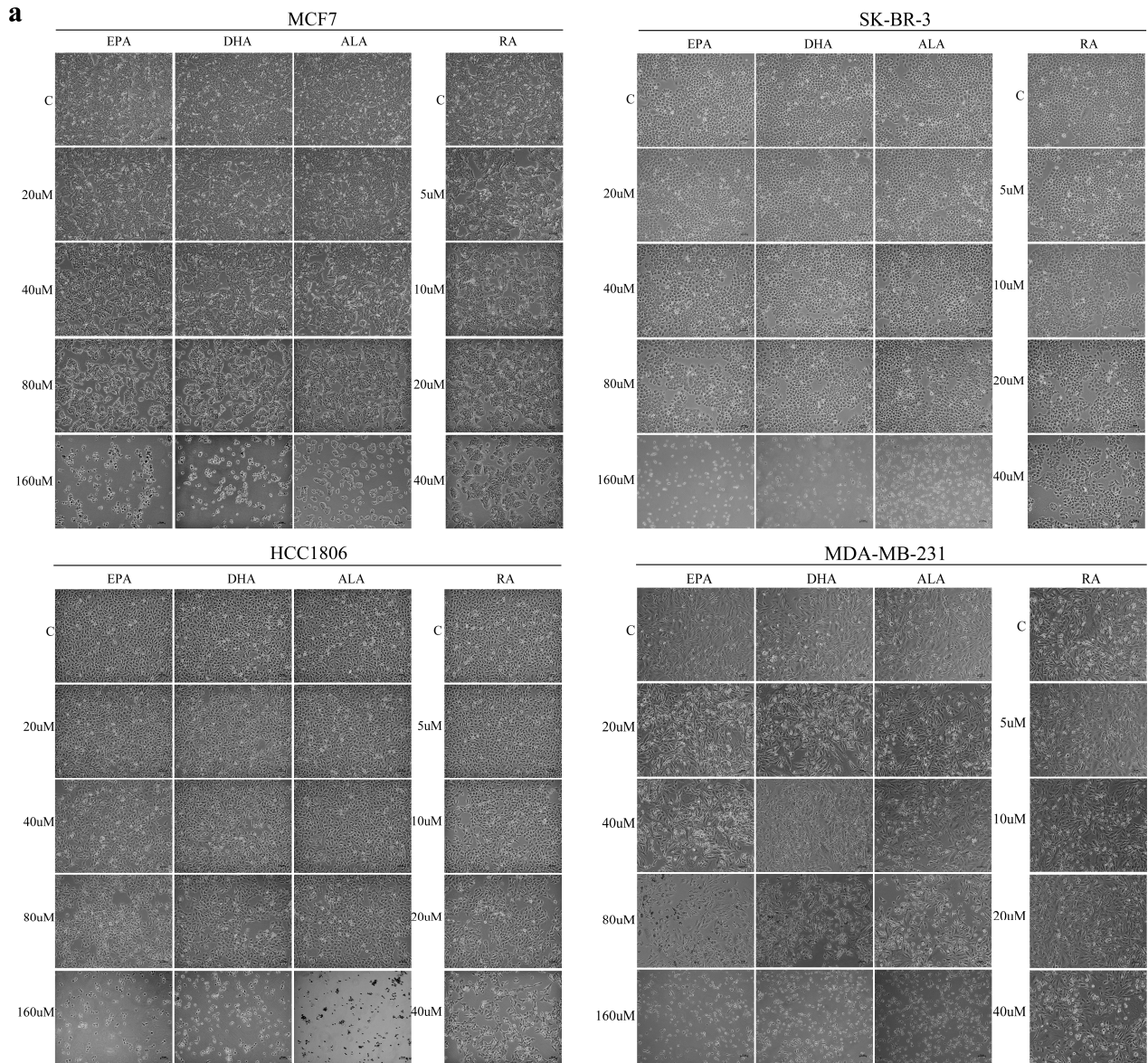


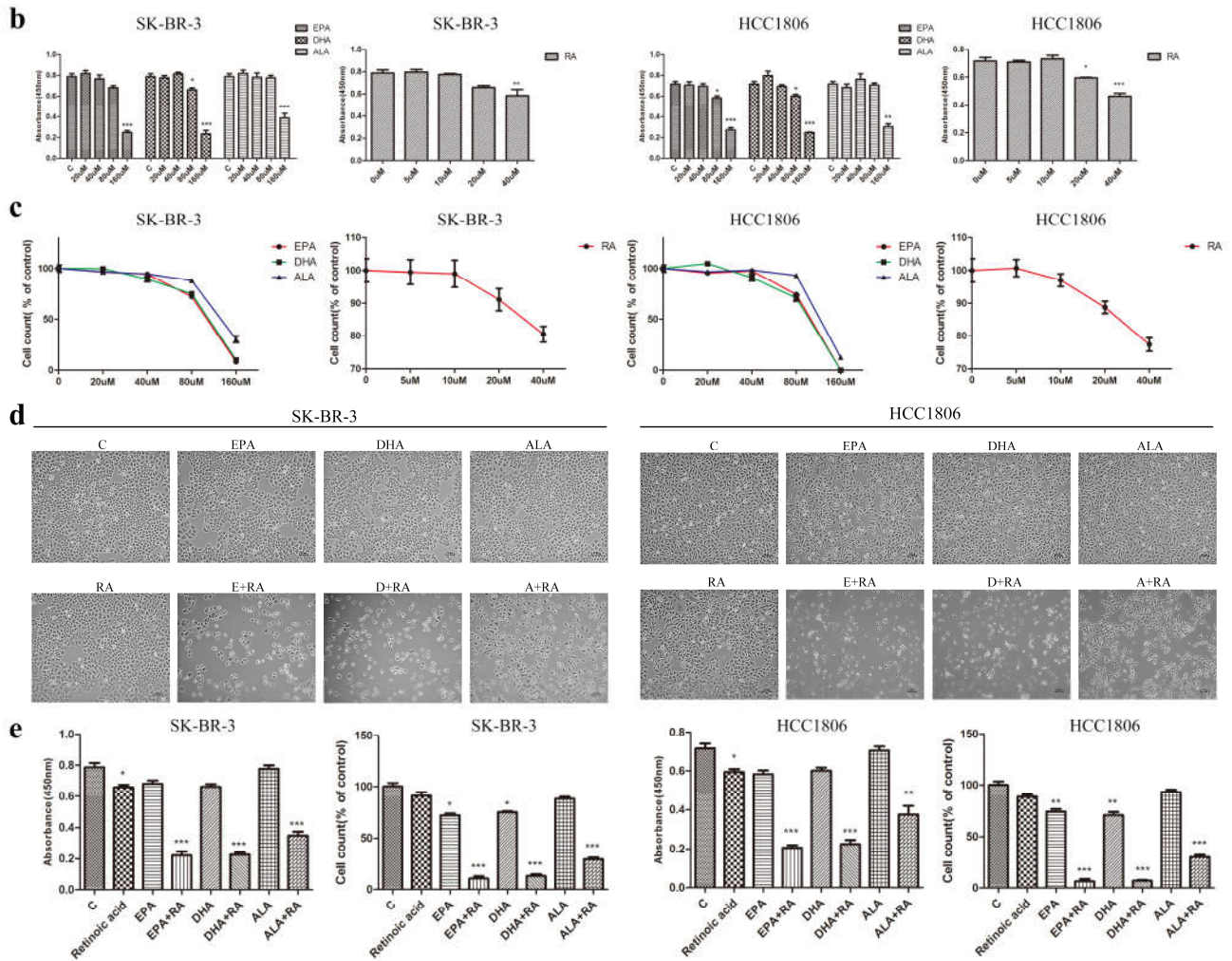
**$\omega$ -3 free fatty acids and all-trans retinoic acid synergistically induce growth inhibition of three subtypes of breast cancer cell lines**

Guangxiao Lin<sup>1,2</sup>, Shenglong Zhu<sup>1,2</sup>, Yikuan Wu<sup>1,2</sup>, Ci Song<sup>1,2</sup>, Wanjing Wang<sup>1,2</sup>, Yuan Zhang<sup>1,2</sup>, Yue-Lei Chen<sup>4\*</sup> and Zhao He<sup>1,2,3\*</sup>.

