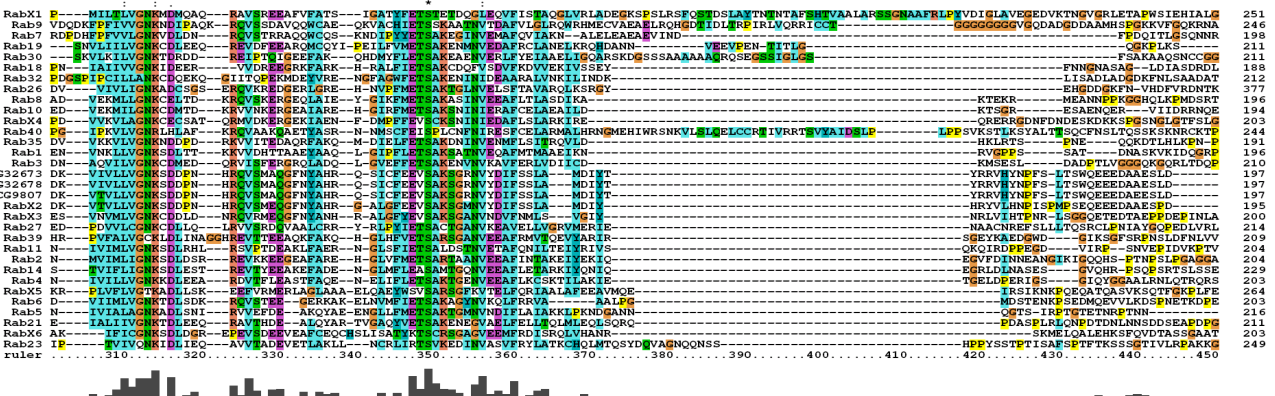
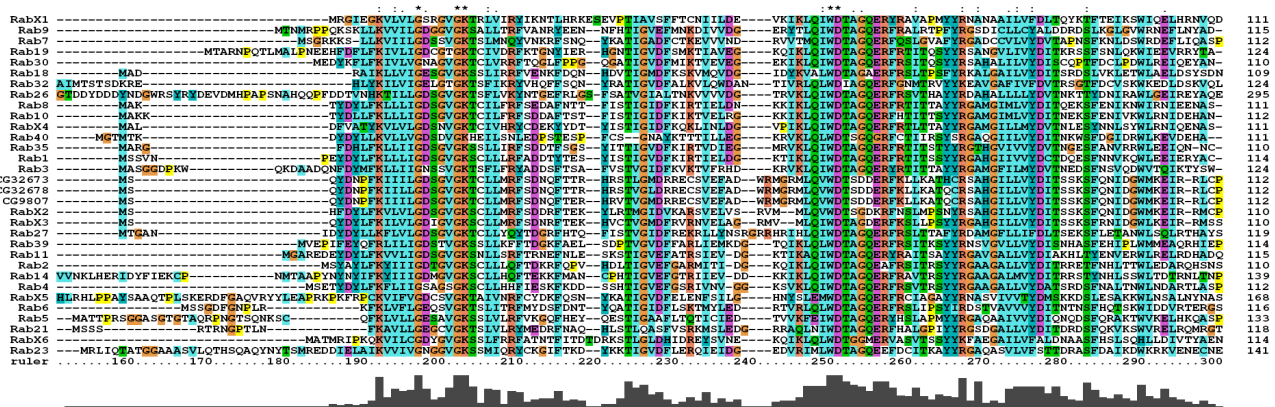
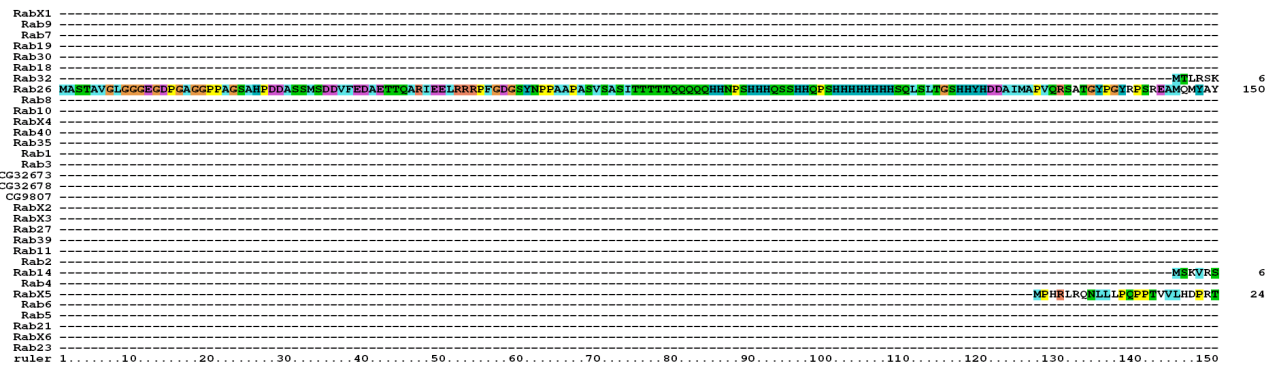


