STF-62247 and pimozide induce autophagy and autophagic cell death in mouse embryonic fibroblasts

Maximilian N. Kinzler[1,3§], Svenja Zielke[1§], Simon Kardo[1], Nina Meyer[2], Donat Kögel[2], Sjoerd J. L. van Wijk*[1], Simone Fulda #*[1,3,4]

[1]Institute for Experimental Cancer Research in Pediatrics, Goethe-University Frankfurt, Komturstr. 3a, 60528 Frankfurt, Germany

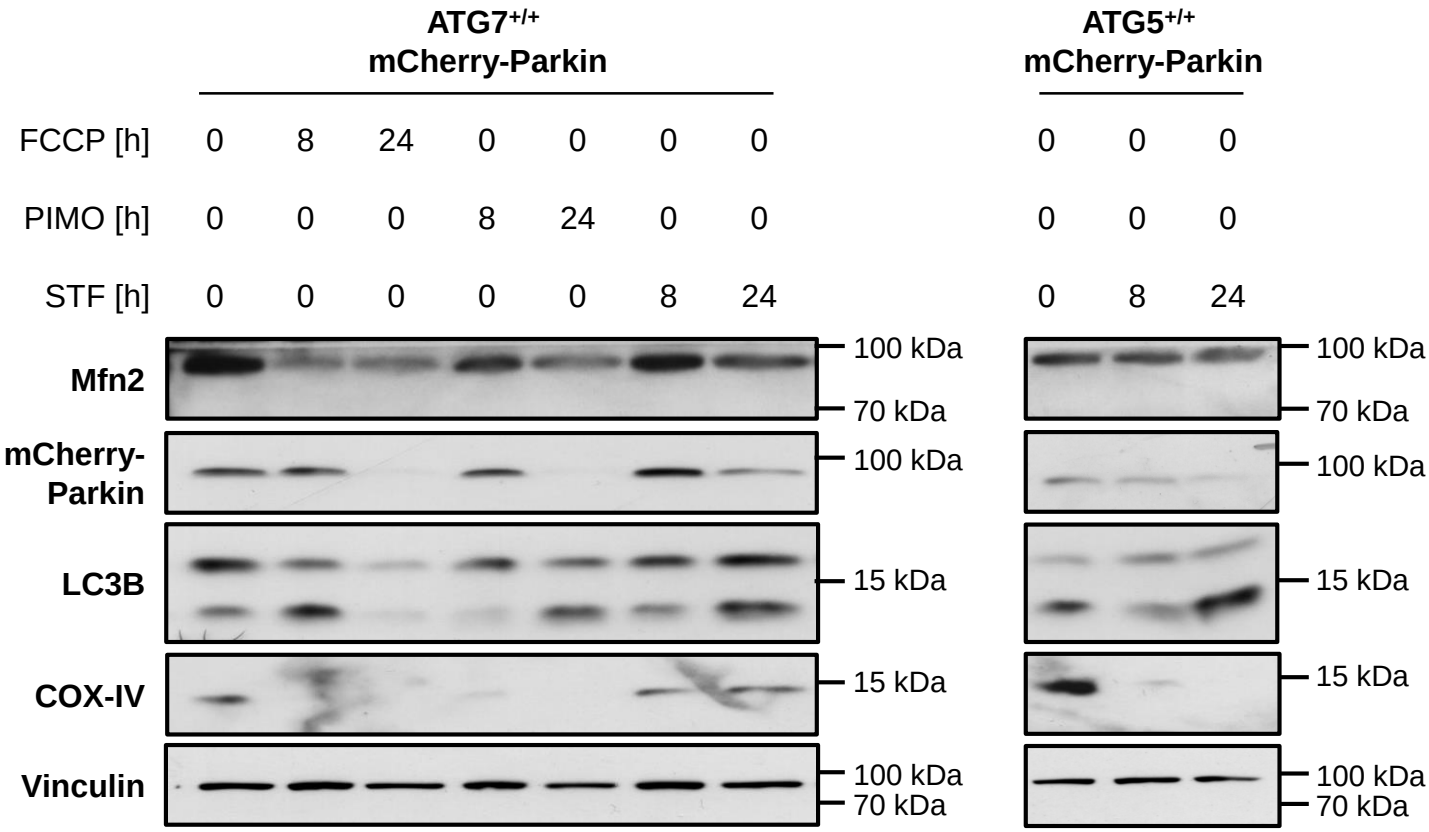
[2]Experimental Neurosurgery, Goethe-University Hospital, Theodor-Stern-Kai 7, 60590 Frankfurt, Germany

[3]German Cancer Consortium (DKTK), Partner Site Frankfurt, Germany

[4]German Cancer Research Center (DKFZ), Heidelberg, Germany

§ shared first authorship, * shared senior authorship, # corresponding author

Pimozide decreases the levels of the mitochondrial proteins Mfn2 and COXIV in *Atg5*+/+ and *Atg7*+/+ cells . STF-26647 decreases levels of COXIV, but not of Mfn2 in *Atg5*+/+ cells, whereas STF-26647 does not decrease levels of COXIV or Mfn2 in Atg7+/+ cells. ATG5$^{+/+}$ and ATG7$^{+/+}$ MEFs were transiently transfected with mCherry-Parkin followed by treatment with 10 µM FCCP, 10 µM pimozide or 10 µM STF-62247 for the indicated time points followed by Western blotting with vinculin as loading control. Uncropped blots are shown in Suppl. Fig. 13. Mfn2 = mitofusin-2



STF-26647 and pimozide do not induce selective autophagy of MG-132-induced aggregates. ATG5+/+ and ATG5-/- MEFs were pre-treated with rapamycin (7.5 uM), STF or PIMO at the indicated concentrations for two hours prior to treatment with MG-132 (3 uM) and the compounds for 18 hours followed by Western Blotting with GAPDH as loading control. Uncropped blots are shown in Suppl. Fig. 14.

