Supplementary methods

T cell isolation

Human PBMCs from healthy blood donors were isolated by density gradient using Lymphoprep™ (Stemcell). CD8+ T cells were isolated using the EasySep™ CD8+ T Cell isolation kit (#17953) and cultured in ImmunoCult™-XF T Cell Expansion medium supplemented with 20 ng/ml IL-2 and 25 µl/ml ImmunoCult™ Human CD3/CD28 activator (all Stemcell)

Oxylipin measurements in ACC tissues:

Oxylipins and polyunsaturated fatty acids (PUFAs) were measured in ACC tissue samples. NSCLC and CRC tissue samples were analyzed as controls. Frozen tissue samples were weighed into Precellys® 24 homogenization tubes containing ceramic beads (1.4 mm; Bertin P000933-LYSK0A tubes). A cooled mixture of ethanol/phosphate buffer (85:15, v/v) was added at a ratio of 3 µL per 1 mg of tissue. Samples were homogenized using a Precellys 24 tissue homogenizer (RRID:SCR_022979) at 5,500 rpm for 3 × 30 seconds at 4 °C with 30-second intervals on ice. 30 µL of each tissue homogenate (equivalent to 10 mg of tissue) were transferred to 1.5 mL Eppendorf tubes. Triplicate QC pool samples were prepared by pooling 10 µL from each study sample. The pooled samples were thoroughly mixed, and 30 µL aliquots were transferred to 1.5 mL Eppendorf tubes. All subsequent steps were conducted as described in the LC-MS/MS section.

Seahorse metabolic phenotyping

At the day of the assay, MΦ were washed with PBS and Seahorse XF RPMI medium, pH 7.4 (Agilent, 103576) supplemented with 2mM L-Glutamine. The metabolic flux was measured using the Agilent Seahorse XFe96 Analyzer (RRID:SCR_019545) according to the manufacturer's protocol. For the metabolic rate test, the following reagents were injected in sequential order for resulting final concentrations: A) 5 mM Glucose; B) 1.5 µM Oligomycin (Sigma Aldrich, 495455); C) 2.5 µM Bam15 (Sigma Aldrich, SML1760-5MG); D) 50 mM 2-DG (Sigma Aldrich, D8375), 0.5 µM Rotenone/Antimycin A (Sigma Aldrich, R8875/A8674) + 8 µM Hoechst3342 (ThermoFisher, 62249). For the flux assay, 3 baseline measurements were followed by the injection of port A-D, while each injection included 3 measurement cycles. Each measurement cycle consisted of 3 min mixing and 3 min measurement time frames. For normalization, cell counts were measured via Hoechst staining in the Cytation1 with the implemented cell measurement software (Agilent Technologies, Santa Clara, CA, USA). Analysis was conducted in the Seahorse Wave software (RRID:SCR_014526).

Viability assay

NCI-H295R, JIL-2266 or CD8+ T cells were seeded into black 96-well plate with clear bottom at a density of 2.5×10^4 cells/well, 1.5×10^4 cells/well or 1×10^4 cells/well, respectively. At the end of the treatment duration, cell viability was detected using CellTiter Glo Assay (Promega) according to the manufacturer's instruction.

Table S1

Table 1: List of antibodies used for IHC, IC, IF, Flow and WB

Primary antibodies					
method	**antigen**	**supplier**	**Cat#**	**RRID**	**dilution**
IHC	CD68	abcam	ab955	RRID:AB_307338	1:10000
	CD163	abcam	ab182422	RRID:AB_2753196	1:200
	COX2	cell signaling	12282	RRID:AB_2571729	1:200
	C1QA	Novus Biologicals	NBP1-87492	RRID:AB_11002443	1:400
IC	CD64	abcam	ab288731	RRID: AB_3675762	1:50
	MerTK	abcam	ab300136	RRID:AB_2936858	1:50
	CD163	R&D Systems	AF1607	RRID:AB_354889	1:100
IF	CD68	abcam	ab955	RRID:AB_307338	1:100
	CD163	R&D Systems	AF1607	RRID:AB_354889	1:100
	C1QA	Novus Biologicals	NBP1-87492	RRID:AB_11002443	1:200
Flow	CD64	abcam	ab288731	RRID: AB_3675762	1:100
WB	CD163	cell signaling	25121	RRID: AB_3675763	1:1000
	CD206	cell signaling	91992	RRID:AB_2800175	1:1000
	COX2	cell signaling	12282	RRID:AB_2571729	1:1000
	GR	cell signaling	12041	RRID:AB_2631286	1:1000
	MerTK	abcam	ab52968	RRID:AB_2143584	1:2000
	C1QA	Novus Biologicals	NBP1-87492	RRID:AB_11002443	1:1000
	GAPDH	sigma	G9545	RRID:AB_796208	1:10000
Secondary antibodies					
AlexaFluor 555 donkey-anti rabbit IgG (H+L)		Invitrogen	A31572	RRID:AB_162543	1:500
AlexaFluor 488 donkey-anti goat IgG (H+L)		Invitrogen	A11055	RRID:AB_2534102	1:500
AlexaFluor 647 donkey-anti mouse IgG (H+L)		Invitrogen	A31571	RRID:AB_162542	1:1000 (IC) 1:200 (Flow)
Goat anti-rabbit peroxidase conjugated		Jackson ImmunoResearch	111-035-003	RRID:AB_2313567	1:10000
Goat anti-mouse peroxidase conjugated		Jackson ImmunoResearch	115-035-003	RRID:AB_10015289	1:10000