Supplementary Figure 1: Alignment of all *Drosophila* Rab proteins.

The entire set of *Drosophila* Rab protein sequences was retrieved by searching the *Drosophila melanogaster* genome sequence (Release 4.3) and aligned using ClustalW 1.83 software.

Supplementary figure 1:

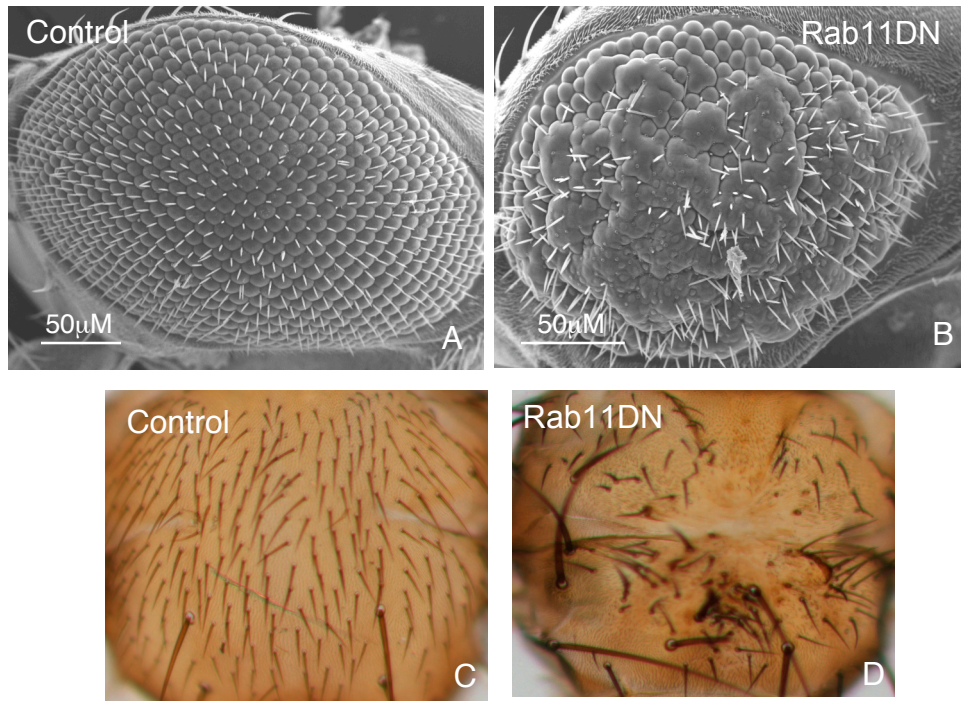


Supplementary Figure 2: Rab11DN causes developmental defects in eyes and bristles.

A-B. A GMR-GAL4 driver was crossed with either *y w* (control) or UAS-YFP-Rab11DN transgenic flies at 25°C. Compared to controls, YFP-Rab11DN expression caused extensive ommatidia fusion and cell death.

