



UNIVERSITÀ DEGLI STUDI DI MILANO
DIPARTIMENTO DI SCIENZE
FARMACOLOGICHE E BIOMOLECOLARI

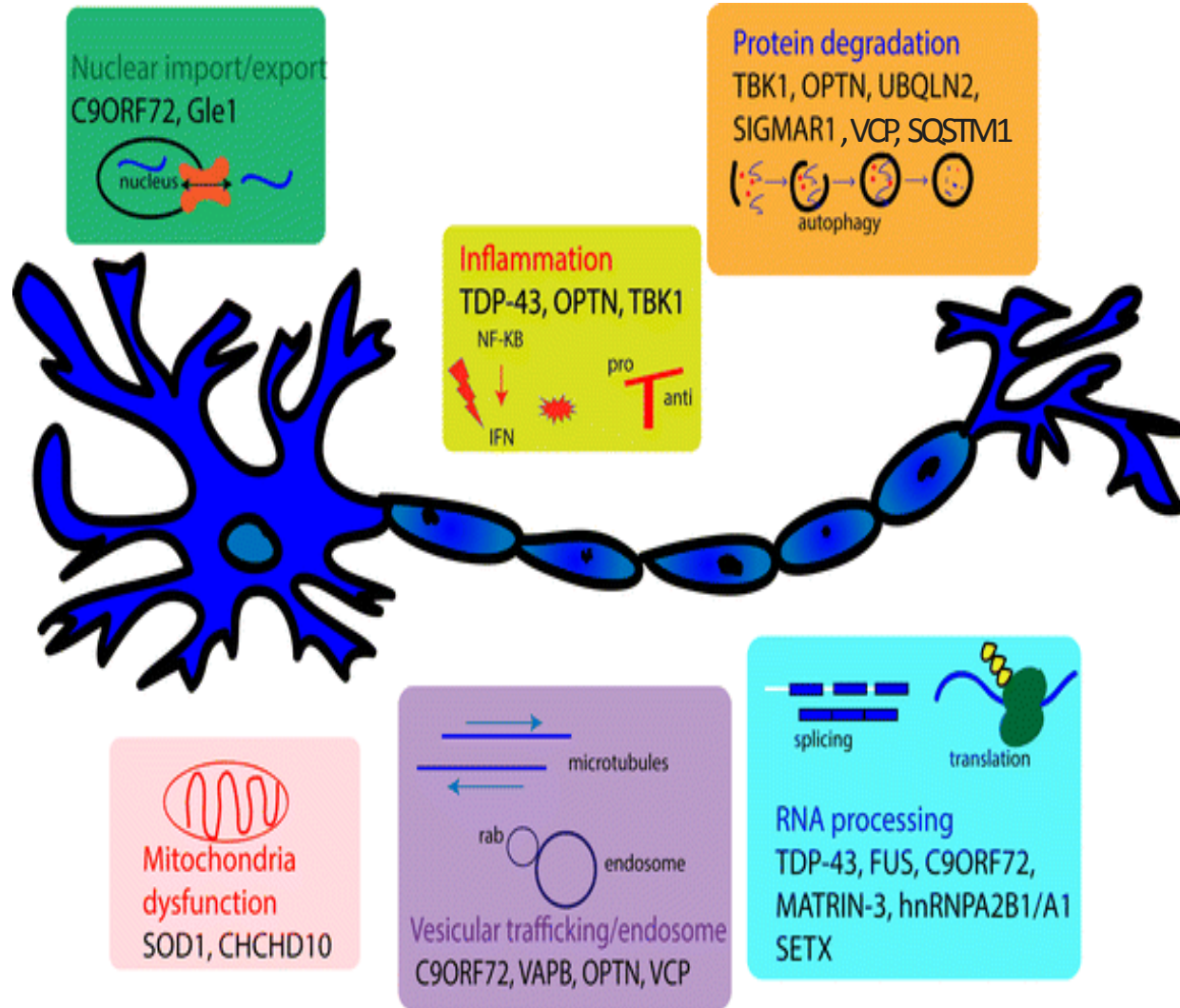
THE ROLE OF VALOSIN CONTAINING PROTEIN (VCP) IN THE CLEARANCE OF TOXIC MISFOLDED PROTEIN AGGREGATES IN AMYOTROPHIC LATERAL SCLEROSIS



