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4 CHARACTERIZATION OF POTENTIAL TRPP2 REGULATING PROTEINS IN EARLY
5 *XENOPUS* EMBRYOS
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ABSTRACT

TRPP2 is a non-specific Ca^{++} -dependent cation channel with versatile functions including control of extracellular calcium entry at the plasma membrane, release of intracellular calcium from internal stores of endoplasmic reticulum, and calcium-dependent mechanosensation in the primary cilium. In early *Xenopus* embryos, TRPP2 is expressed in cilia of the gastrocoel roof plate (GRP) involved in the establishment of left-right asymmetry, and in non-ciliated kidney field (KF) cells, where it plays a central role in early specification of nephron tubule cells dependent on intracellular calcium signaling. Identification of proteins binding to TRPP2 in embryo cells can provide interesting clues about the mechanisms involved in its regulation during these various processes. Using mass spectrometry (MS), we have therefore characterized proteins from late gastrula/early neurula stage embryos co-immunoprecipitating with TRPP2. Binding of three of these proteins, golgin A2, protein kinase-D1 and disheveled-2 has been confirmed by immunoblotting analysis of TRPP2-co-precipitated proteins. Expression analysis of the genes respectively encoding these proteins, *golga2*, *prkd1* and *dvl2* indicates that they are likely to play a role in these two regions. *Golga2* and *prkd1* are expressed at later stage in the developing pronephric tubule where golgin A2 and protein kinase-D1 might also interact with TRPP2. Co-localization experiments using exogenously expressed fluorescent versions of TRPP2 and *dvl2* in GRP and KF reveal that these two proteins are generally not co-expressed, and only co-localized in discrete region of cells. This was observed in KF cells, but does not appear to occur in the apical ciliated region of GRP cells.

INTRODUCTION

Transient receptor potential cation channel (TRPP)-2 also known as polycystin-2 is a non-specific Ca^{++} -dependent cation channel. TRPP2 is encoded by the gene *pkd2*. It can function as a channel regulating entry of extracellular Ca^{++} at the plasma membrane, or release of internal Ca^{++} stores from the endoplasmic reticulum (ER) [Kottgen, 2007]. Yet, the most studied function of TRPP2 concerns its role in the primary cilia. Several *PKD2* mutations affecting the ciliary function of TRPP2 are responsible for autosomal dominant kidney disease (ADPKD) in humans [Ferreira et al., 2015]. TRPP2 can associate with polycystin-1 to form a complex that is thought to act as a mechanosensor upon cilium bending resulting from fluid flow in renal tubules [Paul and Vanden Heuvel, 2014]. Interfering with *pkd2* function causes formation of cysts in developing mouse kidney [Wu et al., 2000] and zebrafish pronephros [Sun et al., 2004], and dilated pronephric tubules in *Xenopus* [Tran et al., 2010]. In addition to its function in kidney tubule cells, TRPP2 plays an important role in the establishment of left-right asymmetry in vertebrate embryos, as shown by the laterality defects observed upon *pkd2* loss of function in mouse [Pennekamp et al., 2002] and zebrafish [Bisgrove et al., 2005]. Laterality defects in mouse *pkd2* mutants, have been related to the asymmetric generation of intracellular calcium ($[\text{Ca}^{++}]_i$) signaling on the left side of the node [McGrath et al., 2003].

In neurula stage *Xenopus* embryos, TRPP2 is expressed in cilia of the gastrocoel roof plate (GRP) [Schweickert et al., 2007], which is involved in the establishment of left-right asymmetry, and in non-ciliated kidney field (KF) cells, where it plays a central role in $[\text{Ca}^{++}]_i$ signaling. KF, also called pronephric field is a region of dorso-lateral mesoderm containing specified kidney precursors that will take part to the development of the pronephros at later stages [Carroll and Vize, 1999]. Several genes including *pax8*, *lhx1*, *osr1* and *osr2* are expressed in KF, and play a major role in the specification of kidney precursors, as evidenced by their respective loss of function, all of which cause severe defects of pronephric development [Buisson et al., 2015; Chan et al., 2000; Tena et al., 2007]. Ca^{++} transients are accumulated in a discrete region of the KF that eventually forms the pronephric tubule. Interfering with $[\text{Ca}^{++}]_i$ signaling with inducible Ca^{++} chelators results in a down-regulation of *pax8* in the KF, and defective tubulogenesis at later stages [Futel et al., 2015; Leclerc et al., 2008]. *Pkd2* knockdown results in a severe inhibition of Ca^{++} transients in the KF, and a dramatic down regulation of *pax8*, indicating that TRPP2 is playing a central role in this process [Futel et al., 2015]. KF specification is dependent on retinoic acid (RA) signals [Cartry et al., 2006], and might also involve Wnt11b [Tetelin and Jones, 2010]. Although Wnt11b could potentially cause Ca^{++} release in the cytosol through the activation of the Wnt-calcium pathway, and Wnts have been recently shown

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3 to activate polycystin-1/TRPP2 complex channel activity [Kim et al., 2016], the potential role of
4 Wnt11b in this process has not been studied. In contrast, we have shown that both $[Ca^{++}]_i$ signaling
5 and TRPP2 expression at the plasma membrane in KF cells are severely impaired upon disruption of
6 RA signaling, pointing to RA-dependent control of TRPP2 trafficking to the plasma membrane as an
7 important regulatory mechanisms of $[Ca^{++}]_i$ signaling in the KF [Futel et al., 2015].
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11 Because of the versatile functions played by TRPP2, several mechanisms involved in the modulation
12 of TRPP2 function have been described, including association with other TRP proteins to form
13 heteromeric channels [Giamarchi et al., 2010; Kottgen et al., 2008], TRPP2 phosphorylation [Cai et al.,
14 2004; Streets et al., 2010] and/or regulation of intracellular trafficking [Hoffmeister et al., 2011;
15 Kottgen et al., 2005], and potential interaction with Wnt-calcium signaling pathway [Kim et al., 2016].
16 Although TRPP2 has been previously shown to be involved in the establishment of left-right
17 asymmetry, as well as in the formation of KF, how TRPP2 function(s) can be controlled in the early
18 embryo is largely ignored. Identification of proteins binding to TRPP2 can provide important
19 information about potential TRPP2 modulatory mechanisms taking place in early *Xenopus* embryos,
20 during processes involving a ciliary function of TRPP2, such as those occurring in the GRP, or where
21 TRPP2 is thought to act at the plasma membrane, as in KF cells. In addition, *Xenopus* developing
22 pronephros is widely used as a model for vertebrate nephrogenesis, and has allowed to obtain
23 important data concerning mir17/bicaudal C control of *pkd2* expression [Tran et al., 2010], or
24 polycystin-1-dependent TRPP2 phosphorylation processes [Streets et al., 2013], that have proved to
25 be important in the study of mammalian nephrogenesis and ADPKD [Lienkamp, 2016]. We have
26 therefore undertaken a characterization of proteins interacting with TRPP2 in late gastrula/early
27 neurula stage embryos, using mass spectrometry (MS) analysis of proteins co-immunoprecipitated
28 with TRPP2.
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41 MATERIALS AND METHODS

42 EMBRYOS

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44 *Xenopus* embryos have been obtained as previously described by human chorionic gonadotropin
45 stimulation of females and in vitro fertilization [Futel et al., 2015], and staged according to
46 [Nieuwkoop and Faber, 1975]. All animal experiments were carried out according to approved
47 guidelines validated by the Ethics Committee on Animal Experiments "Charles Darwin"(C2EA-05) with
48 the "Autorisation de projet" number 02164.02.
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54 IMMUNOPRECIPITATION

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3 Embryo protein extraction has been performed as previously reported [Le Bouffant et al., 2012], in
4 extraction buffer (100mM NaCl, 5mM EDTA, 0.5% NP40,10mM Tris pH 7.5) containing phosphatases
5 and proteases inhibitor cocktails (cOmplete ULTRA, PhosSTOP, Roche). For immunoprecipitation,
6 embryo lysates were incubated during 2h at 4°C with anti-rabbit Ig coated Dynabeads (Dynabeads M-
7 280,Thermo Fisher) previously coupled with the antibody of interest according to manufacturer's
8 instructions. Prior to incubation with lysates, antibodies complexes were crosslinked using 20mM
9 dimethyl pimelimidate according to manufacturer instructions. Crosslinking was not performed when
10 immunoprecipitates were used for MS analysis because they were not eluted, and were directly
11 digested on beads (see below). Beads were washed once with extraction buffer followed by 3 washes
12 with 500mM NaCl, 50mM Tris pH7.5, 1% NP40, and a final wash in phosphate-buffered saline (PBS)
13 before elution of immunoprecipitated proteins in Laemmli sample buffer, or processing for MS
14 analysis (see below).
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22 Western immunoblotting analyses were carried out as previously described [Le Bouffant et al., 2012].
23 Primary antibodies included rabbit polyclonal anti-human TRPP2 (Novus Biochemicals #NB100-
24 92215; 1/1000), anti-human Golgin A2 (Abcam #ab103416; 1/2000), anti-human PrKD1 (Abcam
25 #ab131460, 1/2000), and anti-human dvl2 (Proteintech #12037-1-AP; 1/1000) antibodies. Mouse
26 monoclonal anti- α -tubulin (Sigma T9026; 1:10,000) was used as loading control for input lysates.
27 Secondary peroxidase-conjugated antibodies (goat-anti-rabbit or goat-anti-mouse IgG antisera;
28 Jackson Laboratories) were used at 1:10,000.
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34 MS ANALYSIS

