Supplemental Material and Methods

Zinc Determination in Hsp33 and mutant variants - To determine the amount of zinc bound to reduced and oxidized wild type Hsp33 and the mutant variants, an assay was used, which is based on the formation of a bright red complex between free zinc and the dye 4-(2-pyridylazo) resorcinol (PAR) (ε_{500} of $PAR_2(Zn)$ = 66,000 $M^{-1}cm^{-1}$). The affinity K_a of PAR to zinc is 2 x 10^{12} M^{-1} at pH 7 (1). To assess the amount of free or loosely bound zinc in $Hsp33_{red}$, $Hsp33_{ox\text{-}30°C}$ or $Hsp33_{ox\text{-}43°C}$ (for preparation see Material and Methods in main text), 5 µM of Hsp33 was incubated with 100 µM PAR in 40 mM metal free KH_2PO_4 pH 7.5. To induce zinc release from the high affinity cysteine clusters in Hsp33, 30 µM of the mercury derivative PCMB (para-chloromercurybenzoic acid, ICN Biomedicals) was used instead of the originally described PMPS, which is no longer commercially available. PCMB forms stable mercaptide bonds with Hsp33's cysteines, leading to the release of zinc into the PAR-containing solution and the formation of $PAR_2(Zn)$ complexes, which is monitored at 500 nm.

Thermal MDH aggregation assay to determine Hsp33's chaperone activity – The chaperone activity of Hsp33 was determined by testing the influence of Hsp33 on the thermal aggregation of malate dehydrogenase (L-MDH, Roche). In short, 20 µM MDH was diluted to a final concentration of 300 nM in 40 mM KH_2PO_4 pH 7.5 at 45°C under continuous stirring in the presence or absence of a 4-fold molar excess of wild-type Hsp33 and its mutant variants. Light scattering was monitored using a Hitachi F4500 fluorescence spectrophotometer equipped with a temperature controlled cuvette holder and stirrer at λ_{ex}/ λ_{em} of 360 nm.

Supplemental Figure legends

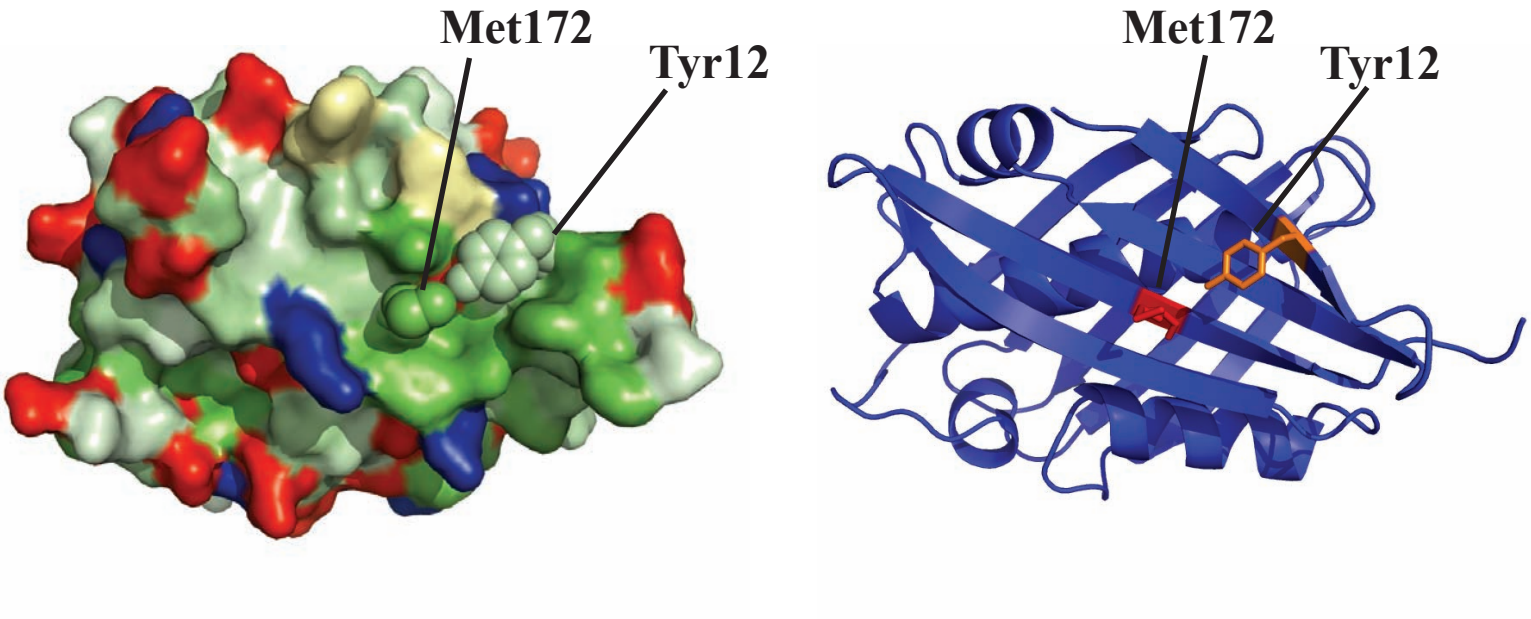
S-Fig. 1. Model of the N-terminal linker-binding platform in oxidized Hsp33
