

Supplemental information

Abcb5-deficient mice show a subtle, pleiotropic phenotype indicating a role for this transporter in intermediary metabolism

Jean-Pierre Gillet, Louise Gerard, Wilfred Vieira, Marie Fourrez, Florence Gaudray, Birgit Rathkolb, Jan Rozman, Tanja Klein-Rodewald, Lore Becker, Antonio Aguilar-Pimentel, Marion Horsch, Nadine Spielmann, Cornelia Prehn, Benoît Bihin, Johannes Beckers, Helmut Fuchs, Valérie Gailus-Durner, Martin Hrabe de Angelis, Eileen Southon, Lino Tessarollo, Di Xia, and Michael M. Gottesman

Supplemental Information

Figure S1. Molecular model of Abcb5. The topology diagram of the ABCB5 is given, showing 2 transmembrane domains (TMDs), each containing 6 helices rendered as cylinders, and a nucleotide binding domain (NBD) following each TMD. The helices of N-terminal half are colored blue and those of C-terminal half are colored coral. The numberings represent boundaries of helices. The stars localize some conserved domains in the respective nucleotide binding domains. **A.** human Abcb5. **B.** mouse Abcb5. **C.** An atomic model of the mouse Abcb5 was constructed based on the sequence alignment of mouse Abcb5 to mouse Abcb1 or P-glycoprotein (PDB:5KPI), for which experimental structures are known.

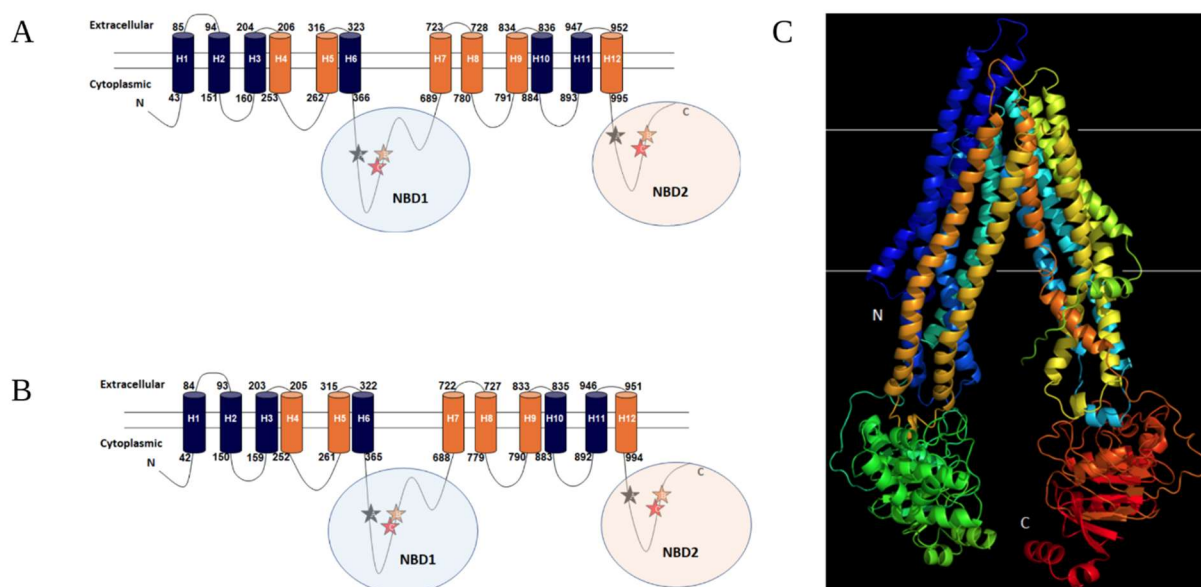


Table S1. Workflow of the analyses

Experimental week			1	2	3	4	5	6	7	8	9
Screens		Methods									
Pipeline 1	Dysmorphology	Anatomical observation									
		DEXA, X-ray									
	Cardiovascular	Blood pressure									
	Energy Metabolism	Indirect calorimetry, body composition									
	Clinical Chemistry	Simplified IpGTT									
		Blood lipid values (fasted mice)									
	Eye	Eye size (LIB: Laser interference biometry)									
	Lung Function	Lung function analysis									
	Molecular Phenotyping	Expression profiling									
Pipeline 2	Behavior	Open field									
		Acoustic startle and PPI (Prepulse inhibition)									
	Neurology	Modified SHIRPA, grip strength, rotarod									
	Nociception	Hot plate									
	Eye	Ophthalmoscopy, slit lamp, otokin. drum									
	Clinical chemistry	Clinical chemical analysis, hematology									
	Immunology, allergy	FACS analysis of PBCs, Ig concentration									
	Steroid metabolism	Corticosteroid, androstenedione, testosterone									
	Cardiovascular	ECG or echocardiography									
	Pathology	Macro- and microscopic analysis									

Table S2. Analyzed parameters.

