

Fig. S1

$\text{*fold change} = (\text{observation} - \text{mean}(\text{Un-Trans})) / (\text{mean}(\text{No Intron}) - \text{mean}(\text{Un-Trans}))$

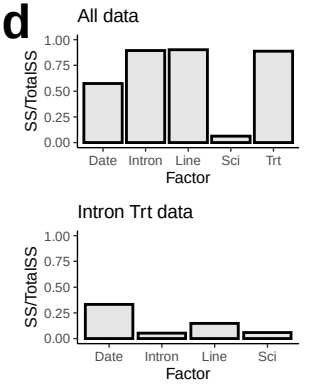
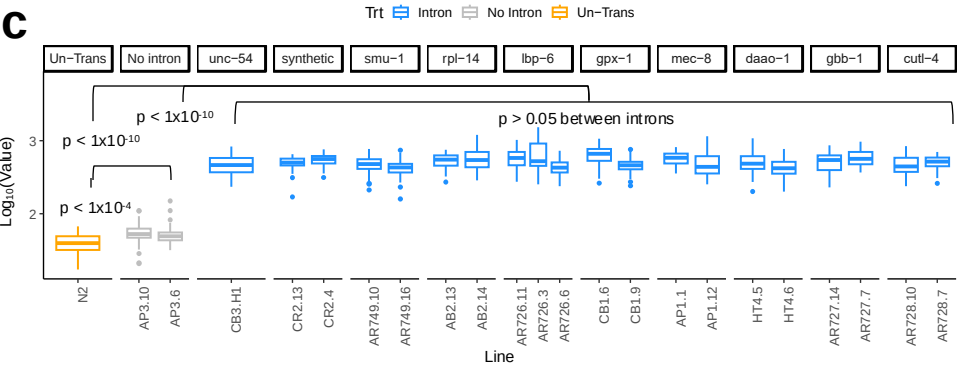
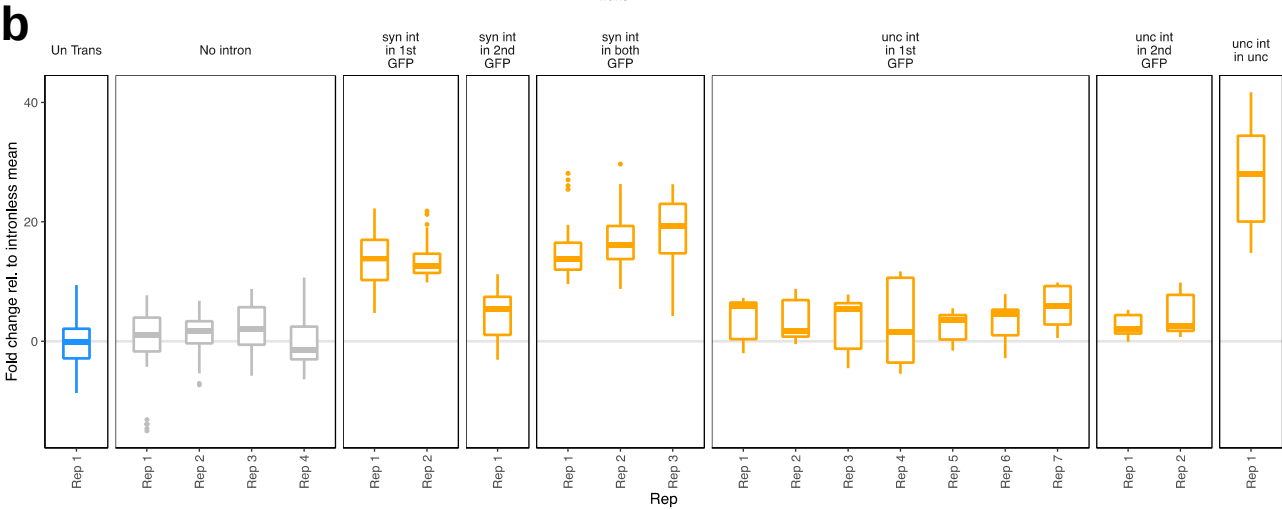
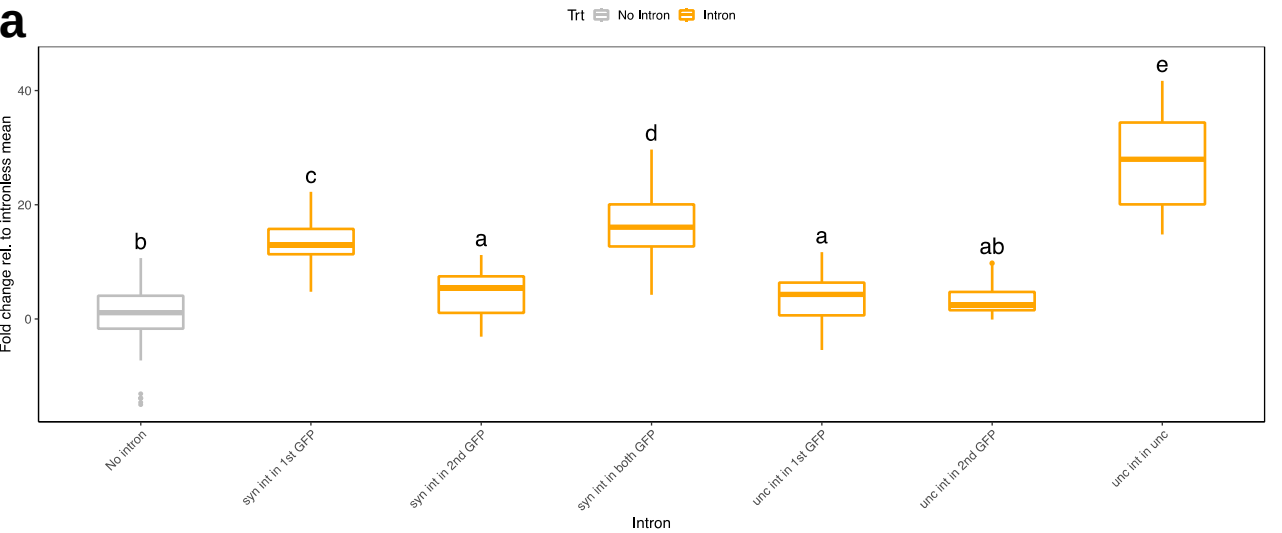
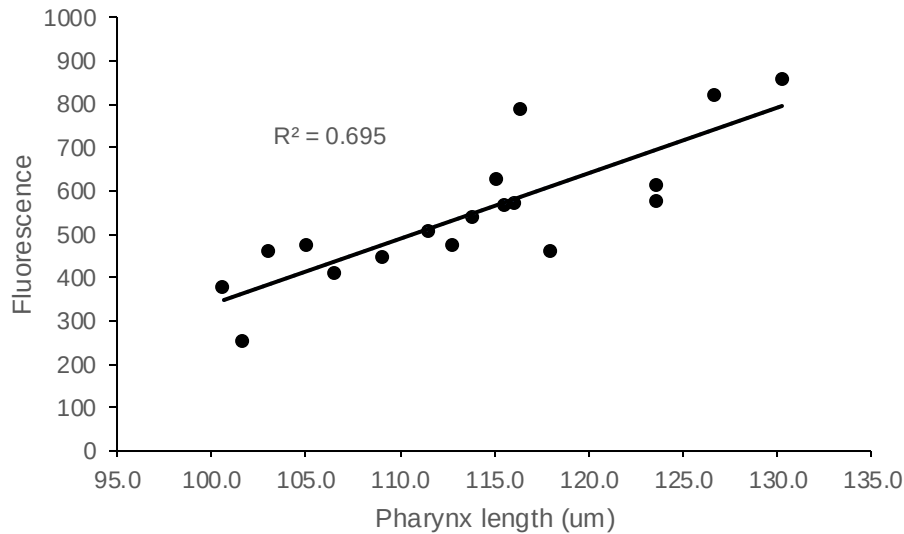


