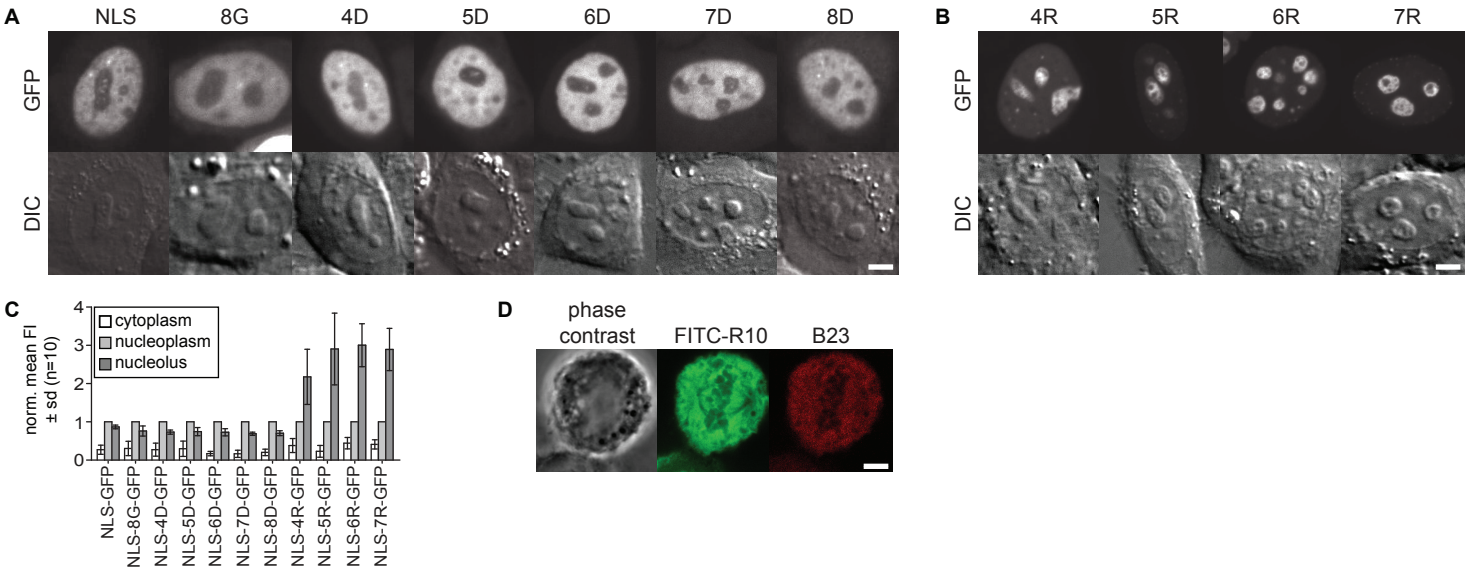
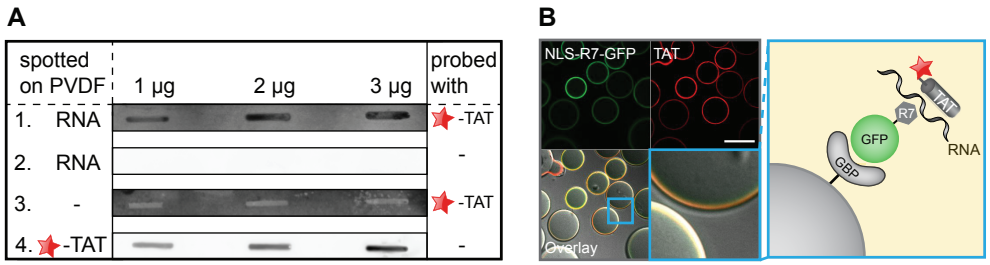
Supplementary Data



Supplementary Figure 1. Intracellular distribution of peptide tagged GFP tracer proteins in living human cells during interphase and metaphase. The images in this figure show live human HeLa cells transfected with NLS-GFP constructs also shown in Figure 2 to validate the same distribution pattern of the charged GFP tracer proteins in a different cell type of a different species. The first panel in A) displays the intracellular distribution of the NLS-GFP control and poly-G coupled NLS-GFP constructs. The second panel shows images of all poly-D containing NLS-GFP constructs. B) The images display the poly-R coupled NLS-GFP constructs. In A) and B) the upper row shows the GFP fluorescence with the corresponding differential interference contrast (DIC) image below. The corresponding quantification of the mean fluorescence intensity for each 10 cells from two independent experiments is plotted in C). Area selection and quantification were done as described for peptides and protein before. The image panel D) shows a fixed HeLa cell in mitosis labeled with FITC-R10 peptide, which highlights the chromosomes as darker regions in the cell by exclusion of the peptide. The cell was further stained with antibodies against the nucleolar antigen B23/nucleophosmin to demonstrate the lack of nucleoli. The FITC-R10 peptide and B23 show a homogeneous distribution throughout the cell with exclusion from the chromosomes aligned in the central plane of the cell. Scalebars: 5 µm.



Supplementary Figure 2. Analyses of total RNA binding to arginine-rich cell penetrating peptides. The slot blot in A) shows the binding of a standard arginine-rich cell penetrating peptide (TAT) in the absence (3) and presence (1) of total RNA.Slots lacking RNA (3) do not show binding of TAT, while slots probed with increasing amounts of RNA show increasing TAT binding (1). Row 4 with spotted TAT peptide shows, when compared to row 2, that only the presence of labeled peptide results in fluorescence detection but not RNA itself. B) In vitro RNA pulldown assay using NLS-R7-GFP immobilized to sepharose beads via the GFP binding protein (GBP) (scheme). RNA was stained using fluorescently labeled TAT. TAT binding to RNA was imaged by confocal microscopy. Scale bars: 100 µm.