C-D. An Eq-GAL4 transgenic line driving expression in a specific area of the notum (Tang and Sun 2002) was crossed with either *y w* (control) or UAS-YFP-Rab11DN transgenic flies at 25°C. Compared to controls, YFP-Rab11DN-expressing flies displayed a strong loss-of-bristle phenotype and defects in the notum epithelium.

Supplementary figure 2:



Supplementary Table 1: Summary of *in situ* hybridization data for *Rab* mRNAs.

<i>Rab</i> gene	Pattern of expression
<i>Rab1</i>	Ubiquitous
<i>Rab2</i>	Central nervous system
<i>Rab3</i>	Central nervous system
<i>Rab4</i>	Ubiquitous, enriched in mesoderm, ectoderm
<i>Rab5</i>	Ubiquitous, enriched in garland cells
<i>Rab6</i>	Ubiquitous
<i>Rab7</i>	Ubiquitous
<i>Rab8</i>	Ubiquitous, enriched in mesoderm, ectoderm
<i>Rab9</i>	Ubiquitous, enriched in central nervous system
<i>Rab10</i>	Ubiquitous, enriched in central nervous system
<i>Rab11</i>	Ubiquitous, enriched in gut
<i>Rab14</i>	Ubiquitous, enriched in salivary gland, central nervous system
<i>Rab18/RP4</i>	Ubiquitous
<i>Rab19/RP3</i>	Ubiquitous
<i>Rab21</i>	Ubiquitous, enriched in gut
<i>Rab23</i>	Stripes
<i>Rab26</i>	Central nervous system
<i>Rab27</i>	Ubiquitous
<i>Rab30</i>	Ubiquitous, enriched in central nervous system
<i>Rab32/RP1</i>	Malpighian tubules
<i>Rab35</i>	Ubiquitous, enriched in central nervous system
<i>Rab39</i>	Ubiquitous
<i>Rab40</i>	Ubiquitous, enriched in central nervous system
<i>RabX1</i>	No strong staining
<i>RabX2</i>	Ubiquitous
<i>RabX3</i>	Ubiquitous
<i>RabX4</i>	Central nervous system
<i>RabX5</i>	Specific dotted pattern, no strong staining in the rest of embryos
<i>RabX6</i>	Ubiquitous
<i>CG9807</i>	No strong staining
<i>CG32673</i>	No strong staining

Supplementary Table 2: Mutations used to generate DN or CA Rab proteins.

The amino acids used to generate the DN or CA form of each Rab protein are summarized here. A conserved T/S in the GTP binding domain or a conserved Q in the GDP binding domain was mutated, with the exception of Rab18, Rab40, RabX2, RabX3, RabX6, CG9807, and CG32673. For these Rab proteins, the amino acid in the corresponding position was mutated.

Rab	DN		CA	
	AA	#	AA	#
Rab1	S	25	Q	70
Rab2	S	20	Q	65
Rab3	T	35	Q	80
Rab4	S	22	Q	67
Rab5	S	43	Q	88
Rab6	T	26	Q	71
Rab7	T	22	Q	67
Rab8	T	22	Q	67
Rab9	S	26	Q	71
Rab10	T	23	Q	68
Rab11	S	25	Q	70
Rab14	S	49	Q	94
Rab18	S	19	A	64
Rab19	T	35	Q	80
Rab21	T	27	Q	73
Rab23	S	51	Q	96
Rab26	T	204	Q	250
Rab27	T	25	Q	74
Rab30	T	21	Q	66
Rab32	T	33	Q	79
Rab35	S	22	Q	67
Rab39	S	23	Q	69
Rab40	H	25	Q	75
RabX1	T	19	Q	66
RabX2	S	21	D	66
RabX3	S	21	D	66
RabX4	T	40	Q	67
RabX5	T	78	Q	123
RabX6	S	22	M	69
CG9807	T	21	D	67
CG32673	T	21	D	67

Supplementary Table 3: Locations of Transgene Inserts.

The coordinates of the insertions, determined by inverse PCR, are shown. Inverse PCR reactions were performed once, and the position was based on the best fit to one or two flanking sequences. The nearest genes to the 5' and 3' of each insertion are also presented. "Location" indicates the boundary coordinate of the nearest gene, irrespective of its orientation, and also specified is the "Distance" between it and the insertion. A negative value is used when an insertion lies less than 1 kb within a gene. "Lethal?" indicates whether the insertion of transgene caused a mutation that prevents homozygotes from surviving. The sequence of each *Rab* construct was verified prior to injection but not *in vivo* for each individual transgenic line.

