Supplementary Data

Mitochondrial metabolism drives hypercholesterolemia-induced breast cancer cell migration

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Extended data Fig. 1. Related to Fig. 1. LDL-exposed TNBC cells show differential invasion potential and metastatic tropism to distant sites in xenotransplanted zebrafish larvae at 4dpi (6dpf). **a**, Representative images of whole zebrafish tile in xenotransplanted larvae at 2dpf and analyzed at 4dpi (6dpf) with invaded organs, as detailed in each image, by DiI-labelled MDA-MB-231 control (red) and Cy5-labelled LDL-exposed (grey) cells. **b**, Representative images of whole DiI-labelled MDA-MB-231 control (red) and Cy5-labelled LDL-exposed (grey) xenotransplanted Tg (*fli1:eGFP*) zebrafish tile (4dpi and 6dpf) with endothelial fli1:eGFP expression (green). Images were captured with a spinning disk inverted confocal microscope Zeiss Cell Observer SD. Nuclei staining with DAPI is in blue. Scale bar, 100 μm. **c**, Quantification of tumor masses (>20 cells) depicted as the number of zebrafish larvae xenografts (*n*=11) with DiI-labelled MDA-MB-231 control (red) and Cy5-labelled LDL-exposed (grey) masses in the indicated organs. **d**-**f**, Total number (**d**), average area (**e**) and mitochondria network (**f**) determined as the Mito-YFP area by the cell tracer DiI or Cy5 area, for control and LDL cells respectively, in control and LDL-exposed MDA-MB-231 cells quantified in the indicated organs of xenotransplanted imunolabelled zebrafish larvae (PVS, *n*=13/26; Int. t., *n*=10/16; S. blad., *n*=7/8, Brain, *n*=5 cells). **g**, **h**, Total area of Mito-YFP (**g**) and cell tracer DiI and Cy5 imunolabelling, for control and LDL respectively, (**h**) in control and LDL-exposed MDA-MB-231 cells (*n*=34/36 and *n*=47/50 cells, respectively) in xenotransplanted zebrafish larvae. Each circle in the plot represents individual cell measurement, lines represent the mean ± sem for each condition. **i**, Chart representing the Mito-YFP-labelled mitochondrial network distribution of control and LDL-exposed

MDA-MB-231 cells in the indicated organs (PVS, *n*=13/26; Int. t., *n*=10/16; S. blad., *n*=7/8, Brain, *n*=5 cells) from xenotransplated zebrafish larvae.
Legend: A. (anal), CHT (caudal hematopoietic tissue), D. (dorsal), H. kidn. (head kidney), Int. t. (intestinal tract), Optic v. (optic vesicle), PVS (perivitelline space), S. blad. (swim bladder), V. (ventral). Statistical analysis was performed by the two-tailed Student's *t*-test (d-h) and Fisher's exact test (i). * $p<0.05$, ** $p<0.01$.

Extended data Fig. 2. Related to Fig. 2. LDL exposed migrating TNBC cells display differential mitochondrial network and increased cristae destabilization.

a, Mitochondrial DNA (mtDNA) content accessed by qPCR analysis of the human mitochondrial ND1 gene relative to the nuclear β2-microglobulin gene in DNA samples from untreated (control) or LDL-exposed MDA-MB-436 cells (*n*=5 each). **b**, Migratory capacity represented as percentage of wound closure at 20h (n=3) and representative images of wounds at 0h and 20h by optical microscopy (4x objective). **c**, Chart representing MitoTracker Deep Red live staining of mitochondrial network distribution of control and LDL-exposed migrating MDA-MB-231 cells (*n*=42 and *n*=39 cells, respectively) and representative images of mitochondrial network distribution acquired in an inverted fluorescent Zeiss Cell Observer Microscope (63x objective, scale bar 20 μm). **d**, Migratory capacity represented as percentage of wound closure at 24h (n=4) and representative images of wounds at 0h and 20h by optical microscopy (4x objective). **e**, Number of TOM-20 labelled mitochondria in control, LDL or LPA-exposed MDA-MB-231 migrating cells (*n*=45/78 cells per condition). **f**, Chart representing TOM-20-labelled mitochondrial network distribution of control, LDL and LPA-exposed