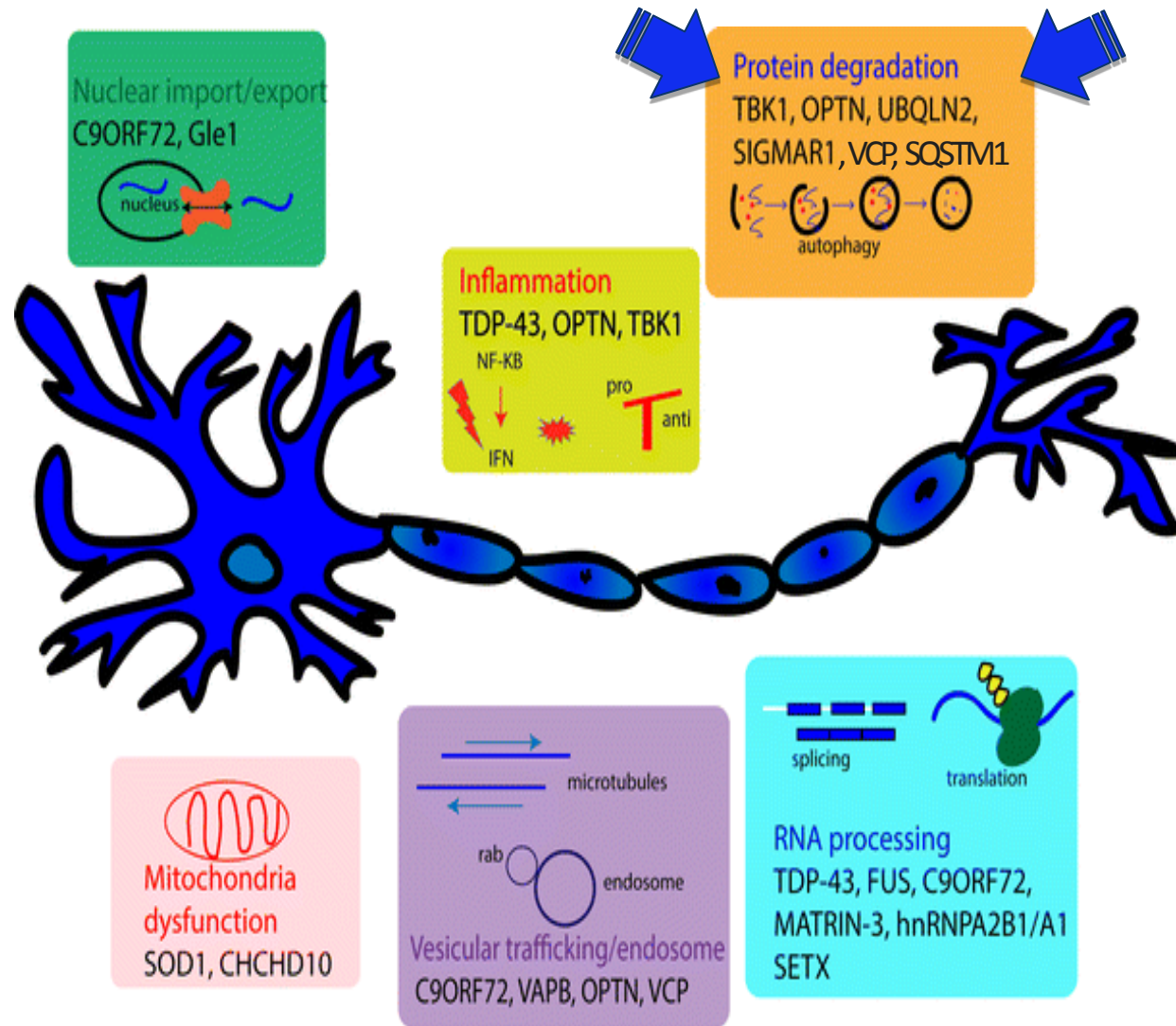
Ljubljana
May 18-20th, 2017

Veronica Ferrari
PhD student

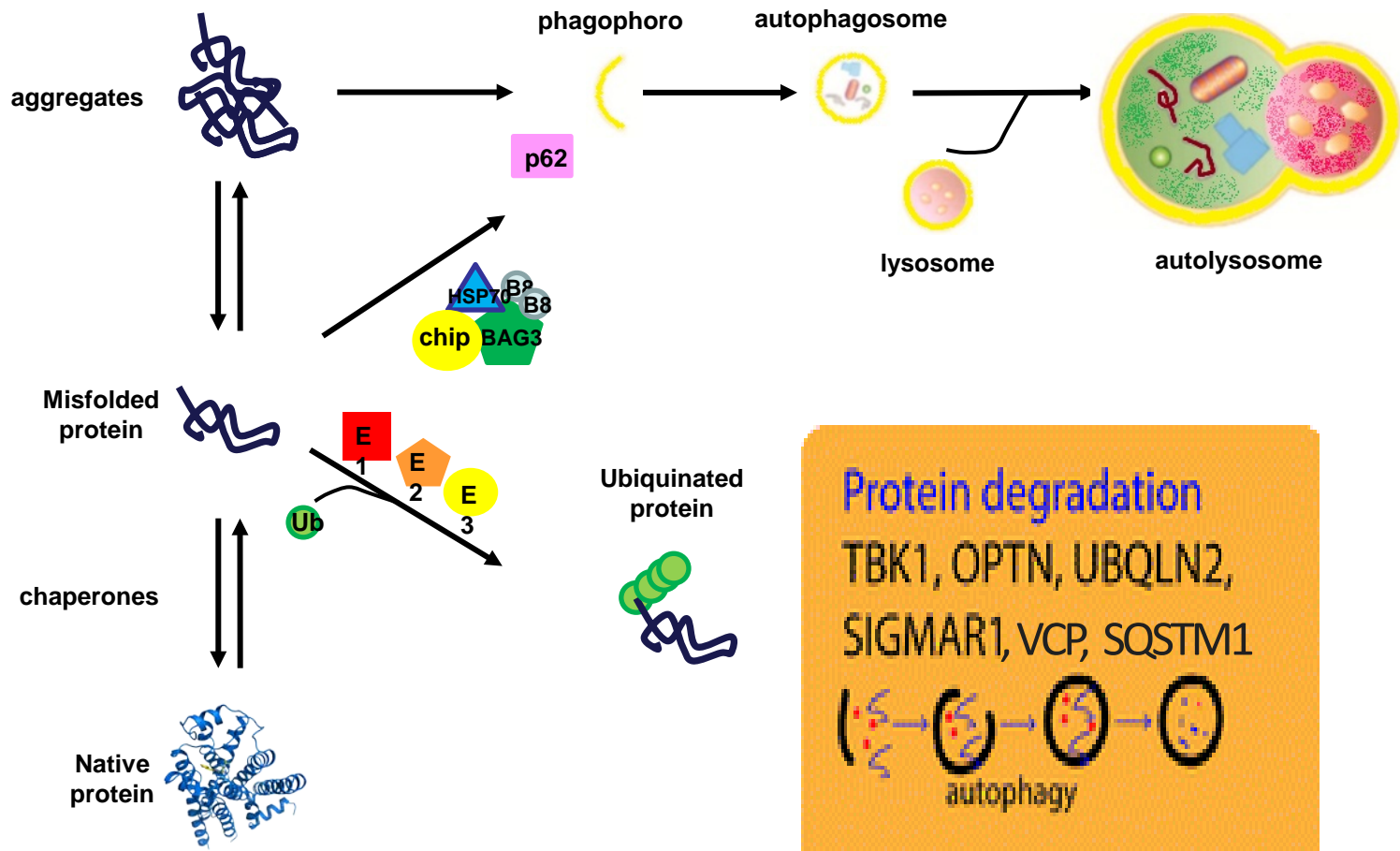
AMYOTROPHIC LATERAL SCLEROSIS



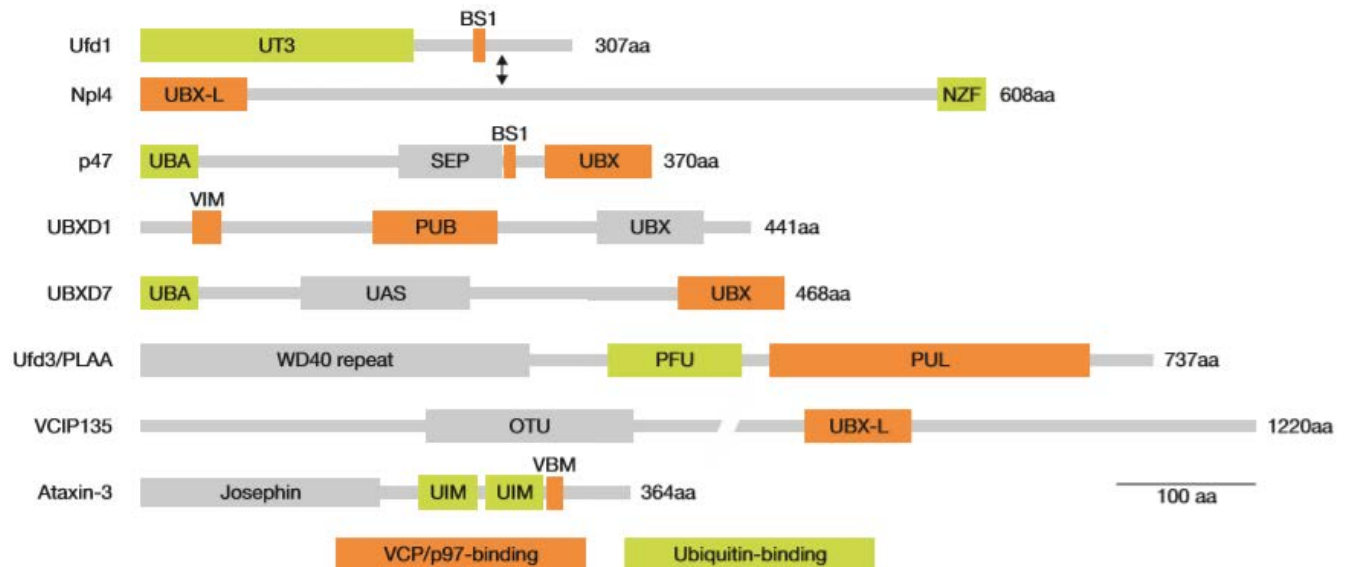
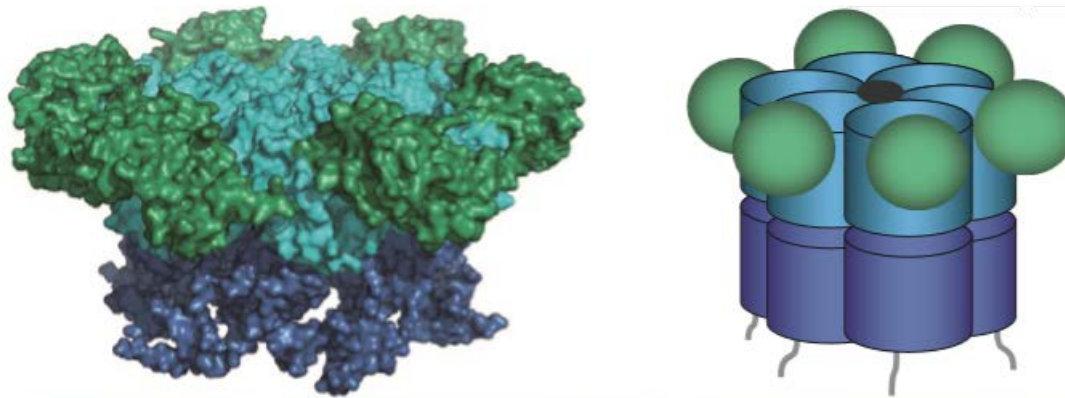
AMYOTROPHIC LATERAL SCLEROSIS



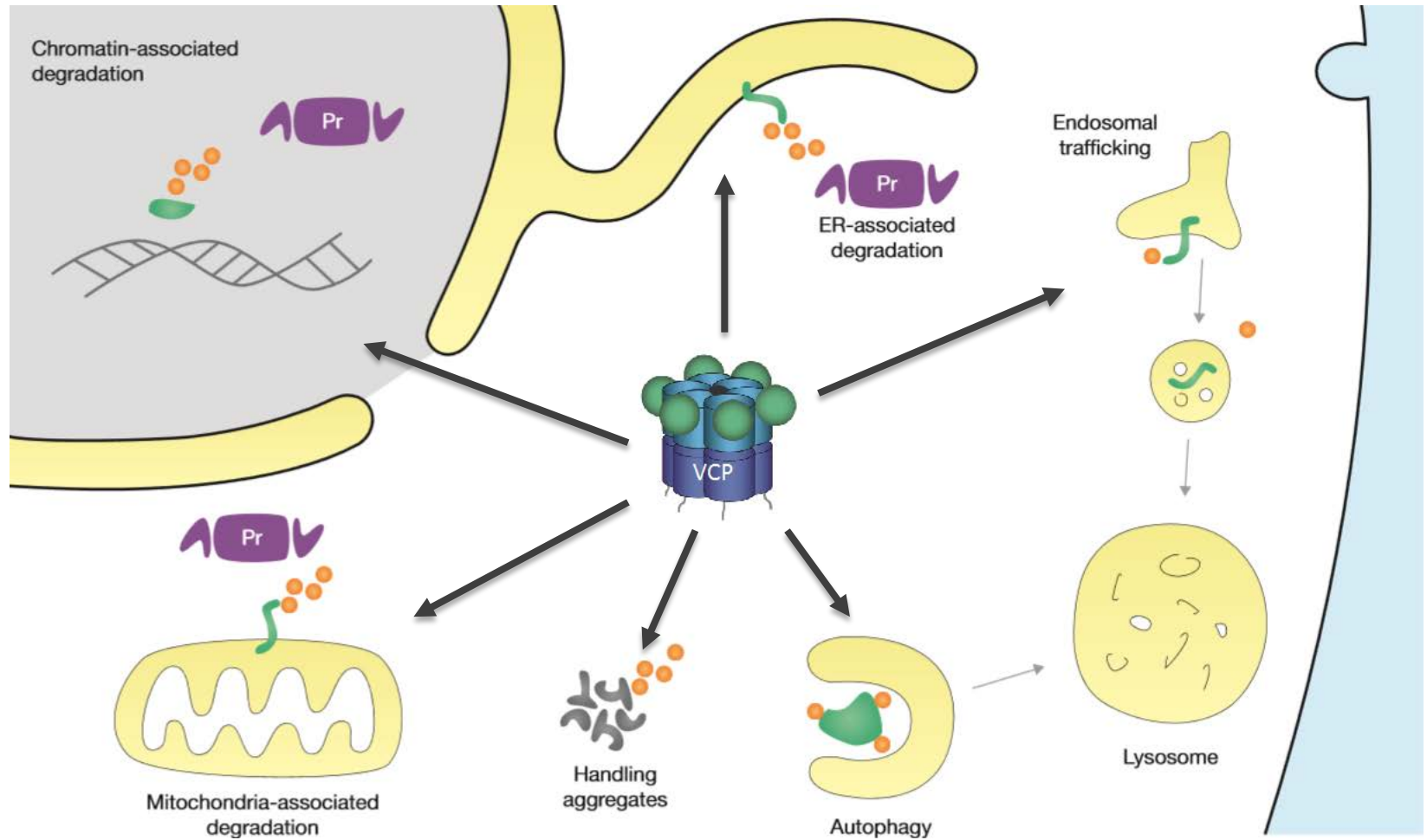
PROTEIN QUALITY CONTROL SYSTEM



VALOSIN CONTAINING PROTEIN



VALOSIN CONTAINING PROTEIN



VALOSIN CONTAINING PROTEIN

Pathogenic mutations in p97.

