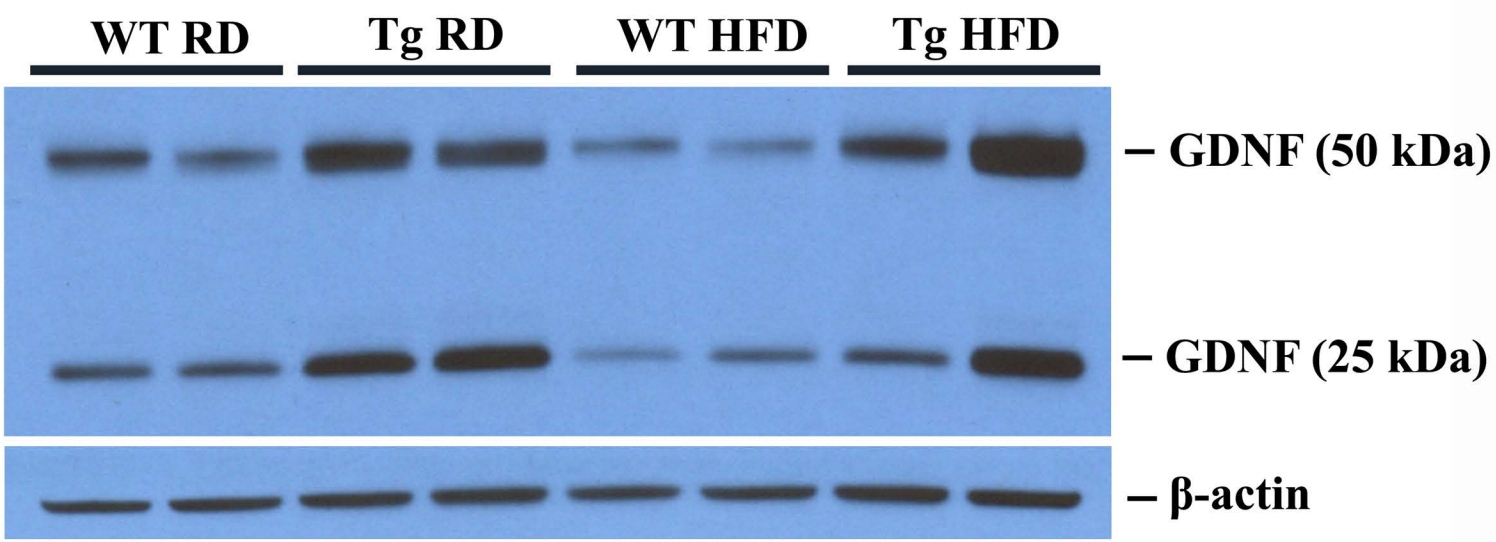
