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Supplemental information

A pair of commissural command neurons induces *Drosophila* wing grooming

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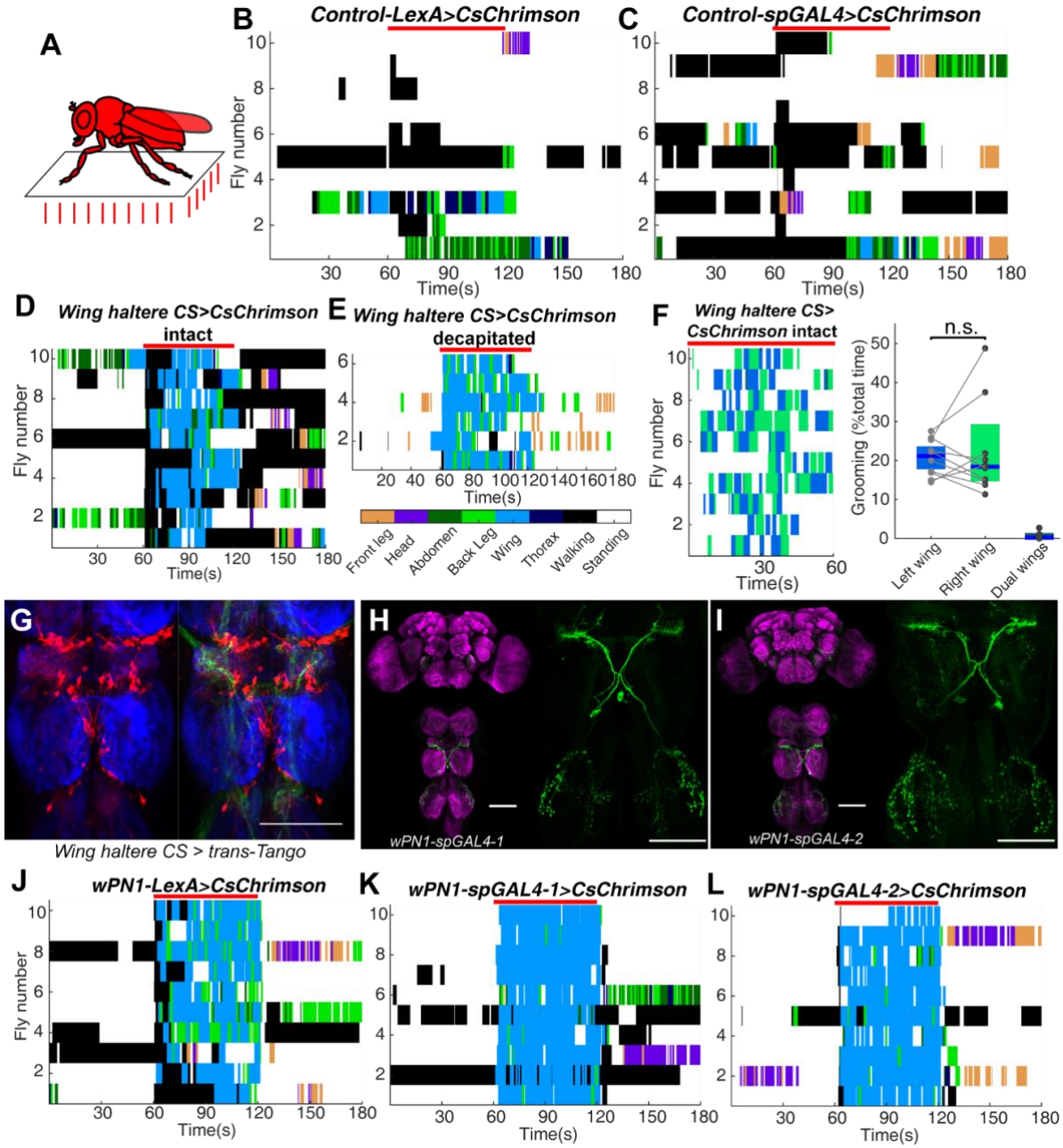

Figure S1. Wing grooming can be induced by optogenetic activation of wing campaniform sensilla and wPN1. Related to Fig. 1.

