

Supplemental data Adipose aP2-hFXR

Supplemental Figure S1

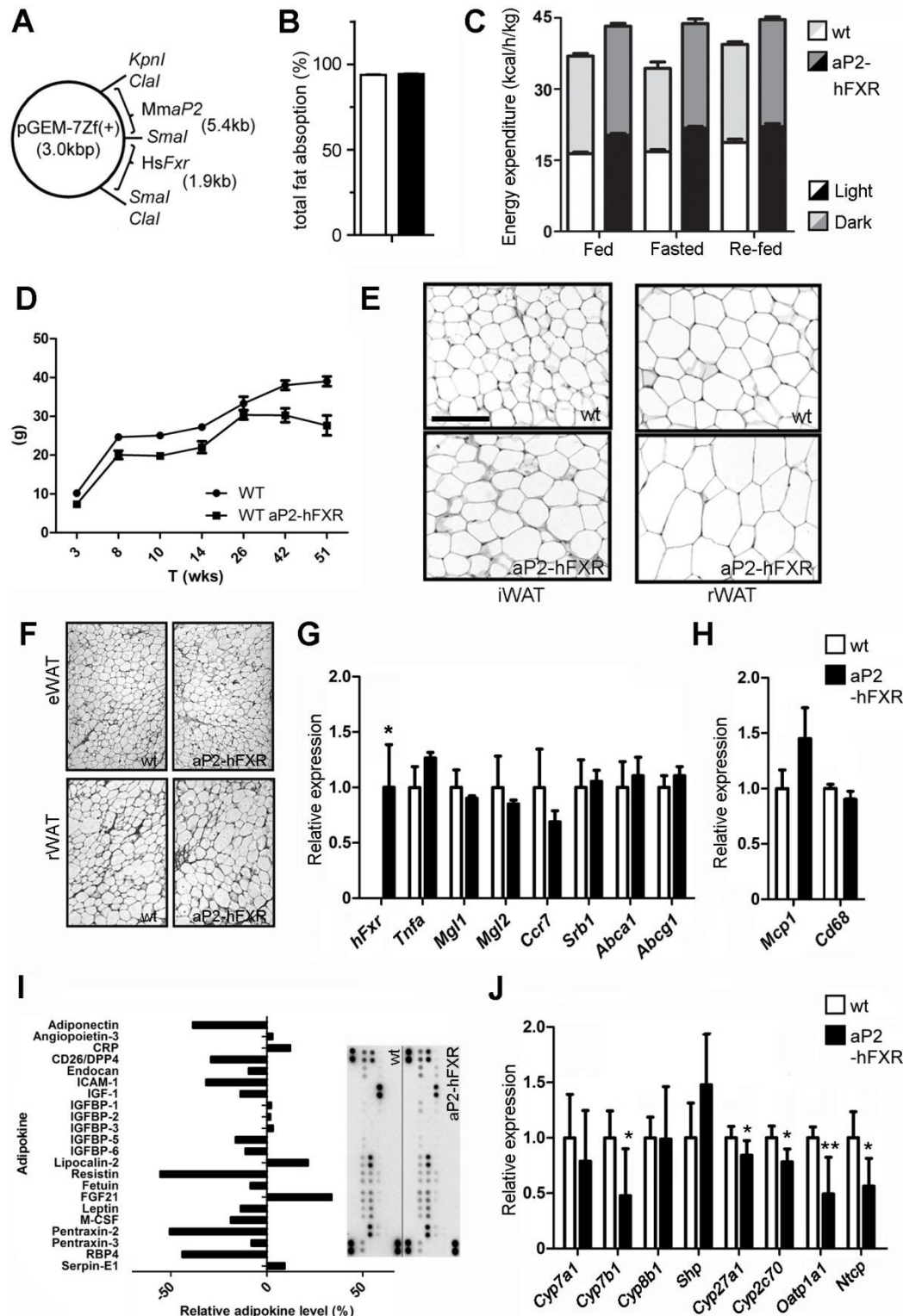


Figure S1 (A) Transgene construct of the human FXR α 2 (NR1H4) ligated to the mouse aP2 (Fabp4) promoter (aP2-hFXR) that was microinjected. (B) Dietary fat absorption and (C) energy expenditure normalized to body weight of chow-fed wild type and aP2-hFXR mice. (D) Body weight development of chow-fed aP2-hFXR compared to wild type controls over time. (E) H&E-stained morphology of retroperitoneal and inguinal WAT (rWAT, iWAT). (F) H&E stained morphology in eWAT and rWAT of 3-week old mice. Relative expression levels of inflammatory markers and FXR targets in (G) isolated macrophages and (H) eWAT of chow-fed aP2-hFXR compared to wild type controls. (I) Plasma adipokines in pooled plasma and adipokine array dotblot of aP2-hFXR mice compared to wild type and (J) Hepatic bile acid synthesis genes. Bar represents 100 μ m. N=6-8/group, G: N=4, data are represented as mean \pm SEM, *p<0.05.