Figure S1

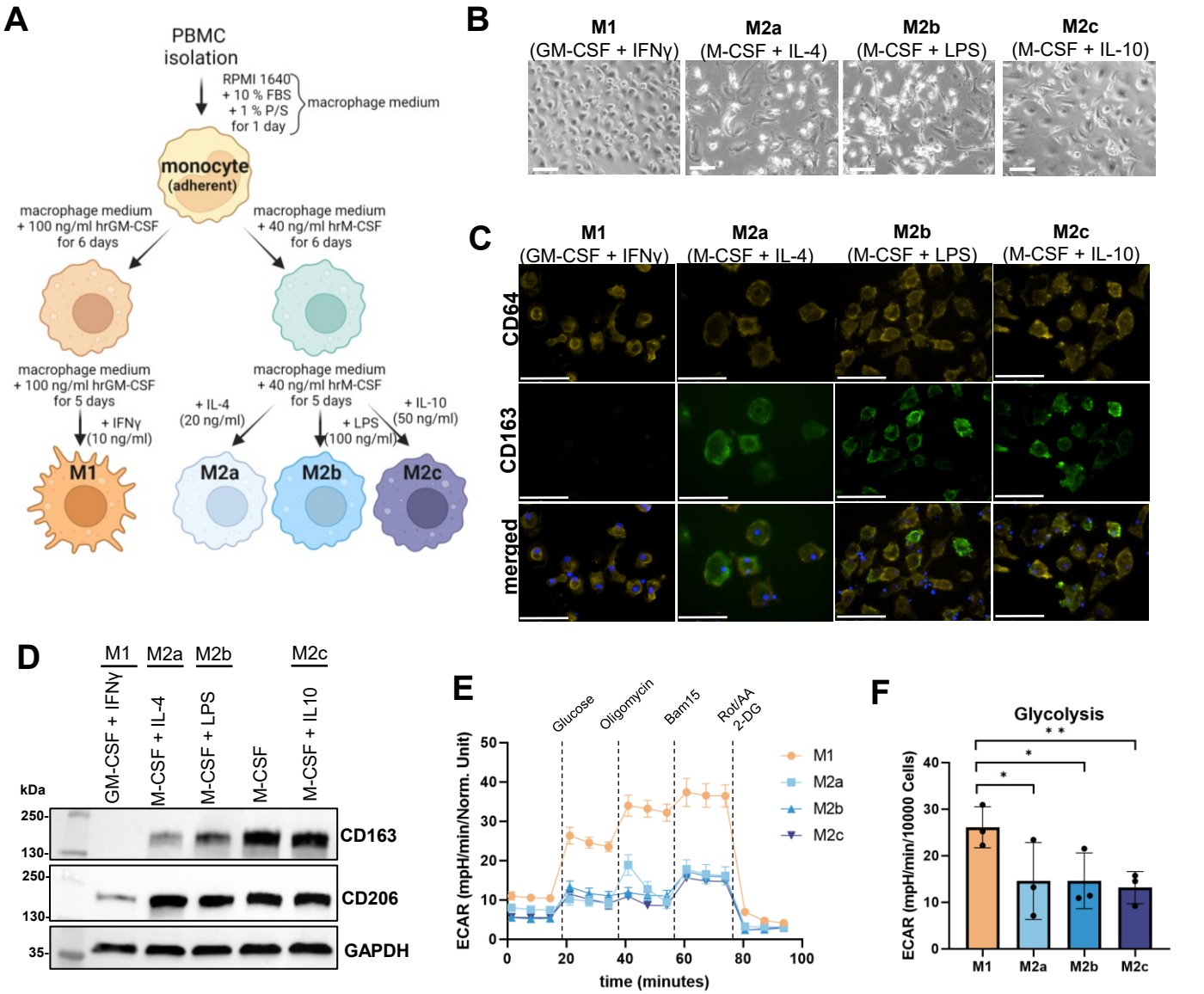

A
PBMC isolation
RPMI 1640 + 10 % FBS + 1 % P/S for 1 day
macrophage medium
monocyte (adherent)
macrophage medium + 100 ng/ml hrGM-CSF for 6 days
macrophage medium + 40 ng/ml hrM-CSF for 6 days
macrophage medium + 100 ng/ml hrGM-CSF for 5 days
+ IFNγ (10 ng/ml)
macrophage medium + 40 ng/ml hrM-CSF for 5 days
+ IL-4 (20 ng/ml)
+ LPS (100 ng/ml)
+ IL-10 (50 ng/ml)
M1
M2a
M2b
M2c
B
M1 (GM-CSF + IFNγ)
M2a (M-CSF + IL-4)
M2b (M-CSF + LPS)
M2c (M-CSF + IL-10)
C
M1 (GM-CSF + IFNγ)
M2a (M-CSF + IL-4)
M2b (M-CSF + LPS)
M2c (M-CSF + IL-10)
CD64
CD163
merged
D
M1
M2a
M2b
M2c
GM-CSF + IFNγ
M-CSF + IL-4
M-CSF + LPS
M-CSF
M-CSF + IL10
kDa
250-
130-
CD163
250-
130-
CD206
35-
GAPDH
E
Glucose
Oligomycin
Bam15
Rot/AA 2-DG
ECAR (mpH/min/Norm. Unit)
time (minutes)
M1
M2a
M2b
M2c
F
Glycolysis
ECAR (mpH/min/10000 Cells)
M1
M2a
M2b
M2c

Figure S2

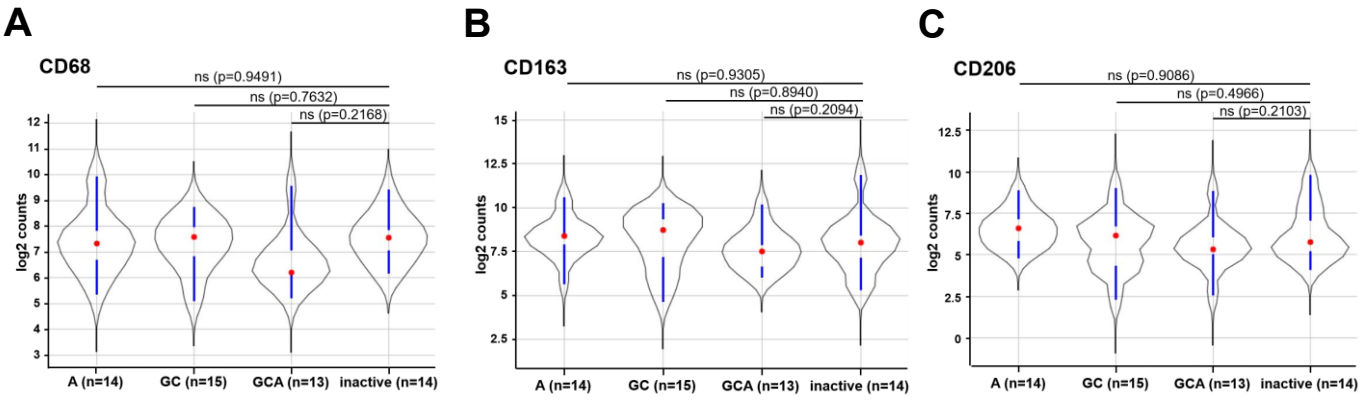

A
CD68
ns (p=0.9491)
ns (p=0.7632)
ns (p=0.2168)
log2 counts
A (n=14)
GC (n=15)
GCA (n=13)
inactive (n=14)
B
CD163
ns (p=0.9305)
ns (p=0.8940)
ns (p=0.2094)
log2 counts
A (n=14)
GC (n=15)
GCA (n=13)
inactive (n=14)
C
CD206
ns (p=0.9086)
ns (p=0.4966)
ns (p=0.2103)
log2 counts
A (n=14)
GC (n=15)
GCA (n=13)
inactive (n=14)

