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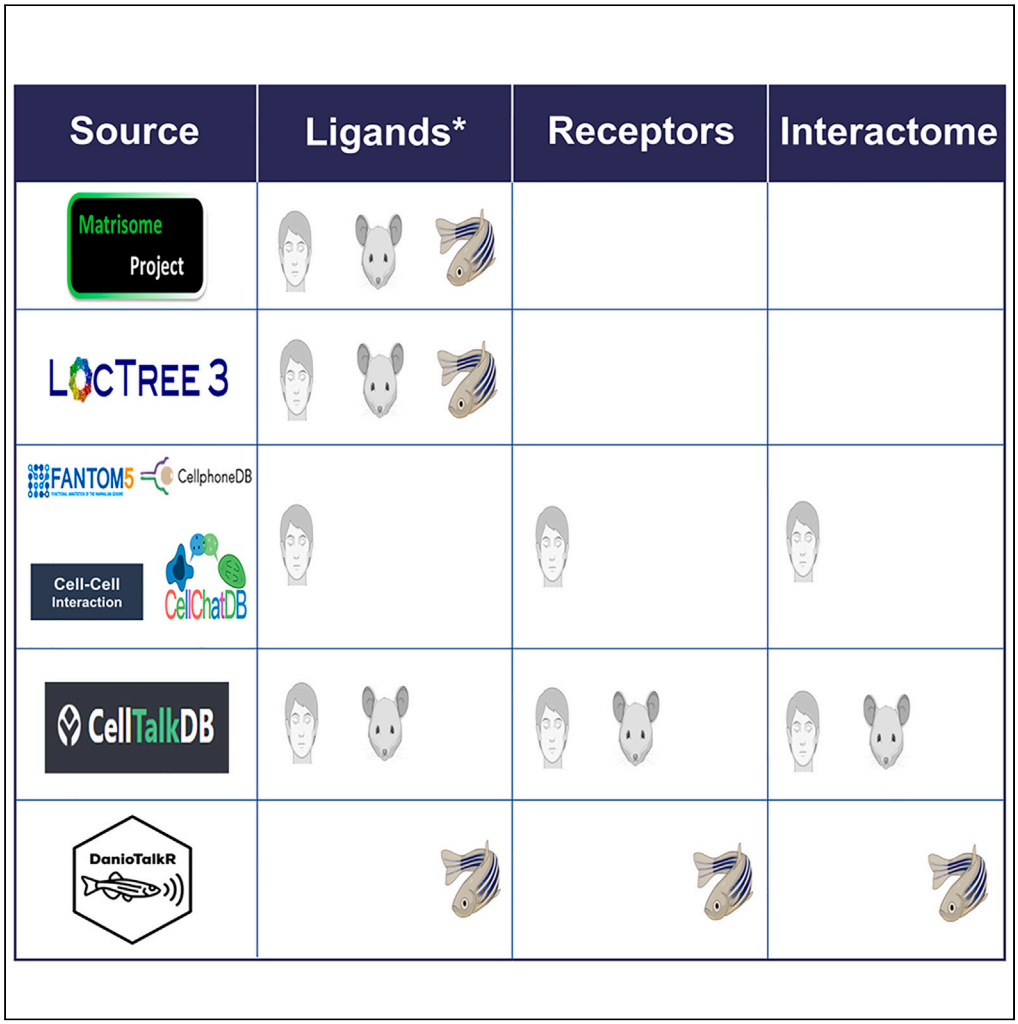

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Highlights

We *de novo* predicted the cellular localization of zebrafish reference proteome

We created zebrafish ligand-receptome interactome atlas & tools to explore them

Our tools also facilitate the identification of drugs against ligands & receptors

Our methodology can be applied to build and explore the interactome of other organisms

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A ligand-receptor interactome atlas of the zebrafish

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SUMMARY

Studies in zebrafish can unravel the functions of cellular communication and thus identify novel bench-to-bedside drugs targeting cellular communication signaling molecules. Due to the incomplete annotation of zebrafish proteome, the knowledge of zebrafish receptors, ligands, and tools to explore their interactome is limited. To address this gap, we *de novo* predicted the cellular localization of zebrafish reference proteome using deep learning algorithm. We combined the predicted and existing annotations on cellular localization of zebrafish proteins and created repositories of zebrafish ligands, membrane receptome, and interactome as well as associated diseases and targeting drugs. Unlike other tools, our interactome atlas is based on both the physical interaction data of zebrafish proteome and existing human ligand-receptor pair databases. The resources are available as R and Python scripts. DanioTalk provides a novel resource for researchers interested in targeting cellular communication in zebrafish, as we demonstrate in applications studying synapse and axo-glial interactome. DanioTalk methodology can be applied to build and explore the ligand-receptor atlas of other non-mammalian model organisms.

INTRODUCTION

Ligand-receptor-mediated cellular communication is essential for diverse processes such as differentiation, homeostasis, tissue morphogenesis, animal development, and behavior.[1–4] Dysregulated cellular communication can lead to a plethora of phenotypes ranging from uncontrolled cellular proliferation and developmental defects to disease states and drug resistance.[5–7] Hence, receptors and ligands are among the leading drug targets for treating human disease conditions.[8,9]

With advances in cryoelectron microscopy and machine learning approaches that enable protein structure prediction, the number of drug targets is expected to increase.[10–12] These developments can aid drug screenings directly using animal model organisms. Due to practical and ethical challenges in using mammalian models in drug screening and limitations of non-animal-based drug screens, larval zebrafish are being increasingly used in high-throughput drug screening.[13–17] However, despite the importance of zebrafish in drug discovery, there are no actively maintained datasets of zebrafish ligands, receptors, and their interactions. Few existing predictions of secretome and membrane proteome are based on incomplete annotation data.[18–21]

With the extensive single-cell RNA sequencing (RNA-seq) datasets of zebrafish, cellular communication in zebrafish can be explored at a holistic level even for rare and transient cells.[22,23] Several tools built on mammalian interactome records have been applied to map the zebrafish ligand-receptor interactions.[24–26] However, tools that depend on ortholog records are likely to ignore zebrafish genes lacking mammalian orthologs or genes with incomplete ortholog records. Hence, it is necessary to build a zebrafish ligand and receptome database that also includes information on ortholog-less genes and genes with incomplete annotation data.

Here, we describe DanioTalk—a repository of zebrafish ligands, membrane receptome, and interactome and tools to explore them from zebrafish-based omics datasets. To address the limitations of existing tools, DanioTalk ligand and membrane receptome datasets are based on deep learning-based subcellular localization predictions allowing the incorporation of orphan zebrafish proteins. DanioTalk can identify ligand-receptor pairs based on physical interaction records of zebrafish proteome and orthologous interactions in

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human ligand-receptor databases. DanioTalk scripts are available on GitHub and allows exploration of ligand-receptor interactions in any zebrafish-based omics datasets. Finally, we present the application of DanioTalk on mapping the ligand-receptor interactions between synaptosome and postsynaptic density (PSD) and axo-glial interactions between oxytocin neurons and glial pituicytes from RNA-seq datasets. We identified novel ligand-receptor interaction pairs with potential functions in axo-glial interactions. DanioTalk provides a novel tool and opportunity for the zebrafish research community to explore ligand-receptor interactions.