(**A**) Experiment setup for optogenetic activation. Free-moving flies were activated by red light delivered from below. (**B-E**) Grooming response induced by optogenetic activation of control lines (**B**, **C**) and the driver line targeting wing campaniform sensilla (**D**, **E**). 60s-optogenetic stimulation is indicated by a red line. (**F**) Quantification of different types of wing grooming during wing campaniform sensilla activation; note that the same videos were used in **D**. (**G**) Expression of *trans*-Tango in campaniform sensilla reveals candidate post-synaptic neurons (red). Green: anti-GFP. Red: anti-HA. Blue: anti-Bruchpilot. Scale bars, 100μm. (**H, I**) Expression pattern of *wPN1-spGAL4-1* (**H**) and *wPN1-spGAL4-2* (**I**) in CNS. (**J**-**L**) Grooming response induced by optogenetic activation of driver lines targeting wPN1.

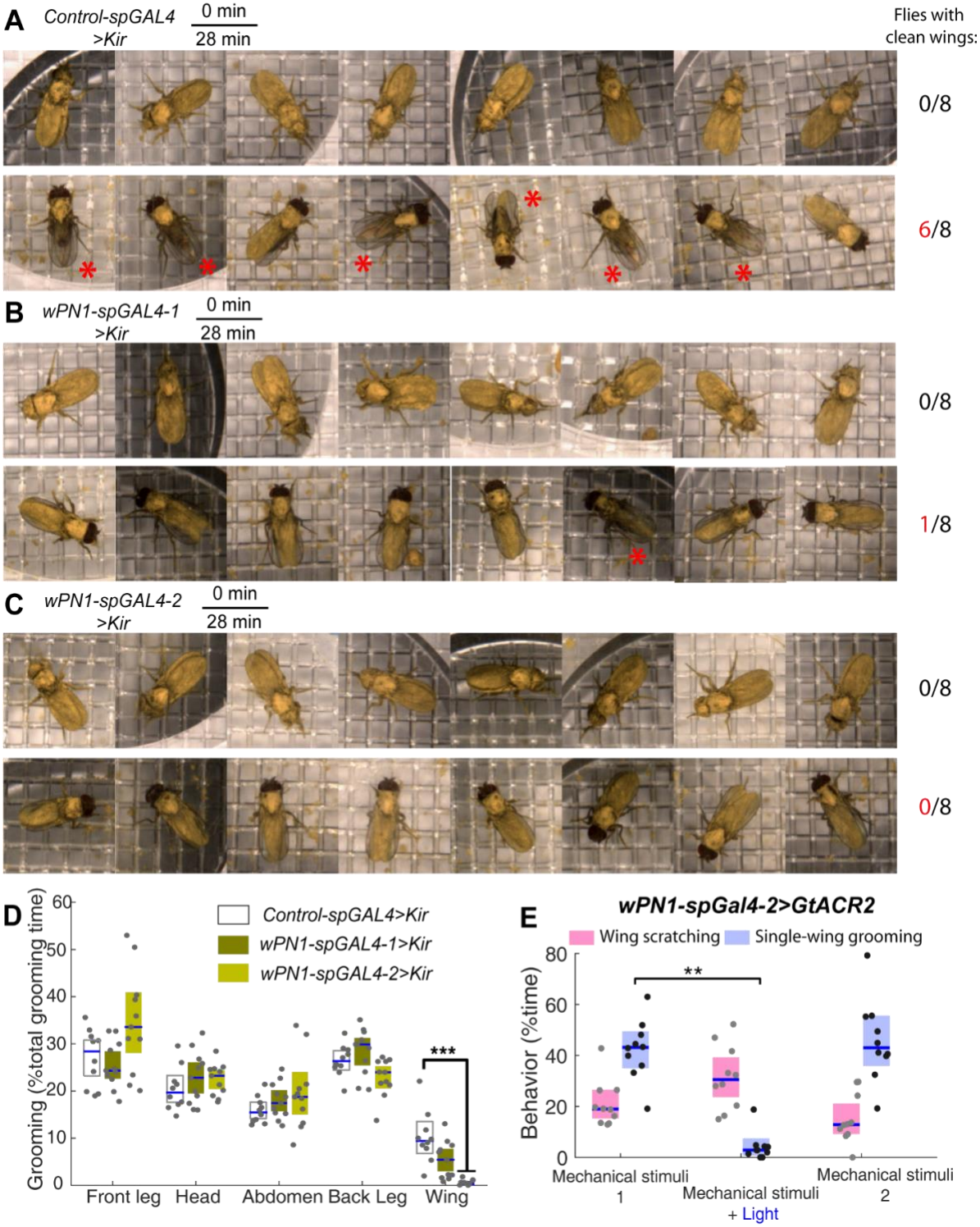

Figure S2. wPN1 is necessary for wing grooming. Related to Fig. 2.

(**A-C**) Video screenshots showing the dust distribution in control flies (**A**) and flies with wPN1 inhibition (**B**, **C**) at different time points. Flies with clean wings are labeled by red asterisks. (**D**) Percent of grooming time each behavior performed by dusted control flies or flies with wPN1 inhibited by Kir. (**E**) Quantification of different wing cleaning behaviors induced by mechanical stimuli with wPN1 inhibition.

