

Supplementary Materials for
**Fatty acid desaturase 2 determines the lipidomic landscape and steroidogenic
function of the adrenal gland**

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Sci. Adv. **9**, eadf6710 (2023)
DOI: 10.1126/sciadv.adf6710

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Supplemental Tables

Table S1. Lipidome composition of adrenal glands of LFD- and HFD-fed mice

	Storage lipids (% mean ± SD)	Non-storage lipids (% mean ± SD)
LFD	86.98 ± 5.43	13.02 ± 5.22
HFD	90.12 ± 1.93	9.87 ± 1.89

Table S2. Lipid class abundance in adrenal glands of LFD- and HFD-fed mice

pmol/μg protein (Aver ± SD)	LFD	HFD	Stat.
CE	246.59 ± 68.57	280.49 ± 41.86	ns
Ceramides	0.86 ± 0.16	1.27 ± 0.35	ns
Cholesterol	37.03 ± 5.65	37.12 ± 3.64	ns
CL	1.35 ± 0.27	1.44 ± 0.31	ns
DAG	12.84 ± 8.06	10.26 ± 3.26	ns
HexCer	0.0781 ± 0.0186	0.0646 ± 0.0089	ns
LPA	0.00430 ± 0.00398	0.00628 ± 0.00148	ns
LPC	3.98 ± 1.25	3.49 ± 0.47	ns
LPE	0.91 ± 0.2	0.84 ± 0.11	ns
LPE O-	0.33 ± 0.12	0.28 ± 0.05	ns
LPG	0.0171 ± 0.0053	0.0246 ± 0.0075	ns
LPI	0.0876 ± 0.0123	0.1330 ± 0.0188	ns
LPS	0.0287 ± 0.0139	0.0395 ± 0.0052	ns
PA	0.0198 ± 0.0200	0.0304 ± 0.0149	ns
PC	68.54 ± 6.82	70.56 ± 12.32	ns
PC O-	2.07 ± 0.15	1.95 ± 0.27	ns
PE	24.72 ± 5.09	29.84 ± 7.06	ns
PE O-	18.23 ± 2.49	17.84 ± 1.65	ns
PG	0.869 ± 0.067	0.766 ± 0.406	ns
PI	12.15 ± 1.94	13.3 ± 2.76	ns
PS	5.9 ± 0.49	5.73 ± 0.72	ns
SM	5.29 ± 0.75	5.56 ± 0.75	ns
TAG	1306.32 ± 767.16	1583.34 ± 338.58	ns
Total	3496.45 ± 1473.05	4128.73 ± 698.88	ns

ns: not significant

Table S3. Double bond index (DBI) and length index (LI) in storage and non-storage lipids in the adrenal glands of LFD- and HFD-fed mice

	STORAGE LIPIDS (Aver ± SD)			NON-STORAGE LIPIDS (Aver ± SD)		
	LFD	HFD	P-value	LFD	HFD	P-value
DBI	2.809 +/- 0.095	3.072 +/- 0.024	***	3.322 +/- 0.049	3.462 +/- 0.042	***
LI	45.291 +/- 4.203	47.537 +/- 1.223	ns	36.828 +/- 0.081	37.110 +/- 0.064	***

*** <0.001; ns: not significant

Table S4. Composition of diets used for feeding of *Fads2*^{-/-} and WT mice

Fatty acid	PUFA-rich diet	Low-PUFA diet
Total sat.	19.62 ± 0.03	47.39 ± 0.01
Total mono.	28.16 ± 0.07	38.02 ± 0.03
18:2n-6	42.02 ± 0.03	9.27 ± 0.01
18:3n-6	0.02 ± 0.01	ND
20:2n-6	0.08 ± 0.001	ND
20:3n-6	0.02 ± 0.01	ND
20:4n-6	0.13 ± 0.003	ND
22:2n-6	ND	ND
22:4n-6	ND	ND
22:5n-6	ND	ND
Total ω6 FAs	42.27 ± 0.03	9.27 ± 0.01
18:3n-3	3.88 ± 0.02	2.43 ± 0.003
20:3n-3	ND	ND
20:5n-3	1.49 ± 0.01	ND
22:5n-3	0.25 ± 0.01	ND
22:6n-3	1.32 ± 0.01	ND
Total ω3 FAs	6.25 ± 0.02	2.43 ± 0.003
Total FA (μg/mg)	47.00 ± 0.68	43.19 ± 1.15

(% of total fatty acids; Mean ± SEM; ND, not detected)

Table S5. Lipidomics in adrenal glands of WT and *Fads2*^{-/-} mice fed a PUFA-rich or low-PUFA diet

Lipid species	Low-PUFA diet			PUFA-rich diet		
	WT (Aver±SD)	<i>Fads2</i> ^{-/-} (Aver±SD)	P value	WT (Aver±SD)	<i>Fads2</i> ^{-/-} (Aver±SD)	P value
C14:1 n-5	0.033333±0.08165	0.083333 ± 0.132916	0.45057	n.d.	n.d.	n.a.
C20:4 n-3 (ETA)	0.95±0.207364	0.5±0.089443	0.00064 (***)	0.983333±0.263944	1.466667±0.338625	0.020215264 (*)
C20:5 n-3 (EPA)	41.6±9.324591	21.23333±2.881435	0.00046 (***)	45.71667±6.546882	66.2±17.14211	0.021037628 (*)
C18:3 n-3 (ALA)	18.93333±6.712575	27.81667±12.06887	0.14619	35.88333±24.94806	37.98333±6.767102	0.846254490

