Forkhead box i2 (Foxi2) transcription factor regulates systemic energy metabolism via neuropeptide AgRP

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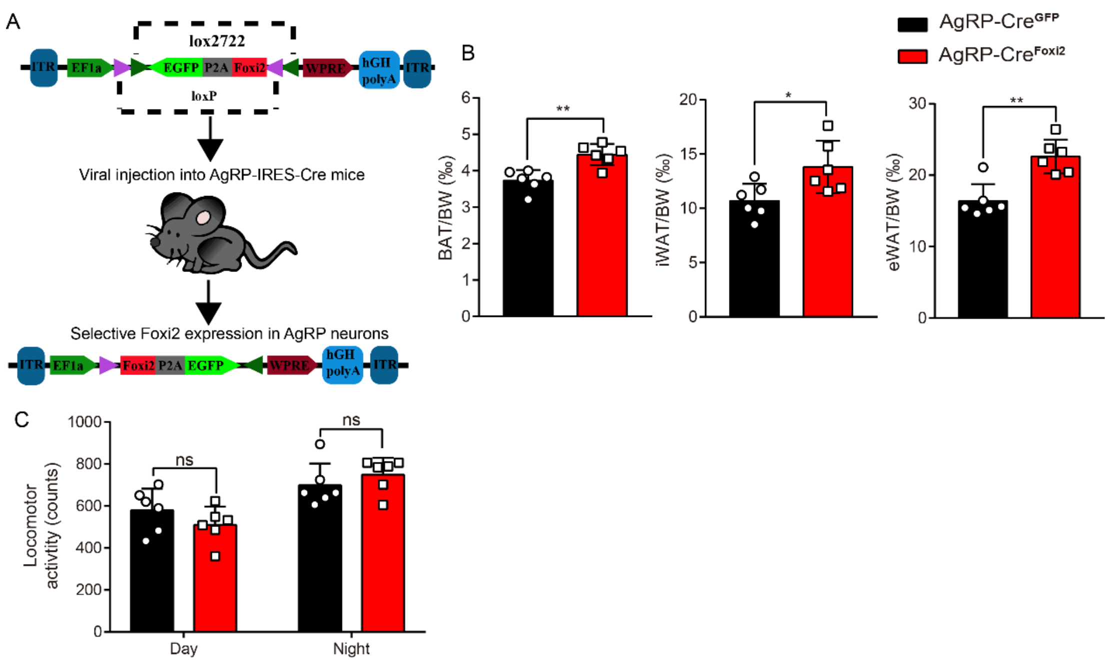
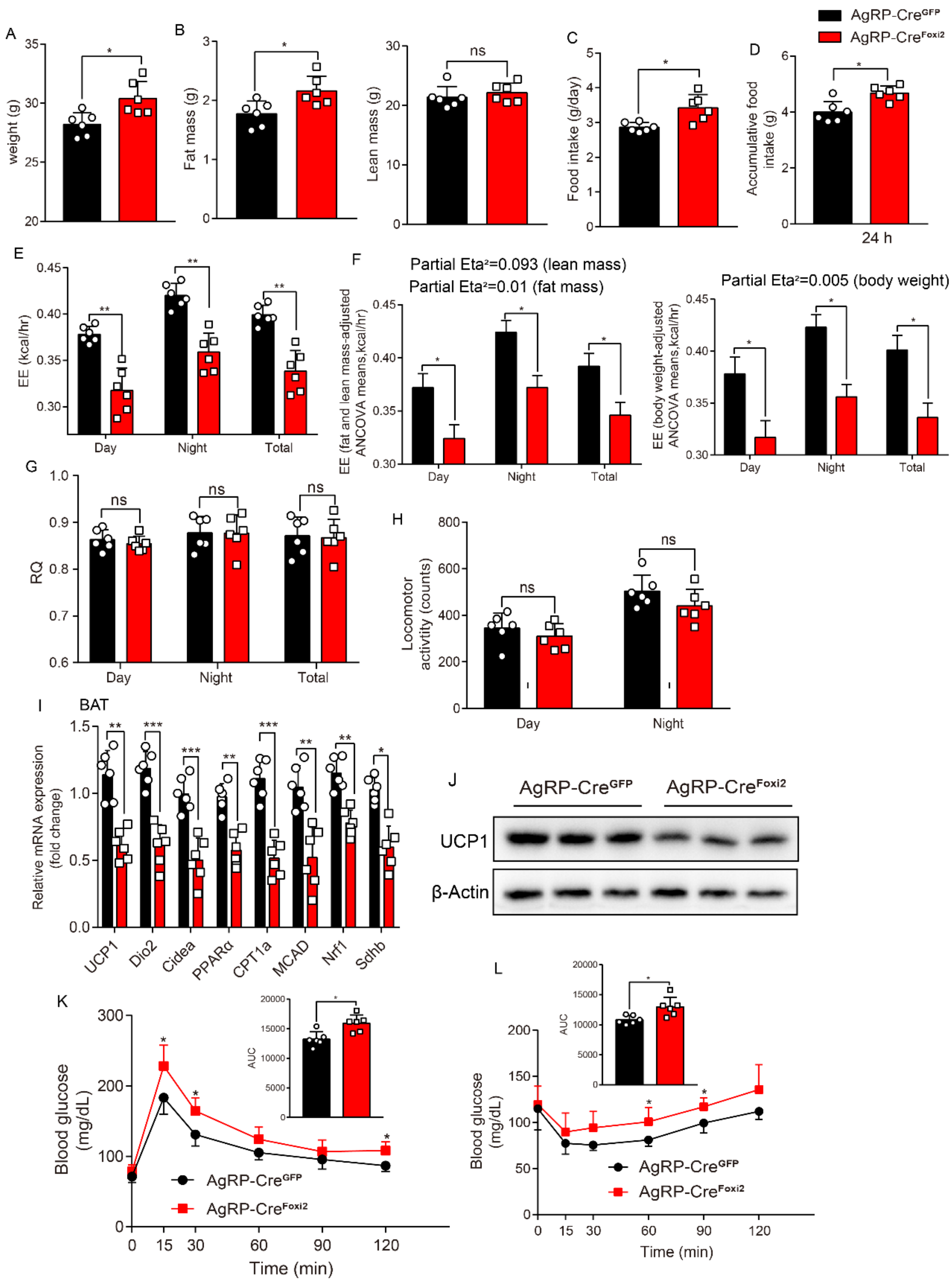
Supplemental Figures

