

Supplementary Material:

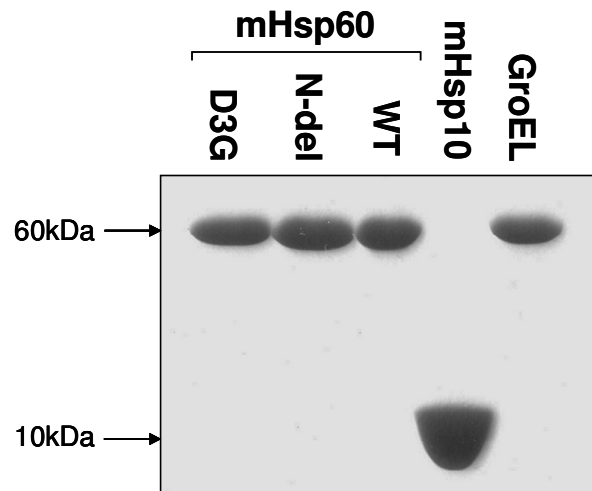


FIGURE 1S. **SDS-PAGE of the various proteins used in this study.** 10 μ g of each protein were analyzed by SDS-PAGE and then stained with Coomassie Brilliant Blue.

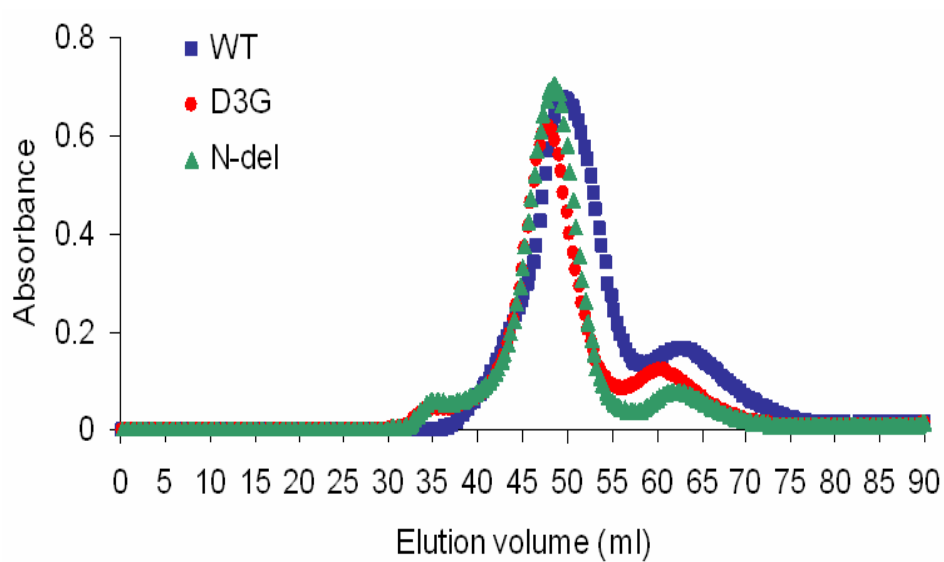


FIGURE 2S. **Gel-filtration chromatography of mHsp60 variants.** The elution pattern of mHsp60 variants from a gel-filtration column following reconstitution of the oligomers as described before (*1*).

