Supplementary Fig.1 Fractionation studies of eWAT.

a, RT-qPCR of *Leptin* expression in SVF and MAF isolated from eWAT from NCD (n=5 biologically independent sample) and HFD (n=6 biologically independent sample) mice. b, RT-qPCR of *CD45* expression in SVF and MAF isolated from eWAT from NCD (n=5 biologically independent sample) and HFD (n=6 biologically independent sample) mice. Throughout, data are presented as means ± SEM. *P* values are determined by unpaired two-tailed Student’s t-test.
Supplementary Fig.2 Metabolic characterization of male mice fed a NCD.

**a**, Representative immunoblot analysis and quantification of GPSM1 expression in BMDMs, Peritoneal macrophages (PMs), monocytes and the indicated tissues from GPSM1<sup>fl/fl</sup>; Lyz2-cre mice and GPSM1<sup>fl/fl</sup> mice (n = 3 biologically independent mice per group). GAPDH and actin were used as loading controls. **b-g**, Male GPSM1<sup>fl/fl</sup>; Lyz2-cre mice and age-matched GPSM1<sup>fl/fl</sup> littermates were fed a NCD for 22 weeks. **b**, Representative image of mice (left) and quantification of tibia length (right) of the indicated genotype (n = 9 biologically independent mice per group). **c**, Body weight (n = 10 biologically independent mice per
d, Percent of fat (left), lean body mass (right). n = 8 biologically independent mice per group. e, ITT and AOC (n = 7 biologically independent mice per group). f, Liver weight (n = 9 biologically independent mice per group). g, Representative images of H&E staining (top) and Oil Red O (bottom) staining of liver sections. Scale bars, 100 μm. h, Quantification of hepatic TG (right, n = 9 biologically independent mice per group). Independent experiments were repeated three times with similar results (g). All data are shown as means ± SEM (a-f, h). P values are determined by unpaired two-tailed Student’s t-test (a, b, d, f and h) or two-way ANOVA with Sidak’s multiple-comparisons test (c, e).
**Supplementary Fig. 3 Metabolic characterization of male mice fed a HFD.**

a, Representative images of 7-week-old mice subjected to a HFD for 12 weeks. b, Lean mass was determined for mice fed a HFD (n = 9 biologically independent mice per group). c, Representative images of the indicated WAT from GPSM1f/f; Lyz2-cre and GPSM1fl/fl mice. d, Frequency distribution of adipocyte size of scWAT and eWAT (n = 5 biologically independent mice per group for scWAT and n = 8 biologically independent mice per group for eWAT). e, Quantification of adipocyte area (left) and cell number (right) of scWAT and eWAT. f, Quantification of pAKT/AKT in eWAT, liver and muscle (n = 4 biologically independent mice per group). g, Representative images of liver section. h, RT-qPCR analysis showing mRNA abundance of fatty acid synthesis and uptake genes and fatty acid synthesis and uptake genes.
β-oxidation genes in liver (n = 10 biologically independent mice per group). All data are shown as means ± SEM. P values are determined by unpaired two-tailed Student’s t-test (b, d, e, and h) or one-way ANOVA with Tukey’s correction for multiple group comparison (f).
Supplementary Fig. 4 GPSM1 deficiency alleviates HFD-induced metabolic disorders in female mice.

