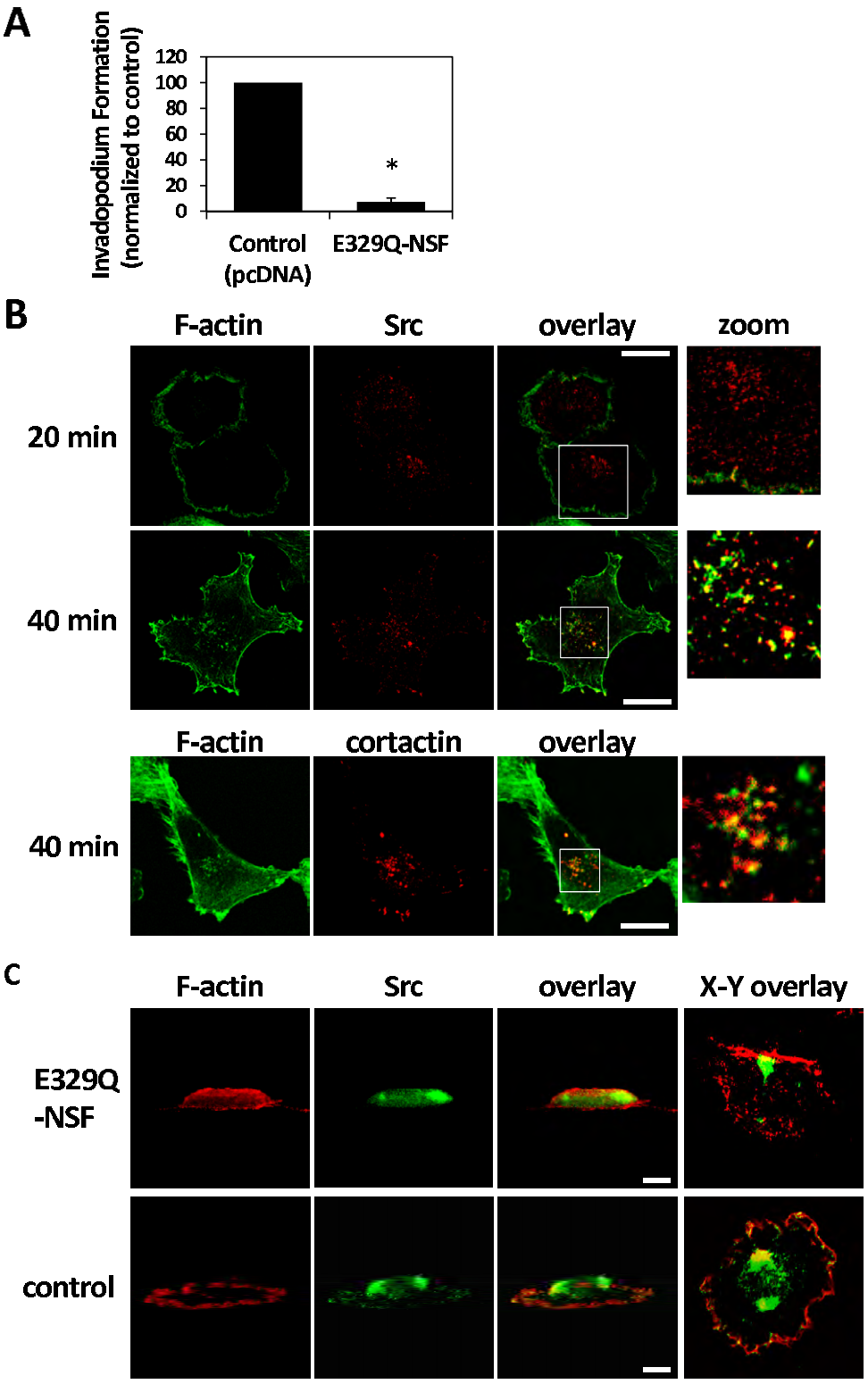

Fig. S1. SNARE-mediated membrane trafficking is required for invadopodium formation and Src trafficking. MDA-MB-231 cells were transfected with either E329Q-NSF or pcDNA3.1. (A) Cells were plated on coverslips coated with Alexa594-labeled gelatin for 5 hours, fixed, permeabilized, stained with anti-NSF antibody and phalloidin, and then F-actin-containing invadopodia were counted using a microscope. Means +/- SEM from 3 independent experiments in which 100 cells per sample were analyzed are shown. Asterisk denotes a value significantly different from wild-type cells; $p < 0.05$. (B) Cells were serum-starved, plated on gelatin-coated coverslips, fixed, permeabilized, and stained using anti-Src or anti-cortactin antibodies, followed by Alexa594-conjugated secondary antibody and Alexa488-phalloidin. Single confocal slices of the ventral membrane of cells are shown. Src co-localises with F-actin at the cell periphery at 20 and 40 mins. Actin/Src punctae are seen in the centre of the cell at 40min. At 40min, cortactin is