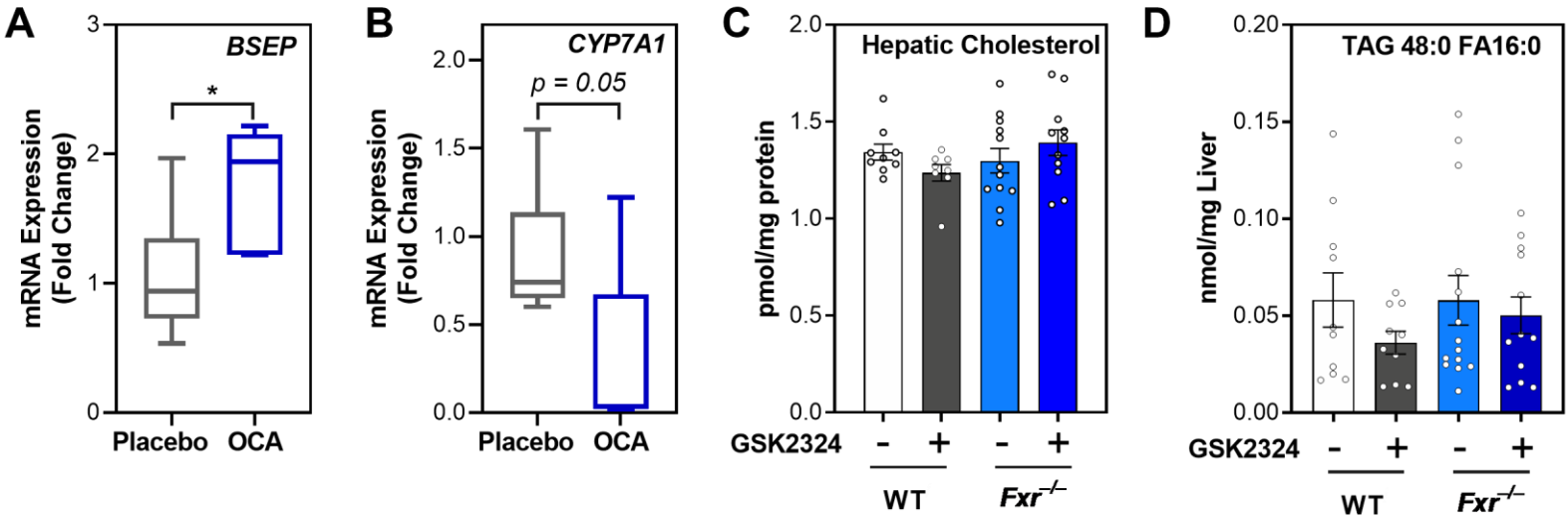

Figure S1. Humans and mice treated with FXR agonists have selective reductions in hepatic TAG containing MUFAs and PUFAs, related to Figure 1: (A, B) Hepatic mRNA expression of (A) BSEP and (B) CYP7A1 in human patients from 1A. (C) Total hepatic cholesterol in livers of mice from 1D. (D) Bar graph of individual TAG containing saturated fatty acids in livers of mice from 1D. Data are represented as mean ± SEM with individual animals noted as dots. *p<0.05

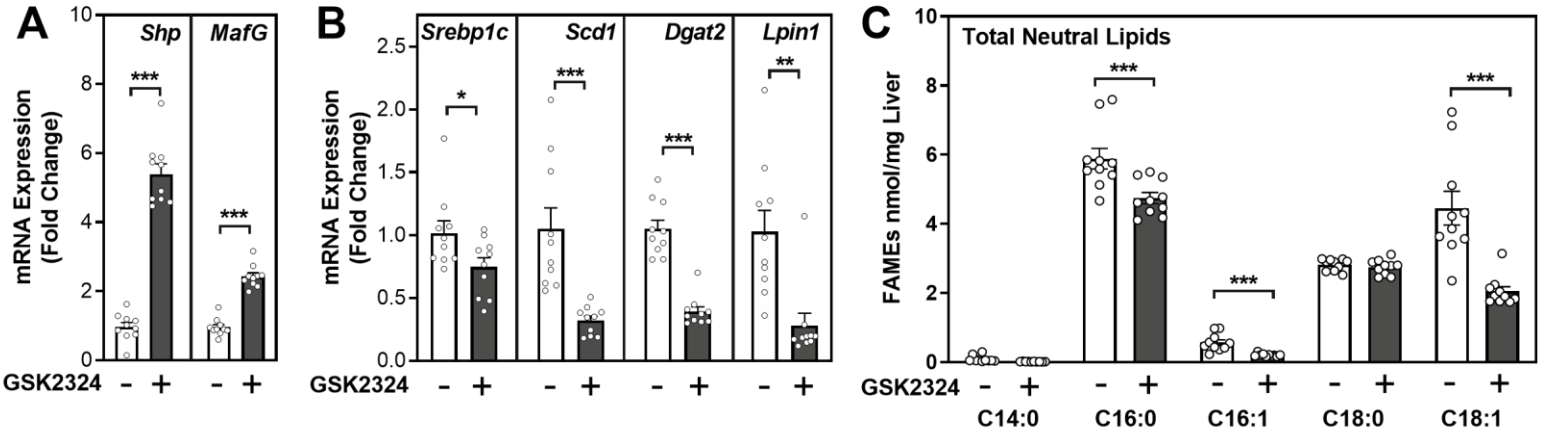

Figure S2. Treatment with GSK2324 selectively reduces the expression of fatty acid and triglyceride synthesis genes, related to Figure 2: (A, B) Hepatic mRNA expression of (A) FXR target genes Shp and MafG and (B) select fatty acid synthesis genes in mice from 2I. (C) Fatty acid methyl ester analysis for total neutral lipids in livers of mice treated as in 2I. Data are represented as mean ± SEM with individual animals noted as dots. *p<0.05, **p<0.01, ***p<0.0001.

