Supporting Information

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SI Materials and Methods

Cell Culture. The mouse kidney collecting duct cell line mpkCCD-clone 11 was supplied by the National Heart, Lung, and Blood Institute (the M.A.K. Laboratory) (1). For maintenance, the cells were cultured in DMEM/Ham's F-12 medium (DMEM/F-12) containing 2% (vol/vol) FBS plus supplements (5 μg/mL insulin; 50 nM dexamethasone; 1 nM triiodotyrosine; 10 ng/mL epidermal growth factor; 60 nM sodium selenite; 5 μg/mL transferrin) at 37° and 5% (vol/vol) CO_2. For experiments, 10,000 cells per square centimeter were seeded onto membrane supports (Transwell; no. 3450; Corning Costar) to allow cell polarization (Fig. 1*A*). When the transepithelial electric resistance (TER) reached 5 $k\Omega \cdot cm^2$ or greater, the basolateral medium was switched to 0% serum DMEM/F-12 containing 60 nM sodium selenite, 5 μg/mL transferrin, and 1 nM vasopressin analog 1-deamino-8-D-arginine vasopressin (dDAVP) to induce endogenous aquaporin-2 (AQP2) expression. The apical medium was similar to the basolateral medium minus dDAVP. Cells were grown for ∼4 d before full polarization and high TER, and an additional 4 d with dDAVP to express an optimal amount of endogenous AQP2.

Immunoblotting. Cell proteins were solubilized in 2% (wt/vol) SDS sample buffer (50 mM Tris, pH 6.8) containing phosphatase inhibitor PhosSTOP (catalog no. 04906845001; Roche) and protease inhibitor mixture (catalog no. 05892791001; Roche). Protein concentrations were determined using the bicinchoninic acid (BCA) assay following the manufacturer's instructions (catalog no. SK3051; Bio Basic). After mixed with loading buffer [5×, 7.5% (wt/vol) SDS, 30% (vol/vol) glycerol, 200 mM DTT, 50 mM Tris, bromophenol blue, pH 6.8], proteins were separated on an SDS/PAGE gel and transferred to a nitrocellulose membrane. The membrane was blocked with 0.1% (wt/vol) bovine serum albumin (BSA) dissolved in phosphate-buffer saline (PBS) (137 mM NaCl, 2.7 mM KCl, 10.1 mM Na_2HPO_4, 1.8 mM KH_2PO_4, pH 7.4) containing 0.05% (vol/vol) Tween-20. After washes, the membrane was incubated with primary antibody overnight at 4 °C. After washes, the membrane was incubated with fluorophore-conjugated secondary antibody for 1 h at room temperature before washing and quantification of the fluorescence signals using a LI-COR Odyssey scanner and software. The AQP2 and protein Mal2 (Mal2) primary antibodies were previously characterized (2–4). Fluorophore-conjugated secondary antibodies were purchased from LI-COR (catalog nos. 92632229 and 92632220). For detection of biotinylated proteins (see below), IRDye800-conjugated streptavidin (catalog no. S11226; Invitrogen) was used.

Immunofluorescence Staining. Cells grown on membrane supports were chilled at 4 °C for 10 min, rinsed three times with ice-cold PBS-CM buffer (PBS plus 0.1 mM $CaCl_2$ and 1 mM $MgCl_2$), and fixed with ice-cold fixation buffer [4% (wt/vol) paraformaldehyde in PBS-CM] for 20 min on a rocking platform. After rinses with PBS-CM, the cells were treated with permeabilization buffer [PBS plus 0.3% (vol/vol) Triton X-100, 0.1% (wt/vol) BSA, and 1 mM NaN_3] for 30 min at room temperature on a rocking platform. To block nonspecific binding, the cells were incubated with blocking buffer [PBS plus 1% (wt/vol) BSA, 0.05% (wt/vol) saponin, 0.2% (wt/vol) gelatin, and 1 mM NaN_3]. The cells were then incubated with primary antibody diluted in the permeabilization buffer overnight at 4 °C on a rocking platform. After washes [PBS plus 0.1% (wt/vol) BSA, 0.05% (wt/vol) saponin, 0.2% (wt/vol) gelatin, and 1 mM NaN_3], the cells were incubated with secondary antibodies diluted in the permeabilization buffer 1 h at room temperature on a rocking platform. After rinses with PBS-CM, the cells were mounted in fluorescence mounting medium (Dako) under a cover glass. Confocal images were acquired with a Leica TCS SP5 microscope and processed with Leica LAS-AF software (Leica Microsystems). The AQP2 and Mal2 antibodies are described above. The zonula occludens protein 1 (ZO1) antibody was purchased from Invitrogen (catalog no. 339100). The secondary antibodies were Alexa488 anti-rabbit IgG (catalog no. A21206), Alexa568 anti-mouse IgG (catalog no. A11031), and Alexa568 anti-chicken IgG (catalog no. A11041) from Invitrogen. For detection of biotinylated proteins, Alexa568-conjugated streptavidin (catalog no. S11226; Invitrogen) was used. Rhodamine-phalloidin (catalog no. R415; Invitrogen) was used to visualize F-actin. Cell nuclei were labeled with PBS buffer containing 1 μg/mL of 4′,6-diamidino-2-phenylindole for 10 min.

