

Supplemental Information

Acetate Promotes T Cell Effector Function during Glucose Restriction

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SUPPLEMENTARY FIGURES AND TABLES

Figure S1

Figure S2

Figure S3

Table S1

Figure S1 (Related to Figure 1)

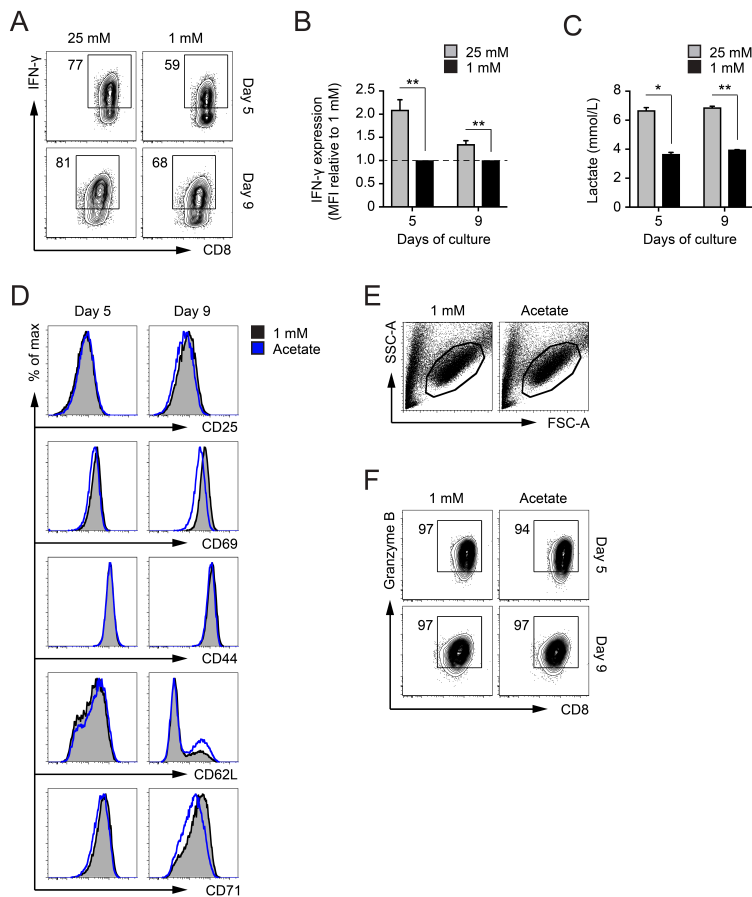


Figure S1. While promoting IFN- γ production, acetate does not affect expression of surface markers, granzyme B, or cell size of glucose-restricted T cells. Related to Figure 1.

A, FACS analysis of IFN- γ production by T cells cultured continuously for 5 or 9 days in 25 mM glucose or switched to 1 mM glucose on day 3 and cultured as described in Fig. 1A. Numbers show percentage of IFN- γ^+ cells. FACS plots are representative of $n=3$ independent experiments. **B**, Quantification of IFN- γ production as MFI of the CD8 $^+$ population as shown in A. Values were normalized to the 1 mM condition. Mean \pm SEM; Student's t test; $n=3$ independent experiments. **C**, Quantification of lactate production, as measure of aerobic glycolysis, in the medium of cells cultured for 24 hours in the indicated conditions. Mean \pm SEM; $n=3$ independent experiments. **D**, FACS analysis of surface expression of the indicated markers on T cells cultured as described in Fig. 1A. FACS plots show representative data of $n=2$ independent experiments. **E**, FACS analysis of the physical parameters forward scatter (FSC, indicating cell size) and side scatter (SSC, indicating the cell shape complexity) of T cells cultured as in Fig. 1A. Analysis was performed at day 5 post-activation. No previous gating strategy was applied before plotting FSC and SSC. The gate indicates the FSC/SSC gate used throughout the manuscript for further analysis. **F**, FACS analysis of Granzyme B production by T cells cultured as described in Fig. 1A. Numbers show percentage of Granzyme B $^+$ cells. FACS plots are representative of $n=2$ independent experiments.