35 Immunoprecipitated proteins were digested directly on beads overnight at 37°C by sequencing grade
36 trypsin (12,5 μ g/ml ; Promega Madison, Wi, USA) in 20 μ l of NH_4HCO_3 25 mmol/L. Peptides were
37 analyzed by an Easy nano-LC Proxeon system (Thermo Fisher Scientific, San Jose, CA) coupled to a
38 LTQ Velos Orbitrap mass spectrometer (Thermo Fisher Scientific, San Jose, CA). Chromatographic
39 separation of peptides was performed with the following parameters: C18 Easy column Proxeon (2
40 cm long, 5 μ m particle size) for peptide pre-concentration, column Easy Column Proxeon C18 (10 cm,
41 75 μ m i.d., 120 A), 300nl/min flow, gradient rising from 95 % solvent A (water - 0,1% formic acid) to
42 25% B (100 % acetonitrile, 0,1% formic acid) in 20 minutes, then to 45% B in 40 min and finally to
43 80% B in 10 min. Peptides were analyzed in the orbitrap in full ion scan mode with a mass resolution
44 of 30000 (at m/z 400) over the range m/z 400-1800. A collision-induced dissociation (CID) activation
45 was used for peptide fragmentation with a collisional energy of 40%, an activation Q of 0,250 for 10
46 ms. MS/MS data were acquired in the LTQ in a data dependent mode in which 20 most intense
47 precursor ions were isolated. Maximum allowed ion accumulation times were set to 100 ms for MS
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3 and 50 ms for MS/MS scans. Data were analyzed using Proteome Discoverer 1.3 software (Thermo
4 Fisher scientific, San Jose, CA) coupled to an in house Mascot search server (Matrix Science, Boston,
5 MA; version 2.3.2). The mass tolerance of fragment ions was set to 10 ppm for precursor ions and 0.6
6 Da for fragments. Oxidation (M) and phosphorylations (STY) were chosen as variable modifications.
7 The maximum number of missed cleavages was limited to 2 for trypsin digestion. Raw files were
8 searched against NCBI nr database with the *Xenopus laevis* taxonomy. A Decoy database approach
9 was used for the False Discovery Rate (FDR) estimation. The target FDR was set to 1%. The Mascot
10 scores were calculated as follows: $-10\log(P)$ where P is the absolute probability that the event is not
11 random.
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18 ANALYSES OF GENE EXPRESSION

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20 Whole mounted in situ hybridization has been carried out as described [Colas et al., 2008]. Embryos
21 were fixed in MEMFA (3.7% formaline in 2mM EGTA, 1mM MgSO₄, 0.1M MOPS pH 7.4), hybridized
22 with digoxigenin-labelled riboprobes, and bound probes further detected with phosphatase-
23 conjugated sheep anti-digoxigenin antibodies according to the method of [Harland, 1991].
24 Chromogenic reaction with alkaline phosphatase was done with BM purple reagent (Roche). DNA
25 sequences used to transcribe antisense riboprobes for *golga2* and *prkd1* were cloned by PCR
26 amplification using the following primers: *golga2* 5'-CACTGAGCCCACACCACCT-3' forward, 5'-
27 TGACTGGACTTCCCGTCTCC-3' reverse, and *prkd1* 5'-CCATCGTGGACCAAAAGTTTC-3' forward, 5'-
28 CCAAAGTGGCTGATCCAAG-3' reverse. They were further subcloned into pCRII vectors (Invitrogen)
29 for antisense probe synthesis.
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37 Real time quantitative PCR (RT-qPCR) has been performed as previously reported [Le Bouffant et al.,
38 2012]. The Comparative Ct method was used to determine the relative quantities of mRNA, using
39 *ornithine decarboxylase (odc)* mRNA as the endogenous reporter. Each RNA sample was analyzed in
40 duplicate. Each data point represents the mean \pm SEM of at least three independent experiments.
41 Data were analyzed using R Commander (R software) by paired Student's t-test. Primers used for RT-
42 qPCR are given in supplemental table 1.
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47 MICROINJECTION OF mRNA AND ANALYSIS OF FLUORESCENT PROTEINS LOCALIZATION

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49 Synthesis of capped mRNA and microinjection has been carried out as described [Colas et al., 2008].
50 A plasmid encoding *Xenopus* dvl2-mcherry (pCS2+-dvl2-mcherry) was obtained by replacing the GFP
51 sequence of pCS2+-dvl2-GFP [Gentzel et al., 2015] by that of mcherry. Plasmids encoding human
52 GFP-TRPP2 (pCS2+-GFP-hPKD2) [Hoffmeister et al., 2011] and arl13b-mcherry (pCS2+-arl13b-
53 mcherry) [Borovina et al., 2010] have been previously described [Futel et al., 2015]. All plasmids were
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3 linearized with asp718 and transcribed with SP6 RNA polymerase. GRP and KF explant dissection
4 procedures have been previously described [Futel et al., 2015; Schweickert et al., 2007]. Dissected
5 explants were immediately transferred into ice-cold 4% paraformaldehyde and fixed overnight at 4°C.
6 After two washes in PBS, explants were mounted as whole-mounts into Mowiol 4-88. Observations
7 were performed on a Zeiss Z1 inverted microscope equipped with ApoTome optical sectioning device.
8 Monochrome images were analyzed using imageJ software. Artificially colorized images and merge
9 images were obtained using Adobe Photoshop CS4.
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15 RESULTS

16 IDENTIFICATION OF GOLGIN A2, PROTEINE KINASE-D1 AND DISHEVELED-2 AS PROTEINS 17 INTERACTING WITH TRPP2 IN EARLY NEURULA EMBRYOS 18 19

20 In an attempt to identify proteins potentially modulating TRPP2 function in *Xenopus* neurulae, we
21 have analyzed proteins binding to immunoprecipitated TRPP2 using MS. The analysis was performed
22 at late gastrula-early neurula stage (NFst.12.5-13) from whole embryo extracts. Proteins
23 immunoprecipitated by the anti-TRPP2 antibody from NFst.12.5-13 embryo extracts have been
24 subjected to MS analysis (Fig1A). Comparison of anti-TRPP2 immunoprecipitates (IPs) and control IPs
25 without primary antibody allowed the identification of 62 proteins specifically detected in TRPP2 IPs
26 and absent from control IPs (table S2). We focused on proteins potentially involved in trafficking,
27 interaction with Wnt-calcium pathway or cilia function (table 1).
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34 Highest Mascot scores were observed for golgin A2 (also known as GM130), protein kinase-D1
35 (PrKD1), also known as PKC- μ) and disheveled-2 (dvl2) (table 1). These three proteins can potentially
36 play a modulatory role of TRPP2 function in *Xenopus* embryo cells. Golgin A2 is a component of the
37 cis-Golgi matrix [Nakamura et al., 1995] involved in TRPP2 trafficking [Hidaka et al., 2004;
38 Hoffmeister et al., 2011]. PrKD1-dependent phosphorylation of TRPP2 Ser801 has been shown to
39 play an important role in the modulation of Ca²⁺ release from ER stores in a non-ciliated MDCK cell
40 line [Streets et al., 2010]. Dvl2 has been recently shown to physically interact with polycystin-1 in
41 mouse embryo fibroblasts, where it can form a complex with TRPP2 through TRPP2 interaction with
42 polycystin-1 [Kim et al., 2016].
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49 Interactions between TRPP2 and golgin A2, PrKD1 or dvl2 were confirmed by co-IP experiments
50 carried out with early neurula stage protein extracts. Western immunoblotting analysis of proteins
51 associated with TRPP2 after TRPP2 immunoprecipitation showed that golgin A2, PrKD1 and dvl2 were
52 all detected in Anti-TRPP2 IPs (Fig.1B). Interactions between PrKD1 and TRPP2, as well as between
53 GolginA2 and TRPP2, were respectively confirmed by anti-PrKD1 and anti-golgin A2 IPs (Fig.1C), but
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3 we were unable to detect TRPP2 in anti-dvl2 IPs (data not shown). It is possible that only a small
4 proportion of total dvl2 is bound to TRPP2, making the amount of TRPP2 co-immunoprecipitated
5 with dvl2 difficult to detect by immunoblotting. Although TRPP2 could be detected both in IPs of
6 anti-Golgin A2 and anti-PrKD1, neither PrKD1 was detected in anti-golgin A2 IPs, nor Golgin A2 in
7 anti-PrKD1 IPs (Fig.1C). This indicates that these three proteins are not forming a single complex.
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10 11 *Golga2*, *prkd1* AND *dvl2* GENES ARE EXPRESSED IN KF AND GRP REGIONS OF THE NEURULA 12 EMBRYOS 13