Supplemental Figure S2

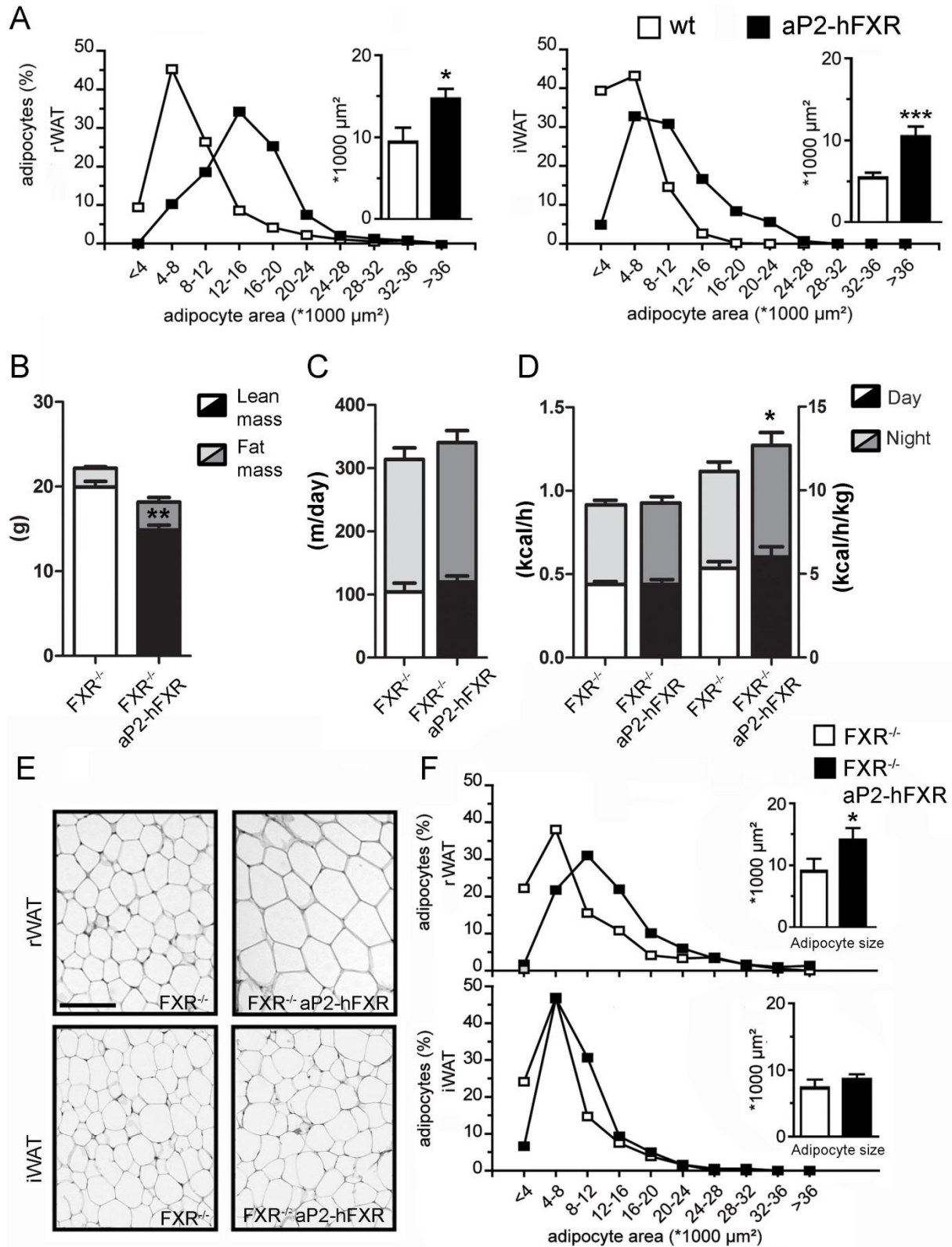


Figure S2 (A) rWAT and iWAT size distribution and average adipocyte size in chow-fed wild type and aP2-hFXR mice. (B) Fat and lean mass, (C) locomotor activity and (D) energy expenditure (unadjusted and adjusted for body weight) of chow-fed $\text{FXR}^{-/-}$ and $\text{FXR}^{-/-}$ aP2-hFXR mice. (E) rWAT and iWAT H&E-stained morphology in $\text{FXR}^{-/-}$ and $\text{FXR}^{-/-}$ aP2-hFXR mice and (F) Distribution and average adipocyte size. $N=6-8/\text{group}$, data are represented as mean \pm SEM, * $p<0.05$, ** $p<0.01$, *** $p<0.001$. Bar represents 100 μm .

