

**Supplemental information**

**Axonal plasticity in response to active forces  
generated through magnetic nano-pulling**

**Alessandro Falconieri, Sara De Vincentiis, Valentina Cappello, Domenica Convertino, Ravi Das, Samuele Ghignoli, Sofia Figoli, Stefano Luin, Frederic Català-Castro, Laura Marchetti, Ugo Borello, Michael Krieg, and Vittoria Raffa**

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**Table S1. qPCR analysis (related to Figure 1)**

Group	Replicate	Cycle, $\beta$ -actin mRNA	Cycle, H1 mRNA
Control, A	1	34.38	-
	2	38.22	-
	3	36.77	-
Stretch, A	1	34.87	-
	2	38.87	-
	3	38.16	-
Control, S	1	19.97	24.92
	2	19.61	24.40
	3	19.80	25.33
Stretch, S	1	19.18	24.54
	2	17.63	23.46
	3	19.68	25.230

**Table S2. GO of cellular Component (CC) (related to Figure 1)**

GOTERM_cc_direct	Term	Gene count	<i>P</i> value
GO:0005737	cytoplasm	313	3.00E-05
GO:0005765	lysosomal membrane	24	4.90E-05
GO:0005794	Golgi apparatus	74	8.80E-05
GO:0005768	endosome	39	4.20E-04
GO:0031902	late endosome membrane	12	6.30E-04
GO:0005764	lysosome	26	1.40E-03
GO:0016020	membrane	314	1.60E-03
GO:0005739	mitochondrion	91	2.70E-03
GO:0005856	cytoskeleton	61	7.40E-03
GO:0005815	microtubule organizing center	14	7.90E-03

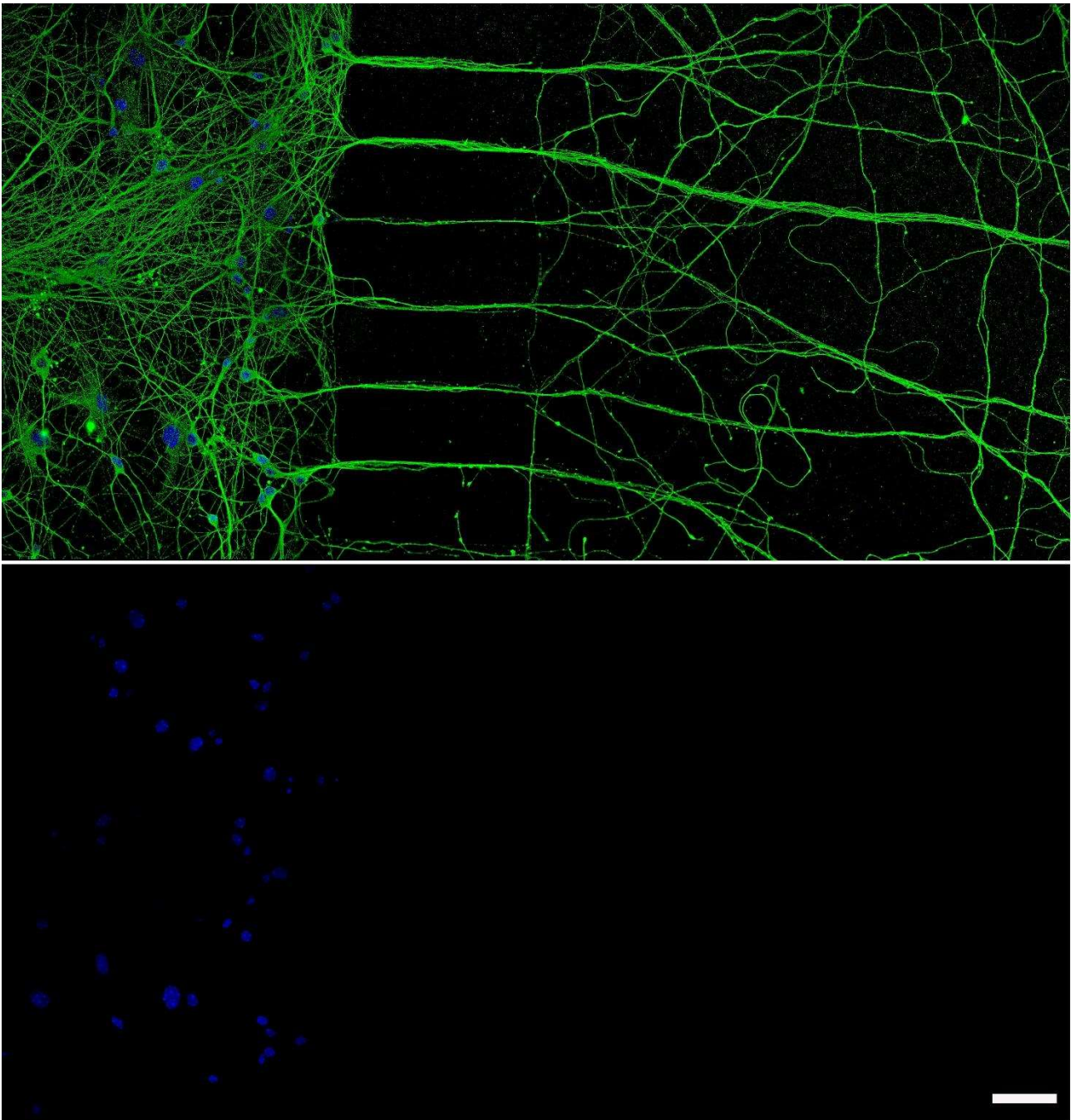
GO:0031225	anchored component of membrane	13	9.40E-03
GO:0005622	intracellular	81	1.30E-02
GO:0005783	endoplasmic reticulum	67	2.40E-02
GO:0005770	late endosome	11	3.30E-02
GO:0045202	synapse	28	6.40E-02
GO:0005829	cytosol	83	7.40E-02

