

CyaA 353 AGKSLFDDGLGAAPGVPSCRSKFSPDVLTPVSP...GLRRPSLGAVERQ
LtxA 103 ERGLTLFAPELDKWIQGNK...HLSNSVGS.TGNLTAKIDKQVSV
PaxA 114 ERGLTLFAPELDKFIQHS...KISNVVGSSTGDTVNNKLAQSQAI
ApXIIIA 114 ERGLTLFAPELDKFIQHS...KISNVVGSSTGDAVSKLAKSQTII
ApXIA 101 ERGLTLFAPELDKFIQHS...KISNVVGSSTGDAVSKLAKSQTII
EhxA 90 ERGLTLFAPELDKFIQHS...KISNVVGSSTGDAVSKLAKSQTII
MmxA 104 ERGLTLFAPELDKFIQHS...KISNVVGSSTGDAVSKLAKSQTII
HlyA 104 ERGLTLFAPELDKFIQHS...KISNVVGSSTGDAVSKLAKSQTII
MbxA 81 ERGLTLFAPELDKFIQHS...KISNVVGSSTGDAVSKLAKSQTII
RtxA 102 ERGLTLFAPELDKFIQHS...KISNVVGSSTGDAVSKLAKSQTII
ApXIIA 105 ERGLTLFAPELDKFIQHS...KISNVVGSSTGDAVSKLAKSQTII
LkTA 102 ERGLTLFAPELDKFIQHS...KISNVVGSSTGDAVSKLAKSQTII
PILkTA 102 ERGLTLFAPELDKFIQHS...KISNVVGSSTGDAVSKLAKSQTII

CyaA 401 DSGYDSLDGVGSRFSLGEVSDMAAVEAAELEMTRQVLHAGARQDDAEFGVSGASAHWQG
LtxA 144 LGTLQAFINTAFSGMDLDA...LTKARQ...NG...KNVTDDVQLAKA...
PaxA 156 ISGVQSVLGSVLGINLNE...AISG...GSELELAKA...
ApXIIIA 156 ISGVQSVLGSVLGINLNE...AISG...GSELELAKA...
ApXIA 143 LSLAQSLFGTALAGMDLDS...LLRRRR...NG...EDVSGSELAKA...
EhxA 132 LSLAQSLFGTALAGMDLDS...LLRRRR...NG...EDVSGSELAKA...
MmxA 146 LSLAQSLFGTALAGMDLDS...LLRRRR...NG...EDVSGSELAKA...
HlyA 146 LSLAQSLFGTALAGMDLDS...LLRRRR...NG...EDVSGSELAKA...
MbxA 124 LSLAQSLFGTALAGMDLDS...LLRRRR...NG...EDVSGSELAKA...
RtxA 145 LSLAQSLFGTALAGMDLDS...LLRRRR...NG...EDVSGSELAKA...
ApXIIA 146 LSLAQSLFGTALAGMDLDS...LLRRRR...NG...EDVSGSELAKA...
LkTA 142 LSLAQSLFGTALAGMDLDS...LLRRRR...NG...EDVSGSELAKA...
PILkTA 142 LSLAQSLFGTALAGMDLDS...LLRRRR...NG...EDVSGSELAKA...

CyaA 461 RALQGAQAVAAQRVLVHAIALMTQFGRAGSTNTPQEAASLSAAVFGLEASSAVAEIVSG
LtxA 182 ...SNNLINELIGTISITN...NVDTFESKQINLKLGEALGQV...HFGS
PaxA 189 ...GVDLASLGVNIAKGT...TIEAFSEQIQNFQKLVQNAK...GLGG
ApXIIIA 189 ...GVDLASLGVNIAKGT...TIEAFSEQIQNFQKLVQNAK...GLGG
ApXIA 181 ...GVDLASLGVNIAKGT...TIEAFSEQIQNFQKLVQNAK...GLGG
EhxA 170 ...GVDLASLGVNIAKGT...TIEAFSEQIQNFQKLVQNAK...GLGG
MmxA 184 ...GVDLASLGVNIAKGT...TIEAFSEQIQNFQKLVQNAK...GLGG
HlyA 184 ...GVDLASLGVNIAKGT...TIEAFSEQIQNFQKLVQNAK...GLGG
MbxA 158 ...GVDLASLGVNIAKGT...TIEAFSEQIQNFQKLVQNAK...GLGG
RtxA 179 ...GVDLASLGVNIAKGT...TIEAFSEQIQNFQKLVQNAK...GLGG
ApXIIA 180 ...GVDLASLGVNIAKGT...TIEAFSEQIQNFQKLVQNAK...GLGG
LkTA 175 ...GVDLASLGVNIAKGT...TIEAFSEQIQNFQKLVQNAK...GLGG
PILkTA 175 ...GVDLASLGVNIAKGT...TIEAFSEQIQNFQKLVQNAK...GLGG

