Supplemental Material to:

Isogambogenic acid induces apoptosis-independent autophagic cell death in human Non-small-cell lung carcinoma cells

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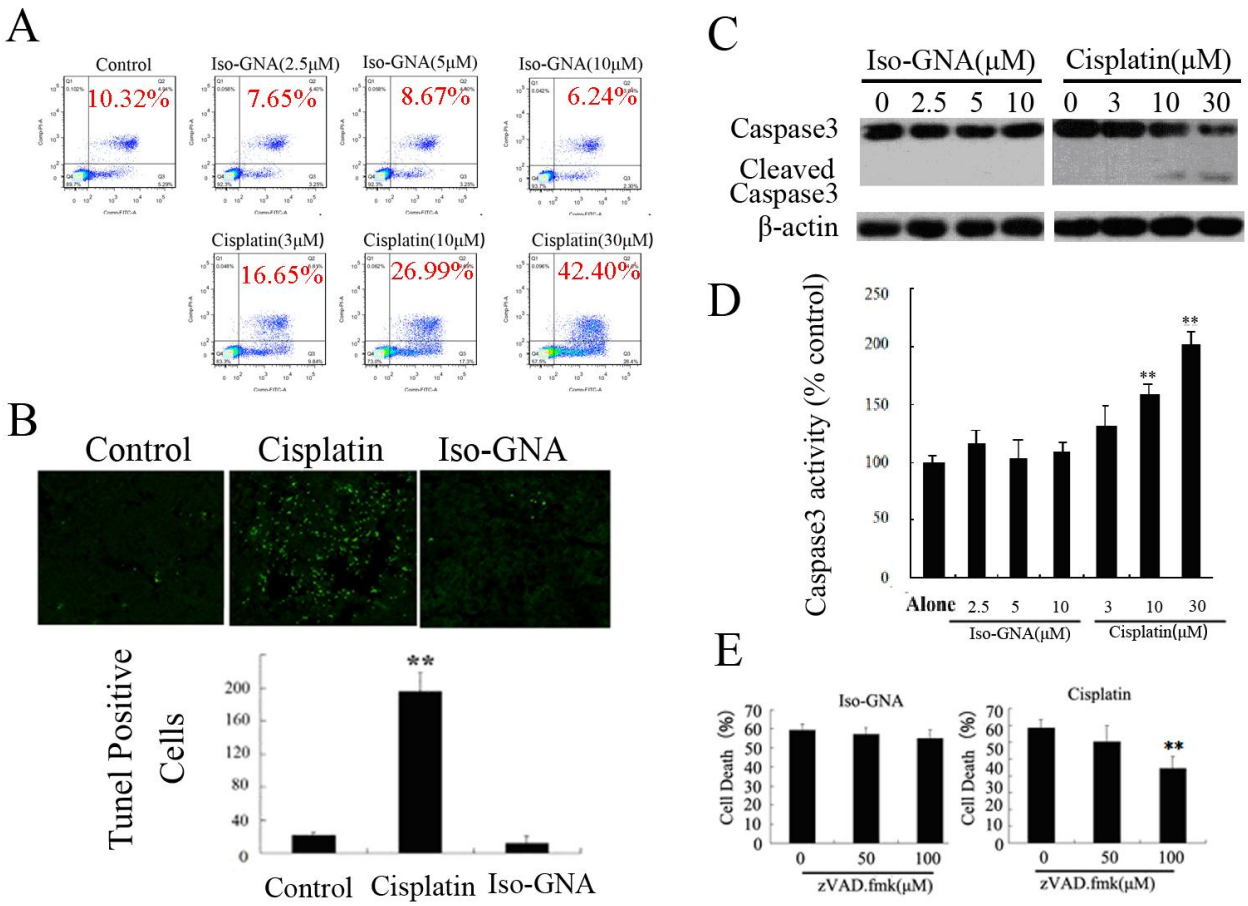

