

Supplementary file 2

Expression vectors used in this study

Table of Contents	Page
Legend to Supplementary files 2A, 2B, 2C and 2D	3
2A: Expression vectors for (modified) bovine cytokines and receptor chains	4
- pRcCMV2- <i>Bos-IL-2-FLAG</i>	4
- pRcCMV2- <i>Bos-IL-15-FLAG</i>	4
- pRcCMV2- <i>Bos-IL-15L-FLAG</i>	5
- pRcCMV2- <i>Bos-IL-15L-(non-tagged)</i>	5
- pRcCMV2- <i>Bos-IL-15Lhyb-FLAG</i>	6
- pcDNA3.1- <i>IL-2-Lead-RLI-bov.IL-15Lhyb</i>	6
- pcDNA3.1- <i>Bos-IL-15Rα-Myc-His</i>	7
- pcDNA3.1- <i>Bos-solIL-15Rα-Myc-His</i>	8
- pcDNA3.1- <i>Bos-IL-2Rα-Myc-His</i>	8
- pcDNA3.1- <i>Bos-solIL-2Rα-Myc-His</i>	9
2B: Expression vector for a genetic fusion of human IL-15R α and IL-15	10
- pcDNA3.1- <i>IL-2-Lead-RLI</i>	10
2C: Expression vectors for (modified) trout cytokines and IL-15R α	11
- pcDNA3.1- <i>trout-IL-2-FLAG</i>	11
- pcDNA3.1- <i>trout-IL-2-(non-tagged)</i>	11
- pcDNA3.1- <i>trout-IL-15-FLAG</i>	12
- pcDNA3.1- <i>trout-IL-15-(non-tagged)</i>	12
- pcDNA3.1- <i>trout-IL-15La-FLAG</i>	13
- pcDNA3.1- <i>trout-IL-15La-(non-tagged)</i>	13
- pcDNA3.1- <i>trout-IL-15Lb-FLAG</i>	14
- pcDNA3.1- <i>trout-IL-15Lb-(non-tagged)</i>	14
- pcDNA3.1- <i>IL-2-Lead-RLI-trout-IL-15La</i>	15
- pcDNA3.1- <i>trout-IL-15Rα-Myc</i>	15
- pcDNA3.1- <i>trout-solIL-15Rα-Myc</i>	16

2D: Vectors for expressing (modified) trout cytokines and trout IL-15R α in insect cells using a baculovirus system.	17
- pFBD-P10Uhis-ieGFP- <i>trout-IL-2-FLAG</i>	17
- pFBD-P10Uhis-ieGFP- <i>trout-IL-15-FLAG</i>	17
- pFBD-P10Uhis-ieGFP- <i>trout-IL-15La-FLAG</i>	18
- pFBD-P10Uhis-ieGFP- <i>trout-solIL-15Rα-Myc</i>	18
- pFBD-P10Uhis-ieGFP- <i>trout-IL-15-RLI</i>	19
- pFBD-P10Uhis-ieGFP- <i>trout-IL-15La-RLI</i>	19
References used in this file	21

Legend to Supplementary files 2A, 2B, 2C and 2D.

This Supplementary file shows the sequence fragments containing bovine (2A), human (2B), and trout (2C) *IL-2*, *IL-15*, *IL-15L*, *IL-2R α* and/or *IL-15R α* sequences, or modifications thereof, which were cloned into DNA plasmid expression vectors behind a CMV immediate early promoter or into a baculovirus transfer vector behind a p10 promoter (2D). The texts for the individual constructs explain what the vectors encode, and how the encoded protein is named in the paper. The pink font sequence fragments indicate the restriction sites used for cloning. The DNA plasmid expression vector used for cloning was either pRc/CMV2 (Invitrogen) or pcDNATM3.1/myc-His B (Invitrogen). The baculovirus transfer vector used for cloning was pFBD-P10Uhis-ieGFP (a derivative of pFastBac-Dual [Invitrogen], see main text Materials and methods section). The cloning strategies varied and were somewhat arbitrarily because experiments were done at different time points and constructs were made in different laboratories.

Several of the expression constructs with bovine *IL-2*, *IL-15L*, *IL-15R α* and *IL-2R α* were already described in detail in references (1) and (2), but for convenience are shown here again.

Because the information on the construction method is not so relevant, the below only briefly mentions whether the construction involved an amplification from cDNA or a purchase of a synthetic gene sequence (Invitrogen) and describes the GenBank accession number of a matching sequence. When relevant, additional information is given.

Green font nucleotide sequence: By choice of primer sequences, or synthetic gene ordering, an ACC motif was added directly upstream of the start codon to assure efficient translation.

Red font amino acid sequence: By choice of primer sequences, or synthetic gene ordering, FLAG tag sequences were added.

Orange font amino acid sequence: By choice of primer sequences, or as part of the commercial vector, Myc tag sequences were added.

Purple font amino acid sequence: As part of the commercial vector, poly-His tag sequences were added.

Other colors are explained in the individual texts.

Supplementary file 2A.
Expression vectors for (modified) bovine cytokines and receptor chains.

pRcCMV2-Bos-IL-2-FLAG

This expression vector encodes bovine FLAG-tagged IL-2 (named IL-2).
Cloned from cDNA. The *IL-2* part of the sequence is identical with GenBank accession EU276068.

[for cloning details see (1)]

AAGCTTACCATGTACAAGATACAACCTCTTGTCTTGCATTGCACTAACTCTTGCACCTCGT
TGCAAACGGTGCACCTACTTCAAGCTCTACGGGGAACACAATGAAAGAAGTGAAGTCAT
TGCTGCTGGATTTACAGTTGCTTTTGGAGAAAGTTAAAAATCCTGAGAACCTCAAGCTC
TCCAGGATGCATACATTTGACTTTTACGTGCCCAAGGTTAACGCTACAGAATTGAAACA
TCTTAAGTGTTTACTAGAAGAACTCAAACCTTCTAGAGGAAGTGCTAAATTTAGCTCCAA
GCAAAAACCTGAACCCAGAGAGATCAAGGATTCAATGGACAATATCAAGAGAATCGTT
TTGGAACCTACAGGGATCTGAAACAAGATTCACATGTGAATATGATGATGCAACAGTAAA
CGCTGTAGAATTTCTGAACAAATGGATTACCTTTTGTCAAAGCATCTACTCAACAATGA
CTGATTATAAGGATGACGACGATAAGTAAAGGGCCC

MYKIQLLSCIALTLALVANGAPTSSSTGNTMKEVKSLLLDLQLLLEKVKNPENLKLSRM
HTDFYVPKVNATELKHKLCLLEELKLLLEVLNLAAPSKNLNPREIKDSMDNIKRVLEL
QGSETRFTCEYDDATVNAVEFLNKWITFCQSIYSTMTDYKDDDDK

pRcCMV2-Bos-IL-15-FLAG

This expression vector encodes bovine FLAG-tagged IL-15 (named IL-15).
The sequence was commercially ordered (Invitrogen). The leader sequence is replaced
with that of bovine IL-2 (shaded blue), and the codons were optimized for expression in
human cells. The encoded IL-15 part is identical to GenBank accession ELR44697.

AAGCTTACCATGTACAAGATCCAGCTGCTGAGCTGTATCGCCCTGACCCTGGCCCTGGT
GGCCAACGGCAATTGGCAGTACGTGATCAACGACCTGAAAACCATCGAGCACCTGATCC
AGAGCATCCACATGGACGCCACCCTGTACACCGAGAGCGACGCCACCCCAACTGCAAA
GTGACCGCCATGCAGTGCTTTTCTGCTGGAAGTGAAGAGTATCCTGCACGAGAGCAAGAA
CGCCACCATCTACGAGATCATCGAGAATCTGACCATGCTGGCCAACAGCAACCTGAGCA
GCATCGAGAACAAGACCGAGCTGGGCTGCAAAGAGTGCAGGAACTGGAAGAGAAGTCC
ATCAAAGAGTTTCTGAAGTCCTTCGTGCACATCGTGCAGATGTTTCATCAACACCAGCGA
CTACAAGGACGACGACGACAAGTGATCTAGAGGGCCC

MYKIQLLSCIALTLALVANGNWQYVINDLKTIEHLIQSIHMDATLYTESDAHPNCKVTA
MQCFLELRVILHESKNATIYEI IENLTMLANSLSS IENKTELGCKECEEELEEKSIKE
FLKSFVHIVQMFINTSDYKDDDDK

pRcCMV2-Bos-IL-15L-FLAG

This expression vector encodes bovine FLAG-tagged IL-15L (named IL-15L). Cloned from cDNA. The *IL-15L* part of the sequence is identical with GenBank accession NM_001301213.

