IRCCS E. Medea, Bosisio Parini (LC)
14 November 2014

Distrofia dei cingoli da deficit di Calpaina 3

Dal gene al paziente

Novel pharmacological approaches to treat protein-misfolding diseases

Dorianna Sandonà

summary of the talk

- brief introduction to protein misfolding diseases
- the pathogenic mechanism of sarcoglycanopathy
- proposed therapeutic intervention for the treatment of sarcoglycanopathy
- results
- few words on a different genetic disorder: Brody's disease
- future work and conclusions

protein-misfolding diseases

genetic disorders in which gene mutations result in folding defective proteins

Quality Control System (QCS)

aggregates
dominant negative mutants

loss of the altered proteins
mis-localization

toxic gain of function

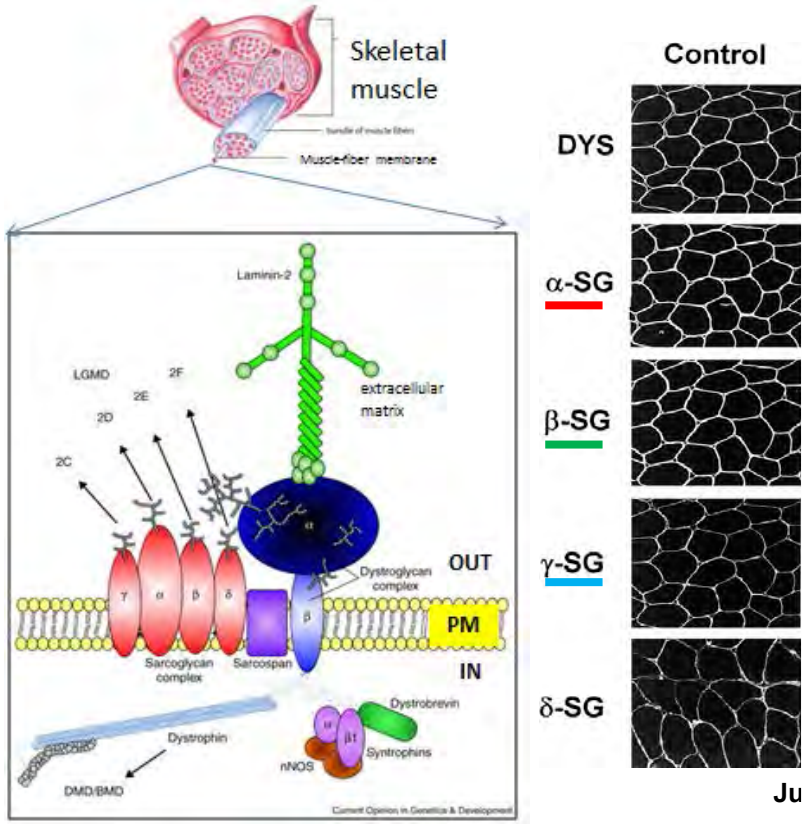
loss of function

Cytoplasm and Endoplasmic Reticulum

Ubiquitin-proteasome system, ERAD and autophagy

Sarcoglycanopathy (LGMD-2C-F):

- Mutations in any sarcoglycan gene leads to a severe reduction/loss of mutated as well as wild type sarcoglycans from DAPC
- Alteration of the structural properties of DAPC



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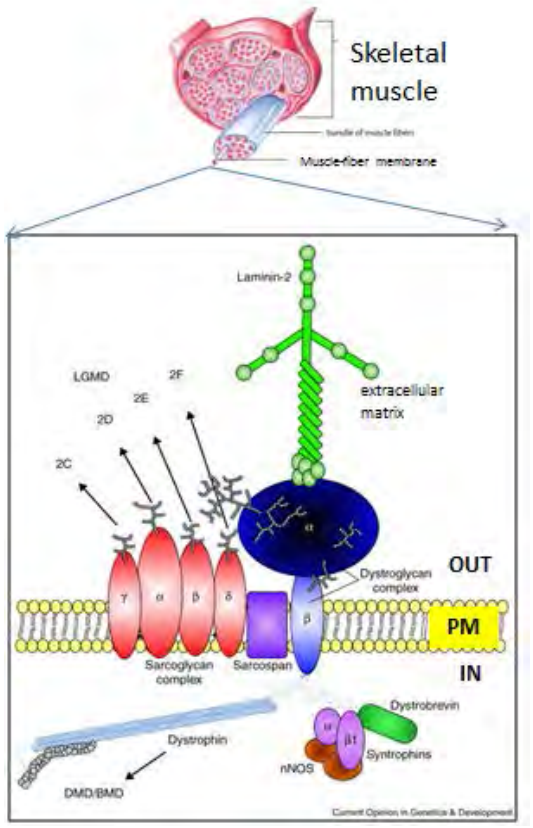
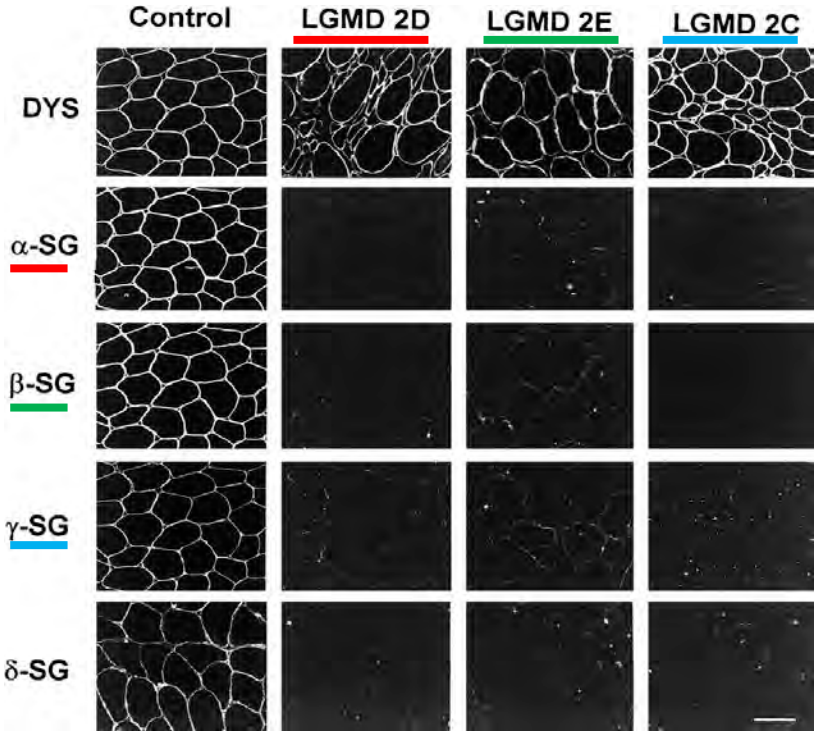
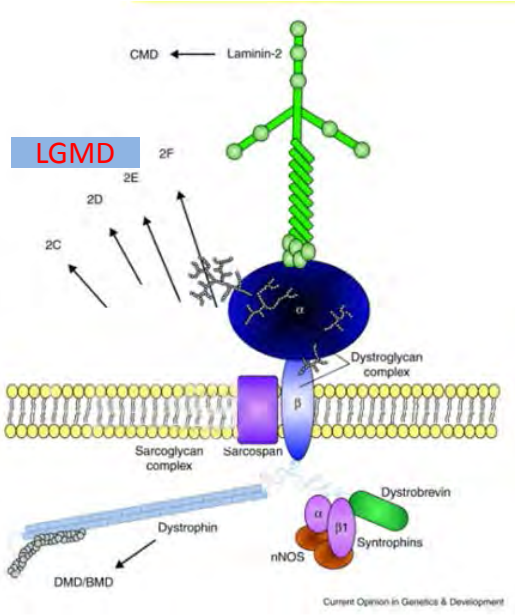
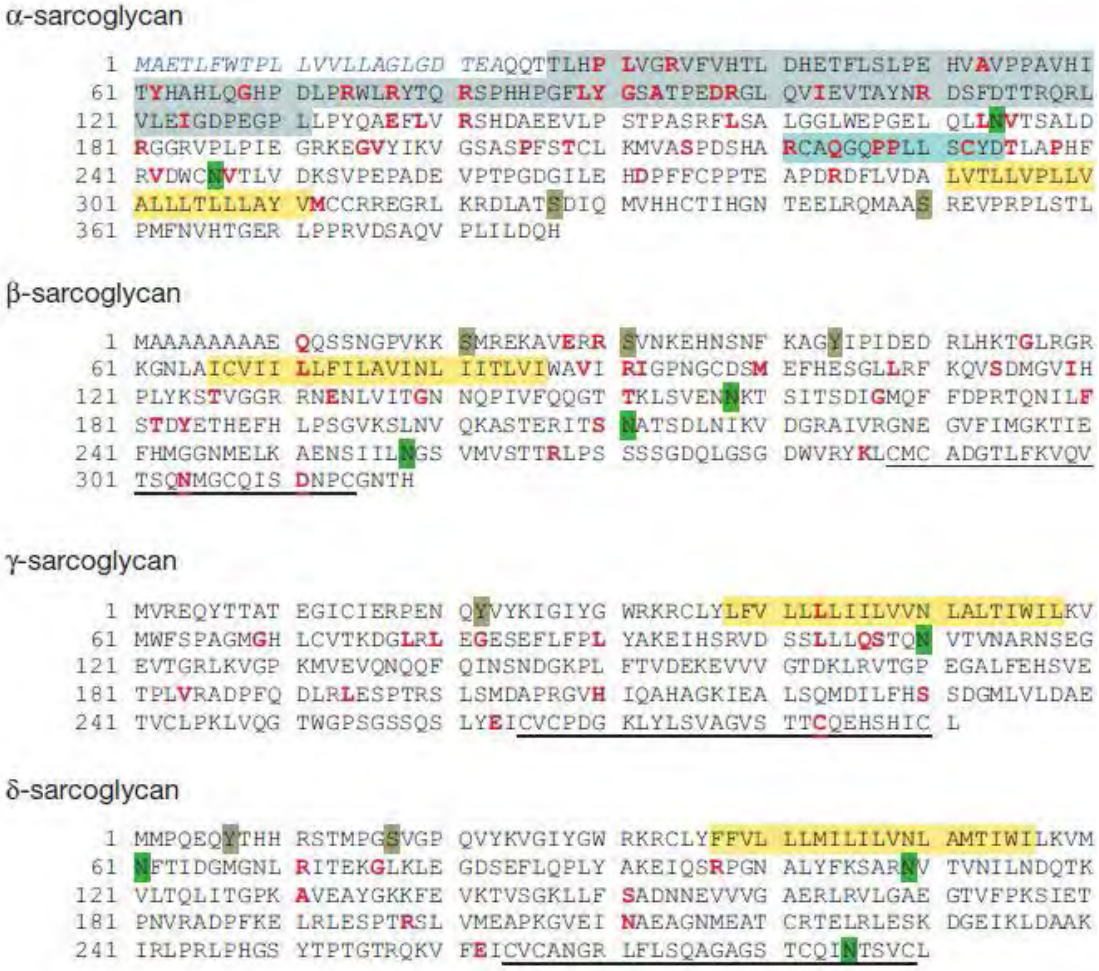




Jung D et al. (1996) J. Biol. Chem.



