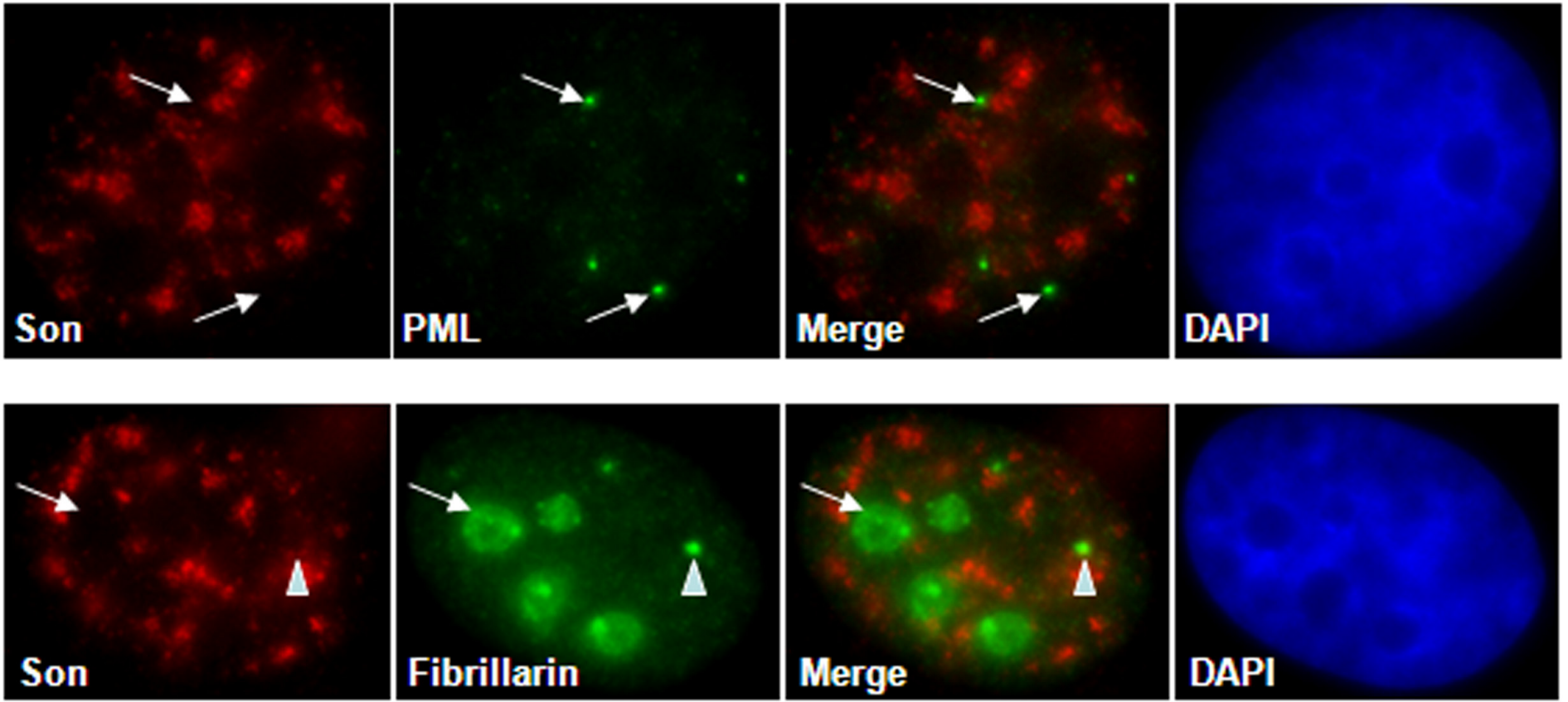
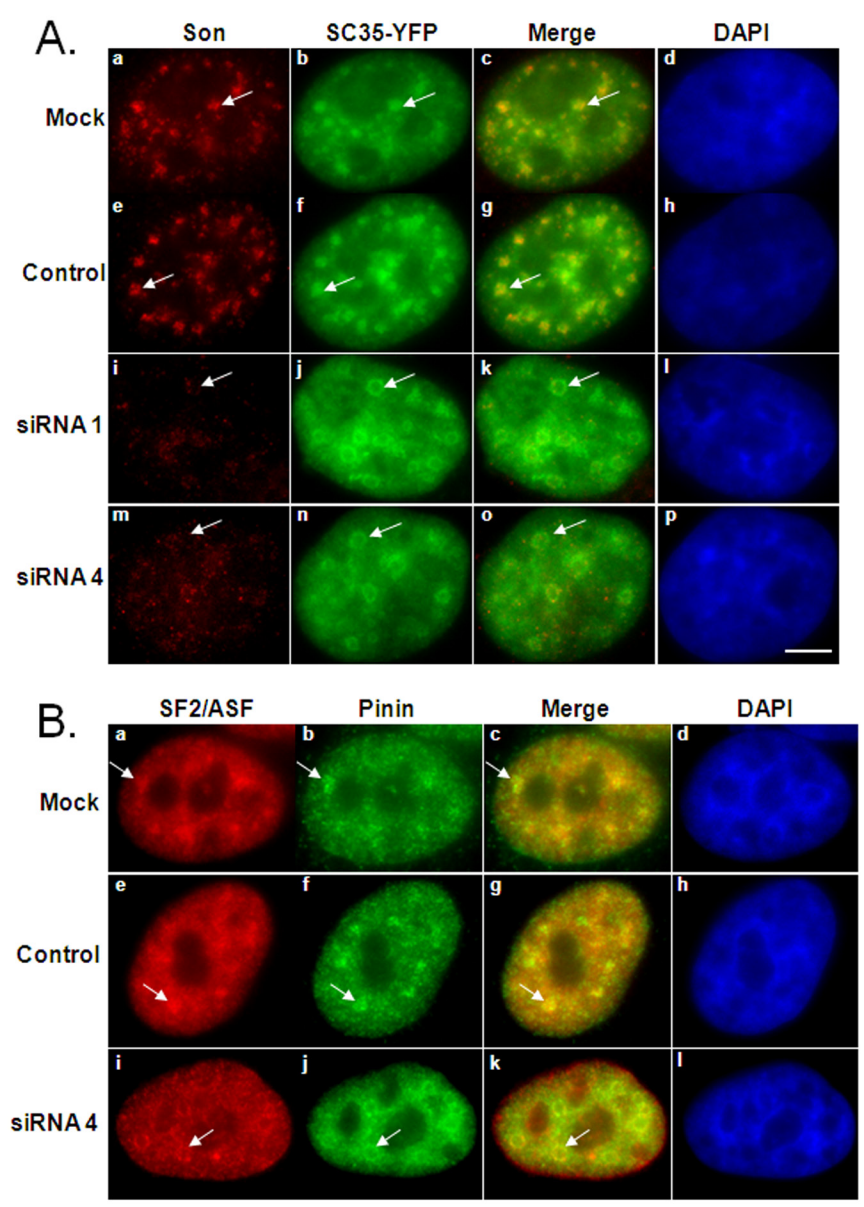
Supplemental Figure 1. Son is not detected in PML bodies, Nucleoli, or Cajal bodies. HeLa cells were processed for dual immunofluorescence labeling of Son and either fibrillarin or PML. Son labeling was absent in regions corresponding to PML bodies (b) nucleoli (e, arrow) and Cajal bodies (e, arrowhead).

