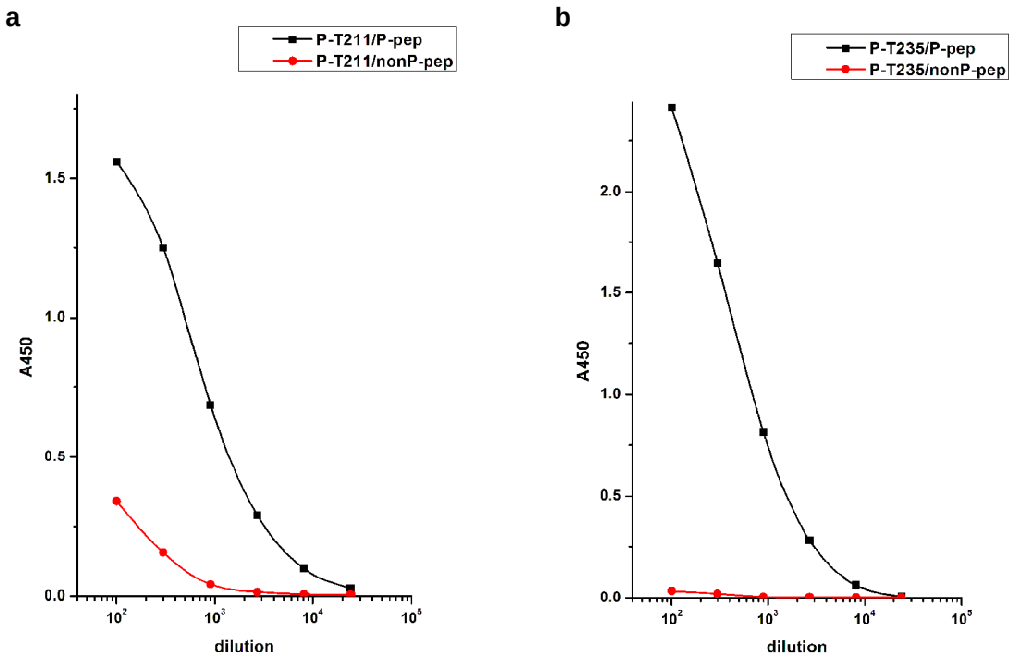
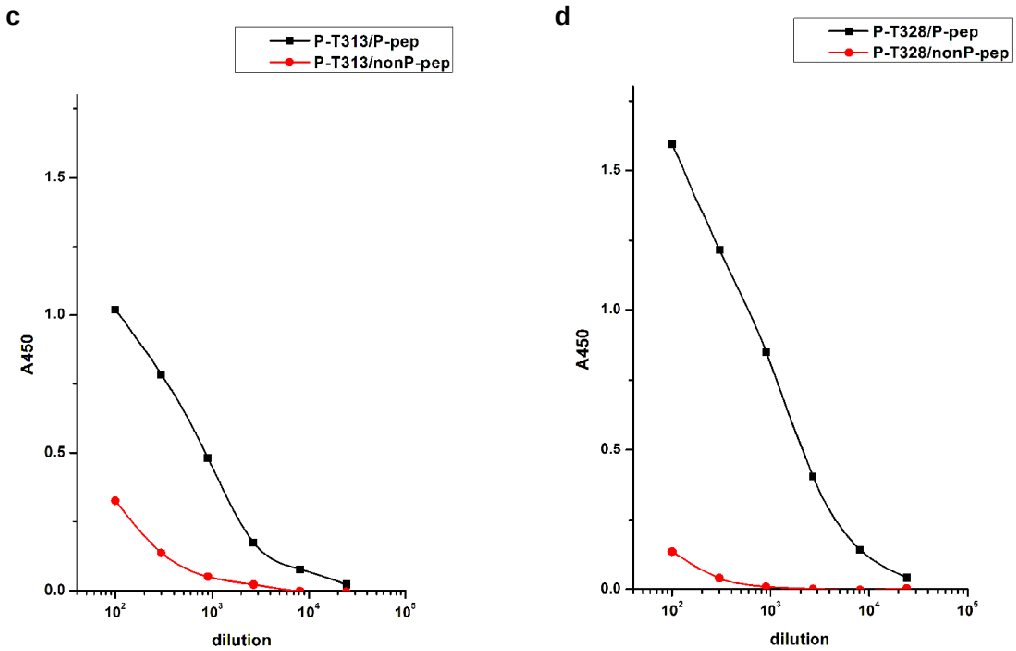


