Sm proteins target partially-assembled spliceosomal snRNPs to Cajal bodies

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Table S1

T7 promoter	5'TAATACGACTCACTATAGGG 3'
U2wt	F: 5´TAATACGACTCACTATAGGG/ATCGCTTCTCGGCCTTTTGG3´ R: 5´ TGGTGCACCGTTCCTGGAGGT3´
U2ΔSLI	F: 5´ TAATACGACTCACTATAGGG/TGTAGTATCTGTTCATCAG3´ R: 5´ TGGTGCACCGTTCCTGGAGGT3´
U2ΔSLIV	F: 5´TAATACGACTCACTATAGGG/ATCGCTTCTCGGCCTTTTGG3´ R: 5´GGAGTGGACGGAGCAAGCTC3´
U2ΔSm	F: 5´GAGCAGGGAGATGGAATAG3´ R: 5´CCATTTAATATATTGTCCTCGG3´
U2altSLIII	F: 5´ TAATACGACTCACTATAGGG/ATCGCTTCTCGGCCTTTTGG3´ R: 5´CGATTGCGTGGAGTATCTCCCTGCTCCAAAAATCCATTTAAT3´
U2ΔSLI,SLIIa,b	F: 5´ TAATACGACTCACTATAGGG/ATATTAAATGGATTTTTGGAACAG3´ R: 5´ TGGTGCACCGTTCCTGGAGGT3´
U2U1Sm	F: 5´GAGCAGGGAGATGGAATAG3´ R:5´ CACAAATTCATTTAATATATTGTCCT3´
U1wt	F: 5' TAATACGACTCACTATAGGG/ATACTTACTGGCAGGGGAG 3' R: 5´CAGGGGAAAGCGCGAACGCAG3'
U1ΔSm	F: 5´ TAGTGGGGGACTGCGTTCGCG3´ R: 5´ATGCAGTCGAGTTTCCCACAT3´
U4wt	F: 5´ TAATACGACTCACTATAGGG/AGCTTTGCGCAGTGGCAGTAT3´ R: 5´ CAGTCTCCGTAGAGACTGTCA3´
U4ΔSm	F: 5´ TAATACGACTCACTATAGGG/AGCTTTGCGCAGTGGCAGTAT3´ R: 5′-CAGTCTCCGTAGAGACTGTGGCCGGCCGCCAATGCCGAC-3′
U5wt	F: 5´ TAATACGACTCACTATAGGG/ATACTCTGGCTTCTCTTCAGAT3´ R: 5´ AGTGCTGGATTAGCCTTGCCAA3´
U5ΔSm	F: 5´CACAAACGTGCCTTGCCTTGG3´ R: 5´GGGTTAAGACTCAGAGTTGTTCCT3´
7SK wt + T7	F: 5´ TAATACGACTCACTATAGGG/GGATGTGAGGGCGATCTGGCTG3´ R: 5´ AGAAAGGCAGACTGCCACATGC3´
7SKSm	R: 5´ AGAAAGGCAGACTGCCACATGCAGCGCCTCATTTGGATGTGCAAAAATCT3´

7SKSMN	R: 5´TGGTACCGGTCATCATATTTACACCCAGTACCTAC3´
7SKSm+SMN	R: 5´ TGGTACCGGTCATCATATTTACACCCAGTACCTACAAAAATTGGT3´
Alu wt + T7	F:5´TAATACGACTCACTATAGGG/CTCCCGCAACGCTACTCTCGT3' R: 5'AGTAGAGACGGGGTTTCACCATGTT3'
Alu + Sm	R: 5'TACCTACAAAAATTGGTCAGCATGGGGGCCCTGCCAGCTACAT 3'
Alu + Sm + SMN	R: 5'TGGTACCGGTCATCATATTTACACCCAG TACCTACAAAAATTGGTCAGCA3'
SRP wt + T7	F: 5´TAATACGACTCACTATAGGG/CTCCCGCAACGCTACTCTCGT3' R: 5'TGGGGGCCCTGCCAGCTACAT 3'
SRP+Sm	R: 5' TACCTACAAAAATTGGTCAGCATGGGGGCCCTGCCAGCTACAT 3'
SRP+Sm+SMN	R: 5' TGGTACCGGTCATCATATTTACACCCAG TACCTACAAAAATTGGTCAGCA 3'

