



**Supplementary figure S1: Overview of the protocols.** Protocol 1 (A) applied in both males and females BBDPN rats; protocol 2 applied on the MS females (B & D) and for the MS males (C). Blue arrows: subcutaneous injections of  $\beta$ -estradiol 3-benzoate or vehicle. VMR: visceromotor response; BBDP-N: BioBreeding diabetes prone normoglycemic rats; PND: post-natal day; MS: maternal separation

**Supplementary table S1: qPCR primers**

| gene         | reverse                  | forward                  |
|--------------|--------------------------|--------------------------|
| <b>F2rl1</b> | GCGTGTCCAATCTGCCAATC     | TGCTGGGAGGTATCACCCCTT    |
| <b>mcpt2</b> | CATGAGCCACACCAGCACAC     | GATGAAAAGGCCTGTGTGGACT   |
| <b>ccl2</b>  | CTAGTTCTCTGTCATACT       | ATGCAGGTCTCTGTCACG       |
| <b>ccl11</b> | AGATGCACGCTGAAAGCCATAGTC | GGTGCCGATATTCTCCCATAGCAT |
| <b>il-4</b>  | CAGACGTCCTTACGGCAACA     | AGCACGGAGGTACATCACGT     |
| <b>gper1</b> | TGGTATGACTGCCTTGAGCG     | CATGCCTACCCCTTGACAGG     |
| <b>gapdh</b> | TCAATGAAGGGGTCGTTGATGG   | TCTCTGCTCCTCCCTGTTCTAGAG |

*F2rl1*: gene coding for *par2* (protease-activated receptor 2); *mcpt2*: mast cell protease 2; *ccl2*: gene coding for *mcp1* (monocyte chemoattractant protein1); *ccl11*: gene coding for *eotaxin-1*; *il4*: interleukin 4; *gper*: G-protein coupled estrogen receptor; *gapdh*: D-glyceraldehyde-3-phosphate dehydrogenase.

**Supplementary table S2: Immune cells activation following ovariectomy in female and  $\beta$ -estradiol in males.**

|                                | female                      |                             |          | male                           |                               |          |
|--------------------------------|-----------------------------|-----------------------------|----------|--------------------------------|-------------------------------|----------|
|                                | Sham                        | Ovx                         | <i>p</i> | vehicle                        | E2                            | <i>p</i> |
| Jejunum EPO                    | 15(6.1-61.6)<br><i>n</i> =6 | 38.5(3-76.6)<br><i>n</i> =8 | 0.3      | 53.2(25.1-57.6)<br><i>n</i> =4 | 37.6(0.8-56.4)<br><i>n</i> =6 | 0.4      |
| Colon EPO                      | 0.3(0.2-0.5)<br><i>n</i> =6 | 0.4(0.3-0.6)<br><i>n</i> =6 | 0.3      | 0.2(0.1-0.2)<br><i>n</i> =4    | 0.2(0.1-0.3)<br><i>n</i> =6   | 0.6      |
| Jejunum $\beta$ -hexoaminidase | 4.2(3.8-7.5)<br><i>n</i> =7 | 7.5(4.6-8.2)<br><i>n</i> =8 | 0.28     | 15.9(7.2-19.7)<br><i>n</i> =4  | 8.6(4.2-10.7)<br><i>n</i> =3  | 0.2      |
| colon $\beta$ -hexoaminidase   | 11.2(7.6-16)<br><i>n</i> =7 | 14.4(6.2-35)<br><i>n</i> =7 | 0.39     | 17.3(9.9-24.8)<br><i>n</i> =5  | 13.6(12-27.3)<br><i>n</i> =4  | 0.2      |

*EPO*: eosinophil peroxidase; *ovx*: ovariectomy; *E2*:  $\beta$ -estradiol. Mann-Whitney *t*-test.