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Functional analysis of co-expression networks of zebrafish *ace2* reveals enrichment of pathways associated with development and disease.

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Abstract

Human Angiotensin I Converting Enzyme 2 (ACE2) plays essential roles in blood pressure regulation and SARS-CoV-2 entry. ACE2 has a highly conserved, one-to-one ortholog, called *ace2*, in zebrafish, which is an important model for human diseases. However, zebrafish *ace2* expression profile has not yet been studied during early development, between genders, across different genotypes, or in disease. Moreover, a network-based meta-analysis for the extraction of functionally enriched pathways associated with differential *ace2* expression is lacking in the literature. Herein, we first identified significant development-, tissue-, genotype- and gender-specific modulations in *ace2* expression via meta-analysis of zebrafish Affymetrix transcriptomics datasets ($n_{\text{datasets}}=107$); and the correlation analysis of *ace2* meta-differential expression profile revealed distinct positively and negatively correlated local functionally enriched gene networks. Moreover, we demonstrated that *ace2* expression was significantly modulated under different

physiological and pathological conditions related to development, tissue, gender, diet, infection, and inflammation using additional RNAseq datasets. Our findings implicate a novel translational role for zebrafish *ace2* in organ differentiation and pathologies observed in the intestines and liver.

Keywords: zebrafish, *ace2*, transcriptome, network analysis, meta-analysis

INTRODUCTION

ACE2 and ACE, involved in angiotensin conversion, are integral elements of renin-angiotensin signaling (RAS) in multiple tissues. Recent studies have shown that RAS is not only present in kidney or adrenal glands but also functional in other tissues with significant roles in multiple pathologies, including cancer (Cheng and Liu, 2019, Nehme et al., 2019, Pinter and Jain, 2017, Rasha et al., 2019). The role of ACE2 and ACE in the regulation of hypertension is well known (Hamming et al., 2007), and recently ACE2 has also become the focus of intense research due to its function as a receptor of SARS-CoV-2 entry in COVID-19 (Ziegler et al., 2020). In addition, *ACE2* expression is tissue-specific; for example, recent findings from single-cell RNA-seq studies show that *ACE2* is highly expressed in type II pneumocytes and intestinal enterocytes, in which SARS-CoV-2 can replicate (Ziegler et al., 2020).

Apart from mammalian models, recently, zebrafish has been used to study *ace2* expression or function. Zebrafish *ace2* is highly conserved in sequence and structure to its mammalian counterparts and exists as the only copy in zebrafish with a duplicated genome (Chou et al., 2006). Furthermore, a recent study, phylogenetically comparing genes co-expressed with *ace2* based on zebrafish single-cell RNA-seq tissue data, has revealed RAS signaling is also conserved between humans and zebrafish (Postlethwait et al., 2021). However, to our knowledge, there is no study investigating the changes in *ace2* expression over zebrafish embryonic and larval developmental stages, between genders, and in the different whole adult tissues as well as across different genotypes, treatments, or pathologies. Accordingly, a meta-analysis of differential

expression of zebrafish *ace2*, performed across public datasets, may reveal important functionally enriched networks (Shehwana and Konu, 2019).

Co-expression analysis is a widely used and effective method based on the guilt-by-association principle to identify the regulatory mechanisms of transcription under certain conditions. Several methods have been proposed to perform co-expression analysis, i.e., effect size combination, rank combination, p-value combination, and random sampling (Evangelou and Ioannidis, 2013, Gur-Dedeoglu et al., 2008, Kolde et al., 2012, Tseng et al., 2012). Since traditional co-expression analysis methods are based on using the expression values from individual samples, preprocessing steps may be required to remove batch effects between datasets. However, data merging with the increasing number of datasets may not be optimal (Cheung and Vijayakumar, 2016); and when combining a heterogeneous set of datasets requires different approaches (Ter Veer et al., 2019). Recent network-based methods have proposed combining the individual co-expression network of each dataset, calculated separately, yet these are computationally expensive for a large collection of datasets (Ter Veer et al., 2019). A simpler and widely used methodology is to use or compare the logFC values (as the mean expression value normalized to internal control) obtained for each dataset that may come from numerous conditions; this methodology has been used in different contexts, including miRNA target prediction, toxicogenomic pattern, and compound similarity matrix (Kramer et al., 2020, Yoon et al., 2019, Zhou et al., 2018, Cheng, 2013). Here, we combined these two methods, i.e., logFC comparison and co-expression network, and performed a pairwise correlation analysis between *ace2* and other genes using logFC values obtained from re-analysis (limma) of public zebrafish Affymetrix datasets (GEO), followed by network enrichment.

Briefly, we hypothesized that significantly enriched functional local networks extracted based on the differential expression profile of *ace2* across divergent contexts can provide a better understanding of zebrafish *ace2* function. Using the wealth of transcriptomics data present in the

zebrafish literature, we aimed to identify the development-, tissue, gender-, and genotype-specific differences in *ace2* expression and extract its co-differentially expressed transcriptome in zebrafish using available datasets. We also investigated whether *ace2* was tissue-specific and found it was expressed the most in intestines followed by liver, although highly variably. We then performed comparisons between relevant datasets using local network signatures (STRING) of genes co-expressed with *ace2*; this helped prioritize the positively and negatively ranked local functional pathways with which *ace2* was associated in development, intestinal differentiation, and variable liver expression. Using additional datasets, we showed that zebrafish *ace2* was also modulated in the different liver and intestinal pathologies, demonstrating its translational importance.

METHODS

Co-differential expression analysis using Affymetrix Zebrafish Chip datasets (GPL1319)

Raw CEL files for all available datasets for GPL1319 were obtained from the NCBI GEO database (Fig. S1) (Barrett et al., 2013). Groups containing at least two samples were used for comparisons, and datasets with no comparable groups were excluded ($n_{\text{datasets}} = 107$). Normalization of CEL files for each study was performed separately, using *rma* in *affy* package (Gautier et al., 2004). From 107 datasets, 344 two-group comparisons were manually generated with the following rules: 1. each experimental group was compared to its corresponding control group; 2. experimental groups with double mutation or morpholino treatment were compared to each of the single mutation/morphant group; 3. only control (or wild type) groups from different tissues, developmental time points or gender were compared with each other; 4. for time series, all other groups were compared to the group with the earliest time point only. Differential expression analysis using *limma* was conducted for each of the manually generated two-group comparisons with the normalized datasets (Ritchie et al., 2015). A matrix of logFC values, $X_{\log\text{FC}}$, was obtained where each row corresponded to a gene and each column one of the *limma* comparisons. The

average logFC values for each gene were calculated across 344 comparisons whose distribution was plotted as a histogram. A Pearson correlation coefficient ($r_{ace2-logFC}$) was then calculated between *ace2* and every other gene found in X_{logFC} . Furthermore, $r_{ace2-logFC}$ values were used to identify STRING enriched local networks, i.e., small protein-protein interaction networks with 5 to 200 members, obtained through hierarchical clustering of the human protein-protein network and named according to the consensus annotations of proteins found in subnetworks (Szkarczyk et al., 2019). In addition, the limma comparisons in which *ace2* was differentially expressed were identified upon setting the FDR and logFC thresholds stringently (adj. p-value < 0.05 and abs(logFC) > 0.5).

Identification of tissue-specific expression patterns of *ace2* in zebrafish adult tissues

Raw data for selected tissue datasets (Table S1) were obtained from SRA (Chen et al., 2020a) and analyzed using Seven Bridges Cancer Genomics Cloud (CGC; <https://www.cancergenomicscloud.org/>). Reads were aligned using the Star Alignment tool (Dobin et al., 2013). HTSeq tool (Anders et al., 2015) was used to retrieve the count data. Raw count data were normalized using the RPKM function from the *edgeR* package in R (McCarthy et al., 2012). Samples from the same tissue and from different datasets were visualized by plotting the scores from the first two dimensions of Principal Component Analysis (PCA) as well as hierarchical clustering. Tau was used as the tissue specificity index as described previously (Kryuchkova-Mostacci and Robinson-Rechavi, 2017).

Dataset collection for investigating sexual dimorphism, and intestinal and liver development and disease

Zebrafish transcriptomics datasets selected from NCBI GEO or Expression Atlas (Papatheodorou et al., 2020) according to their relevance to sexual dimorphism, liver and gut development, and disease/treatments were summarized in Table S2. Series matrix files for four datasets of

GPL14664 (GSE113241 (Alvarez-Rodriguez et al., 2018); GSE112272 (Jia et al., 2019); GSE73233 (Forn-Cuni et al., 2017); and GSE100583 (Holden and Brown, 2018)) and RPKM normalized data of GSE74244 (Aramillo Irizar et al., 2018), GSE118076 (San et al., 2018), and GSE24616 (Domazet-Loso and Tautz, 2010) were obtained from GEO and used for statistical analysis after logarithmic ($\log_2$) transformation. The expression of *ace2* in the E-ERAD-475 dataset was obtained from Expression Atlas (White et al., 2017). ANOVA followed by multiple test correction (Tukey's HSD) was used for statistical analyses as indicated in the figure legends. Raw count data of GSE82200 (Koch et al., 2018), GSE83195 (Schall et al., 2017) and GSE123439 (Chen et al., 2020b) were obtained from NCBI GEO; *Deseq2* package was used for the differential gene expression analysis; and Regularized log (Rlog) normalized expression values were used in visualization (Love et al., 2014).

Condition-specific co-expression analysis

For the generation of co-expression network of *ace2*, a Pearson's correlation coefficient, called r_{ace2} , was calculated between *ace2* and each gene based on the $\log_2$ transformed expression values, generating a vector of r_{ace2} values for each comparison studied, namely, a) larval development, using GSE24616 (2 dpf-8 dpf) and GSE38575 (EtOH treated samples only; 2dpf - 7dpf) datasets; b) intestinal expression, using GSE35889 and GSE12189 (GFP+ samples only); and c) expression in liver, using GSE74244 (liver samples only) and GSE100583, separately (Fig. S2, Table S2). The r_{ace2} value with the minimum p-value was selected for multiple probesets having the same Ensembl ID.

