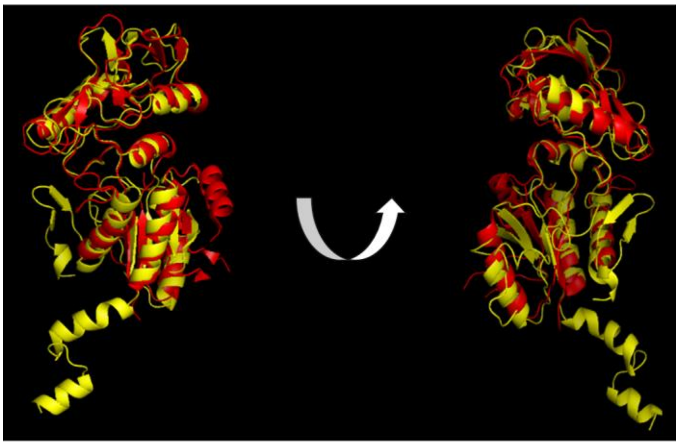
The C-terminus of *Pseudomonas aeruginosa* ExoY effector is crucial for binding to its eukaryotic activator, F-actin

Alexander Belyy[1*], Ignacio Santecchia[2], Louis Renault[3], Blandine Bourigault[3], Daniel Ladant[1] & Undine Mechold[1*]

Supporting Information:

Figure S1. Alignment of ExoY Phyre 2 model (yellow) with ExoY PDB 5XNW (red) performed by PyMol.



Supplementary material and methods

Plasmid preparation

p1653, pB158, pB159, pB160 and p1652 for the expression of WT ExoY and the ExoY variants with C-terminal deletions of 3, 5, 7 and 9 amino acids respectively, in *S. cerevisiae* model, were constructed as follows: Primers #1259 and #1284, #b223 and #b224, #b223 and #b225, #b223 and #b226 or #1259 and #1283 were used to PCR-amplify the according fragments from p1593. The XhoI, NheI digested fragments were cloned into YEpGal555.

p1665, p1666, p1667, p1668, p1669, p1670, p1671, pB187, pB188, pB189, pB216, pB217, pB218, pB219, pB220, pB221, pB222 and pB223 for the expression of ExoY variants with single or double mutations in *S. cerevisiae* (for detailed plasmid description provided in table S1), were constructed as follows: Primers #1259 and #1298, #1259 and #1299, #1259 and #1300, #1259 and #1301, #1259 and #1302, #1259 and #1303, #1259 and #1304, #b223 and #b260, #b223 and #b261, #b223 and #b262, #b223 and #b311, #b223 and #b312, #b223 and #b313, #b223 and #b314, #b223 and #b315, #b223 and #b316, #b223 and #b317, or #b223 and #b318 were used to PCR-amplify the according fragments from p1653. The XhoI, NheI digested fragments were cloned into YEpGal555.

p1664 for the expression of ExoY variant L371A in *S. cerevisiae*, was constructed by two-step overlap PCR, using primers #1259, #1284, #1305 and #1306. The XhoI, NheI digested fragment was cloned into YEpGal555.

pB193, pB194 and pB195 for the expression of ExoY variants with N-terminal deletions of 10, 20 and 30 amino acids respectively in *S. cerevisiae* model, were constructed as follows: Primers #b266 and #b78, #b267 and #b78, or #b268 and #b78 were used to PCR-amplify the according fragments from p1653. The XhoI, NheI digested fragments were cloned into YEpGal555.

pB198 for the expression of ExoY variant from *P. aeruginosa* UCBPP PA14 strain in *S. cerevisiae* model was constructed as follows: Primers #b273 and #b274 were used to PCR-amplify the according fragments from genomic DNA of the strain. The Xho, KpnI digested fragments were cloned into YEpGal555.

pB233 and pB234 for the expression of GFP and GFP-ExoY$_{K81M}$ in *S. cerevisiae* model were prepared as follows: Primers #b327 and #b328 were used to PCR-amplify the GFP gene from pEFGP-C1 plasmid. The Xho digested fragment was cloned into YEpGal555 or into p1594, respectively.

pB247 for the expression of GFP-ExoY$_{K81M}$ with C-terminal deletion of 9 amino acids in *S. cerevisiae* model, was prepared as follows: The BamHI digested fragment from the plasmid pB234 was inserted into p1652.

pB240 and pB256 for expression of ExoY variants with C-terminal deletion of 5 or 9 amino acids, respectively, in *E. coli*, were prepared as follows: Primers #b167 and #b336 and #b167 and #b350 were used to PCR-amplify the according fragments from pUM460. The NdeI, KpnI digested fragments were cloned into pUM460.

pB241 for expression of ExoY variant from *P. aeruginosa* UCBPP PA14 strain in *E. coli* was constructed in two steps: 1) The NcoI/KpnI digested fragment from pB198 was cloned into pUM460. 2) Primers #b78 and #b340 were used to PCR-amplify the C-terminus of the toxin variant from pB198. The NcoI digested PCR-fragment were cloned into the intermediate construction from step 1.

