Supplemental Experimental Procedures

Tumor tissue digestion and single cell collection

For in-vivo molecular analysis, the fresh tumor tissue removed from mice was digested with collagenase and hyaluronidase for 1 h. After grinding with semi-frosted slides and lysis of RBC, the dissociated cells were spun down at 500 r.p.m. for 1 min and then incubated for 1 h to get rid of adhesive cells. The suspended cells were collected and lysis with Trizol (Invitrogen) or RIPA buffer (Beyotime, China).

Western blotting

Cells were collected, lysed in RIPA buffer (Beyotime, China) and sonicated. The protein concentrations were determined by the BCA method (Beyotime, China). Then, protein was run on an SDS-PAGE gel and transferred to nitrocellulose. Nitrocellulose membranes were blocked in 5% bovine serum albumin (BSA) and probed with the following antibodies overnight: anti-β-actin,γH2AX,F-Caspase3, F-Caspase7, Caspase7, Caspase3, P-STAT3, P-STAT1, STAT1 and STAT3 (Cell Signaling Technology, USA). Secondary antibodies conjugated to horseradish peroxidase were followed by enhanced chemiluminescence (Thermo fisher, MA). The results were confirmed by at least three independent experiments.

Cell apoptosis assay

Cell apoptosis was detected using the Annexin-V fluorescein isothiocyanate Apoptosis Kit (BD Biosciences, San Jose, CA, USA). Briefly, cells were stained with Annexin V–fluorescein isothiocyanate and 7-AAD according to the manufacturer's instruction. After incubation in the dark for 15 min at room temperature, stained cells were analyzed by an Accuri C6 flow cytometer (BD Biosciences).

Lentivirus packaging and Stable cell line construction

Stable MPC1/2 expressing cell lines were generated by lentiviral transduction and antibiotic selection. Briefly, Pspax2, pVSV-G and Plvx-IRES-ZsGreen or pLVX-IRES-mcherry or empty vector were transfected into HEK 293T cells using Lipo2000. The viral supernatants were used at MOI = 50 in MC38 cells and MOI = 5 in CaCO-2

cells. Both MPC-1 and MPC2 overexpressing lentivirus were added, 72h post-infection, the infection efficiency of cells was checked with fluorescence microscope and flow cytometry. We furtherly separated the double positive cells (with Zsgreen and mcherry) with flow cytometry-based sorting method and expanded the double positive cells in culture medium and check the expression via Western blot.

Glucose, lactate, ROS, GSSG/GSH, NADP/NADPH ratio and G6P assay

To analyze glucose uptake and lactate secretion, 2×10^4 IFNγ-treated or control cells were plated into a 96-well plate with 100 μl of Minimum Essential Medium for 8 h. The glucose content in the supernatants was assayed with a Glucose Assay Kit (GAGO-20, Sigma) or lactate assay kit (K607, Biovision). For total cellular ROS or Mitochondrial Superoxide detection, the treated cells were stained with CellROX™ Green or MitoSOX™ Red reagents (Invitrogen) and assayed using flow cytometry. For GSSG/GSH,NADP/NADPH and G6P assays, cell extracts were collected and were assayed with the Glutathione Fluorometric Assay Kit (K264, Biovision), NADP/NADPH Quantitation Colorimetric Kit (K347, Biovision) and Glucose-6-Phosphate Colorimetric Assay Kit (K657, Biovision), respectively.

RT-PCR

Total RNA was extracted from cells using Trizol (Invitrogen) and transcribed to cDNA using a high capacity cDNA reverse transcription kit (Applied Biosystems, CA). The primer sequences were as follows: Murine mpc1: 5'-ATGAGTACGCACTTCTGG GG-3'(sense) and 5'-CGCCCACTGATAATCTCTGGA-3' (antisense); Human MPC1:5'-ACTATGTCCGAAGCAAGGATTTC-3' (sense) and 5'-CGCCCACTGA TAATCTCT GGAG-3' (antisense); Murine mpc2: 5'-TACCACCGACTCATGGA TAAAGT-3' (sense) and 5'-CACACACCAATCCCCATTTCA-3' (antisense); Human MPC2: 5'-TACCACCGGCTCCTCGATAAA-3' (sense) and 5'-TATCAGCCAAT CCAGCACACA-3' (antisense); Murine β-actin: 5'-TTCCTTCTTGGGTATGGA ATCCT-3'(sense) and 5'- CACTGTGTTGGCATAGAGGTC-3' (antisense);Human β-actin: 5'-GCACCACACCTTCTACAATGAG-3'(sense), anti-sense: 5'-GGTCTCA AACATGATCTGGGTC-3' (anti-sense). Real-time PCR was performed using ABI