Figure S1: Iso-GNA Induced Apoptosis-independent Cell Death. (**A**) Apoptosis analysis: Approximately 5×10^5 H460 cells were plated on 6-well plates for 24 h and then treated with indicated concentrations of iso-GNA or cisplatin for 24 h, then subjected to flow cytometry for analysis after stained with Annexin V-FITC/ PI. (**B**) Iso-GNA induces no apoptosis *in vivo*. TUNEL assay was performed to measure apoptotic cells in tumor sections from iso-GNA-, cisplatin- and vehicle-treated mice. **P<0.01. as compared with the Control group. (**C**) Expression of caspase3 in H460 cells. Cells were treated with different concentrations of iso-GNA or cisplatin for 24 h, and then western blotting was performed to analyze caspase3 Expression. (D) Caspase3 activity in A549 cells. Cells were treated with different concentrations of iso-GNA or cisplatin for 24 h, and then subjected to analyze Caspase3 activity. **P<0.01. as compared with the Control group. (**E**) Caspase-independent cell death caused by iso-GNA. Different concentrations of zVAD.fmk was added to A549 cells for 6 h before the cells were

treated with iso-GNA or cisplatin for 36 h, then cells were stained with trypan blue to analyze death rate. The data represents the means ± s.d. of three independent tests. **p < 0.01, as compared with the control group.

