Supplementary Figure legends

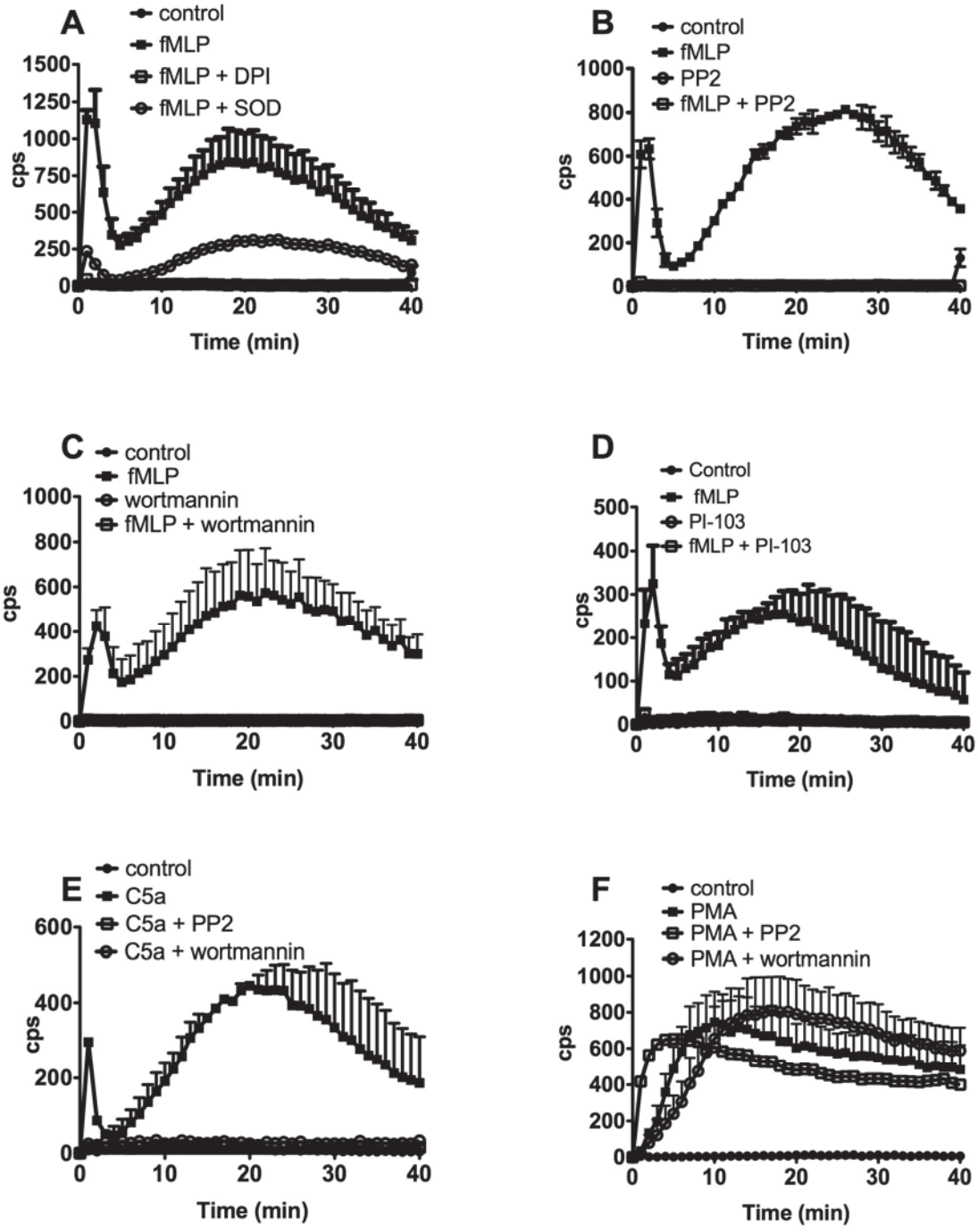
Supplementary Figure 1. **ROS generation by human neutrophils in response to fMLP requires PI3K and SFKs activities.** Human neutrophils suspended in assay buffer containing isoluminol and HRP (see Materials and Methods), in the presence of vehicle (DMSO, <0.1 %), 10 μM PP2, 100 nM wortmannin or 0.5 μM PI-103 were plated in fibrinogen-coated wells and stimulated with 1 μM fMLP (A-D), 1 μM C5a (E) or 20 ng/ml PMA (F). In (A), assays were also run in the presence of 1 μM DPI or 50 U/ml SOD. Chemiluminescence was recorded every one minute and up to forty minutes. Mean results of triplicate assays ± SD of one representative experiment of 4-6 performed is reported.

