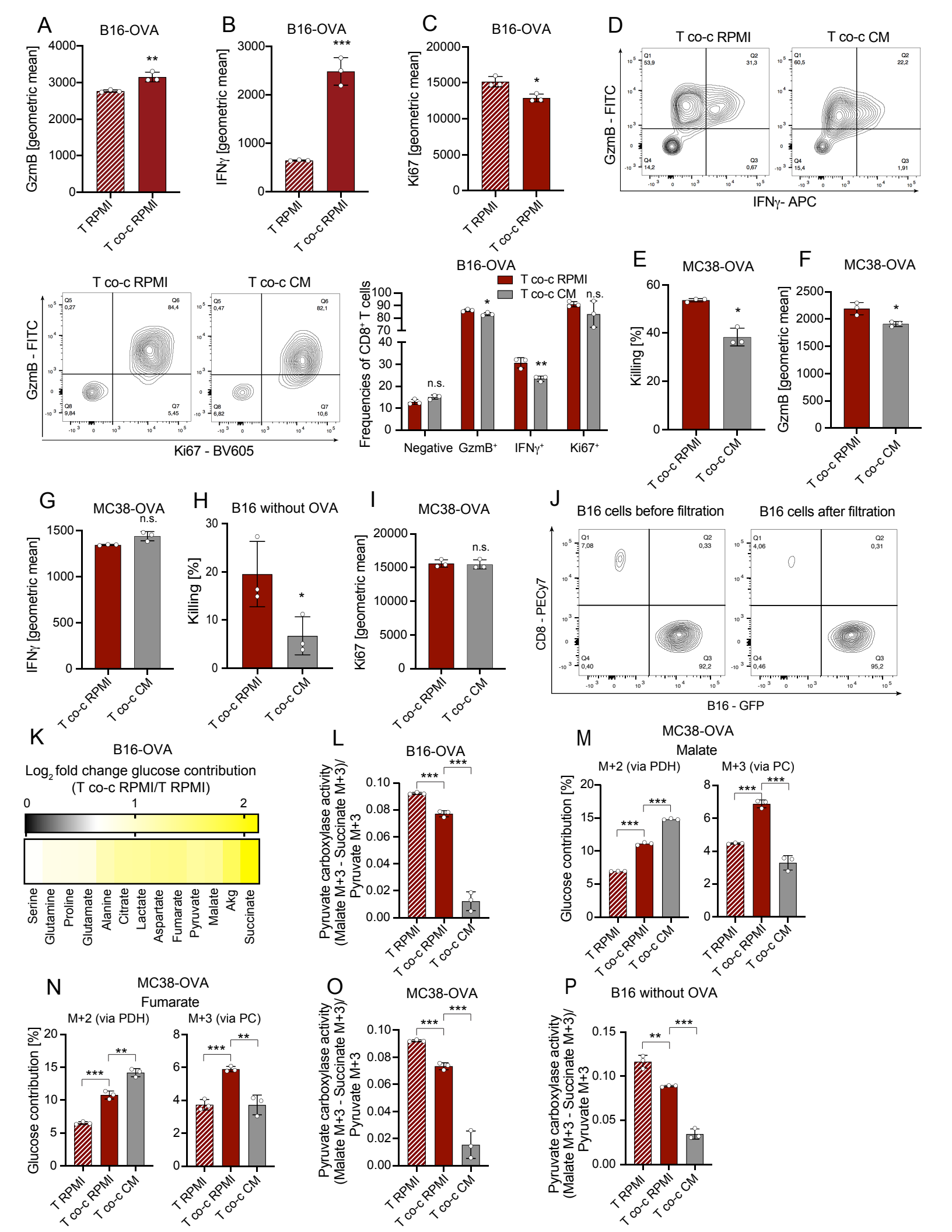
A
B16-OVA
GzmB [geometric mean]
T RPMI
T co-c RPMI
B
B16-OVA
IFNγ [geometric mean]
C
B16-OVA
Ki67 [geometric mean]
D
T co-c RPMI
T co-c CM
GzmB - FITC
IFNγ- APC
T co-c RPMI
T co-c CM
GzmB - FITC
Ki67 - BV605
B16-OVA
T co-c RPMI
T co-c CM
Frequencies of CD8⁺ T cells
n.s.
