

## Supplementary File 2 (A–D). Statistical analysis of FRET data.

### A. T-test values for UNC-70(TSMod) strains.

	TS(Mod) N2 Comma SDR	TS(Mod) N2 Comma Out-SDR	TS(Mod) N2 1.5-fold SDR	TS(Mod) N2 1.5-fold Out-SDR	TS(Mod) <i>pha-1</i> Comma SDR	TS(Mod) <i>pha-1</i> Comma Out-SDR	TS(Mod) <i>pha-1</i> 1.5-fold SDR	TS(Mod) <i>pha-1</i> 1.5-fold Out-SDR
TS(Mod) N2 Comma SDR		P=0.5572 P=0.1683	<b>P=0.0001</b>	P=0.7456	P=1.0000	P=0.4204	P=0.7089	P=0.2554
TS(Mod) N2 Comma Out-SDR			<b>P=0.0004</b>	P=0.7626	P=0.5879	P=0.2288	P=0.5578	P=0.1235
TS(Mod) N2 1.5-fold SDR				<b>P=0.0008</b> <b>P&lt;0.0001</b>	<b>P=0.0002</b>	<b>P&lt;0.0001</b>	<b>P&lt;0.0001</b>	<b>P&lt;0.0001</b>
TS(Mod) N2 1.5-fold Out-SDR					P=0.7650	P=0.2868	P=0.7589	P=0.1593
TS(Mod) <i>pha-1</i> Comma SDR						P=0.3737 P=0.1637	P=0.9745	P=0.3132
TS(Mod) <i>pha-1</i> Comma Out-SDR							P=0.3853	P=0.8861
TS(Mod) <i>pha-1</i> 1.5-fold SDR								<b>P=0.2421</b> <b>P=0.0291</b>
TS(Mod) <i>pha-1</i> 1.5-fold Out-SDR								

P-values in bold are derived from a 2-tailed non-paired T-test. Non-bold P-values are derived from a 2-tailed paired T-test (where applicable). Yellow background indicates very high statistical significance ( $P < 0.001$ ). Green background indicates marginal statistical significance ( $0.01 < P < 0.05$ ). Tests were carried out in the N2 (wild-type) strain or in *pha-1(tm3671)* mutants, as indicated. All tests were performed with the UNC-70(TSMod).

### B. T-test values for controls.

	No Force cont. N2 Comma SDR	No Force cont. N2 Comma Out-SDR	No Force cont. N2 1.5-fold SDR	No Force cont. N2 1.5-fold Out-SDR	5aa-cont. N2 1.5-fold SDR	5aa-cont. N2 1.5-fold Out-SDR	TRAF-cont. N2 1.5-fold SDR	TRAF-cont. N2 1.5-fold Out-SDR
No Force cont. N2 Comma SDR		<b>P=0.6648</b> P=0.1508	<b>P=0.1841</b>	<b>P=0.3688</b>				
No Force cont. N2 Comma Out-SDR			<b>P=0.0726</b>	<b>P=0.1058</b>				
No Force cont. N2 1.5-fold SDR				<b>P=0.6630</b> P=0.0140				
No Force cont. N2 1.5-fold Out-SDR								
5aa-cont. N2 1.5-fold SDR						<b>P=0.6618</b> P=0.0875		
5aa-cont. N2 1.5-fold Out-SDR								
TRAF-cont. N2 1.5-fold SDR								<b>P=0.2452</b> P=0.1080
TRAF-cont. N2 1.5-fold Out-SDR								

P-values in bold are derived from a 2-tailed non-paired T-test. Non-bold P-values are derived from a 2-tailed paired T-test (where applicable). All tests were carried out in the N2 (wild-type) strain. Green background indicates marginal statistical significance ( $0.01 < P < 0.05$ ). Tests were performed with the UNC-70(N-TSMod) (No Force Control), the 5aa spacer, or the TRAF spacer.

