

Supplementary Material

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1 Supplementary protocol

1.1 Affymetrix mRNA and microRNA GeneChip processing

Processing of GeneChip[®] experiments was conducted as recommended by the manufacturer of the GeneChip[®] system (Affymetrix, Santa Clara, CA). For tissue samples, double stranded cDNA was synthesized with a starting amount of 0.1 μ g total RNA. For RNA reverse transcription, the GeneChip[®] 3' IVT Express Labeling Assay (lot ID 0904012, Affymetrix) was used in the presence of a T7-(dT)24 DNA oligonucleotide primer (Affymetrix). The cDNA was then transcribed *in vitro* in the presence of biotinylated ribonucleotides to form biotin-labelled amplified RNA (aRNA). The labelled aRNA was then purified and quantified by UV spectrophotometry at 260 nm and fragmented. 10 μ g of fragmented biotinylated aRNA were hybridized for approximately 16 hrs at 45 °C and 48 °C to the GeneChip[®] Mouse430_2 arrays and GeneChip[®] miRNA2.0 arrays respectively. The arrays were then washed and stained with the GeneChip[®] Hybridization Wash and Stain kit (Affymetrix). The washing and staining steps were performed with GeneChip[®] Fluidics Workstation 450 (Affymetrix). Arrays were then scanned using a solid-state laser scanner (GeneArray[®] Scanner 3000 combined with the GeneChip[®] autoloader, Affymetrix). The Affymetrix GeneChip[®] Operating Software (GCOS) was used to generate the primary and secondary raw data files. The scanned images from miRNA were converted into numerical values of the signal intensity (Signal) and into categorical expression level measurement (Absolute Call) using the Affymetrix AGCC software.

1.2 In situ hybridization (ISH) and Immunohistochemistry (IHC)

Template for Meg3 riboprobe synthesis was generated by RT-PCR on RNA from mouse brain using self-priming oligonucleotide primers flanked in 5' with SP6- and T3-promoter recognition sequences (forward primer: SP6CTCTTCTC CATCGAACGGCT, reverse primer T3-AACAATAAAGAAGCTTGAAGAGGTTTTGAT, amplicon size: 537 bp). The purified PCR product was transcribed using T3-RNA polymerase (anti-sense) and

SP6-RNA polymerase (sense) at 37 °C for 2 hrs using dNTP containing Digoxigenin-UTP according to the manufacturer recommendations (Roche Diagnostics, Schweiz AG, Rotkreuz, Switzerland). The quality and quantity of the riboprobe was evaluated using the 2100 Bioanalyzer. ISH was performed using the fully automated instrument Ventana Discovery Ultra[®] (Roche Diagnostics). All chemicals were also provided by Roche Diagnostics. Briefly, formalin fixed paraffin embedded sections were de-paraffinized and rehydrated under solvent-free conditions (EZprep solution). Pretreatment steps were done with the RiboMap[™] kit following the manufacturers instructions followed by cell conditioning (demasking) performed by heat retrieval cycles in RiboCC solution using option mild followed by a complementary enzymatic digestion (Protease 3 for 16 minutes at 37 °C). Hybridization was performed adding to each slide 200 μ l of RiboHybe solution containing 10 ng of DIG-riboprobe and incubating at 70 °C for 6 hrs. After hybridization section were washed 3 times at 70 °C for 8 min on stringency conditions (2.0 x SSC). DIG-label probe detection was performed using an Alkaline Phosphatase-conjugated Sheep anti-Digoxigenin antibody (Roche Diagnostics) diluted 1/500 in antibody diluent. Antibody incubation was carried out for 30 min at 37 °C followed by chromogenic detection using BlueMap[™] Kit with a substrate incubation time of 4hrs. Counterstaining using ISH nuclear fast red was performed for 2 min. Sections were mounted in Glycerol-gelatin mounting medium (Sigma-Aldrich Chemie GmbH, Buchs, Switzerland) and post-mounted using Pertex[™]. For double staining with Glutamine synthetase (GS), the rabbit anti-GS antibody from Sigma (catalog number G2781) was used at a dilution of 1/20'000 in antibody diluent for 3 hrs and was applied just after the alkaline phosphatase conjugated sheep anti-digoxigenin. The detection step was immediately done using a biotin conjugated donkey anti-rabbit antibody (dilution 1/500 in antibody diluent and incubation time 16 min) followed by application of the DABmap[™] kit according to the provider recommendations. The chromogenic detection for the DIG-labeled probe using the BlueMap Kit was done at the end. IHC for Ki67 was performed using the fully automated instrument Ventana Discovery[®] (Roche Diagnostics). All chemicals were also provided by Roche Diagnostics. Formalin fixed paraffin embedded sections were de-paraffinized and rehydrated under solvent-free conditions (EZprep solution) followed by antigen retrieval (demasking) performed by heat retrieval cycles in a Tris-EDTA based buffer (CC1 solution, option standard). Subsequently slides were blocked using 1x Casein solution in PBS (BioFX laboratories Inc, Catalog number PBSC-0100-5x) and endogenous avidin/biotin activity was quenched for 4 min. Some 100 μ l of a rabbit anti-Ki67 from NeoMarker (catalog number RM-9106S) diluted at 1/200 in antibody diluent were added on slides and incubated for 3 hrs at room temperature. A short post-fixation (glutaraldehyde at 0.05%) was done before applying a biotin conjugated donkey anti-rabbit at 1/500 for 16 min (Jackson Immunoresearch Inc.). Detection was performed with a streptavidin-biotin peroxidase detection system DABMap[®] Kit following the manufacturer recommendations. Slides were counter stained with Hematoxylin and bluing reagent, dehydrated and mounted using Pertex[™] (Biosystems Switzerland AG, Nunningen, Switzerland).

