Supplemental Materials

Molecular Biology of the Cell

Morris et al.

Nck deficiency is associated with delayed breast carcinoma progression and reduced metastasis

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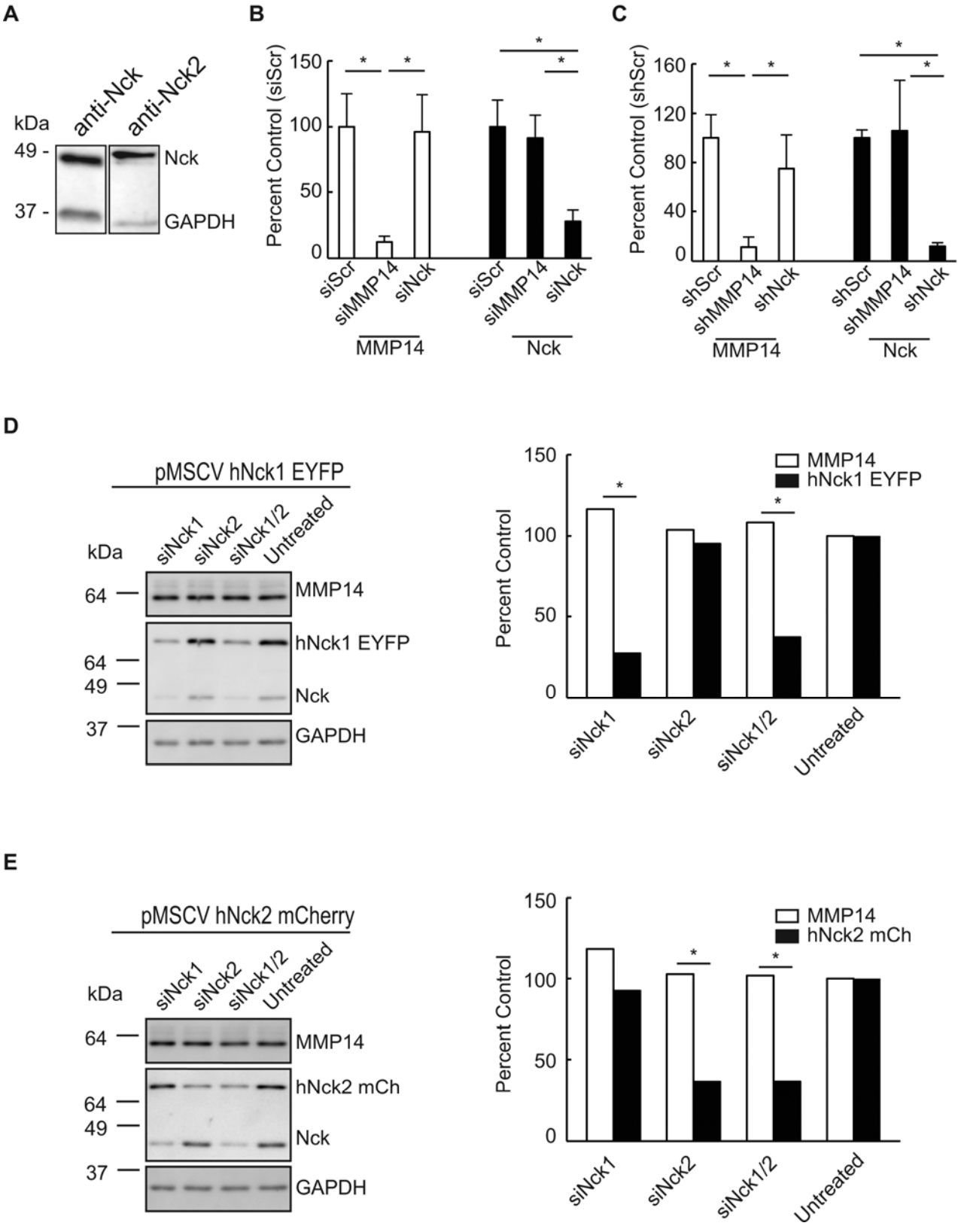
Supplementary Information