Structure of the N-terminal domain (residues 7-178) of *E. coli* Hsp33 (pdb code: 1HW7) is shown. Residues Tyr12 and Met172 are located in the highly hydrophobic β-sheet platform of Hsp33's N-terminal domain, which is covered by the linker region in reduced Hsp33 and predicted to become exposed upon Hsp33's oxidative activation. In surface presentation (**left panel**), positively and negatively charged residues are depicted in blue and red, respectively. The non-charged residues are colored based on their hydrophobicity ranging from most hydrophilic (yellow) to most hydrophobic (green) (2). Residues Tyr12 and Met172 are depicted as spheres colored according to their relative hydrophobicity (2). The ribbon presentation (**right panel**) serves to illustrate the orientation of the depicted structure.

S-Fig. 2. Chaperone activity and zinc coordination of wild-type Hsp33 and mutant variants
A. *In vitro* chaperone activity of Hsp33 and its variants.
Temperature-induced aggregation of 300 nM malate dehydrogenase (MDH) in the absence or presence of a 4-fold molar excess of reduced, zinc-reconstituted Hsp33 ($Hsp33_{red}$) or Hsp33 that was oxidized with 2 mM H_2O_2 at 43°C for 180 min ($Hsp33_{ox43°C}$). Light scattering signal of thermally unfolded MDH after 13 min at 45°C was monitored. 0% activity is defined as the light scattering signal in the absence of any chaperones while 100% activity is defined as the light scattering signal of MDH in the presence of 4-fold molar of fully active wild-type $Hsp33_{ox43°C}$.
B. Zinc coordination of wild type Hsp33 and mutant variants. 5 µM freshly reduced or oxidized Hsp33 wild type and mutants were incubated with 100 µM PAR. No significant zinc release was observed from reduced wild type $Hsp33_{red}$ in the presence of a 20-fold excess of PAR (K_α= 2 x 10^{12} M^{-1} at pH 7) (1) confirming that zinc is tightly associated with all three reduced Hsp33 variants. Only upon addition of 30 µM of the mercury derivative PCMB, release of zinc from the cysteine-coordinated zinc site was detected. The absorbance of $Zn(PAR)_2$ was monitored at 500 nm to calculate the ratio of $Zn(PAR)_2$ complexes to Hsp33. As shown in the figure, zinc binding was detected only for the reduced Hsp33 preparations. These results confirm that oxidation causes loss of zinc binding in all three Hsp33 variants.

