

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21

Supplementary Materials for

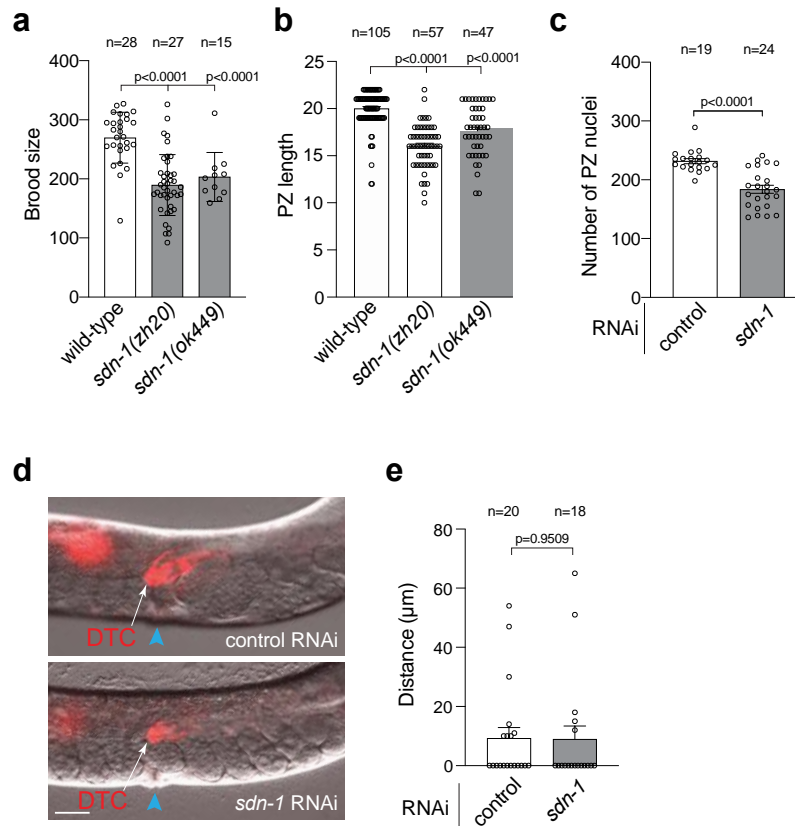
A Somatic Proteoglycan Controls Notch-Directed Germ Cell Fate

Sandeep Gopal*, Aqilah Amran, Andre Elton, Leelee Ng and Roger Pocock*

Correspondence to: roger.pocock@monash.edu and sandeep.gopal@monash.edu

This PDF file includes:

- Figures. S1 to S10
- Table S1



22
23

24 **Fig. S1. *sdn-1* loss-of-function germline analysis**

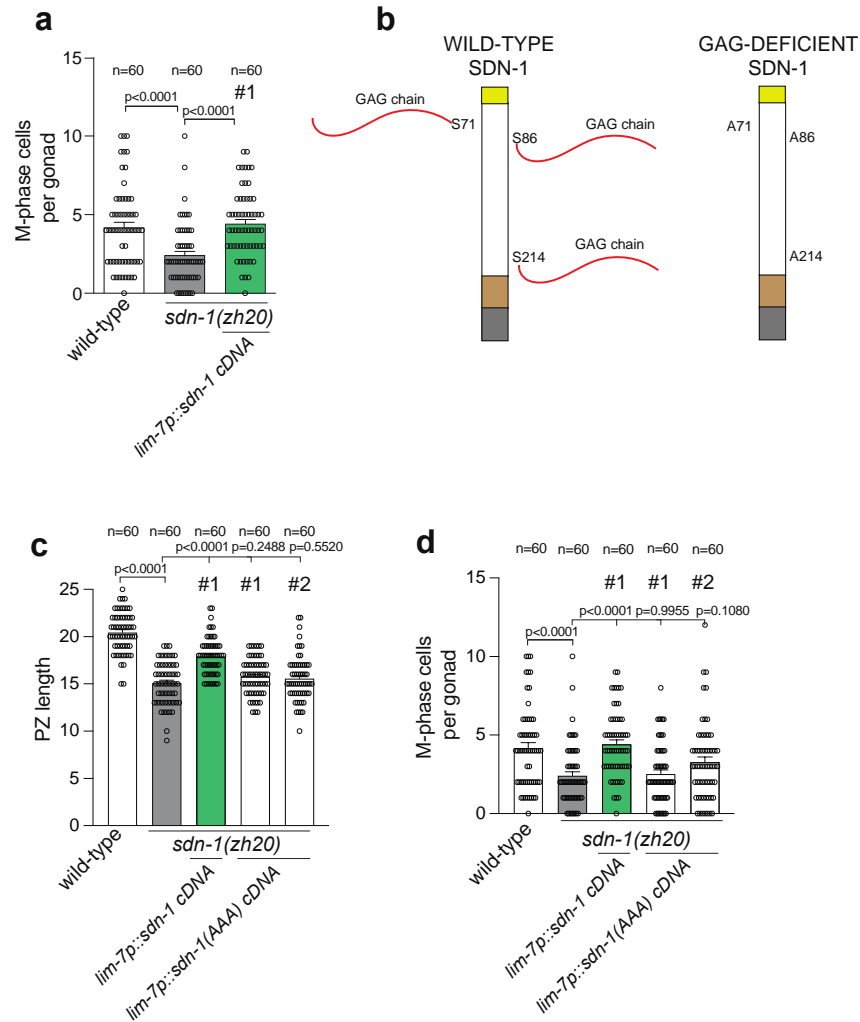
25 (A) Brood size of wild-type, *sdn-1(zh20)* and *sdn-1(ok449)* hermaphrodites. Data are
26 expressed as mean ± s.e.m. and statistical significance was assessed by ordinary one-
27 way ANOVA.

