

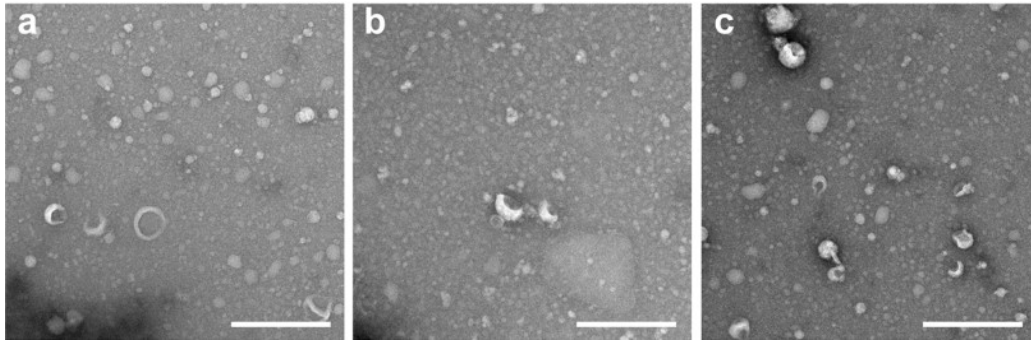
Supplementary Materials

Metabolomic analysis of exosomal-markers in esophageal squamous cell carcinoma

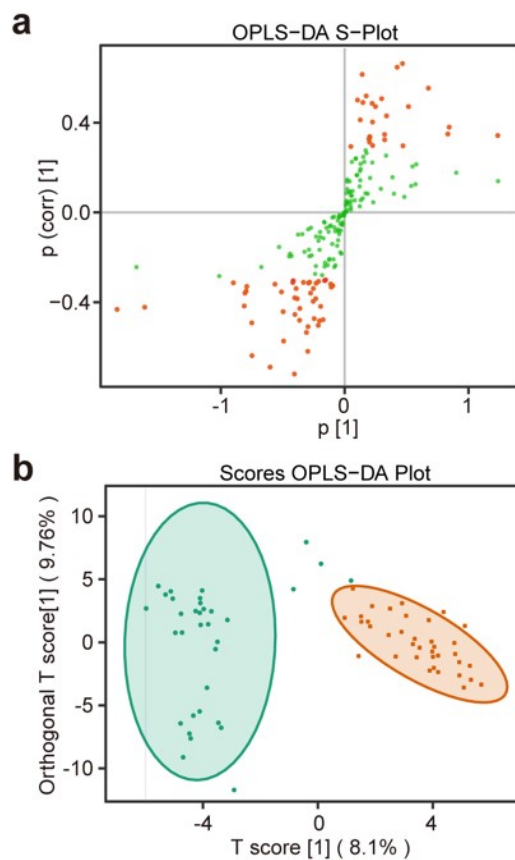
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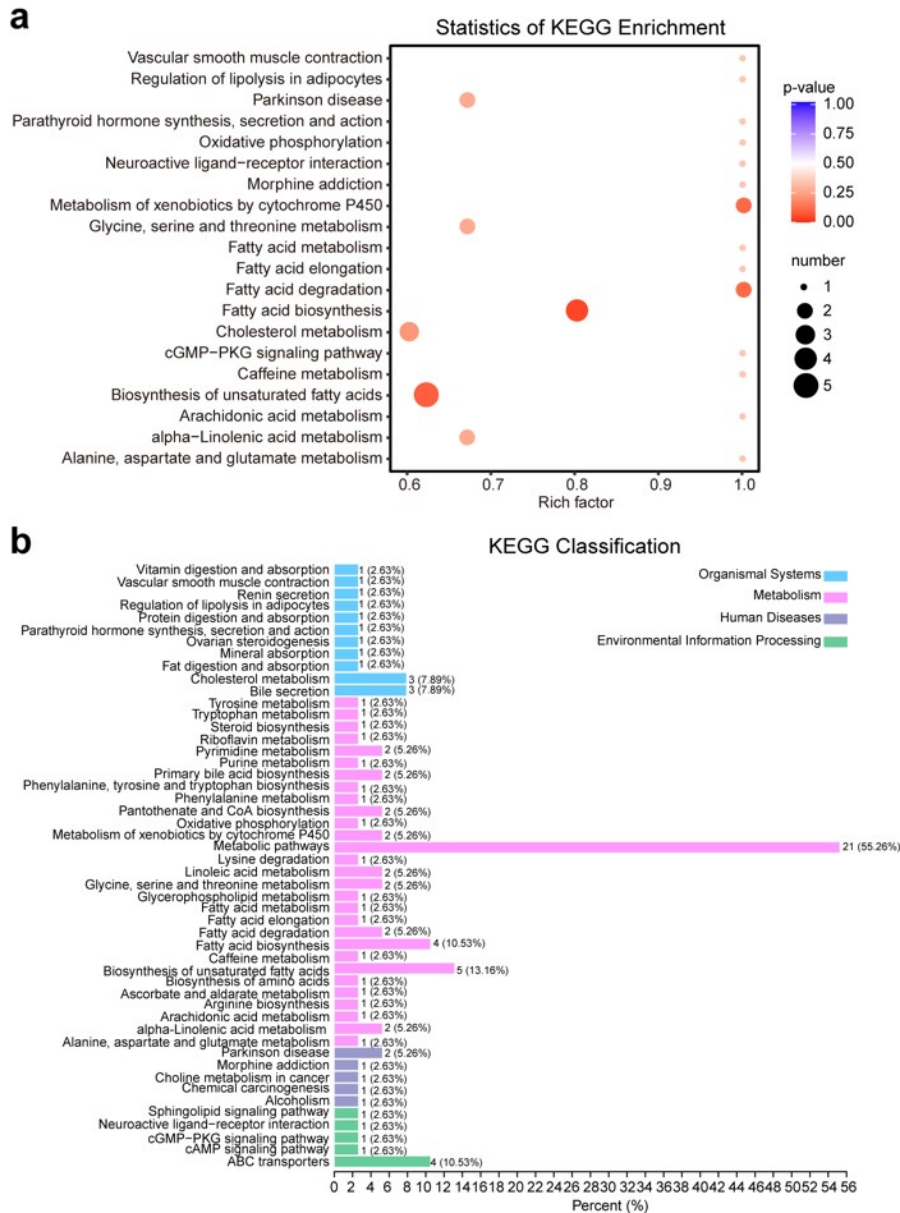