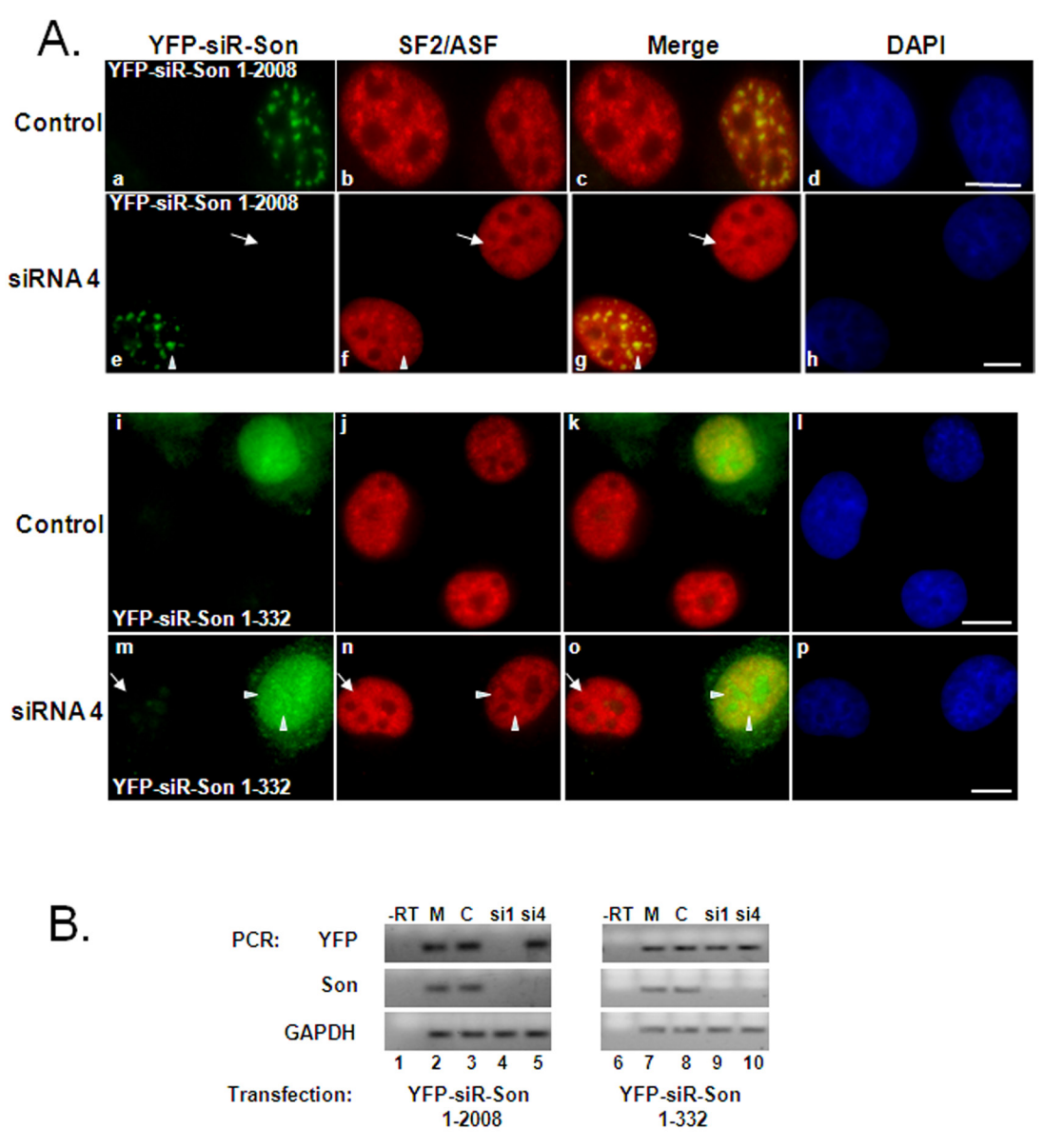
Supplemental Figure 2. Localization of YFP-SC35 (A) and pinin (B) is altered after siRNA-mediated Son depletion. A. HeLa cells stably expressing SC35-YFP were treated with mock transfection, control siRNAs, or Son siRNAs. Cells were fixed and processed for immunolocalization of Son. Son signal is nearly absent after Son depletion (part A: i, m). SC35-YFP localization in nuclear speckles was altered after depletion of Son with siRNAs 1 and 4 (part A: i-j and m-n, arrows), but not with mock transfection (part A: a-b, arrow) or control siRNAs (part A: e-f, arrow). B. HeLa cells were treated with Son siRNA oligos and processed for immunolocalization of pinin. Pinin co-localized with SF2/ASF in doughnut-shaped nuclear speckles in cells treated with Son siRNA 4 (part B: j, arrow). DNA was stained with DAPI. Bar = 5 μm.

