Appendix

APPENDIX A

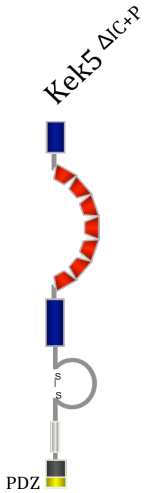
Analysis of Kek5 intracellular deletion variants

This section includes a comprehensive analysis of all the Kek5 variants generated. All constructs were tested with *ptcGAL4* (drives expression in the A/P boundary) to assess viability, crossvein defects and scutellar bristle duplication. In some cases range of crossvein defects or scutellar bristles or viability of a particular variant are depicted in the form of a bar graph for better visualization of any trend.

Representative lines for each Kek5 variant were subsequently used to test for affects on epithelial cell extrusion, 'large-cell' and arm upregulation. Viability was calculated by dividing the number of flies of the right genotype (*ptc>kek5*^{variant}) by the number of balancer flies. Crossvein defects were calculated by dividing the number of wings observed with crossvein defects divided by the total number of wings of the *ptc>kek5*^{variant} scored.

UAS-kek5$^{\Delta IC+PC}$gfp

Simplified nomenclature$^{\theta}$	Transgenic line	Chrom*	GFP$^{\Psi}$	ACV$^{\Phi}$	Bristles$^{\pi}$
1	1F-1M	III	+-	-	
2	8F-1M	III	+	0%	
3	8F-2F	III	++	0%	
4	13M-1M	III	++	26.70%	4-5
5	13M-2M	III	+-	13%	4-5
6	15M-1M	II	+-	0%	
7	15M-2M	II	++-	1.80%	4-5
8	19M-1M	II	++	2.60%	4-5
9	1F-1F	II	++	1.30%	4-5
10	6F-1F	III	++	3.30%	4-5
11	8F-1F	X	++-	5.60%	4-5
12	8F-2Fa	III	++	0%	
13	8F-4M	III	+++	2.80%	4-5



All lines tested were viable.
Simplified nomenclature$^{\theta}$ - Transgenic lines were given numeral nomenclature to simplify the alpha- numeral naming scheme; Chrom* - Chromosome; GFP$^{\Psi}$ - GFP levels were roughly assessed in salivary glands of 3rd instar larvae under a fluorescence dissection microscope; ACV$^{\Phi}$ - Frequency of wings showing crossveins defects (missing or truncated); Bristles$^{\pi}$ - Number of scutellar bristles

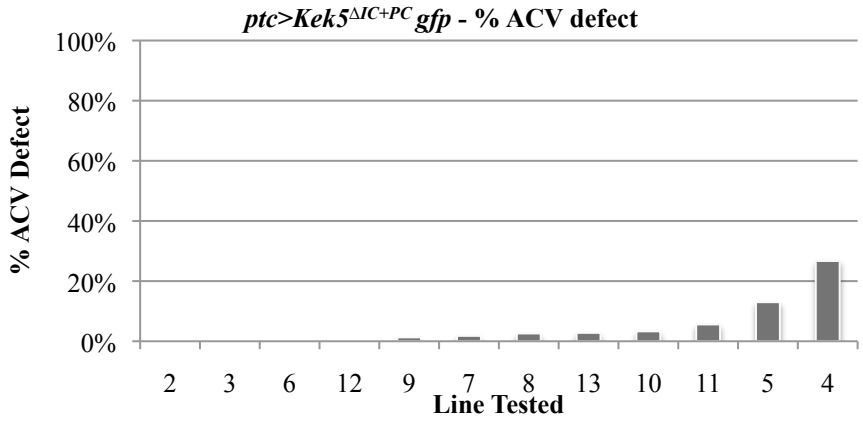


Figure A1: Graph depicting frequency of ACV defects in the tested *ptc>kek5$^{\Delta IC+PC}$* lines. It is clear from the graph that majority of the lines tested show reduces crossvein defects (>5%) indicating that sequence elements in the intracellular region of Kek5 are important for production of crossvein defects.

UAS-kek5$^{\Delta 123}$gfp

Simplified nomenclature$^{\theta}$	Transgenic line	Chrom*	GFP$^{\Psi}$	% Viability	ACV$^{\Phi}$	Bristles$^{\pi}$
1	2F-1M	II	ND	6.25	100%	S
2	3F-1M	II	++	34.5	60%	S
3	4F-1M	II	++-	1.4	100%	S
4	4F-5M	II	ND	LETHAL		
5	7F-2M	II	++	2.6	100%	S
6	10F-2M	II	++	44.4	73%	M-S
7	11F-1Ma	X				
8	11F-1Mb	X	ND	LETHAL		
9	12F-1M	III	++-	LETHAL		
10	16F-3M	III	ND	LETHAL		
11	18F-1Fa	II	+	48	74%	VS (16)
12	18F-1Fb	II	+	45	72%	S
13	23M-1Ma	II	+++	LETHAL		
14	23M-1Mb	III	ND	LETHAL		
15	23M-2M	III	ND	LETHAL		
16	24M-1M	II	+-	18.75	93%	M-S
17	32M-1M	III	++-	LETHAL		
18	34M-1Fa	III	++	LETHAL		
19	34M-1Fb	III	++-	LETHAL		
20	4F-1M	X	ND	LETHAL		
21	7F-2M	II	ND	LETHAL		
22	8F-4Ma	X	ND	LETHAL		
23	8F-4Mb	II	ND	LETHAL		
24	8F-5M	X	ND	LETHAL		
25	13F-1F	X	ND	LETHAL		
26	23M-1M	III	ND	LETHAL		
31	25M-1M	III	ND	LETHAL		
32	26M-2M	II	ND	LETHAL		
34	29M-1Mb	III	ND	LETHAL		
36	30M-3Mb	III	ND	LETHAL		
37	34M-1M	III	ND	LETHAL		
38	35M-1F	X	ND	LETHAL		
39	36M-1M	II	ND	LETHAL		
40	40M-1M	II	ND	LETHAL		
41	43F-1F	X	ND	LETHAL		
42	44F-2M	X	ND	LETHAL		

$^{\theta}$ - Transgenic lines were given numeral nomenclature to simplify the corresponding alpha- numeral naming scheme; Chrom* - Chromosome; GFP$^{\Psi}$ - GFP levels were roughly assessed in salivary glands of 3rd instar larvae under a fluorescence dissection microscope; ACV$^{\Phi}$ - Frequency of wings showing crossveins defects; Bristles$^{\pi}$ - Number of scutellar bristles (W=Weak, M=Moderate, S=Strong bristle duplication), number in parentheses indicates the average number of bristles in flies for that line.

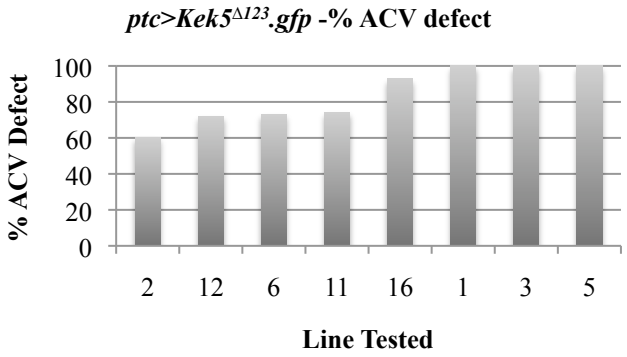


Figure A2: Graph depicting the frequency of crossvein defects in Kek5$^{\Delta 123}$ lines tested with *ptcGAL4*. Among the viable lines tested, frequency of crossveins defects was observed to be ≥60% suggesting that motifs 1,2 and 3 do not participate in induction of crossvein defect by Kek5.

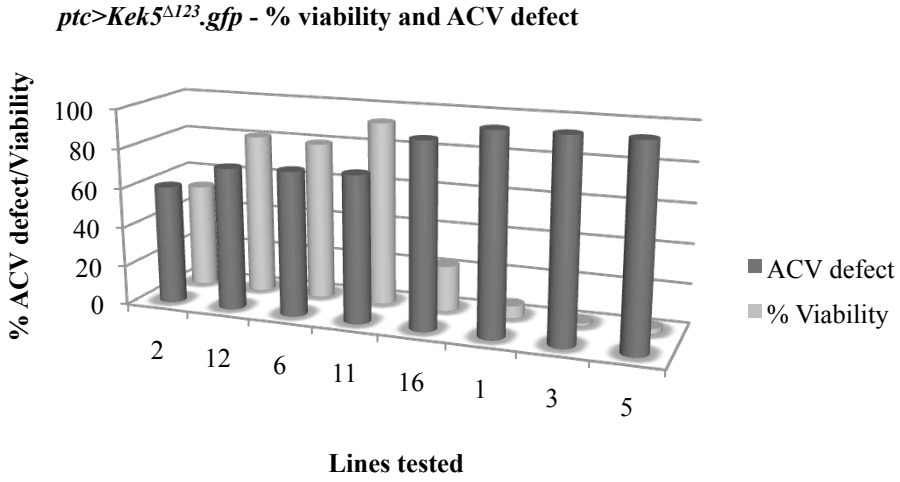
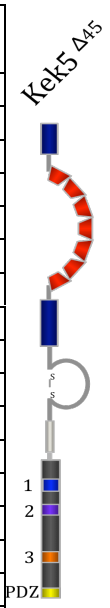


Figure A3: Graph depicting the frequency of crossvein defects in relation to percent viability of the Kek5$^{\Delta 123}$ lines tested with *ptcGAL4*. Among the viable lines tested, frequency of crossveins defects was observed to be ≥60% suggesting that motifs 1,2 and 3 do not participate in induction of crossvein defect by Kek5. Generally, it can be seen that as frequency of crossvein defect increases, percent viability reduces.