C18:3 n-6 (GLA)	6.483333±1.483801	4.383333±2.964737	0.15181	10.35±5.29783	6.283333±2.11605	0.111377473
C14:0	99.383333±16.63171	81.333333±4.507623	0.02809 (*)	59.05±33.32559	73.66667±11.16739	0.332368186
C22:6 n-3 (DHA)	1567.833±281.4653	765.5667±68.03622	0.00005 (****)	1911.067±374.2711	2059.133±373.6225	0.508415986
C16:1 n-7	163.3167±29.67716	163.2±19.59153	0.993746	83.81667±44.24249	91.2±13.55389	0.7041063
C20:4 n-6 (ARA)	7703.533±1051.61	1808.25±236.4437	0.0000001 (****)	7843.15±1187.82	6571.517±769.2027	0.0523427
C18:2 n-6 (LA)	650.3667±128.7274	1498.85±215.0335	0.000009 (****)	1208.883±386.0135	1592.8±208.8327	0.0577698
C22:5 n-3 (DPA)	175.6±27.43545	118.75±16.53273	0.00145 (**)	126.9±30.69111	157.1167±32.01777	0.1261116
C22:5 n-6 (DPA)	53.36667±7.226248	0.066667±0.163299	0.000000 (****)	16.18333±5.177419	n.d.	n.a.
C20:3	126.9667±18.00507	12.06667±5.333542	0.000000 (****)	155.1667±16.56365	31.21667±3.727421	0.0000000 (****)
C16:0 (Palmitic acid)	1049.033±137.3459	1087.883±196.4046	0.699660	791.6833±295.7263	1176.983±237.1646	0.0320074 (*)
C18:1 n-9 cis (OA)	1754±285.4089	2170.517±221.2694	0.0180016 (*)	1056.183±308.9346	1199.933±136.9281	0.3219459
C18:1 n-9 trans (EA)	1887.533±276.9233	2223.267±222.4705	0.043129 (*)	1227.933±321.9364	1330.717±127.5553	0.48386794
C20:2 n-6 (DGLA)	28.18333±4.257895	136.45±19.96034	0.0000001 (****)	41.78333±6.146029	79.81667±19.75605	0.00113822 (**)
C18:0 (Stearic acid)	693.7333±104.6519	657.9167±96.40173	0.55128	580.9833±141.9845	730.2833±127.7511	0.0845414
C20:1 n-9	75.58333±15.21084	162.6±24.82402	0.000025 (****)	29.85±11.24736	40.66667±7.336939	0.0767587
C22:2 n-6	n.d.	0.9±0.745654	n.a.	n.d.	0.183333±0.213698	n.a.
C22:1 n-9	18.65±1.615859	34.36667±9.332452	0.002269 (**)	n.d.	24.96667±5.699006	n.a.
C22:0	254.65±112.2897	269.2167±129.8201	0.8394989	349.5167±219.8333	261.0667±112.668	0.40102768

* <0.05, ** <0.01, *** <0.001, **** <0.0001; n.d.: not detected, n.a.: not applicable

Table S6. List of primers

<i>Cyp11a1_Fwd</i>	5'GCTGAGTACTGGAAAGGGAGC3'
<i>Cyp11a1_Rev</i>	5'TGCCAGCTTCTCCCTGTAA3'
<i>Cyp11b1_Fwd</i>	5'ATAGAGAACTCCGTGGCCTG3'
<i>Cyp11b1_Rev</i>	5'TGCAGTCGGTTGAAGTACCA3'

<i>Cyp11b2_Fwd</i>	5'AGAATGGCGTCTCAACCGAC3'
<i>Cyp11b2_Rev</i>	5'AGTCCCTTGCTACCATGTCC3'
<i>Cyp21a1_Fwd</i>	5'GCTGTGGCTTTCCTGCTTCAC3'
<i>Cyp21a1_Rev</i>	5'GGCCCAGCTTGAGGTCTAACT3'
<i>Fads2_Fwd</i>	5'TCCTCTCGTACTTCGGCACT3'
<i>Fads2_Rev</i>	5'GGTTCACCAGTTGGCTGAG3'
<i>3β-Hsd2_Fwd</i>	5'AGGGAGCTCTCAATTGTGCC3'
<i>3β-Hsd2_Rev</i>	5'GCTTAGAAAGGCTGGTTCTGG3'
<i>Star_Fwd</i>	5'CTGTCCACCACATTGACCTG3'
<i>Star_Rev</i>	5'CAGCTATGCAGTGGGAGACA3'
<i>Tbp_Fwd</i>	5'AGAACAATCCAGACTAGCAGCA3'
<i>Tbp_Rev</i>	5'GGGAAC TTCACATCACAGCTC3'
<i>Mm 18S Fwd</i>	5'GTTCCGACCATAAACGATGCC3'
<i>Mm 18S Rev</i>	5'TGGTGGTGCCCTTCCGTCAAT3'
<i>FADS2 Fwd</i>	5'CACGGCCTTTGTCCTTGCTA3'
<i>FADS2 Rev</i>	5'AGATGTTAGGCTTGGCGTGG3'
<i>STAR Fwd</i>	5'GGGAGTGGAACCCCAATGTC3'
<i>STAR Rev</i>	5'CCAGCTCGTGAGTAATGAATGT3'
<i>Hs 18S Fwd</i>	5'TGCCCTATCAACTTTCGATG3'
<i>Hs 18S Rev</i>	5'GATGTGGTAGCCGTTTCTCA3'

