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Developing new strategies to limit TRIM63/MuRF1-mediated muscle protein loss

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INTRODUCTION

Skeletal muscle has a major role in the control of the whole-body metabolic homeostasis. Under sustained catabolic conditions (injury, ageing, starvation, disuse or disease), and as a result of a synthesis/degradation imbalance, skeletal muscle atrophies and loses mass and strength. During this process, TRIM63/MuRF1, a Ubiquitin (Ub) E3-ligase, has been identified as a key player in skeletal muscle mass loss [1]. Indeed, this E3, when associated with an adequate Ub-conjugating enzyme (E2), targets the contractile proteins (alpha-actin, myosin heavy- and light chains, troponins...) to ubiquitination and subsequent degradation by the 26S proteasome. Among the forty E2 enzymes expressed in mammals, we have previously shown that 5 E2 enzymes functionally interact with MuRF1 in cellulo [2]. MuRF1 is involved in the targeting of several proteins not related to the contractile structure and each MuRF1-E2 couple may be involved in dedicated targets.

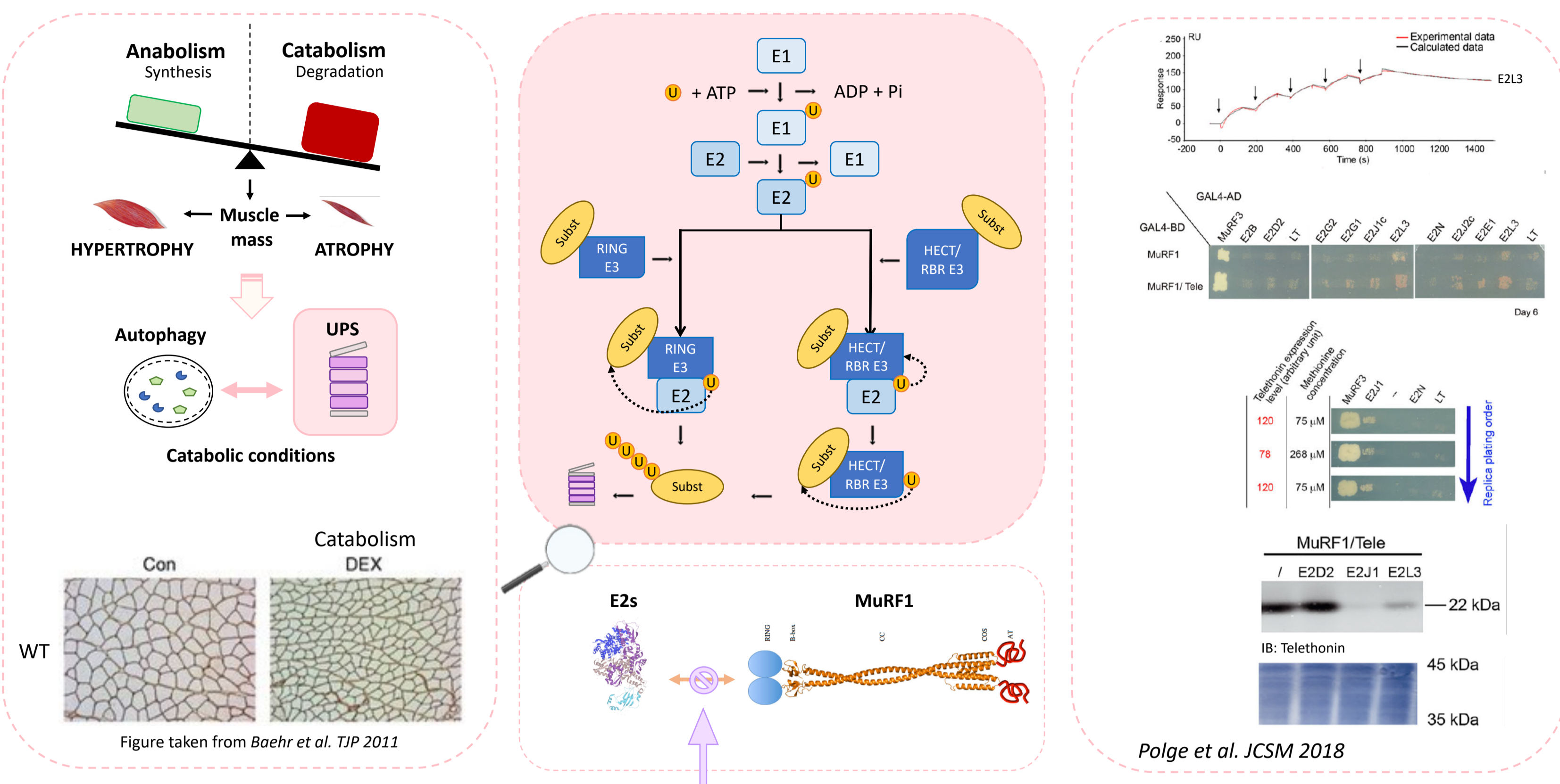
HYPOTHESIS

A reduction in MuRF1 activity during catabolic conditions by targeting MuRF1-E2 interface may protect skeletal muscles from deleterious atrophy

AIMS

To identify MuRF1's cognate E2(s) responsible of the degradation of the contractile proteins.
To design MuRF1-specific inhibitors (chemicals or peptides) able to decrease the interaction between MuRF1 and its E2(s) without full abolition of all MuRF1 functions.

BACKGROUND



APPROACHES

1. Impact of the E2s interacting with MuRF1 in catabolic myotubes (C2C12)

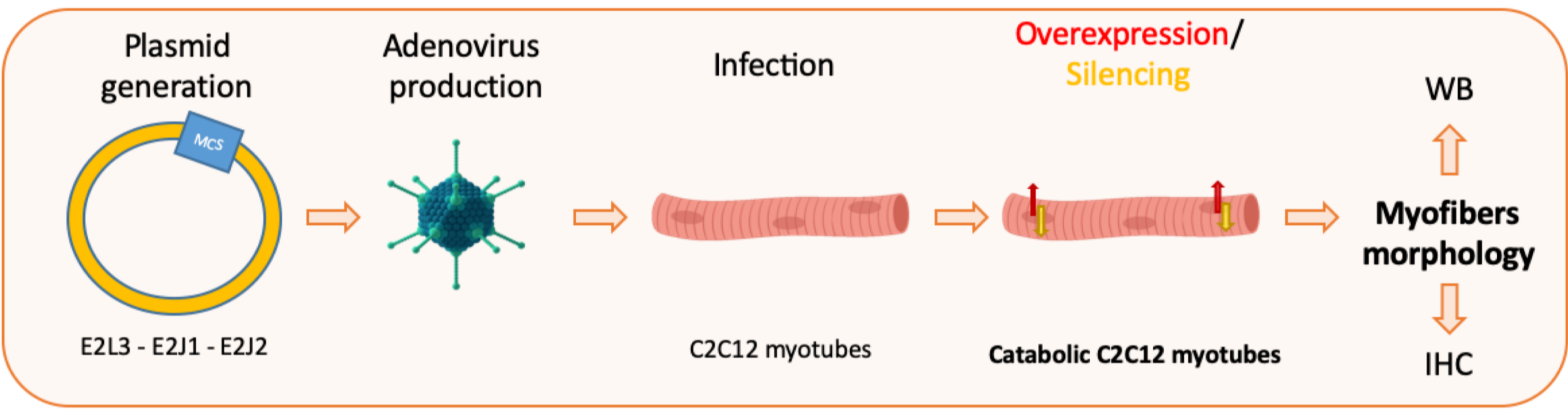
GOAL

To study the muscle contractile structure:

- Protein levels (Western Blotting, WB)
- Myofibers morphology (Immunohistochemistry, IHC)

