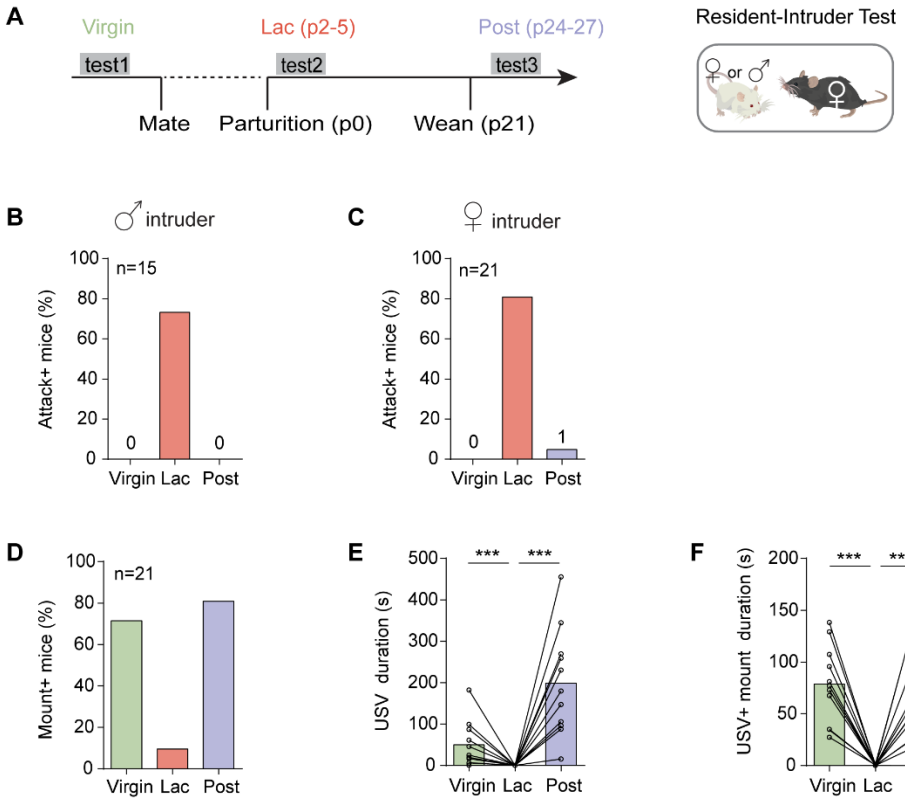
Supplemental information

Figure S1



Related to Figure 1.

Figure S1. Reversible switch of social behaviors in C57BL/6N female mice.

(A) Timeline and schematic of longitudinal behavioral tests. Parturition date counted as p0.

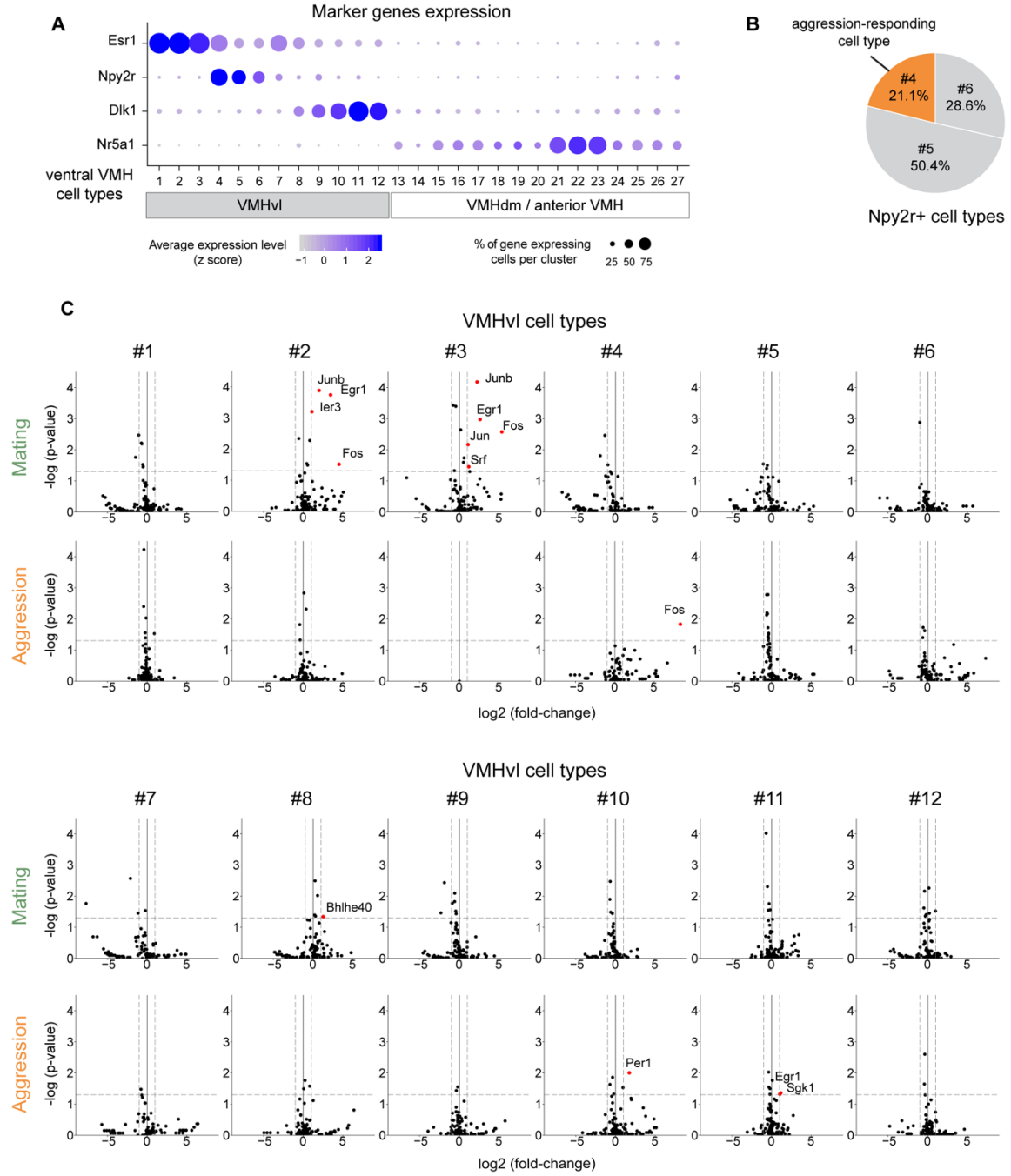
(B-C) Percentage of females exhibiting attack behavior towards (B) male intruder or (C) female intruder in virgin, lactating or post-lactating state.

