

Supplementary Information

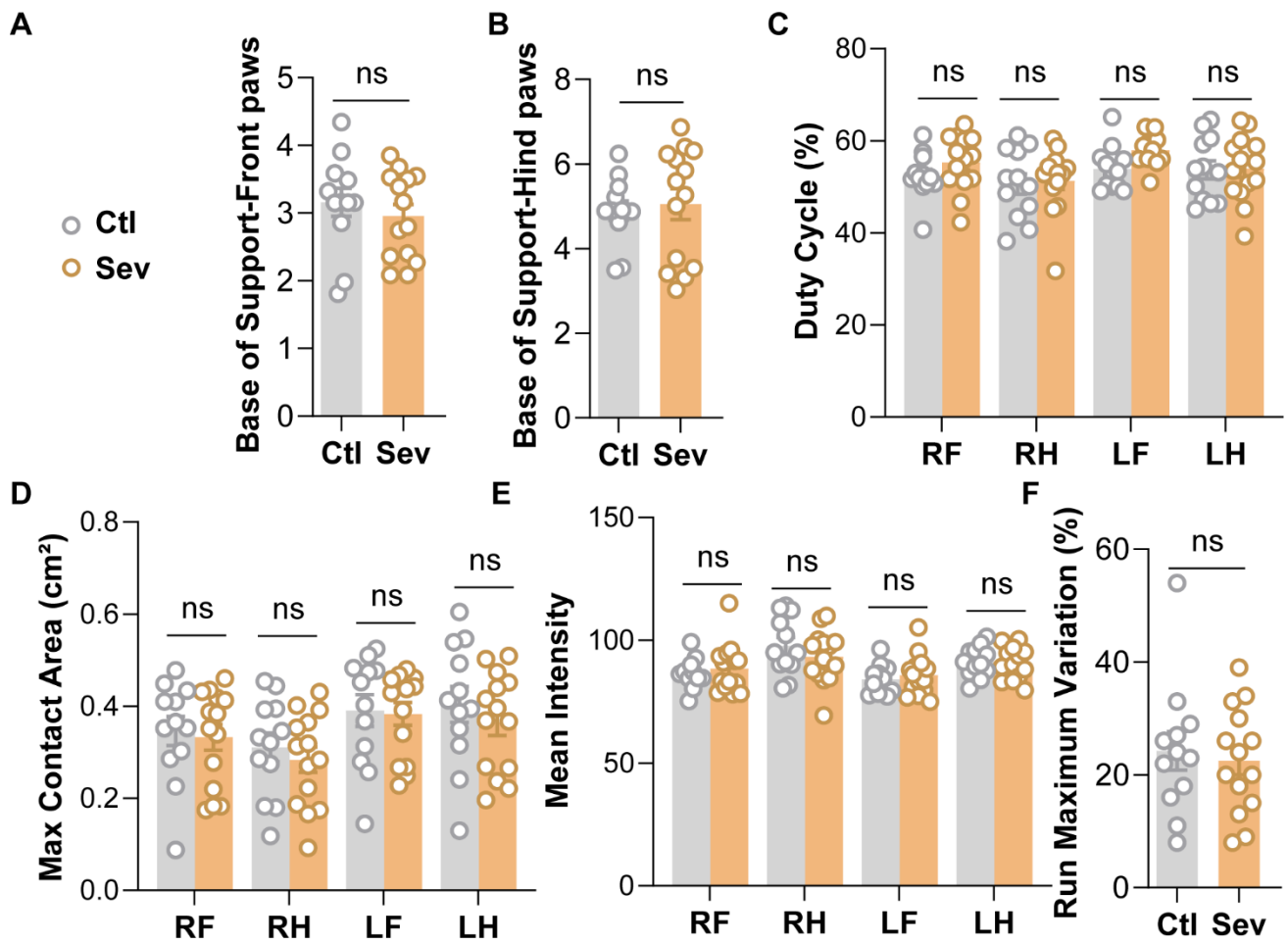


Fig. S1 There was no remarkable difference in other gait indicators of mice after repeated exposure to sevoflurane in the neonatal stage. **A-F**. Gaits analysis of the Ctl ($n = 12$) and Sev group ($n = 14$). The parameters include the base of support-front paws (**A**), base of support-hind paws (**B**), duty cycle (**C**), max contact area (**D**), mean intensity (**E**), and run maximum variation (**F**). The data are denoted as the mean $\pm$ SD. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, $****P < 0.0001$ (unpaired separate variance estimation t test).

