SUPPLEMENTAL MATERIAL

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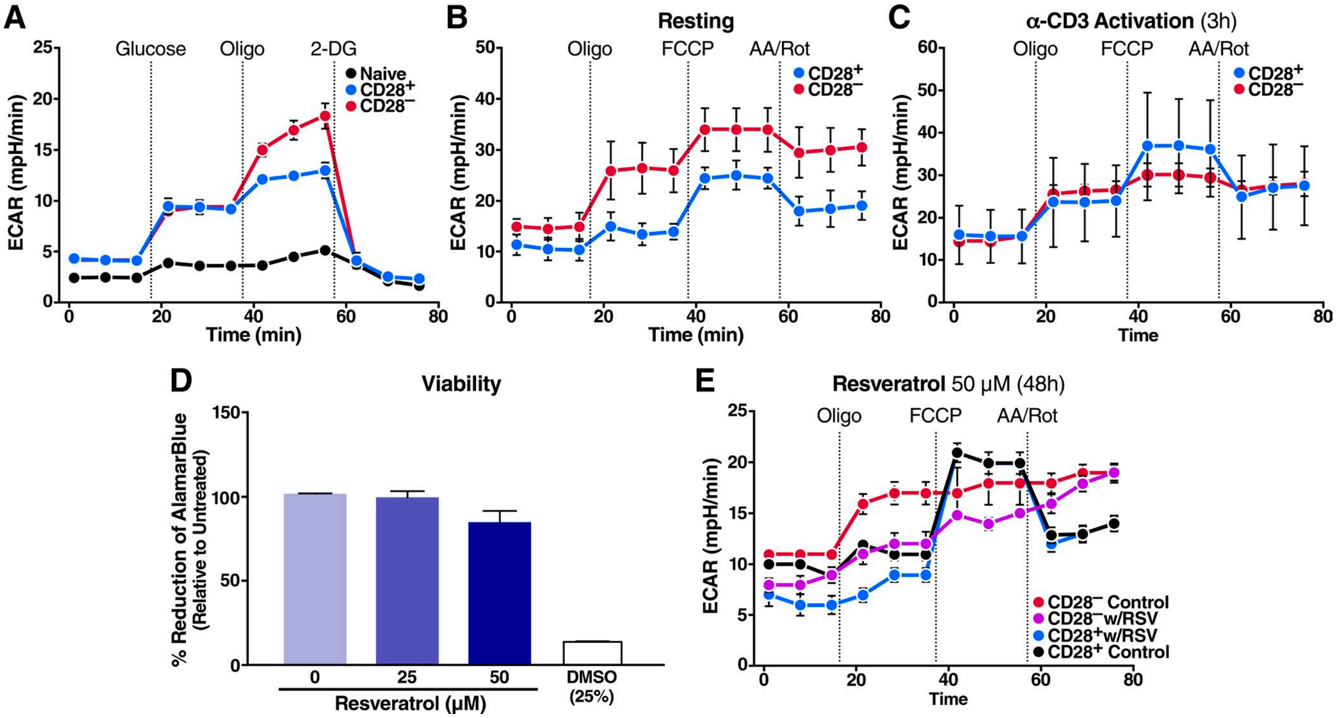

Figure S1. **Glycolytic capacity of resting CD8⁺CD28⁻ T cells depends on glucose and SIRT1.** Metabolism of sorted human T cell populations was assessed using an extracellular flux analyzer. (A) ECAR was measured in freshly isolated and glucose-deprived T cell populations after sequential addition of glucose, oligomycin, and 2-DG (representative, *n* = 2 biological replicates, *n* = 6 technical replicates). (B and C) ECAR measurement of resting (B) and activated (C) T cells (3 h with anti-CD3 antibody; mean of *n* = 2 biological replicates, *n* = 6 technical replicates). mpH/min, milli-pH units per minute. (D) Viability of human CD8⁺ T cells assessed by alamarBlue assay after RSV treatment for 48 h; 25% DMSO was used as toxicity control (*n* = 2 biological in technical triplicates). (E) ECAR analysis of CD28⁺ and CD28⁻ subsets in the presence of 50 µM RSV (representative, *n* = 2 biological replicates, *n* = 6 technical replicates). Data are mean ± SEM of technical replicates and individual donors.

