Supplemental Materials

Video S1. Neutrophil emigration across the vessel wall and tissue in the cremaster muscle of a PTEN KO mouse intrascrotally injected with MIP-2. Magnification is 100X. Frame rate is 5fps. Time is accelerated 20 times.

Video S2. Emigration of WT neutrophils away from a postcapillary venule in response to MIP-2 intrascrotal injection. Magnification is 40X. Caption rate is 5fps.Time is accelerated 20 times.

Video S3. Emigration of PTEN KO neutrophils away from a postcapillary venule in response to MIP-2 intrascrotal injection. Magnification is 40X. Caption rate is 5fps.Time is accelerated 20 times.

Video S4. Fluorescent CD31-outlined endothelium in the postcapillary venule of fMLP-seperfused mouse cremaster muscle.

Table S1. Haemodynamic parameters in saline-superfused cremaster

	Mice/Vessels	Diameter ± SD (μm)	Blood neutrophil count ± SD (million/mL)*	V_{RBC} ± SD (μm/sec)	WSR ± SD (S^{-1})
WT	4/14	24.6 ± 4.1	2.6 ± 1.0	2.48 ± 0.55	1100.8 ± 456.8
KO	4/14	27.9 ± 7.5	2.6 ± 1.3	2.76 ± 0.68**	1113.4 ± 500.4

* N = 9 mice/genotype
** P < 0.5

Table S2. Blood neutrophil count, vessel number and diameter in the differently-stimulated mouse cremaster

A. fMLP superfusion

	Blood neutrophil (million/mL)	Vessel number				Diameter (μm)			
Time (min)		0	20	40	60	0	20	40	60
WT	2.1 ± 0.8	12	11	11	14	26.8 ± 6.7	25.8 ± 7.6	24.2 ± 6.7	26 ± 6.5
KO	3 ± 1.5	10	10	9	7	29.5 ± 6.5	25.2 ± 2	28.6 ± 6.7	25 ± 5.7

B. TNFα intrascrotal injection

	Blood neutrophil (million/mL)	Vessel number		Diameter (μm)	
Time (hrs)		2	4	2	4
WT	1.5 ± 0.4	48	38	28.9 ± 4.5	28.1 ± 6.5
KO	2 ± 0.5	42	19	27.5 ± 5.1	30.6 ± 8.4

C. MIP-2 intrascrotal injection

	Blood neutrophil (million/mL)				Vessel number				Diameter (μm)			
	Saline	MIP-2	LY+MIP-2	PP2+MIP-2	Saline	MIP-2	LY+MIP-2	PP2+MIP-2	Saline	MIP-2	LY+MIP-2	PP2+MIP-2
WT	2.1 ± 0.2	2.9 ± 0.5	2.8 ± 1.9	2.7 ± 0.8	24	39	32	33	32.6 ± 6	30.2 ± 6.7	31.5 ± 7.1	27.9 ± 5.1
KO	3.4 ± 0.8	3.1 ± 0.6	2.5 ± 1.2	2.3 ± 0.3	25	39	25	25	34.2 ± 7	30.3 ± 7.6	31 ± 5.5	27.6 ± 6.6

D. Ischemia/reperfusion

	Blood neutrophil (million/mL)	Vessel number				Diameter (μm)			
Time (min)		0	20	40	60	0	20	40	60
WT	2.8 ± 1	17	18	16	15	31.7 ± 6.2	30 ± 4.6	30.3 ± 5.2	30.7 ± 4.6
KO	2.7 ± 0.8	16	17	15	15	27.8 ± 4.4	30.3 ± 7.1	29.8 ± 7.6	30 ± 7.1

Table S3. The number of vessels and their diameter and the number of cells crawling, crossing the endothelial vessel wall and tissue in response to fMLP or MIP-2 stimulation of the cremaster vessels.

