Supporting Information

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SI Materials and Methods

Venom Collection and Neurotoxin Purification. Adult *Scolopendra subspinipes mutilans* L. Koch (both sexes, $n = 3{,}000$) were purchased from Jiangsu Province, China. Venom was collected manually by stimulating the venom glands, which are located within the first pair of modified legs, known as forcipules or maxillipeds (1), with a 3-V alternating current. Each milking occurred 1 wk after the previous milking. The unique peptide toxin was purified from the venom using a combination of gel filtration and reverse-phase (RP) HPLC, and stored at –20 °C until further use.

Mass Spectrometry. The μ-SLPTX-Ssm6a (hereafter Ssm6a) was dissolved in 0.1% (vol/vol) trifluoroacetic acid (TFA)/water and 0.5 μL was spotted onto a MALDI-TOF plate with 0.5 μL α-cyano-4-hydroxycinnamic acid (CHCA) matrix [10 mg/mL in 60% (vol/vol) acetonitrile]. Spots were analyzed using an UltraFlex I mass spectrometer (Bruker Daltonics) in positive ion mode.

Peptide Sequencing. The complete amino acid sequence of purified neurotoxin was determined by Edman degradation using a pulsed liquid-phase Shimadzu protein sequencer (PPSQ-31A, Shimadzu) according to the manufacturer's instruction.

cDNA Library and Cloning. Total RNA was extracted from the venom glands of 20 centipedes using TRIzol (Life Technologies) and used to prepare cDNA using a SMART PCR cDNA synthesis kit (Clontech). The first strand was synthesized using the 3′ SMART CDS Primer II A [5′ AAGCAGTGGTATCAACGCAGAGTACT (30)N_{-1}N 3′, where n = A, C, G, or T and N_{-1} = A, G, or C] and SMART II A oligonucleotide, (5′ AAGCAGTGGTATCAACGCAGAGTACGCGGG 3′). The 5′ PCR primer II A (5′ AAGCAGTGGTATCAACGCAGAGT 3′) provided in the kit was used to synthesize the second strand using Advantage polymerase (Clontech). A directional cDNA library was then constructed with a plasmid cloning kit (SuperScriptTM Plasmid System, GIBCO/BRL) following the manufacturer's instructions. The resultant cDNA library comprised 2.2×10^5 independent colonies.

A PCR-based method for high stringency screening of DNA libraries was used for screening and isolating cDNA clones. The sense-direction primer was designed according to the amino acid sequence determined by Edman degradation 5′-[GC(A/T/C/G)GA(T/C)AA(T/C)AA(A/G)TG- (T/C)GA(A/G)AA(T/C)TC(A/T/C/G)T-3′]. The primer was used in conjunction with an antisense SMART II A primer II in PCR to screen for transcripts encoding the neurotoxin. PCR was performed using Advantage polymerase (Clontech) using the following conditions: 2 min at 94 °C, followed by 30 cycles of 10 s at 92 °C, 30 s at 50 °C, 40 s at 72 °C. Finally, the PCR products were cloned into the pGEM-T Easy vector (Promega). DNA sequencing was performed on an ABI PRISM 377 DNA sequencer (Applied Biosystems).

hERG Assays. *Xenopus laevis* oocytes were injected with cRNA encoding the hERG channel then incubated at 17 °C in 96 mM NaCl, 2 mM KCl, 5 mM Hepes, 1 mM $MgCl_2$, and 1.8 mM $CaCl_2$, 50 μg/mL gentamycin, pH 7.6 with NaOH. hERG currents were examined at room temperature (~22 °C) 1–2 d after cRNA injection via two-electrode voltage-clamp techniques (Axoclamp 900A; Molecular Devices) using a 90-μL recording chamber. Data were low pass filtered at 2 kHz and digitized at 10 kHz using pCLAMP 10 (Molecular Devices). Microelectrode resistances were 0.1–1 MΩ when filled with 3 M KCl. The external recording solution comprised 96 mM NaCl, 2 mM KCl, 5 mM Hepes, 2 mM $MgCl_2$, 1 mM $CaCl_2$, pH 7.6. Oocytes were voltage clamped at a holding potential of –80 mV, and 1-s pulses to a test potential of 0 mV were applied every 12.5 s until the current magnitude reached a steady-state level. After baseline recordings, the bath solution was displaced with 100 μL of test solution and recording continued until a new steady-state level was achieved. The spider-venom peptide VSTX1, a known inhibitor of hERG, was used as a positive control (2). Data analysis was performed using pCLAMP 10, Prism5 (Graphpad Software), and Microsoft Solver (Microsoft Excel).

