Supplementary information

Nano-immunotraining strategy to enhance tumor targeting of neutrophils through in vivo pathogen-mimicking stimulation

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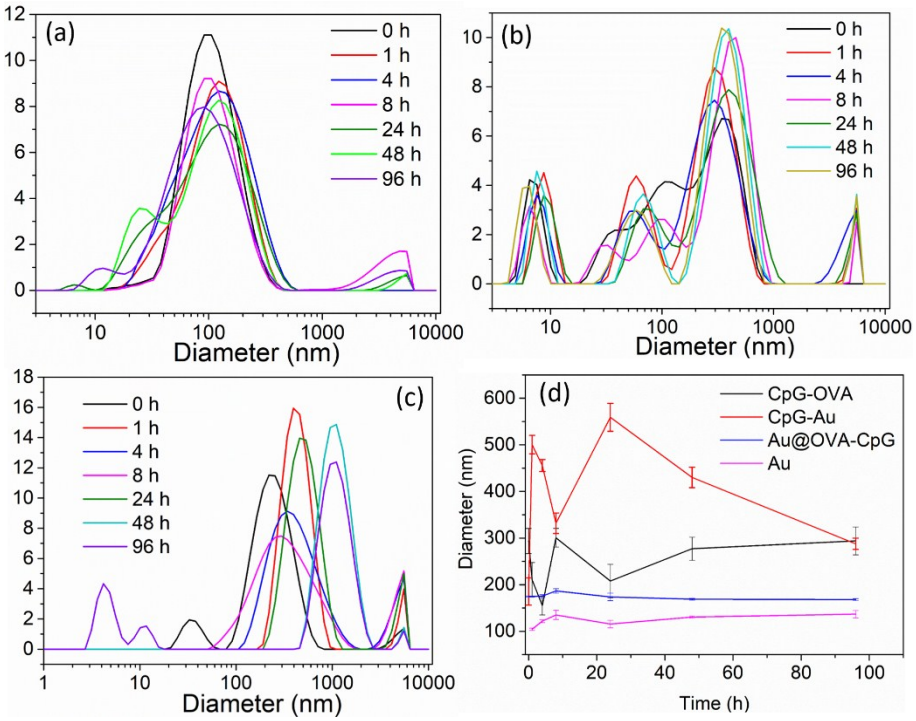
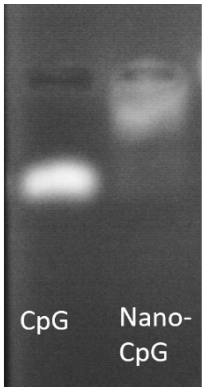
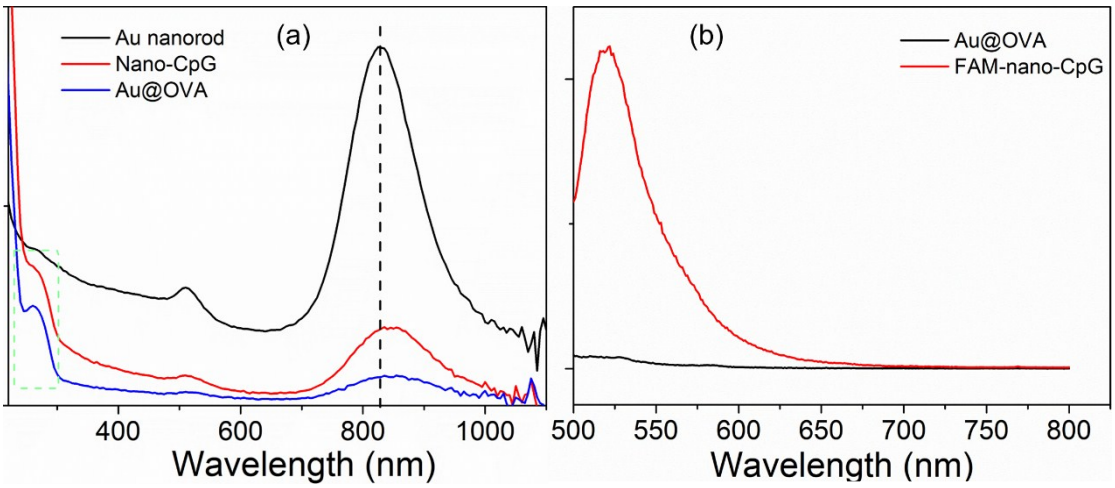


Supplementary Figure 1. The size variation of a) nano-CpG (Au@OVA-CpG) , b) CpG-OVA, c) CpG-Au suspension in vitro monitored by DLS; d) the plot of diameter changes versus time for above three samples and pure Au nanorods.

