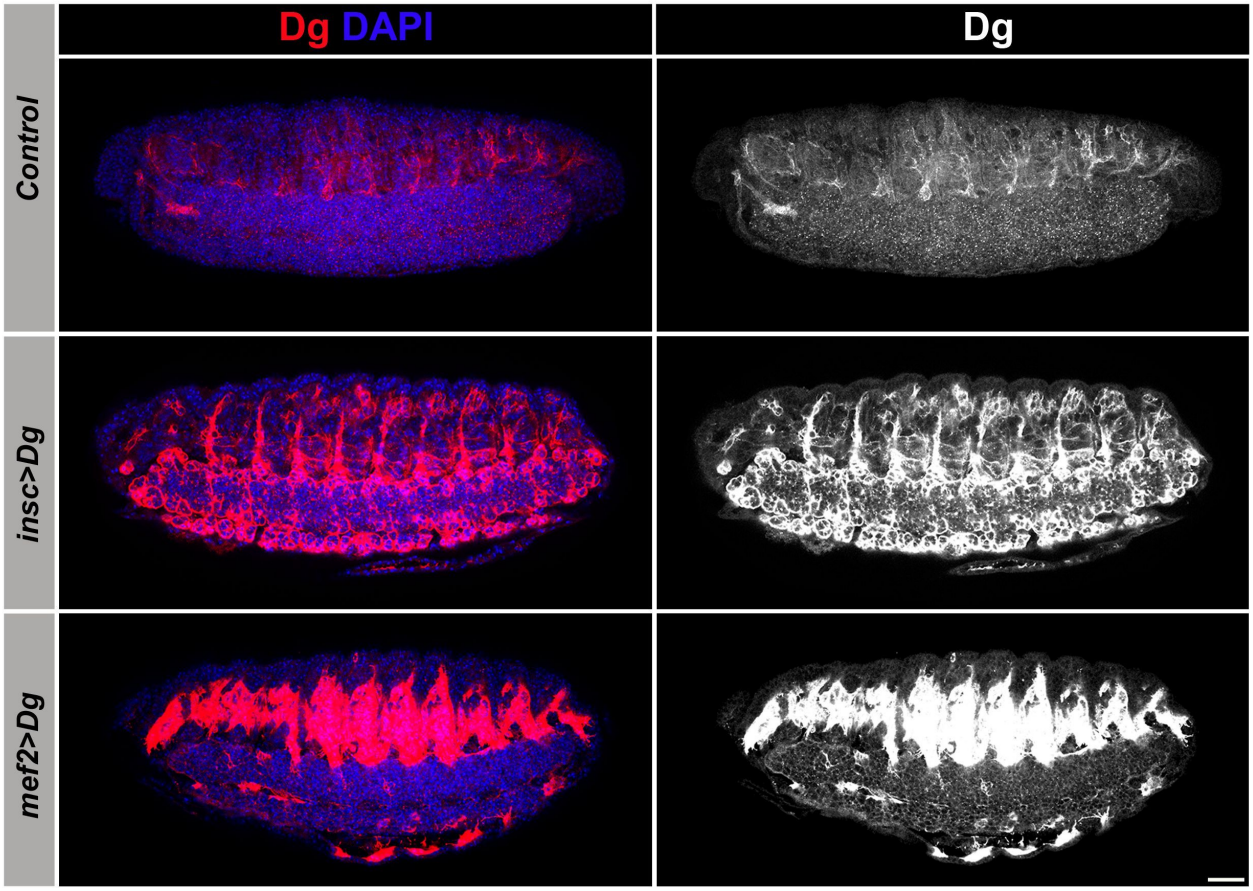
Supplementary Information

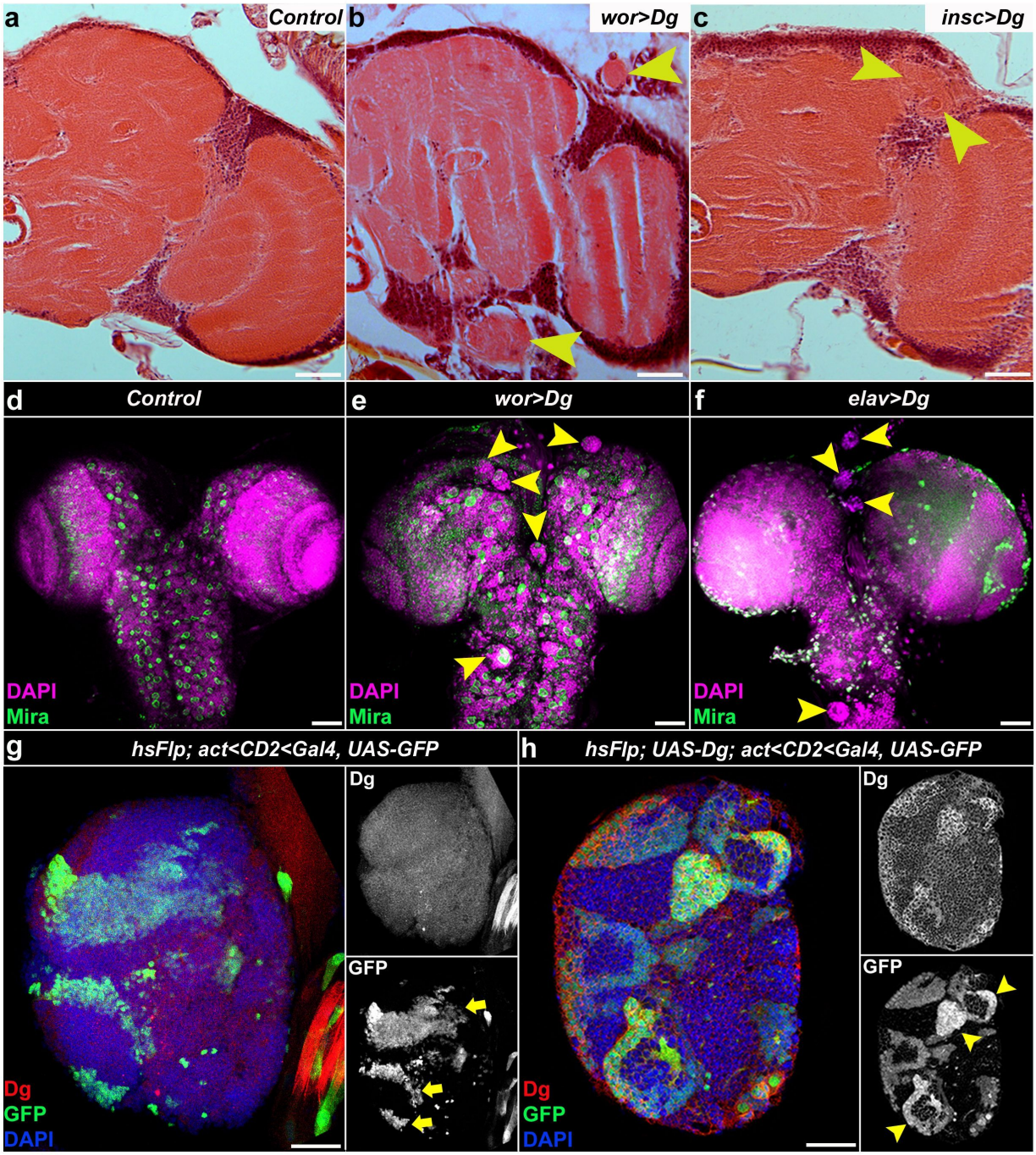
Supplementary Figures and Legends

Supplementary Figure 1



Supplementary Figure 1. Tissue specific overexpression of Dg in *Drosophila* embryo
High levels of Dg can be detected with specific Dg antibody in the nervous system of *insc>Dg* embryo and in muscles of *mef2>Dg* embryo compared to endogenous Dg levels in *Control*. Scale bar equals 25μm.

