58 candidate miRNAs that are low expressed in ESCC tissues were selected from a systematic review of the NCBI database. (85 relevant articles were identified from PubMed up to June 2016)

Criterion 1: 44 miRNAs were excluded. Candidate miRNAs that were not reported in several instances were excluded.

14 candidate miRNAs

Criterion 2: 6 miRNAs were excluded. Candidate miRNAs that were already reported as biomarkers in body fluids such as plasma, serum, and urine.

6 candidate miRNAs: miR-126 / 133b / 143 / 203 / 338-3p / 655

Selection of candidate miRNAs based on a systematic review of the NCBI database (Supplementary Figure S1a).

Test-scale analyses of plasma sample using qRT-PCR to validate the selected miRNA candidates by comparing 10 ESCC patients and 10 healthy volunteers (Supplementary Figure S1c).

Validation-scale analysis of miR-655 plasma level to investigate the correlation of the miR-655 plasma level with clinicopathological characteristics and prognostic outcomes in ESCC patients (Figures 1a, 1b, Tables 1 and Supplementary Table S2).

Evaluation of whether miR-655 overexpression in ESCC cells induced anti-tumour effects in vitro (Figure 1c, 1d, 1e and Supplementary Figure S3).

Investigation of the tumour suppressor function in tumor and lymph node metastasis in vivo (Figure 2).

Plasma miR-655 is a novel candidate therapeutic target and biomarker for screening, monitoring, and predicting prognosis in ESCC.

miR-126

P = 0.3294

Volunteers ESCC

miR-133b

P = 0.4006

Volunteers ESCC

miR-143

P < 0.01

Volunteers ESCC

miR-203

P = 0.3311

Volunteers ESCC

miR-338-3p

P = 0.0624

Volunteers ESCC

miR-655

P < 0.01

Volunteers ESCC

Supplementary Figure S1.