

Supplementary Material

Flubendazole elicits anti-cancer effects via targeting EVA1A-modulated autophagy and apoptosis in triple-negative breast cancer

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Supplementary Figure S1

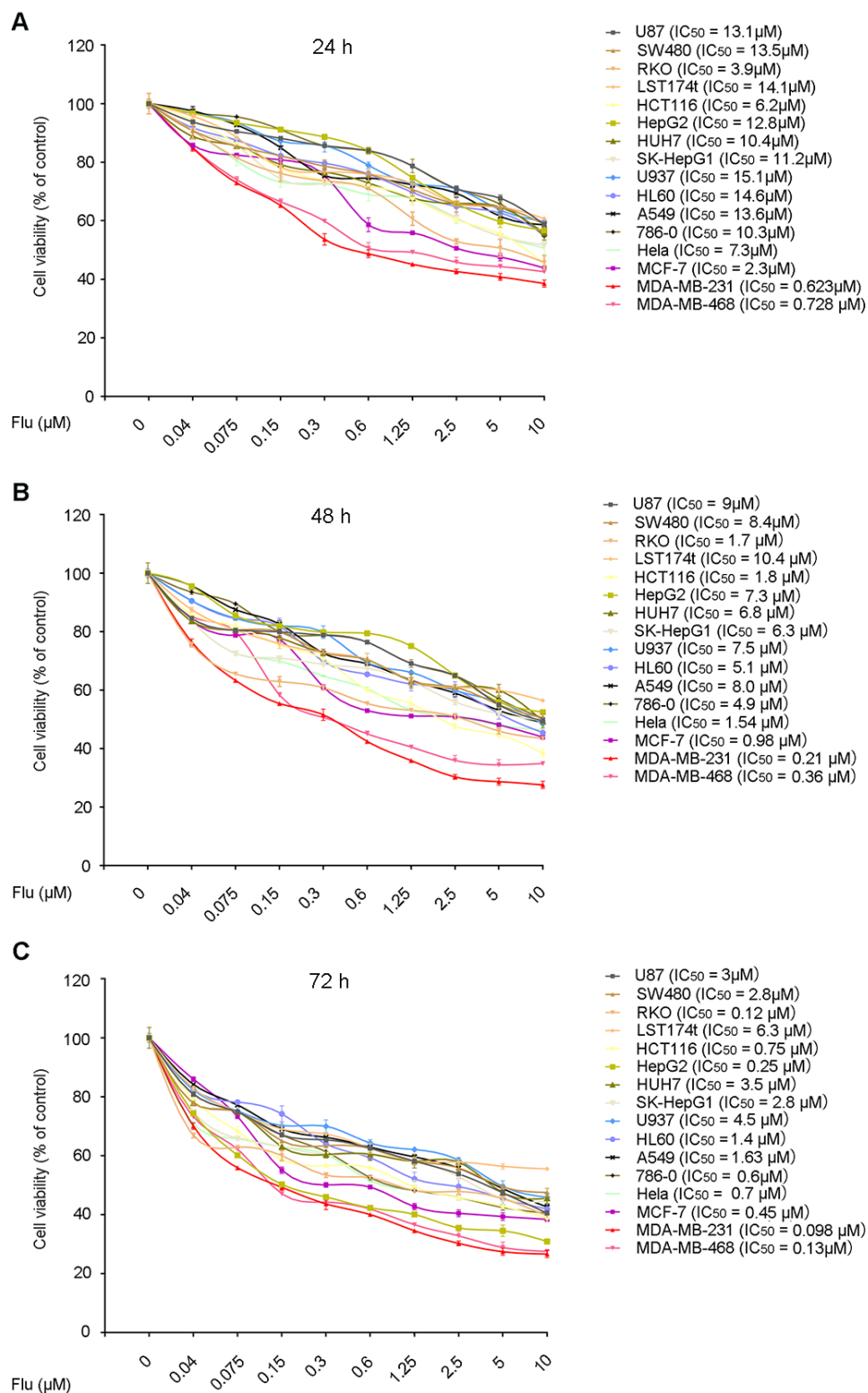


Figure S1. Antitumor effect of flubendazole. (A-C) The cell viability of concentration-dependent and time-dependent treated with flubendazole in various tumor lines. Cell viabilities were

measured by MTT assay, and the IC₅₀ values were calculated by Prism 7.0. Data are expressed as mean ± SEM. All data were representative of at least three independent experiments.