Comparative Network Enrichment Analysis (CNEA)

Ensembl IDs and each of the condition-specific r_{ace2} values was used to identify local network clusters using STRING enrichment analysis (Szklarczyk et al., 2015). After obtaining enrichment results for each dataset, the Comparative Network Enrichment Analysis (CNEA) of STRING local

networks was performed to identify consistently enriched local networks common in both datasets and visualized via scatter plots ($\text{FDR} < 0.01$). Enriched local networks with the largest gene set were selected in the presence of multiple local networks with the same name. A local network interaction graph was then generated using networks significantly ($\text{FDR} < 0.01$) enriched in both datasets where nodes represent local networks and edges, the number of shared genes between local networks. The generated graph was then clustered in Cytoscape using the Markov Cluster Algorithm (MCL) (Shannon et al., 2003). Selected local networks were visualized in detail, where each node on the specific STRING local network data referred to a gene and was colored according to its r_{ace2} , i.e., the correlation with *ace2* expression. In addition, a GO term enrichment analysis was performed for liver datasets using the *clusterprofiler* package in R (Yu et al., 2012).

RESULTS

Re-analysis of zebrafish GPL1319 datasets reveal conditions in which *ace2* expression is significantly modulated

Upon re-analysis of publicly available datasets from the GPL1319 platform ($n=107$ with 344 pairwise group comparisons), *ace2* expression was found differentially expressed in 19 pairwise group comparisons from 11 different datasets relevant to sexual dimorphism, estrogen signaling, embryogenesis, and liver and gut development (Table 1) (Froehlicher et al., 2009, Heiden et al., 2008, Hu et al., 2013, Jacob et al., 2015, MacInnes et al., 2008, Rogers et al., 2011, Small et al., 2009, Soni et al., 2013, Stuckenholtz et al., 2009, Thakur et al., 2014, Okuda et al., 2010). The logFC matrix for all zebrafish genes across 344 comparisons was generated, and row (i.e., gene) mean and coefficient of variation (CV) values were found to be distributed symmetrically around 0 (Fig. S3A-B). The co-differential expression vector of *ace2*, $r_{ace2-\log FC}$, was used to obtain STRING local network clusters, which revealed that genes positively correlated with *ace2* were enriched in carboxypeptidase activity (Figure 1A), intestinal hexose absorption, villin and keratin, and fibrin clot formation associated sub-groups (Fig. S4A) whereas those negatively correlated

belonged to cilium assembly (Figure 1B), microtubule organization pathways, and chromatin-modifying enzymes (Fig. S1A). Ten genes were represented in the GPL1319 platform out of 28 RAS pathway genes (Postlethwait et al., 2021), and eight of them were found to be positively correlated with *ace2*, with a correlation coefficient ranging from 0.46 to 0.85.

Table 1: GPL1319 datasets in which zebrafish *ace2* with a probe set ID “Dr.20290.1.A1_at” was significantly differentially expressed (FDR (adj. p-value) < 0.05 and abs(logFC) > 0.5). MCLR, MPNST, TCDD, LNA, GIT, refer to microcystin-LR, malignant peripheral nerve sheath tumor, 2,3,7,8-Tetrachlorodibenzo-p-dioxin, locked nucleic acid, and gastrointestinal tract.

Dataset ID	logFC	p-value	adj. p-value	Comparison
GSE14979	-3.92	8.7E-06	2.1E-04	Ovary vs Female Body
GSE14979	-3.88	1.0E-06	1.5E-05	Testis vs Male Body
GSE13158	-3.77	5.7E-16	2.1E-13	50uM <i>ERbeta2</i> vs Control morpholino
GSE17711	-1.86	4.8E-06	1.1E-03	<i>cdipt</i> Mutant vs Wild type
GSE55339	-1.43	8.5E-05	1.2E-02	<i>uhrf1</i> Mutant vs Wild type
GSE12214	-1.10	3.0E-04	4.3E-03	1000ug MCLR vs Control ethanol
GSE11493	-1.06	1.6E-03	1.1E-02	MPNST (p53mut) vs Seminoma
GSE14979	-0.90	1.1E-02	2.0E-02	Ovary vs Testis
GSE4859	-0.81	8.3E-05	5.1E-03	Whole ovary 100ppb TCDD vs Whole ovary control
GSE12214	-0.80	4.1E-03	4.2E-02	100ug MCLR vs Control ethanol
GSE32360	-0.64	2.2E-02	3.6E-02	24 hpf mock LNA vs 1 hpf mock LNA

GSE18830	-0.63	2.1E-03	6.0E-03	75% Epiboly vs 30% Epiboly
GSE18830	-0.56	1.0E-03	2.4E-03	Tail Bud vs 30% Epiboly
GSE12189	2.92	7.9E-03	4.8E-02	6 dpf (GIT) vs 2 dpf (GIT)
GSE12189	3.05	9.6E-06	5.4E-04	6 dpf (not-GIT) vs 2 dpf (not-GIT)
GSE12189	3.85	4.5E-06	3.2E-04	3 dpf (GIT) vs 3 dpf (not-GIT)
GSE12189	3.95	9.0E-06	5.6E-03	3 dpf (GIT) vs 2 dpf (GIT)
GSE12189	4.68	5.3E-04	1.2E-02	4 dpf (GIT) vs 2 dpf (GIT)
GSE35889	7.58	5.7E-07	3.6E-04	<i>cdx1b</i> Transgenic vs Wild type

ace2 expression in zebrafish is developmentally modulated

Analysis of GPL1319 datasets revealed *ace2* expression decreased throughout gastrulation (GSE18830, GSE32360; Table1), which was supported by re-analysis of GSE24616 (Figure 2A) and E-ERAD-475 (Fig. S1B). In addition, we found *ace2* was lowly expressed in embryogenesis and started increasing at 3 dpf and thereafter until 4 dpf, after which it was steadily expressed at high levels (Figure 2A, Fig. S4B). Analysis of yet another dataset (GSE38575) supported this finding of a significant increase in *ace2* expression after 3 dpf in zebrafish (Figure 2B). The STRING local network enrichment scores based on r_{ace2} values (between expression profile of *ace2* and that of every other gene), obtained separately for GSE24616 (2dpf - 8dpf) and GSE38575 (untreated; 2dpf - 7dpf) zebrafish embryonic development datasets, were significantly correlated with each other ($r = 0.963$, $p\text{-value} = 7.63e\text{-}127$; Figure 2A-C). Network clustering helped us define pathways modulated at the time of *ace2* induction during the development of early zebrafish larvae. Accordingly, networks enriched with genes positively correlated with *ace2*

included pathways related to intestinal hexose absorption, vitamin D metabolism, carboxypeptidases, interleukin signaling, phenylalanine/tyrosine catabolism, peptide ligand binding, and dopamine receptors, phototransduction, and common fibrin clot/fibrinolysis (Figure 3, Fig. S5). On the other hand, networks enriched with negatively correlated genes included terms such as chromosome segregation, cilium assembly, DNA double-strand break and replication, homeobox, alternative splicing, and endothelial cell proliferation (Figure 3, Fig. S6, Table S3). We then generated graphs with positively and negatively enriched networks separately in which the edge weights representing the number of genes common in both networks to obtain a more comprehensive view of the *ace2*'s functions. Here we found that interleukin-20, -6, -21, and -35 signaling networks formed a network, and some of the genes involved in vitamin D metabolism along with SLC mediated transmembrane transport networks were also enriched (Figure 4). Networks relevant with chromatin segregation clustered together with the checkpoint associated networks, while cilium assembly and microtubule organization formed separate networks as in the case of DNA damage response associated networks (Figure 5).

***ace2* expression in zebrafish is sexually dimorphic and tissue-specific**

One of the most prominent findings of the GPL1319 dataset meta-analysis was the identification of sexual dimorphism in zebrafish *ace2* expression, as shown in Table 1 (GSE14979; Table 1). Interestingly, gonads had lower *ace2* expression when compared with the rest of the body regardless of sex, while testis had relatively higher *ace2* levels than the ovary (GSE14979; Fig. S7A). Moreover, *ace2* expression decreased in the presence of a morpholino for *ERBeta2* (*esr2a*) (GSE13158; Table 1), previously shown to be essential for female sexual maturation and early follicle generation (Lu et al., 2017, Wu et al., 2020). We then re-analyzed the GSE24616 dataset for *ace2* expression in adult zebrafish through time and found *ace2* was highly expressed in juveniles and early adulthood (90 dpf) (Figure 6A). Moreover, males exhibited high *ace2* expression regardless of age while females showed a steady decrease over aging until 9 months

(Figure 6A; adj. p-value <0.0001 (age), adj. p-value <0.0001 (gender), adj. p-value = 0.43 (interaction)); however, after 9 months, *ace2* levels were indistinguishable between males and females. Results obtained from another dataset, GSE123439, were consistent with the observed significance of sexual dimorphism in gonads (Fig. S7B). In addition, we interrogated the tissue-specificity of *ace2* using a collated dataset (Methods for details). Samples from the same tissue and/or anatomically related organs were clustered together in PCA and hierarchical clustering (Fig. S8). We found that the intestines and liver exhibited relatively high *ace2* expression in comparison with the brain, gills, and kidneys in that descending order (Figure 6B). Accordingly, zebrafish *ace2* expression was found to have a high tissue specificity index value (Tau) of 0.99 (Kryuchkova-Mostacci and Robinson-Rechavi, 2017).

