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 signalling molecules. Due to the incomplete annotation of zebrafish proteome, the knowledge of zebrafish receptome, secretome 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 secretome, receptome, and interactome as well as associated diseases and targeting drugs. Unlike other tools, our interactome atlas is primarily based on physical interaction data of zebrafish proteome. The resources are available as R and Python scripts (https://github.com/*DanioTalk*). *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.

Introduction

Ligand-receptor-mediated cellular communication is essential for diverse processes such as differentiation, homeostasis, tissue morphogenesis, animal development, and behaviour (Blockus and Chédotal, 2016; Dufour et al., 2020; Pires-daSilva and Sommer, 2003; Steinhart and Angers, 2018). Dysregulated cellular communication can lead to a plethora of phenotypes ranging from uncontrolled cellular proliferation, developmental defects to disease-states and drug resistance (Kowalczyk et al., 2022; Nisar et al., 2022; Straussman et al., 2012). Hence, receptors and ligands are among the leading drug targets for treating human disease conditions (Attwood et al., 2020; Hauser et al., 2017).

With advances in cryo-electron microscopy and machine learning approaches that enable protein structure prediction, the number of drug targets is expected to increase (Congreve et al., 2020; Lees et al., 2021; Tunyasuvunakool et al., 2021). These developments can aid drug screenings directly using animal model organisms. Due to challenges in using mammalian models in drug screening and limitations of *in vitro* drug screens, larval zebrafish are being increasingly used in high throughput drug screening (Cagan et al., 2019; Hoffman et al., 2016; Patton et al., 2021; Ségalat, 2007). However, despite the importance of zebrafish in drug discovery, there are no actively maintained datasets of zebrafish secreted factors, receptors and their interactions. Few existing predictions of zebrafish secretome and membrane proteome are based on incomplete annotation data (Csályi et al., 2016; Goldberg et al., 2014; Klee, 2008; Nauroy et al., 2018).

With the extensive single-cell RNA-seq datasets of zebrafish, cellular communication in zebrafish can be explored at a holistic level even for rare and transient cells (Dimitrov et al., 2022; Schier, 2020). Several tools built on mammalian interactome data has been previously applied to in zebrafish-based studies to map the ligand-receptor interactions in (Campbell et al., 2021; Holler et al., 2021; Hu et al., 2022). However, only about half of the zebrafish secretome and receptome data have orthologous interaction records for human genes. Tools that depend on such ortholog records are likely to ignore zebrafish genes lacking mammalian orthologs or genes with incomplete orthologue

records. Hence, it is necessary to build a zebrafish secretome 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 secretome, receptome, and interactome and tools to explore them from zebrafish-based omics datasets. To address the limitations of existing tools, *DanioTalk* secretome and receptome datasets are based on deep learning-based subcellular localization predictions allowing the incorporation of orphan zebrafish proteins. *DanioTalk* can identify secretome-receptome pairs primarily based on physical interaction records of zebrafish proteome. *DanioTalk* scripts are available on GitHub and allow exploration of ligand-receptor interactions in any zebrafish-based -omics datasets. Finally, we present the application of *DanioTalk* on mapping the secretome-receptome 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

A bottleneck in using gene ontology (GO) terms or UniprotKB-based cellular localization records of the zebrafish proteome is the incompleteness of such annotations (**Figure 1 – figure supplement 1A**) (Gene Ontology Consortium, 2021; The UniProt Consortium, 2021). 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 (Thumuluri et al., 2022) (**Figure 1A**). We subjected the current zebrafish reference proteome (UP000000437) to DeepLoc 2.0-based prediction (Nevers et al., 2022). Majority of proteins were predicted to be cytoplasmic (*n* = 12186; 26%), nuclear (*n* = 7905; 17%) and multilocalizing (*n* = 12211; 26%) (**Figure 1B-C, Figure 1 – figure**

supplement 1B & Supplementary file 1). DeepLoc 2.0 predicted that 3699 (8%) and 6481 (14%) of proteins were exclusively extracellular- and cell membrane-localized, respectively (Figure 1B & Supplementary file 1). This corresponds to 2571 (10%) and 4002 (15%) genes that code extracellular and membrane-localized proteins, respectively (Figure 1B'). In comparison to previous records, DeepLoc 2.0 identified an additional 1104 and 1953 genes coding for extracellular and membranal proteins, respectively (Figure 1C & D) (Goldberg et al., 2014; Nauroy et al., 2018; The UniProt Consortium, 2021).

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 of pancreatic enzymes (Prss, Cela, Cpa, Amy2a family) as extracellular proteins (coded by 24 genes) (Uhlén et al., 2015) (Supplementary file 2). DeepLoc 2.0 prediction efficiency was also high for 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 (Ornitz and Itoh, 2015). 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 (Supplementary file 2). Hence, although DeepLoc 2.0 is highly reliable in predicting zebrafish secretome, manual curation is required.

Curation of zebrafish secretome and membrane receptome

To create a list of zebrafish secretome and receptome, we first combined our DeepLoc 2.0 predictions with annotations from other existing records (UniprotKB CC, GO terms, and Matrisome) (Gene Ontology Consortium, 2021; Nauroy et al., 2018; The UniProt Consortium, 2021). In addition, we added the zebrafish orthologues of mammalian secretome- and receptor-coding genes based on Cell-Cell interaction

(baderlab.org/CellCellInteractions) and CellTalkDB databases (Figure 2A) (Shao et al., 2021; Ximerakis et al., 2019) and performed manual curation (as detailed in Material sections). Our curated secretome (ligands) and cell membrane receptome database consist of 2788 (10.5%) and 2343 (8.8%) of protein-coding genes respectively (Figure 2B & Supplementary file 3). In comparison to zebrafish matrisome dataset, our curated ligand lists contain 1848 additional genes (1566 DeepLoc 2 predictions and 282 manually curated extracellular-coding genes) (Figure 2C).

