Supporting Information

Roux *et al.* 10.1073/pnas.0808602106

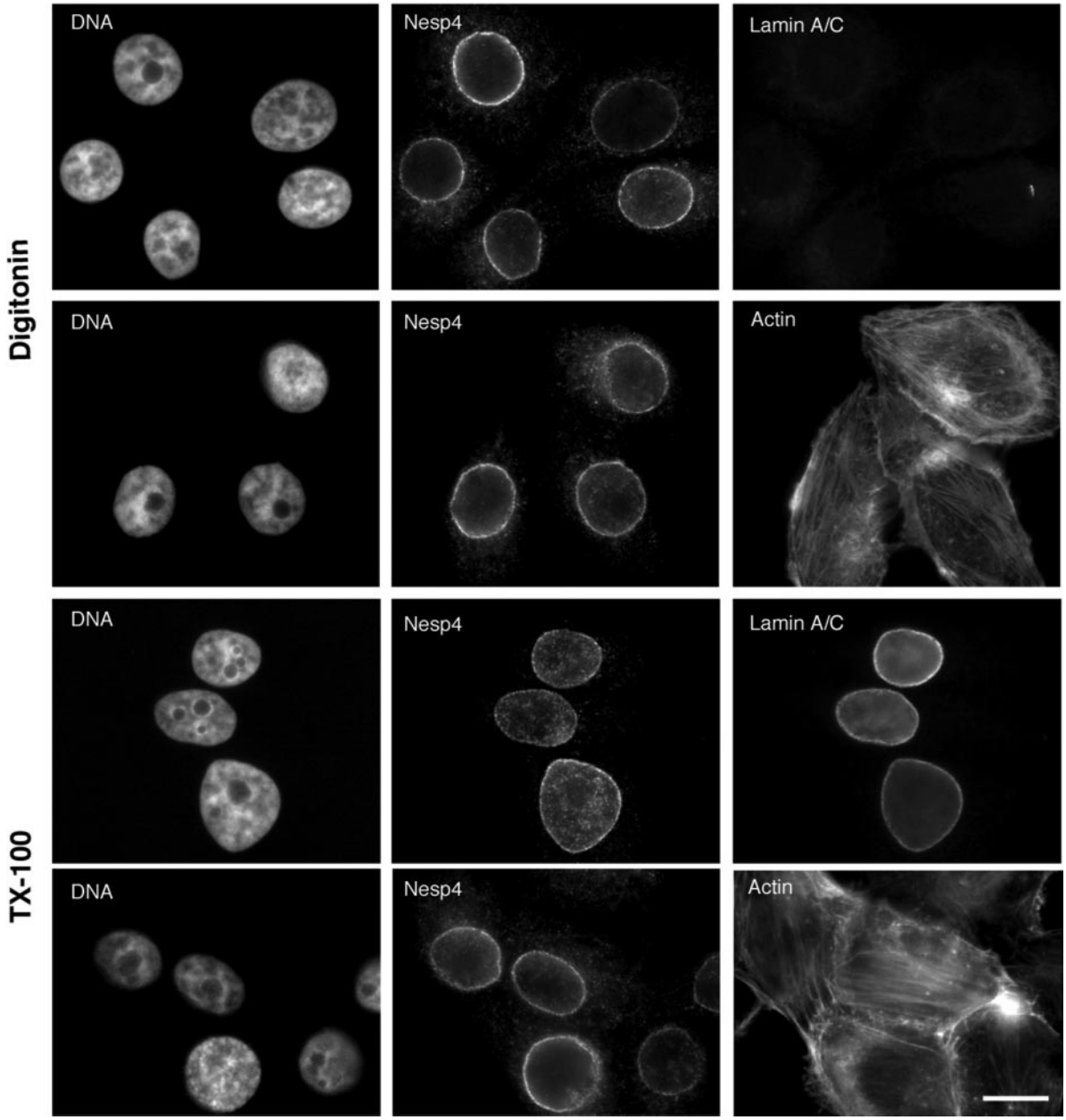

Fig. S1. Nesp4 is exposed on the ONM. Immunofluorescence microsopy of HSG cells stably expressing HA-Nesp4. The cells were fixed with formaldehyde and permeabilized with either an ice-cold solution of digitonin (0.003%) or a room temperature solution of 0.2% Triton X-100. The cells were then double-labeled with a mAb against HA tag and polyclonal antibodies against either lamins A and C or actin. The anti-HA antibody clearly detects HA–Nesp4 (Nesp4) after digitonin permeabilization, whereas lamins A and C (but not actin) remain inaccessible to antibody. The conclusion is that the N terminus of Nesp4 must be exposed on the cytoplamic face of the NE, which is consistent with the view that Nesp4 is a type II ONM protein. DNA is revealed by staining with Hoechst dye. (Bar: 10 μm.)