28 (B) PZ length of wild-type, *sdn-1(zh20)* and *sdn-1(ok449)* adult hermaphrodites. Data
29 are expressed as mean ± s.e.m. and statistical significance was assessed by ordinary
30 one-way ANOVA.

31 (C) 3D germline quantification of PZ nuclei in adult hermaphrodite (+/- *sdn-1* RNAi).
32 Data are expressed as mean ± s.e.m. and statistical significance was assessed by two-
33 tailed unpaired t-test.

34 (D-E) Immunofluorescence images (D) and quantification (E) distal tip cell position in
35 adult hermaphrodites (+/- *sdn-1* RNAi) with respect to vulva (blue arrowhead). Data are
36 expressed as mean ± s.e.m. and statistical significance was assessed by two-tailed
37 unpaired t-test. For all panels, n refers to number of animals analyzed. P-values as
38 indicated.

39
40
41



42

43

44 **Fig. S2. *sdn-1* mutant rescue experiments**

45 (A) Quantification of M-phase cells in distal germlines of wild-type, *sdn-1(zh20)* and *sdn-1(zh20); lim-7p::sdn-1 cDNA* adult hermaphrodites. The *lim-7p::sdn-1 cDNA* rescue line corresponds to the same line in Figure 1F. Data are expressed as mean \pm s.e.m. and statistical significance was assessed by ordinary one-way ANOVA.

49 (B) Schematic of wild-type SDN-1 and GAG-deficient SDN-1. Attachment sites (S71, S86 and S214) are shown with GAG chains as red lines in wild-type. Alanine mutations that prevent GAG attachment are shown in the GAG-deficient SDN-1. Yellow - signal sequence; White - ectodomain; Brown - transmembrane domain; Grey - cytoplasmic domain.

54 (C) Quantification of PZ length in distal germlines of adult hermaphrodites in wild-type, *sdn-1(zh20)* and *sdn-1(zh20); lim-7p* rescue lines expressing wild-type SDN-1 or GAG-chain deficient SDN-1(AAA). Independent rescue lines represented by # (#1 line for wild type SDN-1 corresponds to the same line in Figure 1F). Data are expressed as mean \pm s.e.m. and statistical significance was assessed by ordinary one-way ANOVA.

59 (D) Quantification of M-phase cells in distal germlines of adult hermaphrodites in wild-
60 type, *sdn-1(zh20)* and *sdn-1(zh20); lim-7p* rescue lines expressing wild-type SDN-1 or
61 GAG-chain deficient SDN-1(AAA). Independent rescue lines represented by # (#1 line
62 for wild type SDN-1 corresponds to the same line in Figure 1F). Data are expressed as
63 mean \pm s.e.m. and statistical significance was assessed by ordinary one-way ANOVA.
64 For all panels, n refers to number of animals analyzed. P-values as indicated.

