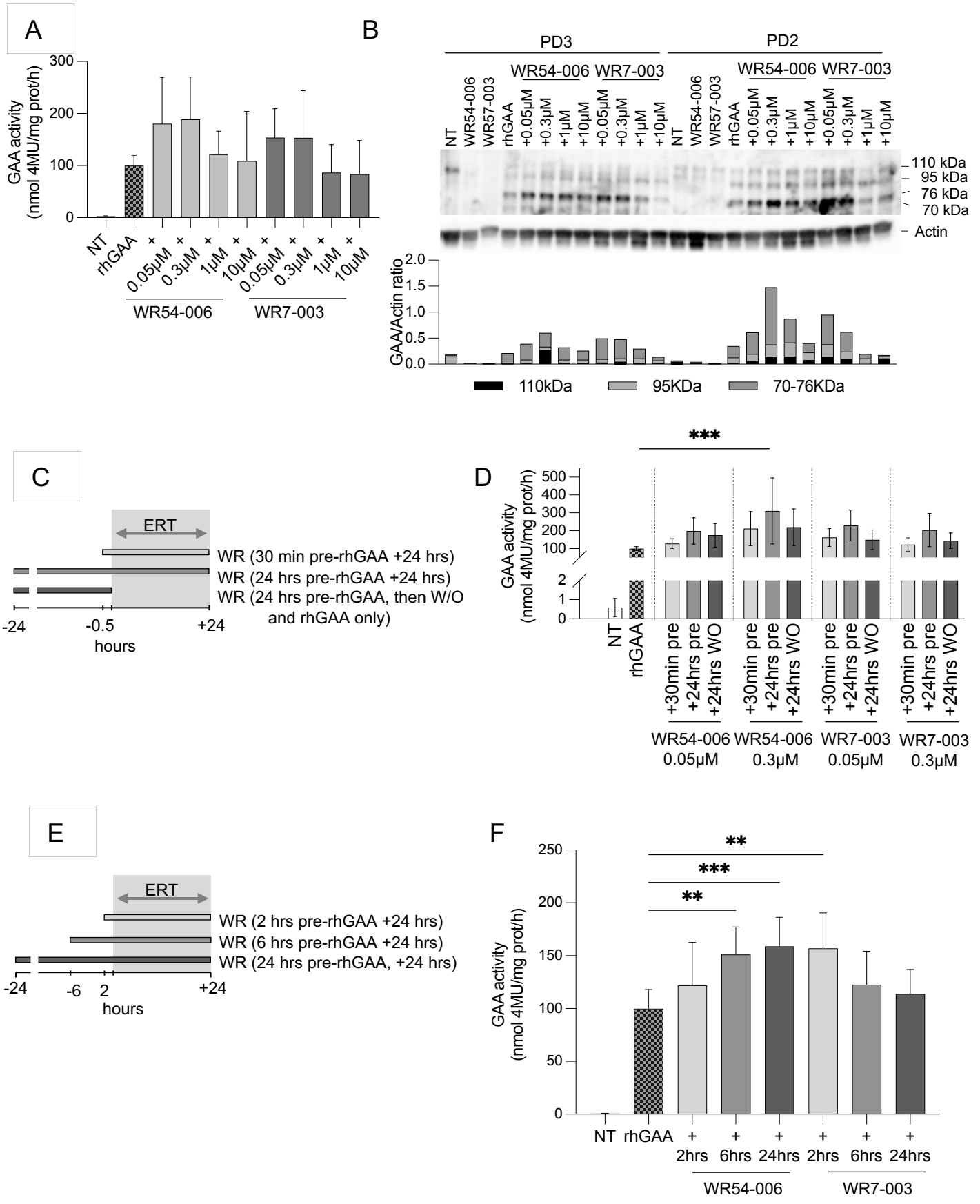
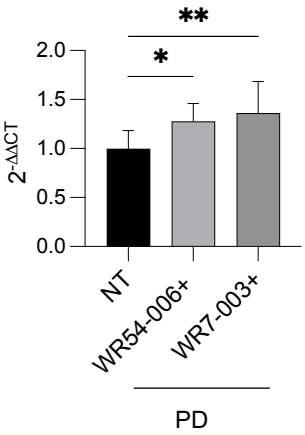
Figure S1 – Optimization of cell incubation protocols



