Supplemental Data

PEROXISOME DEFICIENCY CAUSES A COMPLEX PHENOTYPE DUE TO HEPATIC SREBP/INSIG DYSREGULATION ASSOCIATED WITH ENDOPLASMIC RETICULUM STRESS

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TABLE S1. Activities of cholesterol biosynthetic enzymes in kidneys of P9 BA-fed control and *PEX2* knockout mice

Enzyme	Enzyme activity (pmol/min/mg of protein) in:		Ratio of activities
	Control mice	*PEX2*$^{-/-}$ mice	
HMG-CoA reductase	57.1 ± 4.6 (4)	32.9 ± 3.2 (3) **	0.6
IPP isomerase	218.0 ± 5.0 (4)	343.2 ± 62.7 (3) *	1.6
FPP synthase	297.4 ± 30.0 (4)	466.9 ± 84.5 (3) *	1.6
Squalene synthase	2.1 ± 0.4 (4)	3.1 ± 0.9 (3)	1.5

Each value represents the mean ± standard deviation. Values in parentheses denote the number of samples. The measured activities in BA-fed mice did not significantly differ from that in untreated mice (Kovacs et al., 2004). *, $P < 0.01$; **, $P < 0.001$ (Student's *t* test). Ratio of activities = *PEX2*$^{-/-}$/control.

TABLE S2. Expression of SREBP regulated genes in the liver of P9 *PEX2*$^{-/-}$ mice

Gene	Gene Symbol	Relative expression	GenBank
Cholesterol Synthesis, Uptake, & Metabolism			
Acetoacetyl-CoA synthetase	Aacs	2.4	NM_030210
Acetyl-CoA synthetase 2	Acas2	7.2	NM_019811
ATP citrate lyase	Acly	10.1	NM_134037
HMG-CoA synthase *	Hmgcs1	3.5	NM_145942
HMG-CoA reductase *	Hmgcr	15.7	NM_008255
Mevalonate kinase	Mvk	21.6	NM_023556
Mevalonate pyrophosphate decarboxylase	Mvd	62.7	NM_138656
Isopentenyl-diphosphate Δ isomerase *	Idi1	12.4	NM_145360
Farnesyl diphosphate synthase *	Fpps	11.9	NM_134469
Squalene epoxidase *	Sqle	36.4	NM_009270
Lanosterol synthase *	Lss	44.2	NM_146006
Lanosterol 14α-demethylase	Cyp51	15.8	NM_020010
Sterol-C4-methyl oxidase-like	Sc4mol	27.8	NM_025436
NAD(P) dependent steroid dehydrogenase-like	Nsdhl	70.0	NM_010941
17β-Hydroxysteroid dehydrogenase-7	Hsd17b7	3.5	NM_010476
3β-Hydroxysteroid- Δ8, Δ7-isomerase	Ebp	4.1	NM_007898
Sterol-C5-desaturase	Sc5d	2.0	NM_172769
7-Dehydrocholesterol reductase *	Dhcr7	1.7	NM_007856
24-Dehydrocholesterol reductase	Dhcr24	2.3	NM_053272
Insig-1 *	Insig1	12	NM_153526
Proprotein convertase subtilisin/kexin type 9	Pcsk9	6.9	NM_153565
LDL receptor *	Ldlr	‡	NM_010700
Fatty Acid, TG, & PL Synthesis			
Fatty acid synthase *	Fasn	4.3	NM_007988
Malic enzyme	Mod1	6.9	NM_008615
Acetyl-CoA carboxylase beta	Acc2	16.0	BC022940
Long-chain fatty acyl elongase	Elovl6	3.4	NM_130450
Stearoyl-CoA desaturase 2	Scd2	24.5	NM_009128
Glycerol-3-phosphate acyltransferase	Gpam	4.3	NM_008149
Fatty acid desaturase 2	Fads2	2.6	NM_019699
L-specific multifunctional b-oxidation protein *†	Ehhadh	122.8	NM_023737
Miscellaneous			
Mac30		3	NM_133706
Aldolase C	Aldoc	‡	NM_009657
Retinol dehydrogenase 11	Rdh11	‡	NM_021557
Trefoil factor-3, intestinal	TFF3	2.9	NM_011575
Carbonyl reductase-1	Cbr1	15.2	NM_007620
Aldehyde dehydrogenase family-1, subfamily A7	ALDH1A7	4.7	NM_011921
Diazepam binding inhibitor *	Dbi	2.0	NM_007830
Cysteine sulfinic acid decarboxylase	Csad	1.3	NM_144942

Total RNA was isolated from five P9 wild-type and *PEX2*$^{-/-}$ livers using the RNeasy Mini kit (Qiagen). Equal aliquots of total RNA from each of five mouse livers in each group were pooled and prepared for CodeLink oligonucleotide hybridization. Hybridization, washing, scanning, and analysis of the CodeLink Mouse 20K Bioarray (Amersham Biosciences) were carried out at the Biomedical Genomics Microarray Facility at the University of California San Diego. The relative mRNA expression of each gene in livers from *PEX2*$^{-/-}$ mice compared to the appropriate wild-type controls is shown.

* Gene expression changes that have been verified by one or more methods, i.e. northern gel analysis, quantitative RT-PCR.

