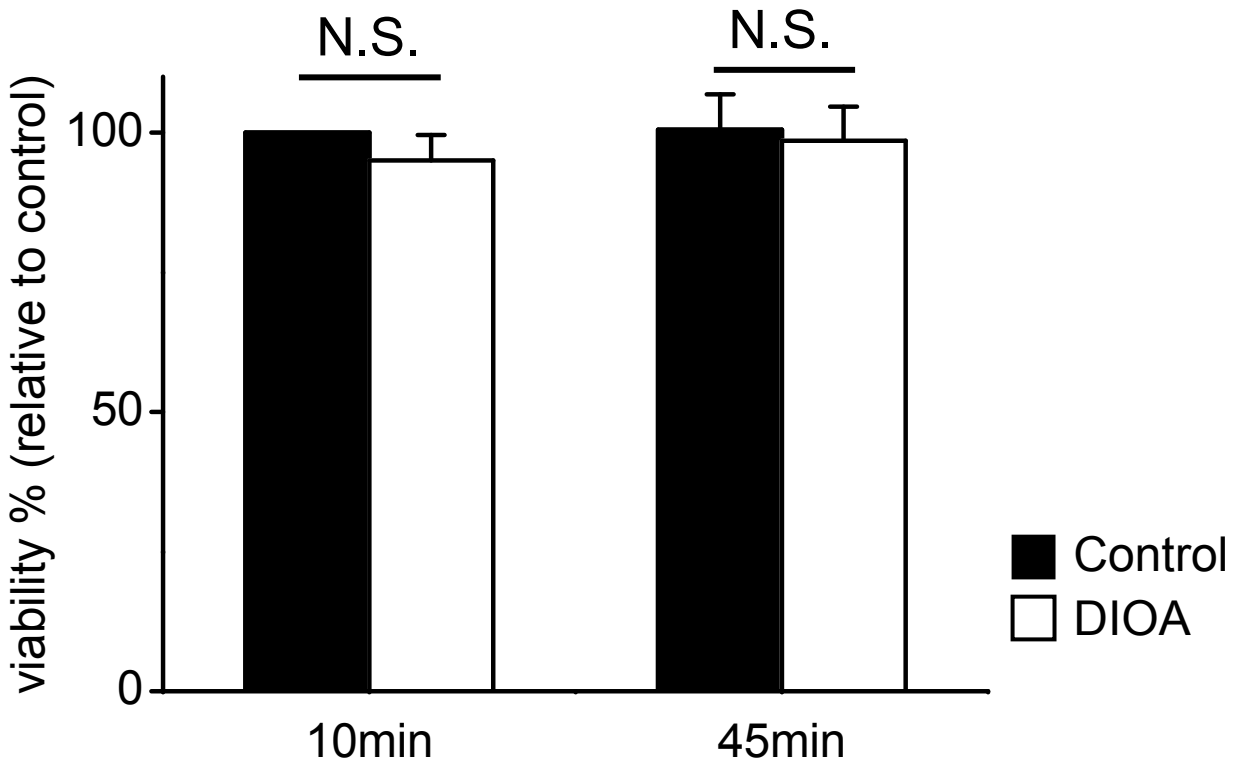

Figure S1. MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3- carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt) assay for determining the viability of human neutrophils incubated with DIOA (100 μM) for 10 minutes and 45 minutes. The PMNs were isolated from three subjects. Each value indicates mean ± SEM. N.S. no significant difference.