***ace2* exhibits the highest expression in intestines**

In accord with the above tissue-specificity analysis, our meta-analysis of GPL1319 datasets also implicated *ace2* in intestinal development. Indeed, *ace2* expression increased the most in the intestines of the zebrafish overexpression model of *cdx1b*, an intestinal differentiation-related transcription factor (GSE35889, Table 1). Moreover, *ace2* expression increased in GFP+ gastrointestinal tract (GIT) cells of transgenic (Tg(XIEef1a1:GFP)s854) zebrafish larvae at and after 3 dpf compared to 2 dpf (GSE12189, Table1; Figure 7A) while in GFP- (non-GIT) cells, *ace2* was significantly upregulated at 6 dpf only. Since *ace2* expression also increased in GFP+ cells when compared to GFP- cells at 3 dpf (GSE12189, Table1; Figure 7A), we further performed a comparative network enrichment analysis (Methods for details of CNEA) of STRING local networks between GSE12189 and GSE35889 datasets. Network enrichment scores of the above-mentioned datasets were highly correlated ($r = 0.798$, p-value = $6.49e-12$; Figure 7B) strengthening the role of *ace2* during intestinal differentiation. The top common networks included those of intestinal hexose absorption, chylomicron assembly, i.e., a key mechanism for lipid transport in the intestines, common pathway of fibrin clot formation and fibrinolysis, fatty acid

degradation, and carboxypeptidases (Figure 7C, Fig. S9). On the other hand, negatively correlated genes were enriched in networks of striated muscle contraction and mRNA splicing (Figure 7C, Fig. S9). Moreover, increases in *ace2* expression were steady overtime in late zebrafish larvae with functional intestines (Fig. S10A).

***ace2* expression is altered with inflammation**

We next focused on available disease model datasets of zebrafish for examining changes in *ace2* expression in inflammation of the intestines and liver. From an intestinal disease perspective, *ace2* was found to be downregulated in the zebrafish model of Short Bowel Syndrome (SBS) (Fig. S10B), which may implicate a possible link between *ace2* expression and inflammatory response (Johnson et al., 2018, Mutanen et al., 2019, Schall et al., 2017). We further re-analyzed two other expression datasets associated with different inflammatory stimuli in relationship with the zebrafish digestive system. No significant alteration in *ace2* expression was observed in response to bacterial colonization (Conventionalization (CONVD), *Exiguobacterium* (Exi), or *Chryseobacterium* (Chrys)), in zebrafish embryos (GSE82200, Figure 8A). However, *ace2* expression significantly increased with immunosuppression in the *myd88* knock-out zebrafish embryos showing significant effects for genotype and treatment with a significant interaction in between these factors (GSE82200; adj. p-value = 4e-07 (Genotype), adj. p-value = 0.03 (Germ), adj. p-value = 0.03 (interaction); Figure 8B). On the other hand, *ace2* expression decreased in kidney with SVCV infection after pre-treatment with β -glucan but not with exposure to lipopolysaccharides (LPS) or Polyinosinic:polycytidylic acid (PolyI:C) exposure (GSE113241, adj. p-value = 0.01 (SVCV), adj. p-value = 0.06 (pre-treatment), adj. p-value = 0.04 (interaction); Figure 8B). LPS treatment was not effective in the liver, muscle, or kidney, either (Fig. S10C).

***ace2* expression is highly variable in the liver and associated with diet and liver disease**

266 Since *ace2* exhibited the second highest yet bimodal expression levels in the liver (Figure 6B;
267 Fig. S11A), we performed GO annotation of genes having high or low r_{ace2} values, obtained
268 analyzing the GSE74244 dataset (the largest RNA-seq zebrafish cohort for zebrafish adult liver
269 tissue in GEO database). Significant GO biological processes of genes whose expression levels
270 were positively correlated with that of *ace2* were enriched in metabolism, and in particular, that of
271 the lipid (Fig. S11B), whereas those negatively correlated included immune response (Fig. S11C).
272 We also showed that liver expression was not sexually dimorphic and could vary regardless of
273 strain and gender using another dataset (GSE100583, Fig. S12A). Results of GO enrichment
274 analysis of GSE100583 were similar in terms of positively correlated genes, yet cilium
275 organization and establishment of cell polarity were found among significantly negatively
276 correlated pathways (Fig. S12B, C). CNEA of STRING local networks between these two
277 independent datasets (GSE74244 vs. GSE100583) were highly concordant for a subset of
278 pathways (Figure 9A, Fig. S13A) and included networks of genes with positive r_{ace2} such as
279 intestinal brush border proteins (villin and keratin) as well as interferon-stimulated genes (ISG15
280 antiviral mechanism), carboxypeptidases, and respiratory electron transport and oxidative
281 phosphorylation (Figure 9A, Fig. S13B). Erythropoietin and hemoglobin network was among those
282 enriched with negative r_{ace2} values in both datasets (Figure 9A, Fig. S13B). However,
283 apolipoprotein A/E genes, known to be functional in fat metabolism and immune response,
284 although enriched in both datasets, acted in the opposite direction (Figure 9A).

285 We further investigated *ace2* expression in zebrafish under conditions of fasting and refeeding in
286 the liver and found *ace2* expression increased when refed after 3 weeks of fasting (Figure 9B) yet
287 it was not altered when given a high carbohydrate diet (GSE8856 (Robison et al., 2008); logFC =
288 0.24, adj. p-value = 0.51 (females) and logFC = -0.67, adj. p-value = 0.99 (males)) or under the
289 condition of overfeeding (GSE48806 (unpublished data); logFC = -1.76, adj. p value = 0.26). In
290 further support of potential involvement of *ace2* co-expressed gene network in liver disease, *ace2*

expression decreased in the zebrafish non-alcoholic fatty liver disease (NAFLD) model (GSE17711; Table 1) and in response to microcystin-LR, previously shown to cause liver damage (GSE11214; Table1, Figure 9C) (Rogers et al., 2011).

Discussion

Zebrafish as a model organism is widely used to understand disease mechanisms due to its high sequence/functional similarity with humans as well as the availability of a wide range of zebrafish genetic models (Gomes and Mostowy, 2020, Logan et al., 2018, Mickiewicz et al., 2019, Willis et al., 2016, Shehwana and Konu, 2019). Conservation and functional similarity of *ace2* and the RAS pathway in zebrafish (Postlethwait et al., 2021) make a disease-focused investigation of zebrafish *ace2* expression essential, yet this has remained unexplored. Our meta-analysis and/or functional network enrichment approaches with respect to various contexts, including age, gender, tissue specificity, genotype, and disease, make the present study unique and timely.

Our meta-analysis has demonstrated that *ace2* is expressed at low levels during early larval development yet starts increasing in expression during differentiation of the liver and intestines (Kimmel et al., 1995). Developmental studies in mice report *Ace2* expression at E12.5 day with an increasing trend over time in kidneys, lung, brain, and heart (Song et al., 2012), while *ACE2* is also expressed in the human intestines, gonads, kidneys, heart, adipose tissue, lung, and liver in accord with its multiorgan functionality (Musavi et al., 2020, Wang et al., 2020). In zebrafish, the *ace2* gene is expressed highly in a subcluster of intestinal epithelial cells, where the *ace* is also expressed (Postlethwait et al., 2021). Herein we have demonstrated that *ace2* is tissue-specifically expressed in zebrafish adult intestines as *ACE2* in humans (Musavi et al., 2020). Our analyses also suggest a potential role for intestinal differentiation for *ace2*, whose expression is higher in the *cdx1b* transgenic strain exhibiting increased intestinal differentiation (Hu et al., 2013). This finding supports that *cdx1b* could be a transcriptional regulator of *ace2* in zebrafish as *Cdx2/CDX2* is in mice (Chen et al., 2020a) and in humans (Cheng et al., 2008, Flores et al., 2008,

Silberg et al., 2000). Moreover, the fish larvae with a mutation in the *uhrf1* gene cause non-functional intestines and also exhibit lower levels of *ace2*, further supporting a potential role for *ace2* in intestinal development (Table 1) (Ganz et al., 2019, Marjoram et al., 2015).

Herein, we compared the local enrichment scores between independent datasets (Yildiz et al., 2013) to increase confidence in our context-dependent analysis, i.e., *ace2* co-expression in intestinal development and liver. Such a comparative analysis has proven to be an effective strategy demonstrating that *ace2* expression is a tightly co-expressed component of carboxypeptidases. Interestingly, this is in accord with the reports proposing the serine protease family of genes, which *ace2* is part of, as a target for COVID-19 treatment (reviewed in (Vargas et al., 2020, Yao et al., 2020). Other networks we identified included fibrinolysis (positive r_{ace2}), and since COVID-19 severity is associated with dysregulation of clot formation (reviewed in (Coccheri, 2020)), we propose that zebrafish larvae can be used to test the effects of SARS-CoV-2 on the fibrinolysis pathway members with which *ace2* is co-expressed during the specific time-window of intestinal development.

Interestingly, several studies have previously reported a higher *ACE2* expression in cilia of well-differentiated epithelial cells compared to undifferentiated cells in humans (Jia et al., 2005, Lee et al., 2020b, Ziegler et al., 2020). *ACE2* expression is present in motile cilia in the human airway epithelial cells (Lee et al., 2020a) and also shown to be affected by SARS-CoV infection (Lee et al., 2020b), which causes loss of cilia (Ziegler et al., 2020). Further studies are needed to understand the dynamicity between *ace2* expression and cilium assembly. Indeed, the significant changes observed in the *ace2* expression levels at the time of intestinal differentiation also provide an effective translational time frame for testing potential drugs affecting intraflagellar transport and the RAS pathway.

Consistently, our analyses of r_{ace2} in zebrafish have also pointed to the enrichment of respiratory chain-related networks. In support of this, *Ace2* knock-out mice have been reported to exhibit

disrupted mitochondrial function in pancreatic islets and skeletal muscle cells (Cao et al., 2019, Shi et al., 2018). Transcriptomic analyses with human cornea, retina and lung datasets propose *ACE2* is significantly co-expressed with mitochondrial genes (Yuan et al., 2020). Indeed, Ang II is known to activate NADPH and can stimulate an increase in reactive oxygen species (ROS) in renal cells, while *ACE2*, as a negative regulator of Ang II, has a protective role against ROS (Gava et al., 2009, Gwathmey et al., 2010, Kim et al., 2012). *Ace2* knock-out mice also exhibit increased insulin resistance which can lead to hepatic and oxidative stress (Cao et al., 2014, Gallagher et al., 2008, Gwathmey et al., 2010, Song et al., 2020). The enrichment of ROS-related pathways in our analysis supports the notion that zebrafish can also serve as a model organism to study the potential link between the modulatory role of *ace2* in mitochondrial function and disease.

