Supplemental methods

Bone marrow smear

Bone marrow was flushed from femurs and tibias of WT and Tph1-/- mice into RPMI buffer and centrifuged at 600g for 10 minutes at RT, followed by resuspension, smearing on glass slides, and May-Gruenwald-Giemsa staining for blinded analysis by light microscopy at 100x (Axiovert 200M, Zeiss) according to standard hematological cell morphology parameters.

Generation of bone marrow chimera

After lethal irradiation with 900 cGy (2x450 cGy), 5 x 10^6 WT or Tph1-/- femur and tibia marrow cells were injected intravenously into WT or Tph1-/- recipient mice, combining the following donor-recipient pairs: WT → WT, Tph1-/- → WT, WT → Tph1-/-, Tph1-/- → Tph1-/-.

Supplemental figure legends

Figure S1. Granulopoiesis is normal in Tph1-/- mice. Bone marrow smear analysis (n = 6, there were no significant differences between WT and Tph1-/- mice).

Figure S2. Decreased leukocyte rolling and adhesion in Tph1-/- mice depends on serotonin produced by non-hematopoietic cells. A) Rhodamine-stained leukocyte rolling in resting mesenteric veins of WT and Tph1-/- mice pre-treated with an intraperitoneal injection of serotonin or vehicle after laparotomy without further stimulation. B) Leukocyte adhesion after LPS challenge in WT and Tph1-/- mice pre-treated with an intraperitoneal injection of serotonin or vehicle. C) Serum serotonin levels in bone marrow chimeric mice. B) Leukocyte rolling in resting mesenteric veins of bone marrow chimeric mice. N = 4-6.