Figure S3

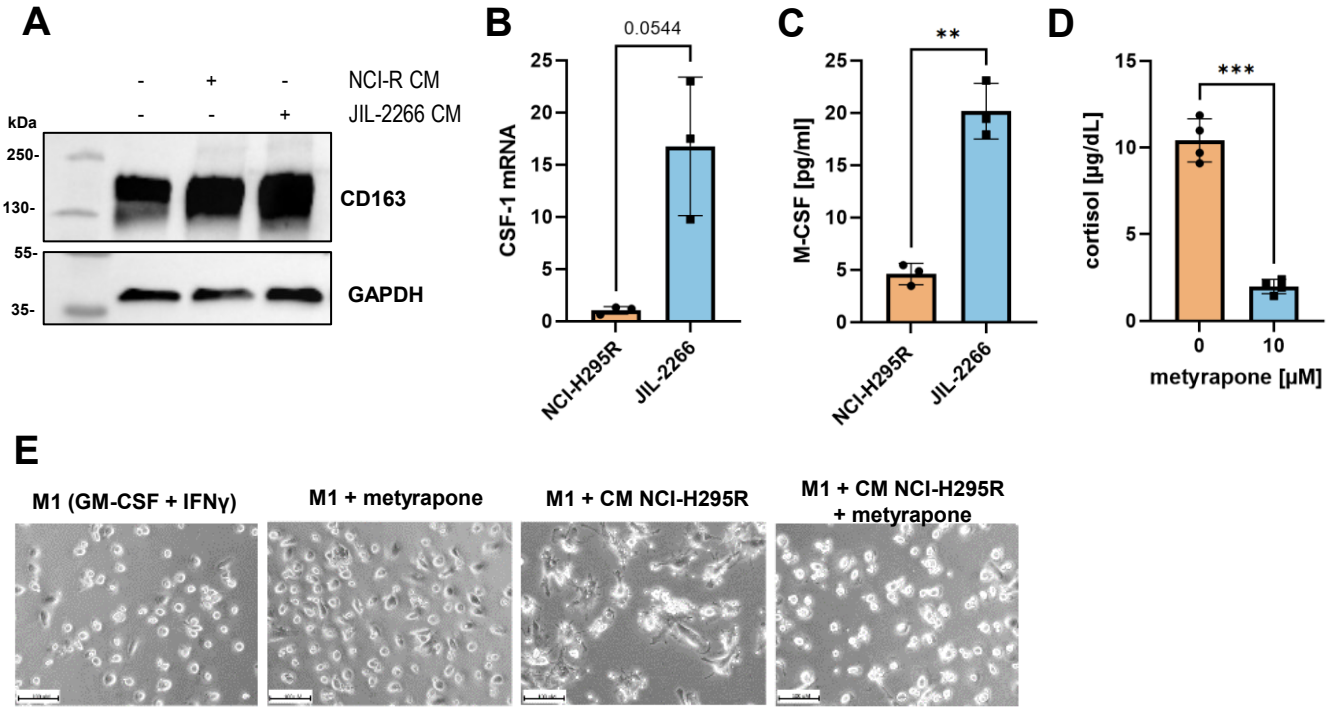

A
NCI-R CM
JIL-2266 CM
kDa
250-
130-
55-
35-
CD163
GAPDH
B
0.0544
CSF-1 mRNA
NCI-H295R
JIL-2266
C
**
M-CSF [pg/ml]
NCI-H295R
JIL-2266
D
***
cortisol [µg/dL]
metyrapone [µM]
E
M1 (GM-CSF + IFNγ)
M1 + metyrapone
M1 + CM NCI-H295R
M1 + CM NCI-H295R + metyrapone

