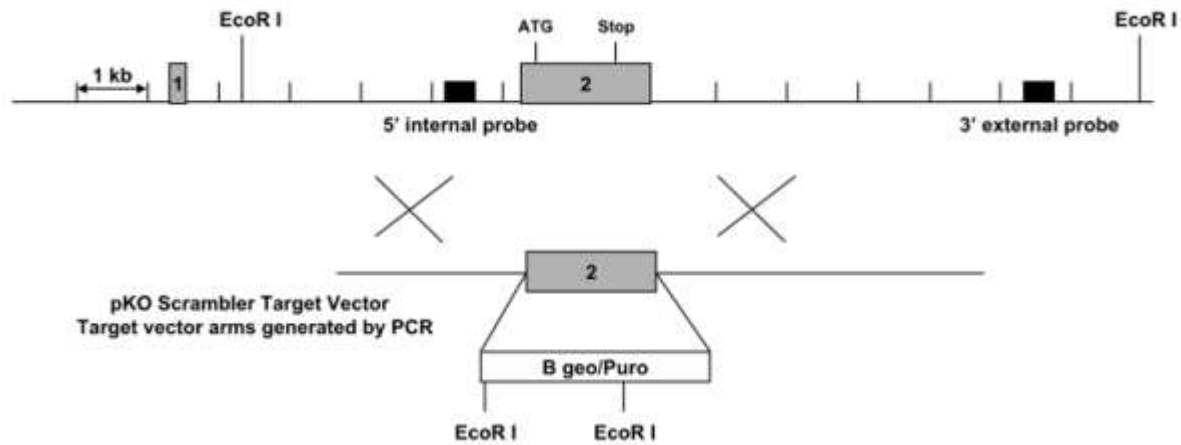
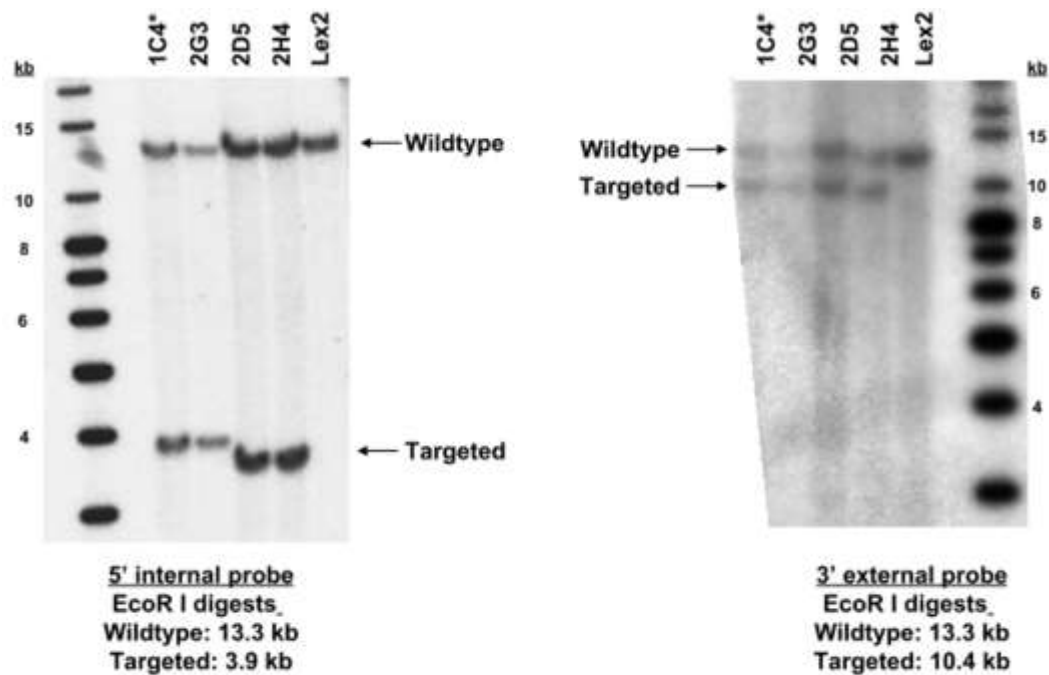
