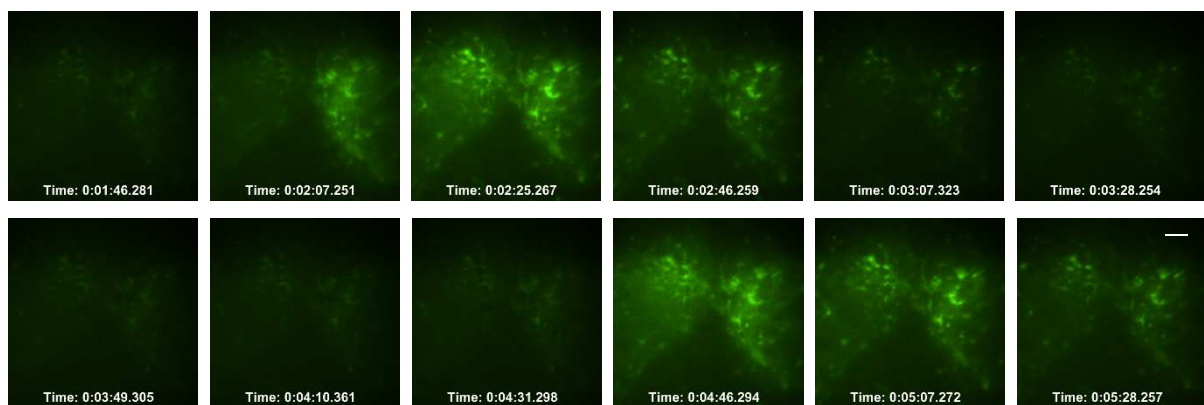


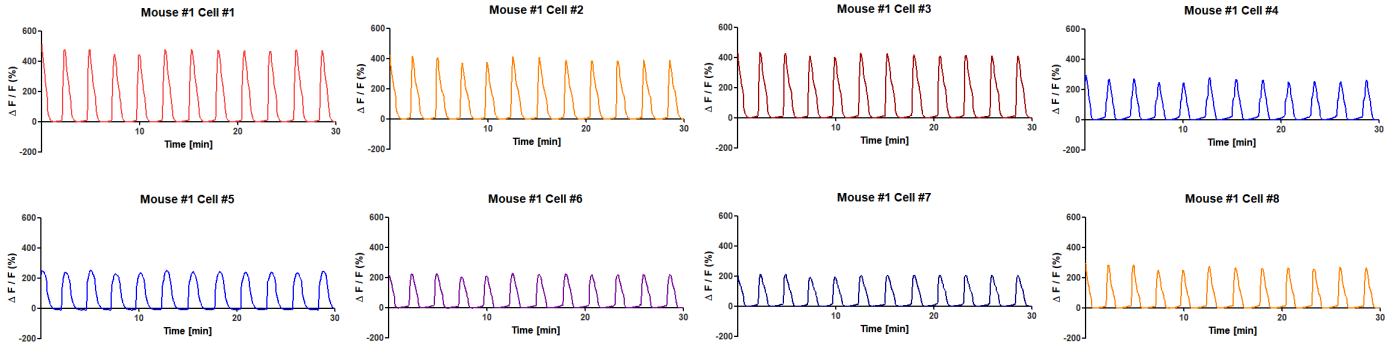
Supplementary Figure S1.

(A) Immunostaining of *Kiss1*-IRES-Cre/tdTomato mice at postnatal day 7 with kisspeptin antiserum (14 slices from 2 mice). Scale bar 20 μ m. (B) FISH performed at the beginning (4 days, 4D) and more than 20 days after culture (>20D). The number of *Kiss1* particles per cell are analyzed using 5 slices from 3 mice (2 males and 1 female) for 4D and 5 slices from 5 mice (3 males and 2 females) for >20D. Positive and negative control experiments are also shown. Scale bar 20 μ m. (C) Analysis of GCaMP-expressing kisspeptin neurons and kisspeptin positive neurons from GCaMP-expressing cells after culture. Percentage was calculated using 11 slices from 9 mice (7 males and 2 females). (D) Immunostaining using kisspeptin antiserum on RGEKO-transduced neuron in the organotypic slice culture. Scale bar 2 μ m.