[for cloning details see (1)]

```
AAGCTTACCATGTGGCTTCTCTGGACCACCCTCCTGCTGGTGCTGCCCTTGGGAGGCCT
AGGACCACTCCTCTGCCCCAAGGGAGCCTTTCTACTTCCATTCATTGCCATCACGAAGATGC
TGGAAAACAAAAATGATGGCAGTCTGTACACCCAGATAATCTATTGGTGTGTCTCTGCT
GAGACTCTCCGATGCTTCCGGCTGGAGTTGTCTGTGATCGGGTTTGAGGAGGGCCCATC
CGTGGGGATCGTTGTGTTCCGCCTACAGCGCCTACTGGATGCCCTGGGGTCCCAGCTGT
GGGTGATTGATCAGGGCCCTTGTCCACCCTGCGAAGGACACCCTCAGAGACCAGTCCCT
CTTTTCTGGCCAACTCTTGGAGTTATTACAGGGGACTTGTGCTCGGGACCTGCCCTC
AGCAGATTACAAGGATGACGACGATAAGTAACTCTAGA
```

```
MWLLWTTLLLVLPLGGLGPLLCPREPFYFLIAITKMLENKNDGSLYTPDNLLVCPAETL
RCFRLELSVIGFEEGPSVGIVVFRLLQRLLDALGSQLWVIDQGPPCEGHPQRPVPLFL
AKLLELLQGT CARDLPSADYKDDDDK
```

pRcCMV2-Bos-IL-15L-(non-tagged)

This expression vector encodes bovine non-tagged IL-15L [named IL-15L(N)]. Cloned from cDNA. The *IL-15L* sequence is identical with GenBank accession NM_001301213.

[for cloning details see (1)]

```
AAGCTTACCATGTGGCTTCTCTGGACCACCCTCCTGCTGGTGCTGCCCTTGGGAGGCCT
AGGACCACTCCTCTGCCCCAAGGGAGCCTTTCTACTTCCATTCATTGCCATCACGAAGATGC
TGGAAAACAAAAATGATGGCAGTCTGTACACCCAGATAATCTATTGGTGTGTCTCTGCT
GAGACTCTCCGATGCTTCCGGCTGGAGTTGTCTGTGATCGGGTTTGAGGAGGGCCCATC
CGTGGGGATCGTTGTGTTCCGCCTACAGCGCCTACTGGATGCCCTGGGGTCCCAGCTGT
GGGTGATTGATCAGGGCCCTTGTCCACCCTGCGAAGGACACCCTCAGAGACCAGTCCCT
CTTTTCTGGCCAACTCTTGGAGTTATTACAGGGGACTTGTGCTCGGGACCTGCCCTC
AGCATAACTCTAGA
```

```
MWLLWTTLLLVLPLGGLGPLLCPREPFYFLIAITKMLENKNDGSLYTPDNLLVCPAETL
RCFRLELSVIGFEEGPSVGIVVFRLLQRLLDALGSQLWVIDQGPPCEGHPQRPVPLFL
AKLLELLQGT CARDLPSA
```

pRcCMV2-Bos-IL-15Lhyb-FLAG

This expression vector encodes bovine FLAG-tagged IL-15L in which a few motifs have been replaced for that of other IL-2/15/15L family members (named IL-15Lhyb).

The part encoding the mature protein was commercially ordered (Invitrogen) with the codons optimized for expression in insect cells, and fused to sequences encoding authentic bovine IL-15L leader sequence and a C-terminal FLAG-tag by using appropriate primers and overlap PCR. The rationale behind the substitutions (shaded yellow) was that *in vitro* handling of bovine IL-15L protein revealed instability (not shown), while two of the modified motifs were different in mammals from IL-2/15/15L family consensus, and we thought that adding of the trout IL-15La glycosylation motif might aid stability. Unfortunately, an improved stability, or a function, was not found for this IL-15Lhyb molecule.

```
AAGCTTACCATGTGGCTTCTCTGGACCACCCTCCTGCTGGTGCTGCCCTTGGGAGGCCT
AGGACCTTTGTTGTGCCCCCGTGAACCCTTCTACTTCTGCGCAAGATCACCAAGATGC
TCGAGAACAAGAACGACGGTTCCTGTACACCCCGACAACCTGCTCGTGTGCCCGCT
GAAACCCTGCGCTGCTTCCGTCTGGAACGTCCGTGATCGGTTTCGAGGAAGGCAACAA
GTCCTCCGTGGGTATCGTGGTGTTCAGGCTGCAGCGTCTGCTGGACGCTCTGGGTTCTC
AGCTGTGGGTACCGACCAGGGACCTTGCCCTCCTTGCAGGGTACCCCTCAACGTCCC
GTGCCTCTGTTCTGGCTAAGCTGCTCGAGCTGCTGCAGGGAACCTGCGCTCGTGACCT
GCCCTCCGCTGATTACAAGGATGACGACGATAAGTAACTAGA
```

```
MWLLWTTLLLVLPLGGLGPLLCPREPFYFLRKITKMLENKNDGSLYTPDNLLVCPAETL
RCFRLELSVIGFEEGNKSSVGIVVFRLLQRLLDALGSQLWVTDQGPPCEGHPQRPVPL
FLAKLLELLQGT CARDLPSADYKDDDDK
```

pcDNA3.1-IL-2-Lead-RLI-bov.IL-15Lhyb

This expression vector is based on the RLI construct described by Mortier *et al.* 2006 (3), and encodes a genetic fusion of a human IL-2 leader, a FLAG-tag, a large part of the human IL-15R α ectodomain, a glycine-serine linker (shaded blue), and the above described bovine IL-15Lhyb sequence (with the modified motifs shaded yellow) (named bov.IL-15Lhyb-h-RLI).

IL-15R α does not interact with the signaling receptor IL-2R β ·IL-2R γ , and is thought to fixate IL-15 into the proper conformation for binding IL-2R β ·IL-2R γ (4); given the observed cross-species activity (main text Fig. 5), we speculated that RLI-versions of bovine and trout IL-15L would have similar stability and signaling advantages as observed for all-human RLI (3, 5, 6).

The linker plus IL-15Lhyb part were commercially ordered (Invitrogen) with the codons optimized for expression in insect cells, and fused to the N-terminal coding part by using plasmid pcDNA3.1-IL-2-Lead-RLI, appropriate primers and overlap PCR.

GGTACCACCATGTACAGGATGCAACTCCTGTCTTGCATTGCACTAAGTCTTGCCTTGT
CACAAACAGTGACTACAAGGATGACGATGACAAGATAGAAGGTAGGATCACATGCCCTC
CCCCATGTCCGTGGAACACGCAGACATCTGGGTCAAGAGCTACAGCTTGTACTCCAGG
GAGCGGTACATTTGTAACCTCTGGTTTCAAGCGTAAAGCCGGCACGTCCAGCCTGACGGA
GTGCGTGTGAACAAGGCCACGAATGTGCGCCACTGGACAACCCCCAGTCTCAAATGCA
TTAGAGACCCTGCCCTGGTTCACCAAAGGCCAGCGCCACCCTCTGGAGGCTCCGGTGG
GGTGGGAGTGGCGGTGGATCCGGTGGCGGAGGCAGCCCTTTGTTGTGCCCCCGTGAACC
CTTCTACTTCCCTGCGCAAGATCACCAAGATGCTCGAGAACAAGAACGACGGTCCCTGT
ACACCCCCGACAACCTGCTCGTGTGCCCCGCTGAAACCCTGCGCTGCTTCCGTCTGGAA
CTGTCCGTGATCGGTTTCGAGGAAGGCAACAAGTCCCTCCGTGGGTATCGTGGTGTTCAG
GCTGCAGCGTCTGCTGGACGCTCTGGGTCTCAGCTGTGGGTACCCGACCAGGGACCTT
GCCCTCCTTGCGAGGGTACCCCTCAACGTCCCGTGCCTCTGTTCCCTGGCTAAGCTGCTC
GAGCTGCTGCAGGGAACCTGCGCTCGTGACCTGCCCTCCGCTTAACTCTAGA

MYRMQLLSICIALSLALVTNSDYKDDDDKIEGRITCPPPMSVEHADIWVKSYSLSRERY
ICNSGFKRKAGTSSLTECVLNKATNVAHWTTPLKCIKIRDPALVHQRPAPPSSGGSGGGGS
GGSGGGGSPLLCPREPFYFLRKITKMLENKNDGSLYTPDNLLVCPAETLRCFRLELSV
IGFEEGNKSSVGIIVFRLQRLLDALGSQLWVTDQGPCPPCEGHPQRPVPLFLAKLLELL
QGTCARDLPSA

pcDNA3.1-Bos-IL-15R α -Myc-His

This expression vector encodes full-length bovine Myc-tagged IL-15R α (named IL-15R α).

