

Supplementary Information

RGS10 controls the metabolic and neuroinflammatory benefits of NLRP3 suppression in diet-induced obesity in male mice.

Declan J. Gresham^{1,2}, Jaegwon Chung¹, Jung Ha Byun^{1,3}, Yu Zhang¹, Nicole Zhang¹, Yoon-Seong Kim⁴, Adetutu Adebawale^{1,2}, Jina Lim¹, Kelly Menees^{1,3}, Nhi Diep¹, Matthew C. Havrda^{5*}, Jae-Kyung Lee^{1,2,3*}

¹Department of Physiology and Pharmacology, University of Georgia College of Veterinary Medicine, Athens, GA, USA.

²Intergrative Physiology and Pharmacology program, University of Georgia, Athens, GA, USA.

³Neuroscience program, University of Georgia, Athens, GA, USA

⁴Robert Wood Johnson Medical School Institute for Neurological Therapeutics, and Department of Neurology, Rutgers Biomedical and Health Sciences, Piscataway, New Jersey 08854, USA

⁵Department of Biomedical Sciences, University of New England College of Osteopathic Medicine, Biddeford, ME, USA

*Corresponding author: jamlee@uga.edu (J.K.L) and mhavrda1@une.edu (M.H)

Supplementary Fig. S1. Purity of BMDM cultures.

Supplementary Fig. S2. RGS10 deficiency enhances NLRP3 inflammasome priming and activation in female BMDMs.

Supplementary Fig. S3. Metabolic phenotypes of female WT, *Rgs10*^{-/-}, *Nlrp3*^{-/-}, dKO mice following HFD.

Supplementary Fig. S4. dKO mice show increased liver weight and hepatic vacuolar area under normal chow.

Supplementary Fig. S5. dKO mice show slightly increased neutrophil frequencies and decreased monocyte/macrophage frequencies in adipose stromal vascular fraction.

Supplementary Fig. S6. dKO mice show increased Iba1+ area in the VMH following HFD.

Supplementary Table 1. Mixed effects model analysis for male GTT/IST tests

Supplementary Table 2. Mixed effects model analysis for female GTT/IST tests

Supplementary Table 3. Primer sequences used for QPCR

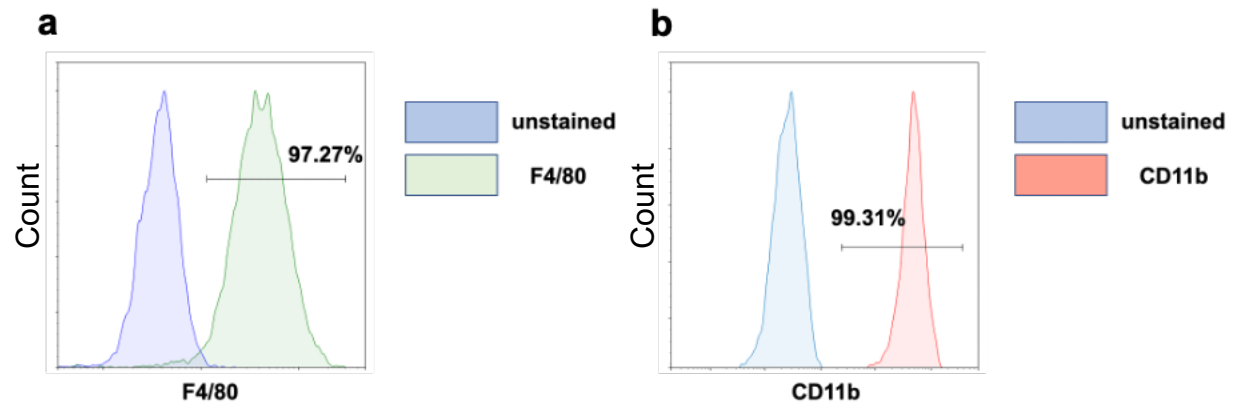


Figure S1. Purity of BMDM cultures. Representative flow cytometry histograms of F4/80 (A) and CD11b (B) in BMDMs after 7 days of differentiation, compared with unstained cells.

