Supplemental Material

Figure S1. (Aa) Immunolocalization of SRSF1 in control and SRSF1 siRNA-treated HeLa cells. (Ab-c) GFP-SF1, GFP-UAP56, CFP-SRSF3, U170K, Sm antigen (Y12 ab) and pan phospho-SR protein (3C5 ab) localization in control and SRSF1-depleted HeLa cells. (Ad) Expression of the tet-repressible HA-SRSF1 and –SRSF2 in SRSF1 and SRSF2 KO MEF total cell extracts in the presence (3 days; +DOX) or absence of Doxycycline (-DOX) (tet-analog). (Ae) Immunolocalization of SRSF1 and SF3a60 in the control and DOX-treated SRSF1 KO MEFs. Note: DOX-treated cells show the absence of SRSF1. The DOX inhibits the transcription of the exogenous Ha-tagged SRSF1 cDNA in the SRSF1 KO MEFs. (Af) RNA-FISH of MALAT1 RNA in the wild type, SRSF1 and SRSF2 KO MEFs. (Ag) Immunolocalization of U170K (green) and SF3a60 (red) in the control siRNA and SRSF1 siRNA incubated control (no drug), DRB-treated and 1hr recovery (1hr rec. post DRB treatment) HeLa cells. DNA is counterstained with DAPI. Scale bar: 5 μm.

(Ba) Western blot to detect SRSF1 and YFP-SRSF1 using GFP and SRSF1 antibodies in asynchronous, control- and SRSF1-siRNA treated HeLa and YFP-SRSF1 stably expressing HeLa cells. (Bb) RNA-FISH in EYFP-SRSF1 stable expressing HeLa cells reveals the co-localization of EYFP-SRSF1 and transiently expressed β-tropomyosin RNA in nuclear speckles (Politz *et al.*, 2006). (Bc) Immunofluorescence staining reveals the normal distribution of B”-U2snRNP and SRSF2 (SC35 ab) in endogenous SRSF1-depleted HeLa cells, which stably express YFP-SRSF1. (Ca) Immunoblot analyses using antibodies against various pre-mRNA processing factors in control and SRSF2-depleted total cellular extracts. (Cb-d) Immunofluorescence staining using antibodies against B”-U2snRNP (green, Cb-c), SRSF1 (green, Cd) and SF3a60 (red, Cb-d) in SRSF2-depleted HeLa cells (Cb) and control and SRSF2 knock out MEFs. (Cc-d). The DNA is counterstained with DAPI. The scale bar represents 5 μm.

Figure S2. SRSF1-depletion results in the stabilization of SRSF2. (A) SRSF1 antibody staining (green) in RFP-SRSF2 (red) stably expressing HeLa cells that were transfected with SRSF1 siRNA. Note that only the cell that is depleted of SRSF1 display increased levels of SRSF2. (B) Immunoblot analysis using GFP antibody from total cell extracts of

control, SRSF1- or SRSF2-depleted HeLa cells (YFP-SRSF2 stable cell line) display increased expression of YFP-SRSF2 upon SRSF1-depletion. Tubulin is used as a loading control. (C) RT-PCR using indicated primers from control or SRSF1- or PRP6-siRNA treated HeLa cells stably expressing RFP-SRSF2. Note that the SRSF1-depleted cells do not show changes in the RFP or endogenous SRSF2 mRNA levels.