Cloned from cDNA. The *IL-15R α* part of the sequence is identical with GenBank accession XM_015465884.

[for cloning details see (1)]

AAGCTTACCATGTCCGGGCGGCTCCGGGGCCGCGGGGCCGCGCCCTCCCCGCGCTGGG
GCTGCTGCTGCTACTACTGCTGCTCGGATCTTCGGCCACGCCGGGCATCACCTGCCCGA
CTCCACATCCGTGGAGCATGCAGACATCCAGGTCAAGAGTTACAGCATCAACTCCAGG
GAGCGGTATGTTTGTAAATCTGGCTTCAAGCGTAAAGCTGGGACTTCCAGCTTGACCCA
GTGTGTGTTAACGAGACCGCGAAAGTCGCCCCACTGGACCACTCCCAACCTCAAGTGCA
TCAGAGACCCCTCCCTGAGTCACCAAAGGCCACCCTCCACAGCAGCGCCTACAGGGTTG
ACCCAGAGCCAGAGAGCCCCACCCCTCCGGAAAAGAGCCAGATCTTACTTCCAAGTC
AGACACCAAAGTGGCCACAAGGCCAGCTACTGGACCAGGCTCCAGGCTGCCATCCACAG
CTCCTCCTGTGGGAACCACAGGGGTAGTCAGTAAGGAGACCACCTACGTCCAGCTCAG
ACAGCAGCCAAGGCTCCGGAACACACATACCCGGCCTTGCAGGACACGCCCGGTGCATA
TCAGAACAATCCCAGAGTTGTGACCGCCGTCTCAACTGTCACTGTGCTCTTTGTAGTAT
GCCTGGTGTCTTCTTCTGGGACGTTGCCTGTGGTCAAGGCGAGCCCACCAGACACCCGGT
GTTGAGATGGAGAGCATGGAGAGTGTGCCAATGACCACGGGGGCGATGCCAGAGGGGA
GGACACAGAAATCCACCCGCATGGCCTAGGAGGCTCCGGGGACGCTGAGGCCAGCAGCG
GCCGAGTGAAGGCCAGCTCTTCCCCAGTCAGAGAGGACCTCGAGTCTAGAGGGCCCG
CGTTTCGAACAAAACCTCATCTCAGAAGAGGATCTGAATATGCATACCCGGTCATCATCA
CCATCACCATTTGA

MSGRLRGRGAGALPALGLLLLLLLLLGGSSATPGITCPTPTSVEHADIQVKSYSINSRERY
VCNSGFKRKAGTSSLTQCVFNETAKVAHWTTPNLKCIRDPSLSHQRPSTAAAPTGLTPE
PESPTPSGKEPDLTSKSDTKVATRPATGPGSRLPSTAPPVGTGTVVSKETTYVPAQTAA
KAPEHTYPALQDTPGAYQNNPRVVTAVSTVTVLVVCLVFLGRCLWSRRAHQTPGVEM
ESMESVPMTTGADARGEDTEIHPHGLGGSGDAEASSGRSEGPALPQSERTSSLEGPRFE
QKLISEEDLNMHGHHHHH

pcDNA3.1-Bos-solIL-15R α -Myc-His

This expression vector encodes bovine soluble Myc-tagged IL-15R α (named soluble IL-15R α or sIL-15R α).

Cloned from cDNA. The *IL-15R α* part of the sequence is identical with GenBank accession XM_015465884.

[for cloning details see (1)]

AAGCTTACCATGTCCGGGCGGCTCCGGGGCCGCGGGCCGGCGCCCTCCCCGCGCTGGG
GCTGCTGCTGCTACTACTGCTGCTCGGATCTTCGGCCACGCCGGGCATCACCTGCCCGA
CTCCACATCCGTGGAGCATGCAGACATCCAGGTCAAGAGTTACAGCATCAACTCCAGG
GAGCGGTATGTTTGTAAATCTGGCTTCAAGCGTAAAGCTGGGACTTCCAGCTTGACCCA
GTGTGTGTTTAAACGAGACCGCGAAAGTCGCCCACTGGACCACTCCCAACCTCAAGTGCA
TCAGAGACCCCTCCCTGAGTCACCAAAGGCCACCCTCCACAGCAGCGCCTACAGGGTTG
ACCCAGAGCCAGAGAGCCCCACCCCTCCGGAAAAGAGCCAGATCTTACTTCCAAGTC
AGACACCAAAGTGGCCACAAGGCCAGCTACTGGACCAGGCTCCAGGCTGCCATCCACAG
CTCCTCCTGTGGGAACCACAGGGGTAGTCAGTAAGGAGACCACCTACGTCCAGCTCAG
ACAGCAGCCAAGGCTCCGGAACACACATACCCGGCCTTGCAGGACACGCCCGGTGCATA
TCAGAACAATCCCAGCTCGAGTCTAGAGGGCCCGCGGTTTGAACAATAACTCATCTCAG
AAGAGGATCTGAATATGCATACCGGTCATCATCACCATCACCATTGA

MSGRLRGRGAGALPALGLLLLLLLLLGGSSATPGITCPTPTSVEHADIQVKSYSINSRERY
VCNSGFKRKAGTSSLTQCVFNETAKVAHWTTPNLKCIRDPSLSHQRPSTAAAPTGLTPE
PESPTPSGKEPDLTSKSDTKVATRPATGPGSRLPSTAPPVGTGTVVSKETTYVPAQTAA
KAPEHTYPALQDTPGAYQNNPSSSLEGPRFEQKLISEEDLNMHGHHHHH

pcDNA3.1-Bos-IL-2R α -Myc-His

This expression vector encodes bovine full-length Myc-tagged IL-2R α (named IL-2R α). Cloned from cDNA. Except for a single nucleotide replacement (shaded yellow), the *IL-2R α* part of the sequence is identical with GenBank accession BC133546.

[for cloning details see (1); through primer choice a silent mutation was introduced at the single yellow-shaded C position in order to delete a gene-internal restriction site]

AAGCTTACCATGGAGCCAGCTTGCTGATGTGGAGGTTCTTCGTATTCATCGTGGTACC
TGGCTGCGTGACAGAGGCTTGTTCATGATGACCCCTCCGAGTCTCAGAAACGCCATGTTCA
AGGTCTTCAGGTACGAGGTGGGCACCATGATAAACTGCGACTGCAAGACAGGCTTCCGC
AGAGTGTGCGCCGTCATGCGCTGCGTGGGGGACTCCAGCCACTCTGCCTGGGAAAACAG
ATGCTTCTGCAACAGCACCTCCCCTGCTAAGAACCCAGTAAAACAAGTCACTCCTGCAC
CCGAAGAACAGAGGGAGAAAAAACCACAGATGCGCAGAACCAAACGCAGCCTCCGGAG
GAAGCTGACCTTCCAGGTCACTGTGAGGAACCGCCACCATGGGAACACGAACGTGAACC
TTTAAAGAGAGTCTACCATTTACGCTGGGGCAGACGGTTCATTACCAGTGCGCCCAGG
GATTCAGGGCCCTACAGACCAGTCCTGCTGAAAGCACCTGCATGATGATCAACGGGGAG
CTGAGGTGGACCAGGCCCAGGCTCAAGTGCATACGTGAAGGGGAGCACGGTCAGGCTTC
AGATGACGCAGAGCCTCAGGAGAGCACGGAAGCTCCCCCTGGGAGTGGAACCTTCTTAC
CAACCAGGATGGCAGGGACCACAGATTTCCAGAAGCCCACAGATGAGATTGCAACGCTG
GATACGTTCATATTTACCACTGAGTACCAGATTGCAGTGGCCGGCTGCACCCTCCTGCT
CGCCAGCATCCTCCTCCTGAGCTGCCTCACCTGGCAGCGGAAATGGAAGAAGAACAGAA
GGACAATCTCGAGTCTAGAGGGCCCGCGGTTCAACAAAACTCATCTCAGAAGAGGAT
CTGAATATGCATACCGGTCATCATCACCATCACCATTGA

MEPSLLMWRFFVFIVVPGCVTEACHDDPPSLRNAMFKVFRYEVTMINCDCKTGFRRV
AVMRCVGDSSSHSAWENRCFCNSTSPAKNPVKQVTPAPEEQREKKPTDAQNQTPPEEAD
LPGHCEEPWPWEHEREPLKRVYHFTLGQTVHYQCAQGFALQTS PAESTCMMINGELRW
TRRLKCI REGEHGQASDDAEPQESTEAPPGSGTFLPTRMAGTTDFQKPTDEIATLDTF
IFTTEYQI AVAGCTLLLASILLLSCLTWQRKWKKNRRTISSLEGPRFEOKLISEEDLNM
HTGHHHHHH

pcDNA3.1-Bos-solIL-2R α -Myc-His

This expression vector encodes bovine soluble Myc-tagged IL-2R α (named soluble IL-2R α or sIL-2R α).