Supplementary Figure 1



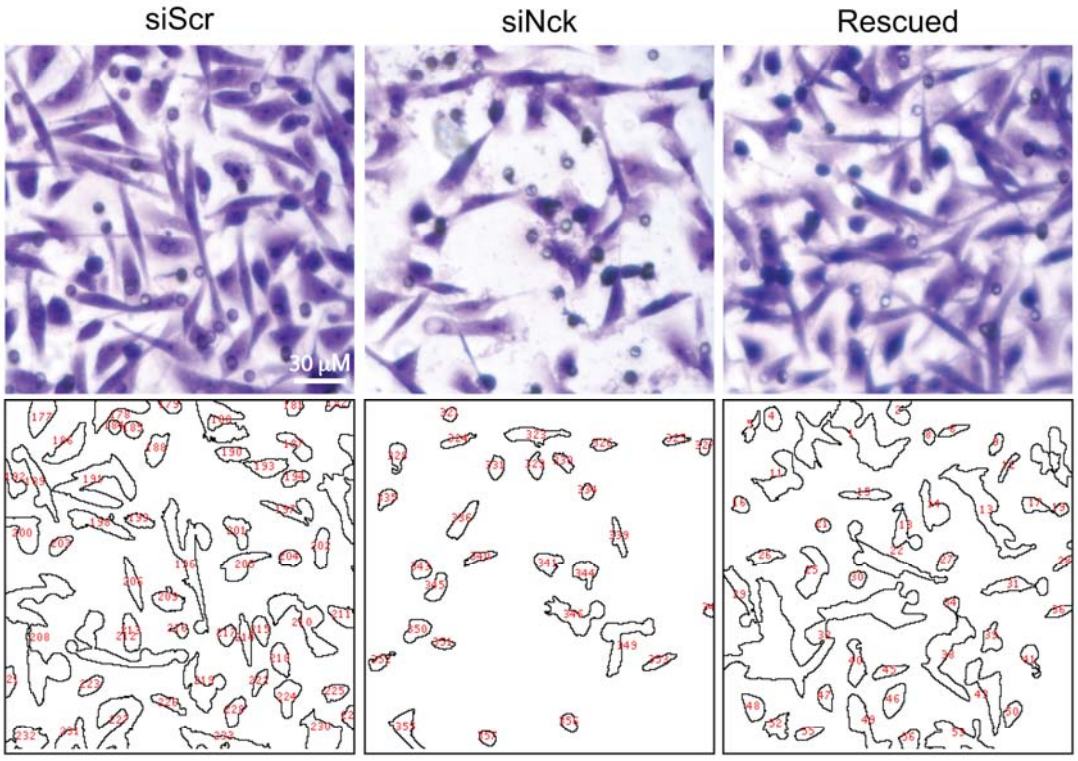
Supplementary Figure 1. Silencing of target proteins in MDA-MD-231 breast carcinoma cells. **A)**Nck expression in the MDA-MB-231 cell line used in the reported experiments. Total cellular levels of Nck were determined by western blotting using a commercially available, anti pan-Nck antibody (left panel) whereas expression of Nck2 was confirmed using a previously validated(Labelle-Cote *et al.*, 2011; Latreille *et al.*, 2011), custom rabbit polyclonal anti Nck2 antibody (right panel). GAPDH levels were used to monitor loading. **B)**Cells were transfected with non-targeting SMARTPool siRNA (siScr) or SMARTPool siRNA targeting MMP14 (siMMP14) or Nck1 and Nck2 (siNck). **C)** Cells were transduced with viruses harboring vectors expressing non-targeting short hairpins (shScr) or short hairpins targeting MMP14 (shMMP14) or Nck1 and Nck 2 (shNck). For experiments in B and C, cell lysates were collected 72 h after transfection/transduction and protein levels determined by western blotting.**D-E)** Western blots (left) and quantification (right) showing specificity/efficacy of SMARTPool siRNA reagents. Cells expressing human Nck1 EYFP (D) or Nck2 mCherry (E) were transfected with relevant siRNAs. A pan-Nck antibody was used to detect levels of endogenous Nck (Nck1 and Nck2) and Nck1 EYFP or Nck2 mCherry fusion proteins.Lack of direct effect of Nck silencing or off target effects of Nck siRNA reagents are suggested by unchanged levels of MMP14. GAPDH levels were used to monitor loading.