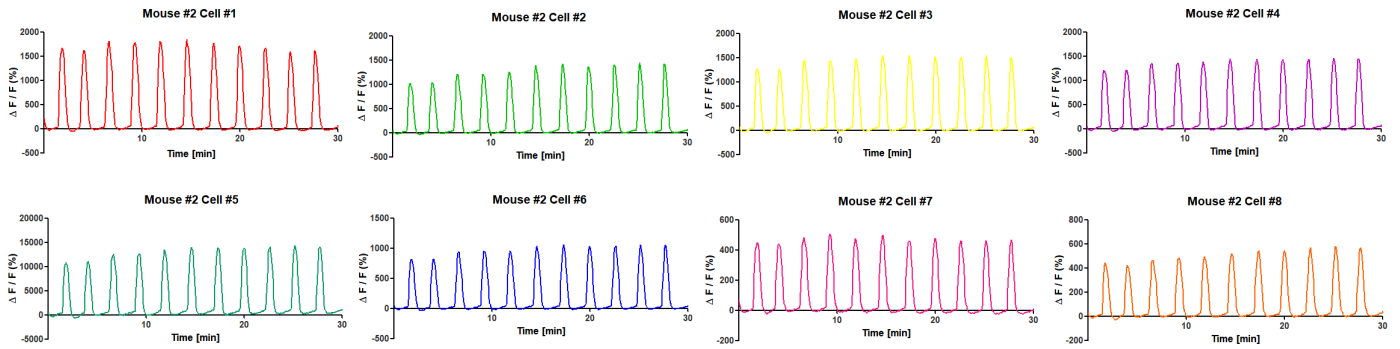


Supplementary Figure S2.
Time-series snapshot of GCaMP activity. Scale bar 100 μm .