Screens	Goal	Methods
Dysmorphology, Bone and Cartilage	Morphological analysis of body, skeleton, bone, and cartilage	Morphological observation, bone densitometry, X-ray
Cardiovascular	Assessment of functional cardiovascular parameters	Awake echocardiography
Energy Metabolism	Measurement of body weight, body temperature, activity, O ₂ consumption, CO ₂ production, respiratory exchange ratio, body composition	Indirect calorimetry, qNMR (MiniSpec)
Clinical Chemistry	Determination of clinical-chemical and hematological parameters in blood glucose tolerance	AU400 autoanalyzer, ABC-animal blood counter, simplified IpGTT
Steroid Metabolism	Analysis of steroid hormones in blood plasma: testosterone, corticosterone and androstenedione	LCMS
Eye	Assessment of morphological alterations of the eye	Slit lamp biomicroscopy fundoscopy, laser interference biometry
Lung Function	Assessment of lung volumes and lung mechanics	Buxco FinePointeRC and forced maneuvers systems
Molecular Phenotyping	Genome-wide transcriptome analysis	Illumina bead array technology
Behavior	Locomotion and anxiety-related behavior, sensory motor gaiting	Open field, acoustic startle & PPI
Neurology	Assessment of basic neurological functions, muscle function and motor coordination/balance	Modified SHIRPA protocol, grip strength, rotarod
Nociception	Detection of altered pain response	Hot plate assay
Immunology	Analysis of peripheral blood samples for immunological parameters	Flow cytometry, bioplex multiplex bead array
Allergy	Analysis of total plasma IgE	ELISA
Pathology	Necropsy and histopathological analysis.	Morphological observation and weigh of visceral organs. Histological analysis using standard histochemical stains.

Table S3. Age of mice when the specific tests were carried out.

GMC module	Parameter set	Age in weeks	Age in days	Age range
Dysmorphology	Dysmorphology	8	62	61-64
Neurology	Grip strength	8	60	59-62
Behaviour	Open field	8	60	59-62
Neurology	Modified SHIRPA	8	60	59-62
Dysmorphology	Body weight	9	69	68-71
Neurology	Rotarod	9	67	66-69
Behaviour	Acoustic startle and PPI	10	74	73-76
Metabolism	Calorimetry	11	83	82-85
Nociceptive	Hotplate	11	81	80-83
Clinical chemistry	Simplified IpGTT	12	90	89-92
Metabolism	Minispec MRI	12	90	89-92
Eyes	Ophthalmoscope	12	88	87-90
Eyes	Slit lamp	12	88	87-90
Eyes	Virtual drum	12	88	87-90
Dysmorphology	DEXA	13	97	96-99
Dysmorphology	X-Ray	13	97	96-99
Clinical chemistry	Clinical chemistry (ad. lib. fed mice)	13	95	94-97
Immunology	FACS analysis of PBCs	13	95	94-97
Clinical chemistry	Hematology	13	95	94-97
Allergy	Immunoglobulin concentration	13	95	94-97
Clinical chemistry	Clinical chemistry (fasting values)	14	104	103-106
Cardiovascular	Echocardiography	14	102	101-104
Eyes	Eye size	16	112	111-113
Steroid	Quantification of steroid hormones in mouse plasma with LC-MS/MS	16	116	115-118
Eyes	Optical coherence tomography	16	112	111-113
Lung	Lung function	17	119	118-120
Molecular phenotyping	Expression profiling	17	120	120-120
Pathology	Macroscopical and histological analysis	16	116	115-118

