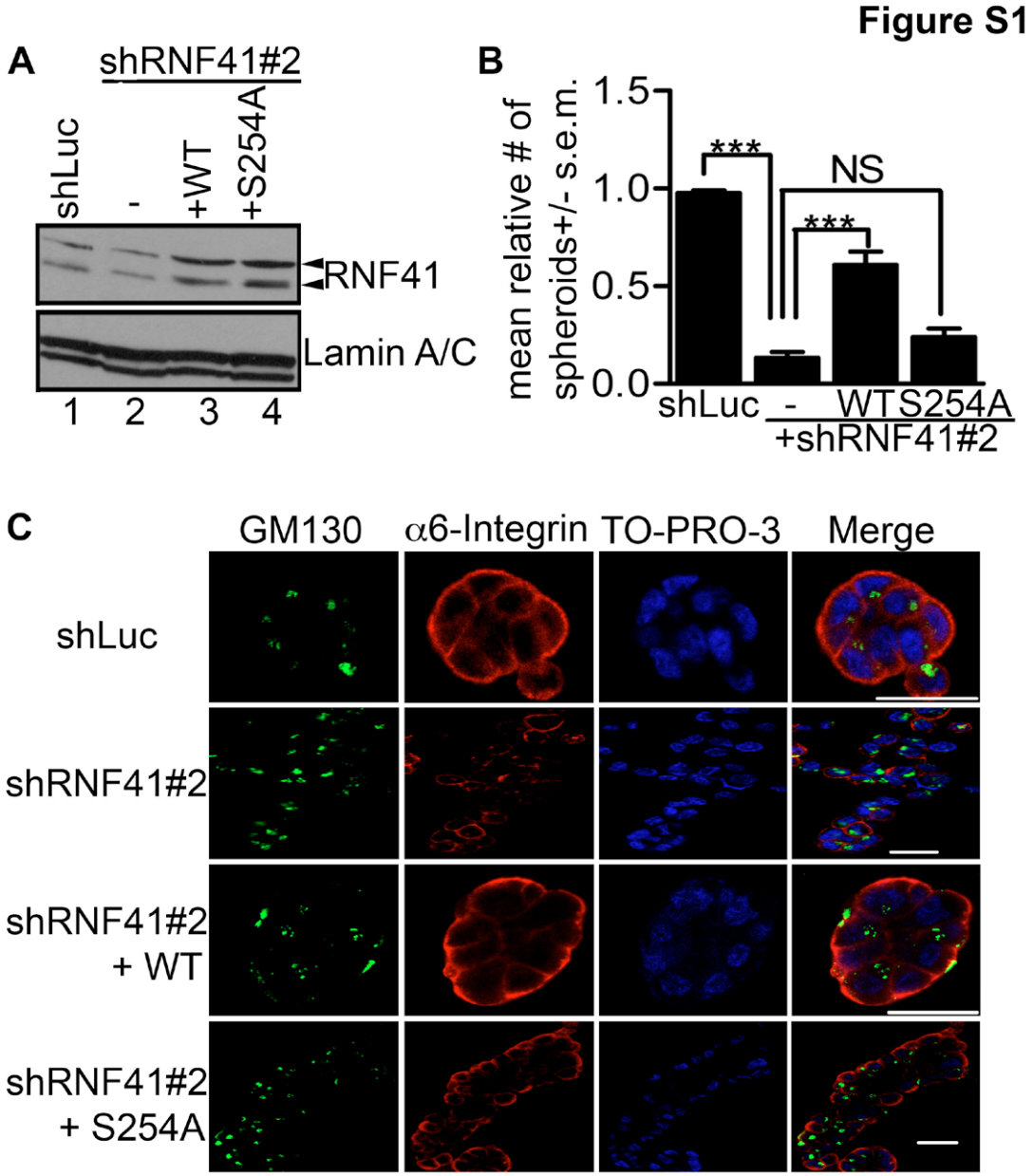

Fig. S1. RNF41 is necessary for establishing apical-basal polarity. (A) MCF10A stable cells expressing control shRNA (shLuc), or a second shRNA specific for RNF41 (shRNF41#2) alone (-) or in the presence of tagged ($FlagHis_6$) WT RNF41 (+ WT) or the tagged-S254A mutant (+ S254A) were cultured in collagen I matrix and harvested after 20 days. Lysates were resolved by SDS-PAGE and analyzed by Western blotting with the indicated antibodies. (B) Quantification of the ratio of round acini-like spheroids generated for cells described in panel A and cultured in a 3D collagen I matrix for 20 days. The number of colonies that formed acini-like spheroids was determined. 500 colonies were counted in each of 3 independent experiments (total=1500 colonies). The data is presented as mean relative number of acini-like spheroids +/- s.e.m. P values from a one-way ANOVA with a Tukey's multiple comparison posttest were determined. Astericks indicate significant p values (*** = $p<0.001$). NS = not significantly different. (C) Representative immunofluorescent confocal images of 3D colonies from 20 day cultures stained with antibody against α6-Integrin or GM130. 3D colonies were visualized with Alexa Fluor 594 (red) and 488 (green) labeled secondary antibodies, respectively, and To-Pro-3 to detect nuclei (blue). A serial immunofluorescent confocal cross section of a representative colony is shown and scale bars represent 20 μm. The data presented is representative of the 500 colonies that were observed in each of 3 independent experiments (total=1500 colonies).