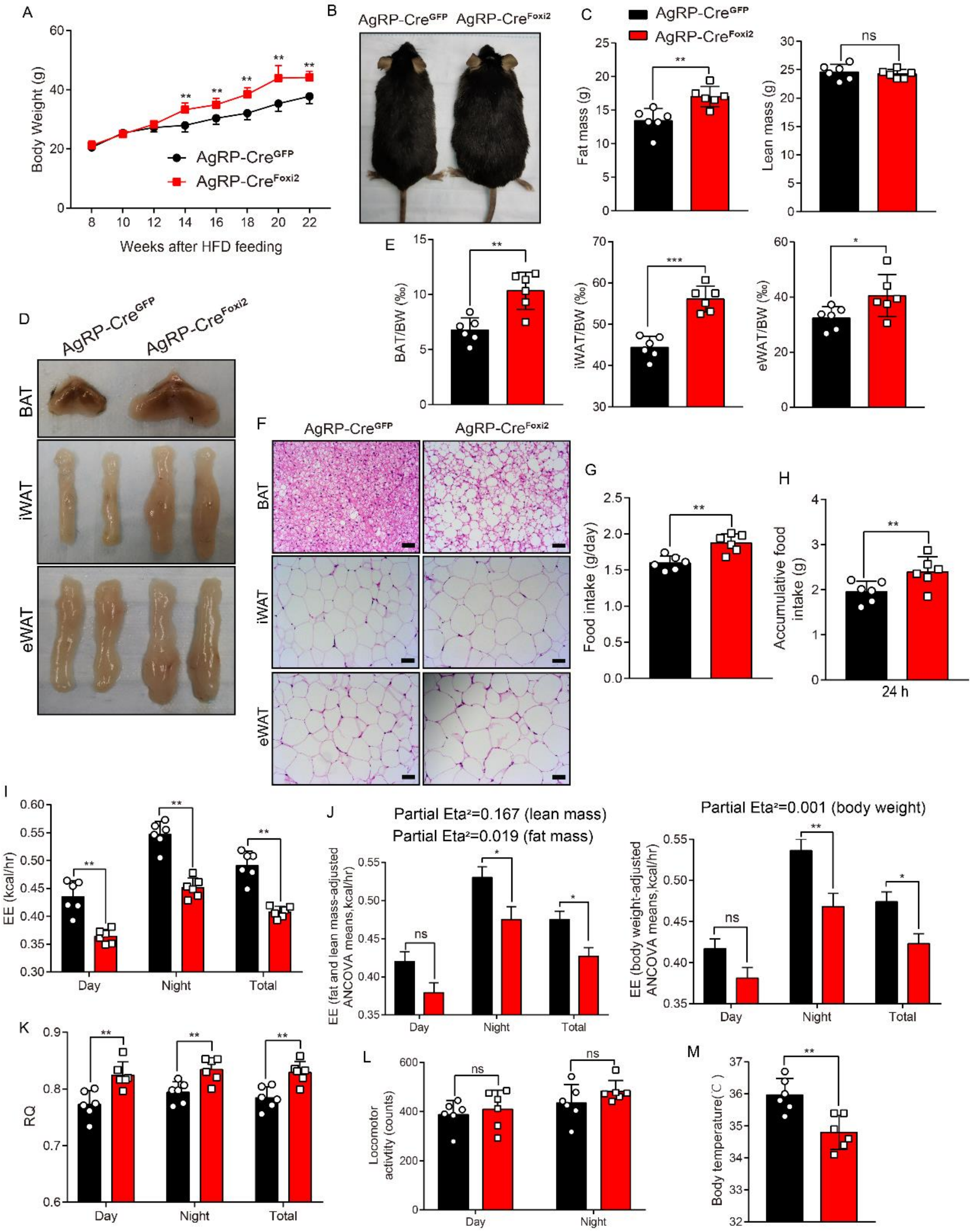


Supplemental Figure 1. Overexpression of Foxi2 in AgRP neurons of male mice causes obesity under a chow diet condition. A: Schematic of Foxi2 overexpression in AgRP neurons in AgRP-IRES-Cre mice by AAV injections. B: Tissue weight of BAT, iWAT and eWAT in AgRP-cre^{GFP} and AgRP-cre^{Foxi2} mice fed a chow diet (n= 6/group). C: Locomotor activity of AgRP-cre^{GFP} and AgRP-cre^{Foxi2} mice (n= 6/group). Data are represented as mean ± SEM. *p<0.05, ** p<0.01, two-tailed Student's t test (B, C). Abbreviations: n.s., not significant.