Female GPSM1f/f; Lyz2-cre mice and age-matched GPSM1f/f littermates were fed a HFD for 14 weeks. HFD feeding started at 7 weeks of age. a, Representative images of mice (left) and body weight (right, n = 10 biologically independent mice per group). b, Percent of fat (left), lean body mass (middle), and lean mass weight (right). n = 9 biologically
independent mice per group. Representative images (left) and weight (right) of the indicated WAT from GPSM1f/f; Lyz2-cre and GPSM1f/f mice. n = 9 biologically independent mice per group. d, Representative H&E staining of scWAT, gonWAT and BAT sections. Scale bars, 100 μm. e, Adipocyte area of scWAT and gonWAT (n = 3 biologically independent mice per group). f, GTT and AOC (n = 10 biologically independent mice for GPSM1f/f and n = 8 biologically independent mice for GPSM1f/f; Lyz2-cre). g, ITT and AOC (n = 8 biologically independent mice per group). h, Representative images of H&E staining (top) and Oil Red O (bottom) staining of liver sections (left). Scale bars, 100 μm. Representative images of liver section (right). i, Liver weight (n = 9 biologically independent mice per group). j, Quantification of hepatic TG (n = 9 biologically independent mice per group). k, Serum levels of TCH, NEFA, ALT, and AST (n = 9 biologically independent mice per group). Independent experiments were repeated three times with similar results (d, h). All data are shown as means ± SEM. P values are determined by unpaired two-tailed Student’s t-test (b, c, e to g, i to k) or two-way ANOVA with Sidak’s multiple-comparisons test (a, f, and g).
Supplementary Fig. 5 Analysis of metabolic inflammation in HFD-fed mice.

a, Flow cytometry quantification of the Lin−Sca1−cKit+ myeloid precursors (MPCs) in the bone marrow of GPSM1f/f and GPSM1f/f, Lyz2-cre mice both under NCD (12-week old) and HFD for 8 weeks (n = 5 biologically independent mice per condition). b, White blood cell counts both under the NCD (n = 5 biologically independent mice per group) and HFD...
settings (n = 6 biologically independent mice per group). NE, neutrophils; MO, monocytes; EO, eosinophils; BA, basophils; LY, lymphocytes. c, The numbers of Ly-6C\text{low} and Ly-6C\text{high} monocytes of \emph{GPSM1}\text{f/f} and \emph{GPSM1}\text{f/f}; Lyz2-cre mice both under the NCD (n = 5 biologically independent mice per group) and HFD (n = 6 biologically independent mice per group) conditions. d to g Male \emph{GPSM1}\text{f/f}; Lyz2-cre mice and age-matched \emph{GPSM1}\text{f/f} littermates were fed a HFD for 12 weeks. HFD feeding started at 7 weeks of age. d, Quantification of the proportion of crown-like structure (n = 5 biologically independent mice per group). e, Gating strategy for analysis of macrophages in eWAT and scWAT. f, The numbers of TIM4\text{+} and TIM4\text{-} macrophages of eWAT and scWAT (n = 3 biologically independent mice per group). g, Quantification of the proportion of Trichrome C\text{*} area (n = 5 biologically independent mice per group). h and i Male \emph{GPSM1}\text{f/f}; Lyz2-cre mice and age-matched \emph{GPSM1}\text{f/f} littermates were fed a HFD for 5 weeks. HFD feeding started at 7 weeks of age. h, GTT and ITT (n = 9 biologically independent mice for \emph{GPSM1}\text{f/f} and n = 10 biologically independent mice for \emph{GPSM1}\text{f/f}; Lyz2-cre). i, Representative H&E and F4/80\text{+} staining of eWAT sections and quantification (n = 4 biologically independent mice per group). Scale bars, 100 μm. All data are presented as means ± SEM. P values are determined by unpaired two-tailed Student’s \emph{t}-test (a to d, f, g, i) or two-way ANOVA with Sidak’s multiple-comparisons test (h).
Supplementary Fig. 6 GPSM1 depletion does not affect inflammatory properties of neutrophils and characterization of CSF1R-administered mice fed a HFD.

**a** and **b** Male *GPSM1*fl/fl; *Lyz2*-cre mice and age-matched *GPSM1*fl/fl littermates were fed a HFD for 12 weeks. **a**, The myeloperoxidase (MPO) activity in eWAT (n = 4 biologically independent sample per group). **b**, RT-qPCR analysis indicating mRNA abundance of neutrophil inflammatory markers from sorted CD11b+Ly6G+ neutrophils from the eWAT by flow cytometry (n = 4 biologically independent sample per group).