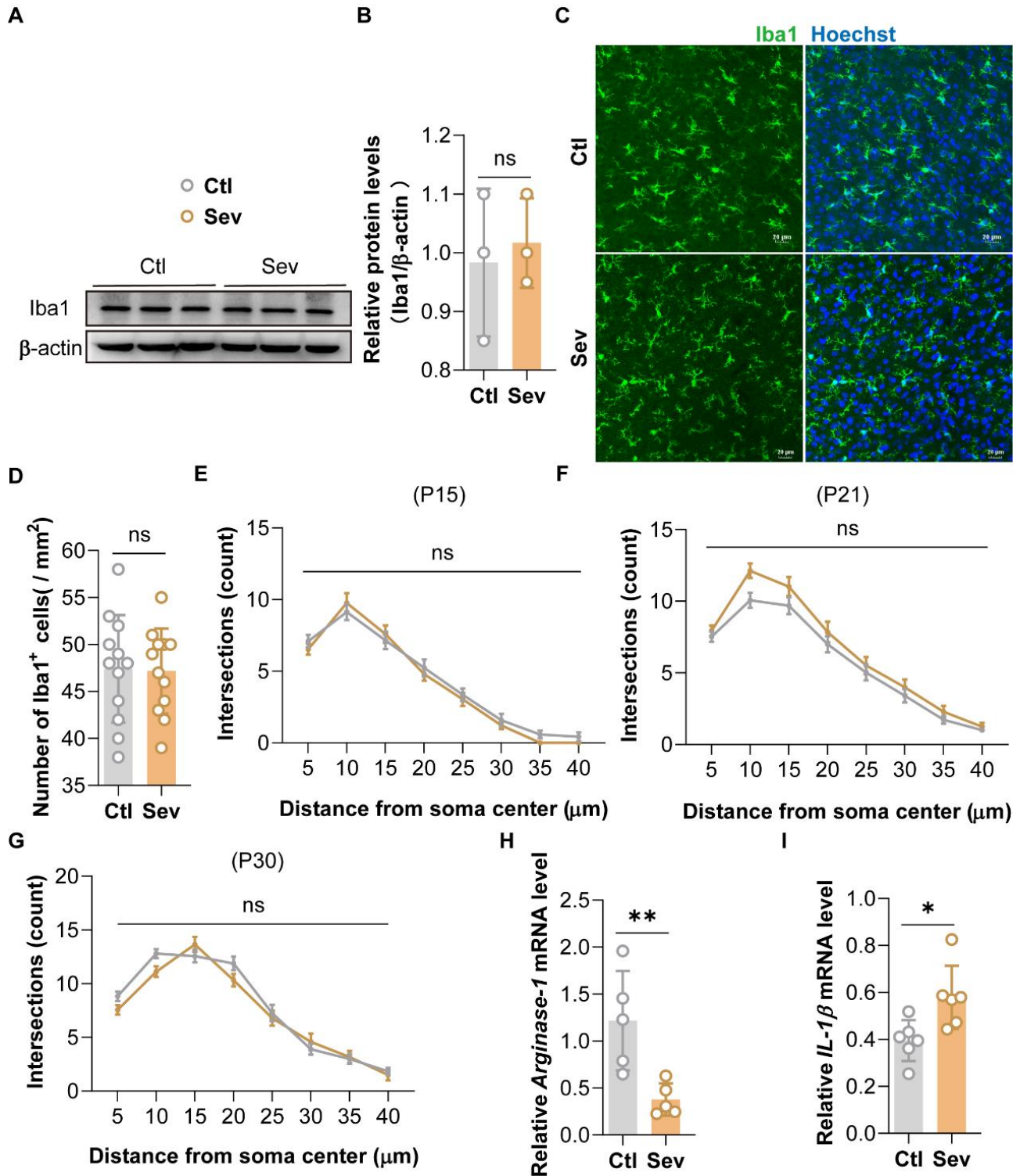


Fig. S2 The number of microglia did not change dramatically after repeated exposure to sevoflurane.