Disease-associated secretome 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 orthologue information available in ZFIN database (Bradford et al., 2022). We identified 51% and 43% of the ligand- and cell membrane receptor-coding genes having human orthologue gene records (Figure 2D & Supplementary file 4). Next, we cross-referenced our dataset with the zebrafish orthologues 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 orthologue genes that are disease-associated (Fig. 2E). Some of the major diseases associated with ligands and cell membrane receptors are related to bone diseases, macular degeneration, diabetes mellitus, and cancer (Fig. 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) (Fig. 2F).

Next, we explored the drug-targetable status of the zebrafish secretome 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 (Avram et al., 2021). We identified 149 ligands and 392 receptors that can be potentially targeted by drugs (Fig. 2G & Supplementary file 5). With respect to the choice of drugs, 311 (13%) and

1023 (44%) of the drugs can potentially target zebrafish ligands and cell membrane receptors respectively (Fig. 2H).

DanioTalk - putative *secretome-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 records of zebrafish (Figure 3A), a subset of which is shown in (Figure 3A') (Szklarczyk et al., 2021). The experimental score in STRING database predicts the confidence in the physical proximity of two proteins. Ligand-receptor pairs with scores with high-confidence scores are very likely to physically interact and result in associated downstream signalling activities. Our interactome database contains data for 370 ligands and 342 receptors at high confidence (Figure 3B). As we observed highly variable STRING physical interaction scores for experimentally validated orthologous human ligand-receptor pairs, we also incorporated medium-ranked pairs and orthologous human ligand-receptors pairs available in protein-protein interaction database IID (Figure 3 – figure supplement 3) (Kotlyar et al., 2022). This resulted in 10,446 ligand-receptor pairs (Figure 3C, 3C' & Supplementary file 6). Based on our analysis, at least 1681 ligands and 1455 receptors lack a high-ranking interacting receptor or ligand, respectively (Figure 3B).

We next probed the accuracy of our ligand-receptor interactome database. We focused on the Nodal-related TGFβ ligands and WNT ligands, key regulators of development (Schier and Shen, 2000; Shen, 2007; Steinhart and Angers, 2018). Our interaction database identified all the previously reported receptors for Ndr2/Cyclops, Ndr1/Squint and Spaw/Southpaw ligands namely Acvr1b/Alk4 (rank 6 and 13), Acvr2a (rank 2 and 11), Acvr2b (rank 1 and 10) respectively (Figure 3D & Supplementary file 7). We also noticed Acvr1c, Tgfbr1 and Tgfbr2 as top ranked receptors. Next, we probed Wnt signalling 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 3D'** & **Supplementary file 7**). Altogether, *DanioTalk* is able to accurately identify major ligand-receptor interactions in zebrafish.

Zebrafish brain synaptic secretome-membrane receptome interactome

Next, we tested the application of *DanioTalk* on existing datasets. We first focused on the zebrafish adult brain synaptosomal and postsynaptic density (PSD) proteome dataset (**Figure 4A**) (Bayés et al., 2017). We identified protein products of 148 ligand- and 184 receptor-coding genes in the synaptosomal fraction. While in the PSD fraction, we identified protein products from 75 ligand- and 88 receptor-coding genes (**Figure 4 – figure supplement 1** & **Supplementary file 8**). Between synaptosome ligands and PSD receptors, we identified 4 and 78 interactions at high and medium confidence rankings, respectively (**Supplementary file 9**). The top-ranked ligand-receptor interacting pairs were Ntn1b-Neo1a, Ntn1b-Dcc, Bdnf-Ntrk3a (**Figure 4B**). While in the case of PSD ligand and synaptosome receptor interactions, we identified 21 and 123 interactions at high and medium confidence ranking respectively (**Supplementary file 9**). The top-ranked PSD ligand- synaptosome receptor pairs were Efnb1-Ephb1/Ephb2b, Agrn/Dag1 (**Figure 4B'**).

Zebrafish Oxytocin Neuron-Glial Pituicytes Axo-Glial Interactome

Axo-glial interactions contribute to synaptic morphogenesis and plasticity (Allen and Eroglu, 2017; Allen and Lyons, 2018). However, the identify of all the axo-glial ligand-receptor interactions of neurohypophysis (NH), a major neuroendocrine interface has not been addressed (Pearson and Placzek, 2013; Wittkowski, 1998). The NH-projecting axonal tracts, NH axo-glial cellular components and their ultrastructure are conserved in zebrafish (Anbalagan et al., 2018; Grinevich and Neumann, 2021; Gutnick et al., 2011; Herget et al., 2017). We mapped the axo-glial interactome of Oxytocin (OXT) neurons and glial pituicytes. Towards this, we used the glial pituicyte bulk RNA-seq data and recently identified OXT neuron scRNA-seq data (**Figure 4C)** (Anbalagan et al., 2018; Shafer et al., 2022)**.** We identified 104 enriched ligands and 66 enriched receptors in OXT neurons, while in glial pituicytes we identified 172 enriched ligands and 82

enriched receptors (Supplementary file 10). The high ranked pituicyte ligand-OXT neuron receptor interactions were Pomca-Mc4r/Mc3r, Slit3-Robo2, Fn1a-Itgb1a while the high ranked OXT neuron ligand-pituicyte receptors were Ihhb-Cdon/Boc, Bdnf-Sort1a, Wnt3a-Fzd2/Fzd8a and Nts-Sort1a (Figure 4D & Supplementary file 11). We also observed several autocrine signalling interactions in glial pituicytes (Fgf10a-Fgfr2, Vegfab-Nrp2b) and in OXT neurons (Slit3/Slit2-Robo2) (Figure 4D & Supplemental file 11).

