Supplementary Data

Neutrophil migration in Dunn Chamber Assay

To better understand the nature of the migratory defect, we performed real time imaging using a Dunn chamber, which allows direct visualization of neutrophil motility and directional migration along a chemokine gradient. Overall, a significantly lower percentage of *Srf* KO than WT neutrophils migrated towards the fMLP gradient (Suppl. Figure 3A; WT 53.44 ± 3.12% vs. KO 18.83 ± 5.11%; p = 0.029). In addition, *Srf* WT neutrophils migrated rapidly towards the fMLP gradient, while those *Srf* KO neutrophils that migrated (with cut-off of minimum distance of 7.5μm) achieved a significantly lower translocation distance (Suppl. Figure 3B; WT 54.29 ± 0.40 μm/30min vs. KO 27.68 ± 0.19 μm/30min; p=0.0003). Movies show WT (Video 1) and KO (Video 2) neutrophil migration. Thus *Srf* KO neutrophils have a severe migration defect *in vitro,* which is likely responsible for the lack of neutrophil accumulation at sites of inflammation *in vivo*.

Cell adhesion is defective in *Srf* KO neutrophils

Integrins LFA1 and Mac1 play distinct roles in neutrophil adhesion. While LFA1 predominantly functions in the initial engagement of the neutrophil with the endothelium leading to rolling followed by arrest, binding of endothelial ICAM-1 to Mac-1 induces neutrophil crawling on the endothelial surface and transmigration through endothelial pores. We tested neutrophil adhesion in response to fMLP. Equal numbers of *Srf* WT and KO neutrophils were allowed to adhere to uncoated or fibrinogen-coated glass coverslips with or without stimulation with fMLP. Non-adherent neutrophils were removed by gentle rinsing. Significantly fewer *Srf* KO neutrophils adhered to uncoated or fibrinogen coated coverslips following stimulation with fMLP. Shown in Supplemental Figure 4.

Supplementary Methods

Dunn chamber assay

Equal numbers of WT and KO neutrophils were allowed to adhere to fibrinogen-coated coverslips, which were inverted onto a Dunn Chamber (Hawksley, Lancing, Sussex). Migration towards an fMLP gradient across the Dunn chamber bridge was imaged every 30 seconds over a period of 30 minutes. Images were analyzed using MetaMorph and Microsoft Excel Software as previously described.[1]

Adhesion Assay

For cellular adhesion isolated neutrophils were stimulated with vehicle or fMLP and allowed to adhere to uncoated of fibrinogen-coated coverslips. Non-adherent cells were gently washed off. Adherent cells were counted on 5 20x fields and averaged. The experiment was repeated 3 times.

Figure S1: (A) Neutrophil morphology from *Srf* WT and KO mice. (B) White cell count (WBC) and white cell differential in primary Dox-inducible Cre *Srf* WT n=11 and

KO n=10 mice. Peripheral blood from Dox-inducible Cre/*Srf* bone marrow transplanted mice aged 4 weeks was obtained after treatment with doxycycline for 8-10 days and WBC and WBC differential assessed on a Hemavet. *Srf* expression in Neutrophils from dox-inducible *Srf* WT and KO mice assessed by qRT-PCR (C) and Western blot (D).

Figure S2: Lung immunohistology of recipients of Dox-inducible *Srf* WT and KO bone marrow probed for the neutrophil specific antibody 7/4 prior to (0hr) and 4 (4hr) and 24 hours (24hr) after LPS nebulization (60x magnification; Size bar=20μm).

Figure S3: CXCR2 expression and migration of *Srf* WT and KO neutrophils in Dunn chamber assay. (A) CXRC2 expression was assessed by flow-cytometry on *Srf* WT and KO neutrophils. (B,C) *Srf* WT and KO neutrophils were directly imaged while exposed to fMLP gradient in a Dunn chamber. (B) The percentage of migrating neutrophils was assessed with a cut-off of 7.5μm, which corresponds to one-half the diameter of an activated neutrophil. (C) The translocation distance in μm was measured for those neutrophils, that migrated at least 7.5μm (*p< .05; ***p< .0005).

