Identification of BRAF as a new interactor of PLCε1, the protein mutated in nephrotic syndrome type 3

Hassan Chaib¹, Bethan E. Hoskins¹, Shazia Ashraf¹, Meera Goyal², Roger C. Wiggins², and Friedhelm Hildebrandt¹, ³
Departments of ¹Pediatrics, ²Internal Medicine, and ³Human Genetics, University of Michigan, Ann Arbor, Michigan 48109, USA

Running head: BRAF interacts with PLCε1

Correspondence to:
Friedhelm Hildebrandt, M.D.
University of Michigan Health System
8220C MSRB III, 1150 West Medical Center Drive
Ann Arbor, MI 48109-5646, USA
Phone: 734-615-7285
Fax: 734-615-1386
Email: fhilde@umich.edu
Steroid resistant nephrotic syndrome is a malfunction of the kidney glomerular filter that leads to proteinuria, hypoalbuminemia, edema, and renal failure. Recently, we identified recessive mutations in the phospholipase C epsilon 1 gene (PLCE1) as a new cause of early-onset nephrotic syndrome and demonstrated interaction of PLCε1 with IQGAP1. To further elucidate the mechanism by which PLCE1 mutations cause nephrotic syndrome, we sought to identify new protein interaction partners of PLCε1. We utilized information from the genetic interaction network of C. elegans. It relates the PLCE1 ortholog (plc-1) to the C. elegans ortholog (lin-45) of human BRAF (v-raf murine sarcoma viral oncogene homolog B1). We hypothesized that this may indicate a functional protein-protein interaction. Using GST pull down of HEK293T cell lysates in vitro and coimmunoprecipitation of mouse kidney lysates in vivo we show that BRAF interacts with PLCε1. By immunohistochemistry in rat kidney we demonstrate that both proteins are coexpressed and colocalize in developing and mature glomerular podocytes, reporting for the first time the expression of Braf in the glomerular podocyte.

**KEYWORDS**

Nephrotic syndrome, glomerulus, immunocytochemistry
INTRODUCTION

Nephrotic syndrome (NS) is a common kidney disease characterized by disruption of the glomerular filtration barrier of the kidney, leading to proteinuria, hypoalbuminemia, and edema. The filtration barrier of the kidney is composed of the interdigitating foot processes of podocytes and fenestrated endothelial cells separated by the glomerular basement membrane.

Monogenic forms of NS have been described and segregate either as autosomal dominant or recessive disorders. Dominant mutations have been identified in $\alpha$-actinin-4 (ACTN4) (12), canonical transient receptor potential 6 ion channel (TRPC6) (23, 34), and Wilms tumor suppressor gene 1 (WT1) (20). Recessive mutations have been identified in nephrin (NPHS1) (16), podocin (NPHS2) (1), and laminin $\beta2$ (LAMB2) (35). All monogenic forms are resistant to treatment and cause focal segmental glomerulosclerosis (FSGS).

Two histologically distinct forms of nephrotic syndrome are diffuse mesangial sclerosis (DMS) and FSGS. DMS is a disorder of glomerular development resulting in global scarring and loss of filtration surface within the first 4 years of life and rapid progression to end-stage kidney disease (ESKD) (6, 7, 8). FSGS is characterized by late onset of proteinuria and slower progression to ESKD.

Recently, we identified mutations in the PLCE1 gene as a new cause of autosomal recessive nephrotic syndrome in children that present with DMS or FSGS (9). Mutations in PLCE1 cause arrest of glomerular podocyte development at the S-shaped stage, thereby halting glomerular development and causing
nephrotic syndrome (9, 22). PLC\(\varepsilon\)1 is a phospholipase enzyme that catalyzes the hydrolysis of phosphatidylinositol-4,5-bisphosphate and generates two second messengers: inositol 1,4,5-triphosphate (IP3) and diacylglycerol (DAG) (33). IP3 releases Ca\(^{2+}\) from intracellular stores, and DAG stimulates protein kinase C. Both messengers initiate a cascade of intracellular responses that result in differential gene expression, cell growth and differentiation (33).

