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Long-term effects of different stem cells in genetic models of Cystic Kidney Disease

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1. Introduction

Kidneys are two bean-like shaped organs located in the abdominal cavity behind the peritoneum, on the left and right side of the spine. A physiological renal function is essential for maintenance of homeostasis, ions regulation in blood or water, hormone production and secretion and, last but not least, formation of urine (**figure 1.1**).

A transversal cut of the kidney reveals, in the parenchyma, three major structures: cortex, medulla and papilla layers, respectively form the outer to the inner part (**figure 1.2**).

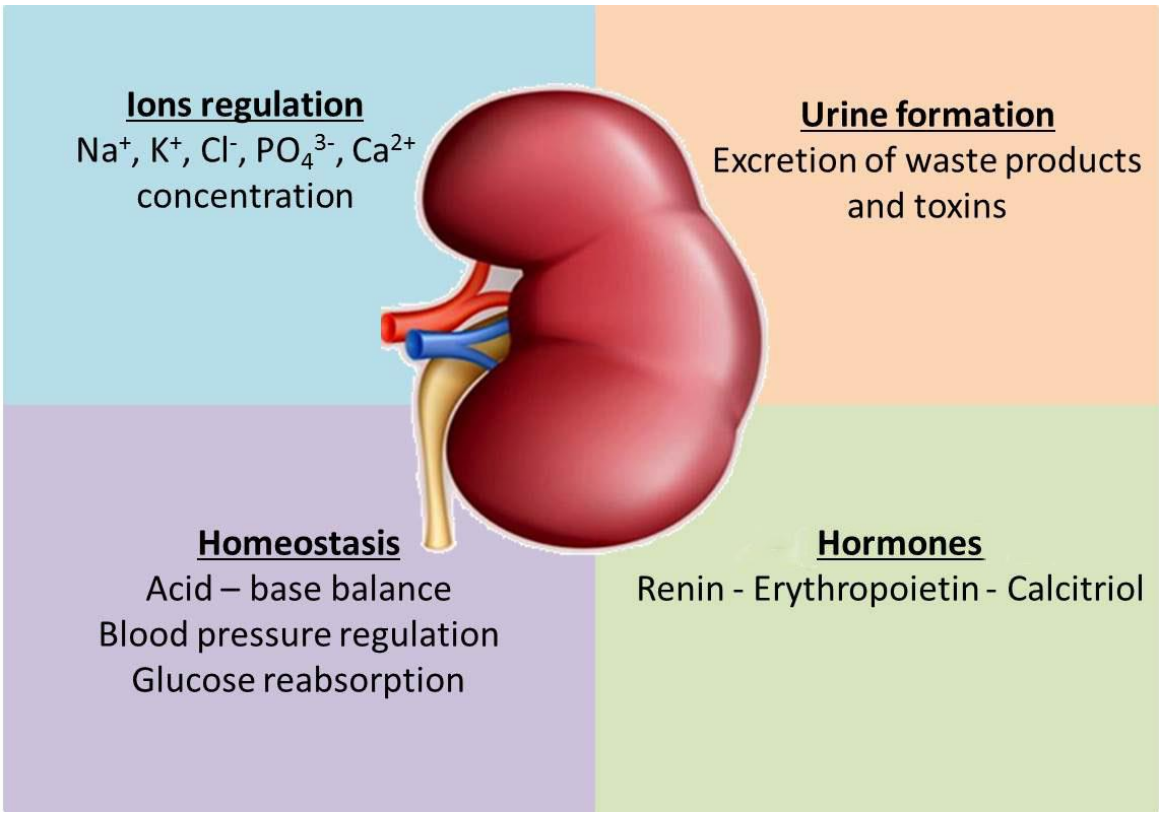


Figure 1.1 The renal system is responsible for maintaining crucial physiological processes such as ions regulation, fluid homeostasis, hormonal balance and excretion of waste products of metabolisms.

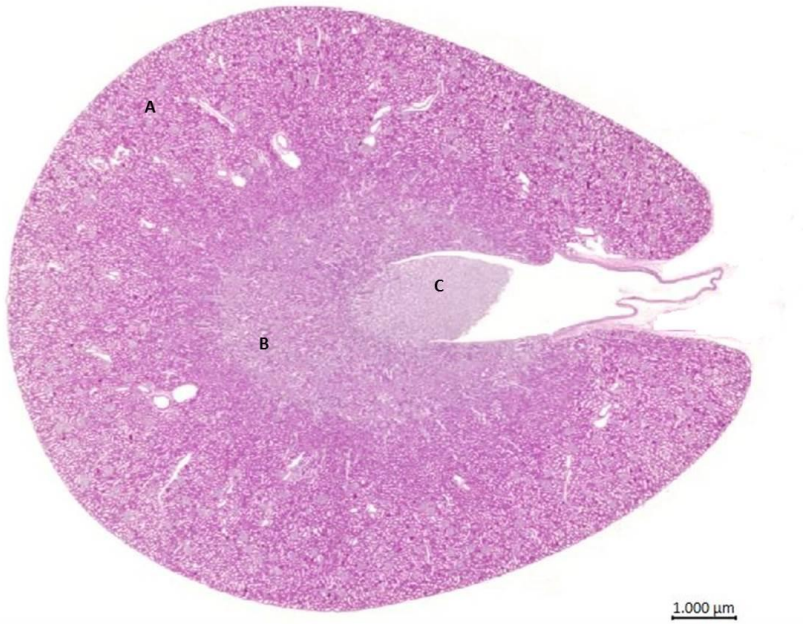


Figure 1.2 Hematoxylin and eosin (H&E) staining of Sprague Dawley (SD) rat kidney. A) cortex layer, B) medulla and C) papillary region. Image acquired with Axio Scan.Z1 microscope (ZEISS), 20x objective.

The nephron is the functional and structural unit of the kidney and it spread between the cortex and the medullary region. Each nephron is composed of a renal corpuscle, consisting of a glomerulus surrounded by Bowman's capsule, and the renal tubule. The nephron is responsible for three essential processes: filtration, reabsorption and secretion (**figure 1.3**).

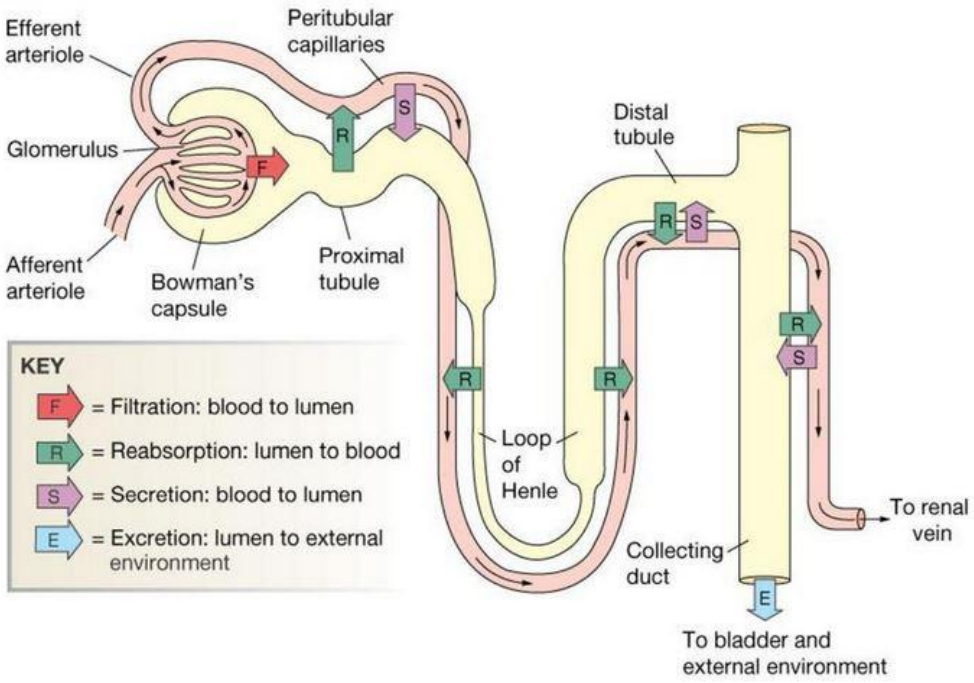


Figure 1.3 Nephron structure and function[1].

Due to the important and fundamental roles, especially in maintaining homeostasis and pH, kidneys are often exposed to a multitude of insults that may compromise their functionality.

1.1 Cystic Kidney Disease

In the last decades, the uprising of chronic kidney diseases (CKD) has become one of the worse and alarming public health problems. An early diagnosis of renal diseases might be beneficial for the quality of life for the patients but often, early stages of the disease are asymptomatic and first evidences appear when the progression of the pathology leads to chronic or end-stage renal disease (ESRD)[2, 3].

Cystic kidney disease is the fourth common cause of ESRD, just after diabetes, hypertension and glomerulonephritis. Cystic disease is a heterogeneous group of chronic disorders with a wide range of manifestations and may be hereditary, or might develop sporadically, as consequence of aging and lifestyle. Genetic cystic kidney disease, such as autosomal dominant polycystic kidney disease (ADPKD), autosomal recessive (AR) PKD and nephronophthisis (NPHP), are the most clinically significant types of cystic renal disease[4].

1.1.1 *ADPKD*

ADPKD is the most common form of cystic kidney disease with an incidence of 1:1000 individuals[5]. ADPKD is a monogenic disorder with a penetrance of 100%[6]. It is caused by the mutation of two different genes: *polycystin 1* (*PKD1*) and *polycystin 2* (*PKD2*), both of which are found on the primary cilia of renal tubules. It is known from literature that the interaction of these two genes promotes the normal development and function of the kidney[7, 8]. Phenotypically, both kidneys appear enlarged due to the development of multiple cysts of different size. Patients with a mutation on the *PKD1* gene present a larger kidney and faster progression to ESRD in comparison to patients with a *PKD2* mutation. They present with a milder disorder. Since, usually, the early stage of the disease is asymptomatic, in the past decades ADPKD was identify as a disease of the adult life but, recently the first symptoms have also been detect in childhood[9]. Early clinical manifestations are the disruption of the inner renal medullary region, polyuria, enlargement of both of the kidneys and hypertension[10]. ADPKD, as a kidney disorder, is affecting primarily the kidney but also other organs might be involved. Extrarenal manifestations of the disease have an impact on liver, consisting of the development of cysts and dilatation of bile ducts, cardiovascular abnormalities and intracranial aneurysm[11-14]. Based on the severe decline of the glomerular filtration rate (GFR),

which describes the efficiency of the renal function, patients with ADPKD are classify into categories. Classification is helpful to identify patients who may benefit most from therapy and patients with a slower progression or late stage of the disease who might not benefit from it[15, 16]. Dialysis and kidney transplantation are usually the therapeutic gold standard, and the only possibility, for the treatment of cystic kidney disease[17, 18]. Clinical trials led to the development of the first FDA approved drug: Tolvaptan, a vasopressin receptor 2 antagonist. Clinical trials demonstrated that Tolvaptan slowed the GFR decline and kidney growth in patients with early ADPKD[19], but further analysis on potential advantages and disadvantages have to be taken into consideration. Other possible targets for therapies are the inhibition of mTOR and CoA reductase activity[20, 21].

1.1.2 *ARPKD*

ARPKD is a rarer disease, with an incidence of about 1:20.000 – 30.000 live births. ARPKD is a congenital hepatorenal syndrome, caused by a single gene mutation[5]. The mutation occurs on the *polycystic kidney and hepatic disease 1* (*PKHD1*) gene. *PKHD1* is located on the cell membrane of kidney cells, primary cilia and, in lower level, in liver and pancreas[22]. ARPKD is typically an infantile disorder with a significant neonatal mortality and childhood morbidity rate[23]. Neonates present a massively enlarged kidneys with the presence of multiple cysts, pulmonary hypoplasia, liver changes like ductal plate malformation, that lead to progressive hepatic fibrosis[24-26]. It is estimated that 20 – 30% of patients dye shortly after birth due to respiratory insufficiency as consequence of pulmonary hypoplasia[24, 26]. In the remaining 70% of patients, the disease progresses to ESRD during adolescence[25, 27]. A minority of patients develop extrarenal and extrahepatic disorders such as pancreatic abnormalities[28]. As consequence of the high mortality rate and the severe and fast progression of the disease, patients usually undergo a combined kidney-liver transplantation within the first decade of life. To this day no pharmacologic therapy has been developed for ARPKD.

1.1.3 *NPHP*

NPHP is a group of autosomal recessive tubulointerstitial cystic kidney diseases, which are genetically heterogeneous. A single gene mutation, on *NPHP* gene, is responsible for the triggering of the disease. Nowadays, about 20 genes have been described to be involved in NPHP[29]. Frequently, NPHP affect patients in the first decade of life or during adolescence and is one of the most common causes of ESRD in childhood[29]. NHPH has been classified into

three different forms: infantile, juvenile, and adolescent NPHP, which develop ESRD respectively, at the ages of 1, 13 and 19 years of age[30, 31]. Usually, the early clinical symptoms are polyuria, polydipsia and anaemia, even if ultrasound scans show normally sized kidneys[30]. As NPHP progresses, cysts start to manifest and kidney loses the corticomedullary differentiation. Further important histological manifestations are the deposition of fibrotic tissue, tubular atrophy and alteration of tubular basement membrane[32]. As for ADPKD, at the beginning of the disease, the symptoms are mild and the diagnosis of the disease is made when the progression is already ongoing. Currently, dialysis and transplantation appear to be the only therapeutic options for the patients.

1.2 Genetic animal models

Nowadays two main animal models of cystic kidney disease have been identified: all are spontaneous hereditary models. These models are often used for the characterization of the disease outcomes, the disease progression and to establish potential therapeutic strategies. In particular, two of the best described spontaneous hereditary models are the PKD/Mhm and PCK rats.

1.2.1 *PKD/Mhm rats*

In 1989 a spontaneous mutation resulting in PKD was noted in a Sprague Dawley (SD) colony in Hannover[33]. A group of animals died after 4 weeks of age, showing an increase in kidney volume and weight, a progressive renal failure, uraemia and hypertension. In the early 1990s in Mannheim, Gretz *et al.* better characterized this animal model and the difference between homozygous (Cy/Cy), heterozygous (Cy/+) and healthy (+/+) rats[34, 35]. These studies highlighted that the cystogenesis process starts during the first week of life. While heterozygous animals develop a slow progression of renal cystic disease, homozygous rats are characterized by massive renal enlargement that leads to an early death in the first postnatal month. Morphologically, in PKD/Mhm (Cy/Cy) rat kidneys the penetrance of the mutation does not depend on the gender of the animals. Both female and male PKD/Mhm (Cy/+) rats manifest in fact, a slowly progressive development of cysts in renal tissues, leading to ESRD. Moreover, male rats display a more severe form of the pathology when compared to females. In 2006 a study was carried out to validate the PKD/Mhm as a model for PKD[36]. The study performed a comparison between the guanidine compound in blood and urine chemistry of humans and rats. Their results pointed out that PKD/Mhm rats present similar disease changes as PKD patients, such as increase in urea concentration and guanidinosuccinic acid[36]. Few

years later, the mutation on the gene leading to the pathology has been found. It was affecting Anks6, a gene located in the proximal cilium[37, 38]. Additional studies suggested that a mutation on both the copy of Anks6 could lead to NPHP16[39, 40]. This is reason why PKD/Mhm rats nowadays are used as animal model for both ADPKD and NPHP16 in order to understand the pathology mechanism of the diseases and to test innovative therapies.

1.2.2 *PCK rats*

At the beginning of the century, in Japan, Katusyama *et al.* identified female rats with both polycystic kidneys and liver cysts derived from a SD colony[41]. The spontaneous mutation developed in these rats was located on the Pkdh1 gene, the orthologous gene affected in human ARPKD[42]. Studies were conducted to better define the model[41, 43, 44]. When compared with SD rats with same age, PCK rats showed an increase of the body weight, related also to the increase of kidney and liver weight. As for other strains, male rats manifested a more severe form of the disease and their survival rate was lower when compared to female[41]. Compared to healthy animals, PCK rats manifested an increase of the creatinine plasma concentration and blood urea nitrogen (BUN) levels[44]. Since the beginning, PCK was not only resembling human ARPKD but also Caroli's disease, a pathology characterized by a congenital intrahepatic biliary dilatation usually associated with congenital hepatic fibrosis of autosomal recessive polycystic kidney disease[41, 43]. Hepatic analysis showed and enlargement of the organ with cyst formation already in the first weeks of age and dilatation of the intrahepatic bile ducts. Also the values of hepatic transaminases in the blood chemistry were increased when compared with SD rats; likewise proteinuria levels[45].
In the last few years PCK rats have been frequently used as a model for the understanding of both kidney and liver disease but also to test potential and innovative therapeutic approaches.

1.3 (Potential) Stem cells therapy for Cystic Kidney Disease

Therapeutic approaches in nephrology are aiming to reduce the injury and delay, if not arrest, the progression of the renal disorders. Unfortunately, especially in CKD, the only available possibilities for patients are dialysis or renal transplantation. Recently, a promising therapeutic approach is the use of stem cells therapy to reduce the renal pathology. Several studies, indeed, assessed the therapeutic potential of mesenchymal stem cells (MSCs) in kidney disorders, likes acute or chronic kidney injury, diabetic nephropathy and autoimmune disease[46-48]. Nowadays, only two published studies show the possible therapeutic effects of allogeneic stem cells in rats demonstrating first of all the safety and tolerability of MSCs and

secondly, the capability of MSCs to ameliorate renal function and limit the cysts formation in PKD animal model[49, 50]. Taken into consideration the dramatic increase of the incidence of diseases and the few available cures, in this study the potential therapeutic effects of human adipose derived stromal cells (ASC) and human ABCB5+ cells will be tested.

1.3.1 *ASC*

Over the years, bone marrow has been the main source for the isolation of MSCs. Nevertheless, the harvesting of bone marrow cells is a highly invasive procedure and is prone with the increasing age to a decline of proliferation and differentiation potential of MSCs[51-53]. Therefore, at the beginning of the century Zuk *et al.* searched for an alternative stem cell source: the adipose tissue[54]. Advantages of this new source are the less invasive method and the possibility to obtain larger quantities. In fact, adipose tissue is collected by needle or liposuction aspiration and, ASC can be easily isolated from the tissues[55] (**figure 1.4**).

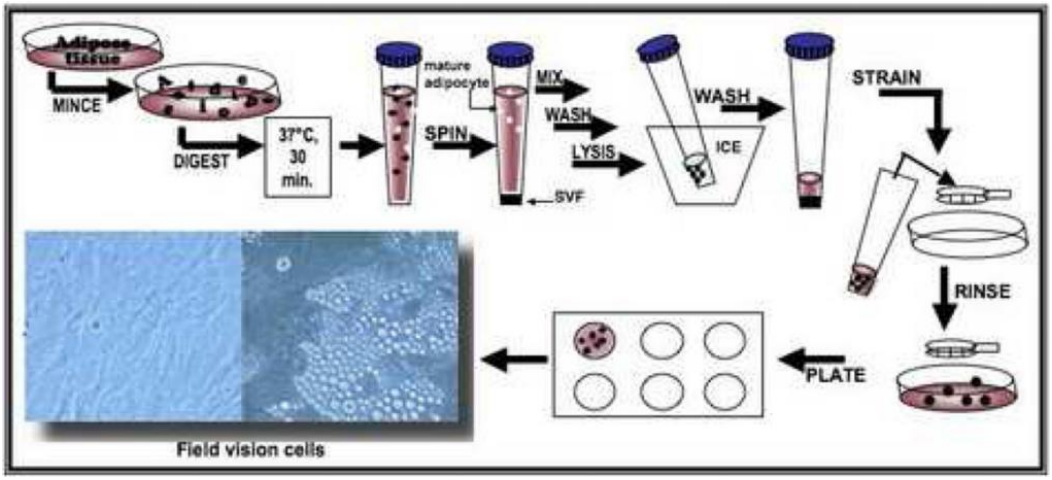


Figure 1.4 Adipose tissue processing and ASC isolation[55].

When tested, ASCs lack the expression of hematopoietic stem cell marker CD45 and CD34 and are positive for stem cell-specific surface markers, such as CD90,CD105, CD73, CD44and CD166[56].

1.3.2 *ABCB5+*

In the last 10 years, Schatton *et al*. characterized a new type of non-hematopoietic cell showing immune-regulatory function similar to stem cells in the human reticular dermis (DIRCs)[57]. These cells express the ATP-binding cassette member B5 (ABCB5). ABCB5+ cells have been tested for the negativity of the CD34, marker of dermal dendritic cells and hematopoietic stem cells, and CD45. Nevertheless, ABCB5+ cells were expressing MSC

markers such as CD29, CD44, CD105 and CD166[58]. The main outcome of this study was that ABCB5+ cells are a phenotypically unique dermal cell subset with multipotent differentiation plasticity (**figure 1.5**). These properties make ABCB5+ cells a valid therapeutic candidate for cell-based immunotherapies in the regenerative medicine field[58].

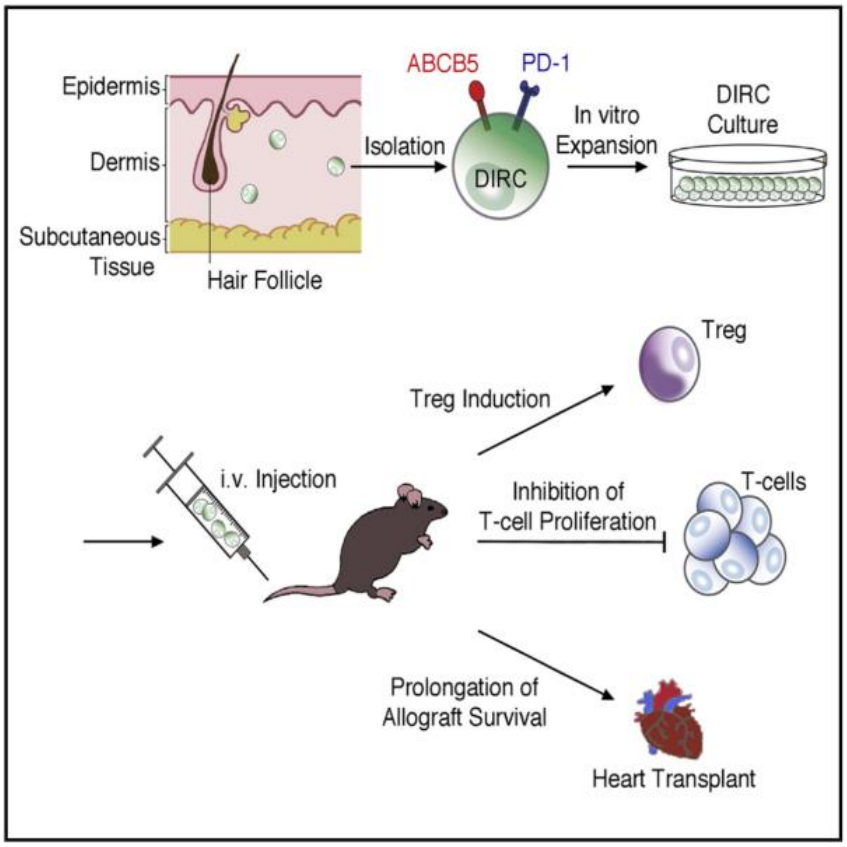


Figure 1.5 ABCB5 identifies immunoregulatory dermal cells[58].

2. Aim of the study

The main goal of this study was to investigate the potential therapeutic effects of different types of human stem cells and their conditioned media on genetic animal models of PKD, since stem cells are not the usual application in monogenic diseases. The aim, in fact, was not to act directly on the genetic mutation characteristics of the pathology but to test whether stem cell therapy could ameliorate the renal function by improving, directly or indirectly, pathways such as metabolism or signal transduction.

In order to achieve this goal, we first characterized two different genetic animal models: the PKD/Mhm (Cy/+) and the PCK rats. In a second step, the long-term effects of human ASC and human $ABCB5^+$ cells, together with ASC conditioned media and a co-culture of $ABCB5^+$ and macrophages media were tested.

PKD/Mhm (Cy/+) and PCK rats were bred in our core facility in accordance with the local authority law and the EC directive 2010/63 EU. To qualify renal damage and disease progression, the principal parameter for renal function, such as creatinine and urea levels, were tested through chemistry analysis and histological analysis were performed.

ASC cells have been provided by Professor Karen Bieback´s group, from the Institute of Transfusion Medicine of Mannheim. Cells were mostly obtained by lipoaspirates from healthy donors undergoing liposuction, according to Mannheim Ethics Commission II (vote numbers 2010-262 N-MA, 2009-210 N-MA, 49/05 and 48/05).

$ABCB5^+$ cells were isolated from different healthy donors, cultured and characterized by Ticeba-RHEACELL GmbH & Co. (Heidelberg, Germany) laboratories and send to us in a ready to use solution.

In the past years, major importance was attached to the effects not only of stem cells but also of the factors they release into the culture medium. For this reason we also tested the effect of two different types of conditioned media: firstly, a medium in which ASC cells were growth, without any external or added stimuli and at a later stage, TICEBA Company provided us with a co-culture of $ABCB5^+$ cell and macrophages M1 stimulated with Interferon γ (INFγ) and lipopolysaccharide (LPS).

To better characterize the effect of the treatments on the kidney function, plasma and urine analysis within GFR measurement were carry out.

3. Animals, material and methods

3.1 Animals

All experiments were conducted in accordance with the German Animal Protection Law and approved by the local authority (Regierungspräsidium Nordbaden, Karlsruhe Germany in agreement with EU guideline 2010/63/EU).

Kidneys of PKD/Mhm animals were palpated at the age of 8 weeks under isoflurane anesthesia. Heterozygous (Cy/+) can be distinguished from the wild type animals by means of the slightly enlarged kidney and altered kidney surface in the heterozygous animals.

A total of 42 male PCK rats and 42 male PKD/Mhm (Cy/+) were engaged in the long term experiment (animal permit G19/17). From these, 36 PCK and 36 PKD/Mhm (Cy/+) rats were used in the cell therapy studies while 6 PCK and 6 PKD/Mhm (Cy/+) rats were assigned to the untreated group as control for the evolution of the pathology.

Animals were housed in pair in individual cages with food and water ad libitum.

3.2 Experimental schedule

Animals were enrolled in the trial at the age of 8 weeks. Therapy began with a single intraperitoneal (i.p.) or intravenous (i.v.) injection of stem cells or i.v. injection of conditioned media. The injections were repeated in monthly intervals. Before the start of the experiment a basal examination of renal function was performed by determination of the GFR measurement and the renal function parameters (plasma and urine samples). These examinations were repeated, for each animal, every 4 weeks up to the age of 8 months (**figure 3.1**). After the baseline assessment, animals were randomly assigned in four different groups depending on the received treatment on day 1: (i) a group that did not received any treatment, (ii) conditioned media group, (iii) stem cells i.p. administration group and (iv) stem cells i.v. administration group. (**table 3.1**).

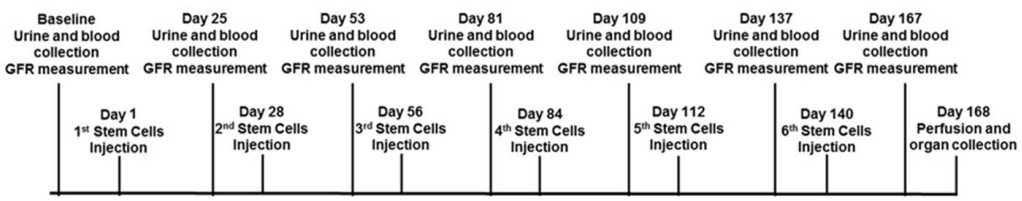


Figure 3.1 6 months experimental set up for the assessment of human ABCB5+ and ASC and derived conditioned media.

Strain	Treatment	Number of animals
PKD/Mhm (Cy/+)		6
	+ CoCM+	6
	+ CM	6
	+ i.p. ABCB5+	6
	+ i.p. ASC	6
	+ i.v. ABCB5+	6
	+ i.v. ASC	6
PCK		6
	+ CoCM+	6
	+ CM	6
	+ i.p. ABCB5+	6
	+ i.p. ASC	6
	+ i.v. ABCB5+	6
	+ i.v. ASC	6

Table 3.1 Number of animals enrolled in the experiment.

3.3 Plasma and urine collection

Plasma samples were collected monthly, the day before starting the trial, via retro-bulbar vein plexus (orbital sinus) under anaesthesia (5% isoflurane at 3 min/L airflow) in lithium-heparinized tubes using capillaries for blood collection. Afterwards, samples were centrifuged and the plasma collected in 1.5 ml vials and stored at -20°C until analysis.

Urine collection was performed by placing the animals overnight into metabolic cages for a period of 16 hours. During this time the animals had free access to water and food. At the end of the 16 hours, urine was weighed and samples centrifuged to get rid of the precipitate. Urine aliquots were then placed in 2 ml collecting tubes and immediately frozen at-20°C until analysed.

Cobas c311 analyzer (Roche Diagnostics GmbH, Mannheim, Germany) was used to evaluate plasma and urine parameters. The urinary albumin level was determined by ELISA and osmolarity was analysed using an osmometer (2020 Multi-Sample Osmometer, Advanced Instruments Inc., Norwood, MA).

3.4 GFR measurement

3.4.1 *ABZWCY-HβCD*

The selected agent for GFR measurement is ABZWCY-HβCD, a zwitterionic agent with an emission wavelength in the near infrared (NIR) region developed by Huang *et al.* in 2017[59]. The kidney can excrete efficiently and fully the agent[59]. The suitable fluorescence, deep

penetration depth, high hydrophilicity and non-toxicity properties of ABZWCY-HβCD make it the ideal candidate for the GFR measurement.

3.4.2 *Device for the GFR measurement*

GFR measurement was performed using a miniaturized electronic device (MediBeacon GmbH, Mannheim, Germany) (**figure 3.2**)[60-62]. The device consists of an optical part formed by two light-emitting diodes (LEDs) with an excitation at 706 nm, an emission at 790 nm (NIR region) and a photodiode detecting the agent's fluorescent light. Energy was supplied by a lithium polymer rechargeable battery with a voltage of 3.7 V and a capacity of 50 mAh. The device holds also an internal memory that records the digital data. By using a micro-USB cable the data can be read out onto a PC for analysis.

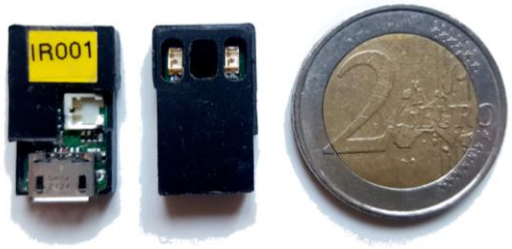


Figure 3.2: Miniaturized device for the transcutaneous GFR measurement.

3.4.3 *Procedure and ABZWCY-HβCD half-life ($t_{1/2}$) determination*

A dosage of ABZWCY-HβCD was 15 mg/100g rat's body weight (BW). Initially a stock solution at a concentration of 160 mg/ml of dye was created. Then it was stored protected from light to minimize the time of the anaesthesia and the potential related risks.

Animals were depilated on part of the back under short isoflurane anaesthesia (5% isoflurane at 3 L/min airflow, decreased to 2% isoflurane at 1.5 L/min airflow). We used an electric shaver for small rodents to ensure a proper fixation of the electronic device. After the shaving, the device with its battery was placed on a double-sided adhesive patch (**figure 3.3A**), fixed on the hairless area and secured with a tubular net bandage plus two strips of adhesive tape to prevent the animal from removing it.

Once the device was correctly fixed, the proper dose of ABZWCY- HβCD was administrated intravenously and the measurement performed for 2 hours. During this period the animal was awake and freely moving (**figure 3.3B**).

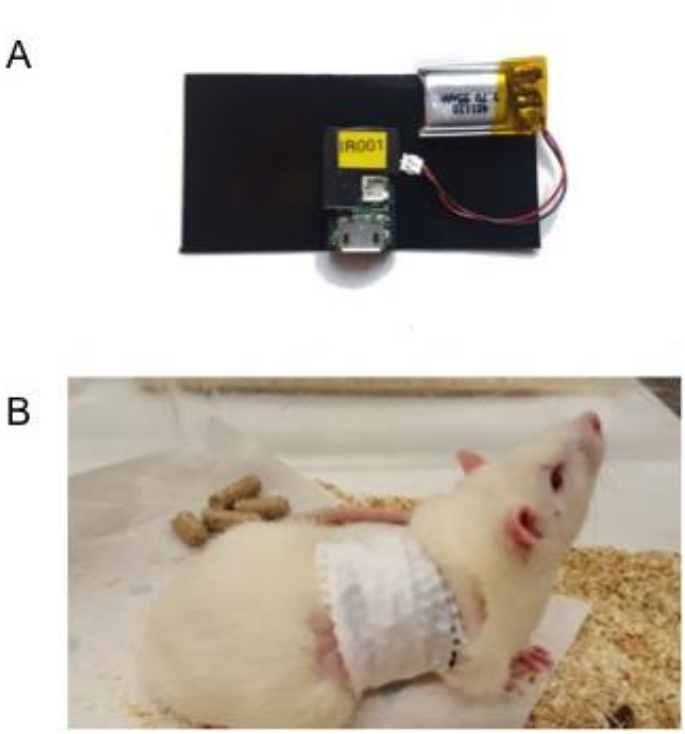


Figure 3.3 Placement of the device. A) Device and battery are placed on a double-sided adhesive patch; B) During the period of measurement, after ABZWCY- HβCD administration, the animal is awake and freely moving.

After the recording period, the device was removed and connected by a USB cable to a PC to download the data using the basic software provided by the company.

To quantify the half-life ($t_{1/2}$) excretion of the administered fluorescent dye, a 3-compartment model was applied using the specific software designed by our group (http://www.mathworks.com/products/compiler/matlab-runtime.html)[63].

3.5 ASC and ABCB5+ administration

3.5.1 *ASC administration*

ASC cells were isolated by Professor Karen Bieback´s group, from the Institute of Transfusion Medicine and Immunology of Mannheim. Cells were obtained from lipoaspirates taken from healthy donors undergoing beauty surgery procedures. In summary, the raw lipoaspirate was washed with sterile PBS 1X, to remove cellular remains and red blood cells. Then it was digested with 0.15% w/v collagenase type I (Sigma-Aldrich, Munich, Germany). DMEM/10% human serum (blood-type AB) (AB-HS) was then added to inhibit the collagenase activity and the sample was centrifuged to obtain the stromal vascular fraction (SVF) pellet. Afterwards, the pellet was re-suspended in a DMEM/10% AB-HS, low glucose medium and filtered through a 100 μm nylon mesh filter to remove cellular fragments. Once

again, the filtrate was centrifuged and the re-suspended SVF cells were plated in cell culture flasks and incubated overnight at 37°C, 5% CO_2. After one day, the culture plates were washed to remove non-adherent and red blood cells. After cells had started to form colonies, they were tested to assess the multipotent MSC criteria. A differentiation assay was performed and the expression of CD90, CD37 and CD105 and lack of expression of CD34 and HLA-DR.81 was tested.

In a second step, cells were cultured in our laboratory. Briefly, cells were re-suspended in DMEM/10% AB-HS medium in T175 cell culture flasks at a concentration of 1.3×10^5 and incubated at 37°C, 5% CO_2. Every 48 hours, cells were washed with PBS 1X to remove cell debris, and fresh media (DMEM/10% AB-HS) was added.

On injections day, ASCs were trypsinized (0.25% trypsin) and incubated for 5 min at 37°C, fresh DMEM/10% AB-HS medium was used to inhibit the trypsin activity and the cells were centrifuged. The supernatant was collected to obtain the conditioned medium (CM), while the cells pellet was re-suspended in fresh medium at a concentration of $1x10^7$ cells/ml.

3.5.2 *ABCB5+ administration*

ABCB5+ ready to use cell suspension ($1x10^7$ cells/ml) and conditioned media were provided by Ticeba-RHEACELL GmbH & Co. (Heidelberg, Germany).

ABCB5+ cells were purified from the skin of healthy donors of different sex, age and nationality by Ticeba-RHEACELL Gmbh.

The conditioned medium was derived by collecting the supernatant of a co-culture of ABCB5+ cell and THP-1 derived macrophages (CoCM+) stimulated with IFN-γ and LPS.

On the seeding day cells were stimulated with 50 IU/ml IFN-γ and, the day after with 50 IU/ml IFN-γ + 20 ng/ml LPS. The third day, conditioned medium was harvested and immediately frozen and stored at -20°C until used. The THP-1 derived macrophages were able to polarize in anti-inflammatory macrophages M2 thanks to the presence of the above mentioned stimuli and ABCB5+ cells.

3.6 Histological analysis

After the 6 months of treatment, the animals underwent perfusion. Prior to the perfusion, animals were weighted and anesthetized by i.p. injection of ketamine/xylazine (xylazin 5mg/kg BW and ketamine 100mg/kg BW). Once the animal was deeply anesthetized, the abdominal cavity was opened through a cut along the linea alba. The left kidney was removed

before starting the perfusion, weighted, cut, placed in a cryotube and deep frozen in liquid nitrogen prior to storage at –80°C for future analysis (section 3.7).

Perfusion was performed through the abdominal aorta: a butterfly cannula was inserted about 0.5 – 1.0 cm from its distal bifurcation and the vena cava was opened. Saline/heparin solution (3 minutes, 280 mbar) was used to wash out the blood from the body of the animals, followed by fixation in 4% PFA pH 7.4 (3 minutes, 230 mbar). After the perfusion portions of right kidney, liver, lungs, heart, spleen and pancreas were collected, placed in embedding plastic boxes for the routine paraffin embedding procedure. Each organ was sliced (3 μm section) and stained with haematoxylin and eosin (H&E) or stored for following immunohistological analysis.

To evaluate the fibrosis level in liver and kidney tissue the Masson-Goldner staining was performed, using light green as a specific stain for the connective tissue.

Immunofluorescence (IF) staining combined with Tunel reaction, was executed to assess the percentage of apoptosis and cell proliferation. Briefly, 3 μm kidney section where pre-treated with Citrate buffer pH 6.0 (20 minutes, 100°C) and incubated first with Tunel mix (In Situ Cell Death Detection Kit – Fluorescein, ROCHE), in the dark, 1h at room temperature (RT) and after overnight at 4°C with the primary rabbit anti-Ki67 antibody (1:50, ab15580, Abcam). After the primary antibody incubation, slides were further incubated with secondary antibody Alexa Fluor® 647 donkey anti-rabbit (1:100, ab150075, Abcam), 45 minutes at RT. Samples were counterstained with 4',6-diamidino-2-phenylindole dihydrochloride (DAPI) for nucleus identification.

All the slides were imaged with the slide scanner Axio ScanZ1 (Zeiss) with a 20x objective. Whole kidney sections were evaluated both with Zen Software (Zeiss) and ImageJ program (Fiji).

3.7 RNA isolation and sequencing

Total RNA of the samples was extracted from whole kidney tissue with the RNeasy mini Kit (QIAGEN).

Approximately 30 mg of frozen tissue were homogenized with lysis buffer. The homogenate was then centrifuged to precipitate any cellular debris and the supernatant were transferred in a 1.5 ml tube. An equal volume of 70% ethanol was added and the total amount transferred into a spin column and centrifuged.

Afterwards, always according to the kit instructions, washing were performed with solutions at decreasing salt concentrations to clean RNA bound to the silica membrane of the columns. Finally, the purified RNA was eluted in RNase-free water.

RNA concentration was measured by using the Spark 10M (TECAN).

To determine RNA quality, the Agilent 2100 Bioanalyzer (Agilent (Waldbronn)) was used and RNA integrity number (RIN) was calculated by a specific Agilent software tool. Samples with low RNA, too much protein-contaminated or with RIN values below 7 were repeated.

RNA sequencing was performed by BGI Tech Solutions Co (Hong Kong) using the BGISEQ-500 method.

R Bioconducor software was applied to analyse RNA sequencing data. For annotation, the ENTREZ-based software package TxDb.Hsapiens.UCSC.hg19.knownGene was used and differential gene expression analysis was performed by a DESeq2 package. As a level of significance $\alpha = 0.05$ with FDR correction was chosen. Gene set enrichment analysis (GSEA) was performed to determine statistically significant differences between treated and untreated animal groups. Pathway analysis was done with the help of the public KEGG (Kyoto Encyclopaedia of Genes and Genome) database to transform the list of individual genes into a set of pathways.

3.8 Statistical analysis

All data are represented as mean ± standard deviation (Std Dev). Values are rounded to 1 digit; this could explain why sometimes same numeric values present a different significant expression.

All the statistical analyses were performed using the software JMP® Genomics 7.

The effect of stem cells or conditioned media therapy was assessed by one-way ANOVA. After confirming a normal data distribution, Tukey-Kramer test of pairwise comparisons was applied to evaluate differences between biochemistry parameters and histologically quantitative parameters in all the groups through the different time points while, in paired measurements, T-test was used to compare the means. If the data distribution was not normal, nonparametric statistics was performed using the Steel-Dwass test. Statistical significance was defined as $p<0.05$.

4. Results

4.1 Characterization of the genetic animal models of Cystic Kidney Disease

4.1.1 *Changes in BW and kidney morphology*

BW was recorded monthly, during the whole experiment. During the six months of investigation, animal's BW progressively increased over time in a hyperbolic pattern. However, already after 25 days the PCK rats show a higher weight gain than PKD/Mhm (Cy/+) rats: 404.0 ± 13.3 *vs*. PCK 455.3 ± 24.1. At day 167, compared to the baseline, the BW was increased 1.5 and 1.6-fold in PKD/Mhm (Cy/+) and PCK respectively (**figure 4.1**).

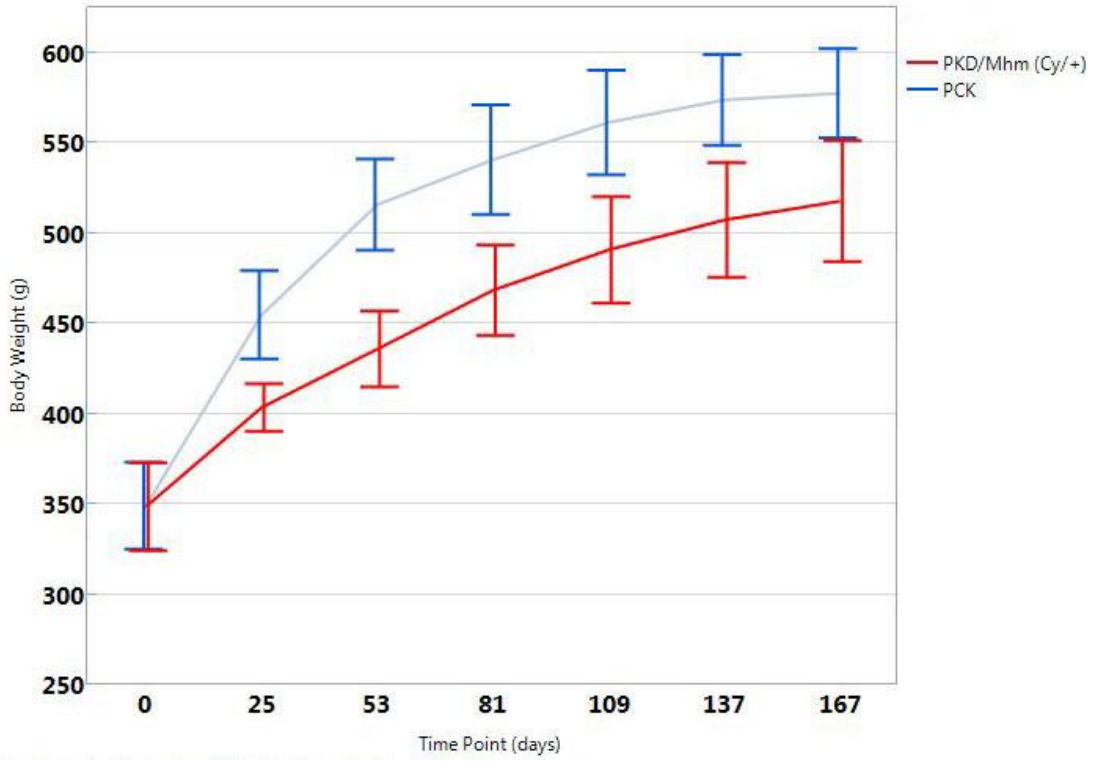


Figure 4.1 Changes over the time in BW in PKD/Mhm (Cy/+) and PCK rats (n=6 in each group). Data are shown as means ± Std.Dev

On the perfusion day, left kidney weight was lower in PKD/Mhm (Cy/+) rats (PKD/Mhm (Cy/+) 3.1 ± 0.8 vs. PCK 5.3 ± 0.3), as well as the kidney/body weight ratio (Kw/BW) (PKD/Mhm (Cy/+) 0.6 ± 0.0 vs. PCK 0.1 ± 0.0).

H&E staining of kidney sections revealed, in both strains, the loss of the renal structures equally distributed all along the cortex and medulla regions, in particular the corticomedullary alteration due to the presence of numerous single layer epithelial cysts of different size. Glomerular and tubular segments appeared to be decreased in number, probably because of interstitial changes. The renal parenchyma appeared tightened. The interstitium presented inflammatory cell infiltration, predominantly mononuclear, and deposition of massive fibrotic tissue (**figure 4.2**).

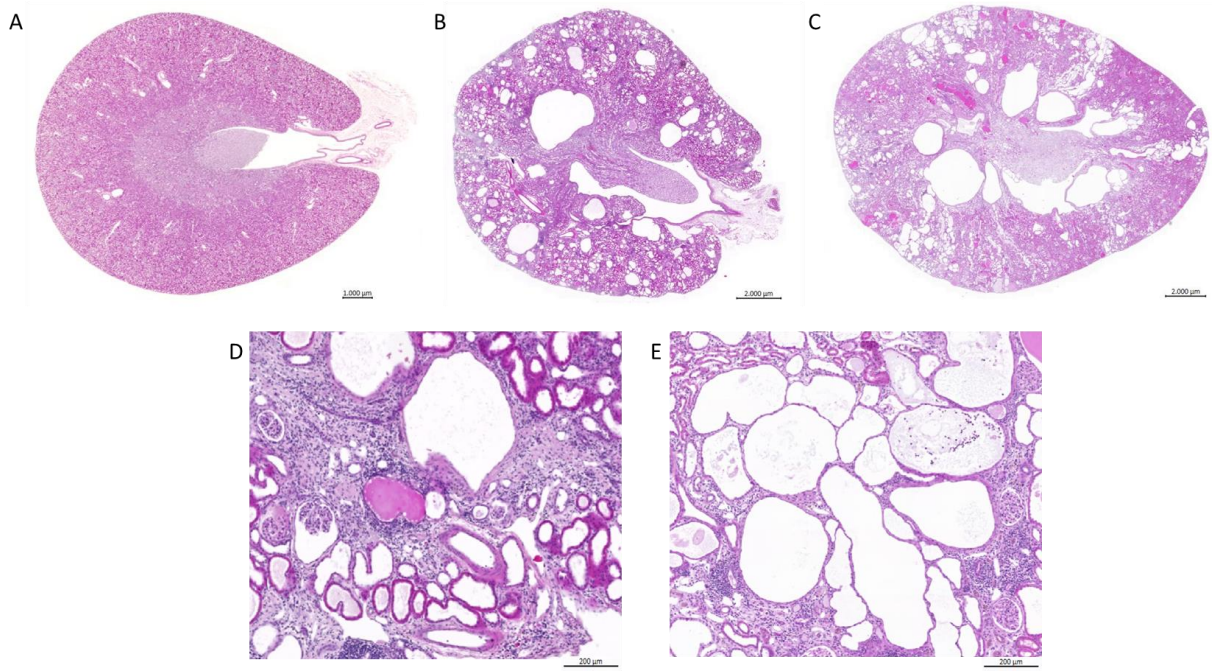
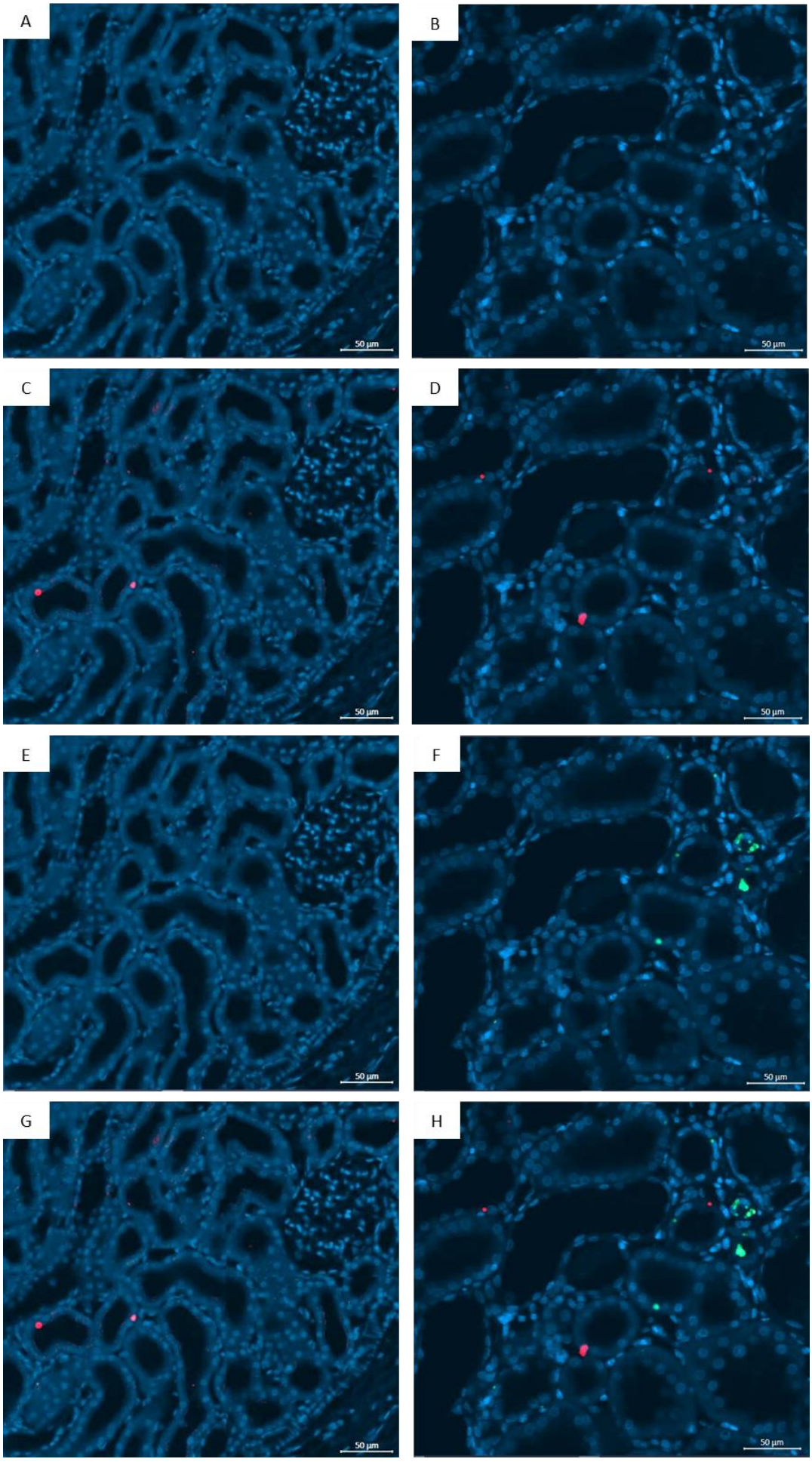


Figure 4.2 Altered renal morphology in cystic animal models. Whole kidney scan of A) SD rat, B) PKD/Mhm (Cy/+) rat, C) PCK rat. D) Fibrotic tissue in PKD/Mhm (Cy/+) rat kidney, E) Single layer epithelium cysts in PCK rat kidney. H&E staining. Images acquired with Axio Scan.Z1 microscope (ZEISS), 20x objective, D) and E) are zoomed in, for the real size check scale bar.

