Induced autophagy ameliorates cardiac proteinopathy

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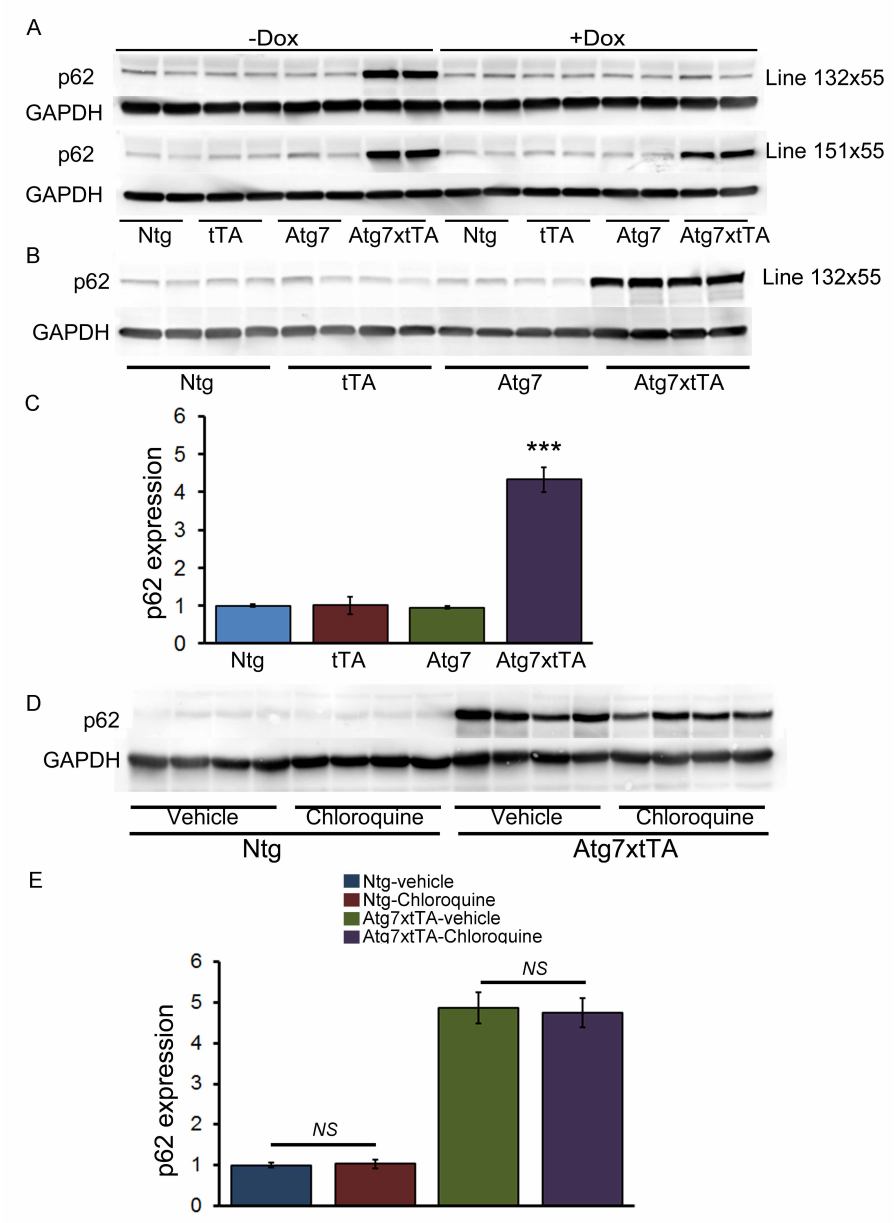
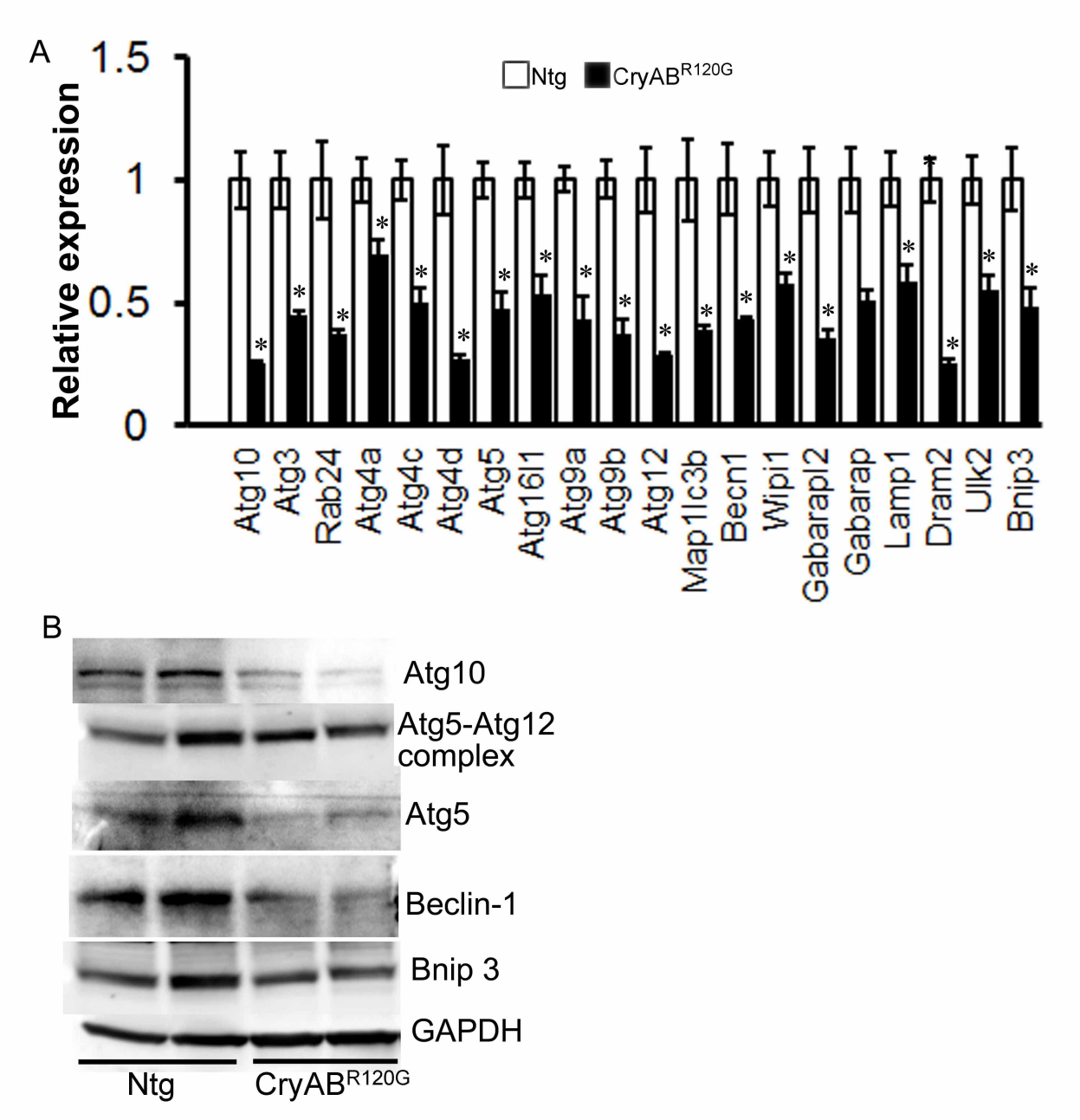
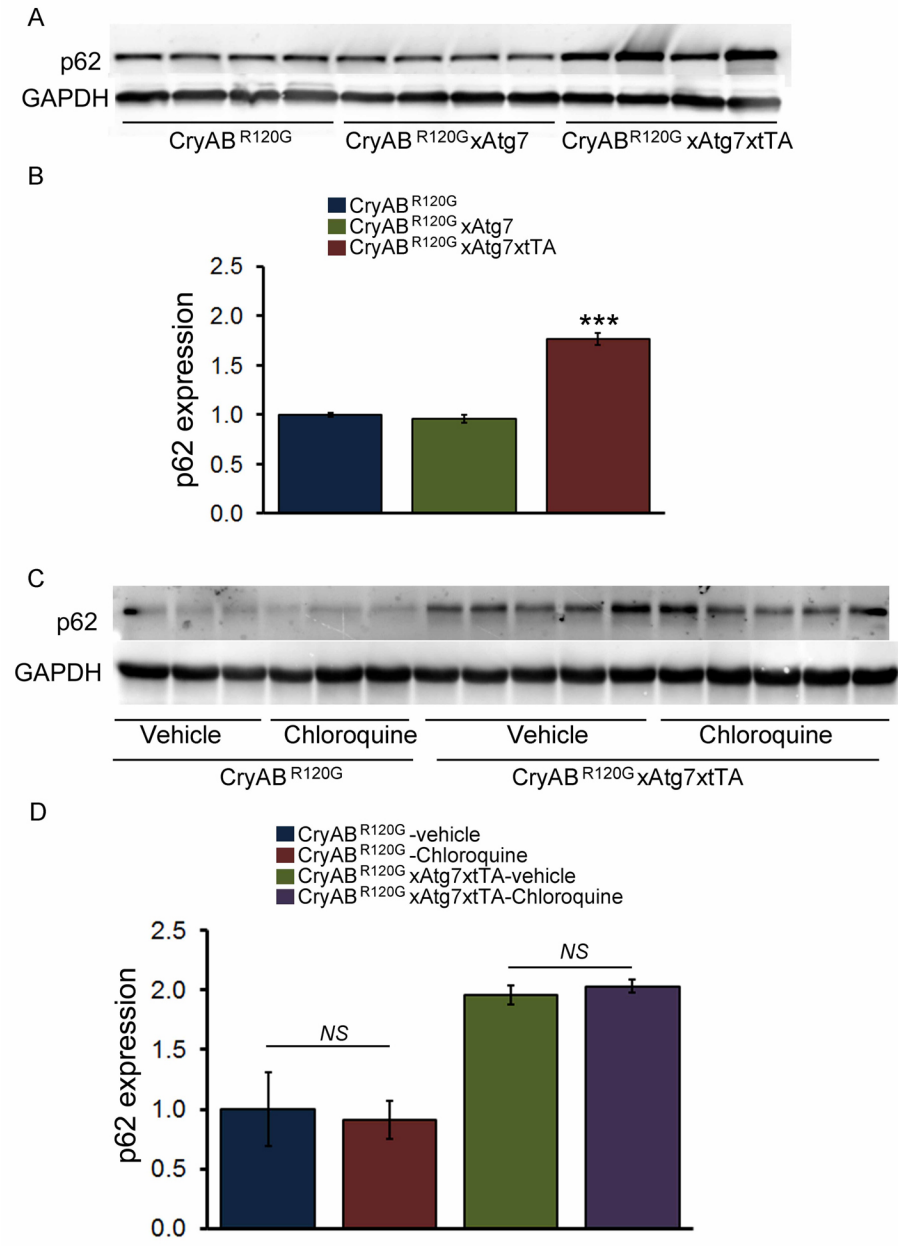
SUPPLEMENTAL DATA



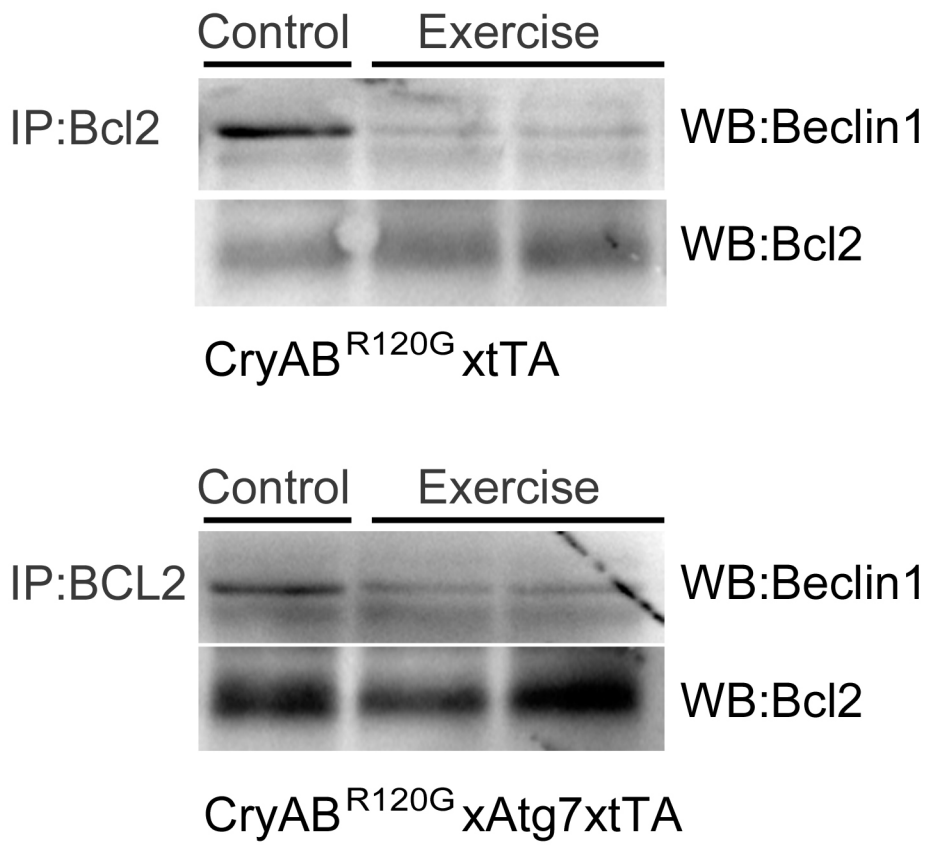
Supplemental Figure 1: Atg7 expression-dependent changes of p62 levels in Atg7xtTA hearts. (**A**) Representative Western blot showing p62 protein expression in cardiac extracts from lines 132 and151 at 3-months as indicated. (**B**) Western blot showing p62 protein expression in the hearts of 3-month-old Atg7xtTA double Tg and control mice. (**C**) Quantitation of p62 expression for line 132. ***, *P < 0.001* versus Ntg, tTA and Atg7 Tg