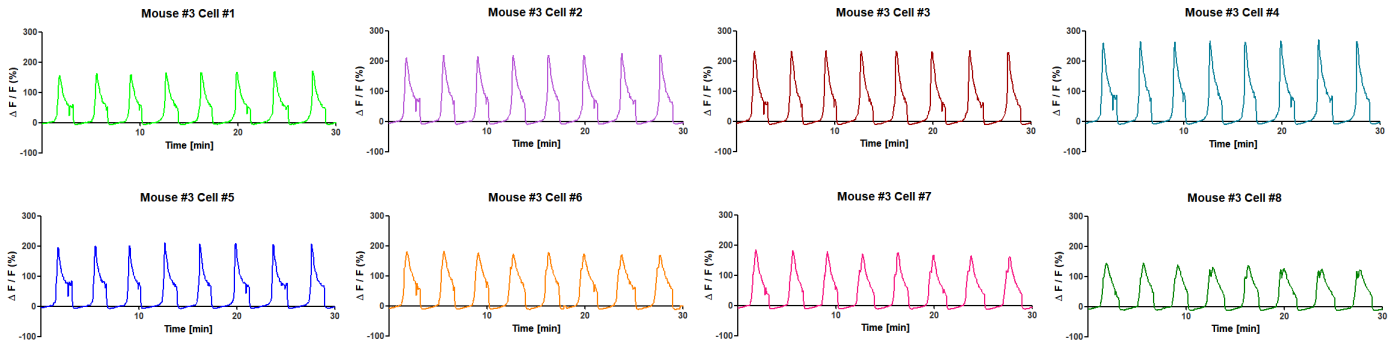
Mouse #1



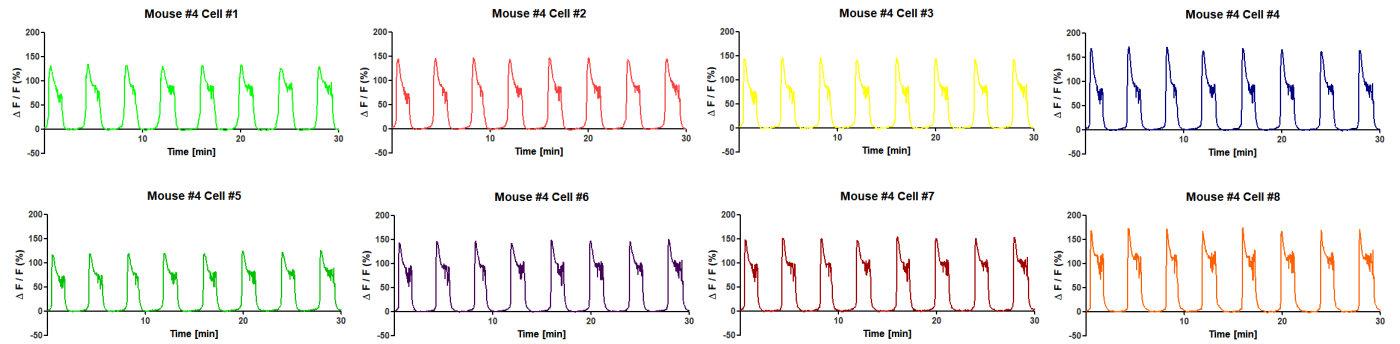