Supplementary Figure 2

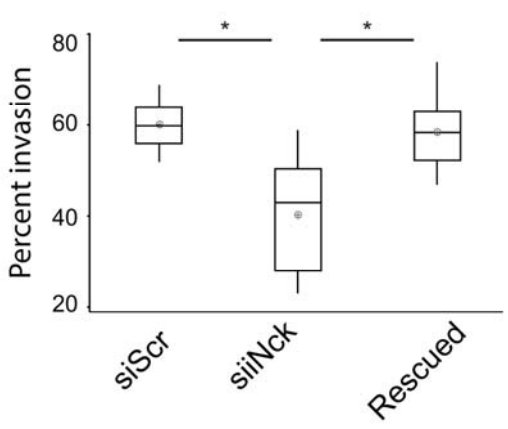
A

human Nck1-targeting siRNAs	human Nck2-targeting siRNAs
siNck1#1: 5' GATTATGGCTTCTGGATGA	siNck2#1: 5' CTTAAAGCGTCAGGGAAGA
mNck2: 5' GGTTGTGGCTGCTAGATGA	mNck2: 5' CTCAAAGCGTCAGGGAGAA
siNck1#2: 5' CCGCTTATGTGAAATTTAA	siNck2#2: 5' CCAACTACGTGGAGCGGAA
mNck2: 5' CCAACTATGTGGAGCGTAA	mNck2: 5' CCAACTATGTGGAGCGTAA
siNck1#3: 5' CGGAAAGCATCTATTGTGA	siNck2#3: 5' CCAACTATGTGGAGCGTAA
mNck2: 5' TGGACAGCGTCTACTGCAT	mNck2: 5' CAACAGGACAGGCTACGTG
siNck1#4: 5' CGAGAAAGGAGATGTAATG	siNck2#4: 5' GAAGTTATTGTGATAGCCA
mNck2: 5' CGAGAAGGGGGAGACCATG	mNck2: 5' GAAGTCATTGTCATAGCCA