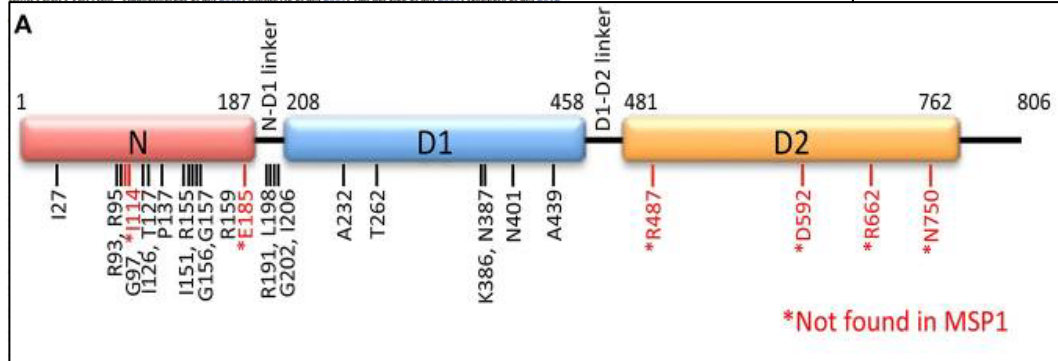
Change in amino acid	Change in gene	Location in protein	Phenotype	References
I27	I27V	79A>G	N domain	IBM, FTD, PDB Rohrer et al., 2011; Majounie et al., 2012; Wehl et al., 2015
R93	R93C	277C>T	N domain	IBM, PDB, FTD Guyant-Maréchal et al., 2006; Hübbers et al., 2007
	R93H	278G>A	N domain	HSP Neveling et al., 2013
R95	R95C	283C>T	N domain	IBM, ALS Wehl et al., 2015
	R95H	284G>A	N domain	AD Kaleem et al., 2007
	R95G	283C>G	N domain	IBM, PDB, FTD, ALS Watts et al., 2004; Kimonis et al., 2008b
G97	G97E	290G>A	N domain	IBM, PDB, FTD Gu et al., 2012; Jerath et al., 2015
I114	I114V	340A>G	N domain	ALS Koppers et al., 2012
I126	I126F	376A>T	N domain	IBM, PDB, FTD Matsubara et al., 2016
T127	T127A	379A>G	N domain	FTD, AD Shi et al., 2016
P137	P137L	410C>T	N domain	IBM, PDB, FTD Stojkovic et al., 2009; Palmio et al., 2011
I151	I151V	451A>G	N domain	IBM, ALS DeJesus-Hernandez et al., 2011; Boland-Freitas et al., 2016
R155	R155S	463C>A	N domain	IBM, PDB, FTD Stojkovic et al., 2009
	R155L	464G>T	N domain	IBM, PDB, FTD Kumar et al., 2010
	R155H	464G>A	N domain	IBM, PDB, FTD, ALS Watts et al., 2004; Hübbers et al., 2007; Kimonis et al., 2008a; Viassolo et al., 2008; Stojkovic et al., 2009; González-Pérez et al., 2012
	R155C	463C>T	N domain	IBM, PDB, FTD, ALS Watts et al., 2004; Schröder et al., 2005; Guyant-Maréchal et al., 2006; Gidaro et al., 2008; González-Pérez et al., 2012
	R155P	464G>C	N domain	IBM, PDB, FTD Watts et al., 2004
G156	G156C	466G>C	N domain	ALS Segawa et al., 2015
	G156S	466G>A	N domain	IBM, PDB, FTD Komatsu et al., 2013
G157	G157R	469G>C	N domain	IBM, PDB, FTD Djamshidian et al., 2009
	G157L	469G>A	N domain	IBM, PDB, FTD Stojkovic et al., 2009
M158	M158V	472A>G	N domain	PDB, ALS Ayaki et al., 2014
R159	R159G	475C>G	N domain	ALS, FTD Johnson et al., 2010
	R159C	475C>T	N domain	IBM, FTD, PD, ALS Bersano et al., 2009; Cham et al., 2012; de Bot et al., 2012; González-Pérez et al., 2012
	R159H	476G>A	N domain	IBM, PDB, FTD, ALS Haubenberger et al., 2005; Stojkovic et al., 2009; van der Zee et al., 2009; Koppers et al., 2012
E185	E185K	553C>T	N domain	CMT2Y Gonzalez et al., 2014
R191	R191G	571C>G	N-D1 linker	IBM, ALS González-Pérez et al., 2012
	R191Q	572G>A	N-D1 linker	IBM, PDB, FTD, ALS Watts et al., 2004; Kimonis et al., 2008b; Stojkovic et al., 2009; Johnson et al., 2010; González-Pérez et al., 2012
L198	L198W	593T>G	N-D1 linker	IBM, PDB, FTD Watts et al., 2007; Kumar et al., 2010
G202	G202W	604G>T	N-D1 linker	IBM, FTD Figuerro-Bonaparte et al., 2016
I206	I206F	616A>T	N-D1 linker	IBM, PDB, FTD Peyer et al., 2013
A232	A232E	695C>A	D1 domain	IBM, PDB Watts et al., 2004; Kimonis et al., 2008b
T262	T262A	784A>G	D1 domain	IBM, PDB, FTD Spina et al., 2008
K386	K386E	1158T>C	D1 domain	IBM Lévesque et al., 2016
N387	N387H	1159A>C	D1 domain	IBM, FTD Watts et al., 2007
	N387S	1160A>G	D1 domain	IBM, PDB, FTD Liewluck et al., 2014
	N387T	1160A>C	D1 domain	ALS Abramzon et al., 2012
N401	N401S	1202A>G	D1 domain	FTD, ALS Shi et al., 2016
A439	A439S	1315G>T	D1 domain	IBM, PDB Stojkovic et al., 2009
	A439P	1315G>C	D1 domain	IBM, PDB, FTD Shi et al., 2012; Kamiyama et al., 2013
	A439G	1316C>G	D1 domain	IBM, FTD Figuerro-Bonaparte et al., 2016
R487	R487H	1460G>A	D2 domain	FTD, ALS Hirano et al., 2015
D592	D592N	1774G>A	D2 domain	ALS Johnson et al., 2010
R662	R662C	1984C>T	D2 domain	ALS Abramzon et al., 2012
N750	N750S	2249A>G	D2 domain	ALS Kenna et al., 2013

IBM, inclusion body myopathy; PDB, Paget's disease of bone; FTD, frontotemporal dementia; PD, Parkinson disease; ALS, amyotrophic lateral sclerosis; CMT2Y, Charcot-Marie-Tooth disease.

VALOSIN CONTAINING PROTEIN

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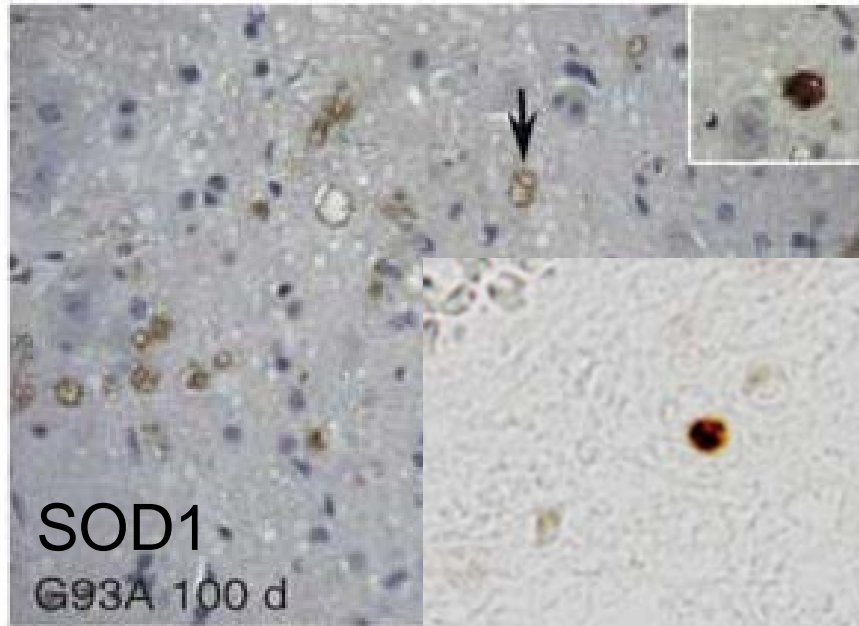
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	N387S	1160A>G	D1 domain	
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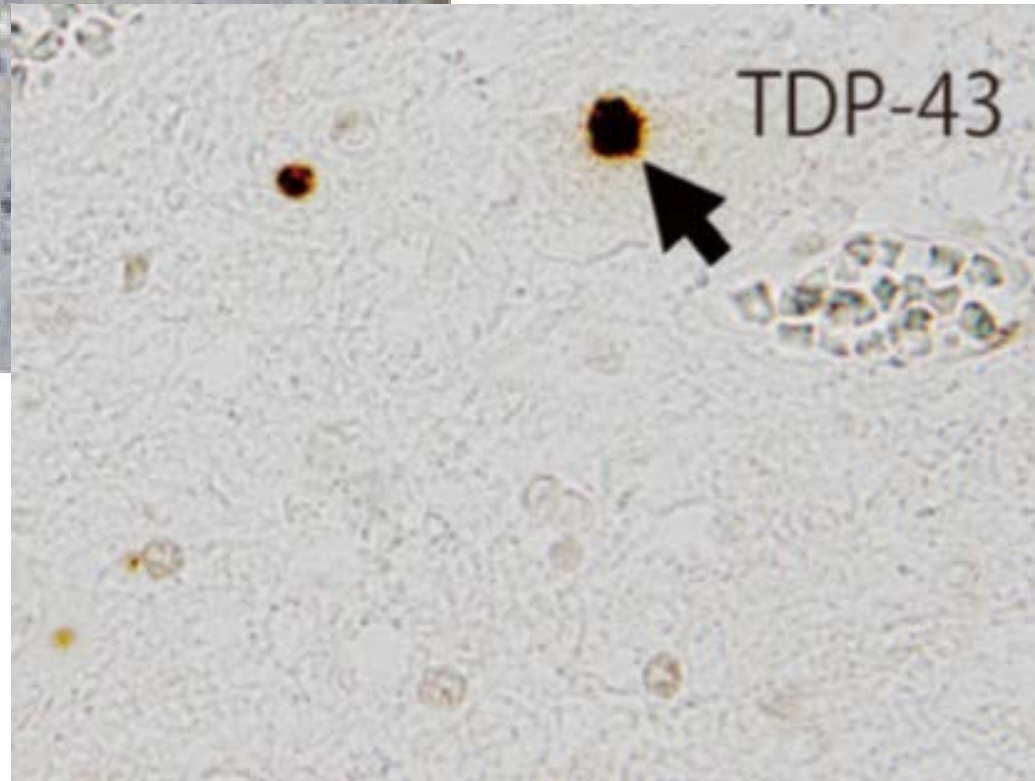
IBM, inclusion body myopathy; PDB, Paget's disease of bone; FTD, frontotemporal dementia; PD, Parkinson disease; ALS, amyotrophic lateral sclerosis; CMT2Y, Charcot-Marie-Tooth disease.