(D) Percentage of females in virgin, lactating or post-lactating state exhibiting mounting behavior towards a female intruder.

(E-F) Duration of (E) ultrasonic vocalizations (USVs) emitted, or duration of (F) male-typical mounting behavior coupled with USVs by the same females, in the virgin, lactating and post-lactating states.

***p<0.001. Mean ± SEM.

Figure S2


A
Marker genes expression
Esr1
Npy2r
Dlk1
Nr5a1
ventral VMH cell types
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27
VMHvl
VMHdm / anterior VMH
Average expression level (z score)
−1 0 1 2
% of gene expressing cells per cluster
25 50 75
B
aggression-responding cell type
#4 21.1%
#6 28.6%
#5 50.4%
Npy2r+ cell types
C
VMHvl cell types
#1
#2
#3
#4
#5
#6
Mating
Aggression
-log (p-value)
Junb
Egr1
Ier3
Fos
Junb
Egr1
Fos
Jun
Srf
Fos
log2 (fold-change)
VMHvl cell types
#7
#8
#9
#10
#11
#12
Bhlhe40
Per1
Egr1
Sgk1
log2 (fold-change)

Related to Figure 1.

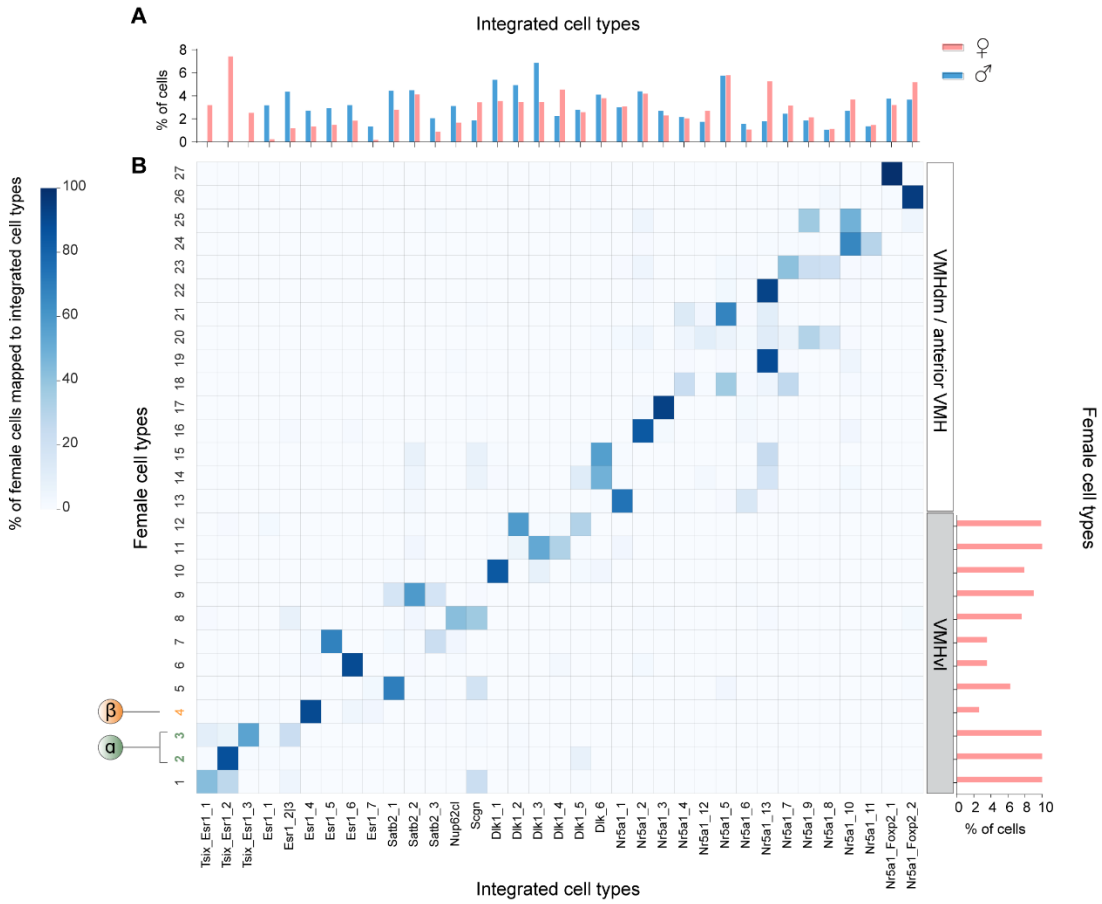
Figure S2. Additional information about female ventral VMH transcriptomic cell types.