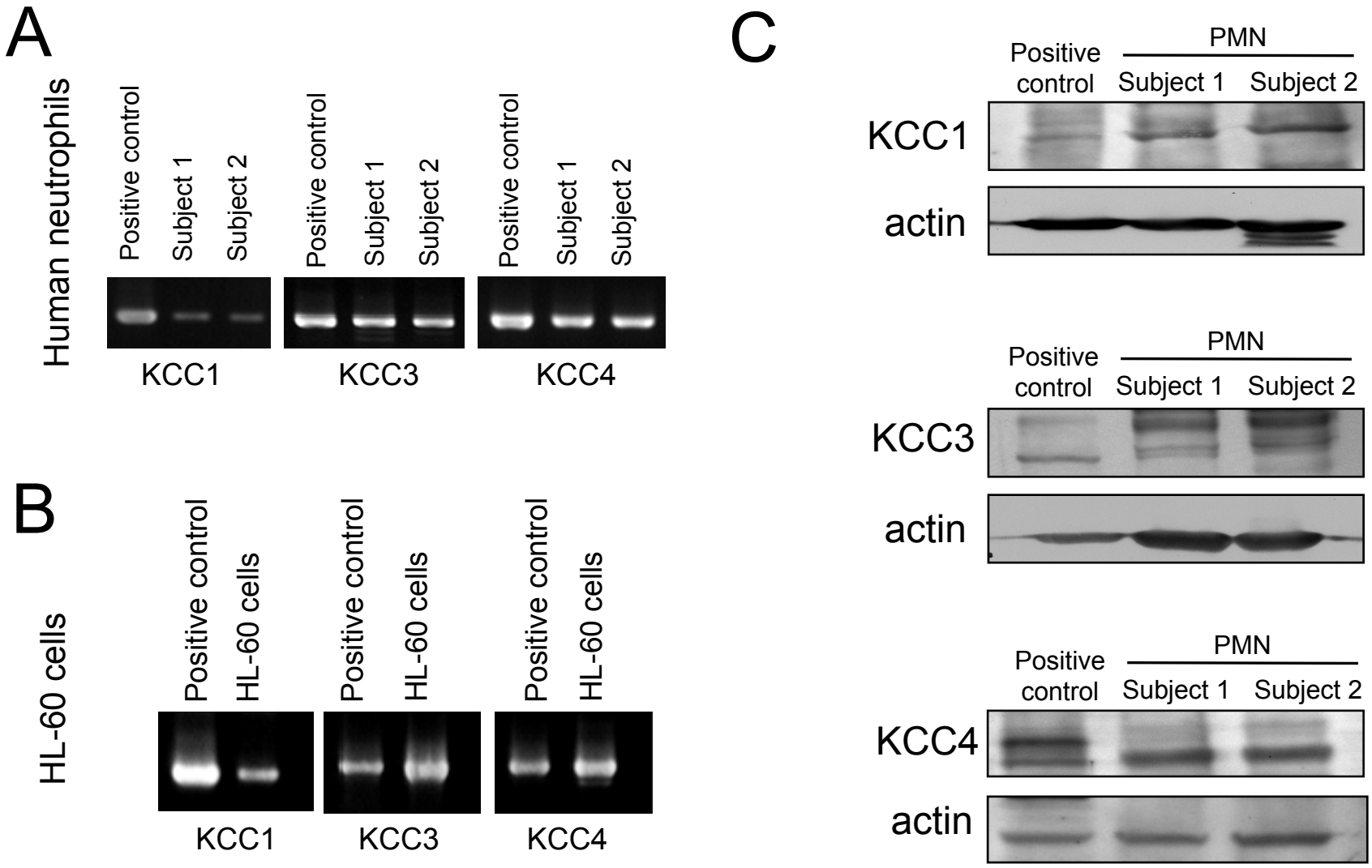

Figure S2. KCC isoforms are identified in human PMNs and HL-60 cells. RT-PCR analysis of individual KCC isoforms shows the presence of KCC1 (421 bp), KCC3 (1099 bp), and KCC4 (783 bp) mRNAs in **(A)** human PMNs and **(B)** HL-60 cells. **(C)** The protein expression of three KCC isoforms in human neutrophils. Positive control: SiHa cells, a cervical cancer cell line.