Genotype		Arm	Location	Cyto	Strand	gene 5'	Location	Distance	gene 3'	Location	Distance	Lethal?
Rab1	WT	01	3L	22,879,289	80C1	Minus	(none)		Mes2	22,879,055	-234	no
	DN	01	3R	8,198,632	87B8	Plus	GstD2	8,198,367	GstD3	8,198,786	154	no
		04	2R	17,630,108	58B9	Minus	CG13503	17,630,130	mei-S322	17,629,683	-425	no
	CA	12a	3L	11,561,084	68C13	Minus	rt		rt			yes
		12c	X	1,091,169	1E4	Minus	CG3638		CG3638			no
Rab2	WT	02	3R	7,603,965	86E18	Minus	l(3)neo38		l(3)neo38			no (semi)
	DN	01	2R	2,250,125	42C7	Minus	l(2)01289		l(2)01289			yes
		05	3R	5,456,535	85E1	Minus	Crc	5,456,607	SpdS	5,457,381	846	no
	CA	02	3L	691,750	61C8	Minus	CG13895	691,733	Ckl1α-i3	692,185	435	no
		25	2R	5,611,474	46E6	Minus	CG1381	5,611,399	14-3-3ζ	5,611,492	18	no
Rab3	WT	02	2R	13,671,196	55B9	Minus	lolal		lolal			no
		05b	3R	19,931,466	95E1	Minus	SyxA	19,931,465	4EHP			yes
	DN	04L	2L	5,108,441	25C6	Plus	CG14035	5,104,074	Msp-300	5,117,577	9,136	no
	CA	11	3L	19,795,273	76D1	Plus	Paps	19,794,774	Rab8	19,801,329	6,056	no
		29a	2R	19,055,570	59E3	Minus	CG3957	19,055,567	CG17280	19,055,840	270	no
Rab4	WT	09	2L	7,576,675	28B1	Plus	CG13790	7,568,818	CG13791	7,578,233	1,558	no
		32	3R	26,714,761	100A7	Plus	Sap-r	26,714,795	CG31101	26,718,636	3,875	no
	DN	37	2L	11,067,085	32D2	Plus	Samuel	11,057,403	CG14914	11,068,776	1,691	no
		46b	3L	16,078,515	72D7	Minus	Zn72D	16,077,647	Taf4	16,078,523	8	no
	CA	13	2L	12,054,932	33C1	Plus	Jhl-21		Jhl-21			no
		28	3L	19,137,106	76A1	Minus	fz2	19,135,885	(none)			no
Rab5	WT	02	3R	18,262,351	94A14	Plus	CG13847	18,253,963	CG12499	18,266,023	3,672	no
		08b	2R	19,174,603	59F5	Plus	Pde8		Pde8			no
	DN	01	2R	3,685,981	44B8	Plus	CG14757	3,685,426	(none)			no
		02	3L	17,936,496	75B2	Minus	Eip75B		Eip75B			yes
	CA	01a	3L	6,192,648	65A9	Plus	LanA	6,191,931	pncr008:3L	6,194,525	1,877	no
		24	2L	3,806,090	24C5	Plus	l(2)k16918		l(2)k16918			no
Rab6	WT	01	2R	17,155,703	57F6	Plus	CG10082		CG10082			no (semi)
	DN	01	2L	3,478,331	23F3	Minus	pgant4	3,475,338	Thor	3,478,434	103	no
		03	3R	15,662,784	92B3	Minus	bnl	15,662,539	CG31459	15,666,567	3,783	no
	CA	05	3L	732,219	61C9	Plus	tRNA:M-i:61D	727,326	emc	732,938	719	no
		23	X	2,646,349	3B6	Plus	CG32795	2,646,098	w	2,646,755	406	no
Rab7	WT	18	3R	20,949,853	96C1	Minus	CG11844		CG11844			no
		21	2L	131,908	21B2	Minus	CG3164	130,791	Gs1	132,060	152	no (semi)
	DN	06	3L	21,813,400	79A2	Plus	(none)		mub	21,813,470	70	no
		19	X	11,647,981	10E6	Minus	CG1578		CG1578			no
	CA	14	3R	27,571,684	100D2	Plus	awd	27,571,755	CG1910	27,571,766	82	yes
		19	2L	1,420,480	22A1	Plus	lea	1,420,449	(none)			no

