Supporting Information

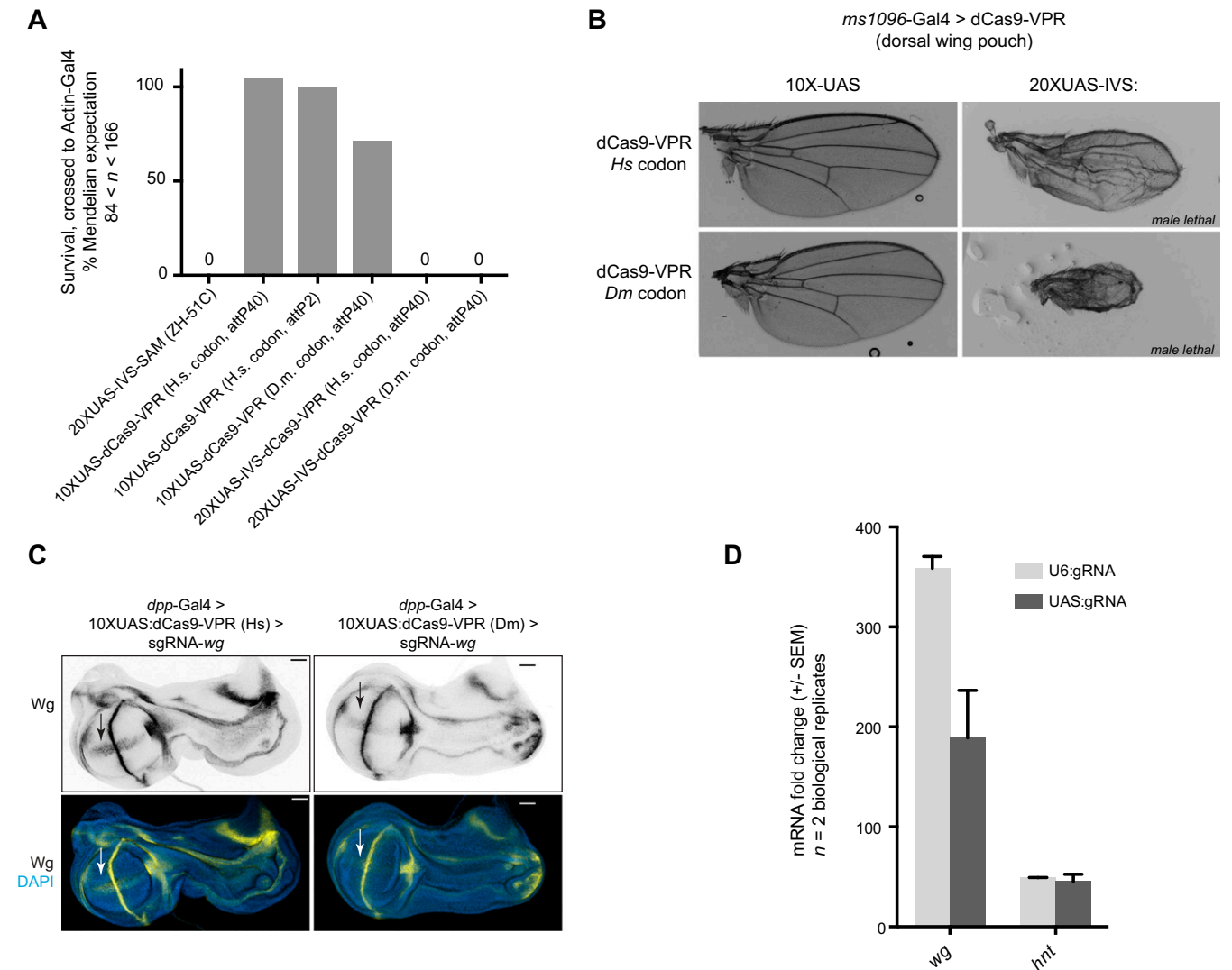## Ewen-Campen et al. 10.1073/pnas.1707635114

Fig. S1. Optimization of expression level and codon use of dCas9-VPR and the sgRNA promoter. (*A*) Ubiquitous expression of 10XUAS-SAM, and three different 10XUAS:dCas9-VPR constructs using *actin*-Gal4 is not lethal, whereas expression of 20XUAS-IVS:dCas9-VPR is 100% lethal regardless of codon use. Dm, *Drosophila melanogaster* codon use; Hs, human codon use. (*B*) Expression of 20XUAS-IVS:dCas9-VPR constructs in the wing (using the *ms1096*-Gal4 line) was highly toxic to wing tissue in the female and caused male lethality. (*C*) Human codon-optimized 10UAS:dCas9-VPR led to higher levels of activation of the target gene *wg* than did *Drosophila* codon use, consistent with previous reports (8). (*D*) Expression of sgRNAs from UAS promoters (in the pCFD6 vector) did not lead to a higher expression than U6-driven sgRNAs (in the pCFD4 vector) in *Drosophila* S2R$^+$ cells.