pB243 and pB244 for expression of ExoY variant with single mutations F367A or L371G, respectively, in *E. coli* were constructed as follows: Primers #b3 and #b334 were used to amplify fragments from pB218 and pB222. The NdeI, KpnI digested fragments were cloned into pUM460.

pB255 for expression of ExoY variant from UCBPP PA14 strain with C-terminus from WT ExoY in *E. coli* was constructed as follows: Primers #b167 and #b349 were used to amplify the fragment from pB241. The NdeI, KpnI digested fragment was cloned into pB241.

pB137 for the expression of 6XHis-tagged maltose-binding protein in *E. coli* was constructed as follows: Primers #b190 and #b191 were used to PCR-amplify the fragment from pMAL-c5X. The NdeI, HindIII digested fragment was cloned into pET28a.

pB138, pB141, pB142, pB152, pB153, pB154, pB155 and pB156 for expression of complete ExoY or ExoY fragments in the fusion with maltose-binding protein in *E. coli* were constructed as follows: Primers #b192 and #b193, #b196 and #b193, #b197 and #b193, #b212 and #b213, #b214 and #b215, #b216 and #b217, #b218 and #b219, or #b220 and #b221 were used to PCR-amplify the ExoY fragment from pUM460. The SacI, HindIII digested fragments were cloned into pB137.

pB182, pB183 and pB184 for expression of cysteine-containing ExoY fragments in the fusion with maltose-binding protein in *E. coli* were constructed as follows: Primers #b197 and #b255, #b197 and #b256, #b197 and #b257 were used to PCR-amplify the fragments from pB142. The SacI, HindIII digested fragments were cloned into pB137.

Table S1. List of *P. aeruginosa* strains encoding PA14-like ExoY variant.

Genome	Genome ID	PATRIC ID	Length (AA)	Identity	ExoU
Pseudomonas aeruginosa ATCC 700888	1163394.3	fig|1163394.3.peg.2931	410	98	yes
Pseudomonas aeruginosa strain TSB 1	287.1875	fig|287.1875.peg.3561	414	100	yes
Pseudomonas aeruginosa strain RW27	287.1876	fig|287.1876.peg.4169	414	100	yes
Pseudomonas aeruginosa strain TSB 3	287.1879	fig|287.1879.peg.5195	414	100	yes
Pseudomonas aeruginosa strain 10% 1	287.1881	fig|287.1881.peg.4280	414	100	yes
Pseudomonas aeruginosa strain 10% 9	287.1882	fig|287.1882.peg.4912	414	100	yes
Pseudomonas aeruginosa strain 12.5% 6	287.1883	fig|287.1883.peg.3981	414	100	yes
Pseudomonas aeruginosa strain 15% 5	287.1884	fig|287.1884.peg.5273	414	100	yes
Pseudomonas aeruginosa strain 15.121c	287.1885	fig|287.1885.peg.3060	414	100	yes
Pseudomonas aeruginosa strain 15.311Ga	287.1886	fig|287.1886.peg.2496	414	100	yes
Pseudomonas aeruginosa strain TSB Pool	287.1887	fig|287.1887.peg.5600	414	100	yes
Pseudomonas aeruginosa strain RW30	287.1888	fig|287.1888.peg.4589	414	100	yes
Pseudomonas aeruginosa strain TSB 4	287.1892	fig|287.1892.peg.3452	414	100	yes
Pseudomonas aeruginosa strain 10% 2	287.1893	fig|287.1893.peg.4854	414	100	yes
Pseudomonas aeruginosa strain 15.211Ga	287.1894	fig|287.1894.peg.4618	414	100	yes
Pseudomonas aeruginosa strain TSB 9	287.1918	fig|287.1918.peg.2590	414	100	yes
Pseudomonas aeruginosa strain 10% 7	287.1920	fig|287.1920.peg.1982	414	100	yes
Pseudomonas aeruginosa strain 12.5% 4	287.1921	fig|287.1921.peg.4712	414	100	yes
Pseudomonas aeruginosa strain 15% 3	287.1922	fig|287.1922.peg.751	414	100	yes
Pseudomonas aeruginosa strain 15.121a	287.1923	fig|287.1923.peg.4455	414	100	yes
Pseudomonas aeruginosa strain 15.221Ga	287.1924	fig|287.1924.peg.4729	414	100	yes
Pseudomonas aeruginosa strain RW18	287.1926	fig|287.1926.peg.3924	414	100	yes
Pseudomonas aeruginosa strain RW192	287.1928	fig|287.1928.peg.3505	414	100	yes
Pseudomonas aeruginosa strain TSB 2	287.1929	fig|287.1929.peg.348	414	100	yes
Pseudomonas aeruginosa strain 10% 8	287.1932	fig|287.1932.peg.4382	414	100	yes

