

## Supplemental Material

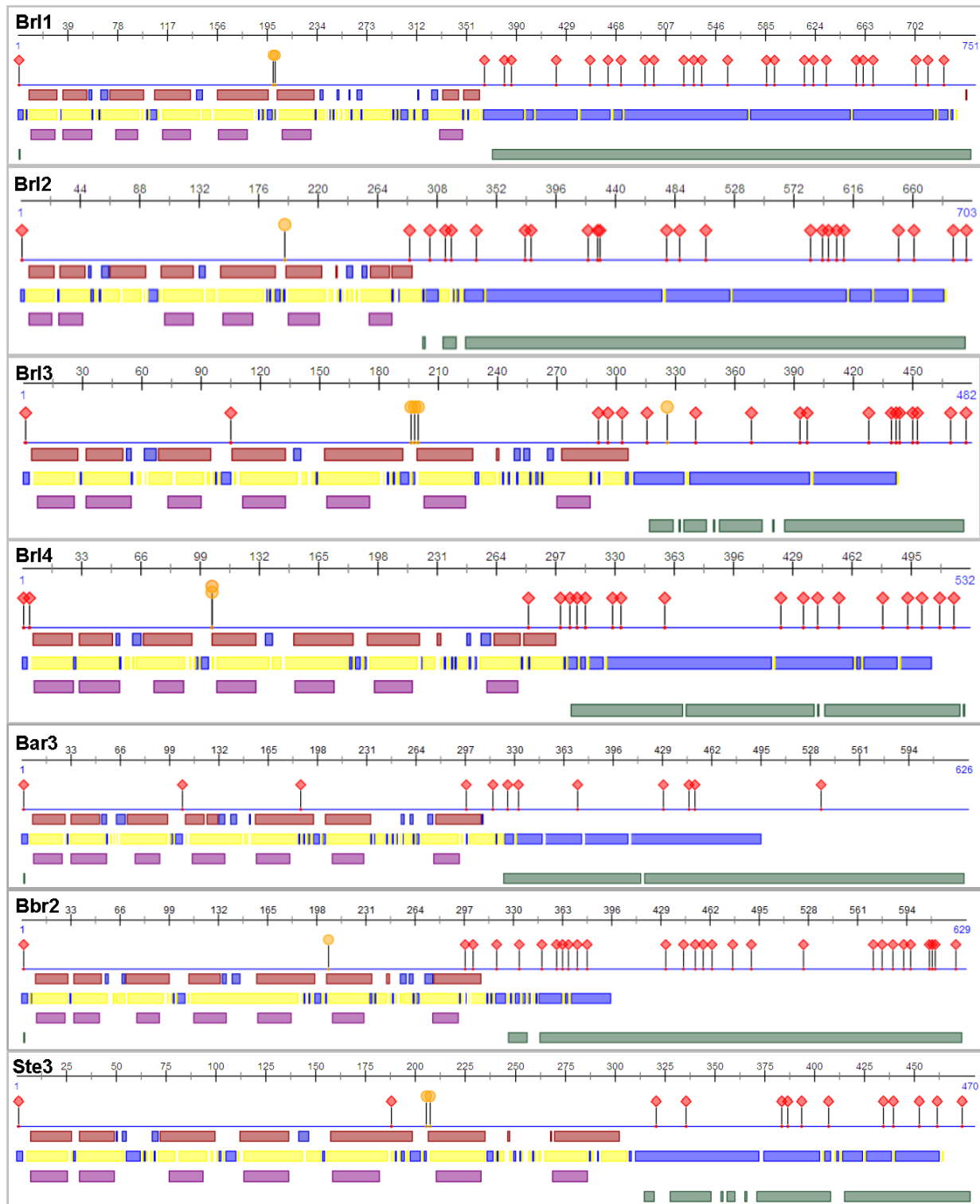
**Table S1: *S. commune* wild-type and mutant strains used in this study.**

| Strain   | Mating type  | Genotype   | Origin         |
|----------|--|--|----------------|
| 12-43    | <i>A</i> <sub>3,5</sub> <i>B</i> <sub>2,2</sub>  | <i>ura</i> <sup>-</sup>  | JMRC, FSU 3214 |
| 4-39     | <i>A</i> <sub>4,6</sub> <i>B</i> <sub>3,2</sub>  |  | JMRC, FSU 2896 |
| T33      | <i>A</i> <sub>3,1</sub> <i>B</i> <sub>4,7</sub>  | <i>trp</i> <sup>-</sup> ; <i>ura</i> <sup>-</sup>  | JMRC, FSU 3446 |
| evc      | <i>A</i> <sub>3,1</sub> <i>B</i> <sub>4,7</sub>  | <i>trp</i> <sup>-</sup> ; <i>ura</i> <sup>+</sup>  | This study     |
| Brl1OE   | <i>A</i> <sub>3,1</sub> <i>B</i> <sub>4,7</sub>  | <i>trp</i> <sup>-</sup> ; <i>tef</i> <sup>P</sup> :: <i>brl1</i>                               | This study     |
| Brl2OE   | <i>A</i> <sub>3,1</sub> <i>B</i> <sub>4,7</sub>  | <i>trp</i> <sup>-</sup> ; <i>ble</i> ; <i>tef</i> <sup>P</sup> :: <i>brl2</i>                  | This study     |
| Brl3OE   | <i>A</i> <sub>3,1</sub> <i>B</i> <sub>4,7</sub>  | <i>trp</i> <sup>-</sup> ; <i>tef</i> <sup>P</sup> :: <i>brl3</i>                               | This study     |
| Brl4OE   | <i>A</i> <sub>3,1</sub> <i>B</i> <sub>4,7</sub>  | <i>trp</i> <sup>-</sup> ; <i>tef</i> <sup>P</sup> :: <i>brl4</i>                               | This study     |
| Brl1-myc | <i>A</i> <sub>3,1</sub> <i>B</i> <sub>4,7</sub>  | <i>trp</i> <sup>-</sup> ; <i>ura</i> <sup>-</sup> ; <i>ble</i> <sup>+</sup> ; <i>brl1</i> -myc | This study     |
| Brl2-His | <i>A</i> <sub>3,1</sub> <i>B</i> <sub>4,7</sub>  | <i>trp</i> <sup>-</sup> ; <i>ura</i> <sup>-</sup> ; <i>ble</i> <sup>+</sup> ; <i>brl2</i> -His | This study     |
| G11      | <i>A</i> <sub>3,5</sub> $\Delta$ <i>matB</i> :: <i>bar2</i> $\Delta$ <i>PstI</i> -HA- <i>gfp</i> ; | <i>trp</i> <sup>-</sup> ; <i>bar2</i> -HA-GFP  | JMRC, FSU      |
| V153-21  | <i>A</i> <sub>3,5</sub> <i>B</i> <sub>null</sub>   | <i>trp</i> <sup>-</sup>  | JMRC, FSU      |

