Supplementary Information

Targeting Membrane Fragility in LGMD R2 through Pharmacological Autophagy Induction

Noëlla Grossi, Emilie Pellier, Quentin Miagoux, Manon Benabides, Jerome Polentes, Celine Leteur, Celine Bruge, Xavier Nissan

Supplementary figure legends

Figure S1. Selected doses of screened molecules and cell viability after 24 hours of treatment. (A) Quantification of the cell viability of S1173X dysferlin myoblasts after 24 hours of treatment with the different drugs relative to the control DMSO 0.1%. **(B)** Table summarizing the doses of compounds tested and cell viability after 24 hours of treatment.

Figure S2. Characterization of autophagic fluxes on the S1173X dysferlin myoblasts treated with all the compounds. (A) Formation of lysosome positive structures under control of different treatments after 24 hours. Representative confocal images depicting lysosomes (green) puncta in *DYSF*^{S1173X} immortalized myoblasts treated with 0.1% DMSO or each compound for 24 hours. Hoechst staining (blue) labels nuclei. Scale bar = 50 µm. **(B)** Formation of p62 positive structures under control of different treatments after 24 hours. Representative confocal images depicting p62 (red) puncta in *DYSF*^{S1173X} immortalized myoblasts treated with 0.1% DMSO or each compound for 24 hours. Hoechst staining (blue) labels nuclei. Scale bar = 50 µm.

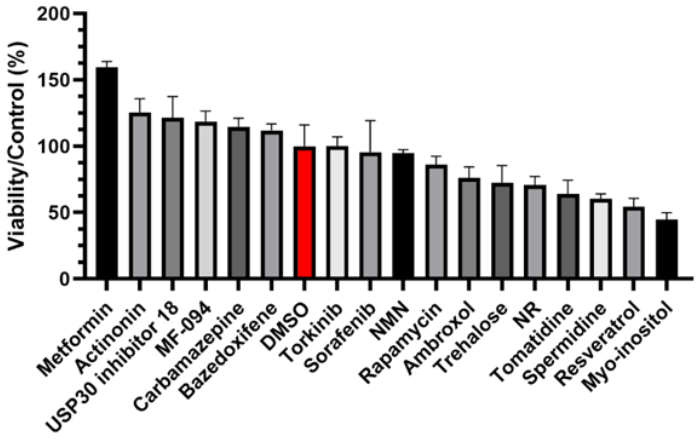
Figure S3. Ability of the molecules tested to modulate autophagy on healthy myoblasts. **(A)** Dot plot representation of the effects of the 17 compounds on lysosome expression after 24 hours of treatment and cell viability. **(B)** Quantification of total lysosome puncta area following treatment relative to the control DMSO 0.1%, using MetaXpress software. Each bar represents the mean ± SD (n=3). **(C)** Dot plot representation of the effects of the 17 compounds on p62 expression after 24 hours of treatment and cell viability. **(D)** Quantification of total p62 puncta area following treatment relative to the control DMSO 0.1%, using HCS Studio software. Each bar represents the mean ± SD (n=3). Abbreviations: NR, Nicotinamide riboside, NMN, nicotinamide mononucleotide.

Figure S4. Characterization of autophagic fluxes on healthy myoblasts treated with all the compounds. (A) Formation of lysosome positive structures under control of different treatments. Representative confocal images depicting lysosomes (green) puncta in *DYSF*^{S1173X} immortalized myoblasts treated with 0.1% DMSO or each compound for 24 hours. Hoechst staining (blue) labels nuclei. Scale bar = 50 µm. **(B)** Formation of p62 positive structures under control or each 6 effective compounds for 24 hours. Representative confocal images depicting p62 (red) puncta in *DYSF*^{S1173X} immortalized myoblasts treated with 0.1% DMSO or each compound for 24 hours. Hoechst staining (blue) labels nuclei. Scale bar = 50 µm.

Supplementary figure S5. Full-length western blot gels shown in figure 5C. (A) Uncropped western blot analysis of p62 expression in myoblasts treated with 0,1% DMSO or each 6 effective compounds for 24 hours. (**B**) Uncropped western blot analysis of Lc3 expression in the same cell extracts. (**C**) Uncropped western blot analysis of p62 and Lc3 expression in the same cell extracts. (**D**) Uncropped western blot analysis of β-actin expression in the same cell extracts.