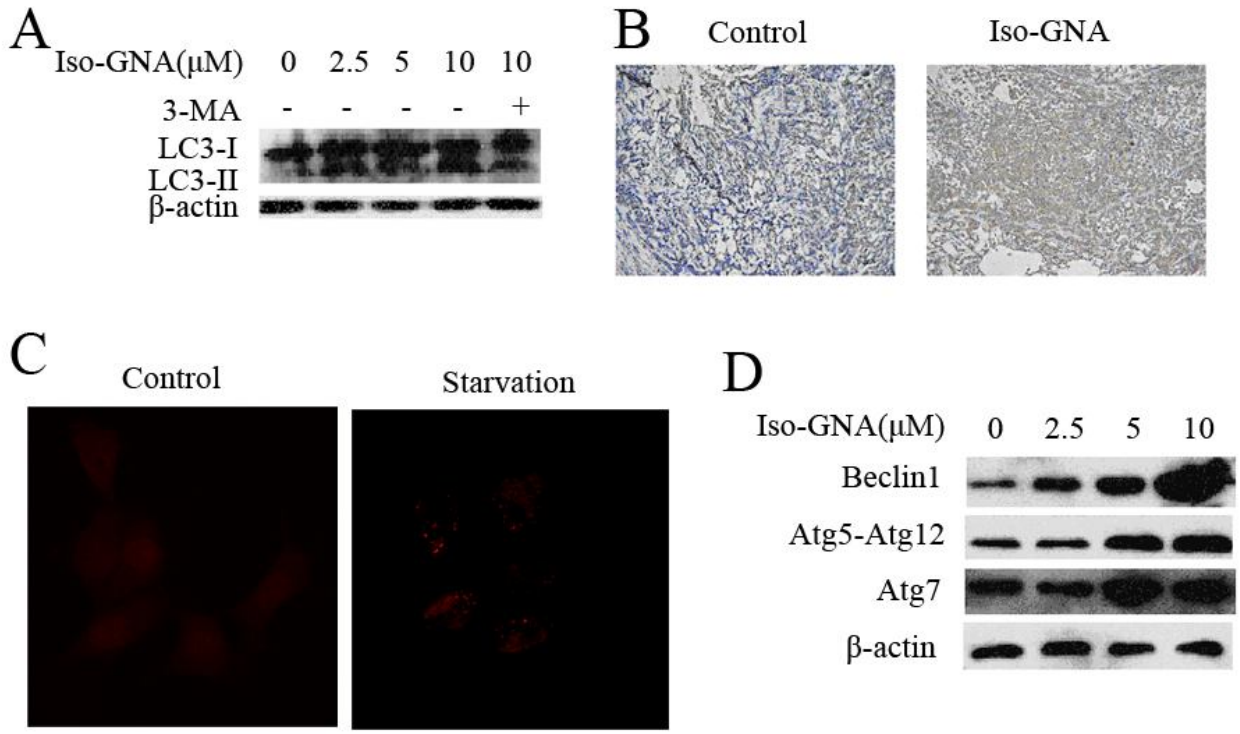

Figure S2. Iso-GNA induces autophagy *in vitro and in vivo*. (**A**) LC3 expression in H460 cells. Cells were treated with different concentrations of iso-GNA for 24 h. and 3-MA (2mM) was treated as an autophagy inhibitor. (**B**) Immunohistochemistry analysis of LC3 in A549 model tumor sections from iso-GNA-treated mice and vehicle-treated control. (**C**) Identification of stably expressing RFP-LC3 A549 cells. Cells were cultured in Earle's balanced salt solution with or without amino acids and fetal bovine serum, The formation of RFP-LC3 proteins was examined by fluorescence microscopy. (**D**) Beclin 1, Atg7 and Atg12-Atg5 complex expression in H460 cells treated with various concentrations of iso-GNA for 24h.

