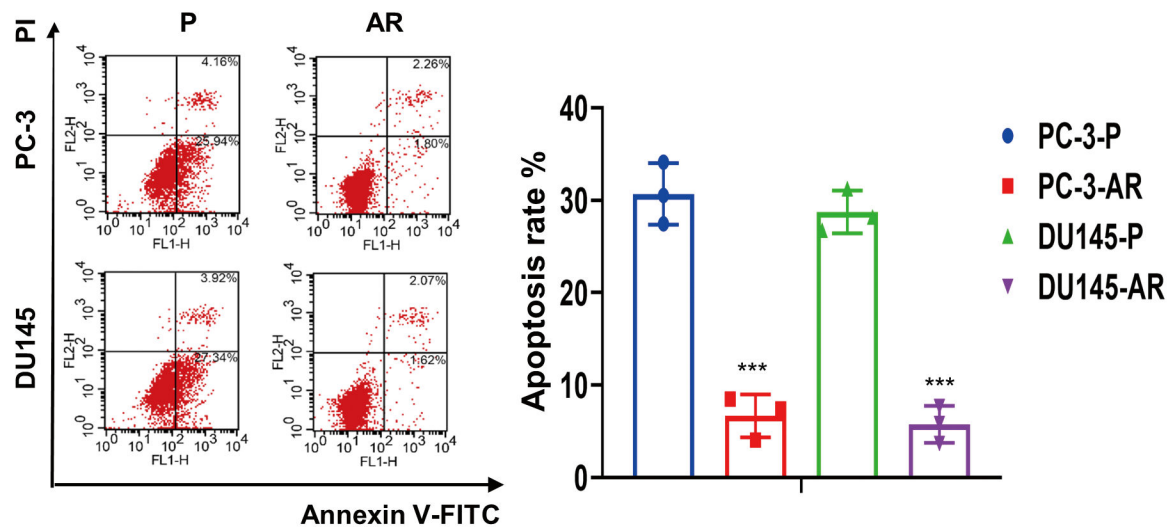
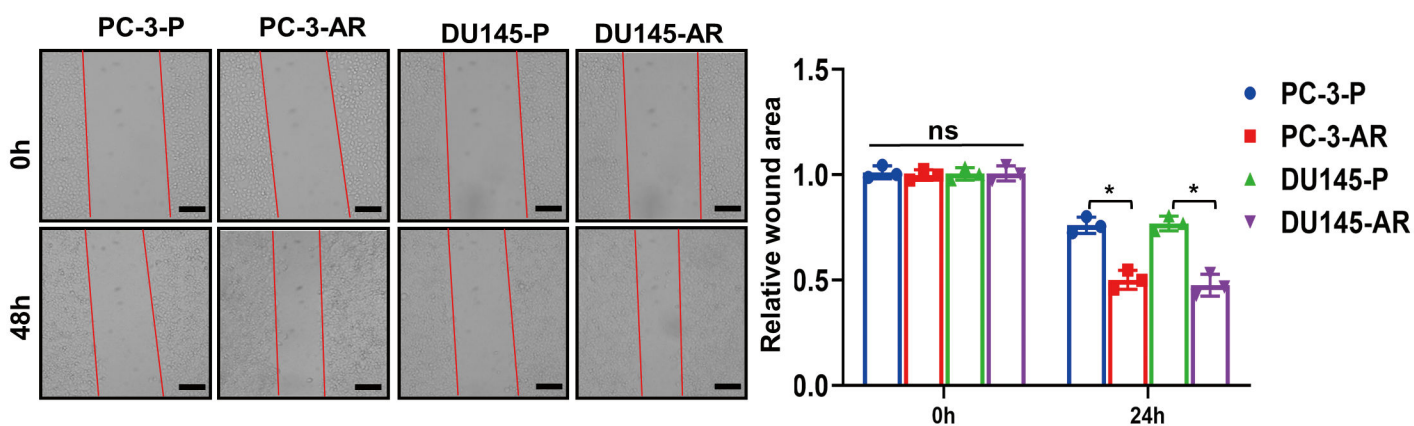