Negative
GzmB⁺
IFNγ⁺
Ki67⁺
E
MC38-OVA
Killing [%]
F
MC38-OVA
GzmB [geometric mean]
G
MC38-OVA
IFNγ [geometric mean]
H
B16 without OVA
Killing [%]
I
MC38-OVA
Ki67 [geometric mean]
J
B16 cells before filtration
B16 cells after filtration
CD8 - PECy7
B16 - GFP
K
B16-OVA
Log2 fold change glucose contribution
(T co-c RPMI/T RPMI)
Serine
Glutamine
Proline
Glutamate
Alanine
Citrate
Lactate
Aspartate
Fumarate
Pyruvate
Malate
Akg
Succinate
L
B16-OVA
Pyruvate carboxylase activity
(Malate M+3 - Succinate M+3)/
Pyruvate M+3
M
MC38-OVA
Malate
M+2 (via PDH)
M+3 (via PC)
Glucose contribution [%]
N
MC38-OVA
Fumarate
M+2 (via PDH)
M+3 (via PC)
Glucose contribution [%]
O
MC38-OVA
Pyruvate carboxylase activity
(Malate M+3 - Succinate M+3)/
Pyruvate M+3
P
B16 without OVA
Pyruvate carboxylase activity
(Malate M+3 - Succinate M+3)/
Pyruvate M+3

Figure S1. $CD8^+$ T cell metabolism and cytotoxic function are impaired in tumor conditioned media, related to Figure 1. (**A-C**) Geometric mean of GzmB, IFNγ, and Ki67 expression by $CD8^+$ T cells in mono-culture (T) and in co-culture with B16-OVA tumor cells (T co-c) in RPMI. Analysis was performed after 24 hours. (**D**) Representative flow cytometry plots and frequencies of $CD8^+$ T cells expressing GzmB, IFNγ and Ki67 in co-culture with B16-OVA tumor cells in RPMI and CM. Analysis was performed after 24 hours. (**E-G**) Killing and geometric mean of GzmB and IFNγ expression by $CD8^+$ T cells in co-culture with MC38-OVA tumor cells in RPMI and CM. Analysis was performed after 24 hours. (**H**) Killing of tumor cells by $CD8^+$ T cells in co-culture with B16 cells not expressing OVA in RPMI and CM. Analysis was performed after 24 hours. (**I**) Geometric mean of Ki67 expression by $CD8^+$ T cells in co-culture with MC38-OVA tumor cells in RPMI and CM. Analysis was performed after 24 hours. (**J**) Flow cytometric measurement of B16-OVA tumor cell populations before and after filtration. (**K**) Heatmap representing differential $^{13}C_6$-glucose contribution to central carbon metabolites in $CD8^+$ T cells in co-culture with B16-OVA tumor cells compared to $CD8^+$ T cells in mono-culture. Analysis was performed after 6 hours. (**L**) PC activity