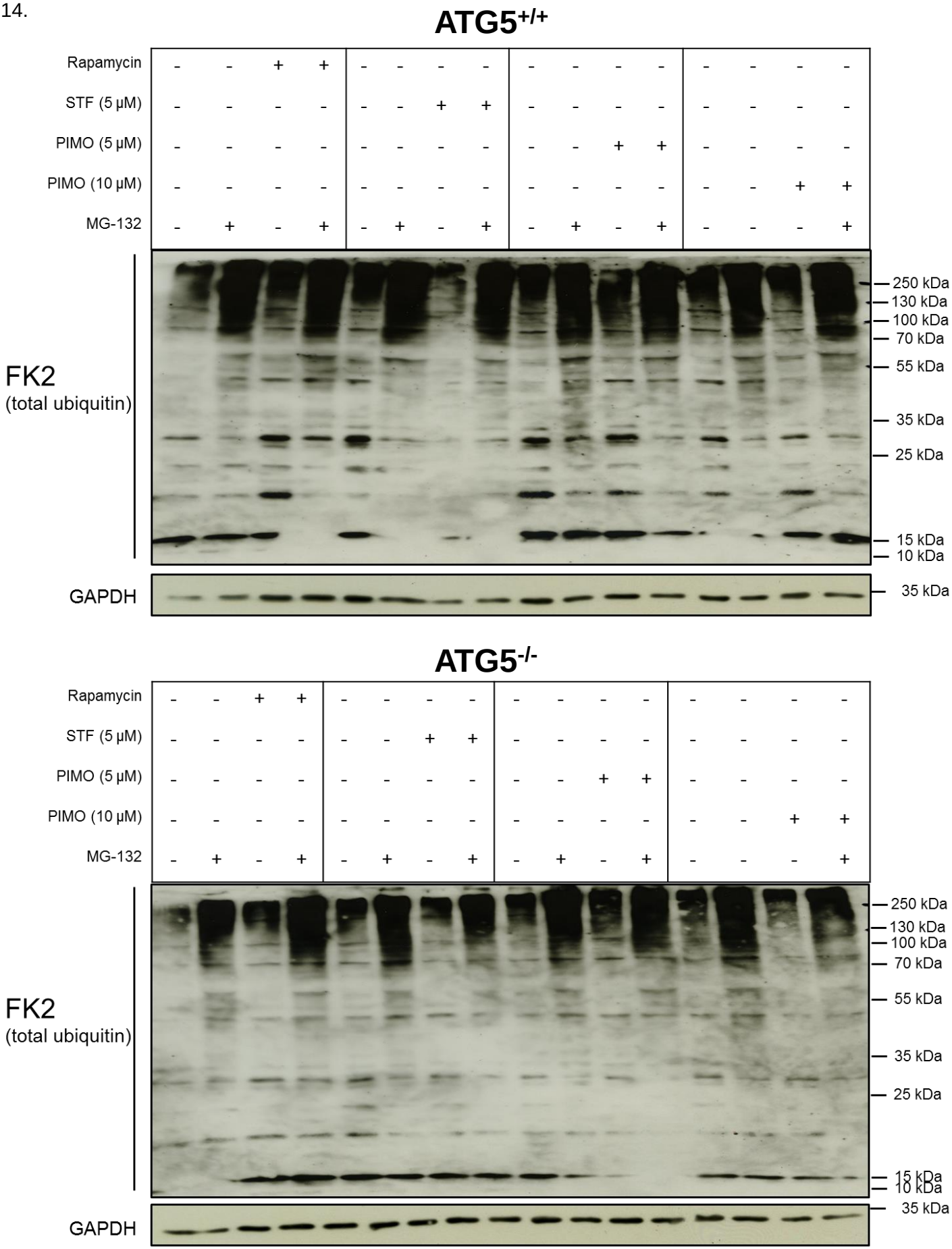
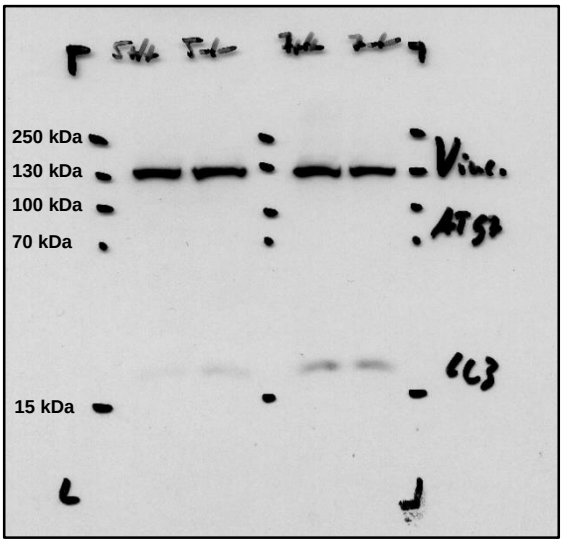
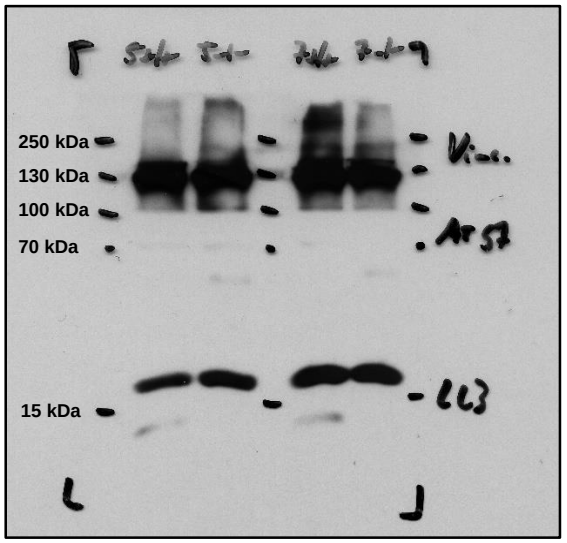