Sarcoglycanopathy (LGMD-2C-F): missense mutations



75%

52

59%

29

40%

20

12%

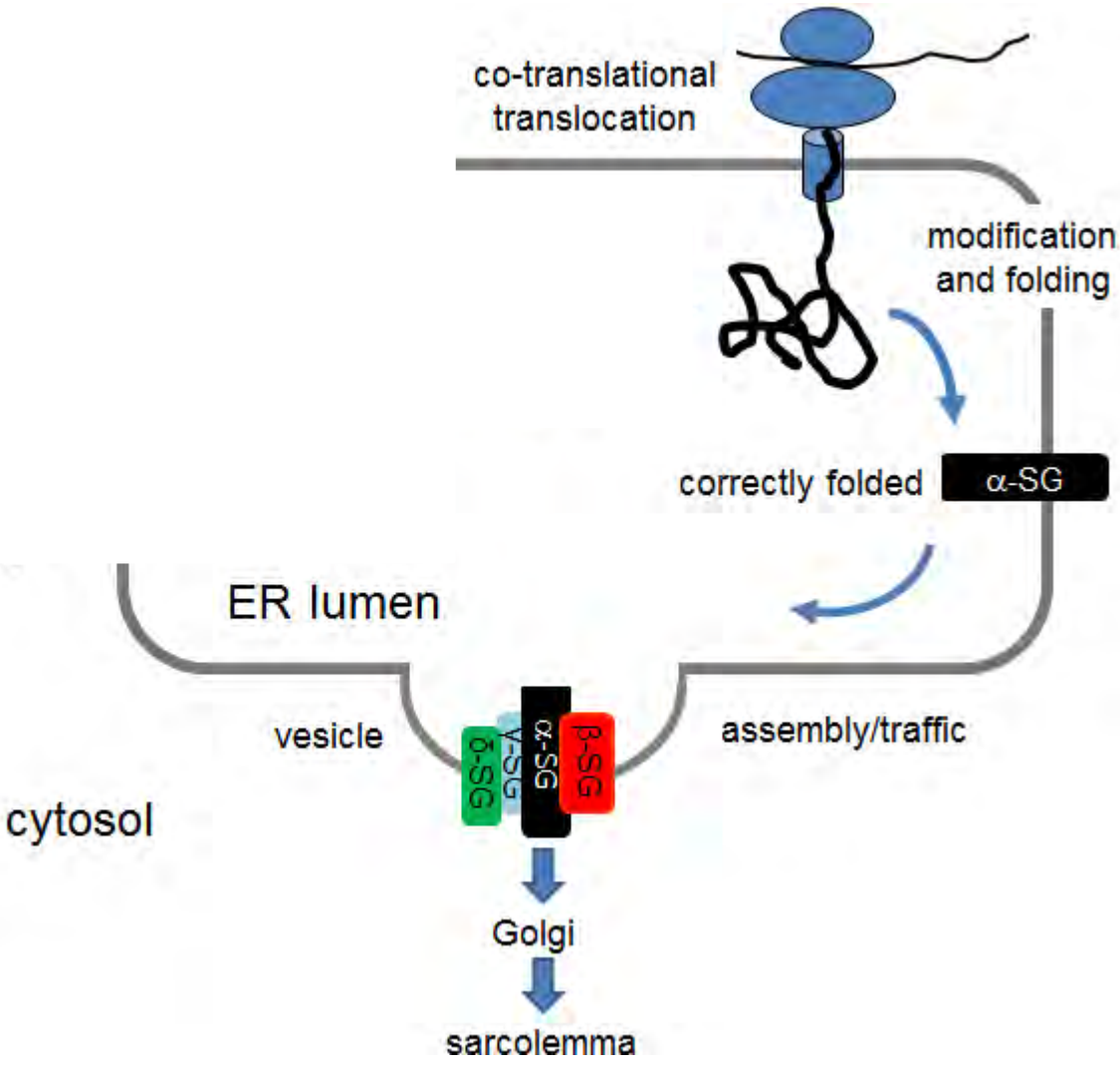
8

Sarcoglycan missense mutations

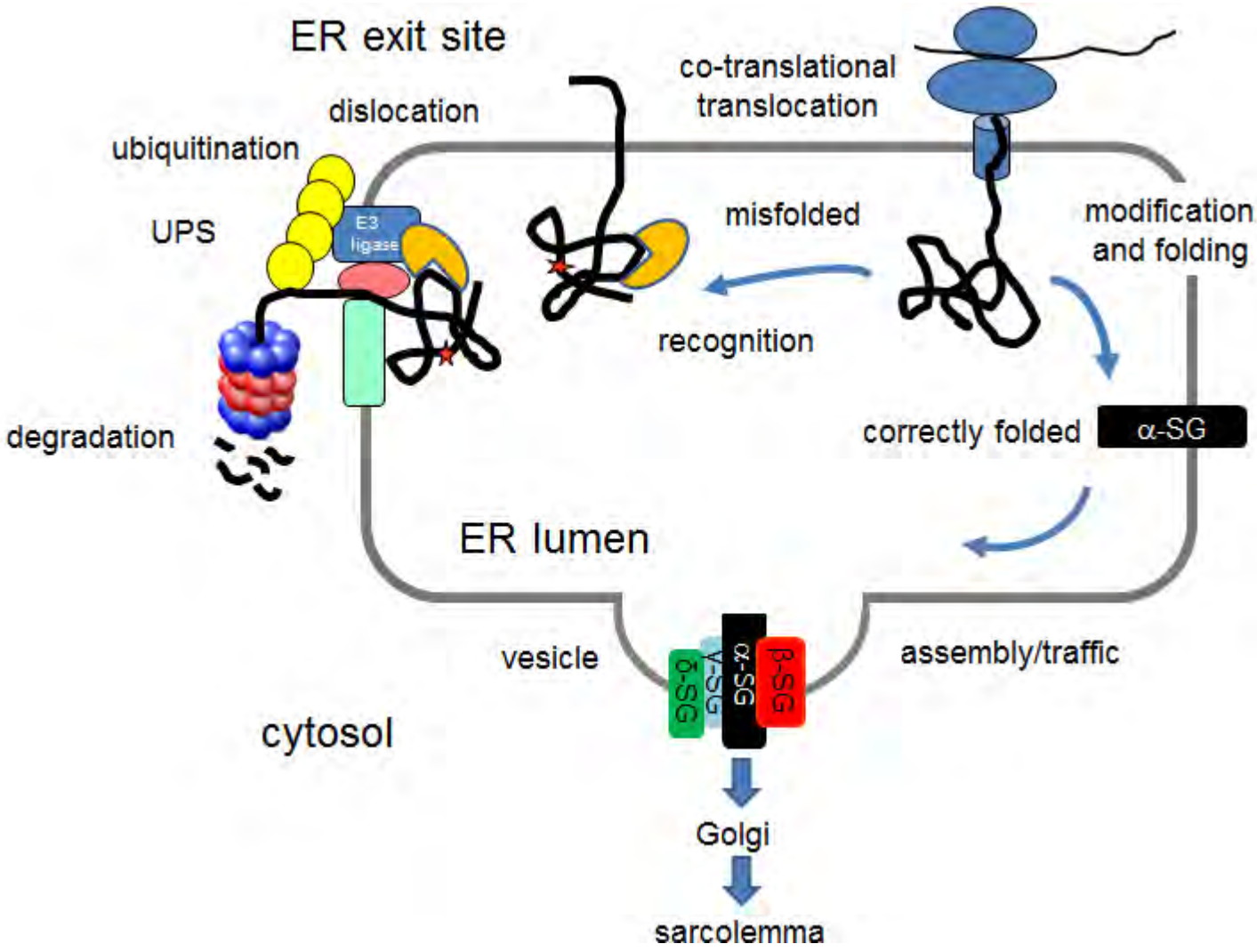
Expert Reviews in Molecular Medicine © Cambridge University Press 2009

Sandonà and Betto

biosynthesis of sarcoglycans

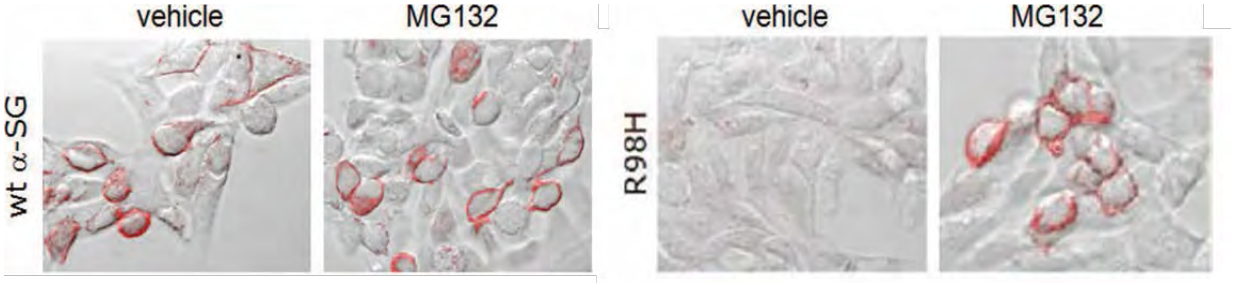


ER-qc and ER associated degradation (ERAD)



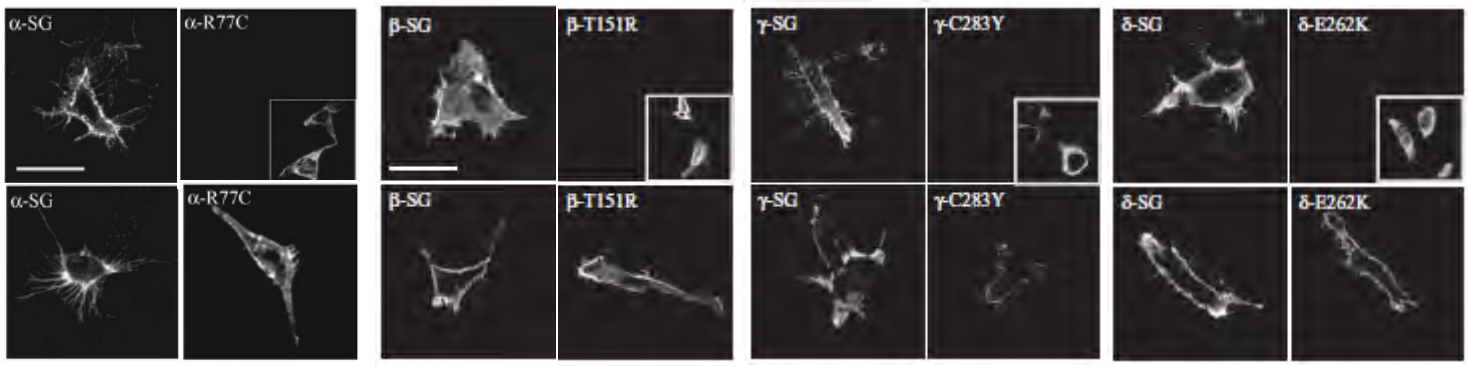
α-sarcoglycan mutants can be rescued by targeting ERAD

proteasomal inhibition leads to quantitative and functional rescue of α-sarcoglycan missense mutants



Gastaldello S. et al. (2008) Am J Path

α-mannosidase inhibition leads to quantitative and functional rescue of different sarcoglycan missense mutants



Bartoli et al. Human Mol Genet 2008; Soheili et al Human Mutation 2012

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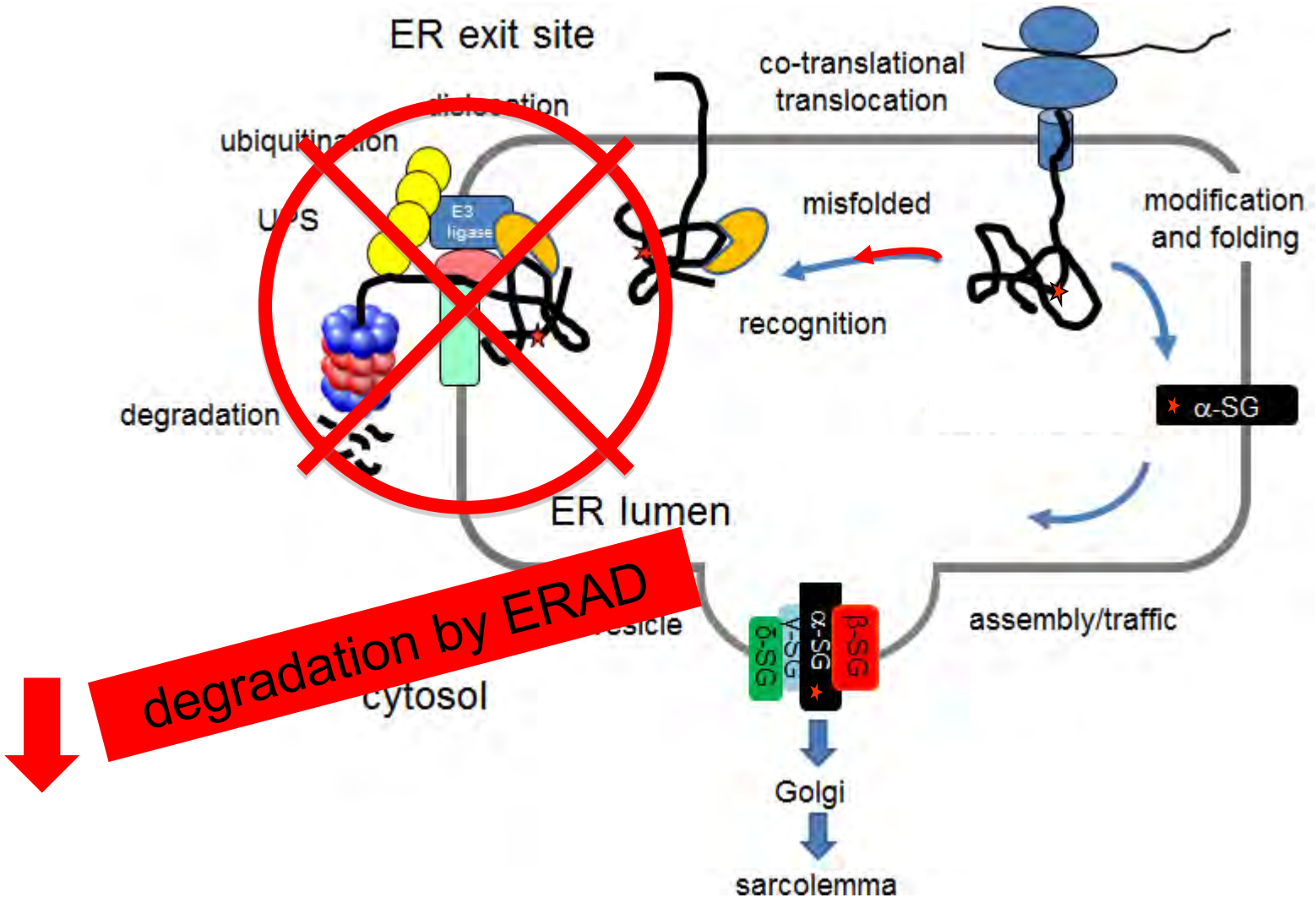
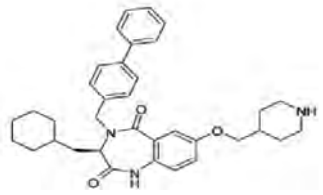