UAS-kek5$^{\Delta 45}$gfp

Simplified nomenclature$^{\theta}$	Transgenic line	Chrom*	GFP$^{\Psi}$	% Viabillity	ACV$^{\Phi}$	Bristles$^{\pi}$
1	5M-1F	II	++	100	20.8%	V
2	6M-1F	III	++	100	1.8%	W
3	11M-1M	II	++	78	15.3%	M (10.3)
4	14M-1Ma	III		100	7.5%	W
6	14M-2M	III	+	100	6.4%	W
7	16M-1F	III	++	100	4.4%	W
8	20M-1F	III	++	100	23.5%	M (10.8)
11	30F-2F	II	++	100	15.9%	M
12	38F-1M	II	+-	94	0.0%	NE
13	40F-1M	III	++	50	100.0%	W (7.6)
14	41F-1F	III	+-	100	1.7%	W (6.9)
16	44M-1M	II	++	80	17.3%	M
17	49M-1F	III	++	100	25.0%	M-S
18	49M-1Fa	II	+	61	1.2%	W-M
20	53F-1M	II	+-	77	15.0%	M (10.8)
21	2F-1F	III	+-	100	4.0%	W
22	2F-3M	III	+++	16	100.0%	S
23	6F-1Fa	III	++	100	33.0%	W
24	6F-1Fb	III	+-	87	15.0%	M
25	6F-2F	II	+-	73	16.9%	M (8.6)
26	10F-1F	X	++	65	25.4%	S (11.3)
27	15F-1M	III	++-	100	0.0%	M-S
29	22M-1M	III	++	100	6.5%	M
30	22M-2F	III	++-	82	13.0%	W (7.2)
31	23M-1M	II	++	100	9.3%	W
32	30M-2M	II	++-	100	25.0%	W-M
33	30M-2M	III	++	100	0.0%	W
35	40M-2Ma	III	+-	53	44.0%	M



$^{\theta}$ - Transgenic lines were given numeral nomenclature to simplify the corresponding alpha- numeral naming scheme; Chrom* - Chromosome; GFP$^{\Psi}$ - GFP levels were roughly assessed in salivary glands of 3rd instar larvae under a fluorescence dissection microscope; ACV$^{\Phi}$ - Frequency of wings showing crossveins defects; Bristles$^{\pi}$ - Number of scutellar bristles (W=Weak, M=Moderate, S=Strong bristle duplication), number in parentheses indicates the average number of bristles in flies for that line.

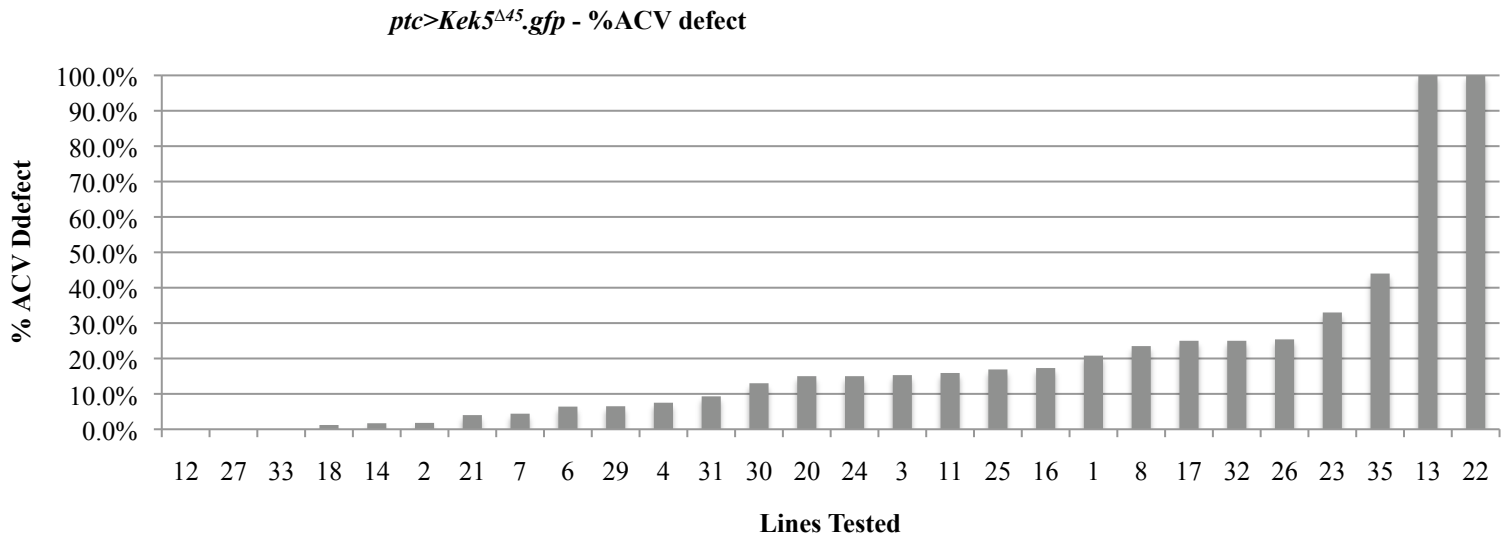
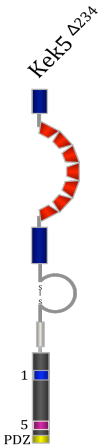


Figure A4: Graph depicting frequency of ACV defects in the tested *ptc>kek5*$^{\Delta 45}$ lines. It is clear from the graph that majority of the lines tested show reduces crossvein defects (~25%). No lethality was observed in any of the *ptc>kek5*$^{\Delta 45}$ crosses.

UAS-kek5$^{\Delta 234}$gfp

Simplified nomenclature$^{\theta}$	Transgenic Line	Chrom*	% Viability	ACV$^{\Phi}$	Bristles$^{\pi}$
1	A2F-2M	II	3	0	4
2	A17F-1F	III	8.7	86.6	4.3
3	A17F-2F	III	Lethal		
4	A20M-2M	II	Lethal		
5	A25M-1F	III	Lethal		
6	A27M-1M	II	Lethal		
7	A27M-2M	II	1.4	16.6	5.3
8	A29M-1M	II	1	0	4
9	A43F-1F	II	100	100	4.4
10	A44M-1M	II	35.5	1.8	4.52
11	A48F-2M	III	Lethal		



$^{\theta}$ - Transgenic lines were given numeral nomenclature to simplify the corresponding alpha- numeral naming scheme; Chrom* - Chromosome; ACV$^{\Phi}$ - Frequency of wings showing crossveins defects; Bristles$^{\pi}$ - Number of scutellar bristles

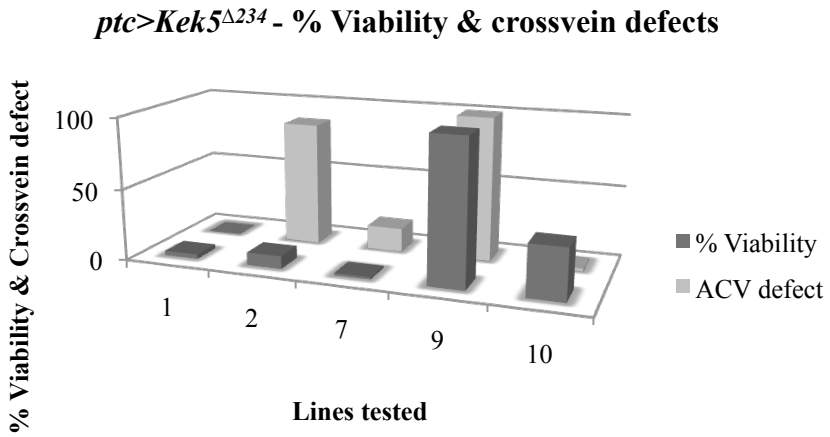


Figure A5: Graph depicting the frequency of crossvein defects in relation to percent viability of the Kek5$^{\Delta 234}$ lines tested with *ptcGAL4*. Among the viable lines tested, no correlation between frequency of crossvein defect and viability could be drawn.

UAS-kek5$^{\Delta 1234}$gfp

Transgenic Line	Chrom*	% Viability	ACV$^{\Phi}$	Bristles$^{\pi}$
HHB1	III	Lethal		
HHB3	II	Lethal		
HHB4	II	Lethal		
HHB6	II	Lethal		
HHB7	II	V	74	15.9
HHB10	I	Lethal		
HHB11	II	Lethal		
HHB12	III	Lethal		
HHB14	I	Lethal		
HHB15	III	V	63	11.15
HHB16	II	Lethal		
HHB19	II	Lethal		
HHB22	III	6	100	9
HHB24	III	Lethal		
HHB26	III	Lethal		
HHB30	II	3	100	7
HHB31	III	Lethal		
HHB32	III	Lethal		
HHB34	II	V	-	15.6
HHB35	II	Lethal		
HHB36	II	V	97	10.9
HHB39	III	Lethal		

$^{\theta}$ - Transgenic lines were given numeral nomenclature to simplify the corresponding alpha- numeral naming scheme; Chrom* - Chromosome; ACV$^{\Phi}$ - Frequency of wings showing crossveins defects; Bristles$^{\pi}$ - Number of scutellar bristles

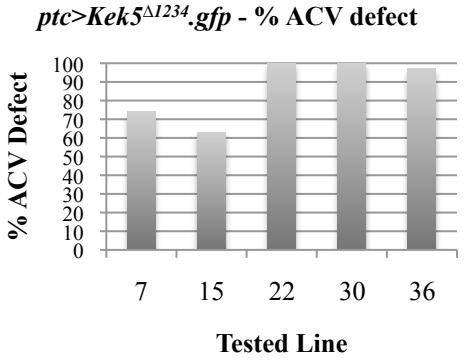


Figure A6: Graph depicting the frequency of crossvein defects in relation to percent viability of the Kek5$^{\Delta 1234}$ lines tested with *ptcGAL4*. High frequency of crossvein defects was observed among the viable lines tested.