Supplementary Figure 2. p38 phosphorylation in response to fMLP is not inhibited by PP2 or wortmannin. Human neutrophils were stimulated with 1 μM fMLP in the presence or the absence of PP2 (A) or wortmannin (B) and lysed as described in Figures 1 and 2 legend and Materials and Methods. Lysates were subjected to immunoblot analysis with antibodies of the indicated specificity as described in Materials and Methods. Histograms at the rights of the immunoblots report densitometric analysis, expressed as ratio between the phosphoprotein versus the total ERK protein signal. Mean results ± SD of 3 independent experiments are reported.

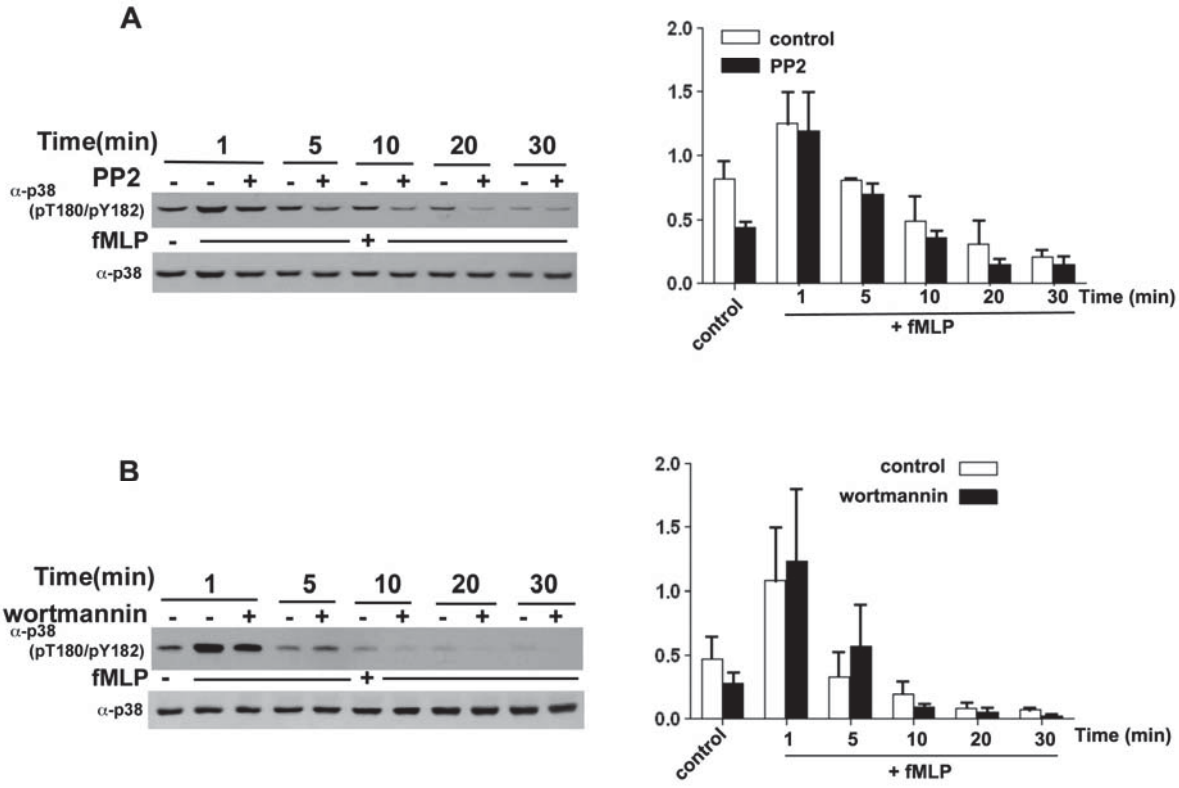
Supplementary Figure 3. TNF-induced ROS generation and AKT activation require both PI3K and SFK activities. (A-B) Human neutrophils were plated in fibrinogen-coated wells and stimulated with 10 ng/ml TNF in the absence or the presence of 10 μM PP2 (A) or 100 nM wortmannin (B). Chemiluminescence was recorded every one minute and up to forty minutes. Mean results ± SD of 3-5 independent experiments are reported. (C-E), Neutrophils were stimulated as in A-B and, after different times of incubation, cells were lysed and lysates subjected to immunoblot analysis with antibodies of the indicated specificity as described in Materials and Methods. Histograms at the right of the immunoblots report densitometric analysis expressed as ratio between the phosphoprotein versus the total specific protein signal. One representative experiment of two performed with identical results is reported. (F-G) Wild type, PI3Kγ-/- or *hck-/-fgr-/-lyn-/-* murine neutrophils were left untreated or stimulated with 5 ng/ml TNF, and lysed at the time points indicated. After different times of

incubation, cells were lysed and lysates subjected to immunoblot analysis with antibodies of the indicated specificity as described in Materials and Methods. Histograms at the right of the immunoblots report densitometric analysis, expressed as ratio between the phosphoprotein versus the total protein signal. One representative experiment of 2-3 performed with identical results is reported.

