Table of content

Table of co	I	
List of abb		
List of figur	VIII	
List of table	es	IX
1 Introdu	iction	1
1.1 P	1	
1.2 T	he F-box Protein O-type 7	3
1.3 S	tructure and functions of the FBXO7 protein	3
1.4 S	CF-E3 ubiquitin ligase complex	5
1.5 T	he Ubiquitin Proteasome System (UPS)	6
1.6 P	arkinsonian-Pyramidal Syndrome (PARK15)	7
1.7 N	ovel interaction partners of FBXO7	7
1.7.1	Proteasomal subunit α 2 (PSMA2)	7
1.7.2	The BAG6-complex	8
1.8 TI	ne CRISPR/Cas9 System	9
2 Aim of	study	12
3 Materia	al and methods	13
3.1 M	aterials	13
3.1.1	Laboratory equipment	13
3.1.2	Antibodies	14
3.1.3	Buffers and solutions	14
3.1.4	Plasmid constructs	16
3.2 M	ethods	17
3.2.1	Cell Culture	17
3.2.2	Culturing HEK293T cells	17
3.2.3	Transfection of HEK293T cells	17
3.2.4	Lysis of HEK293T cells	17
3.2.5	Measurement of protein concentration	18
3.2.6	Co-Immunoprecipitation	18
3.2.7	Cell-based ubiquitination assay	19
3.2.8	Gelelctrophoresis and Western Blot	19
3.2.9	Proteasome activity assay	20

Table of content	- 11

3.2.10		0	Transformation of bacteria with pCMVmyc plasmids (ampicillin resistance)	21	
	3.2.1	1	Primer design	21	
	3.2.1	2	PCR and molecular cloning	22	
4	Resu	ilts		25	
4	.1	Ider	ntification of potential new interaction partners of FBXO7	25	
4	.2	Sett	ting up proof-of-principal experiment for experimental series	25	
4			nere a novel FBXO7 substrate among the subunits of the BAG6 nplex?	26	
4	.4	Ger	nerating FBXO7 knockout using the CRISPR/Cas9 System	32	
4	.5	Tar	geting exon 2 may affect FBXO7 protein levels	36	
4	.6	Mut	tations in the FBXO7 protein seem to affect proteasome activity	36	
5	Disc	ussi	on	39	
5	5.1	ídei	ntification of a novel substrate targeted by FBXO7	39	
	5.1.1	l	Proof-of-principal experiment to verify feasibility of the experiments	39	
5.1.2		2	GET4 is the only subunit of the BAG6 complex acting as a potential substrate in the presence of FBXO7	40	
	5.1.3	3	CRISPR/Cas9 induced gene editing affects FBXO7 expression	41	
	5.1.4	1	CRISPR/Cas9 induced gene editing affects FBXO7 functionality	42	
6	Con	nclusion & Outlook			
7	Refe	References			
Acknowledgement					
Erklärung § 5 Abs. 1 zur Datenaufbewahrung					
Eidesstattliche Erklärung					