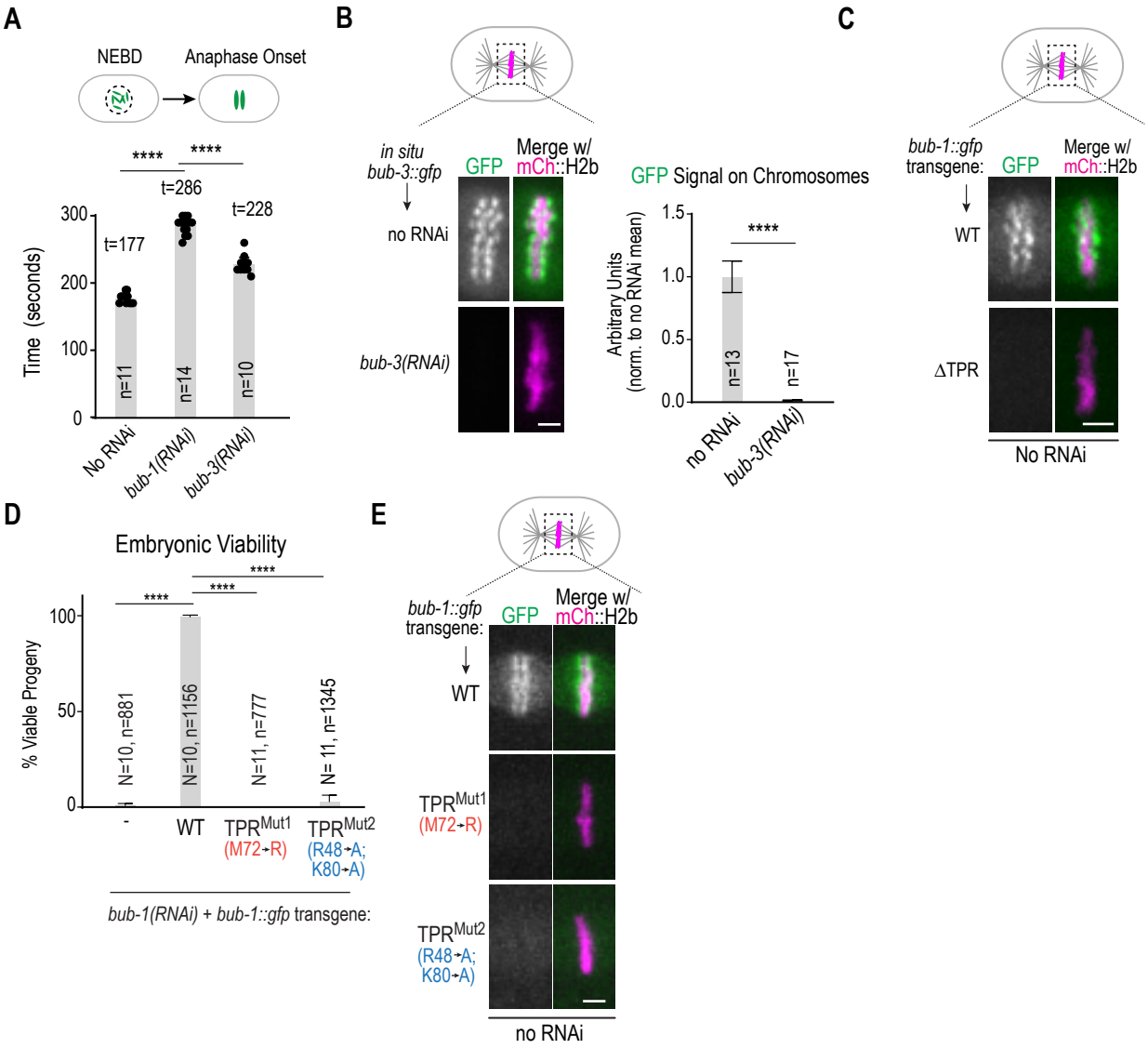
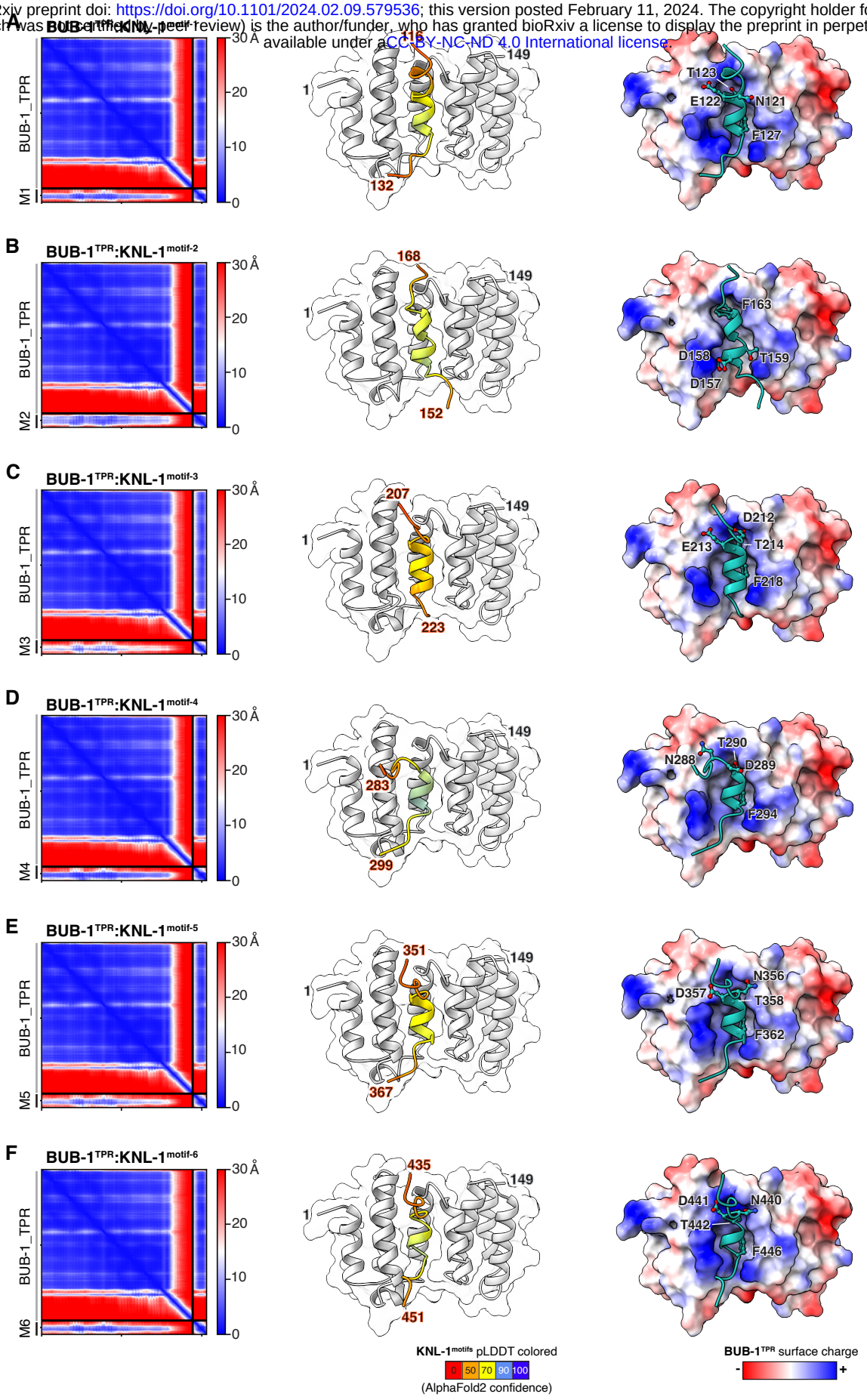
Figure S1