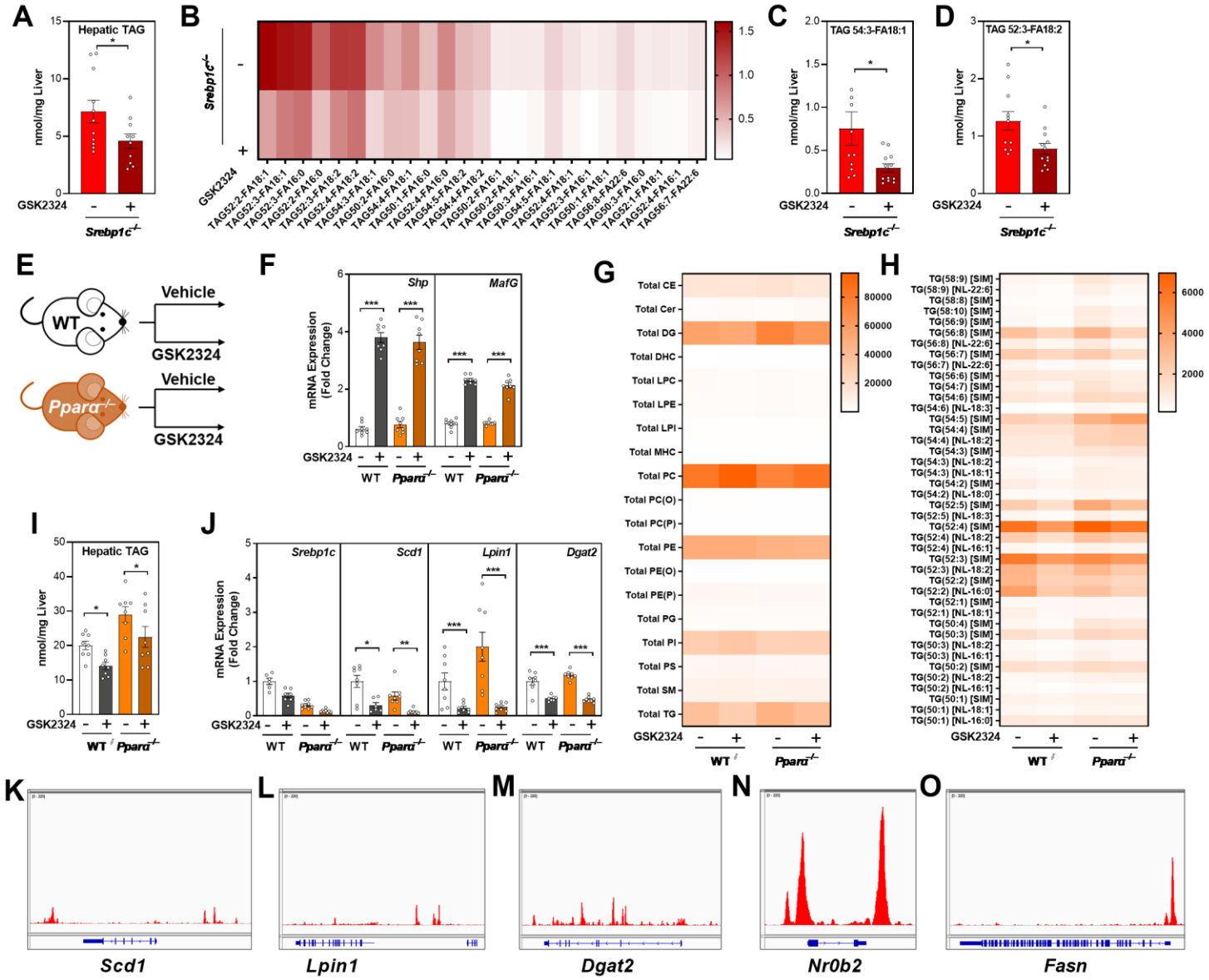

Figure S3, FXR activation reduces hepatic TAG levels independently of SHP, SREBP1c, and PPARα, related to Figure 3: (A-D) Lipidomic analyses showing effect of GSK2324 on (A) total hepatic TAG, (B) hepatic TAG species (nmol/mg liver), (C, D) individual TAG species containing MUFA and PUFA acyl tails in Srebp1c–/– mice. (E) Experiment schematic: Wildtype and Pparα–/– mice (n=7-8/group) fed a standard rodent diet were treated for 3 days with vehicle or FXR agonist GSK2324. (F) Hepatic mRNA expression of FXR target genes Shp and MafG in mice from E. (G-I) Lipidomic analyses showing (G) hepatic lipid classes (nmol/mg liver), (H) hepatic TAG species (nmol/mg liver), and (I) total hepatic TAG in mice from E. (J) Hepatic mRNA expression of select lipogenic genes in mice from E. (K-O) Hepatic ChIP-Seq analysis of FXR binding sites in (K) Scd1, (L) Lpin1, (M) Dgat2, (N) Nr0b2, and (O) Fasn. Data are represented as mean ± SEM with individual animals noted as dots. *p<0.05, **p<0.01, ***p<0.0001.

