

## Supplementary Material

# Plumbagin suppresses breast cancer progression by down-regulating HIF-1 $\alpha$ expression via a PI3K/Akt/mTOR independent pathway under hypoxia condition

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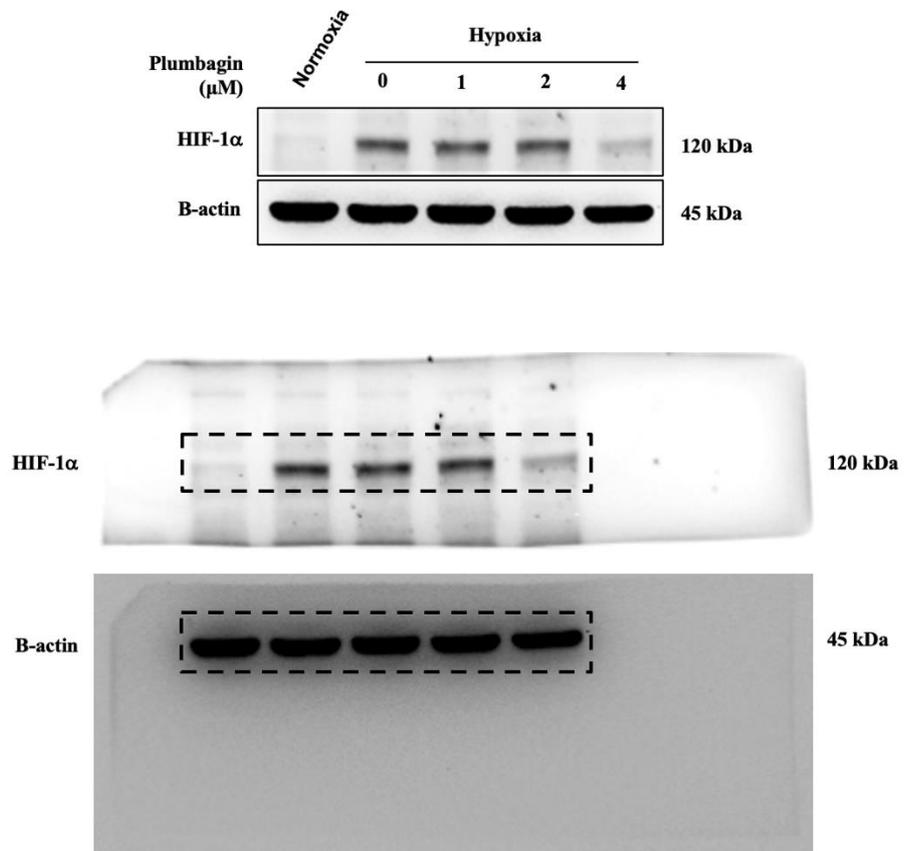
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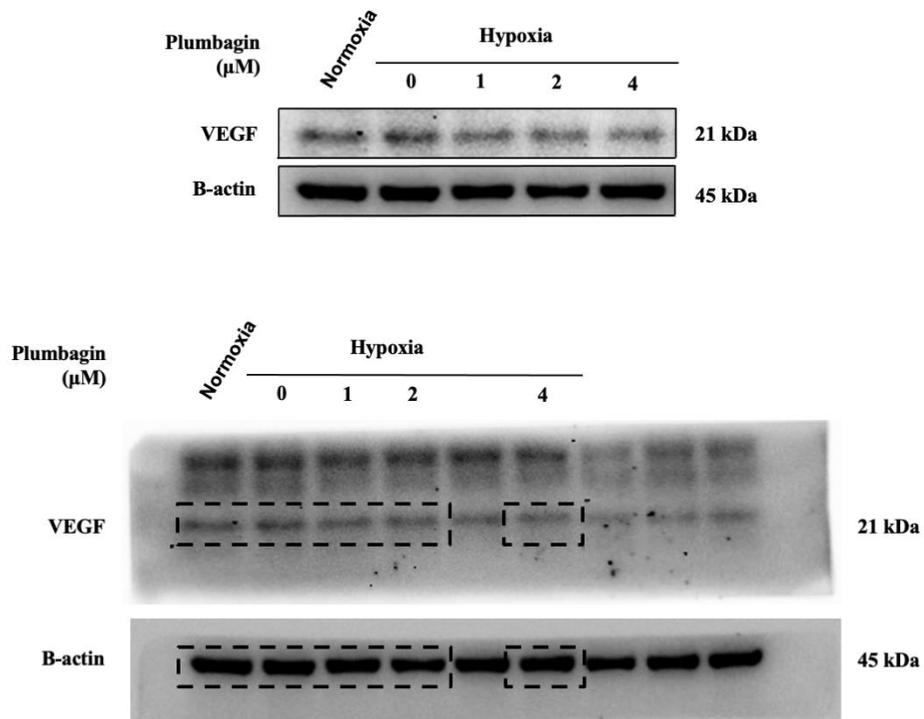
**Table S1.** Lists of primer sequences used for qRT-PCR

