Supplemental Information

Table S1.

Biometric and metabolic parameters of mice fed a chow diet.

Parameter	Genotype	Mean ± SEM	*p*
Body weight (grams)	**WT** **S196A**	23.63 ± 0.6 21.70 ± 0.75	0.132
% Liver weight (Liver g/Body g)	**WT** **S196A**	4.69 ± 0.25 4.41 ± 0.07	0.241
Plasma glucose (mmol/L)	**WT** **S196A**	5.35 ± 0.10 4.63 ± 0.22	0.268
Plasma insulin (ng/ mL)	**WT** **S196A**	0.34 ± 0.05 0.87 ± 0.24	0.103
Hepatic triglycerides (µg / mg protein)	**WT** **S196A**	51.95 ± 5.06 37.63 ± 4.50	0.116
Hepatic total cholesterol (µg / mg protein)	**WT** **S196A**	98.96 ± 10.48 104.43 ± 4 .05	0.688

Table S2.

Biometric and metabolic parameters of mice fed a high fat and high cholesterol diet.

Parameter	Genotype	Mean ± SEM	p-value
Body weight (grams)	WT S196A	21.36 ± 0 .41 19.89 ± 0.35	**0.012**
% Liver weight (Liver g/Body g)	WT S196A	9.30 ± 0.17 6.41 ± 0.18	**3.06E-12**
Plasma glucose (mmol/L)	WT S196A	4.49 ± 0.30 4.61 ± 0.24	0.762
Plasma insulin (ng/ mL)	WT S196A	0.60 ± 0.10 0.87 ± 0.33	0.498

Table S3.

Hepatic unsaturated fatty acid quantification in WT and S196A mice. Significant p values ($p \leq 0.05$) are highlighted in bold.