α-sarcoglycan mutants can be rescued by targeting ERAD

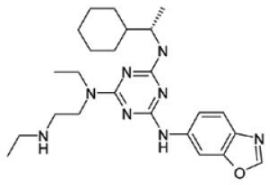
ERAD elements specifically involved in α-sarcoglycan disposal:
E3 ligases: HRD1 and RFP2;
E2 conjugases: UBE2J1;
AAA ATPase: p97;
co-factors: derlin1 and SEL1L
all of them are potential drug targets

Bianchini E. et al (2014) Human Mol Genet

small molecules: inhibitors of the E3 ligase HRD1



LS-101



LS-102

RING-finger type E3 ubiquitin ligase inhibitors as novel candidates for the treatment of rheumatoid arthritis

Yagishita N et al. (2012) Int J Mol Med

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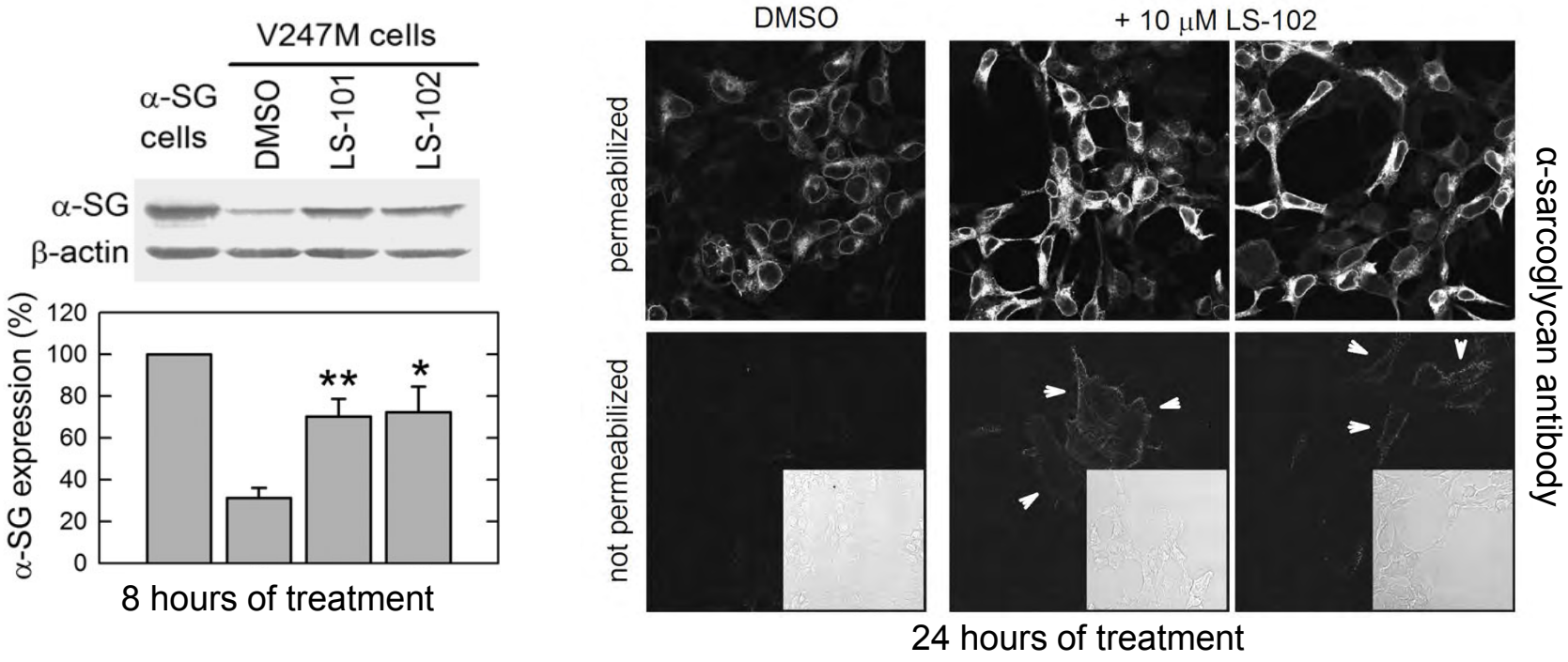
in vitro studies: HEK 293 cells expressing V247M-α-sarcoglycan



Bianchini E. et al (2014) Human Mol Genet

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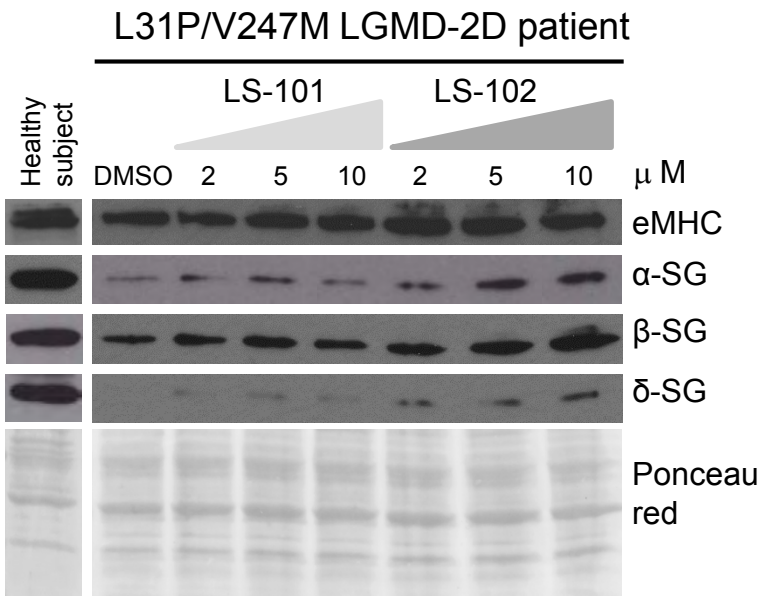

48 hours of treatment

Bianchini E. et al (2014) Human Mol Genet

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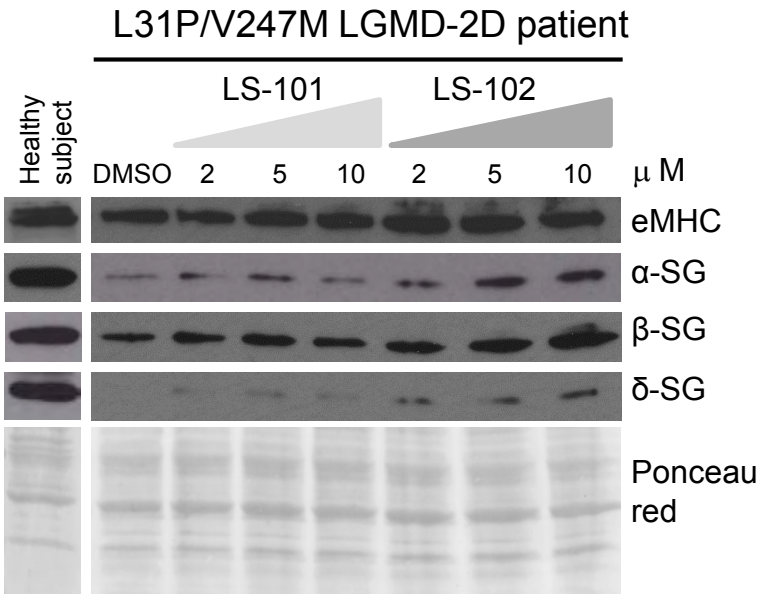

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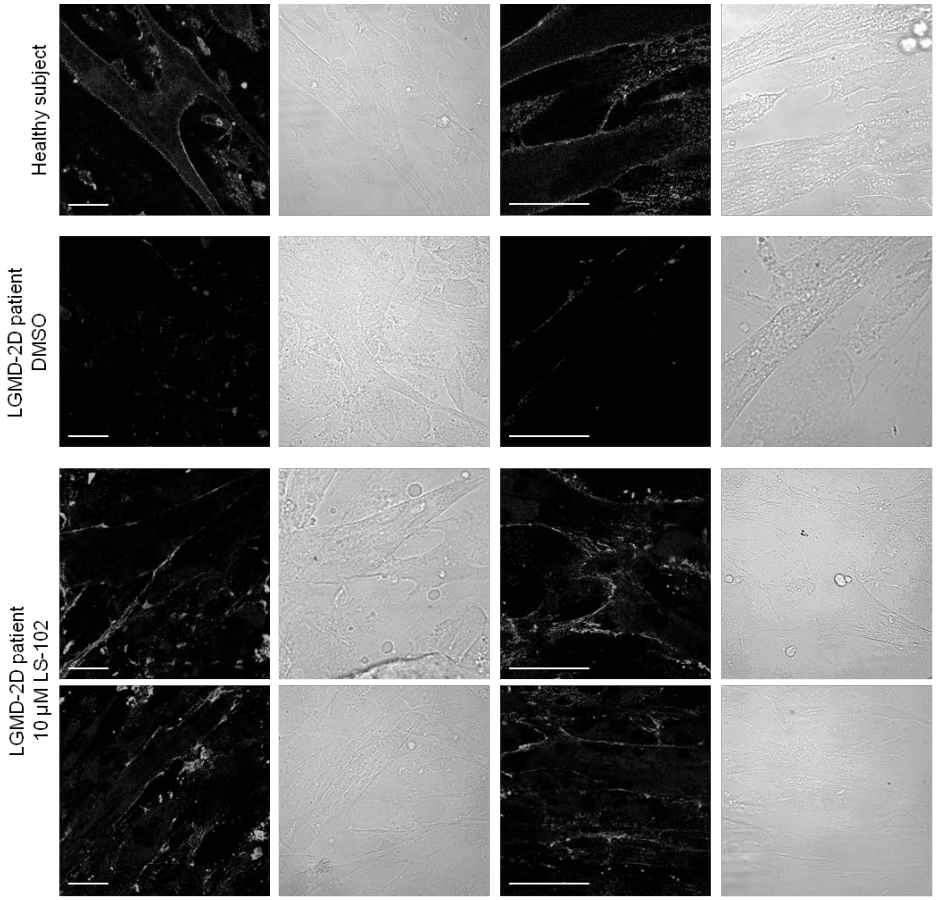

