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Supplemental information

Estradiol cycling drives female obesogenic adipocyte hyperplasia

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Figure S1

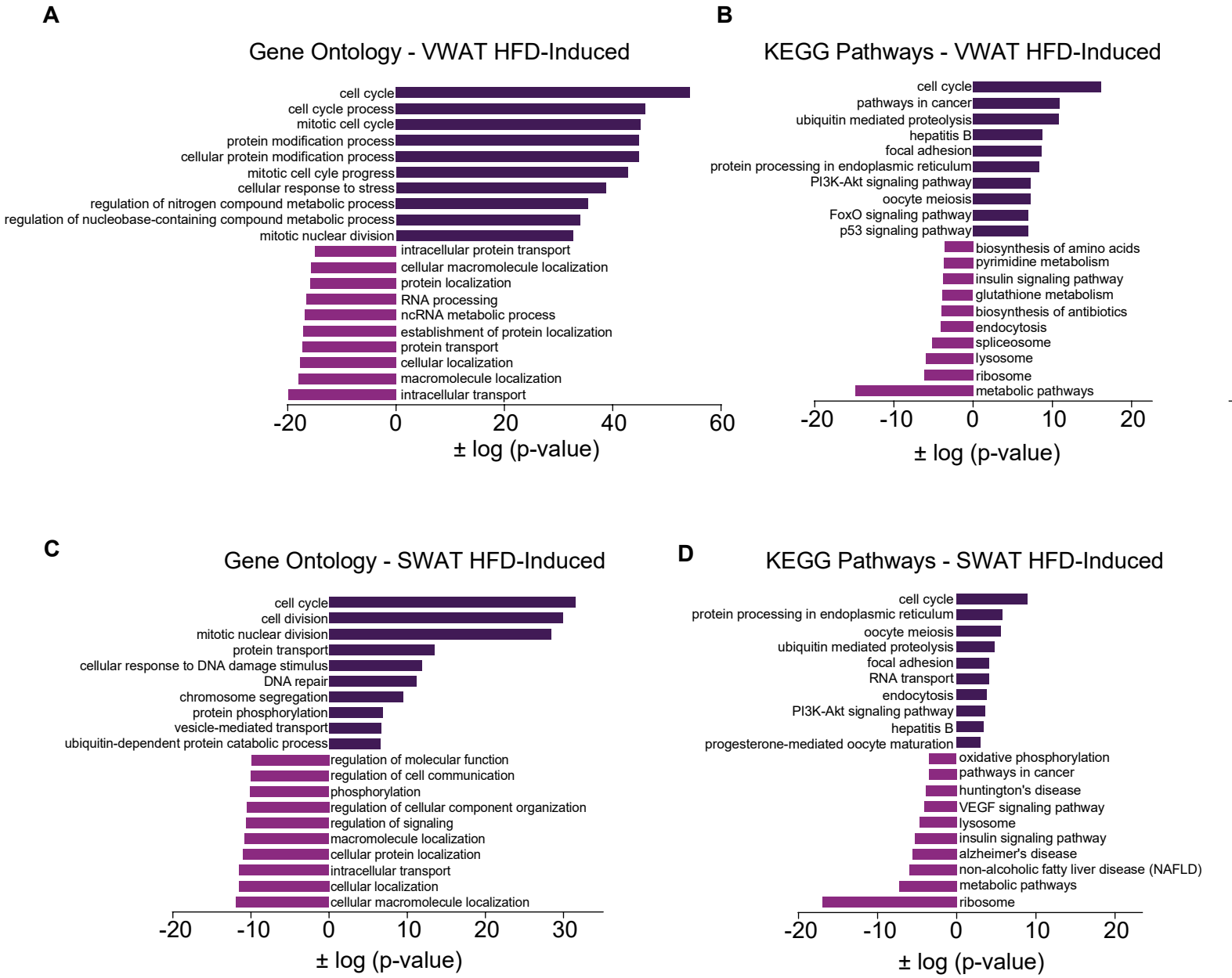
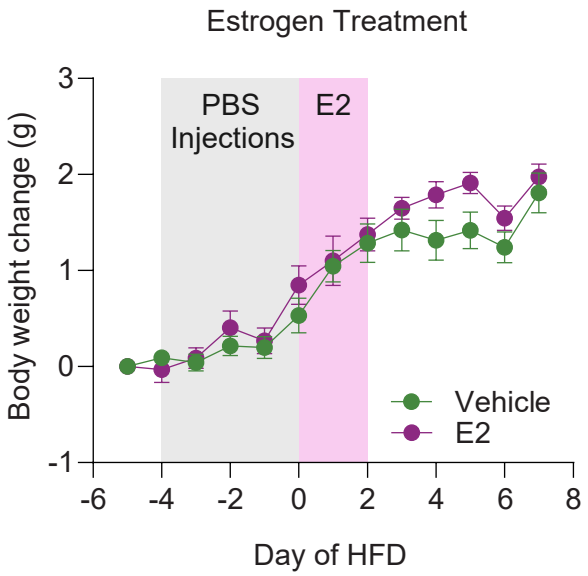

