

1 **Hippo Pathway Elements Co-localize with**
2 **Occludin - a Possible Sensor System in**
3 **Pancreatic Epithelial Cells**

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21 **Keywords: Pancreatic cancer cells; Dobutamine; Tight Junctions; Occludin; YAP.**

22 No potential conflicts of interest are disclosed

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1 **ABSTRACT**

2 External adherens junction-based cell-cell contacts involving E-cadherin interactions function to sense
3 planar cell status and modulate epithelial cell proliferation through Hippo (Hpo) and non-canonical Wnt
4 pathways signaling. We hypothesized these regulatory processes should also be sensitive to a similar
5 cell-cell contact sensor associated with apical-basal polarity events at epithelial surfaces. We used two
6 human pancreatic cancer cell lines to explore this hypothesis: one with the capacity to form functional
7 tight junction structures and polarize (HPAFII) and one lacking this capacity (AsPc1). Occludin (Ocln), a
8 tetraspanning protein associated with TJ structures and capable of establishing external cell-cell
9 contacts, was observed to partially co-localize with Hpo elements YAP (c-yes associated protein) and
10 TEAD (TEA-dependent), which function to drive a proliferative transcription program. Treatment with
11 dobutamine, known to affect YAP, was shown to suppress proliferation in an Ocln-dependent manner.
12 Blockade of protein kinase C-zeta (PKC- ζ) diminished transepithelial electrical resistance (TER) of HPAFII
13 monolayers that was not corrected by dobutamine treatment while the loss of TER resulting from
14 inhibition of ROCK1 could be partially recovered. Examination of normal and cancerous human
15 pancreatic biopsies showed that the cellular localization of Ocln, c-Yes, YAP, and TEAD were similar to
16 HPAFII for normal cells and AsPc1 for cancerous cells. Together, these results suggest a link between
17 Hpo and signals emanating from cell-cell contacts involving Ocln that may regulate pancreatic cell
18 proliferation through the coordination of planar cell polarity with apical-basal polarity events.

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1 INTRODUCTION

2 The Hippo (Hpo) tumor suppressor pathway plays a critical role in controlling organ growth, stem
3 cell function, regeneration, and tumor suppression (Johnson and Halder, 2014). Disrupted regulation
4 of Hpo pathway elements, in particular YAP, correlates with the loss of epithelial cell lateral crowding
5 control that is observed as unregulated proliferation in the context of cancer (Pan, 2010; Poon et al.,
6 2011). Translocation of YAP from the nucleus to the cytoplasm with its subsequent phosphorylation
7 and degradation is correlated with suppression of cell proliferation (Pan, 2010). Regulatory factors
8 including β -catenin, the protein phosphatase PP2A, and the scaffolding protein 14-3-3 have been
9 implicated in processes that suppress cell proliferation through adherens junction (AJ) contacts and
10 Hpo signaling, providing a mechanism of proliferation control based upon lateral cell density within an
11 epithelium (Schlegelmilch et al., 2011). This mechanism links AJ regulatory elements to YAP de-
12 phosphorylation events that control its nuclear localization, where it combines with transcription
13 factors such as TEAD to drive the production of a proliferative program involving connective tissue
14 growth factor (CTGF). Thus, E-cadherin localized at AJ structures provides an extracellular cell-cell
15 contact sensor to regulate nuclear YAP localization and suppress epithelial cell proliferation at high
16 lateral cell density.

17 Epithelial barriers must not only regulate cell-cell planar polarity to restrict lateral growth, they
18 must also coordinate proliferation with apical to basal polarity. Events involving the disorganization
19 and/or dysfunction of TJ components have been correlated with hyper-proliferative responses in the
20 context of inflammation and cancer (Bruewer et al., 2006; Edelblum and Turner, 2009; Martin et al.,
21 2010; Tobioka et al., 2004a; Tobioka et al., 2004b). Previous studies have shown that cytoplasmic
22 components of the TJ, such as zonula occludens-2 (ZO-2), participate in the nuclear localization of YAP
23 in a process that is independent of its phosphorylation state (Spadaro et al., 2012). To date, no TJ-
24 associated element has been identified that might function as a sensor for apical to basal polarity

1 status in a manner similar to the role played by E-cadherin in AJ structures for lateral cell density
2 (Harvey et al., 2013). We conjectured that Hpo pathway signaling could also be involved in sensing TJ
3 status, and thus apical to basal polarity, through a protein sensor that forms cell-cell contacts in
4 association with these structures. Indeed, an extracellular sensor for detection of TJ elements has
5 been previously hypothesized (Farkas, et al., 2012). Our studies suggest the TJ protein occludin (Ocln)
6 as a candidate cell-cell sensor that can fine-tune the suppression of proliferation established by AJs.

7 Although Ocln was the first transmembrane TJ protein identified (Furuse et al., 1993), its role in
8 epithelial cell function is still unclear as it is not required for TJ formation; mice lacking Ocln can
9 produce claudin-based TJs that provide essential barrier function, but these animals exhibit a complex
10 phenotype that suggests a role for this protein in the functional properties of cell-cell interactions
11 (Saitou et al., 2000). Loss of Ocln expression or disruption of its normal cellular organization is
12 associated with inflammatory events (Bruewer et al., 2006; Edelblum and Turner, 2009) and epithelial
13 cell transformation (Sawada, 2013), both of which involve hyper-proliferation. Interestingly, the coiled
14 coil domain of Ocln interacts selectively with PKC- ζ and the non-tyrosine kinase c-Yes (Chen et al.,
15 2002a). PKC- ζ is important for apical to basal polarization events and the c-Yes interaction with Ocln
16 appears to be essential for TJ formation (Chen et al., 2002a; Nusrat et al., 2000). As c-Yes is a *bona fide*
17 binding partner of YAP (Aragona et al., 2013), we examined whether Ocln could function as a potential
18 sensor to regulate proliferation signals involved in apical-basal epithelial cell polarity. We tested this
19 hypothesis using two human pancreatic cancer cells lines with different potentials to polarize *in vitro*:
20 HPAFII and AsPc1 that form robust and compromised TJ barriers, respectively.