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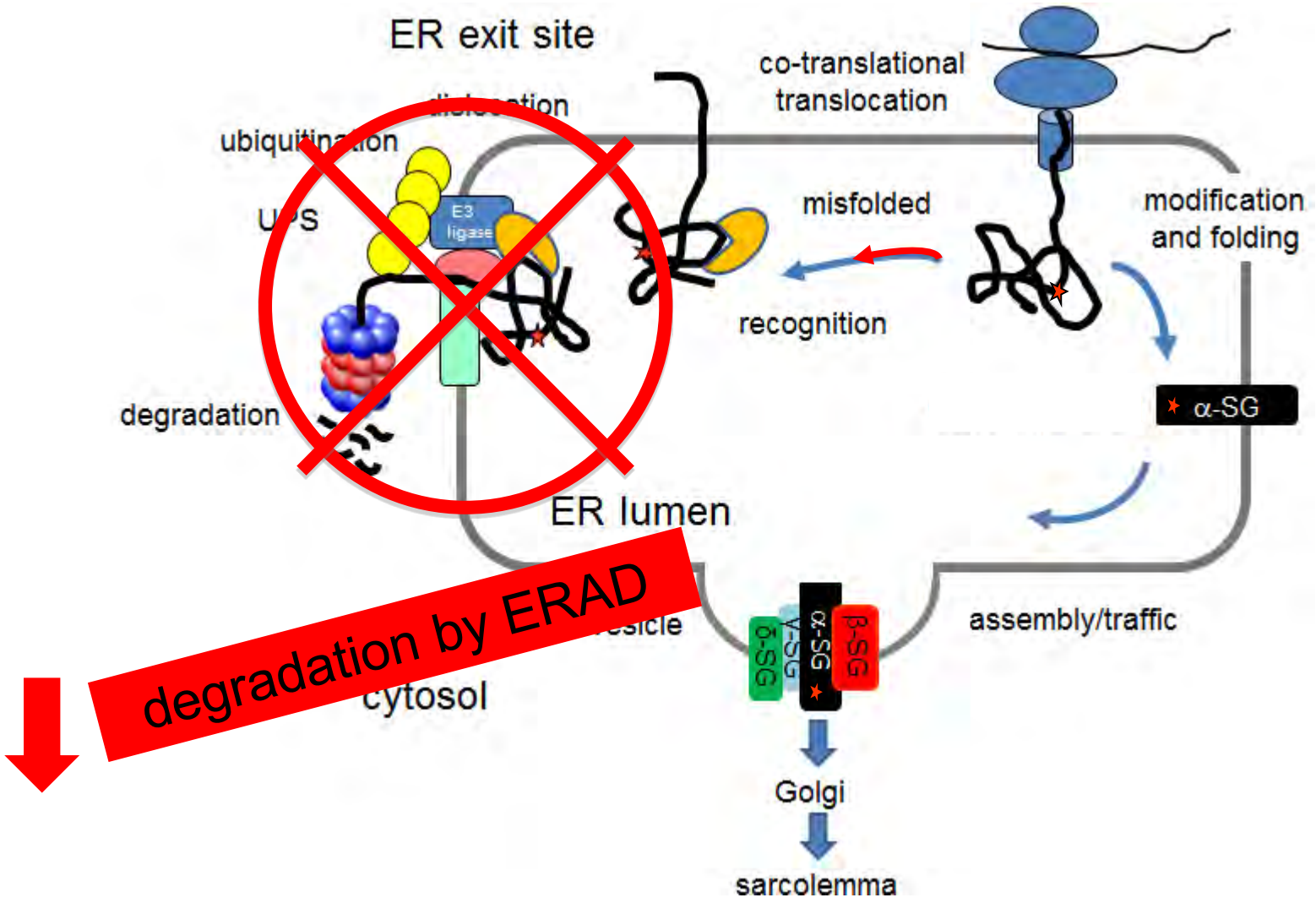
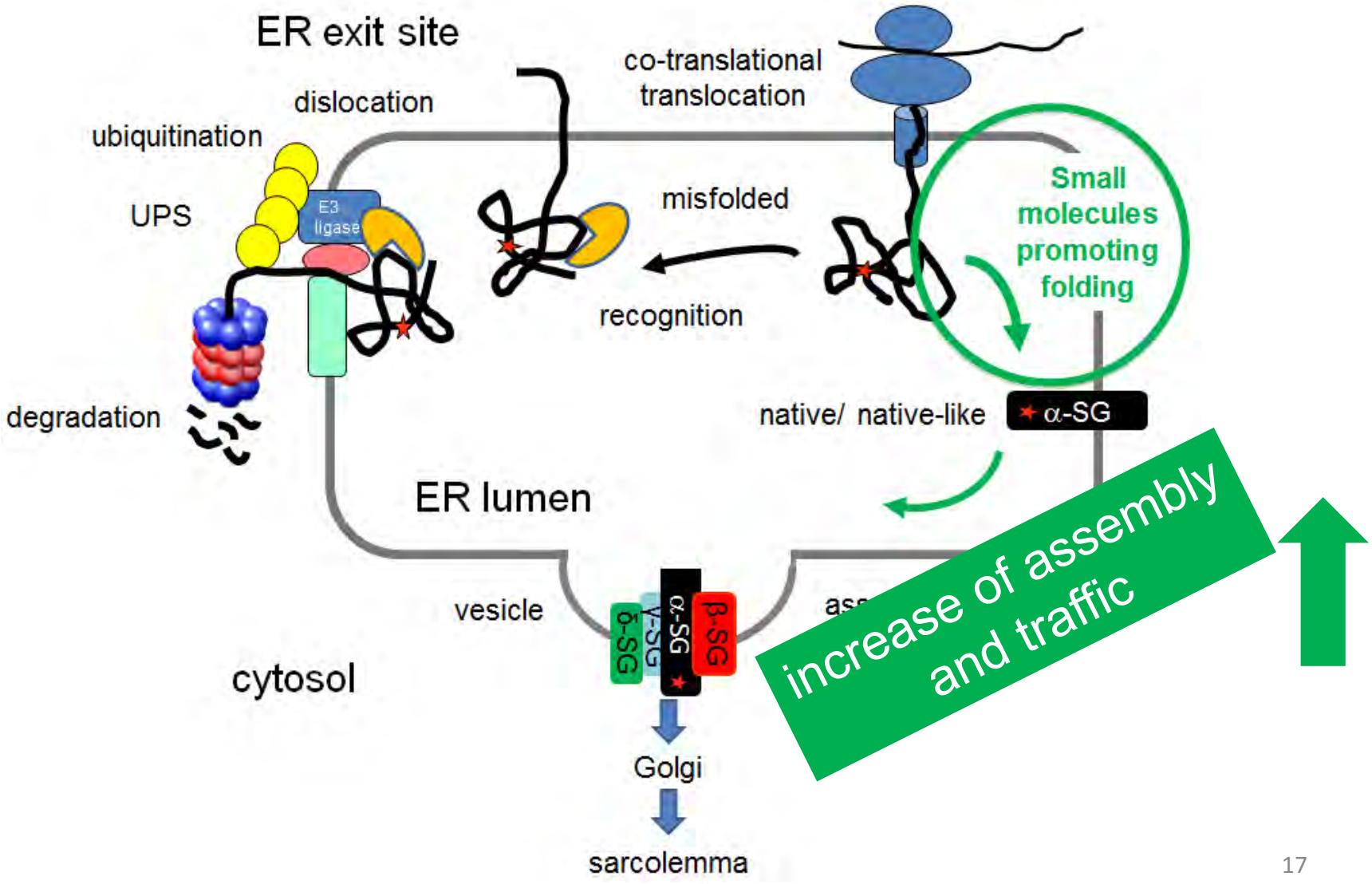


α-sarcoglycan mutants can be rescued by promoting folding



α-sarcoglycan mutants can be rescued by promoting folding

several small molecules tested

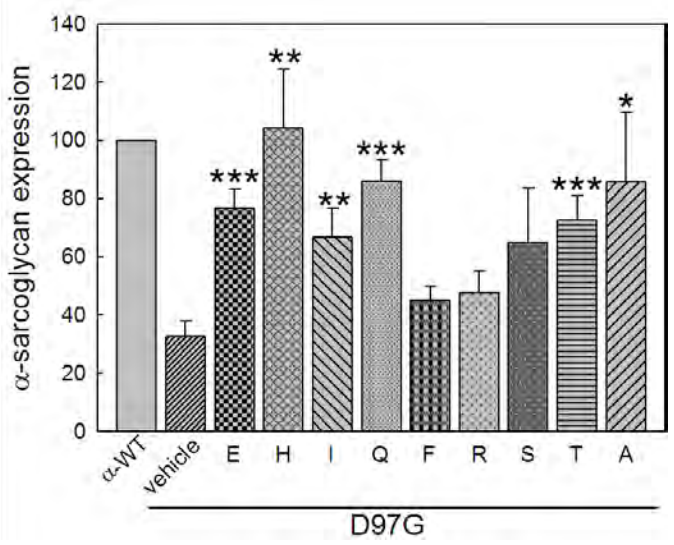
Table 1: list of protein folding correctors

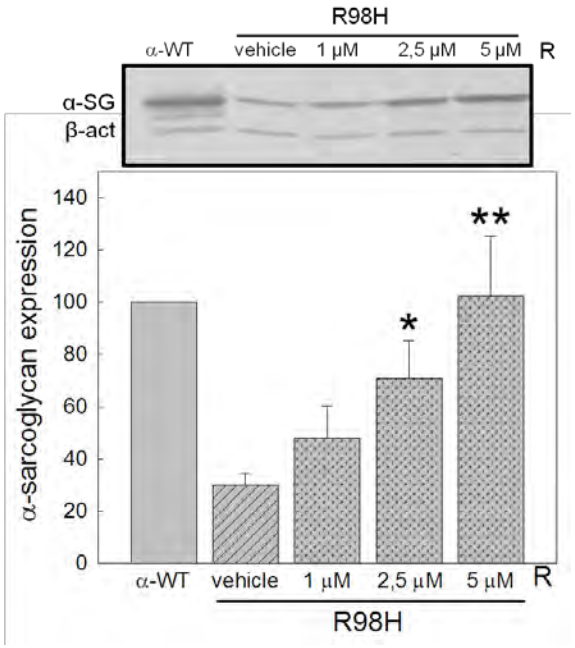
Compounds	Chemical name
A	N-(2-(5-chloro-2-methoxyphenylamino)-4'-methyl-4,5'-bithiazol-2'-yl)pivalamide
B	4-Cyclohexyloxy-2-{1-[4-(4-methoxy-benzensulfonyl)-piperazin-1-yl]-ethyl}-quinazoline
C	2-{1-[4-(4-Chloro-benzensulfonyl)-piperazin-1-yl]-ethyl}-4-piperidin-1-yl-quinazoline
D	1-(benzo[d][1,3]dioxol-5-yl)-N-(5-((S)-(2-chlorophenyl)((R)-3-hydroxypyrrolidin-1-yl)methyl)thiazol-2-yl)cyclopropanecarboxamide
E	N-[2-(5-Chloro-2-methoxy-phenylamino)-4'-methyl-[4,5']bithiazolyl-2'-yl]-benzamide
F	7-chloro-4-(4-(4-chlorophenylsulfonyl)piperazin-1-yl)quinoline
H	4,5,7-trimethyl-N-phenylquinolin-2-amine
I	N-(4-bromophenyl)-4-methylquinolin-2-amine
Q	N-(2-(3-acetylphenylamino)-4'-methyl-4,5'-bithiazol-2'-yl)benzamide
R	N-(2'-(2-methoxyphenylamino)-4-methyl-5,5'-bithiazol-2-yl)benzamide
S	N-phenyl-4-(4-vinylphenyl)thiazol-2-amine
T	2-(6-methoxy-4-methylquinazolin-2-ylamino)-5,6-dimethylpyrimidin-4(1H)-one

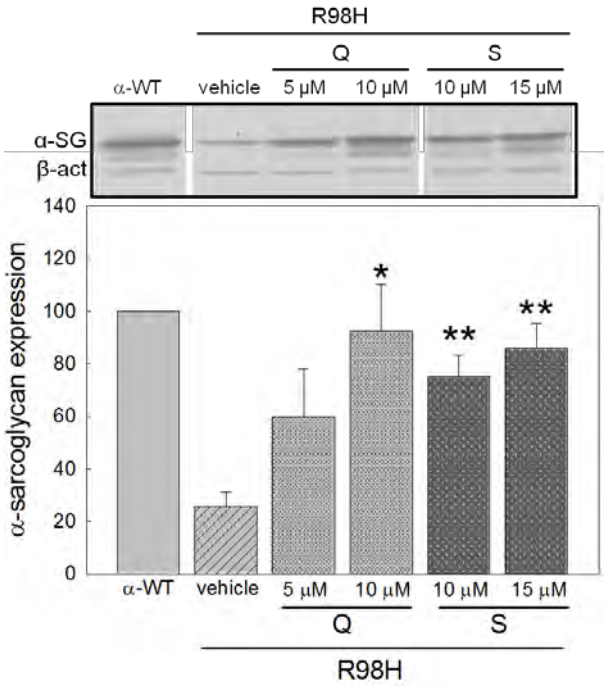
- known as protein folding correctors,
- screened and tested for the treatment of cystic fibrosis
- active on CFTR class II mutations: defective protein processing