A
Gene Ontology - VWAT HFD-Induced
cell cycle
cell cycle process
mitotic cell cycle
protein modification process
cellular protein modification process
mitotic cell cyle progress
cellular response to stress
regulation of nitrogen compound metabolic process
regulation of nucleobase-containing compound metabolic process
mitotic nuclear division
intracellular protein transport
cellular macromolecule localization
protein localization
RNA processing
ncRNA metabolic process
establishment of protein localization
protein transport
cellular localization
macromolecule localization
intracellular transport
-20
0
20
40
60
± log (p-value)
B
KEGG Pathways - VWAT HFD-Induced
cell cycle
pathways in cancer
ubiquitin mediated proteolysis
hepatitis B
focal adhesion
protein processing in endoplasmic reticulum
PI3K-Akt signaling pathway
oocyte meiosis
FoxO signaling pathway
p53 signaling pathway
biosynthesis of amino acids
pyrimidine metabolism
insulin signaling pathway
glutathione metabolism
biosynthesis of antibiotics
endocytosis
spliceosome
lysosome
ribosome
metabolic pathways
-20
-10
0
10
20
± log (p-value)
C
Gene Ontology - SWAT HFD-Induced
cell cycle
cell division
mitotic nuclear division
protein transport
cellular response to DNA damage stimulus
DNA repair
chromosome segregation
protein phosphorylation
vesicle-mediated transport
ubiquitin-dependent protein catabolic process
regulation of molecular function
regulation of cell communication
phosphorylation
regulation of cellular component organization
regulation of signaling
macromolecule localization
cellular protein localization
intracellular transport
cellular localization
cellular macromolecule localization
-20
-10
0
10
20
30
± log (p-value)
D
KEGG Pathways - SWAT HFD-Induced
cell cycle
protein processing in endoplasmic reticulum
oocyte meiosis
ubiquitin mediated proteolysis
focal adhesion
RNA transport
endocytosis
PI3K-Akt signaling pathway
hepatitis B
progesterone-mediated oocyte maturation
oxidative phosphorylation
pathways in cancer
huntington's disease
VEGF signaling pathway
lysosome
insulin signaling pathway
alzheimer's disease
non-alcoholic fatty liver disease (NAFLD)
metabolic pathways
ribosome
-20
-10
0
10
20
± log (p-value)

Figure S1. Cell cycle pathways are upregulated in female APCs at the onset of HFD feeding, **Related to Figure 1.**

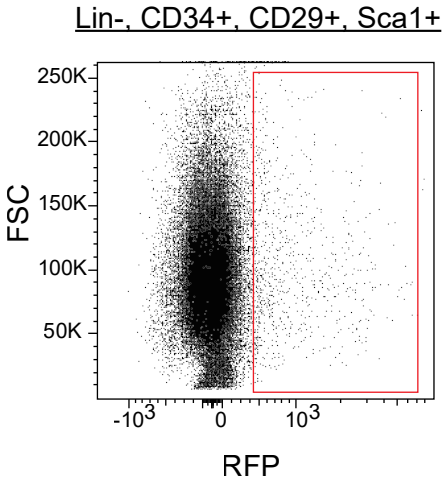
Top diet-induced upregulated (positive) and downregulated (negative) GO terms **(A)** and KEGG pathways **(B)** in VWAT APCs. Top diet-induced upregulated (positive) and downregulated (negative) GO terms **(C)** and KEGG pathways **(D)** in SWAT APCs. N= 5 samples per group, 3 mice pooled for each sample. GO: gene ontology, KEGG: Kyoto encyclopedia of genes and genomes, VWAT: perigonadal fat, SWAT: inguinal subcutaneous fat, HFD: high-fat diet.