Supplemental Figure S3

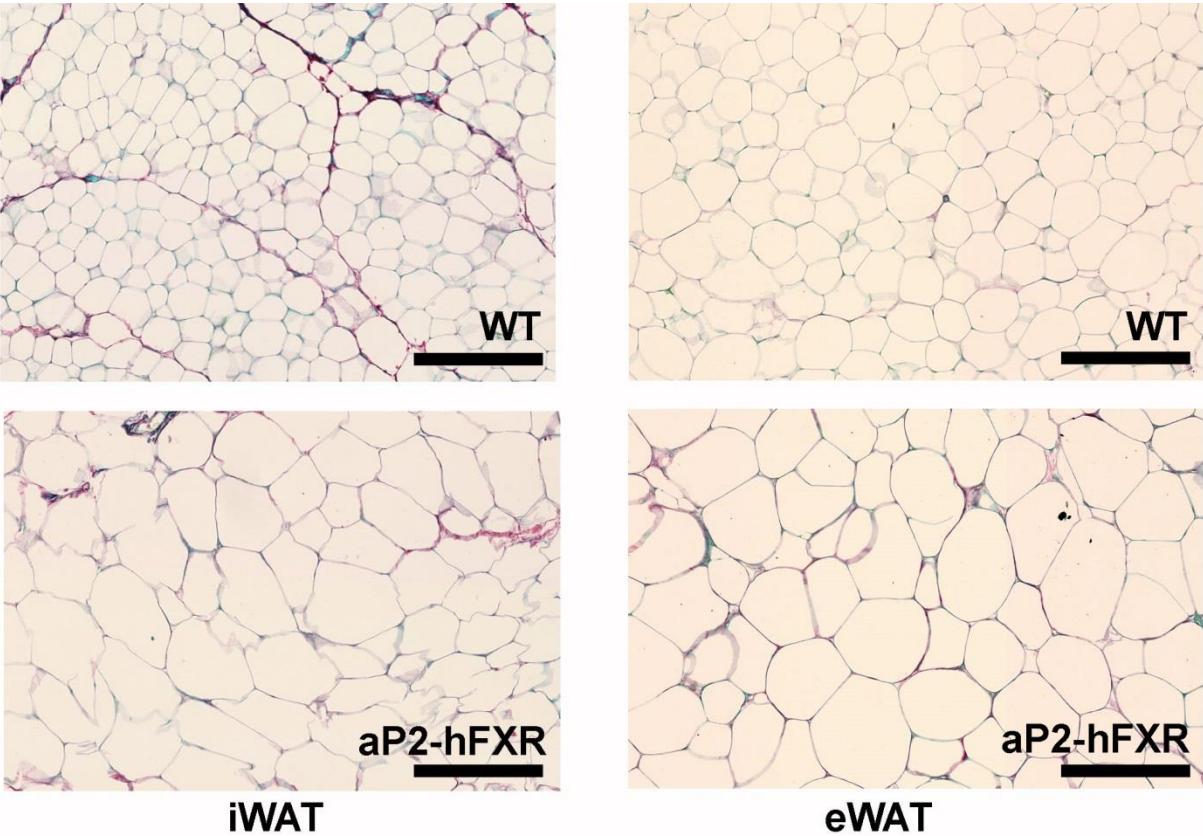


Figure S3 Collagen characterization in iWAT and eWAT of chow-fed wild type and aP2-hFXR mice by picrosirius red staining, showing disorganized tissue architecture in iWAT and pericellular fibrosis in eWAT. Bar represents 200µm.