Pseudomonas aeruginosa strain 12.5% 5	287.1933	fig|287.1933.peg.5202	414	100	yes
Pseudomonas aeruginosa strain 15% 4	287.1934	fig|287.1934.peg.4729	414	100	yes
Pseudomonas aeruginosa strain 15.121b	287.1935	fig|287.1935.peg.5025	414	100	yes
Pseudomonas aeruginosa strain 15.221Ba	287.1936	fig|287.1936.peg.3201	414	100	yes
Pseudomonas aeruginosa strain 12.5% 1.1	287.1938	fig|287.1938.peg.4543	414	100	yes
Pseudomonas aeruginosa strain 12.5% 7	287.1939	fig|287.1939.peg.1213	414	100	yes
Pseudomonas aeruginosa strain 15% 6	287.1940	fig|287.1940.peg.501	414	100	yes
Pseudomonas aeruginosa strain TSB Bead Pool 1	287.1941	fig|287.1941.peg.3114	414	100	yes
Pseudomonas aeruginosa strain RW99	287.1943	fig|287.1943.peg.4558	414	100	yes
Pseudomonas aeruginosa strain RW199	287.1944	fig|287.1944.peg.1423	414	100	yes
Pseudomonas aeruginosa strain TSB 5	287.1945	fig|287.1945.peg.4422	414	100	yes
Pseudomonas aeruginosa strain 10% 3	287.1947	fig|287.1947.peg.286	414	100	yes
Pseudomonas aeruginosa strain 12.5% 1.2	287.1948	fig|287.1948.peg.5965	414	100	yes
Pseudomonas aeruginosa strain 12.5% 8	287.1949	fig|287.1949.peg.3737	414	100	yes
Pseudomonas aeruginosa strain 15.211 Gb	287.1951	fig|287.1951.peg.6017	414	100	yes
Pseudomonas aeruginosa strain 15.311Ba	287.1952	fig|287.1952.peg.4234	414	100	yes
Pseudomonas aeruginosa strain TSB Bead Pool 2	287.1953	fig|287.1953.peg.2806	414	100	yes
Pseudomonas aeruginosa strain TSB 8	287.1962	fig|287.1962.peg.4330	414	100	yes
Pseudomonas aeruginosa strain 10% 6	287.1964	fig|287.1964.peg.3853	414	100	yes
Pseudomonas aeruginosa strain 12.5% 3	287.1965	fig|287.1965.peg.272	414	100	yes
Pseudomonas aeruginosa strain 15% 2	287.1966	fig|287.1966.peg.3237	414	100	yes
Pseudomonas aeruginosa strain 15.111c	287.1967	fig|287.1967.peg.2282	414	100	yes
Pseudomonas aeruginosa strain 15.211Bb	287.1968	fig|287.1968.peg.5183	414	100	yes
Pseudomonas aeruginosa strain ATCC 43731	287.1995	fig|287.1995.peg.4221	414	100	yes
Pseudomonas aeruginosa	287.2562	fig|287.2562.peg.263	414	100	yes
Pseudomonas aeruginosa strain B10W	287.2971	fig|287.2971.peg.3002	410	98	yes
Pseudomonas aeruginosa strain PA14Or_reads	287.2981	fig|287.2981.peg.3047	414	100	yes

Pseudomonas aeruginosa strain 560747	287.3108	fig|287.3108.peg.5175	410	98	yes
Pseudomonas aeruginosa strain CCF_839	287.3111	fig|287.3111.peg.5553	414	100	yes
Pseudomonas aeruginosa strain CL232	287.3115	fig|287.3115.peg.5013	414	100	yes
Pseudomonas aeruginosa strain CLB24226	287.3128	fig|287.3128.peg.1748	410	98	yes
Pseudomonas aeruginosa strain 0511A_66_19	287.3131	fig|287.3131.peg.4963	414	100	yes
Pseudomonas aeruginosa strain 149	287.3262	fig|287.3262.peg.6288	414	100	yes
Pseudomonas aeruginosa strain 150	287.3263	fig|287.3263.peg.144	414	100	yes
Pseudomonas aeruginosa strain 151	287.3264	fig|287.3264.peg.82	414	100	yes
Pseudomonas aeruginosa strain 155	287.3265	fig|287.3265.peg.886	414	100	yes
Pseudomonas aeruginosa strain 152	287.3266	fig|287.3266.peg.2637	414	100	yes
Pseudomonas aeruginosa strain 153	287.3267	fig|287.3267.peg.4232	414	100	yes
Pseudomonas aeruginosa strain 154	287.3268	fig|287.3268.peg.4513	414	100	yes
Pseudomonas aeruginosa strain 156	287.3269	fig|287.3269.peg.2338	414	100	yes
Pseudomonas aeruginosa strain 157	287.3270	fig|287.3270.peg.4242	414	100	yes
Pseudomonas aeruginosa strain 158	287.3271	fig|287.3271.peg.1153	414	100	yes
Pseudomonas aeruginosa strain 159	287.3272	fig|287.3272.peg.139	414	100	yes
Pseudomonas aeruginosa strain 160	287.3273	fig|287.3273.peg.5568	414	100	yes
Pseudomonas aeruginosa strain 161	287.3274	fig|287.3274.peg.5815	414	100	yes
Pseudomonas aeruginosa strain 162	287.3275	fig|287.3275.peg.324	414	100	yes
Pseudomonas aeruginosa strain 163	287.3276	fig|287.3276.peg.6293	414	100	yes
Pseudomonas aeruginosa strain 164	287.3277	fig|287.3277.peg.592	414	100	yes
Pseudomonas aeruginosa strain 167	287.3279	fig|287.3279.peg.558	414	100	yes
Pseudomonas aeruginosa strain 168	287.3281	fig|287.3281.peg.6276	414	100	yes
Pseudomonas aeruginosa strain 169	287.3282	fig|287.3282.peg.2524	414	100	yes
Pseudomonas aeruginosa strain 171	287.3283	fig|287.3283.peg.152	414	100	yes
Pseudomonas aeruginosa strain 170	287.3284	fig|287.3284.peg.1647	414	100	yes
Pseudomonas aeruginosa strain 172	287.3285	fig|287.3285.peg.30	414	100	yes

