

Aus dem Institut für Experimentelle Endokrinologie  
der Medizinischen Fakultät Charité – Universitätsmedizin Berlin

DISSERTATION

Prognostic value of PALB2 expression in pancreatic ductal  
adenocarcinoma

Prognostischer Wert der Expression von PALB2 für Patienten  
mit Pankreaskarzinom

zur Erlangung des akademischen Grades  
Doctor medicinae (Dr. med.)

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## Abbreviations

BRCA1	BReast CAncer 1
BRCA2	BReast CAncer 2
PALB2	PArtner and Localizer of BRCA2
PDAC	pancreatic ductal adenocarcinoma
TMA	Tissue microarrays
IHC	immunohistochemistry
OS	Overall survival
MST	median survival time
DSB	DNA double strand breaks
RPA	replication protein A
RNF	RING finger
CC	Coiled coil
CDK	cyclin-dependent kinases
ROS	reactive oxygen species
FANCN	Fanconi anemia complementation
TCGA	The Cancer Genome Atlas
GSEA	gene set enrichment analysis
CI	confidence intervals
HR	hazard ratio
T	primary tumor site
N	regional lymph node involvement
NES	normalized enrichment score
EMT	epithelial to mesenchymal transition
LV	leucovorin
5-FU	5-fluorouracil
HR	homologous recombination
qRT-PCR	Quantitative real-time PCR

## Abstract

**Introduction:** BRCA1 and BRCA2 interact during DNA repair with the tumor suppressor Partner and Localizer of BRCA2 (PALB2). PALB2 gene mutations are associated with breast and ovary cancer risk, and high PALB2 expression levels associate with poor prognosis in breast cancer. We hypothesized a potential prognostic value of PALB2 expression in pancreatic ductal adenocarcinoma (PDAC).

**Materials and methods:** PALB2 expression was down regulated by siRNA in PDAC cell lines, and effects on growth and migration were studied by cell viability and scratch wound healing assays, respectively. Tissue microarrays (TMAs) from 157 patients who underwent pancreatectomy for PDAC were analyzed by immunohistochemistry (IHC). PALB2 expression in tumors and surrounding tissue was quantified and compared with tumor characteristics and patient outcome using Kaplan-Meier analyses.

**Results:** Reduced PALB2 expression in PDAC cells did not affect proliferation, but was associated with less cell migration in vitro. Increased PALB2 expression was observed in PDAC tissue in comparison to the peritumoral tissues ( $p < 0.01$ ). Overall survival (OS) was inversely related to PALB2 expression ( $p < 0.05$ ). The multivariate analysis indicated that regional lymph node involvement and PALB2 expression levels were independent prognostic factors for OS.

**Conclusion:** Migration of PDAC cells may depend on PALB2 expression, and elevated PALB2 in PDAC tissue indicates poor survival and may constitute a novel prognostic marker that may help in the development and choice of therapeutic strategies in this devastating disease.

## Zusammenfassung

**Einleitung:** BRCA1 (BRCA1) und BRCA2 sind Proteine, die im Rahmen der Brustkrebsforschung identifiziert wurden und bei der Reparatur von DNA-Schäden mit dem Tumorsuppressor Partner and Localizer of BRCA2 (PALB2) interagieren. Genetische Mutationen im Locus von PALB2 sind mit dem Risiko für Brust- und Eierstockkrebs assoziiert; hohe Proteinkonzentrationen von PALB2 sind hierbei ein Zeichen für eine schlechte Prognose. In dieser Arbeit wird die Hypothese verfolgt, dass die PALB2 Expression auch von prognostischer Bedeutung für Adenokarzinome der Bauchspeicheldrüse (PDAC) ist.

**Materialien and Methoden:** In Zellkultur wurde die PALB2 Expression über siRNA in PDAC Zellen reduziert und die Effekte verringerter PALB2 Expression auf Zellproliferation und Zellmigration in Viabilitäts- und Migrationsversuchen analysiert. Kreisförmige, kleine Gewebestanden (Gewebe-Microarrays) von 157 Patienten, die sich einer Operation zur Pankreatektomie unterzogen haben, wurden mittels Immunohistochemie zur Bestimmung der PALB2 Expression analysiert. Die Expression wurde in den Tumorbereichen mit denen des umgebenden Normalgewebes verglichen und zu den Tumorcharakteristika und Überlebensdaten der Patienten durch Kaplan-Meier Analysen in Beziehung gebracht.

**Ergebnisse:** In Zellkultur zeigte sich kein Effekt der PALB2 Expression auf Zellproliferation, wohingegen die Migration durch reduzierte PALB2 Expression negativ beeinflusst wurde. Die PALB2 Expressionsspiegel waren im Tumorgewebe der PDAC Patienten deutlich höher als im benachbarten Normalgewebe ( $p < 0.01$ ). Das mittlere Überleben war invers zu den PALB2 Expressionsspiegeln korreliert, d.h., je höher die PALB2 Expression desto kürzer die verbliebene Überlebenszeit ( $p < 0.05$ ). In der multivariaten Analyse erwiesen sich der Lymphknotenbefall und die PALB2 Expression als unabhängige prognostische Parameter der Lebenserwartung.

**Schlussfolgerung:** Die Migration von PDAC Zellen könnte direkt von PALB2 abhängen. Erhöhte PALB2 Expression im Tumorgewebe von Patienten mit Bauchspeicheldrüsenkrebs stellt einen neuen negativen prognostischen Biomarker der Lebenserwartung da. Die Bestimmung der PALB2 Expression im Tumor könnte die Diagnostik und Prognose bei dieser schrecklichen Erkrankung verbessern und unter Umständen hilfreiche Informationen bei der Entwicklung und Auswahl therapeutischer Maßnahmen liefern.

# 1. Introduction

## 1.1 About pancreatic cancer

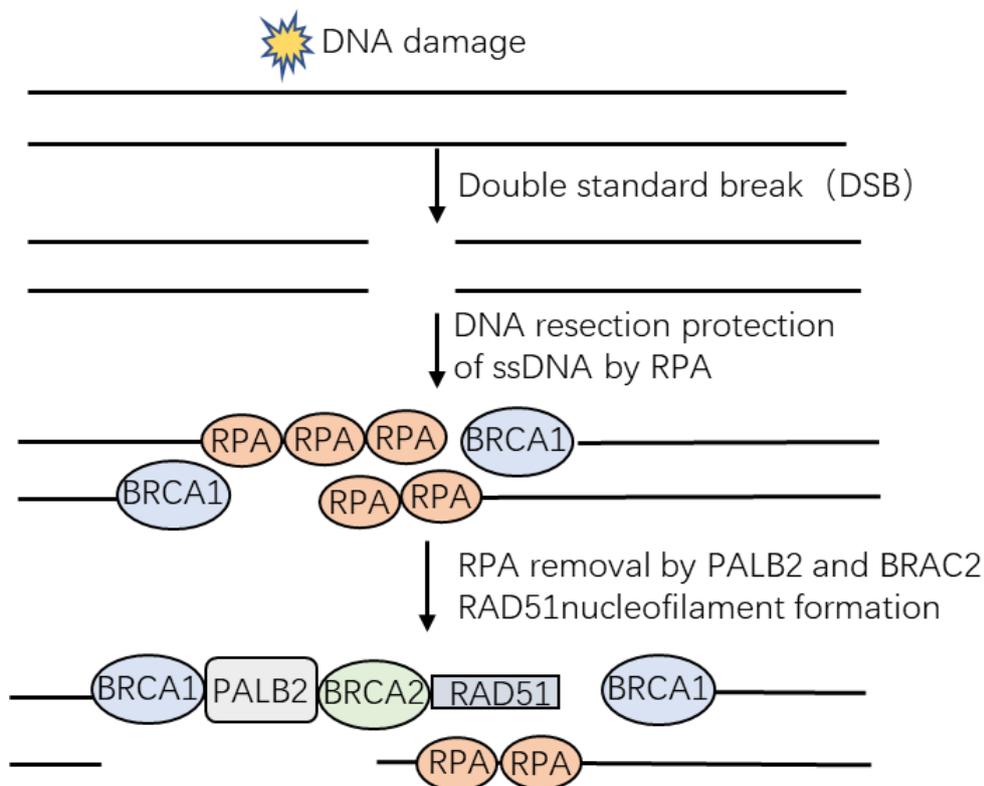
Pancreatic cancer is the fourth leading cause of cancer death worldwide, with an overall 5-year survival rate of <8%. These statistics have not changed in almost 50 years. Its incidence has risen gradually in recent years [1, 2]. About 95% of pancreatic cancer were ductal adenocarcinoma, and the remaining 5% include acinar cells carcinoma, pancreatic blastoma and cystic tumors [3]. Despite the better understanding of PDAC development and improvements in surgical techniques, the survival data have not changed much over the last 80 years. Up to now, surgical resection is still the only potentially curative way to treat for PDAC, but due to lack of early symptoms and a high tendency for metastasis development, about 80% of patients have lost the opportunity of surgical resection at the time of diagnosis [4]. The median survival time (MST) with non-resectable PDAC patients is only 3.5 months. Even after successful surgical resection, the 5-year survival rate is still below 20% [5]. The chemotherapeutic options are also very limited, with FOLFIRINOX (leucovorin (LV), 5-fluorouracil (5-FU), irinotecan, oxaliplatin), gemcitabine plus abraxane (albumin-bound paclitaxel) or gemcitabine plus Xeloda (Capecitabine), which have emerged as the standard of care for pancreatic adenocarcinoma patients [6, 7]. However, median recurrence free survival and median overall survival are still unsatisfactorily poor.

## 1.2 Current diagnostic markers of PDAC

Thus, additional diagnostic markers are needed for both an early detection, and for a better assessment of disease activity and prognosis, in order to guide therapeutic decisions and enable the monitoring and testing of novel treatment options. In recent years, the genomic analyses of PDAC tissues and expression patterns have been extensively conducted. Several new circulating molecules and panels of biomarkers like CA19-9, SYCN, and REG1B have been identified and developed to improve the early identification of first PDAC lesions [8]. Analysis of the genomic landscape of PDAC also revealed several mutations in tumor suppressor genes, such as KRAS, CDKN2A, TP53, and SMAD4 [9, 10], which are inactivated in over half the cases of PDAC. In addition, genes involved in the breast cancer susceptibility gene (BRCA) pathway, namely, BRCA1, BRCA2, and PALB2, have also been identified as being inactivated in PDAC. The relationship of PALB2 mutations with PDAC risk and incidence appears to have population-specific characteristics [11-15].

### 1.3 Functions of PALB2

A number of circulating molecules and panels of biomarkers have been identified and developed to improve the identification of early PDAC lesions [16]. BRCA1 and BRCA2 are two recognized tumor suppressor genes, which encode proteins that take part in transcription and DNA repair [17]. They are widely expressed in breast and other tissue. Partner and localizer of BRCA2 (PALB2) was first identified as colocalizing with BRCA2 in the nucleus [18]. The PALB2 gene is located on chromosome 16p12.2 and comprises 13 exons [19], which encode a protein that acts as a linker between BRCA1 and BRCA2 in the DNA repair processes (Figure 1) [20, 21]. Moreover, PALB2 has also been found to support BRCA2 localization and stability in nuclear structures and promoting homologous recombination (HR). In PALB2-depleted cells, abrogation of BRCA2 causes focus formation and a significant decrease in HR efficiency. In vitro studies also revealed that purified PALB2 is capable of stimulating D-loop and strand invasion using short oligonucleotides or resected DNA double-strand breaks (DSB). In addition, PALB2 uses two DNA-binding domains to interact directly with genomic DNA, and constitutes a key regulator of RAD51-mediated HR. This activity depends on the BRCA1-PALB2-BRCA2 complex formed, that enables PALB2 to interact with RAD51 and stimulate RAD51-mediated D-loop formation.