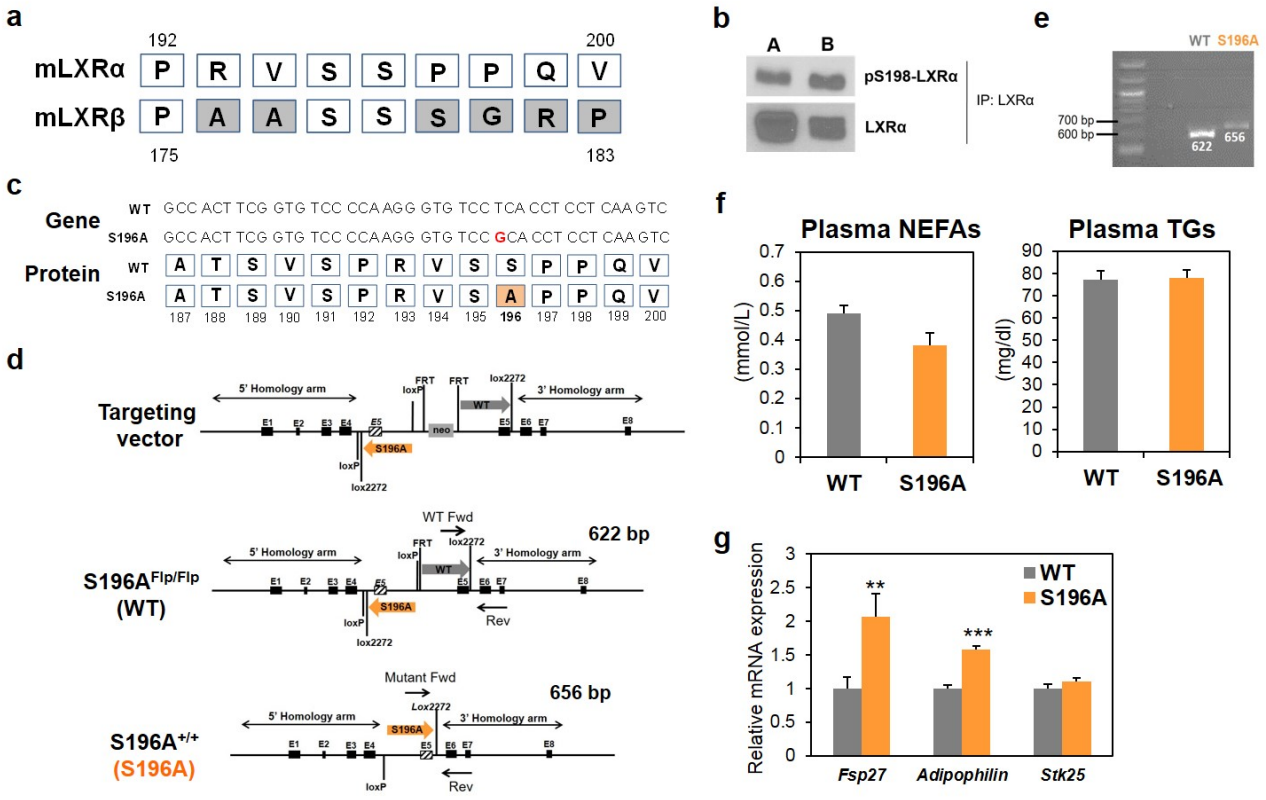
	WT	S196A	*p value*
C16:1, c9	5.28 ± 0.401	6.85 ± 0.297	**0.017**
C18:1, c9	48.69 ± 2.983	62.10 ± 2.631	**0.012**
C18:1, c11	2.29 ± 0.136	2.93 ± 0.128	**0.011**
C18:2, n-6	10.37 ± 0.409	13.11 ± 0.850	**0.024**
C18:3, n-6	0.49 ± 0.028	0.66 ± 0.063	0.054
C18:3, n-3	0.39 ± 0.031	0.47 ± 0.043	0.205
C20:1, n-9	0.38 ± 0.014	0.51 ± 0.021	**0.001**
C20:2, n-6	0.12 ± 0.004	0.14 ± 0.004	**0.037**
C20:3, n-6	0.53 ± 0.028	0.44 ± 0.024	**0.048**
C20:4, n-6	3.95 ± 0.081	4.27 ± 0.049*	**0.011**
C20:5, n-3	0.15 ± 0.006	0.16 ± 0.013	0.559
C22:5, n-3	0.17 ± 0.009	0.17 ± 0.010	0.589
C22:6, n-3	3.15 ± 0.055	3.45 ± 0.128	0.078

Table S4.

Oligonucleotides used to perform ChIP-qPCR analysis.

