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Supplemental Information

**A Brain Module for Scalable Control
of Complex, Multi-motor Threat Displays**

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Figure S1

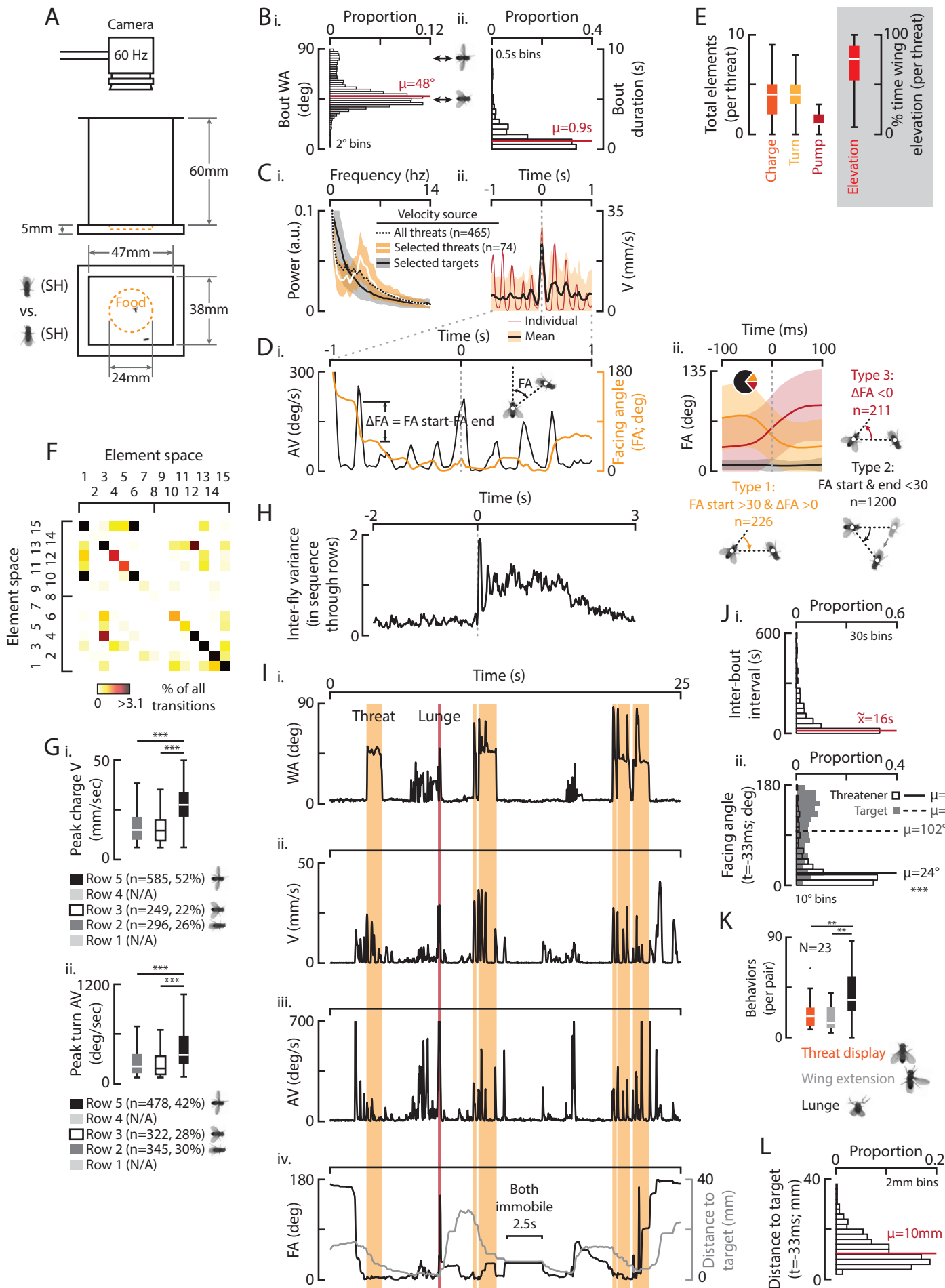


Figure S1: Related to Figure 1. **Natural threat displays are comprised of distinct motor elements.** **A)** A schematic of the behavior arena. **B)** WA during all 465 “bouts” (i), defined as periods when wings are extended or elevated, and a histogram of bout duration (ii). **C)** Average Fourier transform of V (+/- 1s from t=0) for all threats (i, dashed black line), 74 selected threats (white & orange \pm standard deviation (SD) envelope, see Methods), and corresponding selected target V (black & gray \pm SD). An individual example of periodic charging (ii, red) and mean velocity of selected threats (black & orange SD). **D)** An individual AV trace corresponding to Cii (Di, black) plotted with facing angle (FA, orange). Average FA (ii) of turns toward the target where FA start > 30 & Δ FA >0 (Type 1, orange & orange \pm SD), turns where FA start & end <30 (Type 2, black & gray \pm SD), and turns away from the target where Δ FA <0 (Type 3, red & pink \pm SD). A pie chart indicates the relative proportion of each type and examples of each type are schematized but are not all inclusive. **E)** Total motor elements expressed per threat (bout +/- 0.5s). Boxplots contain a line (median), box (25th and 75th percentiles), and whiskers (extremes but not outliers) throughout. **F)** An element space transition matrix derived from all classified threats. **G)** Peak charge V (i) and peak turn AV according to rows with wing pose indicated (Fig. 1F, pairwise and multiple comparison tests). **H)** Inter-fly variance in row assignment as a function of time. **I)** An example trace of WA (i), V (ii), AV (iii), FA (iv), and distance to target (iv, gray). Light orange bars indicate classified threats and the red bar, a lunge. **J)** A histogram of inter-bout interval (i) with median indicated and threatener and target facing angle 33ms before t=0 (ii). **K)** Total threats, wing extensions, and lunges exhibited per pair (pairwise and multiple comparison tests). Images are representative snap-shots during these behaviors as viewed from above. **L)** Distance to target, or inter-centroid distance, 33ms before t=0.

