

Figure S1: Myoblasts on early genital discs undergo mitosis

(A–A'') Genital discs at 16 hours APF. (B–B'') Myoblasts at 16 hours APF. (A, B) Merged photo of A' and A'', and B' and B'', respectively. (A', B') Expression of *DMef2*-driven mCD8-GFP (green) as a marker for myoblasts. (A'', B'') Staining with anti-phospho Histone H3 (Ser10) Mitosis Marker (red). Proliferation occurred in the epithelium of the genital disc as well as in the myoblasts (arrowheads). Scale bars: 20 μ m.

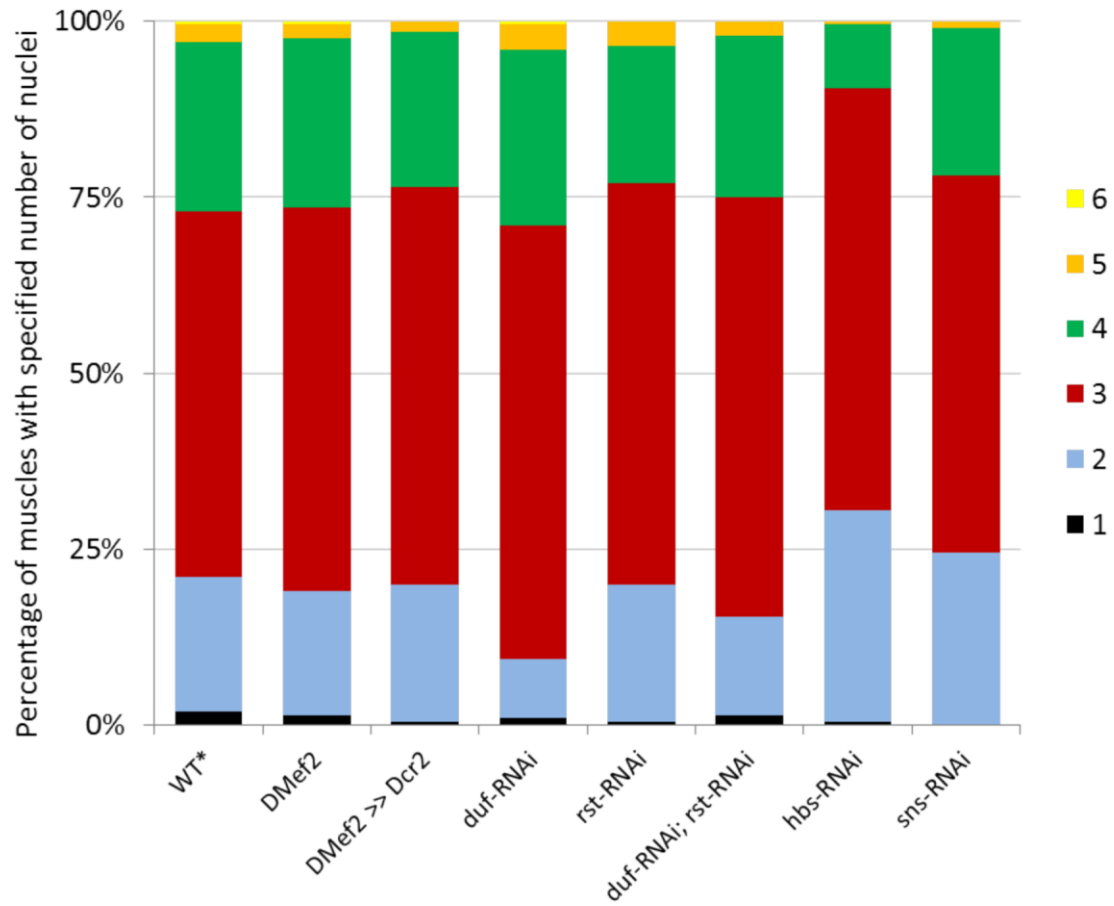


Figure S2: Number of nuclei in testes muscles in control flies

The number of nuclei in nascent myotubes was determined in the given genetic backgrounds at about 42 hours APF. In each case, 200 muscle cells (100% of the cells) were counted; the length of each colour-coded bar in each stack indicates the percentage of muscles with the specified number of nuclei. *Wild-type data are also shown in Fig. 5.