RESULTS

De novo prediction of zebrafish secretome and membrane proteome

To build a zebrafish ligand and receptor database, we require either experimental or *in silico* knowledge on zebrafish protein localization. A bottleneck in using gene ontology (GO) terms or UniprotKB-based cellular localization records of the zebrafish proteome is the incompleteness of such annotations. We identified only 19,281 (41%) of zebrafish reference proteome records (UP000000437) in UniprotKB that contain annotations for protein localization (Figure S1A).[27,28] To address this issue, we used DeepLoc 2.0, a sequence-based deep learning algorithm that can predict cellular localization of proteins with high accuracy[29] (Figure 1A). We subjected the zebrafish reference proteome to DeepLoc 2.0-based prediction.[30] The majority of proteins were predicted to be cytoplasmic (n = 12,186; 26%), nuclear (n = 7,905; 17%), and multilocalizing (n = 12,211; 26%) (Figures 1B, S1B, and Table S1). DeepLoc 2.0 predicted that 3,699 (8%) and 6,481 (14%) of proteins were exclusively extracellular- and cell membrane-localized, respectively (Figure 1B and Table S1). This corresponds to 2,571 (10%) and 4,002 (15%) genes that code extracellular- and membrane-localized proteins, respectively (Figure 1B'). We observe that out of the 2,571 genes whose proteins were predicted by DeepLoc 2.0 to be exclusively extracellular, 1,137 genes have been annotated or reported to be coding for extracellular proteins in UniprotKB and zebrafish matrisome database.[21,28] Furthermore, DeepLoc 2.0 identified an additional 1,104 and 1,953 genes coding for extracellular and membranal proteins, respectively (Figures 1C and 1D).[19,21,28]

Next, we manually assessed how reliable are DeepLoc 2.0 predictions for a subset of proteins. DeepLoc 2.0 successfully predicted the most abundant secreted proteins comprising pancreatic enzymes (Prss, Cela, Cpa, Amy2a family) as extracellular proteins (coded by 24 genes)[31] (Table S2). DeepLoc 2.0 prediction efficiency was also high for the Wnt family of secreted proteins (coded by 24 genes). Next, we assessed the DeepLoc 2.0 predictions for the Fgf protein family, which includes signal peptide-less secreted (Fgf9, Fgf16, Fgf20) and non-secreted Fgf11 subfamily.[32] DeepLoc 2.0 successfully predicted 17 genes coding for canonical signal peptide-containing extracellular Fgf proteins (Fgf 3–8,10, 17–19, 21–23) and all 7 of the intracellular Fgf11 subfamily proteins as non-extracellular proteins. However, when classifying signal peptide-less extracellular Fgf's, DeepLoc 2.0 only classified Fgf9 as extracellular and misclassified Fgf16, Fgf20a, and Fgf20b as cytoplasmic proteins (Table S2). Therefore, although DeepLoc 2.0 is highly reliable in predicting zebrafish secretome, manual curation is required to resolve the misclassified proteins.

Curation of zebrafish ligands and membrane receptome

To create a list of zebrafish ligands and membrane receptome, we first combined our DeepLoc 2.0 predictions with annotations from other existing records (UniprotKB cellular localization, GO terms, and Matrisome).[21,27,28] Furthermore, we added the zebrafish orthologs of curated mammalian ligand- and receptor-coding genes in Cell-Cell Interaction (baderlab.org/CellCellInteractions) and CellTalkDB databases (Figure 2A)[33,34] and performed further manual curation (as detailed in STAR methods). Our curated ligands and membrane receptome database consist of 2,788 (10.5%) and 2,343 (8.8%) of protein-coding genes, respectively (Figure 2B and Table S3). In comparison to zebrafish matrisome dataset, our curated ligand lists contain 1,848 additional genes (1,566 DeepLoc 2 predictions and 282 manually curated extracellular-coding genes) (Figure 2C). In addition, we compared our curated ligand and receptor database with orthologs of ligand and receptor from human ligand-receptor databases (CellChatDB, CellPhoneDB, Ramilowski J.A. et al., CellTalkDB, and Cell-Cell Interactions database).[33,35–39] Out of the 2,788 genes coding the curated ligands, 1,482, i.e., 53% of the genes are in common with human ligand databases (Figures S2A and S2C). Likewise, out of the 2,344 genes coding for receptor in our DanioTalk receptor database, 1,337, i.e., 57% are in common with human receptor databases (Figures S2B and S2D).

Next, as ligands can be also membrane-bound, we analyzed the localization of the ligands in our curated database. Specifically, we compared our curated ligand database with 1) annotations for cell membrane

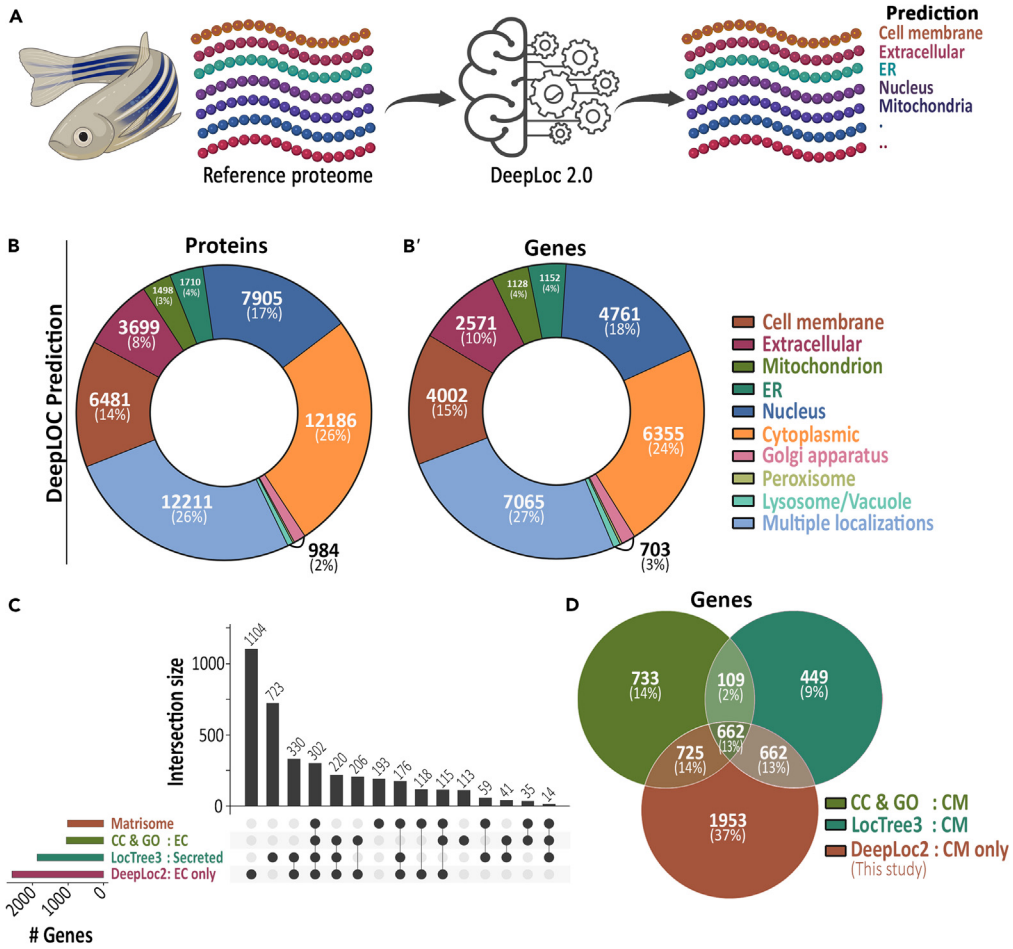

Figure 1. Prediction of cellular localization of zebrafish reference proteome

(A) Graphic describing the DeepLoc 2.0-based prediction of cellular location of the reference zebrafish proteome.

