Figure S1

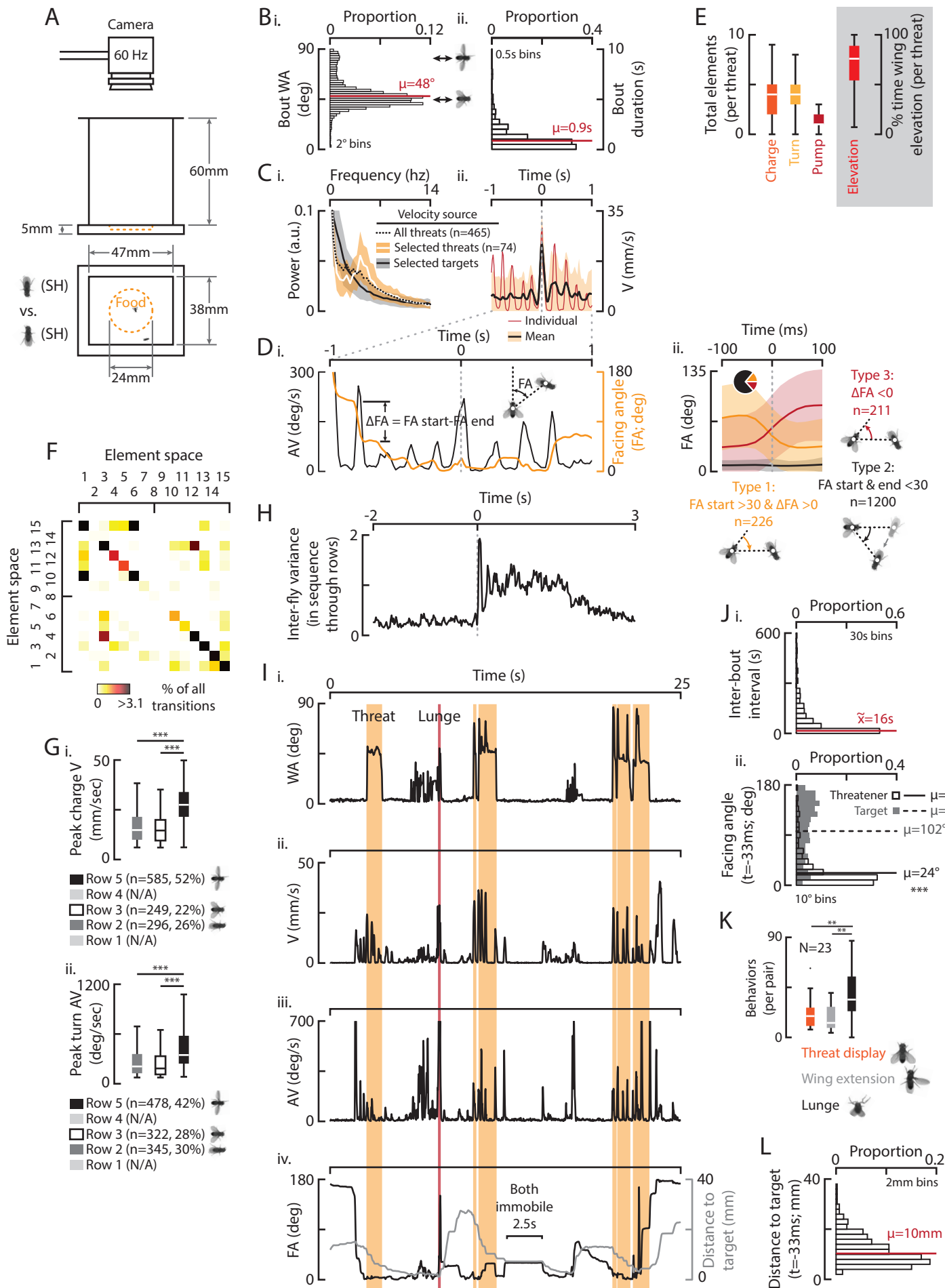**Figure S1:** Related to Figure 1. **Natural threat displays are comprised of distinct motor elements. A)** A schematic of the behavior arena. **B)** WA during all 465 "bouts" (i), defined as periods when wings are extended or elevated, and a histogram of bout duration (ii). **C)** Average Fourier transform of V (+/- 1s from t=0) for all threats (i, dashed black line), 74 selected threats (white & orange ±standard deviation (SD) envelope, see Methods), and corresponding selected target V (black & gray ±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 & ΔFA >0 (Type 1, orange & orange ±SD), turns where FA start & end <30 (Type 2, black & gray ±SD), and turns away from the target where ΔFA <0 (Type 3, red & pink ±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

A

Camera

Actuated dummy magnet

(SH)

vs.

