Supplementary figure legends of '*MCP-1/CCR-2 axis in adipocytes and cancer cell respectively facilitates ovarian cancer peritoneal metastasis*'

Supplementary Figure 1. Conditional medium from adipocytes fascinated ID8 metastasis.

Representative images of the migration of the ID8 cells toward conditional medium from undifferentiated 3T3-L1, or conditional medium from 3T3-L1 differentiated for 5 days, or conditional medium from 3T3-L1 differentiated for 7 days. The corresponding bar graph is displayed on the right side. Date are presented as the mean ± SEM, Student's t test. ***p< 0.001.

Supplementary Figure 2. MCP-1 analysis.

(A) MCP-1 expression level of different tissue in abdominal cavity in GETX database. (B) Representative images of the migration of the ID8 cells toward complete medium, complete medium plus MCP-1 with different concentration gradient. The corresponding bar graph is displayed on the right side. Date are presented as the mean ± SEM, Student's t test. *P<0.05, ***p< 0.001.

Supplementary Figure 3. The migration and invasion ability of cell lines corelates to CCR-2 expression level.

(A) Western blot of CCR-2 in the SKOV3 and SKOV3-M1 and SKOV3-M2 cells. The quantification bar graph of Western blot is displayed on the right panel. (n=3 technical replicates

for three independent experiments) (B) Correlation between CCR-2 expression level and migration/invasion fold change of SKOV3-M1 and SKOV3-M2 cells. (C) Representative images of the migration of the ID8 cells toward conditional medium of differentiated 3T3-L1 cells (mCM). The corresponding bar graph is displayed on the right side. (D) Representative images of the migration of the SKOV3 cells toward conditional medium of primary adipocytes (hCM). The corresponding bar graph is displayed on the right side. (n=3 technical replicates for three independent experiments) (E) Representative images of the migration of the SKOV3-M1 cells toward conditional medium of primary adipocytes (hCM). The corresponding bar graph is displayed on the right side. (F) Representative images of the migration of the SKOV3-M2 cells toward conditional medium of primary adipocytes (hCM). (G) Representative images of the migration of the C13 cells toward conditional medium of primary adipocytes (hCM). (H) Representative images of the migration of the OV2008 cells toward conditional medium of primary adipocytes (hCM). (I) Representative images of the migration of the A2780 cells toward conditional medium of primary adipocytes (hCM). The corresponding bar graph is displayed on the right side. Date are presented as the mean ± SEM, Student's t test. *P<0.05, ***p< 0.001.

Supplementary Figure 4. CCR-2 analysis.

(A) Representative images of the migration of the ID8 cells toward complete medium, complete medium plus MCP-1 (20μg/L), and complete medium, MCP-1 (20μg/L) plus CCR-2 antagonist (RS102895 2μg/L). The corresponding bar graph is displayed on the right side. (B) Dot graph of CCR-2 expression in NCI60 cell lines. (C) GO terms enriched in the cancer cells with a high expression of CCR-2 analyzed in NCI60 cell lines. PR for prostate cancer lines; CNS for central nervous system cancer lines; BR for breast cancer lines; CO for colorectal cancer lines; ME for melanoma lines; LC for non-small-cell lung cancer lines; RE for renal carcinoma lines; OV for ovarian cancer lines. (D) Bar graph of the SKOV3, ID8, C13 and OV2008 cell viability after treatment with cDDP in conditional medium of primary adipocytes (hCM), conditional medium of 3T3-L1 (mCM), or hCM or mCM with CCR2 antagonist (2μg/L), or CCR2 antagonist, or cDDP alone. Date are presented as the mean ± SEM, Student's t test. *P<0.05, ***p< 0.001.

Supplementary Figure 5. Diagram of bioinformatic analysis.

(A) Pie graph of down-regulated pathways in ID8 tumors from MCP-$1^{-/-}$ mice compared with WT mice. (B) GSEA plot of changing pathways between tumors with high or low CCR-2 expression level in TCGA, PMID17290060, GSE17260, GSE26193, GSE9891, GSE32062.

Supplementary Figure 6. Western blot of phosphorylated PI3K/AKT/mTOR pathway, along with HIF-1α and VEGF-A in ID8 cells treated as indicated. CCRa: CCR-2 antagonist (RS102895, 2μg/L). (n=3 technical replicates for three independent experiments).

Supplementary Figure 7. Metformin does not affect the viability of adipocytes.

(A) Representative images of undifferentiated 3T3-L1 cells, 3T3-L1 cells differentiated for 5 days, 3T3-L1 cells differentiated for 7 days, and 3T3-L1 cells differentiated for 7 days and treated with metformin (1mM) for 24 hours. (B) Cell viability of differentiated 3T3-L1 cells treated by metformin (1mM) for 24 hours.

Corresponding author:
Dr. Chen Gang, Cancer Biology Medical Centre, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hubei, 430030, P.R.China., Tel: +86-27-83663351, E-Mail: gumpc@126.com