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Title	Structural, super-resolution microscopy analysis of paraspeckle nuclear body organization
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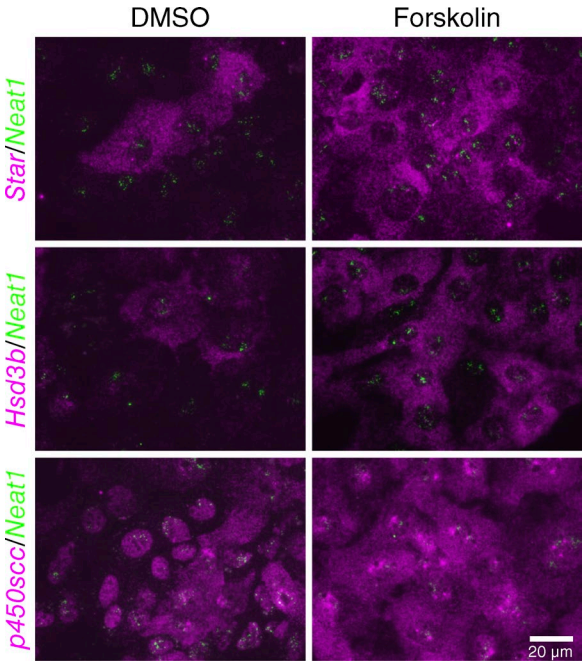

Figure S1. **Marker expression in primary cultures of corpus luteal cells.** FISH detection of *Neat1* and markers for corpus luteal cells, including *Star*, *Hsb3b*, and *p450scc*, in the absence (DMSO) and presence of 10 µg/ml forskolin. Note that the primary culture cells express all three luteal markers upon addition of forskolin. Bar, 20 µm.

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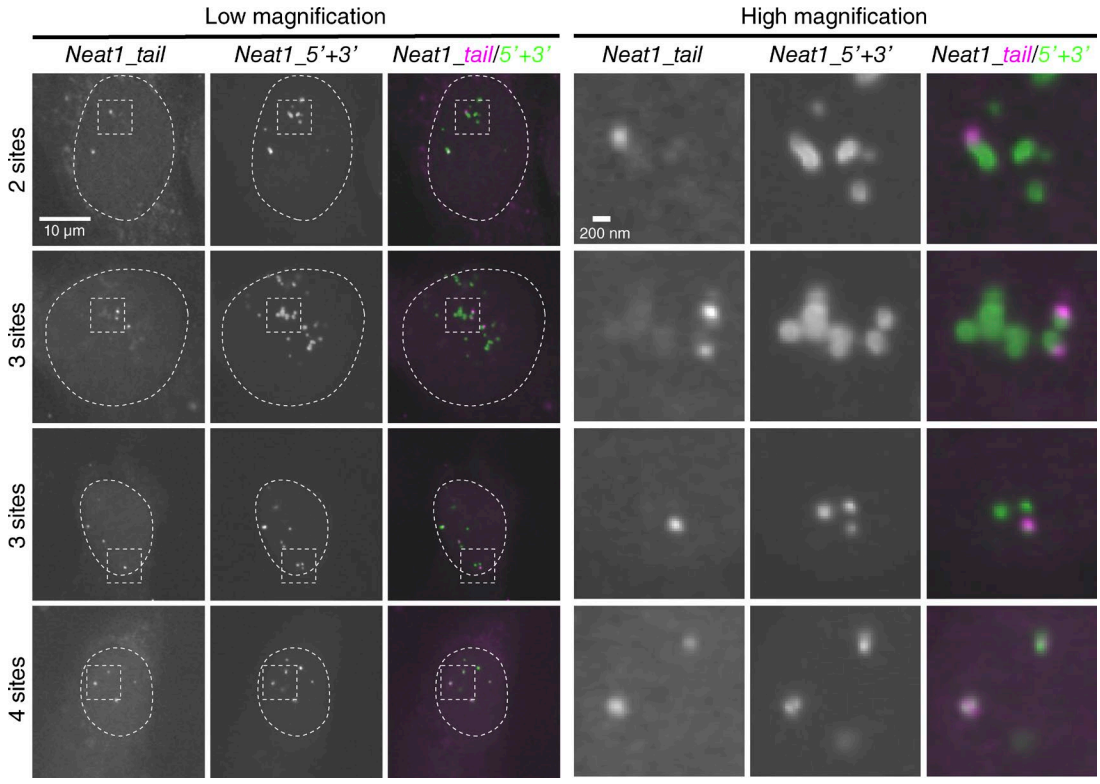

