

## Supplementary Materials

### **A single-sEV analysis identifies plasma EPCAM<sup>+</sup> sEVs as a biomarker for early diagnosis and monitoring postoperative remission of thyroid cancer**

**Simin Yu<sup>1,#</sup>, Yuting Luo<sup>2,#</sup>, Tianfeng Dang<sup>1,#</sup>, Congli Peng<sup>1,#</sup>, Qing Gan<sup>1</sup>, Yuxuan Liang<sup>1</sup>, Jieqing Yu<sup>1</sup>, Ping Long<sup>1</sup>, Wensheng Zhou<sup>1</sup>, Daofeng Dai<sup>1</sup>**

<sup>1</sup>Jiangxi Otorhinolaryngology-Head and Neck Surgery Institute, Department of Otorhinolaryngology-Head and Neck Surgery, The First Affiliated Hospital, Jiangxi Medical College, Nanchang University, Nanchang 330006, Jiangxi, China.

<sup>2</sup>Department of General Thyroid Surgery, The First Affiliated Hospital, Jiangxi Medical College, Nanchang University, Nanchang 330006, Jiangxi, China.

<sup>#</sup>Authors contributed equally to this work.

**Correspondence to:** Dr. Daofeng Dai, Prof. Wensheng Zhou, Prof. Ping Long, Prof. Jieqing Yu, Jiangxi Otorhinolaryngology-Head and Neck Surgery Institute, Department of Otorhinolaryngology-Head and Neck Surgery, The First Affiliated Hospital, Jiangxi Medical College, Nanchang University, Nanchang 330006, Jiangxi, China. E-mail: daofengdai@ncu.edu.cn; zhouwesh@163.com; yifuyuanlongping@163.com; yujieq@ncu.edu.cn

**Supplementary Table 1. The clinicopathological features of TC patients recruited in this study**

| Variable                 | Cohort 1     |               | Cohort 2 | Cohort 3     | Cohort 4      |
|--------------------------|--------------|---------------|----------|--------------|---------------|
| Sex                      |              |               |          |              |               |
| Male                     | 3            | 6             |          | 6            | 4             |
| Female                   | 7            | 22            |          | 28           | 17            |
| Age (years, mean ± SD)   | 44.50 ± 7.56 | 39.25 ± 12.62 |          | 47.32 ± 9.46 | 45.62 ± 11.91 |
| Histological subtype     |              |               |          |              |               |
| PTC                      | 10           | 28            |          | 34           | 21            |
| Size (cm)                |              |               |          |              |               |
| < 1                      | 2            | 2             |          | 26           | 12            |
| ≥ 1                      | 8            | 26            |          | 8            | 9             |
| T stage                  |              |               |          |              |               |
| T1                       | 5            | 21            |          | 33           | 17            |
| T2                       | 4            | 6             |          | 0            | 3             |
| T3                       | 0            | 1             |          | 0            | 0             |
| T4                       | 1            | 0             |          | 1            | 1             |
| N stage                  |              |               |          |              |               |
| N0                       | 6            | 10            |          | 22           | 10            |
| N1-N2                    | 4            | 18            |          | 12           | 11            |
| Clinical stage           |              |               |          |              |               |
| I                        | 9            | 28            |          | 34           | 21            |
| II                       | 0            | 0             |          | 0            | 0             |
| III                      | 1            | 0             |          | 0            | 0             |
| Extrathyroidal extension |              |               |          |              |               |
| Yes                      | 1            | 1             |          | 1            | 9             |
| No                       | 9            | 17            |          | 33           | 12            |

TC, thyroid cancer; PTC, papillary thyroid cancer.

**Supplementary Table 2. The top 20 significantly differentially expressed proteins in Cohort 1**

| <b>Protein</b> | <b>UniProt ID</b> |
|----------------|-------------------|
| CD151          | P48509            |
| CD26           | P27487            |
| CEACAM8        | P31997            |
| EPCAM          | P16422            |
| MADCAM1        | Q13477            |
| NLGN1          | Q8N2Q7            |
| SELE           | P16581            |
| Thy1           | P04216            |
| CAV1           | Q03135            |
| CDH2           | P19022            |
| CDON           | Q4KMG0            |
| CLDN10         | P78369            |
| CLDN11         | O75508            |
| CLEC5A         | Q9NY25            |
| GPA33          | Q99795            |
| GPC1           | P35052            |
| ITGAX          | P20702            |
| LAG3           | P18627            |
| PROM1          | O43490            |
| TPBG           | Q13641            |