CyaA 521 FFRGSSRM.AGGFGVAGGAMALGGGIAAAGVAGMSLT.DDAPAGQKAAAGATLALQTCG
LtxA 223 FGDKLKLNLP...KLGNLGKGLGALSGVLSAISAALLANKDADTATKAAAGATLALQTCG
PaxA 230 VGGQKLQHI.SGSALSKTGLGLDIISLLSGVTASFTLADKNASTSTKVAAGFELSNQVIGG
ApXIIIA 230 VGGQKLQHI.SGSALSKTGLGLDIISLLSGVTASFTLADKNASTSTKVAAGFELSNQVIGG
ApXIA 221 VGGQKLQHI.SGSALSKTGLGLDIISLLSGVTASFTLADKNASTSTKVAAGFELSNQVIGG
EhxA 221 VGGQKLQHI.SGSALSKTGLGLDIISLLSGVTASFTLADKNASTSTKVAAGFELSNQVIGG
MmxA 225 VGGQKLQHI.SGSALSKTGLGLDIISLLSGVTASFTLADKNASTSTKVAAGFELSNQVIGG
HlyA 224 VGGQKLQHI.SGSALSKTGLGLDIISLLSGVTASFTLADKNASTSTKVAAGFELSNQVIGG
MbxA 199 VGGQKLQHI.SGSALSKTGLGLDIISLLSGVTASFTLADKNASTSTKVAAGFELSNQVIGG
RtxA 220 VGGQKLQHI.SGSALSKTGLGLDIISLLSGVTASFTLADKNASTSTKVAAGFELSNQVIGG
ApXIIA 221 VGGQKLQHI.SGSALSKTGLGLDIISLLSGVTASFTLADKNASTSTKVAAGFELSNQVIGG
LkTA 216 VGGQKLQHI.SGSALSKTGLGLDIISLLSGVTASFTLADKNASTSTKVAAGFELSNQVIGG
PILkTA 216 VGGQKLQHI.SGSALSKTGLGLDIISLLSGVTASFTLADKNASTSTKVAAGFELSNQVIGG

CyaA 579 TVELASSIALALAAARGVTSGLQVAGASAGAAAGALAAALSPMEIYGLVQOQSHYADQLDK
LtxA 281 IGKAITQYLLAQRA...AAGLSTTGPVAGLIAASVVSALSPISFLGIAGKQDRARMLEE
PaxA 290 ITKAVSSYILAQR...AAGLSTTGPVAGLIAASVVSALSPISFLGIAGKQDRARMLEE
ApXIIIA 290 ITKAVSSYILAQR...AAGLSTTGPVAGLIAASVVSALSPISFLGIAGKQDRARMLEE
ApXIA 279 ITKAVSSYILAQR...AAGLSTTGPVAGLIAASVVSALSPISFLGIAGKQDRARMLEE
EhxA 269 ITKAVSSYILAQR...AAGLSTTGPVAGLIAASVVSALSPISFLGIAGKQDRARMLEE
MmxA 283 ITKAVSSYILAQR...AAGLSTTGPVAGLIAASVVSALSPISFLGIAGKQDRARMLEE
HlyA 282 ITKAVSSYILAQR...AAGLSTTGPVAGLIAASVVSALSPISFLGIAGKQDRARMLEE
MbxA 256 ITKAVSSYILAQR...AAGLSTTGPVAGLIAASVVSALSPISFLGIAGKQDRARMLEE
RtxA 278 ITKAVSSYILAQR...AAGLSTTGPVAGLIAASVVSALSPISFLGIAGKQDRARMLEE
ApXIIA 279 ITKAVSSYILAQR...AAGLSTTGPVAGLIAASVVSALSPISFLGIAGKQDRARMLEE
LkTA 274 ITKAVSSYILAQR...AAGLSTTGPVAGLIAASVVSALSPISFLGIAGKQDRARMLEE
PILkTA 274 ITKAVSSYILAQR...AAGLSTTGPVAGLIAASVVSALSPISFLGIAGKQDRARMLEE