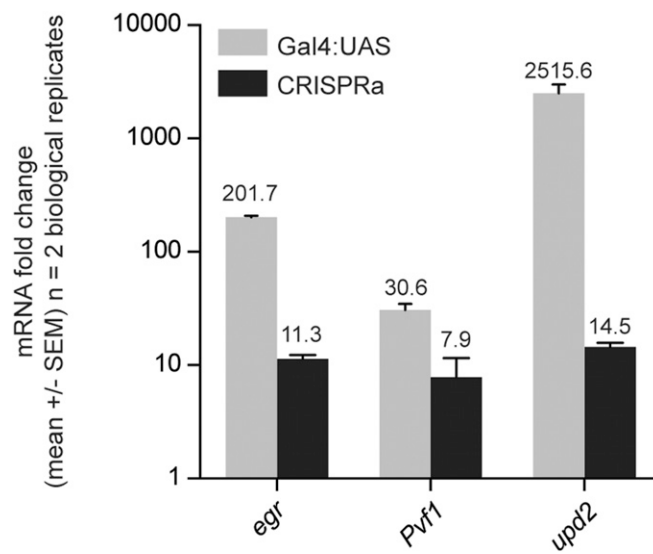
Fig. S2. Comparison of CRISPRa and Gal4-UAS-cDNA in vivo. Three target genes were expressed in the wing using the *nubbin*-Gal4 driver, and their activation level was measured using qPCR of dissected wing discs (~30 wing discs per biological replicate).

Table S1. sgRNA lines used in this study

Target name	FlyBase ID	sgRNA stock ID	sgRNA 1	sgRNA 2	Notes
spitz	FBgn0005672	GS00182	AGGGAGGATGACACTATCCGCAGG	TTAAGTCTGTAATGTTTAGGTGG	sgRNA 2 is equidistant to CG10364
gurken	FBgn0001137	GS00045	GAATGAAAATCGATCGGTGAGCGG	ATAATAGTCGAAGAATCATATGG	
vein	FBgn0003984	GS00144	AGCATTCACAACGTATCTCACGG	GTGCCGAAGATATTCGAACACGG	
Keren	FBgn0052179	GS00186	ATGAAATTAGTTATTAACATCGG	TTAGGCTTATCGATTATATTCGG	sgRNA 2 is equidistant to CG7484
argos	FBgn0004569	GS00057	CACTTCTCGCCGGCTTCAACGG	ATTTGTTCCCAGTGCATGAGCGG	*argos* is within intron of CG33158
PDGF- and VEGF-related factor 3	FBgn0085407	GS00022	CAGTCGCGGCAGATGGCATTGG	ACCAATCCGGCTGATCAGCCTGG	
PDGF- and VEGF-related factor 1	FBgn0030964	GS00032	GCTATTGTTATTGGCGACTGTGG	AGGCCAATGCTGCGACAGCTGGG	
PDGF- and VEGF-related factor 2	FBgn0031888	GS00162	TGTGGATCGCTTCTGTGAAACGG	TCTCTCACAGAGATTGGGAGAGG	
Trunk	FBgn0003751	GS00062	AGCCGAAAAAACTGCTCTCGATGG	CACTCAGTAGTTGTAATACGAGG	sgRNA 2 is >400 bp upstream
Jelly belly	FBgn0086677	GS00159	GTTGAAAACAAGTGGTTGAGCGG	TCACTTTCTCTCATCCCGTTAGG	
branchless	FBgn0014135	GS00107	AGTCTCTGACTAGCACGCTTGGG	GCAAAAAGACCCTGTCGATGAGG	
thisbe	FBgn0033652	GS00134	AGGAAGAATCAGGGAAGAGAGG	GGCCTGGCGGGTGGGCGGGGGGG	
pyramus	FBgn0033649	GS00085	CGGCGGGGGCGGTTCGAAGCGGG	CGCCGCCGACAATCCGAATGCGG	
unpaired 1	FBgn0004956	GS00169	GCGAGCTGAGCGGCCCTCGCCGG	TGCCGAGAGCGGTAGCCCATGG	
unpaired 2	FBgn0030904	GS00171	CAGCAAGCGATTGTGATAGTTGG	TGCTGATGCTGATCGCTCCACGG	
unpaired 3	FBgn0053542	GS00129	TGGAGTGGAGTGTTGTGGAGTGG	GGAGTTCAGTCAGTCTCCGCCGG	
decapentaplegic	FBgn0000490	GS00163	CGTCCAAAGCGGCCGAGGCAAGG	TCTGTGTTGACTGTGACTGTGG	sgRNA 2 is >400 bp upstream, equidistant to CR44912 (ncRNA)