(B) Zebrafish reference proteome cellular localization prediction. Donut chart showing the major DeepLoc 2.0 predictions of zebrafish proteome. The number of proteins (B) and protein-coding genes (B′) are shown.

(C) Upset plot comparing the number of genes with existing extracellular annotation records and DeepLoc 2.0-based 'Extracellular' predictions. CC & GO indicates cellular localization and GO term records, respectively, available in UniprotKB database.

(D) Venn diagram comparing genes with existing cell membrane localization annotation records and DeepLoc-based 'cell membrane' (CM) predictions. CC & GO indicates cellular localization and GO term records, respectively, available in UniprotKB database.

localization in the Zebrafish UniprotKB database; 2) DeepLoc2 cell membrane predictions; and 3) zebrafish orthologs of CellphoneDB membrane-bound extracellular proteins.[35] We identified that out of the 2,788 DanioTalk ligand-coding genes, 10.6%, i.e., 296 ligands are likely to be membrane-bound ligands (Figure S2E).

Disease-associated ligands and membrane receptome

Next, we explored the identity of human diseases in which zebrafish models can be used for understanding disease etiology and/or in drug screening. We cross-referenced our ligand and membrane receptor list with the human ortholog information available in ZFIN (Zebrafish Information Network) database.[40] We identified 51% and 43% of the ligand- and cell membrane receptor-coding genes, respectively, having human ortholog gene records (Figure 2D and Table S3). Next, we cross-referenced our dataset with the zebrafish orthologs of human disease-associated genes in Online Mendelian Inheritance in Man (omim.org) database. We identified 549 ligand- and 397 receptor-coding genes containing records of human ortholog

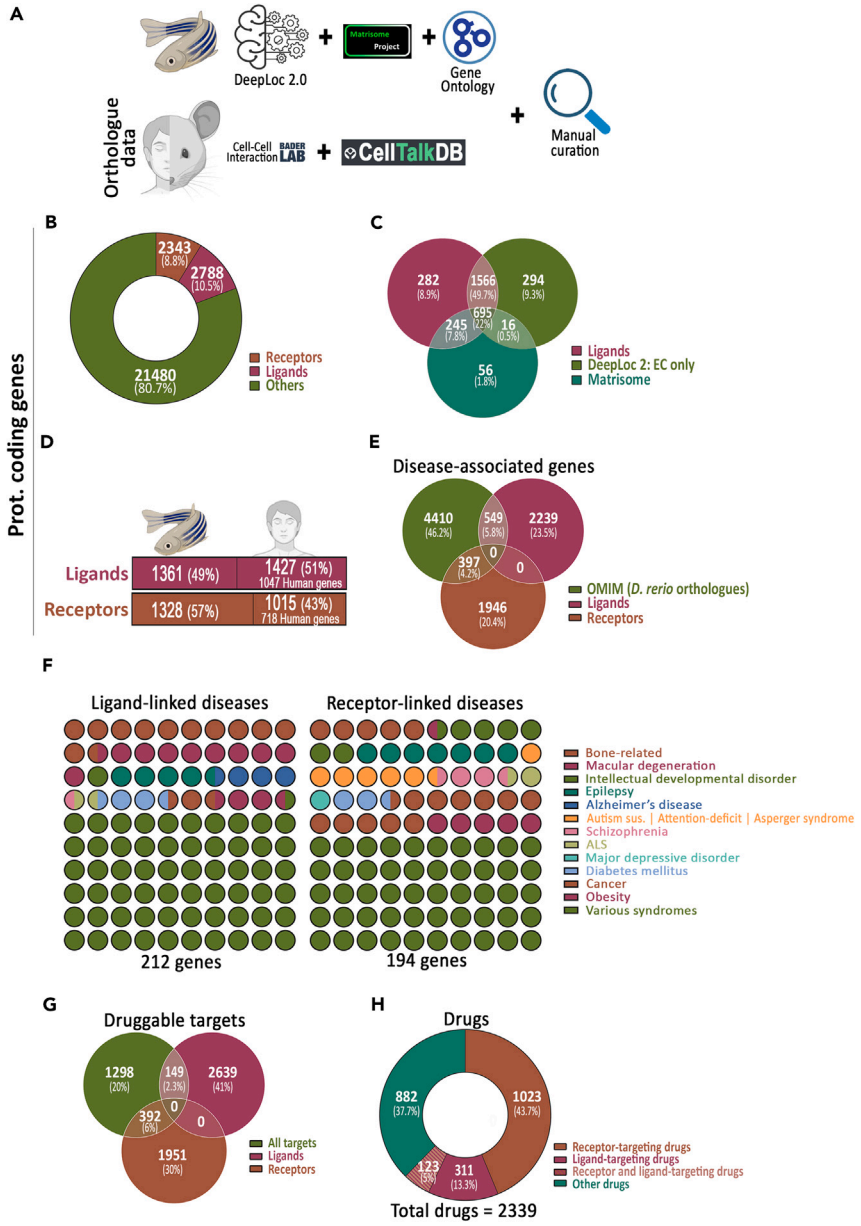

Figure 2. Curated zebrafish ligands and membrane receptors

(A) Scheme describing the steps taken for the creation of curated zebrafish ligands and membrane receptors list.

(B) Donut chart showing the number and percentage of zebrafish genes coding for curated receptor- and ligand-coding genes.

(C) Venn diagram comparing curated ligands with DeepLoc 2.0 extracellular predicted proteins and previously reported Matrisome dataset.

(D) Conservation between zebrafish and human ligand- and membrane receptor-coding genes based on ZFIN records.

(E) Venn diagram comparing the number of curated ligand- and receptor-coding genes with zebrafish orthologs of human disease-linked genes in the OMIM database.

(F) Dot plot showing the diseases associated with human orthologs of some of the zebrafish ligands and membrane receptors.

(G) Venn diagram showing the number and percentage of zebrafish membrane receptors and ligands potentially targetable by drugs listed in the DrugCentralDB.

(H) Donut chart showing the number and percentage of drugs that can potentially target zebrafish membrane receptors and/or ligands.

genes that are disease associated (Figure 2E and Table S4). Some of the major diseases associated with ligands and cell membrane receptors are related to bone diseases, macular degeneration, diabetes mellitus, and cancer (Figure 2F). Nevertheless, several of the ligands and receptors were also associated with numerous syndromes (e.g., Ehlers-Danlos syndrome, Loeys-Dietz syndrome, and Long QT syndrome) (Figure 2F).