TDP-43 PATHOLOGY in ALS



Rakhit et al. 2007

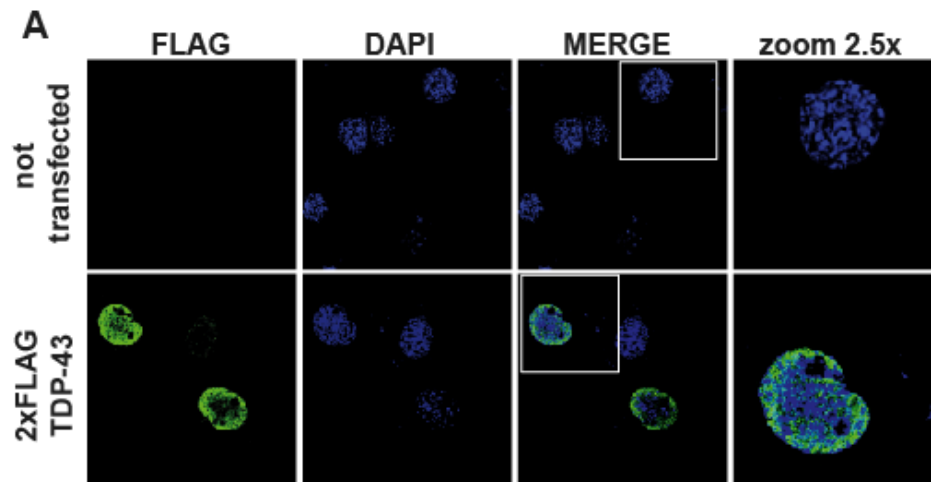


Ayaki et al. 2014

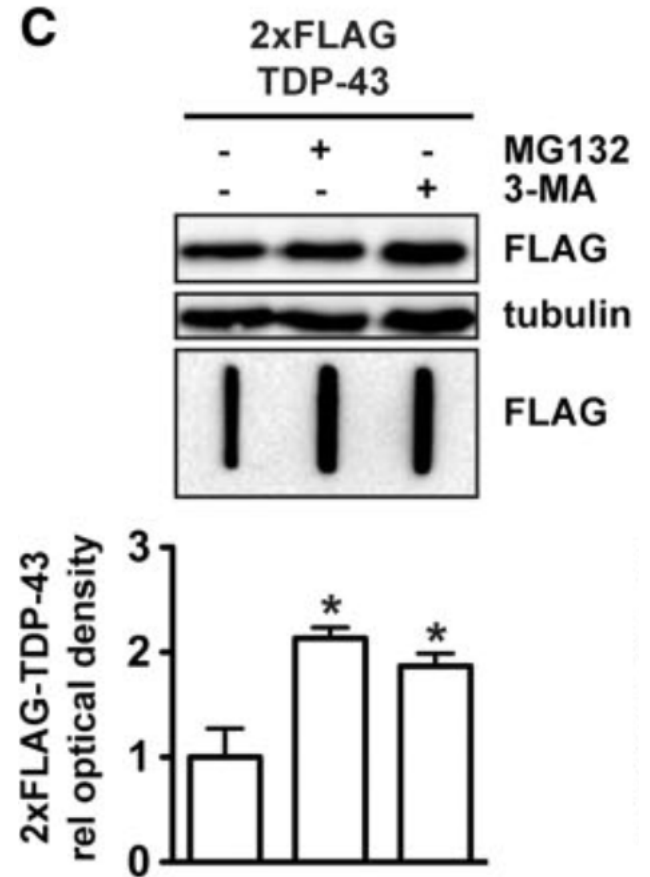
Which is VCP
role in the
clearance of
misfolded
protein
aggregates?



RESULTS

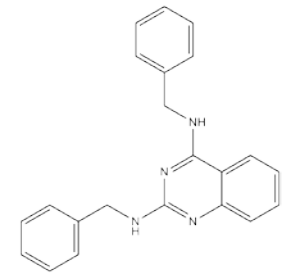
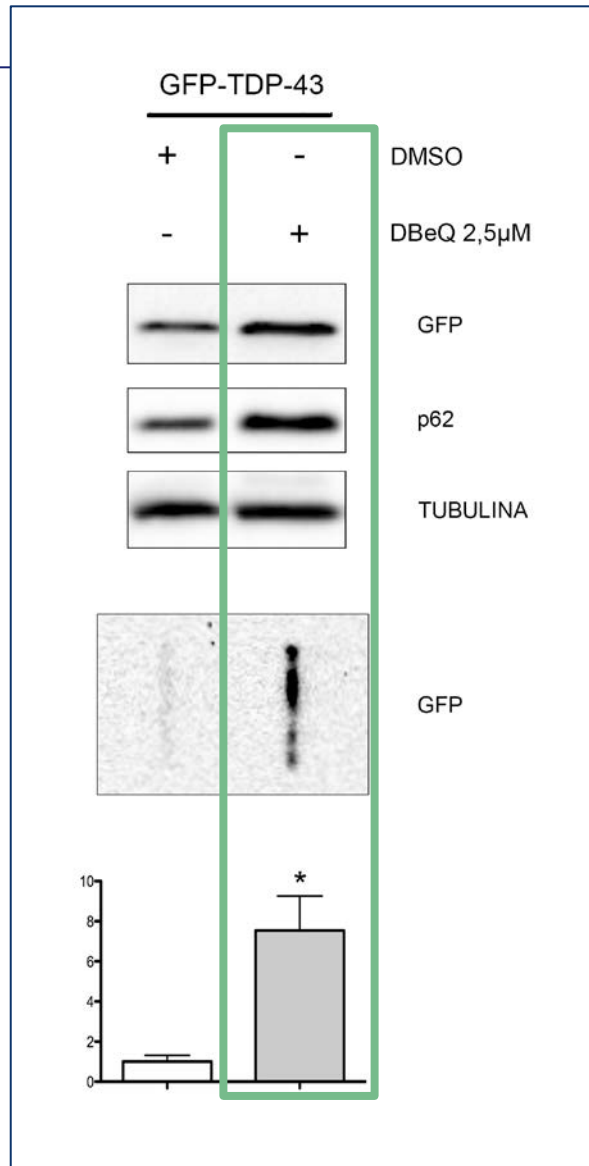