Most of the mutations identified in nephrotic syndrome affect genes expressed in podocytes (30). Therefore, proteins expressed in the podocyte are of interest to elucidate the pathogenesis of nephrotic syndrome. Discovering proteins expressed in podocytes that interact with PLC\(\varepsilon\)1 will help to understand the signaling pathways and molecular mechanisms by which mutations in PLC\(\varepsilon\)1 causes nephrotic syndrome. We set out to identify a new interaction partner of PLC\(\varepsilon\)1. \(PLCE1\) was initially discovered as the \(C.\, elegans\) ortholog \(plc-1\) (29). A database of putative protein interaction partners has been generated for \(C.\, elegans\), using computationally integrated interactome, gene expression, phenotype, and functional annotation data from \(S.\, cerevisiae\), \(C.\, elegans\) and \(D.\, melanogaster\) (36). We hypothesized that proteins suggested by this database as interaction partners of \(C.\, elegans\) \(plc-1\) may also be candidate interaction partners of the human ortholog PLC\(\varepsilon\)1. The database relates \(plc-1\) to the \(C.\, elegans\) ortholog \(lin-45\) of human \(BRAF\) by the criteria of “same \(C.\, elegans\) phenotype (sterile)” and “same \(C.\, elegans\) biological process (signaling)”. We therefore examined whether human PLC\(\varepsilon\)1 and BRAF interact within a protein
complex. We demonstrate that BRAF is expressed in glomerular podocytes and is in fact in a complex with PLC81.
MATERIALS AND METHODS

Cell culture

HEK293T and COS-7 cells between passage 15 and 20 were grown at 37°C as a monolayer culture in a humidified air atmosphere with 5% CO₂ using minimal essential medium or Dulbecco's modified Eagle medium (Invitrogen, Carlsbad, CA.) supplemented with L-glutamine, 10% heat-inactivated fetal bovine serum, and 1% penicillin-streptomycin-neomycin.

Antibodies

Two rabbit polyclonal anti-PLCε1 antibodies, CS117 and RA1 have been characterized previously as described in (9). Mouse monoclonal anti-BRAF (sc-5284), rabbit polyclonal anti-BRAF (sc-166), mouse monoclonal anti-GFP (sc-9996), and mouse monoclonal anti-GST (sc-138-HRP) antibodies were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). The secondary goat anti-rabbit-HRP (cat# 31462) and goat anti-mouse-HRP (cat #31432) antibodies were obtained from Pierce Biotechnology Inc. (Rockford, IL). For cell immunofluorescence studies, the fluorescence conjugated secondary antibodies raised in goat, alexa 488 and alexa 594 were obtained from Molecular Probes (Carlsbad, CA). For immunohistochemistry studies, we used rabbit anti-PLCε1 antibody (RA1), mouse anti-BRAF antibody, and mouse monoclonal antipodocalyxin antibody (2A4) (14, 15). Cy3-conjugated goat anti-rabbit and FITC-conjugated goat anti-mouse antibodies were used as secondary antibodies (Invitrogen).
Cloning vectors

The full length human cDNAs of PLCE1 and BRAF were cloned into pENTR/D-TOPO vectors (Invitrogen). We then used the Gateway Technology to flip both cDNAs into different destination vectors as described below. For yeast-2-hybrid screening, pDEST22 and pDEST32 vectors were used. The full length human cDNA of BRAF and five partial constructs covering different domains of the full length human cDNA of PLCE1 were cloned as bait and prey in pDEST32 and pDEST22 vectors, respectively. For GST pull down and coimmunoprecipitation, we used pDEST27 and pDEST53 in order to express GST-tagged and GFP-tagged proteins, respectively. Empty pDEST27 vector was used as a control.