Figure S4: Integrin Expression and Adhesion. Gene expression was assessed in *Srf* WT and KO neutrophils by qRT-PCR (A). CD11b and CD11a expression was assessed by flow-cytometry in *Srf* WT and KO neutrophils 0, 2, and 5 minutes after fMLP stimulation (B). *Srf* WT and KO neutrophils were allowed to adhere to untreated or fibrinogen coated glass coverslips in the absence or presence of activation with fMLP (C). Representative of 3 independent experiments, number of fields counted per slide (n)
=5. (***p<.0005, *p<.05)

Figure S5: Immunofluorescence quantification. Quantification of cells from Figure 6. (A) Percent of cells with polarized CD11b; n = number of cells: WT 0 min. n=28, WT 15 min. n=32, KO 0 min. n=12, KO 15 min. n=12 cells. (B) Percent of cells with polarized clathrin; WT 0 min. n=25, WT 15 min. n=45, KO 0 min. n=24, KO 15 min. n=30 cells. (C) Percent of cells with polarized kindlin; WT 0 min. n=82, WT 15 min. n=151, KO 0 min. n=64, KO 15 min. n=93 cells.

Video 1 (WT) and Video 2 (KO) neutrophil migration in Dunn chamber assay.

1. Zhang Y, Tang W, Jones MC, Xu W, Halene S, Wu D. Different roles of G protein subunits beta1 and beta2 in neutrophil function revealed by gene expression silencing in primary mouse neutrophils. *J Biol Chem*. 2010;285(32):24805-24814.

Table S1 Actin Cytoskeletal Genes down-regulated

GENE	LOCUS	KO_FPKM	WT_FPKM	Fold Change WT vs KO	p_value
Hunk	chr16:90386396-90499553	0.0243873	2.68032	109.91	0
Srf	chr17:46546838-46556162	0.43898	13.7479	31.32	0
Lhx1	chr11:84519378-84525534	0.13868	3.77373	27.21	0
Acta1	chr8:123891766-123894736	3.48133	47.4917	13.64	0
Lima1	chr15:99778467-99875456	0.78814	8.92323	11.32	0
Actg1	chr11:120345689-120348484	261.927	2246	8.57	0
Myh11	chr16:14194526-14291408	0.0590963	0.496764	8.41	6.14E-13
Fscn2	chr11:120361533-120368173	0.0447551	0.308896	6.90	0.00101364
Clip2	chr5:134489385-134552434	0.914883	5.98154	6.54	0
Tpm4	chr8:72135291-72153129	42.5116	223.3	5.25	0
Cnn2	chr10:79697304-80369637	113.203	586.467	5.18	0.00118772
Lyst	chr13:13590408-13777440	13.2339	67.3001	5.09	0
Fgd3	chr13:49263109-49309208	19.3645	81.0618	4.19	0
Zyx	chr6:42349827-42358395	85.9583	353.862	4.12	0
Wdr1	chr5:38526812-38561595	62.3782	244.644	3.92	0
Numb	chr12:83795438-83842343	14.4483	54.489	3.77	0
Actb	chr5:142903115-142906724	1268.47	4666.63	3.68	2.22E-16
Myh9	chr15:77760588-77842115	106.398	368.155	3.46	0
Acta2	chr19:34241090-34255336	12.8751	43.505	3.38	0
Diap1	chr18:37844824-37935411	34.2191	113.32	3.31	0
Myh10	chr11:68691914-68816624	0.46816	1.48505	3.17	4.01E-10
Egr1	chr18:34861206-34864956	3.77816	11.7991	3.12	4.91E-14
Coro1a	chr7:126699773-126704754	479.265	1231.28	2.57	2.88E-12
Wasf2	chr4:133130632-133198330	17.2757	43.5538	2.52	7.83E-13
Flna	chrX:74223460-74246534	161.403	403.3	2.50	2.79E-10
Ssh2	chr11:77216424-77460219	18.5403	46.313	2.50	3.86E-13
Tln1	chr4:43531512-43562583	64.2728	149.742	2.33	1.93E-10
Pik3cd	chr4:149649167-149701629	37.7274	87.668	2.32	5.23E-09
Pip5k1a	chr3:95059595-95106858	4.85599	10.7838	2.22	1.93E-07