Figure S2

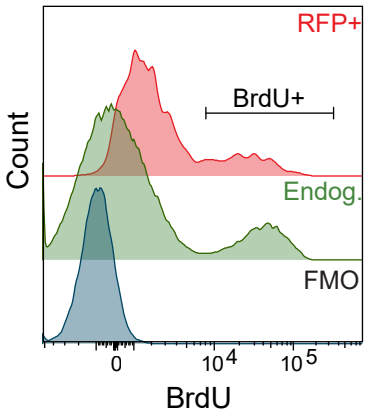
A



B



C



D

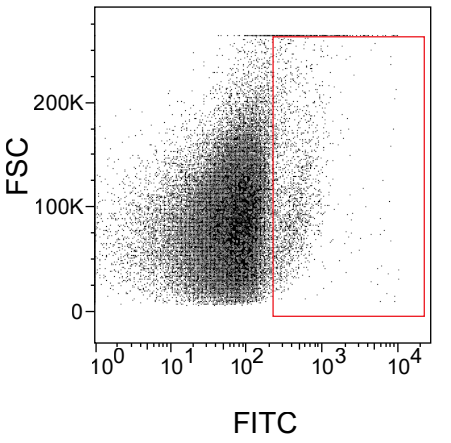


Figure S2. Estradiol drives female APC proliferation in an ERα-dependent manner, Related to Figure 3.

(A) Body weight change during vehicle or E2 treatment (day 0 to 2 of a HFD) in female mice (n= 14-19 mice per group). **(B)** Representative flow cytometry plot identifying transplanted wildtype APCs (RFP+) into wildtype male VWAT. **(C)** Representative histogram of BrdU incorporation into endogenous (RFP -) and transplanted (RFP+) APCs demonstrating that wildtype APCs maintain proliferation capacity after transplantation as described in Jeffery et. al. 2016. **(D)** Representative flow cytometry plot identifying transplanted ERα-KO APCs (GFP+) in female recipient SWAT. Statistical significance was determined by one-way ANOVA with Šidák's tests for panel A. Error bars represent mean ± S.E.M. E2: 17β-estradiol, HFD: high-fat diet, FSC: forward scatter, BrdU: bromodeoxyuridine, Endog: endogenous cells, FMO: fluorescence minus one.