Figure S2. **Neat1 tail probe detects putative transcription sites.** Simultaneous FISH detection of the tail and the 5′/3′ regions of *Neat1*. The tail probe typically detected two to four dots per single nucleus, all of which neighbored the paraspeckles revealed by the probes that detect the 5′ and the 3′ region of *Neat1i*. Dotted curved lines indicate the position of the nuclei, and dotted boxes in the low-magnification panels indicate the region enlarged in the high-magnification panels.

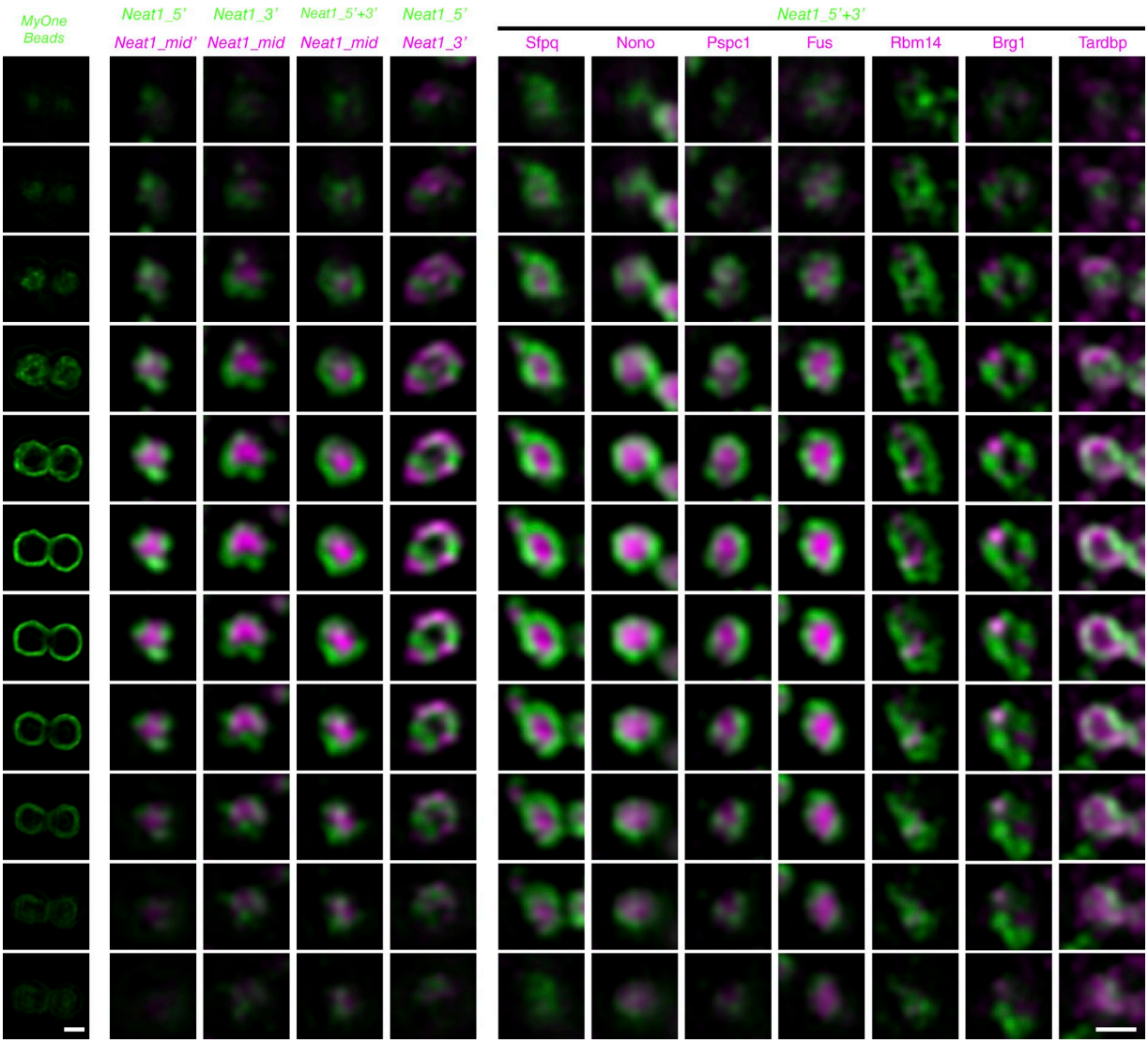

Figure S3. **Series of optical sections of paraspeckles.** Series of optical z-sections of paraspeckles obtained with SIM at the interval of 100 nm (MyOne beads; Thermo Fisher Scientific). Probes and antibodies that detect specific regions of *Neat1* and paraspeckle proteins are shown in the top boxes. To prepare model spheroidal structure, Dynabeads MyOne Streptavidin beads (Thermo Fisher Scientific) were coated with RNA