

## Supplementary Information for

### **FBXO7, a tumor suppressor in endometrial carcinoma, suppresses INF2- associated mitochondrial division**

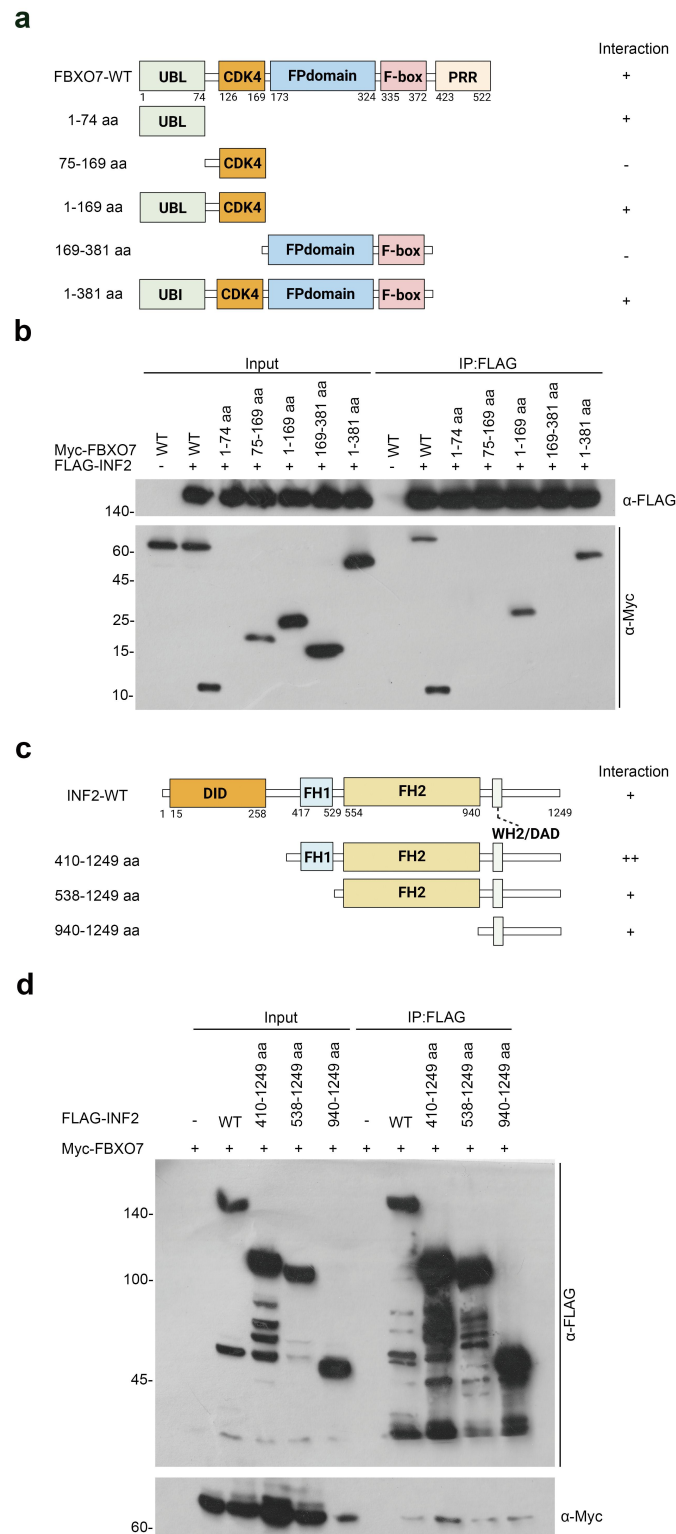
**This PDF file includes:**

Supplementary Fig. 1: Identify the key domains of FBXO7-INF2 interaction

Supplementary Fig. 2: FBXO7 suppresses HEC-1-A cells proliferation and migration

Supplementary Fig. 3: FBXO7 suppresses mitochondrial division in COS-7 cells.