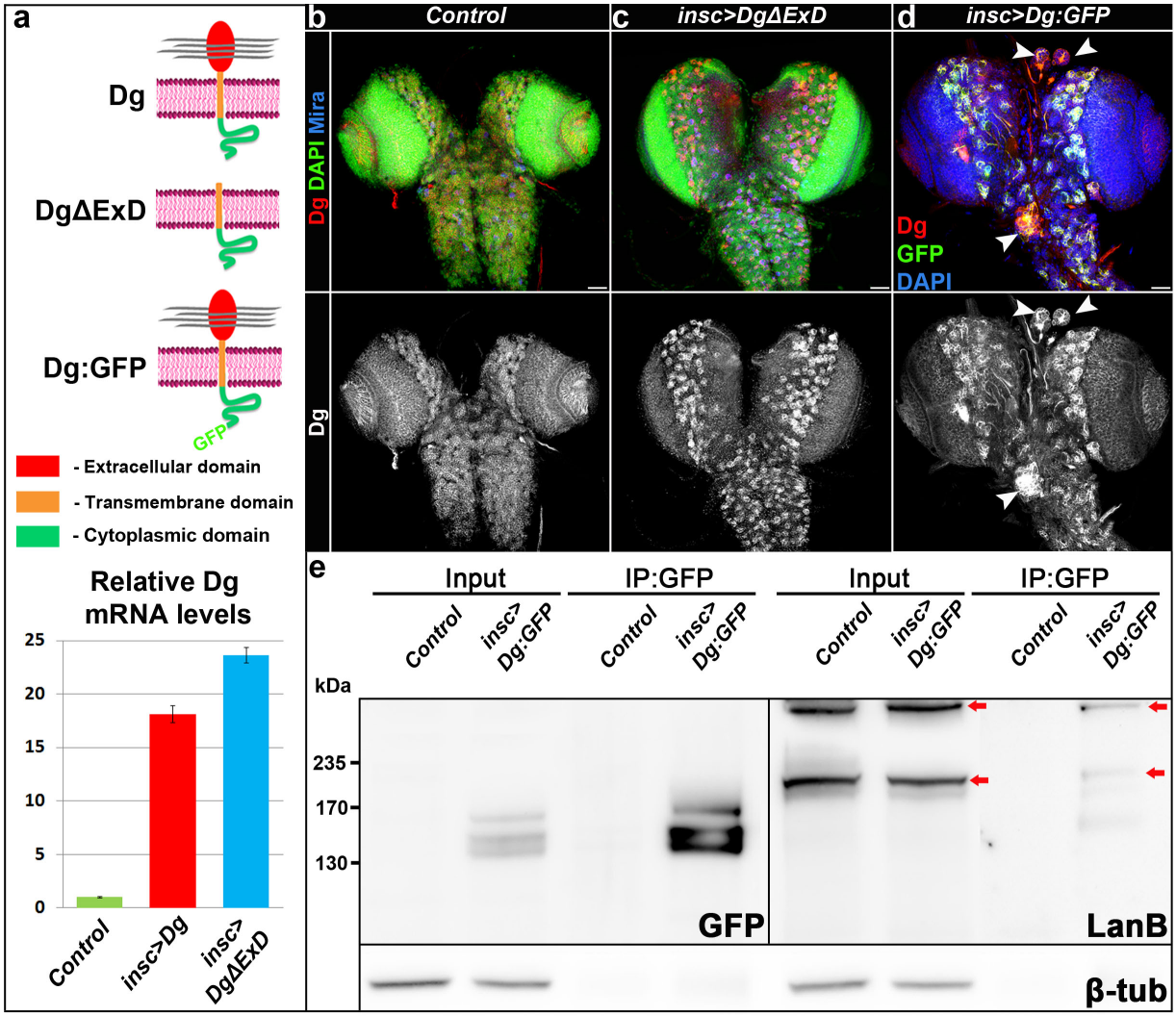
Supplementary Figure 2



Supplementary Figure 2. Dg levels are important for normal brain shape

a-c, Paraffin sections of the adult brains stained with haematoxylin and eosin show normal brain morphology in *control* flies (**a**), while in escapers, overexpression of Dg, using the neuroblast specific (*wor-Gal4*, **b**), and pan-neuronal (*insc-Gal4*, **c**) drivers, results in the appearance of dense round structures that outgrow the normal brain shape (yellow arrowheads). **d**, wild type larval brain has a smooth brain outline. **e-f**, Overexpression of Dg using neuroblast specific (**e**, *wor-Gal4*) or neuron specific (**f**, *elav-Gal4*) drivers results in the appearance of cobblestone-like structures (arrowheads). **g**, Control clones 3 days after clone induction (*hsFlp; act-FRT:CD2:FRT-Gal4, UAS-GFP*) marked by GFP spread uniformly in the brain. **h**, Clones with ectopic expression of Dg (*hsFlp; UAS-Dg; act-FRT:CD2:FRT-Gal4, UAS-GFP*) tend to round up, indicating a difference in cell adhesion relative to the surrounding wild type cells and form the early stage lumps (compare GFP channel in **g** and **h**). This indicates a difference in selective cell adhesion relative to the surrounding wild type cells and provides an explanation for the cause of lump formation. Scale bar equals 50μm in **a-c** and 25μm in **d-h.**