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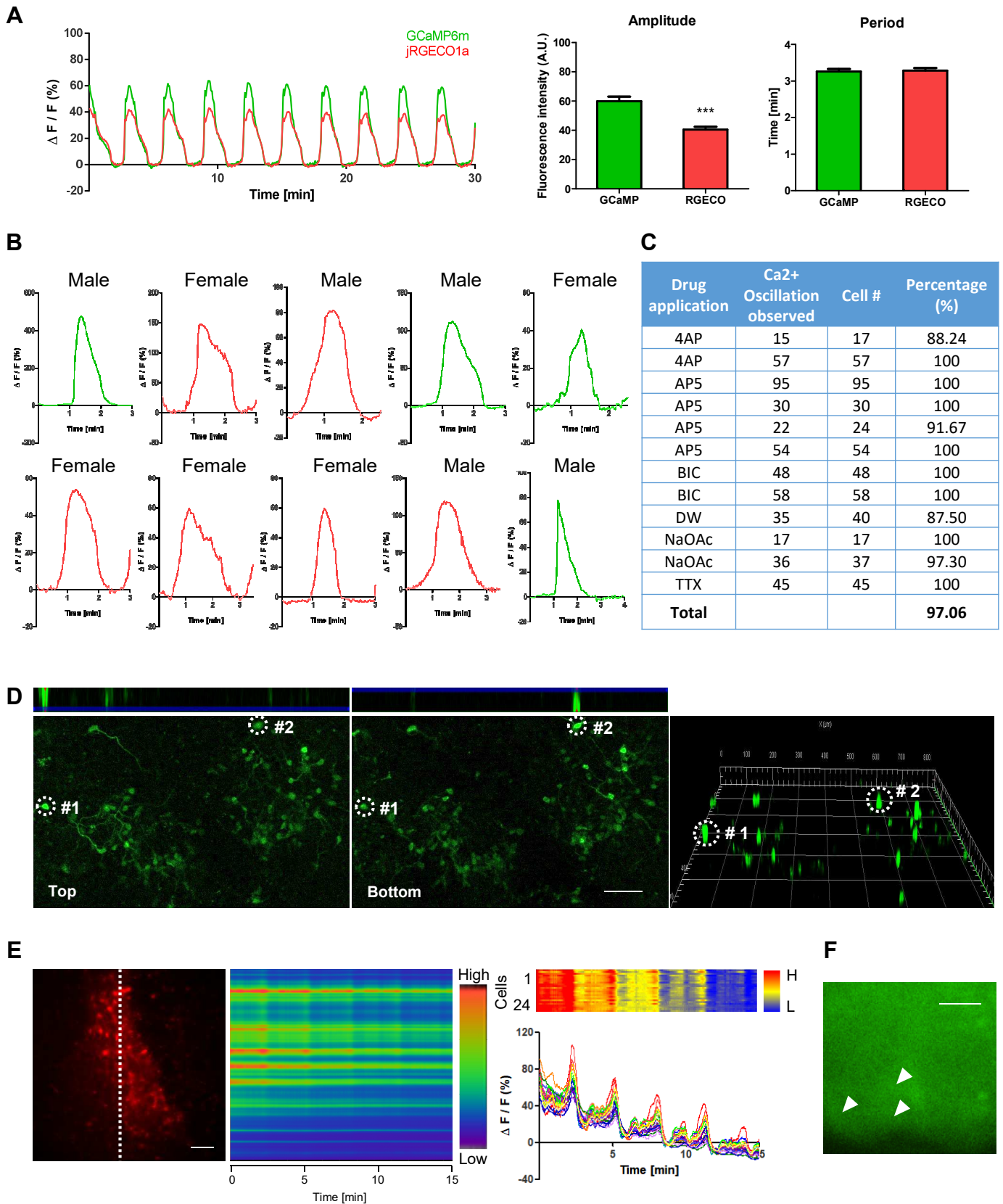
Mouse #3