21 We observed partial intracellular co-localizations between Ocln and c-Yes, YAP, and TEAD that
22 were consistent with multiple, dynamic associations dependent upon cell-cell interactions.
23 Manipulation of Ocln expression could be achieved by treatment with dobutamine, a small molecule
24 known to induce YAP translocation from the nucleus to the cytoplasm through a mechanism

1 independent of Hpo signaling (Bao et al., 2011). Further, dobutamine treatment suppressed cell
2 proliferation of HPAFII and AsPc1 cells in a dose-dependent and Ocln-dependent manner; events
3 associated with reduced nuclear localization of YAP and TEAD. Decreased TJ integrity through the
4 actions a Rho-associated protein kinase (ROCK) inhibitor could be partially corrected by dobutamine to
5 suppress cell proliferation in an Ocln-dependent fashion. Enhanced cell proliferation resulting from
6 the disruption of TJ function using a PKC- ζ inhibitor failed to be affected by dobutamine treatment.
7 Finally, we examined Ocln, c-Yes, YAP, and TEAD cellular distribution in normal and pancreatic cancer
8 biopsies; these results corroborated findings made for the non-proliferative and proliferative formats
9 identified in the HPAFII and AsPc1 cell lines, respectively. Together, our results suggest that Ocln has
10 the characteristics required to function as a sensor of apical to basal polarity events through its ability
11 to co-localize Hpo signaling elements to forming TJ structures and thus provides a potential
12 mechanism to coordinate these processes with AJ-mediated regulation of lateral growth in an
13 epithelial tissue barrier.

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15 **RESULTS**

16 **Assessment of Ocln with Hpo Pathway Elements in Cells Capable of Polarization**

17 HPAFII cells are a human pancreatic cancer cell line that demonstrates the capacity to
18 establish functional TJs and differentiate *in vitro* to form polarized monolayers on permeable supports
19 (Kim et al., 1989). We first characterized the cellular distribution for proteins of interest in HPAFII cells
20 grown at low cell density on plastic where nascent lateral cell-cell contacts had begun to be
21 established. Immunofluorescence demonstrated YAP was extensively co-localized with c-Yes. This co-
22 localization occurred primarily in the cytoplasm; some YAP, but very little c-Yes was observed in the
23 nucleus (Fig. 1A₁). Ocln/c-Yes co-localizations were observed primarily at cell-cell contacts but these

1 appeared to occur to a lesser extent than c-Yes/YAP co-localizations and showed similarities to the
2 Ocln/YAP distribution: mostly cytoplasmic co-localizations (Fig. 1A₂). Co-localization of Ocln with YAP
3 was less striking relative to Ocln/c-Yes and c-Yes/YAP interactions (Fig. 1A₃). Further, c-Yes/YAP co-
4 localizations appeared to be primarily in the cytoplasm while Ocln/YAP co-localizations were observed
5 more frequently at leading edge surfaces of these small cell colonies. TEAD/Ocln co-localizations were
6 predominately in the cytoplasm of HPAFII cells, with limited interactions at the cell surface (Fig. 1A₄).
7 Higher magnification analysis showed TEAD/Ocln co-localizations to be incomplete in their overlap
8 (Fig. 1A₄), unlike the more complete overlay observed for c-Yes/YAP, c-Yes/Ocln and YAP/Ocln co-
9 localizations (Fig. 1A₁-A₃).

10 HPAFII cells grown at high cell density on plastic and treated with dobutamine demonstrated a
11 dose-dependent increase in Ocln immunofluorescence localized to the cell surface and decrease in
12 overall and nuclear YAP content relative to controls (Figs. 2A,C). Dobutamine is a β -adrenergic
13 receptor agonist that induces YAP translocation from the nucleus to the cytoplasm through a
14 mechanism independent of Hpo signaling (Bao et al., 2011). Based upon previous studies, these
15 changes in YAP presumably occurred through its redistribution to the cytoplasm where it is
16 phosphorylated and degraded as a consequence of dobutamine treatment (Chan et al., 2011).
17 Similarly, dobutamine treatment of HPAFII cells resulted in a dramatic reduction in nuclear TEAD
18 content with the remaining TEAD being notable for its apparent co-localizations with Ocln at the
19 plasma membrane (Fig. 2B, D).

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21 **Dobutamine-induced Redistribution of Ocln has Functional Consequences**

22 Dobutamine treatment of HPAFII cells grown at low cell densities resulted in a dose-
23 dependent shift in Ocln immunofluorescence from the cytoplasm to the cell surface (Fig. 2).

1 Concomitant with this shift in Ocln localization, there was an increase in co-localizations involving c-
2 Yes and Ocln that was associated predominantly with the membrane fraction of these cells (Fig. 3A,B).
3 These results are interesting in light of previous findings showing that c-Yes is associated with Ocln at
4 assembling TJ structures and is dissociated from Ocln at times when TJ structures are disassembling
5 (Chen et al., 2002b). Thus, suppression of YAP translocation to the nucleus by dobutamine treatment
6 is associated with increased levels of c-Yes/Ocln co-localization in polarizing HPAFII cells.

7 Besides Ocln, two other MARVEL family proteins localize to TJ structures: MARVELD2
8 (tricellulin: Tric) and MarvelD3 (Md3) (Mariano et al., 2011). While Ocln and MarvelD3 co-localize at
9 bicellular contacts (Kojima et al., 2011), Tric is normally restricted to tricellular contacts (Ikenouchi et
10 al., 2005). Similar to Ocln, down-regulation of Md3 is associated with EMT in pancreatic cancer cells;
11 Tric knockdown studies suggest it to have a role in the size-selective properties of TJ barriers rather
12 than EMT events (Mariano et al., 2011). Others have previously described how functional Ocln
13 expression can drive Tric from bi-cellular to tri-cellular contact sites in developing epithelial barriers
14 (Ikenouchi et al., 2005). We examined the impact of dobutamine on Tric organization in HPAFII cells
15 forming cell-cell contacts at low cell density (Fig. 3C). Untreated HPAFII cells demonstrated Ocln/Tric
16 co-localizations to be present at both bi-cellular contacts and at the external facing cell surfaces of cell
17 colonies. Dobutamine treatment resulted in the exclusion of Tric from bi-cellular contacts that were
18 subsequently dominated by Ocln. Interestingly, Ocln/Tric co-localization remained at externally
19 directed surfaces of cells where no bi-cellular contacts could be established. Immunoprecipitation
20 studies verified the shift of Tric to the membrane following dobutamine treatment but with an
21 increase in its associations with Ocln (Fig. 3D). Together, the findings of c-Yes co-localization and Tric
22 restriction to tri-cellular contact sites suggest that Ocln expression and redistribution to the plasma
23 membrane induced by dobutamine treatment has functional consequences.

