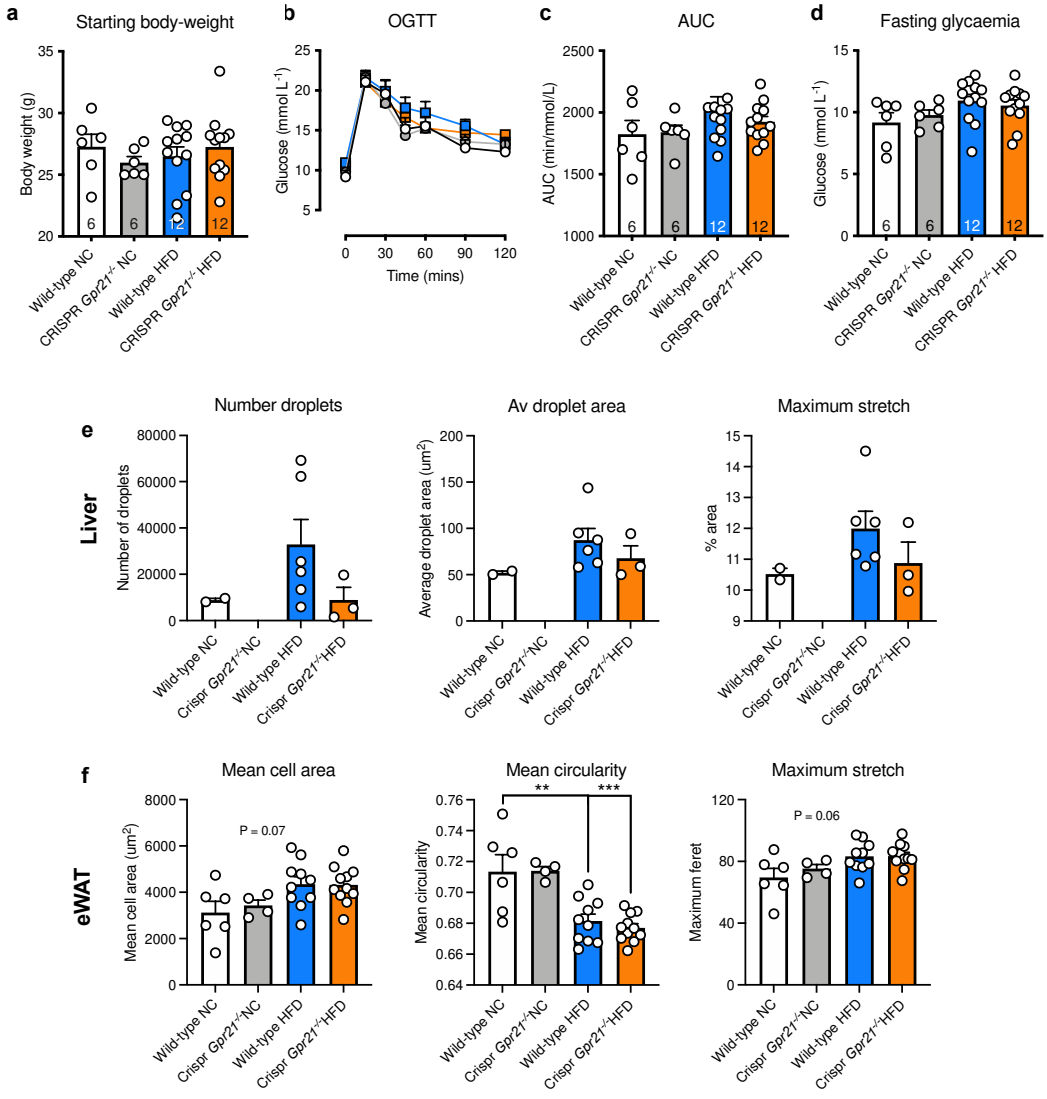