Mouse #4

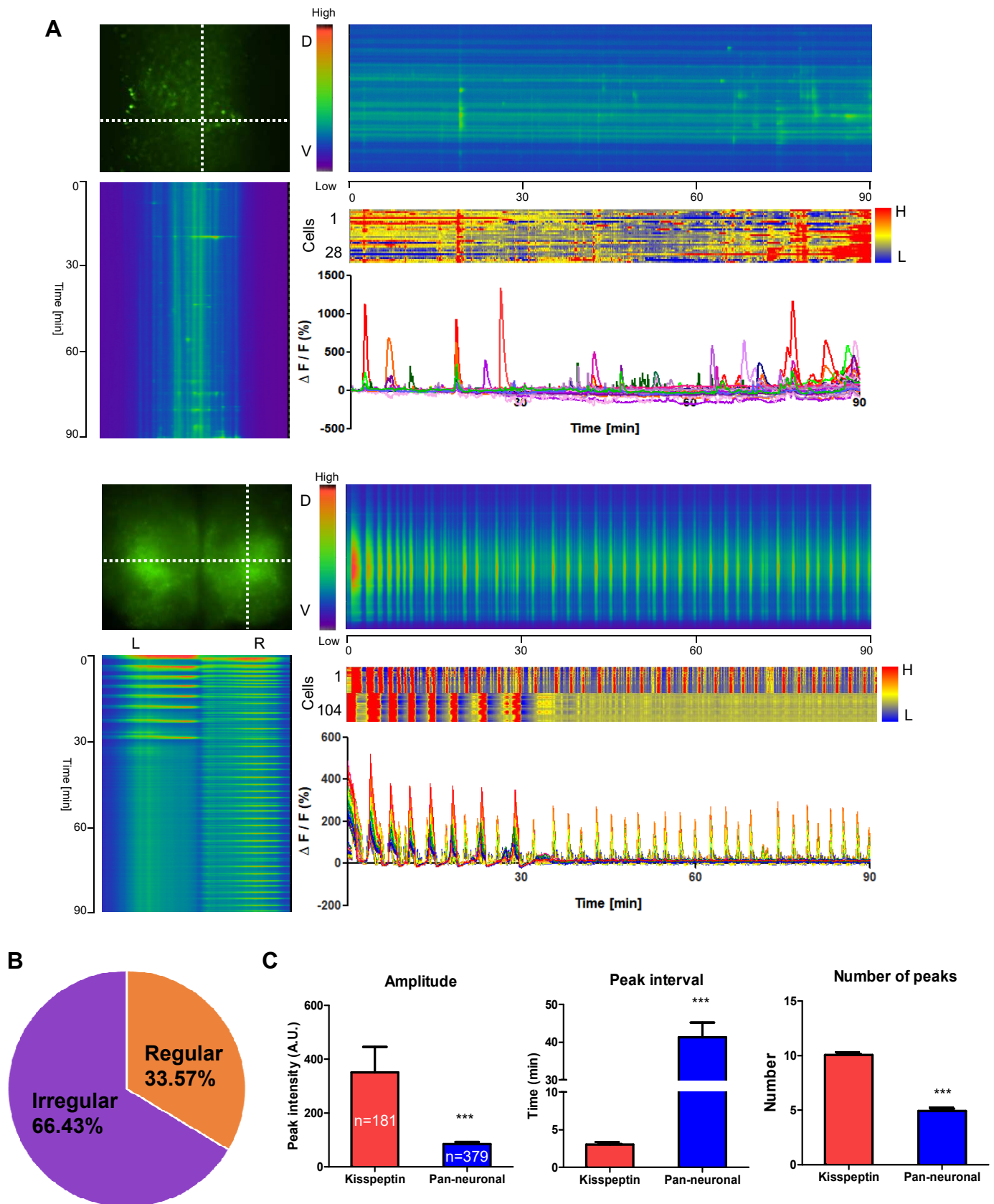


Supplementary Figure S3. Individual Ca^{2+} profile of kisspeptin neurons from 4 different mice.