Genotype			Arm	Location	Cyto	Strand	gene 5'	Location	Distance	gene 3'	Location	Distance	Lethal?
Rab8	WT	09	3L	11,266,102	68C2	Plus	CG6175			CG6175			no (semi)
		45	2R	2,573,584	42E7	Plus	pgant3	2,573,532	52	Tsp42Eq	2,573,920	336	no
	DN	09	2R	2,497,494	42E1	Plus	vimar	2,497,522	-28	CG17002	2,497,541	47	yes
		12	3R	1,414,217	83B4	Plus	CG2926	1,414,097	120	rev7	1,414,665	448	no
	CA	05	2L	7,422,437	27F4	Minus	chm	7,422,389	48	mir-275	7,425,853	3,416	no
		10	3L	3,887,047	63F5	Minus	Ubi-p63E	3,886,670	377	Sc2	3,887,043	-4	yes
Rab9	WT	13	2L	285,734	21B7	Plus	CG3625			CG3625			no
		22	3L	22,341,832	79E4	Minus	Ten-m	22,341,728	104	(none)			yes
	DN	04	3L	14,375,680	70D7	Minus	stwl	14,375,182	498	CG3919	14,375,885	205	no
		11	2L	11,806,543	33A2	Minus	crol			crol			yes
	CA	10	2L	10,506,786	31F4	Minus	Myo31DF	10,506,780	6	CG6094	10,507,117	331	no
		20	3L	732,867	61C9	Plus	tRNA:M-i:61D	727,326	5,541	emc	732,938	71	yes
Rab10	WT	13	2L	13,549,427	34C4	Plus	B4	13,549,323	104	kuz	13,550,141	714	no
		21	3L	6,192,055	65A9	Minus	LanA	6,191,931	124	pncr008:3L	6,194,525	2,470	no
	DN	15	2L	3,056,536	23C5	Minus	v(2)k05816	3,055,601	935	CG3523	3,056,615	79	no (semi)
		25a	3R	22,623,844	97D2	Plus	(none)			TI	22,624,765	921	no
	CA	01	2L	15,329,773	35D1	Plus	(none)			esg	15,329,793	20	yes
		27a	3R	6,586,973	86C5	Plus	MED7	6,586,655	318	CG14685	6,587,032	59	no
Rab11	WT	32	3R	12,013,453	89B7	Minus	Bin1	12,012,615	838	sra	12,012,656	-797	yes
	DN	06	3R	11,154,437	88F1	Plus	CG6236	11,154,000	437	ea	11,154,452	15	no
		35	2R	18,208,550	59A3	Minus	robo	18,208,544	6	CG30259	18,209,026	476	no
	CA	24	2R	3,004,298	43C5	Minus	CG1621	3,004,392	-94	CG1620	3,005,618	1,320	yes
		31	3L	691,428	61C8	Minus	CG13895	691,733	-305	CklIα-i3	692,185	757	no
Rab14	WT	5L	2L	2,311,602	22E1	Plus	CG9967			CG9967			no
		12	3R	27,515,178	100D1	Minus	CG11550	27,505,602		(none)			yes
	DN	01	2L	16,821,059	36B2	Plus	Cas	16,821,135	-76	mdy			yes
		02	3R	26,291,936	99F6	Plus	CDase	26,291,458	478	PH4αEFB	26,291,774	-162	no
	CA	02	2L	13,819,604	34D1	Plus	Sos	13,819,826	-222	b	13,821,203	1,599	no
		07	3L	9,541,946	67C1	Minus	CG32048			CG32048			no
Rab18	WT	01	3R	466,801	82C5	Minus	CG9775	467,273	-472	MED31	467,696	895	no
	DN	02	2L	9,497,568	30B5	Minus	Gdi	9,494,927	2,641	CG33298	9,503,792	6,224	no
		03	3R	8,196,719	87B8	Minus	GstD1	8,194,987	1,732	GstD2	8,197,720	1,001	no
	CA	02	3R	25,283,974	99A11	Minus	Ptp99A			Ptp99A			no
		12	2L	19,133,068	37C1	Plus	brat			brat			no