Supplemental Figure legends

Figure S1. FADS2 is required for steroidogenesis in adrenocortical cells

A. FADS2 protein expression in liver (Liv), adrenal glands (Adr), kidney (Kid), brain (Br) and gonadal adipose tissue (GAT) in two wild-type mice (WT1, WT2) assessed by western blot using α -Tubulin as a loading control. Adrenal glands of *Fads2*^{-/-} mice (KO_{adrenal}) were used as negative control. **B.** PUFA synthesis pathway. **C.** Western blot of cell lysate fractions from NCI-H295R cells, separated by Optiprep density gradient. Same amount of protein from all fractions was blotted per lane. Representative blots are shown. Fractions 10-12 (SDHB positive) were identified as mitochondrial, fractions 4-5 (HSL positive) were identified as lipid droplets. **D.** Cholesterol and CE content in the lipid droplet fractions of NCI-H295R cells treated for 18 h with SC-26196 (10 μ M) or DMSO (Ctrl); results are shown as fold increase in SC-26196-treated compared to DMSO-treated cells. Results of one out of two experiments are shown. **E.** Cell viability of primary adrenal cells treated for 18 h with SC-26196 (10 μ M) or DMSO (n=4). **F.** NCI-H295R cells were treated with SC-26196 (10 μ M) or DMSO (Ctrl) for 18 h followed by 30 min or 24 h treatment with forskolin (FSK, 1 μ M), in presence of SC-26196 or DMSO. Steroids were measured in the cell culture supernatants by LC-MS/MS. **G.** Relative expression of *Star*, *Cyp11a1*, *3 β -Hsd2*, *Cyp11b1* and *Cyp11b2* in primary adrenal cell cultures treated for 18 h with SC-26196 (10 μ M) or DMSO (Ctrl). *Tbp* expression was used as a housekeeping gene. Relative gene expression of DMSO-treated cells was set to 1 (n=11). **H.** FADS2 expression in NCI-H295R cells transfected for 24 h with a FADS2-overexpressing plasmid (pFADS2) or a control plasmid (pCMV); *18S* expression was used as a housekeeping gene (n=4). **I.** FADS2 expression in pFADS2- or pCMV-transfected NCI-H295R cells assessed by western blot (24 h post-transfection). Vinculin was used as a loading control. **J.** 11-Deoxycorticosterone, cortisol and aldosterone levels were measured by LC-MS/MS in cell culture supernatants of pFADS2- or pCMV-transfected NCI-H295R cells treated 24 h post-transfection for 24 h with forskolin (FSK, 1 μ M) (n=5-6). Data in **E-H** and **J** are shown as mean \pm SEM; * p-value<0.05, ** p-value<0.01, *** p-value<0.001; **** p-value<0.0001

Figure S2. Obesity-related changes in lipid composition and gene transcription in the adrenal gland

A. ACTH plasma levels in LFD and HFD mice (n=6-8). **B.** Adrenal gland weight in LFD and HFD mice (7-10). Data in **A** and **B** are shown as mean \pm SEM; * p-value<0.05 **C.** FADS2 protein expression in liver and brain of LFD and HFD mice (n=5). Liver of *Fads2*^{-/-} mice (KO_{liver}) was used as negative control, α -Tubulin was used as a loading control. **D.** Principal component analysis (PCA) performed on all lipids, non-storage and storage lipids in adrenal glands of mice fed for 20 weeks a LFD or HFD. **E-H.** Storage (CE, TAG) (**E**) and non-storage lipids (phospholipids, lysophospholipids and sphingolipids; cholesterol was excluded from the calculation) (**G**) in adrenal glands of mice fed a LFD or HFD were regrouped according to the number of carbon atoms of the acyl chains; only the length of groups with a mean abundance > 1 mol % are shown. Storage (**F**) and non-storage lipids (**H**) were regrouped according to the number of double bonds of their acyl chains. **I,J.** PC (**I**) and PE (**J**) lipid species in adrenal glands of lean and obese mice. Only the species

with a mean abundance > 1 mol % are shown. The mean mol % \pm standard deviation (SD) are shown, (n=7-8 mice per group); * p-value<0.05; ** p-value<0.01; *** p-value<0.001. **K-P.** GSEA based on RNA-Seq in the adrenal cortex of mice fed for 20 weeks a LFD or HFD for genes involved in metabolism of lipids (**K**), mitochondrial biogenesis (**L**), respiratory electron transport (**M**), oxidative phosphorylation (**N**), citrate cycle (**O**) and fatty acid beta oxidation (**P**) (n=4 mice per group).

Figure S3. FADS2 inhibition reduces corticosteroid levels in obese mice

A. 11-Dehydrocorticosterone levels in hair of HFD mice 3 weeks after SC-26196 treatment start (n=8-10 mice per group). **B.** Glucose tolerance test in HFD mice performed at feeding week 14 (n=8-10 mice per group). Data are shown as mean \pm SEM; ** p-value<0.01.

Figure S4. Diet supplementation with icosapent ethyl transcriptionally reprograms mitochondrial metabolism in the adrenal cortex and does not affect ACTH levels

A-C. Body weight (**A**), glucose tolerance test (**B**) and insulin tolerance test (**C**) in mice receiving a LFD, a HFD or a HFD with icosapent ethyl (n=8 mice per group). **D-F.** GSEA based on RNA-Seq in the adrenal cortex of mice fed a HFD supplemented with icosapent ethyl versus mice fed a HFD without icosapent ethyl for genes related to respiratory electron transport (**D**), oxidative phosphorylation (**E**) and citrate cycle (**F**) (n=4 mice per group). **G.** ACTH measurement in plasma of mice fed a HFD containing or not icosapent ethyl (n=8 mice per group). Data in A-C and G are shown as mean \pm SEM.

