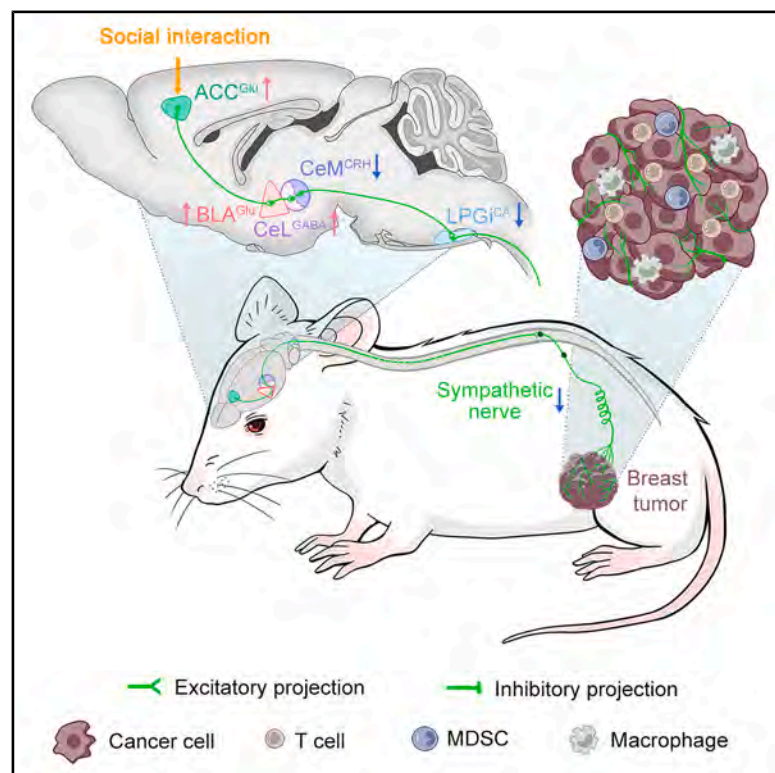


Social interaction in mice suppresses breast cancer progression via a corticoamygdala neural circuit

Graphical abstract



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In brief

Wen et al. reveal that social interaction reduces breast cancer progression by activating $ACC^{Glu} \rightarrow BLA^{Glu}$ circuits, inhibiting intratumoral sympathetic activity. This neural mechanism enhances antitumor immunity, offering new therapeutic insights.

Highlights

- Social interaction inhibits breast cancer progression via neural circuit activation
- ACC^{Glu} neurons are crucial for social interaction's antitumor effect
- $ACC^{Glu} \rightarrow BLA^{Glu}$ circuits mediate the antitumor benefits of social interaction
- Artificial reactivation of social interaction circuits boosts antitumor immunity

Article

Social interaction in mice suppresses breast cancer progression via a corticoamygdala neural circuit

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SUMMARY

Social behaviors are vital for all mammalian species. Clinical observations and epidemiological studies have demonstrated that social interaction (SI) may slow cancer progression and improve patient outcomes. However, the underlying neural mechanisms remain unknown. Here, we found that SI in mouse models suppresses tumor growth through activating glutamatergic inputs from the anterior cingulate cortex (ACC^{Glu}) to the basolateral amygdala (BLA), inhibiting intratumoral sympathetic nerve activity. Chemogenetic inhibition of the SI-activated ACC^{Glu} neurons and ACC^{Glu}→BLA^{Glu} circuits abolished anxiolytic and antitumor effects of SI. Artificial reactivation of these neurons and circuits could mimic the anxiolytic and antitumor effects of SI. Manipulating the ACC^{Glu}→BLA^{Glu} circuits to regulate intratumoral sympathetic activity and norepinephrine (NE) release affected tumor progression by modulating antitumor immunity. Collectively, our study shows that SI activates the corticoamygdala neural circuit, which suppresses cancer progression, offering potential insights into the clinical application of social support in cancer treatment.

INTRODUCTION

Clinicians have long observed that negative emotions can accelerate cancer progression,¹ a finding supported by epidemiological studies.^{2–4} Recent research has revealed that cancer-induced anxiety may accelerate cancer progression via neural pathway.⁵ Social behaviors play an evolutionarily conserved role in mammalian survival and adaptive homeostasis,^{6–10} with human studies revealing robust epidemiological correlations between social engagement patterns and disease outcomes.^{11–13} Central to this framework is social interaction (SI), which clinical studies have associated with both reduced cancer progression rates and improved survival metrics.¹⁴ Notably, several studies in breast cancer demonstrate that SI interventions correlate with decreased anxiety scores and prolonged survival dura-

tions.^{15–17} However, so far there has been little scientific validation of this link, and the mechanisms by which SI mitigates cancer progression remain unknown.

The anterior cingulate cortex (ACC), a subdivision of the medial prefrontal cortex, is particularly critical for emotional responses and social behaviors.¹⁸ The ACC glutamatergic (ACC^{Glu}) neurons project extensively to the basolateral amygdala (BLA), which is involved in anxiety and SI.^{18,19} Previous studies have shown that SI alleviates negative emotions through the medial prefrontal cortex and amygdala.^{10,20} Thus, ACC neurons activated by SI may integrate social information to modulate cancer-induced anxiety and thereby influence cancer progression. In addition, the amygdala contains morphologically and functionally distinct subnuclei with complex interconnectivity, mainly including the BLA, central lateral amygdala (CeL), and

central medial amygdala (CeM). The amygdala microcircuitry is critical for emotion, motivation, and SI.^{18,19,21} Anatomical analyses reveal that BLA neurons are predominantly glutamatergic (BLA^{Glu}), while CeL neurons are GABAergic (CeL^{GABA}).^{22–24} Activation of BLA^{Glu} terminals in the CeL excites CeL^{GABA} neurons, which in turn exert feedforward inhibition on CeM neurons to suppress their activity.⁵ The CeM, which contains corticotropin-releasing hormone (CRH) neurons, is the major output nucleus of the amygdala.^{5,25} Furthermore, our previous study demonstrated that the CeM^{CRH} neurons play an important role in modulating breast cancer progression. Artificial inhibition of CeM^{CRH} neurons markedly reduces anxiety and tumor progression, whereas activation of CeM^{CRH} neurons significantly increases anxiety and tumor progression.⁵ Since BLA^{Glu} neurons receive excitatory input from ACC^{Glu} neurons,^{19,26} we hypothesize that SI suppresses tumor progression via dynamic regulation of the ACC^{Glu}-BLA^{Glu}-CeL^{GABA}-CeM^{CRH} neural circuit.

RESULTS

SI decelerates breast cancer progression

Anxiety is considered a common symptom in patients with breast cancer, which can in turn promote the cancer progression.⁵ Accumulated clinical evidence has suggested that SI could relieve anxious symptoms of breast cancer patients and suppress cancer progression.^{15–17,27} We first quantified SI parameters (interaction bouts and total duration) between tumor-bearing mice and their naive female co-housed familiar littermates (T-N group) or between tumor-bearing mice (T-T group; Figure S1A). Mice in the T-N group exhibited significantly more interaction bouts and total interaction time than those in the T-T group (Figures S1B and S1C). Next, to investigate the mechanistic relationship between SI and tumor progression, we first assessed the impact of SI on anxiety-like behaviors and breast cancer progression in mice. After inoculation with 4T1 cancer cells, mice were randomly assigned to receive daily 1-, 2-, or 24-h SI (accompanied by a naive female co-housed familiar littermate in the home cage) or remain separated and housed singly in the home cage (alone, 0-h SI) for 24 consecutive days (Figure 1A). Notably, daily SI (regardless of duration) significantly reduced anxiety-like behaviors in tumor-bearing mice compared with the 0-h group (Figures 1B–1K). The anxiety-like behaviors were assessed using three validated behavior assays: the light-dark box test (LDT; Figures 1B–1D), the open-field test (OFT; Figures 1E–1G), and the elevated plus maze (EPM) test (Figures 1H–1K).

Given that the intratumor sympathetic activity is positively correlated with anxiety levels and breast tumor progression,⁵ we next assessed the activity of the intratumor sympathetic nerve by examining the tumor tissue NE concentration, which is an indicator of sympathetic activity. ELISA data showed that all three SI protocols (1-, 2-, or 24-h daily interaction) significantly decreased intratumor NE level compared with the alone control group (0-h SI; Figure 1L), suggesting that hyperactive intratumor sympathetic nerve was suppressed by the SI. Histological analyses demonstrated that daily SI (1, 2, or 24 h) markedly decreased the proportion of Ki67⁺ proliferating cells while increasing TUNEL⁺ apoptotic cells (Figure S2). Furthermore,

SI significantly attenuated orthotopic 4T1 tumor growth (Figure 1M), reduced tumor weight (Figures 1N and 1O), and lowered bioluminescence intensity (Figures 1P and 1Q). Consistently, the 1-h SI also significantly decreased the growth (Figures S3A and S3B), weight (Figures S3C and S3D), and luciferase intensity (Figures S3E and S3F) of 4T1 ectopic tumors. Similar results were obtained in female-male SI. We also found that the presence of 1-h SI with a naive male familiar littermate every day also significantly decreased the growth (Figures S4A and S4B), weight (Figures S4C and S4D), and luciferase intensity (Figures S4E and S4F) of 4T1 tumors. Notably, these impressive effects of SI on the tumor progression were not due to the enriched environment (inanimate object) stimulation, since the enriched environment did not significantly affect the tumor progression (Figure S5). Together, these results indicate that the SI leads to decelerated breast cancer progression through inhibition of intratumor sympathetic activity and alleviates the cancer-induced anxiety.

ACC^{Glu} neurons are essential for the antitumor effect of SI

We next investigated the neural mechanism underlying the anti-tumor effect of SI. It is well known that the ACC is located within the dorsomedial prefrontal cortex and contributes to emotional responses and SI.¹⁸ To examine the neural dynamics of ACC neurons during SI, we recorded the neural activity of ACC by performing *in vivo* extracellular recording in freely moving tumor-bearing mice (Figures 2A and 2B). The extracellular recording showed that the firing rate of ACC neurons was increased in response to the SI (Figures 2C–2E). Furthermore, the firing rate of ACC neurons further increased at the onset of social contact during the 1-h SI period (Figure S6). Considering that the glutamatergic neurons were the principal output neuron type in the ACC at both the population and single-cell levels,²⁸ we then specifically focused on investigating the activity of ACC glutamatergic (ACC^{Glu}) neurons of tumor-bearing mice during SI. To determine whether the ACC^{Glu} neurons are involved in the SI, we first assessed the expression of c-Fos (an immediate-early gene marker of neural activity) in the ACC of tumor-bearing mice (Figure S7A). Immunofluorescence staining showed that ACC^{Glu} neurons had significantly higher c-Fos expression in SI mice than in alone control mice (Figures S7B–S7D). Notably, compared with alone naive mice, the SI between naive mice induced more c-Fos expression in the ACC (Figure S8). To further real-time examine the neural dynamics of ACC^{Glu} neurons in tumor-bearing mice during SI, we also performed fiber photometry 24 days after 4T1 cell inoculation (Figures 2F and 2G). Virus rAAV2/9-CaMKIIα-GCaMP6s (expressing the calcium sensor GCaMP6s) or rAAV2/9-CaMKIIα-EGFP was injected into the right ACC, and then one optical fiber was implanted above the virus injection site (Figures 2H and 2I). Similarly, introduction of the naive female co-housed familiar littermate increased the activity of ACC^{Glu} neurons in tumor-bearing mice, and the activity remained elevated during the SI (Figures 2J–2L). Importantly, the activity of ACC^{Glu} neurons further increased at the onset of social contact during 1-h SI period (Figure S9). Thus, these data suggest that the SI activates the ACC^{Glu} neurons in tumor-bearing mice.

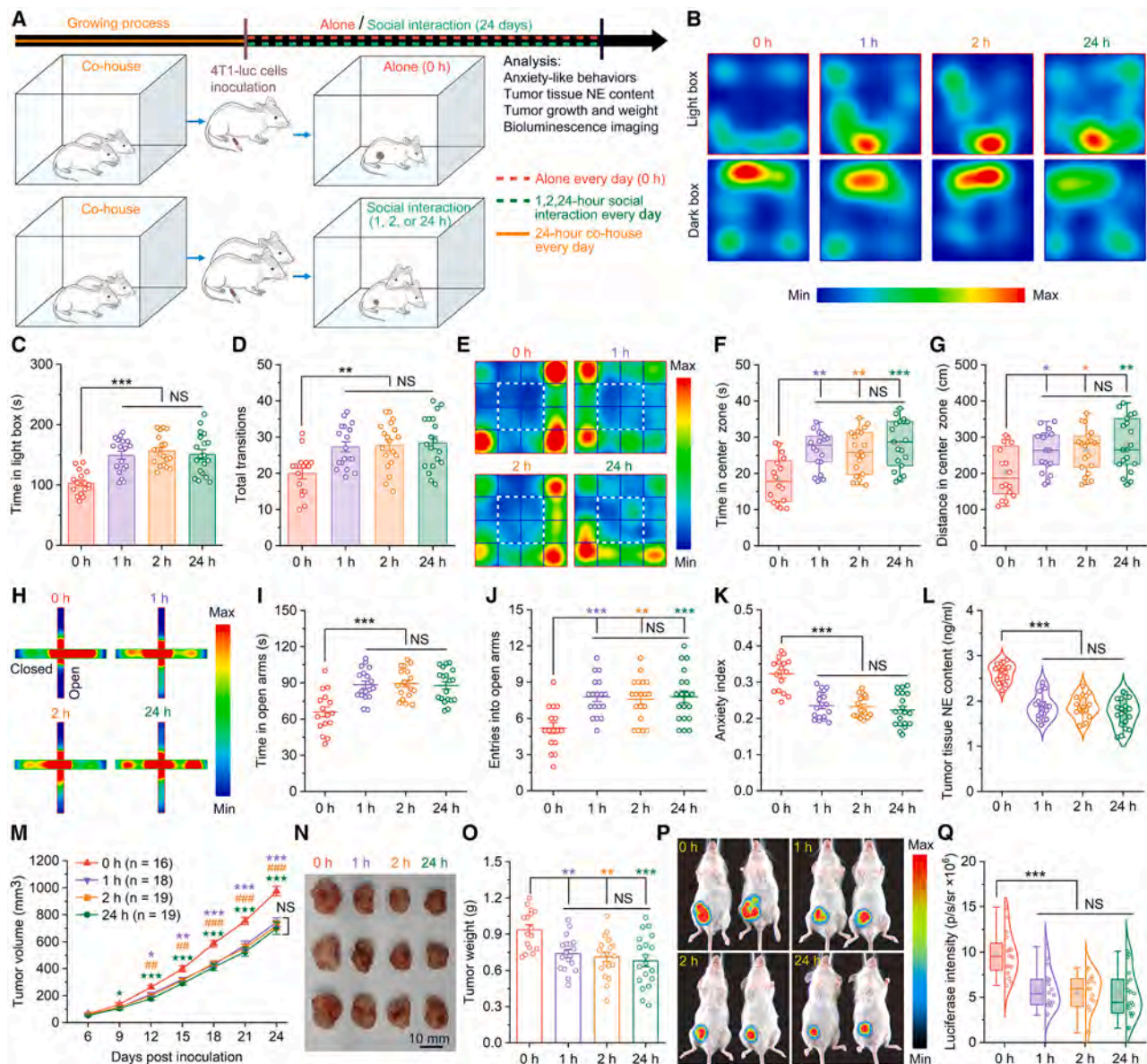


Figure 1. SI relieves anxiety and suppresses breast cancer progression

(A) Schematic showing the SI protocol and timeline.

(B–D) SI increased the time spent in the light box and total number of transitions as measured by LTD.

(E–G) SI increased the time spent in the center zone and distance traveled in the center zone as measured by OFT.

(H–K) SI increased the time spent in the open arms, entries into the open arms, and anxiety index as measured by EPM.

(L) SI decreased NE content of 4T1 tumor tissue.

(M) SI suppressed the tumor growth of 4T1 tumor-bearing mice.

