

## Supplementary Material and Methods

### Real-time PCR

RNA (2-5 $\mu$ g) was treated with DNase I (Promega), then reverse transcribed, using 200 U Superscript II (Invitrogen) and 250ng random primers (Invitrogen), according to the manufacturer's instructions. All samples within an experiment were reverse transcribed at the same time, the resulting cDNA diluted 1:5 in nuclease-free water and stored in aliquots at  $-80^{\circ}\text{C}$  until used. Synthetic RNA standards for standard curve generation were prepared by *in vitro* transcription with T7 polymerase and the yield calculated by incorporation of trace amounts of  $\alpha^{32}\text{P}$ -UTP. Synthetic standards were reverse transcribed as described above and diluted in nuclease-free water using 50 ng/ml yeast tRNA as a carrier. Real-time PCR with SYBR green detection was performed as described previously (1) using an ABI Prism 7700 thermocycler with fluorescence detection (Applied Biosystems) and the following primers: rat PINCF TGGAATAACTGTGTGAAGG, rat PINCR CCAAAGTGAGGATAAGTAG, cyclophilinF TGAGCACTGGGGAGAAAGG, cyclophilin R TTGCCATCCAGCCACTCAG, rat cytokeratin 8F GCAACAGCAGAAGACATCC, rat cytokeratin8R ATCTCGTCGGTCAGTCCTT, mPINC\_1.6F GGGGCTCCTTCCTTGGTT, mPINC\_1.6R GGCTTCTCTTCCCTCTGC, mPINC\_1.0F CTTGGAGCATAGACAGGAC, mPINC\_1.0R CTCAGACGCTTTCCTTCC. Appropriate no-RT and non-template controls were included in each 96-well PCR reaction, and dissociation analysis was performed at the end of each run to confirm the specificity of the reaction. Absolute levels of RNA were calculated from a standard curve established using the cDNA standards described above.

### Fluorescent in situ hybridization (FISH)

Cells were fixed on coverslips using 2% paraformaldehyde (15 mins at 4 °C), treated with proteinase K (20µg/ml for 5 min), post-fixed in 4 % paraformaldehyde, acetylated and prehybridized for 1 h in hybridization buffer (DAKO). Digoxigenin-labelled probes were prepared by *in vitro* transcription using T7 polymerase in the presence of DIG RNA labeling mix (Roche). In each case, both sense and antisense probes were prepared and the sense probe used as a negative control to verify the specificity of the hybridization signal. Hybridization was performed at 50 °C overnight in the presence of ~ 500 ng/ml probe and 0.5 mg/ml sheared salmon sperm DNA. Excess probe was removed by washing in 4X SSC (50 °C, for 5 min), coverslips were washed in 2X SSC (twice for 20 min each at RT), then digested with 40mg/ml RNase A (Roche) (37 °C, 15 min). Coverslips were washed in 0.1X SSC for 15 min at 42 C, then 0.1X SSC for 10 min at RT, then incubated in blocking buffer (5 % BSA in 1 X PBS, for 30 min at room temperature). Immunological detection of digoxigenin-labeled probe was performed by incubating sequentially with anti-digoxigenin (Sigma, clone D1-22), anti-mouse (digoxigenin-conjugated) F(ab')<sub>2</sub> (Roche) and finally anti-digoxigenin-rhodamine Fab fragment (Roche), (all diluted 1:200 in blocking buffer, washing three times for 5 min each between each successive antibody). Nuclei were counterstained using 4', 6-diamidino-2-phenylindole (DAPI; Sigma) and mounted on slides using Slow Fade mounting medium (Molecular Probes).

Deconvolution microscopy was carried out using a Zeiss AxioVert S100 TV microscope and a DeltaVision Restoration Microscopy System (Applied Precision, Inc.). In each case ten to twelve independent fields were captured and representative images selected and subjected to high resolution deconvolution. For high-resolution deconvolved images, captured raw images were deconvolved with the DeltaVision constrained iterative algorithm. All images were digitally processed for presentation with Adobe Photoshop.

CSK extraction of soluble RNA was performed essentially as described in (2) but in the presence of RNase inhibitor (RNasin, Promega). CSK-treated cells were then processed for FISH as described above.

### **SiRNA target sequences**

siRNA against PINC 1.6

1.6A 5' GATCAGTGTCAATGTCTGA 3'

1.6B 5' AACTGTTCCCTGAGACCATT 3'

1.6D 5' TTTCAGATGAGAACGGGCA 3'

siRNA against PINC 1.0

1.0A 5' GCTGCTATGCTACCTGGAA 3'

1.0B 5' CCACCTTTTAGAGTGCTGC 3'

1.0C 5' GGGCAGGAATCAGTCCACC 3'

1.0D 5' TGGAAACGGGAGGGACTTT 3'

### **References**

1. Yuen, T., Zhang, W., Ebersole, B. J. & Sealfon, S. C. (2002) *Methods Enzymol.* **345**, 556-69.
2. He, D. C., Nickerson, J. A. & Penman, S. (1990) *J. Cell Biol.* **110**, 569-80.