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Title	The Sam68 nuclear body is composed of two RNase-sensitive substructures joined by the adaptor HNRNPL
Author(s)	Mannen, Taro; Yamashita, Seisuke; Tomita, Kozo; Goshima, Naoki; Hirose, Tetsuro
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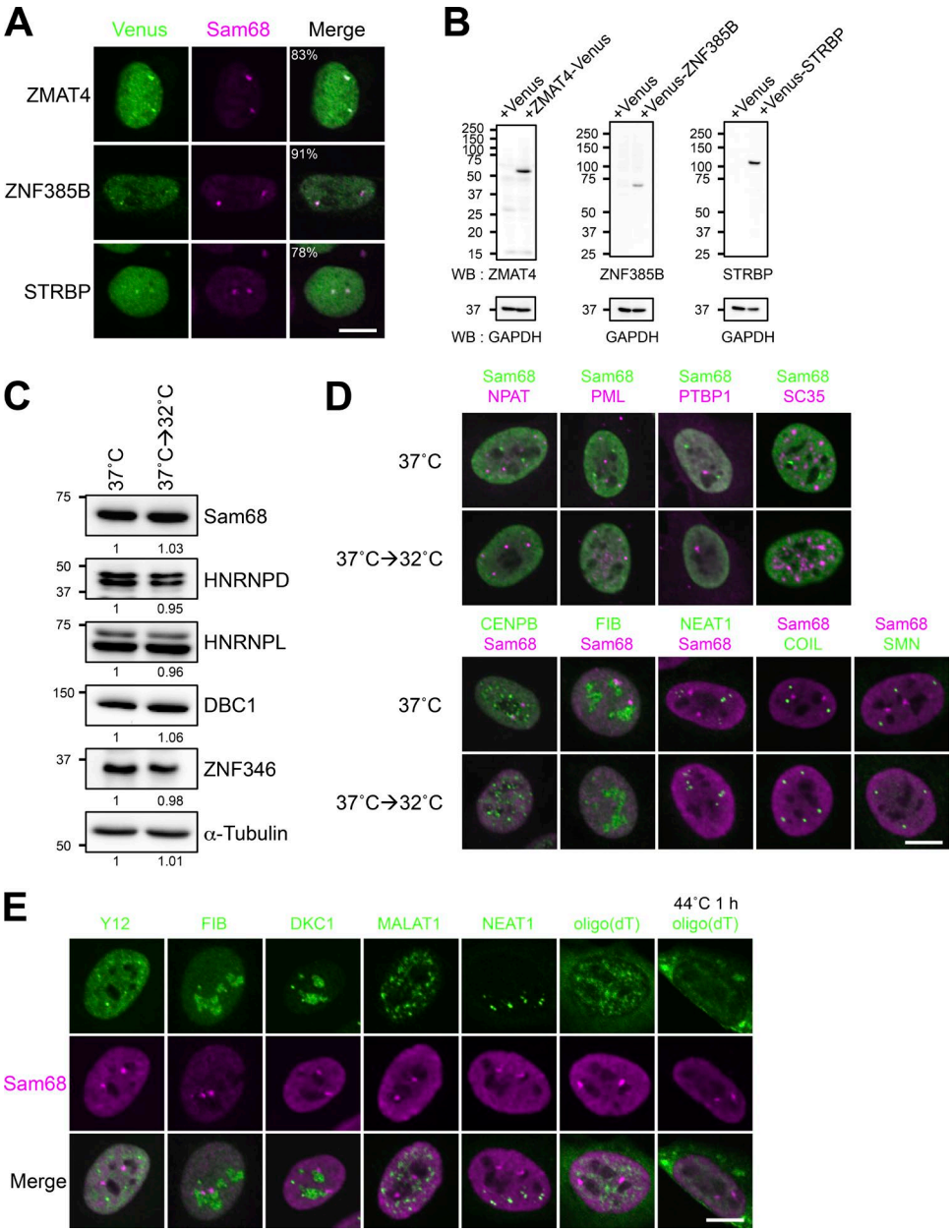