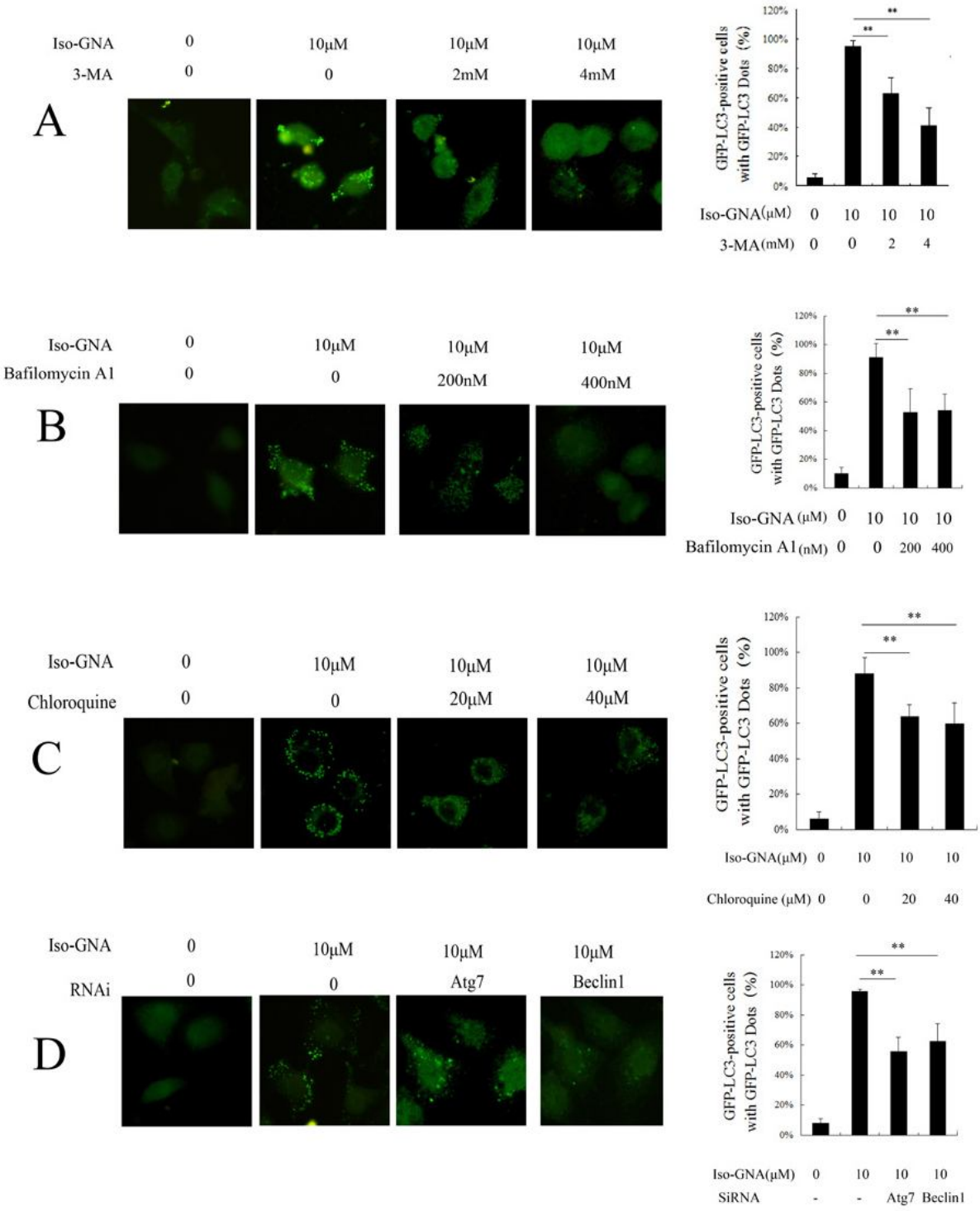

Figure S3. Autophagy induced by iso-GNA in A549 Cells was inhibited by autophagy inhibiting approaches. (**A-D**) A549 cells were transiently transfected with pEGFP-LC3 plasmid and treated with 0，10 μM Iso-GNA or in combination of Iso-GNA（10μM）with (**A**) 2 mM or 4mM 3-MA (**B**) 200nM or 400nM Bafilomycin A1 (**C**)20μM or 40μM Chloroquine (**D**) *Atg7* or *Beclin1* SiRNA for 48 h. GFP-LC3 positive cells were defined as cells that have five or more GFP-LC3 punctate dots. The percentage of GFP-LC3 positive cells with GFP-LC3 dots and the

average number of GFP-LC3 dots per cell were assessed from 100 random fields. ** $p < 0.01$.