2 Supplementary figures

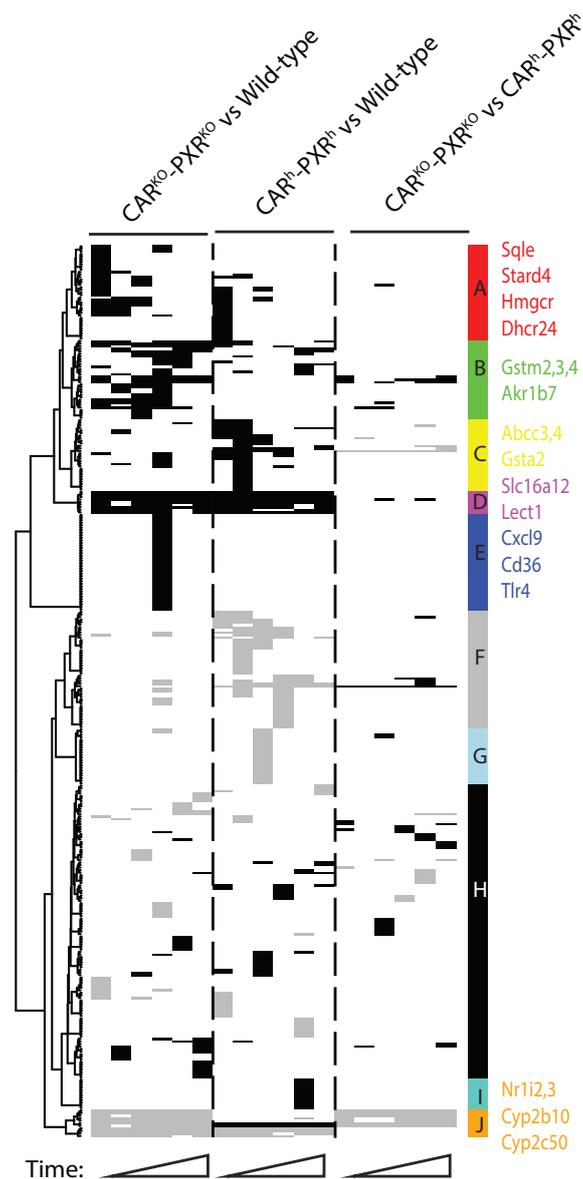


Figure S1: Summary of differential gene expression due to strain effect: comparison of gene expression at corresponding time-point of control CAR^{KO} - PXR^{KO} versus control wild-type, control CAR^h - PXR^h versus control wild-type, and control CAR^{KO} - PXR^{KO} versus control CAR^h - PXR^h mouse livers. Black dots = genes significantly up-regulated, grey dots = genes significantly down-regulated and white dots = no significant change. A gene is considered significantly up-regulated if $|\log_2 FC| > 0.53$ (corresponding to $FC > 1.5$ or $FC < 0.69$) and B.H. (Benjamini and Hochberg) corrected P-Value < 0.01 . Genes are clustered hierarchically by (1) computing Euclidean distance between genes from decision matrix and (2) applying Ward clustering algorithm. Detailed gene list can be consulted in Supplementary material (2).

