Supplementary Materials

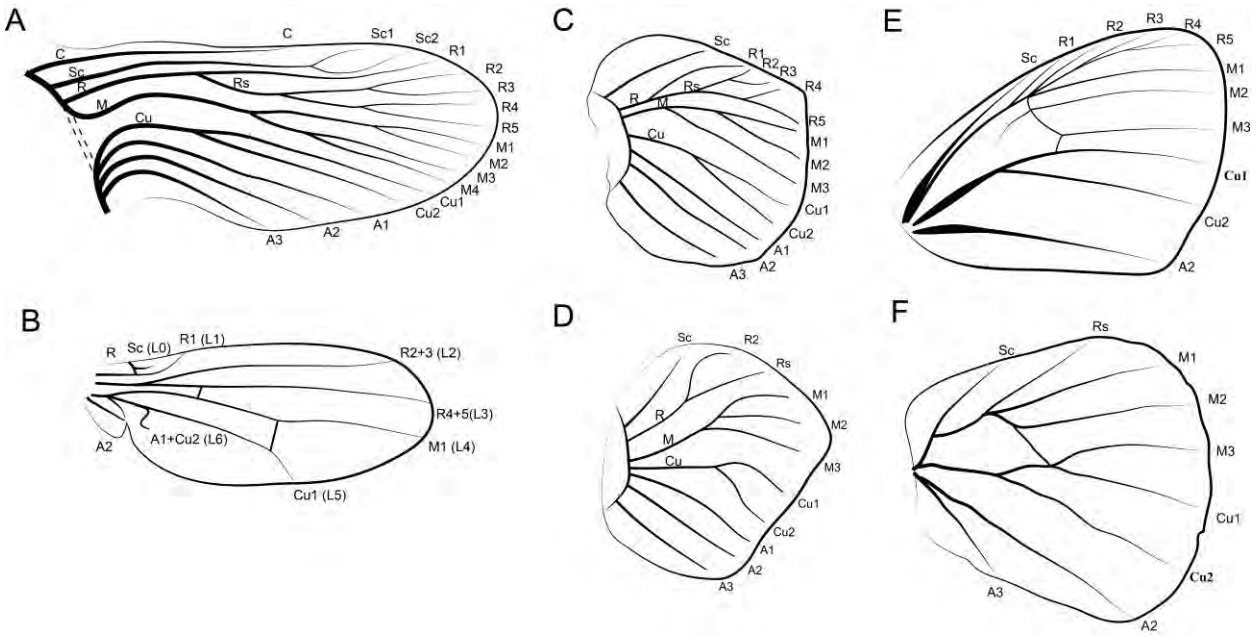

Figure S1. Venation patterns in insects. (A) Comstock-Needham hypothetic venation of primitive insects (redrawn from (Comstock and Needham, 1898)), **(B)** Wing venation of *Drosophila melanogaster* (redrawn from (Blair, 2007)), **(C)** Larval forewing venation and **(D)** hindwing venation of *Bicyclus anynana* butterflies. Larval wings of *B. anynana* were drawn based on methylene blue staining's (**Fig. S5**). **(E)** Adult forewing and **(F)** hindwing venation of *B. anynana*.

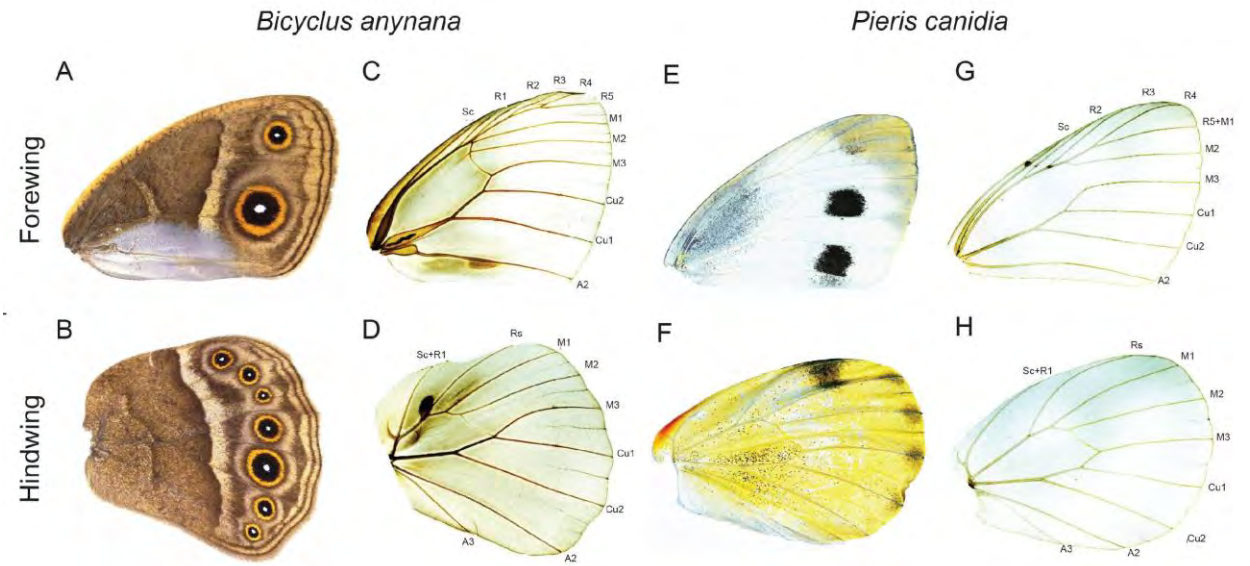

Figure S2. Venation pattern in adult butterflies. (A and C) *Bicyclus anynana* forewing, **(B and D)** and hindwing. **(E and G)** *Pieris canidia* forewing, **(F and H)** and hindwing. **(A, B, E and F)** Adult wings with scales. **(C, D, G, and H)** Adult wings with scales removed.

