

Supplemental Informations

BHLHE22 Expression is Associated with a Proinflammatory Immune Microenvironment and Confers a Favorable Prognosis in Endometrial Cancer

Darmawi, Lin-Yu Chen, Po-Hsuan Su, Phui-Ly Liew, Hui-Chen Wang, Yu-Chun Weng, Rui-Lan Huang and Hung-Cheng Lai

Supplemental tables

Table S1. Patient clinical characteristics

| Clinical characteristics | | n | Mean \pm SEM H-score of BHLHE22 | p-value |
|-------------------------------|--------------|-----|-----------------------------------|--------------------|
| Age (years) | 55 (34-92) | 107 | 42.1 \pm 6.25 | <0.05 (r = 0.21) * |
| Stage | I | 74 | 33.38 \pm 57.37 | n.s ** |
| | II | 12 | 54.17 \pm 77.37 | |
| | III | 14 | 42.14 \pm 64.92 | |
| | IV | 1 | 80 | |
| | N/A | 6 | 119.17 \pm 86.39 | |
| Histologic type | Endometrioid | 95 | 35.26 \pm 60.85 | <0.01 ** |
| | Mixed | 6 | 116.67 \pm 44.57 | |
| | Serous | 6 | 75.83 \pm 89.58 | |
| Grade | 1 | 54 | 28.98 \pm 55.87 | <0.05 ** |
| | 2 | 33 | 41.06 \pm 62.87 | |
| | 3 | 20 | 79.25 \pm 77.41 | |
| Lymphovascular invasion (LVI) | Negative | 62 | 39.19 \pm 60.86 | n.s ** |
| | Positive | 43 | 48.14 \pm 70.97 | |
| | N/A | 2 | 2.5 \pm 3.54 | |
| FIGO | IA | 62 | 35.81 \pm 58.88 | <0.05 ** |
| | IB | 15 | 66 \pm 84.03 | |
| | II | 14 | 57.5 \pm 77.11 | |
| | IIIA | 3 | 11.67 \pm 10.41 | |
| | IIIC | 11 | 34.09 \pm 58.17 | |
| | IVB | 1 | 80 | |
| | N/A | 1 | 0 | |

* Pearson test; ** One-way ANOVA.

Table S2. Differential expressed gene list among HEC1A, Ishikawa and TCGA UCEC related to BHLHE22 expression

| | Gene names | | | |
|---------------|-------------------|----------|-----------|---------|
| Upregulated | BHLHE22 | KLHDC7B | CSTA | CNTN4 |
| | CXCR5 | TMEM158 | HCST | CPZ |
| | HOXC10 | MILR1 | INS-IGF2 | GPSM3 |
| | SPIB | CXCL8 | CLEC4A | ATP2A3 |
| | SELL | HOXC4 | ALPK2 | LTB |
| | NRG1 | SLC12A3 | F5 | LRRK2 |
| | LGALS2 | TMEM37 | IGFL2 | ARHGAP9 |
| | PRKCB | RFTN1 | LINC00494 | SLC7A7 |
| | CD27 | F2R | CD68 | CDH2 |
| | MARCO | SYNE1 | SLC38A5 | TMED6 |
| | IL2RG | RSAD2 | MSLN | PTGES |
| | CD52 | PTGER4 | GLI1 | DPYSL2 |
| | CLRN3 | PAPPA | CSF2RA | ZNF506 |
| | ITGB2 | KCNAB2 | ANKRD44 | CDK6 |
| | EBI3 | ETS1 | ALDH1A3 | NT5E |
| | FGD2 | NXPH4 | FBLN2 | DOCK11 |
| | ISL2 | MMP25 | ALOX5 | SEMA3D |
| | LAMP3 | HLA-DRB1 | ST6GAL2 | RAB39B |
| | CCL3L3 | VNN1 | ADGRD1 | COPZ2 |
| | ACSM5 | SLC9A2 | SUCNR1 | KRT17 |
| | ISM1 | LOXL4 | ATP2C2 | NELL2 |
| | UTS2 | CTSS | ADAM8 | DAB2 |
| | KRT23 | IFI44L | SDS | HERC5 |
| | LINC00882 | RASSF5 | | |
| Downregulated | MAST1 | SERPINA3 | | |
| | ZYG11A | TNNI1 | | |
| | LINC00336 | RPPH1 | | |
| | SERPINA4 | SFTPD | | |
| | GSC | SERPINB5 | | |
| | LEMD1 | SLC34A3 | | |
| | PALM3 | DLX5 | | |
| | GPC2 | CAMK2B | | |
| | ABCC6P2 | NMU | | |
| | BTNL9 | KLK8 | | |