Crippa et al, 2016

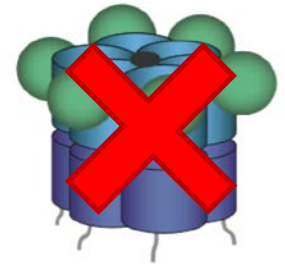


Crippa et al, 2016

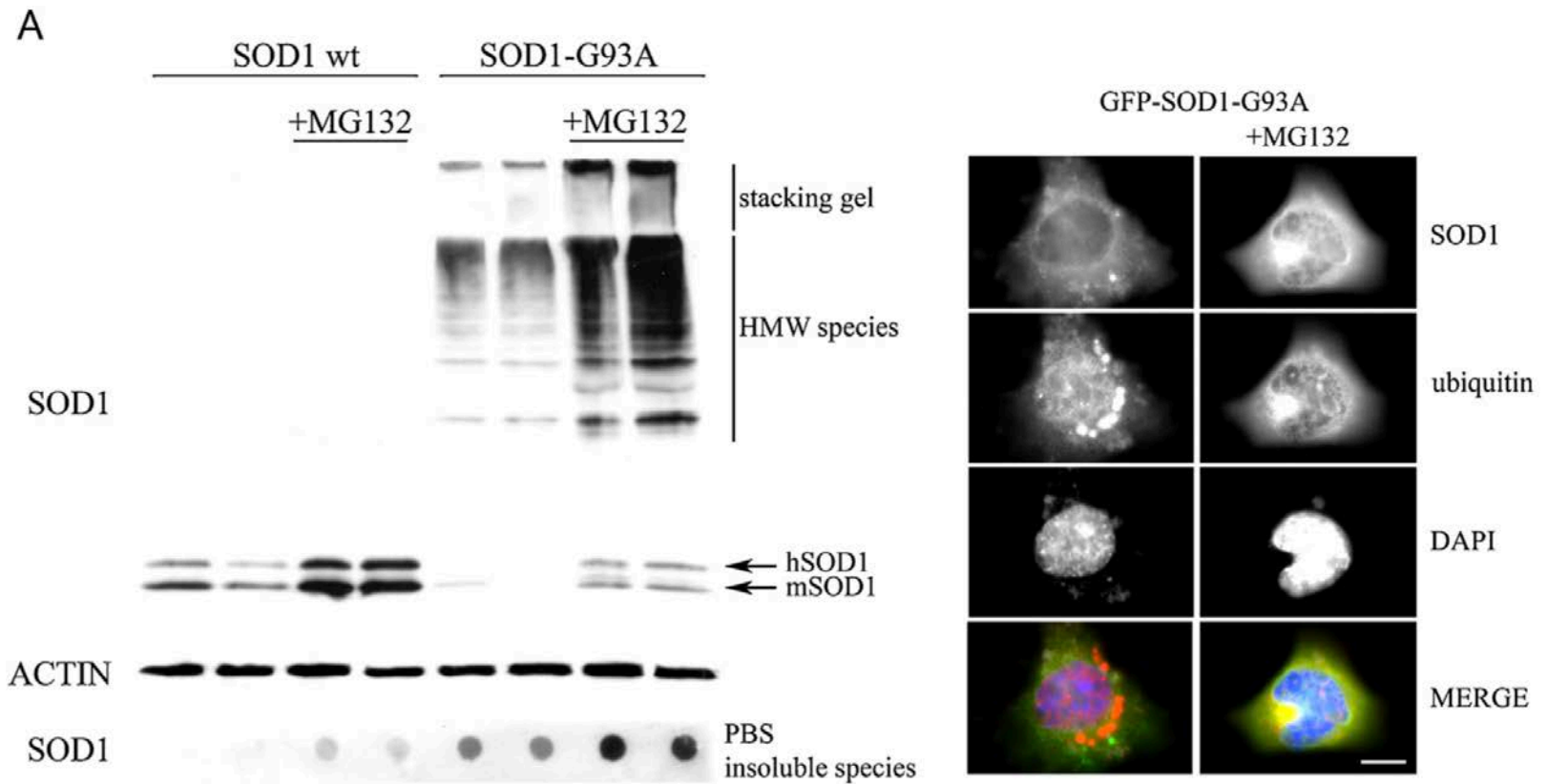
RESULTS



DBeQ

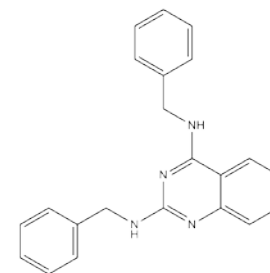
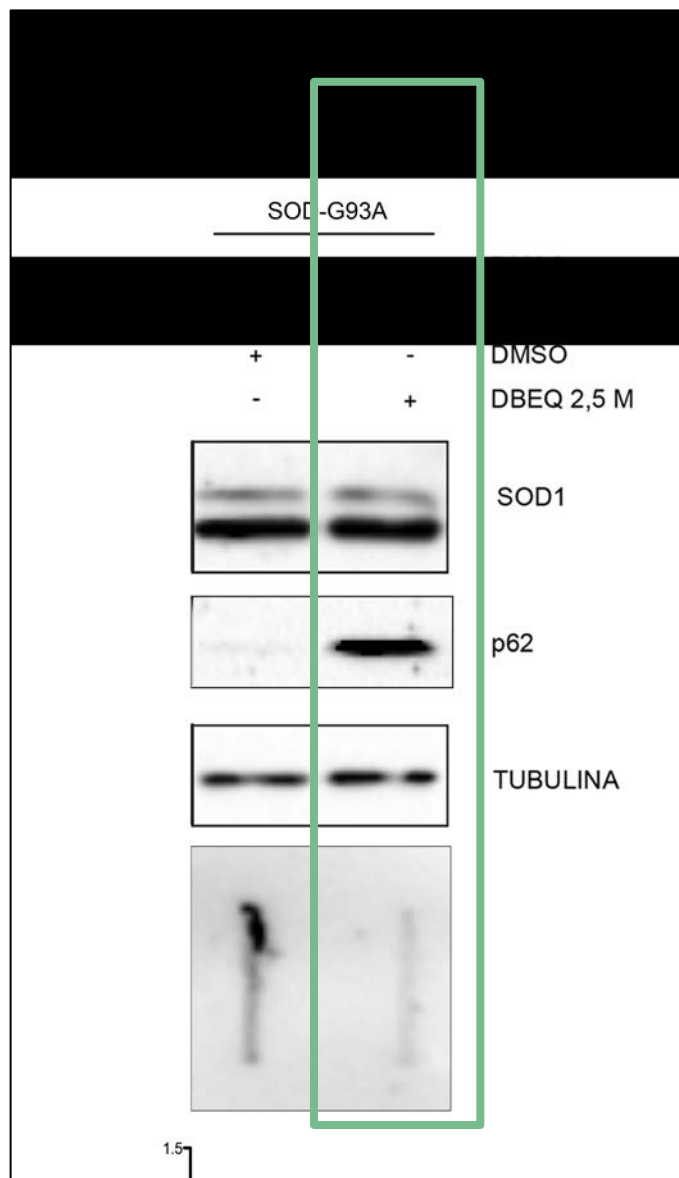


SOD1-ALS

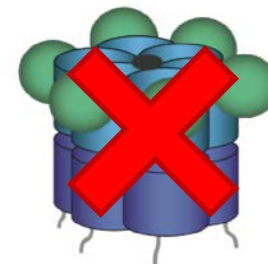


Sau *et al.*, 2007; Crippa *et al.*, 2010a; Bendotti *et al.*, 2012

RESULTS

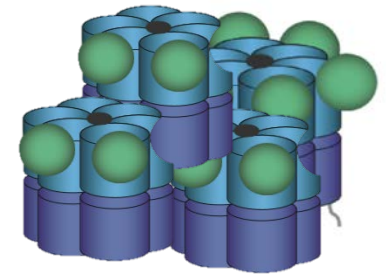
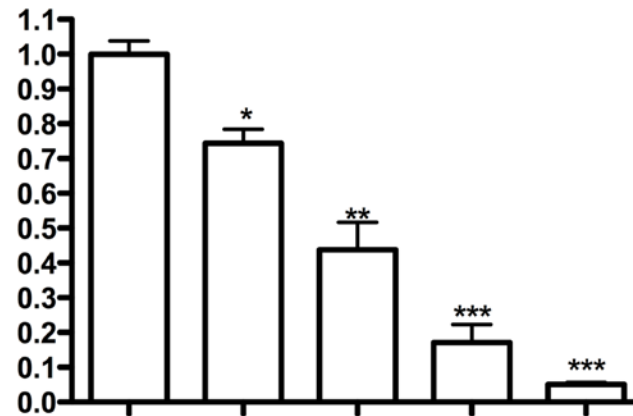
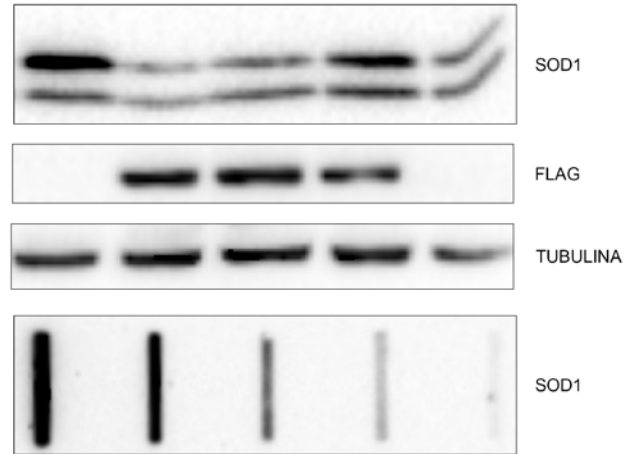


DBEQ



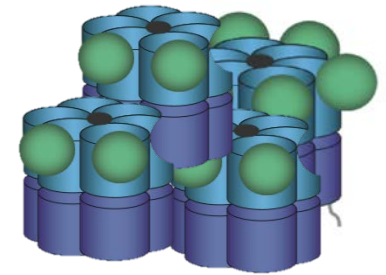
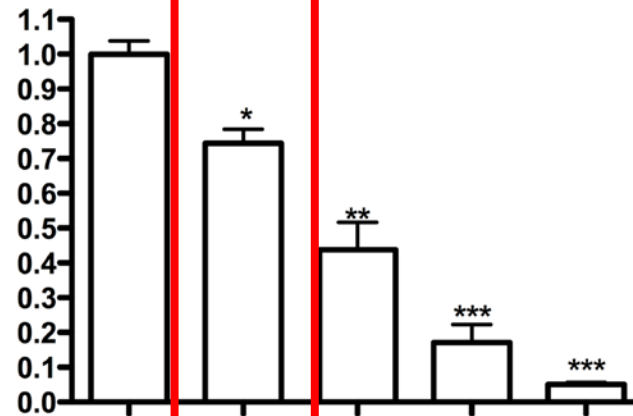
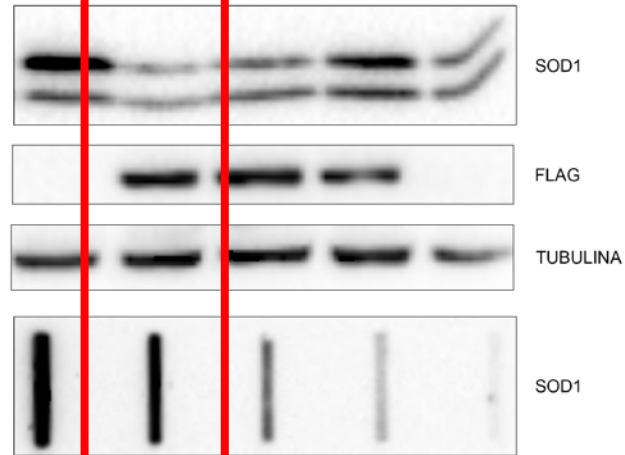
RESULTS

SOD1 G93A					
+	-	-	-	-	pcDNA3
-	+	-	-	-	FLAG-VCP WT
-	-	+	-	-	FLAG-VCP R155H
-	-	-	+	-	FLAG-VCP R191Q
-	-	-	-	+	MYC-SVIP



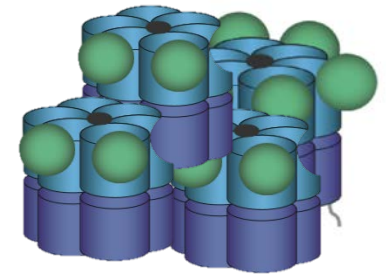
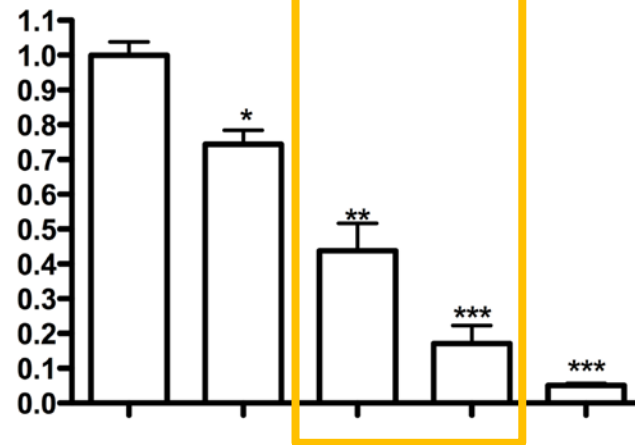
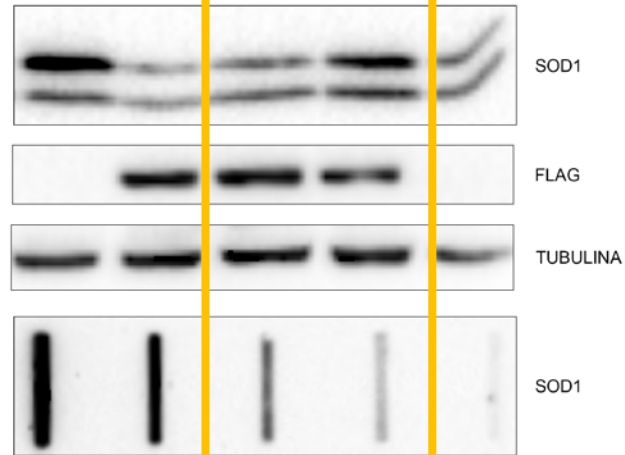
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-	-	-	-	+	MYC-SVIP