The presence of apoptotic bodies was detected in the lumen of tubules and cysts. An increment of apoptotic and proliferation marker positive cells was noticed, after IF staining (as explained in section 3.6) in cystic animals when compared to healthy wild type SD rats (**figure 4.3**, **table 4.1**).


A
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50 µm

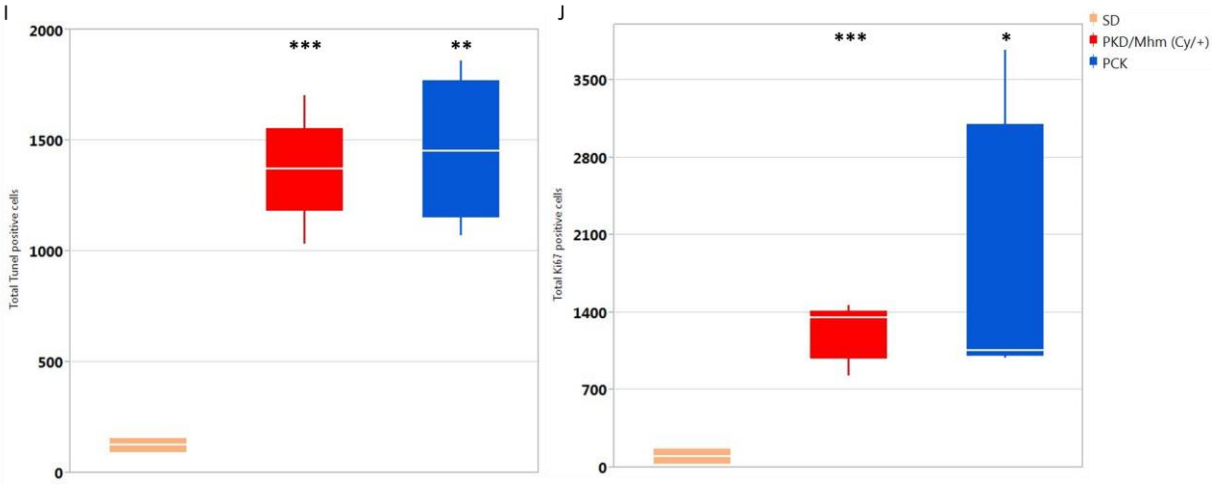

Figure 4.3 Examples for the quantification of apoptotic and proliferative positive marker (histologies for PCK rats are not shown as they are similar to the pictures of PKD/Mhm (Cy/+) rats). A-B) Dapi signal in SD (A) and PKD/Mhm (Cy/+) (B) rats; C-D) Ki67 signal in SD (C) and PKD/Mhm (Cy/+) (D) rats; E-F) Tunel signal in SD (E) and PKD/Mhm (Cy/+) (F) rats; G-H) Merged signals in SD (G) and PKD/Mhm (Cy/+) (H) rats; I-J) Graphic quantification of apoptotic and proliferative positive marker on whole imaged kidney section of SD, PKD/Mhm (Cy/+) and PCK rats (n=6 in each strain). (I) total Tunel positive cells, (J) total Ki67 positive cells. Data are shown as box plots with the median, upper and lower quartile (interquartile range (IQR)) and whiskers (1.5x IQR). Values significantly different from control (SD) are indicated as *p<0.05, **p<0.005, ***p=0.001. Co-staining Tunel-Ki67. Images were acquired with Axio Scan.Z1 microscope (ZEISS), 20x objective, A-H) are zoomed in, for the real size check scale bar.

Strain	Tunel positive cells	Ki67 positive cells
SD	124.7 ± 31.0	101.0 ± 65.6
PKD/Mhm (Cy/+)	**1370.0 ± 237.7*** **	**1239.5 ± 250.7*** **
PCK	**1460.5 ± 324.6** **	**1721.0 ± 1369.8***

Table 4.1 Quantification of Tunel and Ki67 positive nuclei in SD, PKD/Mhm (Cy/+) and PCK rats (n=6 in each group). Analysis was performed on whole 3 µm kidney sections. Data are shown as mean ± Std.Dev. Values significantly different from control (SD) are indicated as *p<0.05, **p<0.005, ***p=0.0001.

While no significant alteration was noted in liver sections of PKD/Mhm (Cy/+) animals, PCK rat livers show a mild deposit of fibrotic tissue and bile duct dilatation (**figure 4.4**).

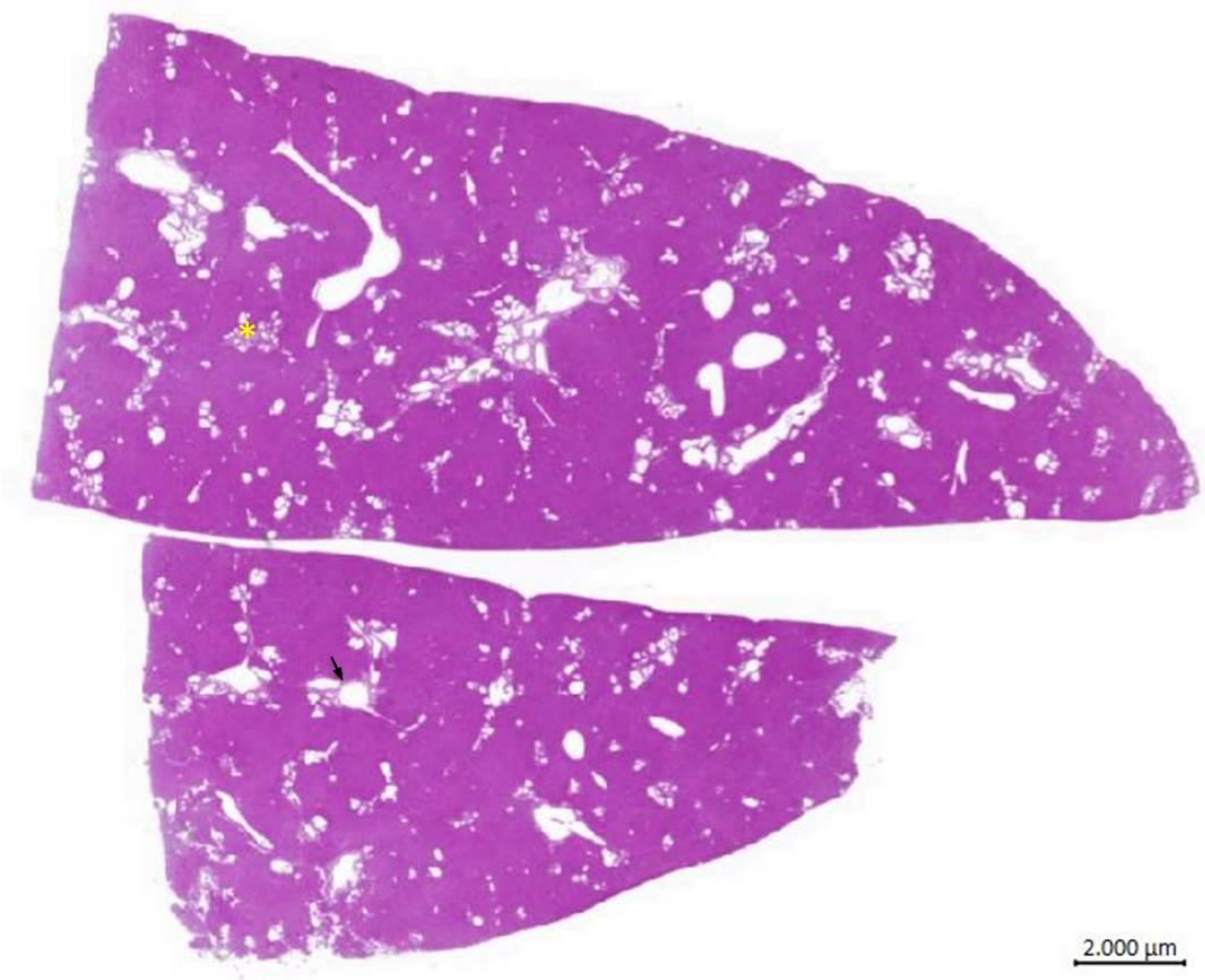

Figure 4.4 Altered hepatic morphology in a PCK rat. Yellow asterisk: fibrotic tissue; black arrow: bile duct dilatation. H&E staining. Images acquired with Axio Scan.Z1 microscope (ZEISS), 20x objective.

4.1.2 *Plasma chemistry, urine analysis and renal function*

The standard plasma indicators to assess renal function are creatinine and urea. Both model strains show a rise in these parameters during the experimental period. Notably, already from the first measurement (baseline) levels of creatinine and urea concentration in PKD/Mhm (Cy/+) rats were higher than in PCK rats ($p<0.05$). However, on day 167, a 2.5-fold increase of plasma creatinine was recorded in both strains (**figure 4.5**).

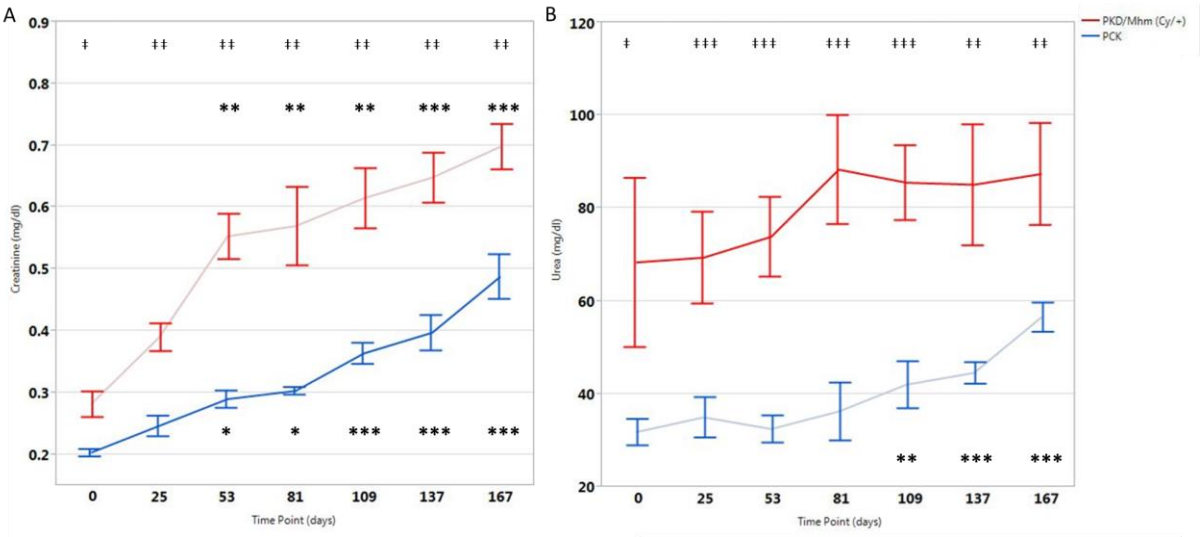

Figure 4.5 Alteration over the time in plasma creatinine and urea levels in PKD/Mhm (Cy/+) and PCK rats (n=6 in each group). (A) plasma creatinine levels, (B) plasma urea levels. Data are shown as means ± Std.Dev. Values significantly different (time point vs baseline) are indicated as *p<0.05, **p<0.005, ***p=0.0001 and (PKD/Mhm (Cy/+) vs PCK) as ‡p<0.05, ‡‡p<0.005, ‡‡‡p=0.0001.

The opposite was true for plasma cholesterol and triglycerides concentration. Indeed, since the beginning of the study, in PCK rats the cholesterol levels were increased by 1.4-fold. Significant differences of glucose (p<0.05) and protein (p<0.005) concentrations were found among the groups at day 167 (**table 4.2**, for individual days see **appendix 1**).

Parameter	Strain	Baseline	Day 167
Creatinine (mg/dl)	PKD/Mhm (Cy/+)	**0.3 ± 0.1‡**	**0.7 ± 0.1*** ‡‡**
	PCK	0.2 ± 0.0	**0.5 ± 0.1*** **
Urea (mg/dl)	PKD/Mhm (Cy/+)	**68.4 ± 18.2‡**	**87.4 ± 11.0‡‡**
	PCK	31.6 ± 2.8	**56.6 ± 3.2*** **
Na (mmol/l)	PKD/Mhm (Cy/+)	**144.3 ± 0.8‡‡‡**	144.8 ±1.9
	PCK	139.2 ± 1.2	**143.8 ± 1.8****
K (mmol/l)	PKD/Mhm (Cy/+)	5.4 ± 0.3	5.1 ± 0.2
	PCK	5.4 ± 0.3	5.1 ± 0.2
Ca (mmol/l)	PKD/Mhm (Cy/+)	2.5 ± 0.2	**2.7 ± 0.1‡**
	PCK	2.7 ± 0.1	2.8 ± 0.1
PO_4 (mmol/l)	PKD/Mhm (Cy/+)	2.4 ± 0.5	2.1 ± 0.3
	PCK	2.3 ± 0.3	2.2 ± 0.1
Cholesterol (mg/dl)	PKD/Mhm (Cy/+)	**97.3 ± 7.5‡‡**	**154.3 ± 15.5*** ‡‡**
	PCK	140.3 ± 15.6	**301.2 ± 62.1*** **
Triglycerides (mg/dl)	PKD/Mhm (Cy/+)	69.2 ± 26.3	**108.8 ± 48.3**
	PCK	86.8 ± 15.0	**160.5 ± 46.9****
Glucose (mg/dl)	PKD/Mhm (Cy/+)	169.0 ± 9.0	**184.3 ± 7.4‡**
	PCK	166.8 ± 10.6	150.3 ± 24.0

Protein (mg/dl)	PKD/Mhm (Cy/+)	62.0 ± 1.7	**65.3 ± 2.7‡‡**
	PCK	62.2 ± 1.8	69.5 ± 2.1

Table 4.2 Plasma biochemistry in PKD/Mhm (Cy/+) and PCK rats (n=6 in each group) at baseline and day 167. Data are shown as mean ± Std.Dev. Values significantly different (time point vs baseline) are indicated as **p<0.005, ***p=0.0001 and (PKD/Mhm (Cy/+) vs PCK) as ‡p<0.05, ‡‡p<0.005, ‡‡‡p=0.0001.

Aspartate-aminotransferase (AST) and alanine-aminotransferase (ALT) plasma concentration are useful parameter to assess a proper liver function. The levels of these parameters were increased, but more or less stable (**table 4.3**).

Time point		AST (U/l)	ALT (U/l)
Baseline	PKD/Mhm (Cy/+)	**88.3 ± 8.8 ‡**	**43.2 ± 6.3 ‡‡**
	PCK	134.0 ± 42.1	82.5 ± 20.4
Day25	PKD/Mhm (Cy/+)	89.3 ± 10.9	53.3 ± 6.2
	PCK	124.4 ± 40.4	69.3 ± 22.1
Day 53	PKD/Mhm (Cy/+)	113.3 ± 69.5	**58.5 ± 13.8 ‡**
	PCK	144.5 ± 36.3	90.0 ± 21.3
Day 81	PKD/Mhm (Cy/+)	92.5 ± 14.9	**57.8 ± 7.1‡**
	PCK	134.3 ± 46.1	90.3 ± 19.2
Day 109	PKD/Mhm (Cy/+)	81.8 ± 5.7	48.7 ± 6.5
	PCK	162.5 ± 84.6	77.5 ± 22.6
Day 137	PKD/Mhm (Cy/+)	138.5 ± 44.6	53.7 ± 8.9
	PCK	113.8 ± 33.3	66.5 ±34.9
Day 167	PKD/Mhm (Cy/+)	72.2 ± 12.4	48.8 ± 10.3
	PCK	135.2 ± 144.9	64.2 ± 53.9

Table 4.3 Changes in plasma AST and ALT in PKD/Mhm (Cy/+) and PCK rats (n=6 in each group). Data are shown as mean ± Std.Dev. Values significantly different (PKD/Mhm (Cy/+) vs PCK) are indicated as ‡p<0.05, ‡‡p<0.005.

Urine was collected overnight during 16 h in metabolic cages, every month before the start of each experiment. Besides diuresis, food and water intake were also measured using metabolic cages. **Table 4.4** shows the changes of these parameters during the time, in both strains.

Time point	Strain	Diuresis (ml)	Food intake (g)	Water intake (ml)
Baseline	PKD/Mhm (Cy/+)	20.3 ± 4.9	15.9 ± 7.0	36.6 ± 8.9
	PCK	16.1 ± 3.1	21.8 ± 5.6	33.8 ± 2.9
Day25	PKD/Mhm (Cy/+)	**29.4 ± 3.5‡**	17.9 ± 2.1	**53.0 ± 9.3‡‡**
	PCK	21.7 ± 4.4	17.4 ± 5.0	37.7 ± 4.5
Day 53	PKD/Mhm (Cy/+)	**26.8 ± 7.4‡**	16.7 ± 3.0	**47.2 ± 10.5‡**
	PCK	17.6 ± 5.0	17.0 ± 3.9	31.3 ± 3.6
Day 81	PKD/Mhm (Cy/+)	**31.2 ± 11.2‡**	13.0 ± 6.6	50.2 ± 29.1
	PCK	18.1 ± 2.7	**14.6 ± 2.6***	26.2 ± 6.4
Day 109	PKD/Mhm (Cy/+)	**44.6 ± 10.4‡**	17.8 ± 5.9	**60.0 ± 25.1‡**
	PCK	44.6 ± 21.6	**14.1 ± 2.7***	32.3 ± 6.4
Day 137	PKD/Mhm (Cy/+)	**44.6 ± 10.4‡‡**	17.6 ± 5.1	**55.5 ± 13.3‡‡**
	PCK	21.4 ± 5.5	15.6 ± 0.7	24.0 ± 11.0
Day 167	PKD/Mhm (Cy/+)	55.8 ± 54.5	12.3 ± 4.3	**56.0 ± 15.6‡**
	PCK	**25.3 ± 2.7****	**10.8 ± 3.5****	33.2 ± 4.4

Table 4.4 Changes in diuresis, food intake and water intake after metabolic cages in PKD/Mhm (Cy/+) and PCK rats (n=6 in each group). Values significantly different (time point vs baseline) are indicated as *p<0.05, **p<0.005 and (PKD/Mhm (Cy/+) vs PCK) as ‡p<0.05, ‡‡p<0.005.

All urinary parameters were normalized to the urine volume of the 16 hours. Urine parameters at baseline and day 167 are summarized in **Table 4.5**. In particular a remarkable increase, in both strains, of proteinuria and albumin levels was detected, in contrast with a decrease of glycosuria levels in PCK (for individual days see **appendix 2**).

Parameter	Strain	Baseline	Day 167
Creatinine (mg/16h)	PKD/Mhm (Cy/+)	8.3 ± 1.7	**16.9 ± 8.6****
	PCK	8.1 ± 1.1	**14.8 ± 3.1****
Urea (mg/16h)	PKD/Mhm (Cy/+)	572.9 ± 178.1	830.5 ± 376.6
	PCK	709.3 ± 120.7	681.6 ± 91.3
Na (mmol/16h)	PKD/Mhm (Cy/+)	1.6 ± 0.7	1.7 ± 1.4
	PCK	1.6 ± 0.6	1.3 ± 0.2
K (mmol/16h)	PKD/Mhm (Cy/+)	3.1 ± 1.0	4.2 ± 2.6
	PCK	3.9 ± 1.0	3.5 ± 0.4
Ca (mmol/16h)	PKD/Mhm (Cy/+)	**0.01 ± 0.01‡**	0.04 ± 0.04
	PCK	0.06 ± 0.04	0.08 ± 0.03
PO_4 (mmol/16h)	PKD/Mhm (Cy/+)	0.1 ± 0.1	0.1 ± 0.1
	PCK	0.1 ± 0.1	0.1 ± 0.1
Glucose (mg/16h)	PKD/Mhm (Cy/+)	**1.2 ± 0.4‡**	1.7 ± 1.7
	PCK	5.2 ± 1.9	1.9 ± 1.5
Protein (mg/16h)	PKD/Mhm (Cy/+)	10.6 ± 5.2	**154.5 ± 177.7‡**
	PCK	9.6 ± 4.9	**436.8 ± 101.5*****
Albumin (mg/16h)	PKD/Mhm (Cy/+)	**2.3 ± 1.6‡**	**103.9 ± 108.5****
	PCK	0.7 ± 0.3	**140.6 ± 71.2****

Table 4.5 Urine biochemistry in PKD/Mhm (Cy/+) and PCK rats (n=6 in each group) at baseline and day 167. Data are shown as mean ± Std.Dev. Values significantly different (time point vs baseline) are indicated as *p<0.05, **p<0.005, ***p=0.001 and (PKD/Mhm (Cy/+) vs PCK) as ‡p<0.05.

Kidney function was assessed via a transcutaneous GFR measurement. A 3-compartment model was used to analyse the ABZWCY-HβCD elimination curve. The analysis shows an impairment of kidney filtration capacity due to the progression of the disease. In both PKD/Mhm (Cy/+) and PCK rats the ABZWCY-HβCD $t_{1/2}$ values were significantly increased, compared to the baseline, already at day 109 respectively of 1.9 and 1.8-fold, and remained high till the end of the experiments (2.5-fold) indicating a decline of the renal function (**figure 4.6**, **table 4.6**).

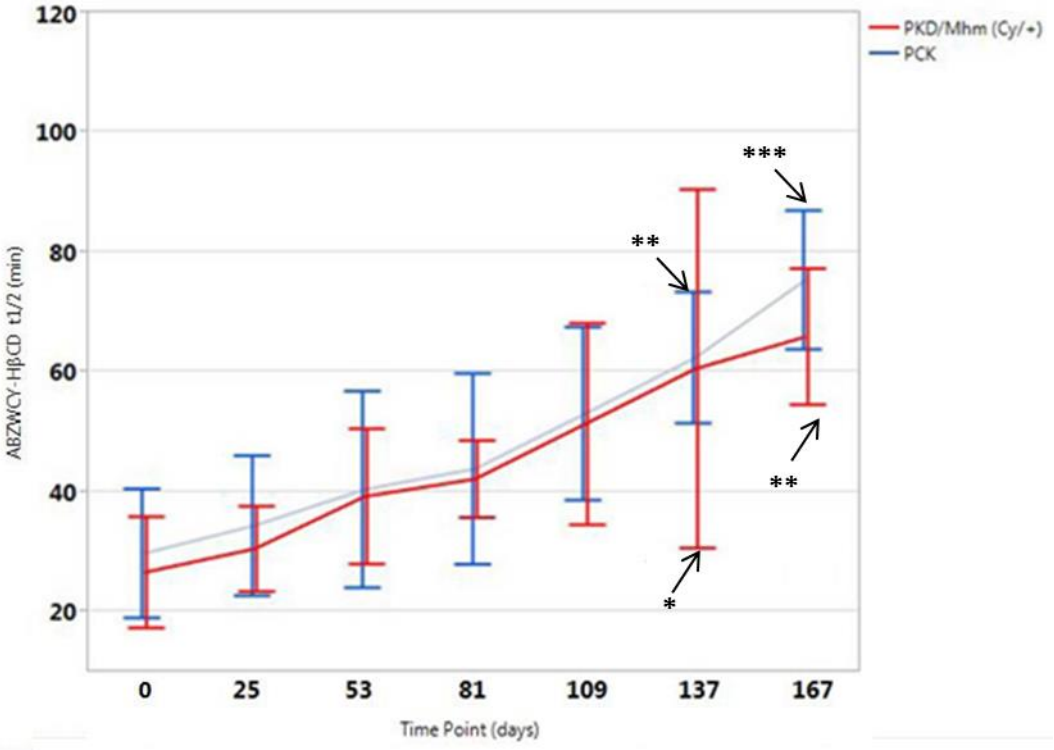

Figure 4.6 Alteration over the time in ABZWCY-HβCD $t_{½}$ levels in PKD/Mhm (Cy/+) and PCK rats (n=6 in each group). Data are shown as means ± Std.Dev. Values significantly different (time point vs baseline) are indicated as *p<0.05, **p<0.005, ***p=0.001.

Time point	Strain	ABZWCY-HβCD $t_{1/2}$ (min)
Baseline	PKD/Mhm (Cy/+)	26.7 ± 9.3
	PCK	29.9 ± 10.8
Day25	PKD/Mhm (Cy/+)	30.6 ± 7.1
	PCK	34.5 ± 11.7
Day 53	PKD/Mhm (Cy/+)	39.3 ± 11.3
	PCK	40.5 ± 16.4
Day 81	PKD/Mhm (Cy/+)	42.3 ± 6.4
	PCK	44.0 ± 15.9
Day 109	PKD/Mhm (Cy/+)	51.4 ± 16.8
	PCK	53.2 ± 14.4
Day 137	PKD/Mhm (Cy/+)	**60.7 ± 29.9***

Day 167	PCK	**62.5 ± 10.9****
	PKD/Mhm (Cy/+)	**66.0 ± 11.4****
	PCK	**75.5 ± 11.6*****

Table 4.6 Changes in ABZWCY-HβCD half-life in PKD/Mhm (Cy/+) and PCK rats (n=6 in each group). Data are shown as mean ± Std.Dev. Values significantly different (time point vs baseline) are indicated as *p<0.05, **p<0.005, ***p=0.001.

At day 167, all the variations of the main parameters of renal function in both animal models show a loss of the kidney function (**table 4.7**).

Parameter	Strain	Day 167
ABZWCY-HβCD $t_{1/2}$ (min)	PKD/Mhm (Cy/+)	66.0 ± 11.4
	PCK	75.5 ± 11.6
Creatinine (mg/dl)	PKD/Mhm (Cy/+)	**0.7 ± 0.1**‡‡
	PCK	0.5 ± 0.1
Urea (mg/dl)	PKD/Mhm (Cy/+)	**87.4 ± 11.0**‡‡
	PCK	56.6 ± 3.2

Table 4.7 ABZWCY-HβCD half-life, plasma creatinine and urea in PKD/Mhm (Cy/+) and PCK rats (n=6 in each group) at day 167. Data are shown as mean ± Std.Dev. Values significantly different (PKD/Mhm (Cy/+) vs PCK) are indicated as ‡‡p<0.005.

4.1.3 *Gene expression profiling*

After isolating the RNA (see section 3.7) gene expression analysis was performed.

The raw data from the RNAseq analyses were investigated in terms of differential expression compared to SD rats. From the 19239 genes identified on average in PKD/Mhm (Cy/+) and PCK, 11326 genes were significantly differentially expressed (adj.p < 0.05) in PKD/Mhm (Cy/+) (5490 upregulated and 5836 downregulated genes) and only 4602 genes were affected in PCK rats (2264 upregulated and 2338 downregulated genes). Venn diagrams in **figure 4.7** show that 1619 and 1817 genes were found in both strains respectively up- and downregulated.

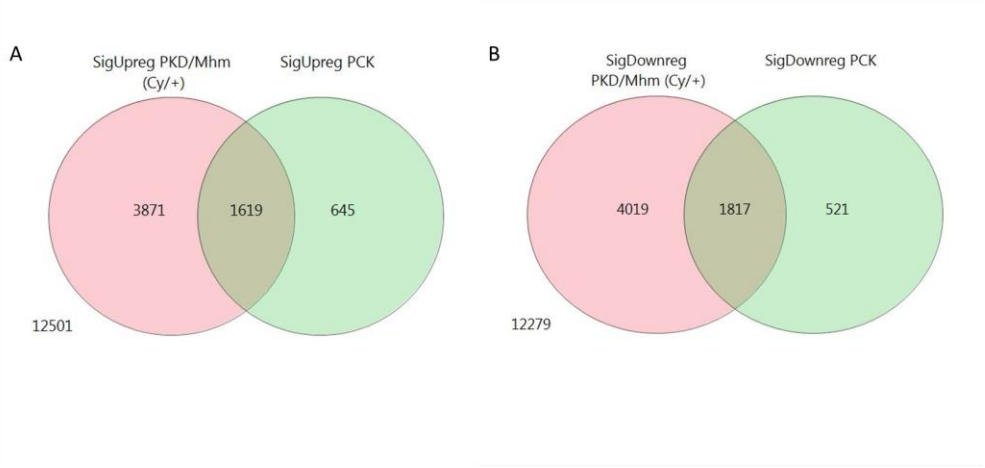

Figure 4.7 Venn diagrams of differentially expressed genes in PKD/Mhm (Cy/+) and PCK rats. A) Upregulated genes, B) Downregulated genes.

GSEA was performed by using the KEGG database. Genes were clustered in pathways and divided into 6 main categories, according to the KEGG database terminology. Significantly differentially expressed pathways were classified with a higher (or lower) normalized enrichment score (NES) (adj.p<0.05). The NES represents upregulated pathways with positive values and downregulated pathways with negative values. **Table 4.8** (**A-B**) and **figure 4.8** show the numbers and main categories of the up- and downregulated pathways.

A	Analysed pathways	305
	Significantly regulated pathways (adj. p-value < 0.05)	199
	Significantly upregulated pathways (adj. p-value < 0.05)	140
	Significantly downregulated pathways (adj. p-value < 0.05)	59

Table 4.8A Overview of GSEA displaying the numbers of up- and downregulated pathways in PKD/Mhm (Cy/+) rats

B	Analysed pathways	305
	Significantly regulated pathways (adj. p-value < 0.05)	156
	Significantly upregulated pathways (adj. p-value < 0.05)	119
	Significantly downregulated pathways (adj. p-value < 0.05)	37

Table 4.8B Overview of GSEA displaying the numbers of up- and downregulated pathways in PCK rats

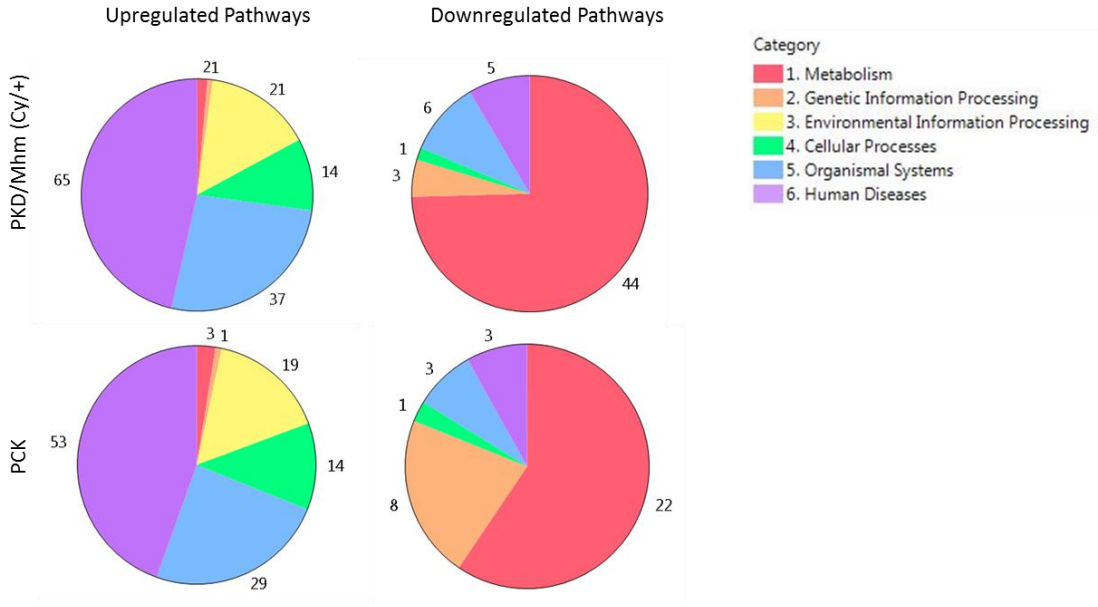

Figure 4.8 Pie charts showing the distribution of pathways containing significantly up- and downregulated genes sorted by main categories of KEGG database. The numbers around the pie charts indicate the number of up- and downregulated pathways of each category.

In both strains, pathways related to the metabolism were downregulated and, above all, a shift from the oxidative phosphorylation to the glycolysis process was detected (**table 4.9 A-B**). As evidence to support these results, **table 4.10** highlights the significantly differentially expressed genes, among all, involved in gluconeogenesis (Gpi, G6pc, Pgm1, Pgk1) and glycolysis (Hk2, Pfkp, Ldha).

A

KEGG Pathways	Main Category	Sub Category	NES PKD/Mhm (Cy/+)
Citrate cycle (TCA cycle)	Metabolism	1.1. Carbohydrate metabolism	-2.22
Ascorbate and aldarate metabolism	Metabolism	1.1. Carbohydrate metabolism	-2.03
Fatty acid degradation	Metabolism	1.3. Lipid metabolism	-2.56
Glycine, serine and threonine metabolism	Metabolism	1.5. Amino acid metabolism	-2.18
Cysteine and methionine metabolism	Metabolism	1.5. Amino acid metabolism	-1.96
Arginine and proline metabolism	Metabolism	1.5. Amino acid metabolism	-1.88
Tryptophan metabolism	Metabolism	1.5. Amino acid metabolism	-2.18
beta-Alanine metabolism	Metabolism	1.6. Metabolism of other amino acids	-2.24
Pyruvate metabolism	Metabolism	1.1. Carbohydrate metabolism	-2.23
Glyoxylate and dicarboxylate metabolism	Metabolism	1.1. Carbohydrate metabolism	-2.27
Propanoate metabolism	Metabolism	1.1. Carbohydrate metabolism	-2.59
Butanoate metabolism	Metabolism	1.1. Carbohydrate metabolism	-2.28
Glycolysis Gluconeogenesis	Metabolism	1.1. Carbohydrate metabolism	-1.78
Steroid hormone biosynthesis	Metabolism	1.3. Lipid metabolism	-1.82
Oxidative phosphorylation	Metabolism	1.2. Energy metabolism	-2.3
Valine, leucine and isoleucine degradation	Metabolism	1.5. Amino acid metabolism	-2.72
Glutathione metabolism	Metabolism	1.6. Metabolism of other amino acids	-1.92
Glycerolipid metabolism	Metabolism	1.3. Lipid metabolism	-1.77
Metabolism of xenobiotics by cytochrome P450	Metabolism	1.11. Xenobiotics biodegradation and metabolism	-1.98
Drug metabolism	Metabolism	1.11. Xenobiotics biodegradation and metabolism	-2.1
Drug metabolism	Metabolism	1.11. Xenobiotics biodegradation and metabolism	-1.84
Carbon metabolism	Metabolism	1.0 Global and overview maps	-2.54
Fatty acid metabolism	Metabolism	1.0 Global and overview maps	-2.32
Biosynthesis of amino acids	Metabolism	1.0 Global and overview maps	-1.93
Fatty acid elongation	Metabolism	1.3. Lipid metabolism	-1.99
Glycosaminoglycan biosynthesis	Metabolism	1.7. Glycan biosynthesis and metabolism	1.81
Steroid biosynthesis	Metabolism	1.3. Lipid metabolism	-1.91
Porphyrin and chlorophyll metabolism	Metabolism	1.8. Metabolism of cofactors and vitamins	-1.77
2-Oxocarboxylic acid metabolism	Metabolism	1.0 Global and overview maps	-1.87
Nicotinate and nicotinamide metabolism	Metabolism	1.8. Metabolism of cofactors and vitamins	-1.79
Selenocompound metabolism	Metabolism	1.6. Metabolism of other amino acids	-1.86
Pantothenate and CoA biosynthesis	Metabolism	1.8. Metabolism of cofactors and vitamins	-1.85
Biosynthesis of unsaturated fatty acids	Metabolism	1.3. Lipid metabolism	-1.78
Histidine metabolism	Metabolism	1.5. Amino acid metabolism	-1.75
Metabolic pathways	Metabolism	1.0 Global and overview maps	-2.25
Fructose and mannose metabolism	Metabolism	1.1. Carbohydrate metabolism	-1.67
Pentose and glucuronate interconversions	Metabolism	1.1. Carbohydrate metabolism	-1.67
Alanine, aspartate and glutamate metabolism	Metabolism	1.5. Amino acid metabolism	-1.67
Glycosaminoglycan degradation	Metabolism	1.7. Glycan biosynthesis and metabolism	1.62
One carbon pool by folate	Metabolism	1.8. Metabolism of cofactors and vitamins	-1.65
Terpenoid backbone biosynthesis	Metabolism	1.9. Metabolism of terpenoids and polyketides	-1.62
Folate biosynthesis	Metabolism	1.8. Metabolism of cofactors and vitamins	-1.6
Retinol metabolism	Metabolism	1.8. Metabolism of cofactors and vitamins	-1.39
Galactose metabolism	Metabolism	1.1. Carbohydrate metabolism	-1.56
Glycerophospholipid metabolism	Metabolism	1.3. Lipid metabolism	-1.34
Amino sugar and nucleotide sugar metabolism	Metabolism	1.1. Carbohydrate metabolism	-1.47

Table 4.9A GSEA analysis using KEGG database sorted by main category**.** Significantly (adj. p<0.05) differentially expressed pathways (PKD/Mhm (Cy/+) vs SD). For each pathway the NES is given. Downregulated pathways are displayed in green, upregulated pathways are displayed in red

B

KEGG Pathways	Main_Category	Sub_Category	NES PCK
Glycosaminoglycan degradation	Metabolism	1.7. Glycan biosynthesis and metabolism	2.19
Other glycan degradation	Metabolism	1.7. Glycan biosynthesis and metabolism	2.11
Glycosaminoglycan biosynthesis	Metabolism	1.7. Glycan biosynthesis and metabolism	1.58
Metabolism of xenobiotics by cytochrome P450	Metabolism	1.11. Xenobiotics biodegradation and metabolism	-1.47
Arginine and proline metabolism	Metabolism	1.5. Amino acid metabolism	-1.48
Drug metabolism	Metabolism	1.11. Xenobiotics biodegradation and metabolism	-1.5
Fatty acid metabolism	Metabolism	1.0 Global and overview maps	-1.58
Glycerophospholipid metabolism	Metabolism	1.3. Lipid metabolism	-1.59
2-Oxocarboxylic acid metabolism	Metabolism	1.0 Global and overview maps	-1.61
Cysteine and methionine metabolism	Metabolism	1.5. Amino acid metabolism	-1.62
Ether lipid metabolism	Metabolism	1.3. Lipid metabolism	-1.62
Citrate cycle (TCA cycle)	Metabolism	1.1. Carbohydrate metabolism	-1.63
Nicotinate and nicotinamide metabolism	Metabolism	1.8. Metabolism of cofactors and vitamins	-1.65
Steroid biosynthesis	Metabolism	1.3. Lipid metabolism	-1.7
Selenocompound metabolism	Metabolism	1.6. Metabolism of other amino acids	-1.74
Metabolic pathways	Metabolism	1.0 Global and overview maps	-1.75
Glutathione metabolism	Metabolism	1.6. Metabolism of other amino acids	-1.79
Carbon metabolism	Metabolism	1.0 Global and overview maps	-1.81
Propanoate metabolism	Metabolism	1.1. Carbohydrate metabolism	-1.84
Glycerolipid metabolism	Metabolism	1.3. Lipid metabolism	-1.86
Glyoxylate and dicarboxylate metabolism	Metabolism	1.1. Carbohydrate metabolism	-1.94
Pyruvate metabolism	Metabolism	1.1. Carbohydrate metabolism	-2.03
Oxidative phosphorylation	Metabolism	1.2. Energy metabolism	-2.32
Fatty acid degradation	Metabolism	1.3. Lipid metabolism	-2.33
Valine, leucine and isoleucine degradation	Metabolism	1.5. Amino acid metabolism	-2.43

Table 4.9B GSEA analysis using KEGG database sorted by main category**.** Significantly (adj. p<0.05) differentially expressed pathways (PCK vs SD). For each pathway the NES is given. Downregulated pathways are displayed in green, upregulated pathways are displayed in red.

SYMBOL	GENE NAME	logFC PKD/Mhm (Cy/+)	log FC PCK
Gpi	glucose-6-phosphate isomerase	0.04	-0.10
G6pc	glucose-6-phosphatase, catalytic subunit	-1.45	0.50
Pgm1	phosphoglucomutase 1	-0.76	-0.07
Pgk1	phosphoglycerate kinase 1	-0.51	0.01
Hk2	hexokinase 2	0.16	-0.01
Pfkp	phosphofructokinase, platelet	-0.29	-0.29
Ldha	lactate dehydrogenase A	-0.36	0.19

Table 4.10 List of significantly differentially regulated genes in PKD/Mhm (Cy/+) and PCK rats. Significantly (adj. p<0.05) differentially regulated genes (model vs SD). Negative log fold changes (logFC) are displayed in green, positive logFC are displayed in red.

Proteasome and ribosome pathways were downregulated in the PCK model. Protein processing pathway was downregulated both in PKD/Mhm (Cy/+) and PCK models, while DNA replication was upregulated (**table 4.11 A-B**).

A

KEGG Pathways	Main Category	Sub Category	NES PKD/Mhm (Cy/+)
Aminoacyl-tRNA biosynthesis	Genetic Information Processing	2.2. Translation	-1.9
SNARE interactions in vesicular transport	Genetic Information Processing	2.3. Folding, sorting and degradation	-1.68
Protein export	Genetic Information Processing	2.3. Folding, sorting and degradation	-1.65
DNA replication	Genetic Information Processing	2.4. Replication and repair	1.5

Table 4.11A GSEA analysis using KEGG database sorted by main category**.** Significantly (adj. p<0.05) differentially expressed pathways (PKD/Mhm (Cy/+) vs SD). For each pathway the NES is given. Downregulated pathways are displayed in green, upregulated pathways are displayed in red.

B

KEGG Pathways	Main_Category	Sub_Category	NES PCK
DNA replication	Genetic Information Processing	2.4. Replication and repair	1.62
Ribosome biogenesis in eukaryotes	Genetic Information Processing	2.2. Translation	-1.47
RNA degradation	Genetic Information Processing	2.3. Folding, sorting and degradation	-1.5
Proteasome	Genetic Information Processing	2.3. Folding, sorting and degradation	-1.51
RNA_polymerase	Genetic Information Processing	2.1. Transcription	-1.65
Ribosome	Genetic Information Processing	2.2. Translation	-1.84
Protein processing in endoplasmic reticulum	Genetic Information Processing	2.3. Folding, sorting and degradation	-1.87
Protein export	Genetic Information Processing	2.3. Folding, sorting and degradation	-1.92
Aminoacyl-tRNA biosynthesis	Genetic Information Processing	2.2. Translation	-2.34

Table 4.11B GSEA analysis using KEGG database sorted by main category**.** Significantly (adj. p<0.05) differentially expressed pathways (PCK vs SD). For each pathway the NES is given. Downregulated pathways are displayed in green, upregulated pathways are displayed in red.

Upregulation of signal transduction pathways was detected in PKD/Mhm (Cy/+) model (**table 4.12A**), likewise in PCK (**table 4.12B**). Also pro-inflammatory mediators such as tumor necrosis factor (TNF)-α and nuclear factor (NF)-κB were upregulated, mainly in the PKD/Mhm (Cy/+) model, as well Janus kinase (JAK)1 and 2 (**table 4.12C**) implying the upregulation of JAK-STAT, NF-κB and TNF signaling pathways.

A

KEGG Pathways	Main Category	Sub Category	NES PKD/Mhm (Cy/+)
MAPK signaling pathway	Environmental Information Processing	3.2. Signal transduction	1.86
Ras signaling pathway	Environmental Information Processing	3.2. Signal transduction	1.72
Rap1 signaling pathway	Environmental Information Processing	3.2. Signal transduction	1.66
Cytokine-cytokine receptor interaction	Environmental Information Processing	3.3. Signaling molecules and interaction	2.1
NF-kappa B signaling pathway	Environmental Information Processing	3.2. Signal transduction	2.19
HIF-1 signaling pathway	Environmental Information Processing	3.2. Signal transduction	1.84
PI3K-Akt signaling pathway	Environmental Information Processing	3.2. Signal transduction	1.96
Notch signaling pathway	Environmental Information Processing	3.2. Signal transduction	1.93
ECM-receptor interaction	Environmental Information Processing	3.3. Signaling molecules and interaction	2.23
Cell adhesion molecules (CAMs)	Environmental Information Processing	3.3. Signaling molecules and interaction	2.32
JAK-STAT signaling pathway	Environmental Information Processing	3.2. Signal transduction	2.07
TNF signaling pathway	Environmental Information Processing	3.2. Signal transduction	2.11
ErbB signaling pathway	Environmental Information Processing	3.2. Signal transduction	1.72
Phospholipase D signaling pathway	Environmental Information Processing	3.2. Signal transduction	1.7
Apelin signaling pathway	Environmental Information Processing	3.2. Signal transduction	1.67
cGMP-PKG signaling pathway	Environmental Information Processing	3.2. Signal transduction	1.61
cAMP signaling pathway	Environmental Information Processing	3.2. Signal transduction	1.58
Wnt signaling pathway	Environmental Information Processing	3.2. Signal transduction	1.5
Hippo signaling pathway	Environmental Information Processing	3.2. Signal transduction	1.48
Sphingolipid signaling pathway	Environmental Information Processing	3.2. Signal transduction	1.46
FoxO signaling pathway	Environmental Information Processing	3.2. Signal transduction	1.44

Table 4.12A GSEA analysis using KEGG database sorted by main category**.** Significantly (adj. p<0.05) differentially expressed pathways (PKD/Mhm (Cy/+) vs SD). For each pathway the NES is given. Downregulated pathways are displayed in green, upregulated pathways are displayed in red.

B

KEGG Pathways	Main_Category	Sub_Category	NES PCK
Cytokine-cytokine receptor interaction	Environmental Information Processing	3.3. Signaling molecules and interaction	2.06
JAK-STAT signaling pathway	Environmental Information Processing	3.2. Signal transduction	2
ECM-receptor interaction	Environmental Information Processing	3.3. Signaling molecules and interaction	1.99
PI3K-Akt signaling pathway	Environmental Information Processing	3.2. Signal transduction	1.94
Cell adhesion molecules (CAMs)	Environmental Information Processing	3.3. Signaling molecules and interaction	1.9
ErbB signaling pathway	Environmental Information Processing	3.2. Signal transduction	1.88
MAPK signaling pathway	Environmental Information Processing	3.2. Signal transduction	1.78
TNF signaling pathway	Environmental Information Processing	3.2. Signal transduction	1.78
Sphingolipid signaling pathway	Environmental Information Processing	3.2. Signal transduction	1.75
Rap1 signaling pathway	Environmental Information Processing	3.2. Signal transduction	1.71
Phospholipase D signaling pathway	Environmental Information Processing	3.2. Signal transduction	1.7
NF-kappa B signaling pathway	Environmental Information Processing	3.2. Signal transduction	1.69
Hedgehog signaling pathway	Environmental Information Processing	3.2. Signal transduction	1.68
Ras signaling pathway	Environmental Information Processing	3.2. Signal transduction	1.66
Apelin signaling pathway	Environmental Information Processing	3.2. Signal transduction	1.61
FoxO signaling pathway	Environmental Information Processing	3.2. Signal transduction	1.61
HIF-1 signaling pathway	Environmental Information Processing	3.2. Signal transduction	1.6
Wnt signaling pathway	Environmental Information Processing	3.2. Signal transduction	1.43
cGMP-PKG signaling pathway	Environmental Information Processing	3.2. Signal transduction	1.37

Table 4.12B GSEA analysis using KEGG database sorted by main category**.** Significantly (adj. p<0.05) differentially expressed pathways (PCK vs SD). For each pathway the NES is given. Downregulated pathways are displayed in green, upregulated pathways are displayed in red.