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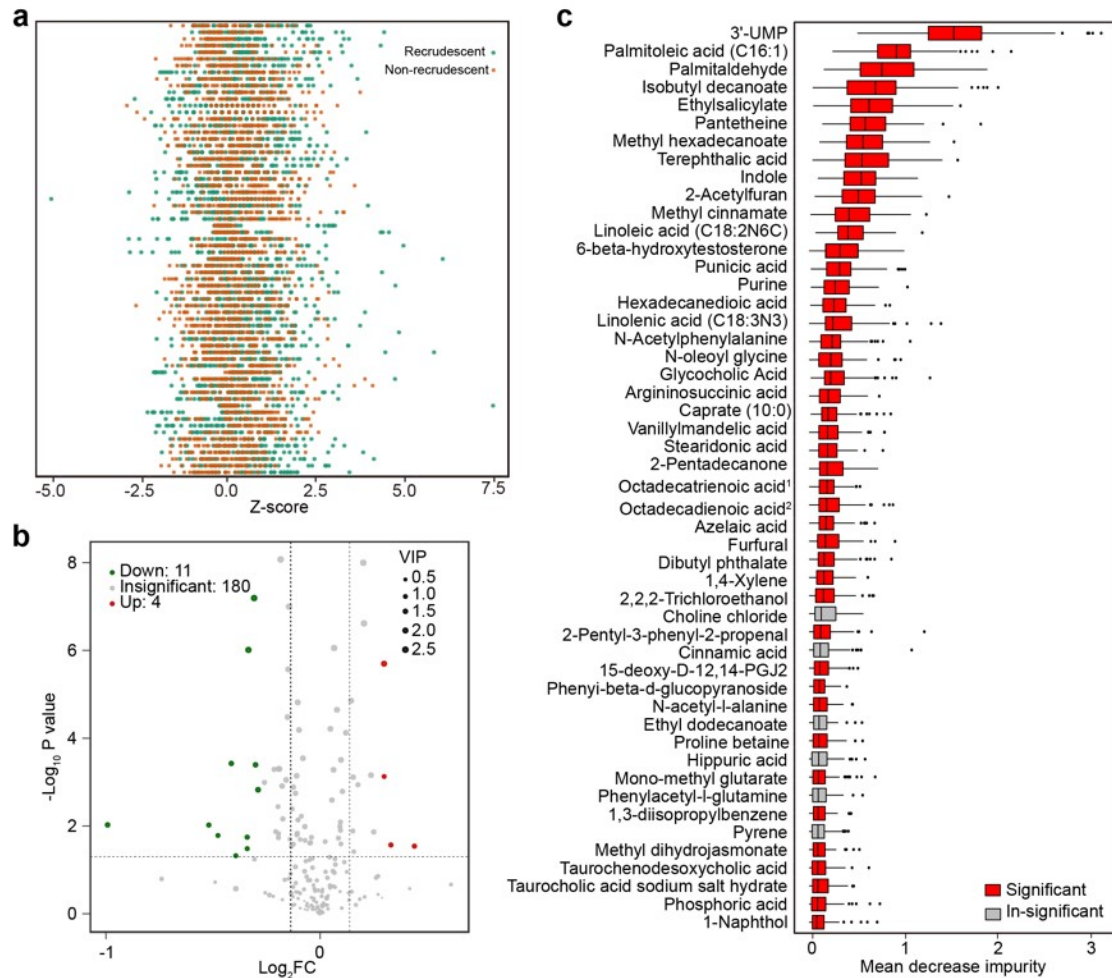
Supplementary Fig. 1: Typical exosome morphologies using TEM analysis from groups of (a) recrudescence patient and (b) non-recrudescence patient and (c) healthy donor. Scale bar is 500 nm.