(A) Expression level of marker genes for VMHvl and VMHdm / anterior VMH in 27 female ventral VMH cell types.

(B) Pie chart illustrating the percentage of different VMHvl transcriptomic cell types that express Npy2r. Only cluster #4 is activated (IEG induction) during aggression (orange sector).

(C) “Volcano plots” showing expression levels of all 139 IEGs in 12 VMHvl cell types from the following animals: lactating females exhibiting attacks vs. control, virgin females exhibiting lordosis vs. control. Colored dots indicate genes with expression fold change >2 (x axis cut-off) and P <0.05 (y axis cut-off; gray dashed lines).

Figure S3



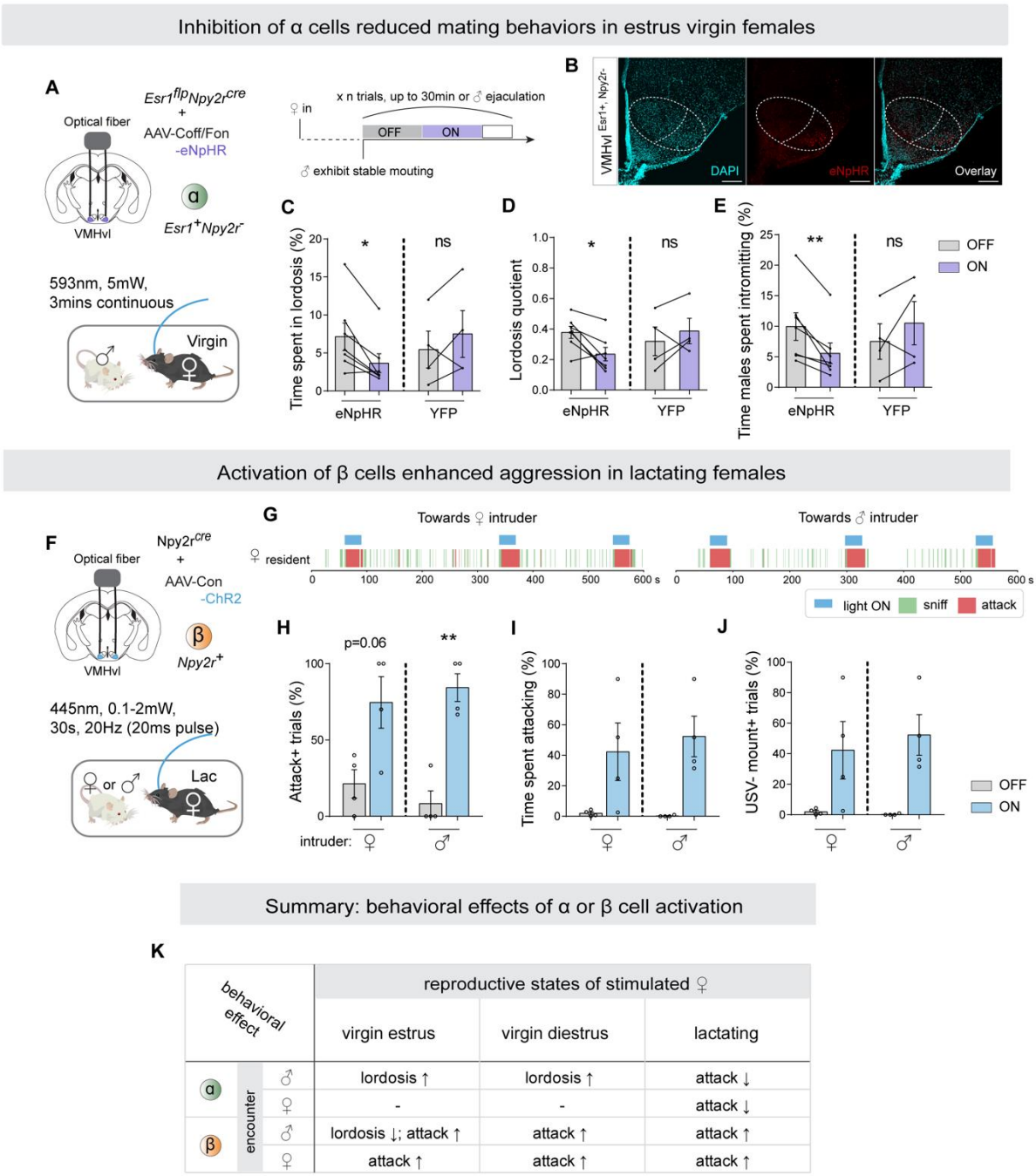
Related to Figure 1.

Figure S3. Comparison between male and female ventral VMH transcriptomes.

(A) Cell number distributions of female (pink) and male (blue) integrated cell types in ventral VMH (previous published data-guided integration (Kim et al., 2019)).

(B) Left, percentage of female cells (from this study) mapped to integrated VMH cell types. Right, cell number distributions of female cells (from this study) in VMHvl. α: female mating-responding cells; β: female aggression-responding cells.

Figure S4



Summary: behavioral effects of α or β cell activation

K

behavioral effect			reproductive states of stimulated ♀		
			virgin estrus	virgin diestrus	lactating
α	encounter	♂	lordosis ↑	lordosis ↑	attack ↓
		♀	-	-	attack ↓
β		♂	lordosis ↓; attack ↑	attack ↑	attack ↑
		♀	attack ↑	attack ↑	attack ↑

Related to Figure 2 and 3.