+/- dead

Actuating magnet

Computer control → Stepper motor

B

i.
Time (s)
0 — 83 — 166

— Threat displays

Dead male — Fly 0–20

ii. Dummy — Fly 0–28

iii. Dead male & dummy — Fly 0–20

Dummy velocity (DV; mm/s): 5 10 16 21 27 32

C

DV (mm/s) 0 — 32

% time threatening 0–20

1-way ANOVAs

	DV	i.	ii.
i.	NA		
ii.	0.549	0.219	
iii.	0.318	<0.001	<0.001

Fly V (mm/s) 0–15

	DV	i.	ii.
i.	0.836		
ii.	<0.001	<0.001	
iii.	0.001	<0.001	0.003

FA (To dummy; Deg) 20–120

	DV	i.	ii.
i.	N/A		
ii.	0.005	N/A	
iii.	0.05	N/A	<0.001

— N/A

i. Dead male   ii. Dummy   iii. Dead male & dummy

D

Time (s)
0 — 83 — 166

% threatening 0–30

— i. — ii. — iii.

Fly V (mm/s) 0–15

FA (To dummy; Deg) 20–120

— N/A

E

% time threatening 0–8

ns

vs. Dummy   Fly pairs

F

Food

4 flies; 1 ant

Ant

Threatener

5mm

N=1

Time (s) 0 — 1.75

G

Wing threats 0–5

a   a   a

Wing extensions 0–40

Lunges 0–5

(b)   (b)   (b)

Column:	1	2	3	4	5
N=	12	12	10	10	10

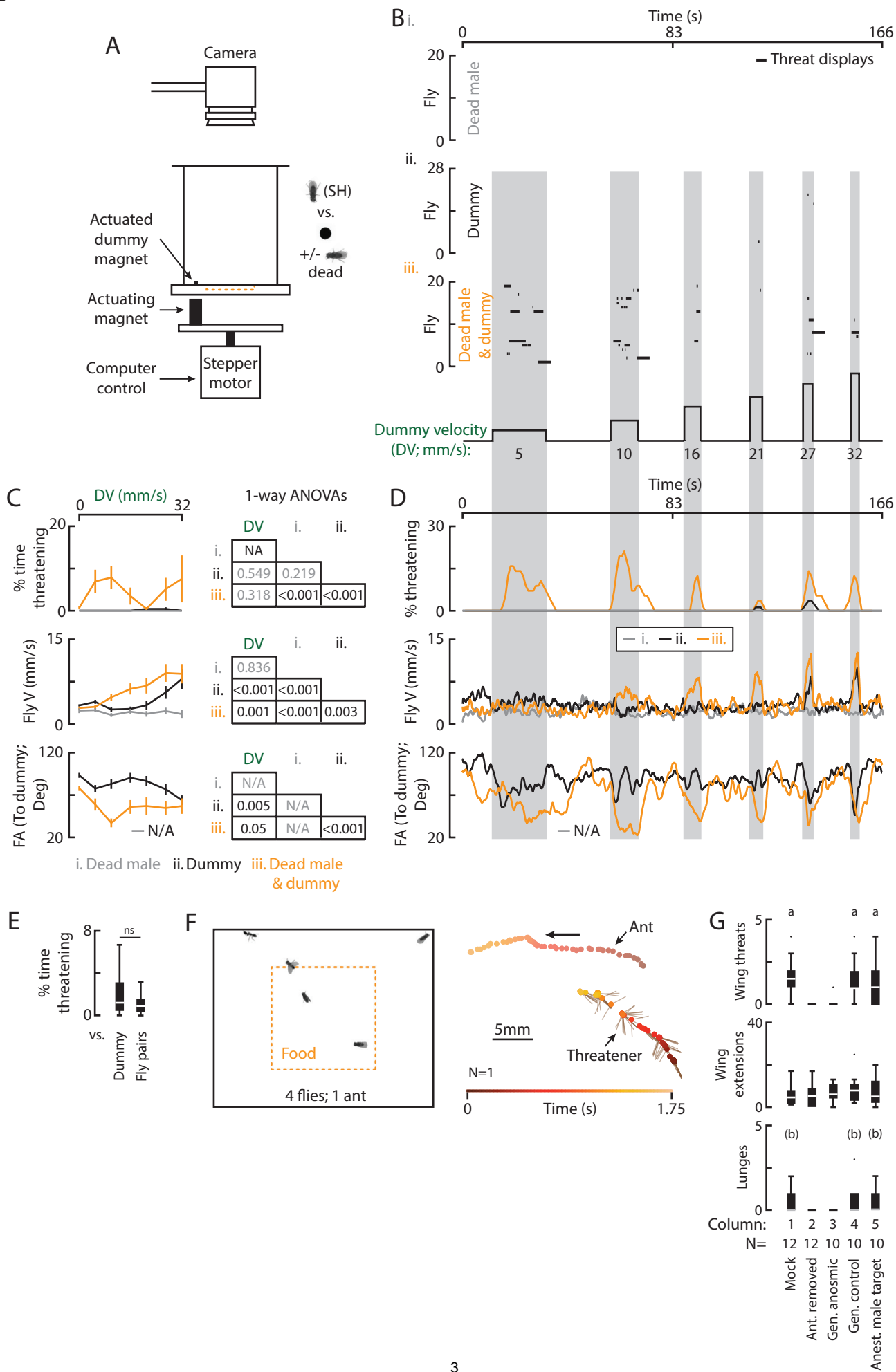Mock   Ant. removed   Gen. anosmic   Gen. control   Anest. male target

3

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

A

| Brain: | - | + | + | - |
| VNC: | - | + | - | + |

Effector
dTrpA1

🪰 vs. 🪰
Group housed (GH)
non-aggressive

i. ii. iii. iv.

