

Supplementary

Table S1 Primers used in this study

Primer set name	Real-time quantitative PCR primer (5' to 3')
<i>SAA1</i>	F: CTGCCTGCCAAATACTGAGAGTC R: CCACTTCCAAGTTCTGTTTATTAC
<i>SAA2</i>	F: TGTGTATCCCACAAGGTTTCAGA R: TTATTACCCTCTCCTCCTCAAGCA
<i>IGF2</i>	F: GTGCTGCATCGCTGCTTAC R: ACGTCCCTCTCGGACTTGG
<i>CELA1</i>	F: GTGGACACAGTACCGAGGAC R: CCAGTTGCTTCGGATGAGGG
<i>HP</i>	F: GCTGACCACGGCCAAAAAC R: CCCACGTAGAGCGTTAGGG
<i>SHH</i>	F: AAAGCTGACCCCTTTAGCCTA R: TTCGGAGTTTCTTGTGATCTTCC
<i>ORM1</i>	F: ACGACACAATAGAGCTTCGGG R: CAGCACTATAAGGTGGGCAA
<i>GAPDH</i>	F: AGGTCGGTGTGAACGGATTTG R: TGTAGACCATGTAGTTGAGGTCA

Table S2 Immune infiltration analysis of all genes in HFD mice compared with ND mice

Cell type	ND	ND	ND	ND	HFD	HFD	HFD	HFD	P value
MCs	0.0337	0.0757	0.2198	0.2393	0.2101	0.0938	0.1612	0.2512	0.5697
Neutrophils	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0
Eosinophils	0.0000	0.0000	0.0000	0.0000	0.0000	0.0083	0.0000	0.0042	0.1666
Memory B cells	0.0000	0.0000	0.0148	0.2683	0.0000	0.0000	0.0271	0.0000	0.3714
Naïve B cells	0.0854	0.1737	0.0000	0.0000	0.0000	0.0084	0.0000	0.2519	0.9972
Plasma cells	1.0000	0.2613	0.7363	0.5023	0.5505	0.2981	0.7662	0.1716	0.4206
Active CD8 T cells	0.0342	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.3559
Naïve CD8 T cells	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0
Memory CD8 T cells	0.1678	0.3839	0.1837	0.2300	0.1578	0.4870	0.2697	0.1542	0.7891
M0 macrophages	0.0000	0.0818	0.1124	0.0000	0.1423	0.0000	0.1541	0.3499	0.1944
M1 macrophages	0.0000	0.1343	0.0000	0.0000	0.0580	0.0872	0.0057	0.0212	0.8131
M2 macrophages	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0
Tregs	0.0000	0.0099	0.0523	0.0000	0.0000	0.0000	0.1539	0.0636	0.3521
Memory CD4 T cells	0.2279	0.2062	0.1594	0.1341	0.2753	0.5320	0.1509	0.1873	0.2827
Naïve CD4 T cells	0.2691	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.3559
Follicular CD4 T cells	0.0000	0.0000	0.0099	0.0000	0.0000	0.0000	0.0000	0.0634	0.4363
Th1 cells	0.0000	0.0114	0.0000	0.0000	0.0207	0.0147	0.0000	0.0744	0.1864
Th17 cells	0.1009	0.2026	0.1799	0.1981	0.3557	0.1106	0.0012	0.0608	0.6544
Th2 cells	0.0499	0.0000	0.0000	0.0000	0.0000	0.0795	0.0000	0.0000	0.7629
Monocytes	0.0982	0.2597	0.2456	0.3158	0.1749	0.1271	0.1182	0.3238	0.5343
Gamma delta T cells	0.0000	0.0367	0.0155	0.0038	0.0000	0.0213	0.0000	0.0000	0.4102
Resting NK cells	0.0039	0.0465	0.0000	0.0000	0.0000	0.0553	0.0000	0.0000	0.9475
Activated NK cells	0.0000	0.0000	0.0000	0.0530	0.0000	0.0000	0.0000	0.0122	0.4821
Activated DCs	0.0485	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.3559
Immature DCs	0.0385	0.2745	0.2284	0.2135	0.2127	0.2347	0.3499	0.1684	0.4459

MC, mast cell; Tregs, regulatory T cells; Th, T helper; NK, natural killer; DC, dendritic cell; HFD, high-fat diet; ND, normal diet.