Pseudomonas aeruginosa strain 177	287.3291	fig|287.3291.peg.4954	414	100	yes
Pseudomonas aeruginosa strain 338	287.3438	fig|287.3438.peg.2904	414	100	yes
Pseudomonas aeruginosa strain 342	287.3442	fig|287.3442.peg.743	414	100	yes
Pseudomonas aeruginosa strain 343	287.3443	fig|287.3443.peg.5123	414	100	yes
Pseudomonas aeruginosa strain 344	287.3444	fig|287.3444.peg.2990	414	100	yes
Pseudomonas aeruginosa strain 345	287.3445	fig|287.3445.peg.220	414	100	yes
Pseudomonas aeruginosa strain 346	287.3446	fig|287.3446.peg.33	414	100	yes
Pseudomonas aeruginosa strain 347	287.3447	fig|287.3447.peg.865	414	100	yes
Pseudomonas aeruginosa strain 348	287.3448	fig|287.3448.peg.564	414	100	yes
Pseudomonas aeruginosa strain 405	287.3503	fig|287.3503.peg.3574	414	100	yes
Pseudomonas aeruginosa strain 406	287.3504	fig|287.3504.peg.3883	414	100	yes
Pseudomonas aeruginosa strain 415	287.3513	fig|287.3513.peg.5514	414	100	yes
Pseudomonas aeruginosa strain 416	287.3514	fig|287.3514.peg.364	414	100	yes
Pseudomonas aeruginosa strain 417	287.3515	fig|287.3515.peg.6005	414	100	yes
Pseudomonas aeruginosa strain 149.1	287.3548	fig|287.3548.peg.3790	414	100	yes
Pseudomonas aeruginosa strain 161.1	287.3550	fig|287.3550.peg.5959	414	100	yes
Pseudomonas aeruginosa strain 163.1	287.3551	fig|287.3551.peg.2700	414	100	yes
Pseudomonas aeruginosa strain COPD2a	287.3736	fig|287.3736.peg.1825	414	100	yes
Pseudomonas aeruginosa strain PASGNDM345	287.3877	fig|287.3877.peg.3379	410	98	yes
Pseudomonas aeruginosa strain PASGNDM699	287.3878	fig|287.3878.peg.3378	410	98	yes
Pseudomonas aeruginosa strain PASGNDM571	287.3884	fig|287.3884.peg.3191	410	98	yes
Pseudomonas aeruginosa strain PASGNDM583	287.3885	fig|287.3885.peg.5453	410	98	yes
Pseudomonas aeruginosa strain PASGNDM544	287.3886	fig|287.3886.peg.5251	410	98	yes
Pseudomonas aeruginosa strain PASGNDM587	287.3887	fig|287.3887.peg.1646	410	98	yes
Pseudomonas aeruginosa strain PASGNDM592	287.3888	fig|287.3888.peg.5154	410	98	yes
Pseudomonas aeruginosa strain PASGNDM586	287.3889	fig|287.3889.peg.2848	410	98	yes
Pseudomonas aeruginosa strain PASGNDM591	287.3890	fig|287.3890.peg.229	410	98	yes