α-sarcoglycan mutants can be rescued by promoting folding

in vitro studies: HEK 293 cells expressing different α-sarcoglycan mutants







24 hours of treatment

P127272.WO.01 pending **Bianchini E. et al in preparation**

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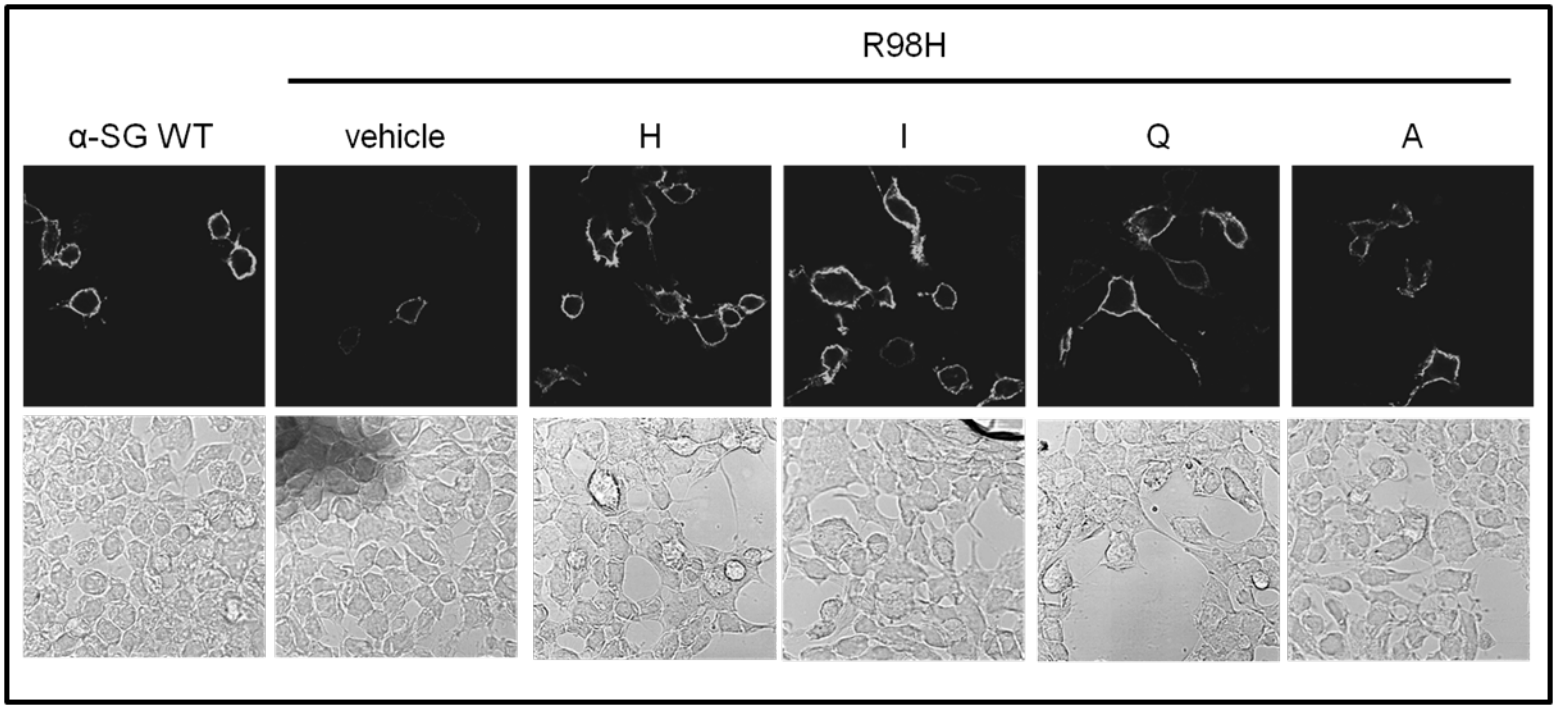

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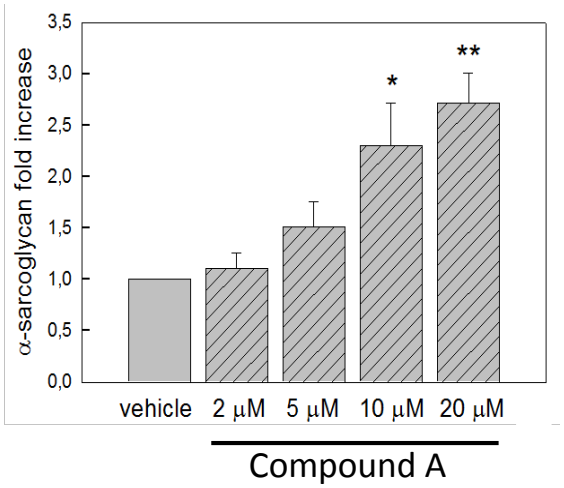

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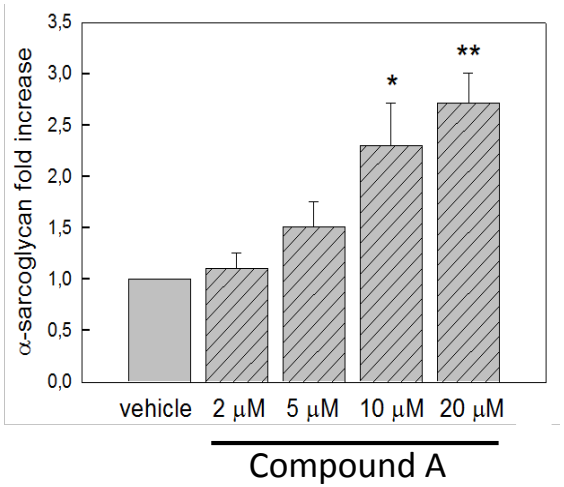

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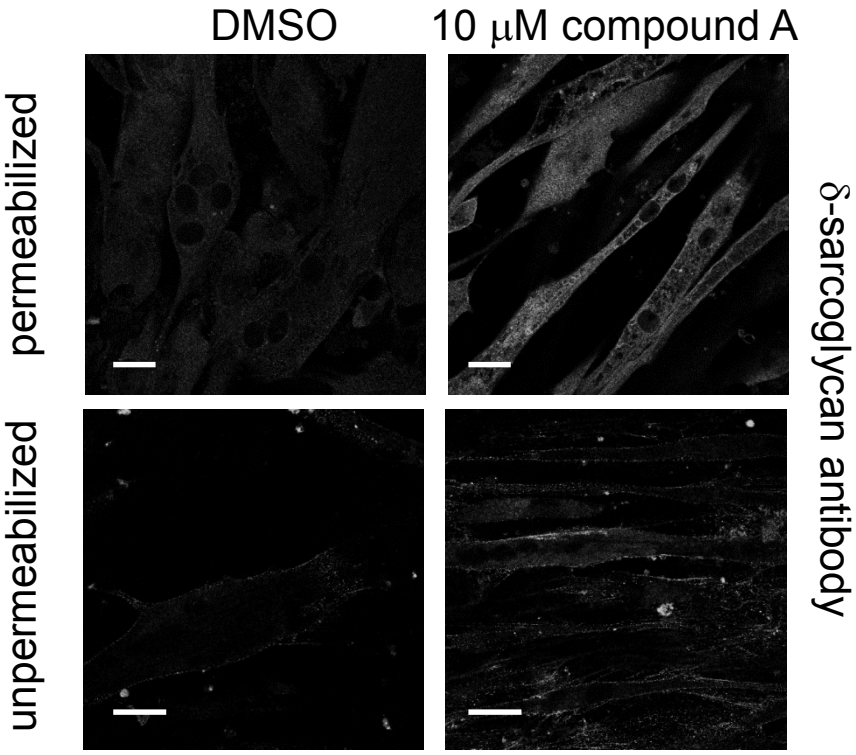

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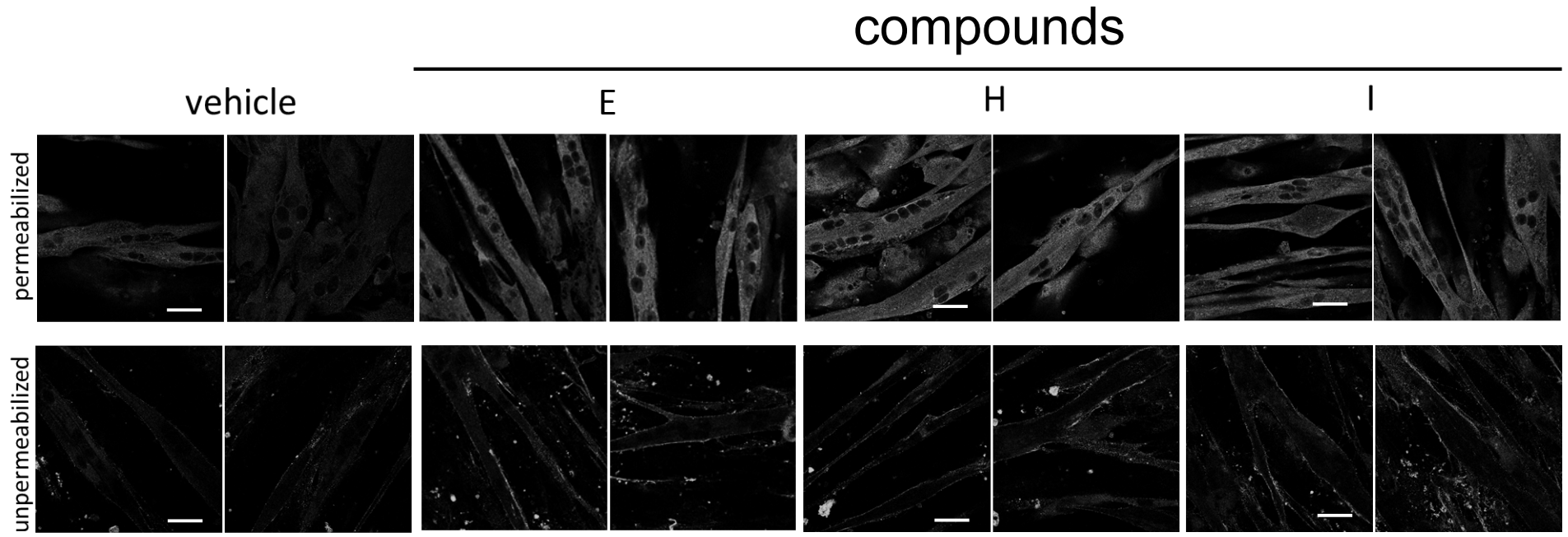

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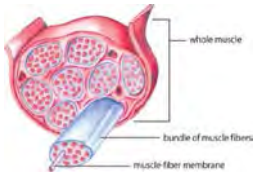
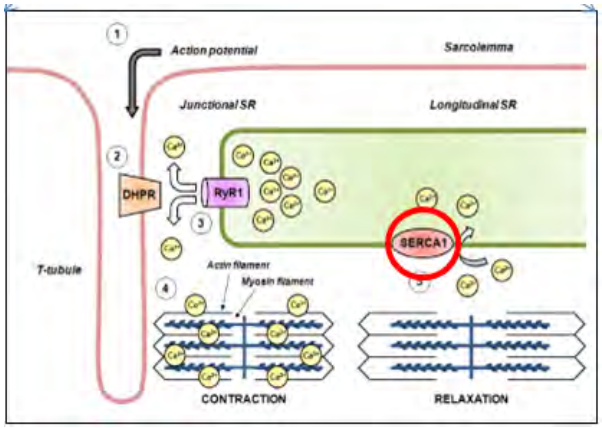
Brody's disease (BD)

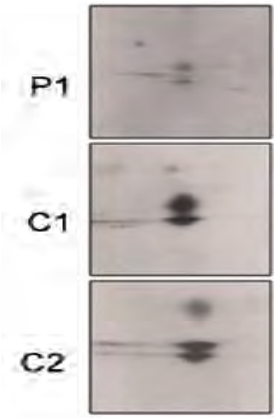




Guglielmi V. et al. (2013) J Genet Syndr Gene Ther

- Autosomal recessive genetic disorder
- Due to mutations in gene encoding Sarco(Endo)plasmic Reticulum Calcium ATPase (SERCA1)
- Both missense-mutations and in frame deletions are known
- Normal level of mRNA encoding mutated SERCA1
- Severe reduction/loss of mutated SERCA1
- Characterized by exercise induced delay in muscle relaxation causing muscle stiffness after contraction
- No effective therapy is at present available

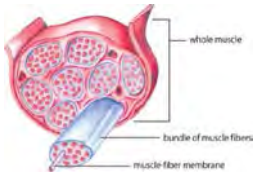
SERCA1a

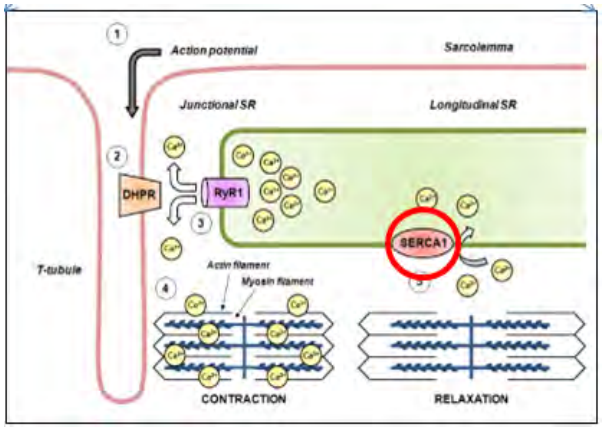


BD patient (P1)
healthy subjects (C1-2)

Vattemi G. et al (2010)
J Neuropat Exp Neurol.