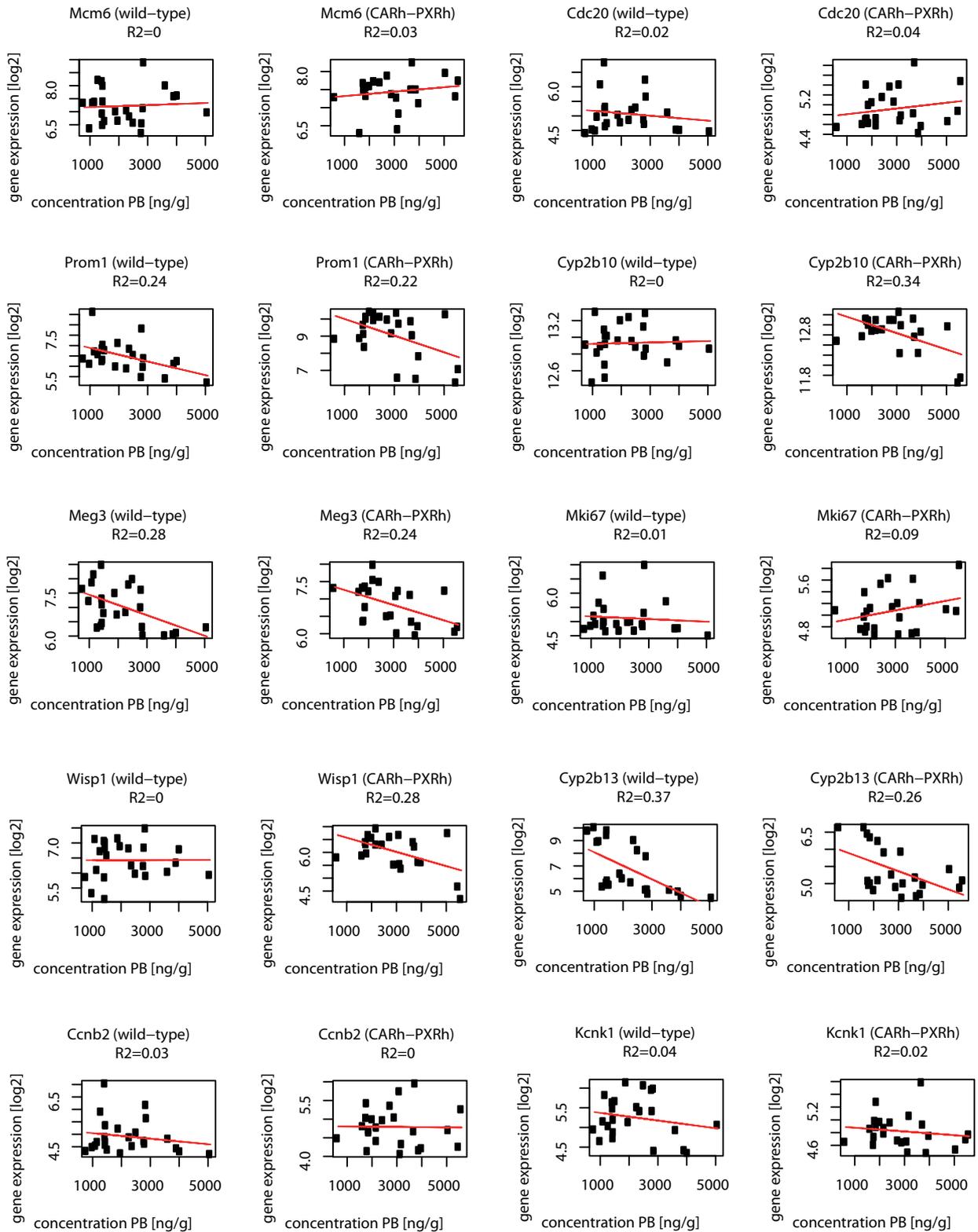


Figure S2: Linear modeling between gene expression and PB liver exposure for the genes of interest. Results do not support significant effect on gene induction upon changes in PB liver exposure. Whilst as for *Cyp2b13*, linear modeling suggests anti-correlation between PB exposure and gene expression, this indeed results from differential expression over time: as PB liver exposure decreases over time, *Cyp2b13* gene expression increases over time.

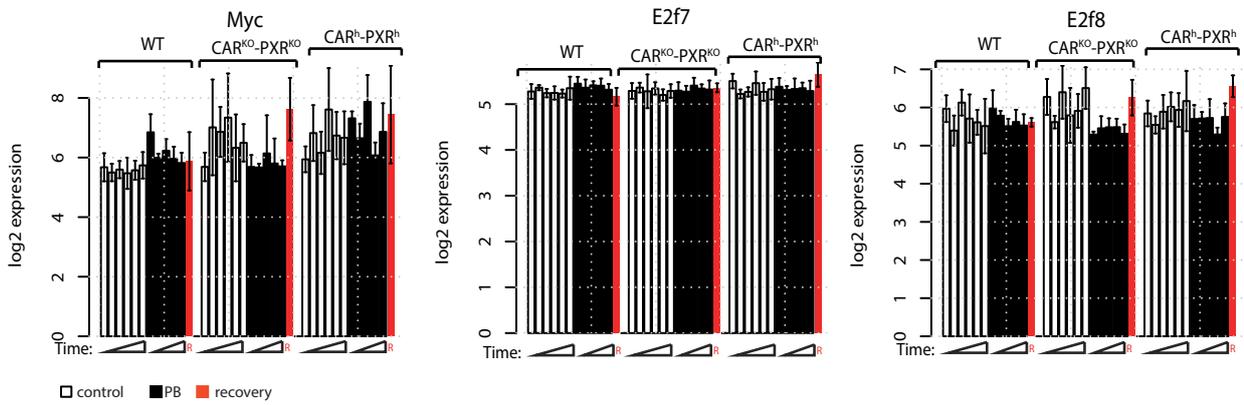


Figure S3: Expression of *E2F7*, *E2F8* and *Myc* upon PB treatment in different strains. Expression (\log_2) in control (open bars), treated (black bars) and recovery time-point (red bars) male animals is given as mean \pm SD (n=3-5 animals per group).

