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Recognition of non-proline N-terminal residues by the Pro/N-degron pathway

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This PDF contains:

Figures S1 and S2, and Tables S1 and S2.

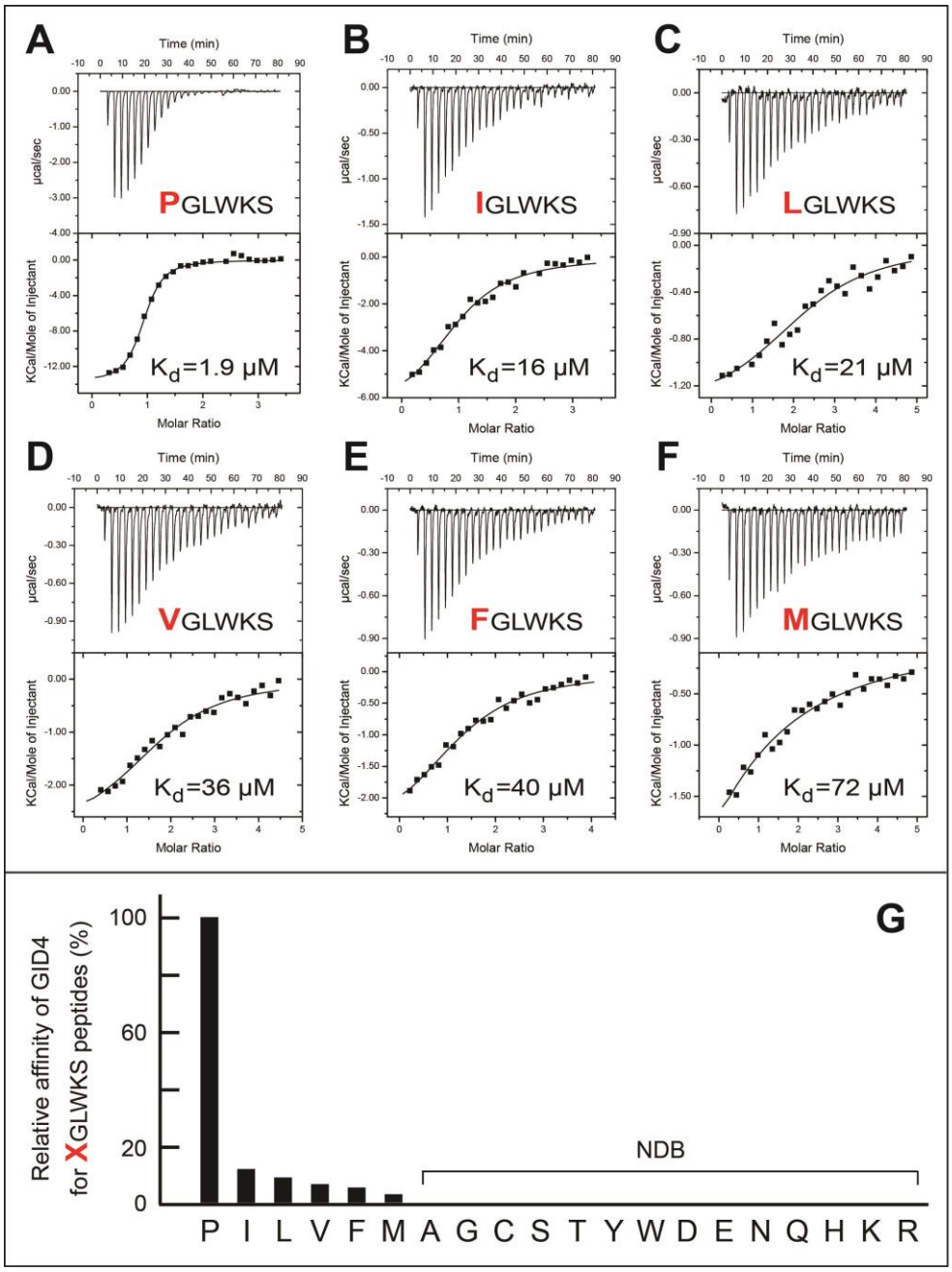

Fig. S1. Affinities (K_d's) of **X**GLWKS peptides (**X** is the set of 20 amino acids in the genetic code), determined using isothermal titration calorimetry (ITC) (see Results, and Materials and Methods), for the binding-competent GID4$^{116-300}$ fragment of human GID4$^{1-300}$. (*A-F*) ITC results and values of K_d for **X**=Pro, Ile, Leu, Val, Phe, and Met. (*G*) Plot of relative GID4 affinities (relative association constants, K_a's) for all 20 **X**GLWKS peptides, with the affinity of GID4$^{116-300}$ for **P**GLWKS taken as 100%. NDB, no detectable binding (K_d > 0.5 mM).

