

Figure S1 – The find of FAM3D in a PBMC chemoattractant platform and FAM3D can not chemoattract HEK293-FPR3 cells.

A The chemoattractant activity of supernatant of HEK293T cells transfected with pcDB-FAM3D or other plasmid of our lab toward PBMC. The 198# represented FAM3D.

B The chemoattractant activity of HEK293-FPR3 cells toward FAM3D. The chemoattractant effects of various concentrations of recombinant FAM3D or WKYMVm (0.1, 1, 10, 100 nM) were evaluated.

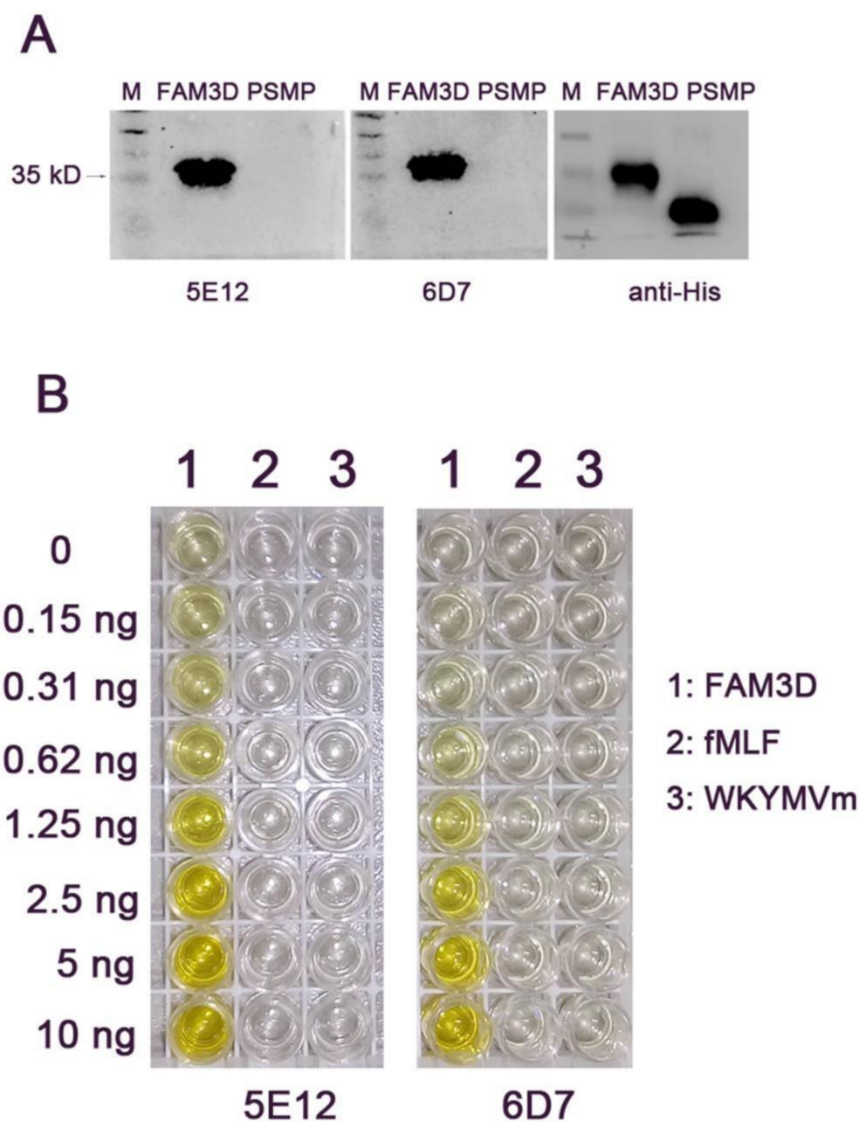


Figure S2 – The mouse monoclonal antibodies of FAM3D, 5E12 and 6D7, recognize FAM3D specifically.

A Both 5E12 and 6D7 could recognize FAM3D (10 ng) specifically, but not PC3-Secreted Microprotein (PSMP) (10 ng) in western blot. Both the recombinant protein of FAM3D and PSMP had the “His” label, the antibody of His could recognize both of them.

B In the direct ELISA experiment, both 5E12 and 6D7 could recognize FAM3D in concentration gradient of FAM3D specifically, but not fMLF or WKYMVm.