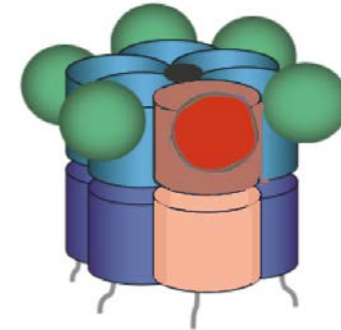
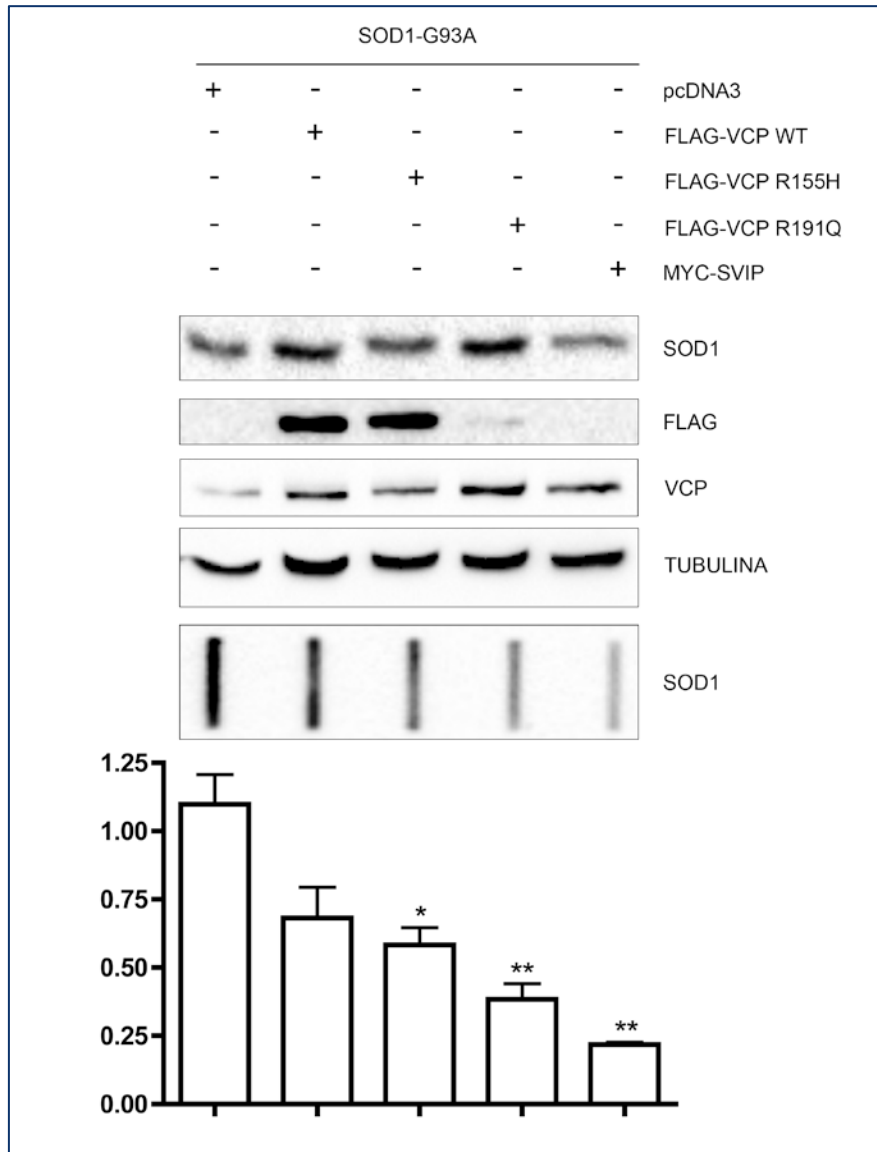


RESULTS

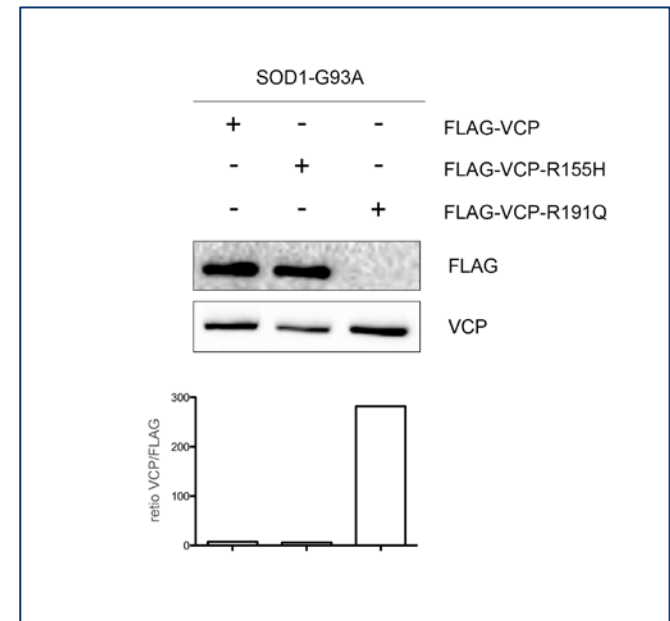
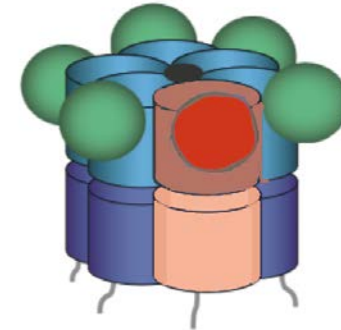
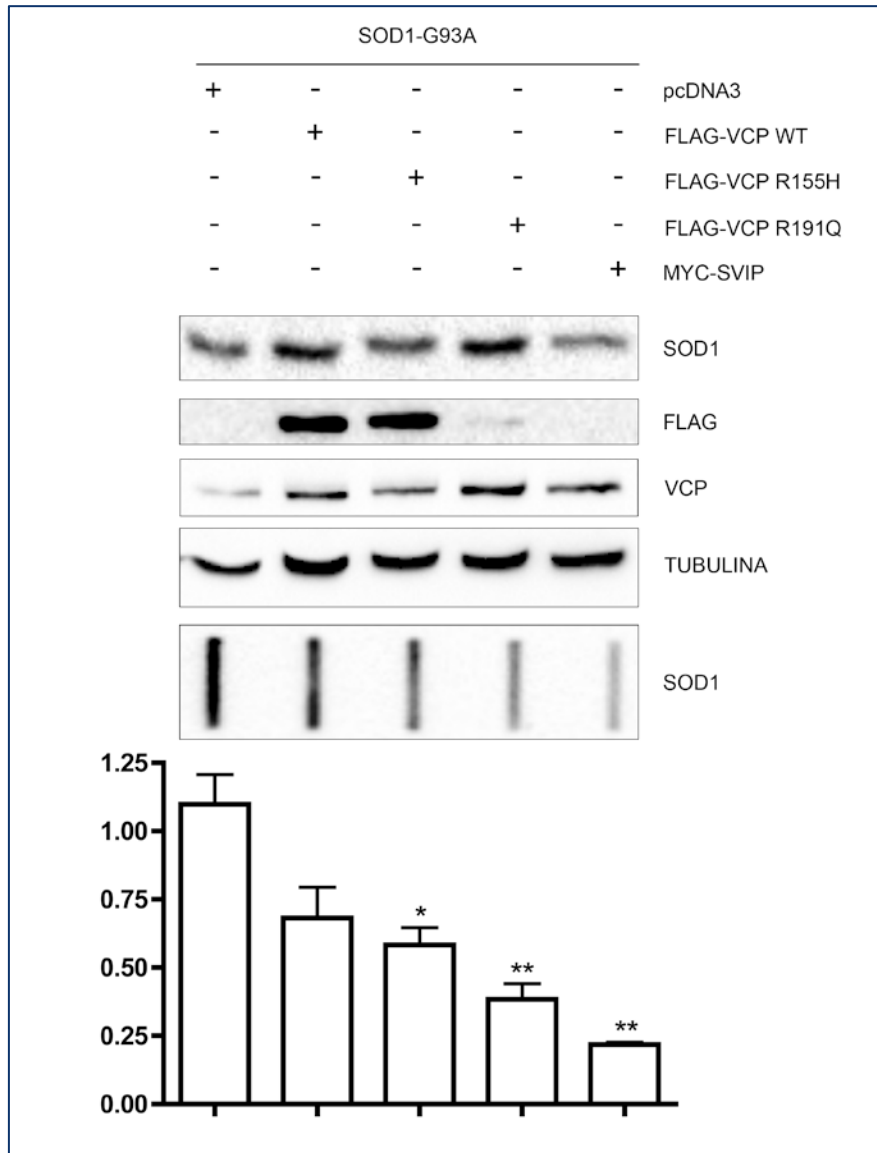
SOD1 G93A					
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-	+	-	-	-	FLAG-VCP WT
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-	-	-	+	-	FLAG-VCP R191Q
-	-	-	-	+	MYC-SVIP