pT211: [C]Q T P G A **pT** P K K L S

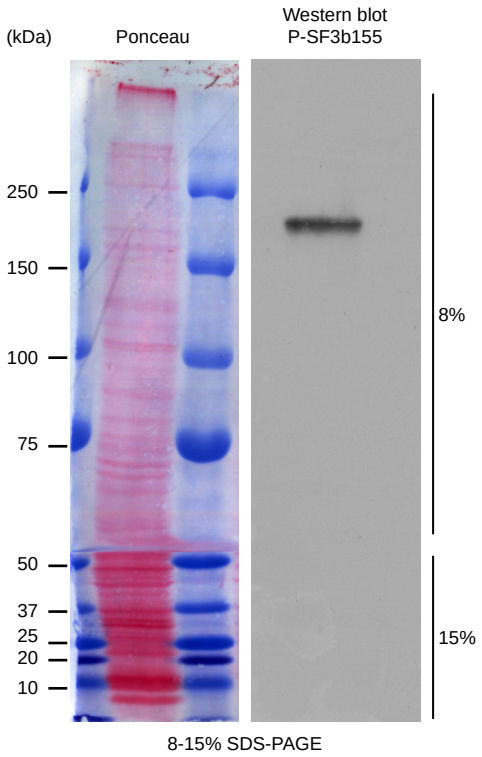
pT235: [C]RWD E **pT** PGRAK



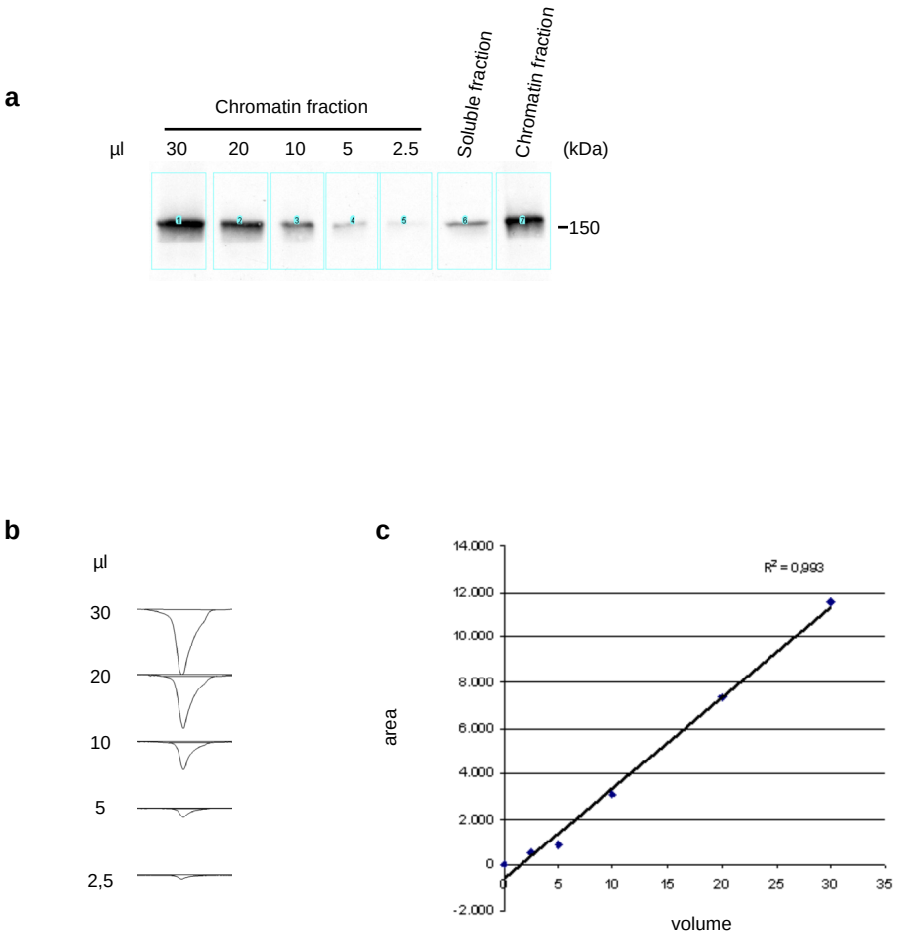
pT313: [C] SGWAE **pT** PRTDR

pT328: [C]IGETP **pT** PGAS

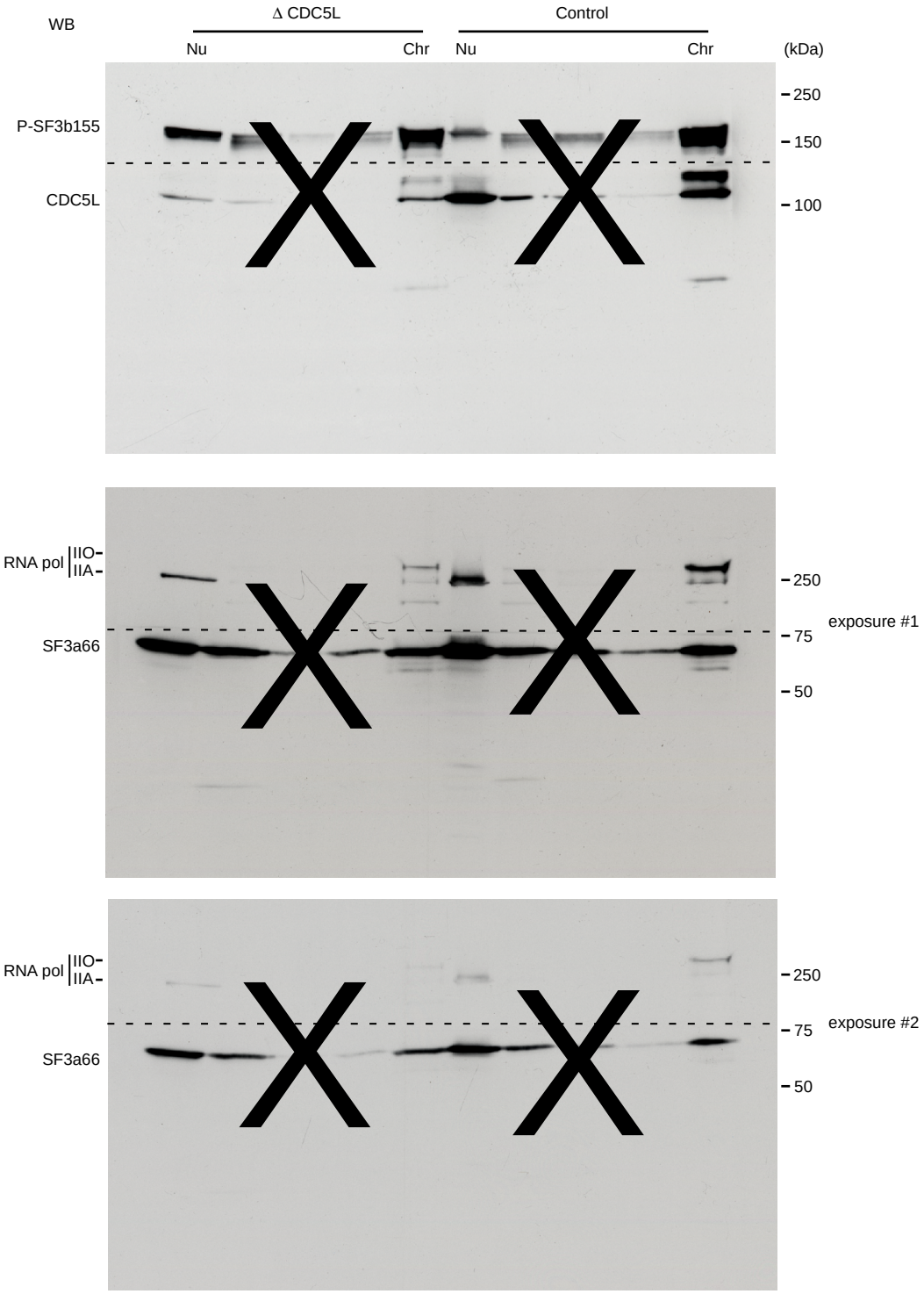
Supplementary Figure S1. ELISA screening of the purified anti-SF3b155 antibodies. The amount of antibody bound to phosphorylated (black dots and lines) or unphosphorylated (red dots and lines) peptides as a function of antibody dilution is shown for four different antibodies (**a**)-(**d**). The target phosphopetides are shown below the respective graphs with the noncoding cysteines shown in brackets.