Threat display: - + + -

B

% threatening

32°C

	i.	ii.	iii.	iv.		
R20E08-Gal4	+	+	+			
Empty (E)-Gal4				+	+	+
Otd-FLP			+	+	+	+
UAS>tdTomato>dTrpA1	+	+			+	
UAS>dTrpA1>tdTomato	+	+	+	+		+
N=	15	14	15	15	15	15	15

C

i. Brain	VNC	ii. Brain	VNC	iii. Brain	VNC	iv. Brain	VNC
TrpA1: OFF	TrpA1: OFF	TrpA1: ON	TrpA1: ON	TrpA1: ON	TrpA1: OFF	TrpA1: OFF	TrpA1: ON
tdTomato: ON	tdTomato: ON	tdTomato: OFF	tdTomato: OFF	tdTomato: OFF	tdTomato: ON	tdTomato: ON	tdTomato: OFF

Brain

50μm

R20E08-Gal4;
UAS>tdTomato>dTrpA1

R20E08-Gal4;
UAS>dTrpA1>tdTomato

R20E08-Gal4; Otd-FLP;
UAS>tdTomato>dTrpA1

R20E08-Gal4; Otd-FLP;
UAS>dTrpA1>tdTomato

Ventral nerve cord (VNC)

50μm

α DsRed
α nc82

D

| Brain: | - | + | + | - |
| VNC: | - | + | - | + |

Effector
Kir 2.1

🪰 vs. 🪰
Single-housed (SH)
aggressive

i. ii. iii. iv.

Threat display: + - - +

E

% threatening

	i.	ii.	iii.	iv.		
R20E08-Gal4	+	+	+	+		
E-Gal4					+	+	+
Otd-FLP			+	+		+	+
UAS>mCherry>Kir::GFP	+		+			+	
UAS>Kir::GFP>mCherry	+	+		+	+		+
N=	18	18	14	14	18	14	15

F

i. Brain	VNC	ii. Brain	VNC	iii. Brain	VNC	iv. Brain	VNC
Kir::GFP: OFF	Kir::GFP: OFF	Kir::GFP: ON	Kir::GFP: ON	Kir::GFP: ON	Kir::GFP: OFF	Kir::GFP: OFF	Kir::GFP: ON
mCherry: ON	mCherry: ON	mCherry: OFF	mCherry: OFF	mCherry: OFF	mCherry: ON	mCherry: ON	mCherry: OFF

Brain

100μm

R20E08-Gal4;
UAS>mCherry>Kir::GFP

R20E08-Gal4;
UAS>Kir::GFP>mCherry

R20E08-Gal4; Otd-FLP;
UAS>mCherry>Kir::GFP

R20E08-Gal4; Otd-FLP;
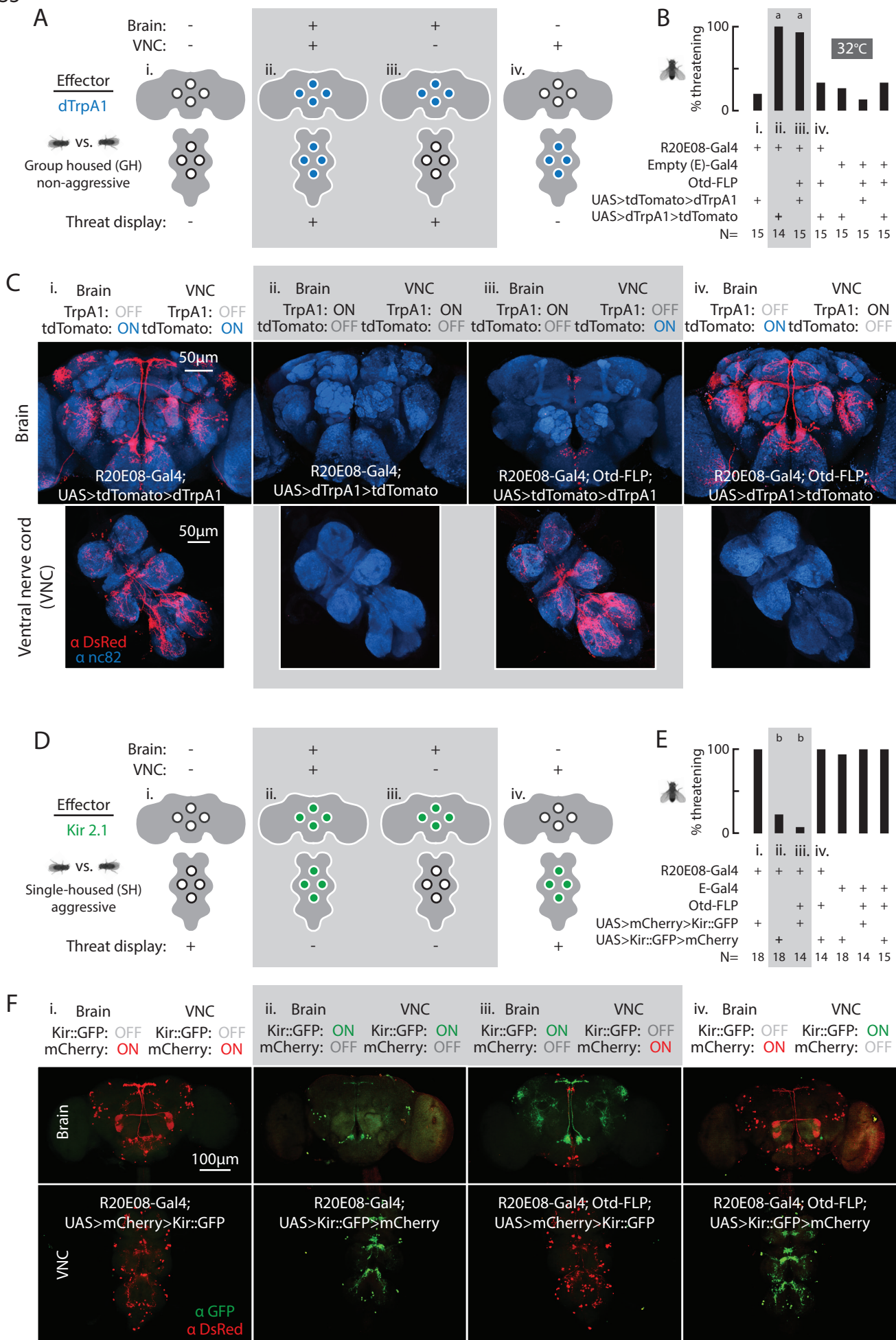UAS>Kir::GFP>mCherry