Figure S3

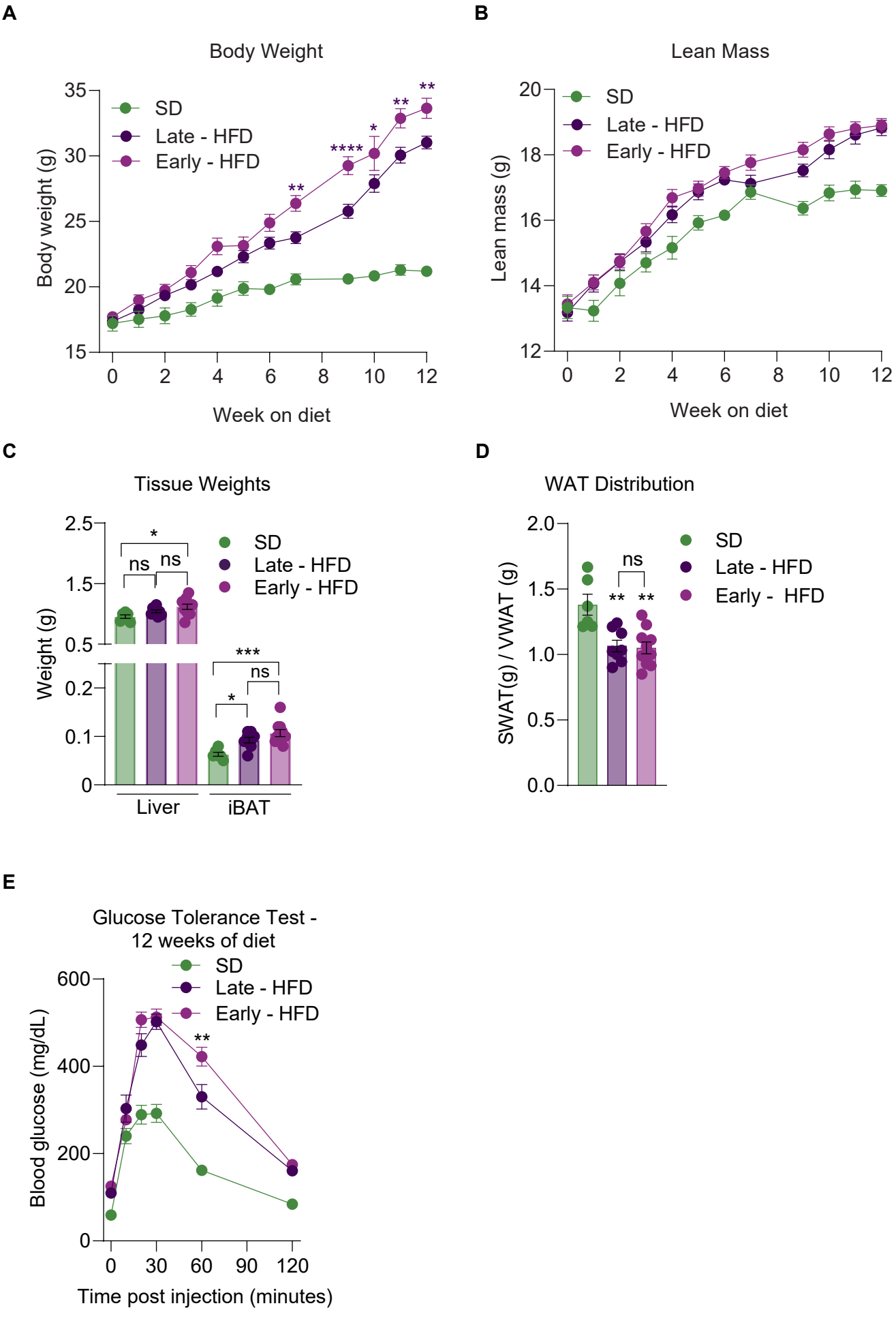

A
Body Weight
Body weight (g)
Week on diet
SD
Late - HFD
Early - HFD
B
Lean Mass
Lean mass (g)
Week on diet
C
Tissue Weights
Weight (g)
Liver
iBAT
D
WAT Distribution
SWAT(g) / VWAT (g)
E
Glucose Tolerance Test -
12 weeks of diet
Blood glucose (mg/dL)
Time post injection (minutes)

Figure S3. Timing of HFD determines hyperplasia in female mice, Related to Figure 4.

(A) Body weight and lean mass weight **(B)** of female mice during 12 weeks of SD or HFD feeding. **(C)** Liver and iBAT weight after 12 weeks of SD or HFD feeding. **(D)** WAT distribution (SWAT/VWAT) after 12 weeks of SD or HFD feeding. **(E)** Blood glucose levels during glucose tolerance test. N= 6-10 mice per group. Statistical significance was determined by ordinary two-way ANOVA with Tukey's test for panels A, B, and E and ordinary one-way ANOVA with Tukey's test for panel C and D. Error bars represent mean ± S.E.M. ns= not significant, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001. SD: standard diet, HFD: high-fat diet, iBAT: interscapular brown adipose tissue.

