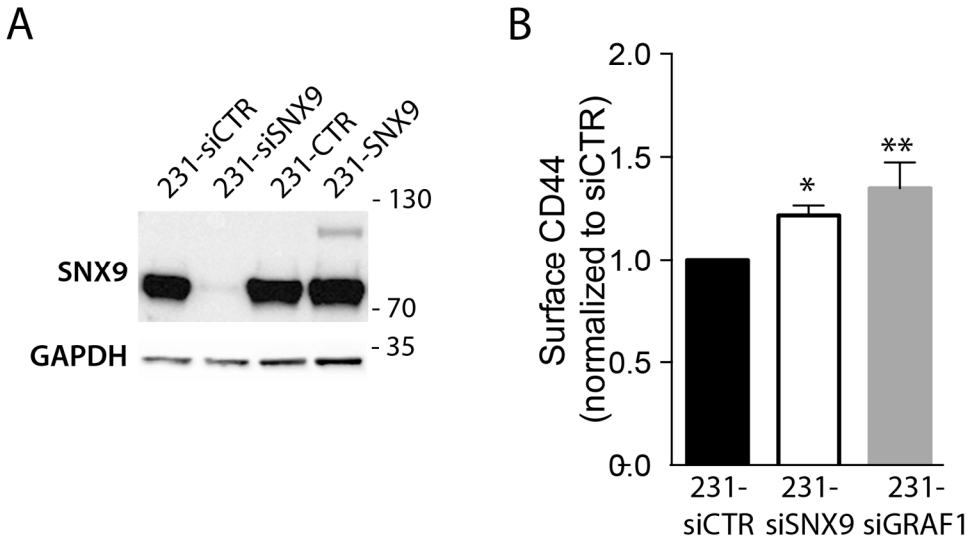
Supplemental Materials

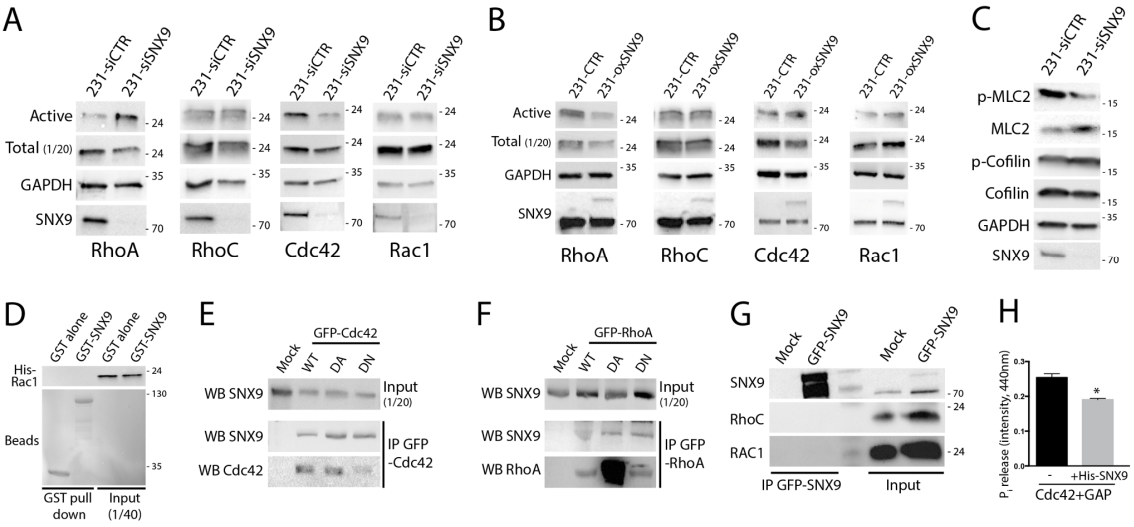
Molecular Biology of the Cell

Bendris et al.

