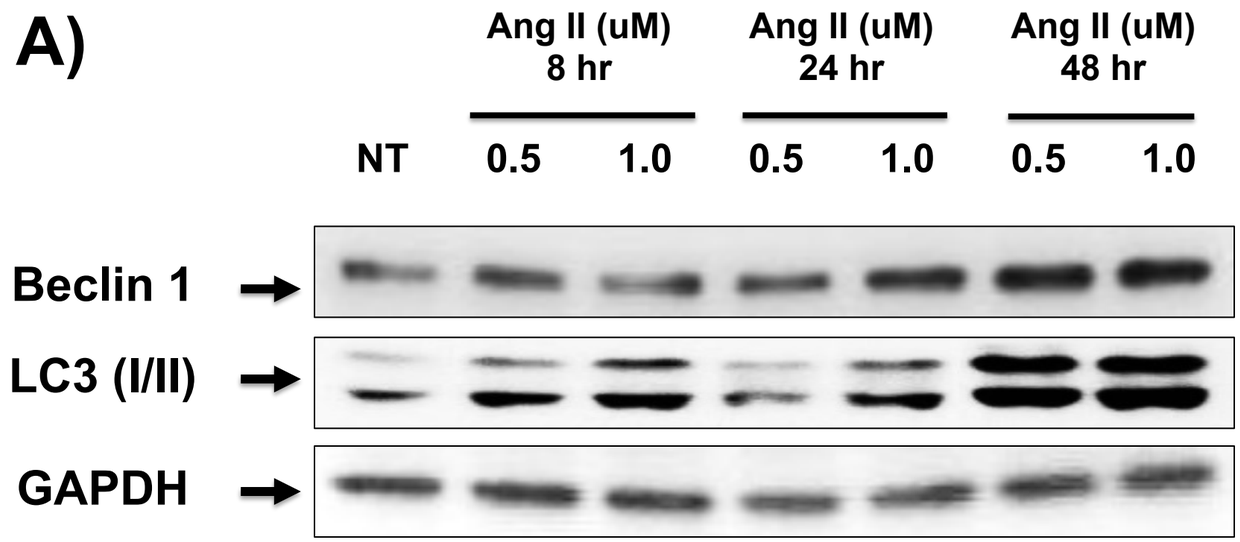
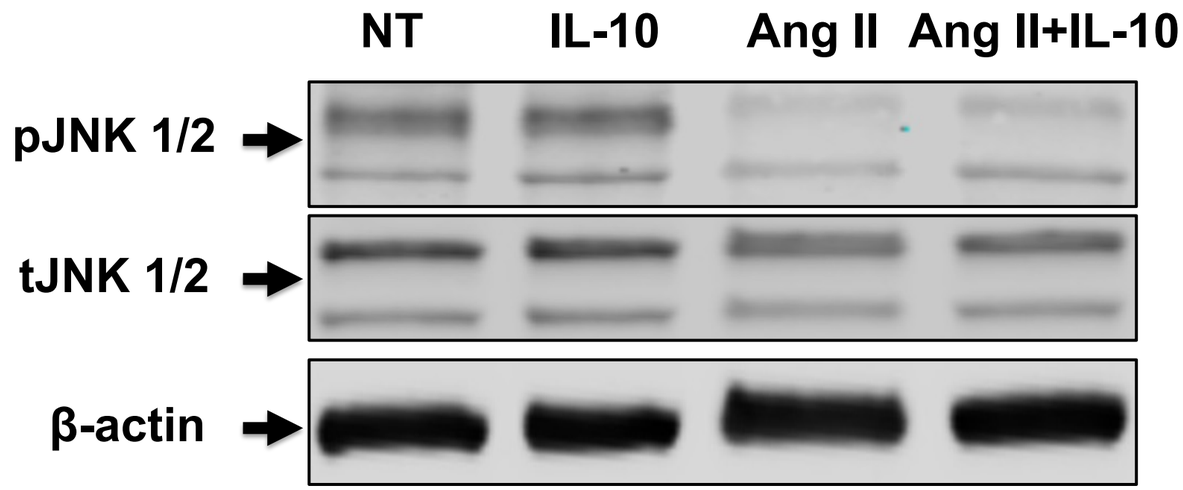
Kishore et. al. Supplementary Figures

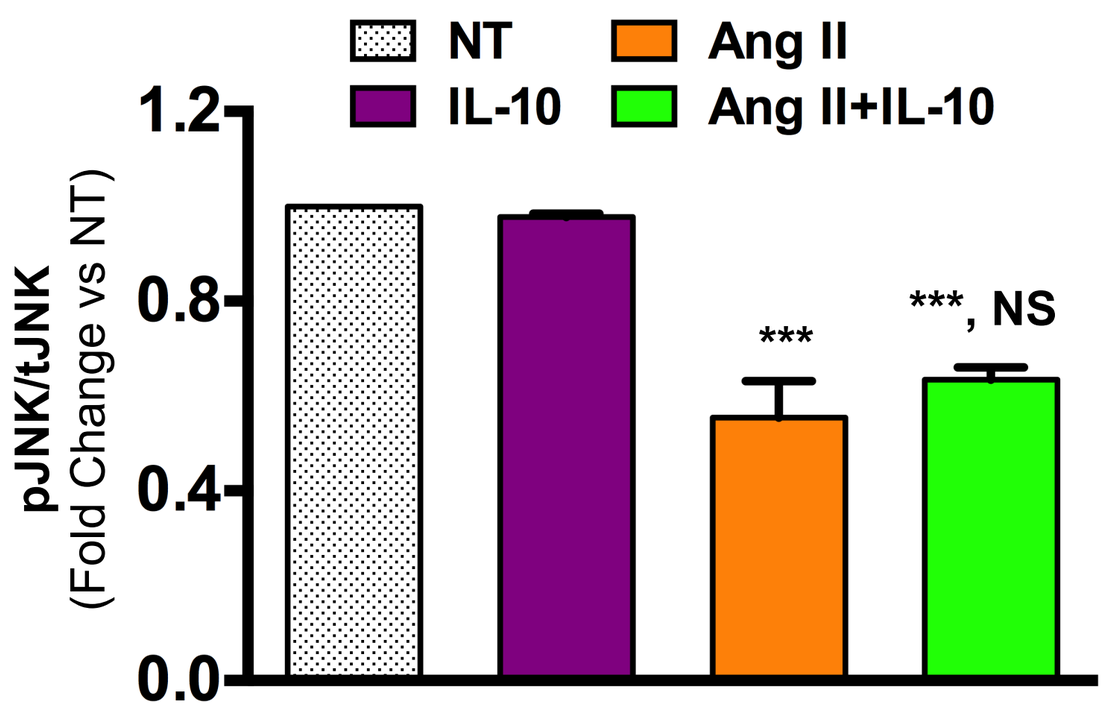
Supplementary Figure S1



Supplementary Figure S1: Acute long-term Ang II treatment induced pathological autophagy in neonatal rat ventricular myocytes (NRVM): NRVM were isolated from 1-2 days old rat pups. Cells were treated with Ang II for 48 hours in low serum (LS) media. **A)** Dose and time response for Ang II in NRVM for autophagy were analyzed by western