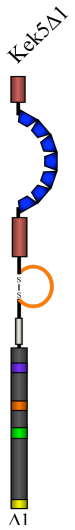
UAS-kek5$^{\Delta 1235}$gfp

Transgenic Line	Chrom*	% Viability	ACV$^{\Phi}$	Bristles$^{\pi}$
HHC3	III	Lethal		
HHC5	II	Lethal		
HHC6	II	Lethal		
HHC8	II	Lethal		
HHC13	III	Lethal		
HHC14	III	Lethal		
HHC15	II	Lethal		
HHC19	II	Lethal		
HHC20	III	V	83	17.3
HHC22	III	very low V		
HHC23	I	Lethal		
HHC24	III	Lethal		
HHC30	II	Lethal		

θ - Transgenic lines were given numeral nomenclature to simplify the corresponding alpha- numeral naming scheme; Chrom* Chromosome; ACV$^{\Phi}$ - Frequency of wings showing crossveins defects; Bristles$^{\pi}$ - Number of scutellar bristles

UAS-kek5$^{\Delta 1}$gfp

Simplified nomenclature$^{\theta}$	Transgenic line	Chrom*	% Viability	ACV$^{\Phi}$	Bristles$^{\pi}$
Δ1-36	Δ1-63F-1M	II	29.5	0	4.2
Δ1-28	Δ1-46F-1M	II	100	3.2	4.3
Δ1-9	Δ1-14M-2M	III	100	15.4	5.4
Δ1-30	Δ1-51F-1M	III	78	21.4	7.5
Δ1-12	Δ1-15M-2Fa	III	21.3	25	8.7
Δ1-35	Δ1-62F-2M	III	100	26	7
Δ1-10	Δ1-14M-3M	III	100	26.8	6.9
Δ1-33	Δ1-56F-2Mb	II	70.6	34.5	12.8
Δ1-13	Δ1-16M-2M	III	100	34.6	7.8
Δ1-16	Δ1-18M-1M	III	100	39.6	6.6
Δ1-31	Δ1-51F-2F	I	94.2	66	5.8
Δ1-4	Δ1-3M-2F	II	56.5	66.6	13.5
Δ1-1	Δ1-1M-1F	I	52	71	10.6
Δ1-11	Δ1-15M-1Fa	II	100	71.7	8.4
Δ1-21	Δ1-28M-1M	II	3.6	75	20.75
Δ1-5	Δ1-6M-1Ma	III	39.7	76.3	10.8
Δ1-15	Δ1-17M-1M	III	18	77.3	11.3
Δ1-18	Δ1-19M-3M	II	48.5	77.7	16.9
Δ1-22	Δ1-36F-2M	I	76.8	80	13.4
Δ1-8	Δ1-10M-1F	III	92	91	13.3
Δ1-32	Δ1-55F-1F	II	71.4	94.3	14.5
Δ1-37	Δ1-63F-2M	II	70.3	96	13.9
Δ1-3	Δ1-3M-1M	III	73.5	97	13.2
Δ1-17	Δ1-19M-1M	III	68.6	97	12.8
Δ1-2	Δ1-2M-1M	II	74	100	17
Δ1-19	Δ1-20M-1M	III	23.8	100	11.2
Δ1-24	Δ1-40F-2M	III	29.3	100	14.8
Δ1-25	Δ1-42F-1M	II	82.6	100	10.1
Δ1-26	Δ1-43F-1M	II	100	100	12
Δ1-29	Δ1-47F-1M	III	51.4	100	7.8
Δ1-6	Δ1-6M-1Mb	II	0		
Δ1-7	Δ1-9M-1F	III	0		
Δ1-14	Δ1-16M-4M	III	0		
Δ1-20	Δ1-22M-1M	III	0		
Δ1-23	Δ1-40F-1M	I	0		
Δ1-27	Δ1-43F-2M	III	0		
Δ1-34	Δ1-62F-1F	I	0		



$^{\theta}$ - Transgenic lines were given numeral nomenclature to simplify the corresponding alpha- numeral naming scheme; Chrom* - Chromosome; ACV$^{\Phi}$ - Frequency of wings showing crossveins defects; Bristles$^{\pi}$ - Number of scutellar bristles

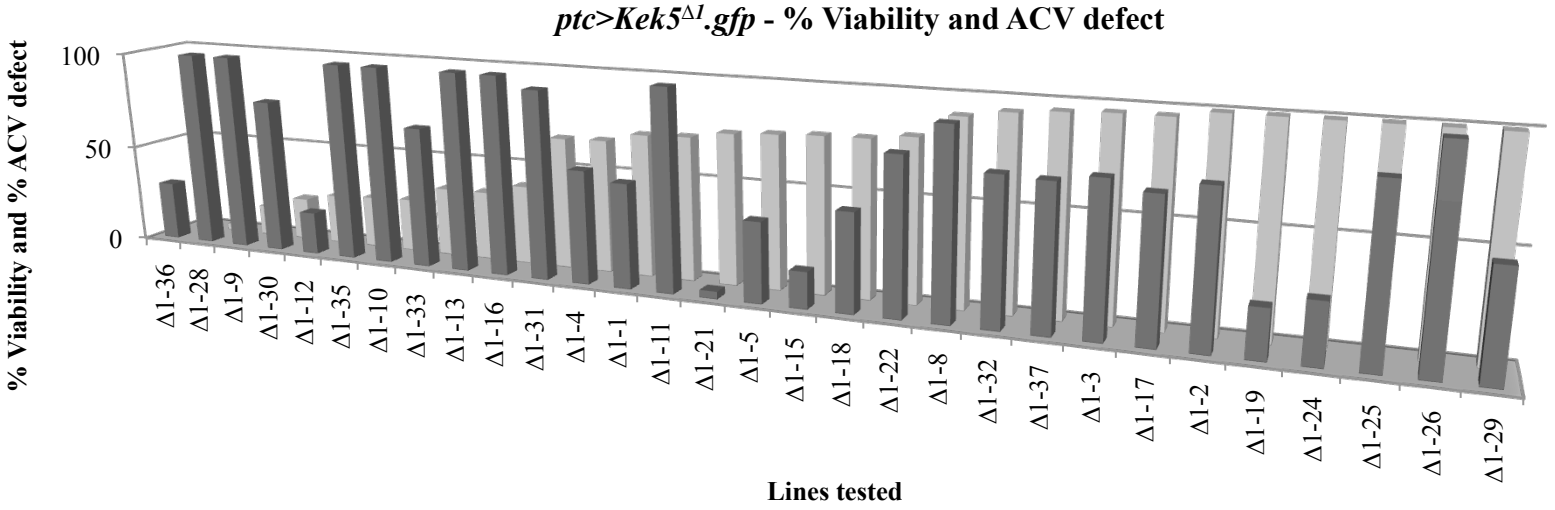
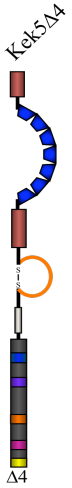


Figure A7: Graph depicting the frequency of crossvein defects in relation to percent viability of the Kek5$^{\Delta 1}$ lines tested with *ptcGAL4*. Two thirds of the viable lines tested displayed high frequency of crossvein defects (>70%) crossvein defects. Although no clear trend between frequency of crossvein defect and % viability can be observed, it can be seen that in many cases increased viability corresponds to low percentage of crossvein defects. Light grey bars indicate the frequency of CV defects while dark grey bars indicate % viability.

UAS-kek5$^{\Delta 4}$gfp

Simplified nomenclature$^{\theta}$	Transgenic Line	Chrom*	% Viability	ACV$^{\Phi}$	Bristles$^{\pi}$
Δ4-6	Δ4- 83F-1M	III	100	0	4.10
Δ4-5	Δ4- 73F-3F	I	81.5	2.3	5.14
Δ4-12	Δ4- 120M-1M	III	100	4.4	4.96
Δ4-9	Δ4- 95F-1M	II	100	6.3	11.64
Δ4-1	Δ4- 42M-1M	III	100	9.7	8.9
Δ4-2	Δ4- 50M-2F	III	100	34.5	9.2
Δ4-3	Δ4- 56M-1M	II	91	52	15
Δ4-4	Δ4- 73F-1M	II	67.6	57.6	13.17
Δ4-8	Δ4- 91F-1M	III	53.8	65.3	12.72
Δ4-11	Δ4- 100F-1M	III	50	90	10.90
Δ4-7	Δ4- 83F-2M	III	53.8	93.6	9.92
Δ4-10	Δ4- 95F-2M	III	8.8	100	9.67



$^{\theta}$ - Transgenic lines were given numeral nomenclature to simplify the corresponding alpha- numeral naming scheme; Chrom* - Chromosome; ACV$^{\Phi}$ - Frequency of wings showing crossveins defects; Bristles$^{\pi}$ - Number of scutellar bristles

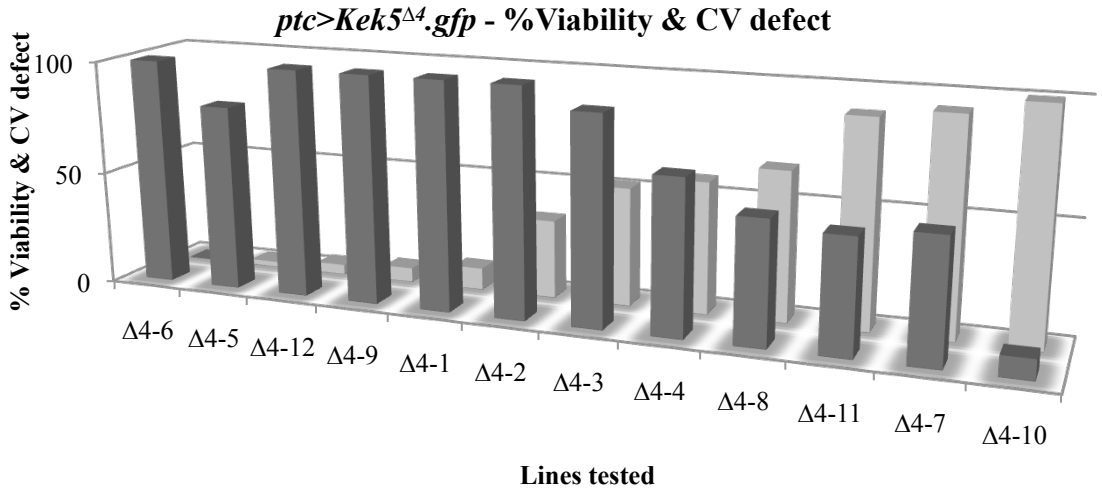
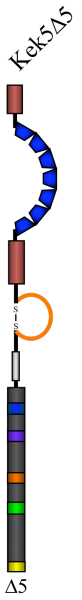


Figure A8: Graph depicting the frequency of crossvein defects in relation to percent viability of the Kek5$^{\Delta 4}$ lines tested with *ptcGAL4*. Two thirds of the viable lines tested displayed <55% crossvein defects. Increased viability appears to correspond to low percentage of crossvein defects and vice versa. Light grey bars indicate the frequency of CV defects while dark grey bars indicate % viability.