Sm proteins

SmD1	ΔGR F: 5' AGCGAATTCTGATGACCCTGAAGAACAGAGAACCT3' R: 5' GCGGGATCCTTCCTGCAACAGCTTCCCTTTTCTTA3'
	Δ1/4GR F: 5' AGCGAATTCTGATGACCCTGAAGAACAGAGAACCT3' R: 5' ATAGGATCC T TCTTCCTCTGCCACGGCCACG3´
	Δ1/2GR F: 5' AGCGAATTCTGATGACCCTGAAGAACAGAGAACCT3' R: 5´ATAGGATCC T GTCCTCTTCCTCTTCCTCTTCCTC3´
SmD2	wt F: 5' AGCGAATTCTGATGAGCCTCCTCAACAAGCCCA3' R: 5' GCGGGATCCTCTTGCCGGCGATGAGCGGGTT3'
	Δhelix F: 5' AGCGAATTCTGATGCAATACCCAAGTGCTCATCAA3' R: 5' GCGGGATCCTCTTGCCGGCGATGAGCGGGTT3'
	Δ1-24 F: 5' AGCGAATTCTGATGAACACCGGTCCACTCTCTGTGC3' R: 5' GCGGGATCCTCTTGCCGGCGATGAGCGGGTT3'
	Δ111-118 F: 5' AGCGAATTCTGATGAGCCTCCTCAACAAGCCCA3' R: 5'GCGGGATCCTCCGCAGGACCACGATGACTG3'
SmD3	wt F: 5'AGCGAATTCATGTCTATTGGTGTGCCGATT3' R: 5'GCGGGATCCGTTCTTCGCTTTTGAAAGATG3'
	ΔCtail F: 5'AGCGAATTCATGTCTATTGGTGTGCCGATT3' R: 5'GCGGGATCCGTTCAAGGCCCAAGTGGCCGCA3'
	Ala F: 5' GCAATGGCAGCGGCAAACATGTTTCAAAACCGAAGA3' R: 5' TGCTGCTGCTGCTGCTGCGGCCACTTGGGCCTTGAGAATA3'
SmB/B'	ΔCtail F: 5'AGCGAATTCTGATGACGGTGGGCAAGAGCAGCA3' R: 5'GCGGGATCCTCAGGTGGGTACTGGGTTGGAG3'

MS2 constructs

MS2 loop	5'TAACATGAGGATCACCCATGTTTT 3'
U2 wt FL	F: 5´AGTCGGATCCGGCAGAGGAACTCCAGCCCCT3´ R: 5´ATAGGAATTCCAAGCCGCCCCGCAGGTGCTACC3´
U2 wt FL MS2	F: 5´AGGAGAACAAATCCGAGGACAATATATTAAAT 3´ R: 5´TTATAGACTATGCAGGAGATACAAGGGTAA3´

U2ΔSLI+IIa,b-MS2	F: 5TAACATGAGGATCACCCATGT3’ R: 5’GCGCTCGCCTTCGCGCCCGCCGTCA3’
U4Δ1-64-MS2	F: 5´AAAACTTTTCCCAATACCCCGC3´ R: 5´GGAAAGGCTTTATTCGCGCC3´

siRNA

SmB/B'	5´UCUACUGUCAUUGAGACCAga3´
SmD1	5´UUAGGUUCAACAUCCACAAgt3´
Tgs1	FR1:5'AAGATTGCCCTTGCTCGCAATAA3' FR2: 5'TATCACCGTATGAAATGGAAACT3'
SMN	AA1: 5’AAGAAGAATACTGCAGCTTCCTT3’ AA2: AA2 5’AAGTGGAATGGGTAACTCTTCTT3’
SmG	5´UACUAUUUCCUCGUAUUACca3´

Supplementary figure legends

Figure S1. Predicted secondary structures of WT U2 snRNA and various U2 deletion mutants.

Figure S2. Microinjected U2 snRNAs with monomethyl-guanosine cap are targeted to Cajal bodies.

