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Supplementary Information for:

Starvation and anti-metabolic therapy promote cytokine release and recruitment of immune cells

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Datasets S1 to S4

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Supplementary Information Text

Includes supplemental methods, qPCR and PCR primers, materials.

Supplemental methods

RayBio© arrays and GSEA

Cells were plated in 6-well plates the day before treatment and the medium switched to glucose-free medium with 0.5 % FBS when they reached a confluence of 70 %. Debris from culture medium was removed by centrifugation at 250 g for 5 min. Supernatants were stored immediately at -80ºC. They were shipped to RayBiotech and subjected to the RayBio© Human Biotin-Label based antibody arrays L-493 and L-507. To identify the most significant deregulated pathways, Gene Set Enrichment Analysis (GSEA) was performed using javaGSEA Desktop Application (Broad Institute). All collections of publicly available gene sets used were extracted from Molecular Signatures Database v6.0 (MSigDB). GSEA was carried out by comparing expression data from secreted protein arrays performed in control cells against arrays from glucose deprived cells. Nominal p-value < 0.05 or FDR < 0.25 were chosen as the cut-off criteria to identify the significantly enriched gene sets.

Secreted Chemokine and Cytokine Arrays

For the cytokine array, A549 cells were treated for 24 h with pyruvate-free DMEM containing 25 mM or 0 mM glucose, 10 % dialyzed FBS and 1 % L-glutamine as described in the main text. After 24 h, the supernatant was collected and centrifuged for 5 min at 3000 g at 4ºC. The supernatants of 3 independent experiments were combined and the Proteome Profiler Human XL Cytokine Array Kit was performed according to manufacturer instructions.
For the chemokine array, A549 cells were transfected with siRNA against a non-targeting sequence (C) or against ATF4 (sequence #1) and deprived of glucose for 24 h. The supernatants of 3 independent experiments were combined and the Human Chemokine Antibody Array was performed according to manufacturer instructions.

Transfection with Small Interfering RNAs (siRNA)

Cells were plated in 6-well plates at a concentration of 450,000 cells/well in 2 ml fresh culture medium. Cells were transfected 5 h later, when adhered. Transfection solution (culture DMEM without supplements) contained 1 µl/ml DharmaFECT and a final concentration of 50 nM siRNA. 40 h post-transfection, cells were washed and medium was replaced by treatment media.

Western Blot

Supernatants were collected in 15 ml tubes and cells were scraped on ice. Afterwards cells were washed once with 1 ml Phosphate Buffered Saline (PBS) and centrifuged for 5 min, 450 g at 4ºC. The supernatant was discarded, and cell pellet was resuspended in 50 µl RIPA Buffer (25 mM Tris-HCL pH 7.6, 150 mM NaCl, 1 % NP-40, 1 % Sodium deoxycholate, 0.1 % SDS) supplemented with protease inhibitors (Roche) and phosphatase inhibitors (Roche) and stored at -20ºC. Cell lysates were sonicated, and quantification was carried out by Pierce BCA Protein Assay Kit©. The standard curve was prepared by using 2 mg/ml bovine serum albumin (BSA). The optical density was measured at an absorbance of 562 nm by BioTek's PowerWave XS microplate spectrophotometer. For the gel electrophoresis, 40 µg protein of each sample were loaded in a final volume of 35 µl containing 4X Laemmli Buffer (63 mM Tris-HCl; 10 % glycerol; 2 % SDS; 0.01 % bromophenol; 5 % β-mercaptoethanol in H_2O). Samples were denaturalized at 95ºC for 10 min, prior to loading the gel. Proteins were separated by Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE). The percentage of the polyacrylamide gel was chosen according to the size of the protein of interest: 7 % resolving gel was prepared for proteins with the size of 80-180 kDa and 12 % resolving gel for proteins with 15-60 kDa. The gel chamber was filled with 500 ml Running Buffer (0.249 M Tris-Base; 1.92 M glycine; 1 % SDS in H_2O; pH 8.3). Proteins were transferred to a nitrocellulose membrane for 1 h, 100 V at RT by using 1 L of Transfer Buffer (25 mM Tris-HCl; 0.2 M glycine; 3.46 mM SDS; 20 % methanol; dest. H_2O). Membranes were blocked in 5 % non-fat dry milk and TBS-T (0.1 M Tris-HCL; 1.5 M NaCl, 0.1 % Tween 20, H_2O, pH 7.5) for 1 h at room

temperature (RT). The fresh membranes were incubated with the primary antibodies O/N at 4ºC in blocking buffer (2.5 % BSA in TBS-T), and washed afterwards 3 times for 10 min each in TBS-T. The second antibody was either a horseradish peroxidase (HRP) or a fluorescent secondary antibody (Li-COR Biosciences) The first was diluted 1:5000 in TBS-T and the latter 1:15-20.0000 in (1:1) TBS:Odyssey blocking buffer (Li-COR Biosciences) and incubated with the membrane for 1 h at RT. Membranes were washed 3 times for 10 min each in TBS-T and signal was obtained by chemiluminescence (ECL) reaction using freshly prepared ECL reagent and ChemiDoc® development. When fluorescent secondary antibodies were used, the third wash was performed with TBS and the membrane was developed with the Odyssey Infrared Imaging System. Protein bands were quantified by ImageJ and values were normalized to actin. Fold induction was calculated by normalizing to each control.

ELISA

Supernatants were collected and centrifuged at 3000 g to remove dead cells and cell debris and stored at -80ºC. Supernatants were diluted when necessary to be in the optimal optical range and analyzed by ELISA using the DuoSet ELISA Development Systems © according to manufacturer's instructions. Optical densities were measured at a BioTek's PowerWave XS microplate spectrophotometer at 450 and 540 nm. Final cytokine concentrations (pg/ml) were normalized to the protein concentration per ml of each sample (after resuspending the total attached protein from a well in 50 µl), measured as described above for WB, and displayed as pg/mg of protein in the figures.

Gene expression analysis by RT-PCR and qPCR

Cells were washed once with PBS and trypsinized using a 0.05 % trypsin EDTA Solution. Cells were pelleted at RT, 450 g for 5 min. Cell pellet was washed once with PBS, centrifuged again at 450 g for 5 min, and lysed in 350 µl RLT buffer completed with β-mercapthoethanol (10 µl/ml) of the RNeasy MINI KIT© (Qiagen). Cell lysate was mixed with 350 µl 70 % ethanol and transferred to a mini spin column placed in a 2 ml collection tube. RNA extraction was performed according to manufacturer instructions. Total RNA was quantified at the NanoDrop and 1 µg per condition of total RNA was retro-transcribed to cDNA using the High-Capacity cDNA Reverse Transcription Kit (Thermo Fisher). For qPCR, 10 ng of cDNA, 1 µM of primer and the Syber-Green Mastermix (Thermo Fisher) was used per reaction. Amplification was performed in a 384 well plate using the Light Cycler480© according to the following protocol: denaturation 1 cycle at 95ºC; amplification: 45 cycles: 95ºC 4 s, 62ºC 30 s, 72ºC 30 s. For the analysis, the ΔCp of the mRNA of interest was calculated by using L32 as the housekeeping gene. The fold increase 2^(-ΔΔCq) was calculated by normalization to the control sample of cells cultured in the presence of glucose at indicated time points.

For RT-PCR cells were collected, and RNA was extracted and retro-transcribed as described for qPCR. Amplification of cDNA was performed in a Thermal Cycler according to the following protocol: 95ºC 5 min, 95ºC 1 min, 55ºC 1 min, 72ºC 1 min for 34 cycles, 72ºC 1 min, 72ºC 5 min, 16ºC to end.

Chromatin Immunoprecipitation (ChIP)

ChIP-qPCR experiments were performed by plating 420,000 cells on 15 cm plates prior to treatment. At a confluence of 80%, cells were treated for 3h with pyruvate-free DMEM supplemented with 1% glutamine, 10% dFBS and 25 mM (Glc+) or 0 mM (Glc-) glucose, or with 4 µM thapsigargin (Tg). Prior to collection of cells, A549 were fixed and intracellular components crosslinked for 15 min with 1 %-formaldehyde on a rocking chamber. Cells were then scratched, pelleted, and cell membrane lysed with cell lysis buffer (10 mM Tris-HCl, pH 8.0; 10 mM NaCl; 0,5 % NP-40 in dH_2O) containing protein inhibitors for 30 minutes at 4ºC on agitation. Nuclei were collected by centrifugation (3500 rpm, 5 min, 4ºC) and lysed with nuclear lysis buffer (1 % SDS; 10 mM EDTA; 50 mM Tris-HCl, pH 8.0 in dH_2O) containing protease inhibitors for 1 h at 4ºC on a rocking chamber. Samples were then sonicated with an M220 Focused-ultrasonicator with Covaris AFA technology (sonicator settings: peak power 75.0 W, duty factor 10.0 cycle / burst 200) for 12 min. Samples were frozen at -20ºC for at least 30 min and then thawed on ice with subsequent centrifugation at 16000 g for 10 min at 4ºC, in which supernatant was kept. Protein concentration

was measured by Pierce BCA Protein Assay Kit© and all the samples diluted to adjust them to the lowest protein concentration. 10 % input was also collected. 40 μL of Magna ChIP beads per sample were washed with ChIP dilution buffer (1 % Triton X-100; 2 mM EDTA; 150 mM NaCl; 20 mM Tris-HCl, pH8.0 in dH_2O) and incubated at 4ºC overnight with 5 % BSA, 5 % herring sperm DNA in PBS 1X and 2.5 μL of capture antibody (rabbit anti-ATF4 antibody #11815, Cell Signaling). The blocked, capture-antibody-conjugated beads were incubated at 4ºC overnight together with the samples. Next day, beads were washed 3 times with TSE-I buffer (0.1 % SDS; 1 % TritonX-100; 2 mM EDTA, pH 8.0; 20 mM Tris-HCl, pH 8.0; 150 mM NaCl in dH_2O) containing protease inhibitors and other 3 times with TSE-II buffer (0.1 % SDS; 1 % TritonX-100; 2 mM EDTA, pH 8.0; 20 mM Tris-HCl, pH 8.0; 500 mM NaCl in dH_2O) containing protease inhibitors and finally, washed once with TSE-III buffer (0.25 M LiCl; 1% NP-40; 1 % Deoxycholate; 1 mM EDTA, pH 8.0; 10 mM Tris-HCl, pH 8.0 in dH_2O) containing protease inhibitors and washed once with TE buffer (10 mM Tris-HCl, pH 8.0; 1 mM EDTA in dH_2O). Samples were eluted in 120 μL elution buffer (1 % SDS; 1 mM EDTA; 150 mM NaCl; 20 mM Tris-HCl, pH 8.0 in dH_2O) and incubated overnight at 65ºC in order to reverse cross-linkage. After DNA extraction and purification, qPCR was run with 2 μL of DNA per sample in Syber Green MasterMix containing 1 μM of primer pair (*see primer table below*). Cp values were analyzed by the % input method: Adjusted input to 100 % was calculated as follows: adjusted input = *Cp Input – log_2 (10)*. Then, percentage input was calculated with the formula: *100*2^ (adjusted input - Cp (sample).*