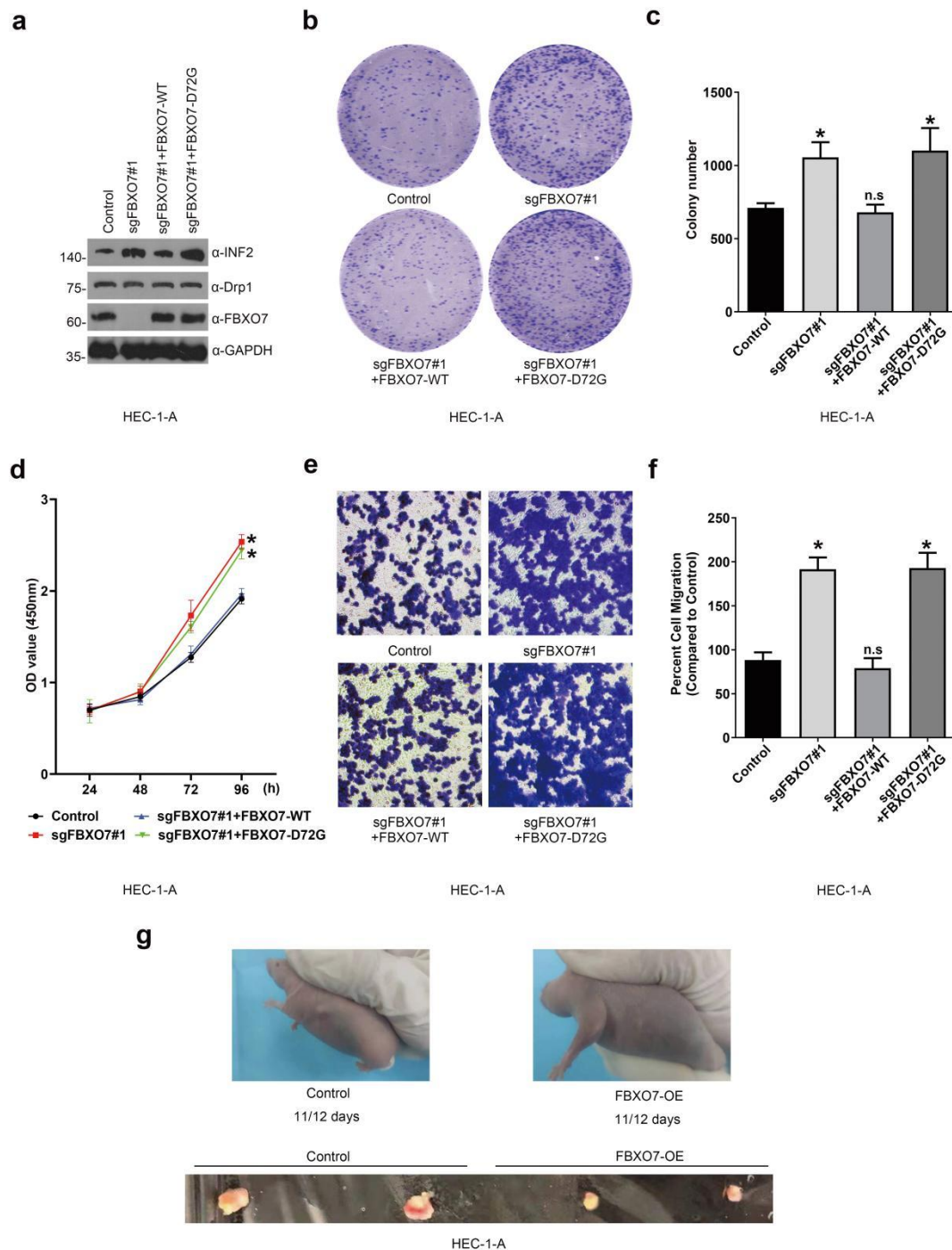
Supplementary Fig. 4: ECa-associated FBXO7 mutants may not have dominant negative effects.



**Supplementary Fig. 1: Identify the key domains of FBXO7-INF2 interaction**

- a) Diagram showing FBXO7-WT and FBXO7 mutants.
- b) Western blotting of WCLs and co-IP samples of anti-FLAG antibody obtained from HEK-293T cells transfected with indicated plasmids and treated with 20  $\mu$ M MG132 for 8 h.