Recombinant Production of μ-SLPTX-Ssm6a. Recombinant Ssm6a was produced via expression in the periplasm of *Escherichia coli*, as reported previously for the spider-venom peptide PcTx1 (3), with minor modifications. A synthetic gene encoding μ-SLPTX-Ssm6a, with codons optimized for high-level expression *E. coli*, was synthesized and inserted into the pLicC-MBP vector (4) by GeneArt (Life Technologies). This vector (pLicC-EU2) encodes a MalE signal sequence for periplasmic export, a His_6 affinity tag for protein purification, a maltose binding protein (MBP) fusion tag for increased solubility (5), and a tobacco etch virus (TEV) protease recognition site preceding the Ssm6a gene. The last residue of the TEV cleavage site was altered to match the first residue of Ssm6a so that cleavage of the fusion protein by TEV yields the native sequence.

E. coli strain BL21(λDE3) was transformed with pLicC-EU2 and grown in Luria-Bertani medium at 37 °C with shaking at 160 rpm. Cultures were cooled to 16 °C and toxin expression was induced with 1 mM isopropyl-β-D-thiogalactopyranoside (IPTG) at an OD_{600} of 1.5–2.0 (Fig. S8, *Inset*). Cells were harvested 12 h later by centrifugation then lysed at 26 kPa using a constant-pressure cell disruptor (TS Series Benchtop Cell Disrupter, Constant Systems). The His_6-MBP-toxin fusion protein was then captured by passing the soluble cell fraction over a Ni-NTA Superflow resin (Qiagen). Recombinant Ssm6a was obtained by cleaving the fusion protein with TEV protease at a concentration of 0.2 mg/mL in a redox buffer comprising 40 mM Tris, 400 mM NaCl, 0.6 mM reduced glutathione (GSH), 0.4 mM oxidized GSH, pH 8. Residual fusion protein was precipitated with 1% TFA before the liberated Ssm6a was purified to >95% purity via RP-HPLC (C_{18} column; Restek) using a gradient of 15–40% solvent B [0.043% TFA in 90% (vol/vol) acetonitrile] in solvent A (0.05% TFA in water) over 30 min (Fig. S8).

The identity of the recombinant Ssm6a was confirmed via MALDI-TOF mass spectrometry with CHCA matrix on a 4700 Proteomics Bioanalyzer (Applied Biosystems), and by coelution with native Ssm6a on RP-HPLC (Onyx Monolithic C_{18} column; Phenomenex). The final yield of Ssm6a was 2.5 mg/L of culture.

Ssm6a Safety Profiling. Groups of five mice were injected intraperitoneally with 1 μmol/kg Ssm6a or the same volume of saline. Blood pressure and heart rate were measured as we described previously (6). A swimming exercise performance test was performed as described previously (7) to examine the effect of Ssm6a on motor function. Mice were injected intraperitoneally with saline or Ssm6a (10, 100, 1,000, or 10,000 nmol/kg); the swim test was initiated 30 min after injection.

1. Undheim EA, King GF (2011) On the venom system of centipedes (Chilopoda), a neglected group of venomous animals. *Toxicon* 57(4):512–524.
2. Redaelli E, et al. (2010) Target promiscuity and heterogeneous effects of tarantula venom peptides affecting Na^+ and K^+ ion channels. *J Biol Chem* 285(6):4130–4142.
3. Saez NJ, et al. (2011) A dynamic pharmacophore drives the interaction between Psalmotoxin-1 and the putative drug target acid-sensing ion channel 1a. *Mol Pharmacol* 80(5):796–808.
4. Cabrita LD, Dai W, Bottomley SP (2006) A family of *E. coli* expression vectors for laboratory scale and high throughput soluble protein production. *BMC Biotechnol* 6:12.
5. Kapust RB, Waugh DS (1999) *Escherichia coli* maltose-binding protein is uncommonly effective at promoting the solubility of polypeptides to which it is fused. *Protein Sci* 8 (8):1668–1674.
6. Liu R, et al. (2013) Human β-defensin 2 Is a novel opener of Ca^{2+}-activated potassium channels and induces vasodilation and hypotension in monkeys. *Hypertension* 62(2): 415–425.
7. Wu RE, et al. (2013) Resveratrol protects against physical fatigue and improves exercise performance in mice. *Molecules* 18(4):4689–4702.