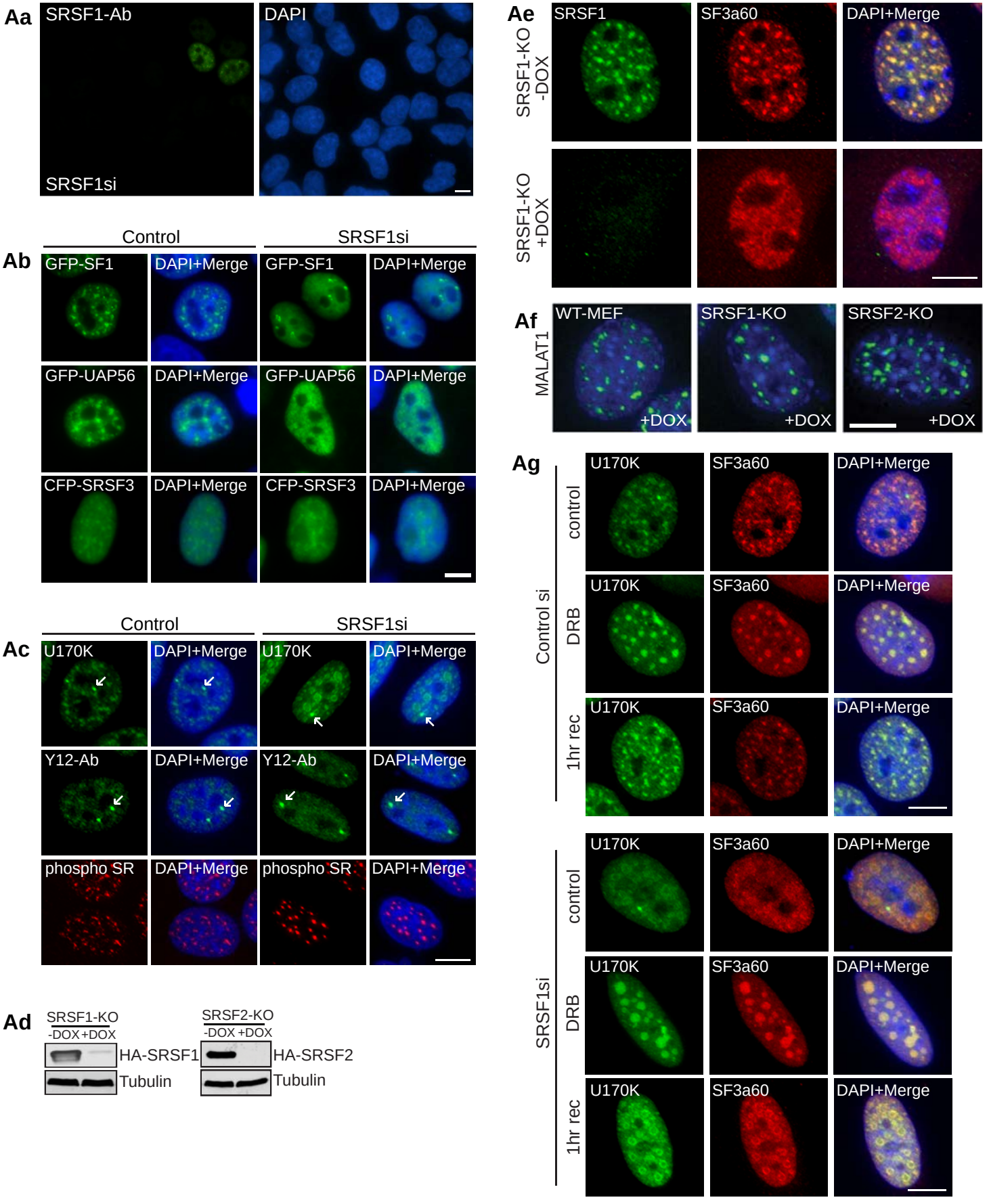
Figure S3Aa-b. Immunolocalization of the endogenous SON (a-a"') and RNA pol II (H5 ab, b-b"'), in CLTon cells, which were transfected with CFP-LacI-SRSF1. Note that SON and RNA pol II do not localize to the *de novo* formed speckles. S3Ac-e. Localization of YFP-SRSF2 in CLTon cells that were transfected with CFP-lacI-SRSF1 (c), CFP-lacI-SRSF1-ΔRS (d) and CFP-LacI-SRSF1-ΔRRM1 constructs. Scale bars, 5 μm.

Figure S3B. Immobilization of SRSF2 on chromatin leads to association with nuclear speckle or *de novo* formation of nuclear speckle. (A) CLTon cells are co-transfected with either YFP-LacI vector (a') or YFP-LacI-SRSF2 (b',c',d' & e') or CFP-LacI-SRSF2 (f') and CFP-SRSF1 (a", b"), -SRSF3 (c"), -U170K (d"), -UAP56 (e") and YFP-SON (f"). Note that the CFP-LacI-SRSF2 fail to recruit YFP-SON to the *de novo* formed speckles. RNA FISH shows the nuclear distribution of endogenous MALAT1 (g", h"), U2snRNA (i") and poly A+ RNA (j") in CLTon cells that are transfected with either YFP-LacI vector (g') or YFP-LacI-SRSF2 (i',j') or CFP-LacI-SRSF2 (h'). Immunolocalization of the RNA pol II (H14 antibody; k") and YFP-Cdk9 (l") in CLTon cells, which are transfected with CFP-LacI-SRSF2 (k', l'). Note that YFP-SON and RNA pol II do not associate to the *de novo* formed speckles. Figures a, b, c, d, e, f, g, h, i, j, k & l represent the stably integrated LacI locus (blue) in the CLTon cells. Scale bars, 5 μm.