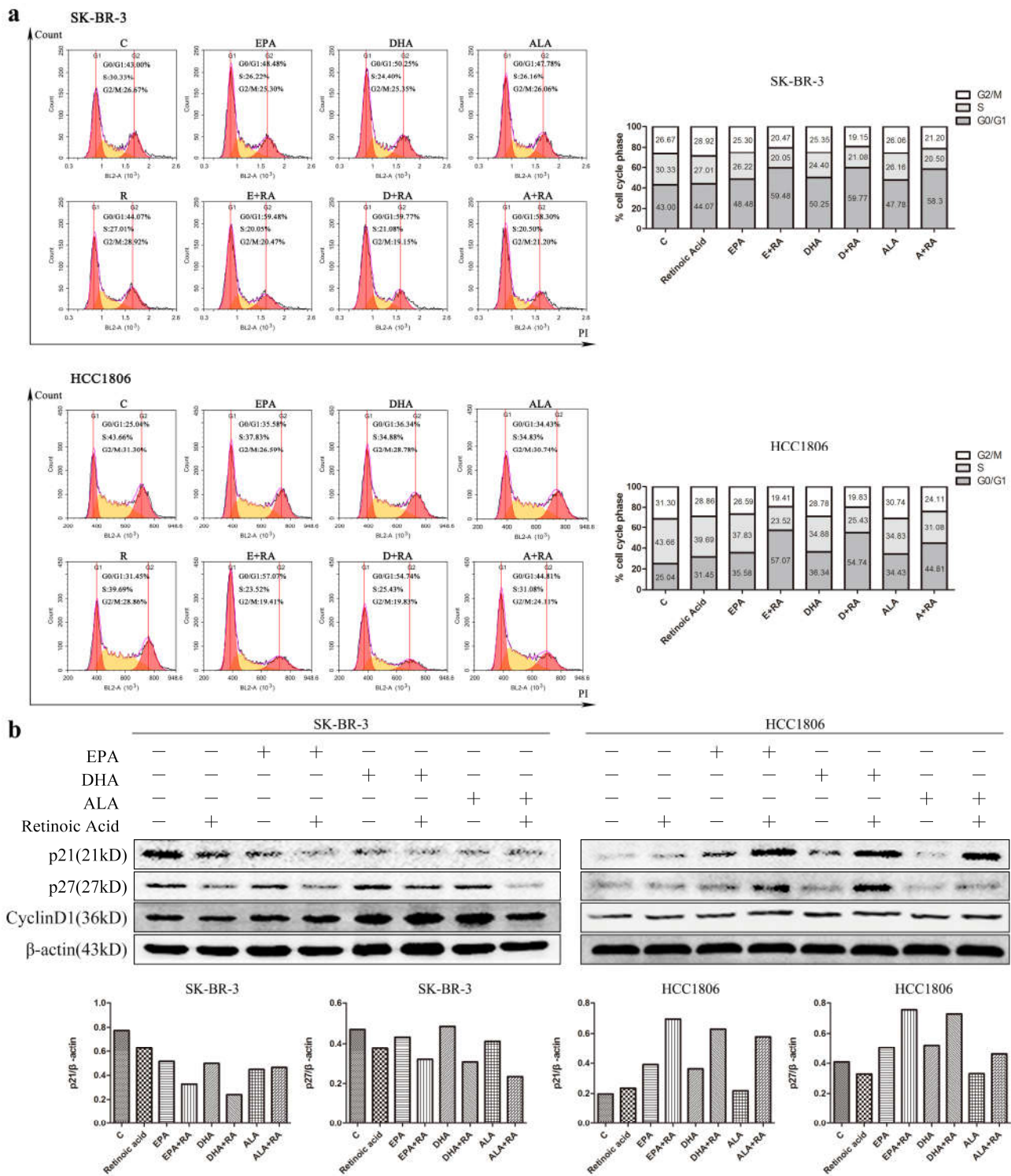
1. State Key Laboratory of Food Science and Technology, School of Food Science and Technology, Jiangnan University, Wuxi, 214122, China.
2. Synergistic Innovation Center for Food Safety and Nutrition, Jiangnan University, Wuxi, 214122, China.
3. School of Medicine, Jiangnan University, Wuxi, 214122, China.
4. Institute of Biochemistry and Cell Biology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai 200031, P.R. China



**Supplementary FigureS1. Cell growth.** MCF7, SK-BR-3, HCC1806 and MDA-MB-231 cells were treated with different concentrations  $\omega$ -3 FFAs and ATRA for 72 h. Cell morphology (a).

