E07-12-1259 Stanek

Supplementary figure legends

Supplementary figure 1. Fluorescently tagged SmB proteins are expressed at levels similar to endogenous and are properly incorporated into snRNPs. (A) HeLa cells were transiently transfected with SmB-YFP or SmB-PA-GFP, cultivated for 24h, total proteins isolated and SmB proteins detected by immunoblotting, using the Y12 monoclonal antibody. Because SmB-YFP signal was 15% of the endogenous SmB signal and transfection efficiency was ~ 15% we conclude that expression of SmB-YFP was similar to expression the endogenous SmB protein. (B) HeLa cells were stably transformed with SmB-GFP tagged on a BAC (a gift of Ralf Kittler and Frank Buchholz). These cells were labeled overnight with P^{32}-orthophosphate, total cell extracts prepared and subjected to immunoprecipitation using anti-GFP or the Y12 antibodies as a positive control. RNA was isolated from pellets and resolved on a denaturing UREA gel, and exposed to a phosphorimager plate. Note that the pattern and intensity of the snRNA signals co-immunoprecipitated with anti-GFP and Y12 were nearly identical.

Supplementary figure 2. snRNPs do not move after cell fixation. To provide a control for snRNP cycling between CBs, SmB-PA-GFP was co-expressed with SART3-CFP to mark CBs. 24h after transfection cells were fixed for 10min in 4% paraformaldehyde/PIPES and kept in Mg-PBS. SmB-PA-GFP was specifically activated in one CB (circle) by short pulse of 405nm laser and images taken every fifteen seconds for 5 minutes. No movement of activated molecules to other CB (arrow) was observed.

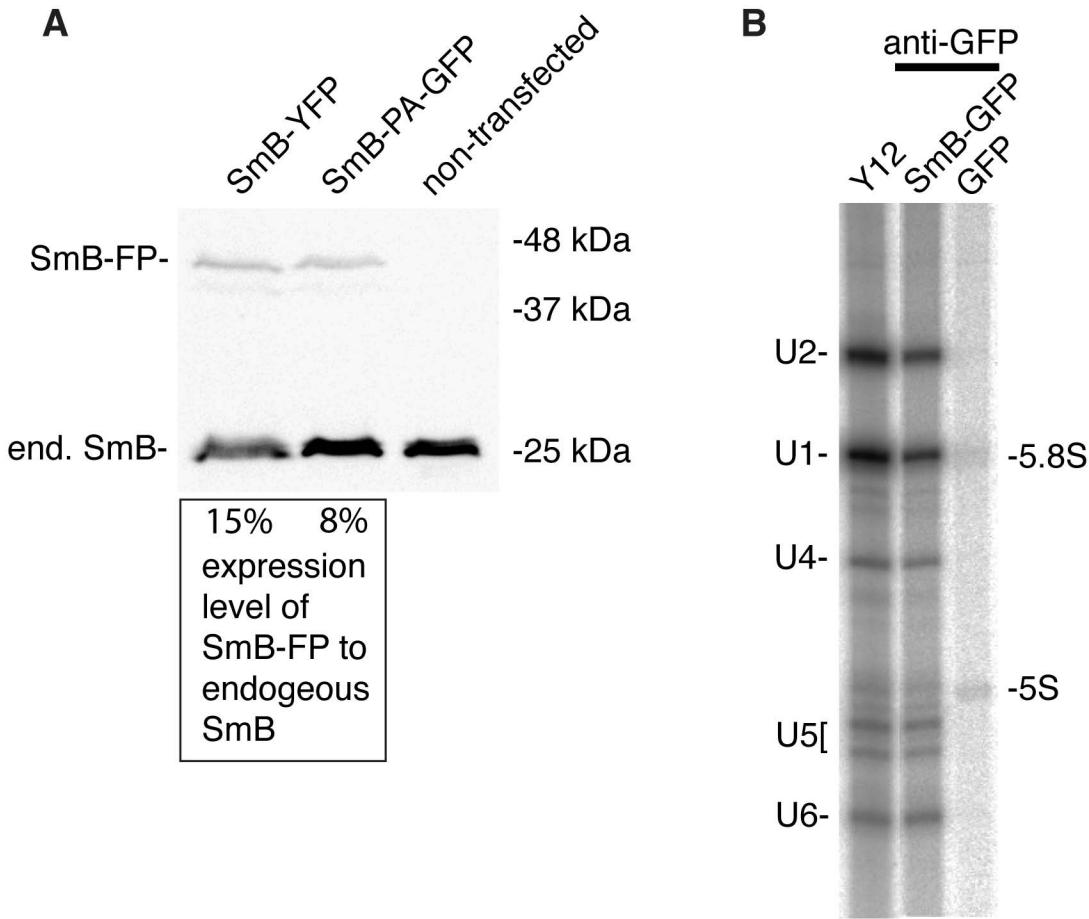
Supplementary figure 3. To determine effects of hPrp22 and hNtr1 knock-down on splicing, cells were treated with siRNAs for 48h, total RNA isolated and level of c-myc, LDHA and tubulin pre-mRNAs and mRNAs determined by RT-qPCR. For each siRNA pre-mRNA to mRNA ratio was determined and normalized to cell treated with neg. control siRNA. Averages of two independent experiments with standard deviation are plotted. For majority of tested siRNAs we observed only a partial increase of pre:mRNA ratio indicating that splicing was not totally blocked.