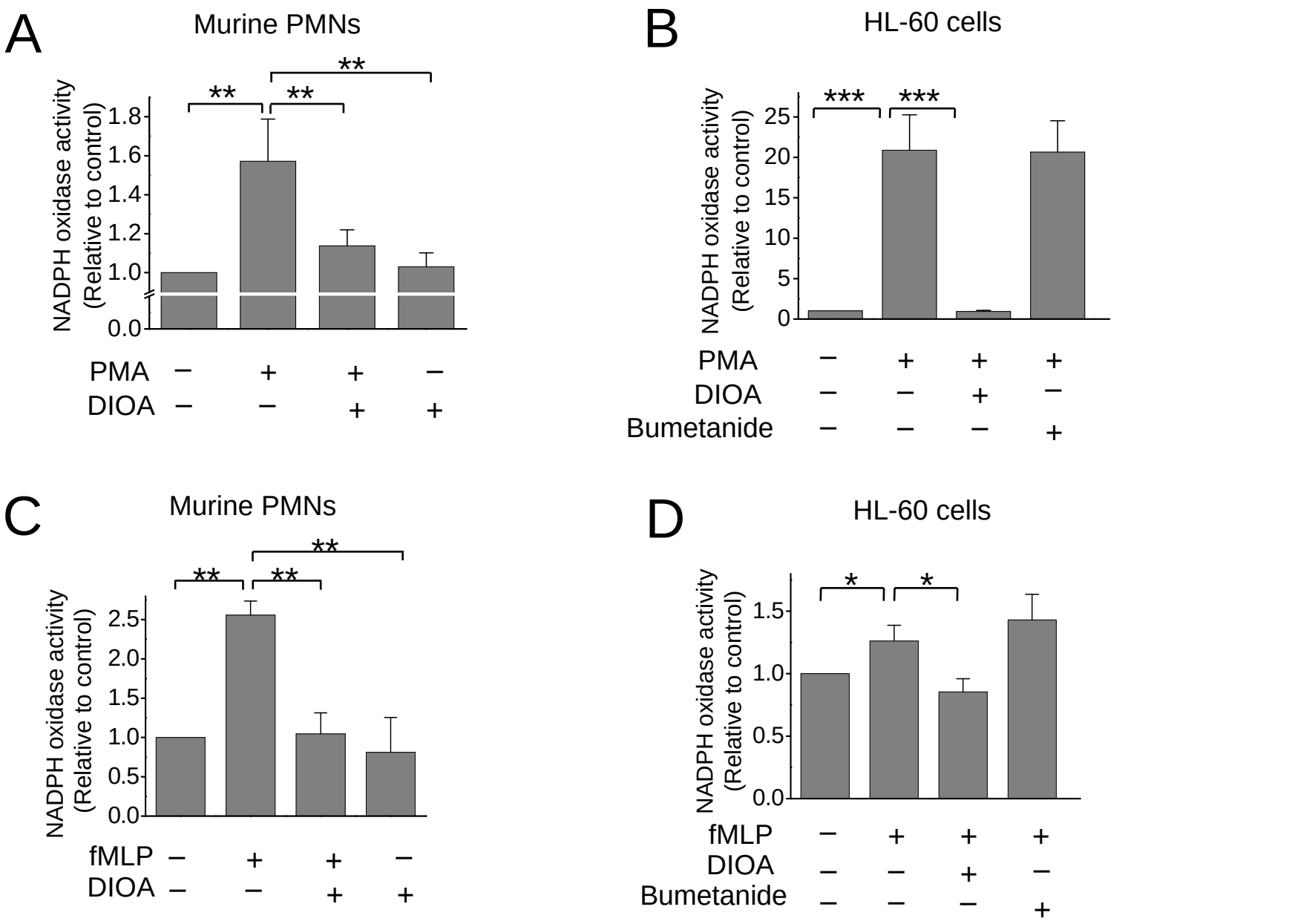

Figure S3. KCC inhibitor suppresses the PMA- and fMLP-induced NADPH oxidase activity in HL-60 cells and murine PMNs. DIOA (50 μM) but not bumetanide (20 μM) significantly inhibited the PMA- **(A,C)** and fMLP- **(B,D)** induced ROS generation in HL-60 cells **(B,D)** and murine PMNs **(A,C)**. The negative control is cells treated with 0.1% DMSO alone. Each bar indicates the mean ± SEM from at least four independent experiments. ***, $p \leq 0.001$; **, $p \leq 0.01$; *, $p \leq 0.05$.