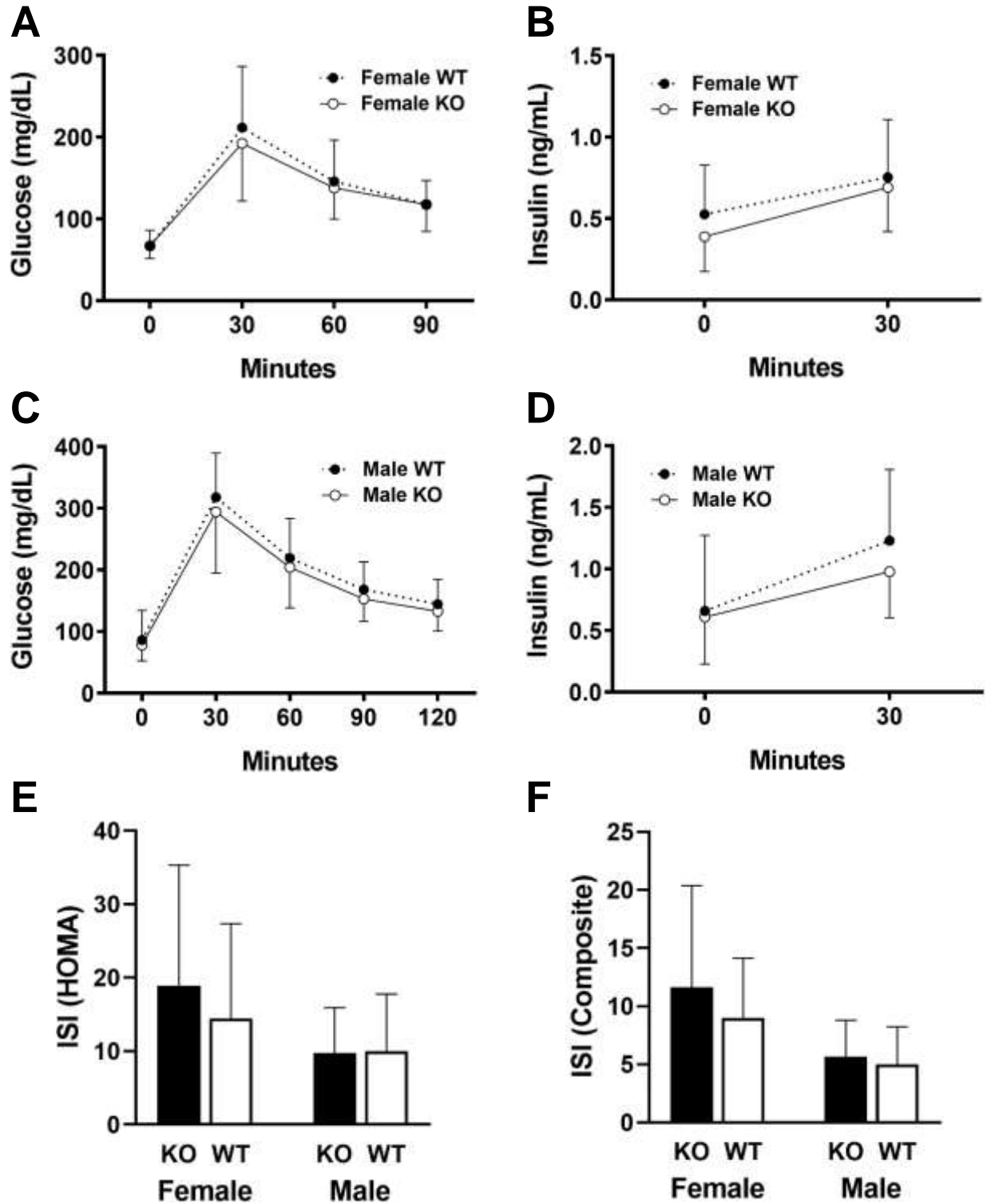