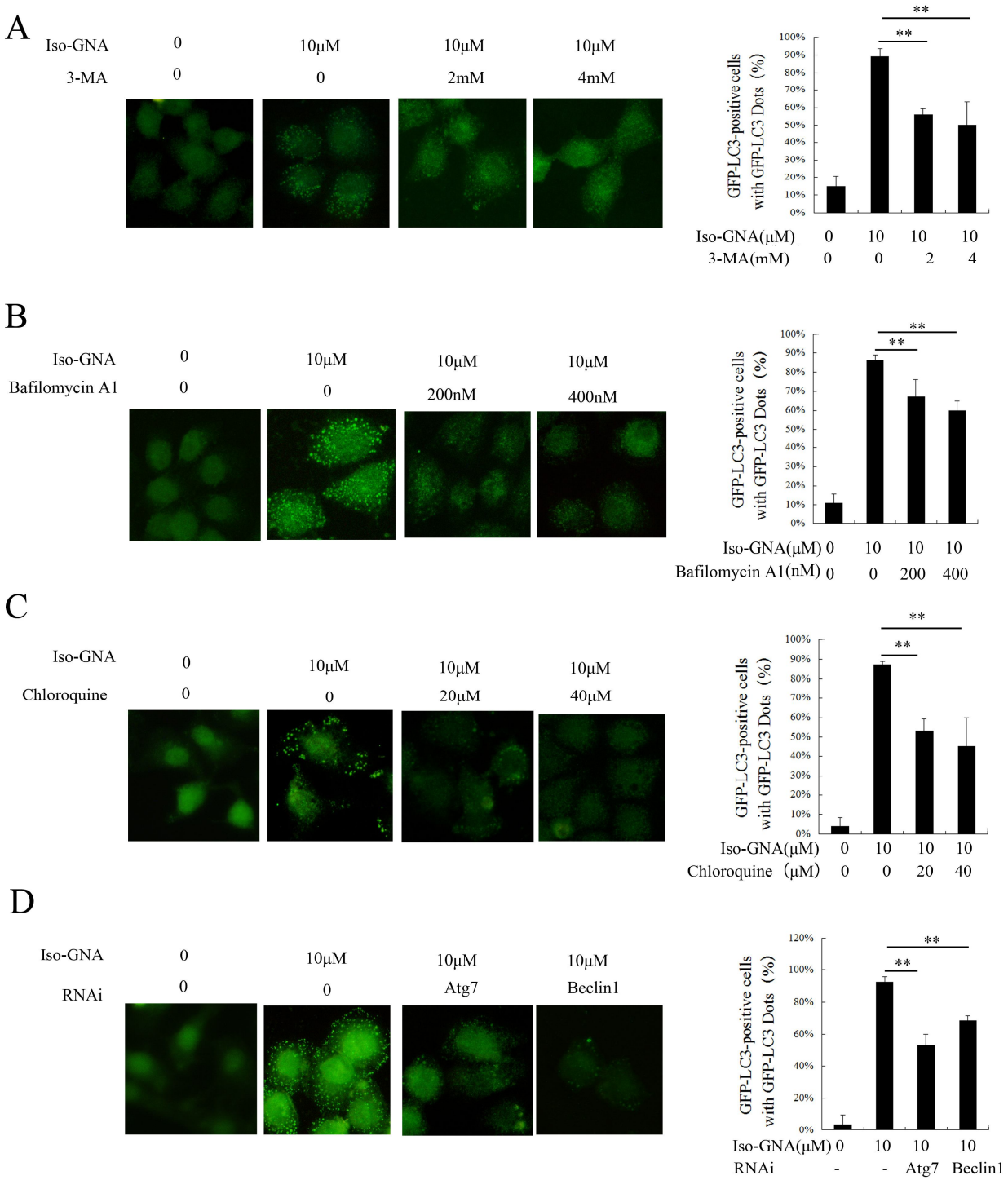

Figure S4: Autophagy induced by iso-GNA in H460 Cells was inhibited by autophagy inhibiting approaches. (**A-D**) H460 cells were transiently transfected with pEGFP-LC3 plasmid and treated with 0，10 μM Iso-GNA or in combination of Iso-GNA（10μM）with (**A**) 2 mM or 4mM 3-MA (**B**) 200nM or 400nM Bafilomycin A1 (**C**)20μM or 40μM Chloroquine (**D**) *Atg7* or *Beclin1* SiRNA for 48 h. GFP-LC3 positive cells were defined as cells that

have five or more GFP-LC3 punctate dots. The percentage of GFP-LC3 positive cells with GFP-LC3 dots and the average number of GFP-LC3 dots per cell were assessed from 100 random fields. ** $p < 0.01$

