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Title	Staufen1, Kinesin1 and microtubule function in cyclin B1 mRNA transport to the animal polar cytoplasm of zebrafish oocytes
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Appendix A. Supplementary data

Supplementary Table 1. Primer sequences used in this study.

Supplementary Figure 1. Specificity of antibodies used in this study. (A) Crude extracts (10 μg, crude) and immunoprecipitates (IP) with (+) or without (-) anti-Stau1 (M) antibody from stages I and II oocytes were immunoblotted with anti-Stau1 (GP) antibody or without the primary antibody (-). (B) Experiments similar to those in (A) using anti-Stau1 (GP) for immunoprecipitation and anti-Stau1 (M) for immunoblotting. (C) Crude extracts (10 μg) from stage IV oocytes were immunoblotted with anti-EB1 antibody or without the primary antibody (-). Asterisks indicate Staufen1 (A, B) and EB1 (C).

Supplementary Figure 2. Inhibition of the *gfp-cyclin B1* reporter mRNA localization by nocodazole and antibodies against Kinesin1 and Staufen1 (anti-Kinesin1 Ab and anti-Stau1 Ab). (A) Water, nocodazole, control anti-GST antibody (control Ab), anti-Kinesin1 Ab or anti-Stau1 Ab was co-injected with the reporter vector into the nucleus and the cytoplasm of full-grown immature (stage IV) oocytes. After injection, oocytes were incubated in culture medium for 4 hr at room temperature. Following whole-mount *in situ* hybridization, oocytes showing localized signals of *gfp-cyclin B1* mRNA were counted. The data are shown as the percentage of the numbers of oocytes with localized signals to the numbers of oocytes examined in each experiment. The total numbers of analyzed oocytes are shown in parentheses. Values are means ± S.D. for three to five independent experiments. Statistical analysis was performed by two-tailed unpaired Student's t-test (*$P < 0.05$, **$P < 0.005$). (B) Total RNA extracted from injected oocytes shown in (A) was subjected to qRT-PCR to quantify *gfp-cyclin B1* reporter mRNAs produced from the injected vectors. The content of *gfp-cyclin B1* reporter mRNA was normalized to that of *β-actin* mRNA. Values are means ± S.D. for three independent experiments.

Supplementary Figure 3. Real-time imaging of EB1-GFP in stage I (A, C) and stage IV (B, D) oocytes isolated from the transgenic zebrafish Tg(-0.5zp3b:Mmu.Mapre1-GFP)/nub1Tg. (A, B) EB1-GFP signals from the whole oocyte of stage I (A) or stage IV (B, the animal pole facing upside) are shown. The signals are evenly distributed in stage I oocyte but accumulated in part at the animal polar cytoplasm of stage IV oocyte. (C, D) Each EB1-GFP signal was traced under a confocal laser microscope LSM 880 with Airyscan (Carl Zeiss) for 1 min at 1 sec intervals, and its movement was shown by a line, the color of which varies according to the time as indicated in the figures. The data were obtained from the whole oocyte of stage I (C) and from a part of the animal polar cytoplasm of stage IV oocyte (D). The EB1-GFP signals in stage I oocyte moved randomly in the cytoplasm as demonstrated by various lines of varying colors, whereas those in stage IV oocyte were almost

immovable as demonstrated by white dots. The size of EB1-GFP signals in stage IV oocytes (D) is significantly larger than that in stage I oocytes (C) (0.61 ± 0.08 μm (n=20) vs. 0.31 ± 0.05 μm (n=20), $P < 0.01$, Student's t-test), suggesting that the plus ends of microtubules are bound to each other and anchored to the animal polar cytoplasm of stage IV oocytes. The original movies from which the data were obtained are provided as Supplementary Movies 1 and 2. Scale bars, 50 μm (A), 200 μm (B), 10 μm (C, D).

Supplementary Movie 1. Real-time imaging of an EB1-GFP-expressing zebrafish stage I oocyte. EB1-GFP signals were monitored under a confocal laser microscope LSM 880 with Airyscan (Carl Zeiss). The EB1-GFP signals move randomly in the oocyte cytoplasm, indicating that the plus ends of microtubules are free in the cytoplasm of young oocytes.

Supplementary Movie 2. Real-time imaging of the animal polar region of EB1-GFP-expressing zebrafish stage IV oocyte. Most of the EB1-GFP signals are immovable, indicating that the plus ends of microtubules are anchored to the animal polar cytoplasm of full-grown oocytes.