Cloned from cDNA. Except for a single nucleotide replacement (shaded yellow), the IL-2R α part of the sequence is identical with GenBank accession BC133546.

[for cloning details see (1); through primer choice a silent mutation was introduced at the single yellow-shaded C position in order to delete a gene-internal restriction site]

AAGCTTACCATGGAGCCAGCTTGCTGATGTGGAGGTTCTTCGTATTCATCGTGGTACC
TGGCTGCGTGACAGAGGCTTGTTCATGATGACCCCTCCGAGTCTCAGAAACGCCATGTTCA
AGGTCTTCAGGTACGAGGTGGGCACCATGATAAACTGCGACTGCAAGACAGGCTTCCGC
AGAGTGTGCGCCGTCATGCGCTGCGTGGGGGACTCCAGCCACTCTGCCTGGGAAAACAG
ATGCTTCTGCAACAGCACCTCCCCTGCTAAGAACCCAGTAAAACAAGTCACTCCTGCAC
CCGAAGAACAGAGGGAGAAAAAACCACAGATGCGCAGAACCAAACGCAGCCTCCGGAG
GAAGCTGACCTTCCAGGTCACTGTGAGGAACCGCCACCATGGGAACACGAACGTGAACC
TTTAAAGAGAGTCTACCATTTACGCTGGGGCAGACGGTTCATTACCAGTGCGCCCAGG
GATTCAGGGCCCTACAGACCAGTCCTGCTGAAAGCACCTGCATGATGATCAACGGGGAG
CTGAGGTGGACCAGGCCCAGGCTCAAGTGCATACGTGAAGGGGAGCACGGTCAGGCTTC
AGATGACGCAGAGCCTCAGGAGAGCACGGAAGCTCCCCCTGGGAGTGGAACCTTCTTAC
CAACCAGGATGGCAGGGACCACAGATTTCCAGAAGCCCACAGATGACTCGAGTCTAGAG

GGCCCGCGGTTCGAACAAAACTCATCTCAGAAGAGGATCTGAATATGCATACCGGTCA
TCATCACCATCACCATTGA

MEPSLLMWRFFVFIVVPGCVTEACHDDPPSLRNAMFKVFRYEVGTMINCDCKTGFRRV
AVMRCVGDSSSHSAWENRCFCNSTSPAKNPVKQVTPAPEEQREKKPTDAQNQTPPEEAD
LPGHCEEPPWEHEREPLKRVYHFTLGQTVHYQCAQGFALQTS PAESTCMMINGELRW
TRPRLKCI REGEHGQASDDAEPQESTEAPP GSGTFLPTRMAGTTDFQKPTDEIATLDTF
IFTTEYQI AVAGCTLLLASILLLSCLTWQRKWKKNRRTISSLEGPRFE **OKLISEEDL**NM
HTGHHHHHH

Supplementary file 2B.

Expression vector for a genetic fusion of human IL-15R α and IL-15.

pcDNA3.1-IL-2-Lead-RLI

Except for the leader sequence which is of human IL-2, the encoded sequence is identical to the RLI molecule described by Mortier *et al.* 2006 (5) which is a genetic fusion subsequently encoding a FLAG-tag, a large part of the human IL-15R α ectodomain, a glycine-serine linker (shaded blue), and human IL-15 (named RLI).

Amplified from commercial cDNA and using appropriate primers and overlap PCR.

GGTACCACCATGTACAGGATGCAACTCCTGTCTTGCATTGCACTAAGTCTTGCACCTTGT
CACAAACAGTGACTACAAGGATGACGATGACAAGATAGAAGGTAGGATCACATGCCCTC
CCCCATGTCCGTGGAACACGCAGACATCTGGGTCAAGAGCTACAGCTTGACTCCAGG
GAGCGGTACATTTGTAACCTCTGGTTTTCAAGCGTAAAGCCGGCACGTCCAGCCTGACGGA
GTGCGTGTGAAACAAGGCCACGAATGTCGCCCACTGGACAACCCCCAGTCTCAAATGCA
TTAGAGACCCTGCCCTGGTTCACCAAAGGCCAGCGCCACCCTCTGGAGGCTCCGGTGG
GGTGGGAGTGGCGGTGGATCCGGTGGCGGAGGCAGCCTGCAGAACTGGGTGAATGTAAT
AAGTGATTTGAAAAAATGAAGATCTTATTCAATCTATGCATATTGATGCTACTTTAT
ATACGGAAAGTGATGTTTACCCCAGTTGCAAAGTAACAGCAATGAAGTGCTTTCTCTTG
GAGTTACAAGTTATTTCACTTGAGTCCGGAGATGCAAGTATTCATGATACAGTAGAAAA
TCTGATCATCCTAGCAAACAACAGTTTGTCTTCTAATGGGAATGTAACAGAATCTGGAT
GCAAAGAATGTGAGGAACTGGAGGAAAAAATATTAAGAATTTTTGCAGAGTTTTGT
CATATTGTCCAAATGTTTCATCAACACTTCTTAA **TCTAGA**

MYRMQLLSICIALSLALVTNS **DYKDDDDK**IEGRITCPPPMSVEHADIWVKSYSLSRERY
ICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIRDPALVHQRPA **SGGSGGGGS**
GGGSGGGGSLQNWVNI SDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLELQ
VISLESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEEELEEKNIKEFLQSFVHIV
QMFINTS

Supplementary file 2C.

Expression vectors for (modified) trout cytokines and IL-15R α .

pcDNA3.1-trout-IL-2-FLAG

This expression vector encodes rainbow trout FLAG-tagged IL-2 (named IL-2). Cloned from cDNA. The *IL-2* part of the sequence is identical with GenBank accession FJ571512.

AAGCTTACCATGGACCGTCGTTACAGGATTTCCTTTTTGACGCTTTTTCTCACCGGTTG
TCTACAAGGAAACCCAATTTCCAGACTCCTAGCTGGAATCGATTATCTAGAAGAAAATA
TTACTTGTCCAGATTCAGTCTTCTATAACCAACTGATGTAGAGGATAGTTGCATTGTT
GCAGCATTGGCCTGTTCCATTAAGGAAGTGGACACTGTGAAAGTAGAATGCCTCGATAA
CGCGGTCCATCTGGAAAGTATGCAACACCACATCAGCATGACTGCCACGGACCTACAAA
AGACGATTGATAAGGAGAACAGCACAACGGACACTTCAGAATGCATCTGTGAAGACAAG
CGGTTGGAAAAGTCTTTCAAGGACTTCATTCAGAACATAAGACATTTAACTCAAGCTCA
TGCTGCAAAGCGTCTAAGTTCAGATTACAAGGATGACGACGATAAGTAACTCGAG

MDRRYRISFLTLFLTGCLQGNPIPRLLAGIDYLEENITCPDSVFYTPTDVEDSCIVAAL
ACSIKELDTVKVECLDNAVHLESMQHHSMTATDLQKTIDKENSTTDTSECICEDKRLE
KSFKDFIQNIRHLTQAHAARKRLSSDYKDDDDK

pcDNA3.1-trout-IL-2-(non-tagged)

This expression vector encodes rainbow trout non-tagged IL-2 [named IL-2(N)]. Cloned from cDNA. The *IL-2* part of the sequence is identical with GenBank accession FJ571512.