C

SYMBOL	GENENAME	logFC PKD/Mhm (Cy/+)	log FC PCK
Nfkb1	nuclear factor kappa B subunit 1	0.66	0.12
Nfkb2	nuclear factor kappa B subunit 2	0.69	0.05
Tnf	tumor necrosis factor	0.06	0.02
Tnfaip1	TNF alpha induced protein 1	0.35	0.04
Jak1	Janus kinase 1	0.63	0.11
Jak2	Janus kinase 2	0.29	0.12
Jak3	Janus kinase 3	0.49	0.05

Table 4.12C List of significantly differentially regulated genes in PKD/Mhm (Cy/+) and PCK rats. Significantly (adj. p<0.05) differentially regulated genes (model vs SD). Positive logFC are displayed in red.

As expected, an upregulation of cell growth and proliferation pathways was seen in both PKD/Mhm (Cy/+) (**table 4.13A**) and PCK (**table 4.13B**) rats.

A

KEGG Pathways	Main Category	Sub Category	NES PKD/Mhm (Cy/+)
Endocytosis	Cellular Processes	4.1. Transport and catabolism	1.63
Phagosome	Cellular Processes	4.1. Transport and catabolism	1.9
Apoptosis	Cellular Processes	4.2. Cell growth and death	1.84
Necroptosis	Cellular Processes	4.2. Cell growth and death	1.81
Cellular senescence	Cellular Processes	4.2. Cell growth and death	1.71
Focal adhesion	Cellular Processes	4.3. Cellular community - eukaryotes	2.1
Adherens junction	Cellular Processes	4.3. Cellular community - eukaryotes	2.08
Gap junction	Cellular Processes	4.3. Cellular community - eukaryotes	1.85
Regulation of actin cytoskeleton	Cellular Processes	4.5. Cell motility	1.95
Peroxisome	Cellular Processes	4.1. Transport and catabolism	-2.56
Lysosome	Cellular Processes	4.1. Transport and catabolism	1.67
Cell cycle	Cellular Processes	4.2. Cell growth and death	1.56
Signaling pathways regulating pluripotency of stem cells	Cellular Processes	4.3. Cellular community - eukaryotes	1.45
Tight junction	Cellular Processes	4.3. Cellular community - eukaryotes	1.39
p53 signaling pathway	Cellular Processes	4.2. Cell growth and death	1.45

Table 4.13A GSEA analysis using KEGG database sorted by main category**.** Significantly (adj. p<0.05) differentially expressed pathways (PKD/Mhm (Cy/+) vs SD). For each pathway the NES is given. Downregulated pathways are displayed in green, upregulated pathways are displayed in red.

B

KEGG Pathways	Main_Category	Sub_Category	NES PCK
Regulation of actin cytoskeleton	Cellular Processes	4.5. Cell motility	2.3
Focal adhesion	Cellular Processes	4.3. Cellular community - eukaryotes	2.19
Lysosome	Cellular Processes	4.1. Transport and catabolism	2.14
Adherens junction	Cellular Processes	4.3. Cellular community - eukaryotes	2.01
Gap junction	Cellular Processes	4.3. Cellular community - eukaryotes	1.97
Endocytosis	Cellular Processes	4.1. Transport and catabolism	1.85
Cell cycle	Cellular Processes	4.2. Cell growth and death	1.8
Phagosome	Cellular Processes	4.1. Transport and catabolism	1.77
p53 signaling pathway	Cellular Processes	4.2. Cell growth and death	1.77
Apoptosis	Cellular Processes	4.2. Cell growth and death	1.65
Cellular senescence	Cellular Processes	4.2. Cell growth and death	1.64
Ferroptosis	Cellular Processes	4.2. Cell growth and death	1.6
Autophagy	Cellular Processes	4.1. Transport and catabolism	1.44
Necroptosis	Cellular Processes	4.2. Cell growth and death	1.41
Peroxisome	Cellular Processes	4.1. Transport and catabolism	-2.15

Table 4.13B GSEA analysis using KEGG database sorted by main category**.** Significantly (adj. p<0.05) differentially expressed pathways (PCK vs SD). For each pathway the NES is given. Downregulated pathways are displayed in green, upregulated pathways are displayed in red.

Inflammatory pathways, such as complement cascade and chemokine signaling pathways, were upregulated in both strains, while PPAR signaling pathway was downregulated either in PKD/Mhm (Cy/+) or PCK (**table 4.14 A-B**).

A

KEGG Pathways	Main Category	Sub Category	NES PCK/Mhm (Cy/+)
Chemokine_signaling_pathway	Organismal Systems	5.1. Immune system	2.09
Axon_guidance	Organismal Systems	5.8. Development	1.88
Osteoclast_differentiation	Organismal Systems	5.8. Development	2.3
Complement_and_coagulation_cascades	Organismal Systems	5.1. Immune system	1.86
Platelet_activation	Organismal Systems	5.1. Immune system	1.92
Antigen_processing_and_presentation	Organismal Systems	5.1. Immune system	2.23
Toll-like_receptor_signaling_pathway	Organismal Systems	5.1. Immune system	2.05
NOD-like_receptor_signaling_pathway	Organismal Systems	5.1. Immune system	1.83
C-type_lectin_receptor_signaling_pathway	Organismal Systems	5.1. Immune system	1.92
Hematopoietic_cell_lineage	Organismal Systems	5.1. Immune system	2.17
Natural_killer_cell_mediated_cytotoxicity	Organismal Systems	5.1. Immune system	2.12
Th1_and_Th2_cell_differentiation	Organismal Systems	5.1. Immune system	2.12
Th17_cell_differentiation	Organismal Systems	5.1. Immune system	2.2
B_cell_receptor_signaling_pathway	Organismal Systems	5.1. Immune system	1.95
Fc_gamma_R-mediated_phagocytosis	Organismal Systems	5.1. Immune system	2.14
Leukocyte_transendothelial_migration	Organismal Systems	5.1. Immune system	1.9
Intestinal_immune_network_for_IgA_production	Organismal Systems	5.1. Immune system	1.97
Neurotrophin_signaling_pathway	Organismal Systems	5.6. Nervous system	1.86
Relaxin_signaling_pathway	Organismal Systems	5.2. Endocrine system	1.76
T_cell_receptor_signaling_pathway	Organismal Systems	5.1. Immune system	1.71
Oxytocin_signaling_pathway	Organismal Systems	5.2. Endocrine system	1.69
Parathyroid_hormone_synthesis,_secretion_and_action	Organismal Systems	5.2. Endocrine system	1.72
PPAR_signaling_pathway	Organismal Systems	5.2. Endocrine system	-1.78
Thermogenesis	Organismal Systems	5.10. Environmental adaptation	-1.59
Prolactin_signaling_pathway	Organismal Systems	5.2. Endocrine system	1.69
GnRH_signaling_pathway	Organismal Systems	5.2. Endocrine system	1.64
Estrogen_signaling_pathway	Organismal Systems	5.2. Endocrine system	1.57
Cholinergic_synapse	Organismal Systems	5.6. Nervous system	1.55
Thyroid_hormone_signaling_pathway	Organismal Systems	5.2. Endocrine system	1.56
Fc_epsilon_RI_signaling_pathway	Organismal Systems	5.1. Immune system	1.61
Circadian_entrainment	Organismal Systems	5.10. Environmental adaptation	1.54
Salivary_secretion	Organismal Systems	5.4. Digestive system	1.55
Vascular_smooth_muscle_contraction	Organismal Systems	5.3. Circulatory system	1.46
Aldosterone_synthesis_and_secretion	Organismal Systems	5.2. Endocrine system	1.5
IL-17_signaling_pathway	Organismal Systems	5.1. Immune system	1.49
Insulin_signaling_pathway	Organismal Systems	5.2. Endocrine system	1.42
Collecting_duct_acid_secretion	Organismal Systems	5.5. Excretory system	-1.63
Cytosolic_DNA-sensing_pathway	Organismal Systems	5.1. Immune system	1.51
Insulin_secretion	Organismal Systems	5.2. Endocrine system	1.45
Glutamatergic_synapse	Organismal Systems	5.6. Nervous system	1.39
Synaptic_vesicle_cycle	Organismal Systems	5.6. Nervous system	-1.37
Aldosterone-regulated_sodium_reabsorption	Organismal Systems	5.5. Excretory system	-1.49
Retrograde_endocannabinoid_signaling	Organismal Systems	5.6. Nervous system	-1.27

Table 4.14A GSEA analysis using KEGG database sorted by main category**.** Significantly (adj. p<0.05) differentially expressed pathways (PKD/Mhm (Cy/+) vs SD). For each pathway the NES is given. Downregulated pathways are displayed in green, upregulated pathways are displayed in red.

B

KEGG Pathways	Main_Category	Sub_Category	NES PCK
Complement and coagulation cascades	Organismal Systems	5.1. Immune system	2.27
Natural killer cell mediated cytotoxicity	Organismal Systems	5.1. Immune system	2.25
Chemokine signaling pathway	Organismal Systems	5.1. Immune system	2.23
B cell receptor signaling pathway	Organismal Systems	5.1. Immune system	2.17
Fc gamma R-mediated phagocytosis	Organismal Systems	5.1. Immune system	2.17
Leukocyte transendothelial migration	Organismal Systems	5.1. Immune system	2.17
Hematopoietic cell lineage	Organismal Systems	5.1. Immune system	2.15
Osteoclast differentiation	Organismal Systems	5.8. Development	2.06
Neurotrophin signaling pathway	Organismal Systems	5.6. Nervous system	2.01
Th17 cell differentiation	Organismal Systems	5.1. Immune system	1.98
Relaxin signaling pathway	Organismal Systems	5.2. Endocrine system	1.9
C-type lectin receptor signaling pathway	Organismal Systems	5.1. Immune system	1.87
Platelet activation	Organismal Systems	5.1. Immune system	1.86
Th1 and Th2 cell differentiation	Organismal Systems	5.1. Immune system	1.82
Intestinal immune network for IgA production	Organismal Systems	5.1. Immune system	1.81
Prolactin signaling pathway	Organismal Systems	5.2. Endocrine system	1.8
Toll-like receptor signaling pathway	Organismal Systems	5.1. Immune system	1.78
GnRH signaling pathway	Organismal Systems	5.2. Endocrine system	1.72
Fc gamma R-mediated phagocytosis	Organismal Systems	5.1. Immune system	1.69
Oxytocin signaling pathway	Organismal Systems	5.2. Endocrine system	1.63
Parathyroid hormone synthesis, secretion and action	Organismal Systems	5.2. Endocrine system	1.6
Cholinergic synapse	Organismal Systems	5.6. Nervous system	1.53
Estrogen signaling pathway	Organismal Systems	5.2. Endocrine system	1.52
Thyroid hormone signaling pathway	Organismal Systems	5.2. Endocrine system	1.52
T cell receptor signaling pathway	Organismal Systems	5.1. Immune system	1.51
Insulin signaling pathway	Organismal Systems	5.2. Endocrine system	1.5
Renin secretion	Organismal Systems	5.2. Endocrine system	1.5
NOD-like receptor signaling pathway	Organismal Systems	5.1. Immune system	1.49
Antigen processing and presentation	Organismal Systems	5.1. Immune system	1.43
PPAR signaling pathway	Organismal Systems	5.2. Endocrine system	-1.46
Thermogenesis	Organismal Systems	5.10. Environmental adaptation	-1.87
Proximal tubule bicarbonate reclamation	Organismal Systems	5.5. Excretory system	-1.92

Table 4.14B GSEA analysis using KEGG database sorted by main category**.** Significantly (adj. p<0.05) differentially expressed pathways (PCK vs SD). For each pathway the NES is given. Downregulated pathways are displayed in green, upregulated pathways are displayed in red.

4.2 Long term effects of stem cells and conditioned media therapy in PKD/Mhm (Cy/+) rats

4.2.1 *Changes in BW and kidney histology*

BW of treated PKD/Mhm (Cy/+) groups was monitored monthly. As outlined for the untreated group, a constant rise of the BW was recorded in the treated groups but no significant differences were observed between them (**figure 4.9**). Moreover, no differences of left kidney weight or Kw/BW ratio were noted among all the groups (**table 4.15 A-B**).

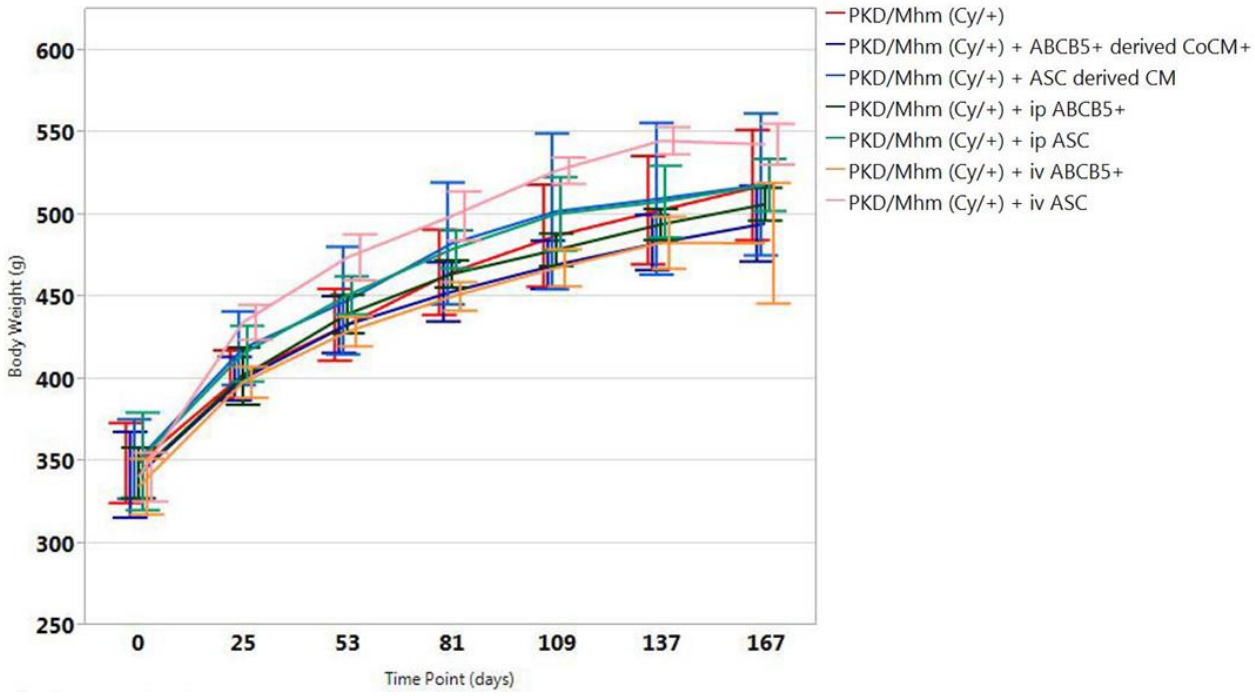

Figure 4.9 Effect on BW of different treatments in PKD/Mhm (Cy/+) (n=6 in each group). Data are shown as means ± Std.Dev.

A	Animal group	Left kidney (g)	Kw/BW ratio
	Untreated	3.1 ± 0.8	0.6 ± 0.1
	+ ABCB5+ derived CoCM+	2.8 ± 0.6	0.6 ± 0.1
	+ i.p. ABCB5+	2.8 ± 0.5	0.6 ± 0.1
	+ i.v. ABCB5+	2.6 ± 0.5	0.5 ± 0.1

Table 4.15A Effect of different treatments on left kidney weight and Kw/BW ratio in PKD/Mhm (Cy/+) groups (n=6 in each group). Comparison between ABCB5+ derived CoCM+, i.p. or i.v. ABCB5+ groups and untreated. Data are shown as mean ± Std.Dev.

B	Animal group	Left kidney (g)	Kw/BW ratio
	Untreated	3.1 ± 0.8	0.6 ± 0.1
	+ ASC derived CM	3.1 ± 0.1	0.6 ± 0.1
	+ i.p. ASC	3.0 ± 0.1	0.6 ± 0.0
	+ i.v. ASC	3.2 ± 0.3	0.6 ± 0.1

Table 4.15B Effect of different treatments on left kidney weight and Kw/BW ratio in PKD/Mhm (Cy/+) groups (n=6 in each group). Comparison between ASC derived CM, i.p. or i.v. ASC groups and untreated Data are shown as mean ± Std.Dev.

H&E and Masson-Goldner trichrome stainings were performed on 3 µm kidney sections. We noted a decrease of cyst number in the i.p. ABCB5+ and ASC derived CM groups ($p<0.05$) and, in the same groups a proportional reduction of the percentage of renal tissue covered by them. A reduction of the percentage of cyst area was observed also in i.v. ABCB5+ and

ABCB5+ derived CoCM+ groups. However, in i.p. and i.v. ABCB5+ and ABCB5+ derived CoCM+ groups, a rise of the average cyst size was registered (**table 4.16A**).

Masson-Goldner staining allowed the quantification of fibrotic tissue on the whole kidney section. All the treated groups presented an increase of the fibrotic tissue when compared to the untreated group, especially i.p. or i.v. ABCB5+ and ABCB5+ derived CoCM+ groups (**table 4.16 A-B**, **figure 4.10**).

Masson-Goldner staining was performed also on 3 μm liver sections. The percentage of fibrosis was only 1.2 ± 0.4 in the untreated group and no differences were noted in the comparison between treated and untreated groups. Indeed, the percentage of cyst area in all the groups was lower than 0.1±0.1 (data not shown).

A

Animal group	Cyst number	Average size (pixels)	% cyst area	% fibrosis
Untreated	4210.6 ± 500.0	426.2 ± 79.0	18.1 ± 3.3	7.9 ± 3.3
+ ABCB5+ derived CoCM+	1682.7 ± 1305.9	648.6 ± 257.1	9.8 ± 5.0	21.9 ± 12.1
+ i.p. ABCB5+	**1072.0 ± 788.8***	826.9 ± 159.8	8.6 ± 5.0	20.4 ± 9.2
+ i.v. ABCB5+	1396.3 ± 1532.3	679.2 ± 232.8	7.9 ± 5.6	16.3 ± 8.3

Table 4.16A Effect of different treatments on cyst number, average size, percentage of cyst and fibrosis area in PKD/Mhm (Cy/+) groups (n=6 in each group). Comparison between ABCB5+ derived CoCM+, i.v. or i.p. ABCB5+ groups and untreated. Analysis was performed on whole kidney section (3μm). Data are shown as mean ± Std.Dev. Values significantly different (treated vs untreated) are indicated as * $p<0.05$.

B

Animal group	Cyst number	Average size (pixels)	% cyst area	% fibrosis
Untreated	4210.6 ± 500.0	426.2 ± 79.0	18.1 ± 3.3	7.9 ± 3.3
+ ASC derived CM	**2606.5 ± 919.9***	376.5 ± 81.3	12.3 ± 5.0	10.3 ± 2.3
+ i.p. ASC	4333.0 ± 713.7	402.0 ± 28.4	17.3 ± 2.1	9.6 ± 3.2
+ i.v. ASC	4117.2 ± 793.5	409.7 ± 59.5	16.2 ± 4.2	10.3 ± 2.8

Table 4.16B Effect of different treatments on cyst number, average size, percentage of cyst and fibrosis area in PKD/Mhm (Cy/+) groups (n=6 in each group). Comparison between ASC derived CM, i.v. or i.p. ASC groups and untreated. Analysis was performed on whole kidney section (3μm). Data are shown as mean ± Std.Dev. Values significantly different (treated vs untreated) are indicated as * $p<0.05$.

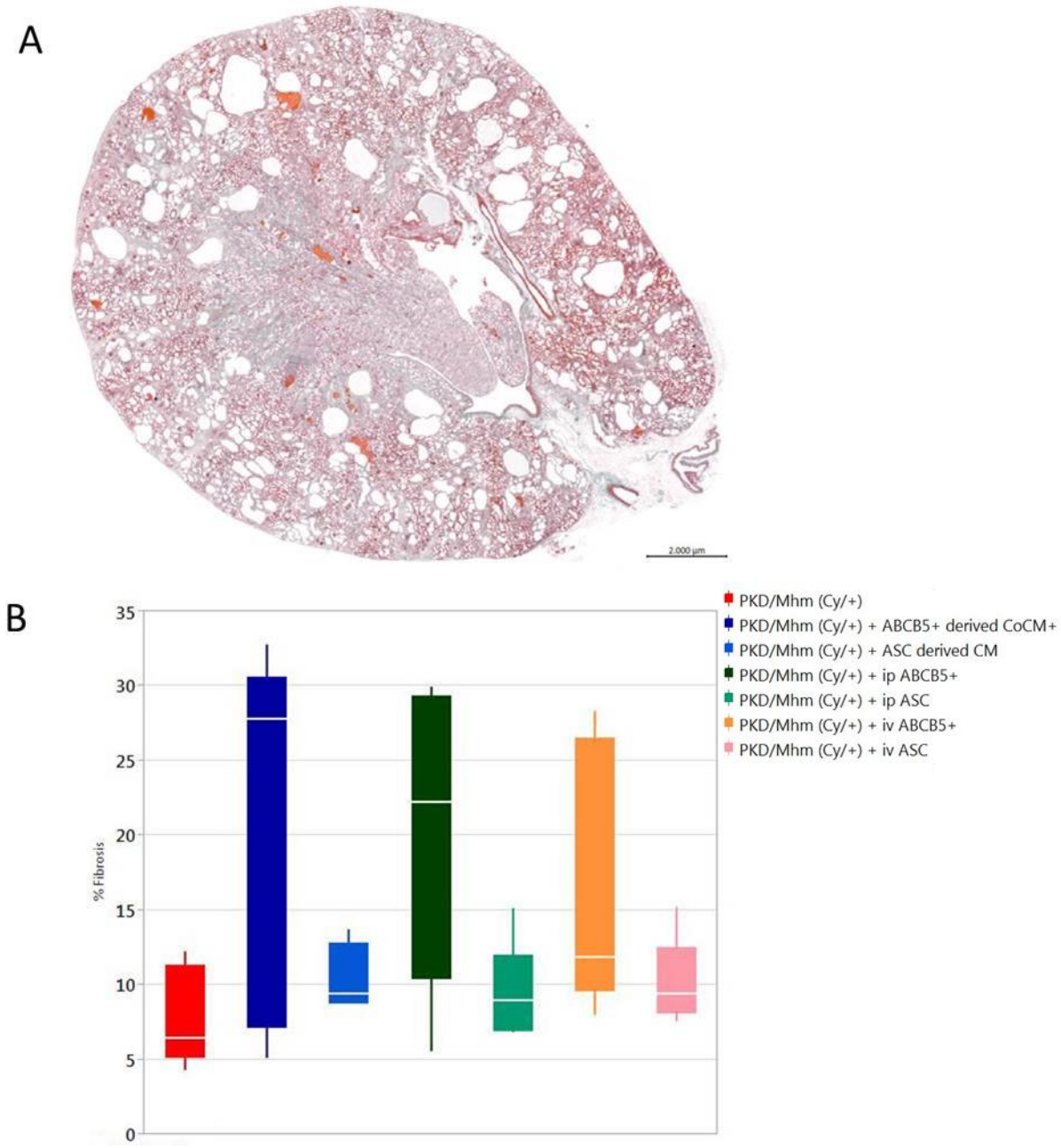

Figure 4.10 Fibrosis in PKD/Mhm (Cy/+) kidneys (n=6 in each group). A) Whole kidney scan of PKD/Mhm (Cy/+) rat, Masson-Goldner trichrome staining, light green stains fibrotic tissue; B) Graphic quantification of fibrosis after different treatments in PKD/Mhm (Cy/+) model. Data are shown as box plots with the median, upper and lower quartile (interquartile range (IQR)) and whiskers (1.5x IQR). Image acquired with Axio Scan.Z1 microscope (ZEISS), 20x objective.

The analysis of the co-staining of Tunel and Ki67 on whole kidney sections outlined a considerable reduction of apoptotic and proliferative positive cells in all treated groups (**table 4.17 A-B**).

A	Animal group	Tunel positive cells	Ki67 positive cells
	Untreated	1370.0 ± 237.7	1239.5 ± 250.7
	+ ABCB5+ derived CoCM+	**977.7 ± 133.3****	**900.2 ± 147.7***
	+ i.p. ABCB5+	**1058.3 ± 106.6***	**880.9 ± 112.0***
	+ i.v. ABCB5+	**1066.4 ± 84.7***	**948.2 ± 145.7***

Table 4.17A Effect of different treatments on Tunel and Ki67 positive nuclei in in PKD/Mhm (Cy/+) groups (n=6 in each group). Comparison between ABCB5+ derived CoCM+, i.p. or i.v. ABCB5+ groups and untreated. Analysis was performed on whole kidney section (3µm). Data are shown as mean ± Std.Dev. Values significantly different (treated vs untreated) are indicated as *p<0.05, ** p<0.005.

B	Animal group	Tunel positive cells	Ki67 positive cells
	Untreated	1370.0 ± 237.7	1239.5 ± 250.7
	+ ASC derived CM	974.5 ± 290.2	**781.0 ± 280.4***
	+ i.p. ASC	1001.4 ± 73.7	976.4 ± 78.3
	+ i.v. ASC	**984.0 ± 287.1***	**804.2 ± 271.5***

Table 4.17B Effect of different treatments on Tunel and Ki67 positive nuclei in in PKD/Mhm (Cy/+) groups (n=6 in each group). Comparison between ASC derived CM, i.p. or i.v. ASC groups and untreated. Analysis was performed on whole kidney section (3µm). Data are shown as mean ± Std.Dev. Values significantly different (treated vs untreated) are indicated as *p<0.05.

4.2.2 *Plasma chemistry, urine analysis and renal function*

From now on, only the results of day 167 will be shown. Further results are displayed in the appendix.

Plasma creatinine levels were reduced in both i.p. ABCB5+ and i.p. ASC groups while no variations were registered in all the others treated groups, when compared to the untreated. Conversely, conflicting results were noted concerning urea levels. In fact, the plasma levels slightly decreased in i.p. and i.v. ABCB5+ groups and increased in i.p. and i.v. ASC and ASC derived CM groups.

A decline of cholesterol and triglycerides levels were seen in i.p. and i.v. ABCB5+ and ASC and ABCB5+ derived CoCM+. The ASC derived CM group showed a reduction of triglycerides levels but no difference concerning the cholesterol levels. A minor decrease of glucose levels was recorded in all the treated groups, in particular in the i.p. ABCB5+ group. Sodium levels visibly decreased in i.p. and i.v. ABCB5+ and i.p. ASC groups ($p<0.05$). Calcium levels were decreased in i.p. ABCB5+ ($p<0.05$) while a significant increment of potassium was detected in i.p. ASC group ($p<0.05$). **Table 4.18** (**A-B**) illustrates the plasma parameters that were analysed for each group (for individual days see **appendix 3**).

A	Parameter	Animal group	Day 167
	Creatinine	Untreated	0.7 ± 0.1
	(mg/dl)	+ ABCB5+ derived CoCM+	0.7 ± 0.2
		+ i.p. ABCB5+	0.6 ±0.1
		+ i.v. ABCB5+	0.7 ± 0.1
	Urea	Untreated	87.4 ± 11.0
	(mg/dl)	+ ABCB5+ derived CoCM+	86.5 ± 23.2
		+ i.p. ABCB5+	79.6 ± 17.7
		+ i.v. ABCB5+	72.3 ± 12.4
	Na	Untreated	144.8 ± 1.9
	(mmol/l)	+ ABCB5+ derived CoCM+	142.2 ± 1.7
		+ i.p. ABCB5+	**141.9 ± 1.6***
		+ i.v. ABCB5+	**142.0 ± 2.0***
	K	Untreated	5.1 ± 0.2
	(mmol/l)	+ ABCB5+ derived CoCM+	5.3 ± 0.4
		+ i.p. ABCB5+	4.9 ± 0.2
		+ i.v. ABCB5+	5.0 ± 0.2
	Ca	Untreated	2.7 ± 0.1
	(mmol/l)	+ ABCB5+ derived CoCM+	2.4 ± 0.2
		+ i.p. ABCB5+	**2.4 ± 0.2***
		+ i.v. ABCB5+	2.5 ± 0.2
	PO_4	Untreated	2.1 ± 0.3
	(mmol/l)	+ ABCB5+ derived CoCM+	2.2 ± 0.2
		+ i.p. ABCB5+	2.1 ± 0.1
		+ i.v. ABCB5+	1.9 ± 0.2
	Cholesterol	Untreated	154.3 ± 15.5
	(mg/dl)	+ ABCB5+ derived CoCM+	138.3 ± 30.9
		+ i.p. ABCB5+	135.1 ± 23.0
		+ i.v. ABCB5+	134.0 ± 29.3
	Triglycerides	Untreated	108.8 ± 48.3
	(mg/dl)	+ ABCB5+ derived CoCM+	69.8 ± 23.4
		+ i.p. ABCB5+	79.3 ± 29.0
		+ i.v. ABCB5+	72.1 ± 29.6
	Glucose	Untreated	184.3 ± 7.4
	(mg/dl)	+ ABCB5+ derived CoCM+	171.7 ± 12.0
		+ i.p. ABCB5+	168.0 ± 14.5
		+ i.v. ABCB5+	178.1 ± 14.6
	Protein	Untreated	65.3 ± 2.7
	(mg/dl)	+ ABCB5+ derived CoCM+	**61.3 ± 2.4***
		+ i.p. ABCB5+	64.1 ± 1.9
		+ i.v. ABCB5+	63.1 ± 1.6
	AST	Untreated	72.2 ± 12.4
	(U/l)	+ ABCB5+ derived CoCM+	88.2 ± 15.1
		+ i.p. ABCB5+	79.6 ± 9.5
		+ i.v. ABCB5+	81.4 ± 14.6
	ALT	Untreated	48.8 ± 10.3
	(U/l)	+ ABCB5+ derived CoCM+	47.7 ± 3.7
		+ i.p. ABCB5+	47.6 ± 8.4
		+ i.v. ABCB5+	46.3 ± 4.3

Table 4.18A Plasma biochemistry in PKD/Mhm (Cy/+) groups (untreated; ABCB5+ derived CoCM+; i.p. ABCB5+; i.v. ABCB5+; n=6 in each group) at day 167. Data are shown as mean ± Std.Dev. Values significantly different (treated vs untreated) are indicated as *p<0.05.

B	Parameter	Animal group	Day 167
	Creatinine	Untreated	0.7 ± 0.1
	(mg/dl)	+ ASC derived CM	0.7 ± 0.2
		+ i.p. ASC	0.6 ± 0.1
		+ i.v. ASC	0.7 ± 0.1
	Urea	Untreated	87.4 ± 11.0
	(mg/dl)	+ ASC derived CM	98.7 ± 21.6
		+ i.p. ASC	90.8 ± 12.7
		+ i.v. ASC	95.6 ± 10.6
	Na	Untreated	144.8 ± 1.9
	(mmol/l)	+ ASC derived CM	145.3 ± 2.2
		+ i.p. ASC	**138.5 ± 5.0***
		+ i.v. ASC	139.5 ± 4.4
	K	Untreated	5.1 ± 0.2
	(mmol/l)	+ ASC derived CM	5.7 ± 0.4
		+ i.p. ASC	**6.1 ± 0.5****
		+ i.v. ASC	5.7 ± 0.4
	Ca	Untreated	2.7 ± 0.1
	(mmol/l)	+ ASC derived CM	2.7 ± 0.1
		+ i.p. ASC	2.6 ± 0.1
		+ i.v. ASC	2.6 ± 0.1
	PO_4	Untreated	2.1 ± 0.3
	(mmol/l)	+ ASC derived CM	2.0 ± 0.4
		+ i.p. ASC	1.8 ± 0.3
		+ i.v. ASC	1.9 ± 0.2
	Cholesterol	Untreated	154.3 ± 15.5
	(mg/dl)	+ ASC derived CM	152.3 ± 22.0
		+ i.p. ASC	144.7 ± 10.6
		+ i.v. ASC	149.5 ± 17.3
	Triglycerides	Untreated	108.8 ± 48.3
	(mg/dl)	+ ASC derived CM	63.3 ± 36.2
		+ i.p. ASC	98.8 ± 42.2
		+ i.v. ASC	63.5 ± 17.2
	Glucose	Untreated	184.3 ± 7.4
	(mg/dl)	+ ASC derived CM	173.0 ± 9.3
		+ i.p. ASC	174.7 ± 17.1
		+ i.v. ASC	174.5 ± 9.9
	Protein	Untreated	65.3 ± 2.7
	(mg/dl)	+ ASC derived CM	66.3 ± 1.7
		+ i.p. ASC	66.3 ± 2.8
		+ i.v. ASC	65.2 ± 1.5
	AST	Untreated	72.2 ± 12.4
	(U/l)	+ ASC derived CM	73.3 ± 5.0
		+ i.p. ASC	98.5 ± 59.0
		+ i.v. ASC	69.3 ± 4.0
	ALT	Untreated	48.8 ± 10.3
	(U/l)	+ ASC derived CM	54.0 ± 14.4
		+ i.p. ASC	51.7 ± 7.5
		+ i.v. ASC	51.0 ± 14.6

Table 4.18B Plasma biochemistry in PKD/Mhm (Cy/+) groups (untreated; ASC derived CM; i.p. ASC; i.v. ASC; n=6 in each group) at day 167. Data are shown as mean ± Std.Dev. Values significantly different (treated vs untreated) are indicated as *p<0.05, ** p<0.005.

Animals were placed in metabolic cages overnight for 16 hours, afterwards urine samples were collected. **Table 4.19** (**A-B**) summarizes diuresis, food and water intake values at day 167 in all the groups (for individual days see **appendix 4**).

A Parameter	Animal group	Day 167
Diuresis (ml)	Untreated	55.8 ± 54.5
	+ ABCB5+ derived CoCM+	28.4 ± 10.1
	+ i.p. ABCB5+	26.3 ± 7.3
	+ i.v. ABCB5+	48.1 ± 20.6
Food intake	Untreated	12.3 ± 4.3
(g)	+ ABCB5+ derived CoCM+	10.1 ± 4.3
	+ i.p. ABCB5+	12.1 ± 3.4
	+ i.v. ABCB5+	11.6 ± 2.4
Water intake	Untreated	56.0 ± 15.6
(g)	+ ABCB5+ derived CoCM+	33.5 ± 15.5
	+ i.p. ABCB5+	32.3 ± 9.8
	+ i.v. ABCB5+	55.9 ± 21.8

Table 4.19A Effect of different treatments on diuresis, food intake and water intake in PKD/Mhm (Cy/+) groups (n=6 in each group) at day 167. Comparison between ABCB5+ derived CoCM+, i.p. or i.v. ABCB5+ groups and untreated. Data are shown as mean ± Std.Dev.

B Parameter	Animal group	Day 167
Diuresis (ml)	Untreated	55.8 ± 54.5
	+ ASC derived CM	28.9 ± 5.6
	+ i.p. ASC	28.4 ± 13.6
	+ i.v. ASC	33.6 ± 13.1
Food intake	Untreated	12.3 ± 4.3
(g)	+ ASC derived CM	9.9 ± 2.7
	+ i.p. ASC	11.5 ± 3.5
	+ i.v. ASC	10.1 ± 2.2
Water intake	Untreated	56.0 ± 15.6
(g)	+ ASC derived CM	40.9 ± 12.5
	+ i.p. ASC	43.1 ± 13.6
	+ i.v. ASC	41.1 ± 14.5

Table 4.19B Effect of different treatments on diuresis, food intake and water intake in PKD/Mhm (Cy/+) groups (n=6 in each group) at day 167. Comparison between ASC derived CM, i.p. or i.v. ASC groups and untreated. Data are shown as mean ± Std.Dev.

Urine biochemistry results showed a decrease in creatinine and urea levels in all the treated groups, likewise the proteinuria and albumin levels. **Table 4.20** (**A-B**) illustrates the analysed urine parameters (for individual days see **appendix 5**).

A Parameter	Animal group	Day 167
Creatinine	Untreated	16.9 ± 8.6
(mg/16h)	+ ABCB5+ derived CoCM+	12.3 ± 2.3
	+ i.p. ABCB5+	12.5 ± 0.6

	+ i.v. ABCB5+	14.2 ± 2.6
Urea	Untreated	830.4 ± 376.6
(mg/16h)	+ ABCB5+ derived CoCM+	552.0 ± 142.1
	+ i.p. ABCB5+	610.5 ± 54.9
	+ i.v. ABCB5+	702.9 ± 149.6
Na	Untreated	1.7 ± 1.4
(mmol/16h)	+ ABCB5+ derived CoCM+	1.5 ± 0.5
	+ i.p. ABCB5+	1.4 ± 0.4
	+ i.v. ABCB5+	1.7 ± 0.6
K	Untreated	4.2 ± 2.6
(mmol/16h)	+ ABCB5+ derived CoCM+	3.1 ± 1.1
	+ i.p. ABCB5+	1.7 ± 1.1
	+ i.v. ABCB5+	3.7 ± 0.8
Ca	Untreated	0.04 ± 0.04
(mmol/16h)	+ ABCB5+ derived CoCM+	0.02 ± 0.01
	+ i.p. ABCB5+	0.01 ± 0.01
	+ i.v. ABCB5+	0.02 ± 0.00
PO_4	Untreated	0.1 ± 0.1
(mmol/16h)	+ ABCB5+ derived CoCM+	0.1 ± 0.1
	+ i.p. ABCB5+	0.0 ± 0.0
	+ i.v. ABCB5+	0.1 ± 0.0
Glucose	Untreated	1.7 ± 1.7
(mg/16h)	+ ABCB5+ derived CoCM+	0.9 ± 0.8
	+ i.p. ABCB5+	1.4 ± 0.9
	+ i.v. ABCB5+	1.4 ± 0.6
Protein	Untreated	154.5 ± 177.7
(mg/16h)	+ ABCB5+ derived CoCM+	49.8 ± 16.4
	+ i.p. ABCB5+	68.4 ± 35.0
	+ i.v. ABCB5+	58.8 ± 36.5
Albumin	Untreated	103.9 ± 108.5
(mg/16h)	+ ABCB5+ derived CoCM+	39.1 ± 16.2
	+ i.p. ABCB5+	58.1 ± 37.5
	+ i.v. ABCB5+	52.6 ± 37.7

Table 4.20A Urine biochemistry in PKD/Mhm (Cy/+) groups (untreated; ABCB5+ derived CoCM+; i.p. ABCB5+; i.v. ABCB5+; n=6 in each group) at day 167. Data are shown as mean ± Std.Dev.

B	Parameter	Animal group	Day 167
	Creatinine	Untreated	16.9 ± 8.6
	(mg/16h)	+ ASC derived CM	13.6 ± 0.6
		+ i.p. ASC	10.9 ± 2.9
		+ i.v. ASC	13.8 ± 1.8
	Urea	Untreated	830.5 ± 376.6
	(mg/16h)	+ ASC derived CM	644.5 ± 99.6
		+ i.p. ASC	557.6 ± 199.5
		+ i.v. ASC	666.2 ± 92.6
	Na	Untreated	1.7 ± 1.4

(mmol/16h)	+ ASC derived CM	1.0 ± 0.2
	+ i.p. ASC	1.6 ± 0.9
	+ i.v. ASC	1.1 ± 0.3
K	Untreated	4.2 ± 2.6
(mmol/16h)	+ ASC derived CM	3.1 ± 0.6
	+ i.p. ASC	2.8 ± 1.0
	+ i.v. ASC	3.0 ± 0.8
Ca	Untreated	0.04 ± 0.04
(mmol/16h)	+ ASC derived CM	0.03 ± 0.02
	+ i.p. ASC	0.01 ± 0.01
	+ i.v. ASC	0.02 ± 0.01
PO_4	Untreated	0.1 ± 0.1
(mmol/16h)	+ ASC derived CM	0.1 ± 0.1
	+ i.p. ASC	0.1 ± 0.1
	+ i.v. ASC	0.1 ± 0.1
Glucose	Untreated	1.7 ± 1.7
(mg/16h)	+ ASC derived CM	1.3 ± 1.4
	+ i.p. ASC	1.9 ± 1.2
	+ i.v. ASC	1.8 ± 0.8
Protein	Untreated	154.5 ± 177.7
(mg/16h)	+ ASC derived CM	81.3 ± 15.8
	+ i.p. ASC	76.0 ± 41.1
	+ i.v. ASC	83.6 ± 25.5
Albumin	Untreated	103.9 ± 108.5
(mg/16h)	+ ASC derived CM	39.0 ± 6.6
	+ i.p. ASC	64.2 ± 34.6
	+ i.v. ASC	60.4 ± 26.1

Table 4.20B Urine biochemistry in PKD/Mhm (Cy/+) groups (untreated; ASC derived CM; i.p. ASC; i.v. ASC; n=6 in each group) at day 167. Data are shown as mean ± Std.Dev.

A significant reduction of ABZWCY-HβCD $t_{1/2}$ in i.p. ABCB5+ and i.p. ASC groups ($p<0.05$) was observed, as shown in **table 4.21** (**A-B**) (for individual days see **appendix 6**). A slight decrease was also recorded in i.v. ABCB5+ and ASC, ABCB5+ derived CoCM+ and ASC derived CM groups (**figure 4.11**).

A	Parameter	Animal group	Day 167
	ABZWCY-HβCD $t_{1/2}$ (min)	Untreated	66.0 ± 11.4
		+ ABCB5+ derived CoCM+	55.0 ± 12.0
		+ i.p. ABCB5+	**41.2 ± 15.5***
		+ i.v. ABCB5+	43.9 ± 19.1

Table 4.21A Effect of different treatments on ABZWCY-HβCD $t_{1/2}$ in PKD/Mhm (Cy/+) groups (n=6 in each group) at day 167. Comparison between ABCB5+ derived CoCM+, i.p. or i.v. ABCB5+ groups and untreated. Data are shown as mean ± Std.Dev. Values significantly different (treated vs untreated) are indicated as *$p<0.05$.

B	Parameter	Animal group	Day 167
	ABZWCY-HβCD $t_{1/2}$ (min)	Untreated	66.0 ± 11.4
		+ ASC derived CM	49.6 ± 15.7
		+ i.p. ASC	**38.8 ± 8.1***
		+ i.v. ASC	43.0 ± 19.8

Table 4.21B Effect of different treatments on ABZWCY-HβCD $t_{1/2}$ in PKD/Mhm (Cy/+) groups (n=6 in each group) at day 167. Comparison between ASC derived CM, i.p. or i.v. ASC groups and untreated. Data are shown as mean ± Std.Dev. Values significantly different (treated vs untreated) are indicated as *p<0.05.

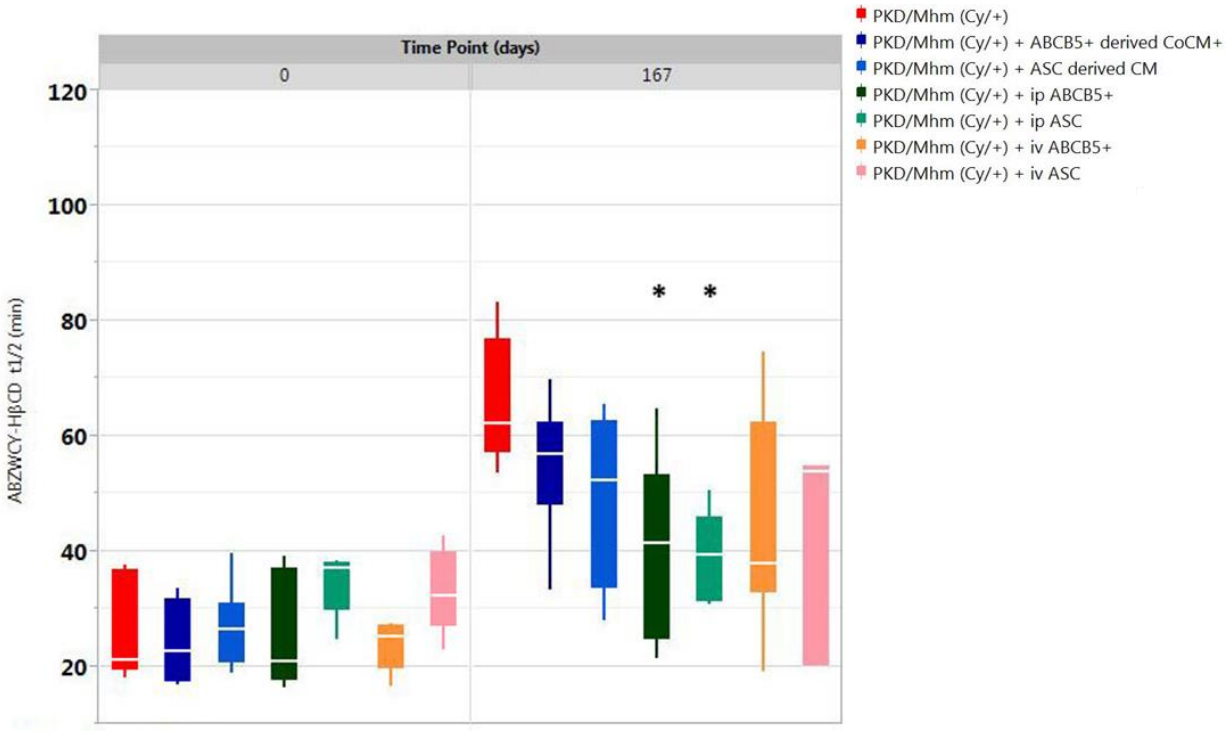

Figure 4.11 Effect of different treatments on ABZWCY-HβCD $t_{1/2}$ in PKD/Mhm (Cy/+) model (n=6 in each group). Data are shown as box plots with the median, upper and lower quartile (interquartile range (IQR)) and whiskers (1.5x IQR). Values significantly different (treatment vs PKD/Mhm (Cy/+)) are indicated as *p<0.05.

Table 4.22 (**A-B**) shows plasma levels of creatinine and urea in correlation with the ABZWCY-HβCD $t_{1/2}$ values.

A	Animal group	ABZWCY-HβCD $t_{1/2}$ (min)	Creatinine (mg/dl)	Urea (mg/dl)
	Untreated	66.0 ± 11.4	0.7 ± 0.1	87.4 ± 11.0
	+ ABCB5+ derived CoCM+	55.0 ± 12.0	0.7 ± 0.2	86.5 ± 23.2
	+ i.p. ABCB5+	**41.2 ± 15.5***	0.6 ±0.1	79.6 ± 17.7
	+ i.v. ABCB5+	43.9 ± 19.1	0.7 ± 0.1	72.3 ± 12.4

Table 4.22A Effect of different treatments on ABZWCY-HβCD $t_{1/2}$, plasma creatinine and urea in PKD/Mhm (Cy/+) groups (n=6 in each group) at day 167. Comparison between ABCB5+ derived CoCM+, i.p. or i.v. ABCB5+ groups and untreated. Data are shown as mean ± Std.Dev. Values significantly different (treated vs untreated) are indicated as *p<0.05.

B	Animal group	ABZWCY-HβCD $t_{1/2}$ (min)	Creatinine (mg/dl)	Urea (mg/dl)
	Untreated	66.0 ± 11.4	0.7 ± 0.1	87.4 ± 11.0
	+ ASC derived CM	49.6 ± 15.7	0.7 ± 0.2	98.7 ± 21.6
	+ i.p. ASC	**38.8 ± 8.1***	0.6 ± 0.1	90.8 ± 12.7
	+ i.v. ASC	43.0 ± 19.8	0.7 ± 0.1	95.6 ± 10.6

Table 4.22B Effect of different treatments on ABZWCY-HβCD $t_{1/2}$, plasma creatinine and urea in PKD/Mhm (Cy/+) groups (n=6 in each group) at day 167. Comparison between ASC derived CM, i.p. or i.v. ASC groups and untreated. Data are shown as mean ± Std.Dev. Values significantly different (treated vs untreated) are indicated as *p<0.05.

4.2.3 *Gene expression profiling*

Gene expression analysis was performed on each treated PKD/Mhm group compared to the untreated. **Table 4.23** (**A**-**B**) summarizes the up- and downregulated pathways identified for each treated group.

