

Table S4. Oligonucleotides Used in This Study

Primer Name ^a	5'-3' Primer Sequence ^b	Description ^c
RitR REC F1	AGGT ATGGGGAAACGGATTTTATTACTTGAG	SUMO-RitR REC/FL
RitR REC F2	ATGGGGAAACGGATTTTATTACTTGAG	SUMO-RitR REC/FL
RitR REC R1	CTAG CCTTCTTGCATGGTATATCCAACACCAC	SUMO-RitR REC
RitR REC R2	CCTTCTTGCATGGTATATCCAACACCAC	SUMO-RitR REC
RitR FL R1	CTAG CCTTCTTGCATGGTATATCCAACACCAC	SUMO-RitR FL
RitR FL R2	CCTTCTTGCATGGTATATCCAACACCAC	SUMO-RitR FL
BS2-HEX-F	Z -ATTTTATTGACAGATTAGAAAAATAATGTTACA	HEX Piu Promoter BS2
BS2-R	TGTAACATTATTTTTCTAATCTGTCAATAAAAT	Piu Promoter BS2
RitR L86A F	GATCATTGGGAAGAC GCG CAAGAAGAGCTGG	Leu86 > Ala mutation
RitR L86A R	CCAGCTCTTCTTG GCG CTCTTCCCAATGATC	Leu86 > Ala mutation
RitR L90A F	GACTTGCAAGAAGAG GCG GAAGTTGTTTCAGCG	Leu90 > Ala mutation
RitR L90A R	CGCTGAACAACCTT C GCC CTCTTCTTGCAAGTC	Leu90 > Ala mutation
RitR V93A F	CAAGAAGAGCTGGAAGTT GCG CAGCGTTTTGCAGTTTC	Val93 > Ala mutation
RitR V93A R	GAAACTGCAAAACGCTG GCG CAACTTCCAGCTCTTCTTG	Val93 > Ala mutation
RitR L86A/V93A F	GGAAGAC GCG CAAGAAGAGCTGGAAGTT GCG CAGCGTTTTG	Leu86 > Ala / Val93 > Ala double mutation
RitR L86A/V93A R	CAAAACGCTG GCG CAACTTCCAGCTCTTCTTG GCG GTCTTCC	Leu86 > Ala / Val93 > Ala double mutation
RitR F96G F	CTGGAAGTTGTTTCAGCGT GGT GCAGTTTCATACATCTATAAG	Phe96 > Gly mutation
RitR F96G R	CTTATAGATGTATGAAACTGC ACC ACGCTGAACAACCTTCCAG	Phe96 > Gly mutation
RitR Y100A F	CAGCGTTTTGCAGTTT CAGCC ATCTATAAGCCAGTCC	Tyr100 > Ala mutation
RitR Y100A R	GGACTGGCTTATAGAT GGCT GAAACTGCAAAACGCTG	Tyr100 > Ala mutation
RitR Y102F F	GTTTTGCAGTTTCATACATC GCG AAGCCAGTCCTTATCG	Tyr102 > Phe mutation
RitR Y102F R	CGATAAGGACTGGCTT GCG GATGTATGAAACTGCAAAAC	Tyr102 > Phe mutation
RitR D81A F	GCCTCAGTCATCATGATTTTA GCT CATTGGGAAGACTTGCAAGAAG	Asp81 > Ala mutation
RitR D81A R	CTTCTTGCAAGTCTTCCCAATG AGC TAAATCATGATGACTGAGGC	Asp81 > Ala mutation
RitR N53A F	GACAGACTATGATTTGATTTTATTG GCC GTTAATCTGGGAGATATG	Asn53 > Ala mutation
RitR N53A R	CATATCTCCAGATTAAC GGCC AATAAAATCAAATCATAGTCTGTC	Asn53 > Ala mutation
RitR N53D F	GACAGACTATGATTTGATTTTATTG GAC GTTAATCTGGGAGATATG	Asn53 > Asp mutation
RitR N53D R	CATATCTCCAGATTAAC GTC CAATAAAATCAAATCATAGTCTGTC	Asn53 > Asp mutation
RitR K10D F	GGAAACGGATTTTATTACTTGAG GAC GAACGAAATCTAGCTC	Lys10 > Asp mutation
RitR K10D R	GAGCTAGATTTCTGTT GTC CTCAAGTAATAAAATCCGTTTCC	Lys10 > Asp mutation
<i>RitR Kan check-F1</i>	5'-GGTGTACCATTGTCACTGATTACTG-3'	5' <i>ritR</i> flank
<i>RitR Kan check-R1</i>	5'-CAATTCCGGTGATATTCTCATTTTAGCC-3'	5' <i>ritR</i> flank
<i>RitR Kan check-F2</i>	5'-GGGATCAAGCCTGATTGGGAG-3'	3' <i>ritR</i> flank
<i>RitR Kan check-R2</i>	5'-CCTAGCTTATTTAAATAATACCAATTGCCG-3'	3' <i>ritR</i> flank
<i>erm-cassette-F</i>	5'-GGTGTACCATTGTCACTGATTACTG-3'	5' <i>erm</i> cassette
<i>erm-cassette-R</i>	5'-CCTAGCTTATTTAAATAATACCAATTGCCG-3'	3' <i>erm</i> cassette
<i>5-FL-check-F</i>	5'-CAATGGTATTGAGTACGGTGATATGC-3'	5' <i>ritR</i> flank
<i>5'-FL-check-R</i>	5'-CTTTCTCAAGTAATAAAATCCGTTTCCC-3'	5' <i>ritR</i> flank
<i>3'-FL-check-F</i>	5'-CCGTTACTTATGAGCAAGTATTGTC-3'	3' <i>ritR</i> flank
<i>3'-FL-check-R</i>	5'-CAAGGTATTGCCATCCTGTTTTTC-3'	3' <i>ritR</i> flank
<i>Ppiu-F1</i>	5'- AATT CTGAAGTTGGCATTCAAAAAATCATGTTC-3'	Piu promoter
<i>Ppiu-F2</i>	5'-CTGAAGTTGGCATTCAAAAAATCATGTTC-3'	Piu promoter
<i>Ppiu(1+2+3)-R1</i>	5'- GATCC GACTAGGAGTAGAAGTAAGCC-3'	Piu promoter
<i>Ppiu(1+2+3)-R2</i>	5'- CGACT AGGAGTAGAAGTAAGCC-3'	Piu promoter
<i>PP2-tet-F</i>	5'-CCTAATCGGAAAGGTTTTCAATCCC-3'	PP2 <i>tet</i> gene
<i>PP2-tet-R</i>	5'-CCGATAACGATACCAAGGATGAAAC-3'	PP2 <i>tet</i> gene
<i>PP2-bga-F</i>	5'-GTCTTGTTGGAACCAACCAG-3'	PP2 <i>bga</i> gene
<i>PP2-bga-R</i>	5'-GTTTCAATCTACTATACAATAAGAGAACG-3'	PP2 <i>bga</i> gene
<i>PP2-seq-F</i>	5'-GCATGCATCGGTACCTGCG-3'	PP2 plasmid
<i>PP2-seq-R</i>	5'-GTAAACGACGGGATCAAGATGTTTC-3'	PP2 plasmid

^aF, forward primer; R, reverse primer; HEX, 5' Hexachlorofluoroscein label; BS2, *piu* promoter Binding Site 2

^bZ, HEX moiety; Bold italicized underlined and **red**, *Bsa*I restriction site; **green**, mutated Gate codon; **blue**, mutated catalytic domain codon.

^cREC, Receiver Domain; FL, Full-length protein; >, Mutated to the residue that follows.