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Supplemental information

Kinin B1 receptor controls maternal adiponectin levels and influences offspring weight gain

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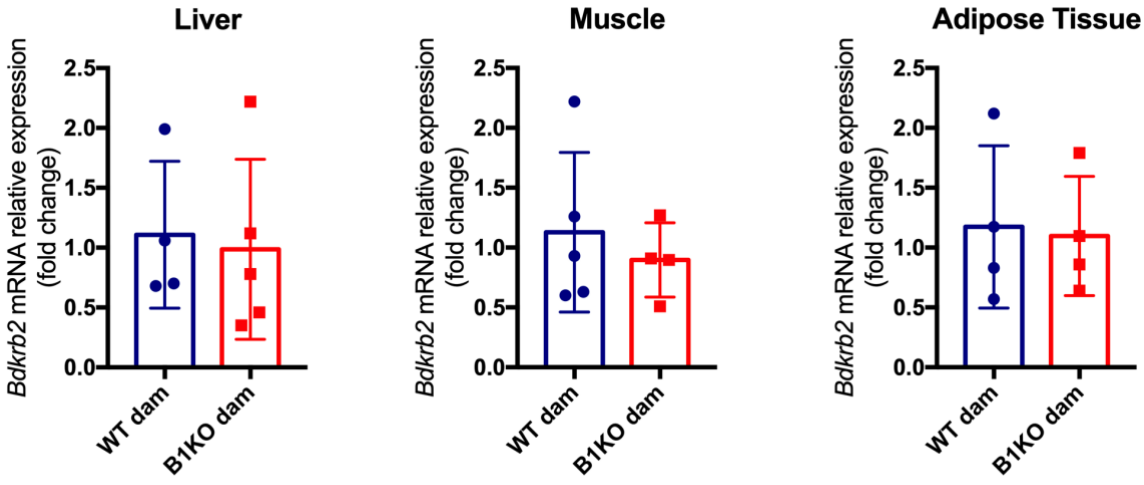

Figure S1. Maternal B1R deficiency does not influence on kinin B2 receptor expression in maternal metabolic tissues, Related to Figure 3. *Bdkrb2* (B2R) gene expression in liver (**a**), muscle (**b**), and adipose tissue (**c**). Maternal B1R deficiency does not affect the kinin B2 receptor expression in maternal metabolic tissues. Data were compared by Mann-Whitney test at GraphPad Prism 7. Values expressed as mean ± SD of 4-5 mice per group.

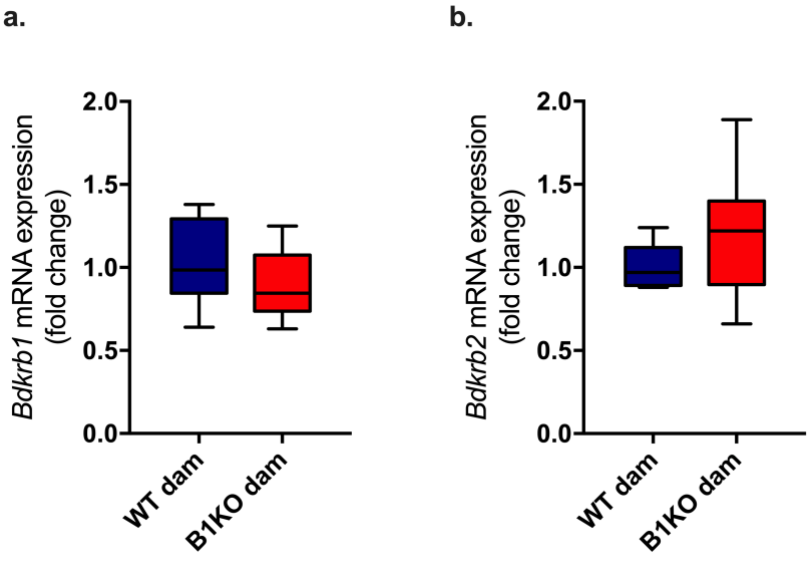

Figure S2. Maternal B1R deficiency does not influence on kinin receptors expression in heterozygous placenta, Related to Figure 2. *Bdkrb1* (B1R) (**a**) and *Bdkrb2* (B2R) (**b**) gene expression. Maternal B1R deficiency does not affect the kinin receptors' expression on the heterozygous placenta. Data were compared by unpaired t-test at GraphPad Prism 7. Values expressed as mean ± SD of 8-10 placentas per group (2 placentas per dam).

