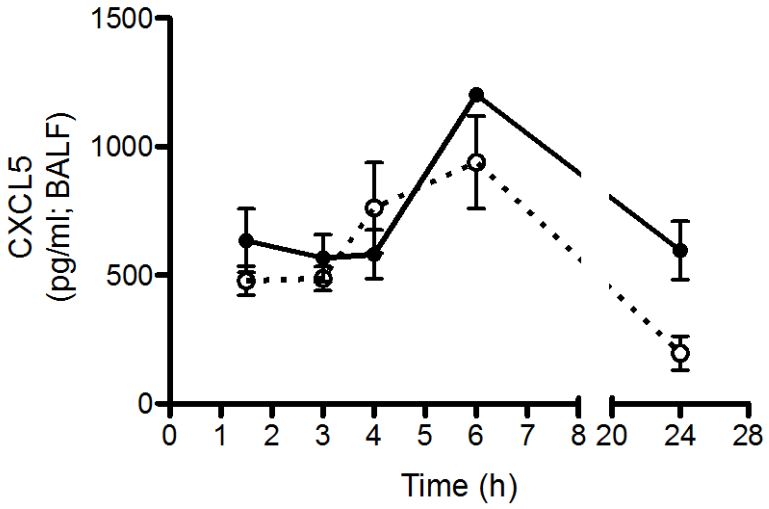
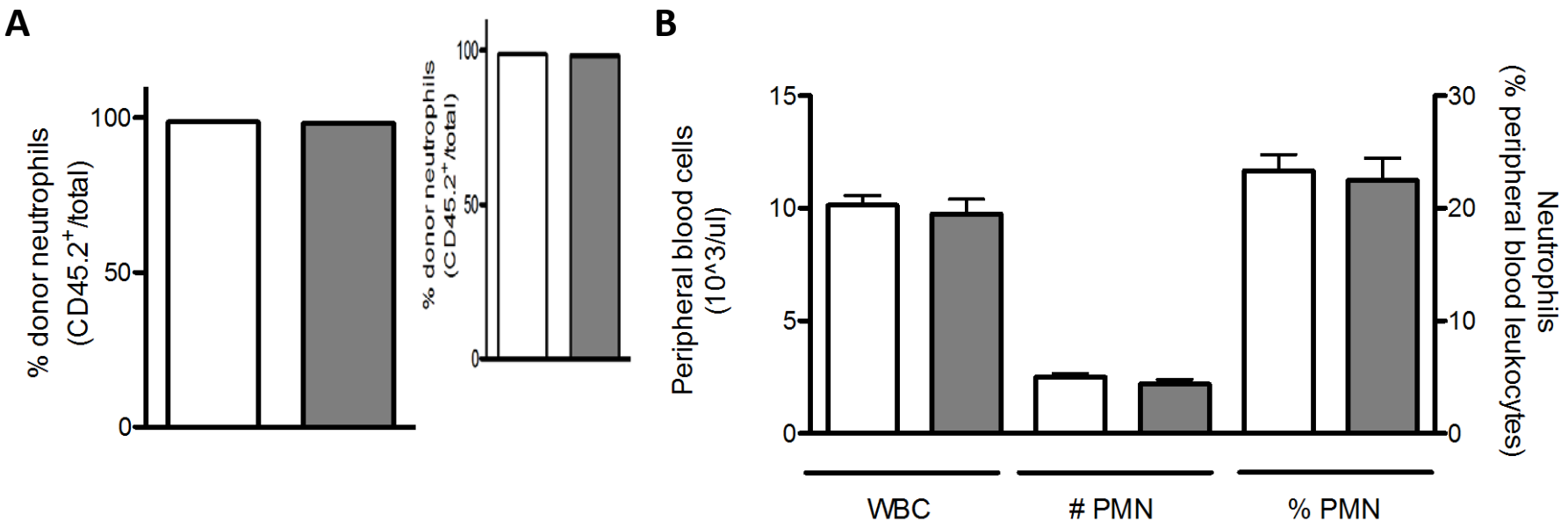
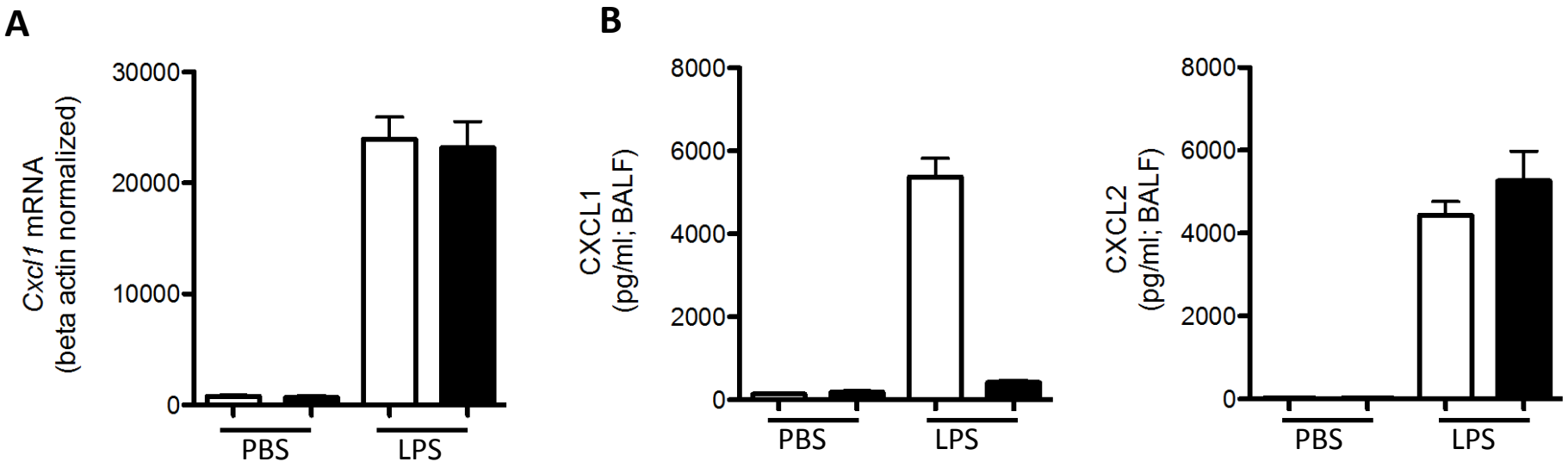


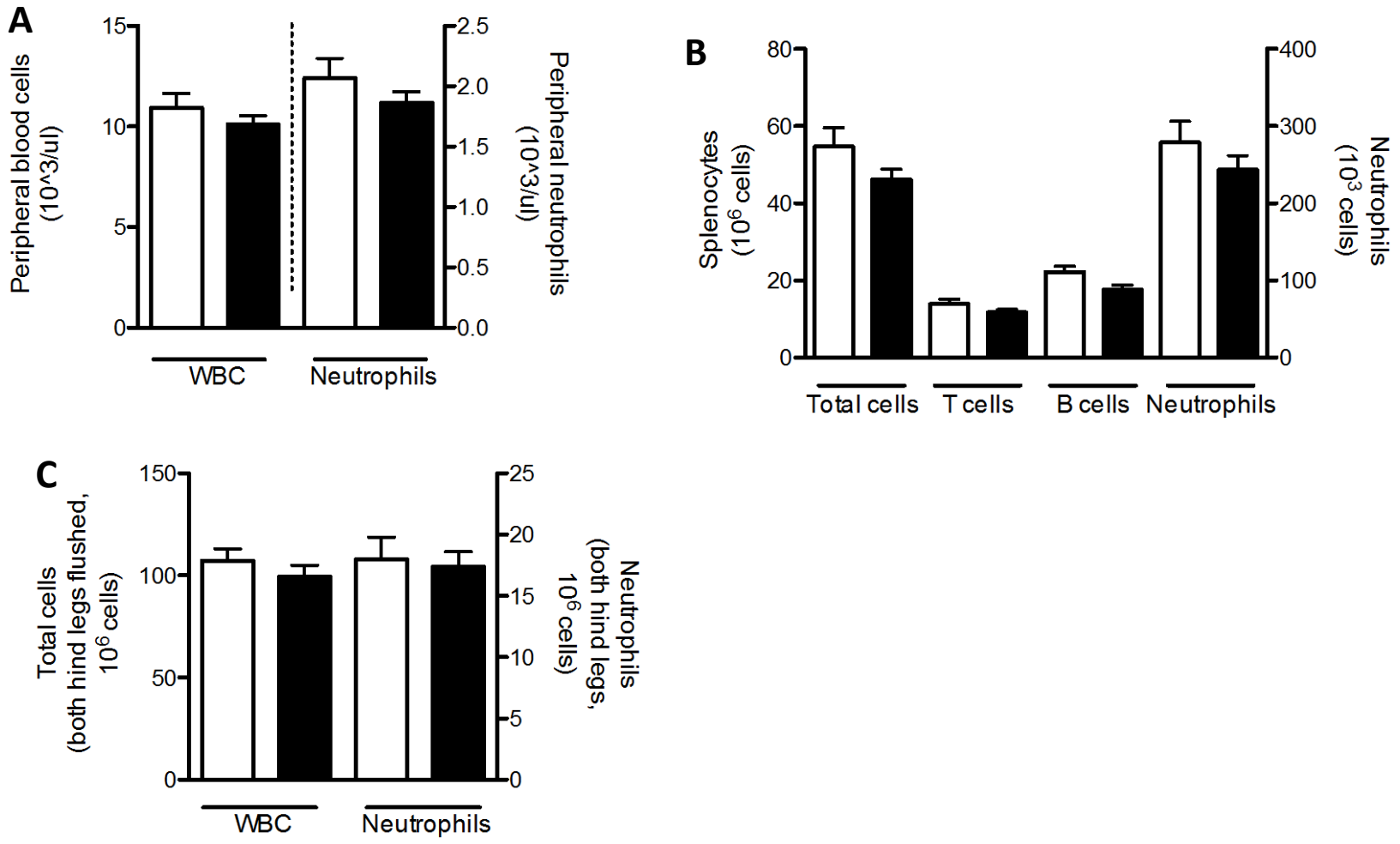
Supplemental Figure 1. LPS-dependent ATF3 regulation is CXCL1-specific. Temporal kinetics of airway production of CXCL5 in WT (dotted lines) or ATF3$^{-/-}$ (solid lines) mice following i.t. LPS challenge. Representative of 7 