



Extended Data Figure 3. A subset of POA *Calb1*⁺ neurons co-express *Adcyap1* and are activated following ejaculation.

a. Schematics of the experimental procedure. C57BL/6 male mice underwent a mating test, during which they either achieved ejaculation or intromission. Thirty minutes after the test, the mice were sacrificed, and their brains were processed for fluorescent *in situ* hybridization to detect *Calb1*, *Adcyap1* and *Fos* signals using RNAscope kits.

b. Example images (left) and quantification (right) show the overlap between *Calb1*⁺ and *Adcyap1*⁺ signals (top row), and *Adcyap1*⁺ and *Fos*⁺ signals (bottom row) under the intromission (left, blue) and ejaculation (right, red) conditions. Scale bar, 50 μ m. n = 4 males/group.

Values are presented as mean \pm SEM. *p < 0.05.