Figure S4

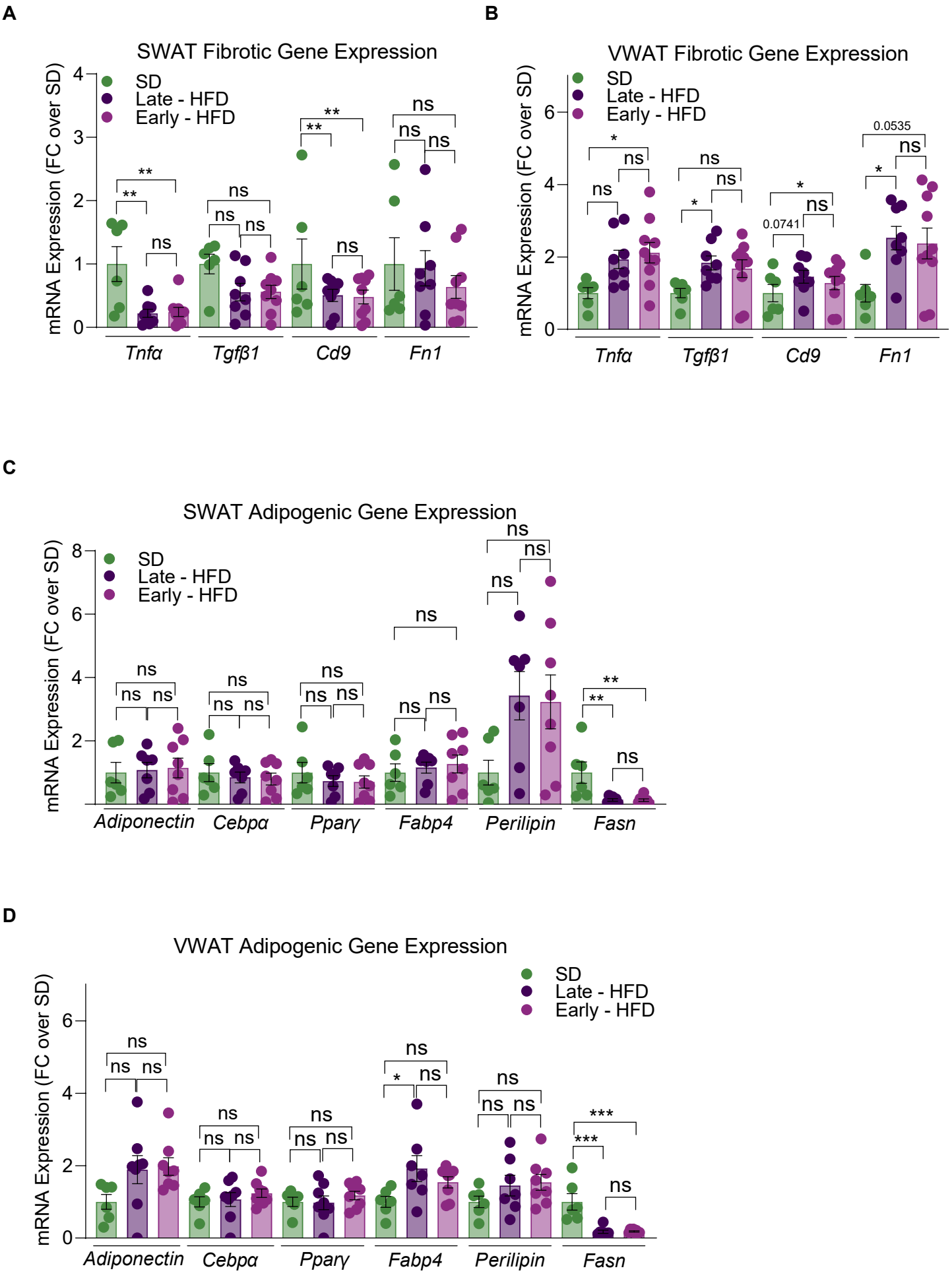

A
SWAT Fibrotic Gene Expression
mRNA Expression (FC over SD)
SD
Late - HFD
Early - HFD
Tnfα
Tgfβ1
Cd9
Fn1
B
VWAT Fibrotic Gene Expression
0.0741
0.0535
C
SWAT Adipogenic Gene Expression
Adiponectin
Cebpα
Pparγ
Fabp4
Perilipin
Fasn
D
VWAT Adipogenic Gene Expression

Figure S4. Timing of HFD does not impact expression of fibrotic or adipogenic genes in WAT, Related to Figure 4.

Gene expression of several fibrotic genes quantified by qPCR in SWAT **(A)** and **(B)** VWAT of female mice after 12 weeks of SD or HFD feeding. Gene expression of several adipogenic genes quantified by qPCR in SWAT **(C)** and **(D)** VWAT of female mice after 12 weeks of SD or HFD feeding. Data presented as fold change (FC) over SD after normalizing to housekeeping gene (*Tbk1)*. N= 6-10 mice per group. Statistical significance was determined by ordinary one-way ANOVA for every gene with Tukey's test. Error bars represent mean ± S.E.M. ns= not significant, *p<0.05, **p<0.01, ***p<0.001. SD: standard diet, HFD: high-fat diet, VWAT: perigonadal fat, SWAT: inguinal subcutaneous fat.

Table S1. Primers used for qPCR analysis, Related to Figure 4 and Figure S4.

Gene name	Forward Primer (5'-3')	Reverse Primer (5'-3')
Tbk1	AGGGCTTTGTGACGGGAACAG	GGCACCCGGTCAAATGAGA
Cd9	ATGCCGGTCAAAGGAGGTAG	GCCATAGTCCAATAGCAAGCA
Tnfα	GCCTGTAGCCCACGTCGTAGC	CGGGGCAGCCTTGTCCCTTG
Fn1	GGCCACCATTACTGGTCTGG	GGAAGGGTAACCAGTTGGGG
Tgfβ1	ACTGGAGTTGTACGGCAGTG	GGGGCTGATCCCGTTGATT
Adiponectin	GGAGATGCAGGTCTTCTTGG	GCGATACACATAAGCGGCTTC
Pparγ	GCCTGCGGAAGCCCTTTGGT	AAGCCTGGGCGGTCTCCACT
Cebpα	CCCAGAGGACCAATGAAATGAAG	TAGCCGGAGGAAGCTAAGAC
Fabp4	GGGATGGAAAGTCGACCACA	CGCCTTTCATAACACATTCCACC
Perilipin	GGGCTGTCTGAGACTGAGGT	TCCTTACTCTCCACGCTGTAAC
Fasn	GGAGGTGGTGATAGCCGGTAT	TGGGTAATCCATAGAGCCCAG