Figure S4. Additional information for optogenetic perturbations for VMHvl α and β cells.

(A-E) Inhibition of α cells reduced mating behaviors in estrus virgin females.

(A) Strategy to inhibit VMHvl$^{Esr1+,Npy2r-}$ cells (α) in virgin females by optogenetics and behavioral paradigm and illustration of stimulation scheme for one session.

(B) Representative eNpHR expression in VMHvl α cells. Scalebar, 200um.

(C) Fraction of time female spent in lordosis,

(D) lordosis quotient (lordosis time/male mounting or intromission time),

(E) fraction of time males spent intromitting during light ON or light OFF periods, in estrus intact (non-ovariectomized) ChR2/YFP-expressing females.

(F-I) Activation of β cells enhanced aggression in lactating females.

(F) Strategy to activate VMHvl^{Npy2r+} cells (β) in lactating females by optogenetics.

(G) Representative raster plots illustrating light-induced behaviors in lactating female towards female or male intruders.

(H) Fraction of trials where lactating female exhibited attack behavior.

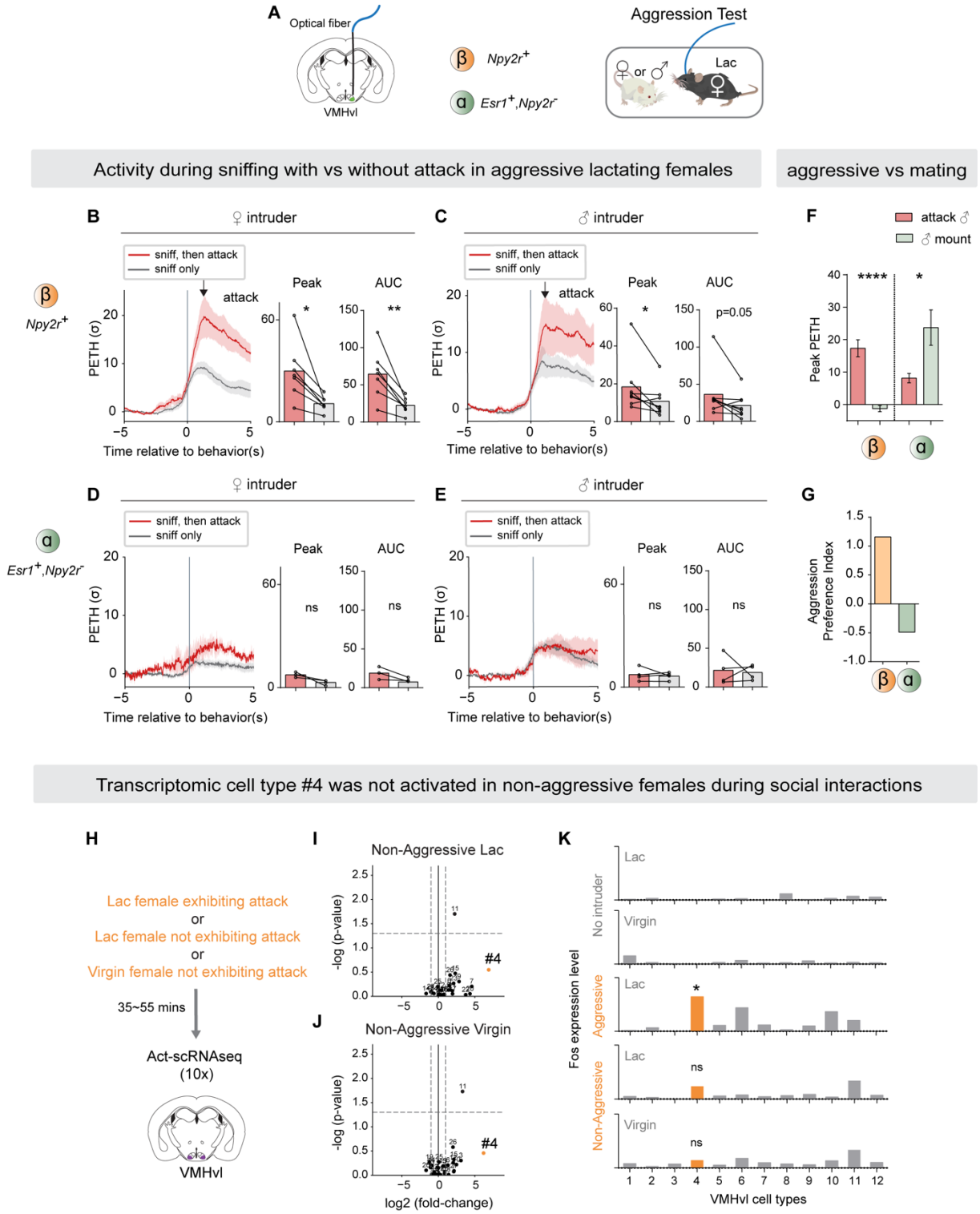
(I) Fraction of time lactating female spent attacking during a trial.

(J) Fraction of trials where lactating females exhibited USV^{-} mounting behavior.

(K) Summary table for behavioral effects of α or β cell activation in females at different reproductive states. ↑ evoke or enhance; ↓ inhibit.

*p<0.05; **p<0.01; Mean ± SEM.