Figure S5. FADS2 deficiency does not affect adrenal gland size, ACTH levels or steroidogenic enzyme expression

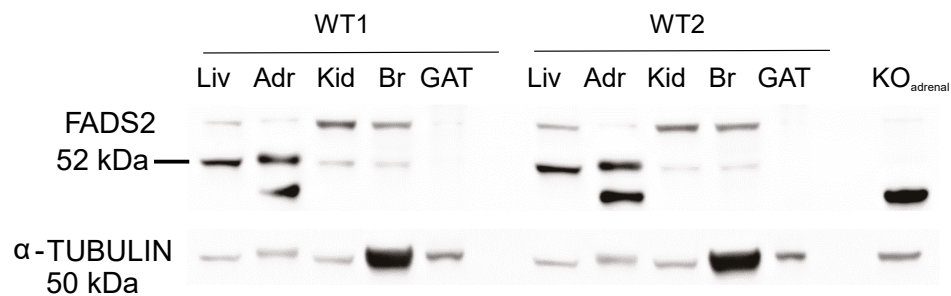
A. Hematoxylin and eosin staining in adrenal glands of WT and *Fads2*^{-/-} mice kept under a PUFA-rich or a low-PUFA diet. Representative photos of one caption (out of 7-8) per adrenal gland of WT and *Fads2*^{-/-} mice are shown (n=5 mice per group). Scale bar, 200 μ m **B.** ACTH measurement in plasma of WT and *Fads2*^{-/-} mice fed a PUFA-rich or low-PUFA diet (n=6-9 mice per group). **C.** Relative *Cyp11a1*, *3 β -Hsd2*, *Cyp11b1* and *Cyp11b2* expression in the adrenal glands of WT and *Fads2*^{-/-} mice fed a PUFA-rich or low-PUFA diet. Gene expression was set as 1 in WT mice fed a low-PUFA diet. *Tbp* was used as housekeeping gene (n=3 mice per group). **D.** Representative electron microscopy images of adrenocortical cells of WT and *Fads2*^{-/-} mice fed a PUFA-rich or low-PUFA diet (magnification 1,200x). Adrenal glands of two WT and two *Fads2*^{-/-} mice fed a low-PUFA and one WT and one *Fads2*^{-/-} mice fed a PUFA-rich diet were examined. Asterisks (*) depict lipid droplets. Scale bar, 1 μ m Data in **B** and **C** are shown as mean \pm SEM.

Figure S6. FADS2 expression correlates with expression of steroidogenic genes in the human adrenal gland

A-G. Correlation of *FADS2* expression with the expression of *STAR* (**A**), *VDAC1* (**B**), *CYP11A1* (**C**), *SF-1* (**D**) and *PRKACA* (**E**) in the human adrenal gland (**A-E**) and with the expression *SF-1* (**F**) and *PRKACA* (**G**)

in adrenocortical carcinoma (**F,G**), based on data from the public GEPIA2 database (<http://gepia2.cancer-pku.cn/#index>).

