Supplementary Information

Macrophages rely on extracellular serine to suppress aberrant cytokine production

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Table S1. Cellular metabolites in macrophages

| Compound name                        | HMDB\text{ID} | Full Average | ΔSG Average | ΔSG/Fold change | p-Value |
|--------------------------------------|----------------|--------------|-------------|-----------------|---------|
| Glycine                              | HMDB00123      | 2560.933     | 198.872     | 0.078           | 0.004   |
| Serine                               | HMDB00187      | 715.341      | 78.786      | 0.110           | 0.005   |
| Serine                               | HMDB03406      |              |             |                 |         |
| N-Acetylglutamic acid                | HMDB01138      | 5.343        | 1.219       | 0.228           | 0.283   |
| Lactic acid                          | HMDB00190      | 3006.492     | 1001.587    | 0.333           | 0.012   |
|                                      | HMDB01311      |              |             |                 |         |
| Malonyl CoA                          | HMDB01175      | 12.268       | 4.909       | 0.400           | 0.003   |
| Dihydroxyacetone phosphate           | HMDB01473      | 23.592       | 10.455      | 0.443           | 0.202   |
| Pyruvic acid                         | HMDB00243      | 159.707      | 73.159      | 0.458           | 0.148   |
| Glutathione (GSH)                    | HMDB00125      | 2359.359     | 1094.667    | 0.464           | 0.002   |
| PRPP                                 | HMDB00280      | 34.109       | 17.240      | 0.505           | 0.066   |
| N-Carboxyamylaspartic acid           | HMDB00828      | 9.154        | 4.788       | 0.523           | 0.145   |
| Total Pyr-related Amino Acids        | -              | 6303.269     | 3319.154    | 0.527           | 0.103   |
| Lactate/Pyruvate                      | -              | 19.235       | 10.241      | 0.532           | 0.069   |
| Total Glutathione                    | -              | 3260.565     | 1808.197    | 0.555           | 0.008   |
| Acetyl CoA                           | HMDB01206      | 13.896       | 7.816       | 0.562           | 0.001   |
| NADP+                                | HMDB00217      | 76.598       | 43.167      | 0.564           | 0.314   |
| CoA                                  | HMDB01423      | 13.446       | 7.821       | 0.582           | 0.011   |
| GSH/GSSG                             | -              | 5.231        | 3.076       | 0.588           | 0.039   |
| IMP                                  | HMDB00175      | 25.094       | 16.002      | 0.638           | 0.021   |
| Adenosine                            | HMDB00050      | 5.241        | 3.432       | 0.655           | -       |
| 2-Hydroxyglutaric acid               | HMDB00606      | 90.754       | 64.572      | 0.712           | 0.015   |
|                                      | HMDB00694      |              |             |                 |         |
| Malate/Asp                           | -              | 0.059        | 0.042       | 0.714           | 0.063   |
| ADP-ribose                           | HMDB01178      | 24.222       | 17.586      | 0.726           | 0.395   |
| Glutamic acid                        | HMDB00148      | 15820.499    | 11791.523   | 0.745           | 0.108   |