(N) Representative tumors dissected from 4T1 tumor-bearing mice.

(O) SI reduced 4T1 tumor weight.

(P) Representative bioluminescence images of 4T1 tumor-bearing mice.

(Q) SI reduced the luciferase intensity of 4T1 tumors.

0 h, *n* = 16 mice; 1 h, *n* = 18 mice; 2 h, *n* = 19 mice; 24 h, *n* = 19 mice. Data are presented as the mean ± SEM, except in box plots (F, G, L, and Q), which denote minimum, first quartile, median, third quartile, and maximum values. The triangles in box plots represent the mean. NS, not significant; **p* < 0.05, ***p* < 0.01, and ****p* < 0.001. (C, D, F, G, I–L, O, and Q) One-way ANOVA followed by Tukey post hoc test. (M) Two-way repeated-measures ANOVA followed by Tukey post hoc test.

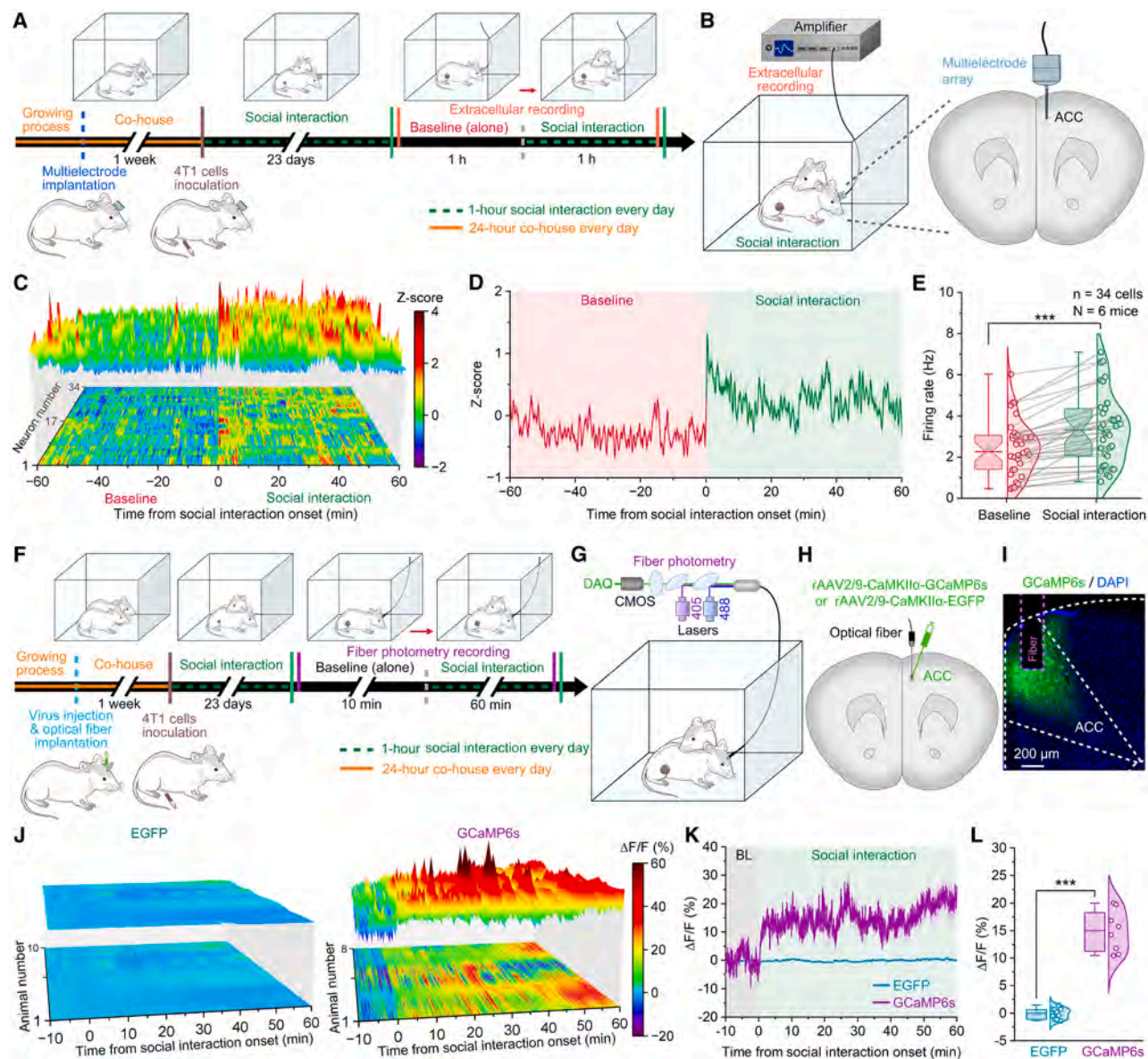


Figure 2. ACC^{Glu} neurons respond to SI in tumor-bearing mice

(A) Experimental protocol of multi-electrode recording.
 (B) Schematic for multi-electrode recording in ACC.
 (C) Heatmaps showing the Z score of all recorded units.
 (D) Averaged Z scored firing rate for ACC neurons showing increased firing rate in response to SI, with shaded areas indicating the SEM.
 (E) Summarized data for averaged Z scored firing rate during the two periods ($n = 34$ cells from 6 mice).
 (F and G) Timeline and schematic for fiber photometry recording of ACC^{Glu} neurons.
 (H) Schematic showing virus injection and optical fiber implantation.
 (I) A representative image of virus expression and fiber position in the ACC.
 (J) Heatmaps show the average changes in fluorescence signal of ACC^{Glu} neurons in the EGFP and GCaMP6s groups.
 (K) Average fluorescence signal of ACC^{Glu} neurons in the EGFP and GCaMP6s groups, with shaded areas indicating the SEM.
 (L) Quantification of the mean $\Delta F/F$ value between the EGFP and GCaMP6s groups (EGFP, $n = 10$ mice; GCaMP6s, $n = 8$ mice).
 Box plots denote minimum, first quartile, median, third quartile, and maximum values. The triangles in box plots represent the mean. *** $p < 0.001$. (E) Two-tailed paired Student's t test. (L) Two-tailed unpaired Student's t test.

In addition, to clarify whether the activity of ACC^{Glu} neurons is required for supporting SI-induced anxiolytic and antitumor effects, we employed a chemogenetic approach to suppress

the ACC^{Glu} neuron activity in tumor-bearing mice during SI. Mice were injected with rAAV2/9-CaMKII α -hM4Di-mCherry or rAAV2/9-CaMKII α -mCherry into bilateral ACC. Approximately

3 weeks later, we inoculated 4T1-luc tumor cells into the mammary fat pad of the virus-expressed mice. Then, the tumor-bearing mice were administered 1-h SI per day following intraperitoneal injection with the hM4Di agent, clozapine-N-oxide (CNO), for 24 consecutive days (Figures S10A–S10C). The behavior results of the LDT, OFT, and EPM demonstrated that chemogenetic inhibition of ACC^{Glu} neurons expressing hM4Di during SI period significantly abolished the SI-induced anxiolytic effect compared with the ACC^{Glu} neurons expressing mCherry (Figures S10D–S10M). The same chemogenetic manipulation also significantly increased the intratumor NE content (Figure S10N). More importantly, immunofluorescence staining showed that chemogenetic inhibition of ACC^{Glu} neurons resulted in a significant increase in the percentage of Ki67⁺ cells but a significant decrease in the percentage of TUNEL⁺ cells in 4T1 tumors (Figure S11). Furthermore, the inhibition of ACC^{Glu} neurons during the SI period not only significantly accelerated the 4T1 tumor growth rate (Figure S10O) but also significantly increased the weight (Figures S10P and S10Q) and luciferase intensity (Figures S10R and S10S) of 4T1 tumors, suggesting that the antitumor effect of SI was reversed. These results indicate that ACC^{Glu} neuron activity is essential for mediating the anxiolytic and antitumor effects of SI.

A subset of ACC^{Glu} neurons mediate the antitumor effect of SI

Because only a subset of ACC^{Glu} neurons was activated by SI, we subsequently employed the c-Fos Tet-Off system to label SI-responsive (i.e., SI-tagged) ACC^{Glu} neurons and further explored the functional role of this ACC subset in the anxiolytic and antitumor effects of SI. The Tet-Off system combined with chemogenetics and optogenetics is used to label and manipulate a subset of event-related neurons, which is particularly advantageous for studying the functional role of specific neuronal populations in complex behaviors like SI.^{29,30} Similar to our previous work,^{30,31} we co-injected rAAV2/9-CaMKII α -Cre and rAAV2/9-c-Fos-DIO-tTA with either rAAV2/9-TRE-tight-jGCaMP7s (expressing the calcium sensor jGCaMP7s) or rAAV2/9-TRE-tight-EGFP (as a control) into the right ACC and implanted an optical fiber above the virus injection site. After surgery, each mouse was co-housed with a naive female familiar littermate (companion mouse) and was fed with a doxycycline (Dox) diet to prevent the expression of jGCaMP7s or EGFP. 2 weeks post-surgery, we inoculated 4T1-luc tumor cells into the mammary fat pad of the virus-expressed mice and provided the tumor mice with 1-h SI per day for 9 days. Then, for labeling SI-tagged ACC^{Glu} neurons with jGCaMP7s or EGFP, all mice were taken off Dox for 3 days, while the tumor mice still experienced 1-h SI per day (Figures S12A–S12D). To record the activity of SI-tagged ACC^{Glu} neurons during SI, we performed fiber photometry 24 days after 4T1 cell inoculation (Figure S12A) and found that the neural activity of SI-tagged ACC^{Glu} neurons in tumor-bearing mice significantly increased during SI (Figures S12E–S12H).

We next asked whether the SI-tagged ACC^{Glu} neurons play a vital role in the SI-induced anxiolytic and antitumor effects. To address this question, we employed chemogenetics to inhibit the activity of SI-tagged ACC^{Glu} neurons during SI. We bilaterally

injected rAAV2/9-CaMKII α -Cre and rAAV2/9-c-Fos-DIO-tTA with either rAAV2/9-TRE-tight-hM4Di-mCherry or rAAV2/9-TRE-tight-mCherry into the rACC. Then the mice underwent the same experimental protocol, and the SI-tagged ACC^{Glu} neurons were labeled with hM4Di or mCherry (Figures 3A–3C). As expected, our results showed that the SI-induced anxiolytic effect (Figures 3D–3M) was significantly disrupted, and the intratumor NE content (Figure 3N) was increased after application of CNO to suppress the activity of SI-tagged ACC^{Glu} neurons during the SI period. The same chemogenetic manipulation significantly increased the percentage of Ki67⁺ cells and decreased the percentage of TUNEL⁺ cells in 4T1 tumors (Figure S13). Moreover, the sustained chemogenetic inhibition also significantly increased the growth rate (Figure 3O), weight (Figures 3P and 3Q), and luciferase intensity (Figures 3R and 3S) of 4T1 tumors, suggesting that the antitumor effect of SI was reversed.

In separate experiments, we also tested whether reactivation of the SI-tagged ACC^{Glu} neurons was sufficient to mimic the anxiolytic and antitumor effects of SI. To reactivate the SI-tagged ACC^{Glu} neurons, we injected rAAV2/9-CaMKII α -Cre and rAAV2/9-c-Fos-DIO-tTA with either rAAV2/9-TRE-tight-hM3Dq-mCherry or rAAV2/9-TRE-tight-mCherry into the bilateral rACC. Then the mice underwent the same experimental protocol, and the SI-tagged ACC^{Glu} neurons were labeled with hM3Dq or mCherry. After SI labeling, we reactivated these SI-tagged ACC^{Glu} neurons by CNO injection once per day for 12 days to mimic SI (Figures 4A–4C). As expected, 12-day chemogenetic reactivation significantly reduced the anxiety-like behaviors of the 4T1 tumor-bearing mice (Figures 4D–4M) and intratumor NE content (Figure 4N). In addition, this artificial reactivation significantly decreased the percentage of Ki67⁺ cells and increased the percentage of TUNEL⁺ cells in 4T1 tumors (Figure S14). Remarkably, the sustained artificial reactivation also decreased the growth rate (Figure 4O), weight (Figures 4P and 4Q), and luciferase intensity (Figures 4R and 4S) of 4T1 tumors, suggesting that the reactivation of the SI-tagged ACC^{Glu} neurons was sufficient to mimic the anxiolytic and antitumor effects of SI. Taken together, these results indicate that the activity of SI-activated ACC^{Glu} neurons is necessary and sufficient for mediating the anxiolytic and antitumor effects of SI.

Neural circuits linking SI and decelerated progression of breast cancer

ACC^{Glu} neurons project axonal terminals into numerous downstream brain areas.³² Thus, the neural circuits that mediate the anxiolytic and antitumor effects of SI remain largely unknown. The BLA is embedded in a highly connected emotional and social brain network and receives robust inputs from the ACC.^{18,19} To further confirm the ACC^{Glu}→BLA projection, we injected anterograde virus rAAV2/9-CaMKII α -mCherry into the rACC (Figures S15A and S15B). Histological results showed that the mCherry-positive projection fibers from rACC^{Glu} neurons were densely distributed in BLA (Figure S15C), suggesting that rACC^{Glu} neurons send strong projections to BLA.

Next, to selectively examine the response of SI-tagged BLA-projecting ACC^{Glu} neurons to SI in tumor-bearing mice, we

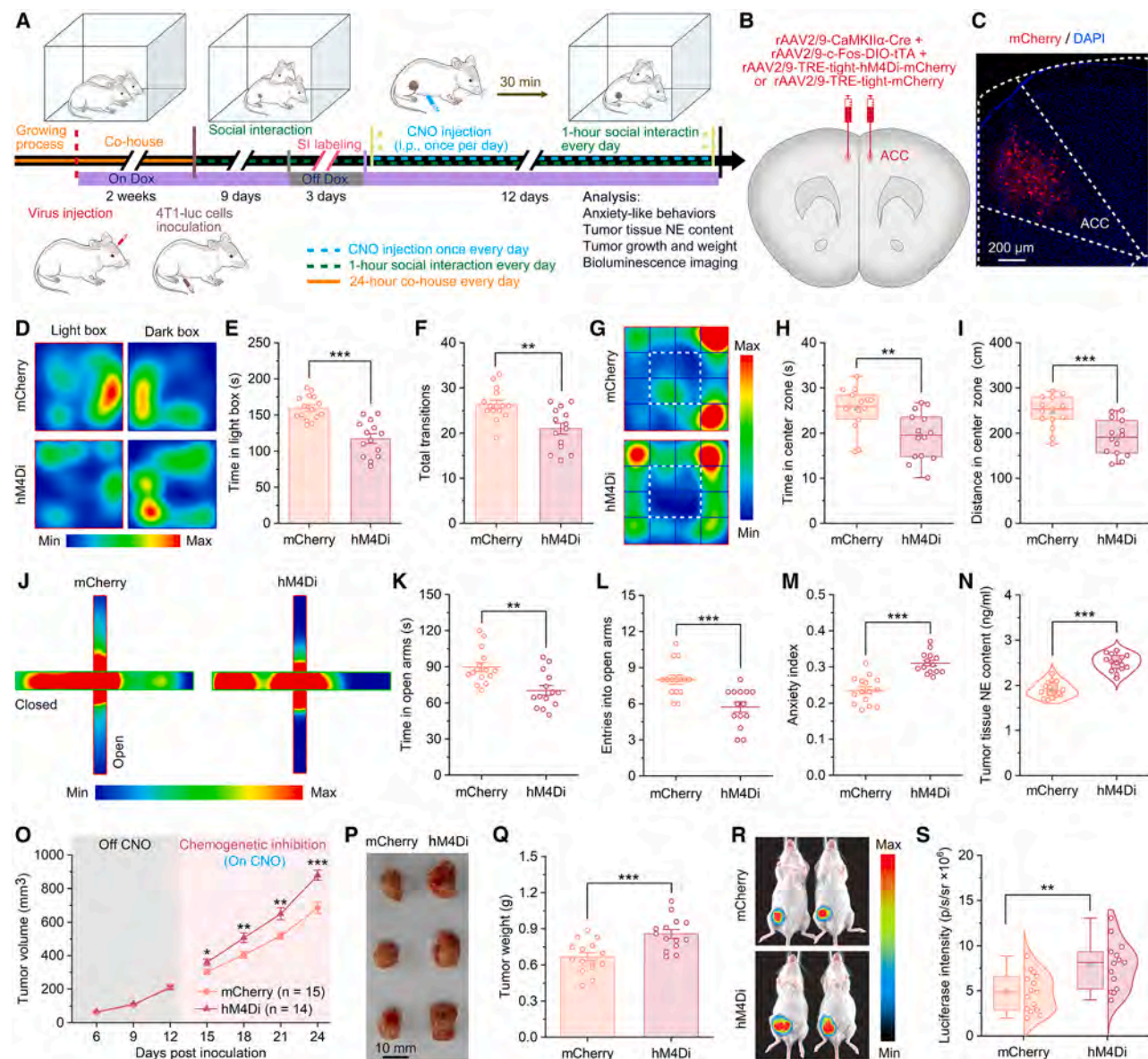


Figure 3. Chemogenetic inhibition of SI-tagged ACC^{Glu} neurons eliminates anxiolytic and antitumor effects of SI

(A) Schematic and timeline for chemogenetic inhibition of SI-tagged ACC^{Glu} neurons.