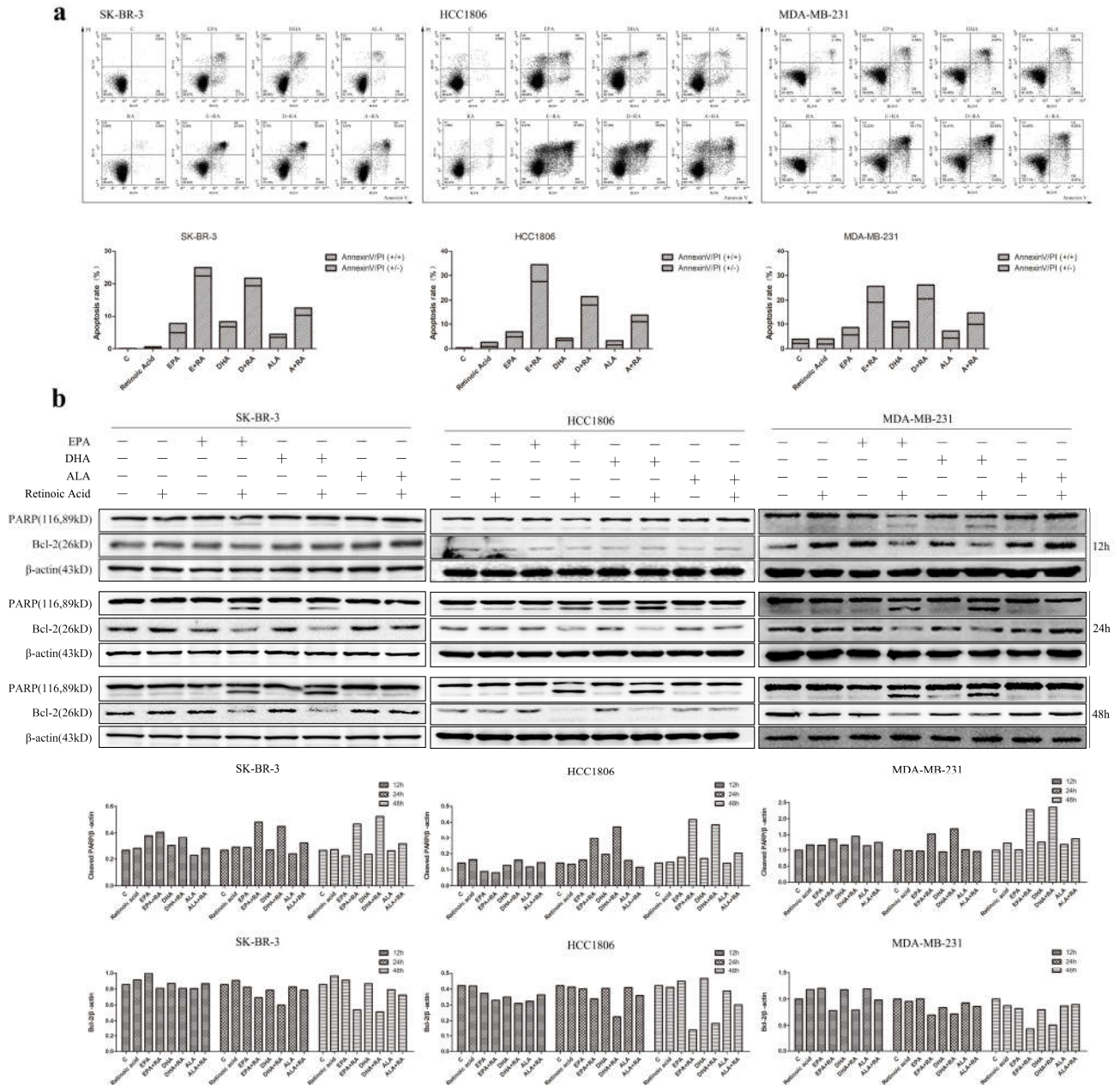


**Supplementary FigureS1. Cell growth.** SK-BR-3 and HCC1806 cells were treated with different concentrations  $\omega$ -3 FFAs and ATRA for 72 h. CCK8 assays (b) and Cell counting assays (c). The inhibitory effects of combined treatment with 80  $\mu$ M  $\omega$ 3-FFAs and 20  $\mu$ M ATRA on SK-BR-3 and HCC1806. Cell morphology (d), CCK8 assays and cell counting assays (e). Values represent the mean  $\pm$  SD (n = 3). \*P < 0.05; \*\*P < 0.01; \*\*\*P < 0.001.

