## Supplementary data

**Table S1**. Oligonucleotides used in this study. Lower case indicates a sequence added for cloning purposes. Bold indicates a mutated sequence. Asterisk indicates fluorescein-labeled (FAM) oligonucleotides. Numbering is considering +1 for the A in the first ATG codon. W, Watson; C, Crick.

| Name | Sequence | Gene | Strand (W/C) | Added site | Hybridization position |
|---|---|---|---|---|---|
| **IXR1 knockout in MW190-9b** | | | | | |
| ECV719 | GGGgcatgcCAGACGGGCCAGACAGC | *KlIXR1* | W | *SphI* | -698 |
| ECV720 | GGGgtcgacCGGAATGTACGTTGGATCTGC | *KlIXR1* | C | *SalI* | +2005 |
| ECV763 | TTTgcggccgcTGTTCGCTATGTCCTTGGTT | *KlIXR1* | C | *NotI* | +12 |
| ECV764 | TTTgcggccgcGCTGAAGCTGCTGCAG | *KlIXR1* | W | *NotI* | +1374 |
| ECV765 | GTCTAGCACTCTCCCAGTG | *KlIXR1* | W | - | -640 |
| ECV766 | GAGAAGTCGTATATGCCTTCATTAC | *KlIXR1* | C | - | +1968 |
| ECV315K2 | CGCCTTAATTAACCCGGGGAT | *KanMX4* | W | - | +1322 |
| ECV314K3 | GCCTCGACATCATCTGCCCAG | *KanMX4* | C | - | +45 |
| ECV808AR | TTAGGAAACCATTTGTACATAGATTTGTG | *KlIXR1* | W | - | -822 |
| ECV809AR | TCCTATTCCACCTTTCAGGATACAAC | *KlIXR1* | C | - | +2241 |
| **Northern blot** | | | | | |
| ECV051 | GGGGTCGACTTGGCAGAAGAACGAATAACAGA | *KlHEM13* | W | | +29 |
| ECV053 | GGGGGATCCCATCGAGGCCTTGAGGAAAA | *KlHEM13* | C | | -764 |
| U3F | CGACGTACTTCAGTATGTAA | *KlSNR17A* | W | | +3 |
| U3R | ATTTGTACCCACCCATAGAG | *KlSNR17A* | C | | +475 |
| **RT-qPCR** | | | | | |
| AVV83q | AAATGGAAGACAACCCGCC | *KlTAF10* | W | - | +217 |
| AVV84q | TTACGAATTTCTGTGTAGCAAGAGC | *KlTAF10* | C | - | +315 |
| AVV85q | ACCTGGAATGCGGACGG | *KlHEM13* | W | - | +558 |
| AVV86q | TGTCCAAAGCATCCTTGTGC | *KlHEM13* | C | - | +662 |
| AJVV164f | GGAATGTTGGTGGTGGTAAGA | *KlSFP1* | W | - | +374 |
| AJVV165r | TGGCCACAGCAGCTATAATC | *KlSFP1* | C | - | +497 |
| AJVV166f | GAGTTACCCAAGCCCAGAAA | *KlABF1* | W | - | +421 |