		Vessel	Diameter (μm) ± SD	Crawling cells	Vessel	Diameter (μm) ± SD	Cells thru EC	Cells thru tissue
fMLP	WT	17	33.1 ± 12.4	39	11	26.3 ± 7.3	21	47
	KO	11	34.9 ± 13.3	43	11	27.5 ± 5.5	18	22
MIP-2	WT	7	23.5 ± 6.1	20	10	24.6 ± 6.1	38	34
	KO	8	31.3 ± 20.5	18	15	23.7 ± 3	59	70

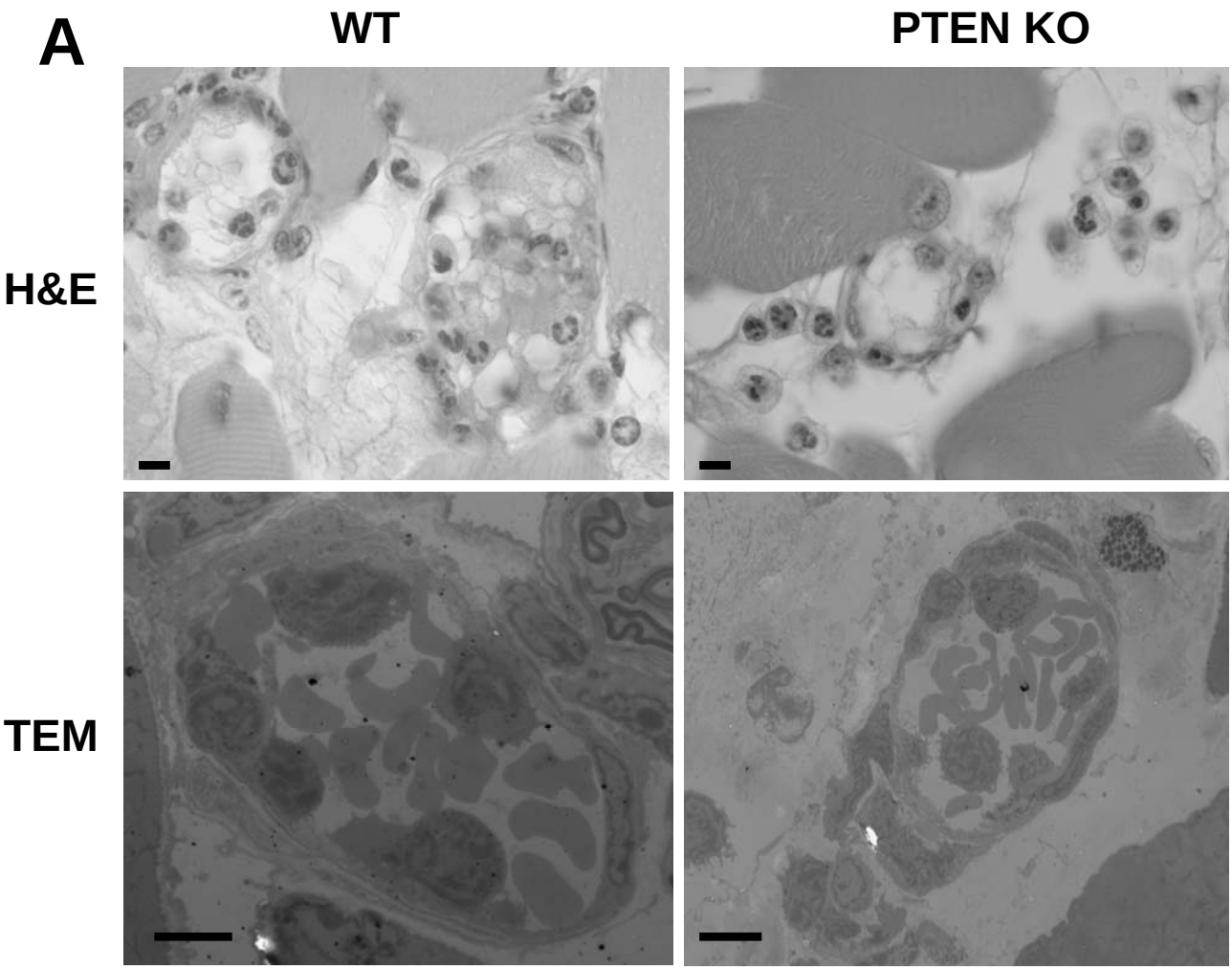
Table S4. Dimensions of poscapillary venules and percentage of emigration junctional pathway

	Vessels	ECs	Diameter (μm) ± SD	EC length (μm) ± SD	EC max width (μm) ± SD	% Junctional ± SEM
WT	22	59	45 ± 14.2	73.7 ± 13.9	19.4 ± 4.8	95.9 ± 1.8
KO	21	39	42.8 ± 14.8	78.6 ± 19.4	21.9 ± 5.9	93.1 ± 1.8