24

1 **Dobutamine Accelerates and Enhances Epithelial Cell Apical-Basal Polarization**

2 Confluent cultures of HPAFII cells grown on plastic demonstrated changes in cell-cell contacts
3 without striking differences in cell number, size, or shape when treated with increasing concentrations
4 of dobutamine (Fig. 4A-C). HPAFII cells grown as confluent monolayers on permeable supports can
5 complete apical-basal polarization, resulting in polarized sheets with trans-epithelial electrical
6 resistance (TER) values of $\sim 300\text{-}500 \Omega\cdot\text{cm}^2$ by day 8 of culture (Fig. 4D). Addition of dobutamine
7 accelerated and enhanced the TER values of HPAFII cell sheets in a dose-dependent manner, with TER
8 values increasing to as high as $\sim 4,000 \Omega\cdot\text{cm}^2$ (Fig. 4D) that typically plateaued at $\sim 1,700 \Omega\cdot\text{cm}^2$ (Fig.
9 4G). Dobutamine treatment altered the localization of OcIn that was consistent with its consolidation
10 at TJ structures in HPAFII cell monolayers (Fig. 4E) that coincided with an increase in total OcIn levels
11 (Fig. 4F,H) and increased TER (Fig. 4G). Further, immunoblot analysis verified that dobutamine
12 treatment increased the apparent molecular weight of OcIn at the membrane (Fig. 4I), consistent with
13 an increased phosphorylation status (Dorfel and Huber, 2012).

14 PKC- ζ and Rho kinase have both been demonstrated to play critical roles in the organization
15 and stabilization of functional TJs (Dodane and Kachar, 1996; Etienne-Manneville and Hall, 2002;
16 Stuart and Nigam, 1995; Suzuki et al., 2001), with PKC- ζ directly interacting with OcIn (Nusrat et al.,
17 2000). We tested the potential involvement of PKC- ζ and ROCK in processes involving dobutamine
18 enhancement of TJ function through pharmacological suppression using the protein kinase C zeta
19 pseudosubstrate (PKC- ζ -PS) peptide inhibitor (Lim et al., 2008) and the p160ROCK inhibitor Y-27632
20 (Ishizaki et al., 2000; Matthews et al., 2006). Incubation of HPAFII monolayers with PKC- ζ -PS ablated
21 TER and treatment with dobutamine failed to have any effect on this outcome (Fig. S.1). Treatment
22 with Y-27632 also ablated TER, but in this case treatment with dobutamine could counteract the
23 actions of Y-27632 (Fig. 4 J, K, L). These data are consistent with the previous roles of PKC- ζ activity
24 being critical for TJ structure/function through its actions on OcIn function while Rho regulation of TJ

1 organization is not as directly aligned with OcIn function at TJs (Ishizaki et al., 2000; Matthews et al.,
2 2006; Nusrat et al., 2000).

3

4 **Assessment of OcIn with Hpo Pathway Elements in Cells Incapable of Polarization**

5 AsPc1 is a cell line established from a human pancreatic ductal adenocarcinoma (PDAC); it is
6 known for its aggressive growth characteristics and inability to form functional TJ structures (Tan and
7 Chu, 1985). When grown at low cell density as previously described for HPAFII cells (Fig. 1), AsPc1 cells
8 expressed OcIn at levels comparable to or even greater than those observed in HPAFII cells with some
9 immunofluorescence being associated with the plasma membrane (Fig. 5A). YAP/c-Yes co-localization
10 occurred mostly in the cytoplasm (Fig. 5A₁). OcIn/c-Yes co-localized predominantly at the membrane
11 similar to that observed in HPAFII cells (Fig. 5A₂). The striking differences between HPAFII and AsPc1
12 cells were primarily related to YAP and TEAD distribution with a much greater relative expression in
13 the nucleus for both in AsPc1 cells (Fig. 5A₃ and 5A₄). OcIn/YAP co-localization was extensive at and
14 adjacent to the plasma membrane (Fig. 5A₃ and Fig.S.2), different from that observed for HPAFII cells.
15 TEAD/OcIn co-localization was almost non-existent (Fig. 5A₄) and failed to show cytoplasmic co-
16 localizations as observed in HPAFII cells.

17 We next examined the actions of dobutamine treatment on AsPc1 cells; a dose-dependent
18 loss of nuclear YAP in AsPc1 cells was associated with an increase in OcIn expression (Fig. S.2). There
19 was also a loss of YAP/OcIn co-localizations at or near the plasma membrane and the distribution of
20 OcIn shifted from being more pronounced at external faces of cells to cell-cell contact locations (Fig.
21 S.2). Dobutamine treatment of AsPc1 cells produced an increase in the extent of c-Yes/OcIn co-
22 localization observed at or near the plasma membrane, similar to HPAFII cells (Fig. S.3). Changes in the
23 cellular distribution of TEAD in AsPc1 cells induced by dobutamine treatment mirrored those observed

1 for YAP (Fig. S.4). It should be noted that we failed to observe such striking changes in YAP/Ocln or
2 TEAD/Ocln co-localizations in HPAFII cells following dobutamine treatment (Fig. 2). We next examined
3 the relative cellular distributions for YAP and Ocln associations by immunoprecipitating YAP followed by
4 immunoblotting for Ocln (Fig. S.5). These studies showed that showed that Ocln in HPAFII cells was
5 more extensively membrane-associated than in AsPc1 cells but that less of the Ocln in HPAFII cells was
6 associated with YAP at the membrane compared to AsPc1 cells. Note that most of the Ocln-antibody
7 reactive materials were of lower molecule weight than full-length Ocln, suggesting the presence of
8 catabolic fragments.

9

10 **Actions of dobutamine on HPAFII and AsPc1 Cell Proliferation**

11 The transcriptional co-activator complex YAP/TEAD is known to affect cell proliferation rates
12 (Chen et al., 2010). Treatment with dobutamine inhibited the proliferation of both HPAFII and AsPc1 in
13 a dose-dependent manner after 24 h and 48 h of exposure (Fig. 6A,B). Transient knockdown of Ocln
14 expression using siRNA did not significantly alter the proliferation rate of HPAFII cells, but it did
15 significantly impede the ability of dobutamine to suppress HPAFII cell proliferation (Fig. 6C). Similar,
16 but less striking, findings were observed for AsPc1 cells (Fig. 6D). It should be noted that the impact of
17 siRNA or control (scrambled) siRNA added at the initiation of these experiments would have taken
18 several hours to affect Ocln expression levels, reducing the potential impact of these treatments over
19 the time course of this study (Fig. 6E). Despite this caveat, these studies suggest that dobutamine
20 reduced proliferation rates in these two pancreatic cancer cell lines, possibly through a mechanism
21 involving Ocln.