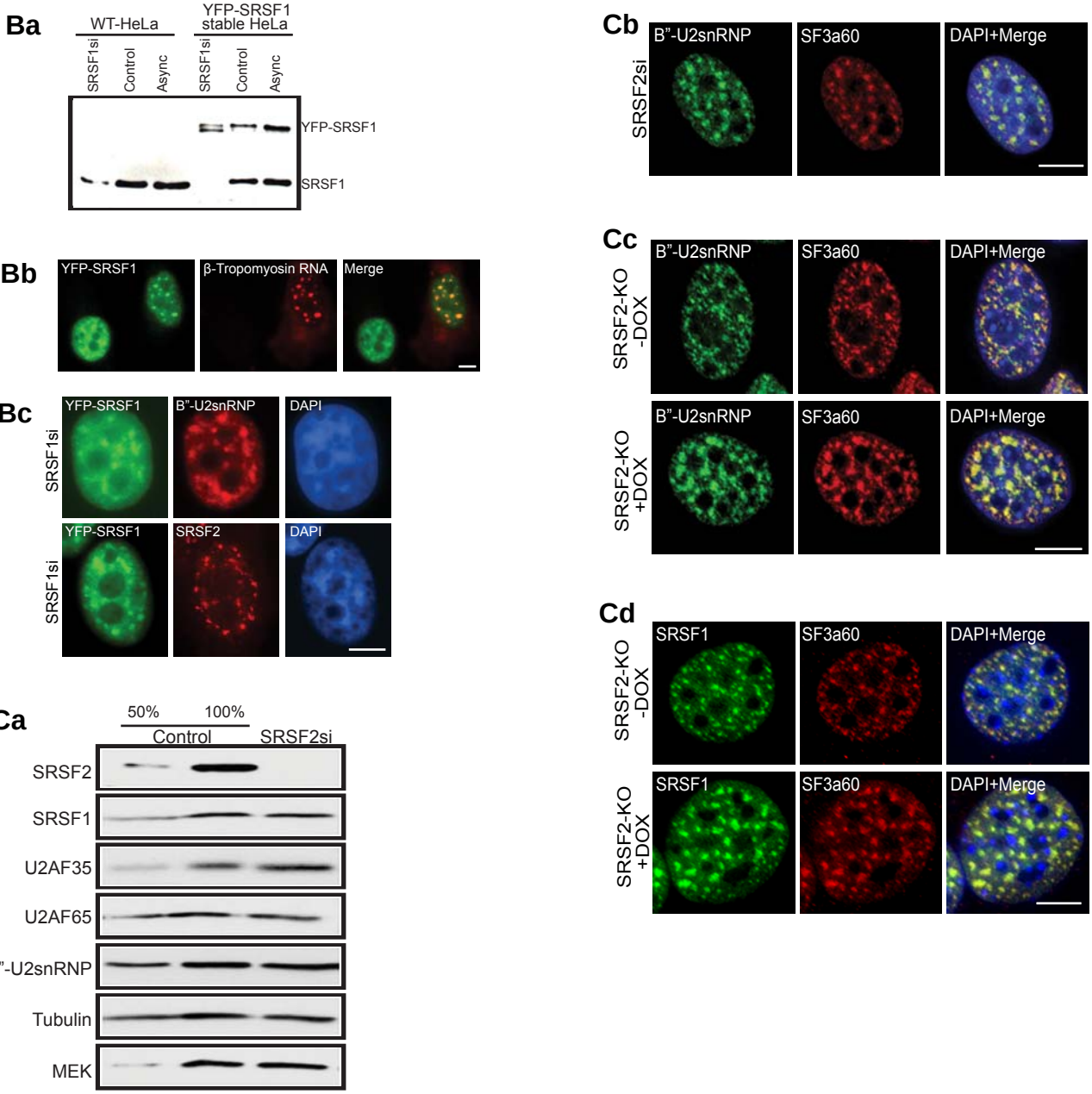
Figure S3C. Immobilization of SRSF2 on chromatin leads to association with nuclear speckle or *de novo* formation of nuclear speckle. Immunolocalization of the endogenous SON (a"), SRSF1 (b"), Cdk9 (c") and RNA pol II (elongation competent form, H5 ab, d") in CLTon cells, which are transfected with CFP-LacI-SRSF2 (a', b', c', d'). (D) CLTon cells are co-transfected with CFP or YFP-LacI vectors and indicated fluorescent-tagged pre-mRNA processing factors. (E) Immunolocalization of RNA pol II, SRSF1, Cdk9, SON or RNA FISH with MALAT1 or U2 snRNA or YFP-Cdk9 localization in CLTon cells that are transfected with CFP or YFP-LacI vectors. Note that the LacI vector

alone does not recruit pre-mRNA processing factors or ncRNA to the chromatin locus. The DNA is counterstained with DAPI. Scale bar represents 5 μm.