Next, we explored the drug-targetable status of the zebrafish ligands and receptome. We cross-referenced our ligand and receptome list with DrugCentral database that contains an up-to-date list of drugs and their targets.[41] We identified 149 ligands and 392 membrane receptors that can be potentially targeted by drugs (Figure 2G and Table S5). With respect to the choice of drugs, 311 (13%) and 1,023 (44%) of the drugs can potentially target zebrafish ligands and membrane receptors, respectively (Figure 2H). Moreover, zebrafish genes coding for the drug-targetable ligand or receptor are highly conserved in comparison to the orthologous human genes based on the orthology score in the Genome Alliance database (Figures S3A and S3B).[42]

DanioTalk—Putative ligand-membrane receptome interactome in zebrafish

Next, to map the ligand-receptome interactions, we identified all pairwise interactions between our ligand and receptome proteins in the STRING (v11.5) physical protein-protein interaction (PPI) records of zebrafish (Figure 3A, Table S6, and Figure S4), a subset of which is shown in (Figure 3A').[43] The experimental score in STRING database predicts the confidence in the physical proximity of two proteins. Ligand-receptor pairs with high-confidence scores are very likely to physically interact and result in associated downstream signaling activities. We identified 70,476 ligand-receptor pairs out of which 1,368 pairs between 370 ligands and 342 receptors were above high confidence cutoff score (Figures 3B–3D).

Next, to estimate the correlation between zebrafish and human ligand-receptor pairs, we compared zebrafish STRING physical interaction scores for the ligand-receptors pairs that have orthologous pairs in human ligand-receptor databases.[33–38] We observed highly variable STRING physical interaction scores, and several hundred pairs were below the medium cutoff score (Figure S5A).

As setting a cutoff value below the medium confidence will result in increase in false-negative ligand-receptor pairs, we also incorporated orthologous human ligand-receptors pairs available in human PPI database IID (Integrated Interactions Database) and other human ligand-receptor databases (Figures S4 and S5B).[33–38] This increased our database to 102,903 ligand-receptor pairs, out of which 22,689 pairs are present in human ligand-receptor databases, and IID experimentally validated PPI records (Figure 3D and Table S6). Even after the addition of orthologous ligand-receptor pairs, we observed 1,605 ligands and 1,368 receptors that lack interacting receptor or ligand, respectively, at medium confidence cutoff (Figures 3B and 3C). DanioTalk contains novel receptors for 183 ligands and novel ligands for 178 receptors (Figures 3B and 3C). DanioTalk contains 3,970 novel zebrafish-specific ligand-receptor pairs (350 high confidence + 3,620 medium confidence pairs) (Figure 3D), and we observe approximately 1–3 ligand per receptor and vice versa (Figure 3E).

We next probed the accuracy of our ligand-receptor interactome database. We focused on the nodal-related transforming growth factor β (Tgfβ) ligands and Wnt ligands, key regulators of development.[4,44,45] Our interaction database identified all the previously reported receptors for Ndr2/Cyclops, Ndr1/Squint, and Spaw/Southpaw ligands namely Acvr1b/Alk4, Acvr2a, and Acvr2b, respectively (Figure 4A and Table S7). We also noticed Acvr1c, Tgfbr1, and Tgfbr2 as top-ranked receptors. Next, we probed Wnt signaling ligand-receptor interactome. Our interaction database identified all the Wnt receptors (Lrp5, Ryk, Fzd, Ror1, and Lrp6) that can interact with the major Wnt ligands (Figure 4B and Table S7). Altogether, DanioTalk can accurately identify major ligand-receptor interactions for Notch and Wnt signaling pathways.

Zebrafish brain synaptic ligand-membrane receptome interactome

Next, we tested the application of DanioTalk on existing datasets. We first focused on the zebrafish adult brain synaptosomal and PSD proteome dataset (Figure 4C).[46] We identified protein products of 145 ligand- and 185 receptor-coding genes in the synaptosomal fraction. While in the PSD fraction, we identified protein products from 74 ligand- and 88 receptor-coding genes (Figures S6A, S6B and Table S8). The top-ranked ligand-receptor interacting pairs between synaptosome ligands and PSD receptors were

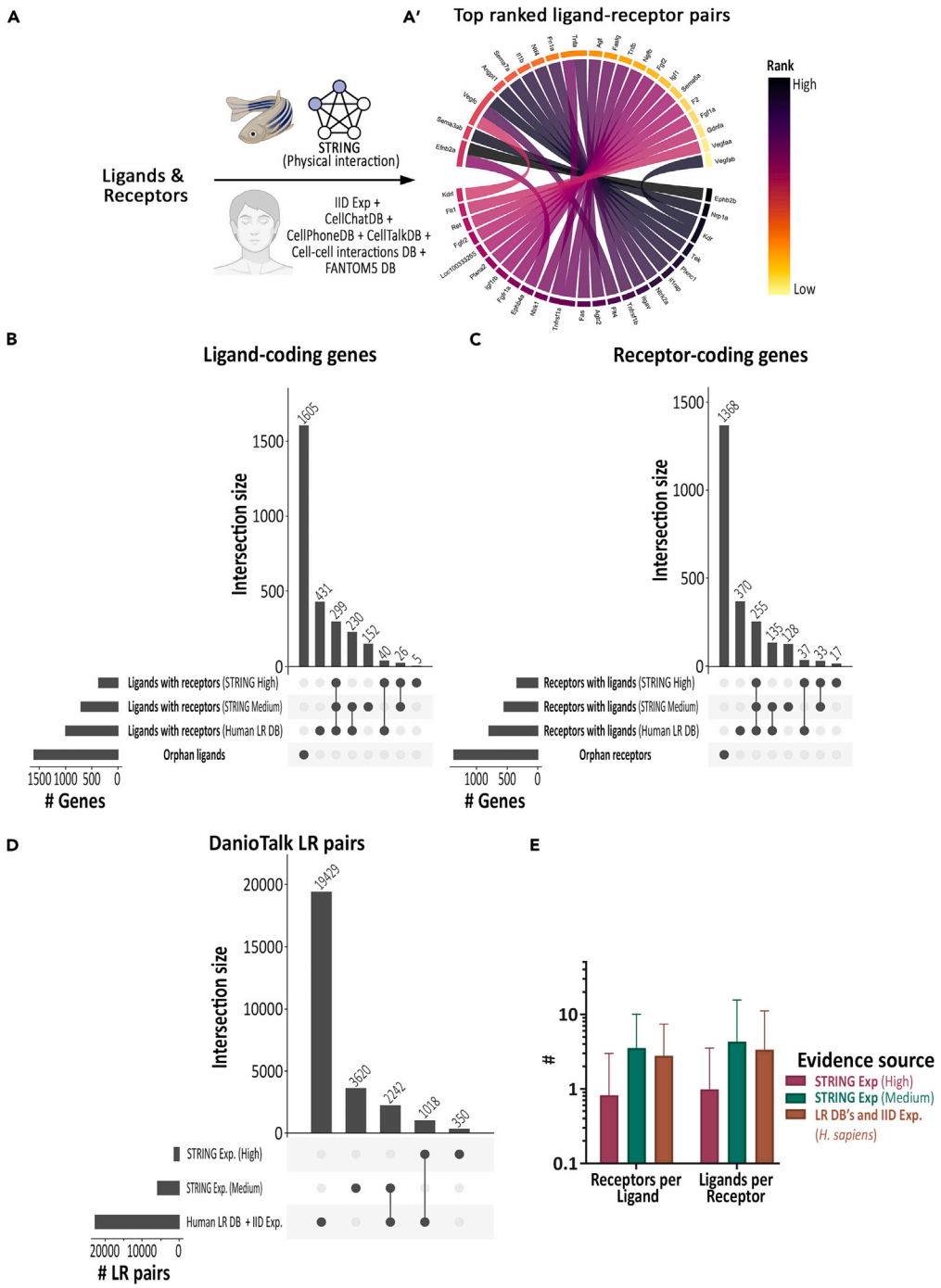

Figure 3. Zebrafish Ligand-Receptor Interactome

(A) Scheme describing steps undertaken to map the zebrafish ligands and membrane receptors interactome (A). Circle plot showing the top 25 ranked ligand-receptor pairs (A').

