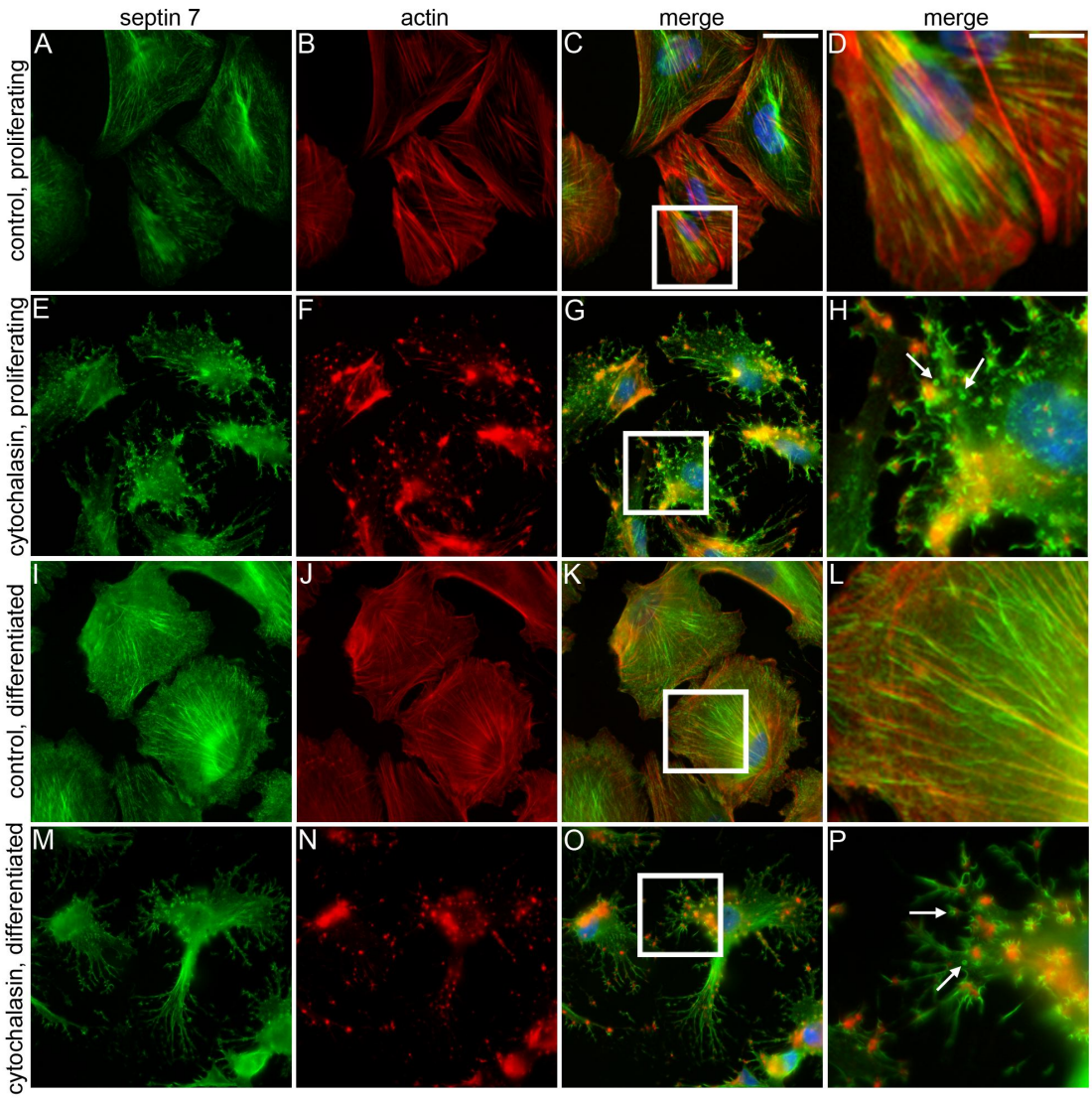
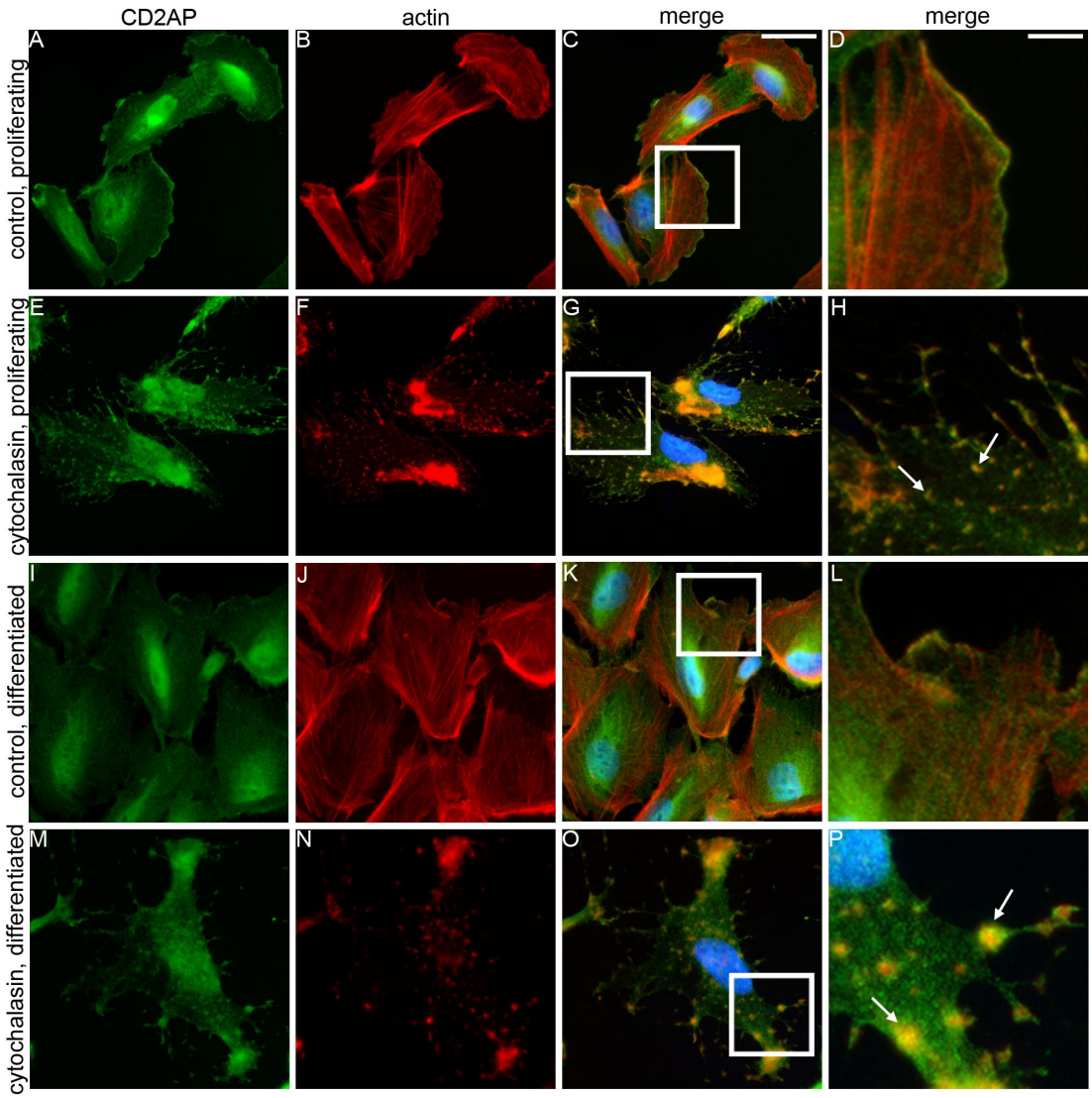
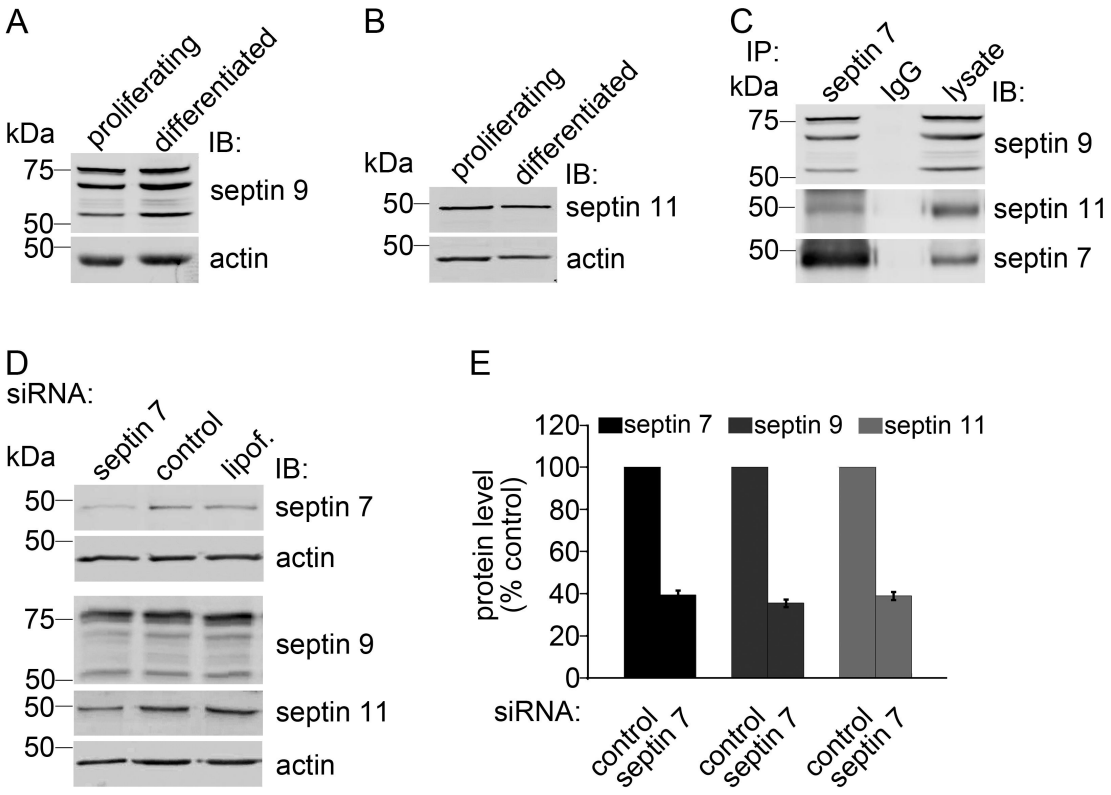


SUPPLEMENTARY FIGURE 1. Localization of septin 7 depends on an intact actin cytoskeleton. Double labeling of proliferating (A-H) and differenciated (I-P) human podocytes for septin 7 (green) and actin (red). Nuclei are visualized with Hoechst (blue). In proliferating podocytes (control, proliferating), septin 7 filaments (A) partially align along actin stress fibers detected with phalloidin (B-D). Septin 7 filaments appear shorter than actin filaments. (E-H) In proliferating podocytes treated with cytochalasin D (cytochalasin, proliferating), septin 7 curled and localized in rings (arrows) (E, G, H). (I-

L) In differenciated podocytes (control, differentiated), septin 7 (I) appears as filaments which occasionally co-localize with actin stress fibers (J-L). In differentiated podocytes treated with cytochalasin D (cytochalasin, differentiated), septin 7 filaments (M) are partially disrupted and form curls and rings (arrows) (M, O, P). Boxed regions indicated in (C, G, K and O) are magnified in (D, H, L and P), respectively. Cultured human podocytes were treated or not with 5 μM cytochalasin D, fixed with PFA, labeled with septin 7 IgG and phalloidin, and examined by fluorescence microscopy. Bar, 20 μm; in D, H, L, P, 7 μM.

