Frindt et al., http://www.jgp.org/cgi/content/full/jgp.201511533/DC1

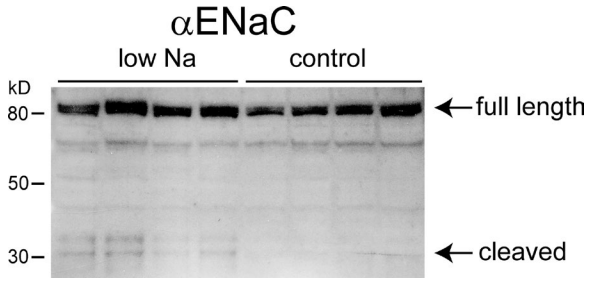

Figure S1. Effect of a low-Na diet on the expression of αENaC in whole kidney microsomes. Each lane was loaded with 75 µg of kidney membrane protein from a different rat. The expression of both full-length (~85 kD) and cleaved (~30 and 35 kD) forms of the subunit increased.

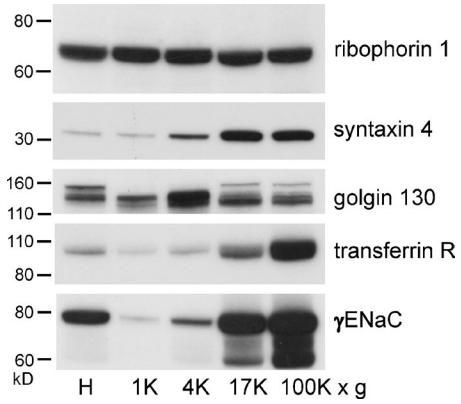

Figure S2. Differential centrifugation of rat kidney homogenates. Each lane was loaded with 30 µg of total protein from the homogenate (H), 1,000-*g* pellet (1K), 4,000-*g* pellet (4K), 17,000-*g* pellet (17K), and 100,000-*g* pellet (100K). The blots were stained with primary antibodies marking different subcellular compartments: ribophorin 1 (ER), syntaxin 4 (plasma membrane), golgin 130 (Golgi), transferrin receptor (endosomes), and γENaC.

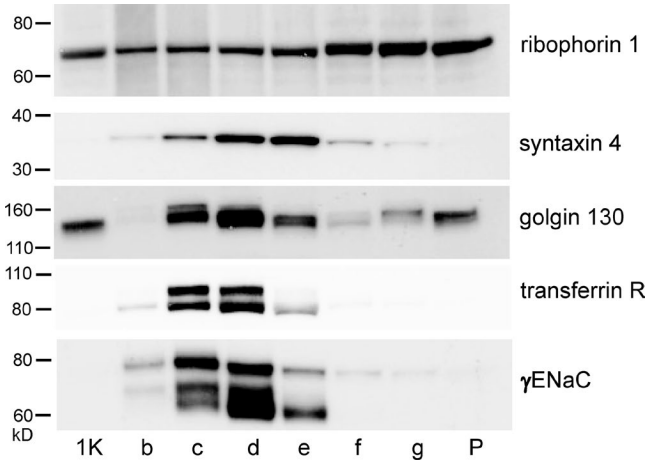

Figure S3. Sucrose density-gradient separation of membranes from the 1,000-*g* pellet. Gels were loaded with 30 µg of total protein from the 1,000-*g* pellet (1K) and from the interfaces of a discontinuous sucrose gradient (b → g, going from the lightest to the heaviest fractions). The blots were stained with primary antibodies as described in Fig. S2.

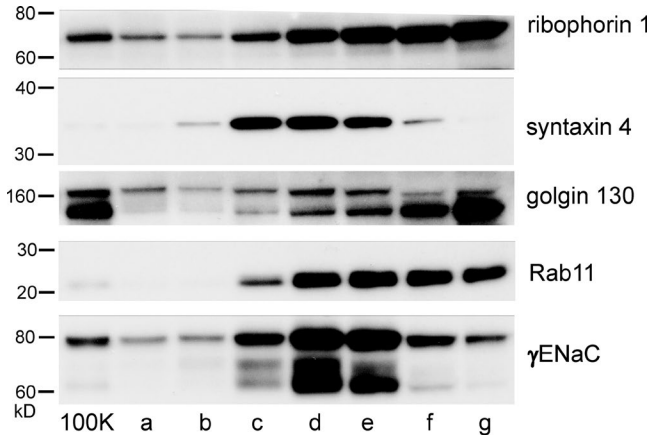

Figure S4. Sucrose density-gradient separation of membranes from the 100,000-*g* pellet. Gels were loaded with 30 µg of total protein from the 100,000-*g* pellet (100K) and from the interfaces of a discontinuous sucrose gradient (a → g, going from the lightest to the heaviest fractions). The blots were stained with primary antibodies as described in Fig. S2. In this case, an antibody against Rab11 was used to mark endosomes.

