Supplementary Information

Dietary obesity-induced Egr-1 in adipocytes facilitates energy storage via suppression of FOXC2

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Table S1. Real-time RT-PCR Primer Sequences

Gene Analyzed	Accession number	QPCR Primer Sequences
Human Egr-1	NM_001964	5'gagcacctgaccgcagagt3'
		5'ccagcaccttctcgttgttc3'
Mouse Egr-1	NM_007913	5'cgagcgaacaaccctatgag3'
		5'cattattcagagcgatgtcagaaa3'
Mouse FOXC2	NM_031519	5'gcttatggaccaaacccatagg3'
		5'gcgcccgtggagaatct3'
Mouse β1-AR	NM_007419	5'cgagctctggacttcggtaga3'
		5'tgacacacagggtctcaatgc3'
PGC-1α	NM_008904	5'gactcagtgtcaccaccgaaa3'
		5'tgaacgagagcgcatcctt3'
PGC-1β	NM_133249	5'gagggctccggcacttc3'
		5'cgtacttgcttttcccagatga3'
UCP-1	NM_009463	5'aagctgtgcgatgtccatgt3'
		5'aagccacaaacccttttgaaaa3'

Figure S1, Zhang et al.

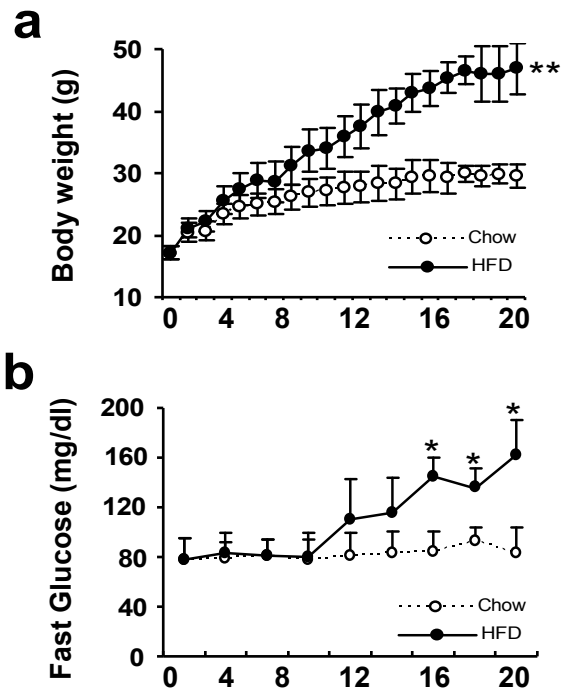

Fig.S1. HFD-induced obesity and diabetes of mice. C57BL/6J mice were purchased from Jackson laboratories and divided into two groups fed with chow-diet or high-fat diet, respectively. Body weight was recorded weekly (**a**) and fasted glucose level was recorded biweekly (**b**). Data represent mean±SD, n=5. *P<0.05, **P<0.01 vs chow group.

Figure S2, Zhang et al.