Figure 1A/B: original blots in different exposures

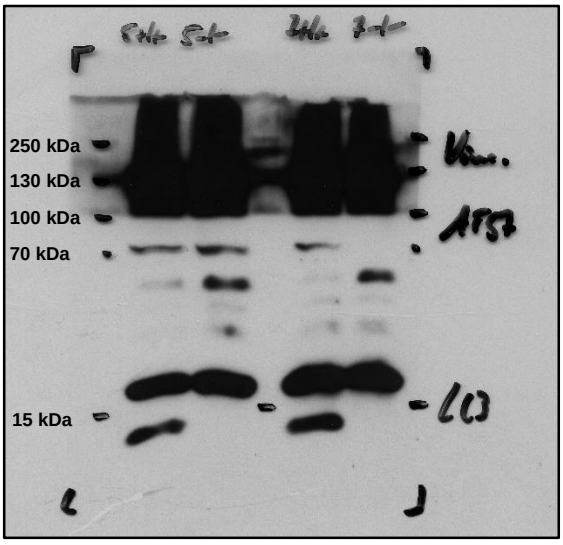
(1) Vinculin, ATG7, LC3B



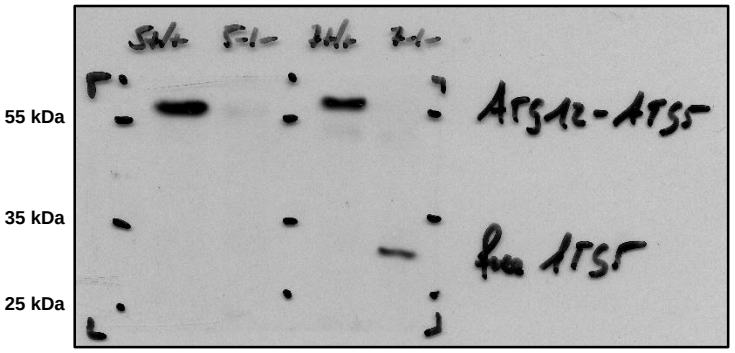
(2) Vinculin, ATG7, LC3B



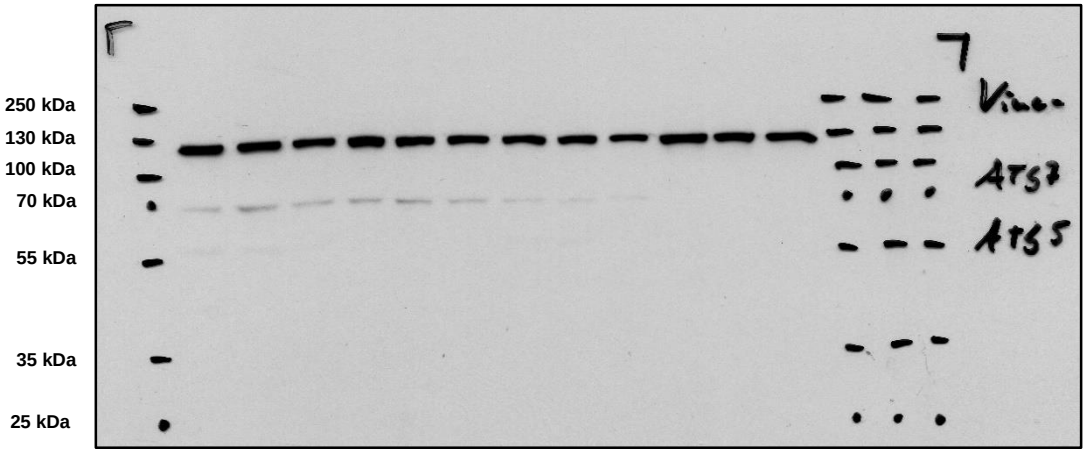
(3) Vinculin, ATG7, LC3B



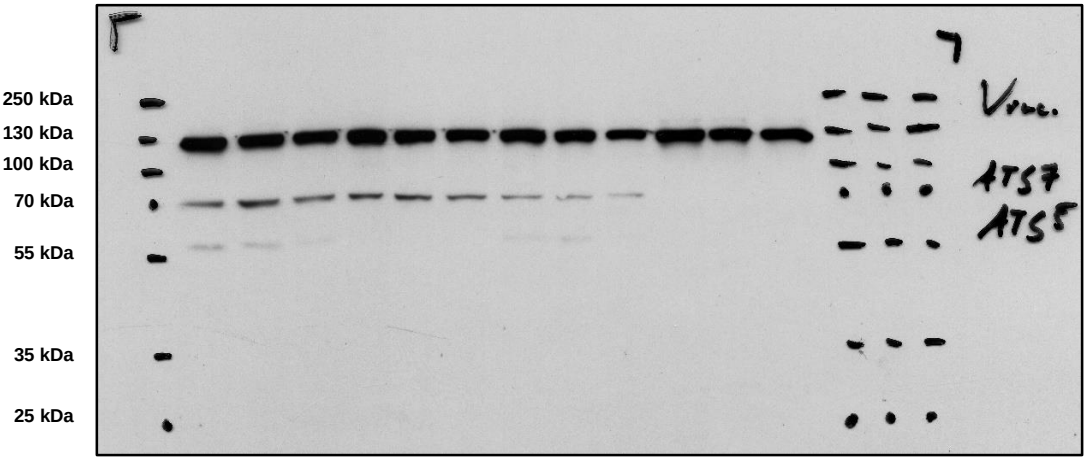
(4) ATG5



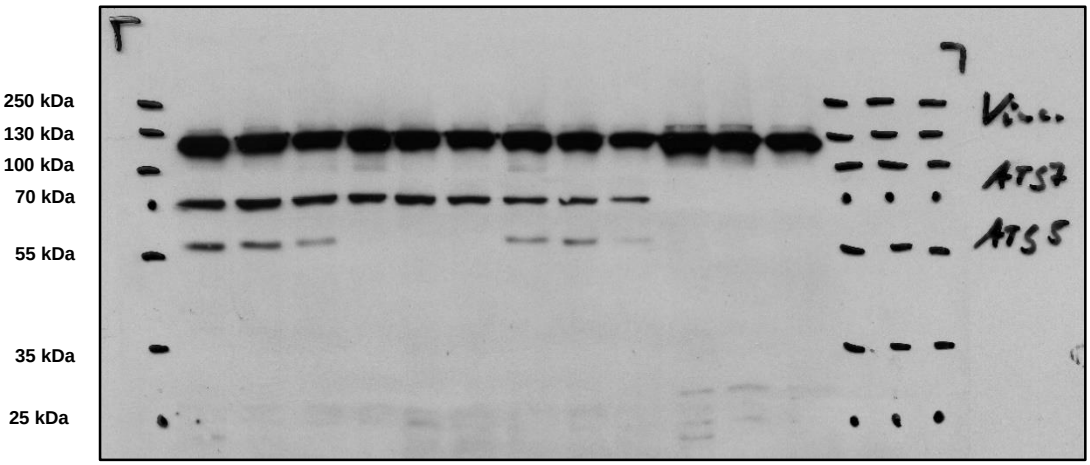
(1) Vinculin, ATG7, ATG5



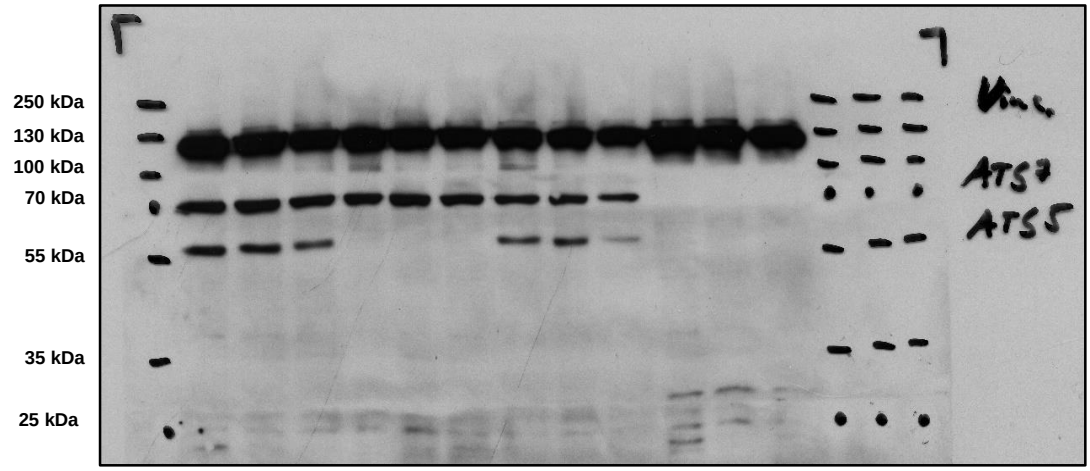