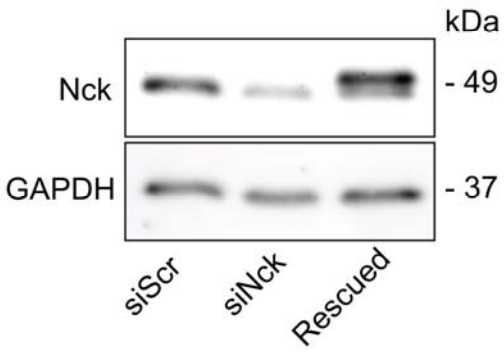
B



C

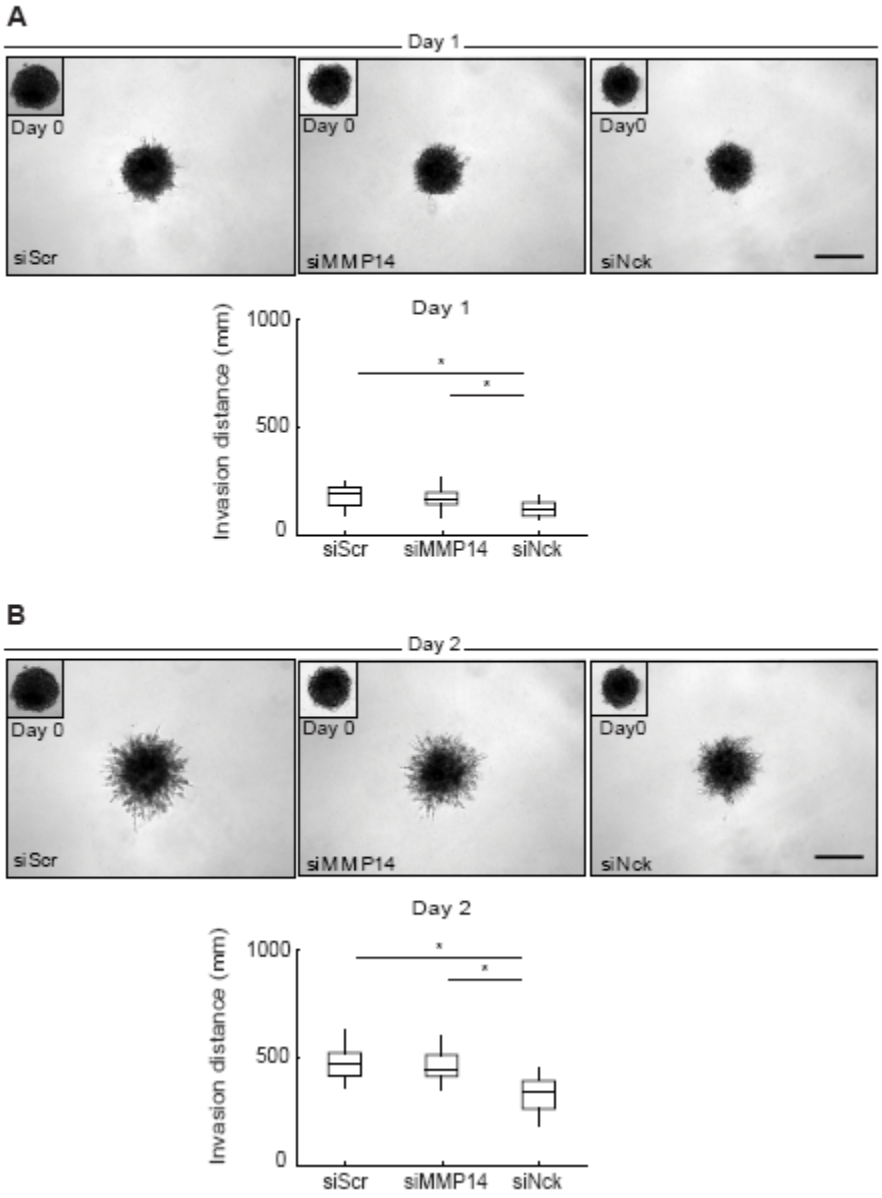


D



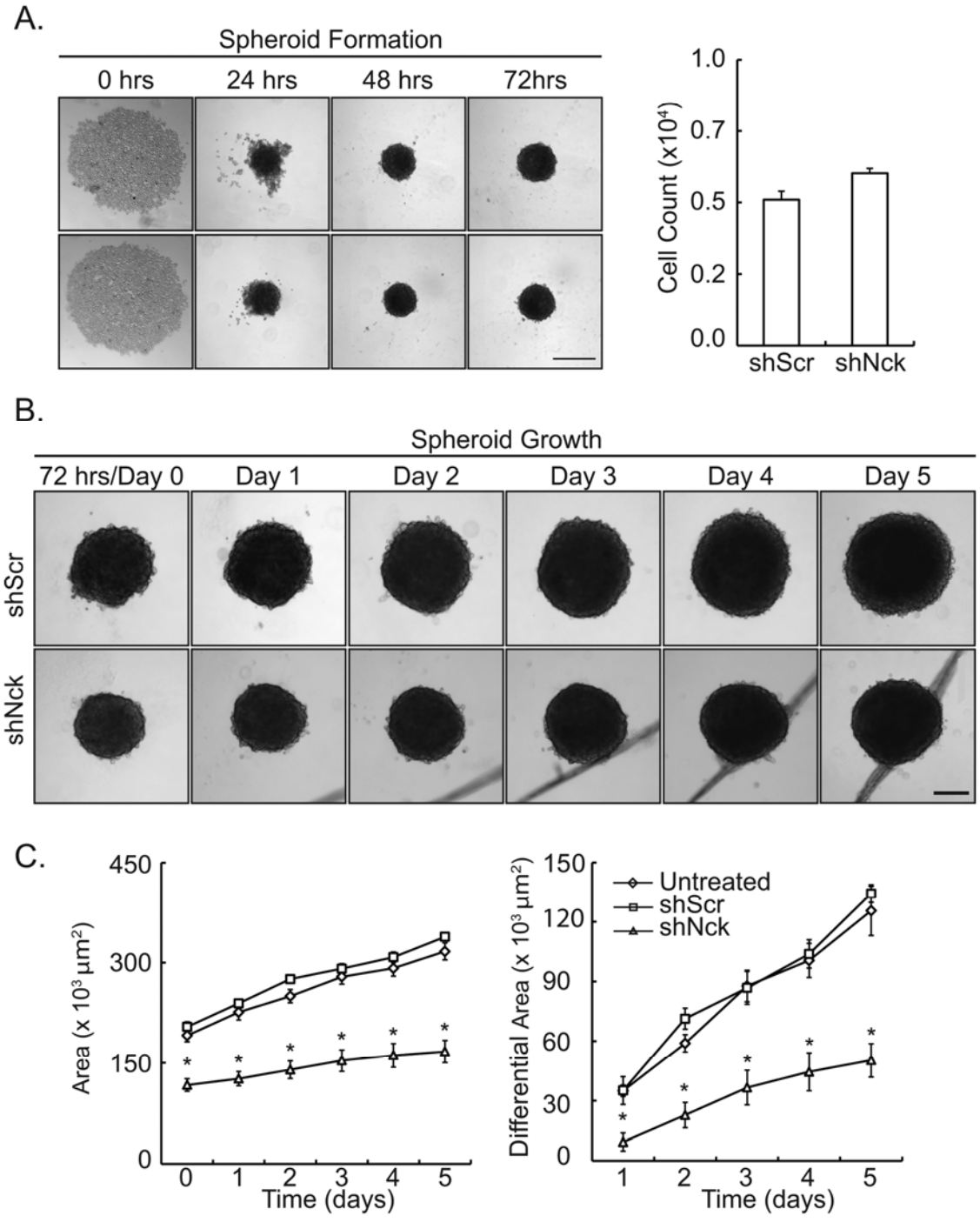
Supplementary Figure 2. Specificity of siRNA oligonucleotides targeting Nck. **A)** Alignment of human Nck1- (left panel) and Nck2-targeting (right panel) siRNA (SMARTPool) oligonucleotides sequences with corresponding segments of the mouse Nck2 cDNA. Base mismatches are highlighted in magenta. **B-C)** Invasiveness of MDA-MB-231 cells transfected with non-targeting (siScr) or Nck1- and Nck2-targeting siRNA oligonucleotides (siNck). A subpopulation of siNck (Nck-silenced) cells was transduced with a virus harboring a vector encoding siRNA-resistant mouse Nck2 (Rescued). Cells were subjected to overnight starvation before seeding onto 8 µm pore filters pre-coated with a laminin-rich matrix (Matrigel). Cells were allowed to invade during 18 h towards bottom chambers of transwell invasion plates loaded with complete medium. Representative wide field images of migrated cells (B, top panels) and cell counts performed with Fiji (B, bottom panels). Quantitative results (n=2 independent experiments) are shown in whisker plots (panel C). Statistically significant differences are indicated (* $p < 0.05$). **D)** Cellular levels of Nck were determined by western blotting using an anti-pan Nck antibody. GAPDH levels were used to monitor loading.