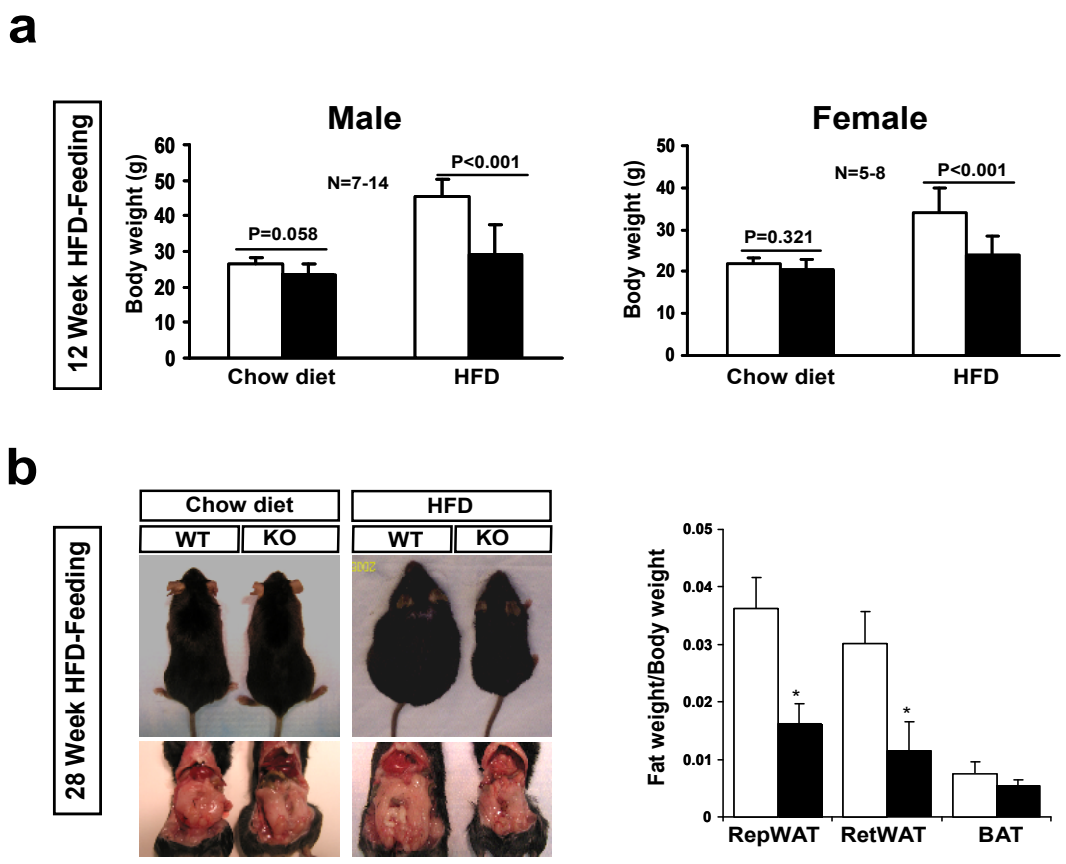

Fig.S2. Both male and female of Egr-1 null mice are resistant to diet-induced obesity. a) Body weight was measured before high-fat feeding and after 12 weeks high-fat feeding for both male and female mice. **b)** Male, wild-type or Egr-1-/- mice were fed with chow or high-fat diet for 20 weeks. Appearance of mice was present on left; the weight ratio of reproductive fat pads, retroperitoneal fat pads and BAT to body weight was presented on right. white bar represent wild-type, black bar represent knockout mice. Data were presented as mean±SD, *P<0.01 vs wild-type.

Figure S3, Zhang et al.