S-Fig. 3. Far-UV CD spectra of wild type Hsp33 and the mutant variants
A. and B. Temperature-induced changes in the secondary structure of wild-type Hsp33 and Hsp33 mutants. **A.** Preparations of freshly reduced $Hsp33_{red}$, Hsp33-$Y12E_{red}$, Hsp33-$M172S_{red}$ or fully oxidized wild type Hsp33 ($Hsp33_{ox43°C}$) were heated from 20°C to 80°C (1°C per minute). Changes in the molar ellipticity were recorded at 222 nm. **B.** Preparations of freshly reduced $Hsp33_{red}$, Hsp33-$Y12E_{red}$, Hsp33-$M172S_{red}$ or fully oxidized wild type Hsp33 ($Hsp33_{ox43°C}$) were heated from 20°C to 50°C (straight lines) and subsequently cooled from 50°C to 20°C (dotted lines). Both heating and cooling rates were set to 1°C per minute. Changes in the molar ellipticity were recorded at 195 nm.
C. Secondary structure of reduced and oxidized wild type Hsp33 and mutant variants. Far-UV CD spectra of (closed squares) freshly reduced Hsp33 preparations ($Hsp33_{red}$) or preparations that were incubated with 2 mM H_2O_2 for 3 h at either (open circles) 30°C ($Hsp33_{ox30°C}$) or (open squares) 43°C ($Hsp33_{ox43°C}$) were recorded. Reduced and oxidized preparations of wild-type Hsp33 and the mutant variants were prepared as described in the Material and Method section of the main text.

S-Fig. 4. Growth defect in *E. coli* strains expressing Hsp33-M172S and Hsp33-Y12E
A and B. *E. coli* strains JH21 (BL21, Δh*slO*) expressing (triangles) wild type Hsp33, (circles) Hsp33-M172S or (squares) Hsp33-Y12E from a pET11a plasmid were cultivated (open symbols) without IPTG or (closed symbols) with 50 µM IPTG in MOPS minimal medium at either (**A**) 37°C or (**B**) 43°C. The OD was monitored at 600 nm. No growth defect was observed when cells were cultivated with 25 µM IPTG. **C.** Expression level of wild type Hsp33 and mutant variants upon cultivation in 0, 25 or 50 µM IPTG in MOPS minimal medium at 37°C. Cells were harvested at OD_{600} of 0.6, resuspended and lysed in 2 x Laemmli buffer and analyzed on reducing SDS PAGE. The amount of protein loaded was adjusted to the same cell OD_{600}. **D.