WT U2 snRNA or deletion mutants thereof were in vitro transcribed in the presence of monomethylated cap analog ($m^7G(5')ppp(5')G$ and microinjected into the cytoplasm or into the nucleus of HeLa cells. U2 snRNA was labeled with UTP-Alexa-488 (green), coilin, a marker of CBs, was immunolabeled by Alexa-647 (red). Dextran-TRITC 70kDa (yellow) was used to monitor nuclear or cytoplasmic injection, DNA was stained by DAPI (blue). Small red box in U2 snRNA scheme represents the Sm site. The scale bar represents 10 μm.

Figure S3. Sm proteins D1 and G are essential for Cajal body targeting of snRNAs.

Microinjection of WT U2 snRNA after depletion of Sm proteins SmD1 (A) SmG (B) and negative control siRNA (C). RNAs were labeled with UTP- Alexa-488 (green), coilin, a marker of CBs, was immunolabeled by Alexa-647 (red). Dextran-TRITC 70 kDa is a marker for injection (yellow). DNA was stained with DAPI (blue). The scale bar represents 10 μm. (D) Western blots assaying the efficiency of the siRNA knockdowns. GAPDH or β actin were used as a loading control.

Figure S4. Deletion mutants of SmB, SmD1 and SmD3 are able to bind snRNA.

Immunoprecipitation of (A) WT SmD3-GFP and deletion mutants thereof, (B) SmB-GFP and the deletion mutant SmBΔCtail-GFP, (C) SmB and SmD1 GR substitution mutants and (D) SmD1-GFP and GR deletion mutants, was performed using anti-GFP antibodies. Precipitated proteins were detected by Western blotting using anti-GFP

antibodies (bottom) and co-precipitated RNAs were resolved on a polyacrylamide gel and visualized by silver staining.

Figure S5. Additional CB markers accumulate in coilin positive foci formed in TGS1-depleted cells upon microinjection of 12S-U2snRNP.

HeLa cells transfected with negative control or anti-Tgs1 siRNAs were microinjected in the cytoplasm with Cy3-labelled native 12S-U2snRNPs and examined by immunofluorescence 2h post microinjection. (A) Cells were stained by antibodies against coilin and box C/D snoRNP marker fibrillarin. In merged pictures, coilin is shown in blue, fibrillarin in green the microinjected native 12S U2 snRNPs are shown in red. (B) Cells were stained by antibodies against coilin and box C/D snoRNP marker NOP58. In merged pictures, coilin is shown in blue, NOP58 in green the microinjected native 12S U2 snRNPs are shown in red. (C) Cells were stained by antibodies against coilin and SART3. In merged pictures, coilin is shown in blue, SART3 in green and the microinjected native 12S U2 snRNPs are shown in red. Insets display a magnification of a single CB. Nuclei of microinjected cells are delineated by dotted white lines, non-microinjected cells by dashed white lines. Scale bars: 10 μm.

