

Figure S1. Successful fractionation of *A. baumannii* as determined by a marker of the inner membrane. 10 μ g of the *A. baumannii* cellular fractions used in Fig. 1C were subjected to immunoblot using a rabbit antibody generated against the known *A. baumannii* inner membrane protein ZrIA.

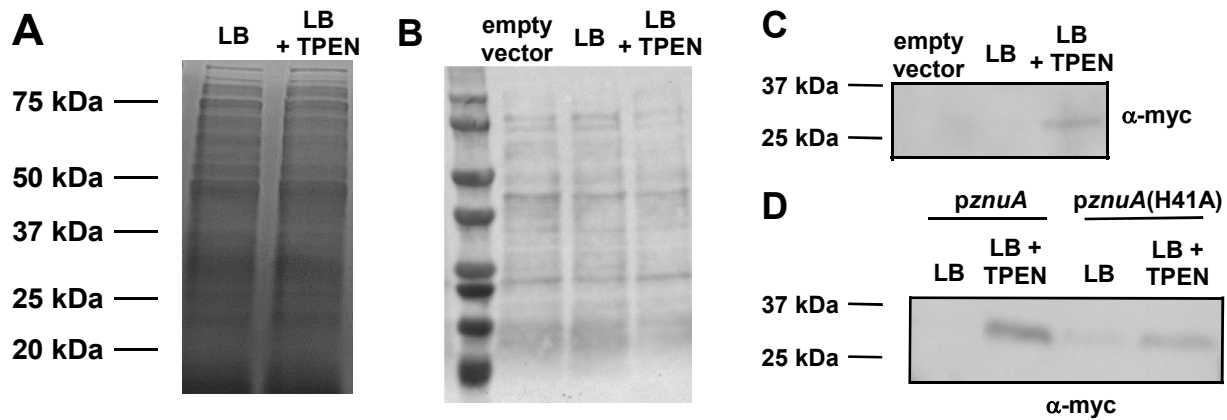


Figure S2. ZnuA is induced in low Zn and ZnuA(H41A) is expressed. (A) Lysates of $\Delta znuA$ *pznua* grown in LB or LB + 30 μ M TPEN were run on an SDS-PAGE gel and stained with Imperial Protein Stain. This gel was run in parallel with Fig. 3A demonstrating loading of 10 μ g protein per lane. (B) Lysates of $\Delta znuA$ containing an empty pWH1266 vector grown in LB + 30 μ M TPEN, as well as $\Delta znuA$ *pznua* grown in LB or LB + 30 μ M TPEN were run on an SDS-PAGE gel and transferred to a nitrocellulose membrane, then stained with Ponceau S. (C) The membranes from B were destained, then probed using an α -myc antibody. (D) Single nucleotide substitution of A for H was made to *znuA* at position 41 on pWH1266. $\Delta znuA$ cells expressing either *pznua*-myc or *pznua*(H41A)-myc were grown in either LB or LB + 30 μ M TPEN and 10 μ g of cell lysate was added to each lane for immunoblotting.

```

E. coli -----VVASLKPVGFIASAIADGVTETEVLLPDGASE H DYSLRPSDVKRLQNADL
S. typhi -----VVASLKPVGFIASAIADGVTDTQVLLPDGASE H DYSLRPSDVKRLQGADL
Y. pestis -----SAAVVT SIRPLGFIAAAIADGVLPTVLLPDGASP H DYALRPSDVQRLRSAEL
P. aeruginosa -----VLTS IKPLQQIAAAVQEGVGSPEVLLPPGASP H HYALRPSDVRRVGDADL
A. baumannii MSTLGSQGLVVSTHPIYLI AKEITKGVVEEPQLLQ-GQSG H DVQLTPAHRKAINDATL
N.menigitidis -----VAKQI GGDRVAVQSLVGANQDS H AYHMTSGDIKKIRSAKL

E. coli VVWVGPEMEAFMQKPVSKLPGAKQVTIAQLE DVKPLLMK-----
S. typhi -VVWVGPEMEAFMEKSVRNIPDNKQVTIAQLADV KPLLMKGADDEDEHAHTGADEEKGDVH
Y. pestis VIWVGPEMEAFLSKPLTQVAENKQIALS QLP SVTPLLKMSDEHDEAE EGESGH HHDHAKDN
P. aeruginosa LYWIGPDMESFLPRVLGSR-SKATVAVQSLP SMKLRHFGEDSH SHEEADHDEHDD-----
A. baumannii VIWLGAHEAPLNKLLSNKKAIALL DSGILS ILPQRNTRG-----
N.menigitidis VLLNGLGLEAADVQRAVKQSKVPFAEATKGIQALKAEEGGHHHHDHDEGH-----

E. coli ----SIHGDFNM H LWLSPEIARATAVAIHGKLVELMPQSRAKLDANLKD FEAQLAST--E
S. typhi ----HHHG EYNM H LWLSPEIARATAVAIHEKLVELMPQSRAKLDANLKD FEAQLAAT--D
Y. pestis PTDDHHHG EYNM H IWLSPAIAKQAAIAIHDRLL ELTPQNKDKLDANLRRFEYQLAQN--E
P. aeruginosa ----HRPGSLDA H LWLSVSNARVIAGKMAADLAQADPANAARYQHNLKVFS DRMDAL--D
A. baumannii ---AALPNTVDT H VWLEPNNAVRI GFFIAALRSQQHPENKAKYWNNA NTFARNMLQA--A
N.menigitidis ----HDHG EFD H WVTDPVLMGTYAQNVANALIQADPEGKTY YQQLGNYQMQLK LHS D

E. coli TQVGNELAPLKGKGYFV H DAYGYFEKQFGLTPLGHFTVNPEIQPGAQRLHEIR-----
S. typhi KQVGNELAPLKGKGYFV H DAYGYEKHYGLTPLGHFTVNPEIQPGAQRLHEIR-----
Y. pestis KNIVTMLKPVQKGYFV H DAYGYFENHFGLSPLGHFTVNPEIQPGAQRLHQIR-----
P. aeruginosa ERIKARVAGIAGKPYFV H EAFDYFEAEYGLKHTGVFSVAS EVQPGAQHVAAMR-----
A. baumannii QAYDSSSN---GKPYWSY H DAYQY LERSLN LKFAGALTDDPHVAPTAAQIKYLNDSRPKA
N.menigitidis AQAAFN AVPAAKR KVL TG H DAF SYMGKRYNIEFIAPQGV SSEAEP SAKQV-----