Genotype			Arm	Location	Cyto	Strand	gene 5'	Location	Distance	gene 3'	Location	Distance	Lethal?
Rab19	WT	02	2L	21,238,542	39B4	Plus	Hr39			Hr39			no
	DN	04	3L	14,516,272	70E2	Plus	CG9238	14,516,230	42	(none)			yes
		05	2R	12,372,190	53D14	Plus	Dek			Dek			yes
	CA	06	3L	1,570,076	62A6	Plus	(none)			CG13917	1,576,794	6,718	no
		15	2R	10,643,285	51E2	Plus	chn			chn			no
Rab21	WT	04	2L	4,852,867	25B1	Minus	CG11926	4,852,640	227	CG31660	4,852,865	-2	no
	DN	01	3R	25,081,343	99A5	Plus	stg	25,081,502	-159	(none)			no
		03	2R	17,379,546	58A3	Plus	comr	17,374,441	5,105	(none)			no
	CA	02	2R	12,612,262	53F8	Minus	GstS1	12,608,116	4,146	CG30456	12,617,804	5,542	no
		09	X	5,009,777	4F2	Minus	(none)			(none)			no
Rab23	WT	01	3R	17,122,206	93D4	Minus	CG10823	17,119,720	2,486	Hsrw	17,122,345	139	no
		02	2L	453,123	21C2	Minus	crq	453,023	100	CG4164	453,111	-12	no (semi)
	DN	01	3R	7,394,893	86E11	Minus	CG6782	7,392,771	2,122	CG14709	7,394,972	79	no
		02	2R	17,927,913	58E1	Plus	mRpS29	17,927,953	-40	rad50	17,928,621	708	yes
	CA	04	3L	8,740,323	66D12	Plus	desert	8,739,434	889	CG32030	8,740,391	68	no
Rab26	WT	01	3L	16,856,365	73D5	Minus	Lmpt			Lmpt			no
		05	2L	15,329,695	35D1	Minus	(none)			esg	15,329,793	98	no (semi)
	DN	02	2R	7,131,618	48A3	Minus	tou	7,131,738	-120	l(2)k14708	7,132,776	1,158	no
		03	3L	17,499,768	74D2	Minus	Nedd4			Nedd4			no
	CA	01	3R	1,627,923	83C4	Plus	CG10979	1,624,348	3,575	CG11000	1,632,725	4,802	no
04		2R	13,238,319	54E2	Plus	CG10934	13,238,244	75	CG10933	13,238,176	-143	no (semi)	
Rab27	WT	01	2L	15,329,688	35D1	Plus	(none)			esg	15,329,793	105	no
		14	3R	7,394,873	86E11	Plus	CG6782	7,392,771	2,102	CG14709	7,394,972	99	no
	DN	02	3R	11,619,543	89A5	Minus	Acyp2	11,619,116	427	CG4699	11,619,518	-25	no
		CA	05	2L	2,752,942	23A3	Minus	Pgk	2,748,522	4,420	CG9894	2,752,799	-143
	07		X	2,646,961	3C1	Minus	CG32795	2,646,098	863	w	2,646,755	-206	no
Rab30	WT	10	2R	12,098,746	53C9	Minus	Cdk4			Cdk4			no
	DN	07	3R	23,407,087	98A7	Plus	wdb	23,407,961	-874	raps	23,408,527	1,440	no
	CA	11	2L	21,639,620	39E3	Plus	Ac3	21,635,560	4,060	cul-2	21,646,345	6,725	no
Rab32	WT	03	2L	7,307,138	27F1	Minus	(none)			wg	7,307,161	23	no
		11	3L	19,863,011	76D5	Minus	CG8004	19,862,327	684	RhoGDI	19,862,648	-363	no
	DN	04	2R	2,261,058	42C8	Plus	CG3267	2,260,767	291	Eb1	2,261,005	-53	no
	CA	03	3R	4,499,927	85A5	Minus	CG8036	4,499,453	474	mRpL19	4,499,839	-88	yes
		08	2R	6,913,334	47F8	Plus	spst	6,913,337	-3	CR30506	6,915,677	2,343	no

