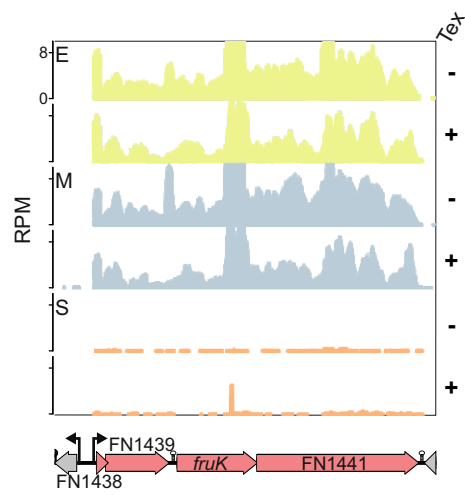
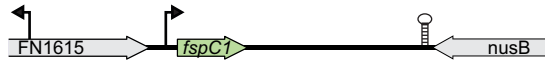


Supplementary Figures



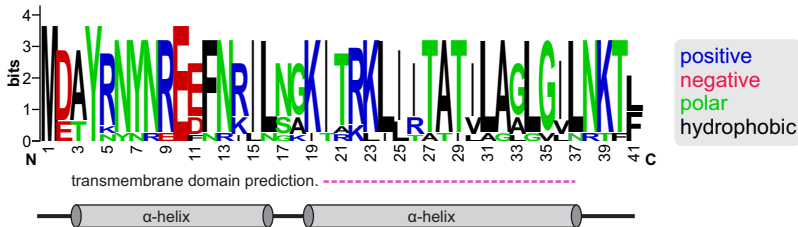
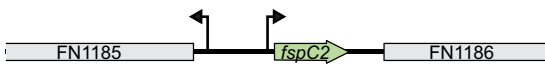
Supplementary Fig. 1 | Example of growth phase dependent transcription of FN1438-FN1441 operon
Normalized read distribution for the FN1438-FN1441 operon including the *fruK* gene likely involved in fructose uptake.

a

fspC1

FNN ATATATATTGACTTCAAATAT-ATAGTGATAAATAAGTATAAAATAAGTAGCTAAAAGATTGGGGTAAAAATTATGGATGCTATAAAAACATAATAGAGAAGAAATTAATAGAATTTAAATGGAAAAATCAAGAAAAATTA
 FNP ATATATATTGACTTCAAATAT-ATAGTGATAAATAAGTATAAAATAAGTAGCTAAAAGATTGGGGTAAAAATTATGGATGCTATAAGAACTATAACAGAGAGGAATTTAATAGAATTTAAATGGAAAAATCAAGAAAAATTA
 FuH ATATATATTGACTTCAAATAT-ATAGTGATAAATAAGTATAAAATAAGTAGCTAAAAGATTGGGGTAAAAATTATGGATGCTATAA---ACTATAATAGAGAAGAAATTAATAGAATTTAAATGGAAAAATCAAGAAAAATTA
 FNA ATATATATTGACTTCAAATAT-ATAGTGATAAATAAGTATAAAATAAGTAGCTAAAAGATTGGGGTAAAAATTATGGATGCTATAAGAACTATAATAGAGAAGAAATTTAATAGAATTTAAATGGAAAAATCAAGAAAAATTA
 FNV ATATATATTGACTTCAAATAT-ATAGTGATAAATAAGTATAAAATAAGTAGCTAAAAGATTGGGGTAAAAATTATGGATGCTATAAGAACTATAATAGAGAAGAAATTTAATAGAATTTAAATGGAAAAATCAAGAAAAATTA
 FuP TTATACCCTTGACTTCAAATATTTTACTATAAATA---GACTTAGATATAGTAGCTAGAAAATAGAGGTAAAAATTAAGAACTATAAGAACTATAATAGAGAAGAAATTTCAATAAAAATCTTTGAGCTAAAAATTA
 Con aTATaTATTGACTTCAAATAT.aTAgTATAATAAAtagTaTAAaTAAaGTAGCTaaaAgATAgGGGTAAAAATTATGGATGCTTATAGaaACTATAATAGAGAAGAAATTTAATAGAATTTTAAATGGAAAAATCAAGAAAAATTA

