

1 **Insight into the substrate specificity of keratinase KerSMD from**
2 ***Stenotrophomonas maltophilia* by site-directed mutagenesis studies in S1 pocket**
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24 **Table S1.** Nucleotide sequences of primers used for site-directed mutagenesis.

Mutagenic site	Primer sequence (5'-3')
S180G	ATGAGCCTGGGTGG <u>GAGG</u> CGGCAGCTGTGAC
E208S	GCGGCCGGCAACT <u>TCGAC</u> CGACAACGCTT
E208N	GCGGCCGGCAACA <u>AACAC</u> CGACAACGCTT
Tyr215 saturation mutagenesis	ACAACGCTTCCAAG <u>NNK</u> CGTCCGGCCAGTTG
R216W	ACGCTTCCAAGTACT <u>TGGC</u> CGGCCAGTTGC
R216Y	ACGCTTCCAAGTACT <u>TACCC</u> GGGCCAGTTGC

25 ^a Nucleotide sequences corresponding to the mutated amino acids are underlined.

26 ^b N represents A, T, G, or C, and K represents G or T.

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38 **Fig. S1.** The SDS-PAGE of the wild type (KerSMD) and its single site mutants. The
39 10% (w/v) gel of SDS-PAGE was used. The concentration of protein sample was 2 μ g.
40 Before uploaded to gel, all samples were heated at 98 °C for 8 min with 5 \times loading
41 buffer (Sangong, Shanghai). After electrophoresis, protein was stained with
42 Coomassie Brilliant Blue R250 and faded with 20% (v/v) acetic acid.