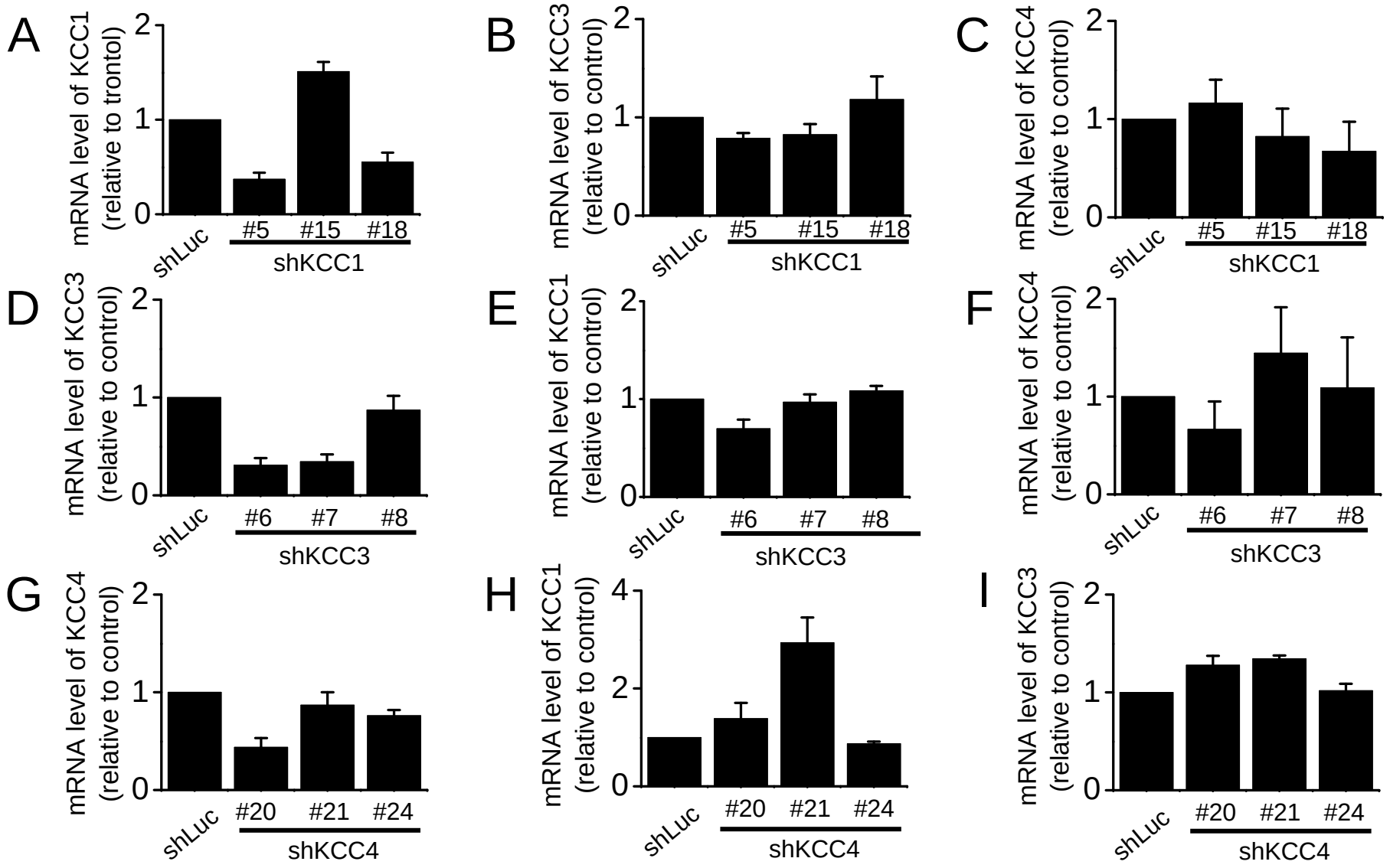

Figure S4.The mRNA expression level of KCC1, KCC3, and KCC4 in each shKCC cell line. (A-C) Three oligonucleotide sequences shKCC1#5, shKCC1#15, and shKCC1#18 were used to generate three stable cell lines with reduced KCC1 expression. KCC1 knockdown efficiency of each sequence was also compared with that of the vector control, shLuciferase oligonucleotide **(A)**. Expression levels of KCC3 **(B)** and KCC4 **(C)** in the three stable KCC1 knockdown cell lines were checked to rule out the possible compensatory expression. **(D)** KCC3 knockdown efficiency of three oligonucleotide sequences, shKCC3#6, shKCC3#7, and shKCC3#8 were compared with that of shLuciferase. Expression levels of KCC1 **(E)** and KCC4 **(F)** in the three KCC3 knockdown cell lines were compared. **(G)** KCC4 knockdown efficiency of three oligonucleotide sequences shKCC4#20, shKCC4#21, and shKCC4#24 were compared with that of the control. Expression levels of KCC1 **(H)** and KCC3 **(I)** were checked in the three KCC4 knockdown cell lines as well.

