

SUPPLEMENTAL INFORMATION:

**Ces1d deficiency protects against high-sucrose diet-induced hepatic triacylglycerol
accumulation**

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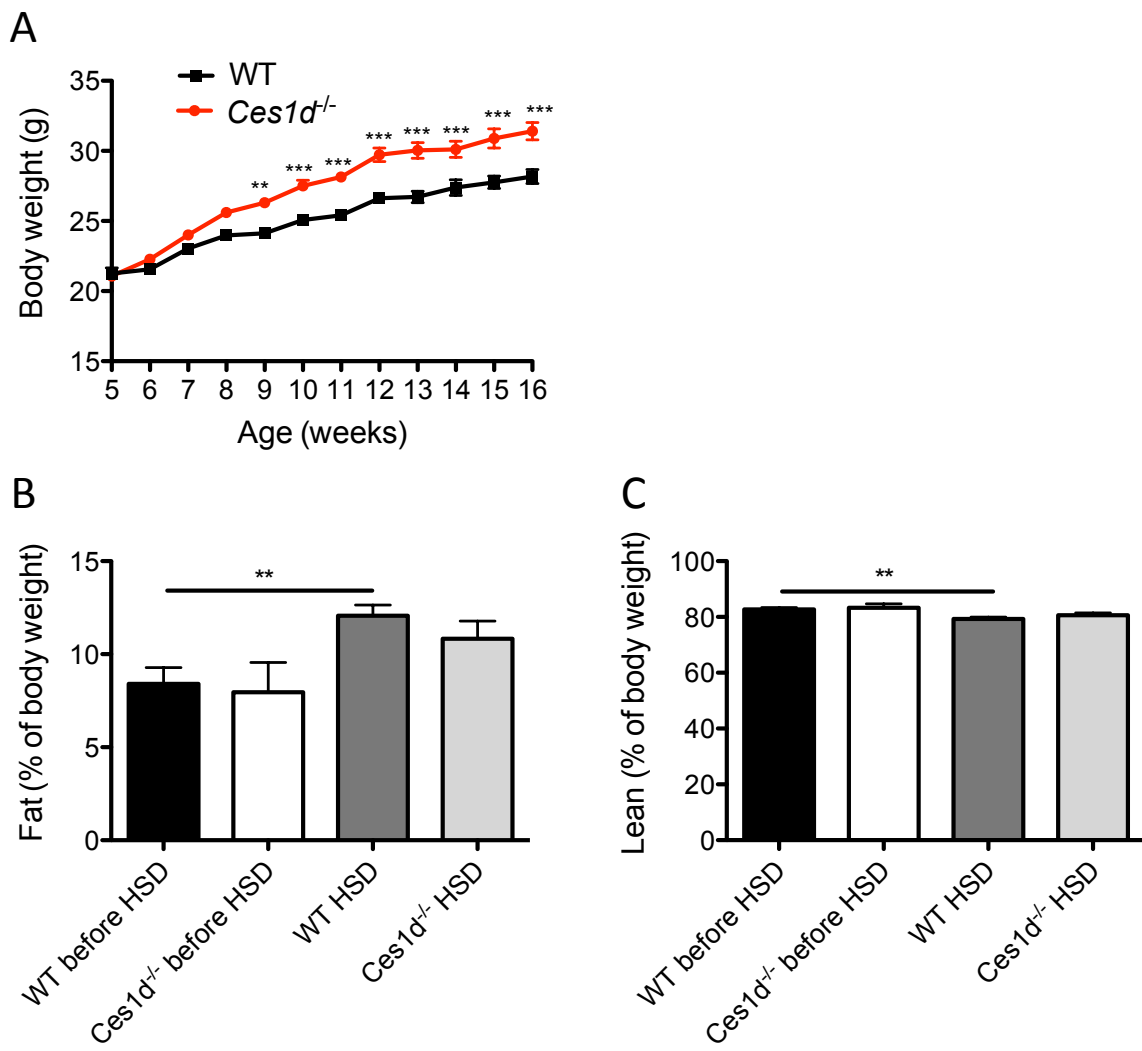
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Supplemental Table S1. Formula of high sucrose diet (HSD)