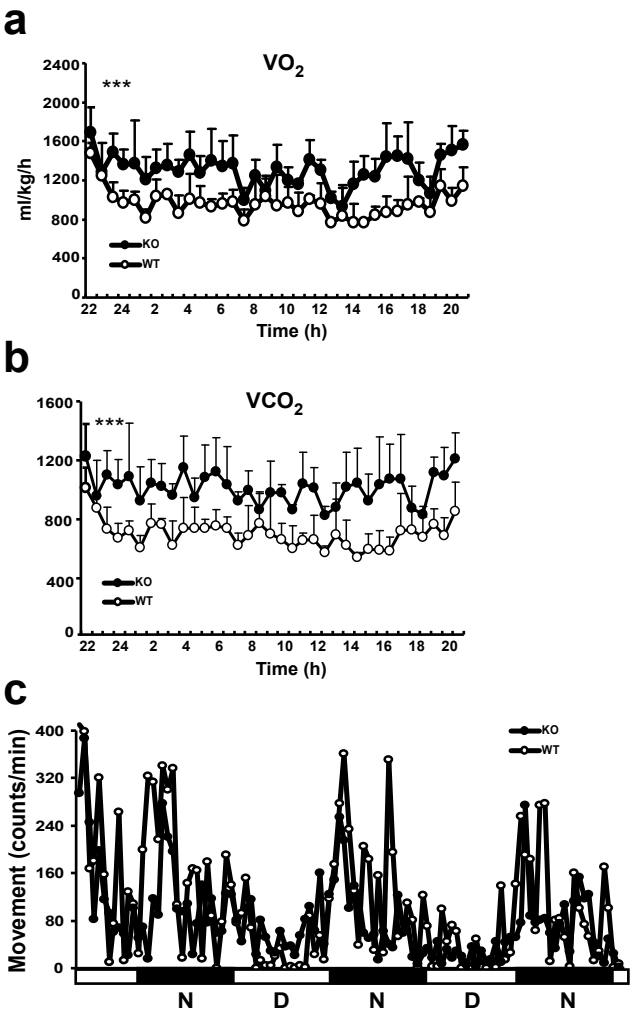

Fig.S3. a & b) Oxygen consumption (VO2) and carbon dioxide production (VCO2) were measured by metabolic cage system (CLAMS, Columbia Instrument) during a period of three days after 16 week of high-fat feeding (n=5). Values from 24 hrs were presented. Values are expressed as the mean±SD. (***, P<0.001). **c)** Physical activity was measured with CLAMS. Representative trace of movement monitoring for high-fat fed mice over a period of 3 days

Figure S4, Zhang et al.