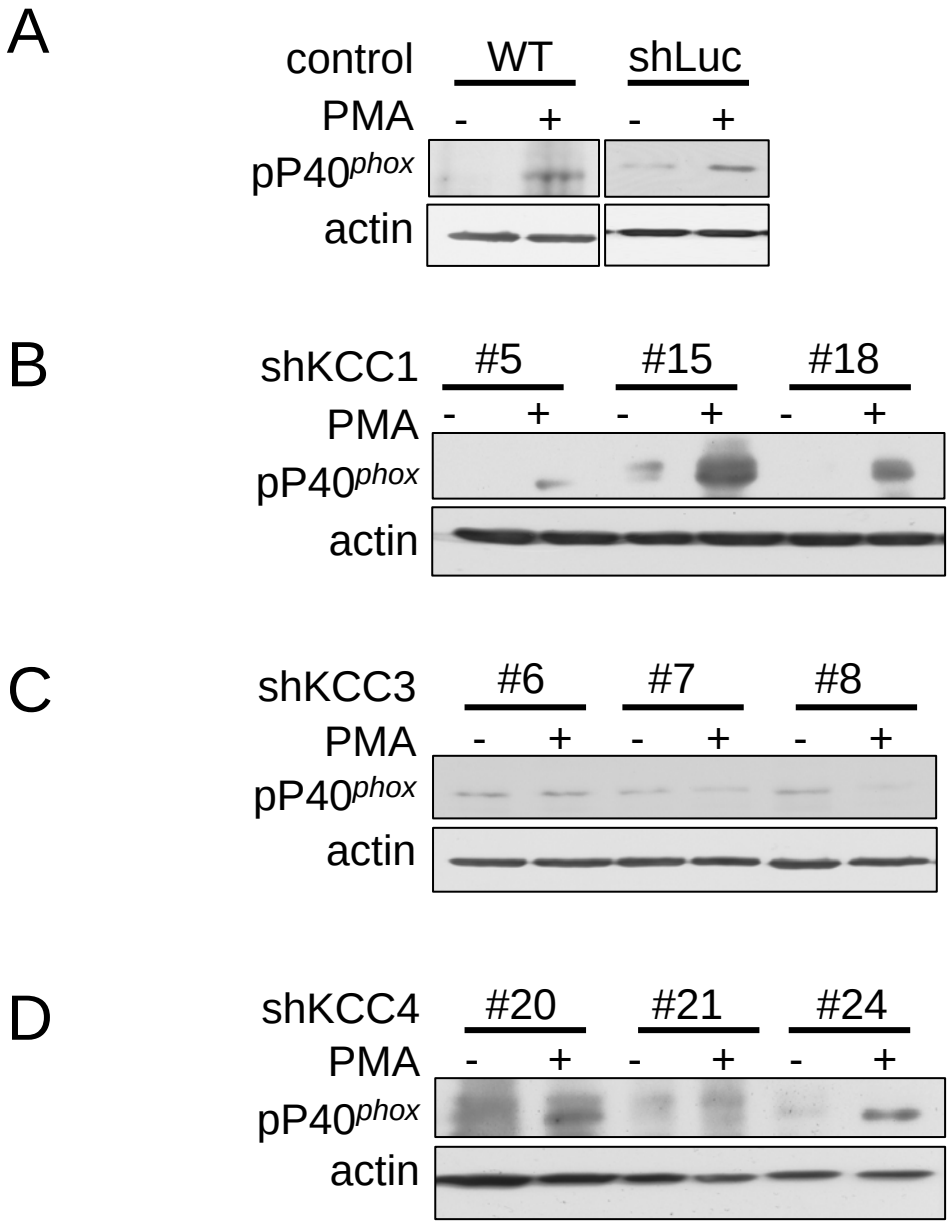

Figure S5. Phosphorylation of p40*phox* in each shKCC cell line in response to PMA stimulation. (A) In wild-type (WT) and shLuciferase (shLuc) cells, PMA induces a remarkably increased phosphorylation of p40*phox*. **(B)** Of the three shKCC1 cell lines, shKCC1#15 and shKCC1#18 showed a marked increase of p40*phox* phosphorylation, whereas shKCC1#5 showed a modest response. **(C)** In three shKCC3 cell lines, PMA failed to provoke a significant phosphorylation of p40*phox*. **(D)** In three shKCC4 cell lines, PMA induced the phosphorylation of p40*phox*.