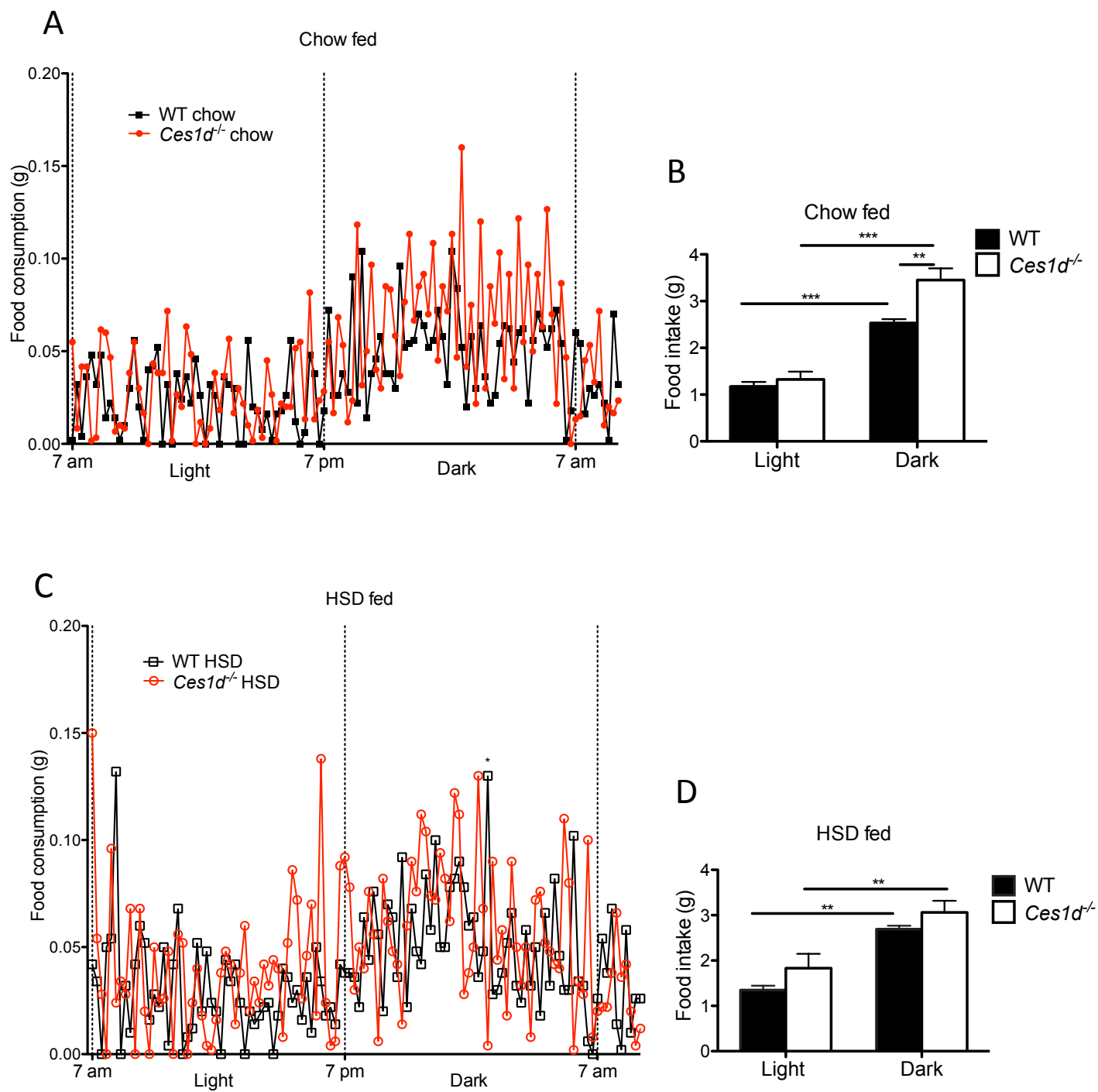
Formula	g/kg diet
Vitamin free casein	211
Alphacel, non-nutritive bulk	164.50
Sucrose	584.50
Salt mixture U.S.P. XIV	40
Vitamin diet fortification mixture	6
Dry vitamin A acetate (500,000U/g)	0.040
Calciferol (850,000U/g)	0.0026
Alpha tocopherol powder 250 I.U.	0.48
Inositol	0.11
Choline chloride	6
Menadione	0.049
Biotin	0.45
P amino benzoic acid	0.11
Ascorbic acid	0.99
Niacin	0.098
Riboflavin	0.022
Pyridoxine HCl	0.022
Thiamine HCl	0.022
Calcium pantothenate	0.066
Folic acid	0.0020
B-12 (trituration 0.1%)	0.030

