Supplementary Information:
Zebrafish as a model to study live mucus physiology

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Supplementary Information

Supplementary Figure S1. Genomic structure of five predicted polymeric secreted mucins in zebrafish.

Supplementary Figure S2. Neighbor-joining phylogenetic tree of mucins in zebrafish, human, and chicken.

Supplementary Figure S3. Alignment of gel-forming secreted mucins from zebrafish, human, and chicken.

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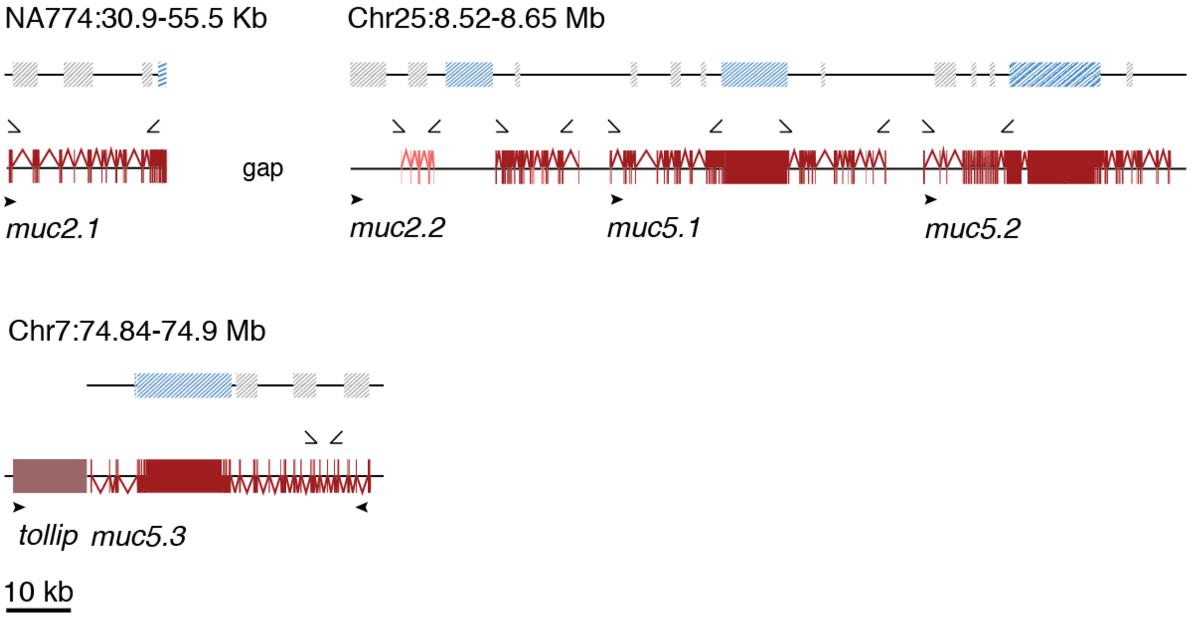
Supplementary Figure S7. Generation of the fluorescent mucin reporter *muc5.1:S-RFP*.

Supplementary Table S1. Additional identified mucin-like fragments in the zebrafish genome.

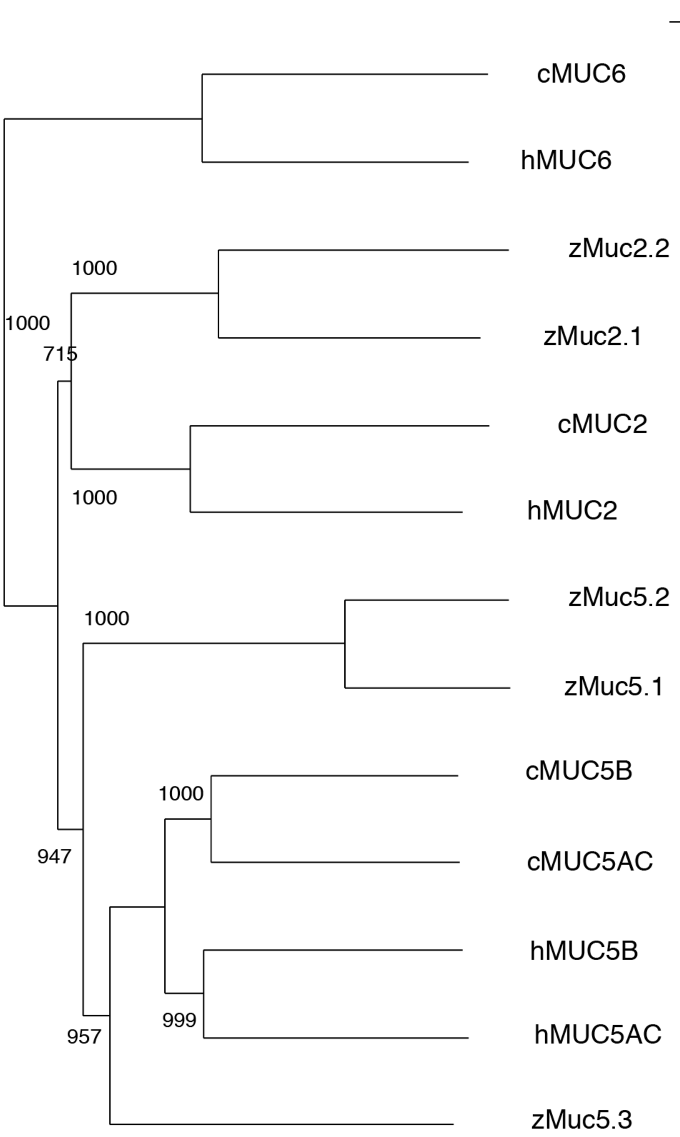
Supplementary Addendum S1. Protein sequences of secreted gel-forming mucins in zebrafish used for the construction of the protein domain models.