glass bottom boat	FBgn0024234	GS00073	TTGTTAAATTTATCATAGTCCGG	CCGATTCATTTTCCAGTCGCGG	sgRNA1 is in 5'intron of YTR, sgRNA2 has U6 terminator
screw	FBgn0005590	GS00080	AGGTAGTTCAATTGCATCAGTGG	CCTGTACCTGACCAATGGGTTGG	*screw* is within intron of CG46244 and CG10443
myoglianin	FBgn0026199	GS00047	ATCGGATACATACGGCTCACCGG	ATATCTTATATAGTTTCTTCGGG	
dawdle	FBgn0031461	GS00051	GCGGCCACAAAAGCCTGGAGCGG	ATGGGTGTGAGTATGGGCCTGGG	
maverick	FBgn0039914	GS00018	TGTTAAGTAAGCATTTGCTGTGG	ACACACCCACCACATCTGCAGTGG	
activin-β	FBgn0024913	GS00028	AAAAAGTCGGCAGATTCAAATGG	AGATAAATCGTTAAGTTTTAGG	
hedgehog	FBgn0004644	GS00191	TATGCCACTGAGAGCCAGATTCGG	TTCCACTTCCCTTGCGCATAAGG	
Wnt oncogene analog 2	FBgn0004360	GS00120	AACACACGACCCTCACTCACTTGG	GCGAGGTTCAAGTGGTTAAAAGG	
Wnt oncogene analog 4	FBgn0010453	GS00048	CCGTGCCTGGCCGTCTTTTGGTGG	ATATTGTGACTGCGATCAATTGG	
Wnt oncogene analog 5	FBgn0010194	GS00148	AGTGAAAATGTGTATGGTGGTGG	GTTGCAGACGACGCAACGCGAGG	
Wnt oncogene analog 6	FBgn0031902	GS00049	TGCCTCTGCTGACCCTCGTTTTGG	ATCCGAGCGCTCAAAATCTGTGG	
wnt inhibitor of Dorsal aka Wnt8	FBgn0038134	GS00104	CGCCCTGGCTTGGGATTTGCAGG	GAGCAGGCCCGAAATTCCCACGG	
Wnt oncogene analog 10	FBgn0031903	GS00034	TTTAATTTGCATTTAGAGGCTGG	AGGACGAACGACGATGTCGATGG	
eiger	FBgn0033483	GS00111	GAGCAAGAGGGACAACGGATTGG	GCATCCTCGCCACGTGAGCGAGG	
spatzle	FBgn0003495	GS00152	GAGTCGAGTCGGCGGCCCTCGGG	TAAGCACTGAGCAATTGTTTGGG	
spatzle 3	FBgn0031959	GS00008	TTTAAGGCGCACGGCGAGAACGG	AAGAGCATTTGCTCTTCGATTGG	
spatzle 4	FBgn0032362	GS00016	CAAGTCCCACCGGCCAAACCGTCGG	AATGCACTCCAAGAAATTGGTGG	
spatzle 5	FBgn0035379	GS00130	TGCCTCTTTTCCCGAGTTTGCGG	GGCAGAGAAAAGCAGAGCTTGGG	
spatzle 6	FBgn0035056	GS00088	CTAGCCAGAGCATGAGAAAGTGG	CGGAAAGTATGCTTCTGTTTTGG	
QUAS (nontargeting control)	—	GS00089	CTCGGGTAATCGCTTATCCTCGG	CGGATAAACAATTATCCTCACGG	Equidistant to CR43009 (miRNA)
