

Supplementary Information

Table S1 Settings used to visualize different sources of fluorescence in confocal intravital microscopy.

Molecule or Dye	λ_{ex} in nm	λ_{em} in nm	stained structure
NAD(P)H	375 to 405	410 to 485	liver tissue
PI	561	570 to 625	necrosis/apoptosis
DY-635(NH ₂)	633	640 to 749	liver function

Microarray pre-processing and analysis

Microarray pre-processing was performed using the lumi package(2) for R. MouseRef-8 (v2_R03_11278551); files provide by Illumina were used for annotation. Pre-processing of raw expression values consisted of background adjustment using the bgAdjust method, variance stabilization transformation and quantile normalization. Detection calls were used to select beads reported present in at least one of the samples for each treatment/time combination. According to Barbosa-Morais we used the illuminaMousev2 package(3) to filter all beads with a quality grade of “good” or “perfect”.(4) This retained a total of 8183 beads for subsequent analysis.

For data analysis based on self-organizing maps (SOMs) we first calculated \log_2 - fold-changes (\log_2 -FCs) for each bead at each time point compared to control using limma.(5) Illumina bead IDs were then mapped to their corresponding Entrez-Gene-ID. \log_2 -FCs of two or more bead-IDs mapping to the same Entrez-Gene-ID were averaged. The oposSOM package(6) for R was then used to create maps of size 40x40. Annotation information used by oposSOM was automatically obtained from Ensembl Biomat.

Differential gene expression was assessed using the limma package for R. For each individual infection, differentially expressed genes over time were identified based on an absolute \log_2 fold-change (\log_2 -FC) > 1 and a Benjamini-Hochberg corrected p-value <0.05.

Identification of regulatory modules

Identification of regulatory modules was performed using the ModuleDiscoverer(7). In brief, ModuleDiscoverer identifies protein communities in large-scale protein-protein interaction networks, that are significantly enriched with proteins associated with differentially expressed genes. The union of all significantly enriched communities then assembles the regulatory module. According to the algorithm's approach, a protein community is defined as a maximal clique, i.e., a maximal group of proteins where all proteins are connected by an edge. Since the enumeration of all maximal cliques in a network is non-deterministic polynomial time (NP)-hard, ModuleDiscoverer employs a randomization-based heuristic that iteratively enumerates maximal cliques starting from a set of random seed nodes in the protein-protein interaction network (PPIN).

To apply the algorithm the PPIN provided by the STRING database was downloaded. All edges with an edge score >700 were filtered to retain 459809 high confidence interactions between 15787 proteins. ModuleDiscoverer was then applied to approximate the protein community structure based on 2,000,002 iterations using a single seed node per iteration. This identified a total of 1,568,921 million maximal cliques enclosing 82,413 unique maximal cliques. For each treatment and time-point, the list of n genes differentially expressed compared to control, together with 10,000 sets of n randomly selected genes drawn from the set of genes measured by the microarray, were selected for further processing. For all gene sets, IlluminaBeadIDs were translated to EntrezGeneIDs using the annotation provided by Illumina and subsequently translated to EnsemblProteinIDs (as used by STRING) using the org.Mm.eg.db annotation package(8) for R. ModuleDiscoverer was then used to test all identified maximal cliques for their enrichment with proteins associated to differentially expressed genes (DEGs). Maximal cliques with a permutation-based p -value <0.001 were selected as significantly enriched with proteins associated to DEGs. All identified regulatory modules were significantly enriched with proteins associated to DEGs (Table S2). The stability of the identified regulatory modules was assessed by pairwise comparisons between the identified regulatory module and 100 regulatory modules identified based on bootstrap

samples of the clique database. The results indicate good stability (Table S2) in that 95% of all comparisons show a similarity (in respect to nodes in the regulatory module) of approximately 96% or higher (CB95).

Table S2 All identified regulatory modules were significantly enriched with proteins associated to DEGs. The stability of the identified regulatory modules was assessed by pairwise comparisons between the identified regulatory module and 100 regulatory modules identified based on bootstrap samples of the clique database and indicate good stability.

treatment	Ca i.v.		Ca i.p.		PCI	
time (in hours)	6	24	6	24	6	24
#sig. enriched max cliques	90	653	130	428	785	523
reg. module #nodes/#edges	106/853	453/4685	315/2288	392/3993	603/4460	515/4357
enrichment with proteins assoc. to DEGs (p-value)	2.57e-02	<1e-10	<1e-10	<1e-10	<1e-10	<1e-10
CB95 node stability in percent	96.21	97.25	96.85	96.89	96.94	96.42

