Supplementary Material for the article:

Reducing the Effects of Intracellular Accumulation of Thermolabile Collagen II Mutants By Increasing Their Thermostability in Cell Culture Conditions.

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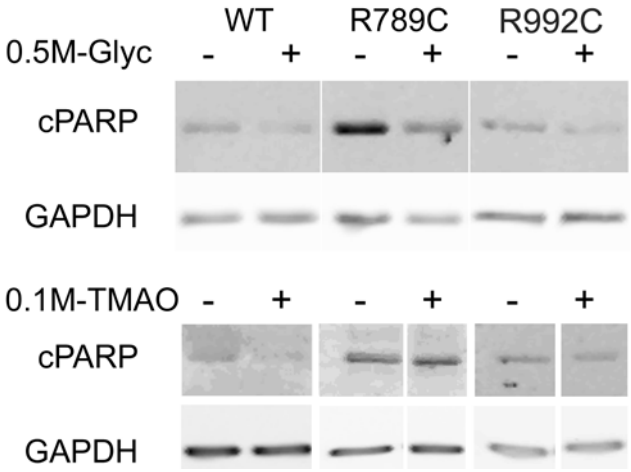
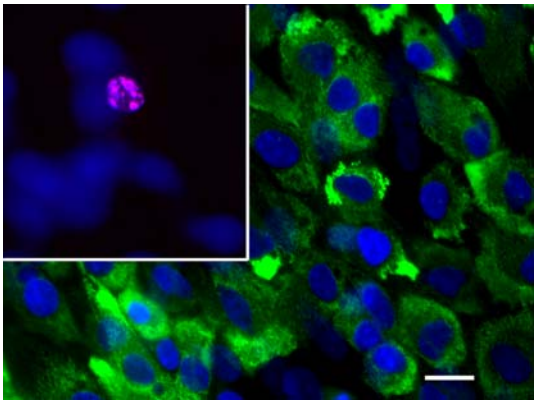


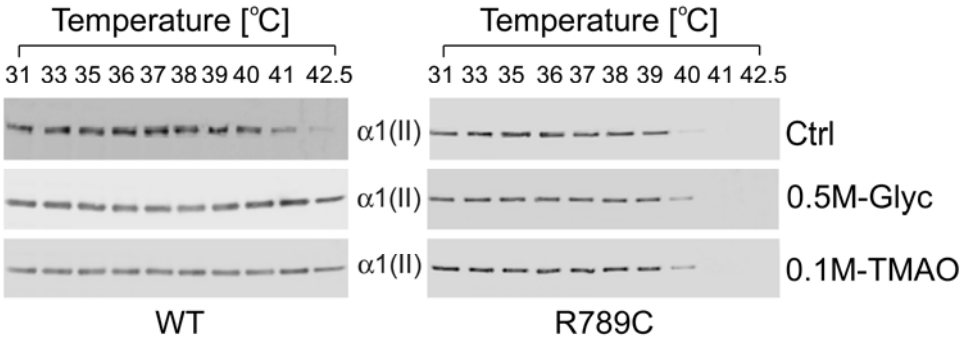
Supplementary Figure S1.

A representative Western blot of cPARP and GAPDH in cells expressing the WT, R789C and R992C collagen II in the presence of 0.5 M glycerol or 0.1 M TMAO. Symbols: cPARP, cleaved PARP; WT, R789C, and R992C, cells that express wild type and respective collagen II mutants; GAPDH, GAPDH-positive bands.



Supplementary Figure S2.

Microscopic analysis of apoptosis in cells expressing collagen II mutants. A representative image of cells expressing the GFP-tagged R992C mutant; the insert depicts an overlay of DAPI-positive and TMR-positive nuclei. Bar = 20 μm.



Supplementary Figure S3. Assays of the thermostability of the collagen II variants.

A representative Western blot assays of thermostabilities of collagen II variants in the presence of glycerol or TMAO. Following preincubation at increasing temperatures, collagen II samples were subjected to brief protease digestion. The digested samples were electrophoresed in 7.5% polyacrylamide gels in reducing conditions and then α1(II) chains were visualized by Western blot. Symbols: WT, wild type collagen II; R789C, collagen II mutant harboring R789C substitution; α1, α1(II) chains.