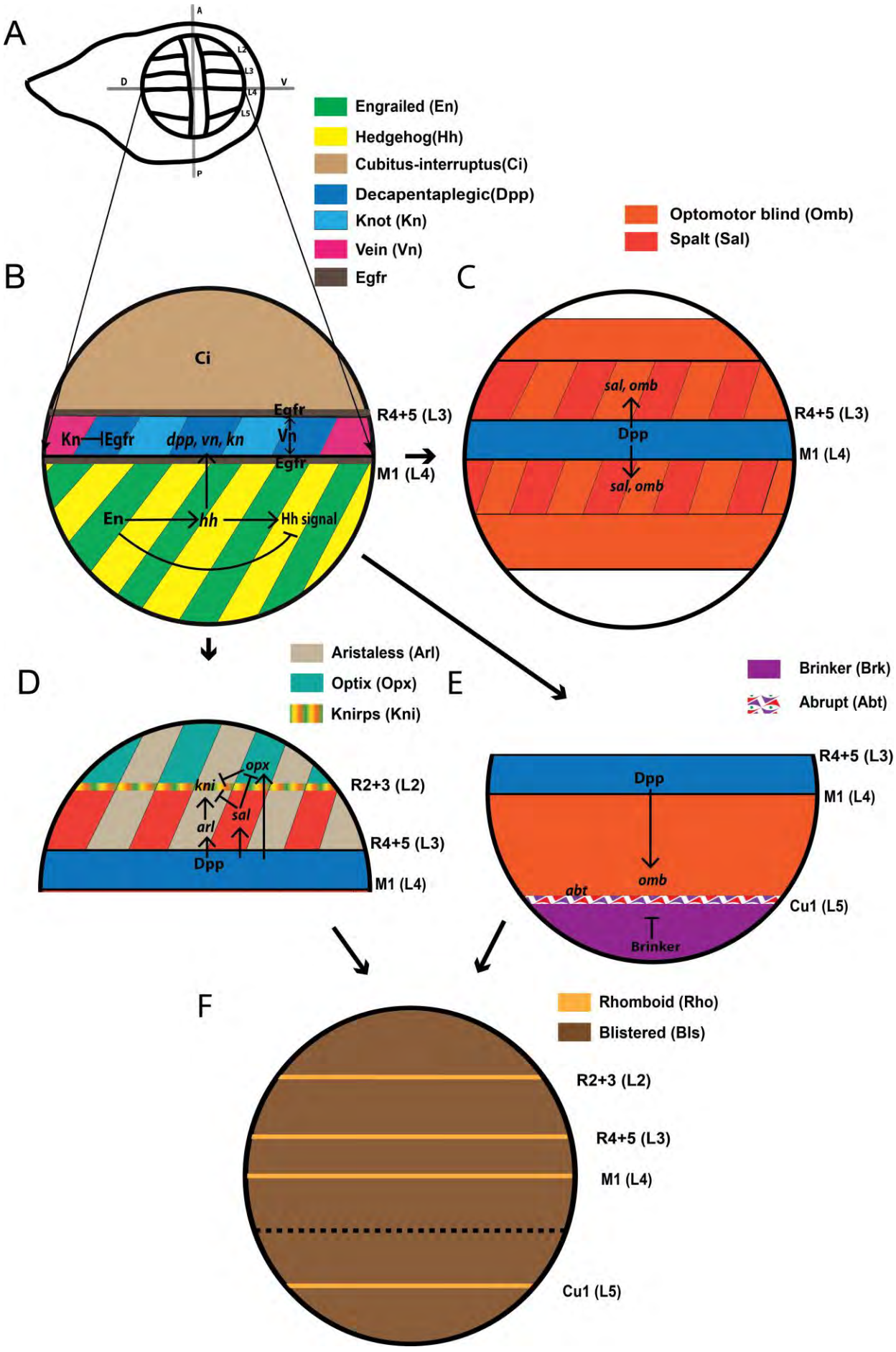

Figure S3. Molecular mechanism involved in venation patterning in *Drosophila melanogaster*. (A) Larval wing disc of *D. melanogaster*. During the larval stage, the wing is divided into two populations of immiscible cells belonging to the Anterior (A) and Posterior (P) compartments. The boundary where these two-populations meets is referred to as the Anterior-Posterior (A-P) boundary (marked by the gray line). **(B)** Venation patterning is initiated by the transcription factors En and Inv in the posterior compartment that activate expression of *hh* while suppressing Hh signaling. Hh is a short-range diffusible morphogen. A small amount of Hh diffuses into the anterior compartment where the presence of Ci activates the BMP ligand *dpp*. Hh also activates the genes *vein* and *knot* overlapping the expression of *dpp*. Knot inhibits Egfr signaling at the R4+5 (L3) and M1 (L4) intervein cells. The veins R4+5 (L3) and M1 (L4) form at the anterior and posterior boundary of the *dpp* and *vein* expression domain due to activation of Egfr signaling via Vein protein. **(C)** Dpp protein then acts as a long-range morphogen activating both *spalt* (*sal*) and *optomotor-blind* (*omb*) at high concentrations, and only *omb* when the concentration falls below the *sal*-inducing threshold. (**D**) The vein R2+3 (L2) forms by the interaction of Al, Opx and Sal. Dpp activates all three transcription factors at different concentration thresholds. Al activates the R2+3 (L2) vein specific gene *knirps*, Sal represses *opx*, and Opx and Sal suppresses *knirps*. (**E**) The vein Cu1 (L5) forms at the boundary of Omb and Brinker where the Cu1 (L5) specific gene *abrupt* is expressed. (**F**) The final step of venation patterning involves expression of Rho in the vein cells and Bls in the intervein cells.

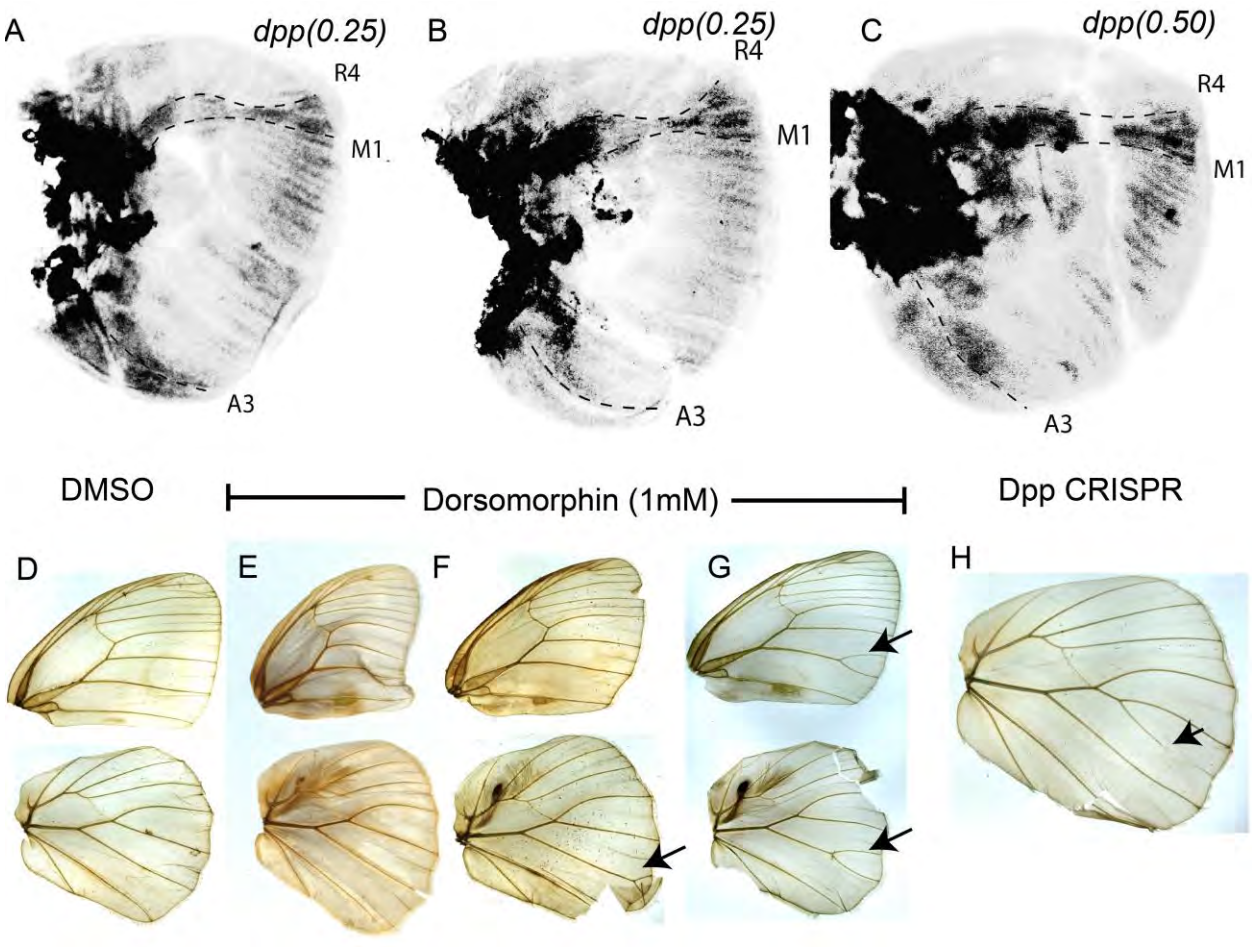

Figure S4. Expression of *decapentaplegic* (*dpp*) and the effect of Dorsomorphin and Dpp CRISPR on the wings of *Bicyclus anynana*. (A-C) *dpp* is expressed in two domains in the larval wings. (**D**) Adult descaled Wings of a control individual injected with DMSO. (**E-G**) Dorsomorphin affects the wing size and venation (black arrow). (**H**) Dpp CRISPR individual with ectopic and missing vein (black arrow).