GST pull down

The full length human cDNAs of PLCE1 and BRAF genes were coexpressed in HEK293T as GFP-tagged proteins together with GST-tagged proteins or with the GST-empty control plasmid. Transfection was carried out using the FuGENE method (Roche Applied Science, Palo Alto, CA.). After 48 hours transiently transfected cells were washed with cold PBS and then proteins were extracted using RIPA buffer with protease inhibitors (cat #11836170001 Roche). Glutathione-Agarose beads (cat #: G4510, Sigma, St Louis. MO) were added to the protein lysates and were incubated overnight at 4°C. After washing several times with cold PBS with protease inhibitors, beads were suspended and denatured in Laemmli sample buffer and loaded onto SDS polyacrylamids gels (4-15%). Gels were transblotted onto nitrocellulose filters. Filters were
immunoblotted with mouse monoclonal anti-GFP antibody. The secondary antibody was an HRP-conjugated goat anti-mouse antibody. Blots were developed with ECL substrate (Santa Cruz sc-2048). For the GST pull down and transfection controls the blots were stripped and re-probed with a mouse anti-GST-HRP antibody.

**Coimmunoprecipitation**

For HEK293T endogenous expression, cells were transfected with full length human *PLCE1* cDNA. After 48 hours, cell lysates were precleared with protein A/G PLUS-Agarose beads from Santa Cruz (sc-2003), and incubated overnight at 4°C with either rabbit polyclonal anti-PLCε1 antibody (CS117) or normal rabbit IgG serum (sc-2027) as control, followed by incubation with A/G PLUS-Agarose beads for 3 hours. The beads were washed extensively with cold PBS containing protease inhibitors, and bound proteins were boiled in sample buffer and resolved on a 4-15% SDG-PAGE gel and transblotted. The blots were immunoprobed with mouse anti-BRAF. For endogenous mouse kidney studies, kidneys obtained from a male adult *129S6* mouse were homogenized in 0.5% triton-X100 with protease inhibitors and cleared by centrifugation. Lysates were further precleared using A/G PLUS-Agarose beads and immunoprecipitated with rabbit anti-PLCε1 (CS117) and immunoblotted with mouse anti-BRAF. For reverse coimmunoprecipitation, lysates were immunoprecipitated with mouse anti-BRAF and blots were immunoblotted with rabbit anti-PLCε1 (CS117).
**Immunofluorescence in HEK293T and COS-7 cells**

For exogenous expression of BRAF and PLCε1 in HEK293T and COS-7 cells, full length human *PLCE1* and *BRAF* cDNA were coexpressed in HEK293T and COS-7 as GST-tagged proteins. Transfection was carried out in four slide chambers using the FuGENE method (Roche Applied Science). After 48 hours, transiently transfected cells were fixed in methanol for 15 mins at -20°C. The cells were washed with PBS and then blocked with TBST containing 0.15% heat inactivated fetal bovine serum for 1 hour at room temperature. Double immunofluorescence staining was performed with polyclonal rabbit anti-PLCε1 (CS117) and mouse monoclonal anti-BRAF. Alexa 488 conjugated goat anti-rabbit and Alexa 594 conjugated goat anti-mouse were used as secondary antibodies. Slides were washed and mounted with Prolong Gold antifade reagent with DAPI (Invitrogen). All images were captured with a Leitz DMRB microscope and an Optronics camera.

For endogenous expression of BRAF in HEK293T cells, cells were methanol fixed, washed and images captured as described in the exogenous studies. Immunofluorescence staining was performed with mouse monoclonal anti-BRAF. Alexa 594 conjugated goat anti-mouse was used as secondary antibody.