Supplemental Figure 3. YFP-siR-Son (1-2008) rescues nuclear speckle morphology but YFP-Son (1-334) does not. HeLa cells were transfected with plasmid encoding YFP-tagged siRNA-resistant Son constructs then plated for siRNA treatment. YFP-Son (1-334) is inherently siRNA-resistant, as it does not contain the region targeted by Son siRNA 4. Treatment of cells with control siRNA (a-d and i-l) did not affect speckle morphology. Treatment with Son siRNA 4 showed the expected nuclear speckle reorganization (e-h, arrow), but neighboring cells protected by YFP-siR-Son (1-

2008) expression showed a normal nuclear speckle phenotype (YFP-positive cells in e-f). Cells expressing YFP-Son (1-334) showed doughnut-like reorganization of nuclear speckles in cells regardless of whether or not they expressed exogenous Son (1-334; m-o).


Son
PML
Merge
DAPI
Son
Fibrillarin
Merge
DAPI


A.
Son
SC35-YFP
Merge
DAPI
Mock
Control
siRNA 1
siRNA 4
a
b
c
d
e
f
g
h
i
j
k
l
m
n
o
p
B.
SF2/ASF
Pinin
Merge
DAPI
Mock
Control
siRNA 4
a
b
c
d
e
f
g
h
i
j
k
l


A.
YFP-siR-Son
SF2/ASF
Merge
DAPI
YFP-siR-Son 1-2008
Control
siRNA 4
YFP-siR-Son 1-2008
YFP-siR-Son 1-332
Control
siRNA 4
YFP-siR-Son 1-332
B.
-RT M C si1 si4
PCR: YFP
Son
GAPDH
1 2 3 4 5
-RT M C si1 si4
6 7 8 9 10
Transfection:
YFP-siR-Son 1-2008
YFP-siR-Son 1-332