Table S4. Summary of Phenotyping results

Screen	Parameter set	Phenotype	Short description
Dysmorphology, Bone and Cartilage	Morphological observation	No	
Dysmorphology, Bone and Cartilage	Clickbox test	No	
Dysmorphology, Bone and Cartilage	DXA	No	
Dysmorphology, Bone and Cartilage	X-Ray	No	
Behaviour	Open Field	No	No differences in total locomotor or rearing activity. No difference in total centre time but there was a significant reduction during the second 5 minutes of the test.
Behaviour	Prepulse Inhibition	Yes	Small increase in prepulse inhibition in the male mutant mice (significant at 81dB and tendency globally).
Neurology	Lactate	Yes	Decreased lactate levels
Neurology	Modified SHIRPA	No	
Neurology	Grip Strength	No	
Neurology	Rotarod	No	
Eye Screen	Slit Lamp	No	
Eye Screen	Eye Size	No	
Eye Screen	Ophthalmoscopy	No	
Eye Screen	OCT	No	
Eye Screen	Virtual Drum	No	
Nociceptive screen	Hot plate	No	
Metabolic screen	Indirect Calorimetry	Yes	Increased maximum VO ₂ in female mutants.
Metabolic screen	Minispec	Yes	Female mutants have more fat and less lean mass.
Clinical Chemistry and Hematology	Clinical Chemistry (ad lib. fed mice)	No	
Clinical Chemistry and Hematology	Hematology	Yes	Increased anisocytosis (RDW), decreased white blood cell count and decreased platelet count in mutant males.
Clinical Chemistry and Hematology	ipGTT	No	
Clinical Chemistry and Hematology	Clinical Chemistry (fasting values)	No	Trend towards increased cholesterol levels in fasted mutant mice.
Immunology screen	Flow cytometry	Yes	Higher frequency of B cells in female mutant mice, higher frequencies of NK-T cells.
Immunology screen	Immunoglobulins	Yes	Slightly higher levels of IgA in blood plasma.
Allergy screen	IgE levels	No	
Steroid screen	Steroid levels	No	
Cardiovascular Screen	Echo	Yes	Significantly increased the diastolic posterior wall width in male animals. Tendency to increased Diastolic posterior wall width in female.
Lung Function Screen	Lung Function	No	
Molecular Phenotyping	Molecular Phenotyping	Yes	Heart and liver were selected. Gene regulation was detected in both organs.
Pathology Screen	Macroscopy Visceral organs weight	Yes	Increase in absolute heart weight and in heart weight normalized to tibia lengths only in male mutants. . Decrease of liver weight normalized to body weight in male mutants
Pathology Screen	Microscopy	No	

Table S5. Indirect calorimetry: means, standard deviation and p-values of a linear model.

The respiratory exchange ratio (RER) is calculated as the ratio $\text{VCO}_2 / \text{VO}_2$.

	Female		Male		Linear Model			
	WT	KO	WT	KO	Sex	Genotype	Body mass	Sex-Genotype
	N=7	N=7	N=7	N=7				
	Mean ± sd				p-value			
Avg. mass [g]	20.2 ± 1.6	21.8 ± 1.4	27.6 ± 1.6	28.1 ± 2.4	< 0.001	0.135	NA	0.384
Body temp. [°C]	36.21 ± 0.67	36.25 ± 0.49	36.14 ± 0.41	35.84 ± 0.23	0.198	0.483	NA	0.367
Food intake [g]	5 ± 0.9	5.2 ± 0.6	5.1 ± 1.4	5.1 ± 0.6	0.767	0.722	0.743	0.608
Avg. VO ₂ [ml/(h animal)]	86.251 ± 8.7	95.14 ± 7.968	99.386 ± 3.837	96.978 ± 9.8	0.031	0.924	< 0.001	0.109
Min. VO ₂ [ml/(h animal)]	53.982 ± 9.352	59.116 ± 8.823	64.906 ± 6.254	62.977 ± 9.329	0.053	0.616	0.001	0.506
Max. VO ₂ [ml/(h animal)]	119.261 ± 14.713	140.567 ± 17.091	141.382 ± 4.16	142.134 ± 15.365	0.007	0.191	< 0.001	0.081
Avg. RER	0.914 ± 0.027	0.915 ± 0.035	0.891 ± 0.037	0.909 ± 0.026	0.241	0.441	NA	0.455
Avg. activity	0.08 ± 0.08	0.13 ± 0.16	0.06 ± 0.05	0.08 ± 0.09	0.388	0.394	NA	0.668

Table S6. Hematology: values measured in EDTA-blood samples. Means, standard deviations and p-values for genotype, sex and genotype-sex interaction effects calculated by a linear model.