Brody's disease (BD)





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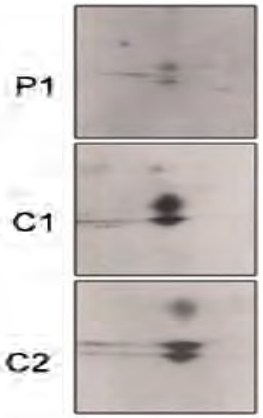

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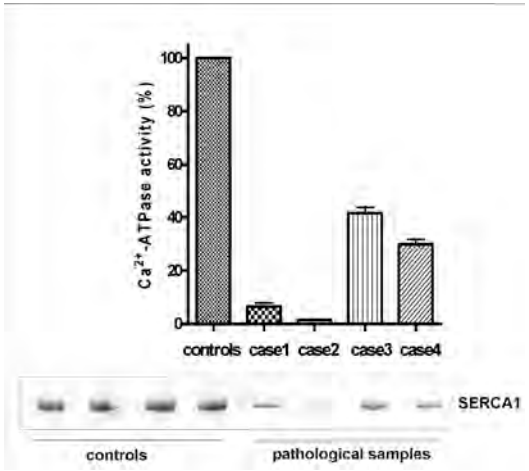
Vattemi G. et al (2010)
J Neuropat Exp Neurol.

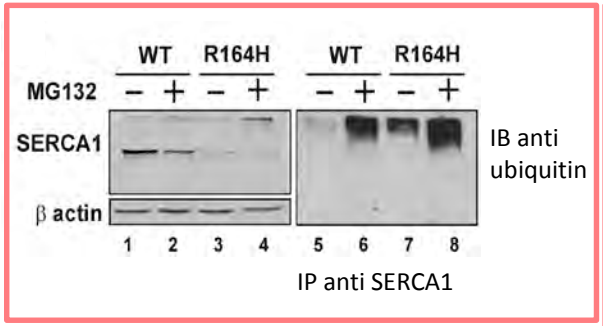
Testoni S. et al (2008) Vet Res



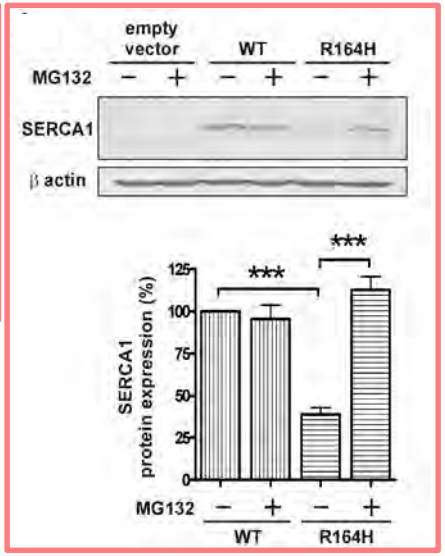
Cattle PMT: the true counterpart of Brody's disease and the animal model of this human pathology

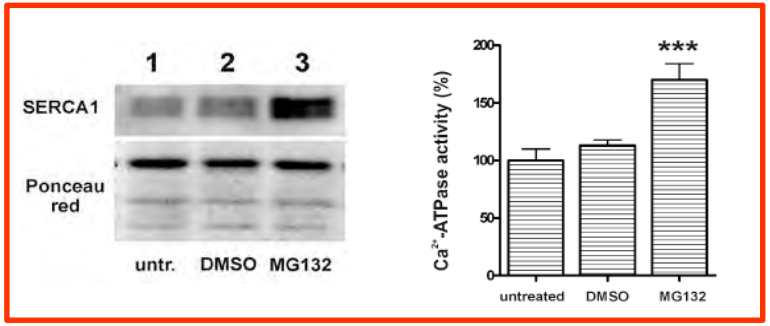
SERCA1 mutants can be rescued by targeting the degradative pathway

SERCA1 mutants are folding defective proteins substrates of the ubiquitin-proteasome system



in vitro studies: HEK 293 cells





ex vivo studies: skeletal muscle fibers from PMT affected cattle

Bianchini E. et al (2014) J Biol Chem

proteasomal inhibition leads to quantitative and functional rescue of SERCA1 mutants

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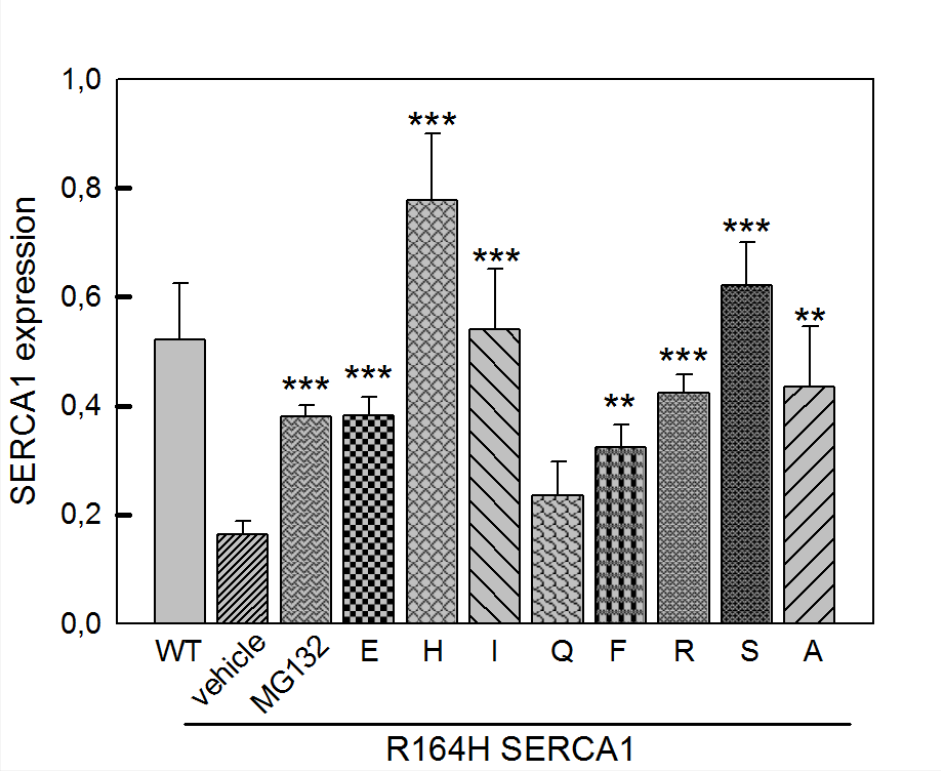


Work in progress:

- testing the rescue of the calcium pump activity
- *ex vivo* experiments with isolated myofibers from PMT affected cow

P127272.WO.01 pending;

proof of principle *in vitro*

α-sarcoglycan / SERCA1 mutants can be rescued by targeting ERAD

α-sarcoglycan / SERCA1 mutants can be rescued by promoting folding

testing *in vivo* efficacy and tolerability of the best molecules of the two pharmacological strategies

different pathologies with a common primary pathogenic event

LGMD-2C-F

BD / PMT

the mutated gene product is recognized by the QC of the cells as folding defective and prematurely degraded by the proteasome

most of the mutated gene products are potentially functional if their removal is avoided

pharmacological approaches acting either on the degradative pathway or the folding process can be useful to rescue these mutants

……and not only for these two diseases

Thanks to:

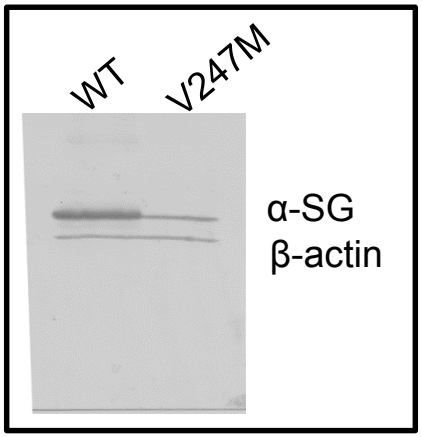
Dr. Elisa Bianchini
Dr. Roberta Sacchetto
Dr. Marcello Carotti
Dr. Chiara Gomiero
Dr. Giorgia Valle
Dr. Romeo Betto
Prof. Francesco Mascarello
Prof. Pompeo Volpe
Dr. Marina Fanin
Prof. Corrado Angelini (IRCCS S. Camillo VE)
Prof. Giacomo Comi (Univerisity of Milano)
Dr. Isabelle Richard (Genethon, Evry)
Prof. Toshihiro Nakajima (University of Tokyo)
Dr. F. Saverio Tedesco (UCL, London)

GFB

..and all of you

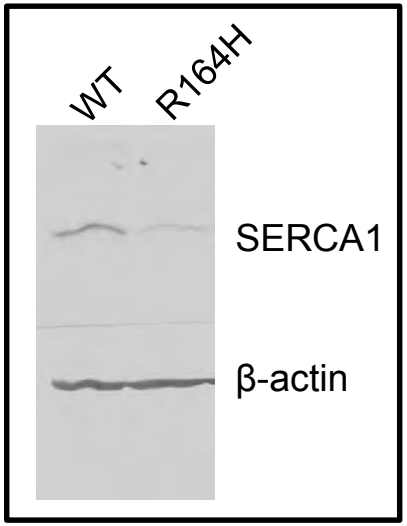
in vitro studies:
HEK 293 cells expressing

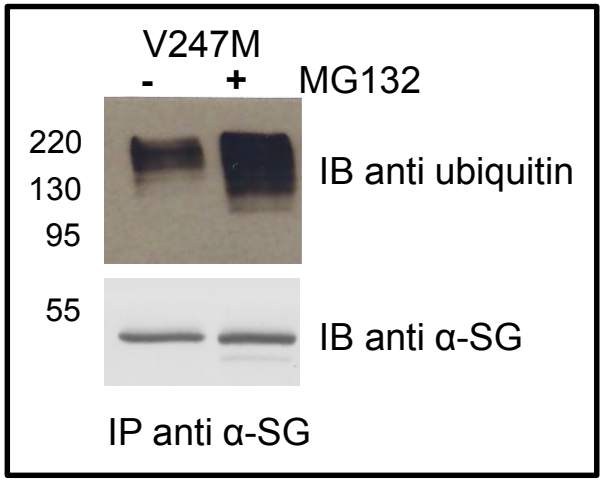
α-sarcoglycan



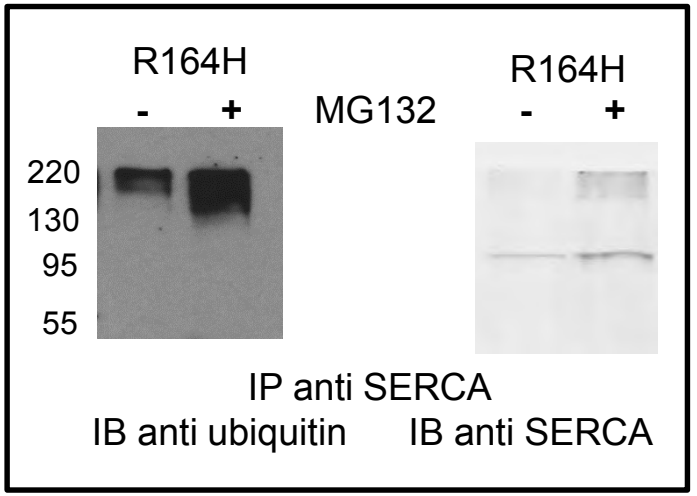
mutant expression levels are lower then those of wild type proteins