Supplementary Materials

A**B**

* Clone achieving germline transmission

Supplementary Figure 1. Targeting strategy and Southern blot confirmation of the *Gpr75* gene mutation. **(A)** Homologous recombination between the pKO Scrambler targeting vector and the *Gpr75* gene resulted in the replacement of exon 2 with the Betagalactosidase-Neomycin/Puromycin (B geo/puro) selection cassette. Restriction enzyme recognition sites are indicated by EcoR I. ATG represents the translation start site. **(B)** Southern hybridization indicating *Gpr75* gene targeting in the embryonic stem cell clones. Clones 1C4, 2G3, 2D5, and 2H4 were analyzed by Southern blot analysis using EcoR I digested genomic DNA with a 5' internal probe and a 3' external probe. Two targeted clones were selected for blastocyst injections. An asterisk (*) indicates the clone that was transmitted through the germ line. Band sizes are indicated in kilobases (kb) using a labeled size marker. Lex2 represents untransfected embryonic stem cell DNA.



Supplementary Figure 2. OGTTs performed in 9-week-old *Gpr75* KO and WT mice fed HFD from weaning. **(A)** Glucose excursions, and **(B)** 0- and 30-minute insulin levels, measured in female *Gpr75* KO (n=16) and WT (n=20) mice. **(C)** Glucose excursions, and **(D)** 0- and 30-minute insulin levels, measured in male *Gpr75* KO (n=21) and WT (n=19) mice. **(E)** HOMA and **(F)** composite insulin sensitivity index (ISI) values for female and male mice.

Supplementary Table 1. Body composition of adult *Gpr75* mouse cohorts

Mouse cohort and diet	Age (weeks)	Sex and Genotype	N	Body Weight (g)	% Body Fat	Body Fat (g)	LBM (g)
Cohort 1, chow	14	Male WT	2	27 ± 1	19 ± 3	5.1 ± 1.0	21 ± 1
		Male KO	4	28 ± 1	15 ± 1	4.3 ± 0.3	23 ± 1
		Female WT	2	21 ± 1	23 ± 1	4.8 ± 0.2	16 ± 1
		Female KO	4	21 ± 3	19 ± 4	4.0 ± 1.1	16 ± 2
Cohort 2, HFD	11	Male WT	8	34 ± 4	23 ± 7	7.9 ± 3.1	26 ± 2
		Male KO	8	26 ± 3 ^{***}	12 ± 4 ^{***}	3.0 ± 0.9 ^{***†}	23 ± 3 [*]
Cohort 3, chow	23-24	Male WT	10	41 ± 4	23 ± 4	9.5 ± 1.9	31 ± 3
		Male KO	4	34 ± 1 ^{**}	13 ± 3 ^{***}	4.2 ± 1.1 ^{***}	30 ± 1
Cohort 4, chow	14-16	Male WT	3	30 ± 2	13 ± 4	4.1 ± 1.3	26 ± 1
		Male KO	5	32 ± 4	9 ± 2	3.0 ± 0.9	28 ± 3
Cohort 5, chow	16-18	Female WT	14	24 ± 2	20 ± 4	4.8 ± 1.3	19 ± 2
		Female KO	16	20 ± 2 ^{***}	14 ± 3 ^{***}	3.0 ± 0.9 ^{***}	18 ± 1
Cohort 6, HFD	23-24	Male WT	14	47 ± 6	35 ± 7	16.5 ± 4.7	30 ± 3
		Male KO	22	36 ± 5 ^{***}	22 ± 7 ^{***}	8.2 ± 3.5 ^{***}	28 ± 2 ^{**}
		Female WT	11	33 ± 3	35 ± 6	11.4 ± 2.6	21 ± 2
		Female KO	18	25 ± 3 ^{***}	16 ± 6 ^{***}	4.1 ± 2.0 ^{***}	20 ± 2
Cohort 7, HFD	15	Male WT	10	40 ± 6	31 ± 7	13.0 ± 4.4	27 ± 2
		Male KO	12	33 ± 4 ^{**}	19 ± 5 ^{***}	6.2 ± 2.2 ^{***†}	27 ± 3
		Female WT	11	28 ± 4	32 ± 7	9.1 ± 3.2	18 ± 1
		Female KO	7	23 ± 2 ^{**†}	22 ± 4 ^{**}	4.9 ± 1.0 ^{**†}	18 ± 1

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Supplementary Table 1, continued

Mouse cohort and diet	Age (weeks)	Sex and Genotype	N	Body Weight (g)	% Body Fat	Body Fat (g)	LBM (g)
Cohort 8, HFD	15	Male WT	9	33 ± 3	29 ± 5	9.8 ± 2.5	23 ± 1
		Male KO	9	26 ± 3 ^{***}	16 ± 5 ^{***}	4.3 ± 1.8 ^{***}	22 ± 2
		Female WT	9	21 ± 3	25 ± 4	5.4 ± 1.6	16 ± 1
		Female KO	9	20 ± 2	16 ± 3 ^{***}	3.4 ± 0.8 ^{**}	17 ± 2