**c** to **f** *GPSM1*fl/fl and *GPSM1*fl/fl; *Lyz2*-cre mice, which had been already HFD-fed for 5 weeks, injected intraperitoneally CSF1R antibody or isotype IgG (10 mg/kg), twice a week, for 5 weeks. **c**, Flow cytometry quantification of total macrophages of eWAT (n = 3 biologically independent mice per group). **d**, Body weight curve (left) and the body weight at 10-week HFD (right). n = 6 biologically independent mice for *GPSM1*fl/fl injected with IgG or CSF1R and *GPSM1*fl/fl; *Lyz2*-cre injected with IgG; n = 7 biologically independent mice for *GPSM1*fl/fl; *Lyz2*-cre injected with CSF1R. **e**, GTT and AOC (n = 5 biologically independent mice per group). **f**, ITT and AOC (n = 5 biologically independent mice for *GPSM1*fl/fl injected with IgG or CSF1R and *GPSM1*fl/fl; *Lyz2*-cre injected with CSF1R; n = 6 biologically independent mice for
GPSM1<sup>1st</sup>, Lyz2-cre injected with IgG). All data are shown as means ± SEM. P values are determined by unpaired two-tailed Student’s t-test (a to c), two-way ANOVA with Tukey’s multiple-comparisons test (d to f) or one-way ANOVA with Tukey’s multiple-comparisons test (d to f).
Supplementary Fig. 7 Metabolic cage studies of NCD- and HFD-fed mice.

**a** Food intake and Activity counts of NCD-fed mice (n = 6 biologically independent mice for \(\text{GPSM1}^{\text{fl}}\) and n = 3 biologically independent mice for \(\text{GPSM1}^{\text{fl}};\ \text{Lyz2-cre}\)) were monitored for a 24 h period. **b** Food intake and Activity counts of HFD-fed mice (n = 7 biologically independent mice for \(\text{GPSM1}^{\text{fl}}\) and n = 5 biologically independent mice for \(\text{GPSM1}^{\text{fl}};\ \text{Lyz2-cre}\)) were monitored for a 24 h period. Throughout, data are presented as means ± SEM. \(P\) values are determined by unpaired two-tailed Student’s \(t\)-test and two-way ANOVA with Sidak’s multiple-comparisons test.
Supplementary Fig. 8 Macrophage GPSM1 regulates TLR4-induced NF-κB inflammatory signaling.

a, BMDMs were infected with Lv-shCON or Lv-shGPSM1 for 72 h and treated with LPS for additional indicated times. Immunoblot analysis of GPSM1 is shown (n= 2 independent samples per condition). b, BMDMs were treated with 250 μM Palmic acid or vehicle control for indicated times. Immunoblot analysis of p-P65 and P65 is shown. c, Quantification of p-P65 positive area to F4/80+ area of eWAT from GPSM1+/+ and GPSM1+/Lyz2-cre mice subjected to HFD for 12 weeks (n = 3 biologically independent mice per group). d, RT-qPCR analysis of indicated genes from sorted F4/80+ macrophages of eWAT (n = 3 biologically independent mice per group). Independent experiments were repeated three times with similar results (a, b). Throughout, data are presented as means ± SEM. P values are determined by unpaired two-tailed Student’s t-test (c, d).
Supplementary Fig.9 TNFAIP3 functions as a GPSM1 target for mediating the NF-κB pathway in macrophages.

a, A20 relative mRNA levels of eWAT from HFD-fed GPSM1<sup>fl/fl</sup> and GPSM1<sup>fl/fl;Lyz2-cre</sup> mice (n = 3 biologically independent sample per group, each sample was obtained from a pool of three mice). b, RT-PCR indicating pro-inflammatory markers in GPSM1<sup>fl/fl</sup> BMDMs infected with Lv-shCON and GPSM1<sup>fl/fl;Lyz2-cre</sup> BMDMs infected with Lv-shCON or Lv-shA20 for 72 h and treated with LPS for 3 h (n= 5 independent samples per condition). c, Quantification of A20 and GPSM1 protein levels in BMDMs from the mice of four genotypes (n = 3 biologically independent mice per group). d, Quantification of the proportion of crown-like structure (n = 3 biologically independent mice per group). e, Quantification of the proportion of F4/80<sup>+</sup> area (n = 3 biologically independent mice per group). All data are presented as means ± SEM. P values are determined by unpaired two-tailed Student’s t-test (a) or one-way ANOVA with Tukey’s multiple-comparisons test (b to e).
**Supplementary Fig. 10** Screen potential GPSM1 inhibitors.

