

**Figure S1:**

Sequence analysis of VgrG1. **(A)** the position of the VgrG1 and MARTX ACDs in their multidomain protein (Satchell, K.J. (2009) *Toxins (Basel)* **1**, 123-133), and the Medor (Lieutaud *et al.* (2008) *BMC Genomics* **9 Suppl 2**, S25) analysis of VgrG1 linker between the puncturing device domain and the ACD. **(B)** Sequence alignment with Multalin (Corpet, F. (1988) *Nucleic Acids Res* **16**, 10881-90) and Esprit (Gouet *et al.* (1999) *Bioinformatics* **15**, 305-8) of the VgrG1 and MARTX ACDs, with the secondary structure of VgrG1 ACD shown. The borders of the residues visible in the electron density map are defined by blue arrows. The catalytic residues are identified by red circles.

**Figure S2:** Three-dimensional superposition and comparison of native VgrG1 ACD with the  $\gamma$ -glutamylcysteine synthetase (GCS, 1VA6 (Hibi *et al.* (2004) *Proc Natl Acad Sci U S A* **101**, 15052-7)). **(A)** Overall superposition using DALI (Holm & Rosenstrom (2010) *Nucleic Acids Res* **38**, W545-9). 150 residues on 503 of GCS are within 4.3 Å from those of VgrG1 ACD ( $Z=3.9$ ). The best superposed area is boxed by green lines. **(B)** Despite this weak structural identity, the three  $\beta$ -strands carrying the ATP and substrate binding sites are perfectly conserved and aligned, as displayed in this stereo view. VgrG1-ACD is coloured blue with atom type coloured side chains and red labels. GCS is coloured red, with red side-chains and blue labels. **(C)** Surface representation of VgrG1 ACD in complex with ATP,  $Mn^{++}$  and  $SO_4^-$ , coloured according to residue conservation with *V. cholerae* MARTX-ACD. The residues with identical types are coloured in blue, the others are coloured in red.

**Figure S3:** Surface local electrostatics potential of VgrG1-ACD in complex with ATP,  $Mn^{++}$  and  $SO_4^-$ .

**Figure S4:** Ribbon views of the structures of G-actin (green) with several actin binding proteins: gelsolin GS1 and the GS1-thym $\beta$ 4 chimera (1YAG, 1T44; violet), ADF-H (3DAW; blue), profilin (2BTF; yellow) and DNaseI (2A42; blue). The positions of actin's Glu 270 and Lys 50 side-chains are indicated.