Quantitative DNA protein interaction (qDPI)-ELISA

qDPI-ELISA was performed as described elsewhere (see SI Ref. 1). A549 cells were plated at 50 % confluence in 100 mm plates. 24 hour later, they were treated with 25 mM or 0 mM glucose. Cells were collected and centrifuged at 1000 rpm for 5 min at 4ºC. Pellets were then resuspended in 500 μl hypotonic buffer (10 mM HEPES; 1.5 mM $MgCl_2$; 10 mM KCl) and then in 500 μl hypotonic buffer containing 0.5 % NP-40 and protease/phosphatase inhibitors, followed by incubation for 30 minutes at 4ºC. Centrifugation at 13000 rpm for 10 min allowed to isolate nuclei that were then further resuspended in 60 μl low salt buffer (10 mM HEPES; 25 % glycerol; 1.5 mM $MgCl_2$; 20 mM KCl; 0.2 mM EDTA; 0.5 mM DTT and protease/phosphatase inhibitors) and in 60 μl high salt buffer (10 mM HEPES; 25 % glycerol; 1.5 mM $MgCl_2$; 800 mM KCl; 0.2 mM EDTA; 0.5 mM DTT and protease/phosphatase inhibitors) for a total of 120 μl. Centrifugation at 12000 rpm for 15 minutes isolated nuclear proteins that were measured by Pierce BCA Protein Assay Kit© . In parallel, forward and reverse single strand-oligonucleotide sequences 3' biotin-tagged for *CXCL8* promoter were annealed at 95ºC for 10 min and gradually cooled down to 4ºC in annealing buffer (5x buffer concentrations: Tris-HCl 0.5 M, pH 7.6; $MgCl_2$ 0.05 M; 1 M DTT). A streptavidin-coated clear 96-well plate (#15124, ThermoFisher Technologies) was coated with ds-oligos for 2 hours at RT on a rocking platform (2 pmol/well; 100 μl/well). Washes were performed with cold 1x Streptavidin wash buffer (20 mM Tris-HCl, 150 mM NaCl; 0.1 % BSA; 0.05 % Tween-20). Nuclear proteins were then loaded onto wells containing ds-oligos at a final concentration of 25 μg/well in 1x DNA binding buffer (12 mM HEPES, pH 7.9; 60 mM KCl; 0.4 mM EDTA; 0.5 mM DTT) 12 % glycerol; 1 μg/μl poly dI/dC) with addition of extra poly dI/dC (1 μg/μl). Nuclear proteins were left overnight at 4ºC on a rocking platform. Wells were washed and blocked with streptavidin wash buffer for 30 min at RT while rocking. Afterwards, wells were incubated for 1 hour at RT with primary antibody (1:1000 in streptavidin wash buffer), followed by 3 washes and finally incubated with HRP-secondary antibody (1:2000 in streptavidin wash buffer) for 30 minutes at RT. Wells were washed 6X and incubated with TMB substrate (50μl/well) for 20 min at RT in the dark. Without washing the plate, 0.176 M sulfuric acid was added to stop the reaction and absorbance was measured at 450 nm using a BioTek's PowerWave XS microplate spectrophotometer. Background value (the signal of wells without oligonucleotides) was subtracted to each well.

Cell death analysis by Propidium Iodide Incorporation

Cells were collected by trypsinization using a 0.05 % trypsin EDTA solution at RT. Cells were centrifuged for 5 min at 450 g and resuspended in 300 μl PBS containing 0.5 μl/ml propidium iodide (PI). The analysis was performed with the Gallios Flow Cytometer Beckman Coulter©. The quantification was done using the FlowJo software.

Proliferation
A549 and HUVEC were plated at 10% confluence 16 h before treatment and incubated for 24 h with the control or conditioned media of other A549 cells. Cells were fixed and stained with crystal violet solution. For quantification cells were lysed in a 10 % SDS solution and the optical density was measured at an absorbance of 595 nm by BioTek's PowerWave XS microplate spectrophotometer.

Conditioned Medium
A549 and H460 cells were treated with DMEM containing 25 mM or 0 mM glucose, 1 % L-glutamine and 0 % dFBS (A549) or 2 % dFBS (H460). After 24 h, supernatants were collected and centrifuged for 5 min at 3000 g to remove remaining dead cells and cell debris.
Conditioned media of A549 cells was prepared by concentrating supernatants 5X using protein concentrators (Thermo Fisher) (cut off 3 kDa) and stored at -80ºC. The elution of the concentrated conditioned media was kept at -80ºC.
Conditioned filtered media was prepared by concentrating supernatants 20X and washing once with fresh glucose-, serum and pyruvate-free DMEM (starting volume). The final concentration was adjusted to a 5X concentration of the supernatant and stored at -80ºC. (see also Figure S7 (A) for detailed methodological workflow).

Purification of PBMCs
Cells were purified from whole blood buffy coats. Buffy coats (10 ml) were diluted in a 1:1 ratio in PBS and layered on top of 15 ml Ficoll in a tube. After centrifugation for 30 min at 750 g the layer containing PBMCs was transferred into a new 50 ml tube. Cells were washed twice in 50 ml PBS 1X by centrifugation at 250 g, 6 min and room temperature. Cells were resuspended in pyruvate-free DMEM containing 25 mM glucose, 0 % FBS and 1 % L-glutamine.

Purification of neutrophils
Cells were purified from whole blood buffy coats. Buffy coats were diluted 1:4 in 0.9 % NaCl before adding a 3 % Dextran - 0.9 % NaCl solution in a ratio of 1:1. After sedimentation for 30-40 min, the upper layer which contained the cells was centrifuged for 6 min at 250 g without breaks and resuspended in calcium and magnesium free HBSS containing 0.25 % BSA (HBSS-BSA) followed by a percoll gradient. The percoll gradient consisted of a 42 % (top) and 51 % (down) percoll in HBSS-BSA. Cell suspension was layered on top and centrifuged for 10 min at 250 g without breaks. Cell pellet was washed twice in HBSS-BSA. Cells were resuspended in pyruvate-free- DMEM containing glucose, 0 % FBS and 1 % L-glutamine. Cell suspension was used immediately for experiments.

Differentiation of THP-1 and HL60 cells
Undifferentiated THP-1 cells were incubated for 24 h with 100 nM Phorbol 12-Myristate 13-Acetate (PMA) in culture media and allowed to recover for 24 h in fresh culture media before using differentiated THP-1 cells for experimental analysis.
Undifferentiated HL60 cells were incubated with 1.25 % DMSO and trans-retinoic acid (ATRA). After 3 days cells were diluted 1:1 with fresh culture media containing 1.25 % DMSO and were incubated for another 4 days before using differentiated HL60 cells for experimental analysis.

Chemotaxis Assay
A549, HUVEC and differentiated THP-1 cells (50,000) were plated on top of an 8 µm Falcon® boyden insert and conditioned media (described before) was placed on the bottom according to manufacturer's instructions. Cells were allowed to migrate for 20 h. For THP-1 cells 1 % dFBS was added to the bottom chamber. Boyden inserts were washed twice with PBS and cells were stained for 3 h with crystal violet. Remaining crystal violet was removed using a cotton swap and 5 pictures of each boyden were taken using an inverted microscope. Cells were counted using ImageJ.
PBMCs (500,000) were plated on top of a 3 µm Millicell® boyden insert and conditioned media was placed in the bottom chamber – no FBS was added. Cells migrated for 20 h. Migrated cells were centrifuged at 250 g for 5 min and resuspended in 100 µl PBS containing markers for CD14, CD56,

CD3 and CD19. After 20 min at RT cells were washed with 1 ml PBS and resuspended in 300 µl PBS and analysed by FACS. Analysis was performed by FlowJo.
Neutrophils and HL60 cells (500,000) were plated on top of a 3 µm Millicell® boyden insert. The bottom chamber contained the conditioned media with no FBS (for neutrophils) or 2 % FBS (for HL60 cells). Cells migrated for 2 h and were counted using Neubauer chamber.

Invasion
A549 cells were plated at 60 % confluence until adherent. Afterwards, cells were treated for 16 h with conditioned media from A549 cells. Cells were harvested and plated on top of a boyden insert of the *QCM ECMatrix Cell Invasion Assay*. Cells were allowed to migrate for 48 h toward DMEM containing 25 mM glucose, 1 % L-glutamine and 10 % FBS. Migration was analysed according to manufacturer instructions.

Wound healing (scratch) assay
A549 cells were plated 24 h before at a confluence of 70 %. A scratch in the monolayer was performed using a pipette and pictures were taken using an inverted microscope. Cells were incubated with serum-free washed conditioned media of other A549 cells to which 25 mM glucose was added. After 24 h pictures were taken using an inverted microscope and wound area was analyzed using ImageJ. Values were normalized to cells grown in regular serum-free culture media containing 25 mM glucose.