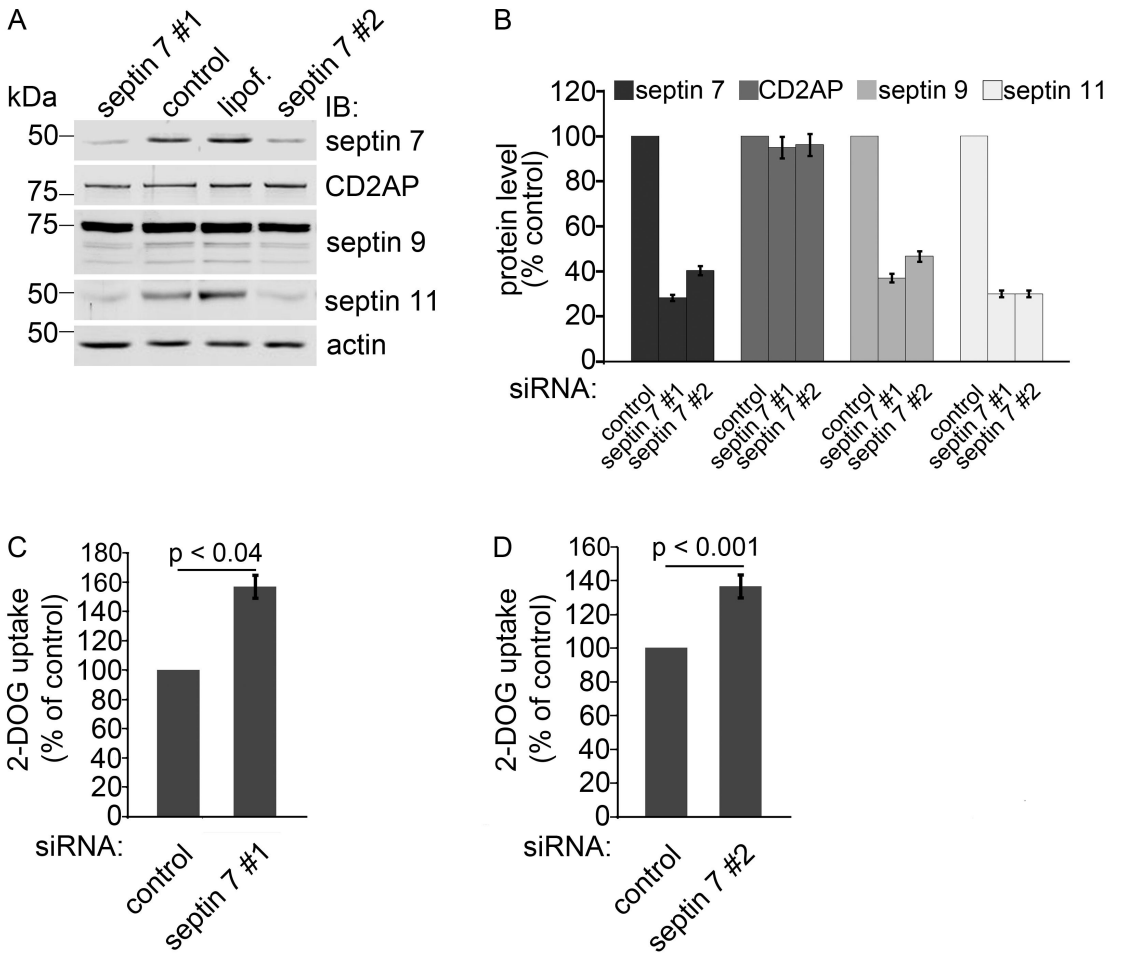


SUPPLEMENTARY FIGURE 2. Localization of CD2AP depends on an intact actin cytoskeleton. Double labeling of proliferating (A-H) and differentiated (I-P) human podocytes for CD2AP (green) and actin (red). Nuclei are visualized with Hoechst (blue). In proliferating (A-D) and differentiated (I-L) podocytes CD2AP localizes perinuclearly in cytosol and in leading edges. In proliferating (E-H) and differentiated (M-P) podocytes treated with cytochalasin D CD2AP appears as aggregates partially colocalizing with aggregated actin (arrows). Cultured human podocytes were treated or not with 5 µM cytochalasin D, fixed with PFA, labeled with CD2AP IgG and phalloidin, and examined by fluorescence microscopy. Bar, 20 µm; in D, H, L, P, 7 µM.