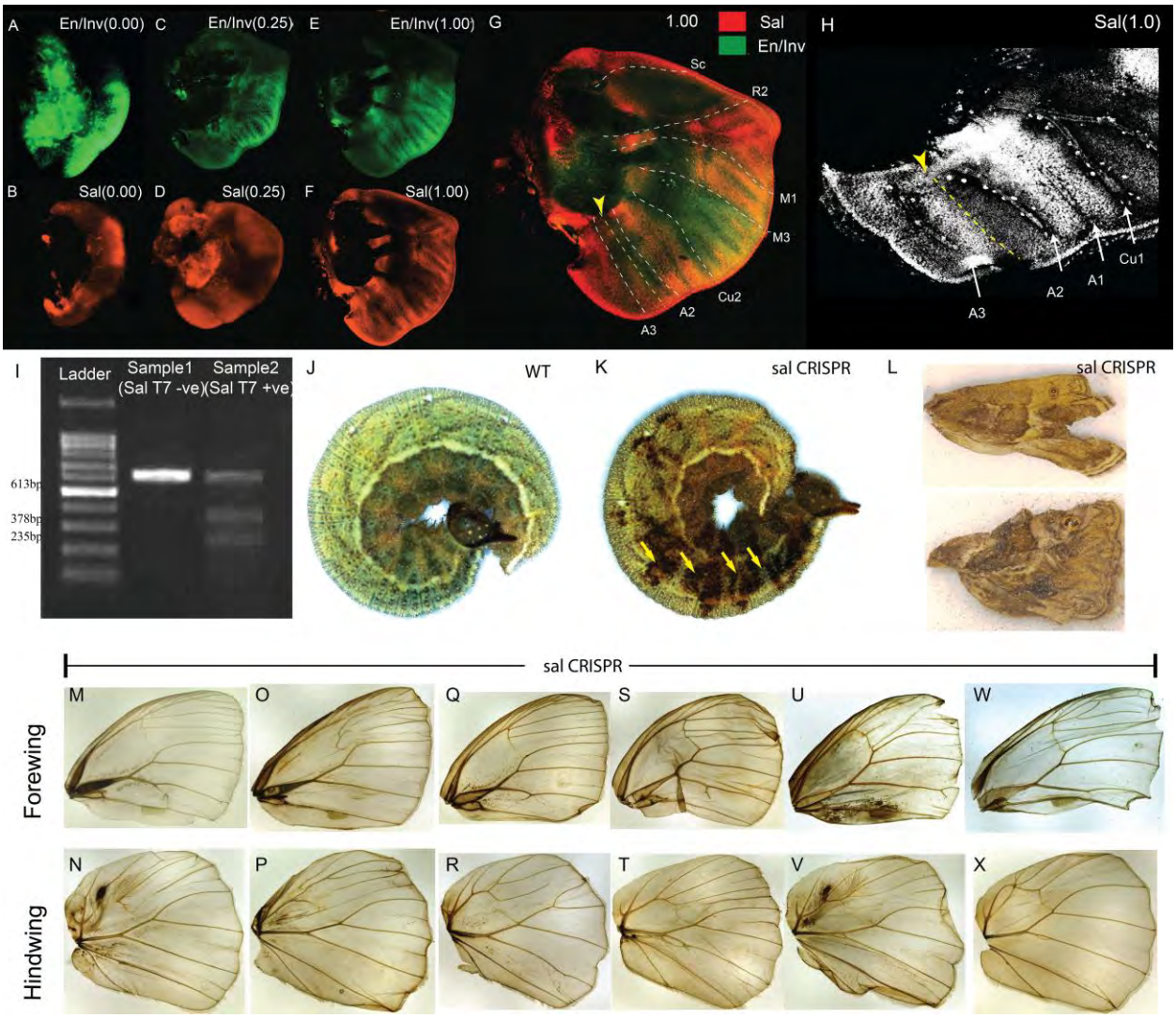

Figure S5. Expression of Spalt (Sal) and Engrailed /Invected (En/Inv); and function of *sal* in *Bicyclus anynana*. (**A, C, and E**) En/Inv staining at different stages of larval wing growth. **(B, D, and F**) Sal staining at different stages of wing growth. (**G**) Merged channels of Sal and En/Inv. **(H)** Closeup of Sal expression showing the anterior boundary of the fourth Sal domain (yellow arrowhead and dotted yellow line). (**I**) T7 endonuclease assay on *sal* guide and Cas9 injected individuals. Sample 2 with T7 endonuclease added shows two shorter DNA bands indicating cleavage of the PCR product. **(J)** A WT fifth instar larva. (**K**) Pigmentation defects on sal CRISPR larva. Spalt has been implicated to be involved in the development of black pigment on the eyespots of *B. anynana* butterflies. (**L**) Severe adult wing patterning defects in some individuals were observed. **(M-X)** Venation defects in *B. anynana* descaled adult forewings and hindwings.

