Mutagenesis of a modular polyketide synthase enoylreductase domain reveals insights into catalysis and stereospecificity

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Supporting Information

Table S1: Primers used

Primer description	Sequence (3′ to 5′)
$RapER_{13}$ N41′A forward primer	CGCCGCAGGCCTGGCCTTCCGCGACGTCG
$RapER_{13}$ N41′A reverse primer	CGACGTCGCGGAAGGCCAGGCCTGCGGCG
$RapER_{13}$ N41′D forward primer	CGCCGCAGGCCTGGACTTCCGCGACGTCG
$RapER_{13}$ N41′D reverse primer	CGACGTCGCGGAAGTCCAGGCCTGCGGCG
$RapER_{13}$ N41′T forward primer	CGCCGCAGGCCTGACCTTCCGCGACGTCG
$RapER_{13}$ N41′T reverse primer	CGACGTCGCGGAAGGTCAGGCCTGCGGCG
$RapER_{13}$ D43′A forward primer	AGGCCTGAACTTCCGCGCCGTCGTCGTGGCCC
$RapER_{13}$ D43′A reverse primer	GGGCCACGACGACGGCGCGGAAGTTCAGGCCT
$RapER_{13}$ D43′N forward primer	AGGCCTGAACTTCCGCAACGTCGTCGTGGCCC
$RapER_{13}$ D43′N reverse primer	GGGCCACGACGACGTTGCGGAAGTTCAGGCCT
$RapER_{13}$ V46′L+V47′I+V52′Y forward primer	AACTTCCGCGACGTCCTCATCGCCCTCGGCATGTAC
$RapER_{13}$ V46′L+V47′I+V52′Y reverse primer	GTACATGCCGAGGGCGATGAGGACGTCGCGGAAGTT
$RapER_{13}$ V52′Y forward primer	CGTGGCCCTCGGCATGTACAATGACAACCGGCCG
$RapER_{13}$ V52′Y reverse primer	CGGCCGCTTGTCATTGTACATGCCGAGGGCCACG
$RapER_{13}$ V52′Y+N53'P forward primer	GTGGCCCTCGGCATGTACCCTGACAACCGGCCGAC
$RapER_{13}$ V52′Y+N53'P reverse primer	GTCGGCCGGTTGTCAGGGTACATGCCGAGGGCCAC
$RapER_{13}$ T122′V forward primer	CCGGTTGTGTTCGCCGTCGCGTACTACGGGCT
$RapER_{13}$ T122′V reverse primer	AGCCCGTAGTACGCGACGGCGAACACAACCGG
$RapER_{13}$ T122′V+Y125′F forward primer	GTTGTGTTCGCCGTCGCGTACTTCGGGCTGGTGGA
$RapER_{13}$ T122′V+Y125′F reverse primer	TCCACCAGCCCGAAGTACGCGACGGCGAACACAAC
$RapER_{13}$ Y124′F forward primer	TCGCCACCGCGTTCTACGGGCTGGTGGATCT
$RapER_{13}$ Y124′F reverse primer	AGATCCACCAGCCCGTAGAACGCGGTGGCGA
$RapER_{13}$ Y124′F+Y125′F forward primer	TCGCCACCGCGTTCTTCGGGCTGGTGGATCT
$RapER_{13}$ Y124′F+Y125′F reverse primer	AGATCCACCAGCCCGAAGAACGCGGTGGCGA
$RapER_{13}$ Y125′F forward primer	TCGCCACCGCGTACTTCGGGCTGGTGGATCT
$RapER_{13}$ Y125′F reverse primer	AGATCCACCAGCCCGAAGTACGCGGTGGCGA
$RapER_{13}$ K236′A forward primer	TTCGTCGAGATGGGCGCGACCGACATCCGTGAC
$RapER_{13}$ K236′A reverse primer	GTCACGGATGTCGGTCGCGCCCATCTCGACGAA
$RapER_{13}$ forward PCR/sequencing primer	CGGATCAACGACGGCCGGTACGAG
$RapER_{13}$ reverse PCR/sequencing primer	CTGGCGGATGTCCCAGGCCTGCAC