Table S3 Immune infiltration analysis of all genes in ob/ob mice compared with WT mice

Cell type	WT	WT	WT	ob/ob	ob/ob	ob/ob	ob/ob	P value
MCs	0.4233	0.4160	0.3117	0.5489	0.6184	0.8125	0.6632	0.0124
Neutrophils	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Eosinophils	0.0000	0.0344	0.0000	0.0216	0.0801	0.0000	0.0000	0.5911
Memory B cells	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Naïve B cells	0.1183	0.5379	0.2516	0.6234	0.0000	0.5477	0.8849	0.4244
Plasma cells	0.3843	0.2109	0.5351	0.5228	0.7269	0.2887	0.3019	0.5924
Active CD8 T cells	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Naïve CD8 T cells	0.0000	0.0273	0.0000	0.2495	0.1104	0.0000	0.0000	0.3031
Memory CD8 T cells	0.2932	0.1008	0.3383	0.0792	0.0929	0.0000	0.3948	0.4305
M0 macrophages	0.6006	0.7763	0.3970	0.4504	0.2477	0.4248	0.3615	0.0927
M1 macrophages	0.0661	0.0000	0.0312	0.0016	0.2294	0.2819	0.0000	0.3330
M2 macrophages	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Tregs	0.6394	0.1225	0.4086	0.3344	0.4317	0.3027	0.0465	0.5134
Memory CD4 T cells	0.7678	0.7946	1.0000	0.5379	0.6677	0.9708	0.5299	0.2499
Naïve CD4 T cells	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Follicular CD4 T cells	0.0000	0.0000	0.0000	0.2422	0.0000	0.0000	0.1944	0.2076
Th1 cells	0.0000	0.0000	0.0807	0.0662	0.0000	0.0000	0.0000	0.7424
Th17 cells	0.8241	0.5822	0.6755	0.3869	0.6848	0.4383	0.3206	0.0864
Th2 cells	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Monocytes	0.4048	0.8761	0.5084	0.2749	0.7307	0.4099	0.7460	0.7723
Gamma delta T cells	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Resting NK cells	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Activated NK cells	0.0000	0.0672	0.0050	0.0000	0.0000	0.0000	0.0000	0.2403
Activated DCs	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Immature DCs	0.4706	0.4462	0.4493	0.6526	0.3719	0.5153	0.5488	0.3770

MC, mast cell; Treg, regulatory T cells; Th, T helper; NK, natural killer; DC, dendritic cell; WT, wild-type.

Table S4 Immune infiltration analysis of 190 genes with consistent trends in HFD mice compared with ND mice

Cell type	ND	ND	ND	ND	HFD	HFD	HFD	HFD	P value
MCs	0.0572	0.1272	0.1642	0.0584	0.5552	0.8033	0.3992	0.9610	0.0041
Neutrophils	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0
Eosinophils	0.0076	0.0000	0.0000	0.0077	0.0000	0.0000	0.0000	0.0000	0.134
Memory B cells	0.0521	0.0438	0.0437	0.0520	0.0000	0.0000	0.0000	0.0000	0.0001
Naïve B cells	0.0606	0.0537	0.0532	0.0604	0.0000	0.0000	0.0088	0.0000	0.0001
Plasma cells	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.356
Active CD8 T cells	0.2544	0.2444	0.2416	0.2537	0.1524	0.0356	0.2026	0.0000	0.0198
Naïve CD8 T cells	0.3662	0.3542	0.3495	0.3652	0.2302	0.0779	0.3023	0.0175	0.0224
Memory CD8 T cells	0.2106	0.2010	0.1991	0.2102	0.1278	0.0247	0.1685	0.0000	0.0215
M0 macrophages	0.0016	0.0000	0.0000	0.0016	0.0000	0.0000	0.0000	0.0000	0.134
M1 macrophages	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0
M2 macrophages	0.0013	0.0000	0.0000	0.0013	0.0000	0.0000	0.0000	0.0000	0.134
Tregs	0.2175	0.2081	0.2061	0.2170	0.1323	0.0274	0.1743	0.0000	0.0214
Memory CD4 T cells	0.0990	0.0912	0.0911	0.0990	0.0502	0.0000	0.0686	0.0000	0.0102
Naïve CD4 T cells	0.4735	0.4579	0.4526	0.4723	0.3174	0.1195	0.4068	0.0444	0.0286
Follicular CD4 T cells	0.0740	0.0663	0.0677	0.0743	0.0528	0.0000	0.0619	0.0000	0.0466
Th1 cells	0.1926	0.1869	0.1842	0.1919	0.1167	0.0390	0.1554	0.0062	0.019
Th17 cells	0.3288	0.3107	0.3109	0.3289	0.2415	0.0542	0.2933	0.0055	0.0507
Th2 cells	0.1058	0.0848	0.0929	0.1079	0.1283	0.0000	0.1238	0.0000	0.3801
Monocytes	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0
Gamma delta T cells	0.0118	0.0065	0.0076	0.0120	0.0000	0.0000	0.0000	0.0000	0.0005
Resting NK cells	0.0300	0.0261	0.0296	0.0305	0.0394	0.0011	0.0342	0.0034	0.3819
Activated NK cells	0.0109	0.0080	0.0089	0.0110	0.0000	0.0000	0.0000	0.0000	0.0001
Activated DCs	0.0000	0.0848	0.0523	0.0000	0.3858	0.4433	0.1558	0.5174	0.0055
Immature DCs	0.0000	0.0000	0.0000	0.0000	0.0256	0.9292	0.0000	1.0000	0.1261