Notes: KO mice different from WT mice, * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; † Mann-Whitney test

Abbreviations: N, number of mice; g, grams; LBM, lean body mass; WT, wild type; KO, knockout; HFD, high fat diet

Supplementary Table 2. Activity of *Gpr75* KO and WT mice in circadian rhythm chambers

Day	Activity counts, dark cycle				Activity counts, light cycle			
	F KO (9)	F WT (9)	M KO (9)	M WT (9)	F KO (9)	F WT (9)	M KO (9)	M WT (9)
1	1289 ± 536	1396 ± 562	1700 ± 752	1929 ± 947	606 ± 193	749 ± 385	838 ± 368	998 ± 350
2	1474 ± 543	1798 ± 796	2083 ± 949	2148 ± 680	558 ± 174	770 ± 411	874 ± 424	940 ± 344
3	1543 ± 551	1663 ± 782	2278 ± 826	2097 ± 566	723 ± 305	801 ± 413	906 ± 394	915 ± 405
4	1934 ± 655	1633 ± 719	2425 ± 1061	2343 ± 600	499 ± 289	547 ± 300	673 ± 361	697 ± 308
5	1816 ± 595	1684 ± 787	2755 ± 1168	2456 ± 513	680 ± 361	563 ± 345	798 ± 400	793 ± 298
6	1821 ± 534	1934 ± 775	2726 ± 1174	2575 ± 657				
7	1159 ± 727	1157 ± 581	1684 ± 847	1532 ± 1249	459 ± 375	519 ± 167	590 ± 397	629 ± 432
8	1168 ± 691	901 ± 375	1311 ± 521	1273 ± 1164	488 ± 429	474 ± 193	429 ± 295	524 ± 231
9	1232 ± 653	1024 ± 526	1246 ± 613	1140 ± 877	449 ± 322	432 ± 179	430 ± 213	503 ± 265
10	1116 ± 574	969 ± 439	1188 ± 553	1143 ± 828	473 ± 270	466 ± 221	447 ± 242	481 ± 177
11	1212 ± 701	1014 ± 431	1276 ± 729	1230 ± 890	289 ± 185	324 ± 161	280 ± 146	306 ± 112
12	1213 ± 620	1092 ± 512	1373 ± 780	1364 ± 818	282 ± 175	312 ± 147	269 ± 143	320 ± 160
13	1117 ± 608	1188 ± 647	1399 ± 659	1335 ± 695				
15	793 ± 372	743 ± 359	1588 ± 996	1386 ± 872				
16	844 ± 471	808 ± 364	1794 ± 1026	1541 ± 1024	316 ± 42	302 ± 114	498 ± 428	658 ± 466
17	844 ± 434	787 ± 455	1861 ± 1134	1478 ± 904	541 ± 123*	412 ± 115	805 ± 482	823 ± 426
18	757 ± 465	702 ± 358	1880 ± 1128	1537 ± 1312	360 ± 88*	251 ± 101	514 ± 436	524 ± 348
19	917 ± 482	713 ± 302	2033 ± 1259	1524 ± 914	308 ± 68	282 ± 150	476 ± 445	579 ± 280
24	836 ± 473	694 ± 312	1211 ± 746	1404 ± 656	694 ± 421	479 ± 204	944 ± 583	975 ± 343
25	1009 ± 519	851 ± 307	1588 ± 1014	1790 ± 980	486 ± 243	384 ± 130	614 ± 379	772 ± 319

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Supplementary Table 2, continued

Day	Activity counts, dark cycle				Activity counts, light cycle			
	F KO (9)	F WT (9)	M KO (9)	M WT (9)	F KO (9)	F WT (9)	M KO (9)	M WT (9)
26	1098 ± 442	850 ± 385	2119 ± 1567	2051 ± 1243	601 ± 390	473 ± 192	717 ± 413	916 ± 328
27	1040 ± 477	954 ± 397	2251 ± 2046	2001 ± 1252	412 ± 219	332 ± 295	526 ± 377	588 ± 269

Notes: Female KO mice different from female WT mice, * $P < 0.05$

Abbreviations: F, female; M, male; KO, knockout; WT, wild type; (N), number of mice