Figure S2

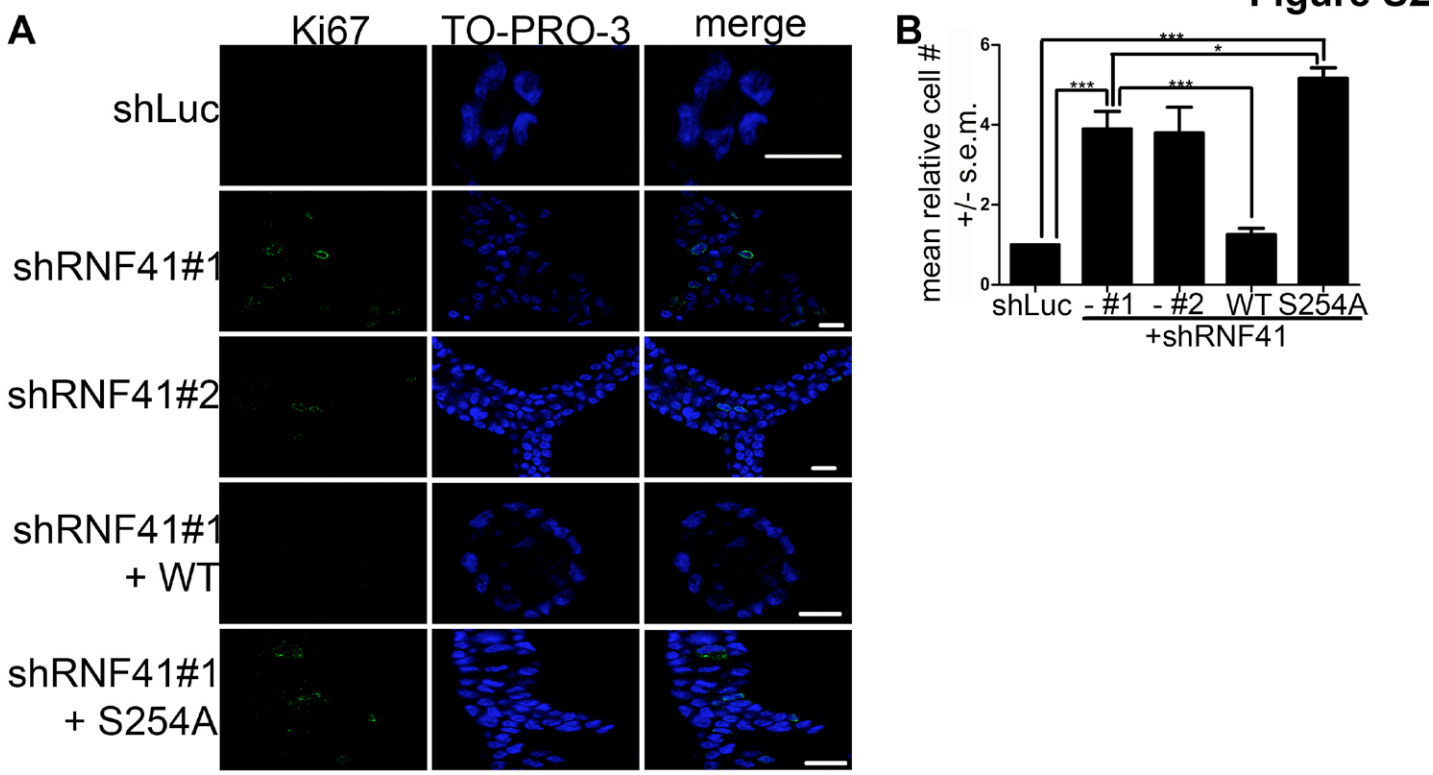

Fig. S2. RNF41 deficient cells continue to proliferate in 3D culture conditions. (A) MCF10A stable cells expressing control shRNA (shLuc), shRNAs specific for RNF41 (shRNF41#1 or #2) alone (-) or in the presence of tagged ($FlagHis_6$) WT RNF41 (+ WT) or the tagged-S254A mutant (+ S254A) were cultured in collagen I matrix for 20 days. Colonies were stained with antibody against Ki67 and visualized with Alexa Fluor 488 (green) labeled secondary antibody and To-Pro-3 to detect nuclei (blue). A serial immunofluorescent confocal cross section of a representative colony is shown and scale bars represent 20 μm. The data presented is representative of the 100 colonies that were observed in each of 3 independent experiments (total=300 colonies). (B) Relative number of cells determined in experiments described in panel A after 20 days of culture. The data is presented as mean relative cell number +/- s.e.m. P values from a one-way ANOVA with a Tukey's multiple comparison posttest were determined. Astericks indicate significant p values (*** = $p<0.001$, * = $p< 0.05$).