experiments, N=3-7 mice/group. BALF, bronchoalveolar lavage fluid.



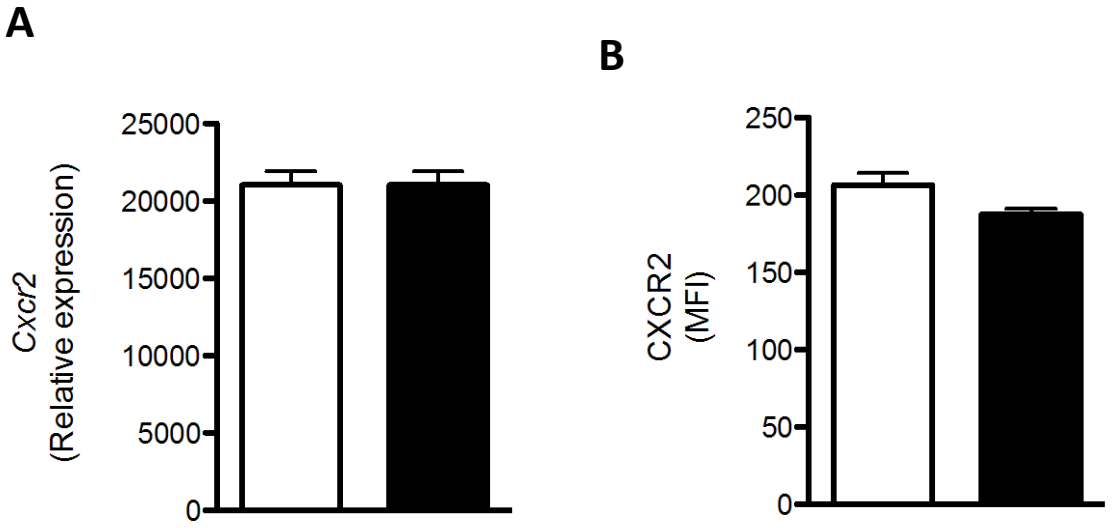
Supplemental Figure 2. WT- and ATF3$^{-/-}$-reconstituted BM chimeras are equivalent. Reconstitution efficiency (A) and leukocyte and neutrophil number and percent (B) in peripheral blood are equivalent between CD45.2^{+} WT (white bars) and ATF3$^{-/-}$ (gray bars) BM-reconstituted