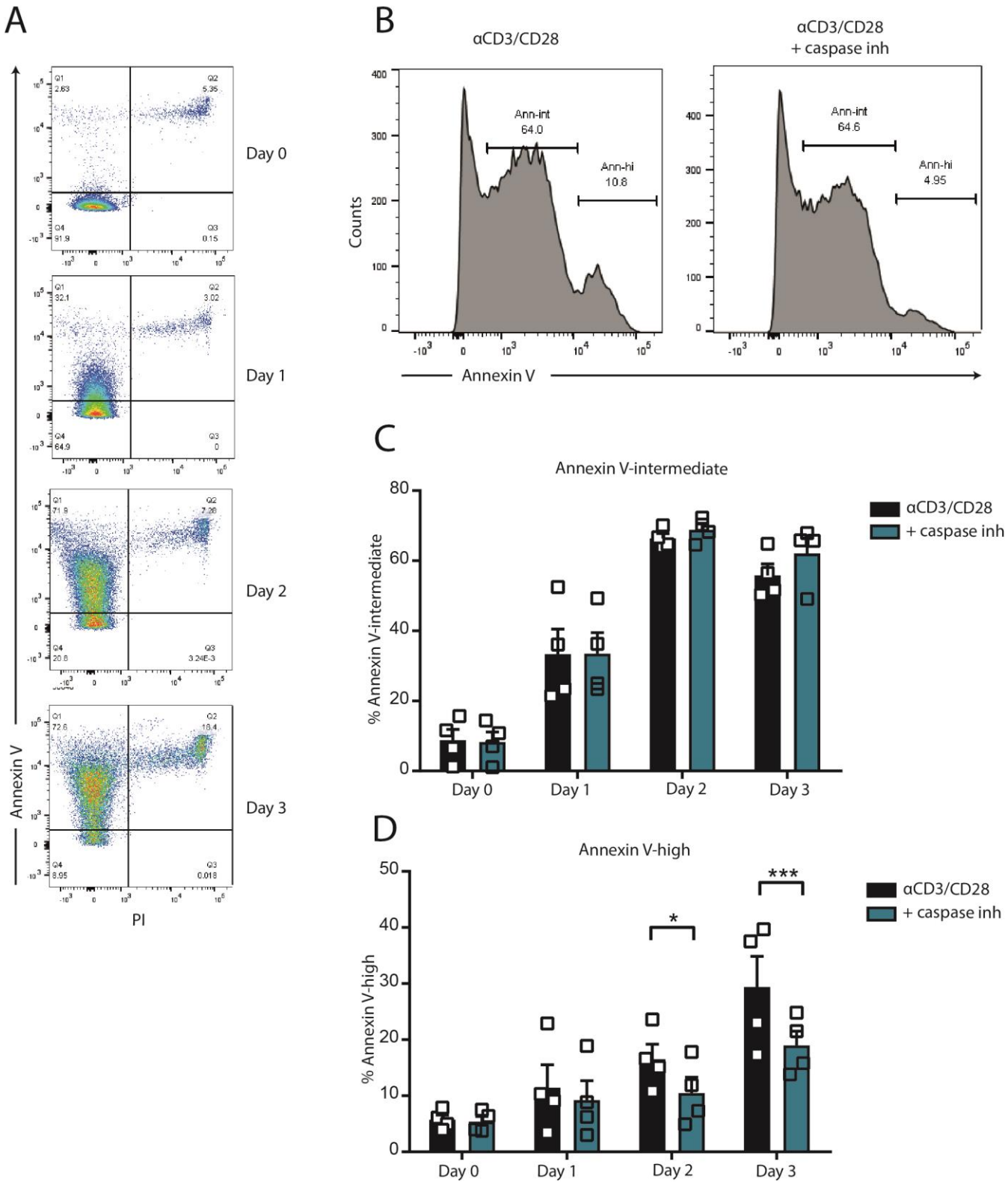
Figure S2



Supplementary Figure S2

TAM receptors TYRO3 and AXL are nearly absent or absent on CD8$^+$ T cells. (A) Representative histogram from MERTK, TYRO3, or AXL surface staining on TAM receptor-positive MDA-MB-231 breast cancer cell line as measured by flow cytometry. (B) Representative histogram of TYRO3 and AXL staining on three-day αCD3/CD28-stimulated PBMCs and gated on CD8. (C) TYRO3 (left, top) and AXL (right, top) protein expression in three-day stimulated PBMCs or CD8$^+$ T cells, with cancer cell lines FM82 or PC3 serving as a positive control. β-actin (bottom) served as a loading control. (B, C and D) are representative of at least 3 experiments with different human healthy donors.