Figure S4

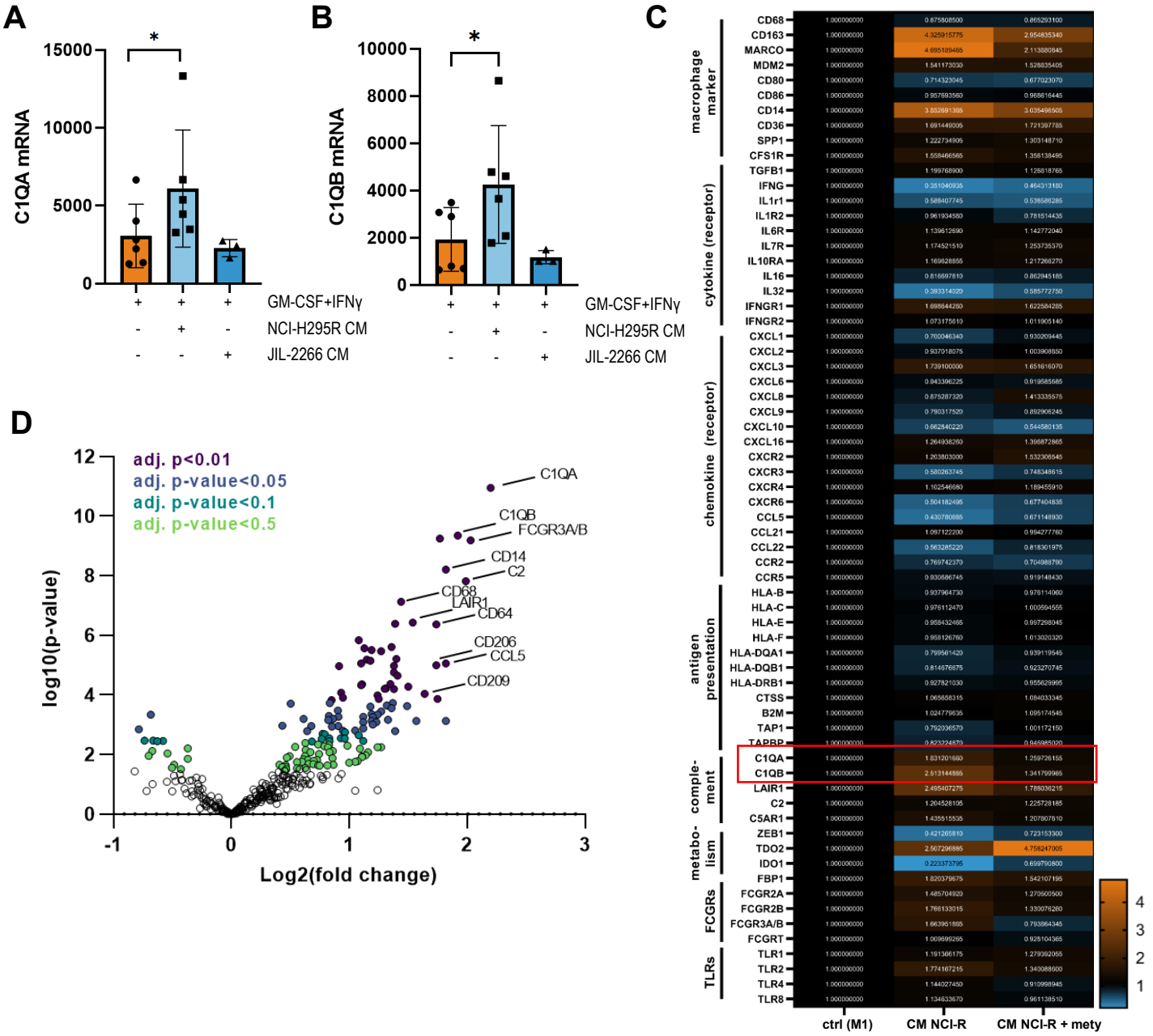
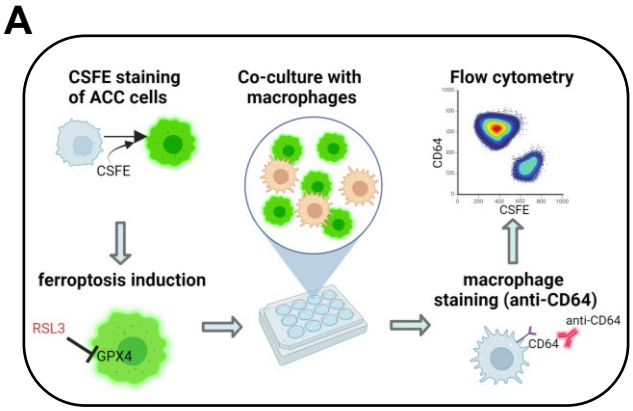
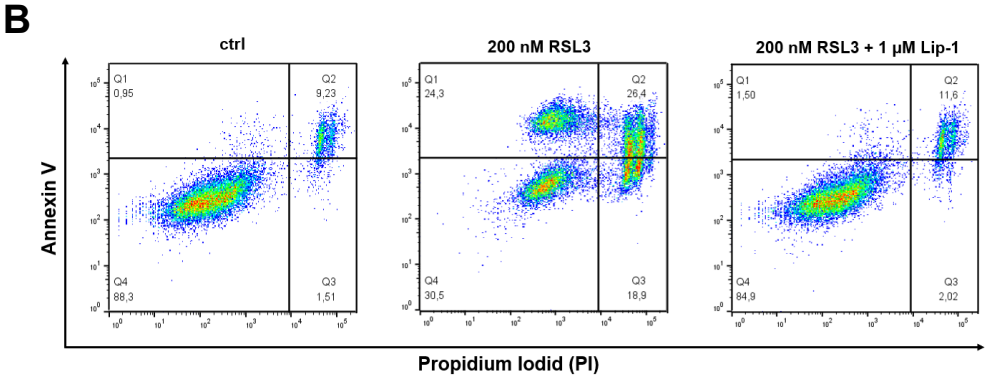
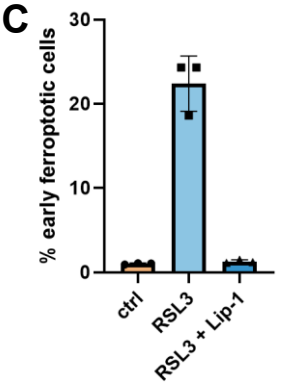
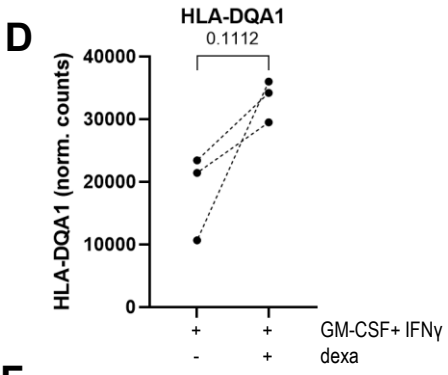

A
B
C
D
C1QA mRNA
C1QB mRNA
GM-CSF+IFNγ
NCI-H295R CM
JIL-2266 CM
adj. p<0.01
adj. p-value<0.05
adj. p-value<0.1
adj. p-value<0.5
log10(p-value)
Log2(fold change)
C1QA
C1QB
FCGR3A/B
CD14
C2
CD68
LAIR1
CD64
CD206
CCL5
CD209
macrophage marker
cytokine (receptor)
chemokine (receptor)
antigen presentation
comple-ment
metabo-lism
FCGRs
TLRs
ctrl (M1)
CM NCI-R
CM NCI-R + mety

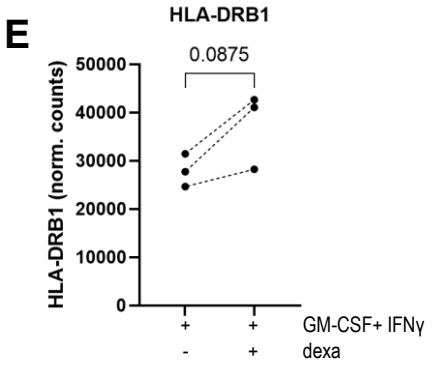
Figure S5


A
CSFE staining of ACC cells
CSFE
Co-culture with macrophages
Flow cytometry
CD64
CSFE
ferroptosis induction
RSL3
GPX4
macrophage staining (anti-CD64)
anti-CD64
CD64