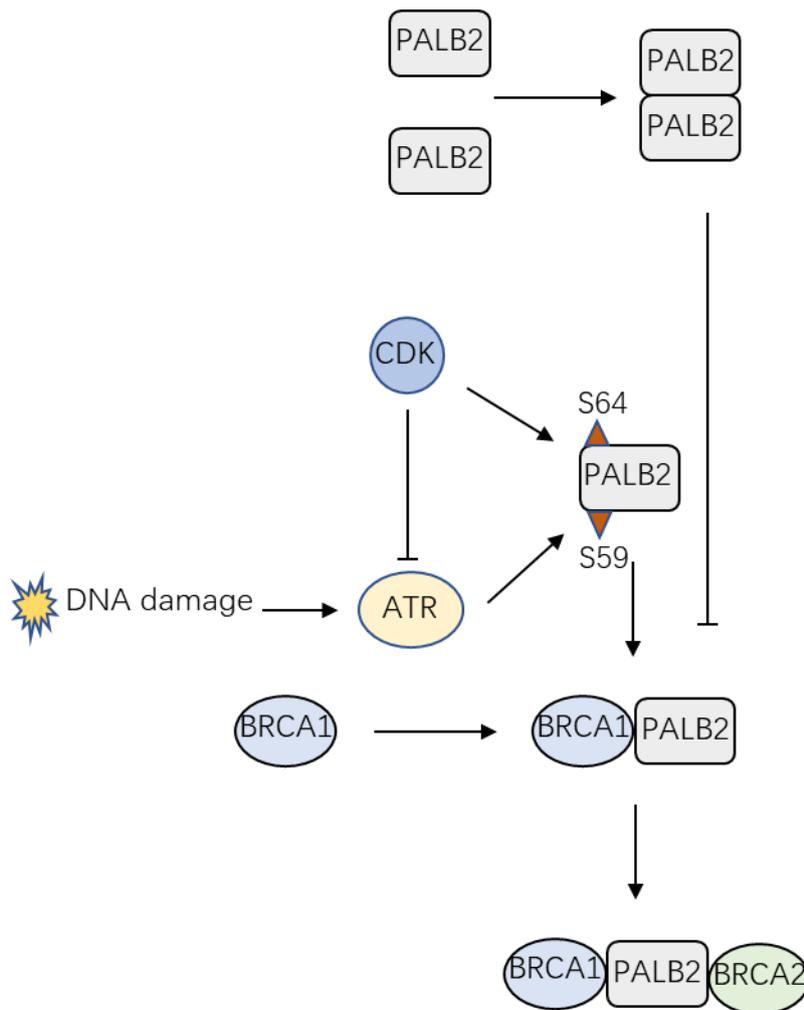
**Figure1.** PALB2 is a mediator in the homologous repair of damaged DNA. In the course of the repair of DNA double strand breaks (DSB), when ssDNA is generated, it becomes protected from

degradation by the single-strand binding protein replication protein A (RPA). Then, BRCA1 recruits PALB2 to the DSB and promotes the recruitment of BRCA2 along with RAD51, that stabilizes strand pairing during the repair process. Acting as homologous recombination mediators, PALB2 and BRCA2 remove RPA and facilitate the assembly of a RAD51 nucleoprotein filament. Picture modified from a template found in literature [20, 21].

#### **1.4 Mechanisms of Regulation of PALB2**

Regulation of PALB2 function involves different kinds of pathways and interactions, including homodimerization, phosphorylation, and ubiquitinylation. It has been demonstrated that PALB2 recruitment to DSBs in S/G2 cells is orchestrated by direct interaction with the RING finger (RNF) E3 ubiquitin ligase RNF168 [22]. A lot of evidence indicates that PALB2 indirectly recognizes ubiquitin marks on histone H2A by physical interaction with RNF168, prompting the assembly of the HR machinery and driving DSB repair in the S/G2 phase. PALB2 seems to accumulate at DSB sites in an RNF168-dependent and BRCA1-independent manner. In other experiments, it has been shown that PALB2 can switch from a low activity oligomer to a BRCA1-PALB2-BRCA2 active complex after DNA damage recognition (Figure 2) [23]. This molecular switch can be controlled by protein phosphorylation, e.g., some kinases like the ATM/ATR can phosphorylate PALB2 in response to genotoxic stress, enabling successive DSB repair [24-27]. Mass spectrometry analysis and immunoprecipitation experiments revealed that MORF related gene on chromosome 15 (MRG15) may constitute the major binding partners of PALB2. MRG15 belongs to the highly conserved family of MRG domain-containing proteins. Several studies indicated a PALB2-dependent role of MRG15 in the repair processes of DSB [28, 29].

Recent studies highlighted that PALB2 regulation is cell cycle-dependent and plays a critical role in the restriction of HR to the S/G2 phase. This activity might be of importance to prevent the potentially deleterious effects of unscheduled DNA recombination. Along this line, Orthwein et al. highlighted a mechanism whereby PALB2 is ubiquitinated during G1 on K20/25/30 residues in the coiled-coil (CC) domain by a complex comprising KEAP1, a PALB2-interacting protein, and CUL3, collectively leading to its degradation [30]. During this process, PALB2 becomes initially phosphorylated at serine 64 (S64) by cyclin-dependent kinases (CDK). Then, the activity of PALB2 promotes DNA end resection and activation of the ATR-Chk1 pathway. The ATR activation drives CDK inhibition and phosphorylation of PALB2 at serine 59 (S59) and stimulates PALB2-BRCA1 interactions and complex formation.

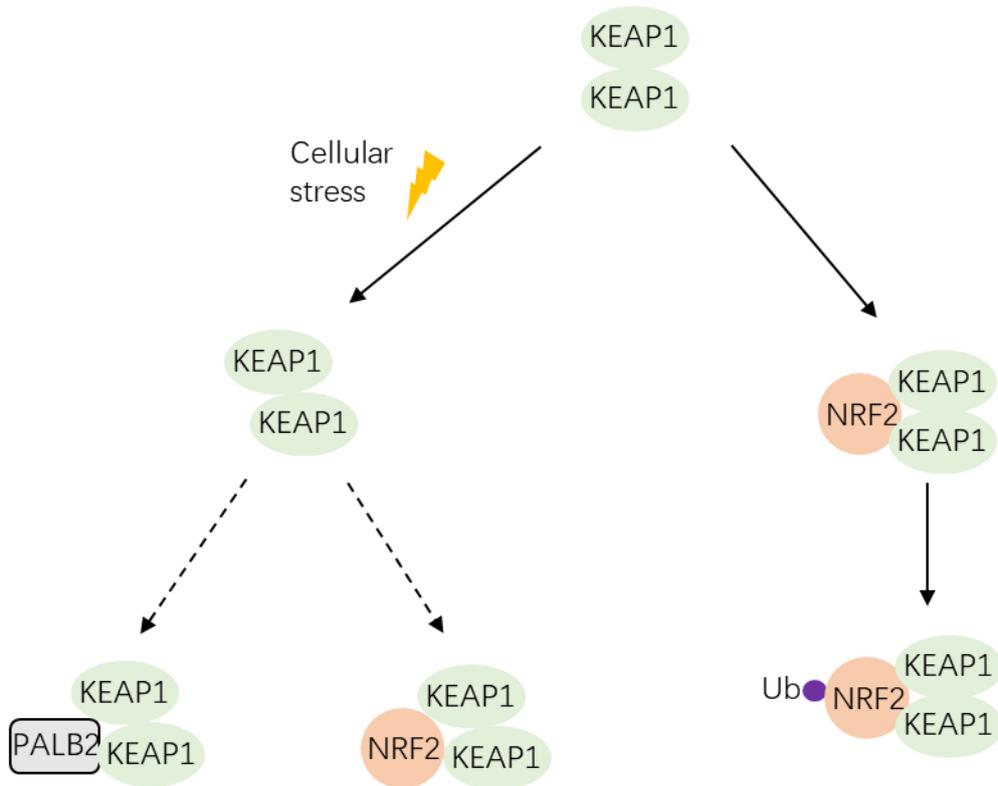


**Figure 2.** In the absence of DNA damage, PALB2 transforms from a low-activity oligomer to a complex with BRCA1 after DNA damage signaling. This process is regulated by a phosphorylation from S64 to S59 by CDK and ATR. Picture modified from a template found in literature [20, 21].

### 1.5 PALB2 and Oxidative Stress

Except for playing a critical role in DSB, the KEAP1–PALB2 interaction also contributes to cellular redox homeostasis (Figure 2). KEAP1 acts as a cysteine-rich oxidative stress sensor. Under normal conditions, it binds to the antioxidant transcription factor NRF2 and marks this protein for ubiquitination and degradation [31]. PALB2 can competitively impede the inhibitory KEAP1–NRF2 interaction and thereby avoid KEAP1-stimulated NRF2 degradation. By this activity, PALB2 is considered crucial for promoting NRF2 accumulation. NRF2 in turn is known to control a set of antioxidant genes, and promote their expression, thereby reducing the burden of intracellular oxidative stress. In agreement with this notion, PALB2 dysfunction has shown to

result in increased levels of reactive oxygen species (ROS) and a pronounced downregulation of a subset of protective and antioxidative NRF2 target genes. These findings indicate a potential and novel connection between oxidative stress and the development of PALB2-associated cancers.



**Figure 3.** In oxidative stress, PALB2 can binds to KEAP1 and enhance the transcriptional activation of antioxidant response element containing genes, this process mediates by NRF2, picture modified from a template found in literature [20].

### 1.6 PALB2 in Disease

Recent research indicated a considerable prognostic value of BRCA mutations for breast, ovarian, and prostate cancers [32-34]. Some epidemiological studies suggested PALB2 mutations are responsible for Fanconi anemia complementation (FANCN) [35, 36], and are associated with childhood cancer [37, 38]. Fanconi anemia is a rare, inherited genome instability-associated syndrome, that it characterized by serval clinical phenotypes, including early-onset bone marrow failure and myeloid leukemia predisposition. PALB2 has also been connected with the risk for breast cancer [39, 40]. Several lines of research confirmed that PALB2 is a high-intermediate breast cancer predisposition gene. In addition, depending on the particular mutation and the

respective population, PALB2 truncating variations confer a relative increased cancer risk in the order of 2 to 30-fold [41-45].

## **2. Aim of the research**

Mutations in PALB2 have been identified in Fanconi anemia complementation group N and are associated with childhood cancer. Moreover, PALB2 mutations and expression levels have also been linked to breast cancer risk and prognosis, respectively. The relationship between hereditary mutations in PALB2 and PDAC risk appears to have population-specific characteristics.

However, little is known about the expression of PALB2 in PDAC tissue and its potential diagnostic value. It is speculated that PALB2 expression is of prognostic relevance in PDAC. To address this hypothesis, PALB2 expression will be studied in human PDAC and peritumoral tissues, and expression levels will be analyzed in relation to disease characteristics and patient survival.