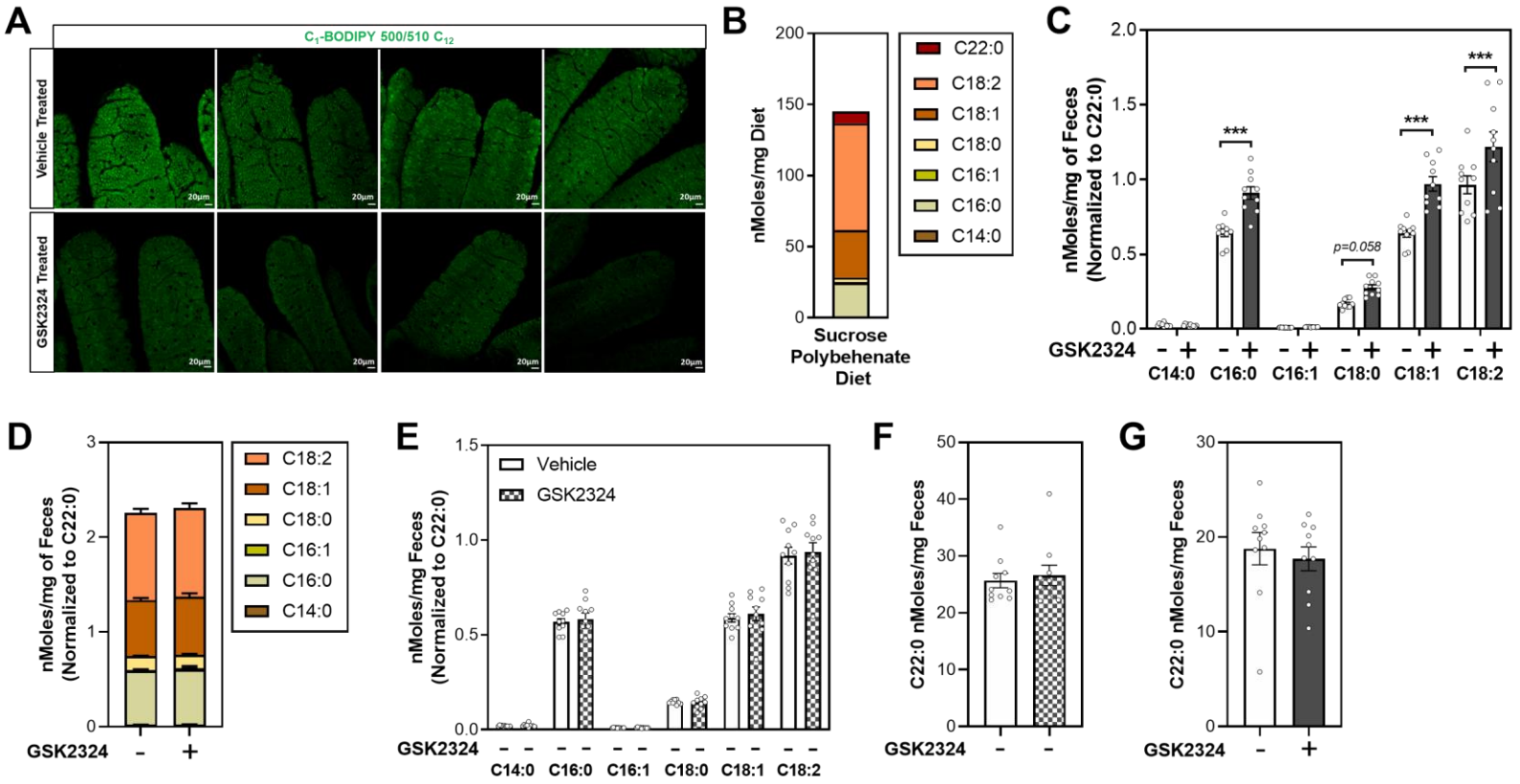

Figure S4. FXR activation decreases intestinal lipid absorption, related to Figure 4: (A) Additional images from intestinal sections of wildtype mice treated with vehicle or GSK2324. (B) Fatty acid composition of custom diet used in 2H. (C-E) Fecal fatty acids from mice in 2H (C) after treatment with GSK2324, (D, E) before treatment with vehicle or GSK2324. (F, G) Fecal C22:0 levels from mice (F) before and (G) after treatment with vehicle or GSK2324 as in 2H. Data are represented as mean ± SEM with individual animals noted as dots. ***p<0.0001.