CD45.1^{+} WT hosts. N=12 ATF3$^{-/-}$, 10 WT, representative of 4 individual experiments. WBC, white blood cells; PMN, neutrophils.



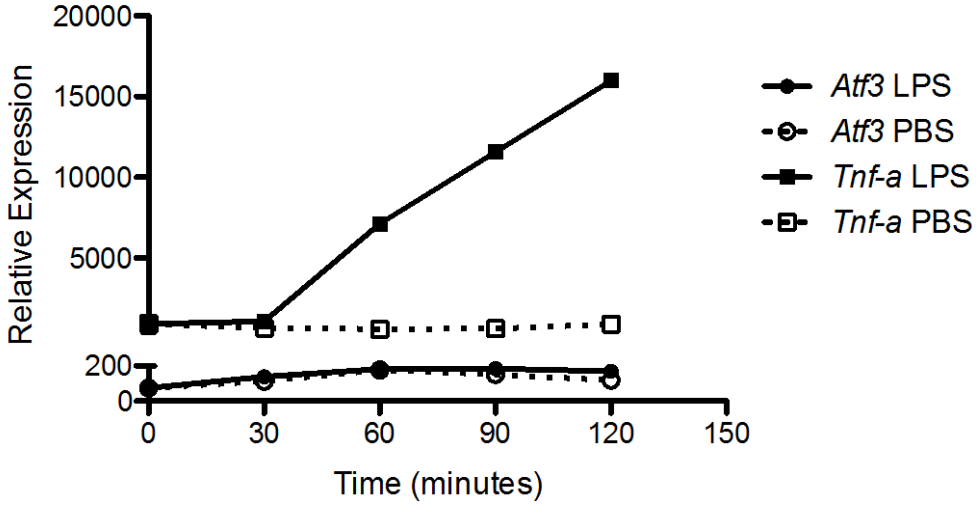
Supplemental Figure 3. Co-administered anti-CXCL1 antibodies specifically neutralize LPS-dependent CXCL1 production. (A) WT mice challenged i.t. with PBS or LPS, and isotype control (white bars) or anti-CXCL1 (black bars) antibodies were sacrificed after 4 hours and their lungs harvested into TRIzol after BAL. *Cxcl1* mRNA transcripts were determined as described in methods. (B) BAL from mice challenged in panel A were evaluated for CXCL1 (left) and CXCL2 (right) production by ELISA. Single experiment, N=6 mice/group. Statistics are unpaired, two-tailed *t* test. ***$p<0.0001$. BALF, bronchoalveolar lavage fluid.



