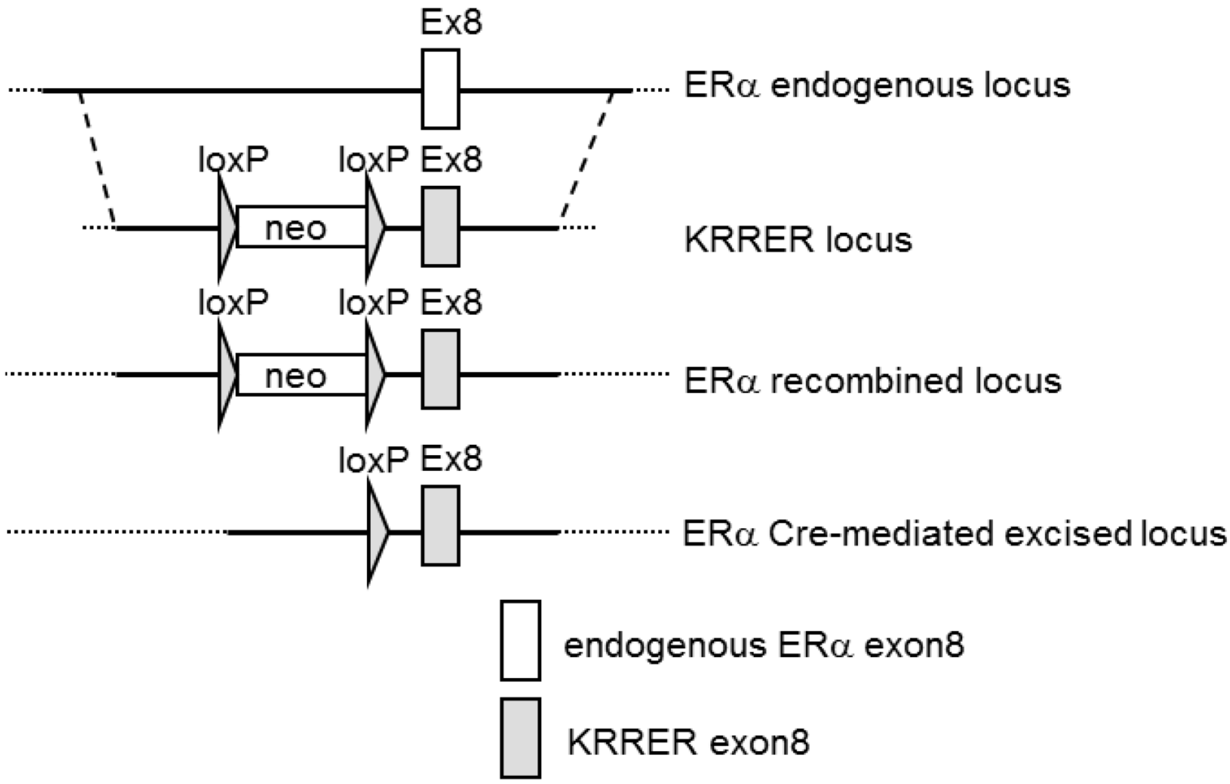
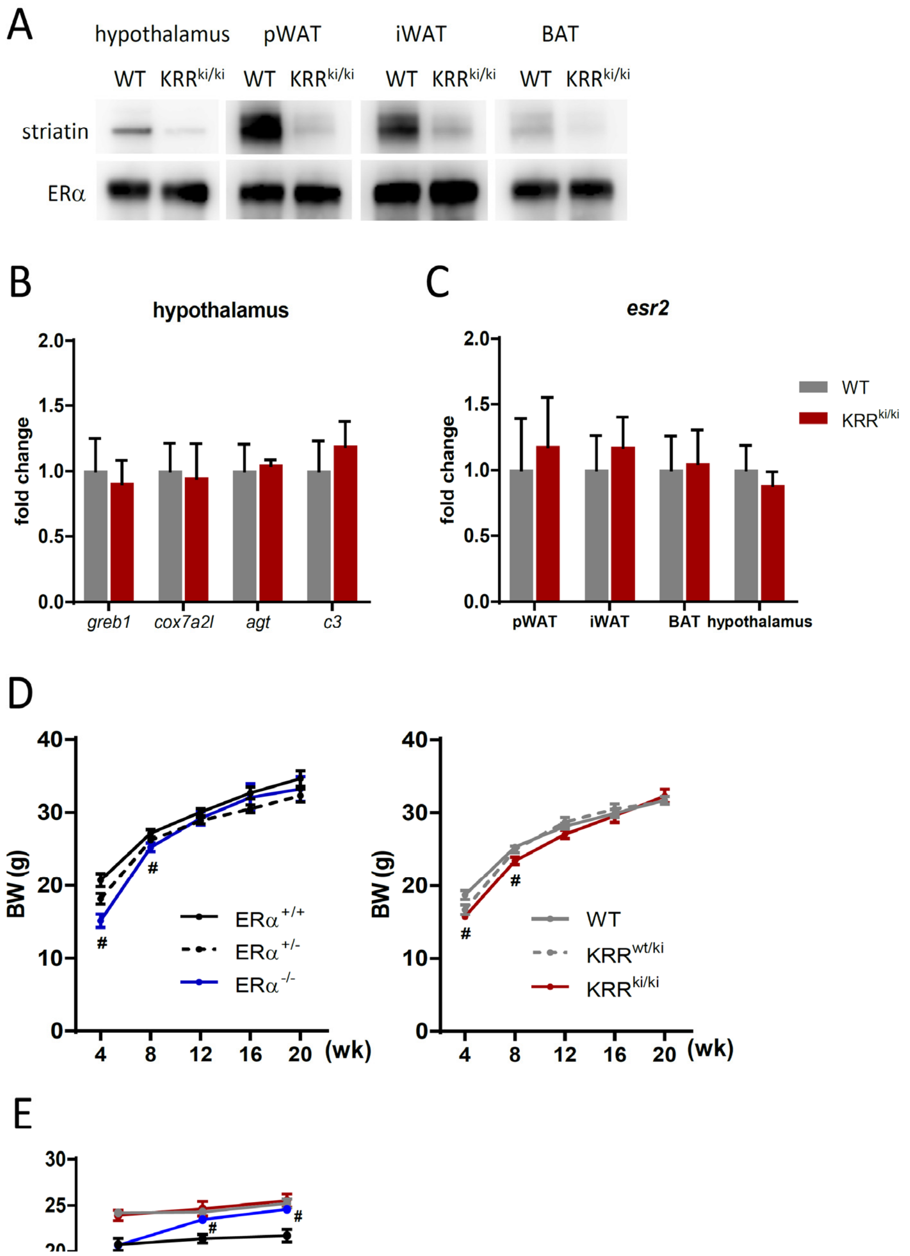
SUPPLEMENTARY DATA