**Table S3. Annotated genes dysregulated in GO:0005856 (cytoskeleton) (related to Figure 1)**

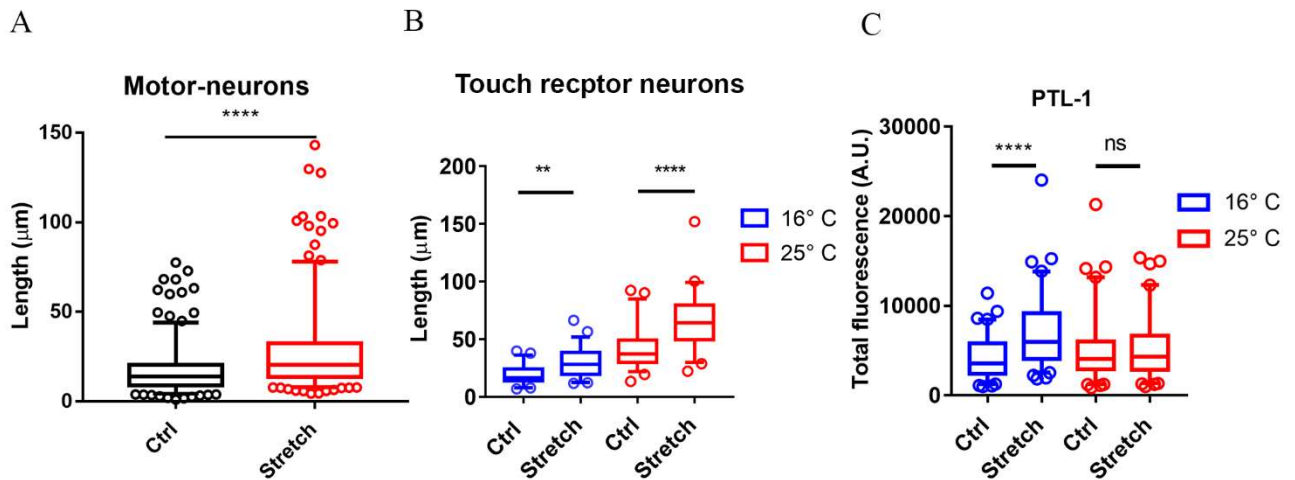
<b>Symbol</b>	<b>Name</b>	<b>Up/Down</b>	<b>Assigned category</b>
Flnb	filamin, beta	up	Actin-binding
Mical1	microtubule associated monooxygenase, calponin and LIM domain containing 1	up	Actin cytoskeleton
Pdlim1	PDZ and LIM domain 1 (elfin)	up	Actin-binding
Svil	supervillin	up	Actin-binding
Tln2	talin 2	up	Actin-binding
Tmod1	tropomodulin 1	up	Actin cytoskeleton
Tpm2	tropomyosin 2, beta	up	Actin cytoskeleton
Tagln	transgelin	up	Actin cytoskeleton
Ahnak	AHNAK nucleoprotein (desmoyokin)	up	Actin cytoskeleton
Axl	AXL receptor tyrosine kinase	up	Actin-binding
Dgkh	diacylglycerol kinase, eta	up	Actin-binding
Trip6	thyroid hormone receptor interactor 6	up	Actin-binding
Cdc42ep1	CDC42 effector protein (Rho GTPase binding) 1	up	Actin cytoskeleton
Rgcc	regulator of cell cycle	up	MT cytoskeleton
Sh3pxd2b	SH3 and PX domains 2B	up	Actin cytoskeleton
Dynlrb2	dynein light chain roadblock-type 2	up	Motor protein / transport
Rab29	RAB29, member RAS oncogene family	up	Motor protein / transport
Zmynd10	zinc finger, MYND domain containing 10	up	Motor protein / transport
Kif18b	kinesin family member 18B	up	Motor protein / transport

