Expanded View Figures

Figure EV1. Characterization of NEAT1 mutants with deletions in the NEAT1_2 3′ terminal regions.

A (left) SRM images of the paraspeckles in HAP1 WT cells detected by NEAT1_5′ (green) and NEAT1_3′ (magenta) FISH probes with MG132 treatment (5 μM for 6 h). Scale bar, 500 nm. (right) Graph showing the proportion of paraspeckles with localization of the NEAT1 3′ ends to the core and shell or the shell in WT cells ($n = 167$).

B Graph showing the proportion of paraspeckles with localization of the NEAT1 5′ ends to the core and shell or the shell in WT, Δ3′, Δ16.6–20.2 kb, and Δ20.2–22.6 kb cells treated with MG132 (5 μM for 6 h; WT: $n = 115$, Δ3′: $n = 21$, Δ16.6–20.2 kb: $n = 161$, and Δ20.2–22.6 kb: $n = 89$).

C (left) Detection of NEAT1_2 by single-molecule FISH (smFISH; magenta) in HAP1 WT, Δ3′, Δ16.6–20.2 kb, and Δ20.2–22.6 kb cells treated with MG132 (5 μM for 6 h). Nuclei were stained with DAPI. Scale bar, 10 nm. (right) Quantitation of area and sum intensity per paraspeckle in each cell line (WT: $n = 407$, Δ3′: $n = 314$, Δ16.6–20.2 kb: $n = 302$, and Δ20.2–22.6 kb: $n = 609$). (***$P = 0.0001$, ****$P < 0.0001$, compared with WT: Kruskal–Wallis test with Dunn's multiple comparison test). Each box plot shows the median (inside line), 25–75 percentiles (box bottom to top), and 10–90 percentiles (whisker bottom to top).

D (left) EM observations of the paraspeckles in MG132-treated (5 μM for 17 h) HAP1 Δ3′ cells using NEAT1_5′ probe. Scale bar, 100 nm. (middle) Graph showing the proportion of the localization of NEAT1_5′ probes (838 gold particles) within the paraspeckles in Δ3′ cells. (right) Graph showing the proportion of localization of NEAT1_5′ probes in each paraspeckle in Δ3′ cells ($n = 21$). The box plot shows the median (inside line), 25–75 percentiles (box bottom to top), and 10–90 percentiles (whisker bottom to top).

E (left) EM observation of the paraspeckles in MG132-treated (5 μM for 17 h) HAP1 WT cells using NEAT1_5′ probe. Scale bar, 100 nm. (middle) Graph showing the proportion of localization of NEAT1_5′ probes (601 gold particles) within the paraspeckles in WT cells. (right) Graph showing the proportion of localization of NEAT1_5′ probes in each paraspeckle in WT cells ($n = 16$). The box plot shows the median (inside line), 25–75 percentiles (box bottom to top), and 10–90 percentiles (whisker bottom to top).

F (left) EM observation of the paraspeckles in MG132-treated (5 μM for 17 h) HAP1 WT cells using the NEAT1_D2 probe. Scale bar, 100 nm. (middle) Graph showing the proportion of localization of NEAT1_D2 probes (461 gold particles) within the paraspeckles in WT cells. (right) Graph showing the proportion of localization of NEAT1_D2 probes in each paraspeckle in WT cells ($n = 24$). The box plot shows the median (inside line), 25–75 percentiles (box bottom to top), and 10–90 percentiles (whisker bottom to top).

G Quantitation of the relative expression levels of NEAT1_1 and NEAT1_2 by RT–qPCR in HAP1 WT, Δ16.6–20.2 kb, and Δ20.2–22.6 kb cells treated with MG132 (5 μM for 6 h). Data are represented as mean ± SD ($n = 3$).

H SRM images of the paraspeckles in MG132-treated (5 μM for 6 h) WT cells detected by NEAT1_5′ (green) and NEAT1_19k (magenta) FISH probes. Scale bar, 500 nm.

I Graph showing the proportion of paraspeckles with localization of the NEAT1_19k probes to the core and shell or the core in WT cells treated with MG132 (5 μM for 6 h; $n = 103$).

Source data are available online for this figure.