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15 In an attempt to evaluate whether TRPP2-interacting proteins detected in whole neurula embryos
16 can potentially play a role in GRP or KF, we have analyzed whether their respective encoding genes
17 *golga2*, *prkd1* and *dvl2* are expressed in these tissues. GRP or KF explants were dissected from early
18 neurula stage embryos (Fig.2A) and their transcript content was subjected to RT-qPCR analysis for
19 *golga2*, *prkd1* and *dvl2* expression, [using *odc* expression for normalization](#). Enrichment of GRP or KF
20 tissue was assessed in parallel by analyzing expression of *brachyury (t)* in GRP samples or *pax8* in KF
21 samples (Fig.2B). *Brachyury (t)* is highly expressed in tailbud and caudal notochord at neurula stage
22 [Gont et al., 1993]. These structures are close to the caudal neural plate where sits the GRP
23 [Schweickert et al., 2007]. *Pax8* is highly expressed in KF cells [Carroll and Vize, 1999]. Results are
24 shown in Fig.2B. As expected, *brachyury (t)* is highly enriched in GRP samples, while it is only
25 detected at very low levels in KF samples. Conversely, *pax8* is strongly expressed in KF samples but is
26 undetectable in GRP samples. Analysis of *golga2*, *prkd1* and *dvl2* expression does not reveal any
27 preferential expression in GRP or KF. All three genes expression are readily detected in both GRP and
28 KF, indicating that their respective encoded proteins are likely to play roles both in ciliated GRP cells,
29 and in non-ciliated cells of the KF.
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39 *Prkd1* AND *golga2* ARE EXPRESSED THROUGHOUT DEVELOPMENT WITH HIGH LEVELS OF 40 EXPRESSION IN THE DEVELOPING KIDNEY. 41

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43 We further investigated how *golga2*, and *prkd1* are expressed during *Xenopus* development. We paid
44 particular attention to the developing pronephros, since it has already been used to study different
45 aspects of *pkd2* function during vertebrate nephrogenesis that can be impaired in ADPKD [Lienkamp,
46 2016; Streets et al., 2013; Tran et al., 2010]. *Dvl2* expression during *Xenopus* development has been
47 studied previously [Gray et al., 2009] and is part of genes of the wnt signaling pathway expressed in
48 the developing pronephros [Zhang et al., 2011]. Our own observations totally confirm these
49 expression patterns, especially the pronephric expression of *dvl2* (data not shown).
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55 Temporal expression was investigated by RT-qPCR. Both genes are expressed at all the stages
56 investigated, ranging from 16-cell stage (NFst.5), when gene expression only relies on maternal
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3 transcripts, to later cleavage stages, gastrulation, neurulation, and tailbud stages until pronephros
4 starts to be functional (NFst.37). *Golga2* and *prkd1* appear to have a very strong maternal expression.
5 Expression is high until mid-gastrula stage (NFst.11), and then progressively drops (Fig.3).
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8 Spatial expression was further investigated using whole-mount in situ hybridization. During
9 gastrulation, both *golga2* and *prkd1* appear to be expressed in all tissues. However, there is higher
10 expression in the marginal zone at early gastrula stage (NFst.10), especially on the dorsal side. At late
11 gastrula stage (NFst.12.5), expression of *golga2* is observed in the entire embryo, but is clearly
12 stronger in axial mesoderm. Likewise, *prkd1* transcripts are detected throughout the late gastrula
13 embryo with a slightly higher expression on the dorsal side of the closing blastopore (Fig.4A). At
14 tailbud stages, *golga2* and *prkd1* are strongly expressed in the developing brain and developing eyes.
15 They are detected in migrating cranial neural crest at early tailbud stage (NFst.24), and later in the
16 developing branchial arches. *Golga2* is expressed in mesoderm in the developing notochord. It is
17 clearly expressed in the pronephric anlage at early tailbud stage. Its expression is maintained at mid
18 and late tailbud stages (NFst.29/30 and 33/34) in the proximal region of the pronephric tubule. *Prkd1*
19 transcripts are also detected in the developing notochord and pronephros. However, *prkd1* is
20 expressed in intermediate and distal segments of the developing pronephric tubule (Fig.4B).
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30 TRPP2 AND DVL2 CO-LOCALIZATION IS RESTRICTED TO DISCRETE REGIONS OF CELL BORDERS

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32 In a further attempt to localize where TRPP2 and dvl2 can interact in potential wnt-dependent
33 processes, we have expressed fluorescent versions of these proteins in GRP and KF regions of
34 neurula stage embryos, and observed their distribution in whole-mounted explants. These
35 fluorescent versions of TRPP2 and dvl2 have been previously used to visualize TRPP2 and dvl2
36 expression in living cells [Hoffmeister et al., 2011; Gentzel et al., 2015]. In GRP explants, we focused
37 on the ciliated apical region of GRP cells. In that case, localization of dvl2-GFP or TRPP2-GFP in the
38 cilium was analyzed in cilia visualized using expression of arl13b-mcherry, as previously described
39 [Futel et al., 2015]. Arl13b is a member of the small GTPases subfamily Arf/Arl localizing to primary
40 cilium axoneme [Borovina et al., 2010].
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47 Localization of dvl2-GFP in GRP cells was mostly restricted to the basis of the cilium revealed by the
48 expression of arl13b-mcherry, and at the level of cell borders. In rare cases, we also observed dvl2-
49 GFP fluorescence in the cilium (Fig.5A-C). In contrast, GFP-TRPP2 co-localized with arl13b-mcherry in
50 many cilia (Fig.5D-F). Expression of dvl2-mcherry with GFP-TRPP2 did not reveal dvl2 and TRPP2 co-
51 localization in GRP cells cilia (Fig.5G-I). Dvl2-mcherry was observed at the basis of cilia, as also
52 observed with dvl2-GFP, but we failed to detect it in cilia expressing GFP-TRPP2. Dvl2-mcherry was
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3 also clearly observed at cell borders, but GFP-TRPP2 was not. Although we cannot rule out that
4 TRPP2 and dvl2 may interact in rare cilia where dvl2-GFP had been observed, our observations
5 suggest that dvl2 interaction with TRPP2 is not significantly taking place in the ciliated apical region
6 of GRP cells.
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10 Co-localization between GFP-TRPP2 and dvl2-mcherry can be observed in KF explants, but only in
11 some discrete regions. Optical sections through the most external part of KF cells rather showed an
12 exclusion of GFP and mcherry fluorescence distribution (Fig.6A-C). As observed in GRP cells, dvl2-
13 mcherry fluorescence was associated with cell borders, while GFP-TRPP2 was not. GFP-TRPP2 was
14 expressed in inner part of the cell as dots, possibly corresponding to traffic vesicle. Dvl2-mcherry was
15 also detected as dots but distinct from GFP-TRPP2, indicating that interaction is not taking place in
16 these structures (Fig.6A-C). Optical sections through deeper part of KF cells did not reveal a general
17 co-localization of dvl2-mcherry and GFP-TRPP2 (fig.6D-F). Still, co-localization could be observed in
18 discrete regions of cell borders, as shown in Fig.6F. These observations show that interaction
19 between TRPP2 and dvl2 is not a general feature. This raises the possibility that it might be only
20 transient, and could be tightly regulated, as for example by signaling events.
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DISCUSSION

In order to provide more information about potential regulators of TRPP2 function(s) during early vertebrate development, it is necessary to establish a better correlation with proteins interacting with TRPP2 that might play an important role in the regulation of these different function(s). These data can be also of potential value for the understanding of the mechanisms underlying cyst formation during ADPKD in human. The early *Xenopus* embryo provides an interesting system where TRPP2 plays critical functions both in ciliated cells of the GRP, and in non-ciliated KF cells. We have therefore characterized proteins interacting with TRPP2 in late gastrula/early neurula stage embryos using MS analysis of proteins co-immunoprecipitated with TRPP2. Interaction has been confirmed by immunoblotting of immunoprecipitated proteins for three of them, *golginA2*, *PrKD1* and *dvl2*. Expression analysis of the genes respectively encoding these proteins, *golga2*, *prkd1* and *dvl2*, indicates that they are likely to play a role in ciliated cells of the GRP involved in the establishment of left-right asymmetry, as well as in non-ciliated cells of the KF, both tissues where TRPP2 is thought to play important functions. At later stages, *golga2* and *prkd1* are strongly expressed in the developing pronephros, as also previously reported for *dvl2* [Zhang et al., 2011], indicating that they might be also involved in later processes involving TRPP2. Further attempt to characterize *dvl2* and TRPP2 interactions, by analyzing *dvl2* and TRPP2 co-localization in KF and GRP cells at neurula stage, reveals that interaction is only taking place in very discrete regions of cell borders, in line with the idea that *dvl2*-TRPP2 interactions might be related with dynamic features of Wnt signaling, rather than with the stable formation of a complex.