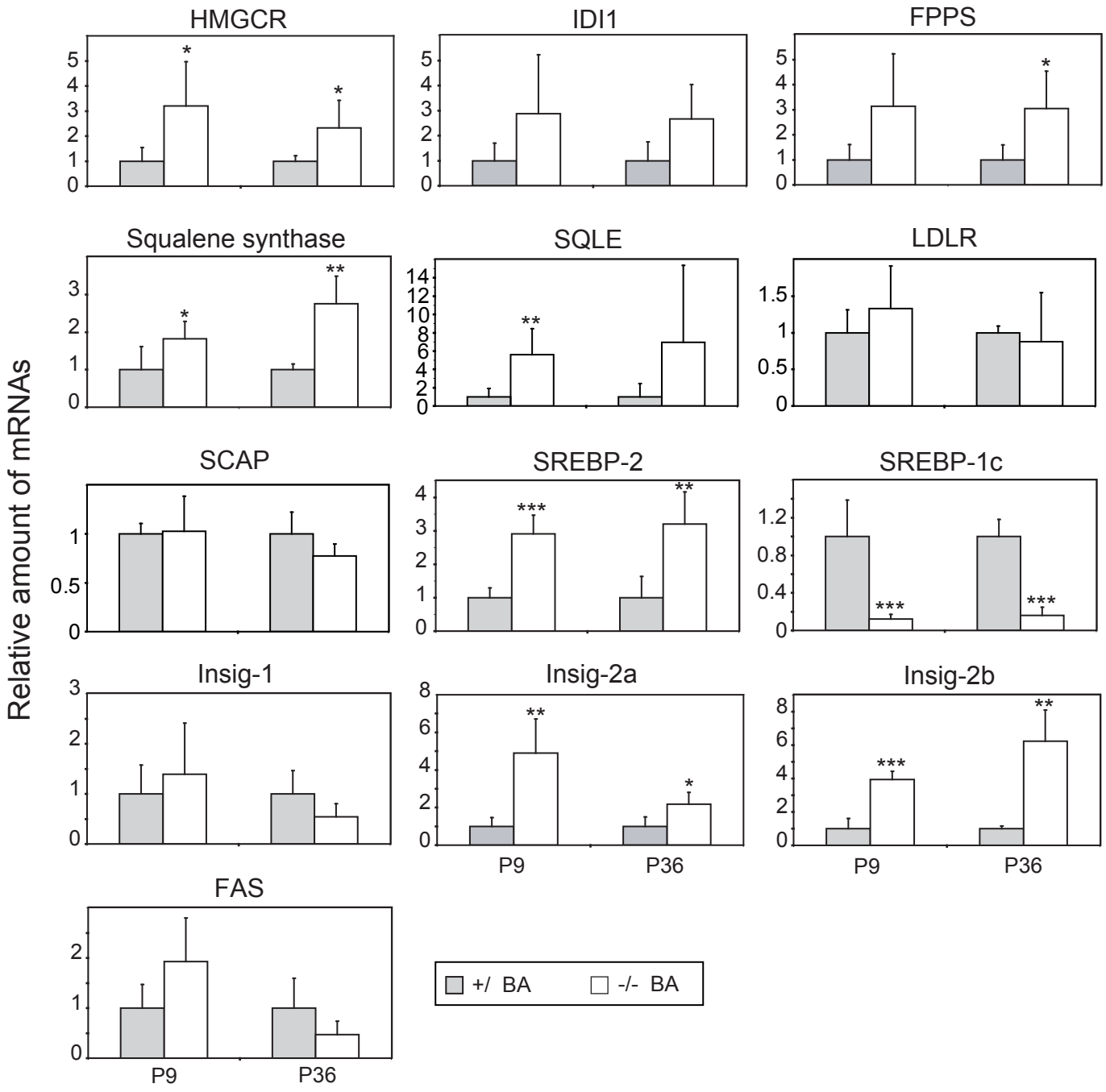
‡ Genes highly expressed in *PEX2*$^{-/-}$ mice but undetectable in wild-type mice.

† L-specific multifunctional β-oxidation protein is a peroxisomal enzyme involved in the oxidation of very long-chain fatty acids and the increased expression in *PEX2*$^{-/-}$ mice probably occurred through the activation of PPARα as a response to increased fatty acid levels. Whether it is a direct target of SREBP-1 is unknown.

Figure S1. Relative amounts of various mRNAs in livers from P9 and P36 BA-fed control and *PEX2*$^{-/-}$ mice. RNA was analyzed by qRT-PCR as described in Fig. 4. Values represent the amount of mRNA relative to that in BA-fed control mice at the respective time point, which was arbitrarily defined as 1. Values are mean ± standard deviation from RNA samples of at least five individual mice, except for P36 untreated *PEX2* knockout, where n = 1. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ vs. BA-fed control mice (Student's *t* test).

Figure S2. Induction of UPR target genes in livers from P9 untreated and BA-fed control and *PEX2*$^{-/-}$ mice. Total RNAs were isolated and subjected to Northern Blot analysis. The same blot was hybridized sequentially with Grp78, Herpud1, CHOP, GADD45, p8, and 28S rRNA probes. The amount of radioactivity in each band was quantified by PhosphorImager analysis and normalized to that in control mice, which was arbitrarily defined as 1.

Supplemental Figure 1



Supplemental Figure 2