Figure S1. **Characterization of SNB components.** (A) SNB localization of Venus-tagged ZMAT4, ZNF385B, and STRBP in HeLa cells. The Venus signals overlap with endogenous Sam68, the marker of SNBs. The cell populations (%) in which Venus and Sam68 signals overlap are shown in the merge panels (>100 cells, n = 3). (B) Absence of ZMAT4, ZNF385B, and STRBP in HeLa cells. Western blotting was performed using antibodies against ZMAT4, ZNF385B, and STRBP with proteins isolated from cells transfected with plasmids harboring Venus and Venus-tagged ZMAT4, ZNF385B, or STRBP, respectively. The antibodies against ZMAT4, ZNF385B, and STRBP detected the exogenously expressed Venus-tagged proteins, but not the endogenous proteins. GAPDH was detected as the control for Western blotting. The molecular mass marker (kD) is shown on the left. (C) Expression of the SNB protein components was unchanged during a temperature shift. Western blotting was performed using antibodies against SNB and proteins isolated from cells cultured under normal (37°C) and cold shock (37– 32°C) conditions. α-Tubulin is a control. Protein signals of cold shock conditions (37–32°C) were quantified from blots using densitometry and normalized to those of normal conditions (37°C). The mean values of the quantified signals (n = 3) are shown below the representative images. The molecular mass marker (kD) is shown on the left. (D) Monitoring other NBs under cold shock conditions. The histone locus body (NPAT), promyelocytic leukemia body (PML), perinucleolar body (PTBP1), nuclear speckle (SC35), centromere (CENPB), nucleolus (FIB), paraspeckle (NEAT1), Cajal body (COIL), and gem (SMN) were detected with SNBs under normal (37°C) and cold shock (37–32°C) conditions. (E) The well-characterized RNA polymerase II nuclear RNAs were not localized in the SNB. The localizations of U-snRNAs, box C/D small nucleolar RNA, and box H/ACA small nucleolar RNAs were monitored by immunofluorescence with Y12, anti-fibrillarin (FIB), and anti-DKC1 (DKC1) antibodies, respectively. Two abundant nuclear lncRNAs (MALAT1 and NEAT1) were monitored by RNA-FISH. Poly(A)$^+$ RNAs were monitored using oligo(dT)$_{50}$ probes. The cytoplasmic stress granules induced by heat shock at 44°C for 1 h are the positive control for oligo(dT)$_{50}$ probes. Bars, 10 µm.

