

Myosin depletion does not significantly alter anillin polarization in embryos with posterior spindles. (A) Schematic and equation used to calculate the polarization index. (B) Quantification of extent of polarized recruitment of GFP::ANI-1 in embryos depleted of ZYG-9 alone or depleted of both ZYG-9 and NMY-2. Error bars represent ± SEM.



MRCK-1 depletion blocks the formation of the polarity cap of NMY-2::GFP and does not significantly alter anillin recruitment during anaphase. (A) Quantification of the GFP intensity in NMY-2::GFP control embryos and MRCK-1-depleted embryos. (B) The distribution of cortical GFP::ANI-1 in control and MRCK-1 depleted embryos are shown during metaphase and anaphase. Cell cycle timing was determined by mCherry::HIS (insets, top right). All images are projections of 5 planes spanning 2  $\mu$ m. (C) Quantification of the degree of polarization of GFP::ANI-1 in control embryos and MRCK-1-depleted embryos. Error bars represent ± SEM; Single asterisk represents p<0.05; double asterisk represents p<0.01 from paired t-tests. 10 (m scale bar.



Anillin promotes the polarized accumulation of myosin. (A) Schematic and equation used to calculate normalized myosin intensity profiles. (B-E) The distribution of cortical NMY-2::GFP are quantified in embryos depleted of MRCK-1 (B), MRCK-1 and ANI-1 (C), MRCK-1 and ZYG-9 (D) and MRCK-1, ZYG-9 and ANI-1 (E). In ANI-1-depleted embryos, there is only slightly more NMY-2::GFP recruitment in the equatorial region (region 6 and 7) and anterior pole (region 2) as compared to the remaining regions. In addition, the intensity gradient along the AP axis is greatly reduced in *mrck-1(RNAi);zyg-9(RNAi);ani-1(RNAi)* embryos as compared to *mrck-1(RNAi);zyg-9(RNAi)* embryos. (F-G) The distribution of NMY-2::GFP from 4.5 s to 7.5 s after anaphase onset calculated as in (B-E).



Depletion of anillin reduces the fraction of bright NMY-2::GFP foci. Fraction of dim (A) and bright (B) NMY-2::GFP foci in control and *ani-1(RNAi)* embryos. The percentage of foci with bright GFP signals is significantly reduced in ANI-1- depleted embryos. (C and D) Lifetime of NMY-2::GFP foci in control embryos (C) and *ani-1(RNAi)* embryos (D). No significant difference on the lifetime of bright and dim NMY-2::GFP foci in control and ANI-1-depleted embryos.



Anillin associates with microtubules. Embryos expressing GFP::ANI-1 were fixed and immunostained to detect endogenous tubulin (red) and GFP::ANI-1 (green). The boxed regions are shown at higher magnification below.



Quantitative analysis of the appearance of cortical invaginations. At 30 s intervals after anaphase onset (t=0) in embryos of the indicated genotypes expressing GFP::PH; mCherry::HIS were quantified as described in materials and methods. Error bars are ±SEM.



Anillin concentrates on meiotic spindles. (A) GFP::ANI-1 distribution in APC/C defective fertilized oocytes (due to depletion of EMB-27 by RNAi or the *mat-1(ax161)* mutation). (B) The distribution of GFP::ANI-1( $\Delta$ CT) and GFP::ANI-1( $\Delta$ NT) in meiotically-arrested fertilized oocytes.

Table S1. *C. elegans* strains used in this study.

Strain #	Marker	Geno	type		
N2	-	Ances	stral N2 Bristol strain; "	wild-type"	
DH244	-	zyg-9(b244)II			
DS77	-	mat-1(ax161) I; him-8(e1489) IV			
AZ212	GFP::HIS	unc-1	19(ed3) III ruls32 [pAZ	132: pie-1::GFP::histoneH2B]	
A7244	GEP::Tubulin	111. rule5	7[nio_1::CED::tubulin_	(100-110/1)	
101550	mCherry::Tubulin	10.57[ple-1011(abdiii) + 0.16-119(+)]			
IH1327		avEv73[nie-1::PIE-1::GEP]			
5111527		unc-119(ed3) III: zuls45 [nmv-2::NMV-2::GFP + unc-			
JJ1473	NMY-2::GFP	119(+	119(+)] V.		
KK866	GFP::PAR-2	itls153 [pie-1::PAR-2::GFP]			
OD38	GFP::ANI-1 unc-1 STag		-119(ed3) III; ItIs28 [pASM14; pie-1::GFP-TEV-		
			Гад::ani-1; unc-119 (+)]		
OD58	GEP: PH unc-		-119(ed3) III; ItIs38 [pAA1; pie-		
	1::0	1::GF	::GFP::PH(PLC1delta1); unc-119 (+)]		
OD70	mCherry::PH unc 1::r	unc-1	ic-119(ed3) III; ItIs44[pAA173; pie-		
		1::mC	1::mCherry::PH(PLC1delta1); unc-119 (+)] V.		
OD120	GFP::ANI-1(∆NT) unc- Stag	unc-1	-119(ed3) III; ItIs58 [pASM16; pie-1/GFP-TEV-		
		Stag:	Stag::ANI-1(aa171-1205); unc-119 (+)]		
OD124	GFP::ANI-1(ACT)		119(ed3) III; ItIs60 [pASM15; pie-1/GFP-TEV-		
04045		Stag::		::AIVI-1(aa2-754); UNC-119 (+)] Obligate het.	
SA245	mCnerry::HIS	nerry::HIS unc-119		19(ea3); tjls57 [pie-1::mCherry::his-48; unc-119(+)]	
Strain #			Allele		
MG390			-	JJ14/3 X AZ212	
MG508	MCnerry::PH; GFP::ANI-1		-	OD70 X OD38	
MG511			-	OD70 x AZ244	
MG529	NMY-2::GFP; GFP::HIS		zyg-9(b244)	MG390 X DH244	
MG535	GFP::PH; mCherry::HIS		-	OD58 x SA245	
MG537	PIE-1::GFP; mCherry::HIS		-	JH1327 x SA245	
MG541	GFP::PH		zyg-9(b244)	OD58 X DH244	
MG542	GFP::ANI-1; mCherry::HIS		-	OD38 x SA245	
MG543	GFP::PAR-2; mCherry::HIS		-	KK866 x SA245	
MG559	GFP::PAR-2; mCherry::HIS		mat-1(ax161)	MG543 x DS77	
MG561	GFP::ANI-1(∆CT); mCherry:Tubulin		mat-1(ax161)	MG583 x DS77	
MG562	GFP::ANI-1(△NT); mCherry::Tubulin		mat-1(ax161)	MG581 x DS77	
MG580	GFP::ANI-1; mCherry::Tubulin		-	OD38 x JA1559	
MG581	GFP::ANI-1(∆NT); mCherry::Tubulin		-	OD120 x JA1559	
MG583	GFP::ANI-1( $\Delta$ CT); mCherry::Tubulin		-	OD124 x JA1559	