Table S2: Plasmids constructed

Plasmid	Marker	Description
pDK28	Apr^R	pDK19.0-based plasmid encoding V52′Y+N53′P TKS-rap13 mutant
pDK29	Apr^R	pDK19.0-based plasmid encoding N41′D TKS-rap13 mutant
pDK30	Apr^R	pDK19.0-based plasmid encoding N41′A TKS-rap13 mutant
pDK31	Apr^R	pDK19.0-based plasmid encoding N41′T TKS-rap13 mutant
pDK32	Apr^R	pDK19.0-based plasmid encoding Y125′F TKS-rap13 mutant
pDK34	Apr^R	pDK19.0-based plasmid encoding D43′N TKS-rap13 mutant
pDK35	Apr^R	pDK19.0-based plasmid encoding D43′A TKS-rap13 mutant
pDK36	Apr^R	pDK19.0-based plasmid encoding D43′T TKS-rap13 mutant
pDK37	Apr^R	pDK19.0-based plasmid encoding Y124′F TKS-rap13 mutant
pDK38	Apr^R	pDK19.0-based plasmid encoding Y124′F+Y125′F TKS-rap13 mutant
pDK39	Apr^R	pDK19.0-based plasmid encoding T122′V TKS-rap13 mutant
pDK40	Apr^R	pDK19.0-based plasmid encoding T122′V+Y125′F TKS-rap13 mutant
pDK41	Apr^R	pDK19.0-based plasmid encoding K236′A TKS-rap13 mutant
pDK42	Apr^R	pDK19.0-based plasmid encoding V46′L+V47′I+V52′Y+N53′P TKS-rap13 mutant

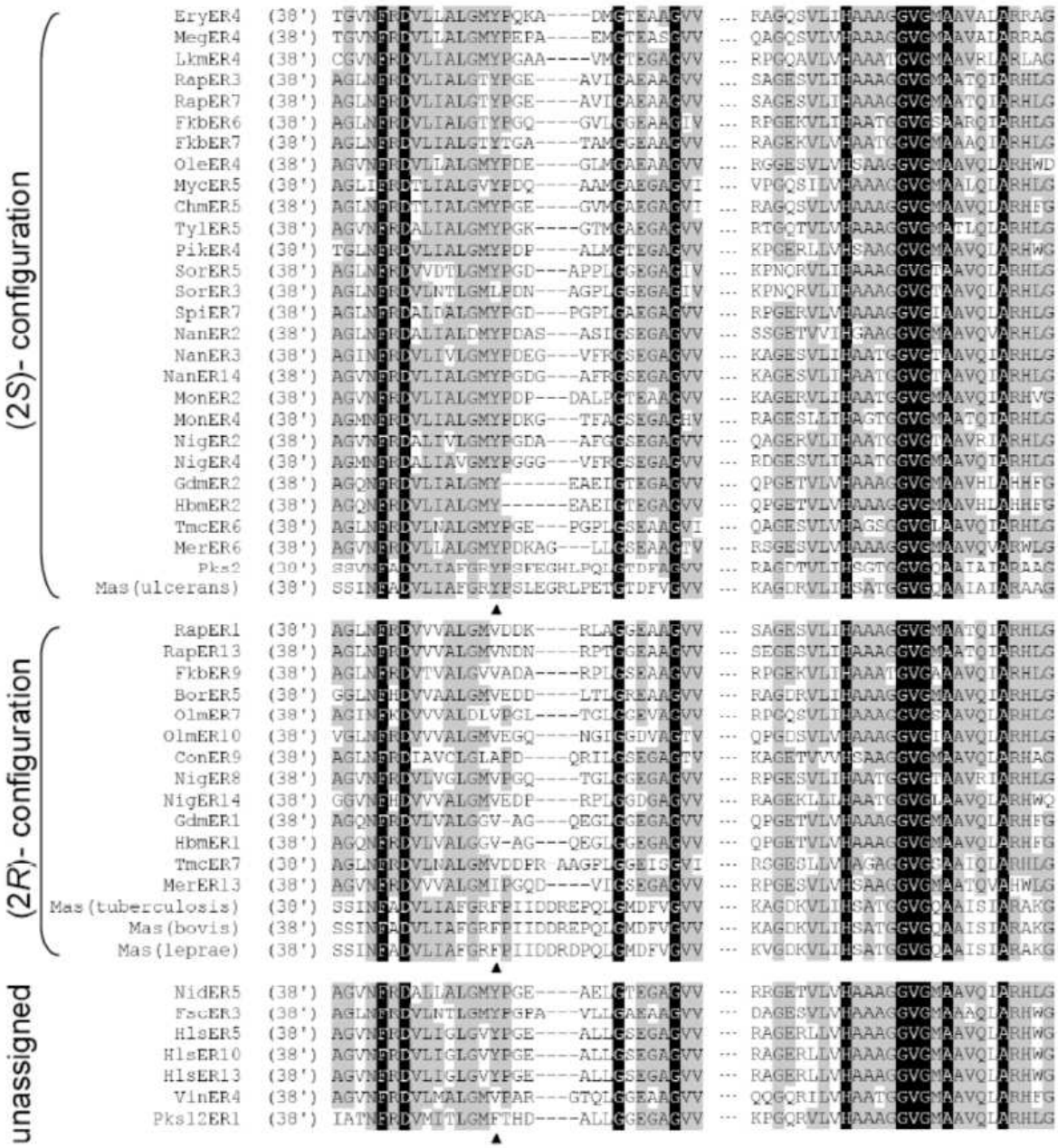

Figure S1: Sequence alignments of ER domains which act on branched extender units