**Immunofluorescence in kidney sections**

Kidneys from 2 month old Fisher 344 rats were perfusion-fixed with periodate-lysine-paraformaldehyde (PLP) as previously described (32). Cryostat-cut kidney sections were treated with Retrieve-All™ target unmasking reagent
(Signet Laboratories, Inc. Dedham, MA) for 2 hrs at 90°C. Two day old Fisher 344 rat kidney sections (n=1) were used for developmental studies. Sections were blocked with 10% goat serum in PBS. Double immunofluoresene staining was performed with anti-PLCє1 antibody (RA1) and mouse anti-BRAF. We also used the 2A4 monoclonal anti-podocalyxin antibody as a marker of the apical surface of podocytes. Cy3-conjugated goat anti-rabbit and FITC-conjugated goat anti-mouse antibodies were used as secondary antibodies. Sections were co-stained with DAPI for nuclear staining.

The animals work was approved by the University Committee on Use and Care of Animals (UCUCA # 7738, # 8608)
RESULTS

BRAF is in a complex of proteins with PLCε1

We evaluated whether BRAF would be part of a signaling complex with PLCε1. We sought to investigate the interaction by glutathione S-transferase (GST) pull down assay. Following cotransfection of HEK293T cells with the full length human cDNA encoding PLCE1 fused with green fluorescent protein (GFP) (GFP-PLCE1) and the full length human cDNA of BRAF fused to GST (GST-BRAF), we demonstrated by GST pull down that GST-BRAF can pull down GFP-PLCε1 (Figure 1, panel A, lane 4). Reciprocally, GST pull down of GST-PLCε1 yielded GFP-BRAF in the pull down as shown in (Figure 1, panel A, lane 10).

We have therefore demonstrated that BRAF interacts with PLCε1 and we conclude that BRAF can form a complex with PLCε1

BRAF and PLCε1 proteins colocalize in HEK293T and COS-7 cell lines

First we evaluated the sub-cellular localization of endogenous BRAF expression in HEK293T cells. Following immunostaining with mouse anti-BRAF we demonstrated a cytoplasmic sub-cellular localization of BRAF in HEK293T cells (Figure 2, panel A, (a,b)). The signal is specific to BRAF as this staining pattern was not seen in the ‘secondary only ‘ control (Figure 2, panel A, (c)).

To further evaluate the identification of BRAF as an interaction partner of PLCε1, we studied the sub-cellular localization of exogenous BRAF and PLCε1 proteins. We evaluated the distribution of both proteins in two cell lines, HEK293T and COS-7. Following the expression of GST-PLCε1 and GST-BRAF
fusion proteins, and double immunofluorescence analysis with rabbit anti-PLC\textsubscript{1} and mouse anti-BRAF, we demonstrated an overlap of cytoplasmic sub-cellular distribution of BRAF and PLC\textsubscript{1} (Figure 2, panel B). The expression patterns of BRAF and PLC\textsubscript{1} proteins were overlapping in both cell lines, compatible with an interaction of BRAF and PLC\textsubscript{1} in both cells.

**Endogenous BRAF interacts with endogenous PLC\textsubscript{1}**

As both studies carried out above are *in vitro* studies we examined protein-protein interaction of PLC\textsubscript{1} and BRAF at the endogenous level by immunoprecipitation. HEK293T cells express endogenous BRAF (Figure 3, panel A), however the endogenous expression of PLC\textsubscript{1} in HEK293T cells is low or undetectable (9). To study the interaction between endogenous BRAF and overexpressed PLC\textsubscript{1}, we expressed GFP-PLC\textsubscript{1} fusion protein in HEK293T cells and immunoprecipitated with rabbit anti-PLC\textsubscript{1} (CS117). Immunoblotting analysis revealed that endogenous BRAF can be immunoprecipitated by the overexpressed PLC\textsubscript{1} but not by a control IgG (Figure 3, panel A). We therefore demonstrated that endogenous BRAF interacts with the exogenous PLC\textsubscript{1}.

