TABLE S1 Sequences of oligonucleotides used for site-directed mutagenesis.

Mutations	Oligonucleotide sequences
T49I	5'-tgattgcctttccggaaatctggattcccggatac-3'
	5'-gtatccgggaatccagatttccggaaaggcaatca-3'
Y85F	5'-gtcatcaacagcgagcaattccgtctcatcgaac-3'
	5'-gttcgatgagacggaattgctcgctgttgatgac-3'
A91S	5'-gcaataccgtctcatcgaacaatcagcagctcgca-3'
	5'-tgcgagctgctgattgttcgatgagacggtattgc-3'
M98K	5'-gcagctcgcaacaagatcaaggtggtgctg-3'
	5'-cagcaccaccttgatcttgttgcgagctgc-3'
I113N	5'-gccggctcgttgtacaactcccaatcgatcatc-3'
	5'-gatgatcgattgggagttgtacaacgagccggc-3'
Y199C	5'-cggcgcagcctgtacgctcggcc-3'
	5'-ggccgagcgtacaggctgcgccg-3'
T310P	5'-gctgctagacccccccgcccaaatctcg-3'
	5'-cgagatttgggcgggggggtctagcagc-3'
R345C	5'-gagccgctcgagtgcgtcacacccg-3'
	5'-cgggtgtgacgcactcgagcggctc-3'

TABLE S2 Analytical conditions for mandelic acid and its derivatives.

Acid Product	Column	Mobile Phase	Flow Speed (mL/min)	Retention Time (min)	
				R	*S*
mandelic acid	CHIRALEL-OJ-H 4.6×150 mm, 5μm	10% 2-propanol; 90% hexane (0.1% TFA)	1.0	19.3	22.7
2-F-mandelic acid	CHIRALEL-OJ-H 4.6×150 mm, 5μm	10% 2-propanol; 90% hexane (0.1% TFA)	1.0	15.8	19.5
2-Cl-mandelic acid	CHIRALEL-OZ-RH 4.6×150 mm, 5 μm	10% 2-propanol; 90% water (0.05% TFA)	0.5	19.0(*S*)	23.8(*R*)
2-Br-mandelic acid	CHIRALEL-OJ-H 4.6×150 mm, 5μm	10% 2-propanol; 90% hexane (0.1% TFA)	1.0	21.3	28.4
2-CH_3-mandelic acid	CHIRALEL-OJ-H 4.6×150 mm, 5μm	10% 2-propanol; 90% hexane (0.1% TFA)	1.0	16.8	24.0
2-OCH_3-mandelic acid	CHIRALEL-OJ-H 4.6×150 mm, 5μm	10% 2-propanol; 90% hexane (0.1% TFA)	1.0	31.4	34.7
3-F-mandelic acid	CHIRALEL-OJ-H 4.6×150 mm, 5μm	5% 2-propanol; 95% hexane (0.1% TFA)	1.0	21.5	26.5
3-Cl-mandelic acid	CHIRALEL-OJ-H 4.6×150 mm, 5μm	5% 2-propanol; 95% hexane (0.1% TFA)	1.0	19.0	25.0
3-Br-mandelic acid	CHIRALEL-OJ-H 4.6×150 mm, 5μm	5% 2-propanol; 95% hexane (0.1% TFA)	1.0	20.3	26.4
4-F-mandelic acid	CHIRALEL-OJ-H 4.6×150 mm, 5μm	5% 2-propanol; 95% hexane (0.1% TFA)	1.0	25.0	28.0
4-Cl-mandelic acid	Chirobiotic R 4.6×100 mm, 5μm	50% methanol;50% water (0.1% HAC)	0.5	1.77(*S*)	2.18(*R*)
4-Br-mandelic acid	Chirobiotic R 4.6×100 mm, 5μm	50% methanol;50% water (0.1% HAC)	0.5	4.17(*S*)	5.25(*R*)

TABLE S3 The enantioselectivity and relative activity of the BCJ2315 mutants based on random mutagenesis and site-directed mutagenesis.