22

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1 **Expression pattern of c-Yes, Ocln, YAP and TEAD in human pancreatic biopsies**

2 Our *in vitro* data with two different human pancreatic cancer cell lines has suggested a
3 potential role for Ocln to regulate cell proliferation through the Hpo signaling cascade. Ocln co-
4 localization with YAP and TEAD suggested a role in the establishment/stabilization/organization of
5 cell-cell contacts associated with TJs. Comparison of epithelial cells capable of undergoing apical-basal
6 polarization (HPAFII) with those that cannot undergo this differentiation (AsPc1) demonstrated that
7 nuclear localization of the YAP/TEAD complex was decreased when Ocln expression was increased by
8 dobutamine treatment. To better understand how these observations might to clinical status, we
9 examined human pancreatic biopsies from four different patients to assess the expression profile and
10 localization of c-Yes, Ocln, YAP and TEAD in cancerous and non-cancerous conditions (Fig. 7, Fig. S.6
11 and Fig. S.7). In normal (non-cancerous, non-inflamed) pancreas we noted that c-Yes was distributed
12 in the cytoplasm and in small amounts at the apical plasma membrane of duct cells. A moderate to
13 strong nuclear c-Yes staining was observed in two pancreatic cancer tissue biopsies and a strong signal
14 was also detected at the apical surface of cells lining duct-like structures, which agrees with the
15 distribution of this non-receptor tyrosine kinase in AsPc1 cells.

16 Ocln expression in normal pancreas samples was very similar to that of c-Yes with localization
17 in the cytoplasm and at the apical plasma membrane of ducts. Ocln expression in PDAC specimens
18 appeared to be substantial but not at the plasma membrane. YAP expression in normal pancreatic
19 epithelial cells was mostly detected in the cytoplasm with minimal nuclear staining observed; stronger
20 YAP staining was detected in biopsies from PDAC patients, which was localized to the cytoplasm and
21 apical surfaces of morphologically altered epithelial cells. TEAD labeling in normal pancreas was
22 restricted to the cytoplasm and very minor amounts of nuclear localization; PDAC samples showed
23 some cytoplasmic staining but also more extensive nuclear localization. Overall, in this limited set of
24 biopsies, we observed c-Yes, Ocln, YAP, and TEAD expression in PDAC that was either altered in its

1 cellular distribution or increased compared to non-cancerous tissue. Ocln, c-Yes, YAP, and TEAD
2 localization in epithelial cells of normal pancreas were more similar to the distribution of these
3 proteins in HPAFII cells while the distribution of these proteins in PDAC biopsies was more consistent
4 with the findings of expression and distribution observed in AsPc1 cells.

5

1 Discussion

2 The Hpo pathway provides important growth regulation of epithelia cells by using homotypic
3 extracellular contacts made by E-cadherin at AJ structures as a sensor to lateral cell-cell contact status
4 (Gumbiner and Kim, 2014). We hypothesized that epithelia could utilize similar homotypic extracellular
5 contacts at TJ structures as a sensor to signal apical-basal polarity status to suppress proliferation in
6 fully polarized conditions. Our studies using two pancreatic cancer cell lines adapted for *in vitro* cell
7 culture provide a potential scenario of how Ocln co-localization with c-Yes, YAP, and TEAD might
8 function as a sensor system for Hpo pathway signaling and build upon the previously established
9 status of c-Yes as a *bona fide* binding partner of YAP and Ocln (Nusrat et al., 2000).

10 In the case of HPAFII cells, where functional TJs can form, it appears that c-Yes/Ocln co-
11 localize at cell-cell contact regions, consistent with the role of this non-tyrosine kinase to facilitate the
12 formation of functional TJs (Chen et al., 2002b) while c-Yes co-localizes with YAP in the cytoplasm
13 adjacent to these cell-cell contact regions (Sudol, 1994). YAP and TEAD both showed co-localization
14 with Ocln that was restricted to the cell surface and cytoplasm of these cells. Treatment with
15 dobutamine increased Ocln expression and distribution at the plasma membrane, events that
16 coincided with enhanced TJ maturation and decreased YAP and TEAD levels. Dobutamine suppressed
17 cell proliferation rates in an Ocln-dependent fashion that required the actions of PKC- ζ , a kinase that
18 promotes apical-basal polarization and interacts with the C-terminal tail of Ocln (Dodane and Kachar,
19 1996; Nusrat et al., 2000; Stuart and Nigam, 1995).

20 In comparison to HPAFII cells, AsPc1 cells fail to form functional TJs and demonstrate distinct
21 co-localization patterns for Ocln with c-Yes, YAP, and TEAD. Most striking, the nuclear levels of YAP
22 and TEAD in AsPc1 cells were increased relative to HPAFII cells. Treatment of AsPc1 cells with
23 dobutamine increased Ocln levels and diminished nuclear YAP and TEAD that coincided with

1 decreased proliferation rates. This suggests that while the sensor function of OcIn might be based
2 upon its localization at TJs, pharmacological intervention with dobutamine can overcome this
3 requirement increasing OcIn expression levels. Thus, the demonstration that OcIn co-localizes with
4 TEAD and YAP may represent a stoichiometric relationship that sequesters critical Hpo elements to
5 limit their access to the nucleus and their actions on proliferation. Previous studies have suggested
6 that TJs not only establish barrier and fence functions associated with mature epithelium, but also act
7 in cell signaling events (Matter and Balda, 2003). Our studies now highlight the potential for a specific
8 TJ element, OcIn, to participate in a signaling event that suppresses proliferation.

9 The function of OcIn has been poorly understood since its identification as the first integral
10 membrane protein of the TJ (Furuse et al., 1993), with functional TJ structures forming in the absence
11 of OcIn (Saitou et al., 2000). Our findings support a role for OcIn in regulating bi-cellular TJ structures
12 (Furuse, 2010). Recent studies support the potential for other TAMP family member, Tric and
13 MarvelD3, to replace OcIn absent at bi-cellular contacts (Cording et al., 2013; Raleigh et al., 2011). This
14 compensation does not always, however, provide a complete correction. While OCLN $-/-$ mice
15 demonstrate morphologically and functionally normal TJs and reach adulthood, they exhibit a complex
16 phenotype that includes reduced growth rates, brain calcifications, testicular atrophy, loss of
17 cytoplasmic granules in salivary gland striated duct epithelial cells, and thinning of compact bone
18 (Saitou et al., 2000). Important to this discussion is the fact that OCLN $-/-$ mice also exhibit chronic
19 inflammation and hyperplasia of their gastric epithelium, indicating that OcIn is involved in the
20 balance between epithelial proliferation and differentiation (Furuse, 2009).

21 Epithelial inflammation is associated with diminished barrier function and the internalization
22 of TJ proteins, such as OcIn, with minimal effects on the cytoplasmic plaque proteins and cytoskeleton
23 associated with TJs (Bruewer et al., 2003). Other studies have shown OcIn to modulate epithelial
24 barrier function (Yu et al., 2005) and expression of OcIn lacking its cytoplasmic domain disrupts apical-

1 basal polarity (Balda et al., 1996). Chronic inflammation and loss of apical-basal polarity are associated
2 with epithelial cell transformation. Such changes, in light of the current observations, are consistent
3 with a potential sensor role for OcIn at TJ structures. This concept is further supported by the
4 demonstration that claudin proteins serve as the essential elements of functional TJs, diffuse slowly in
5 the membrane, and have durable periods of residence at TJs compared to OcIn (Shen et al., 2011),
6 making the more dynamic nature of OcIn membrane residence an appealing aspect for an apical-basal
7 polarity sensor. This combination of rapid dynamics at the plasma membrane and the ability to form
8 homotypic interactions between adjacent cells provides an attractive mechanism for epithelial
9 barriers to assess proliferation *vs.* differentiation programming for the cell.