TRPP2 interaction with *golgin-A2* is probably related with TRPP2 trafficking to the plasma membrane. Golgin family members are important for the specificity and efficiency of traffic at the level of the Golgi. Golgin A2 (GM130) is known to be involved in the tethering of transport vesicles, facilitating ER to Golgi traffic [Seemann et al., 2000; Wong and Munro, 2014]. Although dispensable during mouse development, global knock-out of GM130 causes a developmental delay, a global ataxia and a postnatal death. Its targeted deletion in the brain results in severe neurological defects. It strongly affects secretory trafficking and positioning of Golgi apparatus in Purkinje cells [Liu et al., 2017]. Knockdown of Golgin A2 (GM130) in human hTERT-RPE1 cells does not affect cilium formation [Asante et al., 2013], but this may result from redundancy between members of the golgin family. It has been indeed shown that Golgin A2 (GM130) and golgin GMAP-210 are acting redundantly in anterograde ER to golgi trafficking in HeLa cells [Roboti et al., 2015]. VPS15 whose mutation is causing ciliopathies, including nephropathies, has been recently shown to play a role in ciliogenesis

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3 or cilia function. It can form a complex with golgin A2 (GM130), and it has been proposed that this
4 complex could be involved in IFT20-dependent trafficking to the cilium [Stoetzel et al., 2016].
5 Whether golgin A2 may play also a role in TRPP2 trafficking to the cilium remains unclear. Interaction
6 of golgin A2 with TRPP2 has been previously described. It involves the interaction of the C-terminal
7 tail of TRPP2 with a protein called PIGEA-14 identified in yeast two-hybrid screen (also called chibby-
8 1), which in turn binds to golgin A2. Co-expression of PIGEA-14 and TRPP2 in HeLa cells, as well as in
9 porcine renal epithelial cells LLC-PK1, causes a redistribution of PIGEA-14 and TRPP2 to the trans-
10 Golgi, suggesting that PIGEA-14 might be involved in the control of TRPP2 intracellular localization
11 [Hidaka et al., 2004]. Although interaction with PIGEA-14 is dispensable for TRPP2 trafficking to cilia
12 in LLC-PK₁ cells [Hoffmeister et al., 2011], TRPP2 interaction with golgin A2 might be of importance in
13 TRPP2 trafficking between ER and Golgi. The *chy1* gene encoding chibby-1 in *Xenopus* is expressed
14 throughout development [Shi et al., 2014], but we have not identified chibby-1 among proteins co-
15 precipitated with TRPP2. Whether TRPP2 interacts with golgin A2 in *Xenopus* embryo cells by the
16 mean of chibby-1, or whether this interaction involves a different mechanism still remains unclear.
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26 Our results show that PrKD1 forms complexes with TRPP2 in *Xenopus* neurula cells. PrKD1 has been
27 previously shown to be critical for TRPP2 function as an ER Ca²⁺-release channel in MDKC I cells. It
28 phosphorylates Ser⁸⁰¹ of human TRPP2. Mutation of Ser⁸⁰¹ to glycine results in a loss of Ca²⁺-induced
29 Ca²⁺ release mechanism [Streets et al., 2010]. This Ser residue is conserved in *Xenopus* TRPP2 (Ser⁷⁷⁹),
30 as well as the consensus recognition sequence of PrKD1 around Ser⁷⁷⁹ [Nishikawa et al., 1997],
31 indicating that PrKD1 may modulate TRPP2 function in *Xenopus* embryo cells. Whether PrKD1
32 interacts with TRPP2 in the ER of *Xenopus* embryo cells needs further investigation. However, we
33 observed that Golgin A2 IPs never contained PrKD1, nor PrKD1 IPs contained Golgin A2, although
34 both contained TRPP2. This suggests that these complexes may correspond to different pools of
35 TRPP2, and that PrKD1 is not expressed in the same compartment than golginA2 in *Xenopus* neurula
36 cells. Our previous results indicate that RA-dependent trafficking of TRPP2 to the plasma membrane
37 is critical for the production of calcium transients in KF cells [Futel et al., 2015], suggesting that these
38 transients may be dependent on the entry of extracellular calcium, rather than on mobilization of
39 internal stores. However, TRPP2 might have a potential additional role as an ER Ca²⁺-release channel
40 in *Xenopus* embryo cells. It is remarkable, for instance, that TRPP2 has been shown to interact with
41 the inositol 1,4,5-trisphosphate receptor (IP3R), and that these complexes are responsible for a
42 TRPP2-dependent potentiation of IP3-induced Ca²⁺ release [Sammels et al., 2010], raising the
43 possibility that TRPP2 may also play a role in embryonic cells by potentiating response to
44 Wnt/calcium signaling pathways regulating IP3 [Saneyoshi et al., 2002].
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3 Finally, our results show that dvl2 is forming a complex with TRPP2 in *Xenopus* embryo cells. Co-
4 localization experiments further indicate that this interaction is not a general feature in GRP and KF
5 cells. In GRP cilia, dvl2 and TRPP2 rather appear to be expressed in contiguous parts, with dvl2
6 localized at the base of the cilium, while TRPP2 is rather expressed in the more distal part. It is
7 unclear, however, whether co-localization in KF cells results from a transient interaction that could
8 be dependent on wnt signaling. A truncated form of dvl2 has been used to interfere with tubule
9 elongation at later stages of *Xenopus* pronephros development where it affects mediolateral cell
10 intercalation in a similar manner to that observed in the developing Wnt9b-mutant mouse kidney,
11 and might be related with congenital kidney cyst formation associated with defective Planar Cell
12 Polarity (PCP) signaling [Lienkamp et al., 2012]. Interaction of dvl2 with polycystin-1 has been
13 demonstrated in mouse embryo fibroblasts, and it is therefore possible that dvl2 can co-
14 immunoprecipitate with TRPP2 in a complex made of TRPP2, polycystin-1 and dvl2 [Kim et al., 2016].
15 Interaction of TRPP2 and polycystin-1 has not been described in *Xenopus* embryos, but the *pkd1* gene
16 is expressed at late gastrula stage [Burtey et al., 2005]. TRPP2/dvl2 co-localization experiments in
17 GRP and KF do not argue for a stable formation of TRPP2 and dvl2 complexes. TRPP2/polycystin-1
18 complexes have been shown to form a ligand-induced calcium channel activated by several wnts.
19 Although dvl2 binding to the complex is dispensable for wnt-induced channel activity, it is possible
20 that it is related to more complex aspects of wnt signaling dependent on local calcium release [Kim et
21 al., 2016].
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38 antibody, and for providing dvl2-GFP expression plasmid. We are also extremely grateful to T. Léger
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11 12 13 LEGENDS

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16 Fig.1 Analysis of protein interacting with TRPP2. A. MS workflow. Late gastrula stage embryos (NF.st12.5) were lysed and subjected to anti-TRPP2 IP, or control IP without primary antibody. In
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18 both cases, immunoprecipitated proteins were digested with trypsin on beads. The resulting
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20 peptides were fractionated by chromatography before orbitrap MS/MS analysis and protein
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22 identification. Proteins co-immunoprecipitated with TRPP2 and absent from control IP were scored
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24 for their abundance and selected for further analysis. B. Immunoblotting detection of golgin A2,
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26 PrKD1 and *dvl2* after TRPP2 IP from NF.st12.5 embryos lysates. C. TRPP2 is co-precipitated in anti-
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28 golgin A2 and anti-PrKD1 IPs. PrKD1 is neither detected in anti-golgin A2 IPs, nor is golgin A2 in anti-
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30 PrKD1 IPs, indicating that they do not interact with TRPP2 in the same complex. Inp: NF.st12.5
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32 embryo input lysate; Ctl: control IP lacking primary antibody.