(B) Schematic diagram showing injection of virus into the bilateral ACC.

(C) Representative image of mCherry expression in SI-activated ACC^{Glu} neurons.

(D–M) Representative heatmaps and summary data of the mCherry and hM4Di groups in the LDT (D–F), OFT (G–I), and EPM test (J–M).

(N) Chemogenetic inhibition of SI-tagged ACC^{Glu} neurons increased the intratumor NE content.

(O) Chemogenetic inhibition accelerated 4T1 tumor growth.

(P) Representative tumors dissected from mice in the mCherry and hM4Di groups.

(Q) Chemogenetic inhibition increased 4T1 tumor weight.

(R) Representative bioluminescence images showing the 4T1 tumor-bearing mice of the mCherry and hM4Di groups.

(S) Chemogenetic inhibition increased the luciferase intensity of 4T1 tumors.

mCherry, $n = 15$ mice; hM4Di, $n = 14$ mice. Data are presented as the mean $\pm$ SEM, except in box plots (H, I, N, and S), which denote minimum, first quartile, median, third quartile, and maximum values. The triangles in box plots represent the mean. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$. (E, F, H, I, K–N, Q, and S) Two-tailed unpaired Student's t test. (O) Two-way repeated-measures ANOVA followed by separate one-way ANOVA.

injected retrogradely transported retro-AAV expressing Cre recombinase (rAAV2/retro-CaMKII α -Cre) into the bilateral BLA and rAAV2/9-c-Fos-DIO-tTA together with rAAV2/9-TRE-

tight-jGCaMP7s or rAAV2/9-TRE-tight-EGFP into the bilateral ACC. After SI labeling, we employed fiber photometry 24 days after 4T1 cell inoculation (Figures S15D–S15G) and

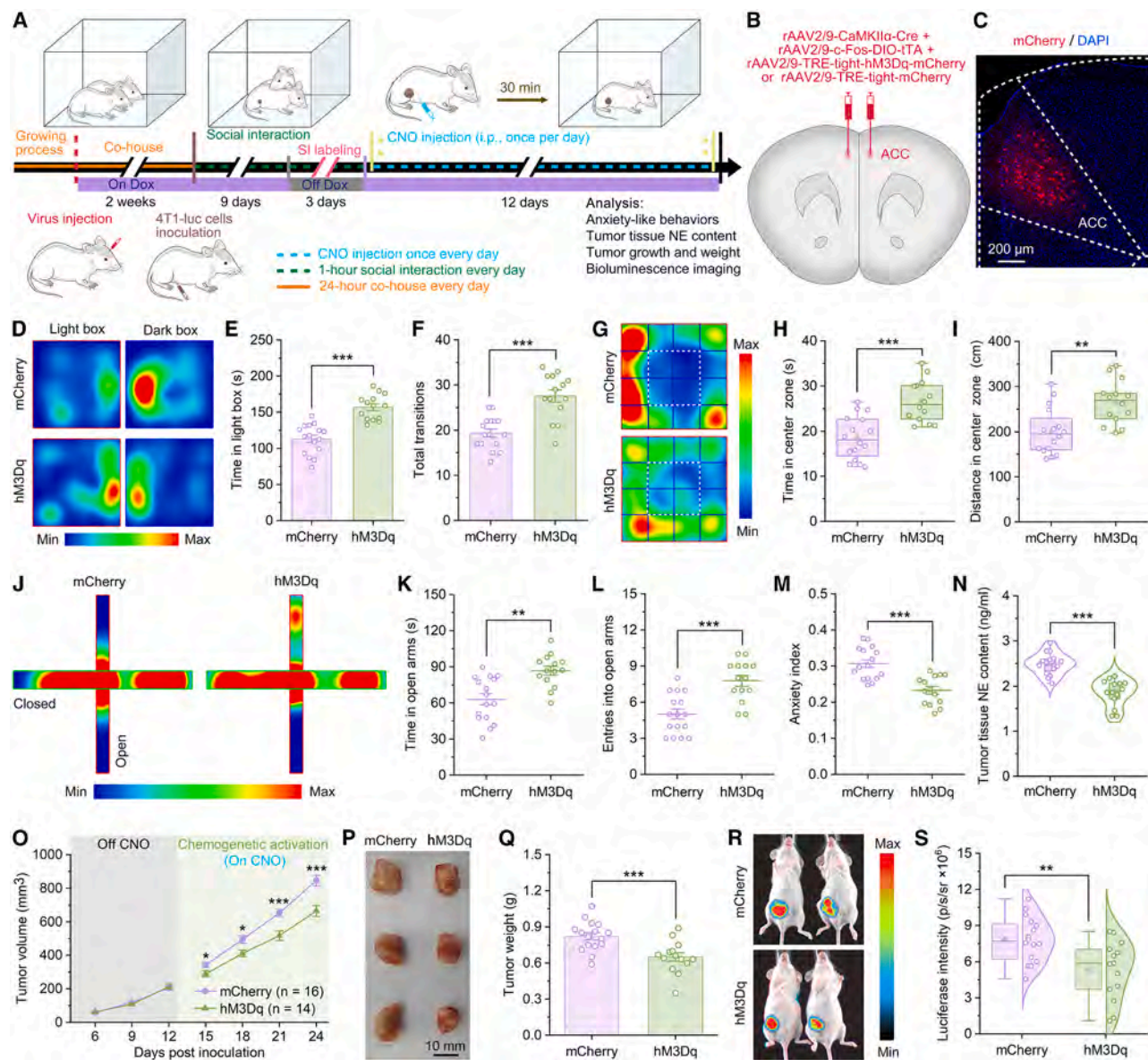


Figure 4. Chemogenetic reactivation of SI-tagged ACC^{Glu} neurons is sufficient to mimic the anxiolytic and antitumor effects of SI

(A) Schematic and timeline of the experimental design.
 (B) Schematic showing injection of virus into the ACC.
 (C) Representative image showing mCherry expression in SI-activated ACC neurons.
 (D–M) Representative heatmaps and summary data of the mCherry and hM3Dq groups in the LDT (D–F), the OFT (G–I), and the EPM test (J–M).
 (N) Chemogenetic reactivation of SI-tagged ACC^{Glu} neurons decreased the intratumor NE content.
 (O) Chemogenetic reactivation decelerated 4T1 tumor growth.
 (P) Representative image of tumors dissected from mice in the mCherry and hM3Dq groups.
 (Q) Chemogenetic reactivation decreased 4T1 tumor weight.
 (R) Representative bioluminescence images showing mice in the mCherry and hM3Dq groups.
 (S) Chemogenetic reactivation significantly decreased the luciferase intensity of 4T1 tumors.
 mCherry, *n* = 16 mice; hM3Dq, *n* = 14 mice. Data are presented as the mean ± SEM, except in box plots (H, I, N, and S), which denote minimum, first quartile, median, third quartile, and maximum values. The triangles in box plots represent the mean. **p* < 0.05, ***p* < 0.01, and ****p* < 0.001. (E, F, H, I, K–N, Q, and S) Two-tailed unpaired Student's *t* test. (O) Two-way repeated-measures ANOVA followed by separate one-way ANOVA.

found that the activity of SI-tagged BLA-projecting ACC^{Glu} neurons in tumor-bearing mice significantly increased during SI (Figures S15H–S15K).

To validate whether the SI-activated ACC^{Glu}→BLA projection participates in the anxiolytic and antitumor effects of SI, we injected rAAV2/retro-CaMKIIα-Cre into the bilateral BLA and

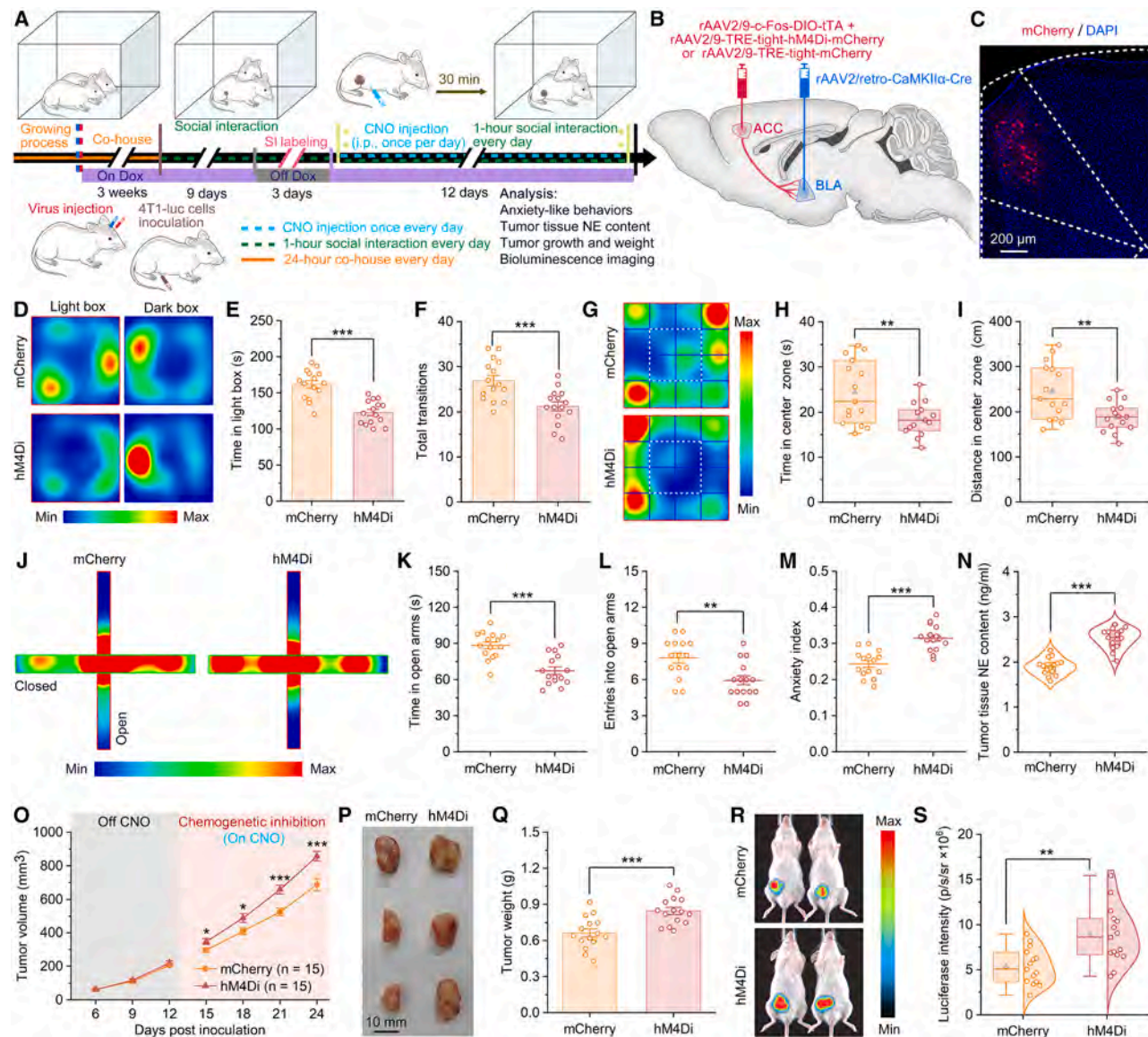


Figure 5. Chemogenetic inhibition of SI-tagged BLA-projecting ACC^{Glu} neurons impairs anxiolytic and antitumor effects of SI

(A) Schematic and timeline for chemogenetic inhibition of SI-tagged BLA-projecting ACC^{Glu} neurons.

(B) Schematic diagram showing virus injection.

(C) Representative image of mCherry expression in SI-activated BLA-projecting ACC^{Glu} neurons.

(D–M) Representative heatmaps and summary data of the mCherry and hM4Di groups in the LDT (D–F), OFT (G–I), and EPM test (J–M).

(N) Chemogenetic inhibition of SI-tagged BLA-projecting ACC^{Glu} neurons increased the intratumor NE content.

(O) Chemogenetic inhibition accelerated 4T1 tumor growth.

(P) Representative image showing tumors dissected from mice in the mCherry and hM4Di groups.

(Q) Chemogenetic inhibition increased 4T1 tumor weight.

(R) Representative bioluminescence images showing the 4T1 tumor-bearing mice of the mCherry and hM4Di groups.

(S) Chemogenetic inhibition increased the luciferase intensity of 4T1 tumors.

n = 15 mice per group. Data are presented as the mean ± SEM, except in box plots (H, I, N, and S), which denote minimum, first quartile, median, third quartile, and maximum values. The triangles in box plots represent the mean. **p* < 0.05, ***p* < 0.01, and ****p* < 0.001. (E, F, H, I, K–N, Q, and S) Two-tailed unpaired Student's *t* test. (O) Two-way repeated-measures ANOVA followed by separate one-way ANOVA.

rAAV2/9-c-Fos-DIO-tTA together with rAAV2/9-TRE-tight-hM4Di-mCherry or rAAV2/9-TRE-tight-mCherry into the bilateral ACC (Figures 5A–5C). As expected, the SI-induced anxiolytic effect (Figures 5D–5M) was significantly blocked, and the intratu-

mor NE content (Figure 5N) was increased after injection of CNO to inhibit the activity of SI-tagged BLA-projecting ACC^{Glu} neurons during the SI period. The suppression of SI-tagged BLA-projecting ACC^{Glu} neurons significantly increased the

percentage of Ki67⁺ cells and decreased the percentage of TUNEL⁺ cells in 4T1 tumors (Figure S16). Moreover, the chemogenetic inhibition also significantly increased the growth rate (Figure 5O), weight (Figures 5P and 5Q), and luciferase intensity (Figures 5R and 5S) of 4T1 tumors, suggesting that the antitumor effect of SI was reversed.

Next, we also tested whether reactivation of the SI-activated ACC^{Glu}→BLA projection was sufficient to mimic the anxiolytic and antitumor effects of SI. To reactivate the SI-tagged BLA-projecting ACC^{Glu} neurons, we injected rAAV2/retro-CaMKIIα-Cre into the bilateral BLA and rAAV2/9-c-Fos-DIO-tTA together with rAAV2/9-TRE-tight-hM3Dq-mCherry or rAAV2/9-TRE-tight-mCherry into the bilateral ACC (Figures 6A–6C). After SI labeling, we reactivated the SI-tagged BLA-projecting ACC^{Glu} neurons by repeated CNO injection once per day for 12 days to mimic SI (Figure 6A). As expected, 12-day chemogenetic reactivation significantly reduced the anxiety-like behaviors of the 4T1 tumor-bearing mice (Figures 6D–6M) and intratumor NE content (Figure 6N). In addition, this artificial reactivation significantly decreased the percentage of Ki67⁺ cells and increased the percentage of TUNEL⁺ cells in 4T1 tumors (Figure S17). Similarly, the sustained artificial reactivation also decreased the growth rate (Figure 6O), weight (Figures 6P and 6Q), and luciferase intensity (Figures 6R and 6S) of 4T1 tumors, suggesting that the reactivation of the SI-activated ACC^{Glu}→BLA projection was sufficient to mimic the anxiolytic and antitumor effects of SI.