Discussion

Zebrafish single-cell RNAseq datasets are used to explore the developmental ligand-receptor interactome (Campbell et al., 2021; Herpelinck et al., 2022; Holler et al., 2021; Hu et al., 2022). However, a bottleneck in using such tools is in the dependence on orthologous mammalian ligand-receptor interactome data while ignoring zebrafish-specific data (Farnsworth et al., 2020; Farrell et al., 2018). Thus, tools to study zebrafish secretome, receptome and interactome independent of orthologue data are essential.

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 (Jumper et al., 2021; Lin et al., 2022; Thumuluri et al., 2022). Here, we applied a deep learning algorithm to predict the cellular localization of the zebrafish proteome and created a curated repository of the zebrafish secretome and membrane receptome. This allows the inclusion of orphan zebrafish secretome or membranal receptor proteins that lack cellular localization or ortholog records. Also, our database of secretome and receptome-linked diseases will aid researchers interested in developing zebrafish models of human disease.

Generally, creating disease models of zebrafish may require the creation of mutations in paralogous genes (Hinman et al., 2021; Howe et al., 2013). Our results suggest that this is not the case for all diseases. For instance, secreted factor-coding genes such as *col13a1, cpe, dll4* and *tgfb2* can be potentially used to model congenital myasthenic

syndrome 19, BDV syndrome, Adam-Oliver syndrome and Loeys-Dietz syndrome 4 respectively (Bosch et al., 2021; Fontana et al., 2014; Logan et al., 2015; Meester et al., 2015). Whereas receptor coding genes such as *gpc4* and *ccln4* can be potentially used for studying Keipert syndrome and Raynaud syndrome respectively (Amor et al., 2019; Palmer et al., 2018).

One of the strengths of research on larval zebrafish is in the pharmacological perturbation experiments which we have also taken advantage in the past (Anbalagan et al., 2018). Some subscription-based tools built for mammalian model organisms can list drugs targeting mammalian proteins (Krämer et al., 2014; Stelzer et al., 2016). Our *DanioTalk* repository and output files contain similar information on drugs that can likely target the zebrafish secretome and receptome.

The potential of *DanioTalk* was revealed in our analysis of the existing dataset on adult zebrafish brain synaptosome-PSD proteomes (Bayés et al., 2017). Several top ligand-receptor pairs were previously studied for their synaptic role in other model organisms. For example, orthologues of our top-ranking Ntn1b-Dcc ligand-receptor pair are well known for their functions in synaptogenesis and synaptic plasticity (Goldman et al., 2013; Horn et al., 2013; Manitt et al., 2009). Another top ligand-receptor pair was Contactin 2 (Cntn2)-Cntnap1 (CASPR). In mice studies, Contactin has been shown to promote synaptic Cntnap1 surface localization via *Cis*-interaction (Murai et al., 2002). But the fact that Cntn2 and Cntnap1 are enriched in synaptosome and PSD fractions suggests an experimental artefact or additional trans-synaptic roles for the Cntn2-Cntnap1 interaction (Dubessy et al., 2019; Ruegg et al., 1989).

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 (Hoffman et al., 2016; Hutson and Chien, 2002; Oprişoreanu et al., 2021). 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 for neuronal cell types of interest (Farnsworth et al., 2020; Raj et al., 2020).

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 (Efremova et al., 2020; Türei et al., 2021). However, such data were not explored to analyse axo-glial ligand-receptor interactions in NH, a major neuroendocrine interface critical in maintaining water balance and reproductive functions (Anbalagan et al., 2018; Brown, 2016; Romanov et al., 2017). Except for few molecular players, the axo-glial interactions in NH were largely unknown (Brown, 2016; Leng et al., 2015; Miyata, 2017; Rosso and Mienville, 2009).

Our analysis validated the Slit3-Robo2 glial pituicyte-OXT neuron interaction, which has previously been reported to promote synaptic OXT levels (Anbalagan et al., 2019). However, we also observed autocrine interactions of Slit2-Robo2 and Slit3-Robo2 in OXT neurons. Slit-Robo autocrine signalling in mice motor neurons has been shown to promote axon fasciculation; however, such defects were not reported for NH-projecting OXT axons in zebrafish (Anbalagan et al., 2019; Jaworski and Tessier-Lavigne, 2012).

Apart from glia to neuronal signalling, neuron to glial signalling are also major players in neuro-glial signalling (Allen and Eroglu, 2017). We observed OXT-neuron-derived Indian hedgehog homolog ligand potentially signalling to glial pituicytes via Cell adhesion molecule-related/down-regulated by oncogenes (Cdon) and Brother of CDO (Boc) receptors. Apart from the role of Hedgehog signalling in midline axonal guidance (Charron and Tessier-Lavigne, 2007; Evans and Bashaw, 2010; Liu and Placzek, 2014), Neuron-derived Hedgehog proteins can regulate astrocyte reactivity and synaptogenesis during mice forebrain development (Chu et al., 2006; Garcia et al., 2010; Xie et al., 2022). It is unknown whether OXT-neuron-derived hedgehog signalling regulates glial pituicytes development and synaptic morphogenesis.