Figure S2 (Related to Figure 2)

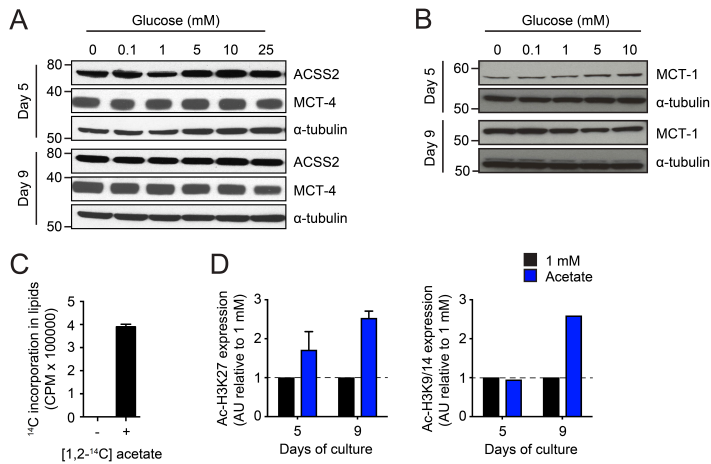


Figure S2. Acetate is incorporated into lipids and histones of glucose-restricted T cells. Related to Figure 2.

A, Western blot analysis of ACSS-2 and MCT-4 in T cells cultured in different glucose concentrations. Data are representative of $n=3$ (for ACSS2) and $n=1$ (for MCT-4) independent experiments. **B**, Western blot analysis of MCT-1 in T cells cultured in different glucose concentrations. Data are representative of $n=1$ experiment. **C**, Quantification of [1,2- ^{14}C] acetate-derived ^{14}C incorporation in lipids extracted from T cells cultured in 10 mM glucose media. Mean \pm SEM; $n=2$ independent experiments. **D**, Quantification of western blots of H3K9/14 and H3K27 acetylation in T cells treated as described in Fig. 1A. Data are representative of $n=2$ ($n=1$ for Ac-H3K9/14) independent experiments.

Figure S3 (Related to Figure 4)

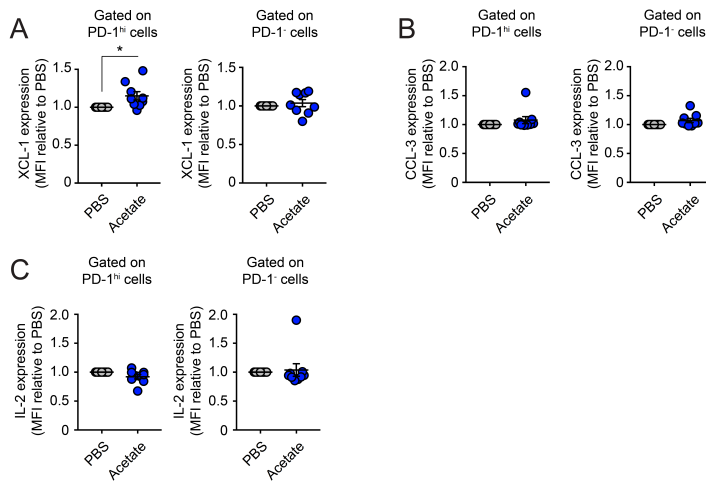


Figure S3. Chemokine and cytokine expression in PBMCs isolated from the blood of patients chronically infected with hepatitis C virus (HCV). Cells were treated overnight with either PBS or 5 mM acetate. Data show MFI of the indicated parameters. Values are normalized to the PBS counterparts. Mean \pm SEM; Paired Student's *t* test; data from n=9 donors.

A-C, Chemokine and cytokine expression in PBMCs isolated from the blood of patients chronically infected with hepatitis C virus (HCV). Cells were treated overnight with either PBS or 5 mM acetate. Data show MFI of the indicated parameters. Values are normalized to the PBS counterparts. Mean \pm SEM; Paired Student's *t* test; data from n=9 donors.

Table S1 (Related to Figure 3)