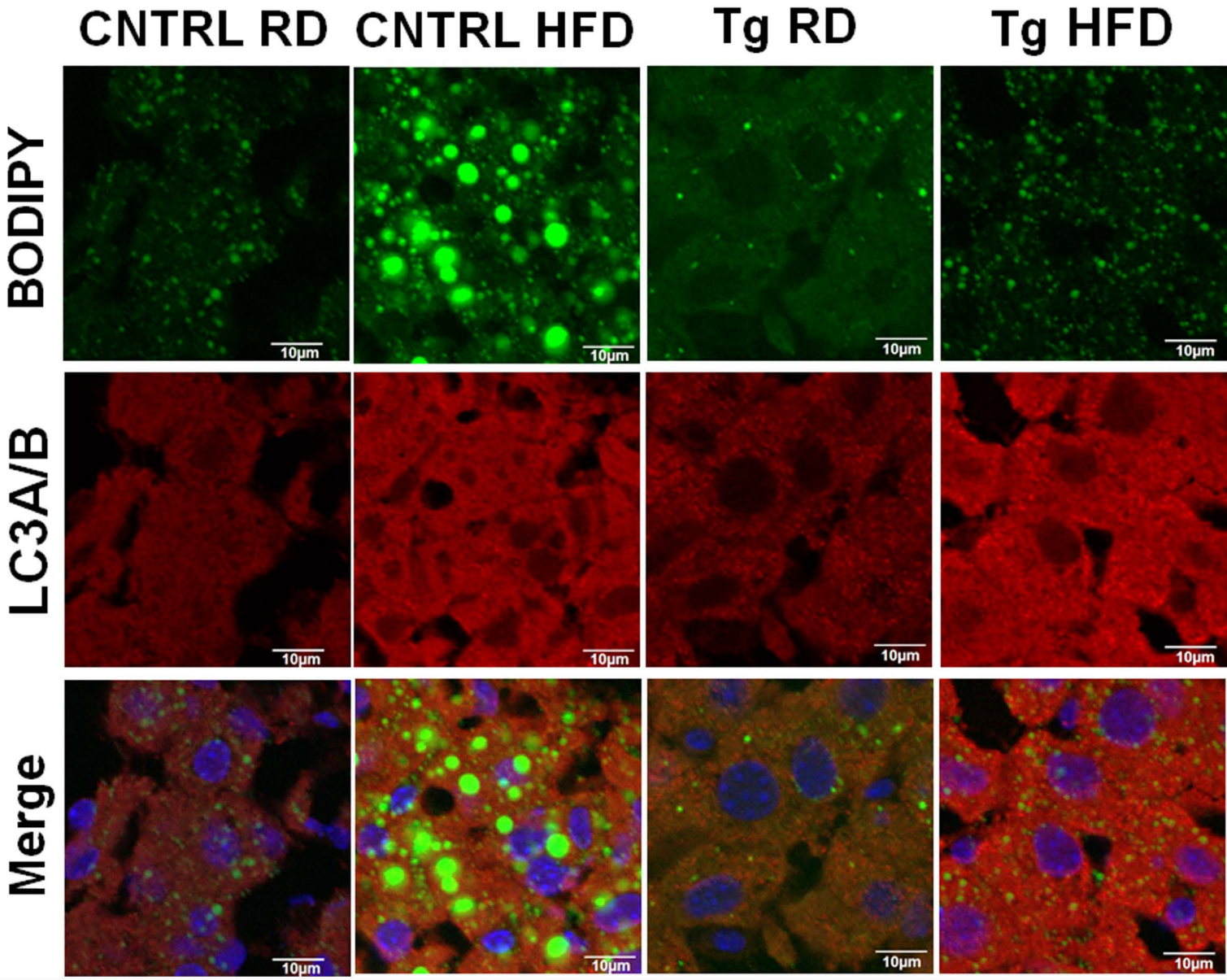