Our findings have also indicated the liver as the second-highest *ace2* expressing tissue in zebrafish yet with a clear bimodal pattern regardless of age (6 months – 42 months) or gender. This bimodality might have arisen from contamination with other tissues, especially the gut and/or pancreas, and/or the presence of intrinsic or extrinsic factors in relationship with diet, inflammation, or infection. GO enrichment analyses of *ace2*'s positively co-expressed genes revealed the involvement of lipid catabolism as a potential discriminator between the low and high *ace2* expressing liver; in partial support of this, our re-analysis shows that *ace2* levels decrease in response to microcystin-LR, a common form of cyanotoxin, which causes liver damage and disruption of lipid metabolism (Liu et al., 2014, Woolbright et al., 2017); and in the NAFLD zebrafish model, in accord with mouse data in the literature (Cao et al., 2016, Yang et al., 2020). In our re-analysis, *ace2* expression in zebrafish refed liver steadily increases after fasting, aiding in the connection between *ace2* expression and diet. *Ace2* levels also have been shown to be elevated in high-fat-fed mice and rats (Gupte et al., 2008, Shoemaker et al., 2019, Zhang et al., 2014), but not in the initial response to lipid exposure in zebrafish digestive organs (Zeituni et al., 2016). This suggests that further experiments are needed to identify the time frame the diet can

alter *ace2* levels in zebrafish. In addition to altered lipid metabolism, interferon pathway modulation (ISG15 antiviral mechanism network) has arisen as a discriminator of the low and high *ace2* expressing liver in zebrafish. The liver, an organ continually subjected to food antigens and pathogens from intestines, can respond to such activators of immune response readily with its residential macrophages (Bleriot and Ginhoux, 2019); and this may result in highly variable interferon activity in the liver (reviewed in (Robinson et al., 2016)). Future studies focusing on embryonic and tissue-specific *ace2* knock-out and overexpression models can help understand the role of adaptive and innate immune system challenges in zebrafish liver expression variability. Moreover, a significant decrease identified in *ace2* levels in response to vitamin D treatment during early development can be studied further in different contexts, including COVID-19 (Benskin, 2020, Bleizgys, 2020, Musavi et al., 2020, Ali et al., 2018, Andersen et al., 2015, Shen et al., 2017).

From a disease perspective, the observed decrease in *ace2* expression in the zebrafish SBS model may point to a link between decreased levels of *ace2* and inflammation as widely observed in human SBS patients (Schall et al., 2017). Our re-analysis has shown that zebrafish *uhrf1* and *cdipt* mutants, with elevated intestinal inflammation, both exhibit a significant decrease in *ace2* expression (Marjoram et al., 2015, Thakur et al., 2014). In human inflammatory bowel disease (IBD), *ACE2* levels exhibit a similar decrease in inflamed ileum yet increases in inflamed colon and rectum (Suarez-Farinas et al., 2020, Verstockt et al., 2020). In addition, the zebrafish embryos deficient in the key innate immunity regulatory factor *myd88* exhibit altered lipid metabolism while expressing *ace2* highly, regardless of the type of microbiome (Koch et al., 2018). Interestingly, in mice, *MyD88* is shown to be essential in protection against SARS-CoV (Hirano and Murakami, 2020, Sheahan et al., 2008). Moreover, our bioinformatics analyses show that preexposure to immune suppressive and lipid modulatory agent β -glucans may lower *ace2*

levels (AbuMweis et al., 2010), all supporting an association between *ace2* levels and modulation of inflammatory pathways, which remains to be tested.

We observed a sexually dimorphic expression pattern of *ace2* in zebrafish, which can provide a model for studying gender-specific *ace2* expression in response to different drugs in regard to hypertension as well as COVID-19 treatment (Cuffe et al., 2016, Goyal et al., 2015). Moreover, *esr2a* knock-out zebrafish, exhibiting a significantly lower female to male offspring ratio (Lu et al., 2017, Wu et al., 2020), also show lower *ace2* levels. Previously, a modulatory role of estrogen on *ACE2* expression through its receptors, *ESR1*, or *GPER1*, has been shown (Feng et al., 2020, Mompeon et al., 2016, Sun et al., 2021). Altogether, our analyses suggest a gender-specific role for *ace2* also in zebrafish.

In conclusion, tissue and context-dependent expression of *ace2* in zebrafish correlate well with the human, non-human primate, and rodent data, as exemplified above. Moreover, via meta-analysis, we provide novel links between *ace2* expression modulation and diseases of the intestines and liver for the first time in the zebrafish literature. Our application of logFC based extraction of co-expressed genes of zebrafish *ace2* translates into functional networks connected with each other via their common components. These networks were obtained based on heterogeneous datasets, hence reveal a relatively wide range of conditions zebrafish *ace2* expression could be modulated. A similar meta-analysis approach has been used in compound networks, and herein to zebrafish *ace2*; hence is easily applicable to species-specific gene networks reflecting differential expression. Furthermore, we provide novel leads for testing zebrafish *ace2* levels against different drugs within a given developmental and physiological time-frame as well as different liver and intestinal pathologies.

Competing interests

The authors declare no competing or financial interests.

References

ABUMWEIS, S. S., JEW, S. & AMES, N. P. 2010. beta-glucan from barley and its lipid-lowering capacity: a meta-analysis of randomized, controlled trials. *Eur J Clin Nutr*, 64, 1472-80.

ALI, R. M., AL-SHORBAGY, M. Y., HELMY, M. W. & EL-ABHAR, H. S. 2018. Role of Wnt4/beta-catenin, Ang II/TGFbeta, ACE2, NF-kappaB, and IL-18 in attenuating renal ischemia/reperfusion-induced injury in rats treated with Vit D and pioglitazone. *Eur J Pharmacol*, 831, 68-76.

ALVAREZ-RODRIGUEZ, M., PEREIRO, P., REYES-LOPEZ, F. E., TORT, L., FIGUERAS, A. & NOVOA, B. 2018. Analysis of the Long-Lived Responses Induced by Immunostimulants and Their Effects on a Viral Infection in Zebrafish (*Danio rerio*). *Front Immunol*, 9, 1575.

ANDERS, S., PYL, P. T. & HUBER, W. 2015. HTSeq--a Python framework to work with high-throughput sequencing data. *Bioinformatics*, 31, 166-9.

ANDERSEN, L. B., PRZYBYL, L., HAASE, N., VON VERSEN-HOYNCK, F., QADRI, F., JORGENSEN, J. S., SORENSEN, G. L., FRUEKILDE, P., POGLITSCH, M., SZIJARTO, I., GOLLASCH, M., PETERS, J., MULLER, D. N., CHRISTESEN, H. T. & DECHEND, R. 2015. Vitamin D depletion aggravates hypertension and target-organ damage. *J Am Heart Assoc*, 4.

ARAMILLO IRIZAR, P., SCHAUBLE, S., ESSER, D., GROTH, M., FRAHM, C., PRIEBE, S., BAUMGART, M., HARTMANN, N., MARTHANDAN, S., MENZEL, U., MULLER, J., SCHMIDT, S., AST, V., CALIEBE, A., KONIG, R., KRAWCZAK, M., RISTOW, M., SCHUSTER, S., CELLERINO, A., DIEKMANN, S., ENGLERT, C., HEMMERICH, P., SUHNEL, J., GUTHKE, R., WITTE, O. W., PLATZER, M., RUPPIN, E. & KALETA, C. 2018. Transcriptomic alterations during ageing reflect the shift from cancer to degenerative diseases in the elderly. *Nat Commun*, 9, 327.

BARRETT, T., WILHITE, S. E., LEDOUX, P., EVANGELISTA, C., KIM, I. F., TOMASHEVSKY, M., MARSHALL, K. A., PHILLIPPY, K. H., SHERMAN, P. M., HOLKO, M., YEFANOV, A., LEE, H., ZHANG, N., ROBERTSON, C. L., SEROVA, N., DAVIS, S. & SOBOLEVA, A. 2013. NCBI GEO: archive for functional genomics data sets--update. *Nucleic Acids Res*, 41, D991-5.

BENSKIN, L. L. 2020. A Basic Review of the Preliminary Evidence That COVID-19 Risk and Severity Is Increased in Vitamin D Deficiency. *Front Public Health*, 8, 513.

BLEIZGYS, A. 2020. Vitamin D AND COVID-19: It is time to act. *Int J Clin Pract*, e13748.

BLERIOT, C. & GINHOUX, F. 2019. Understanding the Heterogeneity of Resident Liver Macrophages. *Front Immunol*, 10, 2694.

CAO, X., LU, X. M., TUO, X., LIU, J. Y., ZHANG, Y. C., SONG, L. N., CHENG, Z. Q., YANG, J. K. & XIN, Z. 2019. Angiotensin-converting enzyme 2 regulates endoplasmic reticulum stress and mitochondrial function to preserve skeletal muscle lipid metabolism. *Lipids Health Dis*, 18, 207.

CAO, X., YANG, F., SHI, T., YUAN, M., XIN, Z., XIE, R., LI, S., LI, H. & YANG, J. K. 2016. Angiotensin-converting enzyme 2/angiotensin-(1-7)/Mas axis activates Akt signaling to ameliorate hepatic steatosis. *Sci Rep*, 6, 21592.

CAO, X., YANG, F. Y., XIN, Z., XIE, R. R. & YANG, J. K. 2014. The ACE2/Ang-(1-7)/Mas axis can inhibit hepatic insulin resistance. *Mol Cell Endocrinol*, 393, 30-8.

CHEN, L., MARISHTA, A., ELLISON, C. E. & VERZI, M. P. 2020a. Identification of Transcription Factors Regulating SARS-CoV-2 Entry Genes in the Intestine. *Cell Mol Gastroenterol Hepatol*.

CHEN, L., WANG, L., CHENG, Q., TU, Y. X., YANG, Z., LI, R. Z., LUO, Z. H. & CHEN, Z. X. 2020b. Anti-masculinization induced by aromatase inhibitors in adult female zebrafish. *BMC Genomics*, 21, 22.