(B) Orphan status of ligands. Upset plot of genes coding for ligands with or without potential receptors in the zebrafish STRING PPI database (High or Medium cutoff) or orthologous receptors in the human ligand-receptor database.

(C) Orphan status of receptors. Upset plot of genes coding for receptors with or without potential ligands in the zebrafish STRING PPI database (High or Medium cutoff) or orthologous ligands in the human ligand-receptor database.

(D) Upset plot of DanioTalk ligand-receptor pair database genes derived from zebrafish STRING PPI database (High or Medium cutoff) or orthologous ligand-receptor pairs in the human ligand-receptor databases.

(E) Zebrafish ligand-receptor interactome stats. Column chart comparing average number of ligand/receptor or receptor/ligand based on zebrafish STRING PPI database or orthologs in the human ligand-receptor database.

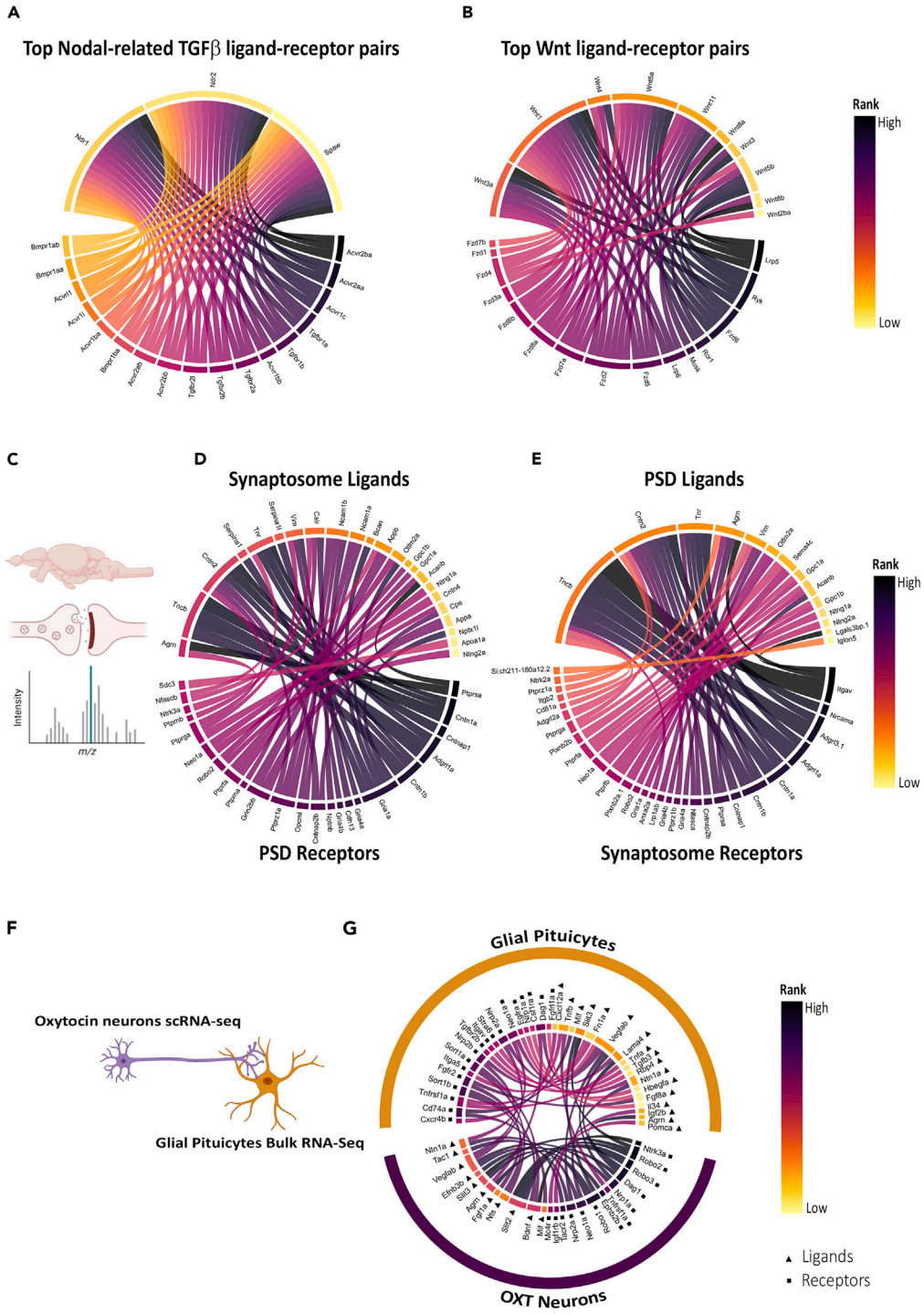

Figure 4. Application of DanioTalk

(A) Receptors for Nodal-related Tgfβ ligands. Circle plot showing the top-ranked receptors for Ndr2, Ndr1, and Spaw ligands. The top 50 ranked interactions at medium confidence (>400) cutoff are plotted.

(B) Receptors for Wnt ligands. Circle plot showing the top-ranked receptors for Wnt ligands. The top 50 ranked interactions at high confidence (>700) cutoff are plotted.

(C) Scheme showing synaptosome-PSD from adult zebrafish brain. Mass spectrometry data were analyzed.[46].

Figure 4. *Continued*

(D) Circle plot showing the top-ranked ligand-receptor interactions between the synaptosome ligands and PSD receptors. Interactions were ranked based on quasi-percentile of peptide expression. The top 50 ranked interactions at medium confidence (>400) cutoff are plotted. Ribbon width indicates interaction scores.

(E) Circle plot showing the top-ranked ligand-receptor interactions between the PSD ligands and synaptosome receptors. Interactions were ranked based on quasi-percentile of peptide expression. The top 50 ranked interactions at medium confidence (>400) cutoff are plotted. Ribbon width indicates interaction scores.

(F) Scheme showing axo-glial interaction between oxytocin neuron and glial pituicytes. Adult oxytocin neuron cluster pseudo-bulk data from scRNA-seq data and adult glial pituicyte bulk RNA-seq data were analyzed.[47,48].

(G) Circle plot showing top-ranked ligand-receptor interactions between oxytocin neurons and glial pituicytes. Interactions were ranked based on quasi-percentile of gene expression. The top 50 ranked interactions at highest confidence cutoff (>900) are plotted.