MDA-MB-231 migrating cells (*n*=51/87 cells per condition). **g**, Mitochondria average area from TEM imaged sections in control and LDL-exposed migrating MDA-MB-231 cells (*n*=99 cells). **h**, qPCR analysis of the relative expression of the indicated genes in untreated (control) or LDL-exposed MDA-MB-231 cells (n=4/5 each). **i**, Quantification of western blot densitometric units for PGC-1α protein expression corrected by β-ACTIN (left) and representative images (right) in untreated (control) or LDL-exposed MDA-MB-231 cells (uncropped images of blots are shown in Extended Data Fig. 7). **j**, Quantification of western blot densitometric units for Drp1, Mfn1 and Mfn2 protein expression corrected by β-ACTIN (related to Fig. 2j).

Data are presented as mean ± s.d. Each circle in the plot represents individual cell measurement. Statistical analysis was performed by the Fisher's exact test (f) and two-tailed Student's *t*-test. * $p<0.05$, ** $p<0.01$, *** $p<0.0001$.

Extended data Fig. 3. Related to Fig. 3. LDL-induced migration of TNBC cells is mediated by the fatty acid transporter CD36.

a, qPCR analysis of the relative expression of the indicated genes in untreated (control) or LDL-exposed MDA-MB-231 cells (n=4/5 each). **b**, Cell count ratio of control or LDL-exposed MDA-MB-231 cells in the absence (vehicle) or presence of sulfosuccinimidyl oleate (SSO, 50 μM) (*n*=4 each). **c**, qPCR analysis of the *CD36* relative expression in untreated shSCR and shCD36 MDA-MB-231 cells (n=5/6 each). **d**, Flow cytometry quantification of BODIPY 493/503 (Bodipy) staining depicted as relative median fluorescence intensity (MFI) of untreated (control) or LDL-exposed shSCR and shCD36 MDA-MB-231 cells (*n*=4). **e-g**, Migratory capacity represented as percentage of wound closure at 24h (**e**), flow

cytometry quantification of lipid droplets by BODIPY 493/503 (Bodipy) staining (**f**, left) and representative histograms (f, right) and cell count ratio (**g**) of untreated (control) or LDL-exposed MDA-MB-231 cells alone (vehicle) or in the presence of an anti-human LDLR antibody (*n*=4). **h**, Number of HSP-60 labelled mitochondria in control or LDL-exposed migrating MDA-MB-231 cells in the absence (vehicle) or presence of SSO (*n*=42/119 cells per condition). **i**, Relative mitochondrial DNA (mtDNA) content from control (untreated) or LDL-exposed shSCR and shCD36 MDA-MB-231 cells (n=4/7 each). **j**, Chart representing HSP-60-labelled mitochondrial network distribution of control or LDL-exposed MDA-MB-231 migrating cells in the absence (vehicle) or presence of SSO (*n*=105/150 cells per condition).

Data are presented as mean ± s.d. Each circle in the plot represents individual cell measurement. Statistical analysis was performed by One-way ANOVA (h), Fisher's exact test (j) and two-tailed Student's *t*-test. * $p<0.05$, ** $p<0.01$, *** $p<0.0001$.

Extended data Fig. 4. Related to Fig. 4. LDL-induced migratory behavior of TNBC cells relies in increased usage of fatty acids and FAO-dependent mitochondrial metabolism.

a, Flow cytometry representative histograms of MitoTracker Deep Red staining in control, PA (0.4 mM) or LDL-exposed MDA-MB-231 cells in the absence (vehicle) or presence of etomoxir (200 µM). **b**, **c**, Flow cytometry quantification of lipid droplets depicted by BODIPY 493/503 (Bodipy) staining depicted as relative median fluorescence intensity (MFI) (**b**) and cell count ratio (**c**) in control, PA (0.4 mM) or LDL-exposed MDA-MB-231 cells in the absence (vehicle) or

presence of etomoxir (200 μM, *n*=4 each). **d**, TOM-20 labelled mitochondria average area in control or LDL-exposed migrating MDA-MB-231 cells in the absence or presence of etomoxir (*n*=61/78 cells per condition). **e,f,g,h,** Flow cytometry quantification of lipid droplets depicted by BODIPY 493/503 (Bodipy) staining as relative median fluorescence intensity (MFI) (**e, f**) and cell count ratio (**g, h**) in the absence (vehicle) or presence of 2-DG (2 mM, *n*=4 each) (**e, g**) and oligomycin (2 μM, *n*=4 each) (**f, h**). **i**, Migratory capacity represented as percentage of wound closure at 24h of control or LDL-exposed MDA-MB-231 cells in the absence (vehicle) or presence of CK666 (50 or 75 μM, *n*=4 each) and representative images of wound closure at 0h and 24h by optical microscopy (4x objective).

