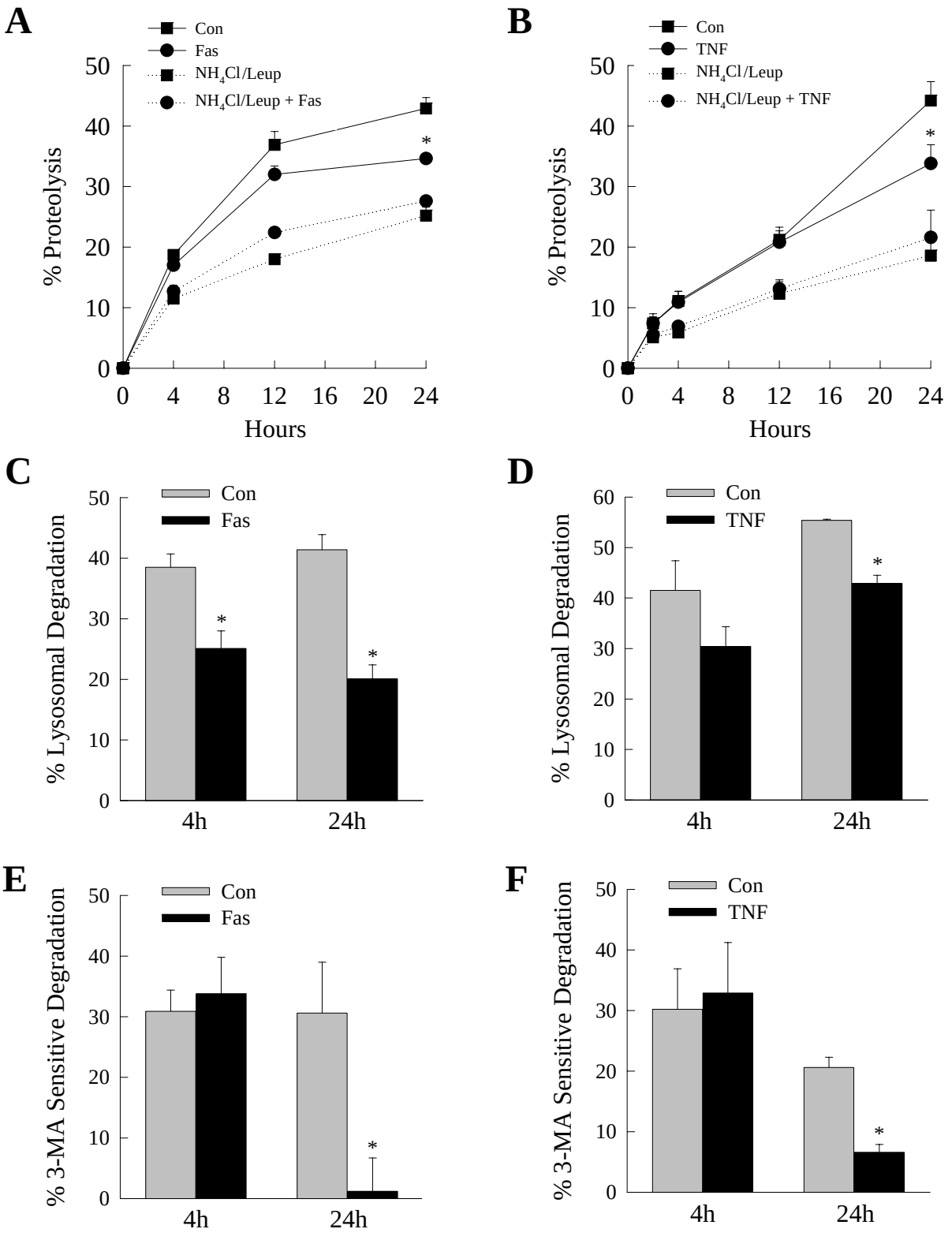
Supplemental Fig. 1. Effect of Jo2 and TNF on autophagic activity. Jo2 and TNF interfere with macroautophagy progression. *A-B,* wild-type MEFs treated with Jo2 (Fas) (*A*) or TNF (*B*) were labeled with [^{3}H]leucine for 48 h. After extensive washing, the cells were incubated in fresh media alone or with Jo2/TNF and rates of protein degradation measured as described in Experimental Procedures. Where indicated, 20 mM ammonium chloride and 100 μM leupeptin or 10 mM 3-MA were added to the incubation media. Values are expressed as a percentage of total radiolabeled protein transformed into soluble amino acids at each time point and are from 4 independent experiments with triplicate wells (* $P<0.01$ as compared to control cells). *C-D,* the percentage of degradation occurring in lysosomes in wild-type MEFs treated with Jo2 (Fas) (*C*) or TNF (*D*) was calculated from the inhibitory effect observed in cells treated with ammonium chloride/leupeptin. Values are expressed as the percentage of total protein degradation and are from 4 independent experiments with triplicate wells (* $P<0.01$ as compared to control cells). *EF,* levels of macroautophagy were calculated in wild-type MEFs treated with Jo2 (Fas) (*E*) or TNF (*F*) as the percentage of lysosomal degradation sensitive to inhibition by 3-MA in the same experiments (* $P<0.005$ as compared to control cells).