Supplementary Figure S2

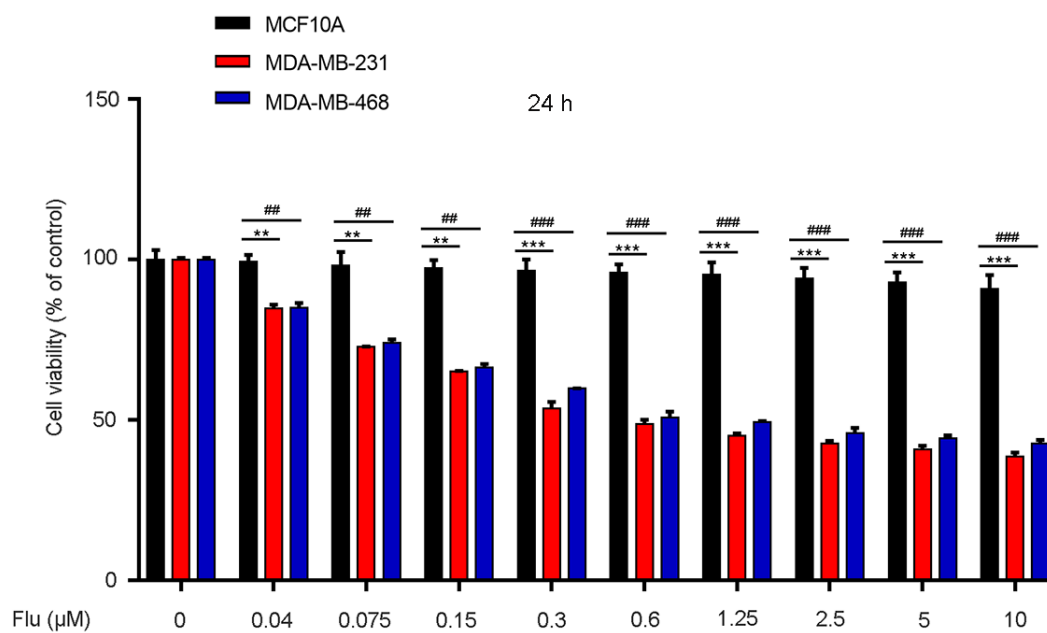


Figure S2. Cell viability of flubendazole on normal breast cells and TNBC cells. (A) The cell viability of concentration-dependent treated with flubendazole for 24 h. Cell viabilities were measured by MTT assay. Data are expressed as mean \pm SEM. All data were representative of at least three independent experiments. **, $P < 0.01$, ***, ###, $P < 0.001$. Statistical significance compared with respective control groups.