VNC

α GFP
α DsRed

5

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 50μm (C) or 100μm (F).

Figure S4

A

i. Split^Thr/+   αGFP αNC82   50μm

ii. E-Gal4;UAS-dTrpA1

Split^Thr; dTrpA1   vs.   same genotype (GH)   28° 29° 30°

B

i. Proportion — 0 to 0.17

Bout WA (deg) — 2° bins — μ=48° — μ=40° — ***

WT □ ····· μ= / Split ■ — μ=

ii. Proportion — 0 to 0.4

Bout duration (s) — 0.5s bins — ** μ=1.3s — μ=0.9s

C

i. Frequency (hz) — 0 to 14

Power (a.u.) — 0.1 to 0

Velocity source
····· All threats (n=489)
■ Selected threats (n=49)
■ Selected targets

ii. Time (s) — −1 to 1

V (mm/s) — 35 to 0

Individual / Mean

D

Time (ms) — −100 to 100

FA (deg) — 135

Type 1: n=294
Type 2: n=1374
Type 3: n=212

E

Total elements (per threat) — 0 to 12

Charge / Turn / Pump *** / Elevation ***

% time wing elevation (per threat) — 0 to 100

····· WT median

F

Element space
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15

Δ% (Split − WT) — −10 to 10

■ > WT pairs ■ <

G

i. Peak charge V (mm/sec) — 0 to 50 — *** *** ***

■ Row 5 (n=418, 39%)
■ Row 4 (N/A)
□ Row 3 (n=325, 30%)
■ Row 2 (n=334, 31%)
■ Row 1 (N/A)

ii. Peak turn AV (deg/sec) — 0 to 1200 — *** *** — ····· WT median

■ Row 5 (n=385, 34%)
■ Row 4 (N/A)
□ Row 3 (n=374, 34%)
■ Row 2 (n=358, 32%)
■ Row 1 (N/A)