FACS Analysis of peritoneal cells
6-week-old Female Balb/C mice were purchased from Envigo and housed in C3M animal facilities (C3M-Nice, France). Animal experiments were performed according to the guidelines of the Institutional Animal Care and Use Committee and the regional ethics committee (France).
Mice (n=5 / group) were intra-peritoneally injected each 24 h for 3 days with 350 µl of pyruvate- and serum-free RPMI containing 10 mM HEPES (as a negative control) or 350 µl of 20 times concentrated conditioned medium from CT26 cells, which were treated for 48 h with pyruvate and serum-free RPMI containing 10 mM HEPES, supplemented with 25 mM or without (0mM) 2-DG. Conditioned media was washed once with pyruvate and serum-free RPMI containing 10 mM HEPES by centrifugation.
To isolate the peritoneal cells, the peritoneal cavity was flushed with 8 ml of ice-cold RPMI. Cells were stained in PBS, 2 % FCS, 2 mM EDTA and with anti-CD45-APC-Vio770, F4/80-PE, Gr1-VB, CD11b-APC, CD11c-VB, CD3-APC-Cy7, CD19-FITC, Ly6C-PE, Ly6G-FITC and NKp46-e450 (see Materials table below)

Supplementary table: qPCR, PCR, ChIP primers and DPI Oligonucleotides

qPCR Primers	Forward 5'-3'	Reverse 5'-3'
CCL2	CAGCCAGATGCAATCAATGC	GCACTGAGATCTTCCTATTGGTGAA
CCL19	CCAGCCCCAACTCTGAGTG	ATCCTTGATGAGAAGGTAGTGGA
CCL20	CCAAGAGTTTGCTCCTGGCT	TGCTTGCTGCTTCTGATTCG
CHOP	AAGGCACTGAGCGTATCATGT	TGAAGATACACTTCCTTCTTGAACA
CTGF	TTGGCCCAGACCCAACTA	GCA GGA GGCGTTGTCATT
CXCL1	ACTGAACTGCGCTGCCAGTG	GGCATGTTGCAGGCTCCTCA
CXCL2	CACACTCAAGAATGGGCAGA	CTTCAGGAACAGCCACCAAT
CXCL3	TCCCCCATGGTTCAGAAAATC	GGTGCTCCCCTTGTTCAGTATCT
CXCL5	TGGACGGTGGAAACAAGG	CTTCCCTGGGTTCAGAGAC
CXCL8	ATACTCCAAACCTTTCCACCC	TCTGCACCCAGTTTTCCTTG
Ecad	CCCAATACATCTCCCTTCACAG	CCACCTCTAAGGCCATCTTTG
Erdj4	TGGTGGTTCCAGTAGACAAAGG	CTTCGTTGACTGACAGTCCTGC
Herpud1	ACTTGCTTCCAAAGCAGGAA	CTCTTGTGCACTTGGTGGTG
IL-6	CAACCTGAACCTTCCAAAGATG	ACCTCAAACTCCAAAAGACCAG

MCS-F	GTTTGTAGACCAGGAACAGTTGAA	CGCATGGTGTCCTCCATTAT
Ncad	CCCAAGACAAAGAGACCCAG	GCCACTGTGCTTACTGAATTG
Snail1	GCTGCAGGACTCTAATCCAGAGTT	GACAGAGTCCCAGATGAGCATTG
Snail2	ACACATTAGAACTCACACGGG	TGGAGAAGGTTTTGGAGCAG
TNFα	ACTTTGGAGTGATCGGCC	GCTTGAGGGTTTGCTACAAC
Twist1	CTCAGCTACGCCTTCTCG	ACTGTCCATTTTCTCCTTCTCTG
Zeb1	ACCCTTGAAAGTGATCCAGC	CATTCCATTTTCTGTCTTCCGC
PCR Primers	**Forward 5'-3'**	**Reverse 5'-3'**
XBP1	TTACGAGAGAAAACTCATGGCC	GGGTCCAAGTTGTCCAGAATGC
ChIP Primers	**Forward 5'-3'**	**Reverse 5'-3'**
CXCL8 promoter amplicon 1,	AAAGAAAACTTTCGTCATACTCCGT	TCAGGGCAAACCTGAGTCATC
CXCL8 promoter amplicon 2,	CATCAGTTGCAAATCGTGGA	TGGTTTCTTCCTGGCTCTTG
CXCL8 promoter 3 (exon 4)	CAGGAATTGAATGGGTTTGC	AAACCAAGGCACAGTGGAAC
CHOP promoter	ATGATGCAATGTTTGGCAAC	AAGAGGCTCACGACCGACTA
qDPI-ELISA Oligos	**Forward 5'-3'**	**Reverse 5'-3'**
Half CRE	GAAAACTTTCGTCATACTCCGTATTTGATAAGGAACAAATAGGAAGTGTGATGACTCA biotin tag	TGAGTCATCACACTTCCTATTTGTTCCTTATCAAATACGGAGTATGACGAAAGTTTTC biotin tag
NF-kappaB CRE	CCTGAGGGGATGGGCCATCAGTTGCAAATCGTGGAATTTCCTCTGACATAATGA biotin tag	TCATTATGTCAGAGGAAATTCCACGATTTGCAACTGATGGCCCATCCCCTCAGG biotin tag
NF-kappaB	GTTTGCCCTGAGGGGATGGGCCATCAGTTGCAAATCGTGGAATTTCCTCTGACA biotin tag	TGTCAGAGGAAATTCCACGATTTGCAACTGATGGCCCATCCCCTCAGGGCAAAC biotin tag

Supplementary table: materials

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
4E-BP1	Cell Signaling	Cat#9452
ATF4	Cell Signaling	Cat#11815, RRID:AB_2616025
p-AMPK	Cell Signaling	Cat#2535
CD19 (PE-Cy7)	BD Pharmingen	Cat#557835
CD3 (APC)	Becton Dickinson	Cat#345767
CD56 (Alexa Fluor 488)	BD Pharmingen	Cat#557699
CHOP	Cell Signaling	Cat#2895
Human IgG Isotype control	R&D System	Cat#MAB002
Human α-IL-8	R&D System	Cat#MAB208-SP
Human α-IL-6	R&D System	Cat#MAB206-SP
HRP-goat anti-rabbit	Zymax	Cat#81-6120

HRP-goat anti-mouse	Zymax	Cat#81-6520
P65	Cell Signaling	Cat#D14E12
phospho-S6	Cell Signaling	Cat#2211
β-actin	Merk Millipore	Cat#MAB1501R
Mouse CD45-APC-Vio770	Miltenyi	Cat#130-110-662
Mouse CD11b-APC	BD Bioscience	Cat#560521
Mouse CD11c-VB	Miltenyi	Cat#130-091-241
Mouse CD3-APC-Cy7	Miltenyi	Cat#130102306
Mouse CD19-FITC	Miltenyi	Cat #130-102-494
Mouse F4-80-PE	Biolegend	Cat#123110
Mouse Gr1-VB	Miltenyi	Cat#130-102-233
Mouse Ly6C-PE	Biolegend	Cat#128007
Mouse Ly6G-FITC	BD Pharmingen	Cat#551460
Mouse NKp46-e450	ThermoFischer	Cat#48335182
Biological Samples		
Human blood samples (buffy coats)	Banc de Sang i Teixits – BST (Blood and Tissue banc)	BB014
Chemicals, Peptides, and Recombinant Proteins		
2-DG	Sigma	Cat#2077
BAY 11-7082	Bionova	Cat#196870
Metformin	Sigma	Cat#D150959
MKC-8866	Fosun Orinove PharmaTech Inc.	N/A
Rapamycin	Merck Millipore	Cat#553210
Torin1	Selleckchem	Cat#S2827
Phorbol 12-Myristate 13 Acetate (PMA)	Sigma	Cat#P1585
All-trans-retinoic acid	Sigma	Cat#R2625
Ficoll-Lymphocyte Isolation Solution (1.077 g/ml)	Rafer	Cat#LN0250
Percoll	Sigma	Cat#P4937
HBSS (with calcium and magnesium)	Life Technologies	24020-091
HBSS (without calcium and magnesium)	Sigma	H6648
RPMI	Sigma	R8758
Dulbecco's Modified Eagle Medium (DMEM)	Thermo Fisher	41965-062
Dulbecco's Modified Eagle Medium (DMEM)- glucose free	Thermo Fisher	11966-025
Dulbecco's Modified Eagle Medium (DMEM)- L-glutamine free.	Thermo Fisher	11960-044
DharmaFECT1	Fisher Scientific	T-2001-02
EGMTM-2 Endothelial Cell Growth Medium-2 BulletKitTM	Lonza	CC-3162
Airway Epithelial Cell Basal Medium	ATCC	PCS-300-030
Bronchial Epithelial Cell Growth Kit	ATCC	PCS-300-040
Critical Commercial Assays		
Human Chemokine Antibody Array	R&D Systems	Cat#ARY017
Proteome Profiler Human XL Cytokine Array Kit	R&D Systems	Cat#ARY022B
DuoSet ELISA Development Systems	R&D Systems	Cat#DY008