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34 Fig.2 *Golga2*, *prkd1* and *dvl2* genes are expressed in both kidney field (KF) and gastrocoel roof plate (GRP). A. Schematic representation of the dissections of GRP and KF explants at neurula stage. B. RT-
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36 qPCR analysis of gene expression in GRP and KF explants. Accurate dissections of GRP and KF have
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38 been validated by checking for *t* expression in GRP and *pax8* expression in KF. *Pkd2*, *golga2*, *prkd1*
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40 and *dvl2* are all expressed in GRP and KF, showing that interaction between their encoded proteins
41
42 can potentially take place in both regions of the embryo. Relative quantities of mRNA are determined
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44 using *odc* mRNA as the endogenous reporter.

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46 Fig.3 Temporal expression of *golga2* and *prkd1* in developing *Xenopus* embryos. RT-qPCR analysis
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48 reveals a strong maternal expression at NFst.5 that progressively declines during later cleavage
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50 stages and gastrulation (NFst9-13), and is stabilized at later stages. Relative quantities of mRNA are
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52 determined using *odc* mRNA as the endogenous reporter.

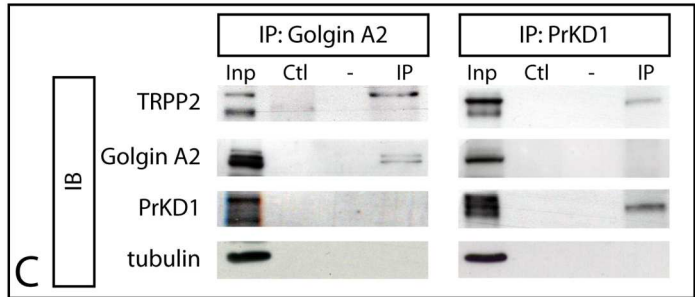
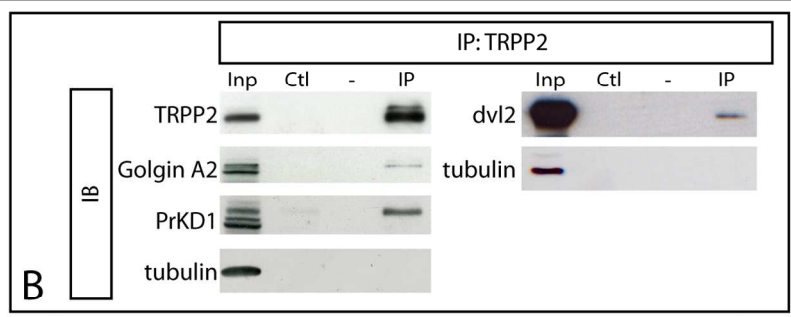
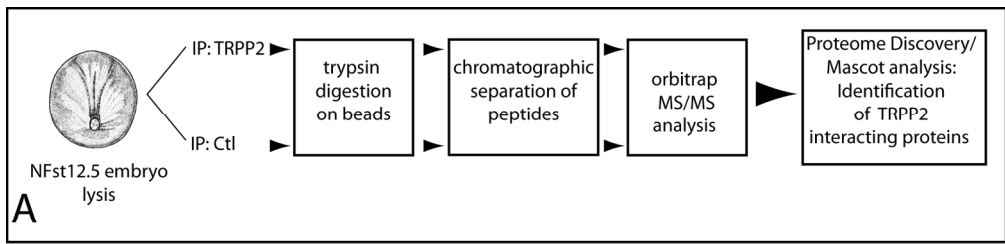
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54 Fig.4 Spatial expression of *golga2* and *prkd1* during *Xenopus* development. A. Gastrula stages. *Golga2*
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56 and *prkd1* are expressed in all tissues. At early gastrula stage, expression is stronger in marginal zone,
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58 especially in the dorsal marginal zone (D). At late gastrula stage, *golga2* has a higher expression in
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60 axial mesoderm (AM). *Prkd1* expression is also stronger on the dorsal side of the closing blastopore

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3 (white arrowhead). B. Tailbud stages. *Golga2* and *prkd1* are strongly expressed in the developing
4 brain (B) and developing eyes (Ey). They are detected in migrating cranial neural crest (CNC) at early
5 tailbud stage (NFst.24), and later in the developing branchial arches (Br). At late tailbud stages *golga2*
6 and *prkd1* expressions are also detected in the developing notochord (Nt). *Golga2*, and to a lesser
7 extent *prkd1* transcripts are detected in the pronephric anlage (black arrowhead) at early tailbud
8 stage. *Golga2* expression is maintained at mid and late tailbud stages in the proximal region of the
9 pronephric tubule (arrows), while *prkd1* transcripts are detected in intermediate and distal segments
10 of the developing pronephric tubule (arrows). Vg : vegetal pole.
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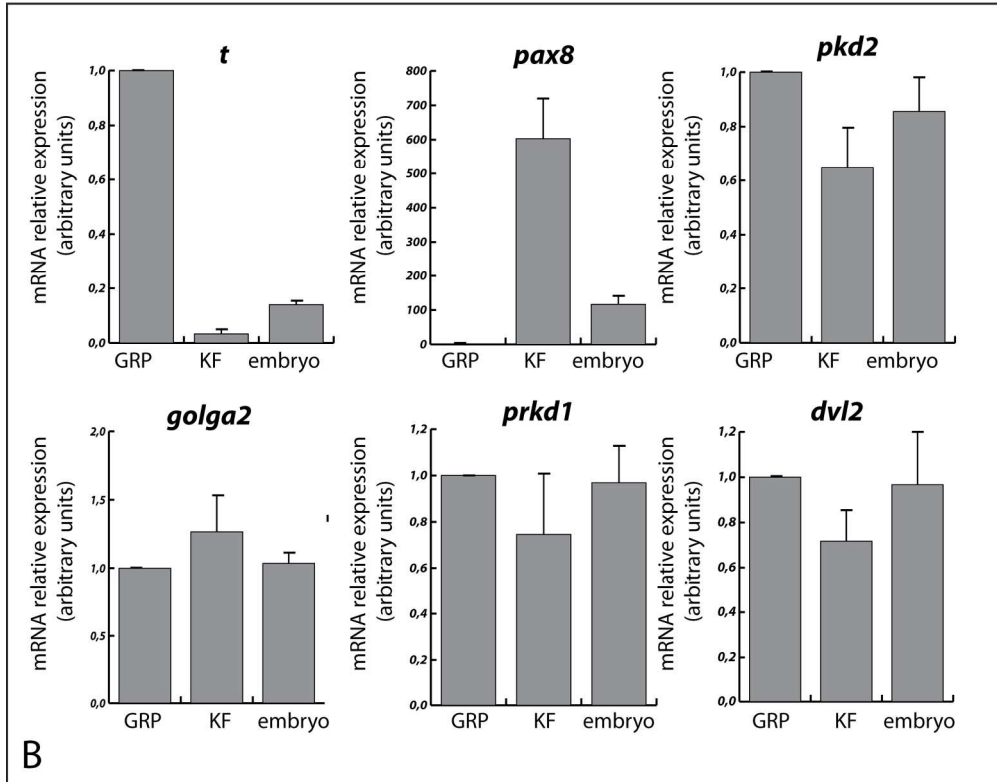
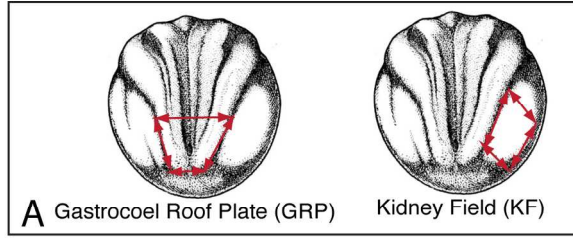
16 Fig.5. Localization of exogenously expressed fluorescent TRPP2 and dvl2 in apical ciliated region of
17 GRP cells. Comparison of GFP- and mcherry-protein expression on optical sections. Dvl2-GFP is
18 generally not expressed in cilia revealed by arl13b-mcherry (A-C). Dvl2 is generally concentrated at
19 the basis of the cilia (arrowheads), but was sometimes co-expressed with arl13b (arrow). In contrast,
20 GFP-TRPP2 is expressed in a vast majority of cilia where it co-localizes with arl13b-mcherry (D-F).
21 Comparison of GFP-TRPP2 and dvl2-mcherry localization (G-I) reveals an exclusive distribution in the
22 cilium with dvl2 concentrated at the basis of the cilium and TRPP2 expressed along the cilium. Both
23 dvl2-GFP and dvl2-mcherry are expressed at cell borders where GFP-TRPP2 is not detected (A,H).
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30 Fig.6. Localization of exogenously expressed fluorescent TRPP2 and dvl2 in KF cells. Comparison of
31 GFP-TRPP2 and mcherry-dvl2 expression on optical sections. (A-C) Optical section tangential to cell
32 surface. Dvl2-mcherry and GFP-TRPP2 exhibit an exclusive distribution. Both appear as dots possibly
33 corresponding to traffic vesicles. (D-F) Deeper section of the same cells as (A-C). Dvl2-mcherry is
34 associated with cell borders. In some places GFP-TRPP2 is co-localized with dvl2-mcherry at cell
35 borders (arrowheads).
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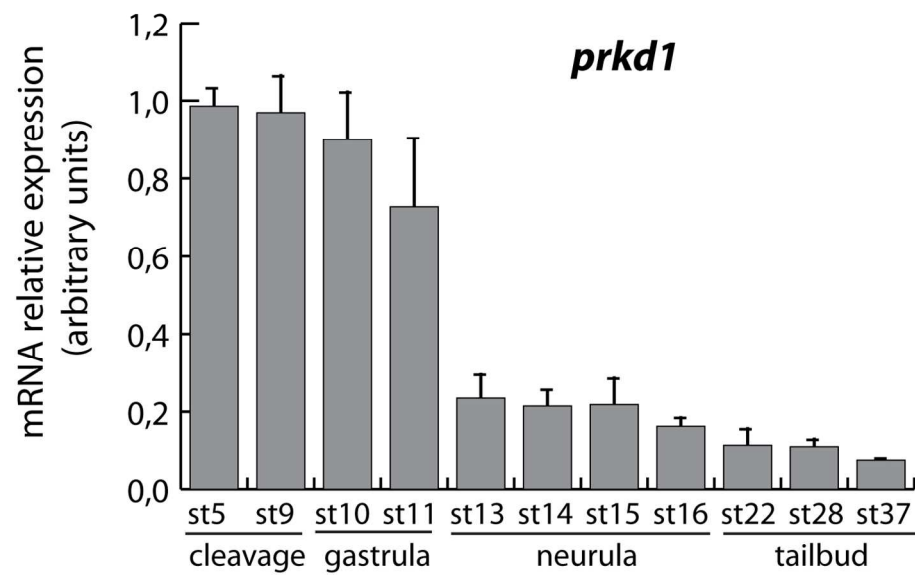
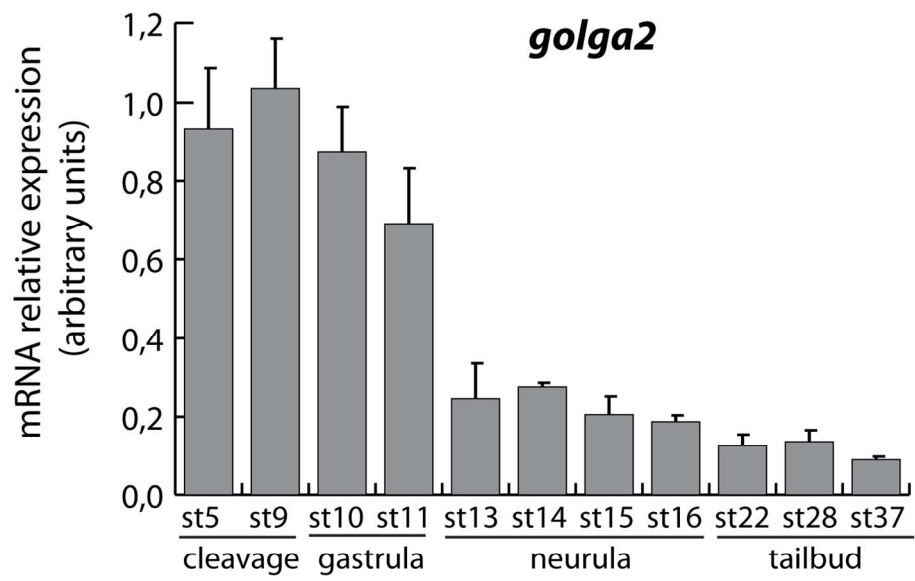