(2) Vinculin, ATG7, ATG5



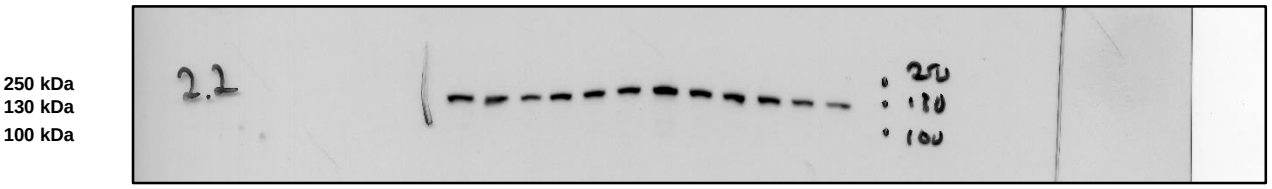
(3) Vinculin, ATG7, ATG5



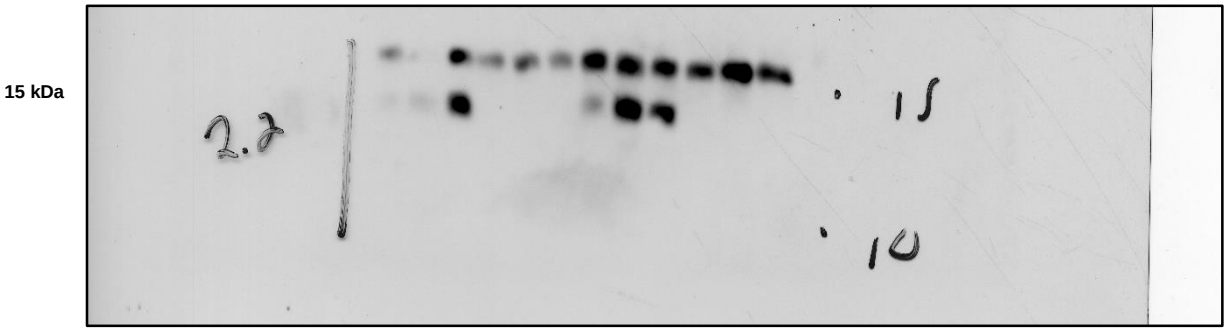
(4) Vinculin, ATG7, ATG5



(5) Vinculin



(6) LC3B



(7) LC3B

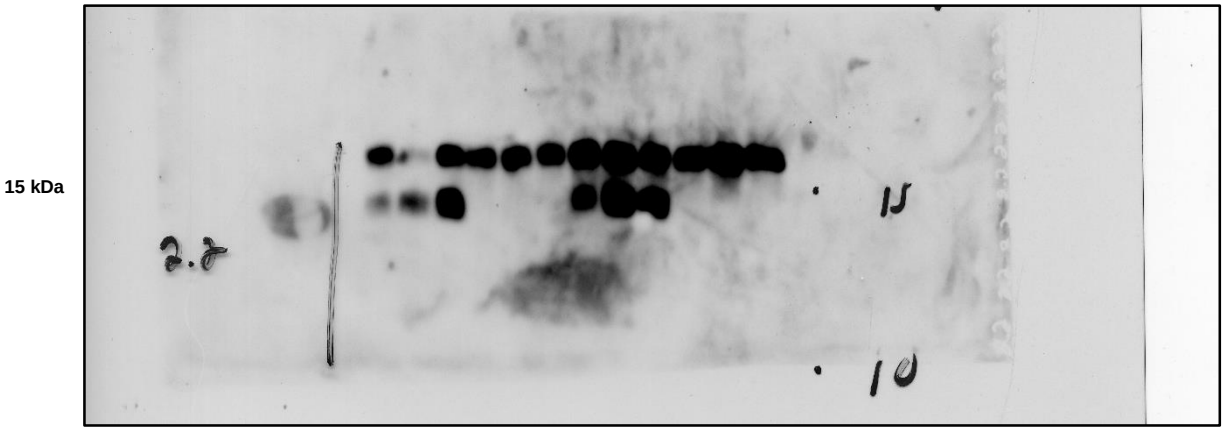
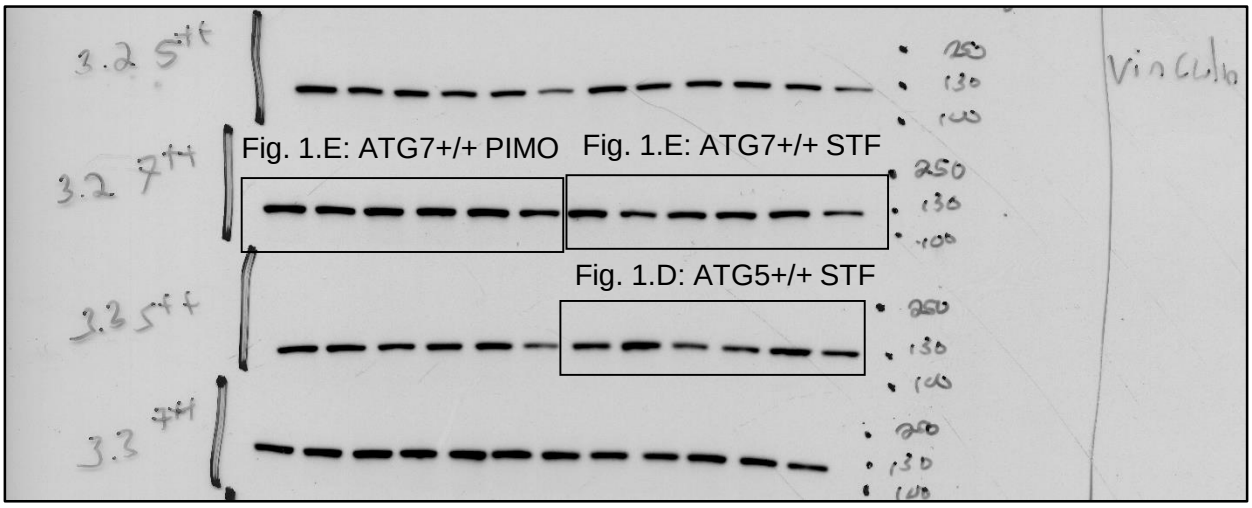


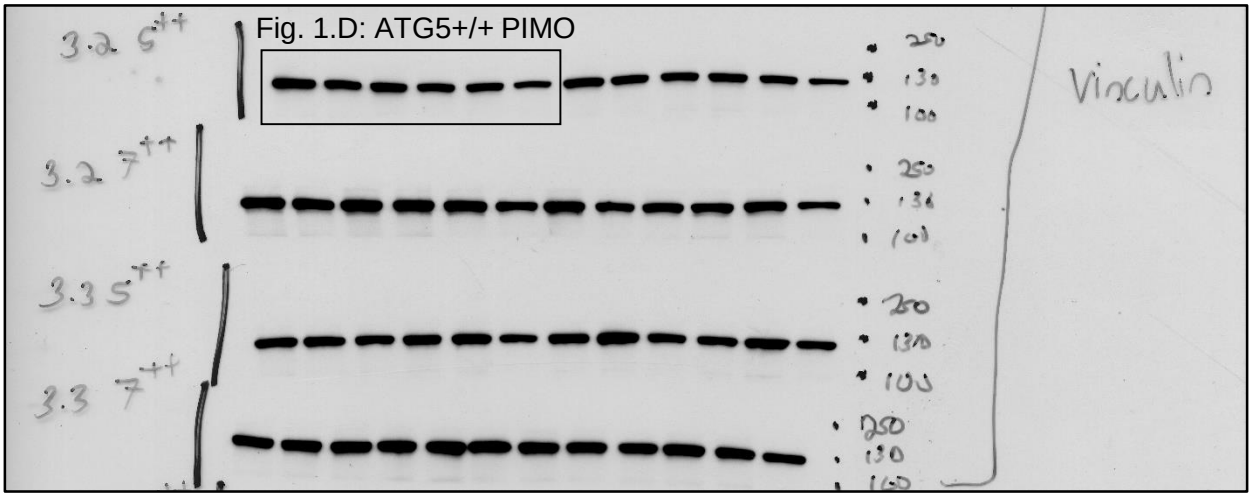
Figure 1D/E: original blots in different exposures

Marked samples are part of the present study.