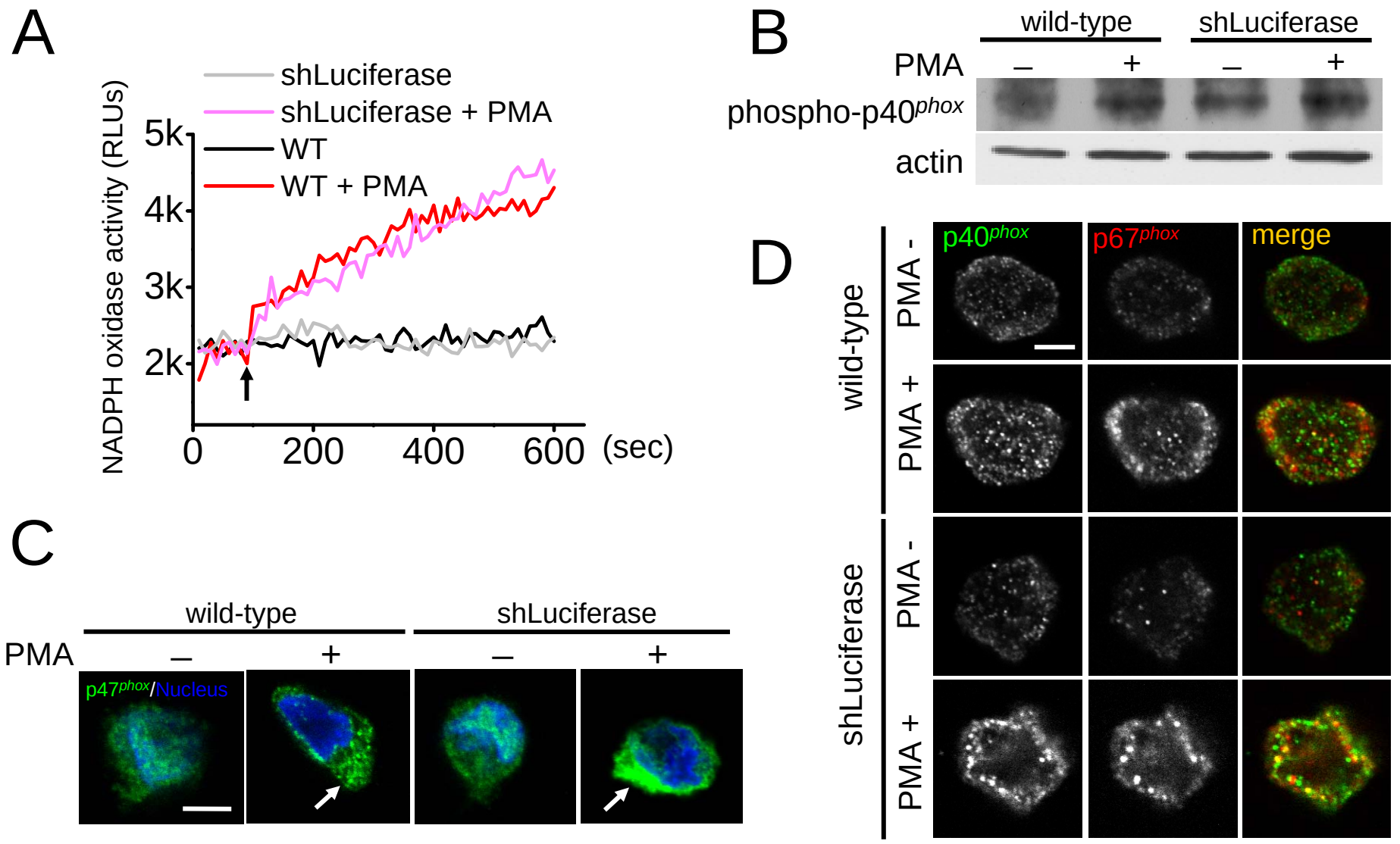

Figure S6. Comparison of NADPH oxidase activity in wild-type (WT) and shLuciferase HL-60 cells. **(A)** ROS generation, **(B)** phosphorylation of p40*phox*, **(C)** translocation of p47*phox* (green), and **(D)** translocation of p40*phox* (green) as well as p67*phox* (red) in response to PMA were similar in wild-type and shLuciferase HL-60 cells. The black arrow in **(A)** indicates the adding of lucigenin. The white arrows in **(C)** indicate the membrane recruitment of p47*phox*. Scale bar, 5 μm.

