SUPPLEMENTAL INFORMATION

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

CD spectroscopy- To prepare the protein sample for circular dichroism, the protein solution was exchanged with 10 mM phosphate buffer (pH 7.5), and 20 mM NaCl to a protein concentration of 1 mg/ml as determined by absorbance at 280 nm. CD spectral data were collected on a Biologic Science Instruments Model MOS450 AF/CD spectrometer at 25 °C. Each spectrum was an average of 10 scans recorded at 2 nm/min, with a slit width of 1.0 mm and path lengths of 0.1 mm and 10 mm for the far UV and the near-UV regions, respectively. The data used for graphical presentation and analysis were each an average of 10 different scans.

FIGURE LEGEND

Fig. S1. Comparison of wild type and mutant MDM2s (aa210-437) by CD spectroscopy. (**A-B**) purity and far-UV CD spectra of MDM2 proteins (**A**), and near UV CD spectra (**B**), respectively. Spectra were acquired at 25 °C in 10 mM phosphate buffer (pH 7.5), 20 mM NaCl. using protein concentrations of 1 mg/ml. Protein purity was confirmed using coomassie-stained SDS–PAGE (inset). The far UV CD spectra show that the mutants did not alter the secondary structure content of the zinc domain. However, the difference in the near UV CD spectra between WT and C322R mutant indicates a change in their tertiary structures and the red shift indicates perturbation of a Trp residue that is situated in an apolar environment (**C**).

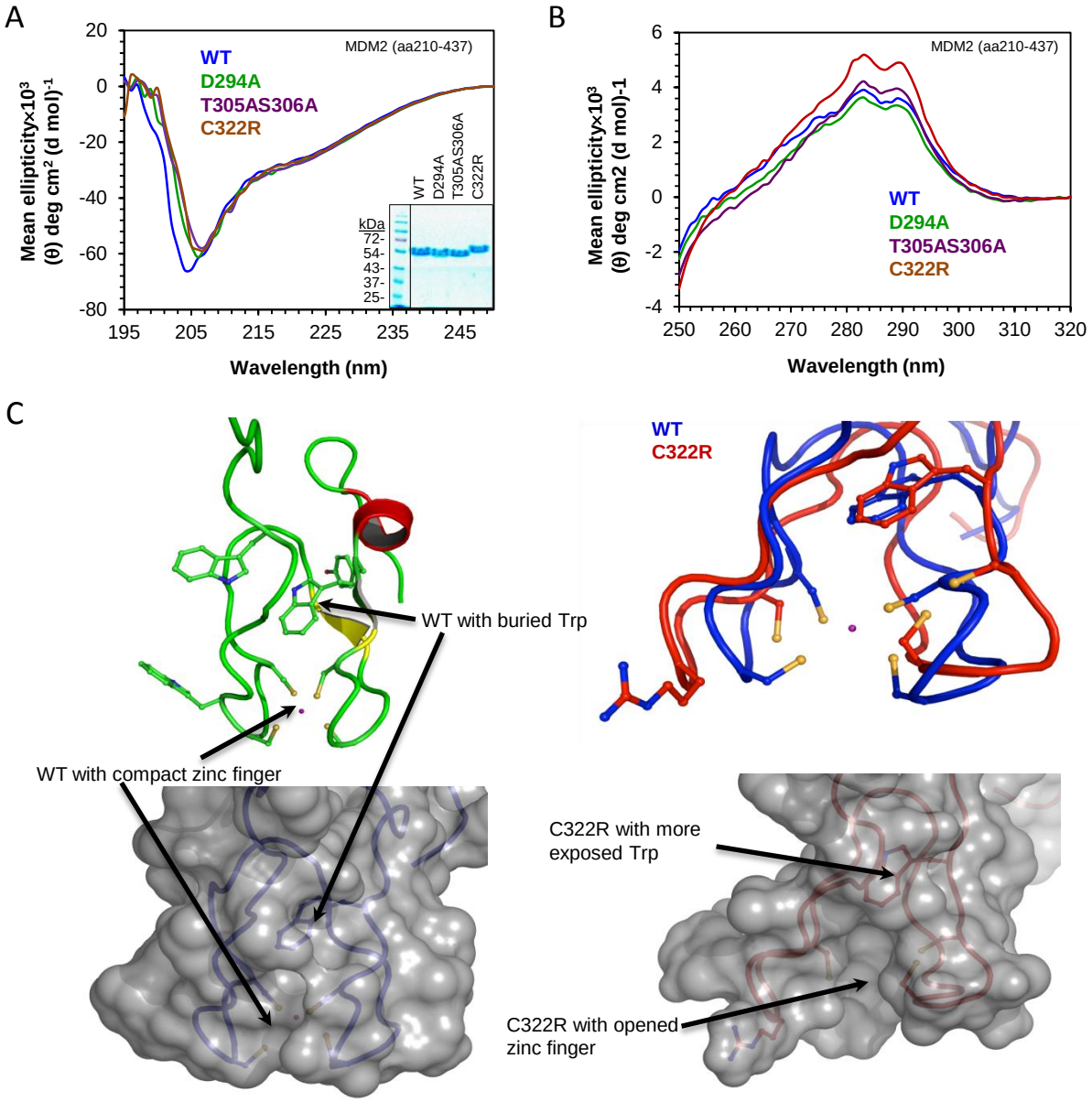
A
MDM2 (aa210-437)
WT
D294A
T305AS306A
C322R
Mean ellipticity×10³ (θ) deg cm² (d mol)⁻¹
Wavelength (nm)
kDa
72-
54-
43-
37-
25-
B
MDM2 (aa210-437)
Mean ellipticity×10³ (θ) deg cm2 (d mol)-1
Wavelength (nm)
C
WT with buried Trp
WT with compact zinc finger
WT
C322R
C322R with more exposed Trp
C322R with opened zinc finger

Figure S1