Figure S2

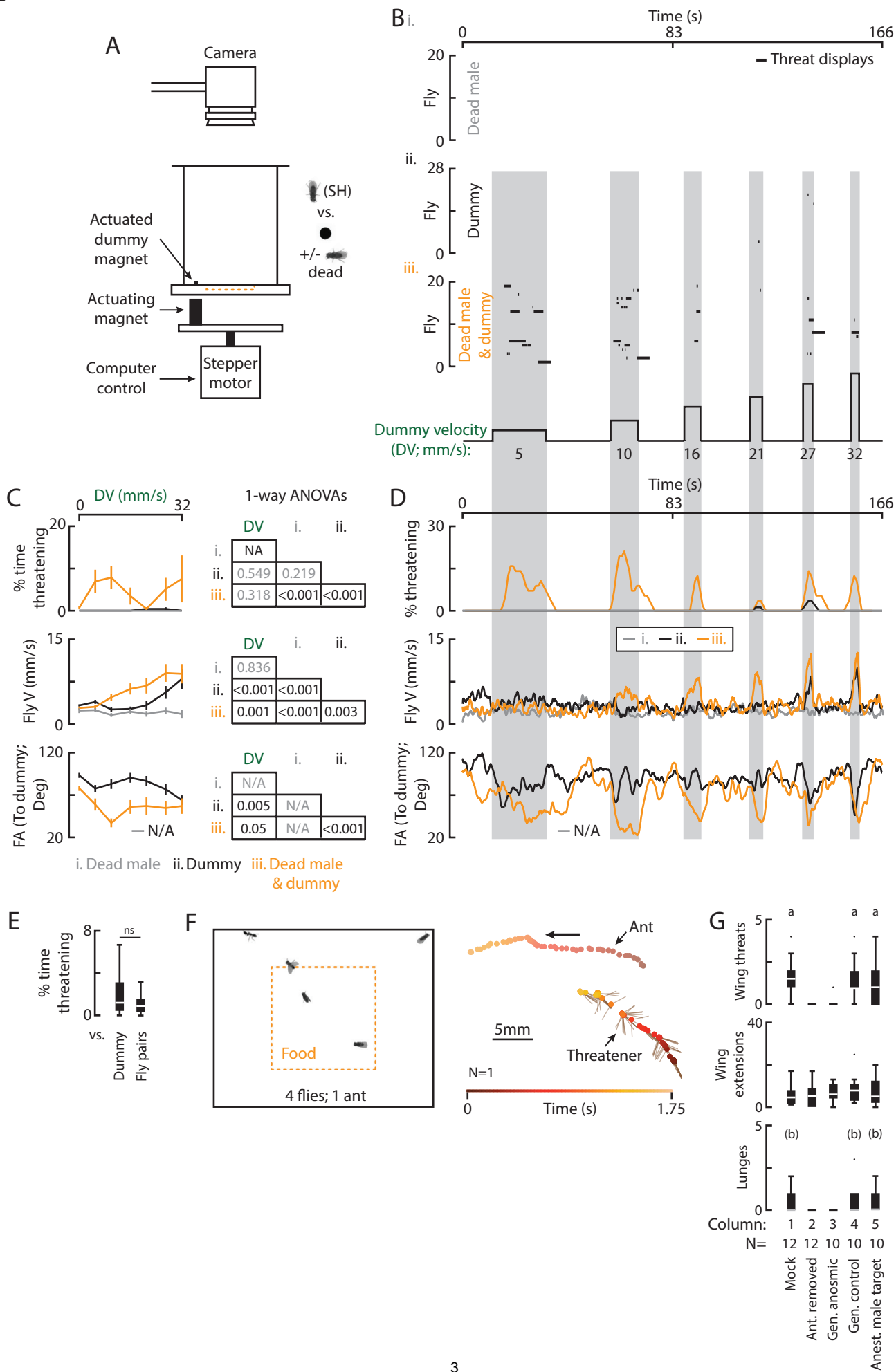


Figure S2: Related to Figure 2. **Chemosensory cues plus a moving object suffice to evoke threats.** **A)** A schematic of the behavior arena. **B)** A raster of threats from conditions described in Fig. 2A in response to increasing dummy velocity (DV). **C)** Average percent of time expressing threats (left, top), average fly V (middle), and average FA toward dummy (bottom) according to DV with SEM bars and p-values from one way ANOVAs versus DV or other conditions (right). **D)** Corresponding percent of flies threatening, average fly V, and average FA toward dummy over time. **E)** Percent time threatening for dummy (Fig. 2) and pair (Fig. 1) experiments. **F)** A threat display directed toward an ant (existence proof). **G)** Total behaviors per fly evoked in condition iii according to manipulations indicated (see methods); mock antennal removal (column 1), antennae removed (2), genetically anosmic (3, *IR8a*, *IR25a*, *OR83b*, *Gr63a*), genetic control (4, anosmic/+), and anesthetized male target (5). “(b)” indicates a significance group from pairwise tests that did not survive multiple comparisons.