### **3. Materials and Methods**

#### **3.1 Patients and sample collection**

The human PDAC tissue microarrays used together with human breast cancer and gastric cancer tissue sections were purchased from the National Engineering Center for BioChips (Shanghai Biochip Co. Ltd., Shanghai, China). The tissue microarray chip contained 157 tumor tissue samples and 121 peritumoral tissues, with a follow up of patients' survival for a time range of 1.2-7 years. TNM staging data were available for 128 patients (Table 1). All of the PDAC cases were diagnosed by positive histology of invasive ductal carcinomas. Overall survival (OS) was calculated as the interval between the date of surgery and the date of death or last follow-up visit. In total, 61 patients were followed up until November 2014, and 96 patients were followed up until December 2011. The use of the human tissues was approved by the Research Ethics Committees, Shanghai and Taizhou Zhejiang, China. Informed consent was obtained from all patients prior to analysis according to the committees' regulations.

#### **3.2 Immunohistochemistry**

The expression of PALB2 in the tissues was evaluated by semi-quantitative immunohistochemistry (IHC) using a rabbit polyclonal serum to PALB2 (diluted 1:2000, ab202970, Abcam, Cambridge, MA) [46]. The IHC procedure was conducted as follows: paraffin-embedded tissue sections were deparaffinized with xylene and rehydrated with a series of different graded ethanol in Autostainer (ST5010, LEICA, Germany). These steps were followed by antigen retrieval and inactivation of endogenous peroxidase using the PT Link pre-treatment system (Dako, USA). After blocking with bovine serum albumin, section slices were incubated with goat anti-PALB2 antibodies at 4 °C overnight. The next day, slices were washed, processed, and signals were developed using the Dako REAL EnVision FLEX+ detection system (K8002, Dako, USA). Images of stained sections were imported into Aperio XT digital microscope (LEICA, Germany) for quantifying positive stained cells.

#### **3.3 Evaluation of immunostaining**

Tumor cells exhibiting a staining signal in their nucleus were categorized as positively stained and the ratio of stained versus unlabeled tumor cells was determined as percentage of PALB2-positive

tumor cells. The percentage of positive cells was classified into five categories; score 0 (0% of positively stained cells), score 1 (1% - 5% of positively stained cells), score 2 (6% - 30% of positively stained cells), score 3 (31% - 60% of positively stained cells) and score 4 (61% - 100% of positively stained cells). The scoring was conducted by two independent pathologists; in case of an inconsistent result (ca. 15% of samples), the sample in question was re-evaluated together and a consensus was achieved. Scores of 0 and 1 were combined and collectively considered as low expression; similarly, scores 2, 3 and 4 were combined and collectively considered as high expression. The reliability of staining with the commercial PALB2 antibody was tested with gastric cancer tissue, known to not expressing PALB2 protein (negative control), and breast cancer tissue with known high expression of PALB2 protein (positive control).

### **3.4 The use of TCGA public database**

The cBio Cancer Genomics Portal (<http://cbioportal.org>) was used to examine the expression of PALB2 across different types of human cancers, based on The Cancer Genome Atlas (TCGA) public database [47, 48]. The level 3 TCGA pancreatic adenocarcinoma datasets encompassing 178 tumors were downloaded from (<https://tcga-data.nci.nih.gov/tcga/>) for expression analysis, evaluating the Root Mean Squared Error (RMSE) normalized mRNA count (“count”)[49], which represents the gene expression of PALB2. For GSEA, the latest official tool was downloaded from <http://software.broadinstitute.org/gsea> (Ver. 3.0).

### **3.5 Cell culture**

Human PANC1, SW1990 and CFPAC1 cell lines were purchased from the American Type Culture Collection (Manassas, USA). Routinely, the cells were maintained in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal bovine serum (FBS) (GIBCO, Carlsbad, CA, USA). Cultures were grown at 37°C in a humidified atmosphere containing 5% CO<sub>2</sub>.

### **3.6 siRNA transfection**

PALB2 knockdown siRNAs and control siRNAs were purchased from Santa Cruz Biotechnology (sc-93396, sc-37007, Dallas, USA). For cell transfection experiments, the following procedure was conducted: seeding of 2 x 10<sup>5</sup> cells per well in 2 ml antibiotic-free normal growth medium supplemented with 10% FBS in a six-well tissue culture plate, incubation of the cells at 37° C in a CO<sub>2</sub> incubator until the cells have grown to 60-80% confluency. Then the siRNA duplex solution

(Solution A) is added directly to the dilute Transfection Reagent (Solution B) at room temperature. Thereafter, the solution is gently mixed by pipetting it up and down and incubating the mixture for 15-45 min at room temperature. Then, the cells are washed once with 2 ml of siRNA Transfection Medium (Santa Cruz Biotechnology). For each transfection, 0.8 ml siRNA Transfection Medium are added to each tube containing the siRNA Transfection Reagent mixture (Solution A + Solution B) are gently mixed and used as overlay for the washed cells. The cells are then incubated for 5-7 h at 37° C in a CO<sub>2</sub> incubator. Finally, 1 ml of normal growth medium containing 2 times the normal serum and antibiotics concentration (2x normal growth medium) are added without removing the transfection mixture, and cells are incubated and grown for 2 days.

### **3.7 Cell proliferation and wound healing assay**

The cell proliferation assay was performed using the cell counting kit 8 (CCK8, Dojindo, Japan). Briefly, cells were seeded in 96-well plates and the substrate, i.e., a water-soluble tetrazolium salt is added. This chemical is reduced to an orange formazan dye by life cells only. Numbers of viable cells can then be quantified at each 24 h interval by measuring OD450 with a microplate reader. For analyzing the cells in a wound healing assay, the cells were seeded and transfected on six-well plates with si-PALB2 or si-NC. 24 h after transfection, an artificial scratch wound is applied to the confluent monolayer of cells by using a 200 µl pipette tip. Thereafter, serum-free medium was added, and cells were imaged at baseline and 24 h after applying the scratch wound.

### **3.8 Quantitative real-time PCR (qRT-PCR)**

Total RNA was isolated with Trizol reagent (Invitrogen) and converted to cDNA with 1 µg RNA using the reverse transcription kit (TOYOBO, Japan). Applied Biosystems SYBR Green Gene Expression Assays were performed using an Applied Biosystems 7500 Real-Time PCR System. GAPDH was used as an endogenous control for normalization. The DDCT method was applied to quantify relative gene expression. The primer used in qRT-PCR are indicated below:

PALB2-F: 5-ACG CGT CGA CAG GCC GAA TGG TGG ATTTA-3  
PALB2-R: 5-CAA GAT ATC GCA CAT GTA CAA ATG TGGGAA-3,  
Vimentin-F: 5- GAC GCC ATC AAC ACC GAG TT-3  
Vimentin-R: 5- CTT TGT CGT TGG TTA GCT GGT-3  
Snail-F: 5-ACC ACT ATG CCG CGC TCT T-3

Snail-R: 5-GGT CGT AGG GCT GCT GGA A-3  
Slug-F: 5-ATG AGG AAT CTG GCT GCT GT-3  
Slug-R: 5-CAG GAG AAA ATG CCT TTG GA-3  
Zeb1-F: 5-GCA CCT GAA GAG GAC CAG AG-3  
Zeb1-R: 5-TGC ATC TGG TGT TCC ATT TT-3

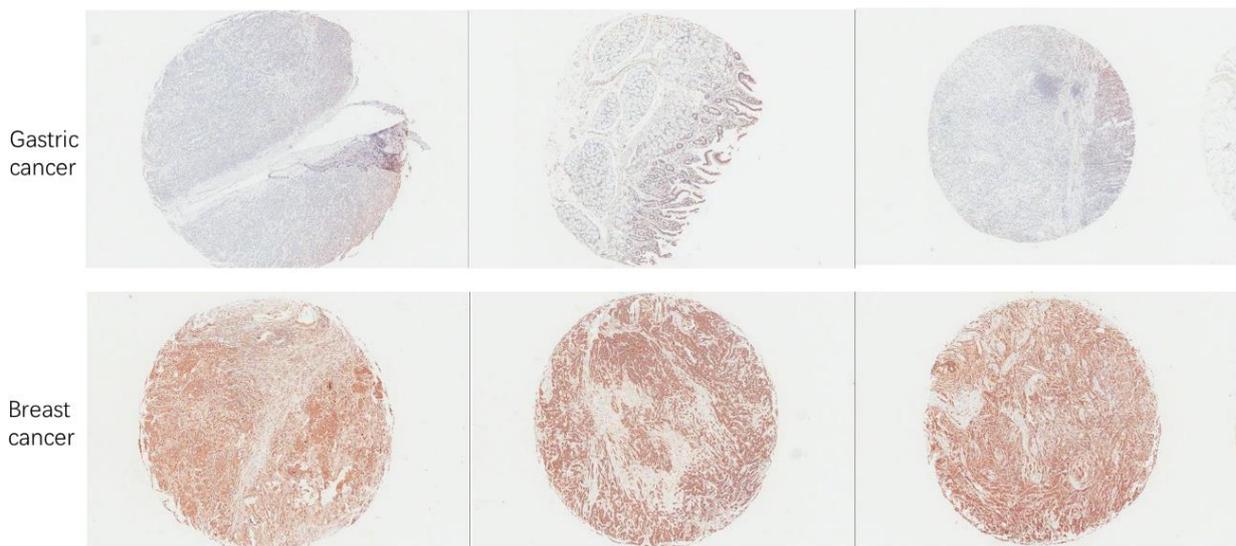
### **3.9 Statistical analysis**

The continuous variables in the different subgroups were compared using an unpaired t-test. Chi-square test was used to analyze the distribution of each categorical variable between PALB2-negative and PALB2-positive groups. OS was plotted using Kaplan-Meier survival curves with 95% confidence intervals (CIs), and the differences between subgroups were compared using the log-rank test. A multivariate COX regression analysis was used to identify independent prognostic factors. All the tests were two sided, and  $p < 0.05$  was considered statistically significant. The statistical analyses were performed using GraphPad Prism 8.0 (Graphpad Software Inc., San Diego, CA) or IBM SPSS Statistics software Version 24.0 (IBM Corp., Armonk, NY, USA).

## 4. Results

### 4.1 Establishment of the immunohistological method and test for antibody specificity

To establish the immunohistological method and test if the commercial antibody yielded congruent data in agreement with current knowledge, gastric and breast cancer tissues were analyzed as negative and positive controls for PALB2 expression, respectively. In agreement with the results from the public TCGA database (see below), no IHC signals were seen for the stomach cancer negative control (Figure 4, upper panel). Strong IHC signals were seen with the breast cancer positive controls, verifying the suitability of the antibody and IHC protocol (Figure 4, lower panel).



**Figure 4.** PALB2 protein expression in breast cancer samples as compared to gastric cancer samples. Surgical samples from stomach and breast cancer (n=3 per tissue) were analyzed by immunohistochemistry and signals were developed in parallel by the analytical technique described. While there was no positive staining in the stomach samples, the breast samples showed a consistent and intensive uniform PALB2 immunoreactivity.

