Supplementary Note 1: ***Whole-cell FLIRT of the AB Cell***

To test whether FLIRT-induced thermal changes calculated from the mCherry bioassay for temperature calibration (see above) can inactivate ts mutant proteins *in vivo*, we used the 27 µm mask to perform whole-cell FLIRT of one cell (AB) in 2-cell *C. elegans* embryos with a fast-acting ts mutation in myosin-II/NMY-2 (*nmy-2(ne3409ts)*[28, 29]. Myosin-II is an actin motor protein required for constriction of the actomyosin contractile ring during cytokinesis, the physical division of one cell into two[30-32]. As expected, thermal upshift of *myosin-II(ts)* mutant embryos from 16ºC to 25-26ºC led to complete failure in cell division in all AB cells (0/10 AB cells divided successfully), whereas upshift to 22ºC did not block cell division in AB (11/11 AB cells divided successfully)[28, 29]. To confirm our IR laser calibrations, we then used FLIRT to target the entire AB cell in control and *myosin-II(ts)* embryos to 22ºC or 26ºC, using a 27 µm diameter mask. We found that 9.8 mW of laser power, corresponding to 26ºC, caused division failure in the AB cell in *myosin-II(ts)* mutant embryos (0/14 AB cells divide successfully). Lower IR laser power (6.1 mW), corresponding to a permissive temperature (22ºC), did not cause cytokinesis failure in the AB cell in *myosin-II(ts)* embryos (7/7 AB cells divide successfully).

Supplementary Note 2: ***Whole-cell upshift of the 2-cell embryo.***

Upon whole-embryo thermal upshift of 2-cell embryos from 16ºC to 26ºC, cytokinesis failed in both AB and P1 cells in *myosin-II(ts)* mutants (0/10 and 0/10 AB and P1 cell divided successfully). Thermal upshift to 26ºC did not disrupt cytokinesis in control embryos (7/7 and 7/7 AB and P1 cell divided successfully).

Supplementary Table 1: *Statistical analysis results*

Figure 2a

Unpaired two-tailed t-test		*Control*	*Delta(ts)*	
		P2-Abp (FLIRT)	P2-ABp (FLIRT)	P2-EMS (FLIRT)
Control	P2-ABp (FLIRT)		1.76915E-09	0.691445126
Delta(ts)	P2-ABp (FLIRT)			7.10651E-09
	P2-EMS (FLIRT)			

Unpaired two-tailed t-test		*Control*	*Delta(ts)*	
		P2-Abp (FLIRT)	P2-ABp (FLIRT)	P2-EMS (FLIRT)
Control	P2-Abp (FLIRT)		****	ns
Delta(ts)	P2-ABp (FLIRT)			****
	P2-EMS (FLIRT)			

Figure 2b

Unpaired two-tailed t-test		p-value	Significance
Control	FLIRT vs adjacent membrane partitions	0.1341	ns
cyk-4(ts)	FLIRT vs adjacent membrane partitions	4.28098E-11	****

Supplemental Figure 6a

Unpaired two-tailed t-test		*during cell division*		*2-cell stage*	
		14°C	16°C	14°C	16°C
during cell division	14°C FLIRT	0.2301			
	16°C FLIRT		0.1037		
2-cell stage	14°C FLIRT			0.3917	
	16°C FLIRT				0.4883

Unpaired two-tailed t-test		*during cell division*		*2-cell stage*	
		14°C	16°C	14°C	16°C
during cell division	14°C FLIRT	ns			
	16°C FLIRT		ns		
2-cell stage	14°C FLIRT			ns	
	16°C FLIRT				ns

Supplemental Figure 7e

One way ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	8160.393656	8	1020.049207	93.97507288	5.48897E-35	2.069831642
Within Groups	781.5215317	72	10.85446572			
Total	8941.915187	80				

Supplemental Figure 7e

Tukey's multiple comparisons test		*Control*			*Delta(ts)*					
		16°C	24°C	26°C	16°C	22°C	24°C	26°C	4-8 cell 24°C	4-8 cell 26°C
Control	16°C		0.8163854	0.9121494	0.9998841	0.1525603	<1.00e-15	<1.00e-15	<1.00e-15	<1.00e-15
	24°C			0.9999974	0.9182599	0.9084777	<1.00e-15	<1.00e-15	<1.00e-15	<1.00e-15
	26°C				0.977038	0.7550296	<1.00e-15	<1.00e-15	<1.00e-15	<1.00e-15
Delta(ts)	16°C					0.1858807	<1.00e-15	<1.00e-15	<1.00e-15	<1.00e-15
	22°C						<1.00e-15	<1.00e-15	<1.00e-15	<1.00e-15
	24°C							0.3968903	0.8098216	0.9999955
	26°C								0.0101035	0.708391
	4-8 cell 24°C									0.6765089
	4-8 cell 26°C									

** Note: Tukey's test for multiple comparisons was conducted in GraphPad Prism, and values are reported to 15 digits.*

Tukey's multiple comparisons test		*Control*			*Delta(ts)*					
		16°C	24°C	26°C	16°C	22°C	24°C	26°C	4-8 cell 24°C	4-8 cell 26°C
Control	16°C		ns	ns	ns	ns	****	****	****	****
	24°C			ns	ns	ns	****	****	****	****
	26°C				ns	ns	****	****	****	****
Delta(ts)	16°C					ns	****	****	****	****
	22°C						****	****	****	****
	24°C							ns	ns	ns
	26°C								*	ns
	4-8 cell 24°C									ns
	4-8 cell 26°C									

Supplemental Figure 8c

Unpaired two-tailed t-test		*Control*	*cyk-4(ts)*	
		26°C	16°C	26°C
Control	26°C		0.8974	5.9435E-18
cyk-4(ts)	16°C			3.67078E-17
	26°C			

Unpaired two-tailed t-test		*Control*	*cyk-4(ts)*	
		26°C	16°C	26°C
Control	26°C		ns	****
cyk-4(ts)	16°C			****