of $CD8^+$ T cells in mono-culture (RPMI) and in co-culture with B16-OVA tumor cells in RPMI and CM. Analysis was performed after 6 hours. (**M-N**) Contribution of $^{13}C_6$-glucose to malate and fumarate M+2 (derived from PDH activity) and M+3 (derived from PC activity) in $CD8^+$ T cells in mono-culture (RPMI) and in co-culture with MC38-OVA tumor cells in RPMI and CM. Analysis was performed after 6 hours. (**O**) PC activity in $CD8^+$ T cells in mono-culture (RPMI) and in co-culture with MC38-OVA tumor cells in RPMI and CM. Analysis was performed after 6 hours. (**P**) PC activity in $CD8^+$ T cells in mono-culture (RPMI) and in co-culture with B16 tumor cells not expressing OVA in RPMI and CM. Analysis was performed after 6 hours. The number of biological replicates for each experiment was n=3. Error bars represent s.d. Two-tailed unpaired Student's T-test was performed. *P<0.05; **P<0.01; ***P<0.001.

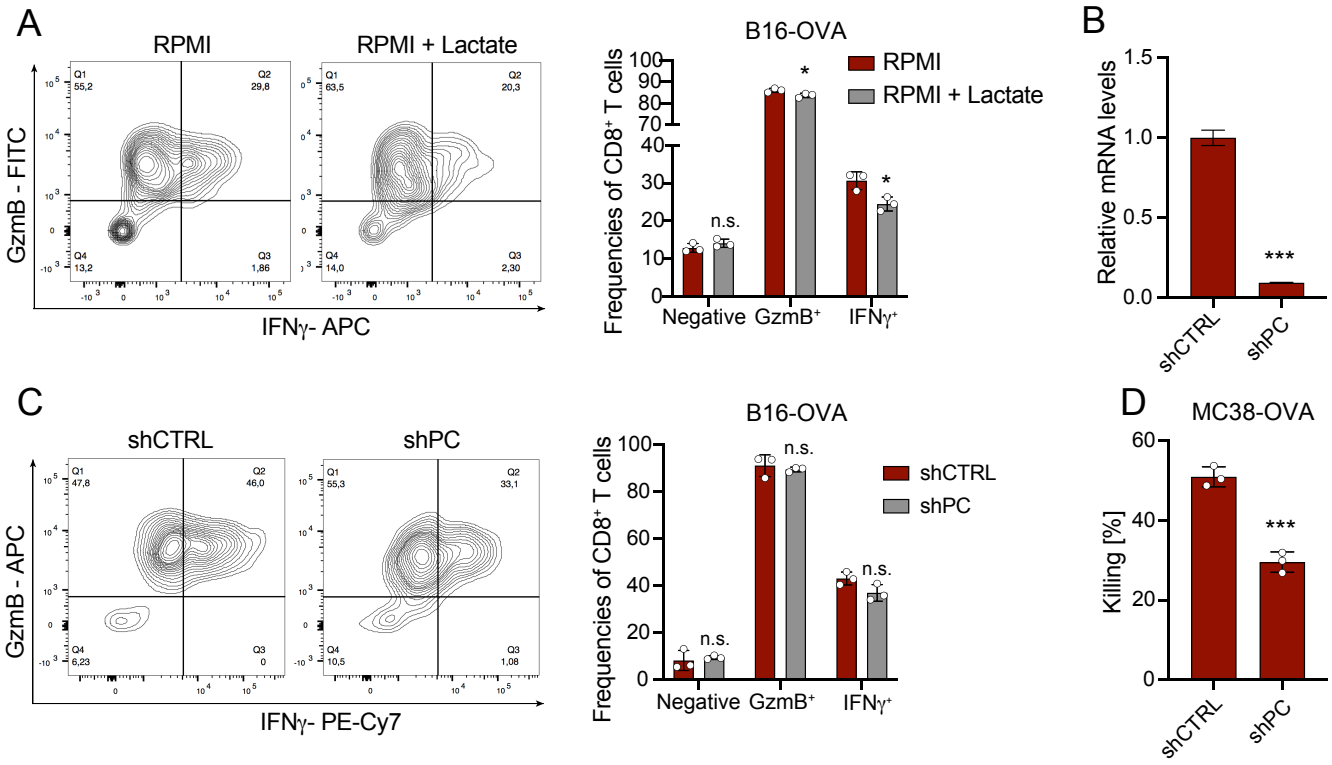

Figure S2. Lactate addition or PC KD impairs CD8⁺ T cell effector function, related to Figure 2 and 3. (**A**) Representative flow cytometry plots and frequencies of CD8⁺ T cells expressing GzmB and IFNγ in co-culture with B16-OVA tumor cells in RPMI and RPMI + Lactate (10mM). Analysis was performed after 24 hours. (**B**) Relative mRNA levels of PC in fold change following transduction with a shRNA for PC and CTRL. (**C**) Representative flow cytometry plots and frequencies of CD8⁺ T cells expressing GzmB and IFNγ after transduction with shRNA for PC or CTRL and co-culture with B16-OVA tumor cells in RPMI. Analysis was performed after 24 hours. (**D**) Killing by CD8⁺ T cells transduced with shRNA for PC or CTRL in co-culture with MC38-OVA tumor cells in RPMI. Analysis was performed after 24 hours. The number of biological replicates for each experiment was n=3. Error bars represent s.d. Two-tailed unpaired Student's T-test was performed. *P<0.05; ***P<0.001.n.s. depicts not significant.

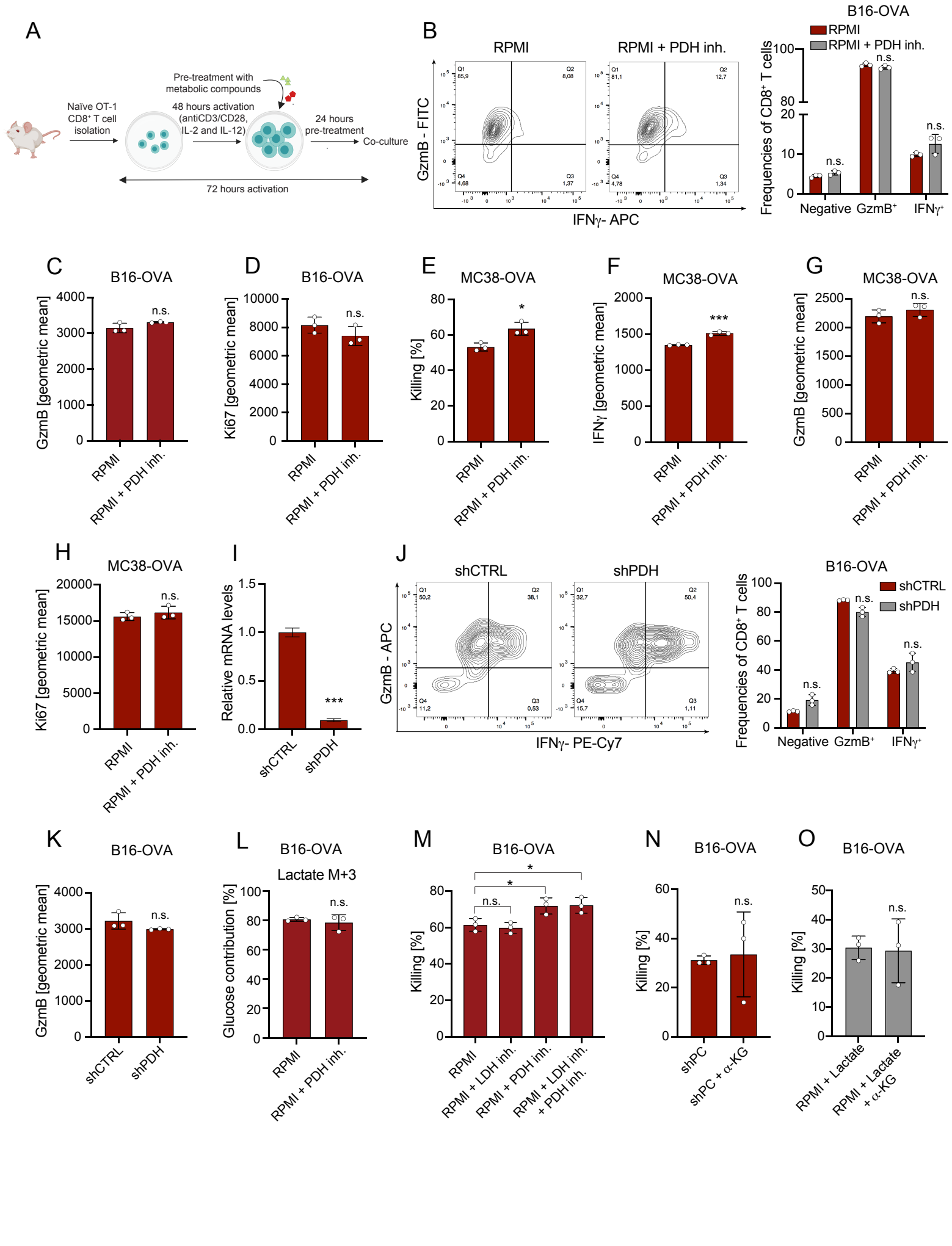
A
Naïve OT-1 CD8+ T cell isolation
Pre-treatment with metabolic compounds
48 hours activation (antiCD3/CD28, IL-2 and IL-12)
24 hours pre-treatment
Co-culture
72 hours activation
B
RPMI
RPMI + PDH inh.