probes that were double-labeled with biotin and FITC, which were subsequently detected with rabbit polyclonal anti-FITC and Cy2-labeled anti-rabbit secondary antibodies. Note that the signals on the transverse Z section located at the center are rather enhanced compared with the signals on the section tangential to the surface of the spheroid. Bars, 500 nm.

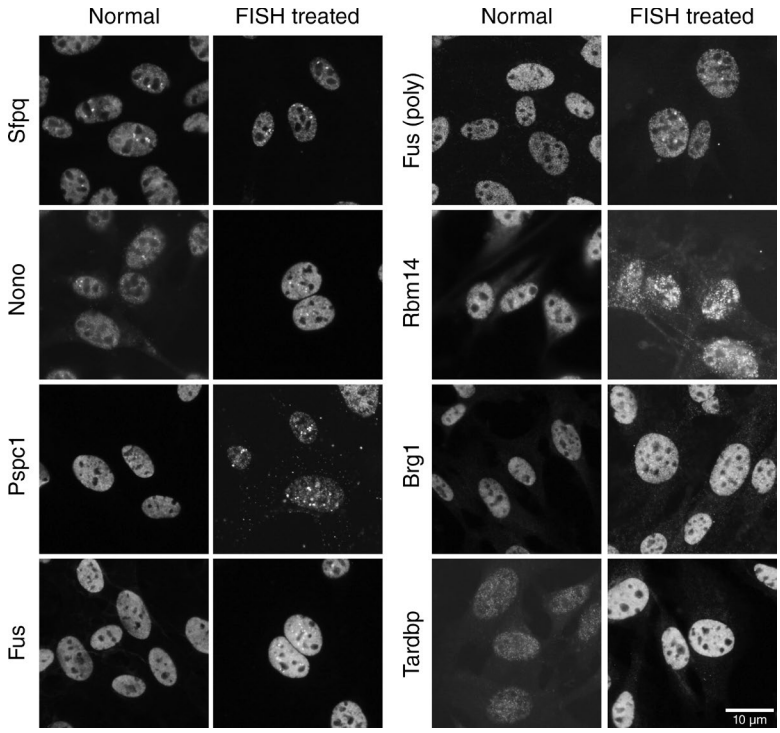

Figure S4. **FISH treatment is compatible with immunohistochemical detection of paraspeckle proteins.** Immunostaining of paraspeckles proteins in FISH-treated cells and control cells permeabilized with 0.5% Triton X-100. Note that the signals were not decreased, but rather enhanced, after the FISH treatment when stained with antibodies against Sfpq, Nono, Fus, Brg1, and Tardbp. In the case of Pspc1 and Rbm14, nucleoplasmic signals became dimmer, whereas the paraspeckle signals were unchanged or became even brighter after the FISH treatment. Bar, 10 µm.