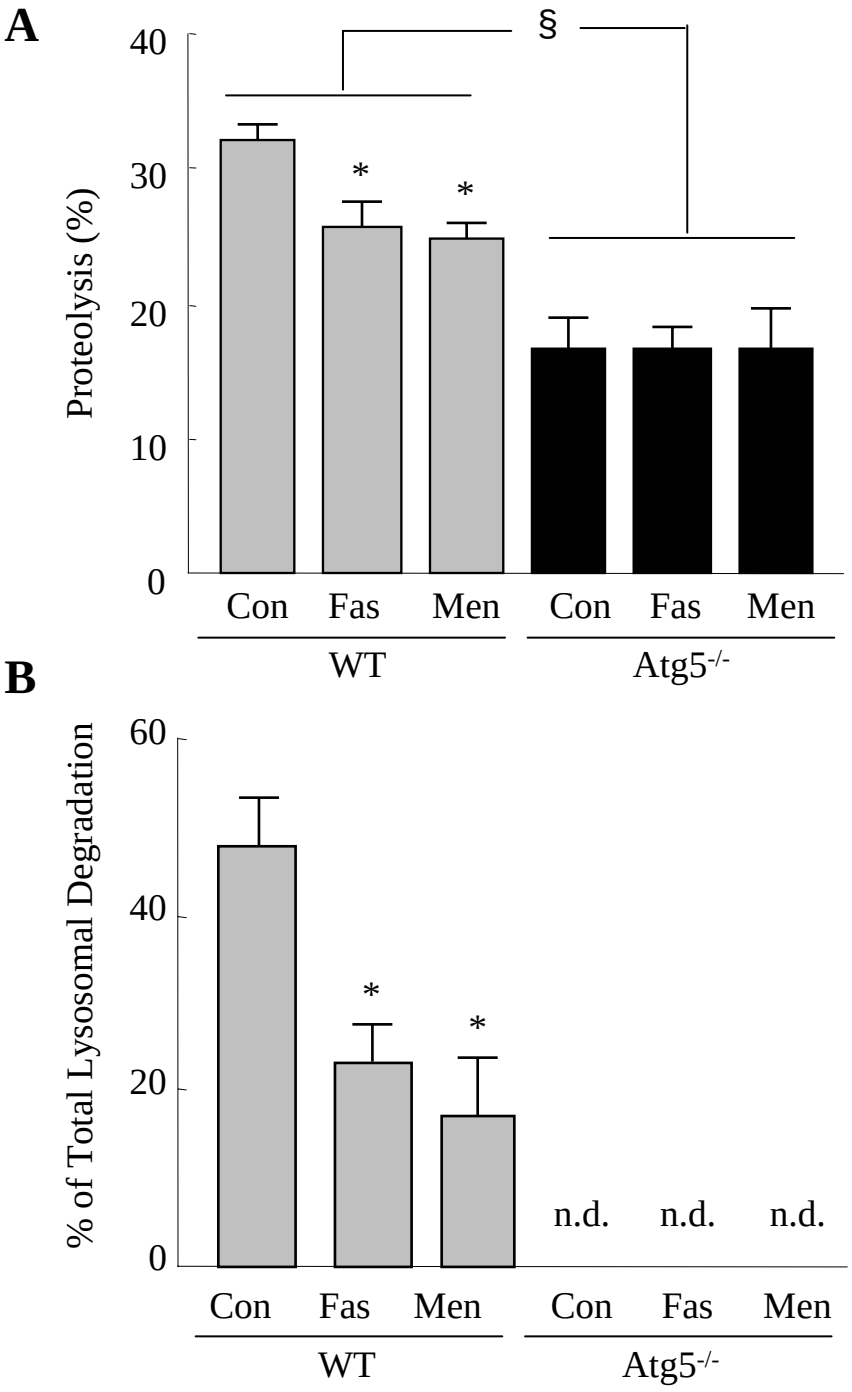
Supplemental Fig. 2. Fas and menadione reduce macroautophagy-dependent proteolysis. Wildtype (WT) and Atg5$^{-/-}$ MEFs were treated with Jo2 (Fas) or menadione (Men) and the total rates of protein degradation (*A*) and the percentage of lysosomal protein degradation attributable to macroautophagy (*B*) were calculated as in Supplemental Fig. 1. Results are from 3-5 experiments with triplicate wells (* $P<0.001$ between control and treated cells; § $P<0.0001$ between WT and Atg5$^{-/-}$). N.d., not detected.