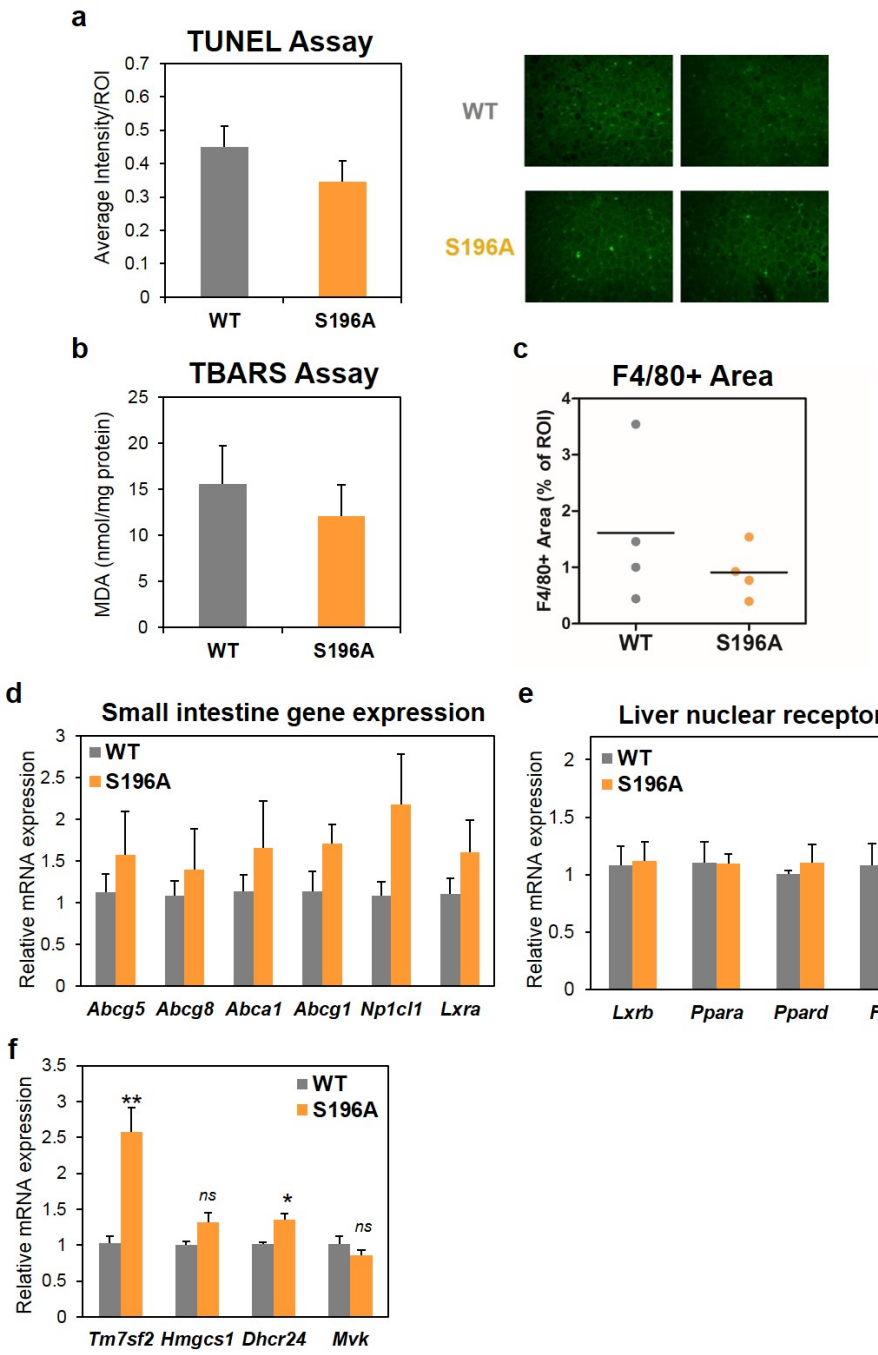
	Forward primer (5' to 3')	**Reverse primer** (5' to 3')
Ces1f DR4	GGTGGTGGCCATTCAATATC	TGTCCACAAACCCTACCTGA
Ces1f TSS	CATTGACTTGGGAGCCTGTC	ACTCACCGCAAATCACACAG
Cyp2c69 DR4	CTACCCACTCCTGCTTCCTG	GGCCTAGTTGGCCATCATTA
Cyp2d69 TSS	TGTCTGGAATGCCTGATCATA	GGATCCATGGAGACCCTTCT
Srebp1c LXRE	AGGCTCTTTTCGGGGATGG	TGGGGTTACTGGCGGTCAC
Srebp1c TSS	GTGGGCCTAGTCCGAAGC	ATCTCGGCCAGTGTCTGTTC

Supplementary Figure S1.