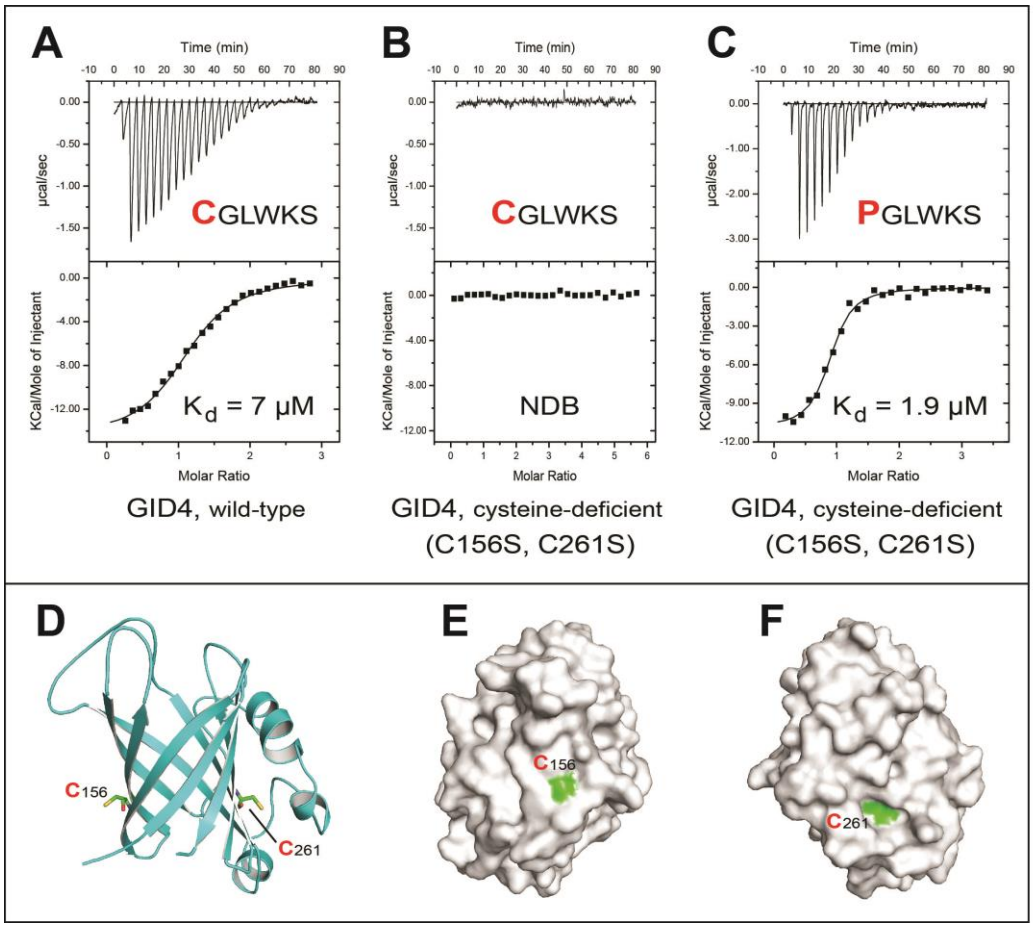

Fig. S2. Affinities (K_d's) of **X**GLWKS peptides (**X**=Cys or Pro) for the "wild-type" GID4$^{116\text{-}300}$ fragment and its cysteine-deficient mutant GID4$^{116\text{-}300,\ C156S,\ C261S}$, determined using isothermal titration calorimetry (ITC) (see Results, and Materials and Methods). (*A*) ITC data and K_d for the binding of the **C**GLWKS peptide to "wild-type" GID4$^{116\text{-}300}$. (*B*) Same as in *A* but with GID4$^{116\text{-}300,\ C156S,\ C261S}$ mutant. NDB, no detectable binding (K_d > 0.5 mM). (*C*) Same as in *B*, but with the **P**GLWKS peptide. (*D*) Ribbon diagram of the human GID4$^{124\text{-}289}$ fragment, with positions of the solvent-exposed Cys-156 and Cys-261 residues indicated. (*E* and *F*) Surface representations of GID4$^{124\text{-}289}$, with the locations of Cys-156 and Cys-261 colored in green. The cited residue numbers refer to full-length GID4$^{1\text{-}300}$.

Table S1. Crystallographic data correction and refinement statistics.

	GID4-VGLWKS	GID4-IGLWKS
Data reduction		
Space group	$P2_1$	P1
Cell	36.8,40.6,56.3,90,98.1,90	40.1,51.2,55.4,68.2,89.1,69.1
Resolution	40.65-1.60 (1.62-1.60)	51.02-1.67 (1.70-1.67)
R_{merge}Overall	0.047 (0.588)	0.033 (0.713)
R_{meas}Overall	0.056 (0.787)	0.044 (0.967)
NumberReflections	21226 (746)	40219 (1972)
MeanIoverSD	13.8 (1.3)	11.6 (0.9)
CChalf	0.998 (0.658)	0.999 (0.588)
Completeness	96.3 (68.5)	91.4 (88.5)
Multiplicity	3.1 (1.7)	2.0 (1.8)