Supplemental Figure 1: Penetrance of BUB-3 depletion and additional analysis of BUB-1 TPR mutants.

(A) The interval from nuclear envelope breakdown (NEBD) to anaphase onset was measured in the indicated conditions; *n* refers to the number of embryos imaged. **(B)** (*left)* One cell embryos expressing *in situ* GFP-tagged BUB-3 were imaged in the indicated conditions. Scale bar is 2 µm. (*right)* Quantification of BUB-3::GFP kinetochore localization indicating that *bub-3(RNAi)* is highly penetrant. Error bars are the 95% confidence interval. **(C)** Images of aligned chromosomes in one cell *C. elegans* embryos expressing the indicated *bub-1::gfp* transgenes in the presence of endogenous BUB-1 (No RNAi). ΔTPR BUB-1 fails to localize even in the presence of endogenous BUB-1. Scale bar is 2 µm. (**D)** Embryonic viability analysis for the indicated conditions. *N* is the number of worms and *n* the number of progeny embryos scored. Error bars are the 95% confidence interval. **(E)** Images of aligned chromosomes in one cell *C. elegans* embryos expressing the indicated *bub-1::gfp* transgenes in the presence of endogenous BUB-1 (No RNAi). Both TPR^{mut1} and TPR^{mut2} BUB-1 fail to localize even when endogenous BUB-1 is present. Scale bar is 2 µm. All *p* values were calculated from unpaired t tests; **** = $p<0.0001$.