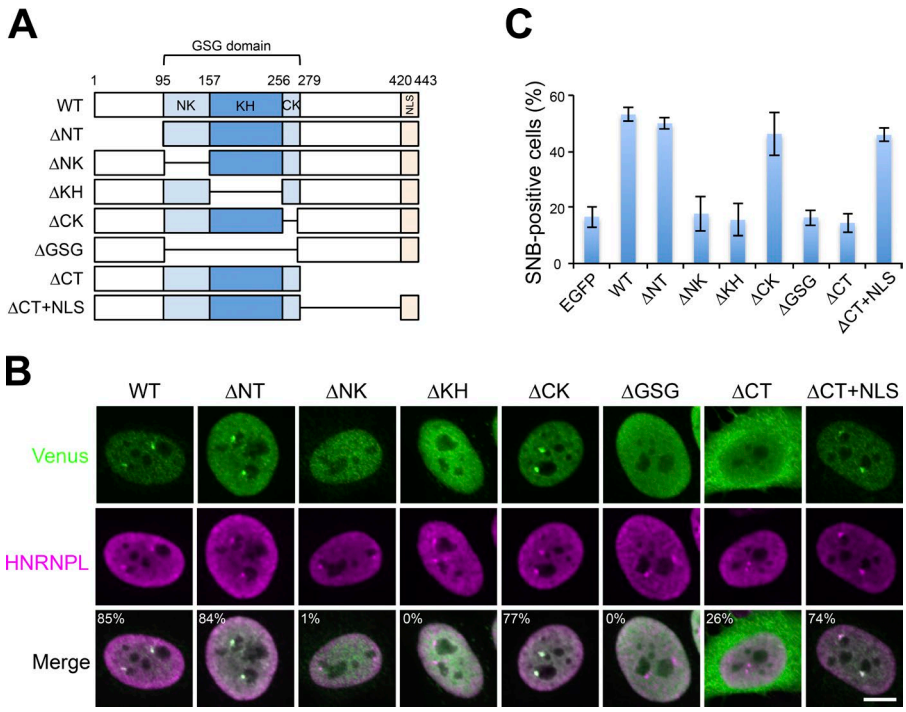

Figure S2. **Dissection of the Sam68 domains that are functional for SNB localization and construction.** (A) Schematics of Sam68 deletion mutants. ΔCT was defective in nuclear localization; therefore, a nuclear localization signal (NLS) was retained at the C terminus of ΔCT to produce ΔCT + NLS. The numbers represent the amino acid numbers of each protein. (B) Localization of Venus-tagged Sam68 deletion mutants shown in A. The cell populations (%) in which Venus and HNRNPL signals overlap are shown in the merge panels (>100 cells, $n = 3$). (C) KH and NK domains are required for SNB formation. Rescue of the defect in SNB formation was performed as described in Fig. 4 E, using the Sam68 deletion constructs shown in A. Sam68 constructs were transfected into HeLa cells in which endogenous Sam68 had been depleted by RNAi, and then SNB-positive cells (HNRNPL foci–positive cells) were counted (>100 cells, ±SD, $n = 3$). As a negative control, the FLAG-EGFP plasmid was transfected (EGFP). P-values for WT versus deletion mutants are 0.0007 (ΔNK), 0.0004 (ΔKH), 6.7×10^{-5} (ΔGSG), and 8.2×10^{-5} (ΔCT; Student's *t* test). Bar, 10 µm.