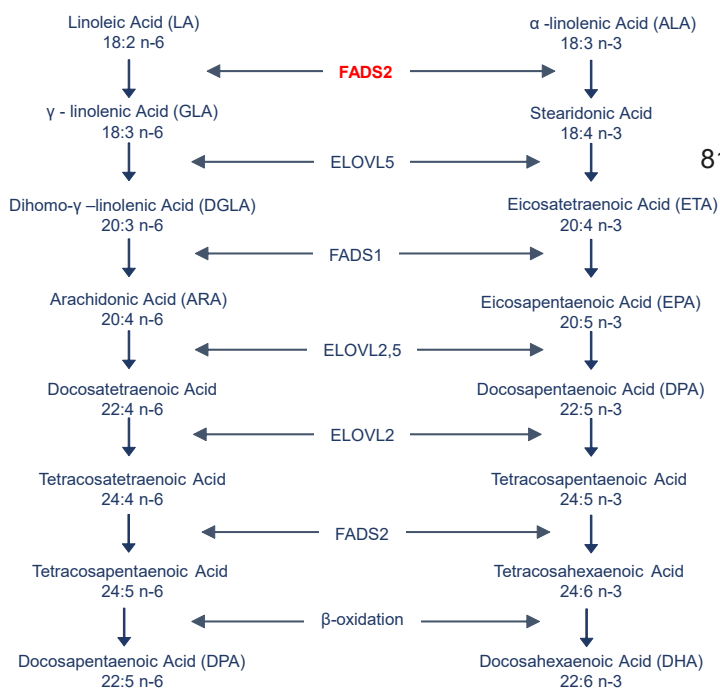
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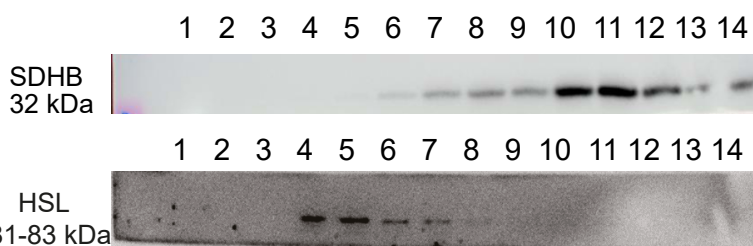
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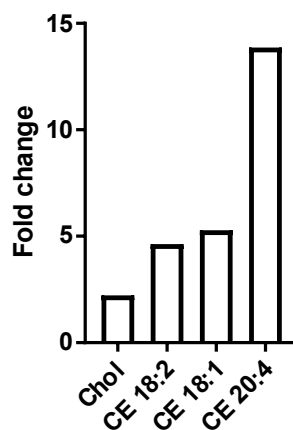
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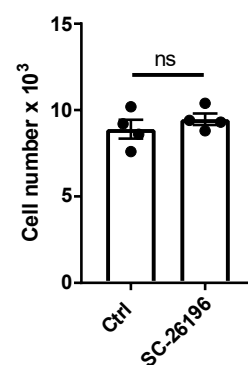
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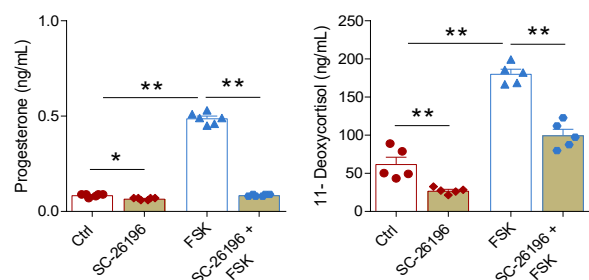
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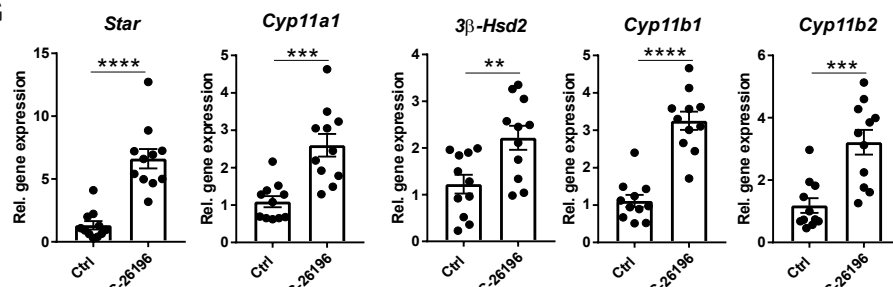
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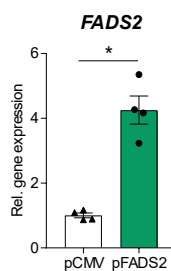
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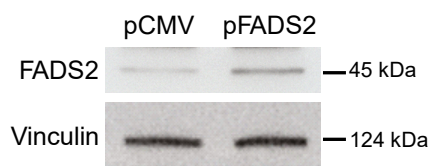
G



H



I



J

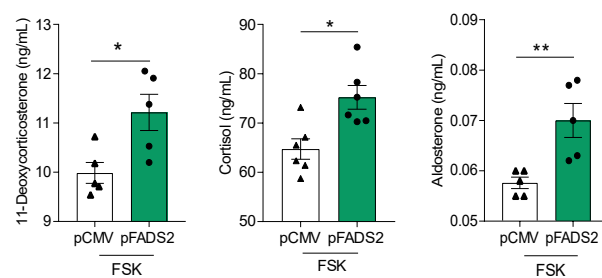


Figure S1

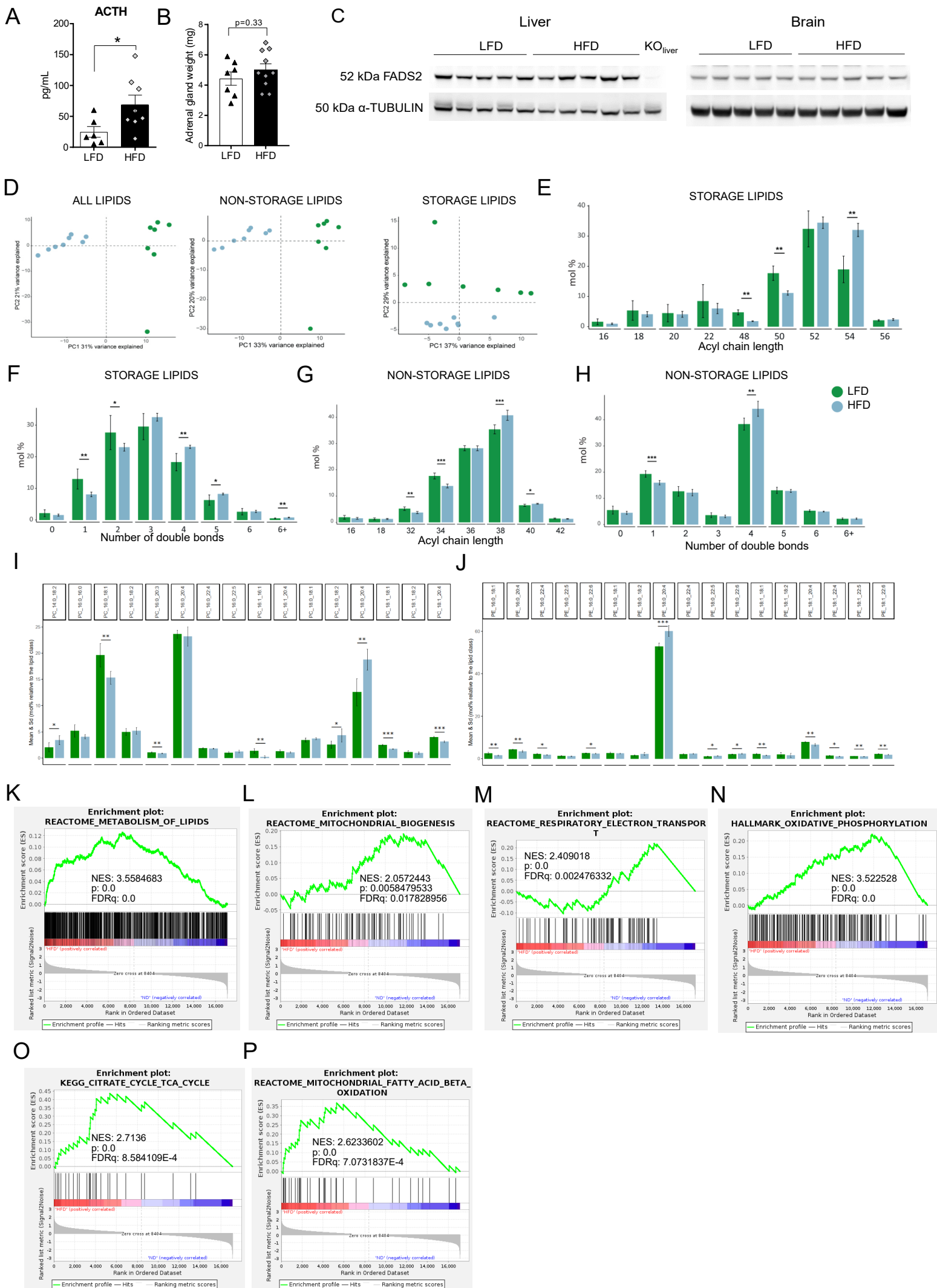
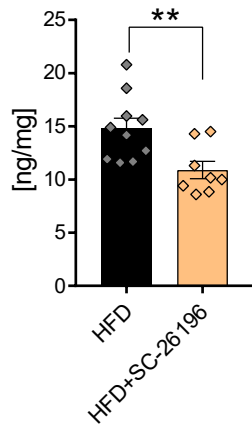