Figure S5


A
Optical fiber
VMHvl
β Npy2r+
α Esr1+,Npy2r-
Aggression Test
♀ or ♂
Lac
Activity during sniffing with vs without attack in aggressive lactating females
aggressive vs mating
B
♀ intruder
C
♂ intruder
F
attack ♂
♂ mount
sniff, then attack
sniff only
attack
Peak
AUC
PETH (σ)
Time relative to behavior(s)
Peak PETH
****
*
**
p=0.05
D
♀ intruder
E
♂ intruder
ns
G
Aggression Preference Index
Transcriptomic cell type #4 was not activated in non-aggressive females during social interactions
H
Lac female exhibiting attack
or
Lac female not exhibiting attack
or
Virgin female not exhibiting attack
35~55 mins
Act-scRNAseq
(10x)
VMHvl
I
Non-Aggressive Lac
-log (p-value)
#4
J
Non-Aggressive Virgin
log2 (fold-change)
K
Fos expression level
No intruder
Aggressive
Non-Aggressive
Lac
Virgin
VMHvl cell types

Related to Figure 6.

Figure S5. VMHvl β cells are highly active in lactating females during aggressive interactions.

(A) Left, schematic illustrating fiber photometry recording in VMHvl β and α cells. Right, schematic illustrating behavioral test of aggressive behaviors in lactating females.

(B-C) Left, PETH of β cells activity aligned to onset of sniffing of (B) female or (C) male followed or not followed by attack. Right, quantification of PETH peak and area under the curve (AUC).

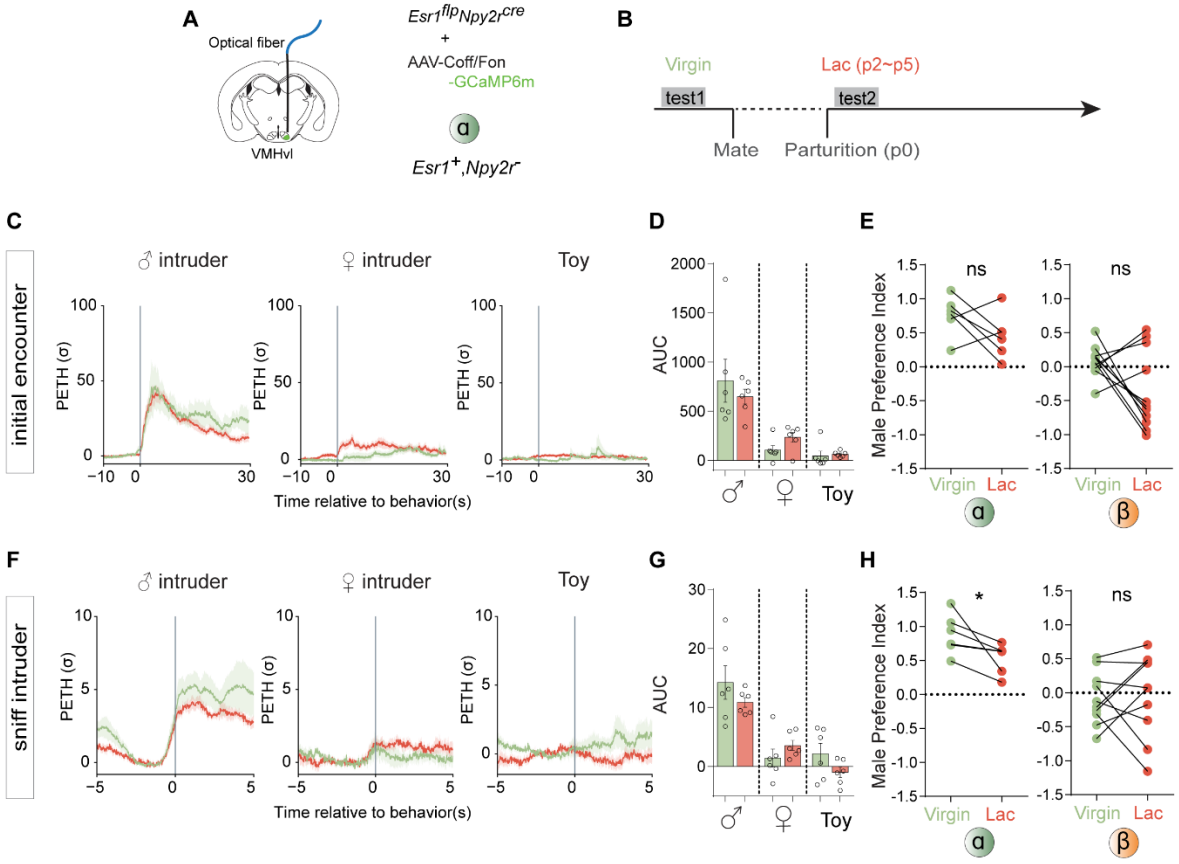
(D-E) Left, PETH of α cells activity aligned to onset of sniffing of (D) female or (E) male followed or not followed by attack.

(F-G) Comparison of neural responses during aggression vs. mating behaviors. (F) PETH peak levels of VMHvl β and α cell activity at consummatory phase during aggressive or mating interactions with male intruder. (G) Aggression preference index = Peak PETH at (attack – mounted) / (attack + mounted).

(H-K) Act-seq results from aggressive vs. non-aggressive females following social interactions with female intruders. (H) Schematic of Act-seq protocol. (I-J) "Volcano plots" showing Fos expression levels in 27 VMH cell types from (I) lactating females not exhibiting attacks vs. control, (J) virgin females not exhibiting attack vs. control. Dashed lines: fold change = 2 (x axis cut-off) and P = 0.05 (y axis cut-off). (K) *Fos* expression levels in 12 VMHvl cell types from control lactating females, control virgin females (No intruder), lactating females exhibiting attacks (Aggressive), lactating and virgin females not exhibiting attack (Non-Aggressive). Orange colored: aggression-responding cluster #4 (See Figure 1F).