Supplementary Figure S3

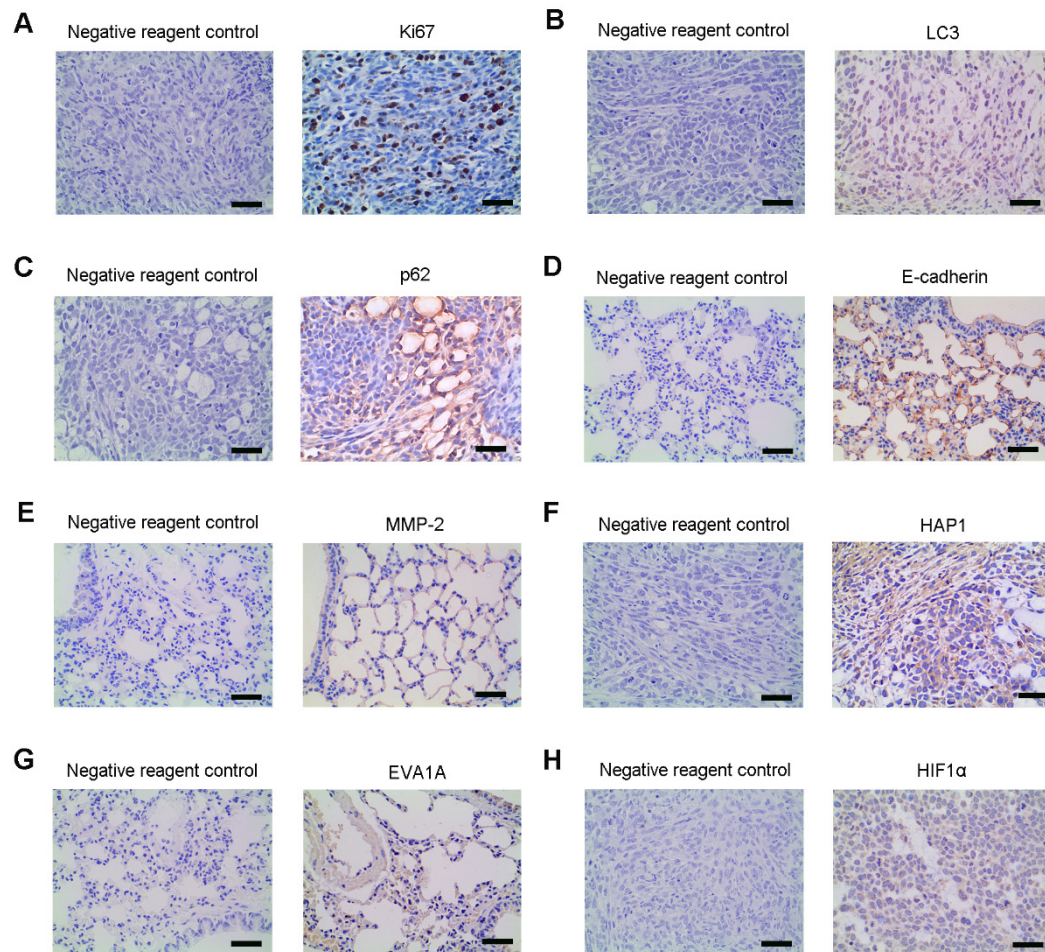


Figure S3. Immunohistochemistry from tumor sections or lung tissue in nude mice. (A-H) Representative images of Ki67, LC3, p62, E-cadherin, MMP-2, EVA1A, HAP1 and HIF1 α in nude mice tumor sections or lung tissues and their respective negative controls, which used as the indication of antibody specificity.

