

Supplemental information

**Pneumolysin promotes host cell necroptosis
and bacterial competence during pneumococcal
meningitis as shown by whole-animal dual RNA-seq**

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SUPPLEMENTAL FIGURES

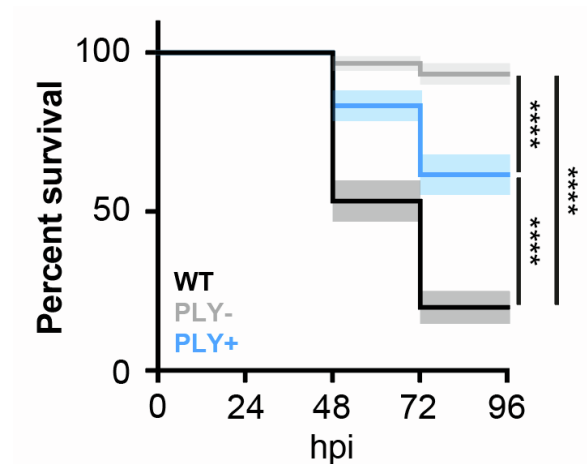


Figure S1. Complementation of the pneumolysin gene partially restores virulence of the pneumolysin-deficient *S. pneumoniae* PLY- strain in a zebrafish larval pneumococcal meningitis model. Survival curves of 2 dpf zebrafish larvae injected with *S. pneumoniae* D39V wildtype, *S. pneumoniae* D39V PLY+ or *S. pneumoniae* D39V PLY- strain in the hindbrain ventricle. Larvae were infected with 300 CFU. The data represent the mean $\pm$ SEM of three biological replicates with 20 embryos in each group; **** p value <0.0001 ; determined by log-rank test.

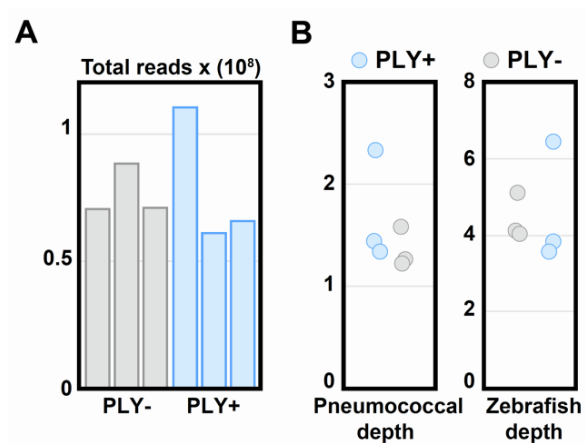


Figure S2. Total reads and depth. (A) On average, each library has 77 million reads (467 million reads in total); equivalent to 6.2 billion nucleotides. **(B)** Average depth of pneumococcal reads is 1.54 and zebrafish depth is 4.54.

	<i>Danio rerio</i>	<i>S. pneumoniae</i>
Total Genes	25,592	2,133
- Unexpressed	3,462	201
- q value > 0.05	15,727	-
- q value > 0.5	-	1.924
Working Libraries	6,403	8
	(25%)	(0.4%)
	vs total genes	vs total genes

Figure S3. Zebrafish and pneumococcal working libraries. Two gene fractions were removed to simplify downstream analysis. After removal of unexpressed genes and non-significant genes the zebrafish working library contained 6,403 genes (25% of *D. rerio* genes) whereas the pneumococcal working library contained 8 genes (0.4% of *S. pneumoniae* genes).