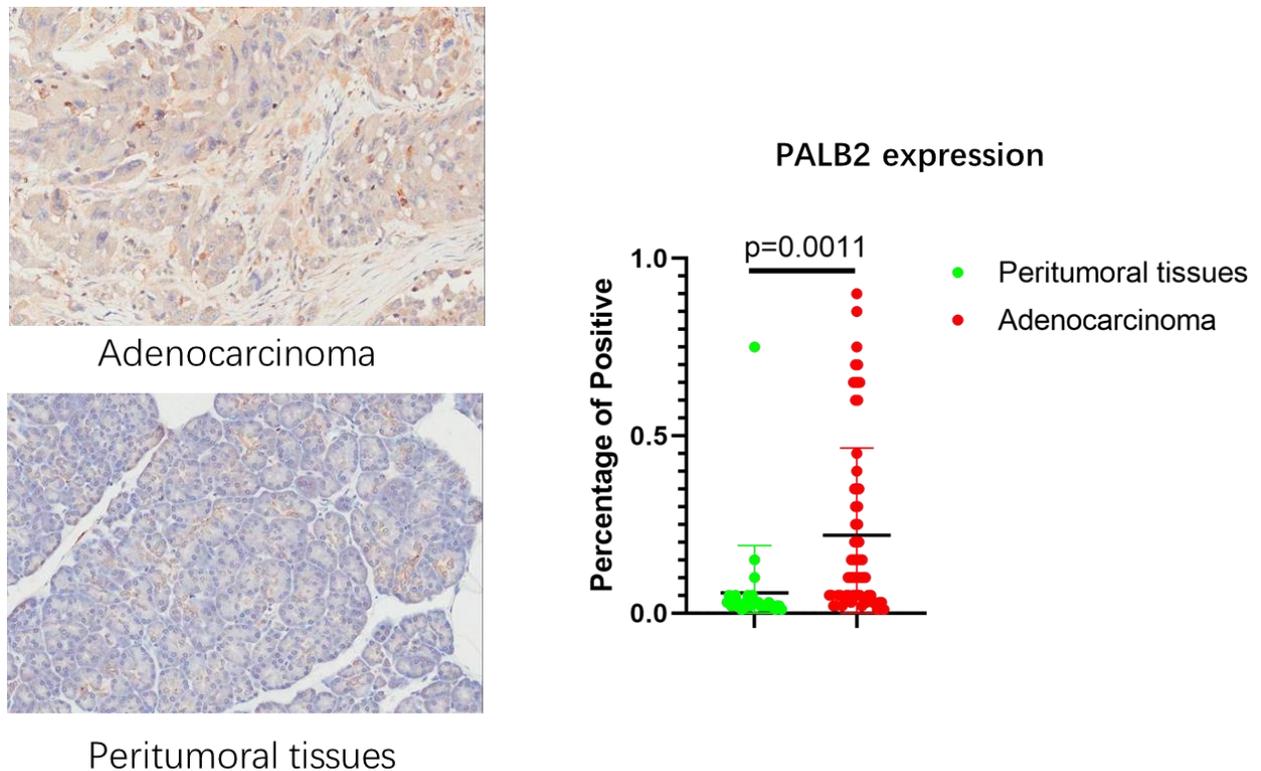
**Table 1.** Clinicopathologic characteristics of patients, and PALB2 expression in PDAC tissue

<b>Features</b>	<b>PALB2 negative</b>	<b>PALB2 positive</b>	<b>p value</b>
<b>Age [years +/- SD]</b>	64.1+/-11.0	62.6+/-10.1	0.3851
<b>Total number</b>	91	60	
<b>Gender</b>			
<b>Male</b>	56 (60.8%)	36 (39.2%)	
<b>Female</b>	35 (59.3%)	24 (40.7%)	
			0.8496
<b>TNM staging n=128</b>			
<b>T (1-2)</b>	54 (66.7%)	27 (33.3%)	
<b>T3</b>	25 (53.1%)	22 (46.9%)	
			0.1306
<b>N0</b>	46 (63.8%)	26 (36.2%)	
<b>N1</b>	33 (58.9%)	23 (41.1%)	
			0.5668
<b>AJCC staging n=128</b>			
<b>I</b>	28 (68.2%)	13 (31.8%)	
<b>IIA</b>	18 (58.1%)	13 (41.9%)	
<b>IIB</b>	33 (60.0%)	22 (40.0%)	
			0.6108
<b>Tumor location</b>			
<b>Head</b>	52 (57.7%)	38 (42.2%)	
<b>Body/Tail</b>	38(63.3%)	22 (36.7%)	
<b>Ki-67 n=89</b>			0.4962
<b>Positive</b>	47 (72.3%)	15 (62.5%)	
<b>Negative</b>	18 (27.7%)	9 (37.5%)	
			0.3718
<b>P53 n=89</b>			
<b>Positive</b>	49 (74.2%)	21 (87.5%)	
<b>Negative</b>	17 (25.8%)	3 (12.5%)	
			0.1810

T: primary tumor site; N: regional lymph node involvement; Chi-square test was used to analyze the distribution of each categorical variable between PALB2-negative and PALB2-positive groups. p-value < 0.05 (bold) indicates a significant difference.

#### 4.2 PALB2 has lower expression in the in the peritumoral tissues than PDAC tissue

Among the tissues analyzed, there were slightly more PALB2-negative cancer tissues than PALB2-positive ones (Table 1). When expression of PALB2 was positive, the staining was lower in the peritumoral tissues as compared to the PDAC tissue (Figure 5).



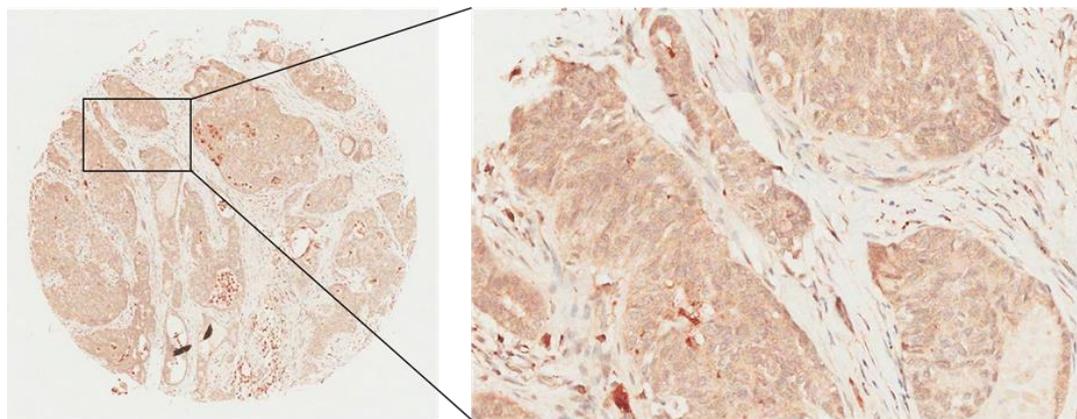
**Figure 5.** IHC for PALB2 expression in PDAC and peritumoral tissue. PALB2 staining was more intense in PDAC as compared to peritumoral tissues. Relative number of positively stained cells was significantly lower in peritumoral tissues (n=30) as compared to tumor (n=60) tissue, unpaired t-test was used to compare positive percentage of PALB2 between tumor and peritumoral tissues, p-value=0.0011 indicates a significant difference.

PALB2-positive cell nuclei in PDAC and in the peritumoral tissues were identified and counted in relation to unlabeled cells. The overall rate of positive labeling of PALB2 in normal pancreas tissue was 24.8% of cells, while 40.8% of cells in PDAC tissue showed positive staining in their nuclei (Table 2).

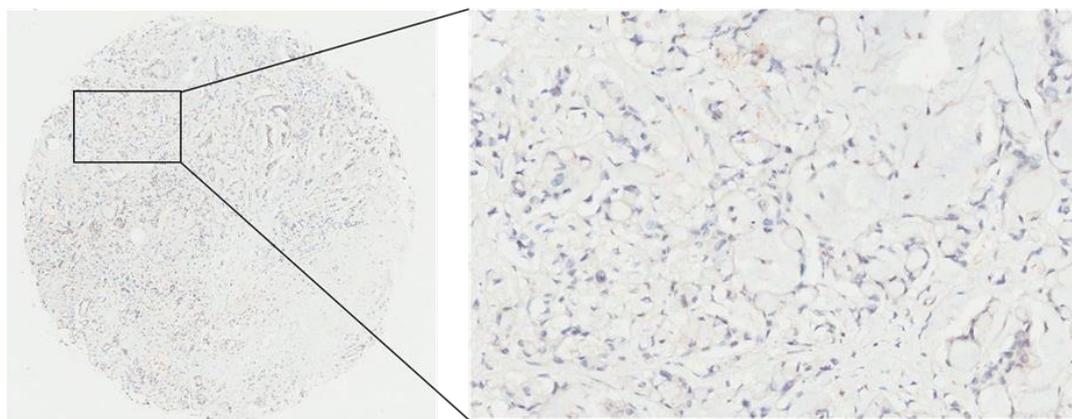
**Table 2.** Nuclear PALB2 expression in peritumoral versus adenocarcinoma tissues

Tissue type	PALB2 positive (rate)
Peritumoral tissues (n=121)	30 (24.8%)
Adenocarcinoma (n=157)	60 (38.2%)

Positively labeled tumor tissues exhibited a dense and intensive staining throughout the malignant area, different to the rather faint signals observed in untransformed tissues (Figure 6).



High expression



Low expression

**Figure 6.** The levels of PALB2 expression were scored into five categories according to the percentage of positively stained cells. An example picture of an intensively stained tissue sample (score of 4) versus a poorly stained sample (score of 1) is shown on the left and right, respectively, along with a magnified tissue area. The magnification is 100- and 1000-fold, respectively.

Next, the relationship between patients' characteristics including PALB2 expression in the tumors and the patients' OS was analyzed. The multivariate Cox regression analysis identified age (p=0.037), N classification (p<0.001) and PALB2 expression levels (p=0.021) as tumor parameters significantly associated with OS in PDAC patients (Table 3).

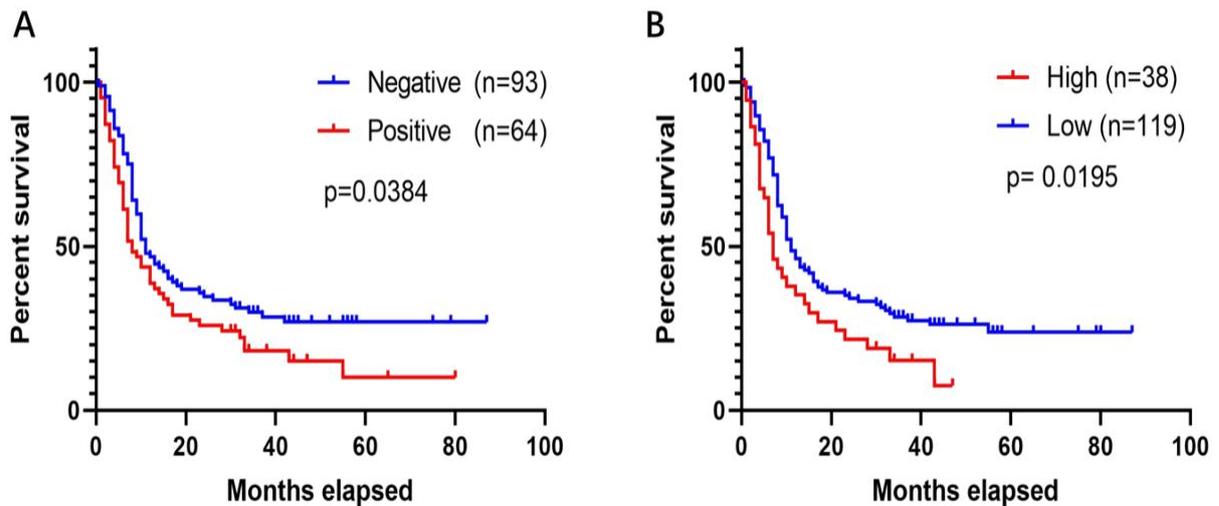
#### 4.3 High expression of PALB2 was associated with poor prognosis in surgical resectable PDAC patients

The relationships between the patient characteristics including PALB2 expression in tumors and patient OS are presented in Table 3. Multivariate Cox regression analysis identified age (p=0.037), N classification (p<0.001) and PALB2 levels (p=0.021) as tumor parameters significantly associated with OS in PDAC patients (Table 3).

