Supplementary material

Supplementary Table S1.

Biometric and metabolic parameters of mice fed a chow diet.

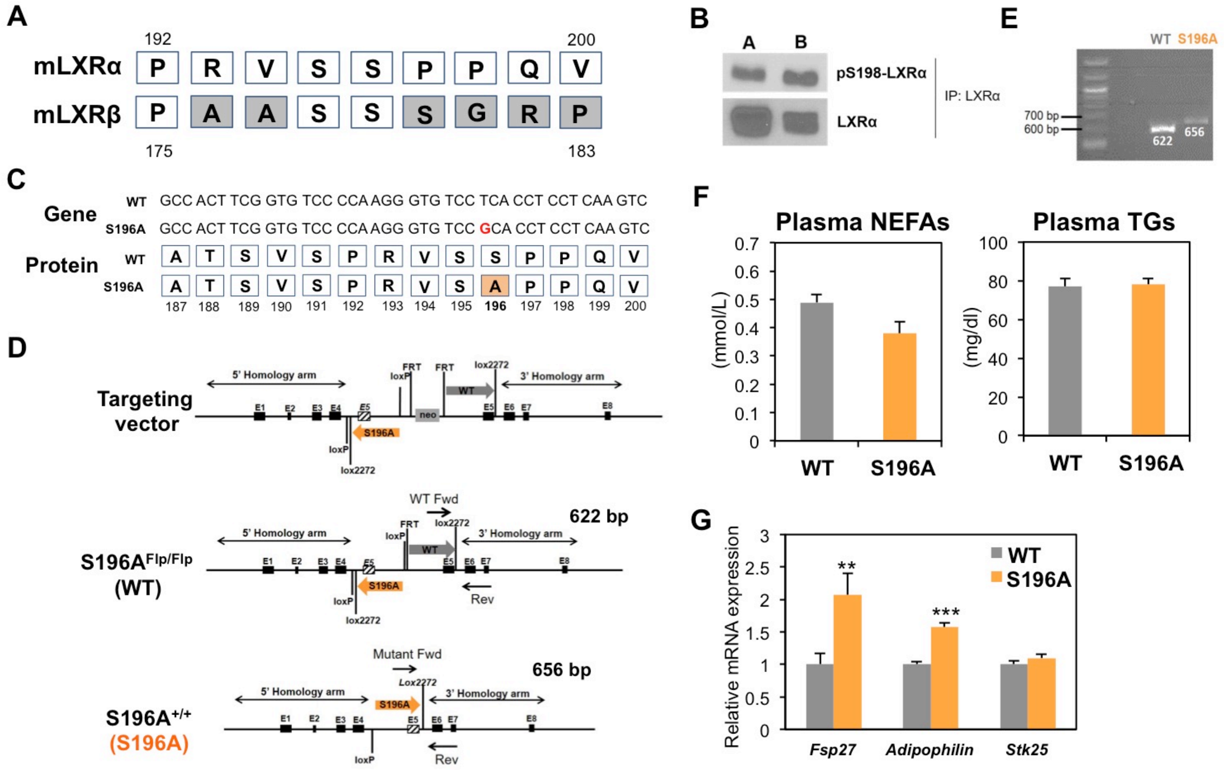
Parameter	Genotype	Mean ± SEM	*p*
Body weight (grams)	WT **S196A**	23.63 ± 0.6 21.70 ± 0.75	0.132
% Liver weight (Liver g/Body g)	WT **S196A**	4.69 ± 0.25 4.41 ± 0.07	0.241
Plasma glucose (mmol/L)	WT **S196A**	5.35 ± 0.10 4.63 ± 0.22	0.268
Plasma insulin (ng/ mL)	WT **S196A**	0.34 ± 0.05 0.87 ± 0.24	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

Supplementary 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

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.

