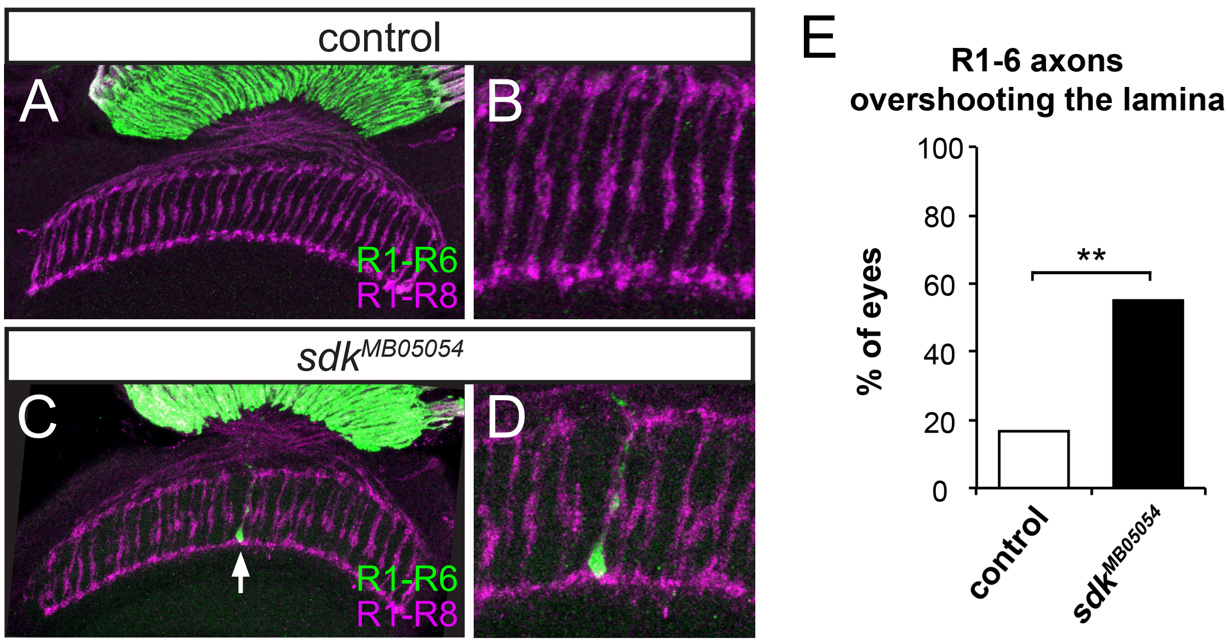

control
A
R1-R6
R1-R8
B
sdk^MB05054
C
R1-R6
R1-R8
D
E
R1-6 axons
overshooting the lamina
100
80
60
40
20
0
% of eyes
**
control
sdk^MB05054

Figure S1

Figure S1: Mistargeted R1-R6 axons are rare in *sdk* mutants. (A-D) show R1-R6 axons labeled with Rh1-lacZ (green) and all photoreceptor axons labeled with anti-Chp (magenta) in control $sdk^{MB05054}$/+ (A, B) or $sdk^{MB05054}$ mutant (C, D) adult head sections. A detail of the R7 and R8 target regions is shown in (B, D). The percentage of eyes in which at least one R1-R6 axon extends beyond the lamina is counted in (E). Control, 16.7%, n=36 eyes; $sdk^{MB05054}$, 55%, n=40 eyes. **p<0.001, Fisher's exact test.