**Table 3.** Multivariate Cox regression analyses of OS of patients with PDAC (n=128)

<b>Factors</b>	<b>HR (95CI%)</b>	<b>p value</b>
<b>Gender</b>	1.357 (0.892-2.064)	0.154
<b>Age (≥64 years/&lt;64 years)</b>	1.586 (1.029-2.445)	<b>0.037*</b>
<b>Tumor location</b>	1.188 (0.757-1.864)	0.454
<b>T Classification</b>	1.106 (0.657-1.596)	0.944
<b>N Classification</b>	2.222 (1.437-3.436)	<b>&lt;0.001*</b>
<b>PALB2 (high/low)</b>	1.736 (1.087-2.773)	<b>0.021*</b>

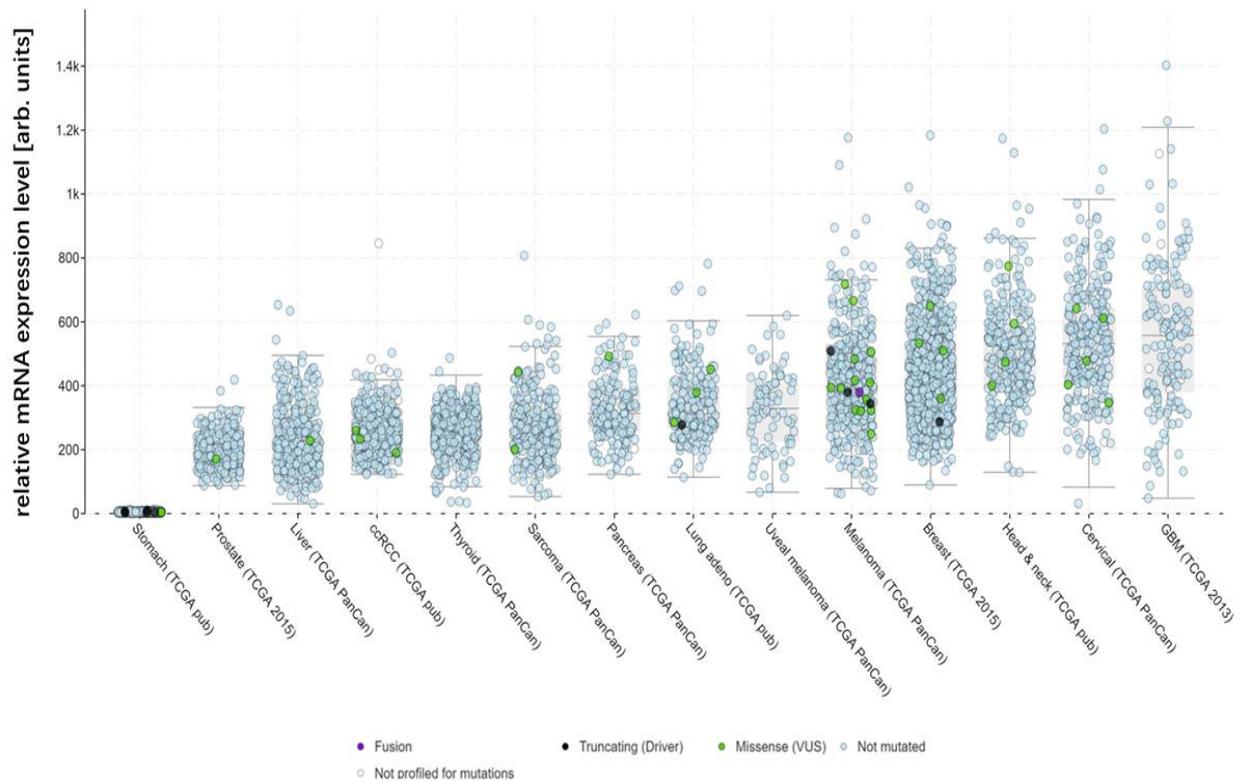
CI: confidence interval; HR: hazard ratio; OS: overall survival; T: primary tumor site; N: regional lymph node involvement; p-value < 0.05 (bold) indicates a significant difference. Survival data were available for most patients. Kaplan-Meier analysis indicated that patients with positive PALB2 expression had reduced OS compared to patients with samples with negative staining (log rank p=0.0384, Figure 7A). Patients with PDAC tissues with positive PALB2 staining were divided into two groups: low PALB2 expression (scores 0 and 1 combined, n=119) versus high PALB2 expression (scores 2, 3 and 4 combined, n=38). A significant negative correlation was observed between PALB2 expression scores and long-term survival rate in Kaplan-Meier analysis (log-rank p=0.0195, Figure 7B). Consequently, a high rate of PALB2 expression may indicate poor prognosis for patients with surgically resectable PDAC.



**Figure 7.** Kaplan-Meier estimates of OS according to the expression levels of PALB2 in patients with resectable PDAC. (A) The PALB2 positive PDAC group exhibited a worse survival rate as compared to the group without detectable PALB2 expression. (B) High expression of PALB2 was associated with a poor OS in comparison to low expression rates. Log-rank test was used to compare differences in OS; p-value < 0.05 indicates a significant difference.

#### 4.4 Upregulation of PALB2 RNA expression was detected in the most types of cancers.

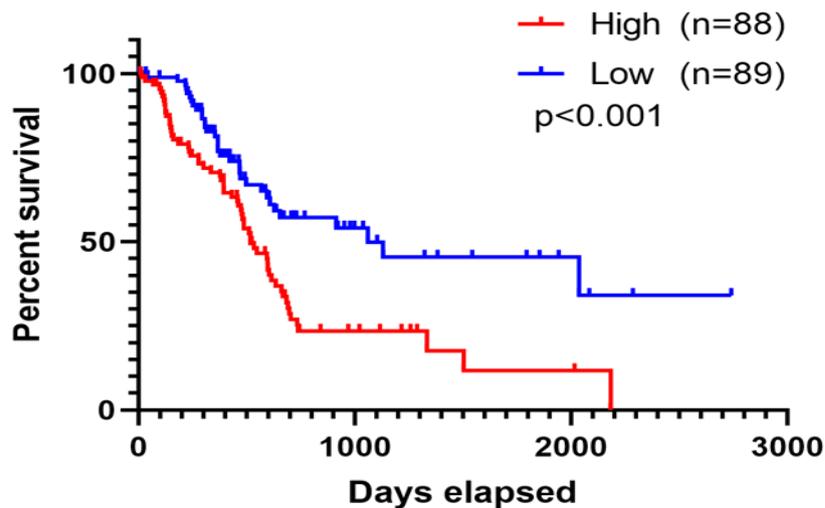
To investigate the spectrum of cancer related PALB2 expression on a larger scale, the TCGA public database was analyzed. Scatterplots of PALB2 mRNA expression across 14 types of cancers were generated by the cBioPortal (Figure 8). The levels of PALB2 mRNA in the cancers were compared to respective normal tissues and plotted in a log-scale format. Many cancer types in the TCGA database showed upregulated PALB2 mRNA, including breast, cervical and pancreatic cancers. The only notable exception in this analysis was stomach cancer, which did not show increased PALB2 mRNA, in agreement with our analysis of stomach cancer as negative control tissue (please compare above, Figure 4).



**Figure 8.** (A) PALB2 expression in 14 types of human cancers. Ascending scatter plots of relative PALB2 mRNA levels in different cancer types were sorted by median PALB2 transcriptional level in comparison to the normal tissues. Stomach showed lowest relative PALB2 expression, whereas strong signals were obtained from pancreas.

#### **4.5 High expression of PALB2 was associated with poor prognosis in the PDAC patients present in the TCGA database**

The 178 PDAC samples were then separated into a relatively high PALB2 expression group (n=88) above the median, and a relatively low PALB2 expression group (n=89) below the median. A Kaplan Meier curve analysis demonstrated a significant negative correlation between PALB2 expression and long-term survival rate (log-rank  $p < 0.001$ , Figure 9).



**Figure 9.** Kaplan-Meier estimates of OS according to the expression levels of PALB2 in PDAC from TCGA database. Separation of the PDAC patients by median PALB2 expression levels (higher 88 vs. lower 89): the group with relatively low PALB2 expression showed a better survival (log-rank  $p$ -value < 0.001). Log-rank test was used to compare differences in OS;  $p$ -value < 0.05 indicates a significant difference.

Multivariate Cox regression analysis identified tumor location ( $p=0.026$ ), N classification ( $p=0.019$ ) and PALB2 levels ( $p=0.004$ ) as tumor parameters significantly associated with OS of the PDAC patients represented in the TCGA database (Table 4).

**Table 4.** Multivariate Cox regression analyses of OS of PDAC samples represented in the TCGA database (May 2019) (n=171)

Factors	HR (95CI%)	p value
Gender	0.963 (0.548-1.693)	0.896
Age ( $\geq 65$ years/ $< 65$ years)	1.005 (0.549-1.841)	0.986
<b>Tumor location</b>	1.970 (1.083-3.585)	<b>0.026*</b>
T Classification	1.032 (0.508-2.097)	0.930
<b>N Classification</b>	1.894 (1.109-3.234)	<b>0.019*</b>
<b>PALB2 (high/low)</b>	1.980 (1.245-3.249)	<b>0.004*</b>

CI: confidence interval; HR: hazard ratio; OS: overall survival; T: primary tumor site; N: regional lymph node involvement;  $p$ -value < 0.05 (bold) indicates a significant difference.

An analysis of the clinicopathological information from the PDAC samples indicated a positive correlation of high levels of PALB2 mRNA expression with the primary tumor site (Table 5).