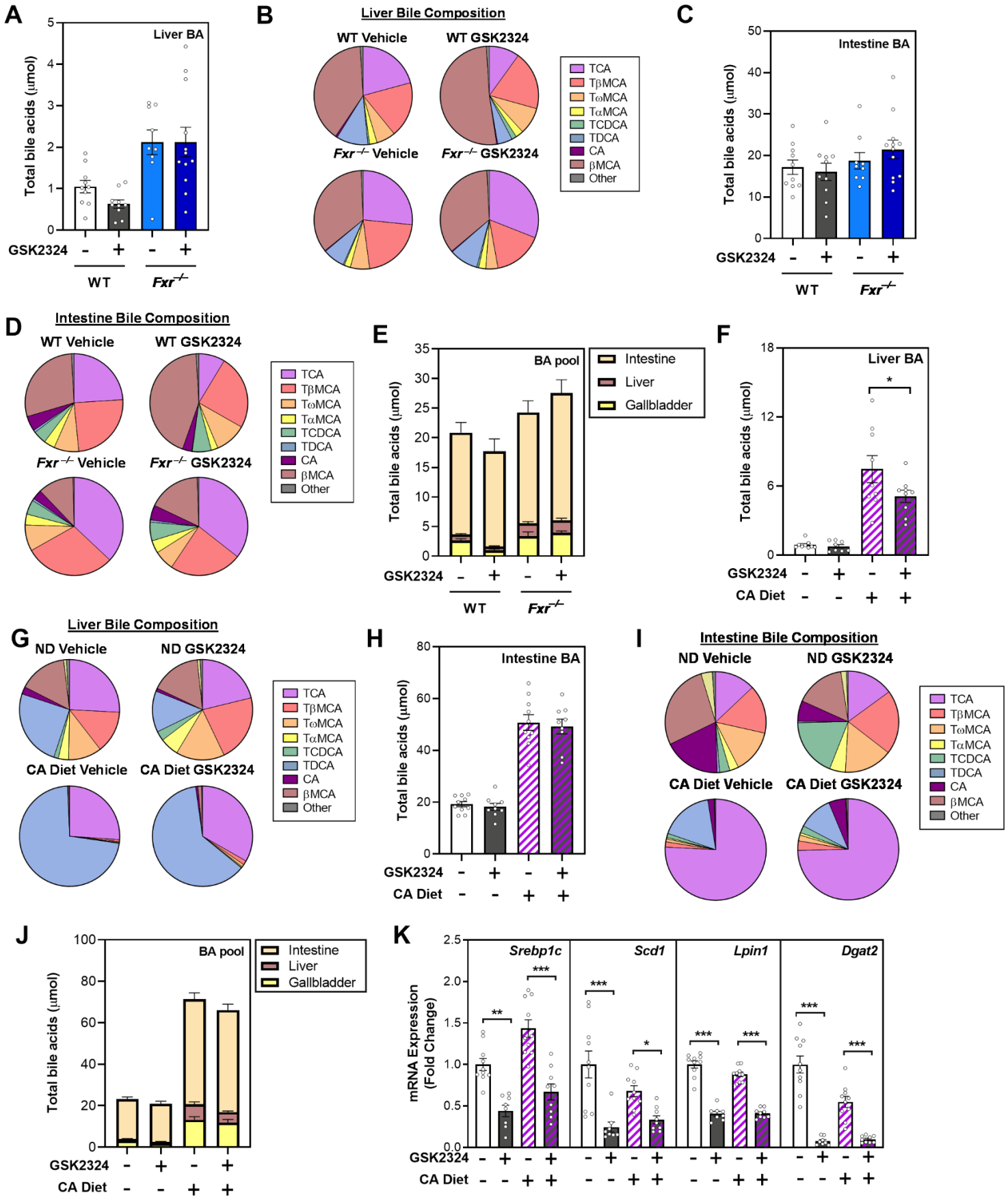

Figure S5. Decreased intestinal lipid absorption requires FXR-dependent changes in bile acids, related to Figure 5: (A) Total liver bile acid amount and (B) liver bile acid composition in mice from 5A. (C) Total intestinal bile acid amount and (D) intestinal bile acid composition in mice from 5A. (E) Total bile acid pool amount in mice from 5A. (F) Total liver bile acid amount and (G) liver bile acid composition in mice from 5E. (H) Total intestinal bile acid amount and (I) intestinal bile acid composition in mice from 5E. (J) Total bile acid pool amount in mice from 5E. (K) Hepatic mRNA expression of select lipogenic genes in mice from 5E. Data are represented as mean ± SEM with individual animals noted as dots. *p<0.05, **p<0.01 ***p<0.0001.

