Figure S1: SEPT9_v1 is overexpressed in breast cancers. (A), Semi-quantitative duplex RT-PCR data was used to define the expression profile of *SEPT9-X* (all isoforms), and multiple *SEPT9* splice variants in BCCs and IHMECs with GAPDH amplification used as an internal loading control. The BCCs marked with an asterisk (*) showed both high genomic copy number and overexpression of the *SEPT9* locus. The 11 BCCs showing overexpression of *SEPT9_v1* are highlighted in bold text. (B), Validation of the anti-SEPT9_v1 antibody used for immunohistochemistry (IHC). BCCs with high and low endogenous SEPT9_v1 expression, as determined previously by RT-PCR and Western blotting in this manuscript, were tested for SEPT9_v1 expression by IHC. Staining by IHC strongly agreed with expression data obtained from other methods. (C), Patient-matched normal (N) and breast cancer tumor (T) tissue pairs were stained for SEPT9_v1 by IHC. Seventy percent of tested tissue pairs showed high expression of SEPT9_v1 in tumors compared to normal mammary epithelial cells in the ducts. This image is the same as the one presented in the manuscript, but is shown here to indicate the color and staining intensity.

Figure S2: SEPT9_v1 overexpression increases cellular invasion and the percentage of aneuploid cells in multiple polyclonal lines, indicating that the phenotypes are not an artifact of cell culture. Invasion (A) and aneuploidy (B) were measured in independent experiments for MCF10A parental cells (n=2), pLNCX2 empty vector control cells (n=4), and SEPT9_v1 overexpressing cells (n=4) in triplicate. (A) SEPT9_v1 promotes invasion in MCF10A cells when compared to parental and empty vector controls. A transwell Matrigel invasion assay was performed in the presence of chemoattractants (serum and growth factors) and the data is presented as the average number of invading cells ± SEM of the independent

experiments. An asterisk (*) denotes $p<0.0001$ by ANOVA. (B) Ectopic expression of SEPT9_v1 in MCF10A cells results in genomic instability in that approximately 45% of cells in the population are aneuploid. The percentage of aneuploid cells from twenty-five metaphases of each independent experiments ± SEM is depicted. An asterisk (*) denotes $p<0.001$ by ANOVA.

Figures S3: SEPT9_v1 is a potent regulator of cellular motility. Representative images of three motility assays are depicted. A wound was created in a confluent culture and wound closure was measured after 20-22 hours as a measure of cellular motility. (A) SEPT9_v1 overexpression resulted in a six- to ten-fold increase in motility compared to SEPT9_v3 overexpressing, empty vector, and parental cell line controls for both HPV4-12 and MCF10A cells. (B) SEPT9_v1 expression knockdown by siRNA in MDA-MB-231 cells (left) or by stable shRNA in BT549 breast cancer cells (right) dramatically decreases cellular motility, as indicated by incomplete wound closure when compared to the parental and negative controls.

Figure S4: SEPT9_v1 immunoprecipitates and colocalizes with vimentin and overexpression of SEPT9_v1 causes an epithelial-to-mesenchymal transition phenotype as determined by increased vimentin expression in MCF10A and Hs578T cell lines. (A) Vimentin co-immunoprecipitated with SEPT9_v1 in Hs578T cells, but not in HPV4-12 cells, only when both proteins were overexpressed (top blots versus bottom blots). SEPT9_v1, alpha tubulin, and vimentin antibodies were used for immunoprecipitation and the samples were subsequently immunoblotted for SEPT9_v1. Whole cell lysates from the parental cell lines were used for immunoprecipation as shown in the blots in the top panel. Whole cell lysates from cells transduced with the SEPT9_v1 construct were used for immunoprecipitation for the blots

represented in the bottom half of the image. (B) Immunofluorescence studies indicate that vimentin co-localizes with SEPT9_v1 in interphase cells and more intense staining of vimentin is observed in SEPT9_v1-overexpressing cells, supporting the hypothesis of an epithelial to mesenchymal transition. The DNA is stained with DAPI (panels a, f, k, and p), SEPT9_v1 is green (panels b, g, l, and q), vimentin staining is shown in purple (panels d, i, n, and s) and the merged images of SEPT9_v1 and vimentin are shown in the last column (panels e, j, o, and t).

Figure S5: Ectopic SEPT_v1 and SEPT9_v3 co-localize with cytoskeletal proteins but with different outcomes and overexpressed SEPT9_v1 causes mitotic defects. (A) Immunofluorescence studies were used to stain cells for DNA (DAPI; panels a, g, and m), SEPT9_v1 (panels b and n), SEPT9-X (panel h), phalloidin/F-Actin (panels c, i, and o), and alpha tubulin (panels d, j, and p). Ectopic expression of the SEPT9_v1 isoform in HPV4-12 caused the microtubule network to break down in interphase cells (panel p) compared to ectopic expression of the SEPT9_v3 isoform (panel j) or the parental cell line (panel d). The merged images of SEPT9_v1 and actin (panels e and q) show the co-localization of actin filaments with SEPT9_v1 and the merged image between SEPT9_v3 and actin (panel k) shows co-localization of SEPT9_v3 with actin rings in the transduced HPV4-12 cells during interphase. The merged images of SEPT9_v1 and alpha tubulin show co-localization of SEPT9_v1 and microtubule filaments in the parental cell line (panel f) and with microtubules bundles in the SEPT9_v1 overexpressing cells (panel r). (B) Immunofluorescence studies of HPV4-12 cells were used to visualize DNA (DAPI; panels a, e, i, and m), and SEPT9_v1 (panels b, f, j, and n), and phalloidin/F-Actin (panels c, g, k, and o). Overexpression of SEPT9_v1 results in nuclear mislocalization (panels j and n) compared with the untransduced cells (panel b) in which

endogenous SEPT9_v1 localizes to the cytoplasm. Ectopic expression of SEPT9_v1 also causes an increase in binucleated giant cells such that they comprise 10% of the population (panels h and l versus d) and results in an increased incidence of the combination of the two defects (panel l). Some of these images are also published in the body of the manuscript, but are presented here for clarity and context.