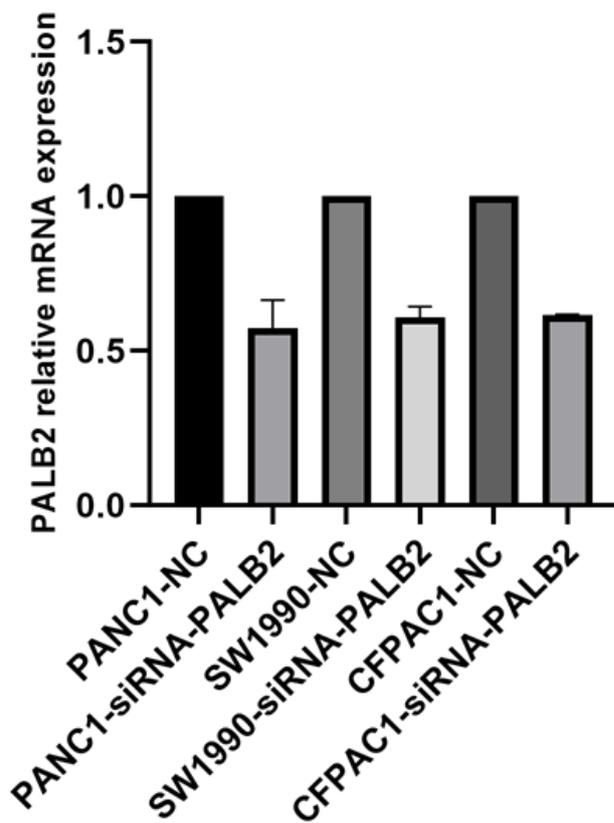
**Table 5.** Clinicopathologic characteristics of patients, and PALB2 expression in PDAC samples represented in the TCGA database (May 2019)

<b>Features</b>	<b>PALB2 high</b>	<b>PALB2 low</b>	<b>p value</b>
<b>Age [years +/- SD]</b>	65.8+/-11.0	63.4+/-10.8	0.1393
<b>Total number</b>	89	89	
<b>Gender</b>			
<b>Male</b>	46 (51.7%)	52 (58.4%)	
<b>Female</b>	43 (48.3%)	37 (41.6%)	0.4513
<b>TNM staging</b>			
<b>T (1-2)</b>	9 (10.1%)	22 (25.2%)	
<b>T (3-4)</b>	80 (89.9%)	65 (74.8%)	<b>0.0099*</b>
<b>N0</b>	24 (27.2%)	25 (29.4%)	
<b>N1</b>	64 (63.8%)	60 (70.6%)	0.8661
<b>AJCC staging</b>			
<b>I-IIA</b>	23 (25.8%)	26 (29.8%)	
<b>IIB-IV</b>	66 (74.2%)	61 (70.2%)	0.6151
<b>Tumor location</b>			
<b>Head</b>	69 (77.5%)	70 (78.6%)	
<b>Body/Tail</b>	20 (22.5%)	19 (21.4%)	>0.9999

T: primary tumor site; N: regional lymph node involvement; p-value < 0.05 (bold) indicates a significant difference. Chi-square test was used to analyze the distribution of each categorical variable between PALB2-negative and PALB2-positive groups. p-value < 0.05 (bold) indicates a significant difference.

#### 4.6 Generating a PALB2 knock down cell model in vitro

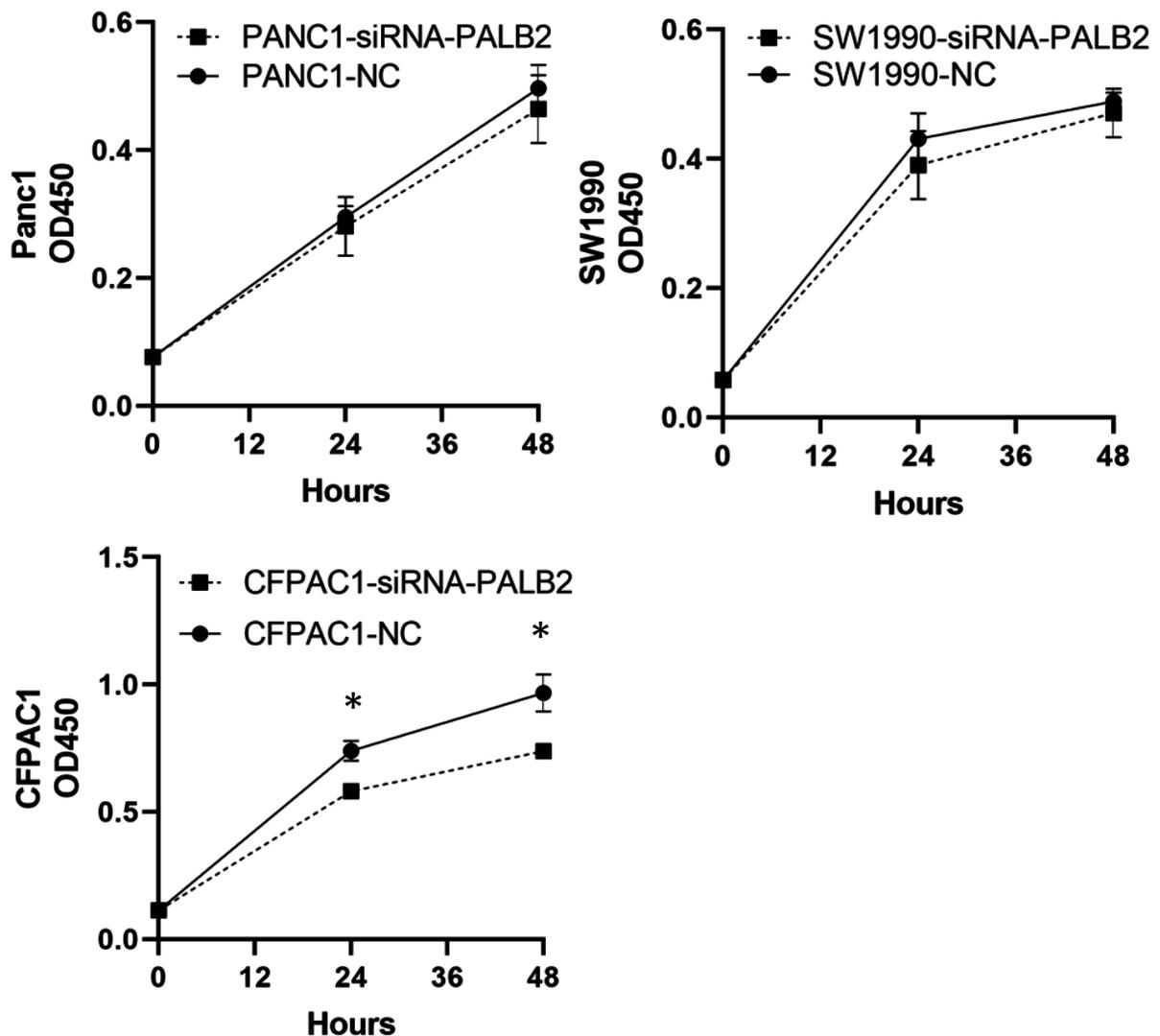
In order to better investigating the biological function of PALB2, we constructed a PALB2 knock down model in vitro. Commercial siRNAs were selected, commercially obtained and used for PALB2 knockdown. As first quality check, we followed mRNA expression of PALB2 in cells transfected with PALB2-specific siRNA in comparison to control-transfected cells. The results of the Realtime-PCR analysis indicated that PALB2-siRNAs were able to effectively knockdown PALB2 mRNA expression levels in the three different PDAC cell lines studied (Figure 10).



**Figure 10.** Quantitative reverse transcription–polymerase chain reaction (qRT-PCR) to test for successful PALB2 knockdown by siRNA. Cells were incubated with PALB2-specific siRNA or control siRNA. After 48 h, cells were harvested, mRNA was prepared and used for qRT-PCR analysis of PALB2 gene expression. The results indicate that PALB2 was successfully knocked down by the PALB2-specific siRNAs in all three human cell lines tested, i.e., in PANC1, SW1990 and CFPAC1 cells. Relative mean values (plus SD) of triplicate analyses are shown.

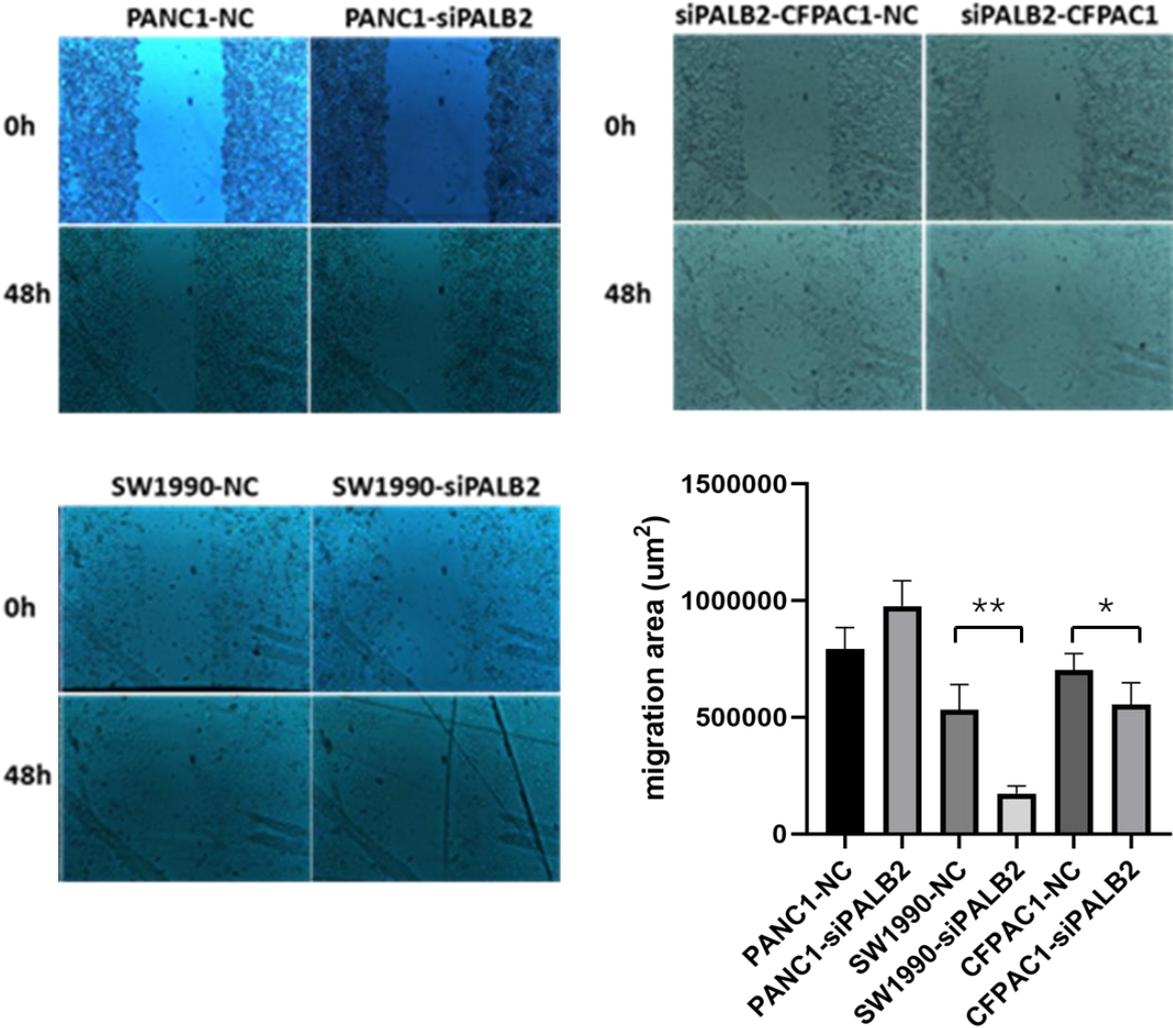
#### 4.7 Analysis of cell growth and cell migration in relation to PALB2 expression

Next, functional readouts of cells with native or reduced PALB2 expression were studied. To this end, wound healing experiments and cell proliferation experiments were conducted in order to investigating the biological function of PALB2 in vitro. Using the three different human cell lines, PALB2 knock down appeared not to affect cell growth and proliferation rates (Figure 11).



