

SUPPLEMENTARY FIGURE LEGENDS

Supplementary Fig. 1. SDS-PAGE of purified recombinant Hsp47s. The final purities of recombinant Hsp47s used in this study were assessed by SDS-PAGE. The molecular sizes are shown in kilodaltons.

Supplementary Fig. 2. Re-separation of the trimer and monomer fraction of biotinylated collagen model peptides (Bio-CP2) by gel filtration chromatography (GFC). The trimer (A) and monomer (B) fraction of Bio-CP2, preserved for 4 months at 4°C, were re-separated by GFC. The re-separation was performed as described in Fig. 2. In Supplementary Figs. 2A and 2B, 5.4 μM and 3.2 μM peptides as a base of monomer were applied, respectively.

Supplementary Fig. 3. Concentration-dependence of trimer formation in biotinylated collagen model peptides. *A-E.* Separation of various concentrations of biotinylated collagen model peptides (Bio-CP2) in PBS by gel filtration chromatography (GFC). Separation was performed as described in Fig. 2. *F.* The ratio of trimer to monomer formation. The ratio was calculated as: Ratio = Area of absorbance at 215 nm in trimer fraction / [(Area of absorbance at 215 nm in trimer fraction) + (Area of absorbance at 215 nm in monomer fraction)].

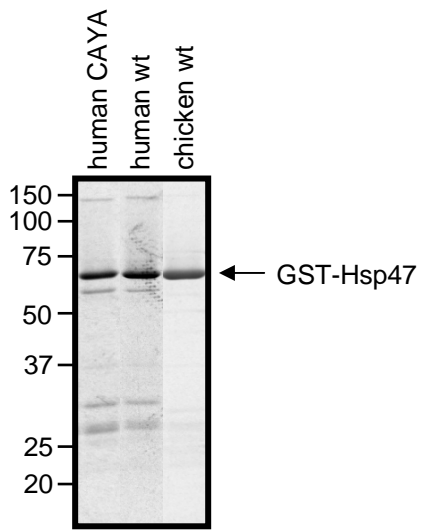
Supplementary Fig. 4. Reversibility of triple helix formation in collagen model peptides, before separation by GFC, by switching between 37°C and 4°C. Using untreated (A) or heat-treated (B) Bio-CP2 mixture after cooling for 4 days at 4°C, we performed the same TR-FRET binding assays as described in Fig. 2. All reaction mixtures were incubated for 2 h at RT.

Supplementary Fig. 5. Reversibility of monomer formation in collagen model peptides by switching between 37°C and 4°C. *A-D.* Heat treatment of the monomer fraction. Monomer fractions of Bio-CP2 mixture, after separation by GFC, were maintained at 4°C (A, B) or heated for 2 h at 37°C (C, D). TR-FRET binding assays (A, C) and GFC (B, D) were performed using each peptide preparation. The separation by GFC and the TR-FRET binding assays were performed as described in Figure 2. Peptides were applied at the concentration of 4.9 μM to GFC.

E-H. Cooling of the heat-treated monomer fraction. TR-FRET binding assays (E, G) and GFC (F, H) were performed as described above using untreated (E, F) or heat-treated (G, H) monomer fractions of Bio-CP2, after cooling for 4 days at 4°C. Peptides were applied at the concentration of 4.9 μM to GFC.

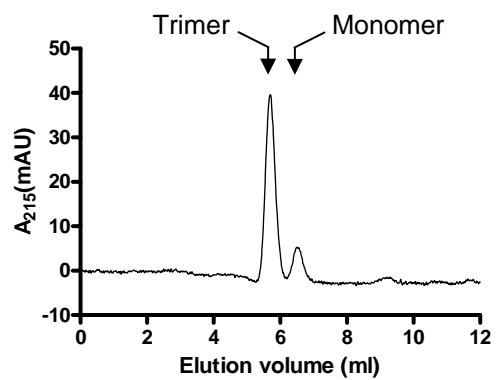
Supplementary Fig. 6. Specific interaction of Hsp47 with the triple helix of collagen model peptides *in vivo*. *A.* Fluorescence images of HeLa cells coexpressing: i) various collagen model peptides fused to foldon and mKG_C, and ii) wild-type or CAYA mutant Hsp47 fused to mKG_N. All constructs also had an ER signal sequence. Three days after transfection, mKG and Hoechst 33342 fluorescence was measured using an IN Cell Analyzer 1000. Bar, 100 μm. *B.* Fluorescence images of HeLa cells coexpressing collagen model peptides fused to foldon and mKG_C and Hsp47 fused to mKG_N, with or without the ER signal sequence. Bar, 100 μm. *C.* Expression levels of Hsp47 (*upper panel*) and collagen model peptides fused to foldon (*lower panel*) in transfected HeLa cells. Whole cell lysates were extracted and analyzed by Western blotting using specific antibodies against Hsp47 and the mKG_C fragment. The asterisk indicates a non-specific band. The molecular sizes are shown in kilodaltons. w, wild-type; C, CAYA mutant.

