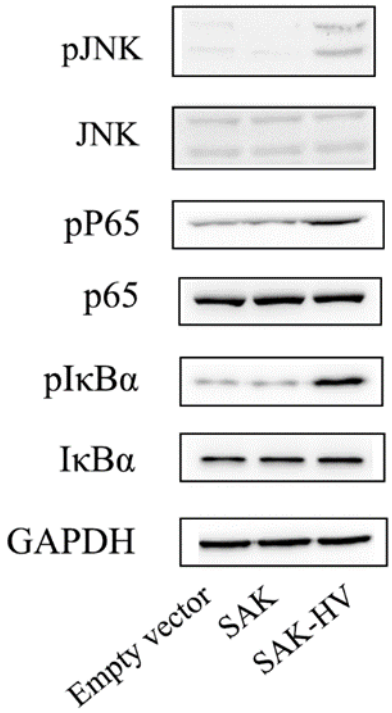
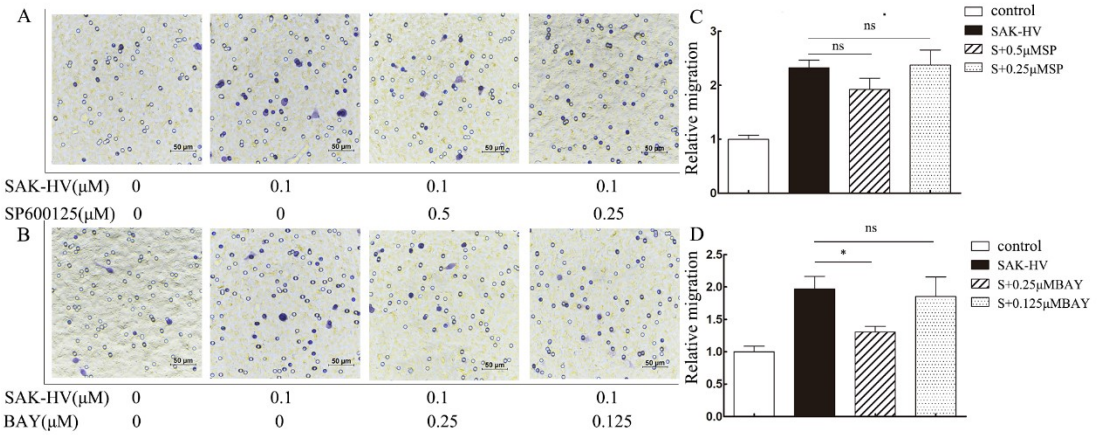
Since SAK-HV is produced in E. coli, it is necessary to control the experiments with a similarly produced protein. Thus, we used the eluant of empty vector, SAK and SAK-HV to treat cells seperately, and detected their effects on the activation of the key JNK and NF-κB signal pathways. The results showed that JNK and NF-κB signal pathways were not activated by the eluant of empty vector and SAK (shown in supplementary materials: Supplementary Figure 1). Therefore, the migration-promoting effect was resulted from SAK-HV, but not the impurities in eluant.

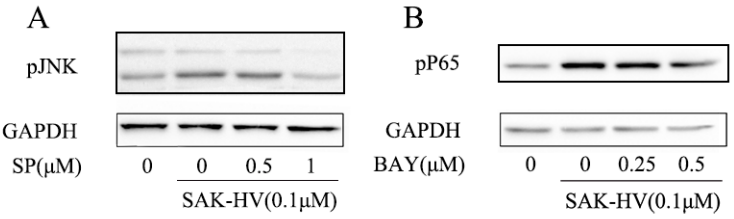
We adopted the doses of 1μM SP600125 and 0.5μM BAY11-7082 in Figure 6 and Figure 8, which might be explained as following. Two lower concentrations of SP600125 (a JNK inhibitor) and BAY11-7082 (a NF-κB inhibitor) were adopted to test the effects of them separately on migration triggered by SAK-HV. As is shown in Supplementary Figure 2, only 0.25μM of BAY reduced the migration stimulated by SAK-HV, whereas 0.125μM of BAY11-7082 could not inhibit migration. Although the migration rate of 0.5μM and 0.25μM of SP600125 treated group presented a tendency of decrease, there were no significant difference compared with the SAK-HV group, indicating that lower doses of inhibitors is not suitable. In addition, Western blot analysis showed that 1μM of SP600125 could attenuate phosphorylation of JNK induced by SAK-HV, but lower concentration had little effect (Supplementary Figure 3). Thus, 1μM of SP600125 was used for further experiments. The doses of other inhibitors were determined in the same way. Taken together, the doses of inhibitors were proper in this manuscript.



Supplementary Figure 1. The effects of eluant of empty vector, SAK and SAK-HV treatment on migration-relative pathways (JNK, and NF-κB) in RAW264.7 cells at 12h time points. Western blot analysis for the JNK, and NF-κB pathways (n=3). The phosphorylation levels of JNK, and NF-κB pathways were upregulated with SAK-HV (1μM) treatment for 12h compared with equal amounts of eluant of empty vector, and SAK(1μM) treatment.



Supplementary Figure 2. SAK-HV-promoted macrophage migration was inhibited by 0.25μM BAY11-7082, but was not affected by lower concentration of BAY11-7082 or SP600125. (**A, B**) The effects of JNK inhibitor SP600125(0.5μM, 0.25μM), and NF-κB inhibitor BAY11-7082(0.25μM, 0.125μM) on the SAK-HV-triggered (0.1μM) migration (n = 3) respectively. The migration was analyzed using the transwell assay, and cells were photographed after SAK-HV and inhibitors treatment. Migration induced by SAK-HV could be inhibited by 0.25μM BAY11-7082, not by 0.125μM BAY11-7082 or SP600125. (**C, D**) The quantification for transwell assay. Abbreviation: SP, SP600125. BAY, BAY11-7082. ** *P*<0.01 versus SAK-HV group, *** *P*<0.001 versus SAK-HV group. ns, no significance.



Supplementary Figure 3. The effects of different doses of SP600125 and BAY11-7082 treatment in the presence of SAK-HV on migration-relative pathways (JNK and P65) in RAW264.7 cells at 12h time points. Western blot analysis of JNK and P65 phosphorylation levels(n=3). The phosphorylation levels of JNK and P65 stimulated by SAK-HV(0.1μM) were inhibited by 1μM SP600125 and 0.5μM BAY11-7082 treatment, while lower concentration of inhibitors had little effect on them. Abbreviation: SP, SP600125. BAY, BAY11-7082.