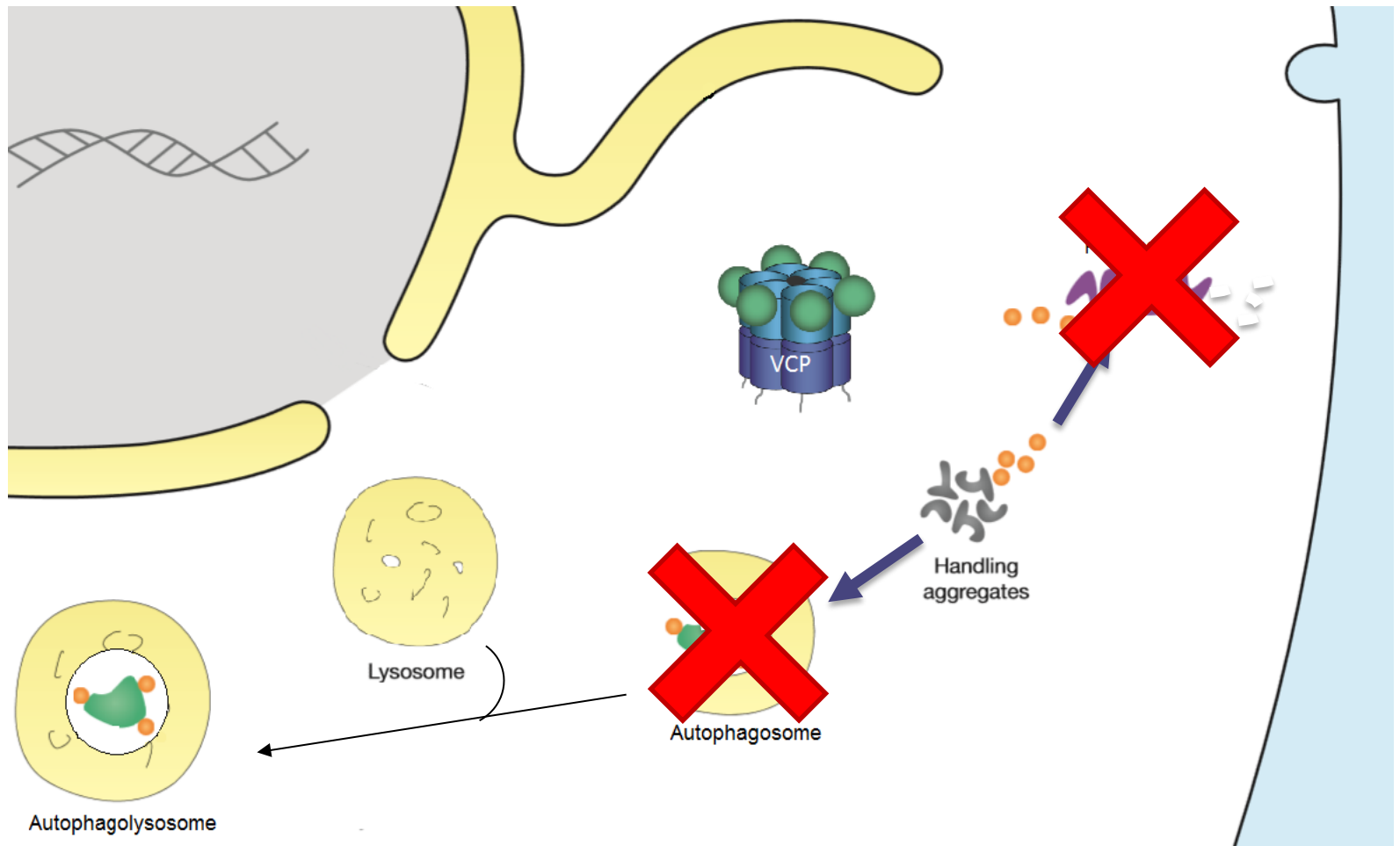
RESULTS



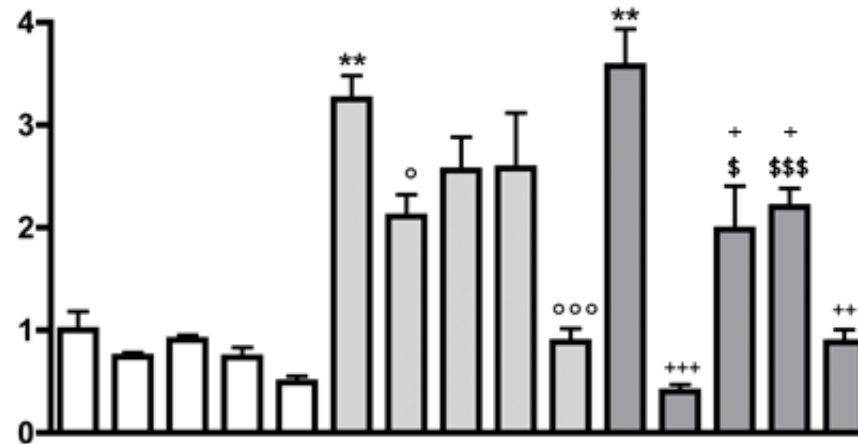
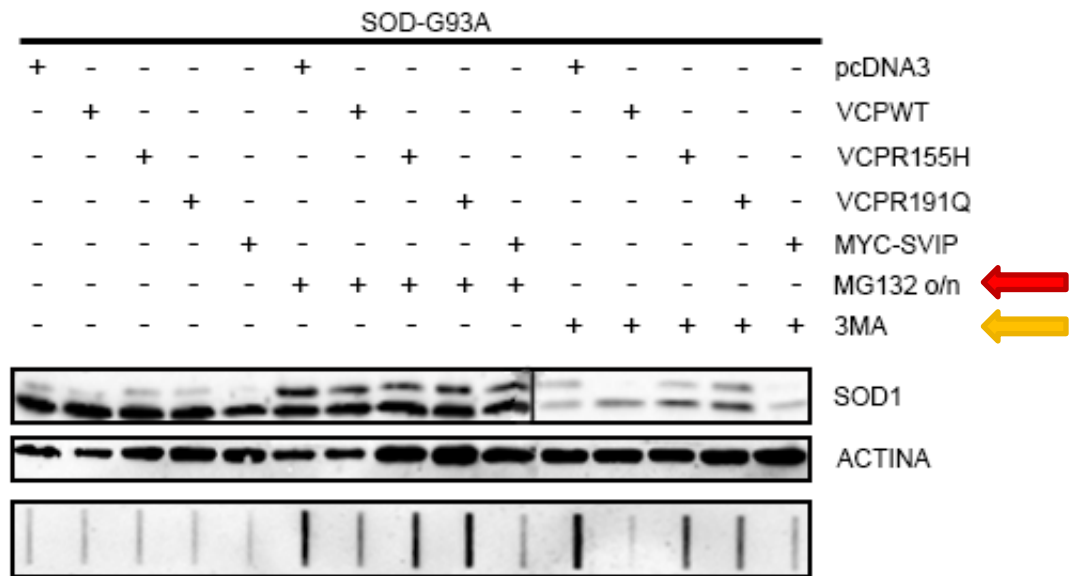
RESULTS



RESULTS



RESULTS

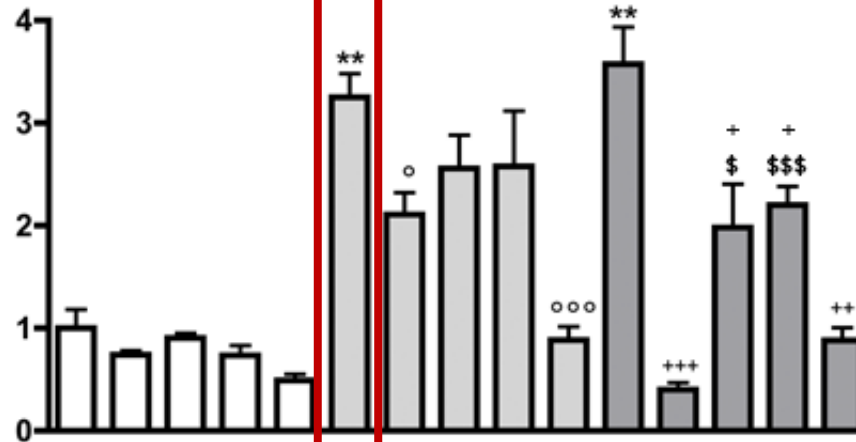
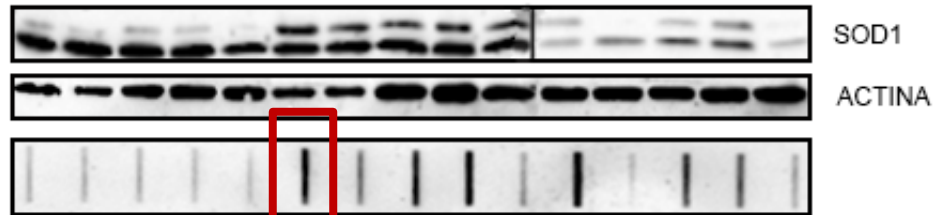


* vs pcDNA3
 ° vs pcDNA3/MG132
 + vs pcDNA3/3MA
 \$ vs VCPWT/3MA

RESULTS



SOD-G93A														
+	-	-	-	-	+	-	-	-	-	+	-	-	-	pcDNA3
-	+	-	-	-	-	+	-	-	-	-	+	-	-	VCPWT
-	-	+	-	-	-	-	+	-	-	-	-	+	-	VCPR155H
-	-	-	+	-	-	-	-	+	-	-	-	-	+	VCPR191Q
-	-	-	-	+	-	-	-	-	+	-	-	-	-	MYC-SVIP
-	-	-	-	-	+	+	+	+	+	-	-	-	-	MG132 o/n
-	-	-	-	-	-	-	-	-	-	+	+	+	+	3MA

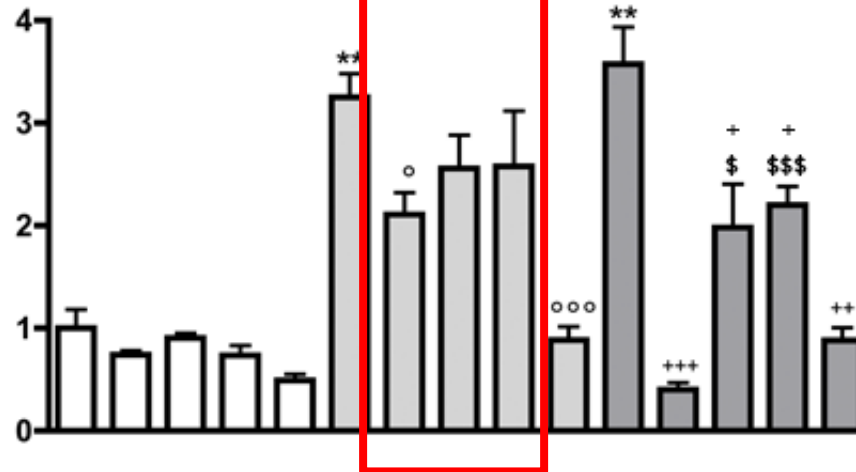
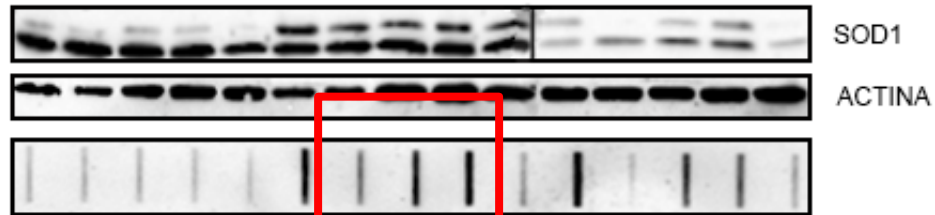