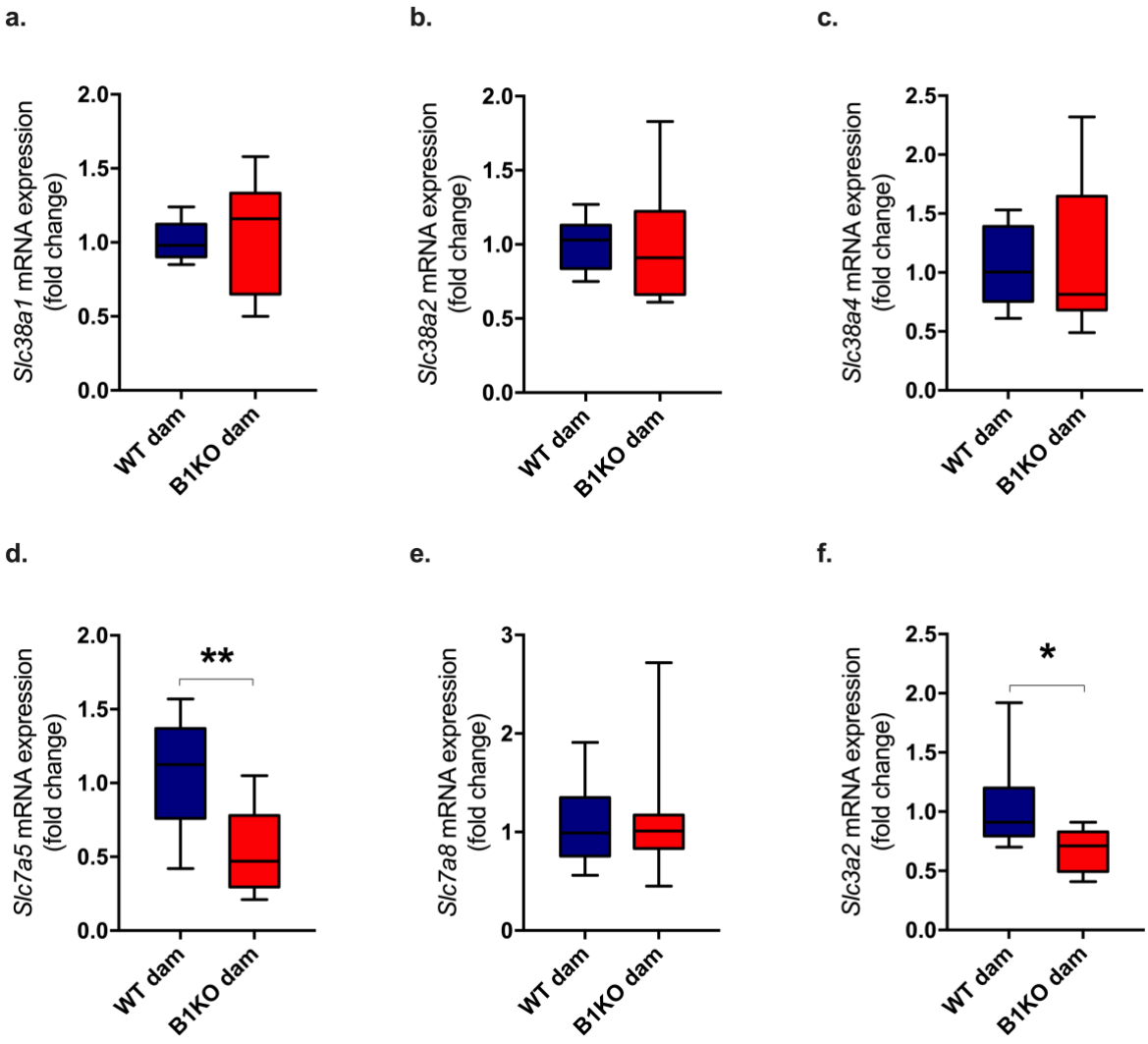

Figure S3. Maternal B1R deficiency represses gene expression of large neutral amino acid transporter 1 (LAT1), Related to Figure 4. *Slc38a1* (SNAT1) (**a**), *Slc38a2* (SNAT2) (**b**), *Slc38a4* (SNAT4) (**c**), *Slc7a5* (LAT1) (**d**), *Slc7a8* (LAT2) (**e**), *Slc3a2* (4F2hc) (**f**). Maternal B1R deficiency represses *Slc7a5* (p=0.0146) and *Slc3a2* (p=0.012) in the heterozygous placenta. *p<0.05, **p<0.01. Data were compared by Mann-Whitney (e, f) and Student's unpaired t-test (a, b, c, d) at GraphPad Prism 7. Values expressed as mean ± SD of 8-10 placentas per group (2 placentas per dam). SNAT – sodium-coupled neutral amino acid transporter; LAT – large neutral amino acid transporter. Blue = WT dam; Red = B1KO dam.