Supplemental Figure S4

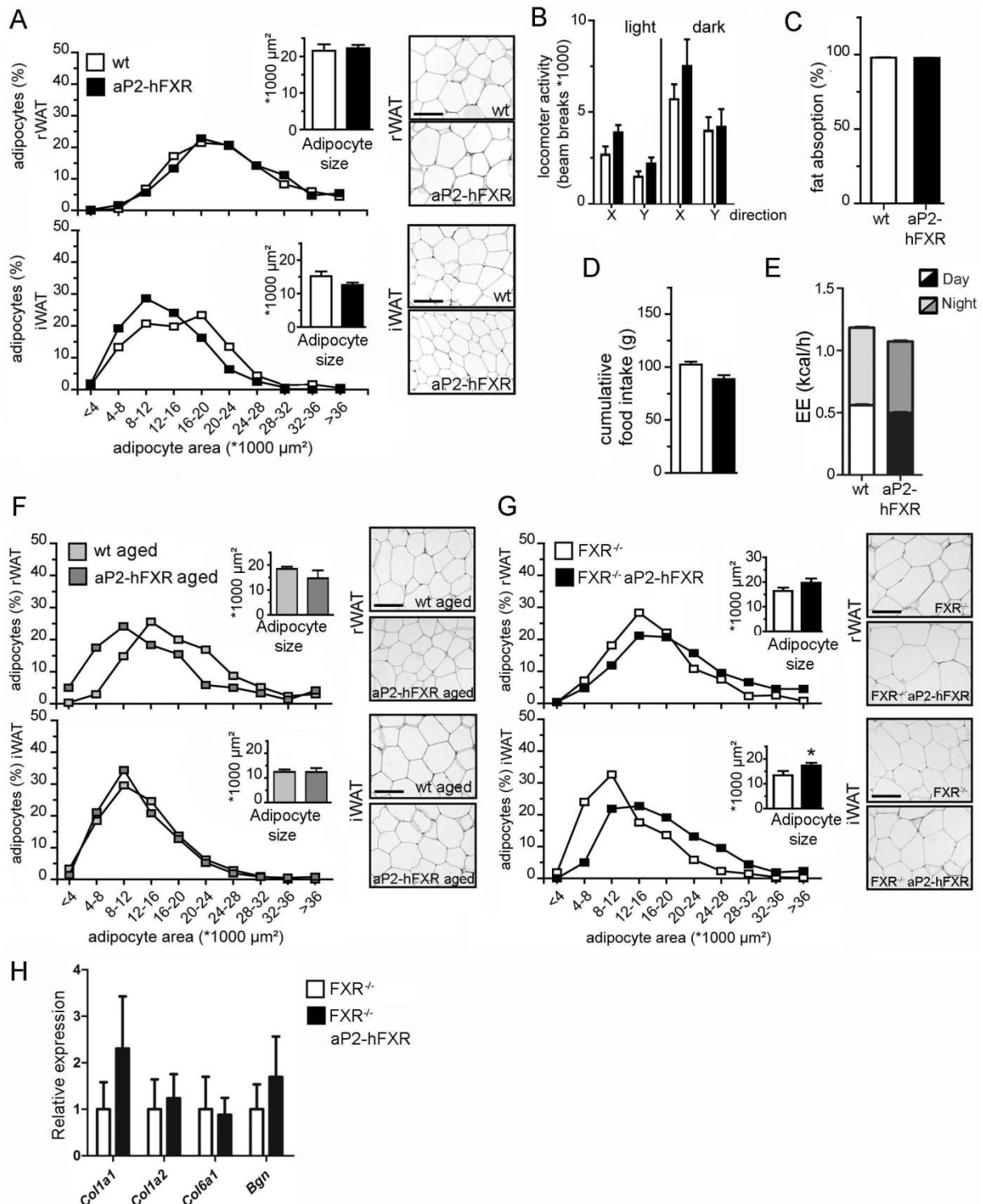


Figure S4 (A) Size distribution, average size and H&E-stained morphology of adipocytes in rWAT and iWAT of wild type and aP2-hFXR mice after 6 weeks HFD. (B) Locomotor activity, (C) Fat absorption, (D) 6-week cumulative food intake and (E) Energy expenditure after 6 weeks HFD. (F) Size distribution, average adipocyte size and H&E stained morphology in rWAT and iWAT of aged wild type and aP2-hFXR mice and (G) rWAT and iWAT of FXR^{-/-} and FXR^{-/-} aP2-hFXR mice fed a HFD for 6 weeks. (I) ECM gene expression in eWAT of 13 weeks HFD-fed FXR^{-/-} and FXR^{-/-} aP2-hFXR mice. N=6-8/group, data are represented as mean \pm SEM, *p<0.05. Bar represents 100 μm .