A

1600020E01Rik	Dlgap1	Gm23701	Myo1d	Slnf10-ps
1700072G22Rik	Dmrt2os	Gm2453	Nampt	Smarcd3
201011101Rik	Dnah3	Gm24819	Ndfip2	Snx20
2610306M01Rik	Dnajb6	Gm25283	Nebi	Spes3
2900011008Rik	Dnajc5b	Gm25505	Nkx1-1	Spice1
4921523L03Rik	Dnase1l3	Gm25916	No10	Srek1ip1
4930447M23Rik	Dnm1t	Gm26686	Notch2	Srsia1
4930519F16Rik	Dock10	Gm26827	Nr4a3	Slk40
4930555B11Rik	Dock6	Gm27007	Nrns	Srsf8
4930565D16Rik	Dok6	Gm37549	Oma1	Syde2
5031415H12Rik	E130102H24Rik	Gm38122	Oxsr1	Syne1
5730508B09Rik	E330017L17Rik	Gm38130	P2ry2	Tbc1d31
9130204K15Rik	Ebf1	Gm38337	Pak6	Tcof1
A330074K22Rik	Eci2	Gm42791	Patl2	Tctex1d1
A930006K02Rik	Efcab2	Gm5544	Pdcd10	Tec
Abi1	Erf2ak3	Gm7895	Pear1	Tespa1
Adam32	Emb	Gm8926	Pgk1	Tg
Add3	Erc6l2	Gna13	Phlpp1	Tmem19
Akap13	Fam105a	Gng2	Pim3	Tmem229b-ps
Aldh18a1	Fam1178b	Gjrl132	Plac5	Tmem70
Angel1	Fam49a	H3f3a	Plydc2	Tnfrsf8
Ankrd44	Fbln1	Haao	Por	Trav15n-1
Ankrd50	Fbxl20	Hbs1l	Ppm1h	Trim69
Aph1a	Fbxo32	Hmgb3	Ppp6r1	Trpm8
Arhgap10	Fcamr	Hmgcs2	Prex1	Ttc27
Ar14a	Fcrl5	Iffng1	Prkch	Uba7
Art2a-ps	Fcrl6	Iqsec1	Psmtd14	Uba2
Asxl1	Fhad1	Itpkb	Ptgs2os2	Ubash3b
Atg9a	Fhl2	Itpri	Ptpn1	Ublcp1
Bbx	Focad	Katnal1	Ptpn5	Ubr2
Bcl9	Foxo2	Katn1	Ptprj	Ucp3
Bhlhe40	Frg1	Kcnd3	Rab31	Utrn
Bod1	Fuom	Kcnh6	Ralgapa2	Vps29b
C230024C17Rik	Fyn	Kdm5a	Ralgs2	Wdr7
Cacna1b	Gad1-ps	Khdclb	Rasgef1b	Wls
Cacl1	Gaint7	Kif1bp	Rassf2	Zochc9
Camk4	Ggnbp1	Kif24	Rbms1	Zdhc14
Car14	Glyr1	Kirrel3	Rhoq	Zfand4
Casp3	Gm11684	Kihl30	Rhoq	Zfp330
Cass4	Gm12536	Larp4b	Ripor2	Zfp365
Ccdc148	Gm12694	Ldocc1	Rnu5g	Zfp407
Cd228	Gm12709	Lpar4	Rps2-ps5	Zfp521
Cd86	Gm12862	Lrrcc5d	Rps21	Zfp629
Cdhr4	Gm13217	Maf	Rtcb	Zfp677
Celf5	Gm14569	Map6	Scube1	Zfp697
Chsy1	Gm14848	Mcf2l	Serpina12	Zmic2
Col15a1	Gm15407	Mepe	Serpina9	Zzz3
Col9a3	Gm15848	Mgat5	Sid1t	
Comm7	Gm16418	Micald	Siglecg	
Cpox	Gm17767	Mirt1	Sik3	
Crrh2	Gm20388	Mllt3	Sia	
Dapp1	Gm20661	Mirps5	Sic22a14	
Def6	Gm21614	Mtra	Sic4a10	
Dera	Gm23218	Mybpc3	Sicc4a1	

B

Gene Ontology Term	Count	p value
GO:0035335-peptidyl-tyrosine dephosphorylation	4	0.001630408
GO:0004721-phosphoprotein phosphatase activity	7	0.002797221
GO:0005085-guanyl-nucleotide exchange factor activity	7	0.004925604
GO:0006470-protein dephosphorylation	6	0.011846165
GO:0005089-Rho guanyl-nucleotide exchange factor activity	4	0.038306049
GO:0016310-phosphorylation	12	0.04000219
GO:0004672-protein kinase activity	11	0.041832928
GO:0008270-zinc ion binding	18	0.042151818
GO:0002250-adaptive immune response	5	0.04865825
GO:0043407-negative regulation of MAP kinase activity	3	0.06691431
GO:0004725-protein tyrosine phosphatase activity	4	0.073957904
GO:0000166-nucleotide binding	27	0.074797927
GO:0004722-protein serine/threonine phosphatase activity	3	0.096974731

Table S1. Acetate enhances chromatin accessibility of genes associated with T cell signaling. Related to Figure 3.

A, List of the 263 genes associated with the 255 open chromatin peaks resulting from acetate treatment of day 9 glucose-restricted cells, as in Fig. 3B. **B**, Pathway enrichment analysis of the genes indicated in A. Gene ontology terms are listed in order of ascending *p* value and the number of genes associated to each gene ontology term is indicated.