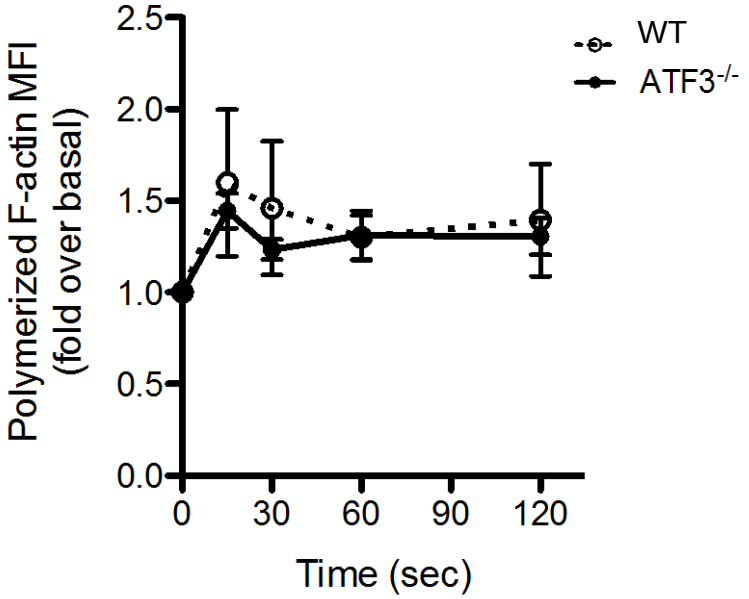
Supplemental Figure 4. No differences in ATF3$^{-/-}$ neutrophil distribution. Neutrophils numbers were assayed by flow cytometry for neutrophil markers 7/4 or Ly6G in peripheral blood (A), spleen (B) or BM (C). Peripheral blood was drawn via tail bleeds into EDTA-chelated tubes. The spleen was harvested, disaggregated through a 100um cell filter and treated with ACK to lyse red blood cells (RBCs). BM was flushed from hind leg bones and disaggregated through a 40um cell filter. Representative of 3 experiments N=8-11 mice/genotype. WBC, white blood cells.



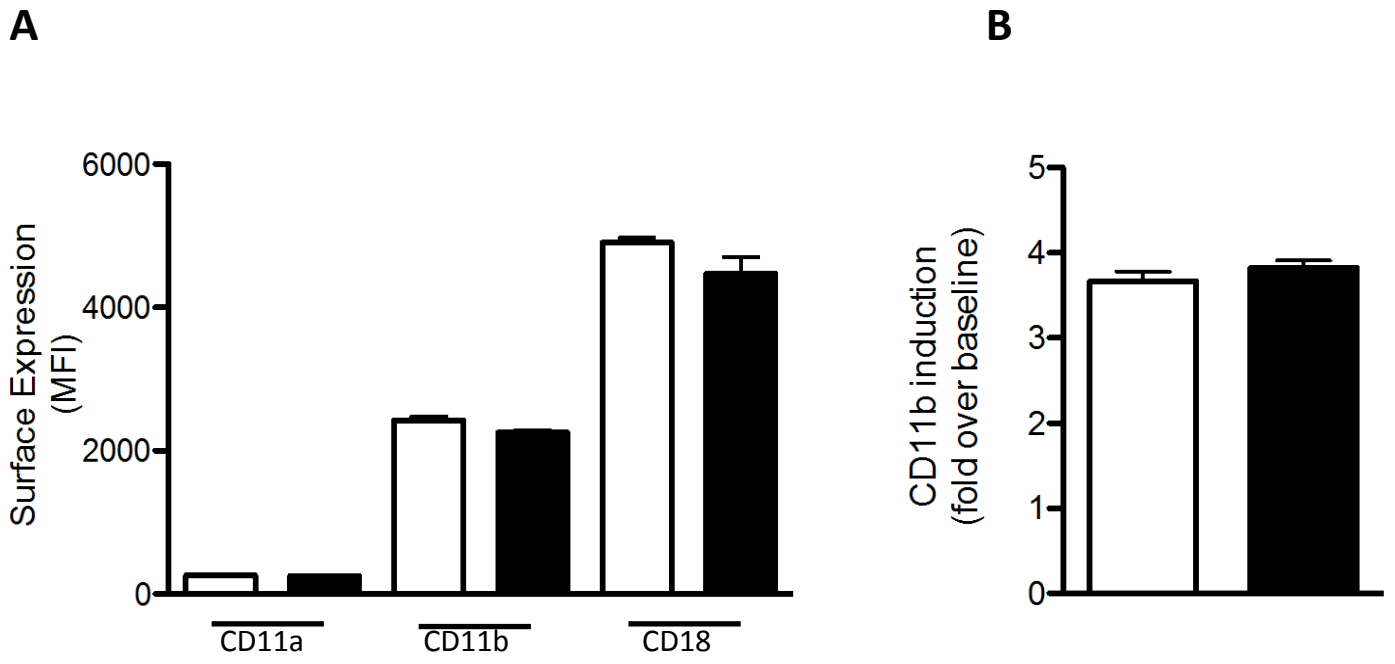
Supplemental Figure 5. ATF3$^{-/-}$ neutrophil show no differences in CXCR2 expression. CXCR2 expression analyzed at the mRNA level by microarray (A) and flow cytometry-based surface protein expression (B) on WT (white bars) and ATF3$^{-/-}$ (black bars) neutrophils. Representative of 1 experiment N=5-6 mice/genotype. MFI, mean fluorescence intensity.