**C. T-test values for UNC-70(TSMod) versus UNC-70(N-TSMod).**

	No Force cont. N2 Comma SDR	No Force cont. N2 Comma Out-SDR	No Force cont. N2 1.5-fold SDR	No Force cont. N2 1.5-fold Out-SDR	TS(Mod) N2 Comma SDR	TS(Mod) N2 Comma Out-SDR	TS(Mod) N2 1.5-fold SDR	TS(Mod) N2 1.5-fold Out-SDR
No Force cont. N2 Comma SDR					<b>P=0.0149</b>	<b>P=0.0087</b>	<b>P&lt;0.0001</b>	<b>P=0.0079</b>
No Force cont. N2 Comma Out-SDR					<b>P=0.0049</b>	<b>P=0.0032</b>	<b>P&lt;0.0001</b>	<b>P=0.0025</b>
No Force cont. N2 1.5-fold SDR					P=0.1168	<b>P=0.0415</b>	<b>P&lt;0.0001</b>	P=0.0529
No Force cont. N2 1.5-fold Out-SDR					P=0.0736	<b>P=0.0434</b>	<b>P&lt;0.0001</b>	<b>P=0.0307</b>
TS(Mod) N2 Comma SDR								
TS(Mod) N2 Comma Out-SDR								
TS(Mod) N2 1.5-fold SDR								
TS(Mod) N2 1.5-fold Out-SDR								

P-values in bold are derived from a 2-tailed non-paired T-test. All tests were carried out in the N2 (wild-type) strain. Green background indicates marginal statistical significance ( $0.01 < P < 0.05$ ). Red background indicates high statistical significance ( $P < 0.01$ ). Yellow background indicates very high statistical significance ( $P < 0.001$ ). The detected differences between UNC-70(TSMod) and UNC-70(N-TSMod) (No Force control) in SDR and Out-SDR regions in early comma stages and in the Out-SDR region at the 1.5-fold stage may indicate that these regions are normally under low-to-moderate levels of tension that are independent of pharyngeal attachment, which are detected by the UNC-70(TSMod).

#### D. Data used for calculations.

Strain Background	TS module	Stage	Region	Mean	SD	N
N2	UNC-70(TSMod)	Early Comma	SDR	0.2591	0.0673	27
N2	UNC-70(TSMod)	Early Comma	Non-SDR	0.2467	0.0858	27
N2	UNC-70(TSMod)	1.5-Fold	SDR	0.1973	0.0517	35
N2	UNC-70(TSMod)	1.5-Fold	Non-SDR	0.2525	0.0772	35
<i>pha-1(tm3671)</i>	UNC-70(TSMod)	Early Comma	SDR	0.2591	0.0630	20
<i>pha-1(tm3671)</i>	UNC-70(TSMod)	Early Comma	Non-SDR	0.2762	0.0764	20
<i>pha-1(tm3671)</i>	UNC-70(TSMod)	1.5-Fold	SDR	0.2585	0.0664	31
<i>pha-1(tm3671)</i>	UNC-70(TSMod)	1.5-Fold	Non-SDR	0.2799	0.0759	31
N2	UNC-70(N-TSMod)	Early Comma	SDR	0.3048	0.0593	23
N2	UNC-70(N-TSMod)	Early Comma	Non-SDR	0.3125	0.0604	23
N2	UNC-70(N-TSMod)	1.5-Fold	SDR	0.2840	0.0576	37
N2	UNC-70(N-TSMod)	1.5-Fold	Non-SDR	0.2901	0.0622	37
N2	UNC-70(TRAF)	1.5-Fold	SDR	0.1068	0.0548	11
N2	UNC-70(TRAF)	1.5-Fold	Non-SDR	0.1337	0.0455	11
N2	UNC-70(5aa)	1.5-Fold	SDR	0.4241	0.0801	21
N2	UNC-70(5aa)	1.5-Fold	Non-SDR	0.4356	0.0888	21