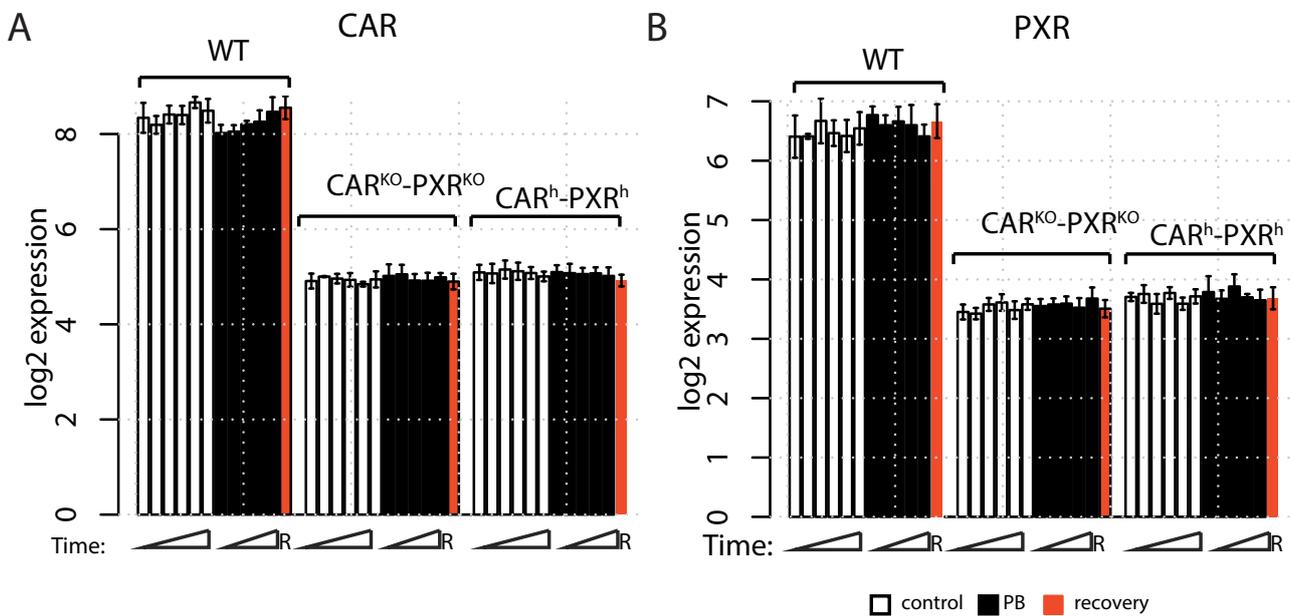


Figure S4: Expression of CAR and PXR in different strains. Log-expression lower than 5.0 is considered as background signal. Expression (\log_2) in control (open bars), treated (black bars) and recovery time-point (red bars) male animals is given as mean \pm SD (n=3-5 animals per group).

3 Supplementary tables

Liver						
Time[day]	Wild-type		CAR^{KO}-PXR^{KO}		CAR^h-PXR^h	
	mean [ng/g]	sd	mean [ng/g]	sd	mean [ng/g]	sd
1	39220	7946	58220	11362	44420	10680
7	16680	6796	50400	12400	26800	7556
14	20260	5533	48520	20617	29160	14417
28	21620	6637	57800	15399	25840	5757
91	11874	2892	36960	9567	14483	5465

Plasma						
Time[day]	Wild-type		CAR^{KO}-PXR^{KO}		CAR^h-PXR^h	
	mean [ng/mL]	sd	mean [ng/mL]	sd	mean [ng/mL]	sd
1	52400	4900	58700	9370	54100	8890
7	19400	3660	71900	10900	28900	5420
14	21100	3860	75300	14500	31500	7730
28	19700	3590	82700	8860	32300	5180
91	11100	3220	52600	6250	17000	7230

Table S1: Plasma and liver PB exposure as measured using LC-MS/MS at each time-point in treated animals (n=3-5 animals per group).