We also observed potential Fgf10a-Fgfr2- and Fgf3-Fgfr2-based autocrine interactions in glial pituicytes (Zhang et al., 2006). This suggests that in addition to the previously reported paracrine role of Fgf10a in neurovascular morphogenesis of NH, Fgf10a and Fgf3 could have additional autocrine functions in glial pituicytes such as reactivity and/or proliferation (Gómez-Pinilla et al., 1995; Goodman et al., 2020; Haan et al., 2013, p. 10; Liu et al., 2013).

Overall, *DanioTalk* offers a comprehensive repository of zebrafish secretome, receptome, secretome-receptome interaction atlas, and a novel data exploratory tool for zebrafish-based researchers.

Methods

Zebrafish dataset:

Zebrafish reference proteome (UP000000437) sequences along with GO-term and cellular localization annotations were downloaded from UniprotKB (The UniProt Consortium, 2021). Gene symbols, orthologue gene symbols, aliases/synonyms, gene descriptions, and ZFIN IDs were downloaded from the ZFIN database (Bradford et al., 2022). 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 terms “plasma membrane”.

Subcellular localization prediction:

Zebrafish reference proteome cellular localization was predicted using DeepLoc 2.0 with default parameters (Thumuluri et al., 2022).

Curated database of the zebrafish secretome and membrane receptome:

Human receptors and ligands were obtained from the Cell-Cell Interaction Database (Gary Bader group, Univ. of Toronto) and the CellTalkDB receptor database (Shao et al., 2021; Ximerakis et al., 2019). In addition human ligands were obtained from MatrisomeDB (Shao et al., 2020). The initial list of zebrafish ligands was obtained by combining i) zebrafish matrisome, ii) zebrafish orthologues from the human ligand

database (see above) and iii) extracellular proteins in DeepLoc 2.0-based extracellular protein coding genes. Initial zebrafish membrane-localized receptors list were obtained by combining zebrafish orthologues of the human receptor database (see above), and DeepLoc 2.0-based membranal protein-coding genes. The list was manually curated based on gene descriptions and localization records of human orthologues available in the Compartments database and the Human Protein Atlas (Binder et al., 2014; Uhlén et al., 2015).

Ligand-receptor interactome database:

The ligand-receptor interaction database was generated based on STRING physical interaction database for zebrafish (v11.5, Ref) and zebrafish orthologues of human protein interaction data available in the IID database (version 2021-05) (Kotlyar et al., 2022; Szklarczyk et al., 2021). Pairs were ranked according to the STRING physical interaction score (High ≥ 700, Medium ≥ 400). Orthologous proteins with experimentally validated interactions in the IID database (*H. sapiens*) database were given a score of 500 for ranking purpose.

Application of *DanioTalk* on zebrafish datasets:

Zebrafish synapse and PSD proteome datasets were extracted from supplementary files in (Bayés et al., 2017). Zebrafish OXT neuron dataset was extracted from Neuronal_04_2 cluster data of Supplemental_data/4-pseudobulk_expression/Subclusters-Danio_rerio.csv reported in (Shafer et al., 2022). Glial pituicytes were extracted from the supplemental table (Log2FC > 1, p-adj. < 0.05, read count ≥ 50) provided in (Anbalagan et al., 2018). 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 (Gu et al., 2014; Team, 2013).

Code availability:

The scripts used in this manuscript are available at https://github.com/*DanioTalk*.

Authors' contributions

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

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Competing interests

The authors declare no competing interests exist.

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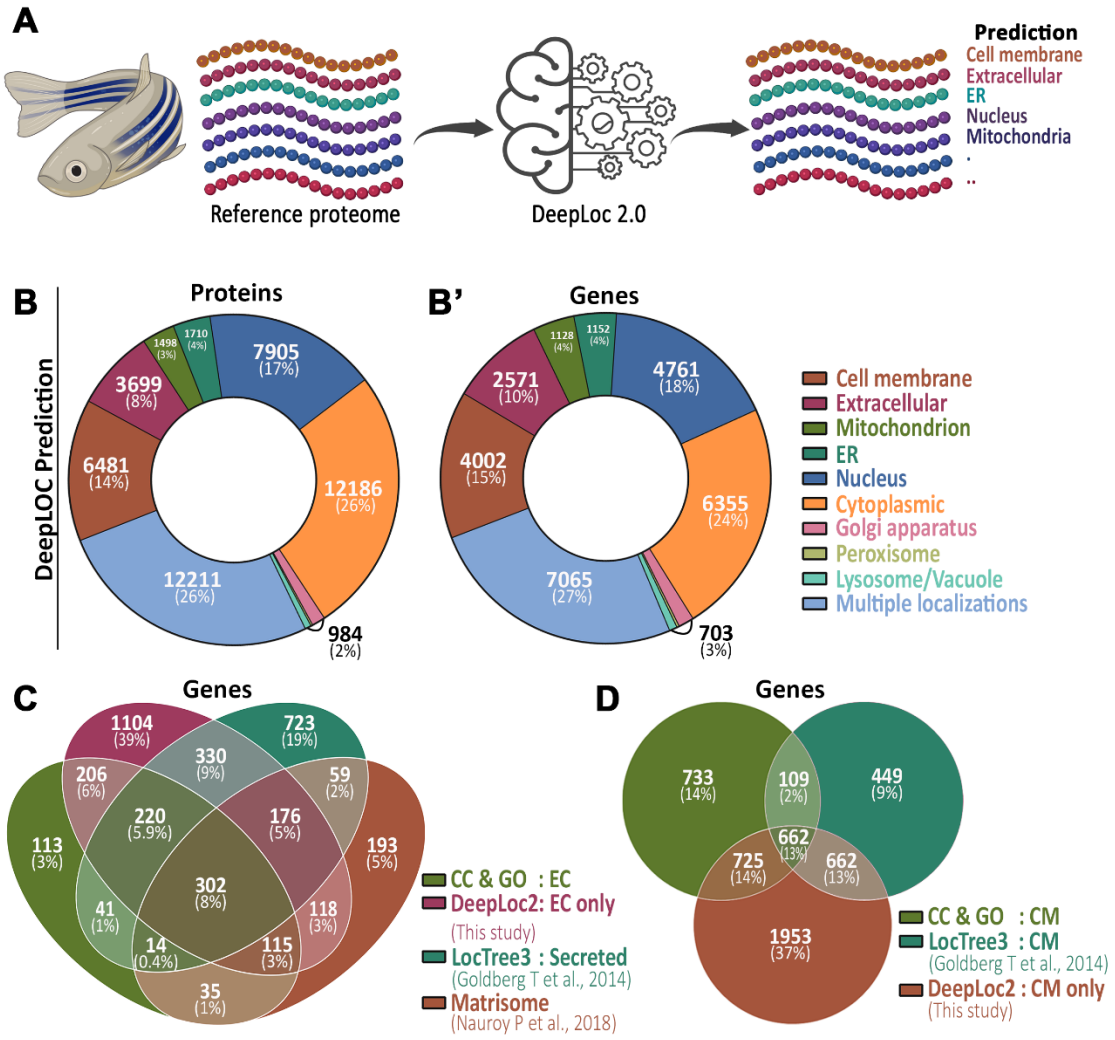