Supplemental Table S2. Primers used for quantitative PCR analysis

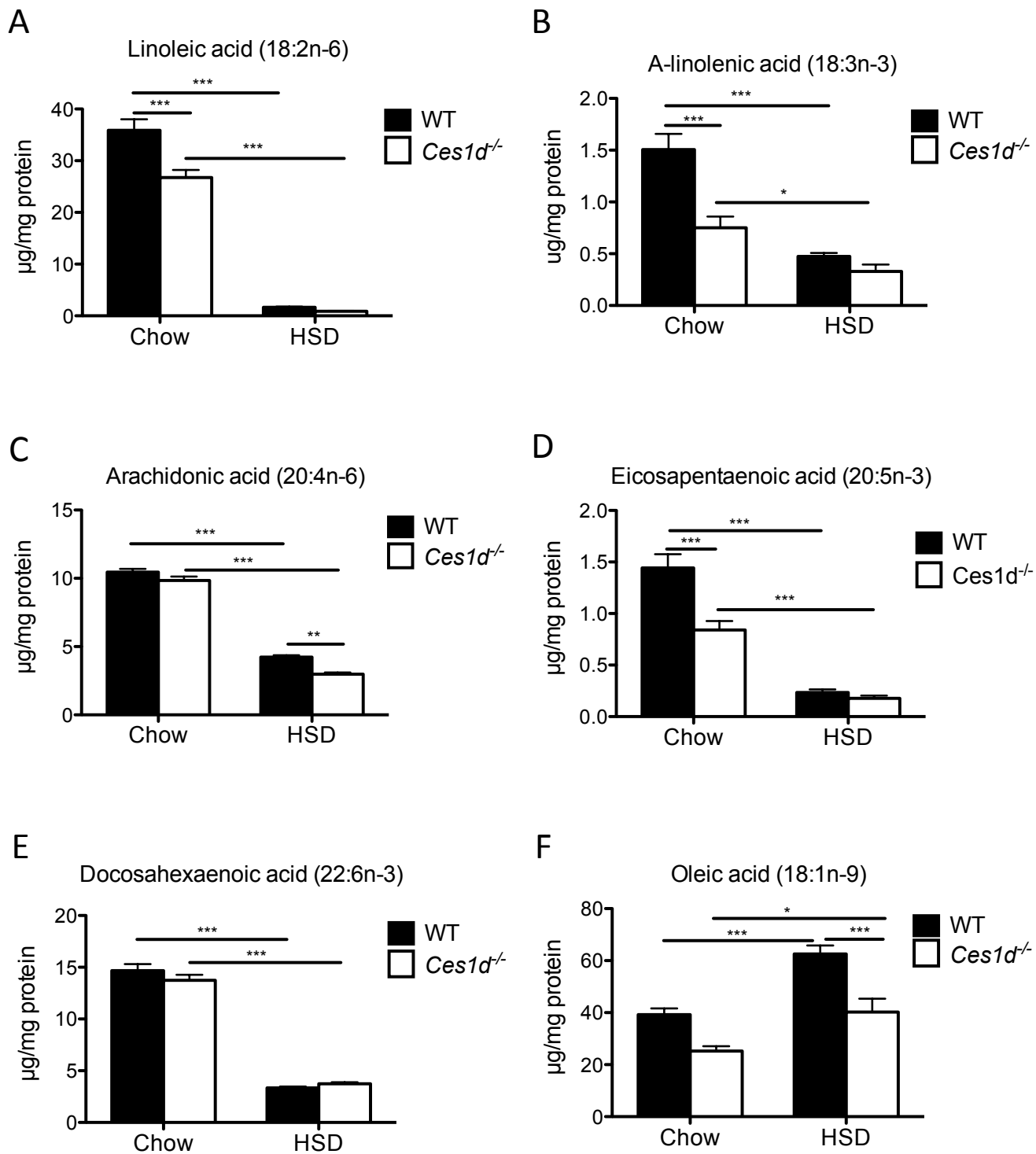
Gene	Sequence
<i>Acox</i>	F: 5'- CAGCAGGAGAAATGGATGCA -3'
	R: 5'- GGGCGTAGGTGCCAATTATCT -3'
<i>Abca1</i>	F: 5'- AGTTTCTGCCCTCTGTGGTC -3'
	R: 5'- GGGTCGGGAGATGAGATGT -3'
<i>Mlxipl (alpha)</i>	F: 5'- CGACACTCACCCACCTCTTC -3'
	R: 5'- TTGTTCA GCCGGATCTTGTC -3'
<i>Mlxipl (beta)</i>	F: 5'- AAGACTGGGATGAACGAGCCAAGA -3'
	R: 5'- AATTTGACGTGGTTTCCACTCGCC -3'
<i>Cd68</i>	F: 5'- GCGGCTCCCTGTGTGTCTGAT -3'
	R: 5'- GGGCCTGTGGCTGGTCGTAG -3'
<i>Cidea</i>	F: 5'- GCCTGCAGGAACTTATCAGC -3'
	R: 5'- AGAACTCCTCTGTGTCCACCA -3'
<i>Cideb</i>	F: 5'- CAGCCTCCAAGAACTGCTAGA -3'
	R: 5'- CCTCCAGGACCAGTGTTAGC -3'
<i>Cidec</i>	F: 5'- GGGTCACAGCTTGGAGGA -3'
	R: 5'- CTCCACGATTGTGCCATCT -3'
<i>Cpt1a</i>	F: 5'- TGAGTGGCGTCCTCTTTGG -3'
	R: 5'- CAGCGAGTAGCGCATAGTCATG -3'