```

Figure S3. ZnuA sequence is conserved among Gram-negative pathogens. *znuA* sequences from 6 different Gram-negative pathogens were aligned using CLUSTALW. Predicted Zn-binding residues are highlighted in red, and H41 mutated in Fig. 3B is boxed.

A. baumannii --MLFYKNILTLSILAAISI PVFAAENENVEKLETIRIKAHPLAQTS---KDFAVADTVVDQKHLTEGAATIGDALNSEVGIYANQFGAGSSRPVIRG
N. meningitidis MAQTTLKPIVLSILLINTPLLAQAHETEQ SVDLET VSVVVGKSRPRAT SGLLHT STASDKIISGDTLRQKAVNLGDALDGVPGIHASQYGGGASAPVIRG

A. baumannii QDGPRVKVLQNSSENVDVSTLSPD **H** AVTVDPVLAKQVEVIRGPSTLLFGAGTVGGLVNVINDNKIPTQMPENGYEQVGLRYNTGSDEKLASAGVTVGL
N. meningitidis QTGRRIKVLNHHGETGDMADFSPD **H** AIMVDTALSQQVEILRGPVTLTYSSGNVAGLV DVADGKIPEKMPENGVSGELGLRLSSGNLEKLTSGGINIGL

A. baumannii GSQVALRVEGLTRDANNYIAPNYIHEGKERRVDNTFAQGDSVNVGLSWIYDRGYTGISYSNRRDQYGLPGHSHEYETCSAHLTGR-----PH
N. meningitidis GKNFVLTHTGLYRKSGDYAVPRYRN----LKRLPDSHADSQTG SIGLSWVGEKGFIVGAYSDDRRDQYGLPAHSHEYDDCHADIIWQKSLINKRYLQLYP

A. baumannii LHCDAHEHDHEEGEEAAH---EEHEHEHGG-PWIDLKSERYDFKTELNDPFAGFQKLRQAAS YTDYQHDEIEEGAIATRFQNKGYDGRIELVHNPIAD
N. meningitidis HLLTEEDIDYDNPGLSCGFHDDDNAHAHTHSGRPWIDLNRNKRYELRAEWKQPFPGFEALRVHLNRNDYRHDEKAGDAVENFFNNQTQNARIELRHQPIGR

A. baumannii WEGVIGTQLGQQK-----LNLTGEEAFMAPTTTCKWSVFALEHKQWKAVHFELSARADQQEIDVDDNSKQD-----FDGSAFSYAGAA
N. meningitidis LKGSWGVQYLQKSSALSAISEAVKQPMLLDNKVQHYSFFGVEQANWDNFTLEGGVRVEKQKASIQYDKALIDRENY YNHPLPLDGAHRQTARSAFALSG

A. baumannii NWEFAPNYKLSFVASHQERLPLAQELYANGA **H** FATNTYELGNDQLSKEKSNVLEGLHFDNDKLDYHLHVYHNFDDYIYAQTLDR-----YKDF
N. meningitidis MWYFTPQHKLSTASHQERLPLSTQELYAHGK **H** VATNTFEVGNKHLNKERSNNIELALGYEGDRWQYNLALYRNRFGNYYIYAQTLNDGRGPKSEDDSEM

A. baumannii RLVOYTQDKARFYGAEGEIGYQITPMYKISAFGDYVRG-----KI DAEG-----NAPRIPAGRLGTKVDA DFGDGFSGSAEY YHIFNQDK
N. meningitidis KLVRYNQSGADFYGAEGEITYFKPTPRYRIGVSGDYVRGRLKNLPSLPGREDAYGNRPFI AQDDQNAPRVPAARLGFHLKASLTDRIDANLDYYRVFAQNK

A. baumannii IAAYETETEGYNMLNLGVAYSQYQYAKTDYRVYLKANNLLDDTVYQHASFVLSNIPQVGRNFTVGVDFSF
N. meningitidis LARYETRTPGHMLNLGANYR-RNTRYGEWNWYVKADNLLNQSVYAHSSFLSDTPQMGRSFTGGVNVKF

Figure S4. ZnuD is a conserved outer membrane protein with predicted Zn binding residues. *znuD* sequence from *A. baumannii* was aligned with *znuD* from *N. meningitidis* using CLUSTALW. Residues shown to bind Zn in *N. meningitidis* are highlighted in purple.