PRINCIPLE

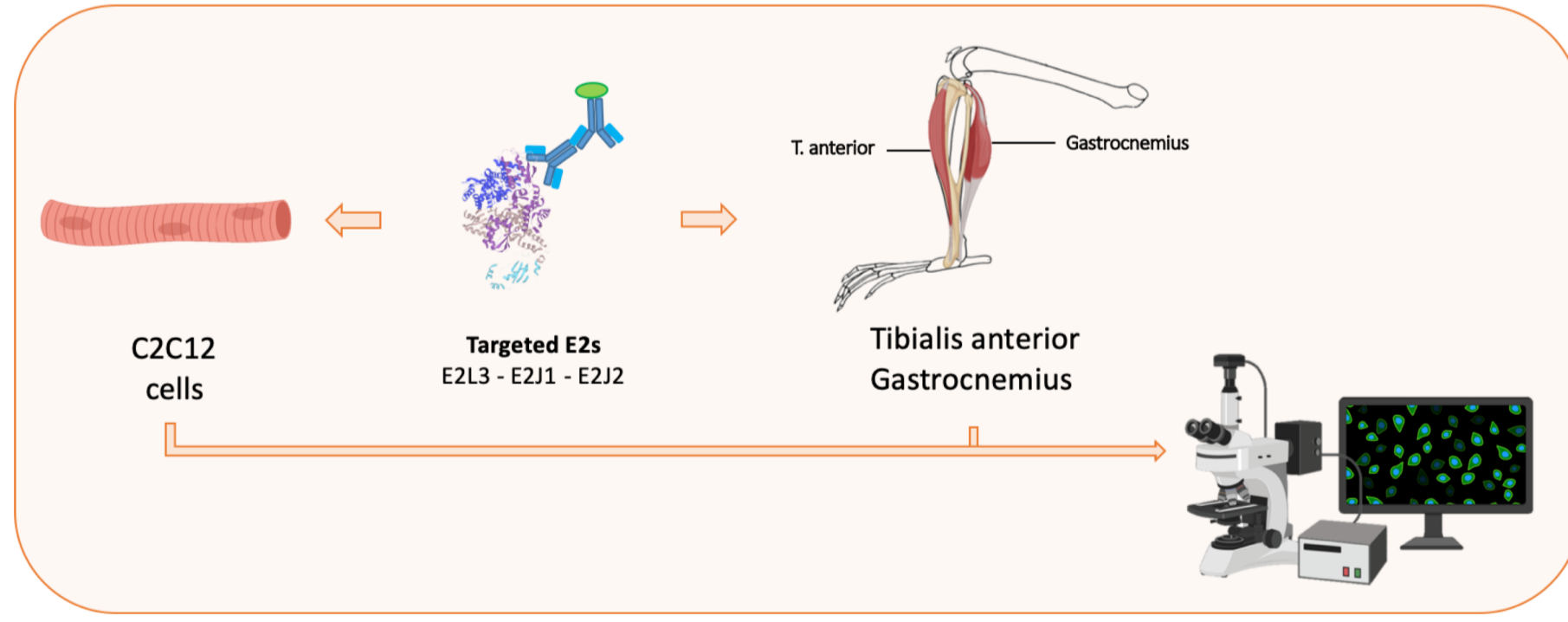
Overexpression
shRNA silencing



2. Cellular location of the E2s interacting with MuRF1

GOAL

- Study the location of E2s in C2C12 cells and muscle tissue (IHC)
- Study the localisation of E2s during atrophy conditions (IHC)