GzmB - FITC
IFNγ- APC
B16-OVA
RPMI
RPMI + PDH inh.
Frequencies of CD8+ T cells
Negative
GzmB+
IFNγ+
C
B16-OVA
GzmB [geometric mean]
D
B16-OVA
Ki67 [geometric mean]
E
MC38-OVA
Killing [%]
F
MC38-OVA
IFNγ [geometric mean]
G
MC38-OVA
GzmB [geometric mean]
H
MC38-OVA
Ki67 [geometric mean]
I
Relative mRNA levels
shCTRL
shPDH
J
shCTRL
shPDH
GzmB - APC
IFNγ- PE-Cy7
B16-OVA
Frequencies of CD8+ T cells
K
B16-OVA
GzmB [geometric mean]
L
B16-OVA
Lactate M+3
Glucose contribution [%]
M
B16-OVA
Killing [%]
RPMI
RPMI + LDH inh.
RPMI + PDH inh.
RPMI + LDH inh. + PDH inh.
N
B16-OVA
Killing [%]
shPC
shPC + α-KG
O
B16-OVA
Killing [%]
RPMI + Lactate
RPMI + Lactate + α-KG

Figure S3. PDH inhibition increases PC activity and CD8$^+$ T cell cytotoxicity without affecting LDH activity, related to Figure 3. (**A**) Schematic representation of CD8$^+$ T cells pre-treatment. After 48 hours activation, CD8$^+$ T cells were pre-treated with specific drugs for additional 24 hours prior co-culture. (**B**) Representative flow cytometry plots and frequencies of CD8$^+$ T cells expressing GzmB and IFNγ in co-culture with B16-OVA tumor cells following pre-treatment with or without the PDH inhibitor CPI-613 in RPMI. CD8$^+$ T cells were pre-treated with the PDH inhibitor for 24 hours (prior co-culture). Analysis was performed after 24 hours. (**C-D**) Geometric mean of GzmB and Ki67 expression by CD8$^+$ T cells in co-culture with B16-OVA tumor cells following pre-treatment with or without the PDH inhibitor CPI-613 in RPMI. CD8$^+$ T cells were pre-treated with the PDH inhibitor for 24 hours (prior co-culture). Analysis was performed after 24 hours. (**E-H**) Killing and geometric mean of GzmB, IFNγ and Ki67 expression by CD8$^+$ T cells in co-culture with MC38-OVA tumor cells following pre-treatment with or without the PDH inhibitor CPI-613 in RPMI. CD8$^+$ T cells were pre-treated with the PDH inhibitor for 24 hours (prior co- culture). Analysis was performed after 24 hours. (**I**) Relative mRNA levels of PDH in fold change following transduction with a shRNA for PDH and CTRL. (**J**) Representative flow cytometry plots and frequencies of CD8$^+$ T cells expressing GzmB and IFNγ following transduction with shRNA for PDH or CTRL in co-culture with B16-OVA tumor cells in RPMI. Analysis was performed after 24 hours. (**K**) Geometric mean of GzmB expression by CD8$^+$ T cells transduced with a shRNA for PDH or CTRL and co-cultured with B16-OVA tumor cells in RPMI. (**L**) Contribution of $^{13}C_6$-glucose to lactate M+3 in CD8$^+$ T cells in co-culture with B16-OVA tumor cells following pre-treatment with or without the PDH inhibitor CPI-613 in RPMI. CD8$^+$ T cells were pre-treated with the PDH inhibitor for 24 hours (prior co-culture). Analysis was performed after 6 hours. (**M**) Killing by CD8$^+$ T cells in co-culture with B16-OVA tumor cells following pre-treatment with either the LDH inhibitor sodium oxamate (10mM) or the PDH inhibitor CPI-613 or the combination of both in RPMI. CD8$^+$ T cells were pre-treated with the PDH inhibitor, LDH inhibitor or both for 24 hours (prior co-culture). Analysis was performed after 24 hours. (**N**) Killing by CD8$^+$ T cells transduced with shRNA for PC and in co-culture with B16-OVA tumor cells in RPMI +/- supplementation with dimethyl-α -ketoglutarate. Analysis was performed after 24 hours. (**O**) Killing by CD8$^+$ T cells in co-culture with B16-OVA tumor cells in RPMI + lactate (10mM) +/- supplementation of dimethyl-α-ketoglutarate. Analysis was performed after 24 hours.The number of biological replicates for each experiment was n=3. Error bars represent s.d. Two-tailed unpaired Student's T-test was performed. *P<0.05; ***P<0.001. n.s. depicts not significant.