Supplementary Figure 1. Generation of KRRKI mice. A targeted strategy was used to insert three point mutations into exon 8 of the mouse ERα gene to replace amino acids at positions 235, 237, and 238 with alanine.



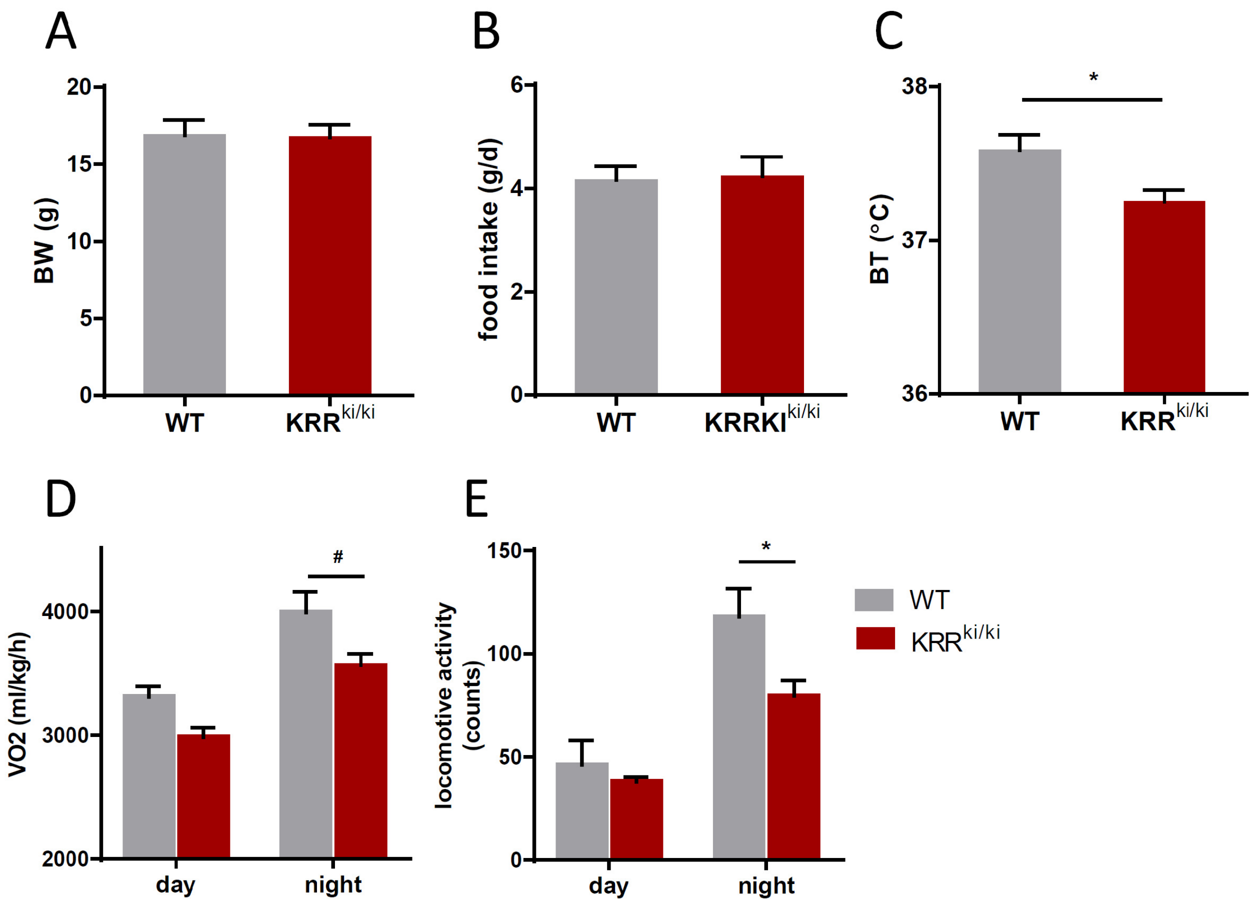
SUPPLEMENTARY DATA

Supplementary Figure 2. A. Co-immunoprecipitation of ERαwith striatin. Proteins were extracted from the hypothalamus, pWAT, iWAT, and BAT tissues of WT and $KRR^{ki/ki}$ mice, immunoprecipitated using an ERα antibody, and immunoblotted using an antibody against striatin. Representative immunoblots are shown. qRT-PCR analysis for ERE-related genes in the hypothalamus (**B**) and ERβ (*esr2*) in various tissues (**C**) (n = 6 per group). * $P < 0.05$. **D**: Body weight of male mice (n = 9-14) over the course of the study. # $P < 0.01$ vs. $ER\alpha^{+/+}$ (left panel) or WT (right panel) mice. **E**: Body weights of WT and $KRR^{ki/ki}$ mice with or without ovariectomy (OVX) (n = 8–10). # $P < 0.01$ vs. WT without ovariectomy. Data are presented as mean ± SEM.