10 We observed differences in the co-localization of c-Yes and YAP with OcIn in HPAFII and AsPc1
11 cells that were consistent with OcIn plasma membrane residence where TJs do or do not form.
12 Importantly, c-Yes, a *bona fide* YAP binding partner is one of the kinases that directly binds to and
13 regulates OcIn function at TJs (Chen and Lu, 2003; Chen et al., 2002b; Nusrat et al., 2000). This is
14 consistent with the observation that epithelial cancers involving dysfunctional Hpo pathway elements
15 have been identified (Pan, 2010) and epigenetic silencing of OcIn promotes tumorigenesis (Osanai et
16 al., 2006). Examination of human normal and PDAC biopsies are consistent for changes previously
17 noted for OcIn and c-Yes in pancreatic cancer (Tan et al., 2004; Kubo et al., 2009). Additionally, YAP
18 and TEAD represent critical component of Hpo signaling, playing a central role in the context of cell
19 proliferation associated with PDAC (Shao et al., 2014). Since YAP does not contain an intrinsic DNA-
20 binding domain but instead regulates target genes by interacting with DNA-binding transcription
21 factors in the nucleus such as TEAD (Yu and Guan, 2013; Vassilev et al., 2001), it is interesting to note
22 that both YAP and TEAD co-localize with OcIn, providing a potential a sensor to relay information
23 relevant to the extent apical-basal polarization through YAP and TEAD to ensure a bias toward
24 polarization *versus* proliferation.

1 These co-localizations with c-Yes, YAP, and TEAD as well as the actions of dobutamine suggest
2 a mechanism whereby OcIn can function as a sensor at TJ structures. OcIn expression enhanced by
3 dobutamine treatment restricted Tric from bi-cellular contacts and increasing epithelial barrier
4 properties, findings consistent with previous studies examining OcIn function (Ikenouchi et al., 2005;
5 Ikenouchi et al., 2008). Dobutamine enhanced interactions with c-Yes and PKC- ζ , and provided a form
6 of OcIn that could co-localize with YAP and TEAD at the plasma cell membrane and within the
7 cytoplasm. Such a mechanism would limit YAP and TEAD access to the nucleus and lead to the
8 suppression of proliferation that is associated with differentiated epithelia. Knockdown of OcIn levels
9 or inhibition of PKC- ζ led to the release of this suppression, consistent with the idea that functional TJ
10 structures involving OcIn are required for this mechanism to work properly under non-pathological
11 conditions. Overall, our data suggest that despite the fact that the role of OcIn and YAP interaction is
12 not necessarily mediated through a functional TJ complex, OcIn is uniquely positioned at TJ structures
13 to register the extent of a functional barrier for the coordination of apical-basal polarity with cell
14 density through co-localization with Hpo pathway elements (YAP and TEAD).

15

1 **Materials and Methods**

2 **Cell culture, TER measurements, paracellular permeability**

3 AsPc1 and HPAF II cell lines were obtained from ATCC, were passaged two months after receipt and
4 were authenticated and characterized through Short Tandem Repeat (STR) profiling. Cells were
5 maintained at 37°C in a humidified atmosphere containing 5% CO₂. AsPc1 cells were maintained in
6 RPMI 1640 media (Fisher[®] / cat. number - 10418243) and HPAFII in MEM (Fisher[®] / cat. number -
7 -11514426), both supplemented with 10% fetal bovine serum FBS (Invitrogen / cat. number -
8 - 10500064) penicillin (100 U/mL) and streptomycin (100 mg/mL) (Sigma[®] / cat.number - P4333). Cells
9 were sub-cultured twice weekly at a 1:2 split ratio. For apical-basal polarity studies, 70,000 HPAFII
10 cells per well (between passage 10 and 12) were seeded onto 0.4-µm pore size, 12 mm Transwell™
11 filters (Fisher[®] / cat. number - CLS3460); TER values were measured by fixed paddle electrodes (World
12 Precision Instruments[®], UK). For paracellular studies, TER measurements were performed on a daily
13 basis and on day 7, cells were treated with 20 µM ROCK inhibitor Y-27632 dihydrochloride (Sigma[®] /
14 cat- number - Y0503) or 50 µM PKC-ζ pseudosubstrate (PKC ζ-PS) (Fisher[®] / cat. number - 10717873)
15 either alone (Cerruti et al., 2013) or in combination with 20 µM Dobutamine (Sigma[®] / cat. number -
16 D0676). Culture media was replaced by fresh MEM without phenol red (Fisher[®] / cat. number - 51200-
17 038) and cultures were then left to equilibrate during at least 30 minutes. FITC Dextran (Sigma[®] / cat.
18 number – 46944) was added to the inner chamber to a final concentration of 1 mg/mL. Cultures were
19 then incubated at 37°C for 4 h. Filters were then removed and the diffused tracer measured by the
20 FLUOstar OPTIMA (FITC-Dextran: Excitation: 485 nm and emission: 544 nm). Each sample was
21 analysed in triplicates from independent experiments.

22

23

1 **Protein extraction and subcellular fractionation**

2 To collect cytoplasm fractions, growth media was removed and cells were washed twice in ice-cold
3 PBS prior to the addition of RIPA buffer (Fisher[®] / cat. number – 10017003). After incubation on ice for
4 five minutes, cells were collected using a cell lifter and the lysate clarified by centrifugation (8000 xg,
5 10 min, 4°C). Preparation of protein concentrations was determined using a NanoDrop 2000
6 instrument (Thermo Scientific). For membrane isolation, cells were washed with PBS containing Ca²⁺
7 and Mg²⁺ and lifted into 50 mM Tris/HCl, pH 7.4 with protease (Fisher[®] / cat. number – 12841640) and
8 phosphatase inhibitors (Fisher[®] / cat. number – 12851650) while on ice. Collected cells were disrupted
9 by freeze-thaw cycles prior to centrifugation (1000 xg, 10 min, 4°C). After centrifugation, nuclear
10 fractions were collected and suspended in lysis buffer. Membrane pellets were isolated from clarified
11 supernatants by ultracentrifugation at 100,000 xg, for 1 h at 4°C (Beckman). Pellets were washed in
12 this way twice in PBS containing Ca²⁺ and Mg²⁺ and then suspended in lysis buffer containing 0.1%
13 Triton-X-100 (Sigma[®] / cat. number - T8787). Each experiment was repeated three times.