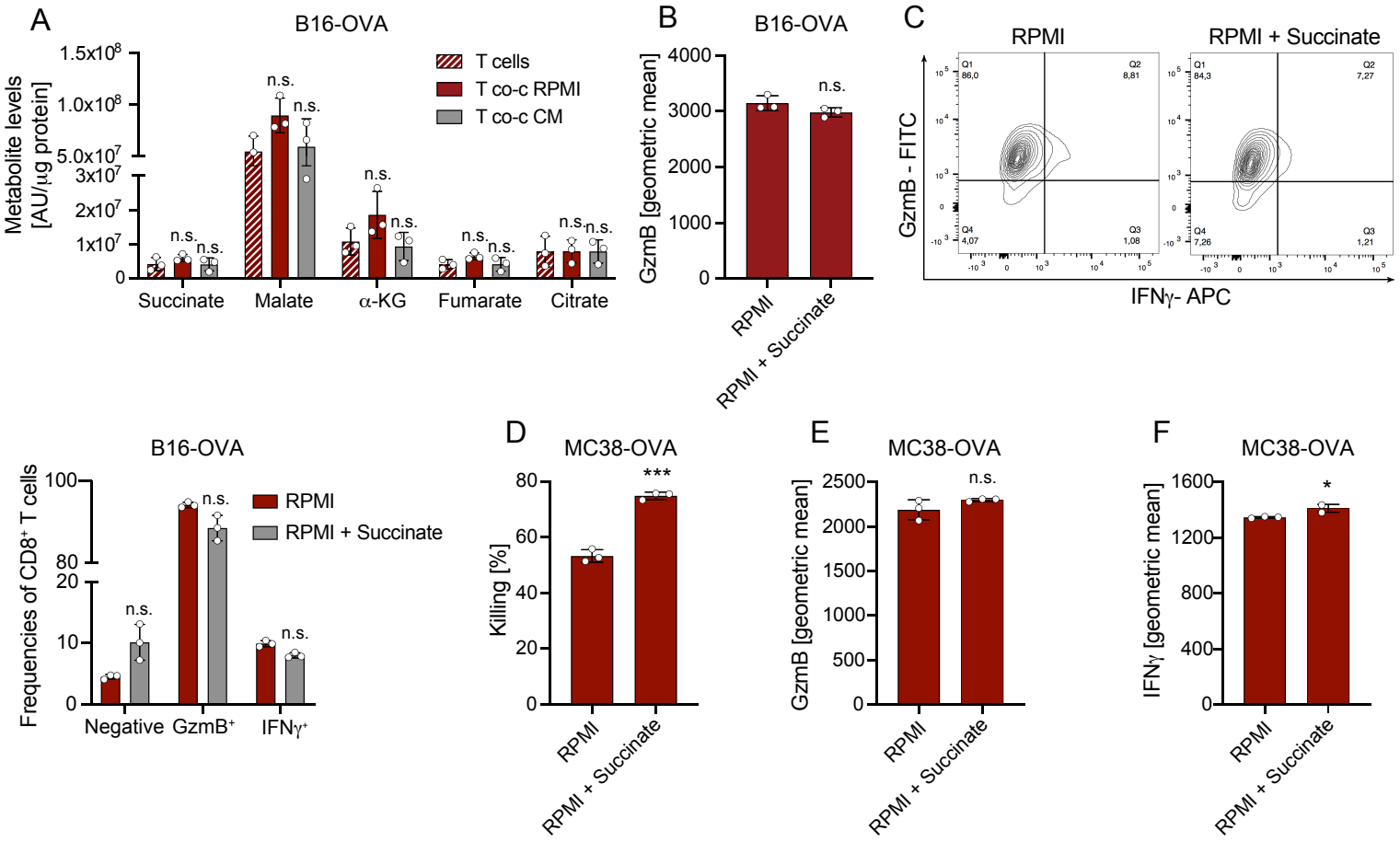

Figure S4. Succinate supplementation increases CD8⁺ T cell cytotoxicity, related to Figure 4. (**A**) Intracellular metabolite levels in RPMI and CM from CD8⁺ T cells in mono-culture or in co-culture with B16-OVA tumor cells. Analysis was performed after 6 hours. (**B**) Geometric mean of GzmB expression by CD8⁺ T cells in co-culture with B16-OVA tumor cells and dimethyl -succinate in RPMI. Analysis was performed after 24 hours. (**C**) Representative flow cytometry plots and frequencies of CD8⁺ T cells expressing GzmB and IFNγ in co-culture with B16-OVA tumor cells and dimethyl-succinate in RPMI. Analysis was performed after 24 hours. (**D-F**) Killing and geometric mean of GzmB and IFNγ expression by CD8⁺ T cells in co-culture with MC38-OVA tumor cells and dimethyl-succinate in RPMI. Analysis was performed after 24 hours. The number of biological replicates for each experiment was n=3. Error bars represent s.d. Two-tailed unpaired Student's T-test was performed. *P<0.05; ***P<0.001. n.s. depicts not significant.