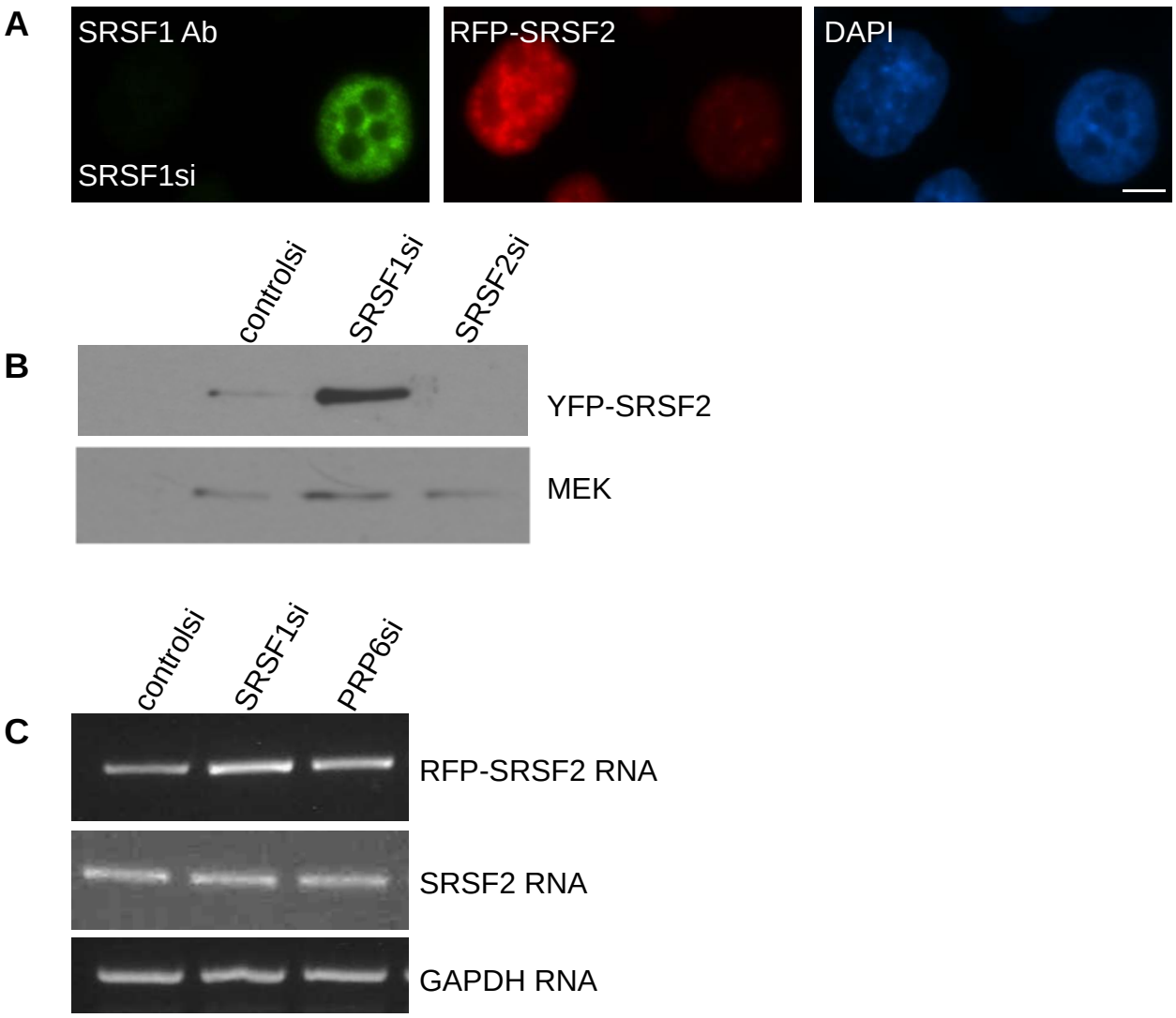
Figure S4. SRSF1 depletion prevents transcription-induced chromatin decondensation of the reporter gene locus. LacI-mCherry localized gene locus (red) in the control and SRSF1-depleted CLTon cells. The DNA is counterstained with DAPI. Scale bars, 5 μm.