14

15 **Immunoprecipitation and immunoblotting**

16 Cells were lysed in RIPA buffer for 10 min at 4°C and the preparation centrifuged (10,000 xg, 10 min,
17 4°C). Lysates containing 500 µg of protein were incubated with a pull-down antibody overnight at 4°C.
18 A/G beads™ (Santa Cruz Biotech / cat. number - sc-2003) were added and the mixture was incubated
19 with rotation for 2 h at 4°C. Beads were washed three times in RIPA buffer by centrifugation at 4000
20 xg, 1 min, RT. 20-35 µg of the protein extract was separated by 12% SDS PAGE (100–150 V, 45 min)
21 and transferred (XCell™ Blot module; Invitrogen) onto a PVDF membrane (Thermo Scientific / cat.
22 number – 10617354) at 30 V for 1.5 h. The PVDF membrane was then rinsed briefly in ddH₂O and
23 blocked in 5% milk dissolved in TBS-T (10 mM Tris- HCl, pH 7.5, 150 mM NaCl and 0.1% Tween 20) for

1 1 h at RT. Blots were then incubated overnight at 4°C in TBS-T containing 5% BSA (Sigma[®] / cat.
2 number - A7906) and a primary antibody at a 1:500 dilution: Ocln polyclonal rabbit (Invitrogen / cat.
3 number - 33-1500), total YAP rabbit monoclonal (Santa Cruz / cat. number - sc-15407), TEAD mouse
4 monoclonal (Cell Signaling / cat. number - 8526), or c-Yes mouse monoclonal (Santa Cruz / cat.
5 number - sc-8403). After three washes in TBS-T at RT, appropriate secondary-horseradish peroxidase
6 (HRP) antibodies (Rabbit: GE Healthcare / cat. number - NA934; Mouse: GE Healthcare / cat. number:
7 NXA931) were applied at a dilution of 1:1000 for 1h at RT in TBS T containing 1% milk. Blots were
8 washed in TBS-T with HRP activity being detected by ECL (GE Healthcare[®] / cat. Number - RPN2232).

9

10 **Immunohistochemistry**

11 Human pancreatic biopsy tissue sections on glass slides were de-waxed in HistoClear (Fisher[®] / HIS-
12 -010-010S) for 10 min, rehydrated in 10 min steps through graded ethanol (100%, 90%, 70% and 50%),
13 and then washed in PBS with gentle shaking for 5 min. Heat induced antigen retrieval was performed
14 using hot 10 mM of Citrate buffer pH 6.0 in a pressure cooker (Morphy Richards) for 15 min followed
15 by a second heating for 5 min at a lower temperature (Shi et al., 1993). Tissue sections were blocked
16 for 30 min at RT with serum blocking reagent and incubated in either a primary antibody or control
17 antibody (Rabbit - DA1E, cat. number - 3900 ; Mouse - G3A1, cat. number – 5415; Cell Signaling).
18 Antibodies were used at 1:100 dilution in PBS with 0.1% BSA and incubated overnight at 4°C in a
19 humid chamber. Slides were then washed in PBS, exposed to a biotin-labeled secondary antibody for
20 30 min at RT, washed in PBS again, and treated with 30% (w/w) H₂O₂ to quench the endogenous
21 peroxidase activity (Veitch, 2004). After being washed in PBS, slides were incubated at RT in
22 avidin:biotin enzyme complex (Vecstatin Elite ABC kit, Vector laboratories, cat. number - PK-6101) for
23 30 min, washed again and incubated with 3,3'-diaminobenzidine (DAB) (Vector Laboratories / cat.

1 number - SK-4100) according to the manufacturer's instructions. Tissue sections were co-stained with
2 Meyer's Haematoxylin (Sigma[®] / cat. number - H9627) for 2 min, rinsed with water, mounted in
3 DePeX glue (Fisher[®] / cat. number - 10050080) and allowed to dry overnight. Images were captured at
4 20x magnification using a DMI 3000B Microscope (Leica Microsystems) and managed using Adobe
5 Photoshop, version 3.0 software (Adobe Systems, Mountain View, CA).

6

7 **Immunofluorescence and confocal microscopy**

8 Cells were grown on coverslips for 48 h, washed three times with 10 mM HEPES buffer containing
9 0.9% CaCl₂, and then fixed with 4% PFA for 20 min. Cells were simultaneously blocked and
10 permeabilized overnight at 4°C in PBS containing 2% BSA and 0.5% Triton X-100. Cells were then
11 washed with 10 mM HEPES buffer containing 0.9% CaCl₂ and incubated for 2 h at 4°C with a primary
12 antibody diluted 1:100 in PBS and then a secondary antibody diluted 1:400 in PBS for 30 min. DAPI
13 (Sigma[®] / cat. number – 32670) was applied for 20 min, and cells were mounted on mowiol and
14 examined using Zeiss Confocal Laser Scanning Microscope using three laser wavelengths: Argon 488
15 nm, HeNe 543 nm and HeNe 633 nm. Images were captured using the 63x oil immersion objective.
16 Results shown are representative of at least three independent experiments.

17

18 **Cell proliferation assay**

19 Cells were plated in white clear bottom 96-well plates, and allowed to attach overnight. Test
20 compounds were added in optimal growth media and incubated for 24 h. For cell proliferation assays,
21 treatment media was removed and 100 µL of 1x dye binding solution of CyQuant (Fisher[®] / cat.
22 number – 10709253) was added. After a 30 min incubation, fluorescent intensity was measured at 22

1 °C using a microplate reader (FLUOstar Omega; BMG Labtech) at 485/530nm excitation/emission
2 wavelengths. Each sample was analysed in triplicates from independent experiments.

3

4 **Ocln Knockdown**

5 We used a pool of 4 individual siRNA (Accell Human OCLN, siRNA-SMARTpool, Fisher[®] / cat. number -
6 -E-187897-00-0005) targeting different sequences in the Ocln gene (details of siRNA used are shown in
7 Table 1). For proliferation assays HPAFII and AsPc1 (passage 12) cells were grown on a 96 well plate
8 approximately 70% confluent. A transfection mixture of 1 µL of HiPerfect, 0.02 µL of siRNA (20 µM
9 stock) or 0.6 µL of scrambled siRNA (Fisher[®] / cat. number – 11841984) were added to 7.5 µL of
10 serum- and antibiotic-free media and rocked for 15 min at RT. 172 µL of fresh media with and without
11 Dobutamine (RPMI or MEM supplemented with 10% FBS, penicillin (100 U/mL) and streptomycin (100
12 mg/mL) was added to each well; the transfection mix was added in a drop-wise fashion and then cells
13 were incubated at 37°C in 5% CO₂ for 24 h. Cells were treated in parallel with a scrambled control
14 siRNA. After removal of treatment media, a cell proliferation assay, previously described, was carried
15 out. For protein levels, cells were harvested in RIPA buffer and run on a gel for western blot analysis.