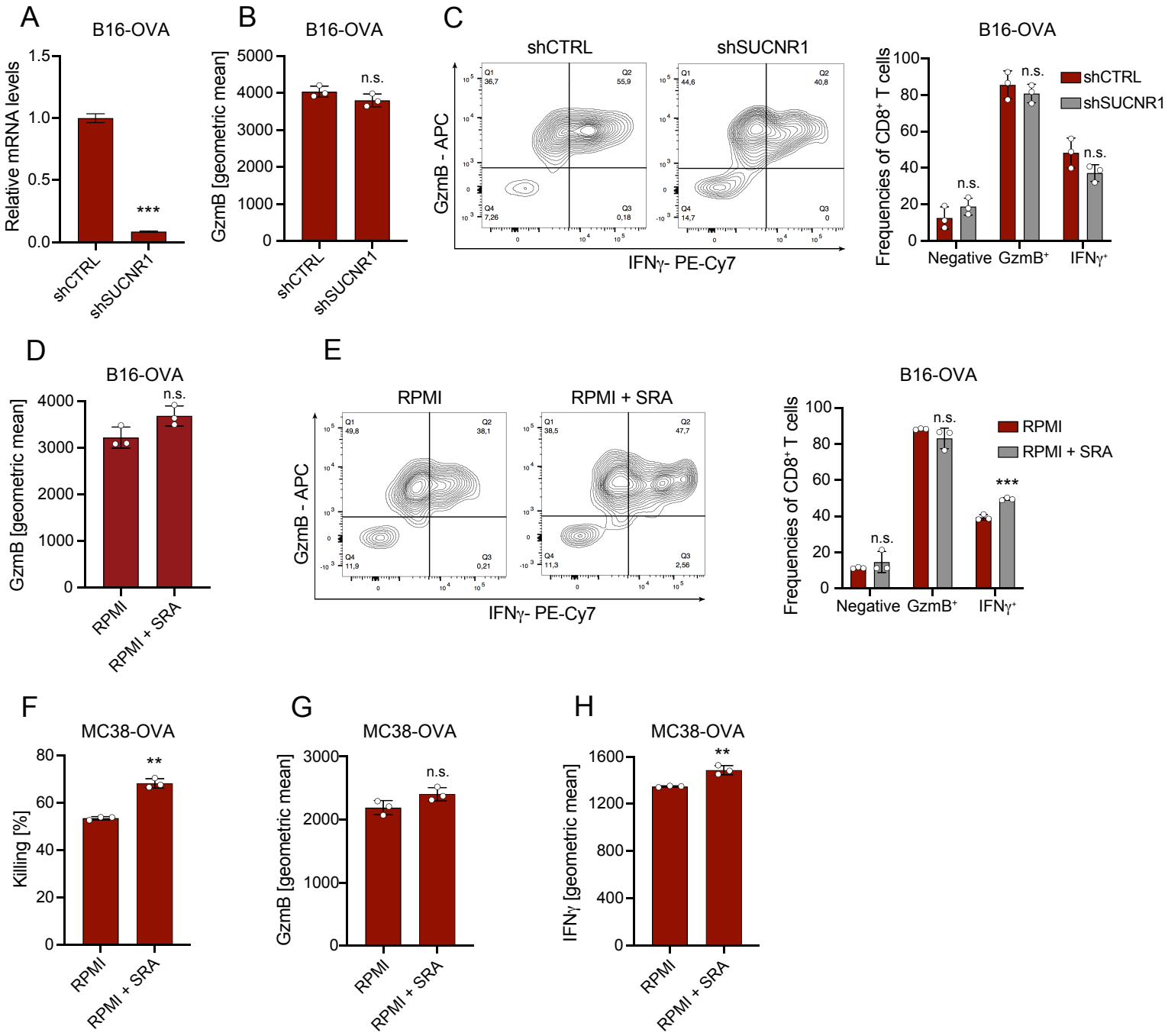

Figure S5. A succinate receptor agonist (SRA) increases CD8⁺ T cell cytotoxicity, related to Figure 5. (**A**) Relative mRNA levels of SUCNR1 in fold change transduced with a shRNA for SUCNR1 and CTRL. (**B**) Geometric mean of GzmB expression by CD8⁺ T cells transduced with a shRNA for SUCNR1 or CTRL and co-cultured with B16-OVA tumor cells in RPMI. Analysis was performed after 24 hours. (**C**) Representative flow cytometry plots and frequencies of CD8⁺ T cells expressing GzmB and IFNγ after transduction with shRNA for SUCNR1 or CTRL and co-culture with B16-OVA tumor cells in RPMI. Analysis was performed after 24 hours. (**D**) Geometric mean of GzmB expression by CD8⁺ T cells in co-culture with B16-OVA tumor cells following pre-treatment with the SRA cis-epoxysuccinic acid in RPMI. CD8⁺ T cells were pre-treated with SRA for 24 hours (prior co-culture). Analysis was performed after 24 hours. (**E**) Representative flow cytometry plots and frequencies of CD8⁺ T cells expressing GzmB and IFNγ in co-culture with B16-OVA tumor cells following pre-treatment with the SRA cis-epoxysuccinic acid in RPMI. CD8⁺ T cells were pre-treated with SRA for 24 hours (prior co-culture). Analysis was performed after 24 hours. (**F-H**) Killing and geometric mean of GzmB and IFNγ expression by CD8⁺ T cells in co-culture with MC38-OVA tumor cells following pre-treatment with the SRA cis-epoxysuccinic acid in RPMI. CD8⁺ T cells were pre-treated with SRA for 24 hours (prior co-culture). Analysis was performed after 24 hours. The number of biological replicates for each experiment was n=3. Error bars represent s.d. Two-tailed unpaired Student's T-test was performed. **P<0.01; ***P<0.001. n.s. depicts not significant.