- c) Diagram showing INF2-WT and INF2 mutants.
- d) Western blotting of WCLs and co-IP samples of anti-FLAG antibody obtained from HEK-293T cells transfected with indicated plasmids and treated with 20  $\mu$ M MG132 for 8 h.



**Supplementary Fig. 2: FBXO7 suppresses HEC-1-A cells proliferation and migration**

- a) Western blotting of WCLs of HEC-1-A cells with FBXO7 knockout and infected with FBXO7-WT

or FBXO7-D72G, or Control. All quantitation were normalized to the protein level of endogenous control GAPDH.

b) Cell colony formation assay of HEC-1-A cells with FBXO7 knockout and infected with FBXO7-WT or FBXO7-D72G, or Control.

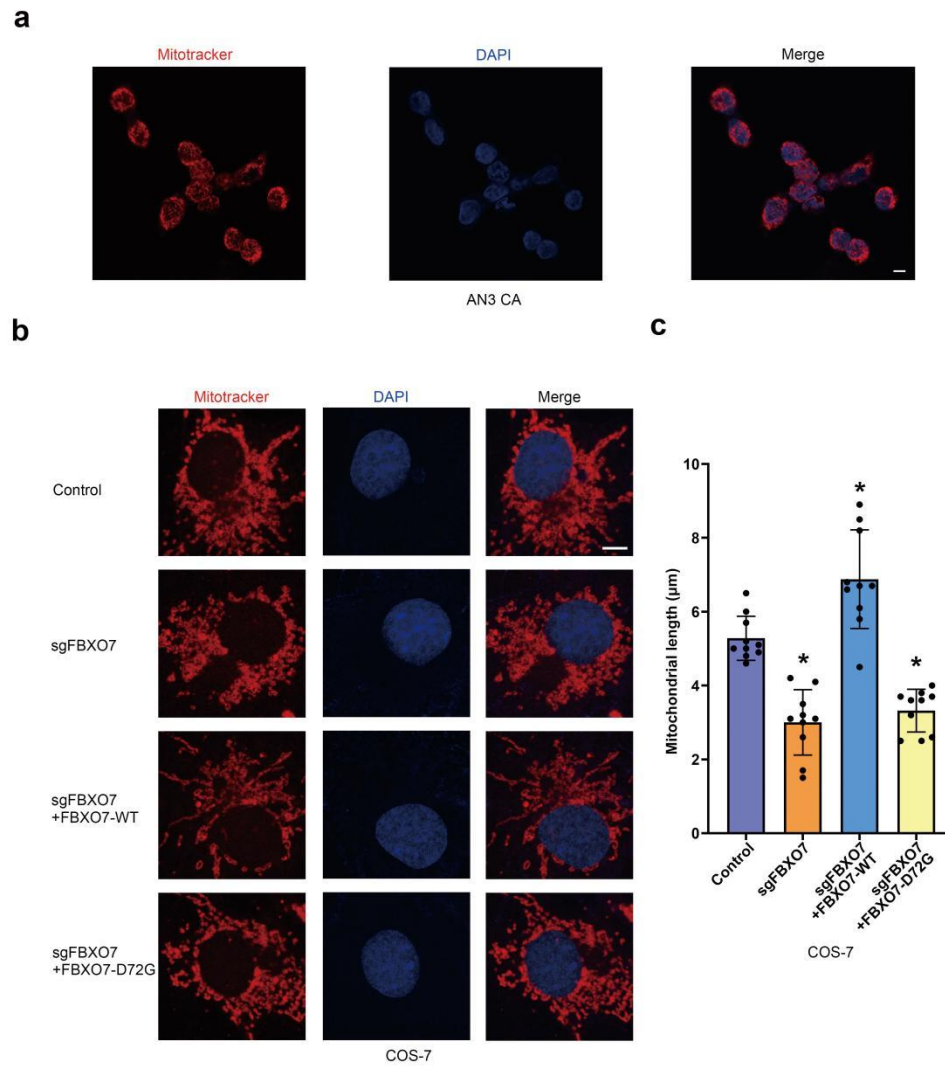
c) Statistics of cell colony formation assay. Data are shown as means  $\pm$  SD (n = 3). \* $p$  < 0.05 vs. the Control group; n.s vs. Control group.