mice by Tukey's *post hoc* test. (**D**) Western blot showing p62 protein expression in the Atg7xtTA and control Ntg hearts after treatment with chloroquine (n = 4/treatment). (**E**) Densitometry analysis (mean ± S.E.M) showed no significant changes (NS) in p62/GAPDH expression as a result of chloroquine treatment.



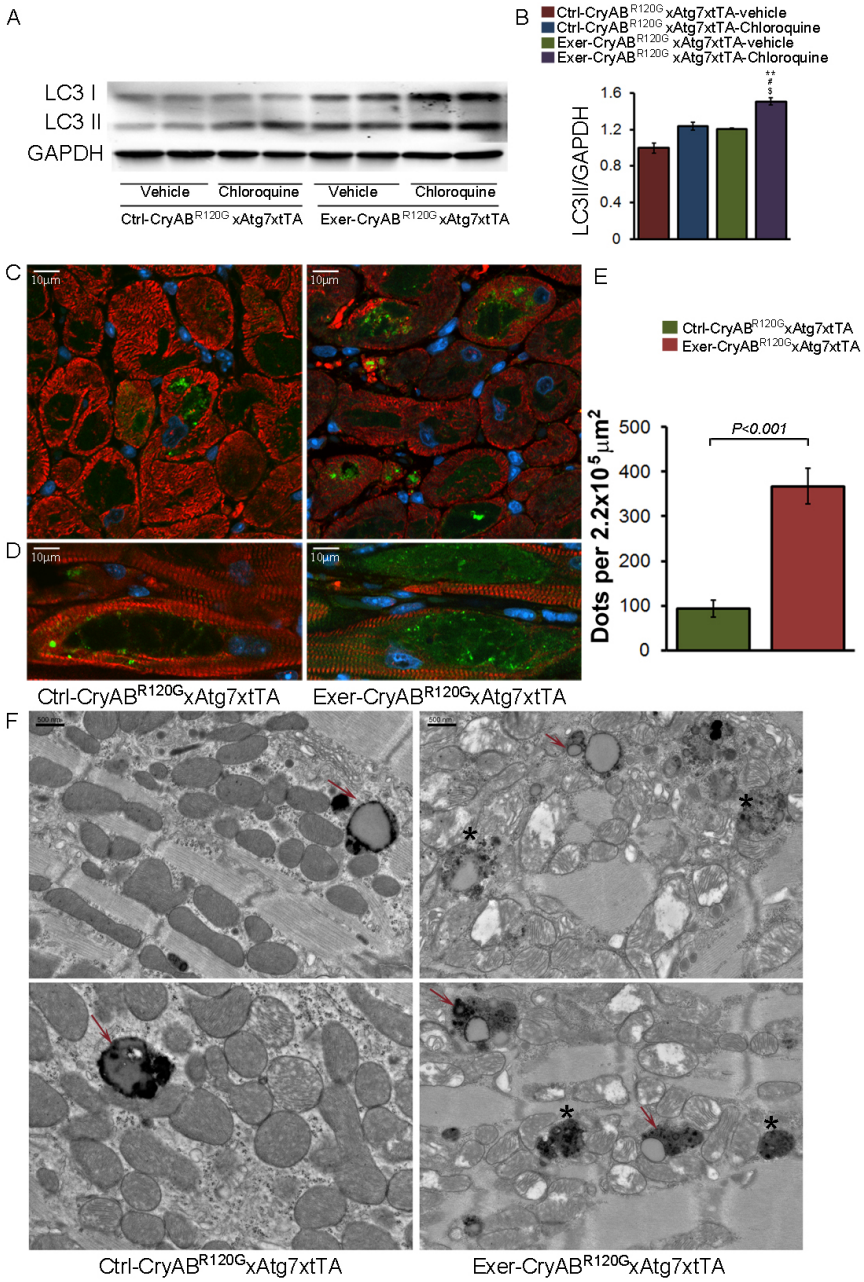
Supplemental Figure 2: Autophagy PCR array in CryAB^{R120G} hearts. (**A**) Graph representing direct group wise comparison of fold change in mRNA levels in male CryAB^{R120G} and control Ntg hearts at 5 months (fold change versus Ntg control, n = 3 per group). All values are reported as mean ± S.E.M. *P < 0.05 by Student's t test. (**B**) Western blot confirmation of the protein-level changes of selected upregulated transcripts detected in Panel A.