Immunoelectron Microscopy. Cells grown on membrane supports were fixed with 4% (wt/vol) paraformaldehyde for 5 min at 37 °C, followed by 40 min on ice. After washing with PBS, the cells were permeabilized and blocked with 0.05% (wt/vol) saponin diluted in PBS containing 10% (vol/vol) goat serum and 1% (wt/vol) BSA for 30 min. The cells were incubated with primary antibody diluted in PBS containing 1% (wt/vol) BSA and 0.05% (wt/vol) saponin for 2 h. The cells were washed with the antibody diluent and incubated with a secondary antibody (Nanogold-IgG and fragment antigen-binding region Fab′ conjugates) for 2 h. After washes with the antibody diluent and PBS, the cells were fixed with 2% (wt/vol) glutaraldehyde dissolved in 0.1 M sodium phosphate buffer at room temperature for 30 min. Silver enhancement was performed following the manufacturer's instructions (catalog no. 2012; Nanoprobes) to increase visibility of the Nanogold particles. The cells were fixed again with 0.2% (wt/vol) osmium oxide solution for 30 min in a fume hood. After washing with water, the cells were incubated with 1% (wt/vol) uranium acetate [2% (wt/vol) uranium acetate/0.2 M sodium phosphate buffer at a 1:1 ratio] for 1 h. After rinsing with 30% (vol/vol) ethanol/water, the cells were dehydrated sequentially with 50%, 70%, 80%, and 90% (vol/vol) ethanol/water (10 min each), followed by 100% ethanol three times (10 min each time). Before embedding, the cells were rinsed with hydroxypropylmethacrylate (HPMA), followed by incubations with 1:3 epon/HPMA (15 min), 1:1 epon/HPMA (30 min), 3:1 epon/HPMA (1 h), and pure epon (overnight). The cells were then molded in pure epon with heating at 65 °C for 3 d. After sectioning into a thickness of 100 nm, the cells were observed and imaged under a transmission electron microscope (JEOL JEM 1400) equipped with an AMT 11-megapixel bottom-mount digital camera and software.

Apical Surface Biotinylation. The apical surface biotinylation method was modified from that of Tamma et al. (5). To minimize intracellular labeling attributable to endocytosis, the cells were chilled on ice for 20 min after experimental treatments. All subsequent apical surface biotinylation steps were done on ice. The apical medium was rinsed with ice cold PBS-CM (pH 6.4) buffer, whereas the basolateral medium remained unchanged. Before biotinylation, the apical medium was switched to freshly prepared oxidation buffer (PBS-CM plus 20 mM $NaIO_4$, pH 6.4) to oxidize exposed hydroxyl groups of the glycosylated proteins in the dark on a rocking platform for 30 min. After washes with PBS-CM (pH 6.4), the apical medium was changed to biotinylation

buffer [PBS-CM (pH 6.4) plus 5 mM biocytin hydrazide (catalog no. 28020; Thermo Scientific)] for 40 min on a rocking platform. After removal of the biotinylation buffer, the apical medium was switched to stabilization buffer (PBS plus 50 mM NH_4Cl, pH 7.4) for 5 min on a rocking platform. The apical and basolateral media were washed three times with PBS (pH 7.4) and the cells were saved for subsequent procedures.

Streptavidin-Affinity Chromatography. Biotinylated cells from one well of a Transwell plate (catalog no. 3450; Corning Costar) were solubilized in 400 μL radioimmunoprecipitation assay (RIPA) lysis buffer [150 mM NaCl, 50 mM Tris (pH 7.4), 5 mM EDTA, 1% (vol/vol) Nonidet P-40, 0.5% (wt/vol) Na-deoxycholate, 0.5% (wt/vol) SDS supplemented with phosphatase inhibitor mixture and protease inhibitor mixture]. The cell lysates were homogenized by sonication and centrifuged at 14,000 × g for 10 min to remove insoluble pellet. Before streptavidin-affinity chromatography, 200 μL Dynabeads (MyOneTM Streptavidin T1; Invitrogen) were added to a 1.5 mL tube and washed with the RIPA lysis buffer. Cell lysate (300 μL; about 1 mg of protein) was added to the beads and incubated overnight at 4 °C on a rotary mixer. After removal of the unbound fraction, the beads were washed 5 times with 300 μL of the RIPA lysis buffer. Bound proteins on the beads were eluted in 30 μL of 2% (wt/vol) SDS sample buffer at 95 °C for 15 min.