blotting. Maximum activation of autophagy was observed at 1 uM dose of Ang II for 48 hours. GAPDH/β-actin were used as internal control. N=3

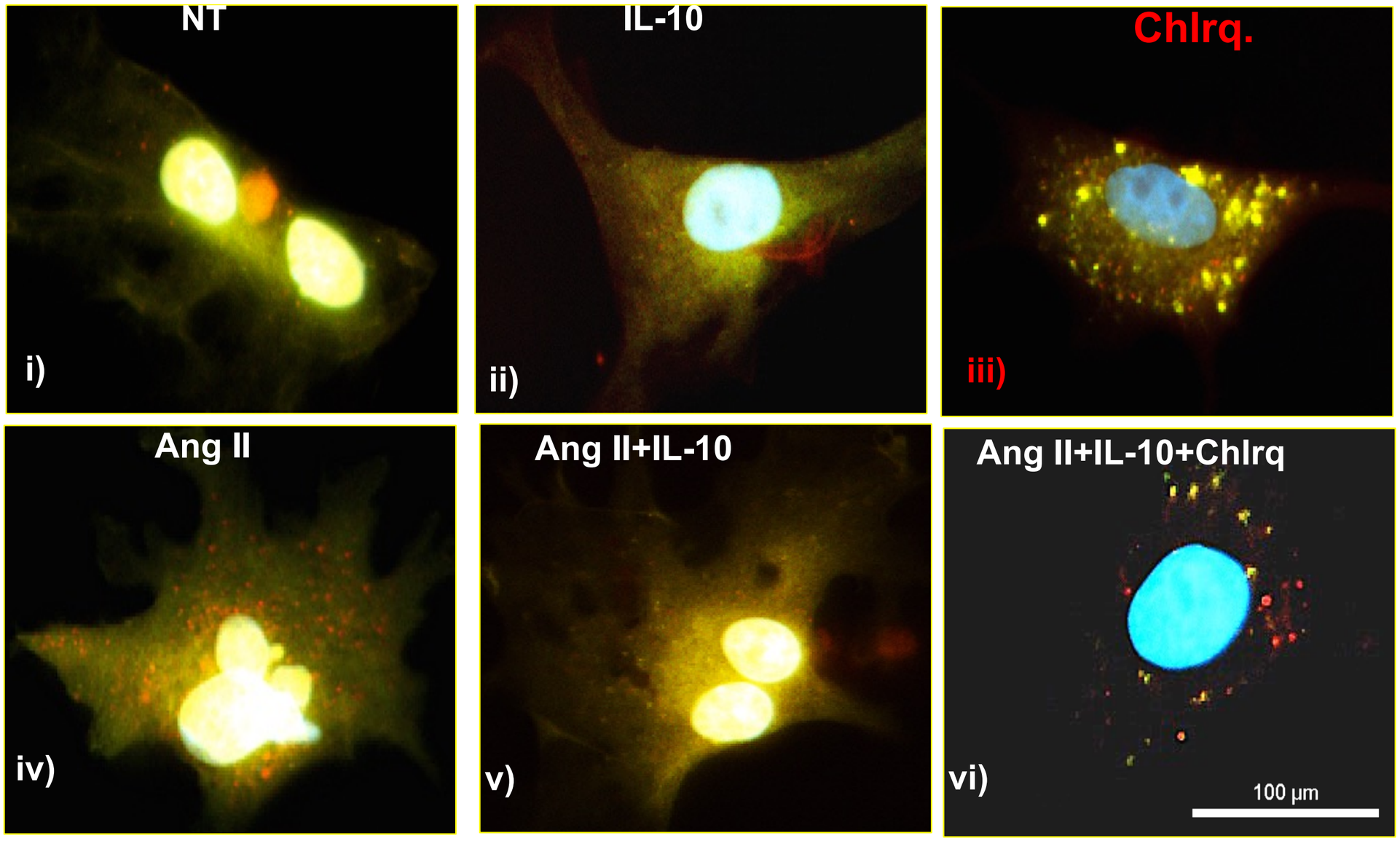
Supplementary Figure S2





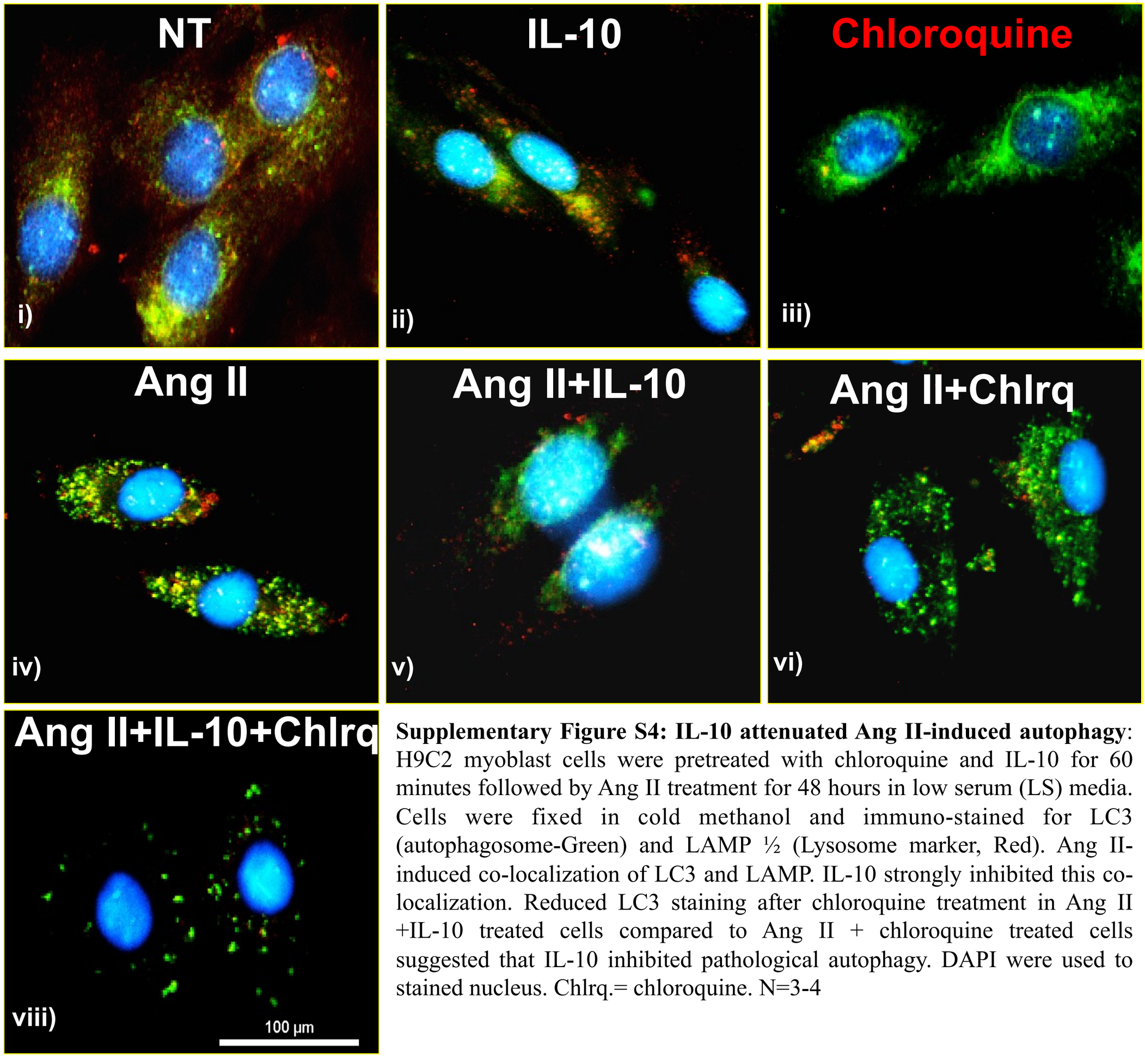
Supplementary Figure S2: Acute long-term Ang II treatment induced inhibits JNK1/2 signaling in neonatal rat ventricular myocytes (NRVM): NRVM were isolated from 1-2 days old rat pups. Cells were treated with Ang II and IL-10 for 48 hours in low serum (LS) media. **A&B)** Cells