Fig. S1

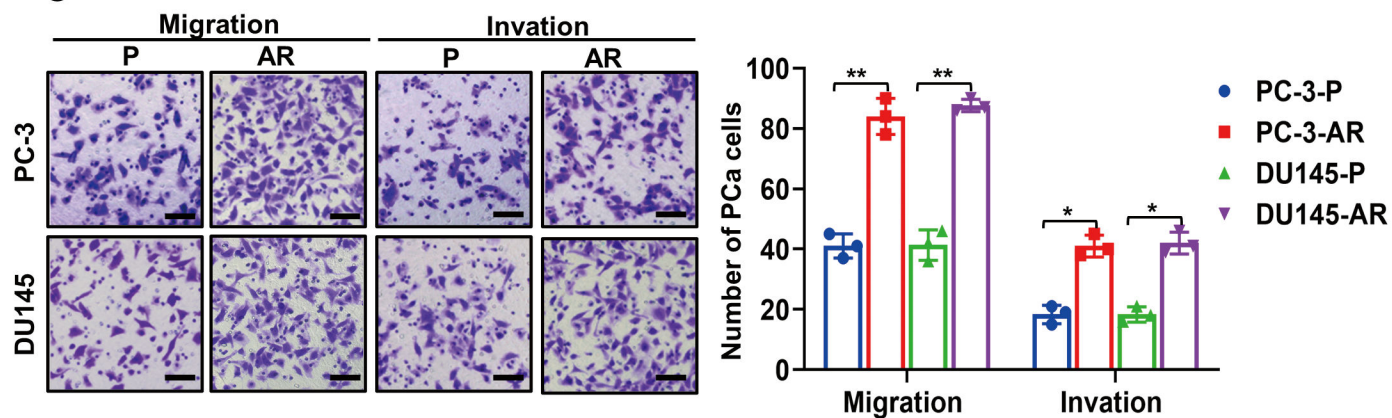
A



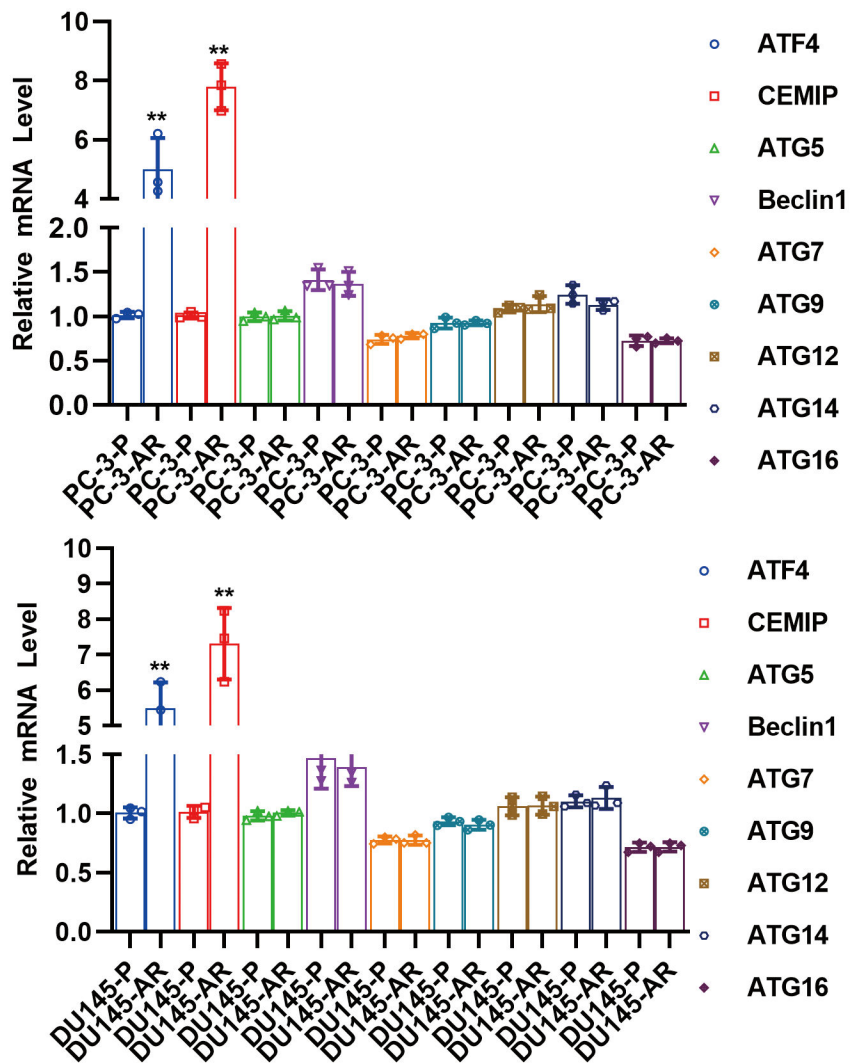
B



C



**A**



**Fig. S3**

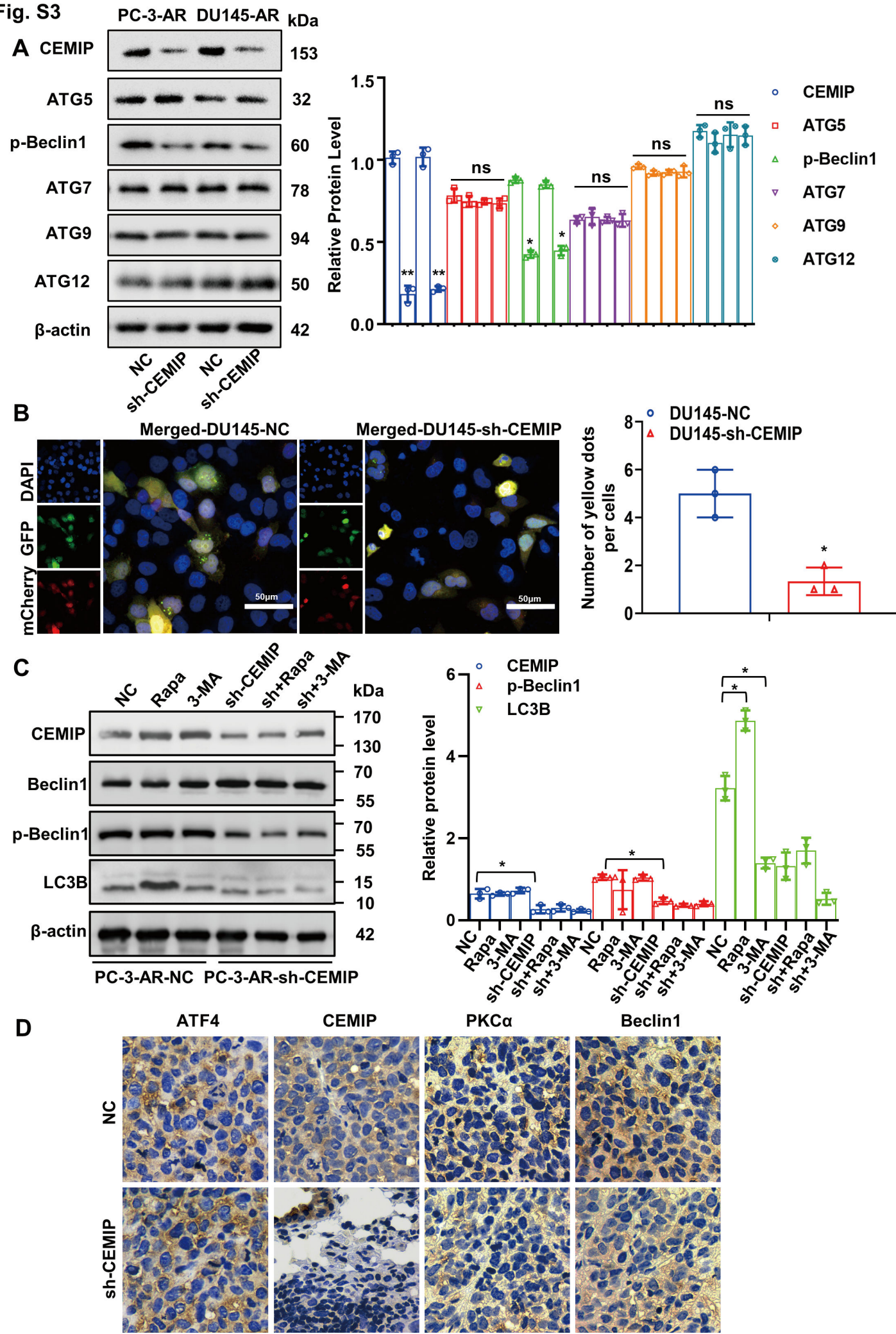
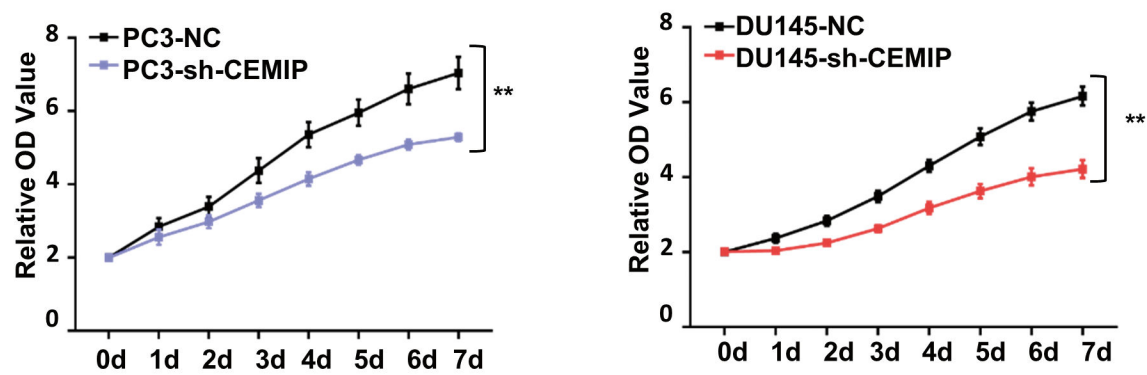
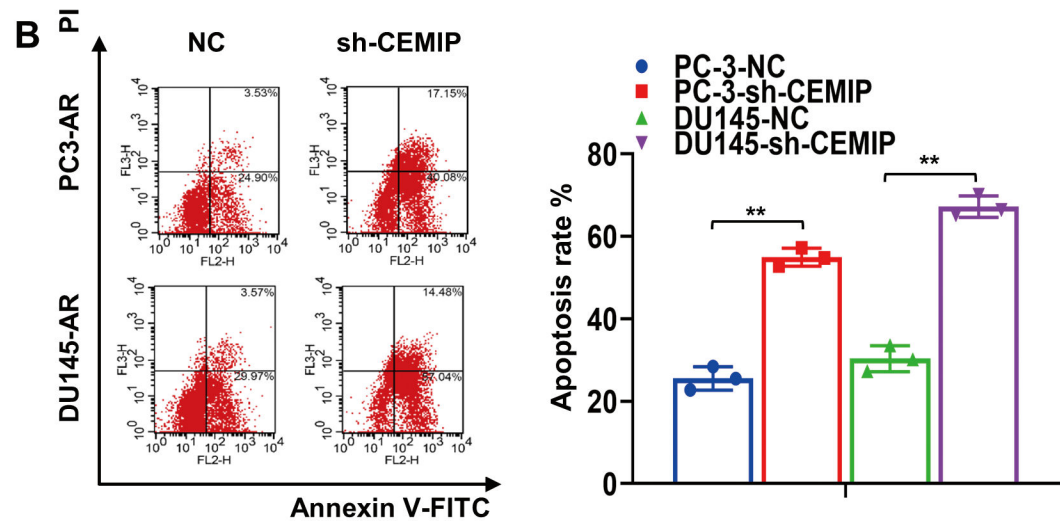