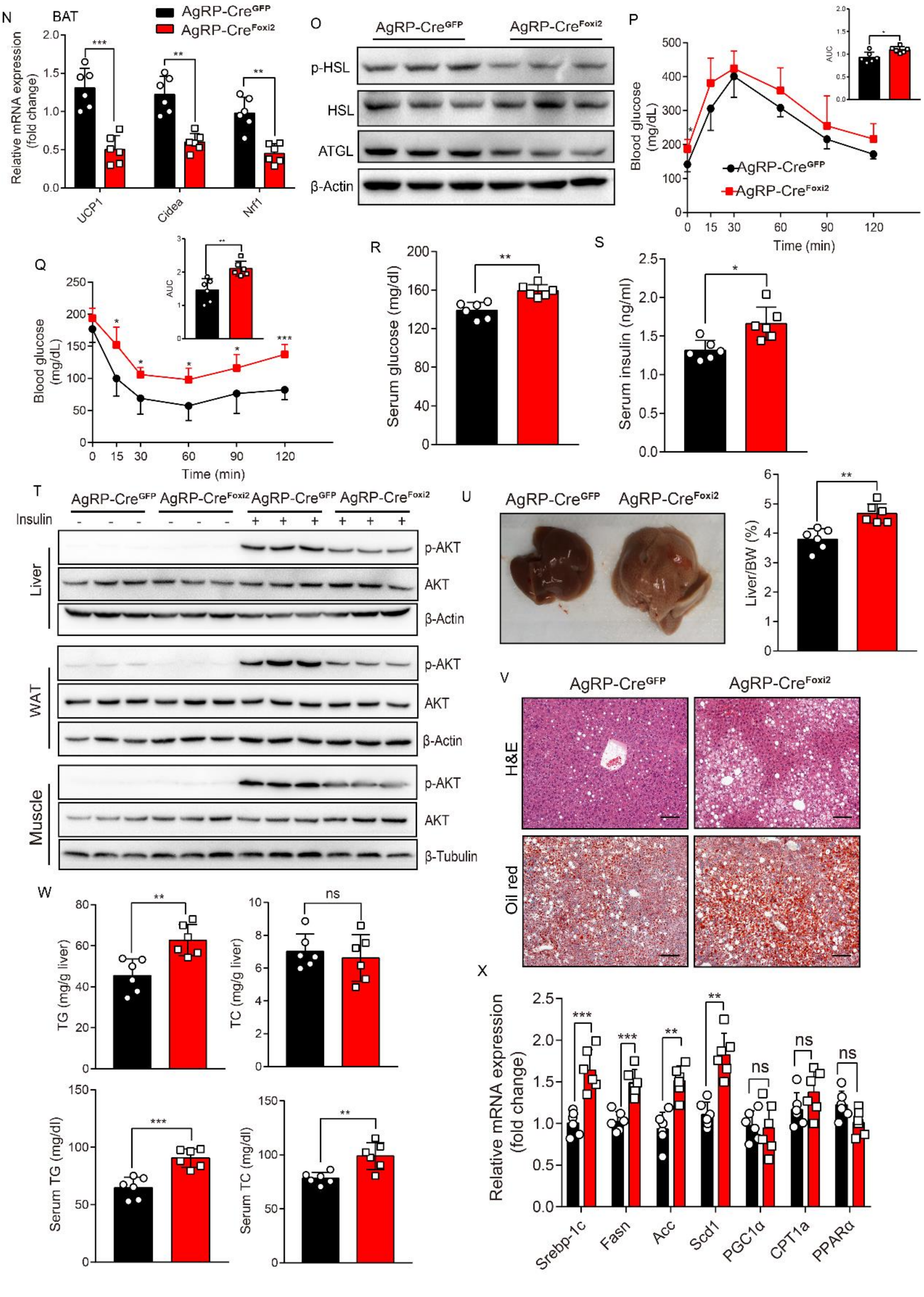


Supplemental Figure 2. Overexpression of Foxi2 in AgRP neurons of female mice causes obesity under a chow diet condition. 14-week-old female AgRP-IRES-Cre mice were received bilateral AAV-Foxi2 injection into ARC. These mice were studied at 22 weeks of age. A: The body weight of AAV-Foxi2 and AAV-GFP-infected mice at

22 weeks of age (n= 6/group). B: Fat and lean mass (n= 6/group). C: Daily food intake (n= 6/group). D: Accumulative food intake of 24 hours in mice fasted overnight (n= 6/group). E and F: Energy expenditure (EE) was measured and analyzed by ANCOVA (n= 6/group). All EE data are expressed as the absolute value (E). All ANCOVA values are expressed as estimated marginal means ± SEM. Estimated effect size of covariates for ANCOVA analysis is presented as partial eta-squared (F). G: Respiratory quotient (VCO_2/VO_2) (n= 6/group). H: Locomotor activity of mice in A (n= 6/group). I: Quantitative PCR analysis of mRNA levels of genes involved in thermogenesis and fatty acid oxidation in BAT from AgRP-cre^{GFP} and AgRP-cre^{Foxi2} mice (n= 6/group). J: Western blotting analysis of UCP1 protein level in BAT of AgRP-cre^{GFP} and AgRP-cre^{Foxi2} mice. K and L: Plots for glucose tolerance tests and insulin tolerance tests (insert graphs represent the area under the curve) from AgRP-cre^{GFP} and AgRP-cre^{Foxi2} mice on a chow diet (n= 6/group). Data are represented as mean ± SEM. *p<0.05, ** p<0.01, *** p<0.001, two-tailed Student's t test (A-I, K, L), ANCOVA (F). Abbreviations: n.s., not significant.