16

17 **Pancreatic tissues and ethics statement**

18 Tissue samples were collected from patients following pancreatic resection for PDAC. Normal
19 pancreatic tissue samples were obtained through an organ donor procurement program whenever
20 there was no suitable recipient for pancreas transplantation. Tissues were fixed in 5%
21 paraformaldehyde solution for 12-24 h and then embedded in paraffin for histological analysis (Erkan
22 et al., 2005). All patients were informed, written consent was obtained, and human protocols were

1 approved by the Ethics Committees of the University of Heidelberg (Germany) and the Technical
2 University of Munich (Germany).

3

4 **Statistical analysis**

5 Data are represented as the mean of 3 independent experiments \pm SEM. Statistical analysis was
6 performed using GraphPad Prism 5 software. Significant differences between experimental and the
7 control group were determined by one-way ANOVA followed by Dunnet's post hoc test for
8 experiments in which three or more groups were compared. A statistically difference was accepted if
9 the p values were less than 0.05. Significance is indicated on the graphs as it follows: * $p < 0.05$, **
10 $p < 0.01$, *** $p < 0.001$.

11

12

13

14

1 **Acknowledgements**

2 We are indebted to the patients who authorized the use of their organs for these studies. We
3 express our thanks to Nadine Wagner for sending the tissue biopsies and Dr. Adrian Rogers for
4 technical assistance with the confocal microscopy pictures. Finally we are grateful to Dr. Ann Hopkins
5 for valuable discussions. Ana Santos Cravo acknowledges support from the University of Bath doctoral
6 training grant. Randy Mrsny thanks The Wellcome Trust for being a VIP recipient.

7

8 **Abbreviation list**

- 9 AJ- adherens junctions;
10 APC - apical polarity complex
11 Dob – Dobutamine
12 EMT – Epithelial Mesenchymal Transition,
13 HDAC - histone deacetylase.
14 Hpo – Hippo;
15 HRP - Horseradish peroxidase;
16 OcIn – Occludin;
17 PDAC – Pancreatic Ductal Adenocarcinoma;
18 PKCζ-PS – PKCζ Pseudosubstrate;
19 PKC-ζ-PS - protein kinase C zeta pseudosubstrate;
20 TEAD - TEA-domain family member.
21 TER - transepithelial electrical resistance;
22 TJ – Tight junction;
23 Tric – Tricellulin;
24 YAP – c-Yes associated protein;
25 ZO-2 - zonula occludens-2;

26

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14

15

16

1 **FIGURE LEGENDS**

2 **Figure 1 – Distribution and co-localization of c-Yes, Ocln, YAP, and TEAD in HPAFII cells *in vitro*.**

3 (A) Immunofluorescence distribution and confocal co-localization of YAP and c-Yes, Ocln and c-Yes, Ocln
4 and YAP, and Ocln and TEAD. Higher magnifications of co-localizations are presented (A1-4). Graphs
5 showing overlapping fluorescence intensity are presented below associated micrographs with red line
6 denoting path of analysis; scale bar, 20 μ m.

7

8 **Figure 2 - Dobutamine induces YAP and TEAD translocation from the nucleus and stabilization of Ocln at**
9 **the cell membrane of HPAFII cells.**

10 (A) Confocal immunofluorescence microscopy showing YAP and Ocln distribution in HPAFII cells. Cells
11 treated with 10 or 20 μ M dobutamine for 24 h were similarly analyzed for YAP and Ocln cell distribution.
12 Scale bar, 20 μ m. (B) Confocal immunofluorescence microscopy showing TEAD and Ocln distribution in
13 HPAFII cells with control buffer addition versus 20 μ M dobutamine for 24 h; scale bar, 20 μ m. (C)
14 Immunoblot analysis of total YAP in nuclear and cytoplasmic fractions of HPAF II cells as a consequence of
15 10 or 20 μ M dobutamine (Dob) exposure. HDAC (histone deacetylase) and β -actin were used as loading
16 controls for nuclear and cytoplasmic fractions, respectively. Quantification of YAP expression values are
17 the mean of 3 independent experiments \pm SEM; ***p < 0.001. (D) Immunoblot analysis of TEAD in the
18 nuclear fractions of HPAF II cells as a consequence of 10 or 20 μ M dobutamine exposure. Quantification of
19 TEAD expression values are mean of 3 independent experiments \pm SEM; *p < 0.05.

20

21 **Figure 3 - Redistribution of Ocln and c-Yes in HPAFII cells and restriction of tricellulin to tri-cellular**
22 **contacts following dobutamine treatment.**

23 (A) Confocal microscopy showing c-Yes and Ocln distribution in HPAFII cells. Cells were treated with 10 or
24 20 μ M dobutamine for 24 h period prior to c-Yes and Ocln cell distribution analysis. Scale bar, 20 μ m. (B)

1 Immunoprecipitation of Ocln followed by an immunoblot analysis for c-Yes in nuclear, cytoplasmic, and
2 membrane fractions of non-treated and 20 μ M dobutamine treated HPAFII cells. (C) Confocal images
3 showing distribution of Ocln and tricellulin (Tric) in HPAFII cells treatment with 20 μ M dobutamine for 24
4 h. Scale bar, 20 μ m. (D) Immunoprecipitation of Ocln followed by an immunoblot analysis for Tric in
5 nuclear, cytoplasmic, and membrane fractions of HPAFII cells following 20 μ M dobutamine exposure.
6 *Abbreviations: Nucl (Nuclear fraction); Cyt (Cytosolic fraction); Memb (Membrane fraction).*

7

8 **Figure 4 - Dobutamine treatment of polarized cell sheets of HPAFII cells.**

9 Bright field light micrograph of confluent HPAFII cells grown on plastic without treatment (A), or after 24 h
10 exposure to 10 μ M dobutamine (B) or 20 μ M dobutamine (C); scale bar, 50 μ m. (D) TER values of HPAFII
11 cell sheets cultured on permeable supports in the absence or presence of dobutamine. (E) Z-stacks of non-
12 treated polarized HPAF II cells (top panel) or HPAFII monolayers exposed to 10 μ M or 20 μ M of
13 dobutamine (middle and bottom panel respectively); red (Ocln), blue (cell nuclei). (F) Immunoblot for total
14 Ocln in HPAFII cell monolayers at day 10. (G) TER values acquired on day 10 – note this is a separate study
15 from data shown in (D). (H) Quantification of total Ocln in HPAFII cells at day 10 culture comparing non-
16 treated with cells exposed to dobutamine; values are mean of 3 independent experiments \pm SEM; *p <
17 0.05. (I) Immunoblot for Ocln in HPAFII cells present in isolated fractions before and after treatment with
18 dobutamine. (J) TER measurements in HPAFII confluent monolayers seeded in Transwell® filters treated on
19 day 7 with 20 μ M Y-27632 or 20 μ M dobutamine, either alone or in combination; values are mean of 3
20 independent experiments \pm SEM; *p < 0.05; (K) Paracellular permeability of HPAFII cells cultured on trans-
21 well filters, using 4 KDa fluorescently labeled FITC-Dextran - 20 μ M of Y-27632 or 20 μ M of dobutamine,
22 either alone or in combination, was added to the apical chamber on day 11; afterwards fluorescence levels
23 were measured in the receiver outer chamber; Values are mean of 3 independent experiments \pm SEM,
24 p* < 0.5, **p < 0.01; (L-left hand side) Confocal microscopy images of HPAFII cells seeded on trans-well