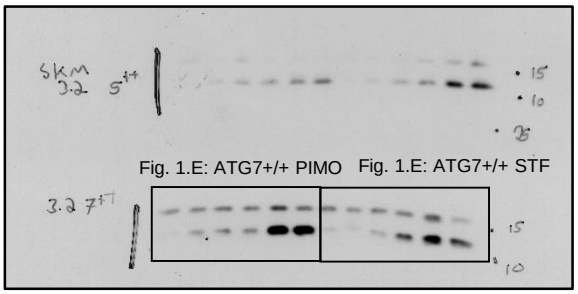
(1) Vinculin



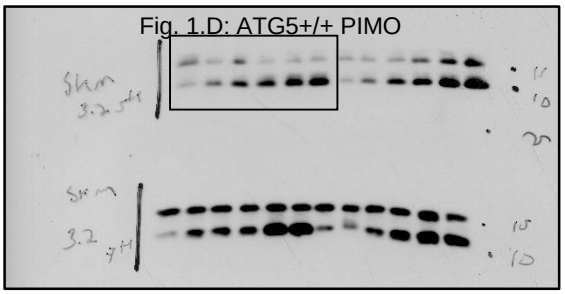
(2) Vinculin



(3) LC3B



(4) LC3B



(5) LC3B

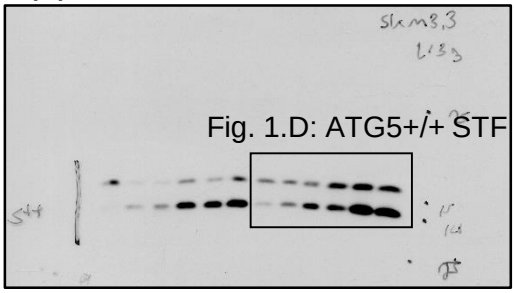
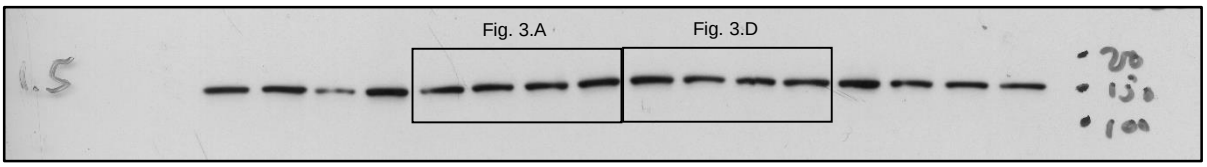


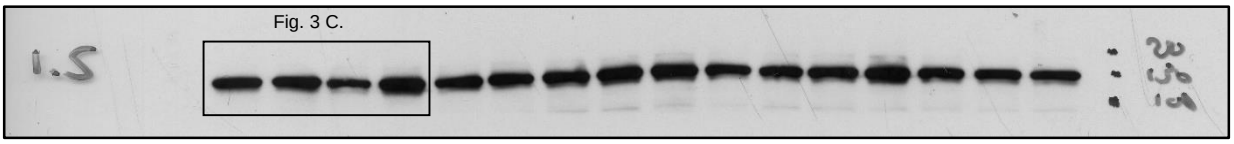
Figure 3A/B/C/D: original blots in different exposures

Marked samples are part of the present study.

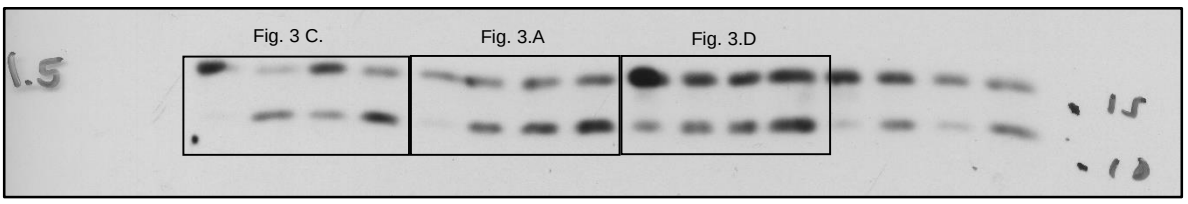
(1) Vinculin



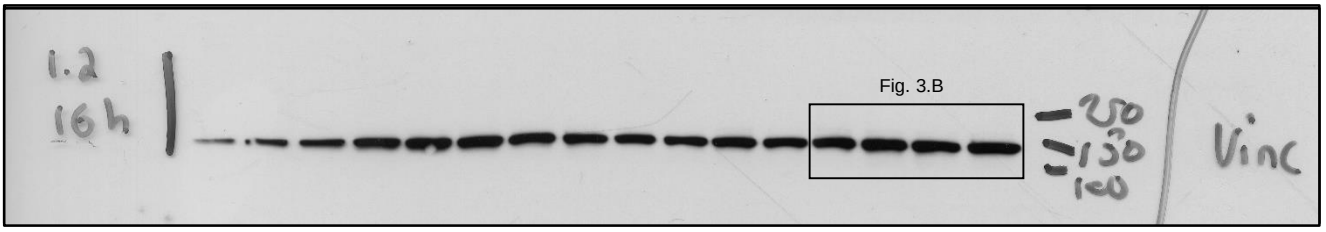
(2) Vinculin



(3) LC3B



(4) Vinculin



(5) LC3B

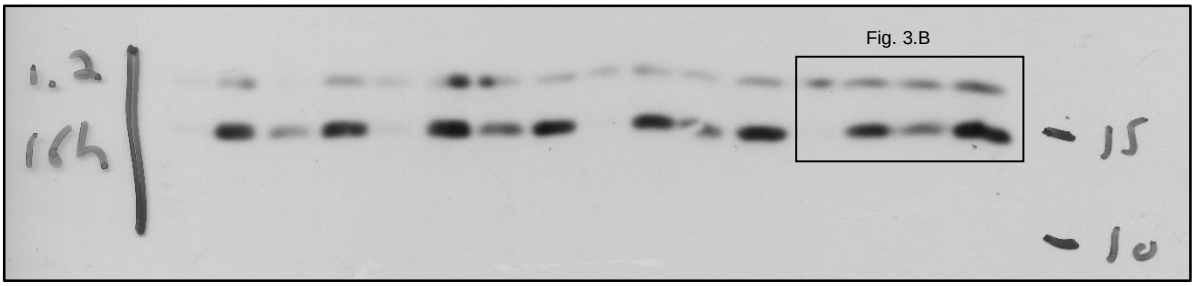
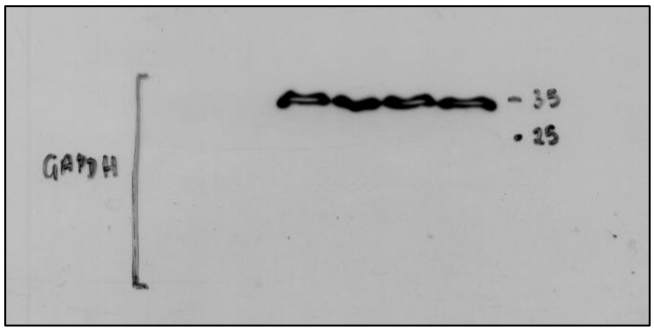
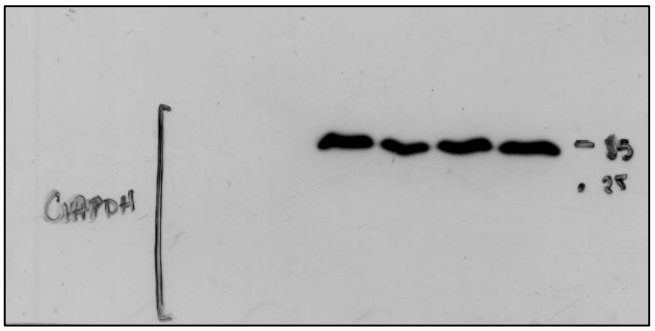