Supplementary Addendum S2. Protein sequences used for the construction of the phylogenetic trees.

Supplementary Addendum S3. Sequences of the verification transcripts.



Supplementary Figure S1. Genomic structure of five predicted polymeric secreted mucins in zebrafish. Four of the mucins have been assigned across chromosomes 25 and 7. For *muc2.1*, the genomic position is unknown and hence, it is arbitrarily depicted left to the mucin gene cluster on chr25, relative to the position of the gap in the current genome assembly. The top bars show the genomic locations of computationally predicted VWD and PTS coding domains. PTS domains (blue boxes) were predicted by PTSPRED [22, 23] and VWD domains (grey boxes) were predicted by Genewise [37]. Ensembl transcript models [36] (in red) for the corresponding genomic regions are aligned below. To confirm the computationally predicted mucin domains we experimentally verified selected regions of each mucin transcript with RT-PCR and sequencing (pointers above transcript models represent the positions of the amplification primers). In pink is an addition to the *muc2.2* transcript that emerged from our experiments and that was missing in the Ensembl transcript model.



Supplementary Figure S2. Neighbor-joining phylogenetic tree of mucins in zebrafish, human, and chicken. The tree uses the same the N-terminal mucin sequences as the MrBayes tree in Fig. 1b. Numbers at branches represent bootstrap values. The tree reveals the same relationship among the mucins as MrBayes and is therefore a confirmation of their phylogenetic status. The only difference is seen with Muc5.3, which appears more distant from Muc5.1 and Muc5.2 and groups closer to the chicken and human mucins.

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danrer_Muc2.2    1 STWESFHFKTFDGDIYQYPGLCEYNLVSDCTS--KQFSVDVKRTQ--TSSGPKISRIVVSINGKMFSISENNVMIDDQ-ITKLPVYESGILIE---ENTIYTN 95
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galgal_Muc2      1 STWGNNHFKTFDGDIYQFPGMCEYNFVSDCQDSYREFSVHIQRALNSNNHPE-IQYILITVTDFTVYLKPKLAVVDGR-IVKTPYYSSGLLIE---SNDIYTK 98
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galgal_Muc5B     1 CTWGNFHFKTFDGDIFRYPGLCNYVFASHCNAPYEEFNIQIRRTL--VENILTIHRIAIKLEGVAIELTKDVVLINSN-RVQLPYSQAGITIE---RSSIYVK 97
danrer_Muc5.3    1 STWGNYHYKTFDGDYFQLPSTCNYILTSHCSSSYEDFNVQLRHEL--LNNEPTVSKITMKLQGTIIELSRDSLSVNGQ-TVTLPYSSAEVFIE---RVTSYIS 97
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galgal_Muc6      1 STWGGGHFSTFDKYQYDFTGTCNYIFATVCDESSPDFNIQFRRGL-----DKKIARIIIELGPSVIIVEKDSISVRSVGVIKLPYASNGIQIAPYGRSVRLVA 98

