Supplementary data:

Protein production and purification

A *Nde*I-*Hin*dIII fragment, containing a *hapB* C-terminal part fused downstream to a His_6-tag region was PCR-amplified from pT7-hapBct (Steidl et al, 1999) using primers HapBct-for and HapB-r, and inserted into the *Nde*I-*Hin*dIII-digested pET-43.1a vector (Novagen) resulting in pET43H6hapBct. A full-length *hapB* PCR product, amplified as a *Bam*HI-*Hin*dIII fragment from pHapB-GFP (Steidl et al, 2004) using the primers HapB-for and HapB-r was cloned into the *Bam*HI/*Hin*dIII sites of pET43H6HapBct to give pET43H6HapB used for expression of HapB.

A gene coding for HapC with an extended N-terminus including a $(His)_6$-tag and a cleavage site for tobacco etch virus (TEV) protease was amplified from pMal-HapC (Kato et al, 1998) by PCR using primers TEVHapC-for and HapC-r and cloned in-frame with the 5' end of the gene of the maltose-binding protein (MBP) in pMAL-c2X (New England Biolabs) to yield pMalC2TEVHapC. A *Bam*HI-*Hin*dIII full-length *hapE* fragment was amplified by PCR using primers TEVHapE-for and HapE-r and pMalE-HapE (Kato et al, 1998) as the template and inserted into the *Bam*HI/*Hin*dIII-digested pET43H6HapB vector to yield pET43H6HapE.

A truncated version of HapE lacking the putative HapX interaction domain was obtained by using pET43H6HapE as the template and primers HapE81f and HapE-r in a PCR experiment. The resulting DNA fragment was inserted into the *Bam*HI/*Hin*dIII-digested pET43H6HapB vector to yield pET43H6HapE-ΔNΔA.

HapX was amplified by PCR from genomic *A. nidulans* DNA using the primers hapX-for and hapX-rev. The resulting *Nco*I-*Nco*I fragment was ligated into pCR2.1 (Invitrogen) resulting in plasmid pCR2.1-hapX. A *Bam*HI-*Hin*dIII full-length *hapX* fragment was PCR-amplified with the primers HapXf and HapXr using pCR2.1-hapX as the template and inserted into the *Bam*HI/*Hin*dIII-digested pET43H6HapBct vector to yield pET43H6HapX.

HapB, MBP-HapC, HapE, HapE-ΔNΔA and HapX proteins were produced by auto-induction in *E. coli* Rosetta 2 (DE3) cells grown at 30°C in 1 l Overnight Express Instant TB Medium (Novagen). Fifteen to 20 g wet weight of cells were collected by centrifugation, re-suspended in 150 ml lysis buffer and homogenized using an Emulsiflex C5 high pressure homogenizer (Avestin).

His_6-HapB-containing cleared cellular extracts in 50 mM NaH_2PO_4, 300 mM NaCl, 10 mM imidazole, 1 mM PMSF, pH 8.0 were applied to a 20 ml Ni-NTA Superflow column

(Qiagen). HapB was eluted with 250 mM imidazole, transferred to 0.1% TFA using a HiPrep desalting column (GE Healthcare) and injected onto a 8 ml SOURCE 15 RPC (GE Healthcare) column. After elution with a gradient from 0% to 60% (v/v) acetonitrile in 0.1% (v/v) TFA, HapB-containing fractions were identified by SDS-PAGE, lyophilized and stored at -80°C.

MBP-HapC fusion protein was isolated using a 50 ml Amylose Resin HF column (New England Biolabs) and digested by adding 4 μg TEV-protease per mg fusion protein at room temperature for two hours. $(His)_6$-MBP was captured on a 25 ml NiSepharose 6 FF column (GE Healthcare) and HapC was further purified on a 20 ml SOURCE 15Q (GE Healthcare) column by elution with a NaCl gradient.

His_6-HapE, His_6-HapE-ΔNΔA- and His_6-HapX-containing inclusion bodies were washed with TBS (20 mM Tris/HCl pH 8.0, 0.15 M NaCl, 1 mM DTT, 1 mM PMSF) re-centrifuged and solubilized in TBS, 8 M urea. After removing the insoluble material by centrifugation, the supernatants were applied to a 25 ml NiSepharose 6 FF column (GE Healthcare). After being washed with TBS, 8 M urea, 10 mM imidazole, proteins were eluted with TBS, 8 M urea, 200 mM imidazole.

His_6-HapE and His_6-HapE-ΔNΔA was transferred to 20 mM Tris/HCl pH 8.5, 6 M urea and His_6-HapX to 50 mM CHES pH 9.5, 8 M urea. Solutions were applied to a 20 ml SOURCE 15Q (GE Healthcare) column, followed by elution with a NaCl gradient. Fractions containing HapC, His_6-HapE, His_6-HapE-ΔNΔA and His_6-HapX were transferred to 10% (v/v) acetonitrile in 0.1% (v/v) TFA and purified to homogeneity on a 20 ml SOURCE 15 RPC column, as described for HapB. For SPR analysis, 0.5 mg lyophilized HapX was dissolved in 10 ml Biacore running buffer supplemented with 1 M arginine and dialyzed overnight against Biacore running buffer.