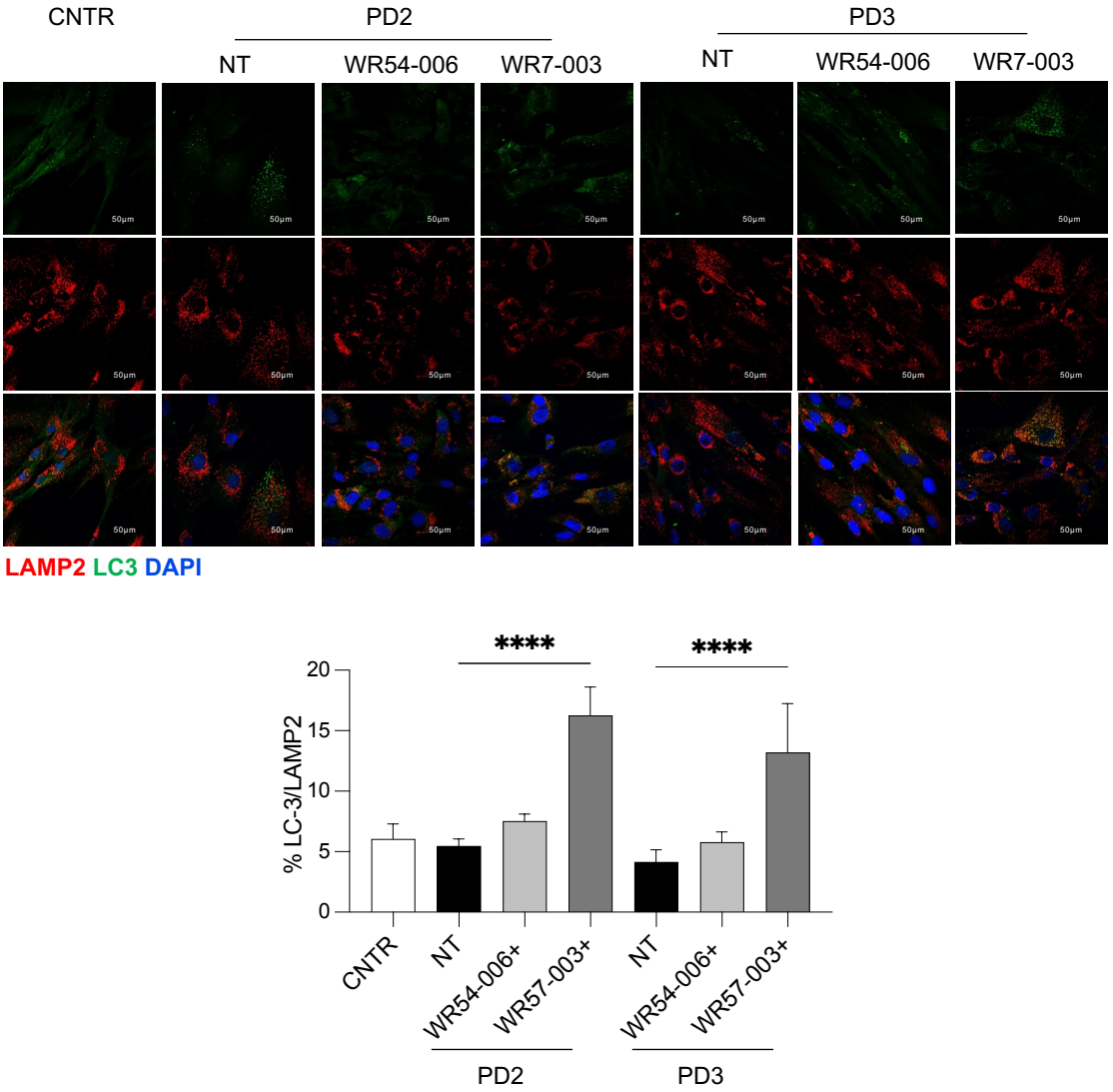
Legend to Figure S1: (A) GAA activity in PD fibroblasts treated with rhGAA in combination with increasing concentrations of WR54-006 or WR7-003. Activity is expressed as nmol 4-MU/mg protein.
(B) Representative western blot analysis of GAA processing in PD3 and PD2 fibroblasts following treatment with rhGAA and TRPML1 agonists. Actin was used as loading control. Densitometric quantification of indicated bands is shown below.
(C-D) Experimental design and corrisponding GAA activity following pre-treatment with TRPML1 agonists before rhGAA exposure or under washout (WO) conditions.
(E-F) Timing-dependent effects of TRPML1 agonists exposure (2, 6, or 24 hours pre-treatment) on rhGAA-mediated enzymatic correction.
Data are presented as mean $\pm$ SD. Statistical significance was determined by one-way ANOVA followed by post hoc multiple comparison testing. Significance is indicated as: **** $p<0.0001$; *** $p<0.0001$; ** $p<0.01$; * $p<0.05$.