1110017D15 Rik	RIKEN cDNA 1110017D15 gene	up	MT-binding
Trim36	tripartite motif-containing 36	up	MT-binding
Rad51d	RAD51 paralog D	up	MT-binding
Ttll9	tubulin tyrosine ligase-like family, member 9	up	MT-binding
Ccsap	centriole, cilia and spindle associated protein	up	MT-binding
Cenpe	centromere protein E	up	MT-binding
Fam83d	family with sequence similarity 83, member D	up	MT-binding
Mtus1	mitochondrial tumor suppressor 1	up	MT-binding
Reep4	receptor accessory protein 4	up	MT-binding
Gramd3	GRAM domain containing 3	up	MT cytoskeleton
Frmd5	FERM domain containing 5	up	MT cytoskeleton
Anapc7	anaphase promoting complex subunit 7	up	MT-binding
Arl6	ADP-ribosylation factor-like 6	up	MT-binding
Aurkb	aurora kinase B	up	MT cytoskeleton
Cenpv	centromere protein V	up	MT-binding
Esrra	estrogen related receptor, alpha	up	MT-binding
Hmmr	hyaluronan mediated motility receptor (RHAMM)	up	MT-binding
Prkci	protein kinase C, iota	up	MT cytoskeleton
Abca2	ATP-binding cassette, sub-family A (ABC1), member 2	up	MT-binding
Pcnt	pericentrin (kendrin)	up	MT-binding
Tubgcp5	tubulin, gamma complex associated protein 5	up	MT cytoskeleton
Bcas3	BCAS3 microtubule associated cell migration factor	up	MT cytoskeleton
Klhl21	kelch-like 21	up	MT cytoskeleton
Tada2a	transcriptional adaptor 2A	up	MT cytoskeleton
Hap1	huntingtin-associated protein 1	up	Motor protein / transport
Arc	activity regulated cytoskeletal-associated protein	down	Actin cytoskeleton
Ccdc66	coiled-coil domain containing 66	down	MT-binding