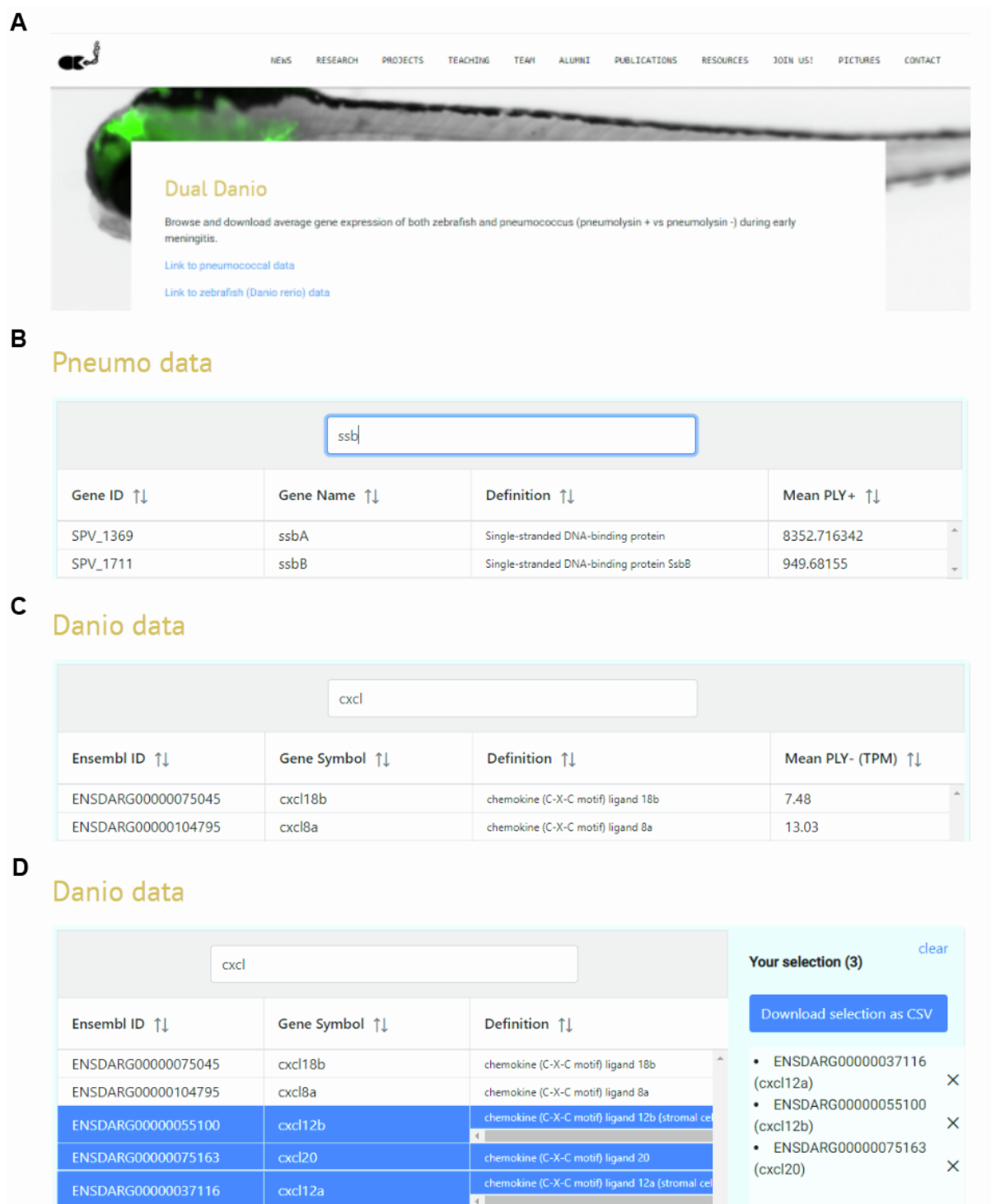


Figure S4. Easy access of the complete dual RNA-seq database. (A) Screenshot of the web-based platform with search result example summary for *D. rerio* **(B)** or *S. pneumoniae* **(C)**. The RNA-seq data of a single gene or multiple genes can be easily exported **(D)**.