Supplementary Figure 1

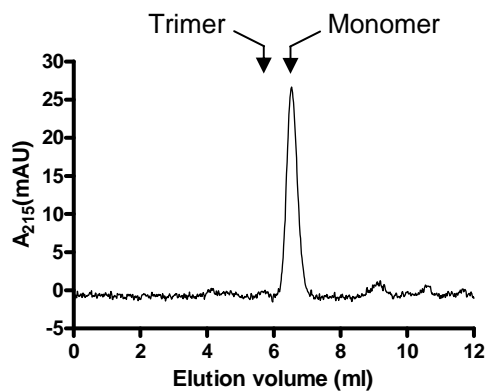


Supplementary Figure 2

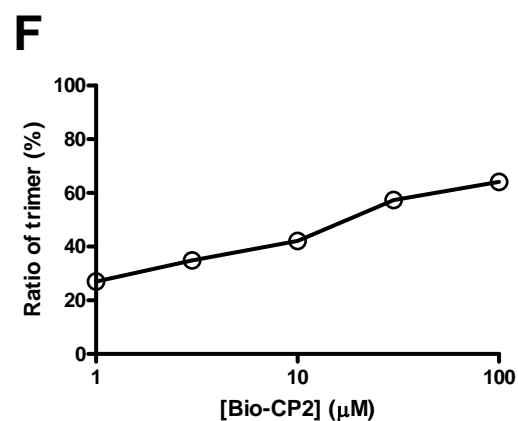
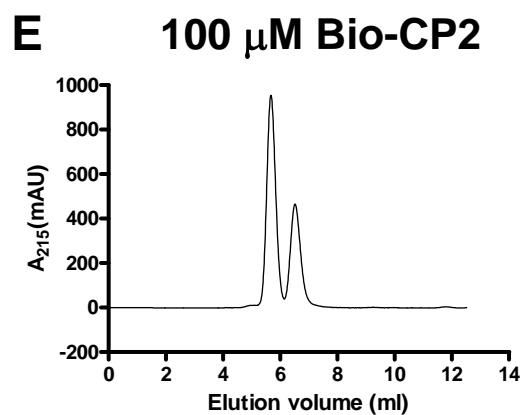
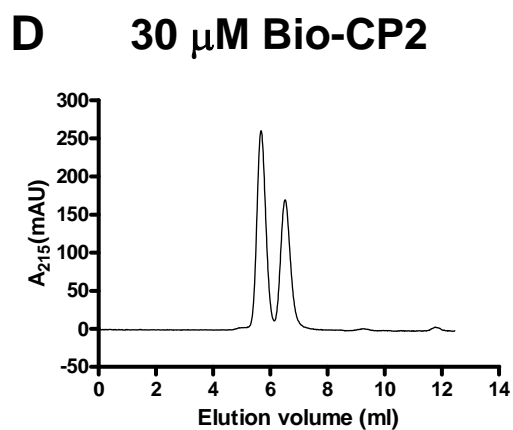
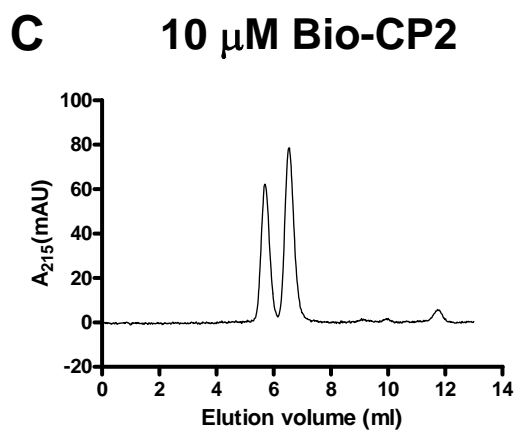
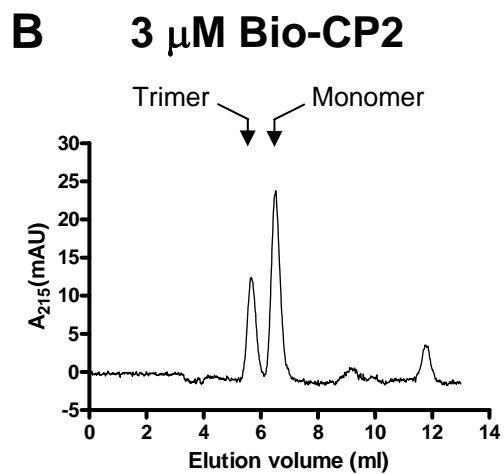
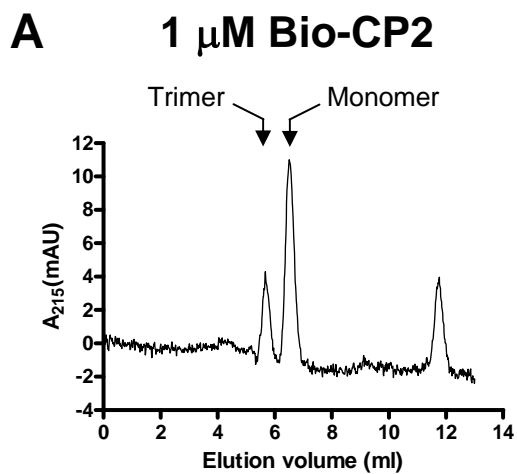
A Trimer fraction