Table S2: Inflammation Genes down-regulated

GENE	LOCUS	KO_FPKM	WT_FPKM	Fold Change WT vs KO	p_value
Ctse	chr1:131638313-131675507	13.8066	199.429	14.44	0
Nlrp3	chr11:59542685-59566956	7.09473	40.9297	5.77	0
Tlr8	chrX:167242731-167263788	4.1957	22.1173	5.27	0
Lyst	chr13:13590408-13777440	13.2339	67.3001	5.09	0
Cxcr1	chr1:74191785-74194631	1.02741	4.93035	4.80	3.45E-13
Il1b	chr2:129364579-129375733	54.5063	257.206	4.72	0
Nlrp12	chr7:3221509-3249740	10.5163	43.4205	4.13	0
Ccl6	chr11:83582060-83623693	260.361	990.904	3.81	0
Tlr5	chr1:182954787-182976044	4.26425	15.362	3.60	0
Il6ra	chr3:89869323-89913162	16.0212	50.4615	3.15	0
Cxcr2	chr1:74153993-74161246	200.837	596.357	2.97	4.44E-16
Tlr13	chrX:106143274-106160493	31.9984	94.2462	2.95	0
Ccr1	chr9:123962125-123968692	72.049	211.234	2.93	0
Tlr6	chr5:64953094-64960034	4.8346	13.8943	2.87	2.27E-12
Csf3r	chr4:126024658-126044975	247.76	686.976	2.77	4.50E-11

Table S3: Integrin expression

GENE	LOCUS	KO_FPKM	WT_FPKM	Fold Change WT vs KO	p_value
Itga8	chr2:12106659-12312315	0.443401	1.31623	2.97	3.61E-08
Itgal	chr7:127296259-127335137	60.3137	149.629	2.48	1.10E-10
Itgam	chr7:128062639-128118491	417.826	694.321	1.66	0.00484506
Itgb7	chr15:102215994-102231935	26.4388	35.9967	1.36	0.0181999
Itga4	chr2:79255425-79428988	13.8089	16.9807	1.23	0.105993
Itga5	chr15:103344285-103366748	3.45827	3.49268	1.01	0.949019
Itgb3	chr11:104607999-104670471	16.0616	16.0745	1.00	0.99505
Itgb1	chr8:128685653-128733579	23.7798	21.9456	0.92	0.531771
Itgb2l	chr16:96422297-96443614	366.189	273.483	0.75	0.0303399
Itgb2	chr10:77530347-77565674	554.921	979.876	0.62	7.77E-05
Itgad	chr7:128173945-128206366	1.90972	1.03751	0.54	0.00195022
Itga9	chr9:118606708-118901003	0.495038	0.222523	0.45	0.00909608
Itga2	chr13:114835911-114932041	0.24647	0.0917129	0.37	0.00247215
Itga2b	chr11:102453296-102469883	3.0518	1.11977	0.37	3.99E-08