Fig. S4

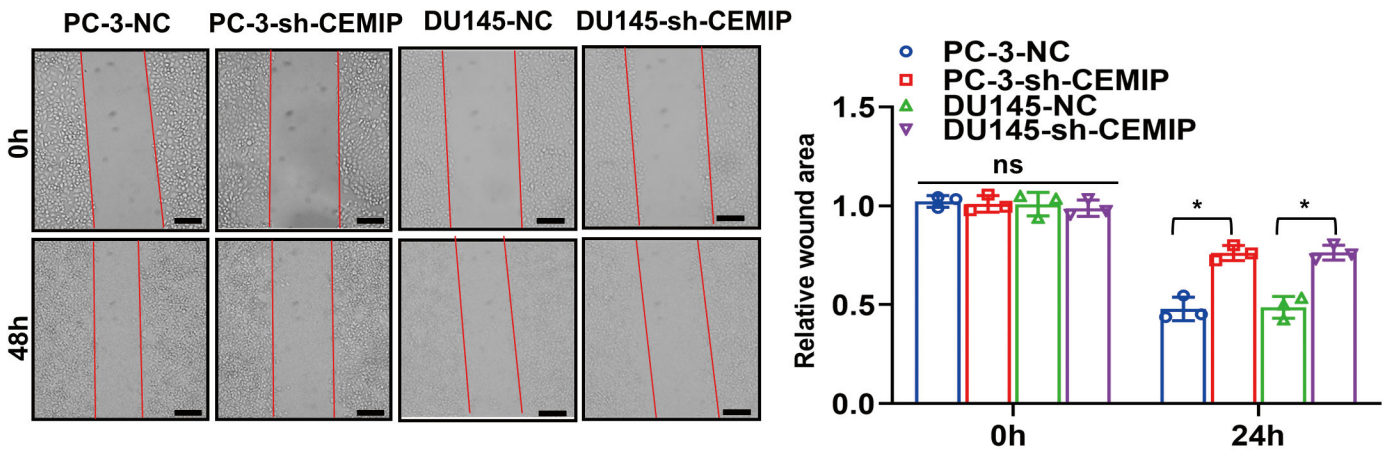
A



B



C



D

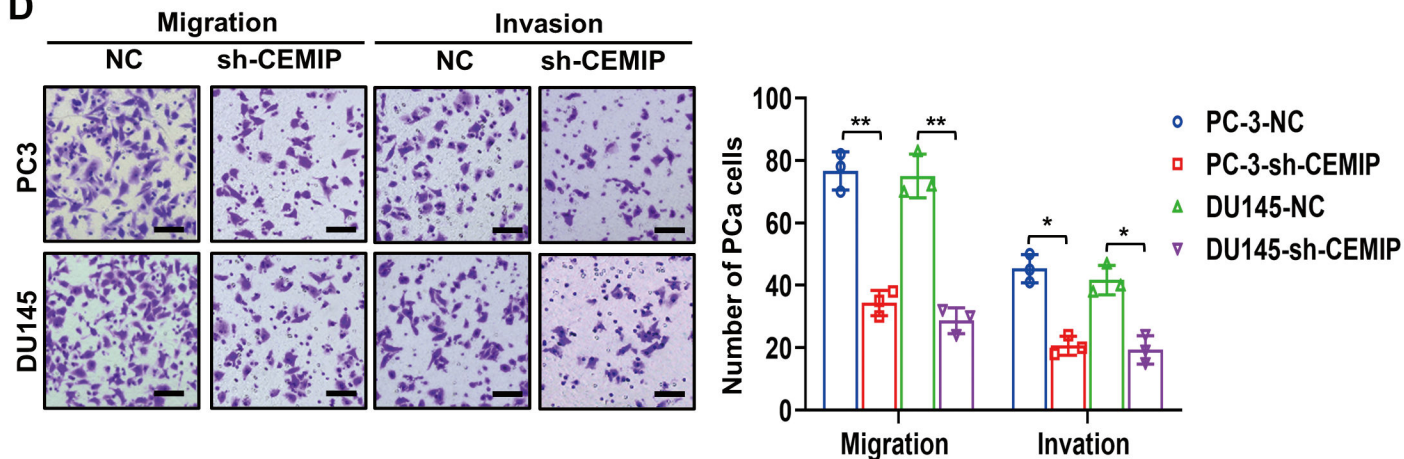


Fig. S5

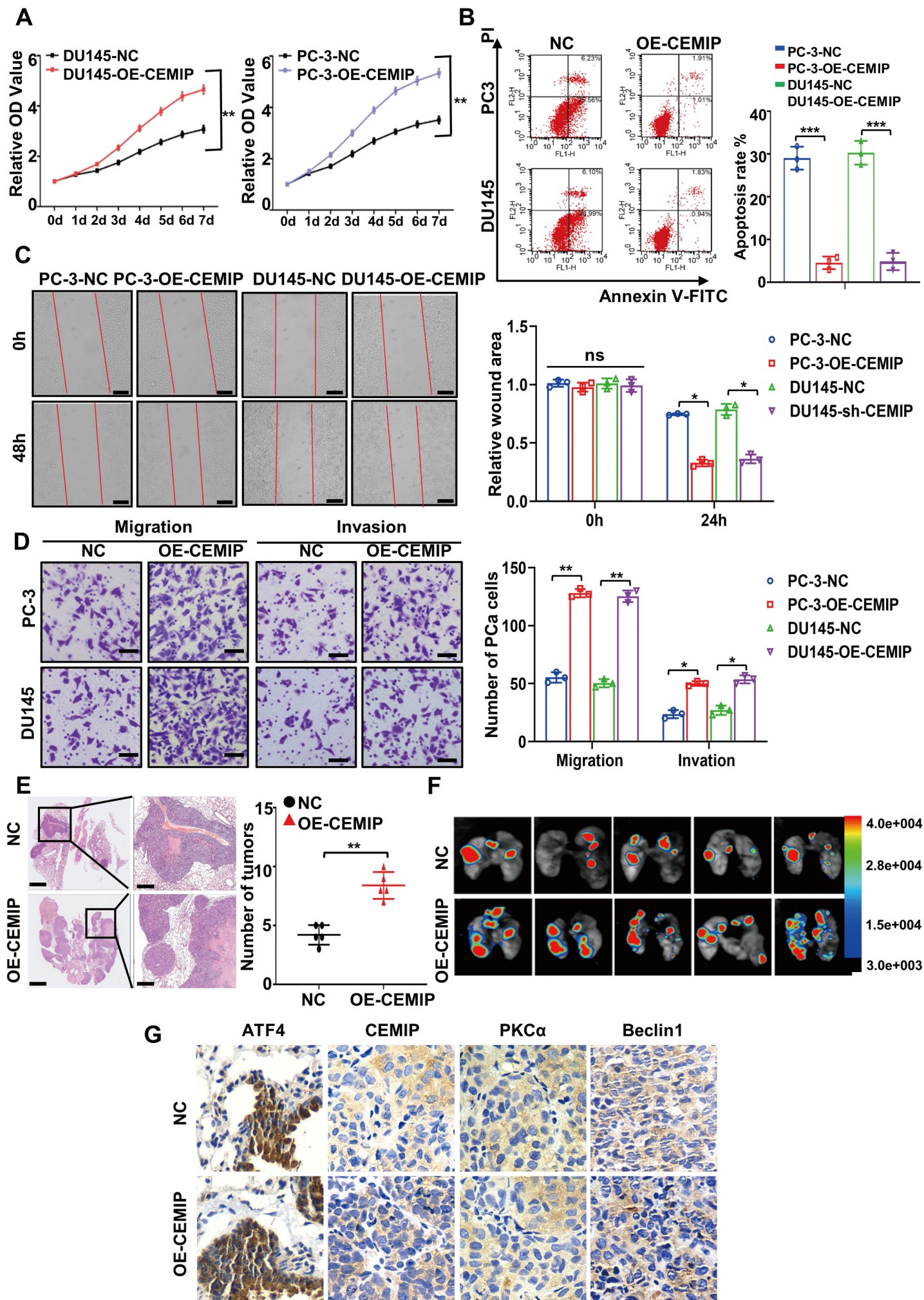