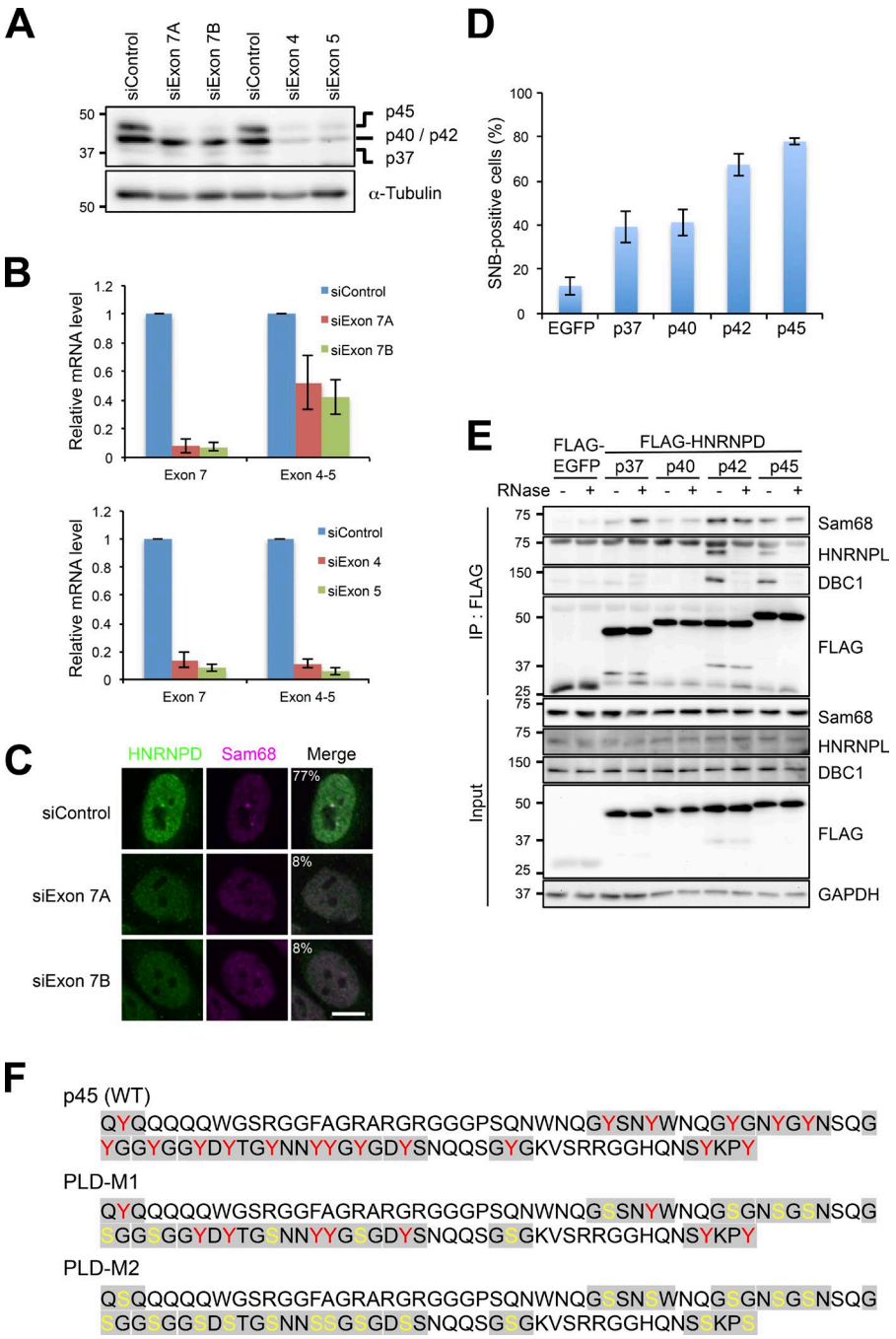

Figure S3. **Characterization of HNRNPD isoforms and mutants.** (A–C) Isoform-specific depletion of HNRNPD. Exon 7–specific siRNAs (siExon 7A and 7B) and siRNAs targeting the common exon (siExon 4 and 5) were used for RNAi. The effects of the siRNAs were monitored at the protein (A) and RNA (B) levels. Error bars in B are ±SD (n = 3). Immunofluorescence analysis of HNRNPD and Sam68 in HeLa cells treated with exon 7–specific siRNAs is shown in C. The cell populations (%) in which HNRNPD and Sam68 signals overlap are shown in the merge panels in C (>100 cells, n = 3). (D) Rescue of the defect in SNB formation with each of the HNRNPD isoforms. The rescue experiment was performed as in Fig. 4 E (>100 cells, ±SD, n = 3). (E) IP of Flag-tagged HNRNPD isoforms to detect the interaction with Sam68, HNRNPL, and DBC1. The presence and absence of RNase treatment are shown above the panels (+ or –). GAPDH was used as the input control. The molecular mass marker (kD) is shown on the left. (F) Sequence of the HNRNPD PLD (262–355) in p45NLS (WT), PLD-M1, and PLD-M2 in Fig. 4 A. The tyrosine residues present in the PLD are shown in red letters. The mutated serine residues are shown in yellow letters. Bar, 10 μm.