MC, mast cell; Tregs, regulatory T cells; Th, T helper; NK, natural killer; DC, dendritic cell; HFD, high-fat diet; ND, normal diet.

Table S5 Immune infiltration analysis of 190 genes with consistent trends in ob/ob mice compared with WT mice

Cell type	WT	WT	WT	ob/ob	ob/ob	ob/ob	ob/ob	P value
MCs	0.1094	0.1516	0.1548	0.5689	0.5639	0.6318	0.7449	0.0002
Neutrophils	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Eosinophils	0.0000	0.0000	0.0047	0.0000	0.0000	0.0000	0.0000	0.2856
Memory B cells	0.0344	0.0333	0.0386	0.0000	0.0000	0.0000	0.0000	0.0001
Naïve B cells	0.0422	0.0408	0.0449	0.0000	0.0000	0.0000	0.0000	0.0001
Plasma cells	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0009	0.4366
Active CD8 T cells	0.1929	0.1872	0.1883	0.1138	0.0993	0.0381	0.0000	0.0101
Naïve CD8 T cells	0.2798	0.2713	0.2710	0.1739	0.1522	0.0707	0.0000	0.0140
Memory CD8 T cells	0.1586	0.1542	0.1559	0.0980	0.0859	0.0282	0.0000	0.0135
M0 macrophages	0.0000	0.0000	0.0005	0.0000	0.0000	0.0000	0.0000	0.2856
M1 macrophages	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
M2 macrophages	0.0000	0.0000	0.0009	0.0000	0.0000	0.0000	0.0000	0.2856
Tregs	0.1642	0.1596	0.1610	0.1008	0.0886	0.0305	0.0000	0.0130
Memory CD4 T cells	0.0717	0.0702	0.0733	0.0381	0.0332	0.0000	0.0000	0.0071
Naïve CD4 T cells	0.3615	0.3511	0.3505	0.2491	0.2210	0.1017	0.0000	0.0265
Follicular CD4 T cells	0.0516	0.0514	0.0551	0.0495	0.0467	0.0000	0.0000	0.1425
Th1 cells	0.1477	0.1431	0.1426	0.0849	0.0738	0.0366	0.0036	0.0076
Th17 cells	0.2442	0.2393	0.2440	0.2077	0.1906	0.0514	0.0000	0.0850
Th2 cells	0.0643	0.0677	0.0796	0.1487	0.1467	0.0000	0.0000	0.9507
Monocytes	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0028	0.4366
Gamma delta T cells	0.0047	0.0052	0.0089	0.0000	0.0000	0.0000	0.0000	0.0024
Resting NK cells	0.0203	0.0228	0.0271	0.0432	0.0427	0.0075	0.0000	0.9980
Activated NK cells	0.0060	0.0066	0.0089	0.0000	0.0000	0.0000	0.0000	0.0002
Activated DCs	0.0572	0.0555	0.0000	0.1340	0.2658	0.4253	0.2584	0.0233
Immature DCs	0.0000	0.0000	0.0000	0.0000	0.0000	0.5886	1.0000	0.2277

MC, mast cell; Tregs, regulatory T cells; Th, T helper; NK, natural killer; DC, dendritic cell; WT, wild-type.