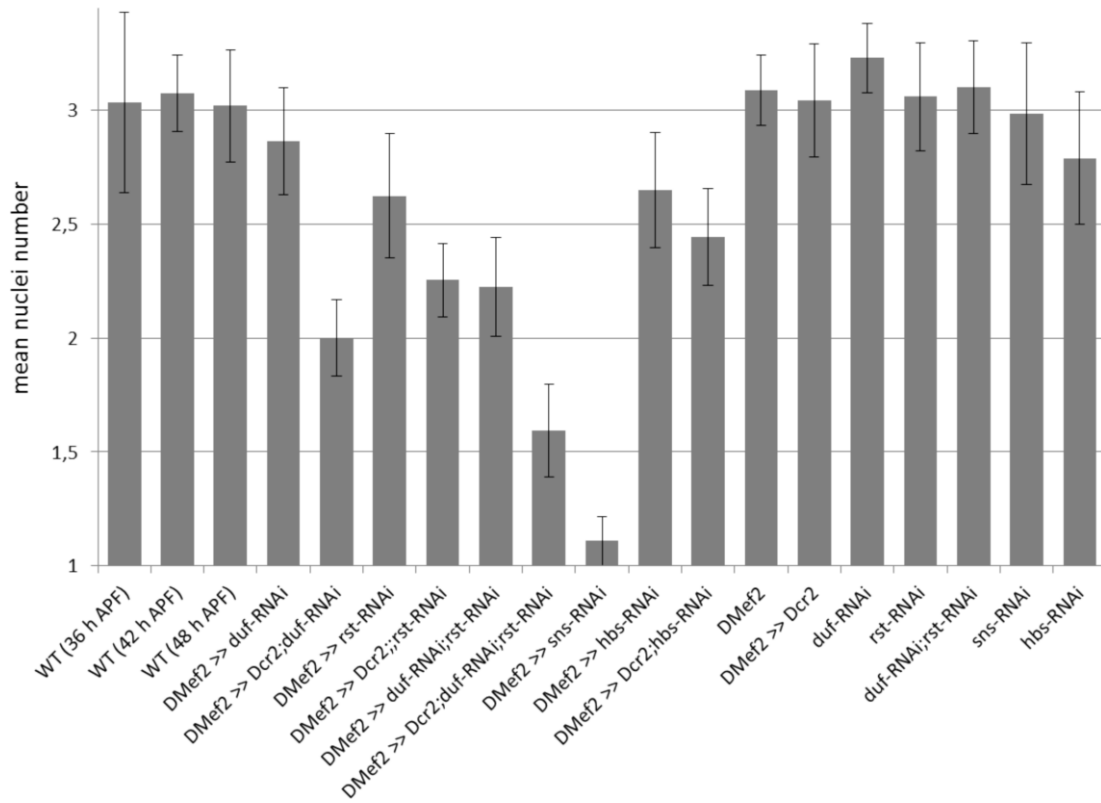


Figure S3: Mean nuclei number in RNAi-mediated knock-down flies and controls

The mean number of nuclei in nascent myotubes in the given genetic backgrounds as listed in Table S2. Error bars represent standard deviation.