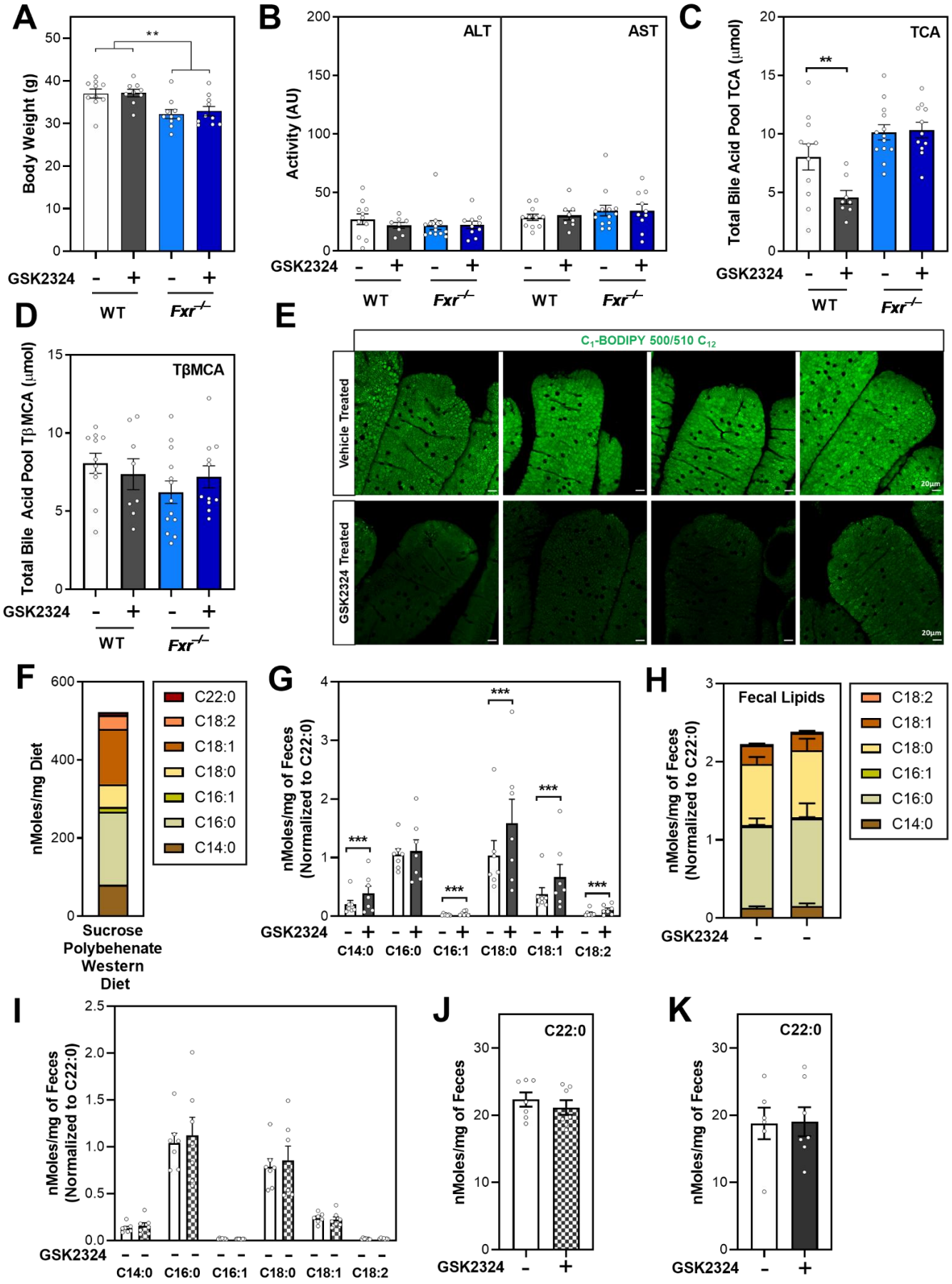

Figure S6. In a NAFLD model, FXR activation dramatically decreases hepatic TAG and intestinal lipid absorption, related to Figure 6: (A) Final body weight of mice treated as in 6A. (B) Plasma ALT/AST measured in mice from 6A. Total amount of (C) taurocholic acid and (D) tauro-β-muricholic acid in the bile acid pool of mice from 6A. (E) Additional images from intestinal sections from mice gavaged with BODIPY as in 6F. (F) Fatty acid composition of custom western diet used in 6J. (G-I) Fecal fatty acids from mice in 6F (G) after treatment with vehicle or GSK2324, and (H, I) before treatment with vehicle or GSK2324. (J, K) Fecal C22:0 levels from mice (J) before and (K) after treatment with vehicle or GSK2324 as in 6J. Data are represented as mean ± SEM with individual animals noted as dots. **p<0.01, ***p<0.0001.

