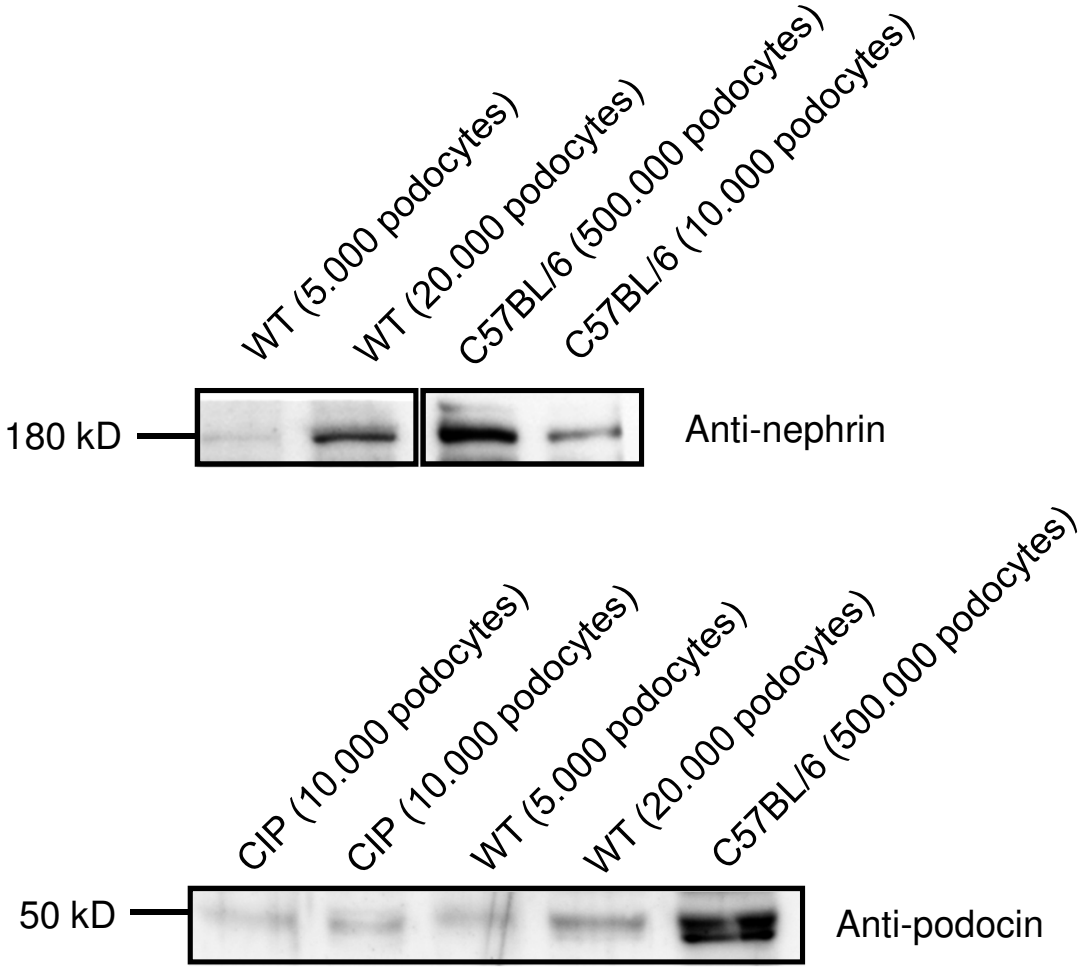