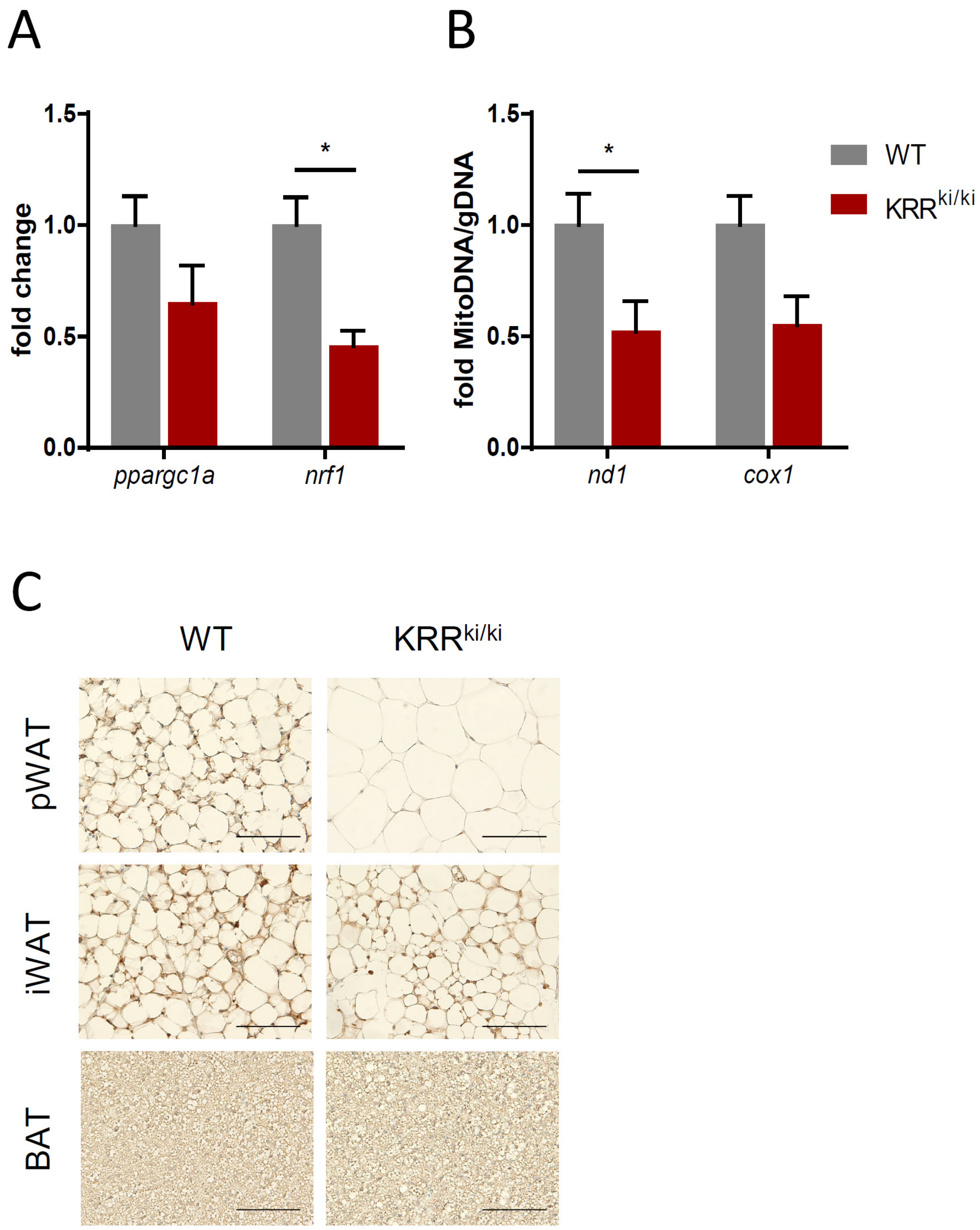
SUPPLEMENTARY DATA

Supplementary Figure 3. Loss of membrane-initiated ERα signaling decreases energy expenditure independently of food intake at 4-weeks of age. Body weight (**A**), daily food intake (**B**), body temperature at ambient temperature (**C**), oxygen consumption (**D**), and locomotor activity (**E**) of WT and $KRR^{ki/ki}$ mice at 4-weeks of age (n = 6 per group). * $P < 0.05$, # $P < 0.01$. Data are presented as mean ± SEM.