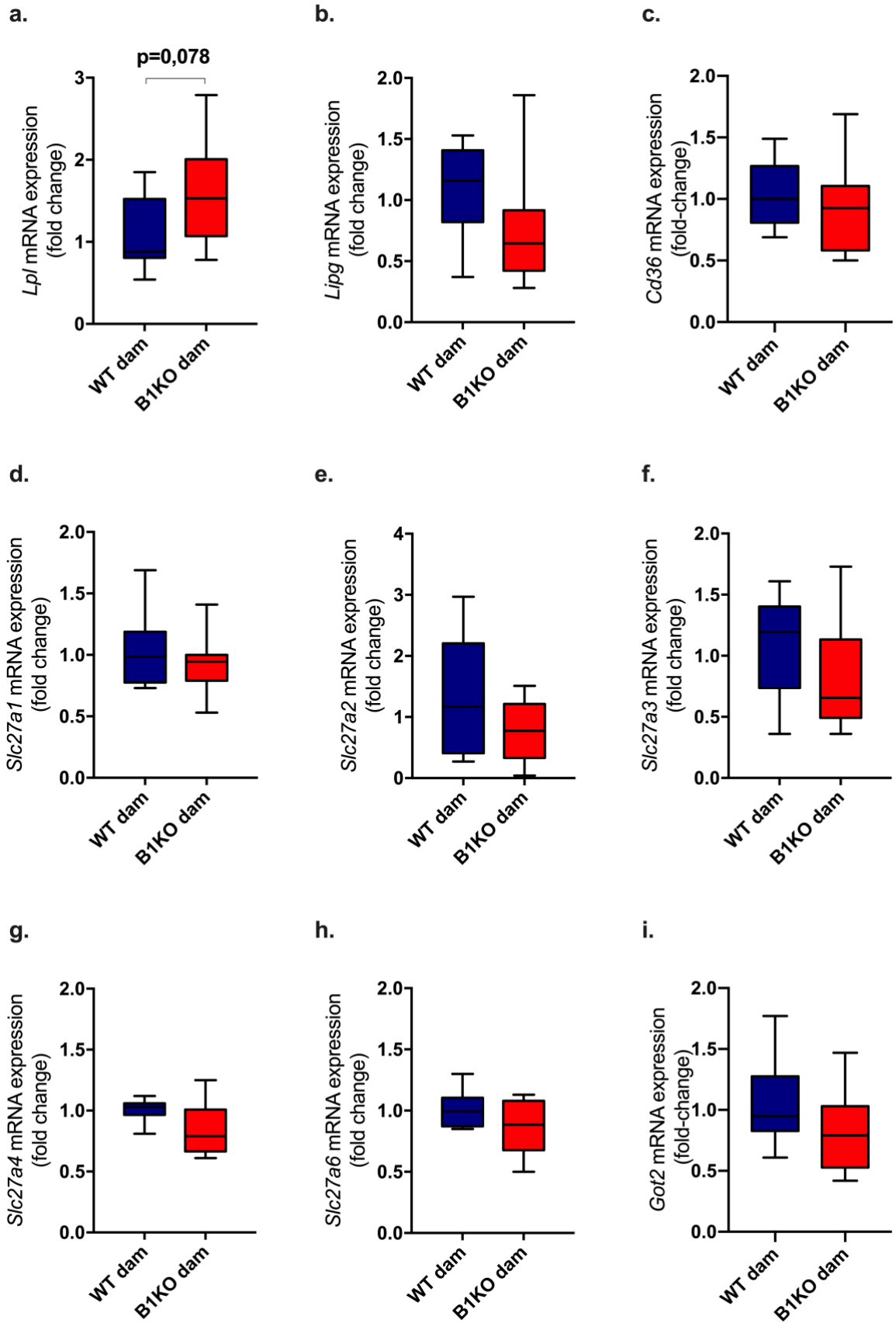

Figure S4. Maternal B1R deficiency does not interfere on placental lipases and free fatty acid transporters expression, Related to Figure 4. *Lpl* (lipoprotein lipase) (**a**), *Lipg* (endothelial lipase) (**b**), *Cd36* (fatty acid translocase) (**c**), *Slc27a1* (FATP1) (**d**), *Slc27a2* (FATP2) (**e**), *Slc27a3* (FATP3) (**f**),

Slc27a4 (FATP4) (**g**), *Slc27a6* (FATP6) (**h**), and *Got2* (pm-FABP) (**i**) gene expression. Maternal B1R deficiency does not affect the lipases and fatty acid transporters expression in heterozygous placenta. Data were compared by unpaired t-test at GraphPad Prism 7. Values