B
ctrl
200 nM RSL3
200 nM RSL3 + 1 µM Lip-1
Annexin V
Propidium Iodid (PI)
Q1 0,95
Q2 9,23
Q4 88,3
Q3 1,51
Q1 24,3
Q2 26,4
Q4 30,5
Q3 18,9
Q1 1,50
Q2 11,6
Q4 84,9
Q3 2,02


C
% early ferroptotic cells
ctrl
RSL3
RSL3 + Lip-1


D
HLA-DQA1
0.1112
HLA-DQA1 (norm. counts)
GM-CSF+ IFNγ
dexa


E
HLA-DRB1
0.0875
HLA-DRB1 (norm. counts)
GM-CSF+ IFNγ
dexa

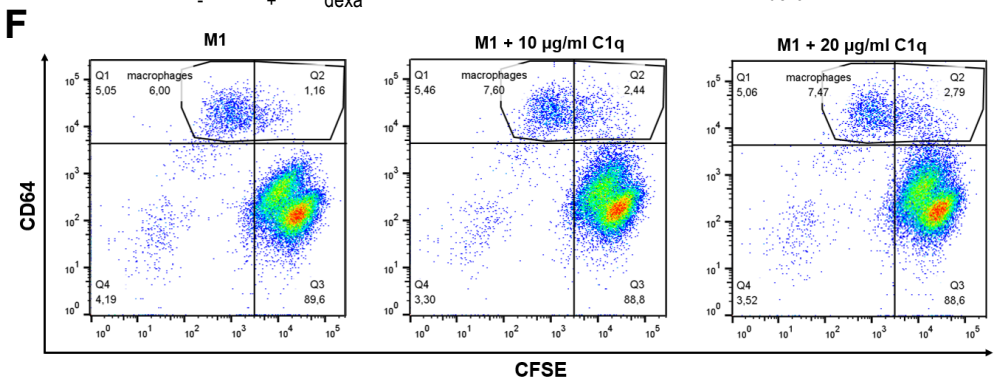

F
M1
M1 + 10 µg/ml C1q
M1 + 20 µg/ml C1q
CD64
CFSE
macrophages
Q1 5,05
Q2 1,16
Q4 4,19
Q3 89,6
Q1 5,46
Q2 2,44
Q4 3,30
Q3 88,8
Q1 5,06
Q2 2,79
Q4 3,52
Q3 88,6