Pseudomonas aeruginosa strain PASGNDM590	287.3891	fig|287.3891.peg.6565	410	98	yes
Pseudomonas aeruginosa strain PASGNDM593	287.3892	fig|287.3892.peg.6239	410	98	yes
Pseudomonas aeruginosa strain W1j	287.4346	fig|287.4346.peg.3848	414	100	yes
Pseudomonas aeruginosa strain U1o-2	287.4351	fig|287.4351.peg.5249	414	100	yes
Pseudomonas aeruginosa strain U1i	287.4352	fig|287.4352.peg.119	414	100	yes
Pseudomonas aeruginosa strain U1j-3	287.4357	fig|287.4357.peg.524	410	98	yes
Pseudomonas aeruginosa strain env065a	287.4422	fig|287.4422.peg.614	410	98	yes
Pseudomonas aeruginosa strain env408	287.4426	fig|287.4426.peg.6260	414	100	yes
Pseudomonas aeruginosa strain env187	287.4449	fig|287.4449.peg.764	414	100	yes
Pseudomonas aeruginosa strain env113b	287.4453	fig|287.4453.peg.298	410	98	yes
Pseudomonas aeruginosa strain L10	287.4463	fig|287.4463.peg.3228	414	100	yes
Pseudomonas aeruginosa strain N002	1194669.3	fig|1194669.3.peg.1295	414	100	yes

Table S2. List of primers, strains and plasmids used in this study.

A. Strains and plasmids

	Description	**Reference**
Bacteria and yeast strains		
E. coli DH5a	F^- Φ80*lac*ZΔM15 Δ(*lac*ZYA-*arg*F) U169 *rec*A1 *end*A1 *hsd*R17(r_k^-, m_k^+) *pho*A *sup*E44 *thi*-1 *gyr*A96 *rel*A1 λ^-	Invitrogen
E. coli BLR	*recA*$^-$: F^- *ompT hsdS*$_B$(r_B^- m_B^-) *gal dcm* Δ(srl-recA)306::Tn*10* (Tet^R)	Novagen
E. coli BL21 DE3	F^- *ompT hsdS*$_B$(r_B^- m_B^-) *gal dcm* (DE3)	Novagen
P. aeruginosa UCBPP-PA14	A strain from international reference panel of *P. aeruginosa* isolates	(1)
S. cerevisiae MH272-3fα	"Wild-type" strain*, ura3, leu2, his3, trp1, ade2*	(2)
S. cerevisiae SC489	*S. cerevisiae* MH272-3fα *act1::*LEU2 + *ACT1*[His3]	(3)
S. cerevisiae SC691	*S. cerevisiae* MH272-3fα *act1::*LEU2 + *ACT1* D25N[His3]	(3)
S. cerevisiae Y70	*S. cerevisiae* MH272-3fα + Vector[Ade2] (YEpGal555)	(4)
S. cerevisiae Y213	*S. cerevisiae* MH272-3fα + ExoY C-9 deletion[Ade2] (p1652)	This study
S. cerevisiae Y214	*S. cerevisiae* MH272-3fα + ExoY[Ade2] (p1653)	(4)
S. cerevisiae Y215	*S. cerevisiae* MH272-3fα + ExoY C-3 deletion[Ade2] (p158)	This study
S. cerevisiae Y216	*S. cerevisiae* MH272-3fα + ExoY C-5 deletion[Ade2] (p159)	This study
S. cerevisiae Y217	*S. cerevisiae* MH272-3fα + ExoY C-7 deletion[Ade2] (p160)	This study
S. cerevisiae Y235	*S. cerevisiae* MH272-3fα + ExoY N-10 deletion[Ade2] (pB193)	This study
S. cerevisiae Y236	*S. cerevisiae* MH272-3fα + ExoY N-20 deletion[Ade2] (pB194)	This study
S. cerevisiae Y237	*S. cerevisiae* MH272-3fα + ExoY N-30 deletion[Ade2] (pB195)	This study
S. cerevisiae Y252	*S. cerevisiae* MH272-3fα + ExoY L371A[Ade2] (p1664)	This study
S. cerevisiae Y253	*S. cerevisiae* MH272-3fα + ExoY D372[Ade2] (p1665)	This study
S. cerevisiae Y254	*S. cerevisiae* MH272-3fα + ExoY F373A[Ade2] (p1666)	This study
S. cerevisiae Y255	*S. cerevisiae* MH272-3fα + ExoY F374A[Ade2] (p1667)	This study
S. cerevisiae Y256	*S. cerevisiae* MH272-3fα + ExoY Q375A[Ade2] (p1668)	This study
S. cerevisiae Y257	*S. cerevisiae* MH272-3fα + ExoY R376A[Ade2] (p1669)	This study
S. cerevisiae Y258	*S. cerevisiae* MH272-3fα + ExoY K377A[Ade2] (p1670)	This study
S. cerevisiae Y259	*S. cerevisiae* MH272-3fα + ExoY V378A[Ade2] (p1671)	This study
S. cerevisiae Y260	*S. cerevisiae* MH272-3fα + ExoY L371A/D372A[Ade2] (pB187)	This study
S. cerevisiae Y261	*S. cerevisiae* MH272-3fα + ExoY D372A/F373A[Ade2] (pB188)	This study
S. cerevisiae Y262	*S. cerevisiae* MH272-3fα + ExoY F373A/F374A[Ade2] (pB189)	This study
S. cerevisiae Y263	*S. cerevisiae* MH272-3fα + ExoY UCBPP-PA14 [Ade2] (pB198)	This study
S. cerevisiae Y276	*S. cerevisiae* MH272-3fα + ExoY E369A[Ade2] (pB216)	This study
S. cerevisiae Y277	*S. cerevisiae* MH272-3fα + ExoY Q368A[Ade2] (pB217)	This study
S. cerevisiae Y278	*S. cerevisiae* MH272-3fα + ExoY F367A[Ade2] (pB218)	This study
S. cerevisiae Y279	*S. cerevisiae* MH272-3fα + ExoY S366A[Ade2] (pB219)	This study
S. cerevisiae Y280	*S. cerevisiae* MH272-3fα + ExoY E369G[Ade2] (pB220)	This study
S. cerevisiae Y281	*S. cerevisiae* MH272-3fα + ExoY A370G[Ade2] (pB221)	This study
S. cerevisiae Y282	*S. cerevisiae* MH272-3fα + ExoY L371G[Ade2] (pB222)	This study
S. cerevisiae Y283	*S. cerevisiae* MH272-3fα + ExoY A370P[Ade2] (pB223)	This study
S. cerevisiae Y284	*S. cerevisiae* MH272-3fα *act1::*LEU2 + *ACT1*[His3] + GFP[Ade2] (pB233)	This study
S. cerevisiae Y285	*S. cerevisiae* MH272-3fα *act1::*LEU2 + *ACT1*[His3] + GFP-ExoY K81M [Ade2] (pB234)	This study
S. cerevisiae Y288	*S. cerevisiae* MH272-3fα *act1::*LEU2 + *ACT1* D25N[His3] + GFP-ExoY K81M [Ade2] (pB234)	This study
S. cerevisiae Y302	*S. cerevisiae* MH272-3fα *act1::*LEU2 + *ACT1*[His3] + GFP-ExoY K81M	This study