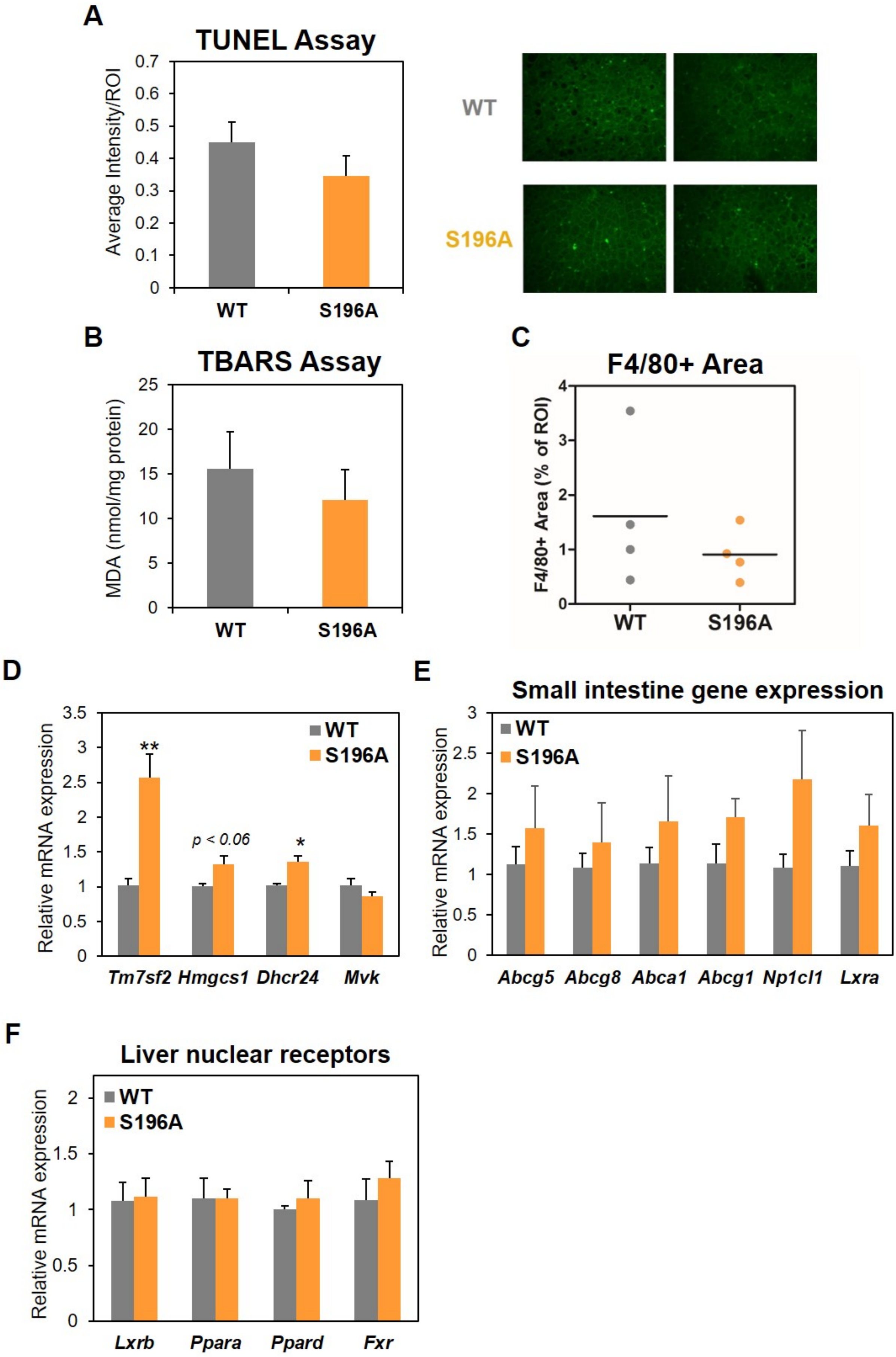
E) Gel electrophoresis of DNA amplified products using the corresponding primers.

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).*

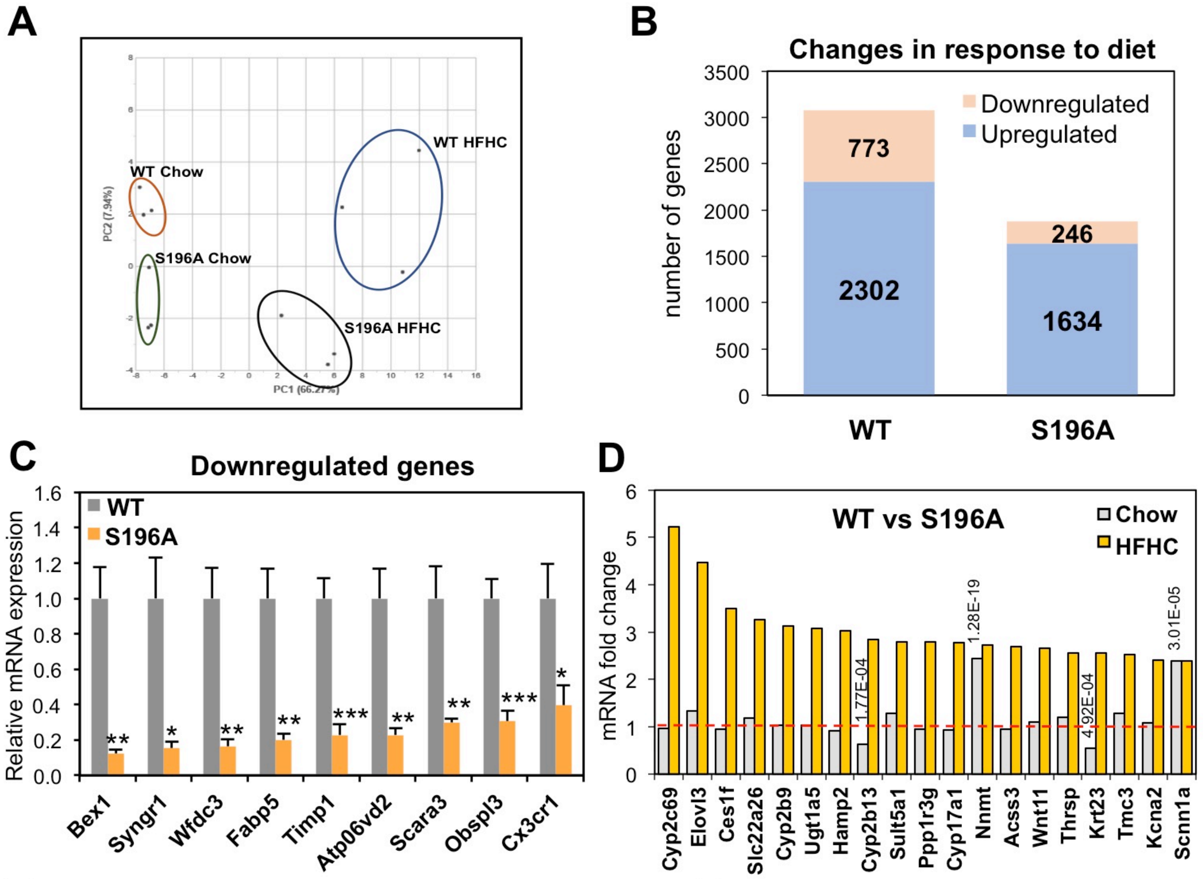
B) Hepatic lipid peroxidation shown as MDA levels in WT and S196A livers (n=6) normalised to protein levels in tissue homogenates.