Stable-Isotopic Labeling with Amino Acids in Cell Culture. Stable-isotopic labeling with amino acids in cell culture (SILAC) advanced DMEM/F-12-Flex media (MS10033) and heavy isotope-labeled arginine and lysine were purchased from Invitrogen and prepared following the manufacturer's instructions. Light SILAC medium contained 100 mg/mL normal L-lysine (12C), 100 mg/mL normal L-arginine (12C and 14N), 15.8 mM glucose, 12.5 mL L-glutamine, 0.8 mL/L phenol red, and 15 mL/L Hepes. The heavy SILAC medium was similar to the light medium, except that normal L-lysine and L-arginine were replaced by heavy L-lysine (13C-labeled) and L-arginine (13C- and 15N-labeled). The doubling time of the mpkCCD cells was estimated at between 20 and 30 h (6). To allow complete isotope incorporation (7), the cells were initially grown in SILAC media containing 2% (vol/vol) dialyzed FBS plus supplements for 12 d. Cells were subcultured in the same SILAC medium when reaching confluence (between 3 and 4 d) to avoid quiescence or contact inhibition of cell growth. Twelve days of culture in the heavy medium resulted in >99% (peptide/peptide) isotope incorporation as measured by quantitative protein mass spectrometry (MS) (Table S4).

Liquid Chromatography–Tandem MS Shotgun Proteomics. Protein samples were reduced with DTT (10 mM) for 1 h at 56 °C, alkylated with iodoacetamide (40 mM) for 45 min at room temperature in the dark, and separated by 1D SDS/PAGE (12.5% wt/vol). The gel was rinsed with distilled deionized water and silver stained (SilverQuest; Invitrogen) before it was sliced into 26 pieces. Each piece was diced into small cubes (~1 mm^3) and destained in a 1.5-mL tube. The gel cubes were dehydrated with 25 mM NH_4HCO_3 in 50% (vol/vol) acetonitrile (ACN) and then allowed to completely dry under vacuum. Proteins in the gel cubes were digested with 12.5 ng/μL trypsin gold (V5280; Promega) overnight at 37 °C. Tryptic peptides were extracted with 50% (vol/vol) ACN/H_2O containing 0.5% (vol/vol) formic acid before drying under vacuum. The dried tryptic peptides were desalted with Supel-Tips C18 pipette tips (Sigma-Aldrich) before being subjected to liquid chromatography–tandem MS (LC-MS/MS) analysis (LTQ-Orbitrap XL; Thermo Scientific) in the Common Mass Spectrometry Facilities located at the Institute of Biological Chemistry, Academia Sinica. With the nanoAcquity system (Waters), tryptic peptides dissolved in solvent A [water with 0.1% (vol/vol) formic acid] were loaded onto a 75-μm inner diameter, 25-cm length C18 BEH column packed with 1.7-μm particles with a pore size of 130 Å (Waters). Peptides were eluted with a segmented gradient in 60 min from 5% (vol/vol) to 40% (vol/vol) solvent B [ACN with 0.1% (vol/vol) formic acid] at a flow rate of 300 nL/min at 35 °C. Eluted peptides were delivered to an LTQ-Orbitrap XL hybrid mass spectrometer using a nanospray interface (Proxeon). Data acquisition was done in a data-dependent mode. For each full-survey mass scan (MS1), the 10 highest intensity precursor peptides were elected for collision-induced dissociation and product peptide scan (MS2). MS1 was acquired in the Orbitrap (m/z 350–1,600) with the resolution set to 60,000 at m/z 400 and automatic gain control (AGC) targeted at 10^6. MS2 was done in the linear ion trap with AGC targeted at 7,000. Dynamic exclusion time was set to 90 s. Peptides with single or unrecognized charge states were excluded. All measurements in the Orbitrap were performed with the "lock mass" option for internal calibration.

LC-MS/MS Data Analysis. Database search and protein quantification were performed using the SEQUEST algorithm included in the Proteome Discover Version 1.3 (Thermo Scientific). The database used for the spectral searching was prepared from the mouse RefSeq database (National Center for Biotechnology Information; released on March 3, 2012 with 50,000 entries) plus common contaminants (common repository of adventitious proteins list; www.thegpm.org/crap/index.html). Precursor ion tolerance was 10 ppm, and product ion tolerance was 0.8 Da. Up to two missed trypsin cleavage sites were allowed. Static modifications included carbamidomethylation of cysteine (+57.021 Da). Dynamic modification included oxidation of methionine (+ 15.995 Da), isotope labeling of lysine (+6.020 Da), isotope labeling of arginine (+10.008 Da), and N-terminal acetylation (+ 42.011 Da). Protein identifications were filtered by the q values (<1%) of each peptide spectrum match (PSM) calculated by the Percolator algorithm (8). The Percolator algorithm used the reversed sequences of the input database to calculate the q values based on a list of PSMs reranked by a semisupervised machine learning method. The q value is the minimal false discovery rate at which a PSM is accepted. Known contaminant peptides were excluded. Quantification was calculated based on precursor peptide area. Only unique peptides matched to the proteins were selected for protein quantitation. The median value of all peptide abundance ratios was used to represent the protein abundance ratio in response to dDAVP vs. vehicle. Raw data can be downloaded from the PRoteomics IDEntifications (PRIDE) database web site (http://proteomecentral.proteomexchange.org, accession number PXD000399). Analyzed data as Microsoft Excel spreadsheets can be downloaded from the mpkCCD Quantitative Apical Membrane Proteome database (http://sbel.mc.ntu.edu.tw/mpkCCDqAMP/qAMP.htm).