Data are presented as mean ± s.d. Each circle in the plot represents individual cell measurement. Statistical analysis was performed by One-way ANOVA with multiple comparison correction (d) and two-tailed Student's *t*-test. * $p<0.05$, ** $p<0.01$, *** $p<0.0001$.

Extended data Fig. 5. Related to Fig. 5. Increased lipid exposure induces metabolic and bioenergetic dependencies in TNBC cells.

a, Gene set enrichment analysis (GSEA) of transcriptomes of MDA-MB-231 control cells compared to LDL-exposed for 48h depicting induced signaling pathways. **b**,**c** Oxygen consumption rate (OCR) (**b**) and Extracellular acidification rate (ECAR) (**c**) of control or LDL-exposed MDA-MB-231 cells cultured in the absence (vehicle) or presence of SSO for 48h (*n*=12/15 each from 3 independent experiments).

Data are presented as mean ± s.d. Statistical analysis was performed by One-way ANOVA with multiple comparison. For (a), statistic cut-off [*-log10 (0.05)*] was applied for Benjamini false discovery rate (FDR) correction.

Extended data Fig. 6. Related to Fig. 6. LDL-induced migratory behavior and mitochondrial adaptations rely in reactive oxygen species signaling.

a,b,c, Flow cytometry quantification of Cell ROX Deep Red staining depicted as relative median fluorescence intensity (MFI) (left) in control, lysophosphatidic acid (LPA, 10 μM) or LDL-exposed MDA-MB-231 cells (*n*=3 each) and representative histograms (right) **(a),** in the presence of SSO (75 μM, *n*=6 each) (**b**) and for shCD36 MDA-MB-231 cells (*n*=3/6 each) (**c**). **d**, qPCR analysis of the relative expression of the indicated genes in untreated (control) or LDL-exposed MDA-MB-231 cells (n=4/5 each). **e,** Migratory capacity represented as percentage of wound closure at 18h of shSCR and shCD36 MDA-MB-231 cells control or LDL-exposed MDA-MB-231 cells in the absence or presence of *N*-acetylcysteine (NAC, 5 mM, *n*=3 each). **f,** TOM-20 labelled mitochondria average area (*n*=36/78 cells per condition) in control or LDL-exposed migrating MDA-MB-231 cells in the absence or presence of *N*-acetylcysteine (NAC, 5 mM) or MitoTEMPO (MitoT, 100 μM). **g**, BODIPY FLC16 (green) and MitoTracker Deep Red (red) staining in LDL-exposed shSCR and shCD36 MDA-MB-231 cells in the absence or presence of etomoxir (200 μM) for 24h (63x objective, scale bar 20 μm).Nuclei are labelled with DAPI (blue).

Data are presented as mean ± s.d. Each circle in the plot represents individual cell measurement. Statistical analysis was performed by One-way ANOVA with

multiple comparison correction (f) and two-tailed Student's *t*-test. ** p<0.01, *** p<0.0001.

Extended data Fig. 7. Uncropped Western blot membranes relative to the Western blot for Drp1, Mfn1, Mfn2 and β-ACTIN proteins displayed in Figure 2 (j).