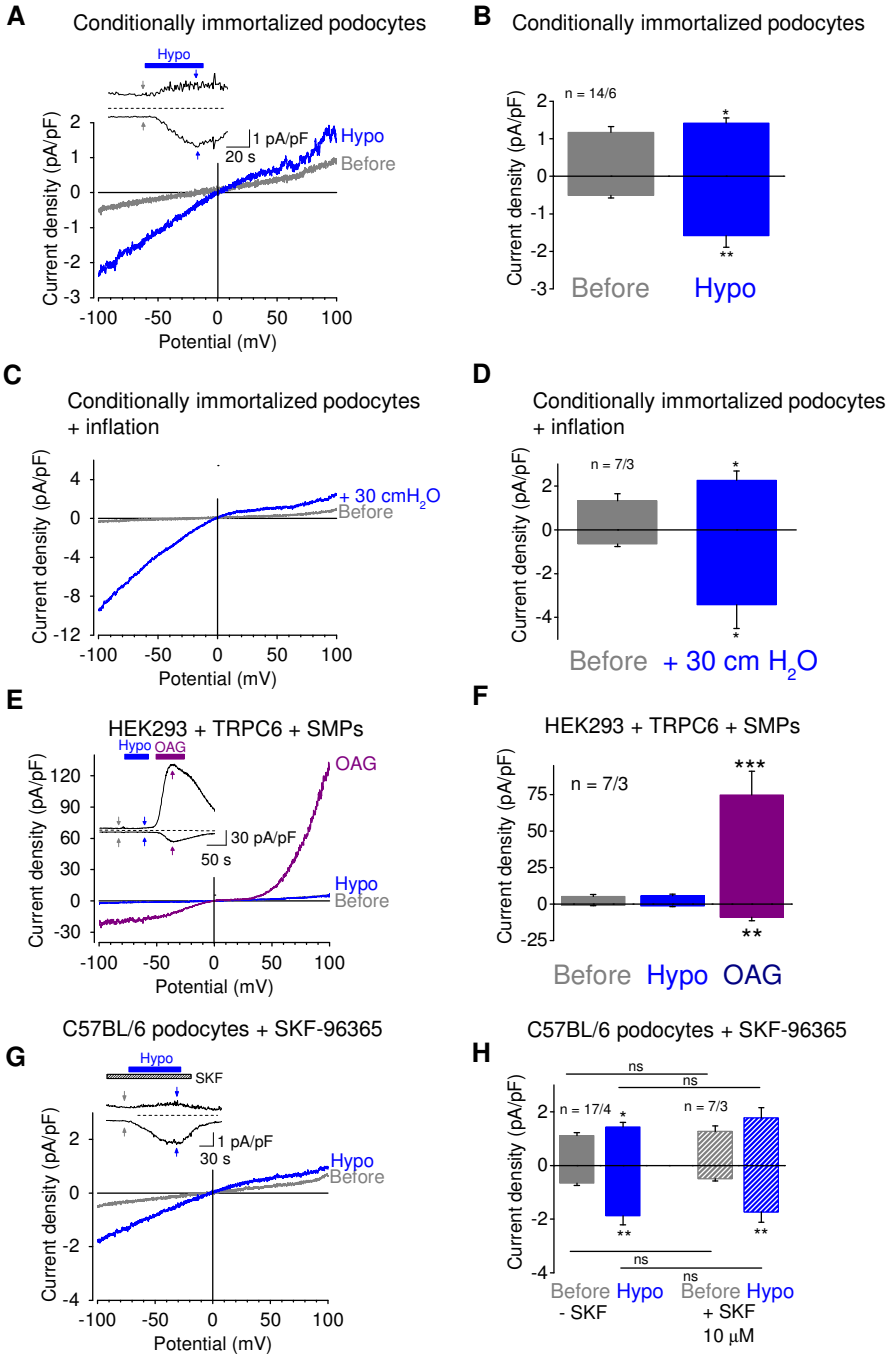
Supplemental Figure 1