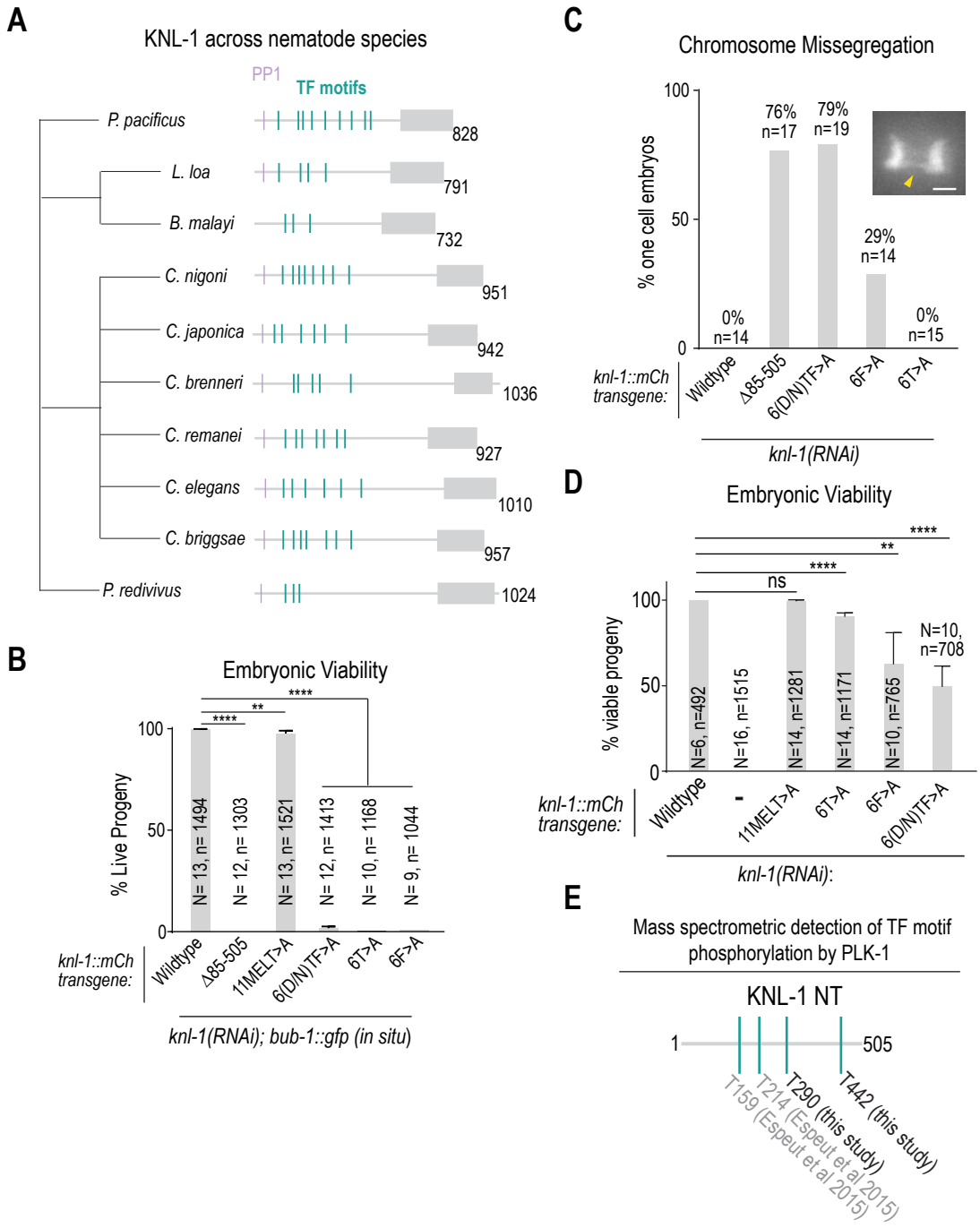
Figure S2



Supplemental Figure 2: AlphaFold models of the BUB-1 TPR with the 6 TF motifs in the KNL-1 N-terminus.

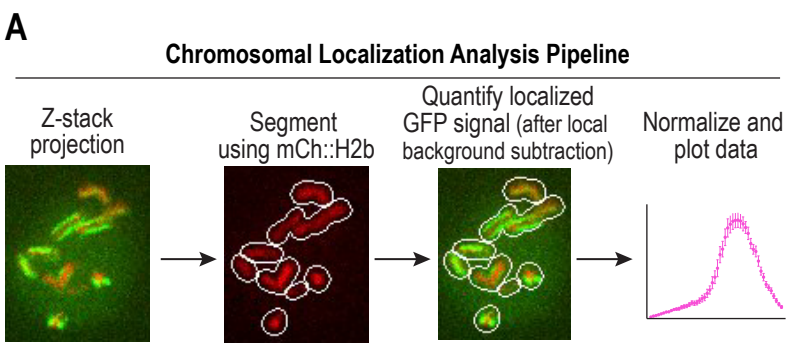
(A) – (F) AlphaFold models of BUB-1 TPR (aa 1-149) interfacing with TF motifs 1-6 (*panels A-F*) in the KNL-1 N-terminus. For each model, 3 elements are shown: *(left*) Predicted Aligned Error (PAE) plot; (*middle*) cartoon model showing the interface of the TPR and the TF motif; the TF motif is color-coded based on the predicted local distance difference test (pLDDT) score; (*right*) BUB-1 TPR surface charge depiction with the modeled bound TF motif; specific residues of the TF motif are highlighted. Aside from motif 2, the F residue of the TF motif occupies the same hydrophobic pocket in the TPR domain. The basic character in the vicinity of the T residue, targeted by PLK-1, likely accounts for the phospho-dependence of the interaction.