Supplemental Figure 3: p62 levels in Atg7 induced CryAB^{R120G} triple Tg hearts. (**A**) Western blot showing p62 protein levels in the hearts of 5-month-old CryAB^{R120G}xAtg7xtTA and control Tg mice. (**B**) Quantitation of p62 levels in CryAB^{R120G}xAtg7xtTA and control Tg mice. ***, *P<0.001* versus CryAB^{R120G} and CryAB^{R120G}xAtg7 Tg mice by Tukey's *post hoc* test. (**C**) Western blot showing p62 protein levels in CryAB^{R120G}xAtg7xtTA and CryAB^{R120G} hearts after treatment with chloroquine (n = 3/treatment). (**E**) Densitometry analysis (mean ± S.E.M) showed no significant changes (NS) in p62/GAPDH values in the CryAB^{R120G}xAtg7xtTA and CryAB^{R120G} mice after treatment with chloroquine.

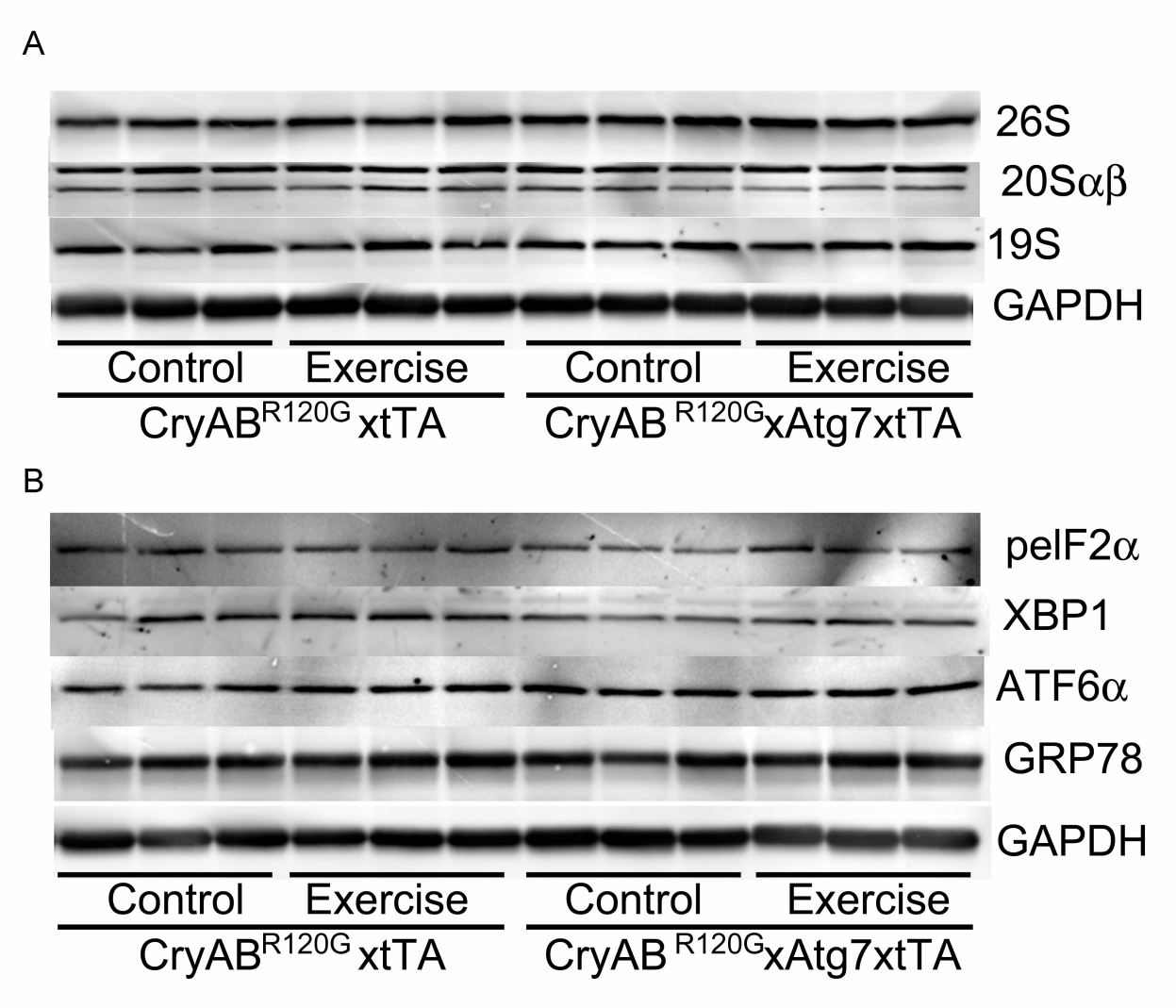


Supplemental Figure 4: Bcl2 and Beclin 1 interaction. Co-immunoprecipitation of Beclin1 with Bcl2 in cardiac tissue from voluntary exercised and control mice from both CryAB^{R120G}xtTA and CryAB^{R120G}xAtg7xtTA mice. IP, immunoprecipitate; WB, western blot.



Supplement Figure 5: Autophagy flux analysis in exercised Atg7 induced CryAB^{R120G} triple Tg hearts. (**A**) An autophagic flux assay showed increased LC3-II levels by Western blot analysis in Atg7 crossed CryAB^{R120G} hearts after voluntary exercise (n =4/treatment). (**B**) Densitometry analysis (mean ± S.E.M) showed increased LC3II/GAPDH values in the