3. Impact of the E2s interacting with MuRF1 in vivo

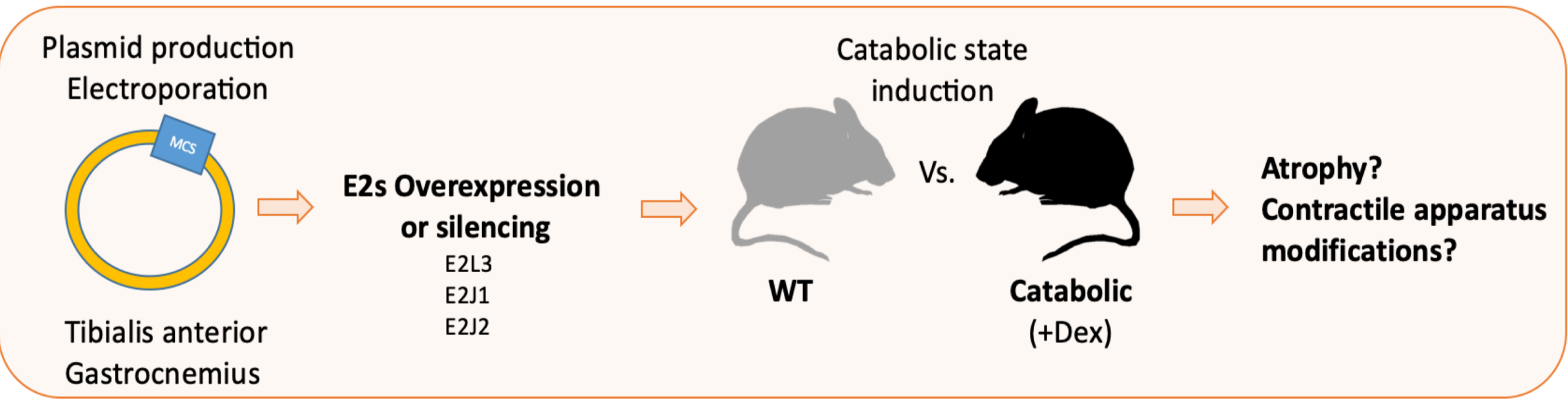
GOAL

To address the impact of the E2s on the muscle contractile structure:

- Protein levels (Western Blotting, WB)
- Myofibers morphology (Immunohistochemistry, IHC)

PRINCIPLE

Overexpression
shRNA silencing



4. Drugs screening in HEK293-GFP1-9 cells

GOAL

Design and/or screening of pharmacological inhibitors capable of disrupting the MuRF1-E2s.

Chemical library



PRINCIPLE

- Inhibition of MuRF1-E2 interaction by targeting amino acids residues involved in MuRF1-E2s interactions
- Inhibition of MuRF1-MuRF1 interaction by synthetic analogues of the α -helical epitopes involved in protein-protein interactions (CC domain)
- Screening of large libraries through collaborations (Pr. M. Reboud-Ravaux, Paris-Sorbonne; Pr. H. Ovaa, LUMC, Leiden, The Netherlands)