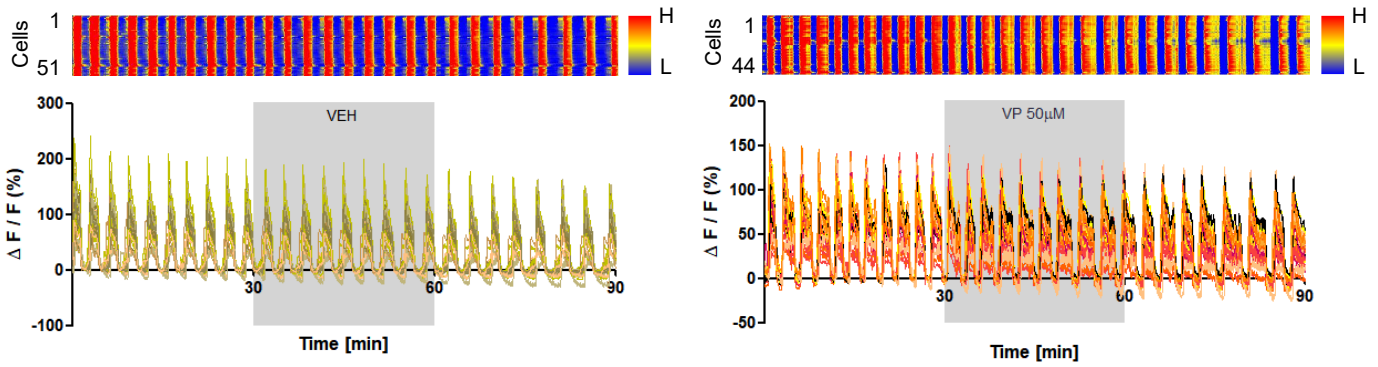
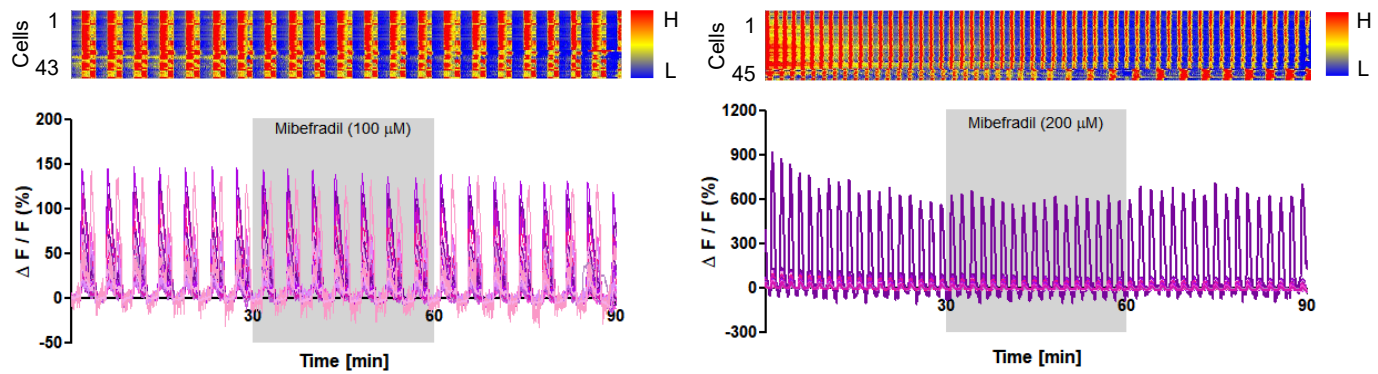
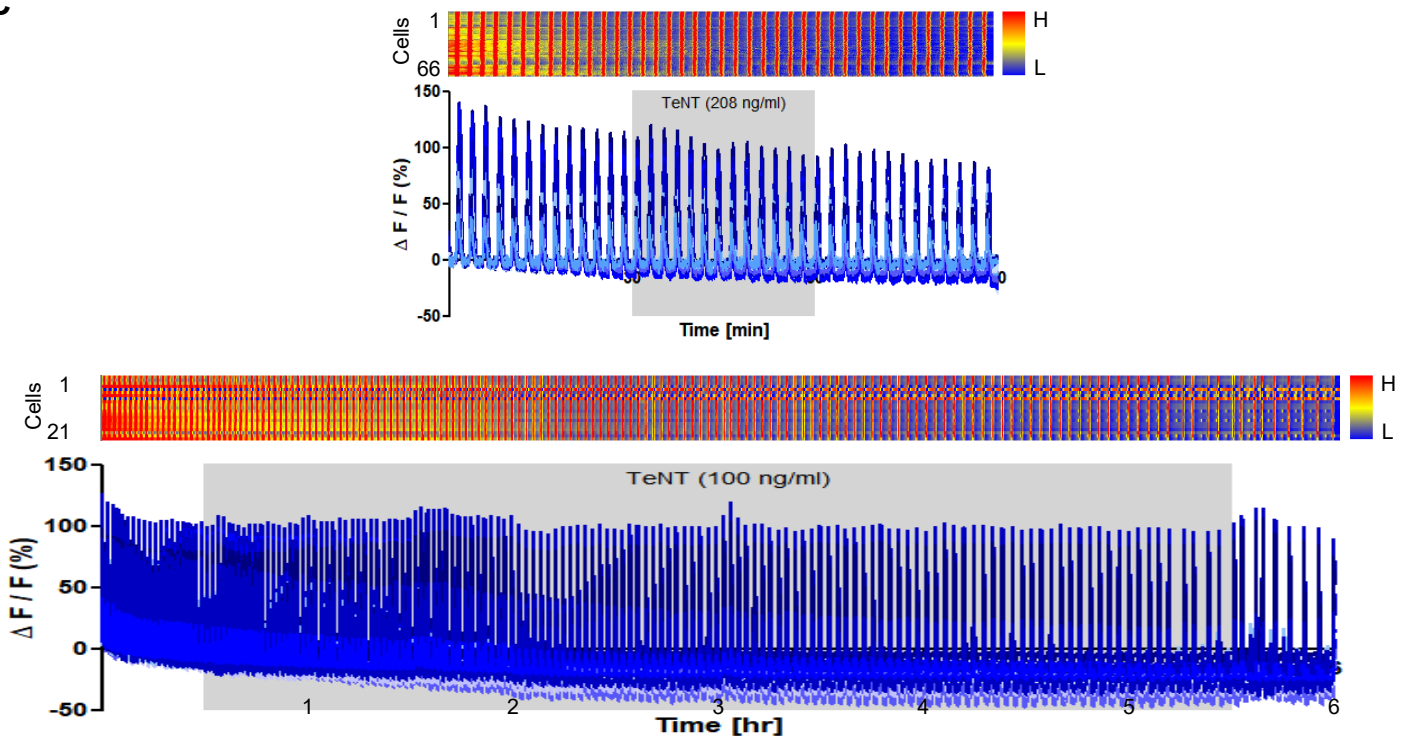
Supplementary Figure S4.