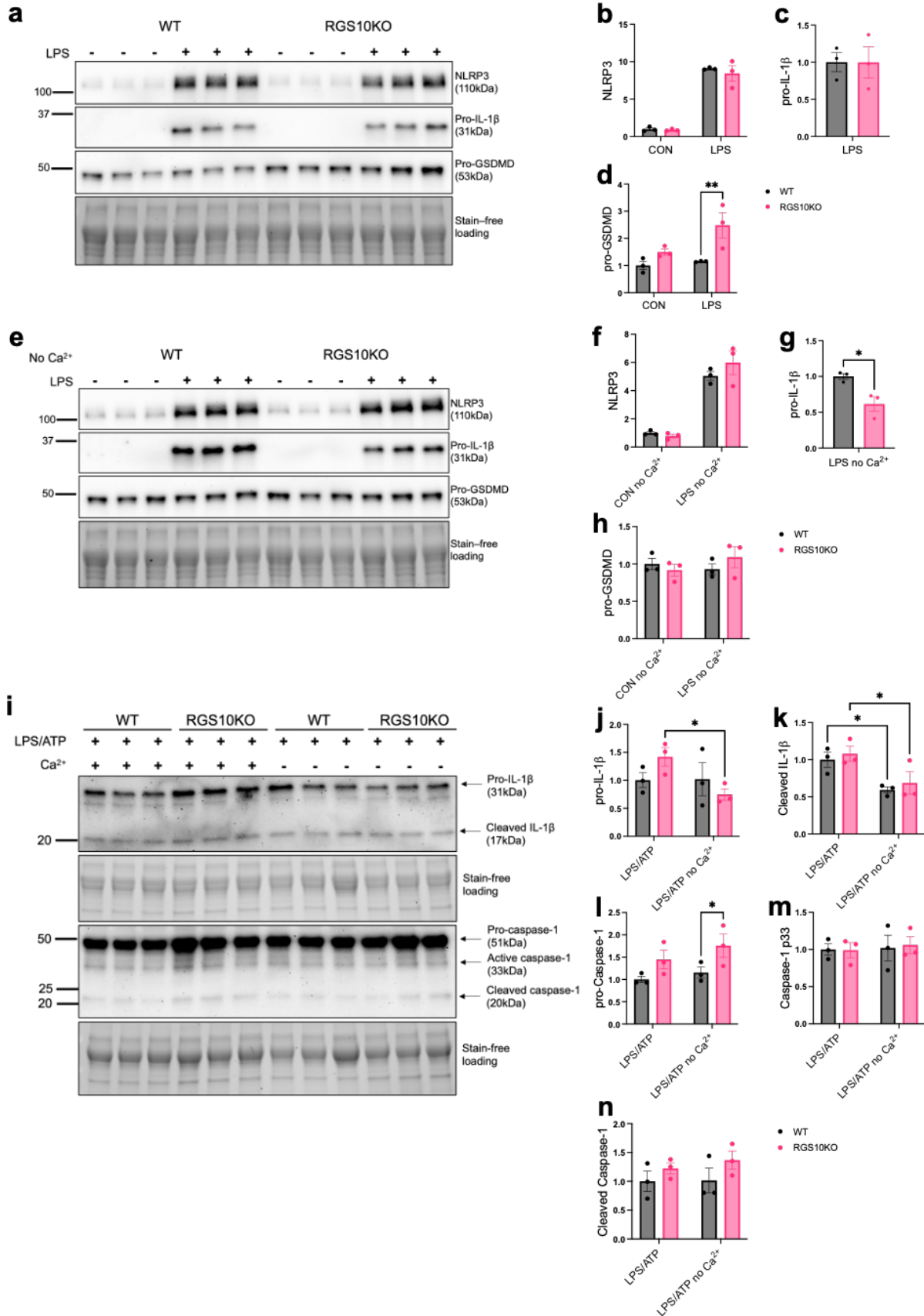


Figure S2. RGS10 deficiency enhances NLRP3 inflammasome priming and activation in female BMDMs. BMDMs were generated from female WT and *Rgs10*^{-/-} mice; bone marrow was pooled from 3 mice per preparation, and n=3 independent preparations per genotype were used. **(A, E)** Representative immunoblots of NLRP3, pro-IL-1 β , and pro-GSDMD in lysates of BMDMs stimulated with LPS (100 ng/mL, 3 h) in complete medium **(A)** or calcium-free medium **(E)**. **(B-D, F-H)** Quantification of NLRP3, **(B, F)**, pro-IL-1 β **(C, G)**, and pro-GSDMD **(D, H)**, normalized to stain-free total protein and expressed relative to [WT CON/WT LPS]. **(I)** Representative immunoblots of supernatants from BMDMs primed with LPS (100 ng/mL, 3 h) and stimulated with ATP (5 mM, 2 h). **(J-N)** Quantification of secreted pro-IL-1 β **(J)**, cleaved IL-1 β p17 **(K)**, pro-caspase-1 **(L)**, caspase-1 p33 **(M)**, and caspase-1 p20 **(N)**, normalized to stain-free gel loading. Statistical analyses were performed using two-way ANOVA with Fisher's LSD **(B, D, F, H, J, K, L, M, N)** or unpaired t-test **(C, G)**. Data are shown as mean $\pm$ SEM. * $p < 0.05$, ** $p < 0.01$.