JMRC: Jena Microbial Resource Collection, Jena, Germany

**Table S2: Primers used in this study.**

| Primer name   | Sequence (5' to 3')                                  |
|---------------|--|
| act-N1        | GTCCGCCCTCGAGAAGAGTTA                                |
| act-N2        | TTGTACGTCGTCTCGTGGATA                                |
| tef-N3        | AGCTTGGCAAGGGTTCCTTCA                                |
| tef-N4        | AACTTCCAGAGGGCGATATCA                                |
| ubi-for       | GAAGGAGTACGATGCGAAGG                                 |
| ubi-rev       | TCCTCCTCTGCCTTCTTGC                                  |
| brl1-for      | GAGCTGTGCAGGACGCGAAGC                                |
| brl1-rev      | ACGGCGGGATCACATTGACA                                 |
| brl2-for      | CACCGAAGCCGCCCAAACCTGCC                              |
| brl2-rev      | TACGAAGGTCGTGGAGGCCG                                 |
| brl3-for      | ACCTGAACCTTCTCGCACGTT                                |
| brl3-rev      | GCACAGACGAAGATGAACCA                                 |
| brl4-for      | TCTCGCTACCTGGATCTCGT                                 |
| brl4-rev      | AACATCCACCTCGACACCTC                                 |
| brl1_for_prom | GCTTGAACGCCGGGTAGTCC                                 |
| brl1_rev_myc  | TTAGAGATCCTTCTGAGATGAGCTTCTGCTCTACCTCTTCGACCTCGCGCT  |
| brl2_for_prom | GATATCGCAATCGTCATCGC                                 |
| brl2_rev_His  | TCAGTGGTGATGGTGATGGTGGAACGCCTGCCAAAAAC               |
| ura2-for      | GACCTGTTCCCCTTTCTTAGC                                |
| ura2-rev      | TCAGCCGGATGCTGGACTA                                  |
| ble-for       | TCTAGAGATCTGACGTGCATTGTG                             |
| ble-rev       | TCTAGACCAGCTTGCTCCAAAGAG                             |
| pRS_tef_fw    | GGCCCCCCTCGAGGTGACGGTATCGATAAGGCGGCGGCTCCGGCTGGGGCGC |
| tef_br11_rev  | GAGGGGATACGTGGGGTTCGTCGAGAGCATTTTGAGTGTTTTCTAAGTGAG  |
| tef_br11_fw   | ACTGACAATCTCACTTAGAAAACACTCAAATGCTCTCGAACGACCCACG    |
| brl1_rev_in   | CGAGTCCGGCCAGGCCTTCTCC                               |
| brl1_for_in   | CGGCCTAGCTGGCGAGGCGAGC                               |
| brl1_pRS      | CGCGGTGGCGGCCGCTCTAGAACTAGTGGACGCCAAAGCGTGAGGAGATCG  |
| tef_br12_rev  | AAATGAGGATACAGGGAATTCGGCGCGCATTTTGAGTGTTTTCTAAGTGAG  |
| tef_br12_fw   | ACTGACAATCTCACTTAGAAAACACTCAAATGCGCGCCGAATTCCTGTATC  |
| brl2_pRS      | CGCGGTGGCGGCCGCTCTAGAACTAGTGGAGCCACACTGGCATGATGCGC   |
| tef_br13_rev  | CATGGAGAAGACCCAGTTGGGATACGACATTTTGAGTGTTTTCTAAGTGAG  |
| tef_br13_fw   | ACTGACAATCTCACTTAGAAAACACTCAAATGTCGTATCCCAACTGGGTC   |
| brl3_pRS_rev  | CGCGGTGGCGGCCGCTCTAGAACTAGTGGAGAGATGAAGGTGGAGGAAAC   |
| tef_br14_rev  | GAACGCAAACGCGACGTTTCGCTGCCGACATTTTGAGTGTTTTCTAAGTGAG |
| tef_br14_fw   | ACTGACAATCTCACTTAGAAAACACTCAAATGTCGGCAGCGAACGTCGC    |
| brl4_pRS_rev  | CGCGGTGGCGGCCGCTCTAGAACTAGTGGAGCAGACAGGCTGCACCAACG   |



**Figure S1: Protein characteristics for Brls, Bar3 and Bbr2 of *S. commune* H4-8 and Ste3 of *S. cerevisiae*.** Amino acid positions are indicated, red rhombi show protein-protein-interaction site (signal peptide cleavage site and C-terminus effected, REPROFSEC prediction), orange circles for polynucleotide/DNA binding sites, auburn boxes show helical structures. Buried (yellow; not able to interact with the outside), and exposed" structures (blue, PROFAC prediction are indicated. Purple boxes indicate helical transmembrane regions (TMSEC prediction). The green bar indicates a disordered region (MD prediction).