Live-Cell Imaging. Cells were transfected with pLifeAct-TagGFP2, a plasmid encoding a GFP-conjugated 17-aa peptide that binds F-actin without interfering F-actin dynamics (no. 60101; ibidi) (9). Transfected cells were grown on membrane supports until polarization before they were excised and placed in modified glass-bottom Petri dishes [PCO-R chamber (catalog no. 0727.300; Zeiss) and glass coverslip (catalog no. 0727.016; PeCon)] with the apical plasma membrane facing downward against the coverslip (Fig. S4). After 50 μL of hormone- or drug-containing medium was applied to basolateral aspect of the cells, the cells were covered with a glass coverslip (catalog no. 0448-948; PeCon) to flatten the membrane support against the glass coverslip of the Petri dish. The Petri dish was transferred to a confocal microscope LSM780 (Zeiss) equipped with a 37 °C chamber for live-cell imaging. Images were taken with a 63× (1.4 NA) plan Apo objective (Zeiss) every 30 s and processed with the ZEN 2011 software.

Water Permeability Measurement. Cell water permeability was measured as described in ref. 10 with modifications. Cells were grown until confluent in six-well filter plates (Corning) and treated for the last 5 d with 1 nM dDAVP (or saline) added to the basolateral compartment. Cells were reseeded into 96-well black plates (Corning) at high density and used for measurements 12 h later. Cells were loaded for 30 min in 100 μL per well of DMEM/F12, 10 μM calcein-AM, and 2.5 mM water-soluble probenecid (Invitrogen). Cells were washed once in prewarmed (37 °C) incubation medium [0.8 mM $MgSO_4$, 5 mM KCl, 1.8 mM $CaCl_2$, 25 mM Na·Hepes (pH 7.5), and adjusted to 285 mOsmol with NaCl] and incubated in 75 μL per well of incubation medium including forskolin (25 μM), latrunculin B, or 0.1% (vol/vol) DMSO solvent controls for 20 min at 37 °C and 5% (vol/vol) CO_2 before measuring water permeability. Fluorescence was recorded on a multimode plate reader (Enspire Multimode Plate Reader; Perkin-Elmer) equipped with a 490-nm band-pass filter for excitation and a 520-nm filter for emission. After baseline fluorescence recording for 5–10 s, cell shrinking was induced by adding 75 μL per well of incubation medium containing 400 mM sucrose to an individual well using the instrument's liquid-handling system while continually monitoring fluorescence. This volume was sufficient to cause instantaneous mixing. The temperature in the reader was maintained at 25 °C, and dynamic changes in fluorescence intensity were recorded for an additional 30 s. All measurements were performed in duplicate on at least 4 individual days. Rate constants and half-life of cell shrinkage were calculated as described previously (10).

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Fig. S1. Workflow for stable isotope-based quantitative analysis of mpkCCD apical membrane proteome in response to the vasopressin analog dDAVP vs. vehicle. $^{m}X_{n}$, m, atomic mass; n, number of atom X in amino acid; X, atom symbol.