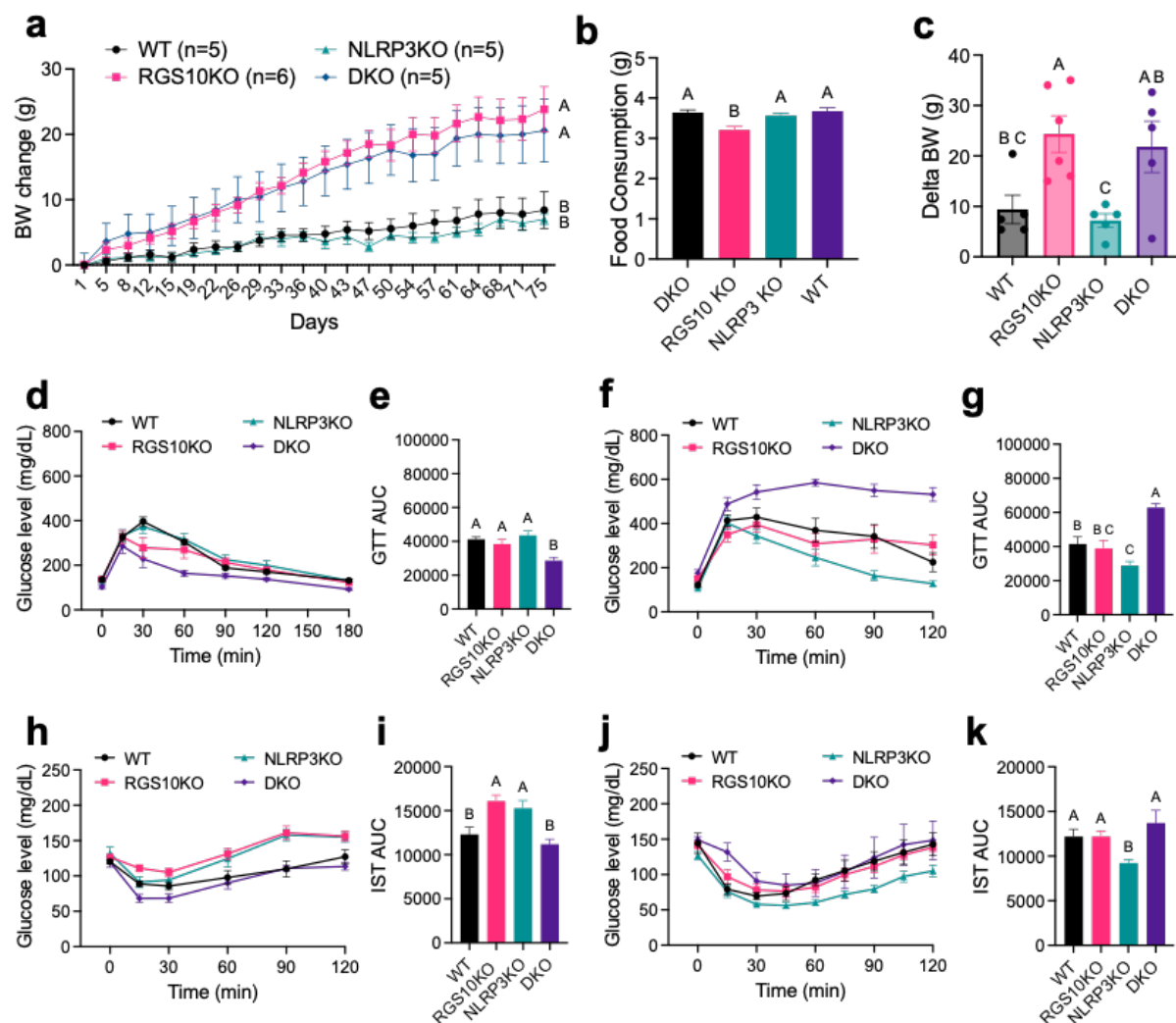


Figure S3. Metabolic phenotypes of female WT, *Rgs10*^{-/-}, *Nlrp3*^{-/-}, dKO mice following HFD.

(A-C) Female mice of each genotype were fed HFD for 12 weeks (n=5-6 per genotype): cumulative body weight gain (A), average daily food intake (B), and final delta bodyweight (C) were recorded. (D-K) Glucose tolerance tests (GTT) (D-G) and insulin sensitivity tests (IST) (H-K) were performed during week 11 after a 3-h fast in mice fed NC (D, E, H, I) or HFD (F, G, J, K) and AUC (E, G, I, K). Per comparison statistics are tabulated in Table S2. Data are mean ± SEM, analyzed by repeated-measures two-way ANOVA with Tukey's post-hoc test (A), Kruskal-Wallis with Dunn's test (B), or one-way ANOVA with Tukey's post-hoc test (C, E, G, I, K); mixed-effects model with Tukey's post-hoc test (D, F, H, J).