	Female		Male		Linear Model		
	WT	KO	WT	KO	Genotype	Sex	Genotype :sex
	N=10	N=10	N=10	N=10			
	Mean ± sd				p-value		
RBC [Mio/mm3]	10.27 ± 0.45	10.44 ± 0.29	10.79 ± 0.37	10.66 ± 0.47	0.865	0.006	0.236
HGB [g/dl]	15.01 ± 0.68	15.1 ± 0.35	15.34 ± 0.51	15.11 ± 0.37	0.659	0.287	0.316
HCT [%]	52.34 ± 3.03	52.47 ± 1.26	54.26 ± 1.67	53.52 ± 1.3	0.624	0.021	0.486
MCV [fl]	50.9 ± 1.73	50.3 ± 1.25	50.2 ± 0.79	50.2 ± 1.62	0.501	0.371	0.501
MCH [pg]	14.64 ± 0.41	14.45 ± 0.4	14.22 ± 0.28	14.21 ± 0.53	0.449	0.016	0.496
MCHC [g/dl]	28.68 ± 0.53	28.75 ± 0.26	28.28 ± 0.41	28.26 ± 0.27	0.838	0.001	0.713
RDW [%]	12.61 ± 0.32	12.83 ± 0.58	12.55 ± 0.33	13 ± 0.3	0.012	0.666	0.368
WBC [103/mm3]	6.28 ± 1.39	6.06 ± 1.01	6.5 ± 1.7	4.18 ± 0.98	0.004	0.051	0.015
PLT [103/mm3]	1095.4 ± 82.45	1045.5 ± 79.53	1199.4 ± 82.26	1064.7 ± 67.86	0.001	0.018	0.095
MPV [fl]	4.9 ± 0.12	4.8 ± 0.12	4.73 ± 0.07	4.77 ± 0.09	0.375	0.005	0.043

RBC: red blood cell count, HGB: hemoglobin, HCT: hematocrit, MCV: mean corpuscular volume, MCH: mean corpuscular hemoglobin, MCHC: mean corpuscular hemoglobin concentrations, RDW: red blood cell distribution width, WBC: white blood cell count, PLT: platelet count, MPV: mean platelet volume.

Table S7. Flow Cytometry: frequencies of leukocyte subpopulations in peripheral blood after erythrocyte lysis [percentage of all leukocytes (CD45+ cells), or corresponding parent gate, respectively]. Medians, first and third quartile and p-values calculated by a Wilcoxon rank-sum test.

	Female		Male		Female	Male	Overall
	WT	KO	WT	KO			
	N=10	N=10	N=10	N=10			
	Median [25%, 75%)				p-value		
CD45+/T cells	32.4 [26.18 , 36.23]	30.75 [27.8 , 32.23]	26.85 [24.07 , 27.48]	25.3 [18.9 , 32.85]	0.516	0.754	0.683
CD45+/CD3+CD4+	17.9 [13.47 , 21.18]	17.05 [14.5 , 18.65]	15.55 [13.55 , 16.6]	15 [10.36 , 17.82]	0.739	0.838	0.794
CD45+/CD3+CD8+	10.85 [10.01 , 12.45]	10.85 [9.62 , 11.53]	9.32 [8.32 , 10.04]	8.26 [6.47 , 11.12]	0.684	0.631	0.763
CD45+/B cells	33.05 [30.6 , 35.52]	39.85 [37.25 , 44.65]	40.25 [34.83 , 47.1]	43.3 [40.77 , 53.27]	0.03	0.315	0.036
CD45+/CD5-NK+	4.23 [3.86 , 4.79]	4.78 [2.87 , 5.17]	2.64 [2.48 , 4.53]	4.03 [2.3 , 4.19]	0.895	0.739	0.862
CD45+/CD11b+Gr1+	5.63 [4.54 , 9.25]	4.19 [3.4 , 5.07]	4.89 [3.79 , 5.37]	4.59 [3.31 , 6.21]	0.063	0.927	0.167
CD45+/NK-Gr1-CD11b+	6.72 [6.09 , 8.38]	5.89 [5.02 , 6.4]	5.54 [5.32 , 5.96]	5.48 [4.63 , 7.47]	0.063	0.853	0.221
CD3+CD4+/CD25+	6.18 [5.55 , 7.32]	6.38 [5.37 , 7.25]	6.44 [5.25 , 7.52]	7.12 [5.71 , 8.24]	0.912	0.542	0.644
CD45+/CD3+gammadeltaTCR+	1.18 [1.12 , 1.23]	0.98 [0.81 , 1.14]	0.64 [0.57 , 0.76]	0.68 [0.64 , 0.75]	0.093	0.54	0.516
CD3+CD4+/CD62L+	56.6 [34.85 , 66.83]	44.75 [30.43 , 62.12]	50.35 [21.02 , 59.95]	33.4 [12.72 , 52.38]	0.579	0.19	0.274
CD3+CD4+/CD44+	98.8 [98.03 , 99.15]	99.25 [98.4 , 99.3]	99.6 [99.42 , 99.6]	99.05 [98.62 , 99.45]	0.269	0.115	0.804
CD3+CD8+/CD62L+	79.25 [65.35 , 82.95]	70.4 [60.58 , 80.22]	70.35 [34.92 , 76.35]	54.8 [28 , 70.28]	0.353	0.353	0.201
CD3+CD8+/CD44+	98.35 [97.1 , 98.72]	98.95 [98.47 , 99.3]	99.4 [99.3 , 99.5]	98.75 [98.4 , 99.03]	0.196	0.159	0.784
CD45+/CD5+NK+	0.36 [0.32 , 0.42]	0.46 [0.41 , 0.5]	0.35 [0.31 , 0.48]	0.42 [0.38 , 0.52]	0.085	0.239	0.044
B cells/IgD+	79.4 [73.33 , 80]	79.2 [77.22 , 81]	83.55 [80.9 , 85.78]	85.25 [80 , 85.67]	0.591	0.928	0.846
B cells/CD5+	4.13 [3.65 , 4.55]	3.44 [3.12 , 3.76]	3.76 [3.23 , 4.14]	2.5 [2.35 , 3.05]	0.105	0.028	0.011
B cells/B220+MHCclassII+	84.55 [83.2 , 87.47]	86.4 [85.25 , 88.25]	89.05 [88.65 , 90.2]	90.65 [89.53 , 93.05]	0.184	0.118	0.204
NK+/CD11b+	88.8 [87.9 , 89.47]	89.65 [86.58 , 91.78]	83.05 [79.17 , 86.1]	84.85 [81.1 , 87.17]	0.403	0.469	0.533
CD3+ rest	3.05 [2.77 , 3.38]	2.65 [2.52 , 3.02]	2.07 [1.84 , 2.18]	2.1 [1.9 , 2.28]	0.108	0.643	0.551
CD4/CD8	1.68 [1.41 , 1.77]	1.62 [1.4 , 1.86]	1.7 [1.47 , 1.84]	1.64 [1.54 , 1.81]	0.955	0.971	1
Granulocytes/Tcells+B cells	0.94 [0.92 , 0.96]	0.9 [0.88 , 0.93]	0.93 [0.89 , 0.95]	0.92 [0.86 , 0.94]	0.027	0.363	0.027