A-B. The protein level (**A**) and quantification (**B**) of Iba1 in the mPFC from the Ctl and Sev group on the 3rd day after repeated exposure to sevoflurane (unpaired separate variance estimation *t*-test), *n* =

3. **C.** Representative images of Iba1 expression in the mPFC from the Ctl and Sev group on the 3rd

day after repeated exposure to sevoflurane (scale bar, 50 μ m), $n = 6$. **D.** Quantitative analysis of Iba1+ cell number (unpaired separate variance estimation t -test). **E-G.** Sholl analysis of microglia morphology in the mPFC from the Ctl and Sev group on the 7th (P15) (**E**), 13th (P21) (**F**), and 22nd (P30) (**G**) days after repeated exposure to sevoflurane (Friedman's M-test). **H-I.** Quantitative mRNA expression level of Arginase-1 (**H**) and IL-1 β (**I**) in the mPFC from the Ctl and Sev group on the 3rd day after repeated exposure to sevoflurane (unpaired separate variance estimation t -test), $n = 6$. The data are denoted as the mean $\pm$ SD. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$.

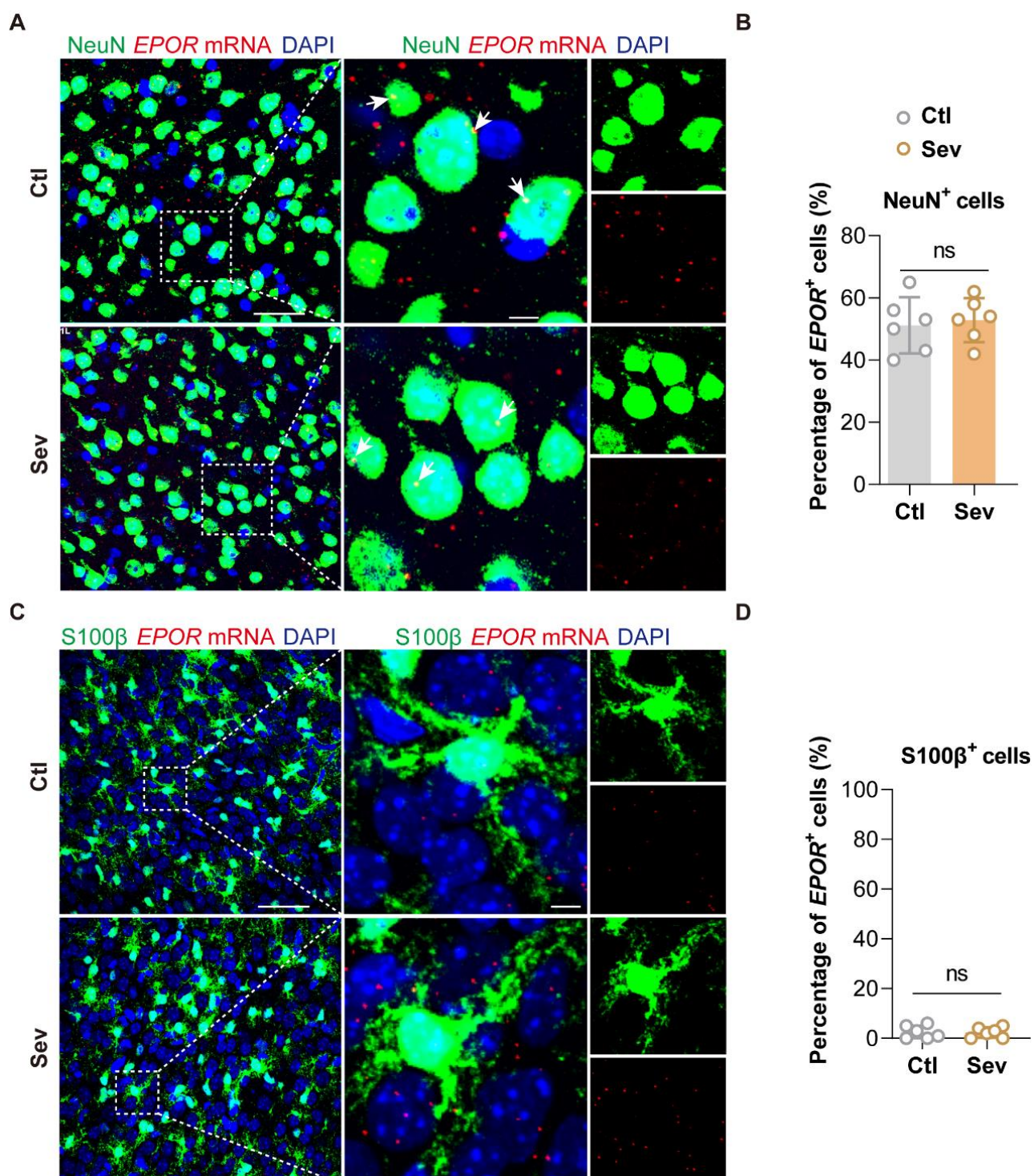


Fig. S3 EPOR expression and changes in neurons and astrocytes in the mPFC after repeated exposure to sevoflurane. **A.** RNAscope in situ hybridization and immunohistochemistry staining showing colocalization of NeuN and *EPOR* mRNA in the mPFC of the Ctl and Sev group on the 3rd day after repeated exposure to sevoflurane, scale bars, left: 25 mm, middle and right: 10 mm. **B.** Quantification

of the percentage of $EPOR^+$ cells in $NeuN^+$ cells, $n = 6$. **C.** RNAscope in situ hybridization and immunohistochemistry staining showing colocalization of S100 β and $EPOR$ mRNA in the mPFC of the Ctl and Sev group on the 3rd day after repeated exposure to sevoflurane, scale bars, left: 25 mm, middle and right: 10 mm. **D.** Quantification of the percentage of $EPOR^+$ cells in S100 β^+ cells, $n = 6$. The data are denoted as the mean $\pm$ SD. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, $****P < 0.0001$ (unpaired separate variance estimation t test).