To study the coimmunoprecipitation of endogenous PLC\textsubscript{1} and endogenous BRAF, we performed coimmunoprecipitation studies in mouse kidney lysates. Lysates from mouse kidneys were immunoprecipitated overnight with polyclonal rabbit anti-PLC\textsubscript{1} antibody (CS117). Immunoblotting analysis revealed that endogenous BRAF can be coimmunoprecipitated by an antibody to endogenous PLC\textsubscript{1} but not by a control IgG (Figure 3, panel B). We therefore
demonstrated that the endogenous PLCε1 co-precipitates with endogenous BRAF. However, we were not able to demonstrate the reciprocal coimmunoprecipitation. The reason for this result is not known, but it may be due to the BRAF antibody masking the interaction epitope by a third protein since the interaction is not direct. Another possibility is that a conformational change in BRAF may occur, making the epitope less accessible to the BRAF antibody.

**BRAF is an indirect interaction partner of PLCε1**

PLCε1 contains multiple known protein-protein interaction domains, suggesting that it may represent a scaffolding protein of signaling processes. To test whether the interaction of PLCε1 and BRAF may be direct, we performed yeast-2-hybrid studies using five different subclones of *PLCE1* as bait and full-length *BRAF* as prey. Analysis of these studies failed to show any direct interaction between the five partial domains of PLCε1 and BRAF (data not shown). No interaction was seen when the clones were reversed, using BRAF as bait and PLCε1 subclones as prey. Therefore we conclude that BRAF does not interact directly with PLCε1 (data not shown).

**Expression and colocalization of endogenous BRAF and PLCε1 protein in rat podocyte glomerulus**

To further examine BRAF expression in renal glomerulus, we performed immunohistochemistry studies in newborn and adult rat kidney sections. In these studies, the podocyte apical marker podocalyxin was used to delineate the
glomerular structure (28). Using a monoclonal mouse anti-BRAF antibody, we demonstrated for the first time that Braf is expressed in podocytes at the S-shaped stage of glomerular development in the newborn rat (Figure 4a-c), and in adult rat glomerulus (Figure 4d-f), as demonstrated by the colocalization with the podocyte podocalyxin marker (Figure 4c, 4f). Braf expression was not limited to podocytes as it was present in tubular epithelial cells in both newborn developing kidneys and to a lesser extend in adult kidneys (Figure 4a, 4d). In contrast, PLCε1 expression was restricted to podocytes in both developing and adult kidneys implying that Braf may have functions other than through its interactions with PLCε1. PLCε1 is known to be expressed in the podocyte, and mutations in *PLCE1* lead to an arrest of glomerular development (9). To further evaluate the identification of BRAF as an interactor of PLCε1, we examined the colocalization of BRAF and PLCε1 in developing podocytes of rat kidney sections (Figure 4g-i). Using mouse anti-BRAF and rabbit anti-PLCε1 (RA1), we demonstrated a colocalization of both proteins in developing podocytes (Figure 4i).
DISCUSSION

We utilized a *C. elegans* genetic interaction database to generate further candidate interaction partners for PLC\(\varepsilon\)1/plc-1 (36). Using a GST pull down assay we were able to demonstrate that BRAF and PLC\(\varepsilon\)1 are in the same protein complex. By immunofluorescence studies we showed sub-cellular colocalization of both proteins in two cell lines, HEK293T and COS-7, supporting the interaction of both exogenous proteins. Furthermore, we demonstrate that exogenous PLC\(\varepsilon\)1 is able to precipitate endogenous BRAF in HEK293T cell lines and endogenous PLC\(\varepsilon\)1 can precipitate endogenous BRAF in mouse kidney, providing evidence of *in vivo* interaction between BRAF and PLC\(\varepsilon\)1. Using immunohistochemistry studies in rat kidney, we demonstrated for the first time that BRAF is expressed in podocytes and colocalizes with PLC\(\varepsilon\)1, supporting their interaction in glomerular podocytes. We thereby identify BRAF as a new interaction partner of PLC\(\varepsilon\)1. A negative result in yeast-2-hybrid interaction studies is compatible with the hypothesis that the interaction of PLC\(\varepsilon\)1 and BRAF may be indirect.