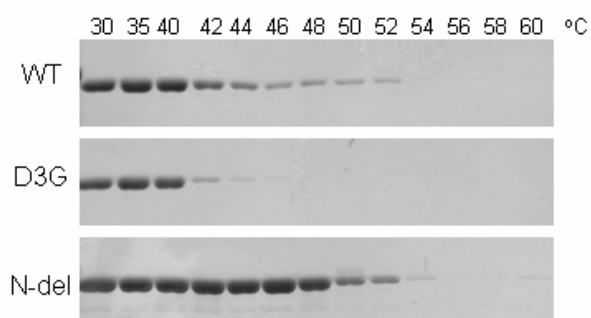
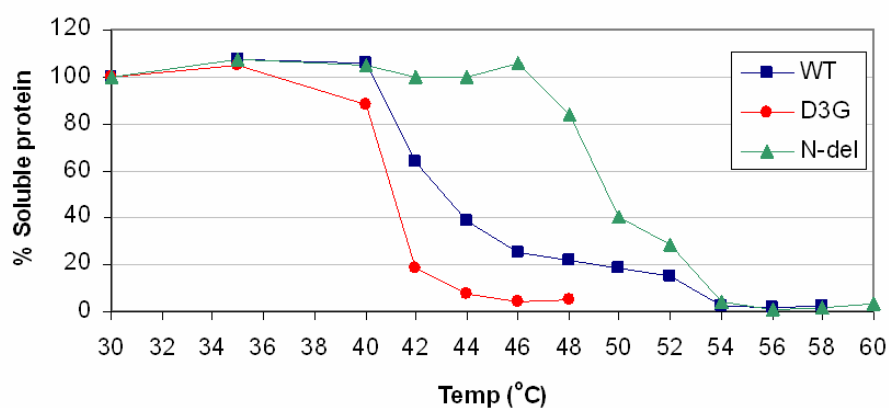
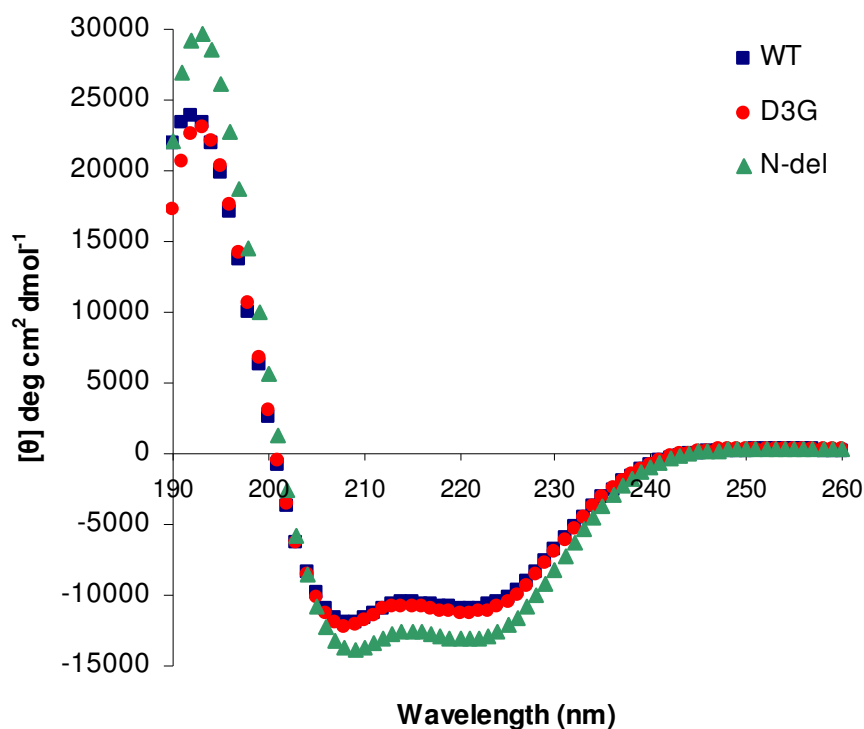
A**B**

FIGURE 3S. Thermal stability of mHsp60 variants. **A**, Samples of 5 μ M of wild-type and mHsp60 mutants in 50 mM Na-HEPES (pH 7.5), 50 mM KCl and 20 mM $MgCl_2$ were incubated for 20 minutes at the indicated temperatures and then centrifuged at 14,000 rpm for 15 min at room temperature. 5 μ l aliquots from supernatant fractions were analyzed by SDS-PAGE and then stained with Coomassie Brilliant Blue. **B**, Quantitative presentation of the results in panel A. Densitometric analysis was carried out using ImageMaster 1D Prime software. 100% is the amount of soluble protein at 30 °C.

A**B**

	WT	D3G	N-del
Helix	34.7 %	35.6 %	42.1 %
Antiparallel	7.9 %	7.6 %	6.2 %
Parallel	8.4 %	8.2 %	6.9 %
Beta-Turn	16.6 %	16.4 %	15.4 %
Rndm. Coil	32.2 %	31.6 %	27.7 %
Total Sum	99.7 %	99.4 %	98.4 %

FIGURE 4S. Circular dichroism analysis of mHsp60 variants. **A**, CD spectra of mHsp60 variants (2 mg/ml), measured at 25 °C in 10 mM Tris (pH 7.7), 10 mM KCl and 5 mM MgCl₂ over the range of 190-260 nm at a scan rate of 1 nm/sec. A cell with 0.1 mm path length was used. All measurements were done with an Aviv CD spectrometer model 202. Each spectrum is an average of 5 scans. The raw data were corrected by subtracting the contribution of the buffer to the signal. Then data were smoothed and converted to molar ellipticity units. **B**, Deconvolution of the results in panel A using CDNN program Version 2 (2).

REFERENCES

1. Viitanen, P. V., Lorimer, G., Bergmeier, W., Weiss, C., Kessel, M., and Goloubinoff, P. (1998) Purification of mammalian mitochondrial chaperonin 60 through in vitro reconstitution of active oligomers, *Methods Enzymol.* 290, 203-217.
2. <http://www.photophysics.com/cdnn.php>