



**Extended Data Figure 8. Validation of the *Pomc-Cre* line and comparison of ejaculation-associated activity in *Pomc*<sup>+</sup> and *Calb1*<sup>+</sup> neurons**

**a, c.** Illustration of the *Pomc-Cre* line maintained on the C57BL/6 background (**a**) and the B6D2F1 hybrid background (**c**). Representative RNAscope fluorescent *in situ* hybridization images with the quantification show overlap between *Cre* expression and *Pomc* signals. Scale bar, 50  $\mu$ m.  $n = 2/\text{group}$ .

**b.** Representative traces of GCaMP6s signals ( $\Delta F/F$ ) recorded in POA *Calb1*<sup>+</sup> neurons (top) and ARC *Pomc*<sup>+</sup> neurons (bottom). GCaMP6s signal duration (left) during ejaculation (Eja.) and latency relative to ejaculation as time '0' (right) were plotted. The quantification showing delayed but more sustained activation of *Pomc*<sup>+</sup> neurons during ejaculation compared to POA *Calb1*<sup>+</sup> neurons.  $n = 10$  *Calb1*<sup>+</sup> males and 6 *Pomc*<sup>+</sup> males.

Values are presented as mean  $\pm$  SEM. \* $p < 0.05$  \*\*\* $p < 0.001$ .