| | | | | | |
|---|---|---|---|---|---|
| AJVV167r | CAGAGGAAGATCTTGGCCTAAC | *KlABF1* | C | - | +566 |
| AJVV168f | GCCACTATCTTCGGGAGTAAAT | *KlTEC1* | W | - | +1452 |
| AJVV169r | CAGTGTTCATGGTTGCAGTTC | *KlTEC1* | C | - | +1555 |
| AJVV170f | CCTCAAGTAGCACAGGTTTCTC | *KlSOK2* | W | - | +199 |
| AJVV171r | CGCATAGCCCAGATACTGATTT | *KlSOK2* | C | - | +324 |
| AJVV172f | GCGACAAAGCGGACATAGA | *KlUME6* | W | - | +761 |
| AJVV173r | CAAGGATCGGCGAAGATGAA | *KlUME6* | C | - | +895 |
| AJVV174f | CGTTAATGGTGGTGACTCTCTC | *KlDAL81* | W | - | +21 |
| AJVV175r | CAACTGAAGTGGTCTCCTTACC | *KlDAL81* | C | - | +139 |
| AJVV176f | AGATATTTCACCCACGCTTCTC | *KlCRF1* | W | - | +1021 |
| AJVV177r | ACCTTCGATGGAATCCTCTTTC | *KlCRF1* | C | - | +1144 |
| AJVV178f | CCTGTTCCTGAGGCTAGAAATG | *KlRNR1* | W | - | +1534 |
| AJVV179r | TTCAAATGGAGTACGGCAGAG | *KlRNR1* | C | - | +1635 |
| AJVV180f | GATACTTCATGGATGCTCTACCC | *KlRNR2* | W | - | +926 |
| AJVV181r | TACCCAAGGCGACCAATAATC | *KlRNR2* | C | - | +1030 |
| AJVV182f | CTGGGATGACGACGAATTAGAT | *KlDUN1* | W | | +1164 |
| AJVV183r | ACGTAAGAAGGTGTTCCACATAG | *KlDUN1* | C | - | +1274 |
| AJVV184f | GACTCGCTCAAGATGGCTTAC | *KLMEC1* | W | - | +3751 |
| AJVV185r | TTCCGTTGGCTGCTTGATAG | *KLMEC1* | C | - | +3850 |
| AJVV218f | GATTGCGTTCGGTAAAGTCAAG | *KlSCH9* | W | - | +1608 |
| AJVV219r | GGCACCAAGTCTATGTTTAGGA | *KlSCH9* | C | - | +1713 |
| AJVV220f | CAGATCCAGTTGCCGAGATTAG | *KlTOR1* | W | - | +1901 |
| AJVV221r | GACTTCGTCATTCAGAGCCATAA | *KlTOR1* | C | - | +2043 |
| AJVV222f | CTGAGTCTTCCGGGTTTGTT | *KlRAP1* | W | - | +320 |
| AJVV223r | CGTTGATCCCTGTTGCTATCT | *KlRAP1* | C | - | +451 |
| AJVV224f | GAAGGAGGTACAATGGCAGTAG | *KlIFH1* | W | - | +370 |
| AJVV225r | GTACTCGAACTGTCGCTATCATC | *KlIFH1* | C | - | +476 |
| AJVV226f | AACACGCCTGCCTCTAATAC | *KlFHL1* | W | - | +687 |
| AJVV227r | CGATCGCCTTCCGATAATAACT | *KlFHL1* | C | - | +819 |
| AJVV228f | CCCATTCCTTGAGAGACCATTC | *KlDOT6* | W | - | +1511 |
| AJVV229r | GTTCATTAGCAGCCTGTGTTTG | *KlDOT6* | C | - | +1630 |

| ***Kl*Ixr1 protein production** | | | | | |
|---|---|---|---|---|---|
| AVV021 | GCGGattaatATGAATCTCAATAACCAA GGACATAGC | *KlIXR1* | W | *VspI* | +1 |
| AVV022 | GGGaagcttTCATTCTGCAGCAGCTTCA GC | *KlIXR1* | C | *HindIII* | +1374 |
| AJVV024f | GGGGacaagtttgtacaaaaaagcaggctTCATG**C ACCATCACCATCACCAT**AATCTCAAT | *KlIXR1* | W | *attB1* | +4 |

| | | | | | |
|---|---|---|---|---|---|
| | AACCAAGGACATAGCGAACATCAGG GGGGaccactttgtacaagaaagctgggtCCTATT | | | | |
| AJVV025r | CTGCAGCAGCTTCAGCTTCATGTTCCG | *KlIXR1* | C | *attB2* | +1374 |
| **EMSA & FA assays** | | | | | |
| | ATCTTGAATGTATGTTGGTTCAGCCTC | | | | |
| AJVV009f* | TATTTCTCTCGTA | *KlHEM13* | W | - | -389 |
| | TACGAGAGAAATAGAGGCTGAACCA | | | | |
| AJVV010r | ACATACATTCAAGAT | *KlHEM13* | C | - | -389 |
| | ATCTTGAATGTATGTGCGCACAGCCT | | | | |
| AJVV011f | CTATTTCTCTCGTA | *KlHEM13* | W | - | -389 |
| | TACGAGAGAAATAGAGGCTG***TGCGC*** | | | | |
| AJVV012r | ***A***CATACATTCAAGAT | *KlHEM13* | C | - | -389 |
| | ATCTTGAATGTATGTTGGTTCAGCCTC | | | | |
| AJVV013f | TTGCGCACTCGTA | *KlHEM13* | W | - | -389 |
| | TACGAG***TGCGCA***AGAGGCTGAACCA | | | | |
| AJVV014r | ACATACATTCAAGAT | *KlHEM13* | C | - | -389 |
| | ATCTTGAATGTATGTTGGTTCAGCCTC | | | | |
| AJVV015f | TATTTCTCTCGTA | *KlHEM13* | W | - | -389 |
| | TTAATTGCAATTGTTACTGTTGATTTT | | | | |
| AJVV029f* | AAA | *KlYCF1* | C | - | -578 |
| | TTAATTGCAATTGTTACTGTTGATTTT | | | | |
| AJVV030f | AAA | *KlYCF1* | W | - | -578 |
| | TTTAAAATCAACAGTAACAATTGCAA | | | | |
| AJVV031r | TTAA | *KlYCF1* | C | - | -578 |
| | ATCTTGAATGTATGTTGGTTCAGCCTC | | | | |
| AJVV045f* | T**CC** | *KlHEM13* | W | - | -389 |
| | **GG**AGAGGCTGAACCAACATACATTC | | | | |
| AJVV046r | AAGAT | *KlHEM13* | C | - | -389 |
| | ATCTTGAATGTATGTTGGTTCAGCCTC | | | | |
| AJVV047f | T**CC** | *KlHEM13* | W | - | -389 |
| | ATCTTGAATGTATG***TGCGCA***CAGCCT | | | | |
| AJVV048f | CT**CC** | *KlHEM13* | W | - | -389 |
| | **GG**AGAGGCTG***TGCGCA***CATACATTCA | | | | |
| AJVV049r | AGAT | *KlHEM13* | C | - | -389 |
| | **TGGCC**CAGCCTCTATTTCTCTCGTA**CC** | | | | |
| AJVV050f* | **GGT** | *KlHEM13* | W | - | -369 |
| | **ACCGG**TACGAGAGAAATAGAGGCTG | | | | |
| AJVV051r | **GGCCA** | *KlHEM13* | C | - | -369 |
| | **TGGCC**CAGCCTCTATTTCTCTCGTA**CC** | | | | |
| AJVV052f | **GGT** | *KlHEM13* | W | - | -369 |
| | **TGGCC**CAGCCTCT***TGCGCA***CTCGTA**C** | | | | |
| AJVV053f | **CGGT** | *KlHEM13* | W | - | -369 |