expressed as mean ± SD of 8-10 placentas per group (2 placentas per dam). FATP – fatty acid transport protein; pm-FABP – plasma membrane fatty acid binding protein.

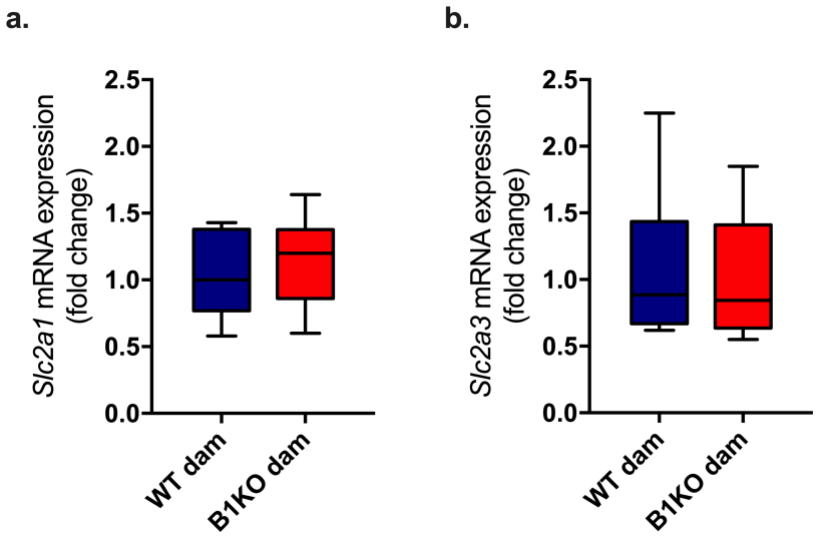

Figure S5. Maternal B1R absence does not change placental glucose transporters, Related to Figure 6. *Slc2a1* (GLUT1) (**a**) and *Slc2a3* (GLUT3) (**b**) gene expression. The lack of maternal B1R does not affect the glucose transporters expression on the heterozygous placenta. Data were compared by unpaired t-test at GraphPad Prism 7. Values expressed as mean ± SD of 8-10 placentas per group (2 placentas per dam). GLUT – glucose transporter.

