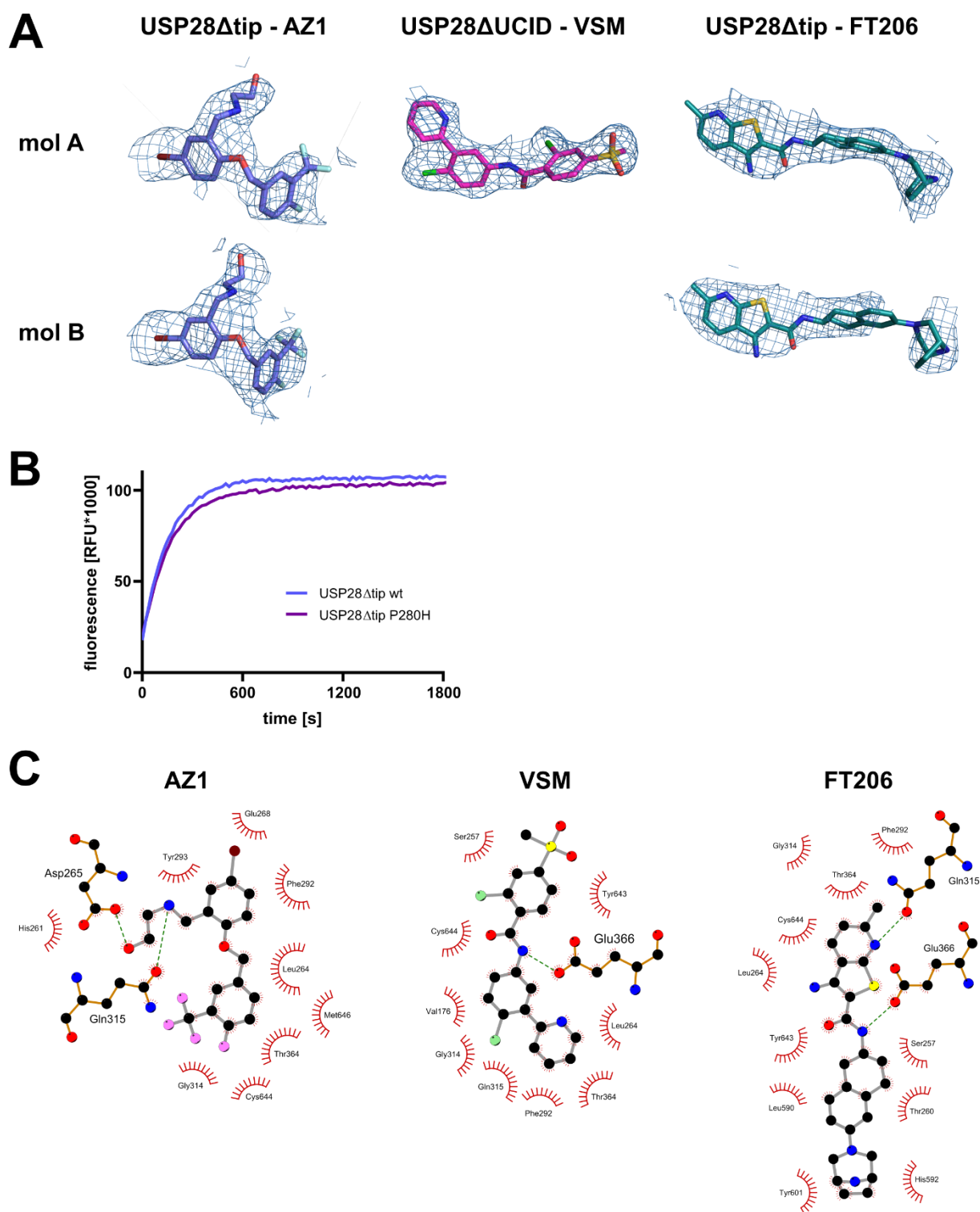


Appendix

Structural basis for the bi-specificity of USP25 and USP28 inhibitors

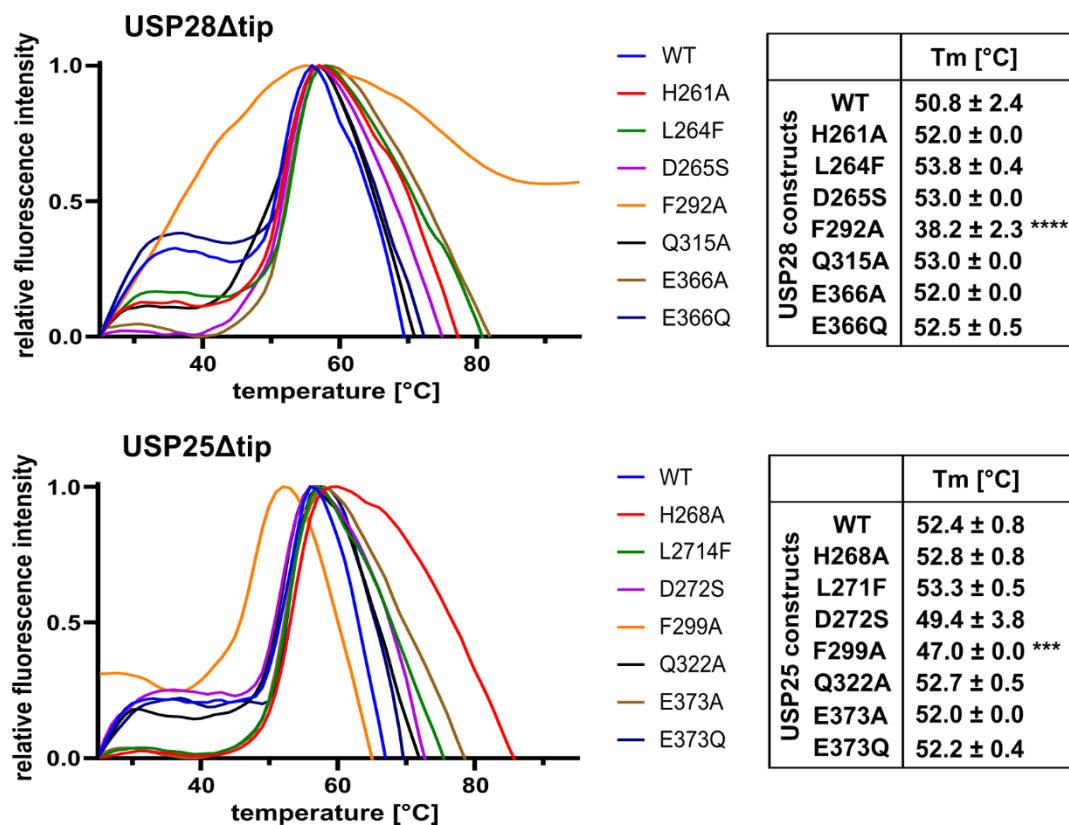
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Appendix Figure S1:

- A) Inhibitor electron density.** 2mFo-DFc simulated annealing (SA) composite omit maps (1σ level) of AZ1, VSM and FT206 bound to USP28 Δ tip (molecules A/B) or USP28 Δ UCID (molecule A). Electron density for the second FT206 molecule (molecule B) is not present for the complete inhibitor.
- B) Catalytic activity of USP28 Δ tip variants:** Representative Ub-Rh110 cleavage assay of USP28 Δ tip variants (wt (blue) and P280H (purple)). The fluorescence signal [RFU* 10^3] is plotted against the time [s].
- C) Ligplot representation of the USP28 inhibitor binding sites.** Ligplot representation of the USP28 binding sites for AZ1 (left), VSM (center) and FT206 (right).



Appendix Figure S2: Structural stability of the cat Δtip variants

Melting curves of USP28 (top) or USP25 (bottom panel) Δtip variants measured by differential scanning fluorimetry. One representative experiment is shown for each variant of both proteins. Fluorescence intensity is plotted against the temperature. Melting points were calculated and the melting temperatures [°C] are given in the Table as averages of $n \geq 5$ independent experiments $\pm$ SD. All the statistics show the results of unpaired Student's *t* tests, with *p*-values (* ≤ 0.05 ; ** ≤ 0.01 ; *** ≤ 0.001 ; **** ≤ 0.0001).