CHENG, J. A. Y., LUN 2013. Comparing gene expression similarity metrics for connectivity map. *2013 IEEE International Conference on Bioinformatics and Biomedicine*, 165-170.

- CHENG, P. Y., LIN, C. C., WU, C. S., LU, Y. F., LIN, C. Y., CHUNG, C. C., CHU, C. Y., HUANG, C. J., TSAI, C. Y., KORZH, S., WU, J. L. & HWANG, S. P. 2008. Zebrafish *cdx1b* regulates expression of downstream factors of Nodal signaling during early endoderm formation. *Development*, 135, 941-52.
- CHENG, Z. & LIU, Z. 2019. Renin-angiotensin system gene polymorphisms and colorectal cancer risk: a meta-analysis. *J Renin Angiotensin Aldosterone Syst*, 20, 1470320319881932.
- CHEUNG, M. W. & VIJAYAKUMAR, R. 2016. A Guide to Conducting a Meta-Analysis. *Neuropsychol Rev*, 26, 121-8.
- CHOU, C. F., LOH, C. B., FOO, Y. K., SHEN, S., FIELDING, B. C., TAN, T. H., KHAN, S., WANG, Y., LIM, S. G., HONG, W., TAN, Y. J. & FU, J. 2006. ACE2 orthologues in non-mammalian vertebrates (*Danio*, *Gallus*, *Fugu*, *Tetraodon* and *Xenopus*). *Gene*, 377, 46-55.
- COCCHERI, S. 2020. COVID-19: The crucial role of blood coagulation and fibrinolysis. *Intern Emerg Med*.
- CUFFE, J. S., BURGESS, D. J., O'SULLIVAN, L., SINGH, R. R. & MORITZ, K. M. 2016. Maternal corticosterone exposure in the mouse programs sex-specific renal adaptations in the renin-angiotensin-aldosterone system in 6-month offspring. *Physiol Rep*, 4.
- DOBIN, A., DAVIS, C. A., SCHLESINGER, F., DRENKOW, J., ZALESKI, C., JHA, S., BATUT, P., CHAISSON, M. & GINGERAS, T. R. 2013. STAR: ultrafast universal RNA-seq aligner. *Bioinformatics*, 29, 15-21.
- DOMAZET-LOSO, T. & TAUTZ, D. 2010. A phylogenetically based transcriptome age index mirrors ontogenetic divergence patterns. *Nature*, 468, 815-8.
- EVANGELOU, E. & IOANNIDIS, J. P. 2013. Meta-analysis methods for genome-wide association studies and beyond. *Nat Rev Genet*, 14, 379-89.
- FENG, Q., LI, L. & WANG, X. 2020. Identifying Pathways and Networks Associated With the SARS-CoV-2 Cell Receptor ACE2 Based on Gene Expression Profiles in Normal and SARS-CoV-2-Infected Human Tissues. *Front Mol Biosci*, 7, 568954.
- FLORES, M. V., HALL, C. J., DAVIDSON, A. J., SINGH, P. P., MAHAGAONKAR, A. A., ZON, L. I., CROSIER, K. E. & CROSIER, P. S. 2008. Intestinal differentiation in zebrafish requires *Cdx1b*, a functional equivalent of mammalian *Cdx2*. *Gastroenterology*, 135, 1665-75.
- FORN-CUNI, G., VARELA, M., PEREIRO, P., NOVOA, B. & FIGUERAS, A. 2017. Conserved gene regulation during acute inflammation between zebrafish and mammals. *Sci Rep*, 7, 41905.
- FROELICHER, M., LIEDTKE, A., GROH, K., LOPEZ-SCHIER, H., NEUHAUSS, S. C., SEGNER, H. & EGGEN, R. I. 2009. Estrogen receptor subtype beta2 is involved in neuromast development in zebrafish (*Danio rerio*) larvae. *Dev Biol*, 330, 32-43.
- GALLAGHER, P. E., FERRARIO, C. M. & TALLANT, E. A. 2008. MAP kinase/phosphatase pathway mediates the regulation of ACE2 by angiotensin peptides. *Am J Physiol Cell Physiol*, 295, C1169-74.
- GANZ, J., MELANCON, E., WILSON, C., AMORES, A., BATZEL, P., STRADER, M., BRAASCH, I., DIBA, P., KUHLMAN, J. A., POSTLETHWAIT, J. H. & EISEN, J. S. 2019. Epigenetic factors *Dnmt1* and *Uhrf1* coordinate intestinal development. *Dev Biol*, 455, 473-484.
- GAUTIER, L., COPE, L., BOLSTAD, B. M. & IRIZARRY, R. A. 2004. affy--analysis of Affymetrix GeneChip data at the probe level. *Bioinformatics*, 20, 307-15.
- GAVA, E., SAMAD-ZADEH, A., ZIMPELMANN, J., BAHRAMIFARID, N., KITTEN, G. T., SANTOS, R. A., TOUYZ, R. M. & BURNS, K. D. 2009. Angiotensin-(1-7) activates a tyrosine phosphatase and inhibits glucose-induced signalling in proximal tubular cells. *Nephrol Dial Transplant*, 24, 1766-73.
- GOMES, M. C. & MOSTOWY, S. 2020. The Case for Modeling Human Infection in Zebrafish. *Trends Microbiol*, 28, 10-18.
- GOYAL, R., VAN-WICKLE, J., GOYAL, D. & LONGO, L. D. 2015. Antenatal maternal low protein diet: ACE-2 in the mouse lung and sexually dimorphic programming of hypertension. *BMC Physiol*, 15, 2.
- GUPTE, M., BOUSTANY-KARI, C. M., BHARADWAJ, K., POLICE, S., THATCHER, S., GONG, M. C., ENGLISH, V. L. & CASSIS, L. A. 2008. ACE2 is expressed in mouse adipocytes and regulated by a high-fat diet. *Am J Physiol Regul Integr Comp Physiol*, 295, R781-8.

- GUR-DEDEOGLU, B., KONU, O., KIR, S., OZTURK, A. R., BOZKURT, B., ERGUL, G. & YULUG, I. G. 2008. A resampling-based meta-analysis for detection of differential gene expression in breast cancer. *BMC Cancer*, 8, 396.
- GWATHMEY, T. M., PENDERGRASS, K. D., REID, S. D., ROSE, J. C., DIZ, D. I. & CHAPPELL, M. C. 2010. Angiotensin-(1-7)-angiotensin-converting enzyme 2 attenuates reactive oxygen species formation to angiotensin II within the cell nucleus. *Hypertension*, 55, 166-71.
- HAMMING, I., COOPER, M. E., HAAGMANS, B. L., HOOPER, N. M., KORSTANJE, R., OSTERHAUS, A. D., TIMENS, W., TURNER, A. J., NAVIS, G. & VAN GOOR, H. 2007. The emerging role of ACE2 in physiology and disease. *J Pathol*, 212, 1-11.
- HEIDEN, T. C., STRUBLE, C. A., RISE, M. L., HESSNER, M. J., HUTZ, R. J. & CARVAN, M. J., 3RD 2008. Molecular targets of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) within the zebrafish ovary: insights into TCDD-induced endocrine disruption and reproductive toxicity. *Reprod Toxicol*, 25, 47-57.
- HIRANO, T. & MURAKAMI, M. 2020. COVID-19: A New Virus, but a Familiar Receptor and Cytokine Release Syndrome. *Immunity*, 52, 731-733.
- HOLDEN, L. A. & BROWN, K. H. 2018. Baseline mRNA expression differs widely between common laboratory strains of zebrafish. *Sci Rep*, 8, 4780.
- HU, B., CHEN, H., LIU, X., ZHANG, C., COLE, G. J., LEE, J. A. & CHEN, X. 2013. Transgenic overexpression of *cdx1b* induces metaplastic changes of gene expression in zebrafish esophageal squamous epithelium. *Zebrafish*, 10, 218-27.
- JACOB, V., CHERNYAVSKAYA, Y., CHEN, X., TAN, P. S., KENT, B., HOSHIDA, Y. & SADLER, K. C. 2015. DNA hypomethylation induces a DNA replication-associated cell cycle arrest to block hepatic outgrowth in *uhrf1* mutant zebrafish embryos. *Development*, 142, 510-21.
- JIA, H. P., LOOK, D. C., SHI, L., HICKEY, M., PEWE, L., NETLAND, J., FARZAN, M., WOHLFORD-LENANE, C., PERLMAN, S. & MCCRAY, P. B., JR. 2005. ACE2 receptor expression and severe acute respiratory syndrome coronavirus infection depend on differentiation of human airway epithelia. *J Virol*, 79, 14614-21.
- JIA, J., QIN, J., YUAN, X., LIAO, Z., HUANG, J., WANG, B., SUN, C. & LI, W. 2019. Microarray and metabolome analysis of hepatic response to fasting and subsequent refeeding in zebrafish (*Danio rerio*). *BMC Genomics*, 20, 919.
- JOHNSON, E., VU, L. & MATARESE, L. E. 2018. Bacteria, Bones, and Stones: Managing Complications of Short Bowel Syndrome. *Nutr Clin Pract*, 33, 454-466.
- KIM, S. M., KIM, Y. G., JEONG, K. H., LEE, S. H., LEE, T. W., IHM, C. G. & MOON, J. Y. 2012. Angiotensin II-induced mitochondrial Nox4 is a major endogenous source of oxidative stress in kidney tubular cells. *PLoS One*, 7, e39739.
- KIMMEL, C. B., BALLARD, W. W., KIMMEL, S. R., ULLMANN, B. & SCHILLING, T. F. 1995. Stages of embryonic development of the zebrafish. *Dev Dyn*, 203, 253-310.
- KOCH, B. E. V., YANG, S., LAMERS, G., STOUGAARD, J. & SPAINK, H. P. 2018. Intestinal microbiome adjusts the innate immune setpoint during colonization through negative regulation of MyD88. *Nat Commun*, 9, 4099.
- KOLDE, R., LAUR, S., ADLER, P. & VILO, J. 2012. Robust rank aggregation for gene list integration and meta-analysis. *Bioinformatics*, 28, 573-80.
- KRAMER, S., BUSCH, W. & SCHUTTLER, A. 2020. A Self-Organizing Map of the Fathead Minnow Liver Transcriptome to Identify Consistent Toxicogenomic Patterns across Chemical Fingerprints. *Environ Toxicol Chem*, 39, 526-537.
- KRYUCHKOVA-MOSTACCI, N. & ROBINSON-RECHAVI, M. 2017. A benchmark of gene expression tissue-specificity metrics. *Brief Bioinform*, 18, 205-214.