UAS-kek5$^{\Delta 5}$gfp

Simplified nomenclature$^{\theta}$	Transgenic Line	Chrom*	% Viability	ACV$^{\Phi}$	Bristles$^{\pi}$
Δ5-1	Δ5 - 7F -2F	II	100	13.3	4.5
Δ5-2	Δ5 - 9F - 1M	II	100	1.5	5.4
Δ5-3	Δ5 - 9F - 2M	III	L		
Δ5-4	Δ5 - 12F - 1M	I	63.8	46.5	9.9
Δ5-5	Δ5 - 16F - 2M	III	23.8	50	12
Δ5-6	Δ5 - 19F - 1M	I	74.6	73.25	11.4
Δ5-7	Δ5 - 22F - 1F	III	100	17.5	8.4
Δ5-8	Δ5 - 26F - 2M	III	L		
Δ5-9	Δ5 - 29F - 1M	III	51.4	70.8	9.9
Δ5-10	Δ5 - 29F - 2M	III	L		
Δ5-11	Δ5 - 32F - 1M	II	100	0	4.1
Δ5-12	Δ5 - 32F - 2M	II	45.7	30.7	13.85
Δ5-13	Δ5 - 32F - 3M	I	46.8	63.3	15.5
Δ5-14	Δ5 - 39M - 1M	III	8.5	83.3	-
Δ5-15	Δ5 - 41M - 1M	II	100	91.2	12.7
Δ5-16	Δ5 - 41M - 2M	II	46.6	81.25	10.12
Δ5-17	Δ5 - 47M - 1M	III	24	75	15.5
Δ5-18	Δ5 - 49M - 2Ma	III	38.6	63.6	11.6
Δ5-19	Δ5 - 49M - 2Mb	II	100	0	4.05
Δ5-20	Δ5 - 50M - 1M	III	22.2	100	13.25
Δ5-21	Δ5 - 53M - 2M	III	59	50	5.05
Δ5-22	Δ5 - 54M - 1M	III	47.5	87.5	13.5
Δ5-23	Δ5 - 54M - 2F	II	100	0	3.96
Δ5-24	Δ5 - 58M - 2Ma	III	100	8.3	7.5
Δ5-25	Δ5 - 58M - 2Mb	II	40.7	45	16.1
Δ5-26	Δ5 - 62M - 1M	III	L		
Δ5-27	Δ5 - 62M - 2M	II	86.5	50	16.05
Δ5-28	Δ5 - 67M - 1M	II	46	16.6	14.04
Δ5-29	Δ5 - 69M - 2F	III	37.5	33.3	13.26



$^{\theta}$ - Transgenic lines were given numeral nomenclature to simplify the corresponding alpha- numeral naming scheme; Chrom* - Chromosome; ACV$^{\Phi}$ - Frequency of wings showing crossveins defects; Bristles$^{\pi}$ - Number of scutellar bristles

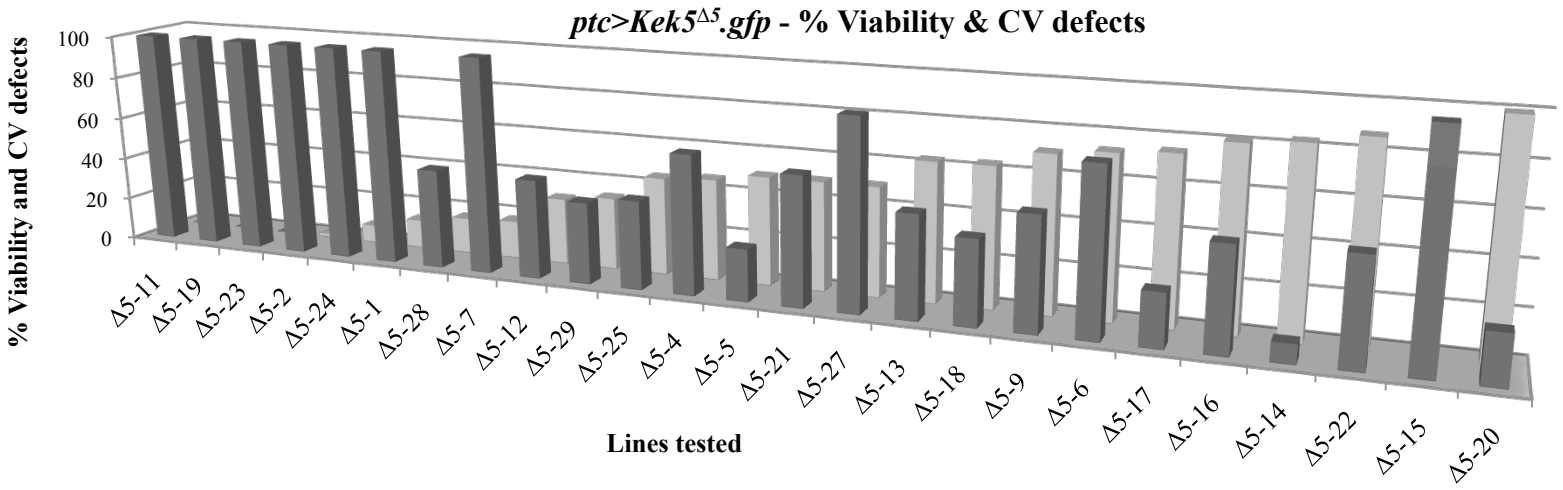


Figure A9: Graph depicting the frequency of crossvein defects in relation to percent viability of the Kek5$^{\Delta 5}$ lines tested with *ptcGAL4*. Majority of the viable lines tested displayed <60% crossvein defects. Despite the lack of a clear trend between percentage of crossvein defect and viability, it can be seen that increased viability corresponds to low frequency of ACV defects. Light grey bars indicate the frequency of CV defects while dark grey bars indicate % viability.

UAS-kek5^{K6PDZ}gfp

Transgenic line	Chrom*	% Viability	ACV$^{\Phi}$	Bristles$^{\pi}$
K5/6.M24.1Ma	III	73.9	0	4.08
K5/6.F25.1Ma	II	69.8	100	9
K5/6.M17.1Ma	II	58.8	100	10
K5/6.F28.1M	III	53.2	100	10.3
K5/6.M25.1Mb	III	33	100	7.6
K5/6.F28.1F	III	16.5	100	8.7

Chrom* - Chromosome; ACV$^{\Phi}$ - Frequency of wings showing crossveins defects; Bristles$^{\pi}$ - Number of scutellar bristles

UAS-kek6^{K5PDZ}gfp

Transgenic line	Chrom*	% Viability	ACV$^{\Phi}$	Bristles$^{\pi}$
K6/5.M2.1M	II	37.5	0	5.2
K6/5.F14.1Mb	I	43.3	0	4.2
K6/5.F18.3M (3/4)	II	53.7	0	4.8
K6/5.F35.1Mb	II	67.6	0	4.5
K6/5.F3.1Ma	I	69.3	0	4.3
K6/5.F14.2Mb	II	73.7	0	4.8
K6/5.F18.1Ma	II	79	0	4.2
K6/5.F20.1M	III	84.7	0	4.4
K6/5.M19.1M	II	85	0	4.6
K6/5.F18.3M (1/2)	III	89.5	0	4
K6/5.M8.1Ma	II	100	0	4.4
K6/5.F4.2Mb	II	100	0	4.1
K6/5.F4.3Mb	III	100	0	4.2
K6/5.F7.1Ma	I	100	0	4.4
K6/5.F17.1F	III	100	0	4.1
K6/5.F18.2Ma	III	100	0	4.7
K6/5.M35.1Ma	II	100	2.4	4

Chrom* - Chromosome; ACV$^{\Phi}$ - Frequency of wings showing crossveins defects; Bristles$^{\pi}$ - Number of scutellar bristles

Table A1: Summary of results obtained from Kek5 variant analyses.

	CV defect (%)	Extrusion	Large cell	Scutellar bristle	Arm upregulation
Kek5	100	+++	+++	15	✔
Kek5$^{\Delta LRR}$	0	NE	NE	4	✖
Kek5$^{\Delta Ig}$	97	++	++	11	✖
Kek5$^{\Delta IC}$	0	NE	NE	4	✔
Kek5$^{\Delta IC+PC}$	<2	NE	NE	4	✔
Kek5$^{\Delta 123}$	93	+	+	16	✔
Kek5$^{\Delta 45}$	16	+	+	9	✔
Kek5$^{\Delta 234}$	-	+	++	5	✔
Kek5$^{\Delta 1234}$	97	+	+	13	✔
Kek5$^{\Delta 1235}$	83	+	+	17	✔
Kek5$^{\Delta 1}$	97	+++	+++	11	✔
Kek5$^{\Delta 4}$	52	+++	+++	10	✔
Kek5$^{\Delta 5}$	50	+++	+++	13	✔
Kek5^{K6PDZ}	100	+++	+++	9	✔
Kek6^{K5PDZ}	0	NE	NE	4	✔

The number of pluses indicates the extent of the phenotype. Tick indicates Arm upregulation while cross indicates no Arm upregulation.