A	Animal group		
		Total analysed pathways	304
	+ABCB5+ derived CoCM+	Significantly regulated pathways (adj. p-value < 0.05)	185
		Significantly upregulated pathways (adj. p-value < 0.05)	37
		Significantly downregulated pathways (adj. p-value < 0.05)	148
	+ i.p. ABCB5+	Significantly regulated pathways (adj. p-value < 0.05)	165
		Significantly upregulated pathways (adj. p-value < 0.05)	46
		Significantly downregulated pathways (adj. p-value < 0.05)	119
	+ i.v. ABCB5+	Significantly regulated pathways (adj. p-value < 0.05)	142
		Significantly upregulated pathways (adj. p-value < 0.05)	47
		Significantly downregulated pathways (adj. p-value < 0.05)	95

Table 4.23A Overview of GSEA displaying the numbers of up- and downregulated pathways in PKD/Mhm (Cy/+) groups (ABCB5+ derived CoCM+; i.p. ABCB5+; i.v. ABCB5+; n=6 in each group).

B	Animal group		
		Total analysed pathways	305
	+ASC derived CM	Significantly regulated pathways (adj. p-value < 0.05)	42
		Significantly upregulated pathways (adj. p-value < 0.05)	31
		Significantly downregulated pathways (adj. p-value < 0.05)	11
	+ i.p. ASC	Significantly regulated pathways (adj. p-value < 0.05)	101
		Significantly upregulated pathways (adj. p-value < 0.05)	55
		Significantly downregulated pathways (adj. p-value < 0.05)	46
	+ i.v. ASC	Significantly regulated pathways (adj. p-value < 0.05)	122
		Significantly upregulated pathways (adj. p-value < 0.05)	48
		Significantly downregulated pathways (adj. p-value < 0.05)	74

Table 4.23B Overview of GSEA displaying the numbers of up- and downregulated pathways in PKD/Mhm (Cy/+) groups (ASC derived CM; i.p. ASC; i.v. ASC; n=6 in each group). Total analysed pathways are 305 in the ASC derived CM; i.p. ASC; i.v. ASC groups while 304 in the ABCB5+ derived CoCM+; i.p. ABCB5+; i.v. ABCB5+ groups because GSEA analyses were performed over two different years.

Figure 4.12 exemplifies the main categories of up- and downregulated pathways taken into account in different stem cells and conditioned media administration. **Figure 4.13** shows the number of common pathways between each pair of the same stem cells administration route and conditioned media.

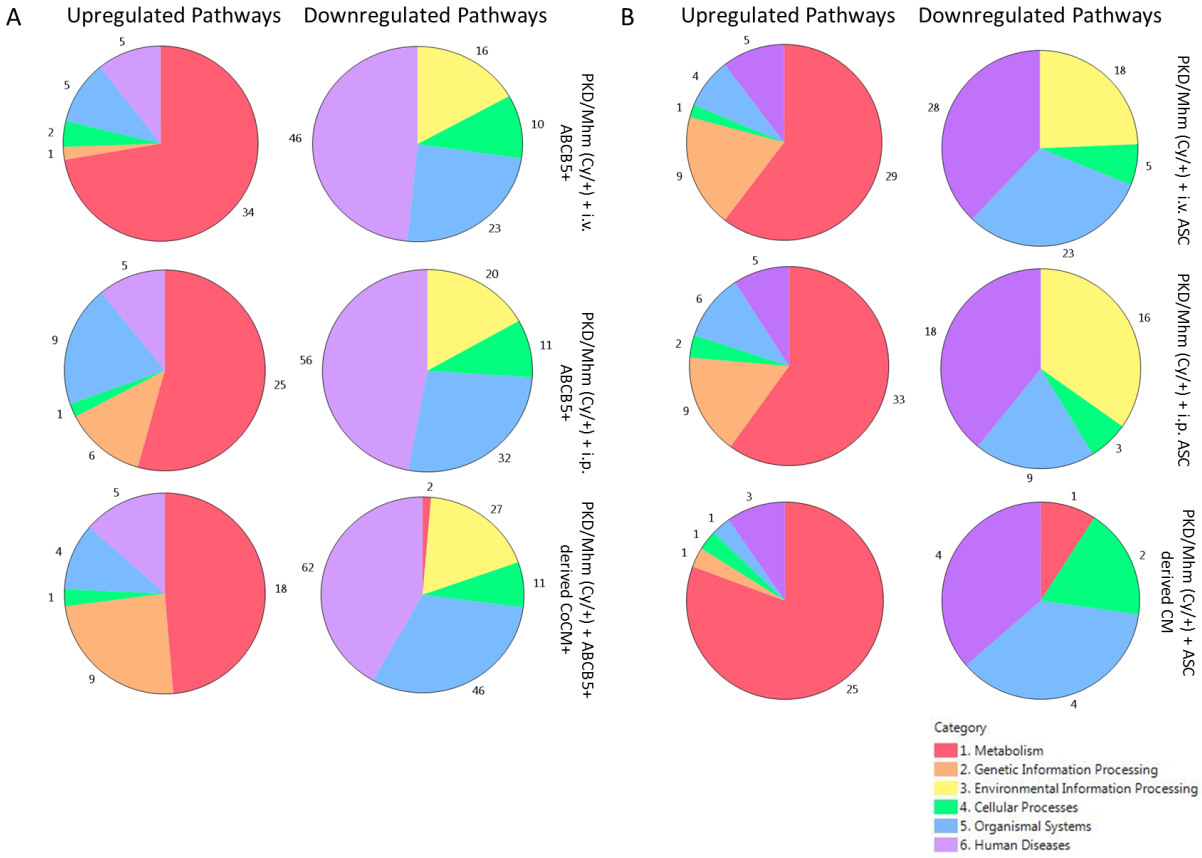

Figure 4.12 Pie charts showing the distribution of pathways containing significantly up- and downregulated genes sorted by main categories of KEGG database. A) ABCB5+ derived CoCM+, i.p.ABCB5+ and i.v. ABCB5+ treatment; B) ASC derived CM, i.p. ASC and i.v. ASC treatment. The numbers around the pie charts indicate the number of up- and downregulated pathways contained in each category.

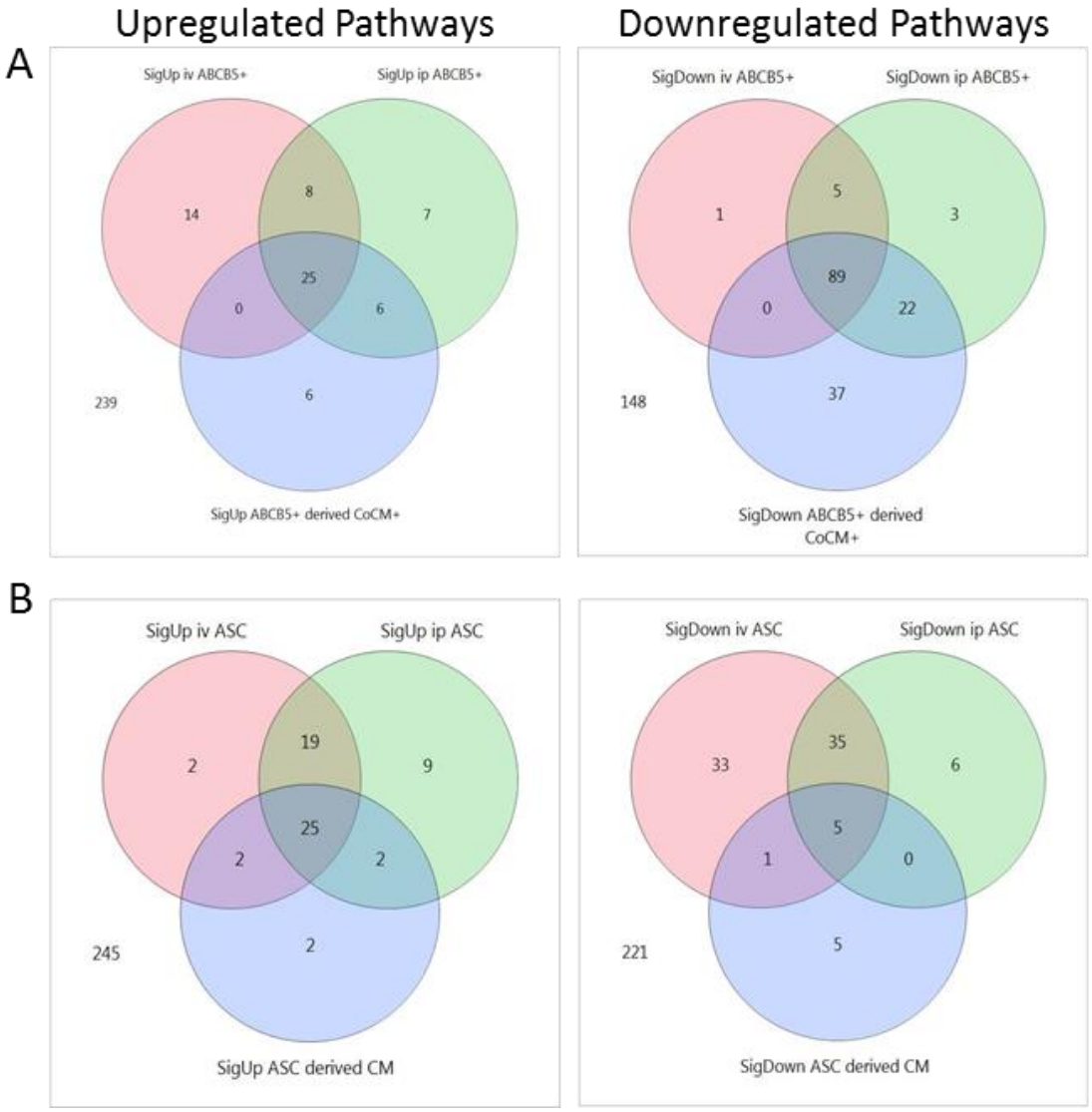

Figure 4.13 Venn diagrams showing the common significant up- and downregulated pathways in PKD/Mhm (Cy/+). A) ABCB5+ derived CoCM+, i.p. ABCB5+ or i.v. ABCB5+ treatment; B) ASC derived CM, i.p. ASC or i.v. ASC treatment. The numbers inside the Venn diagrams indicate the number of un- and downregulated pathways contained in each category.

As described in **figure 4.14**, i.v. ASC and i.v. ABCB5+ groups have in common 32 and 44 pathways respectively up and downregulated, while there are 36 and 37 common pathways among i.p. ASC and i.p. ABCB5+ groups and lastly, ASC derived CM and ABCB5+ derived CoCM+ groups present 17 and 5 up and downregulated common pathways.

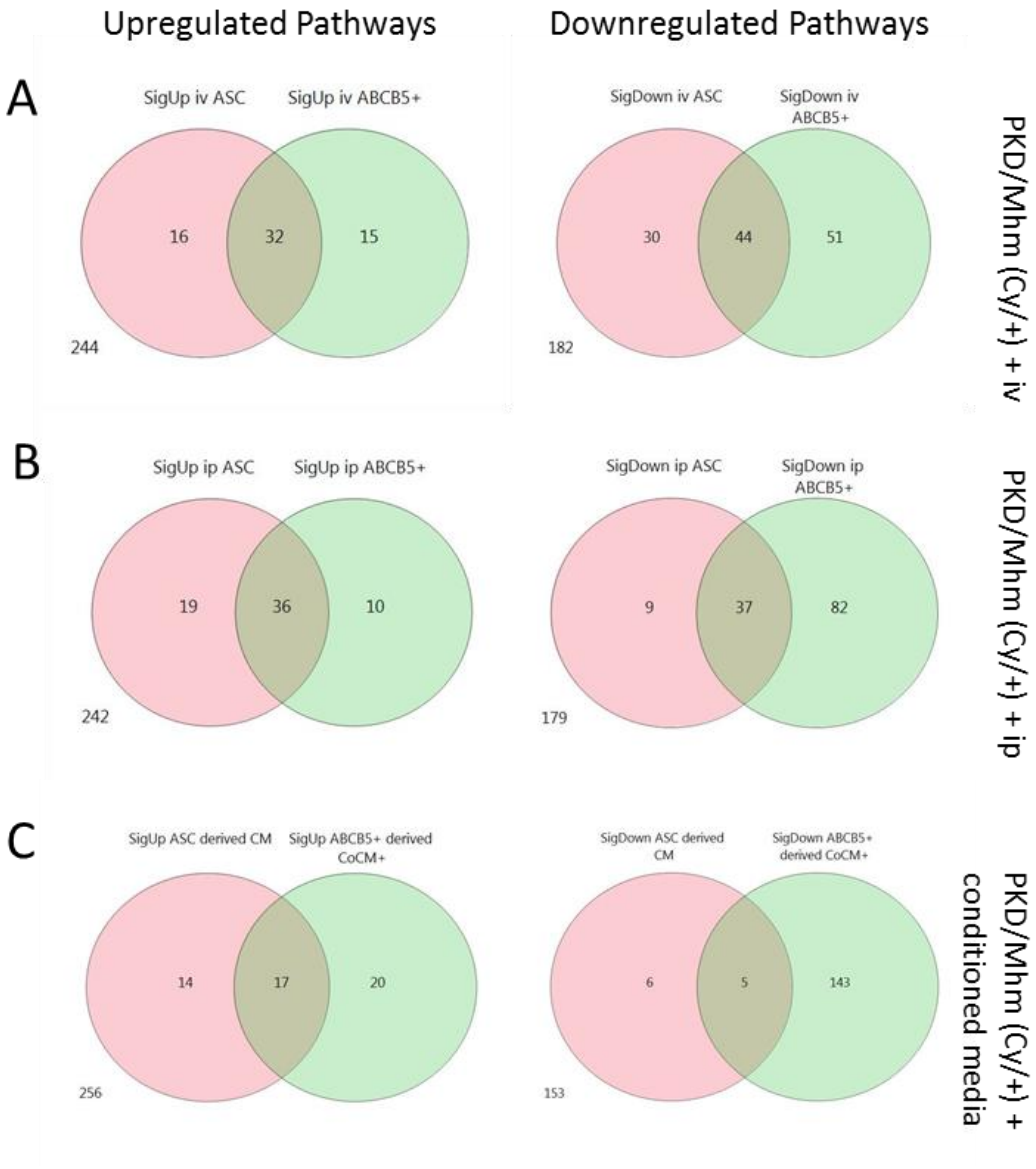

Figure 4.14 Venn diagrams showing the common significant up- and downregulated pathways between PKD/Mhm (Cy/+) treatments. A) i.v. ASC and ABCB5+ treatments, B) i.p. ASC and ABCB5+ treatments, C) ASC derived CM and ABCB5+ derived CoCM+ treatments. The numbers inside the Venn diagrams indicate the number of up- and downregulated pathways contained in each category.

Both ASC and ABCB5+ administrations show an upregulation of the principal metabolism related pathways such as oxidative phosphorylation, citrate cycle and amino acid metabolism, involving a shift of the metabolism pathways from glycolysis to the gluconeogenesis pathway (**table 4.24 A-B**).

A

KEGG Pathway	Sub Category	NES i.v. ABCB5+	NES i.p. ABCB5+	NES ABCB5+ derived CoCM+
Glycolysis Gluconeogenesis	1.1. Carbohydrate metabolism	1.62	1.56	1.62
Citrate cycle (TCA cycle)	1.1. Carbohydrate metabolism	2.45	1.81	1.38
Ascorbate and aldarate metabolism	1.1. Carbohydrate metabolism	1.92	1.51	1.16
Fatty acid degradation	1.3. Lipid metabolism	2.35	1.82	1.42
Steroid hormone biosynthesis	1.3. Lipid metabolism	2.26	1.65	1.81
Oxidative phosphorylation	1.2. Energy metabolism	2.97	3.4	3.08
Pyrimidine metabolism	1.4. Nucleotide metabolism	1.14	1.54	1.34
Alanine, aspartate and glutamate metabolism	1.5. Amino acid metabolism	1.83	1.4	1.05
Glycine, serine and threonine metabolism	1.5. Amino acid metabolism	2.69	1.63	1.39
Cysteine and methionine metabolism	1.5. Amino acid metabolism	2.24	1.86	1.52
Valine, leucine and isoleucine degradation	1.5. Amino acid metabolism	2.47	1.86	1.6
Lysine degradation	1.5. Amino acid metabolism	1.65	-0.6	-1.26
Arginine and proline metabolism	1.5. Amino acid metabolism	1.96	1.33	1.35
Histidine metabolism	1.5. Amino acid metabolism	1.6	0.8	0.62
Tyrosine metabolism	1.5. Amino acid metabolism	1.49	0.77	0.97
Tryptophan metabolism	1.5. Amino acid metabolism	2.5	1.46	1.18
beta-Alanine metabolism	1.6. Metabolism of other amino acids	2.07	1	0.85
Selenocompound metabolism	1.6. Metabolism of other amino acids	2.09	1.49	1.39
Glutathione metabolism	1.6. Metabolism of other amino acids	2.1	1.81	2.07
N-Glycan biosynthesis	1.7. Glycan biosynthesis and metaboli	1.04	1.48	0.91
Glycerolipid metabolism	1.3. Lipid metabolism	1.78	1.46	0.91
Glycosylphosphatidylinositol (GPI)-anchor biosynthesis	1.7. Glycan biosynthesis and metaboli	1.39	2.27	1.79
Arachidonic acid metabolism	1.3. Lipid metabolism	1.31	1.27	1.39
Linoleic acid metabolism	1.3. Lipid metabolism	1.09	1.47	1.83
Pyruvate metabolism	1.1. Carbohydrate metabolism	2.22	1.97	1.74
Glyoxylate and dicarboxylate metabolism	1.1. Carbohydrate metabolism	2.57	2.01	1.76
Propanoate metabolism	1.1. Carbohydrate metabolism	2.29	2.21	1.41
Butanoate metabolism	1.1. Carbohydrate metabolism	2.16	1.75	1.65
Nicotinate and nicotinamide metabolism	1.8. Metabolism of cofactors and vitan	1.73	1.26	1.37
Retinol metabolism	1.8. Metabolism of cofactors and vitan	1.94	1.74	1.53
Porphyrin and chlorophyll metabolism	1.8. Metabolism of cofactors and vitan	1.61	1.23	1.24
Metabolism of xenobiotics by cytochrome P450	1.11. Xenobiotics biodegradation and	2.07	1.85	1.77
Drug metabolism	1.11. Xenobiotics biodegradation and	2	1.53	1.51
Drug metabolism	1.11. Xenobiotics biodegradation and	1.99	1.66	2.09
Metabolic pathways	1.0 Global and overview maps	2.51	2.29	1.83
Carbon metabolism	1.0 Global and overview maps	2.69	2.3	2.01
2-Oxocarboxylic acid metabolism	1.0 Global and overview maps	1.98	1.76	1.54
Fatty acid metabolism	1.0 Global and overview maps	1.8	1.51	0.86
Biosynthesis of amino acids	1.0 Global and overview maps	1.96	1.84	1.65

Table 4.24A GSEA analysis using KEGG database sorted by main category: Metabolism**.** ABCB5+ derived CoCM+; i.p.ABCB5+; i.v. ABCB5+ groups. Significantly (adj. $p<0.05$) differentially expressed pathways (PKD/Mhm (Cy/+) + treatment vs PKD/Mhm (Cy/+)). For each pathway the NES is given. Downregulated pathways are displayed in green, upregulated pathways are displayed in red.

B

KEGG Pathway	Sub Category	NES i.v. ASC	NES i.p. ASC	NES ASC derived CM
Glycolysis Gluconeogenesis	1.1. Carbohydrate metabolism	1.91	2.23	1.57
Citrate cycle (TCA cycle)	1.1. Carbohydrate metabolism	1.6	2.58	1.62
Ascorbate and aldarate metabolism	1.1. Carbohydrate metabolism	2.23	1.63	1.75
Fatty acid degradation	1.3. Lipid metabolism	2.32	2.63	2.39
Steroid hormone biosynthesis	1.3. Lipid metabolism	2.35	1.9	1.77
Oxidative phosphorylation	1.2. Energy metabolism	3.48	3.77	1.15
Pyrimidine metabolism	1.4. Nucleotide metabolism	1.55	1.72	1.41
Alanine, aspartate and glutamate metabolism	1.5. Amino acid metabolism	0.94	1.28	1.52
Glycine, serine and threonine metabolism	1.5. Amino acid metabolism	2.09	2.27	2.17
Cysteine and methionine metabolism	1.5. Amino acid metabolism	2.05	2.25	1.96
Valine, leucine and isoleucine degradation	1.5. Amino acid metabolism	2.44	2.44	2.12
Lysine degradation	1.5. Amino acid metabolism	-0.75	-0.94	1.82
Arginine and proline metabolism	1.5. Amino acid metabolism	1.74	2.25	1.9
Histidine metabolism	1.5. Amino acid metabolism	2.15	1.86	2.24
Tyrosine metabolism	1.5. Amino acid metabolism	1.92	1.47	2.14
Tryptophan metabolism	1.5. Amino acid metabolism	2.18	2.42	2.7
beta-Alanine metabolism	1.6. Metabolism of other amino acids	2.39	2.42	2.68
Selenocompound metabolism	1.6. Metabolism of other amino acids	1.27	1.57	1.93
Glutathione metabolism	1.6. Metabolism of other amino acids	2.48	2.32	2.2
N-Glycan biosynthesis	1.7. Glycan biosynthesis and metaboli	1.47	1.94	1.2
Glycerolipid metabolism	1.3. Lipid metabolism	1.49	1.56	1.67
Glycosylphosphatidylinositol (GPI)-anchor biosynthesis	1.7. Glycan biosynthesis and metaboli	-0.97	0.69	-1.39
Arachidonic acid metabolism	1.3. Lipid metabolism	1.89	1.77	1.73
Linoleic acid metabolism	1.3. Lipid metabolism	2.04	1.83	1.81
Pyruvate metabolism	1.1. Carbohydrate metabolism	2.17	2.36	1.52
Glyoxylate and dicarboxylate metabolism	1.1. Carbohydrate metabolism	2.26	2.29	2.11
Propanoate metabolism	1.1. Carbohydrate metabolism	1.7	2.42	1.6
Butanoate metabolism	1.1. Carbohydrate metabolism	1.93	1.94	1.71
Nicotinate and nicotinamide metabolism	1.8. Metabolism of cofactors and vitan	0.85	1.78	1.79
Retinol metabolism	1.8. Metabolism of cofactors and vitan	2.17	1.56	1.81
Porphyrin and chlorophyll metabolism	1.8. Metabolism of cofactors and vitan	2.05	1.81	1.78
Metabolism of xenobiotics by cytochrome P450	1.11. Xenobiotics biodegradation and	2.84	1.89	2.22
Drug metabolism	1.11. Xenobiotics biodegradation and	2.71	1.72	2.21
Drug metabolism	1.11. Xenobiotics biodegradation and	2.81	2.34	2.24
Metabolic pathways	1.0 Global and overview maps	2.13	2.56	1.8
Carbon metabolism	1.0 Global and overview maps	2.03	2.76	1.65
2-Oxocarboxylic acid metabolism	1.0 Global and overview maps	0.87	1.61	1.06
Fatty acid metabolism	1.0 Global and overview maps	1.49	1.79	1.25
Biosynthesis of amino acids	1.0 Global and overview maps	1.15	1.86	1.38

Table 4.24B GSEA analysis using KEGG database sorted by main category: Metabolism**.** ASC derived CM; i.p. ASC; i.v. ASC groups. Significantly (adj. p<0.05) differentially expressed pathways (PKD/Mhm (Cy/+) + treatment vs PKD/Mhm (Cy/+)). For each pathway the NES is given. Downregulated pathways are displayed in green, upregulated pathways are displayed in red.

Apoptotic pathways, along with cellular senescence, focal adhesion pathways were downregulated in all the treated groups, with the exception of cellular senescence pathway that was upregulated in the ASC derived CM group (**table 4.25 A-B**).

A

KEGG Pathway	Sub Category	NES i.v. ABCB5+	NES i.p. ABCB5+	NES ABCB5+ derived CoCM+
Cell cycle	4.2. Cell growth and death	-1.94	-2.12	-1.31
Endocytosis	4.1. Transport and catabolism	-1.52	-1.41	-1.78
Phagosome	4.1. Transport and catabolism	-2.29	-1.6	-1.41
Peroxisome	4.1. Transport and catabolism	2.68	2.04	1.86
Apoptosis	4.2. Cell growth and death	-1.46	-1.95	-1.59
Necroptosis	4.2. Cell growth and death	-1.58	-1.45	-1.22
Cellular senescence	4.2. Cell growth and death	-1.77	-1.96	-1.81
Focal adhesion	4.3. Cellular community - eukaryotes	-2.15	-2.43	-2.85
Adherens junction	4.3. Cellular community - eukaryotes	-1.85	-1.96	-2.71
Gap junction	4.3. Cellular community - eukaryotes	-1.46	-1.7	-2.16
Signaling pathways regulating pluripotency of stem cells	4.3. Cellular community - eukaryotes	-0.95	-1.32	-1.89

Table 4.25A GSEA analysis using KEGG database sorted by main category: Cellular Processes**.** ABCB5+ derived CoCM+; i.p. ABCB5+; i.v. ABCB5+ groups. Significantly (adj. p<0.05) differentially expressed pathways (PKD/Mhm (Cy/+) + treatment vs PKD/Mhm (Cy/+)). For each pathway the NES is given. Downregulated pathways are displayed in green, upregulated pathways are displayed in red.

B

KEGG Pathway	Sub Category	NES i.v. ASC	NES i.p. ASC	NES ASC derived CM
Cell cycle	4.2. Cell growth and death	-0.86	-1.05	-1.3
Endocytosis	4.1. Transport and catabolism	-0.87	1.01	-0.77
Phagosome	4.1. Transport and catabolism	-0.81	1.24	-1.39
Peroxisome	4.1. Transport and catabolism	2.42	2.62	2.5
Apoptosis	4.2. Cell growth and death	-1.47	-1.19	-1.4
Necroptosis	4.2. Cell growth and death	-0.68	1.16	0.85
Cellular senescence	4.2. Cell growth and death	-1.05	-0.94	0.79
Focal adhesion	4.3. Cellular community - eukaryotes	-1.98	-1.88	-1.64
Adherens junction	4.3. Cellular community - eukaryotes	-1.8	-1.82	-1.41
Gap junction	4.3. Cellular community - eukaryotes	-1.53	-1.14	-1.06
Signaling pathways regulating pluripotency of stem cells	4.3. Cellular community - eukaryotes	-1.5	-1.95	-1.02

Table 4.25B GSEA analysis using KEGG database sorted by main category: Cellular Processes. ASC derived CM; i.p. ASC; i.v. ASC groups. Significantly (adj. p<0.05) differentially expressed pathways (PKD/Mhm (Cy/+) + treatment vs PKD/Mhm (Cy/+)). For each pathway the NES is given. Downregulated pathways are displayed in green, upregulated pathways are displayed in red.

Pathways involved in the translation and transcription were mainly upregulated (**table 4.26 A-B**).

A

KEGG Pathway	Sub Category	NES i.v. ABCB5+	NES i.p. ABCB5+	NES ABCB5+ derived CoCM+
Aminoacyl-tRNA biosynthesis	2.2. Translation	2.37	2.23	1.92
Ribosome biogenesis in eukaryotes	2.2. Translation	1.35	0.84	1.81
Ribosome	2.2. Translation	-1.2	2.1	2.86
RNA transport	2.2. Translation	0.99	0.65	1.68
RNA polymerase	2.1. Transcription	1.24	1.33	2.11
Spliceosome	2.1. Transcription	-0.77	-1.13	1.71
Proteasome	2.3. Folding, sorting and degradation	1.48	2.02	2.63
Protein export	2.3. Folding, sorting and degradation	0.9	1.62	1.7
Protein processing in endoplasmic reticulum	2.3. Folding, sorting and degradation	1.37	1.46	0.98

Table 4.26A GSEA analysis using KEGG database sorted by main category: Genetic Information Processing**.** ABCB5+ derived CoCM+; i.p. ABCB5+, i.v. ABCB5+ groups. Significantly (adj. p<0.05) differentially expressed pathways (PKD/Mhm (Cy/+) + treatment vs PKD/Mhm (Cy/+)). For each pathway the NES is given. Downregulated pathways are displayed in green, upregulated pathways are displayed in red.

B

KEGG Pathway	Sub Category	NES i.v. ASC	NES i.p. ASC	NES ASC derived CM
Aminoacyl-tRNA biosynthesis	2.2. Translation	1.66	2.04	1.18
Ribosome biogenesis in eukaryotes	2.2. Translation	2.21	1.92	0.78
Ribosome	2.2. Translation	3.63	2.31	1.71
RNA transport	2.2. Translation	1.93	1.6	0.74
RNA polymerase	2.1. Transcription	2.01	2.08	0.62
Spliceosome	2.1. Transcription	1.82	1.56	-0.64
Proteasome	2.3. Folding, sorting and degradation	2.94	2.76	0.59
Protein export	2.3. Folding, sorting and degradation	1.95	1.99	-1.17
Protein processing in endoplasmic reticulum	2.3. Folding, sorting and degradation	1.4	2.18	0.89

Table 4.26B GSEA analysis using KEGG database sorted by main category: Genetic Information Processing**.** ASC derived CM; i.p. ASC; i.v. ASC groups. Significantly (adj. $p<0.05$) differentially expressed pathways (PKD/Mhm (Cy/+) + treatment vs PKD/Mhm (Cy/+)). For each pathway the NES is given. Downregulated pathways are displayed in green, upregulated pathways are displayed in red.

Pathways related to the signal transduction were mainly downregulated. **Table 4.27** (**A**-**B**) shows the differentially expressed pathways involved in signal transduction, immune and endocrine system.

A

KEGG Pathway	Sub Category	NES i.v. ABCB5+	NES i.p. ABCB5+	NES ABCB5+ derived CoCM+
MAPK signaling pathway	3.2. Signal transduction	-1.2	-1.99	-2.14
ErbB signaling pathway	3.2. Signal transduction	-0.74	-1.3	-1.82
Ras signaling pathway	3.2. Signal transduction	-1.7	-1.77	-2.4
Rap1 signaling pathway	3.2. Signal transduction	-1.72	-1.96	-2.56
Calcium signaling pathway	3.2. Signal transduction	-1.34	-1.03	-1.65
cGMP-PKG signaling pathway	3.2. Signal transduction	-1.49	-1.52	-2.24
cAMP signaling pathway	3.2. Signal transduction	-1.72	-1.55	-2.14
Cytokine-cytokine receptor interaction	3.3. Signaling molecules and interacti	-2.6	-2.33	-1.91
NF-kappa B signaling pathway	3.2. Signal transduction	-2.24	-2.28	-2.07
FoxO signaling pathway	3.2. Signal transduction	-0.82	-1.63	-1.45
Phosphatidylinositol signaling system	3.2. Signal transduction	-0.89	-0.66	-1.53
Phospholipase D signaling pathway	3.2. Signal transduction	-1.29	-1.42	-2.07
Neuroactive ligand-receptor interaction	3.3. Signaling molecules and interacti	-1.78	-1.57	-1.37
PI3K-Akt signaling pathway	3.2. Signal transduction	-1.58	-1.93	-2.29
Wnt signaling pathway	3.2. Signal transduction	-1.5	-1.62	-2.21
Hedgehog signaling pathway	3.2. Signal transduction	-0.74	-1.47	-2.21
TGF-beta signaling pathway	3.2. Signal transduction	-1.37	-1.52	-1.91
Apelin signaling pathway	3.2. Signal transduction	-1.35	-1.51	-1.92
Hippo signaling pathway	3.2. Signal transduction	-1.65	-1.54	-2.33
Hippo signaling pathway	3.2. Signal transduction	-1.48	-1.53	-2.07
ECM-receptor interaction	3.3. Signaling molecules and interacti	-2.43	-2.37	-2.46
Cell adhesion molecules (CAMs)	3.3. Signaling molecules and interacti	-2.96	-2.11	-2.47
JAK-STAT signaling pathway	3.2. Signal transduction	-1.73	-1.94	-2.04
TNF signaling pathway	3.2. Signal transduction	-1.51	-2.24	-2.07
PPAR signaling pathway	5.2. Endocrine system	1.74	1	0.64
Chemokine signaling pathway	5.1. Immune system	-2.56	-2.27	-2.35
Longevity regulating pathway	5.9. Aging	0.66	-0.68	-1.46
Cardiac muscle contraction	5.3. Circulatory system	0.93	1.86	1.52
Vascular smooth muscle contraction	5.3. Circulatory system	-1.57	-1.46	-1.84
Axon guidance	5.8. Development	-2.07	-1.73	-2.54
Osteoclast differentiation	5.8. Development	-2.12	-2.61	-2.2
Complement and coagulation cascades	5.1. Immune system	-1.79	-2.01	-1.81
Platelet activation	5.1. Immune system	-2.05	-2.12	-2.24
Antigen processing and presentation	5.1. Immune system	-2.54	-2.19	-1.55
Toll-like receptor signaling pathway	5.1. Immune system	-1.68	-2.14	-2.01
NOD-like receptor signaling pathway	5.1. Immune system	-1.7	-2.03	-1.64
RIG-I-like receptor signaling pathway	5.1. Immune system	-0.96	-1.62	-0.84
C-type lectin receptor signaling pathway	5.1. Immune system	-1.75	-2.1	-1.99
Hematopoietic cell lineage	5.1. Immune system	-2.77	-2.3	-1.89
Natural killer cell mediated cytotoxicity	5.1. Immune system	-2.39	-2.52	-2.37
IL-17 signaling pathway	5.1. Immune system	0.82	-1.85	-0.93
Th1 and Th2 cell differentiation	5.1. Immune system	-1.84	-1.92	-1.74
Th17 cell differentiation	5.1. Immune system	-2.1	-2.51	-2.08
T cell receptor signaling pathway	5.1. Immune system	-1.53	-2.08	-2.01
B cell receptor signaling pathway	5.1. Immune system	-1.96	-2.21	-2.22
Fc epsilon RI signaling pathway	5.1. Immune system	-1.4	-1.79	-1.79
Fc gamma R-mediated phagocytosis	5.1. Immune system	-1.72	-1.63	-1.79
Leukocyte transendothelial migration	5.1. Immune system	-2.11	-2.03	-2.06
Intestinal immune network for IgA production	5.1. Immune system	-2.47	-1.79	-1.67
Circadian entrainment	5.10. Environmental adaptation	-1.57	-1.79	-2.07
Thermogenesis	5.10. Environmental adaptation	2.41	2.72	2.16
Synaptic vesicle cycle	5.6. Nervous system	1.47	1.83	1.39
Neurotrophin signaling pathway	5.6. Nervous system	-1.3	-1.6	-1.83
Retrograde endocannabinoid signaling	5.6. Nervous system	1.29	1.83	1.44
Glutamatergic synapse	5.6. Nervous system	-1.45	-1.41	-1.57
Cholinergic synapse	5.6. Nervous system	-1.58	-1.57	-2.08
Taste transduction	5.7. Sensory system	0.5	1.65	-0.86
Insulin signaling pathway	5.2. Endocrine system	-1.03	-1.09	-1.55
Progesterone-mediated oocyte maturation	5.2. Endocrine system	-1.17	-1.57	-1.55
Melanogenesis	5.2. Endocrine system	-1.53	-1.42	-2.11
Prolactin signaling pathway	5.2. Endocrine system	-0.99	-1.47	-1.7
Oxytocin signaling pathway	5.2. Endocrine system	-1.76	-1.78	-2.04
Regulation of lipolysis in adipocytes	5.2. Endocrine system	-1.23	-1.19	-1.81
Aldosterone synthesis and secretion	5.2. Endocrine system	-0.79	-0.88	-1.53
Relaxin signaling pathway	5.2. Endocrine system	-1.37	-1.8	-2.03
Cortisol synthesis and secretion	5.2. Endocrine system	0.82	-1.04	-1.69
Parathyroid hormone synthesis, secretion and action	5.2. Endocrine system	0.69	-1.45	-2.18
Endocrine and other factor-regulated calcium reabsorption	5.5. Excretory system	0.89	1.41	-0.69
Proximal tubule bicarbonate reclamation	5.5. Excretory system	1.95	2.06	1.27
Collecting duct acid secretion	5.5. Excretory system	1.97	2.2	1.98
Bile secretion	5.4. Digestive system	1.65	1.56	0.99

Table 4.27A GSEA analysis using KEGG database sorted by main categories: Environmental Information Processing and Organismal Systems**.** ABCB5+ derived CoCM+; i.p. ABCB5+; i.v. ABCB5+ groups. Significantly (adj. $p<0.05$) differentially expressed pathways (PKD/Mhm (Cy/+) + treatment vs PKD/Mhm (Cy/+)). For each pathway the NES is given. Downregulated pathways are displayed in green, upregulated pathways are displayed in red.

B

KEGG Pathway	Sub Category	NES i.v. ASC	NES i.p. ASC	NES ASC derived CM
MAPK signaling pathway	3.2. Signal transduction	-1.79	-1.4	-0.99
ErbB signaling pathway	3.2. Signal transduction	-1.63	-1.23	-1.01
Ras signaling pathway	3.2. Signal transduction	-1.64	-1.37	-1.12
Rap1 signaling pathway	3.2. Signal transduction	-1.69	-2	-1.24
Calcium signaling pathway	3.2. Signal transduction	-1.61	-1.55	-1.38
cGMP-PKG signaling pathway	3.2. Signal transduction	-1.79	-1.49	-1.2
cAMP signaling pathway	3.2. Signal transduction	-1.8	-1.75	-1.18
Cytokine-cytokine receptor interaction	3.3. Signaling molecules and interactic	-1.17	-1.43	-1.06
NF-kappa B signaling pathway	3.2. Signal transduction	-1.29	-0.97	0.75
FoxO signaling pathway	3.2. Signal transduction	-1.61	-1.49	-1.23
Phosphatidylinositol signaling system	3.2. Signal transduction	-1.61	-1.44	-1.08
Phospholipase D signaling pathway	3.2. Signal transduction	-1.63	-1.32	-0.98
Neuroactive ligand-receptor interaction	3.3. Signaling molecules and interactic	-1.65	-1.57	-1.48
PI3K-Akt signaling pathway	3.2. Signal transduction	-1.7	-1.53	-1.14
Wnt signaling pathway	3.2. Signal transduction	-1.6	-1.49	-1.21
Hedgehog signaling pathway	3.2. Signal transduction	-1.49	-1.75	-0.69
TGF-beta signaling pathway	3.2. Signal transduction	-1.66	-1.69	-1.33
Apelin signaling pathway	3.2. Signal transduction	-1.61	-1.28	-1.28
Hippo signaling pathway	3.2. Signal transduction	-1.61	-1.61	-1.41
Hippo signaling pathway	3.2. Signal transduction	-1.57	-1.68	-1.2
ECM-receptor interaction	3.3. Signaling molecules and interactic	-2.04	-2.02	-1.41
Cell adhesion molecules (CAMs)	3.3. Signaling molecules and interactic	-1.16	-1.21	-1.02
JAK-STAT signaling pathway	3.2. Signal transduction	-1.04	-1.15	-0.78
TNF signaling pathway	3.2. Signal transduction	-1.81	-1.4	-1.06
PPAR signaling pathway	5.2. Endocrine system	1.68	1.89	1.7
Chemokine signaling pathway	5.1. Immune system	-1.25	-1.02	0.72
Longevity regulating pathway	5.9. Aging	-1.61	-1.53	-0.88
Cardiac muscle contraction	5.3. Circulatory system	1.51	1.69	-0.81
Vascular smooth muscle contraction	5.3. Circulatory system	-1.41	-1.05	0.84
Axon guidance	5.8. Development	-2.08	-2.16	-1.66
Osteoclast differentiation	5.8. Development	-1.81	-1.28	-0.94
Complement and coagulation cascades	5.1. Immune system	1.31	1.14	1.35
Platelet activation	5.1. Immune system	-1.02	-1.23	0.73
Antigen processing and presentation	5.1. Immune system	-0.46	1.29	1.18
Toll-like receptor signaling pathway	5.1. Immune system	-1.63	-1.47	0.97
NOD-like receptor signaling pathway	5.1. Immune system	-1.76	-1.18	-0.88
RIG-I-like receptor signaling pathway	5.1. Immune system	-1.78	-0.84	0.76
C-type lectin receptor signaling pathway	5.1. Immune system	-1.5	-1.23	-0.86
Hematopoietic cell lineage	5.1. Immune system	-1.05	-0.9	-1.05
Natural killer cell mediated cytotoxicity	5.1. Immune system	-1.46	-1.09	-0.46
IL-17 signaling pathway	5.1. Immune system	-1.42	-0.93	-1.04
Th1 and Th2 cell differentiation	5.1. Immune system	-1.43	-1.12	0.7
Th17 cell differentiation	5.1. Immune system	-1.34	-0.82	0.73
T cell receptor signaling pathway	5.1. Immune system	-1.32	-1.05	0.79
B cell receptor signaling pathway	5.1. Immune system	-1.5	-1.02	-0.77
Fc epsilon RI signaling pathway	5.1. Immune system	-1.54	-1.26	0.73
Fc gamma R-mediated phagocytosis	5.1. Immune system	-1.59	-1.38	-1.04
Leukocyte transendothelial migration	5.1. Immune system	-1.4	-1.32	-1.27
Intestinal immune network for IgA production	5.1. Immune system	-0.67	-1.31	0.92
Circadian entrainment	5.10. Environmental adaptation	-1.51	-1.38	-0.79
Thermogenesis	5.10. Environmental adaptation	2.39	2.67	1.1
Synaptic vesicle cycle	5.6. Nervous system	0.8	1.45	-1.58
Neurotrophin signaling pathway	5.6. Nervous system	-1.54	-0.93	-0.8
Retrograde endocannabinoid signaling	5.6. Nervous system	1.48	1.94	-0.79
Glutamatergic synapse	5.6. Nervous system	-1.59	-1.62	-1.22
Cholinergic synapse	5.6. Nervous system	-1.63	-1.6	-1.04
Taste transduction	5.7. Sensory system	-1.7	-1.56	-1.76
Insulin signaling pathway	5.2. Endocrine system	-1.55	-1.44	-1.05
Progesterone-mediated oocyte maturation	5.2. Endocrine system	-1.3	-1.17	-0.81
Melanogenesis	5.2. Endocrine system	-1.34	-1.56	-0.92
Prolactin signaling pathway	5.2. Endocrine system	-1.22	-1.23	-0.72
Oxytocin signaling pathway	5.2. Endocrine system	-1.63	-1.67	-1.01
Regulation of lipolysis in adipocytes	5.2. Endocrine system	-1.71	-1.75	-1.36
Aldosterone synthesis and secretion	5.2. Endocrine system	-1.61	-1.2	-1.1
Relaxin signaling pathway	5.2. Endocrine system	-1.84	-1.39	-0.97
Cortisol synthesis and secretion	5.2. Endocrine system	-1.8	-1.34	-1.17
Parathyroid hormone synthesis, secretion and action	5.2. Endocrine system	-1.96	-1.62	-1.18
Endocrine and other factor-regulated calcium reabsorption	5.5. Excretory system	-1.15	-1.07	-1.8
Proximal tubule bicarbonate reclamation	5.5. Excretory system	1.1	1.45	1.04
Collecting duct acid secretion	5.5. Excretory system	1.12	1.82	-1.84
Bile secretion	5.4. Digestive system	-1.01	0.92	1.01

Table 4.27B GSEA analysis using KEGG database sorted by main categories: Environmental Information Processing and Organismal Systems**.** ASC derived CM; i.p ASC; i.v. ASC groups. Significantly (adj. p<0.05) differentially expressed

pathways (PKD/Mhm (Cy/+) + treatment vs PKD/Mhm (Cy/+)). For each pathway the NES is given. Downregulated pathways are displayed in green, upregulated pathways are displayed in red.

4.3 Long term effects of stem cells and conditioned media therapy in PCK rats

4.3.1 *Changes in BW and kidney histology*

A gradual and constant increase in the BW was recorded in all the treated PCK groups (**figure 4.15**), with no significant differences when compared to the BW values of the untreated group.

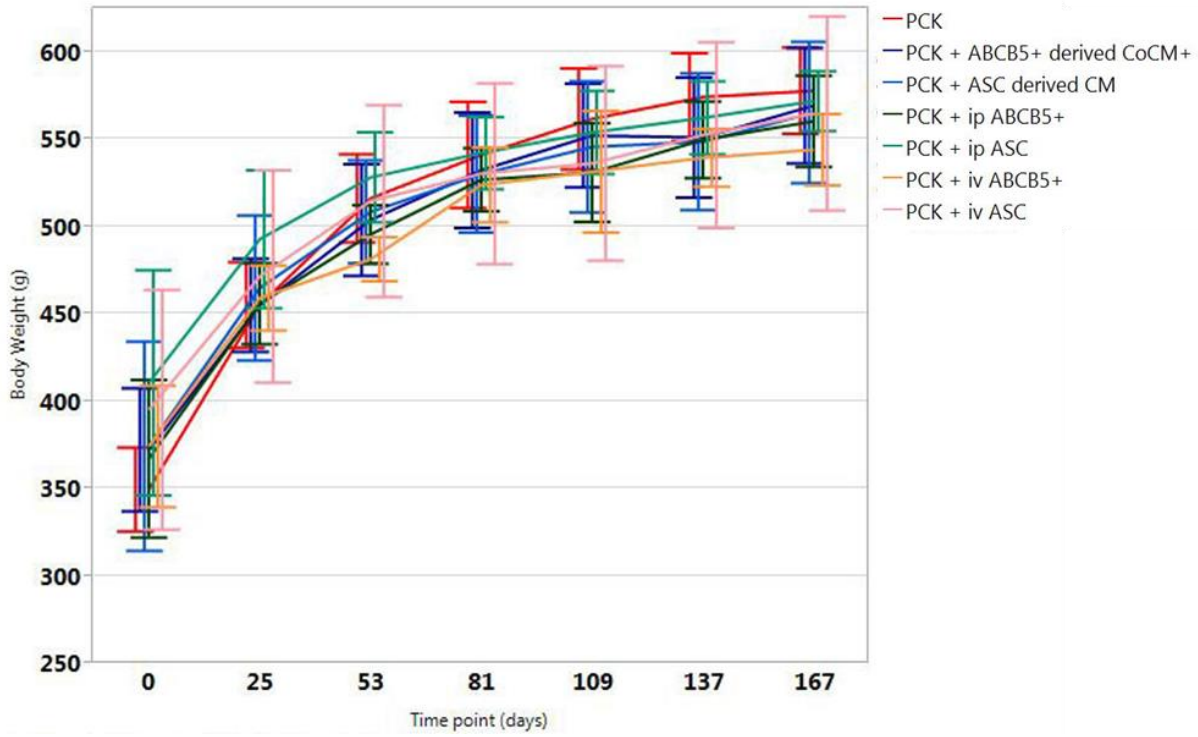

Figure 4.15 Effect on BW of different treatments in PCK (n=6 in each group). Data are shown as means ± Std.Dev.

Left kidney weight and the Kw/BW ratio were slightly increased in ABCB5+ derived CoCM+ and ASC derived CM+ groups in comparison to the untreated group (**table 4.28 A-B**).

A	Animal group	Left kidney (g)	Kw/BW ratio
	Untreated	5.3 ± 0.3	0.9 ± 0.1
	+ ABCB5+ derived CoCM+	5.8 ± 0.9	1.0 ± 0.2
	+ i.p. ABCB5+	5.4 ± 1.4	1.0 ± 0.3
	+ i.v. ABCB5+	5.2 ± 0.8	1.0 ± 0.1

Table 4.28A Effect of different treatments on left kidney weight and Kw/BW ratio in PCK groups (n=6 in each group). Comparison between ABCB5+ derived CoCM+, i.p. or i.v. ABCB5+ groups and untreated. Data are shown as mean ± Std.Dev.

B	Animal group	Left kidney (g)	Kw/BW ratio
	Untreated	5.3 ± 0.3	0.9 ± 0.1
	+ ASC derived CM	6.0 ± 1.0	1.1 ± 0.2
	+ i.p. ASC	5.0 ± 1.2	0.9 ± 0.2
	+ i.v. ASC	5.4 ± 0.4	1.0 ± 0.1

Table 4.28B Effect of different treatments on left kidney weight and Kw/BW ratio in PCK groups (n=6 in each group). Comparison between ASC derived CM, i.p. or i.v. ASC groups and untreated. Data are shown as mean ± Std.Dev.

Histological staining of 3 μm kidney sections highlighted an increase of cysts number in the renal tissue, with the exception of i.p. ABCB5+ treatment. However, the average size was decreased in ASC derived CM, i.v. and i.p. ASC groups. In the same groups, a slight, but no significant, reduction of fibrosis was detected (**table 4.29 A-B**, **figure 4.16**).

A	Animal group	Cyst number	Average size (pixels)	% cyst area	% fibrosis
	Untreated	1320.8 ± 621.7	972.5 ± 607.7	10.0 ± 4.5	13.7 ± 20.8
	+ ABCB5+ derived CoCM+	1499.8 ± 1059.1	982.5 ± 437.2	12.1 ± 4.3	12.1 ± 4.6
	+ i.p. ABCB5+	1284.5 ± 535.9	1016.8 ± 499.3	12.2 ± 5.2	11.0 ± 3.6
	+ i.v. ABCB5+	1847.3 ± 1137.7	1060.9 ± 490.4	15.2 ± 3.9	14.0 ± 2.7

Table 4.29A Effect of different treatments on cyst number, average size, percentage of cyst and fibrosis area in PCK groups (n=6 in each group). Comparison between ABCB5+ derived CoCM+, i.p. or i.v. ABCB5+ groups and untreated. Analysis was performed on whole 3 μm kidney section. Data are shown as mean ± Std.

