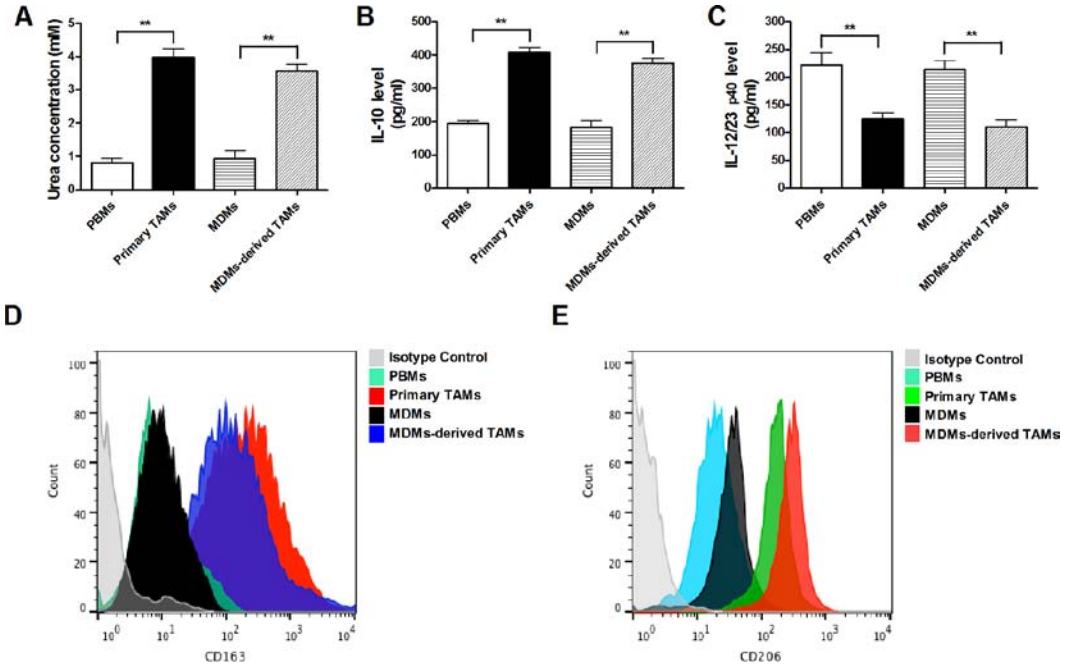
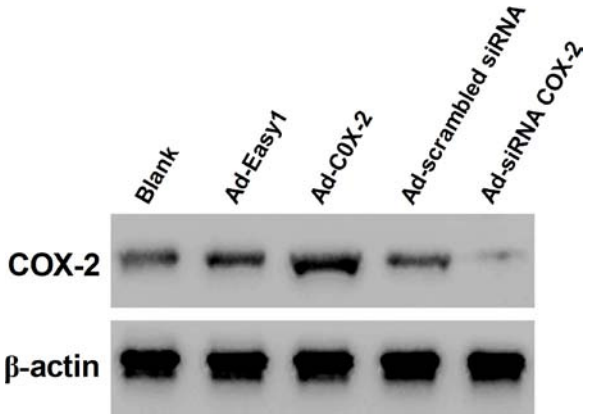