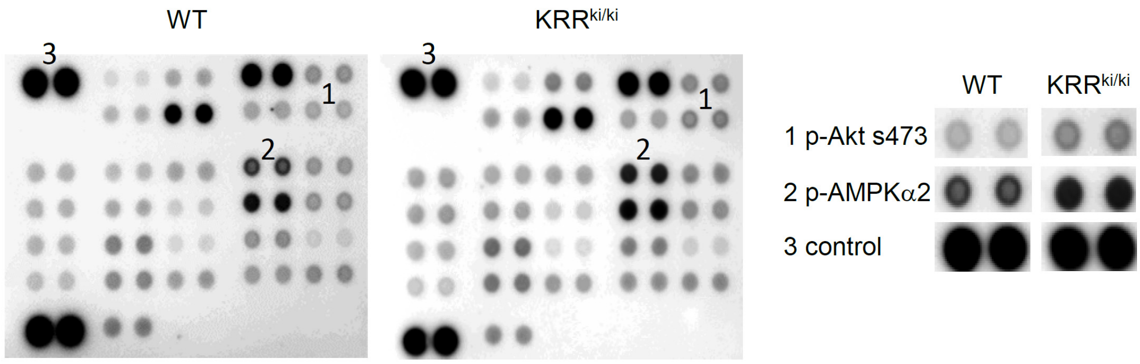
SUPPLEMENTARY DATA

Supplementary Figure 4. qRT-PCR analysis for mitochondrial biogenesis (**A**) and DNA content (**B**) of WT and KRR$^{ki/ki}$ pWAT (n = 6 per group). * $P < 0.05$. **C:** Immunohistochemistry staining for UCP1 of WT and KRR$^{ki/ki}$ pWAT, iWAT, and BAT. Scale bar indicates 100 µm (n = 3-5 per group). Representative pictures are shown.