Gene Symbol	Gene Description	Gene Function	Day 1						Day 7					
			WT			CAR ^{fl} -PXR ^{fl}			WT			CAR ^{fl} -PXR ^{fl}		
			log ₂ FC	B.H.	P-Val	log ₂ FC	B.H.	P-Val	log ₂ FC	B.H.	P-Val	log ₂ FC	B.H.	P-Val
Cenpk	Centromere protein K Gene	Component of the nucleosome distal complex which is involved in assembly of kinetochore proteins, mitotic progression and chromosome segregation.	0.8	1.3E-05	0.2	5.7E-01	0.7	1.0E-03	0.6	5.2E-03				
Ect2	Cct4 oncogene Gene	Binds highly specifically to RhoA, RhoC and Rac proteins, but does not appear to catalyze guanine nucleotide exchange.	0.9	2.0E-02	1.0	7.1E-03	1.8	8.3E-08	1.3	9.5E-05				
Esc2	Establishment of cohesion 1 homolog 2	Required for the establishment of sister chromatid cohesion and couple the processes of cohesion and DNA replication to ensure that only sister chromatids become paired together.	0.7	6.1E-02	0.5	2.8E-01	1.4	1.7E-05	1.3	1.3E-04				
Fign1	Fidgetin-like 1	May regulate osteoblast proliferation and differentiation	1.2	1.0E-10	0.6	5.0E-03	1.0	6.4E-06	0.6	5.5E-03				
Gli25d2	Glycosyltransferase 25 domain containing 2	Has a beta-galactosyltransferase activity; transfers beta-galactose to hydroxylysine residues on collagen	0.9	1.4E-09	0.8	6.3E-07	0.6	6.5E-04	0.7	4.1E-05				
Hells	Helicase, lymphoid specific	Required for de novo or maintenance DNA methylation. May play a role in formation and organization of heterochromatin, implying a functional role in the regulation of transcription and mitosis	1.4	2.2E-08	0.6	8.6E-02	1.0	2.7E-03	0.9	5.4E-03				
Mcm4	Mnichromosome maintenance deficient 4 homolog	Involved in the control of DNA replication	0.7	6.2E-05	0.6	6.3E-03	0.7	9.0E-04	0.8	1.0E-04				
Mcm5	Mnichromosome maintenance deficient 5, cell division cycle 46		1.4	5.4E-07	1.0	1.5E-03	1.1	2.6E-03	1.0	1.4E-03				
Mcm6	Mnichromosome maintenance deficient 6	Binds to chromatin during G1 and detaches from it during S phase, implying that it allows the chromatin to replicate	1.3	3.6E-05	1.1	9.5E-04	1.2	6.0E-04	1.5	1.6E-06				
Mki67	Antigen identified by monoclonal antibody Ki 67		0.6	9.3E-02	0.6	9.5E-02	1.1	7.4E-04	0.8	1.4E-02				
Ncapg2	Non-SMC condensin II complex, subunit G2	Regulatory subunit of the condensin-2 complex	0.8	2.4E-04	0.4	1.7E-01	0.8	9.0E-04	0.5	8.1E-02				
Paps2	3'-phosphoadenosine 5'-phosphosulfate synthase	Bifunctional enzyme with both ATP sulfurylase and APS kinase activity, which mediates two steps in the sulfate activation pathway.	1.0	9.2E-14	0.6	1.5E-05	0.6	4.1E-04	0.1	7.9E-01				
Pbk	PDZ binding kinase	Phosphorylates MAP kinase p38. Seems to be active only in mitosis.	0.7	2.1E-03	0.3	5.3E-01	1.0	8.5E-05	0.8	2.4E-03				
Uhrf1	Ubiquitin-like, containing PHD and RING finger domains, 1	Putative E3 ubiquitin-protein ligase. May participate in methylation-dependent transcriptional regulation. Important for G1/S transition. May be involved in DNA repair and chromosomal stability.	0.8	2.0E-05	0.5	1.2E-02	0.8	1.4E-04	0.7	1.3E-03				

Table S2: PB-mediated differentially expressed genes (from Day 1 until Day 7) functionally enriched in DNA replication. Subset of cluster C of Figure 2 and group (1) of Figure 5. Gene function obtained from **STRING 9.05 - Known and Predicted Protein-Protein Interactions**.