**a**, SPR assay with Biacore to verify the affinity between GPSM1 protein and small-molecular compounds. The compounds were tested for binding with concentration of 50 μmol/L. **b**, The chemical structure of compound 7 (AN-465/42243987). **c**, Docking poses for the top 10 molecules. Ligands (green) and interacted residues (gray) in the receptor are represented as stick. The yellow dash line stands for hydrogen bond and π-π interaction is exhibited as the cyan dash line.
Supplementary Fig. 11 The effects of potential GPSM1 inhibitors in \textit{in vitro} and \textit{in vivo} experiments.

\textbf{a,} Representative immunofluorescence images indicating P65 nuclei translocation of BMDMs treated with 50\textmu M other 9 small-molecular compounds for 16 hours and then stimulated with LPS for 1 hour, showed by high-content screen. BMDMs were stained for P65 (red) and DAPI (blue). \textbf{b,} Quantification of the proportion of P65 nuclear translocation exhibited in \textbf{a} (n= 3 independent samples per group). \textbf{c,} RT-PCR indicating A20 mRNA levels of BMDMs treated with 50\textmu M compounds or vehicle control for 16 hours and then stimulated with LPS for 20 min (n= 4 independent samples per group). \textbf{d,} Percentage of Oil Red O\textsuperscript{+} area of liver from DIO mice treated with either AN-465 or vehicle (n = 4
biologically independent mice per group). e, Quantification of the proportion of crown-like structure and F4/80* area of eWAT (n = 4 biologically independent mice per group). f, CCK8 assays were performed in BMDMs after cells were treated with increasing doses of AN-465/42243987 (n= 3 independent samples per group). g, Serum ALT and AST levels of DIO mice treated with either AN-465 or vehicle (n = 10 biologically independent mice per group). Independent experiments were repeated three times with similar results (a). All data are presented as means ± SEM. P values are determined by two-tailed Student’s t-test (b to g).
Supplementary Fig.12 Assessment of monocyte chemotactic activity in vivo and in vitro.

a, Recruitment monocyte-derived macrophages into implanted Matrigel plugs loaded with MCP-1 of GPSM1f/f and GPSM1f/f; Lys2-cre mice (n = 3 biologically independent mice per group). b, Chemotaxis of WT and GPSM1 KO THP-1 cells (n = 3 or 4 independent samples per condition). All data are presented as means ± SEM. P values are determined by one-way ANOVA with Tukey’s multiple-comparisons test.
### Supplementary Table 1. Clinical characteristics of subjects

| Variable                        | Normal weight (n = 36) | Overweight/Obesity (n = 61) | P value  |
|---------------------------------|------------------------|-----------------------------|----------|
| Male/ female (n)                | 6/30                   | 13/48                       | 0.5823   |
| Age (years)                     | 44.33±10.66            | 41.03±10.60                 | 0.1427   |
| BMI (kg/m²)                     | 21.85±1.56             | 33.01±7.06                  | 4.5082×10⁻¹⁵ |
| SBP (mmHg)                      | 120.86±12.83           | 129.08±17.05                | 0.0152   |
| DBP (mmHg)                      | 80.14±9.47             | 82.50±13.53                 | 0.3662   |
| Fasting plasma glucose (mmol/l) | 4.83 (4.51, 5.61)      | 5.11 (4.75, 5.70)           | 0.2742   |
| HbA1c (%)                       | 5.50 (5.40, 6.40)      | 5.80 (5.10, 6.60)           | 0.7140   |
| Triglyceride (mmol/l)           | 0.94 (0.71, 1.07)      | 1.57 (1.00, 2.31)           | 0.0018   |
| Total cholesterol (mmol/l)      | 4.56 (3.98, 5.31)      | 4.83 (4.48, 5.30)           | 0.2255   |
| ALT (U/L)                       | 16.00 (13.00, 20.00)   | 26.00 (17.50, 52.00)        | 0.0049   |
| AST (U/L)                       | 22.00 (18.00, 26.00)   | 22.00 (18.00, 33.50)        | 0.1422   |
| Low-Density Lipoprotein-c       | 2.59 (2.17, 3.27)      | 2.94 (2.43, 3.53)           | 0.1901   |
| High-Density Lipoprotein-c      | 1.12 (0.88, 1.29)      | 1.06 (0.94, 1.19)           | 0.5238   |

Normal weight: BMI < 24; Overweight/Obesity: BMI ≥ 24

Data are presented as the mean ± SD or median (interquartile range) or n.

