Figure S1. Actin polymerization in VSOP/Hv1<sup>−/−</sup> neutrophils. Neutrophils were allowed to ingest opsonized zymosan for 45 min and then fixed in 3.7% paraformaldehyde, permeabilized with 0.25% Triton X-100, and stained with phalloidin-TRITC. (A) Fluorescence images captured using the same settings showing phagosomes surrounded by actin rings. Bar, 5 µm. (B and C) Quantification of the total cytosolic actin F and of the thickness of phagosomal actin rings. Both parameters were significantly increased in VSOP/Hv1<sup>−/−</sup> neutrophils. Data are mean ± SEM of the indicated number of cells or phagosomes, measured in three independent experiments using cells from two WT and two VSOP/Hv1<sup>−/−</sup> mice.
Figure S2. Effect of fMIVIL on ROS production. ROS production was measured using the L-012 assay. Chemiluminescence was measured every 8 s for 15 min. The amplitude of the changes in chemiluminescence evoked by the addition of DMSO, 10 µM MIVIL, or 10 µM MIVIL + 10 µM DPI (added 5 min before the recording) are shown. Data are means ± SEM of three independent experiments from two WT and two VSOP/Hv1−/− mice.

Video 1. Altered migration of fMIVIL-stimulated VSOP/Hv1−/− neutrophils. VSOP/Hv1+/+ neutrophil migration upon fMIVIL stimulation. The images were acquired every 15 s during 45 min on an ImageXpress Micro automated fluorescence imaging system.

Video 2. Altered migration of fMIVIL-stimulated VSOP/Hv1−/− neutrophils. The clear migration defect in neutrophils lacking VSOP/Hv1 is revealed. Note the inability of VSOP/Hv1−/− neutrophils to detach their tail from the substrate. The images were acquired every 15 s during 45 min on an ImageXpress Micro automated fluorescence imaging system.