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Supplemental Information

Hypothalamic Control of Conspecific Self-Defense

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Supplementary Figure 1

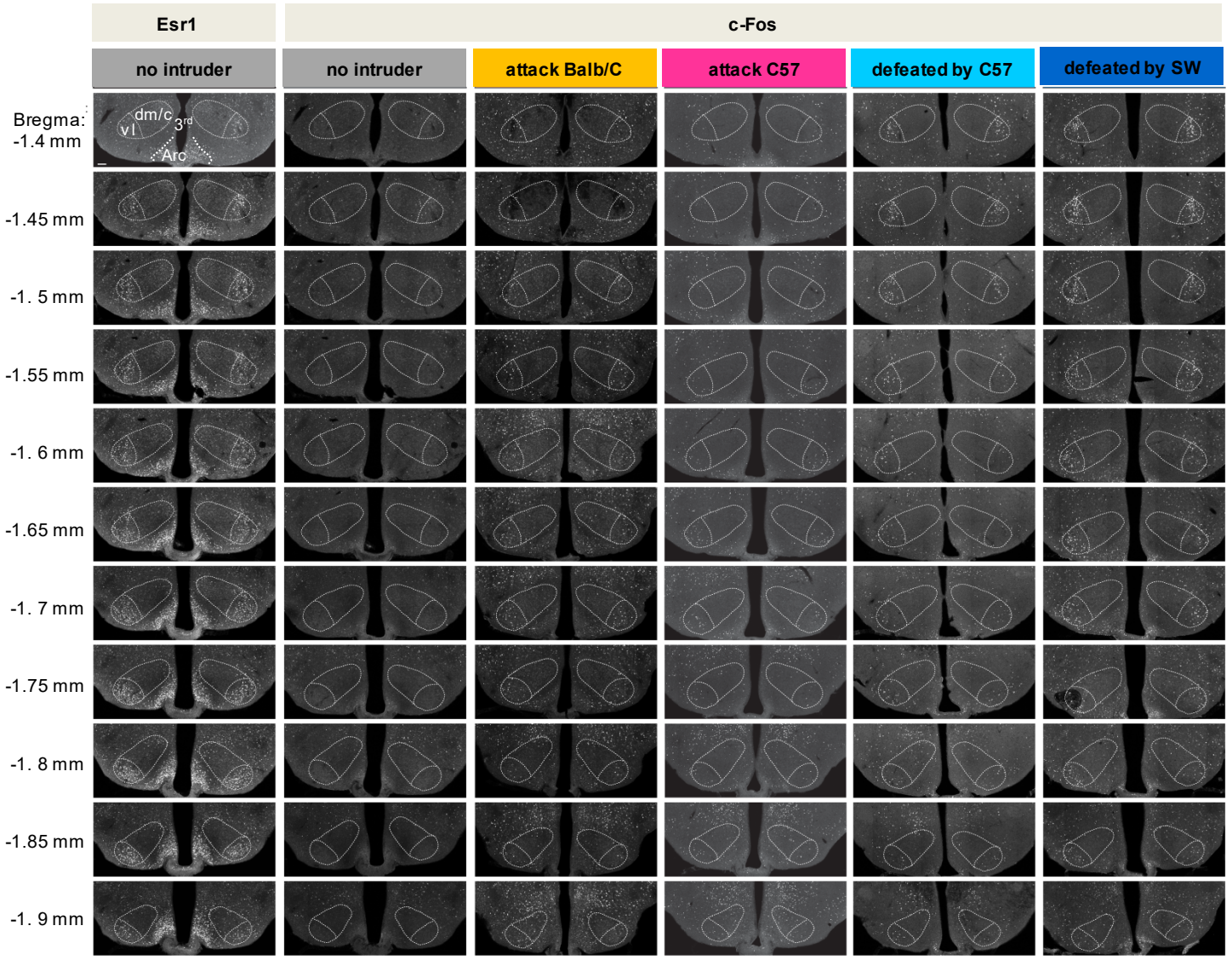

Figure S1. Additional histological images from the c-Fos experiment. Related to Figure 1. Leftmost column shows the Esr1 expression along the anterior-posterior axis of the VMHvl. The remaining columns, from left to right, show Images of c-Fos expression in the VMHvl of a control animal with no intruder exposure and four test animals that interacted with a non-aggressive Balb/C male intruder, a non-aggressive C57BL/6 male intruder, an aggressive C57BL/6 male intruder and an aggressive SW male intruder. Scale bar: 100 µm.