- LEE, I. T., NAKAYAMA, T., WU, C. T., GOLTSEV, Y., JIANG, S., GALL, P. A., LIAO, C. K., SHIH, L. C., SCHURCH, C. M., MCILWAIN, D. R., CHU, P., BORCHARD, N. A., ZARABANDA, D., DHOLAKIA, S. S., YANG, A., KIM, D., CHEN, H., KANIE, T., LIN, C. D., TSAI, M. H., PHILLIPS, K. M., KIM, R., OVERDEVEST, J. B., TYLER, M. A., YAN, C. H., LIN, C. F., LIN, Y. T., BAU, D. T., TSAY, G. J., PATEL, Z. M., TSOU, Y. A., TZANKOV, A., MATTER, M. S., TAI, C. J., YEH, T. H., HWANG, P. H., NOLAN, G. P., NAYAK, J. V. & JACKSON, P. K. 2020a. ACE2 localizes to the respiratory cilia and is not increased by ACE inhibitors or ARBs. *Nat Commun*, 11, 5453.
- LEE, I. T., NAKAYAMA, T., WU, C. T., GOLTSEV, Y., JIANG, S., GALL, P. A., LIAO, C. K., SHIH, L. C., SCHURCH, C. M., MCILWAIN, D. R., CHU, P., BORCHARD, N. A., ZARABANDA, D., DHOLAKIA, S. S., YANG, A., KIM, D., KANIE, T., LIN, C. D., TSAI, M. H., PHILLIPS, K. M., KIM, R., OVERDEVEST, J. B., TYLER, M. A., YAN, C. H., LIN, C. F., LIN, Y. T., BAU, D. T., TSAY, G. J., PATEL, Z. M., TSOU, Y. A., TAI, C. J., YEH, T. H., HWANG, P. H., NOLAN, G. P., NAYAK, J. V. & JACKSON, P. K. 2020b. Robust ACE2 protein expression localizes to the motile cilia of the respiratory tract epithelia and is not increased by ACE inhibitors or angiotensin receptor blockers. *medRxiv*.
- LIU, W., QIAO, Q., CHEN, Y., WU, K. & ZHANG, X. 2014. Microcystin-LR exposure to adult zebrafish (*Danio rerio*) leads to growth inhibition and immune dysfunction in F1 offspring, a parental transmission effect of toxicity. *Aquat Toxicol*, 155, 360-7.
- LOGAN, S. L., THOMAS, J., YAN, J., BAKER, R. P., SHIELDS, D. S., XAVIER, J. B., HAMMER, B. K. & PARTHASARATHY, R. 2018. The *Vibrio cholerae* type VI secretion system can modulate host intestinal mechanics to displace gut bacterial symbionts. *Proc Natl Acad Sci U S A*, 115, E3779-E3787.
- LOVE, M. I., HUBER, W. & ANDERS, S. 2014. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol*, 15, 550.
- LU, H., CUI, Y., JIANG, L. & GE, W. 2017. Functional Analysis of Nuclear Estrogen Receptors in Zebrafish Reproduction by Genome Editing Approach. *Endocrinology*, 158, 2292-2308.
- MACINNES, A. W., AMSTERDAM, A., WHITTAKER, C. A., HOPKINS, N. & LEES, J. A. 2008. Loss of p53 synthesis in zebrafish tumors with ribosomal protein gene mutations. *Proc Natl Acad Sci U S A*, 105, 10408-13.
- MARJORAM, L., ALVERS, A., DEERHAKE, M. E., BAGWELL, J., MANKIEWICZ, J., COCCHIARO, J. L., BEERMAN, R. W., WILLER, J., SUMIGRAY, K. D., KATSANIS, N., TOBIN, D. M., RAWLS, J. F., GOLL, M. G. & BAGNAT, M. 2015. Epigenetic control of intestinal barrier function and inflammation in zebrafish. *Proc Natl Acad Sci U S A*, 112, 2770-5.
- MCCARTHY, D. J., CHEN, Y. & SMYTH, G. K. 2012. Differential expression analysis of multifactor RNA-Seq experiments with respect to biological variation. *Nucleic Acids Res*, 40, 4288-97.
- MICKIEWICZ, K. M., KAWAI, Y., DRAGE, L., GOMES, M. C., DAVISON, F., PICKARD, R., HALL, J., MOSTOWY, S., ALDRIDGE, P. D. & ERRINGTON, J. 2019. Possible role of L-form switching in recurrent urinary tract infection. *Nat Commun*, 10, 4379.
- MOMPEON, A., LAZARO-FRANCO, M., BUENO-BETI, C., PEREZ-CREMADES, D., VIDAL-GOMEZ, X., MONSALVE, E., GIRONACCI, M. M., HERMENEGILDO, C. & NOVELLA, S. 2016. Estradiol, acting through ERalpha, induces endothelial non-classic renin-angiotensin system increasing angiotensin 1-7 production. *Mol Cell Endocrinol*, 422, 1-8.
- MUSAVI, H., ABAZARI, O., BARARTABAR, Z., KALAKI-JOUYBARI, F., HEMMATI-DINARVAND, M., ESMAEILI, P. & MAHJOUB, S. 2020. The benefits of Vitamin D in the COVID-19 pandemic: biochemical and immunological mechanisms. *Arch Physiol Biochem*, 1-9.
- MUTANEN, A., BARRETT, M., FENG, Y., LOHI, J., RABAH, R., TEITELBAUM, D. H. & PAKARINEN, M. P. 2019. Short bowel mucosal morphology, proliferation and inflammation at first and repeat STEP procedures. *J Pediatr Surg*, 54, 511-516.

- 601 NEHME, A., ZOUEN, F. A., ZAYERI, Z. D. & ZIBARA, K. 2019. An Update on the Tissue Renin Angiotensin
602 System and Its Role in Physiology and Pathology. *J Cardiovasc Dev Dis*, 6.
- 603 OKUDA, Y., OGURA, E., KONDOH, H. & KAMACHI, Y. 2010. B1 SOX coordinate cell specification with
604 patterning and morphogenesis in the early zebrafish embryo. *PLoS Genet*, 6, e1000936.
- 605 PAPTAEODOROU, I., MORENO, P., MANNING, J., FUENTES, A. M., GEORGE, N., FEXOVA, S., FONSECA,
606 N. A., FULLGRABE, A., GREEN, M., HUANG, N., HUERTA, L., IQBAL, H., JIANU, M., MOHAMMED,
607 S., ZHAO, L., JARNUCZAK, A. F., JUPP, S., MARIONI, J., MEYER, K., PETRYSZAK, R., PRADA
608 MEDINA, C. A., TALAVERA-LOPEZ, C., TEICHMANN, S., VIZCAINO, J. A. & BRAZMA, A. 2020.
609 Expression Atlas update: from tissues to single cells. *Nucleic Acids Res*, 48, D77-D83.
- 610 PINTER, M. & JAIN, R. K. 2017. Targeting the renin-angiotensin system to improve cancer treatment:
611 Implications for immunotherapy. *Sci Transl Med*, 9.
- 612 POSTLETHWAIT, J. H., MASSAQUOI, M. S., FARNSWORTH, D. R., YAN, Y. L., GUILLEMIN, K. & MILLER, A. C.
613 2021. The SARS-CoV-2 receptor and other key components of the Renin-Angiotensin-
614 Aldosterone System related to COVID-19 are expressed in enterocytes in larval zebrafish. *Biol*
615 *Open*, 10.
- 616 RASHA, F., RAMALINGAM, L., GOLLAHON, L., RAHMAN, R. L., RAHMAN, S. M., MENIKDIWELA, K. &
617 MOUSTAID-MOUSSA, N. 2019. Mechanisms linking the renin-angiotensin system, obesity, and
618 breast cancer. *Endocr Relat Cancer*, 26, R653-R672.
- 619 RITCHIE, M. E., PHIPSON, B., WU, D., HU, Y., LAW, C. W., SHI, W. & SMYTH, G. K. 2015. limma powers
620 differential expression analyses for RNA-sequencing and microarray studies. *Nucleic Acids Res*,
621 43, e47.
- 622 ROBINSON, M. W., HARMON, C. & O'FARRELLY, C. 2016. Liver immunology and its role in inflammation
623 and homeostasis. *Cell Mol Immunol*, 13, 267-76.
- 624 ROBISON, B. D., DREW, R. E., MURDOCH, G. K., POWELL, M., RODNICK, K. J., SETTLES, M., STONE, D.,
625 CHURCHILL, E., HILL, R. A., PAPASANI, M. R., LEWIS, S. S. & HARDY, R. W. 2008. Sexual
626 dimorphism in hepatic gene expression and the response to dietary carbohydrate manipulation
627 in the zebrafish (*Danio rerio*). *Comp Biochem Physiol Part D Genomics Proteomics*, 3, 141-54.
- 628 ROGERS, E. D., HENRY, T. B., TWINER, M. J., GOUFFON, J. S., MCPHERSON, J. T., BOYER, G. L., SAYLER, G.
629 S. & WILHELM, S. W. 2011. Global gene expression profiling in larval zebrafish exposed to
630 microcystin-LR and microcystis reveals endocrine disrupting effects of Cyanobacteria. *Environ Sci*
631 *Technol*, 45, 1962-9.
- 632 SAN, B., ABEN, M., ELURBE, D. M., VOELTZKE, K., DEN BROEDER, M. J., ROUGEOT, J., LEGLER, J. &
633 KAMMINGA, L. M. 2018. Genetic and epigenetic regulation of zebrafish intestinal development.
634 *Epigenomes*, 2, 19.
- 635 SCHALL, K. A., THORNTON, M. E., ISANI, M., HOLOYDA, K. A., HOU, X., LIEN, C. L., GRUBBS, B. H. &
636 GRIKSCHIT, T. C. 2017. Short bowel syndrome results in increased gene expression associated
637 with proliferation, inflammation, bile acid synthesis and immune system activation: RNA
638 sequencing a zebrafish SBS model. *BMC Genomics*, 18, 23.
- 639 SHANNON, P., MARKIEL, A., OZIER, O., BALIGA, N. S., WANG, J. T., RAMAGE, D., AMIN, N.,
640 SCHWIKOWSKI, B. & IDEKER, T. 2003. Cytoscape: a software environment for integrated models
641 of biomolecular interaction networks. *Genome Res*, 13, 2498-504.
- 642 SHEAHAN, T., MORRISON, T. E., FUNKHOUSER, W., UEMATSU, S., AKIRA, S., BARIC, R. S. & HEISE, M. T.
643 2008. MyD88 is required for protection from lethal infection with a mouse-adapted SARS-CoV.
644 *PLoS Pathog*, 4, e1000240.
- 645 SHEHWANA, H. & KONU, O. 2019. Comparative Transcriptomics Between Zebrafish and Mammals: A
646 Roadmap for Discovery of Conserved and Unique Signaling Pathways in Physiology and Disease.
647 *Front Cell Dev Biol*, 7, 5.