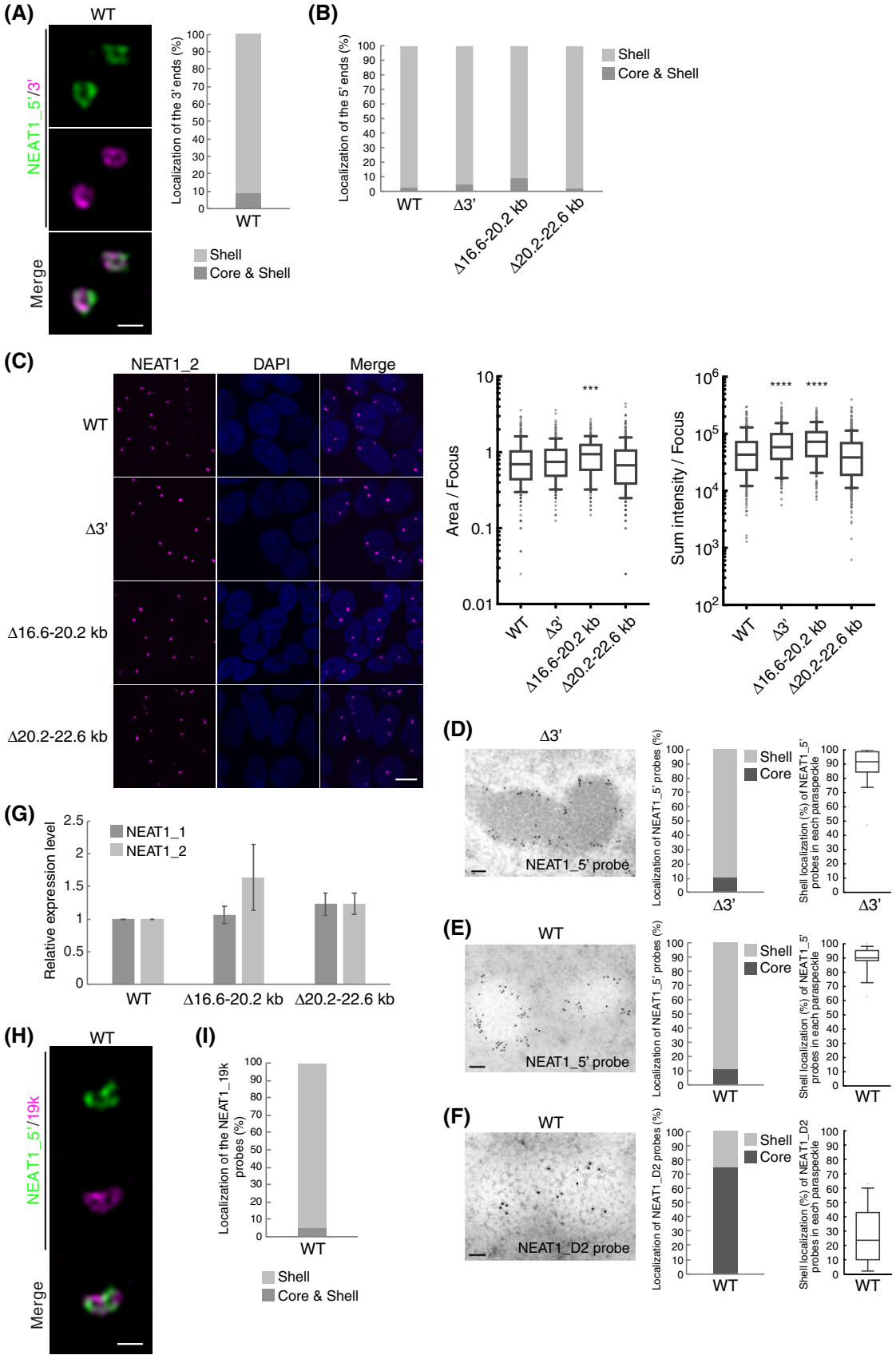
(A)
WT
NEAT1_5'/3'
Merge
Localization of the 3' ends (%)
WT
Shell
Core & Shell
(B)
Localization of the 5' ends (%)
Shell
Core & Shell
WT
Δ3'
Δ16.6-20.2 kb
Δ20.2-22.6 kb
(C)
NEAT1_2
DAPI
Merge
WT
Δ3'
Δ16.6-20.2 kb
Δ20.2-22.6 kb
Area / Focus
Sum intensity / Focus
***
****
****
(D)
Δ3'
NEAT1_5' probe
Localization of NEAT1_5' probes (%)
Shell
Core
Shell localization (%) of NEAT1_5' probes in each paraspeckle
(E)
WT
NEAT1_5' probe
(F)
WT
NEAT1_D2 probe
Localization of NEAT1_D2 probes (%)
Shell localization (%) of NEAT1_D2 probes in each paraspeckle
(G)
Relative expression level
NEAT1_1
NEAT1_2
WT
Δ16.6-20.2 kb
Δ20.2-22.6 kb
(H)
WT
NEAT1_5'/19k
Merge
(I)
Localization of the NEAT1_19k probes (%)
WT
Shell
Core & Shell

Figure EV1.