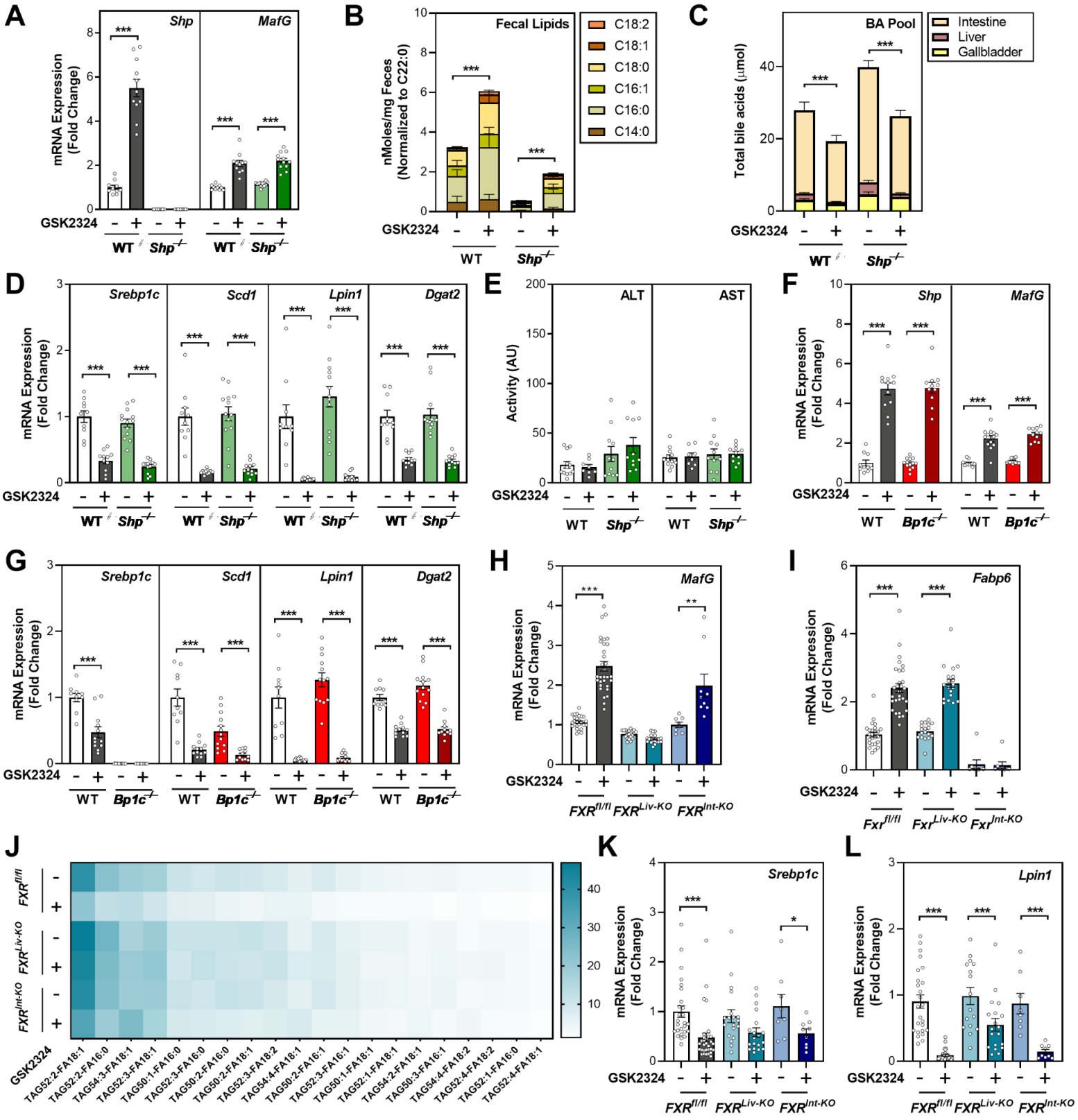

Figure S7. Both intestinal and hepatic FXR are required for changes in hepatic TAG, independent of SHP and SREBP1C, related to Figure 7: (A, F) Hepatic mRNA expression of FXR target genes Shp and MafG in wildtype or (A) Shp–/– mice and (F) Srebp1c–/– mice. (B) Fecal fatty acids after treatment with either vehicle or GSK2324 in mice from 7A. (C) Total bile acid pool amount in mice from 7A. (D, G) Hepatic mRNA expression of select lipogenic genes in wildtype or (D) Shp–/– mice and (G) Srebp1c–/– mice. (H, K, L) Hepatic mRNA expression of (H) MafG, (K) Srebp1c, and (L) Lpin1 in mice from 7E. (I) Ileal mRNA expression of Fabp6 in mice from 7E. (J) Lipidomic analysis of hepatic TAG species (nmol/mg liver) from mice in 7E. Data are represented as mean ± SEM with individual animals noted as dots. *p<0.05, **p<0.01, ***p<0.0001.