Agrn-Ptprsa, Cntn2-Cntnap1/Cntn1a/Cntn1b, and Tncb/Adgrl1a (Figure 4D and Table S9). The top-ranked PSD ligand-synaptosome receptor pairs were Tncb-Itgav/Cntn1b and Cntn2-Nrcama/Cntnap1/Cntn1a (Figure 4E and Table S9).

Zebrafish oxytocin neuron-glial pituicytes axo-glial interactome

Axo-glial interactions contribute to synaptic morphogenesis and plasticity.[49,50] However, the identity of all the axo-glial ligand-receptor interactions in neurohypophysis (NH), a major neuroendocrine interface has not been previously investigated.[51,52] The NH-projecting axonal tracts, NH axo-glial cellular components, and their ultrastructure are conserved in zebrafish.[47,53–55] We aimed to identify the axo-glial interactome of oxytocin neurons and glial pituicytes. Due to the lack of oxytocin neuronal axonal transcriptome, we used the oxytocin neuron scRNA-seq (single-cell RNA-sequencing) data and glial pituicyte bulk RNA-seq data (Figure 4F).[47,48] Using the differentially expressed list of genes from these datasets, we identified only 3 ligand-receptor interactions between oxytocin neurons and glial pituicytes at high confidence (Nts-Sort1a, Bdnf-Ngrfb/Sort1a) (Figures S6C–S6E and Table S10). To incorporate all the genes expressed in oxytocin neurons, we used the pseudo-bulk expression data of oxytocin neuron-enriched cluster. This led to the identification of 282 ligands and 219 receptors in oxytocin neurons. Whereas in glial pituicytes transcriptome, we identified 363 ligands and 186 receptors with average read counts >50 (Figures S6F–S6G and Table S11). Analyzing the ligand-receptor pairs in this dataset led to identification of 38 oxytocin neuron-glial pituicyte ligand-receptor interactions. The top-ranked pituicyte ligand-oxytocin neuron receptor interactions were Slit3-Robo2/Robo1, Lama4-Dag1, and Vegfab-Nrp1a. While the top-ranked oxytocin neuron ligand-pituicyte receptors were Mif-Cd74a, Nts-Sort1a/Sort1b, and Bdnf-Sort1a/Sort1b (Figure 4G and Table S11). We also observed several autocrine signaling interactions in glial pituicytes (Cxcl12a-Cxcr4b, Tnfb-Tnfrsf1a, and Fgf8a/Fgf10a-Fgfr2) and in oxytocin neurons (Slit3/Slit2-Robo2/Robo1/Robo3, Bdnf-Ntrk3a/Sort1a, and Agrn-Dag1) (Figure 4G and Table S11).

DISCUSSION

Zebrafish scRNA-seq datasets are also used to explore and study the ligand-receptor interactome using tools built for mammalian model organisms.[24–26,56] However, a bottleneck in using such tools is in the dependence on incomplete orthologous mammalian ligand-receptor classifications and mammalian ligand-receptor interactome records.[57,58] Thus, tools to study zebrafish ligands, receptome, and interactome independent of ortholog records are essential to help explore ligand-receptor interactions in zebrafish datasets.

In the lack of experimental records, recent studies reported the relatively high success of machine learning-based tools in predicting protein structures and cellular localizations.[29,59,60] Here, we applied a deep learning algorithm to predict the cellular localization of the zebrafish proteome and created a curated repository of the zebrafish ligands and membrane receptome. This allows the incorporation of orphan zebrafish ligands or membranal receptor proteins that previously lacked cellular localization or ortholog records. DeepLoc 2.0 predictions of zebrafish proteome were largely accurate for predicting signal peptide-containing secreted, extracellular protein-based ligands; however, we noticed several instances of misclassified proteins based on literature knowledge on orthologous proteins. Hence, we had to manually curate the database. Our manual curation and inclusion of zebrafish matrisome and mammalian ligand databases resulted in resolving misclassified proteins and inclusion of membrane-bound

ligands. Finally, our database of ligands and receptome-linked diseases will aid researchers interested in developing zebrafish models of human disease.

The DanioTalk algorithms are able to identify top interacting ligands and receptors in major pathways. This suggests that multiple published literature data has observed such PPIs leading to elevated physical interaction score in the zebrafish STRING database. Consequently, for these ligand-receptor pairs with high scores and high confidence, it is likely that the ligand-receptor pairs play a significant role in cellular signaling-mediated functions. High-confidence pairs that have not been previously reported in cellular signaling could be either novel ligand-receptor interactions or potential regulators of gradient formation. Currently, ligand-receptor pairs with low STRING PPI scores, mainly due to limited or less confident experimental evidence, will be assigned lower rankings by the DanioTalk algorithms. We anticipate that as the STRING PPI records are updated and PPI scores increase, the DanioTalk algorithms will be able to identify more high-confidence, zebrafish-specific ligand-receptor pairs.

Due to the inclusion of orthologous records of human ligand-receptor pairs in the DanioTalk ligand-receptor database, the majority of the putative ligand-receptor pairs are currently from human ligand-receptor databases. Hence, in addition to the zebrafish STRING PPI-based ligand-receptor pairs, DanioTalk output files also contain ligand-receptor pairs from multiple human ligand-receptor databases and human IID PPI database. The complementation of orthologous ligand-receptor pairs further strengthens the DanioTalk output files and provides users with the knowledge and database source of orthologous human ligand-receptor interactions. As some ligand-receptor signaling requires dimerization of receptors, to aid users, we also provide such information based on the orthologous records in human ligand-receptor pair databases.[35,36,61] Finally, to provide users with multiple options to explore their datasets, DanioTalk output files contain multiple annotations including GO terms, type of ligand-receptor signaling (secreted vs. cell-contact), and membrane-bound status of the ligands.

One of the strengths of research on larval zebrafish is in the pharmacological perturbation experiments which we have also taken advantage of in the past.[47] Some subscription-based tools built for mammalian model organisms can list drugs targeting mammalian proteins.[62,63] Our DanioTalk repository and output files contain similar information on drugs that can likely target the zebrafish ligands and receptome. In addition, the output files also contain orthology scores and lists all the supporting algorithms. The availability of orthology score will give users the confidence on the drug choice but only at the level of gene conservation. For novel drugs that have not been tested in zebrafish, users will still have to manually determine the conservation of the drug target site at the protein sequence and/or structure level or assess the drug targeting efficiency by experimental analysis or by monitoring the associated phenotype and/or downstream signaling events.