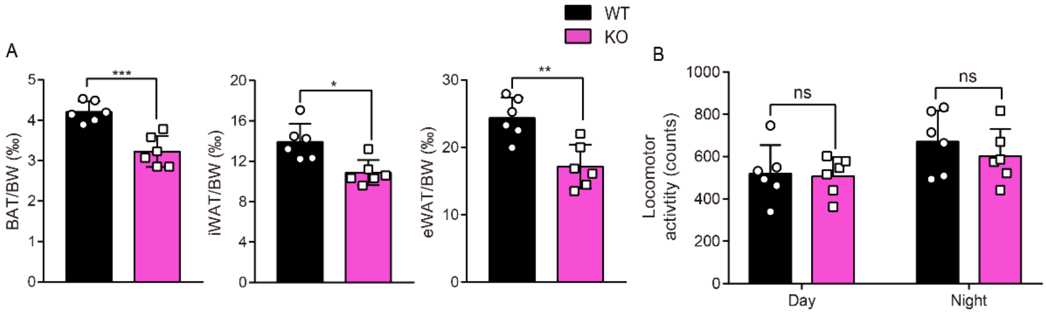
A
Body Weight (g)
Weeks after HFD feeding
AgRP-CreGFP
AgRP-CreFoxi2
B
AgRP-CreGFP AgRP-CreFoxi2
C
Fat mass (g)
Lean mass (g)
ns
D
AgRP-CreGFP
AgRP-CreFoxi2
BAT
iWAT
eWAT
E
BAT/BW (‰)
iWAT/BW (‰)
eWAT/BW (‰)
F
AgRP-CreGFP
AgRP-CreFoxi2
G
Food intake (g/day)
H
Accumulative food intake (g)
24 h
I
EE (kcal/hr)
Day
Night
Total
J
Partial Eta²=0.167 (lean mass)
Partial Eta²=0.019 (fat mass)
EE (fat and lean mass-adjusted ANCOVA means,kcal/hr)
Partial Eta²=0.001 (body weight)
EE (body weight-adjusted ANCOVA means,kcal/hr)
K
RQ
L
Locomotor activity (counts)
M
Body temperature(℃)



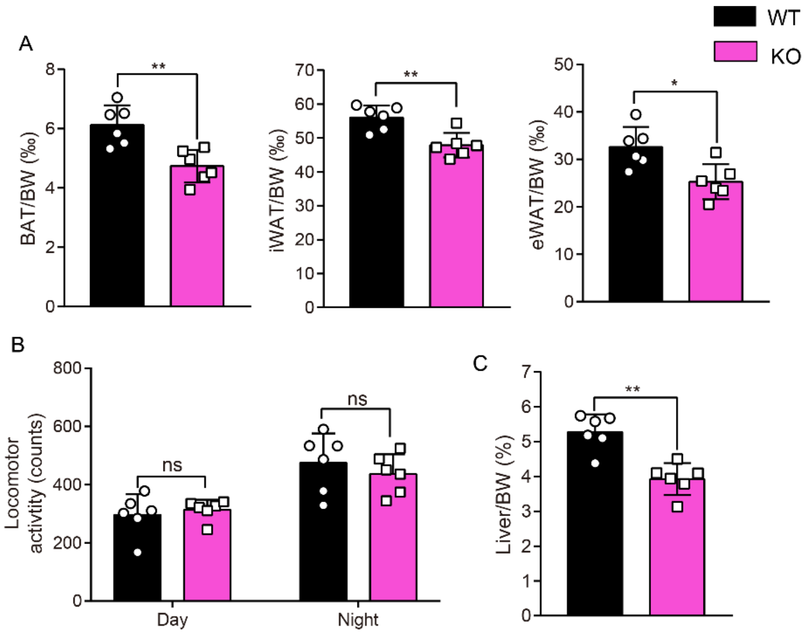
Supplemental Figure 3. Overexpression of Foxi2 in AgRP neurons aggravates HFD-induced obesity. A and B: The growth curve starting at 2 weeks after receiving AAV-GFP or AAV-Foxi2 bilaterally in ARC of 6-week old male AgRP-IRES-Cre mice fed an HFD and a representative photograph of AgRP-cre^{GFP} and AgRP-cre^{Foxi2} mice at

