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Supplemental Material to:

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A nonapoptotic role for CASP2/caspase 2: Modulation of autophagy

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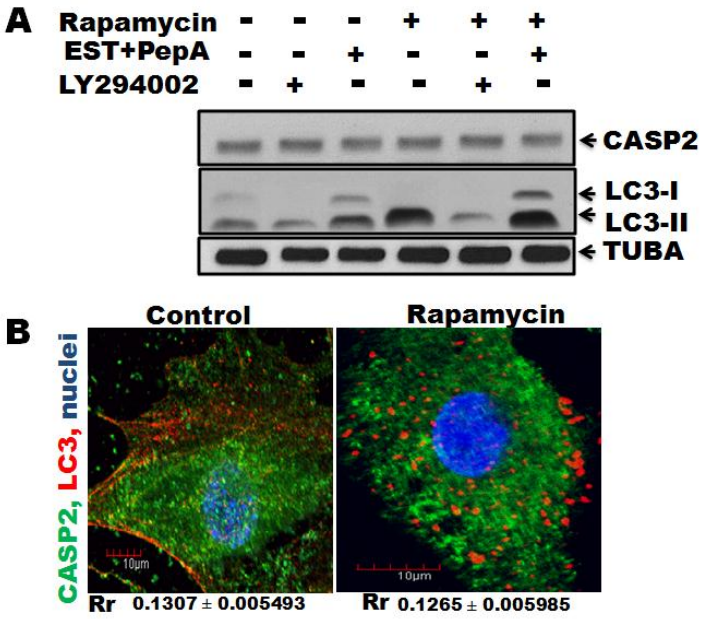

Figure S1. CASP2 levels are not regulated by autophagy. (**A**) WT MEFs were treated with early- and late-stage autophagy inhibitors as well as in the presence of 500 nM rapamycin (autophagy inducer) for 24 h. CASP2 levels were determined by performing western blotting. The effect of the inhibitors was validated by determining the levels of LC3 to detect autophagy. Shown are the representative western blots; the experiment was repeated 3 times. (**B**) Immunocytochemistry was performed to identify localization of CASP2 (green) with LC3 (red). Pearson Coefficient (Rr) was determined using ImageJ and values are presented as ±SEM that indicated lack of colocalization between CASP2 and LC3. At least 20 to 25 cells were analyzed and the experiment was performed in triplicate.

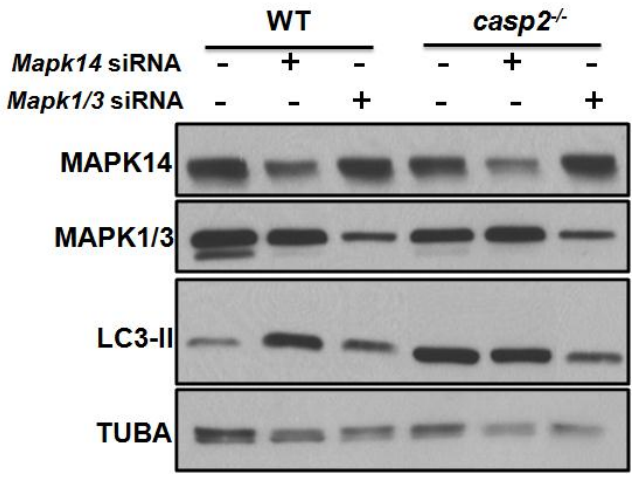

Figure S2. Role of MAPK14 and MAPK1/3 in loss of CASP2-induced autophagy. WT and *casp2*$^{-/-}$ MEFs were cultured in complete medium. MEFs were treated with prevalidated siRNA against *Mapk1/3* and *Mapk14*. Protein lysates were prepared and western blotting was performed to validate the efficiency of the siRNA by probing the blots for protein levels of MAPK14 and MAPK1/3. Modulation of autophagy was detected by determining LC3 levels (LC3-I and LC3-II). The same blots were probed for TUBA that served as loading control. Shown is a representative blot. Each experiment was repeated at least 3 times.

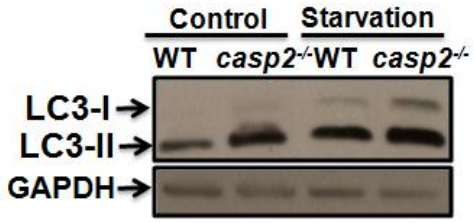

Figure S3. Effect of starvation on loss of CASP2-induced autophagy. WT and *casp2*$^{-/-}$ MEFs were untreated or starved (HBSS for 24 h) in the presence of PepA (1 μM) and EST (10 μM) (for 6 h). Protein lysates were prepared and western blotting was performed to determine LC3 levels (LC3-I and LC3-II). The same blots were probed for GAPDH that served as loading control. Shown is a representative blot. Each experiment was repeated at least 3 times.

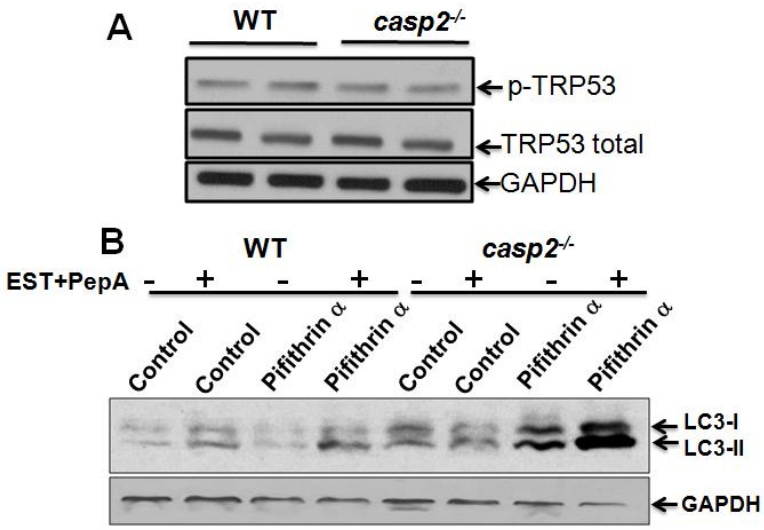

Figure S4. TRP53 is not involved in CASP2-mediated modulation of autophagy in unstressed cells. (**A**) Protein lysates were prepared from WT and *casp2*$^{-/-}$ MEFs cultured in complete medium. Western blotting was performed to detect the levels of active (phosphorylated at serine 15) and total (unphosphorylated) TRP53 using specific antibodies. The same blots were reprobed for GAPDH that served as a loading control. Shown is a representative blot. (**B**) Effect of TRP53 inhibitor (Pifithrin α, 30 μM) on CASP2-mediated autophagy modulation. WT and *casp2*$^{-/-}$ MEFs were incubated with Pifithrin α for 24 h in the presence or absence of PepA and EST (for 6 h). Samples were prepared and autophagy was detected by LC3-I to LC3-II conversion. GAPDH was used as a loading control. The experiment was repeated at least 3 times.