Supplemental Figure S5

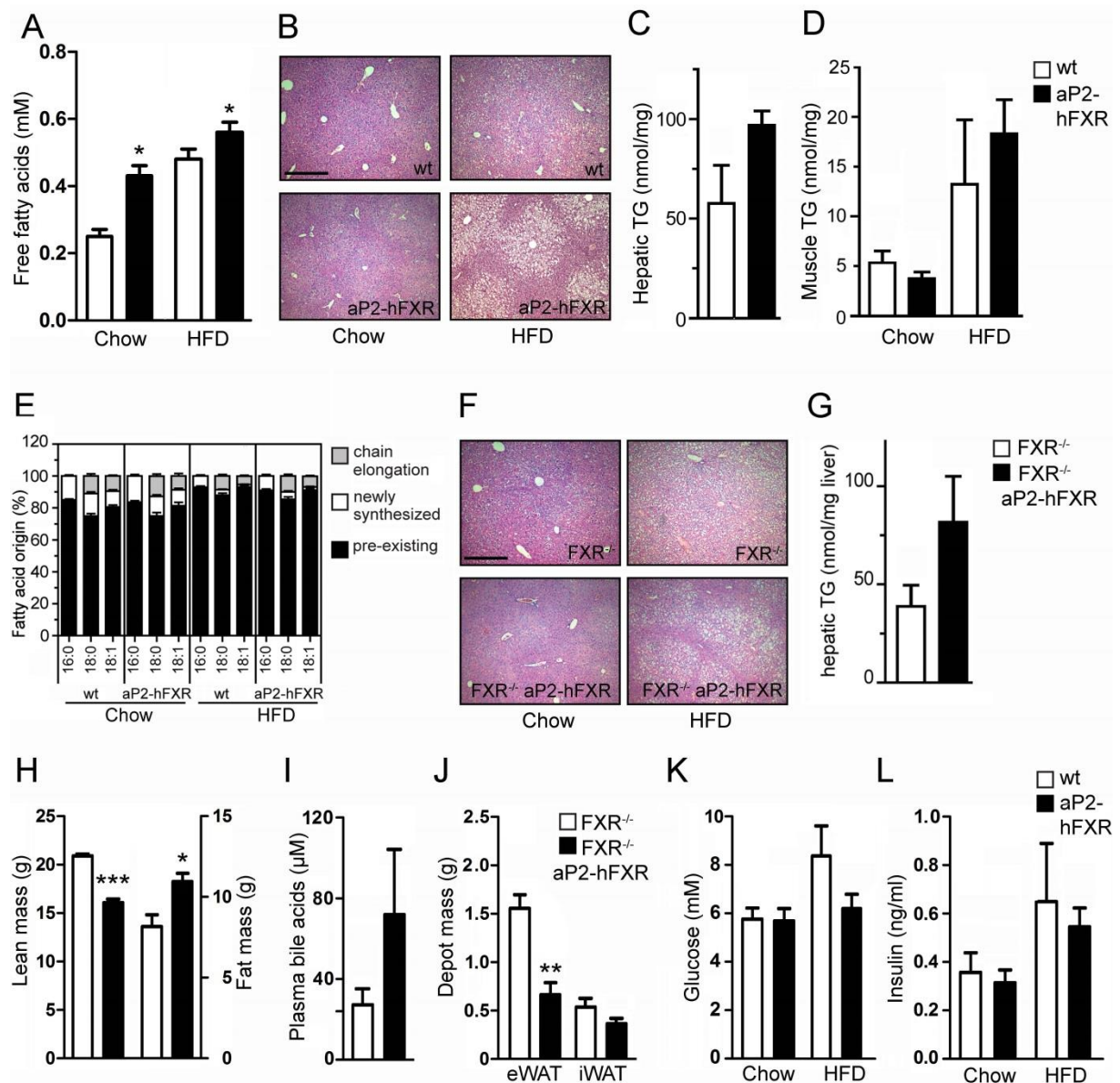


Figure S5 (A) Plasma free fatty acids in chow-fed wild type and aP2-hFXR mice and after 6 weeks on high-fat diet. (B) Liver histology (H&E) in chow-fed wild type and aP2-hFXR mice and after 6 weeks on high-fat diet. (C) Hepatic triglycerides in wild type and aP2-hFXR mice after 6 weeks high-fat diet. (D) Muscle triglycerides in wild type and aP2-hFXR chow-fed mice and after 6 weeks high-fat diet. (E) Origin of C16 and C18 hepatic fatty acids in aP2-hFXR in chow-fed mice and after 6 weeks high-fat diet. (F) Liver histology (H&E) in chow-fed FXR^{-/-} and FXR^{-/-} aP2-hFXR mice and after 6 weeks high-fat diet. (G) Hepatic triglycerides in 6 weeks HFD-fed FXR^{-/-} and FXR^{-/-} aP2-hFXR mice. (H) Lean and fat mass (as also depicted in Figure 6B), (I) plasma bile acids concentrations and (J) adipose depot weight in FXR^{-/-} and FXR^{-/-} aP2-hFXR mice after 3 months high-fat diet. (K) Glucose and (L) insulin levels of chow-fed wild type and aP2-hFXR mice after 6 weeks high-fat diet. N=6-8/group, data are represented as mean \pm SEM, * p <0.05, ** p <0.01, *** p <0.001. Bar represents 200 μ m.

Supplemental Table S1 Differentially expressed extracellular matrix-associated genes in eWAT of aP2-hFXR mice.