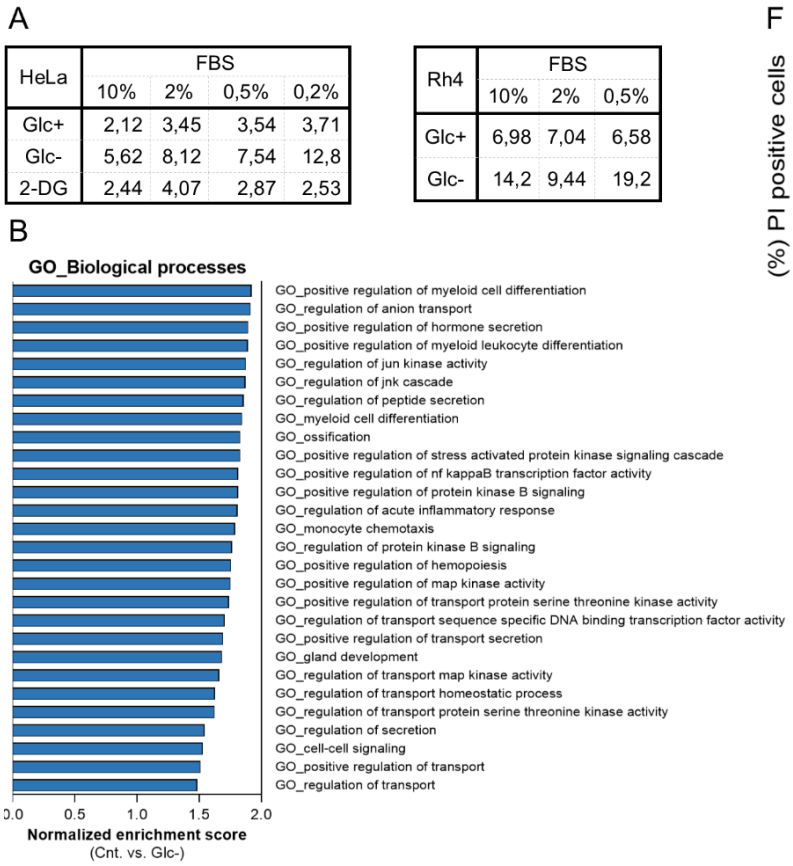
Human IL-6 DuoSet ELISA	R&D Systems	Cat#DY206
Human IL-8/CXCL8 DuoSet ELISA	R&D Systems	Cat#DY208
Human TNF-alpha DuoSet ELISA	R&D Systems	Cat#DY210
Human CXCL1/GRO alpha DuoSet ELISA	R&D Systems	Cat#DY275
Human M-CSF DuoSet ELISA	R&D Systems	Cat#DY216
Human CXCL5/ENA-78 DuoSet ELISA	R&D Systems	Cat#DY254
Human CCL20/MIP-3 alpha ELISA Kit	Novus	Cat#NBP2-31049-1 KIT
QCM ECMatrix Cell Invasion Assay	Millipore	Cat#ECM554
Human L493 Array, membrane https://www.raybiotech.com/human-l493-array-membrane-2/	RayBio	Cat#AAH-BLM-2A-2
Human L507 Array, membrane https://www.raybiotech.com/l-series-507-label-based-human-array-1-membrane-2/	RayBio	Cat# AAH-BLM-1A-2
Pierce™ BCA Protein Assay Kit	Thermo Fisher	Cat#23225
RNeasy MINI KIT	Qiagen	Cat#74104
High-Capacity cDNA Reverse Transcription Kit	Thermo Fisher	Cat#4368814
Experimental Models: Cell Lines		
HeLa		ATCC® CCL-2™
HeLa		ECACC
A549		ATCC-CCL-185
HBTEC (Primary Bronchial/Tracheal Epithelial Cells)		ATCC-PCS-300-010
THP-1		88081201-1VL
Oligonucleotides		
siRNA targeting sequence: ATF4#1 (A#1) 5'-CCAGAUCAUUCCUUUAGUUUA-'3; ATF4#2 (A#2) 5'-GCCUAGGUCUCUUAGAUGA-'3	(León-Annicchiarico et al., 2015), Iurlaro et al (2017). Mol. Cell. Biol. *37*, MCB.00479-16-	
siRNA targeting sequence: XBP1#1 (X#1) 5'-GGAAGCCAUUAAUGAACUA-'3; XBP1#2 (X#2) 5'-AGAAGGCUCGAAUGAGUG-'3		
siRNA targeting sequence: P65#1 (p#1) 5'-GAUUGAGGAGAAACGUAAA-'3; P65#2 (p#2) 5'- GCCCUAUCCCUUUACGUCA-'3	Cullen, S.P. et al (2013), Mol. Cell *49*, 1034–1048.	
Primers for qPCR, PCR, ChIP and qDPI-ELISA see Table above		
Software and Algorithms		
ImageJ	ImageJ	1.44o
Image Lab	Bio-Rad	6.0
Prism	GraphPad	8.0
FlowJo	FlowJo	7.6.4
GSEA-P	http://www.broad.mit.edu/GSEA	RRID:SCR_003199

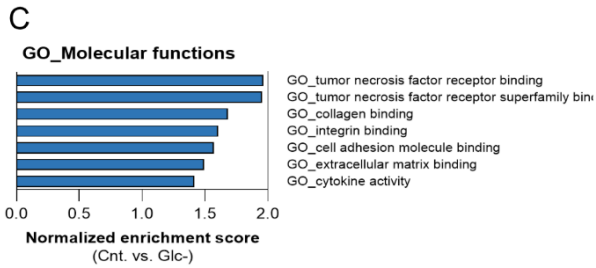
Fig. S1.

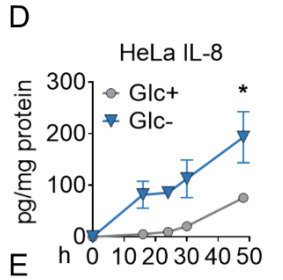
A

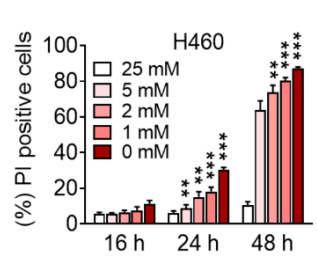
HeLa	FBS			
	10%	2%	0,5%	0,2%
Glc+	2,12	3,45	3,54	3,71
Glc-	5,62	8,12	7,54	12,8
2-DG	2,44	4,07	2,87	2,53

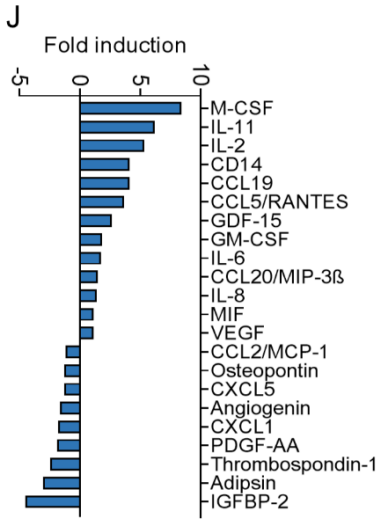
Rh4	FBS		
	10%	2%	0,5%
Glc+	6,98	7,04	6,58
Glc-	14,2	9,44	19,2

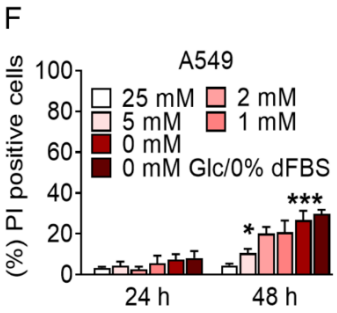


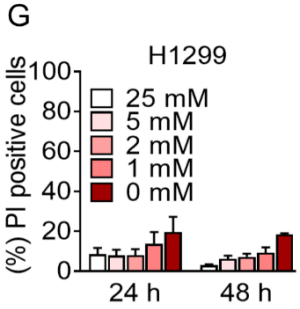


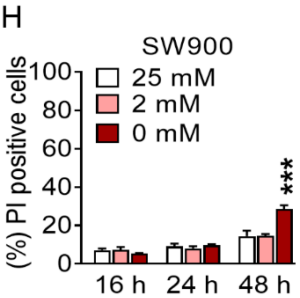


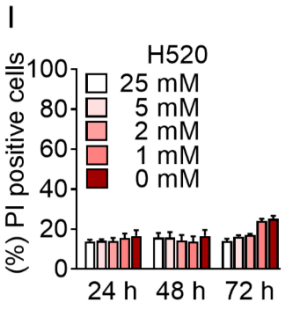












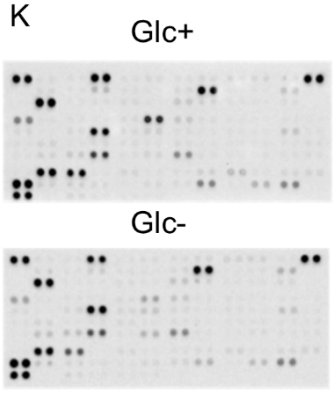


Fig. S1. Analysis of cell death and biological functions of proteins secreted upon glucose deprivation in cancer cells

A. HeLa and Rh4 cells were incubated with 25 mM (Glc+) or 0 mM (Glc-) or in complete medium with 10 mM 2-deoxyglucose (2-DG) for 24 h with indicated FBS concentrations. Cell death was analyzed by FACS. Data are represented as (%) cell death. One experiment is represented.

B-C. HeLa cells were incubated with 25 (Glc+) or 0 mM (Glc-) glucose or with 10 mM 2-Deoxyglucose (2-DG) and 0.5 % dialyzed FBS for 24 h. Supernatants were collected and arrays L-493 and L-507 were performed by RayBiotech (see results in SI1 and SI2). GSEA was performed between Glc+ (Cnt) and Glc-: the graph depicts a plot list of enriched gene sets upon treatment conditions. Gene set collections related to GO terms for Biological processes (A) and Molecular functions (B) were downloaded from MSigDB.v6 and used for the analyses. Corresponding normalized enriched score (NES) regarding each significant gene set with FDR <0.25 or p<0.05 are included. (See also Dataset S3).

D. HeLa cells were treated for indicated times with 25 mM (Glc+) or 0 mM (Glc-), and supernatant was analysed by ELISA for IL-8. Data show mean ± SEM (n=5). Asterisks denote significant differences between Glc- and Glc+, analysed by two-way ANOVA.