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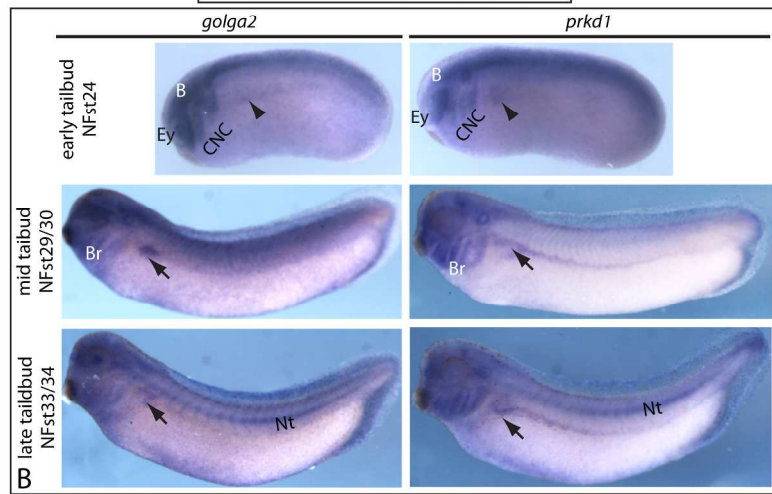
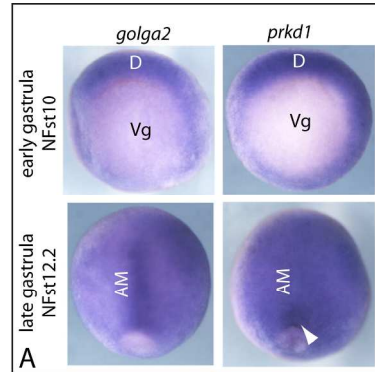


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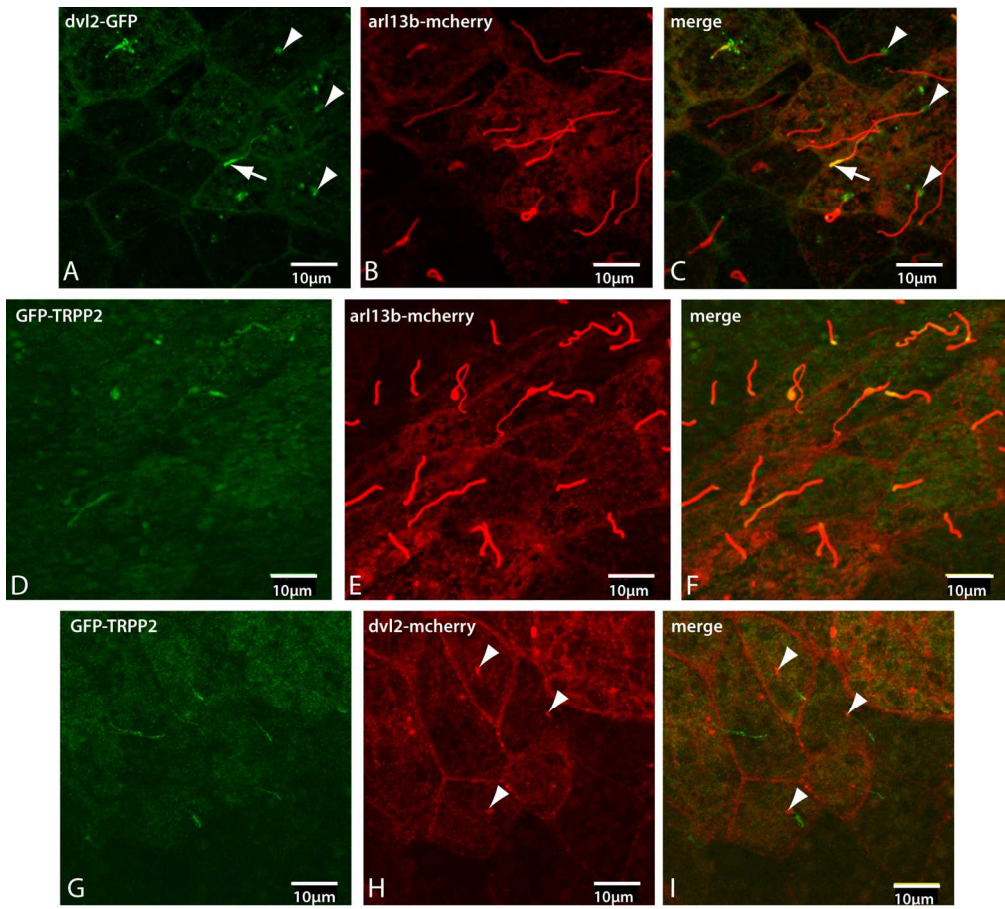
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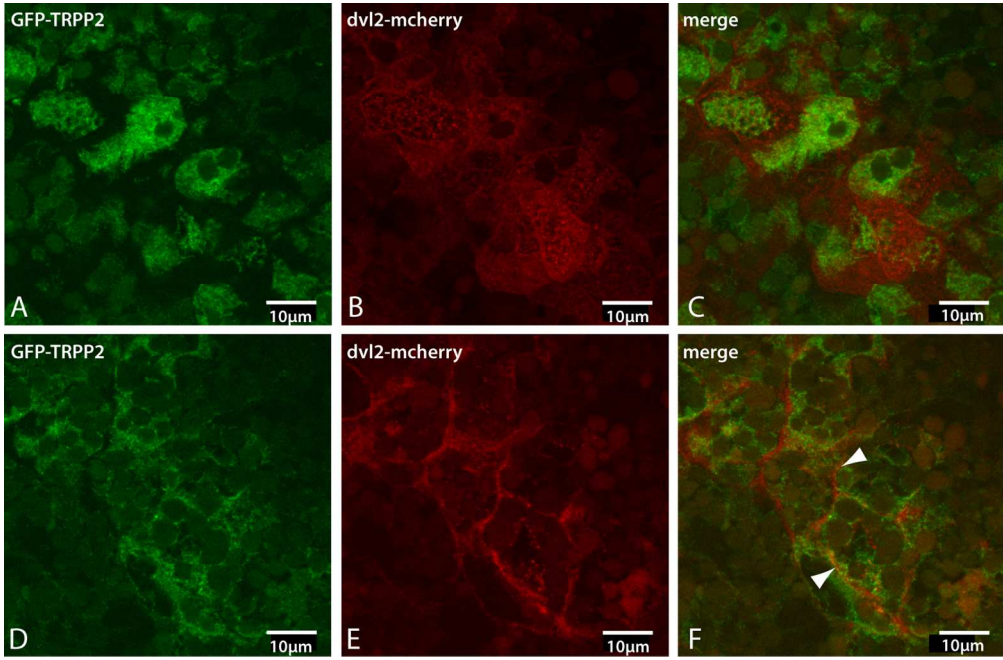
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Accession	Name (encoding gene)	Molecular Function (cellular component)	Score
147906887	golgin A2 (<i>golga2</i>)	transporter activity (golgi)	572.18
148232828	protein kinase D1 (<i>prkd1</i>)	Kinase	193.15
147906757	segment polarity protein dishevelled homolog dvl-2 (<i>dvl2</i>)	protein binding; signal transducer activity (cell surface; cytoplasm; cytoskeleton; membrane; nucleus)	94.15
147899826	14-3-3 protein zeta a (<i>ywhaz</i>)	protein binding	78.00
147902246	microsomal glutathione S- transferase 3 (<i>mgst3</i>)	glutathione-dependent peroxidase activity	71.0
62739367	ninein (gsk3 β -interacting protein) (<i>nin</i>)	metal ion binding; protein binding (Centrosome basal body)	70.13
148224201	adipose differentiation-related protein, perilipin 2 (<i>plin2</i>)	coat intracellular lipid droplets	67.95
156119445	transitional endoplasmic reticulum ATPase (<i>vcp</i>)	catalytic activity; nucleotide binding; RNA binding	36.75
148225959	vesicle (multivesicular body) trafficking 1 (<i>vta1</i>)	synonym LIP5 endosomal EGFR trafficking	31.31
54037970	periplakin (<i>ppl</i>)	desmosomal protein	26.84
147900512	protein disulfide isomerase family A, member 6 precursor (<i>pdia6</i>)	catalyze formation, reduction, and isomerization of disulfide bonds in proteins (endoplasmic reticulum)	26.11
147901815	erlin-2-B precursor (<i>erlin2</i>)	Activated IP3R association/degradation (endoplasmic reticulum)	20.25