Comparisons are done using two-tailed Student’s t-test.
### Supplementary Table 2. Oligonucleotide primers for Loxp sites

| Oligonucleotide primers | Forward primer (5’-3’) | Reverse primer (5’-3’) |
|-------------------------|-------------------------|------------------------|
| GPSM1-Loxp primer1      | CCCAGAAATGCCAGATTACG    | CTTGGGCTGCCAGAATTTTCTC |
| GPSM1-Loxp primer2      | TTACAGTCGGCCAGGCTGAC    | CTTGGGCTGCCAGAATTTTCTC |
| A20-Loxp primer         | CTATCTGTGGTGCAAAAGGCT   | GAATCGCCTACCTAGGAATCAG |
|                         | ACTCTCGG                | CTGTCCAG                |

### Supplementary Table 3. Oligonucleotide primers for ChIP and luciferase reporter assays

| Oligonucleotide primers | Forward primer (5’-3’) | Reverse primer (5’-3’) |
|-------------------------|-------------------------|------------------------|
| ChIP-Tnfaip3            | ACCTATTGCATTCCAGTTCCCA  | GAGAAACTCCTAGGTCCCAG   |
| ChIP-β-globin           | AAGGCTGATTCCGAGGACACAC  | CCCACAGGCAAGAGACAGCAG  |
| PGL4.17- Tnfaip3 (promoter)-WT | CTGAGCTGCCTAGCCTGAGCTTT | ACCGGATTCGCCAGCTTTCCGAAA |
|                         | ACTGGCCAGAGGAGGA        | GTCCCAAGTCCTG          |
| Primer1:                |                         |                        |
|                         | CTGAGCTGCCTAGCCTGAGCTTT | TTTCGGAAGCTGAGGAAAAAAC |
|                         | ACTGGCCAGAGGAGGA        | ATCCATGTTGAAAT         |
| Primer2:                |                         |                        |
|                         | ATTTCCACATGGAGTTTTTTTCCC | ACCGGATTCGCCAGCTTTCCGAAA |
|                         | CAGCTTCCGAAA            | GTCCCAAGTCCTG          |
| Prime1:                 |                         |                        |
|                         | Prime2:                 |                        |
| Gene   | Forward primer (5'-3')          | Reverse primer (5'-3')          |
|--------|--------------------------------|--------------------------------|
| 36b4   | AAGCGCGTCTCCTGGGACATGTGTCT    | CCGCAGGAGGGCAGCATGAGT          |
| Acc1   | ATGCGCGGAATGGTCTCTTCTT       | TGGGGACCTTTGATCTCTCAT          |
| Acta2  | GTCCGCAACGACTGGGAGTAA         | TCGGAAATTCTCCAGGGCAGTTAGGA    |
| Actin  | AGTGTGACGTTGACATTCGCTTCT     | GACGAGACTATATCTCTGGCA         |
| Arg1   | CTCAAGCGCAAGTGCCTTAGAG       | GGAGCTGTCATAGGGACATGCA        |
| Catalase| ACATCGTGG-GAGAGCGGAGAGTAG    | GCTTTGGAACGTTGACAGCATG       |
| Ccl2   | TTAAAAGAGTGACGGCAGACCGCA    | GCATTAGCTTTGAGTTAAGG          |