- SHEN, L., MA, C., SHUAI, B. & YANG, Y. 2017. Effects of 1,25-dihydroxyvitamin D3 on the local bone renin-angiotensin system in a murine model of glucocorticoid-induced osteoporosis. *Exp Ther Med*, 13, 3297-3304.
- SHI, T. T., YANG, F. Y., LIU, C., CAO, X., LU, J., ZHANG, X. L., YUAN, M. X., CHEN, C. & YANG, J. K. 2018. Angiotensin-converting enzyme 2 regulates mitochondrial function in pancreatic beta-cells. *Biochem Biophys Res Commun*, 495, 860-866.
- SHOEMAKER, R., TANNOCK, L. R., SU, W., GONG, M., GURLEY, S. B., THATCHER, S. E., YIANNIKOURIS, F., ENSOR, C. M. & CASSIS, L. A. 2019. Adipocyte deficiency of ACE2 increases systolic blood pressures of obese female C57BL/6 mice. *Biol Sex Differ*, 10, 45.
- SILBERG, D. G., SWAIN, G. P., SUH, E. R. & TRABER, P. G. 2000. Cdx1 and cdx2 expression during intestinal development. *Gastroenterology*, 119, 961-71.
- SMALL, C. M., CARNEY, G. E., MO, Q., VANNUCCI, M. & JONES, A. G. 2009. A microarray analysis of sex- and gonad-biased gene expression in the zebrafish: evidence for masculinization of the transcriptome. *BMC Genomics*, 10, 579.
- SONG, L. N., LIU, J. Y., SHI, T. T., ZHANG, Y. C., XIN, Z., CAO, X. & YANG, J. K. 2020. Angiotensin-(1-7), the product of ACE2 ameliorates NAFLD by acting through its receptor Mas to regulate hepatic mitochondrial function and glycolipid metabolism. *FASEB J*.
- SONG, R., PRESTON, G. & YOSYPIV, I. V. 2012. Ontogeny of angiotensin-converting enzyme 2. *Pediatr Res*, 71, 13-9.
- SONI, K., CHOUDHARY, A., PATOWARY, A., SINGH, A. R., BHATIA, S., SIVASUBBU, S., CHANDRASEKARAN, S. & PILLAI, B. 2013. miR-34 is maternally inherited in *Drosophila melanogaster* and *Danio rerio*. *Nucleic Acids Res*, 41, 4470-80.
- STUCKENHOLZ, C., LU, L., THAKUR, P., KAMINSKI, N. & BAHARY, N. 2009. FACS-assisted microarray profiling implicates novel genes and pathways in zebrafish gastrointestinal tract development. *Gastroenterology*, 137, 1321-32.
- SUAREZ-FARINAS, M., TOKUYAMA, M., WEI, G., HUANG, R., LIVANOS, A., JHA, D., LEVESCOT, A., IRIZAR, H., KOSOY, R., CORDING, S., WANG, W., LOSIC, B., UNGARO, R., DI'NARZO, A., MARTINEZ-DELGADO, G., SUPRUN, M., CORLEY, M. J., STOJMIROVIC, A., HOUTEN, S. M., PETERS, L., CURRAN, M., BRODMERKEL, C., PERRIGOU, J., FRIEDMAN, J. R., HAO, K., SCHADT, E. E., ZHU, J., KO, H. M., CHO, J., DUBINSKY, M. C., SANDS, B. E., NDHLOVU, L., CERF-BENSUSAN, N., KASARSKIS, A., COLOMBEL, J. F., HARPAZ, N., ARGMAN, C. & MEHANDRU, S. 2020. Intestinal inflammation modulates the expression of ACE2 and TMPRSS2 and potentially overlaps with the pathogenesis of SARS-CoV-2 related disease. *Gastroenterology*.
- SUN, X., WANG, H., HODGE, H., WRIGHT, K. N., AHMAD, S., FERRARIO, C. M. & GROBAN, L. 2021. Amplifying effect of chronic lisinopril therapy on diastolic function and the angiotensin-(1-7) Axis by the G1 agonist in ovariectomized spontaneously hypertensive rats. *Transl Res*, 235, 62-76.
- SZKLARCZYK, D., FRANCESCHINI, A., WYDER, S., FORSLUND, K., HELLER, D., HUERTA-CEPAS, J., SIMONOVIC, M., ROTH, A., SANTOS, A., TSAFOU, K. P., KUHN, M., BORK, P., JENSEN, L. J. & VON MERING, C. 2015. STRING v10: protein-protein interaction networks, integrated over the tree of life. *Nucleic Acids Res*, 43, D447-52.
- SZKLARCZYK, D., GABLE, A. L., LYON, D., JUNGE, A., WYDER, S., HUERTA-CEPAS, J., SIMONOVIC, M., DONCHEVA, N. T., MORRIS, J. H., BORK, P., JENSEN, L. J. & MERING, C. V. 2019. STRING v11: protein-protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. *Nucleic Acids Res*, 47, D607-D613.
- TER VEER, E., VAN OIJEN, M. G. H. & VAN LAARHOVEN, H. W. M. 2019. The Use of (Network) Meta-Analysis in Clinical Oncology. *Front Oncol*, 9, 822.

- THAKUR, P. C., DAVISON, J. M., STUCKENHOLZ, C., LU, L. & BAHARY, N. 2014. Dysregulated phosphatidylinositol signaling promotes endoplasmic-reticulum-stress-mediated intestinal mucosal injury and inflammation in zebrafish. *Dis Model Mech*, 7, 93-106.
- TSENG, G. C., GHOSH, D. & FEINGOLD, E. 2012. Comprehensive literature review and statistical considerations for microarray meta-analysis. *Nucleic Acids Res*, 40, 3785-99.
- VARGAS, M., SERVILLO, G. & EINAIV, S. 2020. Lopinavir/ritonavir for the treatment of SARS, MERS and COVID-19: a systematic review. *Eur Rev Med Pharmacol Sci*, 24, 8592-8605.
- VERSTOCKT, B., VERSTOCKT, S., ABDU RAHIMAN, S., KE, B. J., ARNAUTS, K., CLEYNEN, I., SABINO, J., FERRANTE, M., MATTEOLI, G. & VERMEIRE, S. 2020. Intestinal receptor of SARS-CoV-2 in inflamed IBD tissue seems downregulated by HNF4A in ileum and upregulated by interferon regulating factors in colon. *J Crohns Colitis*.
- WANG, Y., WANG, Y., LUO, W., HUANG, L., XIAO, J., LI, F., QIN, S., SONG, X., WU, Y., ZENG, Q., JIN, F. & WANG, Y. 2020. A comprehensive investigation of the mRNA and protein level of ACE2, the putative receptor of SARS-CoV-2, in human tissues and blood cells. *Int J Med Sci*, 17, 1522-1531.
- WHITE, R. J., COLLINS, J. E., SEALY, I. M., WALI, N., DOOLEY, C. M., DIGBY, Z., STEMPEL, D. L., MURPHY, D. N., BILLIS, K., HOURLIER, T., FULLGRABE, A., DAVIS, M. P., ENRIGHT, A. J. & BUSCH-NENTWICH, E. M. 2017. A high-resolution mRNA expression time course of embryonic development in zebrafish. *Elife*, 6.
- WILLIS, A. R., MOORE, C., MAZON-MOYA, M., KROKOWSKI, S., LAMBERT, C., TILL, R., MOSTOWY, S. & SOCKETT, R. E. 2016. Injections of Predatory Bacteria Work Alongside Host Immune Cells to Treat Shigella Infection in Zebrafish Larvae. *Curr Biol*, 26, 3343-3351.
- WOOLBRIGHT, B. L., WILLIAMS, C. D., NI, H., KUMER, S. C., SCHMITT, T., KANE, B. & JAECHKE, H. 2017. Microcystin-LR induced liver injury in mice and in primary human hepatocytes is caused by oncotic necrosis. *Toxicol*, 125, 99-109.
- WU, K., SONG, W., ZHANG, Z. & GE, W. 2020. Disruption of dmrt1 rescues the all-male phenotype of the cyp19a1a mutant in zebrafish - a novel insight into the roles of aromatase/estrogens in gonadal differentiation and early folliculogenesis. *Development*, 147.
- YANG, M., MA, X., XUAN, X., DENG, H., CHEN, Q. & YUAN, L. 2020. Liraglutide Attenuates Non-Alcoholic Fatty Liver Disease in Mice by Regulating the Local Renin-Angiotensin System. *Front Pharmacol*, 11, 432.
- YAO, T. T., QIAN, J. D., ZHU, W. Y., WANG, Y. & WANG, G. Q. 2020. A systematic review of lopinavir therapy for SARS coronavirus and MERS coronavirus-A possible reference for coronavirus disease-19 treatment option. *J Med Virol*, 92, 556-563.
- YILDIZ, G., ARSLAN-ERGUL, A., BAGISLAR, S., KONU, O., YUZUGULLU, H., GURSOY-YUZUGULLU, O., OZTURK, N., OZEN, C., OZDAG, H., ERDAL, E., KARADEMIR, S., SAGOL, O., MIZRAK, D., BOZKAYA, H., ILK, H. G., ILK, O., BILEN, B., CETIN-ATALAY, R., AKAR, N. & OZTURK, M. 2013. Genome-wide transcriptional reorganization associated with senescence-to-immortality switch during human hepatocellular carcinogenesis. *PLoS One*, 8, e64016.
- YOON, S., NGUYEN, H. C. T., JO, W., KIM, J., CHI, S. M., PARK, J., KIM, S. Y. & NAM, D. 2019. Biclustering analysis of transcriptome big data identifies condition-specific microRNA targets. *Nucleic Acids Res*, 47, e53.
- YU, G., WANG, L. G., HAN, Y. & HE, Q. Y. 2012. clusterProfiler: an R package for comparing biological themes among gene clusters. *OMICS*, 16, 284-7.
- YUAN, J., FAN, D., XUE, Z., QU, J. & SU, J. 2020. Co-Expression of Mitochondrial Genes and ACE2 in Cornea Involved in COVID-19. *Invest Ophthalmol Vis Sci*, 61, 13.
- ZEITUNI, E. M., WILSON, M. H., ZHENG, X., IGLESIAS, P. A., SEPANSKI, M. A., SIDDIQI, M. A., ANDERSON, J. L., ZHENG, Y. & FARBER, S. A. 2016. Endoplasmic Reticulum Lipid Flux Influences Enterocyte