Figure S6

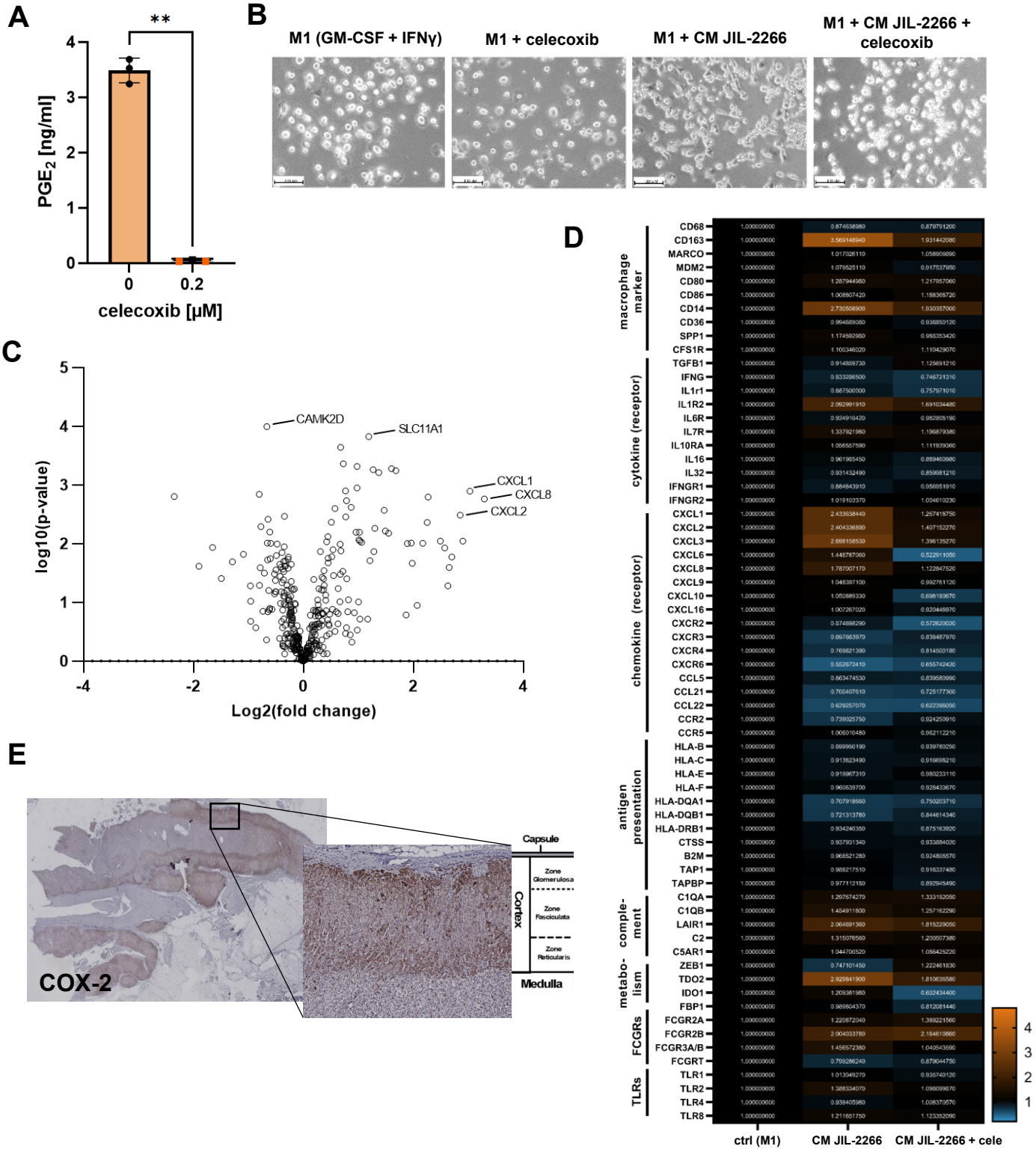

A
**
PGE2 [ng/ml]
celecoxib [µM]
0
0.2
B
M1 (GM-CSF + IFNγ)
M1 + celecoxib
M1 + CM JIL-2266
M1 + CM JIL-2266 + celecoxib
C
CAMK2D
SLC11A1
CXCL1
CXCL8
CXCL2
log10(p-value)
Log2(fold change)
D
macrophage marker
cytokine (receptor)
chemokine (receptor)
antigen presentation
comple-ment
metabo-lism
FCGRs
TLRs
CD68
CD163
MARCO
MDM2
CD80
CD86
CD14
CD36
SPP1
CFS1R
TGFB1
IFNG
IL1r1
IL1R2
IL6R
IL7R
IL10RA
IL16
IL32
IFNGR1
IFNGR2
CXCL1
CXCL2
CXCL3
CXCL6
CXCL8
CXCL9
CXCL10
CXCL16
CXCR2
CXCR3
CXCR4
CXCR6
CCL5
CCL21
CCL22
CCR2
CCR5
HLA-B
HLA-C
HLA-E
HLA-F
HLA-DQA1
HLA-DQB1
HLA-DRB1
CTSS
B2M
TAP1
TAPBP
C1QA
C1QB
LAIR1
C2
C5AR1
ZEB1
TDO2
IDO1
FBP1
FCGR2A
FCGR2B
FCGR3A/B
FCGRT
TLR1
TLR2
TLR4
TLR8
ctrl (M1)
CM JIL-2266
CM JIL-2266 + cele
E
COX-2
Capsule
Cortex
Zone Glomerulosa
Zone Fasciculata
Zone Reticularis
Medulla