cut	FBgn0004198	GS00041	AAGCAACAACATAATGATTAATGG	AGTCGTTTGTTGAATGGTGAGGG	
hindsight aka peb	FBgn0003053	GS00052	GAGAGAAGAGAGAATGAGAAGG	ATTTGAAACGAAGAATGAGAAGG	
scute	FBgn0004170	GS00184	TTCGAGTTCCCTACCTGTGCAGG	TTACAAACCTGATCAATAGATGG	

Table S2. qPCR primers used in this study

Target	5′ primer	3′ primer
spi	TGCGGTGAAGATAGCCGATC	TTCGCATCGCTGTCCCATAA
grk	GTCGCCGTCACAGATTGTTG	GATTGAGCAACTCAACCGCG
vn	GAACGCAGAGGTCACGAAGA	GAGCGCACTATTAGCTCGGA
Krn	CCGCTTTAATCGGCGCTTAC	ATCGGGAAGGTGACATTCGG
aos	TGCTGTTGGGTGAATTTCAGG	CGACTGGTCCAGATGATCCA
Pvf3	TCGTGAAGAGCAGTAAGCATCG	AGGTGCAACTCAGTATGGTGG
Pvf1	CTGTCCGTGTCCGCTGAG	CTCGCCGGACACATCGTAG
Pvf2	GGTGGTCCACATCACGAGAG	CGACTTTGTCGCTGCATCTG
trk	CCAAAATTCTGGGACAGGCAT	AGATGATAGCTCTTCTCCTCGG
jeb	AAATCGAGTGTCCTACCGCC	CATCGCACAGCACATGATCG
bnl	AATGTCGCCCGCTGACAATA	TTGCTGATGGGCGTGTTACT
ths	CGTCCGCAACAACCATGAAG	CATTGCGCACATAGGTCAGC
pyr	GCAACGGATACCAAGTCCCA	TCTGGCTCGAACGCATTAGT
upd1	CAGCGCACGTGAAATAGCAT	CGAGTCCTGAGGTAAGGGGA
upd2	CGGAACATCACGATGAGCGAAT	TCGGCAGGAACTTGTACTCG
upd3	AGCCGGAGCGGTAACAAAA	CGAGTAAGATCAGTGACCAGTTC
dpp	TGGCGACTTTTCAAACGATTGT	CAGCGGAATATGAGCGGCAA
gbb	CATCGACGAGAGCGACATCA	TAGTTGTCGTTGGGCACGTT
scw	TTTCCAACGAGGATCGACAGG	CCAAACTCAAATCCACTGGCA
myo	ATGCTGCGGTTGGAGAAAATA	CGTGACATATCGAGTTACACGG
daw	ATCCTTCGTCCGCATCCTAAG	CGGTTCCAGGTGTTTCAGC
mav	ACGAGGGCCAGGATCTAGG	CGAGTTTCTTGGAAGCCAACAT
Actβ	TGTGGTTGTAAAGTGCTGTTGC	TTGTGGAAATGACTTCCGGGA
hh	GGATTCGATTGGGTCTCCTAC	GGGAACTGATCGACGAATCT
Wnt2	TTAGTGTCCAGCTTTACATCCGT	TCGCGGCACATATTTCGCT
Wnt4	CGGGAACATGAACAGCACGAT	TCACCGAGGGTACACTCGATG
Wnt5	ATATCTCGGCCTCAAGTCCCC	GTCATGTTCAGTGGTATGCTGG
Wnt6	AGACGATTCGCCCGACTTTT	CTGGTCACATTGCACTCCCT
wntD	TTTGCCATCACATTCTTCATGGG	GGGTGTACTGGTAGTAGCTCA
Wnt10	TGGAACTGTTCGTCGCTGAG	CAAACTGGAGGCATGCGGAT
egr	AGCTGATCCCCCTGGTTTTG	GCCAGATCGTTAGTGCGAGA
spz	GACACCTGGCAGTTAATTGTCA	CGAAGTCACAGGGTTGATCCG
spz3	GCCGCAATCGGGTGGTTAT	GCTGCGTCTGCTGTACCTG
spz4	CGCACCCAAATGGATAGGC	GCAATCGTCATTAGGATCAGCA