Figure EV2. Characterization of NEAT1 mutants with deletions in the NEAT1_2 5′ terminal regions.

A Quantitation of the relative expression levels of NEAT1_1 and NEAT1_2 by RT–qPCR in HAP1 WT, Δ5′, and Δ5′/ΔPAS cells treated with MG132 (5 μM for 6 h). Data are represented as mean ± SD ($n = 3$).

B (upper) Detection of NEAT1_2 by smFISH (magenta) in WT, Δ0–0.8 kb, Δ5′, and Δ5′/ΔPAS kb cells treated with MG132 (5 μM for 6 h). Nuclei were stained with DAPI. Scale bar, 10 nm. (lower) Quantitation of area and sum intensity per paraspeckle in each cell line (WT: $n = 839$, Δ0–0.8 kb: $n = 845$, Δ5′: $n = 535$, and Δ5′/ΔPAS: $n = 652$). (*$P = 0.0420$, ****$P < 0.0001$, compared with WT: Kruskal–Wallis test with Dunn's multiple comparison test). Each box plot shows the median (inside line), 25–75 percentiles (box bottom to top), and 10–90 percentiles (whisker bottom to top).

C Graph showing the proportion of paraspeckles with localization of the NEAT1 3′ ends to the core and shell or the shell in WT, Δ0–0.8 kb, and Δ5′ cells treated with MG132 (5 μM for 6 h) by SRM analyses (WT: $n = 167$, Δ0–0.8 kb: $n = 77$, Δ5′: $n = 48$).

D (upper panels) EM observations of the paraspeckles in MG132-treated (5 μM for 17 h) WT and Δ5′ cells using the NEAT1_D2 probe. Scale bar, 100 nm. (bottom, left) Graph showing the proportion of localization (three layers: outer, middle, and inner layers [black circles indicate the boundaries]) of NEAT1_D2 probes (WT: 307 gold particles, Δ5′: 167 gold particles) within the paraspeckles in HAP1 Δ5′ cells. (bottom, right) Graph showing the proportion of localization of NEAT1_D2 probes in each paraspeckle in WT ($n = 22$) and Δ5′ cells ($n = 15$). (***$P = 0.0001$, ****$P < 0.0001$, compared with WT: Mann–Whitney test (two-tailed)). Each box plot shows the median (inside line), 25–75 percentiles (box bottom to top), and 10–90 percentiles (whisker bottom to top).

E Quantitation of the relative expression levels of NEAT1_1 and NEAT1_2 by RT–qPCR in HAP1 WT and Δ0–2.8 kb cells. Data are represented as mean ± SD ($n = 3$).

F Detection of the paraspeckles by smFISH in WT and Δ0–2.8 kb cells. Scale bar, 10 nm.

G (left) The paraspeckles in Δ5′/ΔPAS cells treated with MG132 treatment (5 μM for 6 h) detected with SRM by NEAT1_2k (green) and 3′ (magenta) FISH probes. Scale bar, 500 nm. (right) Graph showing the proportion of paraspeckles with localization of the NEAT1 5′ ends to the core and shell or the shell in Δ5′/ΔPAS cells ($n = 31$).

Source data are available online for this figure.