<i>F4/80</i>	F: 5'- CCCTCGGGCTGTGAGATTGTG -3'
	R: 5'- TGGCCAAGGCAAGACATACCAG -3'
<i>Fgf21</i>	F: 5'- AGATGGAGCTCTCTATGGATCG -3'
	R: 5'- GGGCTTCAGACTGGTACACAT -3'
<i>G0s2</i>	F: 5'- TCTCTTCCCCTGCACCCTA -3'
	R: 5'- TCCTGCACACTTTCCATCTG -3'
<i>G6p</i>	F: 5'- GGATTCCGGTGTTTGAACGTC -3'
	R: 5'- CGGAGGCTGGCATTGTAGATG -3'
<i>Pklr</i>	F: 5'- CATTGTGCTGACAAAGACTGG -3'
	R: 5'- TGGGCAGAACGAGTCACA -3'
<i>Ppia</i>	F: 5'- TCCAAAGACAGCAGAAAACCTTCG -3'
	R: 5'- TCTTCTTGCTGGTCTTGCCATTCC -3'
<i>Nr1h3</i>	F: 5'- CAGAAGAACAGATCCGCTTGAAG -3'
	R: 5'- TGCAATGGGCCAAGGCGTGAC -3'
<i>Pck1</i>	F: 5'- GAACTGACAGACTCGCCCTAT -3'
	R: 5'- TTCCCACCATATCCGCTTC -3'
<i>Srebf1</i>	F: 5'- ATGGATTGCACATTTGAAGAC -3'
	R: 5'- CTCTCAGGAGAGTTGGCACC -3'
<i>Tnfa</i>	F: 5'- GTCTACTGAACTTCGGGGTGA -3'
	R: 5'- CACCACTTGGTGGTTTGCTACGAC -3'
<i>Txnip</i>	F: 5'- ATCCCAGATACCCAGAAGC -3'
	R: 5'- TGAGAGTCGTCCACATCGTC -3'