lysate were prepared from cells after treatment and used for western blot using JNK1/2 antibodies. Prolong Ang II treatment inhibits JNK1/2 phosphorylation. IL-10 treatment were unable to rescued Ang II-induced JNK inhibition. β-actin were used as internal control. N=3

Supplementary Figure S3



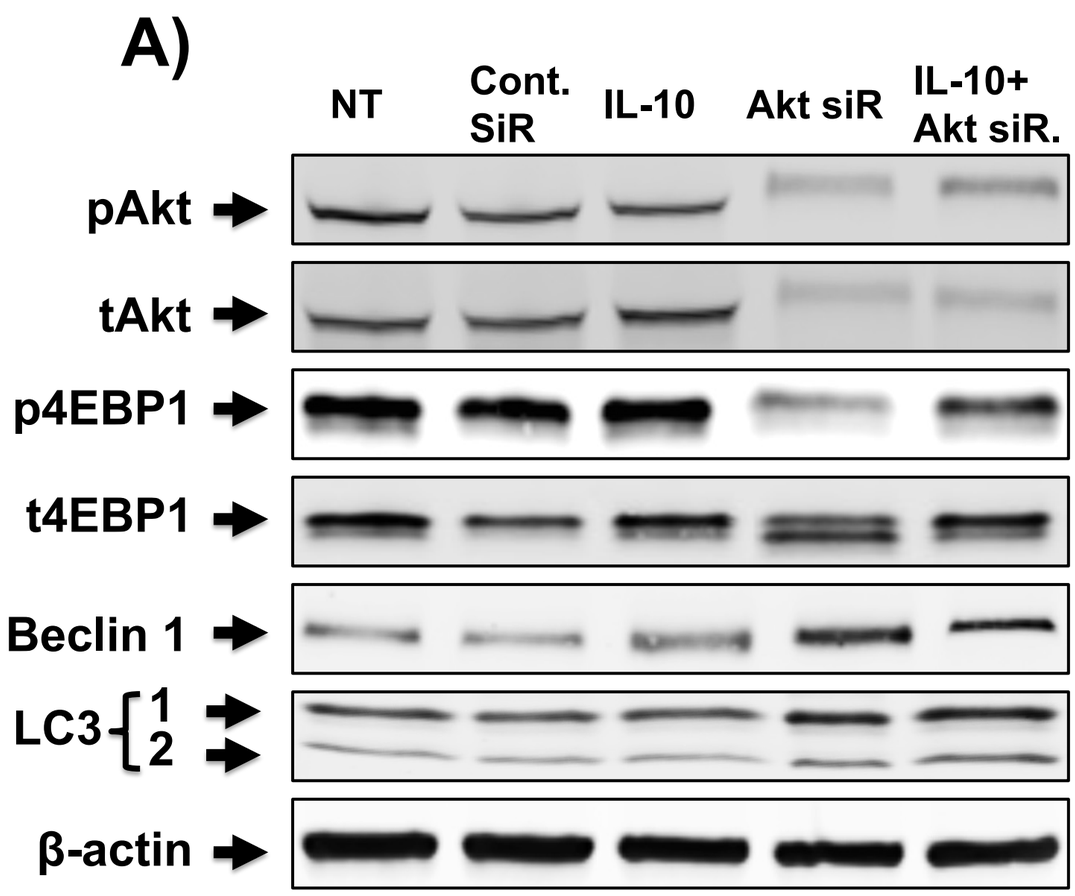
Supplementary Figure S3: IL-10 attenuated Ang II-induced autophagy: **i-vi)** Chloroquine were used to interrupt the progress of autophagy to see the actual role of IL-10. Chloroquine treatment