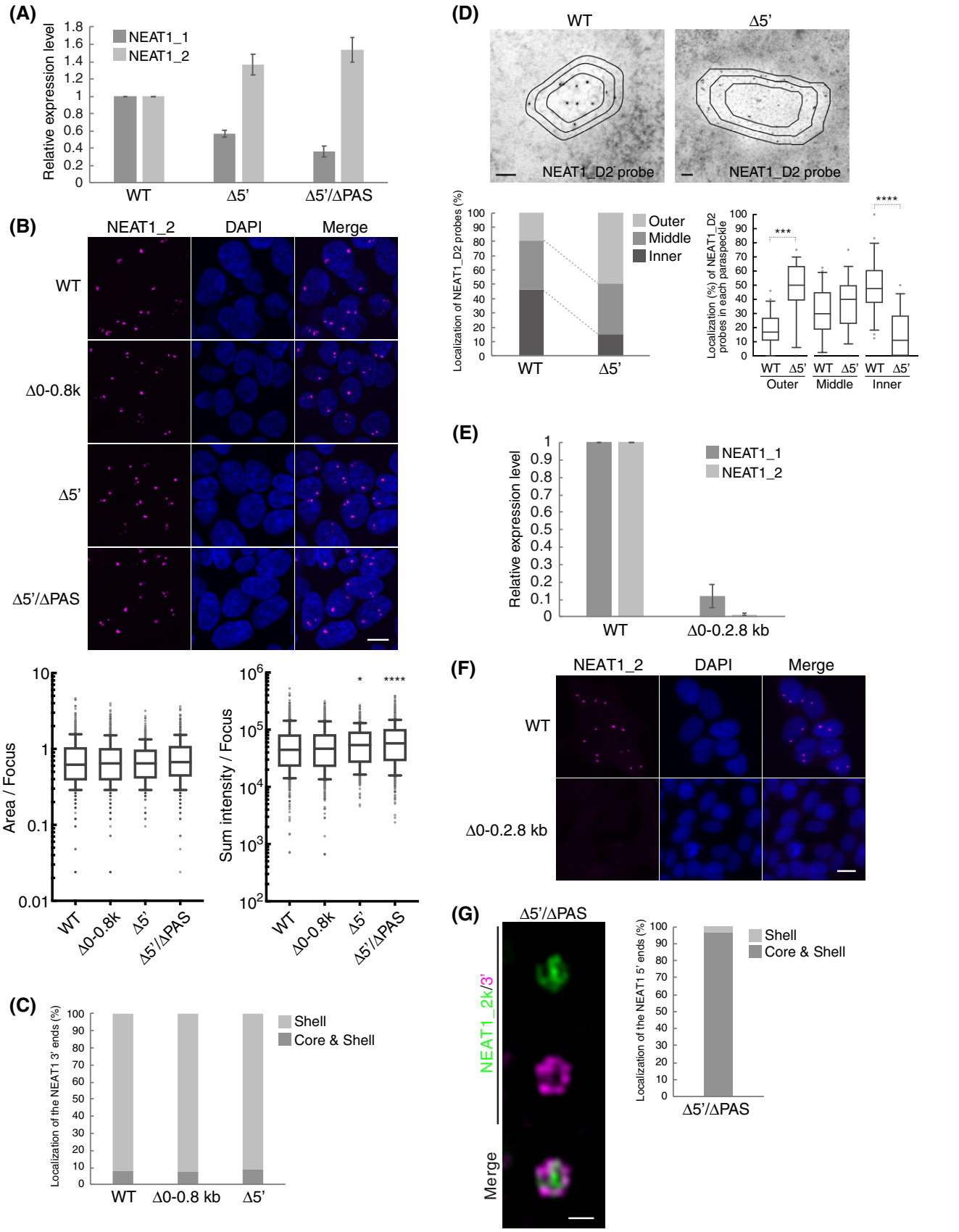
(A)
Relative expression level
NEAT1_1
NEAT1_2
WT
Δ5'
Δ5'/ΔPAS
(B)
NEAT1_2
DAPI
Merge
WT
Δ0-0.8k
Δ5'
Δ5'/ΔPAS
Area / Focus
Sum intensity / Focus
(C)
Localization of the NEAT1 3' ends (%)
Shell
Core & Shell
WT
Δ0-0.8 kb
Δ5'
(D)
WT
Δ5'
NEAT1_D2 probe
Localization of NEAT1_D2 probes (%)
Outer
Middle
Inner
Localization (%) of NEAT1_D2 probes in each paraspeckle
Outer
Middle
Inner
(E)
Relative expression level
NEAT1_1
NEAT1_2
WT
Δ0-0.2.8 kb
(F)
NEAT1_2
DAPI
Merge
WT
Δ0-0.2.8 kb
(G)
Δ5'/ΔPAS
NEAT1_2k/3'
Merge
Localization of the NEAT1 5' ends (%)
Shell
Core & Shell
Δ5'/ΔPAS

Figure EV2.