Fig. S6

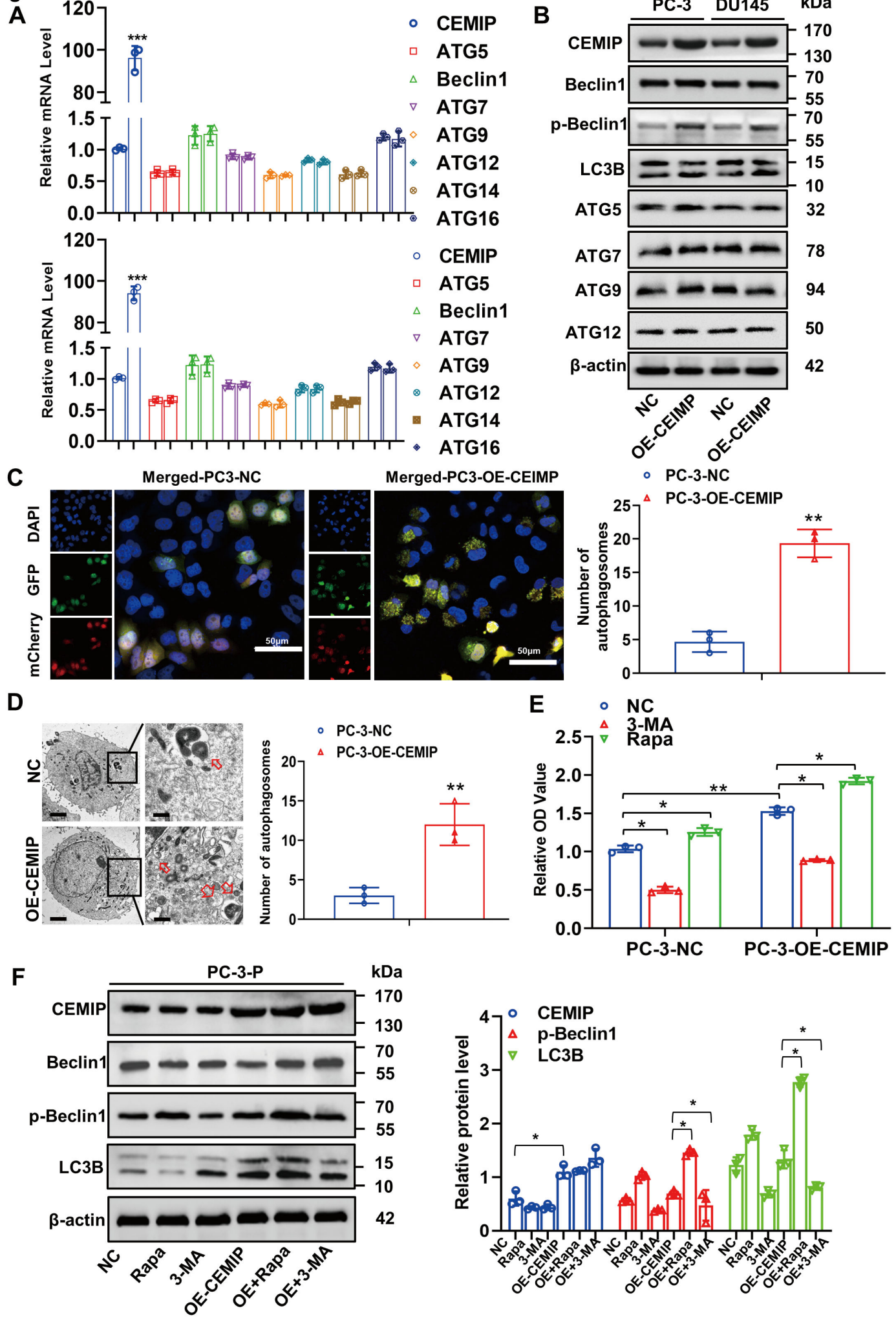


Fig. S7

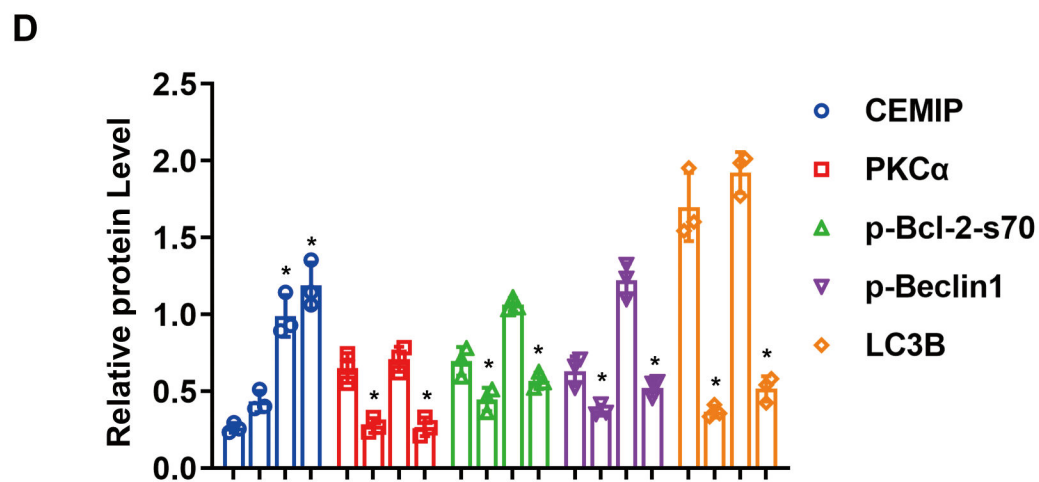
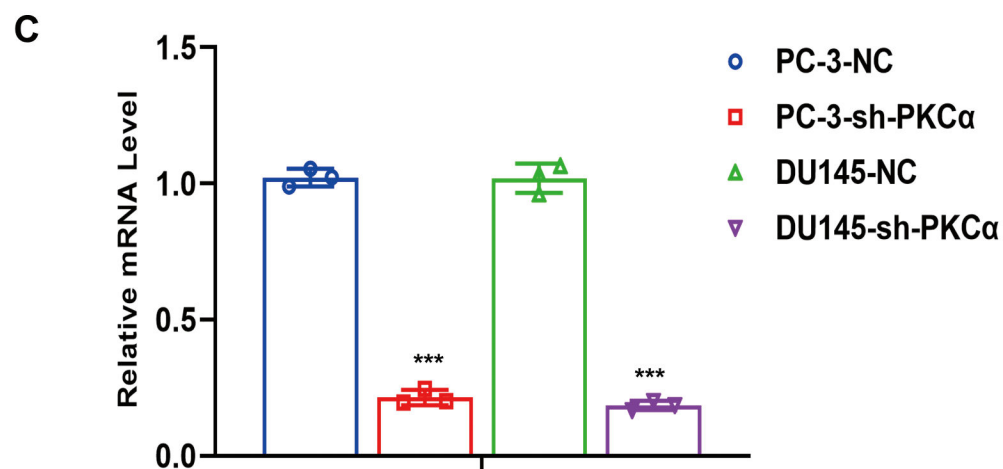