AAGCTTACCATGGACCGTCGTTACAGGATTTCCTTTTTGACGCTTTTTCTCACCGGTTG
TCTACAAGGAAACCCAATTTCCAGACTCCTAGCTGGAATCGATTATCTAGAAGAAAATA
TTACTTGTCCAGATTCAGTCTTCTATAACCAACTGATGTAGAGGATAGTTGCATTGTT
GCAGCATTGGCCTGTTCCATTAAGGAAGTGGACACTGTGAAAGTAGAATGCCTCGATAA
CGCGGTCCATCTGGAAAGTATGCAACACCACATCAGCATGACTGCCACGGACCTACAAA
AGACGATTGATAAGGAGAACAGCACAACGGACACTTCAGAATGCATCTGTGAAGACAAG
CGGTTGGAAAAGTCTTTCAAGGACTTCATTCAGAACATAAGACATTTAACTCAAGCTCA
TGCTGCAAAGCGTCTAAGTTCATAACTCGAG

MDRRYRISFLTLFLTGCLQGNPIPRLLAGIDYLEENITCPDSVFYTPTDVEDSCIVAAL
ACSIKELDTVKVECLDNAVHLESMQHHSMTATDLQKTIDKENSTTDTSECICEDKRLE
KSFKDFIQNIRHLTQAHAARKRLSS

pcDNA3.1-trout-IL-15-FLAG

This expression vector encodes rainbow trout FLAG-tagged IL-15 (named IL-15). Cloned from cDNA. The *IL-15* part of the sequence is identical with GenBank accession NM_001124395.

AAGCTTACCATGACAGGTTTTTTGACAGTGCTCCTTTTTTGCATTCGTTTATTGGAGCG
CAGGACAAAGAAAAGTGTGCGATGGATCTGTCTCTTCTGGGGTTTCCATTACTATCCAC
ACCAGCGTCTGAACATTGAGCTCTGGAATTGCTTCATAATATTGAGCTGCCTGAGTGCC
ACCGCACATTTGCCCATTTGCTGGTGCTGCTGAAACACACGGGATGACAATAGATGACGT
TAAAGAGCTTCAGTCGGAGTTGAAAACTTGAAAAGTACCATAGAAAAATCAGATGCCT
GTTTGTATGCTCCTACCAACGATGACATCTACAATGACCACTGCATCTTTAAGTTCATG
CACTGTTATTTATTGGAGTTGGAGGTTGTCCTGTTTGGAGGACATGTCGGTCCACAGATAA
CTACCATGACGAAATAAAAACATCCATCTACCATCGGAAAAACATTTGGAAGAACATG
AACGCCAATATAACAGTTCTAGATGCTCACCGTGTGAGGCACAGAGAGTTGCCAACTCC
ACAATATTCTCTACAACCTGGAGCGCCTTTTTGGAGAGAATAGGGCAAACCTGTCAGTGA
TTACAAGGATGACGACGATAAGTAACTCGAG

MTGFLT VLLFCIRLLERRTKKSVRWICLFWGFHYYPHQRLNIELWNCFIILSCLSATAH
LPIAGAAETHGMTIDDVKELQSELKNLSTIEKSDACLYAPTNDIYNDHCIFKFMHCY
LLELEVVLFEEDMSVTDNYHDEIKTSIYHRKKHLEEHERQYNSSRCSPCEAQRVANSTIF
LYNLERLLERIGQTVSDYKDDDDK

pcDNA3.1-trout-IL-15-(non-tagged)

This expression vector encodes rainbow trout non-tagged IL-15 [named IL-15(N)]. Cloned from cDNA. The *IL-15* part of the sequence is identical with GenBank accession NM_001124395.

AAGCTTACCATGACAGGTTTTTTGACAGTGCTCCTTTTTTGCATTCGTTTATTGGAGCG
CAGGACAAAGAAAAGTGTGCGATGGATCTGTCTCTTCTGGGGTTTCCATTACTATCCAC
ACCAGCGTCTGAACATTGAGCTCTGGAATTGCTTCATAATATTGAGCTGCCTGAGTGCC
ACCGCACATTTGCCCATTTGCTGGTGCTGCTGAAACACACGGGATGACAATAGATGACGT
TAAAGAGCTTCAGTCGGAGTTGAAAACTTGAAAAGTACCATAGAAAAATCAGATGCCT
GTTTGTATGCTCCTACCAACGATGACATCTACAATGACCACTGCATCTTTAAGTTCATG
CACTGTTATTTATTGGAGTTGGAGGTTGTCCTGTTTGGAGGACATGTCGGTCCACAGATAA
CTACCATGACGAAATAAAAACATCCATCTACCATCGGAAAAACATTTGGAAGAACATG
AACGCCAATATAACAGTTCTAGATGCTCACCGTGTGAGGCACAGAGAGTTGCCAACTCC
ACAATATTCTCTACAACCTGGAGCGCCTTTTTGGAGAGAATAGGGCAAACCTGTCAGTTG
ACTCGAG

MTGFLT VLLFCIRLLERRTKKSVRWICLFWGFHYYPHQRLNIELWNCFIILSCLSATAH
LPIAGAAETHGMTIDDVKELQSELKNLSTIEKSDACLYAPTNDIYNDHCIFKFMHCY
LLELEVVLFEEDMSVTDNYHDEIKTSIYHRKKHLEEHERQYNSSRCSPCEAQRVANSTIF
LYNLERLLERIGQTVS

pcDNA3.1-trout-IL-15La-FLAG

This expression vector encodes rainbow trout FLAG-tagged IL-15La (named IL-15La). Cloned from cDNA. The *IL-15La* part of the sequence is identical with GenBank accession MK619679.

AAGCTTACCATGCTGAGGAGACAGAGGACTGACACTCTTCTAGCCCTTTTGCTGTGGTT
TCTCTTCTTCATCGCCATGACAATGAAACAGGCATATGGAAAATCCATGTGCAGTAAAG
AACTTCCCGGAATTGTGCGAAAATGCATTGAGGAAGTTCACAAGATGGAATCATTGAT
TGCAGACTGTACACCCCACTTTGGCTGATTATCAGAAGTGCCCCACGTCCACACTCAT
ATGCTTTGAAAAAGAAGTGAATGTCCTGGTGTGTTAGAATCTGGGAATAAGTCCTCACCCA
TATACAAGCCAAAACACTATCCATCCGGCTGAAGTCCTTGATCAAACAGAAAGAAGGTGCC
AACTGTCCAGACTGTGAGGCCACAGAGAAAGGGCAGCAAAGGATTTCTTAACAACATT
GCAAACAATTCTGGAGTGGATGAACGATCAGGGGTGTCGGAAGCCATCCAGCCATGATT
ACAAGGATGACGACGATAAGTGACTCGAG

MLRRQRTDTLLALLLWFLFFIAMTMKQAYGKSMCSKELPGIVRKCIEEVHKMESFDCRL
YTPTLADYQKCPTSTLICFEKEVNVLVLESGNKSSPIYKPKLSIRLKSILIKQKEGANCP
DCEAHRERA AKDFLTTLQTILEWMNDQGCRKPSSH DYKDDDDK

pcDNA3.1-trout-IL-15La-(non-tagged)

This expression vector encodes rainbow trout un-tagged IL-15La [named IL-15La(N)]. Cloned from cDNA. The *IL-15La* part of the sequence is identical with GenBank accession MK619679.

AAGCTTACCATGCTGAGGAGACAGAGGACTGACACTCTTCTAGCCCTTTTGCTGTGGTT
TCTCTTCTTCATCGCCATGACAATGAAACAGGCATATGGAAAATCCATGTGCAGTAAAG
AACTTCCCGGAATTGTGCGAAAATGCATTGAGGAAGTTCACAAGATGGAATCATTGAT
TGCAGACTGTACACCCCACTTTGGCTGATTATCAGAAGTGCCCCACGTCCACACTCAT
ATGCTTTGAAAAAGAAGTGAATGTCCTGGTGTGTTAGAATCTGGGAATAAGTCCTCACCCA
TATACAAGCCAAAACACTATCCATCCGGCTGAAGTCCTTGATCAAACAGAAAGAAGGTGCC
AACTGTCCAGACTGTGAGGCCACAGAGAAAGGGCAGCAAAGGATTTCTTAACAACATT
GCAAACAATTCTGGAGTGGATGAACGATCAGGGGTGTCGGAAGCCATCCAGCCATTGAC
TCGAG

MLRRQRTDTLLALLLWFLFFIAMTMKQAYGKSMCSKELPGIVRKCIEEVHKMESFDCRL
YTPTLADYQKCPTSTLICFEKEVNVLVLESGNKSSPIYKPKLSIRLKSILIKQKEGANCP
DCEAHRERA AKDFLTTLQTILEWMNDQGCRKPSSH

pcDNA3.1-trout-IL-15Lb-FLAG

This expression vector encodes rainbow trout FLAG-tagged IL-15Lb (named IL-15Lb). Cloned from cDNA. The *IL-15Lb* part of the sequence is identical with GenBank accession MK619680.