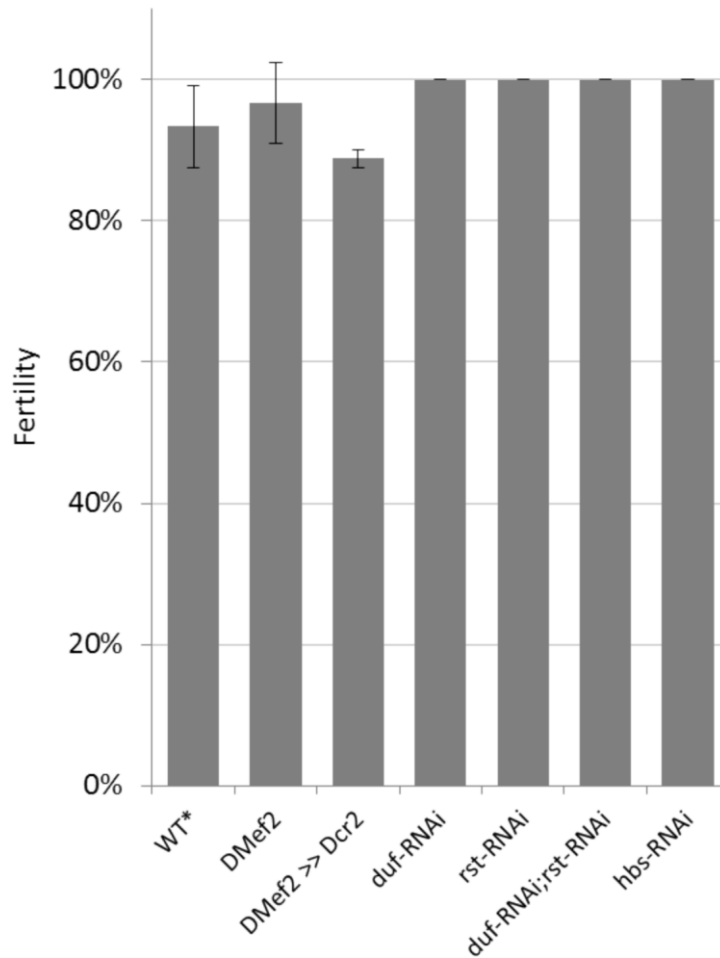


Figure S4: Fertility of control males

The fertility of males in the given different genetic background was tested in up to 30 single crossing with wild-type females. Error bars represent standard deviation. *Wild-type data are also shown in Fig. 6.

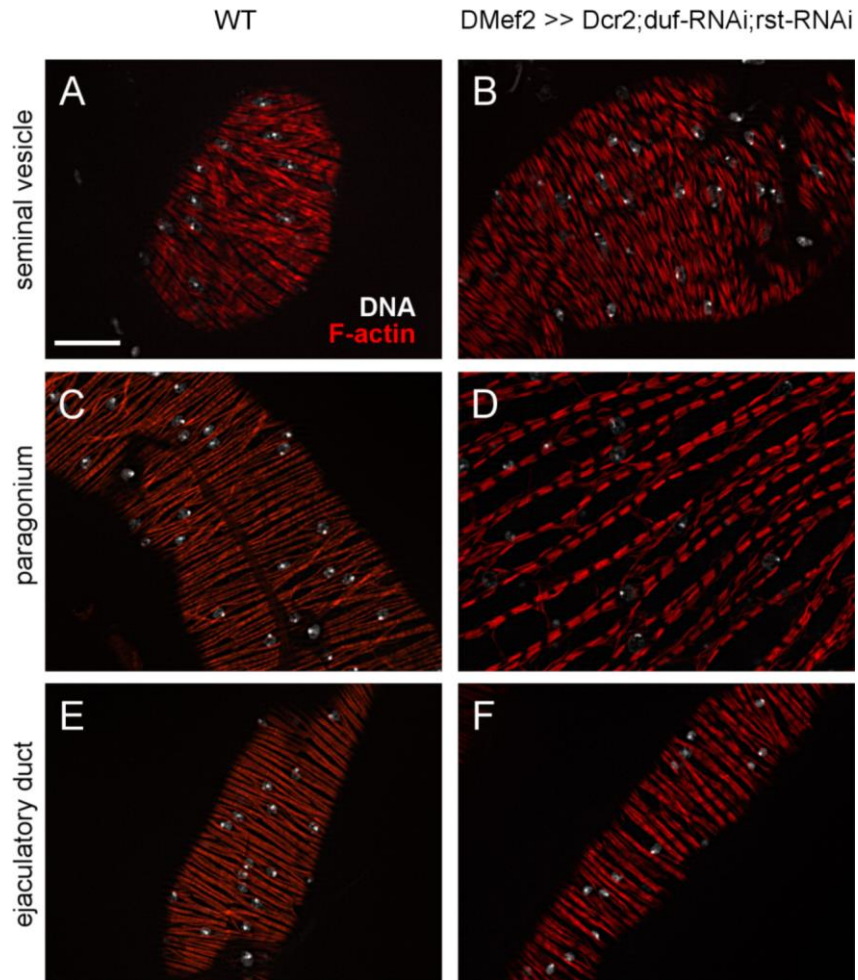


Figure S5: Sarcomere arrangement of reproductive muscles appears normal in *duf* and *rst* knock-down flies