	C-9 deletion[Ade2] (pB247)	
Plasmids for experiments in *S. cerevisiae*		
YEpGal555	*E. coli*/*S. cerevisiae* shuttle vector [ADE2]	(3)
pEGFP-C1	The plasmid was used for the PCR-amplification of the GFP gene	Clontech
p1593	WT ExoY in YEpGal555	(5)
p1594	ExoY K81M in YEpGal555	(4)
p1652	ExoY with C terminal deletion of 9 amino acids in YEpGal555	This study
p1653	WT ExoY in YEpGal555	This study
p1664	ExoY L371A in YEpGal555	This study
p1665	ExoY D372A in YEpGal555	This study
p1666	ExoY F373A in YEpGal555	This study
p1667	ExoY F374A in YEpGal555	This study
p1668	ExoY Q375A in YEpGal555	This study
p1669	ExoY R376A in YEpGal555	This study
p1670	ExoY K377A in YEpGal555	This study
p1671	ExoY V378A in YEpGal555	This study
pB158	ExoY with C terminal deletion of 3 amino acids in YEpGal555	This study
pB159	ExoY with C terminal deletion of 5 amino acids in YEpGal555	This study
pB160	ExoY with C terminal deletion of 7 amino acids in YEpGal555	This study
pB187	ExoY L371A/D372A in YEpGal555	This study
pB188	ExoY D372A/F373A in YEpGal555	This study
pB189	ExoY F373A/F374A in YEpGal555	This study
pB193	ExoY with N terminal deletion of 10 amino acids in YEpGal555	This study
pB194	ExoY with N terminal deletion of 20 amino acids in YEpGal555	This study
pB195	ExoY with N terminal deletion of 30 amino acids in YEpGal555	This study
pB198	ExoY from *P. aeruginosa* UCBPP-PA14 in YEpGal555	This study
pB216	ExoY E369A in YEpGal555	This study
pB217	ExoY Q368A in YEpGal555	This study
pB218	ExoY F367A in YEpGal555	This study
pB219	ExoY S366A in YEpGal555	This study
pB220	ExoY E369G in YEpGal555	This study
pB221	ExoY A370G in YEpGal555	This study
pB222	ExoY L371G in YEpGal555	This study
pB223	ExoY A370P in YEpGal555	This study
pB233	GFP in YEpGal555	This study
pB234	GFP-ExoY K81M fusion in YEpGal555	This study
pB247	GFP-ExoY K81M fusion with C-terminal deletion of 9 amino acids in YEpGal555	This study
Plasmids for protein expression		
pTRCAG	Expression vector controlled by the temperature sensitive cI repressor (cI857), Amp^R	(6)
pUM460	WT ExoY with C-terminal Flag-6XHis tag in pTRCAG	(3)
pB256	ExoY with 5 amino acids C-terminal deletion and C-terminal Flag-6XHis tag in pTRCAG	This study
pB255	ExoY variant from PA14 strain with C-terminus from WT ExoY and C-terminal Flag-6XHis tag in pTRCAG	This study
pB240	ExoY with 9 amino acids C-terminal deletion and C-terminal Flag-6XHis tag in pTRCAG	This study
pB241	ExoY variant from PA14 strain with C-terminal Flag-6XHis tag in pTRCAG	This study
pB243	ExoY F367A with C-terminal Flag-6XHis tag in pTRCAG	This study
pB244	ExoY L371G with C-terminal Flag-6XHis tag in pTRCAG	This study
pET28a	*E. coli* expression vector with T7-promoter, Kan^R	Novagen
pMAL-c5X	The plasmid was used for the PCR-amplification of the MBP gene	NEB