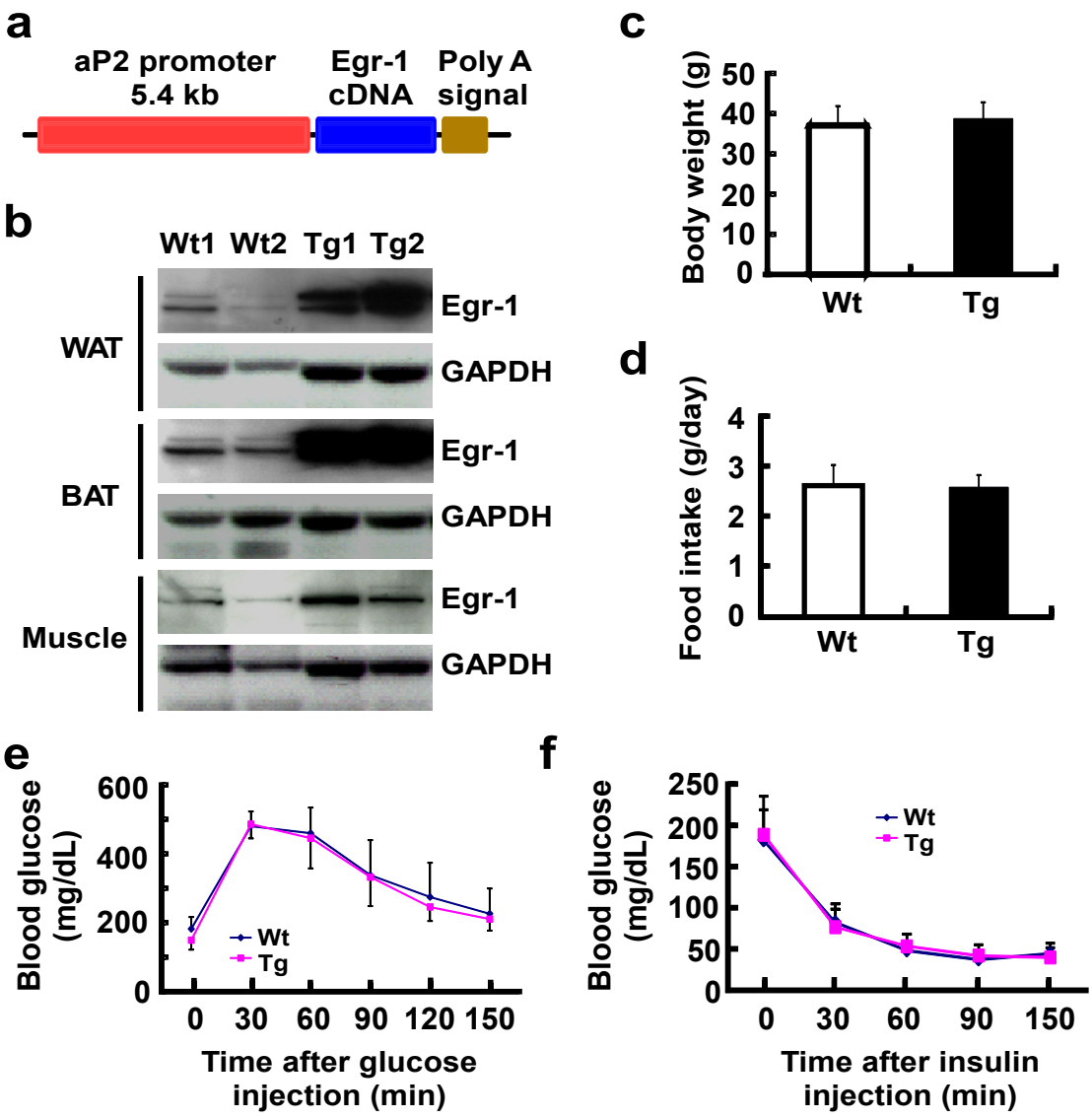

Fig.S4. Overexpression of Egr-1 in adipose does not change the metabolic parameters. a) Construct vector for generation of adipose-specific Egr-1 transgenic mice. aP2 promoter was used to drive rat Egr-1 cDNA expression in adipose tissues. **b)** Two lines of wild-type (Wt) and Egr-1 transgenic (Tg) mice were obtained and the Egr-1 expression in white adipose tissues (WAT), brown adipose tissues (BAT and muscle were detected by western blot. GAPDH was served as loading control. **c&d)** Both body weight and food intake in adult, male wild-type and transgenic mice were measured. n=10. **e&f)** both glucose tolerance (GTT) and insulin tolerance (ITT) tests were performed by injection (i.p.) with 2 g/kg of glucose or 0.75 IU/kg of insulin. The blood glucose levels were recorded at indicated time after injection. Data were presented as mean±SD, n=7.

Figure S5, Zhang et al.