Table S8. Immunoglobulins: levels of immunoglobulins in blood plasma (microgram per milliliter). Changes in the IgG1/IgG2b ratio are indicative of changes in the Th1/Th2 balance. Medians, first and third quartile and p-values calculated by a Wilcoxon rank-sum test. Missing measurements caused by Ig levels above and below measurability are replaced by 0.9*min / 1.1*max of respective Ig measurement. ^aNumber not based on the full number of animals (missing values)

	Female		Male		Female	Male	Overall
	WT	KO	WT	KO			
	N=10	N=10	N=10	N=10			
	Median [25%, 75%)				p-value		
Ig G1	275.75 [195.67 , 341.98]	240.69 [162.79 , 344.33]	148 [134.08 , 289.06]	169.49 [140.65 , 233.01]	0.912	0.739	0.989
Ig G2a	195.51 [98.86 , 344.87]	347.06 [271.24 , 419.55]	130.84 [98.86 , 211.25]	249.13 [179 , 415.33]	0.562	0.108	0.111
Ig G2b	511.7 [324.85 , 570.04]	383.18 [304.24 , 570.61]	159.56 [150.75 , 195.01]	112.4 [97.68 , 144.03]	0.529	0.075	0.289
Ig G3	213.26 [177.85 , 273.58]	217.29 [161.59 , 287.96]	168.22 [151.07 , 190.46]	221.79 [165.81 , 259.94]	0.971	0.604	0.813
Ig M	143.66 [92.37 , 155.41]	178.49 [167.7 , 216.62]	111.51 [79.94 , 122.56]	137.42 ^a [111.83 , 184.26]	0.068	0.105	0.012
Ig A	731.25 [539.85 , 839.03]	1068.31 [850.19 , 1148.46]	666.63 [491.81 , 840.5]	907.16 [619.66 , 1003.98]	0.028	0.114	0.006
Ig G1 / Ig G2b	0.717 [0.336 , 1.011]	0.624 [0.494 , 0.744]	1.007 [0.895 , 1.173]	1.728 [1.217 , 2.031]	0.912	0.105	0.429

Table S9. Echocardiography: medians, first and third quartile and p-values calculated by a Wilcoxon rank-sum test.