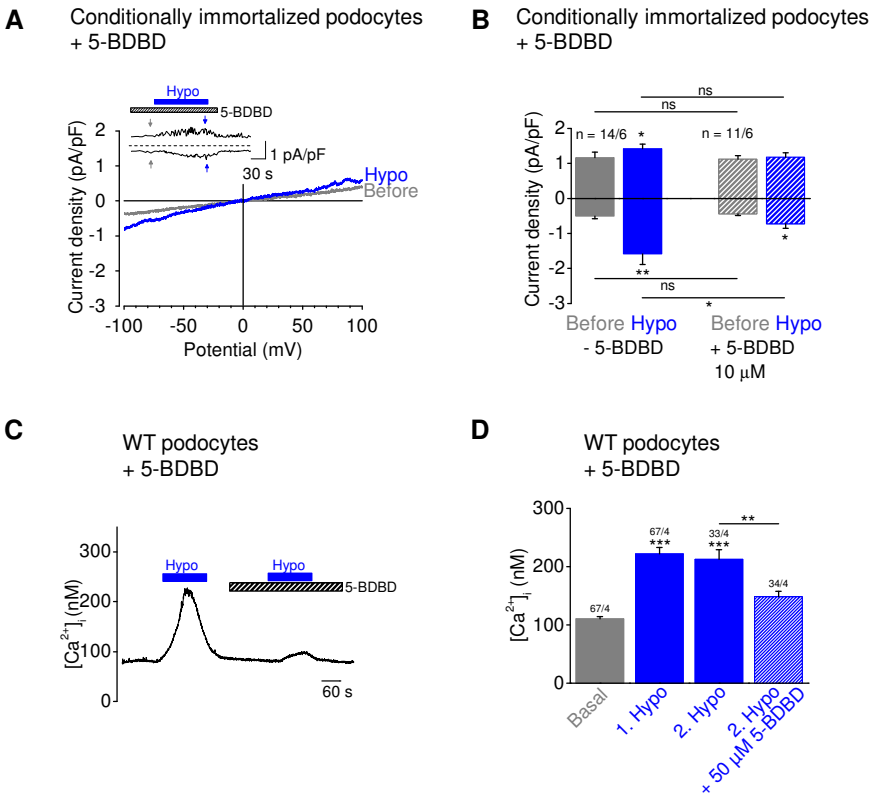
Supplemental Figure 1: Nephrin and podocin are expressed in podocytes on the protein level. Western blot analysis of isolated primary WT and C57BL/6 podocytes and of conditionally immortalized podocytes (CIPs) using guinea-pig anti-nephrin antibodies and rabbit anti-podocin antibodies. Numbers in a parentheses indicate approximate numbers of isolated cells.