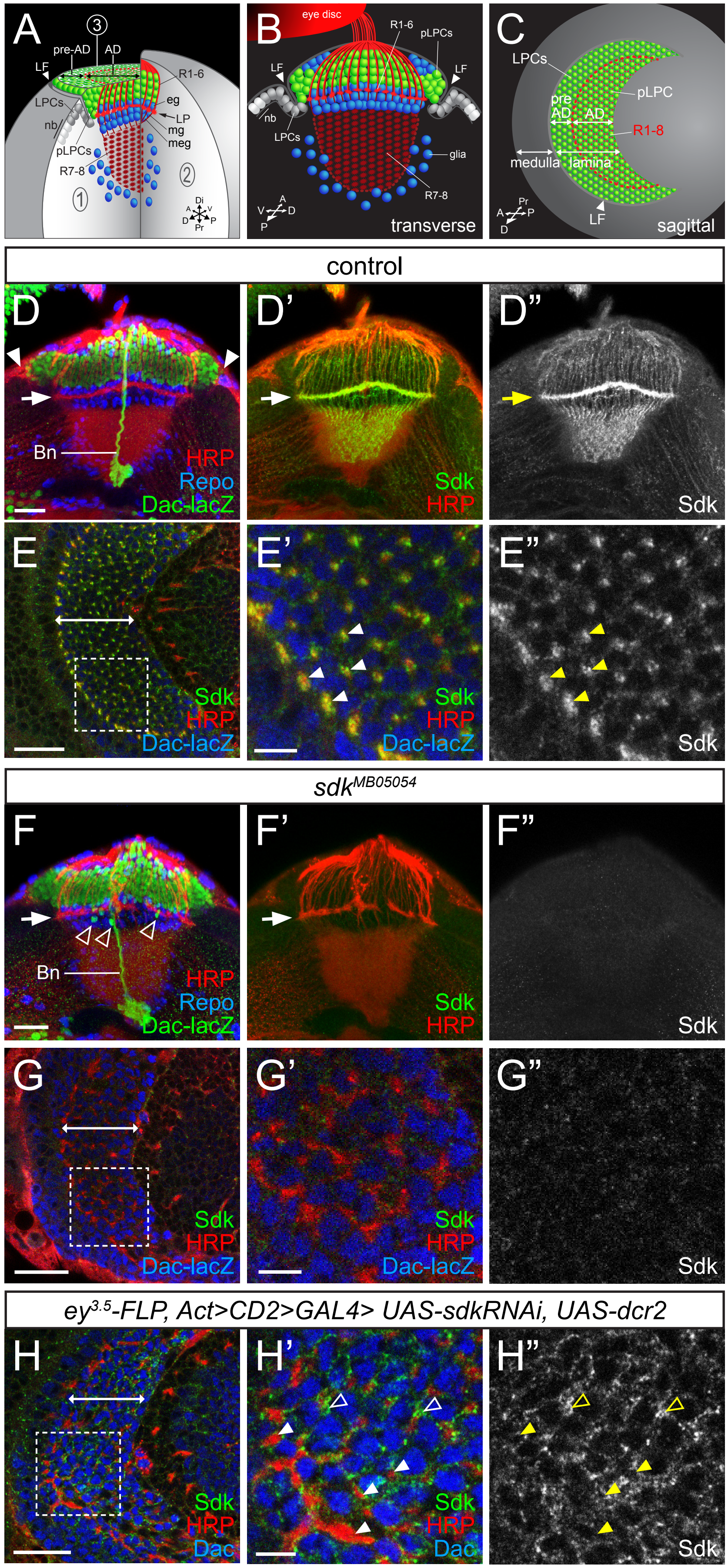

A
pre-AD
AD
LF
R1-6
LPCs
eg
LP
nb
mg
meg
pLPCs
R7-8
B
eye disc
R1-6
pLPCs
LF
nb
LPCs
glia
R7-8
transverse
C
LPCs
pLPC
pre AD
AD
R1-8
medulla
lamina
LF
sagittal
control
D
D'
D''
Bn
HRP
Repo
Dac-lacZ
Sdk
HRP
Sdk
E
E'
E''
Sdk
HRP
Dac-lacZ
Sdk
sdk^MB05054
F
F'
F''
Bn
HRP
Repo
Dac-lacZ
Sdk
HRP
Sdk
G
G'
G''
Sdk
HRP
Dac-lacZ
Sdk
ey^3.5-FLP, Act>CD2>GAL4> UAS-sdkRNAi, UAS-dcr2
H
H'
H''
Sdk
HRP
Dac
Sdk

Figure S2

Figure S2: Sdk expression and function in the larval brain. (A-C) The larval brain in transverse (B, 1 in A), coronal (2 in A), or sagittal (C, 3 in A) sections, labeled as in Fig. 2A. Neuroblasts (nb) give rise to LPCs that become postmitotic in the pre-assembly domain (pre-AD) and differentiate into LN in the assembly domain (AD). The cell bodies of epithelial (eg), marginal (mg), medulla (meg), medulla neuropil and satellite glia are shown in blue. (D, F) Transverse views of control $sdk^{MB05054}$/+ (D) or $sdk^{MB05054}$ mutant (F), labeled with anti-Sdk (D'', F'', green in D', F'), anti-HRP to label photoreceptor axons (red in D, D', F, F'), anti-Repo to label glia (blue in D, F) and anti-β-galactosidase reflecting *dac-lacZ* to label lamina neurons (green in D, F). Bolwig's nerve (Bn) is visible in flies bearing the *Minos* insertion. An arrow indicates the lamina plexus and arrowheads in (D) indicate the lamina furrow. Lamina neurons are seen below the lamina plexus in *sdk* mutants (empty arrowheads in F). (E, G, H) show the sagittal view in control $sdk^{MB05054}$/+ (E), $sdk^{MB05054}$ mutant (G), or *sdk* knockdown in the eye (H). Boxed areas are enlarged in (E', E'', G', G'', H', H''). Brains are labeled with anti-Sdk (E'', G'', H'', green in E, E', G, G', H, H'), anti-HRP (red in E, E', G, G', H, H') and anti-β-galactosidase reflecting *dac-lacZ* (blue in E, G) or Dac (blue in H). Double-headed arrow marks the assembly domain. In control animals, Sdk is present in both photoreceptor axons (arrowheads) and lamina neurons. Note that *sdk* RNAi expression in the eye (H) causes loss of Sdk labeling from photoreceptors (arrowheads), but it is still visible in lamina neurons (empty arrowheads). Scale bars, 20 μm (D, E, F, G, H) and 5 μm (E', G', H').