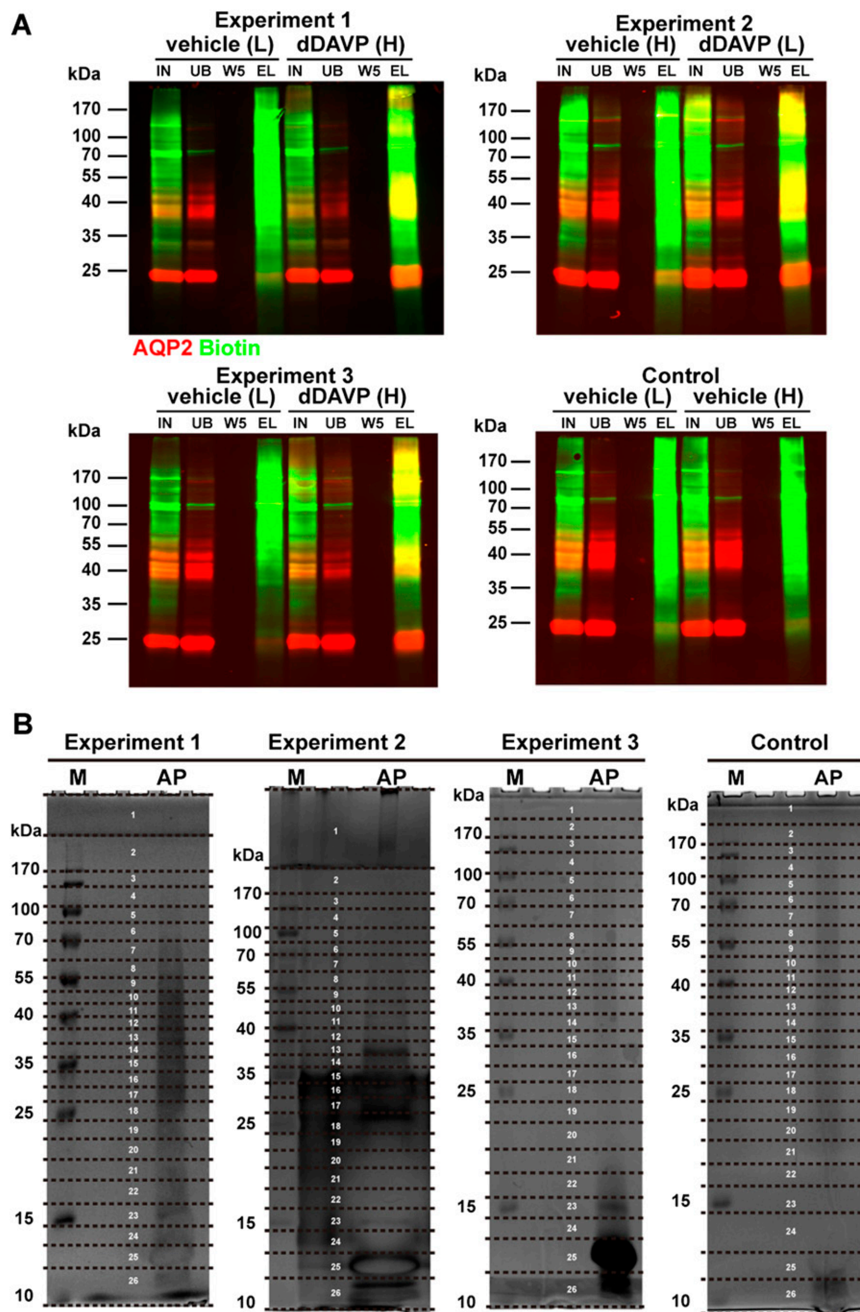


Fig. S2. Preparation of mpkCCD apical membrane proteins for MS. (*A*) Fluorescence staining for biotinylated proteins and immunofluorescence staining for AQP2. Light (L) or heavy (H) isotope labeled mpkCCD cells treated with vehicle or dDAVP (experiments 1 and 3) were biotinylated at the apical membrane surface and subjected to streptavidin-affinity chromatography. In experiment 2, the isotope labeling and the treatment were switched. In the control experiment, light and heavy isotope-labeled cells were both treated with vehicle. EL, eluate; IN, input cell lysate; UB, unbound fraction; W5, fifth wash solution. (*B*) Affinity-isolated proteins from vehicle and dDAVP-treated cells were mixed at 1:1 ratio, separated on an SDS/PAGE gel, and stained with silver before being sliced into 26 pieces for subsequent preparation for MS. AP, apical protein; M, marker.

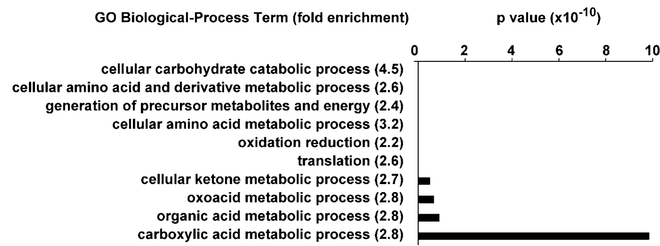


Fig. S3. Gene Ontology (GO) Biological Process terms extracted for 928 apical proteins not regulated by the vasopressin analog dDAVP. *P* values were obtained by Fisher's exact test against all transcripts expressed in mpkCCD cells (1).

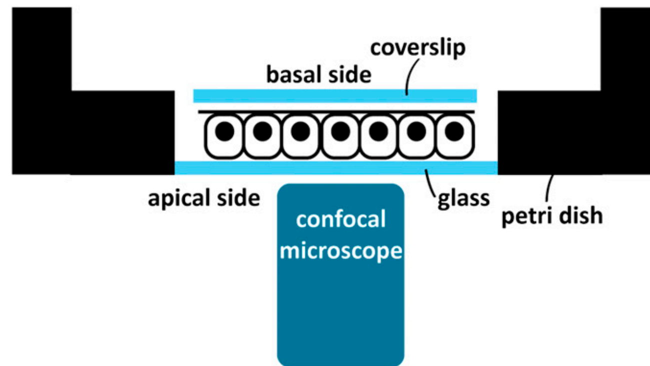


Fig. S4. Live-cell imaging setup for apical F-actin in polarized mpkCCD cells.

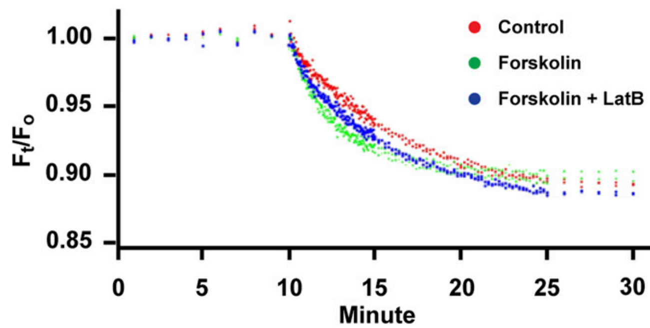


Fig. S5. Sample data from a calcein-quenching experiment. Example of cell fluorescence quenching traces recorded from mpkCCD cells grown in dDAVP (1 nM) for 5 d. The graph demonstrates changes in fluorescence intensity (described as the ratio F_t/F_0 of fluorescence F at time *t* to initial fluorescence F_0) over time. Hyperosmotic challenge induced rapid fluorescence quenching in cell treated with forskolin (green traces), consistent with high water permeability. Control cells or cells pretreated with forskolin and latrunculin B (LatB) combined have significantly slower rates of fluorescence quenching, consistent with reduced water permeability.