E-I Different cell lines were treated at different glucose concentrations as shown and collected at indicated time points. Cell death was analyzed by propidium iodide (PI) incorporation by FACS. Data are represented as mean ± SEM (n=3-4). Asterisks denote significant differences between treated cells and the control sample in Glc+ of each time point, analysed by two-way ANOVA.

J-K. A549 cells were treated for 24 h with media containing 25 (Glc+) or 0 mM (Glc-) glucose. Supernatants of three independent experiments were collected and combined. Proteome Profiler Human XL Cytokine Array Kit was performed according to manufacturer instructions. Mean densities of spots on membranes (K) were analysed by Image J and selected proteins are shown in J. Fold induction of each cytokine was calculated versus Glc+.

Fig. S2.

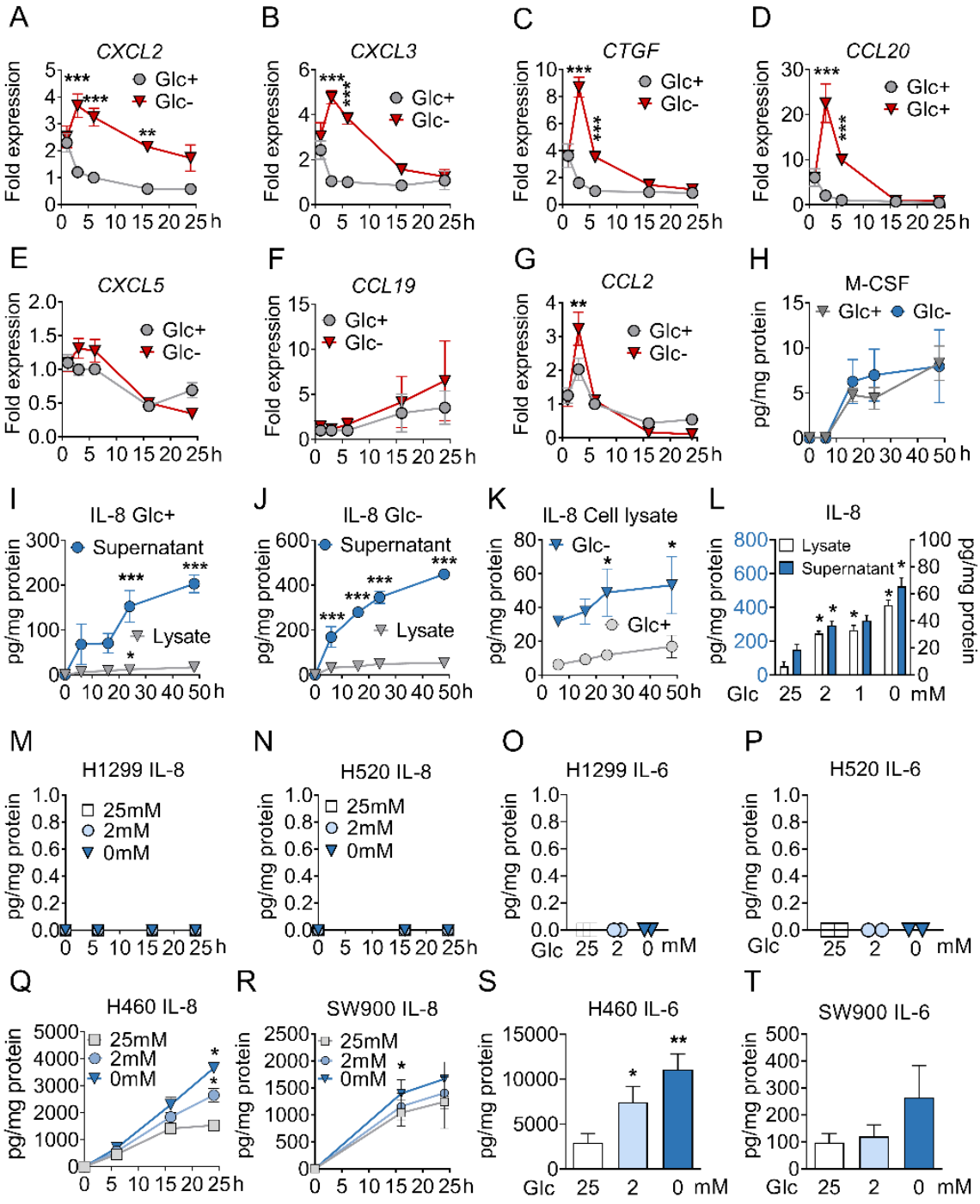


Fig. S2. H460 and SW900, but not H1299 and H520 cells, produce or secrete IL-6 or IL-8

A-G. A549 cells were treated for 1, 3, 6, 16 and 24 h with media containing 0 mM (Glc-) or 25 mM (Glc+) glucose. qPCR of CXCL2 (A), CXCL3 (B), CTGF (C), CCL20 (D), CXCL5 (E), CCL19 (F) and CCL2 (G) is shown. Fold expression was calculated by normalizing to cells treated for 6 h with

Glc+. Data are represented as mean ± SEM (n=3-9). Asterisks denote significance between the Glc+ control sample versus Glc- for each time point analysed by two-way ANOVA.

H. A549 cells were treated as in A for indicated time points. ELISA of M-CSF is shown. Data are represented as mean ± SEM (n=3-6).

I-K. A549 cells were treated for 6, 16, 24 and 48h with 25 mM (Glc+) or 0 mM (Glc-). ELISA of IL-8 of supernatants and cell lysates are shown. Data represent mean ± SEM (n=3). Asterisks denote significant differences between the 25 mM control sample of each series.

L. A549 cells were treated for 24 h with indicated glucose concentrations. ELISA for IL-8 of supernatant and lysate is shown. Data represent mean ± SEM (n=3). Asterisks denote significant differences between the 25 mM control sample of each series.

M-P. H1299 and H520 cells were treated for 6, 16 or 24 h with 25, 2 or 0 mM glucose. Supernatants were analysed for IL-8 and IL-6 release by ELISA. Data represent the mean of 2 experiments.

O-T. H460 and SW900 cells were treated between 6 and 24 h (O-R) or 24 h (S-T) with 25, 2 or 0 mM glucose. ELISA is shown for IL-8 or IL-6 as indicated. Data represent mean ± SEM of H460 (n=3) or SW900 (n=4). Asterisks denote significant differences between the 25 mM control sample versus each treatment for each time point. (Q-R) were analysed by two-way ANOVA for differences versus Glc 25 mM.

Fig. S3.

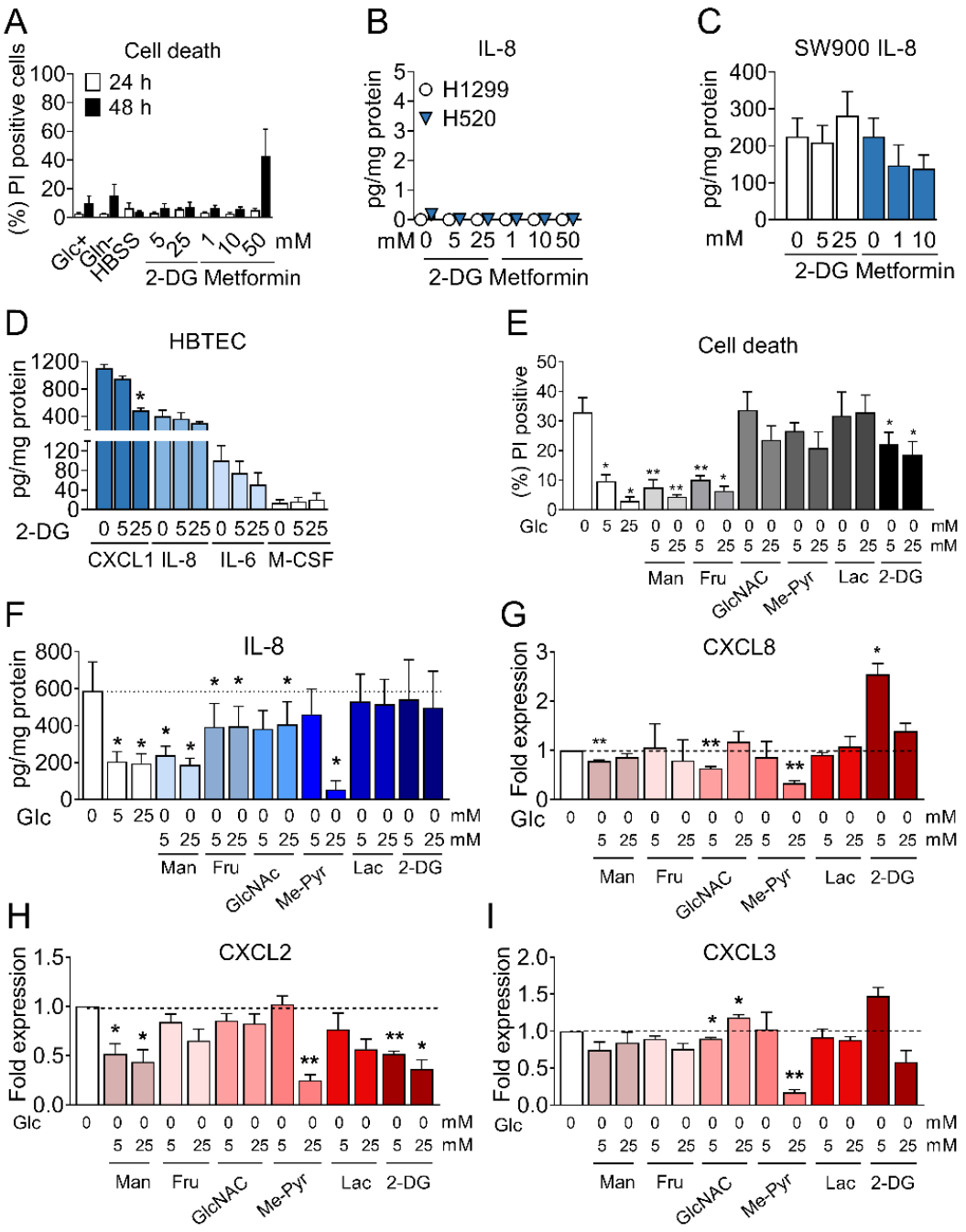


Fig. S3. Effects of 2-DG, metformin and metabolites on cell death and cytokine secretion

A. A549 cells were treated with media containing 25 mM glucose, 0 mM glutamine (Gln-), Hank's Balanced Salt Solution (HBSS), 2-Deoxyglucose (2-DG) or Metformin at indicated time points. Cell death was analysed by PI incorporation by FACS. Data represented mean ±SEM (n=3).