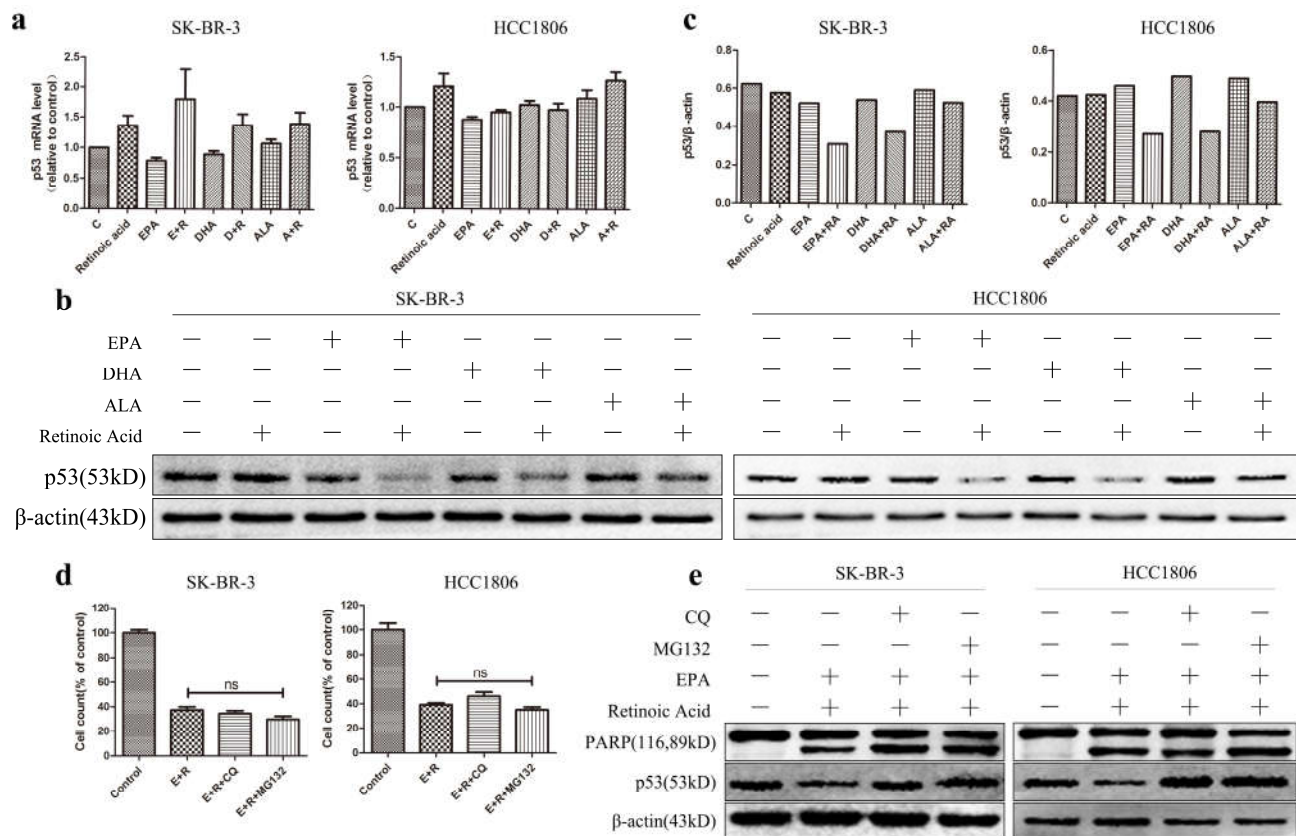


**Supplementary FigureS2. Cell cycle.** SK-BR-3 and HCC1806 cells were treated with 80  $\mu$ M  $\omega$ 3-FFAs and 20  $\mu$ M ATRA alone or in combination for 24 h. (a) Cell cycle was analyzed by flow cytometry (upper panel) and percentages of the total cell population in the different phases of cell cycle. (b) The expression of p21, p27 and cyclin D1 protein and quantification of p21, p27 and cyclin D1 (relative to  $\beta$ -Actin).

