Supporting Information

Structural Elucidation of Peptide Binding to KLHL-12, a Substrate Specific Adapter Protein in a Cul3-Ring E3 Ligase Complex

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E3 Ligase; KLHL-12; Ubiquitination

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Table S1. X-Ray Data collection and refinement statistics (molecular replacement)

	KLHL-12 complex with Dvl3-peptide
Data collection	
Space group	$P2_1$
Cell dimensions	
a, *b*, *c* (Å)	58.84, 182.33, 58.92
α, β, γ (°)	90.00, 105.15, 90.00
Resolution (Å)	2.9 (2.9-2.95) *
R_{sym} or R_{merge}	0.136/0.158 (0.311/0.448)
$I / \sigma I$	5.15 (1.8)
Completeness (%)	91.4 (89.4)
Redundancy	2.6 (2.2)
Refinement	
Resolution (Å)	2.9-29.36
No. reflections	11347
R_{work} / R_{free}	0.207/0.254
No. atoms	
Protein	4226
Peptide	72
B-factors	
Protein	25
Peptide	36
R.m.s. deviations	
Bond lengths (Å)	0.003
Bond angles (°)	0.694

*Values in parentheses are for highest-resolution shell.

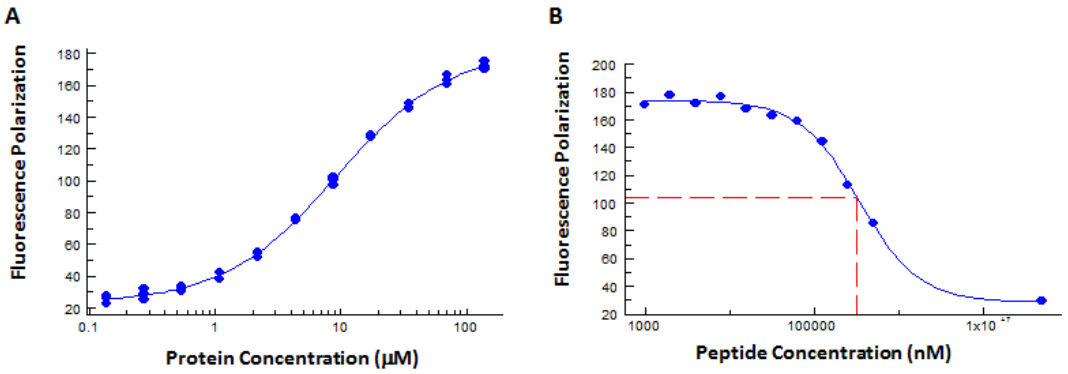

Figure S1. A) Saturation binding assay. 50 nM, Fitc labeled peptide **16**, was mixed with series dilutions of protein KLHL-12 (2-fold dilutions). The mixtures were allowed to mix and equilibrate for 20 min at room temperature on a plate mixer and then measured for fluorescence polarization. A sigmoidal dose-response model was used to calculate binding affinity (Kd = 9.25 μM). B) Competition assay. Series dilutions of unlabeled peptide (peptide **9**, 1 μM to 500 μM) were mixed with 50 nM Fitc-labeled probe (peptide **16**) and 50 μM protein KLHL-12. The mixtures were allowed to mix and equilibrate for 20 min at room temperature on a plate mixer and then measured for fluorescence polarization. A sigmoidal dose-response model was used to calculate binding affinity of peptide **9** (Kd = 43 μM). The last point in the competition binding curves is the signal for a fully displaced FITC labeled peptide.

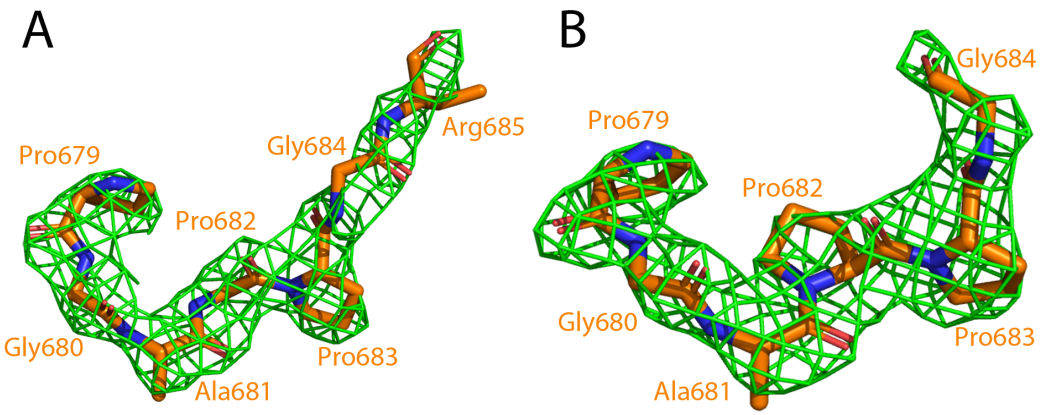

Figure S2. The Fo-Fc omit maps of A) peptide **9** (chain C: **PGAPPGR**DLA, in green mesh) bound with KLHL-12 Kelch domain. B) peptide **9** (chain D: **PGAPPG**RDLA, in green mesh) bound with KLHL-12 Kelch domain. The map contoured at 2.0 σ level. Peptide **9** is shown in orange sticks.