* vs pcDNA3
 ° vs pcDNA3/MG132
 + vs pcDNA3/3MA
 \$ vs VCPWT/3MA

RESULTS



SOD-G93A														
+	-	-	-	-	+	-	-	-	-	+	-	-	-	pcDNA3
-	+	-	-	-	-	+	-	-	-	-	+	-	-	VCPWT
-	-	+	-	-	-	-	+	-	-	-	-	+	-	VCPR155H
-	-	-	+	-	-	-	-	+	-	-	-	-	+	VCPR191Q
-	-	-	-	+	-	-	-	-	+	-	-	-	-	MYC-SVIP
-	-	-	-	-	+	+	+	+	+	-	-	-	-	MG132 o/n
-	-	-	-	-	-	-	-	-	-	+	+	+	+	3MA



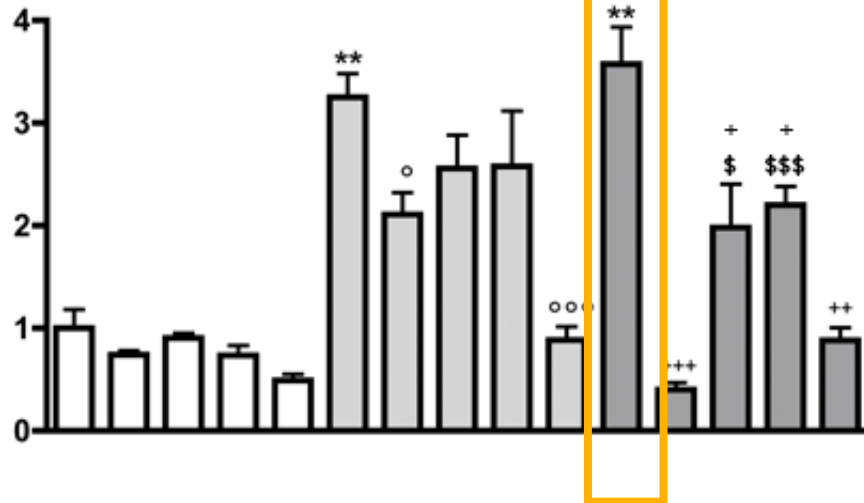
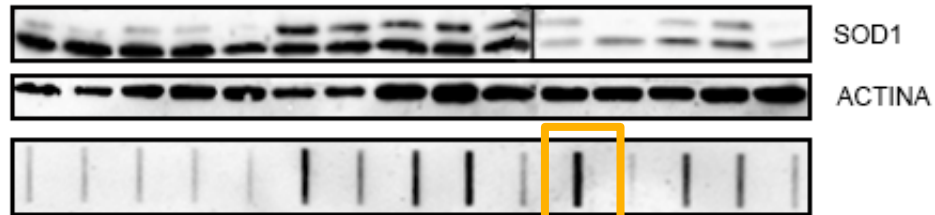
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RESULTS



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-	+	-	-	-	-	+	-	-	-	-	+	-	-	VCPWT
-	-	+	-	-	-	-	+	-	-	-	+	-	-	VCPR155H
-	-	-	+	-	-	-	-	+	-	-	-	+	-	VCPR191Q
-	-	-	-	+	-	-	-	-	+	-	-	-	+	MYC-SVIP
-	-	-	-	-	+	+	+	+	+	-	-	-	-	MG132 o/n
-	-	-	-	-	-	-	-	-	-	+	+	+	+	3MA

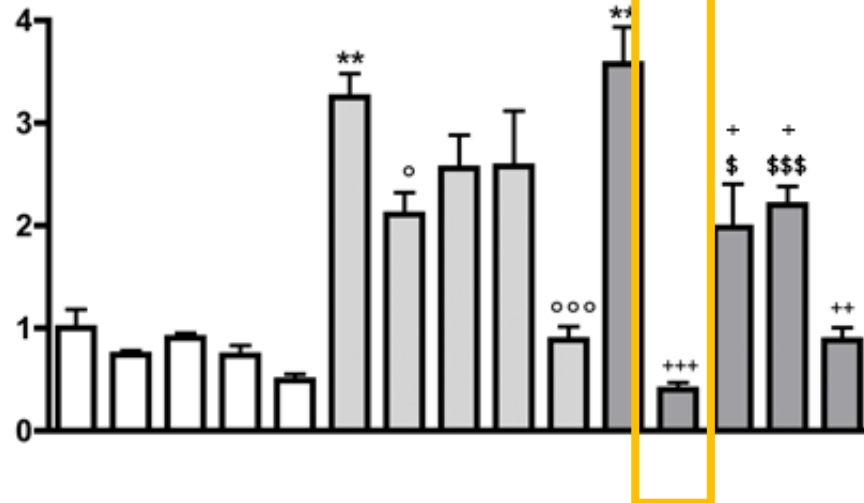
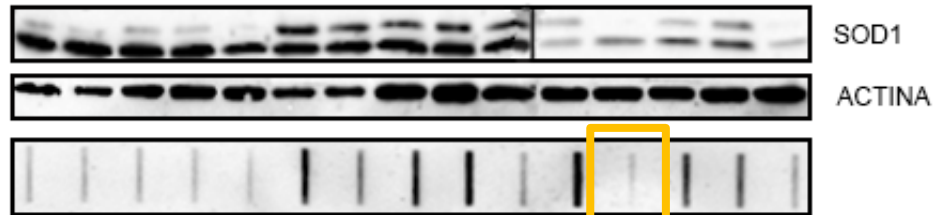


* vs pcDNA3
 ° vs pcDNA3/MG132
 + vs pcDNA3/3MA
 \$ vs VCPWT/3MA

RESULTS



SOD-G93A														
+	-	-	-	-	+	-	-	-	-	+	-	-	-	pcDNA3
-	+	-	-	-	-	+	-	-	-	-	+	-	-	VCPWT
-	-	+	-	-	-	-	+	-	-	-	-	+	-	VCPR155H
-	-	-	+	-	-	-	-	+	-	-	-	-	+	VCPR191Q
-	-	-	-	+	-	-	-	-	+	-	-	-	-	MYC-SVIP
-	-	-	-	-	+	+	+	+	+	-	-	-	-	MG132 o/n
-	-	-	-	-	-	-	-	-	-	+	+	+	+	3MA

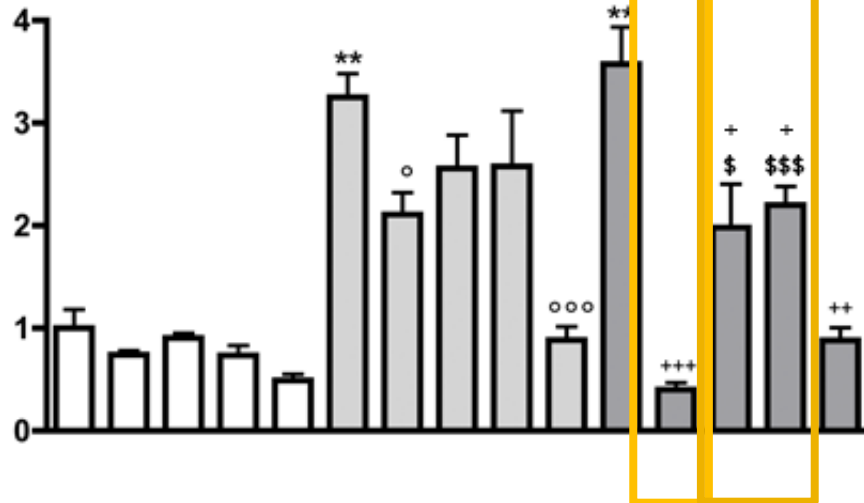
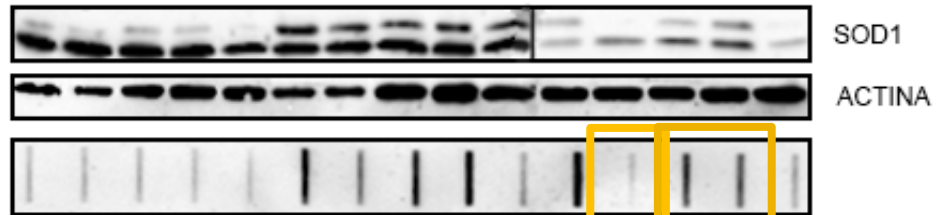


* vs pcDNA3
 ° vs pcDNA3/MG132
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RESULTS



SOD-G93A														
+	-	-	-	-	+	-	-	-	-	+	-	-	-	pcDNA3
-	+	-	-	-	-	+	-	-	-	-	+	-	-	VCPWT
-	-	+	-	-	-	-	+	-	-	-	-	+	-	VCPR155H
-	-	-	+	-	-	-	-	+	-	-	-	-	+	VCPR191Q
-	-	-	-	+	-	-	-	-	+	-	-	-	-	MYC-SVIP
-	-	-	-	-	+	+	+	+	+	-	-	-	-	MG132 o/n
-	-	-	-	-	-	-	-	-	-	+	+	+	+	3MA



* vs pcDNA3
 ° vs pcDNA3/MG132
 + vs pcDNA3/3MA
 \$ vs VCPWT/3MA



CONCLUSION

- VCP overexpression significantly decrease the levels of SOD1-G93A aggregates
- MG132 treatment partially alter the role of VCP
- 3MA does not influence VCP contribute in removing SOD1-G93A aggregates
- DBeQ treatment does not affect the levels of mutated SOD1 aggregates



NEXT STEPS

- Study SOD1-G93A aggregates in condition of depletion of VCP
- Study which are the adaptors and cofactors the cooperate with VCP
- Chemical components that stimulate this pathway



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**THANK YOU
FOR YOUR ATTENTION**