**Table S3: DNA and protein sequence identities.**

| <b>Sequence</b> | <b>Brl1</b>    | <b>Brl3</b>    | <b>Bar3</b>    | <b>Bbr2</b>    | <b>Brl4</b>    | <b>Brl2</b>    |
|-----------------|----------------|----------------|----------------|----------------|----------------|----------------|
| <i>brl1</i>     | ID             | 0.14           | 0.18           | 0.27           | 0.16           | 0.11           |
| <i>brl3</i>     | 0.31           | ID             | 0.25           | 0.21           | 0.19           | 0.1            |
| <i>bar3</i>     | 0.37           | 0.43           | ID             | 0.29           | 0.2            | 0.12           |
| <i>bbr2</i>     | 0.39           | 0.42           | 0.48           | ID             | 0.2            | 0.17           |
| <i>brl4</i>     | 0.28           | 0.35           | 0.33           | 0.31           | ID             | 0.14           |
| <i>brl2</i>     | 0.32           | 0.27           | 0.31           | 0.32           | 0.27           | ID             |
| <b>Sequence</b> | <b>Bar3 N'</b> | <b>Bbr2 N'</b> | <b>Brl1 N'</b> | <b>Brl3 N'</b> | <b>Brl4 N'</b> | <b>Brl2 N'</b> |
| Bar3 N'         | ID             | 0.56           | 0.27           | 0.38           | 0.3            | 0.18           |
| Bbr2 N'         | 0.56           | ID             | 0.36           | 0.36           | 0.29           | 0.21           |
| Brl1 N'         | 0.27           | 0.36           | ID             | 0.27           | 0.23           | 0.2            |
| Brl3 N'         | 0.38           | 0.36           | 0.27           | ID             | 0.37           | 0.22           |
| Brl4 N'         | 0.3            | 0.29           | 0.23           | 0.37           | ID             | 0.25           |
| Brl2 N'         | 0.18           | 0.21           | 0.2            | 0.22           | 0.25           | ID             |
| <b>Sequence</b> | <b>Bar3 C'</b> | <b>Bbr2 C'</b> | <b>Brl1 C'</b> | <b>Brl3 C'</b> | <b>Brl2 C'</b> | <b>Brl4 C'</b> |
| Bar3 C'         | ID             | 0.22           | 0.07           | 0.1            | 0.11           | 0.12           |
| Bbr2 C'         | 0.22           | ID             | 0.15           | 0.09           | 0.14           | 0.09           |
| Brl1 C'         | 0.07           | 0.15           | ID             | 0.05           | 0.06           | 0.09           |
| Brl3 C'         | 0.1            | 0.09           | 0.05           | ID             | 0.05           | 0.09           |
| Brl2 C'         | 0.11           | 0.14           | 0.06           | 0.05           | ID             | 0.06           |
| Brl4 C'         | 0.12           | 0.09           | 0.09           | 0.09           | 0.06           | ID             |

DNA identities are on the left (italics); protein on the right site. 1 = 100 %.