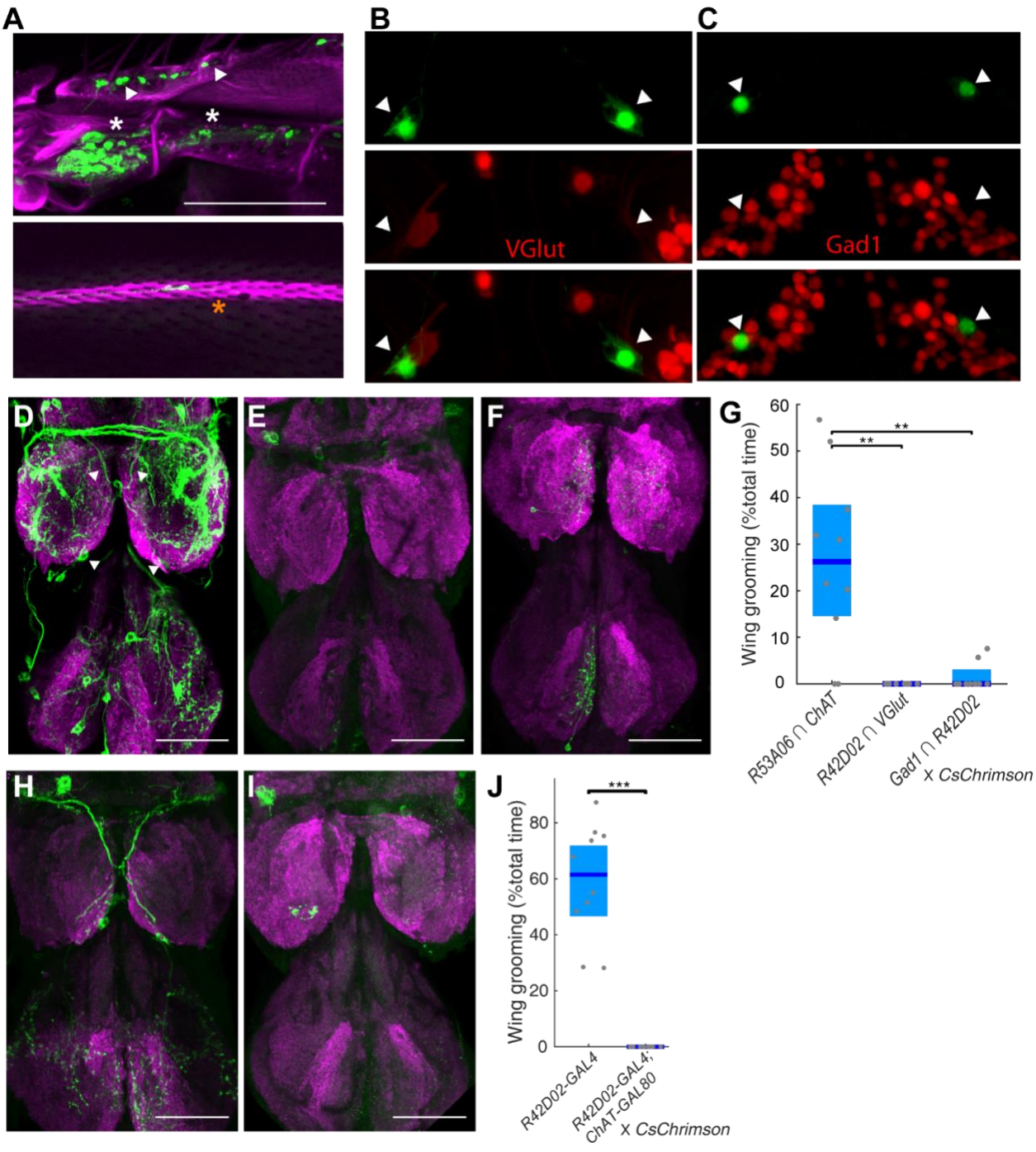

Figure S3. Wing mechanosensory neurons and wPN1 are cholinergic. Related to Fig. 3.

(**A**) Wing bristles (arrow heads) and campaniform sensilla (asterisks) neurons are labelled by *ChAT-LexA*. Scale bars, 100µm. (**B**, **C**) Double labeling of wPN1 (*R42D02-GAL4*, green) with glutaminergic neurons (*VGlut-LexA*, **B**) and GABAergic neurons (*Gad1-LexA*, **C**). (**D-F**) Intersections between wPN1 driver lines and driver lines for cholinergic (**D**), glutaminergic (**E**) or GABAergic (**F**) neurons. Genotype: *R53A06-AD ∩ ChAT-DBD>CsChrimson* (**D**), *R42D02-GAL4 > stop-CsChrimson; VGlut-Flp* (**E**), *Gad1-AD ∩ R42D02-DBD>CsChrimson* (**F**). Scale

bars, 100μm. (**G**) Percent of time flies spend in wing grooming during one-minute photoactivation of corresponding intersection lines. (**H, I**) The expression pattern of the *R42D02-GAL4* driver line alone (**H**) or with *Cha-GAL80* inhibitor (**I**). (**J**) Percent of time flies spend in wing grooming during one-minute photoactivation of corresponding driver lines.