Supplemental figures

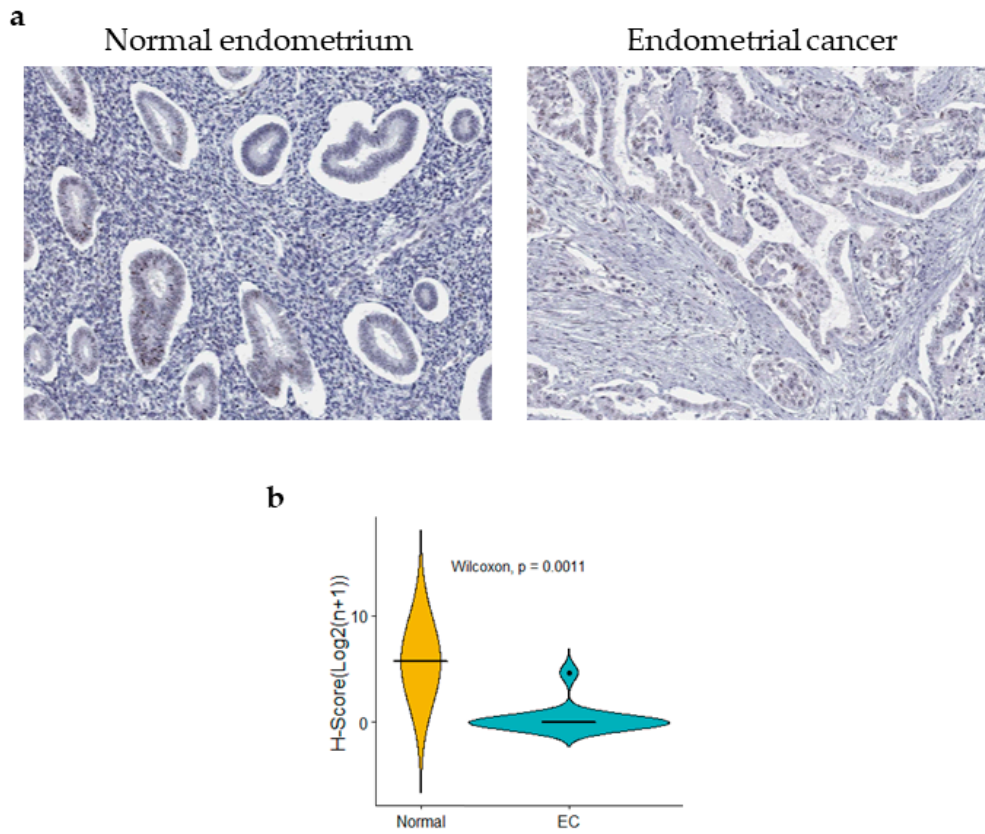


Figure S1. BHLHE22 expression was downregulated in endometrial cancer compare to normal endometrium tissues.

BHLHE22 protein level in normal endometrium and endometrial cancer from IHC of the Human Protein Atlas (HPA) database (a) and quantitatively by H-score (b). The statistical analysis was tested by Wilcoxon-test. IHC, Immunohistochemistry.