SUPPLEMENTARY TABLES

Table S4. Primers

Oligonucleotide name	Sequence (5' to 3')	Description
LK162	CTAGCCTTGACAAC TAGCCAATC	Construction of <i>ply::cam^r</i>
LK163	CTCACAAAAATCCGAGCTCCACC GCTTCTACCTCCTAATAAGTTCT GG	Construction of <i>ply::cam^r</i>
LK164	CCAGGAACTTATTAGGAGGTAGA AGCGGTGGAGCTCGGATTTTTGT GAG	Construction of <i>ply::cam^r</i>
LK165	CGCAAGCATTCTCCTCTCCGCTA GGGCGCTGGCAAG	Construction of <i>ply::cam^r</i>
LK166	CTTGCCAGCGCCCTAGCGGAGA GGAGAATGCTTGCG	Construction of <i>ply::cam^r</i>
LK167	TGCAAATAGAAAGTTTCAGCC	Construction of <i>ply::cam^r</i>
LK283	GCCCGGGATCCTCGCAGGGAGT CTAATAAG	Construction of <i>ply::cam^r</i> complemented
LK284	CTTTATTTGCCATCTTCTACCTCC TTTCATTTACTTTGATAACCATAG TATAAC	Construction of <i>ply::cam^r</i> complemented
LK285	GTTATACTATGGTTATCAAAGTAA ATGAAAGGAGGTAGAAGATGGCA AATAAAG	Construction of <i>ply::cam^r</i> complemented
LK286	GCCCGTCTAGAGACTAGTACACT GGCACTTC	Construction of <i>ply::cam^r</i> complemented
OVL2549	GTGATGCGTCTCGTCTTGGATTT TTGTGAGCTTGGA	Construction of <i>comCDE::ery^r</i>
OVL2771	GTGATGCGTCTCGAGGTACCCG AGCGGC	Construction of <i>comCDE::ery^r</i>
OVL506	ATTCATAGGCGCGTGCTTCTTC	Construction of <i>comCDE::ery^r</i>
OVL2548	GTGATGCGTCTCGAAGAAAAAAG CCGGGAAAA	Construction of <i>comCDE::ery^r</i>
OVL2733	CGCCGCCGCCTGATGAGCCGCC ATTCTTATTGTTTTTGTAAC	Construction of <i>comCDE::ery^r</i>
OVL1667	GTAAATAGAGCTAGTATGAC	Construction of <i>comCDE::ery^r</i>
OVL2772	GTGATGCGTCTCGAGGTACCCGT TACGACGCG	Construction of hlpA- GFP:: <i>trmp^r</i> and hlpA_hlpA-mCherry:: <i>trmp^r</i>
OVL43	AACAAGTCAGCCACCTGTAG	Construction of hlpA- GFP:: <i>trmp^r</i> and hlpA_hlpA-mCherry:: <i>trmp^r</i>
OVL2769	GTGATGCGTCTCGAAGAATATGC CTCCTACTAGTG	Construction of hlpA- GFP:: <i>trmp^r</i> and hlpA_hlpA-mCherry:: <i>trmp^r</i>

OVL2770	GTGATGCGTCTCGACCATTAAAA AGCCTATTGTATC	Construction of hlpA- GFP::trmp ^r and hlpA_hlpA-mCherry::trmp ^r
OVL46	CGTGGCTGACGATAATGAGG	Construction of hlpA- GFP::trmp ^r and hlpA_hlpA-mCherry::trmp ^r
ADP1/45	GATAAAGGCGTAGGAGACACTGA CC	Construction of <i>cbpD</i> ::spec ^r
ADP1/46+SphI	CGATGCATGCGTGTTAACTTCG ACCGCACCTG	Construction of <i>cbpD</i> ::spec ^r
ADP1/47+HindIII	CGATAAGCTTAATATAAAGCGAT GATTGCCTTC	Construction of <i>cbpD</i> ::spec ^r
ADP1/48	CAACTTATCACGGGAACTGGTCG AG	Construction of <i>cbpD</i> ::spec ^r
sPG11+SphI	CGATGCATGCAGGAGGCATATCA AATGAAC	Construction of <i>cbpD</i> ::spec ^r
sPG12+HindIII	CGATAAGCTTTTATAAAAGCCAG TCATTAG	Construction of <i>cbpD</i> ::spec ^r
col1a1b_FW	TGGACAGCCTGGTGCTAAAG	RT-qPCR
col1a1b_RV	CAGCAGGTCCCTGTGGTC	RT-qPCR
pdxp_FW	CTTGTGGGCTACGACGAGAG	RT-qPCR
pdxp_RV	CCAGAACCCGGGGTGATCC	RT-qPCR
nme2b.2_FW	TTGAAGTCGGCAGGAACCTGAT	RT-qPCR
nme2b.2_RV	GTTCTGGAAGTGTCTCTGTTCAC	RT-qPCR
ccl25b_FW	CAGCATCGCACAAGGTTACT	RT-qPCR
ccl25b_RV	TCCGTCTGTTAGCTGCACTC	RT-qPCR
cxcl18b_FW	GCAGGGAGAAGTTCTGGCTAA	RT-qPCR
cxcl18b_RV	ACTTTGTGCGCAGTTTGGTCT	RT-qPCR
mob4_FW	GCTCAATGGCTTGGCAGTTA	RT-qPCR
mob4_RV	AATCAATGGCAGGGCACTCT	RT-qPCR
ripk3_FW	GCAGTTACAAACCCAGCAAAG	RT-qPCR
ripk3_RV	GGCTCCTTCCCAGTGATAATG	RT-qPCR