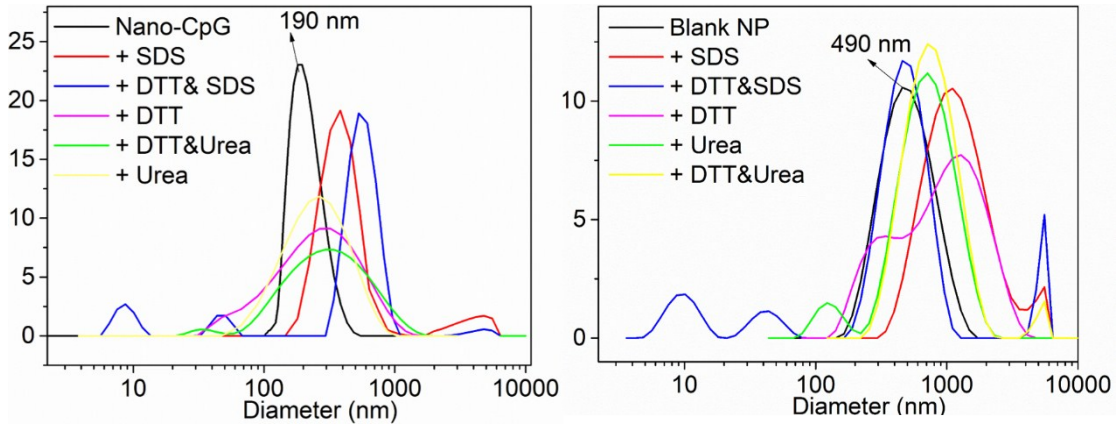


Supplementary Figure 2. The agarose gel electrophoretic analysis of Nano-CpG compared with free CpG. To guarantee the equal CpG amount, no ultrafiltration treatment was performed after preparation of Nano-CpG.

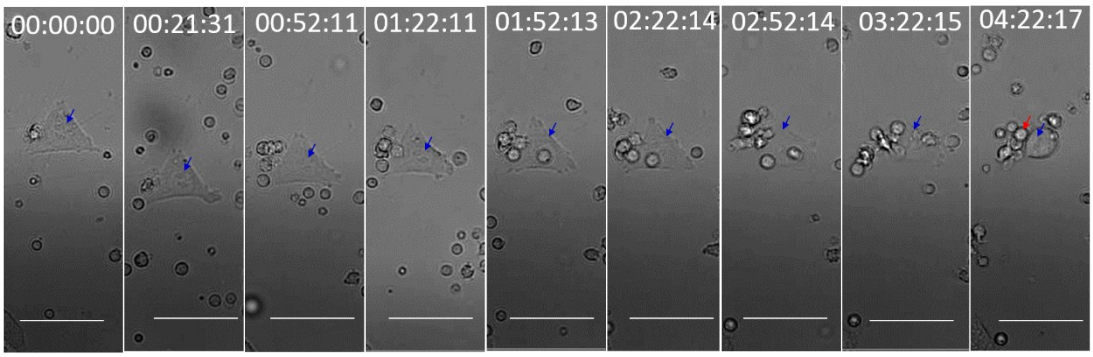


Supplementary Figure 3. a) UV – vis spectra of Au nanorods, Au@OVA, and nano-CpG in water b) Fluorescence spectra of FAM-nano-CpG and Au@OVA in water.

