Structure	GID4 fragment in complex with a peptide
PDB Code	6CDG, 6CDC, 6CD9, 6CD8, 6CCU, 6CCT
Entry clone accession	BC041829.1
Entry clone source	MGC:43491 IMAGE:5268071
SGC clone accession	JMC130-A04
Tag	N-terminal tag: MHHHHHHSSGRENLYFQG MHHHHHHSSGRENLYFQG
Construct sequence	SGSKFRGHQKSKGNSYDVEVVLQHVDTGNSYLCGYLKIKGLTEEYPTLTTFFEGEIISKKHPFL TRKWDADEDVDRKHWGK FLAFYQYAKSFNSDDFDYEELKNGDYVFMRWKEQFLVPDHTIKDISGASFAGFYYICFQKSAA SIEGYYYHRSSEWYQSLNLTHV
Vector	pET28-MHL
Expression host	BL21 (DE3) Codon plus RIL (Stratagene)
Growth method	GID4 was expressed in E.coli BL21 (DE3) codon plus RIL in Terrific Broth (TB) in the presence of 50 µg/mL of kanamycin. Cell were grown at 37 ºC to an OD600 of 1.5 and induced by isopropyl-1-thio-D-galactopyranoside (IPTG), final concentration 0.2 mM, and incubated overnight at 16 ºC. Cell pellets collected by centrifugation and frozen at -80 ºC.
Extraction buffers	**Lysis buffer**: **20 mM Tris-HCl pH 7.5, 400 mM NaCl, 5% glycerol and 2 mM beta-mercaptoethanol**
Extraction procedure	Frozen cell pellet was thawed and suspended in lysis buffer. The cells were lysed by sonication (Virtis408912, Virsonic) on ice: the sonication protocol was 5 sec pulse at half-maximal frequency (5.0), 7 second rest, for 10 minutes total sonication time per pellet. The lysate was centrifuged at 15000rpm for 1h.
Purification	**Wash buffer**: 20 mM Tris pH 7.5, 400 mM NaCl, **5% glycerol** and 25 mM imidazole; **Elution buffer**: 20 mM Tris pH 7.5, 400 mM NaCl, **5% glycerol** and 300 mM imidazole; **Gel filtration buffer**: 20 mM Tris-HCl pH 7.5, 100 mM NaCl and 0.5 mM TCEP

buffers	
Purification procedure	**The fusion proteins were purified by Ni-NTA agarose column. The His tag was cleaved by His-tagged TEV protease (purified in-house) with an approximate molar ratio of 1 : 20 in the dialysis buffer (20 mM Tris-HCl, pH 7.5, 150 mM NaCl and 5 mM beta-mercaptoethanol) at 4 °C overnight. The tag and protease were removed by reloading onto the Ni-NTA. The proteins were further purified by Superdex 200 10/300 (GE Healthcare). The gel filtration buffer contains 20 mM Tris-HCl, pH 7.5, 100 mM NaCl and 0.5 mM TCEP**
Protein stock concentration	**The purified protein was concentrated to 8 mg mL-1** using 15 mL concentrators with a 3,000 molecular weight cut-off (Amicon Ultra-15, UFC900524, Millipore).
Crystallization	The purified GID4 (aa 124-289) proteins were separately incubated with different peptides at a molar ratio of 1 : 1.5 for 1 h on ice before setting up the crystallization trials. The crystals of GID4-PGLW, GID4-PSRW and GID4-PTLV were grown in the precipitant conditions containing 20-25% (v/v) PEG3350, 2-3% (v/v) Tacsimate, pH 7.0 and 0.1 M HEPES, pH 7.5 (Average pH 7.4). The GID4-PGLWKS was crystallized in 20% (v/v) PEG3350, 0.2 M NaBr. The GID4-PSRV was crystallized in 30% (v/v) PEG2000 and 0.1 M KSCN. The GID4-PHRV was crystallized in 20% (v/v) PEG3350 and 0.03 M Citric acid. The crystals were protected in cryoprotectant solution consisting of reservoir solution supplemented with 20% (v/v) glycerol or 20% (v/v) ethylene glycol and flash-frozen in liquid nitrogen before data collection.