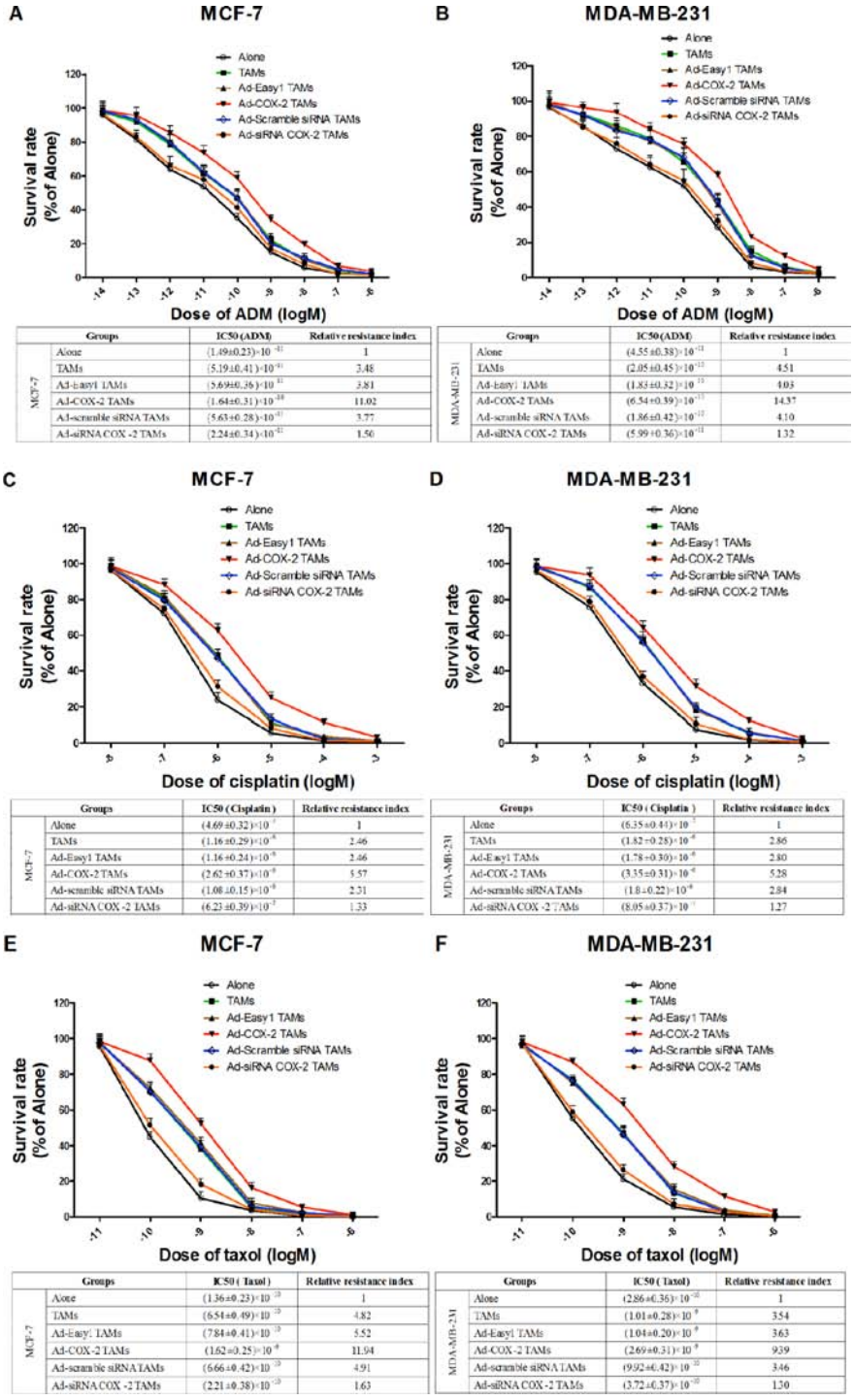
## SUPPLEMENTARY FIGURES AND TABLE