does not affect autophagic flux in IL-10 treated cells conformed the anti-autophagic effect of IL-10. Bar graph indicates mean number of autophagosome (empty box) and autolysosome (filled box) per cell. Scale bar, 100 um and original magnification was 400X with oil emulsion. GFP: green fluorescent protein, RFP: red fluorescent protein, Chlrq: chloroquine. N=3-4

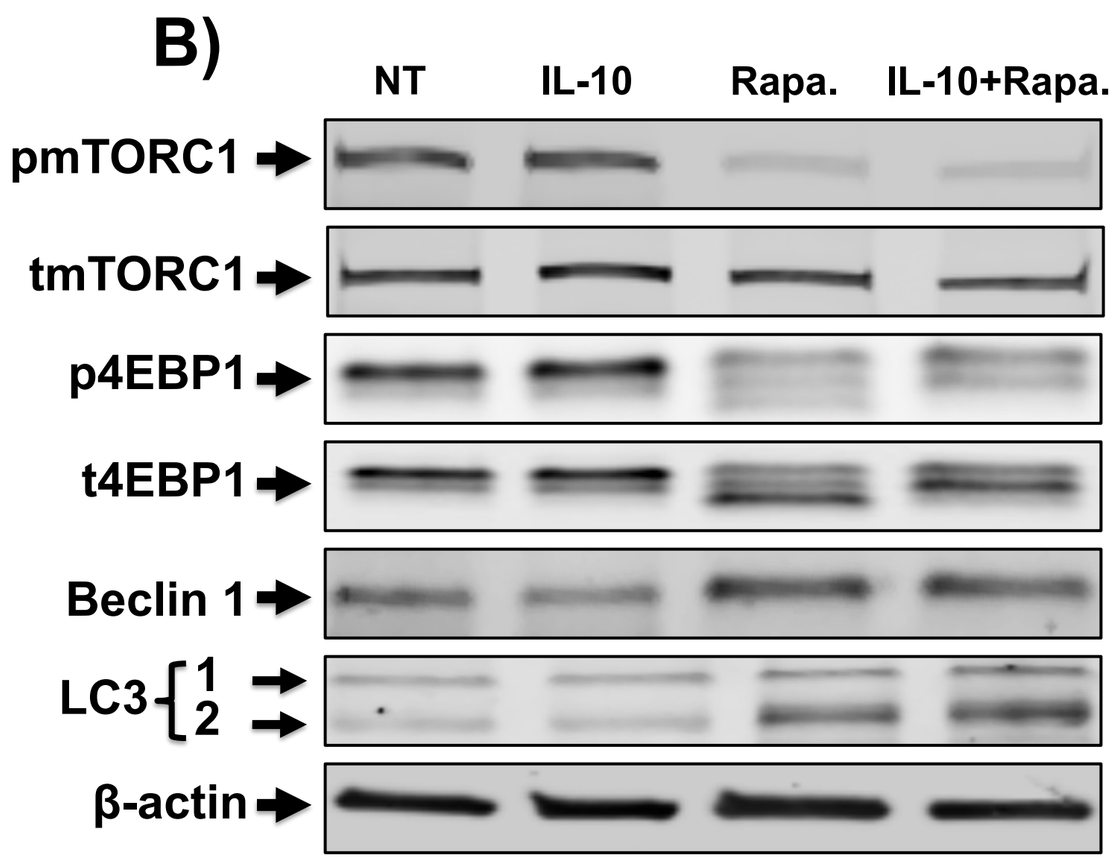
Supplementary Figure S4



Supplementary Figure S4: IL-10 attenuated Ang II-induced autophagy: H9C2 myoblast cells were