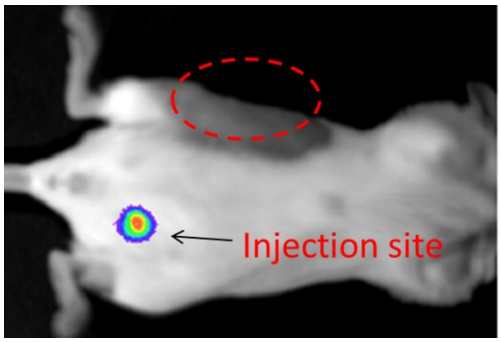
Supplementary Figure 4. The alteration of Z-average hydrodynamic diameters of



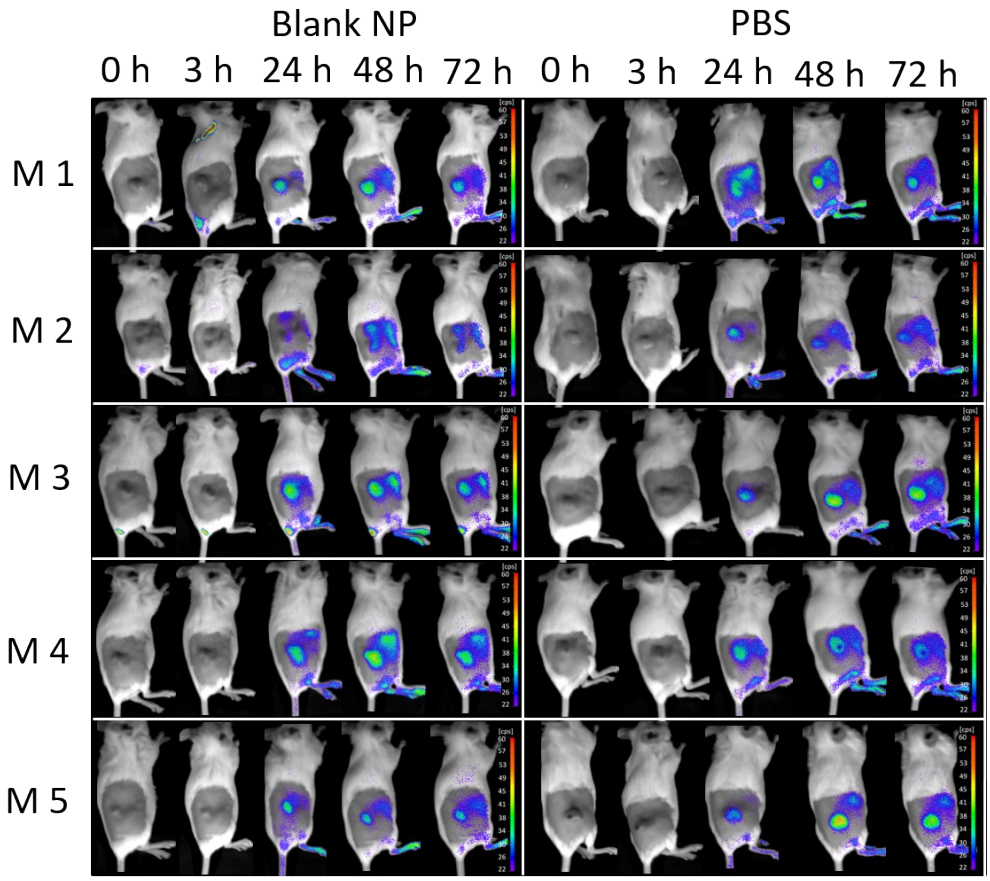
nano-CpG and nanohybrid without CpG (Blank NP) in the presence of 30 $\times 10^{-3}$ M DTT (destroyer of disulfide bond), 1% SDS(destroyer of hydrophobic interaction) and 8.0 M urea (destroyer of hydrogen bond). Upon addition of above destroyer alone or in combination, size alteration of nano-CpG was monitored by DLS. Evidently, DTT & SDS combination can dissipate the nano-CpG heavily rather than DTT & Urea combination or DTT, SDS, urea alone.