**Figure11. Analysis of cell growth and proliferation in relation to PALB2 gene expression.** Using PALB2-specific siRNA, PALB2 was down regulated in PANC1, SW1990 and CFPAC1 cells. Growth and proliferation of the cells was assessed by the analysis of OD450 every 24 h. The data indicate that the knockdown of PALB2 did not affect growth and proliferation of pancreatic ductal adenocarcinoma (PDAC) cells in vitro. The formazan-dye formation assay (CCK8 assay) was used to quantify the effects of PALB2 knockdown on cell proliferation. Biological triplicates were performed and tested by two-sided paired sample t-test (\* $P < 0.05$ ).

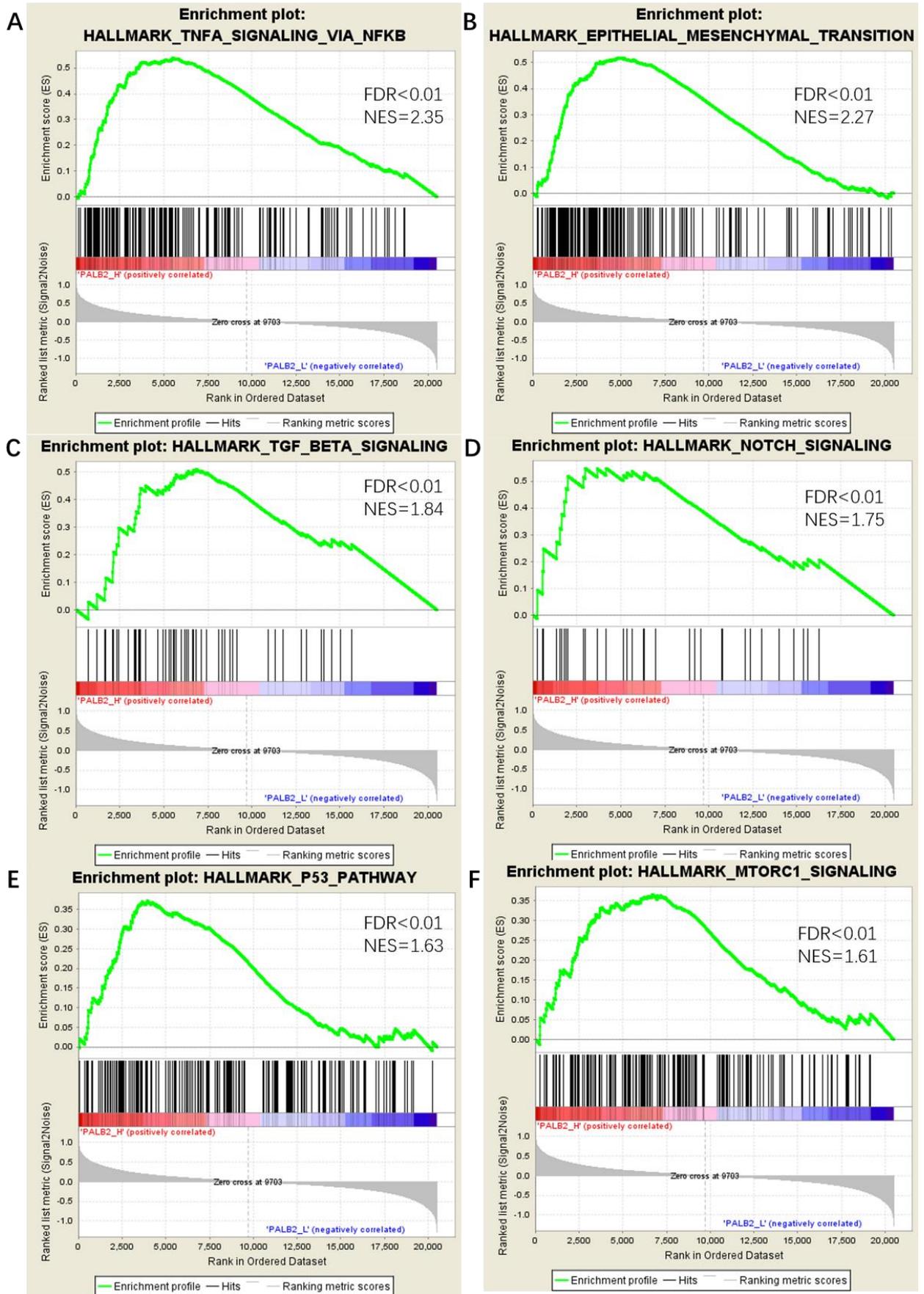
Then as next functional readout, migration of cells with native or reduced PALB2 expression were compared. To this end, a scratch wound was applied to a confluent monolayer of the different cell lines after transfection with PALB2-specific or control siRNA. Migration efficiency into the wound was quantified by live cell imaging using time-lapse microscopy. The results obtained indicate that migration rates are affected by PALB2-knockdown in a cell type specific manner (Figure 12).



**Figure 12. Migration analysis of cells with wildtype or down regulated PALB2 expression in a wound healing test.** Human PDAC cells were transfected with PALB2-specific or control siRNA and grown to confluency. A wound into the monolayer was applied by scratching with a 200  $\mu\text{l}$  pipette tip. Cell migration was analyzed by automatic quantification of the scratch area and distance between the two borders of live cells. The results indicate that down regulation of PALB2 suppressed the migration of pancreatic ductal adenocarcinoma (PDAC) cells in a cell-type specific fashion. Efficient wound healing was observed independent of PALB2 expression in PANC1 cells, whereas cell migration was strongly decreased by PALB2 knockdown in SW1990 cells. the results for CFPAC1 cells were in between the other two human cell lines. Biological triplicates were performed and tested by two-sided paired sample t-test (\* $P < 0.05$ , \*\* $P < 0.01$ ).

#### **4.8 Identification of PALB2 gene associated biological pathways**

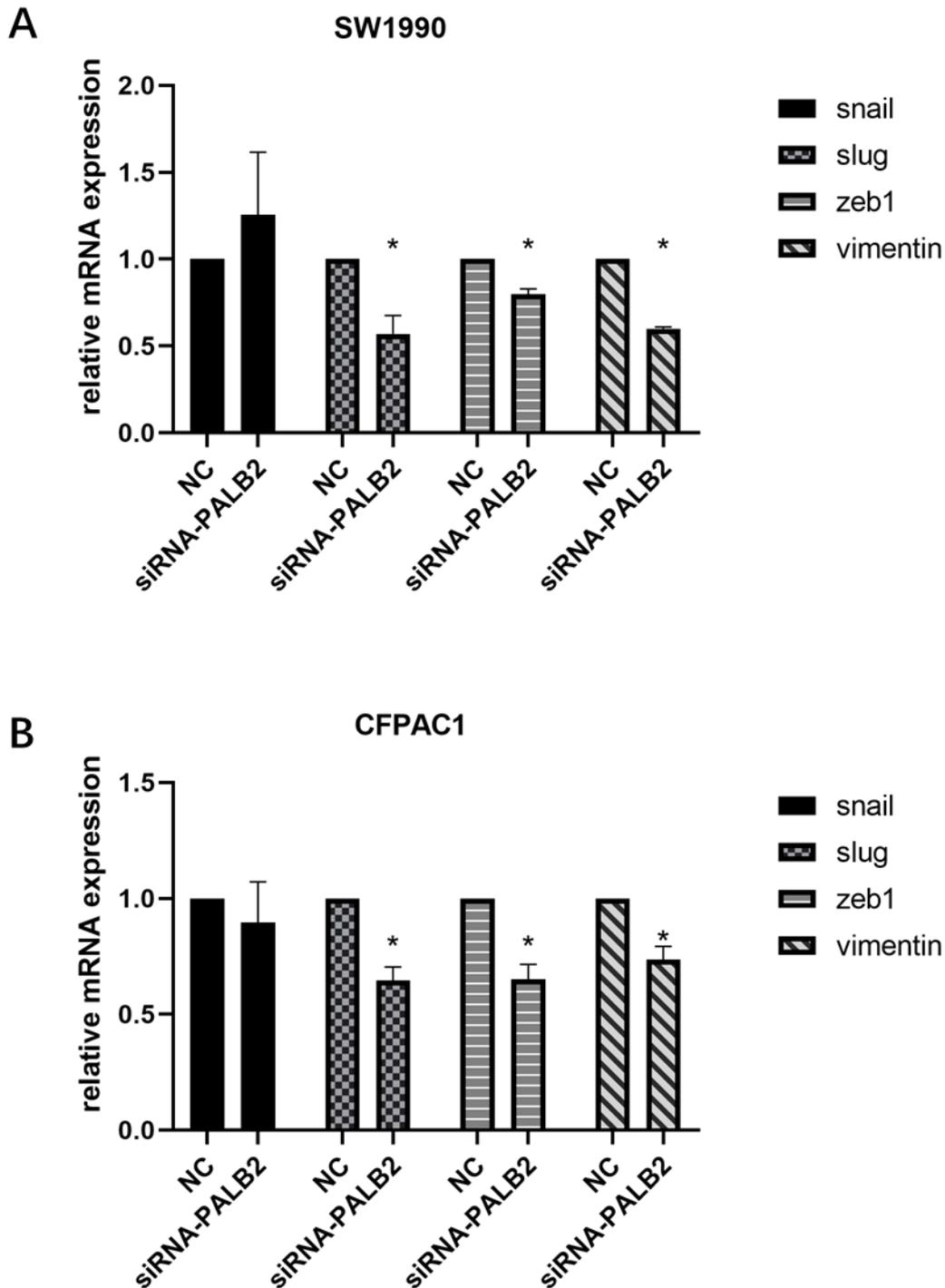
In order to better explore the potential biological function of the PALB2 in PDAC, an in-silico approach was chosen. First, gene set enrichment analysis (GSEA) was used to map the available data into the biocarta pathways database. GSEA is a bioinformatic approach that can analyze the possible correlation of a target gene with known functional gene sets [50, 51]. To this end, the data of the patients with highest PALB2 expression, i.e., those in the top 11% with regards to their PALB2 mRNA levels, were compared to the patients with lowest PALB2 expression, i.e., those localized in the bottom 11% of PALB2 mRNA levels. As information source, The Cancer Genome Atlas (TCGA) gene expression database was used. The most significantly enriched signaling pathways were chosen, based on their normalized enrichment score (NES). The results indicate that high PALB2 expression samples were also enriched for the TNF-alpha, epithelial to mesenchymal transition (EMT), TGF-beta, p53, NOTCH and mTORC1 pathways (Figure 13).



