Supplementary data 2: Cell viability data

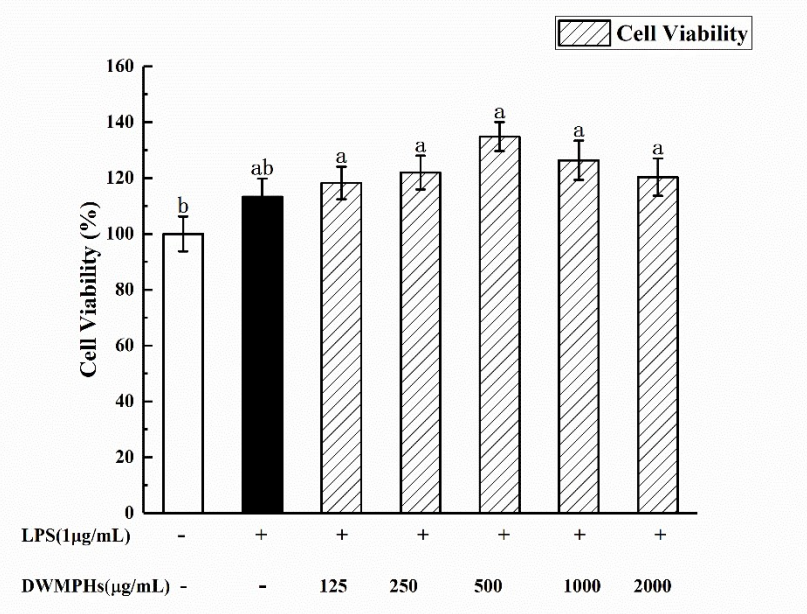

Fig. S10. Effects of different concentrations of DWMPHs on the activity of RAW264.7 cells. Values are means ± SD of three determinations. Different letters (a-b) are means that are significantly different (P<0.05).

The effects of different concentrations of complex protease defatted walnut meal protein hydrolysates (DWMPHs) on RAW264.7 cell viability are as shown in Fig. S10. The cell viability did not decrease but slightly increased under the stimulation of LPS, indicating that the presence of LPS didn't affect the cell viability. The cell viability was higher than the blank group when the hydrolysate concentrations were 0~2000 μg/mL, and with the increase of the concentration, the cell viability showed a trend of increasing first and then decreasing. The maximum activity was observed at concentration of 500 μg/mL, indicating no toxicity to RAW264.7 cells when the concentration of enzymatic hydrolysate was lower than 2000 μg/mL. Therefore, no inhibition on cell viability was observed at hydrolysate concentrations up to 2000 μg/mL in LPS-induced RAW264.7 cells.

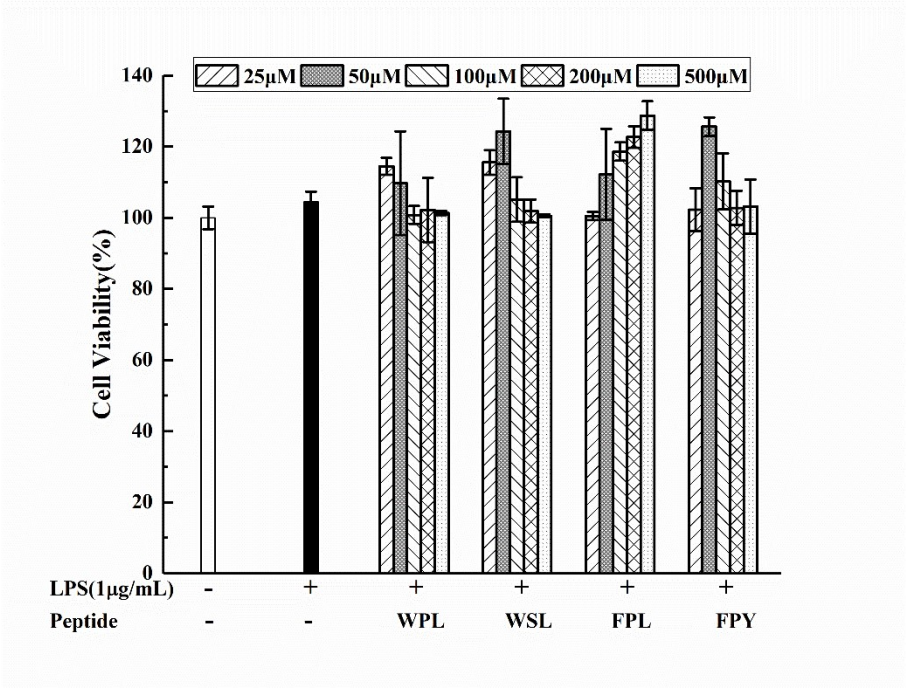

Fig. S11. Effects of four synthetic peptides on the activity of RAW264.7 cells. WPL, (Trp-Pro-Leu),WSL, (Trp-Ser-Leu), FPL, (Phe-Pro-Leu) and FPY, (Phe-Pro-Tyr).

The safety of four synthetic peptides WPL, WSL, FPL and FPY was explored, as shown in Fig. S11. Four synthetic tripeptides can promote cell proliferation in different degree at the tested concentrations (0~500 μM), thus, the peptides did not exhibit any cytotoxic effect on RAW264.7 macrophages.

The effects of WSL and FPY on cell viability was inverted U-shaped with the increase of concentration, and the cell viability was the highest at the concentration of 50 μM, reaching 124.34±9.18% and 125.62±2.63%, respectively. The cell viability decreased gradually with the increase of WPL concentration, but it was still higher than that of blank group. The cell viability was the lowest at the concentration of WPL was 500 μM,, which was 101.34±0.58%. On the contrary, the cell viability increased gradually with the increase of the concentration of FPL, and reached the maximum value of 128.77±3.99% at the concentration of 500 μM.

Therefore, none of the four tripeptides was toxic at concentrations ranging from 0~500 μM and could be used for further determination of anti-inflammatory activity.