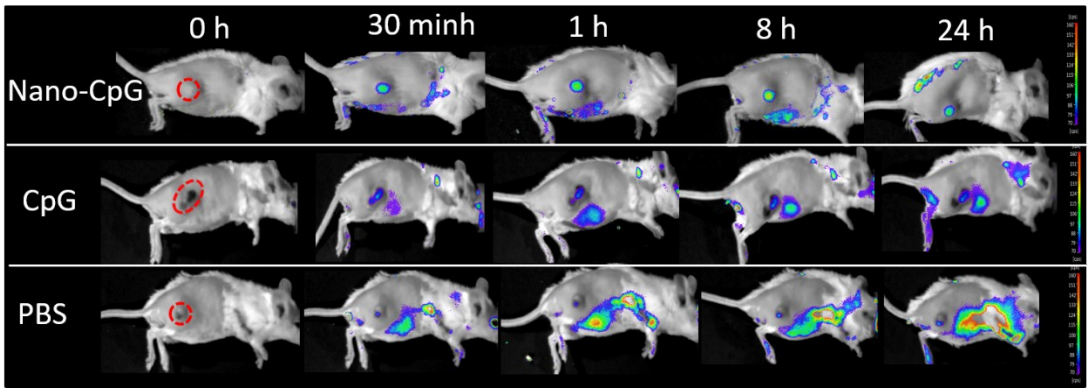
Supplementary Figure 5. Typical migration behavior of neutrophils to tumor cell within 5 h captured by BioTeK Lionheart FX automated live cell imager (neutrophil: small round cell as red arrow pointed; 4T1 cell: blue arrow pointed). After the 4T1 cells adherence, neutrophils were seeded with number ratio of neutrophil to tumor cell of 10:1. The scale bar is 50 μm.



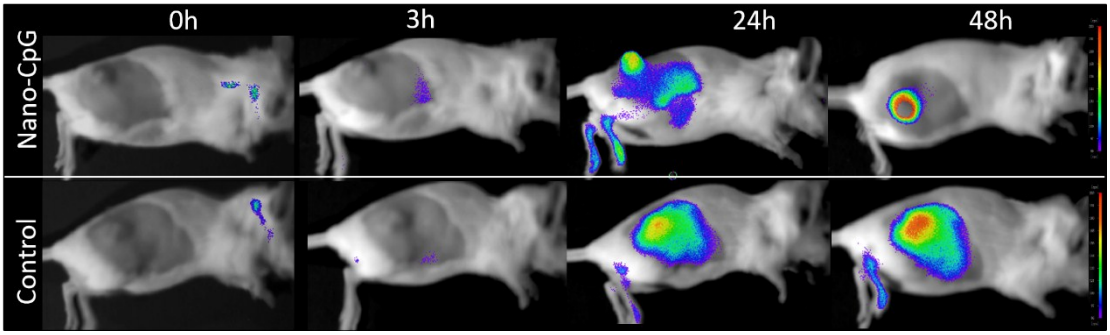
Supplementary Figure 6. Intraperitoneally injection site locates at the opposite side of the tumor in red circled depilatory area.