Figure 7A: original blots in different exposures

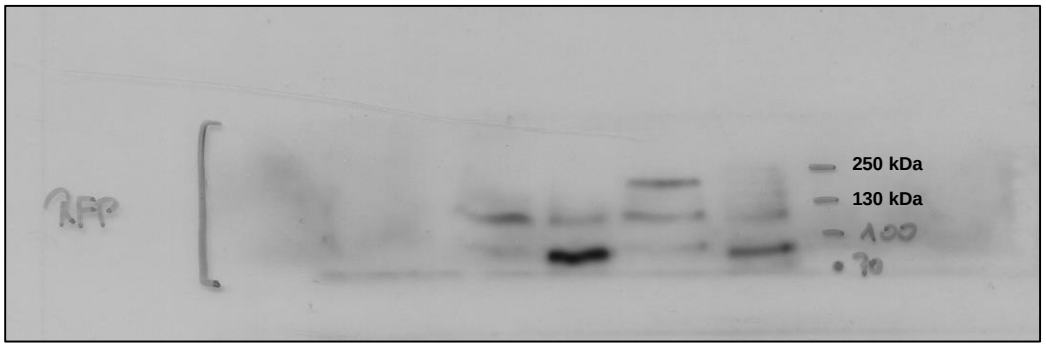
(1) GAPDH



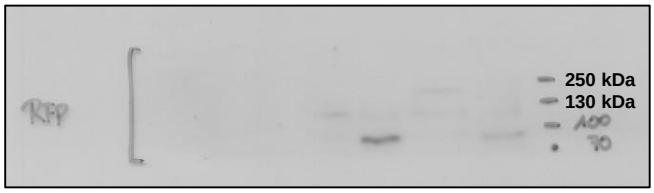
(2) GAPDH



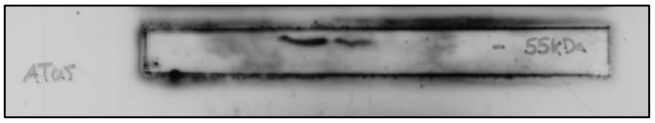
(3) RFP



(5) RFP



(6) ATG5



(4) RFP, ATG5

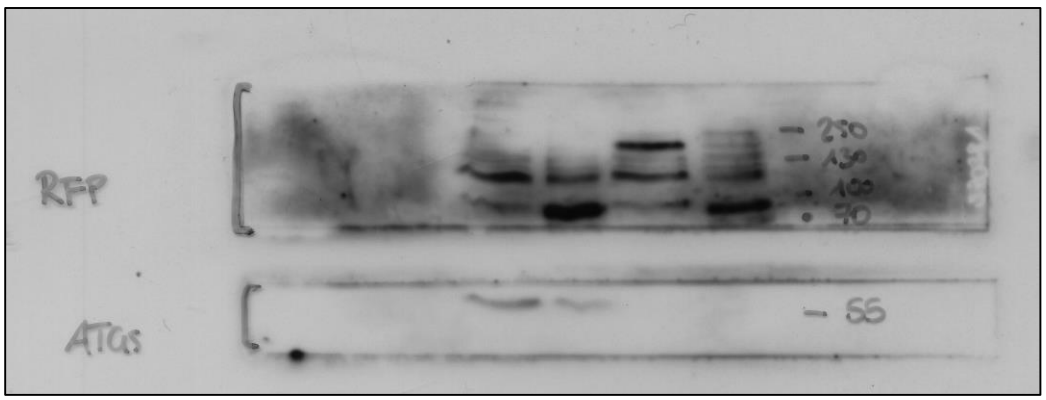
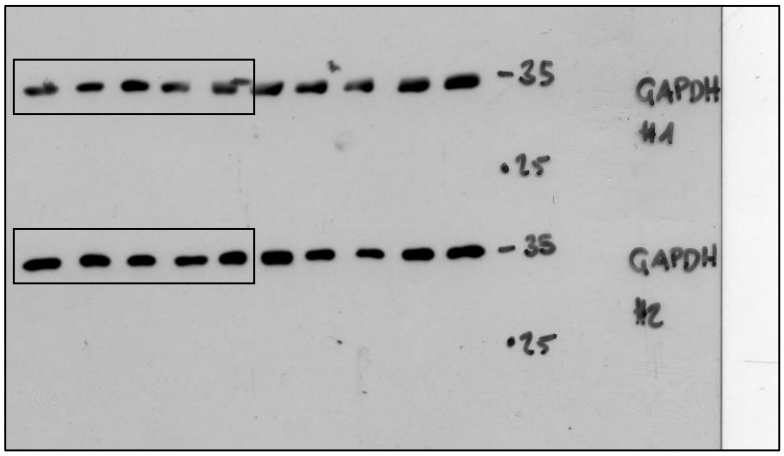


Figure 7C: original blots in different exposures

Marked samples are part of the present study. Lysates were loaded onto two different gels (#1 and #2). #1 and #2 indicates which proteins were detected from the respective gel.

(1) GAPDH (#1), GAPDH (#2)



(2) Mitofusin-2 (#1), GAPDH (#1), GAPDH (#2)

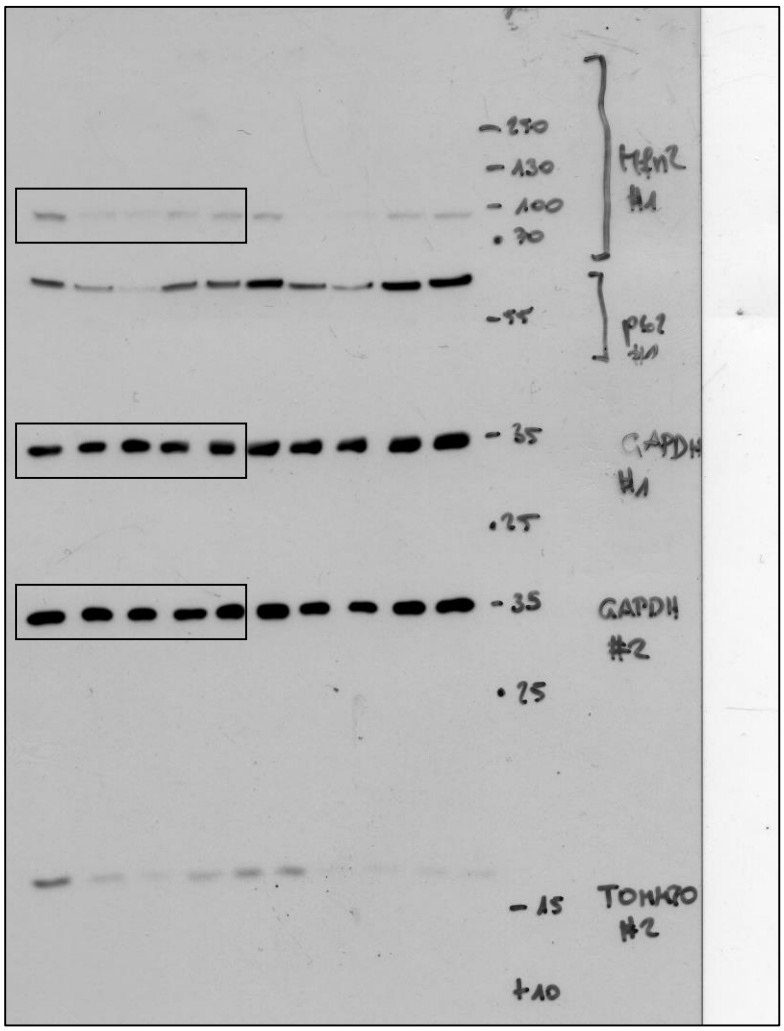
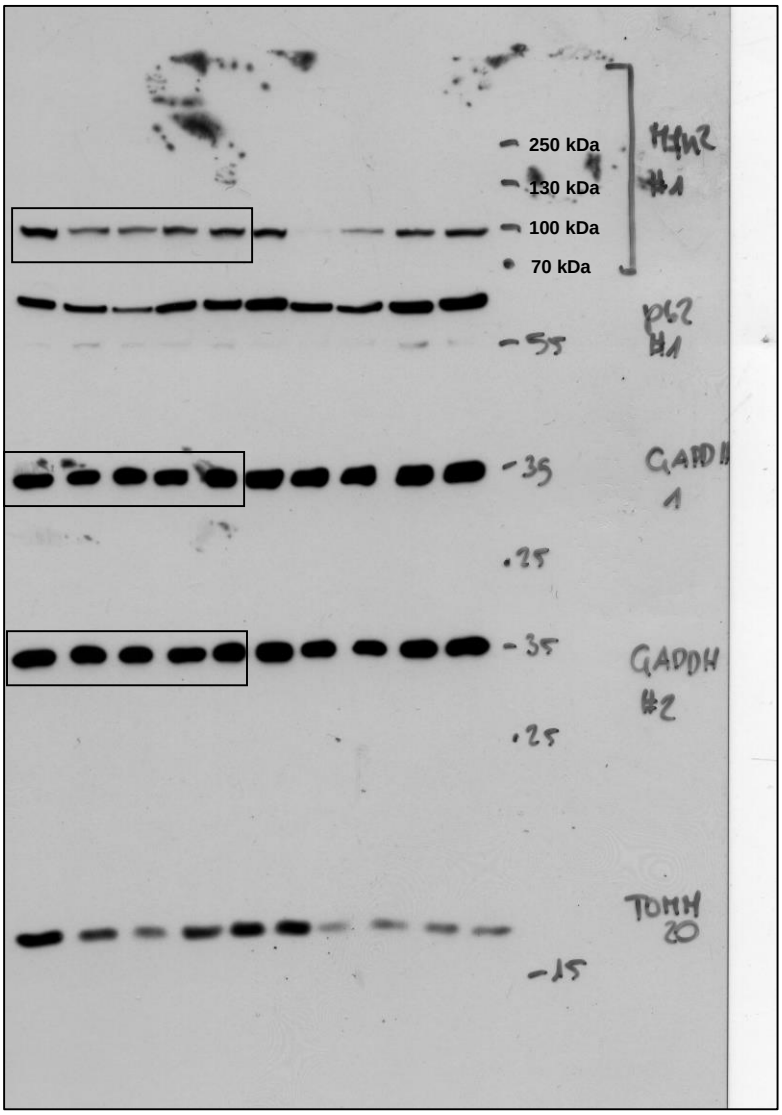