Gene name	Fold change	Gene name	Fold change
<i>Adam19</i>	1,17	<i>Mfap5</i>	1,50
<i>Adamts15</i>	1,37	<i>Mmp12</i>	2,07
<i>Adamts2</i>	1,67	<i>Mmp19</i>	1,16
<i>Adamts5</i>	1,33	<i>Mmp2</i>	1,88
<i>Adamtsl2</i>	1,45	<i>Mmp23</i>	1,12
<i>Ang</i>	1,34	<i>Mmp9</i>	-1,46
<i>Anxa2</i>	1,46	<i>Ncan</i>	1,22
<i>Bgn</i>	2,01	<i>Nid2</i>	1,20
<i>Cilp</i>	2,96	<i>Ntn1</i>	1,18
<i>Col12a1</i>	1,30	<i>Olfml2b</i>	1,59
<i>Col15a1</i>	1,58	<i>Podn</i>	-1,35
<i>Col16a1</i>	1,11	<i>Prelp</i>	1,57
<i>Col1a1</i>	1,80	<i>Slc1a3</i>	-1,86
<i>Col1a2</i>	1,59	<i>Smoc1</i>	-1,25
<i>Col3a1</i>	1,46	<i>Smoc2</i>	1,27
<i>Col4a1</i>	1,36	<i>Sparc</i>	1,35
<i>Col4a2</i>	1,49	<i>Spon1</i>	1,43
<i>Col4a5</i>	1,17	<i>Tgfb3</i>	1,33
<i>Col5a1</i>	1,31	<i>Tgfb1</i>	1,33
<i>Col6a1</i>	1,86	<i>Timp1</i>	1,18
<i>Col6a2</i>	1,85	<i>Tnc</i>	-1,16
<i>Col8a1</i>	1,32	<i>Tnxb</i>	1,44
<i>Crispld2</i>	1,22	<i>Vcan</i>	1,59
<i>Crtac1</i>	1,42	<i>Wnt5a</i>	1,18
<i>Ctgf</i>	1,35		
<i>Ecm1</i>	1,51		
<i>Efemp2</i>	1,13		
<i>Egflam</i>	1,11		
<i>Eln</i>	2,99		
<i>Emilin1</i>	1,18		
<i>Entpd2</i>	1,21		
<i>Fbln1</i>	1,51		
<i>Fbln2</i>	1,91		
<i>Fbn1</i>	1,67		
<i>Gpc1</i>	1,33		
<i>Gpc3</i>	1,25		
<i>Gpc4</i>	1,21		
<i>Hsd17b12</i>	1,21		
<i>Hspg2</i>	1,31		
<i>Lama2</i>	1,30		
<i>Lamb2</i>	1,19		
<i>Lamc1</i>	1,25		
<i>Lepre1</i>	1,17		
<i>Lgals1</i>	1,17		
<i>Lgals3</i>	1,46		
<i>Lox</i>	1,56		
<i>Loxl1</i>	1,45		
<i>Ltp1</i>	1,22		
<i>Ltpb4</i>	1,55		
<i>Lum</i>	1,71		
<i>Matn2</i>	1,59		
<i>Mfap2</i>	1,31		
<i>Mfap4</i>	2,26		

Supplemental Table S2 Differential expression of CEBP α , PPAR γ and WNT pathway genes

Gene name	Fold change	Gene name	Fold change
<u>Regulators</u>		<u>Pparg target genes</u>	
<i>CEBPa</i>	-1,33	<i>Slc5a6</i>	-1,26
<i>Pparg</i>	-1,26	<i>Gys1</i>	-1,31
		<i>Cited2</i>	-1,35
<u>Pparg target genes</u>		<i>Prodh</i>	-1,36
<i>Lep</i>	2,54	<i>Amotl2</i>	-1,37
<i>Lcn2</i>	2,36	<i>Pim3</i>	-1,38
<i>Acta2</i>	1,89	<i>Slc27a1</i>	-1,40
<i>Col1a1</i>	1,80	<i>Mmp9</i>	-1,46
<i>Vcan</i>	1,59	<i>Thbd</i>	-1,52
<i>Col1a2</i>	1,59	<i>Cfd</i>	-1,62
<i>Aldh1a7</i>	1,58		
<i>Col3a1</i>	1,46	<u>Wnt pathway</u>	
<i>Anxa1</i>	1,44	<i>Sfrp5</i>	2,65
<i>Fhl1</i>	1,43	<i>Sfrp1</i>	2,01
<i>Hmgcs1</i>	1,43	<i>Ccnd2</i>	1,26
<i>Ephx1</i>	1,42	<i>Nkd1</i>	1,25
<i>Ctss</i>	1,41	<i>Ppp2r5b</i>	1,24
<i>Vldlr</i>	1,40	<i>Cacybp</i>	1,18
<i>Cx3cr1</i>	1,40	<i>Wnt5a</i>	1,18
<i>Atp2a3</i>	1,39	<i>Sox17</i>	-1,16
<i>Me1</i>	1,37	<i>Myc</i>	-1,2
<i>Plin2</i>	1,30	<i>Ppp2r1b</i>	-1,22
<i>Vcam1</i>	1,29	<i>Tcf7l2</i>	-1,29
<i>Pkm</i>	1,22		
<i>Lpl</i>	1,22		
<i>Calr</i>	1,20		
<i>Lrp1</i>	1,20		
<i>Boc</i>	1,19		
<i>Arntl</i>	1,17		
<i>Ccne1</i>	1,13		
<i>Has2</i>	1,13		
<i>Fam57b</i>	1,10		
<i>Bace1</i>	1,10		
<i>Zfp236</i>	-1,11		
<i>Klf11</i>	-1,11		
<i>Adcy6</i>	-1,13		
<i>Pdpr</i>	-1,13		
<i>Cpt2</i>	-1,14		
<i>Chst11</i>	-1,15		
<i>Slc16a10</i>	-1,16		
<i>Adora2a</i>	-1,17		
<i>Nfkbia</i>	-1,17		
<i>Kank3</i>	-1,18		
<i>Ffar2</i>	-1,18		
<i>Nampt</i>	-1,19		
<i>Pik3r1</i>	-1,20		
<i>Ednra</i>	-1,20		
<i>Akap1</i>	-1,21		
<i>Abca1</i>	-1,21		
<i>Tmem143</i>	-1,21		
<i>Angptl2</i>	-1,22		
<i>Plin1</i>	-1,22		
<i>Hk2</i>	-1,23		