spz5	GGAAAGACGTACTGCGAGCA	AAATCTTCCCAGGTTTCGTCC
spz6	AAGTCTGCCGTGTCCGTTC	CGAGTACATTTTGTCCCAGCG
ct	TGAGGAGAACAAGGATGCGG	TTGTTGGCGCAATCATCGTC
hnt	ACATCCGGTGCCACAATTA	AGGGATGAAGCCGAGGATAGC
Rp49 (control)	ATCGGTTACGGATCGAACAA	GACAATCTCCTTGCGCTTCT
GAPDH (control)	CCAATGTCTCCGTTGTGGA	TCGGTGTAGCCCAGGATT

Table S3. Gal4 + dCas9-VPR toolkit collection

Line	Bloomington stock ID	Full genotype
UAS-dCas9-VPR (II)	66561	w; P{UAS-3XFLAG-dCas9-dCas9-VPR}attp40/CyO; MKRS/TM6B, Tb[1]
UAS-dCas9-VPR (III)	66562	w; Kr[lf-1[/CyO; P{UAS-3XFLAG-dCas9-dCas9-VPR}attp2/TM6b, Tb[1]
elav > dCas9-VPR	67038	P{w[+mW.hs]=GawB}elav[C155]; P{UAS-3XFLAG-dCas9-dCas9-VPR}attp40/CyO
MS1096 > dCas9-VPR	67039	w[1118] P{w[+mW.hs]=GawB}Bx[MS1096]; P{UAS-3XFLAG-dCas9-dCas9-VPR}attp40/CyO
pnr > dCas9-VPR	67040	w[*]; P{UAS-3XFLAG-dCas9-dCas9-VPR}attp40/CyO; P{w[+mW.hs]=GawB}pnr[MD237]/TM6B, Tb[1]
dMef2 > dCas9-VPR	67041	w[*]; P{UAS-3XFLAG-dCas9-dCas9-VPR}attp40/CyO; P{w[+mC]=GAL4-Mef2.R}R1/[TM6B, Tb[1]]
C96 > dCas9-VPR	67042	w[*]; P{UAS-3XFLAG-dCas9-dCas9-VPR}attp40/CyO; P{w[+mW.hs]=GawB}bbg[C96]/TM6B, Tb[1]
Lpp > dCas9-VPR	67043	w[*]; P{UAS-3XFLAG-dCas9-dCas9-VPR}attp40/CyO; P{Lpp-GAL4.B}/TM6B, Tb[1]
Mhc > dCas9-VPR	67044	w[*]; P{UAS-3XFLAG-dCas9-dCas9-VPR}attp40/CyO; P{[ry+]MHC-82-Gal4}/TM6B, Tb[1]
dpp > dCas9-VPR	67045	w[*]; P{UAS-3XFLAG-dCas9-dCas9-VPR}attp40/CyO; P{w[+mW.hs]=GAL4-dpp.blk1}/TM6B, Tb[1]
dpp > dCas9-VPR (SM5 balancer)	In progress	w[*]; P{UAS-3XFLAG-dCas9-dCas9-VPR}attp40; P{w[+mW.hs]=GAL4-dpp.blk1}/SM5; TM6B Tb[1]
hh > dCas9-VPR	67046	w[*]; P{UAS-3XFLAG-dCas9-dCas9-VPR}attp40/CyO; hh-Gal4/TM6B, Tb[1]
tubulin > dCas9-VPR	In progress	w[*]; P{UAS-3XFLAG-dCas9-dCas9-VPR}attp40/CyO; tub-Gal4/TM6B, Tb[1]
tubulin > dCas9-VPR (SM5 balancers)	67048	w[*]; P{UAS-3XFLAG-dCas9-dCas9-VPR}attp40; tub-Gal4/SM5; TM6B Tb[1]
en > dCas9-VPR + GFP	67049	w[*]; P{w[+mW.hs]=en2.4-GAL4}e16E, P{w[+mC]=UAS-2xEGFP}AH2/CyO; UASP{UAS-3XFLAG-dCas9-dCas9-VPR}attp2/TM6B, Tb[1]