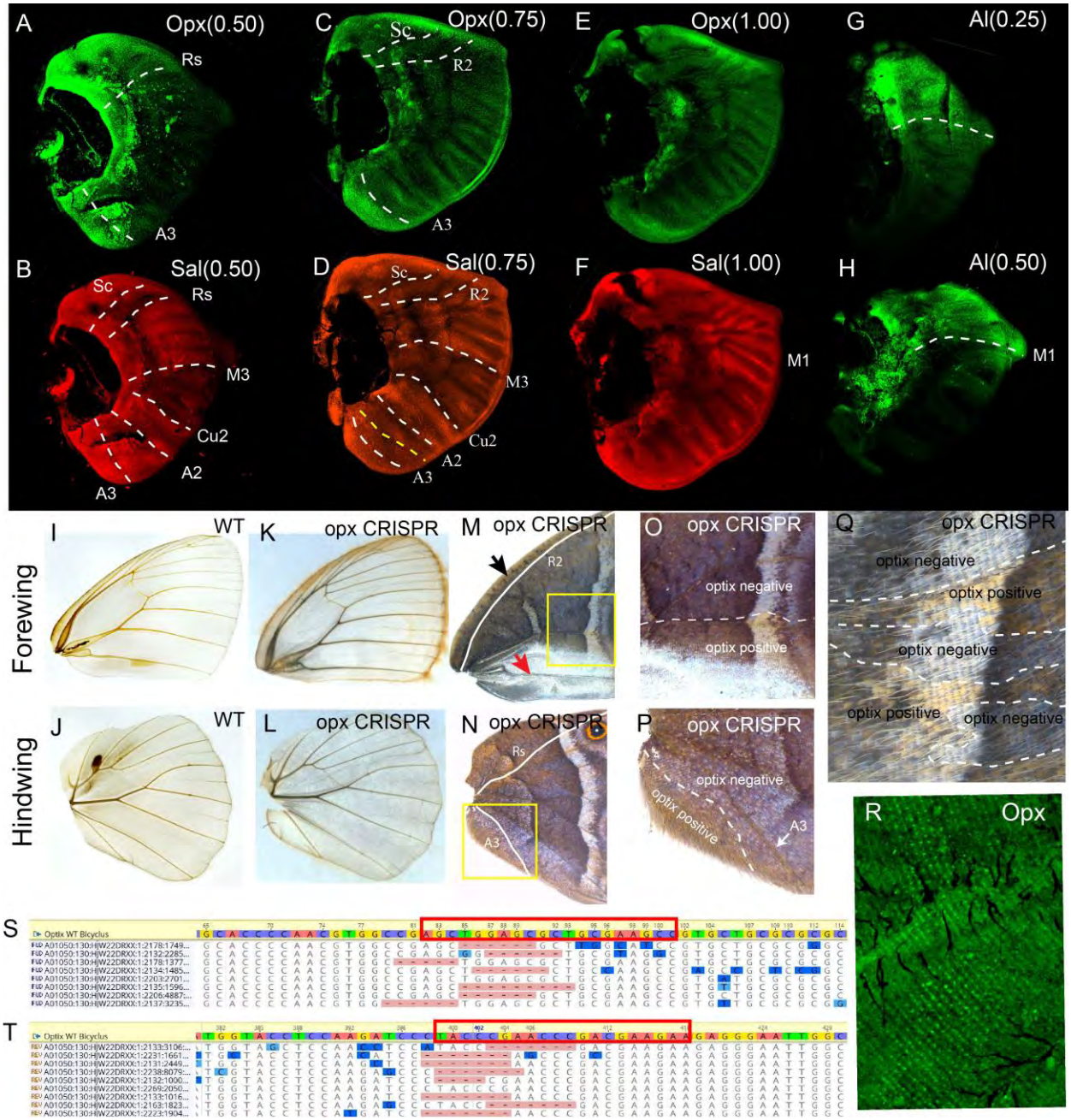

Figure S6. Expression of Optix (Opx) and Aristaless (Al); and function of *opx* in *Bicyclus anynana*. Opx expression at different stages of larval wing growth in the (**A, C and E)**. Sal expression at different stages of larval wing growth **(B, D, and F)**. (**G and H**) Al expression at different stages of larval wing growth. WT descaled adult (**I**) forewing and (**J**) hindwing. Optix CRISPR (**K**) forewing and (**L**) hindwing. No defects in venation are observed. (**M-Q**) Optix CRISPR individuals with loss of scales with ommochrome (orange) pigment. (**R**) Optix expression in the pupal wings. (**S and T**) Deletions in the regions targeted for *optix* CRISPR (red boxes). Black arrow: orange scales in the anterior margin of the forewing overlap the anterior expression of Optix in the larval wing disc. Red arrow: silver scales in the posterior region of the forewing overlap the posterior expression of Optix in the larval wing disc.

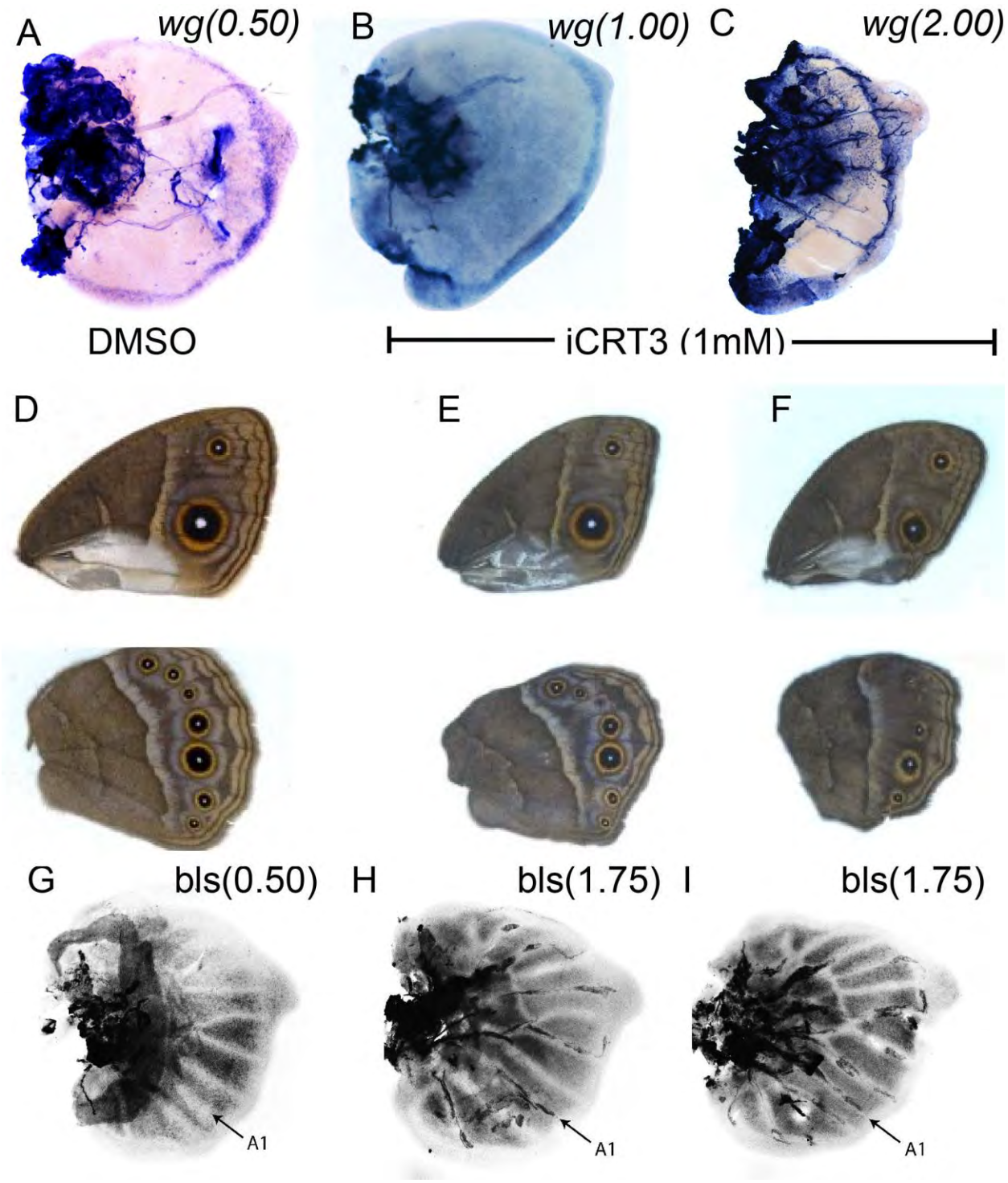

Figure S7. **Expression of *wingless* (*wg*) and *blistered* (*bls*); and the effect of iCRT3 on the wings of *Bicyclus anynana*.** (**A-C**) Expression of *wg* in the larval wing margin. (**D**) Adult wings of a control individual injected with DMSO. (**E and F**) iCRT3 injections reduce the adult wing size relative to DMSO injections. (**G-I**) Expression of *bls* in larval wings. *bls* is absent at the A1 vein at an early stage (0.50). However, during later stages (1.75) *bls* has a stronger expression at the A1 vein.