The potential of DanioTalk was revealed in our analysis of the existing dataset on adult zebrafish brain synaptosome-PSD proteomes.[46] Several top ligand-receptor pairs were previously studied for their synaptic role in other model organisms. For example, in mice CNTNAP1 (CASPR) is well known for its role in synaptic plasticity,[64] and Contactin has been shown to promote synaptic CNTNAP1 surface localization via *Cis*-interaction.[64] But the fact that zebrafish Cntn2 and Cntnap1 are enriched in synaptosome and PSD fractions suggests an experimental artifact or additional *trans*-synaptic roles for the Cntn2-Cntnap1 interaction.[65,66] In addition, another top-ranking ligand-receptor pair was Tenascin (Tncb)-Adhesion G Protein-Coupled Receptor L1 (Adgrl1a). Both TNC and ADGRL1 regulate synaptic development and function, but the role of TNC-ADGRL1 interaction has not been addressed yet.[67–70]

The presence of several conserved ligand-receptor pairs in the zebrafish synapses further stresses the importance of zebrafish in understanding the role of ligand-receptor in synapse biology and in drugs targeting synapses.[14,71,72] Although our synaptosome and PSD ligand-receptor pairs lack spatiotemporal accuracy, DanioTalk can be further combined with zebrafish single-cell brain transcriptome records to identify synaptosome-PSD ligand-receptor interactions in neuronal cell types of interest.[57,73]

In the absence of cell-specific proteome data, RNA-seq data in combination with protein interaction records are largely used to explore ligand-receptor-based cellular interactions.[39,74] However, such data were not explored to analyze axo-glial ligand-receptor interactions in NH, a major neuroendocrine interface critical in maintaining water balance and reproductive functions.[47,75,76] Except for few molecular players, the axo-glial interactions in NH were largely unknown.[75,77–79]

Our analysis identified the Slit3-Robo2 glial pituicyte-oxytocin neuron interaction, which has been reported to promote synaptic oxytocin levels in zebrafish.[80] However, we also observed autocrine Slit-Robo interactions in oxytocin neurons. SLIT-ROBO autocrine signaling in mice motor neurons has been shown to promote axon fasciculation; however, such defects were not reported for NH-projecting oxytocin axons in zebrafish larvae.[80,81] In vertebrates, central release of oxytocin and activity of oxytocin neurons play a key role in animal sociality and behavior.[53,82] It is worth to mention that Robo-Slit expressions are conserved in mammalian oxytocin neurons and mutations in OXTR and ROBO2 are observed in children with social deficits and families with autism spectrum, respectively.[76,83–85] And, in mice, although SLIT-ROBO signaling is essential for the differentiation and positioning of periventricular dopamine neurons, the role in oxytocin neuron development and oxytocin neuron-dependent animal behavior requires further studies.[86]

Neuro-glial signaling are bidirectional.[49] We found oxytocin neuron-derived Neurotensin (Nts) can potentially signal to glial pituicytes via Sortilin (Sort1b) receptor. In rat and teleost brain, neurotensin has been shown to be localized in hypothalamic neurons and in pituitary.[87–89] Neurotensin can regulate intracellular calcium levels in *in vitro* astrocyte cultures from rat ventral tegmental area.[90] But the role of oxytocin neuron-derived Neurotensin in glial pituicytes function or intracellular calcium is unknown.[91]

We also observed potential Cxcl12a-Cxcr4b- and Fgf10a-Fgfr2-based autocrine interactions in glial pituicytes.[92] CXCL12-CXCR4 functions as a chemokine and neurotransmitter during mice brain development and as mitogen for rat cortical astrocytes *in vitro*.[93–96] Although CXCL12-CXCR4 interaction is known to modulate NH-projecting vasopressin neuron firing pattern in rats, the role of CXCL12-CXCR4 interaction in glial pituicytes has not been reported yet.[97,98] We also observed Fgf10a-Fgfr2-based autocrine interactions in glial pituicytes, which suggest that Fgf10a could have additional autocrine functions in glial pituicytes such as reactivity and/or proliferation.[99–103]

Overall, DanioTalk offers a comprehensive repository of zebrafish ligands, membrane receptome, ligand-membrane receptome interaction atlas, and a novel data exploratory tool for zebrafish-based researchers. Our methodology can be also applied by other non-mammalian researchers to build ligand-receptor interactome atlas and tools for their model organism of interest.

Limitations of the study

One limitation of our study is that for the axo-glial ligand-receptor pairs, we used datasets derived from works that used different fish, tissue dissociation protocols, and technologies for generating transcriptome data.[47,48] This could have led to some artifactual ligand-receptor pairs, at least in the case of paracrine ligand-receptor pairs. Another limitation is the dependence of DanioTalk on the zebrafish STRING physical PPI pair database for generating zebrafish-specific ligand-receptor pairs. For zebrafish, in comparison to Pathway Commons, IntAct, and BioGrid, STRING database is the only database with extensive zebrafish physical PPI records.[43,104–106] Another limitation is due to the manual curation of ligands and membrane receptors. Currently, DanioTalk ligand-receptor pair-finder scripts are dependent on the manually curated list of ligands and membrane receptors. However, as we provide the original scripts, it is possible for users to edit the manual list of ligands and membrane receptors and rebuild the interaction database required for executing DanioTalk LR finder scripts. Finally, DanioTalk scripts will ignore receptors localized in other organelles and receptors that only require non-protein- or non-peptide-based ligands for signaling. This will be a disadvantage for researchers interested in such signaling.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.isci.2023.107309.

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AUTHOR CONTRIBUTIONS

S.A. conceived and designed the study. A.Z. contributed to study design and performed DeepLoc 2.0 predictions. M.C. wrote all the scripts. M.C. and S.A. performed the analysis. S.A. performed manual curation of ligands and receptors, wrote the manuscript, and prepared figures. All authors read and approved of the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

INCLUSION AND DIVERSITY

One or more of the authors of this paper self-identifies as an underrepresented ethnic minority in their field of research or within their geographical location.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Software and algorithms		
DanioTalk	This manuscript	https://github.com/DanioTalk and https://doi.org/10.5281/zenodo.7966578
R	R Core Team	Team et al.[107]
DeepLoc 2.0	Thumuluri et al.[29]	Thumuluri et al.[29]
Circlize R package	Gu et al.[108]	Gu et al.[108]
Other		
Zebrafish reference proteome (UP000000437)	UniprotKB	UniProt Consortium[28]
Zebrafish gene symbols, gene descriptions, orthologue records, ZFIN IDs, OMIM disease information	Zebrafish Information Network	Bradford et al.[40]
Zebrafish Matrisome	Nauroy et al.[21]	Nauroy et al.[21] and https://github.com/Matrisome/MatrisomeAnalyzeR/raw/main/data/matrisome.list.rda
Zebrafish protein protein physical interaction data	STRING (v11.5)	Szklarczyk et al.[43]
Zebrafish synaptosome and postsynaptic density proteome	Bayés et al.[46]	Bayés et al.[46]
Zebrafish oxytocin neuron transcriptome	Shafer et al.[48]	Shafer et al.[48]
Zebrafish glial pituicyte transcriptome	Anbalagan et al.[47]	Anbalagan et al.[47]
Zebrafish GO terms	Zebrafish Information Network	Gene Ontology Consortium, Bradford et al.[27,40] and http://current.geneontology.org/annotations/zfin.gaf.gz
Zebrafish protein localization predictions	LocTree3	Goldberg et al.[19]
Human Matrisome	Shao et al.[109]	Shao et al.[109] and https://github.com/Izzilab/MatrisomeAnalyzeR/blob/main/data/matrisome.list.rda
Human protein protein physical interaction data	IID database (v2021-05)	Kotlyar et al.[37]
Human LR pair database and annotations	CellPhoneDB_v4.1.0	Garcia-Alonso et al.[35] and https://github.com/ventolab/cellphonedb-data/archive/refs/tags/v4.1.0.tar.gz
Human LR pair database and annotations	CellChat_1.5.0	Jin et al.[36] and https://github.com/sqjin/CellChat/archive/refs/tags/v1.5.0.tar.gz
Human LR pair database	Ramilowski J.A. et al.,[38] FANTOM5	Ramilowski et al.[38]
Human LR and LR pair database	Cell-Cell Interactions	Ximerakis et al.[34] and https://zenodo.org/record/7589953/files/receptor_ligand_interactions_mitab_v1.0_April2017.txt.gz
Human LR and LR pair database	CellTalkDB	Shao et al.[33] and https://github.com/ZJUFanLab/CellTalkDB/releases/download/v1.0/scsrctdb-1.0.tar.gz
Human GO terms	Gene Ontology	Gene Ontology Consortium[27] and http://geneontology.org/gene-associations/goa_human.gaf.gz
List of drugs	DrugCentralDB	Avram et al.[41] and https://unmtid-shinyapps.net/download/DrugCentral/2021_09_01/drug.target.interaction.tsv.gz