Supplementary Figure 2

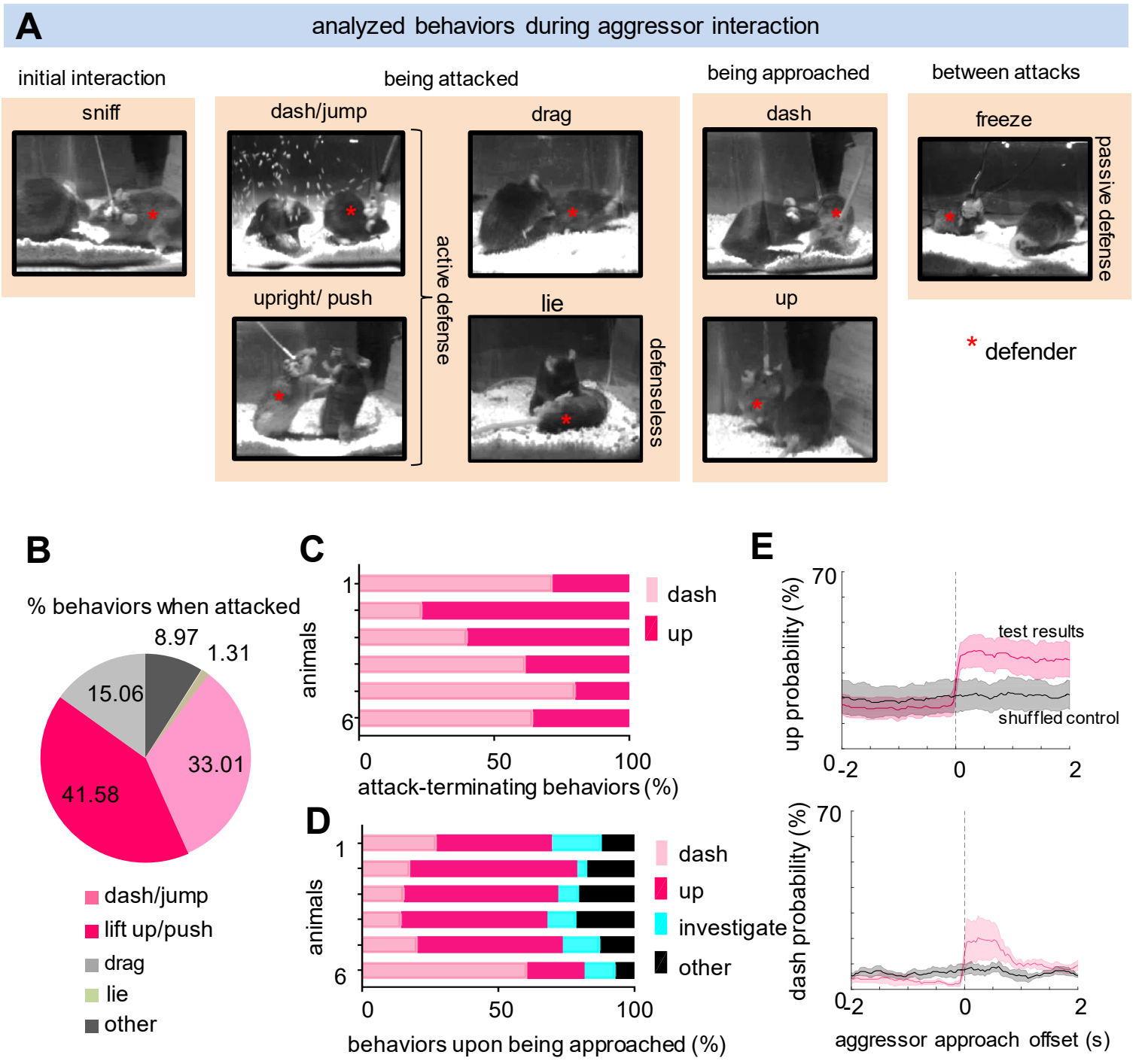

Figure S2. Behavioral characterization of the defending mice. Related to Figure 2.

(A) Images showing the characteristic behaviors during aggressor-defender interaction.

(B) The distribution of behaviors of the defending mouse when being attacked.

(C) The behaviors that were employed by the defending mice to terminate an episode of attack.

(D) The behaviors shown by the defending mice when they were approached by an aggressor.

(E) The probability of upright posture (top) and dashing (bottom) aligned to the aggressor approach offset. Black traces are constructed using shuttled time points. Shades represent ± SEM.