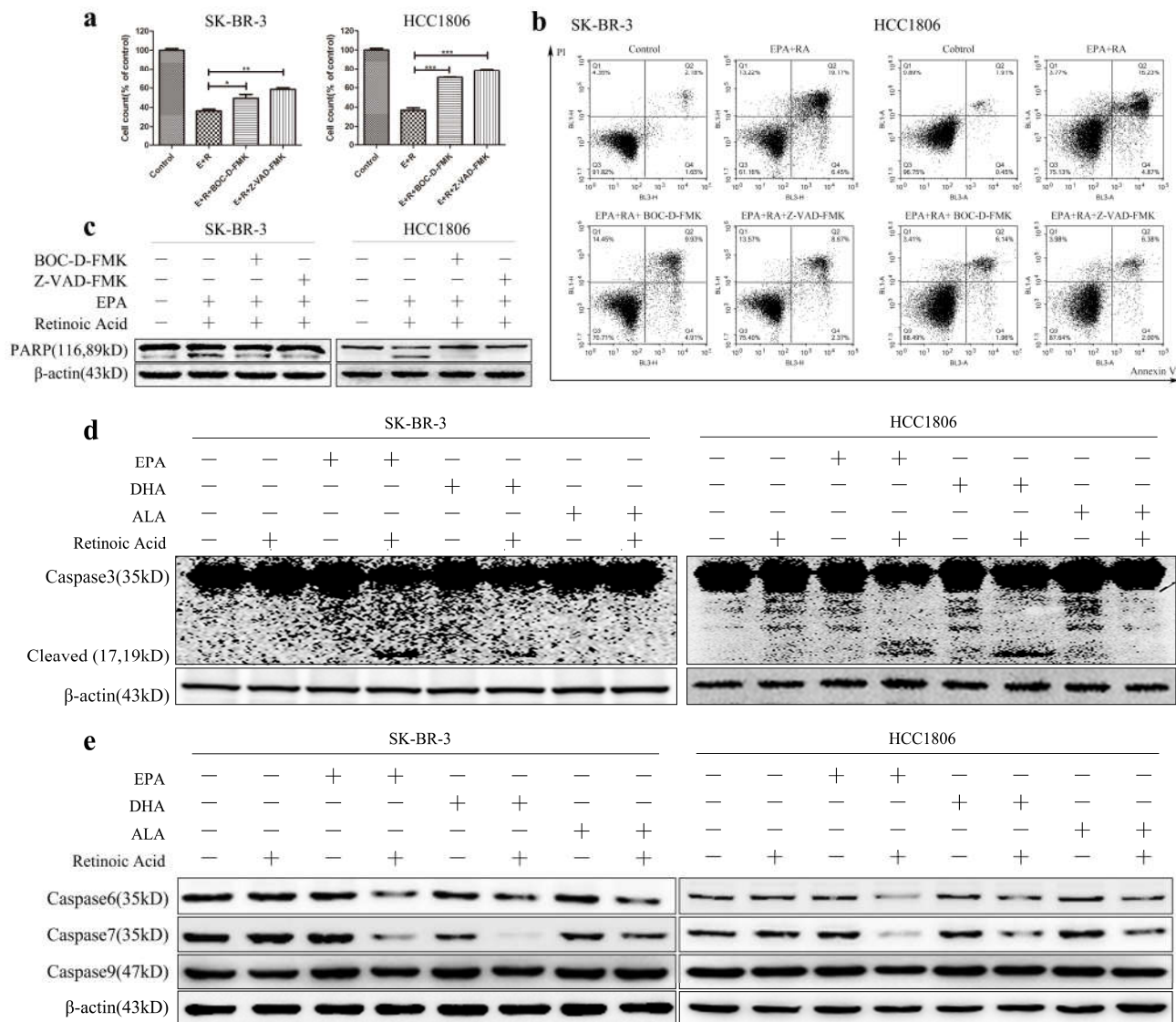




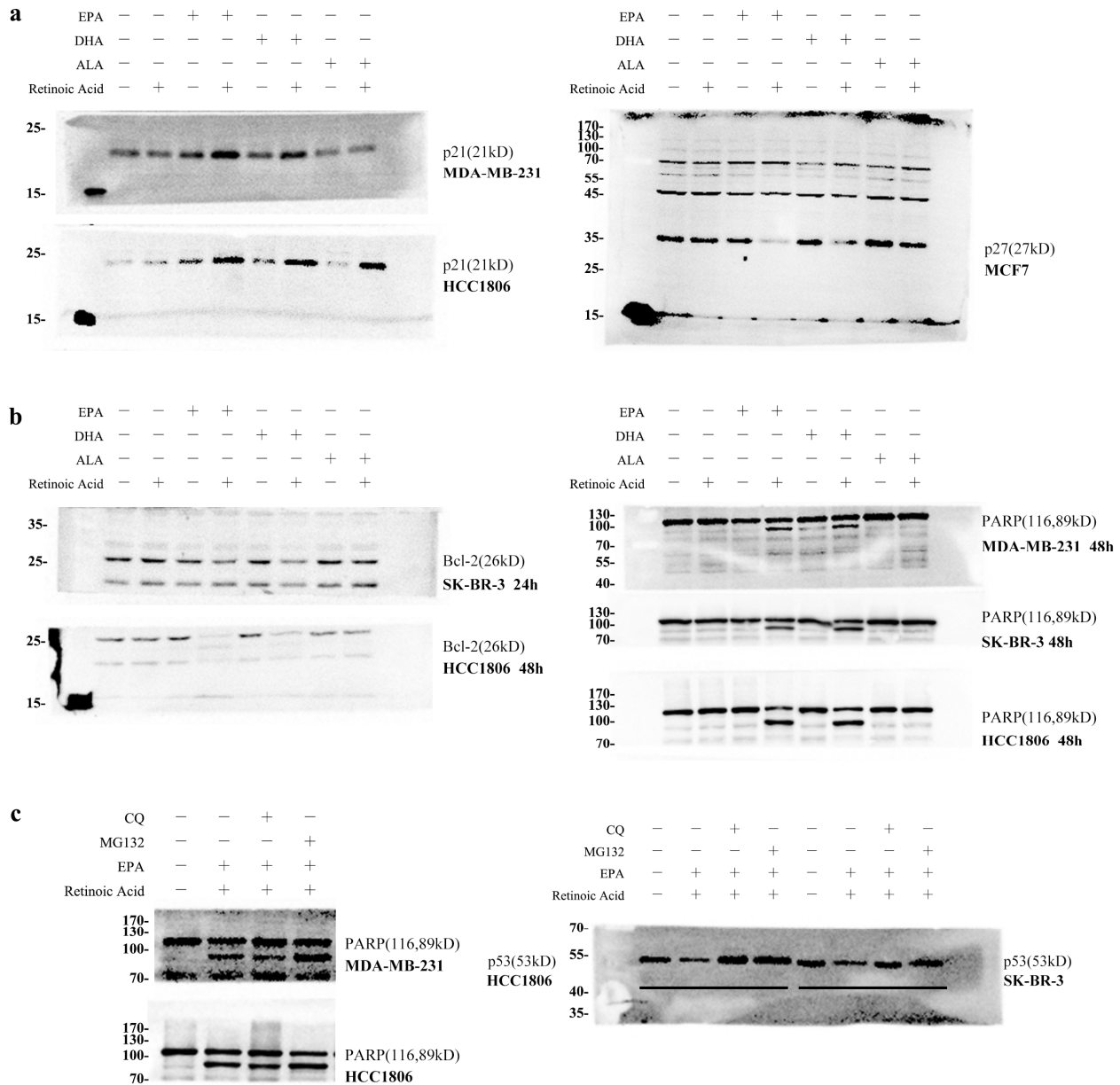
**Supplementary FigureS3. Cell apoptosis.** SK-BR-3, HCC1806 and MDA-MB-231 cells were treated with 80  $\mu\text{M}$   $\omega$ 3-FFAs and 20  $\mu\text{M}$  ATRA. (a) Cell apoptosis was analyzed by flow cytometry with PI and Annexin V-FITC staining and percentage of apoptotic cells at two different stages. (b) PARP and Bcl-2 protein level after treatment from 12 h to 48 h.



**Supplementary FigureS4. p53 and Cell apoptosis.** SK-BR-3 and HCC1806 cells were treated with 80  $\mu\text{M}$   $\omega$ 3-FFAs, 20  $\mu\text{M}$  ATRA alone or in combination for 48 h. (a) Relative expression of p53 was determined by Q-PCR. (b) The expression of p53 protein. (c) Quantification of p53 protein level (relative to  $\beta$ -Actin). SK-BR-3 and HCC1806 cells were treated with 80  $\mu\text{M}$   $\omega$ 3-FFAs and, 20  $\mu\text{M}$  ATRA alone or in combination in the absence or presence of proteasome inhibitor MG132 or lysosome inhibitor CQ. (d) Cell counting assays. (e) The expression of PARP and p53 protein. Values represent the mean  $\pm$  SD (n = 3). \*P < 0.05; \*\*P < 0.01; \*\*\*P < 0.001.