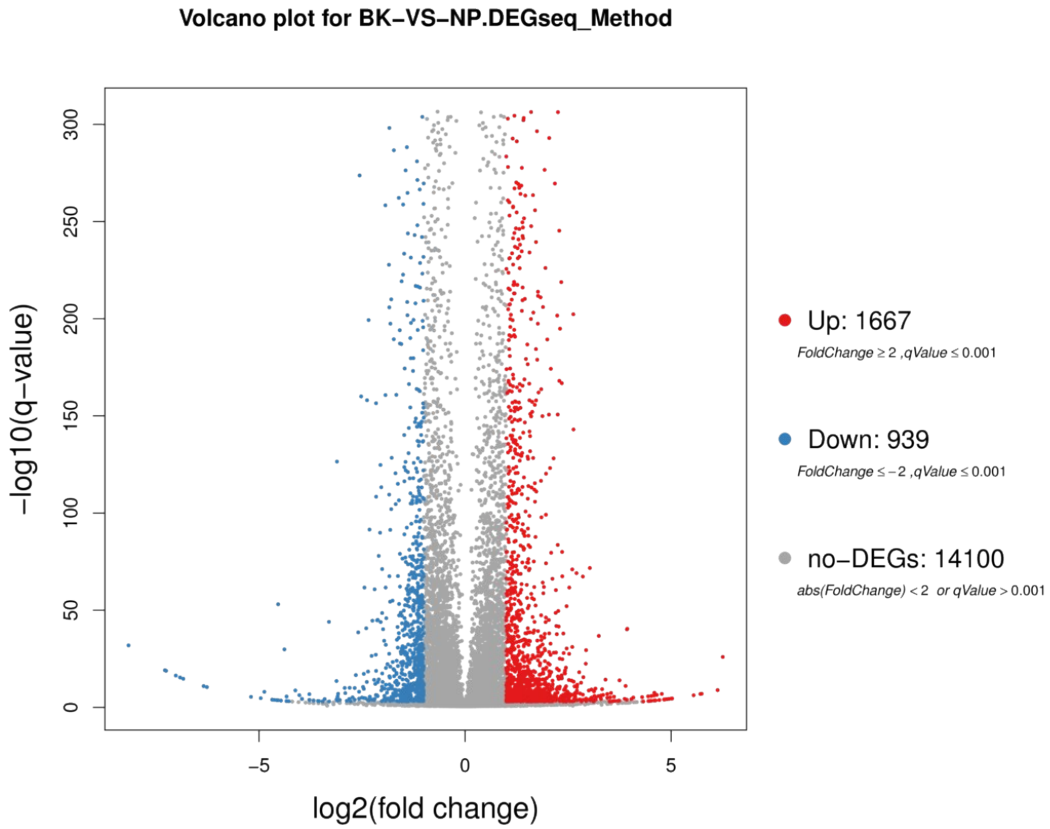
Supplementary Figure 7. In vivo fluorescence imaging of 4T1-bearing BALB/c mice intraperitoneally injected DiR-labeled neutrophils at 0 h, 3 h, 24 h, 48 h, 72 h post-injections. (n=5, i.e. M1, M2, M3, M4, M5 for each group of Blank NP and PBS).