Additionally, in the MMTV-PyMT mouse model, we also examined whether reactivation of the projecting ACC^{Glu} neurons by repeated CNO injection once per day for 15 days to mimic SI (Figures S18A–S18C). Consistently, 15-day chemogenetic reactivation significantly decreased the anxiety-like behaviors of the PyMT tumor-bearing mice (Figures S18D–S18M) and intratumor NE content (Figure S18N). Moreover, this reactivation significantly decreased the percentage of Ki67⁺ cells and increased the percentage of TUNEL⁺ cells in PyMT tumors (Figure S19). More importantly, the sustained reactivation also decreased the growth rate (Figure S18O) and weight (Figures S18P and S18Q) of PyMT tumors. Taken together, these results suggest that the activity of SI-activated ACC^{Glu}→BLA projection is necessary and sufficient for mediating the anxiolytic and antitumor effects of SI.

The amygdala is a collection of nuclei, mainly including the BLA, CeL, and CeM. The amygdala microcircuitry is critical for emotion, motivation, and SI.^{18,19,21} More importantly, the activation of BLA^{Glu} neurons could activate CeL^{GABA} neurons and apply feedforward inhibition onto CeM output neurons.²² Moreover, our previous study has shown that the CeM^{CRH} neurons play a pivotal role in modulating the breast cancer progression.⁵ The BLA^{Glu} neurons receive excitatory afferents from ACC^{Glu} neurons.^{19,26} Therefore, we considered that the SI-activated ACC^{Glu}→BLA projection might increase the activity of BLA^{Glu} neurons and enhance the feedforward inhibition onto hyperactive CeM^{CRH} neurons via CeL^{GABA} neurons. To test this hypothesis, we conducted *in vivo* fiber photometry to measure the activity of BLA^{Glu} (Figures S20A and S20B), CeL^{GABA} (Figures S22A and S22B), and CeM^{CRH} (Figures S24A and S24B) neurons that responded to the SI

by expressing GCaMP6s. During the SI, the BLA^{Glu} (Figures S20C–S20H) and CeL^{GABA} (Figures S22C–S22H) neurons had significant increases in calcium signals, whereas the CeM^{CRH} (Figures S24C–S24H) neurons showed a significant decrease in calcium signals. Importantly, the activity of BLA^{Glu} (Figure S21) and CeL^{GABA} (Figure S23) neurons further increased, whereas the activity of the CeM^{CRH} (Figure S25) neurons further decreased at the time of social contact during the 1-h SI period.

In addition, to further elucidate the effects of reactivation of SI-tagged ACC^{Glu} neurons on the activity of BLA^{Glu}, CeL^{GABA}, and CeM^{CRH} neurons, we employed the optogenetic method to selectively reactivate SI-tagged ACC^{Glu} neurons and fiber photometry to measure the activity of these neurons in freely moving tumor-bearing mice. We injected c-Fos-dependent ChR2-expressing virus into the ACC and the GCaMP6s- or EGFP (EYFP)-expressing virus into BLA (Figures S26A–S26E), CeL (Figures S27A–S27D), or CeM (Figures S28A–S28D). Optogenetics-coupled fiber photometry recordings revealed that optogenetic reactivation of SI-tagged ACC^{Glu} neurons significantly increased the calcium signals of the BLA^{Glu} (Figures S26F–S26I) and CeL^{GABA} (Figures S27E–S27H) neurons, whereas it significantly decreased the calcium signals in the CeM^{CRH} neurons (Figures S28E–S28H). Consistent with these findings, optogenetic reactivation of the SI-tagged BLA-projecting ACC^{Glu} neurons still produced robust increases in calcium signals of the BLA^{Glu} (Figures S26J–S26P) and CeL^{GABA} (Figures S27I–S27N) neurons but produced expected decreases in calcium signals of the CeM^{CRH} neurons (Figures S28I–S28N). Collectively, these findings suggest that the SI-activated ACC^{Glu}→BLA projection could increase the activity of the BLA^{Glu} and CeL^{GABA} neurons, leading to reduced activity of the hyperactive CeM^{CRH} neurons via receiving enhanced feedforward inhibition of CeL^{GABA} neurons.

SI enhances the antitumor immunity

To explore the molecular evolution within tumors induced by SI, we chemogenetically reactivated the SI-tagged BLA-projecting ACC^{Glu} neurons by repeated CNO injection once per day for 12 days to mimic SI and then performed transcriptome analyses in the 4T1 tumor-bearing mice (Figure S29A). Bulk RNA sequencing (RNA-seq) analysis revealed distinct gene expression patterns between hM3Dq and mCherry groups (Figure S29B), with the principal-component analysis (PCA) plot demonstrating complete separation between the two groups (Figure S29C). Notably, the mCherry group exhibited greater transcriptional heterogeneity, while the hM3Dq group showed more uniform gene expression profiles, suggesting that neuro-regulatory strategies reduced tumor heterogeneity. Differential expression analysis identified 763 significant DEGs (69 upregulated and 694 downregulated) in hM3Dq versus mCherry tumors (Figure S29D). Functional enrichment analysis of these DEGs highlighted predominant associations with immune-related pathways, including “regulation of inflammatory response” (GO; Figure S29E) and “Th1/Th2 cell differentiation” and “Th17 cell differentiation” (KEGG; Figure S29F), the latter of which was further validated by gene set enrichment analysis (GSEA) (Figure S29G).

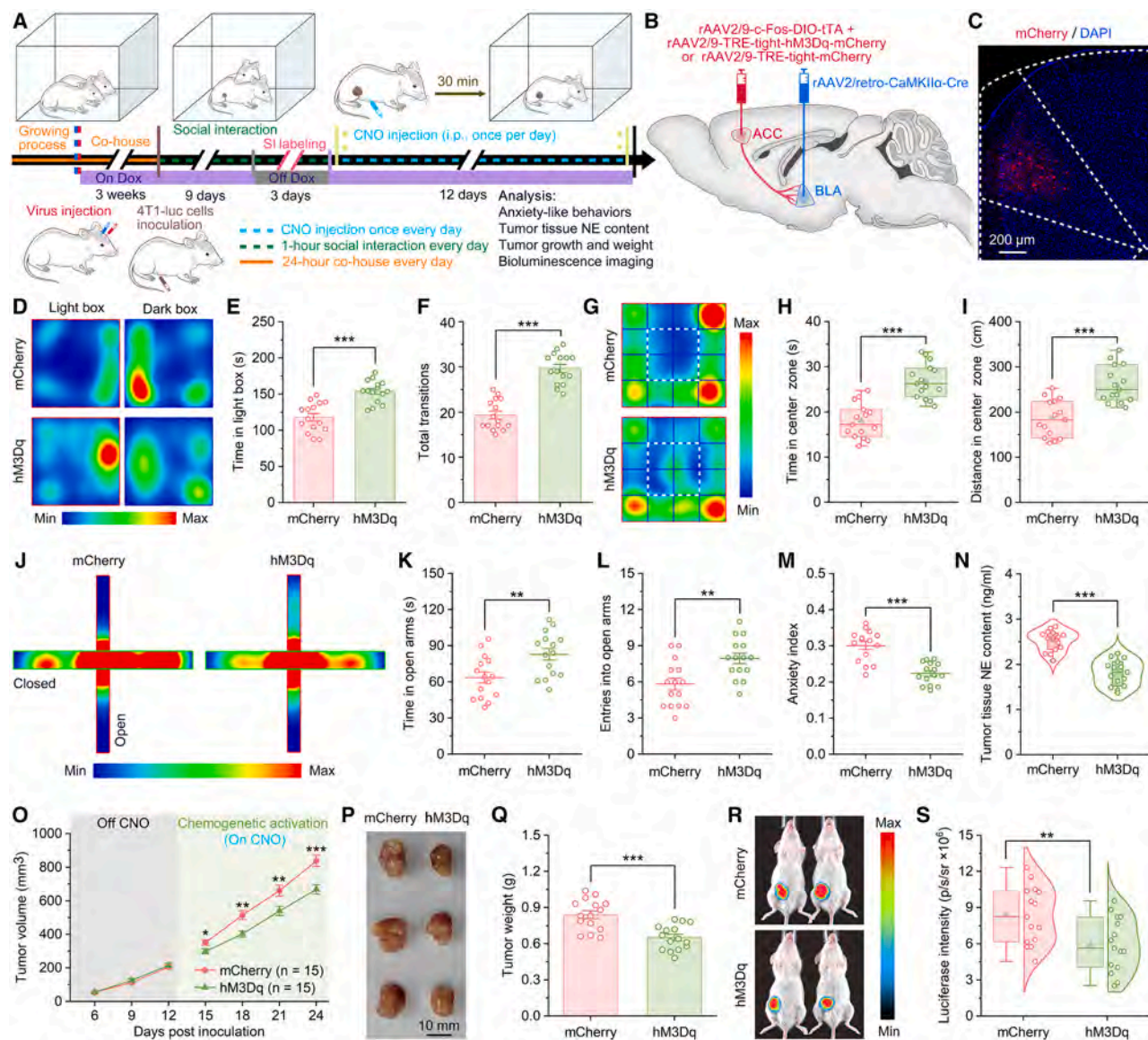


Figure 6. Reactivation of SI-tagged BLA-projecting ACC^{Glu} neurons is sufficient to mimic the anxiolytic and antitumor effects of SI

(A) Schematic and timeline of the experimental design.
 (B) Schematic showing injection of virus into the ACC and BLA.
 (C) Representative image showing mCherry expression in SI-activated BLA-projecting ACC^{Glu} neurons.
 (D–M) Representative heatmaps and summary data of the mCherry and hM3Dq groups in the LDT (D–F), the OFT (G–I), and the EPM test (J–M).
 (N) Chemogenetic reactivation of SI-tagged BLA-projecting ACC^{Glu} neurons decreased the intratumor NE content.
 (O) Chemogenetic reactivation decelerated 4T1 tumor growth.
 (P) Representative tumors dissected from mice in the mCherry and hM3Dq groups.
 (Q) Chemogenetic reactivation decreased 4T1 tumor weight.
 (R) Representative bioluminescence images showing mice in the mCherry and hM3Dq groups.
 (S) Chemogenetic reactivation decreased the luciferase intensity of 4T1 tumors.
 n = 15 mice per group. Data are presented as the mean ± SEM, except in box plots (H, I, N, and S), which denote minimum, first quartile, median, third quartile, and maximum values. The triangles in box plots represent the mean. *p < 0.05, **p < 0.01, and ***p < 0.001. (E, F, H, I, K–N, Q, and S) Two-tailed unpaired Student's t test. (O) Two-way repeated-measures ANOVA followed by separate one-way ANOVA.

In addition, complementary single-cell RNA-seq demonstrated that SI remodeled the tumor immune microenvironment, marked by expanded CD4⁺ and CD8⁺ T cell populations and altered macrophage subset distribution (Figures 7A–7F). Tran-

scriptomic profiling of these T cells revealed enriched programs related to “lymphocyte differentiation” and “T cell activation” (GO; Figures 7G and 7H), while KEGG analysis confirmed coordinated enrichment pathways of Th1/Th2 cell differentiation,

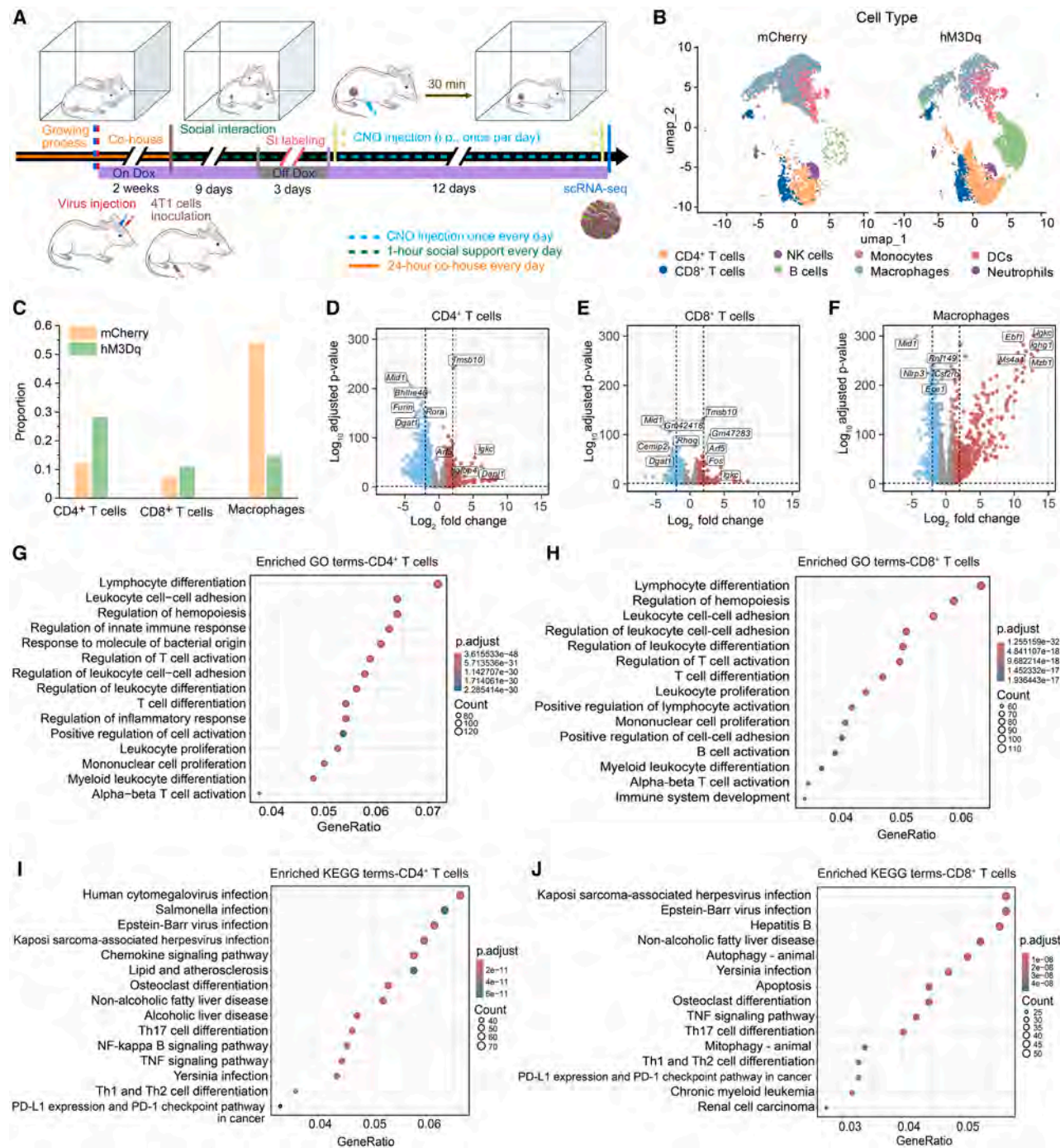


Figure 7. scRNA-seq analysis of tumor evolution under artificial mimicked SI

(A) Experimental design schematic for scRNA-seq analysis.

(B) UMAP visualization of tumor microenvironment composition, annotated by major cell subsets of CD4⁺ T cells, CD8⁺ T cells, natural killer cells (NK), B cells, monocytes, macrophages, dendritic cells (DCs), and neutrophils.

(C) Quantitative analysis of tumor-infiltrating CD4⁺ T cells, CD8⁺ T cells, and macrophage subsets in 4T1 tumors.

(D–F) Volcano plots of DEGs in (D) CD4⁺ T cells, (E) CD8⁺ T cells, and (F) macrophages.