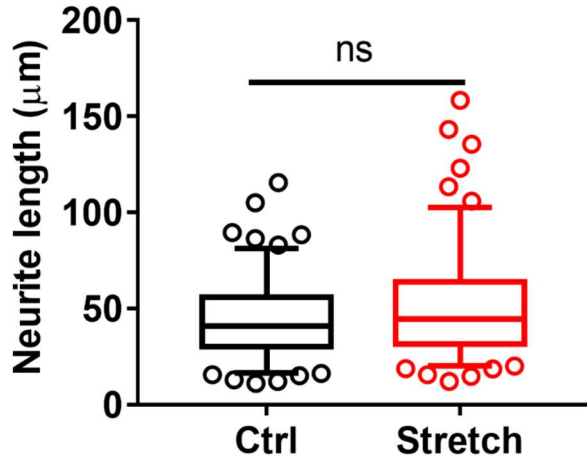
Dapk1	death associated protein kinase 1	down	Actin cytoskeleton
Fntb	farnesyltransferase, CAAX box, beta	down	MT cytoskeleton
Gdpd2	glycerophosphodiester phosphodiesterase domain containing 2	down	Actin cytoskeleton
Katnal1	katanin p60 subunit A-like 1	down	MT cytoskeleton
Klhl3	kelch-like 3	down	Actin-binding
Mark4	MAP/microtubule affinity regulating kinase 4	down	MT cytoskeleton
Mical3	microtubule associated monooxygenase, calponin and LIM domain containing 3	down	Actin cytoskeleton
Rhobtb3	Rho-related BTB domain containing 3	down	Actin cytoskeleton
Shtn1	shootin 1	down	MT cytoskeleton
Spire2	spire type actin nucleation factor 2	down	Actin cytoskeleton
Topbp1	topoisomerase (DNA) II binding protein 1	down	Actin cytoskeleton
Tppp	tubulin polymerization promoting protein	down	MT cytoskeleton
Tsc1	TSC complex subunit 1	down	Actin cytoskeleton
Ttll4	tubulin tyrosine ligase-like family, member 4	down	MT-binding



**Figure S1 (related to Figure 1).** Representative image of the somato-dendritic and the axonal compartment. In bottom panel is shown the absence of somata in the axonal compartment as no nuclei were found; BTUBBIII (green), Hoechst (blue) staining; scale bar: 50  $\mu\text{m}$ .



**Figure S2 (related to Figure 2).** (A) Neurite length of motor-neurons in control and stretched conditions (MSB32 strain). Box plot, 5-95 percentile,  $n=250$  from five independent assays. Mann-Whitney test,  $p<0.0001$ . (B) Axonal length of WT touch receptor neurons for  $\alpha$  and  $\beta$ -tubulin (GN692 strain). Control and stretched axons have been measured both, at 16°C and at 25°C. Box plot, 5-95 percentile,  $n=50$  neurites. Kruskal-Wallis test with post hoc Dunn's test,  $p<0.0001$ . (C) Quantification of mNG total fluorescence in a transgenic model of tagged mNG::PTL-1 at 16°C and at 25°C. Box plot (5-95 percentile),  $n=80$  neurites from four biological replicates. Kruskal-Wallis test with post hoc Dunn's test,  $p<0.0001$ .