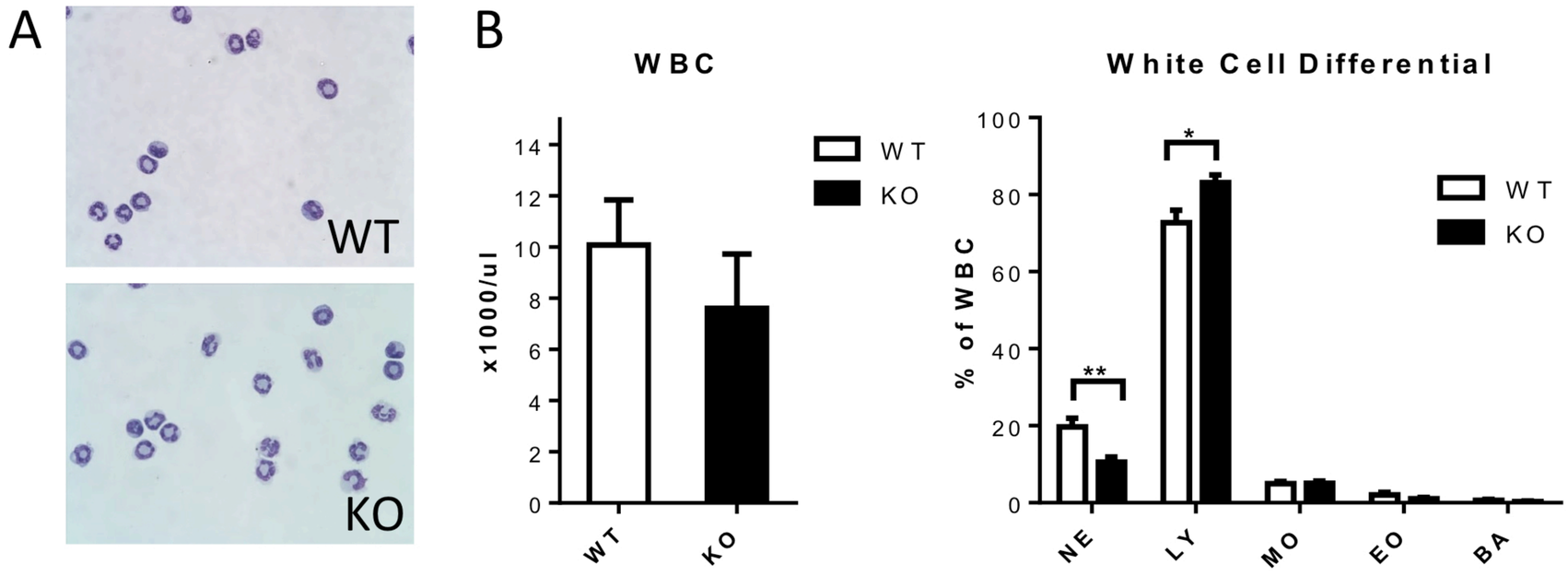

A
WT
KO
B
WBC
x1000/ul
14
12
10
8
6
4
2
0
WT
KO
WT
KO
White Cell Differential
% of WBC
100
80
60
40
20
0
**
*
NE
LY
MO
EO
BA
C
Fold Change
1.5
1.0
0.5
0.0
***
SRF
D
WT
KO
Srf
Gapdh