B-C. H1299, H520 (B) and SW900 (C) cells were treated with indicated metformin and 2-DG concentrations. ELISA for IL-8 is shown. Data represent mean ±SEM (n=3) for SW900 cells and (n=1) for H1299 and H520 cells.

D. HBTEC cells were treated for 24 h with indicated concentrations of 2-DG and supernatants were analysed for CXCL1, IL-8, IL-6 and M-CSF by ELISA. Data are represented as mean ±SEM (n=3-4). Asterisks denote significant differences vs. the 0 mM control sample of each cytokine.

E. A549 cells were treated for 48 h with media containing indicated glucose (Glc) concentrations and resubstituted concentrations of either mannose (Man), fructose (Fru), N-Acetyl-glucosamine (GlcNAC), methyl-pyruvate (Me-Pyr), lactate (Lac) or 2-DG. Cell death was analysed by PI incorporation and FACS after 48 h. Data represented mean ± SEM (n=4). Asterisks denote significant differences versus the 0 mM control sample.

F. A549 cells were treated for 24 h as in E and ELISA for IL-8 is shown. Data represent mean ±SEM (n=4). Asterisks denote significant differences versus the 0 mM glucose control sample.

G-I. A549 cells were treated as in E. qPCR for *CXCL8* (G), *CXCL2* (H) and *CXCL3* (I) is shown. Values were normalized to cells treated without glucose. Data are represented as mean ±SEM (n=3). Asterisks denote significant differences vs. the 0 mM glucose control sample.

Fig. S4.

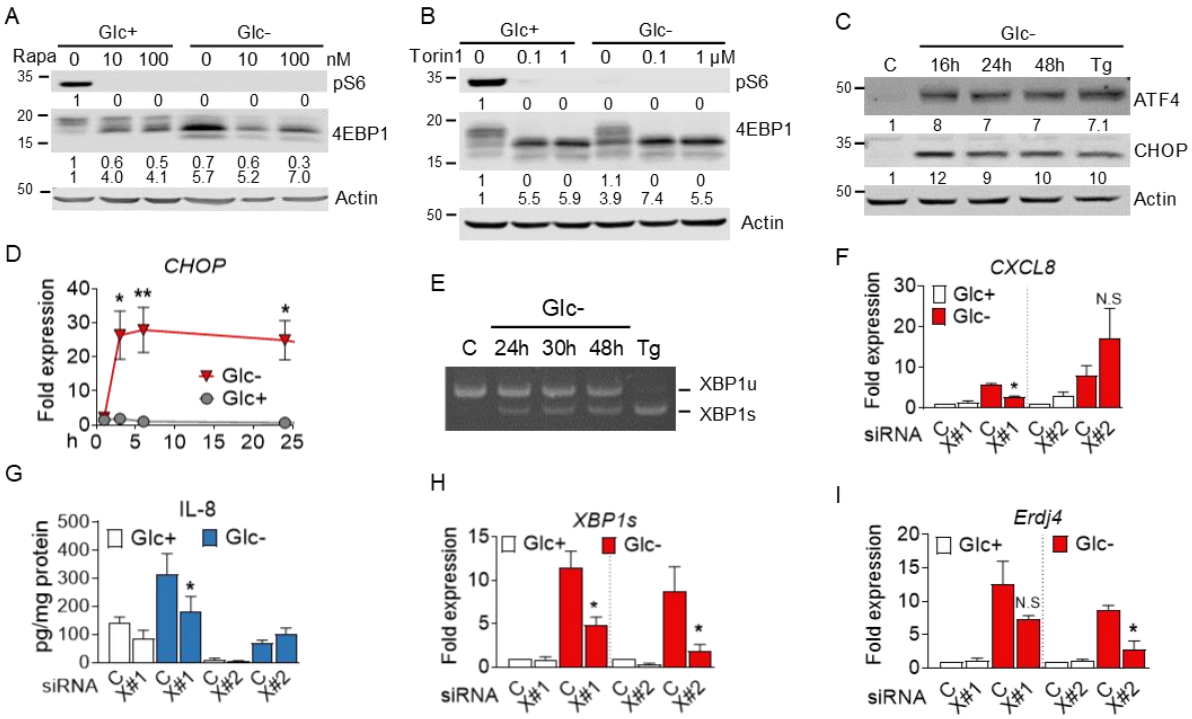


Fig. S4. Starvation responses to glucose deprivation: mTOR and the UPR

A-B. Cells were treated for 24 h with rapamycin (Rapa) (A) or Torin1 (B) in media containing 25 mM (Glc+) or 0 mM (Glc-) glucose. Representative western blot is shown. Numbers underneath each blot indicate band intensity normalized to actin and to Glc(+). In the case of 4E-BP1, they represent upper and center band quantification.

C. Cells were treated for 16 h with 25 mM glucose (C), for indicated time points with 0 mM (Glc-) or for 24 h with 4 µM thapsigargin (Tg). A representative western blot (n=3) for ATF4 and CHOP is shown. A-C Protein bands were quantified and normalized to actin. Fold induction was calculated by normalizing to the control.

D. Cells were treated for indicated times with 25 mM (Glc+) or 0 mM (Glc-) glucose and *CHOP* mRNA was analysed by qPCR. Values were normalized to 6 h Glc+. Data are represented as mean ± SEM (n=3). Asterisks denote significant differences vs. the Glc+ sample of each time point.

E. Cells were treated as described in C. for indicated times and lysed for mRNA extraction. Retro-transcription was performed, followed by RT-PCR for XBP1. A representative PCR is shown out of 3.

F,H-I Cells were transfected for 40 h with non-targeting control siRNA (labeled as ‘‘C’’) or siRNA for XBP1 (labeled as X#1 for sequence 1 and X#2 for sequence 2). qPCR for *CXCL8* (F), *XBP1s* (H) and *Erdj4* (I) is shown. Values were normalized to the control siRNA in Glc+. Data are represented as mean ±SEM (n=3) for X#1 and (n=4) for X#2. Asterisks denote significant differences of XBP1-transfected cells versus the control siRNA in each culture medium.

G. Cells were treated and transfected as in (F) and after 24 h supernatant was analysed by ELISA for IL-8. Data are represented as mean ±SEM (n=3-4). Asterisks denote significant differences of XBP1-transfected cells versus the control siRNA in each culture medium.

Fig. S5.

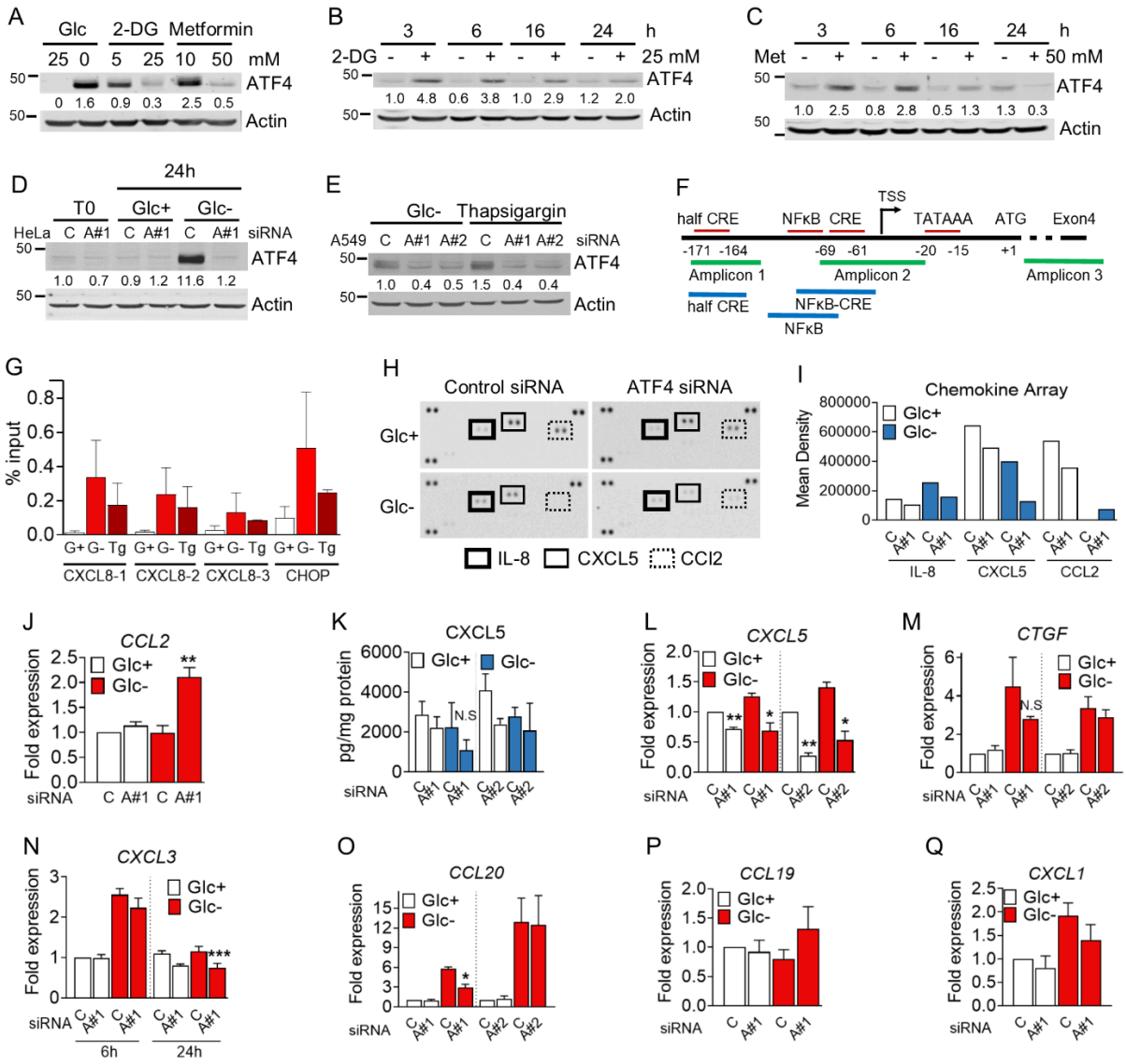


Fig. S5. ATF4 mediates cytokine induction

A. A549 cells were treated for 24 h with indicated concentrations of glucose (Glc), 2-Deoxyglucose (2-DG) or metformin. A representative western blot for ATF4 is shown.