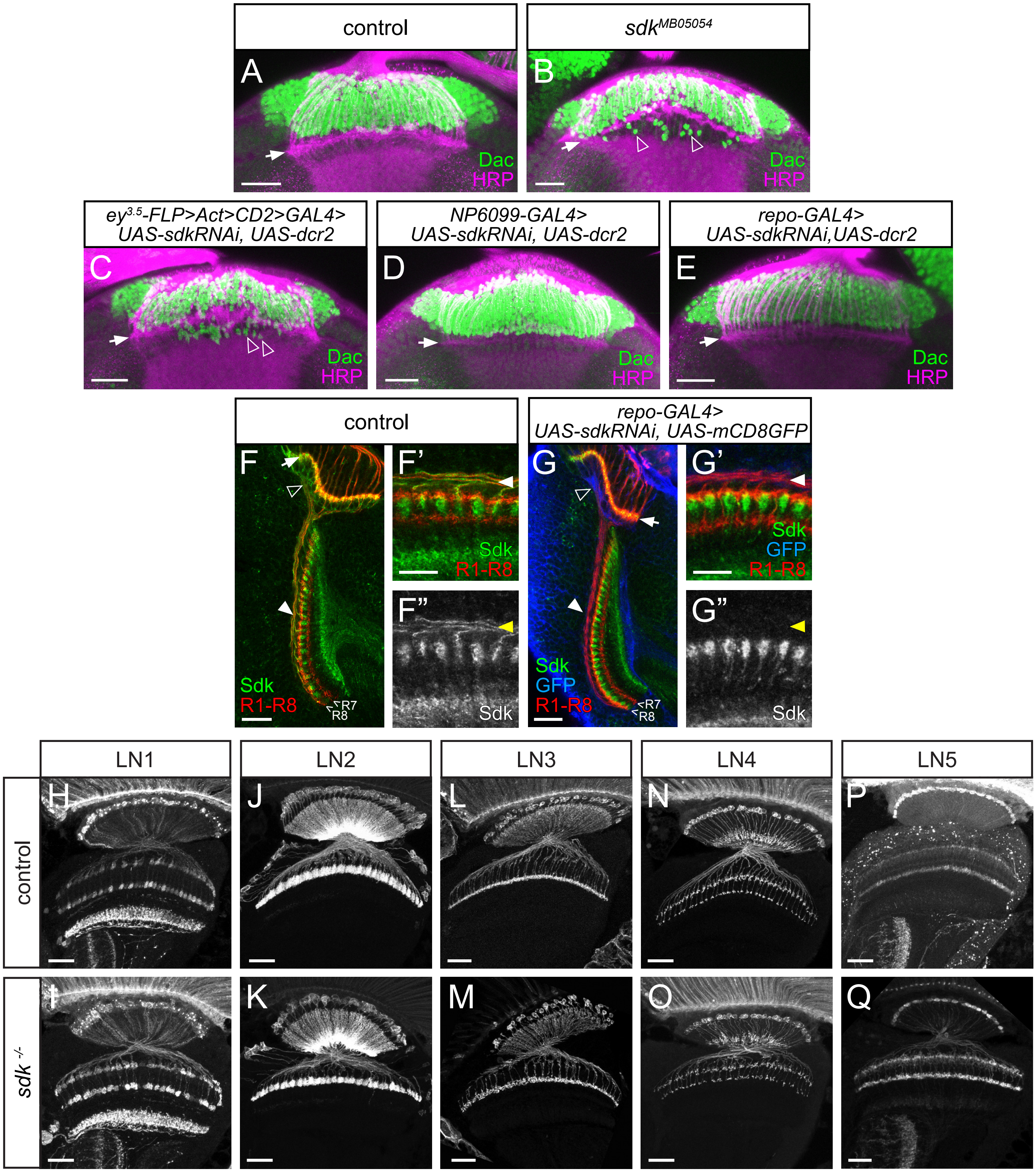


Figure S3

Development • Supplementary information

Figure S3: *sdk* acts in photoreceptors to affect lamina neuron placement. (A-E) Larval brains labeled with anti-Dac (green) and anti-HRP (magenta). (A) $sdk^{\Delta 7}$ (control); (B) $sdk^{MB05054}$; (C) *ey3.5-FLP, Act>CD2>GAL4* driving *UAS-sdk RNAi* in photoreceptors; (D) *NP6099-GAL4* driving *UAS-sdk RNAi* in lamina neurons; (E) *repo-GAL4* driving *UAS-sdk RNAi* in glia. Empty arrowheads indicate Dac-positive cells beneath the lamina plexus (arrow). (F, G) 24 h APF brains labeled with anti-Sdk (F'', G'', green in F, F', G, G'), anti-Chp to mark photoreceptor axons (red) and anti-GFP (blue). (F) control (w^-); (G) *repo-GAL4* driving *UAS-sdk RNAi* and *UAS-mCD8-GFP* in glia. Empty arrowheads indicate glial cell bodies below the lamina plexus (arrow) and filled arrowheads indicate glial processes wrapping the axons of R7 and R8. RNAi expression with *repo-GAL4* effectively removes Sdk from glía. (H-Q) Adult brains showing the five subtypes of lamina neurons labeled with GAL4 or LexA drivers and fluorescent reporters in control (H, J, L, N, P) and *sdk* mutant (I, K, M, O, Q) animals. (H, I) LN1; (J, K) LN2; (L, M) LN3; (N, O) LN4; (P, Q) LN5. Only occasional lamina neurons are missing in *sdk* mutants. Scale bars, 20 µm, except F' and G' (10 µm).