(G and H) Top 15 enriched GO terms from DEGs of (G) CD4⁺ and (H) CD8⁺ T cells.

(I and J) Top 15 enriched KEGG pathways from DEGs of (I) CD4⁺ and (J) CD8⁺ T cells.

Th17 cell differentiation, and “PD-L1 expression and PD-1 checkpoint pathway in cancer” (Figures 7I and 7J). Together, these findings indicate that SI enhances antitumor immunity by concurrently promoting T cell recruitment/differentiation and fine-tuning immune checkpoint interactions. The reactivation of the SI-activated ACC^{Glu}→BLA projection that led to tumor volume reduction may be due to the activation of antitumor pathways in the tumor immune microenvironment.

To functionally validate our transcriptomic findings, we performed flow cytometry analysis to determine whether SI alters immune responses in the tumor microenvironment, with particular focus on tumor-infiltrating lymphocyte populations. Mice were subjected to the same injection of viruses to chemogenetically reactivate SI-tagged BLA-projecting ACC^{Glu} neurons (mimicking the SI) or sham reactivation in the 4T1 tumor-bearing mice (Figures 8A and S30A). Indeed, flow cytometric analysis showed that the 12-day reactivation of the SI-tagged BLA-projecting ACC^{Glu} neurons significantly increased the percentage of infiltrated CD45⁺ leukocytes in 4T1 tumors (Figure S30B). Profiling of the increased infiltrated CD45⁺ leukocytes showed that there were significant increases in the CD4⁺ and CD8⁺ T cell populations following the 12-day mimicking SI (Figures S30C and S30D). It is well known that CD11b⁺Gr-1⁺ myeloid-derived suppressor cells (MDSCs) suppress immune responses in the tumor microenvironment and directly stimulate tumorigenesis, thereby promoting tumor development.³³ The results showed that the percentage of MDSCs was significantly lower in 4T1 tumors of hM3Dq-expressing mice compared with control mice (Figure 8B). We also observed a significant decrease in the percentage of CD4⁺CD25⁺FOXP3⁺ T regulatory cells (Tregs) in 4T1 tumors of hM3Dq-expressing mice (Figure 8C). Moreover, the 12-day mimicking SI significantly decreased the percentage of CD4⁺PD-1⁺ T cells and CD8⁺PD-1⁺ T cells (Figures 8D and 8E), but significantly increased the percentage of CD4⁺IFN- γ ⁺ T cells and CD8⁺IFN- γ ⁺ T cells in 4T1 tumors (Figures S30E and S30F). Additionally, the 12-day mimicking SI also significantly increased the percentage of CD11b⁺F4/80⁺CD86⁺CD206[−] M1 macrophages and the ratio of M1/M2 macrophages, but did not markedly affect the percentage of CD11b⁺F4/80⁺CD86[−]CD206⁺ M2 macrophages in 4T1 tumors (Figure S31).

We also assessed the effects of SI on the immune responses in PyMT tumors. Consistently, flow cytometric analysis showed that the 15-day reactivation of the SI-tagged BLA-projecting ACC^{Glu} neurons (mimicking the SI) resulted in significant increases in infiltrated CD45⁺ leukocytes, CD4⁺ T cells, and CD8⁺ T cells in PyMT tumors (Figures S32A–S32D). The 15-day mimicking SI significantly reduced the percentage of the MDSCs, Tregs, CD4⁺PD-1⁺ T cells, and CD8⁺PD-1⁺ T cells (Figures 8G–8J), whereas the same manipulation significantly increased the percentage of the CD4⁺IFN- γ ⁺ T cells and CD8⁺IFN- γ ⁺ T cells in PyMT tumors (Figures S32E and S32F). Moreover, the 15-day mimicking SI also significantly increased the percentage of CD11b⁺F4/80⁺CD86⁺CD206[−] M1 macrophages and the ratio of M1/M2 macrophages but did not markedly affect the percentage of CD11b⁺F4/80⁺CD86[−]CD206⁺ M2 macrophages in 4T1 tumors (Figure S33). These results indicate that SI can inhibit immunosuppression and promote antitumor immunity in the tumor microenvironment.

DISCUSSION

Over the past decade, a growing body of evidence suggests that both the central and peripheral nervous systems play a crucial role in modulating peripheral malignancies progression.^{5,34–37} Despite existing challenges, the emerging field of cancer neuroscience offers great potential for unraveling the mechanisms of cancer progression, as well as creating innovative treatment approaches.^{34,35,37–39} Our recent findings reveal that the brain, particularly the CeM^{CRH} neurons and downstream circuits, contributes to breast cancer progression via direct sympathetic nerve-tumor crosstalk.⁵ Interestingly, this emerging understanding of neural contributions to cancer progression raises the question of how psychosocial factors, such as SI, might interact with these neural pathways to modulate tumor growth. SI has long been recognized as an important contributor to life quality, significantly enhancing the social, emotional, and functional well-being of cancer patients.^{11,40} Identifying brain neurons and associated circuits activated by SI is crucial for understanding how SI slows down cancer development. So far, the specific circuitry mediating the antitumor effects of SI has remained unclear. Here, our research provides new insights by uncovering the role of corticoamygdala circuits in tumor-bearing mice. Specifically, we found that the ACC^{Glu}→BLA^{Glu}→CeL^{GABA}→CeM^{CRH} neural circuits play distinct roles in mediating the SI's antitumor effects. Artificial manipulation of ACC^{Glu}→BLA neural circuits could mimic or abolish the anxiolytic and antitumor effects of SI. We further confirmed that artificially mimicking SI increased the activity of BLA^{Glu} neurons and then enhanced feedforward inhibition on hyperactive CeM^{CRH} neurons through CeL^{GABA} neurons. In fact, our previous study has confirmed that the hyperactive CeM^{CRH} neurons serve as an important node that leads to the activation of intratumoral sympathetic nerves. Our study showed that SI decreased the intratumoral sympathetic activity and norepinephrine (NE) release by suppressing hyperactive CeM^{CRH} neurons, thereby slowing tumor progression through enhanced antitumor immunity. Our findings highlight the corticoamygdala circuit as a potential target for novel cancer therapies, demonstrating how SI exerts its therapeutic effect through neural mechanisms.

Cancer extends beyond a mere biological pathology, and it also arises from a complex interplay of multiple factors, with psychosocial influences playing a significant role.⁴¹ Emotional support, as a crucial part of SI, buffers cancer patients from psychological distress that can trigger anxiety and other negative emotions.^{42–44} Our study explores how SI affects cancer progression through its influence on neural pathways, focusing on the role of emotional support in modulating tumor growth. We found that SI, such as interacting with siblings, activates glutamatergic neurons in the ACC, a region pivotal in emotional processing. This activation triggers a neural circuit that influences the amygdala, leading to changes in the sympathetic nervous system. As a result, this pathway enhances local antitumor immunity response, thereby slowing cancer progression. Thus, our findings uncover how neural pathways mediate the antitumor effects of SI.

Based on our prior findings that CeM^{CRH} neurons play an important role in tumor growth,⁵ this study investigates how SI

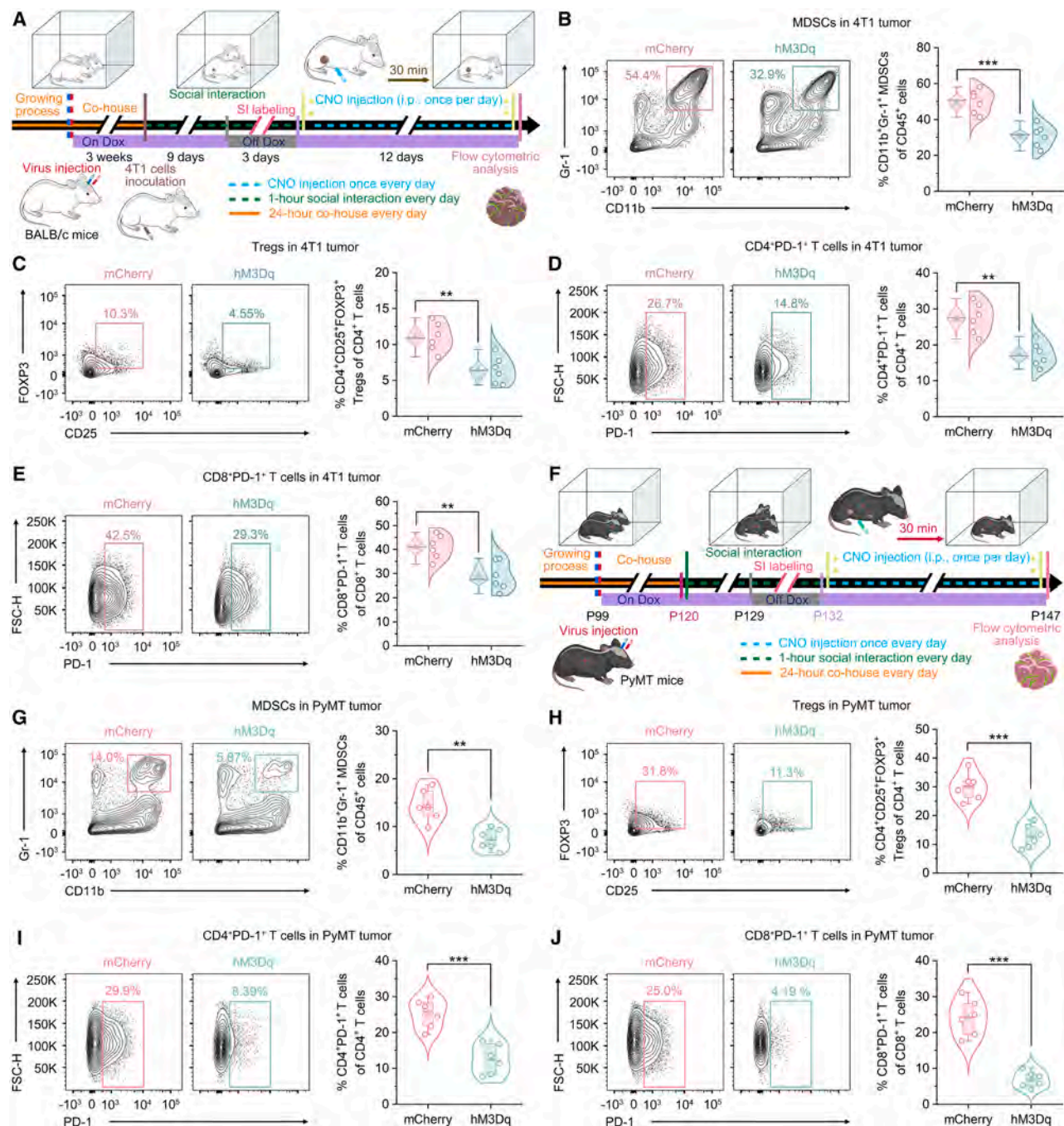


Figure 8. Artificial mimicked SI inhibits immunosuppression in the tumors

(A) Schematic and timeline of the flow cytometry in 4T1 mice.

(B–E) Chemogenetic reactivation of SI-tagged BLA-projecting ACC^{Glu} neurons decreased the percentages of MDSCs (B), Tregs (C), CD4⁺PD-1⁺ T cells (D), and CD8⁺PD-1⁺ T cells (E) in 4T1 tumors.

(F) Schematic and timeline of the flow cytometry in PyMT mice.

(G–J) Chemogenetic reactivation of SI-tagged BLA-projecting ACC^{Glu} neurons decreased the percentages of MDSCs (G), Tregs (H), CD4⁺PD-1⁺ T cells (I), and CD8⁺PD-1⁺ T cells (J) in PyMT tumors.

n = 6 mice per group. Box plots denote minimum, first quartile, median, third quartile, and maximum values. The triangles in box plots represent the mean.

p* < 0.01 and *p* < 0.001. Two-tailed unpaired Student's *t* test.

influences cancer development. We found that SI significantly inhibited the hyperactivity of CeM^{CRH} neurons, which in turn reduced anxiety levels and slowed tumor growth. Taken together, our studies emphasize the central role of the nervous system in cancer development. By manipulating neural circuits associated with SI, we could potentially slow tumor growth and improve treatment responses. This paves the way for innovative cancer therapies targeting neural pathways.

Our research shows a strong connection between SI and anti-tumor immunity within the tumor microenvironment. The artificial mimicked SI via SI-tagged BLA-projecting ACC^{Glu} neurons not only significantly increased antitumor immune response but also inhibited immunosuppression in the tumor microenvironment. Together, these results suggest that the tumor microenvironment becomes more immunostimulatory, which helps suppress tumor growth. While our findings establish a neural circuit basis for SI's antitumor effects, the precise immunological mediators and their cell-type specificity require further investigation. Future studies combining spatial transcriptomics and immune perturbation will be critical to dissect this mechanistic cascade.

Our study demonstrates that SI-driven activation of the corticoamygdala neural circuit elicits antitumor effects in both 4T1 and PyMT tumor mice, providing a scientific basis for exploring SI as a potential cancer treatment in humans.

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Guang-Yan Wu (wgy009@tmmu.edu.cn).

Materials availability

This study did not generate new, unique reagents.

Data and code availability

- Mouse data: bulk RNA-seq data and scRNA-seq data have been deposited into China National GeneBank (CNCB) Sequence Archive (CNCB: <https://doi.org/10.26036/CNP0007628>; CNCB: <https://db.cncb.org/search/project/CNP0007628/>).
- Source data are available from Mendeley Data: <https://data.mendeley.com/preview/cjx8bt9kk4?a=ad7e9f1f-58c4-42cc-82c7-fc8792b2aaa2>. Any additional information required to reanalyze the data reported in this paper is available from the [lead contact](#) upon request. This paper does not report original code.