Supplementary Figure S4

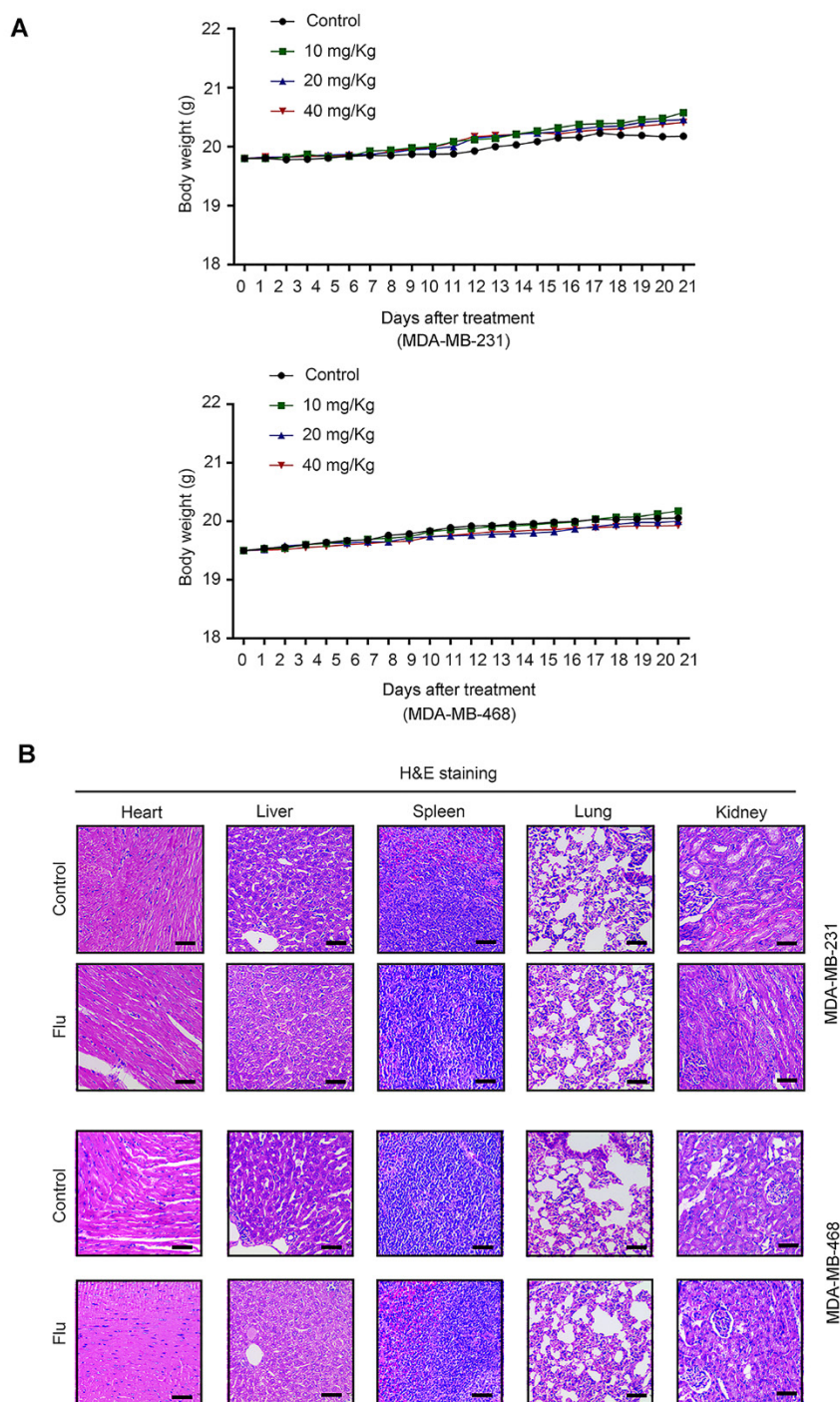


Figure S4. Flubendazole inhibits TNBC *in vivo*. (A) Body weight change of mice in different group, compared with control group. (B) Representative H&E staining in heart, liver, spleen, lung, kidney sections. Scale bar, 40 μ m. Data are expressed as mean \pm SEM. All data were representative of at least three independent experiments.