d) Cell proliferation assay of HEC-1-A cells with FBXO7 knockout and infected with FBXO7-WT or FBXO7-D72G, or Control. Data are shown as means  $\pm$  SD (n = 5). \* $p$  < 0.05 vs. the Control group.

e) Cell migration assay of HEC-1-A cells with FBXO7 knockout and infected with FBXO7-WT or FBXO7-D72G, or Control.

f) Statistics of cell colony formation assay. Data are shown as means  $\pm$  SD (n = 3). \* $p$  < 0.05 vs. the Control group; n.s vs. Control group.

g) The tumor image of HEC-1-A cells with FBXO7 overexpression, or Control implanted tumors from nude mice.

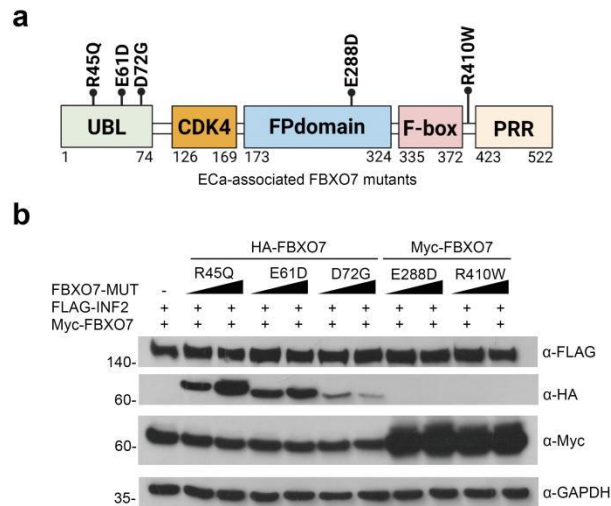


**Supplementary Fig. 3: FBXO7 suppresses mitochondrial division in COS-7 cells**

a) Images of AN3 CA cells. stained with Mitotracker Red and DAPI. Scale bar, 50  $\mu\text{m}$ .

b) Representative images of COS-7 cells with FBXO7 knockout and infected with FBXO7-WT or FBXO7-D72G, or Control. stained with Mitotracker Red and DAPI. Scale bar, 20  $\mu\text{m}$ .

c) Quantification of mitochondria lengths in (b). Data are shown as means  $\pm$  SD ( $n = 10$ ).  $*p < 0.05$  vs. the Control group.



**Supplementary Fig. 4: ECa-associated FBXO7 mutants may not have dominant negative effects.**

a) Diagram showing ECa-associated FBXO7 mutants.

b) Western blotting of WCLs of HEK-293T cells transfected with indicated plasmids. All quantitation were normalized to the protein level of endogenous control GAPDH.