danrer_Muc2.2   96 IRSKTGLTVMWSKDGAVTVELDNKYQNKTCGLCGNFNRIRDEF-ITGNTEKIGYIEFGNKHKVFNPMEFCENPSETDEKNNWKAICGS--------------- 182
danrer_Muc2.1   98 LYSKMGITVMWNKDDAVMVELDSKYSNRTCGLCGDFNGIPVYNEFIQSGRTVGYTEFGNMHRVPNPTHQCEDPFENVDEQNVVDQCEK--------------- 185
homsap_MUC2    100 VYSRAGLTLMWNREDALMLELDTKFRNHTCGLCGDYNGLQSYSEFLSDGVLFSPLEFGNMQKINQPDVVCEDPEEEVAPASCSEHR----------------- 185
galgal_Muc2     99 VYAKLGLILIWNQEDALMVELDSKFGNQTCGLCGDYNGVPIYNEFISGVASYNSITYGNLQKISKPNDECEDPDETRALPSCNEHVSKPNCELRFPGCMETSL 201
homsap_MUC5AC   98 VEARLGLVLMWNHDDSLLLELDTKYANKTCGLCGDFNGMPVVSELLSHNTKLTPMEFGNLQKMDDPTEQCQDPVPEPPRNCSTGF------------------ 182
homsap_MUC5B    98 VSIRLVLTFLWNGEDSALLELDPKYANQTCGLCGDFNGLPAFNEFYAHNARLTPLQFGNLQKLDGPTEQCPDPLPLPAGNCTDEE------------------ 182
galgal_Muc5AC   98 VSAKLGLTFLWNEEDALLVELDKKYANQTCGLCGDFNGIPTNNEFISQNAKLTAVQFGNRQKIDGPTEQCDDPIPSAVLMNCSSEF----------------- 183
galgal_Muc5B    98 VVSKTDIVFMWNQRDSILLELHEKYANQTCGLCGDFNGIPVYNEFYSNNIRMTALQFGNMQKMDGPTEYCEDPTPSSLDN----------------------- 177
danrer_Muc5.3   98 IKAALGLVAMWNEDDSFMIRLDEKYRNQTCGLCGDFNGIPVYNEFVSGGSSLSVSDYGNLWKLDGPTETCTEDTMFLNENCGDEN------------------ 182
danrer_Muc5.1   98 ISNKHGVTVFWEEDNSLSIELPEKYQGQTCGLCGDFNGN-LADDITDNGP--------ATWKVSISTEICEEVTLPSTGPCDELSEQ---------------- 175
danrer_Muc5.2   98 IS-RYGMTIFWEEDNSIAIELAEKYKGLTCGLCGNYNGD-KDDDMPESGP--------ATWKISTPTESCEDVILPPKDQCDQN------------------- 171
homsap_MUC6     99 KQLELELEVVWGPDSHLMVLVERKYMGQMCGLCGNFDGKVTNEFVSEEGKFLEPHKFAALQKLDDPGEICTFQDIPSTHVRQAQH------------------ 183
galgal_Muc6     99 KLMEMELVVMWNNEDYLMVLTEKKYMGKTCGMCGNYDGYELNDFVSE-GKLLDTYKFAALQKMDDPSEICLSEEISIPAIPHKKY------------------ 182

danrer_Muc2.2  183 ----YQADCEELFRAKAWSSCAMSYHP--YVDACVYDRCFSK-----SSSLCATLSEYSRQCSRNGGTPSTWRTKDFCAVTCPYNTVHFESGSPCIDTCSSEN 274
danrer_Muc2.1  186 ----YRADCADLLEDEKWSSCSWVLDPEAYIKACTNDLCNRQPEDEDTTALCATLTEYSRQCSHAGGNPPAWRTAKFCNV-QCPYNMVHSESGSPCMDTCSHK 283
homsap_MUC2    186 ------AECERLLTAEAFADCQDLVPLEPYLRACQQDRCRCPGGD-T--CVCSTVAEFSRQCSHAGGRPGNWRTATLCPK-TCPGNLVYLESGSPCMDTCSHL 278
galgal_Muc2    202 HCEMLREECEKLLTSSAFADCHSRLNLEMYIQACMQDKCACKGNE-DSFCLCSTISEYSRQCSHAGGRPG--------------------------------- 270
homsap_MUC5AC  183 ------GICEELLHGQLFSGCVALVDVGSYLEACRQDLCFCEDTD-LLSCVCHTLAEYSRQCTHAGGLPQDWRGPDFCPQ-KCPNNMQYHECRSPCADTCSNQ 277
homsap_MUC5B   183 ------GICHRTLLGPAFAECHALVDSTAYLAACAQDLCRCP------TCPCATFVEYSRQCAHAGGQPRNWRCPELCPR-TCPLNMQHQECGSPCTDTCSNP 272
galgal_Muc5AC  184 -----ASICKTVLTSEAFTSCNALVDVQDYIDTCIQDLCHCDTSM-ADFCMCNTFAEYSRQCAHAGGQPLNWRTSELC------------------------- 255
galgal_Muc5B       -------------------------------------------------------------------------------------------------------
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danrer_Muc5.1  176 -----ASFCEEYLISPGFSGCYDVMDMRIFQKACVSDLCQCYGN---HDCLCNTLTEISRQCTHAGGQPGTWRTEQLCPK-TCPLNLQYLECGGPCRNTCSAP 269
danrer_Muc5.2  172 -----TMVCQQYLSSPGFSGCYDVMDMKIFEKACVSDMCQCYGS---HDCLCNTLTEISRQCTHAGGQPGTWRTEQLCPK-MCPINLQYMECGGPCKSTCSDP 265
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galgal_Muc6    183 -----AVICSQLLN---LVSPTCSVPKDGFVTRCQLDMQDCSEPG-QKNCTCSTLSEYSRQCAMSHQVVFNWRTENFCSVGKCSANQIYEECGSPCIKTCSNP 276