(A) Comparison of two fluorescent Ca²⁺ indicators, Flex-GCaMP and Flex-RGECO, expressed from the same kisspeptin neuron. Amplitude and period of Ca²⁺ oscillation quantified from GCaMP and RGECO (78 neurons from 3 mice, *** $p < 0.001$). (B) Single peak profiles from different mice. (C) Proportion of the number of cells observing Ca²⁺ oscillation. (D) Confocal imaging of fluorescence intensity difference according to the depth of the cell body located in the organotypic slice. Scale bar 100 μm . 3D representation of the Z-stack image is present at right. (E) Synchronized Ca²⁺ oscillation from horizontal slice kisspeptin neurons. (F) Dopamine transporter (*Slc6a3*; DAT)-IRES-Cre mice transduced with AAV_{2/1}-hSyn-Flex-GCaMP6m. Scale bar 200 μm .



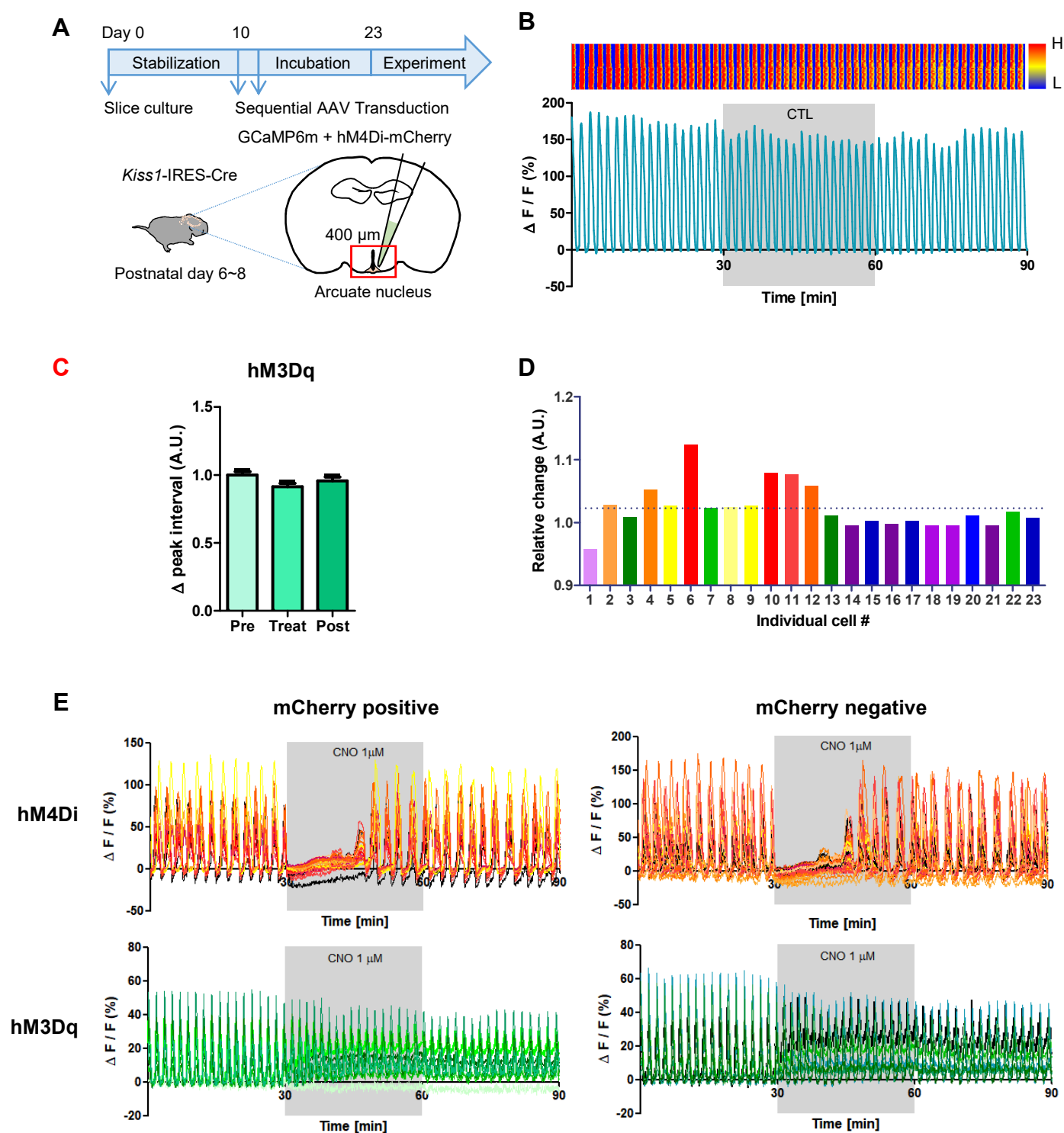
Supplementary Figure S5.

(A) Representative image and change in fluorescence intensity of the indicated horizontal and vertical lines in pan-neuronal imaging from 2 wild type mice. (B) Proportion of cells exhibiting regular and irregular fluctuation of Ca^{2+} concentrations from 5 wild type mice. (C) Amplitude, period, and the number of peaks of Ca^{2+} oscillation in kisspeptin and pan-neuronal population. (181-379 neurons from 4-5 mice, *** $p < 0.001$).