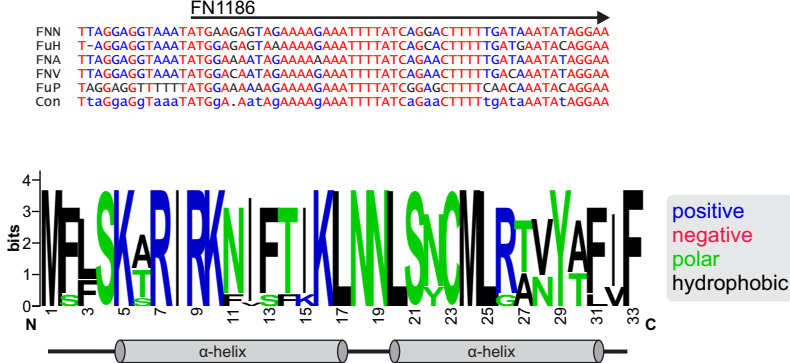
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 FNP AATATCACAGCTACTATTGGCTGGATTAGGAATTTAAACAAAACACTTTAAAT-TTAAAAATTAATAATAACA AAAAAGGAACATTAATAGTTCCTTT-----ATTTTTT-----TCTATATTTGCCAAA
 FuH AATATCACAGCTACTATTGGCTGGATTAGGAATTTGAATAAAACACTTTAAAT-TTATAAATCAATAT-----AAAAAGGAACATTAATAGTTCCTTTTCTATATTTTACTTTTTCTATATTTGCCAAA
 FNA AATATCACAGCTACTATTGGCTGGATTAGGAATTTGAATAAAACACTTTAAAGACTCTT-----AAATTAATAACAAGTAAAAAGGAACATTAATAGTTCCTTT-----ATTTTTTCTTTTTCTATATTTGCCAAA
 FNV AATATCACAGCTACTATTGGCTGGATTAGGAATTTGAATAAAACACTTTAAAGACTCTT-----AAATTAATAACAAGTAAAAAGGAACATTAATAGTTCCTTT-----ATTTTTTCTTTTTCTATATTTGCCAAA
 FuP CTTAGACAGCTACTGACTTGCCTCTTGGTATATTAACAAAACACTTTAAATTAATAAAAAATAGTGT-----GAGATTTTCAACACCATTTATTTTATATTTAGTTTTCTCTATATTTGCCAAA
 Con aTATCACAGCTACTATTGGCTGGATTAGGAATTTAAACAAAACACTTTAAa.ttataAAataAAAAaag.aaaagGAactaTaaatagttcctTTT.....ATTTTTactttttctTATATTTGCCAAA

**b**

fspC2

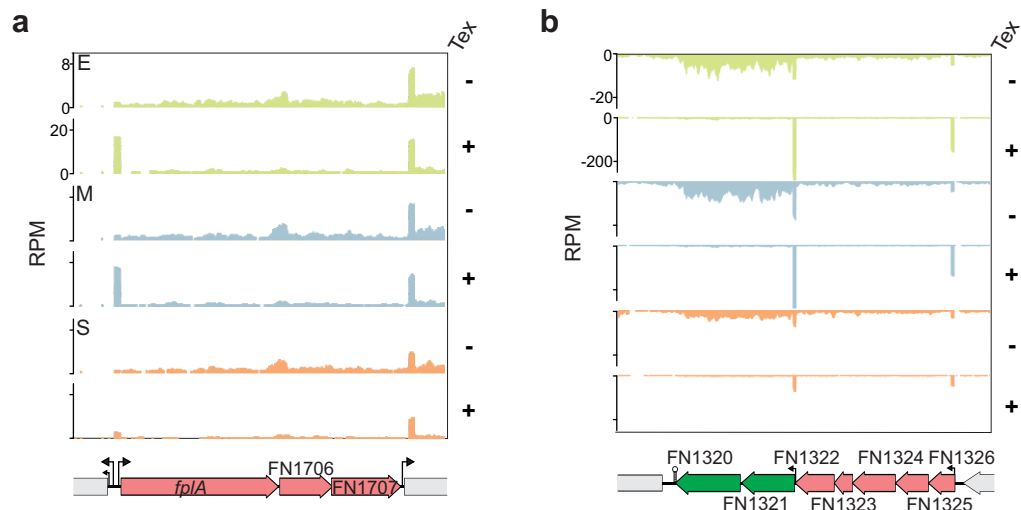
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 FuH ATTTTTTATAATTTATTTGACATTTATTTACAGATGTTATATTAGTAAAAATAAATTTTA--AGGAGGTCAAAATGTTTCTTAGTAAAGCAAGGATAAGGAAAAATTTTTACTATAAAATGAATAACCTGCTAATTGTATGC
 FNA ATATCTAAAAATTTATTTGACATTTATTTACTACTGCTATATTGATTAATAAATAAATTTTGA--GGAGG-TTTAAATGTTTCTTAGTAAAGCAAGGATAAGGAAAAATATAATTAACAATAAACTGAATAACCTTCTAATTGTATGC
 FNV AATATCTAAAAATTTATTTGACATTTATTTATAAATAGCTATATTAGCTAAAAATAAATTTTA--GGAGGTTCTAATGTTTTTTAGCAAAACAAGGATAAGGAAAAATTTTTACTATAAAATGAATAACCTTCTAATTGTATGC
 FuP AAATAATAAAAAAATTTTACTTCTACTGATATTAATGAAATAAATTTTAAAGAGGTTTAAAGATGCTTTTAGTAAATCAAGGATAAGGAAAAATTTATAGTTTTAAAAATGAATAACCTTCTAATTGTATGC
 Con atatt..TaaAattctTATTGCAATTTatTTtaTA.TaTg.TatataTaat.AAAATAAATTTta..ggaGggtTcaaaatGTtCTTAGTAAa..CAAGGATAAGGAAAAATTTctTactATAAAATGAATAACCTTCTAATTGTATGC