stepone plus (Applied Biosystems, MA, USA). The values are presented as the mean ± s.e.m from three independent experiments that were performed in duplicate. Statistical comparisons among groups were performed using ANOVA followed by Fisher's PLSD. The values of all of the parameters were considered statistically significant at $p < 0.05$.

NAD+/NADH ratio detection

For NAD+/NADH ratio detection, detailed procedure is in the cited paper. Briefly, CaCO-2 and mc38 cells were transfected with MPC1/2 and SoNar expression plasmid via electric pulse. About 24h later, the transfected cells were sorted with flow cytometry and resuspended in phenol red free medium and added the indicated reagents. Then, the cells were seeded to a 96-well flat-bottom plate with 4×10^4 cells per well. Fluorescence intensity was measured at 420 nm or 485 nm excitation, and 528 nm emission wavelengths by Synergy 2 Multi-Mode Microplate Reader (BioTek) after 24h incubation. The normalized SoNar signal was defined as the ratio of SoNar fluorescence with excitation at 420 and 490 nm and inversely proportional to NAD+/NADH ratio.

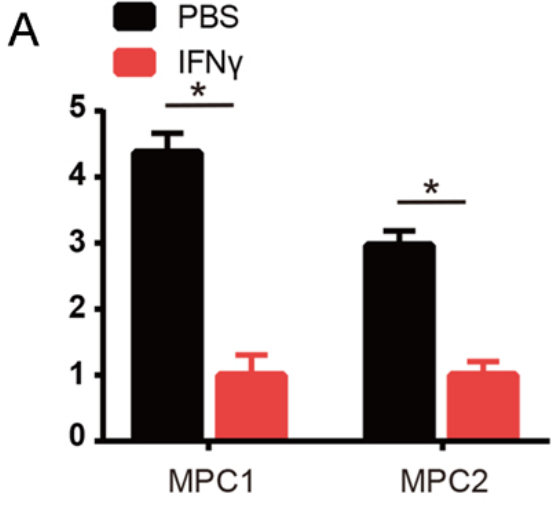
Supplementary figure legends

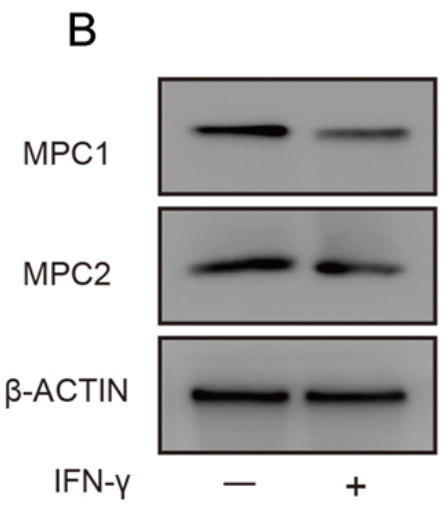
Supplementary Fig.1 (**A-B**) Mice(n=3) were inoculated with 5×10^5 MC38 cells. When tumor size was 7× 7 mm, mice were intratumorally injected with 10 μg IFNγ or PBS once per day for two days. Then the fresh tumor tissue removed from mice was digested with collagenase and hyaluronidase for 1 h. After grinding with semi-frosted slides and lysis of RBC, the dissociated cells were spun down at 500 r.p.m. for 1 min and incubated for 1 h to get rid of adhesive cells. The suspended cells were collected and lysis with Trizol (Invitrogen) or RIPA buffer (Beyotime, China). (**A**) MPC1 and MPC2 expression in tumor treated with PBS or IFNγ were detected with real-time PCR. (**B**) MPC1 and MPC2 expression in tumor treated with PBS or IFNγ were detected with western-blot and β-actin was used as internal control. (**C**) MCF-7 and A375 cancer cells were treated with PBS or IFNγ（50ng/ml）for 48h, the cell lysates were extracted and the levels of MPC1 and MPC2 were detected with western-blot. Data shown are representative of three independent experiments and error bars represent mean ±s.e.m.; *p<0.05, (Student's *t*-test).