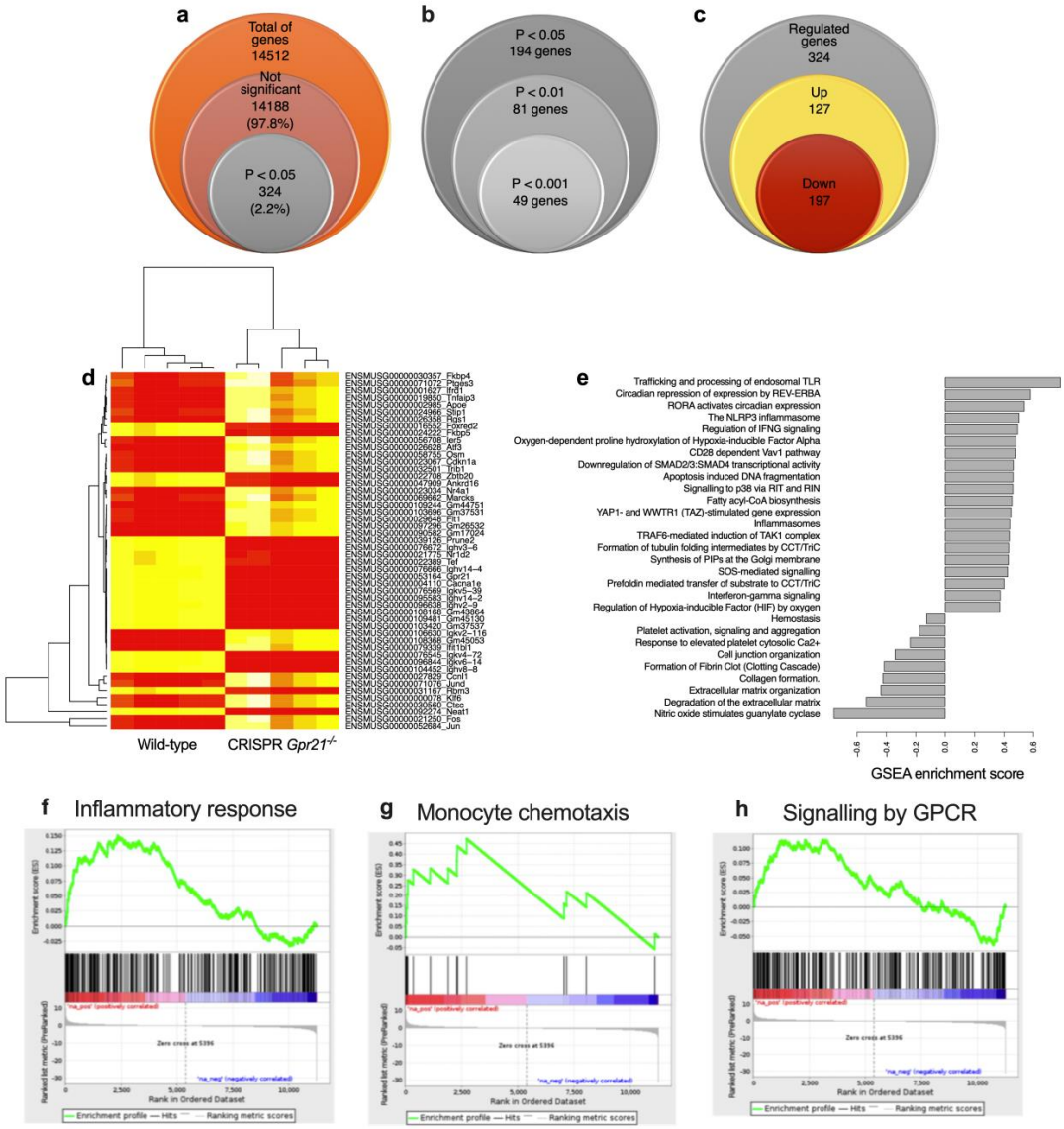
## ONLINE SUPPLEMENTAL MATERIAL



