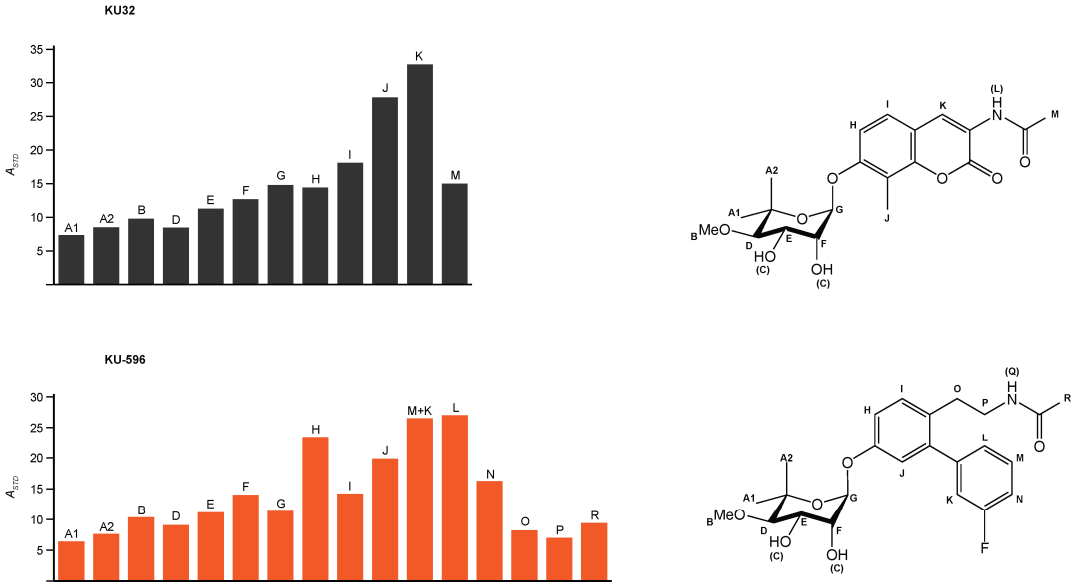
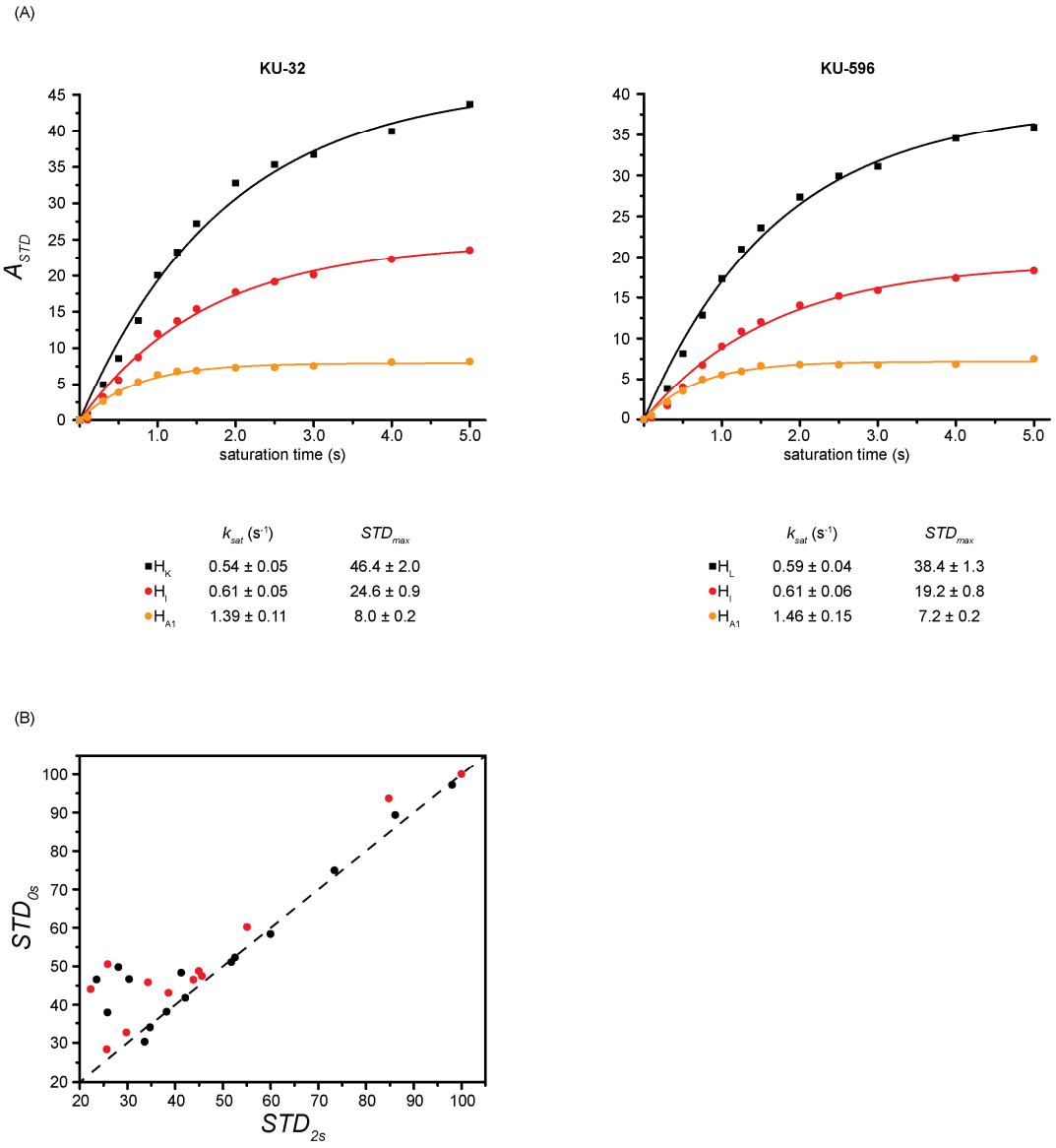
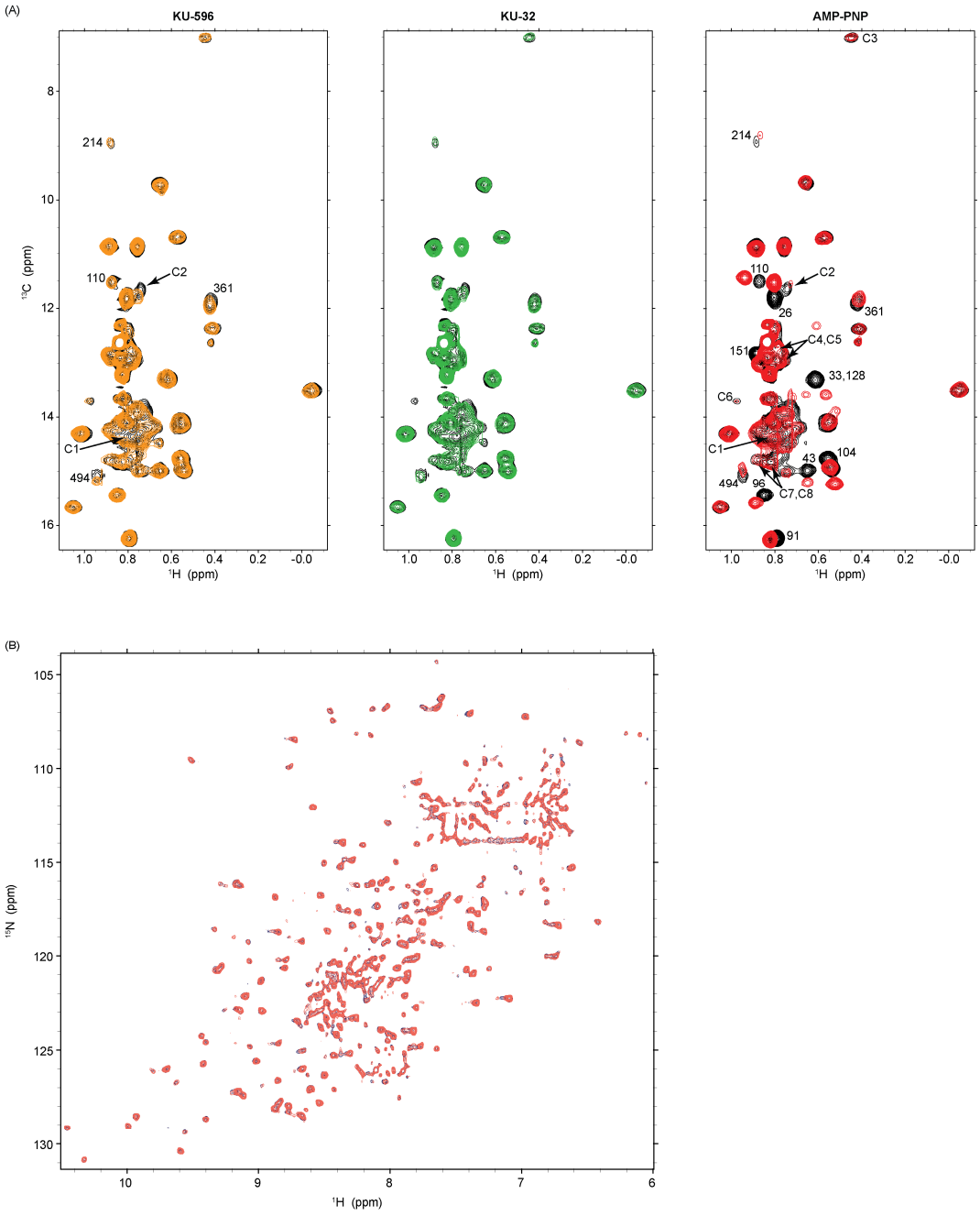