FNN TAAAGGACTGTTTATGCCTTTGTTTTAGTATATTTAGT-----A-----TAACTATTTTTAATATTTAATAA-----TAAAGTAAGTTAAACAATCAGTGTAGCATTCAATGTTTACTGATTTTTTTATTTAATAAT
 FuH TAAAGGACTGTTTATGCCTTTATTTTTAGTATAACTATTTTTTGA-----TATTAATTTTTAATATTTAATAA-----TAAAGTTGGTTAAACAATCAGTGTAGCATTCAATGTTTACTGATTTTTTTATTTAATAAT
 FNA TAAAGGCTAAATATACCTTTATTTTTAGTATAAATCATTTTTTGA-----TA-----TTAATATTTAGTAA-----TAAAGTAAATTAATAATCAGTGTAGCATTCAATGTTTACTGATTTTTTATTT-ATAAAT
 FNV TAAAGGCTGTTTATACCTTTATTTTTAGTATATCTATTTTTTGA-----TA-----TTAATATTTCAATA-----TAAATAAGTTAGCAATCAGTATAGCATTCAATGTTTACTGATTTTTTTATTTAATAAT
 FuP TAAAGGACTAATTTGCCCTTGATTTTTGTATAACTTTTTTAACTTTTAACTTTTAACTATTTTAACTAGTATTTTAACTATCTAATTTGTTTATAAAAAATTAATCAGTTTAGCATTCAATGTTTACTGATTTTTTTAATAAAT
 Con TtaGgaGtTaaATAGgA..AatAgAAAAgAAATTTTTATCaGaaCTTTTtgAtaAAATAGGAA



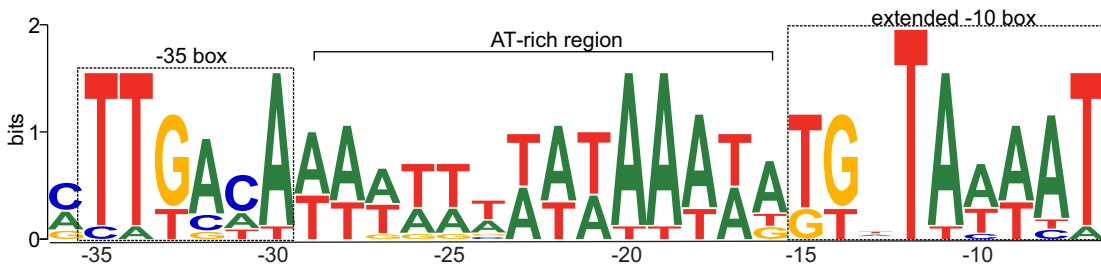
Supplementary Fig. 2 | Features of newly annotated small protein candidates *fspC1* and *fspC2*

Conservation analysis combined with TSS annotation uncovers small protein candidates *fspC1* **a**, and *fspC2* **b**, in intergenic regions. Shown are nucleotide alignments for representative strains of different fusobacteria where we identified the small protein candidates (FNN = *F. n. subsp. nucleatum*; FNA = *F. n. subsp. animalis*; FNP = *F. n. subsp. polymorphum*; FNV = *F. n. subsp. vincentii*; FuH = *F. hwasookii*; FuP = *F. periodonticum*). For protein alignment, the protein sequence of all strains with a complete annotation for the above stated fusobacteria was used. The web-application of jpred 4¹, SignalP (5.0)², TMHMM (2.0)³ was used to predict the secondary structure, signal peptide and transmembrane domain respectively. However, the prediction did not show a signal peptide for either *fspC1* or *fspC2*.



Supplementary Fig. 3 | Annotation of operon structures by dRNA-seq

a, Normalized read distribution for the proposed operon of *fplA* (phospholipase A1 type Vd autotransporter), FN1706 and FN1707. **b**, Normalized read distribution for the operon FN1326-FN1320 including the sub-operon FN1321-FN1320 (green) that is conditionally uncoupled through an additional TSS.



Supplementary Fig. 4 | Promoter motif associated with identified ncRNAs.

MEME analysis identified a promoter motif upstream of 12 ncRNAs. This motif displays an extended -10 box, followed by an AT-rich region and a -35 box similar to the promoter motif found upstream of the majority of pTSS (Fig. 1c).

Supplementary References:

- 1 Drozdetskiy, A., Cole, C., Procter, J. & Barton, G. J. JPred4: a protein secondary structure prediction server. *Nucleic acids research* **43**, W389-394, doi:10.1093/nar/gkv332 (2015).
- 2 Almagro Armenteros, J. J. *et al.* SignalP 5.0 improves signal peptide predictions using deep neural networks. *Nat Biotechnol* **37**, 420-423, doi:10.1038/s41587-019-0036-z (2019).
- 3 Möller, S., Croning, M. D. & Apweiler, R. Evaluation of methods for the prediction of membrane spanning regions. *Bioinformatics* **17**, 646-653, doi:10.1093/bioinformatics/17.7.646 (2001).