Supplementary Table 1: The primers sequence

| RT-qPCR Primers      |                                                                  |
|----------------------|------------------------------------------------------------------|
| Gene                 | Sequence 5'-3'                                                   |
| FBXO7                | F: AGGCCTTTTCCTCCCAGTT                                           |
|                      | R: TCTCTCATCGTATTACACCA                                          |
| INF2                 | F: GGGTCCCCAGCCATCGGCG                                           |
|                      | R: TTGGCATCGAGGAAAGTGA                                           |
| GAPDH                | F: CATGGCCTTCCGTGTTCTTA                                          |
|                      | R: CCCTCAGATGCCTGCTTCA                                           |
| Construction Primers |                                                                  |
| Gene                 | Sequence 5'-3'                                                   |
| FBXO7                | F: AAAGAATTTCGCATGAGGCTGCGGGTGC GGCTTCTGAA                       |
|                      | R: AAAGCGGCCGCTCACATGAATGACAGCCGGCCATCAGTTG                      |
| INF2                 | F: AAAGAATTTCGCATGTCGGTGAAGGAGGGCGCA                             |
|                      | R: AAATCGAGTCACTTGGCCTTGGGCCTGGGCCTGAGGC                         |
| FBXO7<br>Δ UBL       | F: AAAGAATTTCGCTGTTTGATTCTTCAAGATGACATTCCAGC                     |
| FBXO7<br>1-74 AA     | R: AAAGCGGCCGCTCATATCAAGTCCCCAGAAACAA                            |
| FBXO7<br>1-169 AA    | R: CTGCCGTTTCGACGATGCGGCCGCTCACATGGGTTCTGAGGGATAGA               |
| FBXO7<br>169-381 AA  | F: AAAGAATTTCGCCTCTGTAGTGAATCGGTGGAAGGCAA                        |
| FBXO7<br>1-381 AA    | R: CTGCCGTTTCGACGATGCGGCCGCTCGAAAATCACGCAGATATAAAAAC             |
| FBXO7<br>R45Q        | F: CAGTTCTAATACCCAATTTACAATTACAT                                 |
| FBXO7                | R: TAGTTCAATGTAATTGTAAATTGGGTATTAG                               |
| FBXO7                | F: CCCCTCACTGGAGATGAAGACACCTTGGCTTC                              |
| E61D                 | R: CCCATATGAAGCCAAGGTGTCTTCATCTCCA                               |
| FBXO7                | F: TATGGGATTGTTTCTGGGGGCTTGATATGTTTG                             |
| D72G                 | R: AAGAATCAAACATATCAAGCCCCAGAAACAATC                             |
| INF2<br>1-940 AA     | R: AAAGCGGCCGCTTACCTCCTCTCTGCCTTCGCCGCCTG                        |
| INF2<br>V530X        | R: AAAGCGGCCGCTTAGGGGGGGCTGCAGGTGCAGG                            |
| INF2                 | F: GAGATGCTCAGGCCCTGAAGCCCATCAAGTTCTCCAGCA                       |
| L1114I               | R: GCAGGGGGCTGGTTGCTGGAGAACTTGATGGGCTTCAGGGCCTGAG                |
|                      | F: ATGAGGACGAGGACGAGGAGGACATGGCCCCAGAGTCCGCACTG                  |
| INF2<br>T1179M       | R:<br>TTGTCCAGGGATGTGTCCAGTGCGGACTCTGGGGCCATGTCCTCCTCGTCC<br>TCG |

Supplementary Table 2: Sequences of sgRNA and siRNA

| Gene    | Sequence 5'-3'           |
|---------|--------------------------|
| sgFBXO7 | F: GTCTGGTGTGTTGGAATGACG |
| siINF2  | F: ACAAAGAACTGTGTGTGTGA  |

Supplementary Table 3: Antibody and Chemicals

| No. | Name                       | Species | Cat No.    | Source         |
|-----|----------------------------|---------|------------|----------------|
| 1   | Anti-FBXO7                 | Rabbit  | 10696-1-AP | Proteintech    |
| 2   | Anti-INF2                  | Rabbit  | A10038     | Abclonal       |
| 3   | Anti-INF2                  | Mouse   | 66910-1-Ig | Proteintech    |
| 4   | Anti-Drp1                  | Rabbit  | ab184247   | abcam          |
| 5   | Anti-GAPDH                 | Rabbit  | AC001      | Abclonal       |
| 6   | Anti-Myc                   | Mouse   | M192-7     | MBL            |
| 7   | Anti-FLAG                  | Mouse   | M185-7     | MBL            |
| 8   | Anti-HA                    | Mouse   | M180-7     | MBL            |
| 9   | Anti-FLAG M2 agarose beads | Mouse   | M8823      | Sigma          |
| 10  | Anti-Parkin                | Rabbit  | A11172     | Abclonal       |
| 11  | FLAG peptide               |         | F4799      | Sigma          |
| 12  | MG132                      |         | A2585      | APExBIO        |
| 13  | Mdivi-1                    |         | A4427      | APExBIO        |
| 14  | CHX                        |         | HY-B0713   | MCE            |
| 15  | Mitotracker                |         | M7512      | Thermo Fisher, |