

Supporting Information

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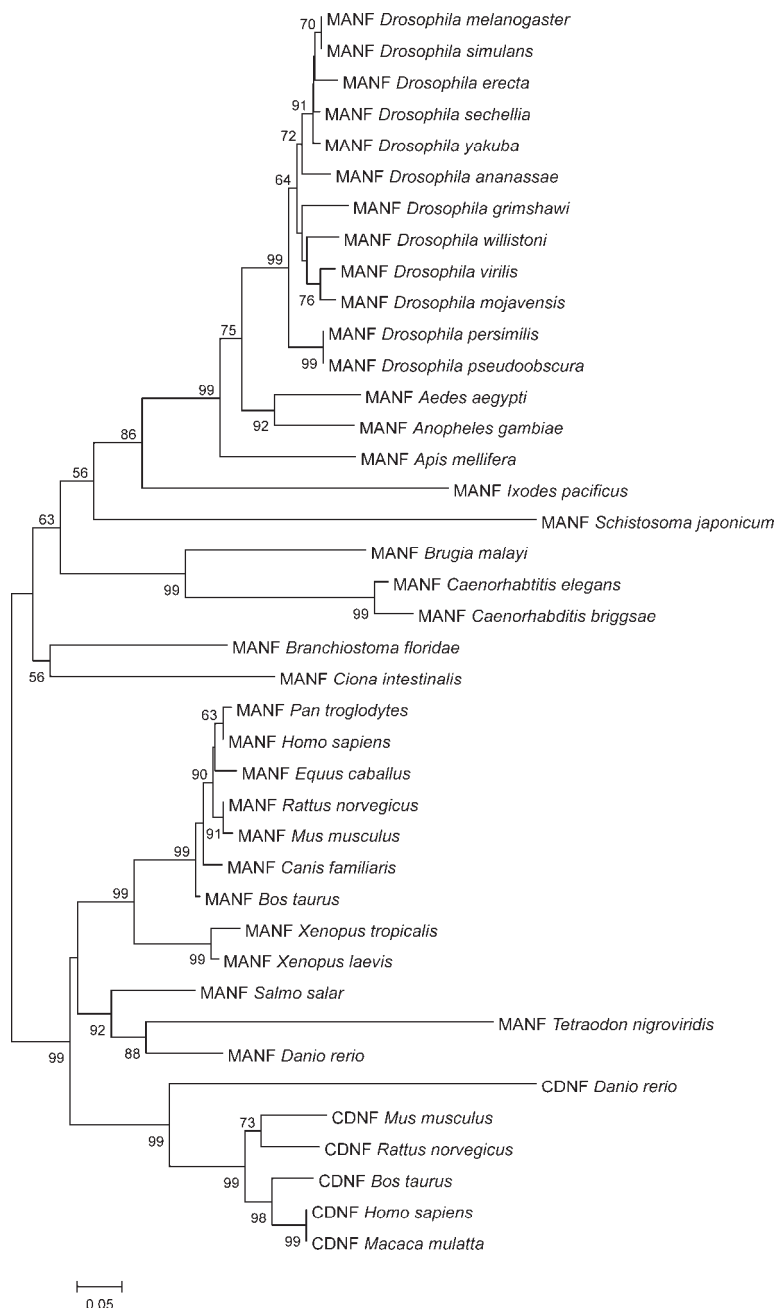