Fig. 1 Chodkowski M. *et al.*,

Figure Legends:

Figure 1. Prediction of cellular localization of reference zebrafish proteome

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

(B) Zebrafish reference proteome cellular localization. Donut chart showing the major DeepLoc2.0 predictions of zebrafish proteome. The number of proteins (B) and protein-coding genes (B′) are shown.

(C) Venn diagram comparing the number of genes with existing extracellular annotation records and DeepLoc 2.0-based 'Extracellular' predictions.

(D) Venn diagram comparing genes with existing cell membrane localization annotation records and DeepLoc-based 'cell membrane' predictions.

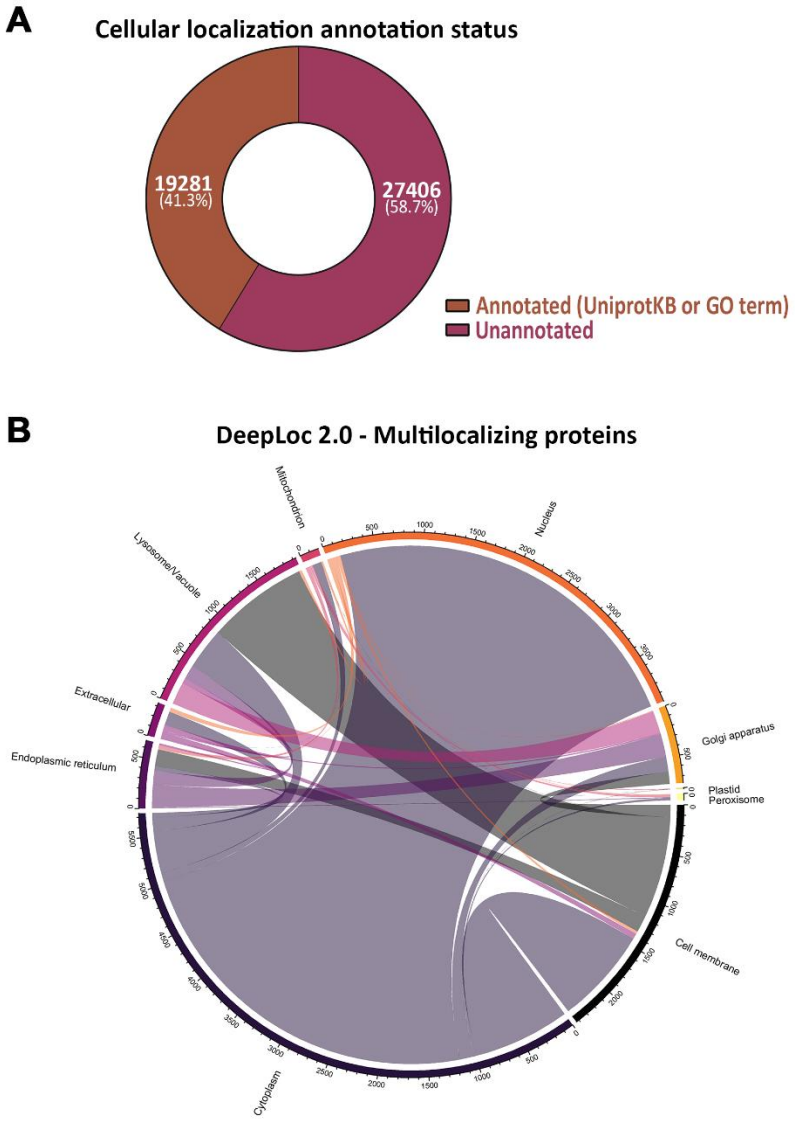

Figure 1 - figure supplement 1
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Figure 1 – figure supplement 1. Annotation status and DeepLoc 2.0 multilocalizing protein localizations

(A) Current status of the cellular localization annotation records of the reference proteome from zebrafish. Donut chart showing number of proteins with and without GO-term based or UniprotKB cellular localization records.