Figure EV3. Characterization of NEAT1 mutants with deletions in both the NEAT1_2 5′ and 3′ regions.

A Quantitation of the relative expression levels of NEAT1_1 and NEAT1_2 by RT–qPCR in HAP1 WT, Δ5′/Δ3′, and Δ5′/Δ3′/ΔPAS cells treated with MG132 (5 μM for 6 h). Data are represented as mean ± SD ($n = 3$).

B (left) Detection of NEAT1_2 by smFISH (magenta) in WT, Δ5′/Δ3′, and Δ5′/Δ3′/ΔPAS cells treated with MG132 (5 μM for 6 h). Nuclei were stained with DAPI. Scale bar, 10 nm. (right) Quantitation of area and sum intensity per paraspeckle in each cell line (WT: $n = 744$, Δ5′/Δ3′: $n = 425$, and Δ5′/Δ3′/ΔPAS: $n = 435$). (****$P < 0.0001$, compared with WT: Kruskal–Wallis test with Dunn's multiple comparison test). Each box plot shows the median (inside line), 25–75 percentiles (box bottom to top), and 10–90 percentiles (whisker bottom to top).

C The paraspeckles detected with SRM by NEAT1_2k FISH probe (green), and NEAT1_15k FISH probe or NONO IF (magenta) in the Δ5′/Δ3′/ΔPAS cells treated with MG132 (5 μM for 6 h). Scale bar, 500 nm.

D Graph showing proportion of paraspeckles with localization of the NEAT1 5′ (left, $n = 41$) and 3′ (right, $n = 14$) ends to the core and shell in Δ5′/Δ3′/ΔPAS cells treated with MG132 (5 μM for 6 h).

Source data are available online for this figure.