Supplemental Figure 2



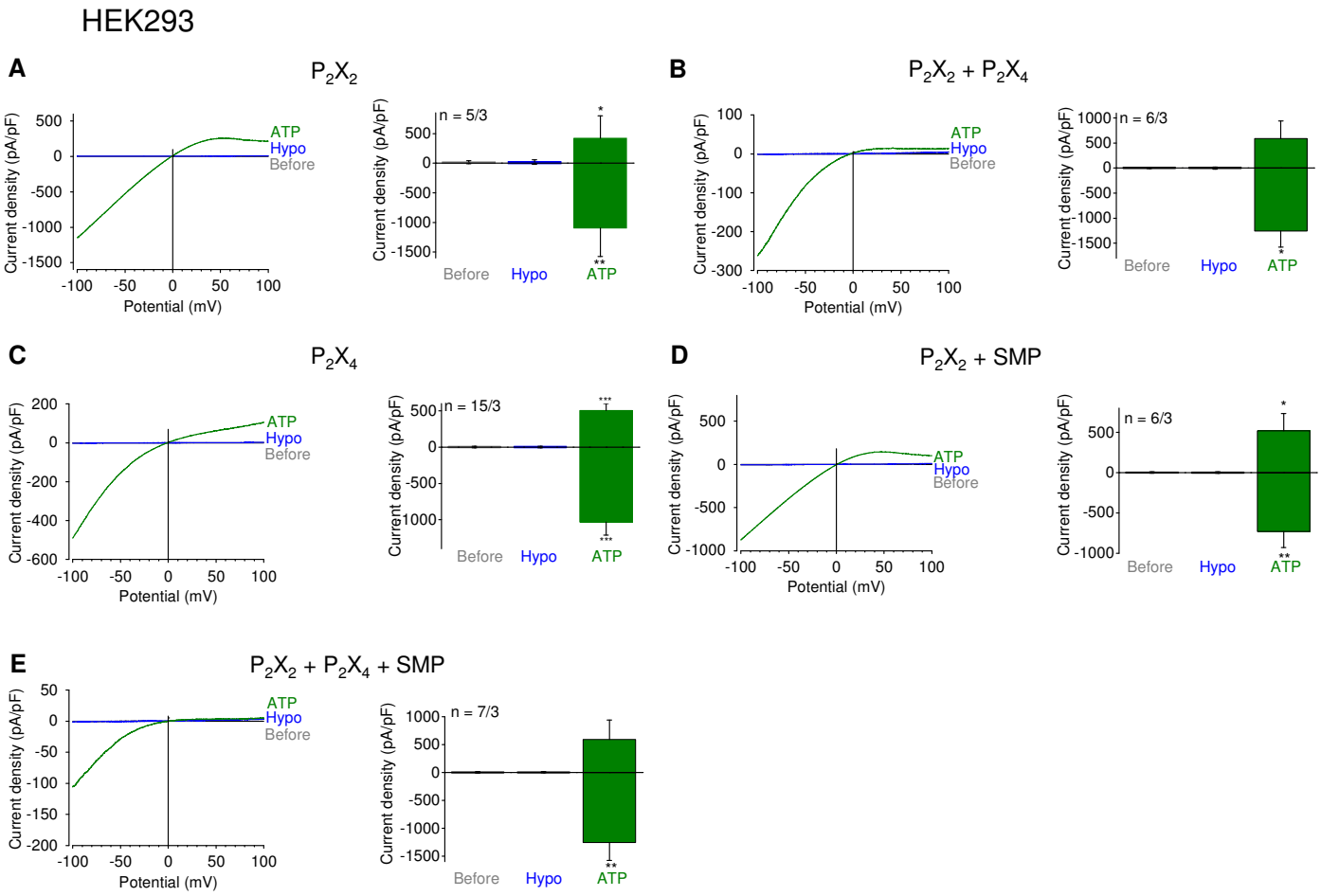
Supplemental Figure 2: Conditionally immortalized podocytes are mechanosensitive and TRPC channels are not mechanotransducing elements in podocytes. (A-D, G and H) Electrophysiological whole-cell measurements of conditionally immortalized murine podocytes (A-D) and of C57BL/6 podocytes in the presence of SKF-96365 (G and H) Exemplary CDV relationships before (grey) and during application of hypoosmotic bath solution (blue) (A, G) and of positive pipette pressure (G). Insets show current density time courses at ±100 mV. Stippled line represents zero current. Bars indicate presence of hypoosmotic solution and of SKF-96365 (hatched bar). Arrows represent the time points of depicted CDV traces. (B, D, H) Summary of current densities before (gray and gray hatched bars) and during application of hypoosmotic bath solution (blue and blue hatched bars) (B, H) or positive pipette pressure (D) in the absence (solid bars) and presence of SKF-96365 (hatched bars) at ±100 mV. Numbers display the number of measured cells and of independent experiments. (E and F) Whole-cell measurements of HEK293 cells over-expressing human TRPC6 and the slit membrane proteins (SMPs) podocin, nephrin and CD2AP with exemplary CDV relationships in the before (grey) and during (blue) hypoosmotic bath solution “Hypo” and in the presence of 100 µM OAG (violet). Insets show current density time courses at ±100 mV. Stippled line represents zero current. Bars indicate application of hypoosmotic solution and of OAG. Arrows represent the time points of depicted CDV traces. (F) Summary of current densities before (gray bars) and during application of hypoosmotic bath solution (blue bar) and of OAG (violet bar) at ±100 mV. Numbers display the number of measured cells and of independent experiments.