AAGCTTACCATGTTGAGGAGACAGAGAAGCTGGCTCTCTTCTGAACGCTTTGCTGTGGTT
TCTCTTCTTCATTGCCATGACAATGAAACAGGCTTATGGACAATCCATTAGCAGTTCAG
AAATTCACCAAATTGTGAAAACATTTATTAAGGAGGAAGTTCACAGGATGGAATCATT
GATTGCAGACTGTACACCCCAACTTTAGCTGATTATAAGAAATGTCCCAGGTCCACACT
CACATGCTTTACGACAGAAGTAAAAGTCCTGATGTTAGAATATGGGAAACGTTCTTCAT
CCTTACACCAGAAAAGACTCACCAAACGACTGACTAAATTGATGTCCTTGATAAAACAG
AAGGATGGTGCCAACACTGTCCACACTGTGAGGTCCACAGAGAACAGGCAGCAAATGATTT
CTTAACAACATTACTAGGGATTCTGGAGTGGATGAACAATCAGGGGTCTCAGTTGCCAG
ACAGCCACGATTACAAGGATGACGACGATAAGTGACTCGAG

MLRRQRTGSLLNALLWFLFFIAMTMKQAYGQSSISSEIHQIVKTFIKEEVHRMESFDCR
LYTPTLADYKKCPRSTLTCTTTEVKVLMLEYGKRSSSLHQKRLTKRLTKLMSLIKQKDG
ANCPHCEVHREQAANDFLTTLGILEWMNNQGSQLPDSHDYKDDDDK

pcDNA3.1-trout-IL-15Lb-(non-tagged)

This expression vector encodes rainbow trout un-tagged IL-15Lb [named IL-15Lb(N)]. Cloned from cDNA. The *IL-15Lb* part of the sequence is identical with GenBank accession MK619680.

AAGCTTACCATGTTGAGGAGACAGAGAAGCTGGCTCTCTTCTGAACGCTTTGCTGTGGTT
TCTCTTCTTCATTGCCATGACAATGAAACAGGCTTATGGACAATCCATTAGCAGTTCAG
AAATTCACCAAATTGTGAAAACATTTATTAAGGAGGAAGTTCACAGGATGGAATCATT
GATTGCAGACTGTACACCCCAACTTTAGCTGATTATAAGAAATGtCCCAGGTCCACACT
CACATGCTTTACGACAGAAGTAAAAGTCCTGATGTTAGAATATGGGAAACGTTCTTCAT
CCTTACACCAGAAAAGACTCACCAAACGACTGACTAAATTGATGTCCTTGATAAAACAG
AAGGATGGTGCCAACACTGTCCACACTGTGAGGTCCACAGAGAACAGGCAGCAAATGATTT
CTTAACAACATTACTAGGGATTCTGGAGTGGATGAACAATCAGGGGTCTCAGTTGCCAG
ACAGCCACTGACTCGAG

MLRRQRTGSLLNALLWFLFFIAMTMKQAYGQSSISSEIHQIVKTFIKEEVHRMESFDCR
LYTPTLADYKKCPRSTLTCTTTEVKVLMLEYGKRSSSLHQKRLTKRLTKLMSLIKQKDG
ANCPHCEVHREQAANDFLTTLGILEWMNNQGSQLPDSH

pcDNA3.1-IL-2-Lead-RLI-trout-IL-15La

This expression vector is based on the RLI construct described by Mortier *et al.* 2006 (3), and encodes a genetic fusion of a human IL-2 leader, a FLAG-tag, a large part of the human IL-15R α ectodomain, a glycine-serine linker (shaded blue), and the rainbow trout IL-15La sequence (named IL-15La-h-RLI; indicated as “RLI” in main text Fig. 8).

The linker plus *IL-15La* part were commercially ordered (Invitrogen) with the codons optimized for expression in insect cells, and fused to the N-terminal coding part by using the above described plasmid pcDNA3.1-*IL-2-Lead-RLI*, appropriate primers and overlap PCR.

GGTACCACCATGTACAGGATGCAACTCCTGTCTTGCATTGCACTAAGTCTTGCACCTTGT
CACAAACAGTGACTACAAGGATGACGATGACAAGATAGAAGGTAGGATCACATGCCCTC
CCCCATGTCCGTGGAACACGCAGACATCTGGGTCAAGAGCTACAGCTTGTACTCCAGG
GAGCGGTACATTTGTAACCTCTGGTTTCAAGCGTAAAGCCGGCACGTCCAGCCTGACGGA
GTGCGTGTGAAACAAGGCCACGAATGTGCGCCACTGGACAACCCCCAGTCTCAAATGCA
TTAGAGACCCTGCCCTGGTTACCAAAGGCCAGCGCCACCCTCTGGAGGCTCCGGTGG
GGTGGGAGTGGCGGTGGATCCGGTGGCGGAGGCAGCAAGCAAGCTTACGGCAAGTCCAT
GTGCTCCAAGGAAGTCCCGGTATCGTGCGCAAGTGCATCGAAGAGGTGCACAAGATGG
AATCCTTCGACTGCCGTCTGTACACCCCCACCCTGGCTGACTACCAGAAGTGCCCTACC
TCCACCCTGATCTGCTTCGAGAAGGAAGTGAACGTCTTGGTCTCGAGTCCGGCAACAA
GTCCTCCCCAATCTACAAGCCCAAGCTGTCCATCCGTCTGAAGTCCCTGATCAAGCAGA
AGGAAGGCGCTAACTGCCCGACTGCGAGGCTCACAGAGAGCGTGCTGCTAAGGACTTC
CTGACCACCCTGCAGACCATCCTCGAGTGGATGAACGACCAGGGTTGCCGCAAGCCCTC
CTCCCACTAACTCTAGA

MYRMQLLSCIALSLALVTNSDYKDDDDKIEGRITCPPPMSVEHADIWVKSYSLSRERY
ICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIRDPALVHQRPAPPSGGSGGGGS
GGSGGGGSKQAYGKSMCSKELPGIVRKCIEEVHKMESFDCRLYTPTLADYQKCPTSTL
ICFEKEVNVLVLESNGKSSPIYKPKLSIRLKSLIKQKEGANCPDCEAHRERAAKDFLTT
LQTILEWMNDQGCRKPSSH

pcDNA3.1-trout-IL-15R α -Myc

This expression vector encodes rainbow trout full-length Myc-tagged IL-15R α (named IL-15R α).

Cloned and polished from cDNA. The *IL-15R α* part of the sequence is, except for 1 silent nucleotide exchange, identical with GenBank accession GFIN01030410.

AAGCTTACCATGCGGCTGCGTCTCCTTTCCCTCCTTCTTATCGTCAAAGTGTGTCGACT
CAATACTGTCTAGTCCAGACGTGTGCCACCCCTGTCTCATTGGAAGCTACCAAGCCTT
TGAACACGAGGGAAATTTATCAAGAAAACGAATCCCTGCGCTACCAGTGTGTGGATGGC
TATGTGAGGAAGGCGGGAACCTCCAATCTCATCAAGTGCAAACGGATTGGTCAGTTCT
TCAGTGGACACTCCCAACACTGATATGCATACCTGACCCATCCATTCCAACACTACGATAG
AACCTTCAACAACCCACAAGACAACACTCAATTTCTACAACGCACCTCTCAGTGCCCA

GAGAGAGTTAGCCCAAGACCAACCACTCTCAGTTCACCTTTTATCTCAAACACTGCATTC
TGTCACAGAGACCCAAGAGGTCATTACTACGGTTACAGTGACAACCACCATCATCAGTG
ACATCACATCAGTGCCAGTGAATAAGTCCAGTGCGGTGACCTCTGTCTCCACGAAGACT
GTTTCTCCATCAGAGAGTTACTGTGTCCCAACAGAGCCCCAACCTCCAGTGACTATCC
AGTTGGCGATGGAAGCACTGGGAAGAGTTCTGCTACAGCCATATATGCAGGTGTAGGTG
GGTGGTAGTAACCATCTTGCTGGTTGCAGCGTTTGGAGTACTAATGTTTTGGAGAAGG
AAACGGATGCAGCGGCTTGATCTGTGCCAACACCAGACGAAATTATGCAAATGAATGT
TGTGACAGAACAAAACTCATCTCAGAAGAGGATCTGTGACTCGAG

MRLRLLSLLLIVKVCRLNTVSPDVCPPLSHWKLTKPLNTREIYQENESLRYQCVDGYVR
KAGTSNLIKCKRIGQVLQWTLPTLICIPDPSIPTTIEPSTTHKTTQFPTHLSVPTERV
SPRPTTSSLLSQTLHSVTETQEVITTVTVTTTIIISDITSVPVNKSSAVTSVSTKTVSP
SESYCVPNRAPTSSDYFVGDGSTGKSSATAIYAGVGGVVVTILLVAAFVLMFWRKRK
QRDLSPPTDEIMQMNVTQKLISEEDL

pcDNA3.1-trout-solIL-15R α -Myc

This expression vector encodes rainbow trout soluble Myc-tagged IL-15R α (named soluble IL-15R α or sIL-15R α).