| Gene           | Sequences (5'-3')       |                      |
|----------------|-------------------------|----------------------|
|                | Forward                 | Reverse              |
| HIF-1 $\alpha$ | TTTTGGCAGCAACGACACAG    | TTTTCGTTGGGTGAGGGGAG |
| VEGF           | ACAACAAATGTGAATGCAGACCA | TACCGGGATTCTTGCGCTT  |
| VEGFR-2        | TGTGTATGTCCCACCCAGCA    | GGGAGGAATGGCATAGACCG |
| GAPDH          | GACAGTCAGCCGCATCTCT     | ACCAAATCCGTTGACTCCGA |

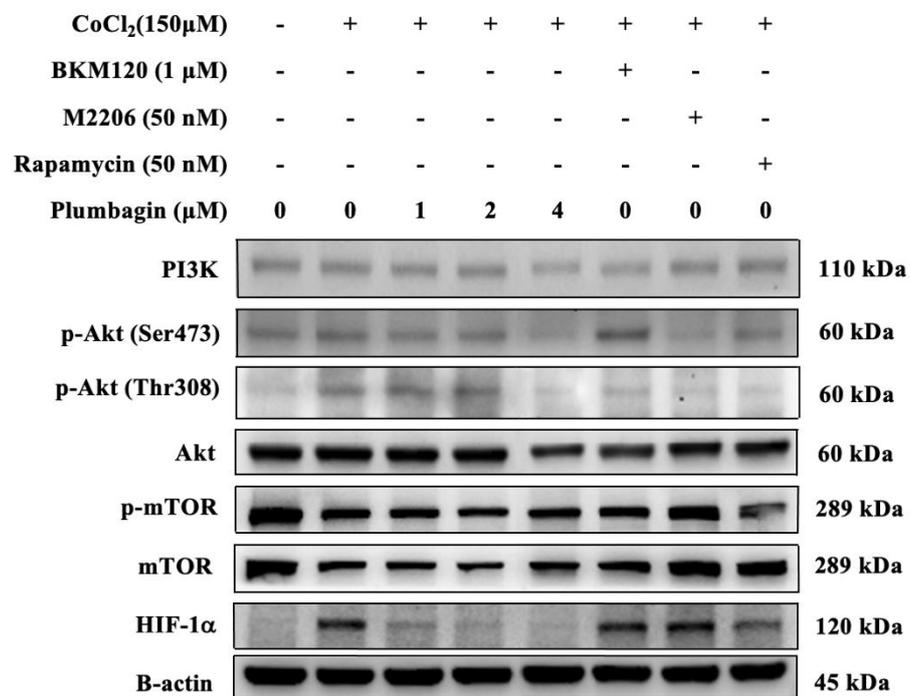
**Figure S1.** Full-length blots representing the protein expression of HIF-1 $\alpha$  and  $\beta$ -actin as shown in Figure 4A of the manuscript.