pB137	6XHis-tag, maltose binding protein and Glycine-serine linker in pET28a	This study
pB138	WT ExoY in pB137	This study
pB141	ExoY S340-V378 in pB137	This study
pB142	ExoY N360-V378 in pB137	This study
pB152	ExoY R2-G41 in pB137	This study
pB153	ExoY A155-S194 in pB137	This study
pB154	ExoY E223-A261 in pB137	This study
pB155	ExoY G299-F339 in pB137	This study
pB156	ExoY V320-W359 in pB137	This study
pB182	ExoY N360-V378 F373C in pB137	This study
pB183	ExoY N360-V378 L371C in pB137	This study
pB184	ExoY N360-V378 +C379 in pB137	This study

B. Primers

Primer ID	**Sequence, 5' to 3'**	**Sense/ Antisense**	**Used for construction(s):**
#1259	CAGCTCGAGCGTATCGACGGTCATCG	S	Numerous
#1283	AAAGTCGCTAGCCTCCTGGAAGCTG	A	p1653
#1284	GGTACCGCTAGCGACCTTACGTTGGAAA	A	p1652, p1664
#1298	ATCTTAGCTAGCGACCTTACGTTGGAAAAAGGCGA	A	p1665
#1299	ATCTTAGCTAGCGACCTTACGTTGGAAAGCGTGG	A	p1666
#1300	ATCTTAGCTAGCGACCTTACGTTGGGCAAAG	A	p1667
#1301	ATCTTAGCTAGCGACCTTACGTGCGAAA	A	p1668
#1302	ATCTTAGCTAGCGACCTTAGCTTGG	A	p1669
#1303	ATCTTAGCTAGCGACCGCACGTTG	A	p1670
#1304	ATCTTAGCTAGCGGCCTTACGT	A	p1671
#1305	CCAGGAGGCGGCCGACTTTTTCC	S	p1664
#1306	GGAAAAAGTCGGCCGCCTCCTGG	A	p1664
#b3	CAGCGAAGCGATGATTTTTG	S	Numerous
#b78	CAGGTTGTCTAACTCCTTCC	S	Numerous
#b167	CCAAGACAGCTAAAGATCCTAGA	S	Numerous
#b223	GACCTCGAGCGTATCGACG	S	Numerous
#b224	TATATAGCTAGCTTGGAAAAAGTCGAGCGC	A	pB158
#b225	TATATAGCTAGCAAAGTCGAGCGCCTCCTG	A	pB159
#b226	TATATAGCTAGCGAGCGCCTCCTGGAAGC	A	pB160
#b260	TCTTAGCTAGCGACCTTACGTTGGAAAAAGGCGGCCGC	A	pB187
#b261	TCTTAGCTAGCGACCTTACGTTGGAAAGCGGCGAG	A	pB188
#b262	TCTTAGCTAGCGACCTTACGTTGGGCAGCGTC	A	pB189
#b266	AGACCTCGAGAACGCAACGGCACA	S	pB193
#b267	AGACCTCGAGCGCCCTGCGGATATG	S	pB194
#b268	AGACCTCGAGCAGGACCTGTTCGATGC	S	pB195
#b273	TATACTCGAGCGTATCGACGGTCATCGTC	S	pB198
#b274	TATAGGTACCGCTTCCCCGCGCC	A	pB198
#b311	TCTTAGCTAGCGACCTTACGTTGGAAAAAGTCGAGCGCCGCCTGGAA	A	pB216
#b312	TCTTAGCTAGCGACCTTACGTTGGAAAAAGTCGAGCGCCTCCGCGAAGCTG	A	pB217
#b313	TCTTAGCTAGCGACCTTACGTTGGAAAAAGTCGAGCGCCTCCTGGGCGCTGGGC	A	pB218
#b314	TCTTAGCTAGCGACCTTACGTTGGAAAAAGTCGAGCGCCTCCTGGAAGGCGGGCCGC	A	pB219
#b315	TCTTAGCTAGCGACCTTACGTTGGAAAAAGTCGAGCGCCCCCTGGAA	A	pB220
#b316	TCTTAGCTAGCGACCTTACGTTGGAAAAAGTCGAGCCCCTCCTG	A	pB221