MRP2 staining

Frozen tissue slices of 8 μ m thickness were fixed in 2% paraformaldehyde solution freshly prepared in PBS. Slides were washed twice in PBS and permeabilized for 1 h at room temperature in PBS containing 10% bovine serum albumin (BSA), 100 mM glycine and 1% TritonX-100. Permeabilization was followed by washing twice and then 1 h incubation with PBS containing 10% BSA, 100 mM glycine. A polyclonal rabbit anti-MRP2 antibody (Sigma Aldrich, M8316) was diluted 1:500 in PBS, 10% BSA. Slides were stained overnight at 4°C. Subsequently, slides were rinsed with PBS (3 times, 15 min) and incubated with Alexa Fluor 488 conjugated donkey-anti rabbit (Thermo Scientific) diluted 1:1000 in PBS, 10% BSA, 50 U ml⁻¹ Alexa Fluor 568 conjugated Phalloidin (Thermo Scientific) for 1 h in the dark at ambient temperature. Slides were again thoroughly washed with PBS (3x15 min) and deionized water (2x15 min). Slices were embedded with a VectaShield Mounting Media with DAPI (VectorLabs) overnight at 4 °C and imaged on a LSM-780 confocal microscope (Zeiss).

Automated image analysis

To objectively quantify the confocal intravital (IVM) and ex-vivo microscopy images, we developed an automated image analysis procedure implemented in the programming language “python” (<http://www.python.org>). The source code for the image analysis is available on request.

Confocal IVM image analysis

Intensities of DY-635 and NAD(P)H autofluorescence were analysed at different time points. In order to exclude contamination from the background, the tissue (hepatocyte) area was segmented based on the autofluorescence channel by the following procedure:

1. Normalization of the image intensity to the 95th percentile
2. Gaussian filtering ($\sigma = 1 \mu\text{m}$) to smooth the image
3. Otsu thresholding of the image intensity
4. Morphological opening and closing to reduce the noise in the thresholded image

To characterize the intensities of DY-635 and NAD(P)H autofluorescence, the medians of the corresponding signals were computed over the tissue area in each image.

The integrated DY-635 intensity was computed for each time series as the average of the median DY-635 intensities over the following time points: 0, 7.5, 10, 30, 40, and 50 min.

Quantification of MRP2 staining

To quantify the MRP2 signal and its colocalization with F-Actin in confocal ex-vivo microscopy images, the following procedure was applied (see exemplary segmentation results in Suppl. Fig. S7):

1. Preprocessing:
 - 1.1. Median filter, 3 px ($\approx 2 \mu\text{m}$)
 - 1.2. Normalization to the 99.9th percentile of the intensity of the whole dataset (for MRP2 and F-Actin channels separately)
2. Background segmentation:
 - 2.1. Gaussian filtering of both channels, $\sigma = 1 \mu\text{m}$
 - 2.2. Assigning to the background the pixels, whose intensities in both channels are less than 15 (out of 255 maximum); correspondingly, the foreground refers to the pixels where at least one channel intensity is greater than or equal to intensity 15
 - 2.3. Morphological opening and closing of the result

3. Segmentation of F-Actin and MRP2 positive regions:
 - 3.1. Computing the median intensity of the foreground in each image (M)
 - 3.2. Thresholding at 2 M
4. Quantitative characterization
 - 4.1. Areas of F-Actin and MRP2 positive regions were quantified and normalized by the total foreground area
 - 4.2. Overlap area was computed as the number of double positive pixels and was normalized by the total foreground area.