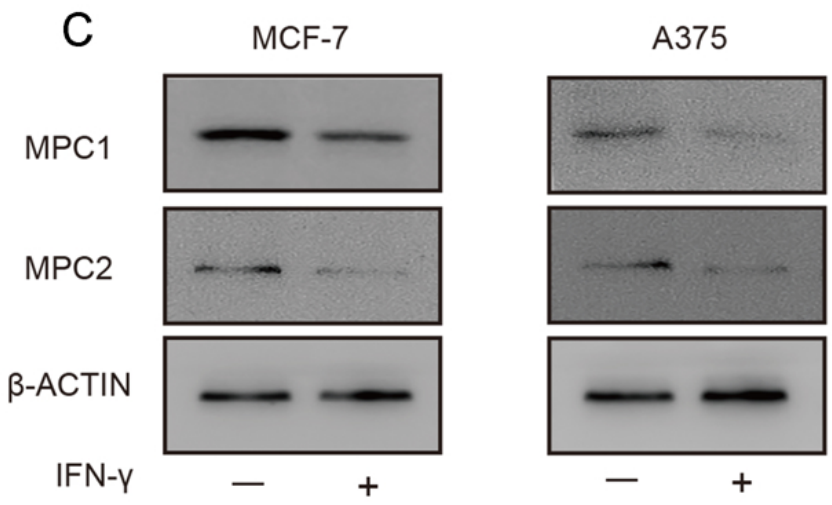
Supplementary Fig.2 (**A**) After treated vector and MPC1/2 overexpressing MC38 and CaCO-2 cells with IFNγ(50ng/ml) or IFNγ (50ng/ml) combined with N-acetylcysteine(NAC,10mM) for 48h.The cells were trypsinized and stained with Annexin-V and 7-AAD.The apoptosis rate was detected with flow cytometry.(**B**) After treated SoNar transfected vector and MPC1/2 overexpressing MC38 and CaCO-2 cells with PBS or IFNγ(50ng/ml) for 24h, the fluorescence intensity were detected with microplate reader. (**C**) After treated vector and MPC1/2 overexpressing MC38 and CaCO-2 cells with IFNγ(50ng/ml) or IFNγ combined with lactate(10mM) or IFNγ combined with pyruvate(10mM) for 48h. The cells were trypsinized and stained with Annexin-V and 7-AAD. The apoptosis rate was detected with flow cytometry. (**D**) After treated vector and MPC1/2 overexpressing MC38 and CaCO-2 cells with PBS or IFNγ(50ng/ml) for 24h, the cells were trypsinized and stained with MitoSOX™ Red (Invitrogen) and detected the mitochondria ROS level with flow cytometry. Data shown are representative of three independent experiments and error bars represent mean ± s.e.m., N.S., no significant difference; *p<0.05, **p<0.01, ***p<0.001 (Student's t-test).

Supplementary Fig.3 (**A-B**) MC38 or CaCO-2 cells were treated with IFNγ(50ng/ml),IFNγ combined with Fludarabine(1uM) or IFNγ combined with Stattic (5uM) for 48h.The total RNA of cells were extracted and the mRNA levels of MPC1 and MPC2were detected with real-time PCR. (**C**) MC38 or CaCO-2 cells were treated with IFNγ combined with Stattic (5uM) for 24h. The fluorescence intensity was detected with microplate reader. (**D**) MC38 or CaCO-2 cells were treated with IFNγ combined with Stattic (5uM) for 48h, the cells were trypsinized and stained with MitoSOX™ Red (Invitrogen) and detected the mitochondria ROS level with flow cytometry. Data shown are representative of three independent experiments and error bars represent mean ± s.e.m., N.S., no significant difference; *p<0.05, **p<0.01, ***p<0.001 (Student's t-test).