Gene Name	Species	Primer Sequence	Source
Shp	Mouse	F: CGATCCTCTTCAACCCAGATG R: AGGGCTCCAAGACTTCACACA	Lee et al., 2008
MafG	Mouse	F: GACCCCCAATAAAGGAAACAA R: TCAACTCTCGCACCGACA	De Aguiar Vallim et al., 2013
Acly	Mouse	F: GCCAGCGGGAGCACATC R: CTTTGCAGGTGCCACTTCATC	This Paper
Acss2	Mouse	F: ACAGAGTCGCCCTTTACGTG R: TCTTCAAAAGTCACCTTCACG	This Paper
Acaca	Mouse	F: TGACAGACTGATCGCAGAGAAAG R: TGGAGAGCCCCACACACA	Liang et al., 2002
Acacb	Mouse	F: TGAATCTCACGCGCCTACTA R: GCCTCTCTTCACCAGATGGA	This Paper
Fasn	Mouse	F: GCTGCGGAAACTTCAGGAAAT R: AGAGACGTGTCACTCCTGGACTT	Liang et al., 2002
Scd1	Mouse	F: CCGGAGACCCCTTAGATCGA R: TAGCCTGTAAAAGATTTCTGCAAACC	Liang et al., 2002
Elovl1	Mouse	F: GTGGCCCAGCCCTACCTT R: TGTGCAGTGAGACCAGGACAA	Yang et al., 2001
Elovl3	Mouse	F: TTCTCACGCGGGTTAAAAATG R: GGGCCTTAAGTCCTGAAACGT	Yang et al., 2001
Elovl2	Mouse	F: TCAATGCTTTCTTGGACAACATG R: GGTAAGAGTCCAGCAGGAACCA	Yang et al., 2001
Elovl5	Mouse	F: ATGGACACCTTTTTCTTCATCCTT R: ATGGTAGCGTGGTGGTAGACATG	Yang et al., 2001
Elovl6	Mouse	F: CAGCAAAGCACCCGAACTA R: AGGAGCACAGTGATGTGGTG	Yang et al., 2001
Gpat1	Mouse	F: AGCAAGTCCTGCGCTATCAT R: CTCGTGTGGGTGATTGTGAC	Li et al., 2009
Gpat2	Mouse	F: AAGAAAGAGGTACAGCGTATCC R: GTGGAGAGCCCTCCTGCACAG	Li et al., 2009
Gpat3	Mouse	F: CTTTGAAATCGGAGGAACCA R: TTTGCAAACTGAACTGCGTC	Li et al., 2009
Gpat4	Mouse	F: GGCATGGTGACGTACCTTCT R: GCTTCAGCCAGCACAAGAC	Li et al., 2009
Agpat1	Mouse	F: GCTGGCTGGCAGGAATCAT R: GTCTGAGCCACCTCGGACAT	Yang et al., 2001
Agpat2	Mouse	F: TTTGAGGTCAGCGGACAGAA R: AGGATGCTCTGGTGATTAGAGATGA	Yang et al., 2001
Agpat3	Mouse	F: GGAGGAAAACACCTGTCCAC R: TCAAGGGTGTCCGACCTG	Li et al., 2009
Agpat5	Mouse	F: CTAGCGAATCATCAAAGCACA R: TCTTTCAGTACGTAGCGCACA	Li et al., 2009
Lpin1	Mouse	F: CCTTCTATGCTGCTTTTGGGAACC R: GTGATCGACCACTTCGCAGAGC	Reue Lab, UCLA
Lpin2	Mouse	F: AGTTGACCCCATCACCGTAG R: CCCAAAGCATCAGACTTGGT	Reue Lab, UCLA
Lpin3	Mouse	F: TGGAATTGGGATGACAAGGT R: CACTGCAAGTACCCCTTGGT	Reue Lab, UCLA
Dgat1	Mouse	F: TCGTGGTATCCTGAATTGGTG R: AGGTTCTCTAAAAATAACCTTGCATT	Yang et al.,2001
Dgat2	Mouse	F: GGCGCTACTTCCGAGACTAC R: TGGTCAGCAGGTTGTGTGTC	Yang et al.,2001
Srebp1c	Mouse	F: GGAGCCATGGATTGCACATT R: GGCCCGGGAAGTCACTGT	Liang et al., 2002
BSEP	Human	F: CAGCCCTCTCATTGGGATT R: TCCGTAAACTTGGACACACTC	This Paper
CYP7A1	Human	F: GCTTATTCTTGGAATTAGGAGAAGG R: TTGGCACCAAATTGCAGAG	This Paper
Tbp	Mouse	F: CTCAGTTACAGGTGGCAGCA R: ACCAACAATCACCAACAGCA	This Paper
36b4	Mouse	F: TTTGACAACGGCAGCATTTA R: CCATTGATGATGGAGTGTGG	This Paper
Fgf15	Mouse	F: CAGGGAGGAAATGGACTGTT R: GGTGAAACACGGGGATAAAG	This Paper
Fabp6	Mouse	F: GAGGCTGTGGGTGACAAGTT R: TACGCGCTCATAGGTCACAT	This Paper

Table S1: Primer sequences used for qPCR, related to STAR methods.

Bile acid	Precursor m/z	Product 1 m/z	Product 2 m/z	Explicit Retention Time	Explicit Collision Energy (precursor, product)
LCA	375.2904			20.8	10
HDCA	391.2853			17.4	10
DCA	391.2853			19.2	10
UDCA	405.2646			8.8	10
3b7a12a	407.2802			13.2	10
αMCA	407.2802			14.1	10
βMCA	407.2802			14.7	10
CA	407.2802			16.8	10
GCDCA	448.3068	74.0248		16.8	10, 35
GCA	464.3017	74.0248		12.7	10, 35
TLCA	482.42	124.0067	79.9578	17.7	10, 55
Tauro 3a7k	496.3	124.0067	79.9578	10.5	10, 55
TUDCA	498.2894	124.0067	79.9578	6.6	10, 55
T-3a6a	498.2894	124.0067	79.9578	9.7	10, 55
T-3a12b	498.2894	124.0067	79.9578	10.7	10, 55
TCDCA	498.2894	124.0067	79.9578	14.2	10, 55
TDCA	498.2894	124.0067	79.9578	14.4	10, 55
T-ωMCA	514.284	124.0067	79.9578	4.3	10, 55
T-βMCA	514.284	124.0067	79.9578	5	10, 55
T-αMCA	514.284	124.0067	79.9578	7.7	10, 55
TCA	514.284	124.0067	79.9578	9.9	10, 55

Table S2: Bile acid retention times and mass-charge ratios used in UPLC-MS/MS methodology, related to STAR methods.