Stapled peptides

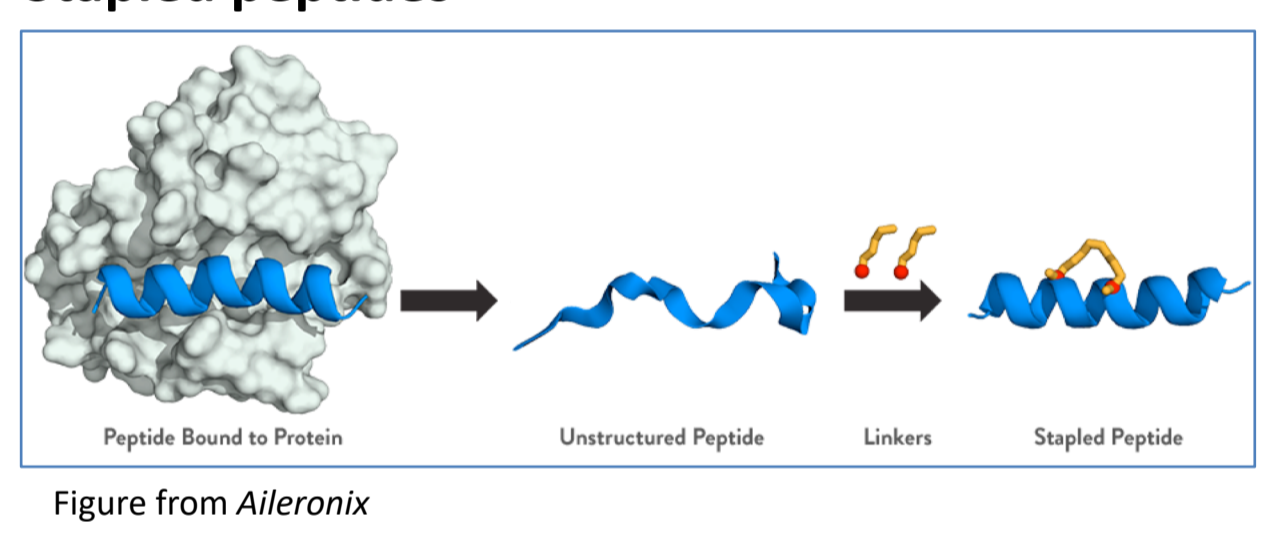


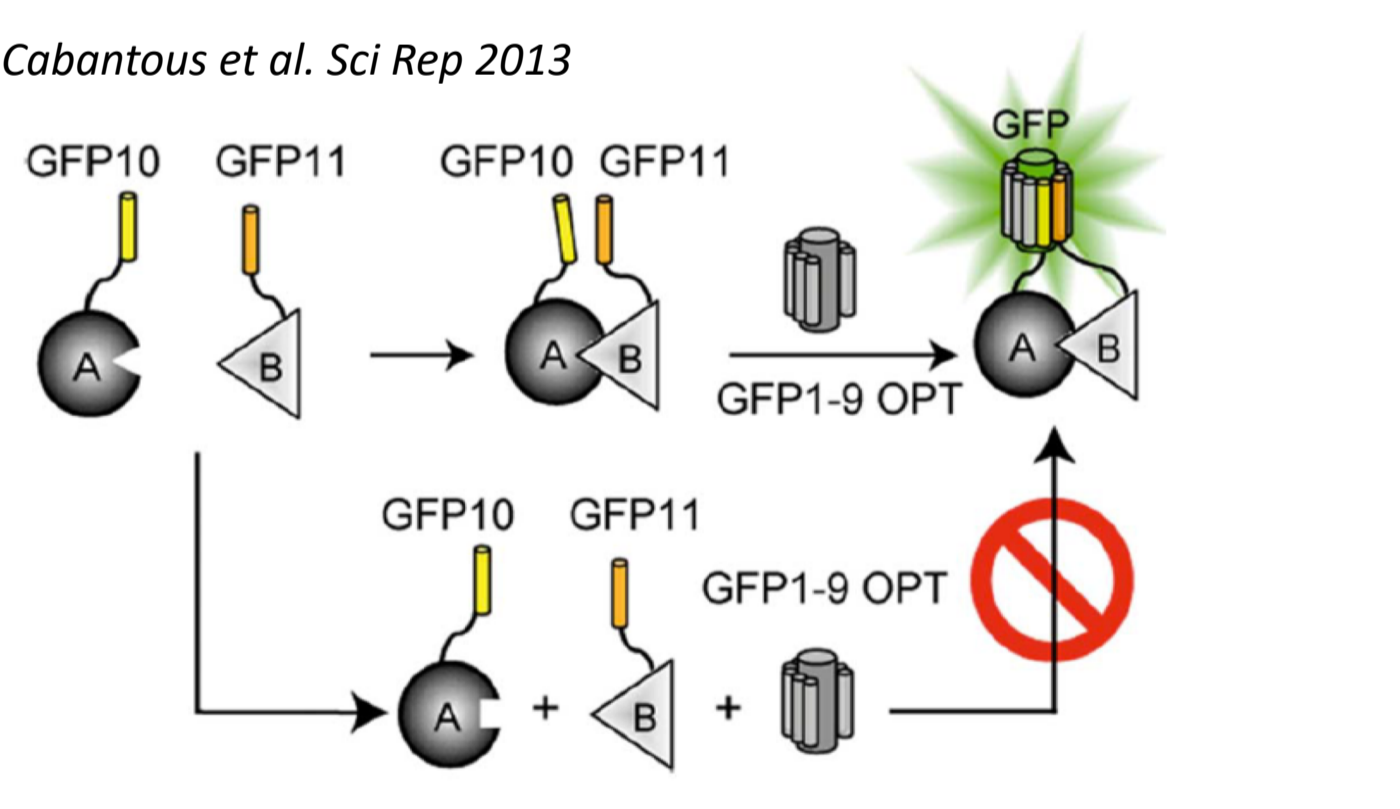
Figure from Alleronix

METHODOLOGY

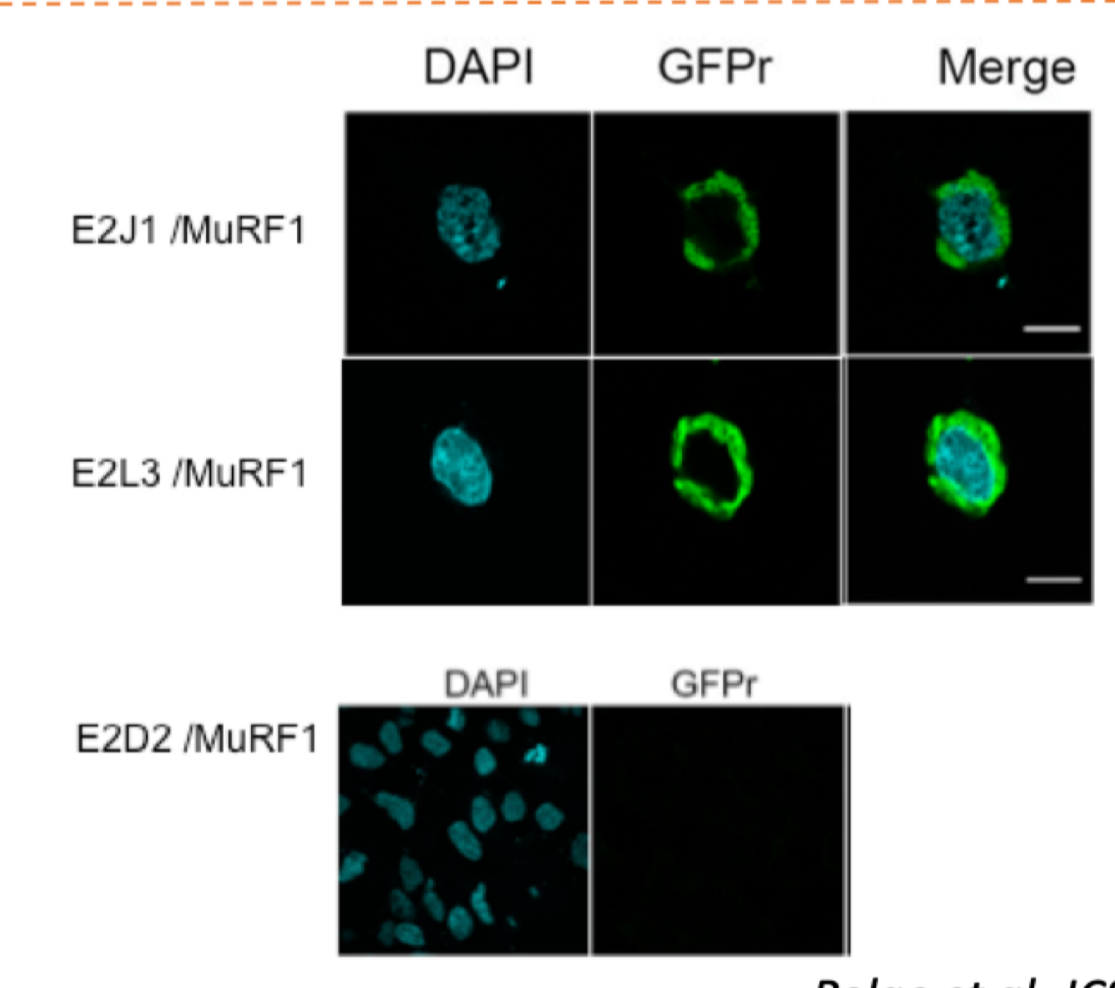
Design a stable system by adapting a split-GFP approach [3]:

- Production of an inducible (Tet-On/Off system) bicistronic vector
- Stable transfection in HEK293 cells and screening through cellular fluorescence signals