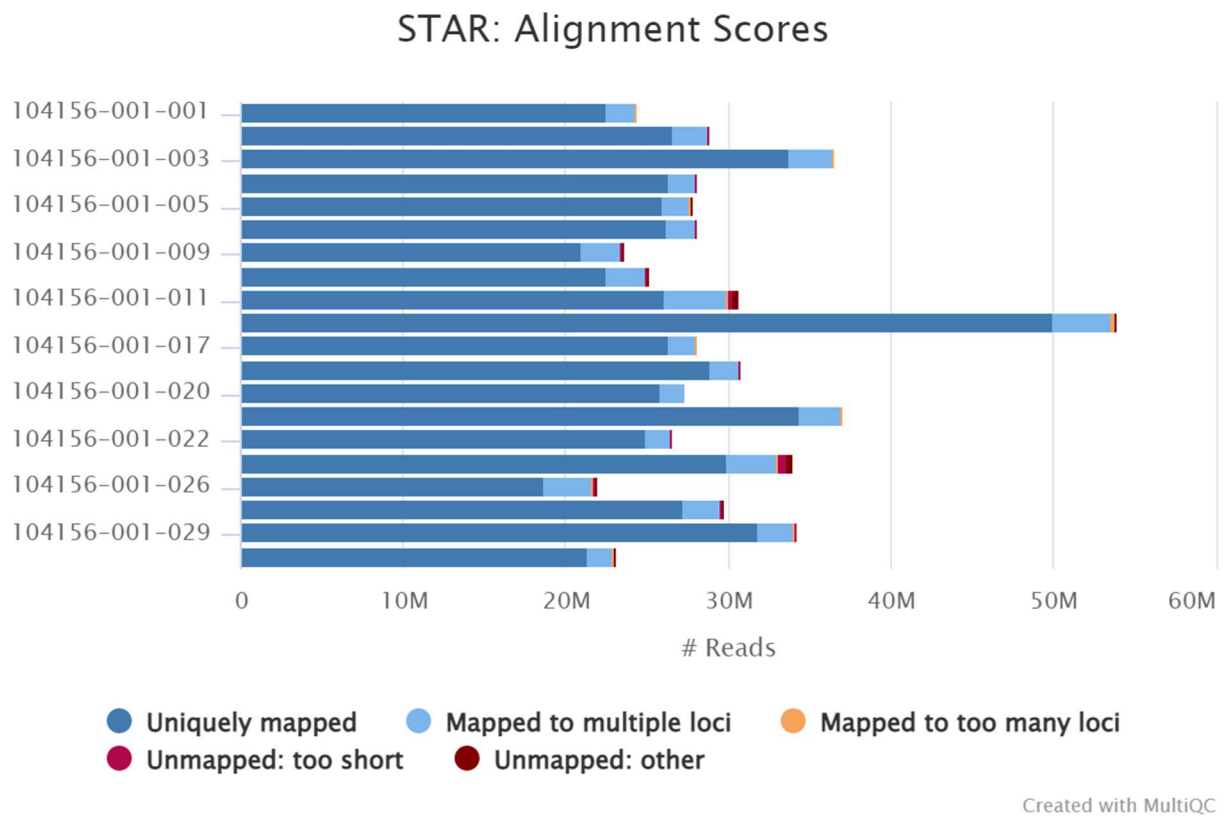


**Figure S3 (related to Figure 4).** Neurite length of touch receptor neurons of a *C. elegans* strain encoding for a fluorescent UNC-104 with Nocodazole treatment ( $1.8 \text{ ng} \cdot \text{ml}^{-1}$ ). Box plot (5-to-95 percentile),  $n=120$  neurites from four biological replicates. Mann Whitney test,  $p=0.08$ .