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Br11_LoeD_artif MFSNDPTYPLFPILAF LSSVLALLPVPFTLQVWNTGACALAVWTATAACLLSFINSVWVWAG
Br11_TatD_ID208 MFSNDPTYPLFPILAF LSSVLALLPVPFALQVWNTGACALAAWTATAACLLSFINSVWVWAG
Br11_H4-8_ID263 MFSNDPTYPLFPILAF LSSVLALLPVPFTLQVWNTGACALAAWAATAACLLSFINSVWVWAG
Br13_LoeD_ID238 MSYPNWVFSMF---MVFGIIMTLLPLPWHLEAWNTGTCLYMLWVAVACLCQLVNSIVWSG
Br13_TatD_ID422 MSYPNWVFSMF---MVFGIIMTLLPLPWHLEAWNTGTCLYMLWVAVACLCQLVNSIVWSG
Br13_H4-8_ID263 -----MF---MVFGIIMTLLPLPWHLEAWNTGTCLYMLWVAVACLCQLVNSIVWSG
Br14_LoeD_ID289 MSAANVAFAFF---AFVTFILVLVPLPWHLQAWNSGVCIIYGWVALGSIILYVDVAVVWNG
Br14_TatD_ID373 MSAANVAFAFF---AFVTFILVLVPLPWHLQAWNSGVCIIYGWVALGSIILYVDVAVVWNG
Br14_H4-8_ID269 MSAANVAFAFF---AFVTFILVLVPLPWHLQAWNSGVCIIYGWVAVGSIILYVDVAVVWNG
Br12_TatD_ID215 MRAE---LLVF---SFLSVCILLVLPFKRVRDIPSTCLLLWLLAWNVIHAVNAVMWAG
Br12_LoeD_ID168 MRAE---FPVF---SFLSVCILFVALPFKRVRDIPSTCLLLWLLALNVIHAVNAVMWAG
Br12_H4-8_ID270 MRAE---FPVS---SFLSVSIIILVALPFKRVRDIPFTCLLLWLLAWNVIHAVNAVMWAG
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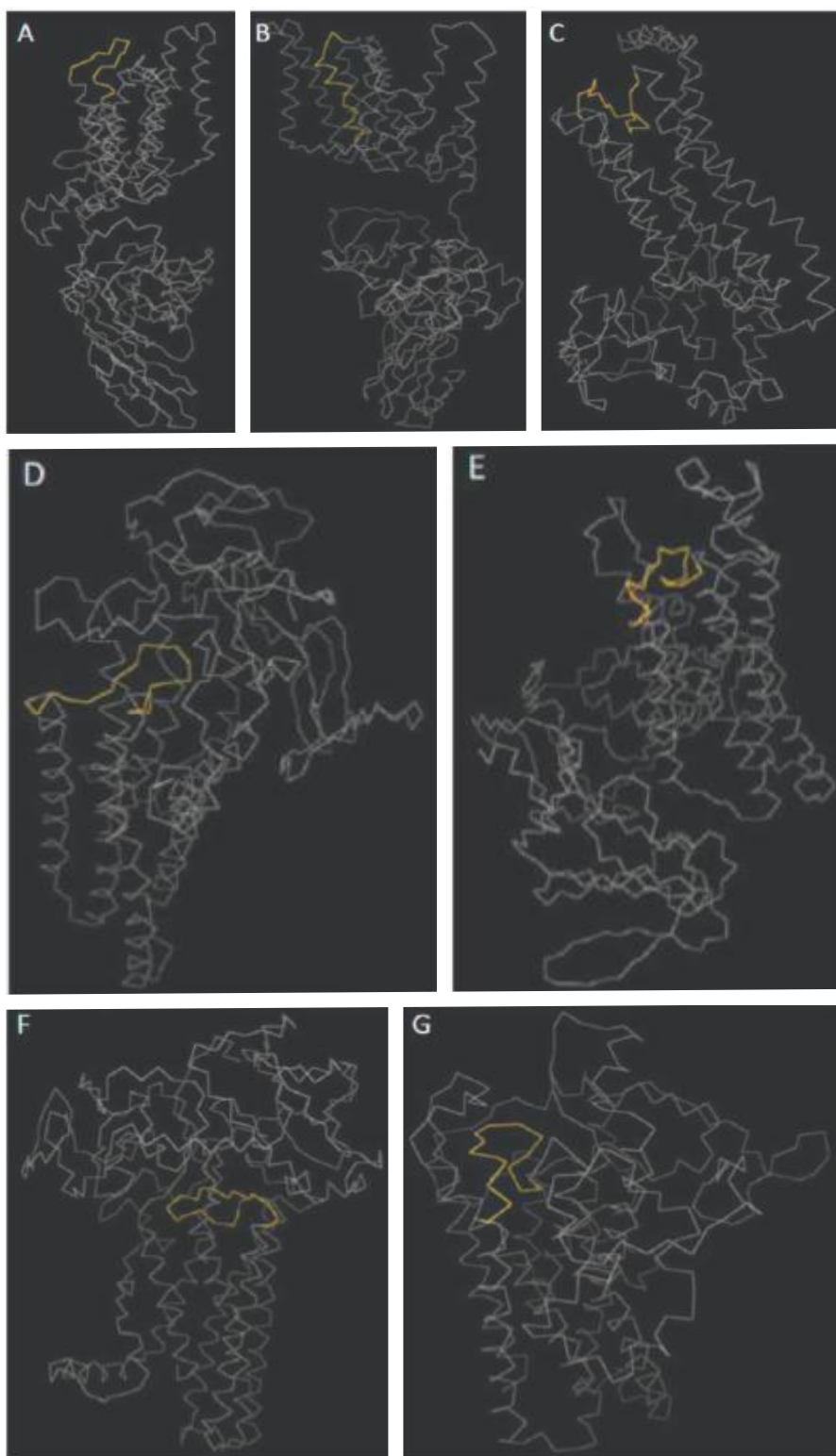
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Br11_H4-8_ID263 NVMNTVPVWCDISSKLI LGASIGIPASGLCISHRLWKVT SMRLVPETRRREILFAIAIDLA
Br13_LoeD_ID238 NAIDWAPVWCDISARVI IGTSVAIPAASLVINRRLYYIA TVRTVTKSKPERRREVMMDDL
Br13_TatD_ID422 NAIDWAPVWCDISARVI IGTSVAIPAASLVINRRLYYIA TVRTVTKSRPERRREVMMDDL
Br13_H4-8_ID263 NAIDWAPVWCDISARVI IGTSVAIPAASLVINRRLYYIA TVRTVTKSKPERRREVMMDDL
Br14_LoeD_ID289 NIIDPMPAWCDFSGRIN VAVPYGLEAATLCIQRRLYHIVRSSDVS-SKTSKRHWIVDVVL
Br14_TatD_ID373 NIIDPMPAWCDFSGRIN VAVPYGLEAATLCIQRRLYHIVRSSDVS-SKTSKRHWIVDVVL
Br14_H4-8_ID269 NINDPIPAWCDFSGRIN VAVPYGLEAATLCIQRRLYHIVRSSDVS-SKTSKRHWIVDVVL
Br12_TatD_ID215 NADVRLPWC DLVTKVM LAALMGVAGCALCMLRVLEFLA SARNP--GPNARRNTVSIEVL
Br12_LoeD_ID168 NADVRLPWC DLVTKVM LAALMGVAGCALCMLRVLEFLA SARNP--GPNARRNTVSIEVL
Br12_H4-8_ID270 NAGVRALAWCDLVTKVM LAALMGVAGCALCMLRVLEFLA SARNP--GPNARRNTVSIEVL