Table S5. Trafficking parameters in saline-superfused cremaster

	Mice/Vessels	Diameter ± SD (μm)	Roll/min ± SEM	Adh/min ± SEM	Transmig ± SEM (Cells/10^4 μm^2)	V ± SEM (μm/sec)
WT	9/45	30.7 ± 6.6	97.8 ± 9.5	3.7 ± 0.5	0.4 ± 0.2	73.8 ± 2.6
KO	9/45	30.1 ± 5.3	62.3 ± 4.5 *	2.5 ± 0.4	0.4 ± 0.2	86.3 ± 3.2 *

* $P < 0.05$



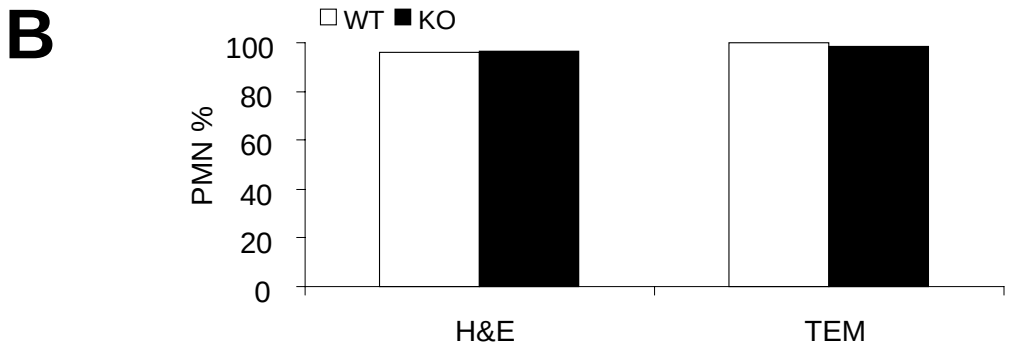


Figure S1. Majority of leukocytes (> 95 %) recruited in the postcapillary venules of the mouse cremaster are neutrophils. (A) Cross sections of the cremaster postcapillary venules as seen under brightfield and transmission electron microscopy. (First row) The cremaster muscle of WT or PTEN KO mouse was superfused with 10 mM fMLP peptide for an hour, fixed with 4 % paraformaldehyde, paraffin-embedded and stained with hematoxylin/eosin. (Second row) WT or PTEN KO mice were intrascrotally injected with 1 mg MIP-2. Three hours later, the cremaster muscle was fixed in a fixing buffer (see Methods) and prepared for electron microscopy. Bars represent 5 mm. TEM magnification is 1200X for WT and 890X for KO mice. N > 3 mice per genotype. **(B)** Quantification of the percentage of neutrophils.