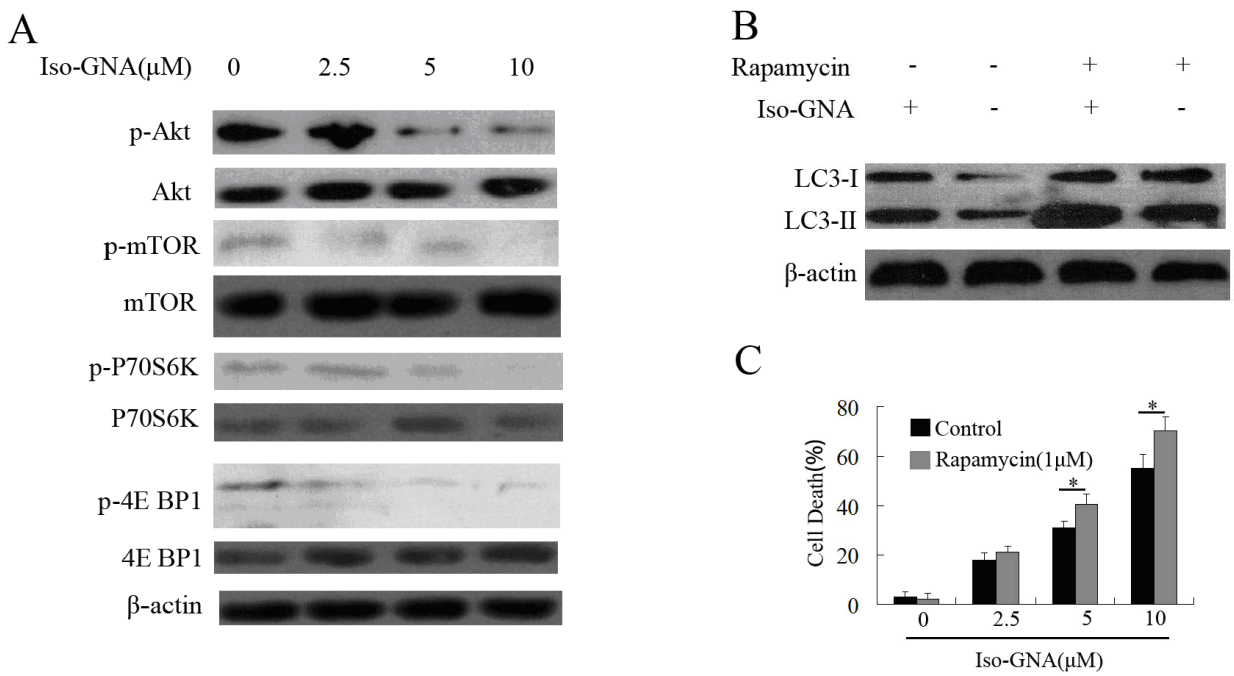

Figure S5: Iso-GNA induces autophagic cell death in H460 cells through Akt-mTOR-dependent signaling pathways. (**A**) Immunoblot analysis of phospho-Akt (S473), total Akt, phospho-mTOR (S2448), total mTOR, phospho-p70 S6K (S424/T421), total p70 S6K, phospho-4E-BP1 (S65/T70) and total 4E-BP1 from lysates of H460 cells treated with various concentrations of iso-GNA for 24 h. (**B**) H460 cells treated with 0, 1μM of rapamycin and/or 10 μM Iso-GNA for 24 h were analyzed by western blot. (C) H460 cells were treated with 0, 2.5, 5 or 10 μM iso-GNA and/or 1 μM rapamycin for 24 h. Cell death was measured by trypan blue dye exclusion assay. *$p<0.05$.

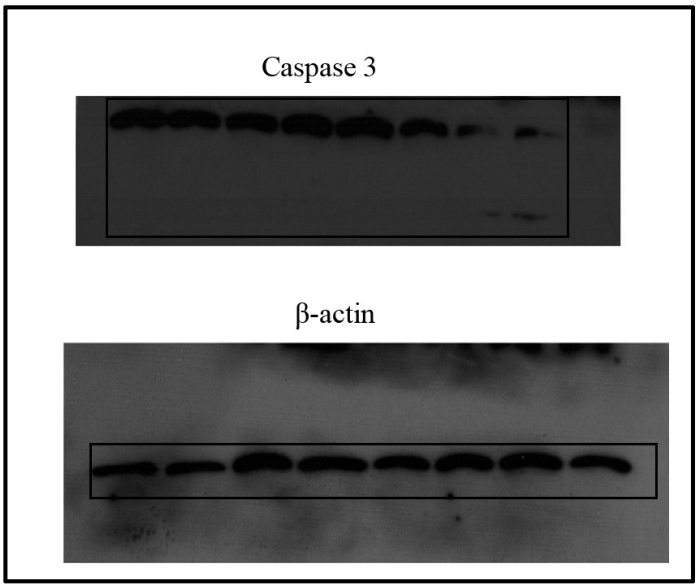

Figure S6: Complete western blotting gels from Fig. 2C.