| | | | | | |
|---|---|---|---|---|---|
| AJVV054r | **ACCGG**TACGAG***TGCGCA***AGAGGCTG **GGCCA** | *KlHEM13* | C | - | -369 |
| AJVV055f | TTAATTGCA**TGCGCAACTGTT**GATTT TAAA | *KlYCF1* | W | - | -578 |
| AJVV056r | TTTAAAATC**AACAGTTGCGCA**TGCAA TTAA | *KlYCF1* | C | - | -578 |
| AJVV057f | TTAATTGCA**ATTGTTTGCGCA**GATTT TAAA | *KlYCF1* | W | - | -578 |
| AJVV058r | TTTAAAATC**TGCGCAAACAAT**TGCAA TTAA | *KlYCF1* | C | - | -578 |

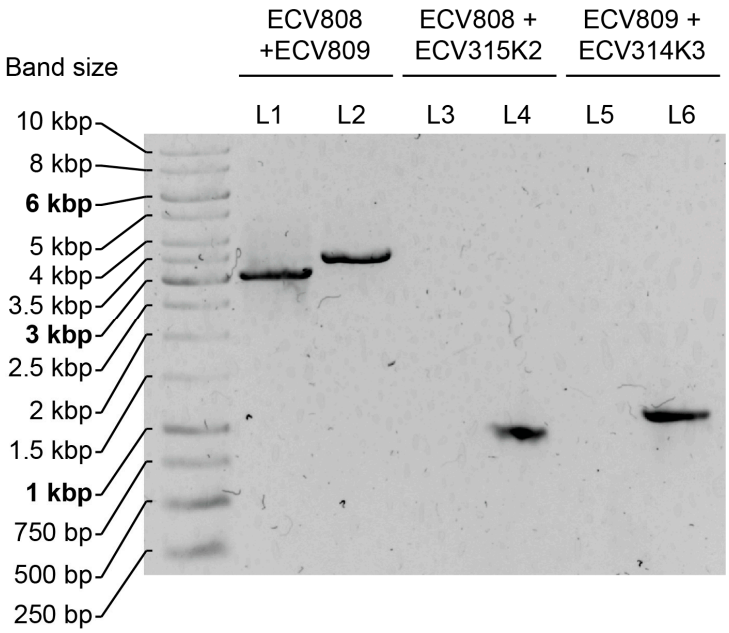


**Figure S1**: Verification of MW190-9b-*klixr1Δ* null strain by PCR and agarose gel electrophoresis. Genomic DNA from the wild type (lanes L1, L3 and L5) or the derivative with the *KlIXR1* gene knocked out by the insertion of the cassette with the kanMX4 resistance gene (lanes L2, L4 and L6) were used as templates. Expected band size: L1: 3088 bp; L2: 3245 bp; L3: no band; L4: 953 bp; L5: no band; L6: 1035 bp.

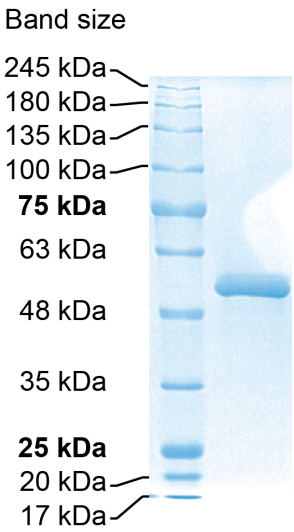


**Figure S2**: SDS-PAGE gel of *Kl*Ixr1 protein. The protein was produced by heterologous expression in *Escherichia coli* and fully isolated up to a high grade of purity. Expected molecular weight: 51.3 kDa.