Figure S1

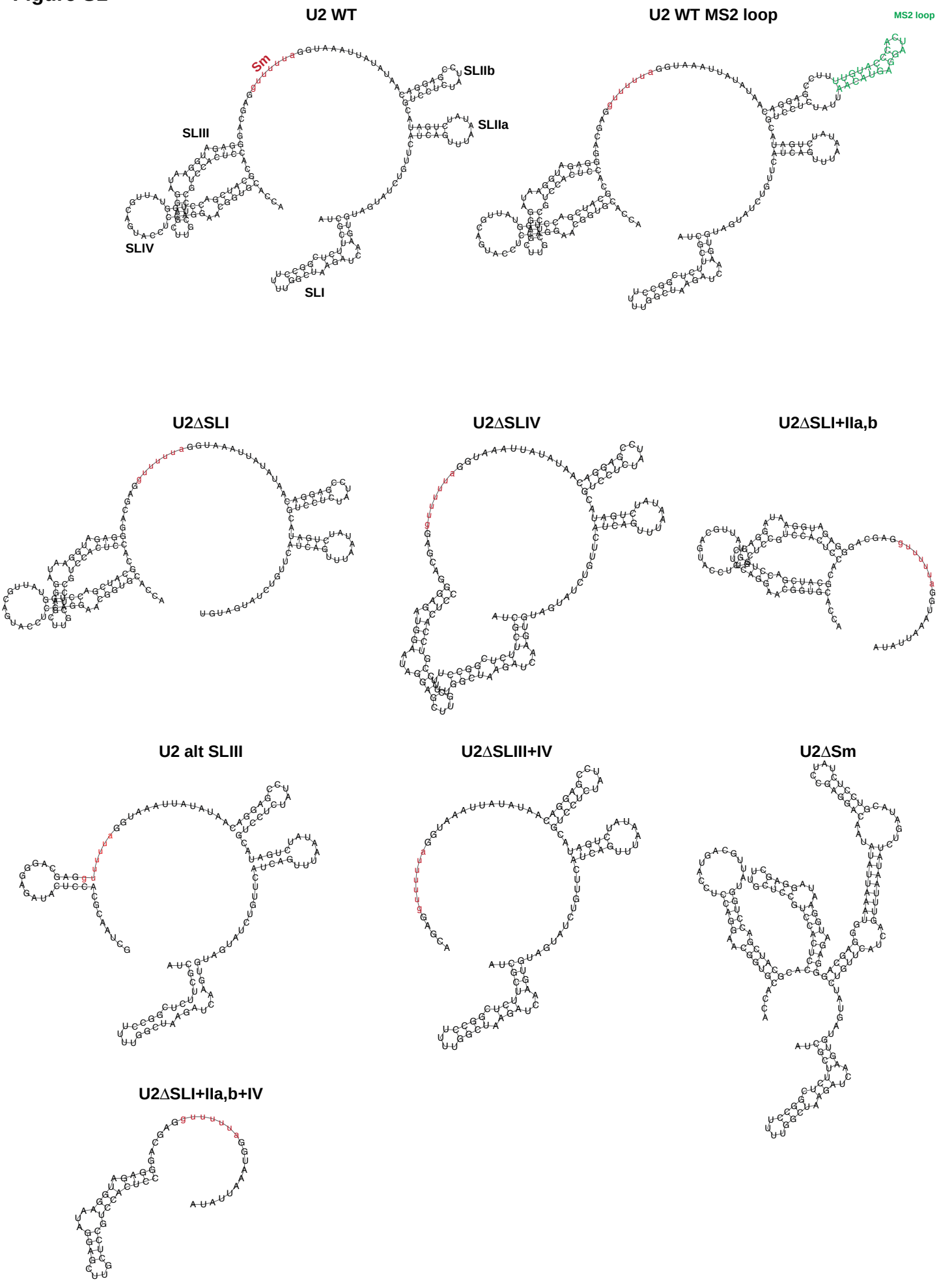
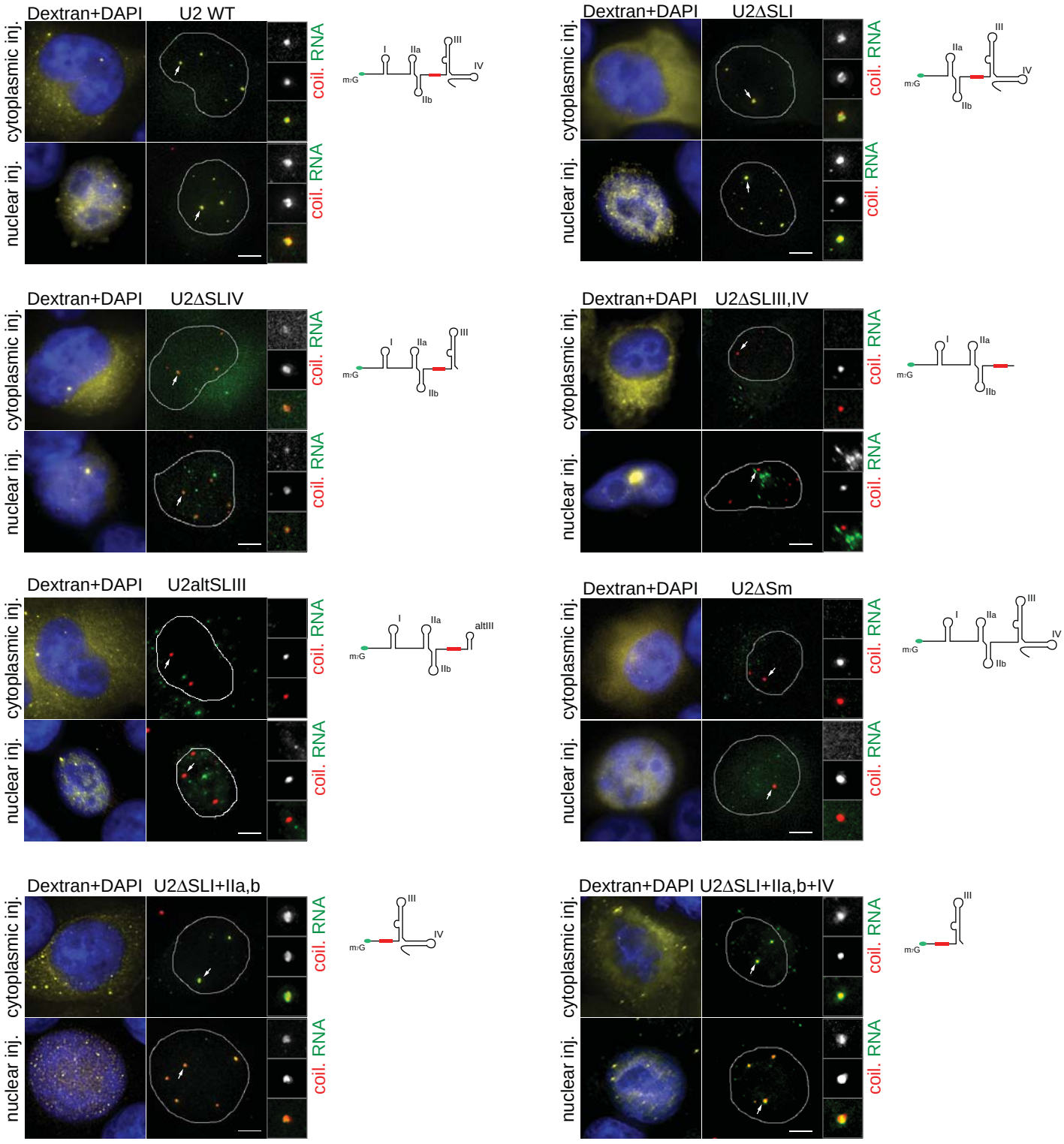