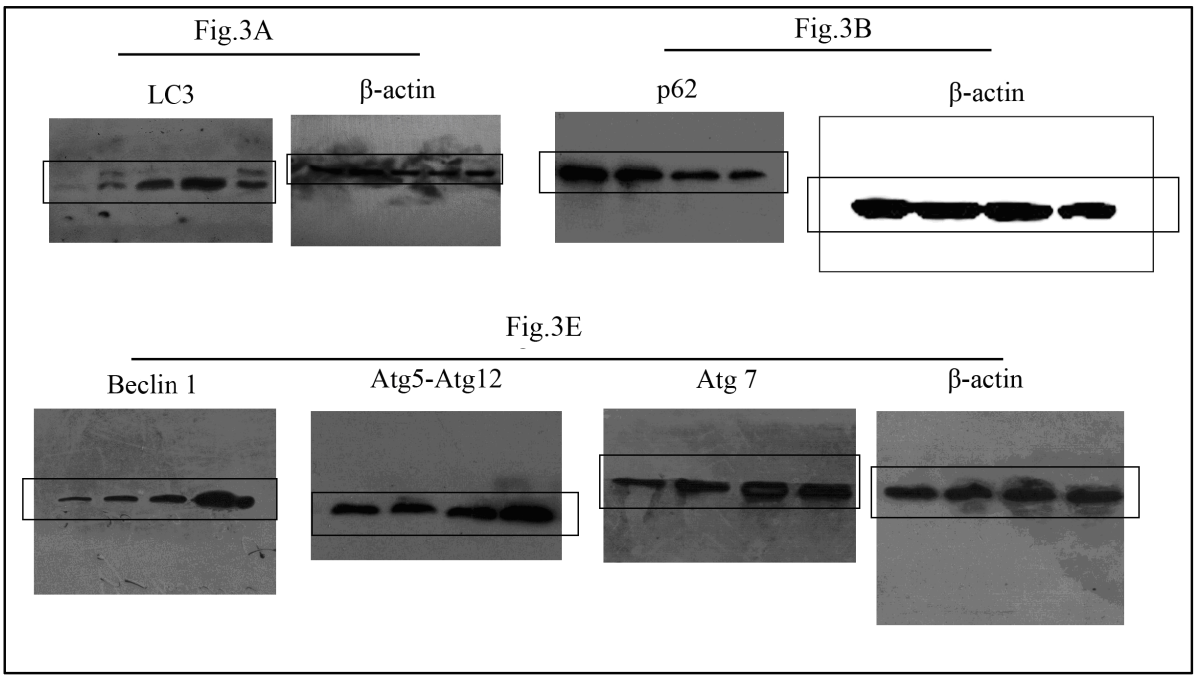

Figure S7: Complete western blotting gels from Fig. 3

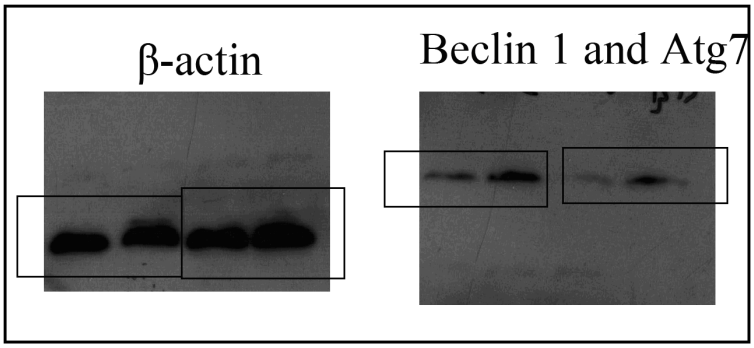

Figure S8: Complete western blotting gels from Fig. 4B.

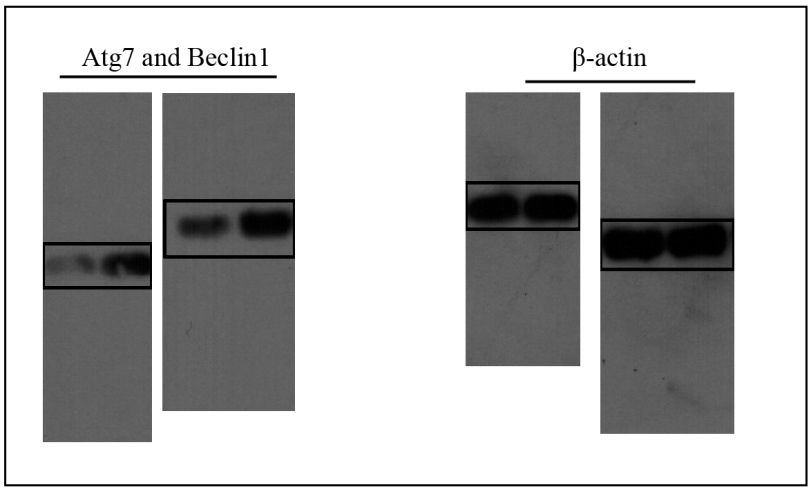

Figure S9: Complete western blotting gels from Fig. 5B.