Supplementary figure 1

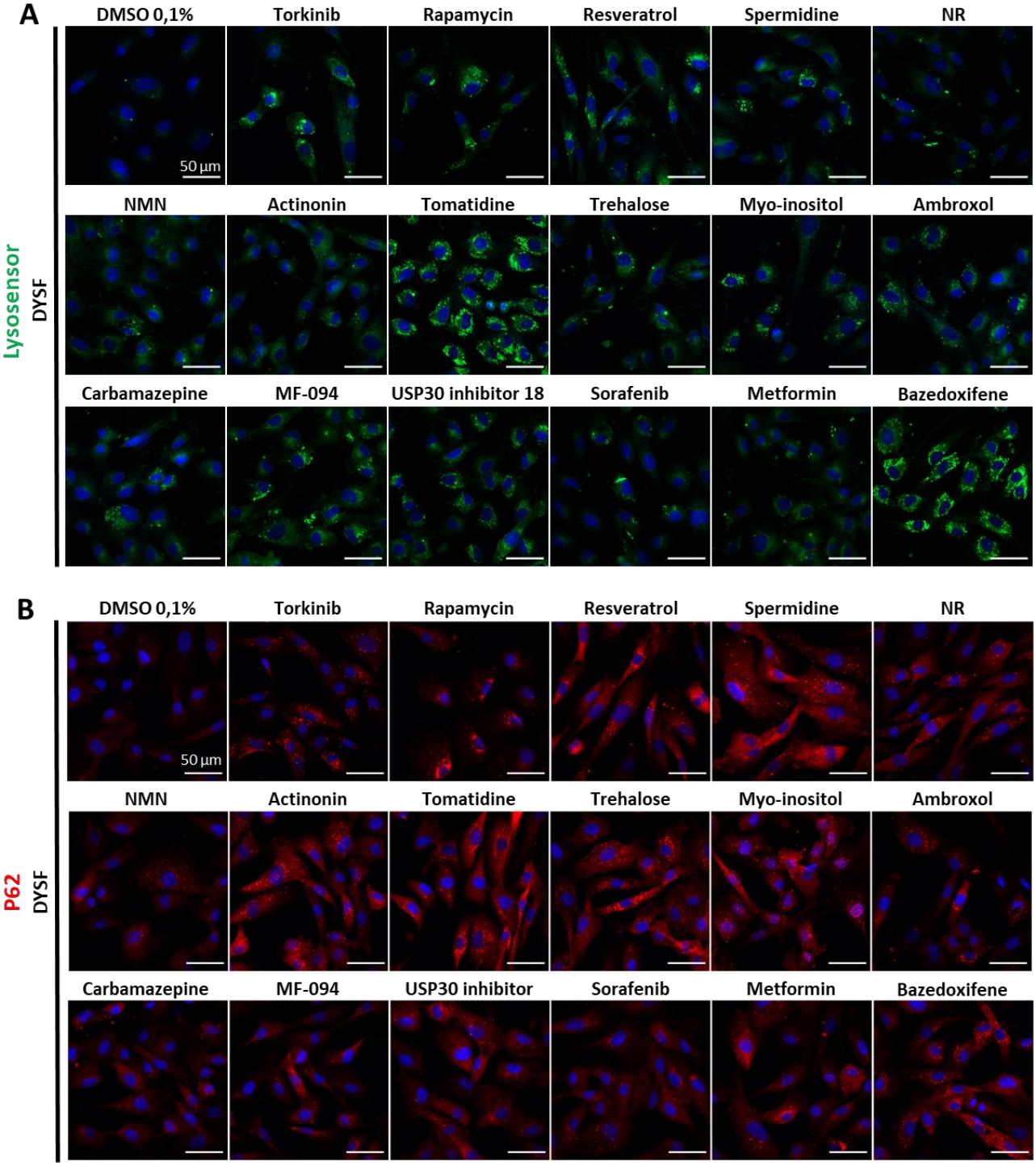
A



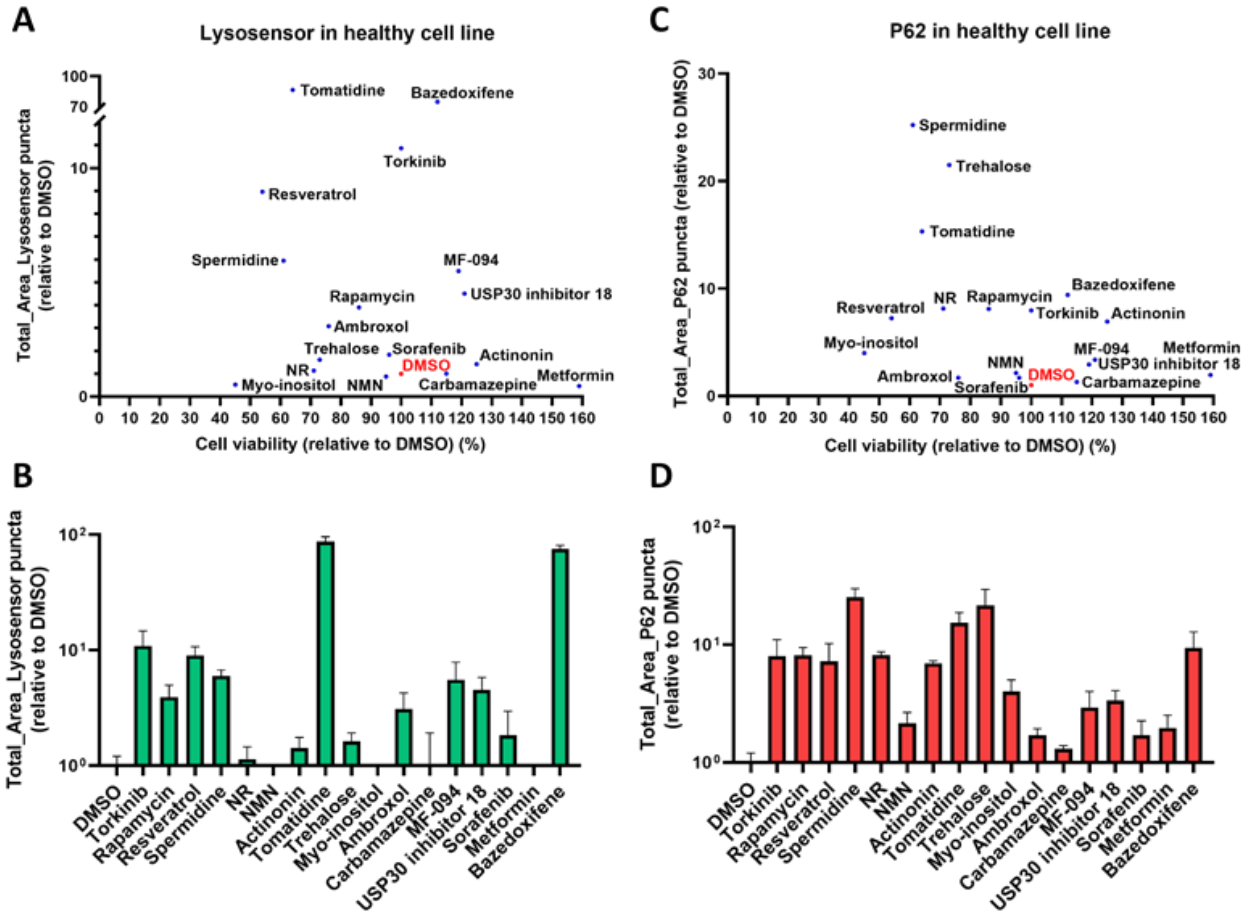
B

Compound	Concentration	Cell viability / Control (%)
DMSO	0,1%	100
Torkinib	10 µM	100
Rapamycin	20 µM	86
Resveratrol	300 µM	54
Spermidine	100 µM	61
NR	100 µM	71
NMN	300 µM	95
Actinonin	10 µM	125
Tomatidine	10 µM	64
Trehalose	50 mM	73
Myo-inositol	50 mM	45
Ambroxol	10 µM	76
Carbamazepine	30 µM	115
MF-094	10 µM	119
USP 30 inhibitor 18	10 µM	121
Sorafenib	10 µM	96
Metformin	200 µM	159
Bazedoxifene	2 µM	112