65

66

67
68
69
70
71
72
73
74
75
76
77
78
79
80
81
82
83
84
85
86
87
88
89
90
91

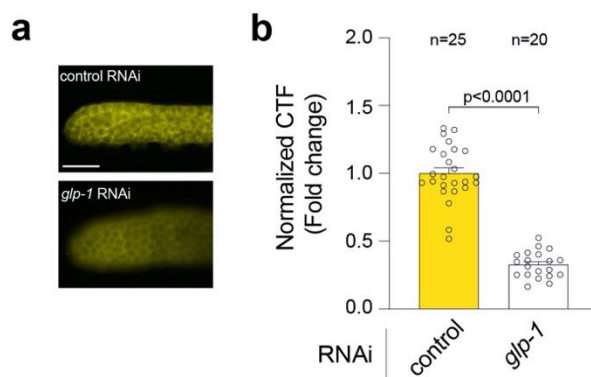
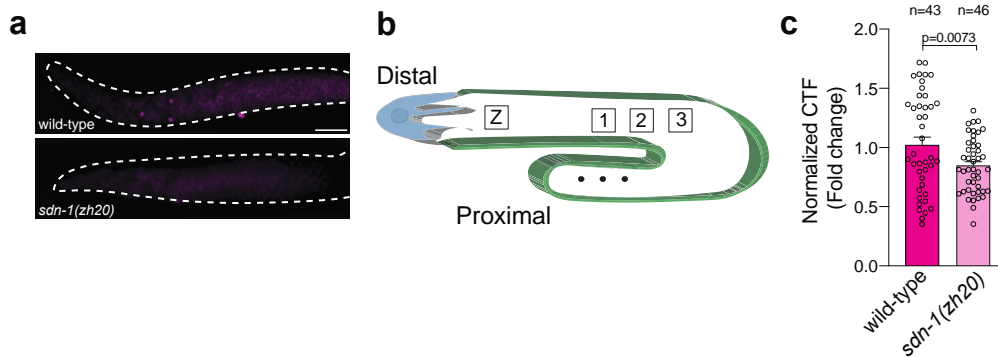


Fig. S3. Validation of *glp-1* RNAi

(A-B) Immunofluorescence images (A) and quantification (B) of GLP-1::V5 expression in the PZ of wild-type (+/- *glp-1* RNAi). Data are expressed as mean \pm s.e.m. and statistical significance was assessed by two-tailed unpaired t-test. Scale bar 20 μ m. n refers to number of animals analyzed. P-values as indicated.

92
93
94
95
96
97
98
99
100
101
102
103
104

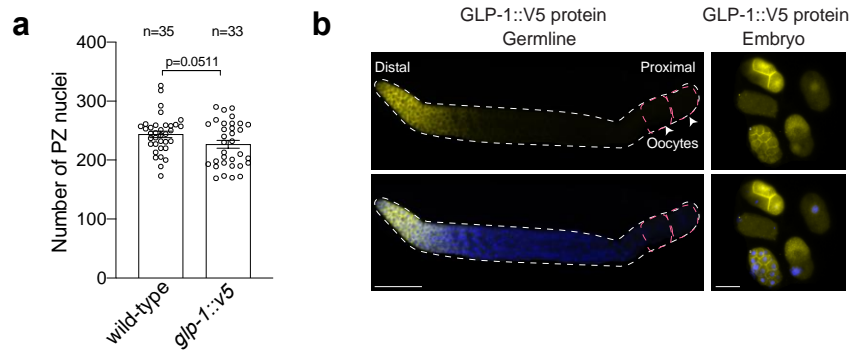


105
106
107
108
109
110
111
112
113
114
115
116
117
118
119

Fig. S4. *glp-1* transcript analysis by smFISH

(A-C) Immunofluorescence images (A), schematic of image analysis (B) and quantification (C) of *glp-1* smFISH expression in the PZ of wild-type and *sdn-1(zh20)* adult hermaphrodites. *glp-1* transcripts = magenta (A). smFISH signal was quantified from three areas (boxes 1-3) while considering an area in the distal end as the background (box Z) (B). Data are expressed as mean \pm s.e.m. and statistical significance was assessed by two-tailed unpaired t-test. Scale bar = 20 μ m. n refers to number of animals analyzed. P-value as indicated.

120
121
122
123
124
125
126
127
128
129



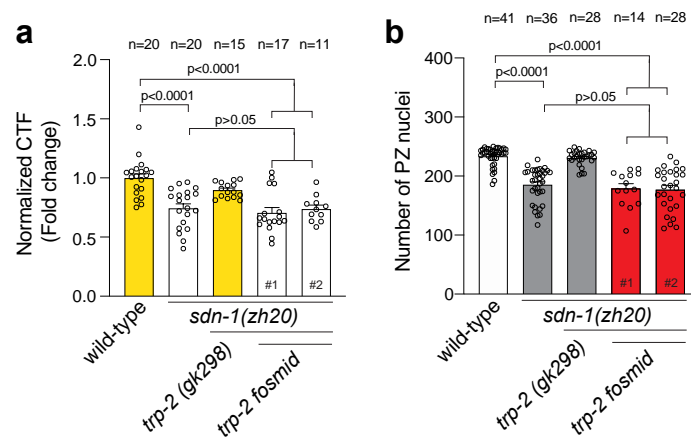
130
131
132
133
134
135
136
137
138
139
140
141

Fig. S5. Analysis of CRISPR-Cas9 generated GLP-1::V5 strain

(A) Quantification of PZ nuclei of wild-type and CRISPR-Cas9-tagged *glp-1::v5* adult hermaphrodites. Data are expressed as mean \pm s.e.m. and statistical significance was assessed by two-tailed unpaired t-test. P-value as indicated. n refers to number of animals analyzed.