Figure S7

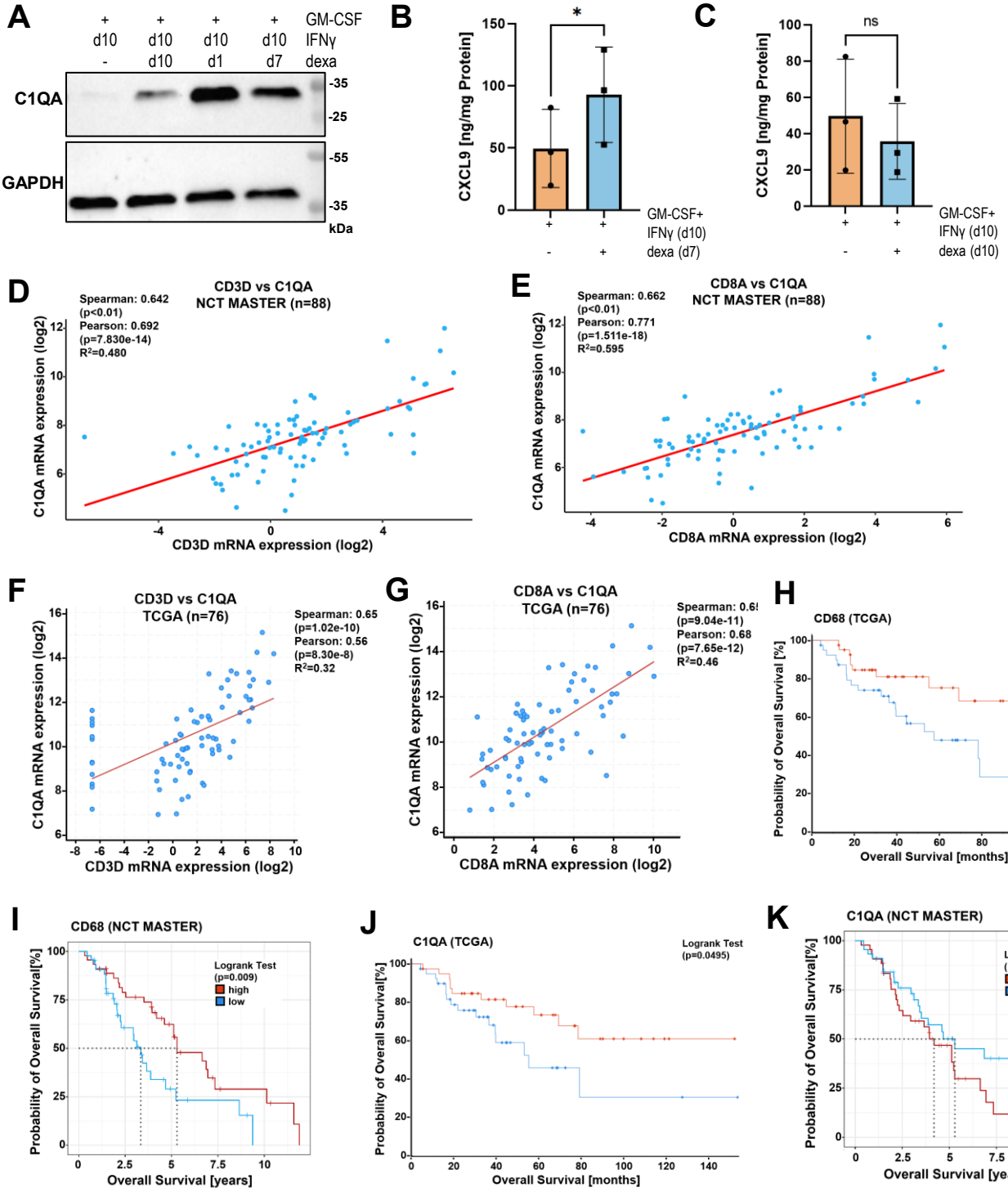

A
GM-CSF
IFNγ
dexa
C1QA
GAPDH
kDa
B
CXCL9 [ng/mg Protein]
GM-CSF+
IFNγ (d10)
dexa (d7)
C
ns
GM-CSF+
IFNγ (d10)
dexa (d10)
D
CD3D vs C1QA
NCT MASTER (n=88)
Spearman: 0.642
(p<0.01)
Pearson: 0.692
(p=7.830e-14)
R²=0.480
C1QA mRNA expression (log2)
CD3D mRNA expression (log2)
E
CD8A vs C1QA
NCT MASTER (n=88)
Spearman: 0.662
(p<0.01)
Pearson: 0.771
(p=1.511e-18)
R²=0.595
C1QA mRNA expression (log2)
CD8A mRNA expression (log2)
F
CD3D vs C1QA
TCGA (n=76)
Spearman: 0.65
(p=1.02e-10)
Pearson: 0.56
(p=8.30e-8)
R²=0.32
C1QA mRNA expression (log2)
CD3D mRNA expression (log2)
G
CD8A vs C1QA
TCGA (n=76)
Spearman: 0.6
(p=9.04e-11)
Pearson: 0.68
(p=7.65e-12)
R²=0.46
C1QA mRNA expression (log2)
CD8A mRNA expression (log2)
H
CD68 (TCGA)
Logrank Test
(p=0.0226)
high
low
Probability of Overall Survival [%]
Overall Survival [months]
I
CD68 (NCT MASTER)
Logrank Test
(p=0.009)
high
low
Probability of Overall Survival[%]
Overall Survival [years]
J
C1QA (TCGA)
Logrank Test
(p=0.0495)
Probability of Overall Survival[%]
Overall Survival [months]
K
C1QA (NCT MASTER)
Logrank Test
(p=0.2)
high
low
Probability of Overall Survival[%]
Overall Survival [years]

Figure S8

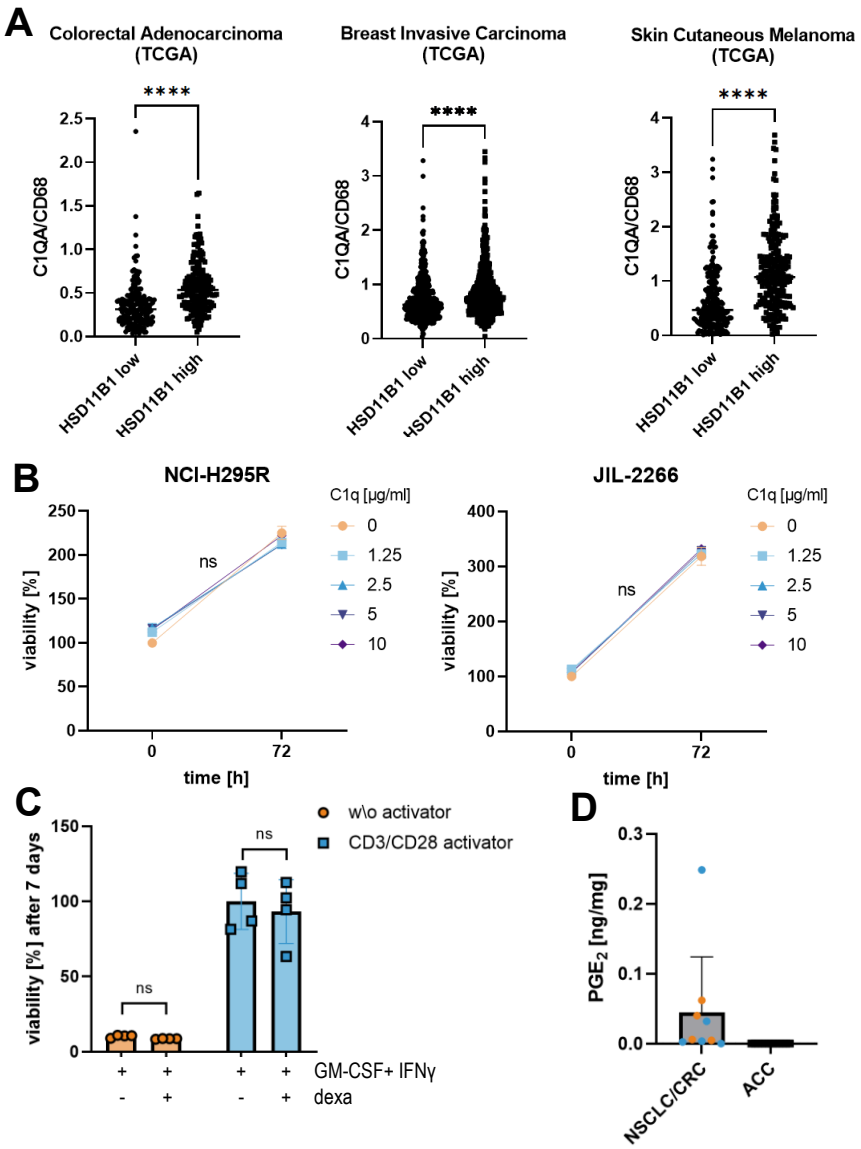

A
Colorectal Adenocarcinoma (TCGA)
Breast Invasive Carcinoma (TCGA)
Skin Cutaneous Melanoma (TCGA)
****
C1QA/CD68
HSD11B1 low
HSD11B1 high
B
NCI-H295R
JIL-2266
C1q [µg/ml]
0
1.25
2.5
5
10
viability [%]
ns
time [h]
C
w\o activator
CD3/CD28 activator
viability [%] after 7 days
GM-CSF+ IFNγ
dexa
D
PGE2 [ng/mg]
NSCLC/CRC
ACC

Figure S1: (A) Overview of the *in vitro* differentiation and polarization of primary human monocytes. Created in BioRender. Triebig, A. (2025) https://BioRender.com/z16d389 **(B)** Morphology of differentiated and polarized MΦ by phase contrast microscopy according to the differentiation status indicated. Representative pictures are shown. Scale bar: 100 µm. **(C)** Expression of the MΦ marker CD64 and CD163 detected by immunofluorescence across the different MΦ phenotypes. **(D)** Expression of the "M2-like" macrophage marker CD163, CD206 according to differentiation status detected by western blot. **(E)** Extracellular acidification rate (ECAR) from metabolic flux assay of polarized MΦ. Representative graph is shown in Mean +/- SEM for technical replicates. **(F)** Quantified normalized ECAR values for glycolysis. N=3 biological replicates are shown in Mean +/- SD.

Figure S2: (A-C) Violin plots displaying the total tumoral expression of (A) *CD68*, (B) *CD163* and (C) *CD206* determined by Nanostring nCounter expression analysis in a total of 56 ACC tumors, stratified by clinical hormone excess; A=androgen excess; GC=glucocorticoid excess; GCA=glucocorticoid and androgen excess. Red dots represent medians, blue lines connect lower adjusted value and lower quartile and upper adjusted value and upper quartile, respectively. FDR adjusted p-values were calculated using the Nanostring nSolver software and Benjamin&Yekulti correction.