H

i. Proportion — 0 to 0.6

Inter-bout interval (s) — 0 to 600 — 30s bins — x̃=21s — x̃=16s *

ii. Proportion — 0 to 0.3

Distance to target (t=−33ms;mm) — 0 to 40 — 2mm bins — μ=10mm — μ=9mm **

I

i. Time (min) — 0 to 10

% copulated — 0 to 100 — 30°

□ Split-G4; +
■ Split-G4; UAS-TRPA1
■ Empty Split; UAS-TRPA1

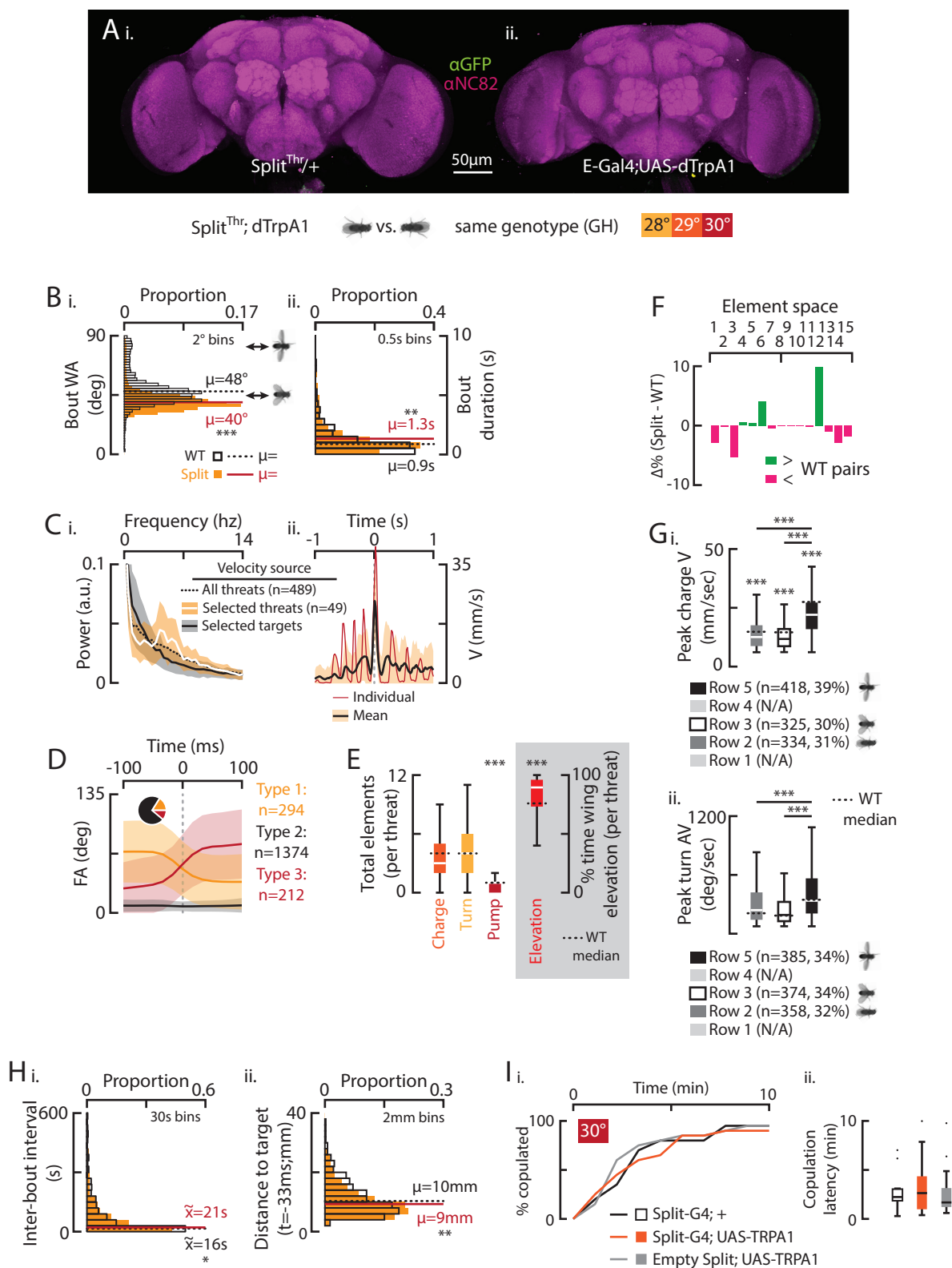ii. Copulation latency (min) — 0 to 10

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 50μm. **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 ±SD envelope), and corresponding selected target V (black & gray ±SD) with (ii) an individual example (red) and mean V for selected threats (black & orange ±SD). **D)** Average FA of type 1 turns toward the target (orange & orange ±SD), type 2 oriented turns (black & gray ±SD), and type 3 turns away from the target (red & pink ±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).

A

Time (s)

22° 27° 28° 29° 30°

Threat displays

Dummy velocity (DV; mm/s): 5 10 16 21 27 32

Fig. 4E

B

i. Time (s)

% threatening

ii. Fly V (mm/s)

—22 —27 —28 —29 —30

iii. FA (To dummy; Deg)

5s

C

i. DV (mm/s)

%time threatening

*** 22 vs. 30

ii. Fly V (mm/s)

*** **

iii. FA (To dummy; Deg)

***

1-way ANOVAs

	DV	22	27	28	29	control
22	N/A					N/A
27	0.393	0.05				0.05
28	0.002	<0.001	<0.001			<0.001
29	0.002	<0.001	<0.001	0.041		<0.001
30	0.062	<0.001	<0.001	<0.001	<0.001	<0.001

	DV					
22	0.644					0.517
27	0.079	<0.001				0.195
28	<0.001	0.184	0.127			0.032
29	<0.001	0.018	0.065	0.91		0.622
30	<0.001	<0.001	0.51	0.037	0.361	0.115

	DV					
22	0.111					0.456
27	0.165	<0.001				<0.001
28	0.21	<0.001	0.358			0.001
29	0.02	<0.001	0.068	0.266		0.008
30	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