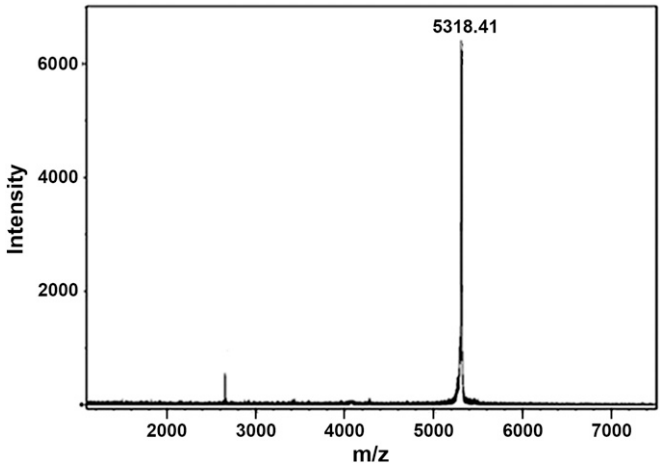

Fig. S1. MALDI-TOF mass spectral analysis of native Ssm6a purified from the venom of *S. subspinipes mutilans* revealed that it has a molecular mass of 5318.41 Da.

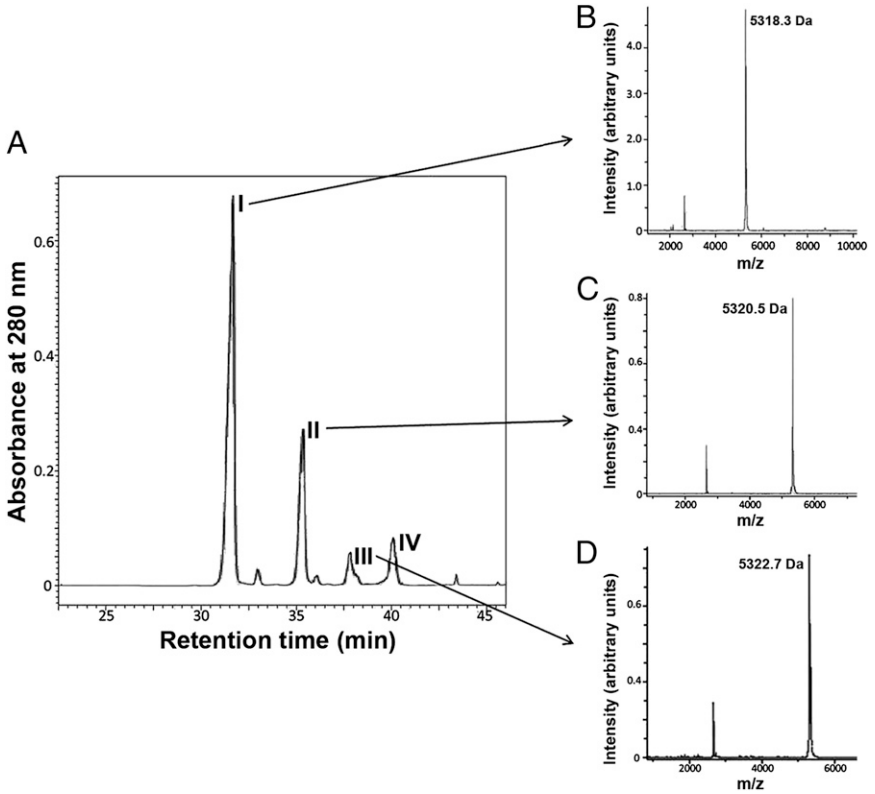

Fig. S2. Partial reduction of disulfide bonds in Ssm6a. (*A*) RP-HPLC chromatogram of Ssm6a after partial reduction with TCEP, which yielded four major species labeled I–IV. (*B–D*) MALDI-TOF mass spectral analysis of peaks I, II, and III indicated that they correspond to nonreduced Ssm6a (peak I), Ssm6a with one disulfide bond reduced (peak II), or Ssm6a with two reduced disulfide bridges (Peak III).