Supplemental Figure 3



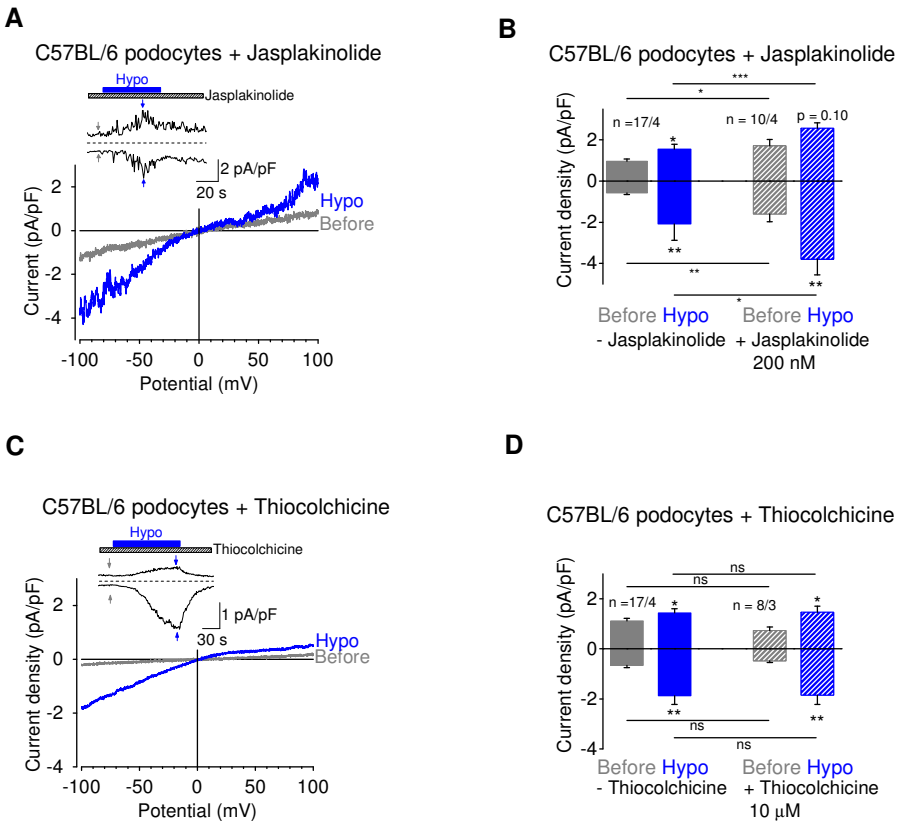
Supplemental Figure 3: P_2X_4 channel blocker 5-BDBD suppresses cell swelling induced calcium increases. (A and B) Electrophysiological whole-cell measurements of conditionally immortalized murine podocytes. (A) Exemplary current density voltage (CDV) relationships before (grey) and during application of hypoosmotic bath solution "Hypo" (blue) in the presence of 10 µM 5-BDBD. Insets show current density time courses at ±100 mV. Stippled line represents zero current. Bars indicate application of hypoosmotic solution and of 5-BDBD (hatched bar). Arrows represent the time points of depicted CDV traces. (B) Summary of current densities before (gray and gray hatched bars) and during application of hypoosmotic bath solution (blue and blue hatched bars) of podocytes in the absence (solid bars) or presence of 5-BDBD (hatched bars) at ±100 mV. Numbers display the number of measured cells and of independent experiments. (C) Representative intracellular calcium concentration trace of fura-2 loaded podocytes repeatedly stimulated with hypoosmotic solution in the presence of absence of 5-BDBD. Applications of hypoosmotic solution (blue bars) and of 5-BDBD (striped bar) are indicated. (D) Summary of calcium concentrations before (gray bar) and during the first application of hypoosmotic solution (blue bar "1. Hypo") and during the second application of hypoosmotic solution "2. Hypo" in the presence (blue striped bar) or absence (blue bar) of 5-BDBD.

Supplemental Figure 4



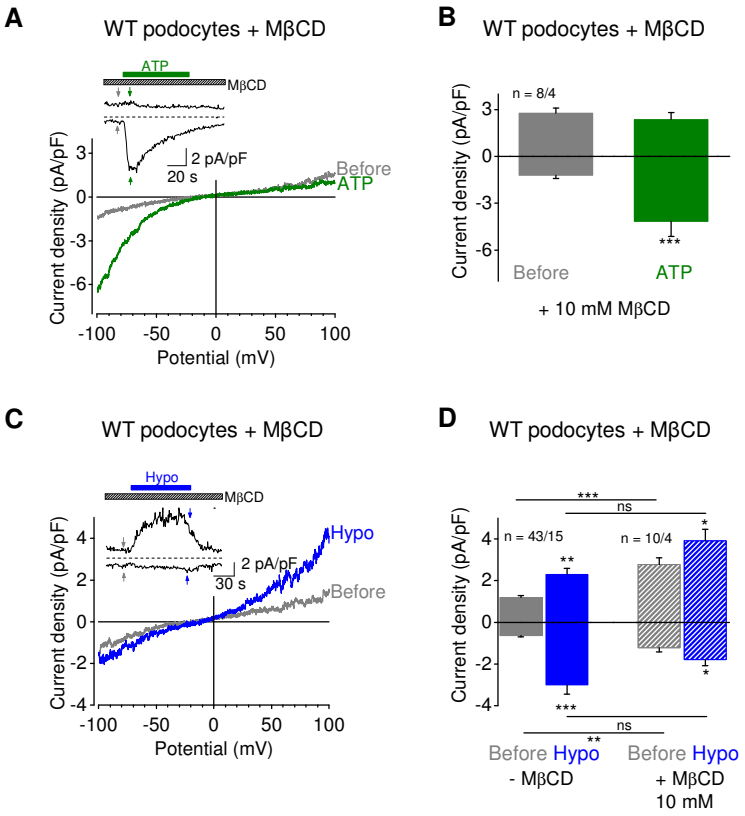
Supplemental Figure 4: Over-expressed P_2X channels are not mechanosensitive. (A-E, left panels) Representative current density voltage relationships of whole-cell recordings of HEK293T cell transiently over-expressing P_2X_2 (A), P_2X_2 and P_2X_4 (B), P_2X_4 (C), P_2X_2 and the slit membrane proteins (SMP) podocin, nephrin and CD2AP (D) or P_2X_2, P_2X_4 and SMP (E) before and during application of hypoosmotic solution and during application of 100 µM ATP (green). (A-E, right panels) Summary of current densities before (gray bars), during the application of hypoosmotic solution (blue bars) and during application of ATP (green bars). Numbers indicate the number of measured cells and of independent experiments.