B	Animal group	Cyst number	Average size (pixels)	% cyst area	% fibrosis
	Untreated	1320.8 ± 621.7	972.5 ± 607.7	10.0 ± 4.5	13.7 ± 20.8
	+ ASC derived CM	1779.3 ± 423.1	760.1 ± 169.8	13.1 ± 4.1	8.2 ± 6.3
	+ i.p. ASC	1917.2 ± 583.3	638.1 ± 258.5	12.1 ± 4.5	8.2 ± 4.3
	+ i.v. ASC	2008.6 ± 322.3	617.0 ± 131.9	12.5 ± 3.3	7.8 ± 3.6

Table 4.29B Effect of different treatments on cyst number, average size, percentage of cyst and fibrosis area in PCK groups (n=6 in each group). Comparison between ASC derived CM, i.p. or i.v. ASC groups and untreated. Analysis was performed on whole 3 μm kidney section. Data are shown as mean ± Std.

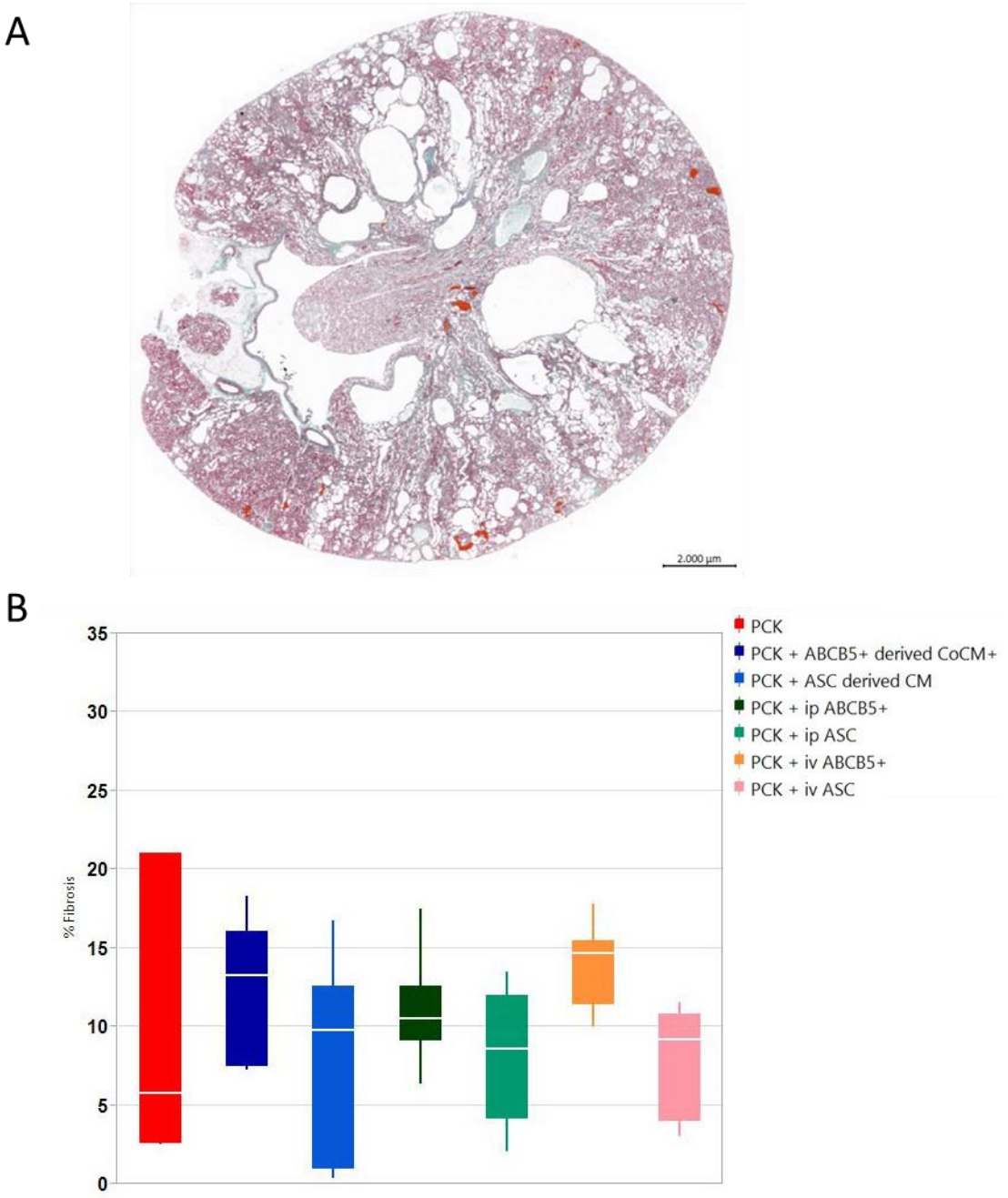

Figure 4.16 Fibrosis in PCK kidneys (n=6 in each group). (A) Whole kidney scan of PCK rat, Masson-Goldner trichrome staining, light green stains fibrotic tissue; (B) Graphic quantification of fibrosis after different treatments on PCK kidneys. Data are shown as box plots with the median, upper and lower quartile (interquartile range (IQR)) and whiskers (1.5x IQR). Images acquired with Axio Scan.Z1 microscope (ZEISS), 20x objective.

Evaluation of the cyst number and fibrosis was performed also on 3 µm liver slices stained with Masson-Goldner.

An increase of the cyst number and size was registered in the i.v. and i.p. ASC and ASC derived CM groups. Cyst number was reduced in i.v. and i.p. ABCB5+ groups but in the same groups an increase of the cyst size was detected. A major increase of fibrotic tissue in the liver was detected in all the treated groups, compared to the untreated (**table 4.30 A-B**).

A	Animal group	Cyst number	Average size (pixels)	% cyst area	% fibrosis
	Untreated	579.0 ± 86.3	673.3 ± 203.2	3.8 ± 0.9	4.8 ± 3.0
	+ ABCB5+ derived CoCM+	547.3 ± 161.1	983.8 ± 372.9	5.2 ± 3.1	16.7 ± 7.6
	+ i.p. ABCB5+	385.8 ± 242.2	1018.1 ± 298.4	3.9 ± 3.0	12.3 ± 8.1
	+ i.v. ABCB5+	379.7 ± 97.6	937.3 ± 356.4	3.6 ± 1.9	10.5 ± 2.6

Table 4.30A Effect of different treatments on cyst number, average size, percentage of cyst and fibrosis area in PCK groups (n=6 in each group). Comparison between ABCB5+ derived CoCM+, i.p. or i.v. ABCB5+ groups and untreated. Analysis was performed on whole 3 μm liver section. Data are shown as mean ± Std.Dev.

B	Animal group	Cyst number	Average size (pixels)	% cyst area	% fibrosis
	Untreated	579.0 ± 86.3	673.3 ± 203.2	3.8 ± 0.9	4.8 ± 3.0
	+ ASC derived CM	958.0 ± 291.9	926.2 ± 254.1	8.1 ± 3.2	12.3 ± 8.0
	+ i.p. ASC	741.0 ± 317.1	801.4 ± 327.5	6.4 ± 4.7	10.2 ± 5.0
	+ i.v. ASC	752.7 ± 305.1	988.6 ± 303.4	7.9 ± 4.0	11.1 ± 6.5

Table 4.30B Effect of different treatments on cyst number, average size, percentage of cyst and fibrosis area in PCK groups (n=6 in each group). Comparison between ASC derived CM, i.p. or i.v. ASC groups and untreated. Analysis was performed on whole 3 μm liver section. Data are shown as mean ± Std.Dev.

A reduction of Tunel signal was noted in all the treated groups, in particular ABCB5+ derived CoCM + ($p<0.005$), ASC derived CM, i.v. and i.p. ABCB+ ($p<0.05$), when compared to the control. The signal of Ki67 positive cells was decreased in all the groups, indicating a reduction of apoptosis and proliferation levels in cells (**table 4.31 A-B**).

A	Animal group	Tunel positive cells	Ki67 positive cells
	Untreated	1460.5 ± 324.6	1721.0 ± 1369.8
	+ ABCB5+ derived CoCM+	**953.8 ± 145.5****	884.5 ± 87.4
	+ i.p. ABCB5+	**1038.5 ± 160.4***	1018.2 ± 271.1
	+ i.v. ABCB5+	**1024.3 ± 131.8***	934.0 ± 118.8

Table 4.31A Effect of different treatments on Tunel and Ki67 positive nuclei in in PCK groups (n=6 in each group). Comparison between ABCB5+ derived CoCM+, i.p. or i.v. ABCB5+ groups and untreated. Analysis was performed on whole 3 μm kidney section. Data are shown as mean ± Std.Dev. Values significantly different (treated vs untreated) are indicated as *$p<0.05$, **$p<0.005$.

B	Animal group	Tunel positive cells	Ki67 positive cells
	Untreated	1460.5 ± 324.6	1721.0 ± 1369.8
	+ ASC derived CM	1121.0 ± 192.5	1025.4 ± 203.3
	+ i.p. ASC	1022.5 ± 331.9	893.0 ± 310.4
	+ i.v. ASC	**926.3 ± 159.2***	730.3 ± 215.2

Table 4.31B Effect of different treatments on Tunel and Ki67 positive nuclei in in PCK groups (n=6 in each group). Comparison between ASC derived CM, i.p. or i.v. ASC groups and untreated. Analysis was performed on whole 3 µm kidney section. Data are shown as mean ± Std.Dev. Values significantly different (treated vs untreated) are indicated as *p<0.05.

4.3.2 *Plasma chemistry, urine analysis and renal function*

Plasma biochemistry results show, at day 167, a slight increase in creatinine levels in i.p. ABCB5+ and ASC derived CM groups. No alterations were recorded in ABCB5+ derived CoCM+, i.v. ABCB5+, i.v. and i.p. ASC groups. Urea levels were slight decreased in ABCB5+ derived CoCM+, i.p. and i.v. ABCB5+ groups when compared to the untreated, while no changes were noted in ASC derived CM, i.p. and i.v. ASC groups. Sodium levels were significantly decreased in i.p. ABCB5+ and ABCB5+ derived CoCM+ groups. AST and ALT concentrations in the plasma were also tested for monitoring the hepatic function. **Table 4.32** (**A-B**) shows the results of the plasma biochemistry analysis (for individual days see **appendix 7**).

A Parameter	Animal group	Day 167
Creatinine	Untreated	0.5 ± 0.1
(mg/dl)	+ ABCB5+ derived CoCM+	0.5 ± 0.1
	+ i.p. ABCB5+	0.6 ± 0.2
	+ i.v. ABCB5+	0.5 ± 0.1
Urea	Untreated	56.6 ± 3.2
(mg/dl)	+ ABCB5+ derived CoCM+	50.7 ± 8.9
	+ i.p. ABCB5+	54.1 ± 16.5
	+ i.v. ABCB5+	50.3 ± 10.6
Na	Untreated	143.8 ± 1.8
(mmol/l)	+ ABCB5+ derived CoCM+	**139.3 ± 2.3***
	+ i.p. ABCB5+	**140.3 ± 2.2***
	+ i.v. ABCB5+	141.0 ± 1.9
K	Untreated	5.1 ± 0.2
(mmol/l)	+ ABCB5+ derived CoCM+	5.1 ± 0.5
	+ i.p. ABCB5+	5.0 ± 0.4
	+ i.v. ABCB5+	4.7 ± 0.5
Ca	Untreated	2.8 ± 0.1
(mmol/l)	+ ABCB5+ derived CoCM+	2.7 ± 0.2
	+ i.p. ABCB5+	2.7 ± 0.1
	+ i.v. ABCB5+	2.7 ± 0.1
PO_4	Untreated	2.2 ± 0.1
(mmol/l)	+ ABCB5+ derived CoCM+	2.1 ± 0.3
	+ i.p. ABCB5+	2.2 ± 0.2
	+ i.v. ABCB5+	2.1 ± 0.2

Cholesterol	Untreated	301.2 ± 62.1
(mg/dl)	+ ABCB5+ derived CoCM+	273.5 ± 76.3
	+ i.p. ABCB5+	294.0 ± 67.9
	+ i.v. ABCB5+	266.2 ± 44.5
Triglycerides	Untreated	160.5 ± 46.9
(mg/dl)	+ ABCB5+ derived CoCM+	143.7 ± 45.0
	+ i.p. ABCB5+	153.5 ± 47.8
	+ i.v. ABCB5+	118.2 ± 34.7
Glucose	Untreated	150.3 ± 24.0
(mg/dl)	+ ABCB5+ derived CoCM+	147.2 ± 18.2
	+ i.p. ABCB5+	145.8 ± 23.3
	+ i.v. ABCB5+	143.8 ± 11.8
Protein	Untreated	69.5 ± 2.1
(mg/dl)	+ ABCB5+ derived CoCM+	69.3 ± 6.4
	+ i.p. ABCB5+	67.5 ± 3.8
	+ i.v. ABCB5+	68.3 ± 4.9
ALT	Untreated	73.3 ± 40.0
(U/l)	+ ABCB5+ derived CoCM+	61.0 ± 17.8
	+ i.p. ABCB5+	69.3 ± 16.3
	+ i.v. ABCB5+	66.1 ± 13.4
AST	Untreated	135.2 ± 144.9
(U/l)	+ ABCB5+ derived CoCM+	144.0 ± 67.3
	+ i.p. ABCB5+	136.8 ± 87.8
	+ i.v. ABCB5+	119.5 ± 59.1

Table 4.32A Plasma biochemistry in PCK groups (untreated; ABCB5+ derived CoCM+; i.p. ABCB5+; i.v. ABCB5+; n=6 in each group) at day 167. Data are shown as mean ± Std.Dev. Values significantly different (treated vs untreated) are indicated as *p<0.05.

B	Parameter	Animal group	Day 167
	Creatinine	Untreated	0.5 ± 0.1
	(mg/dl)	+ ASC derived CM	0.6 ± 0.1
		+ i.p. ASC	0.5 ± 0.1
		+ i.v. ASC	0.5 ± 0.1
	Urea	Untreated	56.6 ± 3.2
	(mg/dl)	+ ASC derived CM	56.7 ± 7.0
		+ i.p. ASC	57.3 ± 10.6
		+ i.v. ASC	56.1 ± 3.5
	Na	Untreated	143.8 ± 1.8
	(mmol/l)	+ ASC derived CM	141.7 ± 1.4
		+ i.p. ASC	141.8 ± 1.5
		+ i.v. ASC	142.3 ± 1.4
	K	Untreated	5.1 ± 0.2
	(mmol/l)	+ ASC derived CM	5.5 ± 0.5
		+ i.p. ASC	5.3 ± 0.2
		+ i.v. ASC	5.0 ± 0.5
	Ca	Untreated	2.8 ± 0.1

(mmol/l)	+ ASC derived CM	2.8 ± 0.0
	+ i.p. ASC	2.8 ± 0.1
	+ i.v. ASC	2.8 ± 0.1
PO_4	Untreated	2.2 ± 0.1
(mmol/l)	+ ASC derived CM	2.0 ± 0.4
	+ i.p. ASC	2.1 ± 0.4
	+ i.v. ASC	1.9 ± 0.3
Cholesterol	Untreated	301.2 ± 62.1
(mg/dl)	+ ASC derived CM	222.8 ± 64.3
	+ i.p. ASC	257.3 ± 52.7
	+ i.v. ASC	252.8 ± 46.7
Triglycerides	Untreated	160.5 ± 46.9
(mg/dl)	+ ASC derived CM	148.2 ± 30.2
	+ i.p. ASC	126.5 ± 16.0
	+ i.v. ASC	143.0 ± 30.7
Glucose	Untreated	150.3 ± 24.0
(mg/dl)	+ ASC derived CM	142.8 ± 23.0
	+ i.p. ASC	141.0 ± 12.0
	+ i.v. ASC	127.0 ± 18.4
Protein	Untreated	69.5 ± 2.1
(mg/dl)	+ ASC derived CM	72.2 ± 4.0
	+ i.p. ASC	69.7 ± 4.1
	+ i.v. ASC	70.5 ± 2.7
ALT	Untreated	73.3 ± 40.0
(U/l)	+ ASC derived CM	81.3 ± 15.4
	+ i.p. ASC	80.9 ± 20.4
	+ i.v. ASC	83.6 ± 15.48
AST	Untreated	135.2 ± 144.9
(U/l)	+ ASC derived CM	325.2 ± 146.4
	+ i.p. ASC	155.7 ± 74.9
	+ i.v. ASC	256.0 ± 127.5

Table 4.32B Plasma biochemistry in PCK groups (untreated; ASC derived CM; i.p. ASC; i.v. ASC; n=6 in each group) at day 167. Data are shown as mean ± Std.Dev.

Urine samples were collected after placing the animals into metabolic cages overnight for 16 hours. **Table 4.33** shows the difference of diuresis, food and water intake values between treated and untreated groups at day 167 (for individual days see **appendix 8**).

A Parameter	Animal group	Day 167
Diuresis (ml)	Untreated	25.3 ± 2.7
	+ ABCB5+ derived CoCM+	24.7 ± 8.8
	+ i.p. ABCB5+	22.4 ± 3.7
	+ i.v. ABCB5+	18.7 ± 5.9
Food intake	Untreated	10.8 ± 3.5

(g)	+ ABCB5+ derived CoCM+	15.6 ± 5.1
	+ i.p. ABCB5+	14.5 ± 2.9
	+ i.v. ABCB5+	15.0 ± 4.2
Water intake	Untreated	33.2 ± 4.4
(g)	+ ABCB5+ derived CoCM+	31.8 ± 9.9
	+ i.p. ABCB5+	30.7 ± 15.9
	+ i.v. ABCB5+	24.2 ± 13.0

Table 4.33A Effect of different treatments on diuresis, food intake and water intake in PCK groups (n=6 in each group) at day 167. Comparison between ABCB5+ derived CoCM+, i.v. or i.p. ABCB5+ groups and untreated. Data are shown as mean ± Std.Dev.

B Parameter	Animal group	Day 167
Diuresis (ml)	Untreated	25.3 ± 2.7
	+ ASC derived CM	29.3 ± 12.2
	+ i.p. ASC	25.4 ± 4.3
	+ i.v. ASC	26.8 ± 6.3
Food intake	Untreated	10.8 ± 3.5
(g)	+ ASC derived CM	17.4 ± 4.7
	+ i.p. ASC	14.9 ± 3.4
	+ i.v. ASC	14.2 ± 5.8
Water intake	Untreated	33.2 ± 4.4
(g)	+ ASC derived CM	38.5 ± 17.0
	+ i.p. ASC	38.3 ± 6.9
	+ i.v. ASC	46.4 ± 19.7

Table 4.33B Effect of different treatments on diuresis, food intake and water intake in PCK groups (n=6 in each group) at day 167. Comparison between ASC derived CM, i.v. or i.p. ASC groups and untreated. Data are shown as mean ± Std.Dev.

Table 4.34 (**A**-**B**) illustrates the urine parameters analysed at day 167 (for individual days see **appendix 9**). Despite no significant differences were detected, proteinuria levels were decreased in all the treated groups when compared to the untreated, while albumin levels increased.

A Parameter	Animal group	Day 167
Creatinine	Untreated	14.8 ± 3.2
(mg/16h)	+ ABCB5+ derived CoCM+	11.9 ±1.8
	+ i.p. ABCB5+	12.0 ± 2.4
	+ i.v. ABCB5+	12.5 ± 1.7
Urea	Untreated	681.6 ± 91.3
(mg/16h)	+ ABCB5+ derived CoCM+	586.1 ± 147.9
	+ i.p. ABCB5+	610.0 ± 148.5
	+ i.v. ABCB5+	571.4 ± 105.2
Na	Untreated	1.3 ± 0.2
(mmol/16h)	+ ABCB5+ derived CoCM+	1.6 ± 0.8

	+ i.p. ABCB5+	1.5 ± 0.6
	+ i.v. ABCB5+	1.4 ± 0.9
K	Untreated	3.5 ± 0.4
(mmol/16h)	+ ABCB5+ derived CoCM+	4.3 ± 1.5
	+ i.p. ABCB5+	3.7 ± 0.9
	+ i.v. ABCB5+	3.6 ± 1.0
Ca	Untreated	0.08 ± 0.03
(mmol/16h)	+ ABCB5+ derived CoCM+	0.10 ± 0.03
	+ i.p. ABCB5+	0.08 ± 0.03
	+ i.v. ABCB5+	0.06 ± 0.03
PO_4	Untreated	0.1 ± 0.1
(mmol/16h)	+ ABCB5+ derived CoCM+	0.1 ± 0.1
	+ i.p. ABCB5+	0.1 ± 0.1
	+ i.v. ABCB5+	0.3 ± 0.3
Glucose	Untreated	1.9 ± 1.5
(mg/16h)	+ ABCB5+ derived CoCM+	2.3 ± 0.6
	+ i.p. ABCB5+	3.0 ± 0.7
	+ i.v. ABCB5+	2.4 ± 0.9
Protein	Untreated	436.8 ± 101.5
(mg/16h)	+ ABCB5+ derived CoCM+	290.1 ± 114.7
	+ i.p. ABCB5+	311.9 ± 161.3
	+ i.v. ABCB5+	288.1 ± 117.3
Albumin	Untreated	140.6 ± 71.2
(mg/16h)	+ ABCB5+ derived CoCM+	200.6 ± 68.5
	+ i.p. ABCB5+	319.8 ± 220.3
	+ i.v. ABCB5+	200.0 ± 52.9

Table 4.34A Urine biochemistry in PCK groups (untreated; ABCB5+ derived CoCM+; i.p. ABCB5+; i.v. ABCB5+; n=6 in each group) at day 167. Data are shown as mean ± Std.Dev.

B Parameter	Animal group	Day 167
Creatinine	Untreated	14.8 ± 3.2
(mg/16h)	+ ASC derived CM	12.3 ± 1.0
	+ i.p. ASC	12.4 ± 1.2
	+ i.v. ASC	12.4 ± 1.7
Urea	Untreated	681.6 ± 91.3
(mg/16h)	+ ASC derived CM	628.7 ± 81.8
	+ i.p. ASC	658.9 ± 111.4
	+ i.v. ASC	659.8 ± 99.8
Na	Untreated	1.3 ± 0.2
(mmol/16h)	+ ASC derived CM	1.8 ± 0.6
	+ i.p. ASC	1.3 ± 0.3
	+ i.v. ASC	1.7 ± 0.8
K	Untreated	3.5 ± 0.4
(mmol/16h)	+ ASC derived CM	4.2 ± 1.4
	+ i.p. ASC	4.0 ± 0.5
	+ i.v. ASC	3.9 ± 0.4

Ca	Untreated	0.08 ± 0.03
(mmol/16h)	+ ASC derived CM	0.07 ± 0.03
	+ i.p. ASC	0.08 ± 0.02
	+ i.v. ASC	0.07 ± 0.01
PO_4	Untreated	0.1 ± 0.1
(mmol/16h)	+ ASC derived CM	0.1 ± 0.1
	+ i.p. ASC	0.1 ± 0.1
	+ i.v. ASC	0.1 ± 0.1
Glucose	Untreated	1.9 ± 1.5
(mg/16h)	+ ASC derived CM	5.9 ± 4.5
	+ i.p. ASC	4.4 ± 1.6
	+ i.v. ASC	3.7 ± 2.2
Protein	Untreated	436.8 ± 101.5
(mg/16h)	+ ASC derived CM	398.2 ± 143.4
	+ i.p. ASC	350.4 ± 79.0
	+ i.v. ASC	320.6 ± 63.2
Albumin	Untreated	140.6 ± 71.2
(mg/16h)	+ ASC derived CM	152.7 ± 66.9
	+ i.p. ASC	197.7 ± 59.3
	+ i.v. ASC	154.8 ± 28.7

Table 4.34B Urine biochemistry in PCK groups (untreated; ASC derived CM; i.p. ASC; i.v. ASC; n=6 in each group) at day 167. Data are shown as mean ± Std.Dev.

Table 4.35 (**A-B**) shows a reduction of ABZWCY-HβCD $_{t1/2}$ in PCK treated groups at day 167. In particular, a significant reduction was recorded in the i.p. ABCB5+ group ($p<0.05$). A minor decline was also detected in ABCB5+ derived CoCM+, i.v. ABCB5+, i.p. and i.v. ASC groups (**figure 4.17**, for individual days see **appendix 10**).

A	Parameter	Animal group	Day 167
	ABZWCY-HβCD $t_{1/2}$ (min)	Untreated	75.5 ± 11.6
		+ ABCB5+ derived CoCM+	66.5 ± 15.1
		+ i.p. A BCB5+	**45.5 ± 22.7***
		+ i.v. ABCB5+	50.9 ± 10.12

Table 4.35A Effect of different treatments on ABZWCY-HβCD $t_{1/2}$ in PCK groups (n=6 in each group) at day 167. Comparison between ABCB5+ derived CoCM+, i.p. or i.v. ABCB5+ groups and untreated. Data are shown as mean ± Std.Dev. Values significantly different (treated vs untreated) are indicated as *$p<0.05$.

B	Parameter	Animal group	Day 167
	ABZWCY-HβCD $t_{1/2}$ (min)	Untreated	75.5 ± 11.6
		+ ASC derived CM	72.5 ± 24.8
		+ i.p. ASC	52.7 ± 4.9
		+ i.v. ASC	54.5 ± 9.3

Table 4.35B Effect of different treatments on ABZWCY-HβCD $t_{1/2}$ in PCK groups (n=6 in each group) at day 167. Comparison between ASC derived CM, i.p. or i.v. ASC groups and untreated. Data are shown as mean ± Std.Dev. Values significantly different (treated vs untreated) are indicated as *$p<0.05$.

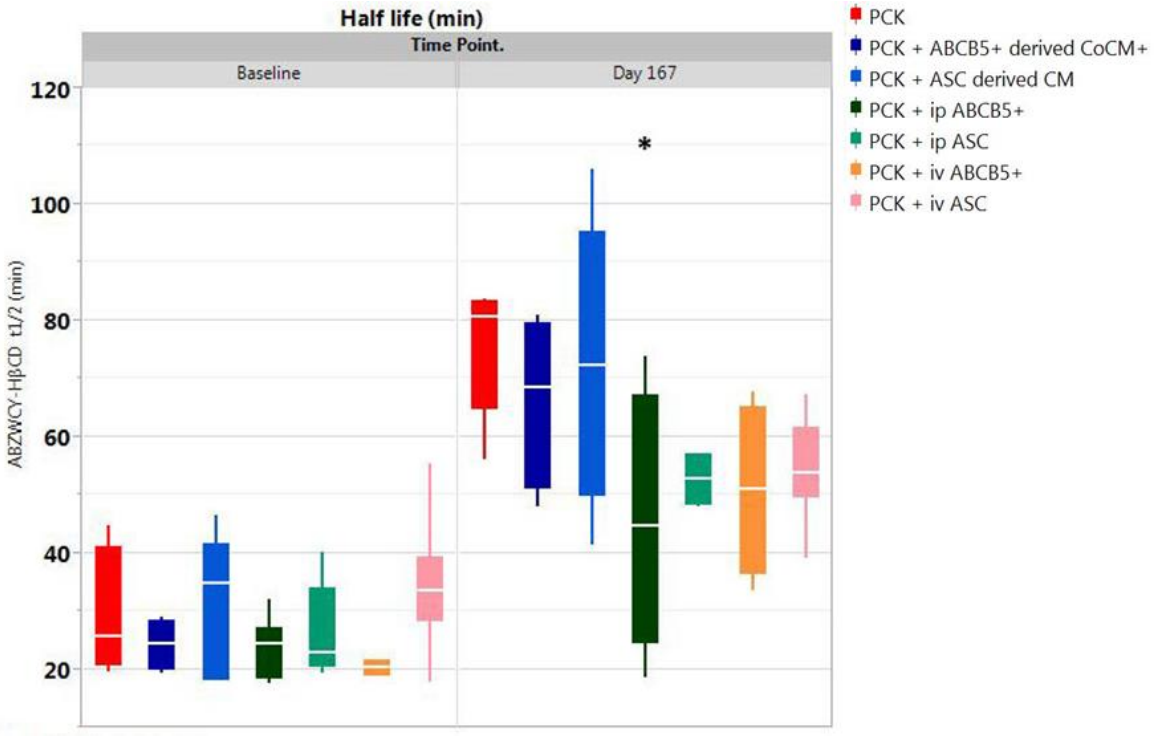

Figure 4.17 Effect of different treatments on ABZWCY-HβCD $t_{1/2}$ in PCK model (n=6 in each group). Data are shown as box plots with the median, upper and lower quartile (interquartile range (IQR)) and whiskers (1.5x IQR). Values significantly different (treatment vs PKD/Mhm (Cy/+)) are indicated as *p<0.05.

Table 4.36 (**A-B**) summarizes the results obtained for ABZWCY-HβCD $t_{1/2}$ and traditional plasma parameters for renal function (creatinine and urea) in each PCK group.

A	Animal group	ABZWCY-HβCD $t_{1/2}$ (min)	Creatinine (mg/dl)	Urea (mg/dl)
	Untreated	75.5 ± 11.6	0.5 ± 0.1	56.6 ± 3.2
	+ ABCB5+ derived CoCM+	66.5 ± 15.1	0.5 ± 0.1	50.7 ± 8.9
	+ i.p. ABCB5+	**45.5 ± 22.7***	0.6 ± 0.2	54.1 ± 16.5
	+ i.v. ABCB5+	50.9 ± 10.1	0.5 ± 0.1	50.3 ± 10.6

Table 4.36A Effect of different treatments on ABZWCY-HβCD $t_{1/2}$, plasma creatinine and urea in PCK groups (n=6 in each group) at day 167. Comparison between ABCB5+ CoCM+, i.p. or i.v. ABCB5+ groups and untreated. Data are shown as mean ± Std.Dev. Values significantly different (treated vs untreated) are indicated as *p<0.05.

B	Animal group	ABZWCY-HβCD $t_{1/2}$ (min)	Creatinine (mg/dl)	Urea (mg/dl)
	Untreated	75.5 ± 11.6	0.5 ± 0.1	56.6 ± 3.2
	+ ASC derived CM	72.5 ± 24.8	0.6 ± 0.1	56.7 ± 7.0
	+ i.p. ASC	52.7 ± 4.9	0.5 ± 0.1	57.3 ± 10.6
	+ i.v. ASC	54.5 ± 9.3	0.5 ± 0.1	56.1 ± 3.5

Table 4.36B Effect of different treatments on ABZWCY-HβCD $t_{1/2}$, plasma creatinine and urea in PCK groups (n=6 in each group) at day 167. Comparison between ASC derived CM, i.p. or i.v. ASC groups and untreated. Data are shown as mean ± Std.Dev.

4.3.3 *Gene expression profiling*

The GSEA analysis was performed on each treated PCK group in comparison with the untreated group. **Table 4.37** (**A-B**) illustrates the number of up and downregulated pathways in each treated group.

A Animal group

	Analysed pathways	304
+ABCB5+ derived CoCM+	Significantly regulated pathways (adjusted p-value < 0.05)	51
	Significantly up-regulated pathways (adjusted p-value < 0.05)	14
	Significantly down-regulated pathways (adjusted p-value < 0.05)	37
+ i.p. ABCB5+	Significantly regulated pathways (adjusted p-value < 0.05)	124
	Significantly up-regulated pathways (adjusted p-value < 0.05)	31
	Significantly down-regulated pathways (adjusted p-value < 0.05)	93
+ i.v. ABCB5+	Significantly regulated pathways (adjusted p-value < 0.05)	27
	Significantly up-regulated pathways (adjusted p-value < 0.05)	12
	Significantly down-regulated pathways (adjusted p-value < 0.05)	15

Table 4.37A Overview of GSEA displaying the numbers of up- and downregulated pathways in PCK groups (ABCB5+ derived CoCM+; i.p. ABCB5+; i.v. ABCB5+; n=6 in each group).

B Animal group

	Analysed pathways	305
+ASC derived CM	Significantly regulated pathways (adjusted p-value < 0.05)	139
	Significantly up-regulated pathways (adjusted p-value < 0.05)	74
	Significantly down-regulated pathways (adjusted p-value < 0.05)	65
+ i.p. ASC	Significantly regulated pathways (adjusted p-value < 0.05)	69
	Significantly up-regulated pathways (adjusted p-value < 0.05)	43
	Significantly down-regulated pathways (adjusted p-value < 0.05)	26
+ i.v. ASC	Significantly regulated pathways (adjusted p-value < 0.05)	57
	Significantly up-regulated pathways (adjusted p-value < 0.05)	15
	Significantly down-regulated pathways (adjusted p-value < 0.05)	42

Table 4.37B Overview of GSEA displaying the numbers of up- and downregulated pathways in PCK groups (ASC derived CM; i.p. ASC; i.v. ASC; n=6 in each group).

Figure 4.18 gives the main categories of pathways taken into account while Venn diagrams in **figure 4.19** shows the number of common pathways between each stem cell treatment and conditioned media.

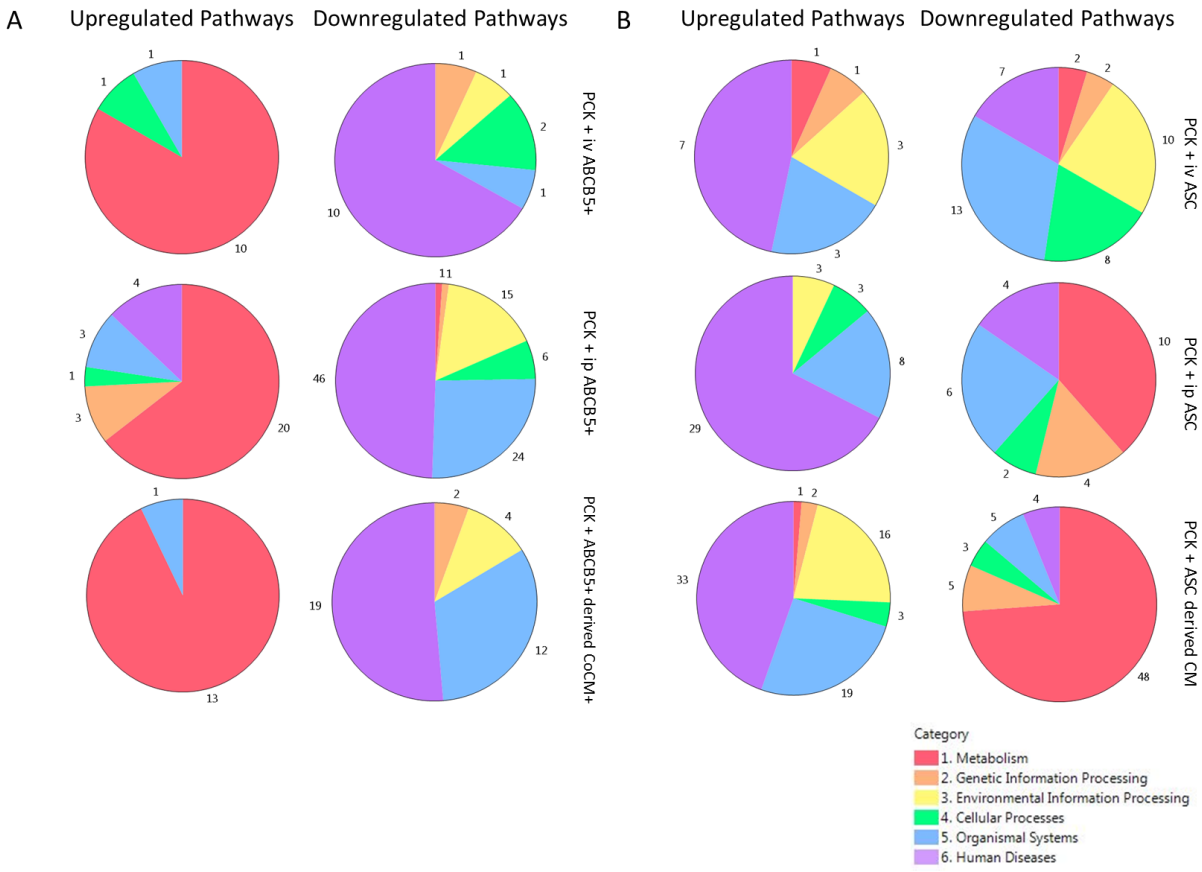

Figure 4.18 Pie charts showing the distribution of pathways containing significantly up- and downregulated genes sorted by main categories of KEGG database. A) ABCB5+ derived CoCM+, i.p. or i.v. ABCB5+ treatment, B) ASC derived CM, i.p. or i.v. ASC treatment. The numbers around the pie charts indicate the number of up- and downregulated pathways contained in each category.

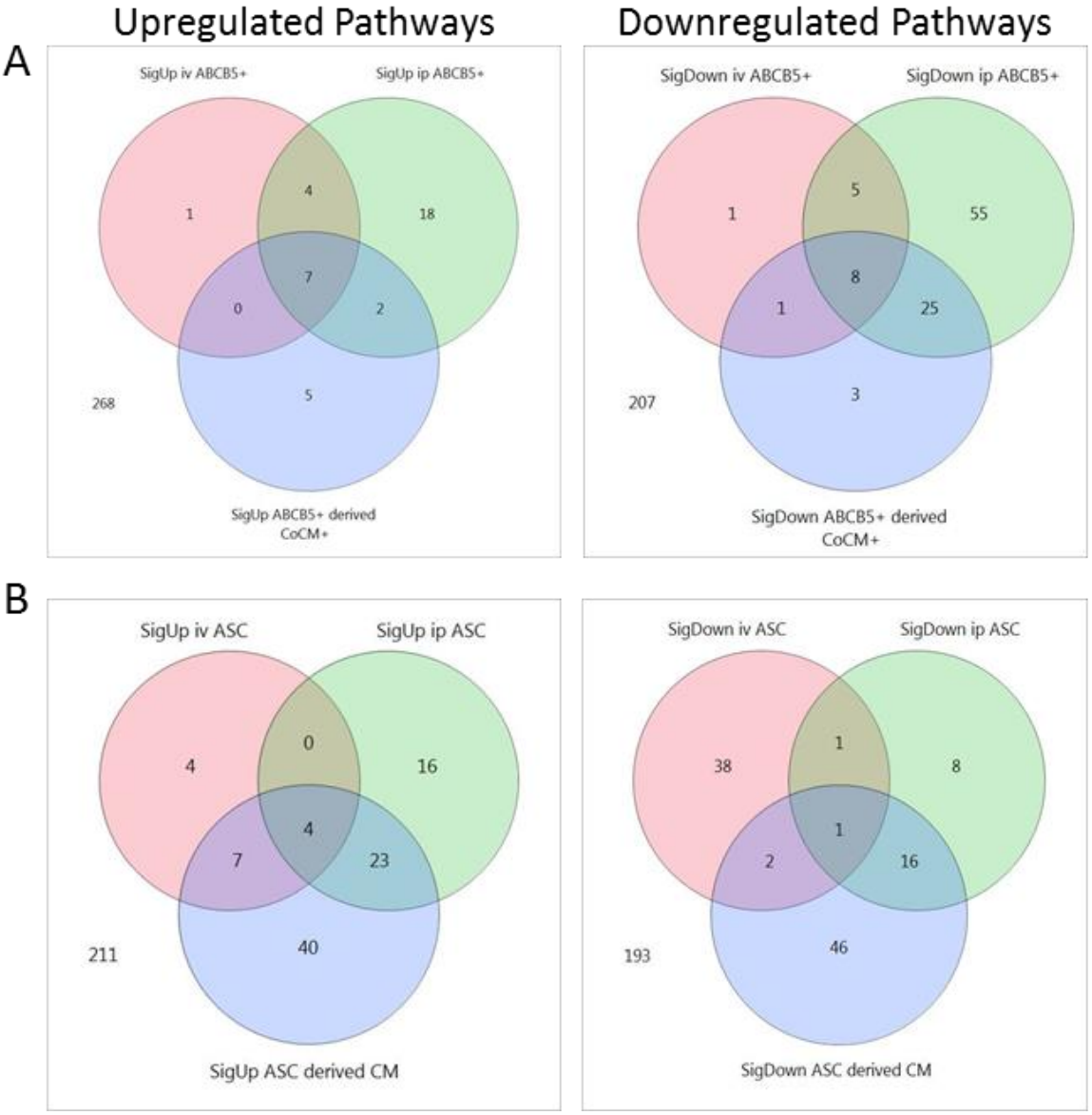

Figure 4.19 Venn diagrams showing the common significant up- and downregulated pathways in PCK. A) ABCB5+ derived CoCM+, i.p. and i.v. ABCB5+ treatment, B) ASC derived CM, i.p. and i.v. ASC treatment. The numbers inside the Venn diagrams indicate the number of un- and downregulated pathways contained in each category

Figure 4.20, shows the common up- and downregulated pathways between the different stem cells and conditioned media administrations. Only 1 common upregulated pathway between ASC derived CM and ABCB5+ derived CoCM+ groups was identified, no one between i.p. ASC and i.p. ABCB5+ or i.v ASC and i.v. ABCB5+. Three common pathways were found downregulated between i.v. ASC and ABCB5+ and no one within i.p. ASC and ABCB5+ or ASC derived CM and ABCB5+ derived CoCM+ groups.

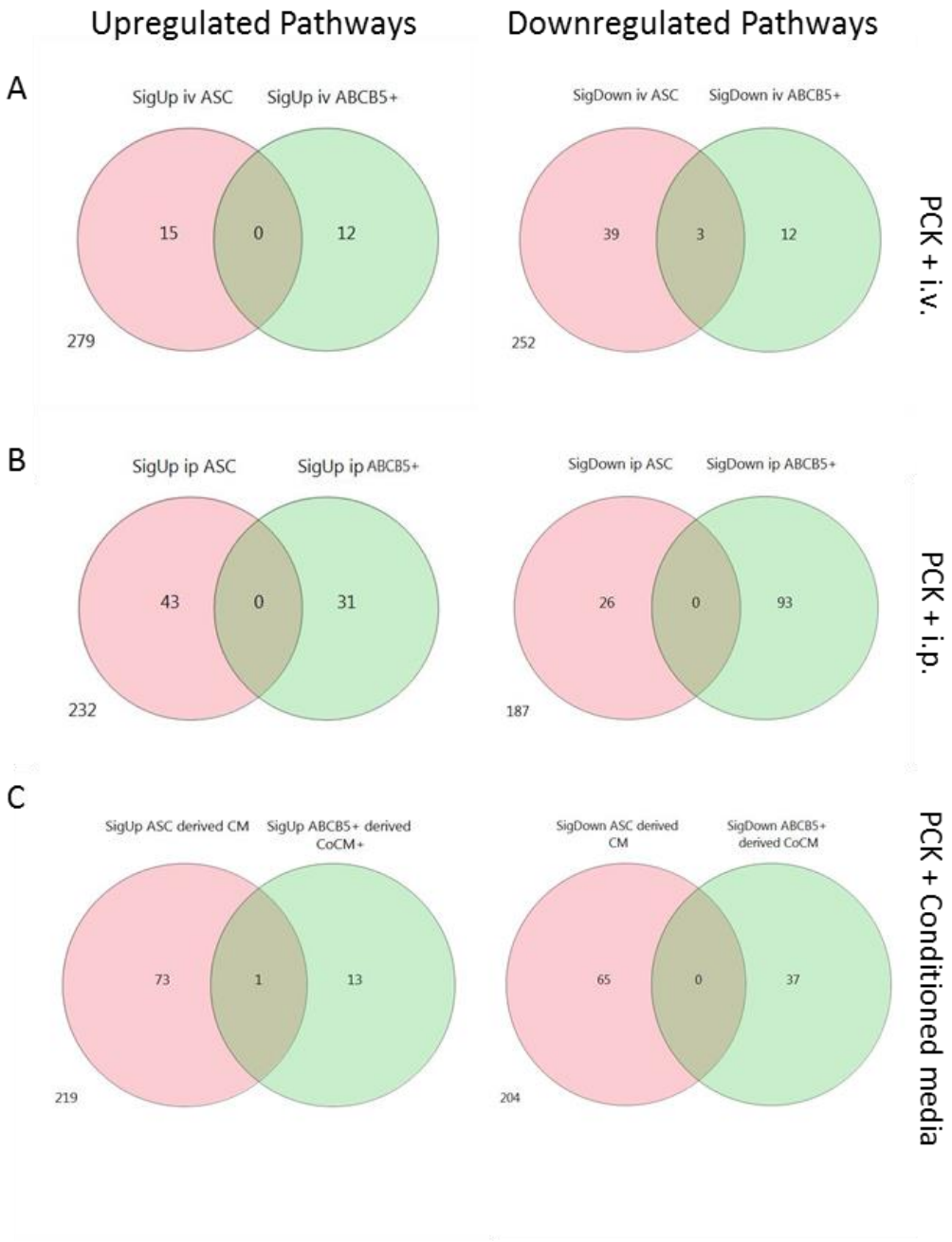

Figure 4.20 Venn diagrams showing the common significant up- and downregulated pathways between PCK treatments. A) i.v. ASC and ABCB5+ treatments, B) i.p. ASC and ABCB5+ treatments, C) ASC derived CM and ABCB5+ derived CoCM+ treatments. The numbers inside the Venn diagrams indicate the number of un- and downregulated pathways contained in each category.

Figure 4.19 and **figure 4.20** emphasized the different outcomes of the treatments in the PCK rats. The principal difference is related to metabolic pathways. While oxidative phosphorylation, citrate cycle and metabolic pathways are upregulated in i.v. and i.p. ABCB5+ and ABCB5+ derived CoCM+ groups, the same pathways were downregulated in the i.v. and i.p. ASC and ASC derived CM groups (**table 4.38 A-B**).

A

KEGG Pathway	Sub Category	NES i.v. ABCB5+	NES i.p. ABCB5+	NES ABCB5+ derived CoCM+
Glycolysis Gluconeogenesis	1.1. Carbohydrate metabolism	-1.21	0.91	1.13
Citrate cycle (TCA cycle)	1.1. Carbohydrate metabolism	1.78	2.57	2.36
Ascorbate and aldarate metabolism	1.1. Carbohydrate metabolism	1.31	1.29	1.6
Steroid biosynthesis	1.3. Lipid metabolism	1.57	1.94	1.95
Steroid hormone biosynthesis	1.3. Lipid metabolism	1.62	1.02	1.29
Oxidative phosphorylation	1.2. Energy metabolism	2.1	3.17	1.38
Glycine, serine and threonine metabolism	1.5. Amino acid metabolism	2.04	2.07	2.12
Cysteine and methionine metabolism	1.5. Amino acid metabolism	1.42	1.63	1.1
Valine, leucine and isoleucine degradation	1.5. Amino acid metabolism	2.22	2.11	1.93
Arginine and proline metabolism	1.5. Amino acid metabolism	1.44	1.44	1.77
Tryptophan metabolism	1.5. Amino acid metabolism	2.28	1.87	2.24
N-Glycan biosynthesis	1.7. Glycan biosynthesis and metabolism	1.47	1.83	1.37
Various types of N-glycan biosynthesis	1.7. Glycan biosynthesis and metabolism	1.57	1.58	1.62
Glycosaminoglycan biosynthesis	1.7. Glycan biosynthesis and metabolism	-0.71	-1.76	0.66
Glycosaminoglycan biosynthesis	1.7. Glycan biosynthesis and metabolism	-0.76	-0.54	0.99
Glycerolipid metabolism	1.3. Lipid metabolism	1.55	1.4	1.65
Glycerophospholipid metabolism	1.3. Lipid metabolism	1.02	-0.97	-0.7
Pyruvate metabolism	1.1. Carbohydrate metabolism	1.68	2.12	1.6
Glyoxylate and dicarboxylate metabolism	1.1. Carbohydrate metabolism	2.2	2.24	2.02
Propanoate metabolism	1.1. Carbohydrate metabolism	1.89	2.35	1.83
Butanoate metabolism	1.1. Carbohydrate metabolism	2.13	1.8	1.67
Drug metabolism	1.11. Xenobiotics biodegradation and metabolism	1.66	1.57	1.11
Biosynthesis of unsaturated fatty acids	1.3. Lipid metabolism	1.03	1.69	1.14
Metabolic pathways	1.0 Global and overview maps	1.95	2.09	1.73
Carbon metabolism	1.0 Global and overview maps	1.52	2	2.12
Fatty acid metabolism	1.0 Global and overview maps	1.31	1.63	1.31
Biosynthesis of amino acids	1.0 Global and overview maps	-1.05	1.04	1.46

Table 4.38A GSEA analysis using KEGG database sorted by main category: Metabolism**.** ABCB5+ derived CoCM+, i.p. or i.v. ABCB5+ groups. Significantly (adj. p<0.05) differentially expressed pathways (PCK + treatment vs PCK). For each pathway the NES is given. Downregulated pathways are displayed in green, upregulated pathways are displayed in red.