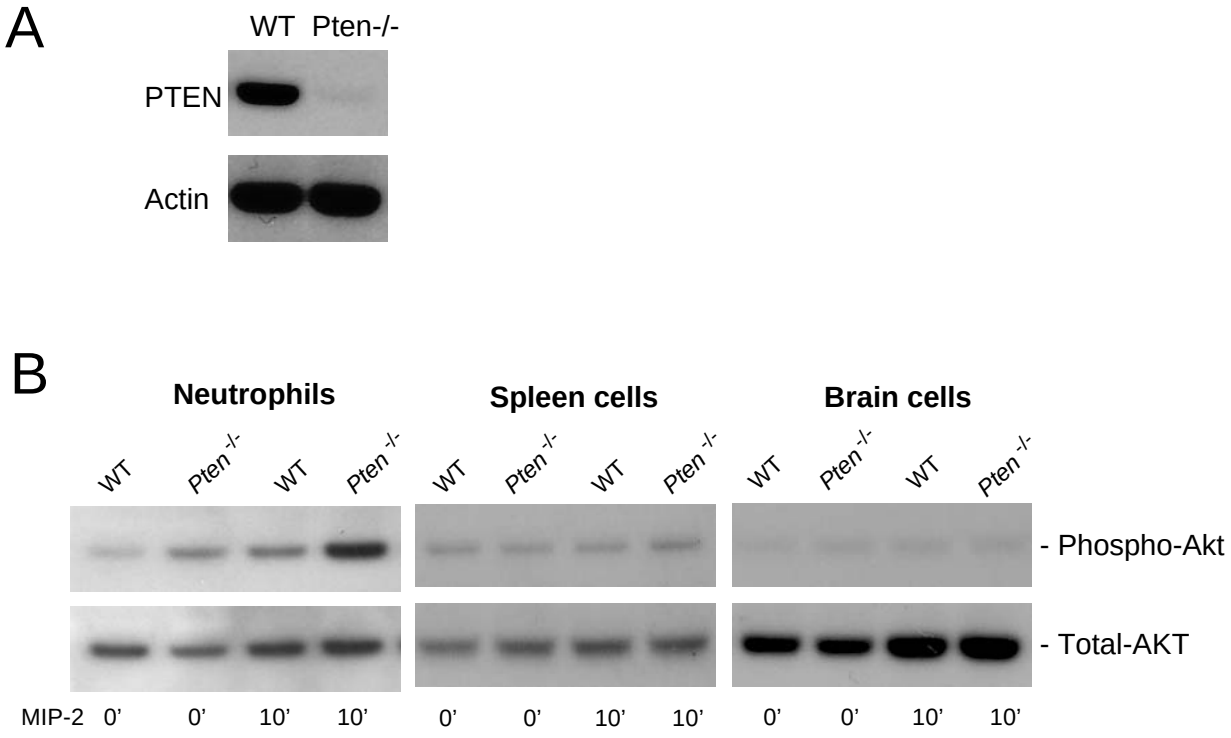


Figure S2. PTEN protein is depleted and PtdIns(3,4,5)P3 signaling is specifically elevated in neutrophils isolated from myeloid-specific PTEN knockout mice. (A) PTEN protein level in purified neutrophils. **(B)** PtdIns(3,4,5)P3 signaling is specifically elevated in PTEN-null neutrophils. Neutrophils, primary spleen cells, and brain cells collected from wild-type and myeloid-specific PTEN-knockout mice were stimulated with 25 ng/mL MIP-2 and lysed at 0 and 10 min. Phosphorylated and total Akt were detected by Western blotting using anti–Phospho-Akt (Ser473) (1:1000) and anti-Akt (1:1000) antibodies (Cell Signaling, Beverly, MA), respectively.