Supplemental Figure 5



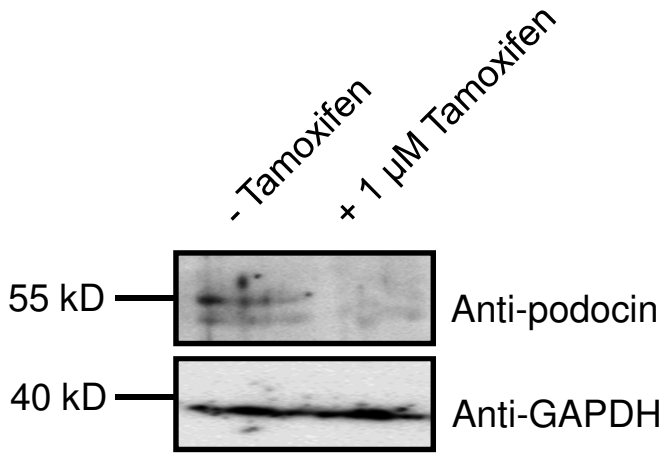
Supplemental Figure 5: Influence of the cytoskeleton on mechanosensation of podocytes (A, C) Representative current density voltage (CDV) relationships of whole-cell recordings of C57BL/6 podocytes treated with 200 nM jasplakinolide (A) or with 10 µM thiochochicine (C) before (gray) and during application of hypoosmotic solution (blue). Insets show current density time courses at ±100 mV. Stippled line represents zero current. Bars indicate application of hypoosmotic solution. Arrows represent the time points of depicted CDV traces. (B, D) Summary of current densities before (gray and gray hatched bars), during the application of hypoosmotic solution (blue and blue hatched bars) of control cells (solid bars) and of podocytes treated with jasplakinolide (B) and with thiocholcicine (D) (hatched bars). Numbers indicate the number of measured cells and of independent experiments.

Supplemental Figure 6



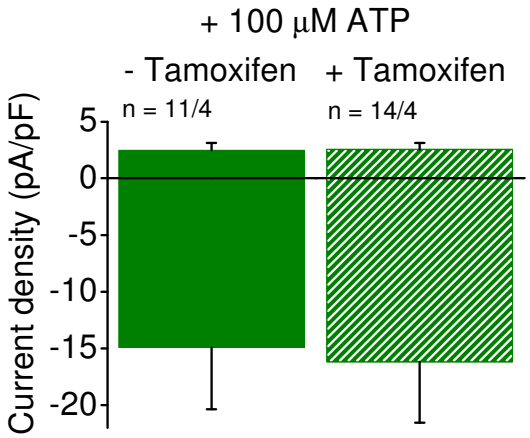
Supplemental Figure 6: Cholesterol depletion abolishes mechanically induced inwardly rectifying cation currents in podocytes. (A-D) Electrophysiological whole-cell measurements of podocytes treated with 10 mM methyl-β-cyclodextrine (MβCD). (A and C) Exemplary current density voltage (CDV) relationships before (grey) and during application of 100 µM ATP (A) (green) and of hypoosmotic solution (blue) (C) in the presence of 10 mM MβCD. Insets show current density time courses at ±100 mV. Stippled lines represent zero current. Application of hypoosmotic solution, of ATP and of MβCD is indicated. Arrows represent the time points of depicted CDV traces. (B and D) Summaries of current densities before (grey and gray hatched bars) and during application of ATP (green bar) (B) or of hypoosmotic solution (blue and blue hatched bars) (D) in the absence (solid bars) and presence of MβCD (hatched bars) at ±100 mV. Numbers indicate the numbers of measured cells and of independent experiments.