B

KEGG Pathway	Sub Category	NES i.v. ASC	NES i.p. ASC	NES ASC derived CM
Glycolysis Gluconeogenesis	1.1. Carbohydrate metabolism	-0.87	-0.88	-1.77
Citrate cycle (TCA cycle)	1.1. Carbohydrate metabolism	-1.99	-2.09	-2.17
Ascorbate and aldarate metabolism	1.1. Carbohydrate metabolism	-1.35	-1.74	-2.39
Steroid biosynthesis	1.3. Lipid metabolism	-1.86	-1.68	-1.92
Steroid hormone biosynthesis	1.3. Lipid metabolism	1.04	-1.7	-2.27
Oxidative phosphorylation	1.2. Energy metabolism	-0.97	-2.7	-1.8
Glycine, serine and threonine metabolism	1.5. Amino acid metabolism	-1.26	-1.72	-2.54
Cysteine and methionine metabolism	1.5. Amino acid metabolism	-1.15	-1.58	-2.3
Valine, leucine and isoleucine degradation	1.5. Amino acid metabolism	-1.18	-1.29	-2.41
Arginine and proline metabolism	1.5. Amino acid metabolism	0.85	-1.03	-1.98
Tryptophan metabolism	1.5. Amino acid metabolism	-0.63	-1.34	-2.42
N-Glycan biosynthesis	1.7. Glycan biosynthesis and metabolism	-1.24	0.87	-2.09
Various types of N-glycan biosynthesis	1.7. Glycan biosynthesis and metabolism	-1.37	-1.11	-1.94
Glycosaminoglycan biosynthesis	1.7. Glycan biosynthesis and metabolism	1.18	1.69	2.36
Glycosaminoglycan biosynthesis	1.7. Glycan biosynthesis and metabolism	-0.63	1.63	1.14
Glycerolipid metabolism	1.3. Lipid metabolism	-1.33	0.68	-1.96
Glycerophospholipid metabolism	1.3. Lipid metabolism	-0.95	0.7	-1.5
Pyruvate metabolism	1.1. Carbohydrate metabolism	-1.16	-1.17	-2.03
Glyoxylate and dicarboxylate metabolism	1.1. Carbohydrate metabolism	-0.93	-1.52	-2.34
Propanoate metabolism	1.1. Carbohydrate metabolism	-1.46	-1.15	-2.27
Butanoate metabolism	1.1. Carbohydrate metabolism	-1.22	-1.28	-2.17
Drug metabolism	1.11. Xenobiotics biodegradation and metabolism	1.09	-1.77	-2.57
Biosynthesis of unsaturated fatty acids	1.3. Lipid metabolism	-1.64	-1.55	-2.15
Metabolic pathways	1.0 Global and overview maps	-1.17	-1.58	-2.47
Carbon metabolism	1.0 Global and overview maps	-1.31	-1.03	-2.38
Fatty acid metabolism	1.0 Global and overview maps	-1.5	-1.5	-2.3
Biosynthesis of amino acids	1.0 Global and overview maps	-1.19	-1.23	-2.01

Table 4.38B GSEA analysis using KEGG database sorted by main category: Metabolism**.** ASC derived CM, i.p. or i.v. ASC groups. Significantly (adj. p<0.05) differentially expressed pathways (PCK + treatment vs PCK). For each pathway the NES is given. Downregulated pathways are displayed in green, upregulated pathways are displayed in red.

Table 4.39 (**A-B**) shows the significantly differentially expressed pathways, in each treated group, involved in the genetic information.

A

KEGG Pathway	Sub Category	NES i.v. ABCB5+	NES i.p. ABCB5+	NES ABCB5+ derived CoCM+
Ribosome	2.2. Translation	-1.35	1.47	-2.86
Spliceosome	2.1. Transcription	-1.91	-2.02	-1.96
Ubiquitin mediated proteolysis	2.3. Folding, sorting and degradation	-0.79	1.67	0.94
Protein processing in endoplasmic reticulum	2.3. Folding, sorting and degradation	1.14	1.43	0.9

Table 4.39A GSEA analysis using KEGG database sorted by main category: Genetic Information Processing**.** ABCB5+ derived CoCM+, i.p. or i.v. ABCB5+ groups. Significantly (adj. p<0.05) differentially expressed pathways (PCK + treatment vs PCK). For each pathway the NES is given. Downregulated pathways are displayed in green, upregulated pathways are displayed in red.

B

KEGG Pathway	Sub_Category	NES i.v. ASC	NES i.p. ASC	NES ASC derived CM
Ribosome	2.2. Translation	3.08	-1.52	-1.09
Spliceosome	2.1. Transcription	1.14	1.06	-0.51
Ubiquitin mediated proteolysis	2.3. Folding, sorting and degradation	-2.04	1.14	-0.97
Protein processing in endoplasmic reticulum	2.3. Folding, sorting and degradation	-1.79	2.05	-2.02

Table 4.39B GSEA analysis using KEGG database sorted by main category: Genetic Information Processing**.** ASC derived CM, i.p. or i.v. ASC groups. Significantly (adj. p<0.05) differentially expressed pathways (PCK + treatment vs PCK). For each pathway the NES is given. Downregulated pathways are displayed in green, upregulated pathways are displayed in red.

Immune system related pathways were mainly downregulated in ABCB5+ derived CoCM+, i.p. and i.v. ABCB5+ groups and upregulated in ASC derived CM, i.p. and i.v. ASC groups. PPAR signalling pathways was upregulated in ABCB5+ derived CoCM+, i.p. and i.v. ABCB5+ groups and downregulated in ASC derived CM and i.v. ASC groups (**table 4.40 A-B**).

A

KEGG Pathway	Sub Category	NES i.v. ABCB5+	NES i.p. ABCB5+	NES ABCB5+ derived CoCM+
MAPK signaling pathway	3.2. Signal transduction	-1.26	-1.66	-1.05
Rap1 signaling pathway	3.2. Signal transduction	0.69	-1.55	1.21
Calcium signaling pathway	3.2. Signal transduction	-0.8	-1.03	1.12
cGMP-PKG signaling pathway	3.2. Signal transduction	-0.89	-1.55	1.14
cAMP signaling pathway	3.2. Signal transduction	-1	-1.52	0.91
Cytokine-cytokine receptor interaction	3.3. Signaling molecules and interaction	-0.8	-2.39	-1.77
NF-kappa B signaling pathway	3.2. Signal transduction	-1.62	-2.21	-1.99
Phospholipase D signaling pathway	3.2. Signal transduction	-0.97	-1.56	1.01
Neuroactive ligand-receptor interaction	3.3. Signaling molecules and interaction	1.22	-1.53	1.13
PI3K-Akt signaling pathway	3.2. Signal transduction	-1.38	-1.59	0.99
Notch signaling pathway	3.2. Signal transduction	-0.82	-1.67	0.73
Cell adhesion molecules (CAMs)	3.3. Signaling molecules and interaction	-1.24	-2.75	-2.05
JAK-STAT signaling pathway	3.2. Signal transduction	-0.94	-1.88	-1.32
TNF signaling pathway	3.2. Signal transduction	-1.33	-1.86	-1.24
PPAR signaling pathway	5.2. Endocrine system	0.86	0.83	1.12
Chemokine signaling pathway	5.1. Immune system	-0.94	-2.23	-1.31
Longevity regulating pathway	5.9. Aging	-0.85	-0.9	1.01
Cardiac muscle contraction	5.3. Circulatory system	-1.07	1.06	-1.89
Vascular smooth muscle contraction	5.3. Circulatory system	-0.94	-1.88	1.24
Axon guidance	5.8. Development	1.01	-1.16	1.47
Osteoclast differentiation	5.8. Development	-1.47	-2.54	-1.62
Platelet activation	5.1. Immune system	-0.98	-2.11	0.67
Antigen processing and presentation	5.1. Immune system	-1.71	-2.62	-2.61
Renin-angiotensin system	5.2. Endocrine system	2.13	1.29	1.54
Toll-like receptor signaling pathway	5.1. Immune system	-0.94	-1.75	-1.24
NOD-like receptor signaling pathway	5.1. Immune system	-1.01	-1.91	-1.54
Cytosolic DNA-sensing pathway	5.1. Immune system	-1.62	-1.57	-1.91
C-type lectin receptor signaling pathway	5.1. Immune system	-1.26	-1.85	-1.38
Hematopoietic cell lineage	5.1. Immune system	0.6	-2.23	-1.8
Natural killer cell mediated cytotoxicity	5.1. Immune system	-1.15	-2.4	-1.96
IL-17 signaling pathway	5.1. Immune system	-1.4	-1.65	-1.02
Th1 and Th2 cell differentiation	5.1. Immune system	-0.71	-2.35	-1.95
Th17 cell differentiation	5.1. Immune system	-0.98	-2.39	-1.91
T cell receptor signaling pathway	5.1. Immune system	-1.25	-2.04	-1.47
B cell receptor signaling pathway	5.1. Immune system	-1.56	-2.32	-1.78
Fc epsilon RI signaling pathway	5.1. Immune system	-0.69	-1.88	-1.14
Fc gamma R-mediated phagocytosis	5.1. Immune system	-1.46	-2.11	-1.18
Leukocyte transendothelial migration	5.1. Immune system	-1.36	-2.17	-1.34
Intestinal immune network for IgA production	5.1. Immune system	-0.58	-1.86	-1.8
GnRH signaling pathway	5.2. Endocrine system	-0.84	-1.08	0.96
Renin secretion*	5.2. Endocrine system	0.77	-0.84	1.12
Aldosterone synthesis and secretion	5.2. Endocrine system	-0.77	-1.15	1.09
Relaxin signaling pathway	5.2. Endocrine system	-1.16	-1.72	1.08
Parathyroid hormone synthesis, secretion and action	5.2. Endocrine system	0.93	-1.25	1.11
Aldosterone-regulated sodium reabsorption	5.5. Excretory system	0.87	0.85	1.04
Bile secretion	5.4. Digestive system	1.39	0.99	1.05
Vitamin digestion and absorption	5.4. Digestive system	1.19	1.1	1.14
Mineral absorption	5.4. Digestive system	1.11	1.13	1.2

Table 4.40A GSEA analysis using KEGG database sorted by main categories: Environmental Information Processing and Organismal Systems**.** ABCB5+ derived CoCM+, i.v. or i.p. ABCB5+ groups. Significantly (adj. p<0.05) differentially expressed pathways (PCK + treatment vs PCK). For each pathway the NES is given. Downregulated pathways are displayed in green, upregulated pathways are displayed in red.

B

KEGG Pathway	Sub Category	NES i.v. ASC	NES i.p. ASC	NES ASC derived CM
MAPK signaling pathway	3.2. Signal transduction	-1.39	1.06	1.36
Rap1 signaling pathway	3.2. Signal transduction	-1.48	0.99	1.62
Calcium signaling pathway	3.2. Signal transduction	-1.17	0.89	1.39
cGMP-PKG signaling pathway	3.2. Signal transduction	-1.45	1.33	1.4
cAMP signaling pathway	3.2. Signal transduction	-1.17	1.78	1.14
Cytokine-cytokine receptor interaction	3.3. Signaling molecules and interaction	1.92	1.2	1.99
NF-kappa B signaling pathway	3.2. Signal transduction	1.52	0.97	1.67
Phospholipase D signaling pathway	3.2. Signal transduction	-1.29	0.83	1.4
Neuroactive ligand-receptor interaction	3.3. Signaling molecules and interaction	1.65	1.16	1.83
PI3K-Akt signaling pathway	3.2. Signal transduction	-1.55	-0.6	1.23
Notch signaling pathway	3.2. Signal transduction	0.89	1.27	2.06
Cell adhesion molecules (CAMs)	3.3. Signaling molecules and interaction	1.36	0.92	1.86
JAK-STAT signaling pathway	3.2. Signal transduction	-1.02	1.36	1.12
TNF signaling pathway	3.2. Signal transduction	-0.88	1.5	1.67
PPAR signaling pathway	5.2. Endocrine system	-1.42	-0.85	-2.23
Chemokine signaling pathway	5.1. Immune system	1.02	0.9	1.83
Longevity regulating pathway	5.9. Aging	-1.73	-1.46	-0.64
Cardiac muscle contraction	5.3. Circulatory system	0.92	-1.93	0.91
Vascular smooth muscle contraction	5.3. Circulatory system	-0.87	1.79	1.26
Axon guidance	5.8. Development	-1.53	1.65	1.77
Osteoclast differentiation	5.8. Development	1.36	1.32	2.09
Platelet activation	5.1. Immune system	-0.96	-1.7	1.76
Antigen processing and presentation	5.1. Immune system	1.35	2.16	0.97
Renin-angiotensin system	5.2. Endocrine system	-1.44	1.55	-1.93
Toll-like receptor signaling pathway	5.1. Immune system	1.06	1.75	1.46
NOD-like receptor signaling pathway	5.1. Immune system	-1.05	1.08	1.31
Cytosolic DNA-sensing pathway	5.1. Immune system	1.42	0.7	-0.6
C-type lectin receptor signaling pathway	5.1. Immune system	-1.04	1.21	1.43
Hematopoietic cell lineage	5.1. Immune system	1.65	1.4	1.68
Natural killer cell mediated cytotoxicity	5.1. Immune system	1.69	1.18	2.09
IL-17 signaling pathway	5.1. Immune system	1	1.11	1.77
Th1 and Th2 cell differentiation	5.1. Immune system	1.03	0.96	1.7
Th17 cell differentiation	5.1. Immune system	1.05	1.51	1.61
T cell receptor signaling pathway	5.1. Immune system	-1.12	1.34	1.46
B cell receptor signaling pathway	5.1. Immune system	-0.97	1.34	1.62
Fc epsilon RI signaling pathway	5.1. Immune system	1.09	0.54	1.57
Fc gamma R-mediated phagocytosis	5.1. Immune system	0.95	1.44	1.81
Leukocyte transendothelial migration	5.1. Immune system	-0.88	-1.55	1.8
Intestinal immune network for IgA production	5.1. Immune system	1.95	0.92	1.49
GnRH signaling pathway	5.2. Endocrine system	-1.73	1.64	0.87
Renin secretion*	5.2. Endocrine system	-1.85	-0.91	1.08
Aldosterone synthesis and secretion	5.2. Endocrine system	-1.33	-1.67	0.98
Relaxin signaling pathway	5.2. Endocrine system	-1.26	1.12	1.59
Parathyroid hormone synthesis, secretion and action	5.2. Endocrine system	-1.67	-2.23	0.86
Aldosterone-regulated sodium reabsorption	5.5. Excretory system	-1.77	-1.64	0.74
Bile secretion	5.4. Digestive system	-1.37	2.25	2.11
Vitamin digestion and absorption	5.4. Digestive system	-0.74	-1.6	1.81
Mineral absorption	5.4. Digestive system	-1.57	-2.07	1.72

Table 4.40B GSEA analysis using KEGG database sorted by main categories: Environmental Information Processing and Organismal Systems**.** ASC derived CM, i.v. or i.p. ASC groups. Significantly (adj. $p<0.05$) differentially expressed pathways (PCK + treatment vs PCK). For each pathway the NES is given. Downregulated pathways are displayed in green, upregulated pathways are displayed in red.

Table 4.41 (**A-B**) exemplifies the results of related cellular processes pathways.

A

KEGG Pathway	Sub_Category	NES i.v. ABCB5+	NES i.p. ABCB5+	NES ABCB5+ derived CoCM+
Cell cycle	4.2. Cell growth and death	-1.46	1.05	0.78
Endocytosis	4.1. Transport and catabolism	-1.53	-1.69	-1.34
Peroxisome	4.1. Transport and catabolism	1.8	2.01	1.12
Apoptosis	4.2. Cell growth and death	-1.44	-1.38	-1.24
Cellular senescence	4.2. Cell growth and death	-1.13	-1.49	-1.19
Focal adhesion	4.3. Cellular community - eukaryotes	-1.68	-1.75	1.15
Adherens junction	4.3. Cellular community - eukaryotes	-1.04	-1.09	1.18
Tight junction	4.3. Cellular community - eukaryotes	-1.02	-1.2	0.88

Table 4.41A GSEA analysis using KEGG database sorted by main category: cellular processes**.** ABCB5+ derived CoCM+, i.p. or i.v. ABCB5+ groups. Significantly (adj. p<0.05) differentially expressed pathways (PCK + treatment vs PCK). For each pathway the NES is given. Downregulated pathways are displayed in green, upregulated pathways are displayed in red.

B

KEGG Pathway	Sub_Category	NES i.v. ASC	NES i.p. ASC	NES ASC derived CM
Cell cycle	4.2. Cell growth and death	-1.32	1.25	1.79
Endocytosis	4.1. Transport and catabolism	-1.57	1.98	1.09
Peroxisome	4.1. Transport and catabolism	0.8	1.19	2.57
Apoptosis	4.2. Cell growth and death	0.99	0.89	1.11
Cellular senescence	4.2. Cell growth and death	-1.24	1.19	1.48
Focal adhesion	4.3. Cellular community - eukaryotes	-1.52	-1.27	1.86
Adherens junction	4.3. Cellular community - eukaryotes	-1.99	1.62	0.84
Tight junction	4.3. Cellular community - eukaryotes	-1.85	1.69	0.88

Table 4.41B GSEA analysis using KEGG database sorted by main category: cellular processes**.** ASC derived CM, i.p. or i.v. ASC groups. Significantly (adj. p<0.05) differentially expressed pathways (PCK + treatment vs PCK). For each pathway the NES is given. Downregulated pathways are displayed in green, upregulated pathways are displayed in red.

5. Discussion

In this study we monitored the progression of cystic disease by analysing plasma and urine biochemistry, GFR transcutaneous measurement and gene expression profiling. Briefly, PKD/Mhm (Cy/+) and PCK rats showed profound alterations of the kidney cytoarchitecture due to the presence of cysts, fibrosis and infiltration of inflammatory cells. We also noted in PCK liver fibrosis. Apoptosis and cell proliferation were found in both strains. Further altered parameters, suggesting a decline of the renal function, were the plasma creatinine, urea, albumin, proteinuria and ABZWCY-HβCD half-life increase in both the strains. The gene expression showed a significant downregulation of the pathways involved in the metabolism and an upregulation of apoptosis and cell cycle pathways.
The same parameters were evaluated to test the potential therapeutic effect of the ABCB5+ and ASC cells and their derived conditioned media. We noted a reduction of cyst number in PKD/Mhm (Cy/+) i.p. ABCB5+ and ASC derived CM groups. A decrease of apoptotic and active proliferative cells was detected in all the PKD/Mhm (Cy/+) treated groups. No significant differences were noted in the urine biochemistry results. ABZWCY-HβCD half-life was decreased in all the treated groups, in particular in i.p. ABCB5+ and i.p. ASC groups. GSEA analyses showed important genetic changes. The metabolism related pathways were upregulated in all the treated groups likewise the PPAR signaling pathway.
No morphological ameliorations were detected in PCK treated groups; instead an increase of hepatic fibrosis was noted. Apoptotic and proliferative positive cells were reduced in all the treated groups. No important ameliorations were noted in the plasma parameters. The proteinuria was slightly decreased in the treated groups. ABZWCY-HβCD half-life was significantly reduced in the i.p. ABCB5+ group. GSEA analyses highlighted different genetic changes between ABCB5+ and ABCB5+ derived CoCM+ groups and ASC and ASC derived CM groups in the metabolic pathways.

Cystic kidney diseases are a heterogeneous group of chronic disorders. Despite the common pathological features, a wide range of manifestations, due to different genes mutations, characterizes these diseases. Within all the cystic diseases, the most common and clinically significant are ADPKD, ARPKD and NPHP[4]. Current therapeutic alternatives for patients include dialysis and transplantation. Because of this and the chronic nature of the diseases,

ADPKD, ARPKD and NPHP have an important socio-economic impact[64-66]. In the last years, experimental approaches and clinical trial were performed in order to discover and validate new medical targets[19, 67-69].

The aim of our study was to examine the therapeutic effects of stem cells and conditioned media in animal models resembling human ARPKD and NPHP16. We investigated the effects of ASC and ABCB5+ cells. ASCs were isolated from lipoaspirates of healthy individuals undergoing liposuction. $ABCB5^+$ cells, obtained from skin of healthy donors of different nationality, age and gender, were provided by Ticeba-RHEACELL GmbH & Co. (Heidelberg, Germany). ASC and ABCB5+ cells were administrated either via i.v. or i.p. injection. It is known from literature that, when injected i.v., cells might be trapped in the lungs with the risk of causing an emboli[70-73]. On this basis, i.p. injection appears a valid and safety alternative.

In the last few years more attention was paid to the mode of action of stem cells. Scientists focused their attention on the possible paracrine action of the cells and on what the cells may release into the medium in which they grow[74-76]. Based on these findings, we decided to test, as an alternative therapy, conditioned media (CM) derived by ASC and conditioned media derived by a co-culture of $ABCB5^+$ cell and macrophages M1 stimulated with INFγ and LPS (CoCM+).

5.1 Genetic animal models of Cystic Kidney Disease

In literature, two different types of animal models for the cystic kidney disease have been described which developed spontaneously cystic disease[77]. For our study we decided to use two spontaneous models: PKD/Mhm (Cy/) and PCK. PKD/Mhm (Cy/) rats resemble the human ADPKD and, as recently discovered, NPHP 16. These animals present a mutation on the Ank6 gene that is correlated with NPHP 16[40, 78]. On the other hand, PCK are an orthologous model for the ARPKD[79]. Despite both the strains present similar features, they differ for severity degrees and penetration of the pathology. These models are characterized by an increased size of the kidney due to the massive presence of cysts in the cortical and medullary region. Besides cysts, an infiltration of inflammatory cells was noted as well as fibrosis. Morphological analyses showed higher cyst number in the PKD/Mhm (Cy/+) rats kidney when compared to the PCK. Fibrotic tissue was almost two times more frequent in the PCK rats compared to PKD/Mhm (Cy/+).

ARPKD is associated with hepatic disorder[28, 80-82]. This is the reason why we evaluated and demonstrated the presence of cysts and fibrosis in hepatic tissue of PCK rats. Our results showed a dilatation of the bile duct, cyst growth and fibrotic tissue surrounding the cystic area in the liver tissue. The fibrosis may be linked to an unbalanced production and reabsorption of extracellular matrix and collagen. Fibrosis and cyst grow was evaluated also in the PKD/Mhm (Cy/+) rats. In this strain, however, 1% of hepatic tissue was occupied by fibrosis and the percentage of cyst area was roughly 0.1%. Further histological analyses highlighted an increase of apoptotic and active proliferative cells. When compared to the SD rats, both strains presented at least 10 times more apoptotic and proliferative positive cells (**table 4.1**). These results are in line with the literature. It is known, that polycystin, present on the primary cilia, are involved in the regulation of cell cycle by regulating apoptosis and cell cycle arrest[83, 84]. Therefore, an alteration of these genes might lead to a misregulation of these cellular processes.

Blood and urine parameters were analysed in order to monitor the progression of the disease. Plasma creatinine and urea are the standard markers for the evaluation of renal function. Both markers showed an increase over time. The creatinine increase was already significant, in both strains, at day 53 and remained altered until the end of the experiment. Although, in the PKD/Mhm (Cy/+) model, at day 25 the creatinine raised 1.4-fold compared to the first measurement (baseline). A rise of urine levels was also detected among the strains, especially in PCK (**figure 4.5**). Our results showed also an alteration of the plasma cholesterol and triglycerides levels. Cholesterol levels increased constantly over time with an 1.5-fold increase at day 53 in PCK and, 1.4-fold at day 109 in PKD/Mhm (Cy/+). In both strains, these levels were considerably increased at day 167, respectively of 1.6 and 2.2-fold in PKD/Mhm (Cy/+) and PCK. Triglycerides levels were stable during the first part of the experimentation but rose in the last measurements. Plasma levels increased 1.6-fold in PKD/Mhm (Cy/+) rats and 1.9-fold in PCK rats (**appendix 1**). Once again our findings are in accordance with previous studies, which already highlighted an increase of cholesterol and triglycerides in PCK rats[85, 86].

Urine analysis of both strains underlined a massive increase of albumin- and proteinuria levels. In PKD/Mhm (Cy/+) rats, protein- and albuminuria levels were increased 6-fold at day 109 and day 53, respectively. PCK rats showed an increase of proteinuria 3-fold already at day 25. In both strains, these levels raised dramatically until the end of the experiment (**appendix 2**). Another important parameter is the urine volume. In PKD/Mhm (Cy/+) rats,

diuresis doubled on day 109 and remained high until the end of the experiment, along with an increase of water intake. Also in PCK rats, diuresis was increased during the entire experiment while no significant fluctuations of water intake were noted (**table 4.4**).

Plasma creatinine level can be affected by numerous factors such as age, gender, hepatic function and muscle mass[87-89]. Therefore, in this study, we evaluated the half-life of ABZWCY-HβCD dye, used for a transcutaneous GFR measurement as GFR is considered one of the best indicators of renal function[90]. The results showed an increase of the half-life levels, indicating a deficit in the renal function, which corroborate what was observed in the plasma biochemistry (**figure 4.6**).

RNAseq was used to perform gene expression analysis. Over 11326 genes were significantly and differentially expressed in PKD/Mhm (Cy/+) and 4602 genes in PCK rats. GSEA analysis was performed on genes defining pathways (KEGG database). The analysis outlined 199 significantly and differently expressed pathways in PKD/Mhm (Cy/+) and 156 significant pathways in PCK. As described, PKD/Mhm (Cy/+) rats exhibit a reprogramming in the metabolism by enhancing the aerobic glycolysis rather than oxidative phosphorylation, known as the Warburg effect[91]. Typical of cancer cells, the Warburg effect is usually associated with a defect in the mitochondrial activity. Several studies highlighted the dysfunctional mitochondrial activity in the pathophysiology of cystic kidney disease[92-95]. Few other studies demonstrated the Warburg effect in other animal models for cystic kidney disease but no one documented it in PCK rats[96, 97]. Unfortunately, in this study it was not possible to test the mitochondria activity. Nevertheless, the oxidative phosphorylation and pyruvate metabolism pathways were found downregulated in both strains (**table 4.9**).

In accordance with the literature, in this study we confirmed, in both models, the upregulation of apoptosis, p53, NF-κB, Notch, cell cycle and cellular senescence pathways (**figure 4.12** and **figure 4.13**)[5, 79, 98-101]. As expected, also MAPK signaling pathway was upregulated. The upregulation of these pathways leads to an alteration of the cellular proliferation and tissue degeneration. Among these pathways, one of the most representatives is NF-κB pathway, which does not only regulate the apoptosis and the cellular growth but also inflammatory genes. Moreover, it was demonstrated that its inhibition may modulated the cystic disease[102]. On the basis of this, it is not surprising that inflammatory pathways were abnormally

regulated in PKD/Mhm (Cy/+) and PCK rats. GSEA analysis outlined also an upregulation of the JAK-STAT, WNT and PI3K-Akt pathways.

In summary, taking into account the morphological, functional and genetic changes, we can assert that both animal models show a severe cystic phenotype. This renal condition, present in both strains, was the starting point to evaluate the therapeutic potential of ASC and ABCB5+ cells and their derived conditioned media.

5.2 Therapeutic effect of stem cells and conditioned media in PKD/Mhm (Cy/+) rats

PKD/Mhm (Cy/+) model was used over the years to better understand and characterized the progression and development of ADPKD and NPHP 16 in terms of morphology, genetics and biochemical analysis[35, 103, 104]. As far as we know, this animal model was never involved in trial for therapeutic treatment with MSCs.

We investigated the treated PKD/Mhm (Cy/+) groups for the same morphological parameter analysed in the untreated group. No remarkable differences were noted concerning the BW, kidney weight or Kw/BW ratio among the groups (**figure 4.9** and **table 4.15**). However, staining performed on whole kidney sections highlighted some histological changes in the treated groups, such as the reduction in cyst number in i.v. and i.p. ABCB5+, ABCB5+ derived CoCM+, i.v. ASC and ASC derived CM groups. On the other hand, i.p. ASC group presented a slight increase of the cyst number but with minor average size (**table 4.16**). Surprisingly, fibrotic tissue deposition in the kidney appeared increased in each treated group with respect to the untreated (**figure 4.10**). The PKD/Mhm (Cy/+) model is characterized by an alteration of the cellular cycle, especially in tubular cells, co-staining of Tunel and Ki67 was performed on 3μm kidney sections. A major reduction of apoptotic positive cells was encountered in all the treated groups, as well as a decrease of proliferation marker positive cells (**table 4.17**).

The same plasma and urine parameters were analysed to monitor the progression of the disease in the untreated and the treated groups. A slight reduction of creatinine levels in both i.p. ABCB5+ and i.p. ASC, and glucose levels in all the treated groups was registered. The cholesterol and triglycerides plasma levels improved (**table 4.18**). One of the mechanisms of cyst formation in ADPKD is the loss of PKD genes function and consequently the disruption

of calcium homeostasis within the cells, which could also lead to an abnormal response of cAMP[105]. In our study, the plasma calcium concentration was reduced in ABCB5+ derived CoCM+ and both i.p. and i.v. ABCB+ and i.p. and i.v. ASC groups. In particular a significant decline was recorded in the i.p. ABCB5+ group. Thus we were not surprised to detect that treated groups expressing a reduction of calcium levels also presented a minor number of cysts in the kidney tissue (**table 4.18**). ABZWCY-HβCD half-life was reduced, at day 167 1.5-fold in both i.v. ABCB5+ and ASC groups, 1.6-fold in i.p. ABCB5+ and 1.7-fold in i.p. ASC groups, revealing an improvement in kidney function. A minor reduction of ABZWCY-HβCD half-life was also recorded in ABCB5+ derived CoCM+ and ASC derived CM groups (**figure 4.11**). A minor reduction of the albumin and protein levels in the urine was detected in all the treated groups, all other urine parameters remained relatively constant (**table 4.20**).

Altogether, analyses of renal function as well as plasma and urine biochemistry show a moderate amelioration of the renal function in ABCB5+ and ASC groups. ABCB5+ derived CoCM+ and ASC derived CM group resulted in milder, but still promising improvements.

GSEA analysis was performed to examine the effects of ABCB5+, ASC, ASC derived CM and ABCB5+ derived CoCM+ on kidney. The analysis revealed major differences between treated and untreated groups. Even though minor variances in the different expression of few pathways among the treated groups were noted, the main trend was unchanged. The analysis showed a profound change of metabolism related pathways in all the treated groups. In fact, oxidative phosphorylation, citrate cycle and gluconeogenesis were upregulated, likewise pyruvate metabolism (**table 4.24**). These results, along with the upregulation of PPAR signalling pathway and the downregulation of cAMP and calcium signalling pathways (**table 4.27**), outlined a potential improvement of the metabolism in the treated animals. Besides that, the downregulation of apoptosis and NF-κB pathways suggest that the new cells lose the tumor-like attitude (Warburg effect) reported in the untreated group. Major changes were noticed also in other pathways involved in the signal transduction like MAPK, PI3K-Akt, TGF-beta and Wnt, which were downregulated (**table 4.27**). Downregulation of apoptosis and cell cycle pathways (**table 4.25**) was also in line with the results noted in the histological analysis. The principal pathways involved in cellular interaction, as focal adhesion, adherens junction and gap junction, were downregulated (**table 4.25**). While an upregulation of these pathways was recorded in the untreated group. This might be also an explanation for the reduced presence of cysts in the kidneys, perhaps leading to a mesenchymal to epithelial

transition of the cells. i.v. and i.p. ABCB5+ along with ABCB5+ derived CoCM+ groups pointed out a downregulation of pathways involved in the immune system. A similar trend was noted also in i.p. and i.v. groups but not in the ASC derived CM, suggesting an attenuation of the inflammation. In line with literature[95], an amelioration of metabolic related pathways could lead to a decrease in the number of cysts and their growth as observed in ABCB5+ derived CoCM+, i.p. and i.v. ABCB5+ treated groups (**table 4.16**).

Taking into account the above-discussed results the treated groups showed important changes, far apart from those observed in the untreated group. Moreover, better results in terms of kidney function improvement were achieved in the i.p. ABCB5+ treated group. When comparing the conditioned media groups, the parameters analysed seem to have a better outcome in the ABCB5+ derived CoCM+ group than the ASC derived CM.

5.3 Therapeutic effect of stem cells and conditioned media in PCK rats

Although, in the last decades, MSCs have represented a promising therapeutic approach, only two preclinical studies testing the potential therapeutic effect of stem cells in cystic kidney disease animal models have been published[49, 50]. Franchi *et al.* demonstrated that a single injection of allogenic Bone Marrow (BM) MSCs was adequate to induce, in PCK rats, renal function amelioration[50]. Conversely, Kelly *et al.* investigated multiple allogenic MSCs injections, demonstrating the beneficial restoration of the kidney function in PCK rats after the treatment[49]. In our study we investigated the putative therapeutic effects of ABCB5+ and ASC cells and derived conditioned media in PCK rats.

The same morphological parameters as analysed in the untreated model were investigated in the treated groups. No differences were noted concerning the BW, kidney weight or Kw/BW ratio values among the groups. Moreover, no other macroscopic ameliorations were identified. Cyst number and percentage of area remained relatively constant among all the groups (**table 4.29**). As an orthologous model of the human ARPKD, PCK rats are characterized by a severe liver disorder. Numerous studies identified the presence of dilated bile ducts, development of cysts and fibrosis[106, 107]. In our study, we confirmed these alterations quantifying cyst number and percentage of fibrosis on 3 μm liver sections. Histological evaluation revealed, however, an increase in cyst number and fibrosis in the treated groups when compared to the untreated. In particular, the percentage of fibrosis was

increased more than 2.5-fold (**table 4.30**). PCK rats are characterized by an increase of apoptotic and proliferative cells. For this reason we performed a co-staining of Tunel and Ki67. Surprisingly, all the treated groups presented a reduction of apoptotic and proliferative positive markers in the kidney (**table 4.31**).

To better characterize the biological changes in the treated groups, plasma and urine biochemistry analysis were performed. Plasma creatinine and urea, albumin- and proteinuria levels were analysed in order to evaluate changes in the kidney function. As previously described PCK model is characterized by hypercholesterolemia and hyperlipidemia. Overall, a slight reduction of these parameters was observed in the treated groups (**table 4.32**). As aforementioned, an unbalance of calcium homeostasis correlates with the cyst formation and development. No fluctuations of plasma calcium levels were encountered within the groups. No significant improvements were observed in urine analysis results although we noted a slight decrease in proteinuria levels, while albuminuria was increased in all the treated groups.

Surprisingly, ABZWCY-HβCD half-life was reduced 1.7-fold in i.p. ABCB5+, 1.5-fold in i.v. ABCB5+ and 1.4-fold in i.v. and i.p. ASC groups. This means GFR improved. A minor decrease of ABZWCY-HβCD half-life was also observed in ABCB5+ derived CoCM+ group. No important fluctuations were noted in the ASC derived CM group (**figure 4.17**). Overall, analyses of renal function, plasma and urine biochemistry performed in treated PCK groups, indicates a slight improvement of the renal function.

The characterization of genetic changes of the treated groups was performed with GSEA analysis using KEGG database. The analysis highlighted different genetic changes when compared ABCB5+ and ABCB5+ derived CoCM+ within ASC and ASC derived CM groups. The different regulation of pathways involved in metabolism is the most obvious change. An upregulation of citrate cycle and oxidative phosphorylation pathways was observed in ABCB5+ derived CoCM+, i.v. and i.p. ABCB5+ groups, likewise for the pyruvate metabolism. In contrast, these pathways were downregulated in ASC derived CM groups, i.v. and i.p. ASC groups (**table 4.38**). cAMP signalling pathway was downregulated in i.v. and i.p. ABCB5+ groups while PPAR was upregulated. These results correlated with the histological cyst analyses. An opposite trend was noted in i.v. ASC group. i.p. ASC and ASC derived CM groups, which exhibited an upregulation of the cAMP pathways and a downregulation of the PPAR pathway (**table 4.40**). Apart from metabolism, another

differential expression was documented for the NF-κB and apoptosis pathways. In fact, while these pathways were downregulated in i.v and i.p. ABCB5+ and ABCB5+ derived CoCM+, we noted an upregulation of the same pathways in ASC derived CM, i.v. and i.p. ASC groups. Instead, MAPK and JAK-STAT signalling pathways were downregulated in i.v. and i.p. ABCB+, i.v. ASC and ABCB5+ derived CoCM+ groups. A similar trend was noted also for cellular processes pathways likewise focal adhesion and adherens and thight junction (**table 4.40** and **table 4.41**). Immune system regulatory pathways were downregulated in i.v and i.p. ABCB5+ and ABCB5+ derived CoCM+ groups, while the opposite trend was observed in i.v and i.p. ASC and ASC derived CM groups (**table 4.40**).

Based on the above-stated results, ABCB5+ and ABCB5+ derived CoCM+ administrations appear to induce beneficial genetic changes in the PCK model, in contrast to ASC and ASC derived CM treatments. In fact, a recovery of metabolism, cell cycle and the cellular interaction activity were observed in ABCB5+ derived CoCM+, i.p. and i.v. ABCB5+ groups. Undoubtedly furthers studies need to be performed, also to better understand the dramatic alteration of the hepatic morphology and function.

5.4 Conclusions

This study provides an extensive description of the differences in outcome obtained by treating PKD/Mhm (Cy/+) and PCK rats with ABCB5+ or ASC cells and derived conditioned media. Untreated and treated groups were characterized on the basis of plasma and urine parameters, ABZWCY-HβCD $t_{1/2}$ and histological changes. Moreover gene expression analysis was performed. The results observed in all the treated groups of PKD/Mhm model, highlighted an amelioration of the principal parameter investigated for renal function. In addition, the genetic profile emphasized changes in the cellular metabolism and behaviour. Conversely, discrepancies were documented in biochemical and genetic results of PCK treated groups. While ABCB5+ and ABCB5+ derived CoCM+ treatments seem to slightly ameliorate the kidney function, the administration of ASC and ASC derived CM appear to improve only few of these factors. The reason why the results are so discordant between the two models and, at the same time, in the PCKs themselves, is not clear yet. Certainly the different severity of the disease and genetic background of the two models played a considerable role in the outcome of the therapeutic approaches. In relation to PCK rats, a valid hypothesis could be a late starting of the therapies. These animals in fact, develop the

disease in the early stage of life. Furthermore, no clinical beneficial were observed, after treatments, in the PCK model concerning the liver function. The suggested mode of action of ABCB5+, ASC and derived conditioned media could be due to a paracrine effect, leading to a rearrangement of the fundamental cellular functions like metabolism, cell cycle and polarity or restoring renal function. Nevertheless, further studies should be performed to better characterize this paracrine effect.

From our results we can assert that ABCB5+ or ASC cells administration, likewise ABCB5+ derived CoCM+ and ASC derived CM might be a valid alternative therapy for cystic kidney disease, in particular i.p. ABCB5+. Future studies have to be carried out in order to better define the mode of action and the side effects. Definitely the interspecies variability and different disease severity between the models is affecting the efficacy of the treatment.

Moreover, we demonstrated that i.p. administration is a valid alternative route compared to the i.v. and certainly less risky. The gene expression analysis performed with RNAseq technology is also an innovation with respect to cystic kidney disease and treatment, as it allowed us to have the most complete overview and dep insight on possible mechanisms.

6. Summary

Cystic kidney diseases are a global public problem, as the population of patients is increasing at a rate of approximately 7% per year[65]. Unfortunately, up to now there is a lack of efficient therapies that can prevent the progressive loss of renal function. Drug treatment is of limited. The only therapeutic alternatives are dialysis or kidney transplantation. Nevertheless, the human and economic impact of the diseases to affected individuals and to medical community and society alike is enormous. Therefore new therapies are urgently needed[64, 65]. Stem cell application represents a promising therapeutic approach. MSC therapies have been used extensively, in the recent years, as a possible therapy for other kidney diseases with the aim of slowing down the course of the disease. However, many aspects remain unclear or are under debate like the finding of an appropriate source of MSCs, the understanding of their modes of action and, not least, the dose and timing of the administration.

Aim of this study was to evaluate the potential therapeutic effects of two different types of stem cells, and the derived conditioned media, in two animal models resembling human cystic kidney diseases. In order to achieve this goal, firstly we characterized, on a long-term basis, two different genetic animal models: the PKD/Mhm (Cy/+) and the PCK rats. Afterwards, we performed a 6 months trial, to test the long-term effects of human ASC and human ABCB5+ cells and ASC derived CM and ABCB5+ derived CoCM+. Animals were classified in four different groups, depending on the treatment received: (i) group that did not received treatment, (ii) ASC derived CM or ABCB5+ derive CoCM+ group, (iii) i.p. ASC or ABCB5+ group and (iv) i.v. ASC or ABCB5+ group. The progression of the disease and the effect of the treatments were determined on the basis of plasma and urine biochemistry, transcutaneous measurement of renal function, histology evaluation and gene expression profiling.

Our results found a different disease severity in the two models. PCK rats presented, since the beginning of the project, worse clinical manifestations compared to PKD/Mhm (Cy/+) probably due to the different genetic background.

ABCB5+ and ASC treatments led to an improvement in renal function reflected by GFR, plasma levels of creatinine, albuminuria and proteinuria, in PKD/Mhm (Cy/+) model. Histological evaluation of the kidney revealed a reduction of apoptosis and cell proliferation. Moreover, comparable genetic changes were reported after these treatments. Also the ABCB5+ derived CoCM+ treatment proved to ameliorate the biochemical parameters, while ASC derived CM treatment had a lesser pronounced outcome. Both after ABCB5+ derived

CoCM+ or ASC derived CM administration the histological changes in apoptosis and proliferation reduction was observed. Ultimately, these animals undergo to genetic changes similar to the one observed after the cells treatment.
Concerning the PCK model. ABCB5+ and ABCB5+ derived CoCM+ treatments slightly ameliorated kidney function, plasma and urine parameter and likewise the levels of apoptotic and proliferative positive markers. Administration of ASC or ASC derived CM improved the renal function and decreased the apoptotic and proliferative positive cells but had only a mild effect on other parameter involved in the kidney function. Additionally, the gene expression profile did not highlight significant genetic changes after those treatments, while ABCB5+ and ABCB5+ derived CoCM+ administrations induced beneficial genetic changes.

In conclusion, our results demonstrate that ABCB5+ or ASC cells administration, either i.v. or i.p., and ABCB5+ derived CoCM+ might be a valid alternative therapy for cystic kidney diseases. However, before a possible application in the clinical field further studies need to be carried out in order to better define the mode of action and the side effects of these therapies.

7. References

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8. Appendix

Appendix 1 Changes in plasma biochemistry in PKD/Mhm (Cy/+) and PCK rats (n=6 in each group). Data are shown as mean ± Std.Dev. Values significantly different (time point vs baseline) are indicated as **p<0.005, ***p=0.0001 and (PKD/Mhm (Cy/+) vs PCK) as ‡p<0.05, ‡‡p<0.005, ‡‡‡p=0.0001.

Parameter	Strain	Baseline	Day25	Day 53	Day 81	Day 109	Day 137	Day 167
Creatinine	PKD/Mhm (Cy/+)	**0.3 ± 0.1‡**	**0.4 ± 0.1‡‡**	**0.6 ± 0 1** ‡‡**	**0.6 ± 0.1** ‡‡**	**0.6 ± 0.1** ‡‡**	**0.7 ± 0.1*** ‡‡**	**0.7 ± 0.1*** ‡‡**
(mg/dl)	PCK	0.2 ± 0.0	0.3 ± 0.0	**0.3 ± 0.0***	**0.3 ± 0.0***	**0.4 ± 0.0*****	**0.4 ± 0.1*****	**0.5 ± 0.1*****
Urea	PKD/Mhm (Cy/+)	**68.4 ± 18.2‡**	**69.4 ± 9.9‡‡‡**	**73.9 ± 8.6‡‡‡**	**88.4 ± 11.7‡‡‡**	**85.6 ± 8.1‡‡‡**	**85.1 ±13.0‡‡**	**87.4 ± 11.0‡‡**
(mg/dl)	PCK	31.6 ± 2.8	35.0 ± 4.3	32.5 ± 2.9	36.3 ± 6.2	**42.1 ± 5.1****	**44.6 ± 2.3*****	**56.6 ± 3.2*****
Na	PKD/Mhm (Cy/+)	**144.3 ± 0.8‡‡‡**	**143.0 ± 3.6‡‡‡**	142.3 ±6.9	141.3 ± 2.3	**144.3 ± 2.3‡‡**	141.8 ± 1.7	144.8 ±1.9
(mmol/l)	PCK	139.2 ± 1.2	140.7 ± 2.7	141.0 ± 3.0	139.7 ± 1.2	139.8 ± 1.1	140.6 ± 1.7	**143.8 ± 1.8****
K	PKD/Mhm (Cy/+)	5.4 ± 0.3	5.1 ± 0.6	5.2 ± 0.7	5.3 ± 0.4	5.3 ± 0.2	5.7 ± 0.4	5.1 ± 0.2
(mmol/l)	PCK	5.4 ± 0.3	5.0 ± 0.5	5.3 ± 0.3	5.2 ± 0.4	5.4 ± 0.4	5.2 ± 0.4	5.0 ± 0.2
Ca	PKD/Mhm (Cy/+)	2.5 ± 0.2	2.6 ± 0.0	**2.6 ± 0.1‡‡**	**2.6 ± 0.1‡‡**	2.6 ± 0.0	2.4 ± 0.1	**2.7 ± 0.1‡**
(mmol/l)	PCK	2.7 ± 0.1	2.7 ± 0.1	2.8 ± 0.1	2.7 ± 0.0	2.7 ± 0.1	2.7 ± 0.1	2.8 ± 0.1
PO^4	PKD/Mhm (Cy/+)	2.4 ± 0.5	2.2 ± 0.1	**2.6 ± 0.2‡‡**	2.1 ± 0.4	2.0 ± 0.3	2.1 ± 0.2	2.1 ± 0.3
(mmol/l)	PCK	2.3 ± 0.3	2.5 ± 0.3	2.0 ± 0.2	1.6 ± 0.5*	2.0 ± 0.4	2.0 ± 0.4	2.2 ± 0.1
Cholesterol	PKD/Mhm (Cy/+)	**97.3 ± 7.5‡‡**	99.7 ± 6.7	**110.0 ± 9.1‡‡**	**107.5 ± 8.5‡‡‡**	**131.7 ± 10.0*** ‡‡‡**	**131.7 ± 10.2***‡‡**	**154.3 ± 15.5*** ‡‡**
(mg/dl)	PCK	140.3 ± 15.6	166.2 ± 16.8	**211.5 ± 29.1***	**237.0 ± 23.7****	**289.5 ± 36.5*****	**312.6 ± 39.0*****	**301.2 ± 62.1*****
Triglycerides	PKD/Mhm (Cy/+)	69.2 ± 26.3	54.3 ± 14.5	67.2 ± 19.4	91.5 ± 21.6	**68.7 ± 10.0‡‡**	**60.2 ± 15.0‡‡**	108.8 ± 48.3
(mg/dl)	PCK	86.8 ± 15.0	79.3 ± 15.1	93.2 ± 28.3	132.2 ± 36.7	126.7 ± 28.2	137.5 ± 29.5	**160.5 ± 46.9****
Glucose	PKD/Mhm (Cy/+)	169.0 ± 9.0	**167.8 ± 9.4‡‡**	**183.8 ± 18.7‡**	195.8 ± 32.1	173.3 ± 12.6	**176.5 ± 19.6‡**	**184.3 ± 07.4‡**
(mg/dl)	PCK	166.8 ± 10.6	143.7 ± 11.4	149.3 ± 20.0	162.7 ± 32.8	172.8 ± 12.9	143.0 ± 16.4	150.3 ± 24.0
Protein	PKD/Mhm (Cy/+)	62.0 ± 1.7	63.0 ± 3.1	63.8 ± 2.9	61.0 ± 1.6	65.5 ± 4.1	62.7 ± 3.8	**65.3 ± 2.7‡‡**
(mg/dl)	PCK	62.2 ± 1.8	66.0 ± 4.2	82.3 ± 31.3	63.2 ± 4.8	64.0 ± 4.0	66.2 ± 3.1	69.5 ± 2.1

Appendix 2 Changes in urine biochemistry in PKD/Mhm (Cy/+) and PCK rats (n=6 in each group). Data are shown as mean ± Std.Dev. Values significantly different (time point vs baseline) are indicated as **p<0.005, ***p=0.0001 and (PKD/Mhm (Cy/+) vs PCK) as ‡p<0.05, ‡‡p<0.005, ‡‡‡p=0.0001.