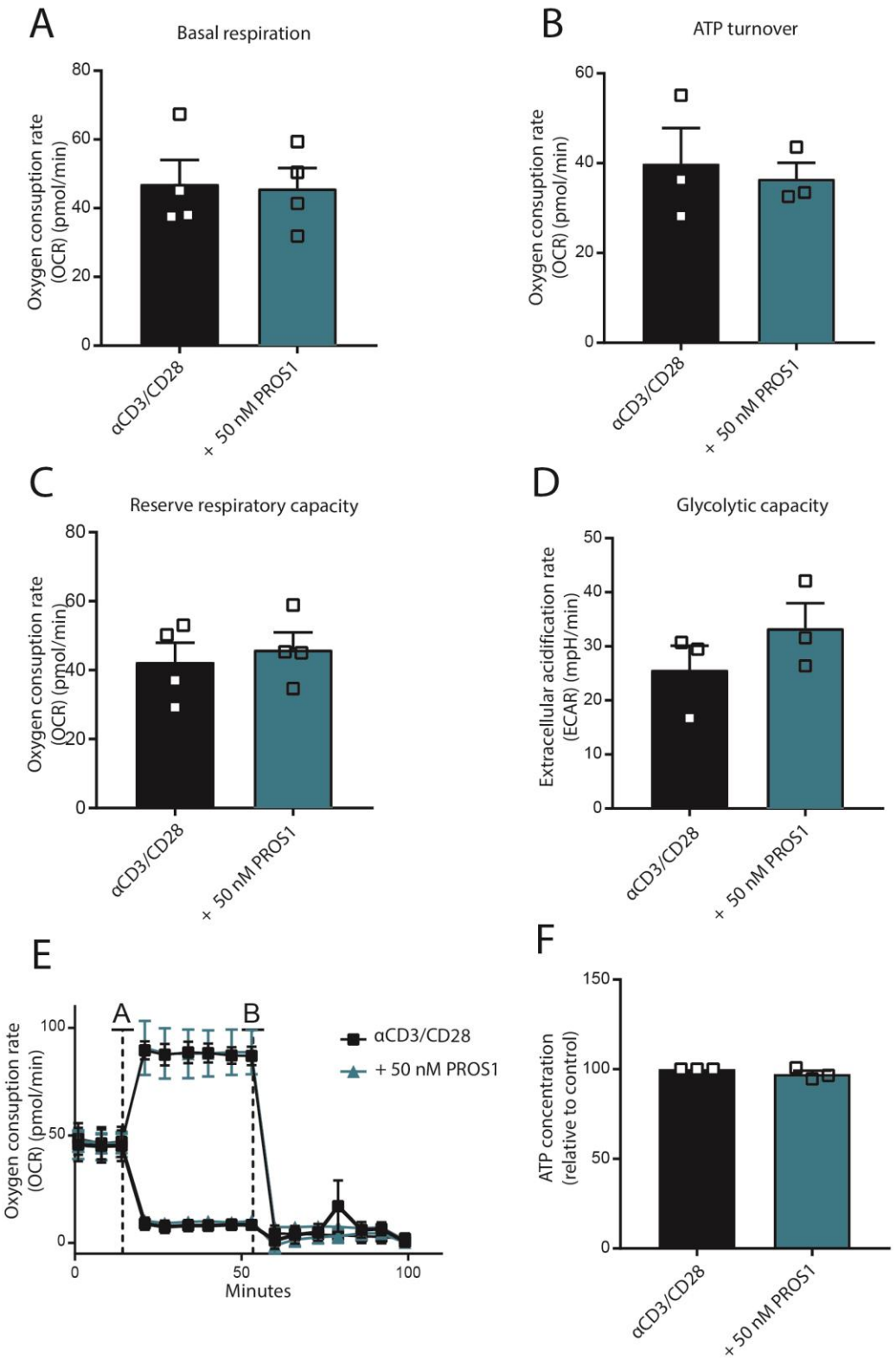
Figure S3


A
Day 0
Day 1
Day 2
Day 3
Annexin V
PI
B
αCD3/CD28
αCD3/CD28
+ caspase inh
Ann-int
64.0
Ann-hi
10.8
Ann-int
64.6
Ann-hi
4.95
Counts
Annexin V
C
Annexin V-intermediate
% Annexin V-intermediate
αCD3/CD28
+ caspase inh
Day 0
Day 1
Day 2
Day 3
D
Annexin V-high
% Annexin V-high
*
***
αCD3/CD28
+ caspase inh
Day 0
Day 1
Day 2
Day 3

Supplementary Figure S3

Phosphatidylserine is not a limiting factor for TAM signaling in CD8$^+$ T cell cultures and is partly non-apoptotic. (A) Representative plots of stimulated CD8$^+$ T cells stained for Annexin V and propidium-iodide (PI). (B) Representative histogram of Annexin V staining divided in 'intermediate' and 'high' staining on stimulated CD8$^+$ T cells, in the presence or absence of caspase-inhibitor Q-VD-OPh. (C,D) Percentage of Annexin V-intermediate (C) or Annexin V-high (D) positive CD8 T cells in the absence or presence of caspase inhibitor Q-VD-OPh over a course of three days (n=4). Data are plotted as mean ± SEM and statistical significance was determined with two-way ANOVA with Bonferroni's multiple comparisons tests (C,D). *$p<0.05$, **$p<0.01$, ***$p<0.001$.