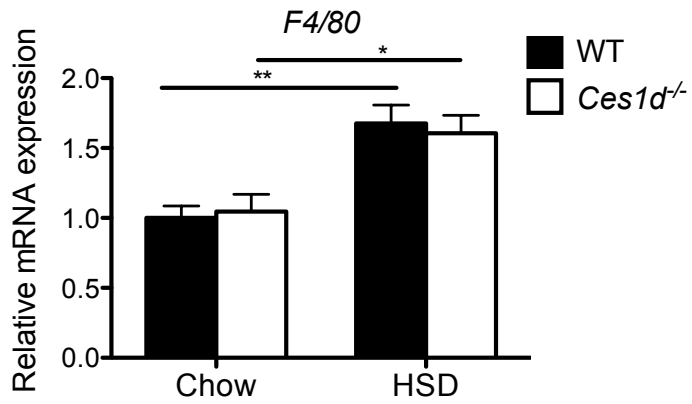
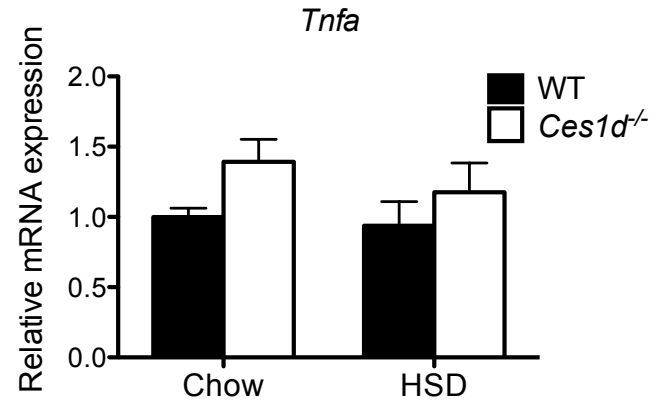
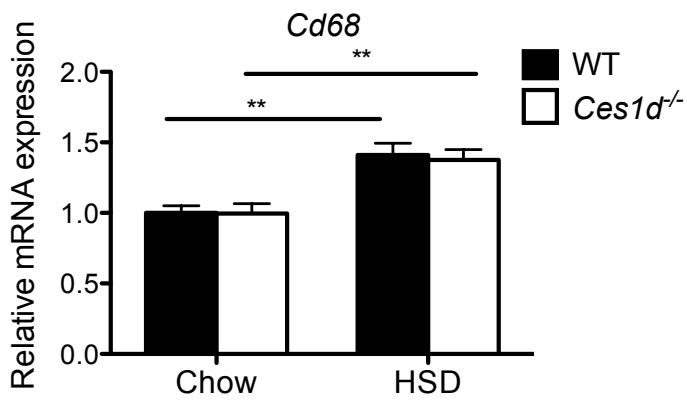
Supplemental Figure S1. Body weight and body composition. (A) body weight of WT and *Ces1d*^{-/-} mice before HSD study initiation age. (B) Fat and (C) lean mass percentage of WT and *Ces1d*^{-/-} mice before or after HSD feeding. N=5-7, values are mean ± SEM. **P<0.01, ***P<0.001.