*p<0. 05; **p<0.01; ***p<0.001; ****p<0.0001; ns, no significance. Mean ± SEM.

Figure S6


A
Optical fiber
VMHvl
Esr1flpNpy2rcre
+
AAV-Coff/Fon
-GCaMP6m
α
Esr1+,Npy2r-
B
Virgin
Lac (p2~p5)
test1
test2
Mate
Parturition (p0)
C
initial encounter
♂ intruder
♀ intruder
Toy
PETH (σ)
Time relative to behavior(s)
D
AUC
♂
♀
Toy
E
Male Preference Index
ns
Virgin
Lac
α
β
F
sniff intruder
♂ intruder
♀ intruder
Toy
PETH (σ)
Time relative to behavior(s)
G
AUC
♂
♀
Toy
H
Male Preference Index
*
ns
Virgin
Lac
α
β

Related to Figure 7.

Figure S6. Activity of VMHvl α cells in social interaction is not state-dependent.

(A) Schematic illustrating fiber photometry recording of VMHvl α cells.

(B) Timeline representing the conducting of longitudinal behavioral tests and recording in the same individuals in virgin and lactating states. Parturition date was counted as p0.

(C) PETH of α cell activity in virgin and lactating state aligned to onset of initial encounters with male, female and toy.

(D) AUC represents timespan 0-30 seconds after initial encounters.

(E) Within-subject comparison of male preference index (MPI) between virgin and lactating state from α and β. MPI = AUC of initial encounter with (male – female) / (male + female).

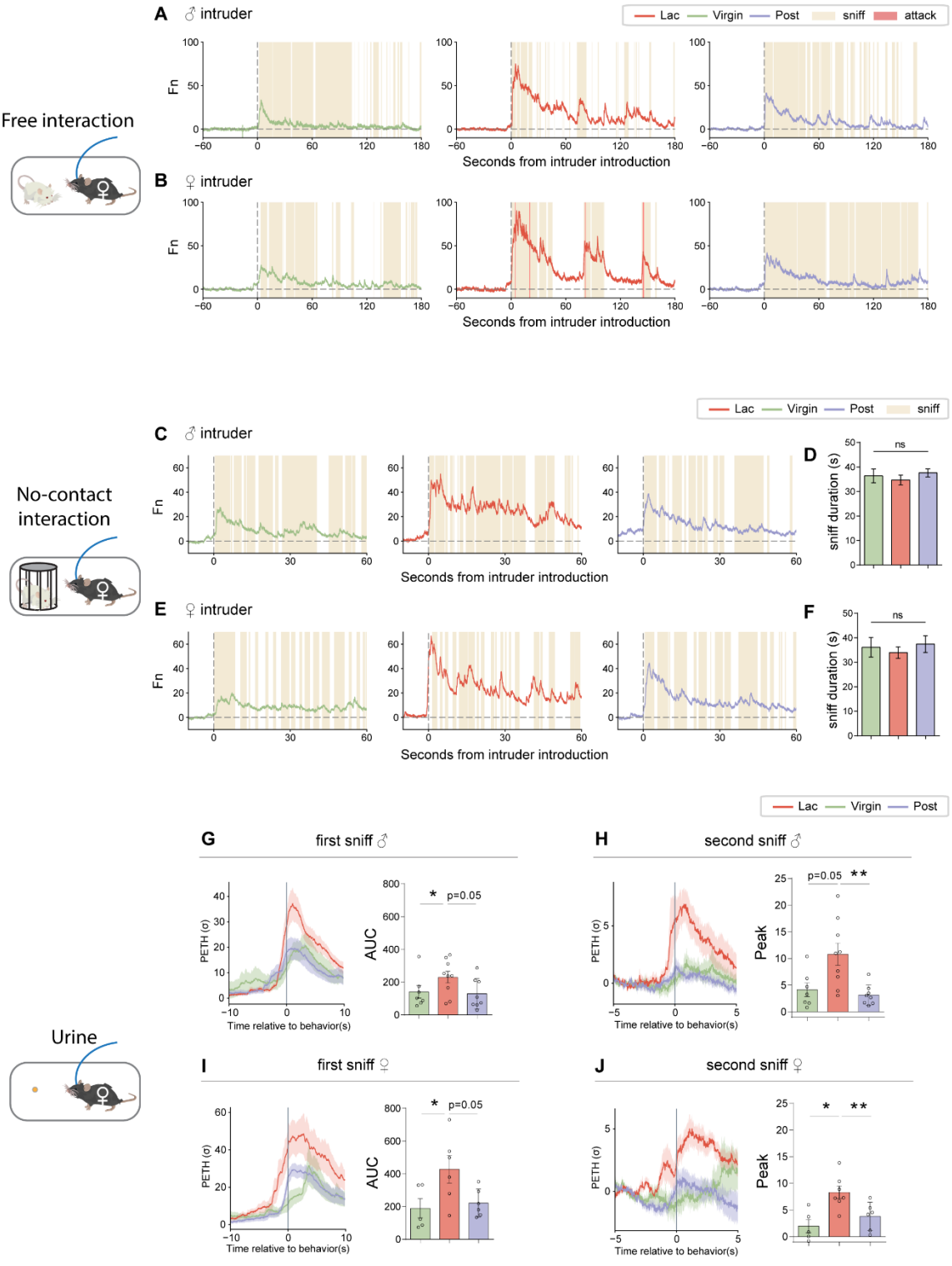
(F) PETH of α cell activity in virgin and lactating state aligned to onset of sniffing of male, female and toy.