CyaA 639 LAQESSAYGYECDALLAQYRDKTAEEGAVAGVSAVLSTVGAAVSIAAAASVVGAPVAVV
LtxA 337 YSKRFKKFGYNGDSLLGQFYANTGIADAAITINTVLSAIAAGVGAASAGSLVGAPIGLL
PaxA 346 FAERFKKFGYNGDSLLGQFYANTGIADAAITINTVLSAIAAGVGAASAGSLVGAPIGLL
ApXIIIA 346 FAERFKKFGYNGDSLLGQFYANTGIADAAITINTVLSAIAAGVGAASAGSLVGAPIGLL
ApXIA 335 YSERFKKFGYNGDSLLGQFYANTGIADAAITINTVLSAIAAGVGAASAGSLVGAPIGLL
EhxA 325 YSERFKKFGYNGDSLLGQFYANTGIADAAITINTVLSAIAAGVGAASAGSLVGAPIGLL
MmxA 339 YSERFKKFGYNGDSLLGQFYANTGIADAAITINTVLSAIAAGVGAASAGSLVGAPIGLL
HlyA 338 YSERFKKFGYNGDSLLGQFYANTGIADAAITINTVLSAIAAGVGAASAGSLVGAPIGLL
MbxA 312 YSERFKKFGYNGDSLLGQFYANTGIADAAITINTVLSAIAAGVGAASAGSLVGAPIGLL
RtxA 334 YSERFKKFGYNGDSLLGQFYANTGIADAAITINTVLSAIAAGVGAASAGSLVGAPIGLL
ApXIIA 335 YSERFKKFGYNGDSLLGQFYANTGIADAAITINTVLSAIAAGVGAASAGSLVGAPIGLL
LkTA 330 YSERFKKFGYNGDSLLGQFYANTGIADAAITINTVLSAIAAGVGAASAGSLVGAPIGLL
PILkTA 330 YSERFKKFGYNGDSLLGQFYANTGIADAAITINTVLSAIAAGVGAASAGSLVGAPIGLL

CyaA 699 TSLTGALNGILRGVQPIIEKIANLADYARKIDELGG...PQAYFEKNLQARHEQLANSNDG
LtxA 397 VSAITSLISGILDASKQAFVEHIANLADYARKIDELGG...PQAYFEKNLQARHEQLANSNDG
PaxA 406 VTGITGLISGILEFASKQPMLEHVASKLGTKEDEWEKK.YGKNYFENGYDARHAKFALE..D
ApXIIIA 406 VTGITGLISGILEFASKQPMLEHVASKLGTKEDEWEKK.YGKNYFENGYDARHAKFALE..D
ApXIA 395 VSAITSLISGILDASKQAFVEHIANLADYARKIDELGG...PQAYFEKNLQARHEQLANSNDG
EhxA 385 VSAITSLISGILEFASKQPMLEHVASKLGTKEDEWEKK.YGKNYFENGYDARHAKFALE..D
MmxA 399 VSAITSLISGILEFASKQPMLEHVASKLGTKEDEWEKK.YGKNYFENGYDARHAKFALE..D
HlyA 398 VSAITSLISGILEFASKQPMLEHVASKLGTKEDEWEKK.YGKNYFENGYDARHAKFALE..D
MbxA 372 VSAITSLISGILEFASKQPMLEHVASKLGTKEDEWEKK.YGKNYFENGYDARHAKFALE..D
RtxA 394 VSAITSLISGILEFASKQPMLEHVASKLGTKEDEWEKK.YGKNYFENGYDARHAKFALE..D
ApXIIA 395 VSAITSLISGILEFASKQPMLEHVASKLGTKEDEWEKK.YGKNYFENGYDARHAKFALE..D
LkTA 390 VSAITSLISGILEFASKQPMLEHVASKLGTKEDEWEKK.YGKNYFENGYDARHAKFALE..D
PILkTA 390 VSAITSLISGILEFASKQPMLEHVASKLGTKEDEWEKK.YGKNYFENGYDARHAKFALE..D

Supplementary Figure S1. Alignment of the pore-forming domain of CyaA against other RTX toxins. Multiple Sequence Alignment (MSA) was performed with Clustal Omega software and plotted with ESPript 3.0. The name of the toxin and the number of the corresponding residues are shown in the left side. The strictly conserved alanine, arginine, aspartic acid, glutamic acid, glutamine, glycine, isoleucine, leucine, lysine, proline, threonine and tyrosine residues are highlighted with a red frame. CRAC motifs are depicted in purple and CARC motifs in blue.