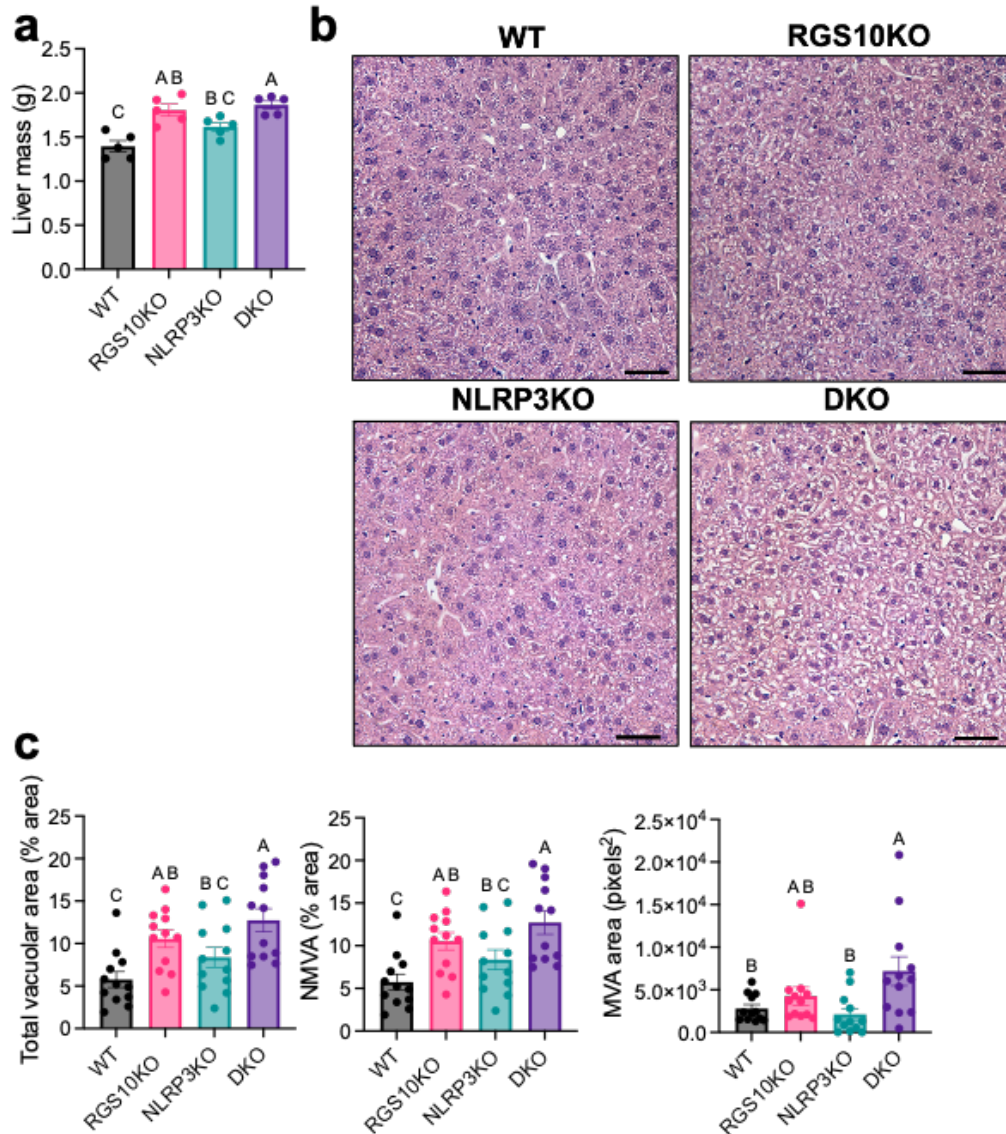


Figure S4. dKO mice show increased liver weight and hepatic vacuolar area under normal chow. (A) Liver weight of male WT, *Rgs10*^{-/-}, *Nlrp3*^{-/-}, and dKO mice after 12 weeks of NC (n=5 mice per group). **(B)** Representative H&E-stained liver sections (40X objective) from the same cohort (n=3 mice per group). Scale bar, 50 μ m. **(C)** Total vacuolar area, MVA, and NMVA, expressed as percent of tissue area. Analysis used the same Labkit classifier and thresholds as in Figure 3, applied blinded to genotype. Data are mean $\pm$ SEM, analyzed by one-way ANOVA with Tukey's post hoc test.