Genotype		Arm	Location	Cyto	Strand	gene 5'	Location	Distance	gene 3'	Location	Distance	Lethal?	
Rab35	WT	15	2R	9,935,014	51A2	Minus	CG10105	9,928,723	6,291	CG17386	9,935,902	888	no
	DN	01	2L	2,999,645	23C4	Minus	CG3558	2,998,614	1,031	(none)			no
		06	3L	11,171,570	68B1	Minus	GlcAT-P	11,171,450	120	CG6199	11,172,113	543	no
	CA	01	3R	20,019,220	95F1	Minus	CG5524	20,019,145	75	CG6204	20,019,588	368	yes
		11	2L	19,060,824	37B13	Plus	CG10495	19,060,655	169	CG17343	19,060,922	98	no
Rab39	WT	13	2R	17,911,405	58D4	Minus	CG3624	17,909,638	1,767	CG6044	17,911,755	350	no
	DN	04	2L	18,819,462	37B1	Minus	CG17324	18,818,509	953	CG17323	18,819,508	46	no
		05	3L	20,043,737	76F1	Plus	CG7385			CG7385			no
	CA	02	X	5,616,232	5C2	Plus	CG3097	5,616,015	217	CG15769	5,618,373	2,141	no
		14	3R	15,662,602	92B3	Minus	bnl	15,662,539	63	CG31459	15,666,567	3,965	no
Rab40	WT	03	3L	323,445	61C1	Minus	Ptpmeg			Ptpmeg			no
		05	2R	20,675,167	60F5	Plus	(none)			CG34038	20,675,534	367	no
	DN	01	3R	14,983,534	91F4	Minus	CG11779			nos			yes
		04	2L	6,411,280	26D1	Plus	(none)			CG13982	6,412,704	1,424	no
	CA	07	2L	2,175,076	22D1	Plus	aop			aop			yes
		13	3L	275,967	61B3	Plus	CG13882	274,816	1,151	CG13883	276,437	470	no
RabX1	WT	10	3R	4,076,136	84F6	Plus	CG15864	4,075,853	283	CG7549	4,082,733	6,597	no
		12	2L	7,010,416	27D3	Plus	(none)			CG13776	7,013,496	3,080	no
	DN	01	2R	3,643,884	44B3	Minus	CG11508	3,643,145	739	Pabp2	3,643,226	-658	yes
		14	3L	1,714,694	62B1	Plus	drpr	1,714,625	69	Oseg4	1,714,525	-169	no (semi)
	CA	02	3R	15,662,784	92B3	Plus	bnl	15,662,539	245	CG31459	15,666,567	3,783	no
		10	2L	5,108,441	25C6	Plus	CG14035	5,104,074	4,367	Msp-300	5,117,577	9,136	no
RabX2	WT	08	3R	15,857,553	92C1	Minus	CG4413	15,857,025	528	CG4936	15,857,542	-11	yes
	DN	02	2L	3,632,183	24A2	Plus	for			for			yes
		05	3L	9,483,196	67B11	Minus	CG3408	9,483,527	-331	RpS9	9,483,828	632	no
	CA	10	2L	1,737,054	22B1	Plus	CG17646			CG17646			no
		21	3R	4,076,190	84F6	Plus	CG15864	4,075,853	337	CG7549	4,082,733	6,543	no
RabX3	WT	24	3R	17,869,926	94A1	Minus	how			how			no
		38	2R	9,138,914	50B6	Plus	(none)			fas	9,138,995	81	no
	DN	01	2R	5,956,098	47A7	Minus	G- α 47A			G- α 47A			yes
		03	3L	13,959,509	70C10	Minus	CG9040	13,953,885	5,624	26-29-p	13,959,494	-15	no
	CA	19	3R	23,406,647	98A7	Plus	wdb			wdb			yes
RabX4	WT	19	3R	17,869,918	94A1	Plus	how			how			no
	DN	02	3R	15,006,292	91F11	Plus	CG5555	15,006,044	248	CG31475	15,006,383	91	yes
		03	2L	3,478,334	23F3	Minus	pgant4	3,475,338	2,996	Thor	3,478,434	100	no
	CA	09	2R	3,686,464	44B8	Minus	CG14757	3,685,426	1,038	(none)			no
		13a	3L	8,649,885	66D10	Plus	(none)			h	8,649,706	-179	yes