D Element space

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
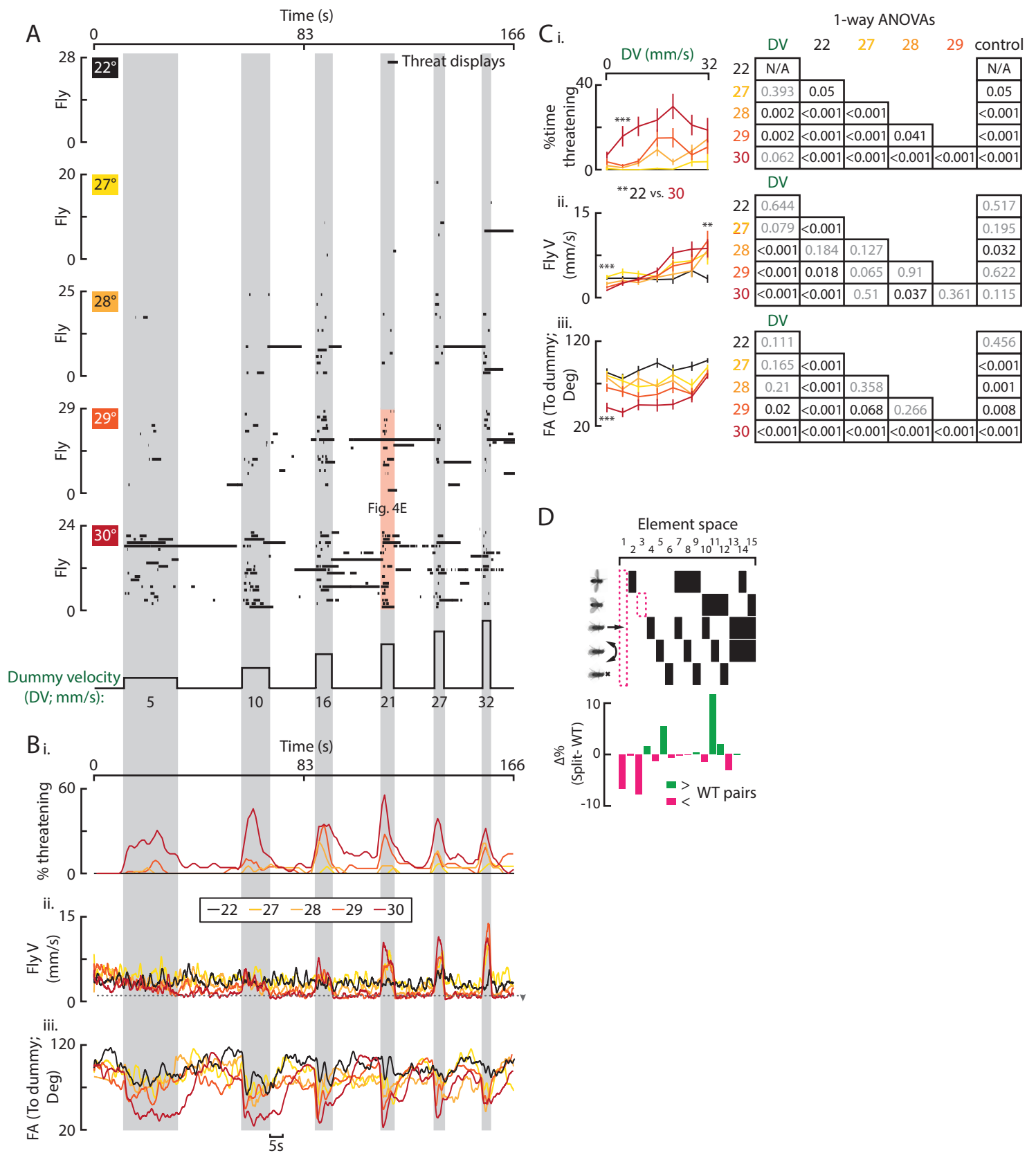
Δ% (Split- WT)

■ > WT pairs
■ <

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).

A

i.

Camera

Red LEDs
685nm
7s

(GH) solitary vs. nothing

Intensity (mW/mm²)	
	0.63
	1.44
	2.27

Diffusing floor

IR backlight

ii.

% time threatening

Wavelength (nm):	530		685	
20E08-Gal4	+	+	+	+
UAS-Chrimson	+		+	
UAS-ReaChR		+		+

N=16

B

i. Time (min) 0 — 5

WA (deg)

Fig. 5C inset   Fig. 5C

ii. V (mm/s)

iii. AV (deg/s)

Stimulation frequency (SF; Hz)
1 5 10 20 40 1 5 10 20 40 1 5 10 20 40

C

i. Proportion 0 — 0.17

Bout WA (deg)

2° bins

μ=48°

μ=39°

***

WT   μ=
Split   μ=

ii. Proportion 0 — 0.6

Bout duration (s)

1s bins

μ=5.6s

μ=0.9s

***

D

i. Frequency (hz) 0 — 14

Power (a.u.)

Velocity source
all threats (n=132)
selected threats (n=66)

ii. Time (s) -1 — 0 — 1

V (mm/s)

Individual
Mean

E

Total elements (per threat)

*** *** *

Charge Turn Pump

% time wing elevation (per threat)

***

Elevation

WT median

F

Element space
1 3 5 7 9 11 13 15
2 4 6 8 10 12 14

Δ% (Split - WT)

> WT pairs
<

G

i.

Peak charge V (mm/sec)

*** ***
*   ***

Row 5 (n=228, 17%)
Row 4 (N/A)
Row 3 (n=1020, 74%)
Row 2 (n=127, 9%)
Row 1 (N/A)

ii.

Peak turn AV (deg/sec)

WT median

Row 5 (n=241, 19%)
Row 4 (N/A)
Row 3 (n=883, 70%)
Row 2 (n=135, 11%)
Row 1 (N/A)

H

i.

Total elements (Light on)

Charge
Turn
Elevation
Pump

% wing elevation (Light on)

Stimulation frequency (SF; Hz)

ii.