Supplementary Figure 3

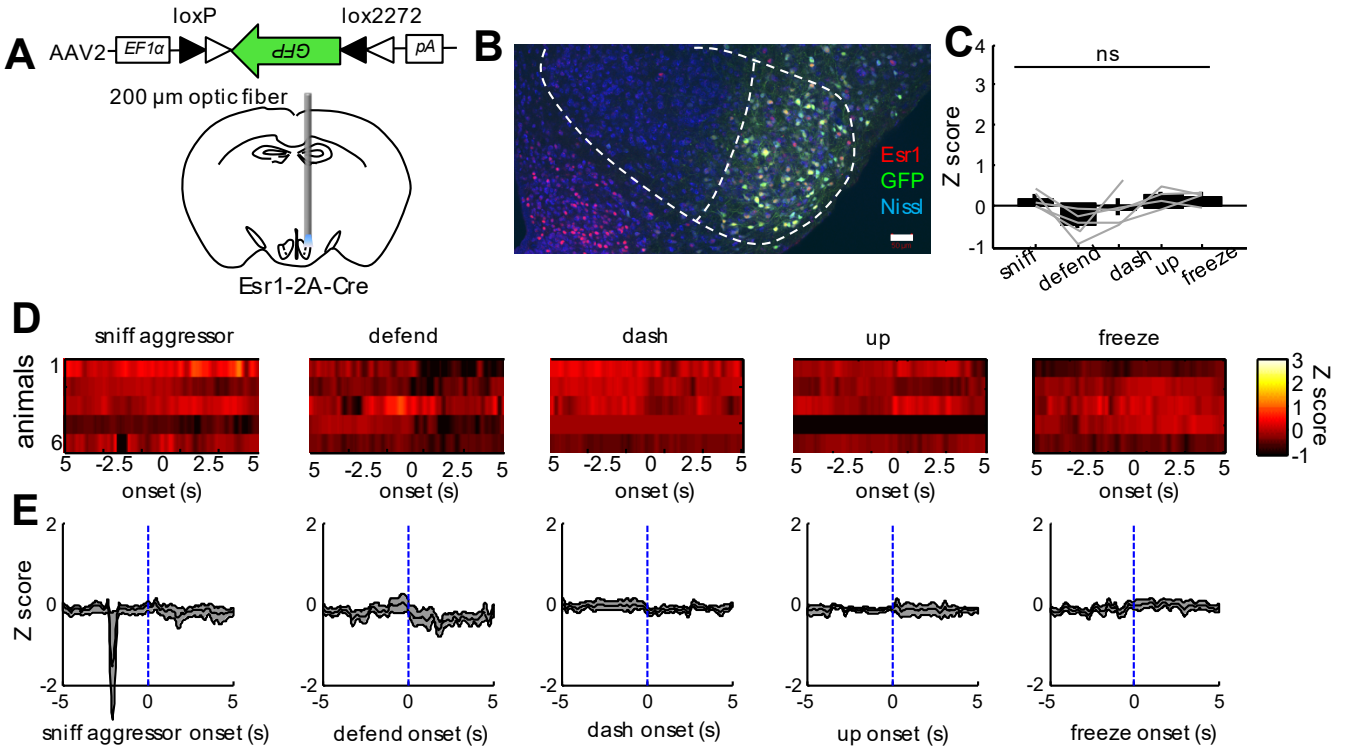

Figure S3. Control data for the fiber photometry recording. Related to Figure 2.

(A) Viral construct and experimental schematics

(B) Expression of GFP (green) is overlapped with Esr1 staining (red). Blue: Nissl. Scale bar: 50 μm

(C) Average GFP signals during various behaviors are not significantly different. One way ANOVA. $p > 0.05$.

(D) Heat maps showing the Z scored GFP signals in the VMHvl of individual animals aligned to the onsets of various behaviors during aggressor interaction.

(E) PETHs showing the average Z scored GFP signals aligned to various behavioral onset across all animals. Shade: ± SEM.