PLC\(\varepsilon\)1 contains several protein domains: RasGEF\_CDC25 (guanine nucleotide exchange factor for Ras-like small GTPases domain), PH domain (pleckstrin homology domain), EF hand, phospholipase catalytic domains (PLC\_X and PLC\_Y), C2 motif (protein kinase C conserved region 2, subgroup 2), and RA1 and RA2 domains (RasGTP binding domain from guanine nucleotide exchange factors). Most of the predicted domains and motifs of human PLC\(\varepsilon\)1 are highly conserved in plce1 orthologs of evolutionarily distant
organisms such as *D. rerio* and *C. elegans*, suggesting a conserved function of the domain assembly within PLC\(\varepsilon\)1 (9). It has been shown that the RA1 and RA2 domains of PLC\(\varepsilon\)1 directly interact with activated H-Ras (13, 33), which is upstream of the MEK1/2, and ERK1/2 signaling within the Ras/MAP kinase pathway. Recently we identified IQGAP1 (IQ motif-containing GTPase-activating protein 1) as an interactor of PLC\(\varepsilon\)1 (9). IQGAP1 has also been shown to interact with the critical slit diaphragm protein nephrin, another protein defective in nephrotic syndrome (16). IQGAP1 is co-expressed with PLC\(\varepsilon\)1 in the S-shaped and capillary loop stages of glomerular development (9, 17,18). Since IQGAP1 is a known scaffold protein for MAP kinase signaling (26, 27), it represents the second protein of this pathway after H-Ras that interacts with PLC\(\varepsilon\)1 (13, 33).

Recently IQGAP1 has been shown to interact directly with BRAF (24).  

**BRAF** is localized on human chromosome 7q34. The *BRAF* gene was initially discovered as a transforming gene in NIH3T3 cell transfection assays with human Ewing sarcoma DNA (10). *BRAF* belongs to the *RAF* family of genes. In mammals, there are three highly conserved *RAF* genes, *ARAF* (otherwise known as *A-Raf*), *BRAF* (*B-Raf*), and *CRAF* (*C-Raf* or *Raf-1*) (5). *BRAF* encodes a serine-threonine kinase protein of 766 amino acid residues that contains a Raf-like Ras-binding domain (RBD) and a protein kinase C conserved region 1 domain (C1). The RBD domain of BRAF binds directly to H-Ras (21). A defect in BRAF is involved in a wide range of cancers (4). The most common mutation, which accounts for more than 90% of cancer cases caused by mutations in *BRAF*, is a glutamic acid for valine substitution at position 600.
(V600E). BRAFV600E is activated and induces constitutive ERK signaling through hyperactivation of RAS-MEK-ERK pathways, and constitutive Nuclear factor kappa-B (NF-κB), stimulating proliferation, survival and transformation (5).

BRAF was identified as a major MEK activator in neuronal tissue (2, 11, 19, 31). It is involved in the transduction of mitogenic signals from the cell membrane to the nucleus. BRAF serves as a central intermediate in many signaling pathways by connecting upstream tyrosine kinases with downstream serine/threonine kinases, such as mitogen-activated protein kinase (MAPK) and MAPK kinase (MKK, also known as MEK) (3, 25). We have identified BRAF, a protein which has been implicated in the MAP kinase pathway, as a third interaction partner of PLCγ1 in addition to H-Ras and IQGAP1. This opens the MAP kinase signaling pathway as an avenue of interest to study how defects in this pathway may be involved in the pathogenesis of nephrotic syndrome. Identification of additional proteins that are expressed in the podocyte and interact directly or indirectly with PLCγ1 will help in the understanding of how mutations in PLCE1 cause nephrotic syndrome.
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Disclosure

The authors have no financial interests to disclose.
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TITLES AND LEGENDS TO FIGURES

Figure 1. GST pull down in HEK293T cells lysates of tagged PLCε1, tagged BRAF and GST-empty vector.