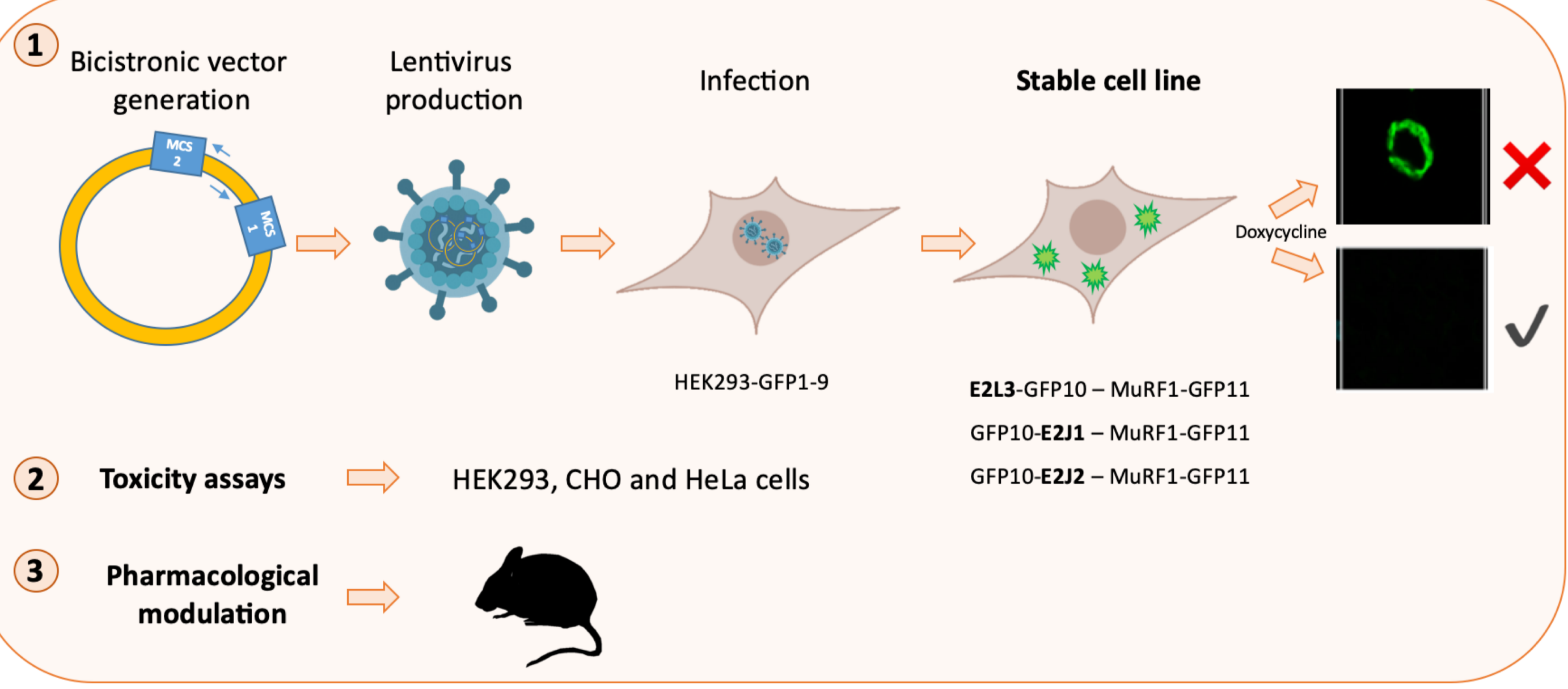
Split-GFP technology



Cabantous et al. Sci Rep 2013



Polge et al. JCSM 2018



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[1] Baehr, L. M., Furlow, J. D., & Bodine, S. C. (2011). Muscle sparing in muscle RING finger 1 null mice: response to synthetic glucocorticoids. *The Journal of physiology*, 589(19), 4759-4776.

[2] Polge, C., Cabantous, S., Deval, C., Claustre, A., Hauvette, A., Bouchenot, C., ... & Taillandier, D. (2018). A muscle-specific MuRF1-E2 network requires stabilization of MuRF1-E2 complexes by telethonin, a newly identified substrate. *Journal of cachexia, sarcopenia and muscle*, 9(1), 129-145.

[3] Cabantous, S., Nguyen, H. B., Pedelacq, J. D., Koraichi, F., Chaudhary, A., Ganguly, K., ... & Waldo, G. S. (2013). A new protein-protein interaction sensor based on tripartite split-GFP association. *Scientific reports*, 3, 2854.