Electronic Supplementary Material (ESI) for Integrative Biology

Supplementary table 1

Cell Line	A549	H460
Low Dyskerin Expression Cells (LDEC)	79% (81 of 103)	65% (63 of 98)
High Dyskerin Expression Cells (HDEC)	21% (22 of 103)	35% (35 of 98)

Supplementary table 1: Number and percentage of HDEC and LDEC detected on the cell lines H460 and A549. The result was obtained using the average telomere length and dyskerin expression of every cell and clustering the results with the Bayesian Schwartz Criteria and Akaike Information Criteria.

Legends of Supplementary figures

Supplementary Figure 1: Antibody validation. A) Double immunofluorescence against dyskerin and coilin –structural component of Cajal bodies- showing occasional colocalization between both proteins (white arrows). B) Double immunofluorescence against dyskerin and the nucleolus component fibrillarin. White arrows point at dyskerin signals, some of which colocalize with fibrillarin (arrowhead), while others are dispersed in the nucleoplasm (arrow). C) Complete dyskerin immunobloting with the Sigma antibody showing a predominant 57 kDa. This molecular weight corresponds to the predicted value for dyskerin protein. D) Immunofluorescence image example showing the dyskerin-staining pattern obtained using the Abcam antibody. The signal distribution matches the Sigma antibody pattern: nucleolar and nucleoplasmatic. E) Sample image of the anti-dyskerin antibody labelling pattern in cells transfected with a plasmid for the expression of DKC1 (DKC+ cells), showing the punctuate foci localized all over the nucleus, with a significant fraction localized in perinucleolar areas. F)

Dyskerin immunobloting in A549 cells transfected with different plasmids. Samples in which dyskerin was silenced with an specific siRNA (siDKC) do not show the corresponding dyskerin protein band in contrast with samples transfected with the dyskerin plasmid (DKC+) and the controls (wt). As expected, dyskerin overexpressing samples also show increased expression of the protein when compared to the controls. G) DKC1 mRNA expression levels in samples treated with a specific siRNA anti DKC1 mRNA (Santa Cruz) and in a sample transfected with siRNA against TERT mRNA. Values for each sample were normalized using two internal control genes, GAPDH and HPRT. Expression levels for the samples treated with the siRNA were normalized to control samples without the siRNA. H) Immunofluorescence against dyskerin with the Sigma antibody in samples transfected with three siRNA anti DKC1 mRNA (Invitrogen) and the siRNA anti DKC1 from Santa Cruz shows a mainly complete reduction in the antibody signal when compared to control samples.

Supplementary Figure 2: Image-based telomere length measurements using image analysis and TRF. A) Sample images of six different cancer cell lines with different average telomere intensities –CY3 PNA telomere probe, top row–; the corresponding counterstained images –DAPI, bottom row–. B) TRF results for the six cell lines shown in (A). C) Regression line between the average telomere length results measured by TRF (y-axis) and image analysis (x-axis), calculated using the six cancer cell lines shown in (A). Each point represents the average telomere length for a given cell line measured by TRF and its correspondent quantitative imaging arbitrary units, and shows the standard error for three experimental triplicates of the TRF (vertical bars) and the image quantification (horizontal bars). D) Example showing the average telomere length and dyskerin expression values of three cell lines of lung cancer with different dyskerin expression levels. Scale bars represent ten microns.

Supplementary Figure 3: Dyskerin expression histograms. Frequency histograms showing the clustering distribution of the dyskerin intensity signal in the four lung cancer cell lines A549, H1299, H460 and H157. The darker shade of red represents the HDEC as clustered by the statistical criteria used by SPSS.

Supplementary Figure 4: Simultaneous immunodetection of dyskerin and Ki67. Simultaneous immunolabelling for dyskerin and Ki67 in the lung cancer cell line A549. High dyskerin cells are both Ki67 positive and negative. Scale bars represent ten microns length.

Supplementary Figure 5: Spatial distribution of dyskerin and telomeres. Frequency histograms showing the telomere length of the telomeres that co-localize with dyskerin signals. The line indicates the average telomere length of all the telomeres for the same sample. 220 out of 321 co-localizing telomeres are shorter than the average telomere length for the cell line A549.

Supplementary Figure 6: Cell proliferation inhibition caused by the telomerase inhibitor MST 312. Clonogenic assay for the lung cancer cell lines A549 and H460 that were grown for 15 days in the presence of the telomerase inhibitor MST 312. The figure clearly shows a reduction both in the number and size of colonies in the treated samples when compared with the controls.

Supplementary Figure 7: Comparison between cell cycle profile and integrated telomere intensity in the A549 cell line. A) FACS analysis of A549 cells showing the distribution of frequencies for the cell cycle G1 (M1), S (M2) and G2/M (M3) phases. B) Histogram for the integrated intensity of the segmented telomeric signals in A549 cells. The distribution of intensities show a single right tailed logistic curve, different from the two tailed binomial observed in the cell cycle analysis.