|                                      | HMDB03339      |              |             |                 |         |
| GMP                                  | HMDB01397      | 48.335       | 36.305      | 0.751           | 0.031   |
| 6-Phosphogluconic acid               | HMDB01316      | 5.321        | 4.014       | 0.754           | 0.056   |
| β-Ala                                | HMDB00056      | 720.881      | 561.348     | 0.779           | 0.097   |
| UDP-glucose                          | HMDB00286      | 311.432      | 245.805     | 0.789           | 0.093   |
| Glutathione (GSSG)                   | HMDB03337      | 450.603      | 356.765     | 0.792           | 0.999   |
| Total Glu-related Amino Acids        | -              | 22170.716    | 17678.615   | 0.797           | 0.678   |
| Total Non-essential Amino Acids      | -              | 32026.606    | 25563.055   | 0.798           | 0.659   |
| Molecule                                    | HMDB ID  | Mean   | SD     | CV   | Mean T      |
|---------------------------------------------|----------|--------|--------|------|-------------|
| cis-Aconitic acid                           | HMDB00072| 29.968 | 24.136 | 0.805| 0.002       |
| Total Glucogenic Amino Acids                | -        | 35908.980 | 29092.146 | 0.810| 0.952       |
| Citric acid                                 | HMDB00094| 1501.179 | 1220.718 | 0.813| 0.007       |
| Total Amino Acids                           | -        | 37024.858 | 30128.808 | 0.814| 0.964       |
| Succinic acid                               | HMDB00254| 73.359  | 61.302 | 0.836| 0.945       |
| Ribose 5-phosphate                         | HMDB01548| 5.018   | 4.195  | 0.836| 0.802       |
| Creatine                                    | HMDB00064| 2238.801| 1903.592| 0.850| 0.702       |
| NADH                                        | HMDB01487| 51.828  | 44.198 | 0.853| 0.154       |
| XMP                                         | HMDB01554| 1.058   | 0.905  | 0.856| 0.264       |
| Malic acid                                  | HMDB00156| 233.752 | 202.486| 0.866| 0.928       |
| Valine                                      | HMDB00883| 885.379 | 767.755| 0.867| 0.507       |
| NAD+                                        | HMDB00902| 803.021 | 706.233| 0.879| 0.066       |
| Cysteine                                    | HMDB00574| 258.835 | 228.813| 0.884| 0.442       |
| 2-Phosphoglyceric acid                      | HMDB03391| 4.369   | 3.884  | 0.889| 0.183       |
| HMG CoA                                     | HMDB01375| 2.744   | 2.444  | 0.891| 0.116       |
| Threonine                                   | HMDB00167| 1310.032| 1170.262| 0.893| 0.562       |
| GDP                                         | HMDB01201| 64.250  | 57.480 | 0.895| 0.138       |
| Total Succinyl CoA-related Amino Acids      | -        | 1973.444| 1771.797| 0.898| 0.486       |
| ATP                                         | HMDB00538| 3828.060| 3441.099| 0.899| 0.241       |
| Total Guanylate                             | -        | 849.425 | 764.637| 0.900| 0.139       |
| AMP                                         | HMDB00045| 168.829 | 151.996| 0.900| 0.069       |
| Uric acid                                   | HMDB00289| 9.699   | 8.744  | 0.902| 0.496       |
| Folic acid                                  | HMDB00121| 1.041   | 0.940  | 0.903| 0.439       |