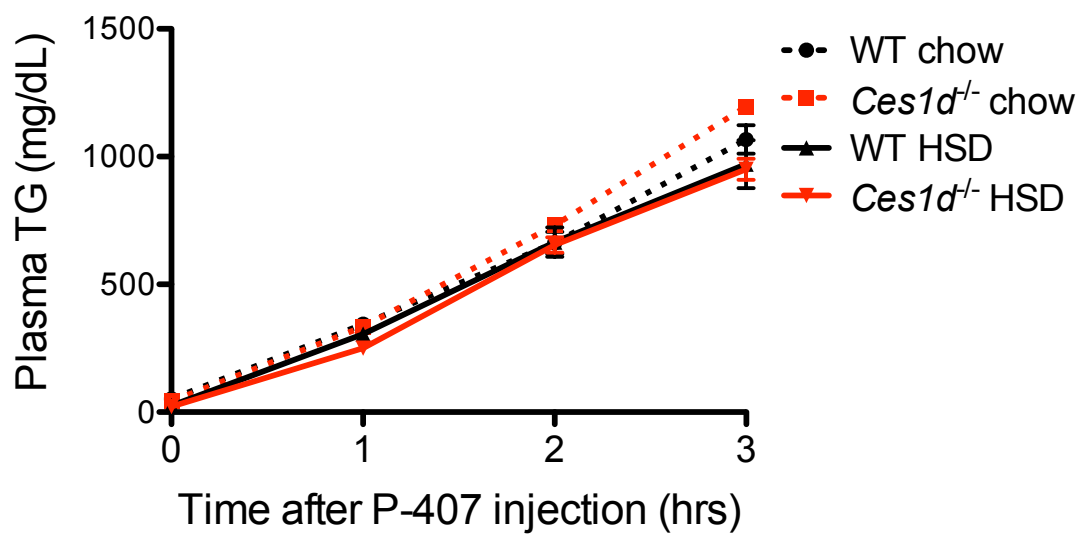
Supplemental Figure S2. Food intake of WT and *Ces1d^{-/-}* mice fed either chow (A-B) or HSD (C-D) in both light and dark phases was measured by metabolic cages. N=6. values are mean \pm SEM. *P<0.05, **P<0.01, ***P<0.001.



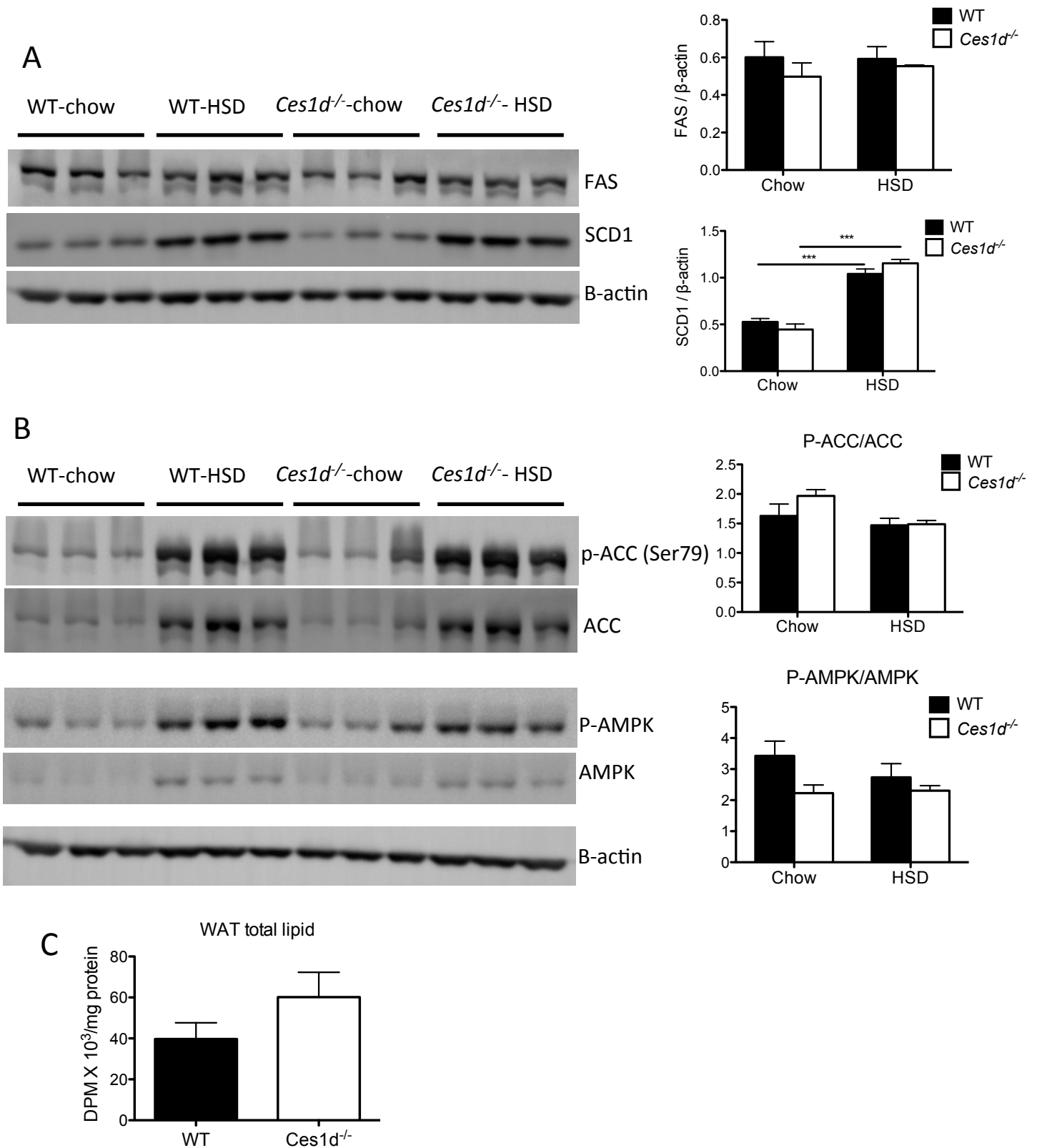
Supplemental Figure S3. Liver essential fatty acids (A and B) and their long-chain metabolites (C-E) in WT and *Ces1d*^{-/-} mice fed either chow or HSD. (F) Liver oleic acid concentration in WT and *Ces1d*^{-/-} mice fed either chow or HSD. N=6. values are mean ± SEM. *P<0.05, **P<0.01, ***P<0.001.