Table S1. Apical proteins of mpkCCD cells up-regulated by dDAVP

Gene symbol	Protein name	IMP	Accession no.	Log_2(d/v)	SD
Aqp2	Aquaporin-2	6TM	160415209	3.36	0.32
Mal2	Protein MAL2	4TM	30725780	0.71	0.08
Clmn	Calmin isoform b	1TM	100816903	0.63	0.15
Mpp5	membrane-associated guanylate kinase p55 subfamily member 5		9625023	0.57	0.19
Frk	Tyrosine-protein kinase FRK		31542823	0.37	0.16
Cand1	Cullin-associated NEDD8-dissociated protein 1		189409138	0.37	0.06
Ptpra	Receptor-type tyrosine-protein phosphatase αiso form 2 precursor	1TM	255304938	0.32	0.13
Sf1	Splicing factor 1 isoform 2		160707945	0.32	0.14
Tnpo1	Transportin-1 isoform 2		115385966	0.30	0.11
Fam129a	Protein Niban		241982745	0.30	0.06
Erap1	Endoplasmic reticulum aminopeptidase 1 precursor	1TM	13507656	0.28	0.10
Plec	Plectin isoform 1hij		256000745	0.27	0.11
Psmd5	26S proteasome non-ATPase regulatory subunit 5		134053913	0.25	0.04
Htra2	Serine protease HTRA2, mitochondrial	1TM	254281222	0.25	0.01
Hdac6	Histone deacetylase 6		194353997	0.24	0.07
Lgals9	Galectin-9 isoform 2		226531139	0.23	0.02
Skp1a	S-phase kinase-associated protein 1		158854016	0.22	0.06
Fubp3	Far upstream element (FUSE) binding protein 3		224922832	0.22	0.07
Cse1l	Exportin-2		12963737	0.21	0.05
Nckap1	nck-associated protein 1	1TM	28395023	0.21	0.09
Cat	Catalase		157951741	0.21	0.02
Ythdf3	YTH domain family protein 3 isoform 2		225543497	0.20	0.09
Gls	Glutaminase kidney isoform, mitochondrial isoform 2		164607135	0.18	0.02
Crip2	Cysteine-rich protein 2		13195646	0.17	0.05
Dbnl	Drebrin-like protein isoform 3		226423873	0.16	0.03
Ndrg1	Protein NDRG1		118150658	0.16	0.02
Cdc42bpb	Serine/threonine-protein kinase MRCK β		283135190	0.15	0.07
Pttg1ip	Pituitary tumor-transforming gene 1 protein-interacting protein precursor	1TM	22122339	0.15	0.04
Rnh1	Ribonuclease inhibitor isoform a		31981748	0.14	0.06
Arhgef2	Rho guanine nucleotide exchange factor 2 isoform 4		312032462	0.14	0.05
Hnrnpul2	Heterogeneous nuclear ribonucleoprotein U-like protein 2		124487099	0.13	0.06
Psme2	Proteasome activator complex subunit 2 isoform 2		71725358	0.13	0.04
Aip	AH receptor-interacting protein		7709982	0.13	0.03
Capn1	Calpain-1 catalytic subunit		6671668	0.12	0.04
Gps1	COP9 signalosome complex subunit 1 isoform 2		295424139	0.11	0.02
Tubb2a	Tubulin β-2A chain		33859488	0.11	0.04
Sumo2	Small ubiquitin-related modifier 2 precursor		19111164	0.10	0.03
Adsl	Adenylosuccinate lyase		29788764	0.09	0.02
Lasp1	LIM and SH3 domain protein 1		6754508	0.06	0.01
Larp7	la-related protein 7		110665742	0.06	0.03
Nt5c2	Cytosolic purine 5′-nucleotidase isoform 3		256665238	0.06	0.01
U2af1	Splicing factor U2AF 35-kDa subunit isoform 1		254939694	0.05	0.02
Gsn	Gelsolin isoform 2		329755243	0.05	0.02
Gnai2	Guanine nucleotide-binding protein G(i) subunit α-2		41054806	0.05	0.01
Scin	Adseverin isoform 1		226246550	<0.01	0.00

All proteins listed passed the two-tailed Student *t* test against $\log_2$ (1) at $P < 0.05$. IMP, integral membrane protein; $\log_2$(d/v), $\log_2$ values of the mean of protein ratios (dDAVP/vehicle) (three independent experiments); TM, transmembrane domain.