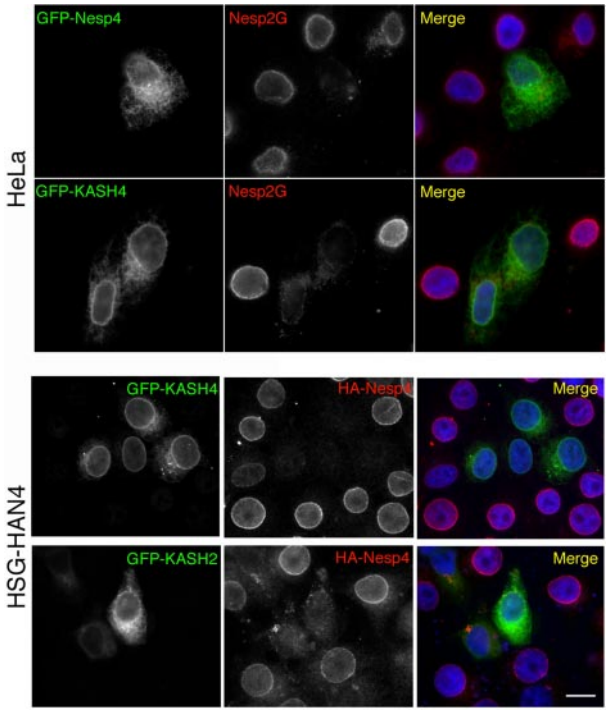

Fig. S2. Competition between KASH domain proteins for tethering at the NE. GFP–Nesp4 or GFP–KASH4 displaces Nesp2G from the NE of HeLa cells. Transient transfection of HSG–HAN4 cells with either GFP–KASH4 or GFP–KASH2 displaces HA–Nesp4 from the NE. DNA is revealed by staining with Hoechst dye. (Bar: 10μm.)

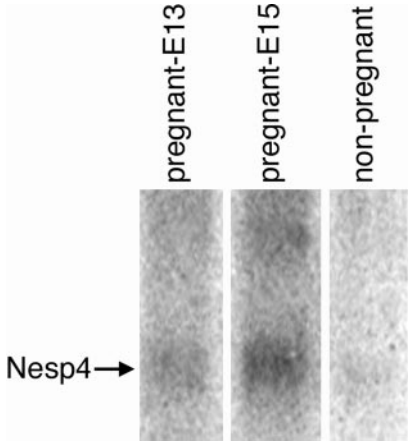

Fig. S3. Nesp4 is expressed in murine mammary tissue. Northern blot analysis of RNA extracted from mammary tissue of nonpregnant or E13 and E15 pregnant mice. The Nesp4 probe labeled a 1.4- to 1.7-kb band that is induced during pregnancy. Transcript size is consistent with mouse cDNA sequences available in GenBank. Each lane contains 20 μg RNA.

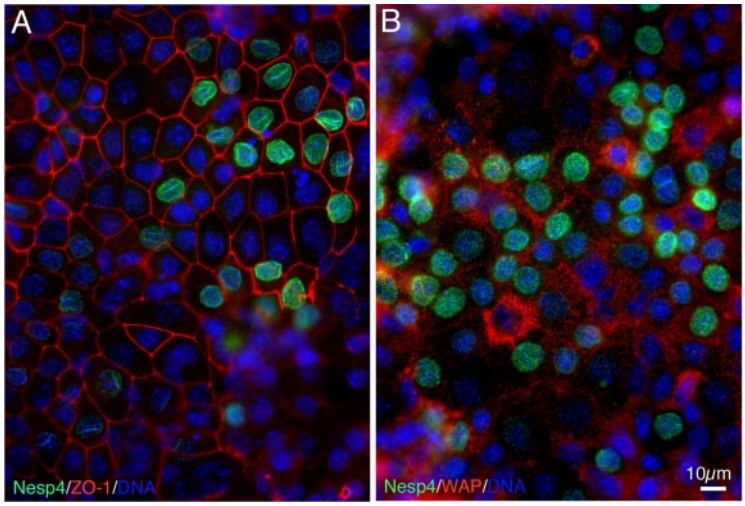

Fig. S4. Differentiated HC11 cells lacking Nesp4 (green) display epithelioid morphology, are delineated by the junctional marker ZO1 (red; *A*), and express whey acidic protein (WAP, red; *B*). After differentiation, the cell population appears highly heterogeneous. DNA (blue) is revealed by staining with Hoechst dye.