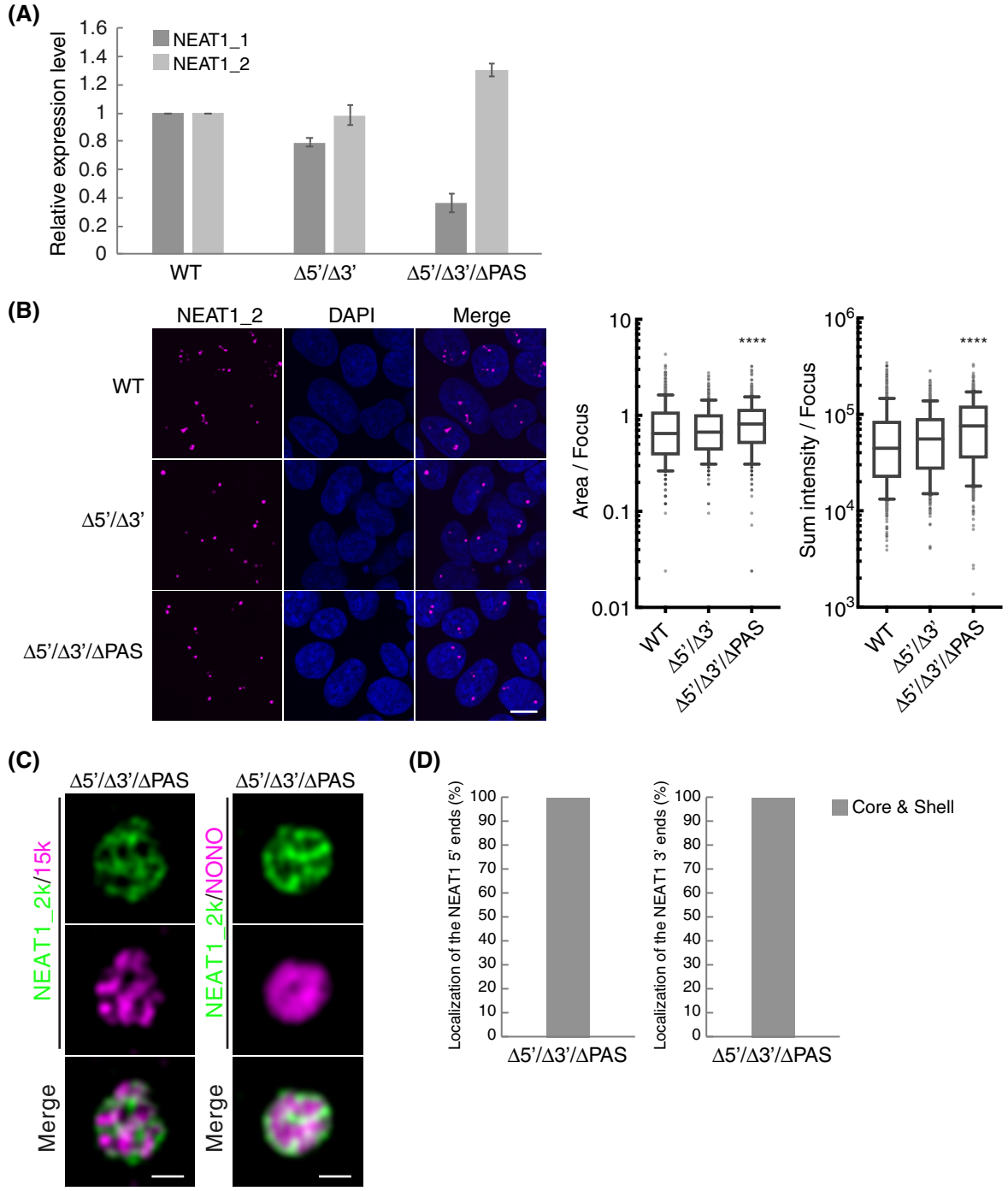

Figure EV3.