Comparison of sarcomere arrangement in (A, C, E) wild-type males and (B, D and F) *DMef2*-driven *Dcr2; duf-RNAi; rst-RNAi* double knock-down males. The filament arrangement of the (A, B) seminal vesicle muscles, (C, D) paragonia muscles and (E, F) ejaculatory duct muscles of the wild-type and the knock-down males are similar. No alterations are visible. F-Actin was stained with Phalloidin; Hoechst was used to label DNA in nuclei. All pictures are optical sections. Scale bar: 20 μ m.



Legend to movie 1:

The mature sperm of *duf;rst* double knock down males are motile. Seminal vesicles of adult males (*DMef2* driven *Dcr-2*; *dufRNAi*; *rstRNAi*) were squashed in PBS, so that numerous sperm were released. To visualize the motility of sperm, a movie was taken at a Zeiss AxioObserver Z.1 at 10fold increased time lapse recording.

Table S1: RT-PCR results of FC-specific determination factors of embryonic myogenesis in genital disc myoblasts

Gene	Transcription ^a				Reference
	8 hours APF	16 hours APF	24 hours APF	30 hours APF	
<i>vestigial</i>	–	–	+	+	Deng et al., 2009
<i>ladybird early</i>	+	+	+	+	Jagla et al., 1998
<i>even skipped</i>	+	+	+	+	Frasch et al., 1987; Su et al., 1999
<i>apterous</i>	+	+	+	+	Bourgouin et al., 1992
<i>slouch (S59)</i>	+	–	–	–	Dohrmann et al., 1990; Knirr et al., 1999
<i>kriippel</i>	–	–	–	–	Ruiz-Gomez et al., 1997
<i>drop</i>	+	+	+	+	Nose et al., 1998
<i>nautilus</i>	+	+	+	+	Balagopalan et al., 2001; Keller et al., 1998

^a +, transcript detected; –, no transcripts detected

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Frasch, M., Hoey, T., Rushlow, C., Doyle, H. and Levine, M. (1987). Characterization and localization of the even-skipped protein of *Drosophila*. *EMBO J* **6**, 749-759.

Jagla, T., Bellard, F., Lutz, Y., Dretzen, G., Bellard, M. and Jagla, K. (1998). ladybird determines cell fate decisions during diversification of *Drosophila* somatic muscles. *Development* **125**, 3699-3708.

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Knirr, S., Azpiazu, N. and Frasch, M. (1999). The role of the NK-homeobox gene slouch (S59) in somatic muscle patterning. *Development* **126**, 4525-4535.

Nose, A., Isshiki, T. and Takeichi, M. (1998). Regional specification of muscle progenitors in *Drosophila*: the role of the msh homeobox gene. *Development* **125**, 215-223.

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Table S2: Detailed summary of nuclei numbers in RNAi-mediated knock-down flies and controls and their fertility