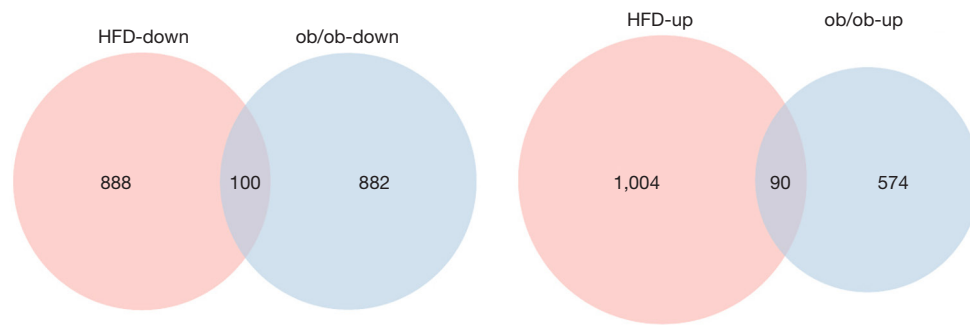


Figure S1 The intersections between DEGs in all groups of mice. (A) The intersections between up-regulated DEGs in all groups. (B) The intersections between down-regulated DEGs in all groups. HFD, high-fat diet; DEGs, differentially expressed genes.

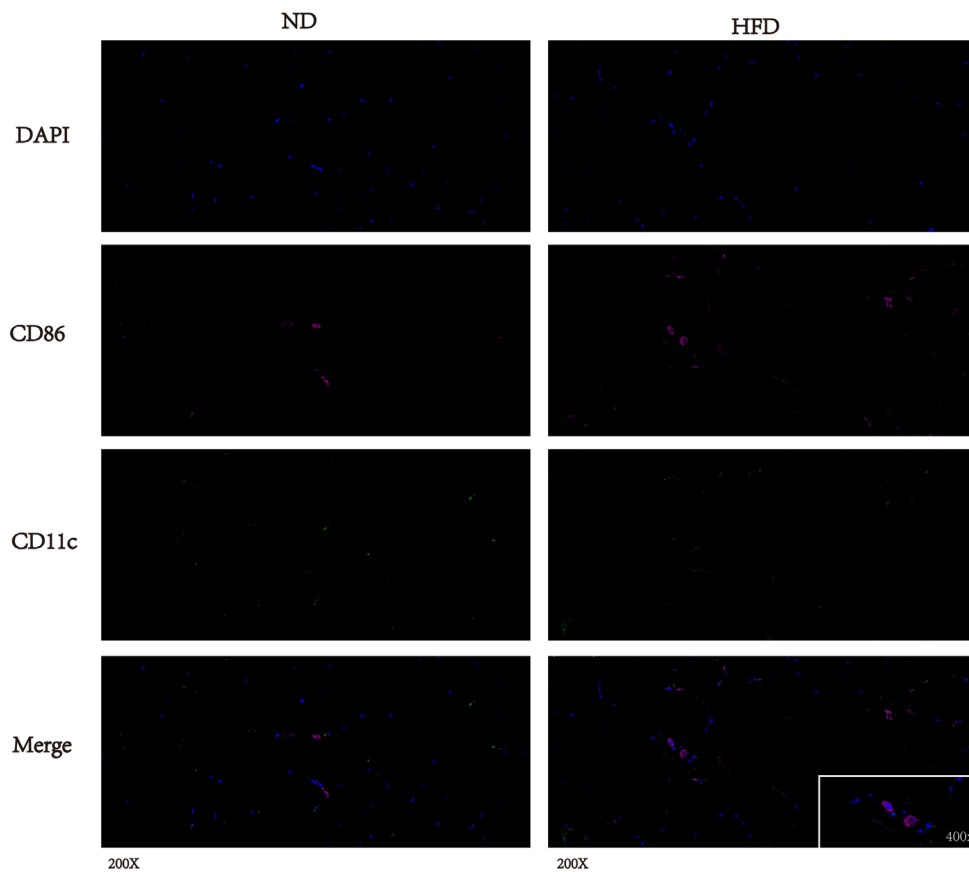


Figure S2 Immunofluorescence staining of active dendritic cells. The co-localization of CD86 (pink) and CD11c (green) is the marker of active dendritic cells. No active dendritic cells were found in the skeletal muscle sections of ND mice or HFD mice. HFD, high-fat diet; ND, normal diet.