Parameter	Strain	Baseline	Day25	Day 53	Day 81	Day 109	Day 137	Day 167
Creatinine	PKD/Mhm (Cy/+)	8.3 ± 1.7	10.8 ± 2.3	10.1 ± 2.6	**9.8 ± 1.6‡**	**12.0 ± 1.2‡**	**15.8 ± 2.9****	**16.9 ± 8.6****
(mg/16h)	PCK	8.1 ± 1.1	11.2 ± 2.0	11.1 ± 2.9	**13.3 ± 2.5***	**13.7 ± 1.3****	**13.9 ± 2.5****	**14.8 ± 3.2****
Urea	PKD/Mhm (Cy/+)	572.9 ± 178.1	793.6 ± 121.1	699.4 ± 220.2	562.3 ± 185.4	741.9 ± 175.6	**890.7 ± 160.2‡**	830.5 ± 376.6
(mg/16h)	PCK	709.4 ± 120.7	1033.9 ± 689.6	729.7 ± 256.7	701.3 ± 85.4	679.7 ± 65.4	662.2 ± 151.2	681.6 ± 91.3
Na	PKD/Mhm (Cy/+)	1.6 ± 0.7	1.6 ± 0.3	1.5 ± 0.4	1.0 ± 0.4	1.8 ± 0.7	**2.2 ± 0.7‡**	1.7 ± 1.4
(mmol/16h)	PCK	1.6 ± 0.6	1.4 ± 0.4	1.0 ± 0.5	1.3 ± 0.4	1.3 ± 0.3	1.3 ± 0.3	1.3 ± 0.2
K	PKD/Mhm (Cy/+)	3.1 ± 1.0	4.3 ± 1.0	3.5 ± 0.9	2.7 ± 1.0	4.2 ± 15.4	4.6 ± 1.0	4.2 ± 2.6
(mmol/16h)	PCK	3.9 ± 1.0	4.5 ± 0.9	2.9 ± 1.4	3.5 ± 1.0	4.3 ± 0.4	3.6 ± 1.3	3.3 ± 0.4
Ca	PKD/Mhm (Cy/+)	**0.01 ± 0.01‡**	0.02 ± 0.01	0.03 ± 0.01	**0.02 ± 0.01‡**	**0.03 ± 0.02‡‡**	**0.04 ± 0.02‡‡‡**	0.04 ± 0.04
(mmol/16h)	PCK	0.06 ± 0.04	0.03 ± 0.01	0.05 ± 0.02	0.07 ± 0.02	0.10 ± 0.02	**0.10 ± 0.02***	0.08 ± 0.03
PO^4	PKD/Mhm (Cy/+)	0.1 ± 0.1	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.1
(mmol/16h)	PCK	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.2	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1
Glucose	PKD/Mhm (Cy/+)	**1.2 ± 0.4‡**	1.3 ± 0.8	2.7 ± 2.2	2.0 ± 1.6	2.5 ± 2.3	2.3 ± 1.2	1.7 ± 1.7
(mg/16h)	PCK	5.2 ± 1.9	3.5 ± 2.8	2.9 ± 1.2	3.0 ± 1.4	3.5 ± 2.3	3.5 ± 1.2	1.9 ± 1.5
Protein	PKD/Mhm (Cy/+)	10.6 ± 5.2	**19.5 ± 5.2‡**	**27.7 ± 9.8‡**	**32.7 ± 4.6‡**	**61.4 ± 16.3‡‡‡**	**96.8 ± 26.5‡‡**	**154.5 ± 177.7‡**
(mg/16h)	PCK	9.6 ± 4.9	31.5 ± 12.5	94.6 ± 44.5*	**212.4 ± 72.4*****	**353.0 ± 64.1*****	**375.1 ± 85.6*****	**436.8 ± 101.5*****
Albumin	PKD/Mhm (Cy/+)	**2.3 ± 1.6‡**	4.3 ± 1.8	**12.4 ± 4.1‡**	**22.4 ± 11.4‡**	**27.4 ± 13.2‡‡**	**25.4 ± 11.3‡‡**	**103.9 ± 108.5****
(mg/16h)	PCK	0.7 ± 0.3	13.5 ± 11.1	69.7 ± 34.8	**103.3 ± 61.1***	**178.8 ± 49.8*****	**173.9 ± 71.9*****	**140.6 ± 71.2*****

Appendix 3A Changes in plasma biochemistry in PKD/Mhm (Cy/+) groups (n=6 in each group). Comparison between ABCB5+ derived CoCM+, i.v. or i.p. ABCB5+ groups and untreated. Data are shown as mean ± Std.Dev. Values significantly different from control are indicated as *p<0.05, **p<0.005.

A Parameter	Animal group	Baseline	Day25	Day 53	Day 81	Day 109	Day 137	Day 167
Creatinine	Untreated	0.3 ± 0.1	0.4 ± 0.1	0.6 ± 0 1	0.6 ± 0.2	0.6 ± 0.1	0.7 ± 0.1	0.7 ± 0.1
(mg/dl)	+ ABCB5+ derived CoCM+	0.3 ± 0.1	0.5 ± 0.2	0.5 ± 0.1	0.5 ± 0.1	0.7 ± 0.2	0.6 ± 0.1	0.7 ± 0.2
	+ i.p. ABCB5+	0.3 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	0.6 ±0.1
	+ i.v. ABCB5+	0.3 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.5 ± 0.2	0.5 ± 0.1	0.5 ± 0.1	0.7 ± 0.1
Urea	Untreated	68.4 ± 18.2	69.4 ± 9.9	73.9 ± 8.6	88.4 ± 11.7	85.6 ± 8.1	85.1 ±13.0	87.4 ± 11.0
(mg/dl)	+ ABCB5+ derived CoCM+	57.5 ± 17.6	68.2 ± 19.9	80.5 ± 15.6	73.9 ± 20.8	74.8 ± 17.2	83.7 ± 20.9	86.5 ± 23.2

	+ i.p. ABCB5+	56.1 ± 10.4	66.6 ± 19.2	67.2 ± 14.7	68.9 ± 17.7	75.3 ± 16.0	74.9 ± 17.0	79.6 ± 17.7
	+ i.v. ABCB5+	52.2 ± 16.2	55.3 ± 17.6	59.3 ± 15.6	65.0 ± 20.2	63.9 ± 18.6	66.8 ± 23.6	72.3 ± 12.4
Na	Untreated	144.3 ± 0.8	143.0 ± 3.6	142.3 ±6.9	141.3 ± 2.3	144.3 ± 2.3	141.8 ± 1.7	144.8 ± 1.9
(mmol/l)	+ ABCB5+ derived CoCM+	141.2 ± 2.9	144.3 ± 3.6	144.0 ± 2.8	142.5 ± 3.1	144.3 ± 1.0	142.3 ± 3.2	142.2 ± 1.7
	+ i.p. ABCB5+	143.3 ± 1.6	142.7 ± 2.3	144.3 ± 2.4	143.3 ± 3.2	144.3 ± 1.4	143.7 ± 2.5	**141.9 ± 1.6***
	+ i.v. ABCB5+	141.6 ± 2.2	142.7 ± 2.0	143.7 ± 2.8	143.1 ± 2.3	143.7 ± 1.3	143.0 ± 2.6	**142.0 ± 2.0***
K	Untreated	5.4 ± 0.3	5.1 ± 0.6	5.2 ± 0.7	5.3 ± 0.4	5.3 ± 0.2	5.7 ± 0.4	5.1 ± 0.2
(mmol/l)	+ ABCB5+ derived CoCM+	5.2 ± 0.2	5.0 ± 0.2	5.0 ± 0.3	5.1 ± 0.4	4.9 ± 0.3	5.2 ± 0.3	5.3 ± 0.4
	+ i.p. ABCB5+	5.3 ± 0.3	4.8 ± 0.1	5.0 ± 0.2	5.3 ± 0.6	**5.2 ± 0.2***	**5.1 ± 0.3***	4.9 ± 0.2
	+ i.v. ABCB5+	5.2 ± 0.2	4.8 ± 0.2	4.9 ± .0.2	5.2 ± 0.3	4.9 ± 0.3	**5.1 ± 0.3***	5.0 ± 0.2
Ca	Untreated	2.5 ± 0.2	2.6 ± 0.0	2.6 ± 0.1	2.6 ± 0.1	2.6 ± 0.0	2.6 ± 0.1	2.7 ± 0.1
(mmol/l)	+ ABCB5+ derived CoCM+	2.6 ± 0.1	2.6 ± 0.1	2.5 ± 0.1	5.5 ± 7.3	**2.6 ± 0.0***	2.6 ± 0.1	2.4 ± 0.2
	+ i.p. ABCB5+	2.5 ± 0.1	**2.5 ± 0.1***	2.5 ± 0.2	2.5 ± 0.2	**2.5 ± 0.1****	2.7 ± 0.0	**2.4 ± 0.2***
	+ i.v. ABCB5+	2.5 ± 0.2	2.5 ± 0.1	2.5 ± 0.2	2.4 ± 0.3	2.6 ± 0.1	2.7 ± 0.5	2.5 ± 0.2
PO_4	Untreated	2.4 ± 0.5	2.2 ± 0.1	2.6 ± 0.2	2.1 ± 0.6	2.0 ± 0.3	2.1 ± 0.2	2.1 ± 0.3
(mmol/l)	+ ABCB5+ derived CoCM+	2.6 ± 0.3	2.5 ± 0.1	2.4 ± 0.2	2.3 ± 0.2	2.1 ± 0.2	2.1 ± 0.2	2.2 ± 0.2
	+ i.p. ABCB5+	2.7 ± 0.2	2.5 ± 0.1	**2.5 ± 0.1***	2.1 ± 0.2	2.1 ± 0.2	2.1 ± 0.1	2.1 ± 0.1
	+ i.v. ABCB5+	2.5 ± 0.3	2.4 ± 0.2	2.3 ± 0.1	2.2 ± 0.2	2.1 ± 0.2	2.0 ± 0.1	1.9 ± 0.2
Cholesterol	Untreated	97.3 ± 7.5	99.7 ± 6.7	110.0 ± 9.1	107.5 ± 8.5	131.7 ± 10.0	131.7 ± 10.2	154.3 ± 15.5
(mg/dl)	+ ABCB5+ derived CoCM+	89.7 ± 5.9	117.4 ± 39.0	102.3 ± 9.7	102.7 ± 18.6	110.5 ± 22.3	121.5 ± 16.7	138.3 ± 30.9
	+ i.p. ABCB5+	88.3 ± 4.6	1010.3 ± 25.8	102.7 ± 12.5	97.7 ± 10.9	**100.3 ± 12.6***	116.0 ± 14.2	135.1 ± 23.0
	+ i.v. ABCB5+	88.6 ± 6.9	105.9 ± 37.4	94.0 ± 14.6	100.9 ± 22.7	**104.4 ± 17.0***	109.9 ± 18.7	134.0 ± 29.3
Triglycerides	Untreated	69.2 ± 26.3	54.3 ± 14.5	67.2 ± 19.4	91.5 ± 21.6	68.7 ± 10.0	60.2 ± 15.0	108.8 ± 48.3
(mg/dl)	+ ABCB5+ derived CoCM+	75.7 ± 34.5	49.8 ± 19.2	44.3 ± 16.0	53.3 ± 24.6	60.7 ± 22.9	71.7 ± 32.0	69.8 ± 23.4
	+ i.p. ABCB5+	77.7 ± 29.8	37.1 ± 7.8	49.0 ± 14.4	53.9 ± 23.3	72. 7 ± 42.7	69.0 ± 28.0	79.3 ± 29.0
	+ i.v. ABCB5+	70.9 ± 30.7	52.0 ± 5.6	50.0 ± 13.2	56.9 ± 20.0	72.7 ± 34.3	72.9 ± 30.4	72.1 ± 29.6
Glucose	Untreated	169.0 ± 9.0	167.8 ± 9.4	183.8 ± 18.7	195.8 ± 32.1	173.3 ± 12.6	176.5 ± 19.6	184.3 ± 7.4
(mg/dl)	+ ABCB5+ derived CoCM+	168.7 ± 6.4	170.0 ± 10.0	168.0 ± 7.3	170.5 ± 7.2	170.2 ± 10.6	174.5 ± 9.3	171.7 ± 12.0
	+ i.p. ABCB5+	168.3 ± 8.9	174.3 ± 14.7	176.4 ± 13.6	171.3 ± 14.4	177.0 ± 27.1	171.3 ± 11.3	168.0 ± 14.5
	+ i.v. ABCB5+	170.0 ± 11.7	168.0 ± 10.1	181.4 ± 13.7	167.6 ± 13.8	166.0 ± 16.0	180.6 ± 19.3	178.1 ± 14.6
Protein	Untreated	62.0 ± 1.7	63.0 ± 3.1	63.8 ± 2.9	61.0 ± 1.6	65.5 ± 4.1	62.7 ± 3.8	65.3 ± 2.7
(mg/dl)	+ ABCB5+ derived CoCM+	60.5 ± 2.1	51.4 ± 24.0	62.1 ± 3.1	63.2 ± 2.4	62.0 ± 3.6	63.3 ± 2.4	**61.3 ± 2.4***
	+ i.p. ABCB5+	59.4 ± 2.2	51.4 ± 21.7	62.6 ± 2.8	62.3 ± 3.0	61.9 ± 3.7	64.1 ± 2.7	64.1 ± 1.9
	+ i.v. ABCB5+	58.7 ± 3.6	44.5 ± 28.6	61.9 ± 1.6	65.0 ± 2.5	64.9 ± 3.7	6.6 ± 3.0	63.1 ± 1.6
AST	Untreated	88.3 ± 8.8	89.3 ± 10.9	113.3 ± 69.5	92.5 ± 14.9	81.8 ± 5.7	138.5 ± 44.6	72.2 ± 12.4
(U/l)	+ ABCB5+ derived CoCM+	78.0 ± 6.7	94.2 ± 23.9	80.8 ± 8.3	86.3 ± 13.6	84.2 ± 11.5	84.5 ± 20.9	88.2 ± 15.1
	+ i.p. ABCB5+	111.9 ± 88.5	88.6 ± 13.4	91.7 ± 9.9	87.9 ± 7.6	85.9 ± 21.3	78.6 ± 13.3	79.6 ± 9.5
	+ i.v. ABCB5+	79.7 ± 9.5	95.9 ± 18.2	83.6 ± 6.7	106.7 ± 39.4	80.1 ± 18.5	85.3 ± 13.1	81.4 ± 14.6
ALT	Untreated	43.2 ± 6.3	53.3 ± 6.2	58.5 ± 13.8	57.8 ± 7.1	48.7 ± 6.5	53.7 ± 8.9	48.8 ± 10.3
(U/l)	+ ABCB5+ derived CoCM+	41.2 ± 6.6	48.0 ± 5.5	47.7 ± 7.4	54.8 ± 7.3	53.8 ± 9.0	53.2 ± 12.1	47.7 ± 3.7
	+ i.p. ABCB5+	39.7 ± 17.7	48.1 ± 9.9	48.0 ± 4.7	47.3 ± 4.1	56.9 ± 18.9	47.9 ± 7.3	47.6 ± 8.4

	+ i.v. ABCB5+	41.6 ± 6.7	54.0 ± 8.6	48.6 ± 5.0	53.3 ± 3.5	59.9 ± 18.4	48.7 ± 9.9	46.3 ± 4.3

Appendix 3B Changes in plasma biochemistry in PKD/Mhm (Cy/+) groups (n=6 in each group). Comparison between ASC derived CM, i.v. or i.p. ASC groups and untreated. Data are shown as mean ± Std.Dev. Values significantly different from control are indicated as *p<0.05, **p<0.005.

B	Parameter	Animal group	Baseline	Day25	Day 53	Day 81	Day 109	Day 137	Day 167
	Creatinine	Untreated	0.3 ± 0.1	0.4 ± 0.1	0.6 ± 0 1	0.6 ± 0.2	0.6 ± 0.1	0.7 ± 0.1	0.7 ± 0.1
	(mg/dl)	+ ASC derived CM	0.3 ± 0.1	0.5 ± 0.1	0.7 ± 0.1	0.7 ± 0.1	0.7 ± 0.0	0.7 ± 0.1	0.7 ± 0.2
		+ i.p. ASC	0.3 ± 0.0	0.5 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	0.5 ± 0.1*	0.6 ± 0.1	0.6 ± 0.1
		+ i.v. ASC	0.3 ± 0.0	0.5 ± 0.1	0.6 ± 0.0	0.7 ± 0.1	0.6 ± 0.0	0.7 ± 0.1	0.7 ± 0.1
	Urea	Untreated	68.4 ± 18.2	69.4 ± 9.9	73.9 ± 8.6	88.4 ± 11.7	85.6 ± 8.1	85.1 ±13.0	87.4 ± 11.0
	(mg/dl)	+ ASC derived CM	67.9 ± 8.2	11.8 ± 13.4	93.3 ± 7.3	97.6 ± 7.8	94.4 ± 7.0	96.4 ± 8.2	98.7 ± 21.6
		+ i.p. ASC	66.0 ± 8.9	74.7 ± 10.7	84.5 ± 7.8	80.8 ± 11.7	81.1 ± 11.5	79.2 ± 9.7	90.8 ± 12.7
		+ i.v. ASC	63.9 ± 3.6	80.1 ± 7.0	91.3 ± 7.4	88.9 ± 12.3	86.8 ± 6.2	90.9 ± 6.5	95.6 ± 10.6
	Na	Untreated	144.3 ± 0.8	143.0 ± 3.6	142.3 ±6.9	141.3 ± 2.3	144.3 ± 2.3	141.8 ± 1.7	144.8 ± 1.9
	(mmol/l)	+ ASC derived CM	144.8 ± 4.0	140.0 ± 3.6	138.6 ± 2.6	140.5 ± 2.5	143.0 ± 2.9	145.0 ± 2.7	145.3 ± 2.2
		+ i.p. ASC	143.2 ± 2.1	141.4 ± 4.9	139.8 ± 3.3	145.3 ± 5.1	144.7 ± 1.9	144.3 ± 0.8	**138.5 ± 5.0***
		+ i.v. ASC	144.0 ± 2.1	144.7 ± 3.5	140.0 ± 3.7	144.0 ± 6.5	143.8 ± 2.8	143.0 ± 0.9	139.5 ± 4.4
	K	Untreated	5.4 ± 0.2	5.1 ± 0.6	5.2 ± 0.7	5.3 ± 0.4	5.3 ± 0.2	5.7 ± 0.4	5.1 ± 0.2
	(mmol/l)	+ ASC derived CM	6.0 ± 1.8	5.2 ± 0.5	5.2 ± 0.3	5.4 ± 0.4	5.3 ± 0.5	5.2 ± 0.4	5.7 ± 0.4
		+ i.p. ASC	5.0 ± 0.3	5.4 ± 0.9	5.4 ± 0.3	5.3 ± 0.4	5.2 ± 0.2	5.2 ± 0.3	**6.1 ± 0.5****
		+ i.v. ASC	5.0 ± 0.4	5.9 ± 0.6	5.3 ± 0.5	5.2 ± 0.3	5.2 ± 0.4	5.0 ± 0.5	5.7 ± 0.4
	Ca	Untreated	2.5 ± 0.2	2.6 ± 0.0	2.6 ± 0.1	2.6 ± 0.1	2.6 ± 0.0	2.6 ± 0.1	2.9 ± 0.1
	(mmol/l)	+ ASC derived CM	1.5 ± 0.2	2.6 ± 0.1	2.6 ± 0.0	2.5 ± 0.1	2.6 ± 0.1	2.6 ± 0.1	2.7 ± 0.1
		+ i.p. ASC	2.6 ± 0.1	2.6 ± 0.1	2.6 ± 0.1	2.6 ± 0.1	2.6 ± 0.1	2.6 ± 0.1	2.6 ± 0.1
		+ i.v. ASC	2.7 ± 0.1	2.6 ± 0.1	2.5 ± 0.1	2.6 ± 0.0	2.6 ± 0.1	2.6 ± 0.1	2.6 ± 0.1
	PO_4	Untreated	2.4 ± 0.5	2.2 ± 0.1	2.6 ± 0.2	2.1 ± 0.4	2.0 ± 0.3	2.1 ± 0.2	2.1 ± 0.3
	(mmol/l)	+ ASC derived CM	2.5 ± 0.3	2.4 ± 0.2	2.3 ± 0.1	2.3 ± 0.1	2.3 ± 0.1	2.2 ± 0.2	2.0 ± 0.4
		+ i.p. ASC	2.6 ± 0.3	2.7 ± 0.3	2.3 ± 0.3	2.2 ± 0.1	2.1 ± 0.1	2.2 ± 0.1	1.8 ± 0.3
		+ i.v. ASC	2.7 ± 0.2	2.5 ± 0.4	2.2 ± 0.3	2.3 ± 0.1	2.2 ± 0.1	2.3 ± 0.2	1.9 ± 0.2
	Cholesterol	Untreated	97.3 ± 7.5	99.7 ± 6.7	110.0 ± 9.1	107.5 ± 8.5	131.7 ± 10.0	131.7 ± 10.2	154.3 ± 15.5
	(mg/dl)	+ ASC derived CM	99.8 ± 7.6	100.3 ± 9.7	107.4 ± 13.7	117.8 ± 12.8	127.0 ± 17.9	138.3 ± 23.4	152.3 ± 22.0
		+ i.p. ASC	95.8 ± 6.5	96.2 ± 7.8	100.5 ± 7.3	111.0 ± 5.4	111.0 ± 8.5	134.3 ± 5.7	144.7 ± 10.6
		+ i.v. ASC	97.0 ± 8.5	103.3 ± 5.1	99.7 ± 6.3	116.5 ± 8.4	119.0 ± 6.5	139.0 ± 13.1	149.5 ± 17.3
	Triglycerides	Untreated	69.2 ± 26.3	54.3 ± 14.5	67.2 ± 19.4	91.5 ± 21.6	68.7 ± 10.0	60.2 ± 15.0	108.8 ± 48.3
	(mg/dl)	+ ASC derived CM	78.5 ± 14.5	82.5 ± 27.0	62.8 ± 28.2	81.3 ± 31.3	66.0 ± 7.0	80.8 ± 21.9	63.3 ± 36.2
		+ i.p. ASC	76.5 ± 17.8	77.0 ± 22.8	64.5 ± 23.1	99.0 ± 43.2	72.0 ± 16.6	78.0 ± 14.2	98.8 ± 42.2
		+ i.v. ASC	94.7 ± 28.1	93.0 ± 16.6	70.3 ± 15.0	70.7 ± 15.0	10.2 ± 30.7	85.7 ± 27.0	63.5 ± 17.2
	Glucose	Untreated	169.0 ± 9.0	167.8 ± 9.4	183.8 ± 18.7	195.8 ± 32.1	173.3 ± 12.6	176.5 ± 19.6	184.3 ± 7.4
	(mg/dl)	+ ASC derived CM	167.0 ± 10.6	163.3 ± 14.3	170.6 ± 13.0	165.6 ± 6.8*	173.0 ± 17.4	166.5 ± 3.1	173.0 ± 9.3

	+ i.p. ASC	165.0 ± 10.8	157.8 ± 14.5	181.8 ± 29.4	174.5 ± 10.1	172.8 ± 13.0	171.3 ± 12.3	174.7 ± 17.1
	+ i.v. ASC	172.5 ± 10.3	169.6 ± 8.0	185.8 ± 19.2	172.5 ± 21.5	174.2 ± 7.0	176.2 ± 31.0	174.5 ± 9.9
Protein	Untreated	62.0 ± 1.7	63.0 ± 3.1	63.8 ± 2.9	61.0 ± 1.6	65.5 ± 4.1	62.7 ± 3.8	65.3 ± 2.7
(mg/dl)	+ ASC derived CM	61.7 ± 3.1	61.5 ± 1.5	61.8 ± 2.2	62.8 ± 2.8	62.8 ± 7.1	65.8 ± 3.5	66.3 ± 1.7
	+ i.p. ASC	59.5 ± 5.6	61.2 ± 2.8	62.3 ± 2.7	66.3 ± 1.0	67.3 ± 1.5	67.5 ± 1.9	66.3 ± 2.8
	+ i.v. ASC	59.0 ± 4.1	62.6 ± 3.2	60.7 ± 1.4	63.7 ± 1.4	64.0 ± 2.0	65.8 ± 4.0	65.2 ± 1.5
AST	Untreated	88.3 ± 8.8	89.3 ± 10.9	113.3 ± 69.5	92.5 ± 14.9	81.8 ± 5.7	138.5 ± 44.6	72.2 ± 12.4
(U/l)	+ ASC derived CM	88.7 ± 11.7	93.0 ± 1.2	84.4 ± 10.8	87.8 ± 14.2	78.0 ± 15.1	83.3 ± 3.7	73.3 ± 5.0
	+ i.p. ASC	82.0 ± 12.1	85.0 ± 6.6	95.7 ± 45.9	79.8 ± 5.9	96.2 ± 25.1	81.2 ± 5.0	98.5 ± 59.0
	+ i.v. ASC	79.7 ± 8.9	81.8 ± 23.0	84.2 ± 18.8	78.2 ± 7.6	83.8 ± 15.9	103.5 ± 66.6	69.3 ± 4.0
ALT	Untreated	43.2 ± 6.3	53.3 ± 6.2	58.5 ± 13.8	57.8 ± 7.1	48.7 ± 6.5	53.7 ± 8.9	48.8 ± 10.3
(U/l)	+ ASC derived CM	46.8 ± 4.3	53.3 ± 5.1	51.0 ± 10.0	47.5 ± 2.6	46.5 ± 9.1	52.5 ± 10.1	54.0 ± 14.4
	+ i.p. ASC	161.7 ± 286.8	51.0 ± 3.6	51.7 ± 12.2	52.0 ± 10.1	52.5 ± 5.4	59.3 ± 5.2	51.7 ± 7.5
	+ i.v. ASC	50.3 ± 3.0	153.8 ± 130.4	51.8 ± 4.0	55.7 ± 7.6	58.2 ± 14.6	60.2 ± 11.6	51.0 ± 14.6

Appendix 4A Changes in diuresis, food intake and water intake after metabolic cages in PKD/Mhm (Cy/+) groups (n=6). Comparison between ABCB5+ derived CoCM+, i.v. or i.p. ABCB5+ groups and untreated. Data are shown as mean ± Std.Dev.

A Parameter	Animal group	Baseline	Day25	Day 53	Day 81	Day 109	Day 137	Day 167
Diuresis (ml)	Untreated	20.3 ± 4.9	29.4 ± 3.5	26.8 ± 7.4	31.2 ± 11.2	44.6 ± 10.4	44.6 ± 10.4	55.8 ± 54.5
	+ ABCB5+ derived CoCM+	21.3 ± 5.8	31.0 ± 7.6	26.0 ± 7.4	32.6 ± 5.2	39.5 ± 11.9	34.6 ± 7.8	28.4 ± 10.1
	+ i.p. ABCB5+	18.7 ± 3.9	31.4 ± 11.4	27.3 ± 4.4	35.3 ± 13.2	65.7 ± 91.5	36.0 ± 11.4	26.3 ± 7.3
	+ i.v. ABCB5+	18.5 ± 6.0	29.2 ± 5.2	42.7 ± 13.3	37.2 ± 12.2	40.9 ± 18.2	33.6 ± 19.5	48.1 ± 20.6
Food intake	Untreated	15.9 ± 7.0	17.9 ± 2.1	16.7 ± 3.0	13.0 ± 6.6	17.8 ± 5.9	17.6 ± 5.1	12.3 ± 4.3
(g)	+ ABCB5+ derived CoCM+	18.0 ± 5.1	17.7 ± 4.7	14.5 ± 4.5	14.5 ± 3.0	14.3 ± 3.4	12.3 ± 2.8	10.1 ± 4.3
	+ i.p. ABCB5+	19.1 ± 3.9	15.1 ± 2.9	15.6 ± 1.9	16.2 ± 3.4	13.7 ± 3.4	12.7 ± 3.1	12.1 ± 3.4
	+ i.v. ABCB5+	18.1 ± 2.7	18.5 ± 2.2	16.0 ± 2.8	14.3 ± 3.3	13.0 ± 2.8	13.3 ± 2.5	11.6 ± 2.4
Water intake	Untreated	36.6 ± 8.9	53.0 ± 9.3	47.2 ± 10.5	50.2 ± 29.1	60.0 ± 25.1	55.5 ± 13.3	56.0 ± 15.6
(g)	+ ABCB5+ derived CoCM+	40.8 ± 8.5	49.6 ± 9.1	37.8 ± 14.8	42.9 ± 6.0	52.7 ± 17.7	43.3 ± 11.4	33.5 ± 15.5
	+ i.p. ABCB5+	39.2 ± 3.8	44.6 ± 13.6	38.9 ± 7.3	52.5 ± 16.4	42.8 ± 12.7	45.7 ± 13.8	32.3 ± 9.8
	+ i.v. ABCB5+	37.3 ± 5.8	47.4 ± 5.1	55.0 ± 14.8	47.6 ± 12.4	51.7 ± 21.4	48.1 ± 18.3	55.9 ± 21.8

Appendix 4B Changes in diuresis, food intake and water intake after metabolic cages in PKD/Mhm (Cy/+) groups (n=6). Comparison between ASC derived CM, i.v. or i.p. ASC groups and untreated. Data are shown as mean ± Std.Dev.

B	Parameter	Animal group	Baseline	Day25	Day 53	Day 81	Day 109	Day 137	Day 167
	Diuresis (ml)	Untreated	20.3 ± 4.9	29.4 ± 3.5	26.8 ± 7.4	31.2 ± 11.2	44.6 ± 10.4	44.6 ± 10.4	55.8 ± 54.5
		+ ASC derived CM	17.0 ± 5.6	30.0 ± 9.5	21.1 ± 10.0	29.5 ± 5.6	42.9 ± 9.0	42.1 ± 6.4	28.9 ± 5.6
		+ i.p. ASC	18.1 ± 6.0	28.8 ± 5.5	38.2 ± 20.2	32.9 ± 10.2	33.6 ± 14.4	35.0 ± 9.4	28.4 ± 13.6
		+ i.v. ASC	13.2 ± 2.3	27.4 ± 6.6	27.0 ± 3.6	30.9 ± 8.3	34.8 ± 9.0	40.3 ± 10.9	33.6 ± 13.1
	Food intake	Untreated	15.9 ± 7.0	17.9 ± 2.1	16.7 ± 3.0	13.0 ± 6.6	17.8 ± 5.9	17.6 ± 5.1	12.3 ± 4.3
	(g)	+ ASC derived CM	17.6 ± 3.7	16.3 ± 6.7	16.0 ± 3.7	19.0 ± 6.6	19.4 ± 4.8	18.0 ± 5.7	9.9 ± 2.7
		+ i.p. ASC	13.5 ± 5.7	18.9 ± 1.8	17.3 ± 3.4	19.3 ± 5.0	21.1 ± 5.8	17.3 ± 5.2	11.5 ± 3.5
		+ i.v. ASC	16.0 ± 4.9	19.3 ± 3.5	16.1 ± 1.6	15.2 ± 1.3	15.1 ± 8.2	17.3 ± 8.5	10.1 ± 2.2
	Water intake	Untreated	36.6 ± 8.9	53.0 ± 9.3	47.2 ± 10.5	50.2 ± 29.1	60.0 ± 25.1	55.5 ± 13.3	56.0 ± 15.6
	(g)	+ ASC derived CM	36.7 ± 7.3	54.9 ± 7.3	43.9 ± 9.7	52.8 ± 12.4	60.0 ± 8.1	55.1 ± 8.6	40.9 ± 12.5
		+ i.p. ASC	32.5 ± 15.0	48.9 ± 5.1	47.2 ± 12.3	49.8 ± 14.6	49.4 ± 12.3	47.8 ± 14.5	43.1 ± 13.6
		+ i.v. ASC	30.7 ± 6.0	47.1 ± 8.2	42.8 ± 3.9	46.9 ± 11.5	50.2 ± 15.8	47.8 ± 22.3	41.1 ± 14.5

Appendix 5A Changes in urine biochemistry in PKD/Mhm (Cy/+) groups (n=6). Comparison between ABCB5+ derived CoCM+, i.v. or i.p. ABCB5+ groups and untreated. Data are shown as mean ± Std.Dev. Values significantly different from control are indicated as *p<0.05.

A	Parameter	Animal group	Baseline	Day25	Day 53	Day 81	Day 109	Day 137	Day 167
	Creatinine	Untreated	8.3 ± 1.7	10.8 ± 2.3	10.1 ± 2.6	9.8 ± 1.6	12.0 ± 1.2	15.8 ± 3.0	16.9 ± 8.6
	(mg/16h)	+ ABCB5+ derived CoCM+	7.8 ± 1.1	11.2 ± 0.8	11.4 ± 1.8	12.2 ± 0.8	13.5 ± 1.1	13.3 ± 1.5	12.3 ± 2.3
		+ i.p. ABCB5+	7.9 ± 1.1	11.3 ± 1.6	11.8 ± 1.3	12.5 ± 1.3	13.1 ± 1.0	13.7 ± 1.2	12.5 ± 0.6
		+ i.v. ABCB5+	8.4 ± 1.1	11.6 ± 1.1	14.5 ± 3.8	13.5 ± 1.1	13.7 ± 1.5	12.5 ± 2.9	14.2 ± 2.6
	Urea	Untreated	572.9 ± 178.1	793.6 ± 121.1	699.4 ± 220.2	562.3 ± 185.4	741.9 ± 175.6	890.7 ± 160.2	830.5 ± 376.6
	(mg/16h)	+ ABCB5+ derived CoCM+	606.2 ± 112.1	754.4 ± 57.6	659.4 ± 155.1	718.0 ± 73.6	729.0 ± 116.8	708.4 ± 110.0	552.0 ± 142.1
		+ i.p. ABCB5+	602.0 ± 78.7	755.8 ± 172.6	718.7 ± 79.6	722.6 ± 126.3	676.2 ± 119.6	705.8 ± 118.3	610.5 ± 54.9
		+ i.v. ABCB5+	619.3 ± 90.7	830.2 ± 111.1	881.9 ± 304.9	**750.4 ± 57.5***	684.1 ± 121.7	628.1 ±184.5	702.9 ± 149.6
	Na	Untreated	1.6 ± 0.7	1.6 ± 0.3	1.5 ± 0.4	1.0 ± 0.4	1.8 ± 0.7	2.2 ± 0.7	1.7 ± 1.4
	(mmol/16h)	+ ABCB5+ derived CoCM+	1.4 ± 0.6	1.5 ± 0.5	1.4 ± 0.5	1.4 ± 0.4	1.5 ± 0.7	1.4 ± 0.6	1.5 ± 0.5
		+ i.p. ABCB5+	1.5 ± 0.2	1.4 ± 0.5	1.5 ± 0.3	1.3 ± 0.5	1.5 ± 0.6	1.4 ± 0.4	1.4 ± 0.4
		+ i.v. ABCB5+	1.5 ± 0.5	1.5 ± 0.5	1.8 ± 0.6	1.6 ± 0.6	1.7 ± 0.5	1.3 ± 0.6	1.7 ± 0.6
	K	Untreated	3.1 ± 1.0	4.3 ± 1.0	3.5 ± 0.7	2.7 ± 1.0	4.2 ± 1.5	4.6 ± 1.0	4.2 ± 2.6
	(mmol/16h)	+ ABCB5+ derived CoCM+	3.7 ± 0.8	3.8 ± 1.2	3.3 ± 0.8	3.9 ± 0.4	3.6 ± 1.6	3.4 ± 0.9	3.1 ± 1.1
		+ i.p. ABCB5+	3.8 ± 0.4	4.0 ± 1.3	3.8 ± 0.7	4.1 ± 0.8	3.5 ± 1.1	3.7 ± 0.6	1.7 ± 1.1
		+ i.v. ABCB5+	3.9 ± 0.6	4.7 ± 1.4	5.0 ± 1.6	4.4 ± 0.6	3.7 ± 0.7	3.0 ± 1.0	3.7 ± 0.8
	Ca	Untreated	0.01 ± 0.01	0.02 ± 0.01	0.03 ± 0.01	0.02 ± 0.01	0.03 ± 0.02	0.04 ± 0.02	0.04 ± 0.04
	(mmol/16h)	+ ABCB5+ derived CoCM+	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01
		+ i.p. ABCB5+	0.01 ± 0.00	0.02 ± 0.01	0.02 ± 0.01	0.01 ± 0.01	0.01 ± 0.05	0.02 ± 0.01	0.01 ± 0.01
		+ i.v. ABCB5+	0.02 ± 0.01	0.02 ± 0.00	0.03 ± 0.01	0.02 ± 0.01	0.02 ± 0.00	0.01 ± 0.01	0.02 ± 0.00
	PO_4	Untreated	0.1 ± 0.1	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.1

(mmol/16h)	+ ABCB5+ derived CoCM+	0.0 ± 0.0	0.1 ± 0.0	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1
	+ i.p. ABCB5+	0.2 ± 0.0	0.1 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.0 ± 0.0
	+ i.v. ABCB5+	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.0	0.1 ± 0.1	0.1 ± 0.0
Glucose	Untreated	1.2 ± 0.4	1.3 ± 0.8	2.7 ± 2.2	2.0 ± 1.6	2.5 ± 2.3	2.3 ± 1.2	1.7 ± 1.7
(mg/16h)	+ ABCB5+ derived CoCM+	1.2 ± 0.9	2.6 ± 1.2	4.0 ± 3.7	3.5 ± 0.7	2.5 ± 1.5	1.5 ± 0.1	0.9 ± 0.8
	+ i.p. ABCB5+	1.3 ± 0.4	1.8 ± 0.8	3.0 ± 1.7	2.4 ± 1.0	1.5 ± 1.1	1.2 ± 0.7	1.4 ± 0.9
	+ i.v. ABCB5+	1.9 ± 0.8	2.4 ± 0.9	3.0 ± 2.6	3.0 ± 2.1	1.9 ± 1.2	**1.0 ± 0.8***	1.4 ± 0.6
Protein	Untreated	10.6 ± 5.2	19.5 ± 5.2	27.7 ± 9.8	32.7 ± 4.6	61.4 ± 16.3	96.8 ± 26.5	154.5 ± 177.7
(mg/16h)	+ ABCB5+ derived CoCM+	12.1 ± 5.5	19.7 ± 6.8	25.4 ± 12.9	33.8 ± 12.2	41.9 ± 16.7	50.2 ± 20.6	49.8 ± 16.4
	+ i.p. ABCB5+	12.2 ± 2.3	19.1 ± 5.2	30.2 ± 12.7	36.4 ± 13.1	47.5 ± 19.5	51.9 ± 22.6	68.4 ± 35.0
	+ i.v. ABCB5+	13.0 ± 3.5	20.3 ± 6.9	26.1 ± 10.2	34.4 ± 17.3	36.9 ± 19.5	**39.4 ± 25.6***	58.8 ± 36.5
Albumin	Untreated	2.3 ± 1.5	4.3 ± 1.7	12.4 ± 4.1	22.4 ± 11.4	27.4 ± 13.2	25.4 ± 11.3	103.9 ± 108.5
(mg/16h)	+ ABCB5+ derived CoCM+	1.8 ± 1.1	4.5 ± 2.0	9.4 ± 4.5	15.9 ± 6.4	24.8 ± 13.1	32.5 ± 26.4	39.1 ± 16.2
	+ i.p. ABCB5+	1.9 ± 0.9	8.6 ± 5.0	14.4 ± 12.1	21.4 ± 13.2	30.6 ± 24.7	38.9 ± 23.0	58.1 ± 37.5
	+ i.v. ABCB5+	1.6 ± 1.5	5.5 ± 3.7	9.1 ± 6.6	14.3 ± 8.7	20.9 ± 15.1	26.5 ± 24.6	52.6 ± 37.7

Appendix 5B Changes in urine biochemistry in PKD/Mhm (Cy/+) groups (n=6). Comparison between ASC derived CM, i.v. or i.p. ASC groups and untreated. Data are shown as mean ± Std.Dev. Values significantly different from control are indicated as *p<0.05.

B	Parameter	Animal group	Baseline	Day25	Day 53	Day 81	Day 109	Day 137	Day 167
	Creatinine	Untreated	8.3 ± 1.7	10.6 ± 2.3	10.1 ± 2.6	9.8 ± 1.6	12.0 ± 1.2	15.8 ± 2.9	16.9 ± 8.6
	(mg/16h)	+ ASC derived CM	7.7 ± 1.9	9.8 ± 2.3	9.2 ± 3.8	11.0 ± 2.8	15.2 ± 4.3	14.0 ± 1.7	13.6 ± 0.6
		+ i.p. ASC	7.7 ± 1.8	9.1 ± 4.2	13.5 ± 2.4	12.7 ± 6.1	13.3 ± 3.4	12.4 ± 1.6	10.9 ± 2.8
		+ i.v. ASC	7.2 ± 2.0	10.4 ± 0.8	11.5 ± 5.3	11.9 ± 1.8	15.7 ± 3.1	16.5 ± 1.5	13.8 ± 1.8
	Urea	Untreated	572.9 ± 178.1	793.6 ± 121.1	699.4 ± 220.1	562.3 ± 185.4	741.9 ± 175.6	890.7 ± 160.2	830.5 ± 376.6
	(mg/16h)	+ ASC derived CM	525.9 ± 127.7	699.8 ± 180.7	564.6 ± 249.6	684.6 ± 194.9	1070.6 ± 388.2	867.8 ± 137.8	644.5 ± 99.6
		+ i.p. ASC	488.3 ± 152.6	789.2 ± 115.7	902.9 ± 163.9	**856.6 ± 435.2***	880.4 ± 243.6	772.2 ± 109.1	557.6 ± 199.5
		+ i.v. ASC	497.2 ± .171.9	726.0 ± 128.7	822.9 ± 119.3	710.3 ± 99.8	971.0 ± 274.4	995.2 ± 243.7	666.2 ± 92.6
	Na	Untreated	1.6 ± 0.7	1.6 ± 0.3	1.5 ± 0.4	1.0 ± 0.4	1.8 ± 0.7	2.2 ± 0.7	1.7 ± 1.4
	(mmol/16h)	+ ASC derived CM	1.7 ± 0.8	1.5 ± 0.4	1.1 ± 0.6	1.3 ± 0.5	2.3 ± 0.5	2.1 ± 1.0	1.0 ± 0.2
		+ i.p. ASC	1.3 ± 0.4	1.7 ± 0.2	1.7 ± 0.2	1.8 ± 0.6	2.4 ± 0.6	1.7 ± 0.4	1.6 ± 0.9
		+ i.v. ASC	1.3 ± 0.7	1.6 ± 0.4	1.4 ± 0.4	1.4 ± 0.5	2.1 ± 0.7	2.3 ± 0.5	1.1 ± 0.3
	K	Untreated	3.1 ± 1.0	4.3 ± 1.0	3.5 ± 0.9	2.7 ± 1.0	4.2 ± 1.5	4.6 ± 1.0	4.2 ± 2.6
	(mmol/16h)	+ ASC derived CM	3.8 ± 1.4	3.7 ± 0.9	2.8 ± 1.4	3.6 ± 1.3	5.3 ± 1.9	5.1 ± 1.7	3.1 ± 0.6
		+ i.p. ASC	3.0 ± 0.6	4.0 ± 0.3	4.5 ± 1.0	4.8 ± 2.2	5.1 ± 1.5	3.9 ± 0.9	2.8 ± 1.0
		+ i.v. ASC	3.1 ± 0.8	3.9 ± 0.8	3.9 ± 1.0	3.9 ± 1.0	4.1 ± 1.1	5.0 ± 1.8	3.0 ± 0.8
	Ca	Untreated	0.01 ± 0.01	0.02 ± 0.01	0.03 ± 0.01	0.02 ± 0.01	0.03 ± 0.02	0.04 ± 0.02	0.04 ± 0.04
	(mmol/16h)	+ ASC derived CM	0.01 ± 0.00	0.02 ± 0.01	0.02 ± 0.01	0.03 ± 0.01	0.05 ± 0.01	0.04 ± 0.01	0.03 ± 0.02
		+ i.p. ASC	0.01 ± 0.00	0.02 ± 0.01	0.03 ± 0.01	0.02 ± 0.01	0.04 ± 0.03	0.02 ± 0.01	0.01 ± 0.01

	+ i.v. ASC	0.01 ± 0.01	0.02 ± 0.00	0.03 ± 0.01	0.02 ± 0.01	0.04 ± 0.04	0.04 ± 0.03	0.02 ± 0.01
PO_4	Untreated	0.1 ± 0.1	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.1
(mmol/16h)	+ ASC derived CM	0.1 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.1	0.1 ± 0.1
	+ i.p. ASC	0.1 ± 0.1	0.1 ± 0.0	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.1
	+ i.v. ASC	0.1 ± 0.1	0.1 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.1 ± 0.1	0.1 ± 0.0	0.1 ± 0.1
Glucose	Untreated	1.2 ± 0.4	1.3 ± 0.8	2.7 ± 2.2	2.0 ± 1.6	2.5 ± 2.3	2.3 ± 1.2	1.7 ± 1.7
(mg/16h)	+ ASC derived CM	1.3 ± 0.8	**5.5 ± 7.3***	3.5 ± 4.8	3.5 ± 2.3	3.3 ± 0.5	1.5 ± 0.8	1.3 ± 1.4
	+ i.p. ASC	0.8 ± 0.6	2.2 ± 1.6	3.0 ± 1.3	2.9 ± 1.2	2.1 ± 1.1	1.8 ± 0.3	1.9 ± 1.2
	+ i.v. ASC	0.8 ± 0.8	2.0 ± 0.9	4.2 ± 3.5	3.0 ± 1.3	3.9 ± 2.6	2.3 ± 0.7	1.8 ± 0.8
Protein	Untreated	10.6 ± 5.2	19.5 ± 5.2	27.7 ± 9.8	32.7 ± 4.6	61.4 ± 16.3	96.8 ± 26.5	154.5 ± 177.7
(mg/16h)	+ ASC derived CM	11.1 ± 3.2	18.6 ± 5.0	21.8 ± 12.0	35.6 ± 15.0	53.3 ± 18.3	63.7 ± 17.9	81.3 ± 15.8
	+ i.p. ASC	8.9 ± 3.2	18.8 ± 6.8	33.6 ± 5.6	38.6 ± 11.5	57.5 ± 15.7	66.2 ± 9.1	76.0 ± 41.1
	+ i.v. ASC	10.6 ± 5.3	14.9 ± 3.8	33.1 ± 13.5	36.2 ± 8.7	70.8 ± 32.0	88.1 ± 21.6	83.6 ± 25.5
Albumin	Untreated	2.3 ± 1.5	4.3 ± 1.8	12.4 ± 4.1	22.4 ± 11.4	27.4 ± 13.2	25.4 ± 11.3	103.9 ± 108.5
(mg/16h)	+ ASC derived CM	2.2 ± 1.9	6.6 ± 4.1	7.3 ± 4.8	16.0 ± 7.2	18.3 ± 16.3	30.2 ± 12.7	39.0 ± 6.6
	+ i.p. ASC	1.4 ± 0.9	4.7 ± 2.6	11.2 ± 6.2	18.0 ± 4.7	27.6 ± 17.2	31.0 ± 14.9	64.7 ± 34.6
	+ i.v. ASC	0.7 ± 0.2	3.5 ± 1.2	10.2 ± 5.4	11.6 ± 5.3	33.3 ± 15.3	28.6 ± 18.7	60.4 ± 26.1

Appendix 6A Changes of ABZWCY-HβCD half-life in PKD/Mhm (Cy/+) groups (n=6). Comparison between ABCB5+ derived CoCM+, i.v. or i.p. ABCB5+ groups and untreated. Data are shown as mean ± Std.Dev. Values significantly different from control are indicated as *p<0.05.

A	Parameter	Animal group	Baseline	Day25	Day 53	Day 81	Day 109	Day 137	Day 167
	ABZWCY-HβCD	Untreated	26.7 ± 9.3	30.6 ± 7.1	39.3 ± 11.3	42.3 ± 6.4	51.4 ± 16.8	60.7 ± 29.9	66.0 ± 11.4
	$t_{1/2}$ (min)	+ ABCB5+ derived CoCM+	24.0 ± 7.6	24.9 ± 5.6	53.3 ± 28.5	47.2 ± 15.1	52.0 ± 22.7	47.1 ± 25.8	55.0 ± 12.0
		+ i.p. ABCB5+	25.3 ± 9.8	31.7 ± 13.2	38.6 ± 10.7	43.6 ± 13.1	32.7 ± 8.6	39.0 ± 18.1	**41.2 ± 15.5***
		+ i.v. ABCB5+	23.8 ± 4.4	27.0 ± 9.5	32.5 ± 17.0	45.0 ± 15.3	35.2 ± 13.8	38.8 ± 11.1	43.9 ± 19.1

Appendix 6B Changes of ABZWCY-HβCD half-life in PKD/Mhm (Cy/+) groups (n=6). Comparison between ASC derived CM, i.v. or i.p. ASC groups and untreated. Data are shown as mean ± Std.Dev. Values significantly different from control are indicated as *p<0.05.

B	Parameter	Animal group	Baseline	Day25	Day 53	Day 81	Day 109	Day 137	Day 167
	ABZWCY-HβCD	Untreated	26.7 ± 9.3	30.6 ± 7.1	39.3 ± 11.3	42.3 ± 6.4	51.4 ± 16.8	60.7 ± 29.9	66.0 ± 11.4
	t1/2 (min)	+ ASC derived CM	26.8 ± 7.3	33.4 ± 11.9	38.5 ± 9.6	42.6 ± 9.1	43.0 ± 10.7	51.2 ± 16.8	49.6 ± 15.7
		+ i.p. ASC	34.6 ± 5.7	28.3 ± 5.2	31.1 ± 8.8	40.8 ± 8.2	36.5 ± 9.6	39.3 ± 10.8	**38.8 ± 8.1***
		+ i.v. ASC	33.2 ± 7.3	40.28 ± 12.2	31.1 ± 10.1	49.9 ± 23.3	43.0 ± 16.8	54.0 ± 11.2	43.0 ± 19.8

Appendix 7A Changes in plasma biochemistry in PCK groups (n=6). Comparison between ABCB5+ derived CoCM+, i.v. or i.p. ABCB5+ groups and untreated. Data are shown as mean ± Std.Dev. Values significantly different from control are indicated as *p<0.05.