Panel A. Following cotransfection of HEK293T cells with GFP-PLCε1 and GST-BRAF (lanes 1, 2, 4, 5), GFP-PLCε1 was detected after GST-BRAF pull down (lane 4). Following cotransfection of HEK293T cells with GFP-BRAF and GST-PLCε1 (lanes 7, 8, 10, 11), GFP-BRAF was detected after GST-PLCε1 pull down (lane 10). Membrane was immunoblotted with mouse anti-GFP.

Panel B. The blot of Panel A was probed with mouse anti-GST antibody as control for loading and pull down efficiency.

Figure 2. Sub-cellular localization of PLCε1 and BRAF in HEK293T and COS-7 cells.

Panel A. Sub-cellular localization of endogenous BRAF in HEK 293T cells. HEK293T cells were immunostained with mouse anti BRAF following with a goat anti-mouse (alexa 594), showing cytoplasmic localization of endogenous BRAF in HEK293T (red,a). A merged image with DNA stained with DAPI (blue) is shown in (b). A staining control with secondary goat anti-mouse only and DAPI is shown in (c).

Panel B. Colocalization of PLCε1 with BRAF in HEK293T and COS-7 cells. Expression of PLCε1 (green a, d) and BRAF (red b,e) was seen in HEK293T and COS-7 cells. Whenever cells are transfected with both proteins there is full colocalization of the two proteins as seen in the merged images (c, f) as yellow. Nuclei (DNA) were stained with DAPI (blue).
Figure 3. Coimmunoprecipitation of PLCε1 and BRAF in HEK293T cells and mouse kidney lysates.

Panel A. Exogenous PLCε1 immunoprecipitates endogenous BRAF in HEK293T cells.

HEK293T cells were transfected with GFP-PLCε1 and endogenous human BRAF was detected after immunoblotting with mouse anti-BRAF. Rabbit IgG was used as control for the IP.

Panel B. Endogenous PLCε1 immunoprecipitates endogenous BRAF in mouse kidney lysates.

Adult mouse kidney lysates were immunoprecipitated with polyclonal rabbit anti-PLCε1 (CS117) and endogenous mouse Braf was detected after immunoblotting with mouse anti-BRAF. Rabbit IgG was used as control for the immunoprecipitation.

Figure 4. Immunofluorescent photomicrographs showing the distribution of Braf in rat glomeruli.

Panels a-c from newborn rat kidney at the S-shaped stage of glomerular development demonstrating that Braf is widely distributed (panel a). Panel b shows podocalyxin (green) which marks the apical surface of developing podocytes (arrowhead). Nuclei are stained in blue with DAPI. The merged image (Panel c) shows that developing podocytes contain Braf. Panels d-f are from adult rat glomerulus. Panel d shows that cells in the glomerulus contain Braf (arrows). Panel e shows nuclei (blue) and podocalyxin (green) to delineate the glomerular structures and to identify podocytes whose cell surface are decorated
with podocalyxin (arrows). **Panel f** (merge) shows that Braf is present in podocytes in the adult glomerulus. **Panels g-i** show colocalization of Braf with PLCε1 in a developing glomerulus. **Panel g** shows the distribution of Braf (green) in podocytes (arrowhead). **Panel h** shows the distribution of PLCε1 (red) and nuclei (blue) in the glomerulus. The merged image **Panel i** shows as yellow color where Braf and PLCε1 codistribute in developing podocytes.
Figure 1

Panel A

Panel B

Cotransfection into HEK293T cells

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WB: Mouse anti-GFP

WB: Mouse anti-GST
Figure 2

Panel A

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Panel B

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Figure 3

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WB: Mouse anti BRAF

Panel B

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WB: Mouse anti BRAF
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<td><img src="image8.png" alt="Image" /></td>
<td><img src="image9.png" alt="Image" /></td>
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Braf
Podocalyxin
Merge
PLCε1
Merge