Table S1: Data collection and refinement statistics for cryo EM structure of LetB, Related to Figure 1.

Data collection	
pixel size (Å):	1.31
electron energy (kV):	300
electron dose (e-/Å/px):	10
total electron dose (e-/Å2):	80
number of frames in each movie:	50
images acquired:	4905
number of particles picked:	731,231
number of particles selected after 2D classification:	534,003
pixel dimension of individual windows:	280 x 280

	Map 1 **Model 1**	**Map 2** **Model 2**
Number of particles:	61,618	57,157
Resolution (Å), Relion:	3.46	3.49
Sphericity of 3DFSC:	0.981	0.980
Map CC:	0.793	0.781
rmsd (bonds):	0.01	0.01
rmsd (angles):	1.28	1.18
All-atom clashscore	3.86	3.96
Ramachandran plot values:		
outliers:	0.00 %	0.00 %
allowed:	2.71 %	2.29 %

favored:	97.29 %	97.71 %
Rotamer outliers:	0.00 %	0.00 %
C-beta deviations:	0	0
Overall score (Molprobity):	1.31	1.25
EMRinger Score:	3.14	2.82
Deposition:		
PDB ID:	6V0C	6V0D
EMDB ID:	EMD-20993	EMD-20994

	Map 3	**Map 4**	**Map 5**
Composite closed model 9:	**Model 3**	**Model 4**	**Model 5**
Number of particles:	101,464	144,219	147,415
Resolution (Å), Relion:	3.06	2.96	3.03
Sphericity of 3DFSC:	0.978	0.989	0.990
Map CC:	0.796	0.800	0.806
rmsd (bonds):	0.01	0.01	0.01
rmsd (angles):	1.24	1.17	1.16
All-atom clashscore	5.69	4.35	6.08
Ramachandran plot values:			
outliers:	0.00 %	0.00 %	0.00 %
allowed:	5.31 %	1.43 %	2.40 %
favored:	94.69 %	98.57 %	97.60 %
Rotamer outliers:	0.00 %	0.00 %	0.00 %
C-beta deviations:	0	0	0
Overall score (Molprobity):	1.68	1.21	1.42

EMRinger Score:	3.32	2.98	3.01
Deposition:			
PDB ID:	6V0E	6V0F	6V0G
EMDB ID:	EMD-20995	EMD-20996	EMD-20997
	Map 6	**Map 7**	**Map 8**
Composite closed model 10:	**Model 6**	**Model 7**	**Model 8**
Number of particles:	71,235	59,687	21,149
Resolution (Å), Relion:	3.60	3.43	3.78
Sphericity of 3DFSC:	0.986	0.958	0.967
Map CC:	0.722	0.789	0.814
rmsd (bonds):	0.01	0.01	0.01
rmsd (angles):	1.16	1.33	1.19
All-atom clashscore	4.20	4.38	5.14
Ramachandran plot values:			
outliers:	0.00 %	0.00 %	0.25 %
allowed:	4.25 %	1.15 %	5.76 %
favored:	95.75 %	98.85 %	93.99 %
Rotamer outliers:	0.00 %	0.00 %	0.00 %
C-beta deviations:	0	0	0
Overall score (Molprobity):	1.50	1.22	1.68
EMRinger Score:	2.34	1.22	1.68
Deposition:			
PDB ID:	6V0H	6V0I	6V0J
EMDB ID:	EMD-20998	EMD-20999	EMD-21000

Table S2: Pairwise percent identity between all MCE domains (from ClustalO), Related to Figure 1.

		residues	LetB							PqiB			MlaD
			MCE 1	MCE 2	MCE 3	MCE 4	MCE 5	MCE 6	MCE 7	MCE 1	MCE 2	MCE 3	MCE 1
LetB	MCE1	46-149	100	19.4	29.7	17.4	20.0	28.4	24.3	37.5	23.3	18.9	21.4
	MCE2	158-272		100	14.6	28.4	28.8	15.4	32.2	19.1	29.6	17.5	17.4
	MCE3	279-382			100	16.5	15.2	29.7	24.3	19.4	19.4	12.1	14.8
	MCE4	391-499				100	21.9	17.4	26.6	18.2	18.4	13.2	24.2
	MCE5	513-625					100	16.0	24.6	20.8	29.0	15.8	17.7
	MCE6	634-737						100	20.2	26.9	18.3	11.1	11.4
	MCE7	746-862							100	21.9	33.1	21.2	20.4
PqiB	MCE1	42-147								100	21.9	12.1	13.5
	MCE2	156-273									100	23.2	21.4
	MCE3	284-412										100	18.9
MlaD	MCE1	38-139											100
		min	*17.4*	*14.6*	*12.1*	*13.2*	*15.2*	*11.1*	*20.2*	*12.1*	*18.3*	*11.1*	*11.4*
		avg	*24.0*	*22.2*	*19.6*	*20.2*	*21.0*	*19.5*	*24.9*	*21.1*	*23.7*	*16.4*	*18.1*
		max	*37.5*	*32.2*	*29.7*	*28.4*	*29.0*	*29.7*	*33.1*	*37.5*	*33.1*	*23.2*	*24.2*

Table S3: Data collection and refinement statistics of LetB MCE2-MCE3 crystal structure, Related to Figure S5 and Figure 4.

Data collection	
Space group:	**$P6_5$**
Cell dimensions:	
a, b, c (Å):	87.56, 87.56, 116.99
α, β, ɣ (°):	90, 90, 120
Resolution (Å):	46.32-2.15 (2.21-2.15)[1]
Wavelength (Å):	1.0000
Observations:	259,635
Unique Reflections:	27,654
Redundancy:	9.4 (4.8)
Completeness (%):	99.8 (97.4)
CC1/2:	1.00 (0.62)
I/σI:/	21.3 (1.2)
R_{meas}:	0.05 (1.26)
Refinement	
Resolution (Å):	41.0 - 2.15
Reflections (work):	26,145
Reflections (free):	1,321
R_{work} / R_{free} (%):	21.4 / 24.4
No. atoms:	
Protein:	3,263
Water:	48
Other:	0

Mean B-factor:	
Protein:	84.1
Water:	61.8
R.M.S. Deviations:	
Bond lengths (Å):	0.003
Bond angles (°):	0.56
Ramachandran plot:	
Favored:	97.2 %
Outliers:	0.2 %
Rotamer outliers:	0.56 %
Molprobity:	
Molprobity score:	1.17
Percentile:	100th
All-atom clashscore:	2.44
Percentile:	99th
PDB ID:	6VCI

[1] Values in parentheses are for highest-resolution shell.