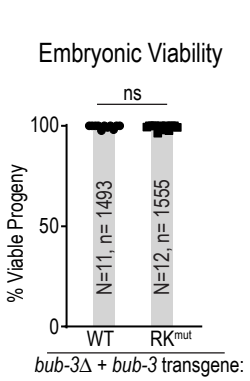
Figure S3

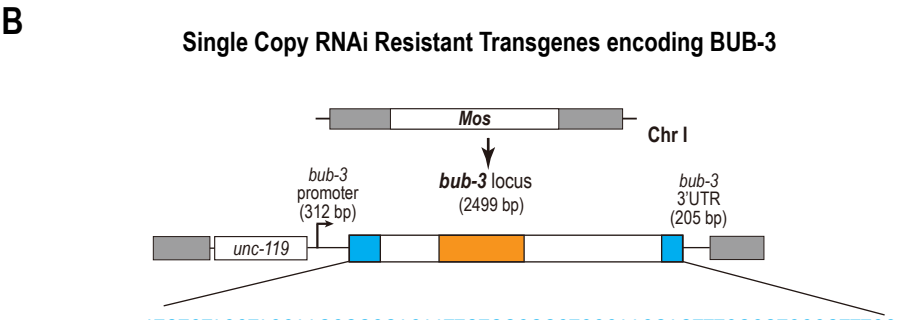


Supplemental Figure 3: Additional analysis of KNL-1 TF motifs.

(A) Cladogram showing TF motifs present in *knl-1* genes across different nematode species. **(B)** Embryonic viability was scored for indicated conditions; note that this analysis was conducted in the presence of *in situ* GFP-tagged BUB-1. *N* is the number of worms and *n* is the number of progeny scored. Error bars are the 95% confidence interval. **(C) & (D)** Chromosome missegregation and embryonic lethality analysis for the indicated KNL-1 variants when the endogenous *bub-1* locus was untagged. *(C)* Chromosome missegregation was quantified for each of the indicated conditions. *n* is the number of embryos imaged. Inset image shows example of missegregating chromosome, highlighted by yellow arrowhead. Scale bar of inset is 2 μm. Example image is the same as shown in *Fig. 1B* and *Fig. 2H*. *(D)* Embryonic viability quantified for indicated conditions. *N* is the number of worms and *n* is the number of progeny scored. Error bars are the 95% confidence interval. **(E)** Schematic of TF motif phosphorylation by PLK-1 detected by mass-spectrometry. Phosphorylation of T290 and T442 (TF motifs 4 and 6 respectively) was identified in this study. Phosphorylation of T159 and T214 (TF motif 2 and TF motif 3 respectively) was identified in a previous study (Espeut et al., 2015). All p values were calculated from unpaired t tests; ns = not significant, ** = $p< 0.01$, **** = $p<0.0001$.







exon 1
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TTCCGGCTGGGATGGCACTTGTCGCGTCTATGAAGTCGGAAAGTTGGGAGATATCTCGGAAA
AACTCGTTTTTACACACGGAAAACCACTTTTGACGTGCACTTTTGCGgtaggggtttcaggttttaggttttt

intron 1 (shortened to remove DNA hairpin)
attaaaaattgctttaaaacatgcccatggggtcacaatgaccgaatatcatgataaaaaaattcaaaaaagttttctagattttatatgatttt
tagaaaattgaaaaaatctcagttttccccgctaattcctatttaaatttccgccaattggatttgttcgatggagcgtgctttcaattggcggaa
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aaattatttttttataaaaacaaaaattccagGGTTACAACAAAGTAGCGTTCGGAGGAGTCGATCACAACGTA

RNAi-Resistant re-encoded exon 2
AAGCTTGCCGATATCGAGACTGGAAATGGTACGCAGctgGGCtcgCATgcgCTCGCGgtcCGTTGT
atgGAGTTTaacCCGatgAGTTCGTTGatcGTAAGTGGCGGAtgggatAGTtcgGTTAAACTAtggGATgc
gCGGTCGTATggaAATGGAGCGATTgagtcgGTGaacGTCTCCTCGAGCGTGTATGCCatggatGTCc
tcAAAcatACAATTCTTGTGGGAACAAAGgatAGAAAGATTTTTatgTACgatAGCCGAAAACTGAGA
gagCCACTCCAGGTGCGCgatTCGccgCTGAAGTACCAAACTCGAgcgGTAcagTTTTTCCCAACA
GGAGAGGCCTTCGTCGTAAGTtcgATTgagggacgggtcGCCGTCGAAtatGTTgatCAAAGCGGCgag
GAAatgatgAAACGGaagtatgcattcaagTGCcatCGTgagAAAgatACCgatGGCACCGAActgATCCACC
CTGTCcatACGGTGGCTTTCcatccgAAGtatGGCactTTCgcgACTGGCGGAGCGGACggaATCgtcA
ACATCtgggatCCGTTTAACAGAaagCGCATCATTCAGCTTCATAAgttagtagtggaggaaaattggctgaaga

intron 2
gcgcggtgtttgccgaagaaaaaactagaaatagctgaatttttgccgaaaatcgaggaaaataggctgaaaactacgaaattcccag
cgttttatgtggatttttgcccaaagtgacgatatttgccaaaaaaaaaaaatggaaattgctgattttattccgaatttgaggaaaaatggct
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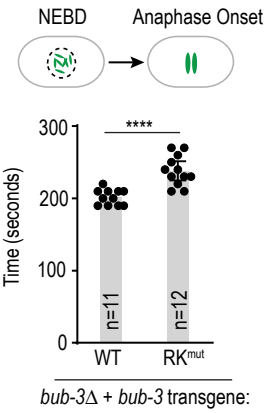
atttttttttcaattttgggcaaaaaaaatgttttttttggagttctgaattttccaaaatttccaaacgttttagctgaaataaccgtgatttctacat

exon 3
attttacccagaaaatttcaaatttttttccagATTCGAAACGTCCATCTCCTCATTATCCTTCAACGAAGACGG
CTCCCAGCTTGCGATAGCCACATCCTATCAATACGAGAAGGAAATTGATCCATCACCACTGCC
AAATAATTCGATCACCATTCGCCACATTACCGATCCGGAGAGCAGACCGAAATAG