Supplementary Figure S5

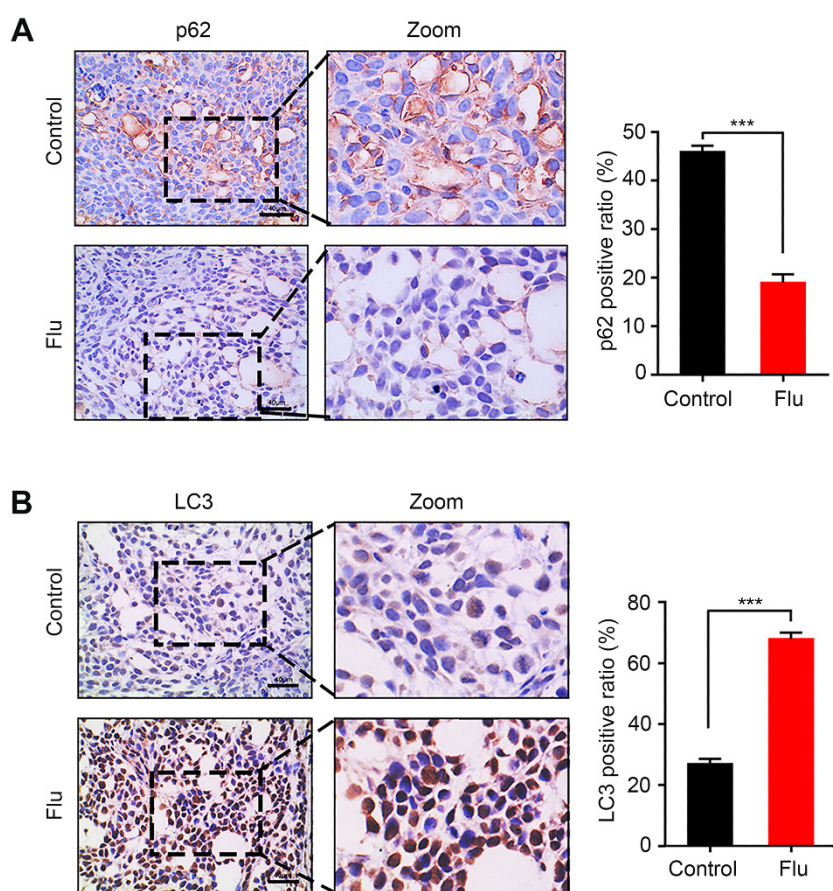


Figure S5. Flubendazole induces autophagy *in vivo*. (A-B) The expression of p62 and LC3 in representative tumor sections of nude mice from control and median dose (20 mg/Kg). Representative images and quantitative analysis of the percentage of positive ratios were shown. Scale bar, 40 μ m. Data are expressed as mean \pm SEM. All data were representative of at least three independent experiments. *, $P < 0.05$, **, $P < 0.01$, ***, $P < 0.001$. Statistical significance compared with respective control groups.

Supplementary Figure S6

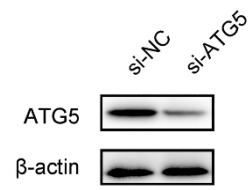


Figure S6. MDA-MB-231 cells were transfected with control or ATG5 siRNA. Then, the expression levels of ATG5 was determined by western blot analysis. β -Actin was measured as the loading control.

Supplementary Figure S7

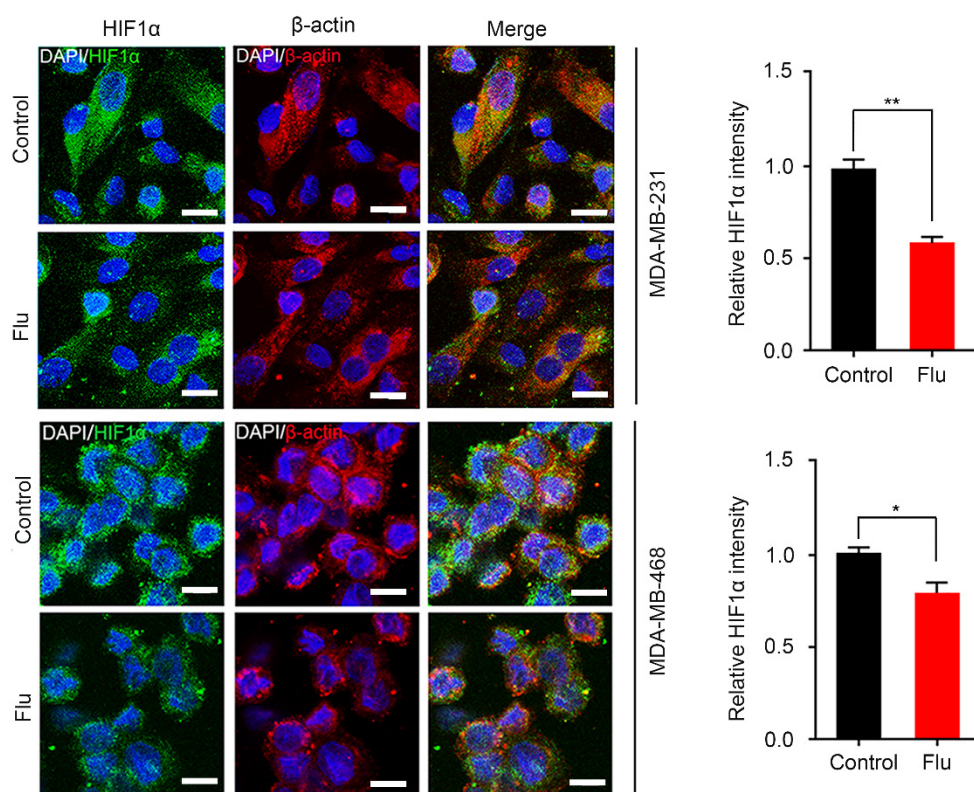


Figure S7. Flubendazole downregulates the expression of HIF1 α *in vitro*. Immunofluorescence analysis of HIF1 α in MDA-MB-231 and MDA-MB-468 cells treated with or without flubendazole (0.5 μ M) for 24 h. Scale bar, 20 μ m. Data are expressed as mean \pm SEM. All data were representative of at least three independent experiments. *, $P < 0.05$, **, $P < 0.01$, ***, $P < 0.001$. Statistical significance compared with respective control groups.