| Total Adenylate                             | -        | 4236.935| 3839.490| 0.906| 0.117       |
| Total BCAA                                  | -        | 2489.684| 2257.947| 0.907| 0.479       |
| GTP                                         | HMDB01273| 736.840 | 670.851| 0.910| 0.349       |
| NADPH                                       | HMDB00221| 46.798  | 42.627 | 0.911| 0.746       |
| Total Essential Amino Acids                 | -        | 4998.253| 4565.754| 0.913| 0.481       |
| S-Adenosylmethionine                        | HMDB01185| 71.002  | 64.898 | 0.914| 0.269       |
| cAMP                                        | HMDB00058| 4.025   | 3.682  | 0.915| 0.315       |
| Lysine                                      | HMDB00182| 349.644 | 320.315| 0.916| 0.454       |
| Methionine                                  | HMDB00696| 249.995 | 230.197| 0.921| 0.479       |
| Ornithine                                   | HMDB00214| 109.002 | 100.418| 0.921| 0.465       |
| Substance                          | HMDB01511 | 1144.803 | 1055.069 | 0.922 | 0.418 |
|-----------------------------------|-----------|----------|----------|-------|-------|
| Phosphocreatine                   | HMDB00172 | 838.070  | 773.844  | 0.923 | 0.467 |
| Fischer's Ratio                   | HMDB00641 | 2.751    | 2.542    | 0.924 | 0.808 |
| Glutamine                         | HMDB01058 | 96.188   | 91.223   | 0.948 | 0.447 |
| Isoleucine                        | HMDB00517 | 63.549   | 60.247   | 0.948 | 0.472 |
| Total Ketogenic Amino Acids       | -         | 4165.709 | 3871.300 | 0.929 | 0.472 |
| Total Acetyl CoA-related Amino Acids | -     | 2058.374 | 1915.545 | 0.931 | 0.461 |
| Leucine                           | HMDB00517 | 63.549   | 60.247   | 0.948 | 0.447 |
| Glutamine                         | HMDB00517 | 63.549   | 60.247   | 0.948 | 0.447 |
| Histidine                         | HMDB00517 | 63.549   | 60.247   | 0.948 | 0.447 |
| NADH/NAD+                         | HMDB00517 | 63.549   | 60.247   | 0.948 | 0.447 |
| 3-Phosphoglyceric acid            | HMDB01058 | 96.188   | 91.223   | 0.948 | 0.085 |
| 2,3-Diphosphoglyceric acid        | HMDB00177 | 175.556  | 166.940  | 0.951 | 0.464 |
| Adenylosuccinic acid              | HMDB00536 | 4.154    | 3.857    | 0.928 | 0.108 |
| Tyrosine                          | HMDB00158 | 478.386  | 470.439  | 0.983 | 0.437 |
| Total Fumarate-related Amino Acids| -         | 797.303  | 785.494  | 0.985 | 0.434 |
| Total Aromatic Amino Acids        | -         | 901.728  | 890.532  | 0.948 | 0.432 |
| Phenylalanine                     | HMDB001058| 96.188   | 91.223   | 0.948 | 0.085 |
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| Total Aromatic Amino Acids        | -         | 901.728  | 890.532  | 0.948 | 0.432 |
| Phenylalanine                     | HMDB001058| 96.188   | 91.223   | 0.948 | 0.085 |
| Total Ketogenic Amino Acids       | -         | 4165.709 | 3871.300 | 0.929 | 0.472 |
| Total Acetyl CoA-related Amino Acids | -     | 2058.374 | 1915.545 | 0.931 | 0.461 |
|           | HMDB00097 | 133.813 | 216.435 | 1.617 | 0.136 |
|-----------|-----------|---------|---------|-------|-------|
| Choline   |           |         |         |       |       |
| Isocitric acid | HMDB00193 | 8.751   | 15.462  | 1.767 | 0.272 |
| Glycerol 3-phosphate/DHAP | - | 4.341   | 12.654  | 2.915 | 0.240 |
| Phosphoenolpyruvic acid | HMDB00263 | 1.037   | 5.637   | 5.435 | -     |