Supplemental Figure 4: Image analysis approach to quantify chromosomal localization, design of the RNAi-resistant bub-3 transgene and additional phenotypic analysis of RK^{mut} BUB-3.

(A) Overview of CellProfiler-based analysis to segment chromosomes based on the mCh::H2b signal and measure chromosomal GFP fluorescence. A Laplacian of Gaussian function was applied to the maximum intensity projection of mCh::H2b images in the timelapse sequence to identify chromosomes and expand a region around them. The GFP signal within the segmented regions was quantified, and the region was further expanded by three pixels to subtract local background. The process was applied to each timepoint to generate dynamic chromosomal localization curves for each condition. **(B)** Schematic of RNAi-resistant *bub-3* transgenes. Exon 2 was reencoded to preserve coding information but make the transgene-encoded mRNA resistant to RNAi triggered by a dsRNA raised to the endogenous sequence. In addition, intron 1 was shortened to remove a DNA hairpin. **(C)** Embryonic viability was quantified for each of the indicated conditions. **(D)** The interval from NEBD-Anaphase onset was quantified for each of the indicated conditions in one cell *C. elegans* embryos. *n* refers to the number of embryos imaged. All p values were calculated by unpaired t tests; ns = not significant, **** = p<0.0001.

Table S1: *C. elegans* Strains

N2	Ancestral
OD334	*unc-119(ed3)?III; ltSi1[pOD809/pJE110; Pknl-1::KNL-1reencoded::RFP; cb-unc-119(+)]II*
OD963	*unc-119(ed3)?III;ltSi251[pOD1940/pTK002; Pbub-1::GFP-Bub1 reencoded; cb-unc-119(+)]II*
OD1702	*unc-119(ed3)?III ltSi560 [oxTi365; oxTi365; pPLG014; Pmex-5::GFP::his-11::tbb-2_3'UTR, tbg-1::gfp::tbb-2_3'UTR; cb-unc-119(+)]V*
OD1904	*bub-3(ok3437)II*
OD1931	*ltSi1[pOD809/pJE110; Pknl-1::KNL-1reencoded::RFP; cb-unc-119(+)]II; ; unc-119(ed3)?III; ltSi560[oxTi365; pPLG014; Pmex-5::GFP::his-11::tbb-2_3'UTR, tbg-1::gfp::tbb-2_3'UTR; cb-unc-119(+)]V*
OD1933	*ltSi44[pOD1039/pJE170; Pknl-1::KNL-1reencoded(Mutant D85-505)::RFP; cb-unc-119(+)]II; unc-119(ed3)?III ltSi560[oxTi365; pPLG014; Pmex-5::GFP::his-11::tbb-2_3'UTR, tbg-1::gfp::tbb-2_3'UTR; cb-unc-119(+)]V*
OD2024	*ltSi268[pOD1951/pTK013; Pbub-1::Bub1 reencoded; cb-unc-119(+)]II; unc-119(ed3)III?;ltSi560[oxTi365; pPLG014; Pmex-5::GFP::his-11::tbb-2_3'UTR, GFP::tbg-1::tbb-2_3'UTR; cb-unc-119(+)]V*
OD2498	*ltSi251[pOD1940/pTK002; Pbub-1::GFP-Bub1 reencoded; cb-unc-119(+)]II; unc-119(ed3)III?; ltIs37 [pAA64; pie-1/mCHERRY::his-58; unc-119 (+)] IV*
OD2802	*unc-119(ed3)III;ltSi1353[pTK084;Pbub-1::ZF1::GFP::BUB-1 reencoded; cb-unc-119(+)]II*
OD2803	*unc-119(ed3)III;ltSi1354[pTK085;Pbub-1::ZF1mut::GFP::BUB-1 reencoded; cb-unc-119(+)]II*
OD2859	*ltSi1353[pTK084;Pbub-1::ZF1::GFP::BUB-1 reencoded; cb-unc-119(+)]II;unc-119(ed3)?III; ltSi560 [oxTi365; pPLG014; Pmex-5::GFP::his-11::tbb-2_3'UTR, tbg-1::gfp::tbb-2_3'UTR; cb-unc-119(+)]V*
OD2860	*;ltSi1354[pTK085;Pbub-1::ZF1mut::GFP::BUB-1 reencoded; cb-unc-119(+)]II;unc-119(ed3)?III; ltSi560 [oxTi365; pPLG014; Pmex-5::GFP::his-11::tbb-2_3'UTR, tbg-1::gfp::tbb-2_3'UTR; cb-unc-119(+)]V*
OD3075	*lt53[knl-1::GFP::tev::loxP::3xFlag)III; ltIs37 [pAA64; pie-1/mCHERRY::his-58; unc-119 (+)] IV*
OD3516	*bub-1(lt82 [bub-1::gfp])I*
OD3598	*lt82 [bub-1::gfp]I;ltSi1[pOD809/pJE110; Pknl-1::KNL-1reencoded::RFP; cb-unc-119(+)]II;unc-119(ed3)?III*
OD3642	*ltSi1353[pTK084;Pbub-1::ZF1::GFP::BUB-1 reencoded; cb-unc-119(+)]II;unc-119(ed3)III?; ltIs37 [pAA64; pie-1/mCHERRY::his-58; unc-119 (+)]IV*
OD3643	*ltSi1354[pTK085; Pbub-1::ZF1mut::GFP::BUB-1 reencoded; cb-unc-119(+)]II;unc-119(ed3)III?; ltIs37 [pAA64; pie-1/mCHERRY::his-58; unc-119 (+)]IV*
OD3849	*ltSi1380[pTK102;Pbub-1::bub-1::gfp T527A reencoded::bub-1 3' UTR; cb-unc-119(+)]II; unc-119(ed3)?III; ltIs37 [pAA64; pie-1/mCHERRY::his-58; unc-119 (+)] IV*
OD4684	*ltSi1468[pOD3592/pJMH3; Pbub-3 R217A,K238A reencoded; cb-unc-119(+)]I; bub-3 (ok3437)II; unc-119(ed3)?III*
OD4689	*ltSi1500[pOD3593/pJMH4; Pbub-3::BUB-3 reencoded; cb-unc-119(+)]I; bub-3 (ok3437)II; unc-119(ed3)?III*
OD4729	*bub-1(lt82 [bub-1::gfp]), ltSi1468[pOD3592/pJMH3; Pbub-3 R217A,K238A reencoded; cb-unc-119(+)]I; bub-3 (ok3437)II; unc-119(ed3)?III*