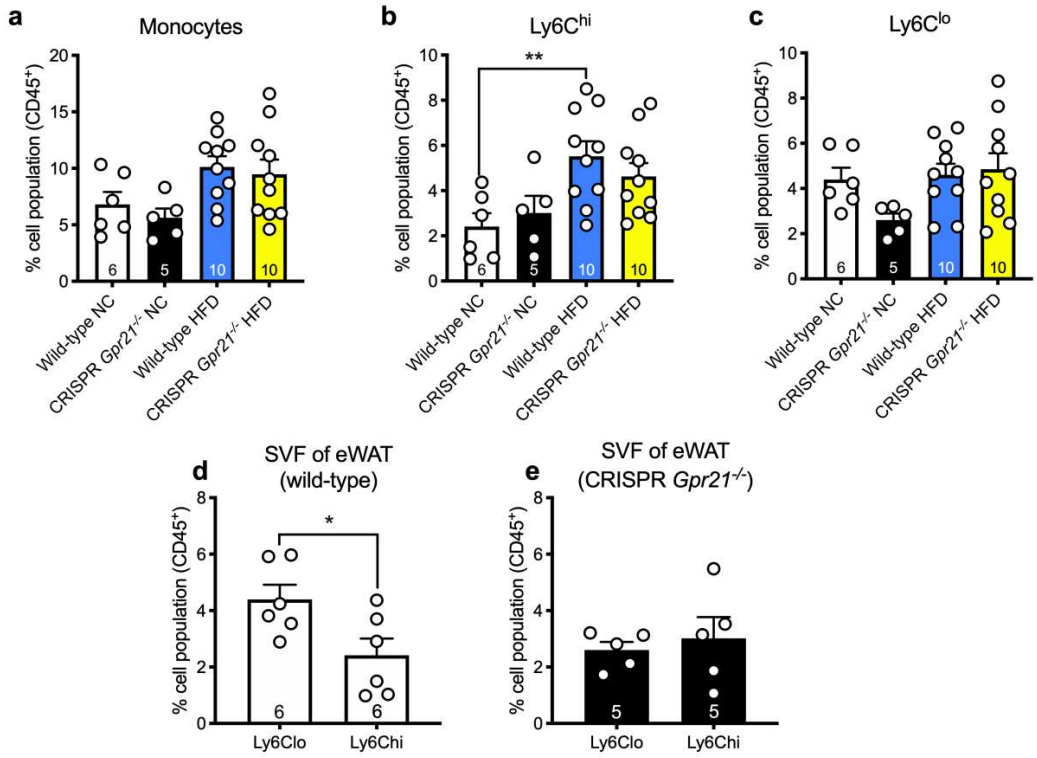

**Supp Fig. 1. Whole-body deletion of *Gpr21* reveals no significant changes in the metabolic phenotype after 6-weeks HFD.** Changes in (**a**) BW (week 6) (**b**) oral glucose tolerance test (OGTT; 3 g/kg lean), (**c**) OGTT area under the curve (AUC), (**d**) fasting glycaemia. Quantitative analysis of H&E staining of histological samples of the (**e**) liver and (**f**) eWAT from wild-type and CRISPR *Gpr21* KO mice fed normal chow or HFD. All data are presented as mean + SEM (n=6-12, unless otherwise stated).




**Supp Fig. 2. RNA-Seq analysis of CD11b+ BMMs identifies significant effects of *Gpr21* deletion on genes and pathways involved in inflammation and GPCR signalling**

Illustration of (**a**) number of genes showing false detection rate < 0.05, (**b**) deconvolution differentially expressed genes (DEGs) by significance, (**c**) separation of DEG rate by up and downregulated genes. Heatmap of the top 50 genes (**d**) with upregulated genes in yellow, and downregulated genes in red (full values shown in **Supp Table 6 & 7**). Gene set enrichment

analysis (GSEA, **e**) using gene sets from Reactome revealed upregulated pathways involved in *Gpr21* function, including (**f**) inflammatory response, (**g**) monocyte chemotaxis, and (**h**) GPCR signalling. All data are presented as mean + SEM, (n=5)




**Supp Fig. 3. Immune cell analysis of bone marrow transplant study.** Analysis of $CD45^+$ (**a**) monocytes, (**b**) $Ly6C^{hi}$, (**c**) $Ly6C^{lo}$, (**d**) Ly6C in wild-type, (**e**) Ly6C in CRISPR *Gpr21*$^{-/-}$, as measured by FACS analysis. All data are presented as mean + SEM. Statistical significance was determined by two-way ANOVA with Tukey's multiple comparison test compared to wild-type NC, with **P*<0.05 and ***P*<0.01 deemed significant.