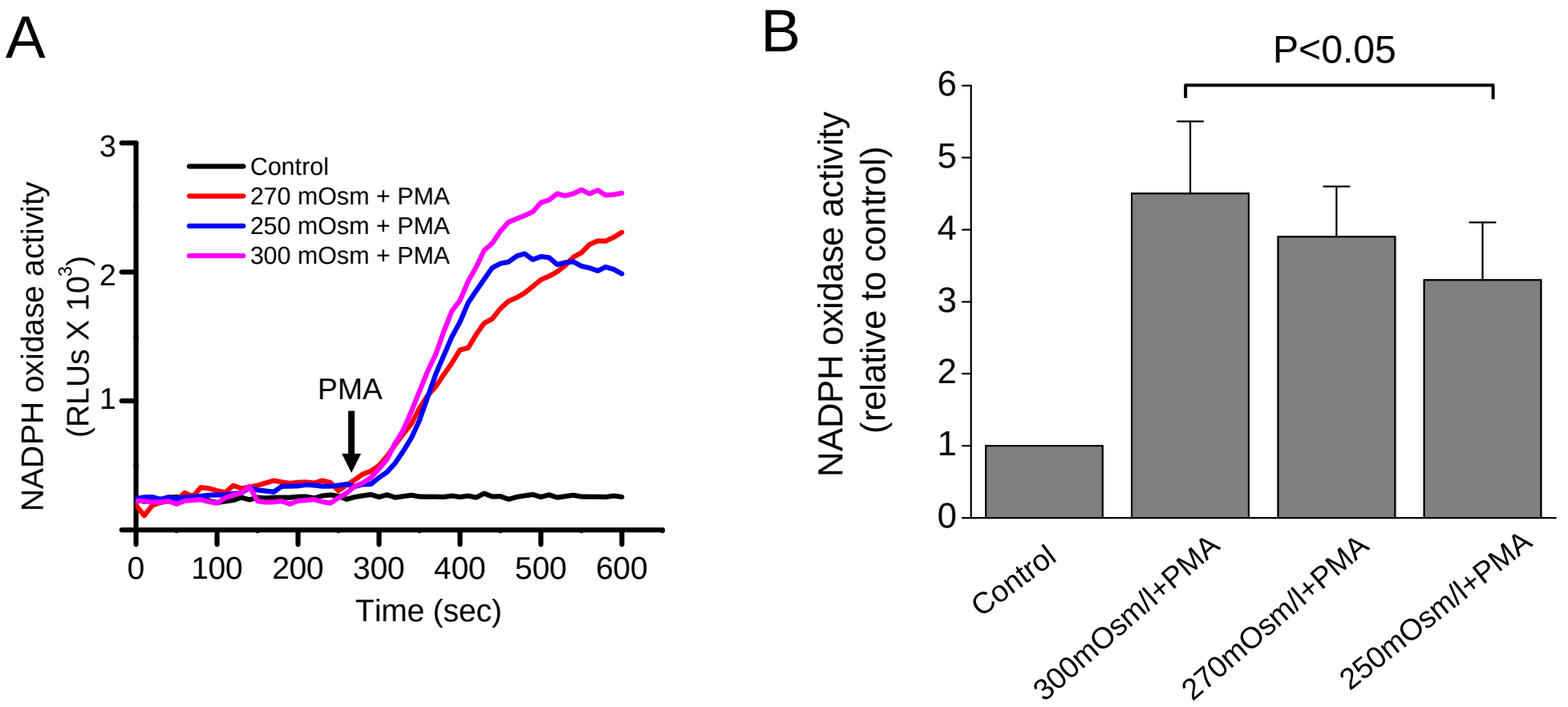

Figure S7. Hypotonic stress reduces the NADPH oxidase activity of human neutrophils. (A) The representative real-time cumulated ROS recordings by the chemiluminescence assay. Neutrophils were pre-incubated with isotonic (300 mOsm/l) or hypotonic (270 or 250 mOsm/l) solutions for 5 minutes prior to 10 nM PMA stimulation. Lucigenin was added before neutrophils were pre-incubated with isotonic or hypotonic solution. The control group was neutrophils pre-incubated with isotonic solution and then without PMA stimulation. (B) Hypotonic stresses mildly to moderately impaired the NADPH oxidase activity of human neutrophil. NADPH oxidase activity is indicated by ROS generation. The ROS production in neutrophils without swelling and PMA stimulation is used as the control and the others are expressed as the relative to the control. Each value represents mean $\pm$ SEM (n=3).