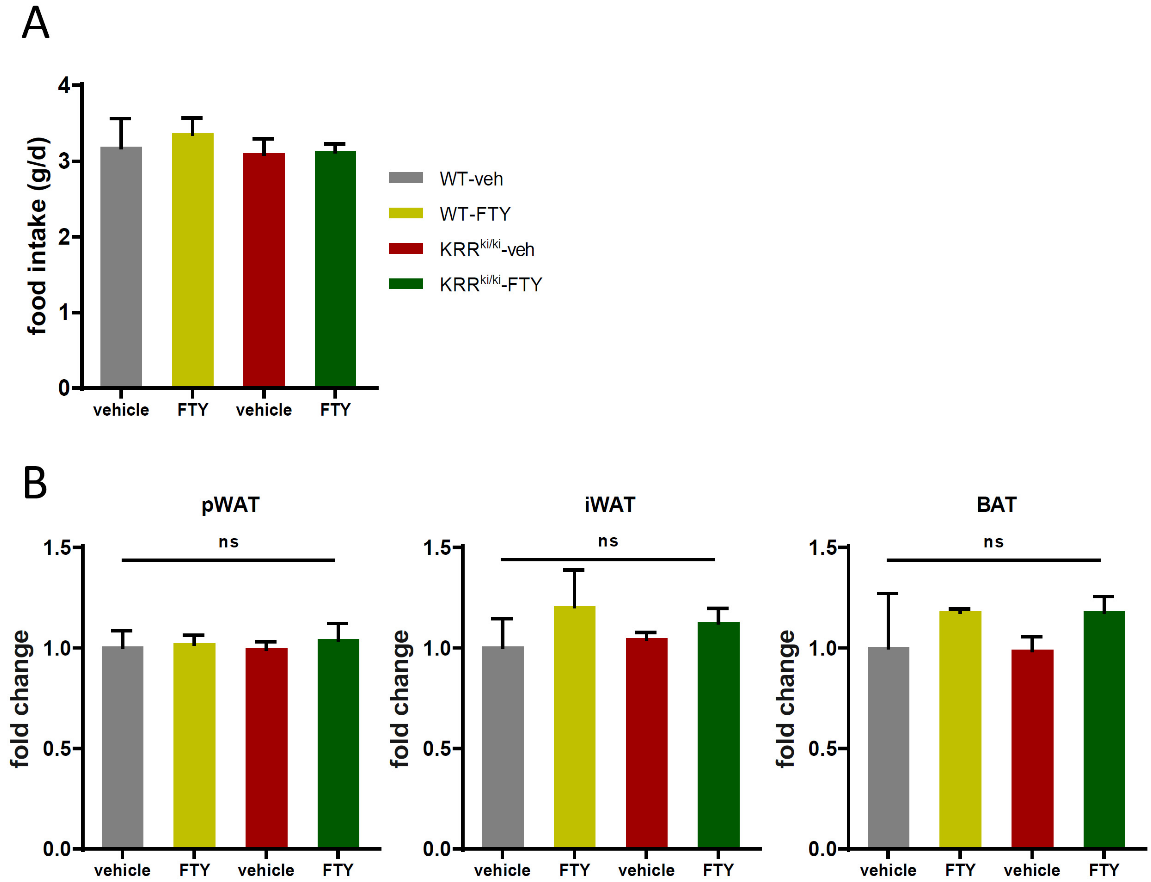
SUPPLEMENTARY DATA

Supplementary Figure 5. Profiling analysis of multiple kinase signaling in hypothalamus. The phospho-kinase array of hypothalamus lysates from WT and KRR$^{ki/ki}$ mice. The right panel shows higher magnification of numbered spots on the left panel.



SUPPLEMENTARY DATA

Supplementary Figure 6. A. Daily food intake of WT and KRR$^{ki/ki}$ mice that received ICV administration of vehicle control (veh) or 2.5 µg FTY720 (FTY) twice a week (n = 6 per group). **B:** PP2A activity in peripheral tissues (n = 4 per group). Data are presented as mean ± SEM.



SUPPLEMENTARY DATA

Supplementary Table 1.

Genes	Primer forward	Primer reverse
tnf	TATGGCCCAGACCCTCAC	GGTTGTCTTTGAGATCCATGC
serpine1	CCTCCTCATCCTGCCTAAGTT	GGCCAGGGTTGCACTAAAC
il6	TGATGGATGCTACCAAACTGG	TTCATGTACTCCAGGTAGCTATGG
ccl2	CCACTCACCTGCTGCTACTCAT	TGGTGATCCTCTTGTAGCTCTCC
adgre1	CTTTGGCTATGGGCTTCCAGTC	GCAAGGAGGACAGAGTTTATCGTG