(G) AUC represents timespan 0-3 seconds after sniffing of intruders.

(H) Within-subject comparison of male preference index (MPI) between virgin and lactating state from α and β cells. MPI = AUC of sniffing of (male – female) / (male + female).

*p<0.05; ns, no significance. Mean ± SEM.

Figure S7


Free interaction
A ♂ intruder
Lac Virgin Post sniff attack
Fn
Seconds from intruder introduction
B ♀ intruder
No-contact interaction
C ♂ intruder
Lac Virgin Post sniff
D
ns
sniff duration (s)
E ♀ intruder
F
Urine
Lac Virgin Post
G first sniff ♂
PETH (σ)
Time relative to behavior(s)
AUC
* p=0.05
H second sniff ♂
Peak
p=0.05 **
I first sniff ♀
J second sniff ♀
* **

Related to Figure 7.

Figure S7. Response of VMHvl β cells to social cues depends on lactation state.

(A-B) Behavioral raster plots of representative normalized GCaMP6m trace from β cells during free-interaction with (A) male intruder and (B) female intruder. Corresponding to Figure S7C,E. Colored shading marks behavioral episodes.

(C,E) Behavioral raster plots of representative normalized GCaMP6m trace from β cells during no-contact-interaction with (C) male intruder and (E) female intruder. Corresponding to Figure S7G,J.

(D,F) Total sniff duration in the first minute of no-contact interaction in three lactation states.

(G-J) β cell responses to urine in three lactation states. (G,I) Left, PETH of β cells activity at first sniff of (G) male urine or (I) female urine. Right, AUC represents timespan 0-10 seconds from behavior onset. (H,J) Left, PETH of β cell activity at second sniff of (H) female urine or (J) male urine. Right, AUC represents timespan 0-3 seconds from behavior onset.

*p<0.05; **p<0.01. Mean ± SEM.

Supplemental Table 1

Related to STAR Methods section QUANTIFICATION AND STATISTICAL ANALYSIS.