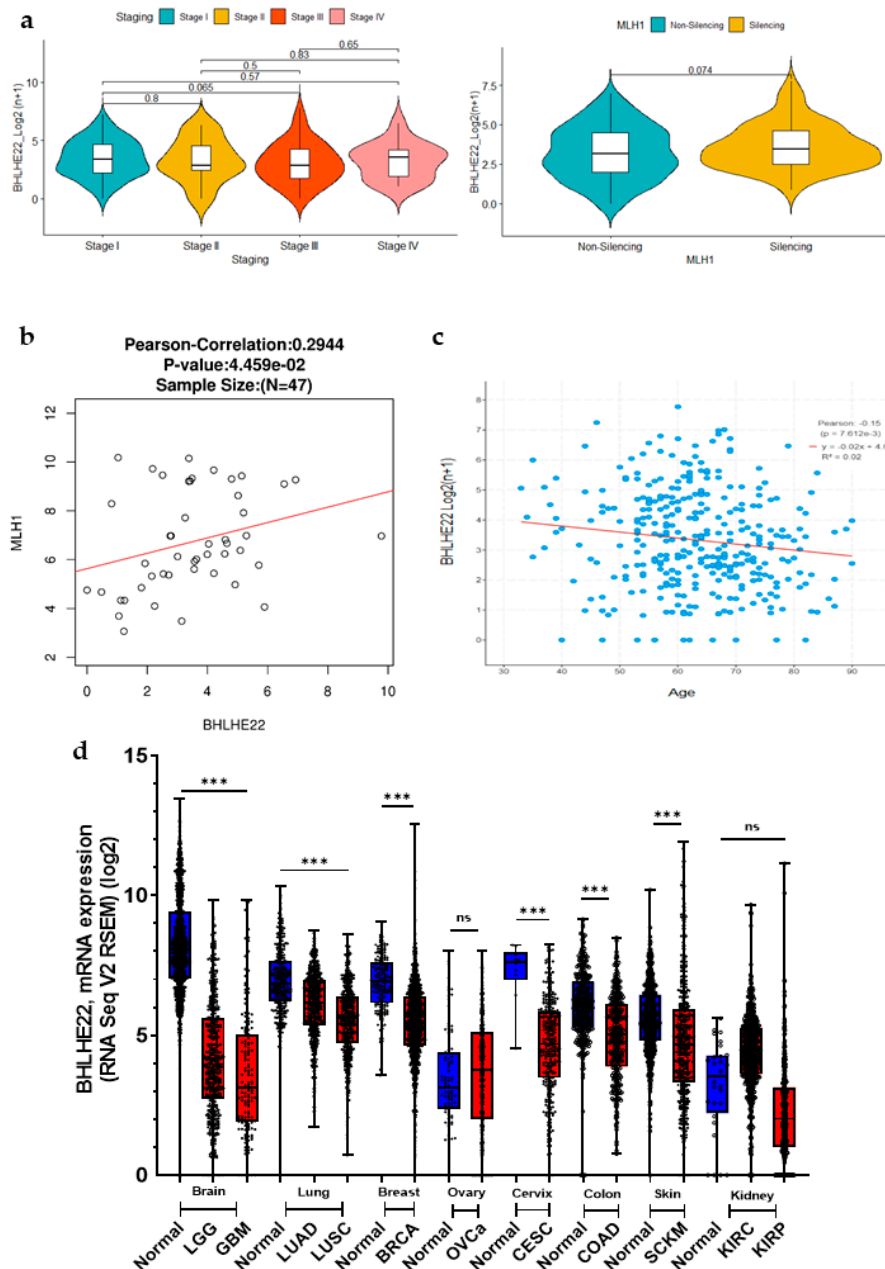


Figure S2. BHLHE22 was associated with clinical characteristics and generally downregulated in tumor compare to normal.

a. Association between clinical characteristic and BHLHE22 expression were assessed in EC patients using UCEC TCGA database. b. Correlation of BHLHE22 expression and MMR related gene, MLH1, were examined in EC patients. c. BHLHE22 expression were negatively correlated with age in EC patients. Pearson-correlation coefficient were shown. d. BHLHE22 mRNA expression in other cancers compared with their normal tissues were tested using Xena browser. TCGA, The Cancer Genome Atlas; LGG, Low Grade Glioma; GBM, Glioblastoma Multiform; LUAD, Lung Adenocarcinoma; LUSC, Lung Squamous Cell Carcinoma; BRCA, Breast Invasive

Carcinoma; OVCa, Ovarian Cancer; CESC, Cervical Squamous Cell Carcinoma and Endocervical Adenocarcinoma; UCEC, Uterine Corpus Endometrial Carcinoma; COAD, Colon adenocarcinoma; SCKM, Skin Cutaneous Melanoma; KIRC, Kidney Renal Clear Cell Carcinoma; KIRP, Kidney Renal Papillary Cell Carcinoma; TPM, transcript per millions; EC, Endometrial Cancer; POLE, DNA polymerase epsilon; MSI, microsatellite instability; MSS, microsatellite stable; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$.