Fig. S1. Statistical analysis of the constructs shown in Fig. 1. a. Combined results for all lines containing the constructs shown in Figs. 1a and 1c. The p-values for all pairwise comparisons between constructs are in Supplementary Table S1. b. Results shown separately for each independent single-copy transgenic line (Rep) containing the constructs shown in Figs. 1a and 1c. c. Summary of fluorescence readings for all single-copy transgenic lines containing the constructs shown in Fig. 1e. Note that the scale is logarithmic to more clearly display the difference between untransformed worms and those containing the intronless control. The source of the intron in each construct is shown along the top of the figure and the name of each line is at the bottom. d. The variability in the data shown in c contributed by the date of the experiment, the intron present in the construct, the variation between lines containing the same construct, the scientist performing the experiment, or the treatment (Trt, meaning untransformed, intronless, or intron-containing). The upper graph compares all lines, while the lower graph shows only lines with an intron-containing transgene. SS is sum of squares.

Fig. S2



Supplementary Fig. S2. GFP fluorescence correlates with pharynx length. Fluorescence measurements of 18 worms of line AR726.3 taken from the same plate on April 27, 2023 are shown relative to the length of the pharynx of each worm measured along the esophagus from the tip of the nose to the base of the grinder.

Table S1. Statistical comparison of fluorescence in worms containing the constructs shown in Figures 1a and 1c.

Pairwise comparison	Difference	Lower	Upper	Adj. p value
No intron vs synthetic introns in 1st GFP	12.57	10.57	14.57	1.92E-10
No intron vs synthetic introns in 2nd GFP	3.25	0.56	5.94	0.007
No intron vs synthetic introns in both GFPs	15.76	14.05	17.47	1.92E-10
No intron vs unc intron in unc sequences	26.54	24.21	28.86	1.92E-10
No intron vs unc intron in 1st GFP	2.59	1.07	4.12	1.33E-05
No intron vs unc intron in 2nd GFP	2.44	-0.04	4.92	0.058
Synthetic introns in 1st GFP vs synthetic introns in 2nd GFP	9.32	6.21	12.43	1.92E-10
Synthetic introns in 1st GFP vs synthetic introns in both GFPs	3.19	0.88	5.51	0.001
Synthetic introns in 1st GFP vs unc intron in unc sequences	13.97	11.17	16.76	1.92E-10
Synthetic introns in 1st GFP vs unc intron in 1st GFP	9.98	7.80	12.16	1.92E-10
Synthetic introns in 1st GFP vs unc intron in 2nd GFP	10.13	7.20	13.06	1.92E-10
Synthetic introns in 2nd GFP vs synthetic introns in both GFPs	12.51	9.58	15.45	1.92E-10
Synthetic introns in 2nd GFP vs unc intron in unc sequences	23.29	19.96	26.62	1.92E-10
Synthetic introns in 2nd GFP vs unc intron in 1st GFP	0.66	-2.17	3.48	0.99
Synthetic introns in 2nd GFP vs unc intron in 2nd GFP	0.81	-2.63	4.25	0.99
Synthetic introns in both GFPs vs unc intron in unc sequences	10.77	8.17	13.37	1.92E-10
Synthetic introns in both GFPs vs unc intron in 1st GFP	13.17	11.25	15.09	1.92E-10
Synthetic introns in both GFPs vs unc intron in 2nd GFP	13.32	10.58	16.07	1.92E-10
Unc intron in unc sequences vs unc intron in 1st GFP	23.94	21.46	26.42	1.92E-10
Unc intron in unc sequences vs unc intron in 2nd GFP	24.10	20.93	27.26	1.92E-10
Unc intron in 1st GFP vs unc intron in 2nd GFP	0.15	-2.48	2.78	0.999998

Table S2. Data for each individual line tested.