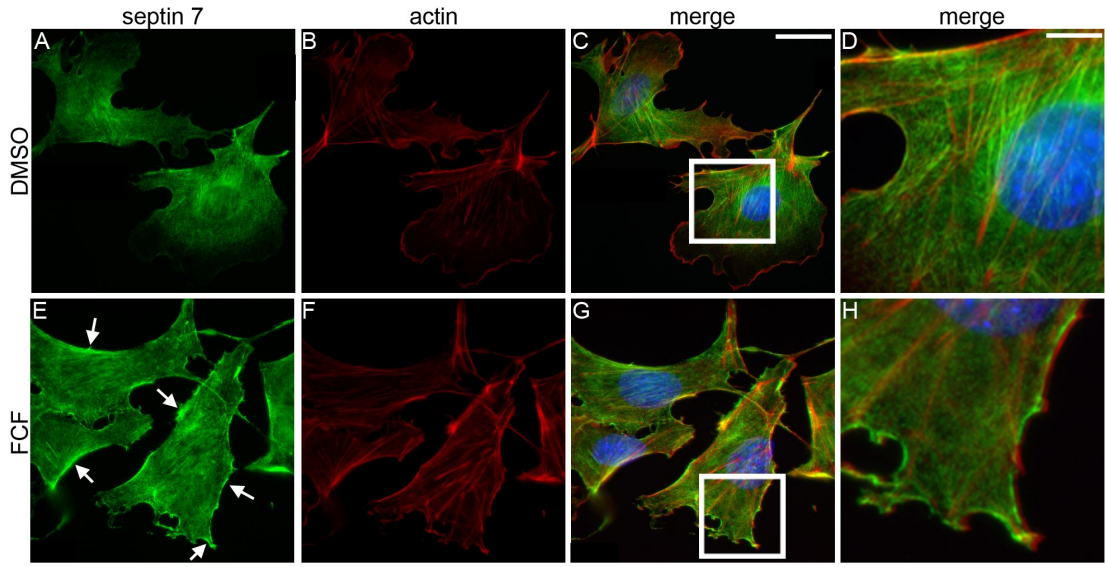
SUPPLEMENTARY FIGURE 3. Septin 7, septin 9 and septin 11 form a complex in cultured human podocytes. (A) Several isoforms of septin 9 are expressed in proliferating and differentiated human podocytes. (B) Septin 11 is expressed in proliferating and differentiated podocytes. In (A-B), cultured podocytes were lysed in 1% NP-40, 20 mM Hepes, pH 7.5, 150 mM NaCl, and 35 µg of lysates were immunoblotted with septin 7, septin 9 and septin 11 IgG. (C) Septin 9 and septin 11 co-immunoprecipitate with septin 7 in differentiated podocytes. No septins can be detected in immunoprecipitations with rabbit IgG. Differentiated podocytes were lysed as in (A), incubated with septin 7 or rabbit IgG, and the immunoprecipitated proteins were immunoblotted with septin 7, septin 9 and septin 11 IgG. Lysate, 10 µg. (D) Septin 7 siRNA leads to 60.5% reduction in septin 7, 64.5% reduction in septin 9 and 61% reduction in septin 11 expression in cultured human podocytes. Proliferating podocytes were transfected with human septin 7

SMARTpool siRNA (septin 7), siCONTROL Non-Targeting Pool siRNA (control) or Lipofectamine 2000 alone (lipof.) and analyzed by immunoblotting 48 h after transfection. (E) Quantification of protein levels of three replicate blots as in (D). The levels of septin 7, septin 9 and septin 11 are presented as % of the control siRNA (set to 100%). Bars show the mean and error bars STDEV, Student's t-test.