Supplementary Figure 4

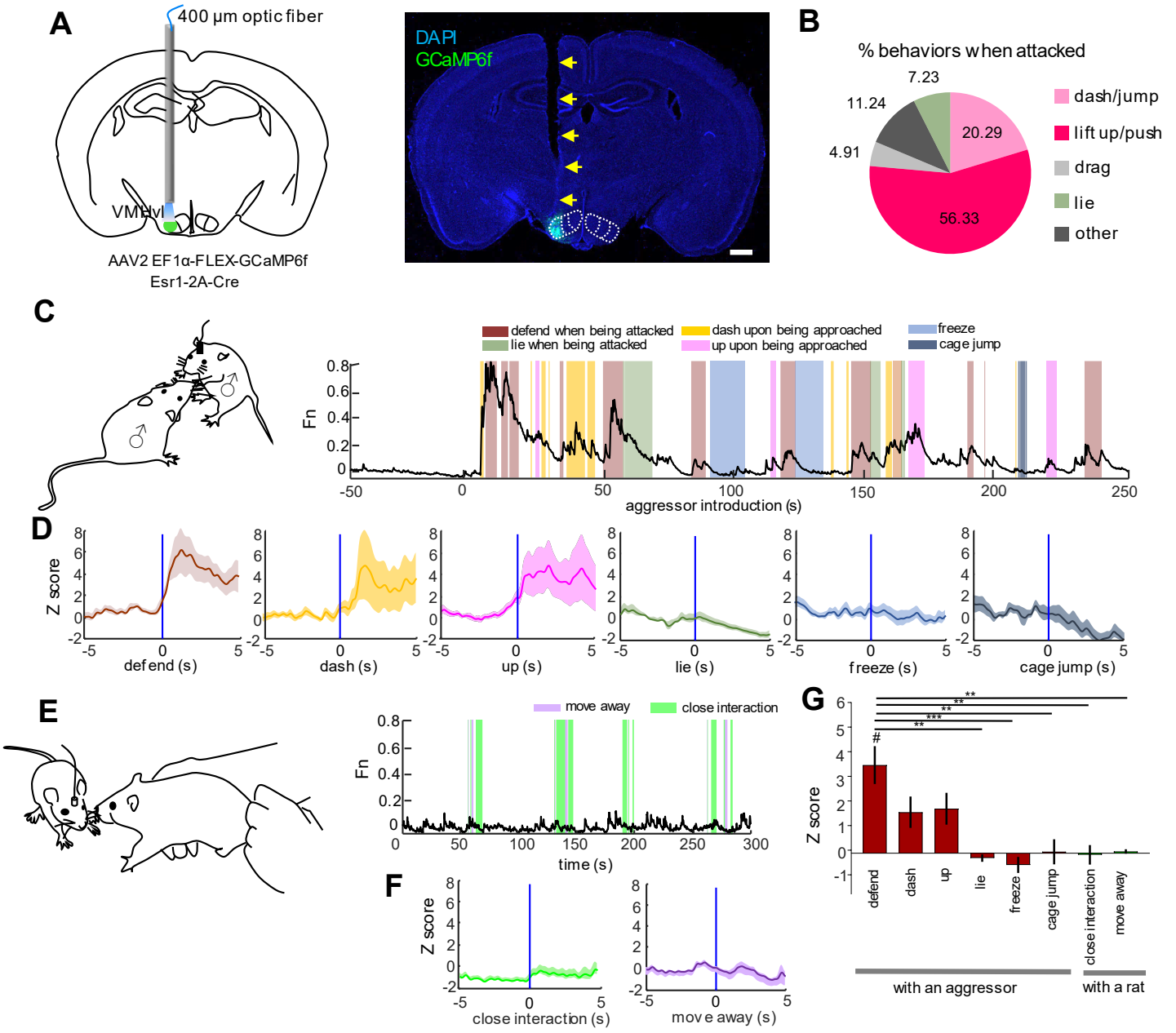

Figure S4: *In vivo* population recording of the VMHvl Esr1+ cells during encounters with an aggressor or a predator. Related to Figure 2.

(A) Schematics of viral injection and implantation location and a representative image showing the optic fiber track (yellow arrows) above the VMHvl and the virally expressed GCaMP6f. Scale bars: 500 μm.

(B) The distribution of behaviors of 3 repeatedly defeated Esr1-2A-Cre C57 mice when they were being attacked.

(C) A representative GCaMP6f trace during encounters with a C57 aggressor. Color shades indicate behavioral events.

(D) PSTHs of Z scored GCaMP6f aligned to various behaviors of the mouse shown in C. Shades represents ± SEM.

(E) The GCaMP6f trace from the same animal as in C and D during close interactions with a hand-held rat. Color shades indicate behavioral events.

(F) PSTHs of Z scored GCaMP6f signal aligned to behavioral onsets of the mouse in the presence of a rat. Shades represent ± SEM.