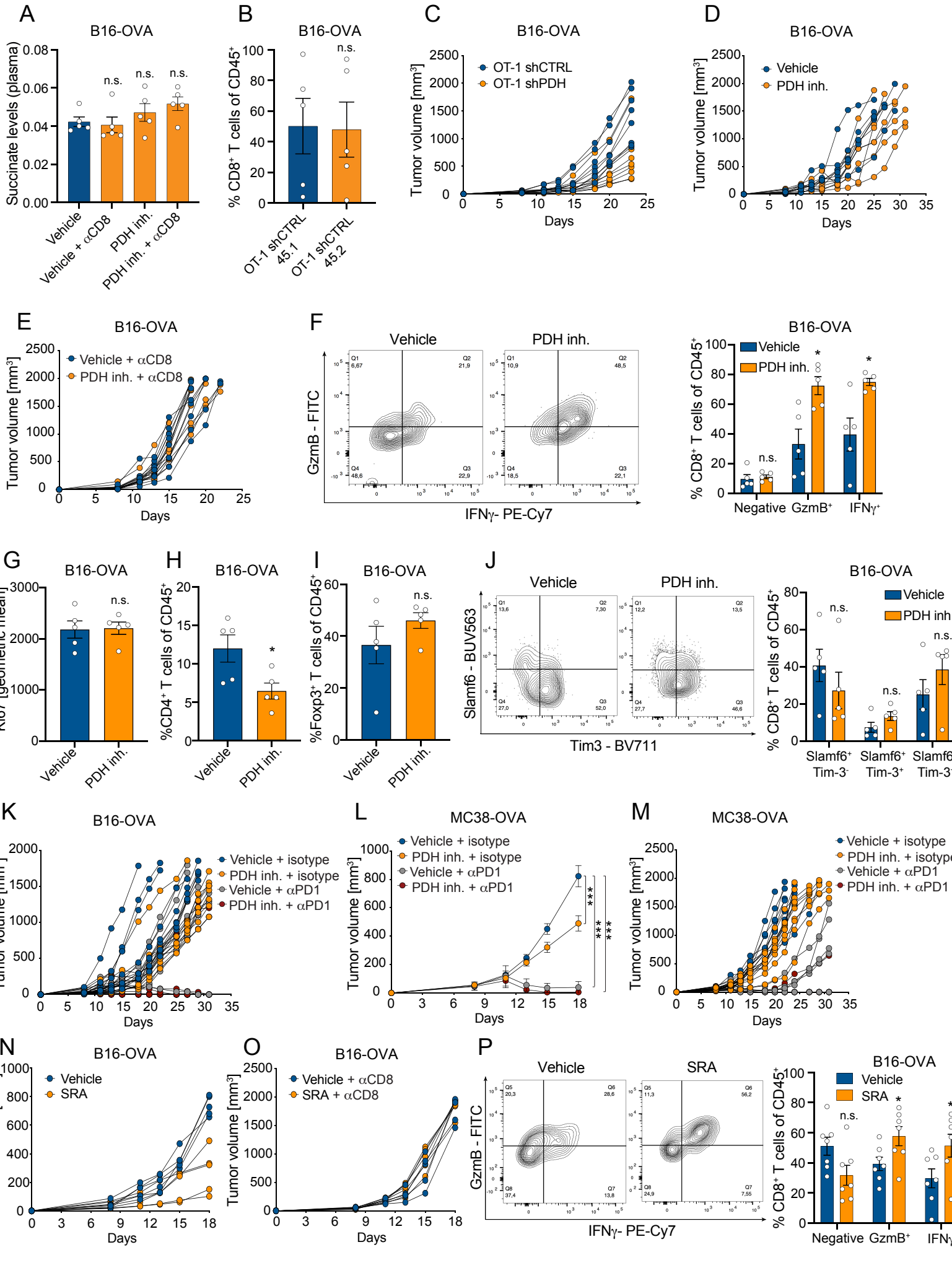
A
B16-OVA
Succinate levels (plasma)
n.s.
Vehicle
Vehicle + αCD8
PDH inh.
PDH inh. + αCD8
B
B16-OVA
% CD8+ T cells of CD45+
n.s.
OT-1 shCTRL 45.1
OT-1 shCTRL 45.2
C
B16-OVA
Tumor volume [mm3]
OT-1 shCTRL
OT-1 shPDH
Days
D
B16-OVA
Tumor volume [mm3]
Vehicle
PDH inh.
Days
E
B16-OVA
Tumor volume [mm3]
Vehicle + αCD8
PDH inh. + αCD8
Days
F
Vehicle
PDH inh.
GzmB - FITC
IFNγ- PE-Cy7
B16-OVA
% CD8+ T cells of CD45+
Vehicle
PDH inh.
n.s.
Negative
GzmB+
IFNγ+
G
B16-OVA
Ki67 [geometric mean]
n.s.
Vehicle
PDH inh.
H
B16-OVA
%CD4+ T cells of CD45+
Vehicle
PDH inh.
I
B16-OVA
%Foxp3+ T cells of CD45+
n.s.
Vehicle
PDH inh.
J
Vehicle
PDH inh.