Supplementary Figure S8

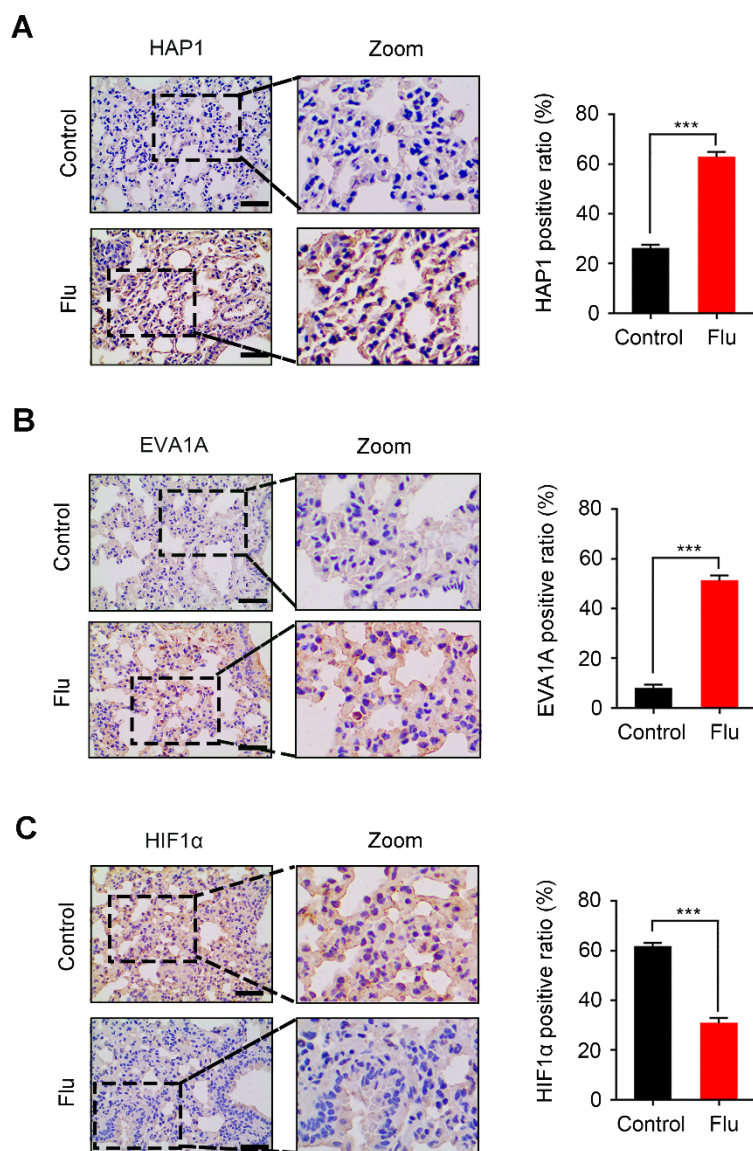


Figure S8. Flubendazole regulates the expression of HAP1, EVA1A and HIF1 α *in vivo*. (A-C)

The expression of HAP1, EVA1A and HIF1 α in representative lung sections of nude mice from vehicle and median dose (20 mg/Kg). Representative images and quantitative analysis of the percentage of positive ratios were shown. Scale bar, 40 μ m. Data are expressed as mean \pm SEM.

All data were representative of at least three independent experiments. *, $P < 0.05$, **, $P < 0.01$,