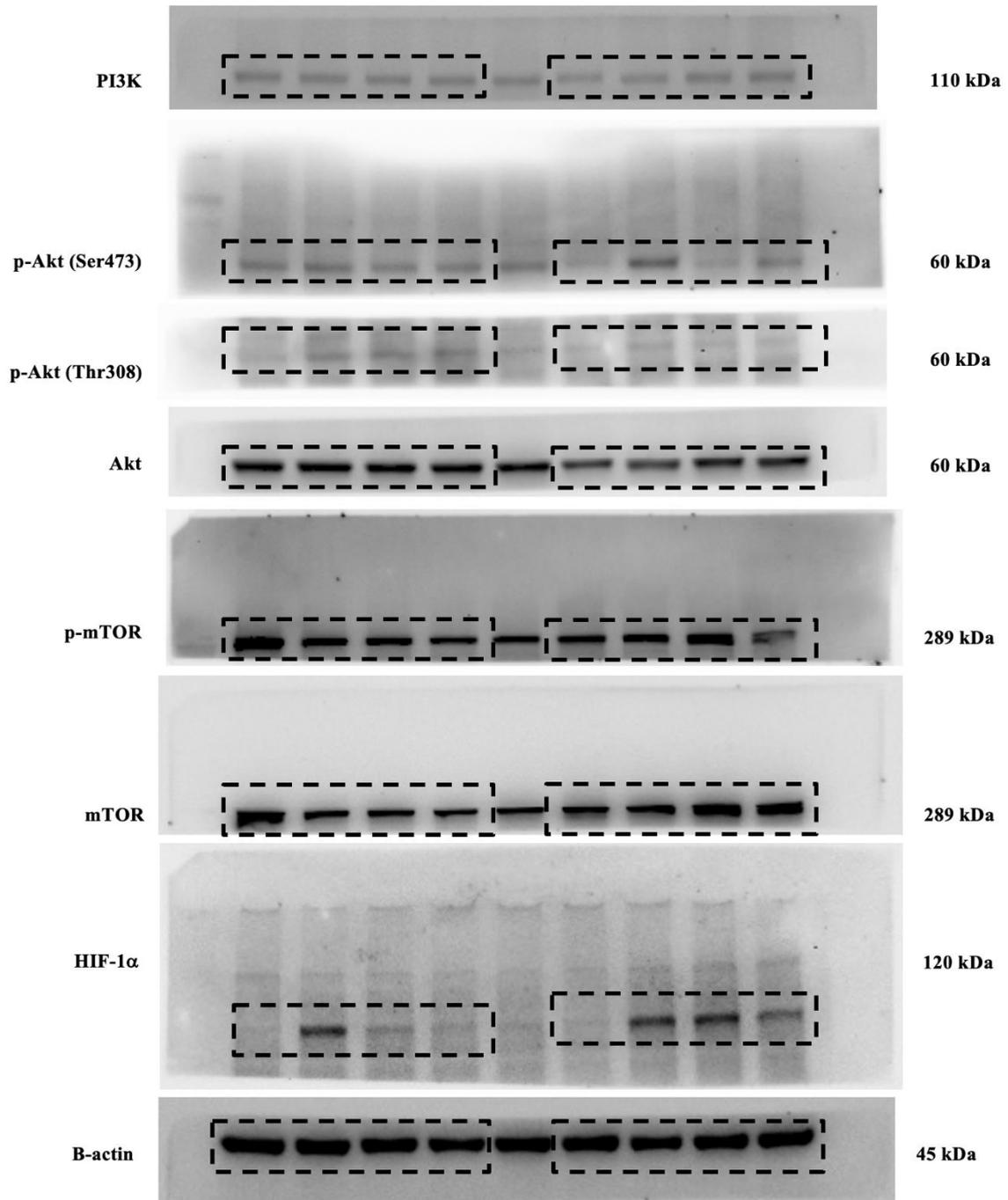
**Figure S2.** Full-length blots representing the protein expression of VEGF and  $\beta$ -actin as shown in Figure 5C of the manuscript.



**Figure S3.** Full-length blots representing the protein expression of PI3K/ Akt/ mTOR/ HIF-1 $\alpha$ , and  $\beta$ -actin as shown in Figure 6A of the manuscript.



|                           |   |   |   |   |   |   |   |   |
|---------------------------|---|---|---|---|---|---|---|---|
| CoCl <sub>2</sub> (150μM) | - | + | + | + | + | + | + | + |
| BKM120 (1 μM)             | - | - | - | - | - | + | - | - |
| M2206 (50 nM)             | - | - | - | - | - | - | + | - |
| Rapamycin (50 nM)         | - | - | - | - | - | - | - | + |
| Plumbagin (μM)            | 0 | 0 | 1 | 2 | 4 | 0 | 0 | 0 |



**Remark:** The membranes were cut separately, according to size of protein marker, in order to incubated with specific 1°Ab, 2°Ab and 5% nonfat dry milk in TBS-T, respectively before detected and imaged the bands with the ChemiDocTMTouch Imaging system.