

Figure 4. Peflin expression suppresses ER export of endogenous collagen I in Rat2 fibroblasts. Rat2 cells were transfected with the plasma membrane marker pCAG-mGFP and grown in the presence or absence of peflin siRNA. Following growth at 41 °C for 24 hours, cells were shifted to 32 °C, with 50µg/ml ascorbate, for 15 minutes, to permit transport, prior to fixation. **(A)** Temperature-shifted R2 cells underwent an ER-to-Golgi transport assay and a transport index for each cell was calculated, and plotted; n = ~200. **(B)** The same cells used in “A” were assigned a value for total cell fluorescence of collagen. Total collagen I content was not significantly affected while ER-to-Golgi transport was significantly enhanced in the absence of peflin in the same cells. **(C)** Validation of immunofluorescence assay for total cell fluorescence of collagen I. Cells were transfected with different concentrations of collagen I siRNA or an siRNA for peflin, demonstrating no significant effect of peflin knockdown on the