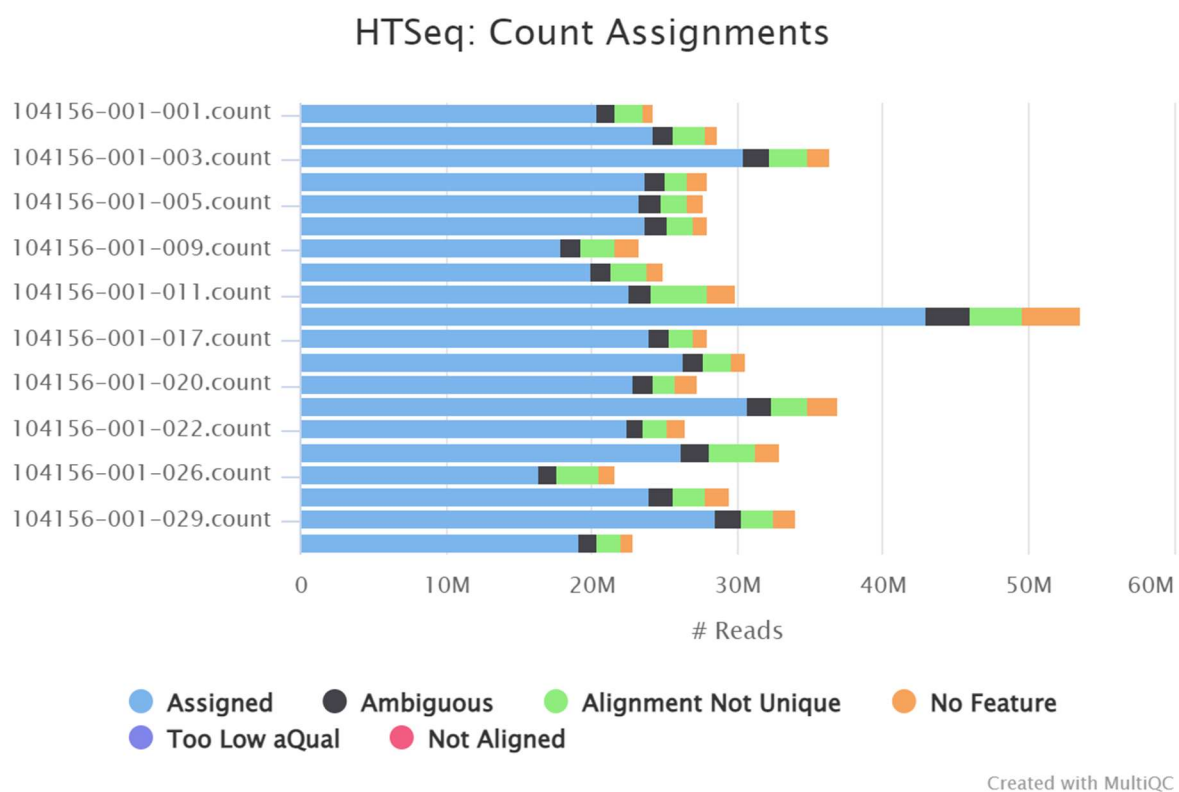


**Figure S4 (related to Figure 1).** Quality control checks on raw sequence datasets.

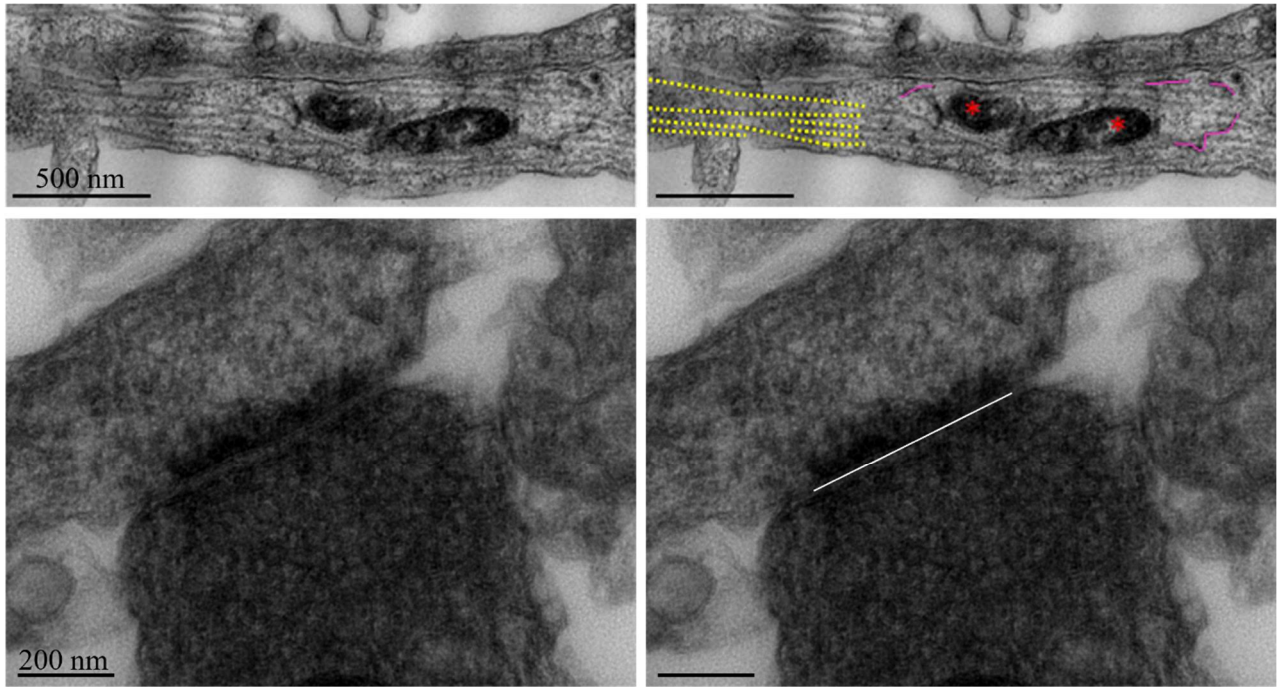




**Figure S5 (related to Figure 1).** Scores of the reads alignment to the reference genome.



**Figure S6 (related to Figure 1).** Count of the reads mapped against the genes of the reference genome.



**Figure S7 (related to Figure 2 and 7).** Representative TEM images. MTs: dashed yellow lines; ER: magenta lines; mitochondrion indicated with “\*”; PSD region: white line.