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Br11_LoeD_artif LSAGTPLLVM LLLHIIVQDHRSTILEDGFGCHAAMPNTLLSYFLVLSWPLGLGLASLVYAVL
Br11_TatD_ID208 LSAGIPLLVM LLLHIIVQDHRFTILEGFGCHAAMPNTLLSYFLVLSWPLGLGLASLVYAVL
Br11_H4-8_ID263 LSAGVPLFV LLLHIIVQSQRFTILEDIGCQAAPNTLLSYFLVLSWPLGLGLASLIYAVL
Br13_LoeD_ID238 IGMGLPVLVMILQYIPQGHRYDIYEDIGCPATYNTWVAIVCVTGWPPIAIGCVSAVYCYML
Br13_TatD_ID422 IGMGLPVLVMILQYIPQGHRYDIYEDIGCPATYNTWVAIVCVTGWPPIAIGCVSAVYCYML
Br13_H4-8_ID263 IGMGLPVLVMILQYIPQGHRYDIYEDIGCPATYNTWVAIVCVTGWPPIAIGCVSAIYCYML
Br14_LoeD_ID289 IAVVLPV LMLGLQYIVQGHRYDIYENYGCMLNTYMTGPAVLILRGPPPIILGVIAGVYCVL
Br14_TatD_ID373 IAVVLPV LMLGLQYIVQGHRYDIYENYGCMLNTYMTGPAVLILRGPPPIILGVIAGVYCVL
Br14_H4-8_ID269 IAVVLPV LMLGLQYIVQGHRYDIYENYGCMLNTYMTGPAVLILRGPPPIILGVIAGVYCVL
Br12_TatD_ID215 FCIGIPVLYMLLHFIVQDQRFIAADIGCVASIHPS SPSLILMWLPPIAIECVAAFALSCI
Br12_LoeD_ID168 FCIVIPVLYMLLHFIVQDQRFIAADIGCVASIHPS SPSLILMWLPPIAIECVAAFALSCI
Br12_H4-8_ID270 FCIVIPVLYMLLHFIVQDQRFIAADIGCVASIHPS SPSLILMWLPPIAIECVAAFALSCI
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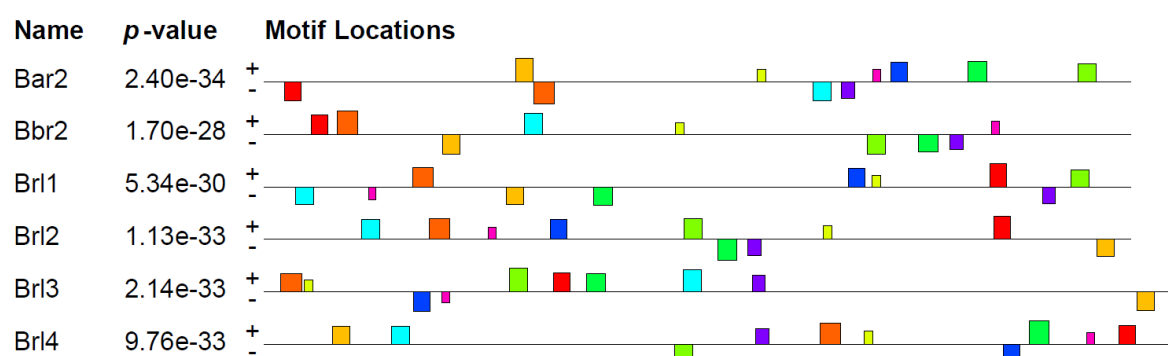
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Br11_TatD_ID208 TLRLSYV-HRLEFDHALAKGLRSLCLRLVLLALVAFTLSVSSSLNIAALARSGPLI
Br11_H4-8_ID263 TLRLSYV-HRLEFDHALAKGTLRTALCLRLVLLALVAFTVSISSSLNIAALARSGPLI
Br13_LoeD_ID238 SIYNFNK-RRRHFN EYLAHNSLTANRYFRLMCLAAIEICLTIPIGSFGLYMNQFSEMQ
Br13_TatD_ID422 SIYNFNK-RRRHFN EYLAHNSLTANRYFRLMCLAAIEICLTIPIGSFGLYMNQFSEMQ
Br13_H4-8_ID263 SIYNFNK-RRRHFN EYLAHNSLTANRYFRLMCLAAIEICLTIPIGSFGLYLNQFSEMQ
Br14_LoeD_ID289 LIRIFTQ-KRRRANT-----GPISRGICLRLIMLSTATLLFTVSLNLYTLIEDTRYG-LA
Br14_TatD_ID373 LIRIFTQ-KRRRANT-----GPISRGICLRLIMLSTATLLFTVSLNLYTLIEDTRYG-LA
Br14_H4-8_ID269 LIRIFTQ-KRRRANT-----GPMSRGICLRLIMLSTATLLFTVSLNLYTLIEDTRYG-LA
Br12_TatD_ID215 AVHHATRLPSTLLDEHLSTRTPISSG LLLRNLCFASTACSLVVI AVLFVGFVSPT----VV
Br12_LoeD_ID168 AVHHATRLPSTLLDEHLSTRTPISSG LLLRNLCFASTACSLVVI AVLFVGFVSPT----VV
Br12_H4-8_ID270 AVHHATRLPSTLLDEHLSTRTPISSG LLLRNLCFASTACSLVVI AVLFVGFVSPT----VV
: : : * : : : : : : : :