Supplemental Table S3 Differentially secreted proteins between isolated eWAT of adipocyte-specific FXR overexpressing mice and controls.

Accession no.	Gene name	Protein name	Fold change
Increased protein level			
P55284	<i>Cdh5</i>	Cadherin-5	1.73
P18760	<i>Cfl1</i>	Cofilin-1	2.54
O35206	<i>Col15a1</i>	Collagen alpha-1(XV) chain	4.22
P31786	<i>Dbi</i>	Acyl-CoA-binding protein	2.79
Q9QZZ6	<i>Dpt</i>	Dermatopontin	1.49
P04117	<i>Fabp4</i>	Fatty acid-binding protein, adipocyte	4.60
P35505	<i>Fah</i>	Fumarylacetoacetase	2.95
P11276	<i>Fn1</i>	Fibronectin	3.06
P10649	<i>Gstm1</i>	Glutathione S-transferase Mu 1	12.10
P97927	<i>Lama4</i>	Laminin subunit alpha-4	4.76
Q61292	<i>Lamb2</i>	Laminin subunit beta-2	2.52
P06151	<i>Ldha</i>	L-lactate dehydrogenase A chain	3.93
P26041	<i>Msn</i>	Moesin	4.18
Q9CPT4	<i>Myd88</i>	myeloid derived growth factor	1.67
Q922R8	<i>Pdia6</i>	Protein disulfide-isomerase A6	3.45
P62962	<i>Pfn1</i>	Profilin-1	2.82
P24369	<i>Ppib</i>	Peptidyl-prolyl cis-trans isomerase B	8.63
P35700	<i>Prdx1</i>	Peroxiredoxin-1	4.29
P99029	<i>Prdx5</i>	Peroxiredoxin-5, mitochondrial	4.46
P62983	<i>Rps27a</i>	Ubiquitin	2.98
Q9JJU8	<i>Sh3bgr1</i>	SH3 domain-binding glutamic acid-rich-like protein	5.02
P08228	<i>Sod1</i>	Superoxide dismutase [Cu-Zn]	4.26
O09164	<i>Sod3</i>	Extracellular superoxide dismutase [Cu-Zn]	1.39
P82198	<i>Tgfb1</i>	Transforming growth factor-beta-induced protein ig-h3	6.22
Q00519	<i>Xdh</i>	Xanthine dehydrogenase/oxidase	2.95
Decreased protein level			
P03953	<i>Cfd</i>	Complement factor D	0.569
Q01149	<i>Col1a2</i>	Collagen alpha-2(I) chain	0.751
P21460	<i>Cst3</i>	Cystatin-C	0.536
P10605	<i>Ctsb</i>	Cathepsin B	0.713
Q9WUU7	<i>Ctsz</i>	Cathepsin Z	0.712
P13020	<i>Gsn</i>	Gelsolin	0.439
Q61646	<i>Hp</i>	Haptoglobin	0.749

Listed proteins are all statistically significant ($p < 0.05$). "Fold change" represents the fold difference in expression between FXR transgenic (aP2-hFXR) and wild-type (WT) mice.

Supplemental Table S4 Secreted proteins specifically induced by adipocyte-specific FXR overexpression in eWAT of adipocyte-specific FXR overexpressing mice.