danrer_Muc2.2  275 TNTQCEEHNIDGCFCPPGTVFDDILNMGCIPREQCHCKHDRIYKPQEELHEVGQDCVCQNGKWNCKIKPVLGRCSVEEGSHFTTFDGQEFTFHGDCTYVLSKD 377
danrer_Muc2.1  284 DTNALCEEHNIDGCFCPPG-TVFDDISDT-GCIPAEKCQCKHDR-VYSSGEVLRKSEEECYCQEGSWVCMSIPGPGLCAVEEGSHFTTFDGKEFTFHGDCNYL 383
homsap_MUC2    279 EVSSLCEEHRMDGCFCPEG-TVYDDIGDS-GCVPVSQCHCRLHGHLYTPGQEITNDCEQCVCNAGRWVCKDLPCPGTCALEGGSHITTFDGKTYTFHGDCYYV 379
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galgal_Muc5B   178 ---------------------------------------CTYN-------------------------------------------------FH--------- 183
danrer_Muc5.3  277 ERGQVCAQHCIDGCFCPPG-TVYDDIHQS-GCIPYTECSCVLGGKIYASGENYTSNCRDCTCDQGQWDCYIKDCPHSCSVEGGSHITTFDGKAYTFHGDCTYV 377
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homsap_MUC6    278 QHS--CSSSCTFGCFCPEG-TVLNDLSNNHTCVPVTQCPCVLHGAMYAPGEVTIAACQTCRCTLGRWVCTERPCPGHCSLEGGSFVTTFDARPYRFHGTCTYI 377
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danrer_Muc2.2  378 SVGSK-FIILGQISPCASLIYDTCLKSIDVIFNSDTVNRLVISEDGTVKHNAAVLLPYTPASRQFVVFSPSSFYILIDT-NFGLRVLVQLVPLMQVYITMDKR 478
danrer_Muc2.1  384 LSKDCEESK-FIILGQIVPCFTHETDTCLKSIVVLFDNDKKNPLFIKADGTVQHNAEVSLPYMTAD--FTVFRPSSFHIILQT-VF-GLQVQVQLVPLMQVYI 481
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galgal_Muc2    353 LAKSAVNDT-HALLAELAPCGSSDGQTCLKTVVLLVDDKKNVVVFRS-DGSVSLNEMTV-NVPHVSASFSVFKPSSYYLIVHT-SFGLQLQIQLSPVMQLFVT 451
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galgal_Muc5AC  339 LTKLCDSND-FTVLGDIHKCGLTDTETCLKGVAISLSGGQTNIVIQP-SGSVFVNMIYT-QLPFSAANVTIFRPSSFFIILQT-TFGLQLQIQIVPLMQLFIN 437
galgal_Muc5B   184 ----CEDEI-FTILVELRKCGMTNTETCLKTVTLSMNKGQTLIEVKT-DGGVFMNSIYT-QLPISAANVTIFRPSSFFVIMHT-NIGIQVEIQITPIMQVFVR 278
danrer_Muc5.3  378 LSKHCSGTE-FTVLGDLVKCGLTETETCLKAITVALFDGITVINVDS-SGNVEVNRIVA-QLPLFTSSVSVFKPSSFYIIIEAPLIGLRLEIQLVPIMQLYIT 477
danrer_Muc5.1  371 LTKHSADSD-FAVVGNLAKCEASRTDTCLHSVTLVISG--TTISFSS-SGDVTLNNINSKELPFSIGPVSIFQPSSSFIIVDL--TSVRLEIQLAPVMQLYIV 467
danrer_Muc5.2  367 LTKHSNDSD-FAVVGNLAKCEPARTDTCLNSVTLVISG--TTIGFTS-DGVVTLNGNSPFNLPAVIGPVSIFQPSLSFIIADL--NSLRLEIQLAPVMQLYIV 463
homsap_MUC6    378 LLQSPQLPEDGALMAVYDKSGVSHSETSLVAVVYLSRQDKIVISQDE----VVTNNGEAKWLPYKTRNITVFRQTSTHLQMAT-SFGLELVVQLRPIFQAYVT 475
galgal_Muc6    377 LMKSSSLPHNGTLMAIYEKSGYSHSETSLSAIIYLSTKDKIVISQNE----LLTDDDELKRLPYKSGDITIFKQSSMFIQMHT-EFGLELVVQTSPVFQAYVK 474