Figure 7C: original blots in different exposures

Marked samples are part of the present study. Lysates were loaded onto two different gels (#1 and #2). #1 and #2 indicates which proteins were detected from the respective gel.

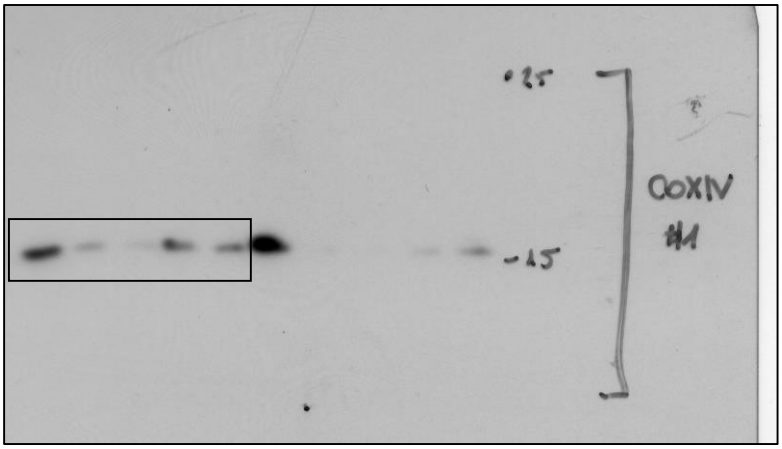
(3) Mitofusin-2 (#1), GAPDH (#1), GAPDH (#2)



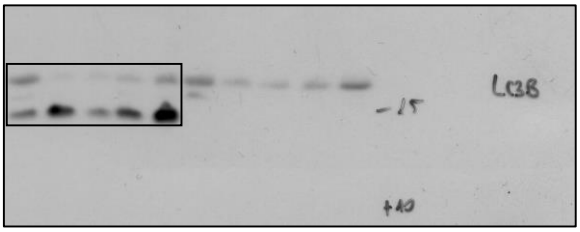
<u>Figure 7C: original blots in different exposures</u> Suppl. Fig. 12

Marked samples are part of the present study. Lysates were loaded onto two different gels (#1 and #2). #1 and #2 indicates which proteins were detected from the respective gel.

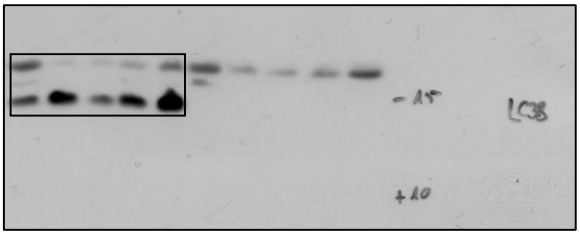
(4) COX-IV (#1)



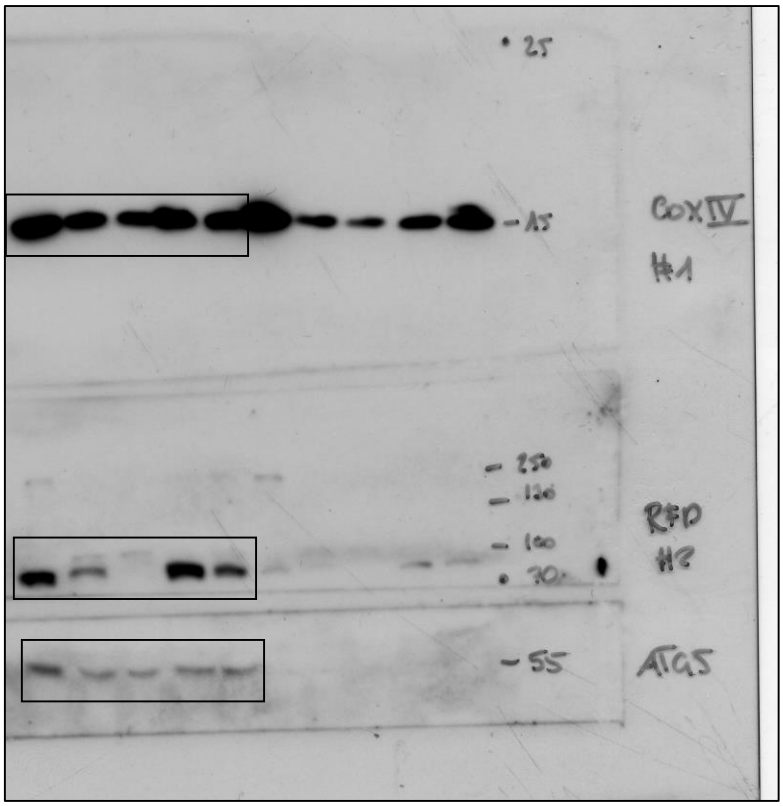
(5) LC3B (#2)



(6) LC3B (#2)



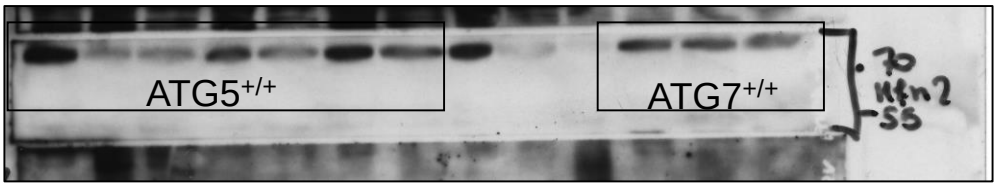
(7) COX-IV (#1), RFP (#2), ATG5 (#2)



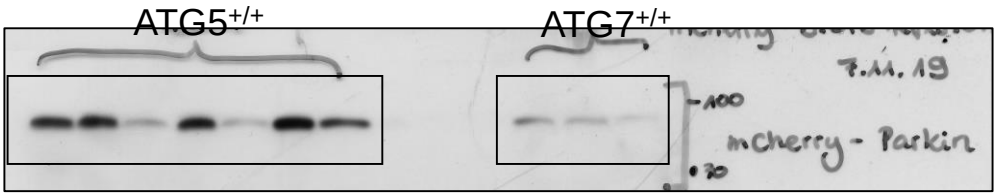
Suppl. Fig. 1: original blots in different exposures

Marked samples are part of the present study.

