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SNVQICTS
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Figure S1. Sequence of wild-type ROL.

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Figure S2. Vector sequence of pFAi2-GAPp-ROL.

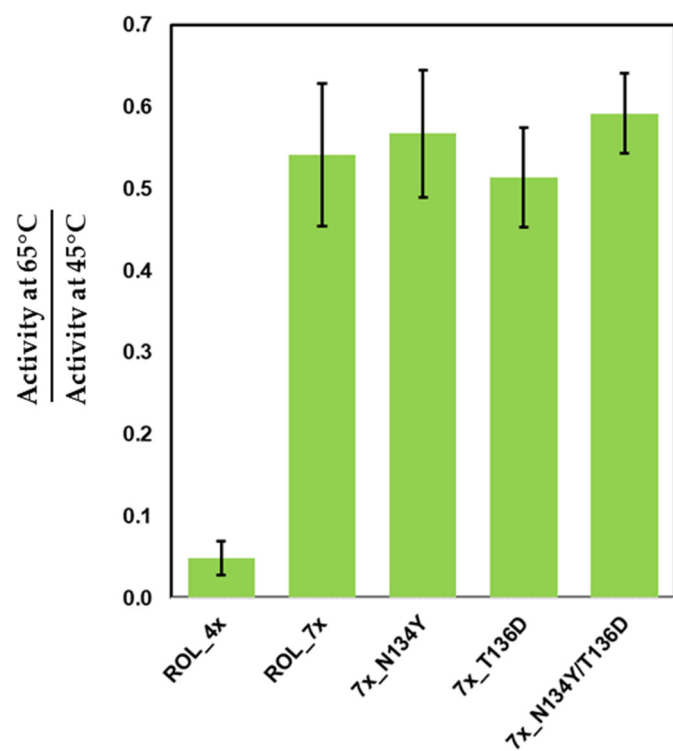


Figure S3. DTNB assay to compare the thermostability of ROL_4x, ROL_7x and ROL_7x mutants.

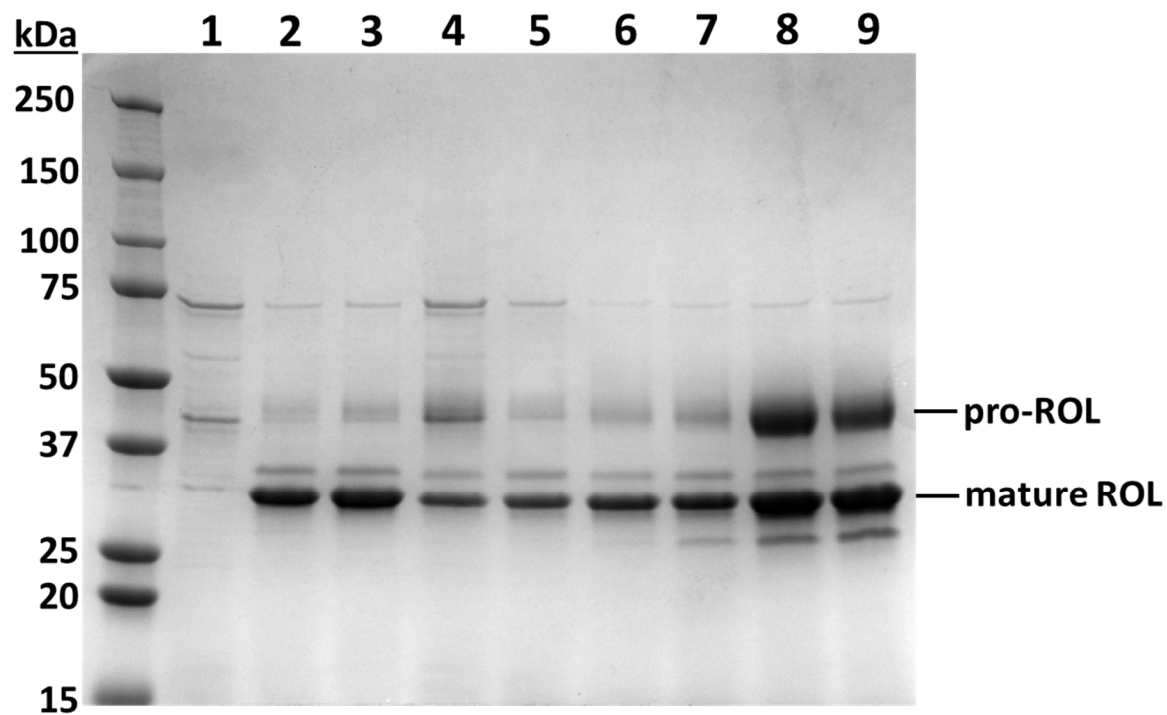


Figure S4. SDS-PAGE analysis of the ROL mutants expressed from *P.pastoris*. (1) Negative Control. (2,3) Wild-type ROL. (4,5) ROL_4x. (6,7) ROL_7x. (8,9) ROL_10x.

Table S1. Relative activity and fold improvement of the single mutants of ROL_4x measured at 60°C and 45°C.

Mutant	Relative activity (60°C/45°C)	Fold Improvement
ROL_4x	24%	1.0
ROL_4x_A8E	38%	1.6
ROL_4x_S31D	22%	0.9
ROL_4x_S114N	33%	1.4
ROL_4x_N134Y	52%	2.2
ROL_4x_T136D	43%	1.8
ROL_4x_P163K	34%	1.4
ROL_4x_F173Y	43%	1.8
ROL_4x_Y187N	30%	1.2
ROL_4x_G193K	30%	1.2
ROL_4x_Q197H	55%	2.3
ROL_4x_Q197Y	47%	1.9
ROL_4x_G228D	34%	1.4
ROL_4x_G228S	22%	0.9
ROL_4x_S267L	57%	2.4

Table S2. Relative activity and fold improvement of the single mutants of ROL_4x and ROL_7x measured at 65°C and 45°C.

Mutant	Relative activity (65°C/45°C)	Fold Improvement
ROL_4x	6%	1.0
ROL_4x_N134Y	16%	2.8
ROL_4x_T136D	3%	0.6
ROL_4x_F173Y	5%	0.9
ROL_4x_Q197H	9%	1.6
ROL_4x_Q197Y	3%	0.5
ROL_4x_S267L	13%	2.3
ROL_7x	45%	8.0

Table S3. Relative activity and fold improvement of the single mutants of ROL_7x measured at 67°C and 45°C.

Mutant	Relative activity (67°C/45°C)	Fold Improvement
ROL_7x	19%	1.0
ROL_7x_A7R	32%	1.7
ROL_7x_L113Y	26%	1.3
ROL_7x_Q150F	3%	0.2
ROL_7x_Q150H	4%	0.2
ROL_7x_G155A	15%	0.8
ROL_7x_S171F	16%	0.8
ROL_7x_V175F	81%	4.2
ROL_7x_G177C	125%	6.4
ROL_7x_T199V	24%	1.2
ROL_7x_F216Y	52%	2.7
ROL_7x_S223Y	4%	0.2
ROL_7x_F261Y	3%	0.2

Table S4. Relative activity and fold improvement of the single mutants of ROL_7x and ROL_10x measured at 70°C and 45°C.

Mutant	Relative activity (70°C/45°C)	Fold Improvement
ROL_7x	2%	1.0
ROL_7x_V175F	10%	4.5
ROL_7x_G177C	14%	6.1
ROL_7x_F216Y	3%	1.2
ROL_10x	121%	54.4