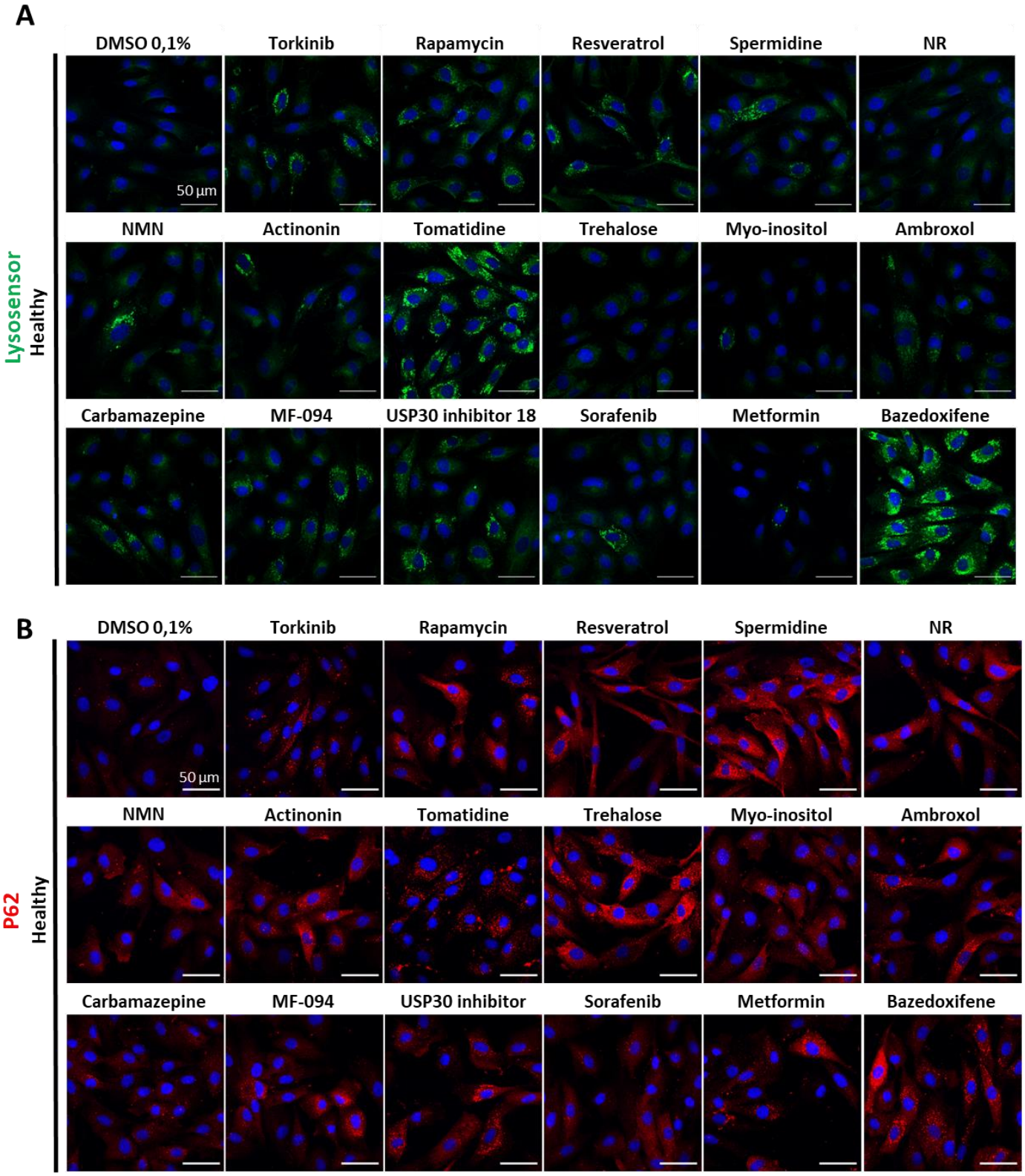
Supplementary figure 2



Supplementary figure 3



Supplementary figure 4



Supplementary figure 5