Supplemental Material SM1: Materials and Methods

Detection of LC3 Protein by Western Blot

Frozen livers were minced and washed in cold lysis buffer [50 mM Tris, 250 mM NaCl, 2 mM EDTA, 10% glycerol + protease inhibitors, 0.5% NP-50, pH 7.2]. The homogenates were then incubated at 4C for 30 min on a rotator, sonicated for 10 sec, and centrifuged at 12,000 x g for 10 minutes (4C). Following centrifugation, the protein concentration of the supernatant was determined using the Bradford method. LC3 protein (50 μg) for each sample was loaded into a 4-20% Tris-HCl Ready Gel and run (60 V, 25C) for 90 min. The protein was then transferred to a nitrocellulose membrane (60 V, 0C, 60 min) and incubated in polyclonal rabbit anti-LC3 antibody (LC3; Medical and Biological Laboratories Co.) for 60 min at 25C. After being washed with Tween Tris-buffered saline (TTBS), the membranes were incubated with 1:10,000 goat anti-rabbit IgG:horseradish peroxidase (StressGen) for 60 min at 25C, developed with an ECL Plus Western Blotting Detection System (Amersham) for five min, and exposed to Kodak Biomax MR film. The films were scanned using an Epson Perfection 1200 U flatbed scanner. The density of the LC3-I and LC3-II bands were measured on each film using computer software (Gel Pro Analyzer). For statistical analysis, protein density was averaged for the young and old rats at each time point. Values obtained from densitometry were analyzed using T-tests. Comparisons with $p<0.05$ were considered statistically significant. Data are presented as mean ± SE.