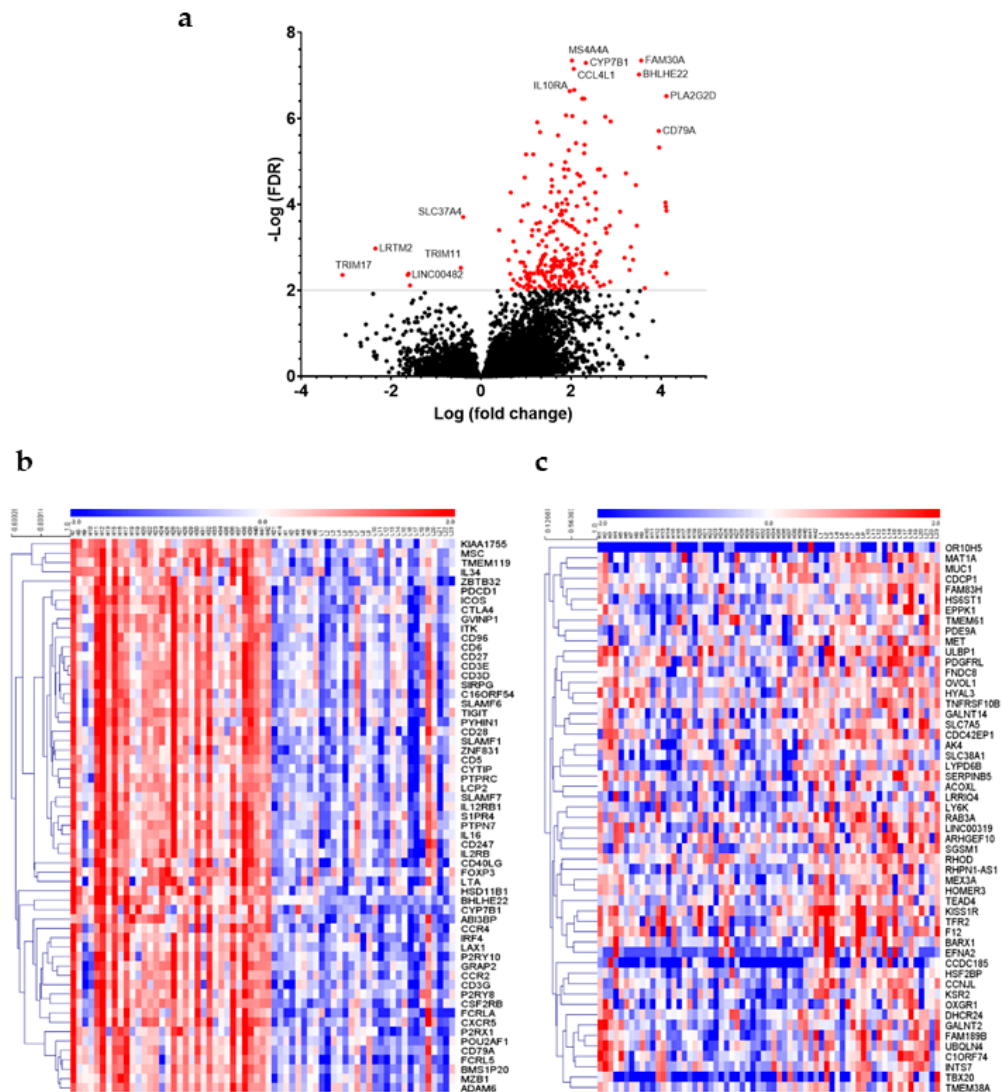


Figure S3. Genes were affected by the high BHLHE22 mRNA expression in EC patients.

a. Differential expressed genes (DEGs) of EC patients using TCGA UCEC dataset based on the BHLHE22 high expression level were shown in volcano plot. Heatmaps showed top 50 DEGs, which were positively (b) or negatively (c) correlated with BHLHE22 expression level in EC patients, respectively.