***, $P < 0.001$. Statistical significance compared with respective control groups.

Supplementary Figure S9

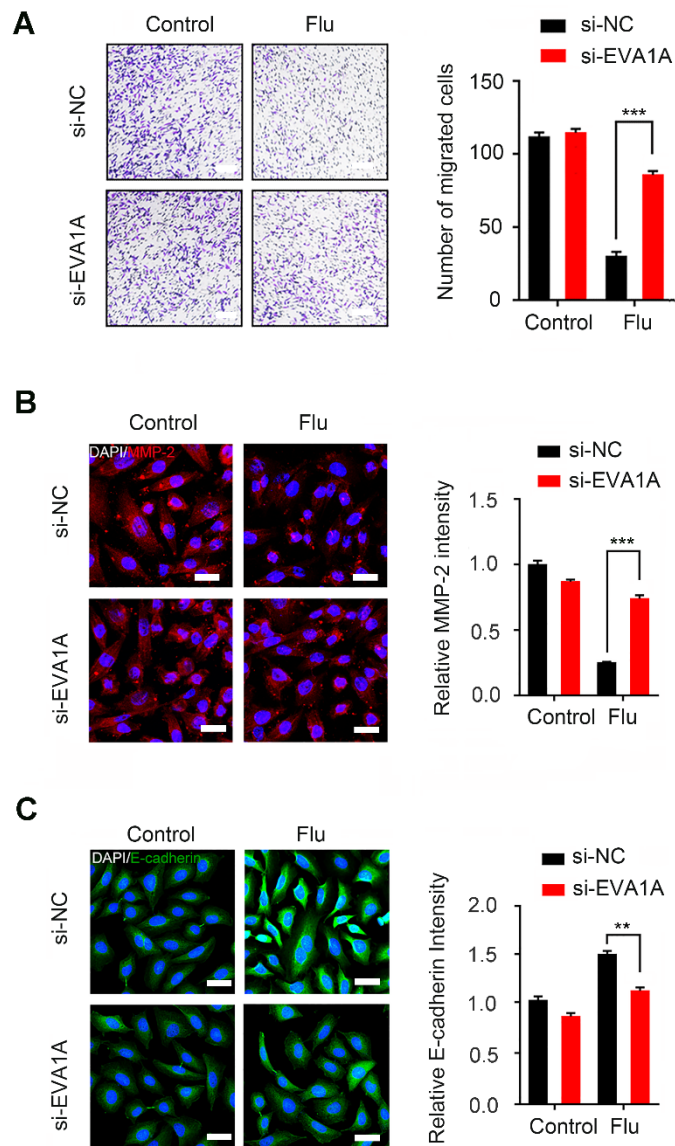


Figure S9. Flubendazole elicits anti-migration potential via targeting EVA1A *in vitro*. (A) The MDA-MB-231 cells were transfected with control or EVA1A siRNA, followed by treatment with or without flubendazole (0.5 μ M) for 24 h. Transwell assay was used to measure migration capabilities of cells. Representative images and statistics were shown. Scale bar, 100 μ m. (B-C) The MDA-MB-231 cells were transfected with control or EVA1A siRNA, followed by treatment with or without flubendazole (0.5 μ M) for 24 h. The expression of MMP-2 and E-cadherin were

analyzed by immunofluorescence. Scale bar, 20 μm . Data are expressed as mean \pm SEM. All data were representative of at least three independent experiments. *, $P < 0.05$, **, $P < 0.01$, ***, $P < 0.001$. Statistical significance compared with respective control groups.

Supplementary Table 2. List of siRNA or cDNA sequences

Gene name	siRNA sense sequences (5'-3')
si-EVA1A	GAGCCTGAATCGCTACTATTA
si-Control	TTCTCCGAACGTGTACGT