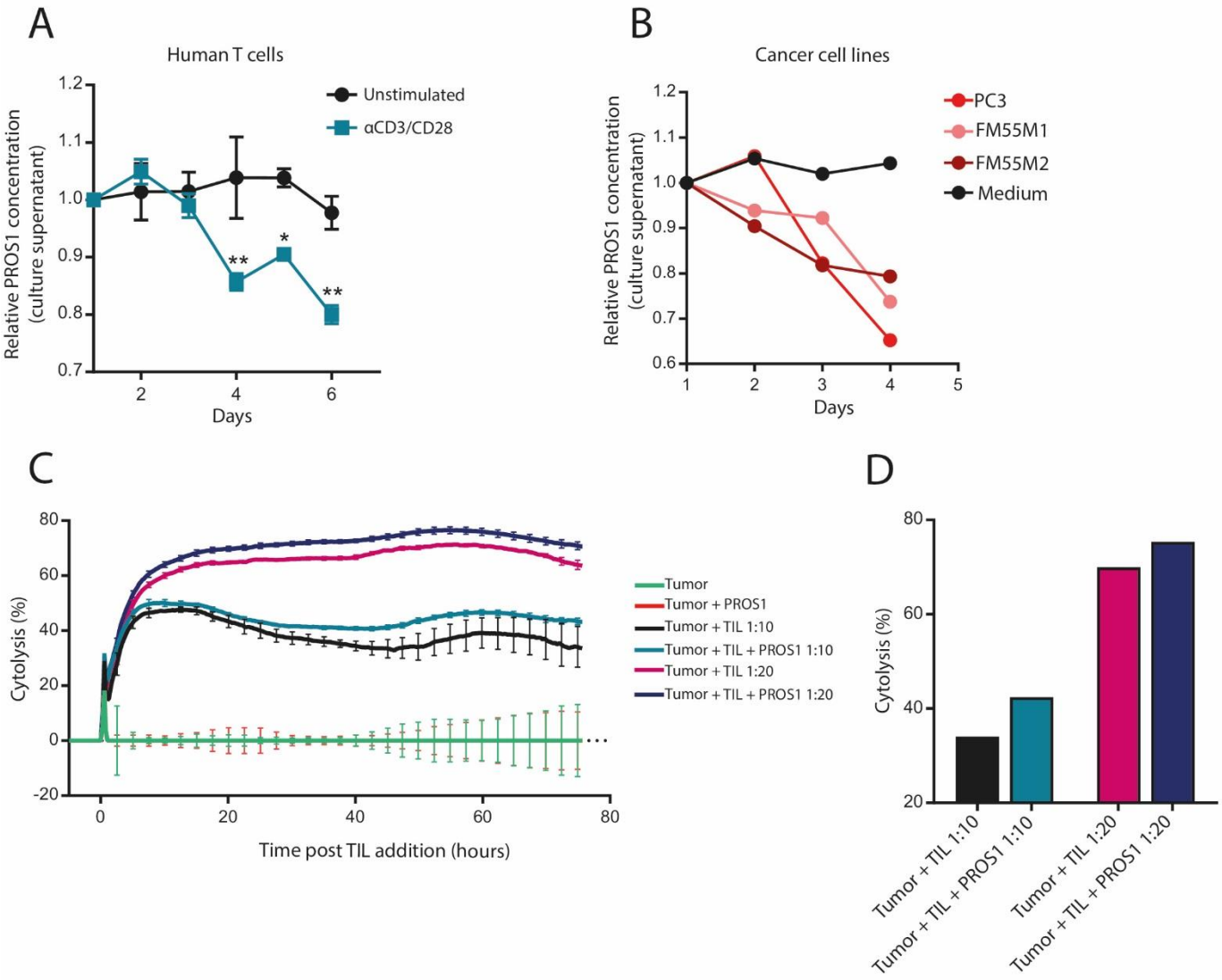
Figure S4


A
Basal respiration
Oxygen consuption rate (OCR) (pmol/min)
αCD3/CD28
+ 50 nM PROS1
B
ATP turnover
C
Reserve respiratory capacity
D
Glycolytic capacity
Extracellular acidification rate (ECAR) (mpH/min)
E
A
B
αCD3/CD28
+ 50 nM PROS1
Minutes
F
ATP concentration (relative to control)

Supplementary Figure S4

Bioenergetic properties of PROS1-supplemented cells. CD3/CD28-stimulated $CD8^+$ T cells were cultured for three days in the presence or absence of 50 nM PROS1. (A) Basal respiration was determined as initial resting consumption of oxygen (n=4). (B) ATP turnover was measured as decrease of oxygen consumption after addition of oligomycin (n=3). (C) Reserve respiratory capacity was measured as percentage of basal respiration, after addition of FCCP (n=4). (D) Glycolytic capacity was measured after addition of oligomycin (n=3). (E) Raw levels of oxygen consumption. Cells were treated with either oligomycin or FCCP at stage A and antimycin A at stage B. (E) Whole-cells levels of ATP normalized to control (n=3). Data are plotted as mean ± SEM and statistical significance was determined with Student's *t* tests.

Figure S5



Supplementary Figure S5

PROS1 consumption by cancer cells and T cells affects TIL-mediated killing. αCD3/CD28-stimulated PBMCs (A, n=3) or cancer cell lines (B) were cultured for four to six days in the presence of 50 nM PROS1. Daily, samples from the culture medium were harvested and PROS1 concentration in culture medium was analyzed. (C) Real-time *in vitro* cytolysis of autologous cancer cells from metastatic melanoma patient 3 after addition of antigen-selected autologous TILs (1:10 or 1:20 target:effector ratio). (D) % Cytolysis 48 hours post TIL addition. Data are plotted as mean ± SEM and statistical significance was determined with two-way ANOVA with Bonferroni's multiple comparisons tests (A). *p<0.05, **p<0.01, ***p<0.001.