Supplemental Information

Supplemental Material and Methods

Cell isolation

Mononuclear cells were isolated using Ficoll-Paque (GE Healthcare, Piscataway Township, NJ). Monocytes and T-cells were isolated using the Pan Monocyte Isolation Kit (Miltenyi Biotec) and Pan T-cell Isolation Kit (Miltenyi Biotec), respectively, according to the manufacturer’s instructions. Cells of at least 90% purity were used for experiments.

In some cases, adherence isolation for monocytes was performed. PBMCs were seeded at 6 x 10^6 per well in a 12-well plate (Greiner bio-one) in IMDM (ThermoFischer Scientific) supplemented with 10% FCS (PAN BioTech) and 40 U/ mL Penicillin-Streptomycin (ThermoFisher Scientific) and left to adhere for 1 h at 37°C. Afterwards, non-adherent cells were removed.

Cell culture

Cells were cultured at a density of 1 x 10^6/ ml in RPMI1640 (ThermoFisher Scientific) supplemented with 2 mM L-Glutamine (Sigma-Aldrich), 10% FCS (PAN BioTech), and 40 U/ mL Penicillin-Streptomycin (ThermoFisher Scientific) at 37°C and 5% CO₂ atmosphere if not stated otherwise. Mycoplasma contamination was regularly assessed using the Venor® GeM Classic Mycoplasma Detection kit (Minerva biolabs, Berlin, Germany) according to the manufacturer’s instructions. Regular cell authentication of cell lines was not performed.

Flow cytometry (FACS)

All surface and intracellular staining included a dead cell exclusion using Zombie AquaDye (Biolegend). Cells were washed in PBS and blocked with FcR Blocking Reagent, human
(Miltenyi Biotec) or human immunoglobulins (Grifols, Gamunex®) prior to the staining. Intracellular staining was performed with a Fixation/Permeabilization Solution Kit (BD Biosciences). For intracellular detection of phosphorylated proteins Phosflow™ Fix Buffer I and Phosflow™ Perm Buffer III (BD Biosciences) were used.

Glucose uptake was analyzed using the fluorescent glucose analog 6-NBDG (ThermoFisher Scientific). Fatty acid uptake was semi-quantified by use of the fluorescently labeled long chain fatty acid, BODIPY™ FL C16 (ThermoFisher Scientific). Mitochondrial superoxide production was assessed by MitoSOX™ Red (Thermo Fisher Scientific) staining according to the manufacturer’s recommendations. To analyze the mitochondrial biomass and fitness MitoTracker® Green (ThermoFisher Scientific) and Tetramethylrhodamine ethyl ester (TMRE, Cayman Chemicals) were used respectively.

**ADP/ATP Assay**

ADP/ATP ratio of in vitro stimulated monocytes was assessed applying the ADP/ATP Ratio Assay Kit (Sigma-Aldrich) with $10^4$ cells/sample as per manufacturer’s instructions.

**Glucose and lactic acid**

Glucose and lactic acid in cell culture supernatants were measured with a HITADO SuperGL compact system according to the manufacturer’s instructions.

**T-cell proliferation assay**

*In vitro* stimulated monocytes were subsequently co-cultured with autologous VPD-labeled T-cells in different ratios. T-cells were stimulated for five days using anti-CD2, -CD3 and -CD28 bead-coupled antibodies (Miltenyi Biotec), and proliferation was assessed by FACS.
Cell sorting
Purified monocytes were stained with CD14-APC, CD16-BV421, and CD137-PE/Cy7. Cell sorting was carried out on a FACS Aria II (BD Biosciences).

IL-10 and IL-12 ELISA and secretion assay
Detection of IL-10 and IL-12 in supernatants of monocytes treated with a control antibody, MCSF (50 ng/ml), GM-CSF (50 ng/ml), or agonistic anti-CD137 mAbs for five days, followed by activation with LPS (100 ng/ml) for 24 hours was performed by Human IL-10 ELISA MAX™ Deluxe (Biolegend) and Human IL-12 ELISA MAX™ Deluxe (Biolegend).
For detection of IL10- and IL12-producing cells after four days of CD137 stimulation, monocytes were harvested and reseeded at 0.4 x 10^6/well in a 24 well plate. Next, cells were either activated with 1 µg/µl LPS and 2 µg/µl Resiquimod for 20 h or left untreated. Subsequently, two-color cytokine secretion assay was performed using IL-10 detection Kit (Miltenyi Biotec) and IL-12 detection Kit (Miltenyi Biotec).

DNA/RNA isolation, quantitative real-time PCR and RNA-sequencing
DNA and RNA were isolated from monocytes using the innuPREP DNA/RNA Mini Kit (analytic Jena). DNA/RNA content was quantified by means of a NanoDrop™ (Thermo Fisher Scientific). For qPCR, RNA was reverse transcribed into cDNA using the SuperScript™ II Reverse Transcriptase (Thermo Fisher Scientific). Gene expression was quantified using the Rotor-Gene SYBR® Green PCR Kit (Qiagen, 204076) and dedicated primers on a Rotor-Gene Q (Qiagen) employing the 2-standard method.
Samples designated for RNA-sequencing (RNAseq) were further processed for removal of residual genomic DNA using the RNase free DNase set (Qiagen).