Supplementary Figure 3



Supplementary Figure 3. Loss of Nck reduces invasion of mammary carcinoma cells in 3D laminin-rich matrices. MDA-MB-231 cells were transiently transfected with small interference (si) RNA oligonucleotides encoding non-targeting sequences (siScr) or sequences targeting MMP14 (siMMP14) or Nck (siNck). Representative images of multicellular tumor spheroids (MTS) and whisker plots showing invasion distance 1 **(A)** or 2 **(B)** days after matrix embedding. Different letters represent statistically different values ($p<0.05$). Results shown summarize data from four independent experiments (n=3-6 spheroids/condition/experiment). Scale bar equals 500 µm.

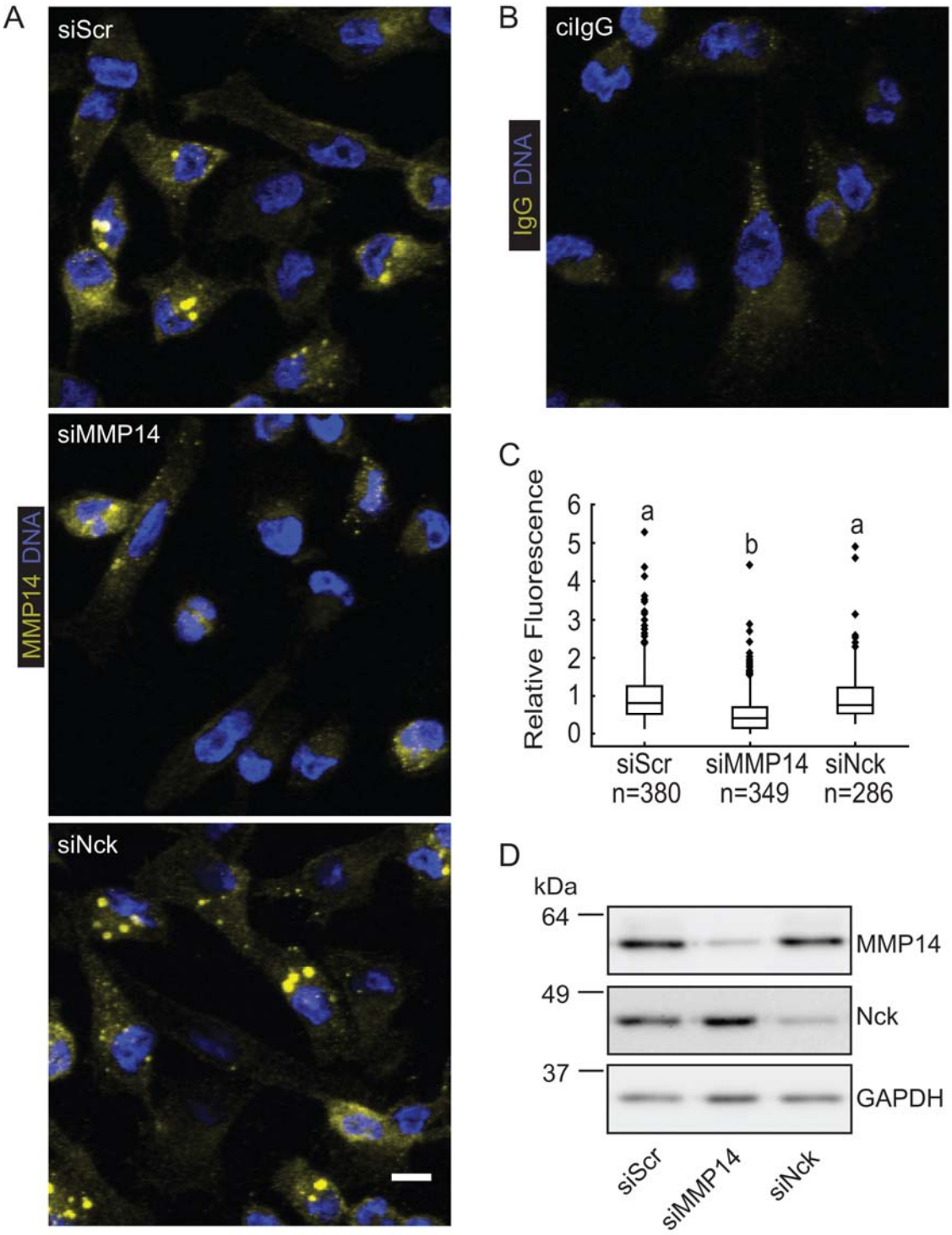
Supplementary Figure 4



Supplementary Figure 4. Loss of Nck reduces growth of mammary carcinoma cell multicellular tumor spheroids (MTS) in laminin-rich matrices. MDA-MB-231 cells expressing

short hairpin (sh) RNAs encoding non-targeting sequences (shScr) or sequences targeting Nck (shNck) were subjected to a spheroid growth assay. **A**) Representative images of spheroid formation. Scale bar equals 500 µm. Bar graph depicting total number of cells (mean ± s.e.m.) per MTS manually counted at 72 hrs.**B)** Representative images of MTS during a 5 day period. Scale bar equals 250 µm. **C)** Total spheroid area (left) and change in spheroid area (right). Values are mean ± s.e.m. (* $p < 0.05$). Results summarize the data from two independent experiments (n=3 speheroids/condition).

Supplementary Figure 5



Supplementary Figure 5. Bulk cell surface exposed MMP14 is not altered by Nck silencing. MDA-MB-231 carcinoma cellstransfected with non-targeting siRNA (siScr) or siRNAs targeting

MMP14 (siMMP14) or Nck (siNck) were plated on poly-l-lysine-coated glass and fixed but not permeabilized. **A)**The endogenous, cell surface exposed MMP14 was visualized by labeling with an antibody that recognizes the extracellular, catalytic domain of MMP14. Pseudocolored Z-projections of confocal stacks were generated after background subtraction. **B)** Background labeling was determined using control isotype IgG (ciIgG). **C)** Fluorescence intensity was normalized to siScr for each experiment and then replicates combined to generate relative fluorescence. Different letters represent statistically different values ($p<0.05$). The total number of cells analyzed (n) is indicated. **D)**Representative western blot showing efficacy of protein silencing. Scale bar equals 10 µm.

References

Labelle-Cote, M., Dusseault, J., Ismail, S., Picard-Cloutier, A., Siegel, P.M., and Larose, L. (2011). Nck2 promotes human melanoma cell proliferation, migration and invasion in vitro and primary melanoma-derived tumor growth in vivo. BMC Cancer *11*, 443.

Latreille, M., Laberge, M.K., Bourret, G., Yamani, L., and Larose, L. (2011). Deletion of Nck1 attenuates hepatic ER stress signaling and improves glucose tolerance and insulin signaling in liver of obese mice. Am J Physiol Endocrinol Metab *300*, E423-434.