U2 WT
Sm
SLIIb
SLIIa
SLIII
SLIV
SLI
U2 WT MS2 loop
MS2 loop
U2ΔSLI
U2ΔSLIV
U2ΔSLI+IIa,b
U2 alt SLIII
U2ΔSLIII+IV
U2ΔSm
U2ΔSLI+IIa,b+IV

Figure S2


Dextran+DAPI
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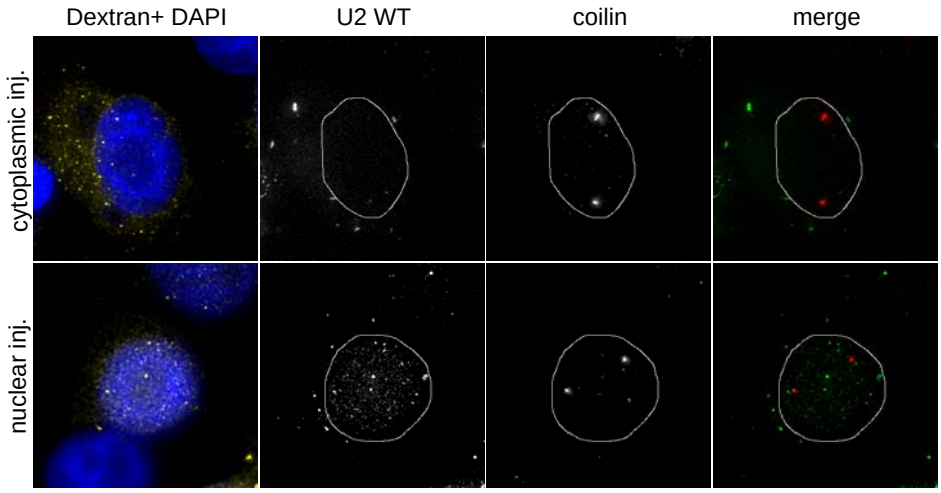
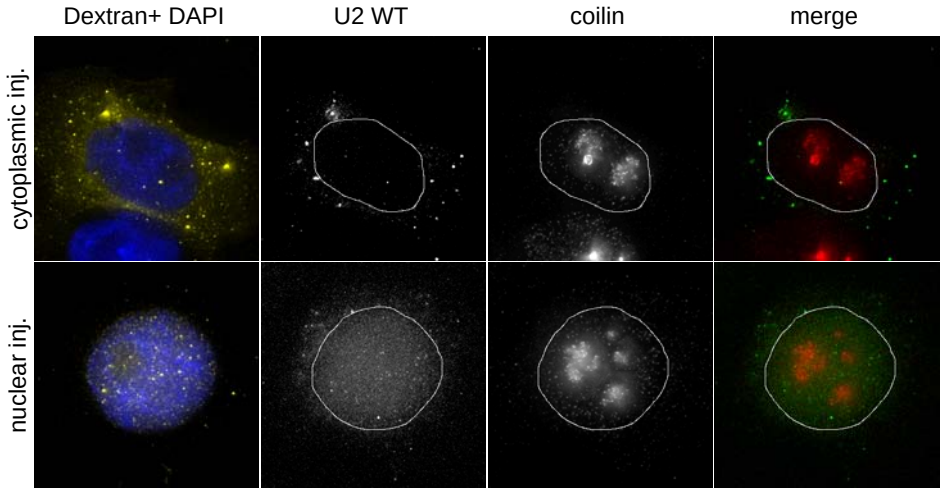
U2ΔSLI
U2ΔSLIV
U2ΔSLIII,IV
U2altSLIII
U2ΔSm
U2ΔSLI+IIa,b
U2ΔSLI+IIa,b+IV
cytoplasmic inj.
nuclear inj.
coil. RNA
m7G
I
IIa
IIb
III
IV
altIII

