

Supplementary Table 1. Percentage of individuals showing handedness defects in various mutants or expression lines.

	embryo					adult			
	foregut		midgut inverse	hindgut inverse	midgut and/or hindgut deformed	hindgut		testis	
	inverse	deformed				inverse	deformed	inverse	deformed
wild-type	0.5(1/200)	2.5(5/200)	0.4(1/247)	0.4(1/247)	2.4(7/247)	0.0(0/55)	0.0(0/55)	0.0(0/56)	7.1(4/56)
<i>Myo31DF^{souther}</i>	0.0(0/51)	0.0(0/51)	75.7(28/37)	75.7(28/37)	5.4(2/37)	40.7(22/54)	29.6(16/54)	73.7(28/38)	18.4(7/38)
<i>Myo31DF^{L152}</i>	1.9(1/51)	0.0(0/51)	82.0(105/128)	82.0(105/128)	6.25(8/128)	85.7(30/35)	2.9(1/35)	78.7(37/47)	19.1(9/47)
<i>Myo31DF^{L152} / DF(2L)J2</i>	—	—	57.7(30/52)	61.5(32/52)	6.0(3/52)	—	—	—	—
<i>Myo31DF^{souther}(+maternal)</i>	—	—	62.8(44/70)	64.2(45/70)	14.2(10/70)	—	—	—	—
<i>Myo31DF^{souther}(-maternal)</i>	—	—	0.0(0/90)	0.0(0/90)	1.1(1/90)	—	—	—	—
<i>UAS-Myo31DF/da-Gal4</i>	97.6(40/41)	2.4(1/41)	0.0(0/41)	0.0(0/41)	0.0(0/41)	—	—	—	—
<i>UAS-2xMyo31DF^{RNAi/+}</i>	—	—	—	—	—	0.0(0/10)	0.0(0/10)	0.0(0/10)	0.0(0/10)
<i>UAS-2xMyo31DF^{RNAi} / byn-Gal4</i>	—	—	0.0(0/20)	0.0(0/20)	0.0(0/20)	82.4(14/17)	5.9(1/17)	0.0(0/17)	0.0(0/17)
<i>UAS-gfp-moesin / byn-Gal4</i>	0.0(0/41)	0.0(0/41)	29.4(5/17)	47.5(28/59)	0.0(0/59)	—	—	—	—
<i>Myo31DF^{L152}; UAS-gfp-moesin / byn-Gal4</i>	0.0(0/8)	0.0(0/8)	60.0(3/5)	52.8(19/36)	0.0(0/36)	—	—	—	—
<i>UAS-gfp-moesin / NP2432</i>	—	—	13.1(8/61)	11.3(9/80)	2.5(2/80)	—	—	—	—
<i>UAS-gfp-moesin / 48Y</i>	—	—	0.0(0/27)	0.0(0/80)	1.3(1/80)	—	—	—	—
<i>UAS-Rac1 N17 / NP2432</i>	—	—	8.2(7/85)	8.2(7/85)	1.2(1/85)	—	—	—	—
<i>UAS-Rho N19 / NP2432</i>	—	—	28.9(22/76)	21.0(16/76)	9.2(7/76)	—	—	—	—
<i>UAS-Cdc42 F89 / NP2432</i>	—	—	1.7(1/58)	3.4(2/58)	3.4(2/58)	—	—	—	—
<i>UAS-Myo61F / byn-Gal4</i>	—	—	100.0(17/17)	100(36/36)	0.0(0/36)	—	—	—	—
<i>UAS-Myo31DF / byn-Gal4</i>	—	—	0.0(0/47)	0.0(0/47)	0.0(0/47)	—	—	—	—
<i>UAS-dsRNA Myo61F / +</i>	0.0(0/92)	0.0(0/92)	0.0(0/97)	0.0(0/97)	1.0(1/97)	—	—	—	—
<i>UAS-dsRNA Myo61F / P{GAL4-nos.NGT}40</i>	1.5(1/65)	0.0(0/65)	7.7(5/65)*	0.0(0/65)	1.5(1/65)	—	—	—	—
Rescue experiments (<i>Myo31DF^{souther}</i> background)									
<i>UAS-Myo31DF / +</i>	—	—	46.3(37/80)	46.3(37/80)	2.5(2/80)	—	—	—	—
<i>UAS-Myo31DF / byn-Gal4</i>	—	—	0.0(0/22)	0.0(0/22)	0.0(0/22)	—	—	—	—
<i>UAS-Myo31DF / NP2432</i>	—	—	0.0(0/40)	0.0(0/40)	7.5(3/40)	—	—	—	—
<i>UAS-Myo31DF / 48Y</i>	—	—	42.1(16/38)	42.1(16/38)	7.9(3/38)	—	—	—	—
<i>UAS-Myo31DF / how^{24B}</i>	—	—	58.1(18/31)	58.1(18/31)	3.2(1/31)	—	—	—	—
<i>UAS-Myo31DF-GFP / byn-Gal4</i>	—	—	6.5(2/31)	9.7(3/31)	3.2(1/31)	—	—	—	—

The percentage of embryos showing defects in handedness (inverse) or deformation of organs (deformed) is shown. Genotypes of the examined embryos and adults are indicated on the left. Actual numbers of scored embryos and adults are shown in parentheses. Dashes: values not determined. Asterisk: embryos showing partial inversion of midgut handedness are included.

Supplementary Table 2. The phenocritical period for inducing the LR defect by knockdown of Myo31DF and by GFP-moesin misexpression.

		<i>GAL80^{ts/+}; UAS-2xMyo31DF^{RNAi} / byn-GAL4</i>	<i>GAL80^{ts/+}; UAS-gfp-moesin / byn-GAL4</i>	<i>GAL80^{ts/+}; + / byn-GAL4</i>
	18°C	0.0 (0/16)	0.0 (0/15)	—
30°C	0-24 HAP	66.6 (12/18)	47.6 (10/21)	0.0 (0/20)
	24-48 HAP	24.0 (6/25)	4.3 (1/23)	—
	48-72 HAP	0.0 (0/19)	0.0 (0/17)	—

The percentage of adults showing defects in handedness is shown. Genotypes of the adults are indicated at the top. The culture conditions are given at the left. Actual numbers of scored adults are shown in parentheses. Dashes: values not determined.