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AUTHOR CONTRIBUTIONS

Conceptualization, G.-Y.W., Y.X., L.-M.D., and Y.Z.; methodology, H.-Z.W., S.-Y.X., Y.-X.L., L.-M.D., and G.-Y.W.; investigation, H.-Z.W., S.-Y.X., Y.-X.L., S.-Z.Y., L.S., B.-Q.T., Q.S., Z.-Q.W., X.-J.Y., Y.-R.W., P.-H.C., X.-W.Q., L.-M.D., and G.-Y.W.; visualization, H.-Z.W., Y.-L.Y., and G.-Y.W.; formal analysis, G.-Y.W., Y.X., L.-M.D., and Y.Z.; funding acquisition,

G.-Y.W., Y.X., Y.Z., and S.-Y.X.; project administration, G.-Y.W., Y.X., L.-M.D., and Y.Z.; supervision, G.-Y.W. and Y.X.; writing – original draft, G.-Y.W., Y.X., L.-M.D., and Y.Z. with the help of all authors; writing – review & editing, G.-Y.W., Y.X., L.-M.D., and Y.Z. with inputs from all authors. All authors read and commented on the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
FITC anti-mouse CD45	Biologend	Cat# 103108
Brilliant Violet 510™ anti-mouse CD3	Biologend	Cat# 100234
PerCP/Cyanine5.5 anti-mouse CD4	Biologend	Cat# 100434
PE/Cyanine7 anti-mouse CD8a	Biologend	Cat# 100722
Brilliant Violet 421™ anti-mouse FOXP3	Biologend	Cat# 126419
Alexa Fluor® 647 anti-mouse IFN-γ	Biologend	Cat# 505814
TruStain FcX™ (anti-mouse CD16/32) blocking	Biologend	Cat# 101320
PerCP/Cyanine5.5 anti-mouse/human CD11b	Biologend	Cat# 101228
Brilliant Violet 510™ anti-mouse Ly-6G/Ly-6C (Gr-1)	Biologend	Cat# 108457
Brilliant Violet 421™ anti-mouse F4/80	Biologend	Cat# 123132
PE anti-mouse CD86	Biologend	Cat# 105008
APC anti-mouse CD206	Biologend	Cat# 141708
Rabbit anti-c-Fos	Synaptic Systems	Cat# 226008
Rabbit anti-Ki67	Abcam	Cat# ab16667
Goat anti-Rabbit conjugated to Alexa Fluor™ Plus 555	Invitrogen	Cat# A32732
Goat anti-Rabbit conjugated to Alexa Fluor™ Plus 488	Invitrogen	Cat# A32731
BV650 Hamster anti-mouse CD279(PD-1)	BD Biosciences	Cat# 744546
BV786 anti-mouse CD25	BD Biosciences	Cat# 564023
Chemicals, Peptides, and Recombinant Proteins		
NE ELISA Kits	Enzyme-linked Biotechnology	Cat# ml063805
Matrigel	Mogengel	Cat# 082724
EZ 488 TUNEL reaction buffer	Life-iLab	Cat# AC12L055
RPMI 1640	Thermo Fisher Scientific	Cat# 11875093
FBS	Thermo Fisher Scientific	Cat# 10437036
D-Luciferin, Sodium Salt	Yeasen, China	Cat# 40901ES01
DMEM	Thermo Fisher Scientific	Cat# C11995500BT
Hyaluronidase	Solarbio	Cat# H8030
DNase I	Solarbio	Cat# D8071
Red Blood Cell Lysis Buffer	Solarbio	Cat# R1010
Collagenase IV	Sangon Biotech	Cat# A004186
Transcription Factor Buffer Set	BD Biosciences	Cat# 562574
Clozapine-N-oxide (CNO)	MedChemExpress	Cat# HY-17366
Doxycycline (Dox)	MedChemExpress	Cat# HY-N0565B
EDTA	MedChemExpress	Cat# HY-Y0682
HEPES	MedChemExpress	Cat# HY-D0857
PFA	Sigma-Aldrich	Cat# P6148
DAPI	Sigma-Aldrich	Cat# D9542
Bacterial and Virus Strains		
rAAV2/9-CaMKIIα-mCherry	Obio Technology	Cat# CN848
rAAV2/9-CaMKIIα-EGFP	Obio Technology	Cat# AOV012
rAAV2/9-CaMKIIα-hM4Di-mCherry	Obio Technology	Cat# H5778
rAAV2/9-CaMKIIα-GCaMP6s	Obio Technology	Cat# H11236
rAAV2/9-CaMKIIα-Cre	Obio Technology	Cat# H14878

(Continued on next page)

Continued

REAGENT or RESOURCE	SOURCE	IDENTIFIER
rAAV2/9-c-Fos-DIO-tTA	Obio Technology	Cat# E16089
rAAV2/9-TRE-tight-EGFP	Obio Technology	Cat# H11501
rAAV2/9-TRE-tight-jGCaMP7s	Obio Technology	Cat# H17808
rAAV2/9-TRE-tight-mCherry	Obio Technology	Cat# H7161
rAAV2/9-TRE-tight-hM4Di-mCherry	Obio Technology	Cat# H8813
rAAV2/9-TRE-tight-hM3Dq-mCherry	Obio Technology	Cat# MB469
rAAV2/9-TRE-tight-hChR2(H134R)-mCherry	Obio Technology	Cat# H6095
rAAV2/retro-CaMKII α -Cre	Obio Technology	Cat# H4873
rAAV2/9-VGAT1-Cre	Obio Technology	Cat# H15944
rAAV2/9-EF1 α -DIO-EYFP	Obio Technolog	Cat# AG20296
rAAV2/9-EF1 α -DIO-GCaMP6s	BrainVTA	Cat# PT-0071
rAAV2/2-CRH-Cre	BrainVTA	Cat# PT-0588

Deposited data

Bulk RNA-seq data and scRNA-seq data	This paper	https://doi.org/10.26036/CNP0007628
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Experimental Models: Cell lines

4T1-CMV-luc-Puro	Obio Technology	Cat# HYC3496
4T1	Obio Technology	Cat# HYC3076

Experimental models: Organisms/strains

Mouse: BALB/c	Laboratory Animal Center, Army Medical University	N/A
Mouse: C57BL/6J	Cyagen Biosciences	C57BL/6J
Mouse: C57BL/6JCya-Tg (MMTV-PyMT)/Cya	Cyagen Biosciences	C001212

Software and Algorithms

Visutrack	Xinruan	https://www.shxinruan.com/
Inper Smart	Inper	https://inper.com/
Image J	NIH	https://imagej.net/ij/
FlowJo	BD Biosciences	https://www.flowjo.com/
Kuant	VILBER	https://www.vilber.com/
Offline Sorter	Plexon	https://plexon.com
SPSS	IBM	https://www.ibm.com

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

Mice

Female and male BALB/c mice (8–9 weeks of age), female C57BL/6JCya-Tg (MMTV-PyMT)/Cya (MMTV-PyMT) transgenic mice (15 weeks of age; C001212, Cyagen Biosciences, China), and female C57BL/6J (MMTV-PyMT negative; 15 weeks of age) mice were used in this study. Mice were housed on a 12/12 h light-dark cycle with food and water available ad libitum. The housing facility had a temperature of 21–23 °C. Paired littermates (two mice per cage) were co-housed before the surgery or behavior experiments. All animal experiments were approved by the Animal Care Committee of Army Medical University, in accordance with the principles outlined in the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

METHOD DETAILS

Stereotaxic surgery and virus injection

Mice were anesthetized with 1.5–2% isoflurane and then fixed in a stereotaxic apparatus (Model 942, David Kopf Instruments, USA). The viruses were injected into the brain at a rate of 20 nL/min using a glass pipette with a tip diameter of approximately 20 μ m, controlled by a programmable nanoliter injector (Nanoject III, Drummond Scientific, USA). Following the injection, the needle was left in place for an additional 5 min and then slowly withdrawn.

For fiber photometry recording of ACC^{Glu} neurons activity during social interaction, the mice were microinjected with 50 nL of rAAV2/9-CaMKII α -GCaMP6s (titer: 3.54×10^{12} vg/mL) or rAAV2/9-CaMKII α -EGFP (titer: 4.04×10^{12} vg/mL) as a control into the right ACC at the following coordinates: +1.60 mm anteroposterior (AP), 0.30 mm mediolateral (ML), and -1.30 mm dorsoventral. Then one

optical fiber was implanted into the right ACC (AP +1.60 mm, ML 0.30 mm, DV -1.20 mm) above the virus injection site, and was cemented to the skull with dental cement. After surgery, the mice were given at least 1 additional week of postoperative recovery time before subsequent experiments were performed (Figures 2H and 2I).

For labeling the ACC^{Glu} neurons with EGFP, the mice were microinjected with 50 nL of rAAV2/9-CaMKII α -EGFP (titer: 4.04×10^{12} vg/mL) into the right ACC (AP +1.60 mm, ML 0.30 mm, DV -1.30 mm; Figure S7).

For global chemogenetic inhibition of ACC^{Glu} neurons, mice were bilaterally microinjected with rAAV2/9-CaMKII α -hM4Di-mCherry (titer: 3.68×10^{12} vg/mL) or rAAV2/9-CaMKII α -mCherry (titer: 3.24×10^{12} vg/mL) into the ACC (AP +1.60 mm, ML $\pm$ 0.30 mm, DV -1.30 mm) in a volume of 50 nL for each side (Figure S10B and S10C).

For labeling and fiber photometry recording of SI-tagged ACC^{Glu} neurons activity during social interaction, the mice were microinjected with 50 nL of rAAV2/9-CaMKII α -Cre (titer: 2.58×10^{12} vg/mL), rAAV2/9-c-Fos-DIO-tTA (titer: 3.57×10^{12} vg/mL) and either rAAV2/9-TRE-tight-jGCaMP7s (titer: 2.25×10^{12} vg/mL) or rAAV2/9-TRE-tight-EGFP (titer: 2.39×10^{12} vg/mL) into the right ACC (AP +1.60 mm, ML 0.30 mm, DV -1.30 mm). Then, one optical fiber was implanted into the right ACC (AP +1.60 mm, ML 0.30 mm, DV -1.20 mm) above the virus injection site (Figures S12C and S12D).

For labeling and chemogenetic inhibition of SI-tagged ACC^{Glu} neurons during social interaction period, mice were bilaterally microinjected with rAAV2/9-CaMKII α -Cre (titer: 2.58×10^{12} vg/mL), rAAV2/9-c-Fos-DIO-tTA (titer: 3.57×10^{12} vg/mL) and either rAAV2/9-TRE-tight-hM4Di-mCherry (titer: 2.25×10^{12} vg/mL) or rAAV2/9-TRE-tight-mCherry virus (titer: 2.39×10^{12} vg/mL) into the ACC (AP +1.60 mm, ML $\pm$ 0.30 mm, DV -1.30 mm) in a volume of 50 nL for each side (Figures 3B and 3C).

For labeling and chemogenetic reactivation of SI-tagged ACC^{Glu} neurons to mimic the effects of social interaction, mice were bilaterally microinjected with rAAV2/9-CaMKII α -Cre (titer: 2.58×10^{12} vg/mL), rAAV2/9-c-Fos-DIO-tTA (titer: 3.57×10^{12} vg/mL) and either rAAV2/9-TRE-tight-hM3Dq-mCherry (titer: 2.36×10^{12} vg/mL) or rAAV2/9-TRE-tight-mCherry (titer: 2.39×10^{12} vg/mL) into the ACC (AP +1.60 mm, ML $\pm$ 0.30 mm, DV -1.30 mm) in a volume of 50 nL for each side (Figures 4B and 4C).

For anterograde tracing, 50 nL of rAAV2/9-CaMKII α -mCherry (titer: 3.24×10^{12} vg/mL) virus was injected into the right ACC (AP +1.60 mm, ML 0.30 mm, DV -1.30 mm; Figures S15B and S15C).

For labeling and fiber photometry recording of SI-tagged BLA-projecting ACC^{Glu} neurons activity during social interaction, the mice were microinjected with 50 nL of rAAV2/9-c-Fos-DIO-tTA (titer: 3.57×10^{12} vg/mL) and either rAAV2/9-TRE-tight-jGCaMP7s (titer: 2.25×10^{12} vg/mL) or rAAV2/9-TRE-tight-EGFP (titer: 2.39×10^{12} vg/mL) into the right ACC (AP +1.60 mm, ML 0.30 mm, DV -1.30 mm). Simultaneously, we injected with 50 nL of retrograde virus rAAV2/retro-CaMKII α -Cre (titer: 3.31×10^{12} vg/mL) into bilateral BLA (AP -1.30 mm, ML $\pm$ 3.40 mm, DV -4.70 mm). Then, one optical fiber was implanted into the right ACC (AP +1.60 mm, ML 0.30 mm, DV -1.20 mm) above the virus injection site (Figures S15F and S15G).

For labeling and chemogenetic inhibition of SI-tagged BLA-projecting ACC^{Glu} neurons during social interaction period, the mice were microinjected with 50 nL of rAAV2/9-c-Fos-DIO-tTA (titer: 3.57×10^{12} vg/mL) and either rAAV2/9-TRE-tight-hM4Di-mCherry (titer: 2.25×10^{12} vg/mL) or rAAV2/9-TRE-tight-mCherry virus (titer: 2.39×10^{12} vg/mL) into the bilateral ACC (AP +1.60 mm, ML $\pm$ 0.30 mm, DV -1.30 mm). Simultaneously, we injected with 50 nL of retrograde virus rAAV2/retro-CaMKII α -Cre (titer: 3.31×10^{12} vg/mL) into bilateral BLA (AP -1.30 mm, ML $\pm$ 3.40 mm, DV -4.70 mm; Figures 5B and 5C).

For labeling and chemogenetic reactivation of SI-tagged BLA-projecting ACC^{Glu} neurons to mimic the effects of social interaction, the mice were microinjected with 50 nL of rAAV2/9-c-Fos-DIO-tTA (titer: 3.57×10^{12} vg/mL) and either rAAV2/9-TRE-tight-hM3Dq-mCherry (titer: 2.36×10^{12} vg/mL) or rAAV2/9-TRE-tight-mCherry virus (titer: 2.39×10^{12} vg/mL) into the bilateral ACC (AP +1.60 mm, ML $\pm$ 0.30 mm, DV -1.30 mm). Simultaneously, we injected with 50 nL of retrograde virus rAAV2/retro-CaMKII α -Cre (titer: 3.31×10^{12} vg/mL) into bilateral BLA (AP -1.30 mm, ML $\pm$ 3.40 mm, DV -4.70 mm; Figures 6B, 6C, S18B and S18C).

For fiber photometry recording of BLA^{Glu} neurons activity during social interaction period, mice were microinjected with 50 nL of rAAV2/9-CaMKII α -GCaMP6s (titer: 3.54×10^{12} vg/mL) or rAAV2/9-CaMKII α -EGFP (titer: 4.04×10^{12} vg/mL) as a control into right BLA (AP -1.30 mm, ML 3.40 mm, DV -4.70 mm). Then, one optical fiber was implanted into right BLA (AP -1.30 mm, ML 3.40 mm, DV -4.60 mm) above the virus injection site (Figures S20C and S20D).

For fiber photometry recording of CeL^{GABA} neurons activity during social interaction period, the mice were microinjected with 50 nL of rAAV2/9-VGAT1-Cre (titer: 2.74×10^{12} vg/mL) together with rAAV2/9-EF1 α -DIO-GCaMP6s (titer: 4.26×10^{12} vg/mL) or rAAV2/9-EF1 α -DIO-EYFP (titer: 3.21×10^{12} vg/mL) into the right CeL (AP -1.30 mm, ML 2.80 mm, DV -4.40 mm). Then, one optical fiber was implanted into the right CeL (AP -1.30 mm, ML 2.80 mm, DV -4.30 mm) above the virus injection site (Figures S22C and S22D).

For fiber photometry recording of CeM^{CRH} neurons activity during social interaction period, the mice were microinjected with 50 nL of rAAV2/2-CRH-Cre (titer: 2.63×10^{12} vg/mL) together with rAAV2/9-EF1 α -DIO-GCaMP6s (titer: 4.26×10^{12} vg/mL) or rAAV2/9-EF1 α -DIO-EYFP (titer: 3.21×10^{12} vg/mL) into the right CeM (AP -1.30 mm, ML 2.50 mm, DV -4.50 mm). Then, one optical fiber was implanted into the right CeM (AP -1.30 mm, ML 2.50 mm, DV -4.40 mm) above the virus injection site (Figures S24C and S24D).

For labeling and optogenetic stimulation reactivation of SI-tagged ACC^{Glu} neurons to mimic the effects of social interaction and fiber photometry recording of BLA^{Glu} neurons activity, mice were microinjected with 50 nL of rAAV2/9-CaMKII α -Cre (titer: 2.58×10^{12} vg/mL), rAAV2/9-c-Fos-DIO-tTA (titer: 3.57×10^{12} vg/mL) and rAAV2/9-TRE-tight-hChR2(H134R)-mCherry (titer: 2.42×10^{12} vg/mL) into the right ACC (AP +1.60 mm, ML 0.30 mm, DV -1.30 mm). Moreover, the right BLA (AP -1.30 mm, ML 3.40 mm, DV -4.70 mm) was injected with 50 nL of rAAV2/9-CaMKII α -GCaMP6s (titer: 3.54×10^{12} vg/mL) or rAAV2/9-CaMKII α -EGFP (titer: 4.04×10^{12} vg/mL). Then, two optical fibers were implanted into the ACC (AP +1.60 mm, ML 0.30 mm, DV -1.20 mm) and BLA (AP -1.30 mm, ML 3.40 mm, DV -4.60 mm) above virus injection site, respectively (Figures S26C–S26E).