also seen at F-actin punctae (bottom row). (C) Total Src localization in untransfected cells and cells transfected with E329Q-NSF for 12 hours, and plated on gelatin for 40min. Src is absent from the cell periphery (marked by F-actin staining) in cells expressing E329Q-NSF, compared to control cells. Scale bar = 10 μm.

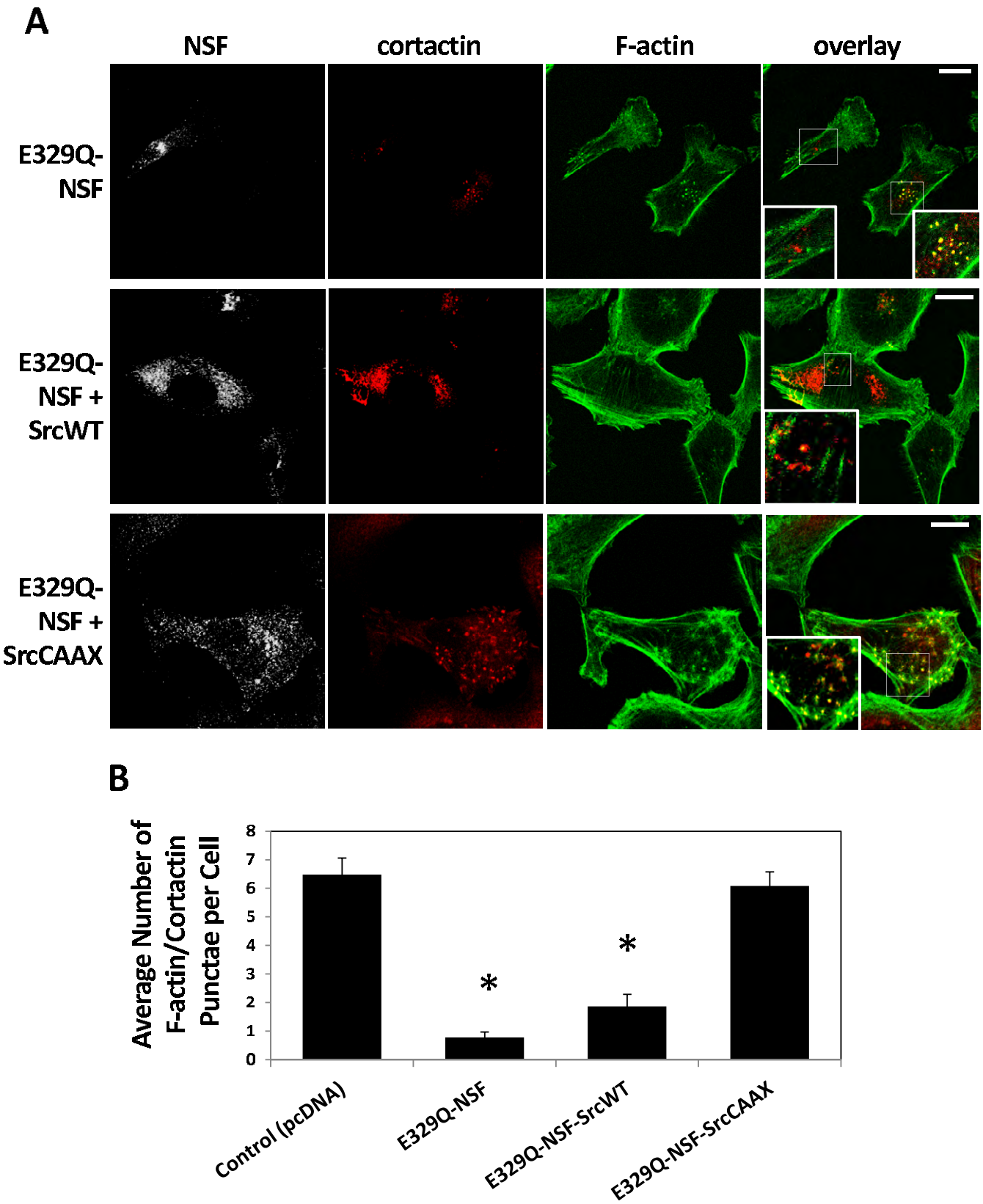

Fig. S2. Membrane-targeted Src restores invadopodia formation. MDA-MB-231 were co-transfected with E329Q-NSF along with either Src-WT or Src-CAAX. Cells were serum-starved, plated on gelatin for 40min, fixed, permeabilized, and stained for NSF, cortactin and F-actin. (A) Cells positive for transfected NSF are shown in the first column, and invadopodia are shown by the co-localization of F-actin and cortactin. E329Q-NSF-expressing cells (upper left cell in top panels) contain fewer F-actin/cortactin punctae than control cells (right-most cell in top panels). Expression of SrcCAAX, but not SrcWT, restores the formation of F-actin/cortactin-containing punctae. All images are single confocal slices at the