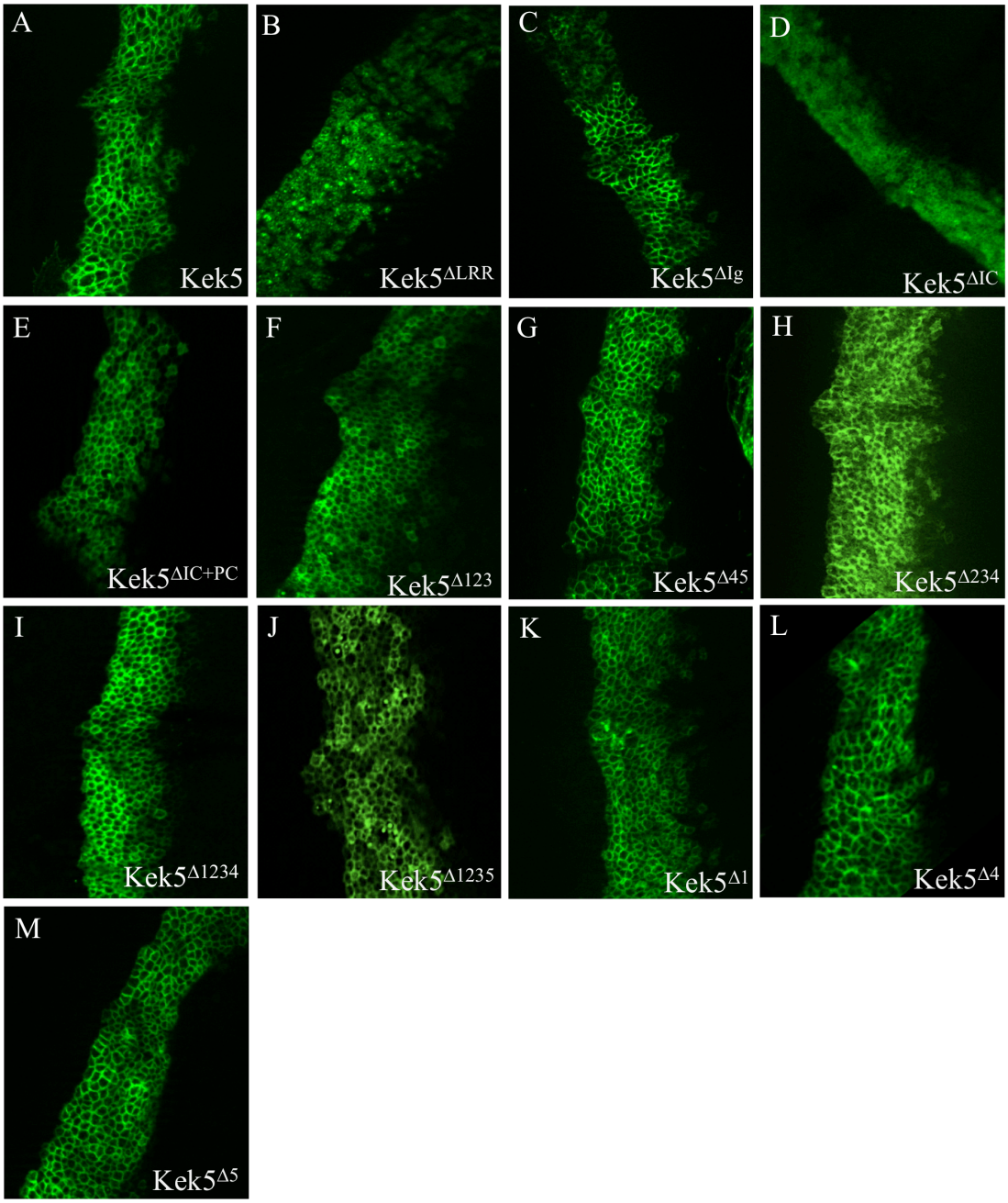


Figure A10: Localization of Kek5 deletion variants. GFP-tagged variants were misexpressed in the in the A/P boundary using *ptcGAL4*. Localization of all the variants to the membrane was normal except Kek5$^{\Delta LRR}$ (B) and Kek5$^{\Delta IC+PC}$ (E). Kek5$^{\Delta 1235}$ in addition to being membrane bound also was vesicular (J). All the wing discs are oriented with their anterior side to the right and ventral side up.

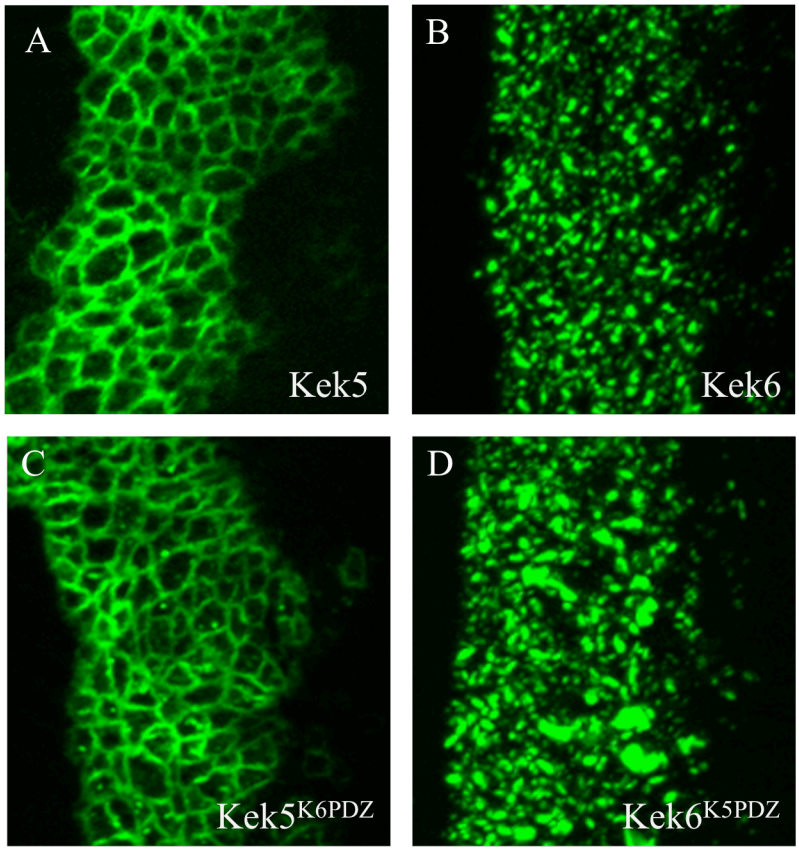


Figure A11a: PDZ domain binding site is sufficient for Kek5 membrane localization. GFP tagged proteins are misexpressed using *ptcGAL4*. Wings discs are oriented with their posterior side towards right. Kek5 localizes to the 3rd instar wing membrane (A) while Kek6 does not (B). Swapping the PDZ domain binding site of Kek5 and Kek6 does not affect this pattern (C, D).