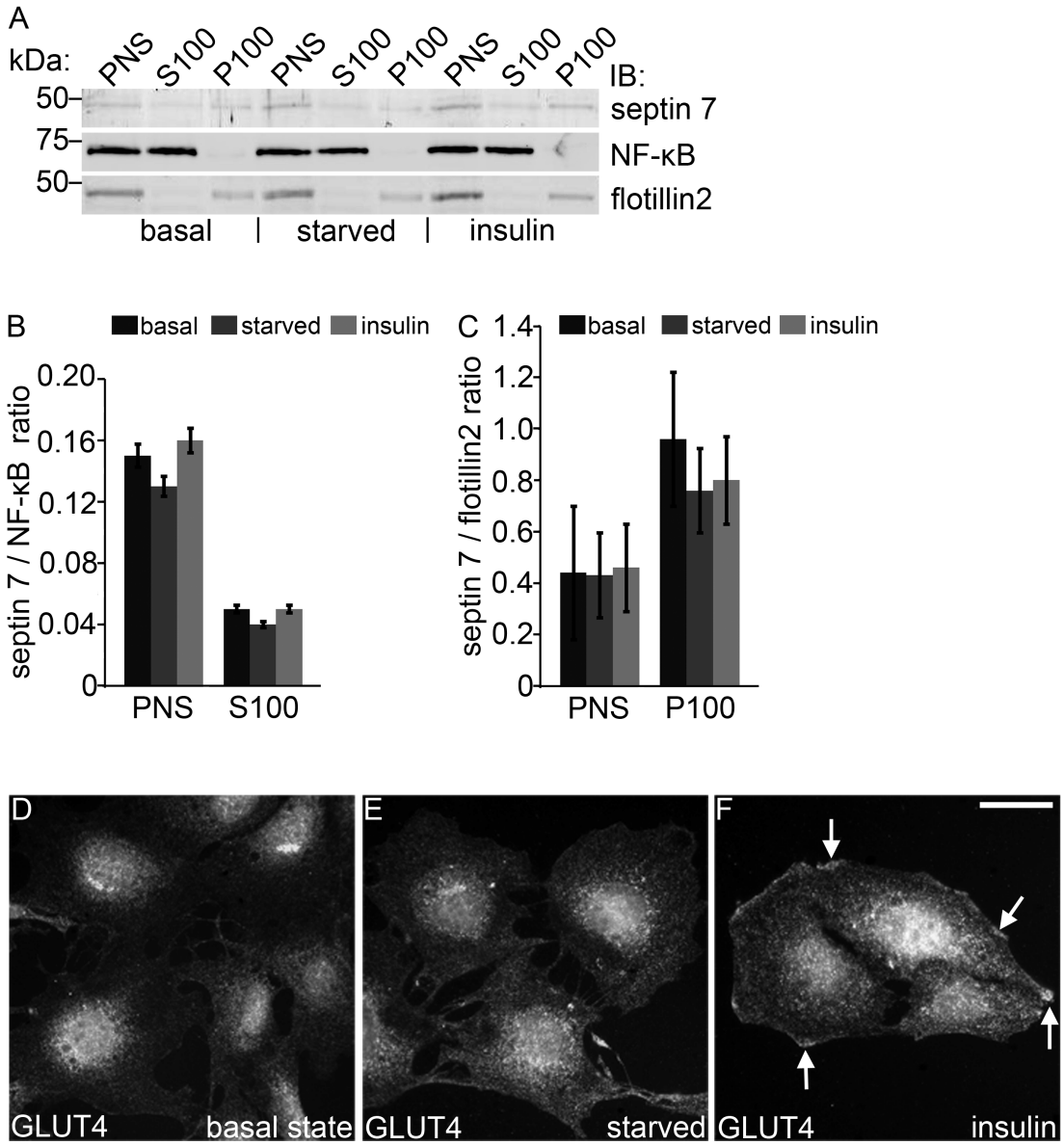


SUPPLEMENTARY FIGURE 4. Knockdown of septin 7 with individual siRNAs increases glucose uptake in HIRc cells. (A) Septin 7 siRNA #1 (ON-TARGET plus J-093922-09) leads to 72% reduction and septin 7 siRNA #2 (ON-TARGET plus J-093922-10) to 60% reduction in septin 7 in HIRc cells. CD2AP expression level remains unchanged whereas septin 9 and septin 11 are reduced by 53-63%% and 72-76% by the individual siRNAs, respectively. HIRc cells were transfected with rat septin-7 siRNA #1 or #2, siCONTROL Non-Targeting Pool siRNA (control) or Lipofectamine 2000 alone (lipof.) and analyzed by immunoblotting 72 h after transfection by Western blotting with the indicated antibodies. (B) Quantification of septin 7, CD2AP, septin 9 and septin 11

levels in three replicate blots as in (A) presented as septin 7/actin, CD2AP/actin, septin 9/actin and septin 11/actin ratio. (C) Septin 7 siRNA #1 increases the glucose uptake activity of HIRc cells by 57% compared to the control siRNA transfected cells (set to 100%) under steady state condition. (D) Depletion of septin 7 by septin 7 siRNA #2 increases the glucose uptake activity of HIRc cells by 37% compared to the control siRNA transfected cells (set to 100%) under steady state condition. Bars show the mean and error bars STDEV of three independent experiments, Student's t-test.