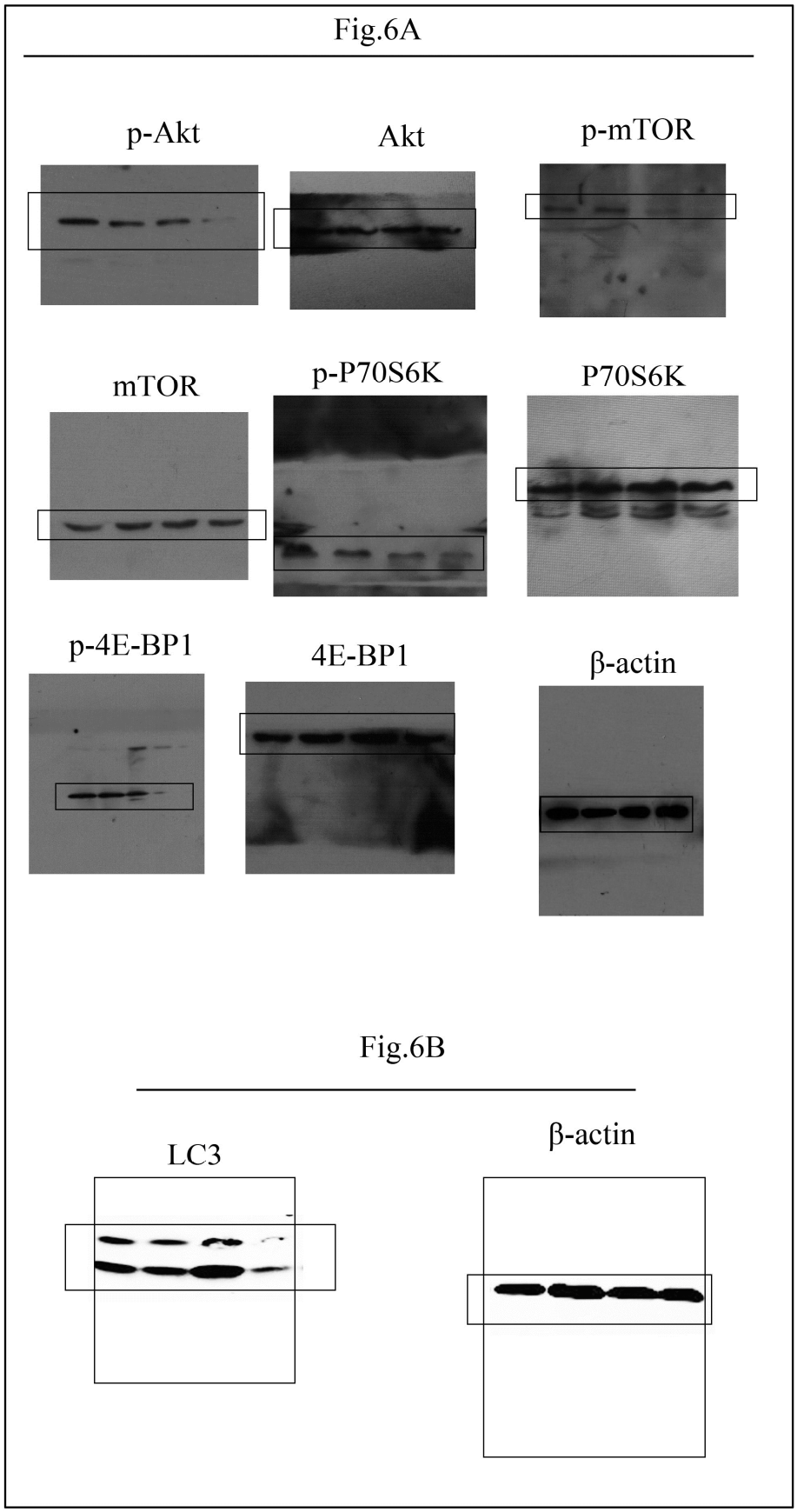

Figure S10: Complete western blotting gels from Fig. 6