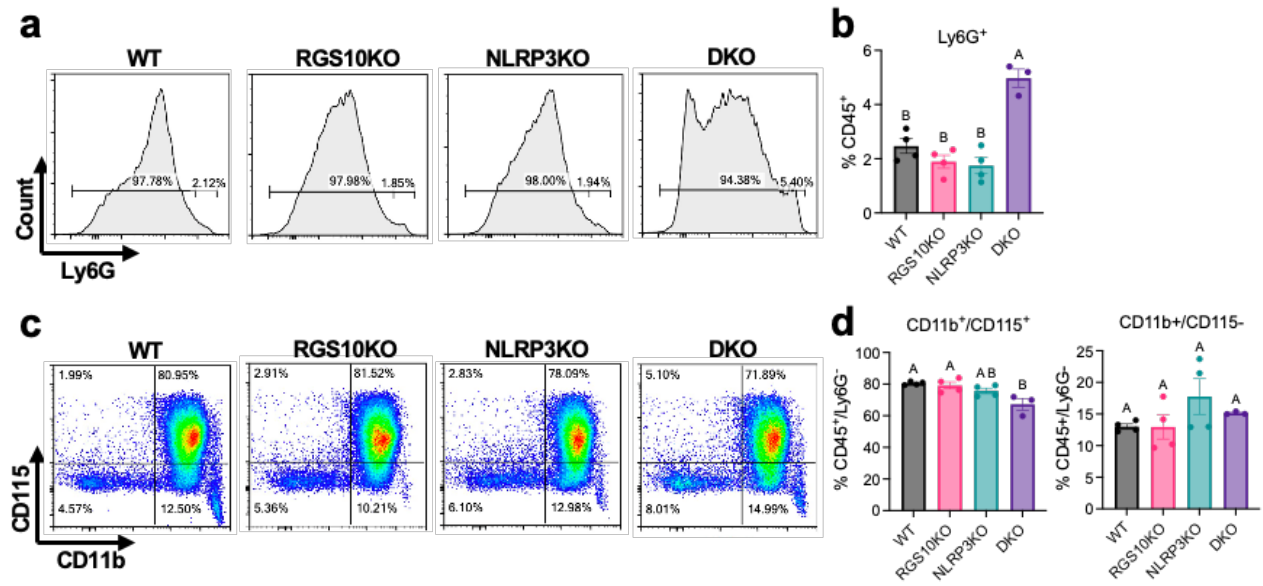


Figure S5. dKO mice show slightly increased neutrophil frequencies and decreased monocyte/macrophage frequencies in adipose stromal vascular fraction. Stromal vascular fractions were isolated from ingWAT and eWAT of male WT, *Rgs10*^{-/-}, *Nlrp3*^{-/-}, and dKO mice after 12 weeks of HFD (n=4,4,4,3); gating strategy as in Figure 6A. **(A-B)** Representative histograms **(A)** and frequency **(B)** of Ly6G cells as a percentage of CD45⁺ cells. **(C)** Representative density plots of CD11b and CD115 within the CD45⁺/Ly6G⁻ gate. **(D)** Frequencies of CD11b⁺/CD115⁺ and CD11b⁺/CD115⁻ as a percentage of the CD45⁺/Ly6G⁻ gate. Data are mean ± SEM, analyzed using one-way (B) or two-way ANOVA (D) with Tukey's post-hoc test.