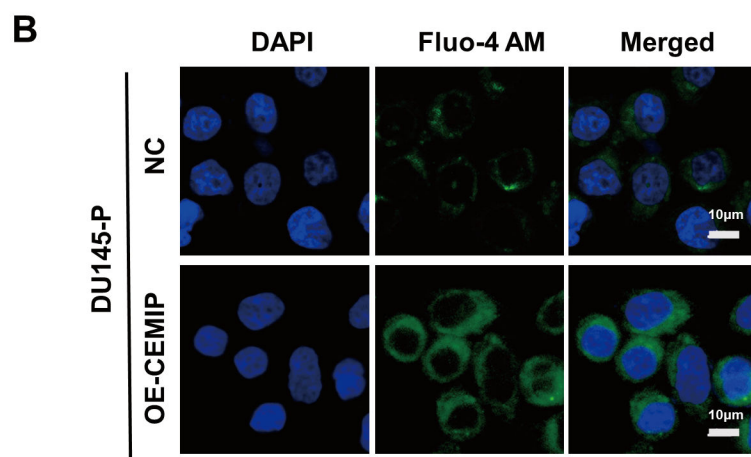
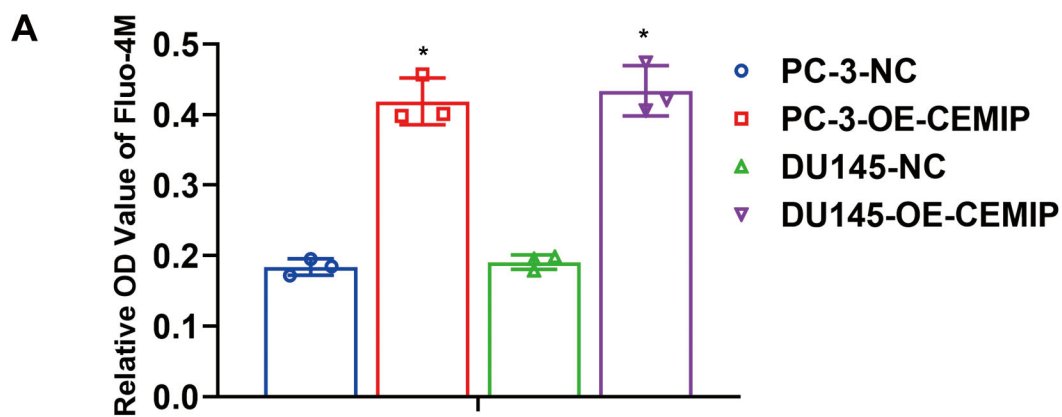
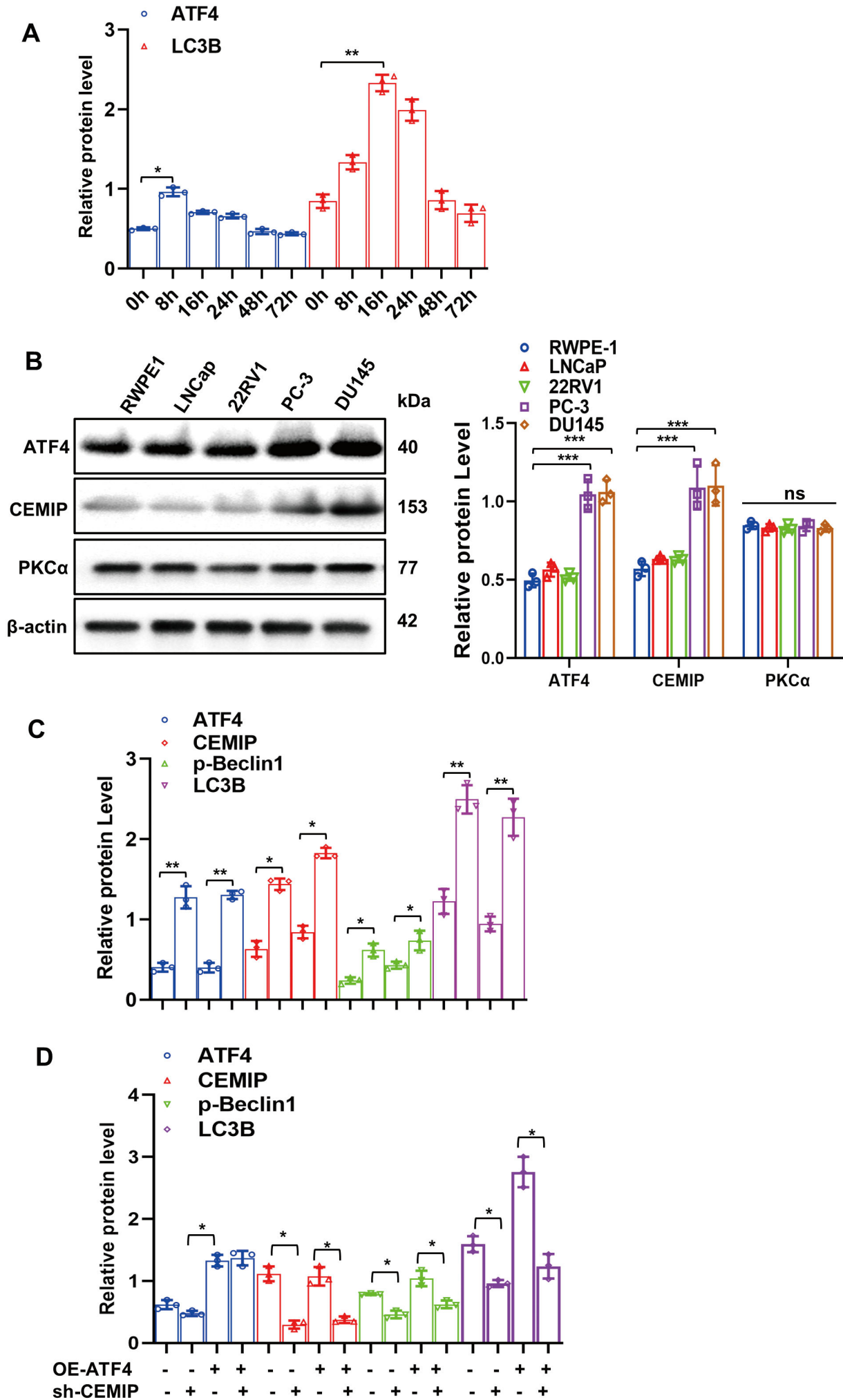


