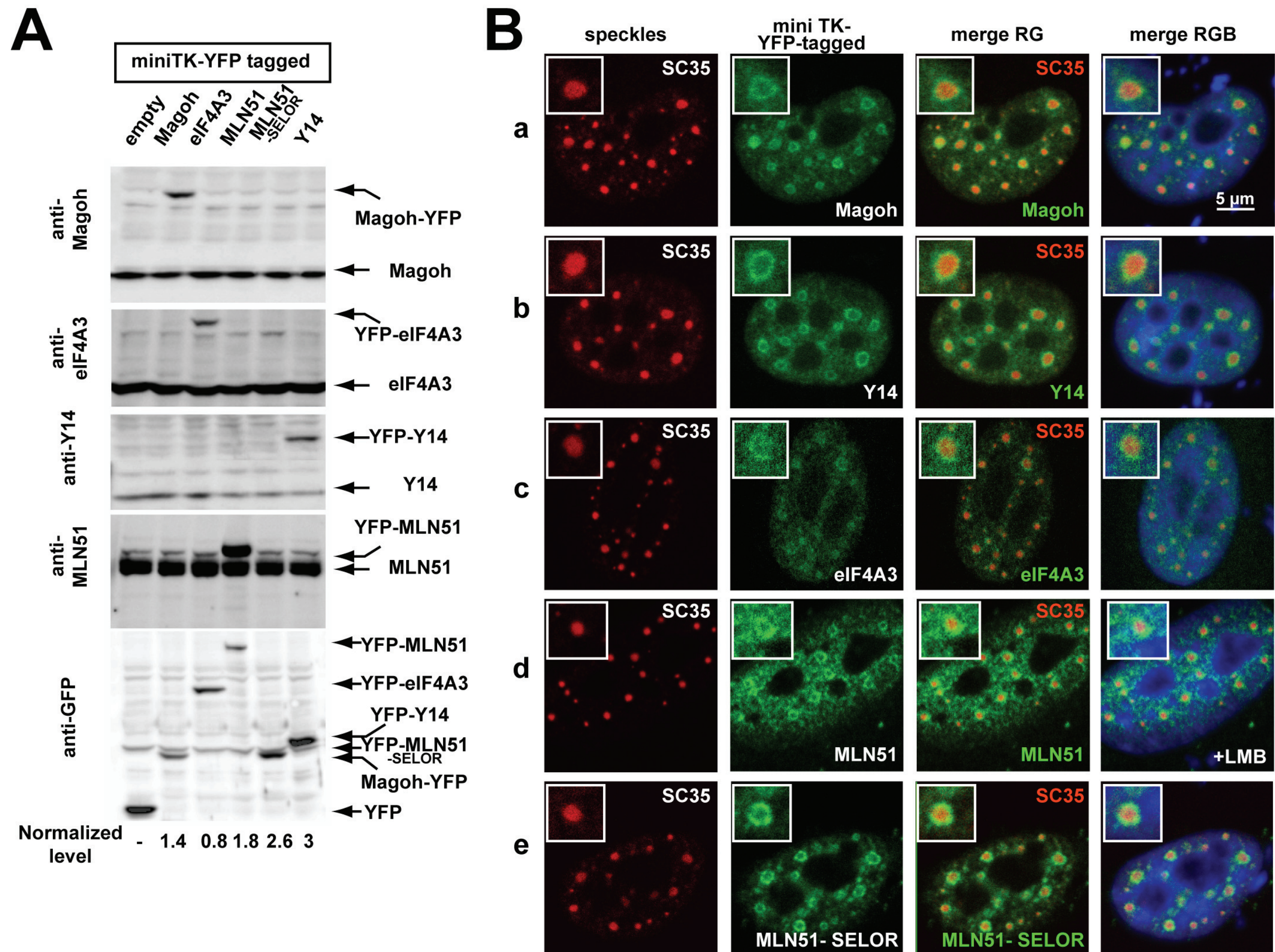
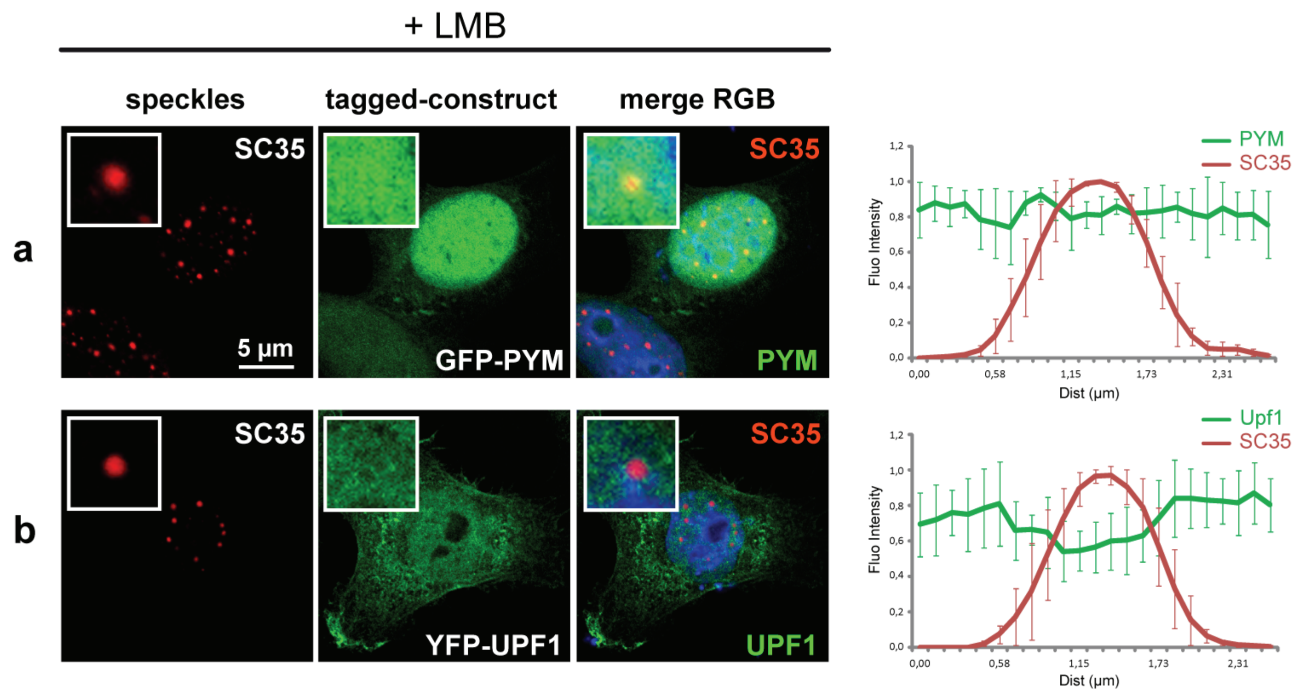