Figure S2

A

11-Dehydrocorticosterone



B

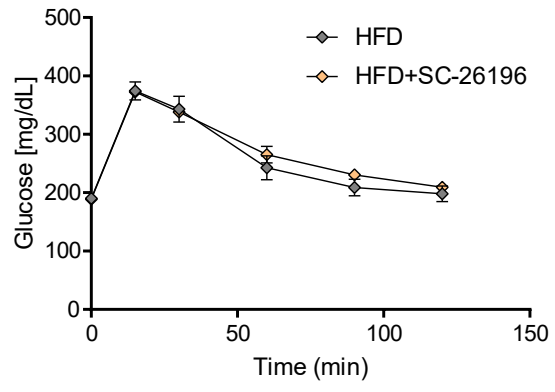


Figure S3

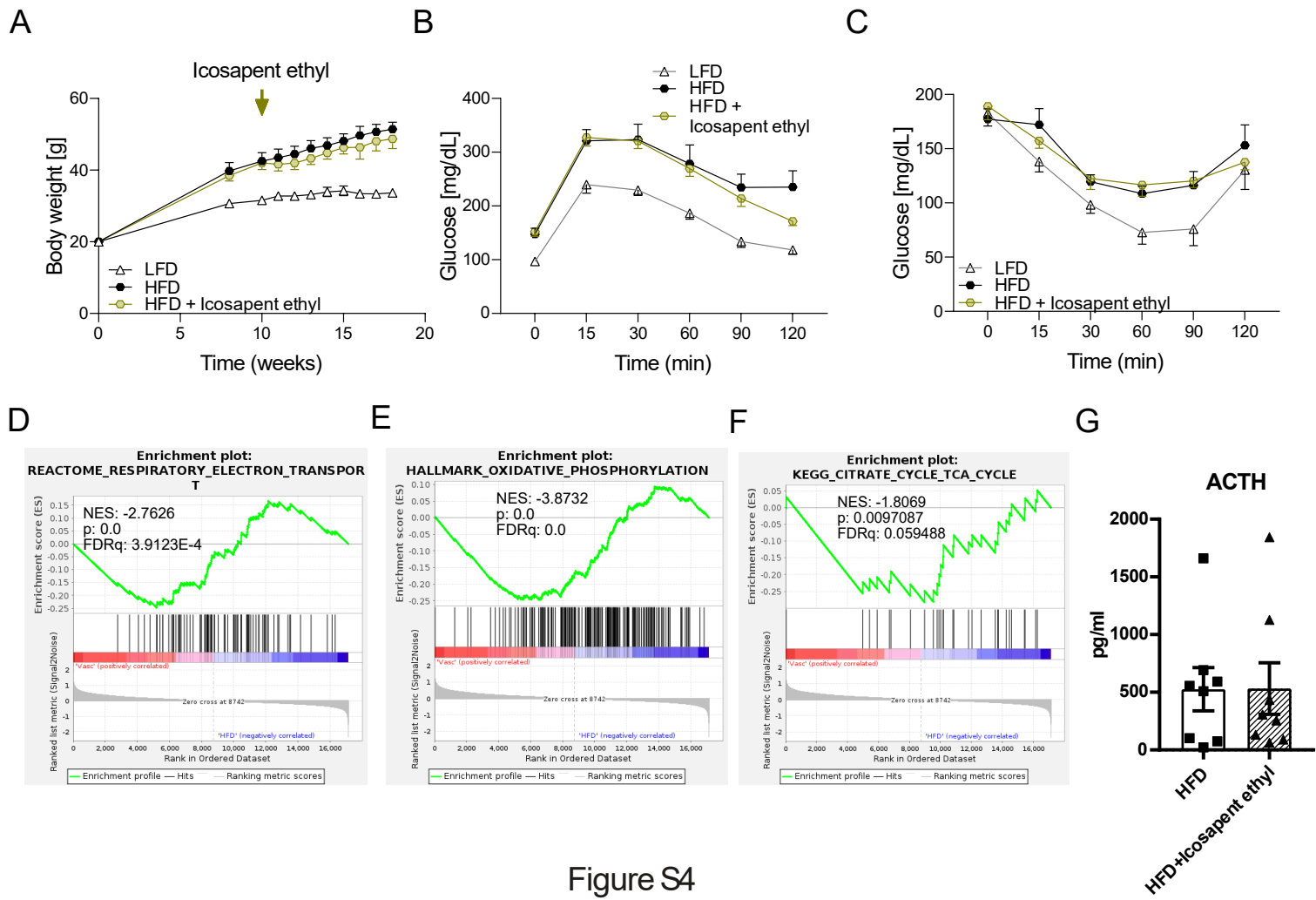


Figure S4