danrer_Muc2.2  479 FQGK------------------------THGLCGNFNKVLADDMKTPQGLVEGTAVSFANAWEVE-TNC-S-RSDKSVIPCSHNSVSENYAEHWCYKIMDKSD 554
danrer_Muc2.1  482 TVDQSFQGK------------------------TCGLCGNFNKVLADDLKTPQGVVEGTAVSFANAWKAQ-SNCPDRSERMDDPCSYSSDSEHFAEHWCSKMK 559
homsap_MUC2    479 LDQASQGQ------------------------VQGLCGNFNGLEGDDFKTASGLVEATGAGFANTWKAQ-STCHDKLDWLDDPCSLNIESANYAEHWCSLLKK 556
galgal_Muc2    452 ADQSVQDIQGKCPLKQEAKPCISTAVKSYILYMGGLCGNFNGMEGDDFKTTNGLVEATGSAFANTWKAQ-PTCADQAEKLEDPCTLSIESANYAEHWCSLLKN 553
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homsap_MUC5B   474 LDPAHQGQ------------------------MCGLCGNFNQNQADDFTALSGVVEATGAAFANTWKAQ-AACANARNSFEDPCSLSVENENYARHWCSRLTD 551
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galgal_Muc5B   279 LDPVFKDQ------------------------TCGLCGNFNNVQTDDFKAISGVVEGTAAAFANTWKTQ-ASCPDIQSSFENPCALSIDNEKYAKHWCGLLTN 356
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danrer_Muc5.2  464 ASTEEKGK------------------------MTGLCGNYNDVQSDDFKTDLGITEGTAISFANFWKKTPYSCPDLENTFDNPCSLSVDTEKLAKDWCSRLTN 542
homsap_MUC6    476 VGPQFRGQ------------------------TRGLCGNFNGDTTDDFTTSMGIAEGTASLFVDSWRAG--NCPAALERETDPCSMSQLNKVCAETHCSMLLR 552
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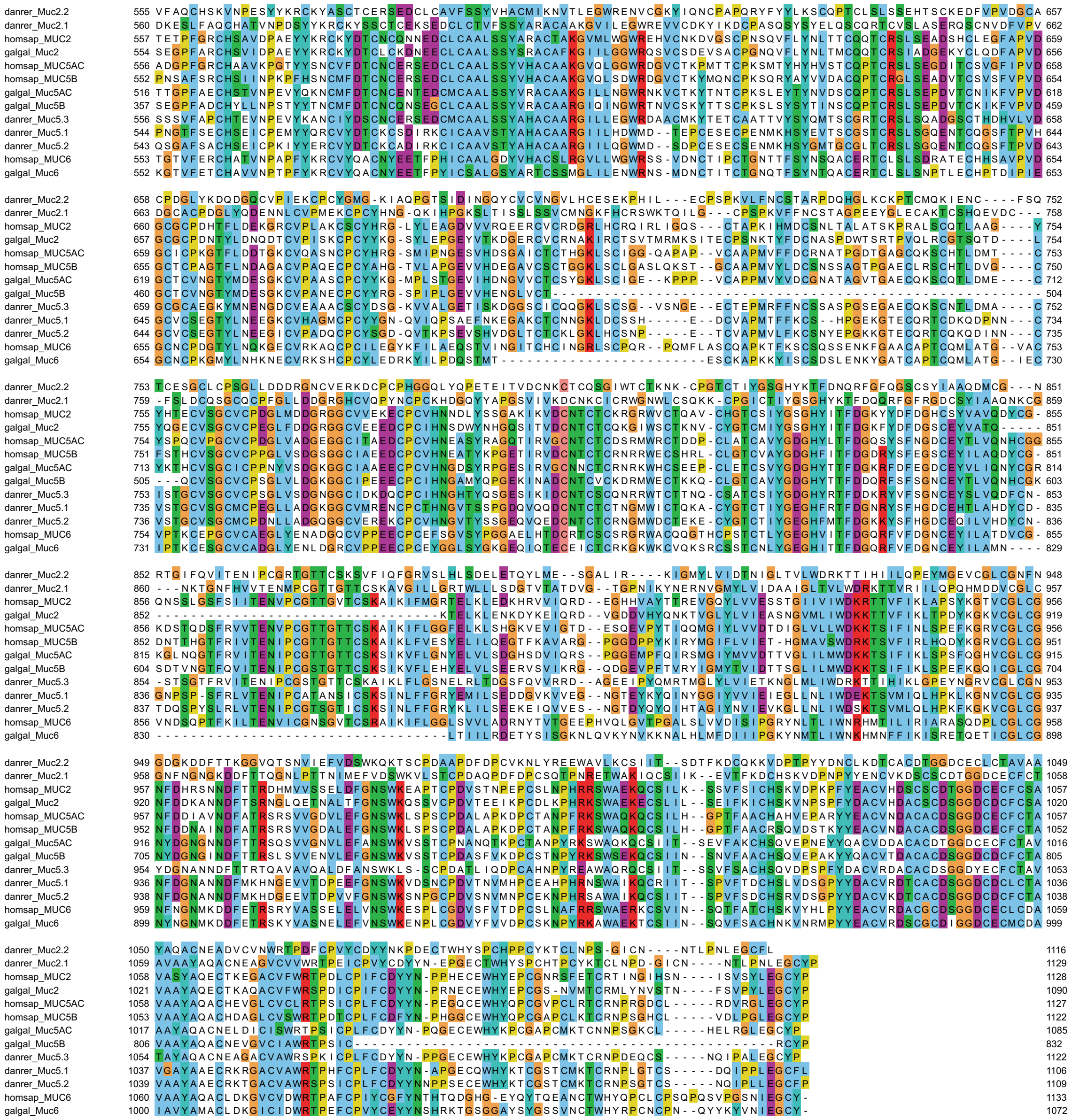
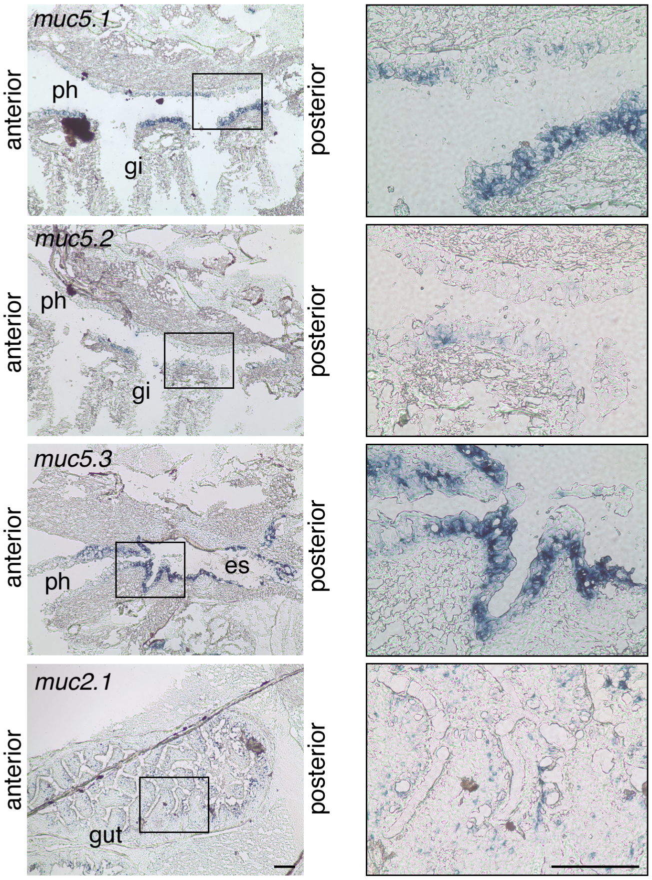
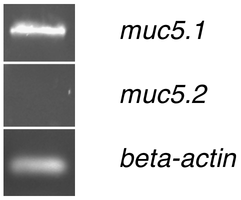