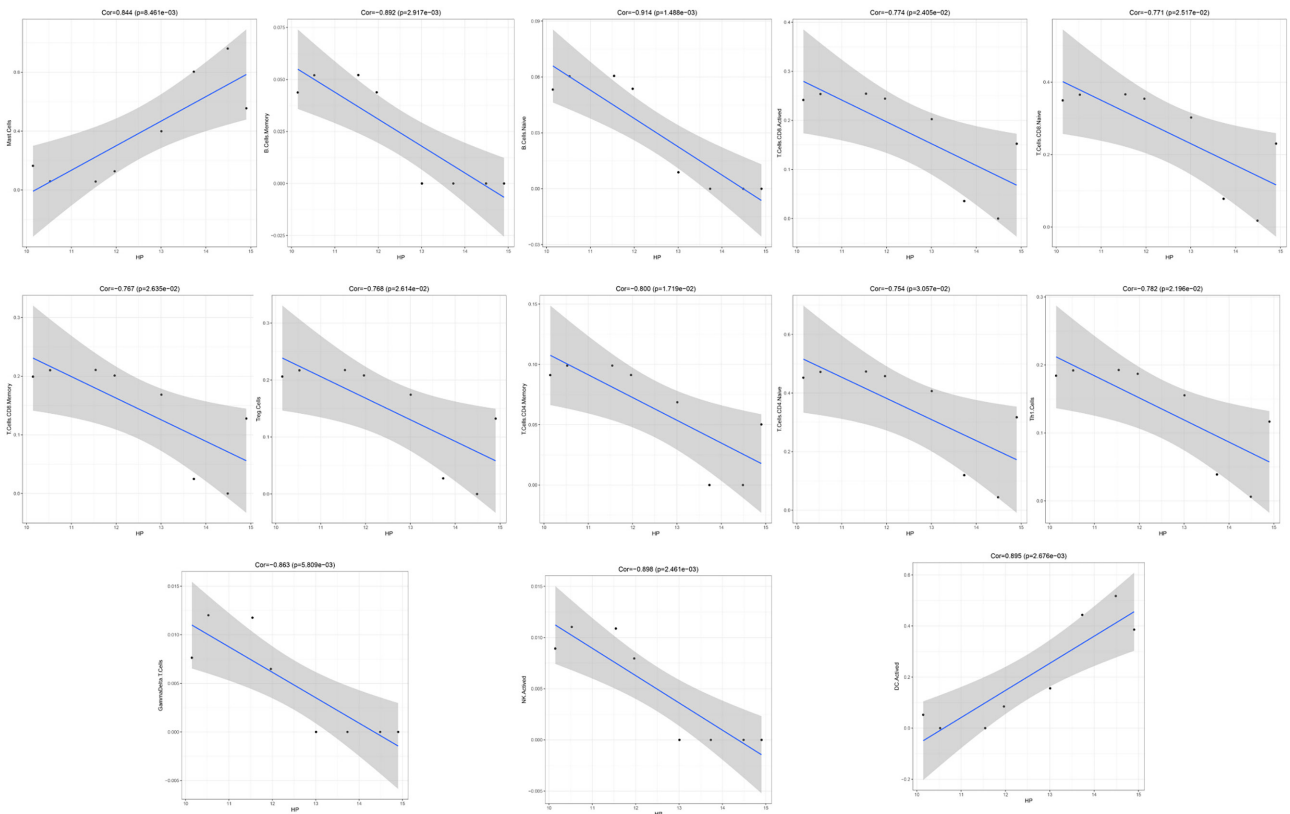


Figure S3 Correlation analysis of the *HP* gene and the proportion of infiltrating immune cells. The abscissa indicates the value of gene expression and the ordinate indicates the proportion of immune cells. Cor, correlation; DC, dendritic cell; NK, natural killer; Tregs, regulatory T cells; Th, T helper.