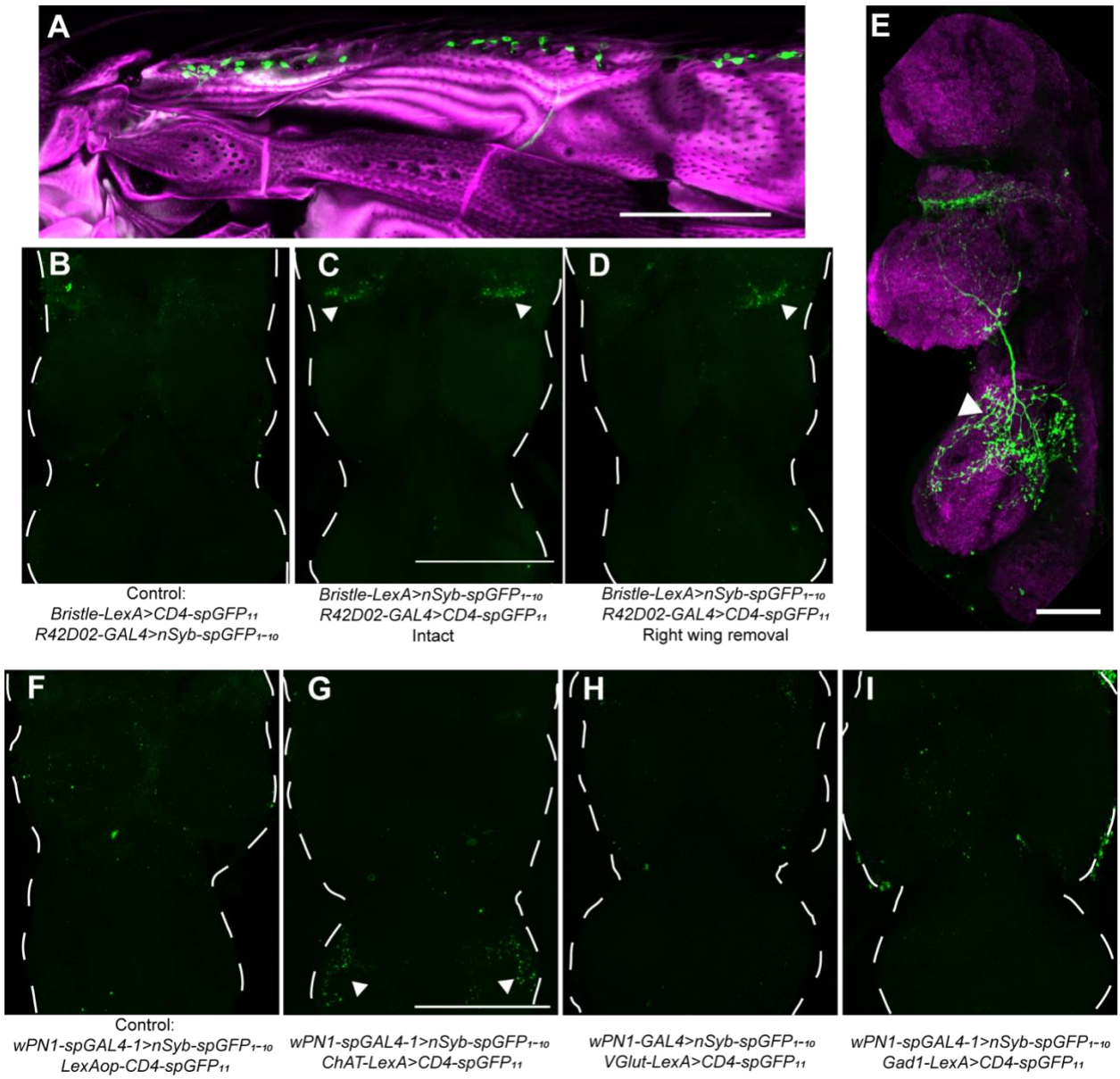

Figure S4. Candidate pre-synaptic and post-synaptic neurons of wPN1. Related to Fig. 3 and 4.

(**A**) Expression pattern of *Bristle-Gal4* in wing. Scale bars, 100μm. (**B**) Lack of GRASP signal in control samples between wPN1 axons and wing bristle neurons. (**C, D**) Synaptic GRASP reconstituted GFP signal (green, arrowhead) was observed between wing bristle neuron axons and wPN1 interneurons. (**E**) Side-view of wPN1, arrowhead indicates the potential connecting areas with downstream commissures. (**F**) Lack of GRASP signal in control samples with only one GFP fragment. (**G**) Synaptic GRASP signal (green, arrowhead) between wPN1 axons and cholinergic neurons. (**H, I**) No GRASP signal is observed between wPN1 and glutaminergic (**H**) or GABAergic (**I**) neurons.