Fig. S8



**Figure S1: Anoikis-resistant PCa cells enhance the capabilities of survival and migration.**

(A) Flow cytometry assay demonstrated the rate of detachment-induced apoptosis in PCa-P and PCa-AR cells.

(B) Wound scratch assay indicated the abilities of migration in parental and anoikis resistant PC-3 and DU145 cells. (Original magnification,  $\times 200$ ).

(C) The migration and invasion capabilities of the parental and anoikis-resistant PCa cells were evaluated by transwell migration and matrigel invasion assays. (Original magnification,  $\times 200$ ).

Data are presented as the means  $\pm$  SD of three independent experiments;  $*p < 0.05$ ,  $**p < 0.01$ .

**Figure S2: Anoikis-resistant PCa cells promote the activity of autophagy.**

(A) Immunofluorescence staining demonstrated the level of autophagic flux in parental and anoikis-resistant DU145 cells. Autophagy flux were detected in 2D culture, and a total of more than 20 cells under each condition were counted for quantification of autophagy (Scale bar, 50  $\mu\text{m}$ ).

(B) RT-PCR assay analysis of the mRNA levels of ATF4, CEMIP, Beclin1 and autophagy related ATGs.  $*p < 0.05$ ,  $**p < 0.01$ .

Data are presented as the means  $\pm$  SD of three independent experiments;  $*p < 0.05$ ,  $**p < 0.01$ .

**Figure S3: Downregulation of CEMIP in anoikis-resistant PCa cells impairs the activity of autophagy.**

(A) The protein expression levels of ATF4, CEMIP, Beclin1, ATG5, ATG7, ATG9 and ATG12 in PC-3 cells with CEMIP knockdown were detected by western blot analysis.

(B) The autophagic flux level of CEMIP-silenced DU145-AR cells was presented by immunofluorescence staining. Autophagy flux were detected in 2D culture, and a total of more than 20 cells under each condition were counted for quantification of autophagy (Scale bar, 50  $\mu$ m).

(C) Western blot analysis of the reversion efficiency in autophagy of CEMIP in PC-3-AR cells on the autophagy related proteins (Beclin1, p-Beclin1, LC3BII/I) after the treatment of Rapamycin or 3-MA.

(D) The protein expression levels of ATF4, CEMIP, PKC $\alpha$  and Beclin1 were detected by immunohistochemistry in the lung metastases of CEMIP knockdown nude mice.

Data are presented as the means  $\pm$  SD of three independent experiments; \* $p < 0.05$ , \*\* $p < 0.01$ .

**Figure S4: Downregulation of CEMIP attenuates cell survival and migration in anoikis-resistant PCa cells.**

(A) Cell viability of CEMIP-downregulated in PCa-AR cells was detected by CCK-8 assay.

(B) Flow cytometry assay certified the detachment-induced apoptosis rate of downregulation of CEMIP in PCa-AR cells.

(C) Wound scratch assay showed the migration capabilities of CEMIP-downregulated in anoikis-resistant PC-3 and DU145 cells (Original magnification,  $\times 200$ ).

(D) The migration and invasion abilities of CEMIP downregulated in anoikis-resistant PC-3 and DU145 cells were evaluated by transwell migration and matrigel invasion assays. (Original magnification,  $\times 200$ ).

Data are presented as the means  $\pm$  SD of three independent experiments;  $*p < 0.05$ ,  $**p < 0.01$ .

**Figure S5: Overexpression of CEMIP enhances migration and survival in parental PCa cells.**

(A) The cell survival ability in CEMIP-overexpressing PC-3-P and DU145-P cells was demonstrated by cell viability assay.

(B) The detachment-induced apoptosis rate in CEMIP-overexpressing PCa-P cells was detected by flow cytometry assay.

(C) Wound scratch assay certified the migration capabilities of CEMIP-overexpressing PC-3 and DU145 cells (Original magnification,  $\times 200$ ).

(D) Transwell migration and matrigel invasion assays certified the abilities of migration and invasion in CEMIP overexpressing PC-3 and DU145 cells. (Original magnification,  $\times 200$ ).

(E) H&E staining demonstrated that CEMIP-overexpressed in PCa cells led to increased lung metastatic colonies. (n = 5 per group, Original magnification,  $\times 10$ ,  $\times 100$ , respectively).

(F) Bioluminescence in vivo imaging showed the number of pulmonary metastasis focuses in CEMIP-overexpressed group and negative control group (n = 5 per group).

(G) The protein expression levels of ATF4, CEMIP, PKC $\alpha$  and Beclin1 were detected by immunohistochemistry in the lung metastases of CEMIP overexpressed nude mice.

Data are presented as the means  $\pm$  SD of three independent experiments; \* $p$  < 0.05.

**Figure S6: Overexpression of CEMIP enhances protective autophagy in PCa cells.**

(A) qRT-PCR analysis of the mRNA level of CEMIP, Beclin-1, and autophagy related ATGs in CEMIP-overexpressing PC-3 and DU145 cells.

(B) The protein expression levels of CEMIP, Beclin1, p-Beclin1, LC3BII/LC3BI, ATG5, ATG7, ATG9, ATG12 and in PC-3-P and DU145-P cells overexpressing CEMIP were detected by western blot analysis.

(C) Immunofluorescence staining certified the level of autophagic flux in CEMIP overexpressing PC-3 and DU145 cells. Autophagy flux were detected in 2D culture, and a total of more than 20 cells under each condition were counted for quantification of autophagy (Scale bar, 50  $\mu$ m).

(D) The number of double-membrane autophagosomes in CEMIP-overexpressing PC-3 cells were detected by transmission electron microscopy. (Original magnification,  $\times$ 1000,  $\times$ 1600, respectively).

(E) Rescue cell viability assay proved the reversion efficiency of cell survival rate by treating with rapamycin or 3-MA after CEMIP overexpressed in PC-3-P and DU145-P cells.

(F) Western blot analysis of the proteins level of CEMIP, p-Beclin1 and LC3BII/LC3BI ratio in CEMIP-overexpressing DU145-P cells after the treatment of Rapa or 3-MA.

Data are presented as the means  $\pm$  SD of three independent experiments; \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ .

**Figure S7: Overexpression of CEMIP enhances PKC $\alpha$  membrane translocation by aggregating of calcium ions in the cytoplasm.**

(A) Immunofluorescence staining demonstrated the levels of cytoplasmic calcium ions in CEMIP-overexpressing and parental DU145 cells. Nuclei were stained blue by DAPI, cytoplasmic calcium ions were stained by Fluo4AM (Scale bar, 10  $\mu$ m).

(B) qRT-PCR assay verified the effective downregulation of CEMIP after transfection for 48h in PC-3 and DU145 cells.

(C) The levels of cytoplasmic calcium ions in CEMIP overexpressing PC-3 and DU145 cells were shown by Enzyme linked immunosorbent assay after the treatment of the calcium ion probe (Fluo-4 AM) was incubated at 37°C for half an hour.

(D) The protein expression levels of CEMIP, PKC $\alpha$ , Beclin1, p-Beclin1, p-Bcl-2-S70 and LC3BII/LC3BI in PC-3 cells co-transformed with overexpressed CEMIP and knockdown PKC $\alpha$  were quantified by histogram.

Data are presented as the means  $\pm$  SD of three independent experiments; \* $p < 0.05$ , \*\* $p < 0.01$ .

**Figure S8: Upregulation of ATF4 promotes CEMIP transcription and autophagy activation**

(A) Quantification of ATF4 and LC3BII/LC3BI protein levels at different suspension time points in PC-3 cells.

(B) The protein expression levels of ATF4, CEMP and PKC $\alpha$  in prostate epithelial cells (RWPE-1) and four common prostate cancer cell lines (LNCaP, 22RV1, PC-3, DU145) were detected by Western blot assay.

(C) Quantification of CEMIP, p-Beclin1 and LC3BII/LC3BI protein levels in PC-3-P cells after stable overexpression of ATF4.

(D) Protein levels of ATF4, CEMIP, p-Beclin1 and LC3B were quantified after down-regulation of CEMIP in PC-3-P cells with stable overexpression of ATF4.

Data are presented as the means  $\pm$  SD of three independent experiments; \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ .

**Supplementary Table 1: qRT-PCR primer sequences in this study**

| <b>Gene names</b> | <b>Primer sequences</b>  |
|-------------------|--------------------------|
| GAPDH-F           | GGTCGGAGTCAACGGATTG      |
| GAPDH-R           | GGAAGATGGTGATGGGATTTC    |
| ATF4-F            | CTCCGGGACAGATTGGATGTT    |
| ATF4-R            | GGCTGCTTATTAGTCTCCTGGAC  |
| ATG5-F            | AAAGATGTGCTTCGAGATGTGT   |
| ATG5-R            | CACTTTGTCAGTTACCAACGTCA  |
| ATG7-F            | ATTGCTGCATCAAGAAACCC     |
| ATG7-R            | GATGGAGAGCTCCTCAGCA      |
| ATG12-F           | TAGAGCGAACACGAACCATCC    |
| ATG12-R           | CACTGCCAAAACACTCATAGAGA  |
| Beclin1-F         | AGGATGATGTCCACAGAAAGTGC  |
| Beclin1-R         | AGTGACCTTCAGTCTTCGGCTG   |
| ATG9L1-F          | TGTTTCTCAATGAATGGAGCCTC  |
| ATG9L1-R          | AAGTTAGCGATGCCAATCCAC    |
| ATG14L-F          | GCAAATCTTCGACGATCCCAT    |
| ATG14L-R          | CACACCCGTCTTTACTTCCTC    |
| ATG16L1-F         | GGCAGTAGCTGGTACCCTCACTTC |
| ATG16L1-R         | CTCTGTCTCTTCCTTCCCAGTCC  |
| PKC $\alpha$ -F   | TGGACTTATCCATCAAGGGATGA  |
| PKC $\alpha$ -R   | AGTGTGATCCATTCCGCAGAG    |
| BCL2-F            | GGTGGGGTCATGTGTGTGG      |
| BCL2-R            | CGGTTTCAGGTACTCAGTCATCC  |
| CEMIP-F           | GGCCGGTGATGTAGACGAAA     |
| CEMIP-R           | CCATTGGAGCCATGGACTGT     |

**Supplementary Table 2: Primary and secondary antibodies**

| Antibody                  | Source      | Identifier | Host   |
|---------------------------|-------------|------------|--------|
| CEMIP antibody            | Abcam       | ab62322    | Rabbit |
| ATF4 antibody             | Proteintech | 10835-1-AP | Rabbit |
| PKC $\alpha$ antibody     | Proteintech | 21991-1-AP | Rabbit |
| Bcl-2 antibody            | CST         | 15071S     | Mouse  |
| p-Bcl-2 antibody          | CST         | 2827S      | Rabbit |
| Beclin1                   | CST         | 3738S      | Rabbit |
| p-Beclin1                 | CST         | 14717S     | Rabbit |
| LC3B                      | Abcam       | ab192890   | Rabbit |
| $\beta$ -actin            | Proteintech | 66009-1-Ig | Mouse  |
| 488 - Anti-Mouse IgG(H+L) | Proteintech | SA00013-1  | Mouse  |
| 594 –Anti-Rabbit IgG(H+L) | Proteintech | SA00013-4  | Rabbit |
| ATG5                      | Proteintech | 10181-2-AP | Rabbit |
| ATG7                      | Proteintech | 10088-2-AP | Rabbit |
| ATG9                      | Proteintech | 26276-1-AP | Rabbit |
| ATG12                     | Proteintech | 11122-1-AP | Rabbit |

**Supplementary Table 3: Drugs and reagents**

| Drug / Reagent                   | Source              | Identifier/<br>formulation |
|----------------------------------|---------------------|----------------------------|
| FITC-Annexin V apoptosis         | BD Biosciences kit  | 556547                     |
| PE-Annexin V apoptosis detection | BD Biosciences kit  | 559763                     |
| CCK-8                            | Vazyme Biotech      | A311-01                    |
| rapamycin                        | Selleck             | S1039                      |
| 3-Methyladenine                  | Selleck             | S2767                      |
| Chromatin immunoprecipitation    | Beyotime (ChIP) kit | P2078                      |
| 7-AAD                            | BD Biosciences kit  | 559925                     |

**Supplementary Table 4: Sequence of Bcl-2 mutations in the study**

| Human Bcl-2 point mutations that mimic phosphorylation sequences                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |  |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--|
| ATGGCGCACGCTGGGAGAACAGGGTACGATAACCGGGAGATAGTGATGA<br>AGTACATCCATTATAAGCTGTCTGCAGAGGGGGCTACGAGTGGGATGCGGG<br>AGATGTGGGCGCCGCGCCCCCGGGGGCCGCCCCCGCACCGGGCATCTTC<br>TCCTCCCAGCCCGGGCACACGCCCCATCCAGCCGCATCCCGGGACCCGG<br>TCGCCAGGACCGATCCGCTGCAGACCCCGGCTGCCCCCGGCGCCGCCGC<br>GGGGCCTGCGCTCAGCCCGGTGCCACCTGTGGTCCACCTGACCCTCCGC<br>CAGGCCGGCGACGACTTCTCCCGCCGCTACCGCCGCGACTTCGCCGAGA<br>TGTCCAGCCAGCTGCACCTGACGCCCTTCACCGCGCGGGGACGCTTTGC<br>CACGGTGGTGGAGGAGCTCTTCAGGGACGGGGTGAAGTGGGGGAGGAT<br>TGTGGCCTTCTTTGAGTTCGGTGGGGTCATGTGTGTGGAGAGCGTCAAC<br>CGGGAGATGTCGCCCCTGGTGGACAACATCGCCCTGTGGATGACTGAGT<br>ACCTGAACCGGCACCTGCACACCTGGATCCAGGATAACGGAGGCTGGGA<br>TGCCTTTGTGGAAGTGTACGGCCCCAGCATGCGGCCTCTGTTTGATTTCT<br>CCTGGCTGTCTCTGAAGACTCTGCTCAGTTTGGCCCTGGTGGGAGCTTGC<br>ATCACCTGGGTGCCTATCTGGGCCACAAGTGA |  |