Figure S1

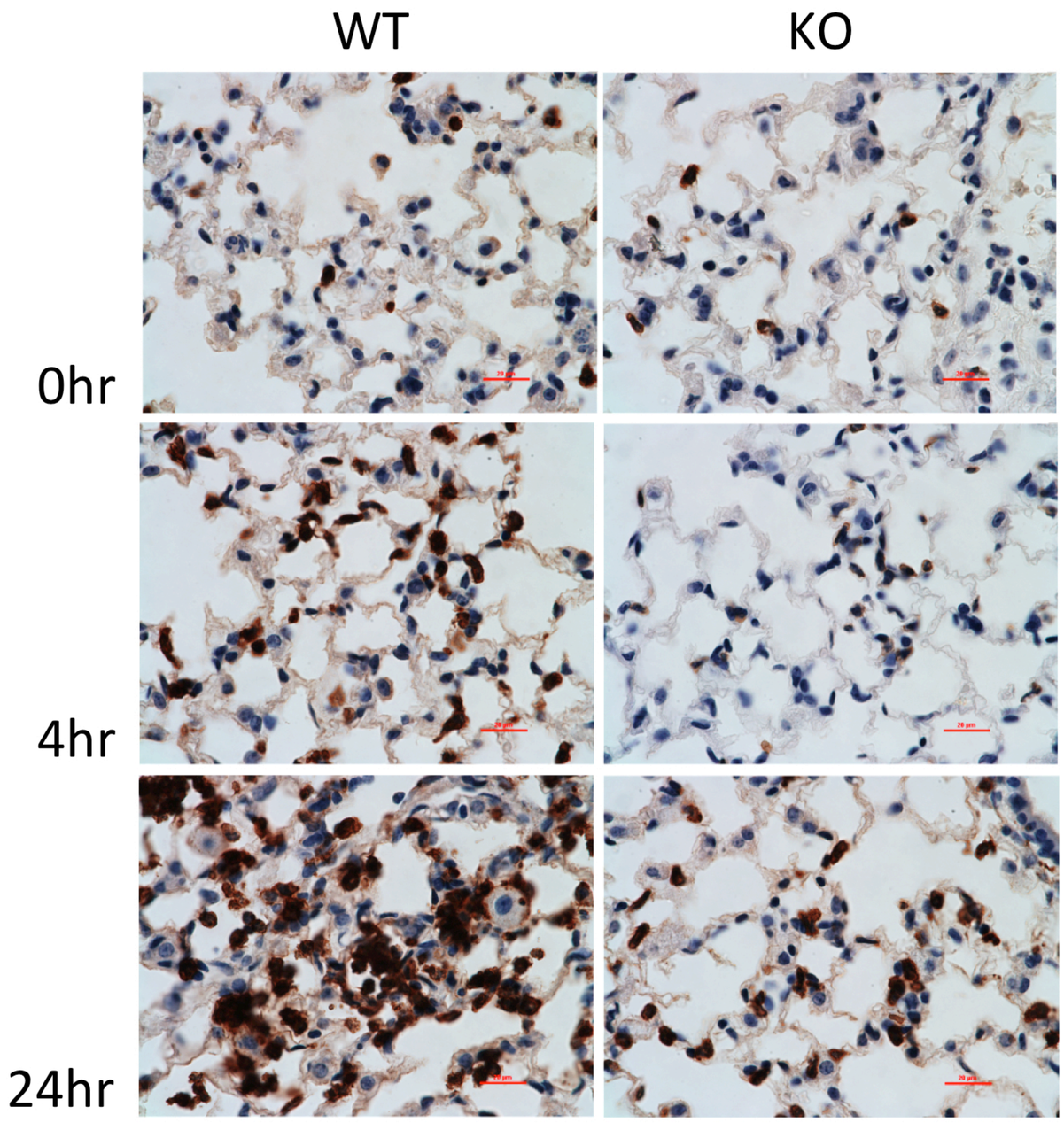


Figure S2

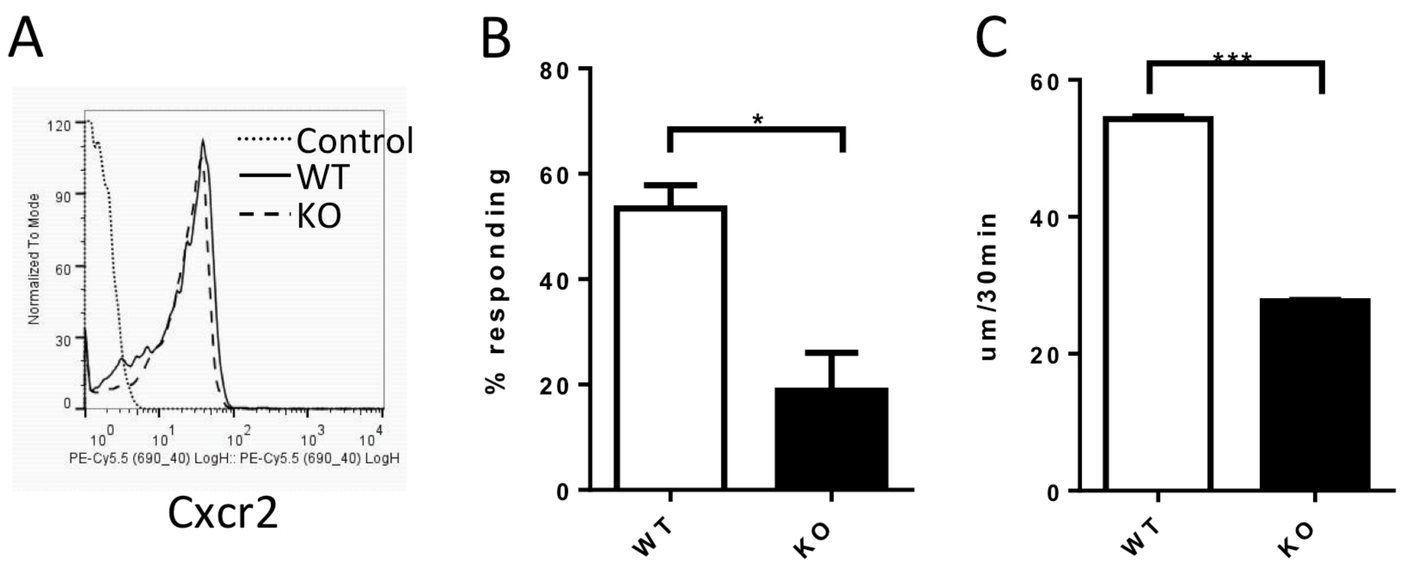

A
Control
WT
KO
Normalized To Mode
PE-Cy5.5 (690_40) LogH:: PE-Cy5.5 (690_40) LogH
Cxcr2
B
% responding
*
WT
KO
C
um/30min
***
WT
KO