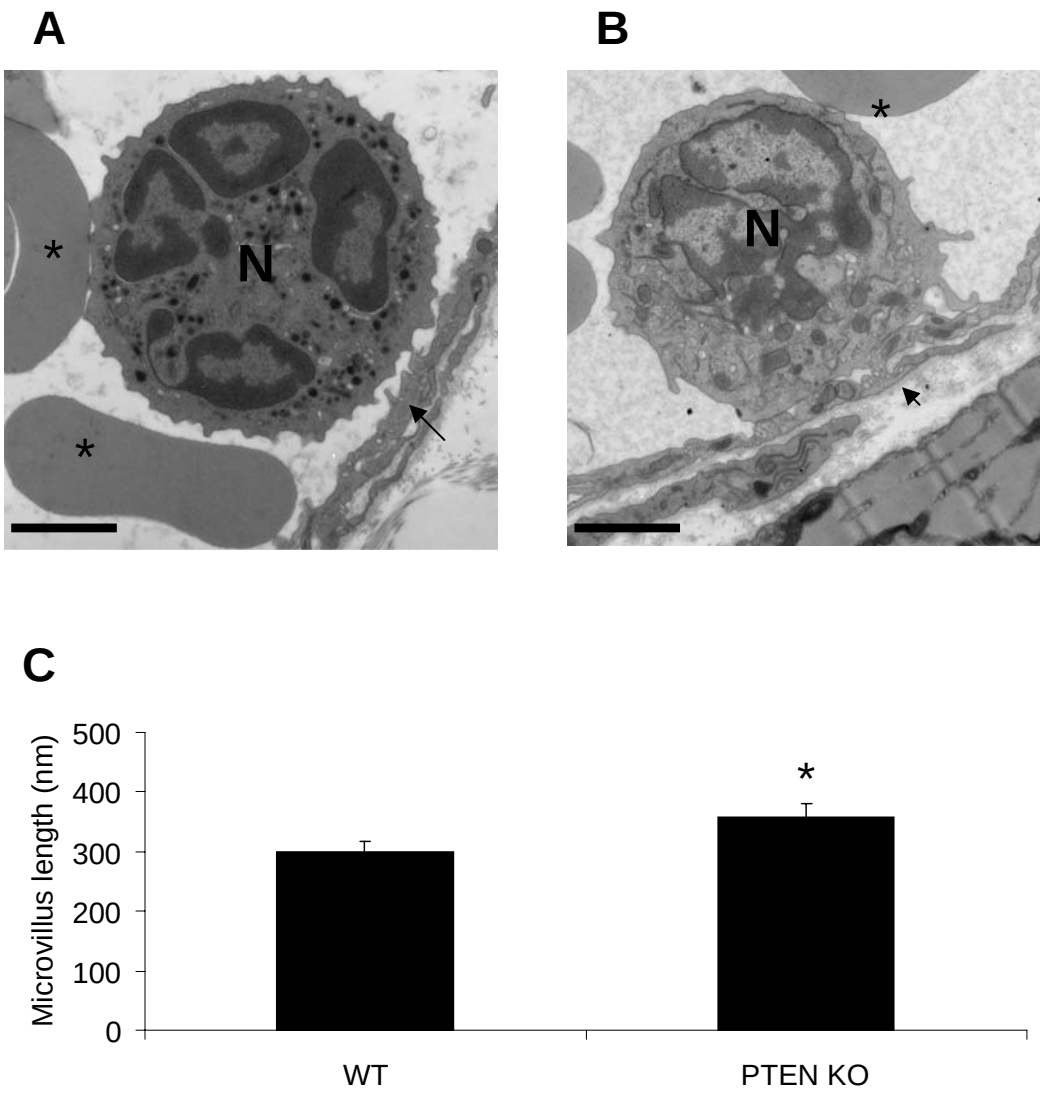


Figure S3. **PTEN KO neutrophils display longer microvillus protrusions**. WT (A) or PTEN KO (B) mice were intrascrotally injected with 1 mg MIP-2. Three hours later, the cremaster muscle was fixed in a fixing buffer (see Methods) and prepared for electron microscopy. Neutrophils (N) are shown touching the vascular wall (arrows). Asterisks indicate red blood cells. **(C)** The length of microvilli in nm. Seventeen WT neutrophils with 132 microvilli and 16 PTEN KO neutrophils with 100 microvilli were analyzed. N = 3 mice per genotype. * $P < 0.05$. Bars represent 2 mm. Magnification is 4800X in A and 2900X in B.