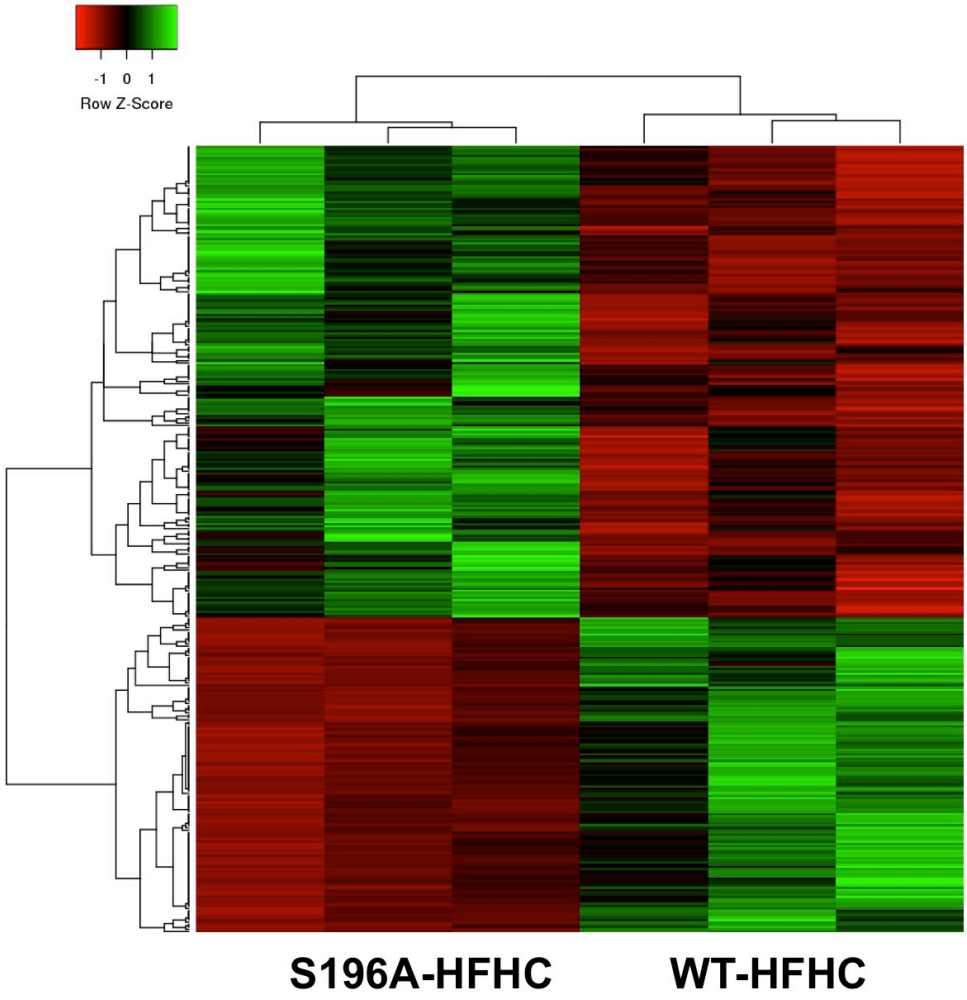
(a) Alignment of the murine LXRα and LXRβ showing differences in S196 phosphorylation motifs. **(b)** LXRα phosphorylation at Ser198 and total LXRα levels in human liver lysates (n=2) by immunoblotting. **(c)** WT and S196A genomic and protein sequence alignment of the murine LXRα depicting the single-site mutation at S196A. **(d)** Targeting construct containing the loxP and FRT sites, the predicted homologous recombinant alleles and the resulting WT and LXRα knock-in locus incorporating the mutated sequence. Diagram also shows oligos used for genotyping and product size. **(e)** Gel electrophoresis of DNA amplified products using the corresponding primers. **(f)** Plasma non-esterified fatty acids (NEFAs) and triglycerides (TGs) levels from WT and S196A mice on HFHC diet (n=5-6). Data are means ± SEM. **(g)** Hepatic gene expression of lipid droplet proteins from WT or S196A mice (n=6). Results shown normalized to cyclophilin and relative to WT set as 1. Data represents means ± SEM. * $p < 0.05$ or ** $p < 0.005$ relative to WT determined by Student's t-test.

Supplementary Figure S2.