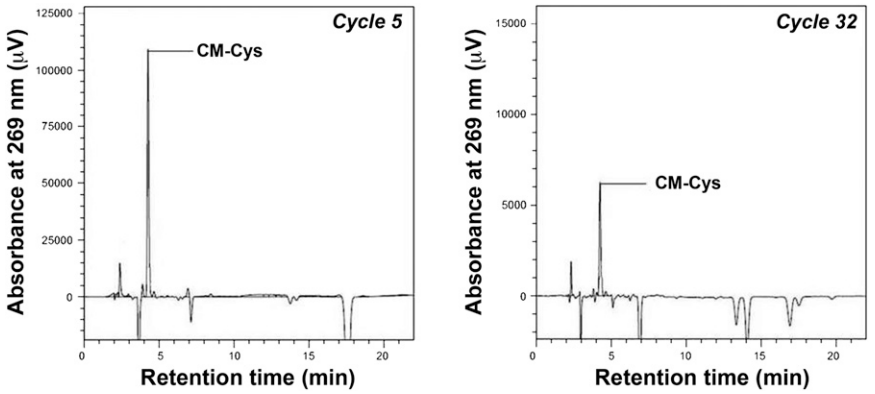

Fig. S3. Partial assignment of the disulfide-bond framework in Ssm6a. A partially reduced peptide containing two disulfide bridges was alkylated with iodoacetamide for 1 min and further purified using C18 RP-HPLC. Edman degradation analysis of the alkylated peptide yielded signals for alkylated Cys residues (Pth-CM-Cys) in cycles 5 and 32, indicative of a Cys5–Cys32 disulfide bond.

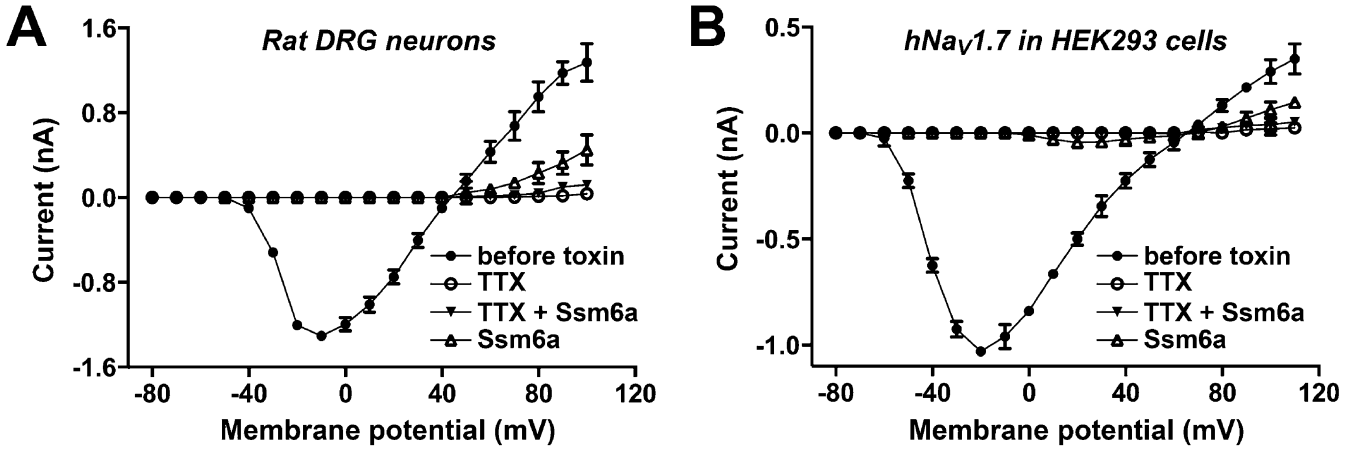

Fig. S4. Ssm6a is a gating modifier. The toxin concentrations used in these experiments were 1 μM Ssm6a and 500 nM TTX. Current–voltage relationships for (*A*) rat dorsal root ganglia (DRG) neurons ($n = 8$ cells) and (*B*) $hNa_V1.7$ currents in HEK293 cells ($n = 12$ cells). At a saturating concentration of Ssm6a (1 μM), the inhibition of TTX-s currents in DRG neurons and HEK293 cells expressing $hNa_V1.7$ was partly overcome by depolarizations to large positive test potentials (>60 mV). In contrast, inhibition of $Na_V1.7$ currents by the pore-blocker TTX was largely voltage-independent.