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**Figure S2: Alignment of N-terminal amino acid sequences of receptors from *S. commune*.** Conserved regions are visible in the lowest rows, if these conserved parts are within the extracellular loops they are highlighted by boxes.



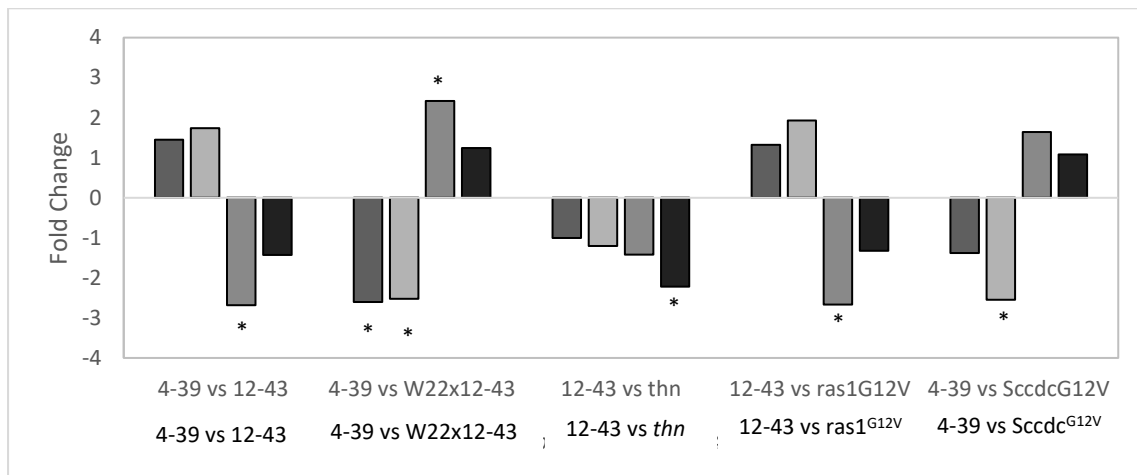
**Figure S3: 3D models of receptor proteins.** Protein secondary structures were modelled with ITASSER-GPCR and the model with highest c-score was taken for visualization. Grey line shows protein backbone e.g. in A with helical transmembrane area at top, intracellular C-terminus at bottom. Conserved extracellular loop is marked in yellow. **A** – Brl1. **B** – Brl2. **C** – Brl3. **D** – Brl4. **E** – Bar3. **F** – Bbr2. **G** – Ste3 of *S. cerevisiae*.



| Motif | Symbol | Motif Consensus          |
|-------|--------|--------------------------|
| 1.    |        | CTTCTTCCTCCCTTCTGCCTT    |
| 2.    |        | TTGTTSGNCRTBCTCWGCCGC    |
| 3.    |        | CTNTYMCDCCGTWMYTCMTCG    |
| 4.    |        | GTGAWGGSBAATGKT          |
| 5.    |        | RKGTGKGS GAWKNSGARGKA    |
| 6.    |        | AACTSGKGMWGCCTYCCTNGCG   |
| 7.    |        | GCAHCTCRTTKTSCSCANA      |
| 8.    |        | ATTGTYCTC                |
| 9.    |        | MGACKYCTRCASACGMMTATCBAT |
| 10.   |        | TVTACTTTAC               |

|             | Motif Consensus |
|-------------|-----------------|
|             | GACGCAaa        |
| <i>bar2</i> | 1               |
| <i>bbr2</i> | 3               |
| <i>brl1</i> | 2               |
| <i>brl2</i> | 3               |
| <i>brl3</i> | 0               |
| <i>brl4</i> | 3               |

**Figure S4: Promoter analyses.** Motifs found in the promoter regions of *brl1*, *brl2*, *brl3*, *brl4*, *bar3* and *bbr2* by MEME and MAST. Motif1 is similar to yeast Tec1 binding site. A motif similar to yeast's Fhl1-binding motif was found by manual sequence inspection in the promoter regions of *bar2*, *bbr2*, *brl1*, *brl2* and *brl4*

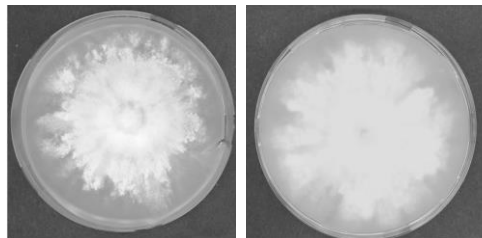


**Figure S5: Expression analysis of *S. commune* *brl* genes in wild-type and mutant strains. (\*)** Significant differences  $\geq 2$ -fold regulation.

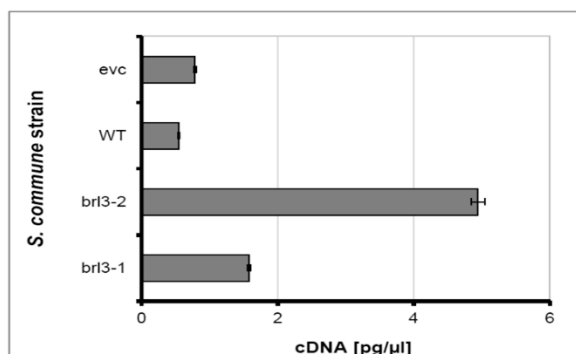
**Table S4: Sequence identity of *brl* genes in several *S. commune* strains compared to the respectively sequence of *S. commune* H4-8.**

| Gene                 | V153-21 | 4-39 | 4-40 | E6   | T2   | T1    |
|----------------------|---------|------|------|------|------|-------|
| <i>brl1</i> (530 bp) | 99.5    | 99.6 | 90.3 | 90.2 | 100  | n.a.  |
| <i>brl2</i>          | 99.4    | 99.6 | n.a. | n.a. | 100  | n.a.  |
| <i>brl3</i>          | 98.7    | 99.6 | n.a. | n.a. | 100* | 96.0* |
| <i>brl4</i>          | 99.7    | 99.4 | 100  | 100° | 100  | 100   |