For labeling and optogenetic stimulation reactivation of SI-tagged ACC^{Glu} neurons to mimic the effects of social interaction and fiber photometry recording of CeL^{GABA} neurons activity, mice were microinjected with 50 nL of rAAV2/9-CaMKII α -Cre (titer: 2.58×10^{12} vg/mL), rAAV2/9-c-Fos-DIO-tTA (titer: 3.57×10^{12} vg/mL) and rAAV2/9-TRE-tight-hChR2(H134R)-mCherry (titer: 2.42×10^{12} vg/mL) into the right ACC (AP +1.60 mm, ML 0.30 mm, DV -1.30 mm). Moreover, the right CeL (AP -1.30 mm, ML 2.80 mm, DV -4.40 mm) was injected with 50 nL of rAAV2/9-VGAT1-Cre (titer: 2.74×10^{12} vg/mL) together with rAAV2/9-EF1 α -DIO-GCaMP6s (titer: 4.26×10^{12} vg/mL) or rAAV2/9-EF1 α -DIO-EYFP (titer: 3.21×10^{12} vg/mL). Then, two optical fibers were implanted into the ACC (AP +1.60 mm, ML 0.30 mm, DV -1.20 mm) and CeL (AP -1.30 mm, ML 2.80 mm, DV -4.30 mm) above the virus injection site, respectively (Figures S27C and S27D).

For labeling and optogenetic stimulation reactivation of SI-tagged ACC^{Glu} neurons to mimic the effects of social interaction and fiber photometry recording of CeM^{CRH} neurons activity, mice were microinjected with 50 nL of rAAV2/9-CaMKII α -Cre (titer: 2.58×10^{12} vg/mL), rAAV2/9-c-Fos-DIO-tTA (titer: 3.57×10^{12} vg/mL) and rAAV2/9-TRE-tight-hChR2(H134R)-mCherry (titer: 2.42×10^{12} vg/mL) into the right ACC (AP +1.60 mm, ML 0.30 mm, DV -1.30 mm). Moreover, the right CeM (AP -1.30 mm, ML 2.50 mm, DV -4.50 mm) was injected with 50 nL of rAAV2/2-CRH-Cre (titer: 2.63×10^{12} vg/mL) together with rAAV2/9-EF1 α -DIO-GCaMP6s (titer: 4.26×10^{12} vg/mL) or rAAV2/9-EF1 α -DIO-EYFP (titer: 3.21×10^{12} vg/mL). Then, two optical fibers were implanted into the ACC (AP +1.60 mm, ML 0.30 mm, DV -1.20 mm) and CeM (AP -1.30 mm, ML 2.50 mm, DV -4.40 mm) above the virus injection site, respectively (Figures S28C and S28D).

For labeling and optogenetic stimulation reactivation of SI-tagged BLA-projecting ACC^{Glu} neurons to mimic the effects of social interaction and fiber photometry recording of BLA^{Glu} neurons activity, mice were microinjected with 50 nL of rAAV2/9-c-Fos-DIO-tTA (titer: 3.57×10^{12} vg/mL) and rAAV2/9-TRE-tight-hChR2(H134R)-mCherry (titer: 2.42×10^{12} vg/mL) into the right ACC (AP +1.60 mm, ML 0.30 mm, DV -1.30 mm). Moreover, the right BLA (AP -1.30 mm, ML 3.40 mm, DV -4.70 mm) was injected with 50 nL of retrograde virus rAAV2/retro-CaMKII α -Cre (titer: 3.31×10^{12} vg/mL) together with rAAV2/9-CaMKII α -GCaMP6s (titer: 3.54×10^{12} vg/mL) or rAAV2/9-CaMKII α -EGFP (titer: 4.04×10^{12} vg/mL). Then, two optical fibers were implanted into the ACC (AP +1.60 mm, ML 0.30 mm, DV -1.20 mm) and BLA (AP -1.30 mm, ML 3.40 mm, DV -4.60 mm) above virus injection site, respectively (Figures S26J–S26L).

For labeling and optogenetic stimulation reactivation of SI-tagged BLA-projecting ACC^{Glu} neurons to mimic the effects of social interaction and fiber photometry recording of CeL^{GABA} neurons activity, mice were microinjected with 50 nL of rAAV2/9-c-Fos-DIO-tTA (titer: 3.57×10^{12} vg/mL) and rAAV2/9-TRE-tight-hChR2(H134R)-mCherry (titer: 2.42×10^{12} vg/mL) into the right ACC (AP +1.60 mm, ML 0.30 mm, DV -1.30 mm). Moreover, the right BLA (AP -1.30 mm, ML 3.40 mm, DV -4.70 mm) was injected with 50 nL of retrograde virus rAAV2/retro-CaMKII α -Cre (titer: 3.31×10^{12} vg/mL). Simultaneously, the right CeL (AP -1.30 mm, ML 2.80 mm, DV -4.40 mm) was injected with 50 nL of rAAV2/9-VGAT1-Cre (titer: 2.74×10^{12} vg/mL) together with rAAV2/9-EF1 α -DIO-GCaMP6s (titer: 4.26×10^{12} vg/mL) or rAAV2/9-EF1 α -DIO-EYFP (titer: 3.21×10^{12} vg/mL). Then, two optical fibers were implanted into the ACC (AP +1.60 mm, ML 0.30 mm, DV -1.20 mm) and CeL (AP -1.30 mm, ML 2.80 mm, DV -4.30 mm) above the virus injection site, respectively (Figures S27I and S27J).

For labeling and optogenetic stimulation reactivation of SI-tagged BLA-projecting ACC^{Glu} neurons to mimic the effects of social interaction and fiber photometry recording of CeM^{CRH} neurons activity, mice were microinjected with 50 nL of rAAV2/9-c-Fos-DIO-tTA (titer: 3.57×10^{12} vg/mL) and rAAV2/9-TRE-tight-hChR2(H134R)-mCherry (titer: 2.42×10^{12} vg/mL) into the right ACC (AP +1.60 mm, ML 0.30 mm, DV -1.30 mm). Moreover, the right BLA (AP -1.30 mm, ML 3.40 mm, DV -4.70 mm) was injected with 50 nL of retrograde virus rAAV2/retro-CaMKII α -Cre (titer: 3.31×10^{12} vg/mL). Simultaneously, the right CeM (AP -1.30 mm, ML 2.50 mm, DV -4.50 mm) was injected with 50 nL of rAAV2/2-CRH-Cre (titer: 2.63×10^{12} vg/mL) together with rAAV2/9-EF1 α -DIO-GCaMP6s (titer: 4.26×10^{12} vg/mL) or rAAV2/9-EF1 α -DIO-EYFP (titer: 3.21×10^{12} vg/mL). Then, two optical fibers were implanted into the ACC (AP +1.60 mm, ML 0.30 mm, DV -1.20 mm) and CeM (AP -1.30 mm, ML 2.50 mm, DV -4.40 mm) above the virus injection site, respectively (Figures S28I and S28J).

These viruses were obtained from Obio Technology (Shanghai, China) and BrainVTA (Wuhan, China). For all experiments, animals with incorrect injection sites were excluded from further analysis. The efficacy of viruses in pharmacogenetic and optogenetic experiments has been confirmed in our previous works.^{5,30,45}

Cell culture

The 4T1 breast cancer cell line (4T1-CMV-luc-Puro, HYC3496; 4T1, HYC3076) was obtained from Obio Technology (Shanghai, China). The cells were cultured in RPMI 1640 medium (11875093, Thermo Fisher Scientific) containing 10% FBS (10437036, Thermo Fisher Scientific, USA) in a humidified incubator at 37°C with 5% CO₂ (Forma CO₂ Incubator, Thermo Fisher Scientific). Transfected cells at the logarithmic growth stage were collected and diluted to the desired concentration with Matrigel (082724, Mogenel, China).⁵

Breast tumor induction

The 4T1-luc or 4T1 breast cancer cells (2×10^5 cells per mouse, 100 μ L) were injected into the mammary fat pad (e.g., Figure 1) or into the flank (Figure S2) of one female BALB/c mouse of the paired co-housed familiar littermates (two mice per cage). After 6 days of 4T1

cells inoculation, the breast tumor sizes were measured every 3 days using a digital caliper. The tumor volume was calculated as $(\text{length} \times \text{width}^2)/2$. The maximally allowed tumor length 20 mm and the maximally allowed tumor volume 2000 mm³ were not exceeded in all experiments.

Social interaction protocol

Pairs of female littermates that were co-housed (two mice per cage) before surgery or behavior experiments were used to exam the effects of social interaction. In 4T1 transplanted breast cancer model, one of the paired co-housed familiar female littermates was inoculated with 4T1 cells. After this, the 4T1 tumor-bearing mouse was accompanied by a respective naive female co-housed familiar littermate in home cage for 1, 2, or 24 hours every day, for 24 consecutive days (i.e., 1, 2, or 24-hour social interaction). Moreover, the 0-hour social interaction (alone) was one of the paired co-housed familiar littermates, which was inoculated with 4T1 cells and then separated and housed singly in home cage for 24 consecutive days (Figure 1A). Additionally, to test the effects of female-male interaction on tumor progression, the female-male littermates were used. The 4T1 tumor-bearing mouse was accompanied by a respective naive male co-housed familiar littermate in home cage for 1 hours every day, for 24 consecutive days (Figure S4). The protocol of 1-hour social interaction every day for all experiments was same (e.g., Figure 2A).

In MMTV-PyMT mouse model, the protocol of 1-hour social interaction every day for PyMT tumor-bearing mouse was also accompanied by a respective female naive co-housed familiar littermate (MMTV-PyMT negative mouse) in home cage for 1 hour every day for 9 consecutive days from P121 (121 days after birth; e.g., Figure S18A). In MMTV-PyMT mouse model, the protocol of 1-hour social interaction every day for PyMT tumor-bearing mouse was also accompanied by a respective female naive co-housed familiar littermate (MMTV-PyMT negative mouse) in home cage for 1 hour every day for 9 consecutive days from P121 (121 days after birth; e.g., Figure S18A).

Social interaction test

To examine the strength of social interaction behaviors, one tumor-bearing mice and one naive female co-housed familiar littermate (T-N social interaction), or two tumor-bearing mice (T-T social interaction; Figure S1A) were positioned within a 30 × 30 × 35 cm opaque black plastic behavioral box, and their interactions were recorded for a period of one hour using cameras. Social interaction behaviors were delineated in accordance with established scholarly literature, with the emphasis being on documenting social contact exhibited by pairs of mice, such as sniffing, allogrooming, and crawling under. The video recordings were subsequently analyzed using Inper Smart (Inper, China) to calculate the (Figure S1).^{46,47}

Enriched environment stimulation

To examine the effect of enriched environment (inanimate object) stimulation on the tumor progression, each 4T1 tumor-bearing mouse was placed in an enriched environment cage for 1 hours every day, for 24 consecutive days. The enriched environment cage contained climbing platforms, plastic tubes and tunnels, triangular object and small boxes (Figure S5).⁴⁸

The labeling of SI-tagged ACC^{Glu} neurons or SI-tagged BLA-projecting ACC^{Glu} neurons

The procedure for labeling SI-activated neurons was conducted similarly to our previous studies.^{30,31} Mice were fed with diet containing 100 mg/kg Dox (HY-N0565B, MCE, USA) after the c-Fos-dependent virus injection. In the 4T1 transplanted breast cancer model, on day 9 post 4T1 cells inoculation, the tumor-bearing mice were subjected to 1-hour social interaction, and then, 60 min after the social interaction, they were switched to a regular diet without Dox for 3 days to allow activity-dependent labeling for a window of time. During the off Dox period, the tumor-bearing mice were still administered the daily 1-hour social interaction. On day 12 post 4T1 cells inoculation, 60 min after 1-hour social interaction, the tumor-bearing mice were put back on the Dox diet (e.g., Figures 3A and S15A).

Similarly, in the MMTV-PyMT mouse model, 60 min after 1-hour social interaction at P129 (day 129 after birth), the PyMT tumor-bearing mice switched to regular diet without Dox for 3 days to allow activity-dependent labeling for a window of time. During the off Dox period, the PyMT tumor-bearing mice were still administered the daily 1-hour social interaction. 60 min after 1-hour social interaction at P132 (day 132 after birth), the tumor-bearing mice were put back on the Dox diet (Figure S18A).

Pharmacogenetic manipulations

For in vivo chemogenetic inhibition of the ACC^{Glu} neurons, the 4T1 tumor-bearing mice were subjected to intraperitoneal injection of CNO (1 mg/kg; diluted with 5% dimethyl sulfoxide and saline; HY-17366, MCE) once daily for 24 consecutive days from 1 day after 4T1 cells inoculation (Figures S10 and S11). Then the tumor-bearing mice were administered the daily 1-hour social interaction 30 min after daily CNO injection.

For in vivo chemogenetic inhibition of SI-tagged ACC^{Glu} neurons (Figures 3 and S13), and SI-tagged BLA-projecting ACC^{Glu} neurons (Figures 5 and S16), the 4T1 tumor-bearing mice were intraperitoneally injected with CNO once daily for 12 consecutive days from day13 after 4T1 cells inoculation. The tumor-bearing mice were then given the daily 1-hour social interaction 30 min after daily CNO injection.

For in vivo chemogenetic reactivation of SI-tagged ACC^{Glu} neurons (Figures 4 and S14), or SI-tagged BLA-projecting ACC^{Glu} neurons (Figures 6 and S17) to mimic the effects of social interaction, the 4T1 tumor-bearing mice were intraperitoneally injected with

CNO once daily for 12 consecutive days from day 13 after 4T1 cells inoculation. Moreover, in the MMTV-PyMT mouse model, the PyMT tumor-bearing mice were intraperitoneally injected with CNO once daily for 15 consecutive days from P133 (day 133 after birth; [Figures S18 and S19](#)).

Optogenetic manipulations

For optogenetic reactivation of SI-tagged ACC^{Glu} neurons (e.g., [Figures S26B and S26C](#)), or SI-tagged BLA-projecting ACC^{Glu} neurons (e.g., [Figures S26B and S26J](#)) to mimic the effects of social interaction during fiber photometry recording, tumor-bearing mice expressing ChR2 received laser illumination (473 nm, ~5 mW, 20 Hz, 15-ms pulse duration, 30 epochs of 5 s, separated by an interval of 20–40 s) delivered from a 473-nm laser (QAXK-LASER-B2, Thinkerbiotech, China).

In vivo extracellular recording

For in vivo extracellular recording of ACC neural activity during the social interaction, a 4-channel electrode array was implanted into the ACC (AP +1.60 mm, ML 0.30 mm, DV -1.30 mm). A ground wire was soldered to the skull screws. The mice were inoculated with 4T1 cells 1 week after surgery. Then the tumor-bearing mice received daily 1-hour social interaction for 23 days. We performed the in vivo extracellular recording 24 days after 4T1 cells inoculation ([Figure 2A](#)). It is worth noting that the mice were handled to adapt to extracellular recording procedures at least three times (2 hours each time) per day for three days before the extracellular recording. The neural signals were amplified and acquired using a Omniplex Neural Data Acquisition System (Plexon, USA). Spiking activity was digitized at 40 kHz and bandpass filtered (250–8000 Hz). Spike sorting was performed using Offline Sorter 4 (Plexon). The firing rates of sorted units were calculated using Neuroexplorer 5 (Nex Technologies, USA). The Curves graph of firing rates were computed over a bin width of 5 s for each unit between -60 and 60 minutes.

Fiber photometry recording

The fiber photometry recording has been described in our previous studies.^{5,30} For recording of neural activity during social interaction, the tumor-bearing mouse was accompanied by a respective naive female co-housed familiar littermate in home cage for 1 hour 24 days after 4T1 cells inoculation ([Figure 2F](#)). Fluorescence signals of neurons were recorded by a fiber photometry recording system (Inper, China). In addition, fluorescence signals of the neurons were also recorded by a fiber photometry recording system during optogenetic reactivation of SI-tagged ACC^{Glu} neurons (e.g., [Figures S26B and S26C](#)), or SI-activated ACC^{Glu}→BLA projection (e.g., [Figures S26B and S26J](#)). Fluorescence signal excitation was provided by a 488-nm laser. Moreover, a 405-nm laser was concurrently used to isolate the movement-corrected signals from the channel. The laser power was adjusted at the tip of the optical fiber to a low level of 20–40 μ W to minimize bleaching of GCaMP6s/ jGCaMP7s and EGFP/ EYFP.