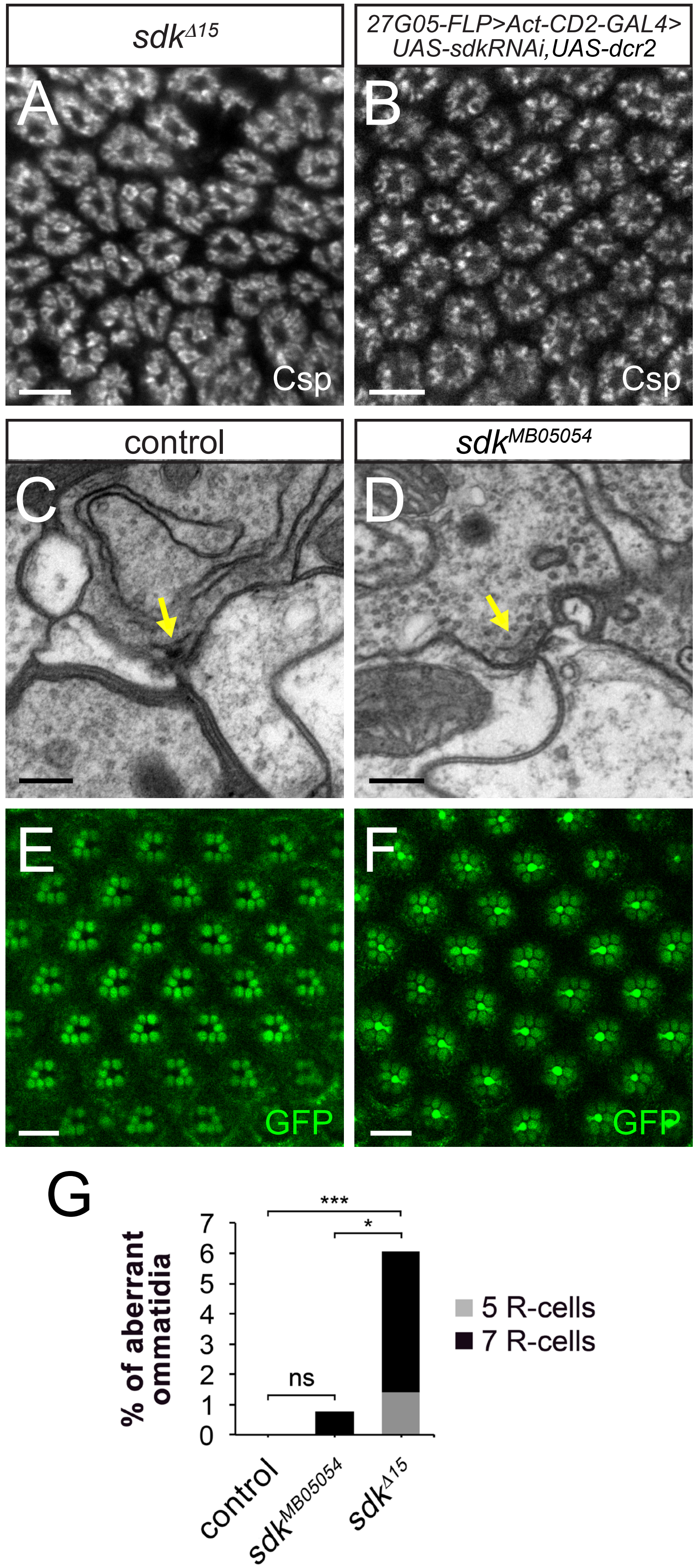

sdkΔ15
27G05-FLP>Act-CD2-GAL4>
UAS-sdkRNAi,UAS-dcr2
A
B
Csp
Csp
control
sdkMB05054
C
D
E
F
GFP
GFP
G
% of aberrant ommatidia
***
*
ns
7
6
5
4
3
2
1
0
5 R-cells
7 R-cells
control
sdkMB05054
sdkΔ15

Figure S4

Figure S4: Sdk is required for R1-R6 axon sorting but not to form tetrad synapses. (A-B) Anti-Csp labels R1-R6 in adult laminas from $sdk^{\Delta 15}$ mutants (A) and *27G05-FLP, Act>CD2>GAL4* driving *UAS-sdk RNAi* in lamina neurons (B). (C, D) tetrad synapses in which L1 and L2 neurons are postsynaptic to a single T-bar (arrows) in wild type controls (Meinertzhagen and O'Neil, 1991) (C) and $sdk^{MB05054}$ mutants (D). (E, F) Adult retinas in which R1-R6 are marked with Rh1-GFP. (E) $sdk^{\Delta 7}$ control; (F) $sdk^{MB05054}$; GFP from the *Minos* element is also visible in R7. Only a few ommatidia contain extra or missing photoreceptors (quantified in (G)). n=258 ommatidia, 4 retinas ($sdk^{\Delta 7}$), n=262 ommatidia, 4 retinas ($sdk^{MB05054}$), n=215 ommatidia, 3 retinas ($sdk^{\Delta 15}$). *p<0.01, ***p<0.0001, Fisher's exact test. $sdk^{MB05054}$ is not significantly different from the control. Scale bars, 5 μm (A, B), 200 nm (C, D), 10 μm (E, F).