(B) Images of GLP-1::V5 in adult germlines and embryos. 30 germlines and 20 embryos were analysed. Performed in duplicate. Scale bars = 25 μ m.

142
143
144
145
146
147
148
149
150
151
152
153
154
155
156
157



158
159
160

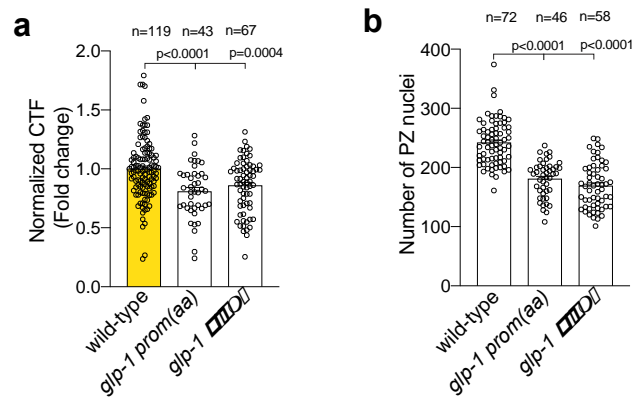
Fig. S6. *trp-2* fosmid rescue analysis

161 (A) Quantification of GLP-1::V5 protein in wild-type, *sdn-1(zh20)* and *sdn-1(zh20); trp-*
162 *2(gk298)* (+/- *trp-2* fosmid) in adult hermaphrodite germlines. Data are expressed as
163 mean ± s.e.m. and statistical significance was assessed by ordinary one-way ANOVA.

164 (B) Quantification of PZ nuclei in wild-type, *sdn-1(zh20)* and *sdn-1(zh20); trp-2(gk298)*
165 (+/- *trp-2* fosmid) in adult hermaphrodite germlines. Data are expressed as mean ±
166 s.e.m. and statistical significance was assessed by ordinary one-way ANOVA. n refers
167 to number of animals analyzed. P-values as indicated.

168

169
170
171
172
173
174
175
176
177
178



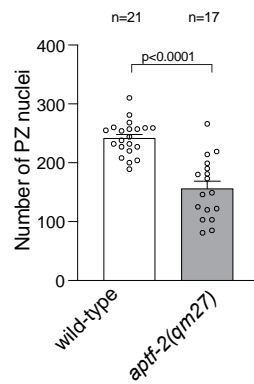
179 **Fig. S7. *glp-1* promoter analysis**

180 (A) Quantification of GLP-1::V5 protein in wild-type, *glp-1prom(aa)* and *glp-1promΔ*
181 adult hermaphrodite germlines. Data are expressed as mean \pm s.e.m. and statistical
182 significance was assessed by ordinary one-way ANOVA.

183 (B) Quantification of PZ nuclei in wild-type, *glp-1prom(aa)* and *glp-1promΔ* adult
184 hermaphrodites. Data are expressed as mean \pm s.e.m. and statistical significance was
185 assessed by ordinary one-way ANOVA. n refers to number of animals analyzed. P-
186 values as indicated.