(G) The average Z scored GCaMP6 responses during various behaviors observed in the presence of a conspecific aggressor or a rat (n = 3). One way ANOVA, Post-hoc pairwise comparison with Tukey-Kramer correction. **p< 0.01, ***p<0.001. One sample *t-test*. # p< 0.05. Error bars: ± SEM.

Supplementary Figure 5

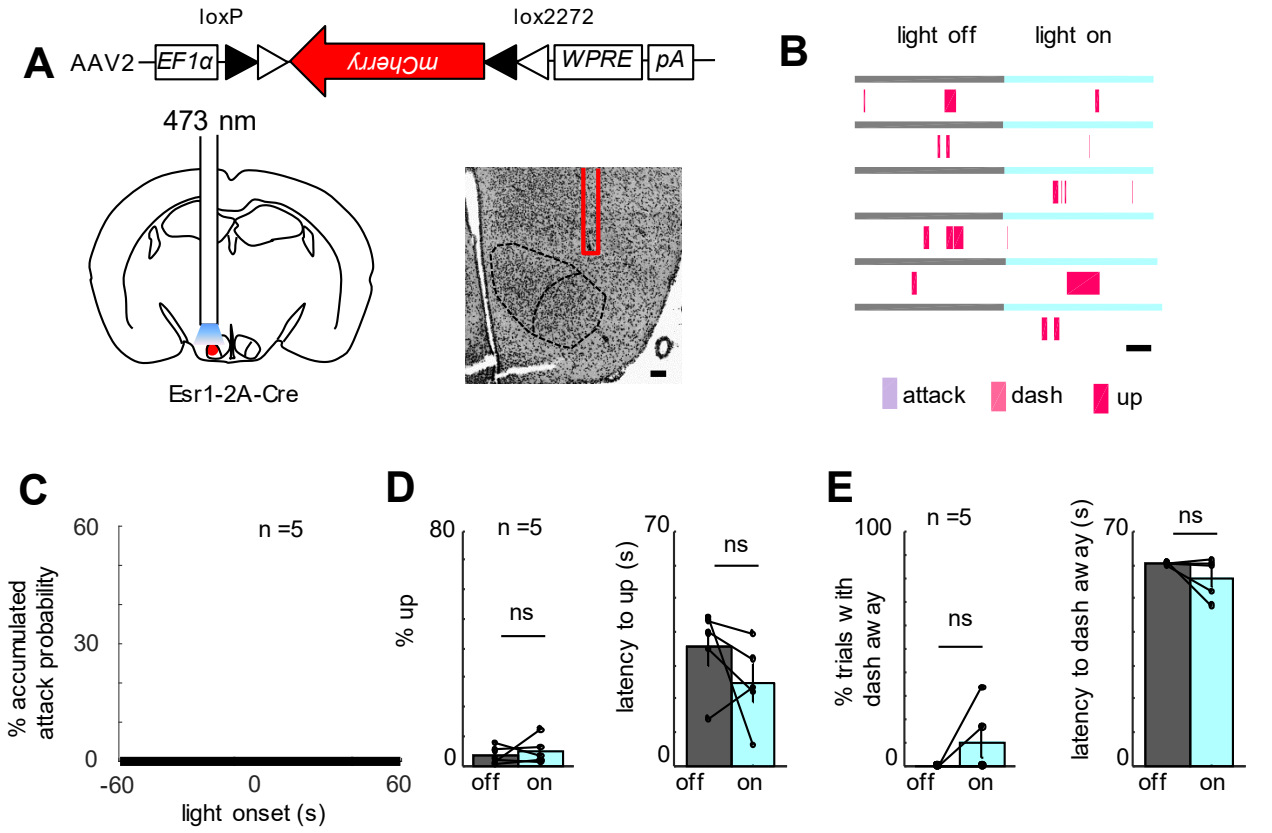

Figure S5. Control data for optogenetic activation experiment. Related to Figure 4.

(A) Viral construct and experimental deign. Image showing the track of a cannula. Scale bar: 100 μm.
(B) Behavior raster during light-on and light-off period from a representative animal. Scale bar: 10 s.
(C) Accumulated probability of attack from 60 s before VMHvl stimulation to the stimulation offset.
(D) Comparison of percentage of upright posture (Left) and latency to upright postures (right) between light-on and light-off periods. Paired t-test.
(E) Comparison of the percentage of trials that animals showed dashing behaviors (left) and latency to dash between light-on and light-off periods. Paired t-test.

Error bars: ± SEM.

