

Supplementary Materials for

Identification of miR-515-3p and its targets vimentin and MMP3 as a key regulatory mechanism in esophageal cancer metastasis: functional and clinical significance

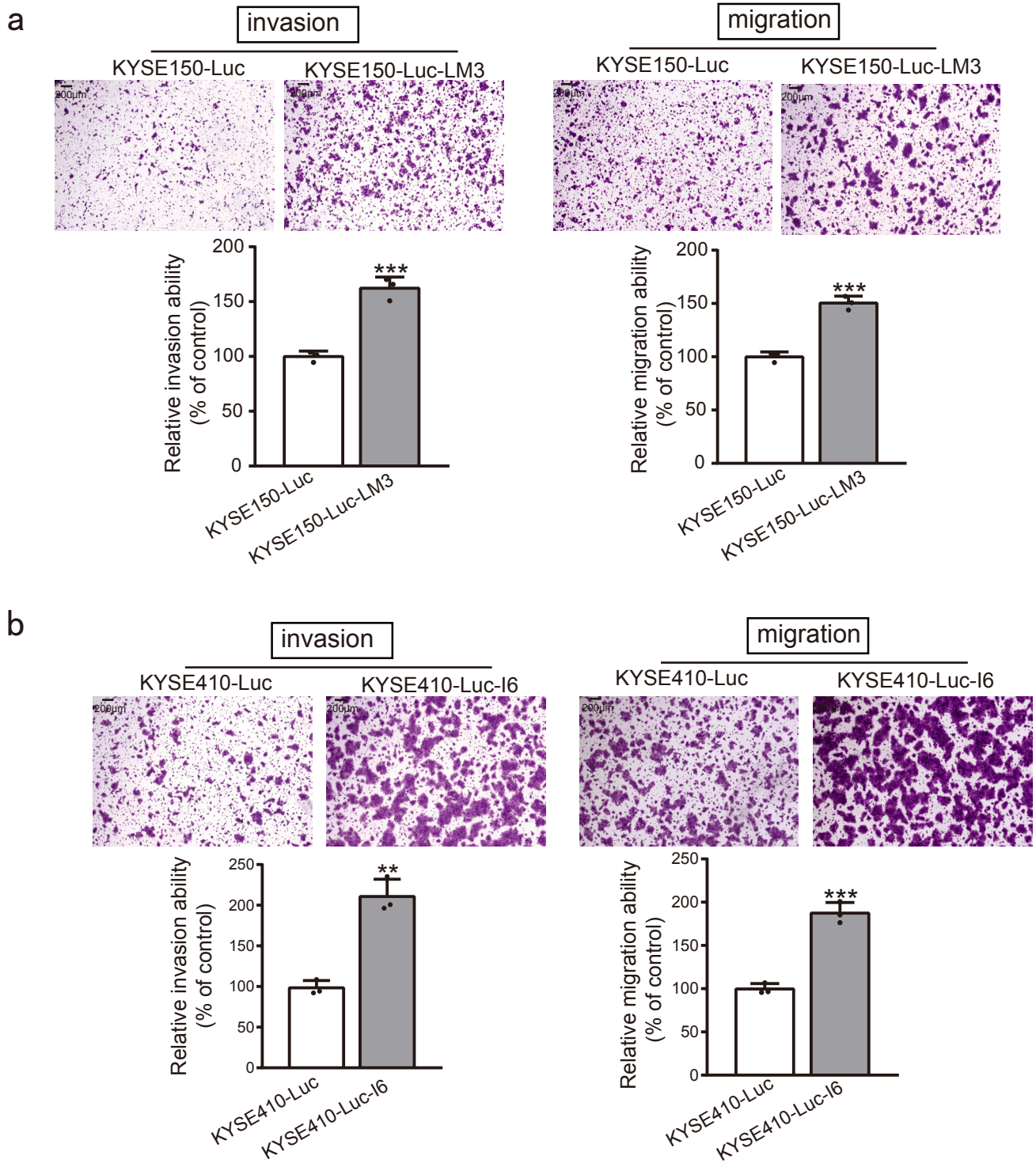