Figure S3

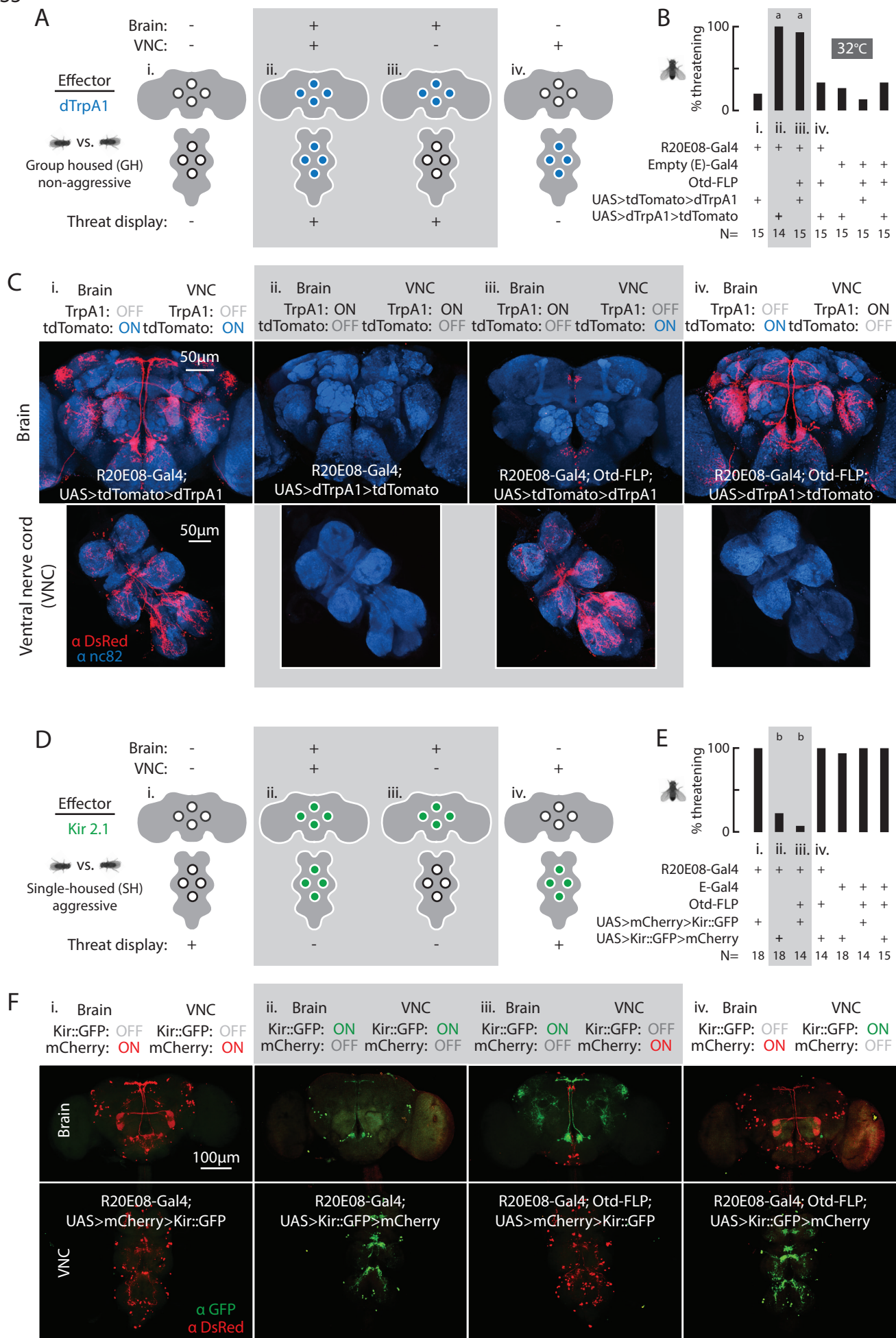


Figure S3: Related to Figure 3. **Identification of neurons that control threat displays.** A cartoon representation of experimental genotypes and brain and ventral nerve cord (VNC) expression of dTrpA1 (**A**) and Kir 2.1 (**D**). **B & E** Percent of flies, with genotypes indicated, expressing at least one threat. ("a, b, c" indicate significance groups after multiple comparison tests throughout). **C**) Brain images corresponding to Fig. S3A expressing tdTomato (red) or untagged dTrpA1 (no red) with blue neuropil. **F**) Brain images corresponding to Fig. S3D expressing mCherry (red) or Kir 2.1::GFP (green). Scale bars are 50 μ m (C) or 100 μ m (F).