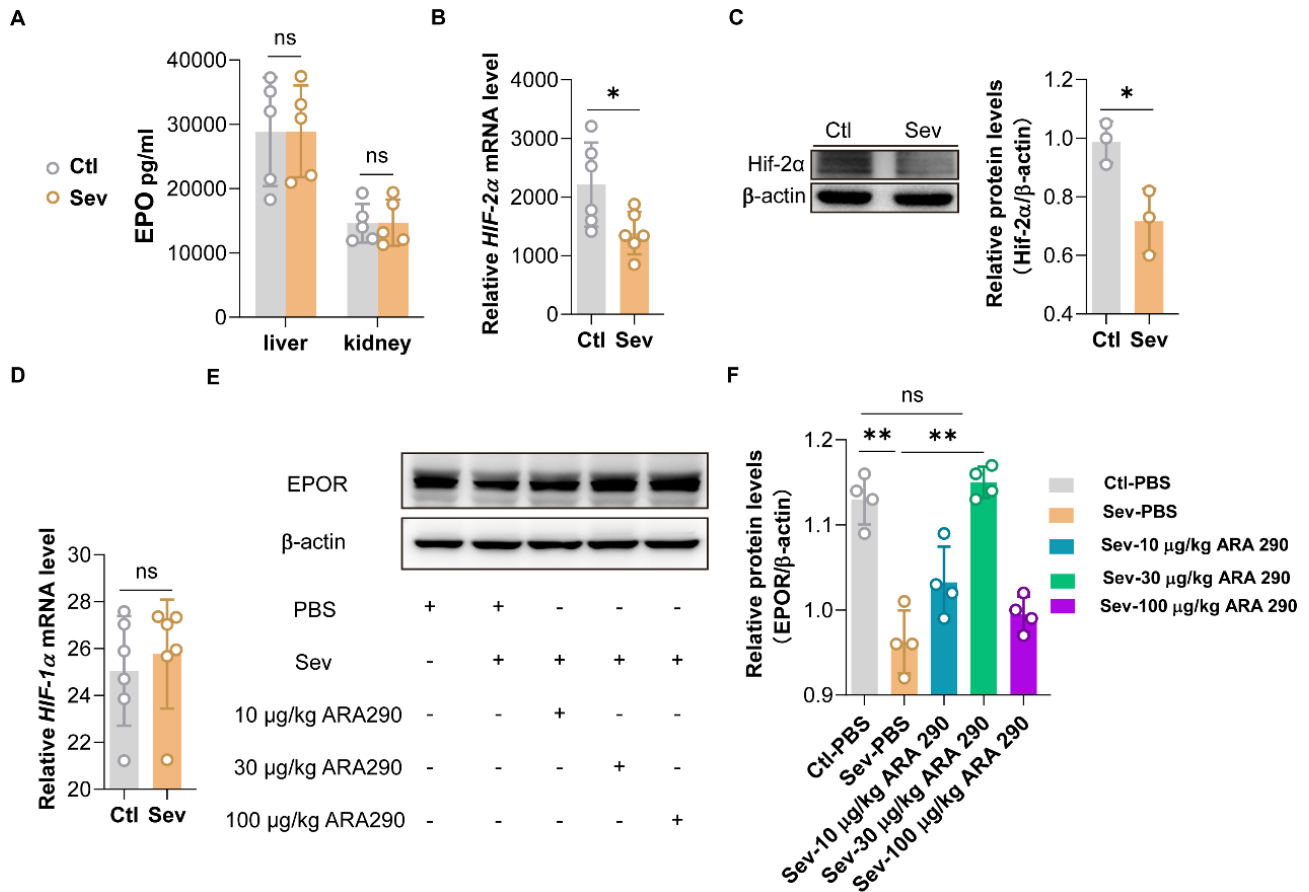


Fig. S4 The expression of HIF-2 α , rather than HIF-1 α , was decreased in the mPFC after repeated exposure to sevoflurane. **A**. The expression level of EPO in the liver and kidneys of mice between the Ctl and Sev group (unpaired separate variance estimation *t*-test), *n* = 5. **B** and **D**. Quantitative mRNA expression level of HIF-2 α (**B**) and HIF-1 α (**D**) in the mPFC from the Ctl and Sev group on the 3rd day after repeated exposure to sevoflurane (unpaired separate variance estimation *t*-test), *n* = 6. **C**. The protein level and quantification of HIF-2 α in the mPFC from the Ctl and Sev group on the 3rd day after repeated exposure to sevoflurane (unpaired separate variance estimation *t*-test), *n* = 3. **E**. Expression of EPOR in the mPFC under different concentrations of ARA290. **F**. Quantification of EPOR protein levels (Kruskal-Wallis H test with Nemenyi's multiple-comparison test), *n* = 4. The data are denoted as the mean $\pm$ SD. **P* < 0.05, ***P* < 0.01, ****P* < 0.001, *****P* < 0.0001.