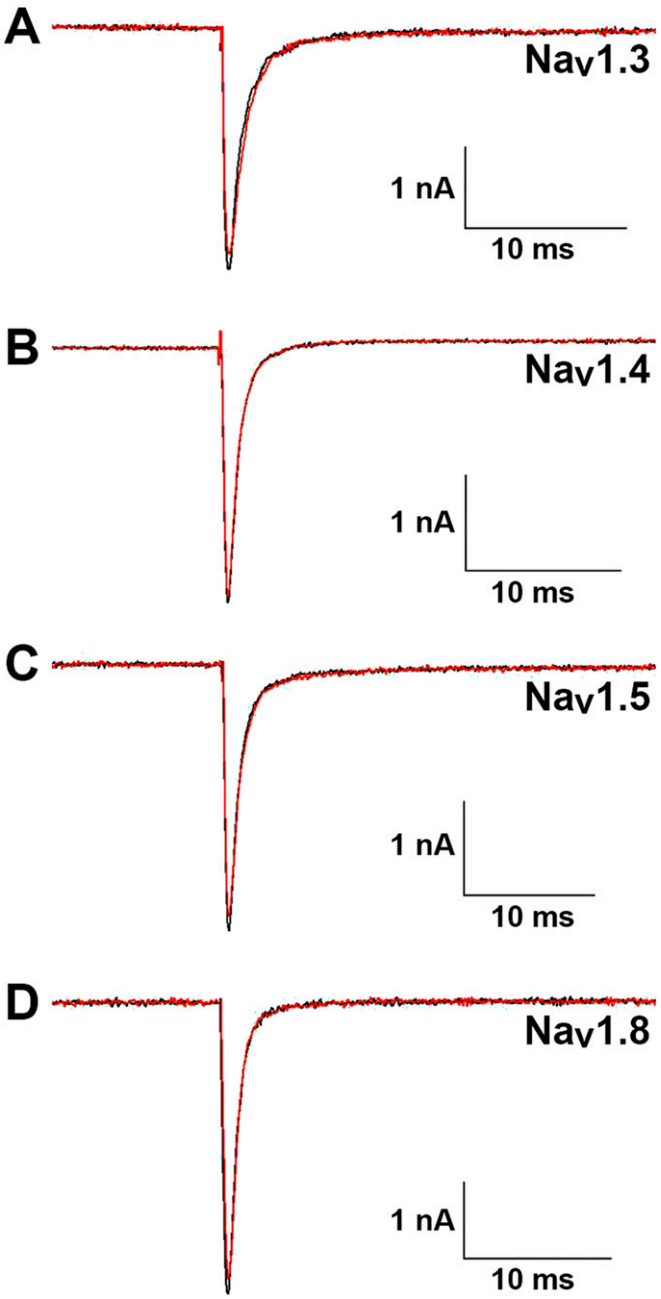

Fig. S5. Effect of Ssm6a on selected Na_V channel subtypes. Ssm6a (10 μM, red traces) had no effect on currents mediated by (*A*) $hNa_V1.3$, (*B*) $hNa_V1.4$, (*C*) $hNa_V1.5$, or (*D*) $hNa_V1.8$.