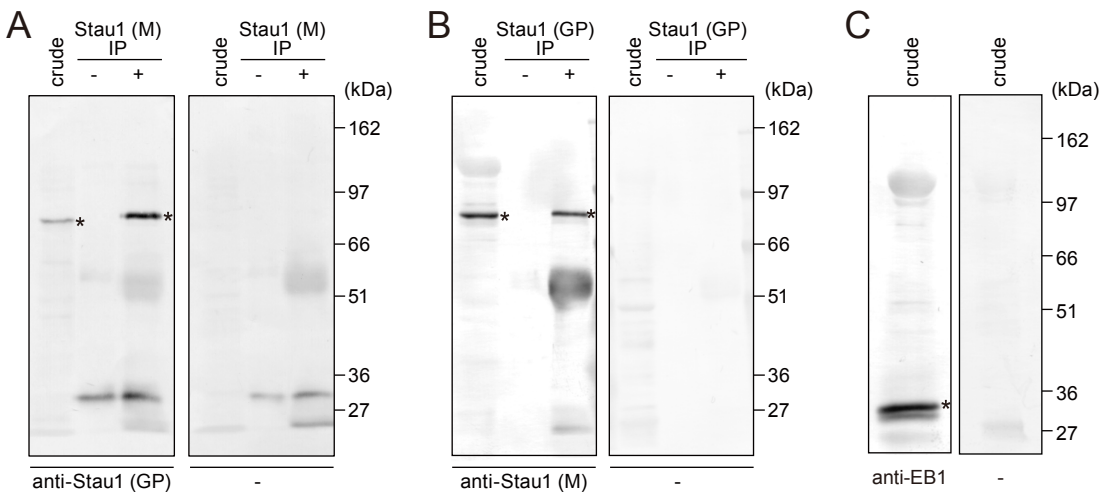
Takahashi et al. Supplementary Table 1

Primer sequences used in this study

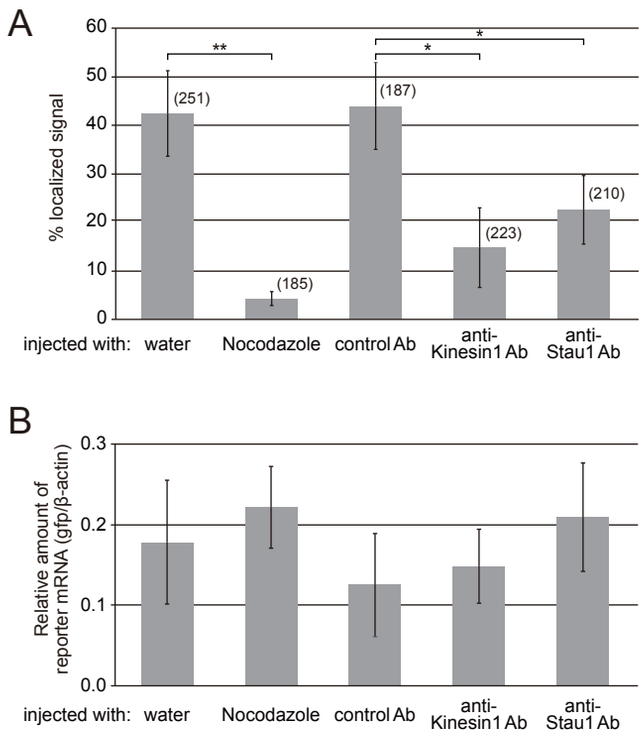
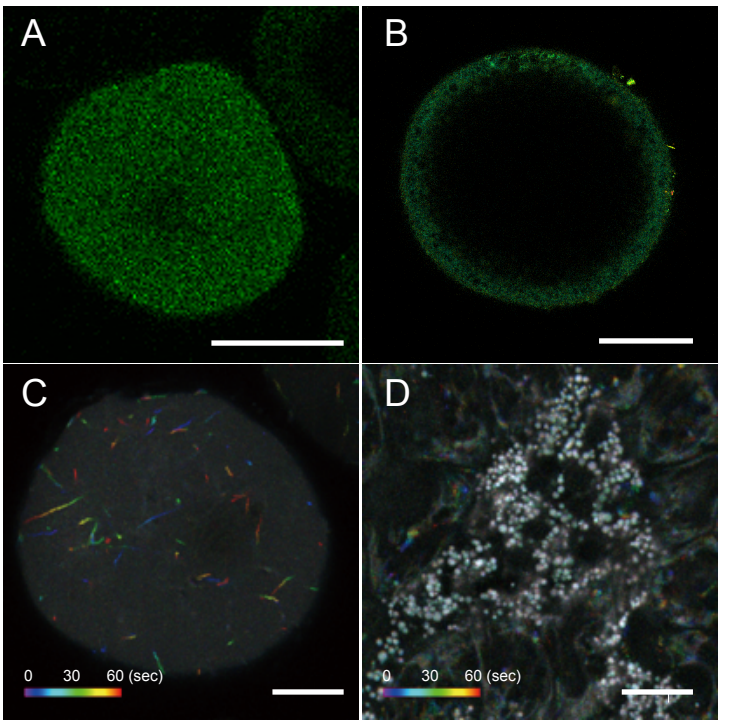
Purpose	Name/Target		Sequence (5'-3')
Construction	Stau1_exF		caccAACCCCATCAGCAGACTGG
	Stau1_exR		GGCTGTCCCATCCAGAGTC
	Stau1_F		ccatcgattcgaattTGGCAAACCCTAAAGAGAAGACC
	Stau1_R		gagaggccttgaattCTAGGCTGTCCCATCCAGAGTC
	xVg1VLE_IF_F		aagcagatctcatagACTTTATTTCTACACTGTTATGTC
	xVg1VLE_IF_R		tatcttcgcagatctGCTCAAGTCATATGGACTATTATAT
	cyclinB1_ORF_R		CTATGAGATCTGCTTAGCCAG
	pT2KXIGΔin_BglII_F		AGATCTGCGAAGATACGG
	EB1_exF		caccATGGCTGTGAACGTATATTC
	EB1_exR		GAACTCCTCCTGGTCC
RT-PCR	*cyclin B1*	Forward	GACAGGCTTTGAAGAAGAAGGAGG
		Reverse	GGAAGGCTCAGACACAACCTTAA
	β-actin	Forward	AAATCGCTGCCCTGGTCGTT
		Reverse	CTGTCCCATGCCAACCATCA
	gfp	Forward	CGACCACTACCAGCAGAACA
		Reverse	ACGAACTCCAGCAGGACCAT

Additional sequences required for TOPO cloning or In-Fusion cloning are indicated by lowercase letters.

Takahashi et al. Supplementary Figure 1



Takahashi et al. Supplementary Figure 2



Takahashi et al. Supplementary Figure 3