**Supplementary Materials**

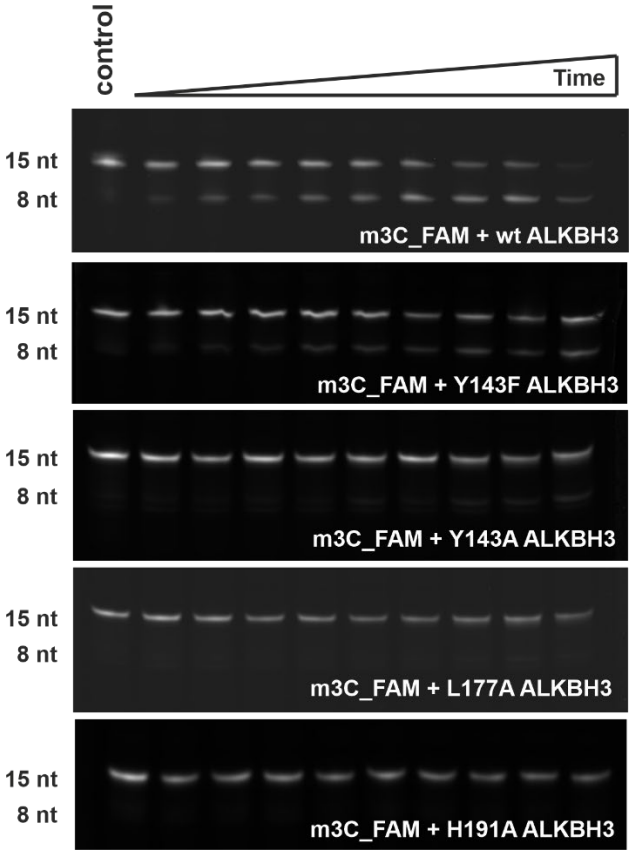


**Figure S1.** PAGE analysis of ALKBH3 repair activity towards the m3C-containing substrate. ALKBH3 at 2 μM was incubated with an equimolar amount of a FAM-labelled single-stranded substrate m3C_FAM. The reaction was quenched at each time point by the addition of an equal volume of 0.2 M NaOH.