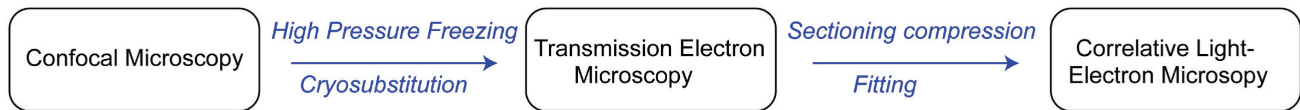
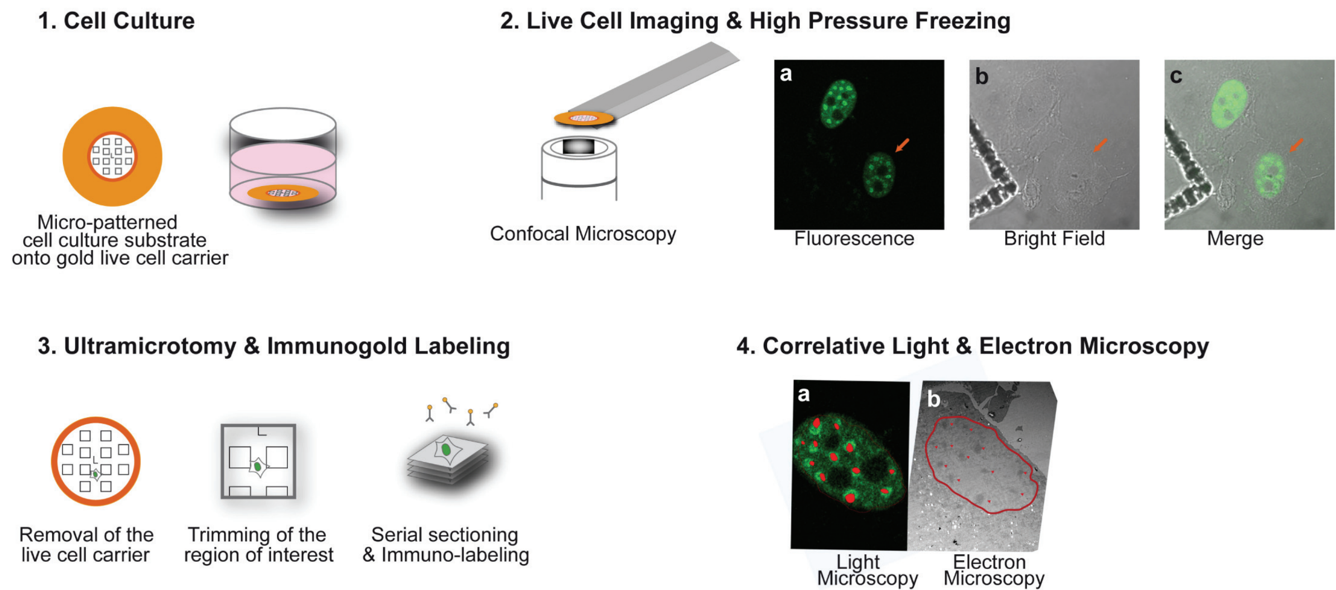