Fig. S1. Phylogenetic tree of MANF and CDNF family members. Phylogenetic and molecular evolutionary analyses were conducted using *MEGA* version 4 (Tamura K, Dudley J, Nei M & Kumar S (2007) *MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0*. *Mol Biol Evol* 24:1596–1599). Polypeptides without signal sequences were aligned with Clustal W and Minimum Evolution (Construct Phylogeny) method was used to create unrooted tree by Close-Neighbor-Interchange (CNI) algorithm. Bootstrap values higher than 50% are shown for each branch point. GenBank accession numbers for amino acid sequences used in alignment (from the top to the bottom, first initials of each name of the species in latin shown) of MANFs are: AF132912 *Dme*; EDX12700 *Dsi*; EDV49132 *Der*; EDW44880 *Dse*, EDW97329 *Dya*; EDV43440 *Dan*; EDV94929 *Dgr*; EDW83752 *Dwi*; EDW66730 *Dvi*; EDW15128 *Dmo*; EDW34412 *Dpe*; EAL28603 *Dps*; ABF18222 *Aa*; EAA07858 *Ag*; XP_625023 *Am*; AAT92198 *Ip*; AAW24701 *Sj*; EDP33404 *Bm*; AAK93864 *Ce*; XP_001672075 *Cb*; EEA72749 *Bf*; XP_002127331 *Ci*; XP_001169644 *Pt*; P55145 *Hs*; XP_001495433 *Ec*; P0C5H9 *Rn*; Q9CXI5 *Mm*; XP_850540 *Cf*; P80513 *Bt*; AAI70889 *Xt*; AAH43846 *Xl*; ACI69956 *Ss*; CAF93651 *Tn*; AAI24317 *Dr*; and of CDNFs are: NP_001116753 *Dr*; Q8CC36 *Mm*; P0C5I0 *Rn*; XP_591544 *Bt*; AAH37872 *Hs*; XP_001088321 *Mam*.

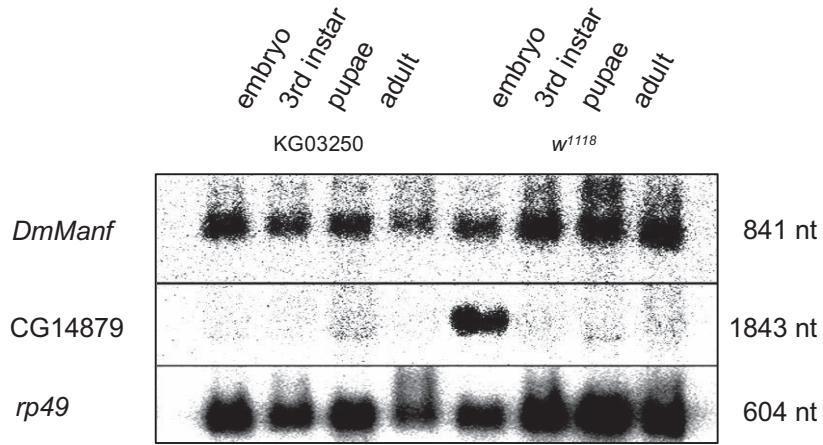


Fig. S2. Northern analysis of *DmManf* and the neighboring gene CG14879 in the P-element line KG03250 and wild type line *w*¹¹¹⁸ during *Drosophila* development. P-element homozygous viable line KG03250 shows *DmManf* levels indistinguishable from wild type but lack embryonic expression of CG14879, while very weak pupal expression of CG14879 is still present. *rp49* serves as loading control.