pretreated with chloroquine and IL-10 for 60 minutes followed by Ang II treatment for 48 hours in low serum (LS) media. Cells were fixed in cold methanol and immuno-stained for LC3 (autophagosome-Green) and LAMP ½ (Lysosome marker, Red). Ang II-induced co-localization of LC3 and LAMP. IL-10 strongly inhibited this co-localization. Reduced LC3 staining after chloroquine treatment in Ang II +IL-10 treated cells compared to Ang II + chloroquine treated cells suggested that IL-10 inhibited pathological autophagy. DAPI were used to stained nucleus. Chlrq.= chloroquine. N=3-4

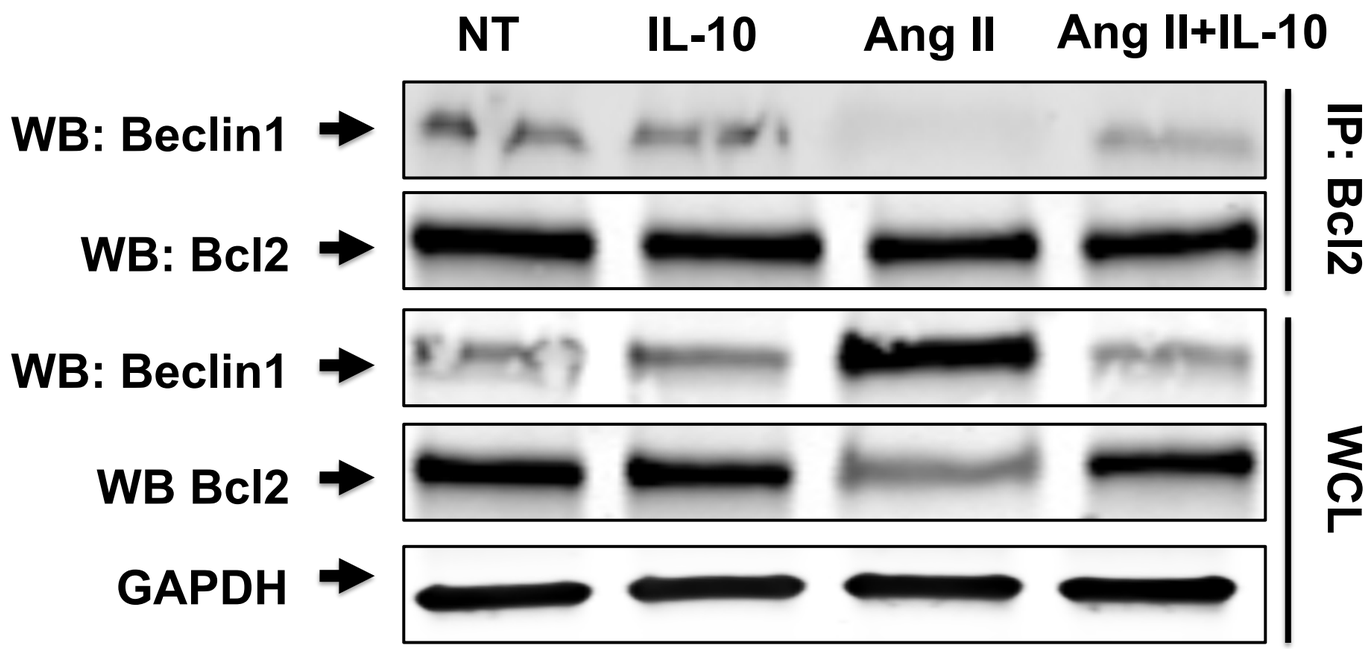
Supplementary Figure S5





Supplementary Figure S5: Rapamycin and Akt siR actiates basal autophagy in neonatal rat ventricular myocytes (NRVM): NRVM were isolated from 1-2 days old rat pups. Cells were treated with rapamycin or Akt siR and IL-10 as described in method. **A&B)** Cells lysate were prepared from cells after treatment and used for western blot. Inhibition of AKT/mTORC1 signaling activates autophagic markers. IL-10 treatment was unable to rescued this effect. β-actin were used as internal control. N=3

Supplementary Figure S6



<u>Supplementary Figure S6:</u> IL-10 restored physical association of Bcl2 with beclin 1 and thus reduced Ang II-induced autophagy: Cells were lysed and used for co-immunoprecipitation (IP) using Pierce co-immunoprecipitation kit. Western blot was performed on immunoprecipitated proteins and whole cells lysate (WCL) for Bcl2 and beclin1. Co-immunoprcipitation data indicate IL-10-induced beclin1 association with bcl2.