Figure S3

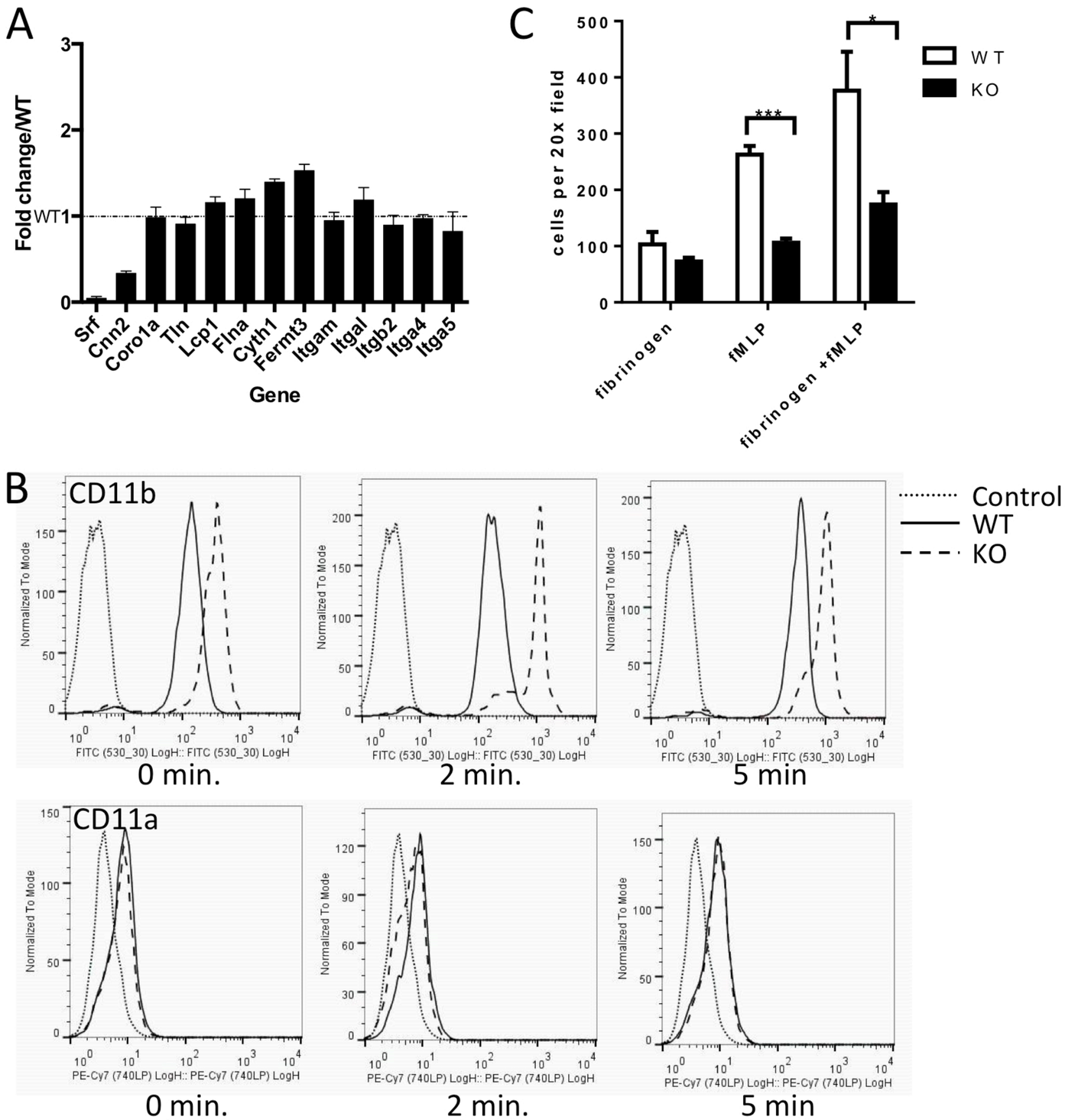
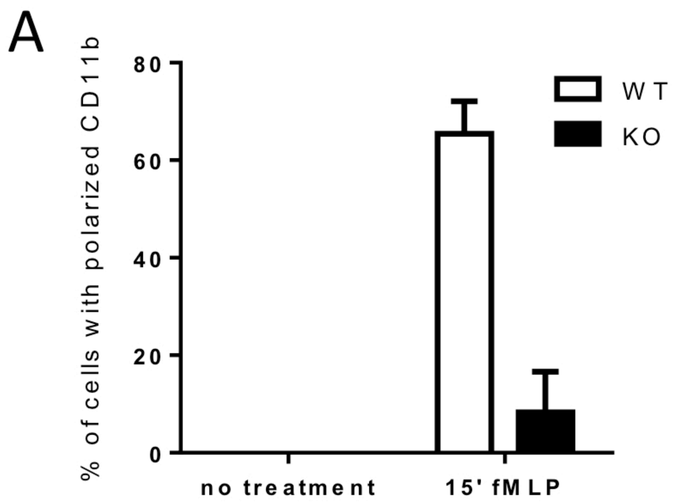