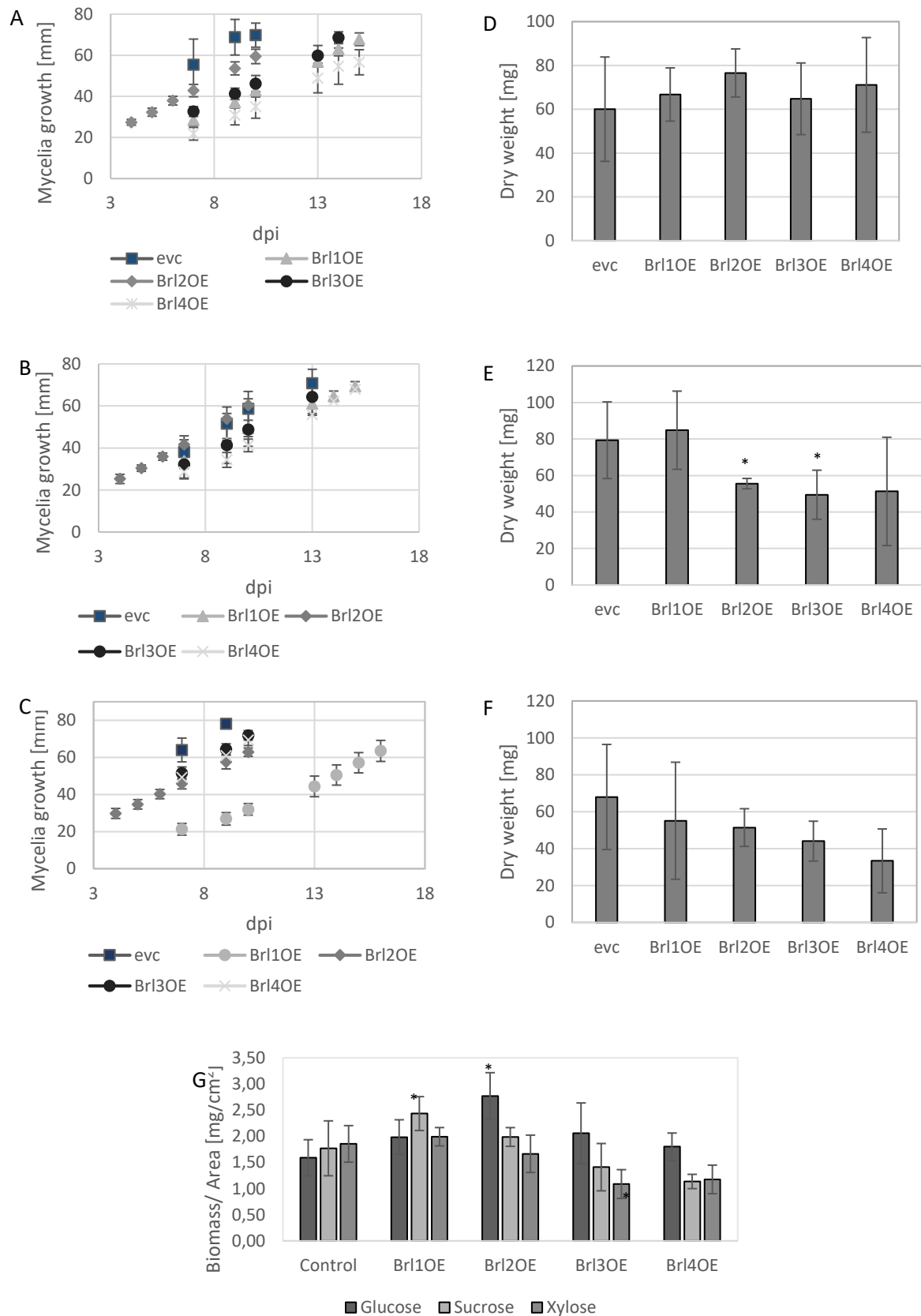
n.a. no amplicon; \* 500 bp amplicon of *brl3*; ° 736 bp amplicon of *brl4*



**Figure S6: Morphology of dikaryotic *S. commune* strain 12-43x4-39.**

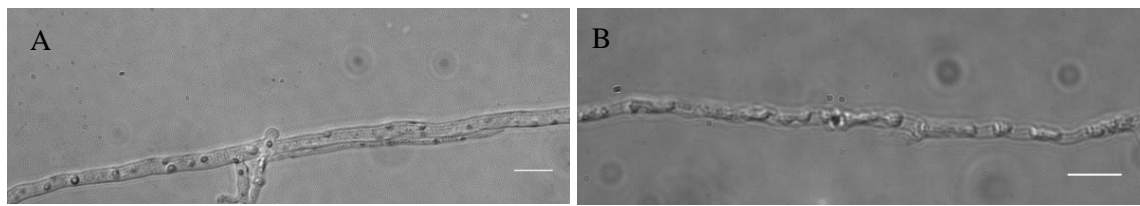


**Figure S7: Absolute quantification by qRT-PCR of *brl3* expression.** Overexpression of *brl3* was measured in strain brl3-1 (1.58 pg/μl), strain brl3-2 (4.94 pg/μl), WT (0.55 pg/μl) and evc (0.78 pg/μl).

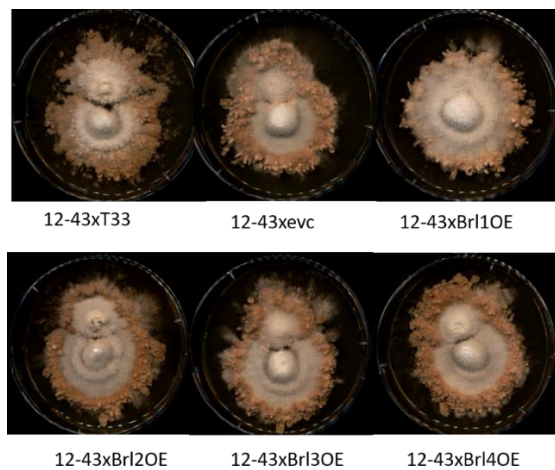


**Figure S8: Growth (A-C) and biomass (D-F) of the empty vector control strain and *brl* overexpression strains using glucose (A, D), sucrose (B, E), and xylose (C, F) as carbon source. (G) shows the formed biomass/cm<sup>2</sup> on glucose, sucrose and xylose. (\*) The student's T-test was used to determine the *P* value between control and transformants (\*) *P* < 0.05.**