Fly strain	Nuclei number in testes muscles						Mean nuclei number ± s.d.	Male fertility		Viability	Ability to fly
	1	2	3	4	5	6		Number of crossings	Crossings with progeny		
WT (36 h APF)	0	46	106	44	3	1	3,035±0.40				
WT (42 h APF)	4	38	104	48	5	1	3.075±0.17	30	28	viable	yes
WT (48 h APF)	1	44	108	44	3	0	3.02±0.25				
DMef2 >> duf-RNAi	2	63	97	36	2	0	2.865 ±0.23	30	29	viable	yes
DMef2 >> Dcr2;duf-RNAi	31	140	27	2	0	0	2 ±0.17	29	29	viable	yes
DMef2 >> rst-RNAi	4	79	105	12	0	0	2.625 ±0.27	30	30	viable	yes
DMef2 >> Dcr2;;rst-RNAi	13	127	56	4	0	0	2.255 ±0.17	28	26	viable	yes
DMef2 >> duf-RNAi;rst-RNAi	17	124	56	3	0	0	2.225 ±0.22	30	29	viable	yes
♂ DMef2 >> Dcr2;duf-RNAi;rst-RNAi	95	91	14	0	0	0	1.595 ±0.20	29	9	viable	flightless
♀ DMef2 >> Dcr2;duf-RNAi;rst-RNAi	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	28	2	viable	flightless
DMef2 >> sns-RNAi	181	16	3	0	0	0	1.11 ±0.11	n.d.	n.d.	pupal lethal	n.d.
DMef2 >> hbs-RNAi	11	72	100	16	1	0	2.62 ±0.25	30	30	viable	yes
DMef2 >> Dcr2;hbs-RNAi	10	102	77	11	0	0	2.445 ±0.21	30	30	viable	yes
DMef2	3	35	109	48	4	1	3.09 ±0.15	30	29	viable	yes
DMef2 >> Dcr2	1	39	113	44	3	0	3.045 ±0.25	27	24	viable	yes
duf-RNAi	2	17	123	50	7	1	3.23 ±0.15	29	29	viable	yes
rst-RNAi	1	39	114	39	7	0	3.06 ±0.24	29	29	viable	yes
duf-RNAi;rst-RNAi	3	28	119	46	4	0	3.1 ±0.20	30	30	viable	yes
sns-RNAi	0	49	107	42	2	0	2.985 ±0.31	n.d.	n.d.	n.d.	n.d.
hbs-RNAi	1	60	120	18	1	0	2.79 ±0.29	30	30	viable	yes

n.d.: not determined; ± s.d.: standard deviation

Table S3: Oligonucleotides used for RT-PCR analyses

Oligonucleotide	Sequence
NotchRTfwd	CGAGCTATGAGGATTGCATC
NotchRTrev	CATGCCTCATCGCCTGAATGTG
DeltaRTfwd	GTACCTTCTCGCTGATCGTCG
DeltaRTfwd	GTGACAGTAATCGCCCTGCC
LmdRTfwd	GCAATAAAGCATTCTCGCGTTTG
LmdRTrev	GCAATTGCTGCGGTCTGTCGTC
dufRT1fwd	GCACGAGATTCAACAGTCGCAG
dufRT1rev	GCTGCTGGCGGTCAAGTTGG
rstRT1fwd	TGCCACCGAGGATCGCAAAG
rstRT1rev	GCAGCGGTATCACCGTGTAC
snsRT1fwd	CTGCACATCAAGGACGCGAAG
snsRT1rev	CTCGTAGGTCAGCGAATCAATC
hbsRT1fwd	GTGTGGAGCGCAGTGATATTGG
hbsRT1rev	CTTGAGATCCTTGCCATGACGAC
Tplsense	GAGTGCATCACGTGAATGGG
Tplantisense	GCCACGCTGATCCGCATTC
VgRTfwd	GACTTGTATGGCACCGCGAC
VgRTrev	GATTCCTGTACTTGCGCCACCTG
KrRTfwd	GATGTCCATATCAATGCTTC
KrRTrev	CCATGAATGCGGCAGCTTGAC
LbeRTfwd	GAGCGTTCCCAGCGATCGAG
LbeRTrev	CGTTCGACAGTCCCAGTGAG
EveRTfwd	CTACAACATGGAGAGCCACCATG
EveRTrev	GTGCTCTCCGGGAGGTTTCAG
ApRTfwd	CATCCAGTATGAGCGCCACCTAC
ApRTrev	CATGGTACGCAGCTGGTGATGC
SlouRTfwd	GACATGGATCAGGACGATATG
SlouRTrev	GTGGGGGAGTTGACATCCATGC
NauRTfwd	GCAGTCTCAGCTCCGAGGAGC
NauRTrev	CGATATACTCGATGGCATTGC
Dr-mshRTfwd	CTCTCACATGCAATCCACGATAC
Dr-mshRTrev	GTTCGGCGATGCTCAGGTTACTG