| Ccl3   | CATGACACTCTGCAACAAATGCTTCTCT| GACGACAGGCTGCTGGTATTTGAC     |
| Ccl7   | CAAAGTCTCCACATGTGGCTCTA     | GACACCTTCTGGATGAGGCGTCT       |
| CD36   | ATGGGCTGTGATCCGAGAAGCTG     | GTCTTCCCCAAATACGATCTCC       |
| CD45   | TCCAGGAGTGTATTACACGC         | TGTGGTATTGCTACGAGTGC         |
| Col1a1 | TGACTCGGAAAGAGCGGAGAGTAG    | GATGCTGTTTGTAGTTGCTA         |
| Col3a1 | GTGTCTCGGCCAGACATTTGGT      | CACCGTTTACCCCTGGACC         |
| Col4a1 | GCCAAGTGCTGATGGAGAGGAGGAGA | AGCAGGGGTGTGTTAGTTACG        |
| Col6a1 | GATGAGGAGTGAATGGGAGAGGA    | CAGCAAGGAAGGATGTCGCA         |
| Col6a2 | ATGAGGAGGACGTGGTGGA         | TGTGCTGTTTGTAGTTGCTA         |
| Col6a3 | CAGAACCATTTTCTCCTACT        | AGAGCTACATCTTTTCCCA          |
| Cpt-1a | CTCAAGTGGGGAGGGACGTCTTTCA   | GGCCTCTGGTGATCAGCAGCA         |
| Cxcl1  | TGGACCCAAAGGGCAGCTCTTTCA    | GTCGAGGAGCGTTGACCACC         |
| Fabp4  | CAGGCTTATAAGGGGATTGGG       | CCGCATTACAGTTAATGGTGC         |
| Fasn   | GGAGTTGCAGTGATAGCCGATG      | TGGTAGATCCATAGAGACCCAG        |
| Gpsm1  | CTTTCTTCCAGCGGTCTGCTG       | TACAGAATCGGGCGCTGTGA         |
| IL-10  | GCTATGCTGACGTGCTTCTACT      | CCTGCTGATCCTGCATGCAA         |
| IL-1β  | GCAACTGTTTCTGCAACTCACT      | ATCTTTTGGGGTGCTGCTA          |
| IL-6   | CGACGGGCCTCCCTACCTTTC       | TGGGGAGTGTTACGCTCCTGGA        |
| Lcad   | GCGAAATCTGGGCACTGTTAAGA    | TCAGGAGGTTGCGCACCAT         |
| Leptin | GACACCAAAAAACCTCTCCT        | CAGTGTCTGCTGCATCA           |
| Mcad   | GACATTTGGGAAACTGCTGATG      | TCAAGAGCTATAGCTACGGCTCTG     |
| Mmp2   | TAACCTGAGTGGCCTGGTT         | TCCAGTAAATACGACCTCTGGA       |
| Mmp9   | CGTCGTGATCCCGACTTACT       | AACACACAGGTTGTCCTCTC         |
| Mrcl   | CTCTGTTCCAGATGTTGAGCA      | TGGCACTCCTCAAAACATATATGGA    |
| Nos2   | GTTTCTGAGCCCCAAATACACAAGA | GTGGACGAGGTGTCGATGCAC       |
| Pdk4   | TTCACACCTTACCCACAGT          | AAAGGGCGGTTTCTTGGG          |
| Retnla | CCAATACGCTAATCATTCCCCTCC | ACCAGTACGCTGCTGCTCC         |
| S100A8 | GGAATACCATGCCCCCTCTA       | TGCGTGTCTTTGAGAGATC         |
| Scd1   | GCTGAGTGCAGTGGGAGAGA       | TCGCAGGATCAGGCTGTTAGG        |
| Srebp-1c| GAGGCGACATCAGCTTCTCC      | CAGCAGTGAGTCGCTGCTGCTG     |
| Tgfb1  | GTGGTGGGCAACATGTGGGACTCTA | TGGTGGCAGCAGCTGCGTA          |
| TNF-a  | GACGCTGAGACGTGGGCAAGAGAG   | ACCGGCTGGAGTTGCTGGA          |
| Ucp2   | GCTGAGTGGGTGTCGAGGATA      | ACTGCCCAGGGCAGAGTT          |
Gel source data

Supplemental figure 3a

Supplemental figure 9a

Supplemental figure 9b