Supplemental Figure 7



Supplemental Figure 7: Tamoxifen induced podocin down-regulation in podocytes from *Nphs2*$^{flox/flox}$,*Cre*$^{+/+}$ mice. Exemplary western blot showing expression of podocin in podocytes isolated from *Nphs2*$^{flox/flox}$,*Cre*$^{+/+}$ mice treated with 1 µM tamoxifen and untreated control podocytes. Probe was isolated from approximately 2000 primary podocytes.

Supplemental Figure 8



Supplemental Figure 8: ATP-induced currents are similar in podocin knock-down and control podocytes. Summary of ATP-induced current density increases at ±100 mV in podocin knock-down (green hatched bar) and control podocytes (green bar). Numbers indicate the numbers of measured cells and of independent measurements.

Supplemental Material and Methods

Quantitative Real-time Polymerase Chain Reaction (qPCR) analysis

Total RNA from primary mouse podocytes was isolated using the Trizol Reagent (Qiagen). First-strand synthesis was carried out with random hexamers as primers using REVERTAID reverse transcriptase (Fermentas, Sankt Leon-Roth, Germany). The following primers pairs were used for the amplification of specific fragments from the first-strand synthesis: TRPC1, C1for (GATGTGTCTTTGCCAAGC), C1rev (CTGGACTGGCCAGACATCTAT); TRPC2, C2for (ACTTCACTACATATGATCTGGGTCAC), C2rev (CACGTCCAGGAAGTTCCAC); TRPC3, C3for (TTAATTATGGTCTGGGTTCTTGG), C3rev (TCCACAACTGCACGATGTACT); TRPC4, C4for (AAGGAAGCCAGAAAGCTTCG), C4frev (CCAGGTTCCTCATCACCTCT); TRPC5, C5for (GCTGAAGGTGGCAATCAAAT), C5rev (AAGCCATCGTACCACAAGGT); TRPC6, C6for (ACTGGTGTGCTCCTTGCAG), C6rev (GAGCAGCCCCAGGAAAAT); TRPC7, C7for (CCCAAACAGATCTTCAGAGTGA), C7rev (TGCATTCGGACCAGATCA); nephrin, Nephrinfor (CCATCCTCCCAGAGATGTTC), Nephrinrev (TTCTCCATGTCGTCCAGGTT); Neph1, Neph1for (CAGTGCCAACCTTTCTACGTC), Neph1rev (TGTTTGCACTGTGGTTGACA); Neph2, Neph2for (GCTTCTTCCTCTTCAGTCAAGG), Neph2rev (GCTGTCCTGACACCACCAC); Neph3, Neph3for (ATCCACTTGGGCCGTAGA), Neph3rev (AGAGAGGTGGCTGCAGATG); CD2AP, CD2APfor (GAAGGGGAGATTATCCATTTGA), CD2APrev (TCCTTCTTTACCGTTCAGTTCAC); Synaptopodin, Synpofor (GTAGCCAGGTGAGCCAAGG), Synporev (TTTTCGGTGAAGCTTGTGC); podocin, Podofor (CCATCTGGTTCTGCATAAAGG), Podorev (CCAGGACCTTTGGCTCTTC); endothelin receptor type A, $Et_A$for (TGTGAGCAAGAAATTCAAAAATTG), ET_Arev (ATGAGGCTTTTGGACTGGTG); endothelin receptor type B, $ET_B$for (CGGTATGCAGATTGCTTTGA), ET_Brev (AACAGAGAGCAAACACGAGGA); angiotensin II type 1a receptor, $AT_{1A}$for (ACTCACAGCAACCCTCCAAG), AT_{1A}rev (CTCAGACACTGTTCAAAATGCAC); angiotensin II type 1b receptor, $AT_{1B}$for (CAGTTTCAACCTCTACGCCAGT), AT_{1B}rev (GGGTGGACAATGGCTAGGTA); angiotensin II type 2 receptor, $AT_2$for (TGTTCTGACCTTCTTGGATGC), AT_2rev (GCCAGGTCAATGACTGCTATAACT); arginine vasopressine V1 type A receptor, $V_{1a}$for (GGGATACCAATTTCGTTTGG), V_{1a}rev (AAGCCAGTAACGCCGTGAT); arginine vasopressine V1b receptor, $V_{1b}$for (TGATGTGGGACCTGATACCTAGT), V_{1b}rev (CCATCCTGTGGTAAGGGTGA); arginine vasopressine receptor