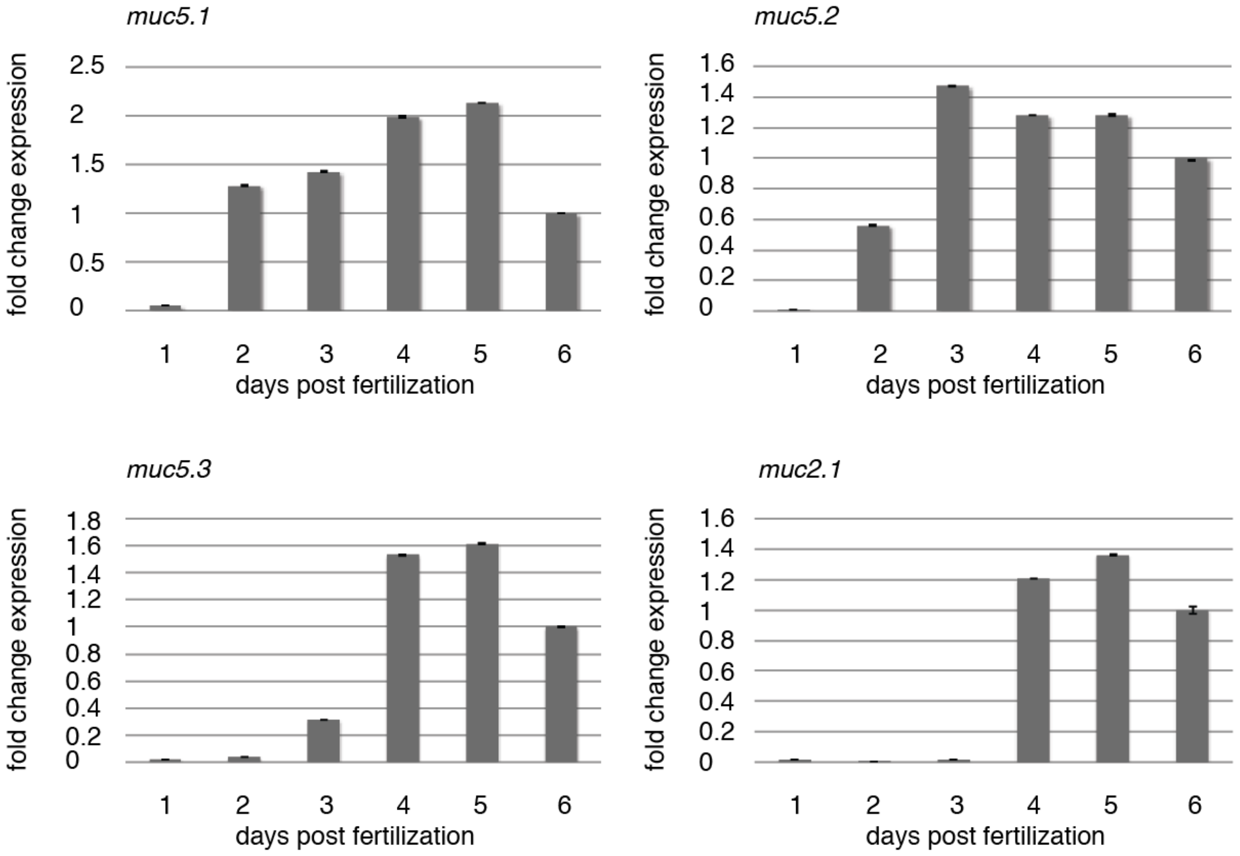
Supplementary Figure S3. Alignment of gel-forming secreted mucins from zebrafish, human, and chicken. Mucin sequences containing the three N-terminal VWD domains were aligned using ClustalW and visualized in Jalview. These sequences are the same as those used for the construction of the phylogenetic trees in Fig. 1b and Supplementary Fig. S2.