22 weeks of age under an HFD (n= 6/group). C: MRI assay of body composition of AgRP-cre^{GFP} and AgRP-cre^{Foxi2} mice (n= 6/group). D: Gross appearance of interscapular BAT, inguinal and epididymal fat pads from mice in B. E: Tissue weight of BAT, iWAT and eWAT in AgRP-cre^{GFP} and AgRP-cre^{Foxi2} mice (n= 6/group). F: Hematoxylin and eosin staining of paraffin-embedded BAT, iWAT and eWAT sections in AgRP-cre^{GFP} and AgRP-cre^{Foxi2} mice. Scale bar, 50 μm. G: Daily food intake (n= 6/group). H: Accumulative food intake of 24 hours in AgRP-cre^{GFP} and AgRP-cre^{Foxi2} mice fasted overnight (n= 6/group). I and J: Energy expenditure (EE) was measured and analyzed by ANCOVA (n= 6/group). All EE data are expressed as the absolute value (I). All ANCOVA values are expressed as estimated marginal means ± SEM. Estimated effect size of covariates for ANCOVA analysis is presented as partial eta-squared (J). K: Respiratory quotient (VCO_2/VO_2) (n= 6/group). L: Locomotor activity of mice (n= 6/group). M: The rectal temperature of AgRP-cre^{GFP} and AgRP-cre^{Foxi2} mice subjected to acute cold exposure for 6 hours (n= 6/group). N: Quantitative PCR analysis of mRNA levels of UCP1, Cidea and Nrf1 in BAT of AgRP-cre^{GFP} and AgRP-cre^{Foxi2} mice (n= 6/group). O: Western blotting analysis of lipolytic enzymes in eWAT from AgRP-cre^{GFP} and AgRP-cre^{Foxi2} mice. P and Q: Plots for glucose tolerance tests and insulin tolerance tests (insert graphs represent the area under the curve) from AgRP-cre^{GFP} and AgRP-cre^{Foxi2} mice on an HFD (n= 6/group). R and S: Blood glucose and insulin levels in AgRP-cre^{GFP} and AgRP-cre^{Foxi2} mice under an ad libitum condition (n= 6/group). T: Western blotting analysis of AKT and p-AKT (Ser473) in the liver, adipose tissue, and muscle 15 min after administration of insulin (1 U/kg). U: Representative photograph

and liver weight in AgRP-cre^{GFP} and AgRP-cre^{Foxi2} mice (n= 6/group). V: Representative images of H&E- and Oil-red O-stained hepatic sections of AgRP-cre^{GFP} and AgRP-cre^{Foxi2} mice. Scale bar, 100 μm. W: Hepatic TG, TC content and serum TG, TC content of AgRP-cre^{GFP} and AgRP-cre^{Foxi2} mice (n= 6/group). X: Quantitative PCR analysis of expression of Srebp-1c, Fasn, Acc, Scd1, PGC1α, CPT1a and PPARα in the livers of AgRP-cre^{GFP} and AgRP-cre^{Foxi2} mice (n= 6/group). Data are represented as mean ± SEM. *$p<0.05$, **$p<0.01$, ***$p<0.001$, two-tailed Student's t test (A, C, E, G-N, P-S, U, W, X), ANCOVA (J). Abbreviations: n.s., not significant.



Supplemental Figure 4. Global Foxi2 KO mice are lean under a chow diet condition. A: Tissue weight of BAT, iWAT and eWAT in WT and KO mice fed with a chow diet (n= 6/group). B: Locomotor activity of mice (n= 6/group). Data are represented as mean ± SEM. *p<0.05, ** p<0.01, *** p<0.001, two-tailed Student's t test (A, B). Abbreviations: n.s., not significant.



Supplemental Figure 5. Foxi2 KO mice are resistant to HFD-induced obesity. A: Tissue weight of BAT, iWAT and eWAT in WT and KO mice (n= 6/group). B: Locomotor activity of mice (n= 6/group). C: Liver weight of WT and KO mice (n= 6/group). Data are represented as mean ± SEM. *p<0.05, ** p<0.01, two-tailed Student's t test (A-C). Abbreviations: n.s., not significant.