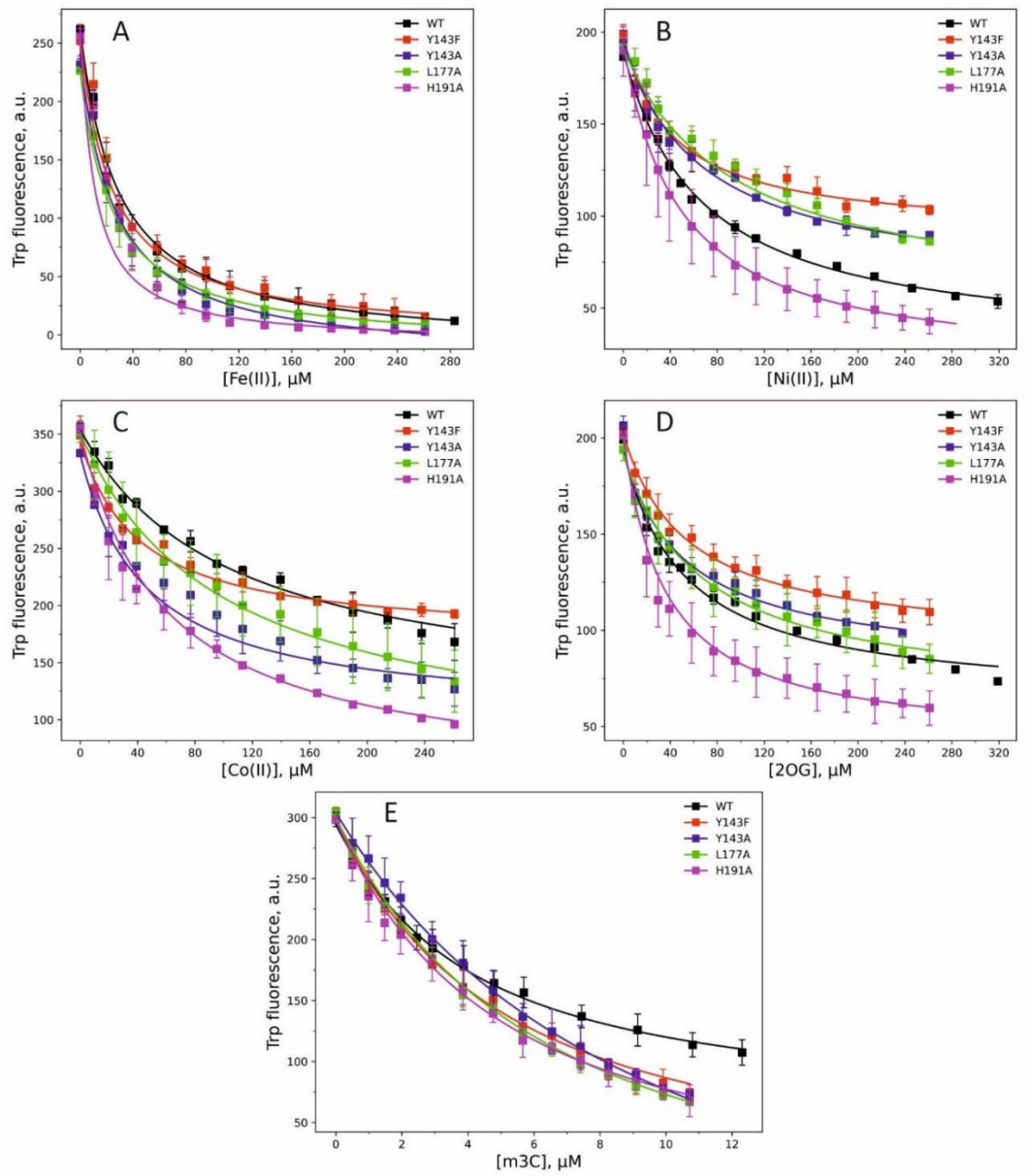


**Figure S2.** Equilibrium binding of the wild-type and mutant ALKBH3 proteins to transition metals, 2OG, or m3C-containing DNA. The quenching of the enzyme fluorescence is shown as a function of Fe(II) (A), Ni(II) (B), Co(II) (C), 2OG (D), and single-stranded m3C substrate (E). The protein concentration was 1 μM. In the course of titration by Fe(II), a sodium ascorbate (1mM) was added to keep the metal in a reduced state. Experimental data points were fitted to a single-site binding model (see "Materials and Methods"). Each trace represents the average of three technical replicates.