Total elements (Light on)

Charge
Turn
Elevation
Pump

% wing elevation (Light on)

SF (Hz)

iii. 1-way ANOVAs

	SF	Control
Charge	<0.001	<0.001
Turn	<0.001	<0.001
Elevation	<0.001	<0.001
Pump	<0.05	<0.001
Immobile	<0.001	0.521
Immobile	<0.001	<0.001

I

i.

Normalized occurrences

Turns   Elevation
Charges   Pumps
Immobility

Threshold frequency (per fly; Hz)
1 5 10 20 40

ii.

Normalized occurrences

*** ***   ***
              ***

Trials with:				
Charges	+		+	+	+
Turns		+	+	+	+
Wing elevation		+		+	+

All trials (N=20; n=200)

Equal probability

J

(GH; solitary)

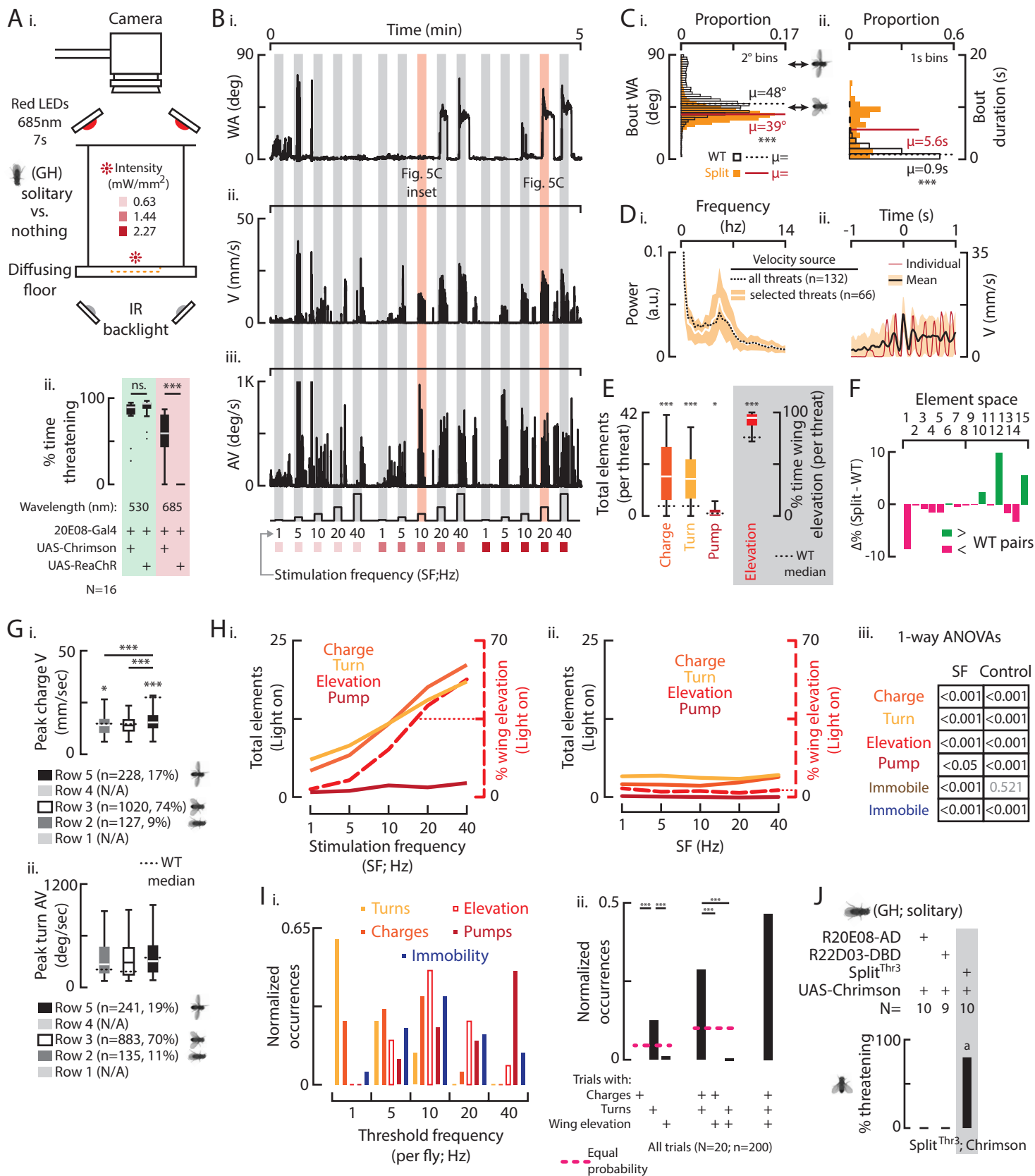|			
R20E08-AD	+		
R22D03-DBD		+	
SplitThr3			+
UAS-Chrimson	+	+	+
N=	10	9	10