Extended data Table 1 – List of primers used for measurements of gene expression levels and mtDNA content by quantitative real-time PCR.

name	sequence
h*ND1*-F	5'-CCCTAAAACCCGCCACATCT-3'
h*ND1*-R	5'-GAGCGATGGTGAGAGCTAAGGT-3'
hβ2-microglobulin-F	5'-TCGCTCCGTGGCCTTAGCTGT-3'
hβ2-microglobulin-R	5'-CTTTGGAGTACGCTGGATAGCCTCC-3'
h*18S*-F	5'-GCCCTATCAACTTTCGATGGT-3'
h*18S*-R	5'-CCGGAATCGAACCCTGATT-3'
h*LDHA*-F	5'-ACCCAGTTTCCACCATGATT-3'
h*LDHA*-R	5'-CCCAAAATGCAAGGAACACT-3'
h*PFKFB3*-F	5'-ATTGCGGTTTTCGATGCCAC-3'
h*PFKFB3*-R	5'-GCCACAACTGTAGGGTCGT-3'
h*PKM2*-F	5'-CCACTTGCAATTATTTGAGGAA-3'
h*PKM2*-R	5'-GTGAGCAGACCTGCCAGACT-3'
h*FASN*-F	5'-CGACAGCACCAGCTTCGCCA-3'
h*FASN*-R	5'-CACGCTGGCCTGCAGCTTCT-3'
h*HMGCR*-F	5'-CCAAACCCCGTAACCCAAAG-3'
h*HMGCR*-R	5'-AGCGACTATGAGCGTGAACAA-3'
h*CPT1A*-F	5'-ATGCGCTACTCCCTGAAAGTG-3'
h*CPT1A*-R	5'-GTGGCACGACTCATCTTGC-3'
h*ATP5g1*-F	5'-GCTGTTGTACCAGGGGTCTAA-3'
h*ATP5g1*-R	5'-CTGGCGTGGGAAGTTGCTGT-3'
h*COX5b*-F	5'-GCTGCATCTGTGAAGAGGACAAC-3'
h*COX5b*-R	5'-CAGCTTGTAATGGGTTCCACAGT-3'
h*NDUFB5*-F	5'-CTTCCTCACTCGTGGCTTTC-3'
h*NDUFB5*-R	5'-TTTCCCATGGTCTCCACTGT-3'
h*ACADVL*-F	5'-ACGGGCGTACTGGGTGTT-3'
h*ACADVL*-R	5'-ATGGTGGAGGAGACCACTTG-3'
h*PGC-1α*-F	5'-CACCAGCCAACACTCAGCTA-3'
h*PGC-1α*-R	5'-GTGTGAGGAGGGTCATCGTT-3'
h*PGC-1β*-F	5'-GGCAGGCCTCAGATCTAAAA-3'
h*PGC-1β*-R	5'-TCATGGGAGCCTTCTTGTCT-3'
h*NRF1*-F	5'-CCATCTGGTGGCCTGAAG-3'
h*NRF1*-R	5'-GTAGTGCCTGGGTCCATGA-3'
h*ERRα*-F	5'-GGCGGCAGAAGTACAAGC-3'
h*ERRα*-R	5'-ATTCACTGGGGCTGCTGT-3'
h*TFAM*-F	5'-GAACAACTACCCATATTTAAAGCTCA-3'
h*TFAM*-R	5'-GAATCAGGAAGTTCCCTCCA-3'
h*PPARα*-F	5'-AGAGTGGGCTTTCCGTGTC-3'
h*PPARα*-R	5'-GCCGCCTTCAGGTACAGTAG-3'
h*CD36*-F	5'-GGTGTGGTGATGTTTGTTGC-3'
h*CD36*-R	5'-CAGGGCCTAGGATTTGTTGA-3'
h*LDL-R*-F	5'-GCTTGTCTGTCACCTGCAAA-3'
h*LDL-R*-R	5'-AACTGCCGAGAGATGCACTT-3'
h*SRBP1*-F	5'-CTGTGGGTGAGATCATGTGG-3'
h*SRBP1*-R	5'-GCCAGAAGTCAACCTTGCTC-3'
h*SOD2*-F	5'-GCTCCGGTTTTGGGGTATCTG-3'
h*SOD2*-R	5'-GCGTTGATGTGAGGTTCCAG-3'
h*Cat*-F	5'-TGTTGCTGGAGAATCGGGTTC-3'
h*Cat*-R	5'-TCCCAGTTACCATCTTCTGTGTA-3'
h*GPX*-F	5'-CAGTCGGTGTATGCCTTCTCG-3'
h*GPX*-R	5'-GAGGGACGCCACATTCTCG-3'

h*HIF-1α*-F	5’-CATAAAGTCTGCAACATGGAAGGT-3’
h*HIF-1α*-R	5’-ATTTGATGGGTGAGGAATGGGTT-3’