Cloned and polished from cDNA. The *IL-15R α* part of the sequence is, except for 1 silent nucleotide exchange, identical with GenBank accession GFIN01030410.

AAGCTTACCATGCGGCTGCGTCTCCTTTCCCTCCTTCTTATCGTCAAAGTGTGTCGACT
CAATACTGTCAGTCCAGACGTGTGCCACCCCTGTCTCATTGGAAGCTCACCAAGCCTT
TGAACACGAGGGAAATTTATCAAGAAAACGAATCCCTGCGCTACCAGTGTGTGGATGGC
TATGTGAGGAAGGCGGGAACCTCCAATCTCATCAAGTGCAAACGGATTGGTCAGGTTCT
TCAGTGGACACTCCCAACACTGATATGCATACCTGACCCATCCATTCCAACACTACGATAG
AACCTTCAACAACCCACAAGACAACCTCAAGAACAAAACTCATCTCAGAAGAGGATCTG
TGACTCGAG

MRLRLLSLLLIVKVCRLNTVSPDVCPPLSHWKLTKPLNTREIYQENESLRYQCVDGYVR
KAGTSNLIKCKRIGQVLQWTLPTLICIPDPSIPTTIEPSTTHKTTQKLISEEDL

Supplementary file 2D.

Vectors for expressing (modified) trout cytokines and trout IL-15R α in insect cells using a baculovirus system.

pFBD-P10Uhis-ieGFP-trout-IL-2-FLAG

This expression vector encodes rainbow trout FLAG-tagged IL-2 (named IL-2). Cloned from cDNA. The *IL-2* part of the sequence is identical with GenBank accession FJ571512.

CCCGGGACCATGGACCGTCGTTACAGGATTTCCTTTTTGACGCTTTTTTCTCACCGGTTG
TCTACAAGGAAACCCAATCCCAGACTCCTAGCTGGAATCGATTATCTAGAAGAAAATA
TTACTTGTCCAGATTCAGTCTTCTATACACCAACTGATGTAGAGGATAGTTGCATTGTT
GCAGCATTGGCCTGTTCCATTAAGGAAGTGGACACTGTGAAAGTAGAATGCCTCGATAA
CGCGGTCCATCTGGAAAGTATGCAACACCACATCAGCATGACTGCCACGGACCTACAAA
AGACGATTGATAAGGAGAACAGCACAACGGACACTTCAGAATGCATCTGTGAAGACAAG
CGGTTGGAAAAGTCTTTCAAGGACTTCATTTCAGAACATAAGACATTTAACTCAAGCTCA
TGCTGCAAAGCGTCTAAGTTCAGATTACAAGGATGACGACGATAAGTAAACCCGGG

MDRRYRISFLTLFLTGCLQGNPIPRLLAGIDYLEENITCPDSVFYTPPTDVEDSCIVAAL
ACSIKELDTVKVECLDNAVHLESMQHHISMTATDLQKTIDKENSTTDTSECI CEDKRLE
KSFKDFIQNIRHLTQAHAARKRLSSDYKDDDDK

pFBD-P10Uhis-ieGFP-trout-IL-15-FLAG

This expression vector encodes rainbow trout FLAG-tagged IL-15 (named IL-15). The sequence was commercially ordered (Invitrogen). The signal peptide is replaced with that of rainbow trout IL-2. The *IL-2* part of the sequence is identical with GenBank accession FJ571512. The *IL-15* part of the sequence is identical with GenBank accession NM_001124395. The encoded IL-2 leader sequence is shaded blue.

CCCGGGACCATGGACCGTCGTTACAGGATTTCCTTTTTGACGCTTTTTTCTCACCGGTTG
TCTACAAGGAGCTGAAACACACGGGATGACAATAGATGACGTTAAAGAGCTTCAGTCGG
AGTTGAAAAACTTGAAAAGTACCATAGAAAAATCAGATGCCTGTTTGTATGCTCCTACC
AACGATGACATCTACAATGACCACTGCATCTTTAAGTTCATGCACTGTTATTTATTGGA
GTTGGAGGTTGTCCTGTTTGGAGACATGTCGGTACAGATAACTACCATGACGAAATAA
AAACATCCATCTACCATCGGAAAAAACATTTGGAAGAACATGAACGCCAATATAACAGT
TCTAGATGCTCACCGTGTGAGGCACAGAGAGTTGCCAACTCCACAATATTCCTCTACAA
CCTGGAGCGCCTTTTGGAGAGAATAGGGCAAACGTTCAGTGATTACAAGGATGACGACG
ATAAGTGACCCCGG

MDRRYRISFLTLFLTGCLQGAETHGMTIDDVKELQSELKNLSTIEKSDACL YAPTND
IYNDHCIFKFMHCYLLLELVLFEDMSVTDNYHDEIKTSIYHRKKHLEEHERQYNSSRC
SPCEAQRVANSTIFLYNLERLLERIGQTVSDYKDDDDK

pFBD-P10Uhis-ieGFP-trout-IL-15La-FLAG

This expression vector encodes rainbow trout FLAG-tagged IL-15La (named IL-15La). Cloned from cDNA. The *IL-15La* part of the sequence is identical with GenBank accession MK619679.

CCCGGGACCATGCTGAGGAGACAGAGGACTGACACTCTTCTAGCCCTTTTGCTGTGGTT
TCTCTTCTTCATCGCCATGACAATGAAACAGGCATATGGAAAATCCATGTGCAGTAAAG
AACTTCCCAGGAATTGTGCGAAAATGCATTGAGGAAGTTCACAAGATGGAATCATTGAT
TGCAGACTGTACACCCCACTTTGGCTGATTATCAGAAGTGCCCCACGTCCACACTCAT
ATGCTTTGAAAAAGAAGTGAATGTCCTGGTGTTAGAATCTGGGAATAAGTCCACCCA
TATACAAGCCAAAACACTATCCATCCGGCTGAAGTCCTTGATCAAACAGAAAGAAGGTGCC
AACTGTCCAGACTGTGAGGCCACAGAGAAAGGGCAGCAAAGGATTTCTTAACAACATT
GCAAACAATTCTGGAGTGGATGAACGATCAGGGGTGTCGGAAGCCATCCAGCCATGATT
ACAAGGATGACGACGATAAGTAACCCGGG

MLRRQRTDLLLALLLWFLFFIAMTMKQAYGKSMCSKELPGIVRKCIEEVHKMESFDCRL
YPTTLADYQKCPTSTLICFEKEVNVLVLESGNKSSPIYKPKLSIRLKSILIKQKEGANCP
DCEAHRERA AKDFLTTLQTILEWMNDQGCRKPSSH DYKDDDDK

pFBD-P10Uhis-ieGFP-trout-solIL-15R α -Myc

This expression vector encodes rainbow trout soluble Myc-tagged IL-15R α (named soluble IL-15R α or sIL-15R α).

Cloned from cDNA. The *IL-15R α* part of the sequence is, except for 1 silent nucleotide exchange, identical with GenBank accession GFIN01030410.