**Supplementary Figure S1: The molecular phenotype of macrophages in human breast cancer or co-cultured with breast cancer cells. A.** Arginase activity (urea concentration) in peripheral blood monocytes (PBMs), primary tumor-associated macrophages (TAMs), monocyte-derived macrophages (MDMs), or MDMs-derived TAMs. **B.** and **C.** Expression of IL-10 and IL-12/23 in macrophages was detected by ELISA. **D.** and **E.** Expression of CD163 and CD206 in macrophages was analyzed by flow cytometry. All the experiments were performed thrice in triplicate. Mean ± SD, *$p < 0.05$ and **$p < 0.01$.




**Supplementary Figure S2: Western blot analysis of COX-2 in TAMs.** TAMs were transfected with control Ad-Easy1, Ad-COX-2, control Ad-scrambled siRNA or Ad-siRNA COX-2 for 48 h. COX-2 protein was detected by western blot. β-actin was used as a loading control, and the blots shown are representative of six independent experiments.




| | Groups | IC50 (ADM) | Relative resistance index |
|---|---|---|---|
| MCF-7 | Alone | $(1.49\pm0.23)\times10^{-6}$ | 1 |
| | TAMs | $(5.19\pm0.41)\times10^{-6}$ | 3.48 |
| | Ad-Easy1 TAMs | $(5.69\pm0.36)\times10^{-6}$ | 3.81 |
| | Ad-COX-2 TAMs | $(1.64\pm0.31)\times10^{-5}$ | 11.02 |
| | Ad-scramble siRNA TAMs | $(5.63\pm0.28)\times10^{-6}$ | 3.77 |
| | Ad-siRNA COX -2 TAMs | $(2.24\pm0.34)\times10^{-6}$ | 1.50 |

| | Groups | IC50 (ADM) | Relative resistance index |
|---|---|---|---|
| MDA-MB-231 | Alone | $(4.55\pm0.38)\times10^{-7}$ | 1 |
| | TAMs | $(2.05\pm0.45)\times10^{-6}$ | 4.51 |
| | Ad-Easy1 TAMs | $(1.83\pm0.32)\times10^{-6}$ | 4.03 |
| | Ad-COX-2 TAMs | $(6.54\pm0.39)\times10^{-6}$ | 14.37 |
| | Ad-scramble siRNA TAMs | $(1.86\pm0.42)\times10^{-6}$ | 4.10 |
| | Ad-siRNA COX-2 TAMs | $(5.99\pm0.36)\times10^{-7}$ | 1.32 |

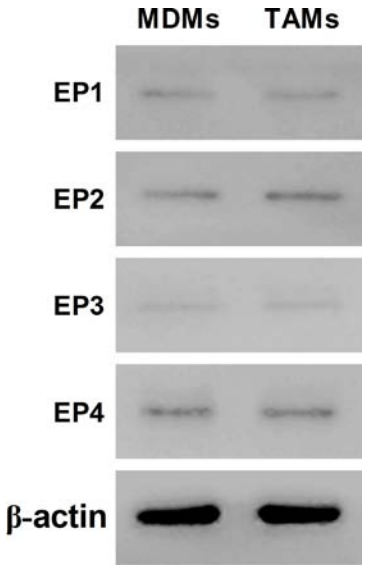
| | Groups | IC50 ( Cisplatin ) | Relative resistance index |
|---|---|---|---|
| MCF-7 | Alone | $(4.69\pm0.32)\times10^{-7}$ | 1 |
| | TAMs | $(1.16\pm0.29)\times10^{-6}$ | 2.46 |
| | Ad-Easy1 TAMs | $(1.16\pm0.24)\times10^{-6}$ | 2.46 |
| | Ad-COX-2 TAMs | $(2.62\pm0.37)\times10^{-6}$ | 5.57 |
| | Ad-scramble siRNA TAMs | $(1.08\pm0.15)\times10^{-6}$ | 2.31 |
| | Ad-siRNA COX -2 TAMs | $(6.23\pm0.39)\times10^{-7}$ | 1.33 |

| | Groups | IC50 ( Cisplatin ) | Relative resistance index |
|---|---|---|---|
| MDA-MB-231 | Alone | $(6.35\pm0.44)\times10^{-7}$ | 1 |
| | TAMs | $(1.82\pm0.28)\times10^{-6}$ | 2.86 |
| | Ad-Easy1 TAMs | $(1.78\pm0.30)\times10^{-6}$ | 2.80 |
| | Ad-COX-2 TAMs | $(3.35\pm0.31)\times10^{-6}$ | 5.28 |
| | Ad-scramble siRNA TAMs | $(1.8\pm0.22)\times10^{-6}$ | 2.84 |
| | Ad-siRNA COX -2 TAMs | $(8.05\pm0.37)\times10^{-7}$ | 1.27 |