#b317	TCTTAGCTAGCGACCTTACGTTGGAAAAAGTCGCCCGCCTCC	A	pB222
#b318	TCTTAGCTAGCGACCTTACGTTGGAAAAAGTCGAGCGGCTCCTGG	A	pB223
#b327	TATACTCGAGGTGAGCAAGGGCGAGG	S	pB233, 234
#b328	TATACTCGAGCTTGTACAGCTCGTCCATGC	A	pB233, 234
#b349	TATAGGTACCGACCTTACGTTGGAAAAAGTCGAGCG	A	pB255
#b350	TATAGGTACCAAAGTCGAGCGCCTCCTGGAAGC	A	pB256
#b336	TATAGGTACCCTCCTGGAAGCTGGGCC	A	pB240
#b340	TATACCATGGGCCGTATCGACGGTCATC	A	pB241
#b334	TTCGGTTAGAGCGGATCTTAGGTACCGACCTT	A	pB243, 244
#b190	TATACATATGAAAATCGAAGAAGGTAAACTG	S	pB137
#b191	TATAAAGCTTCAACCAGAGCTCCCGCTCGAGCCCGAATTAGTCTGCGCGTCTT	A	pB137
#b192	TATAGAGCTCTGGTCGTATCGACGGTCATCGTCA	S	pB138
#b193	TATAAAGCTTCAGACCTTACGTTGGAAAAAGTC	A	Numerous
#b196	TATAGAGCTCTGGTTCCTTGCTGGTCGAGTGC	S	pB141
#b197	TATAGAGCTCTGGTAACGTGCCGCTGCGGC	S	Numerous
#b212	TATAGAGCTCTGGTCGTATCGACGGTCATCG	S	pB152
#b213	TATAAAGCTTCACCCCACCCCCTGC	A	pB152
#b214	TATAGAGCTCTGGTGCAGACGGTGTCGGG	S	pB153
#b215	TATAAAGCTTCACGACCGCCCCAGG	A	pB153
#b216	TATAGAGCTCTGGTGAGGCGCATGGCAG	S	pB154
#b217	TATAAAGCTTCAGGCCATATCCTCCCTGC	A	pB154
#b218	TATAGAGCTCTGGTGGCTCCCATATGGGTGA	S	pB155
#b219	TATAAAGCTTCAAAAGCTCTTCCGATCCGC	A	pB155
#b220	TATAGAGCTCTGGTGTCGGAGAAGAGTCCGTTC	S	pB156
#b221	TATAAAGCTTCACCAGTCGGGATGGGC	A	pB156
#b255	ATATAAAGCTTCAGACCTTACGTTGGAAACAGTCGAG	A	pB182
#b256	ATATAAAGCTTCAGACCTTACGTTGGAAAAAGTCGCACGC	A	pB183
#b257	ATATAAAGCTTCAACAGACCTTACGTTGGAAAAAGTC	A	pB184

References for supporting information

1. De Soyza, A., Hall, A. J., Mahenthiralingam, E., Drevinek, P., Kaca, W., Drulis-Kawa, Z., Stoitsova, S. R., Toth, V., Coenye, T., Zlosnik, J. E. A., Burns, J. L., Sa-Correia, I., De Vos, D., Pirnay, J.-P., Kidd, T. J., Reid, D., Manos, J., Klockgether, J., Wiehlmann, L., Tummler, B., McClean, S., and Winstanley, C. (2013) Developing an international Pseudomonas aeruginosa reference panel. *Microbiologyopen*. **2**, 1010–1023
2. Peisker, K., Braun, D., Wolfle, T., Hentschel, J., Funfschilling, U., Fischer, G., Sickmann, A., and Rospert, S. (2008) Ribosome-associated complex binds to ribosomes in close proximity of Rpl31 at the exit of the polypeptide tunnel in yeast. *Mol Biol Cell*. **19**, 5279–5288
3. Belyy, A., Tabakova, I., Lang, A. E., Jank, T., Belyi, Y., and Aktories, K. (2015) Roles of Asp179 and Glu270 in ADP-Ribosylation of Actin by Clostridium perfringens Iota Toxin. *PLoS One*. **10**, e0145708
4. Raoux-Barbot, D., Belyy, A., Worpenberg, L., Montluc, S., Deville, C., Henriot, V., Ladant, D., and Mechold, U. Differential regulation of actin-activated nucleotidyl cyclase virulence factors by filamentous and globular actin. *Submitted*
5. Belyy, A., Raoux-Barbot, D., Saveanu, C., Namane, A., Ogryzko, V., Worpenberg, L., David, V., Henriot, V., Fellous, S., Merrifield, C., Assayag, E., Ladant, D., Renault, L., and Mechold, U. (2016) Actin activates Pseudomonas aeruginosa ExoY nucleotidyl cyclase toxin and ExoY-like effector domains from MARTX toxins. *Nat Commun*. **7**, 13582
6. Gmira, S., Karimova, G., and Ladant, D. (2001) Characterization of recombinant Bordetella pertussis adenylate cyclase toxins carrying passenger proteins. *Res Microbiol*. **152**, 889–900