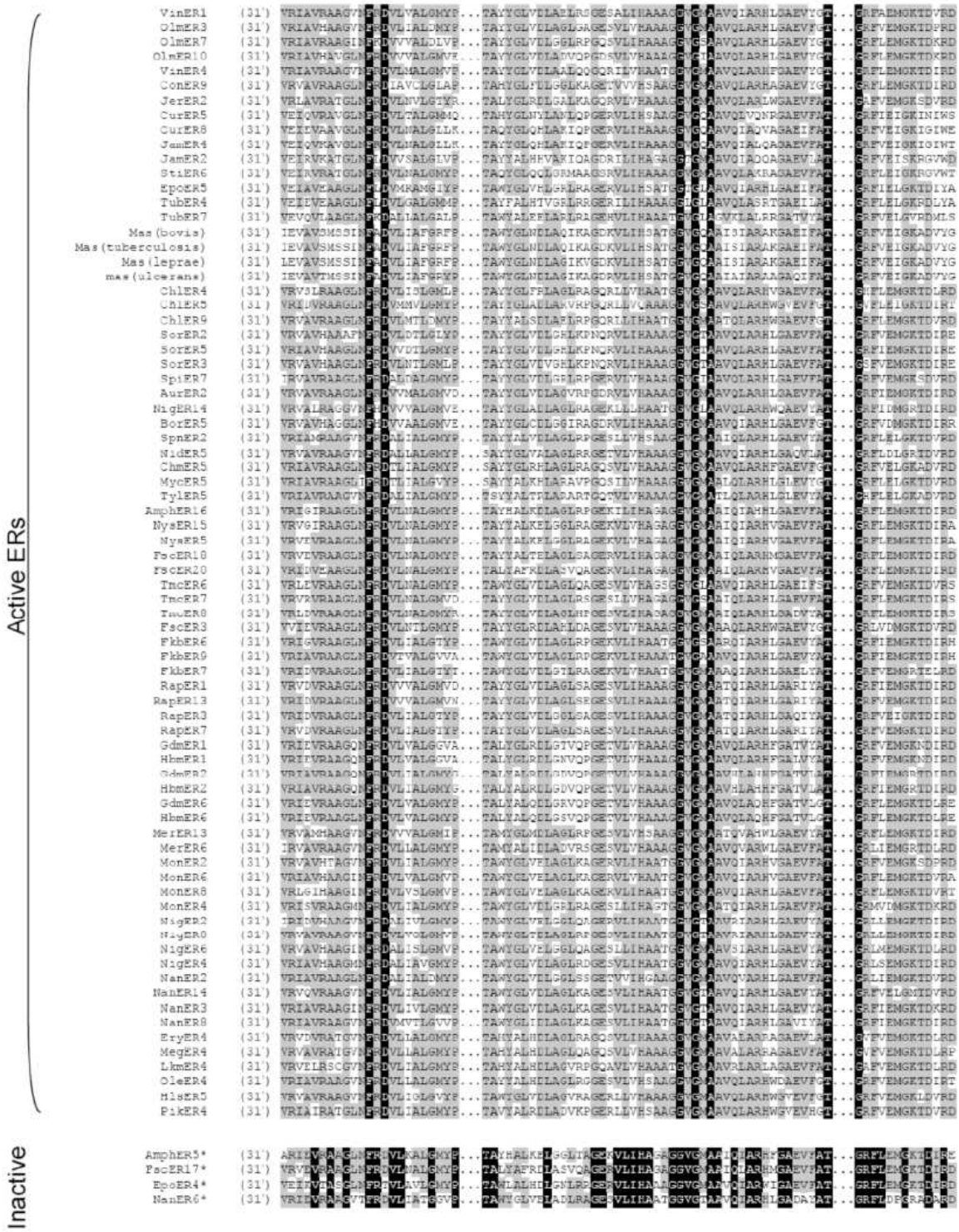

Figure S2: Sequence alignments of active and inactive ERs