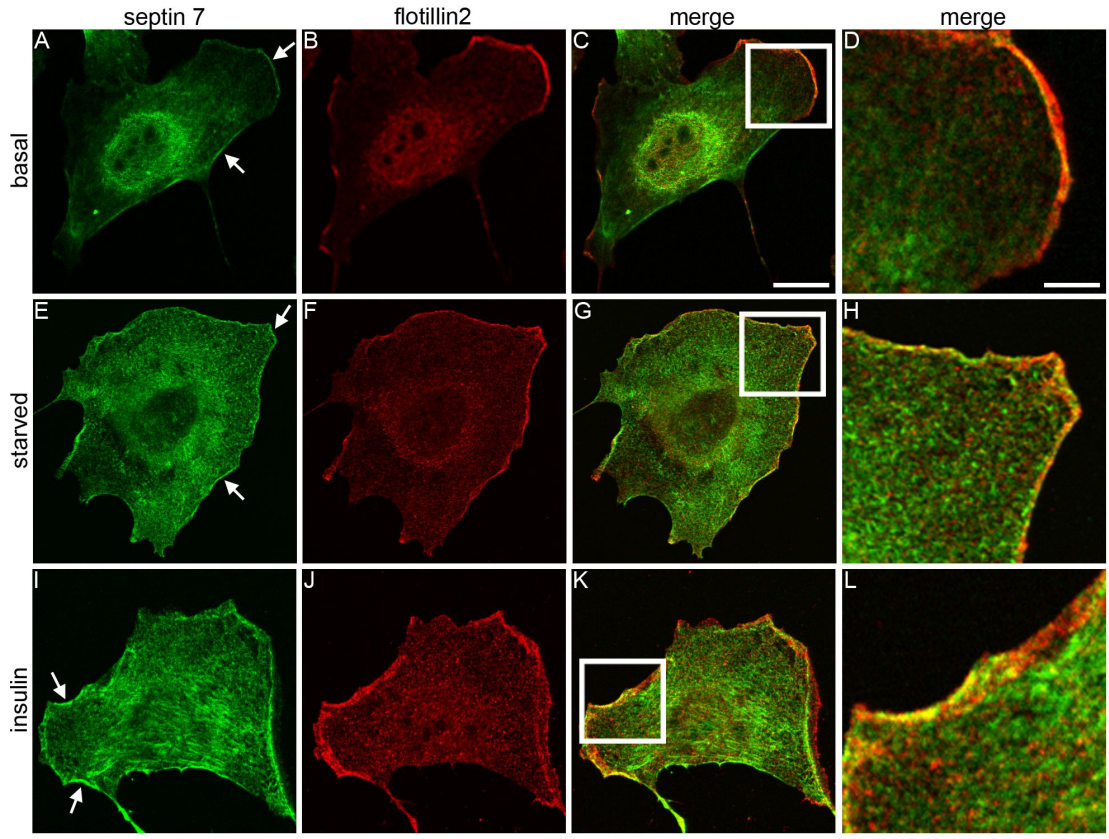


SUPPLEMENTARY FIGURE 5. Forchlorfenuron alters septin 7 organization in mouse podocytes. (A-D) Immunofluorescence images of mouse podocytes treated with solvent only (DMSO) show filamentous septin 7 (A) and actin (B) organization. Septin 7 filaments partially align along actin stress fibers detected with phalloidin (C-D). (E-H) In forchlorfenuron (FCF) treated podocytes septin 7 filaments cumulate at the cell periphery (arrows). Cultured mouse podocytes were treated or not with 50 µM FCF for 4 h, fixed with PFA, labeled with septin 7 IgG and phalloidin, and examined by fluorescence microscopy. Bar, 20 µm; in D, H, 7 µM.