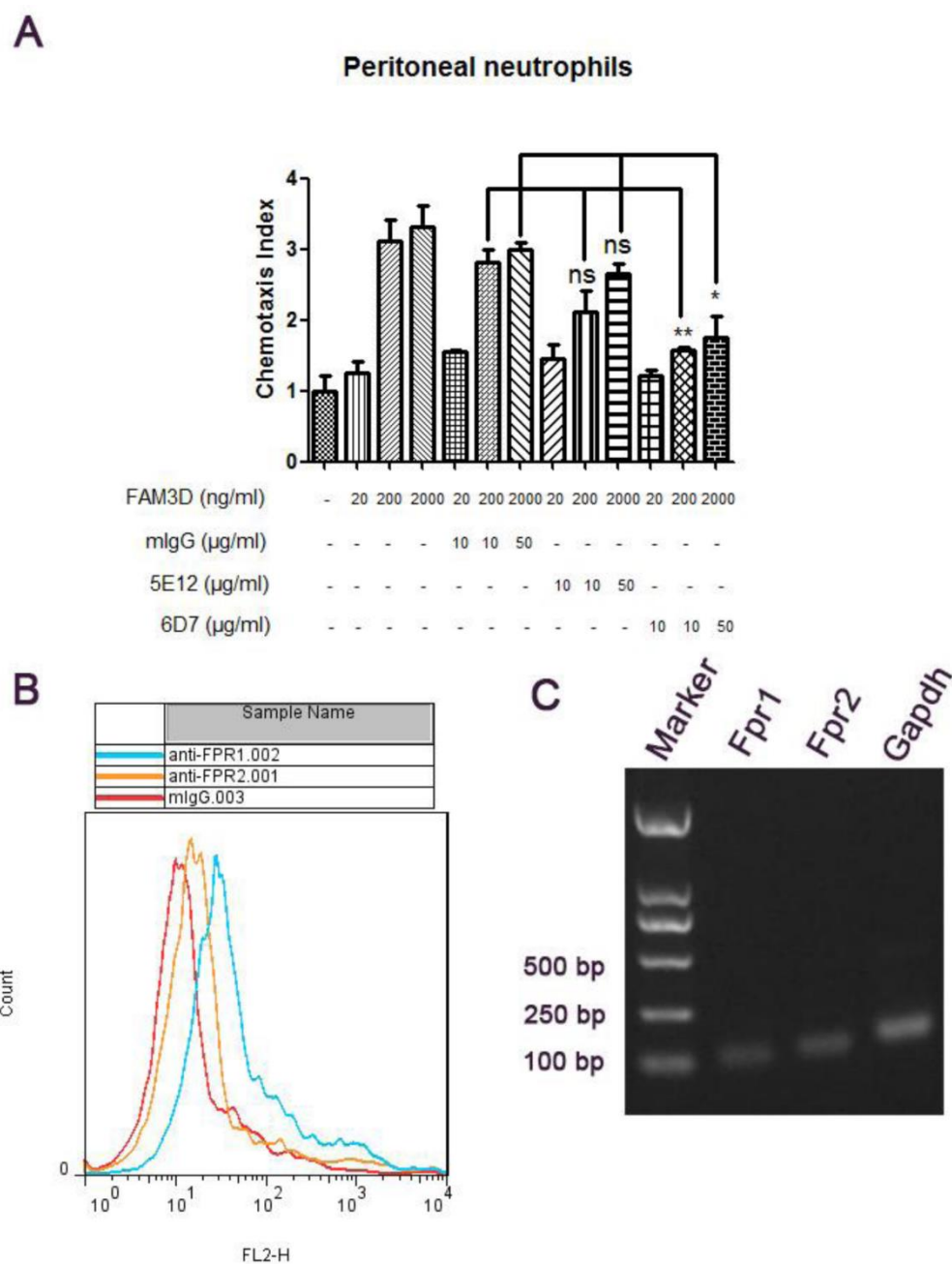


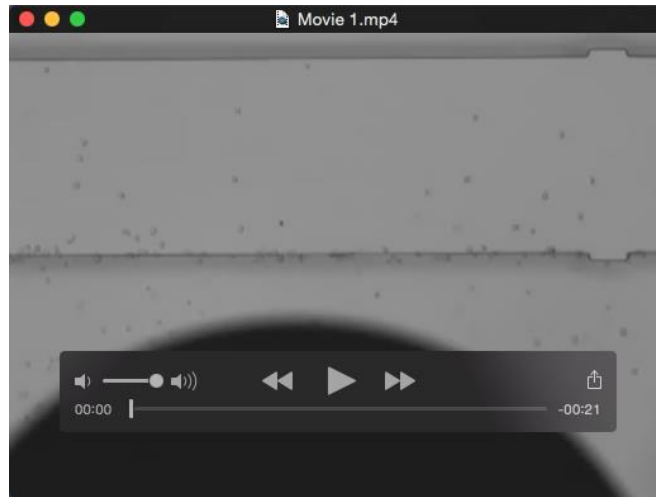
Figure S3- The mouse monoclonal antibody of FAM3D, 6D7 can block the chemotaxis of mouse neutrophils to FAM3D completely.

A Mouse monoclonal antibodies targeting FAM3D (5E12 and 6D7) or mouse IgG were applied in the chemotaxis assay. FAM3D (20, 200, or 2000 ng/ml) was pretreated

with 5E12 or 6D7 or mIgG (10 or 50 $\mu\text{g/ml}$) for 30 min, and the chemotaxis index was calculated for peritoneal neutrophils. $*0.01 < P < 0.05$, $**0.001 < P < 0.01$, and $***P < 0.001$.

B Flow-cytometric analysis using mouse anti-FPR1, mouse anti-FPR2 or mouse IgG as the primary antibody, the PE anti-mouse IgG as the secondary antibody, to detect the expression of receptors on the surface of peritoneal neutrophils.

C The expression of Fpr1 and Fpr2 of peritoneal neutrophils by RT-PCR. We use the same condition to amplify them with 35 cycles.



Movies 1–3. Mouse neutrophils migrating directionally towards the chemoattractant are observed using the TAXIScan-FL Cell dynamic analysis system from Japan. 10

nM WKYMVm is a positive control. In the experiment, we set the peritoneal neutrophils in one side, medium control, 10 nM FAM3D, or 10 nM WKYMVm in the other side. FAM3D and WKYMVm were diluted in RPMI 1640 medium supplemented with 0.5% BSA. The distance from the cells toward chemotactic agent is 24 μ m. Cells migrating from the side to the protein staying side can be seen. Movie 1 shows the cells dynamic change of medium control, Movie 2 shows FAM3D 10 nM, Movie 3 shows WKYMVm 10 nM.