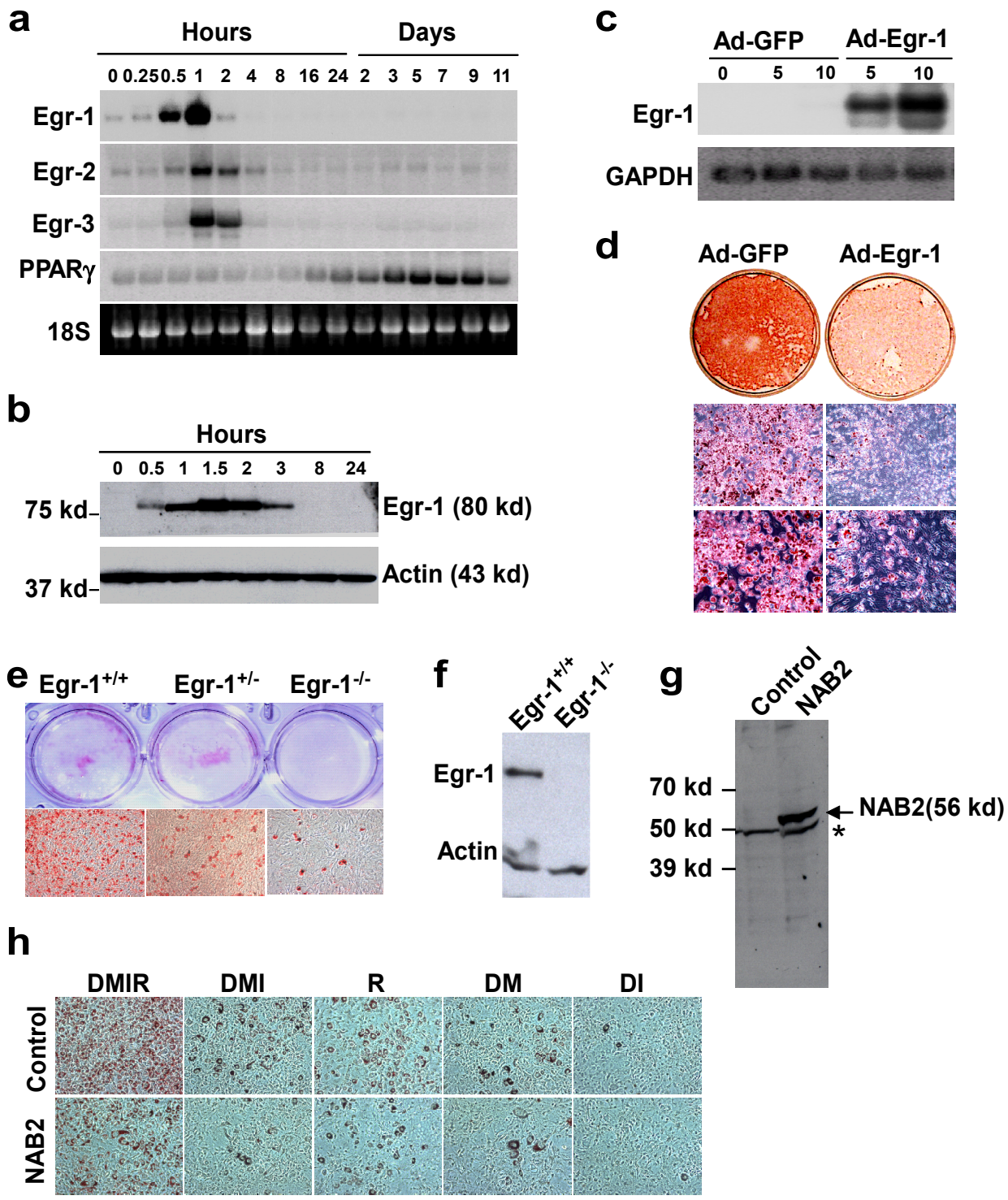

Fig.S5. Transient expression of Egr-1 is required for 3T3-L1 adipogenesis. **a)** Northern blot showed Egr-1, Egr-2 and Egr-3 were transient expressed during 3T3-L1 adipogenesis. PPARγ is a marker of adipogenesis. 18S was served as loading control. **b)** Western blot showed Egr-1 protein was also transient expressed during 3T3-L1 adipogenesis. **c&d)** Adenovirus infection-mediated sustained overexpression in 3T3-L1 cells reduced adipogenesis. Western blot confirmed adenovirus infection with 5, 10 MOI mediated overexpression of Egr-1 in 3T3-L1 cells. GAPDH was served as loading control **(c)**. Adenovirus-infected 3T3-L1 cells were treated with DMI for 7 days and subjected to Oil O staining **(d)**. **e)** Mouse embryonic fibroblasts isolated from Egr-1+/+, Egr-1+/- and Egr-1-/- mice were induced with DMI for 9 days and subjected to Oil O staining. **f)** Western blot confirmed Egr-1 is not expressed in the MEFs from Egr-1-/- mice. Actin was served as loading control. **g&h)** NAB2 (a Egr-1 inhibitor) stable cell line and control 3T3-L1 cell line were induced by different combinations of inducer (D: dexamethasone; M: IBMX; I: insulin; R: rosiglitazone) for 7 days and subjected Oil O staining. Overexpression of NAB2 was confirmed by Western blot **(g)**. All experiments were repeated three times and showed similar results.