type 2, $V_2$for (GACAGGTGTGCCGCTCAT), V_2rev (GAGAGCTAGGGGACGAAAGG); P_2X_1, $P_2X_1$for (TCCGAAGCCTTGCTGAGA); P_2X_1rev (GGTTTGCAGTGCCGTACAT); P_2X_2, $P_2X_2$for (CTTCACAGAACTGGCACACAA), P_2X_2rev (TATTTGGGGTTGCACTCTGA); P_2X_3, $P_2X_3$for (AGCATCCGTTTCCCTCTCTT), P_2X_3rev (TTTATGTCCTTGTCGGTGAGG); P_2X_4, $P_2X_4$for (CCAACACTTCTCAGCTTGGAT), P_2X_4rev (TGGTCATGATGAAGAGGGAGT); P_2X_5, $P_2X_5$for (CACAGTCATCAACATTGGTTCC), P_2X_5 (AGGTAGATAAGTACCAGGTCACAGAAG); P_2X_6, $P_2X_6$for (CCTGGACACGAAAGGCTCT), P_2X_6rev (CACCAGTGATTGGCTGTCC); P_2X_7, $P_2X_7$for (TCTTCCGACTAGGGGACATC), P_2X_7rev (TACCCATGATTCCTCCCTGA); P_2Y_1, $P_2Y_1$for (GCAGTCCAGTCTTTGGCTAGA), P_2Y_1rev (AGTTTCAACCTTTCCATACCACA); and three references, hypoxanthin phosphoribosyltransferase 1, Hprt1for (TCCTCCTCAGACCGCTTTT), Hprt1rev (CCTGGTTCATCATCGCTAATC), tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, ζ-polypeptide, Ywhazfor (TAAAAGGTCTAAGGCCGCTTC), Ywhazrev (CACCACACGCACGATGAC), and succinate dehydrogenase complex, subunit A, Sdhafor (CCCTGAGCATTGCAGAATC), Sdharev (TCTTCTCCAGCATTTGCCTTA) giving predicted product sizes of 127 bp for TRPC1, 111 bp for TRPC2, 91 bp for TRPC3, 92 bp for TRPC4, 90 bp for TRPC5, 101 bp for TRPC6, 94 bp for TRPC7, 76 bp for Nephrin, 86 bp for Neph1, 78 bp for Neph2, 74 bp for Neph 3, 81 bp for CD2AP, 93bp for synaptopodin, 94 bp for podocin, 72 bp for ETA, 92 bp for ETB, 62 bp for AT1A, 75 for AT1B, 66 bp for V1A, 75 bp for V1B, 92 bp for V2, 76 bp for AT2, 90 bp for Hprt1, 60 bp for Ywhaz and 70 bp for Sdha. Real-time polymerase chain reaction (RT-PCR) was performed using the master mix from the Absolute QPCR SYBR Green Mix kit (Abgene, Epsom, UK).

Supplemental Material and Methods

Quantitative Real-time Polymerase Chain Reaction (qPCR) analysis (2)

Ten picomoles of each primer pair and 0.2 µl of the first-strand synthesis was added to the reaction mixture, and PCR was carried out in a light-cycler apparatus (Light-Cycler 480, Roche Applied Science) using the following conditions: 15 min of initial activation and 45 cycles of 12 s at 94 °C, 30 s at 50 °C, 30 s at 72 °C, and 10 s at 80 °C each. Fluorescence intensities were recorded after the extension step at 80 °C after each cycle to exclude fluorescence of primer dimers melting lower than 80 °C. All primers were tested by using diluted complementary DNA (cDNA) from the first-strand synthesis (10–1000×) to confirm linearity of the reaction and to determine particular efficiencies. Data were calculated as percentage of the geometric mean expression of the three references (Hprt1, Ywhaz and Sdha) which showed the highest tissue-independent transcription. Samples containing primer dimers were excluded by melting curve analysis and identification of the products by agarose gel electrophoresis. Crossing points were determined by the software program. All experiments were performed in quadruplets, and experiments were repeated at least three times.