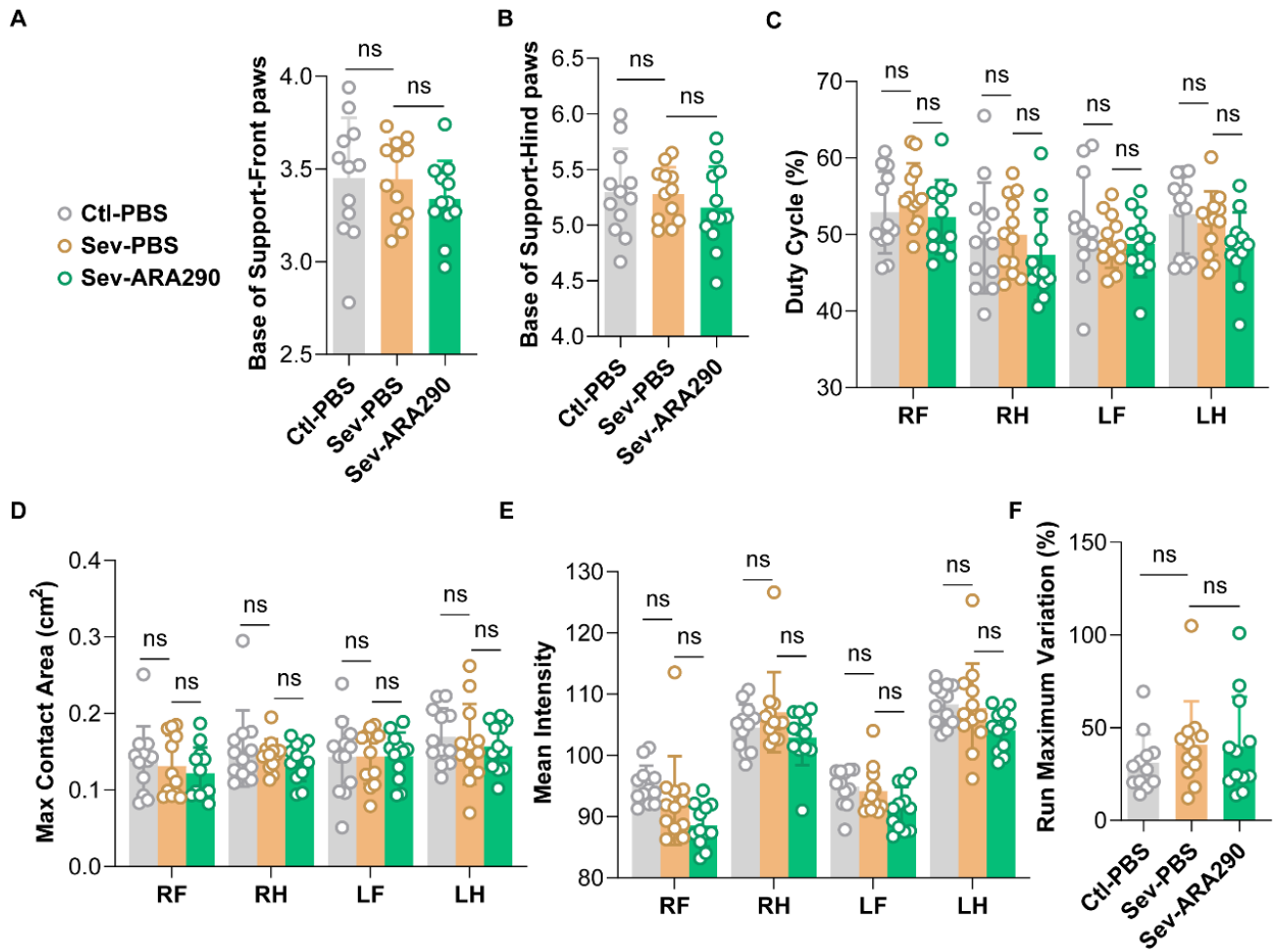


Fig. S5 There was no remarkable difference in other gait indicators of mice after supplementing ARA290. **A-F.** Gaits analysis of three groups, $n = 12$. The parameters include the base of support-front paws (**A**), base of support-hind paws (**B**), duty cycle (**C**), max contact area (**D**), mean intensity (**E**), and run maximum variation (**F**). The data are denoted as the mean $\pm$ SD. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, $****P < 0.0001$ (Kruskal-Wallis H test with Nemenyi's multiple-comparison test).

Table S1 The primers used in this study

Gene Name	Sense (5'-3')	Anti-sense (5'-3')
GADPH	GGTGAAGGTCGGTGTGAACG	CTCGCTCCTGGAAGATGGTG
EPOR	GGGCTCCGAAGAAGTTCTGTG	ATGACTTTCGTGACTCACCT
IL-1 β	GCAACTGTTCTGAACTCAACT	ATCTTTTGGGGTCCGTCAACT
Arginase-1	CTCCAAGCCAAAGTCCTTAGAG	AGGAGCTGTCATTAGGGACATC
EPO	ACTCTCCTTGCTACTGATTCCT	ATCGTGACATTTTCTGCCTCC
HIF-1 α	ACCTTCATCGGAAACTCCAAAG	CTGTTAGGCTGGGAAAAGTTAGG
HIF-2 α	CTGAGGAAGGAGAAATCCCGT	TGTGTCCGAAGGAAGCTGATG