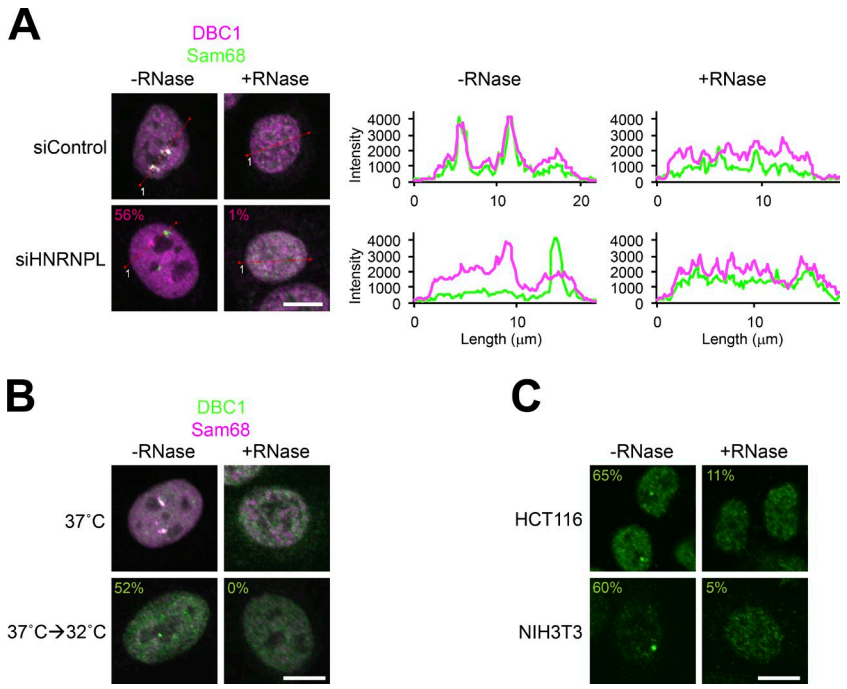

Figure S4. **RNase sensitivity of DBC1 foci.** The RNase sensitivity of DBC1 foci was monitored in HNRNPL-depleted HeLa cells (A), HeLa cells under cold shock conditions (B), and two cell lines (HCT116 and NIH3T3; C). RNase treatment was performed as shown in Fig. 1 A. The line scan data in A were obtained as in Fig. 5 A. The cell populations (%) in which DBC1 foci are detectable are shown in A, B, and C by magenta and green numbers (>100 cells, $n = 3$). Bars, 10 μm.