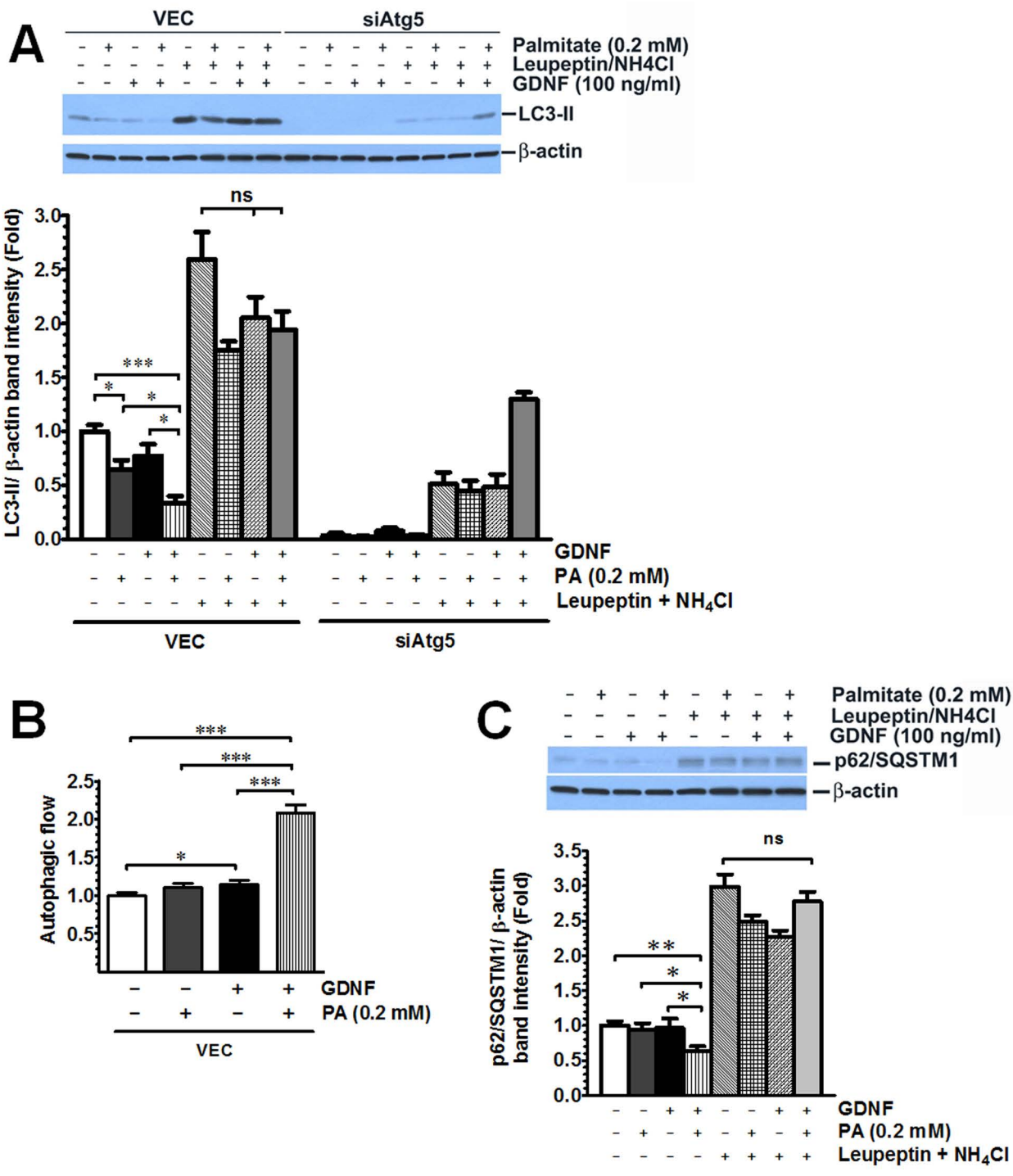