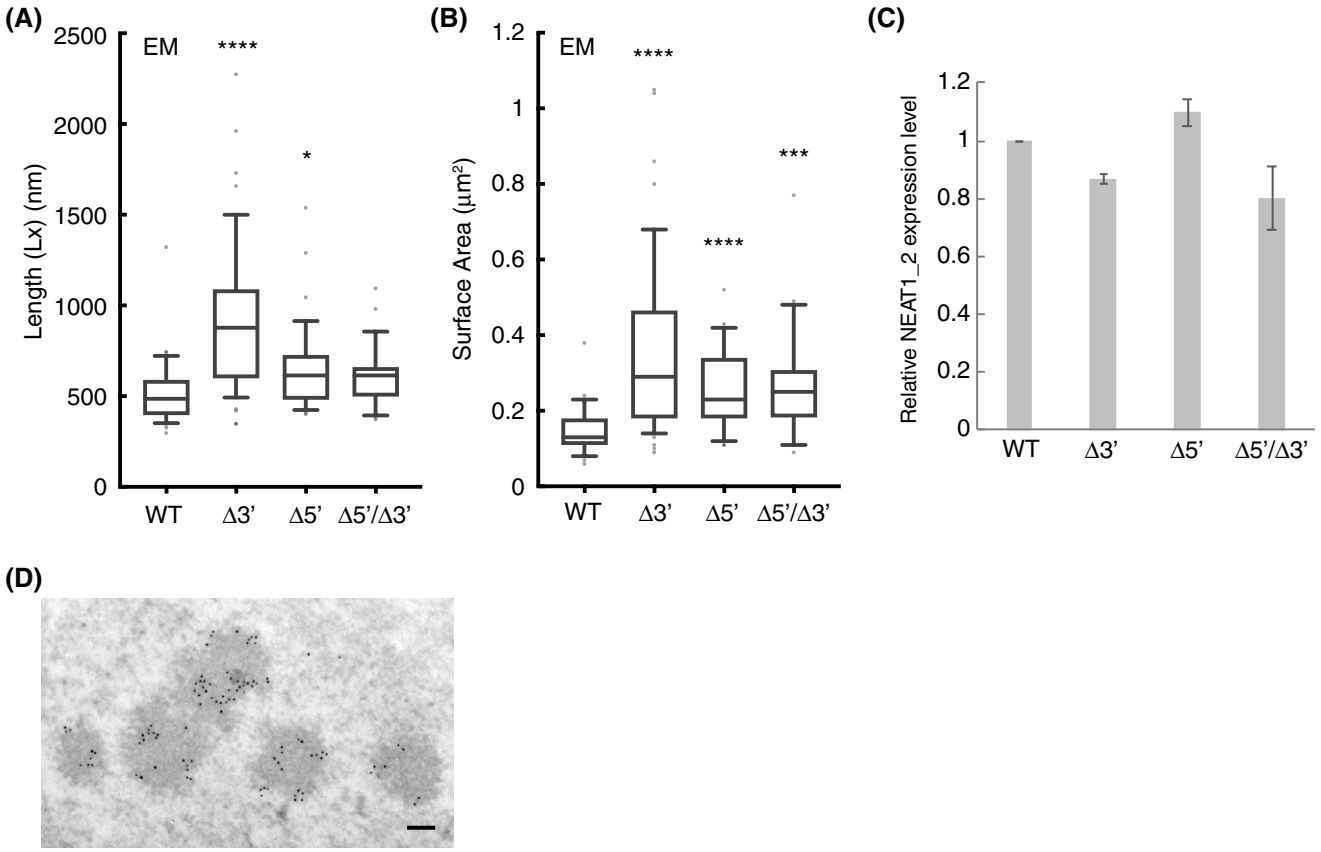

Figure EV4. Additional size and structure parameters determined by EM for the paraspeckles of NEAT1 mutants.

A Length (Lx) of the paraspeckles determined by EM in HAP1 WT, Δ3′, Δ5′, and Δ5′/Δ3′ cells treated with MG132 (5 μM for 17 h). WT (mean size: 518.6 nm, $n = 35$), Δ3′ (mean size: 937.1 nm, $n = 49$), Δ5′ (mean size: 648.1 nm, $n = 39$), and Δ5′/Δ3′ (mean size: 608.9 nm, $n = 28$). (*$P = 0.0451$, ****$P < 0.0001$, compared with WT). Each box plot shows the median (inside line), 25–75 percentiles (box bottom to top), and 10–90 percentiles (whisker bottom to top).

B Surface area of the paraspeckles determined by EM in WT, Δ3′, Δ5′, and Δ5′/Δ3′ cells treated with MG132 (5 μM for 17 h). WT (mean area: 0.1497 μm^2, $n = 35$), Δ3′ (mean area: 0.3684 μm^2, $n = 49$), Δ5′ (mean area: 0.2582 μm^2, $n = 39$), and Δ5′/Δ3′ (mean area: 0.2657 μm^2, $n = 28$). (***$P = 0.0008$, ****$P < 0.0001$, compared with WT). Each box plot shows the median (inside line), 25–75 percentiles (box bottom to top), and 10–90 percentiles (whisker bottom to top).

C An analysis of the NEAT1_2 expression levels for quantification of the NEAT1_2 number per paraspeckle. Data are represented as mean ± SD ($n = 3$).

D A representative image of a cluster of paraspeckles detected by paraspeckle marker proteins, BRG1 (gold particles) under EM in the presence of MG132. In this image, five paraspeckles form a cluster. Scale bar, 100 nm.

Figure EV5. Contributions of stretching free energy of B blocks in the core to the structure of paraspeckles.

A Energetic contributions of triblock copolymer micelle model of the paraspeckle considering elastic free energy of B blocks (see the Materials and Methods for details).

B Theoretical calculation of the fraction α of the A blocks in the shell versus the length of the A blocks (represented by the number of segments) of spherical paraspeckles in the steady state with (magenta) and without (black) the stretching free energy of B blocks in the core. The parameters used for the calculations are shown in the Materials and Methods (the broken curves are results for the excluded volume $v_A/b^3 = 0.5$ and $v_C/b^3 = 0.5$). These data are related to Fig 4D.

C Theoretical calculation of the radius of paraspeckles versus the length of the A blocks (represented by the number of segments) of spherical paraspeckles in the steady state with (magenta) and without (black) the stretching free energy of B blocks in the core. The radius was rescaled by the segment length. The parameters used for the calculations are shown in the Materials and Methods (the broken curves are the results for the excluded volume $v_A/b^3 = 0.5$ and $v_C/b^3 = 0.5$). These data are related to Fig 5A.

D Theoretical calculation of the number of NEAT1_2 transcripts versus the length of the A blocks (represented by the number of segments) of spherical paraspeckles in the steady state with (magenta) and without (black) the stretching free energy of B blocks in the core. The parameters used for the calculations are shown in the Materials and Methods (the broken curves are the results for the excluded volume $v_A/b^3 = 0.5$ and $v_C/b^3 = 0.5$). These data are related to Fig 5B.