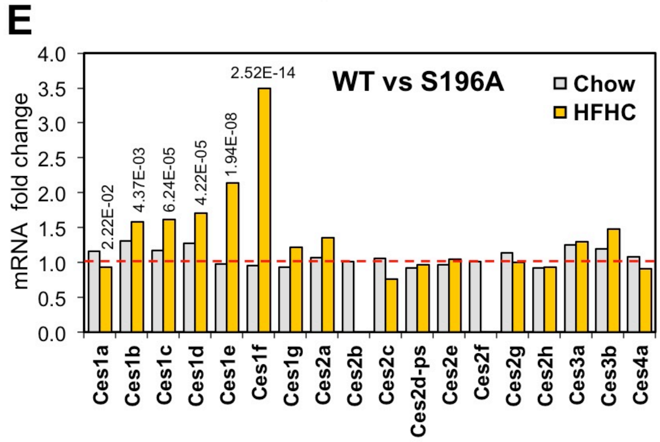
C) Quantification of F4/80-positively stained areas in liver sections of WT and S196A mice (n=4) at 200x magnification. Dots represent average of three independent areas per animal.

D,F) Hepatic and **E)** Small intestine 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.

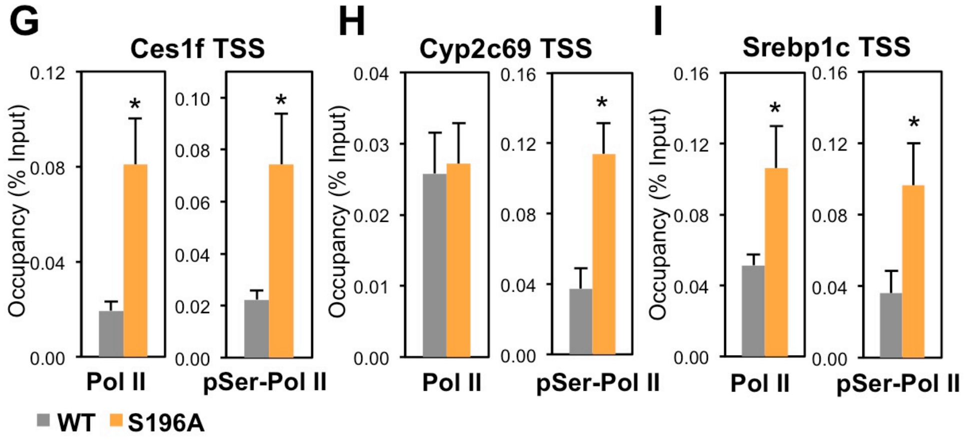
Supplementary Figure S3.





F

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



A) Principal Component (PC) Analysis plot showing RNAseq samples analysed by diet and genotype.

B) Number of genes differentially expressed between chow and HFHC-fed livers by RNAseq (n=3).

C) qPCR validation of top downregulated genes on experimentally-independent HFHC-fed WT and S196A livers (n=6). Results shown normalized to cyclophilin and relative to WT.

D) Fold-change of hepatic RNAseq gene counts of top upregulated genes comparing genotypes by diet (n=3). Shown are p values of genes differentially expressed on a chow diet.

E) Fold-change of hepatic RNA-Seq gene counts for Ces family members comparing genotypes by diet (n=3). Shown are p values of genes differentially expressed on a HFHC diet.

F) Total spectral counts obtained from immunoprecipitates of wild type LXRα (LXRα), phospho-mutant (S198A) and not expressing LXR (VO) cells identified by mass spectroscopy.

G-I) RNA Pol II and pSer2-Pol II occupancy at Ces1f, Cyp2c69 TSS and Srebp1c TSS in livers of WT and S196A mice fed a HFHC (n=3-6).

Data represents mean ± SEM. * $p < 0.05$ relative to WT determined by Student's t-test