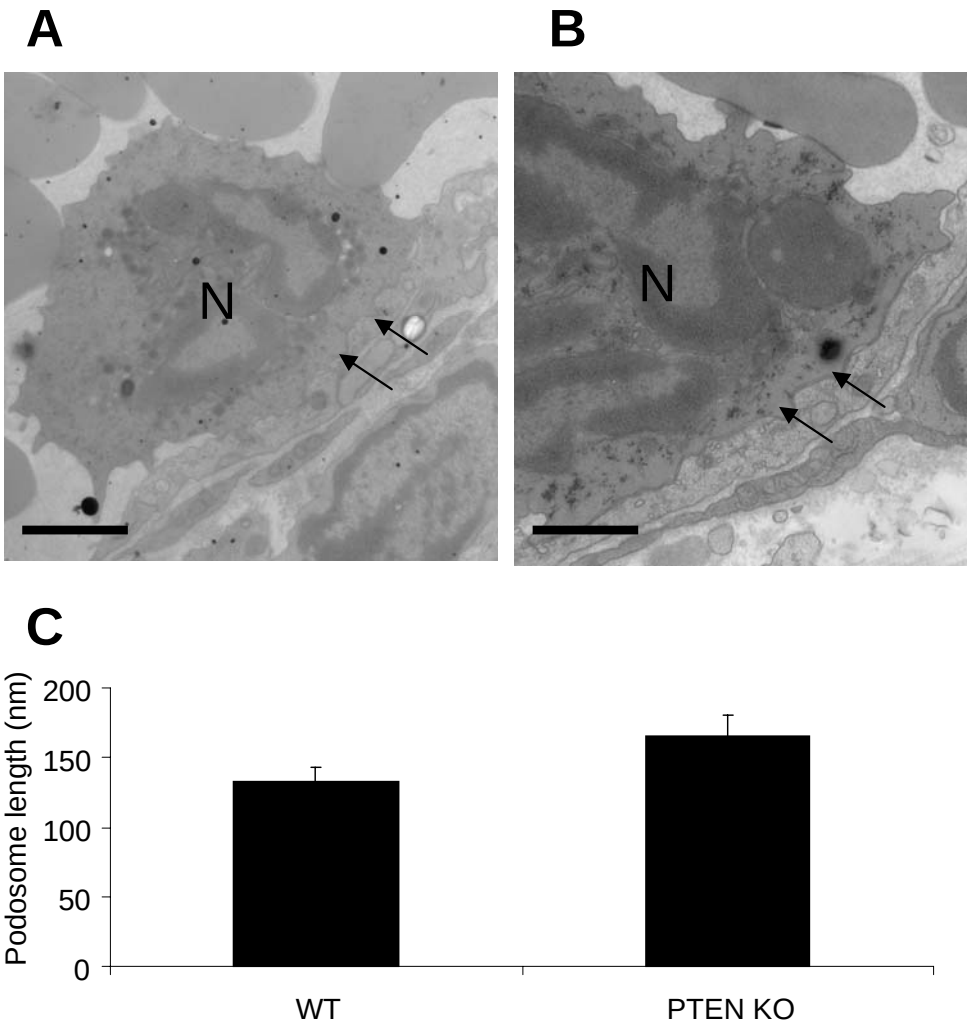


Figure S4. Neutrophils display podosomes in contact with vascular endothelium. WT (A) or PTEN KO (B) mice were intrascrotally injected with 1 mg MIP-2. Three hours later, the cremaster muscle was fixed in a fixing buffer (see Methods) and prepared for electron microscopy. Neutrophils (N) are shown touching the vascular wall. Podosomes are pointed to by arrows. **(C)** The length of podosomes is indicated in nm. Thirteen WT neutrophils with 54 podosomes and 12 PTEN KO neutrophils with 57 podosomes were analyzed. N = 3 mice per genotype. Bars represent 2 mm in A and 0.5 mm in B. Magnification is 4800X in A and 9300X in B. * $P < 0.05$.

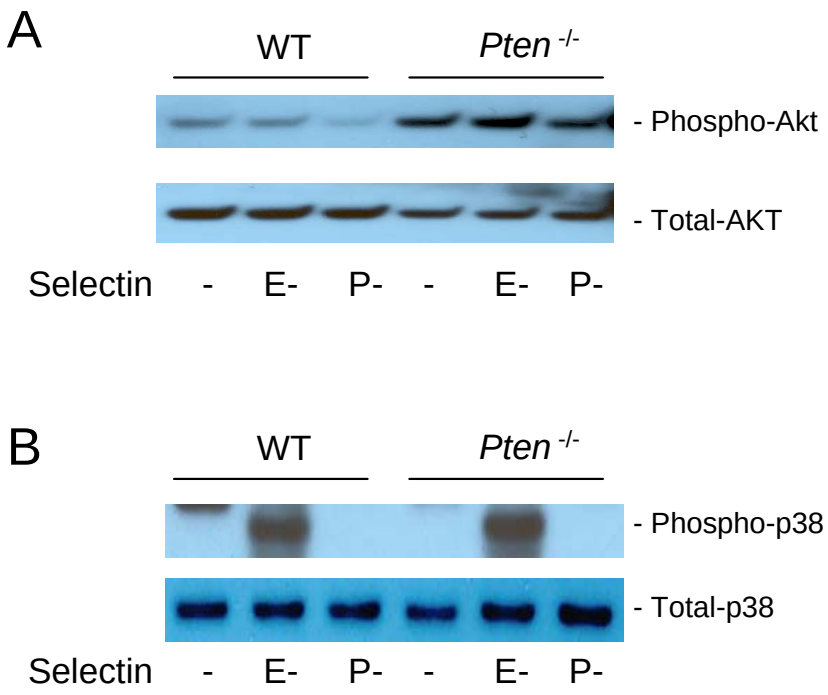


Figure S5. PtdIns(3,4,5)P3 signaling can not be activated by E- or P- selectin. (A) Neutrophils collected from wild-type and myeloid-specific PTEN-knockout mice were stimulated with 20 µg/mL E- or P-selectin for 10 min. Phosphorylated and total Akt were detected by Western blotting using anti–Phospho-Akt (Ser473) (1:1000) and anti-Akt (1:1000) antibodies (Cell Signaling, Beverly, MA), respectively. (B) E-selectin can activate MAPK (p38) pathway. Phosphorylated and total p38 were detected by Western blotting using anti–Phospho-p38 (1:1000) and anti-p38 (1:1000) antibodies (Cell Signaling, Beverly, MA), respectively.