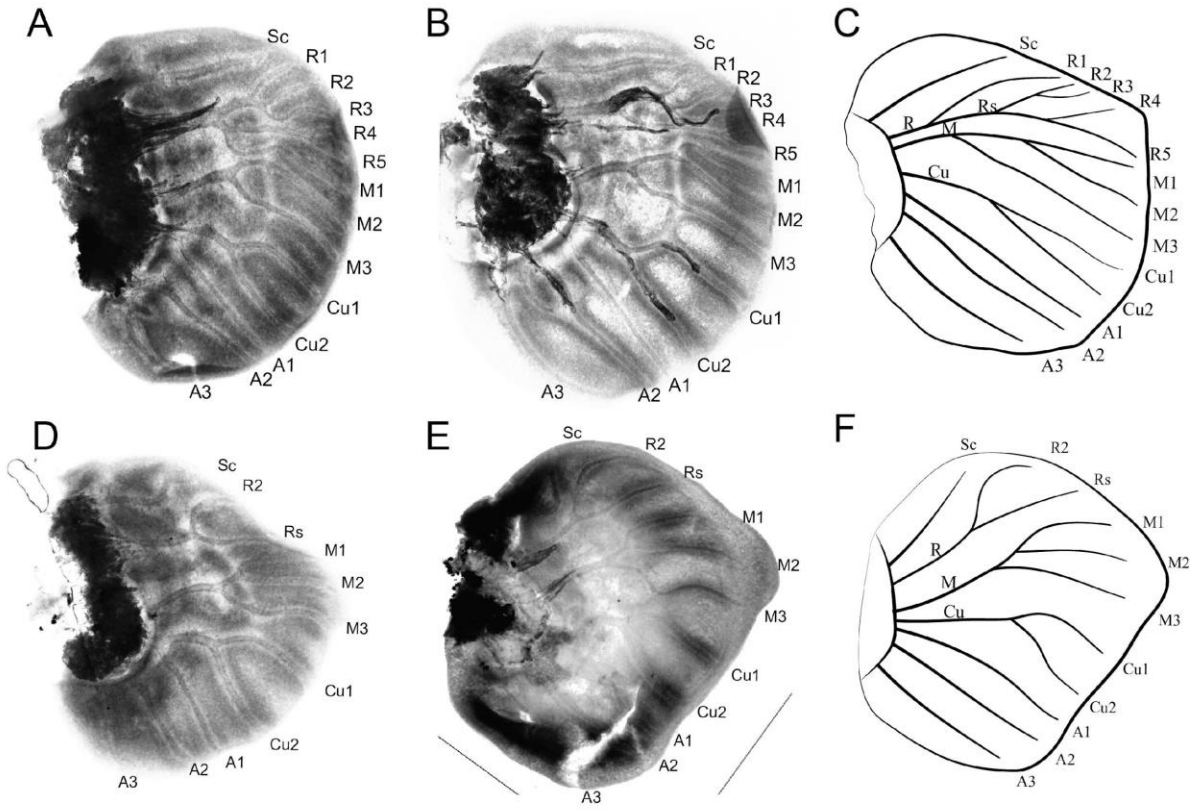

Figure S8. Methylene blue staining of *Bicyclus anynana* larval wings. (A and B) Forewing stained with methylene blue; **(D and E)** Hindwing stained with methylene blue; Illustration of (**C**) forewing and (**F**) hindwing venation.

Table S1. Primer table

No.	Primer Name	Sequence	Description
1.	Dpp_insitu_F	GTTCTTCAACGTAAGCGGCG	Forward primer to amplify *dpp* for in-situ hybridization
2.	Dpp_insitu_R	CCACAGCCTACCACCATCAT	Reverse primer to amplify *dpp* for in-situ hybridization
3.	En_insitu_F	TTGAAGACCGTTGCAGTCC	Forward primer to amplify *en* for in-situ hybridization
4.	En_insitu_R	TAGATTGCTGTTCCCGCTTT	Reverse primer to amplify *en* for in-situ hybridization
5.	Inv_insitu_F	GGACCAAAGTGACGAAGAGC	Forward primer to amplify *inv* for in-situ hybridization
6.	Inv_insitu_R	TCCGGCACTCTAGCCTCTAC	Reverse primer to amplify *inv* for in-situ hybridization
7.	Bls_insitu_F	CTGACCGGCACCCAAGTGAT	Forward primer to amplify *bls* for in-situ hybridization
8.	Bls_insitu_R	CGTTGCGGGTGGTGAGACAT	Reverse primer to amplify *bls* for in-situ hybridization
9.	Sal_CRISPR_Seq_F	GCATCGACAAGATGCTGAAA	Forward primer to amplify *sal* for CRISPR-Cas9 invitro cleavage assay
10.	Sal_CRISPR_Seq_R	TTCATTTAGGGACGGTGGAG	Reverse primer to amplify *sal* for CRISPR-Cas9 invitro cleavage assay
11.	Sal_CRISPR_Guide	GAAATTAATACGACTCACTATAGGTGATCGAGCCGGCGTTGAGTTTTAGAGCTAGAAATAGC	Forward primer for guide synthesis to knockout *sal*
12.	Optix_CRISPR_Guide_1	GAAATTAATACGACTCACTATAGGGGCTTCGCAGCGCTCCAGCTGTTTTAGAGCTAGAAATAGC	Forward primer 1 for guide synthesis to knockout *optix*
13.	Optix_CRISPR_Guide_2	GAAATTAATACGACTCACTATAGGTTCTTCGTCGGGTTCGGGTAGTTTTAGAGCTAGAAATAGC	Forward primer 2 for guide synthesis to knockout *optix*
14.	Dpp_CRISPR_Guide	GAAATTAATACGACTCACTATAGGGAGACTGTTGTTGTACGACGTGGGTTTTAGAGCTAGAAATAGC	Forward primer for guide synthesis to knockout *dpp*
15.	CRISPR_Guide_R	AAAAGCACCGACTCGGTGCCACTTTTTCAAGTTGATAACGGACTAGCCTTATTTTAACTTGCTATTTCTAGCTCTAAAAC	Reverse primer for guide synthesis CRISPR guides
16.	Wg_insitu_F	CAGCAGCTGGATTTTGTCAG	Forward primer to amplify *wg* for in-situ hybridization
17.	Wg_insitu_R	TATTGTGCCGTTGTCATCGT	Reverse primer to amplify *wg* for in-situ hybridization
18.	Sal_qPCR_F	TGTATGCCATCGCGTATTGT	Forward primer to amplify *sal* for qPCR
19.	Sal_qPCR_R	TAGTGGTAAACGCACGACCA	Reverse primer to amplify *sal* for qPCR
20.	FK506_qPCR_F	AAACTAACCTGCAGCCCTGA	Forward primer to amplify FK506 for qPCR
21.	FK506_qPCR_R	CAAGACGGAGAAGTTCCACA	Reverse primer to amplify FK506 for qPCR
22.	UBQL40_qPCR_F	CGGTAAACAATTGGAAGATGG	Forward primer to amplify UBQL40 for qPCR
23.	UBQL40_qPCR_R	CGAAGTCTGAGGACAAGATGC	Reverse primer to amplify UBQL40 for qPCR

