

Supplementary information

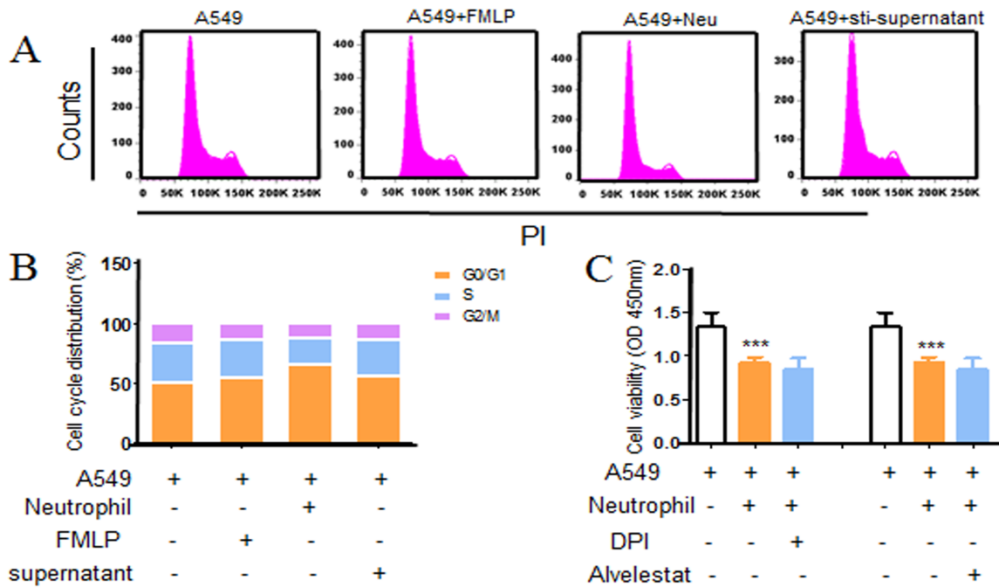


Figure S1 The effect of degranulation, respiratory burst or NETs on neutrophil anti-tumor activity. Challenged with FMLP-stimulated neutrophil supernatant, A549 cells cell cycle was evaluated by flow cytometry (A, B). Neutrophil antitumoral effect was assayed in the presence or absence of DPI and alvelestat at 20:1 E:T ratios using A549 as target cells (C). Data are representative of four independent experiments. Mean and SD are presented. Statistical analysis was performed by one way ANOVA; *** $P < 0.001$, compared with control group.

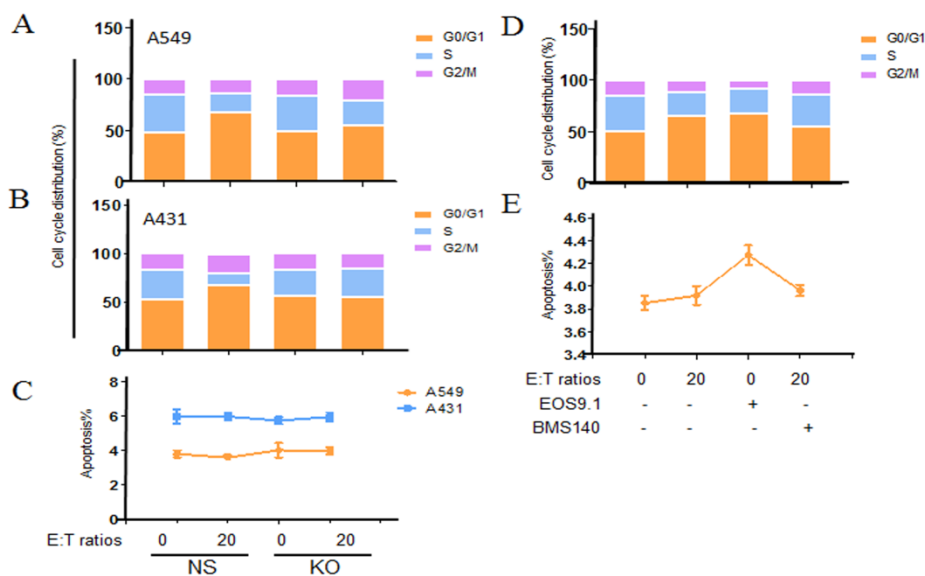


Figure S2 The effect of Fas agonist, antagonist and Fas knock-out tumor cells on neutrophils

antitumoral activity. Fas was knock out in A549, A431 cells. The relevant empty lentivectors were used to induce control nonspecific (NS) expressing cells. Knock out tumor cells co-incubated with neutrophils for 24 hours at E:T ratio of 20:1, cell cycle and apoptosis of tumor cells were examined (A, B, C). A549 cancer cells co-incubated with human neutrophils from healthy donors in the presence or absence of the Fas antagonist and agonist at effector to target (E:T) cell ratio of 20:1. Cell cycle and apoptosis of A549 cells were detected by flow cytometry (D, E). Data are representative of four independent experiments. Mean and SD are presented. Statistical analysis was performed by one way ANOVA; *** $P < 0.001$, compared with control group.