Figure S3: (A) Western blot of CD163 expression in MΦ after exposure to CM in the absence of exogenous growth factors. **(B)** Relative mRNA expression of *CSF1* in NCI-H295R and JIL-2266 cells measured by qPCR. **(C)** Total M-CSF in CM of NCI-H295R and JIL-2266 cells quantified by ELISA. **(D)** Total cortisol in supernatants of NCI-H295R after treatment with metyrapone (10 µM) or DMSO control. Cortisol was measured by a chemiluminescence immunoassay (DiaSorin; REF:313261). N=4 biological replicates. **(E)** Morphology of MΦ after exposure to NCI-H295R CM or control as indicated. Representative pictures are shown. Scale bar: 100 µm. CM=conditioned medium

Figure S4: (A-B) *C1QA and* (B) *C1QB* mRNA expression in MΦ exposed to CM of NCI-H295R (N=6), JIL-2266 (N=3) cells or control (N=6). Biological replicates are shown in Mean +/- SD. Statistical analysis was performed using paired t-test. **(B)** Heatmap of macrophage-related genes differentially expressed in MΦ after exposure to CM of NCI-H295R cells treated with 10 µM metyrapone or DMSO control. **(C)** Nanostring nCounter analysis of RNA isolated from 58 ACCs. Tumors were classified according to tumoral *CD163* expression into high and low expression; the median was used as cutoff. Adjusted p-values were calculated using Benjamin&Yekulti correction. CM=conditioned medium

Figure S5: (A) Schematic representation of phagocytosis assay. CSFE stained early ferroptotic ACC cells were co-cultured with MΦ overnight. MΦ were stained for CD64 and phagocytosis was analyzed by flow cytometry. Created in BioRender. Triebig, A. (2025) https://BioRender.com/j94t809 **(B)** Representative flow cytometry plots and **(C)** quantification of AnnexinV+/PI- populations (early ferroptotic cells) after treatment with the ferroptosis inducer RSL3 (200 nM), co-treatment with RSL3 and Lip-1 (1 µM) or DMSO control. **(D-E)** RNA expression of the HLA class II genes (D) *HLA-DQA1* and (E) *HLA-DQR1* in MΦ exposed to dexamethasone or control. N=3 biological replicates are shown in Mean +/- SD. Statistical analysis was performed using paired t-test. **(F)** Representative flow cytometry plots of MΦ phagocytosis after exposure to different concentrations of purified human C1q. Dexa=dexamethasone

Figure S6: (A) Total PGE_2 in supernatants of JIL-2266 cells after treatment with 200 nM celecoxib or DMSO control after 48h detected by ELISA. For measurements below the limit of detection (LOD), values were substituted with LOD/2 and are plotted in red. **(B)** Morphology of MΦ after exposure to JIL-2266 CM or medium control as indicated. Representative pictures are shown. Scale bar: 100 µm. **(C)** Volcano plot showing DEGs in MΦ exposed to GM-CSF+IFNγ vs GM-CSF+IFNγ+PGE_2 (concentration of PGE_2: 100 nM). Analysis of mRNA expression was performed with Nanostring nCounter. Adjusted significances were calculated using Benjamin&Yekulti correction. **(D)** Heatmap of macrophage-related genes differentially expressed in MΦ after exposure to CM of JIL-2266 cells treated with 200 nM celecoxib or DMSO control. **(E)** Immunohistochemical staining of a normal adrenal gland for COX-2. One representative image has been enlarged. Cele=celecoxib; CM=conditioned medium

Figure S7: (A) C1QA protein expression in MΦ treated with GM-CSF (day 1), IFNy (day 10) and dexamethasone (100 nM; varying timepoints as indicated). **(B)** CXCL9 secretion by MΦ exposed to GM-CSF+IFNγ vs GM-CSF+IFNγ and dexamethasone (day 7). N=3 biological replicates are shown in Mean +/- SD. Statistical analysis was performed using paired t-test. **(C)** CXCL9 secretion by MΦ exposed to GM-CSF+IFNγ vs GM-CSF+IFNγ and dexamethasone (day 10). N=3 biological replicates are shown in Mean +/- SD. Statistical analysis was performed using paired t-test. **(D-E)** Correlation of *C1QA* and (D) *CD3D* or (E) *CD8A* expression in ACC tumours within the NCT MASTER dataset. N=88. **(F-G)** Correlation of *C1QA* and (F) *CD3D* or (G) *CD8A* expression in ACC tumours within the TCGA dataset. N=76. **(H-I)** Kaplan-Meier plots of OS according to *C1QA* expression in ACC tumors. Data was obtained from the (H) TCGA or (I) NCT MASTER dataset. **(J-K)** Kaplan-Meier plots of OS according to *C1QA* expression in ACC tumors. Data was obtained from the (J) TCGA or (K) NCT MASTER dataset. Dexa=dexamethasone

Figure S8: (**A**) *C1QA/CD68* gene expression ratio in Colorectal Adenocarcinoma, Breast Invasive Carcinoma and Skin Cutaneous Melanoma. Gene expression data was obtained from the TCGA dataset. Tumors were classified according to tumoral *HSD11B1* expression into high and low expression; the median was used as cutoff. Significances were calculated using Mann-Whitney-U-Test. (**B**) Viability of the ACC cell lines NCI-H295R and JIL-2266 after 48-hour treatment with different concentrations of human purified C1q or DMSO control. To test for effects of different C1q concentrations on cell growth, a two-way ANOVA was performed. **(C)** Viability of CD8+ T cells after 7 days of culture in the CM of MΦ polarized with GM-CSF+IFNγ or GM-CSF+IFNγ+dexamethasone, respectively, with or without the addition of CD3/CD28 activator. N=4 biological replicates are shown in Mean +/- SD. Statistical analysis was performed using paired t-tests. **(D)** Total amount of PGE_2 measured in non-small lung cell carcinoma (NSCLC), colorectal carcinoma (CRC) and ACC tissue samples by LC-MS/MS. Anonymized NSCLC and CRC samples served as controls and were obtained from the Human Tissue and Cell Research (HTCR) foundation.