Table S2. Spalt CRISPR-Cas9 injection table

Sl. No.	Concentration	Date	Eggs Injected	Hatchlings	% Hatchlings
1.	300 ng/µl	28th Sept 2018	302	48	15.9
2.	300 ng/µl	10th Oct 2018	306	25	8.2
3.	300 ng/µl	11th Nov 2018	120	18	15.0
4.	300 ng/µl	9th Feb 2019	135	8	5.9

Table S3. Optix CRISPR-Cas9 injection table

Sl. No.	Concentration	Date	Eggs Injected	Hatchlings	% Hatchlings
1.	300 ng/µl	11th March 2020	785	85	10.8
2.	300 ng/µl	12th March 2020	398	47	11.9
3.	300 ng/µl	6th June 2020	326	65	19.9

Table S4. Dpp CRISPR injection table

Sl. No.	Concentration	Date	Eggs Injected	Hatchlings	% Hatchlings
1.	300 ng/µl	23rd Jan 2020	623	89	14.3
2.	300 ng/µl	3rd Mar 2020	923	117	12.7
3.	300 ng/µl	11th Mar 2020	427	64	14.9

Table S5. *In-situ* hybridization Buffers

Buffers	Chemicals	Amount
10X PBS (500 ml) * Sterilize by autoclaving.	K_2HPO_4	5.34 g
	KH_2PO_4	2.64 g
	NaCl	40.9 g
	DEPC treated H_2O	To 500 ml
1X PBST (50 ml)	1X PBS	50 ml
	Tween® 20	50 µl
20X SSC (1000 ml) *Adjust the pH to 7.0 with 1M HCl and sterilize by autoclaving.	NaCl	175.3 g
	Trisodium citrate	88.2 g
	DEPC treated H_2O	Till 1000 ml
Pre-hybridization buffer (40 ml)	Formamide	20 ml
	20X SSC	10 ml
	DEPC treated water	10 ml
	TWEEN20	40 µl
Hybridization buffer (40 ml)	Formamide	20 ml
	20X SSC	10 ml
	DEPC treated water	10 ml
	TWEEN20	40 µl
	Salmon sperm	40 µl
	Glycine (100mg/ml)	40 µl
Block buffer (50 ml)	1X PBS	50 ml
	TWEEN20	50 µl
	BSA	0.1 gm
Alkaline phosphatase buffer (20 ml)	Tris-HCl (pH 8.0)	2 ml
	NaCl (5M)	400 µl
	$MgCl_2$ (200mM)	250 µl
	DEPC treated water	Till 20 ml
	TWEEN20	20 µl

Table S6. Immunohistochemistry Buffers

Buffers	Chemicals	Amount
Fix buffer (30 ml)	M PIPES pH 6.9 (500 mM)	6 ml
	mM EGTA pH 6.9 (500mM)	60 µl
	% Triton x-100 (20 %)	1.5 ml
	mM $MgSO_4$ (1M)	60 µl
	37% Formaldehyde	55 µl per 500 µl of buffer
	dH_2O	22.4 ml
Block buffer (40 ml)	50 mM Tris pH 6.8 (1 M)	2 ml
	150 mM NaCl (5 M)	1.2 ml
	0.5% IGEPAL (NP40) (20%)	1 ml
	5 mg/ml BSA	0.2 gr
	H2O	35.8 ml
Wash buffer (200 ml)	50mM Tris pH 6.8 (1 M)	10 ml
	150 mM NaCl (5 M)	6 ml
	0.5% IGEPAL (20 %)	5 ml
	1 mg/ml BSA	0.2 gr
	dH_2O	179 ml
Mounting media	Tris-HCl (pH 8.0)	20 mM
	N-propyl gallate	0.5%
	Glycerol	60%

Table S7. Raw Cq data on the Dorsomorphin and DMSO treated samples

Biological replicates	Raw Cq (Dorsomorphin treated)			Raw Cq (DMSO treated)		
	spalt	FK506	UBQL40	*spalt*	FK506	UBQL40
1 (5th July 2019)	28.36	22.50	20.89	28.10	22.69	21.05
	28.36	22.61	20.96	28.05	22.55	20.94
	28.20	22.54	20.76	27.76	22.67	20.94
2 (14th April 2019)	31.54	22.57	20.89	30.43	22.22	20.64
	31.80	22.56	20.90	30.59	22.16	20.47
	31.23	22.50	20.87	30.58	22.19	20.67
3 (21st May 2019)	32.02	22.76	21.15	30.40	22.23	20.60
	31.67	22.72	21.11	30.55	22.16	20.44
	31.39	22.62	21.00	30.32	22.29	20.72

Supplementary Materials and Methods

Peptides used for antibody development (Highlighted in green)

Spalt (XP_023939142.1)

MPRVKPACVRRVSIGESSGSCSEEDVGNAMPDEARDRPEAHMCPRCQEQFENLHDFLYHKRLCDEKAMQM
GEERMHSDPEDMVVSGDEEMDGPNKRLEQVRRHRQDAENNNSLEDGEAEIPEADMPPVGLPFPLAGHVTL
EALQNTRVAVAQFAATAMANNANNEAAIQELQVLHNTLYTLQSQQVFQLQLIRQLQNQLSLTRRKEDDPH
SPPPSEPEQNAPSTPARSPSPPRPPREPSPVIPSPPTSQSLPSTHTHHTPKTEQISIPKIPTSSPSLMTH
PLYSSISSSLASSIITNNDPPPSLNEPNTLEMLQKRAQEVLDNASQGLLANNLADELAFRKSGKMSPYDG

KSGGRNEPFFKHRCRYCGKVFGSDSALQIHIRSHTGERPFKCNVCGSRFTTKGNLKVHFQRHTSKFPHVK
MNPNPVPEHLDKYHPPLLAQLSPGPIPGMPPHPLQFPPGAPAPFPPNLPLYRPPHHDLLPPRPLGDKPLS
HHPLFAMREEQDAPADLSKPSAPSPPRPASDIFKSEPQDEESQRDSSFEETDRISPKREIEDNDIGQDAE
QDRYPSTSPYDDCSMDSKYSNEDQIGRDSPHVKPDPDQPENLSSKTSSISGPISIATGLRTFPSFPLFPH
SPPSSVSSGSLTPFHHHPNSTMDSALTRDPLFYNAILPRPGSNDNSWESLIEITKTSETTKLQQLVDNIN
NKVSDPNECIVCHRVLSCKSALQMHYRTHTGERPFRCKLCGRAFTTKGNLKTHMGVHRIKPPSQLLHQCP
VCHKKFSDPSMLHQHIRLHTGERNNVFFNQFHDNEINSQSLPGSDVTEYNSFHSIPPPIFPTPSTPGDRR
ADSRGTDDESGRDEREPATREFDDEPDIKDRRTSPLSVCASASEFEVKTITTTASLPSATGSESGRSARG
SPPSPSPSPSALSTPPRLPHHSPLPSPPTPLAALGALGGSPFSPLGLAFPPAVRGNTTCTICYKTFACNS
ALEIHYRSHTKERPFKCTVCDRGFSTKSSGGGCQCGRRARAPRPPHATALDLWNAFVYPGNMKQHMLTHK
IRDMPPGFDKGPGGPSGPPSEEGRDPSPDRRSSPEKLDLKRSPPVHPPPPMSHPPIDMPPLPKRPTVPSI
PSHPPPSASSKHLCGVCRKNFSSSSALQIHMRTHTGDKPFRCAVCQKAFTTKGNLKGLLLPATRLISRST
NQATALFGTLGPFIYRLSELYAPPSATSALRLVELSDFGSADFR