exercised Atg7 crossed mice. **, *P < 0.01* versus non-exercised CryAB^{R120G}xAtg7xtTA-vehicle treated group; #, *P < 0.05* versus non-exercised CryAB^{R120G}xAtg7xtTA-chloroquine treated group; $, *P < 0.05* versus exercised CryAB^{R120G}xAtg7xtTA-chloroquine treated group by Tukey's *post hoc* test. (**C, D**) Immunofluorescence analysis of heart sections showing punctate LC3 staining (green) in 7-month-old voluntary exercised CryAB^{R120G}xAtg7xtTA and control non-exercised CryAB^{R120G}xAtg7xtTA hearts. TnI (red) was used to identify cardiomyocytes. DAPI (blue) was used to identify nuclei (60X). (**C**) Quantification of LC3 dots per microscopic field (220,000 µm^2) in LV. (**E**) Representative transmission electron micrographs of hearts from control and

voluntary exercised Atg7-induced CryAB^{R120G} triple Tg mice showing increased amphisomes (*) and autophagosomes (denoted by arrow).



Supplemental Figure 6: Expression of Ubiquitin Proteasome System (UPS) and Unfolded Protein Response (UPR) markers. (**A**) Representative Western blot showing UPS proteins 19S, 20S and 26S expression levels in cardiac extracts from exercised and control non-exercised CryAB^{R120G}xtTA as well as CryAB^{R120G}xAtg7xtTA mice. (**B**) Representative Western blot showing UPR marker proteins Atf6α, peIF2α, XBP1 and GRP78 expression levels in cardiac extracts from exercised and control non-exercised CryAB^{R120G}xtTA as well as CryAB^{R120G}xAtg7xtTA mice.