**Figure 13.** Gene set enrichment analysis (GSEA) associated with PALB2 expression. The gene sets of TNF-alpha, EMT, TGF-beta, NOTCH, p53 and mTORC1 pathways were enriched in PDAC samples with high expression levels of PALB2. (A) TNF-alpha pathway (NES=2.35, FDR<0.01) (B) EMT pathway (NES=2.27 FDR<0.01) (C) TGF-beta pathway (NES=1.84, FDR<0.01) (D) NOTCH pathway (NES=1.75, FDR<0.01) (E) p53 pathway (NES=1.63, FDR<0.01) (F) mTORC1 pathway (NES=1.61, FDR<0.01). For GSEA, the latest official tool was downloaded from <http://software.broadinstitute.org/gsea> (Ver. 3.0). The results of GSEA that had a false discovery rate (FDR) < 0.25, and Normalized Enrichment Score (NES) > 1 were considered to indicate a statistically significant difference.

#### **4.9 PALB2 is associated with epithelial mesenchymal transition (EMT) in PDAC**

As the GSEA results indicated, the epithelial mesenchymal transition (EMT) constitutes one of the pathways most strongly associated with the PALB2 gene expression level. In order to verify this notion, PALB2 expression was reduced (knockdown) by PALB2-specific siRNA, and gene expression was compared to control cells exposed to control siRNA application. In order to analyze the effects, gene expression of several transcription factors known to be of central importance for EMT were quantified, such as the Snail family. Hence, expression of several EMT markers were compared between PALB2 knockdown and negative control cells. The results indicate that knock down of PALB2 was not affecting mRNA levels of Snail in SW1990 or CFPAC1 cells, whereas Slug and Zeb1 was efficiently down regulated in both SW1990 and CFPAC1 cells. Importantly, mRNA levels of the mesenchymal marker Vimentin were down regulated in both cell lines, indicating a direct association of PALB2 levels with vimentin gene expression (Figure 14).



**Figure 14. Effects of siRNA-mediated down regulation of PALB2 expression on the epithelial–mesenchymal transition (EMT).** SW1990 and CFPAC1 cells showed reduced migration behavior in the scratch wound test upon PALB2 down regulation. In order to test for effects of PALB2 on EMT markers, gene expression of snail, slug, zeb1 and vimentin were analysed in relation to siRNA medicated PALB2 knock down. Down regulation of PALB2 was associated with reduced gene expression of the EMT markers slug, zeb1 and vimentin. Biological triplicates were performed and tested by two-sided paired sample t-test (\* $P < 0.05$ , \*\* $P < 0.01$ ).

## 5. Discussion

In this study, the expression and biological function of the tumor suppressor gene PALB2 was studied in several PDAC cell lines. In addition, the expression of PALB2 was quantified in peritumoral and tumoral tissue samples, and compared to the clinicopathological characterization of the patients. The experiments were intended to support the attempts for better diagnostic methods, given that PDAC belongs to the leading causes of cancer-related mortality [52]. The results indicate that PALB2 affects important cellular processes like EMT and tumor cell migration. In the clinical samples, the protein levels were increased in the tumor as compared to the peritumoral tissues. Notably, high PALB2 expression was negatively associated with OS of surgical resectable PDAC patients. These findings qualify elevated PALB2 expression levels as a negative prognostic marker for survival in surgical resectable PDAC. Interestingly, PALB2 expression appears to become upregulated in most tumor types, with the notable exception of stomach cancer. This notion was verified when analyzing stomach in comparison to breast cancer tissue slices. In PDAC, the three parameters age, N classification and PALB2 expression levels may be combined for obtaining a valuable marker of OS and mortality. Knockdown of PALB2 in vitro supported this notion, as the results indicated that PALB2 knockdown inhibited the migration ability of pancreatic cancer cells, i.e., high PALB2 expression may support cancer cell migration. On the molecular level, the gene expression of several EMT-related transcription factors were down regulated by PALB2 knock down, indicating that PALB2 expression is positively associated with EMT-related transcription factors and the transition process. These data indicate that PALB2 may directly affect EMT via up regulation of crucial transcription factors, thereby promoting the ability to metastasis of PDAC tumor cells, finally contributing to the poor outcomes of PDAC patients and their low overall survival rates.

In terms of function, PALB2 is known to colocalize with BRCA1 and BRCA2 and to take part in error-free homologous recombination repair [53]. Dysfunctions of BRCA1 and BRCA2 are known to promote carcinogenesis [54]. PALB2 mutations also have been reported in other types of cancer, such as lung or breast and ovarian cancer [55, 56]. Mutations in PALB2 appear to associate with a high risk of breast cancer in both men and women [57], and the increased risks seem to be particularly resulting from protein truncation mutations [58]. This was most evident in a mutation screening study conducted in Finland and identifying c.1592delT as a recurrent mutation in familial breast cancer cases, encoding a truncated protein with impaired BRCA2-binding capacity

[59]. Notably, loss-of-function mutations in PALB2 are reported to confer an eight to nine times increased breast cancer risk for females below 40 years of age, partly overlapping with increased breast cancer risk from BRCA2 mutations [60].

In patients with pancreatic cancer, germline truncating mutations in PALB2 were also detected supporting PALB2 as a susceptibility gene for tumorigenesis of the pancreas [61]. A European study provided evidence that truncating mutations of PALB2 predispose to both pancreatic and breast cancer risk [62]. Despite these convincing lines of evidence for a causal involvement of PALB2 for tumorigenesis, the relative importance of dysregulated PALB2 expression for disease course and mortality risk are poorly known.

Missense polymorphisms in BRCA1 have been described to exert only a very moderate effect on prognosis of PDAC patients [63]. Certain mutations in other BRCA pathway genes were in general associated with better prognosis of PDAC patients [64]. In terms of expression levels, elevated BRCA2 mRNA expression predicts poor prognosis in breast cancer patients [65], and similarly, increased PALB2 expression was associated with poor overall survival of patients with advanced breast cancer [66]. The relationship between PALB2 expression levels and prognosis of patients with PDAC remained uncharacterized.

The data from the analyses presented here now indicate a considerable prognostic significance of PALB2 overexpression in PDAC. The Kaplan Meier survival analysis highlighted a strong association of elevated PALB2 expression levels with poor OS odds. The expression of PALB2 was not associated with the other clinicopathological parameters analyzed. PALB2 may thus constitute an independent prognostic factor of OS in resectable PDAC patients and may serve as an additional marker besides classical parameters of cancer staging and grading. Notably, the results were obtained from patients residing in Asia (our immunohistochemical analysis of tissue arrays from Chinese patients) and in America (the TCGA database is mainly composed of US American patients), respectively, indicating a population-independent finding.

The occurrence and development of pancreatic cancer is associated with a dysregulation of a variety of signaling pathways. In order to gain some additional insight into the potential biological function of PALB2, a GSEA using TCGA gene expression data was conducted to identify those pathways that may be directly affected by an increased or decreased PALB2-expression. Many of the signaling pathways associated with an upregulated expression of PALB2, i.e., those enriched

in the PALB2 high expression group, were related to cell cycle control and proliferation, including the TNF-alpha, EMT, TGF-beta, p53, NOTCH and mTORC1 pathways. This notion is in agreement with previous research indicating that PALB2 might be involved in tumor cell proliferation, invasion and migration, particularly via the P53 and NF- $\kappa$ B signaling pathways [67]. In the vitro experiments, PALB2 knockdown affected the PDAC cells' migration ability, but not the proliferation rates. According to the GSEA results, EMT emerged as one strongly associated pathway that could be a downstream mechanism affected by PALB2 knockdown. EMT is a multistep process during which the epithelial phenotype of cells is progressively lost and their differentiated characteristics fade while undergoing characteristic changes in adhesion and motility [68-70]. The mesenchymal cancer cells acquire a more invasive, metastatic and chemo resistant phenotype [71-74]. In order to testing this hypothesis, the expression levels of EMT relevant transcription factors were quantified in relation to PALB2 expression. It became obvious that PALB2 knockdown caused reduced gene expression of Slug, Zeb1 and Vimentin. These findings were consistent with previous reports that RNA silencing of PALB2 is capable of inhibiting breast cancer cell proliferation and migration, and may lead furthermore to a downregulated activity of the epithelial to mesenchymal transition (EMT) pathways [66]. However, the underlying regulatory alterations and effects require further clarification, and the findings presented should next be verified by an in-depth analysis of clinical data sets.

In the Kaplan Meier based analysis, the relation between PALB2 expression and OS was relatively strong. This relation was found both on the mRNA and protein expression levels. The analyses conducted were of sufficient size to identifying these interactions, however, some limitations need to be mentioned. As a retrospective study, not all data of the PDAC patients were available for analysis, and due to the observational type of our study, mechanistic conclusions from our data cannot be drawn. Additional functional studies have not yet been conducted but are essentially needed to better highlight the biological role exerted by elevated PALB2 protein levels in tumor cells, and their relevance for cell proliferation and the tumor's susceptibility to oncostatic medication. These analyses have been initiated and hopefully will contribute further to a better understanding of the proteins and its relevance for diagnosis and prognosis in this most devastating disease, i.e., PDAC.

## **6. Conclusion**

In conclusion, the experiments presented above indicate a functional role of PALB2 for cancer cell migration and EMT. Increased expression levels of PALB2 were observed in PDAC tissues, and an association of high PALB2 levels with reduced survival odds was found. These findings suggest that PALB2 may serve as a diagnostic marker to better categorize the PDAC patients into subgroups of OS chances, better predicting their mortality risk and potentially to help in selecting the most suitable treatment plan. The findings may eventually aid in the management of PDAC patients in clinical practice and in the choice of individualized therapy strategies in the future.

## 7. Outlook

The results obtained in this study indicate an increased expression level of PALB2 in most PDAC tumor tissues, and an association of high expression levels of PALB2 with poor prognosis. Besides, knockdown of PALB2 inhibited the PDAC cells' migration in vitro. However, the mechanisms underlying the phenotype and the molecular effects of PALB2 in PDAC are still unclear. Based on the TCGA public database, an analysis the top 20 PALB2 patients with respect to expression levels in comparison to the respective bottom 20 PALB2 expression patients was conducted. The results indicate that several pathways, including TNF-alpha, EMT, TGF-beta, p53, NOTCH and mTORC1 are potentially involved in the phenotype resulting from an altered PALB2 expression. It is thus assumed that the epithelial to mesenchymal transition (EMT) might be a central and most important downstream mechanism of PALB2 dysregulation in PDAC. PALB2 may also act as an independent factor to regulate the metastasis of the pancreatic cancer cells. The roles of PALB2 in pancreatic cancer progression need further investigation.

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„Ich, Ouyang Ge, versichere an Eides statt durch meine eigenhändige Unterschrift, dass ich die vorgelegte Dissertation mit dem Thema: Prognostic value of PALB2 expression in pancreatic ductal adenocarcinoma ("Prognostischer Wert der Expression von PALB2 für Patienten mit Pankreaskarzinom"), selbstständig und ohne nicht offengelegte Hilfe Dritter verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel genutzt habe.

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Meine Anteile an etwaigen Publikationen zu dieser Dissertation entsprechen denen, die in der untenstehenden gemeinsamen Erklärung mit dem/der Erstbetreuer/in, angegeben sind. Für sämtliche im Rahmen der Dissertation entstandenen Publikationen wurden die Richtlinien des ICMJE (International Committee of Medical Journal Editors; [www.icmje.org](http://www.icmje.org)) zur Autorenschaft eingehalten. Ich erkläre ferner, dass mir die Satzung der Charité – Universitätsmedizin Berlin zur Sicherung Guter Wissenschaftlicher Praxis bekannt ist und ich mich zur Einhaltung dieser Satzung verpflichte.

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