(B) Circle plot showing the number of proteins predicted to localize to multiple cellular regions and organelles.

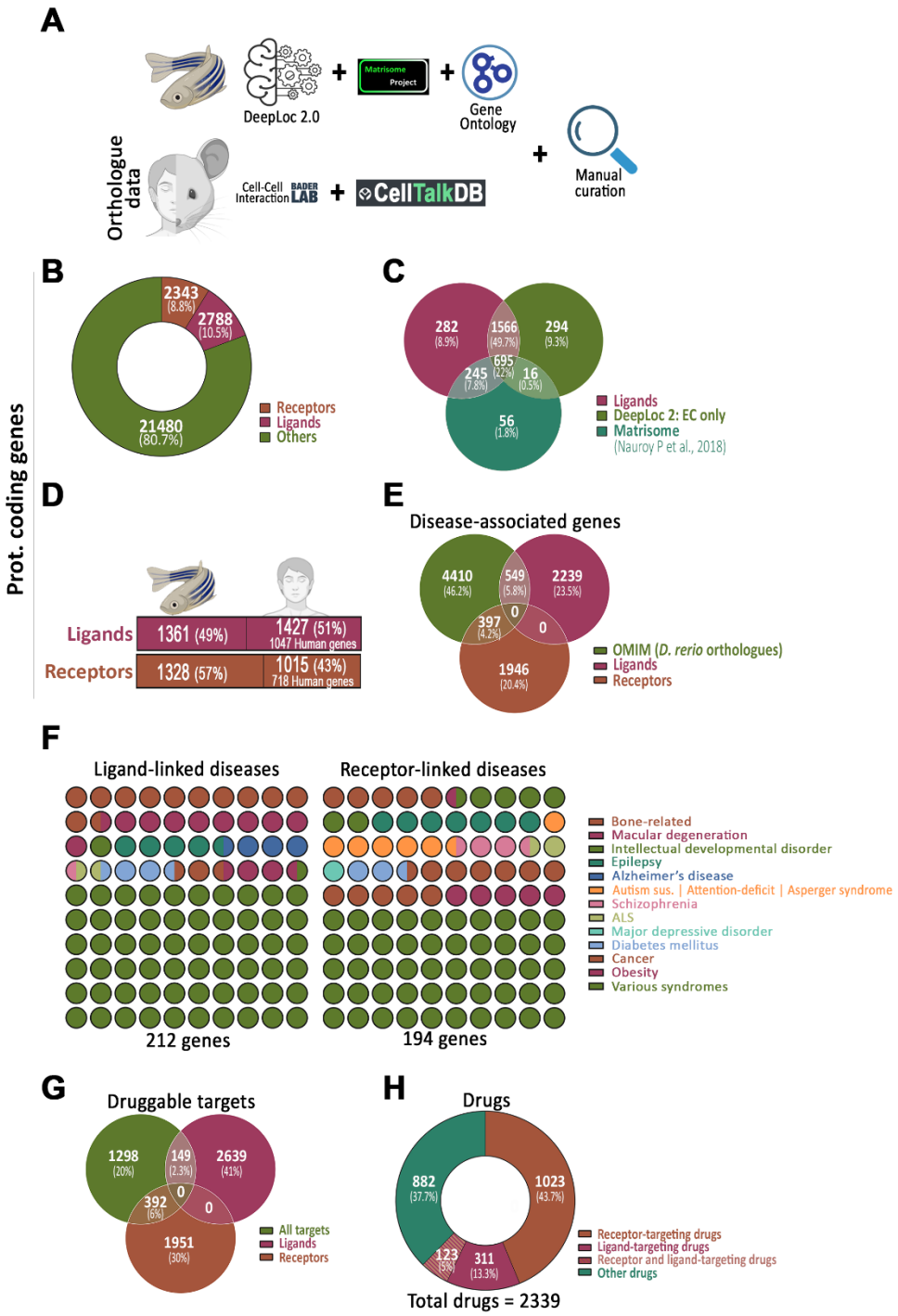

Fig. 2 Chodkowski M. *et al.*,

Figure 2. Curated zebrafish ligands and membrane receptors

(A) Scheme describing the steps taken for the creation of 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 DeepLoc2.0 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 orthologues of human disease-linked genes in the OMIM database.