Supplementary Figure S1. Localization of the four EJC factors under low expression conditions. (A) Quantification of low expression YFP-tagged constructs. HeLa cells were transfected with the indicated YFP-tagged expression vectors under the control of the minimal thymidine kinase promoter (mini TK). Twenty-four hours after transfection, the level of each YFP-tagged protein expression was analyzed using specific antibodies as indicated on the left. In addition, on the bottom panel, expression of all YFP-tagged proteins was detected using an anti-GFP antibody. For the quantification of all constructs excepted MLN51-SELOR, signals otained using a specific antibody and corresponding to the YFP-fusion and to the endogenous counterpart on the same lane were measured. For MLN51-SELOR, signals obtained using the anti-GFP antibody was measured in the YFP-MLN51-SELOR and in the YFP-MLN51 lanes and used for quantification. The normalized expression levels of the YFP-tagged constructs as indicated on the bottom were measured using the endogenous expression level set to one. Note that YFP-eI4A3 is less expressed that the endogenous protein, while YFP-tagged Magoh and MLN51 are expressed less than twice the endogenous level. Finally, tagged-Y14 and MLN51-SELOR are expressed around 3 fold. (B) Hela cells expressing YFP-tagged fusion proteins Magoh (a), Y14 (b), eIF4A3 (c), MLN51 (d) and MLN51-SELOR (e) under the minimal TK promoter control were labeled with the anti-SC35 antibody (red). On the RGB (red, green and blue) merge panels, DNA was counterstained in blue. Cells in (d) were treated with cyclo-heximine (6 hours at 10µg/ml) and leptomycine B (LMB) (5 hours at 20ng/ml). Insets show a 2X magnification. Note that all the fours EJC core factors are enriched at the periphery of nuclear speckles. Likewise, a truncated MLN51 construct, MLN51-SELOR, that is devoid of its nuclear export signal, also accumulates at the periphery of speckles. Scale bar, 5µm.



