

Supplementary Table 1. List of oligonucleotides used to generate the GFP fusions.

GFP version	primer	sequence (5'-3')
4R-GFP	Fw	CCGGTGCACCGCCGGCGCCGG
	Rev	CCGGCCGGCGCCGGCGGTGCA
5R-GFP	Fw	CCGGTGCACCGCCGGCGCCGG
	Rev	CCGGCCGGCGCCGGCGGTGCA
6R-GFP	Fw	CCGGTGCACCGCCGGCGCCGGCGCCGG
	Rev	CCGGCCGGCGCCGGCGCCGGCGGTGCA
7R-GFP	Fw	CCGGTGCACCGCCGGCGCCGGCGCCGGCGC
	Rev	CCGGCCGGCGCCGGCGCCGGCGCCGGCGGTGCA
4D-GFP	Fw	CCGGTCGACGATGACGAT
	Rev	CCGGATCGTCATCGTCGA
5D-GFP	Fw	CCGGTCGACGATGACGATGAC
	Rev	CCGGGTCATCGTCATCGTCGA
6D-GFP	Fw	CCGGTCGACGATGACGATGACGAT
	Rev	CCGGATCGTCATCGTCATCGTCGA
7D-GFP	Fw	CCGGTCGACGATGACGATGACGATGAC
	Rev	CCGGGTCATCGTCATCGTCATCGTCGA
8D-GFP	Fw	CCGGTCGACGACGACGACGACGACGACGAC
	Rev	CCGGGTCGTCGTCGTCGTCGTCGTCGTCGA
8G-GFP	Fw	CCGGCCGGCGCCGGCGCCGGCGCCGGCGGTGCA
	Rev	CCGGTGCACCGCCGGCGCCGGCGCCGGCGCCGGC

Supplementary Table 2. Lists of normalized mean fluorescence intensity values measured for the fluorescent peptides and proteins in cytoplasm, nucleoplasm and nucleoli and their statistical significance (P-values).

A

	K5	K6	K7	K8	K9	K10	K11	K12
cytoplasm	0.73	0.73	0.76	0.77	0.72	0.73	0.73	0.73
nucleus	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
nucleolus	1.06	1.06	1.04	1.04	1.06	1.02	1.05	1.06
p-value	0.068	0.199	0.290	0.326	0.199	0.762	0.096	0,016*

B

	R5	R6	R7	R8	R9	R10	R11	R12
cytoplasm	0.613	0.702	0.706	0.715	0.732	0.676	0.649	0.663
nucleus	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
nucleolus	1.045	1.178	1.155	1.162	1.223	1.162	1.123	1.141
p-value	0.406	0,002**	0,010**	0,005**	0,004**	0,034*	0,010**	0,041*

C

	L-R10	D-R10
cytoplasm	0.68	2.88
nucleus	1.00	1.00
nucleolus	1.16	3.72
p-value	0,034*	0,0002**

D

	GFP	NLS-GFP	8G-GFP	4D-GFP	5D-GFP	6D-GFP	7D-GFP	8D-GFP	4R-GFP	5R-GFP	6R-GFP	7R-GFP
cytoplasm	0.90	0.39	0.25	0.19	0.23	0.15	0.23	0.27	0.25	0.08	0.09	0.10
nucleus	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
nucleolus	0.70	0.68	0.63	0.67	0.66	0.60	0.64	0.51	1.74	2.45	2.67	2.71
p-value	0,0101*	0,0002**	0,0039**	0,0233*	0,0101*	0,0101*	0,0002**	0,0163*	0,0304*	0,0005**	0,0002**	0,0090**

E

		NLS-GFP	8G-GFP	4D-GFP	5D-GFP	6D-GFP	7D-GFP	8D-GFP	4R-GFP	5R-GFP	6R-GFP	7R-GFP
cytoplasm		0.30	0.30	0.30	0.30	0.20	0.20	0.20	0.40	0.20	0.40	0.40
nucleus		1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
nucleolus		0.90	0.80	0.70	0.70	0.70	0.70	0.70	2.20	2.90	3.00	2.90
p-value		0.44935	0.13057	0.1986	0.17362	0.010*	0.0587	0.09618	0.0139*	0.0025**	0.0002**	0.0003**

The p-value was calculated for the nucleoplasm versus the nucleolus mean intensity measurements only with a non-parametric Kruskal-Wallis-ANOVA test. The p-values represent a measure of significance for the mean intensity values from the nucleoplasm and nucleolus belonging to the same or different population of measurements. Significant different distributions are found for nucleolar accumulation but also nucleolar exclusion of proteins. At the level of $p < 0.05$ the measurements of mean fluorescence intensity in nucleoplasm and nucleoli for one peptide or protein are significantly different and indicated by asterisk (*). Highly significant differences ($p < 0.01$) are indicated by double asterisk (**). A) shows the values for the FITC labeled poly-K peptides K5 to K12. B) lists values for the FITC labeled poly-R peptides R5 to R12. C) shows the mean fluorescence intensity and p-values for the comparison of L-R10 versus D-R10 peptide distribution. Note that the high levels of accumulation of the D-R10 peptide in cytoplasm and nucleolus reflect the low intensity measured in the nucleoplasm, which is used as the reference level to normalize for nucleolar accumulation. D) shows the normalized fluorescence intensity and p-values for the mean fluorescence of GFP and all versions of it with NLS as well as additional fusion of poly-G, poly-D and poly-R sequences in mouse C2C12 cells. In E) are the corresponding values for the mean fluorescence in human HeLa cells.