HMDB: The human metabolome database

Metabolic profiling in periMΦs cultured in Full or ΔSG medium for 24 h (n = 3).

Statistical analysis was performed with unpaired t-test.
Table S2. Key resources

| Reagent or resource                          | Source                               | Identifier   |
|---------------------------------------------|--------------------------------------|--------------|
| **Chemicals, peptides, and recombinant proteins** |                                      |              |
| 4-Hydroxy-L-phenylglycine                    | Sigma-Aldrich                        | Cat# 56160   |
| Acetyl-CoA                                   | Sigma-Aldrich                        | Cat# A2056   |
| Apocynin                                     | Tokyo Chemical Industry Co., Ltd.    | Cat# H0261   |
| Glutathione reduced ethyl ester             | Merck                                | Cat# G1404   |
| Hoechst33342                                 | Thermo Fisher Scientific             | Cat# H1399   |
| LPS                                          | Sigma-Aldrich-Aldrich                | Cat# L4391   |
| Malonyl-CoA                                  | Sigma-Aldrich                        | Cat# M4263   |
| Methyl L-(-)-Lactate                         | Tokyo Chemical Industry Co., Ltd.    | Cat# L0163   |
| Methyl Pyruvate                              | Tokyo Chemical Industry Co., Ltd.    | Cat# P0580   |
| MitoSOX Red                                  | Thermo Fisher Scientific             | Cat# M36008  |
| MitoTEMPO                                    | Sigma-Aldrich                        | Cat# SML0737 |
| MitoTrackerRedCMH2XROS                       | Thermo Fisher Scientific             | Cat# M7513   |
| PEP (Phosphoenolpyruvic Acid Monopotassium Salt) | Wako                                | Cat# 164-14761 |
| Recombinant Human M-CSF                      | Peprotech                            | Cat# 300-25  |
| Recombinant Murine IL-10                     | Peprotech                            | Cat# 210-10  |
| S1QEL1.1                                     | Cayman                               | Cat# 20982   |
| S3QEL-2                                      | Cayman                               | Cat# 18556   |
| UK5099                                       | Merck                                | Cat# 5.04817.0001 |
| Thioglycollate                               | BD Biosciences                       | Cat# 225640  |
| Lipofectamine RNAiMAX Transfection Reagent   | Thermo Fisher Scientific             | Cat# 13778075|
| **Critical Commercial Assays**               |                                      |              |
| Fast SYBR Green Master Mix                   | Thermo Fisher Scientific             | Cat# 4385612 |
| Mouse IL-10 Quantikine ELISA Kit             | R&D Systems                          | Cat# M1000B  |
| Mouse IL-6 Quantikine ELISA Kit              | R&D Systems                          | Cat# M6000B  |
| Seahorse Xfp Cell Mito Stress Test Kit       | Agilent                              | Cat# 103010-100 |
| **Deposited Data**                           |                                      |              |
| Microarray database                          | GEO: GSE156325                       |              |
| **Experimental Models: Cell Lines**          |                                      |              |
| J774.1 cell line                             | RIKEN BioResource Center             | RRID:CVCL_4770 |
| Oligonucleotides | Source | Cat# |
|------------------|--------|------|
| siAsct1          | Thermo Fisher Scientific | 1320001 |
| siSnat2          | Thermo Fisher Scientific | 1320001 |
| GFP-22 siRNA     | Qiagen | 1022064 |
| All primers are available in Table S3 | Hokkaido System Science Co., Ltd. | |

| Software and Algorithms | Source | RRID|
|-------------------------|--------|-----|
| FlowJo software v.10    | BD     | SCR_008520 |
| Seahorse XFe96 Software Wave Desktop | Agilent | SCR_014526 |
| Prism v.6.0c            | GraphPad | SCR_002798 |
Table S3. Primers for Q-PCR