187

188
189
190
191
192
193
194
195
196
197

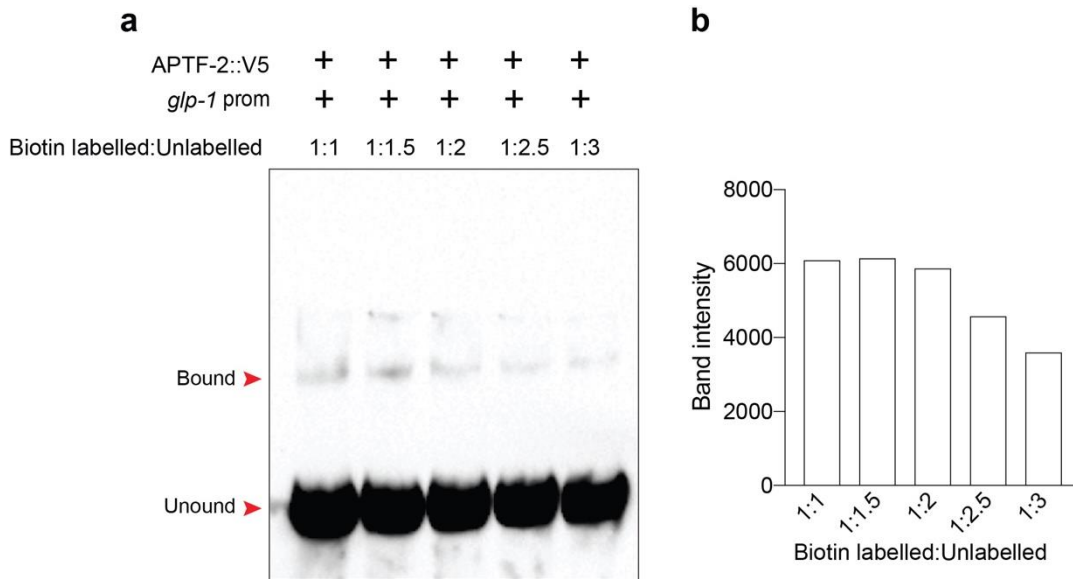


198
199
200
201
202
203
204
205
206

Fig. S8. PZ nuclei analysis of *aptf-2(qm27)* animals

Quantification of PZ nuclei in wild-type and *aptf-2(qm27)* adult hermaphrodites. Data are expressed as mean \pm s.e.m. and statistical significance was assessed by two-tailed unpaired t-test. n refers to number of animals analyzed. P-value as indicated.

207
208
209
210
211
212
213
214

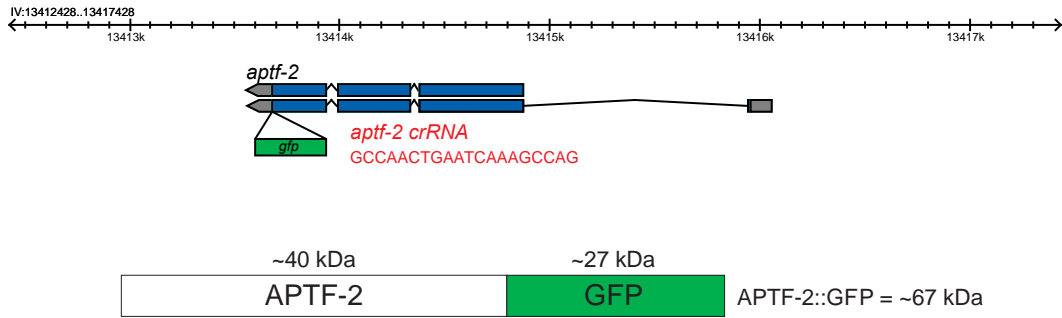


215
216
217
218
219
220
221
222
223
224
225

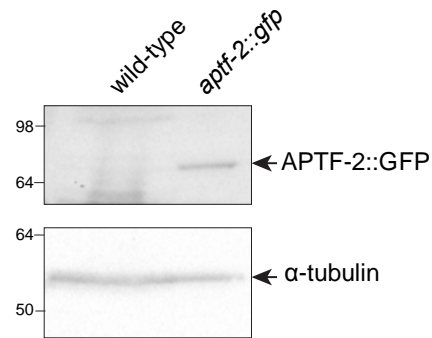
Fig. S9. *glp-1* EMSA analysis

(A-B) EMSA competition experiments. APTF-2::V5 protein produced in HEK293T cells and the TFAP2 consensus motif in the *glp-1* promoter (A). DNA bound and unbound with APTF-2::V5 is marked. Biotin-labelled wild-type *glp-1* promoter is competed away with increasing amount of unlabeled *glp-1* promoter. Quantification of shifted *glp-1* promoter with increasing amount of unlabeled *glp-1* promoter (B).

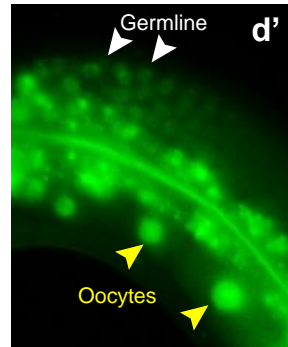
a



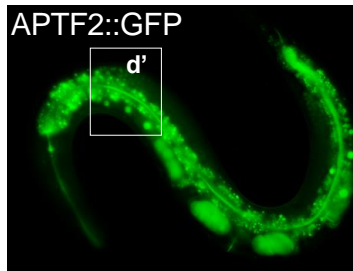
b



c



d



226

227 **Fig. S10. APTF-2::GFP expression analysis**

228 (A) *aptf-2* genomic locus (upper image) showing 3'-end tagging of the gene (crRNA
229 used for Cas9 cleavage in red). Schematic of APTF-2 protein (lower image) showing

230 approximate molecular weight of the fusion protein following CRISPR/Cas9 insertion of
231 GFP.