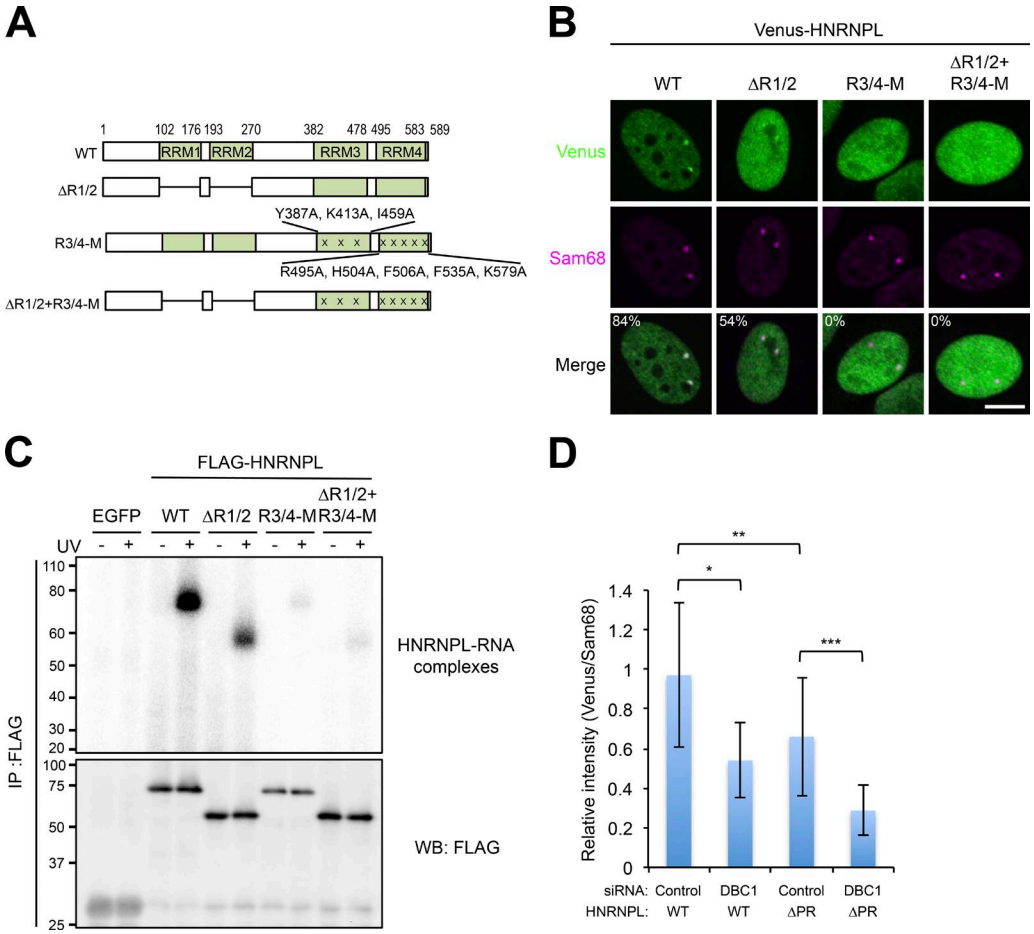

Figure S5. **Roles of the four RRMs and PR domain of HNRNPL.** (A) Schematics of the HNRNPL RRM mutants are shown. Functional RRM sets were abolished by deletion of RRM1 and RRM2 in ΔR1/2 or mutations of the critical residues for RNA binding of RRM3 and RRM4 in R3/4-M. ΔR1/2+R3/4-M lacks all four functional RRMs. (B) Localizations of the Venus-tagged HNRNPL RRM mutants shown in A. The cell populations (%) in which Venus and Sam68 signals overlap are shown in the merge panels (>100 cells, $n = 3$). (C) UV cross-linking and IP of Flag-tagged HNRNPL WT and the RRM mutants. ^{32}P-labeled HNRNPL-RNA complexes were visualized by ^{32}P-labeling as in Fig. 6 F (top). Immunoprecipitated Flag-HNRNPLs were detected by Western blotting (WB; bottom). The molecular mass marker (kD) is shown on the left. (D) DBC1 depletion by RNAi results in a moderate reduction in the SNB localization of HNRNPL WT and ΔPR. The HNRNPL levels were normalized by the Sam68 levels in SNBs. *, $P = 2.6 \times 10^{-5}$; **, $P = 0.0022$; ***, $P = 1.2 \times 10^{-6}$ (Student's *t* test). Bar, 10 μm.

Table S1. **Antibodies used in this study**

Antigen	Animal	Manufacturer (catalog no.)	Application	Dilution
COIL	Mouse IgG1	Abcam (ab11822)	IF	1:1,000
SMN	Mouse IgG1	BD (610647)	IF	1:1,000
PML	Mouse IgG1	Santa Cruz Biotechnology, Inc. (sc-966)	IF	1:1,000
SC35	Mouse IgG1	Sigma-Aldrich (S4045)	IF	1:1,000
DKC1	Mouse IgG2a	Santa Cruz Biotechnology, Inc. (sc-373956)	IF	1:1,000
Y12	Mouse IgG3	Abcam (ab3138)	IF	1:300
PTBP1	Mouse IgG1	Abcam (ab30317)	IF	1:1,000
NPAT	Mouse IgG2b	BD (611344)	IF	1:100
CENPB	Rabbit	Abcam (ab25734)	IF	1:1,000
C23	Mouse	Santa Cruz Biotechnology, Inc. (sc-17826)	IF	1:1,000
FIB	Rabbit	Santa Cruz Biotechnology, Inc. (sc-25397)	IF	1:100
H3K9me3	Rabbit	Abcam (ab8898)	IF	1:1,000
Sam68	Mouse	Santa Cruz Biotechnology, Inc. (sc-1238)	IF, WB	1:100, 1:1,000
Sam68	Rabbit	Santa Cruz Biotechnology, Inc. (sc-333)	IF, WB	1:100, 1:1,000
HNRNP L	Mouse IgG1	Abcam (ab6106)	IF, WB	1:100, 1:1,000
HNRNP L	Rabbit	Abcam (ab32680)	WB	1:1,000
HNRNP D	Rabbit	EMD Millipore (07-260)	WB	1:1,000
HNRNP D	Rabbit	Abcam (ab50692)	IF	1:30
DBC1	Rabbit	Bethyl Laboratories, Inc. (A300-432A)	IF, WB	1:1,000, 1:1,000
DBC1	Mouse IgG1	Cell Signaling Technology (#5857)	IF, WB	1:100, 1:1,000
ZNF346	Rabbit	Generated by MBL International	IF	1:1,000
ZNF346	Mouse	Abcam (ab88450)	IF	1:30
ZNF346	Rabbit	Abcam (ab96198)	WB	1:100
ZMAT4	Rabbit	Abcam (ab173873)	WB	1:100
ZNF385B	Mouse	Abcam (ab70931)	WB	1:500
STRBP	Rabbit	Abcam (ab111567)	WB	1:500
FLAG	Rabbit	Sigma-Aldrich (F7425)	IF, WB	1:1,000, 1:1,000
FLAG	Mouse	Sigma-Aldrich (F1804)	IF, WB	1:1,000, 1:3,000
GFP	Rabbit	MBL International (598)	IF, WB	1:1,000, 1:1,000
GAPDH	Rabbit	Abcam (ab37168)	WB	1:10,000
GAPDH	Mouse	Abcam (ab8245)	WB	1:10,000
α-Tubulin	Rabbit	Abcam (ab15246)	WB	1:10,000
α-Tubulin	Mouse	Abcam (ab7291)	WB	1:10,000
DIG	Mouse IgG1	Abcam (ab420)	FISH	1:1,000

IF, immunofluorescence; WB, Western blotting.