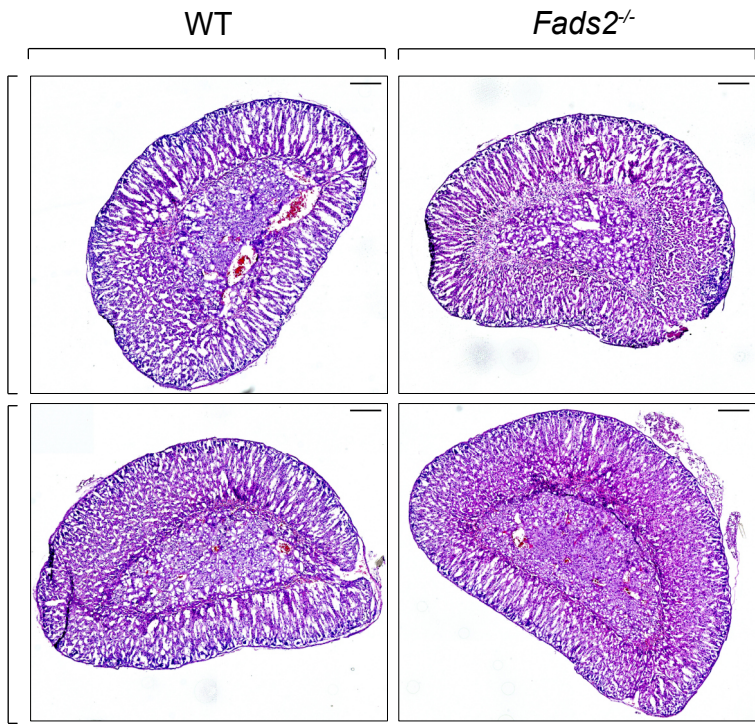
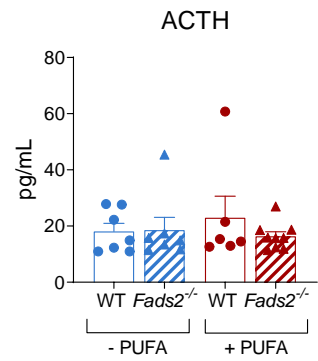
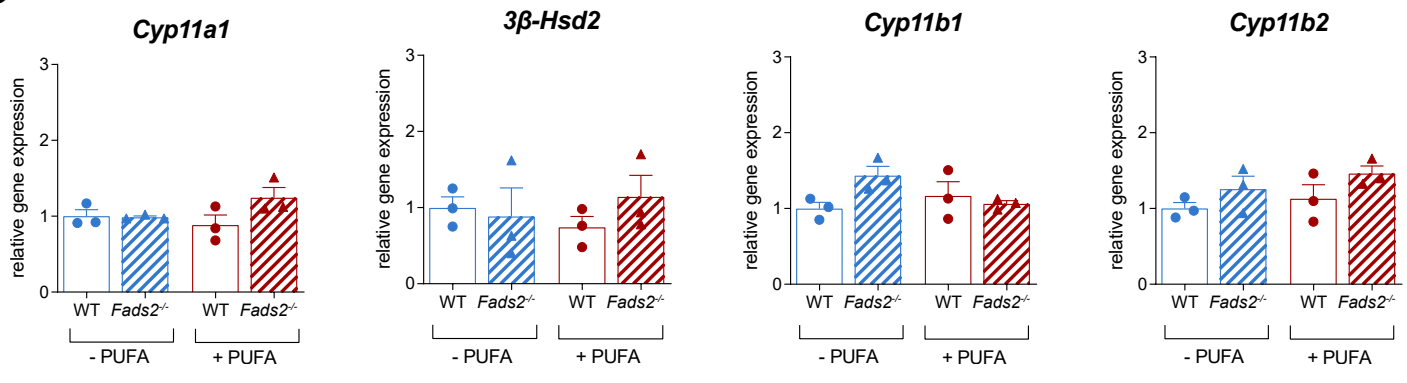
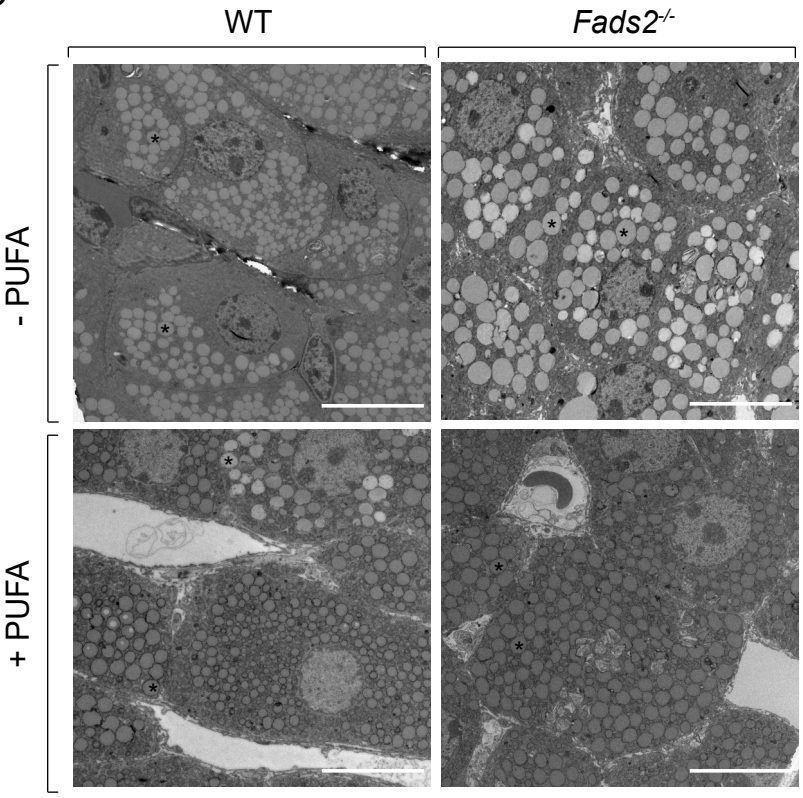
A**B****C****D**