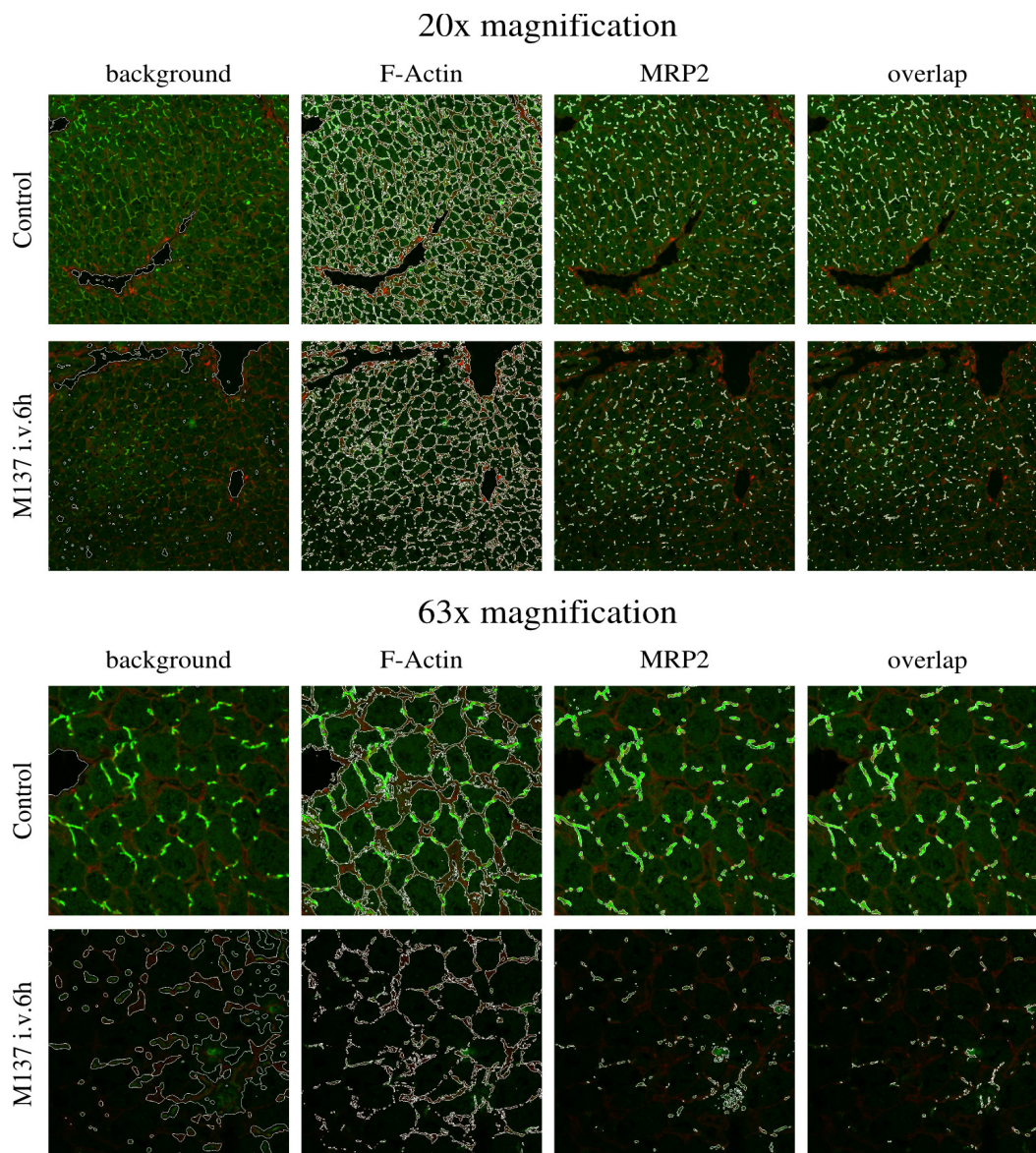


Figure S7: Exemplary segmentation results

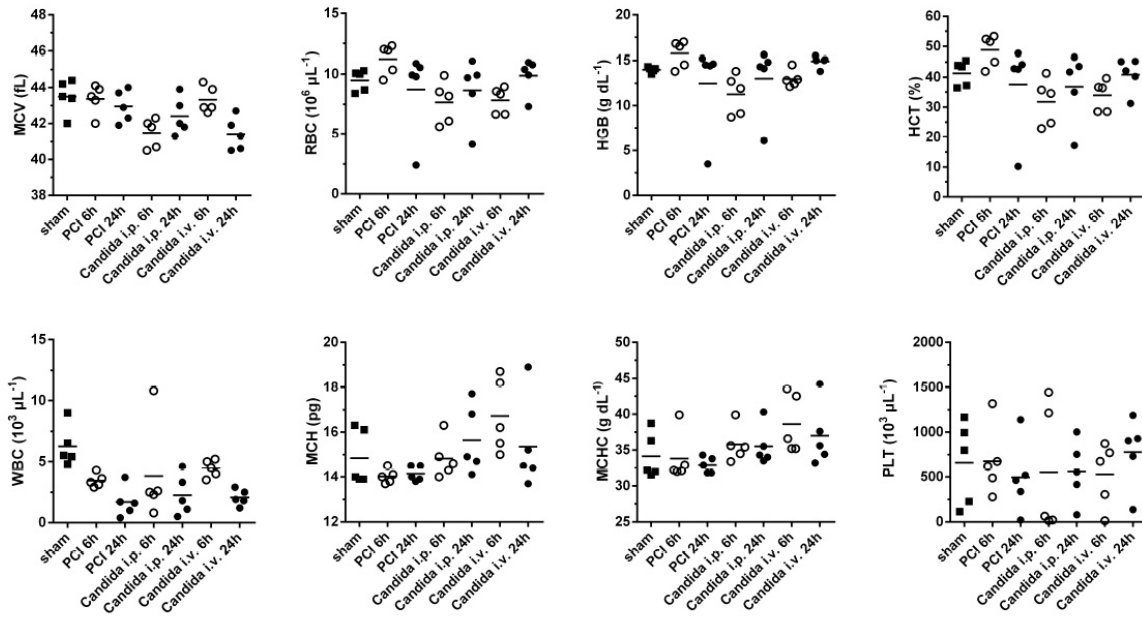


Figure S1: Hemogram of mice after systemic polymicrobial and fungal infection. Full blood count showing mean corpuscular volume (MCV), red blood cell (RBC) count, hemoglobin (HGB), hematocrit (HCT), white blood cell count (WBC), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and platelets (PLT) from healthy animals (sham) and different infection models.

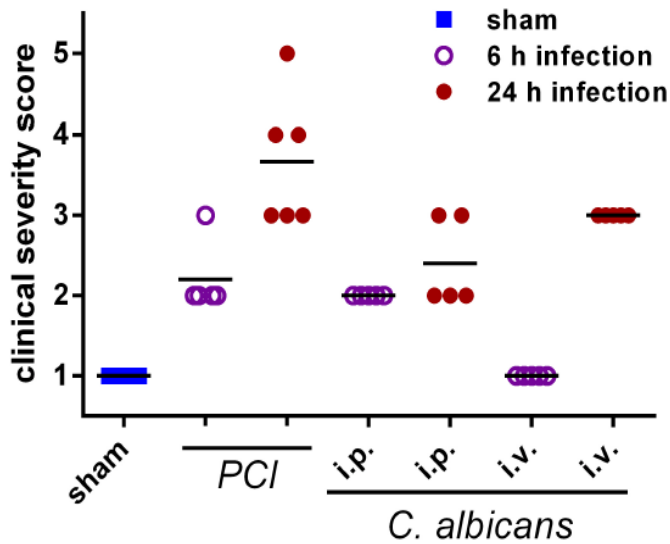


Figure S2: Phenotypic scoring of animals after infection. The clinical severity score (CSS) combines sign of illness (diarrhea, posture, agility, response to stimuli) to a score correlating with the overall condition of the animal during infection. CSS only accepts integral numbers between 1 and 5, where 1 refers to "no signs of illness" and 5 to "death".