OD4730	*bub-1(lt82 [bub-1::gfp]), ltSi1500[pOD3593/pJMH4; Pbub-3::BUB-3 reencoded; cb-unc-119(+)]I; bub-3 (ok3437)II; unc-119(ed3)?III*
OD4745	*bub-1(lt82 [bub-1::gfp]), ltSi1500[pOD3593/pJMH4; Pbub-3::BUB-3 reencoded; cb-unc-119(+)]I; bub-3 (ok3437)II; unc-119(ed3)?III; ltIs37 [pAA64; pie-1/mCHERRY::his-58; unc-119 (+)] IV*
OD4752	*bub-1(lt82 [bub-1::gfp]), ltSi1468[pOD3592/pJMH3; Pbub-3 R217A,K238A reencoded; cb-unc-119(+)]I; bub-3 (ok3437)II; unc-119(ed3)?III; ltIs37 [pAA64; pie-1/mCHERRY::his-58; unc-119 (+)] IV*
OD4775	*ltSi1528[pJMH8/pOD3597; Pknl-1::rfp reencoded MELT->MELA motif mutant (T88A, T112A, T202A, T231A, T258A, T281A, T326A, T349A, T376A, T405A, T431A) ; cb-unc119(+)]II*
OD4777	*ltSi1530[pJMH10/pOD3599; Pknl-1::rfp reencoded 6T mutant (T123A, T159A, T214A, T290A, T358A, T442A); cb-unc119(+)]II*
OD4967	*ltSi1468[pOD3592/pJMH3; Pbub-3 R217A,K238A reencoded; cb-unc-119(+)]I; bub-3 (ok3437)II; unc-119(ed3)?III; ltSi560 [oxTi365; oxTi365; pPLG014; Pmex-5::GFP::his-11::tbb-2_3'UTR, tbg-1::gfp::tbb-2_3'UTR; cb-unc-119(+)]V*
OD4968	*ltSi1500[pOD3593/pJMH4; Pbub-3::BUB-3 reencoded; cb-unc-119(+)]I; bub-3 (ok3437)II; unc-119(ed3)?III; ltSi560 [oxTi365; oxTi365; pPLG014; Pmex-5::GFP::his-11::tbb-2_3'UTR, tbg-1::gfp::tbb-2_3'UTR; cb-unc-119(+)]V*
OD5035	*bub-1(lt82 [bub-1::gfp])I; unc-119(ed3)? III; ltIs37 [pAA64; pie-1/mCHERRY::his-58; unc-119 (+)] IV*
OD5064	*ltSi1604[pOD4379/pPLG415; Pbub-1::gfp(PATC enriched) reencoded (1-189); cb-unc-119(+)]II*
OD5135	*ltSi1604[pOD4379/pPLG415; Pbub-1::gfp(PATC enriched) reencoded (1-189); cb-unc-119(+)]II; unc-119(ed3)? III; ltIs37[pAA64; pie-1/mCHERRY::his-58; unc-119 (+)] IV*
OD5276	*ltSi1340[pTK077;Pbub-3::BUB-3 reencoded::GFP; cb-unc-119(+)]I; unc-119(ed3)? III; ltIs37 [pAA64; pie-1/mCHERRY::his-58; unc-119 (+)] IV*
OD5288	*ltSi1340[pTK077;Pbub-3::BUB-3 reencoded::GFP; cb-unc-119(+)]I; ltSi268[pOD/pTK013; Pbub-1::bub-1 reencoded::bub-1 3' UTR; cb-unc-119(+)]II; unc-119(ed3)? III; ltIs37 [pAA64; pie-1/mCHERRY::his-58; unc-119 (+)] IV*
OD5335	*ltSi1806 [Pbub-1:::bub-1::gfp R48A K80A::bub-1 3' UTR reencoded::bub-1 gfp; cb-unc-119(+)]II*
OD5348	*ltSi1734[pOD3940/pJMH30; Pbub-1::bub-1::gfp M72R::bub-1 3' UTR; cb-unc-119(+)]II;*
OD5360	*ltSi1732[pOD3939/pJMH29; Pbub-1:::bub-1::gfp R48A K80A::bub-1 3' UTR; cb-unc-119(+)]II; ltIs37 [pAA64; pie-1/mCHERRY::his-58; unc-119 (+)]IV;*
OD5361	*ltSi1734[pOD3940/pJMH30; Pbub-1::bub-1::gfp M72R::bub-1 3' UTR; cb-unc-119(+)]II; ltIs37 [pAA64; pie-1/mCHERRY::his-58; unc-119 (+)]IV;*
OD5462	*lt148[bub-3::GFP])II ; unc-119(ed3) III; ltIs37 [pAA64; pie-1/mCHERRY::his-58; unc-119 (+)] IV*
OD5481	*ltSi1340[pTK077;Pbub-3::BUB-3 reencoded::GFP; cb-unc-119(+)]I; ltSi1806 [Pbub-1:::bub-1 reencoded R48A K80A::bub-1 3' UTR; cb-unc-119(+)]II ; unc-119(ed3)? III; ltIs37 [pAA64; pie-1/mCHERRY::his-58; unc-119 (+)] IV*
OD5482	*ltSi1340[pTK077;Pbub-3::BUB-3 reencoded::GFP; cb-unc-119(+)]I; ltSi1807 [Pbub-1:::bub-1 reencoded M72R::bub-1 3' UTR; cb-unc-119(+)]II ; unc-119(ed3)? III; ltIs37 [pAA64; pie-1/mCHERRY::his-58; unc-119 (+)] IV*
OD5520	*ltSi1840[pOD5283/pJMH93; Pknl-1::knl-1::rfp reencoded 6(D/N)TF mutant (N121A, T123A, F127A, D157A, T159A, F163A, D212A, T214A, F218A, D288A,*