Genotype		Arm	Location	Cyto	Strand	gene 5'	Location	Distance	gene 3'	Location	Distance	Lethal?	
RabX5	WT	13b	2L	7,010,423	27D3	Minus	(none)			CG13776	7,013,496	3,073	no
		22b	3L	20,283,341	77B4	Plus	eRF1			eRF1			no (semi)
	DN	01	2R	6,335,822	47C1	Plus	CG12342	6,335,780	42	Prosβ5	6,335,871	49	no
	CA	10	X	6,552,255	6C8	Minus	CG14440	6,543,739	8,516	CG14441	6,554,956	2,701	no
RabX6	WT	03	3L	21,813,415	79A2	Plus	(none)			mub	21,813,470	55	no
		04	2L	21,238,362	39B4	Plus	Hr39			Hr39			no
	DN	01	2L	10,506,779	31F4	Plus	Myo31DF	10,506,780	-1	CG6094	10,507,117	338	no
		02	3R	435,389	82C3	Plus	(none)			5-HT2	438,597	3,208	no
	CA	13	2R	19,358,687	60A3	Minus	ytr	19,358,696	-9	gbb	19,359,183	496	no
		27	3L	16,626,881	73B5	Minus	Baldspot	16,626,886	-5	Gα73B	16,627,816	935	no
CG9807	WT	21	2	unmapped									yes
		25	3L	18,800,040	75E2	Plus	Indy			Indy			no
	DN	01	2R	8,746,085	49F11	Minus	CG4663	8,746,135	-50	CG12765	8,746,490	405	no
		03	3R	629,366	82D5	Minus	CG14656	627,445	1,921	CG31529	629,873	507	yes
	CA	04	2R	15,777,715	56F11	Minus	mus209	15,777,720	-5	plu	15,777,821	106	yes
		14b	3R	21,463,573	96E6	Minus	(none)			CG17383	21,463,549	-24	no
CG32673	WT	35	3R	22,741,453	97D7	Minus	Ets97D	22,738,608	2,845	sda	22,741,455	2	no
		42	2L	7,790,585	28C4	Plus	CG7149	7,790,557	28	CG7144	7,791,406	821	no
	DN	01	2R	16,887,109	57E1	Minus	(none)			CG10795	16,890,809	3,700	no
		02	3R	7,775,489	87A2	Plus	(none)			CG31211	7,775,402	-87	no
	CA	05	3L	22,769,560	80A4	Plus	jim	22,767,229	2,331	CG11226	22,770,909	1,349	no
		17	2L	5,999,636	26B2	Plus	lid	5,999,483	153	Tsp26A	6,000,229	593	no

Supplementary Material and Methods

Tissue preparation and scanning EM

Adult eyes were fixed in 2% glutaraldehyde in 0.1M PO₄ buffer and 1% OsO₄. The samples were dehydrated in an ethanol series followed by critical-point drying in CO₂, mounted on an SEM stub, and coated in Argon atmosphere with 16 nm of gold. SEM observations were made in a Philips 525 microscope equipped with Semicaps digital image acquisition.

Adult thorax dissection, fixation and imaging

For adult images, animals were boiled briefly in 10% KOH, dissected, and mounted in 70% glycerol in PBS with spacers to preserve the shape of the thorax. Images were taken using a Zeiss Axioplan 2 microscope.