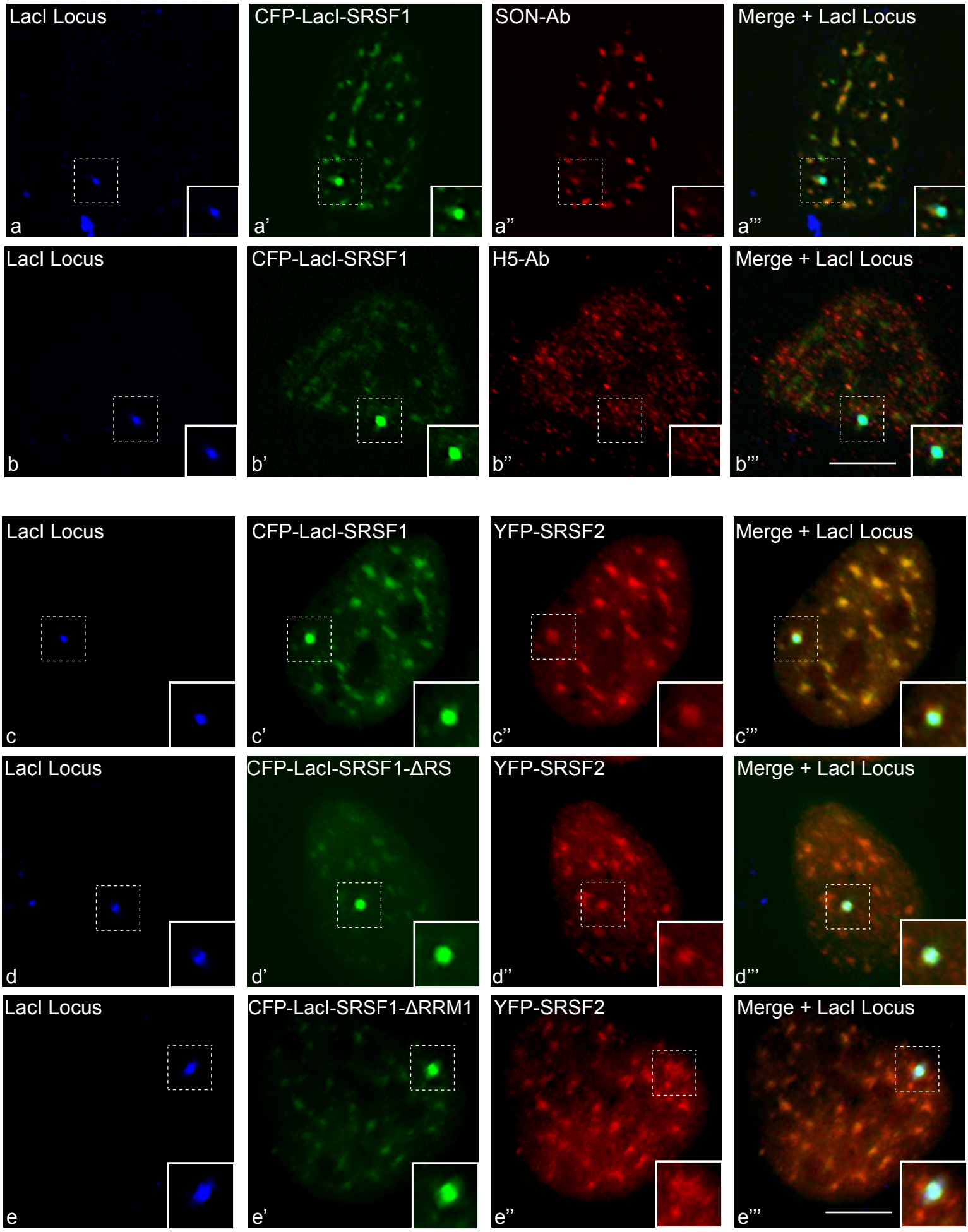
Tripathi et al., Fig: S1A



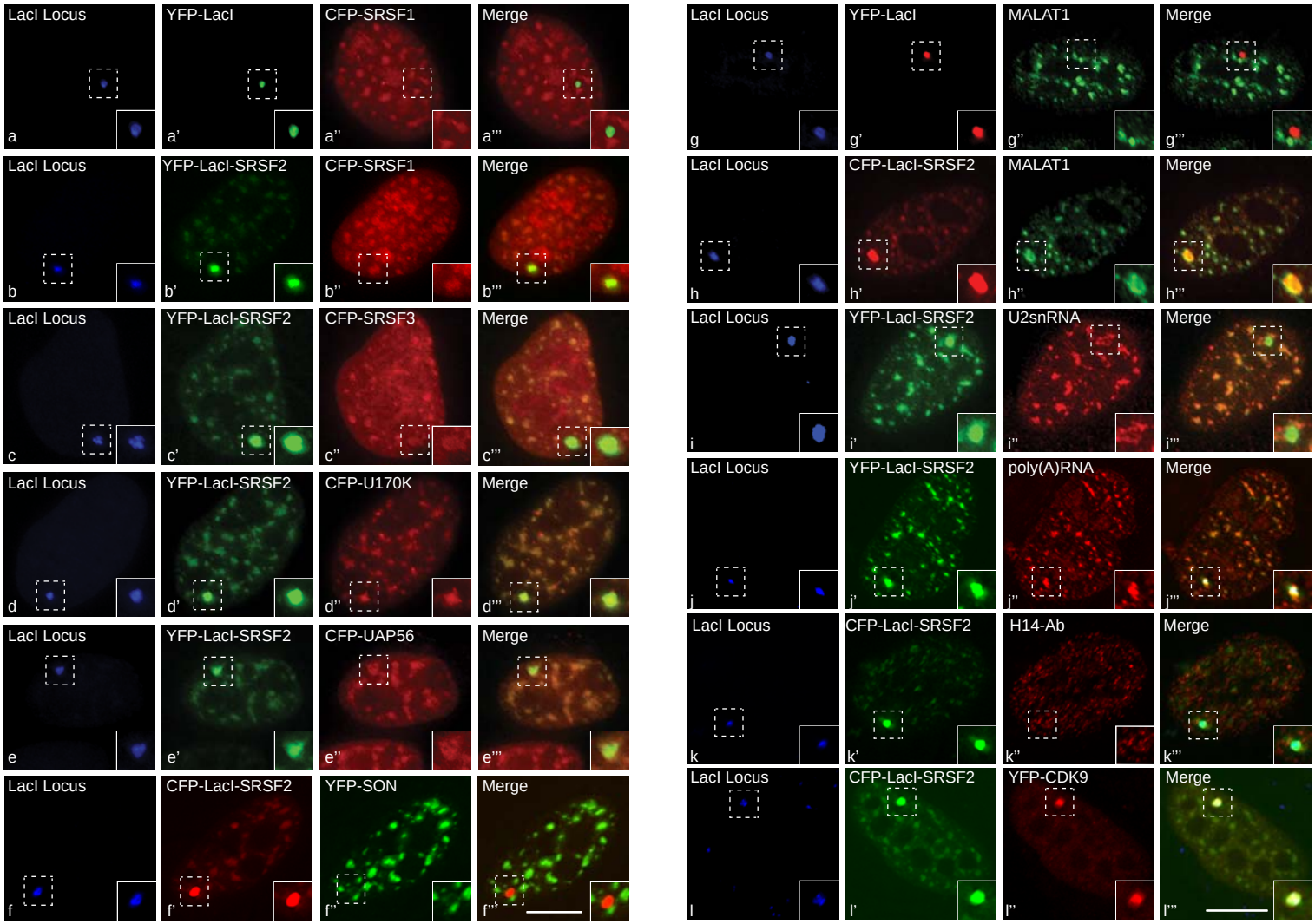