	T290A, F294A, N356A, T358A, F362A, N440A, T442A, F446A)::knl-1 3'UTR; cb-unc119(+)]II
OD5557	*bub-1(lt82 [bub-1::gfp])I; ltSi1840[pOD5283/pJMH93; Pknl-1::knl-1::rfp reencoded 6(D/N)TF mutant (N121A, T123A, F127A, D157A, T159A, F163A, D212A, T214A, F218A, D288A, T290A, F294A, N356A, T358A, F362A, N440A, T442A, F446A)::knl-1 3'UTR; cb-unc119(+)]II; unc-119(ed3)? III;*
OD5559	*bub-1(lt82 [bub-1::gfp])I; ltSi1530[pJMH10/pOD3599; Pknl-1::rfp reencoded 6T mutant (T123A, T159A, T214A, T290A, T358A, T442A); cb-unc119(+)]II; unc-119(ed3)? III;*
OD5560	*bub-1(lt82 [bub-1::gfp])I; ltSi44[pOD1039/pJE170; Pknl-1::KNL-1reencoded(Mutant D85-505)::RFP; cb-unc-119(+)]II; unc-119(ed3)? III;*
OD5561	*ltSi1865[pOD5286/pJMH96; Pknl-1::knl-1::rfp reencoded 6F mutant (F127A, F163A, F218A, F294A, F362A, F446A)::knl-1 3' UTR; cb-unc-119(+)]II*
OD5570	*bub-1(lt82 [bub-1::gfp])I; ltSi1528[pJMH8/pOD3597; Pknl-1::rfp reencoded MELT->MELA motif mutant (T88A, S112A, T202A, T231A, T258A, T281A, T326A, T349A, T376A, T405A, T431A) ; cb-unc119(+)]II; unc-119(ed3)? III;*
OD5571	*bub-1(lt82 [bub-1::gfp])I; ltSi1865[pOD5286/pJMH96; Pknl-1::knl-1::rfp reencoded 6F mutant (F127A, F163A, F218A, F294A, F362A, F446A)::knl-1 3' UTR; cb-unc-119(+)]II; unc-119(ed3)? III;*
OD5587	*ltSi1806 [pOD5287;Pbub-1:::bub-1 reencoded R48A K80A::bub-1 3' UTR; cb-unc-119(+)]II; unc-119(ed3)?III; ltSi560 [oxTi365; oxTi365; pPLG014; Pmex-5::GFP::his-11::tbb-2_3'UTR, tbg-1::gfp::tbb-2_3'UTR; cb-unc-119(+)]V*
OD5588	*ltSi1807 [pOD5288; Pbub-1:::bub-1 reencoded M72R::bub-1 3' UTR; cb-unc-119(+)]II; unc-119(ed3)?III; ltSi560 [oxTi365; oxTi365; pPLG014; Pmex-5::GFP::his-11::tbb-2_3'UTR, tbg-1::gfp::tbb-2_3'UTR; cb-unc-119(+)]V*
OD5590	*ltSi1840[pOD5283/pJMH93; Pknl-1::knl-1::rfp reencoded 6(D/N)TF mutant (N121A, T123A, F127A, D157A, T159A, F163A, D212A, T214A, F218A, D288A, T290A, F294A, N356A, T358A, F362A, N440A, T442A, F446A)::knl-1 3'UTR; cb-unc119(+)]II; unc-119(ed3)?III; ltSi560 [oxTi365; oxTi365; pPLG014; Pmex-5::GFP::his-11::tbb-2_3'UTR, tbg-1::gfp::tbb-2_3'UTR; cb-unc-119(+)]V*
OD5591	*ltSi1865[pOD5286/pJMH96; Pknl-1::knl-1::rfp reencoded 6F mutant (F127A, F163A, F218A, F294A, F362A, F446A)::knl-1 3' UTR; cb-unc-119(+)]II; unc-119(ed3)?III; ltSi560 [oxTi365; oxTi365; pPLG014; Pmex-5::GFP::his-11::tbb-2_3'UTR, tbg-1::gfp::tbb-2_3'UTR; cb-unc-119(+)]V*
OD5592	*ltSi1530[pJMH10/pOD3599; Pknl-1::rfp reencoded 6T mutant (T123A, T159A, T214A, T290A, T358A, T442A); cb-unc119(+)]II; unc-119(ed3)?III; ltSi560 [oxTi365; oxTi365; pPLG014; Pmex-5::GFP::his-11::tbb-2_3'UTR, tbg-1::gfp::tbb-2_3'UTR; cb-unc-119(+)]V*
OD5608	*ltSi1895[pOD5305/pJMH98; Pbub-1::bub-1::GFP(PATC enriched) del2-189 reencoded::bub-1 3'UTR; cb-unc-119(+)]II*
OD5634	*ltSi1898[pOD5304/pJMH97; Pbub-1::bub-1::GFP(PATC enriched) reencoded::bub-1 3'UTR; cb-unc-119(+)]II*
OD5643	*ltSi1895[pOD5305/pJMH98; Pbub-1::bub-1::GFP(PATC enriched) del2-189 reencoded::bub-1 3'UTR; cb-unc-119(+)]II; unc-119(ed3)? III; ltIs37 [pAA64; pie-1/mCHERRY::his-58; unc-119 (+)] IV*
OD5655	*ltSi1898[pOD5304/pJMH97; Pbub-1::bub-1::GFP(PATC enriched) reencoded::bub-1 3'UTR; cb-unc-119(+)]II; unc-119(ed3)? III; ltIs37 [pAA64; pie-1/mCHERRY::his-58; unc-119 (+)] IV*