Supplementary Figure 1. Absolute STD effects for KU-32 (top) and KU-596 (bottom). For both experiments, the ligand and Hsp90 concentration was 770 μM and 4.7 μM, respectively, while a saturation transfer of 2 s was used.



Supplementary Figure 2. Determination of STD_{0s}. (A) Representative examples of build-up curves obtained for protons of KU-32 (left) and KU-595 (right), from which k_{sat} and STD_{max} were determined by fitting to equation (2). STD_0 was subsequently calculated as $k_{sat} \times STD_{max}$. (B) Comparison of relative STD_{2s} and STD_{0s} for each of the protons of KU-32 (red) and KU-596 (black), shown in Fig. 3 of the main text. For some signals (particularly of the methyl protons A1 and A2) STDs determined using a single saturation time (2s) are underestimated as compared to those determined as STDs at zero time.



Supplementary Figure 3. The interaction of Hsp90 with small ligands followed by NMR. (A) Methyl-TROSY spectra of Hsp90 in the free-state (black), and in the presence of excess KU-596 (yellow), KU-32 (green) and AMP-PNP (red). Signals for which magnifications are provided in the main text (Fig. 4) are labelled on the spectrum of KU-596. A more extensive assignment is shown in the spectrum of AMP-PNP. (B) The TROSY ^{15}N HSQC spectrum of N-Hsp90a (120 μM) acquired in the absence (blue) or presence of KU-596 (0.5 mM).