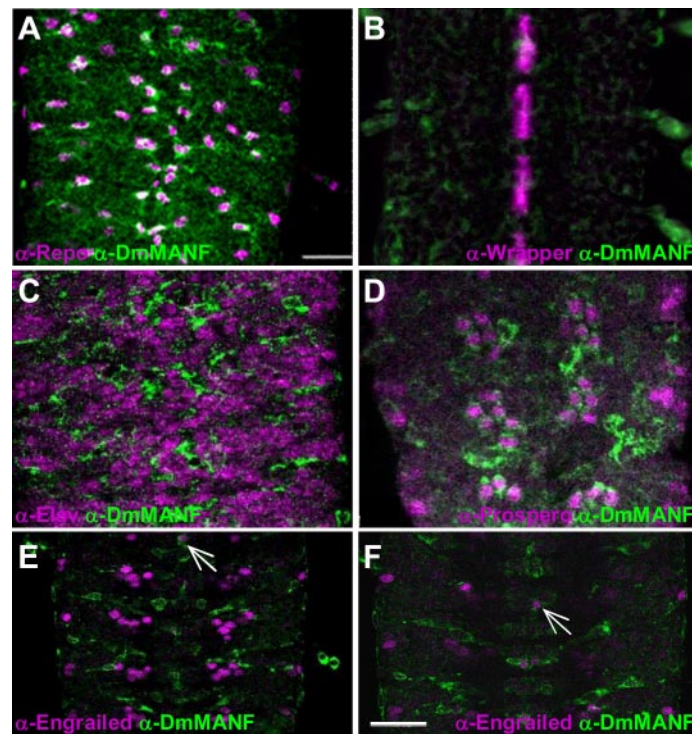


Fig. S3. DmMANF co-localizes with glial but with no neuronal markers. A, Partial co-localization of anti-DmMANF and anti-Repo labeling all glial nuclei except midline glia, ventral view. Scale bar, 20 μm . B, Anti-DmMANF shows no co-localization with anti-Wrapper positive midline glia. C, Anti-DmMANF shows no co-localization with pan-neuronal marker anti-Elav. D, Dorsal plane shows partial co-localization with anti-Prospero positive longitudinal glia. E, F, Anti-Engrailed positive neurons from the middle part of the ventral cord showing no co-localization with anti-DmMANF, but at midline there is co-localization in small channel glia (arrow). Scale bar, 15 μm .

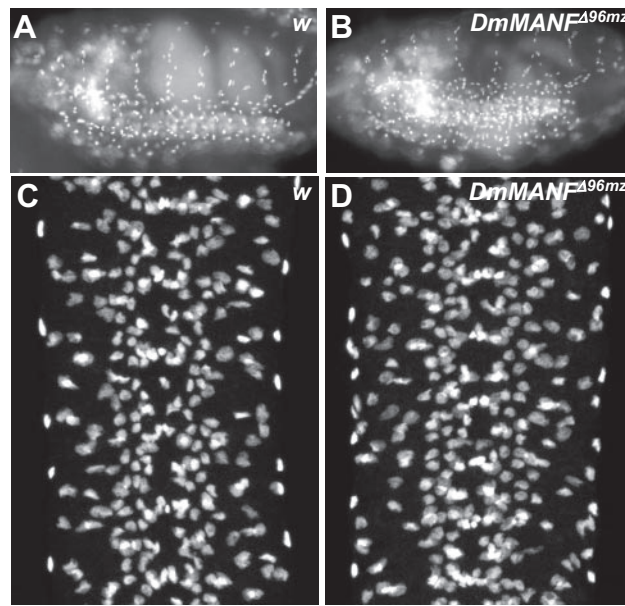


Fig. S4. In *DmMANF^{Δ96mz}* mutants glial numbers and location are unaffected. Repo-positive glia in whole mount embryos (A and B) and in VNC (C and D).

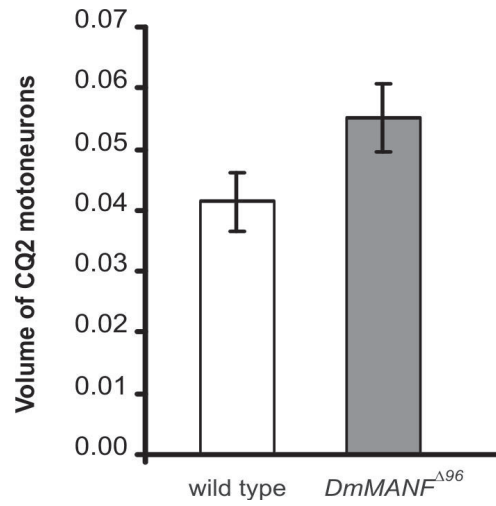


Fig. S5. Volume quantification of CQ-motoneurons. In y axis relative volume of neurites to total volume of the neuropile is presented. Analyzed genotypes: wild type, CQ2> τ -LacZ ($n = 5$); *DmMANF*^{Δ96}, CQ2> τ -LacZ ($n = 6$). Mean \pm SEM. $P = 0,086$ versus wild type, Student's t-Test.