| | Groups | IC50 ( Taxol ) | Relative resistance index |
|---|---|---|---|
| MCF-7 | Alone | $(1.36\pm0.23)\times10^{-9}$ | 1 |
| | TAMs | $(6.54\pm0.49)\times10^{-9}$ | 4.82 |
| | Ad-Easy1 TAMs | $(7.84\pm0.41)\times10^{-9}$ | 5.52 |
| | Ad-COX-2 TAMs | $(1.62\pm0.25)\times10^{-8}$ | 11.94 |
| | Ad-scramble siRNA TAMs | $(6.66\pm0.42)\times10^{-9}$ | 4.91 |
| | Ad-siRNA COX -2 TAMs | $(2.21\pm0.38)\times10^{-9}$ | 1.63 |

| | Groups | IC50 ( Taxol) | Relative resistance index |
|---|---|---|---|
| MDA-MB-231 | Alone | $(2.86\pm0.36)\times10^{-10}$ | 1 |
| | TAMs | $(1.01\pm0.28)\times10^{-7}$ | 354 |
| | Ad-Easy1 TAMs | $(1.04\pm0.20)\times10^{-7}$ | 363 |
| | Ad-COX -2 TAMs | $(2.69\pm0.31)\times10^{-7}$ | 939 |
| | Ad-scramble siRNA TAMs | $(9.92\pm0.42)\times10^{-8}$ | 346 |
| | Ad-siRNA COX -2 TAMs | $(3.72\pm0.37)\times10^{-8}$ | 130 |

**Supplementary Figure S3: COX-2 in macrophages enhances multi-drug resistance in breast cancer cells.** Breast cancer cells which were co-cultured with or without TAMs transfected with adenoviral COX-2 or siRNA COX-2 for 7 days were treated with different concentrations of chemotherapeutic drugs including ADM (A and B), cisplatin (C and D) and taxol (E and F) and incubated for 48 h. At the end of the drug exposure duration, cell viability was measured according to the protocol of CCK-8 (KeyGEN Biotech, Nanjing, China). All plates had control wells containing medium without cells to obtain a value for background spectrometric absorbance which was subtracted from the test sample readings. Data were expressed as ratios of treated to control cells, mean ± SD for three replications.




**Supplementary Figure S4: Western blot analysis of EP1–4 in macrophages.** EP1–4 proteins in MDMs and TAMs were detected by western blot. β-actin was used as a loading control, and the blots shown are representative of six independent experiments.

**Supplementary Table S1: Antibodies used in this study**

| Antibody | Clone | Company | Isotype/Source | Dilution |
|---|---|---|---|---|
| Ki-67 | MX006 | Maixin-Bio | Mouse IgG | 1:200 |
| cleaved caspase 3 | 5A1E | Cell Signaling Technology | Rabbit IgG | 1:200 |
| Bcl-2 | 50E3 | Cell Signaling Technology | Rabbit IgG | 1:1000 |
| Bcl-xl | 54H6 | Cell Signaling Technology | Rabbit IgG | 1:1000 |
| Bax | D2E11 | Cell Signaling Technology | Rabbit IgG | 1:1000 |
| Bad | D24A9 | Cell Signaling Technology | Rabbit IgG | 1:1000 |
| Bid | 7A3 | Cell Signaling Technology | Mouse IgG | 1:1000 |
| P-gp | EPR10364–57 | Abcam | Rabbit IgG | 1:2000 |
| MRP1 | EPR4658(2) | Abcam | Rabbit IgG | 1:3000 |
| LRP | EPR13227(B) | Abcam | Rabbit IgG | 1:3000 |
| BCRP | EPR2099(2) | Abcam | Rabbit IgG | 1:2000 |
| COX-2 | D5H5 | Cell Signaling Technology | Rabbit IgG | 1:1000 |
| total Akt | C67E7 | Cell Signaling Technology | Rabbit IgG | 1:1000 |
| p-Akt (Ser473) | D9E | Cell Signaling Technology | Rabbit IgG | 1:1000 |
| EP1 | Polyclonal | Abcam | Rabbit | 1:1000 |
| EP2 | EPR8030(B) | Abcam | Rabbit IgG | 1:2000 |
| EP3 | 5F5 | Abcam | Mouse IgG | 1:1000 |
| EP4 | Polyclonal | Abcam | Rabbit | 1:1000 |
| β-actin | D6A8 | Cell Signaling Technology | Rabbit IgG | 1:1000 |