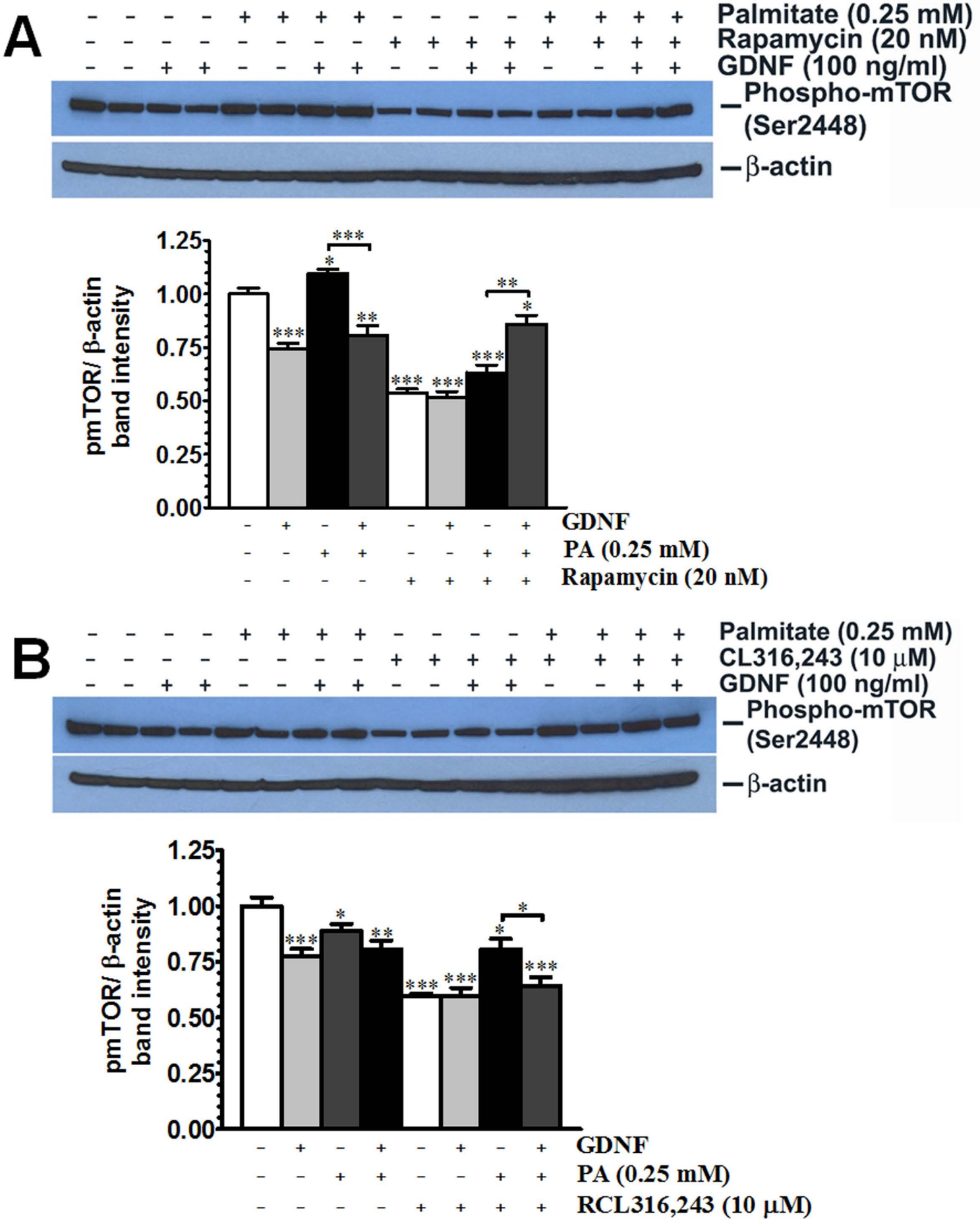
Supplementary Fig 1



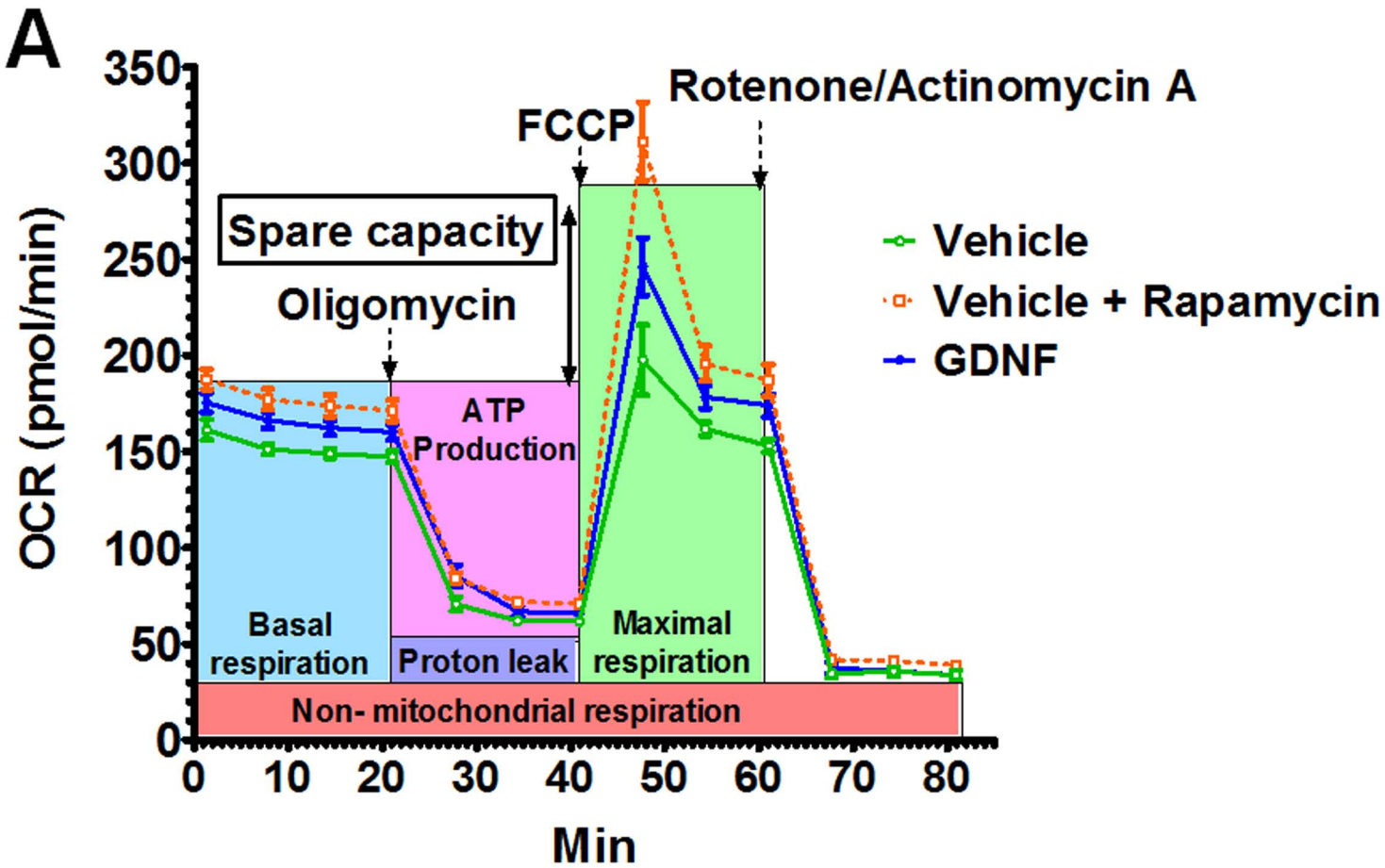
Supplementary Fig 2

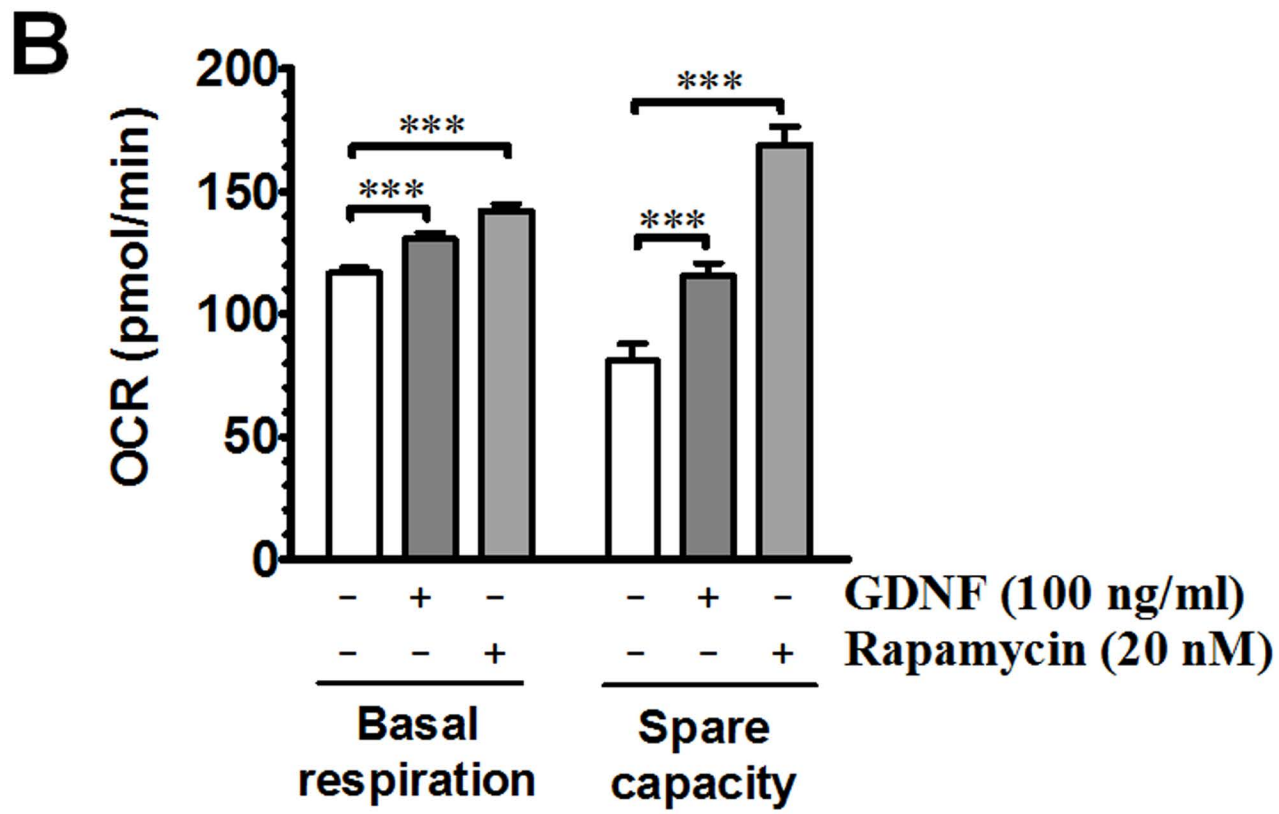


Supplementary Fig. 3



Supplementary Fig 4





Supplementary Fig 5

Glial Cell Line Derived Neurotrophic Factor Enhances Autophagic Flux in Mouse and Rat Hepatocytes and Protects Against Palmitate Lipotoxicity

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Supplementary Information

Supplementary Fig. 1

GDNF transgenic (Tg) mice have higher hepatic GDNF expression levels than control (CNTRL) mice. Western blot analysis of hepatic GDNF levels in CNTRL and Tg mice fed a RD or HFD for 12 weeks.

Supplementary Fig. 2

High fat diet-fed GDNF transgenic (Tg) mice less hepatic steatosis than HFD-fed control mice. Representative images of staining of liver sections from 12 weeks RD and HFD-fed CNTRL and Tg mice for triglycides (BODIPY) and LC3A/B). Nuclei are stained blue. Scale 10 μm.