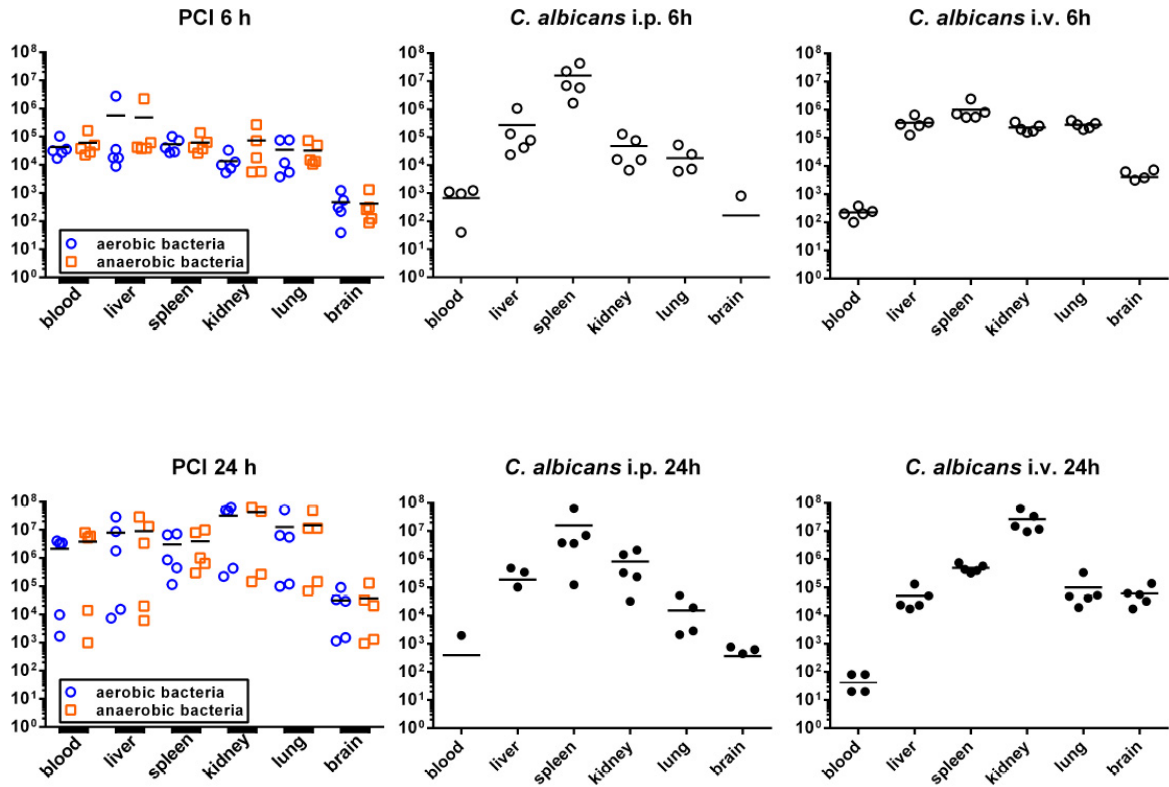


Figure S3: Pathogen load in different organs. Bacterial burden differentiated into aerobic and anaerobic bacteria for PCI model, and fungal burden in *C. albicans* models. Systemic