1 filters, after being exposed to 20 μ M Y-27632 either alone or in combination with 20 μ M dobutamine
2 compared to non-treated cells; Scale bar, 20 μ m; (L-right hand side) Bright field images of HPAFII cells
3 seeded on 6 well plates after being treated with 20 μ M Y-27632 or 20 μ M dobutamine, either alone or in
4 combination, compared to non-treated cells; scale bar, 50 μ m. *Abbreviations: Nucl (Nuclear fraction); Cyt*
5 *(Cytosolic fraction); Memb (Membrane fraction).*

6
7 **Figure 5 – Distribution and Co-localization of c-Yes, Ocln, YAP, and TEAD in AsPc1 cells *in vitro*.**

8 (A) Immunofluorescent distribution and confocal co-localization of YAP and c-Yes, Ocln and c-Yes, Ocln and
9 YAP, and Ocln and TEAD. Higher magnifications of co-localizations are presented (A1-4). Graphs showing
10 overlapping fluorescence intensity are presented below associated to the micrograph with red line
11 denoting path of analysis; scale bar, 20 μ m.

12
13 **Figure 6 – Dobutamine suppresses proliferation through Ocln.**

14 Cell proliferation of HPAF II cells (A) and AsPc1 cells (B) exposed to 10, 20 and 50 μ M Dobutamine over 24
15 h and 48 h; values are expressed as mean \pm SEM from 3 independent experiments; *p < 0.05; **p < 0.01
16 compared to non-treated cells. HPAFII (C) and AsPc1 (D) cells treated with siRNA targeting Ocln or a
17 scrambled, control siRNA at the same time as dobutamine was added to initiate the time course of the
18 study. Cell proliferation was determined using a live cell fluorescent label. Values are mean of 3
19 independent experiments compared to siRNA treated levels \pm SEM; *p < 0.05; ***p < 0.001. (E)
20 Immunoblot showing Ocln knockdown and quantification of protein expression.

21
22 **Figure 7 - Immunostaining of normal and cancerous pancreatic tissue biopsies.** Individual biopsies,
23 obtained from donation (normal) or surgical biopsy (PDAC), were fixed, embedded, sectioned, mounted

1 on slides, and stained with specific antibodies following an antigen-retrieval protocol. Each picture
2 represents one image field per patient sample biopsy. Antibody labeling was detected using a peroxidase
3 reaction prior to counter-staining with Meyer's Haematoxylin. Scale bar, 50 μ m.

4

5 **SUPPLEMENTARY FIGURES**

6 **Figure S.1** – Incubation of HPAFII monolayers with PKC ζ -PS ablated TER and treatment with dobutamine
7 failed to have any effect on this outcome. Related to Figure 4. (A) Bright field images of HPAFII cells
8 treated with dobutamine either alone or in combination with 50 μ M PKC ζ -PS; (B) TER measurement of
9 HPAFII cells treated with dobutamine either alone or in combination with 50 μ M PKC ζ -PS; (C) Confocal
10 microscopy of HPAFII cells treated with dobutamine either alone or in combination with 50 μ M PKC ζ -PS;
11 scale bar, 50 μ m.

12 **Figure S.2** - YAP trans locates to the cytoplasm and Ocln formation gets stabilized at the cell membrane of
13 AsPc1 cells; Related to Figure 5. (A) Confocal images showing YAP trans-location from the nucleus to the
14 cytoplasm and Ocln formation at the cell membrane as cells are treated with 10 or 20 μ M dobutamine for
15 a 24h period. Scale bar, 20 μ m;

16 **Figure S.3** – c-Yes trans-locates from the cytosol to the cell membrane of AsPc1 cells; Related to Figure 3.
17 (A) Confocal images showing co-localization of Ocln and c-Yes as cells are treated with 10 and 20 μ M
18 dobutamine for a 24h period. Scale bar, 20 μ m; (B) Immunoprecipitation of Ocln followed by an
19 immunoblot analysis for c-Yes in nuclear, cytoplasmic, and membrane fractions of non-treated and 20 μ M
20 dobutamine treated HPAFII cells. *Abbreviations: Nucl. – Nuclear fraction; Cyt. – Cytosolic fraction; Memb.*
21 *– Membrane fraction; WCL – Whole cell lysate input.*

22

1 **Figure S.4** - Reduced nuclear TEAD signal after dobutamine treatment in AsPc1 cells; Related to Figure 2.
2 Confocal images showing a progressively weaker TEAD labeling as cells are treated with dobutamine for a
3 24h period. Scale bar, 20 μm

4 **Figure S.5** – Ocln interacts with YAP at the cell membrane of AsPc1 and not in HPAFII cells.
5 Immunoprecipitation of YAP followed by an immunoblot analysis for Ocln in cytoplasmic and membrane
6 fractions of AsPc1 and HPAFII cells. Related to Figures 1 and 5. *Abbreviations: Nucl (Nuclear fraction); Cyt*
7 *(Cytosolic fraction); Memb (Membrane fraction).*

8 **Figure S.6** – c-Yes and YAP distribution in pancreatic biopsies from five different patients diagnosed with
9 PDAC compared to normal pancreatic sections. Each picture represents one image field per patient sample
10 biopsy. Related to Figures 1 and 3. Scale bar, 50 μm

11 **Figure S.7** – TEAD and Ocln distribution in pancreatic biopsies from five different patients diagnosed with
12 PDAC compared to normal pancreatic sections. Each picture represents one image field per patient sample
13 biopsy. Related to Figures 1 and 2. Scale bar, 50 μm

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1 **TABLES**

2 TABLE I - Sequences of siRNA used to knockdown human Ocln were prepared as SMARTpool and used for
3 transfections.

4

Species	Target	Sequence
Human	Ocln	GCAUUAACUAGCUGGAUGU
Human		CCCGUUUGGAUAAAGAAUU
Human		GUCAGAGGUUUAGAUUAGA
Human		CUCUUC AACUGUUAGAUUC

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