Figure S3

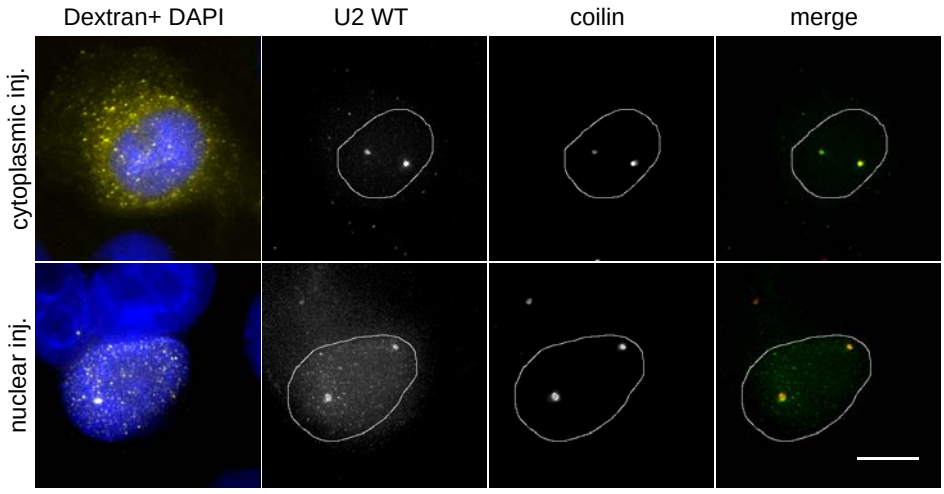
A **SmD1 siRNA**



B **SmG siRNA**



C **Negative control siRNA**



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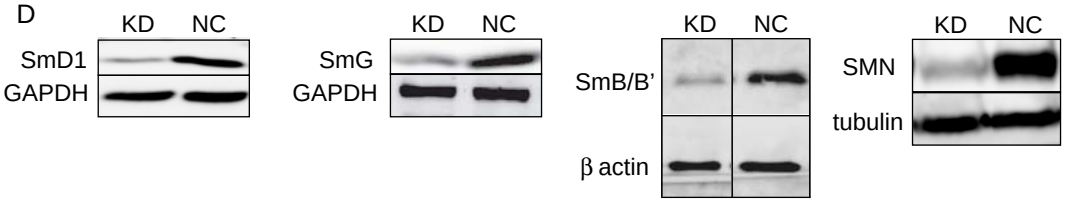