level of the ventral membrane. Scale bar = 10μm. (B) Quantification of the number of F-actin/cortactin punctae per cell. Means +/- SEM from 3 independent experiments in which 30-50 cells per sample were analyzed are shown. Asterisk denotes a value significantly different from wild-type cells; $p < 0.05$.

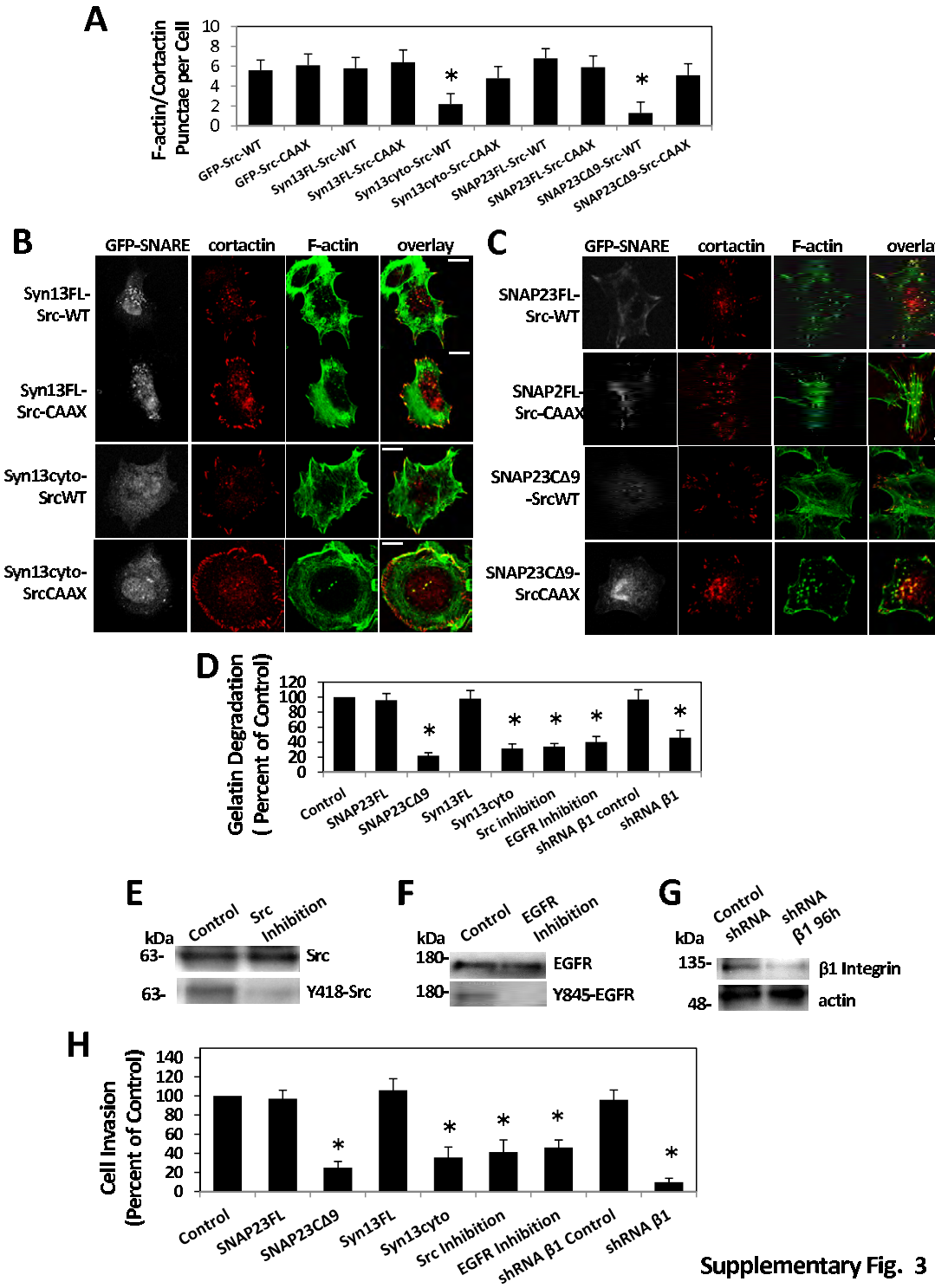

Fig. S3. Inhibition of SNAP23, Syntaxin13, Src, EGFR and β1 integrin impairs invadopodium formation, matrix degradation and invasion in HT-1080 cells. (A-C) Cells were cotransfected with GFP, GFP- SNAP23FL/CΔ9 or GFP-Syntaxin13FL/cyto and either Src-WT or Src-CAAX for 20 hrs. (A-C) Cells were serum starved overnight followed by culturing on gelatin coated coverslips for 40min in serum free media. Cells were fixed, permeabilized, and stained for anti-cortactin and actin (phalloidin). (A) Quantification of the number of actin/cortactin punctae demonstrate that invadopodia formation is rescued by Src-CAAX but not Src-WT. (B and C) Cells expressing Src-WT or Src-CAAX and, GFP-SNAP23FL/CΔ9, GFP-Syntaxin13FL/cyto, are represented in the first column in grey. Invadopodia formation is represented by co-localization of actin (green) and cortactin (red) in the overlay (yellow). (D and H) Cells were either transfected as in (A) or treated with 10μM PP2 (Src inhibitor), 11nM AG1478 (EGFR inhibitor)or transfected with shRNA control or shRNA β1 integrin. (D) Cells were plated on Alexa594-labeled gelatin for 3 hrs and scored as for presence or absence of matrix degradation. (E) Cells were treated with PP2, lysed and Western blotted for Y418-Src, stripped and reprobed for Src. (F) Cells were treated with AG1478, lysed and Western blotted for Y845-EGFR, stripped and reprobed for EGFR. (G) shRNA control and shRNA β1 integrin transfected cells were lysed 96hrs post transfection and probed for total β1 integrin; actin represents a loading control. (H) Cells were collected and subjected to transwell invasion assay. Cells invaded through matrigel towards 10% FBS for 18 hours and were then fixed and counted. Asterisk denotes a value significantly different from control cells ($p < 0.05$).