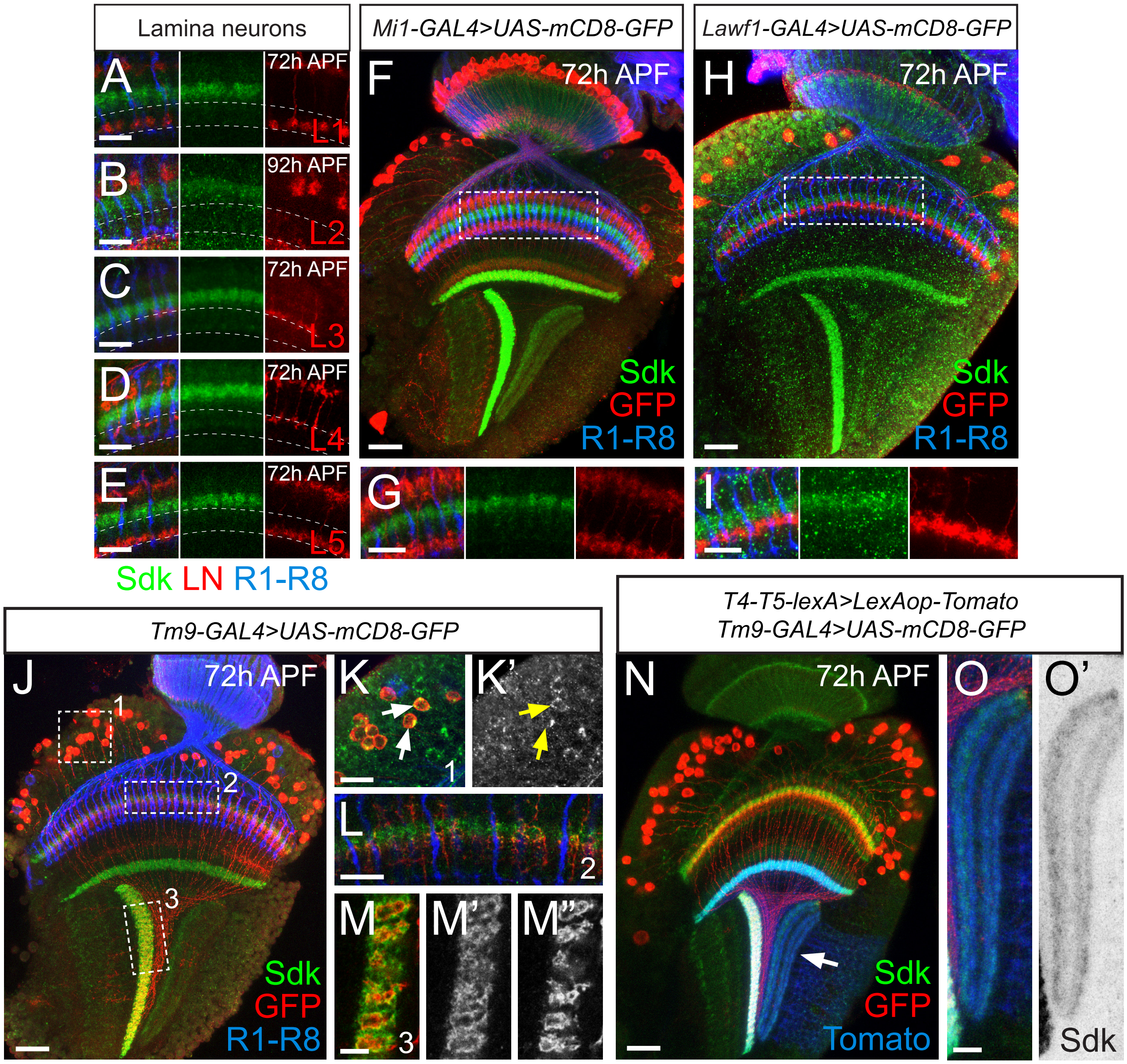
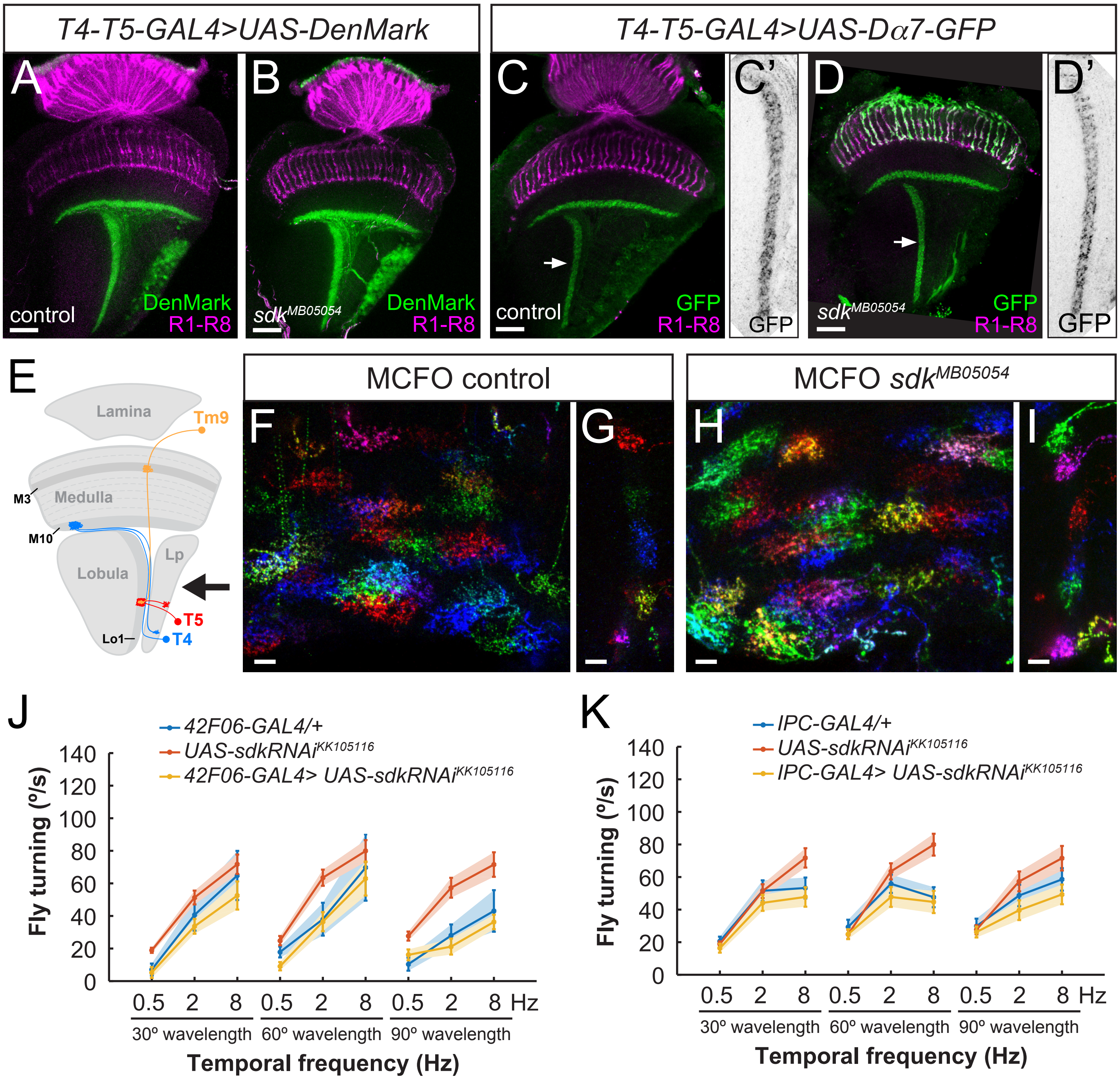