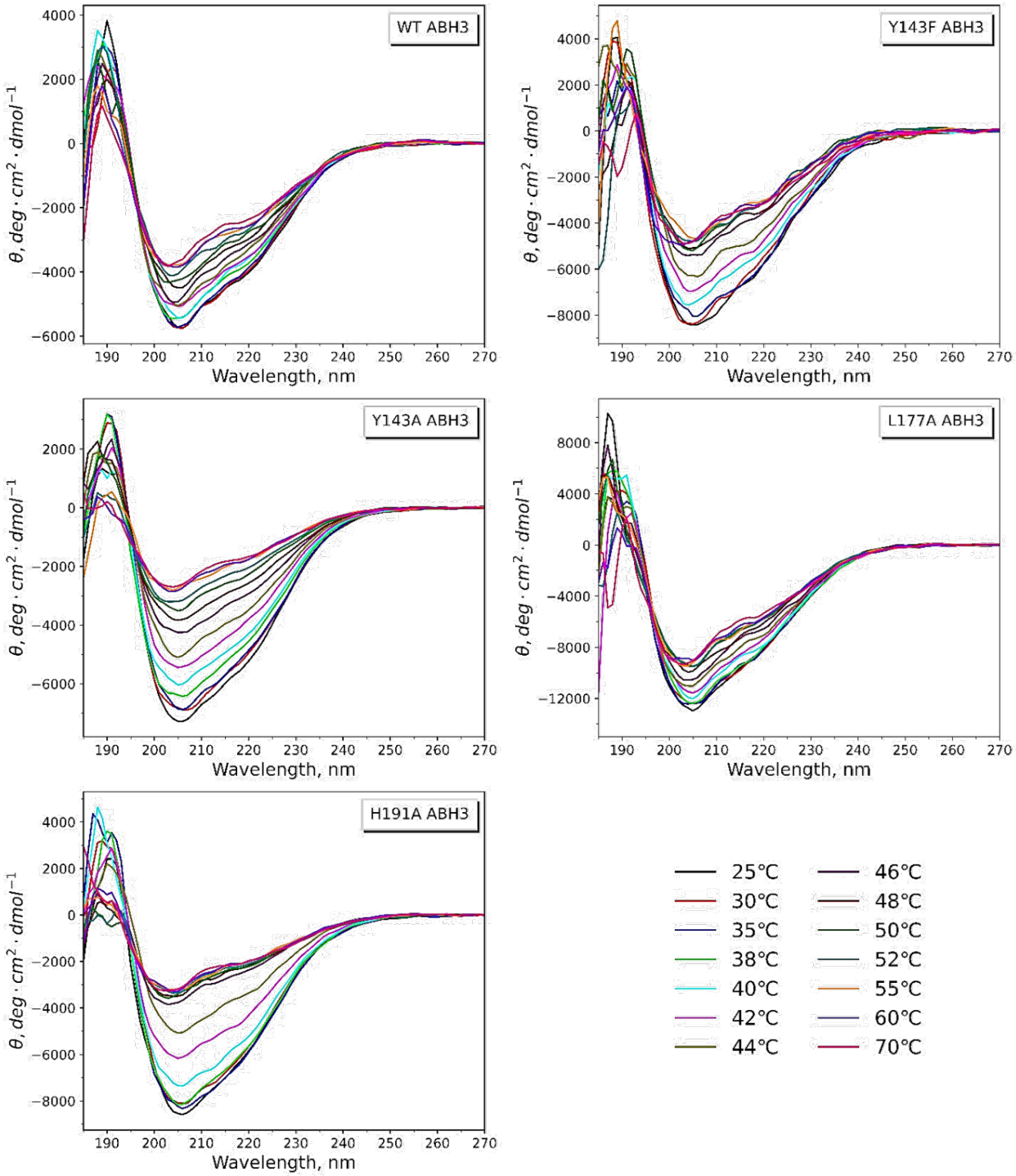


**Figure S3.** CD spectra of the wt, Y143F, Y143A, L177A, and H191A ALKBH3 proteins scanned at various temperatures and expressed in mean residue ellipticity (θ) units. Spectra were collected in a 0.1 cm cell with 0.16–0.20 mg/ml proteins from 25 to 70°C over a wavelength range of 185 and 270 nm. The data were corrected for background signal and smoothed.

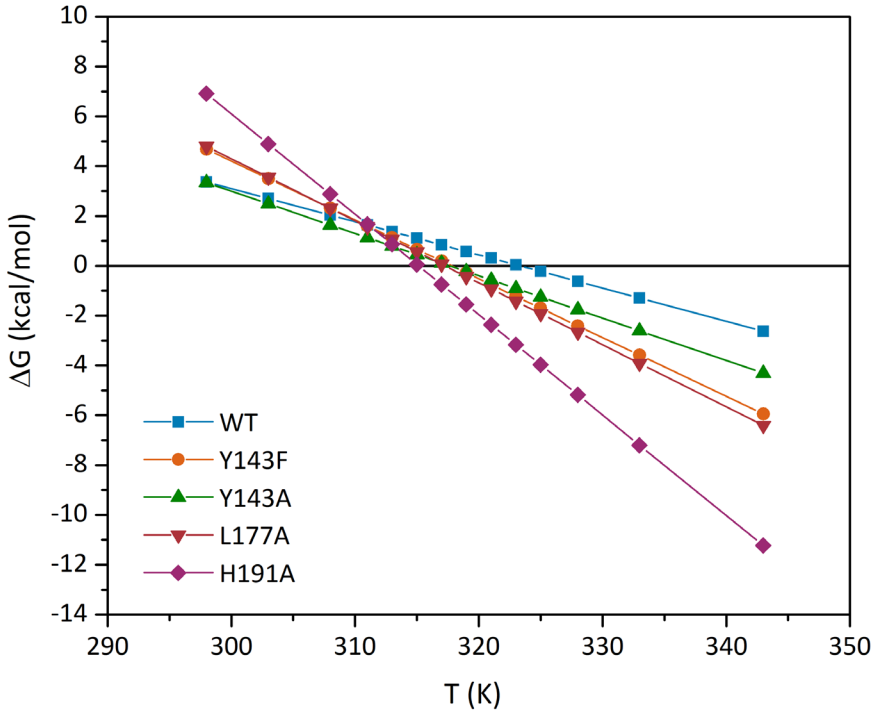


**Figure S4.** Temperature dependence of free energy ($\Delta G$) of wt, Y143F, Y14.3A, L177A, and H191A ALKBH3 unfolding. The plots were built using the following equation $\Delta G = \Delta H - T^{*}\Delta S$.