- Nuclear Morphology and Lipid-dependent Transcriptional Responses. *J Biol Chem*, 291, 23804-23816.
- ZHANG, W., XU, Y. Z., LIU, B., WU, R., YANG, Y. Y., XIAO, X. Q. & ZHANG, X. 2014. Pioglitazone upregulates angiotensin converting enzyme 2 expression in insulin-sensitive tissues in rats with high-fat diet-induced nonalcoholic steatohepatitis. *ScientificWorldJournal*, 2014, 603409.
- ZHOU, X., WANG, M., KATSYV, I., IRIE, H. & ZHANG, B. 2018. EMUDRA: Ensemble of Multiple Drug Repositioning Approaches to improve prediction accuracy. *Bioinformatics*, 34, 3151-3159.
- ZIEGLER, C. G. K., ALLON, S. J., NYQUIST, S. K., MBANO, I. M., MIAO, V. N., TZOUANAS, C. N., CAO, Y., YOUSIF, A. S., BALS, J., HAUSER, B. M., FELDMAN, J., MUUS, C., WADSWORTH, M. H., 2ND, KAZER, S. W., HUGHES, T. K., DORAN, B., GATTER, G. J., VUKOVIC, M., TALIAFERRO, F., MEAD, B. E., GUO, Z., WANG, J. P., GRAS, D., PLAISANT, M., ANSARI, M., ANGELIDIS, I., ADLER, H., SUCRE, J. M. S., TAYLOR, C. J., LIN, B., WAGHRAY, A., MITSIALIS, V., DWYER, D. F., BUCHHEIT, K. M., BOYCE, J. A., BARRETT, N. A., LAIDLAW, T. M., CARROLL, S. L., COLONNA, L., TKACHEV, V., PETERSON, C. W., YU, A., ZHENG, H. B., GIDEON, H. P., WINCHELL, C. G., LIN, P. L., BINGLE, C. D., SNAPPER, S. B., KROPSKI, J. A., THEIS, F. J., SCHILLER, H. B., ZARAGOSI, L. E., BARBRY, P., LESLIE, A., KIEM, H. P., FLYNN, J. L., FORTUNE, S. M., BERGER, B., FINBERG, R. W., KEAN, L. S., GARBER, M., SCHMIDT, A. G., LINGWOOD, D., SHALEK, A. K., ORDOVAS-MONTANES, J., LUNG-NETWORK@HUMANCELLATLAS.ORG, H. C. A. L. B. N. E. A. & NETWORK, H. C. A. L. B. 2020. SARS-CoV-2 Receptor ACE2 Is an Interferon-Stimulated Gene in Human Airway Epithelial Cells and Is Detected in Specific Cell Subsets across Tissues. *Cell*, 181, 1016-1035 e19.

Figure Legends

Figure 1. Carboxypeptidase (A) and Intraflagellar (B) networks colored according to $r_{ace2-logFC}$ values across GPL1319 datasets ($n_{dataset} = 107$; $n_{comparison} = 344$).

Figure 2. *ace2* expression patterns during developmental stages from GSE24616, where min, d, and h refer to minutes, days, and hours, respectively (A) and GSE38575, where EtOH, VD, and dpf refer to ethanol, vitamin D, and days post-fertilization, respectively (B); and correlation between significantly modulated STRING local network enrichment scores obtained using r_{ace2} values using developmental datasets (C).

Figure 3. Top 15 positively and negatively enriched networks using r_{ace2} values obtained STRING local network analysis of GSE24616 and GSE38575 focusing on zebrafish early development.

Figure 4. Selected common positively enriched networks using r_{ace2} values during zebrafish development. Each node represents an enriched network, and edge weights represent the number of shared genes. They are colored according to the enrichment score.

774 **Figure 5.** Selected common negatively enriched networks with r_{ace2} values during zebrafish
 775 development. Each node represents an enriched network, and edge weights represent the
 776 number of shared genes. They are colored according to the enrichment score.

777 **Figure 6.** Sexual dimorphic expression pattern in adulthood based on GSE24616 (Simple effect
 778 analysis following Two-Way ANOVA results were represented. *, p-value < 0.05) (A). Tissue-
 779 specific expression of *ace2* (log2(RPKM+1)) in the intestines (731.1) and liver (432.8) and the
 780 relatively low expression in the gills (1.57), brain (1.08), kidney (0.83), heart (0.62), and muscle
 781 (0.49) in the six-month-old zebrafish cohort (B).

782 **Figure 7.** *ace2* expression from GSE12189 where GFP+ and GFP- refer to cells in and out of
 783 digestive tract, respectively (A, Limma results were represented. *, adj. p-value < 0.05; **, adj. p-
 784 value < 0.01; ***, adj. p-value < 0.001; ****, adj. p-value < 0.0001). Correlation between the
 785 significantly modulated STRING local networks obtained using r_{ace2} values in GSE12189 and
 786 GSE35889 intestine-specific datasets (B). Bar plots of significant STRING local network
 787 enrichment scores for GSE12189 and GSE35889 (C).

788 **Figure 8.** *ace2* expression during immune response in *myd88* knock-out (Myd88KO) model with
 789 bacterial colonization (Conventionalization (CONVD), *Exiguobacterium* (Exi) and
 790 *Chryseobacterium* (Chrys)) (Tukey HSD corrected One-way ANOVA results were represented. *,
 791 adj. p-value < 0.05; ****, adj. p-value < 0.0001) (A); in viral infection (SVCV: Spring Viraemia Carp
 792 Virus) after pre-treatment with the viral medium (MEM), β -glucans, lipopolysaccharide (LPS), or
 793 Polyinosinic:polycytidylic acid (PolyI:C) (Simple effect analysis following Two-Way ANOVA results
 794 were represented. *, p-value < 0.05) (B).

795 **Figure 9.** Bar plots of significant STRING local network enrichment scores for GSE74244 and
 796 GSE100583 liver datasets (A); *ace2* expression in zebrafish livers during fasting and refed
 797 conditions (B); in microcystin-LR (MCLR) exposure (*, p-value < 0.05; **, p-value < 0.01) (C).

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Draft

205x100mm (300 x 300 DPI)

254x254mm (300 x 300 DPI)

Figure 3. Top 15 positively and negatively enriched networks using race2 values obtained STRING local network analysis of GSE24616 and GSE38575 focusing on zebrafish early development

423x254mm (300 x 300 DPI)

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Figure 6. Sexual dimorphic expression pattern in adulthood based on GSE24616 (Simple effect analysis following Two-Way ANOVA results were represented. *, p-value < 0.05) (A). Tissue-specific expression of ace2 ($\log_2(\text{RPKM}+1)$) in the intestines (731.1) and liver (432.8) and the relatively low expression in the gills (1.57), brain (1.08), kidney (0.83), heart (0.62), and muscle (0.49) in the six-month-old zebrafish cohort (B).

279x254mm (300 x 300 DPI)

Figure 7. ace2 expression from GSE12189 where GFP+ and GFP- refer to cells in and out of digestive tract, respectively (A, Limma results were represented. *, adj. p-value < 0.05; **, adj. p-value < 0.01; ***, adj. p-value < 0.001; ****, adj. p-value < 0.0001). Correlation between the significantly modulated STRING local networks obtained using race2 values in GSE12189 and GSE35889 intestine-specific datasets (B). Bar plots of significant STRING local network enrichment scores for GSE12189 and GSE35889 (C).

202x221mm (300 x 300 DPI)

Figure 8. *ace2* expression during immune response in *myd88* knock-out (*Myd88KO*) model with bacterial colonization (Conventionalization (CONVD), *Exiguobacterium* (Exi) and *Chryseobacterium* (Chrys)) (Tukey HSD corrected One-way ANOVA results were represented. *, adj. p-value < 0.05; ****, adj. p-value < 0.0001) (A); in viral infection (SVCV: Spring Viraemia Carp Virus) after pre-treatment with the viral medium (MEM), β -glucans, lipopolysaccharide (LPS), or Polyinosinic:polycytidylic acid (PolyI:C) (Simple effect analysis following Two-Way ANOVA results were represented. *, p-value < 0.05) (B).

279x127mm (300 x 300 DPI)

Figure 9. Bar plots of significant STRING local network enrichment scores for GSE74244 and GSE100583 liver datasets (A); ace2 expression in zebrafish livers during fasting and refeed conditions (B); in microcystin-LR (MCLR) exposure (*, p-value < 0.05; **, p-value < 0.01) (C).

338x355mm (300 x 300 DPI)