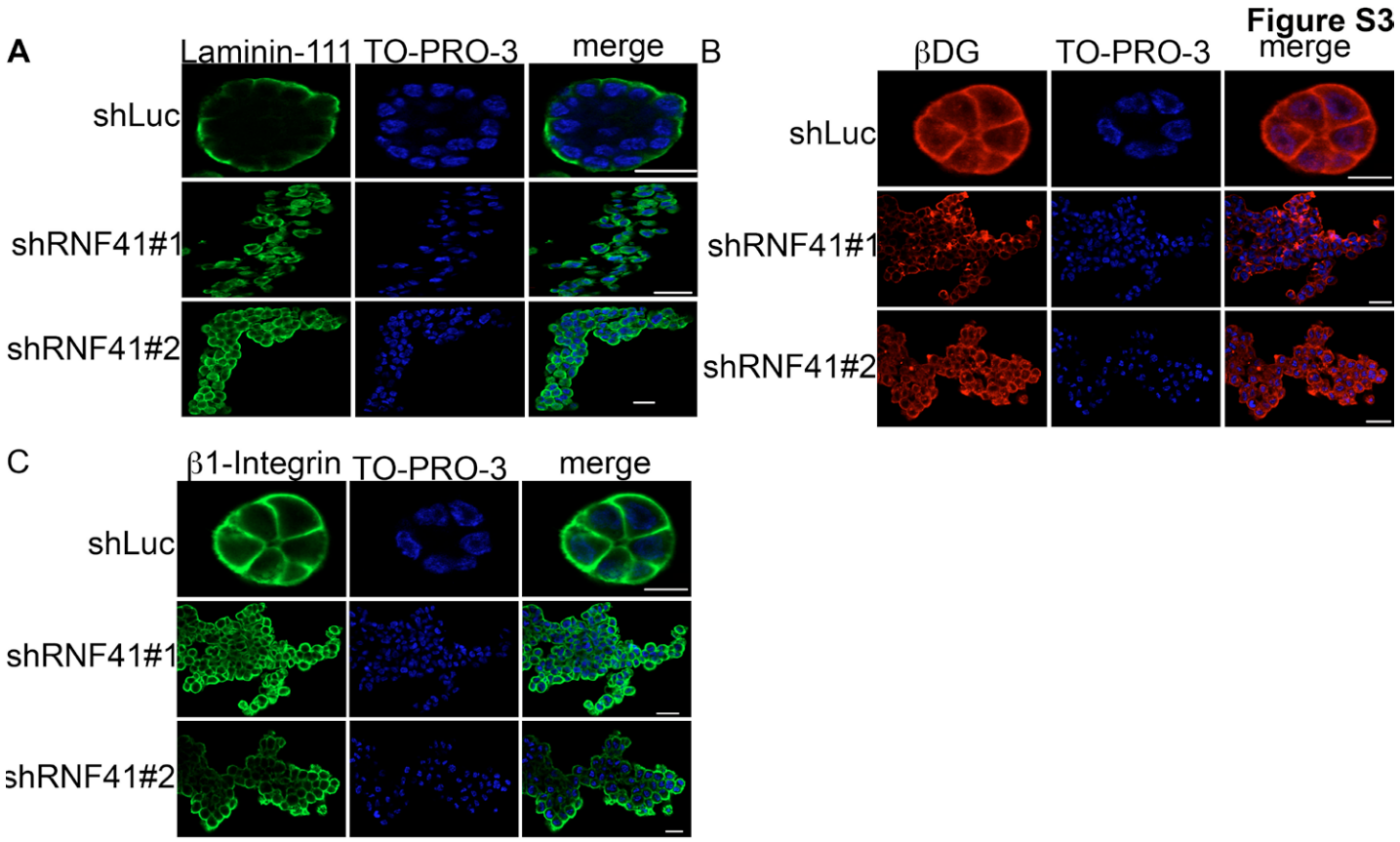

Fig. S3. Phosphorylation of RNF41 by Par-1b required for laminin-111 deposition and polarized localization of laminin-111 receptors at day 14. (A, B) MCF10A stable cells expressing control shRNA (shLuc or shRNAs specific for RNF41 (shRNF41#1 or #2) were cultured in a 3D collagen I matrix and on day 14, colonies were stained with antibody against laminin-111 (panel A), βDG (panel B) or β1-Integrin (panel C). Colonies were visualized with Alexa Fluor 488 (green) and Alexa Fluor 594 (red) labeled secondary antibody and To-Pro-3 to detect nuclei (blue). A serial immunofluorescent confocal cross section of a representative colony is shown and scale bars represent 20 μm. The data presented is representative of the 500 colonies that we observed in each of 3 independent experiments for a total of 1500 colonies.