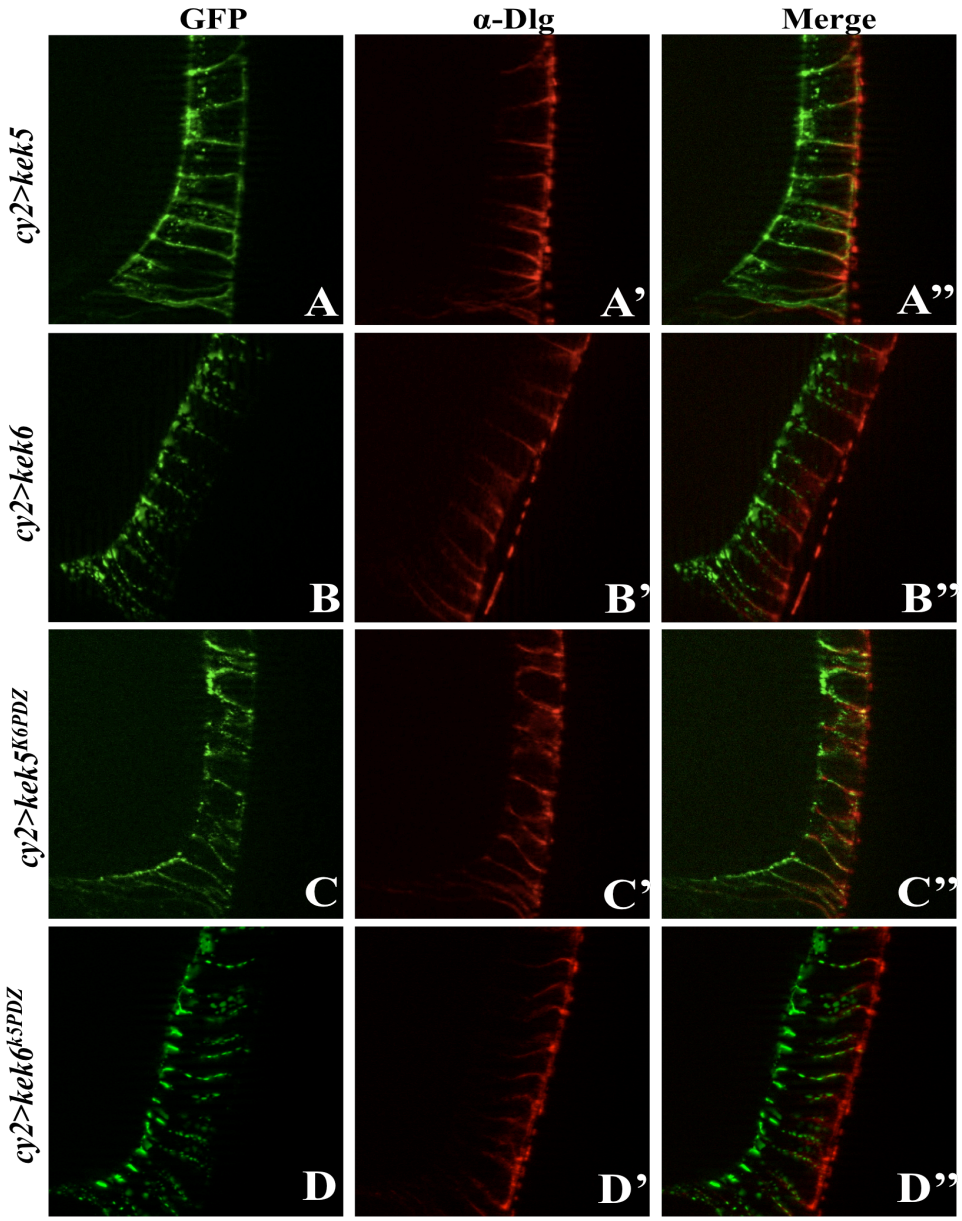


Figure A11b: Kek5 PDZ domain binding site appears to be a generic protein localization domain. Stage 10 egg chambers of the respective GFP tagged variants stained with α-Dlg to mark the baso-lateral cell surface. Substitution of the PDZ domain of Kek6 with Kek5 does not seem to alter the localization of Kek5 (compare A-A'' and C-C''). Replacing the PDZ domain of Kek6 with that of Kek5 likewise does not appear to alter the localization pattern of Kek6 (compare B-'' to D-D''). Apical side in each panel is towards left.

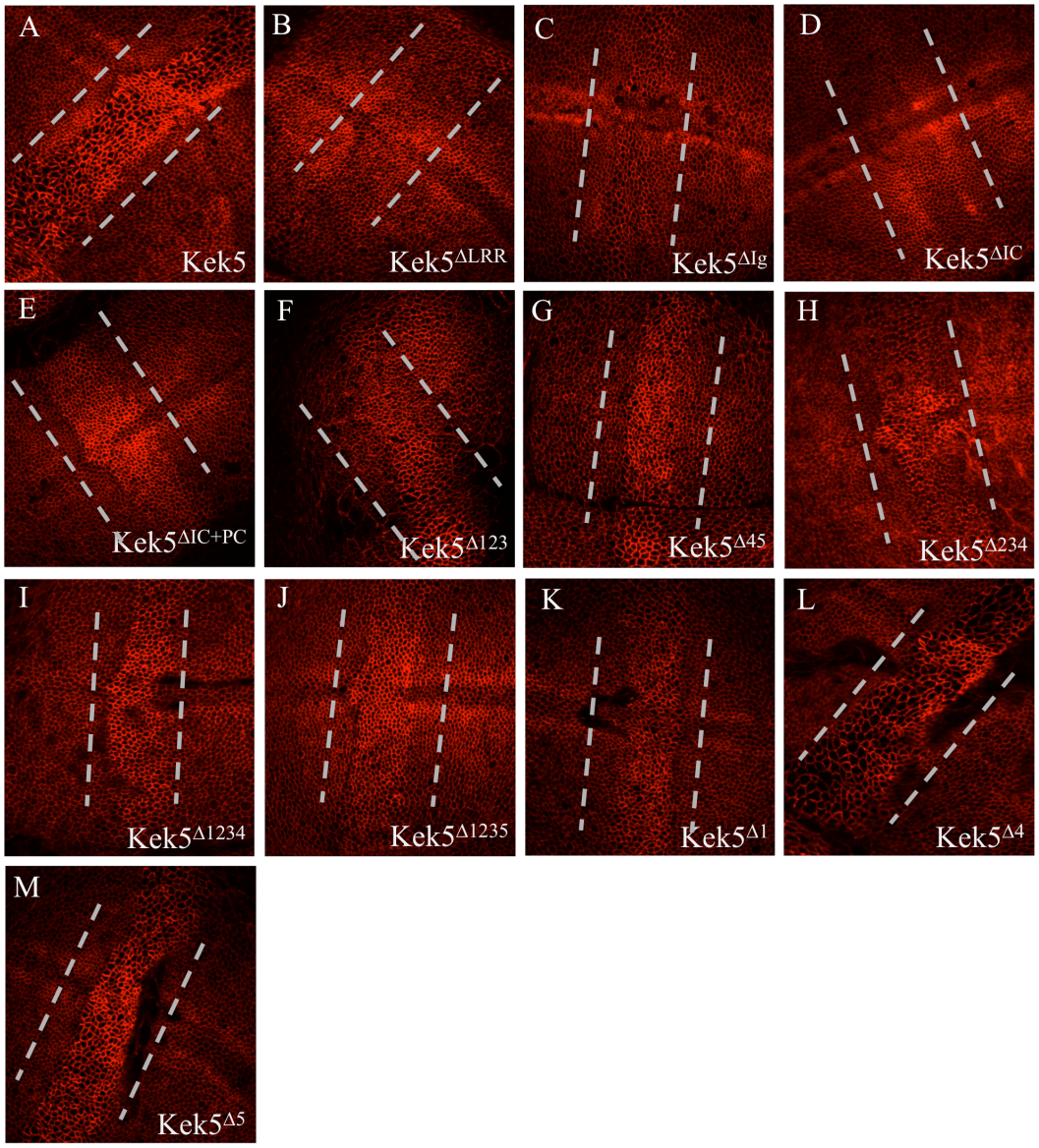


Figure A12: Analysis of Arm upregulation by Kek5 deletion variants. Kek5 variants were examined for Arm upregulation after misexpression in the A/P boundary. Arm upregulation was observed in all variants except Kek5$^{\Delta LRR}$ (B), Kek5$^{\Delta Ig}$ (C) and Kek5$^{\Delta IC}$ (D). Dotted line represents the region of Kek5 (*ptcGAL4*) expression. All the wing discs are oriented with their anterior side to the right and ventral side up.

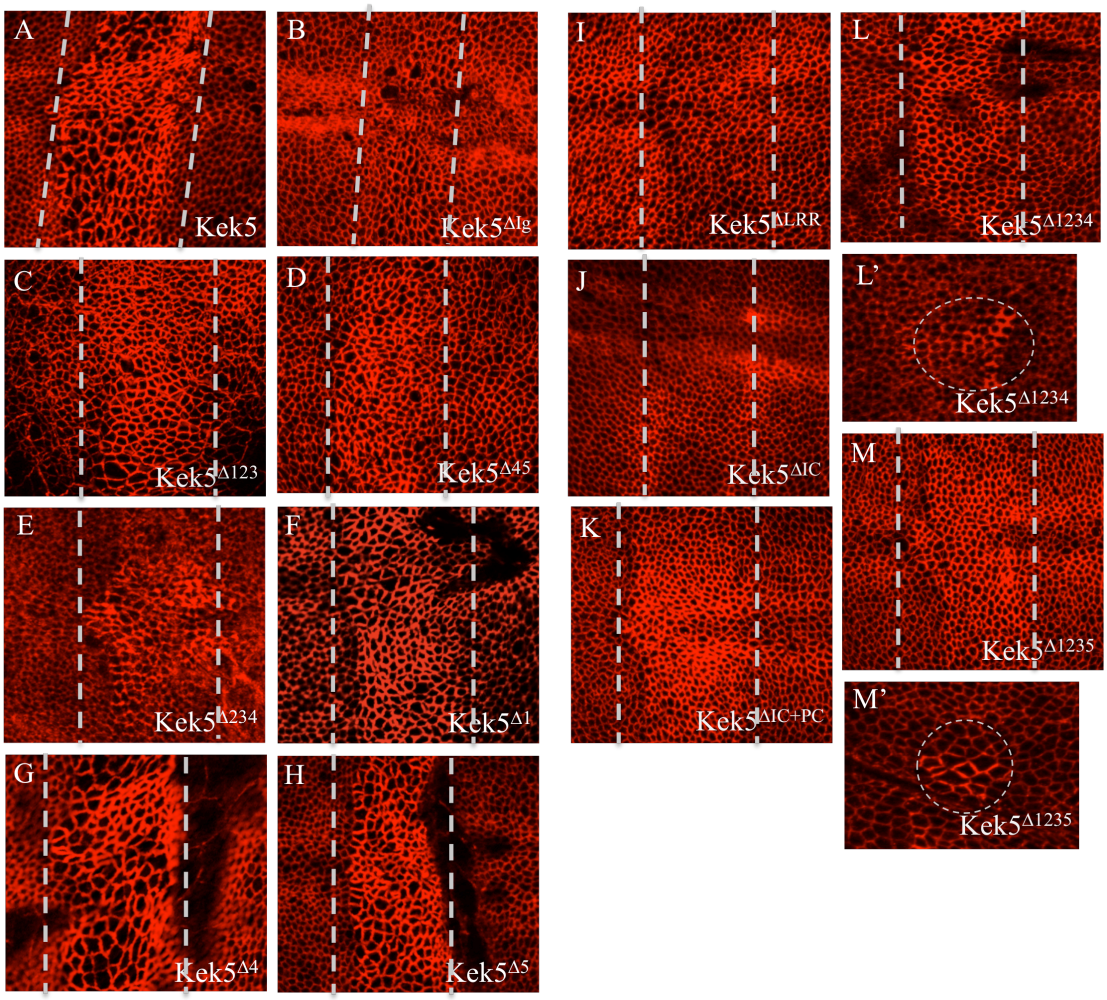


Figure A13: Analysis of 'Large cell' phenotype by Kek5 deletion variants. Kek5 variants were expressed in the A/P boundary using *ptc>GAL4* and the discs stained with anti-Armadillo to mark the cell membranes. Misexpression of Kek5 results in enlargement of cells at the apical region (A). This enlargement is seen with all other variants (B-H) except Kek5$^{\Delta LRR}$ (I), Kek5$^{\Delta IC}$ (J), Kek5$^{\Delta IC+PC}$ (K), Kek5$^{\Delta 1234}$ (L, L') and Kek5$^{\Delta 1235}$ (M, M'). Dotted lines enclose the region of *ptcGAL4* expression. All the wing discs are oriented with their anterior side to the right and ventral side up. L' and M' are Kek5$^{\Delta 1234}$ and Kek5$^{\Delta 1235}$ misexpression clones, respectively where the circle indicates the clone.