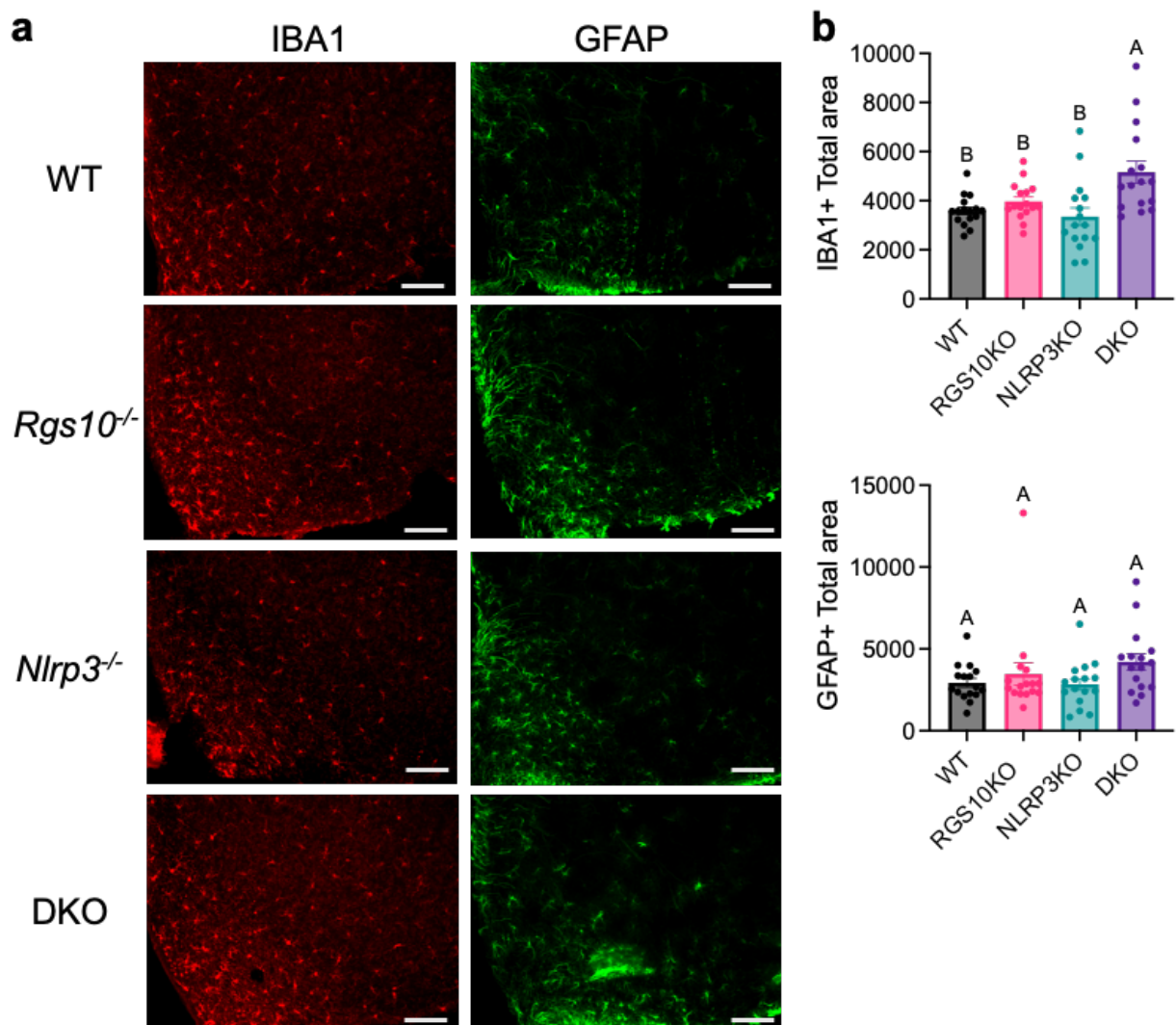


Figure S6. dKO mice show increased Iba1+ area in the VMH following HFD.

(A) Representative immunofluorescence images of Iba1 (microglia) and GFAP (astrocyte) in the VMH (20X objective) of male WT, *Rgs10*^{-/-}, *Nlrp3*^{-/-}, and dKO mice after 12 weeks of HFD (n=4). Scale bar, 100 μ m. **(B)** Percent Iba1⁺ area or GFAP⁺ immunoreactive area in the VMH. n=3-4 images per animal; n=4 mice per genotype. Images were acquired with identical exposure settings and analyzed with a fixed threshold, blinded to genotype, as in Figure 7. Data are mean $\pm$ SEM, analyzed using one-way ANOVA with Tukey's post-hoc test.

Table S1. Mixed effects model analysis for male GTT/IST tests