B-C. A549 cells were treated for indicated times with 0 or 25 mM 2-DG (B) or 0 or 50 mM metformin ("Met") (C). Representative western blots are shown.

D. HeLa cells were transfected for 24 h with non-targeting siRNA (labelled as ''C" for Control siRNA) or siRNA against ATF4 (labelled "A#1" for ATF4 sequence 1). After 24 h, cells were washed and incubated in 25 (Glc+) or 2 mM glucose (Glc-). A representative western blot of ATF4 is shown.

E. Representative western blot for ATF4 of A549 cells transfected for 40 h with non-targeting siRNA (labelled as "C" for Control siRNA) or siRNA against ATF4 (labeled ''A#1'' for sequence 1 or ''A#2'' for sequence 2) which were treated for 24 h with media containing 0 mM glucose ("Glc-") or 25 mM glucose in combination with 4 µM thapsigargin. A-E Protein bands were quantified and normalized to actin. Fold induction was calculated by normalizing to each control.

F. Scheme for ChIP primers (green) and qDPI-ELISA oligonucleotide (blue) localization in the *CXCL8* promoter. A half CRE and full CRE binding sites were previously described (supplementary references 2,3).

G. A549 cells were treated for 3 h with media containing 25 mM (Glc+) or 0 mM (Glc-) glucose, or 4 μM thapsigargin (Tg). Nuclei were isolated, lysed and sonicated for IP for ATF4. DNA was extracted from the eluted samples and qPCR for CXCL8 promoter was performed. Fold expression was calculated by the % of input loaded. Data are represented as mean ± SEM (n=3).

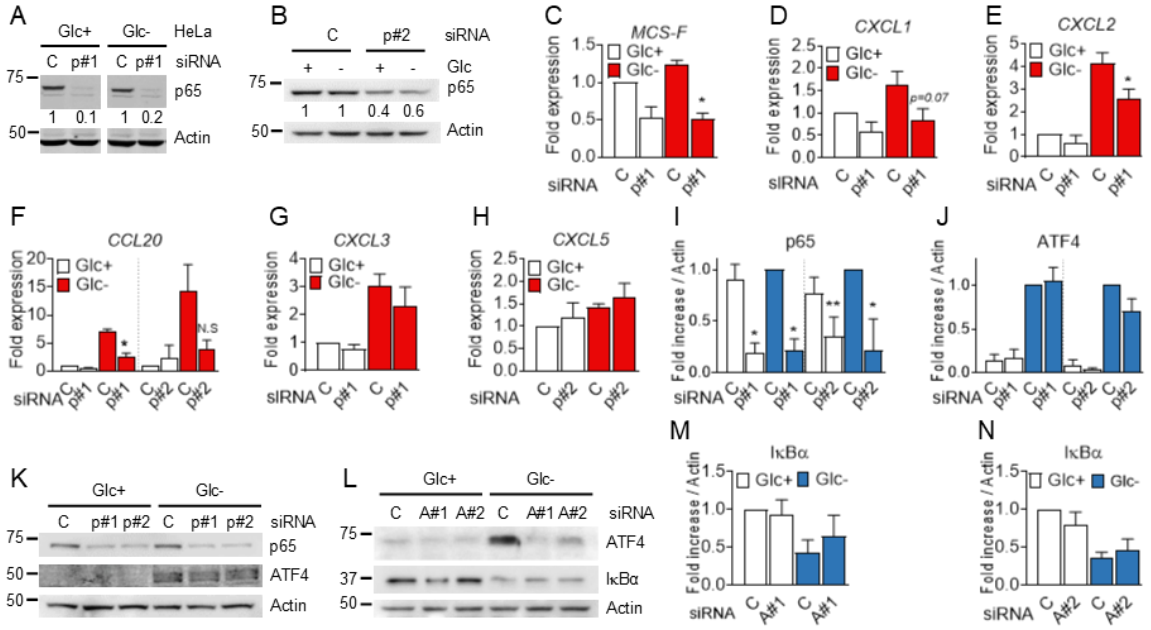
H. A549 cells were transfected as described in E with siATF4#1. and supernatants of three independent experiments were collected and combined. Human Chemokine Antibody Array was performed. Spots on membranes are presented.

I. Mean densities of spots on membrane shown in H for IL-8, CXCL5 and CCL2 were analysed by ImageJ.

J-L. A549 cells were transfected for 40 h with non-targeting siRNA (labelled as “C" for Control siRNA) or siRNA against ATF4 (labelled ’’A#1’’ for sequence 1 or ‘’A#2’’ for sequence 2) as described in E and treated with 25 mM (Glc+) or 0 mM (Glc-) glucose for 6 h (J,L) or 24 h (K). qPCR data for CCL2 (J) and CXCL5 (L) were normalized to the control siRNA of each treatment. ELISA is shown for CXCL5 (K). Data are represented as mean ±SEM (n=3-5). Asterisks denote significant differences of ATF4-transfected cells versus the control siRNA in each culture medium.

M-Q. A549 cells were transfected for 40 h as described in J and treated for 6 h with media containing 0 mM (Glc-) glucose or 25 mM (Glc+). qPCR is shown for CTGF (M), CXCL3 (N), CCL20 (O), CCL19 (P) and CXCL1 (Q). Values were normalized to control siRNA of each treatment. Data are represented as mean ± SEM (n=3-5). Asterisks denote significant differences of ATF4-transfected cells versus the control siRNA for each treatment.

Fig. S6.



The NF-κB p65 subunit regulates cytokine induction

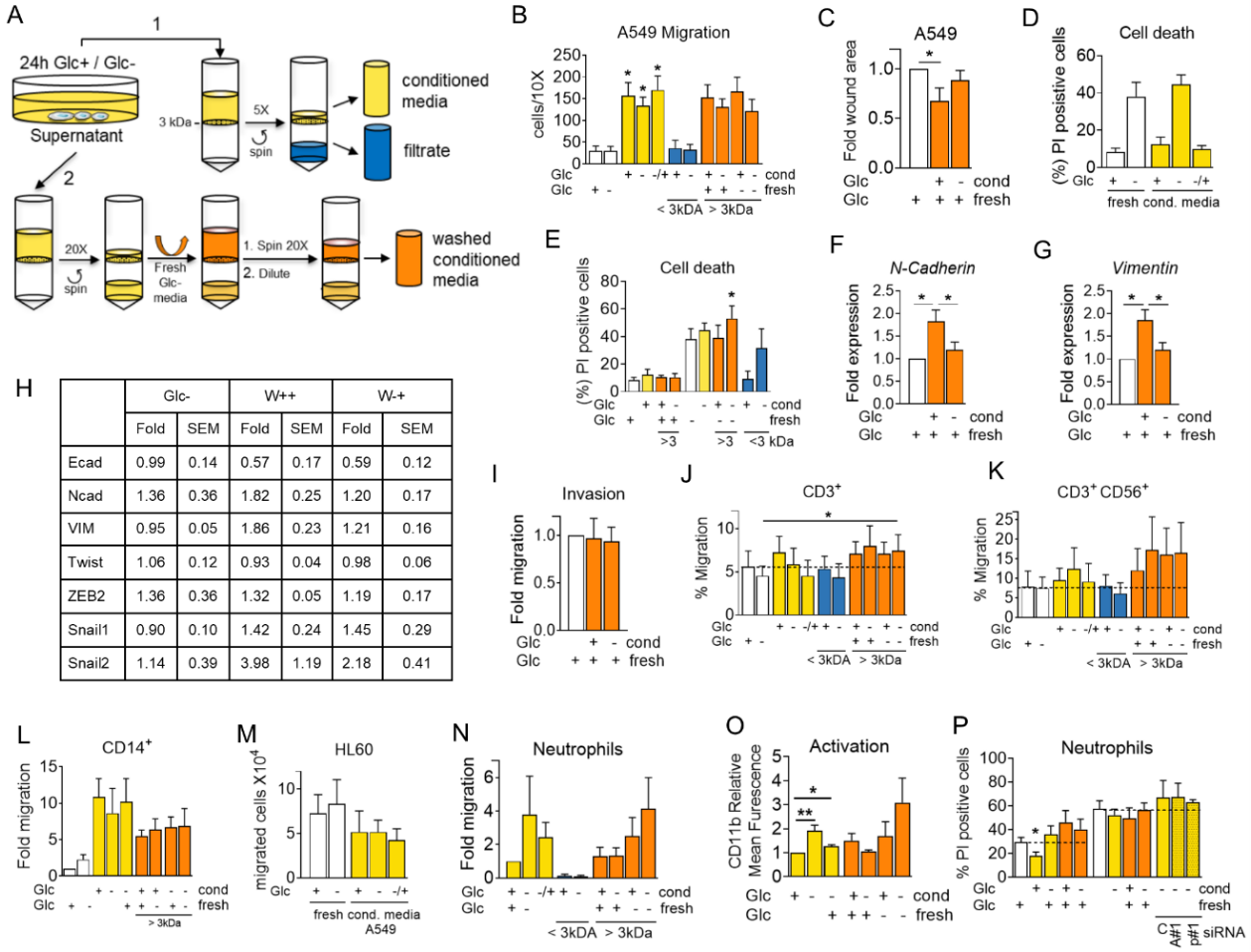
A-B. HeLa cells were transfected for 24h with control non-targeting siRNA (labelled as ''C'') or siRNA for p65 (labelled as ''p#1'' for sequence 1 and ''p#2'' for sequence 2). Cells were treated for 24 h with 25mM (Glc+) or 0 mM (Glc-) glucose. A representative western blot for p65 is shown. Bands were quantified and normalized to actin. Fold induction was calculated by normalizing toward the control sample in Glc+.