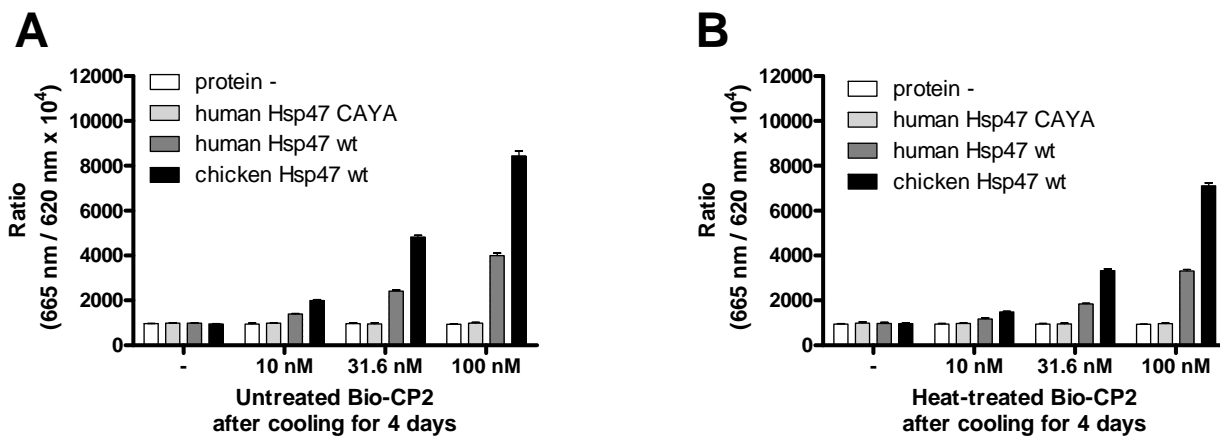
B Monomer fraction



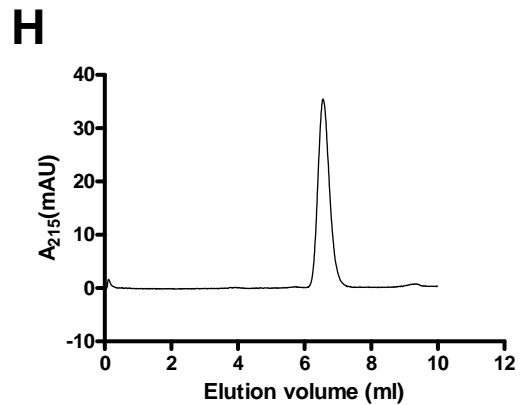
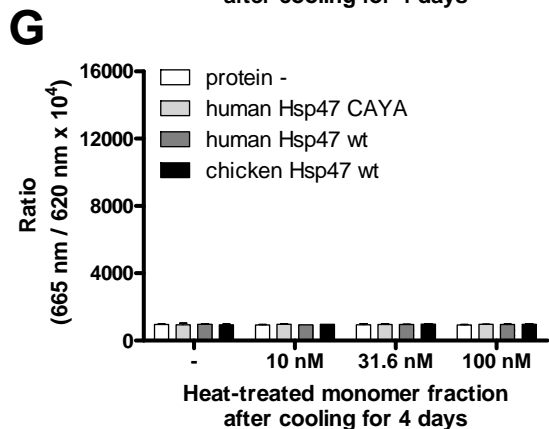
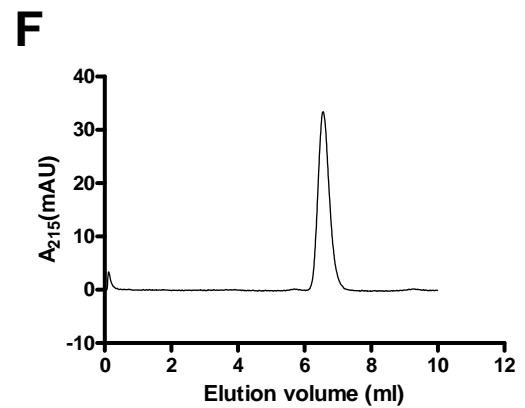
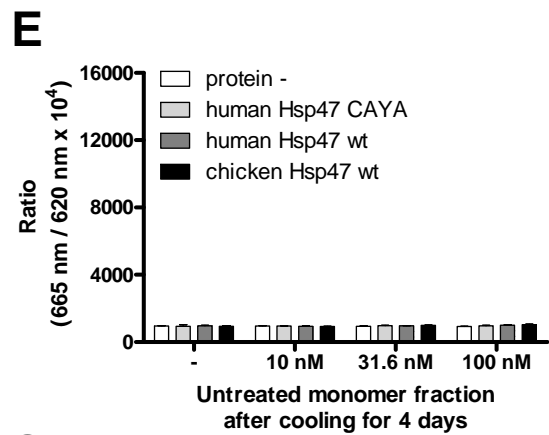