Figure	Compared group	Test used	P value	n
1E	Lac attack vs Lac control	Wilcoxon Rank Sum	\	\
1F	Virgin lordosis vs Virgin control	Wilcoxon Rank Sum	\	\
1G	Virgin control vs Lac control	Wilcoxon Rank Sum	\	\
2D	ON vs OFF, Estrus	Wilcoxon Rank Sum	0.002	10
2D	ON vs OFF, Diestrus	Paired t test	0.0005	9
2E	ON vs OFF, Estrus	Paired t test	0.0006	10
2E	ON vs OFF, Diestrus	Wilcoxon Rank Sum	0.0078	9
2F	ON vs OFF, Estrus	Paired t test	<0.0001	10
2F	ON vs OFF, Diestrus	Paired t test	0.0007	9
2G	ON vs OFF, Estrus	Paired t test	0.0006	10
2G	ON vs OFF, Diestrus	Wilcoxon Rank Sum	0.0078	9
2K	ON vs OFF	Paired t test	0.0021	5
2K	ON vs OFF, YFP	Paired t test	0.9204	5
3E	ON vs OFF	Paired t test	<0.0001	6
3E	ON vs OFF, YFP	Paired t test	0.9123	5
3L	ON vs OFF, Female	Paired t test	<0.0001	5
3L	ON vs OFF, Male	Paired t test	<0.0001	5
3M	ON vs OFF, Female	Paired t test	<0.0001	5
3M	ON vs OFF, Male	Paired t test	0.0002	5
3N	ON vs OFF, Female	Paired t test	0.0192	5
3N	ON vs OFF, Male	Paired t test	0.0067	5
4E	Male vs Female	Paired t test	0.0027	8
4E	Male vs Toy	Wilcoxon Rank Sum	0.0078	8
4E	Female vs Toy	Wilcoxon Rank Sum	0.0781	8
4H	Male vs Female	Paired t test	0.0025	8
4H	Male vs Toy	Paired t test	0.0013	8
4H	Female vs Toy	Paired t test	0.9927	8
4K	Male vs Female	Paired t test	0.0004	6
4K	Male vs water	Mann-Whitney U	0.0095	6,5
4K	Female vs water	Mann-Whitney U	0.019	6,5
4L	Male vs Female	Wilcoxon Rank Sum	0.7402	35
4L	Male vs water	Mann-Whitney U	<0.0001	35,19
4L	Female vs water	Mann-Whitney U	<0.0001	35,19
4F	Male vs Female	Wilcoxon Rank Sum	0.0537	11
4F	Male vs Toy	Paired t test	0.0001	11
4F	Female vs Toy	Paired t test	0.0002	11
4I	Male vs Female	Paired t test	0.8065	8
4I	Male vs Toy	Paired t test	0.0414	8
4I	Female vs Toy	Paired t test	0.0026	8
4G	alpha vs beta	Mann-Whitney U	0.0018	8,11
4J	alpha vs beta	Mann-Whitney U	0.0003	8
4M	alpha vs beta	Welch-corrected t test	<0.0001	6,35
5E	appetitve vs consummatory	Unpaired t test	0.0119	7
5I	appetitve vs consummatory	Unpaired t test	0.0002	11,5
6G	AGG vs non-AGG	Mann-Whitney U	0.0033	14,5
6G	AGG vs non-AGG	Mann-Whitney U	0.0012	14,5
6H	AGG vs non-AGG	Mann-Whitney U	0.0112	9,4
6H	AGG vs non-AGG	Mann-Whitney U	0.0196	9,4
7D	Virgin vs Lac, peak	Wilcoxon Rank Sum	0.0039	9
7D	Virgin vs Lac(highest value removed)	Paired t test	0.0002	8
7D	Post vs Lac, peak	Wilcoxon Rank Sum	0.0078	8
7D	Post vs Lac(highest value removed)	Paired t test	0.0004	7
7D	Virgin vs Lac, AUC	Wilcoxon Rank Sum	0.0039	9
7D	Virgin vs Lac(highest value removed)	Paired t test	0.0004	8
7D	Post vs Lac, AUC	Wilcoxon Rank Sum	0.0078	8
7D	Post vs Lac(highest value removed)	Paired t test	0.0014	7
7F	Virgin vs Lac, peak	Paired t test	0.0057	7
7F	Virgin vs Lac(highest value removed)	Paired t test	0.0037	6
7F	Post vs Lac, peak	Paired t test	0.0024	6
7F	Post vs Lac(highest value removed)	Paired t test	0.0025	5
7F	Virgin vs Lac, AUC	Paired t test	0.0051	7
7F	Virgin vs Lac(highest value removed)	Paired t test	0.002	6
7F	Post vs Lac, AUC	Paired t test	0.0035	6
7F	Post vs Lac(highest value removed)	Paired t test	0.0026	5
7K	Virgin vs Lac	Paired t test	0.0018	10
7K	Post vs Lac	Paired t test	0.0019	10
7H	Virgin vs Lac	Wilcoxon Rank Sum	0.0002	14
7H	Post vs Lac	Paired t test	0.0002	12
7I	Virgin vs Lac, AUC	Two-way ANOVA	<0.0001	10
7I	Post vs Lac, AUC	Two-way ANOVA	<0.0001	10
7I	Virgin vs Lac, peak	Two-way ANOVA	<0.0001	10
7I	Post vs Lac, peak	Two-way ANOVA	<0.0001	10
7L	Virgin vs Lac, AUC	Two-way ANOVA	<0.0001	10
7L	Post vs Lac, AUC	Two-way ANOVA	<0.0001	10
7L	Virgin vs Lac, peak	Two-way ANOVA	<0.0001	10
7L	Post vs Lac, peak	Two-way ANOVA	<0.0001	10
S1E	Virgin vs Lac	Wilcoxon Rank Sum	0.001	11
S1E	Post vs Lac	Wilcoxon Rank Sum	0.001	11
S1F	Virgin vs Lac	Wilcoxon Rank Sum	0.001	11
S1F	Post vs Lac	Wilcoxon Rank Sum	0.001	11
S2C	Lac attack vs Lac control	Wilcoxon Rank Sum	\	\
S2C	Virgin lordosis vs Virgin control	Wilcoxon Rank Sum	\	\
S4C	ON vs OFF, eNpHR	Wilcoxon Rank Sum	0.0313	7
S4C	ON vs OFF, YFP	Wilcoxon Rank Sum	0.375	4
S4D	ON vs OFF, eNpHR	Paired t test	0.0155	7
S4D	ON vs OFF, YFP	Wilcoxon Rank Sum	0.625	4
S4E	ON vs OFF, eNpHR	Paired t test	0.007	7
S4E	ON vs OFF, YFP	Wilcoxon Rank Sum	0.5	4
S4H	Female	Paired t test	0.0656	4
S4H	Male	Paired t test	0.0025	4
S4I	Female	Paired t test	0.1237	4
S4I	Male	Paired t test	0.0282	4
S4J	Female	Paired t test	0.4226	3
S4J	Male	Paired t test	0.3061	4
S5B	Peak	Paired t test	0.0129	7
S5B	AUC	Paired t test	0.0048	7
S5C	Peak	Wilcoxon Rank Sum	0.0391	8
S5C	AUC	Wilcoxon Rank Sum	0.0547	8
S5D	Peak	Wilcoxon Rank Sum	0.25	4
S5D	AUC	Wilcoxon Rank Sum	0.25	4
S5E	Peak	Wilcoxon Rank Sum	>0.9999	4
S5E	AUC	Wilcoxon Rank Sum	0.875	4
S5F	Beta	Welch-corrected t test	<0.0001	9,5
S5F	Alpha	Welch-corrected t test	0.0284	4,7
S6D	Male	Paired t test	0.3224	6
S6D	Female	Paired t test	0.208	6
S6D	Toy	Paired t test	0.1508	6
S6E	Alpha	Paired t test	0.1747	6
S6E	Beta	Paired t test	0.0609	11
S6G	Male	Paired t test	0.554	6
S6G	Female	Wilcoxon Rank Sum	0.1563	6
S6G	Toy	Wilcoxon Rank Sum	0.4375	6
S6H	Alpha	Wilcoxon Rank Sum	0.0313	6
S6H	Beta	Paired t test	0.8165	10
S7G	Virgin vs Lac	Paired t test	0.015	4
S7G	Post vs Lac	Paired t test	0.0522	6
S7H	Virgin vs Lac	Paired t test	0.0198	5
S7H	Post vs Lac	Paired t test	0.0022	6
S7I	Virgin vs Lac	Paired t test	0.028	7
S7I	Post vs Lac	Paired t test	0.0524	8
S7J	Virgin vs Lac	Paired t test	0.0515	7
S7J	Post vs Lac	Paired t test	0.0036	8