Supplementary video legends

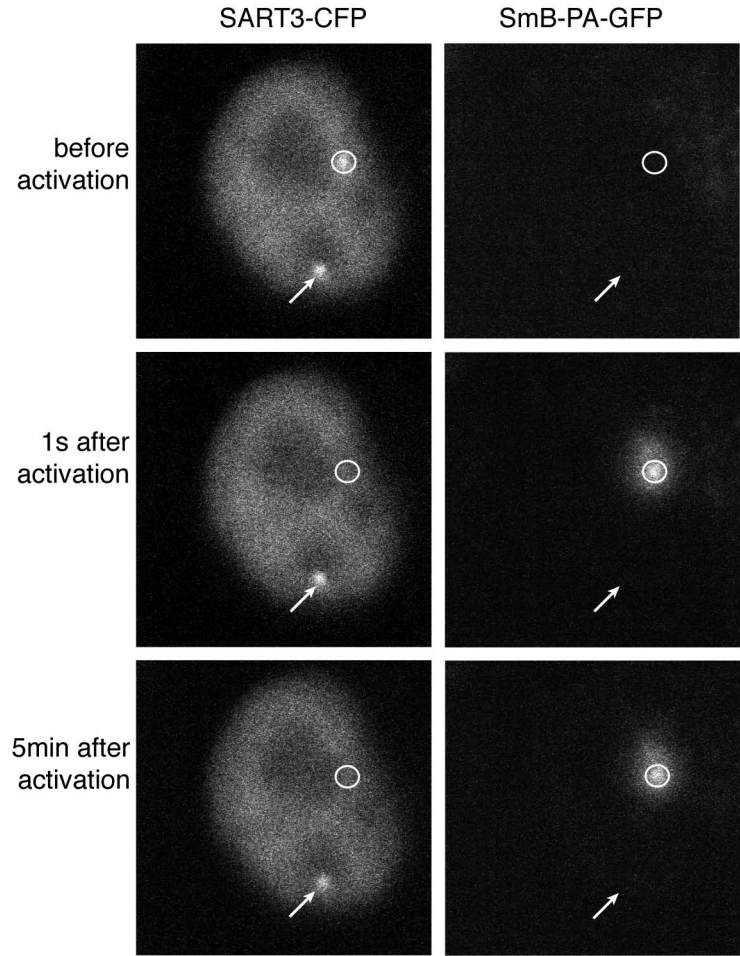
Video 1. snRNPs cycle between CBs. To observe movement of snRNPs in the cell nucleus, SmB-PA-GFP was co-expressed with SART3-CFP (not shown - see also fig. 4) in HeLa cells and activated by short pulse of 405nm laser specifically in one CB. Images were taken every 15s for 5min. Activated molecules moved throughout the whole nucleoplasm and accumulated in other CBs in the same nucleus. The detection system was adjusted to detect very low signals of PA-GFP but using this set up we also detected cell autofluorescence in the cytoplasm. Rate: 4 f/s.

Video 2. snRNPs cycle between CBs. To observe movement of snRNPs in the cell nucleus, SmD1-PA-GFP was co-expressed with coilin-CFP (not shown - see also fig. 4) in HeLa cells and activated by short pulse of 405nm laser specifically in one CB. Images were taken every 15s for 5min. Activated molecules moved throughout the whole nucleoplasm and accumulated in other CBs in the same nucleus. The detection system was adjusted to detect very low signals of PA-GFP but using this set up we also detected cell autofluorescence in the cytoplasm. Rate: 4 f/s.

Supplementary fig. 1



Supplementary fig. 2



Supplementary figure 3