Figure S1

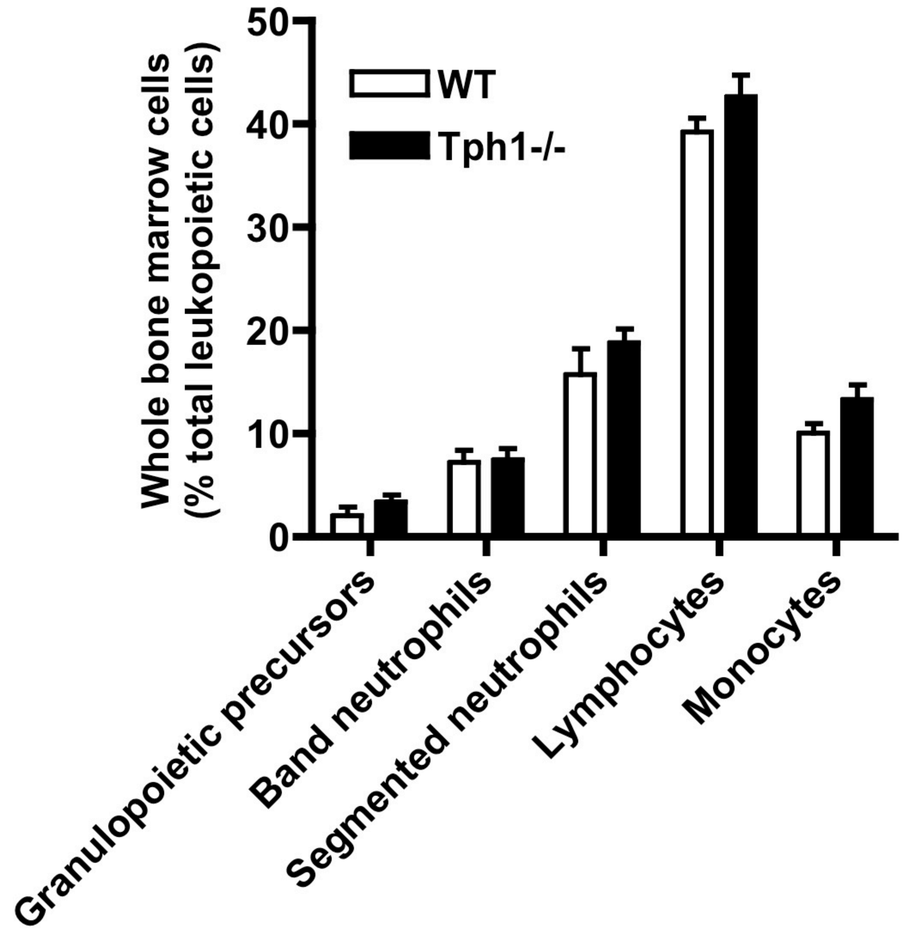
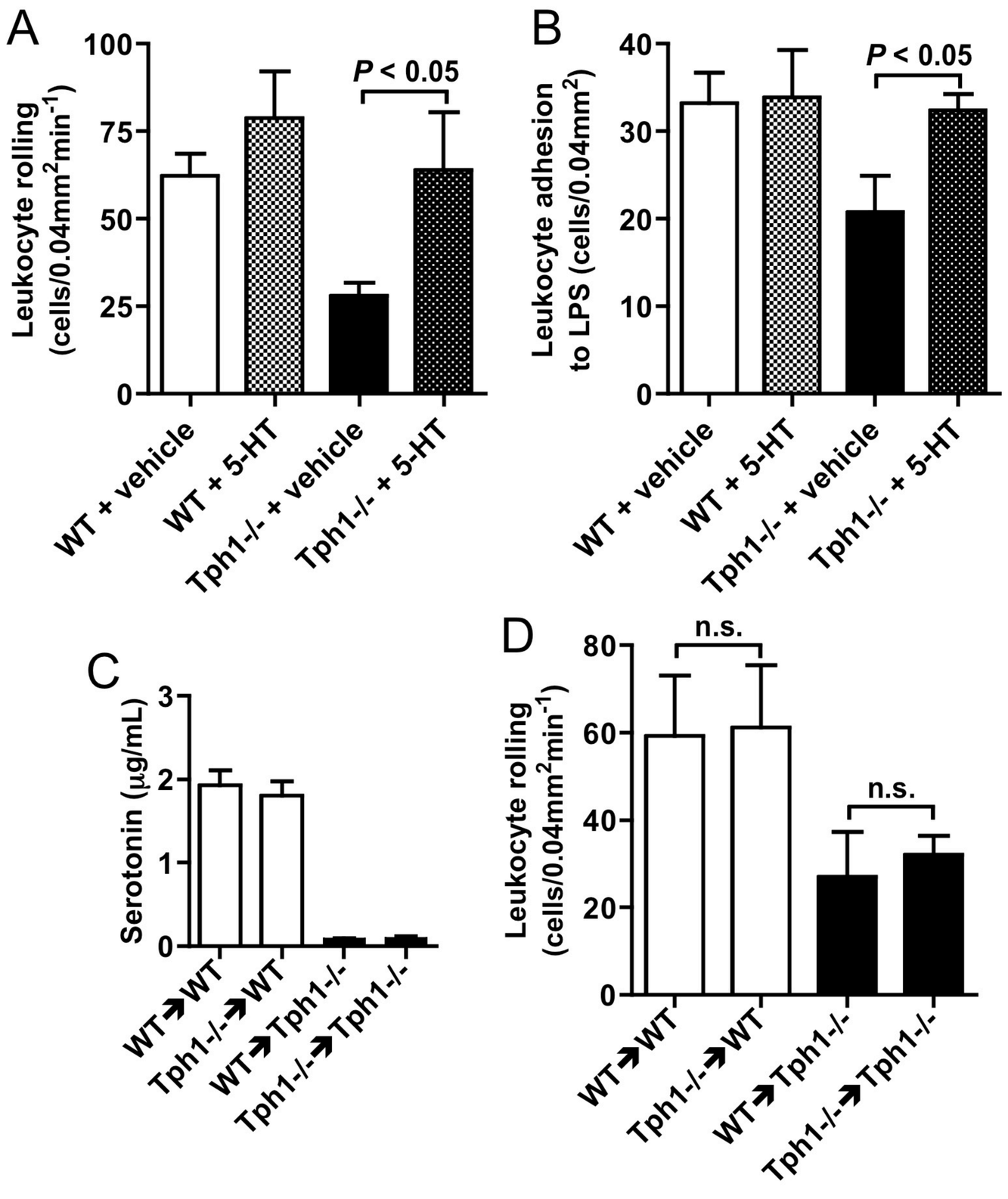

Whole bone marrow cells
(% total leukopoietic cells)
50
40
30
20
10
0
WT
Tph1-/-
Granulopoietic precursors
Band neutrophils
Segmented neutrophils
Lymphocytes
Monocytes

Figure S2


A
Leukocyte rolling (cells/0.04mm²min⁻¹)
100
75
50
25
0
P < 0.05
WT + vehicle
WT + 5-HT
Tph1-/- + vehicle
Tph1-/- + 5-HT
B
Leukocyte adhesion to LPS (cells/0.04mm²)
40
30
20
10
0
P < 0.05
WT + vehicle
WT + 5-HT
Tph1-/- + vehicle
Tph1-/- + 5-HT
C
Serotonin (μg/mL)
3
2
1
0
WT➔WT
Tph1-/-➔WT
WT➔Tph1-/-
Tph1-/-➔Tph1-/-
D
Leukocyte rolling (cells/0.04mm²min⁻¹)
80
60
40
20
0
n.s.
n.s.
WT➔WT
Tph1-/-➔WT
WT➔Tph1-/-
Tph1-/-➔Tph1-/-