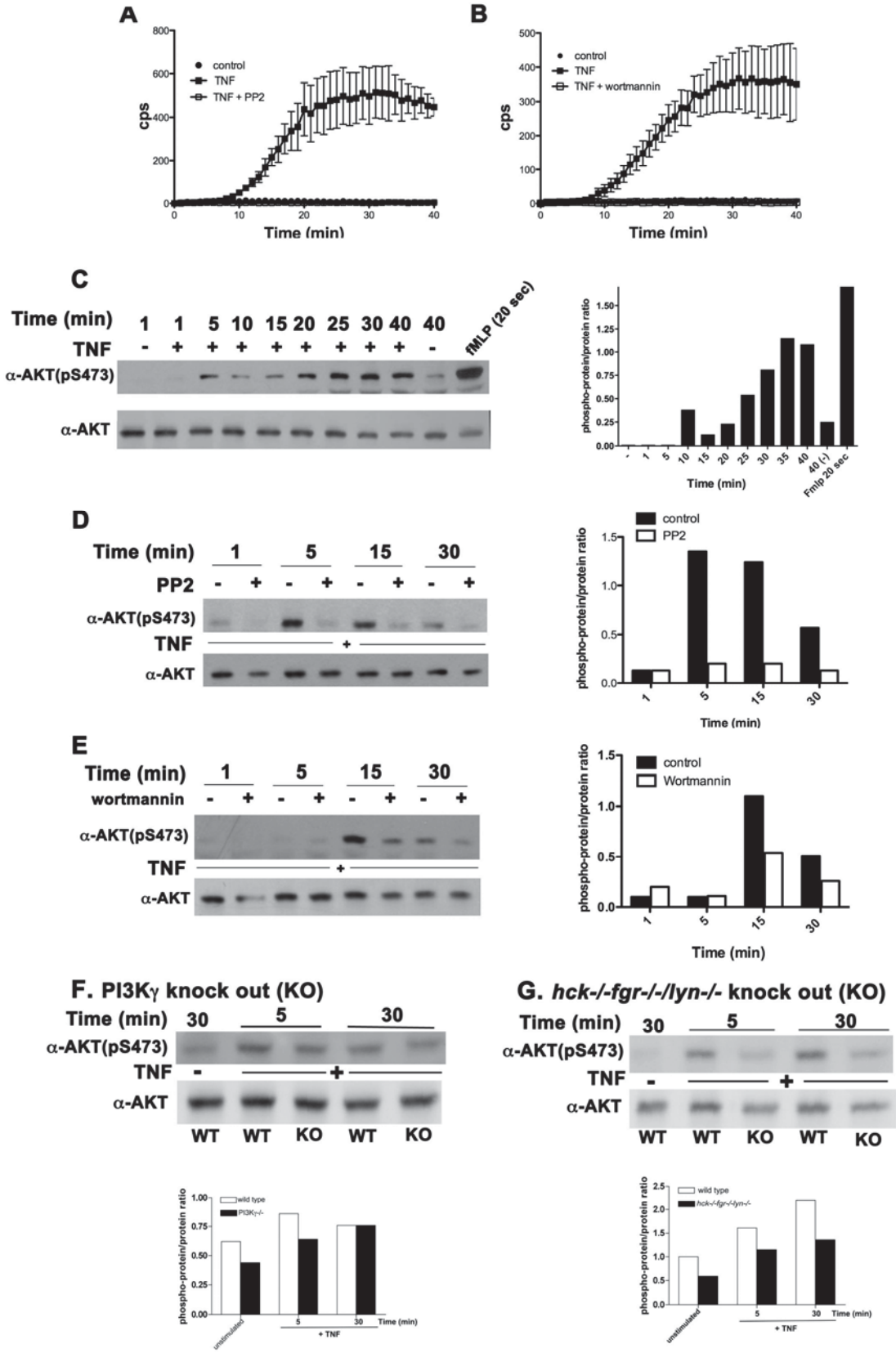
Supplementary Figure 4. Class IA PI3K inhibitors suppress human neutrophil spreading. Human neutrophils were isolated as described in *Materials and Methods*, and plated in tissue culture plastic wells in the absence of any stimulus or in fibrinogen-coated wells in the presence of 10 ng/ml TNF. Cells were left untreated or incubated with 100 nM wortmannin, 0.1 μM Compound 15e, 0.5 μM IC87114 or 5 μM AS604850. Photos were taken with a 40x objective after 30 minutes on plastic and after 40 minutes on fibrinogen in the presence of TNF. On plastic or on fibrinogen plus TNF, respectively, per cent of spread cells was: control, 62 or 74%; plus wortmannin, 26 or 3%; plus Compound 15e, 19 or 2%; plus IC87114, 25 or 4 %; plus AS604850, 74 0r 45%. One reprsentative experiment of 2-3 performed with comparable results is shown.