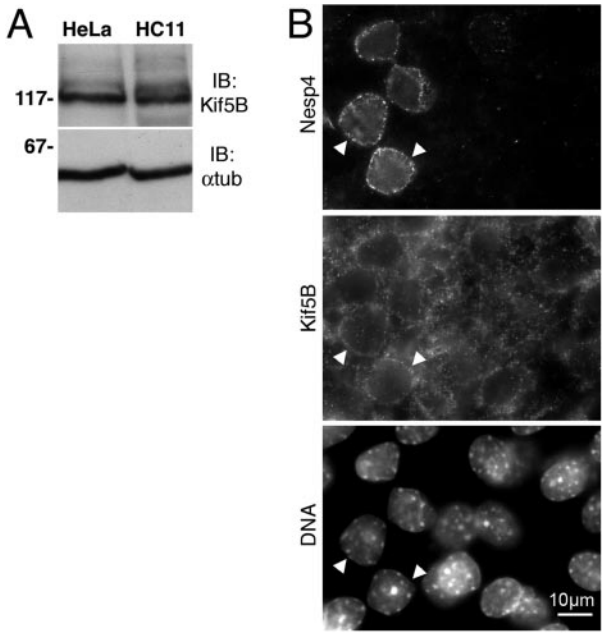

Fig. S5. Differentiated HC11 cells express abundant kinesin-1 heavy chain (Kif5B) as revealed by Western blot analysis. (*A*) Relative to α-tubulin, the level of Kif5B in HC11 cells is comparable with that seen in HeLa cells. (*B*) In those HC11 cells expressing Nesp4, Kif5B can be detected at the NE (arrowheads). In contrast, cells lacking Nesp4 display little or no enrichment of Kif5B at the NE. DNA is revealed by staining with Hoechst dye.

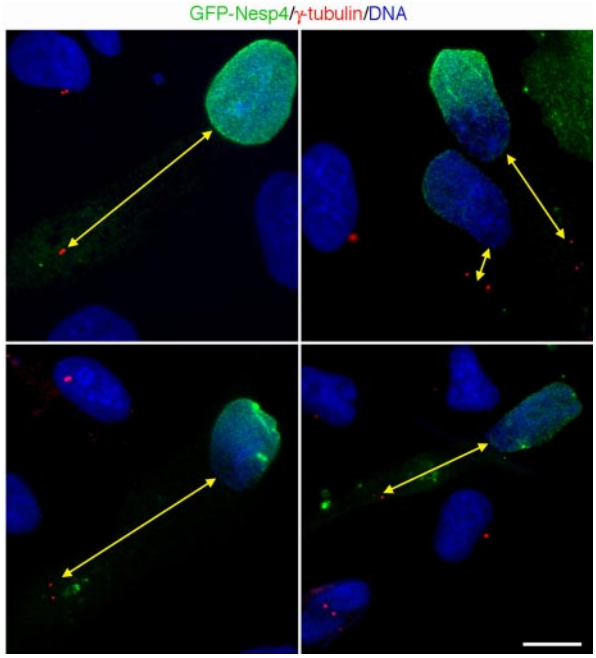

Fig. S6. Exogenous Nesp4 induces separation of the nucleus from the centrosome in HeLa cells. Immunoflorescence microscopy of HeLa cells transiently transfected with GFP–Nesp4. An anti-γ-tubulin antibody was used to detect the centrosomes (red). The transient expression of GFP-Nesp4 (green) is associated with dramatic displacement (yellow arrows) of the nuclei (blue) from the centrosomes. In comparison, the centrosomes of nontransfected cells are immediately adjacent to the nuclei. DNA is revealed by staining with Hoechst dye. (Bar: 10 μm.)