Slamf6 - BUV563
Tim3 - BV711
B16-OVA
% CD8+ T cells of CD45+
Vehicle
PDH inh.
n.s.
Slamf6+ Tim-3-
Slamf6+ Tim-3+
Slamf6- Tim-3+
K
B16-OVA
Tumor volume [mm3]
Vehicle + isotype
PDH inh. + isotype
Vehicle + αPD1
PDH inh. + αPD1
Days
L
MC38-OVA
Tumor volume [mm3]
Vehicle + isotype
PDH inh. + isotype
Vehicle + αPD1
PDH inh. + αPD1
***
***
Days
M
MC38-OVA
Tumor volume [mm3]
Vehicle + isotype
PDH inh. + isotype
Vehicle + αPD1
PDH inh. + αPD1
Days
N
B16-OVA
Tumor volume [mm3]
Vehicle
SRA
Days
O
B16-OVA
Tumor volume [mm3]
Vehicle + αCD8
SRA + αCD8
Days
P
Vehicle
SRA
GzmB - FITC
IFNγ- PE-Cy7
B16-OVA
% CD8+ T cells of CD45+
Vehicle
SRA
n.s.
Negative
GzmB+
IFNγ+

Figure S6. PDH inhibition promotes tumor regression, also in a MC38 syngeneic mouse model, related to Figure 6. (**A**) Relative succinate levels in the plasma of B16 syngeneic mice. Analysis was performed at day 18. n=5. Two-tailed unpaired Student's T-test was performed. (**B**) % of OT-1 $CD8^+$ T cells of $CD45^+$ with the congenic markers 45.1 and 45.2 transduced with a shRNA for CTRL in B16 tumors. OT-1 shCTRL 45.1 and OT-1 shCTRL 45.2 were adoptively transferred using a ratio 1:1, one day prior B16-OVA tumor implantation. Analysis was performed at day 16. (**C**) Individual tumor growth curves showing tumor volume in mice upon adoptive transfer of OT-1 $CD8^+$ T cells transduced with a shRNA for PDH and CTRL. Adoptive transfer was performed 9 days after B16-OVA tumor implantation (relative to Figure 6D). (**D**) Individual tumor growth curves showing tumor volume in mice treated with either vehicle or the PDH inhibitor CPI-613 (relative to Figure 6E). (**E**) Individual tumor growth curves showing tumor volume in mice treated with either vehicle or the PDH inhibitor CPI-613 with antibodies to specifically deplete $CD8^+$ T cells (relative to Figure 6F). (**F**) Representative flow cytometry plots and % of $CD8^+$ T cells expressing GzmB and IFNγ from mice implanted with B16 cells and treated with either vehicle or the PDH inhibitor CPI-613. Analysis was performed at day 18. Two-tailed unpaired Student's t-test was performed. (**G**) Geometric mean of Ki67 expression in $CD8^+$ T cells from mice implanted with B16 tumor cells and treated with either vehicle or the PDH inhibitor CPI-613. n=5. Analysis was performed at day 18. Two-tailed unpaired Student's t-test was performed. (**H**) % of $CD4^+$ T cells of $CD45^+$ in B16 tumors from mice treated with either vehicle or the PDH inhibitor CPI-613. Analysis was performed at day 18. Two-tailed unpaired Student's t-test was performed. (**I**) % of $Foxp3^+$ T cells of $CD45^+$ in B16 tumors from mice treated with either vehicle or the PDH inhibitor CPI-613. Analysis was performed at day 18. Two-tailed unpaired Student's t-test was performed. (**J**) Representative flow cytometry plots and % of $CD8^+$ T cells of $CD45^+$ expressing Slamf6 and Tim-3 from mice implanted with B16 cells and treated with either vehicle or the PDH inhibitor CPI-613. Analysis was performed at day 18. Two-tailed unpaired Student's t-test was performed. (**K**) Individual tumor growth curves showing tumor volume in mice treated with either vehicle or the PDH inhibitor CPI-613 and isotype control or αPD-1 (Relative to Figure 6I). (**L**) Tumor volume overtime of mice implanted with MC38-OVA cells and treated with either vehicle or the PDH inhibitor CPI-613 with isotype control or αPD-1. Vehicle + isotype: n=9; PDH inh. + isotype: n=10; Vehicle + αPD1: n=5; PDH inh. + αPD1: n=5. A two-way Anova with Sidak's multiple comparison test was performed. (**M**) Individual tumor growth curves of mice implanted with MC38-OVA cells and treated with either vehicle or the PDH inhibitor CPI-613 with isotype control or αPD-1 (Relative to Supplementary Figure 7L). (**N**) Individual tumor growth curves of mice treated with either vehicle or the SRA cis-epoxysuccinic acid (Relative to Figure 6K). (**O**) Individual tumor growth curves of mice treated with either vehicle or the SRA cis-epoxysuccinic acid with antibodies to specifically deplete $CD8^+$ T cells (relative to Figure 6L). (**P**) Representative flow cytometry plots and % of $CD8^+$ T cells of $CD45^+$ expressing GzmB and IFNγ from mice implanted with B16 cells and treated with either vehicle or the SRA cis-epoxysuccinic acid. Analysis was performed at day 18. Two-tailed unpaired Student's t-test was performed. Error bars represent s.e.m. *P<0.05; ***P<0.001. n.s. depicts not significant.