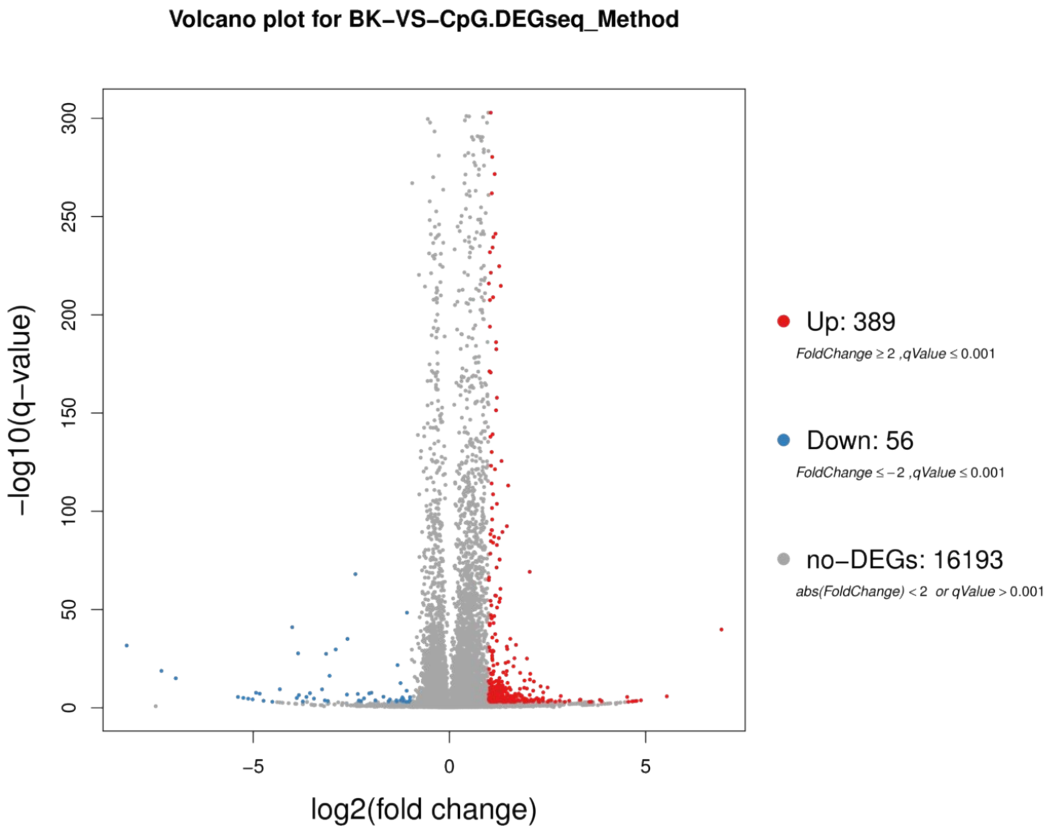


Supplementary Figure 8. Typically in vivo fluorescence imaging of intravenously injected DiR-labeled neutrophils, harvesting from different BALB/c mice groups of Nano-CpG, CpG and PBS, in 4T1-bearing BALB/c mice at 0 h, 30 min, 1 h, 8 h，24 h post-injections. Red circle indicates the location of tumor site.