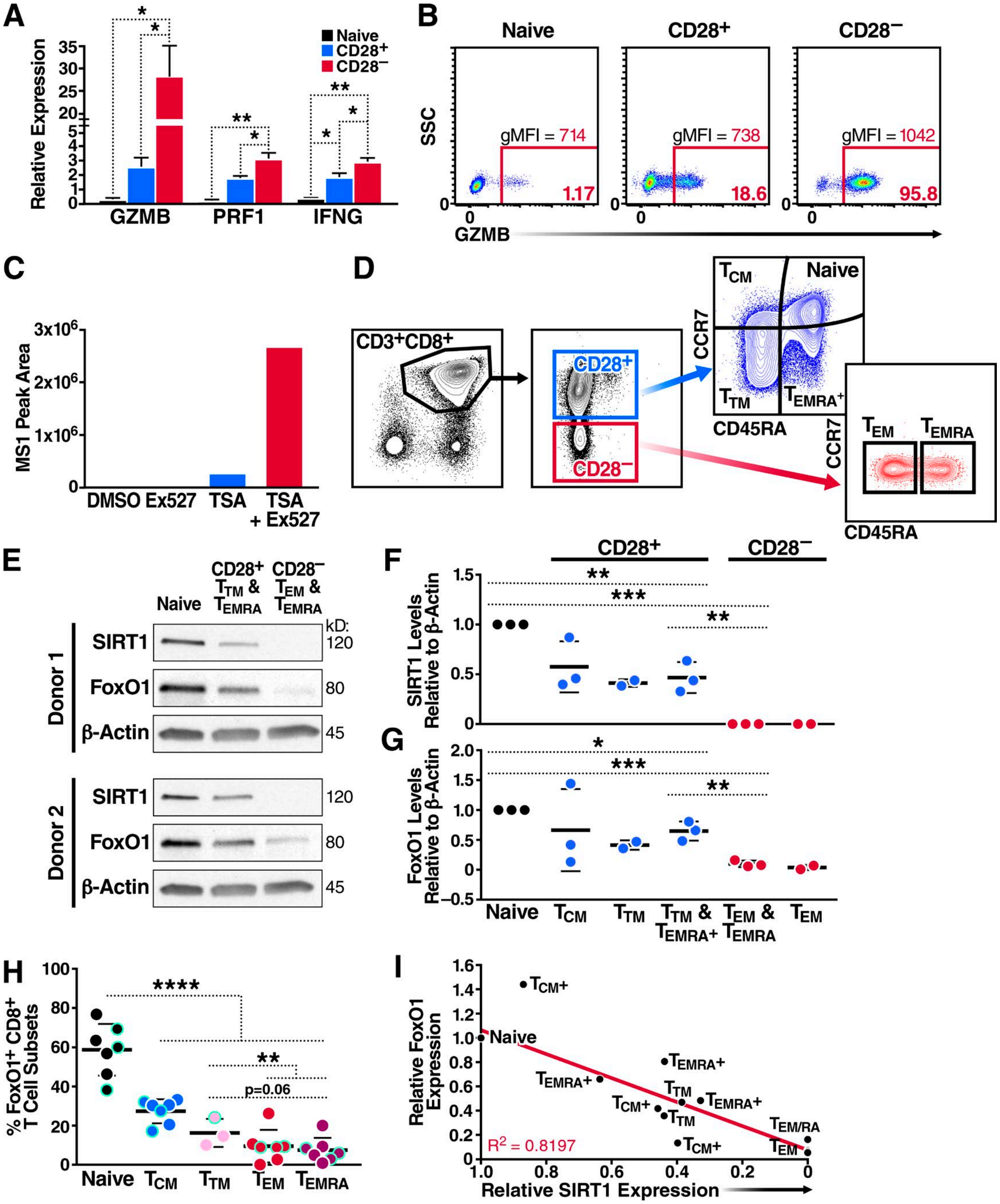

Figure S2. **Cytotoxicity and FoxO1 status in CD8$^+$ T cell subsets.** (A) Cytotoxic gene expression assessed by qRT-PCR in freshly isolated human T cells relative to the mean expression in CD28$^+$ cells (n = 7, two-way ANOVA). (B) GZMB intracellular staining was measured by flow cytometry in resting T cell populations treated with brefeldin A for 4 h (representative, n = 3, numbers show geometric median fluorescence intensity). (C) Freshly isolated CD8$^+$ T cells were analyzed by mass spectrometry for AcK peptides (treated with 400 nM TSA and 25 µM Ex-527 for 4 h). The integrated MS1 intensity for peptide AASMDNNSK(ac)FAK is shown (n = 1). (D) Representative sorting flow plot of CD8$^+$CD28$^+$ and CD28$^-$ T cells stained for memory markers CCR7 and CD45RA. (E–G) Naive, CD28$^+$ T$_{CM}$, T$_{TM}$ + T$_{EMRA}$$^+$, and CD28$^-$ T$_{EM}$ + T$_{EMRA}$ were sorted as shown in D, probed for SIRT1 and FoxO1 expression by Western blot, and quantified by densitometry (F and G; n = 3 biological replicates). (H) CD8$^+$ memory T cell subsets from CMV$^-$ and CMV$^+$ (green circles) blood donors were stained for intracellular FoxO1 protein and analyzed by flow cytometry (n = 7 biological replicates and n = 3 for T$_{TM}$, paired one-way ANOVA). (I) Linear regression analysis of SIRT1 and FoxO1 Western blots in sorted subsets (R^2 = 0.819). Data are mean ± SEM of individual donors. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$.