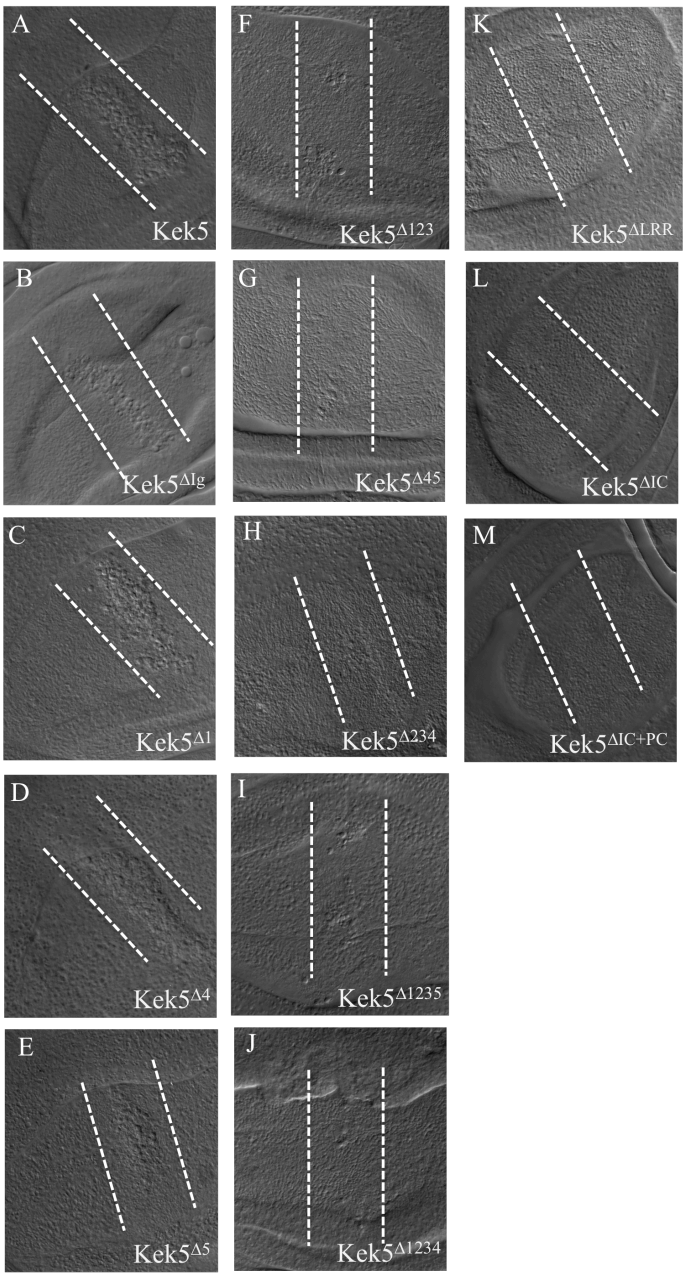


Figure A14: Examination of epithelial cell extrusion caused by Kek5 variants. Kek5 variants were expressed in the A/P boundary using *ptcGAL4* and showed varying degrees of extrusion. Variants in panels B-E displayed extrusion comparable to Kek5 (A). Multi motif intracellular deletions caused minimal extrusion (F-J) while Kek5$^{\Delta LRR}$ (B), Kek5$^{\Delta IC}$ (D) and Kek5$^{\Delta IC+PC}$ (E) displayed no extrusion. Dotted line indicates the area of *ptc* expression domain. All the wing discs are oriented with their anterior side to the left and ventral side up.

APPENDIX B

Table B1: Primers used for generation of various Kek5 intracellular deletions variants

Kek5 Variant	WPI oligo #	Oligo sequence	Note
Kek5$^{\Delta 234}$	5' W140	GCGTATGCCAATAGCTTGCCAGCCGGCGGCAACTCCACCC	SP
	3' W139	GGGTGGAGTTGCCGCCGGCTGGCAAGCTATTGGCATACGC	SP
Kek5$^{\Delta 1234}$	5' W138	GTACTGtCGTCGCATCAAGACCATCGCCGGCTCACAGGGaGGC	SP
	3' W137	GCCtCCCTGTGAGCCGGCGATGGTCTTGATGCGACGaCAGTAC	SP
Kek5$^{\Delta 1235}$	5'W136	GTGACTCTCCGAAGGCCGCCATGTCCGTGACGACGACGCGC	SP
	3'W135	GCGCGTCGTCGTCACGGACATGGCGGCCTTCGGAGAGTCAC	SP
Kek5$^{\Delta 1}$	5' W200	GAAGAGCCTGCTCAACGAGCGCACGGACATCGAGAGCGTGGATGG	SDM
	3' W201	CCATCCACGCTCTCGATGTCCGTGCGCTCGTTGAGCAGGCTCTTC	SDM
Kek5$^{\Delta 4}$	5' W202	CCACCGCGGAACTGCAGGCGATCGCCGGCTCACAGGGGGG	SDM
	3' W203	CCCCCCTGTGAGCCGGCGATCGCCTGCAGTTCCGCGGTGG	SDM
Kek5$^{\Delta 5}$	5' W204	GGTGACTCTCCGAAGGCCGCCATGTCCGTGACGACGACG	SDM
	3' W205	CGTCGTCGTCACGGACATGGCGGCCTTGGAGAGTCACC	SDM

SDM – Site directed mutagenesis
SP – Stitching PCR

Table B2: Primers used for generation of tagged BMP receptor constructs

BMP receptor	WPI oligo #	Oligo sequence
Thickveins	5' W370	GGGGacaaCtttgtacaaaaaagTTGGAAAATGGCGCCGAAATCCAGAAAG
	3' W371	GGGGacAactttgtacaagaaagTtgCGACAATCTTAATGGGCACATC
Saxophone	5' W372	GGGGacaaCtttgtacaaaaaagTTGGAAAATGGAGCTCTCCTCCGCC
	3' W373	GGGGacAactttgtacaagaaagTtgCAACGCAGACCTCGTCGAAGTC
Punt	5' W374	GGGGacaaCtttgtacaaaaaagTTGGAAAATGTCCAAATACGATCTG
	3' W375	GGGGacAactttgtacaagaaagTtgCTAAGCAATTCGTAGATTCCTTGGC

APPENDIX C

Results from *ptc>kek5* deficiency screen

Table C1: Components of various signaling pathways and cell adhesion complexes tested in the *ptc>kek5* deficiency screen. NT=Not Tested; NE=No Effect; L=Lethal; WS=Weak Suppressor

	Genes	Cytological location	Df tested in the screen (BS #)	Average # of scutellar bristles
Components of BMP pathway	*Decapentaplegic (dpp)*	22F1-22F3	NT	-
	glass bottom boat (gbb)	60A3-60A4	NT	-
	screw (scw)	38A1-38A1	8679	8.48
	twisted gastrulation (tsg)	11A1-11A1	9217	7.33
	short gastrulation (sog)	13E1-13E1	9219	10.45
	tolloid related (tlr-2)	96A-96A	9211???	9.9
	crossveinless2 (cv2)	57D12-57D13	7556	NE
	thickveins (tkv)	25D1-25D2	7497	L
	saxophone (sax)	43E18-43E18	8941	8.75
	punt (put)	88C9-88C9	9090	6.94
	wishful thinking (wit)	64A5-64A5	8060	8.64
	mothers against dpp (mad)	23D3-23D3	NT	-
	medea (med)	100C7-100D1	NT	-
	smad anchor for receptor activation (sara)	57E6-57E6	7556	NE
	daughters against dpp (dad)	89E11-89E11	7655??	WS
	dsmurf (lack)	54C12-54D1	7890	8.29
Components of Hippo pathway	*hippo (hpo)*	56D13-56D13	9067	10.1
	fat (ft)	24D8-24D8	7498	WS
	dachsous (ds)	21E2-21E2	8908	9.71
	dachs (d)	29D1-29D1	NT	-
	yorkie (yki)	60B7-60B8	NT	-
	expanded (ex)	21C2-21C2	NT	-
	merlin (mer)	18E1-18E1	7721	8.43
	salvador (sav)	94D10-94D10	8963	6.6
	warts (wts)	100A5-100A5	7997	8.3
	mob as tumor suppressor (mats)	94A12-94A12	8923	10.4
	kibra (kibra)	88D1-88D2	NT	-
Components of Cadherin-Catenin complex	*shotgun (shg)*	57B15-57B16	7554	L
	armadillo (arm)	2B14-2B14	NT	-
	α-catenin (α-cat)	80F1-80F2	NT	-
	polychaetoid (pyd)	85B3-85B7	9077	14
	p120 catenin (p120ctn)	41B1-41B1	NT	-
	echinoid (ed)	24D4-24D6	NT	-
	canoe (cno)	82F4-82F6	8967	9.41
	cortactin	93B8-93B9	7739	NE
Other interesting genes	*patched (ptc)*	44D5-44E1	9276	NT
	epidermal growth factor receptor (egfr)	57E9-57F1	7556	NE
	spitz (spi)	37F2-37F2	8679	8.48
	frizzled (fz)	70D4-70D5	8073	8.2
	fz2	75F9-76A1	8082	10.1
	fz3	1C4-1C4	9053	8
	inflated (if)	15A5-15A7	8954	8.7
	multiple edematous wings (mew)	11E3-11E8	8898	7.2
	tiggrin (tig)	26D1-26D1	9341	11.38