**Supp Table 1.** Summary of the guide RNAs used to generate the *Gpr21*$^{-/-}$

| Region | Sequence |
|---|---|
| 5' end of exon | 5' TCCAAAGTAAGGGCCGTTTA 3' |
| 3' end of exon | 5' TTTAGATTAACATATCAGCT 3' |

**Supp Table 2.** Summary of the primers used to genotype the *Gpr21*$^{-/-}$ cohorts

| Gene | Forward | Reverse |
|---|---|---|
| *CRISPR 3'UTR* | *n/a* | *AGTCTGTGCACCAAAAAGCAA* |
| *CRISPR 5'UTR* | TCAGCATGCAGAATCACAGGTA | *TGGAATAGGGAAAGCCAACA* |

**Supp Table 3.** Summary of the primers used in this study

| Gene | Forward | Reverse |
|---|---|---|
| *Actb2* | CATTGCTGACAGGATGCAGAAGG | TGCTGGAAGGTGGACAGTGAGG |
| *Gapdh* | AGGTCGGTGTGAACGGATTTG | TGTAGACCATGTAGTTGAGGTCA |
| *Ccl2* | GCTACAAGAGGATCACCAGCAG | GTCTGGACCCATTCCTTCTTGG |
| *Ccr2* | GCTGTGTTTGCCTCTCTACCAG | CAAGTAGAGGCAGGATCAGGCT |
| *Cd68* | ATCCCCACCTGTCTCTCTCA | ACCGCCATGTAGTCCAGGTA |
| *Gpr21* | TGTGGCTTTTGTTTGGATTTC | GGGCAGAGGGAGGAAGATTA |
| *F4/80* | CGTGTTGTTGGTGGCACTGTGA | CCACATCAGTGTTCCAGGAGAC |
| *Il1β* | TGGACCTTCCAGGATGAGGACA | GTTCATCTCGGAGCCTGTAGTG |
| *Ltb4r* | TGCCCATTGTTCTACTGTCTG | GCGTTTCTGCATCCTTTTCAG |
| *Nlrp3* | CTCCAACCATTCTCTGACCAG | ACAGATTGAAGTAAGGCCGG |
| *Tnfα* | GGTGCCTATGTCTCAGCCTCTT | GCCATAGAACTGATGAGAGGGAG |
| *Rabgap1 5-6* | TCAGGATACATGTCTTTCGCTG | GTAAAGATGTCGCTGTCAGGAG |
| *Rabgap1 19-20* | CGCTCAGAAGAAAATGCAAA | CTGCTCCCTCATGGTATGGT |

**Supp Table 4.** Summary of the antibodies used in this study

| Antibody | Clone | Supplier | Catalogue number |
|---|---|---|---|
| PB-CD45 | 104 | Australian Biosearch, AUS | 109819 |
| Alexa 488-Ly-6C | HK1.4 | Thermo Fisher, AUS | 53-5932-82 |
| Alexa 647-Ccr2 | 475301 | In Vitro Technologies, AUS | FAB5538R |
| PE-Cx3cr1 | n/a | In Vitro Technologies, AUS | FAB5825P |

**Supp Table 5.** RNASeq full data set; attached as excel worksheet – available on request

**Supp Table 6.** Top significant genes upregulated in *Gpr21*$^{-/-}$ CD11b$^{+}$ BM monocytes

| Row names | logFC | logCPM | FDR |
|---|---|---|---|
| ENSMUSG00000097296 Gm26532 | 1.2 | 4.1 | 3.36E-18 |
| ENSMUSG00000090582 Gm17024 | 1.1 | 3.2 | 2.27E-11 |
| ENSMUSG00000001627 Ifrd1 | 0.5 | 6.8 | 1.50E-08 |
| ENSMUSG00000026628 Atf3 | 0.9 | 7.3 | 2.62E-07 |
| ENSMUSG00000019850 Tnfaip3 | 0.5 | 6.7 | 2.95E-06 |
| ENSMUSG00000026358 Rgs1 | 0.5 | 6.4 | 2.95E-06 |
| ENSMUSG00000023034 Nr4a1 | 0.5 | 5.9 | 2.95E-06 |
| ENSMUSG00000056708 Ier5 | 0.5 | 7.4 | 8.36E-06 |
| ENSMUSG00000058755 Osm | 0.5 | 5.3 | 1.22E-05 |
| ENSMUSG00000052684 Jun | 0.5 | 10.4 | 3.07E-05 |
| ENSMUSG00000106630 Igkv2-116 | 5.3 | -1.0 | 3.30E-05 |
| ENSMUSG00000027829 Ccnl1 | 0.5 | 8.5 | 3.45E-05 |
| ENSMUSG00000032501 Trib1 | 0.6 | 5.5 | 4.34E-05 |
| ENSMUSG00000021250 Fos | 0.5 | 11.1 | 6.50E-05 |