232 (B) Western blot of endogenously tagged APTF-2::GFP from mixed-stage worms.
233 APTF-2::GFP protein is the correct size (APTF-2 = ~40kDa, GFP = 27kDa, combined =
234 ~67kDa). Top blot - anti-GFP antibody; bottom blot - anti-tubulin loading control.
235 Performed in duplicate.

236 (C-D) Endogenous APTF-2::GFP expression in the adult hermaphrodite germline. DIC
237 image of a young adult hermaphrodite (C), fluorescence image of the same worm (D)
238 and zoomed image of the boxed region (D') showing expression in the germline (white
239 arrowheads) and oocytes (yellow arrowheads). Scale bar = 50µm.

240
241
242
243

Table S1. Materials used.

Reagents	Company	Catalogue number
Formaldehyde (37%)	Sigma	F8775
Lipofectamine 2000	Thermo Fisher	11668019
UltraPure™ Agarose	Thermo Scientific	16500-500
Ampicillin sodium salt	Sigma	A9518
DMEM	Life Technologies	11960044
L-glutamine	Life Technologies	25030-081
Fluoroshield	Sigma Aldrich	F6182
Formamide, Deionized	Merck Millipore	S4117
DAPI readymade solution	Sigma Aldrich	MBD0015
Alexa Fluor™ Plus 555 Phalloidin	Thermofisher	A30106
Goat serum (10mL)	Sigma Aldrich	G9023
Epstein-Barr Nuclear Antigen (EBNA)	Thermofisher	Part of 20148
Antibody	Company	Catalogue number
Mouse monoclonal anti-V5 (clone SV5-pk1) (1:1000)	BioRad	MCA1630
Mouse monoclonal anti- α -tubulin (clone 12G10) (1:200)	Developmental Studies Hybridoma Bank	AB1157911
Mouse-polyclonal anti-GFP (1:1000)	Sigma Aldrich	11814460001
Rabbit-monoclonal anti-pH3 (1:1000)	Sigma Aldrich	06570
Goat anti-Mouse IgG (H+L) Secondary Antibody, HRP conjugate (1:1000)	Life Technologies	32430
Bacterial strain	Source	Identifier
Bacteria <i>Escherichia coli</i> HT115	CAENORHABDITIS GENETICS CENTER (CGC)	N/A
Bacteria <i>Escherichia coli</i> OP50	CGC	N/A
<i>C. elegans</i> strain	Source	Identifier
<i>sdn-1(zh20)</i>	CGC	AH205
<i>trp-2(gk298)</i>	CGC	VC602
<i>glp-1::v5(q1000)</i>	Kimble lab	JK5933
<i>sdn-1(ok449)</i>	CGC	RB690
<i>sdn-1(zh20); trp-2(gk298) glp-1::v5(q1000)</i>	This study	RJP3846
<i>sdn-1(zh20); rpls121(lim-7p::sdn-1::gfp::tbb-2)</i> line 1	This study	RJP3867
<i>sdn-1(zh20); rpls121(lim-7p::sdn-1::gfp::tbb-2)</i> line 2	This study	RJP3866
<i>sdn-1(zh20); rpls154(mex-5p::sdn-1::gfp::tbb-2)</i> line 1	This study	RJP3857
<i>sdn-1(zh20); rpls154(mex-5p::sdn-1::gfp::tbb-2)</i> line 2	This study	RJP3855