Figure S4

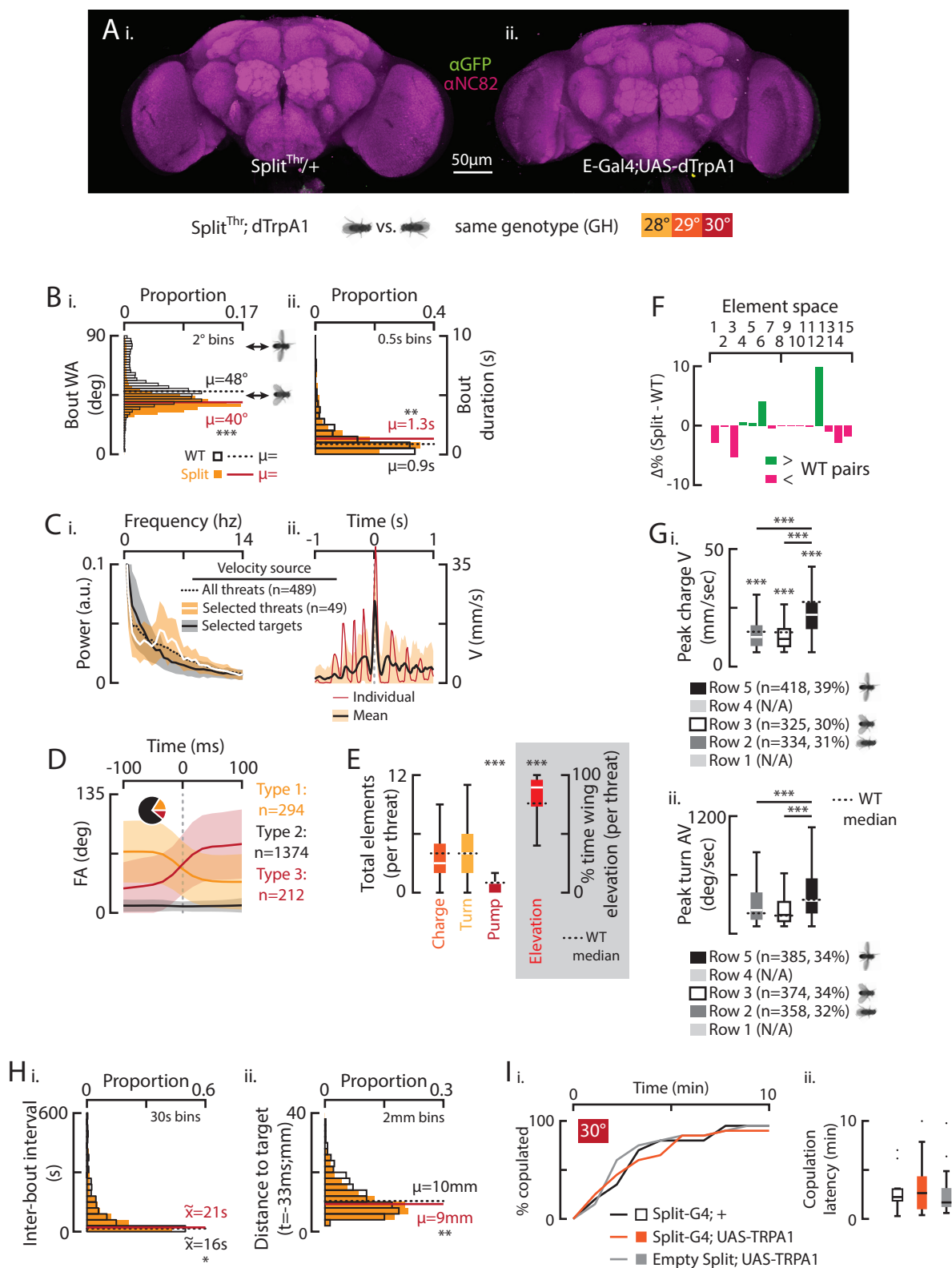


Figure S4: Related to Figure 3. **Thermogenetic activation of Split^{Thr} neurons induces WT-like threats in fly pairs.** **A)** Representative control brains (i, ii) express no GFP. Scale bar is 50 μ m. **B)** A histogram of WA (i) and duration (ii) for induced bouts (orange) pooled from 28°, 29°, and 30°, with WT data overlaid (open). **C)** Average Fourier transform of V (+/- 1s from t=0) for all 489 threats (i, dashed black line), 49 selected threats (white & orange \pm SD envelope), and corresponding selected target V (black & gray \pm SD) with (ii) an individual example (red) and mean V for selected threats (black & orange \pm SD). **D)** Average FA of type 1 turns toward the target (orange & orange \pm SD), type 2 oriented turns (black & gray \pm SD), and type 3 turns away from the target (red & pink \pm SD). A pie chart indicates the relative proportion of each type. **E)** Total motor elements expressed per threat (bout +/- 0.5s). **F)** The relative differences in element space from WT threats (Fig. 1Eii). **G)** Peak charge V (i) and peak turn AV according to rows (Fig. 3F). **H)** A histogram of inter-bout intervals (i) and distance to target (ii) for induced (orange) and WT (open) threats. (Significance from pairwise and multiple comparisons tests as indicated or vs. WT). **I)** % of flies that copulated with virgin females over time (i) and copulation latency according to genotype (ii).