Armadillo (XP_023941962.1)

MSYQIPSSQSRTMSHSNYGGSDVPMAPSKEQQTLMWQQNSYLVDSGINSGAATQVPSLTGKEDDEMEGDQ
LMFDLDQGFAQGFTQEQVDDMNQQLSQTRSQRVRAAMFPETLEEGIEIPSTQLDPAQPTAVQRLSEPSQM
LKHAVVNLINYQDDADLATRAIPELIKLLNDEDQVVVSQAAMMVHQLSKKEASRHAIMNSPQMVAALVRA
ISNSNDLETTKGAVGTLHNLSHHRQGLLAIFKSGGIPALVKLLSSPVESVLFYAITTLHNLLLHQDGSKM
AVRLAGGLQKMVALLQRNNVKFLAIVTDCLQILAYGNQESKLIILASQGPIELVRIMRSFDYEKLLWTTS
RVLKVLSVCSSNKPAIVEAGGMQALAMHLGNPSGRLVQNCLWTLRNLSDAATKVEGLEGLLQSLVQVLAS
TDVNIVTCAAGILSNLTCNNQRNKVTVCQAGGVDALVRTVVSAGDREEITEPAVCALRHLTSRHVESEMA
QNAVRLHYGLPVIVKLLQPPSRWPLVKAVVGLVRNLALCPANHAPLREHGAVHHLVRLLLRAFNDTQRQR
GSVSGGGGAGGAYADGVRMEEIVEGAVGALHILAREGLNRALIRQQNVIPIFVQLLFNEIENIQRVAAGV
LCELAADKEGAEMIEAEGATAPLTELLHSRNEGVATYAAAVLFRMSEDKPHDYKKRLSMELTNSLFRDDH
QMWPNDLAMQPDLQDMLGPEQGYEGLYGTRPSFHQQGYDQIPIDSMQGLEIGSGFGMDMDIGEADGGGAA
SADLAFPEPPLDNNNVAAWYDTDL

Rhomboid (XP_023940805.1)

MANQQEHNKRYMSGKRTRSYRCAVHQRDREVSSENDFHLLLEDPTLFARMVHLVAMEVLPEERDRKYYQE
RYTCCPPPFFIICVTLLELGVFAWYAWGAGGVAAAAGPVPVDSPLVYRPDRRRELWRFLTYSVVHAGWLH
LAFNLLVQLAVGLPLEMVHGAVRCGAVYLAGVLGGSLAASVLDPDVCLAGASGGVYALLAAHLANALLNF
HAMRYGAVRLVAALAVASCDVGFAVHARYTKQEAPPVSYAAHVAGALAGLTIGLLVLKHAQQRLWERLLW
WAALGAYAACTLFAVLYNVFSAPVDELHYMPPDPPPDAGF

Sequences

Sequence of *engrailed* used for *in-situ* hybridization (XM_024092264.1)

TTGAAGACCGTTGCAGTCCGAACCAGGCCAACAGCCCCGGTCCGGTCACCGGCAGAGTCCCTGCGCCTCA
CTCCGAAGTAAGAAACGNGTACCAAAGTCAATACACTTGCACGACTATCGATCAAAGGTTTGACAGAACG
ATGACAGTGGTGAAAGTGCAGCCGAATTCACCACCGATGAGTCCACTGACGTGAAGCCCATAATCCCTGA
GTTTGAAGACAAGAGAAACCGACAACCACCACCAACCATACCCTTCTCTATCAGCAACATATTACACCCA
GAATTCGGTTTGACAGCGATTCGAAAAACGAACAAAATCGAAGGACCAAAACACGTCGGCCCCAACCACA
GCATTTTGTACAAACCTTATTTGTCGAACGAGTTATCGAGTTCGAAATTCAATTTCGATTATTTAAAATC
TAAGGATGATTTCGGTGCATTACCTCCACTTGGCGGTTTGAGGCAGACCGTGTCGAATATTGGAGAACAG
AAGGAGGCACCAAAGATTATAGAGCAGCAGAAGAGGCCAGATTCAGCCAGCTCTATTGTCTCTTCCACAT
CTAGCGGGGCTTTATCGACGTGTGGCAGCACTGACGCCAACAGCAGTCAAAGCGGGAACAGCAATCTA

Sequence of *invected* used for *in-situ* hybridization (XM_024092263.1)

GGACCAAAGTGACGAAGAGCACGACCCCTACTCGCCCAACACTAGAGACACCATCACACCAGACTTCATA
GAAGAAGACAAACAAGACAGGCCTATACACACATCCTCTTTCTCCATACACAATGTCCTTAAGAAGGAAA
GAGACAGTAATAGTCCTGAGAACGTCTTCTCAACTGAAAAGTTGTTGCAAAGTACACCGAACTTTGAAGA
TTCTAGGAACTCTGAAAGCGTTAGTCCGAGACTTGAAGATGATCACAATGAAAGAGCTGATATAAGTGTT

GATGACAACTCTTGTTGTAGTGATGATACTGTGCTATCTGTTGGCAATGAAGCCTTACCAACCAATTACC
CAAACGACAAAGATCCGAACCAAGGCTTAACCTCCTTCAAACATATACAAACTCATTTGAACGCAATATC
ACAGTTAAGTCAAAATTTAAACATAAACCAACCAATCCTCCTACGACCCAACCCAATAACACCAAACCCG
TTAATGTTCCTAAACCAACCGTTGTTATTCCAAAACCCTTTAATAAACCAAGTGGATTTAAAATCAGGGT
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AACCAAGGATGTTTGAAATTTAGCATTGATAATATACTGAAAGCTGATTTTGGTAGACGAATTACTGATC
CGTTGACAAAGAGAAAAACGAAGACGAGGCAGTATGAGGCAAAATCTACCCCTGTCAAAGAGGTTCAGTC
TCCCCCTAAAGAGGTAGAGGCTAGAGTGCCGGA

Sequence of *decapentaplegic* used for *in-situ* hybridization (XM_024080858.1)

GTTCTTCAACGTAAGCGGCGTACCGGCCGACGAGGTGGCGCGCGGCGCCGACCTCTCGTTCCAACGAGCC
GTCGGCACCACCGGCAGACAGAGACTGTTGTTGTACGACGTGGTGCGCCCTGGCCGCCGCGGCCACTCCG
AGCCGATCCTGCGGCTGCTGGACTCCGTTCCGCTCCGGCCCGGGGAGGGAATCGTCAACGCCGACGCTCT
GGGAGCGGCGCGACGGTGGCTCAAAGAGCCCAAACATAATCACGGACTATTAGTGCGAGTGTTAGAAGAA
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CCACCTCAACGGCACGAACCACGCGATAGTGCAGACTCTGGTCAACTCAGTGAACCCCGCGACGGTGCCC
AAAGCGTGCTGCGTGCCGACGCAACTCTCATCTATATCTATGTTATATATGGACGAAGTGAACAATGTGG
TGCTTAAAAACTATCAGGACATGATGGTGGTAGGCTGTGG

Sequence of *blistered* used for *in-situ* hybridization (XM_024084428.1)