| Gene       | Primer 5'                  | Primer 3'                  |
|------------|-----------------------------|-----------------------------|
| Atf4       | 5'-CTATGGATGATGGCTTGGCC     | 3'                          |
| Atf4       | 5'-CCGGAAAAGGCACTCCTCC-3'   |                             |
| Atp5a1     | 5'-TGGGCGTGTGTTAAGCATTG-3'  |                             |
| Atp5a1     | 5'-TTCCCCAAACACGACAACTCC-3' |                             |
| Ccl2       | 5'-CCACTCACCTGCTGCTACTCAT-3' |                             |
| Ccl2       | 5'-TGGGTGATCTCTTGTAGCTCTCC-3' |                             |
| Ccl3       | 5'-AACCAAGTCCTTCTCAGCGCC-3' |                             |
| Ccl3       | 5'-GGAATCTTCCGGGCTGTAGGAG-3' |                             |
| Il10       | 5'-GCGCTGTCATCGATTTCCTCC-3' |                             |
| Il10       | 5'-CACCTGCTCCACTGCTCTG-3'   |                             |
| Il12b      | 5'-ACATCTACCGAAGTCCAATGCA-3' |                             |
| Il12b      | 5'-GGAATTGTAATAGCGATCCTGAGC-3' |                             |
| Il1b       | 5'-CTGGTGATGACGTTCCCATTA-3' |                             |
| Il1b       | 5'-CCGACAGCAGAGGCTTTT-3'    |                             |
| Il6        | 5'-CCAGAGATACAAAGAAATGATGG-3' |                             |
| Il6        | 5'-ACTCCAGAAGACCAGGAAAT-3'  |                             |
| Ndufb8     | 5'-AACCGATCACAGCATGAGAGG-3' |                             |
| Ndufb8     | 5'-TATCGGTTCACCCCAGTTCATC-3' |                             |
| Phgdh      | 5'-GGCCACCCGAATGCAAT-3'     |                             |
| Phgdh      | 5'-CGGCCACTTACGAGAGATG-3'   |                             |
| Asct1(Slc1a4) | 5'-GGGAACGTGACCAAAGAACAGA-3' |                             |
| Asct1(Slc1a4) | 5'-GGCCTCATTGAAGGAATTAGAAG-3' |                             |
| Asct2(Slc1a5) | 5'-TGCTTTCTGGACCTCTTCT-3'   |                             |
| Asct2(Slc1a5) | 5'-CCCGTTTTAGTGTGTGCAGTG-3' |                             |
| Tnfa       | 5'-ACCCTCACACTCAGATCATCTTC-3' |                             |
| Tnfa       | 5'-TGGTGTTTTGCATCAGAGCT-3'  |                             |
| Uqcre      | 5'-TGGGCGTGTGTAAGCATTG-3'   |                             |
| Uqcre      | 5'-TTCCCCAAACACGACAACCTCC-3' |                             |
Figure S1

**Figure S1. (Related to Fig. 1)**

**Effect of culture with the serine- and glycine-depleted medium on cellular amino acid content in peritoneal macrophages**

Cellular content of amino acids in periMΦs analyzed by CE-TOF MS. PeriMΦs were cultured in Full or ΔSG medium for 24 h, followed by stimulation with LPS (100 ng/ml) for 6 h (n = 3). Cellular contents of serine and glycine are shown in Fig. 1g.

Values are means ± 95% CI. Statistical analysis was performed with unpaired t-test. *p < 0.05; **p < 0.01 Related to Fig. 1.
Figure S2. (Related to Fig. 1)
Effect of culture with the serine- and glycine-depleted medium on cellular amino acid content in bone marrow–derived macrophages
Relative cellular content of amino acids in BMDMs analyzed by GC/MS. BMDMs were cultured in Full or ΔSG for 24 h (n = 4).
Values are means ± 95% CI. Statistical analysis was performed with unpaired t-test. **p < 0.01. Related to Fig. 1.
Figure S3

a

**ΔSG**

| Conc. (pg/ml) | IL10 | IL6 | TNFα |
|---------------|------|-----|------|
| LPS (+)       | 0    | 0   | 0    |

**IL10**

| Conc. (pg/ml) | IL10 | IL6 | TNFα |
|---------------|------|-----|------|
| LPS (+)       | 0    | 0   | 0    |

**IL6**

| Conc. (pg/ml) | IL10 | IL6 | TNFα |
|---------------|------|-----|------|
| LPS (+)       | 0    | 0   | 0    |

**TNFα**

| Conc. (pg/ml) | IL10 | IL6 | TNFα |
|---------------|------|-----|------|
| LPS (+)       | 0    | 0   | 0    |

b

**ΔSG**

| perIFNαs | II1β | II12 | Ccl2 | Ccl3 |
|-----------|------|------|------|------|
| LPS (−) (+) | N.S. | N.S. | N.S. | N.S. |

**II1β**

| perIFNαs | II1β | II12 | Ccl2 | Ccl3 |
|-----------|------|------|------|------|
| LPS (−) (+) | N.S. | N.S. | N.S. | N.S. |