Figure S5

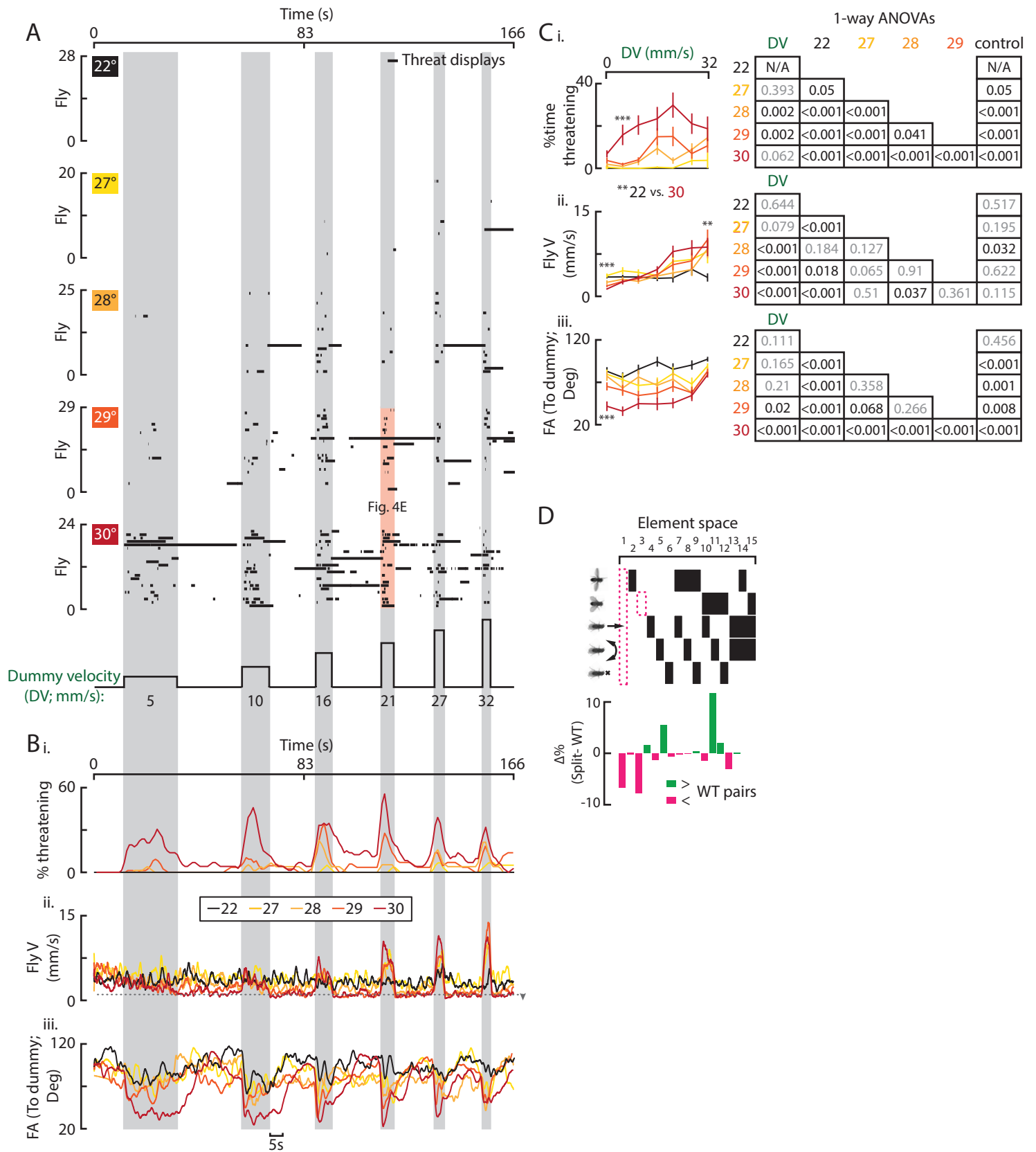


Figure S5: Related to Figure 4. **Thermogenetic activation of solitary Split^{Thr} flies substitutes for male cues and enhances sensitivity to dummy motion.** **A)** A raster of threats according to temperature in response to increasing dummy velocity (DV). **B)** Percent of flies threatening (i), average fly V (ii), and average FA toward dummy (iii) over time and color coded to temperature. **C)** Corresponding average percent of time expressing threats (i), average fly V (ii), and average FA toward dummy (iii) according to DV, with notable, but not all, pairwise significance (left), with SEM error bars and p-values from one way ANOVAs versus DV or other conditions (right). **D)** The relative differences in element space from WT threats (Fig. 1Eii).