SERCA1A





mutant proteins are ubiquitinated

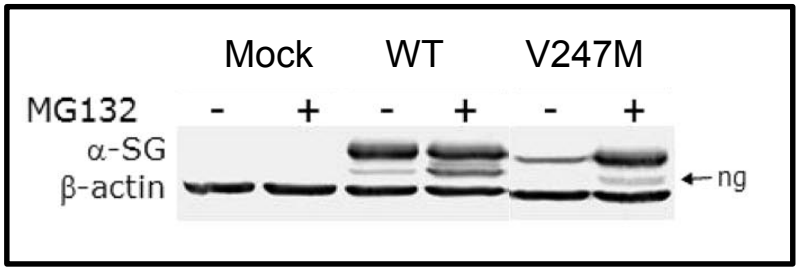


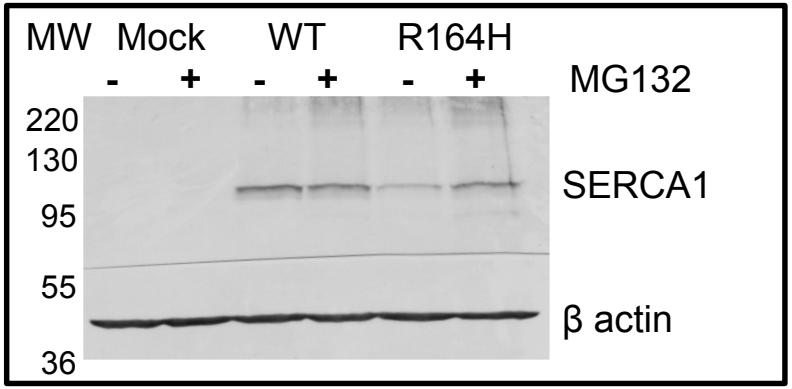
in vitro studies:
HEK 293 cells expressing

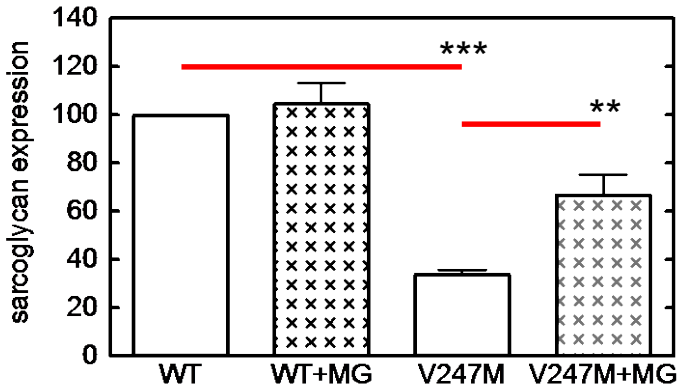
α-sarcoglycan

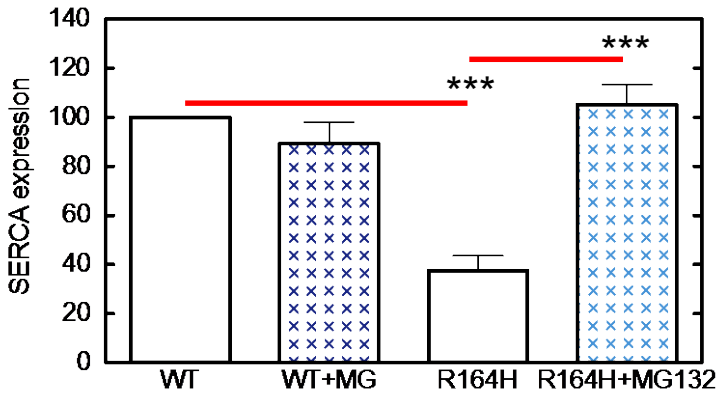
SERCA1A

proteasomal inhibition results in:
accumulation of mutant proteins





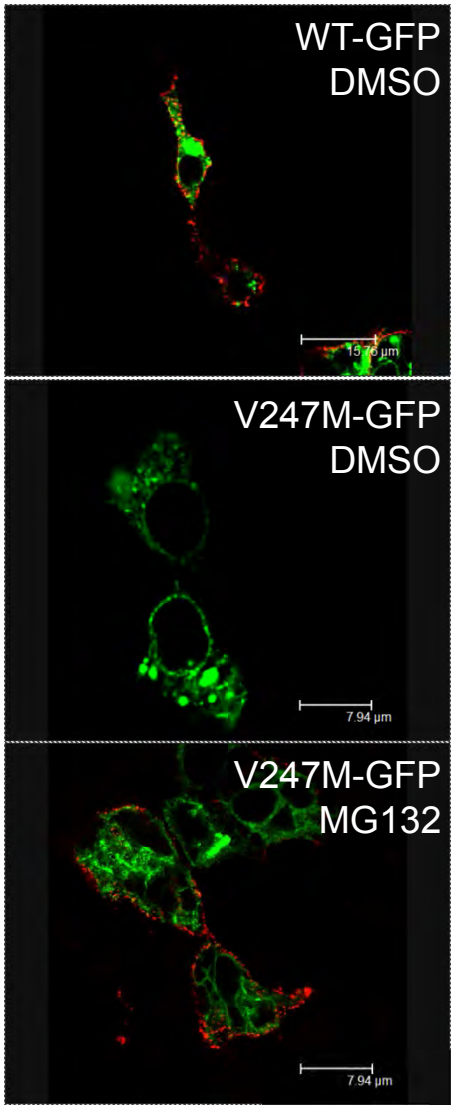




quantitative recovery of mutants

in vitro studies:
HEK 293 cells expressing

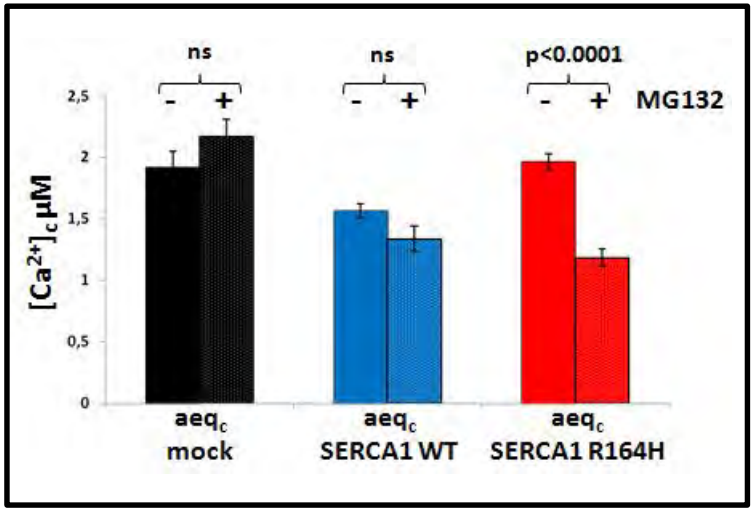
α-sarcoglycan



proteasomal inhibition results in:

proper localization of V247M α-SG mutant at plasma membrane

SERCA1A



rescue of the calcium pump activity of R164H SERCA1 mutant

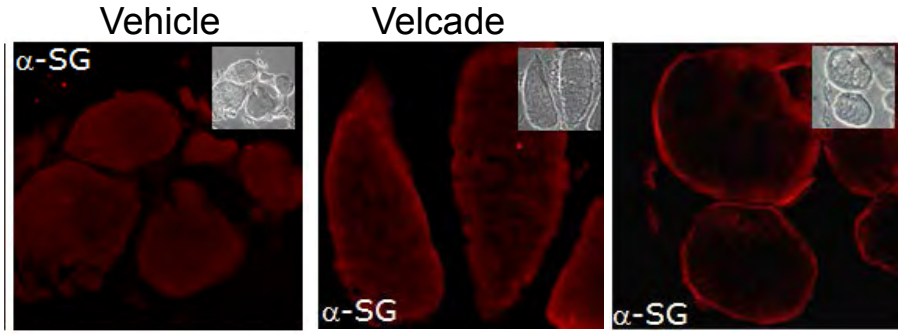
qualitative and functional recovery of mutants

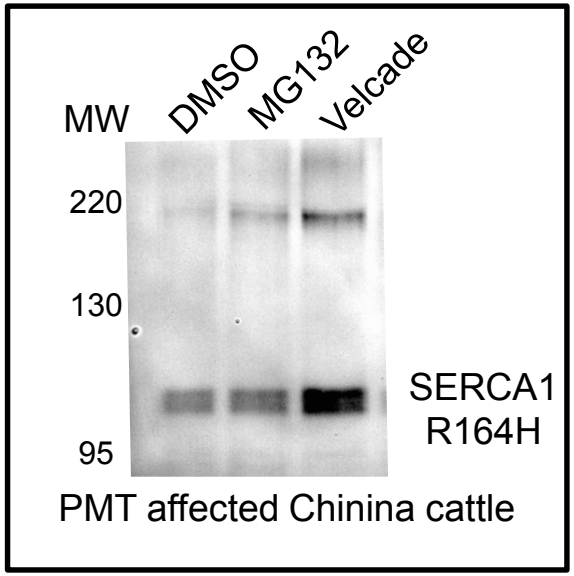
ex vivo studies:
skeletal muscle biopsy

α-sarcoglycan

treatment with proteasome inhibitors:

SERCA1A



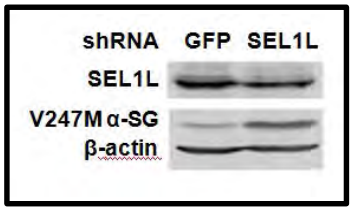


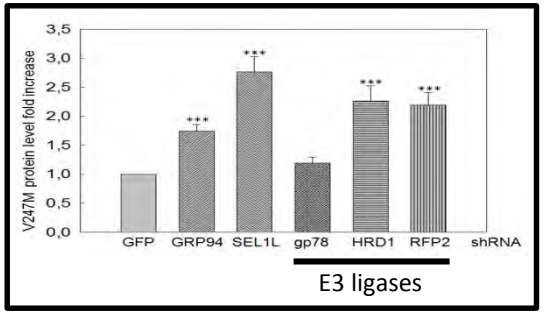
protein mutant recovery in the physiological site of muscle fibers

at present we are performing experiments in which the intermediate steps of the degradative pathway are manipulated

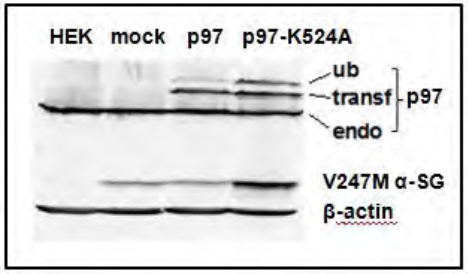
the ERAD pathway responsible for α-sarcoglycan mutant disposal has been deciphered

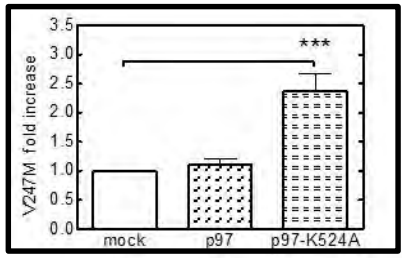
in vitro studies: HEK 293 cells expressing the V247M mutant of α-sarcoglycan



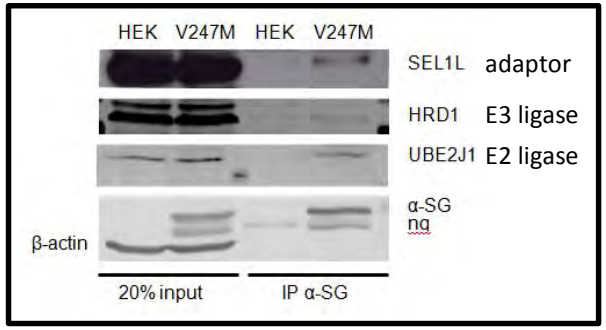


RNA interference of specific ERAD components





overexpression of DN forms of specific ERAD factors



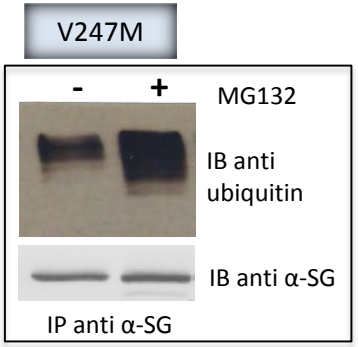
different ERAD factors specifically interacts with V247M-α-sarcoglycan

Bianchini et al (2014) Human Mol Genet

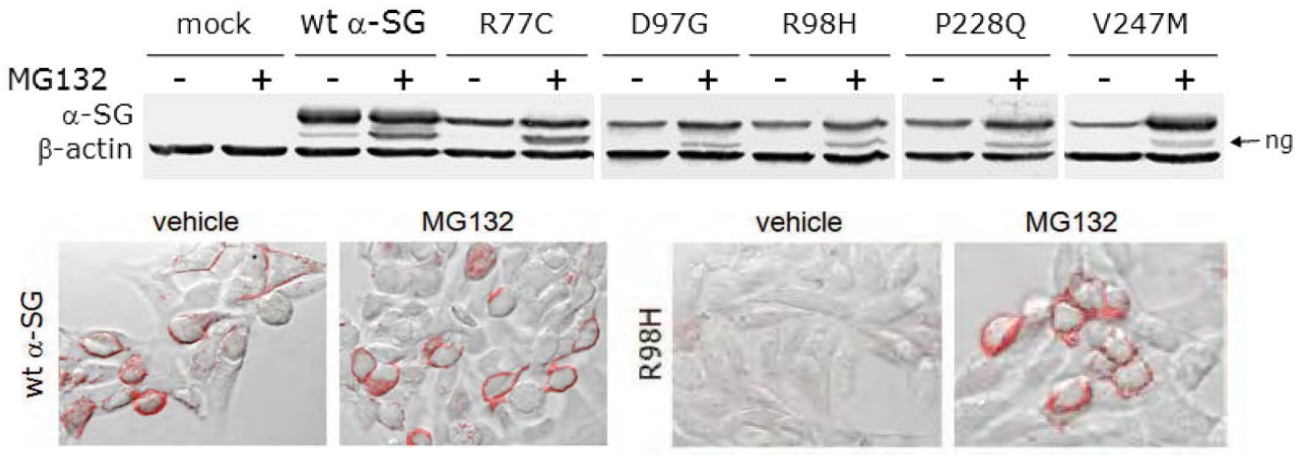
Sarcoglycanopathy (LGMD-2D)