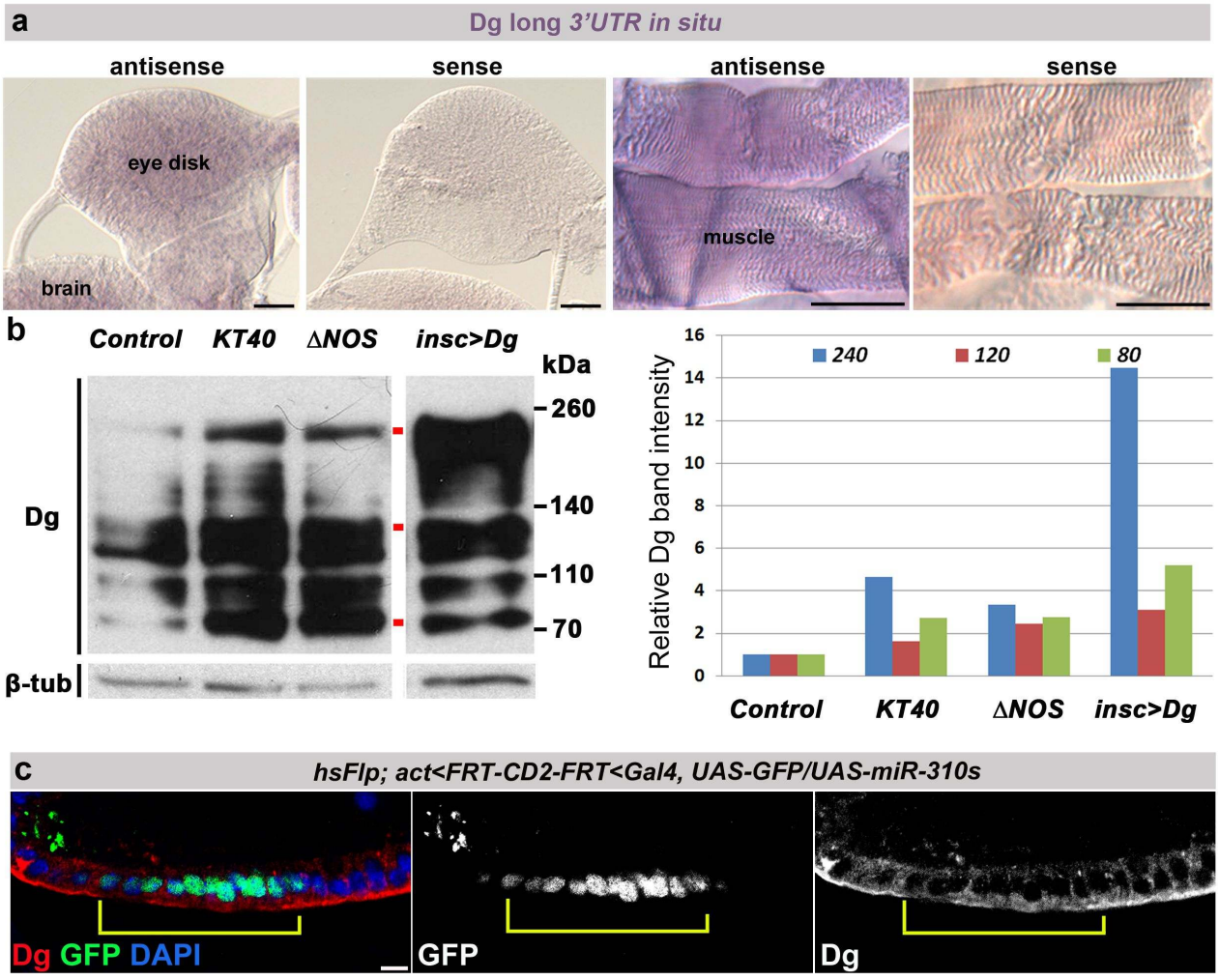
Supplementary Figure 3



Supplementary Figure 3: Dg malformed brain phenotype results from abnormal interaction of the ECM-binding domain of Dg with neural lamella

a, The vertebrate Dg protein originates a single mRNA precursor that is cleaved post-translationally into α- and ß-Dg subunits[1]. ß-Dg is a transmembrane protein that connects to the actin cytoskeleton via Dys and is associated with molecules involved in various signalling pathways[2]. α-Dg is a peripheral membrane protein that connects to the ECM via its direct binding to extracellular molecules, such as laminins, perlecan, agrin, neurexins and via non-covalent interaction with the ß-Dg subunit forms the link between the ECM and the cytoskeleton. Drosophila Dg does not undergo posttranslational cleavage; however it contains both functional domains. Overexpression of the ECM binding domain-depleted Dg isoform shows a similar increase in *Dg* mRNA levels, detected with primers against the region encoding for the C-terminal end of the Dg protein. Data represent two biological replicates (three technical replicate per each). See also Supplementary Table 4. **b-c**, Overexpression of truncated version of Dg that lacks the extracellular domain (*DgΔExD*) using the *insc-Gal4* neuronal driver has no effect on the brain shape. **d,** Overexpression of GFP-tagged version of Dg using the *insc-Gal4* neuronal driver (*insc>Dg:GFP*) causes brain malformations (arrowheads). **e**, Western blot analysis on the samples from larval brains that express GFP-tagged Dg under control of the neuronal driver (*insc>Dg:GFP*). Pull-down using beads against the GFP tag showed that LanB can be detected in the Dg:GFP co-immunoprecipitation samples. This analysis shows that in *Drosophila* developing central nervous system, like in vertebrates, the ECM receptor Dg binds the ECM protein laminin. Scale bar equals 25μm in **b-d**.

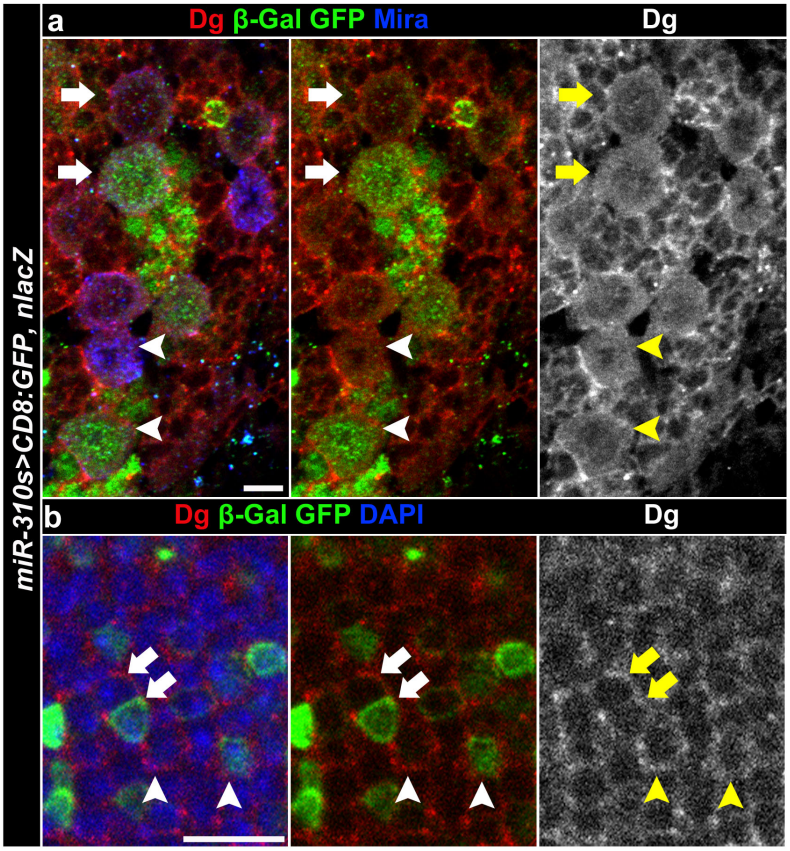
Supplementary Figure 4



Supplementary Figure 4. *Dg* mRNA with the long *3'UTR* can be detected in total mRNA extracts and regulated by the *miR-310s in vivo*

a, *in situ* hybridization against the long *3'UTR* region shows that this variant of *Dg* mRNA is present in larval muscle and eye disks. **b**, Western blot analysis from the whole larva protein extracts reveals that main Dg protein isoforms are upregulated in *miR-310s* and Δ*NOS* mutants, compared to *Control*, measured by Image J software. Ectopic Dg expression in the nervous system leads to dramatic upregulation of Dg protein levels (~20 times). **c**, Ovarian follicle cell clones that ectopically express the *miR-310s (hsFlp; act<FRT-CD2-FRT<Gal4/+, UAS-GFP/UAS-miR-310s)* are used to measure *miR-310s* effect on Dg protein levels. Dg normally is localized to the basal side of the follicular epithelium; however, in miR-310s overexpressing cells, Dg expression levels at the basal side are decreased by approximately 30%, analysed by Image J software. Scale bar equals 25μm in **a** and 5μm in **c.**