Lamina neurons
Mi1-GAL4>UAS-mCD8-GFP
Lawf1-GAL4>UAS-mCD8-GFP
A
72h APF
L1
B
92h APF
L2
C
72h APF
L3
D
72h APF
L4
E
72h APF
L5
Sdk LN R1-R8
F
72h APF
Sdk
GFP
R1-R8
G
H
72h APF
Sdk
GFP
R1-R8
I
Tm9-GAL4>UAS-mCD8-GFP
T4-T5-lexA>LexAop-Tomato
Tm9-GAL4>UAS-mCD8-GFP
J
72h APF
1
2
3
Sdk
GFP
R1-R8
K
K'
L
M
M'
M''
N
72h APF
Sdk
GFP
Tomato
O
O'
Sdk

Figure S5

Figure S5: Sdk expression in lamina and medulla neurons. All panels are labeled with anti-Sdk (green) and anti-GFP (red). (A-M) show anti-Chp in blue. mCD8-GFP (A, C-O) or mTomato (B) expression is driven in L1 with *C202a-GAL4* (A), L2 with *GMR48E07-GAL4* (B), L3 with *GMR14B07-GAL4* (C), L4 with *31C06-GAL4* (D), L5 with *6-60-GAL4* (E), Mi1 and L5 with *bsh-GAL4* (F, G), Lawf1 with *GMR52H01-AD/+; GMR17C1-DBD* (H, I), or Tm9 with *GMR24C08-GAL4* (J-O). In (N, O), T4/5-LexA is used to drive mTomato (blue). (B) is 92h APF and the other panels are 72h APF. (A-E, G, I, L) Enlargements of medulla layer M3a. The growth cones of lamina neurons and Lawf1 and the dendrites of Mi1 do not colocalize with Sdk to this layer. Sdk is present in the cell bodies (arrows in K), dendrites (L) and axon terminals (M) of Tm9. (J, K, N, O) show maximum intensity projections of confocal stacks, while (L, M) show single confocal sections corresponding to the regions boxed in (J). (O) is an enlargement of the T4/T5 axon terminal layers in the lobula plate, showing Sdk labeling (O', green in O) of all four layers. Scale bars, 10 μm (A-E, G, I, K, L), 20 μm (F, H, J, N), and 5 μm (M, O).



T4-T5-GAL4>UAS-DenMark
T4-T5-GAL4>UAS-Dα7-GFP
A
B
C
C'
D
D'
control
DenMark
R1-R8
sdkMB05054
GFP
E
Lamina
Tm9
M3
Medulla
M10
Lobula
Lp
T5
T4
Lo1
MCFO control
MCFO sdkMB05054
F
G
H
I
J
42F06-GAL4/+
UAS-sdkRNAiKK105116
42F06-GAL4> UAS-sdkRNAiKK105116
Fly turning (º/s)
140
120
100
80
60
40
20
0
0.5
2
8
Hz
30º wavelength
60º wavelength
90º wavelength
Temporal frequency (Hz)
K
IPC-GAL4/+
UAS-sdkRNAiKK105116
IPC-GAL4> UAS-sdkRNAiKK105116

Figure S6

Figure S6: *sdk* is not required for T4 or T5 to arborize in the correct layers, accumulate postsynaptic receptors, or mediate motion vision. (A, B) The label DenMark (green) was driven in T4 and T5 with *GMR42F06-GAL4* in wildtype (A) or $sdk^{MB05054}$ (B) to mark their dendritic arbors. (C, D) The GFP-tagged Dα7 AChR subunit was expressed in T4 and T5 (C', D', green in C, D) in control (C) and $sdk^{MB05054}$ (D). Photoreceptors are labeled with anti-Chp (magenta in A-D) and in (D), express GFP from the P3-GFP marker in the $sdk^{MB05054}$ Minos element. The Lo1 layer (arrow) is enlarged in (C', D'). (E-I) The multi-color FLP-out system in combination with *GMR42F06-GAL4* was used to label the dendrites of individual T5 cells with anti-HA (red), anti-V5 (green) and anti-FLAG (blue) in $sdk^{\Delta 7}$ controls (F, G) and $sdk^{MB05054}$ mutants (H, I). (F, H) show views of the Lo1 layer from the direction indicated by the arrow in the diagram in (E), and (G, I) show the Lo1 layer in the same orientation as (E). (J, K) Behavioral optomotor responses to square wave contrast gratings were measured as in Figure 4G, H. *sdk RNAi* was expressed in T4 and T5 neurons with GMR*42F06-GAL4* (J) or *IPC-GAL4* (K). We observed no significant differences ($p<0.05$) between experimental genotype and control genotypes, as measured by a rank sum test, Bonferroni corrected for 9 comparisons for each genotype. To be considered significant, the experimental genotype must be different from both genotype controls. n=7, *42F06-GAL4/+;* n=28, *UAS-sdkRNAi/+;* n=9, *42F06-GAL4 X UAS-sdk RNAi*; n=19, *IPC-GAL4/+;* n=26, *IPC-GAL4 X UAS-sdkRNAi*. Scale bars, 20 μm (A-D) and 5 μm (F-I).