Figure S2 – qRT-PCR of TRPML1 in PD cells incubated with agonist compounds



Legend to Figure S2: Quantitative real-time PCR analysis of TRPML1 mRNA levels in PD fibroblasts patients (n=3) treated with TRPML1 agonists (WR54-006 and WR7-003) compared with untreated cells. Gene expression was normalized to GAPDH and calculated using the $2^{-\Delta\Delta}$Ct method. Each biological replicate was analyzed in techical duplicate. Data are presented as mean $\pm$ SD. Statistical significance was determined by one-way ANOVA followed by post hoc multiple comparison testing. ** $p<0.01$; * $p<0.05$.

Figure S3 - IF LAMP2 LC3 in controls and in single PD fibroblast cell lines and quantitative analysis



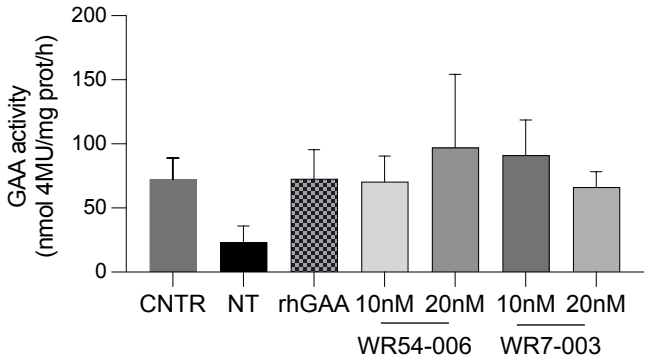
Legend to Figure S3: Representative immunofluorescence image of control (CNTR) and PD fibroblasts (PD2 and PD3) treated with WR54-006 and WR7-003 or left untreated (NT). Cells were stained for LAMP2 (red), LC3 (green), and nuclei (DAPI,blue). (Brightness +20%; contrast +30%) Scale bar: 50µm. LC3-LAMP2 colocalization was quantified using ImageJ and expressed as percentage of LC3 signal overlapping with LAMP2. Data are presented as mean ± SD. Statistical significance was determined by one-way ANOVA followed by post hoc multiple comparison testing: **** $p<0.0001$.

Figure S4 - Enhancement of GAA correction in patient-derived myoblasts

A

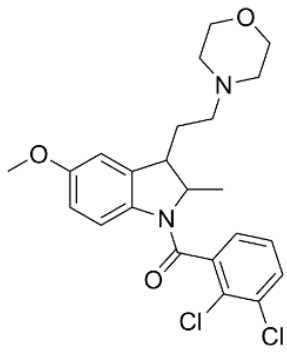
Pt ID	Genotype (DNA)	Genotype (protein)	Phenotype	Residual GAA activity (nmol 4-MU/mg prot/h)
PD7 myoblasts	c.-32-13T>G / c.989G>A	abnormal splicing / p.W330*	Late onset	12.60
PD8 myoblasts	c.-32-13T>G / c.1551+1G>C	abnormal splicing / abnormal splicing	Late onset	34.08

B



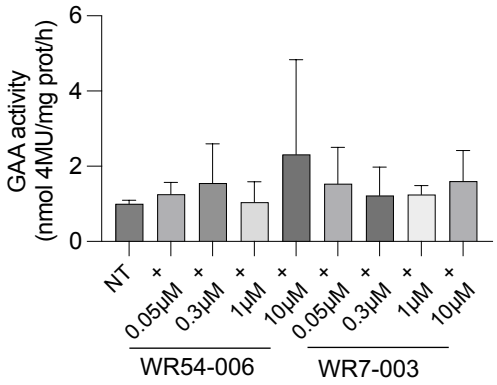
Legend to Figure S4: (A) Clinical and molecular characterization of PD patient-derived myoblast lines (PD7–PD8), including genotype, predicted protein effect, phenotype, and residual GAA enzymatic activity. (B) GAA enzymatic activity measured in human myoblasts under untreated conditions (NT) and following treatment with rhGAA alone or in combination with WR54-006 and WR7-003 at the indicated concentrations. GAA activity is expressed as nmol 4-MU/mg protein/h. Data are presented as mean ± SD.