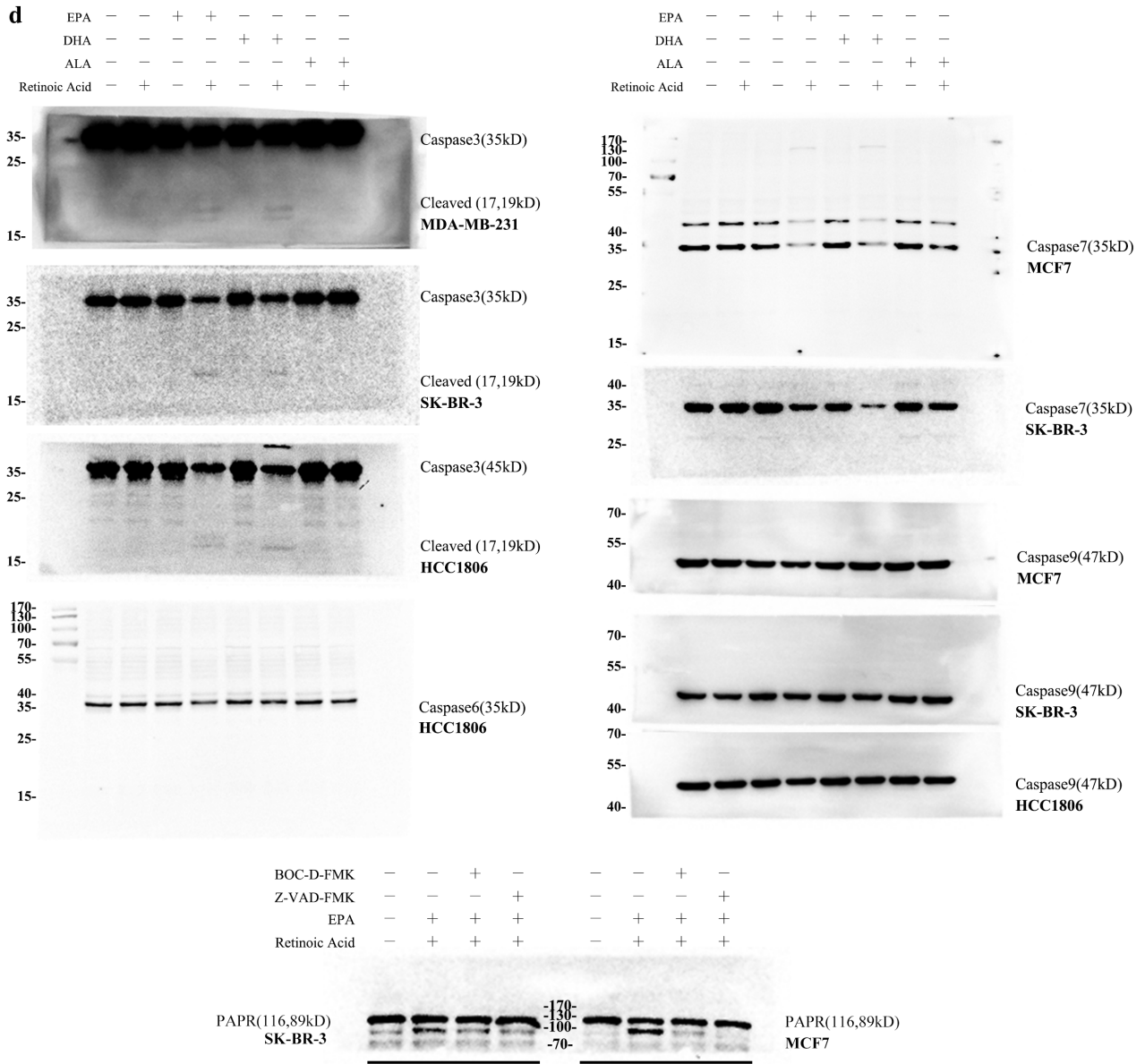


**Supplementary FigureS5. Caspase signaling pathway.** SK-BR-3 and HCC1806 cells were pretreated with 10  $\mu$ M Z-VAD-FMK and BOC-D-FMK for 1 h and then exposed to 80  $\mu$ M  $\omega$ 3-FFAs and 20  $\mu$ M ATRA for 48 h. (a) Cell counting assays. (b) Cell apoptosis was analyzed by flow cytometry with PI and Annexin V-FITC staining. (c) The expression of PARP protein. (d) The expression of Caspase-3 protein. (e) The expression of Caspase-6, -7 and -9 protein.  $\beta$ -Actin was used as an internal control. Values represent the mean  $\pm$  SD (n = 3). \*P< 0.05; \*\*P< 0.01; \*\*\*P< 0.001.



**Supplementary FigureS6. Scanned images of immunoblotting.** Originals for cropped bands in: (a) Figure 2 panel b and Supplementary FigureS2 panel b. (b) Figure 3 panel b and Supplementary FigureS3 panel b. (c) Figure 4 panel e and Supplementary FigureS4 panel e. (d) Figure 5 panel c, d, e and Supplementary FigureS5 panel c, d, e.





**Supplementary FigureS6. Scanned images of immunoblotting.** Originals for cropped bands in: (a) Figure 2 panel b and Supplementary FigureS2 panel b. (b) Figure 3 panel b and Supplementary FigureS3 panel b. (c) Figure 4 panel e and Supplementary FigureS4 panel e. (d) Figure 5 panel c, d, e and Supplementary FigureS5 panel c, d, e.