Supplemental Table 1. Primers used in quantitative-PCR, ChIP assay and plasmids construction.

Gene symbol	Forward primer	Reverse primer
Foxi2	5'-TTCCGAAGGAAGAGGAGACGG-3'	5'-CAAGTAGTGGTGGCCTCAGACG-3'
Ucp1	5'-AGGCTTCCAGTACCATTAGGT-3'	5'-CTGAGTGAGGCAAAGCTGATTT-3'
Cidea	5'-GCCGTGTTAAGGAATCTGCTG-3'	5'-TGCTCTTCTGTATCGCCCAGT-3'
Dio2	5'-CAGTGTGGTGCACGTCTCCA ATC-3'	5'-TGAACCAAAGTTGACCACCAG-3'
PGC1α	5'-TGATGTGAATGACTTGGATACA GACA-3'	5'-GCTCATTGTTGTACTGGTTGGAT ATG-3'
Nrf1	5'-GCACCTTTGGAGAATGTGGT-3'	5'-CTGAGCCTGGGTCATTTTGT-3'
Sdhb	5'-AATTTGCCATTTACCGATGGGA-3'	5'-AGCATCCAACACCATAGGTCC-3'
Srebp1c	5'-GGAGCCATGGATTGCACATT-3'	5'-GGCCCGGGAAGTCACTGT-3'
Fasn	5'-GTAAGTTCTGTGGCTCCAGAG-3'	5'-GCCCTCCCGTACACTCACTC-3'
Acc	5'-AGGAAGATGGCGTCCGCTCTG-3'	5'-GGTGAGATGTGCTGGGTCAT-3'
Scd1	5'-CCTGCCTCTTCGCGTTTGT-3'	5'-GGCGTGCCTTGTACGTTCT-3'
CPT1a	5'-GAACCCCAACATCCCCAAAC-3'	5'-TCCTGGCATTCTCCTGGAAT-3'
PPARα	5'-ACAAGGCCTCAGGGTACCA-3'	5'-GCCGAAAGAAGCCCTTACAG-3'

MCAD	5'-AACACTTACTATGCCTCGATTGCA-3'	5'-CCATAGCCTCCGAAAATCTGAA-3'
AgRP	5'-ATGCTGACTGCAATGTTGCTG-3'	5'-CAGACTTAGACCTGGGAACTCT-3'
NPY	5'-TGGCCAGATACTACTCCGCT-3'	5'-AGTGTCTCAGGGCTGGATCT-3'
POMC	5'-GGCTTGCAAACTCGACCTCT-3'	5'-CGTACTTCCGGGGGTTTTCA-3'
CART	5'-AAGAAGTACGGCCAAGTCCC-3'	5'-CAGTCACACAGCTTCCCGAT-3'
36B4	5'-GAGGAATCAGATGAGGATATGGGA-3'	5'-AAGCAGGCTGACTTGGTTGC-3'
Foxi2-shRNA primers	CCGGGCTCAGCCAGATCTACCAGTACTCGAGTACTGGTAGATCTGGCTGAGCTTTTTG	AATTCAAAAAGCTCAGCCAGATCTACCAGTACTCGAGTACTGGTAGATCTGGCTGAGC
AgRP ChIP primers	5'-CTTCGTGGGTACTGGCCATG-3'	5'-TTTTGACAGAATCTTACTATGTAAGGCC-3'
AgRP promoter-1795Luc	5'-CTAGCTAGCCTTGTTTACCATTGCGCGAC-3'	5'-CCGCTCGAGTATCACCATGCAGCAGTGAA-3'
AgRP promoter-545Luc	5'-CTAGCTAGCACTGGGGTATATCCCTTCTC-3'	5'-CCGCTCGAGTATCACCATGCAGCAGTGAA-3'
AgRP promoter-	5'-CTAGCTAGCCTTCGTGGGTACTGGCCATG-3'	5'-CCGCTCGAGTATCACCATGCAGCAGTGAA-3'

345Luc		
AgRP promoter-195Luc	5’-CTAGCTAGCTCTGTAAATTGGAGGCCAGC-3’	5’-CCGCTCGAGTATCACCATGCAGCAGTGAA-3’