il1a	TTGGTTAAATGACCTGCAACA	GAGCGCTCACGAACAGTTG
il1b	AGTTGACGGACCCCAAAAG	AGCTGGATGCTCTCATCAGG
il10	CAGAGCCACATGCTCCTAGA	TGTCCAGCTGGTCCTTTGTT
ucp1	GGCCTCTACGACTCAGTCCA	TAAGCCGGCTGAGATCTTGT
elovl3	GCCTCTCATCCTCTGGTCCT	GCTTGAGGCCCACTGTAAAC
cidea	GGCCGTGTTAAGGAATCTGC	CATGAACCAGCCTTTGGTGC
cox8b	CCAGCCAAAACTCCCACTT	GCTCTCCAAGTGGGCTAAGA
gapdh	CACTGAAGGGCATCTTGG	CATTGTCATACCAGGAAATGAG
ppp2ca	CCTCACGTTGGTGTCCAGA	GTTACTACGTTCCGGTCATGG
ppp2cb	CCACTTACAGCTTTAGTAGATGGACA	GCGATCCAGGGCTCTTATG
esr2	GACCCTCACTGGCACGTT	AATCCCTTCCACGCACTTC
greb1	GACCGTCTACTACCTCGTCCA	GCCAGGAGCGTAGGAAGAT
cox7a2l	TATTTGCCACACCAACCAAA	TCAGGTGGAAACCATCAGC
agt	CGGAGGCAAATCTGAACAAC	TCCTCCTCTCCTGCTTTGAG
c3	CGGCATAGAGAAGAGGCAAG	AAGGCAGCATAGGCAGAGC
ppargc1a	CCCTGCCATTGTTAAGACC	TGCTGCTGTTCCTGTTTTC
nrf1	TGGAGTCCAAGATGCTAATGG	GCGAGGCTGGTTACCACA
nd1	AATCGCCATAGCCTTCCTAACAT	GGCGTCTGCAAATGGTTGTAA
cox1	CCCAATCTCTACCAGCATC	GGCTCATAGTATAGCTGGAG
lpl	CGAGTCGTCTTTCTCCTGATGAT	TTCTGGATTCCAATGCTTCGA