Table S2. Apical proteins of mpkCCD cells down-regulated by dDAVP

Gene symbol	Protein name	IMP	Accession no.	$\log_2$(d/v)	SE
Tnfrsf10b	Tumor necrosis factor receptor superfamily member 10B precursor	1TM	31981095	−0.56	0.23
Mcam	Cell surface glycoprotein MUC18 precursor	1TM	160333901	−0.46	0.18
Abhd14b	Abhydrolase domain-containing protein 14B		171460960	−0.42	0.18
Magt1	Magnesium transporter protein 1	4TM	298676496	−0.36	0.10
D10Wsu52e	tRNA-splicing ligase RtcB homolog		21703842	−0.31	0.08
Cryz	Quinone oxidoreductase		33859530	−0.31	0.12
Psmd14	26S proteasome non-ATPase regulatory subunit 14		145966883	−0.29	0.13
Cyb5b	Cytochrome b5 type B-like	1TM	149275191	−0.27	0.01
Gdi2	rab GDP dissociation inhibitor β		116089273	−0.27	0.09
Arpc4	Actin-related protein 2/3 complex subunit 4 isoform 1		13386054	−0.26	0.04
Polr2h	DNA-directed RNA polymerases I, II, and III subunit RPABC3		21704118	−0.25	0.04
Cops5	COP9 signalosome complex subunit 5		7304971	−0.25	0.11
Prps2	Ribose-phosphate pyrophosphokinase 2		13386146	−0.24	0.08
Cdh13	Cadherin-13 precursor	GPI	110625609	−0.24	0.10
Rab1b	ras-related protein Rab-1B		21313162	−0.24	0.02
Pgam5	Serine/threonine-protein phosphatase PGAM5, mitochondrial isoform 2	1TM	254587960	−0.22	0.08
Phb2	Prohibitin-2		126723336	−0.21	0.07
Itga7	Integrin α-7	1TM	112293269	−0.21	0.07
Arpc2	Actin-related protein 2/3 complex subunit 2		112363072	−0.18	0.05
Psmd3	26S proteasome non-ATPase regulatory subunit 3		19705424	−0.18	0.06
Eif3h	Eukaryotic translation initiation factor 3 subunit H		18079341	−0.18	0.08
Rod1	Polypyrimidine tract-binding protein 3 isoform 2		30039680	−0.17	0.05
Bphl	Valacyclovir hydrolase precursor		21624609	−0.17	0.04
Timm50	Mitochondrial import inner membrane translocase subunit TIM50 precursor	1TM	22094989	−0.17	0.05
Cdcp1	CUB domain-containing protein 1 precursor	1TM	61098143	−0.16	0.07
Slc25a5	ADP/ATP translocase 2	6TM	22094075	−0.16	0.02
Rab6a	ras-related protein Rab-6A isoform 2		13195674	−0.16	0.06
Arcn1	Aoatomer subunit δ		148747410	−0.15	0.03
Ahsa1	Activator of 90-kDa heat shock protein ATPase homolog 1		22122515	−0.15	0.05
Aldoa	Fructose-bisphosphate aldolase A isoform 2		6671539	−0.14	0.06
Idh3b	Isocitrate dehydrogenase 3, β subunit		18700024	−0.13	0.03
Slc7a5	Large neutral amino acids transporter small subunit 1	12TM	31982764	−0.13	0.00
Rpl23	60S ribosomal protein L23		12584986	−0.13	0.04
Pygb	Glycogen phosphorylase, brain form		24418919	−0.13	0.05
Rpl37a	60S ribosomal protein L37a		6677785	−0.13	0.05
Preb	Prolactin regulatory element-binding protein	1TM	158749640	−0.12	0.04
Glud1	Glutamate dehydrogenase 1, mitochondrial precursor		6680027	−0.12	0.04
Eif4a3	Eukaryotic initiation factor 4A-III		20149756	−0.12	0.04
Rab5c	ras-related protein Rab-5C		113866024	−0.11	0.02
Got2	Aspartate aminotransferase, mitochondrial		6754036	−0.11	0.04
Hadhb	Trifunctional enzyme subunit β, mitochondrial precursor		21704100	−0.11	0.04
Epha2	Ephrin type A receptor 2 precursor	1TM	32484983	−0.10	0.01
Rab10	ras-related protein Rab-10		7710086	−0.10	0.03
Ppp2r2a	Serine/threonine protein phosphatase 2A 55 kDa regulatory subunit B α isoform isoform 1		110625886	−0.10	0.04
Sec61a1	Protein transport protein Sec61 subunit α isoform 1	10TM	8394252	−0.10	0.04
Ccdc109a	Calcium uniporter protein, mitochondrial precursor	2TM	168823441	−0.10	0.00
Rab5b	ras-related protein Rab-5B isoform 1		28916687	−0.09	0.03
Rpl24	60S ribosomal protein L24		18250296	−0.08	0.03
Actb	Actin, cytoplasmic 1		6671509	−0.08	0.01
Rps15a	40S ribosomal protein S15a		24762230	−0.08	0.03
Capzb	F-actin capping protein subunit β isoform b		6753262	−0.06	0.02
Akr7a5	Aflatoxin B1 aldehyde reductase member 2		240120054	−0.06	0.02
Atp6v0d1	V-type proton ATPase subunit d 1		31981304	−0.06	0.02
Phb	Prohibitin		6679299	−0.05	0.02
Rps6	40S ribosomal protein S6		6677809	−0.05	0.01

All proteins listed passed the two-tailed Student t test against $\log_2$ (1) at $P < 0.05$. GPI, GPI anchor; IMP, integral membrane protein; $\log_2$(d/v), $\log_2$ values of the mean of protein ratios (dDAVP/vehicle) (three independent experiments); TM, transmembrane domain.