SUPPLEMENTARY FIGURE 6. Septin 7 is found in cytoplasmic and membrane fractions in mouse podocytes. Mouse podocytes at basal state, serum starved for 20 h or starved and stimulated with 20 nM insulin for 15 min were washed twice in ice-cold HES buffer (20 mM Hepes, pH 7.4, 1 mM EDTA, 255 mM sucrose) and scraped into HES supplemented with 50 mM NaF, 1 mM Na_3VO_4 and 1x Complete proteinase inhibitor cocktail (Roche). Cells were homogenized by passing 10 times through a 25G needle and

the homogenate was centrifuged 1000 x g at 4°C for 5 min to obtain post-nuclear supernatant (PNS). PNS was ultracentrifuged 100 000 x g at 4°C for 1h using SW55 Ti rotor to obtain cytosol (S100) and membrane (P100) fractions. Equal volumes of the fractions were separated in SDS-PAGE for immunoblotting. (A) Septin 7 is present in both membrane and cytosolic fractions in basal state, starved and insulin stimulated mouse podocytes. Insulin stimulation does not change the proportion of septin 7 in the fractions. NF-κB (Abcam, Cambridge, United Kingdom), a cytosolic protein, and flotillin2 (Santa Cruz Biotechnology), a membrane protein, are detected as expected exclusively in S100 and P100 fractions, respectively. (B) Quantification of septin 7 level in the PNS and cytosolic (S100) fractions in basal state, starved and insulin stimulated podocytes in three replicate blots as in (A) presented as septin 7/NF-κB ratio. (C) Quantification of septin 7 level in the PNS and membrane (P100) fractions in basal state, starved and insulin stimulated podocytes in three replicate blots as in (A) presented as septin 7/flotillin2 ratio. In B-C, bars show the mean and error bars STDEV of three independent experiments, Student's t-test. (D-F) Immunofluorescence images of mouse podocytes stained for GLUT4. GLUT4 localizes in the perinuclear region in basal (D) and starved (E) mouse podocytes and partially translocates to the plasma membrane (arrows) after insulin stimulation (F). Cultured mouse podocytes were starved for 20 h, treated or not with 20 nM insulin, fixed with 2% PFA for 30 min, blocked, incubated with GLUT4 antibody (Millipore, Billerica, MA) diluted in 2% FCS, 2% bovine serum albumin, 0.2% fish skin gelatin, 0.1% saponin at room temperature for 1 h. Detection was with AlexaFluor 488 donkey anti-rabbit IgGs (Molecular Probes). Samples were examined with Zeiss Axioplan2 microscope (Thornwood, NY). Bar, 20 μm.



SUPPLEMENART FIGURE 7. Septin 7 partially co-localizes with the membrane marker flotillin2 in mouse podocytes. Confocal microscopy images of mouse podocytes stained for septin 7 (green) and flotillin2 (red). Nuclei are visualized with Hoechst (blue). Septin 7 localizes in the cytoplasm in filamentous and punctate pattern and on the plasma membrane (arrows) in basal state (A), starved (E) and insulin stimulated (I) mouse podocytes. Flotillin2 localizes in vesicles in the cytoplasm and on the plasma membrane in basal state (B), starved (F) and insulin stimulated (J) podocytes. Septin 7 and flotillin2 partially co-localize on the plasma membrane (C-D, G-H, K-L). Cultured mouse podocytes were starved for 20 h, treated or not with 20 nM insulin, fixed with PFA and methanol, labeled with septin 7 and flotillin2 IgG (Santa Cruz Biotechnology), and examined by Leica SP2 confocal microscope (Wetzlar, Germany). Bar, 20 μm; in D, H, L, 7 μM.