C-H. A549 cells were transfected for 40 h with control non-targeting siRNA (labelled as ''C'') and siRNA for p65 (labelled as ''p#1'' for sequence 1 and ''p#2'' for sequence 2). Cells were treated for 6 h with media containing 25 mM (Glc+) or 0 mM (Glc-) glucose. qPCR is shown for MCSF (C), CXCL1 (D), CXCL2 (E), CCL20 (F), CXCL3 (G) and CXCL5 (H). Values were normalized versus control siRNA in Glc+. Data are represented as mean ± SEM (n=3-4). Asterisks denote significant differences of p65-transfected cells versus the control siRNA in each condition.

I-K. A549 cells were transfected and treated for 24 h as described in C. A representative western blot of p65 and ATF4 is shown (K). p65 (I) and ATF4 (J) (n=3-4) were quantified and normalized to actin. Fold induction was calculated vs. Glc- siRNA control sample. Asterisks denote significant differences of p65-transfected cells versus the control siRNA in each condition.

L-N A549 cells were transfected for 40 h with control non-targeting siRNA (labelled as ''C'') or siRNA for ATF4 (labelled as ''A#1'' for sequence 1 and ''A#2'' for sequence 2). Cells were treated for 24 h with 25 mM (Glc+) or 0 mM (Glc-) glucose. A representative western blot of ATF4 and IκBα is shown. Bands were quantified and normalized to actin. Fold induction of IκBα (M-N) was calculated by normalizing toward the control siRNA in Glc+. n=4 for ATF4#1 and n=3 for ATF4#2.

Fig. S7.



H

	Glc-		W++		W-+	
	Fold	SEM	Fold	SEM	Fold	SEM
Ecad	0.99	0.14	0.57	0.17	0.59	0.12
Ncad	1.36	0.36	1.82	0.25	1.20	0.17
VIM	0.95	0.05	1.86	0.23	1.21	0.16
Twist	1.06	0.12	0.93	0.04	0.98	0.06
ZEB2	1.36	0.36	1.32	0.05	1.19	0.17
Snail1	0.90	0.10	1.42	0.24	1.45	0.29
Snail2	1.14	0.39	3.98	1.19	2.18	0.41

Figure S7. Conditioned medium exerts minor effects on cell death and cell migration of A549 cells

A. Scheme of medium preparation. Cells were treated for 24 h with 25 mM (Glc+) or 0 mM (Glc-) glucose. Conditioned media (yellow) and elution (blue) was stored. Filtered media tubes were refilled with fresh Glc- medium (orange) and washed once by centrifugation. For experiments, 25 mM glucose was supplemented when indicated.

B. A549 cells were plated on top of a Boyden insert. Cells were allowed to migrate for 20 h toward fresh (white bars) glucose-containing (25 mM) or glucose-free medium, to conditioned media (yellow bars) or to washed conditioned media (orange) from A549 cells grown in glucose-containing or glucose-free medium for 24h. -/+ indicates conditioned, glucose-free medium to which 25 mM glucose was added before the experiment. Data are represented as mean ± SEM (n=3). Asterisks denote significant differences of cells migrated toward fresh Glc+ media (white bar).

C. A549 cells were treated for 24 h with fresh glucose containing (25 mM) media or with conditioned media (orange) as described in B. Scratch Assay was performed and wound area was calculated using ImageJ. Data are represented as mean ± SEM (n=6).

D-E. A549 cells were treated for 48 h with fresh media (white bars) or conditioned media (yellow, orange bars) prepared as described in B. Cell death was analyzed with propidium iodide (PI)

staining by FACS. Data are represented as mean ±SEM (n=5). Asterisks denote significant differences between cells treated with fresh Glc- media versus the conditioned media (E).

F-G. Analysis of N-Cadherin (F) and Vimentin (G) mRNA expression in A549 cells after incubation for 24 h with fresh media containing 25 mM glucose (no FBS, white bars) or with washed conditioned media (orange bars) derived from other A549 cells which had been treated for 24 h either in the presence (+) or absence (-) of glucose. Fresh (25 mM) glucose was added to washed conditioned media when indicated. Data are represented as mean ± SEM (n=4). Asterisks denote significant differences between the Glc+ control sample or as indicated.

H. A549 cells were treated for 6 h with media containing 25 mM (Glc+) or 0 mM (Glc-) or for 24 h with washed conditioned media as described in F. W-+ means conditioned medium from starved cells washed with fresh glucose containing medium. W++ means conditioned medium from cells incubated with 25 mM Glc containing medium and washed with fresh glucose containing medium. qPCR was performed and fold induction versus Glc+ is shown. Data are represented as mean ± SEM Glc- (n=4) and (W++, W-+) (n=6-7).

I. A549 cells were allowed to migrate for 48 h toward fresh or washed conditioned media prepared as described in B using the QCM ECMatrix Cell Invasion Assay. Data are represented as mean ± SEM (n=4).

J-L. Human PBMC were allowed to migrate for 20 h toward media as described in B. Migrated cells were stained for indicated markers and analysed by FACS. Data are represented as mean ± SEM $CD3^+$ (n=4-5), $CD3^+CD56^+$ (n=4-6) and CD14+ (n=4-5). Asterisks denote significant differences between cells migrated toward fresh Glc- media (J).

M. HL60 cells were differentiated and cells migrated 2 h toward media described in B were counted. Data are represented as mean ± SEM (n=3).

N. Neutrophils were extracted from human blood and allowed to migrate for 2 h toward conditioned media described in B. Data are represented as mean ± SEM (n=9)

O-P. Neutrophils were purified from human blood and incubated for 16 h with fresh and conditioned media of A549 cells prepared as described in B in the presence of 10 % FBS. Cells were stained for CD11b and analysed by FACS for activation (O) or stained with PI and analysed for cell death by FACS (P). Data are represented as mean ± SEM. n=4-8 for O, n=4-10 for P.

Fig. S8.

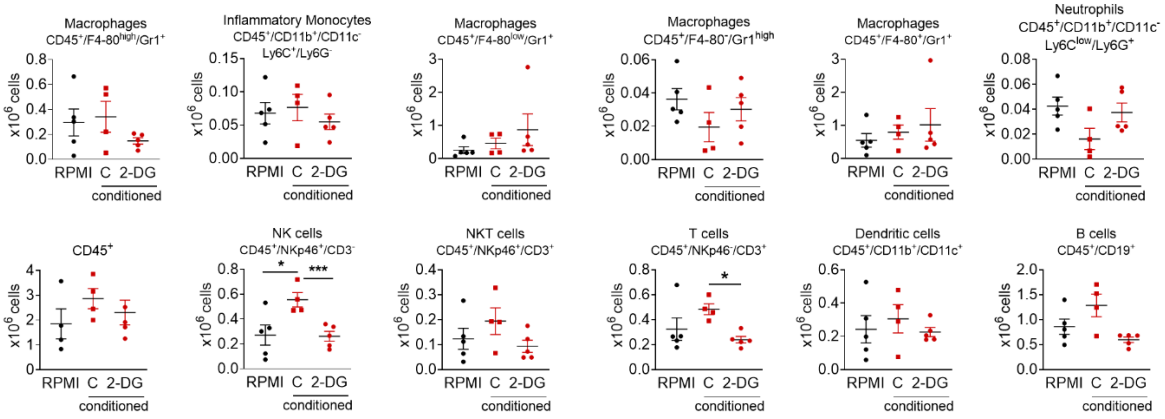


Figure S8. Conditioned medium from CT26 cells alters immune cell composition in the peritoneal cavity

Conditioned media from CT26 cells that had been incubated for 48 h with (2-DG) or without (C) 25 mM 2-DG was cleared by centrifugation and further concentrated 20 times. 350 µl of this medium and control medium (RPMI) were injected intra-peritoneally each 24 h for 3 days. 72 h after the first injection peritoneal cells were isolated and subjected to FACS analysis using the indicated markers. Asterisks denote significance using Student T test ($p<0.05$).

Dataset S1 (separate file). RAYBIO Human 507 array.

HeLa and Rh4 cells were subjected to 25 or 0 mM glucose or to treatment with 2-deoxyglucose in the presence of 0.5 % dialyzed FBS, and array L-507 was performed. Raw data: Raw values (duplicates) and averages are presented before background subtraction; Average: only averages are presented

Dataset S2 (separate file). RAYBIO Human 493

HeLa and Rh4 cells were subjected to 25 or 0 mM glucose or to treatment with 2-deoxyglucose in the presence of 0.5 % dialyzed FBS, and array L-493 was performed. Raw data: Raw values (duplicates) and averages are presented before background subtraction; Average: only averages are presented

Dataset S3 (separate file). GSEA GO-Pathway analysis

Dataset S4 (separate file). XL Cytokine Proteome Profiler array results

SI References

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3. Valentino Bezzerri, Monica Borgatti, Alessia Finotti, Anna Tamanini, Roberto Gambari, Giulio Cabrini. The Journal of Immunology December 1, 2011, 187 (11) 6069-6081