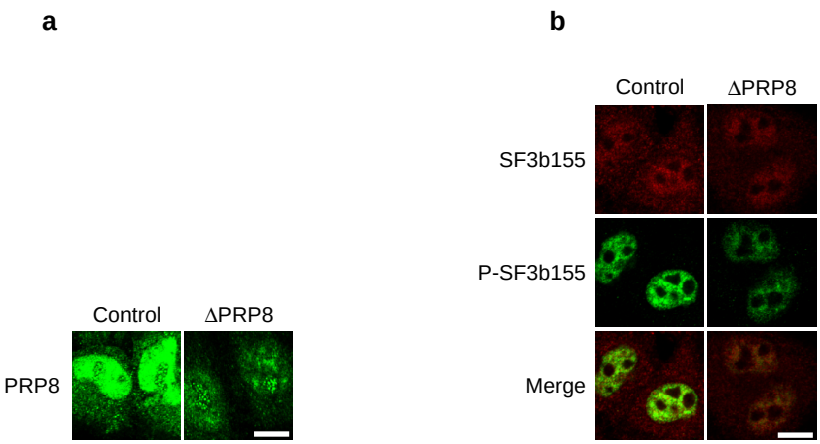
Supplementary Figure S2. **p-T313-SF3b155 antibody recognizes a unique band by western blot.** Total HeLa cell extracts were resolved on a 8-15% SDS-PAGE gel and transferred to a nitrocellulose membrane.The membrane was stained with ponceau (left panel) followed by western blotting with a p-T313-SF3b155 (right panel).



Supplementary Figure S3. Quantitation of the amount of P-SF3b155 in soluble nucleoplasmic and insoluble chromatin fractions of HeLa cells. The soluble and the chromatin-associated P-SF3b155 signal was quantified by western blot. To avoid any potential leakage from the nucleoplasm into the cytoplasm during extract preparation, which would have led to an underestimation of nucleoplasmic P-SF3b155, HeLa cells were directly lysed: Cells were fractionated into (i) a soluble fraction comprising the cytoplasm and the nucleoplasm and (ii) an insoluble fraction corresponding to the chromatin. The chromatin fraction was then resuspended in an equivalent volume of lysis buffer, briefly sonicated to shear the DNA, and an identical volume of the soluble fraction and chromatin fraction were analysed by SDS-PAGE followed by P-SF3b155 immunoblot (Panel **a**). Band intensities were assessed by using image J (panel **b**). To perform accurate measurements, it was necessary to remain within the dynamic range of the film used for ECL detection. To this aim, several different amounts of the chromatin fraction was analysed on the same gel and the intensities of the P-SF3b155 band were plotted on a graph to ensure the linearity of the signal (panel **c**).

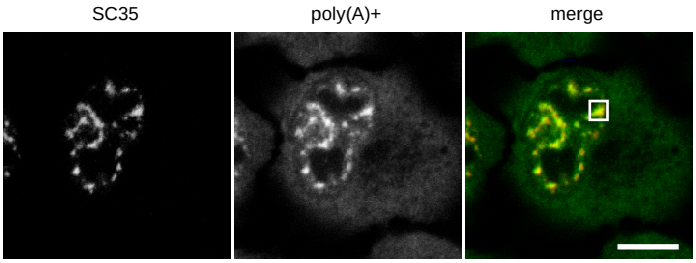


Supplementary Figure S4. Full western blots used Fig 5a.
Lanes crossed out contained unrelated samples analyzed on the same gels. Dashed lines mark the positions where the membrane was cut to allow concomitant incubations with different antibodies. Antibodies used for western blot are indicated on the left hand side.

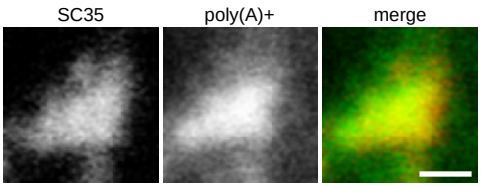


Supplementary Figure S5. **hPRP8 knockdown inhibits SF3b155 phosphorylation**. Double immunofluorescence of hPrp8 RNAi knockdown HeLa cells with (**a**) an anti-PRP8 antibody (green), or (**b**) the MC-epitope specific monoclonal SF3b155 (red) and anti-pT313-SF3b155 antibodies (green). Scale bars: 10 µm.