Figure	Panel	Stage	Genotype
1	E, F	Larva	*yw, FRT19A/ Y; ey$^{3.5}$-FLP, UAS-lacZ*
	G	Larva	*ey-FLP, ubi-GFP, FRT19A/ Y*
	H	Larva	*ey-FLP, ubi-GFP, FRT19A/ sdk^{MB05054}, FRT19A*
2	B	Larva	*sdk^{MB05054}/ w; dac-lacZ* (heterozygous female)
	C	Larva	*sdk^{MB05054}/ Y; dac-lacZ* (hemizygous male)
	D	Larva	*ey$^{3.5}$-FLP/ w; Act>CD2>GAL4, UAS-lacZ/ UAS-sdkRNAi$^{P\{KK105116\}VIE-260B}$; UAS-dcr2*
	E	Larva	*NP6099-GAL4/ w; UAS-sdkRNAi$^{P\{KK105116\}VIE-260B}$/ UAS-NLS-GFP; UAS-dcr2*
	F	Larva	See Fig. S3
3	A,D	Adult	*sdk$^{\Delta 7}$*
	B,E	Adult	*sdk^{MB05054}*
	C,F	Adult	*ey$^{3.5}$-FLP/ w; Act>CD2>GAL4, UAS-lacZ/ UAS-sdkRNAi$^{P\{KK105116\}VIE-260B}$; UAS-dcr2*
	G	Adult	*sdk$^{\Delta 7}$* *sdk^{MB05054}* *ey$^{3.5}$-FLP/ w; Act>CD2>GAL4, UAS-lacZ/ UAS-sdkRNAi$^{P\{KK105116\}VIE-260B}$; UAS-dcr2* *Act>CD2>GAL4/ w; 27G05-FLP, UAS-lacZ/ UAS-sdkRNAi$^{P\{KK105116\}VIE-260B}$; UAS-dcr2*
	H	Adult	*FRT19, tub-GAL80, ey-FLP/FRT19,sdk^{MB05054}; lGMR-GAL4/UAS-lacZ*
4	A	30h APF	*sdk$^{\Delta 7}$; GH146-GAL4/UAS-myr-tdTomato*
	B	38h APF	*sdk$^{\Delta 7}$; UAS-myr-td-Tomato/+; E(spl)mδ-GAL4/+*
	C	40h APF	*sdk^{MB05054}; UAS-myr-td-Tomato/+; E(spl)mδ-GAL4/+*
	D	40 h APF	*UAS-myr-td-Tomato/ UAS-sdkRNAi$^{P\{KK105116\}VIE-260B}$; E(spl)mδ-GAL4/+*
	E	40 h APF	*sdk$^{\Delta 7}$; UAS-myr-td-Tomato/+; E(spl)mδ-GAL4/+* *UAS-myr-td-Tomato/ UAS-sdkRNAi$^{P\{KK105116\}VIE-260B}$; E(spl)mδ-GAL4/+*
	G	Adult	*sdk$^{\Delta 7}$; sdk^{MB05054}*
	H	Adult	*ey3.5FLP, Act>CD2>GAL4/ y,w; UAS-sdkRNAi$^{P\{KK105116\}VIE-260B}$ / +; UAS-dcr2/+* *Act>CD2>GAL4/ y,w; UAS-sdkRNAi$^{P\{KK105116\}VIE-260B}$/ +; UAS-dcr2/+* *ey3.5FLP, Act>CD2>GAL4/ y,w; attP^{60100}/ +; UAS-dcr2/+*
5	A-C	24, 42, 55h APF	*w*
	D	72h APF	*48E07-GAL4, UAS-mTomato*
	F	adult	*NP3507-GAL4; hs-FLP; UAS-FlyBow1.1*
	G	55h APF	*UAS-sdkRNAi$^{P\{KK105116\}VIE-260B}$; GMR42F06-GAL4/ UAS-dcr2*
	H	Adult	*sdk$^{\Delta 7}$/Y; LexAop-myr-tdTomato/GMR24C08-GAL4; UAS-FLP, BRP-FRT-stop-FRT V5-2A-LexA/+*
	I	Adult	*sdk^{MB05054}/Y; LexAop-myr-tdTomato/ GMR24C08-GAL4; UAS-FLP, BRP-FRT-stop-FRT, V5-2A-LexA/ +*
	K	Adult	*sdk$^{\Delta 7}$/Y; GMR42F06-LexA, UAS-Syb-GFP1-10; GMR24C08-GAL4, LexAop-CD4::spGFP11*
	L	Adult	*sdk^{MB05054}/Y; GMR42F06-LexA, UAS-Syb-GFP1-10; GMR24C08-GAL4, LexAop-CD4::spGFP11*
Suppl. Figures			
S1	A	Adult	*sdk^{MB05054}/+;; Rh1-lacZ*
	B	Adult	*sdk^{MB05054};; Rh1-lacZ*
S2	D, E	Larva	*sdk^{MB05054}/ w; dac-lacZ* (heterozygous female)
	F, G	Larva	*sdk^{MB05054}/ Y; dac-lacZ* (hemizygous male)
	H	Larva	*ey$^{3.5}$-FLP/ w; Act>CD2>GAL4, UAS-lacZ/ UAS-sdkRNAi$^{P\{KK105116\}VIE-260B}$; UAS-dcr2*