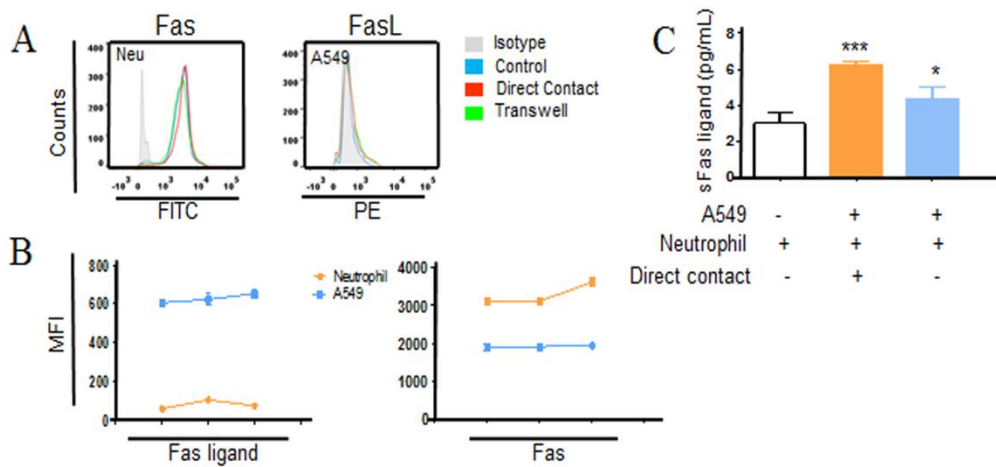


Figure S3 The expression of Fas and Fas ligand on neutrophils or tumor cells in co-culture condition. A549 cancer cells co-incubated with human neutrophils from healthy donors in the presence or absence of the Fas antagonist and agonist at effector to target (E:T) cell ratio of 20:1. Fas L and Fas expression level were further examined by using flow cytometry (A, B) and ELISA (C). Data are representative of four independent experiments. Mean and SD are presented. Statistical analysis was performed by one way ANOVA; *** $P < 0.001$, ** $p < 0.01$, * $p < 0.05$, compared with control group.

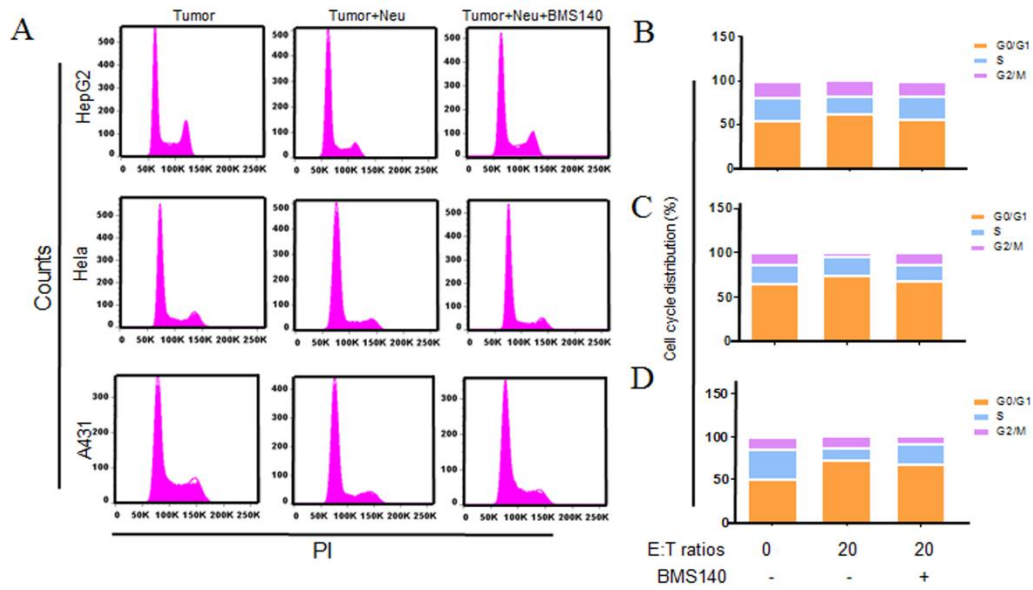


Figure S4 Neutrophils antitumoral activity on A431, Hela and HepG2. Neutrophil antitumor activity was assayed by co-incubating A431, Hela and HepG2 cancer cells with human neutrophils from healthy donors at effector to target (E:T) cell ratio of 20:1. Cells were incubated at 37°C in a humidified 5% CO₂ incubator for 24h. In the presence or absence of Fas antagonist, the tumor cells cell cycle was assessed by flow cytometry (A, B, C, D). Data are representative of four independent experiments. Mean and SD are presented. Statistical analysis was performed by one way ANOVA; *** $P < 0.001$, compared with control group.