GCATACGAGCTATCAACGCTGACCGGCACCCAAGTGATGCTGCTGGTCGCGTCGGAGACCGGCCACGTGT
ACACGTTCGCGACACGCAAACTGCAGCCGATGATCACGTCCGACTCGGGCAAGCGGCTCATACAGACGTG
CCTCAACTCGCCCGACCCGCCCACCACCAGCGAGCAGCGCATGGCCGCCACCGGCTTCGAGGAGACCGAG
CTCACGTATAACGTTGTAGACGACGAGATGAAGGTGAGACAACTGGCGTACGCTAACCAGTACCCCATAG
AGCACCACCCGGGGTTGGCGCCGTCGCCACTGCAGCAGTACCACCAGCACCCGCCCTGCCCCTCGCCCCT
CCCCCTCGGCTCGCTGGGCCAGCCGTACTCGCACGCGCATCTATCGCACCCCCACATGTCTCACCACCCG
CAACG

Sequence of *wingless* used for *in-situ* hybridization (XM_024099417.1)

CAGCAGCTGGATTTTGTCAGTCCAGCTAGGAAGGGGGGCATAGCAAAGGCAGGCGAACCAAATAACTTAT
CACCCTTGTCTCCAAGTGTCCTATACATGGACCCGGCTGTTCACGCCACCTTGAGGAGGAAACAGAGAAG
GCTAGCGAGGGAGAACCCTGGGGTCCTCGCAGCAATATCCAAGGGAGCCAGCATGGCTGTGGCCGAATGC
CAGCATCAGTTCAAATACAGGAGATGGAACTGTTCTACAAGAAATTTTTTGCGAGGGAAGAATCTATTTG
GAAAAATTGTTGACAGAGTTTCGCCGGACAAAGCCCCCCCGGCCGGGGCGGCTATAATTACTAATATACA
CGTCGACACGCCATTGACGATTGACGCGACATCTTCATTTCATTGTGGTGTAAACCTCAAGGATCGCATT
AACACGGACGATGACAACGGCACAATA

CRISPR targets

Region of *spalt* targeted by CRISPR-Cas9 (location of guide RNA highlighted in red) (XM_024083374.1)

GCATCGACAAGATGCTGAAAATAATAATAGTCTCGAAGACGGCGAGGCCGAAATACCTGAAGCCGACATG
CCCCCCGTGGGTCTGCCGTTCCCTTTGGCAGGACACGTTACTCTTGAGGCTCTACAAAATACGAGAGTAG
CGGTCGCCCAATTCGCTGCAACAGCGATGGCAAATAATGCGAATAACGAAGCTGCTATACAAGAATTACA
AGTGTTACACAACACTCTATACACTTTACAGTCACAACAAGTATTTCAACTTCAGTTAATACGTCAGCTT
CAGAATCAGTTATCTCTAACTCGACGGAAAGAAGACGATCCACACAGCCCACCGCCAAGTGAACCAGAAC
AGAATGCCCCGTCAACGCCGGCTCGATCACCGTCGCCGCCGCGTCCGCCACGGGAGCCGTCGCCTGTTAT
ACCCTCTCCTCCTACTAGCCAAAGTTTGCCGTCGACTCACACACATCACACACCCAAAACTGAACAGATA

TCTATCCCTAAGATTCCAACTTCCTCACCATCTTTAATGACCCACCCACTTTATAGTTCAATTTCTTCGT
CATTAGCATCTTCCATCATAACAAACAATGATCCTCCACCGTCCCTAAATGAA

Region of *optix* targeted by CRISPR-Cas9 (location of guide RNAs highlighted in red) (XM_024080404.1)

ATGCGCGGCTCCTGGGACGAGTCCACGACGGCGGCGCTGCACGCGCGCATCCTGGAGGCGCACCGCGGGT
CCGCCGCGCCCGACCGCGCCGAGCCCGCGTGCGAGCCTCCGCCGCTGACGCTGGGCGCGCTGGAGCTGGC
GGCGCCCACGCCGCTGCTGCCGCTGCCCACGCTGAGCTTCAGCGCCGCGCAGGTGGCCACCGTGTGCGAG
ACGCTGGAGGAGAGCGGCGACGTGGAGCGCCTGGCGCGCTTCTTGTGGTCGCTGCCCGTGGCGCACCCCA
ACGTGGCCGAGCTGGAGCGCTGCGAAGCCGTGCTGCGCGCGCGCGCCGTCGTCGCCTTCCACGCCGGCCG
CCACCGCGAGCTGTACGCCATCCTCGAGCGCCACCGCTTCCAGCGCTCCAGCCACGCCAAGCTGCAAGCG
CTGTGGCTGGAGGCGCACTACCAGGAGGCTGAGCGCCTGCGCGGCCGTCCGCTGGGCCCCGTCGACAAGT
ACCGCGTGCGGAAGAAGTTCCCGCTCCCGAGGACGATCTGGGACGGCGAGCAGAAGACGCACTGTTTCAA
GGAGCGGACGCGATCTCTACTCCGAGAATGGTACCTCCAAGATCCCTACCCGAACCCGACGAAGAAGAGG
GAATTGGCGGCGGCGACGGGTCTGACGCCGACGCAAGTCGGCAACTGGTTCAAAAACCGACGGCAAAGAG
ACCGAGCGGCCGCCGCCAAGAACCGCTCCGCCGTGCTGGGCAGAGGATAA

Region of *dpp* targeted by CRISPR-Cas9 (location of guide RNA highlighted in red) (XM_024080858.1)

GTTCTTCAACGTAAGCGGCGTACCGGCCGACGAGGTGGCGCGCGGCGCCGACCTCTCGTTCCAACGAGCC
GTCGGCACCACCGGCAGACAGAGACTGTTGTTGTACGACGTGGTGCGCCCTGGCCGCCGCGGCCACTCCG
AGCCGATCCTGCGGCTGCTGGACTCCGTTCCGCTCCGGCCCGGGGAGGGAATCGTCAACGCCGACGCTCT
GGGAGCGGCGCGACGGTGGCTCAAAGAGCCCAAACATAATCACGGACTATTAGTGCGAGTGTTAGAAGAA
GACGCCGCGAGTGCGAGCAGGGACGCGAAGTTCCCGCACGTGCGCGTGCGCAGACGCGTCACGGACGAGG
AGGAGGAGTGGCGGACGGCGCAGCCGCTGCTCATGCTGTACACGGAGGACGAGCGCGCGCGCGCGTCGCG
GGAGACGAGCGAGCGGCTGACGCGCAGCAAGCGCGCGGCGCAGCGGCGGGGGCACCGCGCGCACCACCGC
CGCAAGGAGGCGCGCGAGATCTGCCAGCGCCGCCCGCTGTTCGTCGACTTCGCGGACGTGGGCTGGAGCG
ACTGGATCGTGGCCCCGCACGGCTACGACGCGTACTACTGCCAGGGCGACTGCCCCTTCCCGCTGCCGGA
CCACCTCAACGGCACGAACCACGCGATAGTGCAGACTCTGGTCAACTCAGTGAACCCCGCGACGGTGCCC
AAAGCGTGCTGCGTGCCGACGCAACTCTCATCTATATCTATGTTATATATGGACGAAGTGAACAATGTGG
TGCTTAAAAACTATCAGGACATGATGGTGGTAGGCTGTGG