	Female		Male		Female	Male	Overall
	WT	KO	WT	KO			
	N=9	N=9	N=10	N=10			
	Median [25%, 75%)				p-value		
IVSs [mm]	0.63 [0.61 , 0.65]	0.62 [0.59 , 0.67]	0.61 [0.57 , 0.63]	0.61 [0.57 , 0.63]	0.982	1	0.983
IVSd [mm]	0.53 [0.51 , 0.56]	0.56 [0.48 , 0.59]	0.49 [0.47 , 0.55]	0.56 [0.52 , 0.58]	0.813	0.093	0.169
LVPWs [mm]	0.65 [0.6 , 0.67]	0.68 [0.65 , 0.71]	0.64 [0.62 , 0.67]	0.63 [0.61 , 0.67]	0.329	0.643	0.501
LVPWd [mm]	0.59 [0.55 , 0.64]	0.6 [0.59 , 0.63]	0.56 [0.54 , 0.59]	0.62 [0.57 , 0.64]	0.654	0.011	0.029
LVIDs [mm]	1.43 [1.17 , 1.57]	1.31 [0.95 , 1.55]	1.34 [1.04 , 1.56]	1.44 [1.16 , 1.65]	0.666	0.436	0.868
LVIDd [mm]	2.65 [2.41 , 2.97]	2.53 [2.45 , 2.98]	2.71 [2.32 , 3.08]	2.67 [2.38 , 2.89]	0.796	0.853	0.751
Weight [g]	22.2 [20.3 , 23.5]	22.1 [21.7 , 23]	27.3 [27.2 , 29.7]	29.9 [29.3 , 31.3]	0.814	0.197	0.431
Heart rate Echo [bpm]	451.41 [401.11 , 631.58]	590.16 [356.44 , 611.11]	653.09 [598.92 , 680.18]	663.82 [560.02 , 696.64]	0.931	1	0.846
Respiration Rate Echo [l/min]	205.71 [169.81 , 253.52]	215.57 [197.8 , 279.07]	259.11 [216.15 , 310.46]	290.8 [237.82 , 317.89]	0.622	0.591	0.414

IVSs: interventricular septum in systole, IVSd: interventricular septum in diastole, LVPWs: left ventricular posterior wall in systole, LVPWd: left ventricular posterior wall in diastole, LVIDs: left ventricular internal dimension in systole, LVIDd: left ventricular internal dimension in diastole. Echo: echocardiography.

Table S10. Ingenuity pathway analysis of significantly regulated genes between Abcb5-deficient and WT mice livers.

Category	Genes
Quantity of Lipid	Abca3, Apom, Asah1, Avpr1a, Bmp7, Dhcr24, Egr1, Fgf21, Gfer, Insig1, Ppp1r3c, Rdh16, Stard4
Transcription	Arntl, Bmp7, Cern4l, Cdkn1a, Creb3l2, Dbp, Ddx5, Egr1, Gadd45g, Hes1, Id1, Id2, Lgals1, Lpin2, Ncoa4, Nfil3, Per2, Rbbp7, Rfx4, Tef, Tgif1, Tsc22d1, Usp2, Wnt2
Hepatic system disorder	Avpr1a, Bmp7, Ddc, Ddx5, Insig1, Insig2, Lpin2, Mak16, Rdh16, Slco1a2, Usp18, Wnt2
Quantity of steroid	Apom, Avpr1a, Bmp7, Dhcr24, Egr1, Gfer, Insig1, Stard4
Cell Proliferation	Arntl, Asah1, Avpr1a, Bmp7, Cdkn1a, Ctsl2, Cxadr, Cxcl9, Ddx5, Dhcr24, Egr1, Fgf21, Gadd45g, Gfer, Hes1, Hla-E, Id1, Id2, Insig1, Lgals1, Ncoa4, Osgin1, Prdx2, Rbbp7, Tgif1, Tsc22d1, Usp18, Wnt2
Cell death	Asah1, Asns, Atg3, Bfar, Bmp7, Cdkn1a, Creb3l2, Ctse, Ctsl2, Cxcl9, Cyb5r3, Ddx5, Dhcr24, Egr1, Gadd45g, Gpc1, Gsta5, Hes1, Hla-E, Id1, Id2, Lgals1, Ncoa4, Nfil3, Osgin1, Ppm1b, Prdx2, Raet1b, S100a10, Tsc22d1, Usp18, Usp2, Wnt2
Fatty acid metabolism	Asah1, Avpr1a, Cyp4a14, Dbp, Gpc1, Insig1, Insig2, Prkag2, Slco1a2, Tef

The significantly regulated genes in liver were classified by their molecular functions using Ingenuity pathway analysis (IPA) software. Table S10 summarizes those categories that are over-represented and selected due to their p-value (<0.01).

