

**Table S3.** Normalized activities of AP and BG WaaL truncation fusions to PhoALacZ $\alpha$ 

Residue <sup>a</sup>	Avg AP <sup>b</sup>	Avg BG <sup>c</sup>	%AP <sup>d</sup>	%BG <sup>e</sup>	NAR <sup>f</sup> (%AP÷%BG)	Colony Colour <sup>g</sup>	Localization <sup>h</sup>
<i>Random</i>							
R40	170.9	0.0	90.5	0.1	>100	Blue	P
R64	-4.1	4.7	-2.2	14.9	<0.01	Red	C
M86	189.0	-1.6	100.0	-5.0	>100	Blue	P
L135	125.2	-1.3	66.3	-4.0	>100	Blue	P
L157	-1.8	5.6	-1.0	17.8	<0.01	Red	C
S169	-2.7	5.8	-1.4	18.6	<0.01	Red	C
S198	68.5	2.4	36.3	7.8	4.64	Purple	TM
L199	63.6	1.2	33.7	3.9	8.55	Purple	TM
L206	56.1	0.5	29.7	1.7	17.24	Blue	P
A208	78.3	2.0	41.4	6.4	6.46	Purple	TM
L225	10.8	2.4	5.7	7.8	0.73	Purple	TM
G229	65.0	1.3	34.4	4.2	8.15	Purple	TM
K233	15.4	2.8	8.2	9.0	0.90	Purple	TM
I256	31.6	3.1	16.7	10.0	1.67	Purple	TM
R259	162.0	-2.4	85.7	-7.7	>100	Blue	P
S262	110.0	-1.0	58.2	-3.3	>100	Blue	P
W280	64.8	-1.6	34.3	-5.0	>100	Blue	P
P302	38.9	-1.2	20.6	-3.9	>100	Blue	P
L307	34.3	4.2	18.2	13.4	1.36	Purple	TM
K334	-1.1	2.8	-0.6	8.9	<0.01	Red	C
G359	174.8	-4.7	92.5	-15.1	>100	Blue	P
D397	-3.6	31.3	-1.9	100	<0.01	Red	C
<i>Targeted</i>							
A178	-4.2	5.5	-7.2	14.7	<0.01	Red	C
W180	-2.4	2.4	-4.1	6.4	<0.01	Red	C
F371	58.8	23.0	100.0	61.6	1.62	Purple	TM
P401	-3.5	37.3	-6.0	100.0	<0.01	Red	C

As purpose-built truncations had higher absolute activities than random truncations, the two sets were normalized independently against the maximum activity within the respective subset of residues.

<sup>a</sup> Position of the terminal amino acid of WaaL followed by reporter.

<sup>b</sup> and <sup>c</sup> AP and BG activities of the fusions in Miller units, measured as described in Materials and Methods; average of four independent experiments.

<sup>d</sup> and <sup>e</sup> Percentage of AP and BG activities of the fusions in relation to the maximum measured activity within the set, calculated separately for targeted and random truncations.

<sup>f</sup> Normalized %AP ÷ %BG activity ratio (NAR), rounded to two decimal places.

<sup>g</sup> Colony colour of expressed clone on dual-indicator plate.

<sup>h</sup> Localization of terminal amino acid on the topology map: periplasm (P), cytoplasm (C), transmembrane (TM).