Supplementary Information

The Establishment of a Mouse Model of Deep Endometriosis

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Induction of endometriosis

We used an established mouse model of endometriosis by intraperitoneal (i.p.) injection of endometrial fragments (Somigliana et al., 1999) as used in our previous studies (Ding et al., 2015; Long et al., 2016). Briefly, after 1 week of acclimatization, donor mice were given an intramuscular injection of 3 μg/mouse estradiol benzoate (Animal Medicine Factory, Hangzhou, China) to stimulate the growth of endometrium. One week later they were sacrificed and their uteri were harvested. The uterine tissues were seeded in a Petri dish containing warm sterile saline, and split longitudinally with a pair of scissors.

Two uterine horns from each mouse were minced with scissors, ensuring that the maximal diameter of each fragment was consistently smaller than 1 mm. The fragments were then injected i.p. to recipient mice. To eliminate any potential bias, the endometrial tissue fragments from two donor mice were mixed together and then divided into four parts, each injected i.p. to one mouse from each one of the four groups. By this approach, any individual variation was minimized.

Hotplate test

The hotplate test was performed with a commercially available Hotplate Analgesia Meter (Model BME-480, Institute of Biomedical Engineering, Chinese Academy of Medical Sciences, Tianjin, China) as reported previously (Lu et al., 2010). Since mice are not vocal about their pain severity, and since central sensitization has been well documented in women with endometriosis as well as rodents with induced endometriosis (He et al., 2010; Lu, Nie, Liu, Zheng and Guo, 2010), the hotplate latency can be used as a surrogate measure for the severity of endometriosis-related generalized hyperalgesia (Bannon and Malmberg, 2007). Mice were brought to the testing room and allowed to acclimatize for 10 min before the test. The withdrawal latencies of the hind paws to thermal stimulations were determined in seconds. From the moment when the mouse was placed into the cylinder, the criteria of withdrawal included shaking or licking of its hind paws, or jumping on the hotplate. The latency was calculated as the mean of two sessions separated by a 60-min interval.

References