Table S2: Primers for dsRNA synthesis

Gene No.	Name	Oligonucleotide (5'-3'), #1	Oligonucleotide (5'-3') #2	Template
R06C7.8	*bub-1*	AATTAACCCTCACTAAAGGCCTCATTGAACTTGGAAACC	TAATACGACTCACTATAGGGATCCGAATTGGCACATAA	Genomic DNA
Y54G9A.6	*bub-3*	AATTAACCCTCACTAAAGGACAGTAAACGCAGGGAATGC	TAATACGACTCACTATAGGCAAAGTAGCGTTCGGAGGAG	Genomic DNA
C02F5.1	*knl-1*	AATTAACCCTCACTAAAGGAATCTCGAATCACCGAAATGTC	TAATACGACTCACTATAGGTTCACAAACTTGGAAGCCGCTG	Genomic DNA

Table S3: Antibody list

Antibody Name	Target	Host Species	Stock Concentration (mg/mL)	Source
OD31-B	BUB-1 (aa 287-665)	Rabbit	2.8	Oegema et al. 2001
OD194	GFP	Goat	15	Hyman Lab
DM1α	α-tubulin	Mouse	1	Sigma (T9026)
OD144	BUB-3	Rabbit	2.98	Essex et al, 2009
M2	FLAG	Mouse	1	Sigma (F1804)
OD34	KNL-1 (1-150)	Rabbit	3.8	Desai et al 2003
	Anti-Rabbit IgG, HRP conjugated	Goat	0.8	Jackson Immunoresearch (111-035-003)
	Anti-Mouse IgG, HRP conjugated	Donkey	0.8	Jackson Immunoresearch (715-035-150)
	Anti-Mouse Heavy Chain Specific, HRP conjugated	Goat	0.8	CST (96714)
	Anti-Rabbit IgG, HRP conjugated	Donkey	0.8	Jackson Immunoresearch (711-035-152)
	Anti-Goat IgG, HRP conjugated	Donkey	0.5	Jackson Immunoresearch (705-035-003)
	Anti-Mouse IgG, AP conjugated	Donkey	0.8	Jackson Immunoresearch (715-055-150)