Intron	Description	Construct	Line	Fluorescence units			Relative fluorescence	Relative RNA
				Ave.	St. Dev.	n		
	Wild-type untransformed worms		N2	40	12	47	0	
none	Intronless control	AP3		53	16	149	1 +/- 1.2	1.03 +/- 0.07
			RL381 AP3.6	52	16	76	0.9 +/- 1.2	1
			RL382 AP3.10	55	16	73	1.1 +/- 1.2	1.05 +/- 0.09
unc-54	In natural location	CB3		487	149	59	33.6 +/- 11.2	16.7 +/- 2.9
			RL377 CB3.H1	487	149	59	33.6 +/- 11.2	16.7 +/- 2.9
			RL421 CB3.10 *2-copy	1402	447	41	102.2 +/- 33.5	27.0 +/- 2.8
Synthetic	Small synthetic intron	CR2		526	95	112	36.5 +/- 7.1	14.2 +/- 2.9
			RL401 CR2.4	547	95	58	38.0 +/- 7.1	14.8 +/- 3.9
			RL386 CR2.13	504	90	54	34.8 +/- 6.8	13.4 +/- 0.6
From smu-1	PATC intron	AR749		455	116	111	31.1 +/- 8.7	9.8 +/- 2.1
			RL394 AR749.10	484	129	47	33.3 +/- 9.7	10.7 +/- 2.3
			RL395 AR749.16	433	101	64	29.5 +/- 7.6	9.0 +/- 2.3
From rpl-14	From gene expressed at high level	AB2		561	170	97	39.1 +/- 12.7	13.8 +/- 2.3
			RL390 AB2.13	544	125	48	37.8 +/- 9.4	13.5 +/- 3.2
			RL392 AB2.14	578	205	49	40.4 +/- 15.4	14.2 +/- 0.9
From lbp-6	From gene expressed at high level	AR726		559	243	146	38.9 +/- 18.1	13.8 +/- 9.0
			RL425 AR726.3	695	344	41	49.1 +/- 25.8	13.3 +/- 5.0
			RL379 AR726.6	442	118	60	30.2 +/- 8.9	11.9 +/- 8.1
			RL423 AR726.11	590	176	45	41.3 +/- 13.2	15.8 +/- 12.7
From gpx-1	From gene expressed at medium level	CB1		553	170	105	38.5 +/- 12.8	12.4 +/- 2.7
			RL420 CB1.6	651	186	47	45.8 +/- 14.0	11.4 +/- 4.3
			RL385 CB1.9	473	102	58	32.5 +/- 7.6	13.4 +/- 0.1
From mec-8	From gene expressed at medium level	AP1		546	180	92	38.0 +/- 13.5	13.2 +/- 3.7
			RL399 AP1.1	575	111	49	40.1 +/- 8.3	11.8 +/- 5.4
			RL422 AP1.12	514	233	43	35.5 +/- 17.5	14.6 +/- 2.1
From daao-1	From gene expressed at low level	HT4		477	141	129	32.8 +/- 10.6	13.1 +/- 3.6
			RL387 HT4.5	508	143	71	35.1 +/- 10.7	15.4 +/- 3.3
			RL388 HT4.6	439	130	58	29.9 +/- 9.8	10.8 +/- 2.6
From gbb-1	From gene expressed at low level	AR727		567	160	104	39.6 +/- 12.0	16.1 +/- 5.3
			RL378 AR727.7	600	157	60	42.0 +/- 11.8	16.9 +/- 7.9
			RL383 AR727.14	522	154	44	36.2 +/- 11.6	15.3 +/- 4.5
From cutl-4	From gene expressed at very low level	AR728		502	119	101	34.6 +/- 8.9	15.1 +/- 6.9
			RL400 AR728.7	518	95	55	35.9 +/- 7.2	18.1 +/- 10.2
			RL449 AR728.10	482	142	46	33.2 +/- 10.6	12.1 +/- 1.2

The relative fluorescence and relative RNA are given as the mean +/- standard deviation.

The numbers in bold include all the data from single-copy worms containing the same construct.