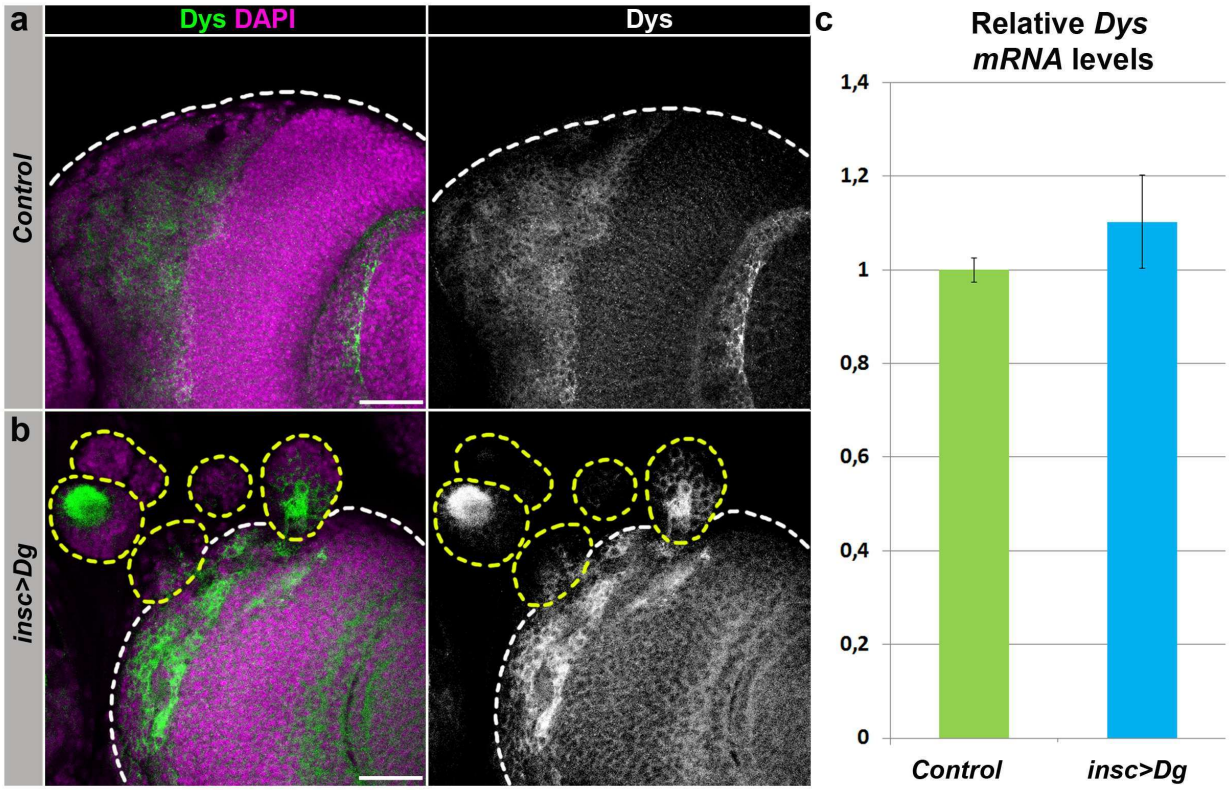
Supplementary Figure 5



Supplementary Figure 5. Dg and *miR-310s* levels are dynamic in the larval NBs and photoreceptor cells

a, NBs that express *miR-310s* in the *Drosophila* larval brain have either high (arrowheads) or low (arrow) levels of Dg protein. **b,** Photoreceptor cells in the eye disk have high (arrowheads) or low (arrows) levels of Dg protein. Scale bar equals 5μm**.**

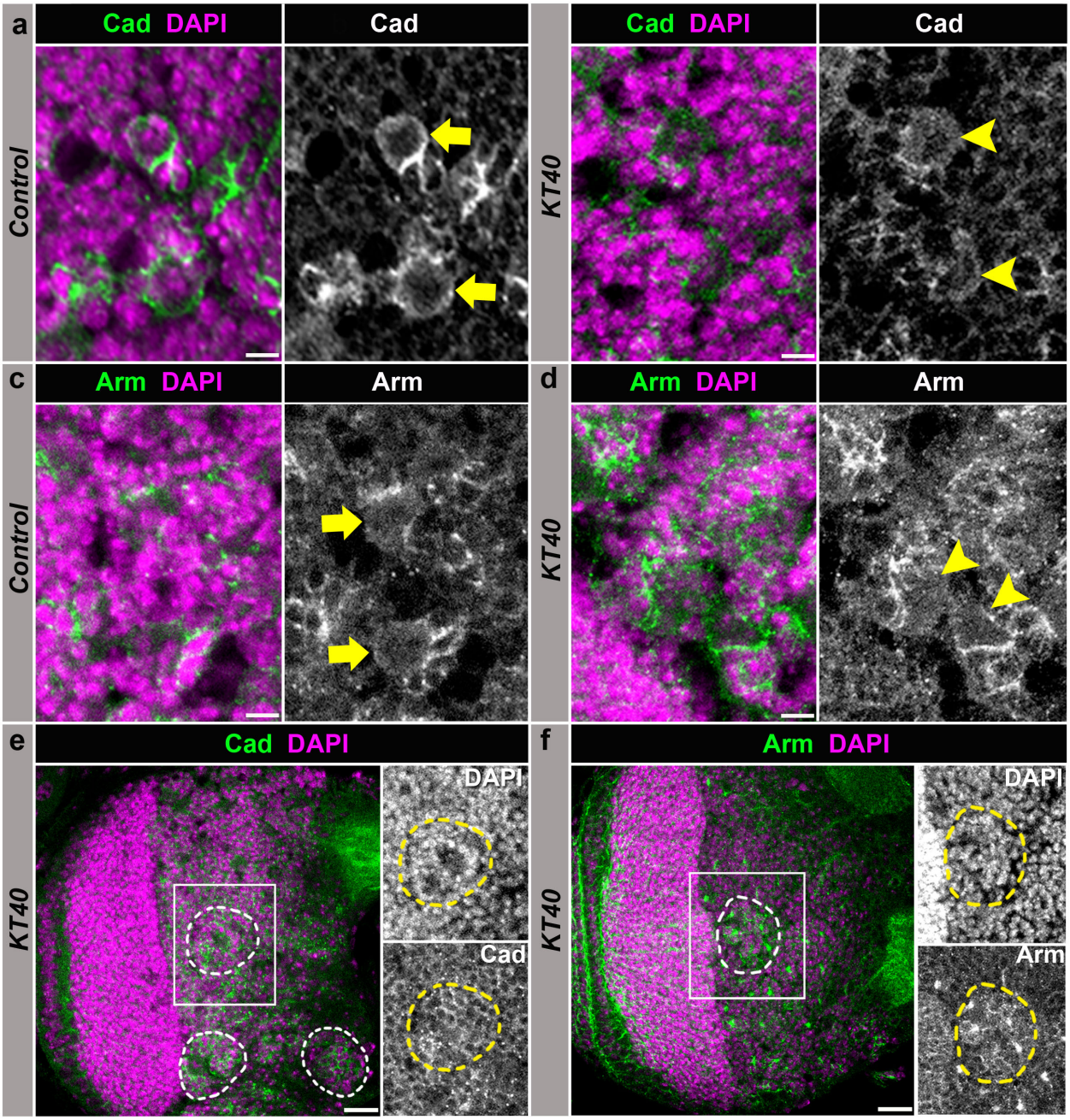
Supplementary Figure 6



Supplementary Figure 6. Dys protein, but not mRNA is upregulated in the brain lumps caused by Dg overexpression in the nervous system

a-**b**, Dys protein levels are upregulated in the lumps caused by Dg overexpression (**b**, *insc>Dg*) when compared to its endogenous levels in *Control* (**a**, *wt*). **c**, *Dys* mRNA levels from the whole larva mRNA extracts are not increased upon Dg overexpression in the nervous system. Data represent AVE±SD from two biological replicates (three technical replicates per each). See also Supplementary Table 4. Scale bar equals 25μm.