<i>sdn-1(zh20) rpls114(lag-2p::sdn-1::gfp::tbb-2)</i> line 1	This study	RJP3863
<i>sdn-1(zh20); rpls114(lag-2p::sdn-1::gfp::tbb-2)</i> line 2	This study	RJP3858
<i>sdn1(zh20); rpls157(lim-7p::sdn-1SA::gfp::tbb-2)</i> line 1	This study	RJP3746
<i>sdn1(zh20); rpls157(lim-7p::sdn-1SA::gfp::tbb-2)</i> line 2	This study	RJP3747
<i>sdn-1(zh20); glp-1::v5(q1000)</i>	This study	RJP3845
<i>trp-2(gk298) glp-1::v5(q1000)</i>	This study	RJP4118
<i>glp-1::v5(q1000)promΔ</i>	This study	RJP4729
<i>sdn1(zh20); glp-1::V5(q1000)promΔ</i>	This study	RJP4730
<i>glp-1::v5(q1000)prom(aa)</i>	This study	RJP4744
<i>glp1::v5(q1000); aptf-2::gfp</i>	This study	RJP4035
<i>sdn-1(zh20); glp-1::V5(q1000); aptf-2::gfp</i>	This study	RJP4731
<i>glp-1::v5(q1000); rpEx2183(sdn-1p::gfp)</i>	This study	RJP5186
<i>glp-1::v5(q1000); rpEx2184(lim-7p::gfp)</i>	This study	RJP5187
<i>sdn-1(zh20); glp-1::v5(q1000); rpEx2184(lim-7p::gfp)</i>	This study	RJP5188
<i>trp-2p::mCherry (IskEx383)</i>	PMID: 29883446	KHK667
<i>sdn-1(zh20); trp-2(gk298) glp-1::v5(q1000); rpEx2185(lim-7p::trp-2)</i> line 1	This study	RJP5189
<i>sdn-1(zh20); trp-2(gk298) glp-1::v5(q1000); rpEx2186(lim-7p::trp-2)</i> line 2	This study	RJP5190
<i>qls154 [lag-2p::MYR::tdTomato + ttx-3p::GFP] V</i>	PMID: 24586318	JK4472
Mammalian Cell Lines	Source	Identifier
HEK293T	N/A	N/A
Wild-type mouse embryonic fibroblast (MEF)	N/A	N/A
Syndecan-4 knockout MEF	N/A	N/A
Commercial Kit	Company	Catalogue number
LightShift™ Chemiluminescent EMSA Kit	Thermofisher	20148
Pierce™ Biotin 3' End DNA Labelling Kit	Thermofisher	89818
NE-PER™ Nuclear and Cytoplasmic Extraction Reagents	Thermofisher	78833
Plasmid	Source	Identifier
<i>pCDNA3.2-aptf-2-v5</i>	This study	N/A
<i>minimos-mex-5p::sdn-1::tbb-2-UTR</i>	Bulow lab	N/A
<i>minimos-lag-2p::sdn-1::tbb-2-UTR</i>	Bulow lab	N/A
<i>minimos-lim-7p::sdn-1::tbb-2-UTR</i>	Bulow lab	N/A
<i>rab-3p::mCherry::unc-54UTR</i>	This study	N/A
<i>myo-2p::mCherry::unc-54UTR</i>	This study	N/A
<i>myo-3p::mCherry::unc-54UTR</i>	This study	N/A

<i>eft-3p::mos1transposase::tbb-2UTR</i>	This study	N/A
<i>hsp16.41p::peel-1::tbb-2UTR</i>	This study	N/A
<i>sdn-1p::gfp</i>	This study	N/A
<i>lim-7p::gfp</i>	This study	N/A
<i>lim-7p::trp-2 cDNA</i>	This study	N/A
Oligonucleotide	Source	Identifier
<i>glp-1-prom</i> F 5'aacacactcagaaaatgggCGGAGTATGCCACCCGGCCTTCATTCGTCGCT CCTGTCTT3'	This study	oSG168
<i>glp-1-prom</i> R 5'aagacaaggagcgacgaatgaaggccgggtggcactactccgccattttc tgagtgttt3'	This study	oSG169
<i>glp-1-prom</i> Δ F 5'aacacactcagaaaatgggCGGAGTATGCGGCCTTCATTCGTCGCTCCTGTCTT TT3'	This study	oSG170
<i>glp-1-prom</i> Δ R 5'aagacaaggagcgacgaatgaaggccgcactactccgccattttctgagt gtgtt3'	This study	oSG171
Control DNA duplex 5'tagcatatgcta3' 3'atcgatacga5'	Thermofisher	Part of 20148
crRNA for <i>aptf-2-gfp</i> gccaaactgaatcaaagccag	This study	N/A
crRNA for <i>glp-1</i> mutations aatgggCGGAGTATGCCACC	This study	N/A
<i>sdn-1</i> promoter F 5'gaaatgaaataagctttcacctgatagcgcgtaacg3'	This study	N/A
<i>sdn-1</i> promoter R 5'ccaatcccggggatccattgtctgttggtgtaacacc3'	This study	N/A
<i>lim-7p::gfp</i> reporter F 5'cgactctagaggatcagtactgtgccttgattctcctg3'	This study	N/A
<i>lim-7p::gfp</i> reporter R 5'tcatttttctaccgccaatcgacagccggcac3'	This study	N/A
<i>lim-7p::trp-2</i> F 5'aggacccttggtagcatgacatcatcagaagcatccgag3'	This study	N/A