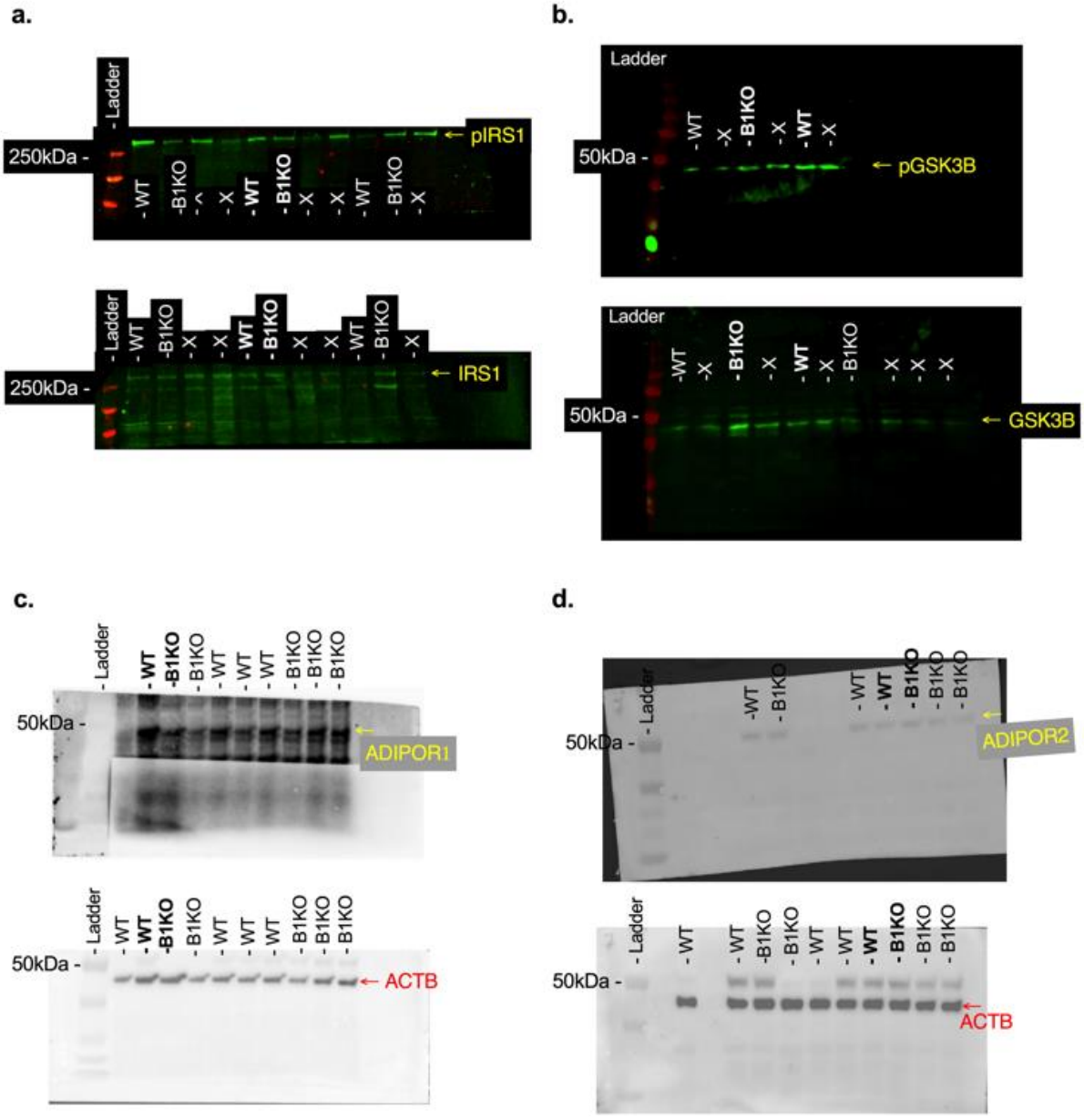

Figure S6. Maternal B1R deficiency reduces placental glycogenesis and adiponectin signaling, Related to Figures 5 and 6. Expression of pIRS1/IRS1(**a**), pGSK3B/GSK3B (**b);** ADIPOR1 (**c**) and ADIPOR2 (**d**) normalized by ACTB, and their respective representative western blot images. Maternal B1R deficiency reduces phosphorylated and total IRS1 ratio (p=0.045) (Figure 6.d), phosphorylated and total GSK3B ratio (p=0.032) (Figure 6.**e**), and ADIPOR1 protein content (p=0.012) (Figure 5.c) in the heterozygous placenta, with no change on ADIPOR2 protein content (Figure 5.d). We suggest the increased molecular weight of (p)IRS1 could be due to a complex formation with p85 subunit of phosphoinositide 3-kinase monomer. Representative bands are identified in bold. X – samples not used in this manuscript. ACTB – beta-actin; ADIPOR1 – adiponectin receptor 1; ADIPOR2 – adiponectin receptor 2; GSK3B – glycogen synthase kinase 3 beta; pGSK3B – phosphorylated GSK3B; IRS1 – insulin receptor substrate 1; pIRS1 – phosphorylated IRS1.