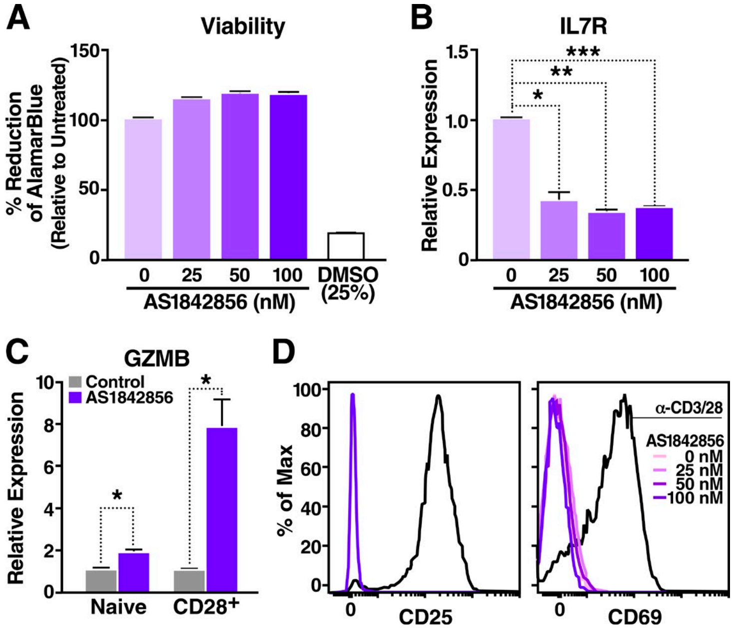

Figure S3. **FoxO1 inhibitor increases GZMB expression without T cell activation.** (A) Viability of human CD8$^+$ T cells was assessed with an alamarBlue assay after AS1842856 treatment for 48 h; DMSO (25%) served as toxicity control (*n* = 2 biological replicates in technical duplicates). (B) IL7R mRNA was measured by qRT-PCR, normalized to RPL13A mRNA and to untreated control, in human CD8$^+$ T cells after increasing doses of AS1842856 for 48 h (*n* = 3, paired two-tailed Student's *t* test). (C) GZMB mRNA was measured in sorted CD8$^+$ naive and CD8$^+$CD28$^+$ memory T cells after treatment with 100 nM AS1842856 for 48 h (*n* = 3, paired two-tailed Student's *t* test). (D) Activation markers CD25 and CD69 were assessed by flow cytometry in CD8$^+$ T cells after 48-h treatment of AS1842856 or α-CD3/28 activation (representative, *n* = 2). Data are mean ± SEM of individual donors. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Table S1. **Microarray identifies 159 differentially regulated genes between CD8$^+$CD28$^+$ and CD8$^+$CD28$^-$ T cells**