Model refinement		
Resolution	36.40-1.60	51.01-1.75
Reflections / free	21165 / 1049	35093 / 1737
Rwork / Rfree	0.186 / 0.220	0.193 / 0.227
All (num atoms / mean B)	2782 / 23.7	5347 / 38.9
Protein	2625 / 23.4	5143 / 38.8
Ligand	94 / 27.7	132 / 45.6
Water	50 / 28.0	56 / 35.7
Others	13 / 25.9	16 / 32.4
rmsd bonds / angles	0.010 / 1.7	0.011 / 1.7

Values in parentheses are for highest-resolution shell.

Table S2. Affinities (K_d's, in μM), determined using isothermal titration calorimetry (ITC) (see Results, and Materials and Methods) of the indicated mutants of human GID4 (its $GID4^{116-300}$ fragment) for the Nt-Ile-bearing **I**GLWKS peptide. "Wild type", the unmodified $GID4^{116-300}$ fragment. NDB, no detectable binding (K_d > 0.5 mM). The cited residue numbers refer to full-length $GID4^{1-300}$.

$GID4^{116-300}$	K_d (μM)
wild type	16
V141A	NDB
L159A	~200
I161A	65
L164A	NDB
T173A	3.8
F254A	NDB
L171A	50
L240A	NDB
I249A	NDB

Table S3. Affinities (K_d's, in μM), determined using isothermal titration calorimetry (ITC) (see Results, and Materials and Methods) of the indicated peptides, **I**GLWKS and **P**GLWKS, for human GID4 (its $GID4^{116-300}$ fragment) that had been mutated, as indicated, at the position of Thr-173. "Wild type", unmodified $GID4^{116-300}$ fragment. The cited residue numbers refer to full-length $GID4^{1-300}$.

$GID4^{116-300}$	K_d (μM)	
	PGLWKS	**I**GLWKS
wild type	1.9	16
T173A	1.2	3.8
T173I	1.1	11.7
T173L	1.5	10.6
T173V	0.5	5.9