Supplementary Figure 6

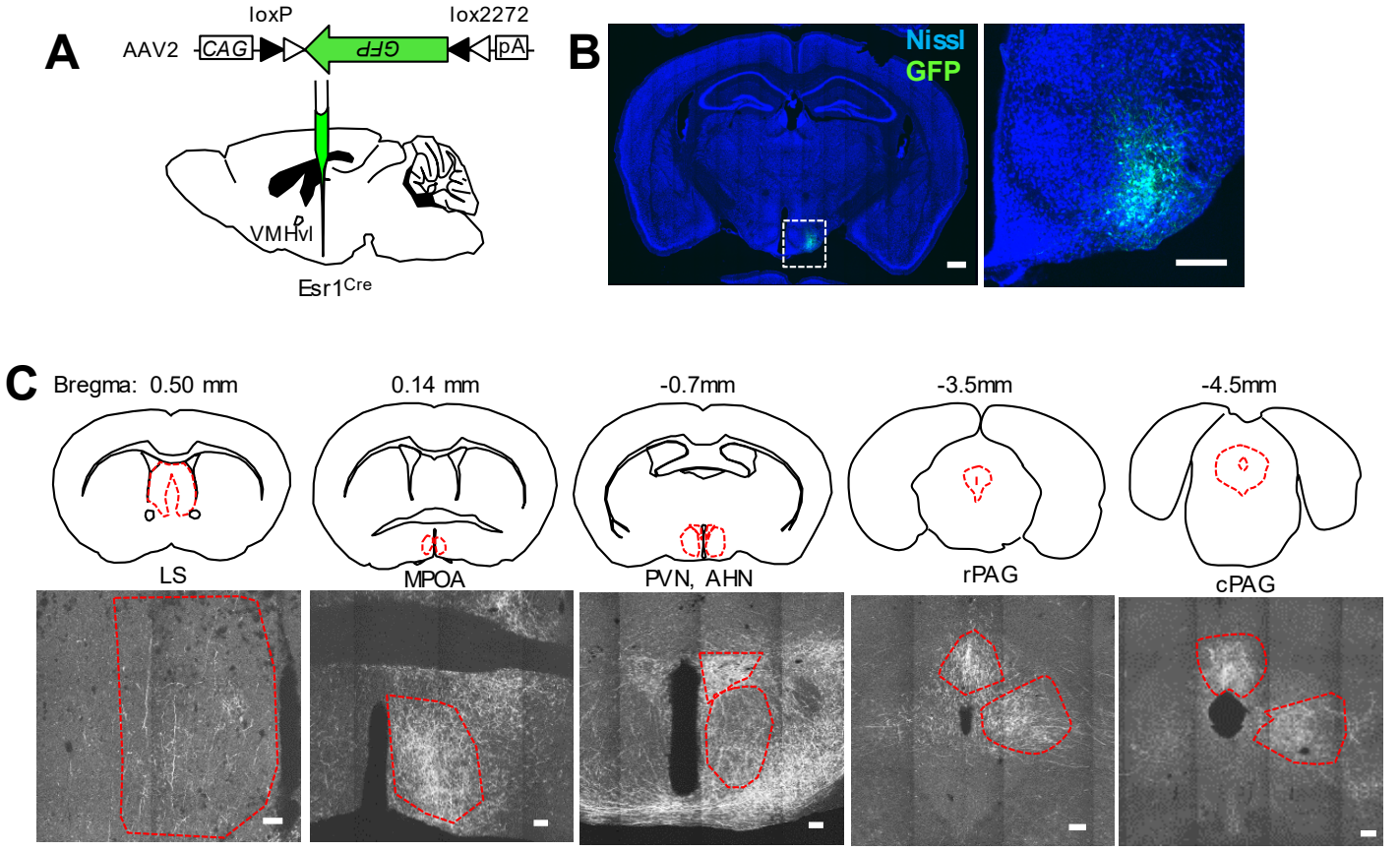

Figure S6. Projection pattern of the VMHvl Esr1⁺ cells. Related to Figure 5.

(A) Viral construct and experimental design.

(B) Example images showing GFP expressing cells in the VMHvl. Right shows the enlarged view of the boxed area. Scale bars: 500 μm (left) and 100 μm (right).

(C) The axons from the VMHvl Esr1+ cells in the LS, MPOA, AHN, PVN, aPAG and cPAG. Dashed red lines indicate the regions that were targeted in the retrograde experiment. Scale bars: 100 μm.

LS: lateral septum; MPOA: medial preoptic area; AHN: anterior hypothalamic nucleus; PVN: periventricular nucleus; rPAG and cPAG: rostral and caudal periaqueductal gray.