** Expression of Hsp33-M172S at 43°C causes protein aggregation *in vivo*. *E. coli* strains JH21 (BL21, Δh*slO*) expressing either wild type Hsp33 or Hsp33-M172S were cultivated in 50 µM IPTG for 5 hours at 43°C. Cells were harvested by centrifugation and soluble and insoluble fractions were prepared. The insoluble protein fraction was loaded onto a reducing SDS-PAGE to visualize cellular proteins that co-precipitate with Hsp33-M172S. The amount of protein loaded was adjusted to the same OD_{600}. The lane with purified Hsp33 was added to the image for clarity.

References
1. Hunt, J. B., Rhee, M. J., and Storm, C. B. (1977) *Analytical biochemistry* **79**(1-2), 614-617
2. White, S. H., and Wimley, W. C. (1998) *Biochimica et biophysica acta* **1376**(3), 339-352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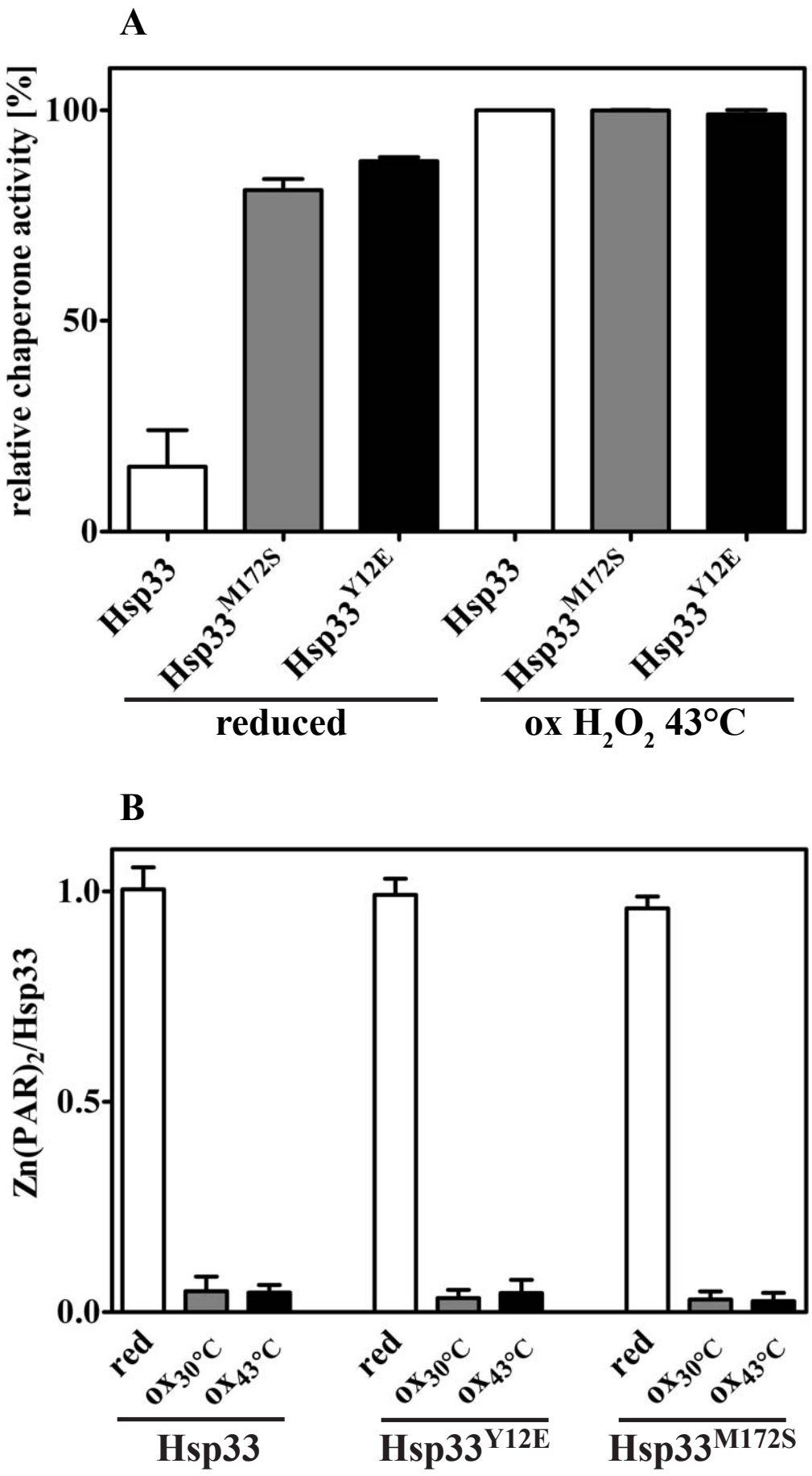
Tyr12
Met172
Tyr12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50
0
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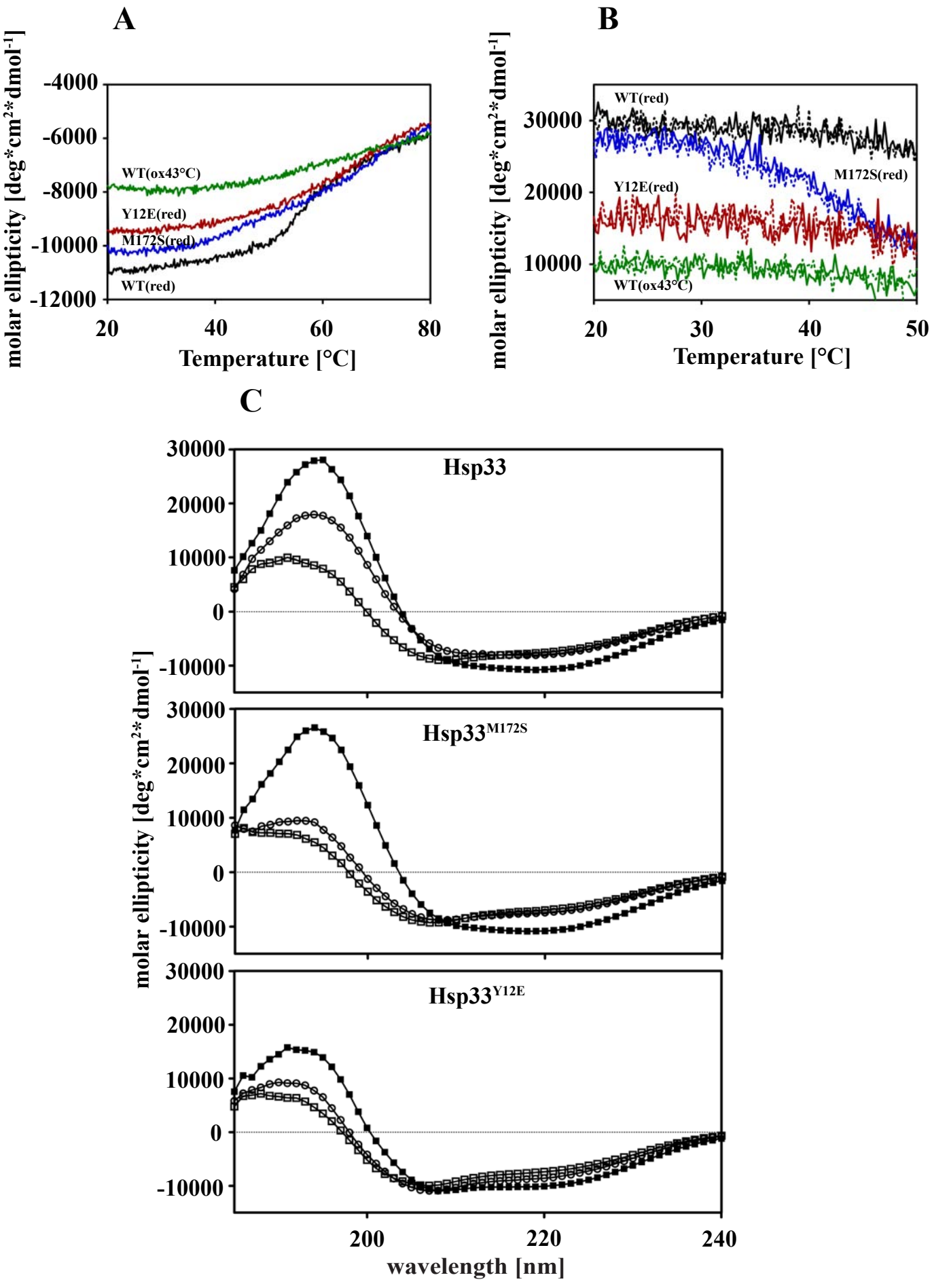