For RNAseq of ex vivo sorted monocytes “INVIEW Transcriptome” (Eurofins/GATC Biotech) product was purchased and RNAseq was performed by Eurofins/GATC Biotech. A strand-specific cDNA library was prepared and sequencing was performed on a HiSeq4000 with >30 x 10⁶ 50bp single end reads.

RNA-sequencing of in vitro stimulated monocytes was performed by the next generation sequencing core unit of the FAU Erlangen-Nürnberg. The cDNA library was prepared using TruSeq Stranded mRNA Kit (Illumina). Libraries were sequenced on a HiSeq2500 sequencer (Illumina) with 95bp single end reads.

All samples were processed as follows: First, reads were mapped against the human genome (GENCODE GRCh38.p13) using STAR v2.7.3a. Second, all non-multimapping and non-multi-overlapping reads mapping to exons were counted using featureCounts v2.0.0. Differential gene expression analysis was performed in R v3.6.1 using DESeq2 v1.26.0. The “Approximate Posterior Estimation for GLM” model was used to assess differential expression for each gene (apeglm v1.8).

Gene set enrichment analysis v4.0.3 (GSEA, http://broadinstitute.org/gsea) was performed to determine enriched gene sets in ex vivo CD137HI/LO and in vitro CD137-triggered monocytes. Preranked GSEA was performed on gene set collections from MSigDB v7.1 (https://www.gsea-msigdb.org/gsea/msigdb/index.jsp) using Log₂ fold-change, as determined by DESeq2, as ranking metric. Collections used were: HALLMARK, KEGG, and GO as well as selected gene sets for monocyte/macrophage differentiation.
Supplemental Tables

### Supplemental Table 1: Cell lines

| Cell lines | Provider | Order No. |
|------------|----------|-----------|
| **Myeloma** |          |           |
| OPM-2      | DSMZ     | ACC50     |
| RPMI-8226  | DSMZ     | ACC402    |
| U-226      | DSMZ     | ACC9      |
| MM.1S      | ATCC     | CRL-2974  |
| **CLL**    |          |           |
| EHEB       | DSMZ     | ACC67     |
| Mec-1      | DSMZ     | ACC497    |
| HG-3       | DSMZ     | ACC765    |
| **Burkitt**|          |           |
| Raji       | Kind gift from the Malmberg group at the Oslo university hospital | |
| BL-41      | Kind gift from the Malmberg group at the Oslo university hospital | |
| BJAB       | Kind gift from the Malmberg group at the Oslo university hospital | |
| Raji-Null  | Invivogen | raji-null |
| Raji-hPD-L1| Invivogen | raji-hpd1 |

### Supplemental Table 2. Antibodies for flow cytometry

| Antibodies                  | Fluorochrome | Clone       | Manufacturer | Order No.   |
|-----------------------------|--------------|-------------|--------------|-------------|
| CPT1a                       | AF488        | 8F6AE9      | Abcam        | ab171449    |
| HK2                         | -            | EPR20839    | Abcam        | ab209847    |
| Goat Anti-Rabbit IgG        | AF647        | polyclonal  | Abcam        | ab150079    |
| HLA-DR                      | FITC         | G46-6       | BD           | 555811      |
| CD11b                       | APC          | ICRF44      | Biolegend    | 301310      |
| CD11b                       | PE           | ICRF44      | Biolegend    | 301305      |
| Antigen   | Color  | Antibody   | Catalog Number |
|----------|-------|------------|----------------|
| CD11b    | FITC  | ICRF44     | 301330         |
| CD14     | PerCP | HCD 14     | 325622         |
| CD14     | APC   | HCD 14     | 325608         |
| CD16     | BV421 | 3G8        | 302038         |
| CD16     | FITC  | 3G8        | 302006         |
| CD32     | PE    | FUN-2      | 303206         |
| CD36     | FITC  | 5-271      | 336204         |
| CD36     | PE    | 5-271      | 336206         |
| CD38     | APC   | HIT2       | 303509         |
| CD64     | APC   | 10.1       | 305014         |
| CD86     | PE    | IT2.2      | 305438         |
| CD86     | PE/Cy7| IT2.2      | 305422         |
| CD137    | PE/Cy7| 4B4-1      | 309818         |
| CD137    | APC/Cy7| 4B4-1     | 309830         |
| CD163    | BV421 | GHI/61     | 333612         |
| CD274    | PE    | 29E.2A3    | 329706         |
| CD274    | APC   | 29E.2A3    | 329708         |
| CD279    | PE    | EH12.2H7   | 329906         |
| HLA-DR   | APC/Cy7| L243     | 307618         |
| IL6      | PE    | MQ2-13A5   | 501107         |
| pERK     | PE    | 6B8B69     | 369505         |
| TNFa     | AF647 | MAb11      | 502916         |
| Mfn-2    | -     | D2D10      | 9482s          |
| GLUT1    | APC   | 202915     | FAB1418G       |
| GLUT2    | PE    | 199017     | FAB1414P       |
| GLUT3    | FITC  | 202017     | FAB1415G       |
Supplemental Table 3: Primers for mtDNA content