The fiber photometry data were analyzed using built-in software. The fluorescence value during social interaction, each social contact, or each optogenetic stimulation was derived. The change in the fluorescence values ($\Delta F/F$) was calculated by $(F - F_0)/F_0$, where F refers to the fluorescence values at each time point (-10–60 min relative to the social interaction onset or -2–10 s relative to each social contact and optogenetic stimulation onset) and F_0 refers to the median of the fluorescence values during the baseline period (-10–0 min relative to the social interaction onset or -2–0 s relative to the social contact onset and optogenetic stimulation onset). To visualize the fluorescence change, the $\Delta F/F$ values are presented with heatmaps or plots with shaded areas indicating SEM. To statistically quantify the change in fluorescence values across the scratching train or optogenetic stimulus, the average amplitude of $\Delta F/F$ was defined as the average fluorescence amplitude change from the baseline during the peak period (0–60 min relative to the social interaction onset, 0–10 s relative to the social contact onset, or 0–5 s relative to the optogenetic stimulation onset; e.g., [Figures 2L, S9C and S26F](#)).

Behavioral experiments

The anxiety-like behaviors of mice were evaluated using light-dark box test (LDT), open field test (OFT), and elevated plus maze (EPM) test at 24 days after tumor cell inoculation. During the scoring process, the operator was blinded to experimental groups.⁵

Light-dark box test

The apparatus consists of a covered box (34 × 17 × 17 cm), which is equally divided into two compartments by a partition with a hole at floor level. The light compartment is brightly illuminated by white diodes (390 lux), while the dark compartment is completely dark. During test, each mouse was placed into light compartment and allowed to move freely between the light and dark compartments for 5 min. The transition behavior between light and dark compartments was monitored with a digital camera during experiment, and data were acquired and analyzed by VisuTrack (Xinruan, Shanghai, China).

Open field test

The OFT was performed in a square chamber (50 × 50 cm) with 45 cm high walls. The floor of the chamber is evenly divided into sixteen squares; the central four squares are named center zone, and the others are named peripheral zones. During the test, each mouse was placed in center zone and then allowed to freely move for approximately 5 min. The motion trials were recorded by a video camera during experiment, and the data were acquired and analyzed by VisuTrack (Xinruan, China).

Elevated plus maze test

The EPM is a crossed shape platform elevated 60 cm above the floor and consists of a central intersection platform (5 × 5 cm), two open arms (35 × 5 cm) and two closed arms (35 × 5 × 16 cm). During test, each mouse was placed on the central intersection platform and left to explore the maze for 10 min. The measures of EPM test were recorded by video camera during experiment, and data were acquired and analyzed by VisuTrack 2020 (Xinruan, China). The anxiety index was calculated using the following equation: $(1 - ((\text{open arm time}/\text{total time on the maze}) + (\text{open arm entry}/\text{total entry}))/2)$.

Bioluminescence imaging

The growth and metastasis of breast tumors were observed using an in vivo bioluminescence imaging system (NEWTON 7.0, VILBER, France). Before observation, each mouse was injected (i.p., 150 mg/kg) with fluorescein substrate (D-Luciferin, Sodium Salt, 40901ES01, Yeasen, China). After anesthesia with 1.5–2% isoflurane, mice were placed into an in vivo bioluminescence imaging system to obtain bioluminescence images and data. Data were analyzed using the Kuant software (VILBER).

Breast tumor procurement

After the behavioral experiments, the tumor-bearing mice were deeply anesthetized with isoflurane, and the primary breast tumor tissues were quickly removed and placed on ice. The breast tumor tissues were washed with precooled phosphate-buffered saline (PBS, 0.01 M, pH = 7.4) to remove residual blood, and then the weight of the tumors was measured as quickly as possible.

NE assessment

For the analysis of NE levels in breast tumor tissues in different groups, NE enzyme-linked immunosorbent assay (ELISA) kits (ml063805, Enzyme-linked Biotechnology Co., Ltd., Shanghai, China) were used. The tumor tissues (0.1 g) were weighed, 0.9 ml PBS (0.01 M, pH = 7.4) was added and then they were fully homogenized by a homogenizer. Next, the supernatants were carefully collected after centrifugation for 15 min at 2000 × g. Fifty microliters of different concentrations of the standard and 50 μl of different sample supernatants were added to the well according to the manufacturer's instructions. Then, 100 μL of HRP-conjugated reagent was added and incubated for 60 min at 37°C. The plates were washed with washing buffer 5 times, and the mixed substrate (100 μL) was added to each well. After incubation for 15 min at 37°C, 50 μL termination solutions were added to each well, and the optical density (OD) value was measured by a microplate reader (Imark, Bio-Rad, California, United States) at 450 nm, 3 times.

Isolation of lymphocytes and macrophages from tumor tissues

The fresh tumor tissues from 4T1 or PyMT tumor-bearing mice were cut into ~1 mm³ pieces and digested with 25 ml of DMEM (C11995500BT, Thermo Fisher Scientific) supplemented with 1 mg/ml collagenase IV (A004186, Sangon Biotech, China), 3% FBS, 0.1 mg/ml hyaluronidase (H8030, Solarbio, China) and 0.01 mg/ml DNase I (D8071, Solarbio, China) at 37 °C for 60 minutes. The dissociated cells were collected into a 15-ml tube and centrifuged at 500 × g for 5 min. The cells were resuspended in 5 ml of Red Blood Cell Lysis Buffer (R1010, Solarbio, China), kept still for 5 min at 37 °C, centrifuged at 500 × g for 5 min and washed with Buffer A (PBS with 2 mM EDTA, 0.5% FBS, and 25 mM HEPES) twice before filtration with a 70-μm filter. These cells were collected and resuspended in Buffer A for further analysis.

Bulk RNA sequencing (Bulk RNA-seq)

The bulk RNA-seq of tumor samples was performed by Knorigene Technologies (Chongqing, China). Total RNA purity and integrity were assessed using a NanoDrop 2000 (Thermo, USA) and Agilent 2100 bioanalyzer (Agilent, USA). The cDNA library was constructed using the NEBNext® UltraTM RNA Library Prep Kit (New England Biolabs, USA). To preferentially select cDNA fragments 250~300 bp in length, the library fragments were purified using the AMPure XP system (Beckman Coulter, Beverly, USA). Indexed samples were clustered on a cBot Cluster Generation System using the TruSeq PE Cluster Kit v3-cBot-HS (Illumina) following the manufacturer's instructions. After cluster generation, the library preparations were sequenced on an Illumina HiSeq platform. The sequenced reads were aligned to the GRCh37 (hg19) reference genome using HISAT2. Principal component analysis (PCA) was performed to assess sample variability. Differentially expressed genes (DEGs) were identified based on $|\log_2 \text{fold change}| > 1$ and adjusted p-value < 0.05. Functional enrichment analysis of DEGs was conducted using Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG), and Gene Set Enrichment Analysis (GSEA).

Single-cell RNA sequencing (scRNA-seq)

Fresh tissue specimens were dissected into 0.5 mm³ fragments in ice-cold PBS and enzymatically digested at 37°C for 20 min (100 rpm) using a solution containing 0.35% collagenase IV, 2 mg/ml papain, and 120 U/ml DNase I. The reaction was quenched with 10% FBS, and the resulting cell suspension was sequentially filtered through 70 μm and 40 μm strainers. Erythrocytes were lysed using RBC lysis buffer (MACS 130-094-183), followed by live cell enrichment with Dead Cell Removal MicroBeads (MACS 130-090-101). CD45⁺ cells were isolated by fluorescence-activated cell sorting (FACS), and viability was confirmed (>85% by trypan blue exclusion). Single-cell libraries were prepared using the 10X Genomics Chromium Single Cell 3' Kit (v3) according to the manufacturer's protocol, targeting 8,000 cells at a concentration of 700–1200 cells/μL. Paired-end sequencing (PE150) was performed on an Illumina NovaSeq platform (OBio Technology). Raw sequencing data (FASTQ files) were processed using Cell Ranger (v7.0.1) for

alignment and initial quantification, with unique molecular identifier (UMI) counts summarized for each barcode. Downstream analysis was conducted with Seurat (v4.1.3), applying the following quality control thresholds: cells with < 200 genes, < 1000 UMIs, >15% mitochondrial UMIs, or >5% hemoglobin UMIs were excluded. Potential doublets were identified and removed using DoubletFinder (v2.0.4). Gene expression data were normalized via the “LogNormalize” method (scaling factor = 10000) and log-transformed. Cell clusters were identified using graph-based clustering and visualized via a 2-dimensional Uniform Manifold Approximation and Projection (UMAP) algorithm with the RunUMAP function. DEGs were defined as those with $|\log_2FC| > 0.58$ and adjusted P-value < 0.05. Functional enrichment analysis (GO and KEGG pathways) was performed using R (v4.1.3).

Fluorescence-activated cell sorting (FACS)

The tumor tissue-derived cells in Buffer A were counted. An aliquot of cells was pelleted and resuspended in the Buffer A at 10^7 cells/mL. A total of 1×10^6 cells per sample were incubated with FVS780 according to the manufacturer's protocol (565388, BD Biosciences, USA) for live/dead discrimination. For lymphocytes analysis, cells were stained with antibodies for 30 min at 4 °C. The antibodies included FITC anti-mouse CD45 antibody (103108, Biolegend, China), Brilliant Violet 510™ anti-mouse CD3 antibody (100234, Biolegend), PerCP/Cyanine5.5 anti-mouse CD4 antibody (100434, Biolegend), PE/Cyanine7 anti-mouse CD8a antibody (100722, Biolegend), BV650 Hamster anti-mouse CD279 antibody (PD-1; 744546, BD Biosciences), BV786 anti-mouse CD25 antibody (564023, BD Biosciences). Then, the antibodies of Brilliant Violet 421™ anti-mouse FOXP3 (126419, Biolegend) and Alexa Fluor® 647 anti-mouse IFN- γ (505814, Biolegend) were incubated according to the manufacturer's protocol of Transcription Factor Buffer Set (562574, BD Biosciences).

For myeloid derived suppressor cells (MDSCs) analysis, cells were incubated with TruStain FcX™ (anti-mouse CD16/32) blocking antibody (101320, Biolegend) prior to staining with fluorochrome-conjugated antibodies for 15 min at 4 °C. The fluorochrome-conjugated antibodies included FITC anti-mouse CD45 antibody (103108, Biolegend), PerCP/Cyanine5.5 anti-mouse/human CD11b antibody (101228, Biolegend), Brilliant Violet 510™ anti-mouse Ly-6G/Ly-6C (Gr-1) (108457, Biolegend).

For macrophages analysis, cells were incubated with TruStain FcX™ (anti-mouse CD16/32) blocking antibody (101320, Biolegend) prior to staining with fluorochrome-conjugated antibodies for 15 min at 4 °C. The fluorochrome-conjugated antibodies included FITC anti-mouse CD45 antibody (103108, Biolegend), PerCP/Cyanine5.5 anti-mouse/human CD11b antibody (101228, Biolegend), Brilliant Violet 421™ anti-mouse F4/80 antibody (123132, Biolegend), PE anti-mouse CD86 antibody (105008, Biolegend), and APC anti-mouse CD206 antibody (141708, Biolegend).

The cells were resuspended in Buffer A for FACS analysis on a BD LSRFortessa™ Cell Analyzer (BD Biosciences) or CytoFLEX LX flow cytometer (Beckman Coulter). The CD4⁺ T cells were assessed as CD45⁺CD3⁺CD4⁺ cells. The CD8⁺ T cells were assessed as CD45⁺CD3⁺CD8⁺ cells. T regulatory cells (Tregs) were assessed as CD4⁺CD25⁺FOXP3⁺ cells. MDSCs were assessed as CD45⁺CD11b⁺Gr-1⁺ cells. M1-like macrophages were assessed as CD45⁺CD11b⁺F4/80⁺CD86⁺CD206[−] cells. The M2-like macrophages were assessed as CD45⁺CD11b⁺F4/80⁺CD86[−]CD206⁺ cells.

Immunofluorescence staining

To evaluate c-Fos expression, the tumor-bearing mice were deeply anesthetized with isoflurane 90 min after 1, 2, or 24-hour social interaction or alone (Figure S2). Then the mice were perfused intracardially with preheated (37 °C) physiological saline and precooled (4 °C) 4% paraformaldehyde (PFA, P6148, Sigma-Aldrich, USA). The brains were removed and stored in 4% PFA at 4 °C for 24 h and then transferred to 20% sucrose solutions at 4 °C for 48 h.

To evaluate tumor cell proliferation, tumor-bearing mice were deeply anesthetized with isoflurane and perfused intracardially with preheated (37 °C) physiological saline and precooled (4 °C) 4% PFA. The tumors were removed and stored in 4% PFA at 4 °C for 24 h and then transferred to 20% sucrose solutions at 4 °C for 48 h.

Next, the brains or tumors were sectioned using a freezing microtome (CM1900, Leica, Germany). Slices were blocked in solution containing 10% normal goat serum and 0.5% Triton X-100 for 30 min at 37 °C. Next, the slices were incubated with primary antibodies, including Rabbit anti-c-Fos antibody (1:500, 226008, Synaptic Systems) and Rabbit anti-Ki67 antibody (1:1000, ab16667, Abcam, USA) at 37 °C for 1 h and then transferred to a slow shaker at 4 °C overnight. On the following day, the slices were washed with 0.01 M PBS (3 × 5 min) and then incubated with corresponding fluorescent secondary antibodies, including Goat anti-Rabbit conjugated to Alexa Fluor™ Plus 555 antibody (1:500, A32732, Invitrogen, USA), and Goat anti-Rabbit conjugated to Alexa Fluor™ Plus 488 antibody (1:1000, A32731, Invitrogen) at room temperature for 2 h. Slices were washed with 0.01 M PBS (3 × 5 min), incubated for 10 min with DAPI (1:2000, D9542, Sigma-Aldrich), and then subjected to wash steps in PBS, followed by mounting and cover-slipping on microscope slides. The images were acquired using a scanning laser microscope (SpinSR, Olympus, Japan). Quantification of the images was performed using Image J software with standardized parameters.

TUNEL assay

To examine tumor cell apoptosis, the terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick end (TUNEL) assay was performed. Briefly, after washing with PBS, the tumor slices were permeabilized with proteinase K (20 μ g/mL) for 20 min. Then, the slices were washed in 0.01 M PBS (3 × 5 min) and incubated with a EZ 488 TUNEL reaction buffer (AC12L055, Life-iLab, China) for 2 h. After incubation, the slices were washed in 0.01 M PBS (3 × 5 min) and then stained with DAPI (1:2000) for 10 min to label the cell nucleus. All slices were mounted in pre-cleaned slides with antifade mounting medium, then imaged with a scanning

laser microscope (SpinSR, Olympus). Quantification of the images was performed using Image J software with standardized parameters.

Histology

To examine the expression of viruses, the placement of the optical fiber and electrode array, mice were deeply anesthetized with isoflurane and transcardially perfused with preheated (37°C) physiological saline and precooled (4°C) 4% PFA. The brains were removed and stored in 4% PFA at 4°C for 24 h and then transferred to 20% sucrose solutions at 4°C for 48 h. The brains or tumors were sectioned at a thickness of 30 μ m using a freezing microtome (CM1900, Leica). Slices were washed with 0.01 M PBS (3 $\times$ 5 min), incubated for 10 min with DAPI (1:2000), and then subjected to wash steps PBS, followed by mounting and cover slipping on microscope slides. The virus expression and the placement of the optical fiber and wireless optogenetic probe were analyzed using an Olympus VS200 virtual slide system (Japan).

QUANTIFICATION AND STATISTICAL ANALYSIS

Data analysis and statistics

Information regarding statistical analyses can be found in the figure legends. All statistical tests were performed using SPSS software for Windows (v. 25.0). To compare two groups, a two-tailed unpaired Student's *t* test or two-tailed paired Student's *t* test (Figures 2E and S6B) was conducted. For multiple comparisons between more than two groups, a one- or two-way ANOVA followed by Tukey's post hoc test was performed. Data are presented as the mean $\pm$ SEM, except in box plots, which denote minimum, first quartile, median, third quartile, and maximum values. A value of *P* < 0.05 was considered statistically significant.