Supplemental Figure 6. *Atf3* is not expressed in stimulated mouse neutrophils. WT neutrophils stimulated *in vitro* were analyzed by qRT-PCR for *Atf3* (circles) and *Tnf-*α (squares) in response to 10ng/ml LPS (solid lines) or PBS control (dotted lines). Representative of a single experiment, N=2 wells/condition.



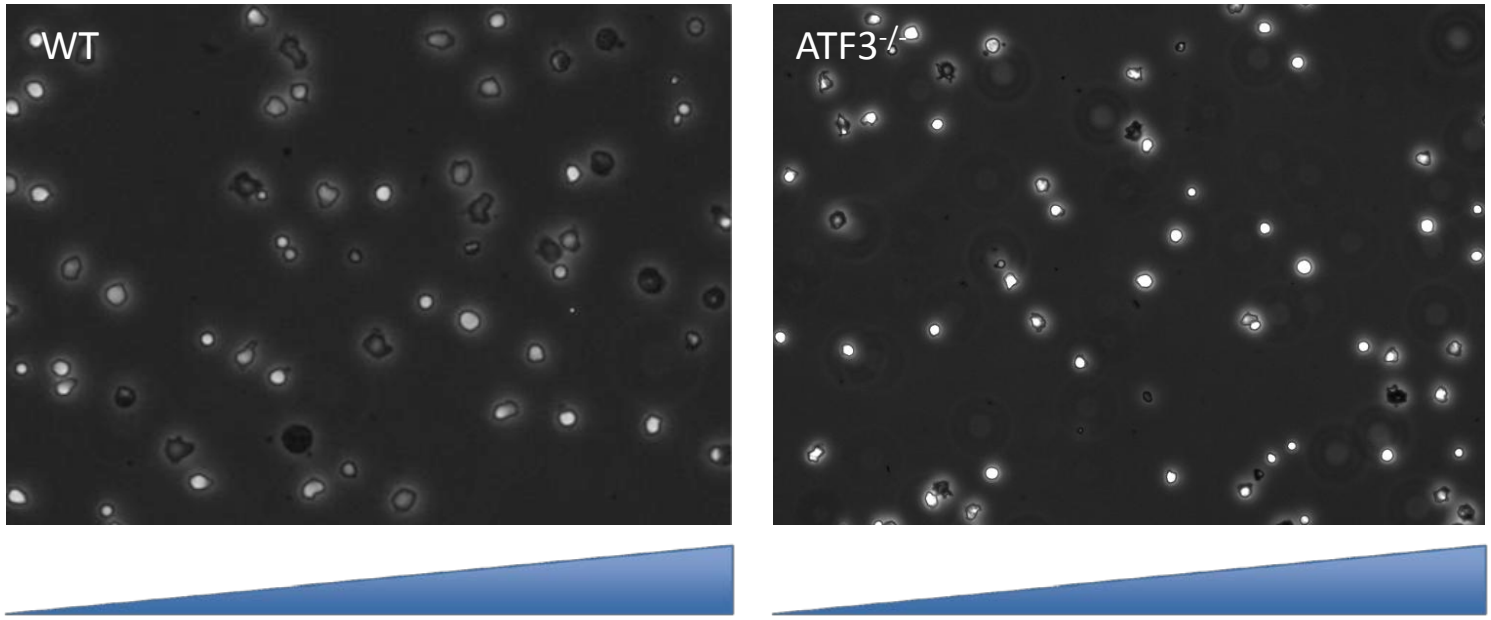
Supplemental Figure 7. ATF3-mediated differences in F actin polymerization are adhesion-dependent. Neutrophils were stimulated with fMLP for the indicated times in a 37°C water bath and quenched with 2% PFA. F-actin polymerization was determined by flow cytometry and reported as fold increase in phalloidin-rhodamine MFI over baseline. Results pooled from 3 independent experiments. 1 mouse/genotype/experiment. MFI, mean fluorescence intensity.