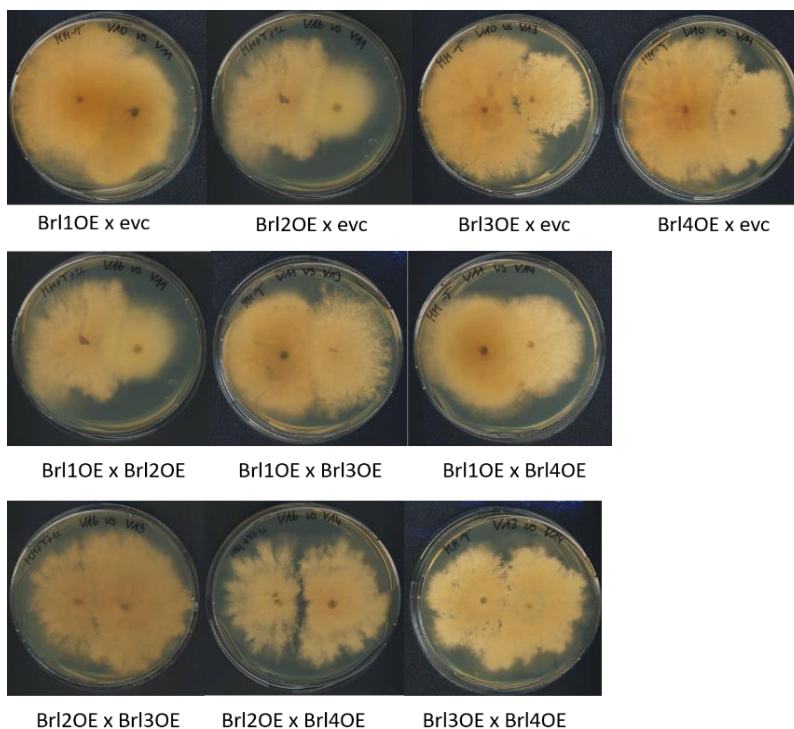




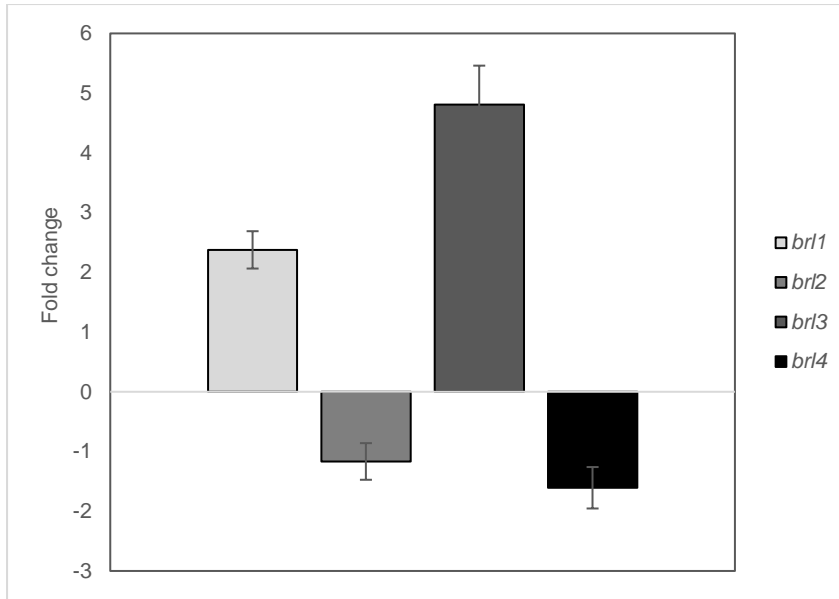
**Figure S9:** *S. commune* brl1OE (A) and brl4OE (B) form vacuole-rich wide hyphae. Bar represents 10 μm.



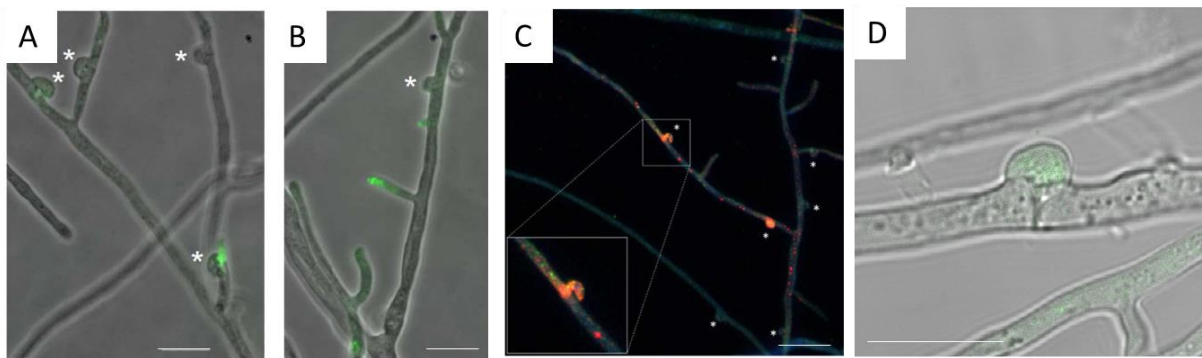
**Figure S10:** Mating interactions of T33, evc and *brl*-overexpressing strains with the compatible partner 12-43.



**Figure S11:** Confrontation of Brl1OE, Brl2OE, Brl3OE and Brl4OE with each other did not result in any significant growth reduction. Only confrontation of Brl2OE with Brl4OE resulted in a minor growth gap (not significant).



**Figure S12: Expression of *brl1*, *brl2*, *brl3* and *brl4* in *S. commune* V153-21 (*B<sub>null</sub>*).** The data were normalized to the expression in the monokaryon 12-43 and relatively quantified to three reference genes.



**Figure S13. Localization of Brl2::his (A-C) and Bar2::egfp in dikaryon** Localization of His-tagged Brl2 close to the hyphal tip and most likely membrane associated (A-B). Co-localization of Brl2::his and Bar2::HA is presented in dikaryotic hyphae (C). The labelling for Bar2 is shown in red, Brl2 in green and DAPI signal in blue. Localization of Bar2::egfp in dikaryon (D). Pseudoclamps are marked with asterisks. Bar represents 10  $\mu$ m.