Mutants	*ee* (%) (mutant)	Relative activity(%) (mutant)	Mutations	*ee*(%) (mutations)	Relative activity(%) (mutations)
WT	89.2%	100	-	-	-
M18	95.9±0.2	87±5	T49S	95.9±0.2	87±5
M338	98.3±0.2	82±4	A91S	89.2±0.4	98±4
			I113N	98.4±0.1	81±4
M101	99.3±0.3	75±5	M98K	89.3±0.3	102±3
			Y199C	94.3±0.4	201±5
			T310P	98.1±0.2	33±6
			R345C	90.1±0.3	99±3
M1332	99.3±0.2	72±7	T49I	95.9±0.3	78±6
			Y85F	88.9±0.5	102±3
			I113N	98.4±0.1	81±4

TABLE S4 The enantioselectivity and relative activity of the mutants based on site-saturation mutagenesis at selected four 'hot spots'.

Mutations	49		113		199		310	
	RA[a](%)	*ee*(%)	RA[a](%)	*ee*(%)	RA[a](%)	*ee*(%)	RA[a](%)	*ee*(%)
A	84±3	96.3±0.4	102±2	94.1±0.7	273±12	95.6±0.8	104±4	89.5±1.2
C	47±7	88.3±0.3	75±4	80.9±0.4	231±15	94.3±0.4	117±6	88.7±1.8
D	52±7	92.8±0.5	2±1	>99.9	222±12	94±0.3	103±2	90.3±0.9
E	26±4	99.0±0.2	23.9±1	90.6±0.4	191±15	95.1±0.4	118±7	90.1±0.8
F	24±5	97.1±0.4	45±4	93.7±0.8	105±5	93.4±0.9	103±8	89.3±0.4
G	72±4	97.3±0.7	6±3	>99.9	306±17	96.7±0.4	89±4	90.2±0.4
H	80±5	88.8±0.6	2±1	89.7±0.8	114±8	89.1±0.3	280±13	89.7±0.4
I	83±6	95.6±0.3	100	89.2±0.3	117±5	91.4±0.4	126±11	88.6±0.5
K	16±8	94.8±0.5	1±1	>99.9	148±9	93.1±0.3	103±7	89.2±0.5
L	98±3	92.8±0.7	188±11	77.1±1.2	177±7	92.3±0.4	132±9	89.9±0.7
M	46±3	97.2±0.2	138±7	97.2±0.3	145±6	92.3±1.3	116±3	89.1±1.2
N	105±2	76.1±0.8	74±5	98.4±0.3	140±7	90.9±0.5	100±3	88.9±0.4
P	4±2	87.1±0.9	61±9	93.2±0.2	179±9	92.3±0.5	38±8	98.1±0.2
Q	96±5	93.7±0.3	29.7±3	96.2±0.3	230±11	93.8±0.7	99±4	89.5±1.1
R	55±9	90.2±0.2	1±1	95.8±0.2	217±6	89.1±0.3	17±9	92.5±0.5
S	79±7	94.8±0.2	2±1	>99.9	324±13	93.5±0.4	125±3	88.2±1.1
T	100	89.2±0.9	32±3	96.1±0.1	185±9	93.4±0.9	100	89.2±0.9
V	45±3	92.6±0.2	99±4	89.5±0.2	145±8	92.5±1.2	118±5	88.4±0.4
W	87±3	90.6±1.0	1±1	>99.9	121±7	93.5±1.5	96±7	89±1.2
Y	16±5	96.6±0.5	2±1	98.1±0.3	100	89.2±0.9	119±4	88.9±1.0

[a] RA=Relative Activity

TABLE S5 The enantioselectivity and relative activity of the double mutants generated by combination of mutations.

I113M+199	Relative activity(%)	*ee* (%)
A	286±11	98.4±0.2
C	176±8	98.4±0.1
D	231±5	98.1±0.2
E	249±16	98.3±0.1
F	110±3	97.6±0.3
G	376±21	98.7±0.1
H	153±16	97.1±0.3
I	180±11	97.6±0.4
K	218±9	98.0±0.2
L	154±6	97.9±0.4
M	199±11	97.8±0.3
N	102±4	97.8±0.3
P	225±16	97.5±0.2
Q	219±9	98.1±0.4
R	186±7	97.5±0.3
S	263±8	98.6±0.3
T	263±9	98±0.4
V	243±14	97.6±0.4
W	147±8	97.5±0.3
Y	138±7	97.2±0.2