Supplementary Fig 3

GDNF enhances autophagic flux in rat hepatocytes

A) Western blot analysis of LC3 levels in VEC (empty vector-infected) and siAtg5 (Atg5 siRNA infected) RALA255-10G rat hepatocytes cultured in medium supplemented with or without GDNF and palmitate (PA) for 6 days and with or without the lysosomal inhibitors leupeptin and ammonium chloride during the last 4 h of culture and (B) plot of autophagic flow for VEC cells

exposed to GDNF and PA for 6 days. Plotted are means + SEM (***, P<0.001; *, P<0.05. n=3). (C) Western blot analysis of p62/SQSTM1 levels in VEC cells cultured for 6 days with or without GDNF and palmitate and with or without the lysosomal inhibitors leupeptin and ammonium chloride during the last 4 h of culture. Plot of p62/SQSTM1 band intensity adjusted to β-actin. Plotted are means + SEM (**, P<0.01; *, P<0.05. n = 3).

Supplementary Fig. 4

GDNF dephosphorylates mTOR in HepG2 cells. Western blot analysis of phospho-mTOR (Ser2448) levels in HepG2 cells cultured for 48h in medium supplemented with or without GDNF (100 ng/ml) and palmitate (0.25 mM) and during the last 24h with or without (A) rapamycin (20 nM) and (B) the β_3-adrenergic receptor agonist CL316,243 (10 µM). (C) Mitochondrial respiration profile and (D) comparisons of basal respiration and spare respiratory capacity in HepG2 cells cultured for 6 days in medium supplemented with or without GDNF and with or without rapamycin during the last 24h of culture. Plotted are means + SEM (***, P<0.001; **, P<0.01; *, P<0.05. n = 5).

Supplementary Fig.5

GDNF enhances mitochondrial respiration in HepG2 cells. (A) Mitochondrial respiration profile and (B) comparisons of basal respiration and spare respiratory capacity in HepG2 cells cultured for 6 days in medium supplemented with or without GDNF and with or without rapamycin during the last 24h of culture. Plotted are means + SEM (***, P<0.001. n = 5).