Supplementary Figure S2. Localization of two peripheral and cytoplasmic EJC factors under nuclear export inhibition. The fluorescent-tagged EJC constructs (PYM (a) and UPF1 (b), green) were transfected into HeLa cells, and the endogenous SC35 protein was detected by immunofluorescence (red). Twenty-four hours after transfection, cells were treated with cycloheximide (10µg/mL, 6 hours) and LMB (20ng/mL, 5 hours) and then processed for immunofluorescence. On the RGB panel, DNA was counterstained in blue. Enlargements of selected speckles are shown with a 2.5X magnification. Right panels show mean fluorescence intensity line scans of the images accross representative speckles. Note that PYM and UPF1 are retained in the nucleus, without any enrichment at the perispeckle region. Scale bar, 5µm.

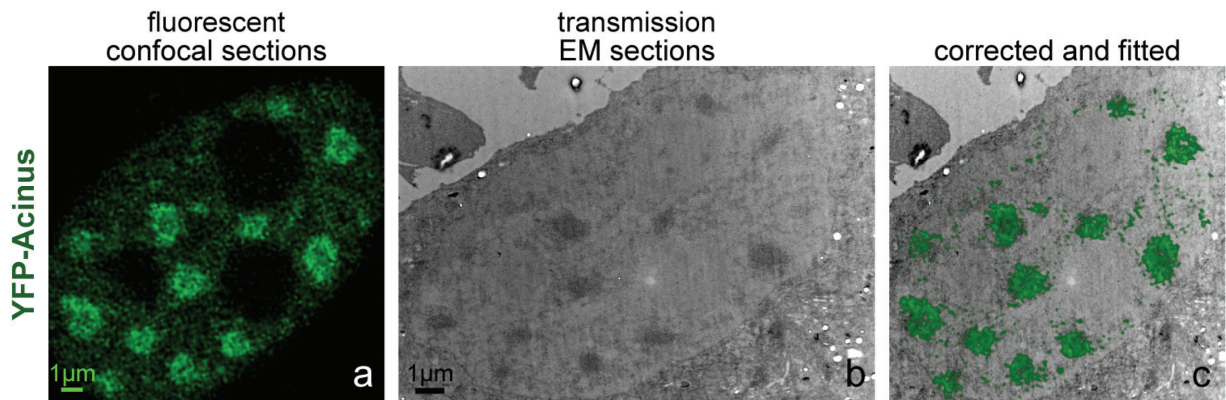




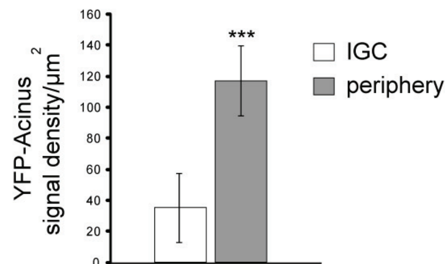
Supplementary Figure S3

Experimental outline of the Correlative Light-Electron Microscopy (CLEM) method on living cells
The CLEM method combines two types of imaging approaches: light microscopy (fluorescence on living cells) and transmission electron microscopy (immunolabeling on serial thin sections).
1. The culture substrates were prepared from aclar films on which a reference grid was micro-patterned and mounted onto a gold plated live cell carrier. Cells transfected with GFP-tagged SELOR construct were seeded on these collagen pre-coated montages and cultured under normal conditions.
2. For light microscopy, the montage was installed on the rapid loader of the EMPACT-2. Cells are imaged by confocal microscopy, then fixed by high pressure freezing. The reference coordinates are used to record the position of the selected cell with fluorescence (a), bright field (b) or both (c).
3. After cryo-substitution and embedding, the carrier was removed from the block. Trimming was performed around the area of the selected cell followed by serial thin sectioning of the block. Serial immunolabeling was achieved using anti-GFP antibody coupled to gold particles.
4. Coordinates still visible in the first sections allowed the retrieval of the selected cell. The structures observed by transmission electronic microscopy after anti-GFP immunogold labeling were easily fitted with the confocal fluorescent image (red dots, nuclear speckles in fluorescent microscopy; red arrowheads, nuclear speckles in electron microscopy). Red lines surround the nuclear envelope.

A



B



Supplementary Figure S4. Ultrastructural characterization of the perispeckle by CLEM analysis. MCF7 cells were transiently transfected with YFP-Acinus construct and then processed for microscopy analysis and immunogold labeling by Correlative Light and Electron Microscopy (CLEM). (A) YFP-Acinus is present at the periphery of IGCs. a, YFP signal from one live confocal z section; b, transmission EM image; c, fitting between light and electron microscopy acquisition. Scale bar, 1µm. (B) The YFP signal is enriched at the periphery of IGC. Graph representing the preferential distribution of YFP-Acinus at the periphery of IGC. The mean labeling density of gold particles per surface unit μm^2, that marks the presence of YFP-Acinus, was quantified in speckle *versus* periphery ± standard deviations. P-value was obtained from Student's statistical t-test, ** indicates $P < 0.001$.