A	Parameter	Animal group	Baseline	Day25	Day 53	Day 81	Day 109	Day 137	Day 167
	Creatinine	Untreated	0.2 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.4 ± 0.0	0.4 ± 0.1	0.5 ± 0.1
	(mg/dl)	+ ABCB5+ derived CoCM+	0.2 ± 0.0	0.3 ± 0.0	0.3 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.5 ± 0.1
		+ i.p. ABCB5+	0.2 ± 0.0	0.3 ± 0.0	0.4 ± 0.1	0.4 ± 0.1	0.4 ± 0.2	0.4 ± 0.1	0.6 ± 0.2
		+ i.v. ABCB5+	0.2 ± 0.0	0.3 ± 0.0	0.3 ± 0.1	0.4 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	0.5 ± 0.1
	Urea	Untreated	31.9 ± 2.8	35.0 ± 4.3	32.5 ± 2.9	36.3 ± 6.2	42.1 ± 5.1	44.6 ± 2.3	56.6 ± 3.2
	(mg/dl)	+ ABCB5+ derived CoCM+	32.8 ± 3.1	33.5 ± 2.1	34.6 ± 1.8	37.9 ± 4.7	39.3 ± 5.9	46.5 ± 9.7	50.7 ± 8.9
		+ i.p. ABCB5+	33.7 ± 5.0	36.1 ± 2.8	40.1 ± 10.0	43.8 ± 14.3	46.0 ± 13.2	50.9 ± 14.1	54.1 ± 16.5
		+ i.v. ABCB5+	30.9 ± 2.6	33.2 ± 3.3	35.1 ± 3.3	37.1 ± 5.1	40.9 ± 6.3	43.4 ± 8.2	50.3 ± 10.6
	Na	Untreated	139.2 ± 1.2	140.7 ± 2.7	141.0 ± 3.0	139.7 ± 1.2	139.8 ± 1.1	140.6 ± 1.7	143.8 ± 1.8
	(mmol/l)	+ ABCB5+ derived CoCM+	140.3 ± 3.1	138.7 ± 1.4	141.3 ± 1.6	141.7 ± 1.6	138.8 ± 2.4	140.8 ± 2.0	**139.3 ± 2.3***
		+ i.p. ABCB5+	140.9 ± 1.4	139.7 ± 1.7	141.9 ± 2.0	141.7 ± 1.5	140.0 ± 2.2	140.3 ± 1.5	**140.3 ± 2.2***
		+ i.v. ABCB5+	141.0 ± 1.5	139.1 ± 1.6	140.9 ± 1.6	142.7 ± 1.5	141.7 ± 3.2	141.4 ± 1.8	141.0 ± 1.9
	K	Untreated	5.4 ± 0.3	5.0 ± 0.5	5.3 ± 0.3	5.2 ± 0.4	5.4 ± 0.4	5.2 ± 0.4	5.1 ± 0.2
	(mmol/l)	+ ABCB5+ derived CoCM+	5.2 ± 0.4	5.0 ± 0.4	4.7 ± 0.3	4.7 ± 0.2	4.7 ± 0.2	4.7 ± 0.4	5.1 ± 0.5
		+ i.p. ABCB5+	5.3 ± 0.4	4.7 ± 0.6	4.8 ± 0.5	5.0 ± 0.4	5.3 ± 1.0	4.7 ± 0.4	5.0 ± 0.3
		+ i.v. ABCB5+	5.1 ± 0.3	4.9 ± 0.6	4.7 ± 0.2	4.7 ± 0.2	4.7 ± 0.4	4.8 ± 0.4	4.7 ± 0.5
	Ca	Untreated	2.7 ± 0.1	2.7 ± 0.1	2.8 ± 0.1	2.7 ± 0.0	2.7 ± 0.1	2.7 ± 0.1	2.8 ± 0.1
	(mmol/l)	+ ABCB5+ derived CoCM+	2.7 ± 0.2	2.7 ± 0.1	2.7 ± 0.1	2.7 ± 0.1	2.7 ± 0.1	2.7 ± 0.1	2.7 ± 0.2
		+ i.p. ABCB5+	2.7 ± 0.1	2.9 ± 0.4	2.7 ± 0.2	2.7 ± 0.1	2.7 ± 0.1	2.7 ± 0.1	2.7 ± 0.1
		+ i.v. ABCB5+	2.7 ± 0.1	2.8 ± 0.3	2.7 ± 0.1	2.7 ± 0.1	2.7 ± 0.1	2.7 ± 0.1	2.7 ± 0.1
	PO4	Untreated	2.3 ± 0.3	2.5 ± 0.3	2.0 ± 0.2	1.6 ± 0.5	2.0 ± 0.4	2.0 ± 0.4	2.2 ± 0.1
	(mmol/l)	+ ABCB5+ derived CoCM+	2.3 ± 0.2	2.4 ± 0.2	2.0 ± 0.2	2.2 ± 0.2	2.2 ± 0.2	2.1 ± 0.2	2.1 ± 0.3
		+ i.p. ABCB5+	2.2 ± 0.3	2.1 ± 0.2	2.2 ± 0.2	2.2 ± 0.1	2.2 ± 0.1	2.1 ± 0.2	2.2 ± 0.2
		+ i.v. ABCB5+	2.3 ± 0.5	2.2 ± 0.2	2.2 ± 0.4	2.2 ± 0.1	2.1 ± 0.3	2.1 ± 0.3	2.1 ± 0.2
	Cholesterol	Untreated	140.3 ± 15.6	166.2 ± 16.8	211.5 ± 29.1	237.0 ± 23.7	289.5 ± 36.5	312.6 ± 39.0	301.2 ± 62.1
	(mg/dl)	+ ABCB5+ derived CoCM+	155.3 ± 41.6	175.7 ± 41.5	205.0 ± 55.9	254.3 ± 64.8	273.3 ± 61.0	297.3 ± 79.0	273.5 ± 76.3
		+ i.p. ABCB5+	143.1 ± 25.5	170.6 ± 29.9	189.3 ± 28.7	223.1 ± 38.9	246.4 ± 48.1	274.3 ± 58.5	294.0 ± 67.9
		+ i.v. ABCB5+	156.3 ± 37.6	176.1 ± 37.5	185.7 ± 32.2	226.6 ± 45.0	265.0 ± 37.6	280.9 ± 57.3	266.2 ± 44.5
	Triglycerides	Untreated	86.8 ± 15.0	79.3 ± 15.1	93.2 ± 28.3	132.2 ± 36.7	126.7 ± 28.2	137.5 ± 29.5	160.5 ± 46.9
	(mg/dl)	+ ABCB5+ derived CoCM+	85.0 ± 12.3	70.5 ± 12.1	104.8 ± 30.8	111.8 ± 45.7	133.7 ± 39.7	157.5 ± 57.6	143.7 ± 45.0
		+ i.p. ABCB5+	89.3 ± 15.9	73.1 ± 20.5	103.1 ± 29.4	102.4 ± 27.9	129.9 ± 40.4	129.0 ± 19.0	153.5 ± 47.8
		+ i.v. ABCB5+	91.5 ± 13.6	83.1 ± 15.3	97.4 ± 28.2	98.3 ± 17.7	113.3 ± 20.0	175.3 ± 56.9	118.2 ± 34.7
	Glucose	Untreated	166.8 ± 10.6	143.7 ± 11.4	149.3 ± 20.0	162.7 ± 32.8	172.8 ± 12.9	143.0 ± 16.4	150.3 ± 24.0
	(mg/dl)	+ ABCB5+ derived CoCM+	165.3 ± 26.4	155.2 ± 25.8	144.5 ± 18.0	151.3 ± 14.6	147.8 ± 21.6	130.3 ± 16.9	147.2 ± 18.2
		+ i.p. ABCB5+	169.4 ± 24.7	157.1 ± 6.2	142.3 ± 14.2	149.0 ± 16.6	146.9 ± 37.7	139.0 ± 12.1	145.8 ± 23.3

	+ i.v. ABCB5+	161.3 ± 18.7	153.9 ± 19.7	139.7 ± 8.5	152.0 ± 13.6	143.7 ± 15.3	136.9 ± 11.5	143.8 ± 11.8
Protein	Untreated	62.2 ± 1.8	66.0 ± 4.2	82.3 ± 31.3	63.2 ± 4.8	64.0 ± 4.0	66.2 ± 3.1	69.5 ± 2.1
(mg/dl)	+ ABCB5+ derived CoCM+	64.7 ± 5.3	65.0 ± 4.3	68.7 ± 2.0	67.5 ± 2.9	68.0 ± 1.6	69.5 ± 2.0	69.3 ± 6.4
	+ i.p. ABCB5+	62.7 ± 4.0	65.6 ± 2.0	69.9 ± 5.9	68.1 ± 4.3	66.7 ± 2.7	68.0 ± 2.5	67.5 ± 3.8
	+ i.v. ABCB5+	66.1 ± 3.0	69.0 ± 5.3	68.1 ± 3.4	67.7 ± 4.5	67.1 ± 3.8	68.8 ± 2.7	68.3 ± 4.9
ALT	Untreated	82.5 ± 20.4	69.3 ± 22.1	90.0 ± 21.3	90.3 ± 19.2	77.5 ± 22.6	66.5 ± 34.9	73.3 ± 40.0
(U/l)	+ ABCB5+ derived CoCM+	68.3 ± 13.1	76.8 ± 15.4	85.3 ± 31.4	78.2 ± 31.0	74.0 ± 26.8	57.3 ± 26.2	61.0 ± 17.8
	+ i.p. ABCB5+	77.1 ± 6.2	78.0 ± 16.1	93.9 ± 20.5	68.7 ± 28.0	74.0 ± 15.9	60.2 ± 18.6	69.3 ± 16.3
	+ i.v. ABCB5+	71.9 ± 10.0	81.8 ± 22.1	88.1 ± 14.4	75.7 ± 10.5	70.0 ± 14.2	55.0 ± 14.0	66.1 ± 13.4
AST	Untreated	134.0 ± 42.1	124.4 ± 40.4	144.5 ± 36.3	134.3 ± 46.1	162.5 ± 84.6	113.8 ± 33.3	135.2 ± 144.9
(U/l)	+ ABCB5+ derived CoCM+	101.0 ± 22.9	179.0 ± 70.7	134.0 ± 44.0	152.7 ± 78.2	152.0 ± 91.5	129.0 ± 93.2	144.0 ± 67.3
	+ i.p. ABCB5+	117.0 ± 27.6	135.6 ± 27.5	170.0 ± 54.6	167.1 ± 62.2	166.0 ± 88.5	126.0 ± 106.2	136.8 ± 87.8
	+ i.v. ABCB5+	119.3 ± 35.4	162.1 ± 33.2	162.7 ± 47.0	150.7 ± 47.9	150.7 ± 47.2	100.7 ± 84.0	119.5 ± 59.1

Appendix 7B Changes in plasma biochemistry in PCK groups (n=6). Comparison between ASC derived CM, i.v. or i.p. ASC groups and untreated. Data are shown as mean ± Std.Dev.

B	Parameter	Animal group	Baseline	Day25	Day 53	Day 81	Day 109	Day 137	Day 167
	Creatinine	Untreated	0.2 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.4 ± 0.0	0.4 ± 0.1	0.5 ± 0.1
	(mg/dl)	+ ASC derived CM	0.2 ± 0.1	0.3 ± 0.0	0.3 ± 0.1	0.4 ± 0.0	0.4 ± 0.1	0.5 ± 0.1	0.6 ± 0.1
		+ i.p. ASC	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.1	0.4 ± 0.0	0.4 ± 0.0	0.5 ± 0.1
		+ i.v. ASC	0.3 ± 0.1	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.4 ± 0.1	0.4 ± 0.1	0.5 ± 0.1
	Urea	Untreated	31.9 ± 2.8	35.0 ± 4.3	32.5 ± 2.9	36.3 ± 6.2	42.1 ± 5.1	44.6 ± 2.3	56.6 ± 3.2
	(mg/dl)	+ ASC derived CM	34.4 ± 3.2	37.4 ± 4.7	36.8 ± 6.5	42.1 ± 7.4	47.9 ± 8.9	51.2 ± 7.9	56.7 ± 7.0
		+ i.p. ASC	35.1 ± 0.7	35.4 ± 2.6	34.6 ± 4.5	37.3 ± 4.7	43.4 ± 9.7	46.7 ± 11.5	57.3 ± 10.6
		+ i.v. ASC	35.6 ± 2.6	35.2 ± 6.0	35.8 ± 4.2	38.7 ± 5.0	44.6 ± 6.3	47.1 ± 5.7	56.1 ± 3.5
	Na	Untreated	139.2 ± 1.2	140.7 ± 2.7	141.0 ± 3.0	139.7 ± 1.2	139.8 ± 1.1	140.6 ± 1.7	143.8 ± 1.8
	(mmol/l)	+ ASC derived CM	139.9 ± 2.7	140.9 ± 2.0	139.7 ± 3.6	139.1 ± 1.6	141.7 ± 3.7	141.7 ± 1.5	141.7 ± 1.4
		+ i.p. ASC	140.5 ± 2.4	141.2 ± 1.7	140.5 ±3.3	140.0 ± 1.7	143.8 ± 4.7	142.8 ± 0.8	141.8 ± 1.5
		+ i.v. ASC	140.2 ± 3.1	141.3 ± 2.5	140.7 ± 3.4	139.7 ± 1.5	143.7 ± 4.1	142.5 ± 1.1	142.3 ± 1.4
	K	Untreated	5.4 ± 0.3	5.0 ± 0.5	5.3 ± 0.3	5.2 ± 0.4	5.4 ± 0.4	5.2 ± 0.4	5.1 ± 0.2
	(mmol/l)	+ ASC derived CM	5.3 ± 0.4	4.9 ± 0.3	5.1 ± 0.3	5.3 ± 0.4	5.5 ± 0.4	5.7 ± 0.7	5.5 ± 0.5
		+ i.p. ASC	5.2 ± 0.3	4.8 ± 0.3	5.0 ± 0.3	5.5 ± 0.8	5.3 ± 0.4	5.1 ± 0.4	5.3 ± 0.2
		+ i.v. ASC	5.1 ± 0.4	4.8 ± 0.2	5.1 ± 0.5	5.1 ± 0.7	5.5 ± 0.3	5.2 ± 0.4	5.0 ± 0.5
	Ca	Untreated	2.7 ± 0.1	2.7 ± 0.1	2.8 ± 0.1	2.7 ± 0.0	2.7 ± 0.1	2.7 ± 0.1	2.8 ± 0.1
	(mmol/l)	+ ASC derived CM	2.7 ± 0.1	2.8 ± 0.1	2.8 ± 0.0	2.7 ± 0.1	2.7 ± 0.1	2.8 ± 0.1	2.8 ± 0.0
		+ i.p. ASC	2.8 ± 0.1	2.8 ± 0.1	2.7 ± 0.1	2.7 ± 0.1	2.8 ± 0.1	2.8 ± 0.1	2.8 ± 0.1
		+ i.v. ASC	2.8 ± 0.1	2.8 ± 0.1	2.7 ± 0.1	2.6 ± 0.2	2.7 ± 0.1	2.8 ± 0.1	2.8 ± 0.1

PO4	Untreated	2.3 ± 0.3	2.5 ± 0.3	2.0 ± 0.2	1.6 ± 0.5	2.0 ± 0.4	2.0 ± 0.4	2.2 ± 0.1
(mmol/l)	+ ASC derived CM	2.3 ± 0.3	2.2 ± 0.2	2.1 ± 0.3	1.7 ± 0.4	2.0 ± 0.2	2.1 ± 0.3	2.0 ± 0.4
	+ i.p. ASC	2.2 ± 0.2	2.4 ± 0.2	2.1 ± 0.2	1.9 ± 0.3	2.0 ± 0.2	2.1 ± 0.3	2.1 ± 0.4
	+ i.v. ASC	2.2 ± 0.2	2.1 ± 0.2	2.1 ± 0.2	2.0 ± 0.3	1.9 ± 0.1	2.0 ± 0.3	1.9 ± 0.3
Cholesterol	Untreated	140.3 ± 15.6	166.2 ± 16.8	211.5 ± 29.1	237.0 ± 23.7	289.5 ± 36.5	312.6 ± 39.0	301.2 ± 62.1
(mg/dl)	+ ASC derived CM	146.3 ± 17.4	169.6 ± 22.0	200.1 ± 20.2	206.7 ± 56.1	236.0 ± 58.2	238.3 ± 75.9	222.8 ± 64.3
	+ i.p. ASC	161.3 ± 33.3	199.8 ± 38.2	239.5 ± 47.6	264.5 ± 38.5	272.2 ± 48.6	264.7 ± 46.4	257.3 ± 52.1
	+ i.v. ASC	164.5 ± 32.2	186.2 ± 25.6	225.5 ± 32.6	233.0 ± 35.7	208.3 ± 55.4	244.5 ± 28.5	252.8 ± 46.7
Triglycerides	Untreated	86.8 ± 15.0	79.3 ± 15.1	93.2 ± 28.3	132.2 ± 36.7	126.7 ± 28.2	137.5 ± 29.5	160.5 ± 46.9
(mg/dl)	+ ASC derived CM	82.5 ± 12.6	77.0 ± 16.1	108.9 ± 16.5	118.6 ± 37.1	131.1 ± 31.2	160.4 ± 48.0	148.2 ± 30.2
	+ i.p. ASC	94.0 ± 11.9	92.5 ± 21.6	114.5 ± 25.7	137.8 ± 38.5	126.0 ± 18.5	124.3 ± 22.9	126.5 ± 16.0
	+ i.v. ASC	82.8 ± 12.1	96.0 ± 14.9	124.3 ± 22.0	109.2 ± 21.6	103.3 ± 29.8	118.5 ± 27.5	143.0 ± 30.7
Glucose	Untreated	166.8 ± 10.6	143.7 ± 11.4	149.3 ± 20.0	162.7 ± 32.8	172.8 ± 12.9	143.0 ± 16.4	150.3 ± 24.0
(mg/dl)	+ ASC derived CM	148.6 ± 13.4	142.7 ± 5.9	146.7 ± 16.2	141.6 ± 17.6	143.7 ± 27.0	131.4 ± 22.1	142.8 ± 23.0
	+ i.p. ASC	157.7 ± 9.9	164.2 ± 13.8	161.0 ± 26.8	161.0 ± 7.0	136.5 ± 8.5	146.3 ± 12.5	141.0 ± 12.0
	+ i.v. ASC	157.8 ± 13.3	144.0 ± 2.8	148.0 ± 14.5	149.5 ± 14.8	145.5 ± 23.1	145.0 ± 12.4	127.0 ± 18.4
Protein	Untreated	62.2 ± 1.8	66.0 ± 4.2	82.3 ± 31.3	63.2 ± 4.8	64.0 ± 4.0	66.2 ± 3.1	69.5 ± 2.1
(mg/dl)	+ ASC derived CM	65.3 ±3.4	69.3 ± 5.2	69.1 ± 7.1	67.9 ± 6.0	69.4 ± 4.6	72.4 ± 4.7	72.2 ± 4.0
	+ i.p. ASC	63.7 ± 1.9	64.0 ± 1.3	63.7 ± 4.2	63.0 ± 2.7	70.0 ± 4.0	69.8 ± 2.4	69.7 ± 4.1
	+ i.v. ASC	64.2 ± 2.3	65.2 ± 2.6	66.2 ± 4.9	64.5 ± 4.6	68.0 ± 4.2	69.7 ± 3.4	70.5 ± 2.7
ALT	Untreated	82.5 ± 20.4	69.3 ± 22.1	90.0 ± 21.3	90.3 ± 19.2	77.5 ± 22.6	66.5 ± 34.9	73.3 ± 40.0
(U/l)	+ ASC derived CM	85.0 ± 16.6	89.9 ± 17.3	93.7 ± 10.6	88.0 ± 25.7	99.4 ± 31.2	85.7 ± 27.6	81.3 ± 15.4
	+ i.p. ASC	91.5 ± 21.7	92.5 ± 12.1	72.3 ± 7.3	82.8 ± 25.4	87.7 ± 15.2	81.7 ± 19.3	80.9 ± 20.4
	+ i.v. ASC	87.2 ± 16.2	94.8 ± 13.1	98.2 ± 14.3	90.8 ± 19.0	93.2 ± 21.8	95.0 ± 20.6	83.6 ± 15.5
AST	Untreated	134.0 ± 42.1	124.4 ± 40.4	144.5 ± 36.3	134.3 ± 46.1	162.5 ± 84.6	113.8 ± 33.3	135.2 ± 144.9
(U/l)	+ ASC derived CM	154.2 ± 54.9	196.4 ± 76.6	245.1 ± 102.7	270.7 ± 120.5	328.4 ± 198.8	368.6 ± 210.1	325.2 ± 146.4
	+ i.p. ASC	147.7 ± 41.4	163.0 ± 44.3	126.0 ± 26.2	115.2 ± 60.6	178.7 ± 86.9	149.8 ± 58.1	155.7 ± 74.9
	+ i.v. ASC	154.7 ± 45.6	171.3 ± 48.8	202.2 ± 86.0	208.7 ± 88.0	255.2 ± 120.6	248.3 ± 111.3	256.0 ± 127.5

Appendix8A Changes in diuresis, food intake and water intake after metabolic cages in PCK groups (n=6). Comparison between ABCB5+ derived CoCM+, i.v. or i.p. ABCB5+ groups and untreated. Data are shown as mean ± Std.Dev.

A	Parameter	Animal group	Baseline	Day25	Day 53	Day 81	Day 109	Day 137	Day 167
	Diuresis	Untreated	16.1 ± 3.1	21.7 ± 4.4	17.6 ± 5.0	18.1 ± 2.7	44.6 ± 21.6	21.4 ± 5.5	25.2 ± 2.7
	(ml)	+ ABCB5+ derived CoCM+	19.5 ± 10.5	15.8 ± 3.6	16.7 ± 2.9	16.1 ± 5.8	**19.3 ± 3.0***	20.4 ± 5.8	24.7 ± 8.8
		+ i.p. ABCB5+	20.6 ± 11.0	19.2 ± 4.0	16.5 ± 4.3	21.6 ± 5.2	55.1 ± 86.2	19.5 ± 5.1	22.4 ± 3.7
		+ i.v. ABCB5+	20.0 ± 13.2	16.0 ± 4.6	16.5 ± 5.1	17.9 ± 3.7	55.1 ± 5.9	19.5 ± 4.5	18.7 ± 5.9
	Food intake	Untreated	21.8 ± 5.6	17.3 ± 5.0	17.0 ± 3.9	14.6 ± 2.6	14.1 ± 2.7	15.6 ± 0.7	10.8 ± 3.5

(g)	+ ABCB5+ derived CoCM+	19.2 ± 5.9	14.0 ± 1.8	13.6 ± 3.1	13.4 ± 4.3	15.0 ± 4.8	12.5 ± 5.1	15.6 ± 5.1
	+ i.p. ABCB5+	21.9 ± 3.4	17.4 ± 3.2	14.1 ± 2.9	14.3 ± 5.1	12.8 ± 5.6	11.3 ± 3.9	14.5 ± 2.9
	+ i.v. ABCB5+	19.9 ± 3.8	18.4 ± 3.8	14.9 ± 4.6	14.7 ± 4.3	12.6 ± 3.4	13.4 ± 5.0	15.0 ± 4.3
Water intake	Untreated	33.8 ± 2.9	37.7 ± 4.5	31.3 ± 3.6	26.2 ± 6.4	32.3 ± 6.4	24.0 ± 11.0	33.2 ± 4.4
(g)	+ ABCB5+ derived CoCM+	28.4 ± 13.1	23.4 ± 11.7	25.0 ± 3.5	24.3 ± 4.3	29.8 ± 8.6	26.7 ± 13.7	31.8 ± 9.9
	+ i.p. ABCB5+	32.8 ± 8.3	31.2 ± 4.9	24.0 ± 5.8	29.9 ± 6.9	28.6 ± 11.8	22.2 ±9.8	30.7 ± 15.9
	+ i.v. ABCB5+	30.2 ± 12.8	28.7 ± 4.1	24.9 ± 8.2	28.1 ± 4.7	24.4 ± 9.8	26.4 ± 10.0	24.2 ± 13.0

Appendix8B Changes in diuresis, food intake and water intake after metabolic cages in PCK groups (n=6). Comparison between ASC derived CM, i.v. or i.p. ASC groups and untreated. Data are shown as mean ± Std.Dev. Values significantly different from control are indicated as *p<0.05.

B	Parameter	Animal group	Baseline	Day25	Day 53	Day 81	Day 109	Day 137	Day 167
	Diuresis	Untreated	16.1 ± 3.1	21.7 ± 4.4	17.6 ± 5.0	18.1 ± 2.7	44.6 ± 21.6	21.4 ± 5.5	25.3 ± 2.7
	(ml)	+ ASC derived CM	13.9 ± 2.7	20.1 ± 7.9	19.3 ± 4.8	18.0 ± 3.5	21.2 ± 3.6	26.8 ± 2.3	29.3 ± 12.2
		+ i.p. ASC	21.2 ± 11.7	23.3 ± 10.2	20.7 ± 7.2	20.2 ± 5.5	22.9 ± 4.0	23.8 ± 8.2	25.4 ± 4.3
		+ i.v. ASC	16.2 ± 8.9	21.1 ± 8.0	22.5 ± 3.2	16.8 ± 5.1	**17.2 ± 6.5***	25.0 ± 4.3	26.8 ± 6.3
	Food intake	Untreated	21.8 ± 5.6	17.3 ± 5.0	17.0 ± 3.9	14.6 ± 2.6	14.1 ± 2.7	15.6 ± 0.7	10.8 ± 3.5
	(g)	+ ASC derived CM	22.8 ± 6.0	15.8 ± 3.5	16.7 ± 4.5	13.9 ± 3.2	16.5 ± 2.8	19.6 ± 3.0	17.4 ± 4.7
		+ i.p. ASC	17.3 ± 5.2	15.7 ± 1.9	16.7 ± 9.4	16.7 ± 2.1	17.5 ± 1.7	14.3 ± 3.9	14.9 ± 3.4
		+ i.v. ASC	17.3 ± 2.7	18.5 ± 3.8	19.2 ± 5.6	15.3 ± 3.5	18.9 ± 8.5	16.3 ± 3.5	14.2 ± 5.8
	Water intake	Untreated	33.8 ± 2.9	37.7 ± 4.5	31.3 ± 3.6	26.2 ± 6.4	32.3 ± 6.4	24.0 ± 11.0	33.2 ± 4.4
	(g)	+ ASC derived CM	30.3 ± 5.3	34.5 ± 7.9	32.0 ± 7.7	29.9 ± 3.8	29.9 ± 6.8	36.3 ± 8.8	38.5 ± 17.0
		+ i.p. ASC	36.2 ± 10.0	36.0 ± 10.8	31.6 ± 14.4	32.5 ± 5.1	34.4 ± 5.8	32.8 ± 11.1	38.3 ± 6.8
		+ i.v. ASC	30.3 ± 11.6	41.2 ± 9.7	38.7 ± 7.2	31.4 ± 6.1	30.9 ± 9.6	36.3 ± 1.0	46.4 ± 19.7

Appendix 9A Changes in urine biochemistry in PCK groups (n=6). Comparison between ABCB5+ derived CoCM+, i.v. or i.p. ABCB5+ groups and untreated. Data are shown as mean ± Std.Dev.

A)	Parameter	Animal group	Baseline	Day25	Day 53	Day 81	Day 109	Day 137	Day 167
	Creatinine	Untreated	8.1 ± 1.1	11.2 ± 2.0	11.1 ± 2.9	13.3 ± 2.5	13.7 ± 1.3	13.9 ± 2.5	14.8 ± 3.2
	(mg/16h)	+ ABCB5+ derived CoCM+	8.9 ± 1.2	11.8 ± 0.7	12.8 ± 0.9	11.4 ± 2.8	12.3 ± 1.2	12.2 ± 1.4	11.9 ±1.8
		+ i.p. ABCB5+	9.1 ± 1.5	12.3 ± 0.7	11.6 ± 1.6	12.1 ± 0.9	12.6 ± 1.8	12.3 ± 1.1	12.0 ± 2.4
		+ i.v. ABCB5+	9.1 ± 1.1	11.7 ± 0.6	12.1 ± 1.0	13.1 ± 1.1	12.2 ± 1.3	12.5 ± 1.3	12.5 ± 1.7
	Urea	Untreated	709.4 ± 120.7	1033.9 ± 689.6	729.7 ± 256.7	701.3 ± 85.4	679.7 ± 65.4	662.2 ± 151.2	681.6 ± 91.3
	(mg/16h)	+ ABCB5+ derived CoCM+	701.7 ± 152.7	717.1 ± 67.1	724.4 ± 101.1	573.4 ± 151.8	627.2 ± 79.0	557.1 ± 107.6	586.1 ± 147.9
		+ i.p. ABCB5+	749.4 ± 136.8	824.5 ± 204.2	677.2 ± 145.6	662.9 ± 89.6	648.0 ± 91.0	591.3 ± 119.6	610.0 ± 148.5

	+ i.v. ABCB5+	709.8 ± 104.6	809.0 ± 128.8	708.5 ± 44.3	735.6 ± 94.8	619.2 ± 135.7	617.6 ± 115.6	571.4 ± 105.2
Na	Untreated	1.6 ± 0.6	1.4 ± 0.4	1.0 ± 0.5	1.3 ± 0.4	1.3 ± 0.3	1.3 ± 0.3	1.3 ± 0.2
(mmol/16h)	+ ABCB5+ derived CoCM+	1.7 ± 0.4	1.2 ± 0.3	1.1 ± 0.2	1.1 ± 0.3	1.3 ± 0.3	1.4 ± 0.2	1.6 ± 0.8
	+ i.p. ABCB5+	1.9 ± 0.6	1.6 ± 0.5	1.3 ± 0.5	1.6 ± 0.5	1.7 ± 0.9	1.5 ± 0.4	1.5 ± 0.6
	+ i.v. ABCB5+	1.5 ± 0.4	1.5 ± 0.6	1.3 ± 0.4	1.2 ± 0.3	1.0 ± 0.3	1.6 ± 0.6	1.4 ± 0.9
K	Untreated	3.9 ± 1.0	4.5 ± 0.9	2.9 ± 1.4	3.5 ± 1.0	4.3 ± 0.4	3.6 ± 1.3	3.5 ± 0.4
(mmol/16h)	+ ABCB5+ derived CoCM+	4.2 ± 1.1	3.9 ± 0.5	4.0 ± 0.3	3.2 ± 0.7	4.0 ± 0.6	3.7 ± 0.6	4.3 ± 1.5
	+ i.p. ABCB5+	5.0 ± 1.3	4.8 ± 1.1	4.0 ± 0.8	4.0 ± 0.5	4.4 ± 0.8	3.7 ± 0.7	3.7 ± 0.9
	+ i.v. ABCB5+	4.2 ± 0.6	4.5 ± 0.9	4.1 ± 0.5	3.9 ± 0.6	3.7 ± 0.5	3.8 ± 0.7	3.6 ± 1.0
Ca	Untreated	0.06 ± 0.04	0.03 ± 0.01	0.05 ± 0.02	0.07 ± 0.02	0.10 ± 0.02	0.10 ± 0.02	0.08 ± 0.03
(mmol/16h)	+ ABCB5+ derived CoCM+	0.03 ± 0.02	0.03 ± 0.00	0.04 ± 0.01	0.05 ± 0.02	0.08 ± 0.02	0.09 ± 0.04	0.10 ± 0.03
	+ i.p. ABCB5+	0.05 ± 0.02	0.05 ± 0.04	0.05 ± 0.07	0.07 ± 0.03	0.08 ± 0.02	0.07 ± 0.03	0.08 ± 0.03
	+ i.v. ABCB5+	0.03 ± 0.01	0.04 ± 0.02	0.06 ± 0.02	0.06 ± 0.02	0.06 ± 0.03	0.06 ± 0.03	0.06 ± 0.03
PO4	Untreated	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.2	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1
(mmol/16h)	+ ABCB5+ derived CoCM+	0.2 ± 0.2	0.1 ± 0.2	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.2 ± 0.2	0.1 ± 0.1
	+ i.p. ABCB5+	0.1 ± 0.1	0.2 ± 0.2	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.2 ± 0.1	0.1 ± 0.1
	+ i.v. ABCB5+	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.2 ± 0.2	0.2 ± 0.1	0.2 ± 0.9	0.3 ± 0.3
Glucose	Untreated	5.2 ± 1.9	3.5 ± 2.8	2.9 ± 1.2	3.0 ± 1.4	3.5 ± 2.3	3.5 ± 1.2	1.9 ± 1.6
(mg/16h)	+ ABCB5+ derived CoCM+	3.0 ± 0.6	2.9 ± 0.5	2.4 ± 1.0	1.8 ± 0.8	2.4 ± 0.5	2.0 ± 1.1	2.3 ± 0.6
	+ i.p. ABCB5+	3.8 ± 0.3	2.9 ± 0.8	2.6 ± 0.7	3.3 ± 0.8	3.1 ± 2.5	2.5 ± 0.7	3.0 ± 0.7
	+ i.v. ABCB5+	3.0 ± 0.8	3.1 ± 0.5	2.8 ± 1.2	2.6 ± 0.6	2.4 ± 0.4	1.9 ± 0.8	2.4 ± 0.9
Protein	Untreated	9.6 ± 4.9	31.5 ± 12.5	94.6 ± 44.5	212.4 ± 72.4	353.0 ± 64.1	375.1 ± 85.6	436.8 ± 101.5
(mg/16h)	+ ABCB5+ derived CoCM+	13.3 ± 10.0	63.3 ± 65.1	117.0 ± 88.3	219.1 ± 59.5	318.2 ± 89.9	328.0 ± 149.7	290.1 ± 114.7
	+ i.p. ABCB5+	14.1 ± 11.2	66.3 ± 77.4	116.8 ± 66.5	244.0± 107.2	313.2 ± 140.7	256.7 ± 116.8	311.9 ± 161.3
	+ i.v. ABCB5+	19.1 ± 24.1	74.5 ± 83.7	131.2 ± 87.5	200.6 ± 51.1	251.8 ± 40.2	281.8 ± 160.9	288.1 ± 117.3
Albumin	Untreated	0.7 ± 0.3	13.5 ± 11.1	69.7 ± 34.8	103.3 ± 61.1	178.8 ± 49.8	173.9 ± 71.9	140.6 ± 71.2
(mg/16h)	+ ABCB5+ derived CoCM+	5.8 ± 9.5	36.4 ± 48.5	78.6 ± 43.6	124.5 ± 70.9	166.4 ± 63.1	201.6 ± 67.4	200.6 ± 68.5
	+ i.p. ABCB5+	6.8 ± 13.0	36.4 ± 54.4	64.5 ± 27.3	123.7 ± 27.1	181.7 ± 85.9	227.1 ± 141.3	319.8 ± 220.3
	+ i.v. ABCB5+	6.1 ± 10.9	39.5 ± 56.0	59.5 ± 25.7	136.3 ± 40.8	194.9 ± 56.7	298.3 ± 107.2	200.0 ± 52.9

Appendix 9B Changes in urine biochemistry in PCK groups (n=6). Comparison between ASC derived CM, i.v. or i.p. ASC groups and untreated. Data are shown as mean ± Std.Dev. Values significantly different from control are indicated as *p<0.05.

B) Parameter	Animal group	Baseline	Day25	Day 53	Day 81	Day 109	Day 137	Day 167
Creatinine	Untreated	8.1 ± 1.1	11.2 ± 2.0	11.1 ± 2.9	13.3 ± 2.5	13.7 ± 1.3	13.9 ± 2.5	14.8 ± 3.2
(mg/16h)	+ ASC derived CM	7.8 ± 1.8	11.6 ± 4.0	12.4 ± 2.1	11.6 ± 1.7	12.0 ± 1.8	12.5 ± 3.6	12.3 ± 1.0
	+ i.p. ASC	8.9 ± 2.2	11.7 ± 1.3	13.9 ± 2.3	12.3 ± 1.2	12.3 ± 1.6	11.6 ± 2.3	12.4 ± 1.2
	+ i.v. ASC	8.5 ± 1.8	11.0 ± 1.1	12.9 ± 2.9	10.6 ± 3.2	10.0 ± 1.2	11.4 ± 2.1	12.4 ± 1.7
Urea	Untreated	709.4 ± 120.7	1033.9 ± 689.6	729.7 ± 256.7	701.3 ± 85.4	679.7 ± 65.4	662.2 ± 151.2	681.6 ± 91.3

(mg/16h)	+ ASC derived CM	613.1 ± 77.2	609.1 ± 326.3	765.3 ± 219.6	666.6 ± 84.1	617.9 ± 72.3	665.5 ± 125.6	628.7 ± 81.8
	+ i.p. ASC	606.5 ± 48.0	675.1 ± 66.1	823.2 ± 269.7	750.3 ± 118.1	681.4 ± 99.7	575.5 ± 121.2	658.9 ± 111.4
	+ i.v. ASC	610.6 ± 89.6	709.4 ± 116.5	830.4 ± 227.2	620.8 ± 198.5	538.5 ± 118.6	640.9 ± 54.3	659.8 ± 99.6
Na	Untreated	1.6 ± 0.6	1.4 ± 0.4	1.0 ± 0.5	1.3 ± 0.4	1.3 ± 0.3	1.3 ± 0.3	1.3 ± 0.2
(mmol/16h)	+ ASC derived CM	1.3 ± 0.3	1.5 ± 0.4	1.3 ± 0.4	1.4 ± 0.3	1.8 ± 0.5	1.9 ± 0.5	1.8 ± 0.6
	+ i.p. ASC	1.4 ± 0.5	1.5 ± 0.4	1.5 ± 0.5	1.5 ± 0.5	1.7 ± 0.5	1.6 ± 0.7	1.3 ± 0.3
	+ i.v. ASC	1.4 ± 0.4	1.6 ± 0.6	1.4 ± 0.6	1.2 ± 0.6	1.3 ± 0.7	1.6 ± 0.3	1.7 ± 0.8
K	Untreated	3.9 ± 1.0	4.5 ± 0.9	2.9 ± 1.4	3.5 ± 1.0	4.3 ± 0.4	3.6 ± 1.3	3.5 ± 0.4
(mmol/16h)	+ ASC derived CM	3.1 ± 1.3	3.7 ± 0.9	3.7 ± 1.9	3.8 ± 0.8	4.3 ± 0.7	3.6 ± 0.9	4.2 ± 1.4
	+ i.p. ASC	3.4 ± 0.3	3.7 ± 0.5	4.2 ± 1.9	4.6 ± 0.7	3.9 ± 0.3	3.4 ± 1.0	4.0 ± 0.5
	+ i.v. ASC	3.4 ± 0.3	4.0 ± 0.7	4.1 ± 0.9	3.4 ± 1.2	2.9 ± 0.7	4.0 ± 0.6	3.9 ± 0.4
Ca	Untreated	0.06 ± 0.04	0.03 ± 0.01	0.05 ± 0.02	0.07 ± 0.02	0.10 ± 0.02	0.10 ± 0.02	0.08 ± 0.03
(mmol/16h)	+ ASC derived CM	0.04 ± 0.03	0.04 ± 0.02	0.06 ± 0.02	0.08 ± 0.04	0.07 ± 0.02	0.07 ± 0.03	0.07 ± 0.03
	+ i.p. ASC	0.04 ± 0.02	0.03 ± 0.01	0.07 ± 0.05	0.10 ± 0.04	0.10 ± 0.03	0.08 ± 0.03	0.08 ± 0.02
	+ i.v. ASC	0.03 ± 0.01	0.04 ± 0.02	0.08 ± 0.03	0.08 ± 0.03	0.07 ± 0.03	0.07 ± 0.0.3	0.07 ± 0.01
PO4	Untreated	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.2	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1
(mmol/16h)	+ ASC derived CM	0.1 ± 0.1	0.1 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.2	0.2 ± 0.2	0.1 ± 0.1
	+ i.p. ASC	0.1 ± 0.1	0.1 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	0.1 ± 0.1
	+ i.v. ASC	0.1 ± 0.1	0.0 ± 0.0	0.1 ± 0.1	0.1 ± 0.0	0.2 ± 0.2	0.1 ± 0.1	0.1 ± 0.1
Glucose	Untreated	5.2 ± 1.9	3.5 ± 2.8	2.9 ± 1.2	3.0 ± 1.4	3.5 ± 2.3	3.5 ± 1.2	1.9 ± 1.5
(mg/16h)	+ ASC derived CM	4.0 ± 1.8	1.5 ±1.1	3.6 ± 1.7	4.0 ± 2.0	4.1 ± 2.8	4.8 ± 1.4	5.9 ± 4.5
	+ i.p. ASC	2.6 ± 1.5	1.5 ± 1.5	4.6 ± 3.0	5.0 ± 2.1	4.4 ± 1.1	3.3 ± 1.4	4.4 ± 1.6
	+ i.v. ASC	2.6 ± 2.2	1.0 ± 1.1	3.6 ± 1.2	3.5 ± 1.6	4.0 ± 1.3	3.3 ± 2.7	3.7 ± 2.2
Protein	Untreated	9.6 ± 4.9	31.5 ± 12.5	94.6 ± 44.5	212.4 ± 72.4	353.0 ± 64.1	375.1 ± 85.6	436.8 ± 101.5
(mg/16h)	+ ASC derived CM	18.3 ± 18.6	70.4 ± 40.9	162.2 ± 84.1	186.0 ± 75.9	344.3 ± 65.7	361.9 ± 99.8	398.2 ± 143.4
	+ i.p. ASC	33.8 ± 35.0	107.5 ± 71.5	256.7 ± 122.0	201.6 ± 121.7	341.2 ± 72.3	295.1 ± 102.7	350.4 ± 79.0
	+ i.v. ASC	29.1 ± 23.5	104.3 ± 70.7	219.8 ± 129.4	131.3 ± 80.6	211.7 ± 59.6	284.6 ± 45.3	320.6 ± 63.2
Albumin	Untreated	0.7 ± 0.3	13.5 ± 11.1	69.7 ± 34.8	103.3 ± 61.1	178.8 ± 49.8	173.9 ± 71.9	140.6 ± 71.2
(mg/16h)	+ ASC derived CM	5.8 ± 8.7	28.1 ± 16.1	82.2 ± 30.7	99.0 ± 34.4	110.4 ± 18.0	137.8 ± 35.0	152.7 ± 66.9
	+ i.p. ASC	17.3 ± 18.4	54.4 ± 38.5	101.1 ± 43.3	209.0 ± 75.7	155.2 ± 38.8	163.1 ± 51.8	197.7 ± 59.3
	+ i.v. ASC	12.0 ± 11.7	50.9 ± 37.6	83.5 ± 24.8	115.1 ± 36.1	**86.0 ± 31.9***	158.1 ± 54.3	154.8 ± 28.7

Appendix 10A Changes of ABZWCY-HβCD half-life in PCK groups (n=6). Comparison between ABCB5+ derived CoCM+, i.v. or i.p. ABCB5+ groups and untreated. Data are shown as mean ± Std.Dev. Values significantly different from control are indicated as *p<0.05.

A) Parameter	Animal group	Baseline	Day25	Day 53	Day 81	Day 109	Day 137	Day 167
ABZWCY-HβCD	Untreated	29.7 ± 10.8	34.5 ± 11.7	40.5 ± 16.4	44.0 ± 15.9	53.2 ± 14.4	62.5 ± 10.9	75.5 ± 11.6
$t_{1/2}$ (min)	+ ABCB5+ derived CoCM+	24.3 ± 4.6	31.9 ± 4.0	32.3 ± 5.0	31.3 ± 12.5	50.7 ± 21.6	47.4 ± 14.8	66.5 ± 15.1

+ i.p. ABCB5+	23.8 ± 5.2	25.1 ± 14.3	42.7 ± 15.4	33.2 ± 13.4	35.5 ± 7.8	44.6 ± 25.6	**45.5 ± 22.7***
+ i.v. ABCB5+	20.3 ± 1.4	26.3 ± 4.4	33.0 ± 12.8	32.6 ± 7.5	40.4 ± 20.5	38.6 ± 16.6	50.9 ± 10.1

Appendix 10B Changes of ABZWCY-HβCD half-life in PCK groups (n=6). Comparison between ASC derived CM, i.v. or i.p. ASC groups and untreated. Data are shown as mean ± Std.Dev. Values significantly different from control are indicated as *p<0.05.

B) Parameter	Animal group	Baseline	Day25	Day 53	Day 81	Day 109	Day 137	Day 167
ABZWCY-HβCD	Untreated	29.9 ± 10.8	34.5 ± 11.7	40.5 ± 16.4	44.0 ± 15.9	53.2 ± 14.4	62.5 ± 10.9	75.5 ± 11.6
$t_{1/2}$ (min)	+ ASC derived CM	31.3 ± 11.3	30.9 ± 3.9	42.9 ± 14.6	32.3 ± 11.2	53.0 ± 16.2	72.2 ± 31.5	72.5 ± 24.8
	+ i.p. ASC	26.4 ± 8.1	29.2 ± 6.2	35.4 ± 5.1	36.6 ± 8.1	31.6 ± 10.5	56.4 ± 29.6	52.7 ± 4.9
	+ i.v. ASC	34.4 ± 12.0	35.6 ± 10.8	35.8 ± 10.4	32.0 ± 9.6	54.7 ± 13.8	52.5 ± 4.5	54.5 ± 9.3

9. Curriculum vitae

Personal Information

Name:	Daniela Nardozi
Date of Birth:	22.07.1989
Place of Birth:	Rome, Italy
Nationality:	Italian
Civil Status:	Unmarried
Father's Name:	Giampaolo Nardozi
Mother's Name:	Nadia Miotto

Academic qualification

September 2016 – Today Mannheim, Germany	Ph.D student, Medical Research Center, University of Heidelberg, Germany. Thesis title:
October 2013 – October 2015 Rome, Italy	Master of Medical Biotechnology, University of Rome Tor Vergata, Rome, Italy. Thesis title: Inflammation and bone metabolism: an archaic bond mediated by PTX3
October 2008 – July 2013 Rome, Italy	Bachelor of Biotechnology, Università Cattolica del Sacro Cuore , Rome, Italy Thesis title: Methylation of MGMT in cancer diagnostics: study methods and therapeutic significance

Publications

Torres Crigna A, Daniele C, Gamez C, Medina Balbuena S, Pastene DO, **Nardozi D**, Brenna C, Yard B, Gretz N, Bieback K.
Stem/Stromal Cells for Treatment of Kidney Injuries With Focus on Preclinical Models.
Frontiers Medicine 2018; 5:179

Daniele C, **Nardozi D**, Torelli A, Khan AUM, Gretz N
Transcutaneous Measurement of Glomerular Filtration Rate in Rodents.
Methods in Molecular Biology 2020; 129-137

Patent

Methods and products for treating renal disease / Inventors: TICEBA, N. Gretz, C. Daniele, **D. Nardozi** / US-Provisional number 62/838062

Abbreviations

ABCB5	ATP-binding cassette member B5
ABCB5+	Cells expressing ABCB5
AB-HS	Human serum (blood-type AB)
ADPKD	Autosomal Dominant Polycystic Kidney Disease
ALT	Alanin-aminotransferase
ARPKD	Autosomal Recessive Polycystic Kidney Disease
ASC	Adipose Derived Stromal Cells
AST	Aspartat-aminotransferase
BUN	Blood Urea Nitrogen
BW	Body Weight
CKD	Chronic kidney disease
CM	ASC derived conditioned medium
CoCM+	Co-culture of ABCB5+ cell and THP-1 derived macrophages
DAPI	4',6-diamidino-2-phenylindole dihydrochloride
DIRCs	Dermal Immunoregulatory Cells
ESRD	End-stage renal disease
GFR	Glomerular Filtration Rate
GSEA	Gene Set Enrichment Analysis
H&E	Hematoxylin and eosin
IF	Immunofluorescence
INFγ	Interferon γ
JAK	Janus kinase
KEGG	Kyoto Encyclopedia of Genes and Genome database
Kw/BW	Kidney to body weight
LEDs	Light-Emitting Diodes
LogFC	Log Fold Changes
LPS	Lipopolysaccharide
MCP-1	Monocyte chemoattractant protein-1
MSC	Mesenchymal Stromal Cells
NF-κB	Nuclear factor-κB

NIR	Near Infrared
NPHP	Nephronophthisis
PKD	Polycystic Kidney Disease
PKD 1	*polycystin 1*
PKD 2	*polycystin 2*
PKHD 1	*polycystic kidney and hepatic disease 1*
RIN	RNA integrity number
RT	Room temperature
SD	Sprague Dawley
SOCS	Suppressors of cytokine signaling
Std Dev	Standard deviation
TNF-α	Tumor necrosis factor-α

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