infection was achieved in all animals. Data shown as colony forming units per ml or g organ weight.

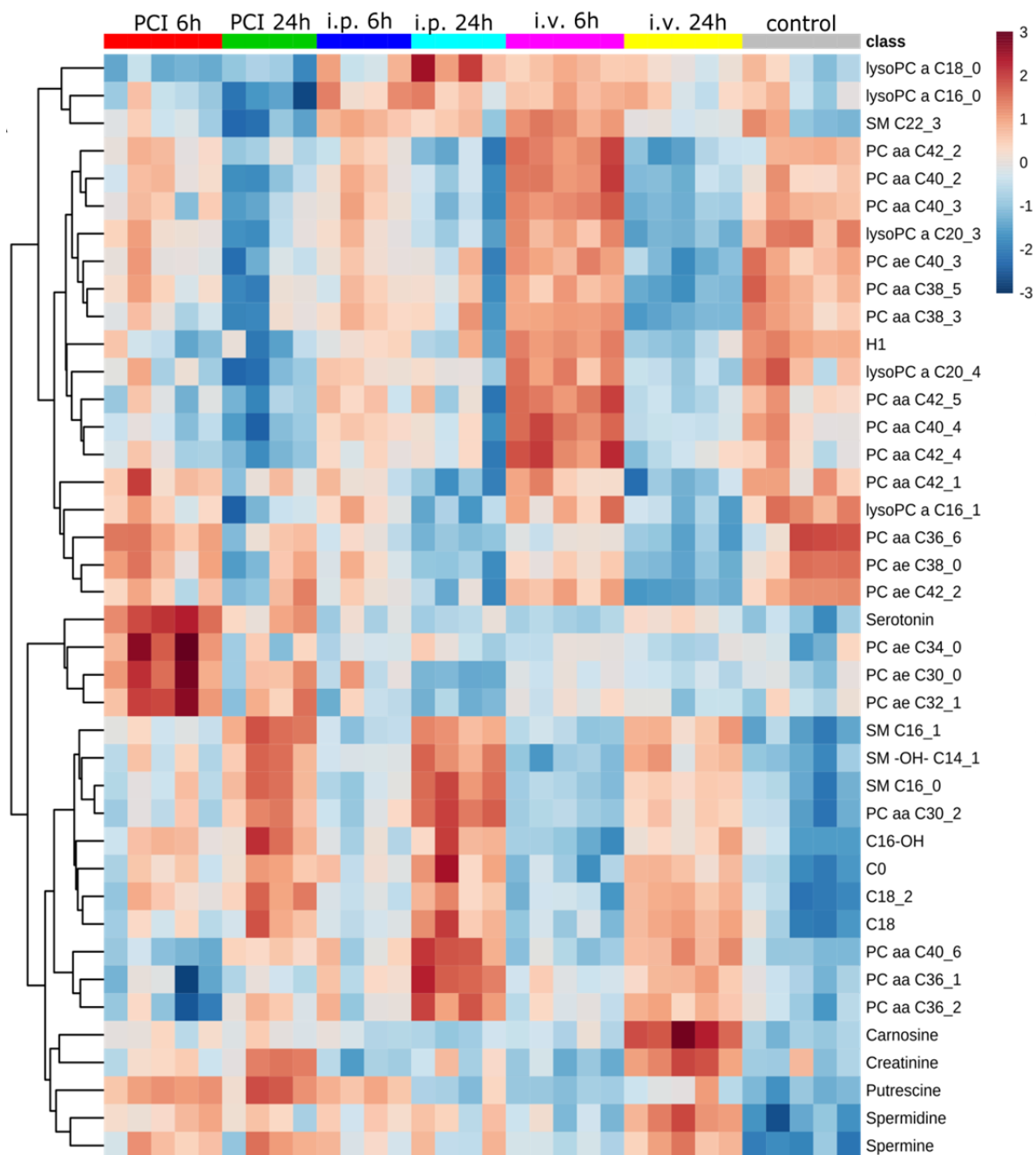
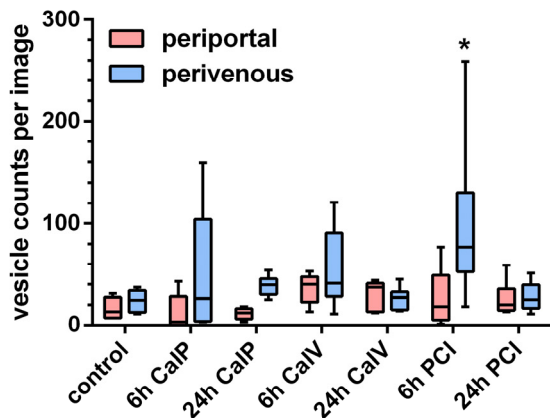
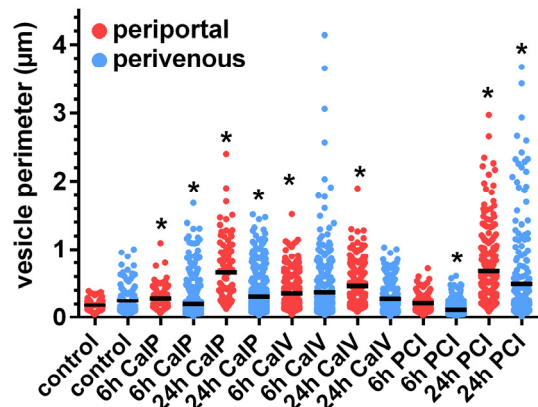