Gene name	entrez_ids	$\log_2$ fold change	P-value	FDR
Up-regulated in CD28$^-$				
GPR141	353345	3.04618	0.00000	0.00000
FCGR3A	2214	2.91839	0.00002	0.00012
CX3CR1	1524	2.91351	0.00000	0.00001
KLRF1	51348	2.70094	0.00002	0.00008
TXNDC3	51314	2.38590	0.00000	0.00000
GNLY	10578	2.31302	0.00000	0.00005
PLEK	5341	2.04459	0.00032	0.00190
PDGFD	80310	2.02545	0.00011	0.00068
ZEB2	9839	2.01872	0.00000	0.00001
FCRL6	343413	1.81571	0.00329	0.00986
GZMB	3002	1.78378	0.00357	0.01430
KLRD1	3824	1.68624	0.00094	0.00564
GZMH	2999	1.65432	0.00000	0.00003
KIR2DS2	100132285	1.59739	0.00076	0.00306
KIR2DL1	3802	1.56286	0.00031	0.00186
PRSS23	11098	1.51952	0.00001	0.00004
TGFBR3	7049	1.49051	0.00002	0.00013
NKG7	4818	1.47231	0.00083	0.00500
TNFRSF9	3604	1.47152	0.01948	0.04680
EPB41L4A	64097	1.45895	0.00288	0.01730
KLRA1	10748	1.34042	0.00529	0.01590
FGFBP2	83888	1.33460	0.00013	0.00077
LYN	4067	1.33105	0.00000	0.00000
GPR56	9289	1.32119	0.00116	0.00464
PTPN12	5782	1.30337	0.00006	0.00034
FAM49A	81553	1.29781	0.00108	0.00648
SUSD1	64420	1.29745	0.00028	0.00170
ST8SIA6	338596	1.29096	0.00579	0.02090
GALNT3	2591	1.27587	0.00325	0.01950
RHOBTB3	22836	1.27035	0.00042	0.00316
PIK3R3	8503	1.26994	0.00358	0.01430
CD244	51744	1.26005	0.00034	0.00207
TOX	9760	1.23303	0.00000	0.00002
BHLHE40	8553	1.22762	0.01181	0.03540
PRF1	5551	1.22406	0.00001	0.00007
RAP2A	5911	1.21786	0.00184	0.01100
UBE2F	140739	1.20307	0.00046	0.00279
SLCO4C1	353189	1.16089	0.00062	0.00248
DUSP10	11221	1.15745	0.00120	0.00360
CMKLR1	1240	1.14464	0.00023	0.00091
LYST	1130	1.13964	0.00068	0.00407
PIK3AP1	118788	1.13478	0.00087	0.00524
PRDM1	639	1.13389	0.01228	0.04760
RCAN2	10231	1.12531	0.01148	0.03450
PDLIM1	9124	1.11957	0.00217	0.01620
EGR2	1959	1.09724	0.00768	0.01840
F2R	2149	1.09415	0.00027	0.00163
OR7E13P	10820	1.08994	0.00730	0.02920
DDI1	414301	1.07559	0.00056	0.00477
USP28	57646	1.06786	0.00007	0.00040
KLRG1	10219	1.04885	0.01903	0.04570
PTMS	5763	1.04800	0.00001	0.00004
PTGDR	5729	1.03258	0.01848	0.03700
PRR5L	79899	1.03002	0.00000	0.00002
B4GALT5	9334	1.02674	0.00057	0.00341
KLRC4	8302	1.01029	0.00058	0.00232
JAKMIP2	9832	1.00682	0.00187	0.01120
Down-regulated in CD28$^-$				
CD28	940	−3.21368	0.00000	0.00000
RCAN3	11123	−3.09246	0.00000	0.00000
LRRN3	54674	−3.01606	0.00001	0.00003