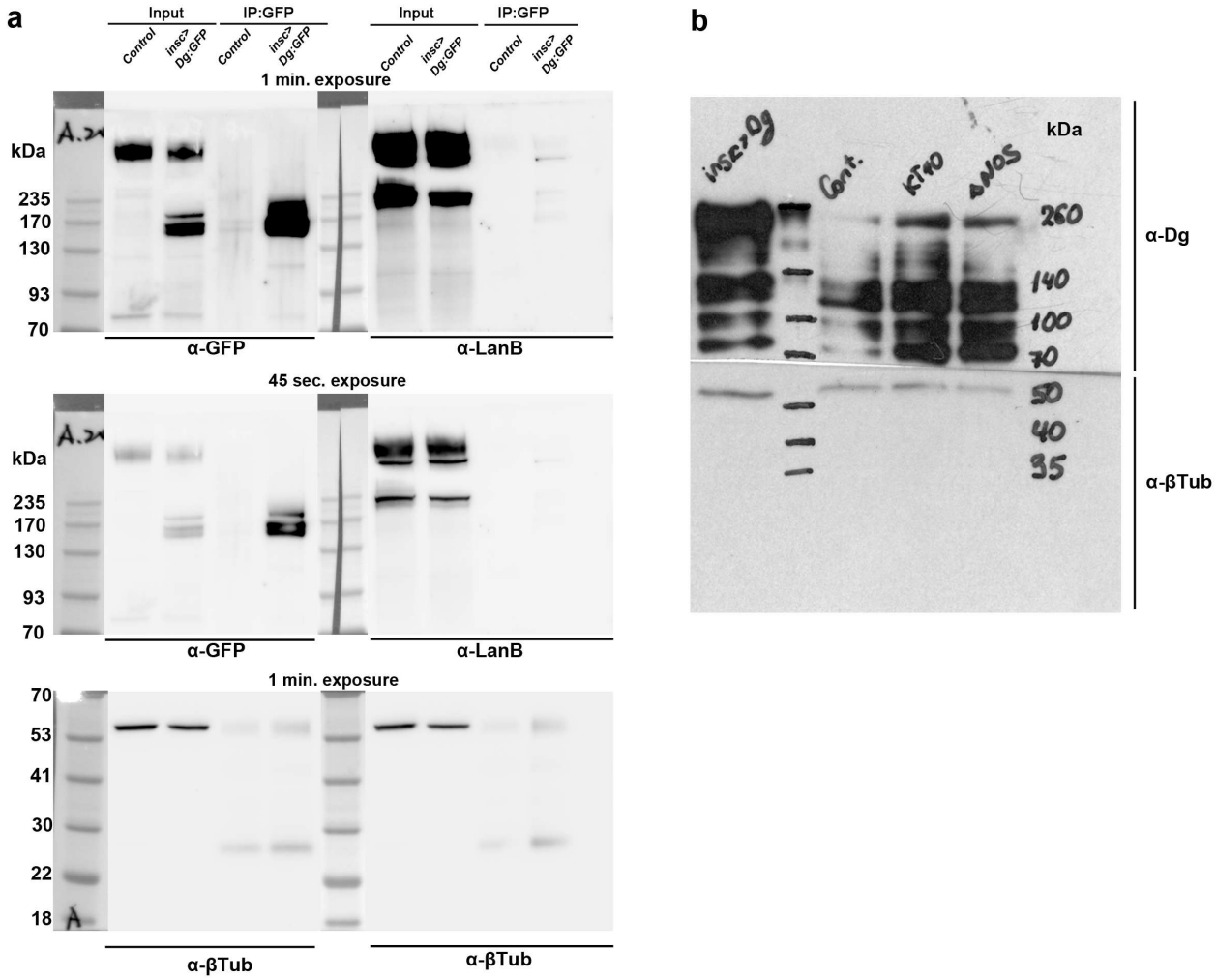
Supplementary Figure 7



Supplementary Figure 7. The cell adhesion proteins DE-Cadherin (Cad) and Armadillo (Arm) are misexpressed in the brain of *miR-310s* mutants

a-**b**, In *KT40* mutants, Cad levels are decreased in NBs (**b**, arrowheads), when compared to *Control* (**a**, arrows). **c**-**d**, In *KT40* mutants, Arm levels are increased in NBs (**d,** arrowheads), when compared to *Control* (**c**. arrows). **e**-**f**, The mild brain overgrowth phenotype in the *miR-310s* loss-of-function mutants. Brain lumps (indicated by dashed lines) in *miR-310s* mutants have abnormal localization of Cad (**e**) and Arm (**f**). Rectangles indicate magnified areas shown as separate single channel images to the right. Scale bar equals 5μm in **a-d** and 25μm in **e-f.**

Supplementary Figure 8



Supplementary Figure 8. Original uncropped Western blots

a, Related to Supplementary Figure 3. **b**, Related to Supplementary Figure 4.

Supplementary Tables

Supplementary Table 1: Dg levels are important for *Drosophila* survival

Cross	number of eclosed non Cy adults	number of eclosed Cy adults	Survival, %	AVE±AD Survival, %	*p*-value
Control (CyO /+ x CyO /+)	30	60	100	98.8±1.3	
	39	80	97.5		
CyO/Dg^{055} x *CyO/Dg*^{055}	42	209	40.2	37.4±2.8	2.0x10^{-6}***
	39	226	34.5		
CyO/Dg^{055} x *CyO/ Dg*^{038}	18	123	29.3	29.7±0.4	4.0x10^{-7}***
	22	146	30.1		
CyO/Dg^{055} *x CyO/Dg*^{043}	25	122	41.0	41.4±5.4	1.3x10^{-3}***
	63	243	51.9		
CyO/Dg^{055} x *CyO/ Dg*^{086}	11	120	18.3	13.5±4.9	0***
	4	93	8.6		

Numbers reported are the AVE±AD. Statistics were calculated using two-way tables and significance was calculated using the chi-squared test: *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001

Genotype	number of biological replicates	number of embryos	% embryonic lethality	*p*-value
Control (*Oregon/ w*^{1118})	4	435	16.8±2.3	-
tub>Dg (*UAS-Dg/+; tub-Gal4/+*) **ubiquitous**	2	-	100	-
mef2>Dg (*UAS-Dg/+; mef2-Gal4/+*) **muscle**	2	222	14.5±5.0	0.46
insc>Dg (*insc-Gal4/UAS-Dg)* **neuroblast**	3	779	99.2±1.1	3.2x10^{-8}***
elav>Dg (*elav-Gal4/+; UAS-Dg/+*) **pan-neuronal**	3	677	59.7±15.4	0.002 **
wor>Dg (*UAS-Dg/+; wor-Gal4/+*) **neuroblast**	4	847	80.0±7.6	5.2x10^{-6}***

Numbers reported are the AVE±SD. Statistics were calculated using the two-tailed Student's t-test: *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001.