A**B****C**

Supplemental Figure S4. Hepatic mRNA expression of inflammatory markers in WT and *Ces1d*^{-/-} mice fed either chow or HSD. N=6. values are mean \pm SEM. *P<0.05, **P<0.01.

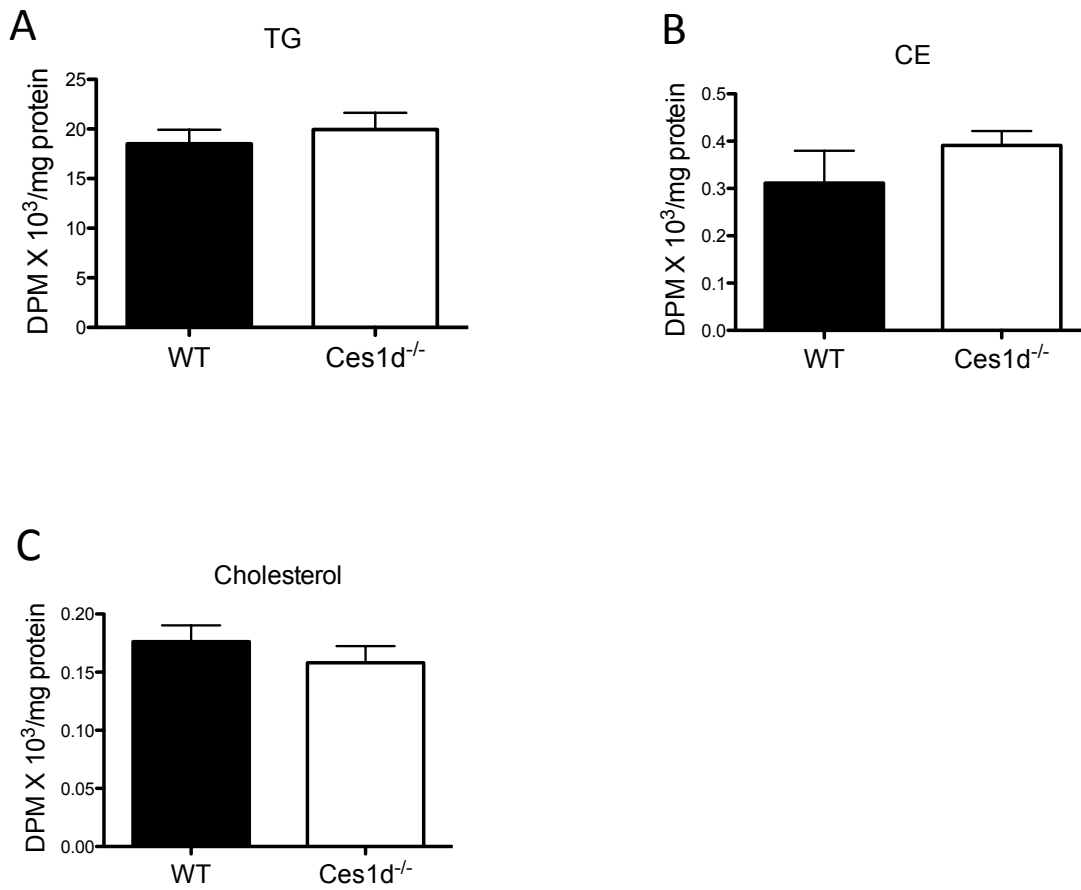


Supplemental Figure S5. VLDL-TG secretion rates of WT and *Ces1d*^{-/-} mice fed chow diet or HSD. N=4-6, values are mean ± SEM.



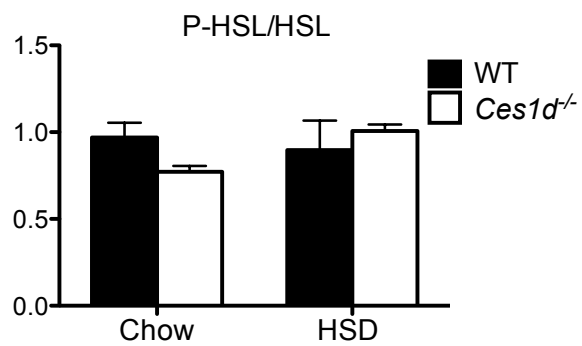
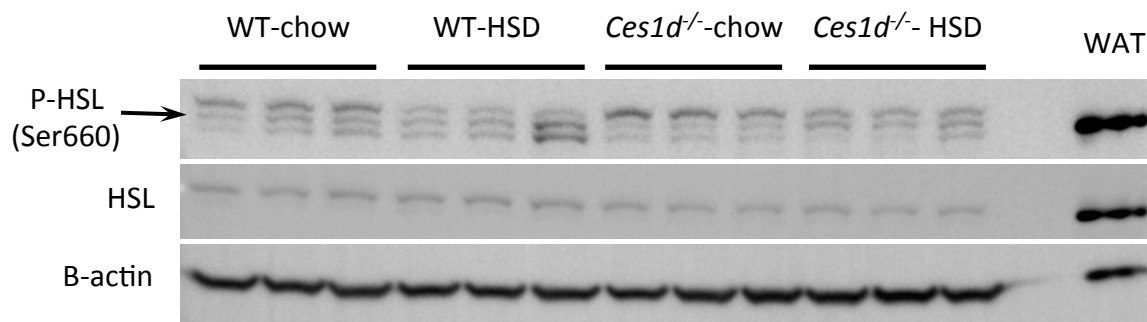
Supplemental Figure S6. Effects of HSD and *Ces1d* deficiency on lipogenic enzymes and AMPK in white adipose tissue (WAT). Abundance of (A) FAS, SCD-1, (B) p-ACC/ACC, and p-AMPK/AMPK in chow or HSD fed WT and *Ces1d*^{-/-} mice was assessed by immunoblotting. Values of relative band intensities were shown as

mean \pm SEM. (C) Lipogenic rate in the WAT of HSD fed WT and *Ces1d*^{-/-} mice was evaluated by the incorporation of ¹⁴C-acetate precursor into total lipids during refed state. N=6. values are mean \pm SEM.



Supplemental Figure S7. Lipogenic rate in the livers of HSD fed WT and *Ces1d*^{-/-} mice was evaluated by the incorporation of ¹⁴C-acetate precursor into liver (A) TG, (B) CE, and (C) cholesterol during refed state.

N=6. values are mean ± SEM.



Supplemental Figure S8. Protein abundance of liver p-HSL/HSL in chow or HSD fed WT and *Ces1d*^{-/-} mice was assessed by immunoblotting. Values of relative band intensities were shown as mean \pm SEM.