**II12**

| perIFNαs | II1β | II12 | Ccl2 | Ccl3 |
|-----------|------|------|------|------|
| LPS (−) (+) | N.S. | N.S. | N.S. | N.S. |

**Ccl2**

| perIFNαs | II1β | II12 | Ccl2 | Ccl3 |
|-----------|------|------|------|------|
| LPS (−) (+) | N.S. | N.S. | N.S. | N.S. |

**Ccl3**

| perIFNαs | II1β | II12 | Ccl2 | Ccl3 |
|-----------|------|------|------|------|
| LPS (−) (+) | N.S. | N.S. | N.S. | N.S. |

b

**ΔSG**

| BMDMs mRNA levels | II1β | II12 | Ccl2 | Ccl3 |
|-------------------|------|------|------|------|
| LPS (−) (+) | N.S. | N.S. | N.S. | N.S. |

**II1β**

| BMDMs mRNA levels | II1β | II12 | Ccl2 | Ccl3 |
|-------------------|------|------|------|------|
| LPS (−) (+) | N.S. | N.S. | N.S. | N.S. |

**II12**

| BMDMs mRNA levels | II1β | II12 | Ccl2 | Ccl3 |
|-------------------|------|------|------|------|
| LPS (−) (+) | N.S. | N.S. | N.S. | N.S. |

**Ccl2**

| BMDMs mRNA levels | II1β | II12 | Ccl2 | Ccl3 |
|-------------------|------|------|------|------|
| LPS (−) (+) | N.S. | N.S. | N.S. | N.S. |

**Ccl3**

| BMDMs mRNA levels | II1β | II12 | Ccl2 | Ccl3 |
|-------------------|------|------|------|------|
| LPS (−) (+) | N.S. | N.S. | N.S. | N.S. |

b

**ΔSG**

| mRNA levels | II10 | II6 |
|-------------|------|-----|
| LPS (ng/ml) | 0    | 1   | 10  | 100 |

**II10**

| mRNA levels | II10 | II6 |
|-------------|------|-----|
| LPS (ng/ml) | 0    | 1   | 10  | 100 |

**II6**

| mRNA levels | II10 | II6 |
|-------------|------|-----|
| LPS (ng/ml) | 0    | 1   | 10  | 100 |

**ΔSG**

| Concentration (pg/ml) | IL10 | IL6 |
|-----------------------|------|-----|
| Phenylglycine (−) (+) | *    | *   |

**IL10**

| Concentration (pg/ml) | IL10 | IL6 |
|-----------------------|------|-----|
| Phenylglycine (−) (+) | *    | *   |

**IL6**

| Concentration (pg/ml) | IL10 | IL6 |
|-----------------------|------|-----|
| Phenylglycine (−) (+) | *    | *   |
Figure S3. (Related to Fig. 2)
Effect of serine and glycine deprivation on cytokine production in macrophages
(a) Production of cytokines in BMDMs. BMDMs were cultured in Full or ΔSG medium for 24 h, followed by stimulation with LPS (100 ng/ml) for 24 h (n = 4-6).
(b,c) Gene expression of cytokines in periMΦs (c) and BMDMs (d). PeriMΦs and BMDMs were cultured in Full or ΔSG medium for 24 h, followed by stimulation with LPS (100 ng/ml) for 8 h and 24 h, respectively (n = 3–4).
(d) Gene expression of cytokines in BMDMs. BMDMs were cultured in Full or ΔSG medium for 24 h, followed by stimulation with LPS (1, 10 ng/ml) for 8 h (n = 4).
(e) Production of cytokines in BMDMs. BMDMs were cultured in Full medium with or without 4-hydroxy-L-phenylglycine (5mM, an inhibitor of ASCT1) for 24 h, followed by stimulation with LPS (100 ng/ml) for 8 h (n = 4).
Values are means ± 95% CI. N.D., not detected. Statistical analysis was performed with unpaired t-test. *p < 0.05; **p < 0.01; N.S., not significant. Related to Fig. 2.
Figure S4. (Related to Fig. 2)
Serine and glycine deprivation results in impaired production of glutathione in macrophages

(a) Schema of glutathione metabolism.