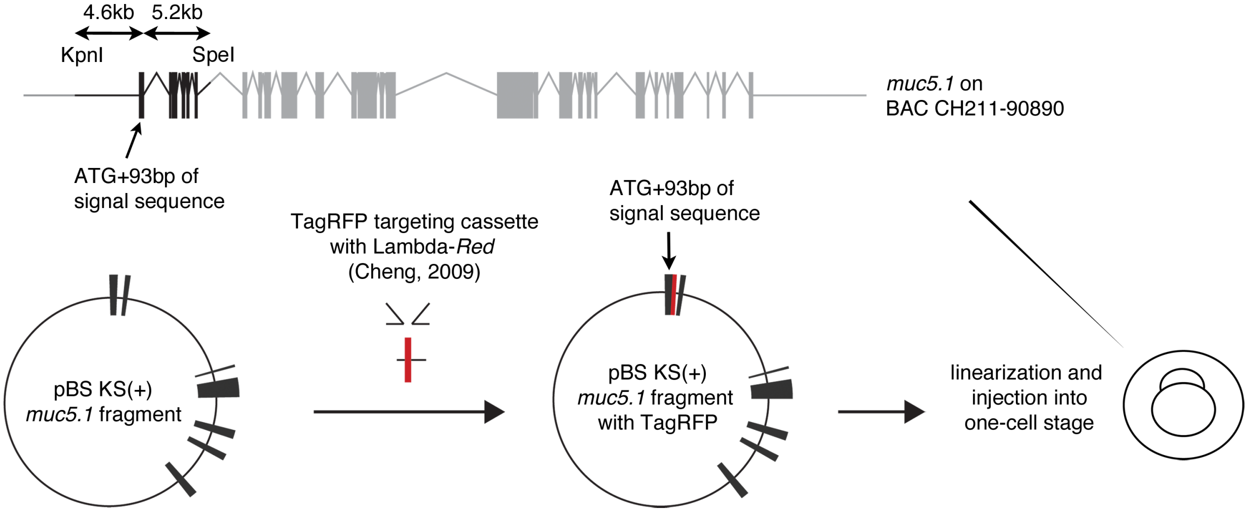
Supplementary Figure S4. *In situ* hybridization of the secreted mucins in sagittal sections of adult zebrafish. Image on the right shows an enlargement of the boxed area on the left. ph - pharynx, gi - gills, es - esophagus. DIG-UTP antisense RNA probes were used and positives are visualized in blue via NBT/BCIP reaction. The *in situ* hybridization signal suggests the localization of *muc5.1* and *muc5.2* in the pharynx, *muc5.3* in the esophagus, and *muc2.1* in the gut, supporting the RT-PCR data on isolated tissues in Fig 1c. Scale bar: 100 μm.



Supplementary Figure S5. Differential expression of *muc5.1 and muc5.2* in gill lamellae. The gill lamellae were separated from the gill arches. The total RNA was extracted from the lamellae, transcribed into cDNA and mucin transcripts were amplified by PCR. *muc5.1* but not *muc5.2* is expressed in the lamellar part of the zebrafish gills.



Supplementary Figure S6. qRT-PCR analysis of mucin gene expression during zebrafish development. The expression of individual mucin genes was normalized to *beta-actin* expression and compared to the expression at day six for each individual mucin. The timing of expression of each mucin correlates with the development of the respective organs in which they are found in the adult fish, namely the skin for *muc5.1* and *muc5.2*, the esophagus for *muc5.3* and the gut for *muc2.1* [27, 28].



Supplementary Figure S7. Generation of the fluorescent mucin reporter *muc5.1:S-RFP*. The reporter consists of the Tag-RFP (Tag-labeled Red Fluorescent Protein) fused in frame with the mucin signaling sequence downstream of the *muc5.1* promoter (S). A 9.8kb KpnI/SpeI fragment, which contains the mucin promoter region and part of the 5' region of *muc5.*1, was excised from BAC CH211-90890 and cloned into the pBSII KS(+) vector. The Tag-RFP targeting cassette was generated with high fidelity PCR and inserted behind the mucin signaling sequence in the first exon by Lambda-*Red* homologous recombination (see [42, 43] and Methods for details). The resulting construct contains the *muc5.1* promoter region (4.6 kb in the promoter and 5.2 kb in the 5' genomic sequence) and RFP fused in-frame with ATG. RFP is terminated with the stop codon.

Supplementary Table S1. Additional mucin-like fragments from the zebrafish genome identified in the bioinformatics searches. Their classification was determined with MrBayes. It appears that the zebrafish genome might contain at least one more *muc5*- and one more *muc2*-like mucin.