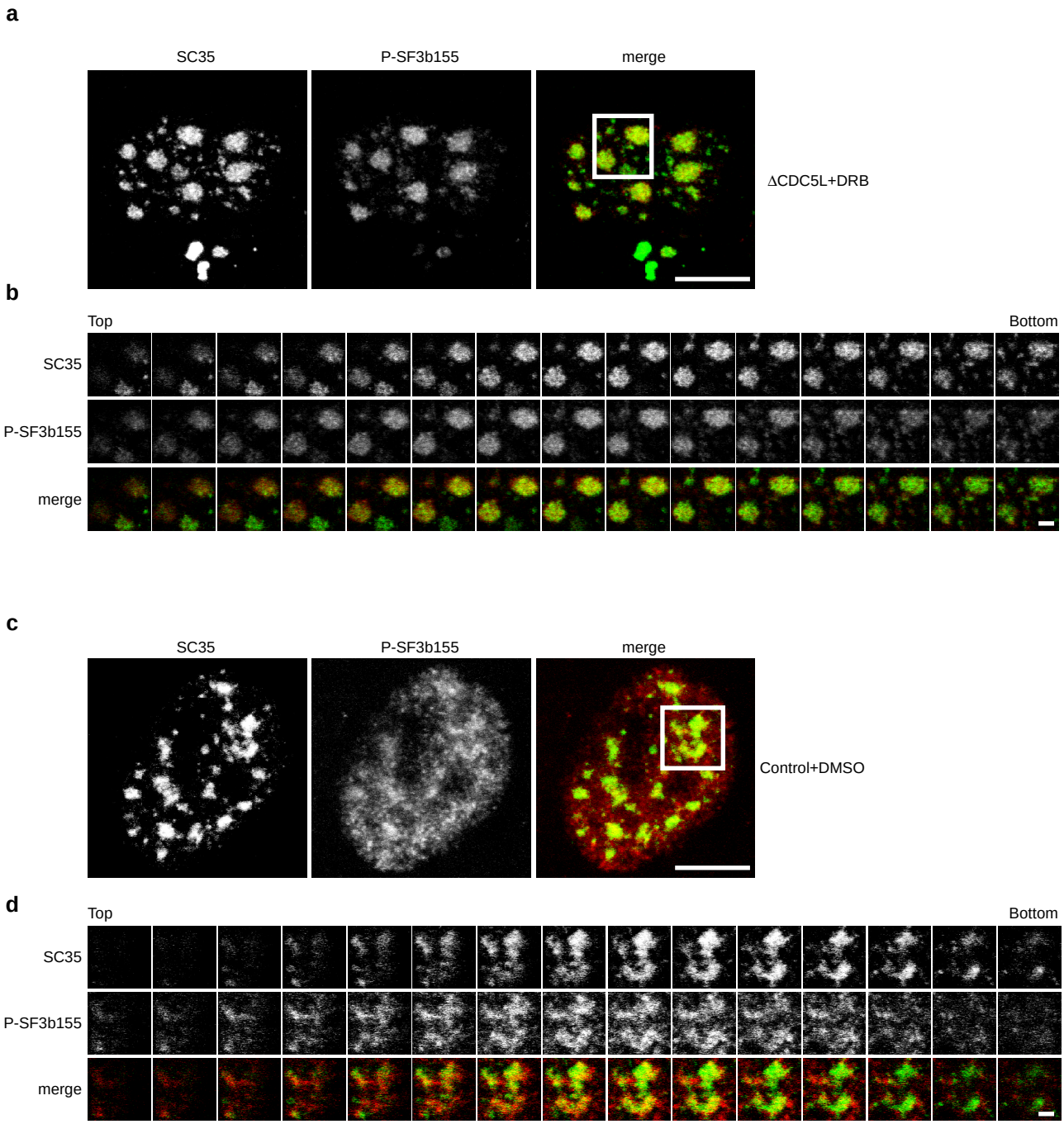
a



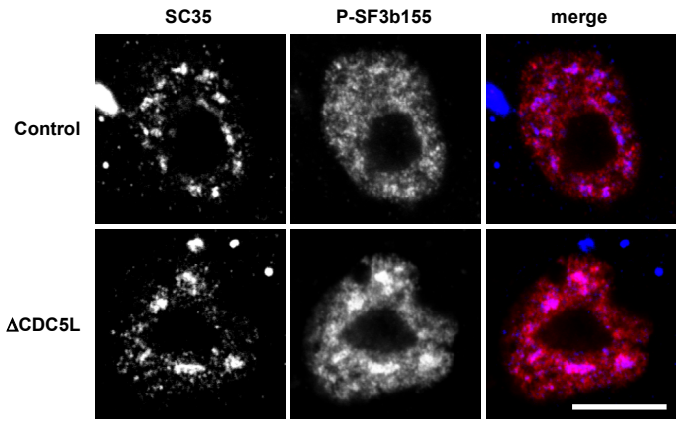
b



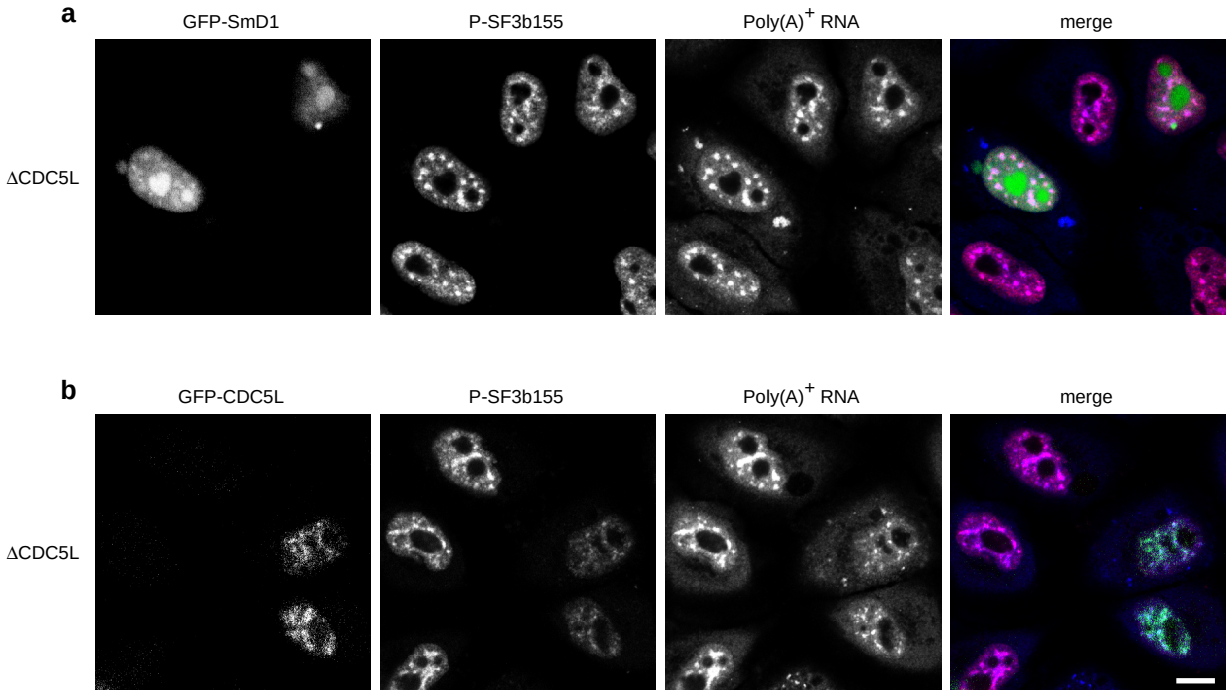
Supplementary Figure S6. Poly(A)+ RNA accumulates within speckles. (**a**) HeLa cells were labeled by immuno-fluorescence with an anti-SC35 antibody (a speckle marker) (red) and by FISH with a poly(A)+ RNA specific probe (green). Scale bar: 10 µm. (**b**) Magnification of speckles corresponding to the white-framed area shown in A. Scale bar: 1 µm.