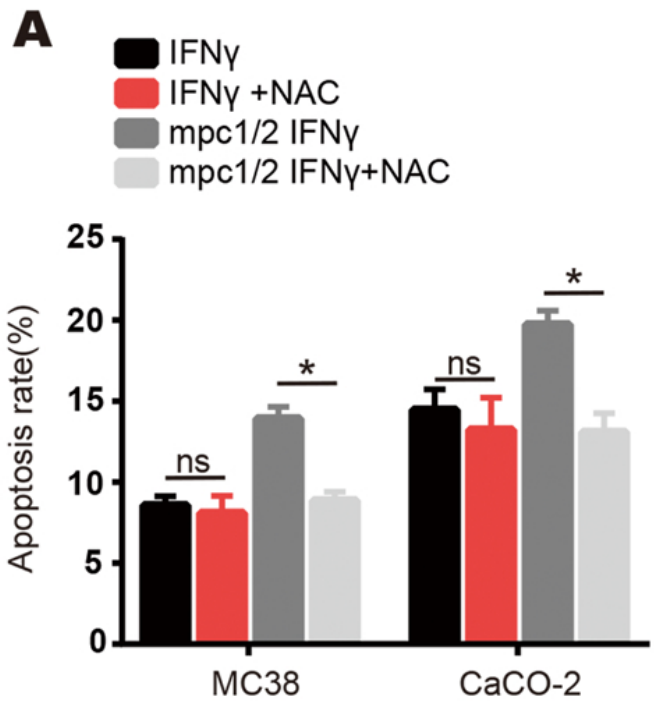
Supplementary Fig.1

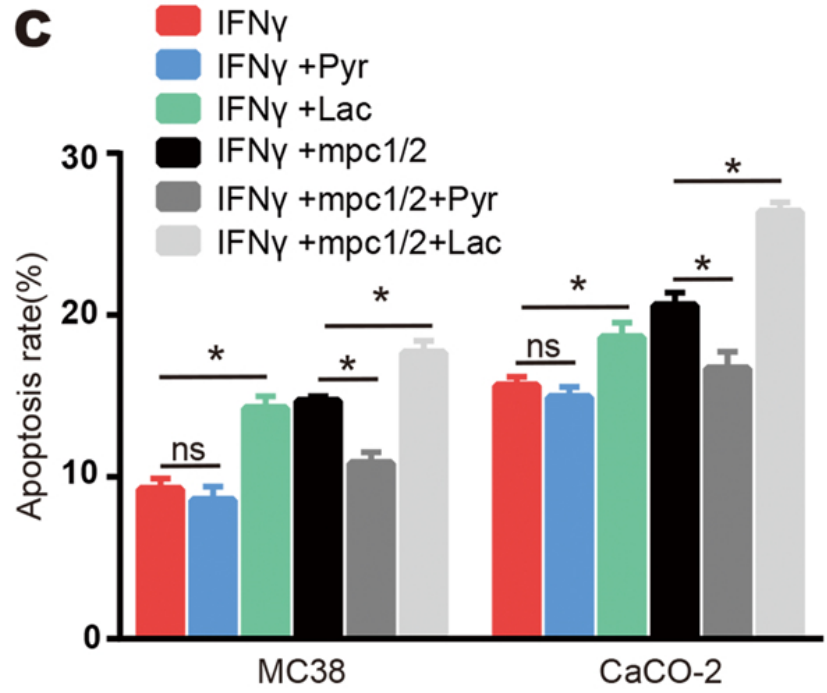


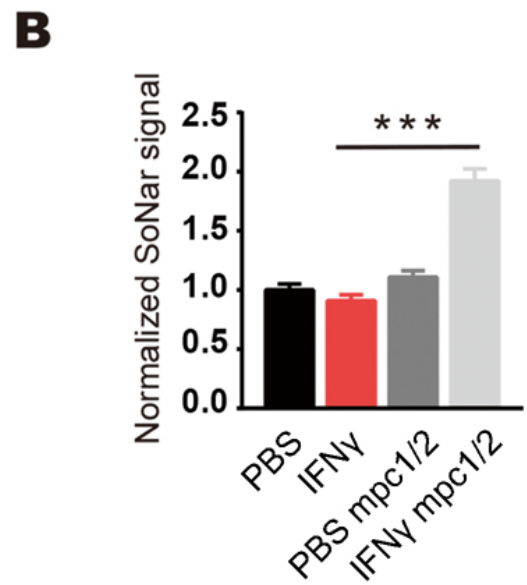