Table S1. **Microarray identifies 159 differentially regulated genes between CD8$^+$CD28$^+$ and CD8$^+$CD28$^-$ T cells** *(Continued)*

Gene name	entrez_ids	$\log_2$ fold change	P-value	FDR
LEF1	51176	-2.88079	0.00000	0.00000
SCML1	6322	-2.85338	0.00000	0.00001
NELL2	4753	-2.75817	0.00069	0.00414
CCR7	1236	-2.75189	0.00000	0.00000
CCR4	1233	-2.73984	0.00010	0.00123
CD62L (SELL)	6402	-2.72432	0.00068	0.00407
VSIG1	340547	-2.64089	0.00000	0.00000
IFI44L	10964	-2.44298	0.00643	0.03860
SERINC5	256987	-2.40756	0.00000	0.00000
TXK	7294	-2.40674	0.00002	0.00012
ITGA6	3655	-2.25528	0.00000	0.00000
FCER1G	2207	-2.20898	0.00232	0.01390
HOOK1	51361	-1.98196	0.00001	0.00002
GCNT4	51301	-1.97421	0.00061	0.00147
GZMK	3003	-1.95871	0.00108	0.00647
ZNF204	7754	-1.93824	0.00005	0.00021
NRCAM	4897	-1.88703	0.00307	0.01230
AK5	26289	-1.85673	0.00018	0.00055
DSC1	1823	-1.83981	0.00094	0.00376
SESN3	143686	-1.83407	0.00321	0.03850
LOC100288114	100288114	-1.83211	0.00000	0.00003
SLC4A10	57282	-1.82461	0.00021	0.00246
SH3BGRL2	83699	-1.77375	0.00002	0.00022
IFI44	10561	-1.75209	0.00018	0.00109
CNKSR2	22866	-1.67434	0.00000	0.00000
FAAH2	158584	-1.63792	0.00020	0.00122
ABLIM1	3983	-1.63051	0.00138	0.00829
RASGRF2	5924	-1.56132	0.00002	0.00018
MAL	4118	-1.55220	0.00000	0.00000
NT5E	4907	-1.54542	0.00099	0.00396
DENND5A	23258	-1.54457	0.00002	0.00011
IL6ST	3572	-1.48902	0.00003	0.00016
IGF1R	3480	-1.46375	0.00001	0.00005
OR10AD1	121275	-1.42079	0.00000	0.00000
LASS6	253782	-1.41802	0.00196	0.01170
DOCK9	23348	-1.39609	0.00000	0.00000
KLF7	8609	-1.38395	0.00005	0.00056
SULT1B1	27284	-1.37986	0.00802	0.03210
IL26	55801	-1.37830	0.00093	0.01120
CAMK4	814	-1.36832	0.00001	0.00006
CCR2	729230	-1.34807	0.00097	0.01170
APP	351	-1.33761	0.00004	0.00026
C9orf135	138255	-1.33327	0.00771	0.04620
EFHA2	286097	-1.33119	0.00034	0.00202
INADL	10207	-1.32465	0.00000	0.00000
USP6NL	9712	-1.32055	0.00003	0.00042
LMO7	4008	-1.31889	0.00018	0.00109
GPR82	27197	-1.30788	0.00133	0.01300
PDE3B	5140	-1.30030	0.00060	0.00362
USP53	54532	-1.29765	0.00001	0.00004
CAMSAP1L1	23271	-1.29739	0.00006	0.00037
FAM117B	150864	-1.29393	0.00093	0.00560
PRKCA	5578	-1.28151	0.00001	0.00006
SLC16A10	117247	-1.26315	0.00699	0.04190
ACVR1C	130399	-1.25999	0.00001	0.00007
SLAMF1	6504	-1.25618	0.00177	0.01060
PDK1	5163	-1.25416	0.00006	0.00035
RAB3GAP1	22930	-1.25248	0.00001	0.00004
KDSR	2531	-1.24796	0.00027	0.00320
ALPK1	80216	-1.24721	0.00018	0.00217
GPR34	2857	-1.24673	0.00208	0.02500
SAMD12	401474	-1.24492	0.00001	0.00006
C9orf72	203228	-1.24421	0.00002	0.00017