| | | | |
|---|---|---|---|
| ENSMUSG00000071076 Jund | 0.4 | 8.3 | 6.50E-05 |
| ENSMUSG00000002985 Apoe | 0.6 | 6.6 | 8.32E-05 |
| ENSMUSG00000030560 Ctsc | 0.4 | 9.2 | 1.16E-04 |
| ENSMUSG00000029648 Flt1 | 0.6 | 4.1 | 1.18E-04 |
| ENSMUSG00000108368 Gm45053 | 1.1 | 2.0 | 1.18E-04 |
| ENSMUSG00000069662 Marcks | 0.4 | 5.8 | 1.42E-04 |
| ENSMUSG00000000078 Klf6 | 0.4 | 9.6 | 1.73E-04 |
| ENSMUSG00000109244 Gm44751 | 0.8 | 4.0 | 3.36E-04 |
| ENSMUSG00000023067 Cdkn1a | 0.5 | 5.1 | 3.95E-04 |
| ENSMUSG00000103696 Gm37531 | 0.7 | 3.9 | 3.95E-04 |
| ENSMUSG00000024966 Stip1 | 0.4 | 6.6 | 3.95E-04 |
| ENSMUSG00000071072 Ptges3 | 0.3 | 6.8 | 4.57E-04 |
| ENSMUSG00000030357 Fkbp4 | 0.3 | 6.8 | 9.52E-04 |
| ENSMUSG00000079339 Ifit1bl1 | 0.8 | 2.0 | 1.01E-03 |

**Supp Table 7.** Top significant genes downregulated in *Gpr21*$^{-/-}$ CD11b$^{+}$ BM monocytes

| **Row names** | **logFC** | **logCPM** | **FDR** |
|---|---|---|---|
| ENSMUSG00000092274 Neat1 | -1.5 | 9.9 | 3.49E-78 |
| ENSMUSG00000053164 Gpr21 | -3.6 | 0.7 | 3.90E-28 |
| ENSMUSG00000024222 Fkbp5 | -0.7 | 7.0 | 3.57E-17 |
| ENSMUSG00000016552 Foxred2 | -0.4 | 6.3 | 4.21E-06 |
| ENSMUSG00000076666 Ighv14-4 | -2.6 | 1.0 | 1.75E-05 |
| ENSMUSG00000021775 Nr1d2 | -0.6 | 3.7 | 3.55E-05 |
| ENSMUSG00000096638 Ighv2-9 | -2.9 | 0.3 | 5.39E-05 |

| | | | |
|---|---|---|---|
| ENSMUSG00000047909 Ankrd16 | -0.4 | 5.4 | 5.39E-05 |
| ENSMUSG00000076545 Igkv4-72 | -2.3 | 2.0 | 1.16E-04 |
| ENSMUSG00000004110 Cacna1e | -1.1 | 1.0 | 1.42E-04 |
| ENSMUSG00000039126 Prune2 | -0.9 | 3.2 | 1.44E-04 |
| ENSMUSG00000095583 Ighv14-2 | -1.5 | 0.4 | 1.44E-04 |
| ENSMUSG00000103420 Gm37537 | -1.8 | -1.1 | 2.10E-04 |
| ENSMUSG00000096844 Igkv6-14 | -4.6 | 1.7 | 2.39E-04 |
| ENSMUSG00000076569 Igkv5-39 | -1.5 | 1.5 | 2.66E-04 |
| ENSMUSG00000109481 Gm45130 | -1.8 | -0.8 | 4.10E-04 |
| ENSMUSG00000022389 Tef | -0.5 | 4.2 | 4.57E-04 |
| ENSMUSG00000022708 Zbtb20 | -0.3 | 5.6 | 4.87E-04 |
| ENSMUSG00000031167 Rbm3 | -0.3 | 8.1 | 4.87E-04 |
| ENSMUSG00000076672 Ighv3-6 | -1.1 | 3.0 | 6.15E-04 |
| ENSMUSG00000108168 Gm43864 | -1.2 | 0.3 | 7.92E-04 |
| ENSMUSG00000104452 Ighv8-8 | -1.5 | 2.3 | 8.34E-04 |