**Supplementary Table 5: Sequences of knockdown in the study**

| Gene names          | Sequences                                                     |
|---------------------|---------------------------------------------------------------|
| CEMIP shRNA1        | CCAGGAATGTTGAATGTCTTTTTCAAGAGAAAAGACA<br>TTCAACATTCCTGGTTTTTT |
| CEMIP shRNA2        | CGAATGAAGATCATCAAGAATTTCAAGAGAATTCTTG<br>ATGATCTTCATTCGTTTTTT |
| CEMIP shRNA3        | GATCCTTACTATGGTCTGATTCAAGAGATCAGACCATA<br>GTAAGGATCTTTTTT     |
| PKC $\alpha$ shRNA1 | GCGTCCTGTTGTATGAAATTTCAAGAGAATTTCATACA<br>ACAGGACGCTTTTTT     |
| PKC $\alpha$ shRNA2 | AAGCTCCATGTACAGTACGATTCAAGAGATCGTACT<br>GTGACATGGAGCTTTTTTT   |
| PKC $\alpha$ shRNA3 | CCATCCGCTCCACACTAAATTCAAGAGATTTAGTGTGG<br>AGCGGATGGTTTTTT     |

**Supplementary Table 6: Predicted ATF4 binding sites on CEMIP promoter**

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NC\_000015.10: 80777273-80779525 Homo sapiens chromosome 15, RCh38.p12

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ATGGATTGCGAAAGTGTGGGCAGTAAATCCACCAGTGT**TCATGTTGCTATAT**  
TTATCCATAAAGAATTACGCGGCACGCCCCGTTCACTGAGTGCTGAAT  
GGCAGTGCTGAATGGAGAACTGGCTGGAGAATCTGGTTTTAGACATTTCGG  
AGCCTGTTTGT**TAATTCATCAGCA**CTGCTCTTTTCCCCTGGGCATTGGTCTGT  
GATAAAGAAAACCTGGATACCAGAGGAATGTGCTGGGCATTT**CAGTGCTTT**  
GAGAAAACATAGCCAAGCACTTCTCAGGGCCCATCCCTAGACAGGACCCT  
GCCTTTACCACTTCATCTCCCAGATGGCTGTGGAGCCAGCCCCTCACCAGA  
GAGCT**TACCTGGCTCCACTCAGGCCTCAAATCTACTGTCTGTTGATGGAAAA**  
TCCTGCATGTCGTGCCAAGCC**AGGCTGATTCAAGG**TCGACCTTTCTCCATT  
TGGGCCAAATGCCCAGCACCCCCAAACCCCTAACACACAACCTCTTTGTGG  
GTCTGGTTTCTGGGAGCTCCTTAAGGAAGAAAAACATAAACAGGAATCAT  
GGTGAGGACTGGGGAGAGGGGCCCTGGGTCGGGGGGGCTCCATTTTATTC  
GGCTGGAAAGAAAAGGTTGGGGAGGAGGGCTTGCCATTGGATCCAGTTC  
ATTACTTCCCAATAAATCAAAGAAGGGCTGAGAATCCTGACCACCACCCCC  
AGTAAGGATCAAGTTCCGCTTTCTGGAAGAGGCCTAGAGGTGGATTGCAG  
GACTTCCCAGTTTAACCAGGTGACAAAGGCTTCTGCTTCAGCCCAGATGT  
GATCACAGACTTCCACAGCTTCTGCCGACCACAGCCTGGCCTTAAAGTCC  
AGACCTAGAGTCCCGGAGCTAGTGTGTACTACACAGAATGGAGACCTTGC  
CTGAGGAGCTGGTTCTCAGAGAACACTGGCAGGAAGGAAGAAAGAAATG  
TATATTCTAGGAGGCCTCTGCTAACCAGGAAAGGGGGCTGAGTCACACTCT  
GAAAGGTGGAAGAGGCAGAGAGCAGGTATCAACGCCCTCATTTTACAGAT  
TAAACAAAACAAGAAAAACAAACACTCCTCACCTCCCTGCAAAAAAAC  
CACCCTGAGGCTCAGACAGGGGAAGTAGCTGGCCCTGGTCACACAGTAG  
GTTAGCGGCAGAGTTGAGAGGAGACTCCATATGCCCTAGGGATGTGTTGT  
GATGAACTTTTCTACTGGTACTGTTTCCTCCCGCGAGGGAATGTCTAGAC  
CAGCCGCACCTTCTTGCTTTGACCCTTCAGAACTTTGGCCTGTCCAGTTAA  
AGAGGCACAGAGCCCTCCTACCCACAGGGA**ATGTTTCTAACTTACCAAGC**

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ACCTATTATGTGCTTAGAACTTGGACTTTCCTGATCTCATTTAAGCCTCACC  
ATTCTAGTGAGGAAGCATCACAGAACCCATTTTCC**TGATGAGGAA**ACTGAG  
ACTGGGAGGGGTTAAGATCCTGTACACAGTTGTAAC TGGCATGTCTGAATC  
CAGTTTCAAACCAAGGTGCCTTTCAGTGAACACCCAAGCATCTTCCTCTC  
GGATTAAC TGGCTAGGCCAATAACCAAAATCTCTTTCTCGGGCTACTCTTTTGC  
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GCGATTCTGTGGCAGGCGCAGACCTTTTCTCCTGTCCTCCACTGCCTCTTT  
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CCTTGGAGGCCCGGGGACTTGCTCTCCGCGGTCCGCCGCGGCCGCTCCAC  
CGAGTCCTGGCCTCCAGCGCCCTCACCTGGCTGCTGCGGGCGGGACTCTG  
CGCACCCGGGCTCCGCGGGGCTCAGGTCTGCGTCTCCCAGAGCCGCCGCCA  
GAGCCCGCCCCGGTCCGGCCCCGGGCACAGGGCCCCCGCCCCACTTGGGC  
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GCTTCGGTGCCGCGCGGGCGTCAGGGGCTCCTCGGGGGAGGAGTCAGTA  
AAAGTGGCTATAAAAGCGGCCTCTCGGCTGAGGGCCGGGGAGCTAGCGCT  
CAAGCAGAGCCCAGCGCGGTGCTATCGGACAGAGCCTGGCGAGCGCAAG  
CGGCGCGGGGAGCCAGCGGGGCTGAGCGCGGCCAGGGTCTGAACCCAGA  
TTTCCCAGACTAGCTACCACTCCGCTTGCCACGCCCCGGGAGCTC

the 3 site sequence: GGCTGATTCAAGG

the 3 site mutant: GGCCCGTCCGAAG

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