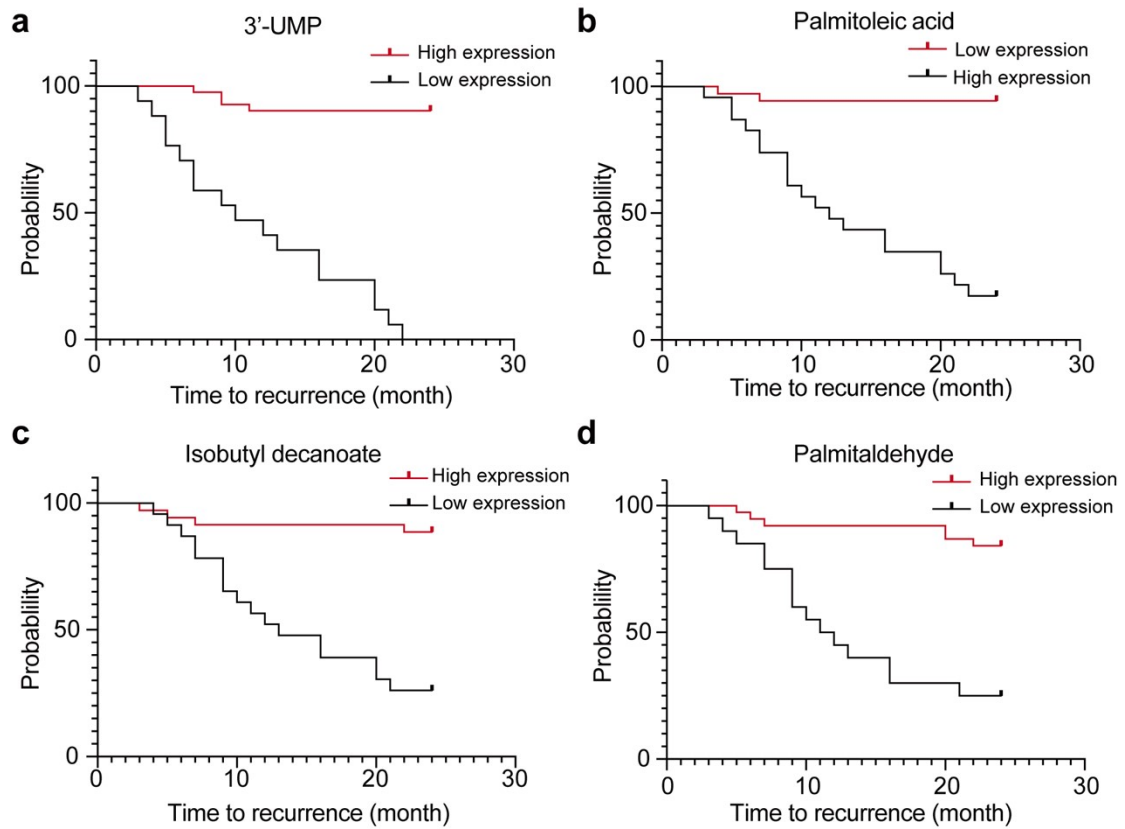
Supplementary Figure 2: OPLS-DA analysis of the groups of recrudescence patients and non-recrudescence patients. (a) OPLS-DA S plot of two groups. The VIP values of green points are below 1, and VIP of red points are above 1. (b) The score of OPLS-DA plot. The parameters are: $R^2X(\text{cum})$, 0.179; $R^2Y(\text{cum})$, 0.866; $Q^2(\text{cum})$, 0.716; pre, 1; ort, 1.