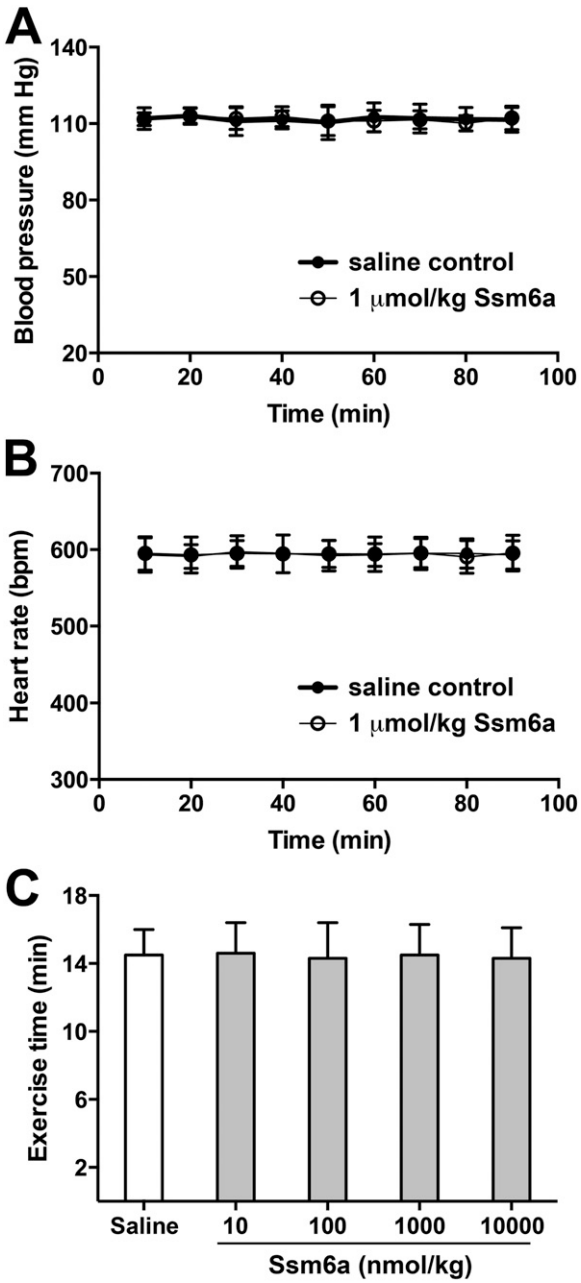

Fig. S6. Intraperitoneal injection of Ssm6a had no effect on (*A*) blood pressure, (*B*) heart rate, or (*C*) motor function (as judged by a swim test) at doses up to 1–10 μmol/kg. In each experiment, cohorts of five treated animals were compared with five saline-treated mice. Data are mean ± SE.

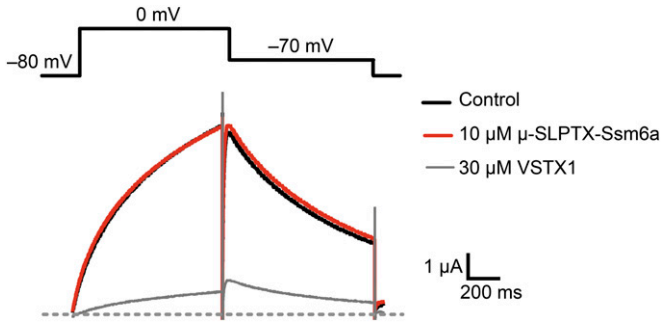

Fig. S7. Ssm6a has no effect on human ERG (Kv11.1) channels expressed in *Xenopus* oocytes. Representative K^+ currents before (black) and after addition of Ssm6a (10 μM, red) or the positive control peptide VSTX1 (30 μM, gray). The voltage-clamp protocol is shown above the current traces. Ssm6a had no effect on the outward activating current or the deactivating tail current of hERG channels (n = 3).

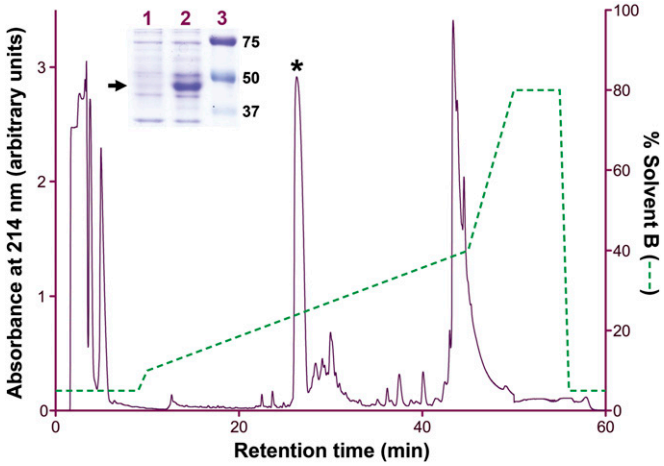

Fig. S8. Expression and purification of recombinant Ssm6a. RP-HPLC chromatogram showing purification of recombinant Ssm6a after liberation from the His_6-MBP fusion tag by TEV protease. The peak corresponding to Ssm6a is highlighted with an asterisk. (*Inset*) A SDS/PAGE gel showing *E. coli* cells before (lane 1) and after (lane 2) induction of Ssm6a expression with IPTG. Lane 3 contains molecular mass standards, with masses indicated in kilodaltons on the right of the gel. The arrow indicates the running position of the MBP-toxin fusion protein.