

**Table S2 Summary of the ITC data for AT3-UIM12 binding with diUb chains by different fitting models**

Binding Pair	$K_{d(1)}; K_{d(2)}$ ( $M^{-1}$ )	Chi <sup>2</sup> /DOF	Fitting model	Evaluation
UIM12/Linear diUb	$5.95 \times 10^{-5}; 2.47 \times 10^{-3}$	$4.1 \times 10^2$	Sequential <sup>a</sup>	+ <sup>b</sup>
UIM12/K48-diUb	$1.43 \times 10^{-5}; 2.92 \times 10^{-4}$	$6.5 \times 10^3$	Sequential	+
UIM12/K63-diUb	$1.02 \times 10^{-5}; 1.47 \times 10^{-4}$	$1.1 \times 10^4$	Sequential	-
GS-Sub/K48-diUb	$1.81 \times 10^{-4}; 7.69 \times 10^{-7}$	$9.4 \times 10^3$	Conventional	+/-
GS-Ins/K48-diUb	$4.85 \times 10^{-6}; 1.10 \times 10^{-4}$	$1.4 \times 10^3$	Conventional	+
RAP80L/K48-diUb	$9.43 \times 10^{-7}; 2.79 \times 10^{-4}$	$1.0 \times 10^3$	Conventional	+

<sup>a</sup> Conventional, conventional two-site binding model; Sequential, sequential two-site binding model.

<sup>b</sup> +, good fitting; +/-, moderate; -, bad fitting.