

Supplementary Figure 1. Distribution of mappable reads (million) and mappable (million) bases across different cancer types

BRCA (n = 942), KIRC (n = 515), LUSC (n = 237), OV (n = 412), GBM (n = 154), CRC (n = 228), and UCEC (n = 320). The boxes show the median ± 1 quartile, with whiskers extending to the most extreme data point within 1.5 interquartile range from the box boundaries.



Correlation Coefficient

Supplementary Figure 2. Correlations between pseudogenes and their WT cognate genes in different cancer types

The boxes show the median ± 1 quartile, with whiskers extending to the most extreme data point within 1.5 interquartile range from the box boundaries.



Supplementary Figure 3. Global pattern of pseudogenes expression across different cancer types Unsupervised clustering based on all pseudogenes surveyed. BRCA (n = 837), KIRC (n = 448), LUSC (n = 220), OV (n = 412), GBM (n = 154), CRC (n = 228), and UCEC (n = 316).



Supplementary Figure 4. Tumor-lineage-specific pseudogenes expression across different cancer types

(a) Pair-end sequencing for BRCA (n = 837), KIRC (n = 448), LUSC (n = 220), OV (n = 412), and GBM (n = 154), (b) Single-end Sequencing for CRC (n = 228) and UCEC (n = 316). Tumor-lineage-specific pseudogenes were identified based on supervised analysis (ANOVA, corrected *P*-value < 0.05, >1.5 fold change). The color intensity represents the expression level (red, high; blue, low).



Supplementary Figure 5. Differentially expressed pseudogenes between normal and tumor samples

(a) BRCA (pink, n = 837) and breast non-tumor (cyan, n = 105) samples. (b) KIRC (pink, n = 448) and kidney non-tumor (cyan, n = 67). (c) LUSC (pink, n = 220) and lung non-tumor (cyan, n = 17) samples. (d) Overlap of cancer-related differentially expressed pseudogenes in (a)-(c). Cancer-specific pseudogenes were identified based on supervised analysis (ANOVA, corrected *P*-value < 0.05, >1.5 fold change). The color intensity represents the expression level (red, high; blue, low).



Supplementary Figure 6. The predictive power of mRNA expression in classifying endometrial subtypes

(a) The ROC curves of the three classifiers based on the cross-validation within the training set (RF: random forest, SVM: support vector machine, LR: logistic regression.). (b) The ROC curve by applying the best-performing classifier (RF) built from the whole training set to the test set.



Supplementary Figure 7. Non-negative matrix factorization (NMF) consensus cluster in breast cancer samples

(a) Distribution of cophenetic correlation based on the number of clusters (K) =2 to 8, that running multiple ranks consecutively can allow for the comparison between differing numbers of clusters using cophenetic correlation. (b) Heatmap for four clusters based on NMF consensus clustering (n = 942).



Supplementary Figure 8. Potential functional mechanisms of expressed pseudogenes in KIRC

(a) The analysis summary for inferring the mechanisms through which the expressed pseudogenes may function. (b) Heatmap of up-regulation of better prognosis gene and down-regulation of worse prognosis genes in the pseudogene-expression subtype (subtype 1, with a better-survival, n = 234).