Figure S5- Chemical structure of the TRPML1 inhibitor ML-SI1



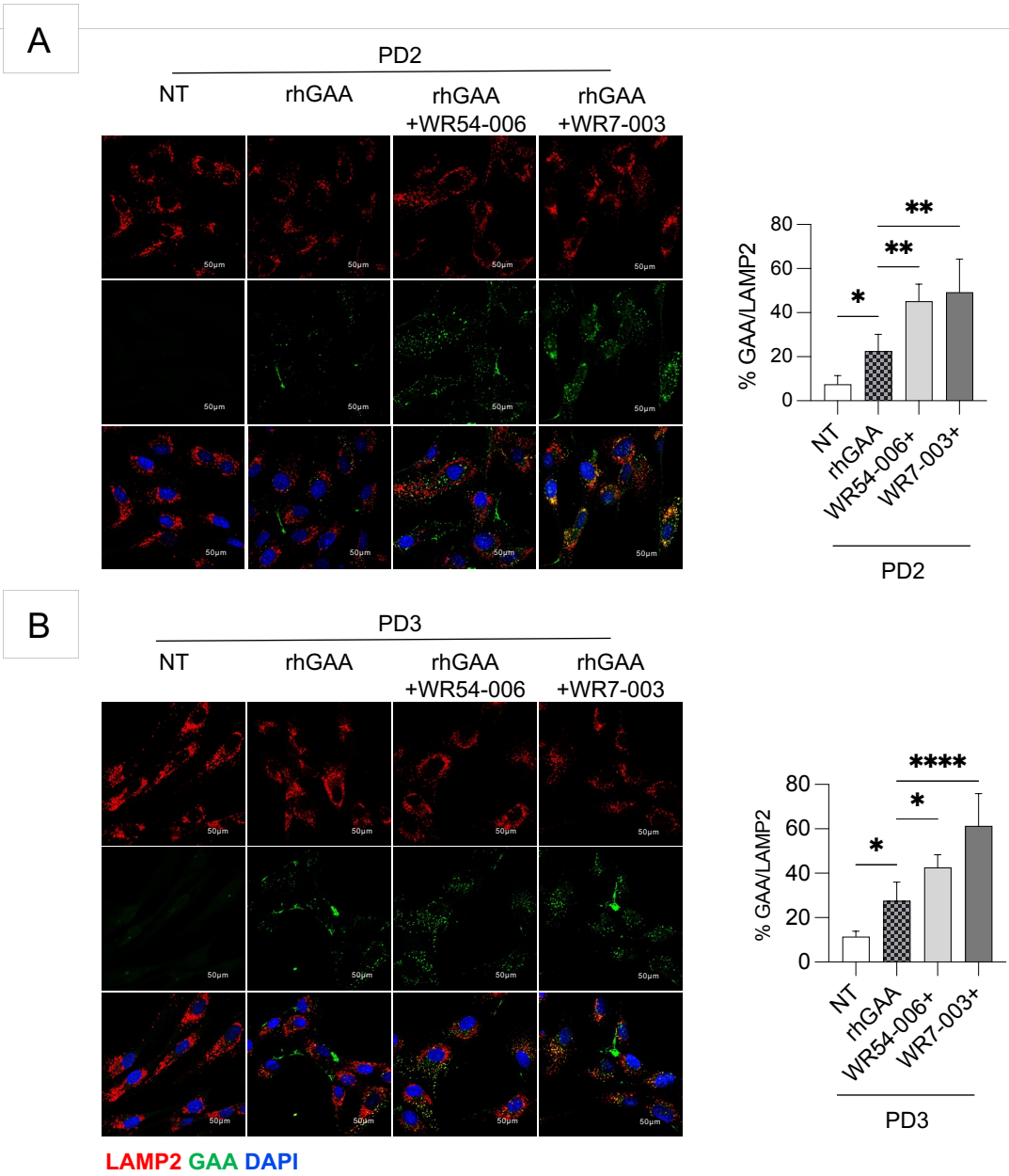
Legend to Figure S4: Chemical structure of ML-SI1, a selective inhibitor of lysosomal TRPML1 channel.

Figure S6 – Dose-dependent effects of TRPLM1 agonists on endogenous GAA activity



Legend to Figure S5: Endogenous GAA enzymatic activity was measured in PD fibroblasts (n=6) following 48-hour treatment with increasing concentrations (0.05-10 μM) of WR54-006 and WR7-003 compared with untreated cells (NT). GAA activity is expressed as nmol 4-MU/mg protein/hour. Data are presented as mean ± SD. Normal control values are 64.4+22.9 nmol 4-MU/mg protein/hour.

Figure S7– TRPML1 agonists improve rhGAA lysosomal trafficking in additional PD fibroblast lines



Legend to Figure S6: Representative immunofluorescence images of PD fibroblasts from PD2 (A) and PD3 (B) treated with rhGAA alone or in combination with WR54-006 and WR7-003. Cells were stained for LAMP2 (red), GAA (green), and nuclei (DAPI,blue). (Brightness +20%; contrast +30%) Scale bar: 50µm. Quantitative analysis of GAA-LAMP2 colocalization was performed using ImageJ and is expressed as percentage of GAA signal overlapping with LAMP2. Data are presented as mean + SD. Statistical significance was determined by one-way ANOVA followed by post hoc multiple comparison testing. Significance is indicated as: **** p<0.0001; *** p<0.001; ** p<0.01; * p<0.05.