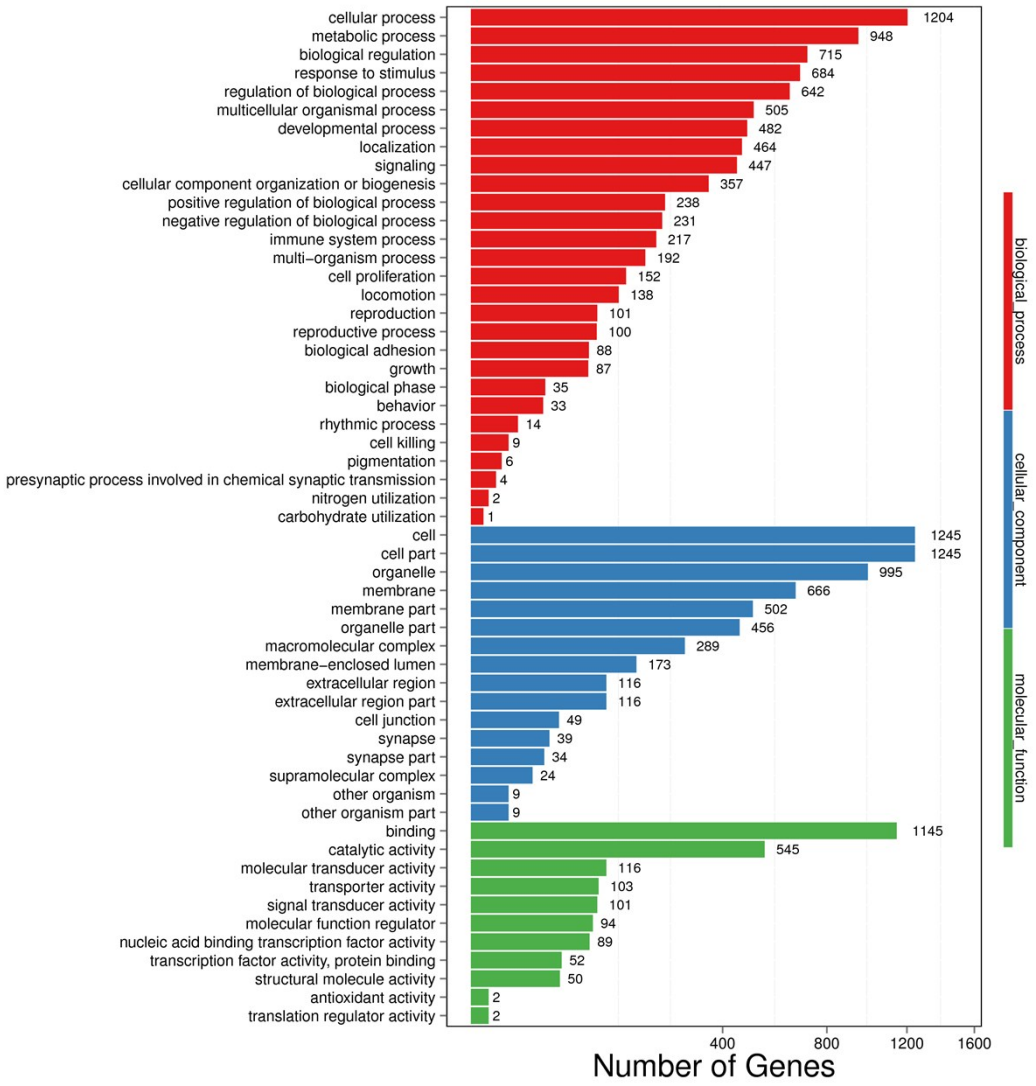


Supplementary Figure 9. Typical in vivo fluorescence images of CT26-bearing BALB/c mice intraperitoneally injected DiR-labeled neutrophils that harvested from Nano-CpG groups and PBS control mice groups at 0 h, 3 h, 24 h, 48 h post-injections.

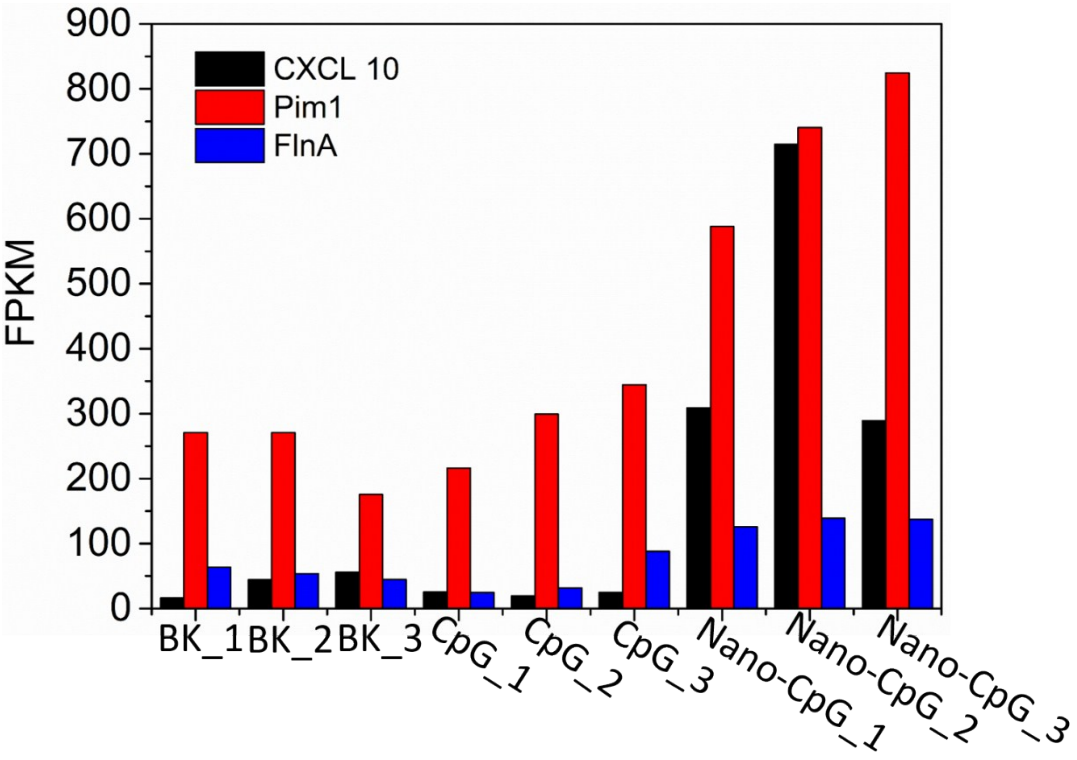




Supplementary Figure 10. Volcano plots for the neutrophil harvested from different treated mice groups, down with blue color and up with red color.



Supplementary Figure 11. Typical GO classification of DEGs



Supplementary Figure 12. Typical genes variation in pathway of immune response for different mice groups, FPKM denotes fragments per kilobase million.