Table S3. Effects of dDAVP, forskolin, and latrunculin B on osmotically induced mpkCCD cell shrinkage using a calcein quenching-based method

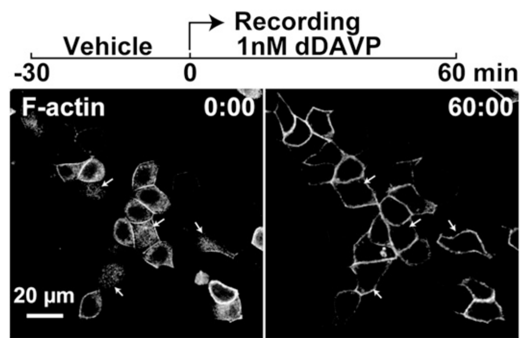
Growth condition	Rate constant (k)			Half-life time of shrinkage (s)		
	Control (DMSO)	Forskolin	Forskolin plus latrunculin B	Control (DMSO)	Forskolin	Forskolin plus latrunculin B
-dDAVP	0.087 ± 0.006	0.073 ± 0.010	0.077 ± 0.008	8.059 ± 0.598	9.806 ± 1.146	9.205 ± 0.991
+dDAVP	0.165 ± 0.008	0.372 ± 0.028*	0.200 ± 0.009†	4.230 ± 0.199	1.896 ± 0.145*	3.484 ± 0.141†

Cells were grown in the presence or absence of 1 nM dDAVP, and cell shrinkage assays were performed as described in *SI Materials and Methods*. Data were fitted to a one-phase exponential decay function in Prism 5 (GraphPad), and rate constants (k) or half-life time of cell shrinking after sucrose addition was determined. Smaller half-life times of cell shrinkage are indicative of greater osmotically induced water transport rates. Values are means ± SE. *$P < 0.05$ compared with control; †$P < 0.05$ compared with forskolin.

Table S4. Isotope-labeling efficiency of mpkCCD cells grown in heavy SILAC medium

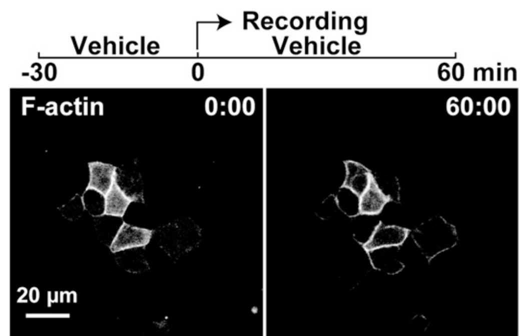
Types of peptides	No. of peptides	Percentage
Heavy-labeled peptides	6,456	99.4
Light-labeled peptides	29	0.4
Incompletely labeled peptides	10	0.2

The cells were cultured in heavy SILAC medium for 12 d. Heavy-labeled peptides, all lysine and arginine residues are labeled with heavy isotopes; incompletely labeled peptides, some lysine and/or arginine residues are labeled with heavy isotopes; light-labeled peptides, all lysine and arginine residues are labeled with light isotopes.



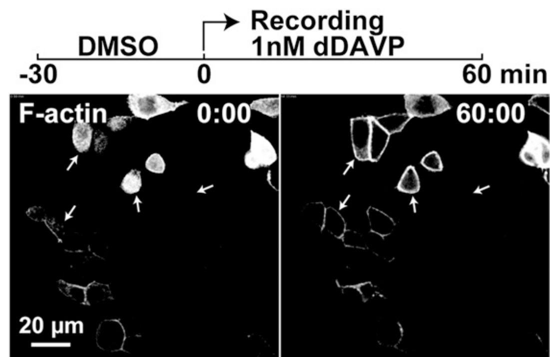
Movie S1. Live-cell imaging of apical F-actin in mpkCCD cells in the presence of vasopressin.

Movie S1



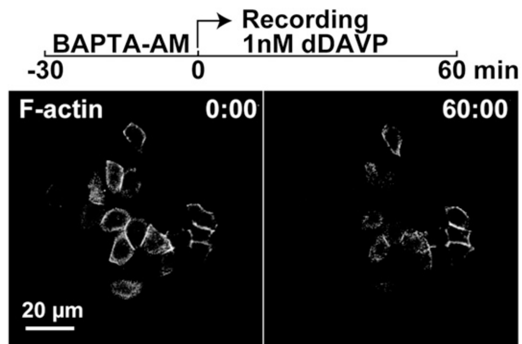
Movie S2. Live-cell imaging of apical F-actin in mpkCCD cells in the absence of vasopressin.

Movie S2



Movie S3. Live-cell imaging of apical F-actin in mpkCCD cells pretreated with vehicle (DMSO) before the cells were exposed to dDAVP.

Movie S3



Movie S4. Live-cell imaging of apical F-actin in mpkCCD cells pretreated with BAPTA-AM before the cells were exposed to dDAVP.

Movie S4