Tripathi et al., Fig: S1B & S1C



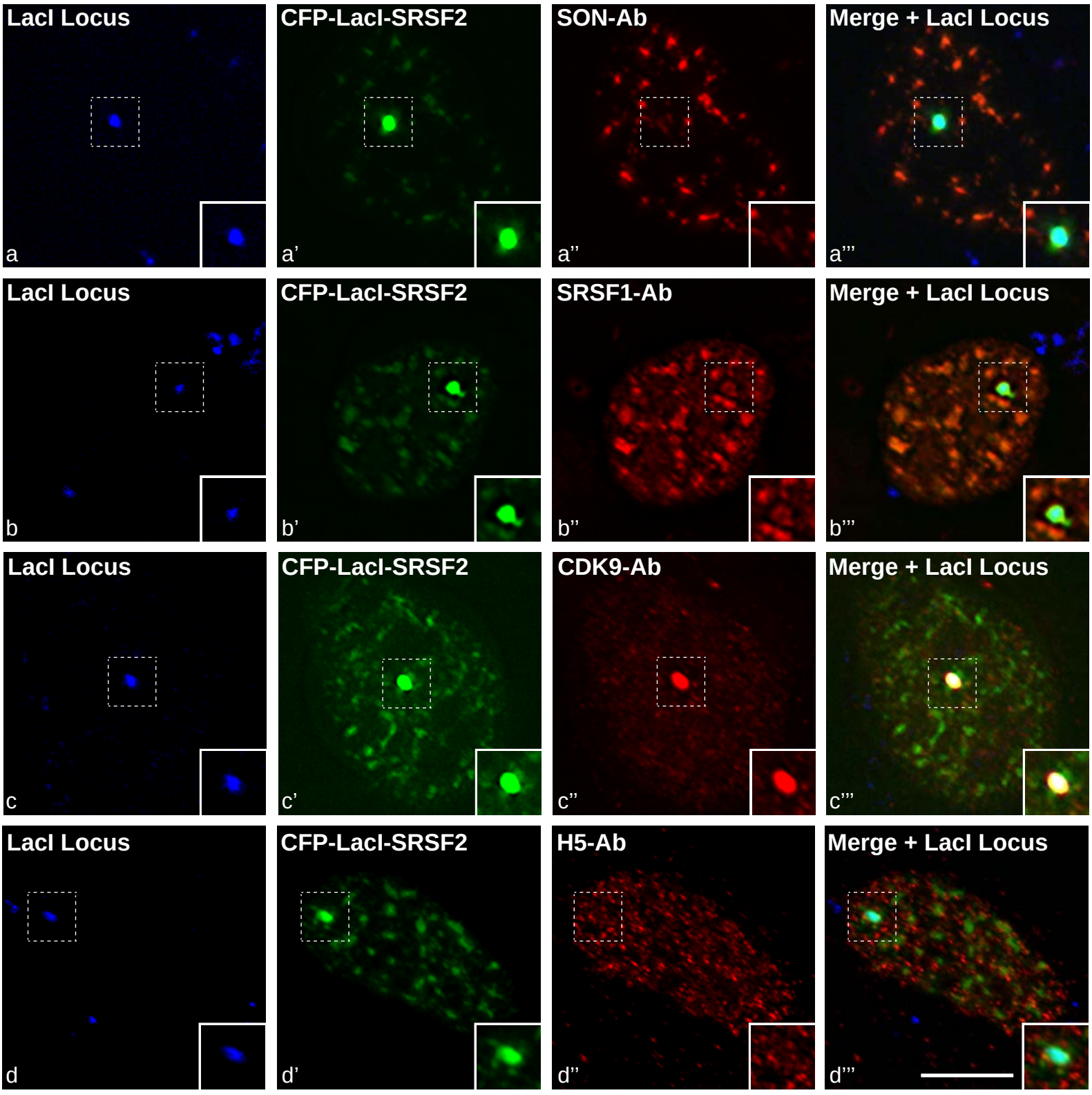
Tripathi et al., Fig: S2



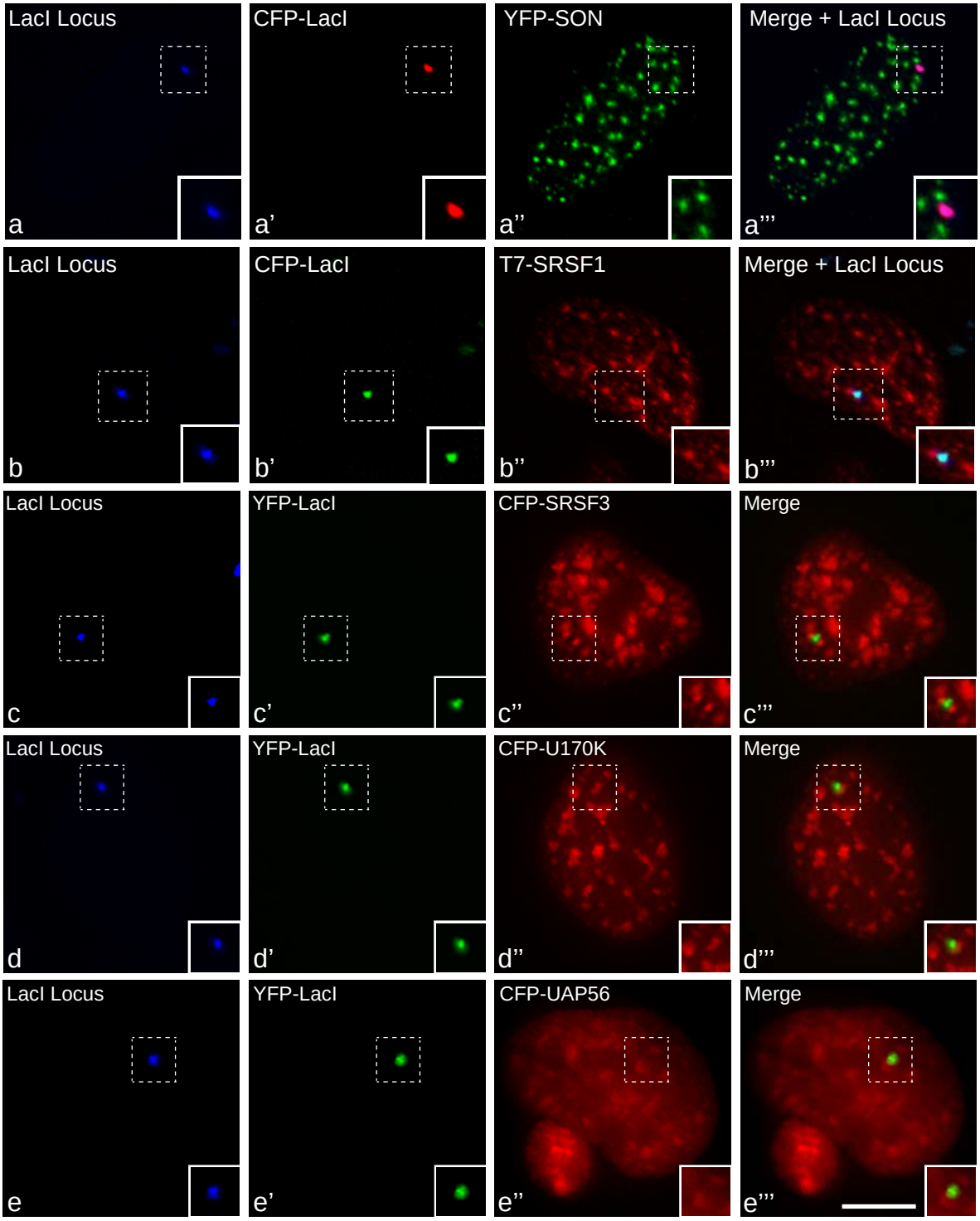