Supplementary Figure S7. Posttranscriptional spliceosomes are retained in speckles in ΔCDC5L cells. ΔCDC5L cells were incubated with 100 µM DRB for 3.5h. Cells were analyzed by double immunofluorescence with anti-SC35 (green) and anti-pT313-SF3b155 antibodies (red). Z-stack pictures were acquired; each signal was recorded in a 500-600nm z-section with a 150 nm step in z. (**a**) Projection along the z-axis of all single z-sections in one plane. Each staining is shown individually in black and white; the merge panel represents the superposition of both immunofluorescence signals. (**b**) Single z-sections from the framed area shown in panel a are shown from the top to the bottom of the Z-stack. Both channels are shown individually in black and white.(**c**) As in A but with control knockdown cells treated with DMSO for 3.5h. (**d**) As in B with control knockdown cells. Scale bars: 10 µm in panels (**a**) and (**c**), 2 µm in panels (**b**) and (**d**).



Supplementary Figure S8. P-SF3b155 accumulates in speckles already after 48h of CDC5L RNAi-mediated knockdown. CDC5L-depleted and control cells were analyzed after 48h of incubation either with a CDC5L siRNA or control siRNA with no known target in mammalian cells. Cells were stained with anti-P-SF3b155 and anti-SC35 antibodies. Each staining is shown individually in black and white; in the merge panels SC35 is shown in blue and P-SF3b155 in red. Scale bar: 10 μm.



Supplementary Figure S9. **Transfection of GFP-CDC5L into ΔCDC5L cells abolishes the accumulation of Poly(A)+ RNA and P-SF3b155 in speckles, while GFP-SmD1 has no effect.** Cells depleted of CDC5L by RNAi (ΔCDC5L**)** were transfected with either GFP-CDC5L or GFP-SmD1 48h post-siRNA transfection and cultured for an additional12h. Next, cells were fixed and examined by immunofluorescence microscopy using the anti-P-SF3b155 antibody (red) and by FISH to visualize the poly(A)+ RNA (blue); GFP-CDC5L and GFP-SmD1 are shown in green. (**a**) ΔCDC5L cells transfected with GFP-SmD1. (**b**) ΔCDC5L cells transfected with GFP-CDC5L. Scale bar: 10 μm.

Supplementary Table S1. Peptides from SF3B155 identified by in-gel trypsin digestion of the B^{act} spliceosome separated in 1D SDS-PAGE followed by Fe3+-IMAC enrichment and LC/MS/MS analysis. See the method section for details.

Peptide sequences	Observed (m/z)	Charges	Mr(expt)	Mr(calc)	Error (ppm)	Score	Homology	Identity
R.GGDSIGETPTPGASK.R	687,2972	2+	1372,5798	1372,647	-48,96	44	22	43
R.GGDSIGE<u>T</u>PTPGASK.R + Phospho (ST)	727,2799	2+	1452,5452	1452,6134	-46,9	31	27	43
R.T<u>M</u>II<u>S</u>PER.L + Oxidation (M); Phospho (ST)	521,7307	2+	1041,4468	1041,4566	-9,34	30	26	42
K.GSETPGA<u>T</u>PGSK.I + Phospho (ST)	584,7423	2+	1167,47	1167,4809	9,29	24	31	42
R.GGDSIGETPTPGASK.R	687,3262	2+	1372,6378	1372,647	-6,7	43	26	43
R.GGD<u>S</u>IGETPTPGASK.R + Phospho (ST)	727,3088	2+	1452,603	1452,6134	-7,11	37	25	43
R.WDQTADQTPGA<u>T</u>PK.K + Phospho (ST)	798,3409	2+	1594,6672	1594,6665	0,49	34	21	43

Supplementary Table S2. Peptides from SF3B155 identified by in-solution trypsin digestion of the B^{act} spliceosome followed by Fe3+-IMAC enrichment and LC/MS/MS analysis. See the method section for details.

Peptide sequences	Observed (m/z)	Charges	Mr(expt)	Mr(calc)	Error (ppm)	Score	Homology	Identity
K.LLVDVDESTLSPEEQKER.K	696,3468	3+	2086,0186	2086,043	-11,7	44	28	44
R.WDE<u>T</u>PGR.A + Phospho (ST)	470,6786	2+	939,3426	939,3488	-6,5	24	26	43
R.GGDSIGETP<u>T</u>PGASK.R + Phospho (ST)	727,3112	2+	1452,6078	1452,6134	-3,8	22	28	41