spalt > dCas9-VPR	67050	w[*]; P{w[+mW.hs]=GawB}salm[LP39]/CyO; P{UAS-3XFLAG-dCas9-dCas9-VPR}attp2/TM6B, Tb[1]
blistered > dCas9-VPR	67051	w[*]; P{w[+mC]=bs-GAL4.Term}G1/CyO; UAS:P{UAS-3XFLAG-dCas9-dCas9-VPR}attp2/TM6B, Tb[1]
nos > dCas9-VPR	67052	w[*]; P{w[+mC]=GAL4-nos.NGT}40/CyO; P{UAS-3XFLAG-dCas9-dCas9-VPR}attp2/TM6B, Tb[1]
twist > dCas9-VPR	67053	w[*]; P{w[+mC]=GAL4-twi.2xPE}1/CyO; P{w[+mC]=UAS-3xFLAG.dCas9.VPR}attP2
esg > dCas9-VPR + GFP	67054	w[*]; esg-Gal4, P{ UAS-GFP}/CyO; P{UAS-3XFLAG-dCas9-dCas9-VPR}attp2/TM6B, Tb[1]
nubbin > dCas9-VPR	67055	w[*]; P{w[+mW.hs]=GawB}nubbin-AC-62/CyO; P{UAS-3XFLAG-dCas9-dCas9-VPR}attp2/TM6B, Tb[1]
nubbin > dCas9-VPR (SM5 balancers)	In progress	w[*]; P{w[+mW.hs]=GawB}nubbin-AC-62; P{UAS-3XFLAG-dCas9-dCas9-VPR}attp2/SM5; TM6b Tb[1]
Myo1A > dCas9-VPR	67057	w[*]; P{GawB}Myo31DFNP0001/CyO; P{UAS-3XFLAG-dCas9-dCas9-VPR}attp2/TM6B, Tb[1]
DE-Gal4 > dCas9-VPR	In progress	w[*]; P{UAS-3XFLAG-dCas9-dCas9-VPR}attp40; P{GawB}mirr[DE]/TM6b Tb[1]
GMR-Gal4 > dCas9-VPR	In progress	w[*]; "GMR-Gal4"/CyO; CyO; P{UAS-3XFLAG-dCas9-dCas9-VPR}attp2/TM6B, Tb[1]
elav[ts] > dCas9-VPR	67058	P{w[+mW.hs]=GawB}elav[C155]; P{UAS-3XFLAG-dCas9-dCas9-VPR}attp40, P{w[+mC]=tubP-GAL80[ts]}10]/CyO
MS 1096[ts] > dCas9-VPR	67059	P{w[+mW.hs]=GawB}Bx[MS1096]; P{UAS-3XFLAG-dCas9-dCas9-VPR}attp40, P{w[+mC]=tubP-GAL80[ts]}10]/CyO
pnr[ts] > dCas9-VPR	67060	w[*]; P{UAS-3XFLAG-dCas9-dCas9-VPR}attp40, P{w[+mC]=tubP-GAL80[ts]}10]/CyO; P{w[+mW.hs]=GawB}pnr[MD237]/TM6B, Tb[1]]
Lpp[ts] > dCas9-VPR	67061	w[*]; P{UAS-3XFLAG-dCas9-dCas9-VPR}attp40, P{w[+mC]=tubP-GAL80[ts]}10]/CyO; P{Lpp-GAL4.B}/TM6B, Tb[1]
Delta[ts] > dCas9-VPR	In progress	w[*]; P{UAS-3XFLAG-dCas9-dCas9-VPR}attp40/CyO; P{w[+mW.hs]=GawB}Dl[05151], P{w[+mC]=tubP-GAL80[ts]}[*]/TM6B, Tb[1]
Lpp[ts] > dCas9-VPR (SM5 balancer)	In progress	w[*]; P{UAS-3XFLAG-dCas9-dCas9-VPR}attp40, P{w[+mC]=tubP-GAL80[ts]}10]; P{Lpp-GAL4.B} SM5; TM6b Tb[1]