Figure S4

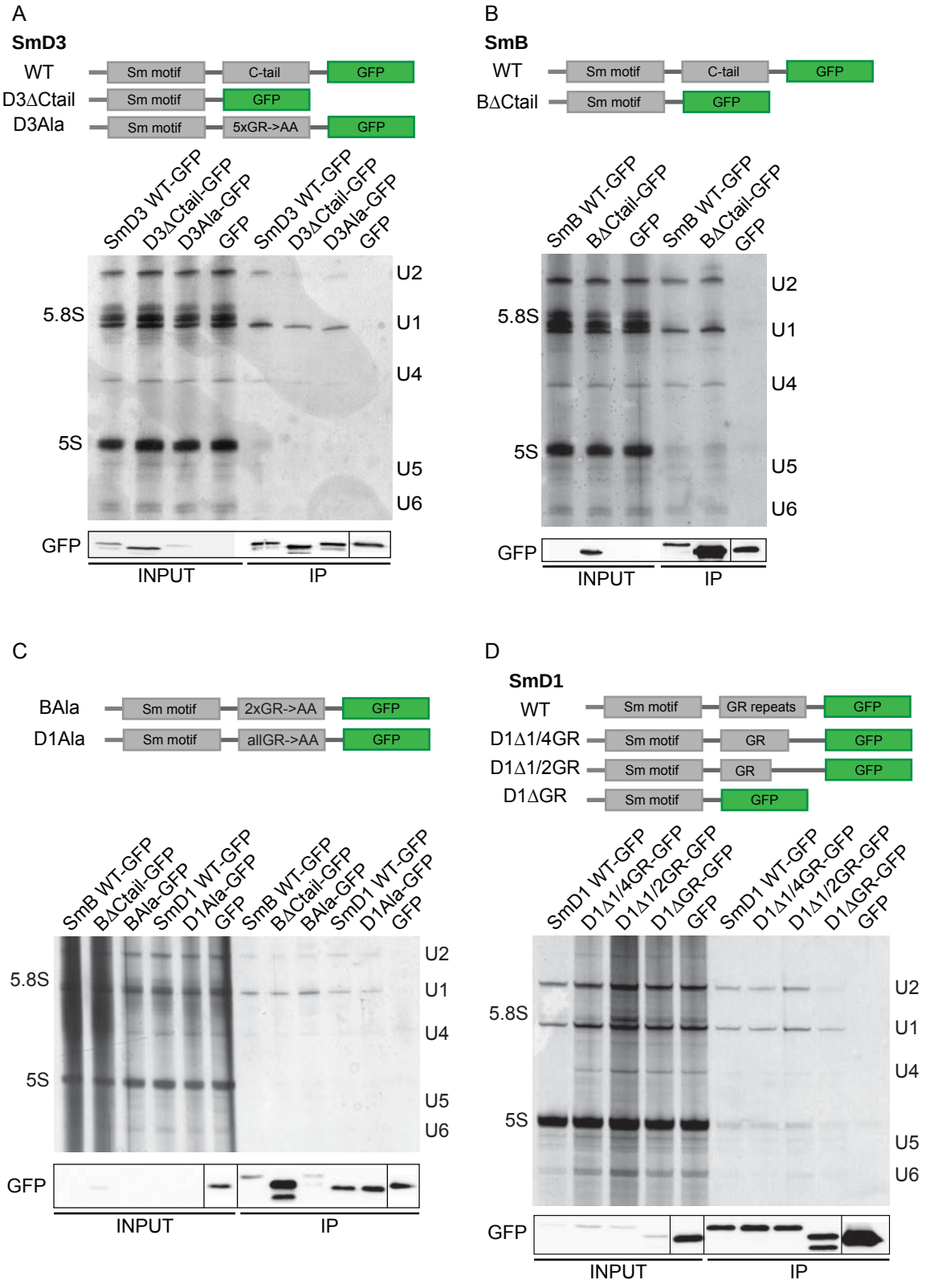
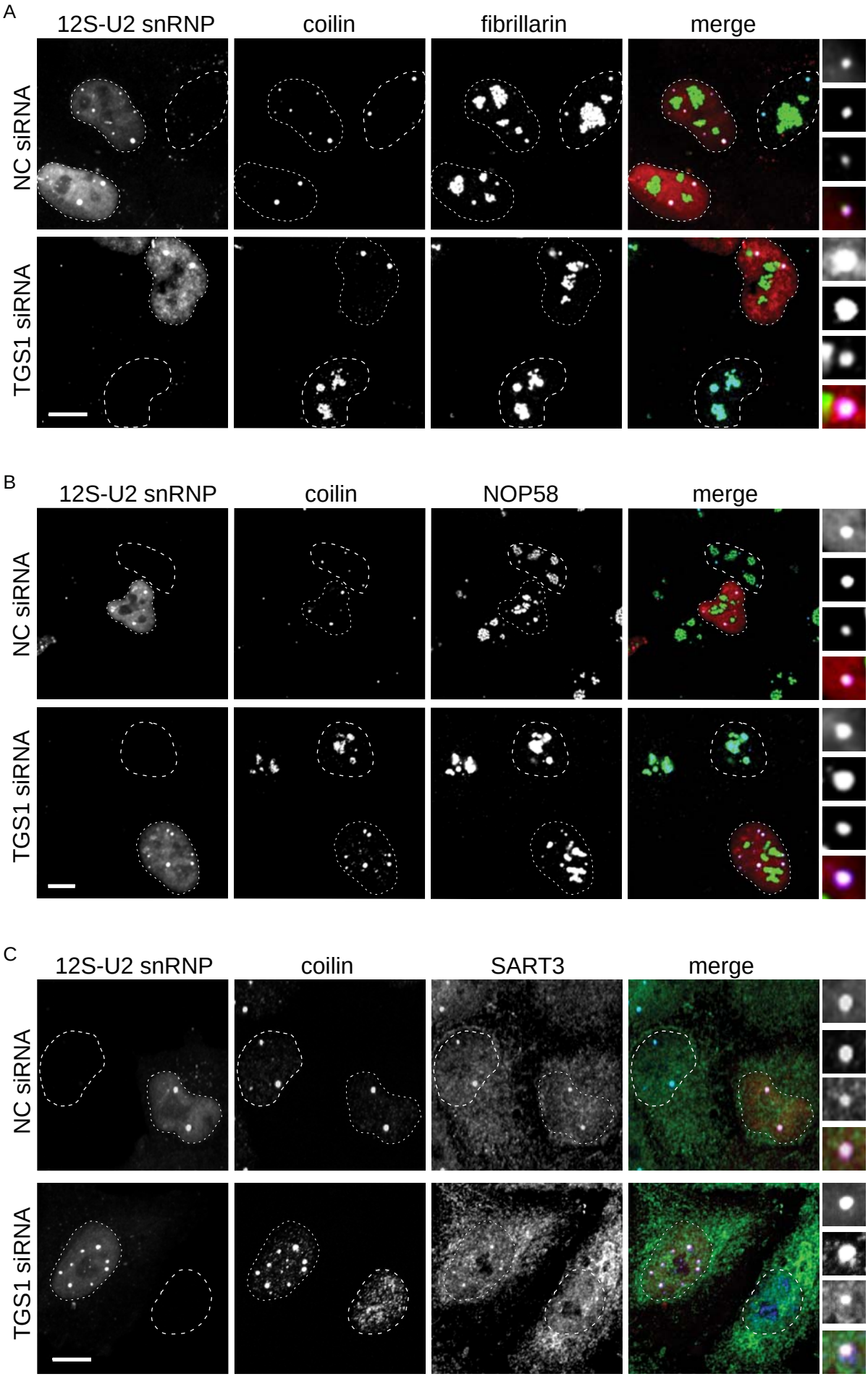

A
SmD3
WT
D3ΔCtail
D3Ala
Sm motif
C-tail
GFP
5xGR->AA
SmD3 WT-GFP
D3ΔCtail-GFP
D3Ala-GFP
GFP
5.8S
5S
U2
U1
U4
U5
U6
INPUT
IP
B
SmB
BΔCtail
SmB WT-GFP
BΔCtail-GFP
C
BAla
D1Ala
2xGR->AA
allGR->AA
BAla-GFP
D1Ala-GFP
D
SmD1
D1Δ1/4GR
D1Δ1/2GR
D1ΔGR
GR repeats
GR
SmD1 WT-GFP
D1Δ1/4GR-GFP
D1Δ1/2GR-GFP
D1ΔGR-GFP

Figure S5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fibrillarin
merge
NC siRNA
TGS1 siRNA
B
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coilin
NOP58
merge
NC siRNA
TGS1 siRNA
C
12S-U2 snRNP
coilin
SART3
merge
NC siRNA
TGS1 siRNA