Gene Symbol	Gene Description	Gene Function	Day 1						Day 7					
			WT		CAR ⁺ -PXR ^R		WT		WT		CAR ⁺ -PXR ^R		CAR ⁺ -PXR ^R	
			log ₂ FC	B.H. P-Val	log ₂ FC	B.H. P-Val	log ₂ FC	B.H. P-Val	log ₂ FC	B.H. P-Val	log ₂ FC	B.H. P-Val	log ₂ FC	B.H. P-Val
Arhgap11a	Rho GTPase activating protein 11A		0.2	4.4E-01	0.2	5.4E-01	0.6	1.1E-03	0.6	1.1E-03	0.5	8.0E-03		
Aspm	Abnormal spindle-like, microcephaly associated	Probable role in mitotic spindle regulation and coordination of mitotic processes.	0.3	2.2E-01	0.1	7.1E-01	0.6	2.2E-03	0.4	6.8E-02				
Birc5	Baculoviral IAP repeat-containing 5	Component of the chromosomal passenger complex (CPC), a complex that acts as a key regulator of mitosis.	0.4	3.7E-01	0.4	4.3E-01	1.4	1.0E-07	0.9	3.9E-03				
Ccnm2	Cyclin A2	Essential for the control of the cell cycle at the G1/S (start) and the G2/M (mitosis) transitions	0.4	1.0E-01	0.4	1.1E-01	1.0	1.5E-05	0.9	7.2E-05				
Ccnb2	Cyclin B2	Essential for the control of the cell cycle at the G2/M (mitosis) transition	0.2	8.0E-01	0.2	7.8E-01	1.9	2.8E-10	1.3	6.3E-06				
Cdc20	Cell division cycle 20 homolog	Required for full ubiquitin ligase activity of the anaphase promoting complex/cyclosome (APC/C)	0.3	3.7E-01	0.4	1.9E-01	1.6	9.4E-15	1.0	1.1E-06				
Cdc3	Cell division cycle associated 3	F-box-like protein which is required for entry into mitosis.	0.2	7.2E-01	0.4	1.4E-01	1.1	2.4E-08	0.9	2.5E-06				
Cdk1	Cyclin-dependent kinase 1	Required in higher cells for entry into S-phase and mitosis. p34 is a component of the kinase complex that phosphorylates the repetitive C-terminus of RNA polymerase II	0.3	3.4E-01	0.3	3.4E-01	0.7	5.7E-03	0.8	9.6E-04				
Cdk3	Cyclin-dependent kinase inhibitor 3		0.2	2.4E-01	0.2	3.8E-01	0.7	5.0E-08	0.4	3.2E-04				
Clbp2	Cytoskeleton associated protein 2	Possesses microtubule stabilizing properties. Involved in regulating aneuploidy, cell cycling, and cell death in a p53- dependent manner	0.3	1.2E-01	0.2	6.3E-01	1.0	2.0E-10	0.8	7.3E-07				
Dtl	Denticleless homolog	Seems to be necessary to ensure proper cell cycle regulation of DNA replication.	0.5	2.0E-02	0.4	3.3E-02	0.6	3.1E-03	0.5	7.1E-03				
Gsta2	Glutathione S-transferase, alpha 2	Conjugation of reduced glutathione to a wide number of exogenous and endogenous hydrophobic electrophiles	0.3	4.6E-01	0.3	4.3E-01	0.7	8.8E-03	0.8	6.2E-04				