**Supplementary Table 3. The top 20 significantly differentially expressed proteins in Cohort 2**

| <b>Protein</b> | <b>UniProt ID</b> |
|----------------|-------------------|
| CD44           | P16070            |
| CEACAM5        | P06731            |
| CEACAM7        | Q14002            |
| CPM            | P14384            |
| EPCAM          | P16422            |
| ITGB3          | P05106            |
| NT5E           | P21589            |
| PCDH1          | Q08174            |
| SIGLEC6        | O43699            |
| TENM1          | Q9UKZ4            |
| ALDH1A1        | P00352            |
| CDH13          | P55290            |
| CDH6           | P55285            |
| CLDN11         | O75508            |
| EMCN           | Q9ULC0            |
| FN1            | P02751            |
| IL1RAPL1       | Q9NZN1            |
| ILK            | Q13418            |
| ITGAX          | P20702            |
| LAG3           | P18627            |

**Supplementary Table 4. The top 20 significantly differentially expressed sEV in Cohort 1**

| sEV                       | UniProt ID |
|---------------------------|------------|
| CD26 <sup>+</sup> sEV     | P27487     |
| EPCAM <sup>+</sup> sEV    | P16422     |
| SIGLEC11 <sup>+</sup> sEV | Q96RL6     |
| PCDH17 <sup>+</sup> sEV   | O14917     |
| CEACAM8 <sup>+</sup> sEV  | P31997     |
| CDON <sup>+</sup> sEV     | Q4KMG0     |
| CLDN10 <sup>+</sup> sEV   | P78369     |
| LAG3 <sup>+</sup> sEV     | P18627     |

**Supplementary Table 5. The top 20 significantly differentially expressed sEV in Cohort 2**

| sEV                       | UniProt ID |
|---------------------------|------------|
| TENM2 <sup>+</sup> sEV    | Q9NT68     |
| EPCAM <sup>+</sup> sEV    | P16422     |
| ESAM <sup>+</sup> sEV     | Q96AP7     |
| FN1 <sup>+</sup> sEV      | P02751     |
| CDH13 <sup>+</sup> sEV    | P55290     |
| SIGLEC11 <sup>+</sup> sEV | Q96RL6     |
| MUC1 <sup>+</sup> sEV     | P15941     |
| MMP9 <sup>+</sup> sEV     | P14780     |
| CDH1 <sup>+</sup> sEV     | P12830     |
| TENM4 <sup>+</sup> sEV    | Q6N022     |
| LAG3 <sup>+</sup> sEV     | P18627     |
| EMCN <sup>+</sup> sEV     | Q9ULC0     |
| Thy1 <sup>+</sup> sEV     | P04216     |

**Supplementary Table 6. Featured proteins in exosome clusters in Cohort 1**

| <b>Cluster</b> | <b>Protein</b> |
|----------------|----------------|
| Cluster 1      | TENM2          |
| Cluster 2      | ESAM           |
| Cluster 3      | CDH13          |
| Cluster 4      | CD36           |
| Cluster 5      | SIGLEC9        |
| Cluster 6      | ITGB4          |
| Cluster 7      | MUC1           |
| Cluster 8      | PCDH17         |
| Cluster 9      | EPCAM          |
| Cluster 10     | CLDN4          |
| Cluster 11     | CD36, ITGA1    |
| Cluster 12     | ULBP1          |
| Cluster 13     | SIGLEC11       |
| Cluster 14     | NECTIN1        |
| Cluster 15     | LICAM          |
| Cluster 16     | DSC1           |
| Cluster 17     | ITGA1          |
| Cluster 18     | FN1            |

**Supplementary Table 7. Featured proteins in exosome clusters in Cohort 2**

| <b>Cluster</b> | <b>Protein</b> |
|----------------|----------------|
| Cluster 1      | ITGA1, ITGAV   |
| Cluster 2      | DSC1, ITGB4    |
| Cluster 3      | DSC1, FN1      |
| Cluster 4      | ITGA1, DSC2    |
| Cluster 5      | ITGB4          |
| Cluster 6      | DSC1           |
| Cluster 7      | MUC1           |
| Cluster 8      | ADAM10         |
| Cluster 9      | DSGI           |
| Cluster 10     | FOLH1          |
| Cluster 11     | TENM2          |
| Cluster 12     | LICAM          |
| Cluster 13     | ESAM           |
| Cluster 14     | ULBP1          |
| Cluster 15     | EPCAM          |
| Cluster 16     | CLDN4          |
| Cluster 17     | SIGLEC9        |
| Cluster 18     | PCDH17         |
| Cluster 19     | CDH13          |
| Cluster 20     | SIGLEC11       |