Supplementary Table 2: Deregulation of DGC-NOS signalling leads to the brain malformation phenotypes

Genotype	Brains with lumps (%)	Strength of observed phenotype	n, lobes analyzed	*p*-value[t]
Control (*w^{1118}*)	0.00	---	16	
hsFlp;FRT42B GFP/ FRT42B Dg^{086}	42.11	weak	19	0.0063 **
tub>DgRNAi (*tub-Gal4*, *UAS-DgRNAi)*	5.88	weak	18	0.263
insc>Dg (*insc-Gal4/UAS-Dg*)	100.00	strong	16	$1.1x10^{-6}$***
insc>Dg +NG (*insc-Gal4/UAS-Dg* + 0.2ng/µl NG 5% sucrose)	84.21	strong	19	$4.8x10^{-5}$ (*w^{1118}*) *** 0.3609 (*insc>Dg*)
KT40 (*KT40/KT40)*	45.24	weak	84	0.0015 **
KT40; tub>DgRNAi (*KT40/KT40; tub-Gal4, UAS-DgRNAi)*	21.74	weak	23	0.027 (*w^{1118}*) * 0.025 (*KT40*) *
ΔNOS	34.26	weak	108	0.003 **
ΔNOS+NG (*ΔNOS* + 0.2ng/µl NG 5% sucrose)	28.00	weak	25	0.012 (*w^{1118}*) * 0.554 (*ΔNOS*)

Statistics were calculated using two-way tables and significance using the chi-squared test:
*P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001.
For each experiment 3-5 independent crosses were analysed.
[t] Statistical comparisons were made to genotype *w^{1118}* unless there is a genotype in parenthesis, in which case the comparison was made to that genotype.

Supplementary Table 3: Luciferase activity assays show that the extended Dg *3'UTR* can be targeted by the *miR-310s* family of miRNAs

Experiment	miRNA expression plasmid	Renilla /Firefly luciferase ratio (psiCHECK-2)	Renilla /Firefly luciferase ratio (psiCHECK-2-Dg-P1,P2, P3-3'UTR)		Relative Luciferase levels (psiCHECK-2 Dg- P1,P2, P3 or P4-3'UTR /psiCHECK-2)	Relative Luciferase levels [a]	p-value
I	none[a]	0.18 ± 0.02	**P1**	0.10 ± 0.08	0.60 ± 0.45	1.00 ± 0.13	
			P2	0.09 ± 0.00	0.51 ± 0.01	1.00 ± 0.00	
			P3	0.22 ± 0.01	1.28 ± 0.06	1.00 ± 0.01	
	miR-310s	0.30 ± 0.07	**P1**	0.20 ± 0.02	0.67 ± 0.07	1.12 ± 0.03	0.800
			P2	0.20 ± 0.11	0.67 ± 0.23	1.31 ± 0.21	0.601
			P3	0.35 ± 0.14	1.17 ± 0.13	0.92 ± 0.03	0.407
II	none[a]	0.30 ± 0.06	**P4**	0.22 ± 0.08	0.71±0.16	1.00 ± 0.23	
	miR-310s	1.93 ± 0.59		0.35 ± 0.04	0.19±0.06	0.27 ± 0.08	0.0095**
	miR-310	1.43 ± 0.90		0.25 ± 0.05	0.21±0.08	0.35 ± 0.01	0.0031**
	miR-311	1.88 ± 0.05		0.41 ± 0.11	0.22±0.06	0.31 ± 0.08	0.016*
	miR-312	1.63 ± 0.22		0.53 ± 0.07	0.33±0.04	0.46 ± 0.05	0.0067**
	miR-313	2.59 ± 0.43		0.39 ± 0.11	0.15±0.03	0.21 ± 0.04	0.0018**
III	none[a]	0.68 ± 0.08	**P4**	0.15 ± 0.03	0.22±0.02	1.00 ± 0.09	
	miR-92a	2.04 ± 0.36		0.34 ± 0.07	0.17±0.01	0.77 ± 0.05	0.015*
	***miR-92b* (mutant)[c]**	1.72 ± 0.30		0.47 ± 0.08	0.28±0.03	1.27 ± 0.13	0.045*

The Firefly luciferase expression is used as an endogenous control where the Dg long *3'UTR* was cloned into the *Renilla luciferase* gene.

[a] Values in the presence of miRNA expressing plasmids were normalized to the downregulation that occured in cells with no miRNA expressing plasmid present.

[b] *miR-92a* and *miR-92b* were tested on a different day and therefore were compared to a different control sample.

[c] Note that *miR-92b* mutant plasmid has a point mutation in the seed sequence. Failure of *miR-92b (mutant)* to reduce the luciferase activity confirms the specificity of targeting.

AVE ± SD reported and significance was tested using a two-tailed Student's t-test: *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001.

Supplementary Table 4: RT-qPCR analysis of *Dg*, *Dys* and miRNAs transcript levels