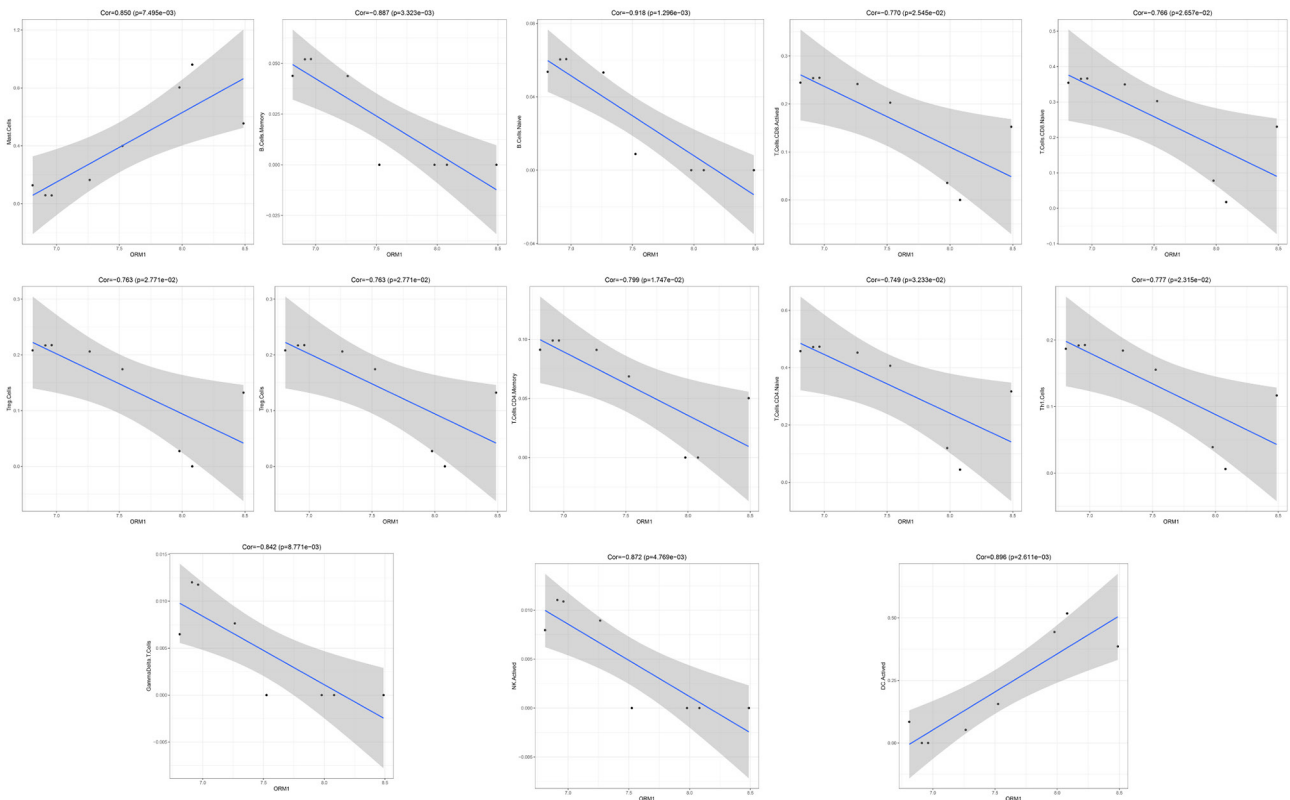


Figure S4 Correlation analysis of the *ORM1* gene and the proportion of infiltrating immune cells. The abscissa indicates the value of gene expression and the ordinate indicates the proportion of immune cells. Cor, correlation; DC, dendritic cell; NK, natural killer; Tregs, regulatory T cells; Th, T helper.

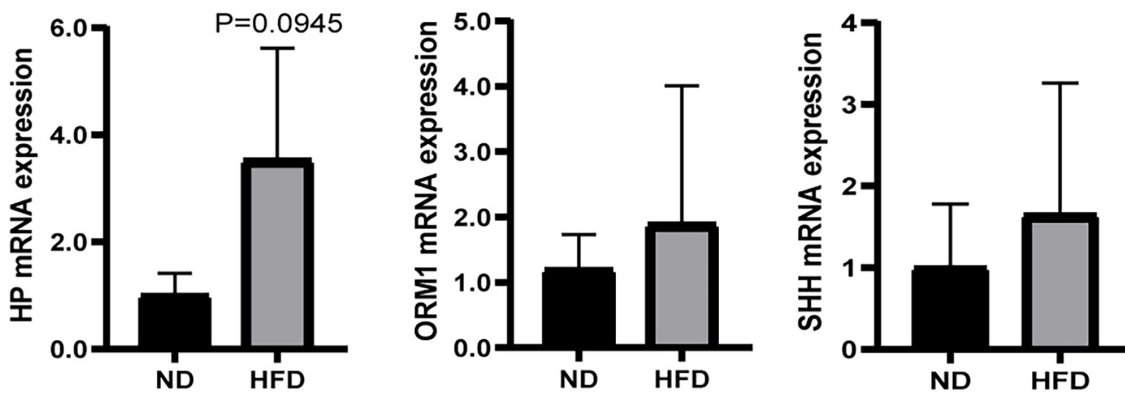


Figure S5 Determination of *HP*, *ORM1*, and *SHH* gene expression by quantitative reverse transcription PCR. The results show that the expression levels of the *HP*, *ORM1* and *SHH* genes in HFD mice were higher than those in ND mice, but there was no statistical difference between the two groups. HFD, high-fat diet; ND, normal diet.