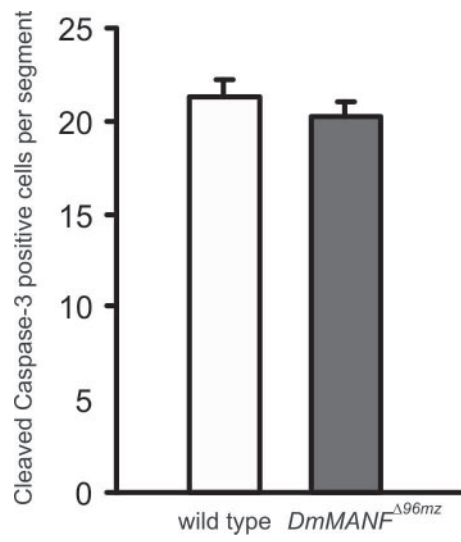


Fig. S6. Quantification of apoptotic cells in embryonic stage 16 VNC. The number of cleaved Caspase-3 positive cells counted per segment of middle stage 16 VNC of wild type ($n = 35$) and *DmMANF^{Δ96mz}* ($n = 49$). Mean \pm SEM.

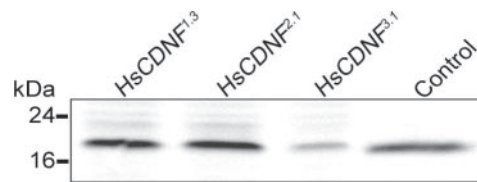


Fig. S7. Western blot analysis of larval homogenates overexpressing human (Hs) CDNF. *da*-GAL4 driven expression of three independent UAS-*HsCDNF* transgenic fly lines probed with anti-CDNF. Lysate from cells transfected with HsCDNF served as positive control.

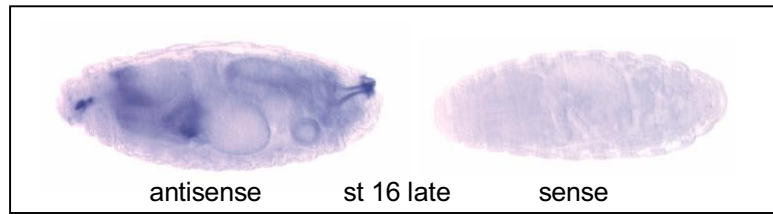


Fig. S8. *In situ* hybridization with *DmManf* antisense (left) and sense (right) probe on late stage 16 wild type embryos.

Table S1. Identical amino acids between species of evolutionarily conserved MANF proteins

	<i>D.m.</i> MANF	<i>D.p.</i> MANF	<i>A.m.</i> MANF	<i>A.g.</i> MANF	<i>A.a.</i> MANF	<i>H.s.</i> CDNF	<i>H.s.</i> MANF	<i>M.m.</i> MANF	<i>X.l.</i> MANF	<i>D.r.</i> MANF	<i>C.e.</i> MANF
<i>D.m.</i> MANF	x	92	75	78	79	49	52	51	54	57	50
<i>D.p.</i> MANF	92	x	75	76	79	47	54	54	56	56	53
<i>A.m.</i> MANF	75	75	x	74	73	48	52	51	52	55	48
<i>A.g.</i> MANF	78	76	74	x	85	49	52	51	51	53	51
<i>A.a.</i> MANF	79	79	73	85	x	48	51	51	53	53	51
<i>H.s.</i> CDNF	47	47	48	49	48	x	59	61	56	56	46
<i>H.s.</i> MANF	52	54	52	52	51	59	x	98	82	72	48
<i>M.m.</i> MANF	51	54	51	51	51	59	98	x	82	72	50
<i>X.l.</i> MANF	54	56	52	51	53	58	82	82	x	78	50
<i>D.r.</i> MANF	57	56	55	53	53	56	72	72	78	x	48
<i>C.e.</i> MANF	50	53	48	51	51	46	48	50	50	48	x

Calculated percentages by Blossum 62 matrix between amino acid composition of proteins without the signal sequence. *D.m.*, *Drosophila melanogaster*, fruit fly; *D.p.*, *Drosophila pseudoobscura*, North American fruit fly; *A.m.*, *Apis mellifera*, honeybee; *A.g.*, *Anopheles gambiae*, anopheline mosquito; *A.a.*, *Aedes aegypti*, yellow fever mosquito; *H.s.*, *Homo sapiens*, human; *M.m.*, *Mus musculus*, mouse; *D.r.*, *Danio rerio*, zebrafish; *X.l.*, *Xenopus laevis*, African clawed frog; *C.e.*, *Caenorhabditis elegans*, nematode.