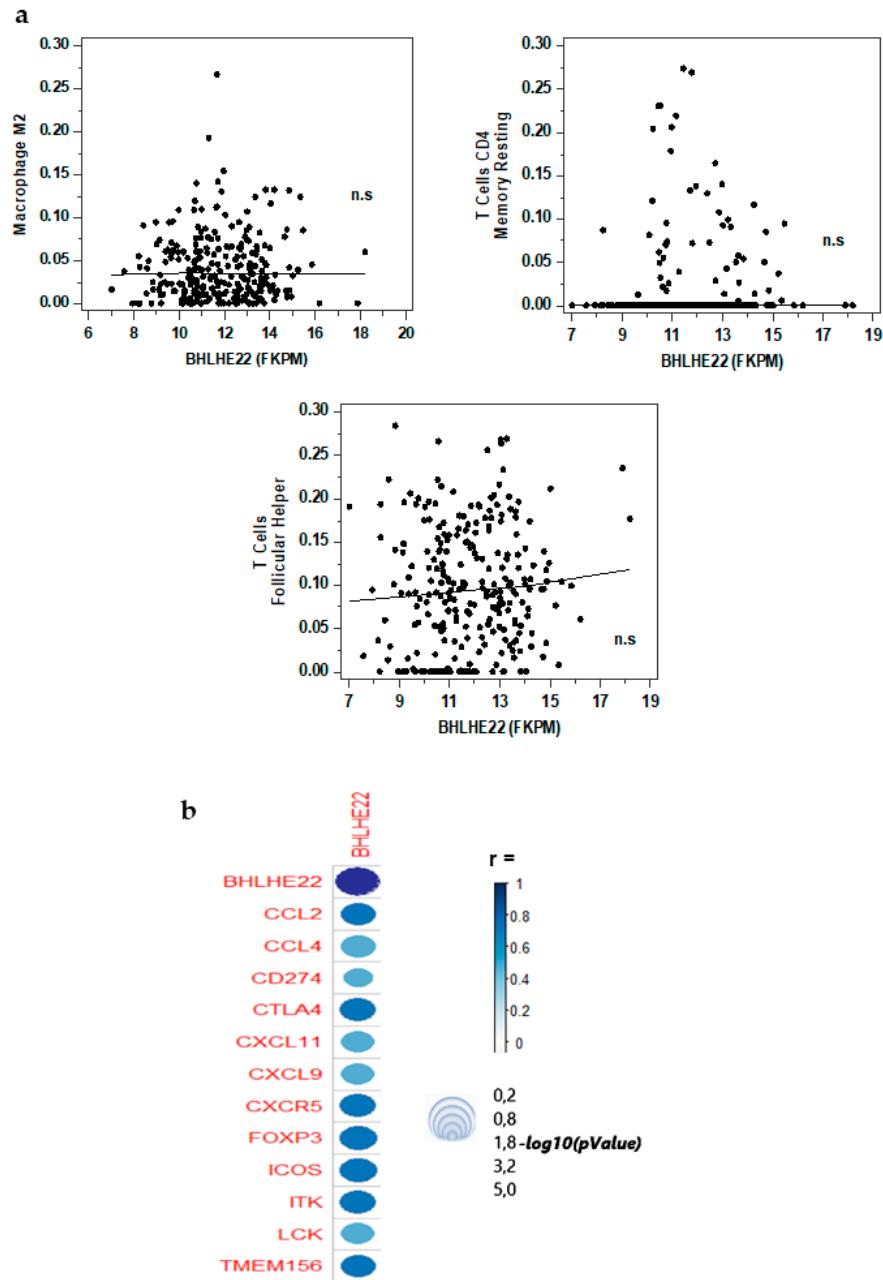


Figure S4. The association between BHLHE22 expression and other immune cells in EC patients were as-sessed by TIMER algorithm.

a. BHLHE22 expression level was unsignificantly correlated with macrophage M2, T cells memory resting and T cell follicular helper. Statistical analysis with Pearson correlation test were shown. b. BHLHE22 expression had a strong positive correlation with immune-related genes expression in EC patients using UCEC TCGA database. Circle size represented the $-\log_{10}(\text{p-value})$ and color represented r value.