Recombinant DNA	DNA sense sequences
C-terminal Flag-tagged EVA1A ^{WT}	ATGAGGCTGCCCCTCAGCCACAGCCCAGAGCACGTGGAGATGGCTTTG CTCAGCAACATCCTAGCGGCCTATTCCCTTTGTCTCAGAAAATCCTGAGC GAGCAGCTCTGTACTTTGTTTCTGGCGTGTGCATCGGGCTGGTGCTGAC CCTGGCTGCTCTGGTGATAAGGATCTCTTGCCACACAGACTGCAGGCGG CGTCCCGGGAAGAAGTTCCTGCAGGACAGAGAGAGCAGCAGCGACAG CAGCGACAGCGAGGATGGCAGTGAGGACACCGTGTCCGATCTCTCCGT GCGGAGACACCGCCGCTTCGAGAGGACTTTGAACAAGAATGTGTTTAC CTCTGCGGAGGAGCTGGAGCGCGCCAGCGGCTGGAGGAGCGCGAGC GCATCATCAGGGAGATCTGGATGAATGGCCAGCCTGAGGTGCCCGGGA CCAGGAGCCTGAATCGCTACTATTAGGACTACAAGGATGACGATGACAA GGATTACAAAGACGACGATAAGGACTATAAGGATGATGACGACAAA
C-terminal Flag-tagged EVA1A ^{N110A}	ATGAGGCTGCCCCTCAGCCACAGCCCAGAGCACGTGGAGATGGCTTTG CTCAGCAACATCCTAGCGGCCTATTCCCTTTGTCTCAGAAAATCCTGAGC GAGCAGCTCTGTACTTTGTTTCTGGCGTGTGCATCGGGCTGGTGCTGAC CCTGGCTGCTCTGGTGATAAGGATCTCTTGCCACACAGACTGCAGGCGG CGTCCCGGGAAGAAGTTCCTGCAGGACAGAGAGAGCAGCAGCGACAG CAGCGACAGCGAGGATGGCAGTGAGGACACCGTGTCCGATCTCTCCGT GCGGAGACACCGCCGCTTCGAGAGGACTTTGAACAAGGCTGTGTTTAC CTCTGCGGAGGAGCTGGAGCGCGCCAGCGGCTGGAGGAGCGCGAGC GCATCATCAGGGAGATCTGGATGAATGGCCAGCCTGAGGTGCCCGGGA CCAGGAGCCTGAATCGCTACTATTAGGACTACAAGGATGACGATGACAA GGATTACAAAGACGACGATAAGGACTATAAGGATGATGACGACAAA
C-terminal Flag-tagged EVA1A ^{W135A}	ATGAGGCTGCCCCTCAGCCACAGCCCAGAGCACGTGGAGATGGCTTTG CTCAGCAACATCCTAGCGGCCTATTCCCTTTGTCTCAGAAAATCCTGAGC GAGCAGCTCTGTACTTTGTTTCTGGCGTGTGCATCGGGCTGGTGCTGAC CCTGGCTGCTCTGGTGATAAGGATCTCTTGCCACACAGACTGCAGGCGG CGTCCCGGGAAGAAGTTCCTGCAGGACAGAGAGAGCAGCAGCGACAG CAGCGACAGCGAGGATGGCAGTGAGGACACCGTGTCCGATCTCTCCGT GCGGAGACACCGCCGCTTCGAGAGGACTTTGAACAAGAATGTGTTTAC CTCTGCGGAGGAGCTGGAGCGCGCCAGCGGCTGGAGGAGCGCGAGC GCATCATCAGGGAGATCGCGATGAATGGCCAGCCTGAGGTGCCCGGGA CCAGGAGCCTGAATCGCTACTATTAGGACTACAAGGATGACGATGACAA GGATTACAAAGACGACGATAAGGACTATAAGGATGATGACGACAAA
C-terminal Flag-tagged EVA1A ^{T113A}	ATGAGGCTGCCCCTCAGCCACAGCCCAGAGCACGTGGAGATGGCTTTG CTCAGCAACATCCTAGCGGCCTATTCCCTTTGTCTCAGAAAATCCTGAGC GAGCAGCTCTGTACTTTGTTTCTGGCGTGTGCATCGGGCTGGTGCTGAC CCTGGCTGCTCTGGTGATAAGGATCTCTTGCCACACAGACTGCAGGCGG CGTCCCGGGAAGAAGTTCCTGCAGGACAGAGAGAGCAGCAGCGACAG CAGCGACAGCGAGGATGGCAGTGAGGACACCGTGTCCGATCTCTCCGT GCGGAGACACCGCCGCTTCGAGAGGACTTTGAACAAGAATGTGTTTAC CTCTGCGGAGGAGCTGGAGCGCGCCAGCGGCTGGAGGAGCGCGAGC GCATCATCAGGGAGATCGCGATGAATGGCCAGCCTGAGGTGCCCGGGA CCAGGAGCCTGAATCGCTACTATTAGGACTACAAGGATGACGATGACAA GGATTACAAAGACGACGATAAGGACTATAAGGATGATGACGACAAA

CAGCGACAGCGAGGATGGCAGTGAGGACACCGTGTCCGATCTCTCCGT GCGGAGACACCGCCGCTTCGAGAGGACTTTGAACAAGAATGTGTTTCGC CTCTGCGGAGGAGCTGGAGCGCGCCCAGCGGCTGGAGGAGCGCGAGC GCATCATCAGGGAGATCTGGATGAATGGCCAGCCTGAGGTGCCCGGGA CCAGGAGCCTGAATCGCTACTATTAGGACTACAAGGATGACGATGACAA GGATTACAAAGACGACGATAAGGACTATAAGGATGATGACGACAAA