Table 1: TRPP2-interacting proteins potentially involved in trafficking, interaction with Wnt-calcium pathway or cilia function.

	Forward	Reverse
<i>actin b</i>	5'-TCTATTGTGGGTGCGCCAAG-3'	5'-TTGTCCCATTCCAACCATGAC-3'
<i>odc</i>	5'-GGGCAAAGGAGCTTAATGTGG-3'	5'-TGCCAACATGGAAACTCACAC-3'
<i>pax8</i>	5'- CAGCAATTTCAATATAGGTCACGG-3'	5'- TCCATTACAAAAGCCCCAC-3'
<i>t</i>	5'- CACCAAGAATGGAAGACGAATG -3'	5'- CCCGACATGCTCACCTTCA -3'
<i>golga21</i>	5'- CAGGTGGAACAGTTGGAGACAA -3'	5'- TGGTGTGGGCTCAGTGTCTG -3'
<i>prkd1</i>	5'- AGCTGATCCATTCCCGCAG -3'	5'- ATGCGAGCAAAGCCGAAAT - 3'
<i>dvl2</i>	5'- TGAGACTGAGCGTTTCCCATG -3'	5'- AGAGGCTGAAGAGTGACACCG -3'

Table S1: Primers used for RT-QPCR

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Accession	Description	Encoding gene	Molecular Function	Cellular Component	Biological Process	Score A3	MW [kDa]
147906887	golgin A2 [Xenopus laevis]	<i>gopa2</i>	transporter activity	cytoplasm; Golgi; membrane	metabolic process; transport	572.18	111.4
148232828	protein kinase D1 [Xenopus laevis]	<i>prkd1</i>	catalytic activity; metal ion binding; nucleotide binding; protein binding		metabolic process; regulation of biological process	193.15	71.4
148236420	60S ribosomal protein L19 [Xenopus laevis]	<i>rpl19</i>	structural molecule activity	cytoplasm; ribosome	metabolic process	103.08	23.5
147906757	segment polarity protein dishevelled homolog DVL-2 [Xenopus laevis]	<i>dvl2</i>	protein binding; signal transducer activity	cell surface; cytoplasm; cytoskeleton; membrane; nucleus	cellular homeostasis; cell organization and biogenesis; development; metabolic process; regulation of biological process	94.15	79.7
148234571	ribosomal protein S19 [Xenopus laevis]	<i>rps19</i>	structural molecule activity	cytoplasm; ribosome	metabolic process	82.24	16.0
147899826	14-3-3 protein zeta A [Xenopus laevis]	<i>yhaz</i>	protein binding			78.00	27.7
189217492	uncharacterized protein LOC100158287 [Xenopus laevis]	<i>egrs</i>	catalytic activity; nucleotide binding	cytoplasm	metabolic process	77.76	73.8
148234611	acyl-CoA dehydrogenase family, member 9 [Xenopus laevis]	<i>acsd9</i>	catalytic activity; nucleotide binding		metabolic process	76.61	68.4
28278397	LOC448495 protein [Xenopus laevis]	<i>ef4a1</i>	catalytic activity; nucleotide binding			74.75	45.8
117558137	LOC100306792 protein [Xenopus laevis]	<i>imnt</i>		cytoplasm; membrane; mitochondrion		74.13	54.5
147902246	microsomal glutathione S-transferase 3 [Xenopus laevis]	<i>mgs3</i>				71.10	16.3
62739367	LOC733210 protein [Xenopus laevis]	<i>nin</i>	metal ion binding; protein binding	cytoplasm	cell organization and biogenesis	70.13	149.3
148224201	uncharacterized protein LOC494638 [Xenopus laevis]	<i>pln2</i>				67.95	44.4
52346118	ribosomal protein L26 [Xenopus (Silurana) tropicalis]	<i>rpl26</i>	structural molecule activity	cytoplasm; ribosome	metabolic process	67.86	17.2
148227748	pyruvate dehydrogenase (lipoamide) alpha 1 [Xenopus laevis]	<i>pdha1</i>	catalytic activity		metabolic process	65.96	44.5
148232054	heat shock protein 90kDa alpha (cytosolic), class B member 1 [Xenopus laevis]	<i>hsp90ab1</i>	nucleotide binding; protein binding		metabolic process; response to stimulus	65.19	82.9
147903835	ribosomal protein L12 [Xenopus laevis]	<i>rpl12</i>	structural molecule activity	cytoplasm; ribosome	metabolic process	62.44	17.8
148227920	chaperonin containing TCP1, subunit 7 [Xenopus laevis]	<i>cc7</i>	nucleotide binding; protein binding	cytoplasm	metabolic process	61.08	59.2
147899563	NADH dehydrogenase (ubiquinone) Fe-S protein 1, 75kDa (NADH-coenzyme Q reductase) [Xenopus laevis]	<i>nduf51</i>	catalytic activity; metal ion binding	membrane	metabolic process	56.29	79.5
147904284	ATP synthase, H+ transporting, mitochondrial F1 complex, delta subunit [Xenopus laevis]	<i>atp5d</i>	catalytic activity; transporter activity	membrane	metabolic process; transport	53.60	16.9
238963	single-stranded DNA binding protein s chain, mtSSBs [Xenopus laevis, oocyte, Mitochondrial Peptide, 125 aa]		DNA binding			53.13	14.1
147902974	ribosomal protein L29 [Xenopus laevis]	<i>rpl29</i>	structural molecule activity	cytoplasm; ribosome	metabolic process	52.86	8.6
148227463	acetyl-CoA acetyltransferase A, mitochondrial precursor [Xenopus laevis]	<i>acat1</i>	catalytic activity; metal ion binding	cytoplasm; mitochondrion	metabolic process	52.53	44.1
148226821	cofilin-1-B [Xenopus laevis]	<i>chl1</i>	protein binding	cytoplasm; nucleus; organelle lumen	cell division; cell organization and biogenesis; regulation of biological process	51.86	19.1
52346106	60S ribosomal protein L36 [Xenopus (Silurana) tropicalis]	<i>rpl36</i>	structural molecule activity	cytoplasm; ribosome	development; metabolic process; regulation of biological process	51.01	12.3
147902489	chaperonin containing TCP1, subunit 5 (epsilon) b [Xenopus laevis]	<i>cc5b</i>	nucleotide binding; protein binding	cytoplasm	metabolic process	50.22	58.6
148235541	ribophorin II precursor [Xenopus laevis]		catalytic activity	cytoplasm; endoplasmic reticulum; membrane	metabolic process	48.66	69.2
189217812	glutamyl-prolyl-tRNA synthetase [Xenopus laevis]	<i>egrs</i>	catalytic activity; nucleotide binding	cytoplasm	metabolic process	47.66	169.3
148227968	ribosomal protein L34 [Xenopus laevis]	<i>rpl34</i>	structural molecule activity	cytoplasm; ribosome	metabolic process	47.43	13.3
148223127	mg-bb02e05 [Xenopus laevis]	<i>gapdh</i>	catalytic activity; nucleotide binding	cytoplasm	metabolic process	47.41	35.9
148223359	mitochondrial ATP synthase beta subunit [Xenopus laevis]	<i>atp5b</i>	catalytic activity; nucleotide binding; transporter activity	membrane	metabolic process; transport	45.38	56.3