Genotype	*Dg exon 14-15* Average C_T	*RpL32* Average C_T	ΔC_T *Dg* – *RpL32*[1]	$\Delta\Delta C_T$ (ΔC_T-$\Delta C_{T,control}$[2])	Average *Dg* relative to control[3]	*p*-value two-tailed Student's t-test
insc-Gal4 (larvae)	20.79±0.13	15.59±0.04	5.21±0.13	0.00±0.13	1.00±0.09	
insc-Gal4/UAS-Dg (larvae)	16.83±0.07	15.81±0.14	1.03±0.07	-4.18±0.07	18.14±0.83	$3.7x10^{-6}$***
insc-Gal4/UAS-DgExD (larvae)	16.43±0.05	15.79±0.12	0.64±0.05	-4.57±0.05	23.76±0.75	$8.2x10^{-7}$***
w^{1118} (whole flies)	21.60±0.13	16.28±0.21	5.37±0.25	-0.17±0.26	1.01±0.16	
Oregon R (whole flies)	21.72±0.18	15.87±0.04	5.11±0.24	-0.22±0.50	0.90±0.12	0.41
Dg^{O55}/Dg^{O86} (whole flies)	23.36±0.72	15.70±0.67	7.66±0.38	2.00±0.45	0.26±0.08	0.0017**
KT40/KT40 (whole flies)	19.51±0.18	15.58±0.06	3.94±0.12	-1.60±0.12	3.03±0.25	$3.4x10^{-6}$***
Oregon R (whole flies)	21.24±0.12	15.41±0.08	5.82±0.03	0.00±0.03	1.00±0.02	
ΔNOS (whole flies)	21.04±0.33	15.64±0.25	5.40±0.24	-0.43±0.24	1.35±0.21	0.0455*
w^{1118} (heads)	23.15±0.34	16.06±0.09	7.09±0.32	0.02±0.45	1.02±0.22	
KT40/KT40 (heads)	21.06±0.06	16.48±0.32	4.630.41	-2.44±0.58	5.66±1.57	0.0071**
w^{1118} (bodies)	20.80±0.49	16.26±0.16	4.53±0.34	0.00±0.33	1.02±0.22	
KT40/KT40 (bodies)	20.81±0.44	16.28±0.30	4.53±0.14	0.00±0.14	1.01±0.10	0.93
Oregon R (whole flies, 29˚C)	19.62±0.11	14.74±0.09	4.87±0.01	0.00±0.01	1.00±0.01	
miR-310s-Gal4/UAS-Dg (whole flies, 29˚C)	19.05±0.08	15.06±0.08	4.00±0.04	-0.88±0.04	1.84±0.05	$2.1x10^{-4}$***
Oregon R (larvae)	20.72±0.09	15.24±0.14	5.49±0.07	0.00±0.07	1.00±0.05	
miR-310s-Gal4/UAS-Dg (larvae)	15.38±0.05	14.22±0.16	1.15±0.14	-4.33±0.15	20.23±2.01	7.8e-5***
Genotype	***Dg-long* Average C_T**	***RpL32* Average C_T**	**ΔC_T *Dg-long* – *RpL32*[1]**	**$\Delta\Delta C_T$ (ΔC_T-$\Delta C_{T,control}$[2])**	**Average *Dg-long* relative to control[3]**	***p*-value two-tailed Student's t-test**
w^{1118} (heads)	27.06±0.90	14.01±0.23	13.04±0.76	-0.00±0.76	1.10±0.58	
KT40/KT40 (heads)	23.63±0.38	13.66±0.05	9.96±0.34	-3.09±0.34	8.63±1.98	0.0032**
w^{1118} (bodies)	25.65±0.18	13.88±0.23	11.78±0.04	0.00±0.04	1.00±0.03	
KT40/KT40 (bodies)	24.65±0.16	14.42±0.05	10.22±0.18	-1.55±0.18	2.95±0.37	0.0059**
w^{1118} (ovaries)	28.33±0.16	13.10±0.45	15.22±0.29	0.00±0.28	1.01±0.20	
KT40/KT40 (ovaries)	27.11±0.26	12.77±0.18	14.33±0.28	-0.89±0.28	1.88±0.36	0.057
Genotype	***Dg-short* and *long* Average C_T**	***RpL32* Average C_T**	**ΔC_T *Dg-short* – *RpL32*[1]**	**$\Delta\Delta C_T$ (ΔC_T-$\Delta C_{T,control}$[2])**	**Average *Dg-short* relative to control[3]**	
w^{1118} (heads)	22.97±1.22	14.18±0.43	8.48±0.68	0.00±0.68	1.06±0.48	
KT40/KT40 (heads)	20.17±0.63	13.99±0.13	6.19±0.53	-2.29±0.53	5.12±1.67	0.049*
w^{1118} (bodies)	20.21±0.43	14.64±0.23	5.62±0.19	0.00±0.20	1.01±0.13	
KT40/KT40 (bodies)	20.25±0.43	14.83±0.26	5.36±0.72	-0.25±0.72	1.28±0.57	0.45
Genotype	***Dys* Average C_T**	***Rpl32* Average C_T**	**ΔC_T *Dys* – *Rpl32*[1]**	**$\Delta\Delta C_T$ (ΔC_T-$\Delta C_{T,control}$[2])**	**Average *Dys* relative to control[3]**	***p*-value two-tailed Student's t-test**
insc-Gal4 (larvae)	21.27±0.04	15.59±0.04	5.68±0.04	0.00±0.04	1.00±0.03	
insc-Gal4/UAS-Dg (larvae)	21.36±0.13	15.81±0.14	5.55±0.13	-0.13±0.13	1.10±0.10	0.278

Genotype	*miR-312* Average C_T	*2S rRNA* Average C_T	ΔC_T *miR-312 – 2s rRNA*[1]	$\Delta\Delta C_T$ (ΔC_T-$\Delta C_{T,control}$[2])	Average *miR-312* relative to control[3]	*p*-value two-tailed Student's t-test
Oregon R (whole flies, 29˚C)	25.26±0.13	8.51±0.38	16.75±0.25	0.00±0.25	1.01±0.18	
miR-310s-Gal4/UAS-Dg (whole flies, 29˚C)	24.94±0.03	9.74±0.26	15.2±0.30	-1.55±0.30	2.96±0.61	0.0056**
Oregon R (larvae)	28.55±0.15	8.88±0.19	19.67±0.21	0.00±0.21	1.01±0.15	
miR-310s-Gal4/UAS-Dg (larvae)	26.11±0.19	8.88±0.18	17.23±0.20	-2.44±0.20	5.47±0.78	$8.5x10^{-5}$***
Oregon R (whole flies)	25.27±1.01	6.89±1.08	18.38±0.32	0.00±0.32	1.02±0.23	
Dg^{O55}/Dg^{O86} (whole flies)	24.97±0.78	6.18±0.55	18.78±0.24	0.41±0.24	0.76±0.12	0.037*
KT40/KT40 (whole flies)	31.75±1.48	6.01±0.25	25.74±1.25	7.36±1.25	0.01±0.01	$8.2x10^{-5}$***
Oregon R (whole flies, +5% sucrose)	25.24±0.13	8.53±0.14	16.72±0.13	0.00±0.13	1.00±0.09	
Oregon R (whole flies, +0.2ng/µl NG 5% sucrose)	24.40±0.16	8.19±0.15	16.21±0.16	-0.52±0.16	1.44±0.15	0.0006***
Oregon R (larvae, +5% sucrose)	26.26±0.11	7.60±0.02	18.66±0.09	0.00±0.09	1.00±0.06	
Oregon R (larvae, +0.2ng/µl NG 5% sucrose)	25.27±0.02	7.64±0.06	17.63±0.08	-1.03±0.08	2.04±0.11	0.0073**
w^{1118} (larvae)	26.11±0.13	6.93±0.10	19.18±0.17	0.00±0.18	1.00±0.11	
ΔNOS (larvae)	26.79±0.28	6.84±0.22	19.95±0.23	0.76±0.23	0.60±0.10	0.0037**
insc-Gal4 (larvae)	25.66±0.02	5.64±0.12	20.02±0.02	0.00±0.02	1.00±0.01	
insc-Gal4/UAS-SynRNAi (larvae)	26.14±0.03	5.76±0.21	20.38±0.03	0.36±0.03	0.78±0.02	0.0036**
Oregon R (larvae)	24.44±0.06	5.76±0.07	18.68±0.06	0.00±0.06	0.96±0.04	
insc-Gal4/UAS-Dg (larvae)	23.08±0.05	5.75±0.06	17.52±0.26	-1.16±0.26	2.26±0.40	0.0001***
Genotype	***miR-310*** **Average C_T**	***2S rRNA*** **Average C_T**	**ΔC_T** ***miR-310 – 2s rRNA***[1]	**$\Delta\Delta C_T$ (ΔC_T-$\Delta C_{T,control}$[2])**	**Average *miR-310* relative to control[3]**	***p*-value two-tailed Student's t-test**
Oregon R (whole flies, +5% sucrose)	23.74±0.14	8.53±0.14	15.22±0.14	0.00±0.14	1.00±0.09	
Oregon R (whole flies, +0.2ng/µl NG 5% sucrose)	22.68±0.58	8.19±0.15	14.49±0.58	-0.72±0.58	1.76±0.73	0.0048**
Oregon R (larvae, +5% sucrose)	25.53±0.00	7.63±0.06	17.93±0.00	0.00±0.00	1.00±0.00	
Oregon R (larvae, +0.2ng/µl NG 5% sucrose)	24.34±0.03	7.64±0.07	16.70±0.03	-1.23±0.03	2.34±0.04	0.0005***
w^{1118} (larvae)	23.27±0.18	6.73±0.16	16.54±0.18	0.00±0.18	1.00±0.13	
ΔNOS (larvae)	23.67±0.43	6.66±0.12	17.02±0.43	0.48±0.43	0.74±0.22	0.0540*
insc-Gal4 (larvae)	24.86±0.07	5.64±0.12	19.22±0.06	0.00±0.06	1.00±0.05	
insc-Gal4/UAS-SynRNAi (larvae)	25.76±0.06	5.76±0.21	20.00±0.06	0.78±0.06	0.58±0.02	0.0001***
Oregon R (larvae)	24.90±0.03	5.78±0.09	19.13±0.03	0.00±0.03	1.00±0.02	
insc-Gal4/UAS-Dg (larvae)	23.10±0.12	5.77±0.04	17.33±0.12	-1.80±0.12	3.48±0.29	0.0001***