Figure S4: Metabolomics. Heatmap shows the top 40 significant metabolite concentrations as classified by ANOVA (p -val $4.08E-0.5$). Concentrations were log-transformed and normalized to the mean of the control group. The dendrogram for metabolite groups uses Euclidean distance measure and the average algorithm for clustering. The color-coding represents the metabolite concentration increase from blue to red, scaled to the average of each metabolite. a = acyl, aa = diacyl, AC = acylcarnitine, ae = acyl-alkyl, Cx:y = where x is the number of carbons in the fatty acid side chain; y is the number of double bonds in the fatty acid side chain, H1 = hexose, OH = hydroxyl, PC = phosphatidylcholine, SM = sphingomyeline.



Kruskal Wallis Test, Dunn's multiple comparison
 $\alpha < 0.05$ against respective control (PP or PV)



Kruskal Wallis Test, Dunn's multiple comparison
 $\alpha < 0.05$ against respective control (PP or PV)

Figure S5: Quantification and size of vesicles

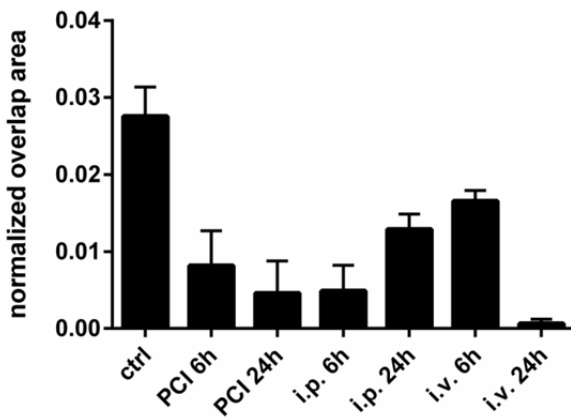


Figure S6: Overlap of quantified MRP2-stained and F-Actin-stained areas at 20x magnification depict the loss of membrane-associated MRP2 protein leading to cholestasis.

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