Gstt1	Glutathione S-transferase, theta 1	Conjugation of reduced glutathione to a wide number of exogenous and endogenous hydrophobic electrophiles.	0.4	7.3E-06	0.2	1.2E-01	0.6	4.9E-10	0.2	7.3E-02				
Hmnr	Hyaluronan mediated motility receptor	Involved in cell motility.	0.3	7.7E-02	0.3	3.7E-02	0.8	7.0E-11	0.7	2.8E-09				
Kif20b	Kinesin family member 20B	Plus-end-directed motor enzyme that is required for completion of cytokinesis	0.4	6.6E-02	0.1	7.9E-01	0.7	1.1E-03	0.5	3.7E-02				
Nsk2	Never in mitosis gene e-related expressed kinase 2	Protein kinase involved in mitotic regulation. May have a role at the G2-M transition.	0.2	3.5E-01	0.0	1.0E+00	0.8	1.1E-05	0.7	1.0E-04				
Nuf2	NUF2, NDC80 kinetochore complex component	Acts as a component of the essential kinetochore-associated NDC80 complex, which is required for chromosome segregation and spindle checkpoint activity.	0.3	2.2E-01	0.4	1.0E-01	0.6	1.9E-03	0.4	1.0E-01				
Nusap1	Nucleolar and spindle associated protein 1	Microtubule-associated protein with the capacity to bundle and stabilize microtubules.	0.3	2.6E-01	0.3	3.6E-01	0.7	8.7E-03	0.2	5.4E-01				
Part3b	Par-3 partitioning defective 3 homolog B	Putative adaptor protein involved in asymmetrical cell division and cell polarization processes	0.5	1.5E-04	0.3	1.7E-01	0.6	5.1E-05	0.3	1.3E-01				
Rapg4f	Rap guanine nucleotide exchange factor	Guanine nucleotide exchange factor (GEF) for RAPIA, RAP1B and RAP2A small GTPases that is activated by binding cAMP.	0.5	3.7E-01	-0.1	9.4E-01	1.2	2.5E-03	0.1	9.4E-01				
Stmn1	Stathmin 1	Involved in the regulation of the microtubule filament system by destabilizing microtubules.	0.2	7.9E-01	0.1	9.4E-01	0.8	2.3E-03	0.8	1.5E-03				
Ubc2c	Ubiquitin-conjugating enzyme E2C	Acts as an essential factor of the anaphase promoting complex/cyclosome (APC/C), a cell cycle-regulated ubiquitin ligase that controls progression through mitosis.	0.4	3.4E-01	0.2	6.6E-01	0.9	4.4E-04	0.7	7.4E-03				
Zwch1	Zwisch, kinetochore associated, homolog	Essential component of the mitotic checkpoint, which prevents cells from prematurely exiting mitosis.	0.5	3.2E-02	0.4	1.8E-01	0.9	2.5E-05	0.6	7.3E-03				