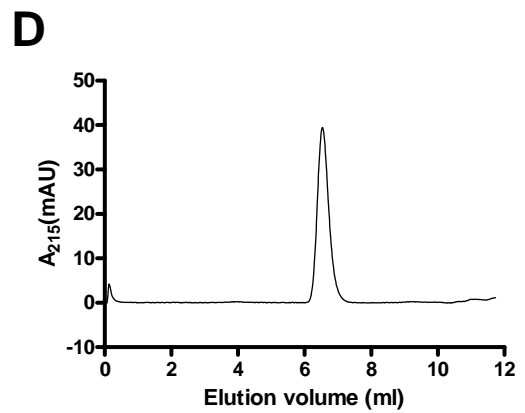
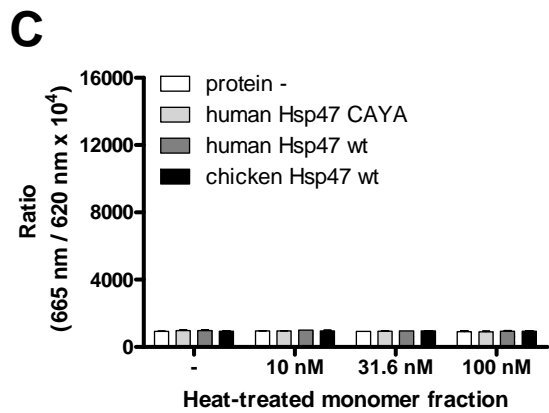
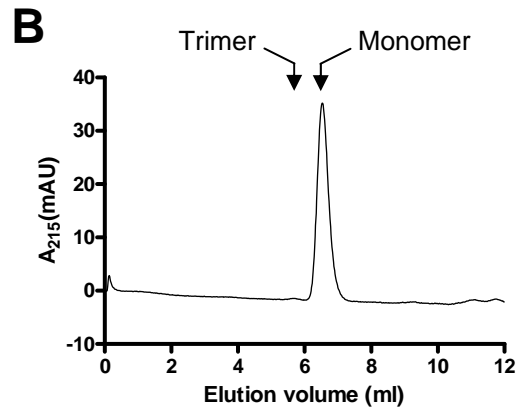
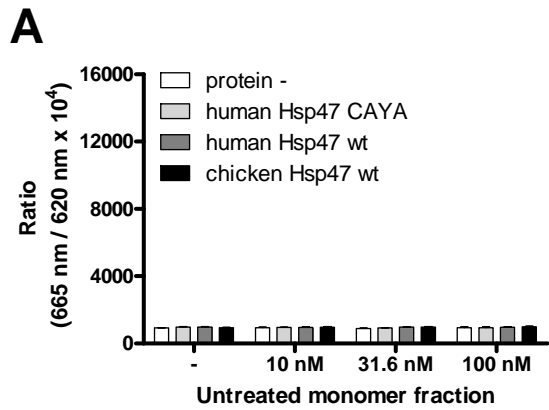
Supplementary Figure 3



Supplementary Figure 4



Supplementary Figure 5



Supplementary Figure 6