Hsp33M172S
Hsp33Y12E
Hsp33
Hsp33M172S
Hsp33Y12E
reduced
ox H2O2 43°C
B
Zn(PAR)2/Hsp33
1.0
0.5
0.0
red
ox30°C
ox43°C
red
ox30°C
ox43°C
red
ox30°C
ox43°C
Hsp33
Hsp33Y12E
Hsp33M172S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-6000
-8000
-10000
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WT(ox43°C)
Y12E(red)
M172S(red)
WT(red)
20
40
60
80
Temperature [°C]
B
molar ellipticity [deg*cm²*dmol⁻¹]
30000
20000
10000
WT(red)
M172S(red)
Y12E(red)
WT(ox43°C)
20
30
40
50
Temperature [°C]
C
molar ellipticity [deg*cm²*dmol⁻¹]
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Hsp33M172S
Hsp33Y12E
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10000
0
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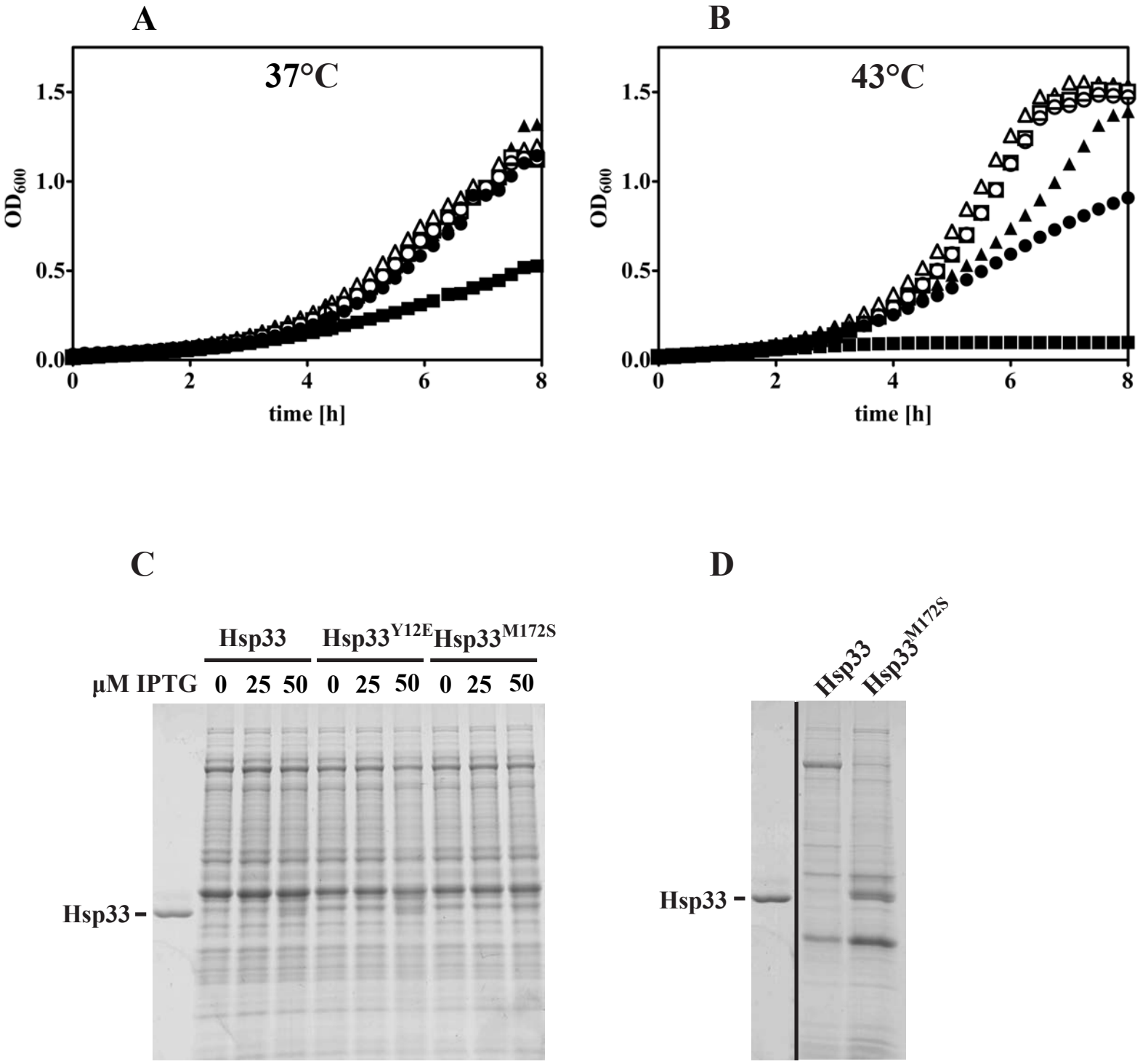
wavelength [nm]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1.0
0.5
0.0
OD600
0
2
4
6
8
time [h]
B
43°C
1.5
1.0
0.5
0.0
OD600
0
2
4
6
8
time [h]
C
Hsp33
Hsp33Y12E
Hsp33M172S
µM IPTG 0 25 50 0 25 50 0 25 50
Hsp33
D
Hsp33
Hsp33M172S
Hsp33