Table S3: PB-mediated differentially expressed genes (around Day 7) functionally enriched in mitosis. Subset of cluster C of Figure 2 and group (2) of Figure 5. Gene function obtained from **STRING 9.05 - Known and Predicted Protein-Protein Interactions**.

Table S4: **Set of genes differentially expressed in at least one time point due to strain effect.** Selected gene-set and their corresponding differential gene expression analysis due to differences in genetic background (wild-type, CAR^{KO} - PXR^{KO} and CAR^h - PXR^h) in the absence of PB treatment. Genes are selected if they are significantly differentially expressed in at least one of the following contrasts: 1) wild-type versus CAR^{KO} - PXR^{KO} mouse livers, 2) wild-type versus CAR^h - PXR^h mouse livers and 3) CAR^{KO} - PXR^{KO} versus CAR^h - PXR^h mouse livers at corresponding time-point. A gene is considered differentially expressed if $|\log_2 FC| > 0.53$ (corresponding to $FC > 1.5$ or $FC < 0.69$) and B.H. (Benjamini and Hochberg) corrected P-Value < 0.01 . This list and the clusters correspond to Supplementary Figure S1.

Table S5: **Set of genes differentially expressed in at least one time point due to PB-treatment effect.** Selected gene-set and their corresponding differential gene expression analysis due to PB treatment in corresponding genetic backgrounds (wild-type, CAR^{KO} - PXR^{KO} and CAR^h - PXR^h). Genes are selected if they are significantly differentially expressed in at least one of the following contrasts: 1) PB-treated versus non-treated mouse livers from wild-type mice, 2) PB-treated versus non-treated mouse livers from CAR^h - PXR^h mice and PB-treated versus non-treated mouse livers from CAR^{KO} - PXR^{KO} mice at corresponding time-points. A gene is considered differentially expressed if $|\log_2 FC| > 0.53$ (corresponding to $FC > 1.5$ or $FC < 0.69$) and B.H. (Benjamini and Hochberg) corrected P-Value < 0.01 . This list and the clusters correspond to Figure 2A.

Table S6: **Set of genes differentially expressed in at least one time point due to the interaction between the strain and the PB-treatment effects.** Selected gene-set and their corresponding differential gene expression analysis due to interaction between PB-treatment and genetic background (wild-type, CAR^{KO} - PXR^{KO} and CAR^h - PXR^h). Genes are selected if they are significantly differentially expressed in at least one of the following contrasts: 1) PB-treatment effect in wild-type versus PB-treatment effect in CAR^{KO} - PXR^{KO} mouse livers, 2) PB-treatment effect in wild-type versus PB-treatment effect in CAR^h - PXR^h mouse livers and 3) PB-treatment effect in CAR^h - PXR^h versus PB-treatment effect in CAR^{KO} - PXR^{KO} mouse livers at corresponding time-points. A gene is considered differentially expressed if $|\log_2 FC| > 0.53$ (corresponding to $FC > 1.5$ or $FC < 0.69$) and B.H. (Benjamini and Hochberg) corrected P-Value < 0.01 .

Table S7: **Set of genes differentially expressed at recovery time-point.** Selected gene-set and their corresponding differential gene expression analysis due to PB-treatment in corresponding genetic backgrounds (wild-type, CAR^{KO} - PXR^{KO} and CAR^h - PXR^h). Genes are selected if they are significantly differentially expressed at recovery time-point in at least one strain. A gene is considered differentially expressed if $|\log_2 FC| > 0.53$ (corresponding to $FC > 1.5$ or $FC < 0.69$) and B.H. (Benjamini and Hochberg) corrected P-Value < 0.01 .