Figure	Comparison		Significant difference?
2F NC GTT	WT	RGS10KO	ns
	WT	NLRP3KO	ns
	WT	DKO	t=0 (p=0.0021)
	RGS10KO	NLRP3KO	ns
	RGS10KO	DKO	t=0 (p=0.0073)
	NLRP3KO	DKO	ns
2H HFD GTT	WT	RGS10KO	ns
	WT	NLRP3KO	ns
	WT	DKO	ns
	RGS10KO	NLRP3KO	t=120 (p=0.0259)
	RGS10KO	DKO	ns
	NLRP3KO	DKO	t=120 (p=0.0346), t=180 (p=0.0290)
2J NC IST	WT	RGS10KO	t=15 (p=0.0095), t=30 (p=0.0253)
	WT	NLRP3KO	t=0 (p<0.0098), t=30 (p<0.0319)
	WT	DKO	ns
	RGS10KO	NLRP3KO	t=0 (p=0.0281)
	RGS10KO	DKO	t=15 (p=0.0053)
	NLRP3KO	DKO	ns
2L HFD IST	WT	RGS10KO	ns
	WT	NLRP3KO	t=0 (p=0.0120), t=15 (p=0.0194), t=30 (p=0.0118)
	WT	DKO	ns
	RGS10KO	NLRP3KO	t=15 (p=0.0018)
	RGS10KO	DKO	ns
	NLRP3KO	DKO	ns