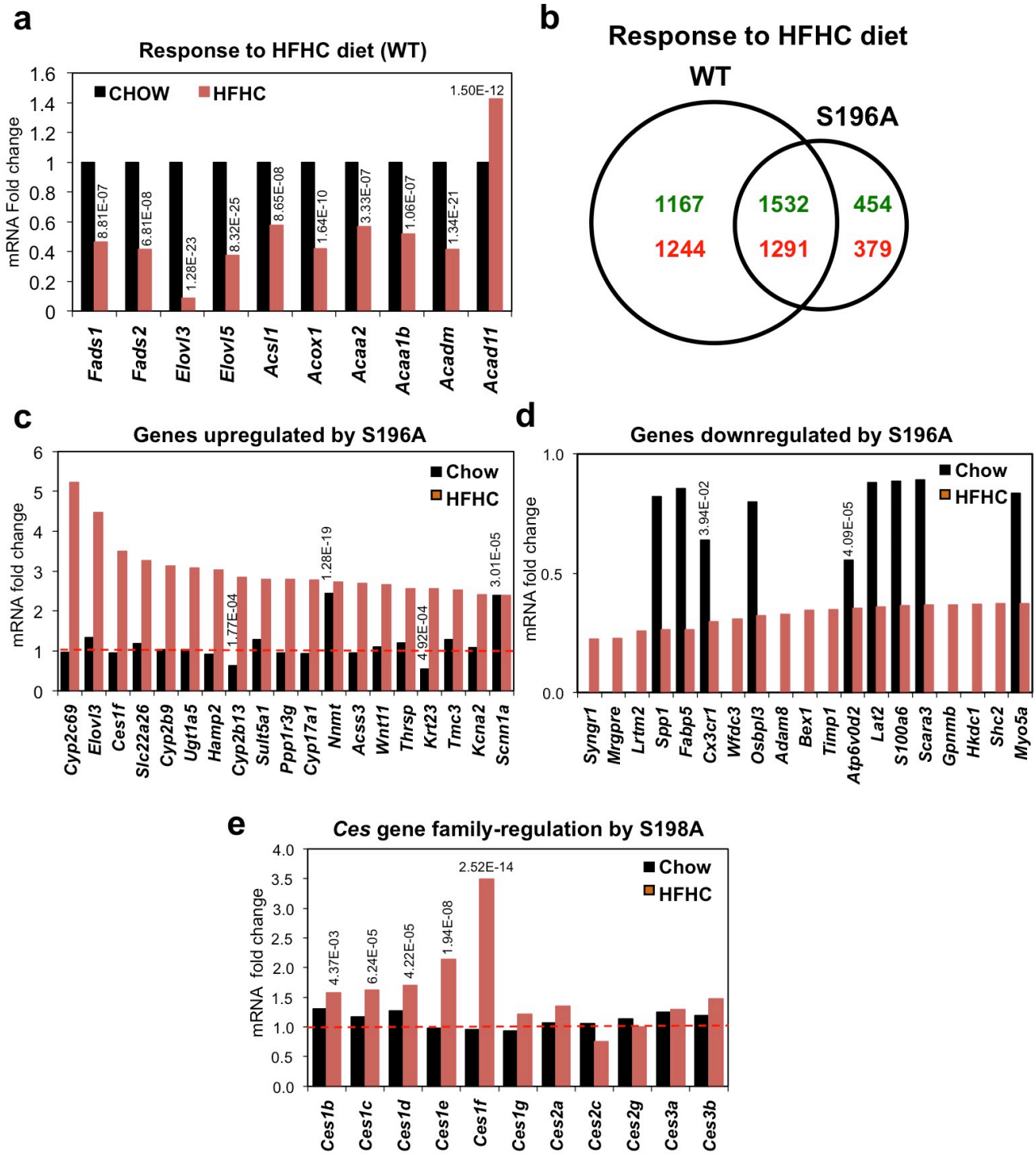
(a) Hepatic cell apoptosis assessed *in situ* by Direct DNA Fragmentation (TUNEL) Assay (n=6) *(Right)*. Representative images of TUNEL-stained liver sections from WT and S196A mice at 200x magnification (*Left).* **(b)** Hepatic lipid peroxidation shown as MDA levels in WT and S196A livers (n=6) normalised to protein levels in tissue homogenates. **(c)** Quantification of F4/80-positively stained areas in liver sections of WT and S196A mice (n=4) at 200x magnification. Dots represent average of three independent areas per animal. **(d)** Small intestine and **(e)** (**f)** hepatic gene expression from WT or S196A mice fed a HFHC diet for 6 weeks (n=6). Results shown normalized to cyclophilin levels and relative to WT. Data represents means ± SEM. * $p < 0.05$ or ** $p < 0.005$ relative to WT determined by Student's t-test.