Supplementary Material

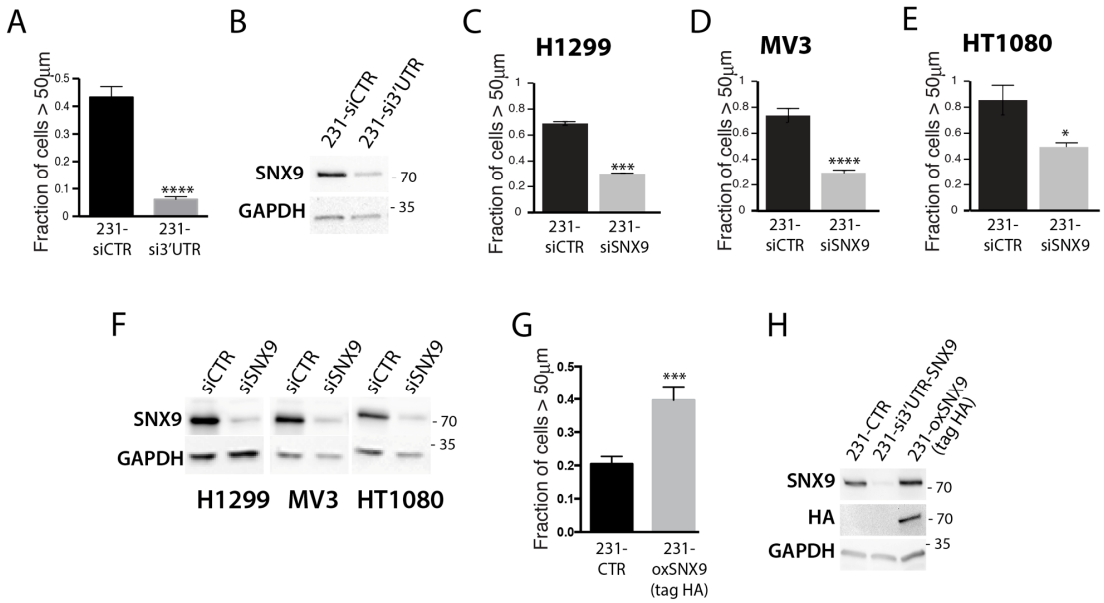


Supplementary Fig S1:SNX9 depression increases surface expression of CD44.
A. Representative Western blot illustrating SNX9 overexpression or depletion levels in MDA-MB-231 cells.GAPDH was used as loading control.Blotis representative of 3 independent experiments.**B.**231-siCTR, -siSNX9 and –siGRAF1 cells were incubated with anti-CD44 antibody at 4°C, to prevent its internalization. Cells were then washed with cold PBS to remove unbound antibody and HRP signal proportional to surface expression of CD44 was quantified as in Methods section. n=3, p*<0.05, p**<0.005.



Supplementary Fig S2:SNX9 expression modulates the activity of RhoGTPases.

A-B. Active forms of GTPases were pulled down using beads coupled to respective effector domains that can only bind the GTP-bound, *i.e.* active, form of the GTPases (see Methods). Blots shown here are examples from 3 independent experimentsillustrating quantifications of active GTPases in 231-siSNX9 (**A**) and in 231-oxSNX9 (**B**) compared to 231-siCTR and 231-CTR cells, respectively. **C.** Representative Western blots illustrating quantification of phosphorylated cofilin and MLC2 in 231-siSNX9 compared to 231-siCTR. **D.** Western blots comparing the interaction of His-Rac1 with GST alone or with GST-SNX9 beads, *in vitro*. We used the GTPase Rac1 as a specificity control of RhoA and Cdc42 direct binding to SNX9 (see Fig 2D). **E, F**. Western blots showing interaction of endogenous SNX9 with GFP-Cdc42 mutants (**E**) or with GFP-RhoA mutants (**F**) expressed in MDA-MB-231 cells. WT=wild type, DA=dominant active, and DN=dominant negative. **G**. We used Rac1 and RhoC as a specificity control of endogenous RhoA and Cdc42 binding to GFP-SNX9 in cells, compared to Supplementary Fig 2E-F. Western blots of endogenous Rac1 or RhoC showing no interactions with pulled down GFP-SNX9, expressed in MDA-MB-231 cells. **H.** P_i production after GTP hydrolysis by Cdc42 incubated with p50GAP with or without His-SNX9, *in vitro*. *p=0.035.
Blots are representative of 3 independent experiments.



Supplementary Fig S3: SNX9 depletion impairs cell invasiveness of cancer cell lines
A. Quantification of invasion assays for control cells or cells treated with a siRNA against the 3'UTR of SNX9. n=3. ****p<0.0001. **B**. Western blot analysis of SNX9 expression in conditions used in (**A)**. GAPDH was used as loading control.Blotis representative of 3 independent experiments.**C-E.** After appropriate siRNA depletions, H1299 (lung cancer), HT1080 (fibrosarcoma) or MV3 (melanoma) cell lines were subjected to an inverted invasion assay. Bar charts represent quantification of invading cells of H1299 (**C**), MV3 (**D**) or HT1080 (**E**). n=3. *p=0.02, ***p=0.001, ****p=0.0002.

G. Quantification of invasion assays for control cells or cells overexpressing HA-tagged SNX9. n=3. ***p=0.0007. **F**. Western blot analysis of SNX9 expression in conditions used in (**C-E)**. GAPDH was used as loading control.Blotis representative of 3 independent experiments.**G**. MDA-MB-231 cell line stably expressing HA-SNX9 was subjected to an inverted invasion assay, bar chart represents quantification of invading cells. n=3, ***p=0.002. **H.** Western blot analysis of SNX9 protein levels in 231-oxSNX9 expressing HA-tagged SNX9, compared to parental 231-CTR cells. Given that HA-SNX9 co-migrated with endogenous SNX9, we used si3UTR as a negative control and also used an antibody against the HA tag.GAPDH was used as loading control.Blotis representative of 3 independent experiments.