Table S1. **Microarray identifies 159 differentially regulated genes between CD8$^+$CD28$^+$ and CD8$^+$CD28$^-$ T cells** *(Continued)*

Gene name	entrez_ids	log$_2$ fold change	P-value	FDR
NBEA	26960	−1.23812	0.00012	0.00073
ZNF238	10472	−1.22531	0.00055	0.00333
C1orf162	128346	−1.21798	0.00012	0.00047
RALGPS2	55103	−1.21599	0.00003	0.00018
ZNF208	7757	−1.21537	0.00055	0.00659
CSGALNACT1	55790	−1.21343	0.00008	0.00033
LRRC16A	55604	−1.21062	0.00026	0.00105
CLUAP1	23059	−1.19804	0.00000	0.00000
GTSCR1	220158	−1.18521	0.00379	0.01520
ZFP28	140612	−1.17834	0.00001	0.00003
BACH2	60468	−1.17753	0.00064	0.00386
EPHX2	2053	−1.16847	0.00349	0.02090
SPG20	23111	−1.16119	0.00029	0.00115
OCM	654231	−1.15669	0.00057	0.00170
LOC283174	283174	−1.12311	0.00013	0.00078
OCM2	4951	−1.11977	0.00071	0.00213
RIC3	79608	−1.09937	0.00035	0.00211
RBM11	54033	−1.09596	0.00001	0.00005
ZFYVE9	9372	−1.09436	0.00000	0.00001
FAM102A	399665	−1.08628	0.00192	0.01150
IGSF9B	22997	−1.08560	0.00034	0.00409
CNN3	1266	−1.07971	0.00327	0.00981
NPCDR1	246734	−1.06681	0.01193	0.04770
VWDE	221806	−1.05085	0.00060	0.00241
OXNAD1	92106	−1.04769	0.00000	0.00003
C13orf15	28984	−1.03962	0.00896	0.03580
FAM134B	54463	−1.02815	0.00001	0.00004
TMEM14C	51522	−1.02783	0.00001	0.00006
DAPP1	27071	−1.02770	0.00000	0.00000
FLJ44048	401024	−1.02506	0.00068	0.00408
ME1	4199	−1.02438	0.00307	0.03680
ACVR2A	92	−1.02148	0.00056	0.00220
CASP10	843	−1.02041	0.00004	0.00025
DSEL	92126	−1.01814	0.00003	0.00013
P2RY14	9934	−1.01522	0.01979	0.03960
LY96	23643	−1.01413	0.00364	0.02190

Table S2. **Primer list for qRT-PCR**

Human gene	qRT-PCR forward primers (5′–3′)	qRT-PCR reverse primers (5′–3′)
RPL13A	CCTGGAGGAGAAGAGGAAAGAGA	TTGAGGACCTCTGTGTATTTGTCAA
SIRT1	GCCTCACATGCAAGCTCTAGTGAC	TTCGAGGATCTGTGCCAATCATAA
GZMB	CCCTGGGAAAACACTCACACA	GCACAACTCAATGGTACTGTCG
PRF1	GGCTGGACGTGACTCCTAAG	CTGGGTGGAGGCGTTGAAG
IFNG	TCGGTAACTGACTTGAATGTCCA	TCGCTTCCCTGTTTTAGCTGC
CCR7	TGAGGTCACGGACGATTACAT	GTAGGCCCACGAAACAAATGAT
CD62L	CTTTCACCAAGGGCGATTTA	GGCATTTATCATTTGGCTGG
IL7R	CCCTCGTGGAGGTAAAGTGC	CCTTCCCGATAGACGACACTC
KLRG1	TCCATGTTAGAGTTGCCTACGG	AAGTGGAGTAGTTGGAGCCCT
FOXO1	TCGTCATAATCTGTCCCTACACA	CGGCTTCGGCTCTTAGCAAA