CCCGGGACCATGCGGCTGCGTCTCCTTTCCCTCCTTCTTATCGTCAAAGTGTGTCGACT
CAATACTGTCAGTCCAGACGTGTGCCACCCCTGTCTCATTGGAAGCTCACCAAGCCTT
TGAACACGAGGGAAATTTATCAAGAAAACGAATCCCTGCGCTACCAGTGTGTGGATGGC
TATGTGAGGAAGGCGGGAACCTCCAATCTCATCAAGTGCAAACGGATTGGTCAGGTTCT
TCAGTGGACACTCCCAACACTGATATGCATACCTGACCCATCCATTCCAACACTACGATAG
AACCTTCAACAACCCACAAGACAACACTCAAGAACAAAACTCATCTCAGAAGAGGATCTG
TGACCCGGG

MRLRLLSLLLIVKVCRLN TVSPDVC PPLSHWKLTKPLN TREIYQENESLRYQCVDGYVR
KAGTSNLIKCKRIGQVLQWTLPTLICIPDPSIPTTIEPSTTHKTTQEQKLISEEDL

pFBD-P10Uhis-ieGFP-trout-IL-15-RLI

This expression vector is based on the RLI construct described by Mortier *et al.* 2006 (3), and encodes a genetic fusion of a human IL-2 leader, a FLAG-tag, a large part of the trout IL-15R α ectodomain, a glycine-serine linker (shaded blue), and the rainbow trout IL-15 sequence (named IL-15-RLI).

The sequence was commercially ordered (Invitrogen). The *IL-15R α* part of the sequence is, except for 1 silent nucleotide exchange, identical with GenBank accession GFIN01030410. The *IL-15* part of the sequence is identical with GenBank accession NM_001124395.

CCCGGGACCATGTACAGGATGCAACTCCTGTCTTGCATTGCACTAAGTCTTGCACCTTGT
CACAAACAGTGACTACAAGGATGACGATGACAAGAATACTGTCAGTCCAGACGTGTGCC
CACCCCTGTCTCATTGGAAGCTCACCAAGCCTTTGAACACGAGGGAAATTTATCAAGAA
AACGAATCCCTGCGCTACCAGTGTGTGGATGGCTATGTGAGGAAGGCGGGAAACCTCCAA
TCTCATCAAGTGCAAACGGATTGGTTCAGGTTCTTCAGTGGACACTCCCAACACTGATAT
GCATACCTGACCCATCCATTCCAACACTACGATAGAACCTTCAACATCTGGAGGCTCCGGT
GGAGGTGGGAGTGGCGGTGGATCCGGTGGCGGAGGCAGCGAAACACACGGGATGACAAT
AGATGACGTTAAAGAGCTTCAGTCGGAGTTGAAAAACTTGAAAAGTACCATAGAAAAAT
CAGATGCCTGTTTGTATGCTCCTACCAACGATGACATCTACAATGACCACTGCATCTTT
AAGTTCATGCACTGTTATTTATTGGAGTTGGAGGTTGTCCTGTTTGAGGACATGTCCGT
CACAGATAACTACCATGACGAAATAAAAACATCCATCTACCATCGGAAAAAACATTTGG
AAGAACATGAACGCCAATATAACAGTTCTAGATGCTCACCGTGTGAGGCACAGAGAGTT
GCCAACTCCACAATATTCCTCTACAACCTGGAGCGCCTTTTGGAGAGAATAGGGCAAAC
TGTCAGTTGACCCGGG

MYRMQLLSICIALSLALVTNSDYKDDDDKNTVSPDVCPLSHWKLTKPLNTREIYQENES
LRYQCVDGYVRKAGTSNLIKCKRIGQVLQWTLPTLICIPDPSIPTTIEPSTSGGSGGGG
SGGSGGGGSETHGMTIDDVKELQSELKNLKSTIEKSDACLYAPTNDIDIYNDHCIFKFM
HCYLLELEVVLFEEDMSVTDNYHDEIKTSIYHRKKHLEEHERQYNSSRCSPCEAQRVANS
TIFLYNLERLLERIGQTVS

pFBD-P10Uhis-ieGFP-trout-IL-15La-RLI

This expression vector is based on the RLI construct described by Mortier *et al.* 2006 (3), and encodes a genetic fusion of a human IL-2 leader, a FLAG-tag, a large part of the rainbow trout IL-15R α ectodomain, a glycine-serine linker (shaded blue), and the rainbow trout IL-15La sequence (named IL-15La-RLI).

The sequence was commercially ordered (Invitrogen). The *IL-15R α* part of the sequence is, except for 1 silent nucleotide exchange, identical with GenBank accession GFIN01030410. The *IL-15La* codons were optimized for expression in insect cells.

CCCGGGACCATGTACAGGATGCAACTCCTGTCTTGCATTGCACTAAGTCTTGCACCTTGT
CACAAACAGTGACTACAAGGATGACGATGACAAGAATACTGTCAGTCCAGACGTGTGCC
CACCCCTGTCTCATTGGAAGCTCACCAAGCCTTTGAACACGAGGGAAATTTATCAAGAA
AACGAATCCCTGCGCTACCAGTGTGTGGATGGCTATGTGAGGAAGGCGGGAAACCTCCAA

TCTCATCAAGTGCAAACGGATTGGTCAGGTTCTTCAGTGGACACTCCCAACACTGATAT
GCATACCTGACCCATCCATTCCAACACTACGATAGAACCCTTCAACATCTGGAGGCTCCGGT
GGAGGTGGGAGTGGCGGTGGATCCGGTGGCGGAGGCAGCAAGCAAGCTTACGGCAAGTC
CATGTGCTCCAAGGAACTGCCCCGTATCGTGCGCAAGTGCATCGAAGAGGTGCACAAGA
TGGAATCCTTCGACTGCCGTCTGTACACCCCCACCCTGGCTGACTACCAGAAGTGCCCT
ACCTCCACCCTGATCTGCTTCGAGAAGGAAGTGAACGTCCTGGTCCTCGAGTCCGGCAA
CAAGTCCTCCCAATCTACAAGCCCAAGCTGTCCATCCGTCTGAAGTCCCTGATCAAGC
AGAAGGAAGGCGCTAACTGCCCCGACTGCGAGGCTCACAGAGAGCGTGCTGCTAAGGAC
TTCCTGACCACCCTGCAGACCATCCTCGAGTGGATGAACGACCAGGGTTGCCGCAAGCC
CTCCTCCCCTAA**CCCGGG**

MYRMLLSICIALSLALVTNS**DYKDDDDK**NTVSPDVCPPLSHWKLTkPLNTREIYQENES
LRYQCVDGYVRKAGTSNLIKCKRIGQVLQWTLPTLICIPDPSIPTTIEPST**SGGS****SGGGG**
SGGS**SGGGG**KQAYGKSMCSKELPGIVRKCIIEVHKMESFDCRLYTPTLADYQKCPST
LICEFEKVNVLVLESGNKSSPIYKPKLSIRLKSLIKQKEGANCPDCEAHRERAADFLT
TLQTILEWMNDQGCRKPSSH

References used in this file

1. Dijkstra JM, Takizawa F, Fischer U, Friedrich M, Soto-Lampe V, Lefèvre C, et al. Identification of a gene for an ancient cytokine, interleukin 15-like, in mammals; interleukins 2 and 15 co-evolved with this third family member, all sharing binding motifs for IL-15Ra. *Immunogenetics*. (2014) 66:93–103. doi: 10.1007/s00251-013-0747-0
2. Yamaguchi T, Schares S, Fischer U, Dijkstra JM. Identification of a fourth ancient member of the IL-3/IL-5/GM-CSF cytokine family, KK34, in many mammals. *Dev Comp Immunol*. (2016) 65:268–79. doi: 10.1016/j.dci.2016.07.018
3. Mortier E, Quéméner A, Vusio P, Lorenzen I, Boublik Y, Grötzinger J, et al. Soluble interleukin-15 receptor alpha (IL-15R alpha)-sushi as a selective and potent agonist of IL-15 action through IL-15R beta/gamma. Hyperagonist IL-15 × IL-15R alpha fusion proteins. *J Biol Chem*. (2006) 281:1612–9. doi: 10.1074/jbc.M508624200
4. Ring AM, Lin JX, Feng D, Mitra S, Rickert M, Bowman GR, et al. Mechanistic and structural insight into the functional dichotomy between IL-2 and IL-15. *Nat Immunol*. (2012) 13:1187–95. doi: 10.1038/ni.2449
5. Bessard A, Solé V, Bouchaud G, Quéméner A, Jacques Y. High antitumor activity of RLI, an interleukin-15 (IL-15)-IL-15 receptor alpha fusion protein, in metastatic melanoma and colorectal cancer. *Mol Cancer Ther*. (2009) 8:2736–45. doi: 10.1158/1535-7163.MCT-09-0275
6. Vincent M, Bessard A, Cochonneau D, Teppaz G, Solé V, Maillason M, et al. Tumor targeting of the IL-15 superagonist RLI by an anti-GD2 antibody strongly enhances its antitumor potency. *Int J Cancer*. (2013) 133:757–65. doi: 10.1002/ijc.28059