α-sarcoglycan mutants are substrates of the ubiquitin-proteasome system

in vitro studies: HEK 293 cells



α-sarcoglycan mutants are poly-ubiquitinated



Gastaldello et al. Am J Path 2008

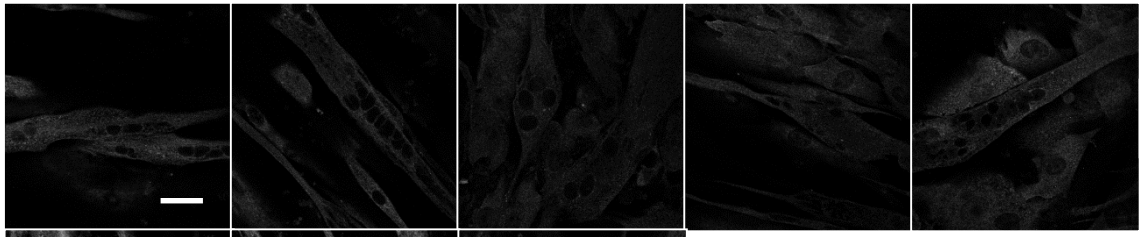
proteasomal inhibition leads to quantitative and functional rescue of α-sarcoglycan mutants

IF d-SG 1:100 2h 4°C 6813 cells FIX

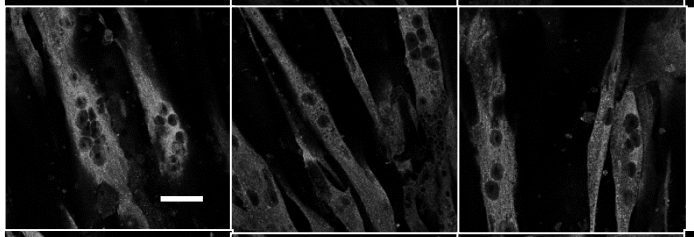
48 h trattamento da d5 a d7

tutte stesso zoom/gain

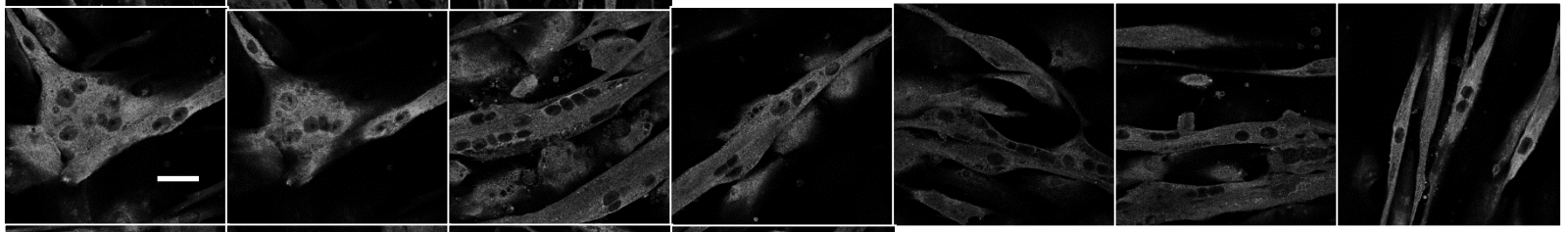
barra 31.75 µM



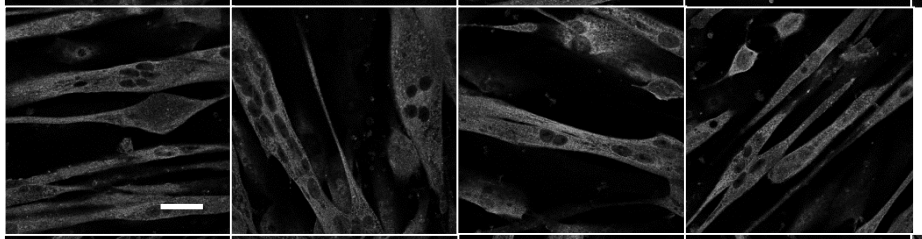
dmso



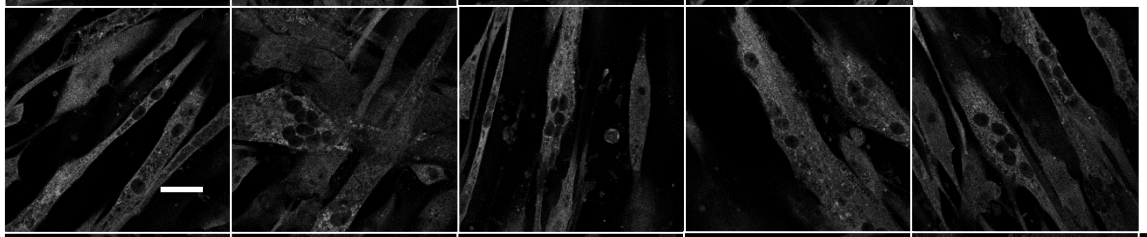
E 10 µM



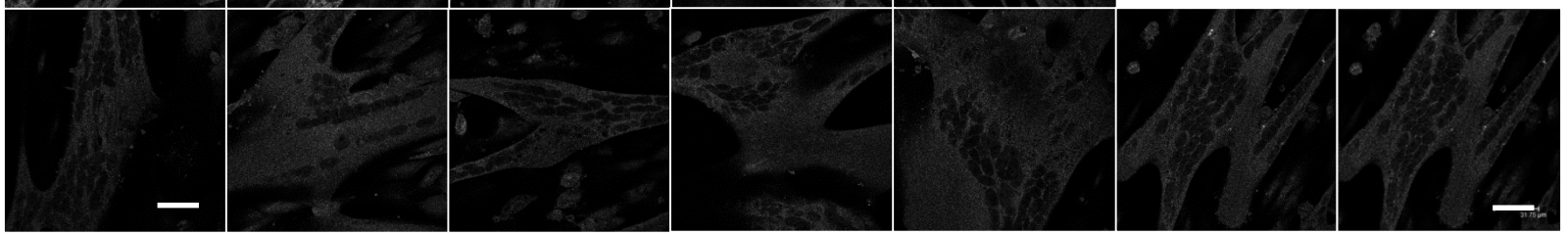
H 5 µM



I 5µM



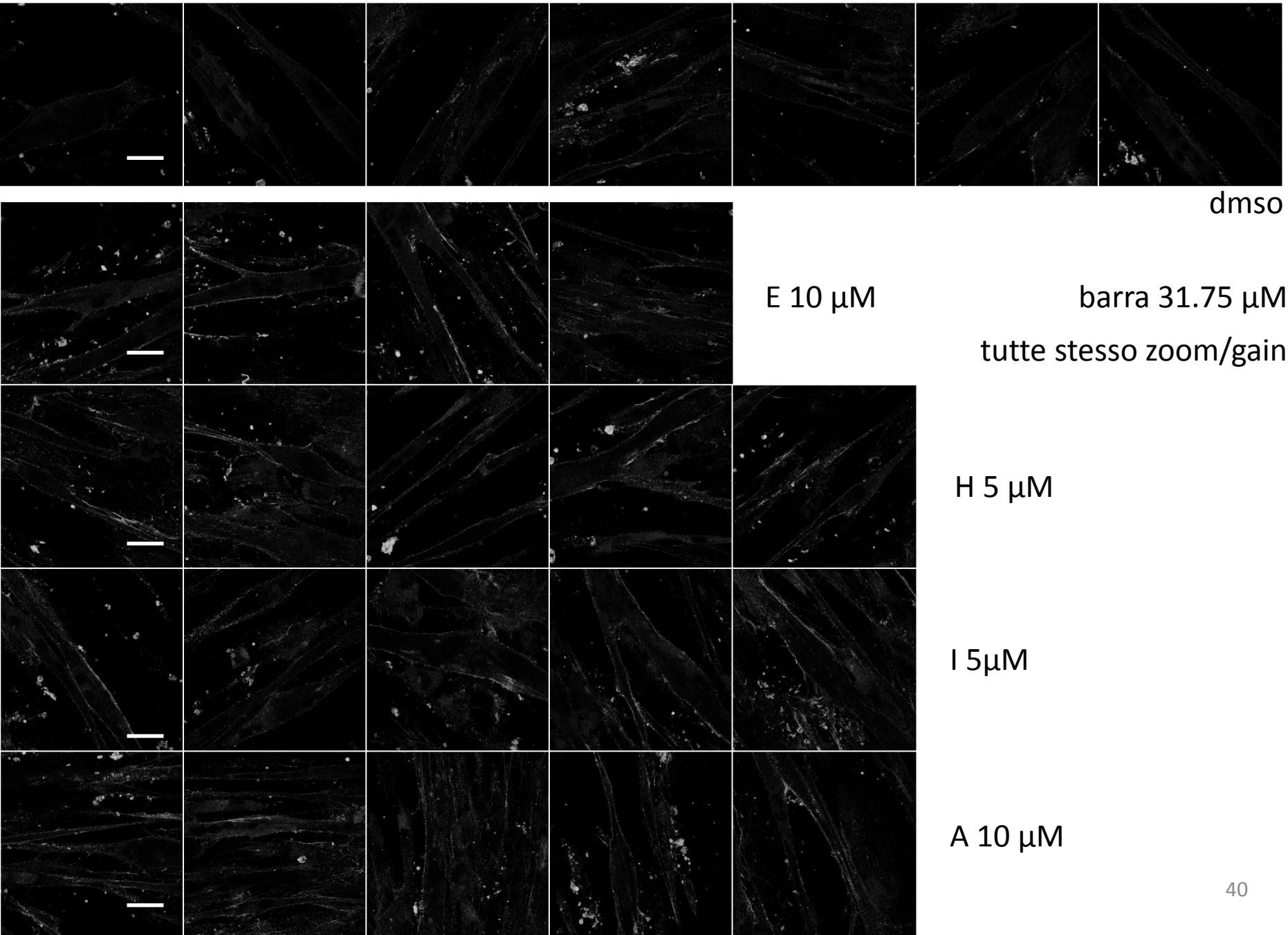
A 10 µM



LHCNM2 dmso

IF d-SG 1:100 2h 4°C 6813 cells in vivo

48 h trattamento da d5 a d7



dmso

E 10 µM

barra 31.75 µM

tutte stesso zoom/gain

H 5 µM

I 5µM

A 10 µM

Sarcoglycanopathy (LGMD-2C-F) missense mutants

animal models:
rodents

- KO mice for each sarcoglycan (not suitable)
- α-sarcoglycan$^{H77C/H77C}$ KI mice (no phenotype, probably the residues is not fundamental in mouse) (two independently generated mice)
- β-sarcoglycan$^{T151R/T151R}$ KI mouse (available from Sept. 2015)

goodness of the phenotype based on preliminary data

Sarcoglycanopathy (LGMD-2C-F) missense mutants

animal models:
zebrafish

Why this fish?

Because of the high number of fishes in each progeny
Because of the transparency of the first development stages
Because of the ability to absorb drugs dissolved in the fish-water
Because its skeletal muscles appear nearly identical to human ones
Because it has reproducible and easily measurable motor behaviors
Because any alteration in muscle function can be easily observed and measured
and so on…..

Sarcoglycanopathy (LGMD-2C-F) missense mutants

animal models:
zebrafish

- δ and β sarcoglycan will be targeted
- predicted muscle phenotype (morpholino-δ-sarcoglycan KD) [Cheng et al. 2006]
- ERAD and UPS are working in zebrafish [Imai et al. 2010; Chen et al. 2011; Imamura et al 2012]
- availability of δ-sarcoglycan null mutants in which to inject mutated human δ-sarcoglycan
- easy way to generate sarcoglycan mutant fishes by CRISPR/cas technology