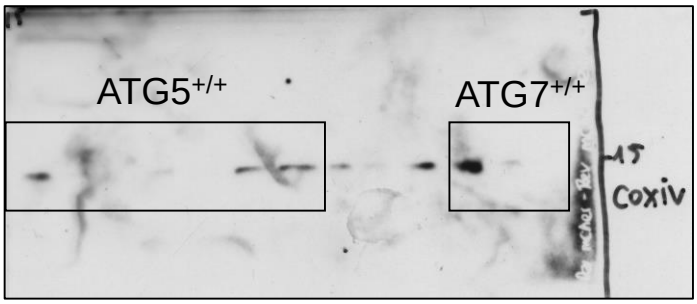
(1) Mitofusin-2



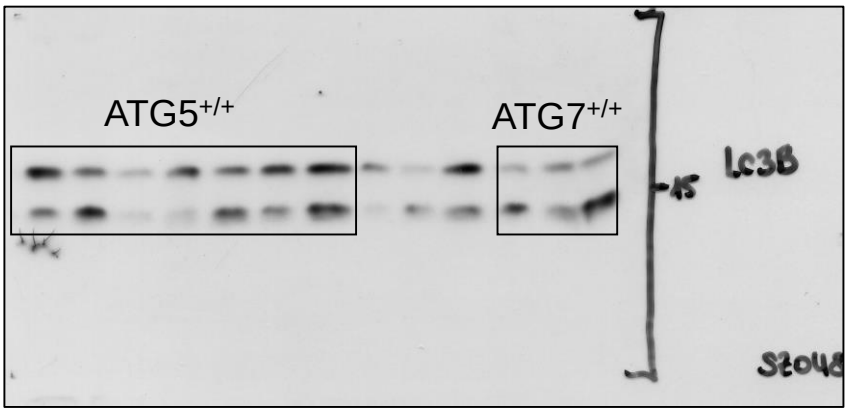
(2) mCherry-Parkin



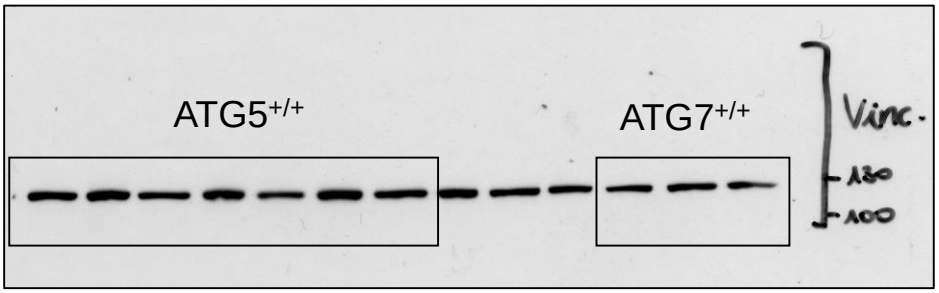
(3) COXIV



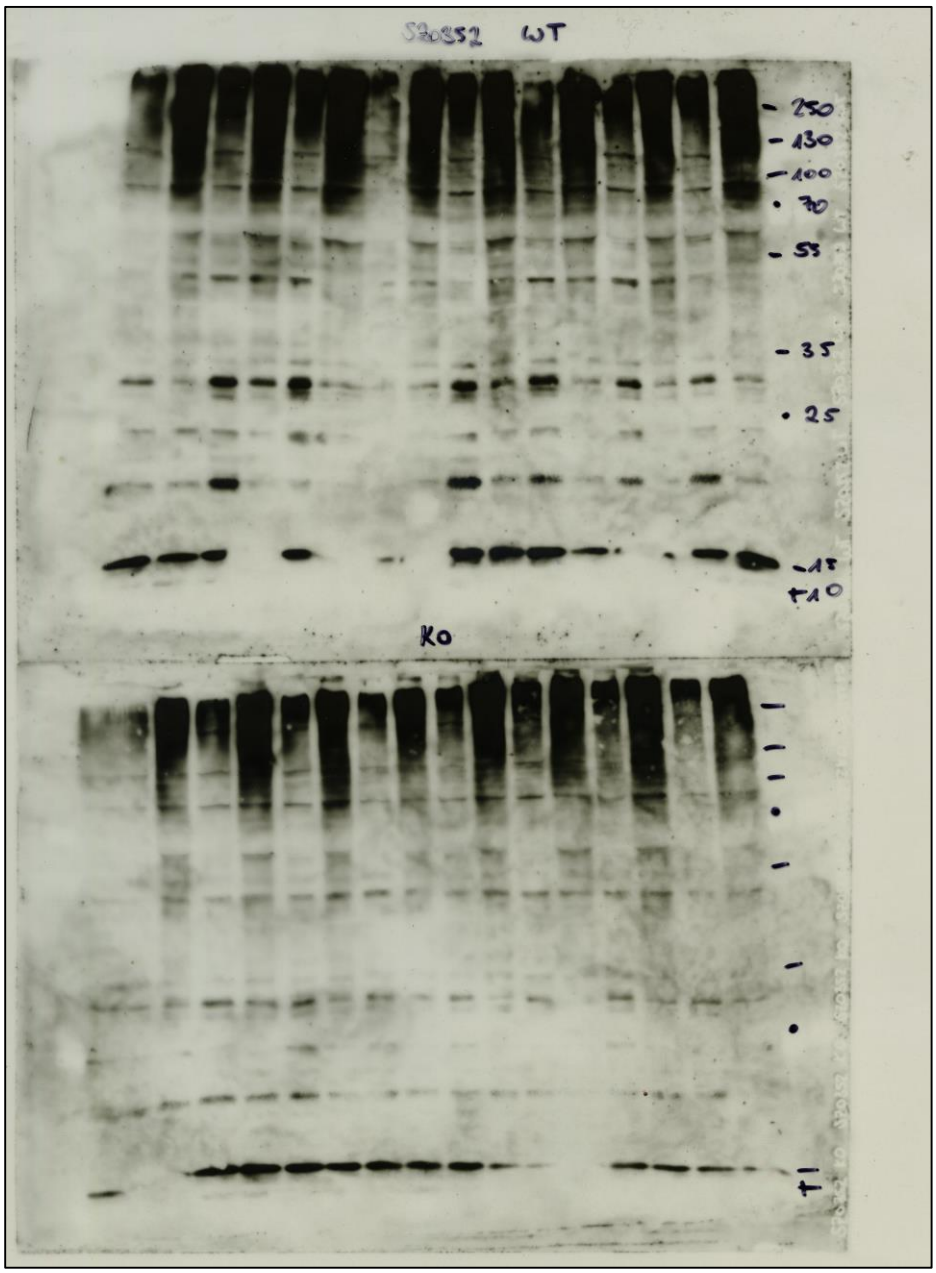
(4) LC3B



(5) Vinculin



(1) FK2 (total ubiquitin)



(2) GAPDH