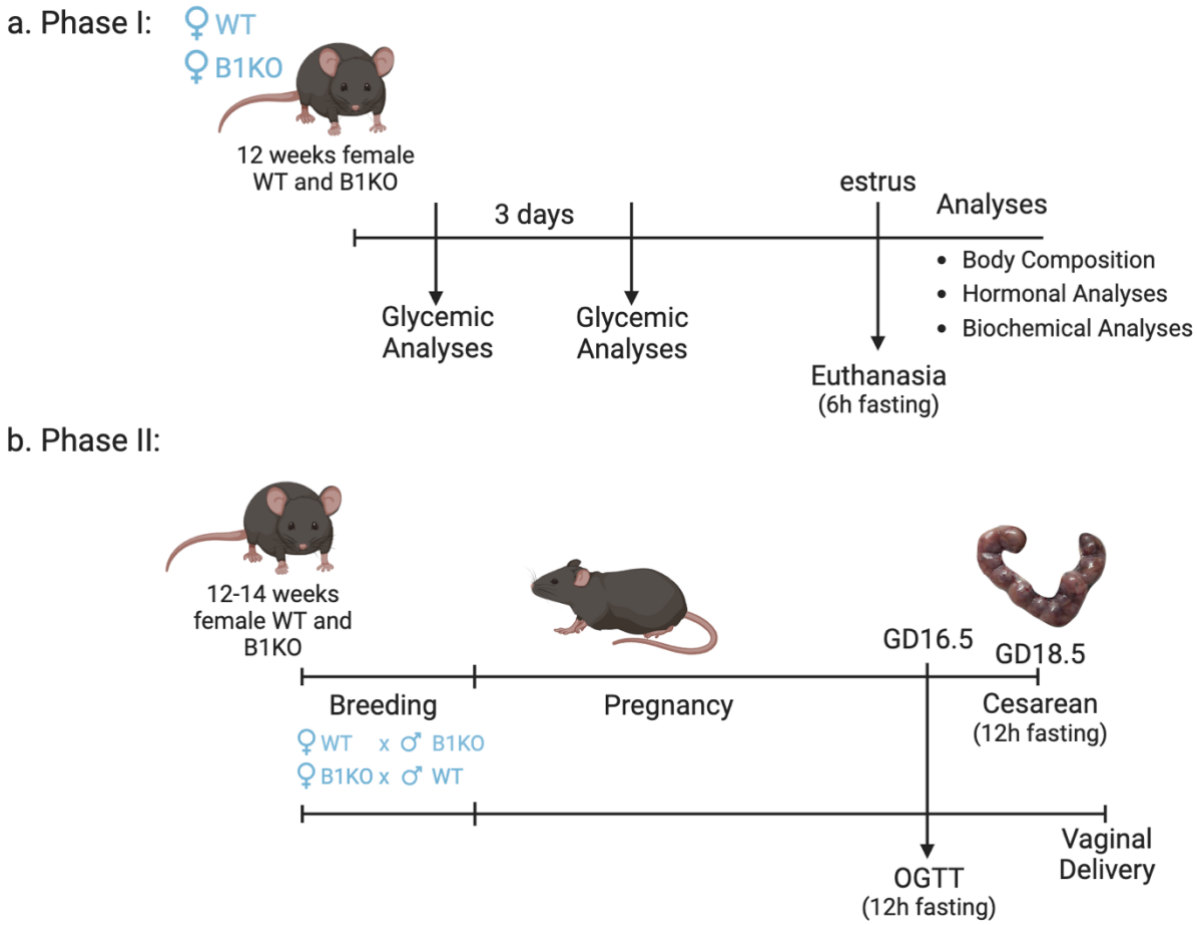

Figure S7. Experimental Design, Related to STAR Methods. 12-week-old female C57Bl/6 WT and B1KO mice were randomly submitted to Phase I (**a**) or Phase II (**b**) of the experimental design. In Phase I (**a**), the virgin females were submitted to glycemic analyses as GTT, ITT, and blood glucose and β-ketone levels measurement. The virgin females were euthanized by cervical dislocation after 6-hour fasting at the estrus stage, and serum, liver, gastrocnemius, and adipose tissue were collected for several analyses. In Phase II (**b**), at gestational day 16.5, the glucose tolerance test (GTT) was performed 12 hours after fasting in pregnant females. At gestational day 18.5, some of the pregnant mice were submitted to cesarean section and euthanized by cervical dislocation 12h after fasting, as well as the serum, placenta, adipose tissue, gastrocnemius, and liver were collected for several analyses. Other pregnant mice underwent vaginal delivery, and the offspring were analyzed until 30 days after birth.

Table S1. RT-qPCR Primer Sequences, Related to STAR Methods

Adiponectin	F – TGTTCCTCTTAATCCTGCCCA
	R – CCAACCTGCACAAGTTCCCTT
Bdkrb1	F – TGGAGTTGAACGTTTTGGGTTT
	R – GTGAGGATCAGCCCCATTGT
Bdkrb2	F – GGTGCTGAGGAACAACGAGA