(b) Cellular content of total glutathione, GSH, GSSG, and GSH/GSSG in periMΦs. PeriMΦs were cultured in Full or ΔSG medium for 24 h, followed by stimulation with LPS (100 ng/ml) for 6 h (n = 3).

(c) Gene expression of Il10 and Il6 in BMDMs. BMDMs were cultured in Full medium with GSH (1 mM) for 24 h, followed by stimulation with LPS (100 ng/ml) for 24 h (n = 4).

Values are means ± 95% CI. Statistical analysis was performed with unpaired t-test (b) and Dunnett’s test (c) (compared to ΔSG + GSH (-)). *p < 0.05; **p < 0.01; N.S., not significant. Related to Fig. 2.
Replenishment of PEP, lactate, acetyl-CoA, or malonyl-CoA has little effect on the aberrant cytokine expression caused by serine and glycine deprivation

(a-d) Gene expression levels of *Il10* in BMDMs. BMDMs were cultured in Full or ΔSG medium with the indicated concentrations of phosphoenolpyruvate (PEP) (a), lactate (b), acetyl-CoA (c), or malonyl-CoA (d) for 24 h, followed by stimulation with LPS (100 ng/ml) for 24 h (n = 4).

Values are means ± 95% CI. Statistical analysis was performed with Dunnett's test (compared to ΔSG + PEP 0 mM (a); ΔSG + lactate 0 mM (b); ΔSG + acetyl-CoA 0 µM (c); ΔSG + malonyl-CoA 0 µM (d)). N.S., not significant. Related to Figs. 3 and 4.
Figure S6. (Related to Figs. 3 and 4)

*Srebp1* deficiency does not affect cytokine expression caused by serine and glycine deprivation

Gene expression of *Il10* and *Il6* in BMDMs obtained from wild-type (WT) or *Srebp1*-deficient (KO) mice. BMDMs were cultured in Full or ∆SG medium for 24 h, followed by stimulation with LPS (100 ng/ml) for 24 h (n = 4).

Values are means ± 95% CI. Statistical analysis was performed unpaired t-test. *p < 0.05; **p < 0.01 ; N.S., not significant. Related to Figs. 3 and 4.
Figure S7

Effect of pyruvate treatment or inhibition of mitochondrial pyruvate transporter on cellular amino acid content in macrophages

(a) Relative cellular content of amino acids in BMDMs analyzed by GC/MS. BMDMs were cultured in ∆SG medium with or without pyruvate (10 mM) for 24 h (n = 4).

(b) Relative cellular content of amino acids in BMDMs analyzed by GC/MS. BMDMs were cultured in Full medium with or without UK5099 (10 µM, an inhibitor of mitochondrial pyruvate carrier) for 24 h (n = 4). Values are means ± 95% CI. Statistical analysis was performed with unpaired t-test. *p < 0.05; **p < 0.01. Related to Fig. 4.
Figure S8.

Graphical summary: Potential molecular mechanisms underlying extracellular serine–regulated inflammatory cytokine production

In most cell types, there are 3 major pathways providing serine: de novo serine synthesis, uptake of extracellular serine, and conversion from glycine (left). However, the role of serine uptake has been largely unknown. Deletion of serine in the culture media remarkably reduced the cellular serine content in macrophages, indicating that macrophages largely depend on extracellular serine (right). Under serine deprivation, macrophages stimulated with LPS exhibited aberrant cytokine expression patterns, among which expression of anti-inflammatory Il10 was markedly inhibited. In terms of the underlying mechanism, serine deprivation reduced pyruvate transport into mitochondria, impairing mitochondrial production of ROS. Supplementation of IL10 suppressed the sustained expression of Il6. Thus, this study demonstrates that macrophages rely on extracellular serine to suppress aberrant cytokine production.