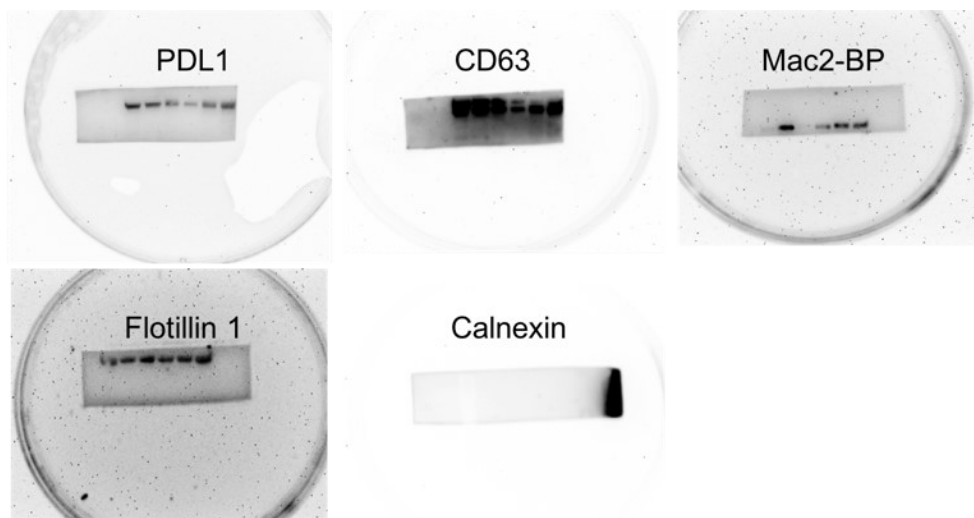
Supplementary Figure 3: Enrichment analysis of the differential metabolites between the groups of recrudescence patients and non-recrudescence patients. (a) Statistics of KEGG pathways based on differential metabolites. (b) Percentages of pathway classifications based on KEGG annotations.



Supplementary Figure 4: Selection of significant metabolites for classifying recrudescent and non-recrudescent patients. (1) Calculation of the Z scores to show the normalized abundance of the differential metabolites between two groups. (2) Volcano plot showing the significant metabolites considering VIP value, p-value, and FC value. (3) Random forest modeling of the importance of marker candidates according to their mean decrease impurity (octadecatrienoic acid¹, 13-HOTrE [13S-hydroxy-9Z,11E,15Z-octadecatrienoic acid]; octadecatrienoic acid², 9-oxoODE [9-oxo-10E,12Z-octadecadienoic acid]).



Supplementary Figure 5: Evaluation of the selected metabolic markers from random forest by the Kaplan-Meier analysis. From a-b are: 3'-UMP, palmitoleic acid, isobutyl decanoate and palmitaldehyde.



Supplementary Figure 6: Original WB scans for Figure 2.c