Mhc[ts] > dCas9-VPR	67062	w[*]; P{UAS-3XFLAG-dCas9-dCas9-VPR}attp40, P{w[+mC]=tubP-GAL80[ts]}10]/CyO; P{[ry+]MHC-82-Gal4}/TM6B, Tb[1]
dMef2[ts] > dCas9-VPR	67063	w[*]; P{UAS-3XFLAG-dCas9-dCas9-VPR}attp40, P{w[+mC]=tubP-GAL80[ts]}10]/CyO; P{w[+mC]=GAL4-Mef2.R}R1/(TM6B, Tb[1]
dMef2[ts] > dCas9-VPR (SM5 balancer)	In progress	w[*]; P{UAS-3XFLAG-dCas9-dCas9-VPR}attp40, P{w[+mC]=tubP-GAL80[ts]}10]; P{w[+mC]=GAL4-Mef2.R}R1/SM5; TM6b Tb[1]
C96[ts] > dCas9-VPR	67064	w[*]; P{UAS-3XFLAG-dCas9-dCas9-VPR}attp40, P{w[+mC]=tubP-GAL80[ts]}10]/CyO; P{w[+mW.hs]=GawB}bbg[C96]/TM6B, Tb[1]
tub[ts] > dCas9-VPR	67047	w[*]; P{UAS-3XFLAG-dCas9-dCas9-VPR}attp40, P{w[+mC]=tubP-GAL80[ts]}10]/CyO; tub-Gal4/TM6B, Tb[1]

Table S3. Cont.

Line	Bloomington stock ID	Full genotype
tub[ts] > dCas9-VPR (SM5 balancer)	In progress	w[*]; P{UAS-3XFLAG-dCas9-dCas9-VPR}attp40, P{w[+mC]=tubP-GAL80[ts]}10; tub-Gal4/SM5; TM6B, Tb[1]
dpp[ts] > dCas9-VPR	67066	w[*]; P{UAS-3XFLAG-dCas9-dCas9-VPR}attp40, P{w[+mC]=tubP-GAL80[ts]}10/CyO; P{w[+mW.hs]=GAL4-dpp.blk1}TM6B, Tb[1]
Myo1A[ts] > dCas9-VPR	67067	w[*]; P{GawB}Myo31DFNP0001/CyO; P{UAS-3XFLAG-dCas9-dCas9-VPR}attp2, P{w[+mC]=tubP-GAL80[ts]}2/TM6B, Tb[1]
en[ts] > dCas9-VPR + GFP	67069	w[*]; P{w[+mW.hs]=en2.4-GAL4}e16E, P{w[+mC]=UAS-2xEGFP}AH2/CyO; P{UAS-3XFLAG-dCas9-dCas9-VPR}attp2, P{w[+mC]=tubP-GAL80[ts]}2/TM6B, Tb[1]
spalt[ts] > dCas9-VPR	67070	w[*]; P{w[+mW.hs]=GawB}salm[LP39]/CyO; P{UAS-3XFLAG-dCas9-dCas9-VPR}attp2, P{w[+mC]=tubP-GAL80[ts]}2/TM6B, Tb[1]
blistered[ts] > dCas9-VPR	67071	w[*]; P{w[+mC]=bs-GAL4.Term}G1/CyO; P{UAS-3XFLAG-dCas9-dCas9-VPR}attp2, P{w[+mC]=tubP-GAL80[ts]}2/TM6B, Tb[1]
esg[ts] > dCas9-VPR + GFP	67072	w[*]; esg-Gal4,P{UAS-GFP}/CyO; P{UAS-3XFLAG-dCas9-dCas9-VPR}attp2, P{w[+mC]=tubP-GAL80[ts]}2/TM6B, Tb[1]
FLP-out Gal4 > dCas9-VPR	NA (N.P. laboratory stock)	hsFlp; Ay-Gal4, UAS:RFP-myr; UAS:dCas9-VPR/SM6, TM6b