A
Fold change/WT
WT
Srf
Cnn2
Coro1a
Tln
Lcp1
Flna
Cyth1
Fermt3
Itgam
Itgal
Itgb2
Itga4
Itga5
Gene
C
cells per 20x field
WT
KO
***
*
fibrinogen
fMLP
fibrinogen +fMLP
B
CD11b
Control
WT
KO
Normalized To Mode
FITC (530_30) LogH:: FITC (530_30) LogH
0 min.
2 min.
5 min
CD11a
PE-Cy7 (740LP) LogH:: PE-Cy7 (740LP) LogH
0 min.
2 min.
5 min

Figure S4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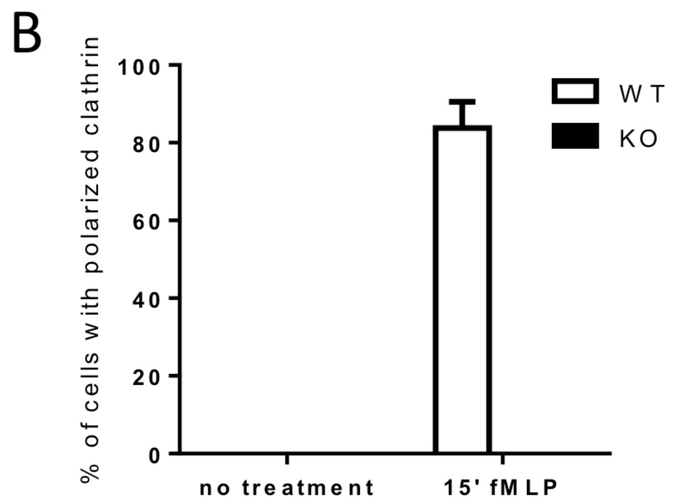
% of cells with polarized CD11b
80
60
40
20
0
WT
KO
no treatment
15' fMLP


B
% of cells with polarized clathrin
100
80
60
40
20
0
WT
KO
no treatment
15' fMLP

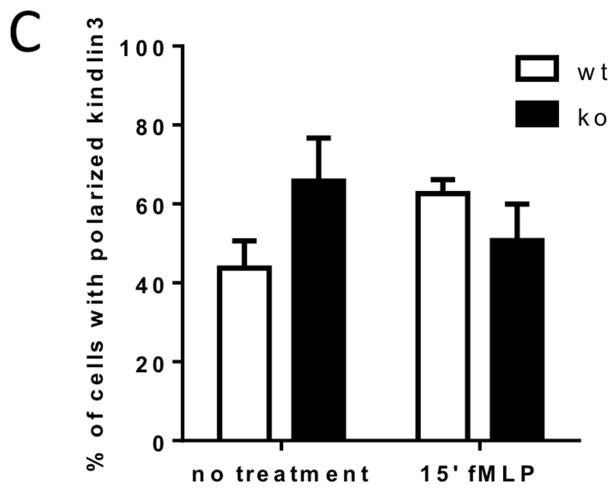

C
% of cells with polarized kindlin3
100
80
60
40
20
0
wt
ko
no treatment
15' fMLP

Figure S5