

A

	1	2	3	4
DM14-1	100.0	23.33	43.33	21.67
DM14-2	23.33	100.0	21.67	33.33
DM14-3	43.33	21.67	100.0	21.67
DM14-4	21.67	33.33	21.67	100.0

B

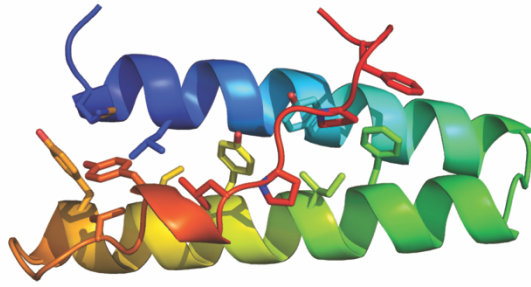
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          *   *
DM14-1  139 VDTLSIIKQRLEMYKQAEANAKTAGDSGKARRFGRGLKTLKDLHRQAAAGKSINVDDIPP198
DM14-2  257 NPLVTQMRSRQTDYKAAALQSKRSGDISTALQFLKVVKQFDVVIKMCEDGQEVDLSDMPP316
DM14-3  359 TNMLEALQQRLEKYQSVEAAAKAENNSGKARRFGRIVKQYEDAIKLYKAGKPVPYDELPV418
DM14-4  496 EQQMLLLLERQKEFKLAAIEAKKAGEIDQAKEYLKIFKGFDSLLNAASSGLPVDLSTLPV555

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Figure S1. Analysis of DM14 sequences from Lgd. Related to Figure 1. (A) Percent sequence identity among DM14 repeats. (B) primary sequence alignment of the four DM14 repeats from Lgd. Repeats 1 and 3, but not 2 or 4, contain basic residues (blue) at the starred positions.

A



B

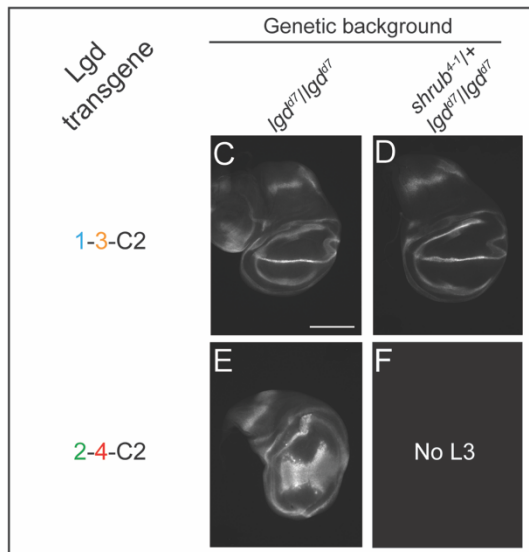
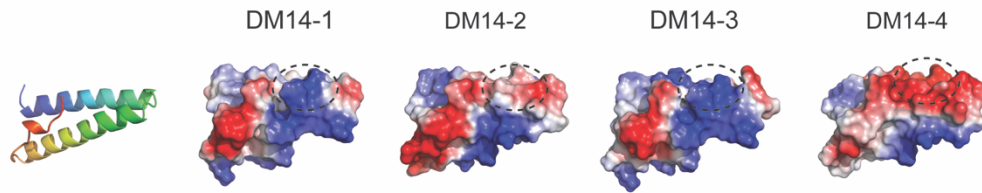


Figure S2. Comparison among DM14 domains. Related to Figures 1 and 2. (A) Packing between the C-terminal tail and the helical hairpin of DM14-3. (B) Homology models based on the structure of the third DM14 repeat, colored by electrostatic potential on a sliding scale from negative (red) to positive (blue). Circled areas highlight starred residues in the alignment of Figure S1. (C-F) The indicated constructs of Lgd were transgenically expressed in *lga* null and *lga* null/*shrub* heterozygous genetic backgrounds. The fly imaginal discs were stained for the Notch target gene, *wingless* (*wg*), as a surrogate for MVB dysfunction. Lethality in early L3 stage of development was observed in panel (F). Scale bar: 200 μ M.

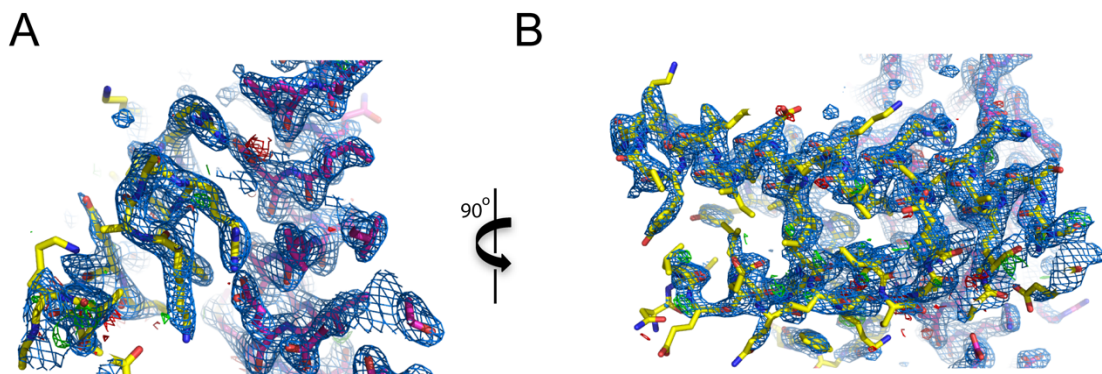


Figure S3. Lgd-Shrub complex electron density. Related to Figure 3. (A) 2F_o-F_c electron density map (blue mesh) contoured at 1.5σ, showing electron density at the Lgd-Shrub interface. Lgd is in yellow, and Shrub is in magenta. An F_o-F_c map contoured at ± 3σ (green mesh, positive density and red mesh, negative density) is also shown. Excess density is observed around the first Lgd helix, consistent with the presence of a populated alternative conformation. (B) Zoomed in view on the Lgd helices, oriented after a 90-degree rotation from the view shown in panel (A).

Table S1. Summary of Biacore binding analysis. Related to Figures 1 and 4..

Figure	1O	1O	1O	4A	4A	4A
<i>Ligand (Shrub)</i>	6-106	6-106	6-106	6-106	6-106	6-106 E86R
<i>Analyte (LGD)</i>	359-423	359-560	496-560	359-423	359-423 R393A	359-423
<i>Best-fit values</i>						
Bmax	96.88	244.20	ND	104.50	ND	ND
Kd (micromolar)	2.96	1.57	ND	2.81	ND	ND
Non-specific	0.34	1.20	ND	0.40	ND	ND
<i>Std. Error</i>						
Bmax	1.55	8.89	ND	2.80	ND	ND
Kd	0.10	0.15	ND	0.17	ND	ND
Non-specific	0.03	0.24	ND	0.06	ND	ND
<i>Goodness of Fit</i>						
Degrees of Freedom	6	ND	ND	6	ND	ND
R square	0.9998	ND	ND	0.9995	ND	ND
Absolute Sum of Squares	2.2890	ND	ND	8.0910	ND	ND
Sy.x	0.6176	ND	ND	1.1610	ND	ND
<i>Number of points</i>						
# of X values	9	9	9	9	9	9
# Y values analyzed	9	9	9	9	9	9