Figure S6

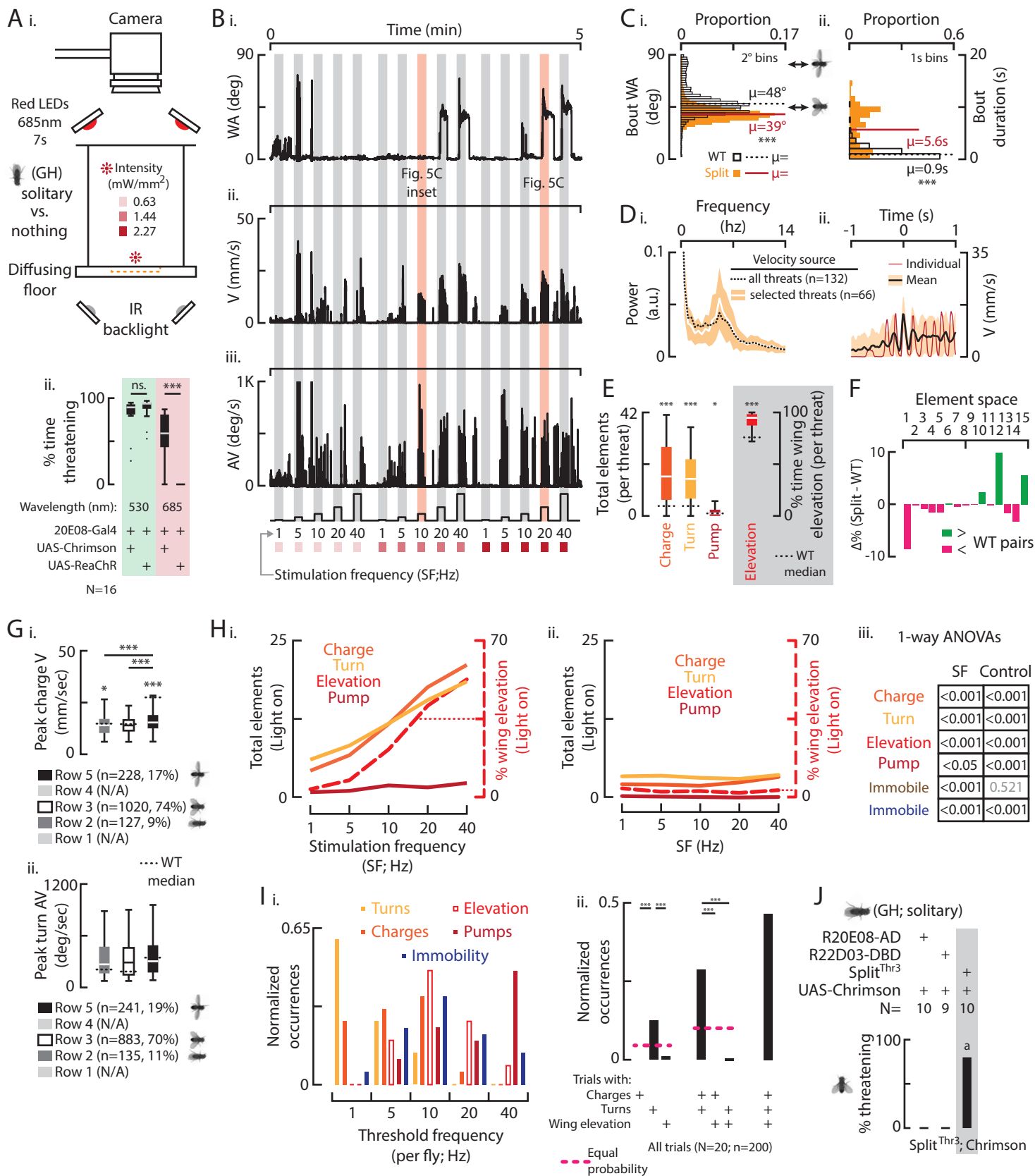


Figure S6: Related to Figure 5. **Optogenetic activation of Split^{Thr} neurons substitutes for sensory cues and induces different threat motor elements in a threshold-dependent manner.** **A)** A schematic of the behavior arena (i) and a comparison of ReaChR and Chrimson (ii) for light induced threat induction. **B)** An individual WA (i), V (ii), and AV (iii) trace from an experiment where the frequency and intensity of photostimulation increases over time. Flies were often generally active at the beginning. **C)** A histogram of bout WA (i) and bout duration (ii). **D)** Average Fourier transform of V (+/- 1s from t=0) for all 132 threats (i, dashed black line), 66 selected threats (white & orange, \pm SD envelope), and (ii) an individual example (red) and mean V for selected threats (black & orange \pm SD). **E)** Total motor elements expressed per threat (bout +/- 0.5s). **F)** The relative differences in element space from WT threats (Fig. 1Eii). **G)** Peak charge V (i) and peak turn AV according to rows (Fig. 5E). **H)** Total motor elements expressed vs. stimulation frequency (SF) for experimental flies (i) and controls (ii). Right y-axis corresponds to % wing elevation. One-way ANOVAs (iii) of experimental data vs. SF or controls. **I)** Histograms of threshold SF for the indicated motor elements (i, see Fig. 5Gi) and normalized occurrences of photostimulation trials containing charges, turns, and/or wing elevation individually or in combination (ii, see Methods). **J)** Percent of flies with indicated genotypes that expressed at least one light induced threat.