S3	A	Larva	*sdk*$^{\Delta 7}$
	B	Larva	*sdk*^{MB05054}
	C	Larva	*ey*$^{3.5}$*-FLP/ w; Act>CD2>GAL4, UAS-lacZ/ UAS-sdkRNAi*$^{P\{KK105116\}VIE\text{-}260B}$*; UAS-dcr2*
	D	Larva	*NP6099-GAL4/ w; UAS-sdkRNAi*$^{P\{KK105116\}VIE\text{-}260B}$*; UAS-dcr2*
	E	Larva	*UAS-sdkRNAi*$^{P\{KK105116\}VIE\text{-}260B}$*; repo-GAL4/ UAS-dcr2*
	F	24 h APF	*w*^{1118}
	G	24 h APF	*UAS- sdkRNAi*$^{P\{KK105116\}VIE\text{-}260}$*, UAS-mCD8GFP; repo-GAL4/UAS-dcr2*
	H	Adult	*sdk*^{MB05054}*/+; c202a-GAL4/UAS-myrRFP/+*
	I	Adult	*sdk*^{MB05054}*/Y; c202a-GAL4/UAS-myrRFP/+*
	J	Adult	*sdk*^{MB05054}*/+; +/+; GMR48E07-GAL4, UAS-myrTomato/+*
	K	Adult	*sdk*^{MB05054}*/Y; +/+; GMR48E07-GAL4, UAS-myrTomato/+*
	L	Adult	*sdk*$^{\Delta 15}$*/+; GMR22E09-LexA, LexAop-myrTomato/+; +/+*
	M	Adult	*sdk*$^{\Delta 15}$*/Y; GMR22E09-LexA, LexAop-myrTomato/+; +/+*
	N	Adult	*sdk*^{MB05054}*/+; UAS-mCD8GFP/+; GMR31C06-GAL4/+*
	O	Adult	*sdk*^{MB05054}*/Y; UAS-mCD8GFP/+; GMR31C06-GAL4/+*
	P	Adult	*sdk*$^{\Delta 15}$*/+; UAS-myrTomato/+; 6-60-GAL4/+*
	Q	Adult	*sdk*$^{\Delta 15}$*/Y; UAS-myrTomato/+; 6-60-GAL4/+*
S4	A	Adult	*sdk*$^{\Delta 15}$
	B	Adult	*Act>CD2>GAL4/ w; 27G05-FLP, UAS-lacZ/ UAS-sdkRNAi*$^{P\{KK105116\}VIE\text{-}260B}$*; UAS-dcr2*
	C	Adult	Canton S
	D	Adult	*sdk*^{MB05054}
	E	Adult	*sdk*$^{\Delta 7}$*/Y; Rh1-GFP/+*
	F	Adult	*sdk*^{MB05054}*/Y; Rh1-GFP/+*
S5	A	72h APF	*c202a-GAL4; UAS-mCD8GFP*
	B	92h APF	*GMR48E07-GAL4, UAS-myrTomato*
	C	72h APF	*GMR14B07-GAL4/UAS-mCD8GFP*
	D	72h APF	*UAS-mCD8GFP/ +; GMR31C06-GAL4/ +*
	E	72h APF	*6-60-GAL4, UAS-Syb-GFP/ UAS-mCD8-GFP*
	F, G	72h APF	*UAS-mCD8GFP/+; bsh-GAL4/+*
	H, I	72h APF	*GMR52H01-AD/+; GMR17C1-DBD/UAS-mCD8GFP*
	J-M	72h APF	*UAS-mCD8-GFP/GMR24C08-GAL4*
	N-O	72h APF	*UAS-mCD8GFP/+; GMR42F06-LexA/LexAop-myrTomato; GMR24C08-GAL4/+*
S6	A	Adult	*UAS-mCD8-GFP/UAS-DenMark; GMR42F06-GAL4*
	B	Adult	*sdk*^{MB05054}*; UAS-mCD8-GFP/UAS-DenMark; 42F06-GAL4*
	C	Adult	*sdk*$^{\Delta 7}$*/Y;; GMR42F06-GAL4/UAS-D*α*7-GFP*
	D	Adult	*sdk*^{MB05054}*/Y;; GMR42F06-GAL4/UAS-D*α*7-GFP*
	F, G	Adult	*sdk*$^{\Delta 7}$*, hs-FLP-PEST/Y; MCFO UAS-HA-V5-FLAG/ GMR42F06-GAL4*
	H, I	Adult	*sdk*^{MB05054}*, hs-FLP-PEST/Y; MCFO UAS-HA-V5-FLAG/ GMR42F06-GAL4*
	J	Adult	*w*$^{+}$*/ y,w; UAS-sdkRNAi*$^{P\{KK105116\}VIE\text{-}260B}$*/ +; GMR42F06-GAL4, UAS-dcr2/+*
			w^{+}*/ y,w; attP*^{60100}*; GMR42F06-GAL4, UAS-dcr2*
			w^{+}*/ y,w; UAS-sdkRNAi*$^{P\{KK105116\}VIE\text{-}260B}$*/ +; UAS-dcr2/+*
	K	Adult	*w*$^{+}$*/ y,w; IPC-GAL4/attP*^{60100}*; UAS-dcr2/+*
			w^{+}*/ y,w; UAS-sdkRNAi*$^{P\{KK105116\}VIE\text{-}260B}$*/ IPC-GAL4; UAS-dcr2/+*
			w^{+}*/ y,w; UAS-sdkRNAi*$^{P\{KK105116\}VIE\text{-}260B}$ *V +; UAS-dcr2/+*

Table S1: Genotypes for the experiments shown in each figure.