RESOURCE AVAILABILITY

Lead contact

Further information and requests for scripts should be directed to and will be fulfilled by the lead contact, Dr. Savani Anbalagan (savanb@amu.edu.pl).

Materials availability

Software code generated in this study have been deposited to Github: https://github.com/DanioTalk and Zenodo https://doi.org/10.5281/zenodo.7966578.

Data and code availability

- The DanioTalk scripts are open-source and publicly available from Zenodo https://doi.org/10.5281/zenodo.7966578 Or Github: https://github.com/DanioTalk
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.
- Codes use to analyze the data are available in this paper's supplemental information.

METHOD DETAILS

Zebrafish, mouse and human datasets

A complete list of datasets used are listed in the key resources table. Zebrafish reference proteome (UP000000437) sequences along with GO-term and cellular localization annotations were downloaded from UniprotKB.[28] Gene symbols, ortholog gene symbols, aliases/synonyms, gene descriptions, and ZFIN IDs were downloaded from the ZFIN database.[40] The extracellular protein-coding gene was identified based on the annotation terms 'extracellular' or 'secreted' or 'ligands'. Cell membrane protein-coding genes were identified based on the terms "plasma membrane". Human ligand-receptor databases were downloaded from Cell-cell InteractionDB, CellChatDB, CellPhoneDB, CellTalkDB and Ramilowski et al.[33–36,38] Human PPI database was downloaded from IID.[37]

Subcellular localization prediction

For machine learning-based cellular localization data, zebrafish reference proteome was subjected to DeepLoc 2.0 algorithm with default parameters to obtain protein localization predictions and associated scores.[29]

Curated zebrafish ligands and membrane receptome database

For an initial list of zebrafish ligands, the extracellular protein-coding genes were identified based on DeepLoc 2.0-based extracellular protein coding gene predictions. The list was supplemented with genes with GO terms or subcellular location annotation matching 'extracellular' or 'secreted' or 'ligands'. These lists were further expanded with secreted and extracellular matrix-associated genes from zebrafish Matrisome database, extracellular proteins from LocTree3 database, zebrafish orthologs from human matrisome database, CellTalkDB ligands, curated Cell-Cell Interaction database ligands.[19,21,33,34,109]

For an initial list of zebrafish membrane receptors, membrane protein-coding genes were identified on DeepLoc 2.0-based membranal protein-coding gene predictions. The list was supplemented with genes with GO terms or subcellular location annotation matching "plasma membrane". These lists were further expanded with cell membrane proteins from LocTree3 database, zebrafish orthologs from human CellTalkDB receptors and in curated Cell-Cell Interaction database receptors.[19,33,34]

Both the initial ligand and membrane receptor lists were manually curated based on gene descriptions and localization records of human orthologs available in the Compartments database and the Human Protein Atlas.[31,110]

To attest the credibility of ZFIN listed human orthologs of zebrafish genes, we also incorporated Genome Alliance database provided human orthology score and identity of algorithms that support the orthology.[42]

Ligand-receptor interactome database

The ligand-receptor (LR) interaction database was generated based on zebrafish protein-protein interaction in STRING physical interaction database (v11.5). To supplement human LR pairs, we also added orthologous pairs from multiple human LR databases namely CellChatDB, CellPhoneDB, CellTalkDB, Cell-Cell Interactions DB, Ramilowski_FANTOM5 database and also orthologous human PPI data available in the IID database (version 2021-05).[33–38] Human LR pairs annotated to be interacting in complexes in CellPhoneDB and CellChatDB were split into individual interactions.

Annotations of LR interactome database

Ligand and receptor type (Secreted or ECM or membrane-bound and etc.) were annotated based on the annotations available in matrisome database or GO terms or zebrafish orthologs of human genes in CellChat database.[21,27,28,36,109] LR signaling that work via dimers or complexes were annotated based on zebrafish orthologs of human genes in CellChat and CellPhoneDB database. In addition, the type of LR interactions (Secreted signaling vs. Cell-Cell contact) was also annotated based on zebrafish orthologs of human genes in CellChat database.[36]

Scoring of LR pairs in datasets

LR pairs were scored either according to the STRING physical interaction score (Highest $\geq$900, High $\geq$700, Medium $\geq$400) or evidence of orthologous human LR interaction in any of the human databases listed in the key resources table. For scoring purposes, LR pairs reported in human LR pair databases (CellTalkDB, CellPhoneDB, CellChatDB, Ramilowski et al.) were given a score of 500. Pairs from human IID PPI with "exp" (individual or in combination with "ortho" or "pred") were given a score of 400. Pairs from Cell-Cell interactions or IID PPI without "exp" were given a score of 250. These scores were chosen to prioritize the zebrafish high ranking STRING PPI-based LR pairs (>700).

Application of DanioTalk on zebrafish datasets

For identifying Nodal-related TGFβ and Wnt receptors, DanioTalk LR finder script were run for selected ligands and across all curated receptors (using pseudo-cell type label, fold change and p-adj values in the input files).

Zebrafish synapse and PSD proteome were obtained from quantitative mass spectrometry data previously reported by the Seth Grant group (University of Edinburgh, Scotland).[46] For peptide expression, the average peptide count for synaptosome and post synaptic density was utilized (count >0.05).

Zebrafish oxytocin neuron marker genes and pseudo-bulk expression were downloaded from Supplemental_data/3-marker_gene_lists/Drerio_zebrafish.markers.sub.csv (Neuronal_04_2 cluster, avg. $Log_2FC > 0.25$, p-adj. < 0.05) and Supplemental_data/4-pseudobulk_expression/Subclusters-Danio_rerio.csv (Neuronal_04_2 cluster, expression >0.05) respectively.[48] Glial pituicytes genes were downloaded from the Table S1 (For differentially expressed genes: $Log_2FC > 1$, p-adj. < 0.05, average AMCA+ read count $\geq$50 and for all expressed genes average: AMCA+ read count $\geq$50).[47] Gene symbols were updated based on ZFIN alias database. The ligand and receptors were compared and ranked based on DanioTalk interaction database and plotted using Circlize R package (Data S1–S6).[108,107]