Table C2: Analysis of the interesting chromosomal regions for possible Kek5 interactors

	Original Df from the screen	Cytology of overlapping region	Interesting genes/allele	Average number of scutellar bristles
ENHANCERS	**BS# 9177 (21B3-21B7) ANB*=15.30**	21B3-21B3 (BS# 9193)	-	11.64
		21B3-21B7 (BS# 8901)	-	11.92
		21B7-21B8 (BS# 24958)	-	12.60
		-	*split ends/spen*^{1401} (BS# 5808)	10.81
		-	*split ends/spen*^{16H1} (BS# 5809)	9.75
		-	*kismet/kis*^{1} (BS# 431)	13.37
		-	*smoothened/smo*^{119B6} (BS# 24772)	9.20
	BS# 9077 (85A5-85D1) ANB*=16.32	84F6-85C3 (BS# 9338)	-	11.48
		85A3-85A10 (BS# 24981)		8.89
		85A5-85A9 (BS# 9623)	-	8.42
		85A5-85B6 (BS# 7629)	-	7.48
		85B1-85C2 (BS# 25010)	-	7.46
		85C2-85D11 (BS# 26518)	-	10.89
		85C3-85C3 (BS# 9225)	-	9.5
		85C3-85D1 (BS# 9203)	-	10.68
		85C3-85C11 (BS# 7630)	-	7.93
		85C11-85D2 (BS# 7631)	-	7.97
		-	*Map kinase kinase 4/mkk4*^{e01485} (BS# 17956)	7.74
		-	*hunchback/hb*^{4} (BS# 5339)	8.48
		-	*hunchback/hb*^{1} (BS# 6259)	14.68
			polychaetoid/pyd^{KG02008} (BS# 14253)	10.60
		-	*polychaetoid/pyd*^{1} (BS# 562)	7.60
		-	*polychaetoid/pyd*^{J4} (BS# 8850)	7.40
		-	*polychaetoid/pyd*^{ex147} (Gift from Mark Peifer)	10.40
		-	*polychaetoid/pyd*^{ex180} (Gift from Mark Peifer)	8.50
		-	*polychaetoid/pyd*^{B12} (Gift from Mark Peifer)	11.00
		-	*hyrax/hyx*^{EY06898} (BS# 16768)	10.39
		-	*relish/rel*^{E20} (BS# 9457)	11.88
SUPPRESSORS		66A10-66A19 (BS# 25722)	-	10.25
		66A17-66B12 (BS# 27367)	-	11.59
		66A17-66C8 (BS# 26830)	-	10.0
		66A17..20-66C1..5 (BS# 5877)	-	9.2

	BS# 7745 (66A17-66B5) ANB*=3.31	66A19-66A20 (BS# 32017)	-	12.06
		66A22-66C5 (BS# 8065)	-	9.04
		-	*pebble/pbl*^{3} (BS# 9358)	13.88
		-	*nemo/nmo*^{P1} (BS# 27897)	9.07
		-	*sunday driver/syd*^{A2} (BS# 32017)	12.06
	BS# 7984 (89B9-89B13) ANB*=6	89B6-89B16 (BS# 30592)	-	5.73
		89B7-89B18 (BS# 9481)	-	9.75
		89B12-89B18 (BS# 7736)	-	8.9
		-	*Cadherin89D/cad89D*^{e03186} (BS# 18129)	9.27
		-	*taranis/tara*^{1} (BS# 6403)	11.47
		-	*taranis/tara*^{03881} (BS# 11613)	8.43
		-	*belphegor/bor*^{c05496} (BS# 17709)	11.8
		-	*gilgamesh/gish*^{04895} (BS# 11790)	9.67

ANB= Average number of bristles*

APPENDIX D

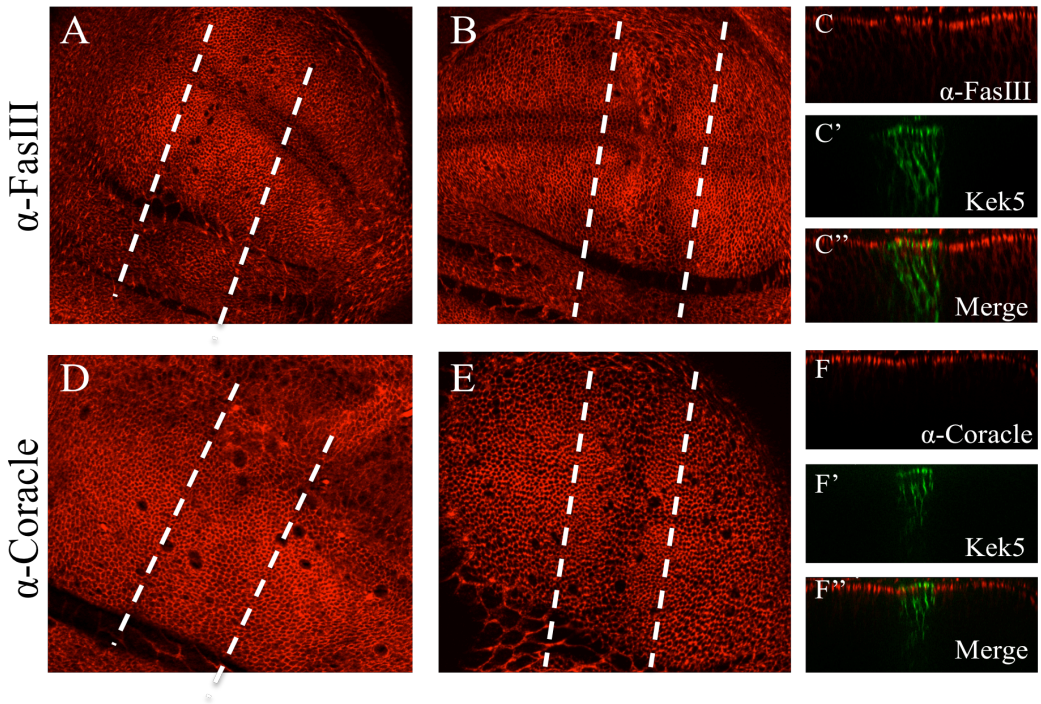


Figure D1: Kek5 does not alter components of the septate junction. Third instar wing discs from *ptc>mCD8* and *ptc>kek5* were stained with septate junction markers FasIII (A-C) and Coracle (D-F). There was no detectable change in the levels of these proteins in *ptc>kek5* discs when compared to *ptc>mCD8*. C-C'', F-F'' indicate the Z planes. Wing disc are oriented with their ventral side upwards. Broken lines indicate the region of Kek5.GFP expression.

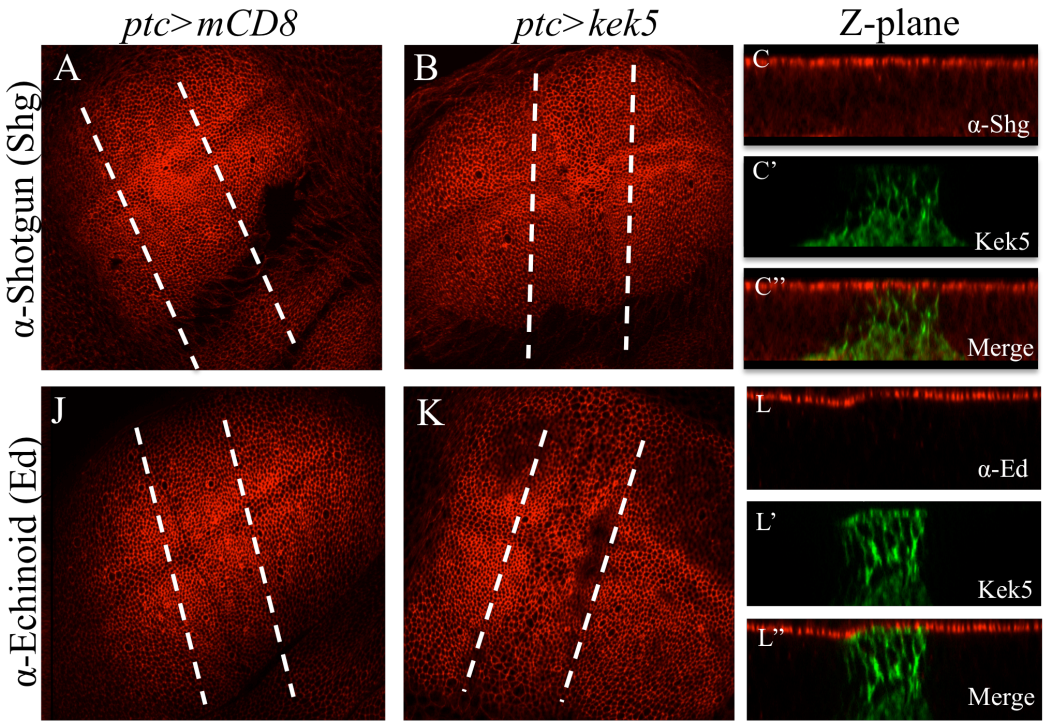


Figure D2: Kek5 does not affect AJ components Shotgun and Echinoid. Third instar wing discs from *ptc>mCD8* and *ptc>Kek5* larvae were dissected and stained with antibodies for Shg and Ed. No affect on Shg or Ed was seen in wild type discs, *ptc>mCD8* (A, D) or *ptc>kek5* discs (B-C'', E-F''). Broken white line indicates the region of GFP expression. Discs are oriented with their ventral side upwards.