Figure S7

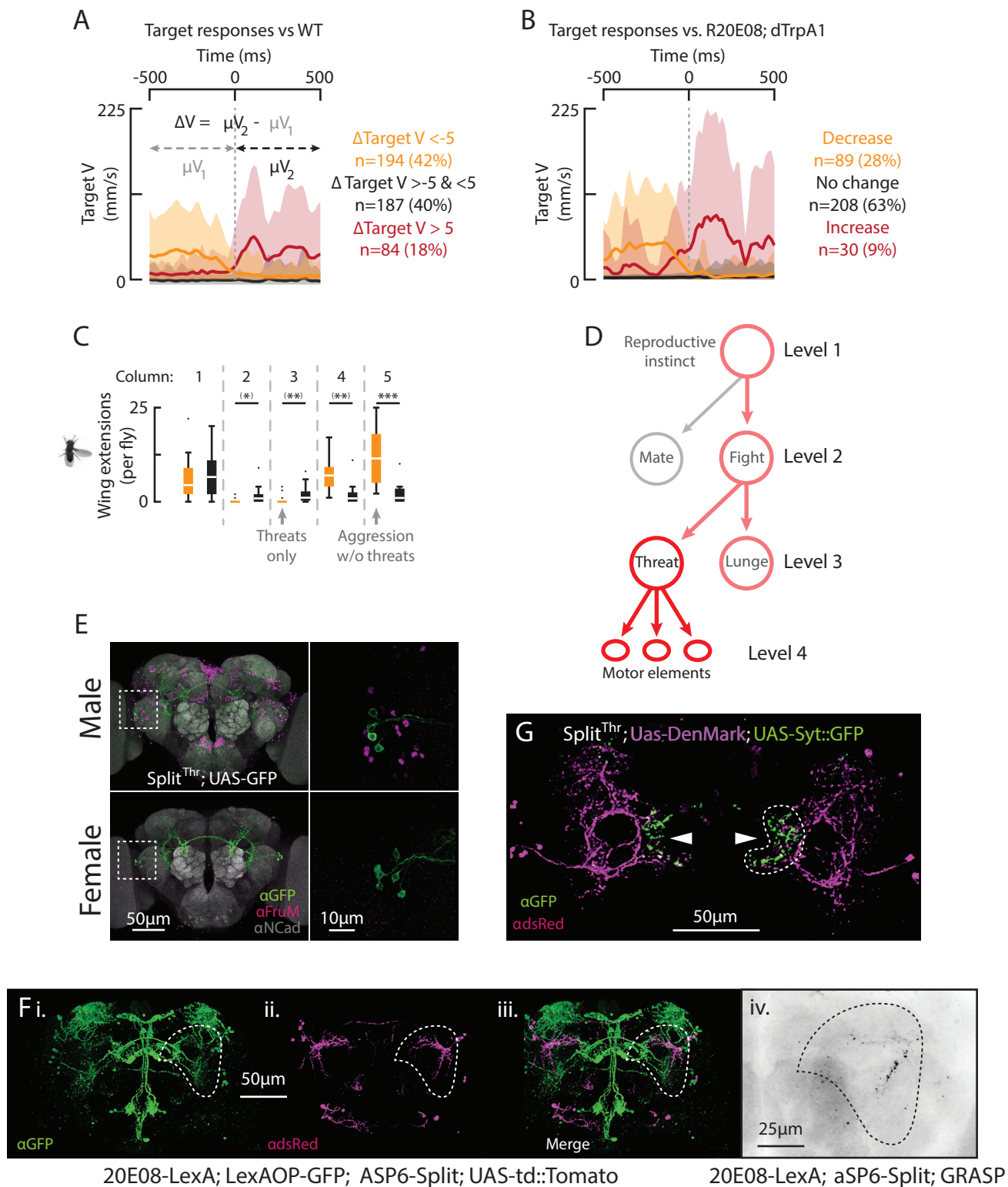


Figure S7: Related to Figure 7. **Threats function to repel conspecifics and are mediated by a scalable brain module.** **A)** A classification of WT Target V (\pm SD) co-occurring with WT threats (Fig. 1). **B)** WT Target V (\pm SD) co-occurring with thermogenetically induced threats (Fig. 7C, 3rd column). **C)** Total wing extensions exhibited by genotypes indicated in Fig. 7C (Significance from pairwise and multiple comparison tests). **D)** AIP neurons may constitute a “threat” module in a hypothetical neural hierarchy (Tinbergen 1950). **E)** Male and female AIP expression (green) co-labeled with α FruM (red). **F)** Double fluorescence (i-iii) between AIP neurons (20E08-LexA; green) and aSP6 neurons (11F03-DBD; 71D08-AD, magenta), and GRASP proximity labeling (iv, native). **G)** Split^{Thr} expression of ~dendritic (UAS-DenMark, magenta) and synaptic (UAS-syt::GFP, green) markers. One of two bilateral outputs (arrowheads) is outlined (dashed white). Scale bars are 50 μ m (E, left, F, G) or 10 μ m (E, right).