Bile acid (abbreviation)	Common Name	Systematic Name	Source	Standard Concentration Range (nM)	R-squared value	Sample dilution for quantification
LCA	Lithocholic acid	3α-Hydroxy-5β-cholan-24-oic acid	Kind gift from Dr. Lee Hagey	500-8	0.9678	1 to 100
HDCA	Hyodeoxycholic acid	3α,6α-Dihydroxy-5β-cholan-24-oic acid	Kind gift from Dr. Lee Hagey	500-0.2	0.9915	1 to 100
DCA	Deoxycholic acid	3α,12α-Dihydroxy-5β-cholan-24-oic acid	Sigma-Aldrich	500-0.2	0.9987	1 to 100
UDCA	Ursodeoxycholic acid	3α,7β-Dihydroxy-5β-cholan-24-oic acid	Kind gift from Dr. Lee Hagey	500-8	0.9669	1 to 100
3b7a12a		3β,7α,12α-Trihydroxy-5β-cholan-24-oic acid	Kind gift from Dr. Lee Hagey	1000-15	0.998	1 to 100
αMCA	α-muricholic acid	3α,6β,7α-Trihydroxy-5β-cholan-24-oic acid	Kind gift from Dr. Lee Hagey	1000-0.5	0.9974	1 to 100
βMCA	β-muricholic acid	3α,6β,7β-Trihydroxy-5β-cholan-24-oic acid	Kind gift from Dr. Lee Hagey	1000-4	0.997	1 to 10,000
CA	Cholic acid	3α,7α,12α-trihydroxy-5β-cholan-24-oic acid	Sigma-Aldrich	1000-1	0.9995	1 to 10,000
GCDCA	Glyco chenodeoxycholic acid	3α,7α-Dihydroxy-5β-cholan-24-oic acid N-(carboxymethyl)-amide	Sigma-Aldrich	500-0.2	0.9979	1 to 100
GCA	Glyco cholic acid	3α,7α,12α-trihydroxy-5β-cholan-24-oic acid N-(carboxymethyl)-amide	Sigma-Aldrich	1000-4	0.9989	1 to 100
TLCA	Tauro Lithocholic acid	3α-Hydroxy-5β-cholan-24-oic acid N-(2-sulphoethyl)-amide	Kind gift from Dr. Lee Hagey	1000-0.5	0.9986	1 to 100
7-oxo-TLCA	7-oxolithocholic acid	3α-Hydroxy-7-oxo-5β-cholan-24-oic Acid N-(2-sulphoethyl)-amide	Kind gift from Dr. Lee Hagey	1000-0.5	0.9935	1 to 10,000
TUDCA	Tauro Ursodeoxycholic acid	3α,7β-Dihydroxy-5β-cholan-24-oic acid N-(2-sulphoethyl)-amide	Kind gift from Dr. Lee Hagey	1000-2	0.9717	1 to 10,000
T-HDCA	Tauro Hyodeoxycholic acid	3α,6α-Dihydroxy-5β-cholan-24-oic acid N-(2-sulphoethyl)-amide	Kind gift from Dr. Lee Hagey	1000-2	0.9945	1 to 10,000
T-3a12b	Tauro Lagodeoxycholic	3α,12β-Dihydroxy-5β-cholan-24-oic acid N-(2-sulphoethyl)-amide	Kind gift from Dr. Lee Hagey	1000-4	0.993	1 to 10,000
TCDCA	Tauro Chenodeoxycholic acid	3α,7α-Dihydroxy-5β-cholan-24-oic acid N-(2-sulphoethyl)-amide	Sigma-Aldrich	1000-0.5	0.9985	1 to 10,000
TDCA	Tauro deoxycholic acid	3α,12α-Dihydroxy-5β-cholan-24-oic acid N-(2-sulphoethyl)-amide	Sigma-Aldrich	1000-0.5	0.9995	1 to 10,000
T-ωMCA	Tauro ω-muricholic acid	3α,6α,7β-Trihydroxy-5β-cholan-24-oic acid N-(2-sulphoethyl)-amide	Kind gift from Dr. Lee Hagey	see TCA		1 to 10,000
T-βMCA	Tauro β-muricholic acid	3α,6β,7β-Trihydroxy-5β-cholan-24-oic acid N-(2-sulphoethyl)-amide	Kind gift from Dr. Lee Hagey	see TCA		1 to 10,000
T-αMCA	Tauro α-muricholic acid	3α,6β,7α-Trihydroxy-5β-cholan-24-oic acid N-(2-sulphoethyl)-amide	Kind gift from Dr. Lee Hagey	see TCA		1 to 10,000
TCA	Tauro cholic acid	3α,7α,12α-trihydroxy-5β-cholan-24-oic acid N-(2-sulphoethyl)-amide	Sigma-Aldrich	10000-40	0.9959	1 to 10,000

Table S3: Bile acid standard information and catalogue numbers, related to STAR methods.