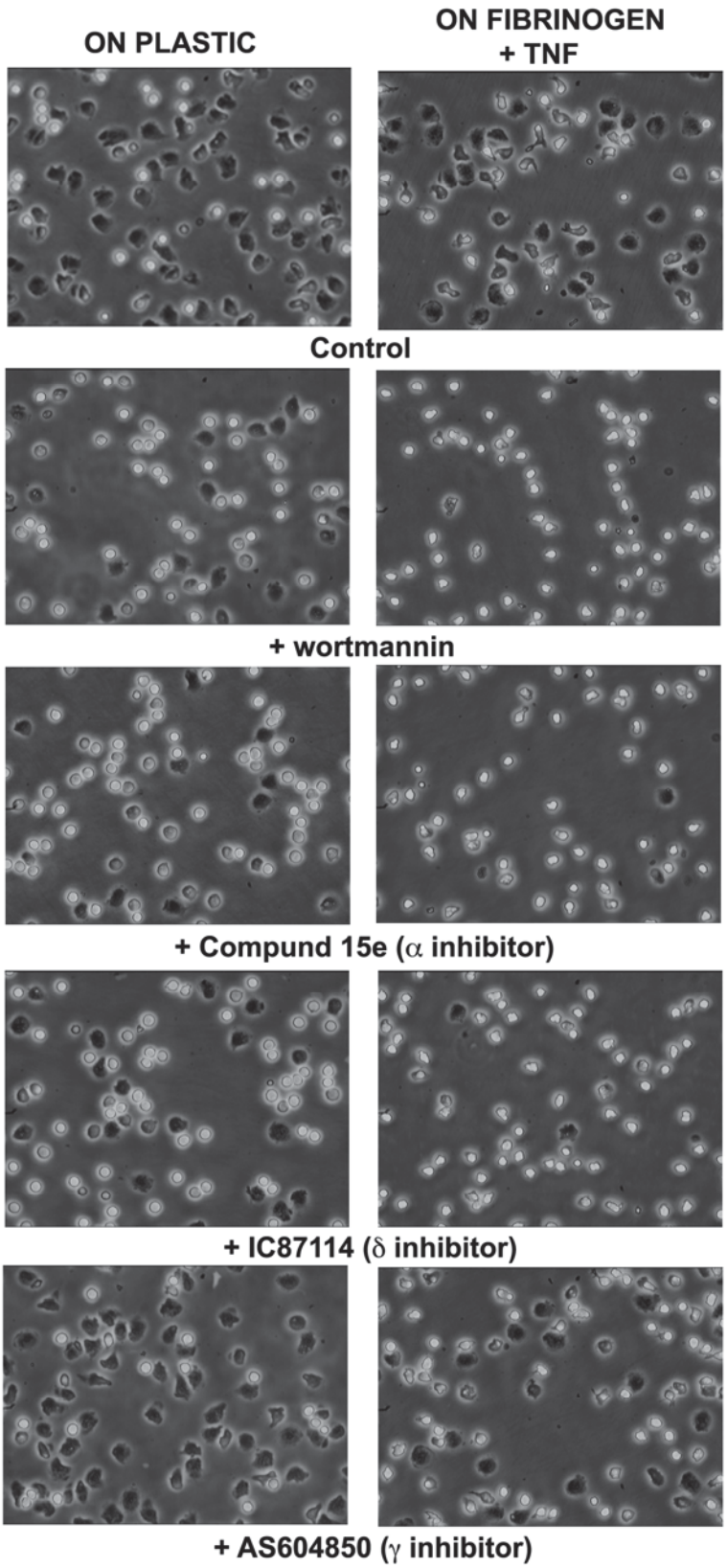
SUPPLEMENTARY FIGURE 1



SUPPLEMENTARY FIGURE 2



SUPPLEMENTARY FIGURE 3



SUPPLEMENTARY FIGURE 4