Accession no.	Gene name	Protein name	Average H/L ratio
<i>only identified in aP2-hFXR-TG adipose tissue</i>			
Q8BWT1	<i>Acaa2</i>	3-ketoacyl-CoA thiolase, mitochondrial	0.064
P51174	<i>Acadl</i>	Long-chain specific acyl-CoA dehydrogenase, mitochondrial	0.052
Q8BNJ2	<i>Adamts4</i>	A disintegrin and metalloproteinase with thrombospondin motifs 4	5.65
P11859	<i>Agt</i>	Angiotensinogen	4.66
P97449	<i>Anpep</i>	Aminopeptidase N	0.189
P28653	<i>Bgn</i>	Biglycans	2.10
Q8R016	<i>Blmh</i>	Bleomycin hydrolase	0.054
Q923D2	<i>Blvrb</i>	Flavin reductase	0.221
P14106	<i>C1qb</i>	Complement C1q subcomponent subunit B	1.78
P27784	<i>Ccl6</i>	C-C motif chemokine 6	3.25
P06909	<i>Cfh</i>	Complement factor H	2.96
Q9DBP5	<i>Cmpk1</i>	UMP-CMP kinase	0.167
Q80X19	<i>Col14a1</i>	Collagen alpha-1(XIV) chain	0.101
P11087	<i>Col1a1</i>	Collagen alpha-1(I) chain	8.73
Q3U962	<i>Col5a2</i>	Collagen alpha-2(V) chain	8.08
Q04857	<i>Col6a1</i>	Collagen alpha-1 (VI) chain	2.88
A6H584	<i>Col6a5</i>	Collagen alpha-5(VI) chain	0.054
P97821	<i>Ctsc</i>	Dipeptidyl peptidase 1 (Cathepsin C)	4.58
P16406	<i>Enpep</i>	Glutamyl aminopeptidase	0.216
Q9WVH9	<i>Fbln5</i>	Fibulin-5	0.054
Q8VCM7	<i>Fgg</i>	Fibrinogen gamma chain	0.047
P13707	<i>Gpd1</i>	Glycerol-3-phosphate dehydrogenase [NAD+], cytoplasmic	0.066
P46412	<i>Gpx3</i>	Glutathione peroxidase 3	0.049
P28798	<i>Grn</i>	Granulins	2.53
Q99LP6	<i>Grpel1</i>	GrpE protein homolog 1, mitochondrial	0.091
O09131	<i>Gsto1</i>	Glutathione S-transferase omega-1	0.317
Q9WVLO	<i>Gstz1</i>	Maleylacetoacetate isomerase	0.038
Q88844	<i>ldh1</i>	Isocitrate dehydrogenase [NADP]	0.106
P11438	<i>Lamp1</i>	Lysosome-associated membrane glycoprotein 1	0.111
P11672	<i>Lcn2</i>	Neutrophil gelatinase-associated lipocalin	3.65
Q61233	<i>Lcp1</i>	Plastin-2	0.070
P24527	<i>Lta4h</i>	Leukotriene A-4 hydrolase	0.138
P27046	<i>Man2a1</i>	Alpha-mannosidase 2	0.744
P33434	<i>Mmp2</i>	72 kDa type IV collagenase	6.32
P09103	<i>P4hb</i>	Protein disulfide-isomerase	0.047
Q99LX0	<i>Park7</i>	Protein DJ-1	0.174
P30412	<i>Ppic</i>	Peptidyl-prolyl cis-trans isomerase C	0.127
Q91VI7	<i>Rnh1</i>	Ribonuclease inhibitor	0.342
Q9QUR8	<i>Sema7a</i>	Semaphorin-7A	2.82
P07758	<i>Serpina1a</i>	Alpha-1-antitrypsin 1-1	0.035
Q00896	<i>Serpina1c</i>	Alpha-1-antitrypsin 1-3	0.058
O88968	<i>Tcn2</i>	Transcobalamin-2	3.90
P21981	<i>Tgm2</i>	Protein-glutamine gamma-glutamyltransferase 2	0.039
P63028	<i>Tpt1</i>	Translationally-controlled tumor protein	0.302
Q88342	<i>Wdr1</i>	WD repeat-containing protein 1	0.168

“Average H/L ratio” represents the average heavy/light ratio of the particular protein in the secretome of epididymal WAT of FXR transgenic (aP2-hFXR) mice and is directly related to the protein synthesis rate of that protein.

Supplemental Table S5 Expression levels of inflammatory markers
in eWAT and liver of FXR^{-/-} aP2-hFXR and Crown-like structure abundance

Gene name	Fold change (SEM)
eWAT	
Chow	
<i>Tnfq</i>	1.24 (0.49)
<i>Mcp-1</i>	1.10 (0.40)
<i>Il10</i>	1.15 (0.55)
HFD (3 months)	
<i>Cc68</i>	0.76 (0.35)
<i>Tnfq</i>	1.51 (0.02)
<i>Mcp-1</i>	1.16 (0.48)
<i>IL10</i>	1.29 (0.25)
Liver	
Chow	
<i>Cd68</i>	1.49 (0.36)
<i>Tnfq</i>	1.14 (0.20)
<i>Mcp-1</i>	1.51 (0.26)
<i>IL10</i>	1.15 (0.57)
HFD (3 months)	
<i>Tnfq</i>	0.72(0.42)
<i>Mcp-1</i>	1.58 (0.26)
<i>Il10</i>	1.17 (0.57)
CLS abundance (chow) (CLS/mm²)	2,25 (0.95)*

Relative expression level of inflammatory markers in eWAT and Liver of FXR^{-/-} aP2-hFXR compared to FXR^{-/-} mice (N=5-6). * Crown-like structure abundance was not different between groups (N=5), however a single sample (5,9-fold) had a large impact (1,32-fold average without outlier).