Tripathi et al., Fig: S3A



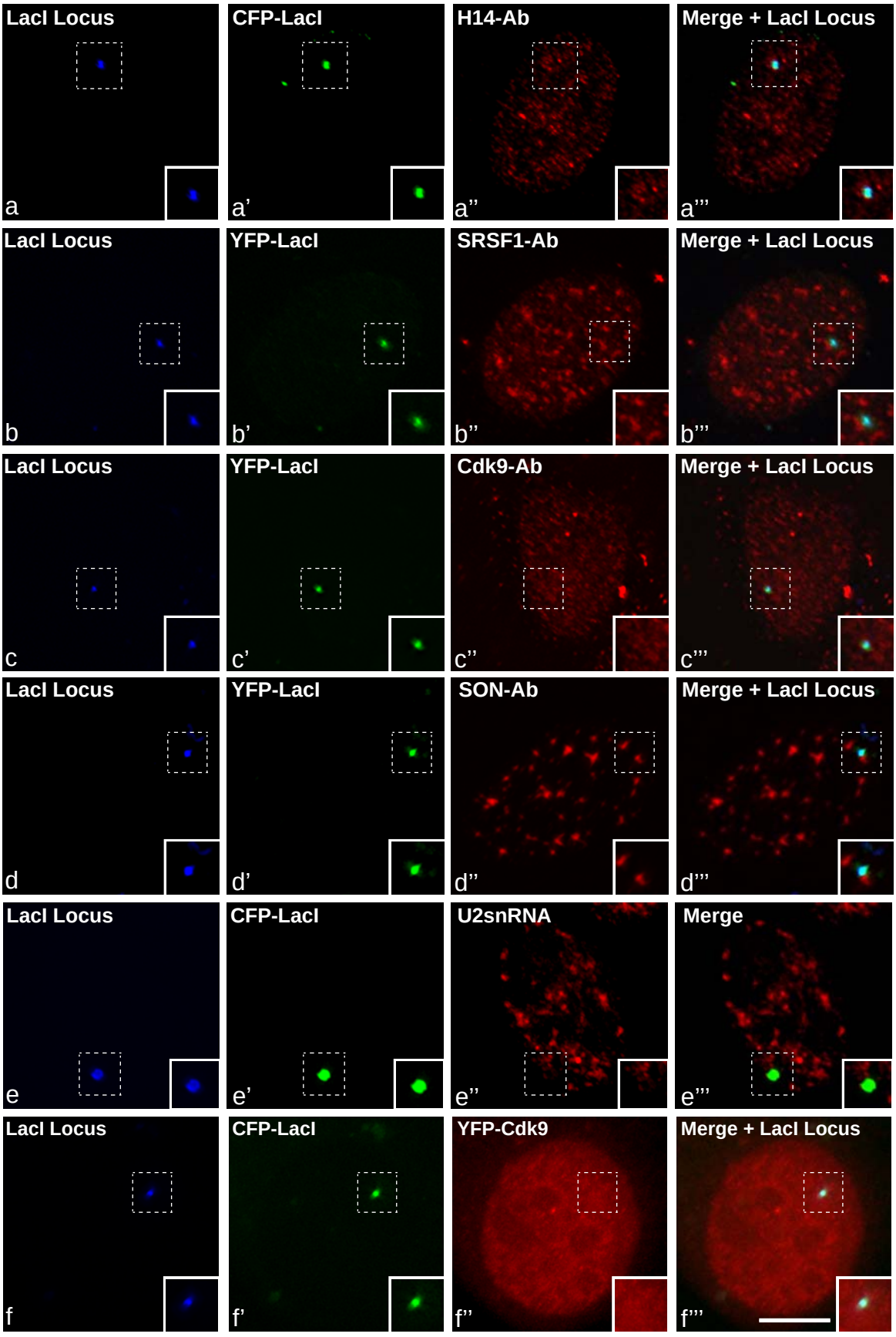
Tripathi et al., Fig: S3B



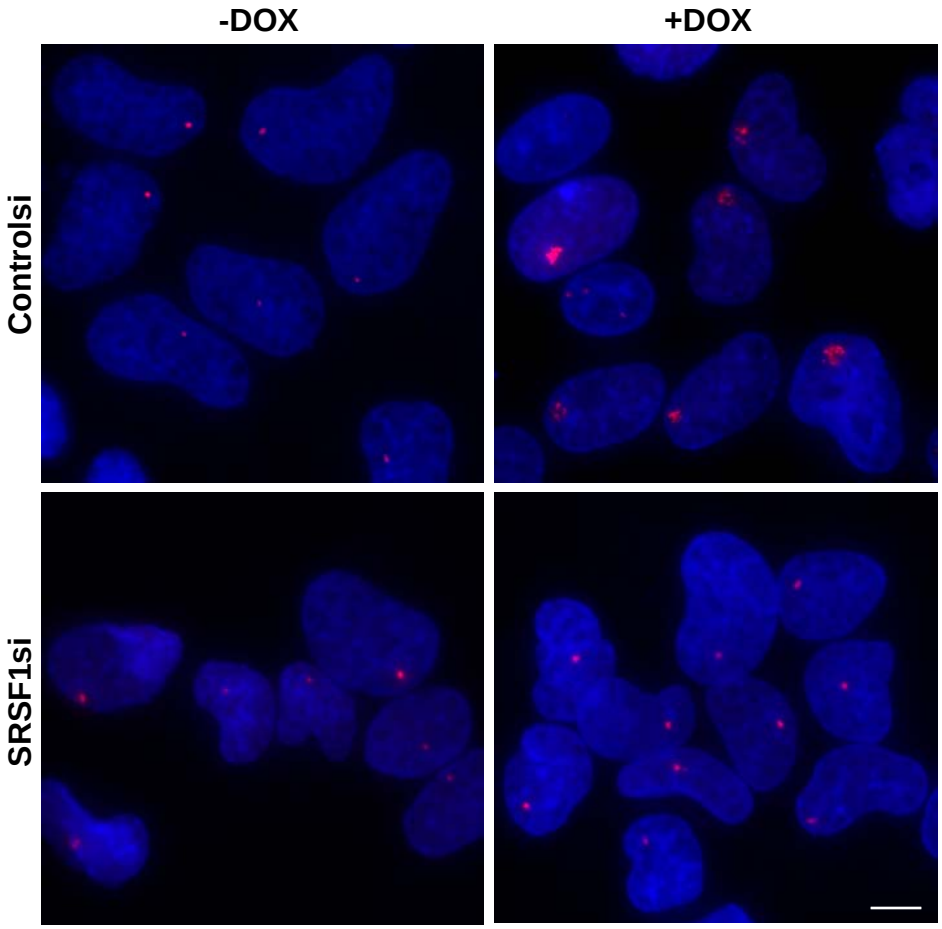
Tripathi et al., Fig: S3C



Tripathi et al., Fig: S3D



Tripathi et al., Fig: S3E



Tripathi et al., Fig: S4