% threatening

a

SplitThr3; Chrimson

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, ±SD envelope), and (ii) an individual example (red) and mean V for selected threats (black & orange ±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.
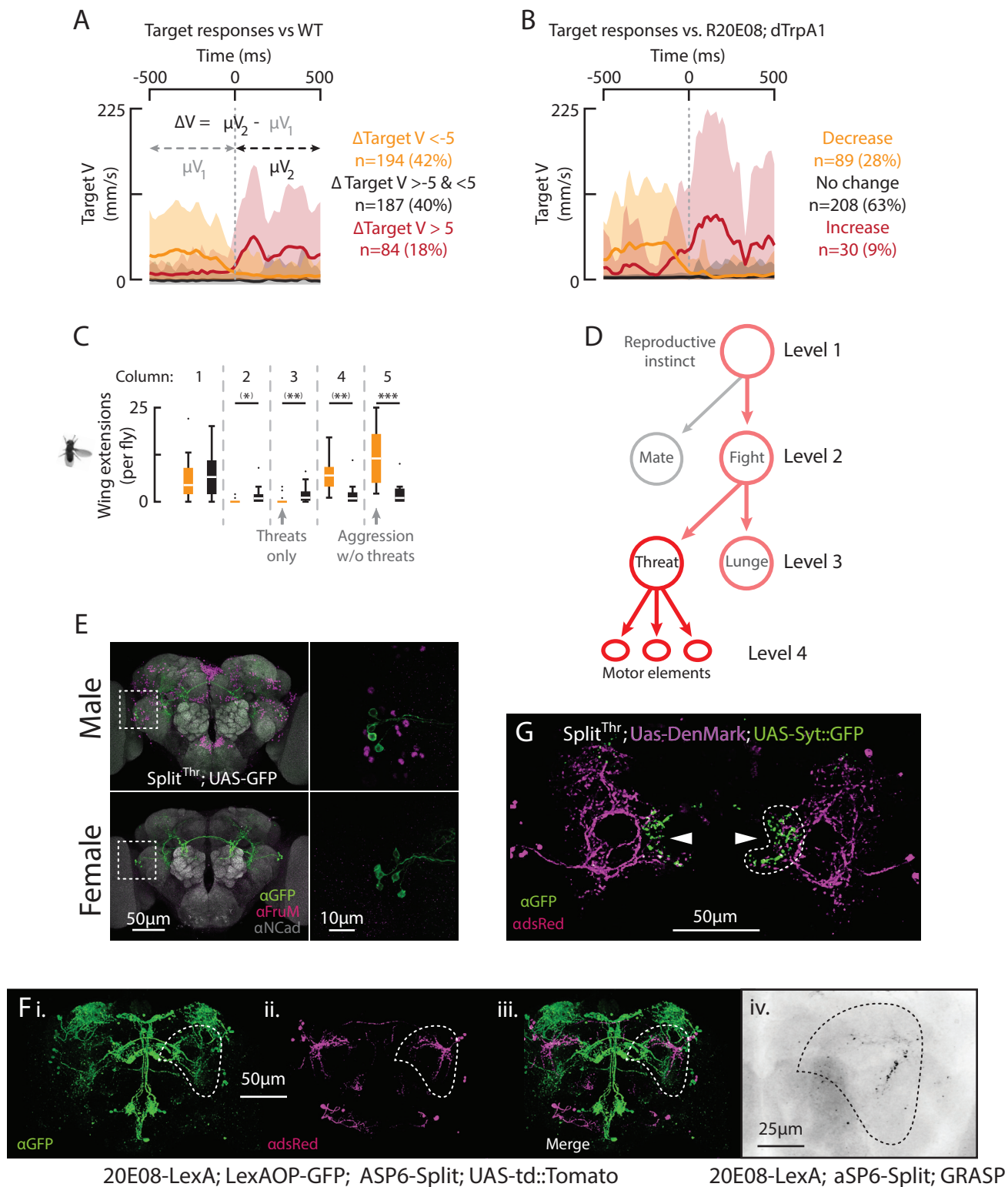
A

Target responses vs WT

Time (ms)

-500   0   500

$\Delta V = \mu V_2 - \mu V_1$

μV_1 μV_2

ΔTarget V <-5
n=194 (42%)

Δ Target V >-5 & <5
n=187 (40%)

ΔTarget V > 5
n=84 (18%)

B

Target responses vs. R20E08; dTrpA1

Time (ms)

-500   0   500

Decrease
n=89 (28%)

No change
n=208 (63%)

Increase
n=30 (9%)

C

Column: 1   2   3   4   5

(*)   (**)   (**)   ***

Wing extensions (per fly)

Threats only

Aggression w/o threats

D

Reproductive instinct — Level 1

Mate   Fight   Level 2

Threat   Lunge   Level 3

Motor elements   Level 4

E

Male

Female

Split^{Thr}; UAS-GFP

αGFP
αFruM
αNCad

50μm   10μm

G

Split^{Thr}; Uas-DenMark; UAS-Syt::GFP

αGFP
αdsRed

50μm

F

i.   ii.   iii.   iv.

αGFP   αdsRed   Merge

50μm   25μm

20E08-LexA; LexAOP-GFP; ASP6-Split; UAS-td::Tomato   20E08-LexA; aSP6-Split; GRASP

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 (±SD) co-occurring with WT threats (Fig. 1). **B)** WT Target V (±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 αFruM (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 50µm (E, left, F, G) or 10µm (E, right).