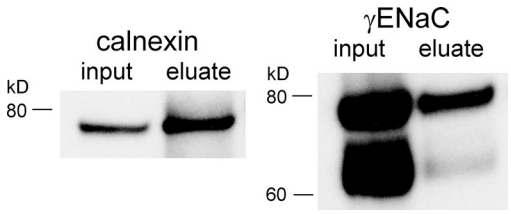

Figure S5. Recovery of protein with calnexin immunoisolation. Starting material represents 60 µg of protein from fraction f of the sucrose density-gradient fractionation of a 1,000-*g* pellet (Fig. S3). 500 µg of protein was absorbed onto anti-calnexin–coated beads, eluted with Triton X-100 to release γENaC, and then re-eluted with SDS to release calnexin. The eluate lanes were loaded with 50% of the eluates representing 250 µg of the starting material.

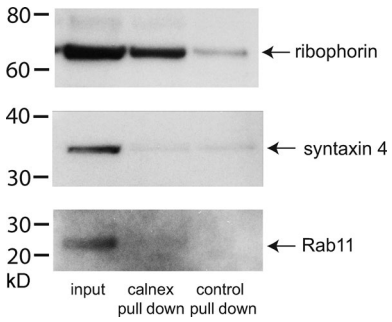

Figure S6. Specificity of calnexin immunoisolation. Starting material represents 60 µg of protein from fraction f of the sucrose density-gradient fractionation of a 1,000-*g* pellet (Fig. S3). 500 µg of protein was absorbed onto anti-calnexin–coated beads and eluted with Triton X-100. The eluate lanes were loaded with 50% of the eluates representing 250 µg of the starting material. Blots were probed with antibodies against ribophorin (top), syntaxin 4 (middle), or Rab11 (bottom). The bottom panel has a higher background, as the blot was stripped and reprobed with anti-Rab11 antibody. The eluates contained significant amounts of ribophorin but not of the other two proteins.

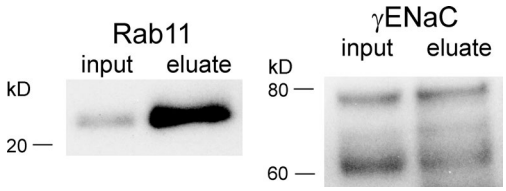

Figure S7. Recovery of protein with Rab11 immunoisolation. Starting material represents 10 µg of protein from fraction e of the sucrose density-gradient fractionation of a 100,000-*g* pellet (Fig. S4). 250 µg of protein was incubated with 2.5 µg anti-Rab11 antibody, and the complexes were captured with protein G–coated magnetic beads. The absorbed material was eluted with sample buffer, and the eluate lanes were loaded with 50% of the eluates representing 125 µg of the starting material.

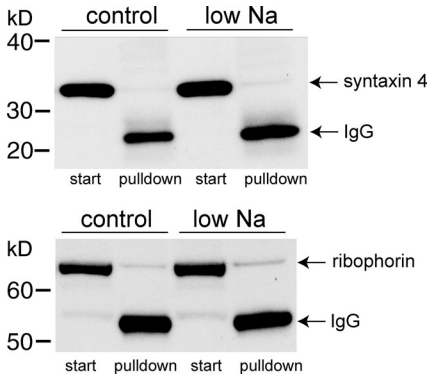

Figure S8. Specificity of Rab11 immunoisolation. Starting material represents 10 µg of protein from fraction e of the sucrose density-gradient fractionation of a 100,000-*g* pellet (Fig. S4). 250 µg of protein was incubated with 2.5 µg anti-Rab11 antibody, and the complexes were captured with protein G–coated magnetic beads. The absorbed material was eluted with sample buffer, and the eluate lanes were loaded with 50% of the eluates representing 125 µg of the starting material. Blots were probed with antibodies against syntaxin 4 (top) or ribophorin (bottom). The eluates contained minimal amounts of these proteins. The bands at 55 kD and 22 kD represent IgG heavy chains and light chains, respectively.

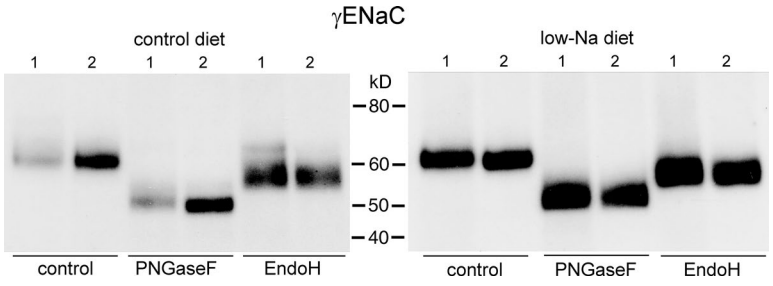

Figure S9. Effects of PNGaseF and EndoH on γENaC from urinary exosomes. Exosome preparations from two rats on control diet and two on low-Na diet were incubated with no enzyme (control), PNGaseF, or EndoH as in Fig. 1. In all cases, the decrease in apparent molecular mass was less with EndoH than with PNGase, indicating mature glycosylation trees.