E Theoretical calculation of the fraction of A (or C) blocks in the shell vs the logarithm of the transcription rate (rescaled by the rate at which the transcripts are spontaneously incorporated in the paraspeckle). The numbers of segments of A blocks were 5.0 (cyan), 7.5 (orange), 8.15785 (brown), and 8.86482 (black). The parameters used for the calculations are shown in the Materials and Methods (the broken curves are the results for the excluded volume $v_A/b^3 = 0.5$ and $v_C/b^3 = 0.5$). These data are related to Fig 6E.

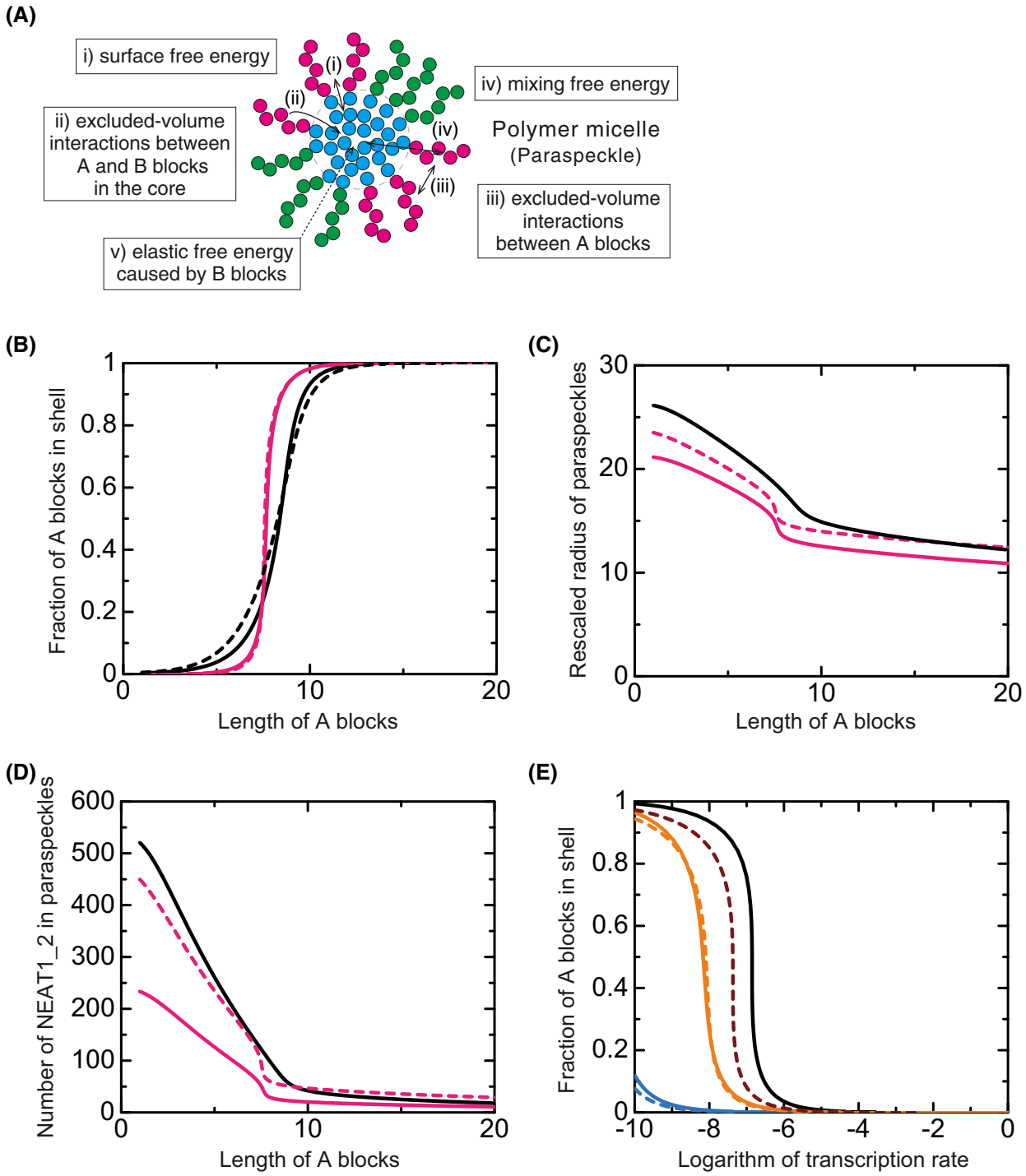

Figure EV5.