A**B****C**

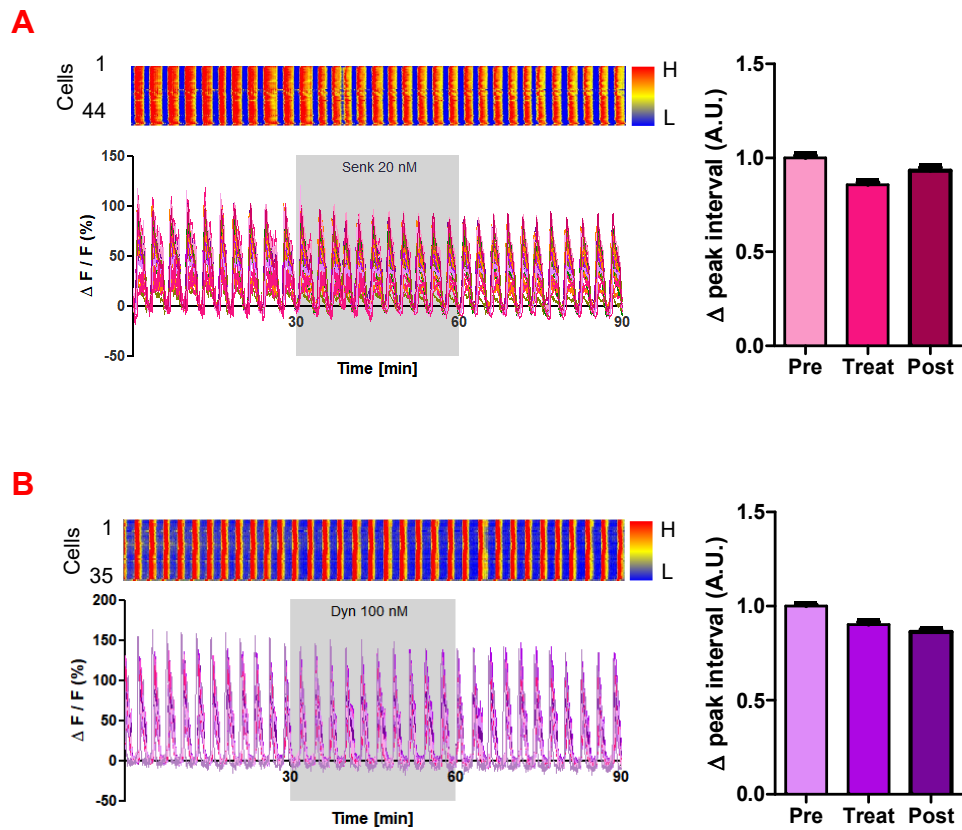
Supplementary Figure S6.

(A) Effect of administration of vehicle (0.03% methanol) and L-type voltage-gated Ca^{2+} channel blocker (verapamil, VP, 50 μ M). (B) Administration of T-type voltage-gated Ca^{2+} channel blocker with two doses (Mibefradil, 100 μ M and 200 μ M). (C) Raster plot and quantified graph of Ca^{2+} oscillation with synaptic release blocker tetanus toxin (TeNT) administration 208 ng/ml for 30 min (upper) and 100 ng/ml for 5 hr (bottom).



Supplementary Figure S7.

- (A) Experimental scheme for chemogenetic modulation of ARN kisspeptin neurons and Ca^{2+} imaging. (B) Control (CTL, 0.01% distilled water in recording media) treatment for DREADD experiment. (C) Δ peak interval in hM3Dq (62 neurons from 2 mice) experiment. (D) Fold change of individual neurons transduced with hM3Dq after CNO treatment (activation). Dotted line indicates the average value. (E) mCherry-positive and -negative neurons distinguished.



Supplementary Figure S8.

(A) NK3R agonist senktide administered for longer time at lower dose (Senk 20nM). Δ Peak interval in the pre-, treat, and post- phases are analyzed from 103 neurons from 3 mice (1 male and 2 females). (B) Dyn administered for longer time at lower dose (100nM). Δ Peak interval in the pre-, treat, and post- phases are analyzed from 89 neurons from 2 female mice.