Supplemental Fig. 3. TNF blocks the ability of rapamycin to induce macroautophagy. Wildtype cells were untreated or treated with TNF and the total rates of protein degradation were calculated and the percentage of macroautophagy determined as in Supplemental Fig. 1. Cells were treated with 100 μM rapamycin during the chase to stimulate macroautophagy. Values are expressed as fold-increase in macroautophagy in response to rapamycin treatment as compared to untreated cells. Results are from 2 experiments with triplicate wells.

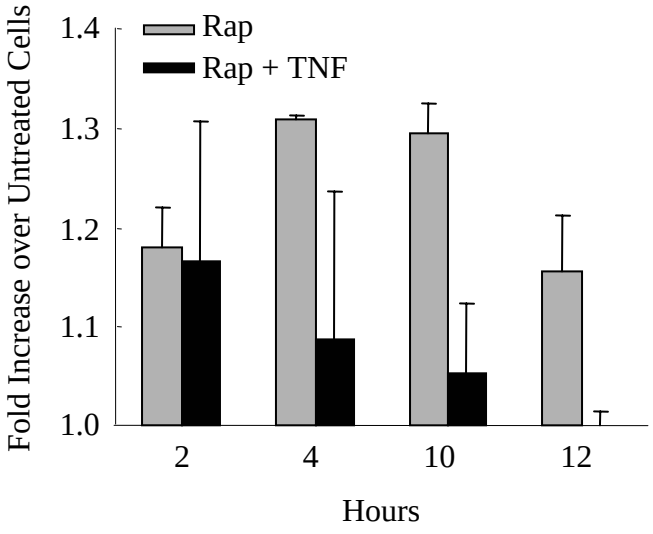
Supplemental Fig. 4. Atg5$^{-/-}$ cells have lower levels of oxidized proteins upon exposure to oxidative stress. Cytosol (20 μg protein) from wild-type (WT) and Atg5$^{-/-}$ MEFs untreated (Con) or treated with menadione (Men) were derivatized, subjected to SDS-PAGE and immunoblotted for DPN moieties. Membranes were stripped and reblotted for β-actin.



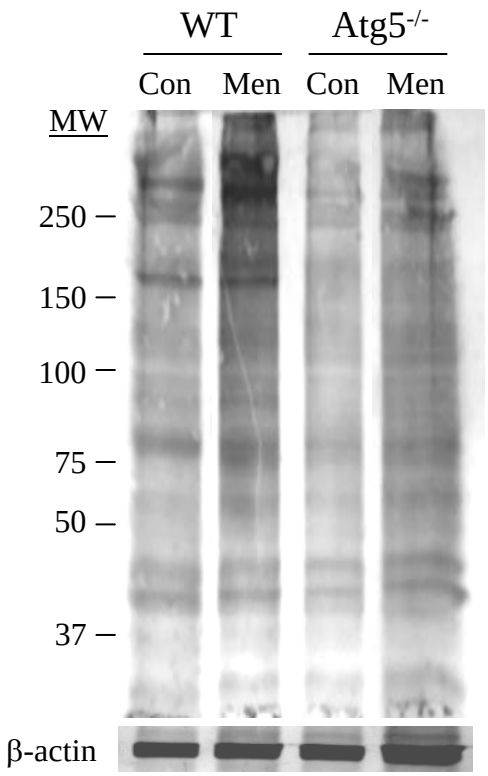
Supplemental Fig. 1



Supplemental Fig. 2



Supplemental Fig. 3



Supplemental Fig. 4