<i>lim-7p::trp-2</i> R 5'gatatcaataccatgggcataaaagttcatcagtgctgc3'	This study	N/A
Software	Source	Identifier
ImageJ 1.47n	National Institute of Health, USA	N/A
Imaris 9.1.2	Bitplane	N/A

246

247

248

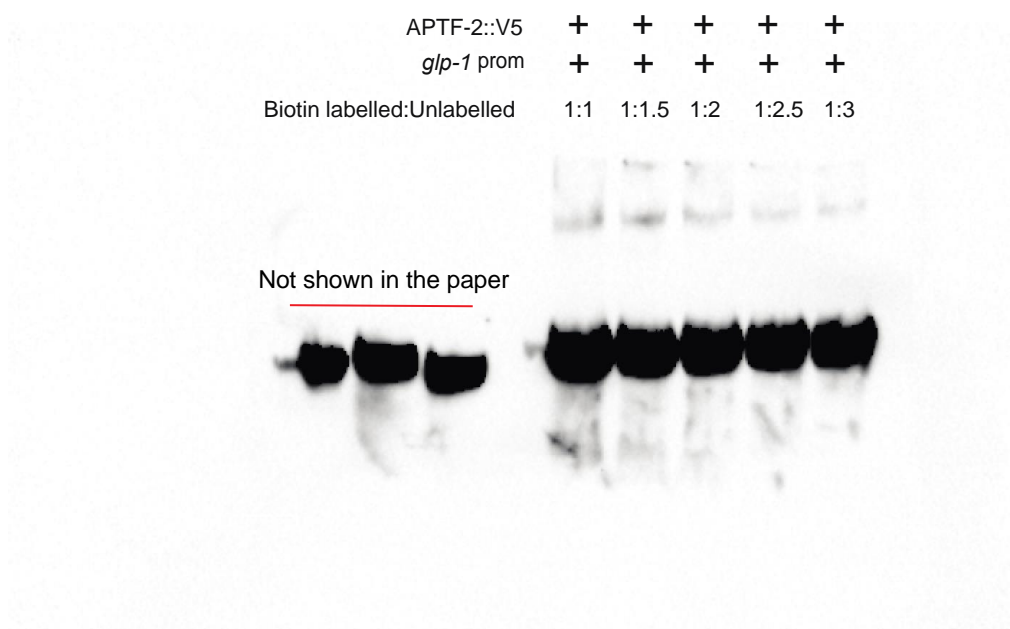
249

250

Uncropped blots

All blots were detected by biotin labelling of oligos and chemiluminescent EMSA kit as explained in supplementary materials.

Figure S9A

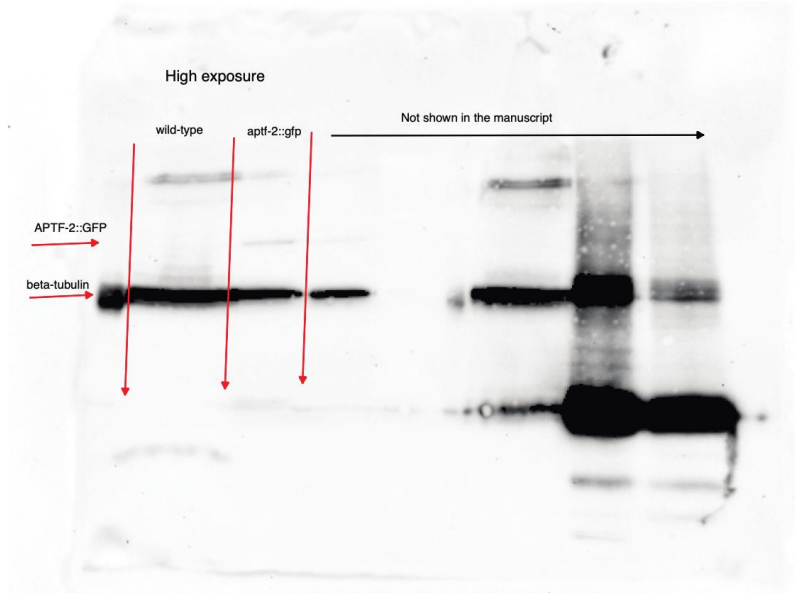


251

252

Uncropped images of Western blots detecting APTF-2::GFP

Supplementary Figure 10B - Top panel high exposure



Supplementary Figure 10B - Bottom panel low exposure