Table S1. The primer sequences of *KCC1*, *KCC3*, and *KCC4* for semi-quantitative PCR and real-time PCR. The shRNA oligonucleotide sequence for knocking down KCC1, KCC3, and KCC4.

Semi-quantitative PCR	
gene	Sequence of primers
KCC1	5’-TGGGACCATTTTCCTGACC-3’ 5’-CATGCTTCTCCACGATGTCAC-3’
KCC3	5’-CTATCCTTGCCATCCTGACC-3’ 5’-GCAGCAGTTGTCACTCG AAC-3’
KCC4	5’-GACTCGTTTCCGCAAAACC-3’ 5’-AGAGTGCCGTGAT GCTGTTGG-3’
Realtime PCR	
KCC1	5’-GTGATCATCTCCATCCTCTCCATCT-3’ 5’- GTTGCCCAGCATGCATACC-3’ Probe: 5’-CCCTCCCGTGTTTCC-3’
KCC3	5’-ACATTGACGTTTGCTCTAAGACCAA-3’ 5’-ACTCGAGTTACAGAAGAATCCCCATA-3’ Probe: 5’-CTGTCATG TTGTTAATTTC-3’
KCC4	5’-TGCGCCTGACGTGGAT-3’ 5’-GGCCACGATGAGGAAGGA-3’ Probe 5’-CCAGGACACCAGCCACC-3’
Oligonucleotide sequence for knocking down KCC1, KCC3 and KCC4	
Target & No.	Sequence of oligonucleotides
shKCC1#5 shKCC1#15 shKCC1#18	5’-CGCCCAAAGGTATCGTCT CTT-3’ 5’-GGACCATTCTGGCCATCATTA-3’ 5’-CCAGAGGAATTGACTACTATG-3’
shKCC3#6 shKCC3#7 shKCC3#8	5’-CGGACATAAGAAAGCTCGAAA-3’ 5’- CCGAAACTCAATGCTACGATT -3’ 5’-CCGGTTTGCTTTGCTTCG ATT-3’
shKCC4#20 shKCC4#21 shKCC4#24	5’-GCCATAGTGACGACGTCTTTC-3’ 5’-TGATCACCATCTACTCCTAAT-3’ 5’- CCCAGTGG TTAGGTTGCATTT-3’
shLuciferase	5’-CAAATCACAGAATCGTCGTAT-3’

Table S2. Hematological indices of *KCC3*$^{+/+}$ and *KCC3*$^{-/-}$ mice.

	KCC3$^{+/+}$	*KCC3* $^{-/-}$
WBC (10^3/mm^3)	5 ± 1.4	9 ± 1.2 *
Neutrophil (%)	34.3 ± 9.9 %	44 ± 5.4 %
Lymphocyte (%)	65.7 ± 9.9 %	56 ± 5.4 %
RBC (10^6/mm^3)	8.2 ± 0.2	9.0 ± 0.2
Hb (g/dl)	12.1 ± 0.2	13.5 ± 0.2
Hct (%)	39.1 ± 0.4	44.5 ± 0.3
MCV (fL)	47.7 ± 0.6	49.4 ± 0.6
MCH (pg)	14.8 ± 0.2	15 ± 0.2
MCHC (g/dl)	30.9± 0.3	30.3± 0.3
RDW-CV (%)	15.8 ± 0.2	14.9 ± 0.2
Platelet (10^6/mm^3)	8.2 ± 0.4	8.7 ± 0.4

WBC, indicates white blood cell count; RBC indicates red blood cell count; Hb indicates hemoglobin; Hct indicates hematocrit; MCV indicates mean cell volume; MCH indicates mean corpuscular Hb; MCHC indicates mean corpuscular hemoglobin concentration; RDW-CV, coefficient of variation of the red cell width; WT indicates wild type.

*$P \leq 0.05$ compared with *KCC3*$^{+/+}$

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