Table S11. Ingenuity pathway analysis of significantly regulated genes between Abcb5-deficient and WT hearts.

Category	Genes
Tumorigenesis	Alas2, Ang, Aqp9, Bcl11b, Bdh1, Bnip3l, Ca2, Ccndbp1, Cdc25b, Cdkn1a, Col3a1, Col6a1, Csf3r, Ctse, Cyp2a6, Cyr61, Diaph3, E2f2, Fah, Fbn1, Fech, Fos, Gpx1, Hbb, Hpn, Hr, Il1b, Isg20, Jmjd5, Lep, Lgals3, Lgals4, Loxl1, Lphn1, Mest, Mkks, Mpp1, Myocd, Ncoa4, Nusap1, Parp2, Psmb1, Psme3, Ptgds, Retnla, Rorc, S100a8, S100a9, Scgb1a1, Sfrp5, Slc4a1, Spon2, Stk35, Syt12, Thra, Timp1, Tpm2, Trim10, Tspan33
Cell death	Ahsp, Ang, Aqp9, Atg3, Bcl11b, Bnip3l, Ca2, Cd6, Cdc25b, Cdkn1a, Col6a1, Creb3l2, Csf3r, Ctse, Cyr61, Dbh, E2f2, Erc1, Fah, Fbn1, Fos, Gpx1, Gsta1, Hbb, Hpn, Il1b, Isg20, Lep, Lgals3, Lgals4, Ncoa4, Nr1d1, Parp2, Psmb1, Psme3, Ptgds, Retnla, Rorc, S100a8, S100a9, Scgb1a1, Sfrp5, Sh3gl2, Slc1a1, Slc4a1, Snca, Thra, Timp1, Trim10
Immune response	Aqp9, Bcl11b, Ca2, Cdkn1a, Col3a1, Csf3r, Ctse, Dhx58, E2f2, Fah, Fbn1, Fos, Gpx1, Il1b, Isg20, Lep, Lgals3, Lgals4, Mpp1, S100a8, S100a9, Scgb1a1, Slpi, Snca, Spon2, Timp1, Ube2l6, Uts2
Quantity of blood cells	Ahsp, Bcl11b, Bnip3l, Cdkn1a, Csf3r, Ctse, E2f2, Fos, Hbb, Il1b, Lep, Lgals3, Pabpc1, Psme3, Rorc, S100a8, S100a9, Slc4a1, Snca, Spon2, Thra, Timp1, Tspan33
Glucose metabolism	Ca2, Ccdc68, Cdkn1a, Dbh, E2f2, Fah, Gpx1, Hbb, Il1b, Lep, Lgals3, Ptgds, Thra, Timp1, Trim10
Heart disease	Bnip3l, Ca2, Col3a1, Csf3r, Fos, Gpx1, Il1b, Lep, Prkag2, Rorc, Thra, Timp1, Uts2
Myelopoiesis	Ahsp, Alas2, Bnip3l, Cdkn1a, Csf3r, E2f2, Fech, Hbb, Il1b, Thra, Timp1, Trim10
Proliferation of muscle cells	Ang, Cdkn1a, Gpx1, Il1b, Lep, Myocd, Ptgds, Timp1, Uts2
Blood pressure	E2f2, Gpx1, Il1b, Lep, Mkks, Timp1, Tpm2, Uts2, Uts2d
Atherosclerosis	Cdkn1a, Fos, Il1b, Lep, Lgals3, S100a8, S100a9, Timp1

The significantly regulated genes in liver were classified by their molecular functions using Ingenuity pathway analysis (IPA) software. Table S11 summarizes those categories that are over-represented and selected due to their p-value (<0.01).

Table S15. Plasma concentrations of minerals, iron and alkaline phosphatase activity of ad libitum fed mice. Means, standard deviations and p-values for genotype, sex and genotype-sex interaction effects calculated by a linear model. ALP: Alkaline phosphatase.