Table S2. Mixed effects model analysis for female GTT/IST tests

Figure	Comparison		Significant difference?
S3D NC GTT	WT	RGS10KO	ns
	WT	NLRP3KO	ns
	WT	DKO	t=30 (p=0.0319), t=60 (p=0.0002), t=180 (p=0.0159)
	RGS10KO	NLRP3KO	ns
	RGS10KO	DKO	t=0 (p=0.0289), t=180 (p=0.0376)
	NLRP3KO	DKO	t=60 (p=0.0100), t=180 (p=0.0031)
S3F HFD GTT	WT	RGS10KO	ns
	WT	NLRP3KO	ns
	WT	DKO	t=60 (p=0.0490), t=120 (p=0.0026)
	RGS10KO	NLRP3KO	t=0 (p=0.0064), t=120 (p=0.0415)
	RGS10KO	DKO	t=30 (p=0.0462), t=60 (p=0.0056), t=120 (p=0.0132)
	NLRP3KO	DKO	t=0 (p=0.0286), t=30 (p=0.0107), t=60 (p=0.0013), t=90 (p<0.0001), t=120 (p=0.0002)
S3H NC IST	WT	RGS10KO	t=15 (p=0.0345), t=90 (p=0.0367)
	WT	NLRP3KO	t=90 (p=0.0417)
	WT	DKO	ns
	RGS10KO	NLRP3KO	ns
	RGS10KO	DKO	t=15 (p=0.0023), t=30 (p=0.0103), t=60 (p=0.0317), t=90 (p=0.0128), t=120 (p=0.0071)
	NLRP3KO	DKO	t=90 (p=0.0079), t=120 (p=0.0090)
S3J HFD IST	WT	RGS10KO	ns
	WT	NLRP3KO	ns
	WT	DKO	t=15 (p=0.0461)
	RGS10KO	NLRP3KO	t=90 (p=0.0483), t=120 (p=0.0463)
	RGS10KO	DKO	ns
	NLRP3KO	DKO	t=15 (p=0.0368)

Table S3. Primer sequences used for QPCR

Gene	Forward (5'-3')	Reverse (5'-3')
<i>Gapdh</i>	CAAGGTCATCCATGACAACTTTG	GGCCATCCACAGTCTTCTGG
<i>Ppia</i> (<i>Cyclophilin</i>)	TGGAGAGCACCAAGACAGACA	TGCCGGAGTCGACAATGAT
<i>18s rDNA</i>	TAGAGGGACAAGTGGCGTTC	CGCTGAGCCAGTCAGTGT
<i>Fasn</i>	GCTGCGGAACTTCAGGAAAT	AGAGACGTGTCACTCCTGGACTT
<i>Tgfb1</i>	GGTTCATGTCATGGATGGTGC	TGACGTCACTGGAGTTGTACGG
<i>Col1a1</i>	CCAAGGGTAACAGCGGTGAA	CCTCGTTTTCTTCTTCTCCG
<i>Col4a1</i>	TTAAAGGACTCCAGGGACCAC	CCCACTGAGCCTGTCACAC
<i>Acta2</i>	GGCTCTGGGCTCTGTAAGG	CTCTTGCTCTGGGCTTCATC
<i>Cd68</i>	GGAATACATGGCGGTGGAATA	GATGAATTCTGCGCCATGAA
<i>Cd11b</i>	TGCCAAGACGATCTCAGCAT	GCCTCCCACCACCAAAGT
<i>Ccl2</i>	CTTCCTCCACCACCATGCA	CCAGCCGGCAACTGTGA
<i>Tnf</i>	CTGAGGTCAATCTGCCCAAGTAC	CTTCACAGAGCAATGACTCCAAAG
<i>Pparg</i>	CCCACCAACTTCGGAATCA	TGCGAGTGGTCTTCCATCAC
<i>Adipoq</i>	TCACGGTGTACATGAAAGATGTG	GAGAACGGCCTTGTCTTCT
<i>Glut4</i>	CCTTTCTCATTGGCATCATTTT	CACGGCCAAGACATTGTTG
<i>Prdm16</i>	AGCCCTCGCCCACTTGC	TGACCCCCGGCTTCCGTTCA
<i>Ucp1</i>	GAGGTGTGGCAGTGTTTCATTG	GGCTTGCATTCTGACCTTCA