## Contents

	Zusa	mmenfas	sung .					•		•			•		•			•	•				i
	Abst	ract				•••		•••		·			•		•		• •	•		•			iii
	List	of Abbre	eviation	15						•		•	•				•			•			v
1	Intr	oductio	n																				1
	1.1	Liver ar	natomy	and pl	iysiolo	og <b>y</b> .				•						 •				•	• •		1
	1.2	Hepatic	lipid 1	netabo	lism .		• • •					•	•	• •			• •						2
		1.2.1	Hepati	c lipoge	nesis					•			•		•	 •							2
		1.2.2	Hepati	c fatty	acid u	ptak	e.			•		•		• •					•	•			3
		1.2.3	Fatty a	icid oxi	dation	ι.	•••					•	•										3
	1.3	Hepatic	lipid :	netabo	lism ir	n live	er in	jur	у.			•						•					3
	1.4	Cell dea	th in ;	general	•••							•				 •		•		•			4
		1.4.1	Apopte	osis	•••	•••						•	•			 •			•				5
		1.4.2	Necrop	tosis .	• • •										•					•			8
		1.4.3	Pyropt	osis							• •	•			•		•			•		•	9
	1.5	Ferropt	osis.									•											10

		1.5.1	Ferroptosis inducers	10
		1.5.2	Morphology	11
		1.5.3	Biochemical hallmarks of ferroptosis	12
		1.5.4	Ferroptosis inhibitors	15
		1.5.5	The key players of ferroptosis	16
		1.5.6	The role of ferroptosis in liver diseases	17
2	Ain	ı of th	e study	21
3	Mat	terial		23
	3.1	Lab e	uipment and Consumables	23
	3.2	Reage	nts	27
	3.3	Kits a	nd enzymes	30
	3.4	Antib	odies	31
	3.5	FACS	Antibodies	32
	3.6	Buffer	s and Solutions	32
		3.6.1	Genotyping	32
		3.6.2	Protein isolation and Western Blot	33
		3.6.3	Immunohistochemistry staining	34
		3.6.4	Immunofluorescence staining	34
	·	3.6.5	Fluorescence activated cell sorting (FACS)	35
		3.6.6	Isolation of primary hepatocytes	36

		3.6.7	Primer-Sequences	36
4	Met	hods		39
	4.1	Anima	ıls	39
		4.1.1	Chronic mouse models	40
		4.1.2	Acute mouse models	42
	4.2	Cell ci	ulture	43
		4.2.1	Hepa1-6 cells	43
		4.2.2	Isolation of primary mouse hepatocytes	43
		4.2.3	Treatment of primary mouse hepatocytes	44
		4.2.4	Determination of the cell number	44
	4.3	Mice	dissection	44
	4.4	Serun	1 Analysis	45
	4.5	Fluor	escence-activated cell sorting (FACS)	45
	4.6	Geno	typing of genetically modified mice	46
		4.6.1	DNA isolation	46
		4.6.2	Genotype PCRs	47
		4.6.3	Gel electrophoresis	49
	4.7	RNA	Isolation and qRT-PCR	49
		4.7.1	RNA Isolation of liver tissue	49
		4.7.2	RNA concentration determination	50
		4.7.3	Complementary DNA (cDNA) synthesis	50

		4.7.4	Quantitative Real-time PCR (qRT-PCR)	51
	4.8	Protei	n Isolation and Western Blot	51
		4.8.1	Isolation of total protein from liver samples	51
		4.8.2	Protein concentration determination	52
		4.8.3	SDS Polyacrylamide gel electrophoresis (SDS PAGE)	52
		4.8.4	Western Blot	53
	4.9	Lipid	Peroxidation (MDA) Assay	54
	4.10	Lipid	Peroxidation (BODIPY 665/676)	54
	4.11	Histol	Dgy	54
		4.11.1	Immunohistochemistry	54
		4.11.2	Immunofluorescencent stainings of cryosections	56
	4.12	Statist	ical analysis	58
	<b>4.13</b>	Softwa	ле	58
5	Res	ults		59
Ū	1000			
	5.1	In vitr	v experiments	59
		5.1.1	Hepa1-6 cell line experiments	59
		5.1.2	Primary mouse hepatocytes	60
	5.2	The ro	le of ACSL4 in chronic liver injury	63
		5.2.1	Development of NASH	63
		5.2.2	Liver tumorigenesis using a NASH-HCC model	64

		5.2.3	Liver tumorigenesis using a fibrosis- and inflammation-associated	. 78	2							
			HCC model	. 70	,							
	5.3	3 The role of ferroptosis in acute liver failure										
		5.3.1	Drug-induced liver injury	. 85	,							
		5.3.2	Necrosis is reduced in GPX4 <sup><math>\Delta</math>hepa</sup> mice $\dots \dots \dots \dots \dots$	. 86	;							
		5.3.3	Cell death is increased in GPX4 <sup><math>\Delta</math>hepa</sup> mice	. 91								
6	Dise	cussior	a	93	;							
	6.1	Prima	ary mouse hepatocytes are susceptible towards ferroptosis induction	. 93	3							
	6.2	ACSL	4 and its role for NASH development	. 94	ł							
	6.3	ACSL	4-dependent ferroptosis suppresses tumor formation	. 95	ò							
	6.4		ole of ACSL4-dependent pathways for tumor progression in a NASH- model	. 97	7							
	6.5	Dimin	nished fibrosis upon $ACSL4$ deletion alters tumor progression	. 98	3							
	6.6	GPX4	4-dependent ferroptosis aggravates acute liver injury in DILI model	. 100	)							
7	Cor	ıclusio	n	103	3							
B	iblioį	graphy	,	v	r							
$\mathbf{L}_{\mathbf{i}}$	ist of	Figur	res	xxi	i							
$\mathbf{L}_{\mathbf{i}}$	ist of	f Table	35	xxv	i							
	Ack	nowled	gements	. xxi	x							
	Eide	esstaatl	liche Erklärung	. xxx	xi							

Publicati	ons	• •	 •	• •			•		 •			•	 •		•		 •	. >	cxxii
Scie	tific papers .	• •					•			•				•	•	•		. х	xxii
Post	er presentatio	ns		• •		•	•	 •	 •					•				. х	xxii
Oral	presentations			• •							•	• •						. x	xxii