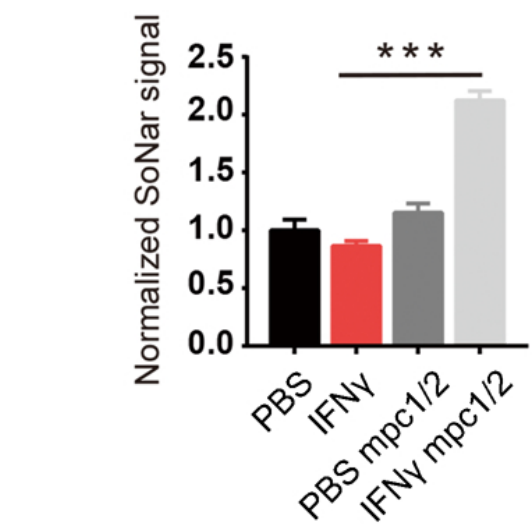


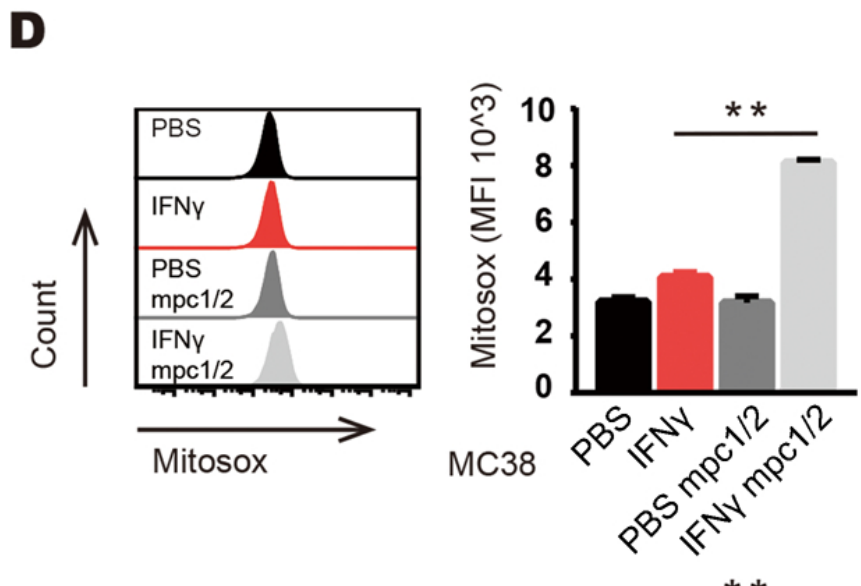
Supplementary Fig. 2

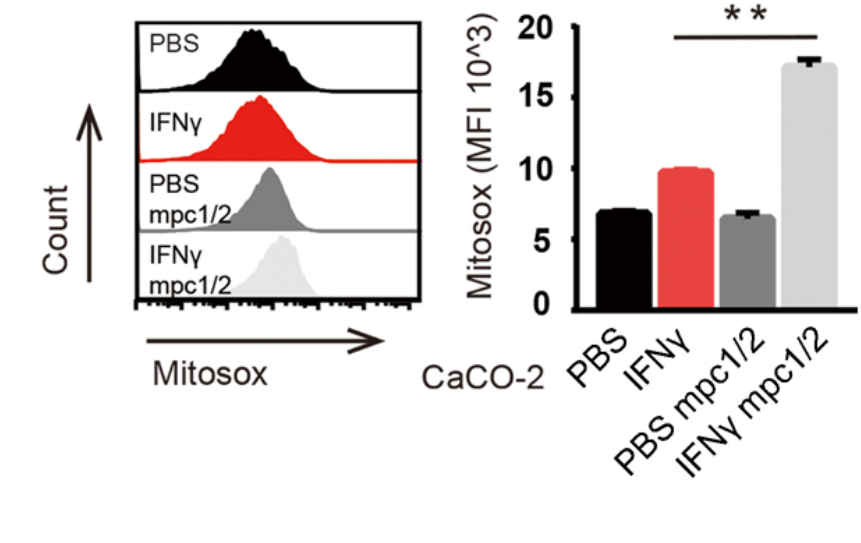












Supplementary Fig. 3