| Gene  | Forward          | Reverse                        |
|-------|------------------|--------------------------------|
| MT-TL1| CACCCAAGAACAGGGTTTG | TGGCCATGGGTATGTTGT TA          |
| MT-   | GCCTCCCCCGTAAATGATA | TTATCGGATTACCGGGCTCT          |
| RNR2  |                   |                                |
| B2M   | TGCTGTCTCCATGTITT- | TCTCTGCTCCCACCTCTAG T         |
|       | GATGTATCT         |                                |
Supplemental Figures

**Supplemental Figure 1:** Venn diagram comparing the overlap of the differentially expressed genes (p<0.05) between CD137\(^{\text{High}}\), CD137\(^{\text{Low}}\) with classical and non-classical monocytes derived from Gene Expression Omnibus Series GSE107011.

**Supplemental Figure 2:** Expression of target antigens for therapeutic antibodies on tumor cell lines used for phagocytosis experiments, as determined by FACS (red=stained, gray= unstained). Upper panel depicts representative histograms of CD20 staining on Burkitt lymphoma cell lines BL-41, BJAB, and Raji as well as CLL cell lines Mec-1 and HG-3. Lower panels show CD38 expression on multiple myeloma cell lines OPM-2, MM.1S, RPMI8826, and U-266.
Supplemental Figure 3: Expression of CD11b, CD32, CD64, CD86, CD120b, glucose transporter 1 (GLUT1), hexokinase 2 (HK2), CD36, carnitine palmitoyltransferase I (CPT1) as well as mitochondrial mass (i.e. Mitotracker) and mitochondrial membrane potential (i.e. TMRE) were determined by FACS in CD137HI and CD137LO monocytes from patients with (A) chronic lymphocytic leukemia (CLL) or with (B) multiple myeloma. Abbreviations: *, p<0.05; **, p<0.01; ***, p<0.001.
Supplemental Figure 4: (A) Depicted are basal glycolysis after glucose administration of control vs. anti-CD137 mAb-treated monocytes (left panel) and glycolytic reserve capacity after oligomycin administration (right panel). (B) Glucose consumption (left panel) and lactate production (right panel) were determined in supernatants of monocytes cultured with either control or anti-CD137 mAb for four days. (C) Graph shows basal respiration of control vs. anti-CD137 mAb-treated monocytes. Abbreviations: **, p<0.01; ***, p<0.001.

Supplemental Figure 5: (A) Viability of CD20+ B-cell-derived Non-Hodgkin Lymphoma cell lines (HG-3 and Raji) and CD38+ multiple myeloma cell lines (MM1S and OPM2) in presence of Rituximab and Daratumumab respectively. (B) Killing of CPD-stained Raji cells in presence or absence of a therapeutic antibody (Ritux=Rituximab) by monocytes pretreated with either control or anti-CD137 mAb for 3 days. Survival of tumor cells co-cultured with control mAb-treated monocytes without therapeutic antibody are set to 100%. (C) Expression of PD-L1 on Raji cell lines as determined by FACS. Red histogram shows PD-L1 expression on Raji PD-
L1 cell line, orange histogram represents PD-L1 expression on Raji null cell line and grey histogram is unstained control. Abbreviations: **, p<0.01.

Supplemental Figure 6: (A) Principal component analysis of differentially expressed genes between isotype and agonistic anti-CD137 mAb-treated monocytes shows distinct clustering of samples according to treatment. Monocytes were cultured with either control antibody or the anti-CD137 mAb 4B4-1 for four days and subsequently RNA extraction and sequencing were performed (n=3). (B) Gene set enrichment analysis of differential gene expression between isotype and anti-CD137 mAb-treated monocytes. Graph shows significantly enriched pathways found in MSigDB hallmark (orange) and biological process ontology (green) gene sets. Size of gene set is annotated to the right. Bar length corresponds to normalized enrichment score (NES).
Supplemental Figure 7: Expression of CD11b, CD14, and CD163 was quantified by FACS on monocytes from patients with (A) CLL or with (B) multiple myeloma treated for four days with a control antibody (i.e. isotype) or an agonistic anti-CD137 mAb. Abbreviations: *, p<0.05.
Supplemental Figure 8: (A) Expression of CD11b, CD14, and CD163 was determined on monocytes treated with either control antibody, anti-CD137 mAb or a combination of anti-CD137 mAb and the indicated concentrations (in nM) of SCH772984 for four days. (D) Glucose consumption and lactate production were determined in the supernatants of monocytes treated with either control antibody, anti-CD137 mAb or a combination of anti-CD137 mAb and the indicated concentrations (in nM) of SCH772984 for four days. Bars indicate the mean. Abbreviations: *, p<0.05; **, p<0.01.