Figure S5

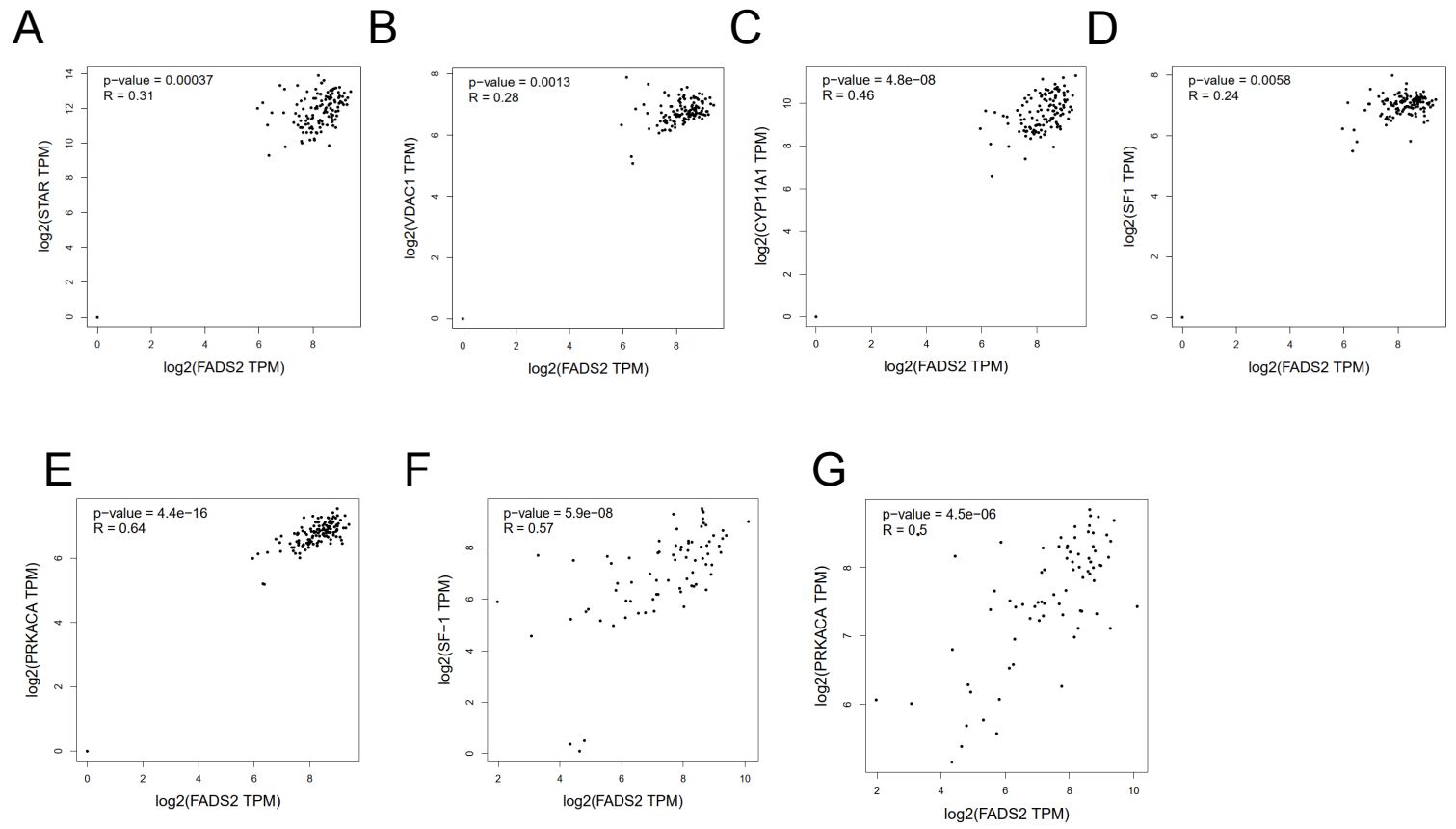


Figure S6