

## **AUTHOR INFORMATION**

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Notes:

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## **SUPPORTING INFORMATION**

Table S1, Observed melting temperatures from differential scanning fluorimetry (DSF) experiments;

Figure S1, Indole rescues FITC-E2 V<sub>H</sub> W118G activity;

Figure S2, Validation of SPR ISA setup;

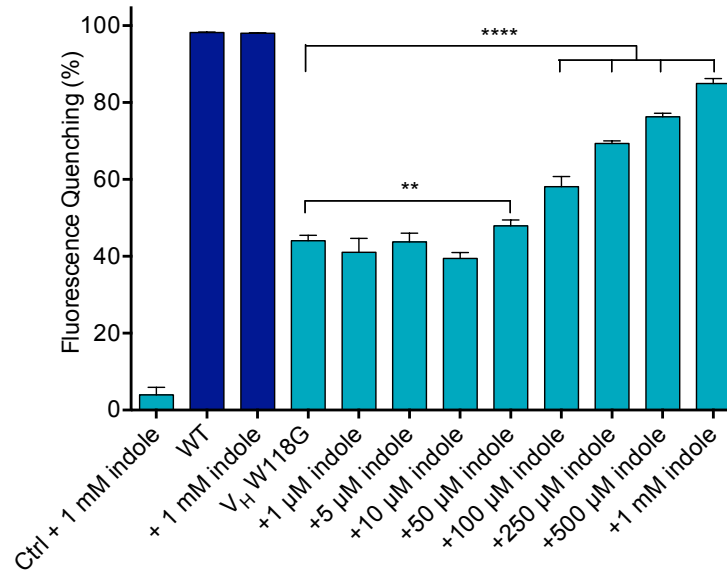
Figure S3, Differential scanning fluorimetry (DSF) thermal unfolding transitions.

**SUPPLEMENTARY TABLE**

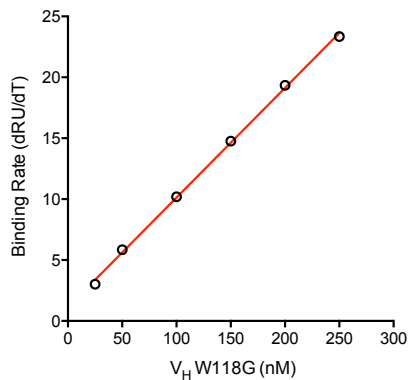
<b>Sample</b>	<b>Condition</b>	<b>T<sub>m</sub> (°C)</b>	<b>S.E.M. (°C)</b>
WT	Apo	64.1	0.06
	1000X indole	63.5	0.06
	1:1 Fluor	69.9	0.05
	1:1 Fluor + 1000X indole	69.3	0.10
V <sub>H</sub> W118G	Apo	48.9	0.26
	1000X indole	47.0	0.15
	1:1 Fluor	52.4	0.21
	1:1 Fluor + 1000X indole	53.7	0.06
V <sub>H</sub> W52G	Apo	53.5	0.07
	1000X indole	52.6	0.01
	1:1 Fluor	53.7	0.07
	1:1 Fluor + 1000X indole	52.9	0.07
V <sub>L</sub> W107G	Apo	59.9	0.07
	1000X indole	59.2	0.01
	1:1 Fluor	59.9	0.13
	1:1 Fluor + 1000X indole	59.3	0.07

**Table S1: Observed melting temperatures from differential scanning fluorimetry (DSF) experiments.** Values were obtained by fitting to the data presented in Figure S3. All data were collected in triplicate, experimental uncertainty corresponds to standard error of the mean.

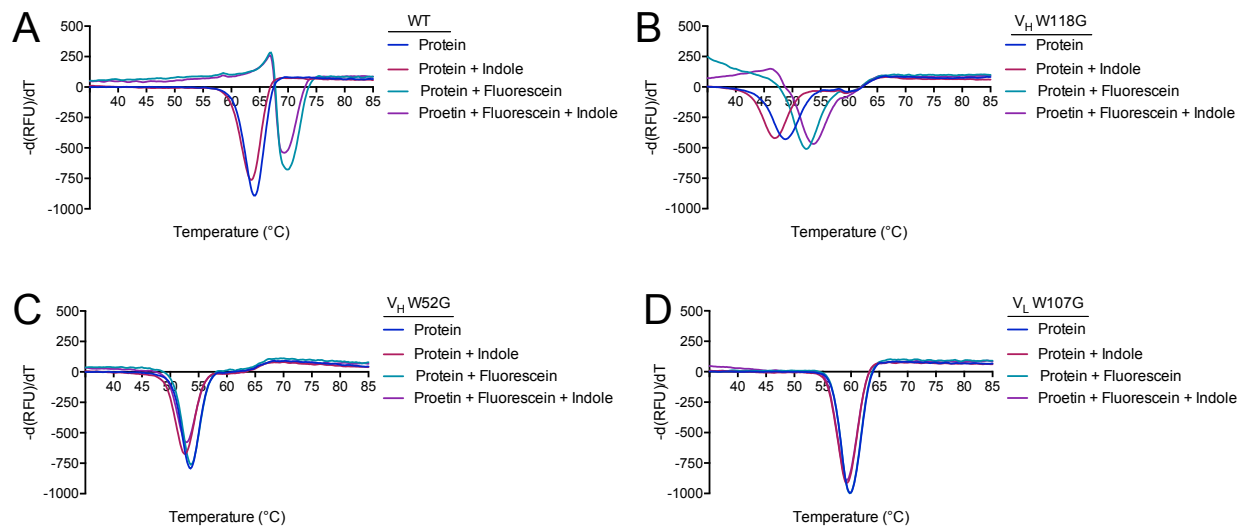
## SUPPLEMENTARY FIGURES



**Figure S1: Indole rescues FITC-E2 V<sub>H</sub> W118G activity.** Addition of indole rescues antigen-binding of FITC-E2 V<sub>H</sub> W118G in a dose-dependent manner. Data are presented as mean  $\pm$  SEM; \*\* $p < 0.01$ , \*\*\*\* $p < 0.0001$  for V<sub>H</sub> W118G with indole versus no indole;  $n = 4$ .



**Figure S2: Validation of SPR ISA setup.** This plot shows the concentration dependence between the initial binding rate  $d(RU)/dT$  and the protein concentration: this confirms that our experimental setup indeed reports on diffusion limited conditions (and not on interaction kinetics).



**Figure S3: Differential scanning fluorimetry (DSF) thermal unfolding transitions.** Samples include 5  $\mu$ M protein (*blue*); 5  $\mu$ M protein + 5 mM indole (*magenta*); 5  $\mu$ M protein + 5  $\mu$ M fluorescein (*teal*); and 5  $\mu$ M protein + 5  $\mu$ M fluorescein + 5 mM indole (*purple*). **(A)** Wild-type FITC-E2 **(B)** V<sub>H</sub> W118G **(C)** V<sub>H</sub> W52G **(D)** V<sub>L</sub> W107G.