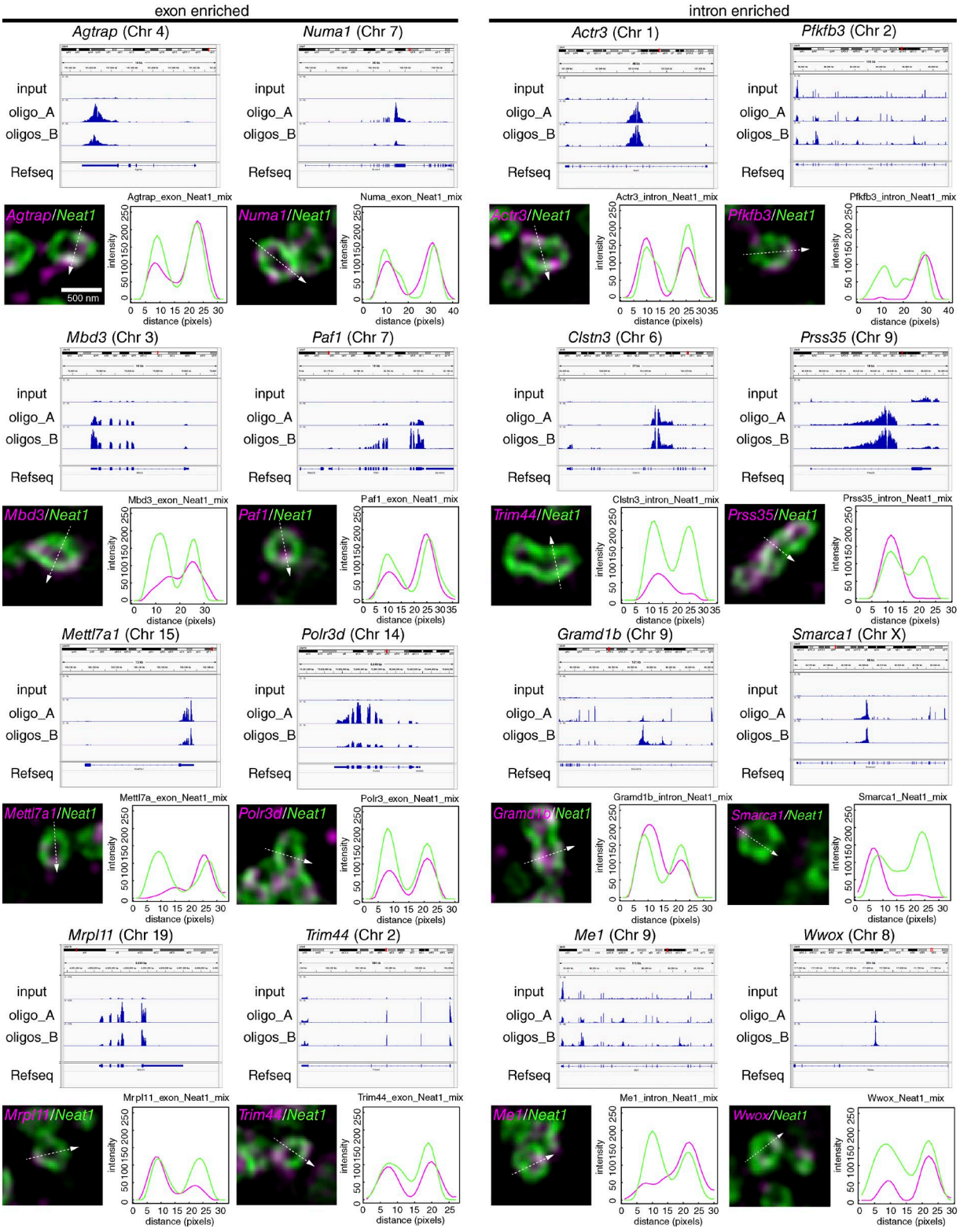

Figure S5. **Mapping of RNA sequencing reads and the shell distribution of CHART-enriched AG-rich RNAs.** Mapping of CHART-enriched RNAseq reads at miscellaneous genes. Note that the CHART reads are enriched at exons (left) or introns (right) of particular genes. Images show simultaneous detection of AG-rich RNAs and *Neat1* in corpus luteal cells using SIM. Intensity profiles along the dashed line are shown in the graphs adjacent to the images. Note that all exon-enriched (left) and intron-enriched (right) RNAs are located at the paraspeckle shells detected with the *Neat1_5′+3′* probe. Bar, 500 nm.

Provided online are three Excel tables. Table S1 is a list of the number of CHART RNAseq reads mapped to introns of Refseq genes. Table S2 is a list of the number of CHART RNAseq reads mapped to mRNAs of Refseq genes. Table S3 is a list of primers and oligonucleotides used in this study.