Supplemental Procedures

Plasmids

Sequences for IbpA-COOH, Fic1, and Fic2 were amplified from *H. somni* pHS139 ((Tagawa et al., 2005), AB087258) and inserted into pET41a (IbpA-COOH, Novagen) and pET-GSTx (Fic1, Fic2) for bacterial protein expression. IbpA-COOH contains the indicated amino acids of the protein formerly designated p76, while Fic1 and Fic2 contain the indicated amino acids of DR1 and DR2, respectively (Figure 1, Cole et al., 1993). For expression in mammalian cells, the amplified sequences were inserted into EGFP-N1 (BD Biosciences). The HYPE coding sequence was amplified from cDNA (Origene; accession NM_007076.2) and inserted into pET-GSTx. Mammalian expression vectors for activated and dominant negative forms of 3xHA-tagged RhoA, Rac, and Cdc42 were provided by Dr. Lorena Navarro, University of California, Davis. Wild type Rac and Cdc42 (pGEX-2T), wild type RhoA (pGEX-4T3), Rhotekin-PBD and PAK-PBD bacterial expression constructs were provided by Dr. Gary Bokoch, Scripps Research Institute, California. FLAG-tagged RhoA was constructed by cutting and ligating a BHI/NOTI fragment from RhoA (pGEX-4T3) into pCDNA3.1 N-FLAG (Gregory Taylor, University of Nebraska). All point mutations were generated by the QuikChange Site-Directed Mutagenesis kit (Stratagene).

Mass spectrometric analyses

Proteins were incubated with 0.25μg of trypsin or chymotrypsin overnight at 37°C for trypsin digests or at 25°C for chymotrypsin digests. Protease reactions were stopped with 100% formic acid (final 5%). 2μl of the peptide mixture was analyzed by automated microcapillary liquid chromatography-tandem mass spectrometry. Fused-silica capillaries (100μm i.d.) were pulled using a P-2000 CO_2 laser puller (Sutter Instruments, Novato, CA) to a 5μm i.d. tip and packed with 10cm of 5μm Magic C18 material (Agilent, Santa Clara, CA) using a pressure bomb. This column was then placed in-line with a Dionex 3000 HPLC equipped with an autosampler. The column was equilibrated in buffer A (2% acetonitrile, 0.1% formic acid), and the peptide mixture was loaded onto the column using the autosampler. The HPLC separation at a flow rate of 300nl/min was provided by a gradient between Buffer A and Buffer B (98% acetonitrile, 0.1% formic acid). The HPLC gradient was held constant at 100% buffer A for 5 min after peptide loading followed by a 30-min gradient from 5% buffer B to 40% buffer B. The gradient was then switched from 40% to 80% buffer B over 5 min and held constant for 3 min. Finally, the gradient was changed from 80% buffer B to 100% buffer A over 1 min, and then held constant at 100% buffer A for 15 minutes. The application of a 1.8 kV distal voltage electrosprayed the eluted peptides directly into a Thermo LTQ ion trap mass spectrometer equipped with a custom nanoLC electrospray ionization source. Full masses (MS) spectra were recorded on the peptides over a 400-2000 *m*/*z* range, followed by five tandem mass (MS/MS) events sequentially generated in a data-dependent manner on

the first, second, third, fourth and fifth most intense ions selected from the full MS spectrum (at 35% collision energy). Mass spectrometer scan functions and HPLC solvent gradients were controlled by the Xcalibur data system (ThermoFinnigan, San Jose, CA). MS/MS spectra were extracted from the RAW file with ReAdW.exe (http://sourceforge.net/projects/sashimi). The resulting mzXML file contains all the data for all MS/MS spectra and can be read by the subsequent analysis software.

Preparation of CCS

BHI broth supplemented with 0.1% Tris-base and 0.01% thiamine monophosphate was inoculated with *H. somni* scraped from an 18 hr BHI blood agar culture at 10^7 CFU/ml, and incubated at 37^{o}C for 6 hr with shaking at 200 RPM. Bacteria were then pelleted by centrifugation (4,000 g, 15 min, 4^{o}C) and the supernatant filtered through a 0.22μm pore filter (Millipore, Billerica, MA). The supernatant was concentrated using Amicon Centriplus columns with a 10,000 molecular mass pore size (Millipore, Billerica, MA). Retentant was washed once in PBS and the volume adjusted to yield a 40X concentrate. Cytoxicity was determined by treating HeLa cell monolayers with live *H. somni* or CCS. Log phase bacterial cultures at a final density of 2 x 10^8 CFU/ml were added to HeLa cells at MOI of 100. Bacterial counts were confirmed by plating on BHI blood agar.

Supplemental Reference

Cole, S.P., Guiney, D.G., and Corbeil, L.B. (1993). Molecular analysis of a gene encoding a serum-resistance-associated 76 kDa surface antigen of Haemophilus somnus. J Gen Microbiol *139*, 2135-2143.