Supplementary Figure S3.



Clustered heatmap of hepatic RNAseq gene counts in WT and S196A mutant mice (n=3/genotype) of regulated genes in response to a HFHC diet.

Supplementary Figure S4



(a) Fold change of hepatic RNAseq gene counts of fatty acid genes in response to the HFHC diet compared to chow (set as 1) in WT mice (n=3/group). Adjusted *p* values are shown. **(b)** Venn diagram of genes induced (green) or reduced (red) in response to HFHC diet in WT or S196A mice. Significance was set at p<0.05. **(b)** Fold change of hepatic RNAseq gene counts of top upregulated genes in S196A compared to WT mice (from Fig. 4) on chow or HFHC diet (n=3/genotype). Shown are p values of genes differentially expressed between WT and S196A mice on chow. All genes shown are significantly regulated on the HFHC diet (p<0.05). Red bar set

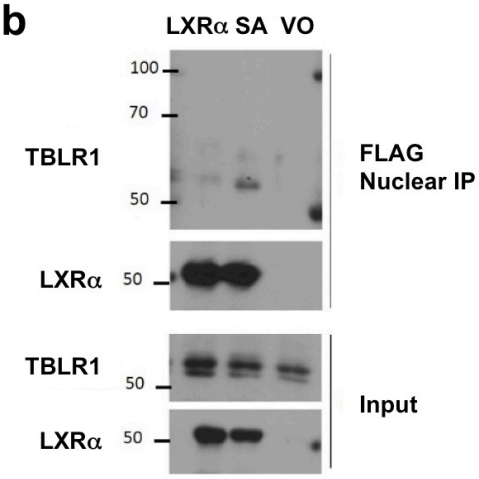
at fold change=1 indicates no change in gene expression between WT and S196A mice. **(c)** Fold change of hepatic RNAseq gene counts of top downregulated genes in S196A compared to WT mice (from Fig. 4) fed chow or HFHC diet (n=3/genotype). For gene expression on chow, p values of genes differentially expressed between WT and S196A are shown. Data is not shown for those genes minimally expressed. For gene expression on the HFHC diet, all genes depicted are significantly reduced ($p<0.05$). **(d)** Fold change of hepatic RNAseq gene counts for Ces gene family members comparing WT and S1986A genotypes by diet (n=3/group). Shown are p values of genes differentially expressed on a HFHC diet. Red bar set at fold change=1 indicates no change in gene expression between WT and S196A mice.

Supplementary Figure S5.

a

VO	LXRα	S198A	Peptide
0	13	37	NCoR
0	4	22	TBLR1

b



(a) Total spectral counts obtained from immunoprecipitates of wild-type human LXRα (LXRα), phospho-mutant (S198A) and control cells (expressing only the empty retroviral vector, VO) identified by mass spectroscopy. **(b)** Immunoprecipitation assays with cells expressing GLAG-tagged wild-type human LXRα (LXRα) and S198A (SA) mutant or vector only (VO). Wild-type and mutant LXRα were immunoprecipitated with anti-FLAG agarose beads followed by immunoblotting with specific TBLR1 antibodies. Expression of TBLR1 and LXRα in protein extracts prior to immunoprecipitation analysis (input) are shown.