Reported numbers are AVE ± SD, and significance was tested using a two-tailed Student's t-test: *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001.

Supplementary Table 5: Dg levels correlate with the mitotic index of larval NBs

Genotype	Number of lobes analysed	# EdU positive NBs	# EdU negative NBs	% EdU Positive NBs	*p*-value
Control (w^{1118})	14	14.3 ± 1.6	16.3 ± 2.4	46.8 ± 3.9	
Dg^{055}/Dg^{055}	17	8.4 ± 2.4	18.0 ± 3.4	31.4 ± 5.7	$2.8x10^{-9}$***
AVE ± SD are reported and significance was tested using a two-tailed Student's t-test: *p ≤ 0.05 **p ≤ 0.01 ***p ≤ 0.001.					
Genotype	**Number of lobes analysed**	**# PH3 positive NBs**	**# PH3 negative NBs**	**% PH3 positive NBs**	***p*-value[t]**
Control (w^{1118})	8	18.1 ± 5.1	23.9 ± 6.2	42.9 ± 2.6	
KT40 (*KT40/KT40*)	8	19.2 ± 4.6	19.1 ± 5.5	50.4 ± 9.3	0.048*
KT40/DfExcel6070 (*KT40/DfExcel6070*)	8	23.8 ± 6.5	25.0 ± 5.1	48.3 ± 6.1	0.039*
KT40; tub>DgRNAi (*KT40/KT40*; *tubGal4, UAS-DgRNAi*)	8	14.6 ± 2.7	31.4 ± 3.9	32.0 ± 6.0	$3.0x10^{-3}$***
tub>DgRNAi (*tubGal4, UAS-DgRNAi*)	6	15.7 ± 3.4	30.3 ± 8.0	34.6 ± 7.3	0.015*
miR-310s>Dg (*UAS-Dg/+; miR-310s-Gal4/+*)	6	29.0 ± 6.9	19.0 ± 5.3	60.5 ± 6.5	$1.4x10^{-5}$***
miR-310s> Khc-73 (*miR-310s-Gal4/UAS-Khc-73*)	7	16.3 ± 3.1	31.1 ± 5.0	34.5 ± 1.5	0.0068**
miR-310s>DgRNAi (*miR-310s-Gal4/ UAS-DgRNAi*)	10	9.9 ± 0.7	33.6 ± 1.1	22.7 ± 4.7	$8.3x10^{-9}$***
Dg^{055}/Dg^{055}	8	13.0 ± 3.7	29.5 ± 8.3	31.2 ± 9.2	0.0040**
Sister clones hs Flp; FRT42B GFP/ FRT42B GFP	17	2.0 ± 0.7	2.82 ± 0.8	40.9 ± 5.8	0.449 (w^{1118})
Dg clones hs Flp; FRT42B GFP/ FRT42B Dg^{086}	17	2.2 ± 0.75	4.6 ± 1.4	33.3 ± 8.6	0.020* (*sister clones*)
AVE ± SD are reported and significance was tested using the two-tailed Student's t-test: *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001. [t] Statistical comparisons were made to genotype w^{1118} unless there is a genotype in parenthesis, in which case the comparison was made to that genotype.					

Supplementary Table 6: *miR-310s* and *Dg* misexpression leads to the premature termination of photoreceptor axon projections

Genotype	Defective lamina plexuses (%)	n, analyzed optic lobes	*p*-value[t]
Control (*w^{1118}*)	5.9 ± 5.6	52	
Control (*KT40/+*)	6.1 ± 5.3	55	0.49
KT40 (*KT40/KT40*)	62.4 ± 3.7	61	$6.4x10^{-5}$***
KT40; GMR>miR-310s (*KT40/KT40*; *GMR-Gal4/UAS-miR-310s*)	3.6 ± 5.1	25	$3.0x10^{-4}$*** (*KT40*) 0.33 (*w^{1118}*)
GMR-Gal4 (Control)	8.2 ± 1.2	60	0.27
GMR>miR-310s (*GMR-Gal4/UAS-miR310s*)	7.6 ± 1.2	40	0.29
miR-310s>Dg (*UAS-Dg/+*; *miR-310s-Gal4/+*)	40.8 ± 8.7	39	0.0022**
GMR>Dg (*UAS-Dg; GMR-Gal4)*	75.1 ± 2.5	96	$2.8x10^{-4}$***
KT40; *GMR>DgRNAi (KT40/KT40*; *GMR-Gal4/UAS-DgRNAi)*	36.2 ± 8.0	49	0.0034** (*KT40*) 0.0029** (*w^{1118}*)
GMR>DgRNAi (*GMR-Gal4/UAS-DgRNAi*)	12.1 ± 3.0	51	0.258

The experiment was conducted using three different bouts of progeny from independent crosses. Each experimental round consisted of between 5 and 20 optic lobe examinations being marked as disturbed or normal to generate an experimental percentage per biological replicate.
AVE ± SD reported and statistical significance was tested using the one-tailed Student's t-test:
*P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001.
[t] Statistical comparisons were made to genotype *w^{1118}* unless there is a genotype in parenthesis, in which case the comparison was made to that genotype.

Supplementary Table 7: NO amounts are altered in *Dg* and *NOS* mutants

Genotype	Relative NO levels (Total Nitrite/Nitrate)	*p*-value
Control (*insc-Gal4*)	1.00 ± 0.07	
NG (*insc-Gal4* +0.2ng/µl NG in 5% sucrose)	1.20 ± 0.17	0.049*
ΔNOS	0.75 ± 0.19	0.028*
insc>Dg (*insc-Gal4/UAS-Dg*)	1.25 ± 0.12	0.001**

AVE ± SD reported from the measurements done from 3-6 independent experiments and statistical significance was tested using two sample z-statistics: *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001.

Supplementary References

1. Barresi, R. & Campbell, K.P. Dystroglycan: from biosynthesis to pathogenesis of human disease. *J Cell Sci* **119**, 199-207 (2006).
2. Cohn, R.D. Dystroglycan: important player in skeletal muscle and beyond. *Neuromuscul.Disord.* **15**, 207-217 (2005).