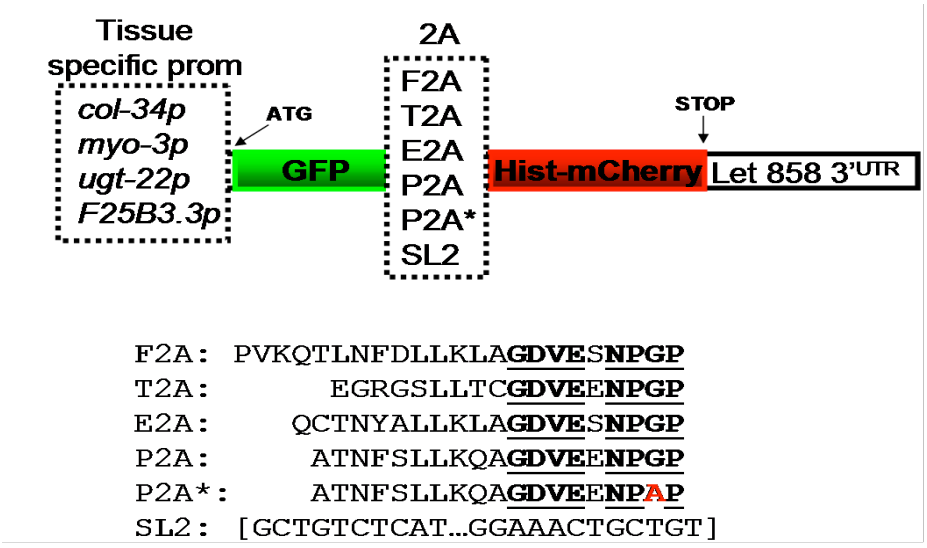
a-



b-

2a sequence	**correct localization**	2 markers in the nucleus	2 markers in the whole cell	GFP only expressed	mCherry only expressed
F2A (n=104)	98,15%±1,66%	0%±0%	1,08%±1,86%	0%±0%	0,78%±1,34%
T2A (n=115)	96,86%±2,73%	0%±0%	0%±0%	1,63%±2,82%	1,52%±2,62%
E2A (n=120)	94,35%±8,28%	0%±0%	0%±0%	0%±0%	5,65%±8,28%
P2A (n= n=144)	88,11%±3,05%	9,62%±4,29%	2,27%±2,71%	0%±0%	0%±0%
P2A* (n=96)	4,30%±7,45%	94,62%±9,31%	1,08%±1,86%	0%±0%	0%±0%
SL2 (n=150)	95,31%±1,92%	0%±0%	0%±0%	0,98%±1,70%	3,71%±1,75%

Figure S1 **a-** Schematic representation of the constructs generated to test the canonical 2A (F2A, T2A, E2A and P2A) peptides in *C. elegans*. P2A* (Hahn and Palmenberg 1996) was used as a negative control and SL2 (Macosko *et al.* 2009; Kagias *et al.* 2012) was used as a positive control. **b-** Quantification of the number of cells exhibiting a given sub-cellular localization pattern for the GFP and HISTONE::mCherry proteins in transgenic lines expressing each of the 4 canonical 2A sequences ("correct localization", GFP in the whole cell, HISTONE::mCherry in the nucleus). The very high % of cells exhibiting the expected localization pattern suggests a remarkable efficiency in *C. elegans*. In addition, note that the "self-cleavage" efficiency is not correlated with the length of the 2A peptide used. Although still very robust, we found the peptide P2A to be slightly less efficient in generating two distinct sub-cellular localizations for GFP and mCherry compared to the other 2A peptides; this however may not be due to a lesser production of 2 independent polypeptides (see Supp Fig. 2A and the western blots in Fig. 1). As expected, the "cleavage-inefficient" P2A* variant leads to the colocalization of the two fluorescent proteins in the nucleus. n= total number of cells scored.