Ensembl ID	**Position**	**Genomic size (bp)**	**Domains**	**Mucin family**
ENSDARG00000089256	Zv9_NA551	13024	Cys-knot	*muc5*
ENSDARG00000090240	Zv9_NA551	2222	VWD	*muc5*
ENSDARG00000060302	Zv9_NA551	500	VWD	*muc5*
ENSDARG00000089023	Zv9_NA447	9544	VWD	*unknown*
ENSDARG00000076684	Zv9_NA447	18758	VWD	*muc2*
ENSDARG00000073938	Zv9_NA774	22126	PTS, VWD, Cys-knot	*muc2*
ENSDARG00000089990	chr25:8,583,497-8,592,403	8907	none	*muc5*

Supplementary Addendum S1. Protein sequences of secreted gel-forming mucins in zebrafish used for the construction of the protein domain models.

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>danrer_Muc5.1 | chr25 cluster | IDENTICAL TO ENSEMBL : ENSDARG00000070331 |
ENSDART00000153708| ENSDARP00000127611

MGFDSEGTVRMPQMWMLRWVFLLAGLQSIQAGFMGDYRDMENPMTPMWPTTPAISMTSVPVITVEPNPDHRSTICST
WGNFHFKTFDGHFFQLPDTCNYVLAVMCDAASSDFNIQMQRETVNGSISFSTVLIKLDGTVIKVTDSDITMGEETVT
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GTVYKSGESYQQACKSCTCDAGHWTCTYLDCPGTCSVVGGSHVTTFDGKSFTFSGNCDYILTKHSADSDFAVVGNLA
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>danrer_Muc5.2 | chr25 cluster | IDENTICAL TO ENSEMBL ENSDARP00000075869 ENSDART00000081428 ENSDARG00000058556 comment: missing parts of pts domain as compared to genewise result below

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>danrer_Muc5.2_alt_genewise | chr25 cluster | obtained by genewise alignment with muc5.3 protein to chr25 region encoding muc5.2.

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>danrer_Muc2.2_chr25_cluster | chr25 cluster | IDENTICAL TO ENSEMBL
ENSDARG00000078994 |ENSDART00000112246| ENSDARP00000098665
comment: incomplete, without 4th vwd of muc2

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VISGLFQSTFIRS


>danrer_Muc2.2_chr25_cluster_genewise | chr25 cluster | Same as ENSEMBL
ENSDARG00000078994, but more complete version obtained by alignment of muc2.1
to genomic region of ENSDARG00000078994
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>danrer_Muc2.1 | Zv9_NA774 | IDENTICAL TO ENSEMBL ENSDARG00000074142
|ENSDART00000046576 | ENSDARP00000046575 comment: no 4th vwd domain

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>danrer_Muc5.3 | chr7 | IDENTICAL TO ENSEMBL ENSDARG00000089847
|ENSDART00000148421 | ENSDARP00000124449

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```
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```

```
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```

```
>danrer_alternative_Muc5.3 | chr7 | obtained by genewise alignment, may not
be reliable
```

```
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```

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```

Supplementary Addendum S2. Protein sequences of gel-forming secreted mucins used for the construction of the phylogenetic trees. Multiple alignment is in FASTA format and covers only the N-terminal part of the secreted mucins including three VWD domains, before the PTS region.

```
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TIYTNIRSKTGLTVMWSKDGAVTVELDNKYQNKTCGLCGNFNRIRDEF-I
TGNTEKIGYIEFGNKHKVFNPMEFCENPSETDEKNNWKAICGS-------
------------YQADCEELFRAKAWSSCAMSYHP--YVDACVYDRCFSK
-----SSSLCATLSEYSRQCSRNGGTPSTWRTKDFCAVTCPYNTVHFESG
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LPNLEGCFL

>danrer_Muc2.1
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Supplementary Addendum S3. Sequences of transcripts obtained by RT-PCR and sequencing to verify the expression of mucin genes. The primers used for amplification of each transcript are also shown.

muc5.1

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R1 – IJ4 – tggtggtgttgaaaatgtga

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AAGGGCAATGTGTGTGGACTGTGTGGAAACTTTGATGGCAATGCAAACAATGACTTCATGAAGCACAATGGGGAAGT
GGTGACAGATCCAGAGGAGTTTGGCAATAGCTGGACGGTGGATTCAAATTGTCCTGATGTGACAAATGTGATGCATC
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F3 - IJ7 – catccctaggcacatccact
R3 - IJ63 – tgttttgcgtcgcttaagaa

CATCCCTAGGCACATCCACTGTTACACCAACTACTGGAACTTCGGCACCAACTTCTACAGAAACACCATCCACAACA
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muc5.2

F1 - IJ68 – ctgagatgggtcatcctgct
R1 - IJ71 – gtctcgggtgatgaaggtgt

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ACAAAA

muc5.3

F1 - IJ80 – cgagcaatatgcacagcact
R1 - IJ81 – gctcgagcagttgaaaaacc

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ACTGCAAGTGTGCGCATTATCATC

muc2.1

F1 – IJ125 – tgctgcttctggcgctttctggt
R1 - GY2 – cataaccaatttccccatcg

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muc2.2

F1 - IJ111 – actgtccgtgtcctcatggt
R1 - IJ112 – tctcctccagtgtcacatgc

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F2 - IJ113 – accacaaccaaacccatgtt
R2 - IJ114 – cctctcgaatgctggatctc

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