	Female		Male		Linear Model		
	WT	KO	WT	KO	Sex	Genotype	Sex-Genotype
	N=10	N=10	N=10	N=10			
	Mean ± sd				p-value		
Calcium [mmol/l]	2.36 ± 0.07	2.42 ± 0.07	2.41 ± 0.05	2.4 ± 0.07	0.645	0.258	0.133
Inorganic phosphate [mmol/l]	1.3 ± 0.6	1.5 ± 0.4	1 ± 0.1	1.1 ± 0.1	0.001	0.131	0.529
Iron [μmol/l]	29.1 ± 4	28.8 ± 4.2	28.3 ± 5.4	28.5 ± 3.8	0.709	0.957	0.834
ALP [U/l]	103 ± 28	103 ± 22	65 ± 8	67 ± 10	< 0.001	0.914	0.862

Table S16. Mouse ABC Transporter TaqMan Assays

ABC genes	TaqMan Assays
Abca1	Mm00442646 ml
Abca2	Mm00431553 ml
Abca3	Mm00550501 ml
Abca4	Mm00492004 ml
Abca5	Mm00461656 ml
Abca6	Mm00461636 ml
Abca7	Mm00497010 ml
Abca8a	Mm00462440 ml
Abca8b	Mm00457361 ml
Abca9	Mm00461704 ml
Abca12	Mm00613683 ml
Abca13	Mm00624342 ml
Abca14	Mm00509570 ml
Abca15	Mm00623451 ml
Abca16	Mm01163245 ml
Abca17	Mm01299670 ml
Abcb1a	Mm00440761 ml
Abcb1b	Mm00440736 ml
Abcb2	Mm00443188 ml
Abcb3	Mm00441668 ml
Abcb4	Mm00435630 ml
Abcb5	Mm01225815 ml
Abcb6	Mm00470049 ml
Abcb7	Mm01235258 ml
Abcb8	Mm00472410 ml
Abcb9	Mm00498197 ml
Abcb10	Mm00497927 ml
Abcb11	Mm00445168 ml
Abcc1	Mm00456156 ml
Abcc2	Mm00496899 ml
Abcc3	Mm00551550 ml
Abcc4	Mm01226380 ml
Abcc5	Mm00443360 ml
Abcc6	Mm00497685 ml
Abcc7	Mm00445197 ml
Abcc8	Mm00803450 ml
Abcc9	Mm00441638 ml
Abcc10	Mm00467403 ml
Abcc12	Mm00556685 ml
Abcd1	Mm00431749 ml
Abcd2	Mm00496455 ml
Abcd3	Mm00436150 ml
Abcd4	Mm00436180 ml
Abce1	Mm00649858 ml
Abcf1	Mm01275245 ml
Abcf2	Mm00457400 gl
Abcf3	Mm00658695 ml
Abcg1	Mm00437390 ml
Abcg2	Mm00496364 ml
Abcg3	Mm00446072 ml
Abcg4	Mm00507250 ml
Abcg5	Mm00446249 ml
Abcg8	Mm00445970 ml
18S	Hs99999901_s1 (species : eukaryotic, rat, mouse, human)

Methods S1.

The mice were acclimatized at the GMC facility for two weeks and then divided into two groups to enter two pipelines. The mice in the first pipeline were subjected to a morphological whole-body checkup in the dysmorphology screen and were passed to the cardiovascular screen. Following blood pressure reading, the energy metabolism was analyzed by calorimetry. One week later, a simplified IpGTT (intraperitoneal glucose-tolerance test) was performed by the clinical chemical screen. Following this analysis, the mice re-entered the dysmorphology screen for X-ray analysis and bone densitometry by DEXA (dual energy X-ray absorption). Blood samples for the measurement of fasted plasma values were collected by the clinical chemical screen. Following the determination of eye size parameters, the mice underwent lung function analysis. The second pipeline started with the behavior screen. The initial screening included neurological tests and lasted three weeks. The modified SHIRPA analysis (SmithKline Beecham Pharmaceuticals, Harwell, MRC Mouse Genome Center and Mammalian Genetics Unit, Imperial College School of Medicine at St Mary's Royal London Hospital, St Bartholomew's and the Royal London School of Medicine Phenotype Assessment <http://www.har.mrc.ac.uk/services/phenotyping/neurology/shirpa.html>), grip strength, and rotarod were carried out. Later on, the animals were tested in a nociceptive screen and went through eye screen tests. When the mice were at least 14 weeks old, blood was taken and samples distributed to the blood-based screens for clinical chemistry, immunology, cardiovascular, and allergy. Then, the mice were passed to the cardiovascular screen, wherein the mice stayed two weeks. Determination of ANP (atrial natriuretic peptide) level in the plasma samples, ECG (electrocardiography), or echocardiography analysis were performed on request. Three weeks after testing of the first blood sample, a second sample was taken to confirm the findings and analyze the steroid levels. After completion of the primary screen, all animals of the second pipeline were analyzed macro- and microscopically, including determination of heart weight in the pathology laboratory.