	R – CCCAACACAGCACAAAGAGC
Gys1	F – TCAGAGCAAAGCACGAATCCAG
	R – CATAGCGGCCAGCGATAAAGA

Lepr, isoform a	F – GAAGTCTCTCATGACCACTACAGATGA
	R – TTGTTTCCCTCCATCAAAATGTAA
Lepr, isoform b	F – GCATGCAGAATCAGTGATATTTGG
	R – CAAGCTGTATCGACACTGATTTCTTC
Lipg	F – ATGCGAAACACGGTTTTCCTG
	R – GTAGCTGGTACTCCAGTGGG
Lpl	F – GGGAGTTTGGCTCCAGAGTTT
	R – TGTGTCTTCAGGGGTCCTTAG
Prl3b1	F – CACCAGACAACATCGGAAGAC
	R – TGACAGCAGAGTATCAGGTACA
Rn18s	F – TCAACTTTCGATGGTAGTCGCCGT
	R – TCCTTGGATGTGGTAGCCGTTTCT
Slc2a1	F – CAGTTCGGCTATAACACTGGTG
	R – GCCCCCGACAGAGAAGATG
Slc2a3	F – ATGGGGACAACGAAGGTGAC
	R – GTCTCAGGTGCATTGATGACTC
Slc27a1	F – TCTGTTCTGATTCGTGTTCGG
	R – CAGCATATACCACTACTGGCG
Slc27a2	F – TCCTCCAAGATGTGCGGTACT
	R – TAGGTGAGCGTCTCGTCTCG
Slc27a3	F – CATTGGGGAGTTGTGCCGATA
	R – GCCAAGCGCACCTTATCCT
Slc27a4	F – ACTGTTCTCCAAGCTAGTGCT
	R – GATGAAGACCCGGATGAAACG
Slc27a6	F – GCAGCATGGGTCCTGAAAG
	R – ACGGGAGAACTAAGATAGCAGC
Slc3a2	F – TGATGAATGCACCCTTGTACTTG
	R – GCTCCCCAGTGAAAGTGGA
Slc38a1	F – CCTTCACAAGTACCAGAGCAC
	R – GGCCAGCTCAAATAACGATGAT
Slc38a2	F – TAATCTGAGCAATGCGATTGTGG

	R – AGATGGACGGAGTATAGCGAAAA
Slc38a4	F – GCGGGGACAGTATTCAGGAC
	R – GGAACTTCTGACTTTCGGCAT
Slc7a5	F – ATATCACGCTGCTCAACGGTG
	R – CTCCAGCATGTAGGCGTAGTC
Slc7a8	F – TGTGACTGAGGAACTTGTGGA
	R – GTGGACAGGGCAACAGAAATG