62859151	ribosomal protein L27 [Xenopus (Silurana) tropicalis]	<i>rpl27</i>	structural molecule activity	cytoplasm; ribosome	metabolic process	44.92	15.8
147904294	GTP-binding nuclear protein Ran [Xenopus laevis]	<i>ran</i>	nucleotide binding		regulation of biological process	44.08	24.3
148230444	prohibitin 2 [Xenopus laevis]	<i>phb2</i>		membrane		42.74	33.3
76779525	LOC733343 protein [Xenopus laevis]	<i>imnt</i>		cytoplasm; membrane; mitochondrion		40.34	54.6
65047	ribosomal protein L14 [Xenopus laevis]	<i>rpl18</i>				39.66	17.7
64659	elongation factor 1-alpha (454 AA) [Xenopus laevis]	<i>ee1a1o</i>	catalytic activity; nucleotide binding			38.49	49.4
148229058	elongation factor 1-beta [Xenopus laevis]	<i>ee1b2</i>	RNA binding	cytoplasm	metabolic process	38.29	25.2
44968271	chaperonin subunit 8 theta [Xenopus laevis]	<i>cc8</i>	nucleotide binding; protein binding	cytoplasm	metabolic process	37.82	33.4
156119445	transitional endoplasmic reticulum ATPase [Xenopus laevis]	<i>vcp</i>	catalytic activity; nucleotide binding; RNA binding		metabolic process; reproduction; response to stimulus	36.75	89.1
148234340	ATP synthase, H+ transporting, mitochondrial Fo complex, subunit B1 [Xenopus laevis]	<i>atp5f1</i>	transporter activity	cytoplasm; membrane; mitochondrion	metabolic process; transport	34.02	28.2
27882460	Atg5c1 protein [Xenopus laevis]	<i>atp5c1</i>	catalytic activity; transporter activity	membrane	metabolic process; transport	33.71	30.0
147904130	aconitase 2, mitochondrial [Xenopus laevis]	<i>aco2</i>	catalytic activity		metabolic process	33.45	85.2
147902483	hydroxysteroid dehydrogenase-like protein 2 [Xenopus laevis]	<i>hsdl2</i>	catalytic activity; nucleotide binding	cytoplasm	metabolic process	31.59	44.5
148225959	uncharacterized protein LOC432221 [Xenopus laevis]	<i>vta1</i>				31.31	33.8
148230575	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 9, 39kDa [Xenopus laevis]	<i>ndufa9</i>	catalytic activity; nucleotide binding		metabolic process	30.61	42.9
148227702	ribosomal protein S10 [Xenopus laevis]	<i>rps10</i>				30.37	18.9
147907116	importin subunit alpha-1 [Xenopus laevis]	<i>kpa1</i>	protein binding; transporter activity	cytoplasm; nucleus	transport	28.84	57.7
148233826	chaperonin containing TCP1, subunit 6A (zeta 1) [Xenopus laevis]	<i>cc6a</i>	nucleotide binding; protein binding	cytoplasm	metabolic process	28.84	57.8
148234619	carbamoyl-phosphate synthase 1, mitochondrial [Xenopus laevis]	<i>cps1</i>	catalytic activity; metal ion binding; nucleotide binding		metabolic process	28.67	164.0
21952442	glutathione S-transferase [Xenopus laevis]	<i>hgsd5</i>	catalytic activity; protein binding			27.58	22.4
148224070	sorting and assembly machinery component 50 homolog B [Xenopus laevis]	<i>samm50</i>		cytoplasm; membrane; mitochondrion		26.92	52.0
54037970	LOC495053 protein [Xenopus laevis]	<i>ppl</i>		desmosome		26.84	153.7
148228991	MGC80163 protein [Xenopus laevis]		structural molecule activity	cytoplasm; ribosome	metabolic process	26.48	11.6
148232341	ribosomal protein S2 [Xenopus laevis]	<i>rps2</i>	RNA binding; structural molecule activity	cytoplasm; ribosome	metabolic process	26.42	30.3
147900512	protein disulfide isomerase family A, member 6 precursor [Xenopus laevis]	<i>pdisa6</i>	catalytic activity		cellular homeostasis; metabolic process; regulation of biological process	26.11	47.8
47123004	MGC8285 protein [Xenopus laevis]	<i>slc25a10</i>		membrane	transport	25.17	31.6
148232533	chaperonin containing TCP1, subunit 2 (beta) [Xenopus laevis]	<i>cc2</i>	nucleotide binding; protein binding	cytoplasm	metabolic process	23.48	57.6
148228666	X-ray repair complementing defective repair in Chinese hamster cells 6 [Xenopus laevis]	<i>xrcc6</i>	catalytic activity; DNA binding; protein binding	nucleus	metabolic process; response to stimulus	22.98	69.2
148235207	MGC82977 protein [Xenopus laevis]	<i>tra2</i>	nucleotide binding			22.66	32.2
148236843	ribosomal protein L15 [Xenopus laevis]	<i>rpl15</i>	structural molecule activity	cytoplasm; ribosome	metabolic process	21.88	24.0
147901815	erlin-2-B precursor [Xenopus laevis]	<i>erln2</i>		cytoplasm; endoplasmic reticulum; membrane		20.25	36.8

	calc. pl	rank
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2	5.02	1
3	6.81	2
4	11.47	3
5	6.02	4
6	10.26	5
7	4.81	6
8	8.05	7
9	7.31	8
10	5.48	9
11	6.33	10
12	9.42	11
13	4.91	12
14	5.76	13
15	10.55	14
16	7.80	15
17	5.05	16
18	9.14	17
19	6.49	18
20	6.55	19
21	4.79	20
22	9.09	21
23	11.60	22
24	8.70	23
25	7.75	24
26	11.56	25
27	6.49	26
28	5.24	27
29	8.09	28
30	11.47	29
31	8.25	30
32	5.40	31
33	10.62	32
34	7.88	33
35	9.91	34
36	7.65	35
37	11.71	36
38	9.11	37
39	4.79	38
40	5.25	39
41	5.30	40
42	9.55	41
43	9.41	42
44	7.12	43
45	7.39	44
46	6.46	45
47	9.45	46
48	10.11	47
49	5.41	48
50	6.68	49
51	6.38	50
52	5.83	51
53	6.70	52
54	6.01	53
55	4.50	54
56	10.15	55
57	5.21	56
58	9.60	57
59	6.21	58
60	6.35	59
61	11.37	60
62	11.40	61
63	6.67	62

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3 Table S2: proteins specifically detected in TRPP2 immunoprecipitates and absent from control
4 immunoprecipitates. Proteins are ranked according to their mascot score (scoreA3). For each
5 accession number, protein sequence was obtained from NCBI database, and blasted on Xenbase *X.*
6 *laevis* proteins. In some cases where NCBI description was incomplete, further protein identification
7 obtained from Xenbase is added in the column "description". Gene names from Xenbase gene models
8 are given in a separate column. Details about discriminating peptides are accessible by selection of
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