Supplemental Figure 8. ATF3$^{-/-}$ neutrophils display normal β_2 integrin expression. (A) The major β_2 integrins CD11a (LFA-1), CD11b (Mac-1) and the common β_2 subunit, CD18, were quantified by flow cytometry on unstimulated neutrophils from WT (white bars) or ATF3$^{-/-}$ (black bars) mice. Data are representative of 2 experiments, N=4-6 mice/genotype. (B) WT (white bars) and ATF3$^{-/-}$ (black bars) neutrophils were stimulated with fMLP and CD11b surface expression enrichment compared to baseline was measured by flow cytometry. Data are from 1 experiment, N=4-6 mice/genotype. MFI, mean fluorescence intensity.

Gene ID	Gene name	Accession #	Fold-change	Corrected *p* value
Tiam2	T-cell lymphoma invasion and metastasis 2	NM_011878	-284.039	0.000
Erd1	Erythroid differentiation regulator 1	NM_133362	-24.121	0.004
Odz3	Odd Oz/ten-m homolog 3	AK050784	5.838	0.024
Igh-VJ558	Immunoglobulin heavychain (J558 family)	BC019425	5.544	0.033
Igj	Immunoglobulin joining chain	NM_152839	3.505	0.031
Efna2	Ephrin A2	NM_007909	2.498	0.046
Sympo	Synaptopodin	AK020250	2.493	0.024
Susd4	Sushi domain containing 4	NM_144796	2.433	0.020
Mtus2	Microtubule associated tumor suppressor candidate 2	NM_029920	2.083	0.027

Supplemental Table 1. Significantly differentially expressed protein encoding genes identified by microarray analysis comparison of WT and ATF3$^{-/-}$ neutrophils. Table represents significantly differentially expressed genes independently co-identified by analysis of microarray data as described in "Methods." Indicated fold change and corrected *p* values are of ATF3$^{-/-}$ neutrophils relative to WT neutrophils from RMA-normalized probes and Benjamini-Hochberg post-test to correct for multiple comparisons.



Supplemental videos. ATF3$^{-/-}$ neutrophils exhibit defective chemotaxis. WT (left video) or ATF3$^{-/-}$ (right video) 0.1x10^{6} neutrophils were plated and allowed to adhere to uncoated glass coverslips for 30 minutes at 37°C. Non-adherent cells were washed with 0.1% gelatin in HBSS + 1 mM HEPES and then coverslips were mounted in a Zigmond chamber in a gradient of 10 uM fMLP in HBSS + 1 mM HEPES on the right. Images were acquired every 5 seconds for 20 minutes on a Zeiss Axiovert 200 microscope at 10X/0.3 NA objective (original magnification x100), equipped with an ORCA-ER-C4742-95 camera driven by OpenLab software version 5.5.0.