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

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

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

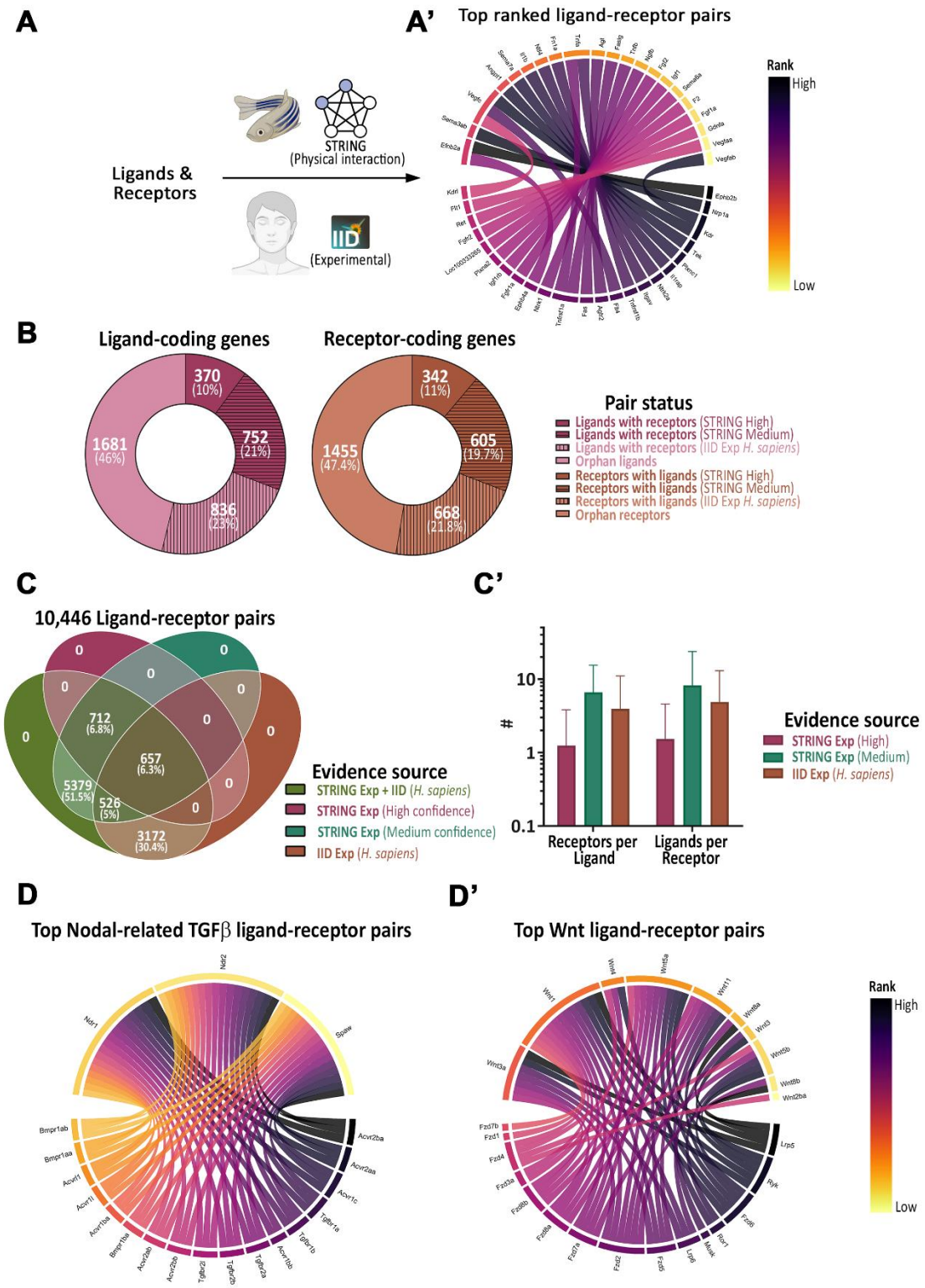

Fig. 3 Chodkowski M. *et al.*,

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 and membrane receptors. Donut chart showing the number and percentage of genes coding for ligands and receptors with or without potential receptors and ligands respectively.

(C) Zebrafish ligand-receptor interactome stats. Venn diagram comparing ligand-receptor pairs across different ranking categories (C). Graph showing # of putative receptors/ligand and ligands/receptor (C′).

(D) Receptors for Nodal-related TGF-beta ligands and Wnt ligands. Circle plot showing the top ranked receptors for Ndr2, Ndr1 and Spaw ligands (D) and Wnt ligands (D′).

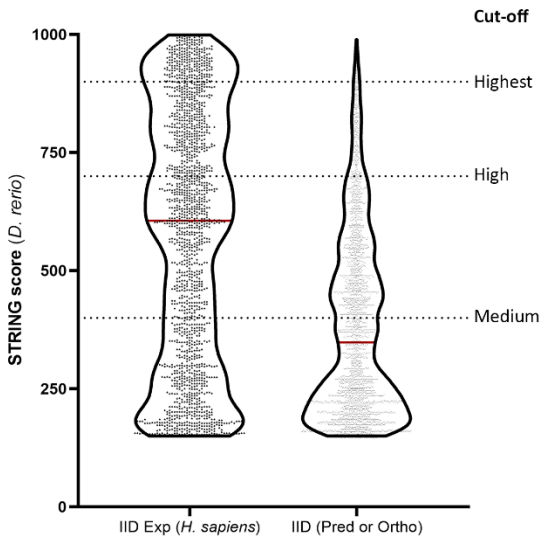

Figure 3 - figure supplement 1
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Figure 3 – figure supplement 1.

Conservation of zebrafish ligand-receptor interactions. Violin plot showing STRING database zebrafish ligand-receptor interactions scores to their human orthologue ligand-receptor records in IID database. 'Pred' denotes predicted interactions.

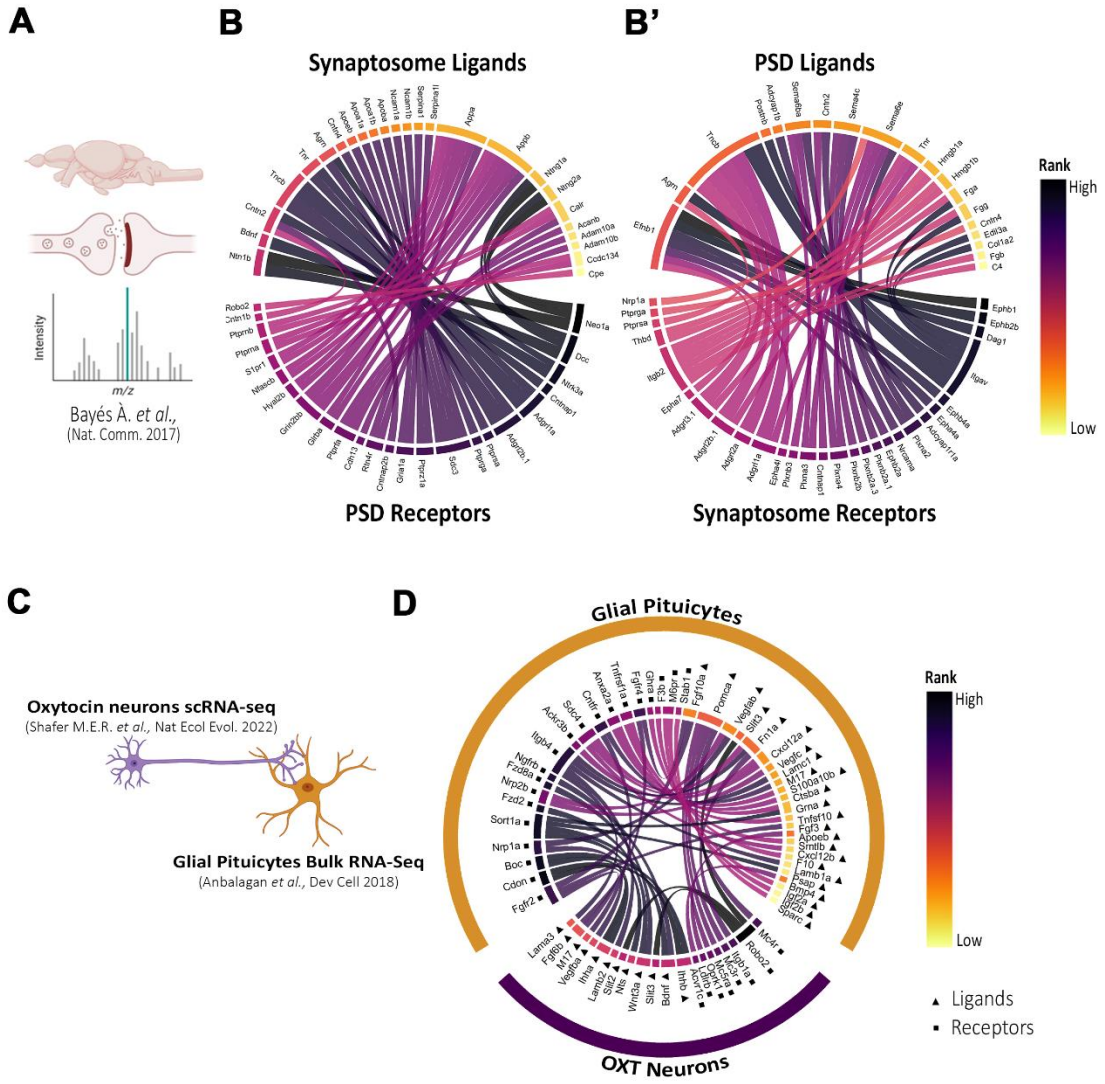

Fig. 4 Chodkowski M. *et al.*,

Figure 4. Application of *DanioTalk*

(A) Scheme showing synaptosome-PSD from adult zebrafish brain.

(B) Circle plot showing the top-ranked ligand-receptor interactions between the synaptosome and PSD (B and B′).

(C) Scheme showing axo-glial interaction between OXT neuron and glial pituicytes.

(D) Circle plot showing top-ranked ligand-receptor interactions between OXT neurons and glial pituicytes.

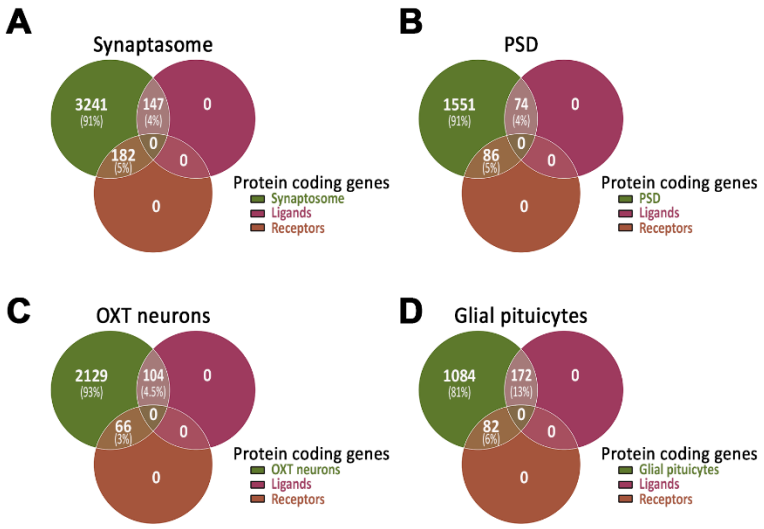

Figure 4 - figure supp 1
Chodkowski M. *et al.,*

Figure 4 – figure supplement 1. Application of *DanioTalk*

(A) Venn diagram showing the number and percentage of genes coding for synaptosome-enriched ligands and receptors.

(B) Venn diagram showing the number and percentage of genes coding for PSD-enriched ligands and receptors.

(C) Venn diagram showing the number and percentage of genes coding for OXT neuron-enriched ligands and receptors.

(D) Venn diagram showing the number and percentage of genes coding for Glial pituicyte-enriched ligands and receptors.