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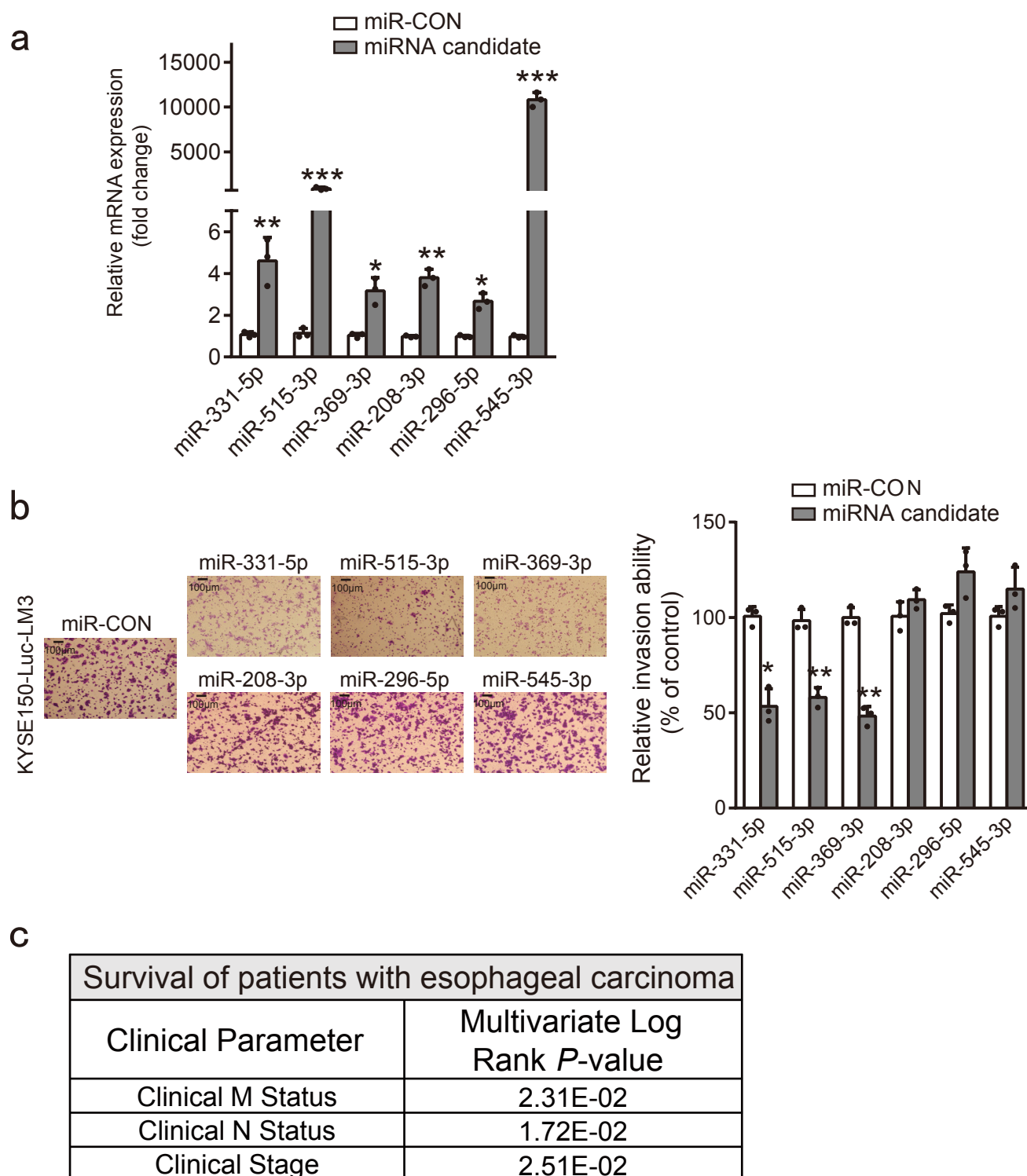
This PDF file includes:

Figures. S1 to S8

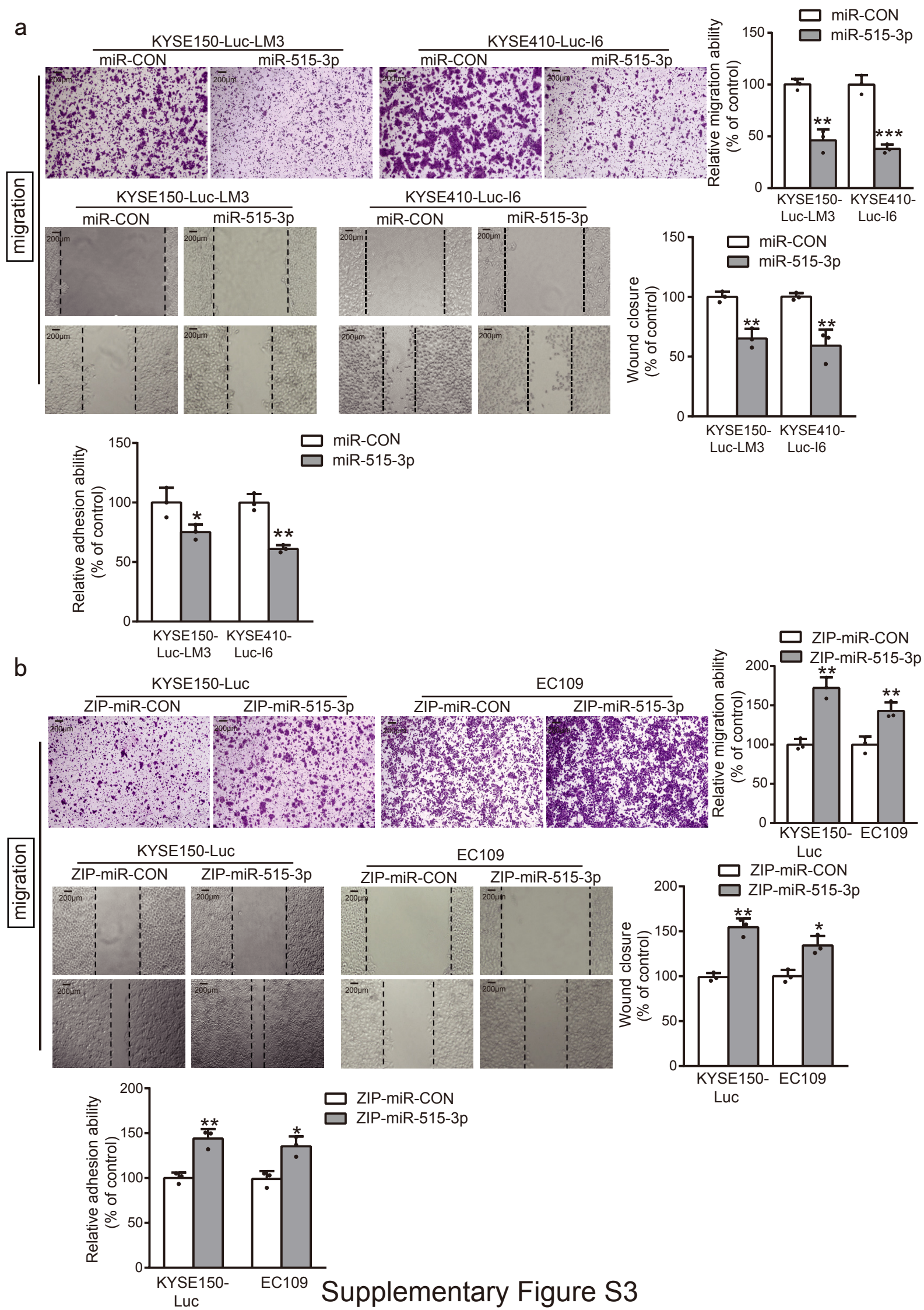
Tables S1 to S2



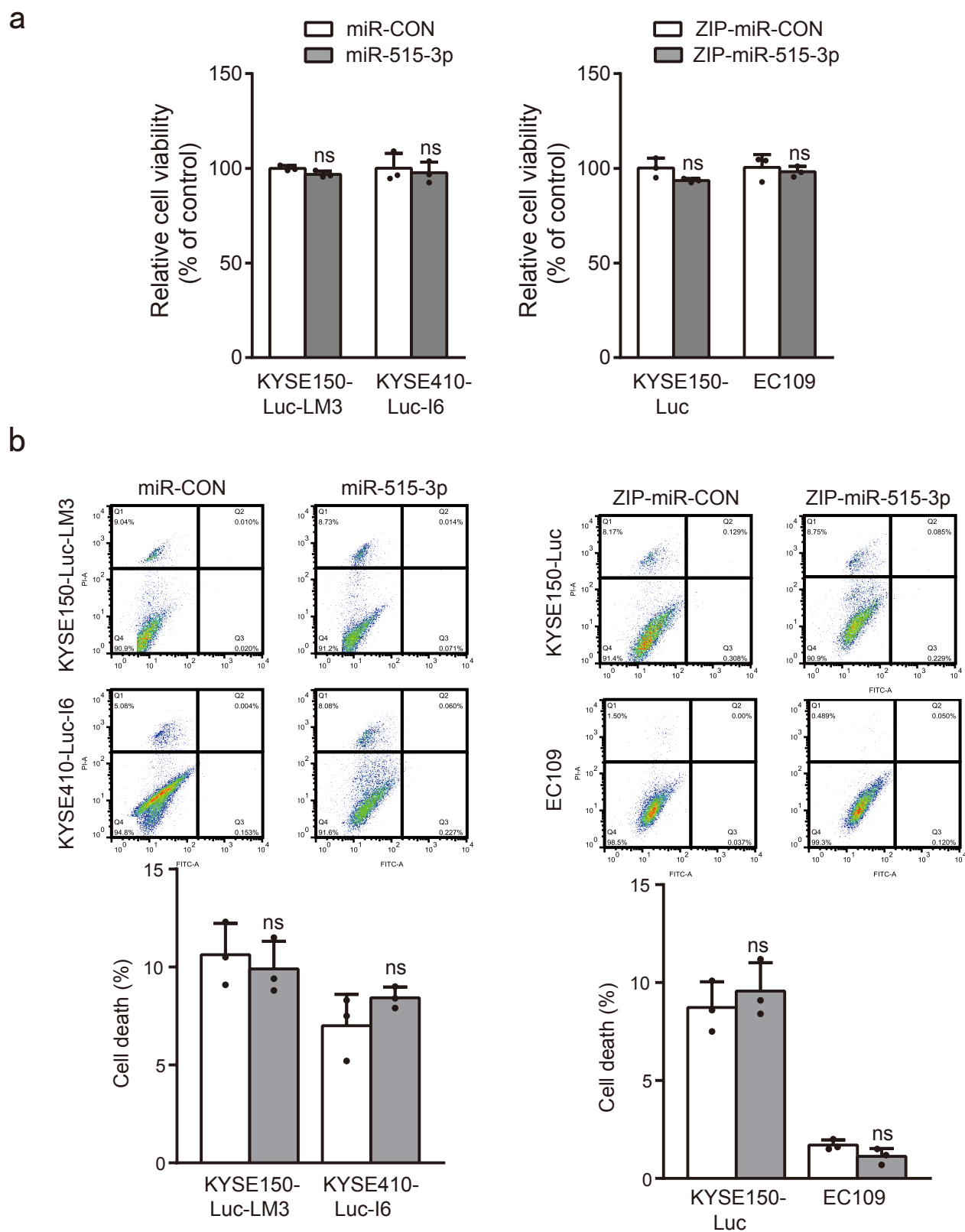
Supplementary Figure S1. Comparison of invasion and migration abilities of KYSE150-Luc-LM3 and KYSE150-Luc as well as KYSE410-Luc-I6 and KYSE410-Luc cells. (a, b) The invasion and migration abilities of KYSE150-Luc-LM3 and KYSE150-Luc cells (a) as well as KYSE410-Luc-I6 and KYSE410-Luc cells (b) were analyzed by chamber invasion and migration assays, and increased invasion and migration potential was observed in KYSE150-Luc-LM3 and KYSE410-Luc-I6 cells.



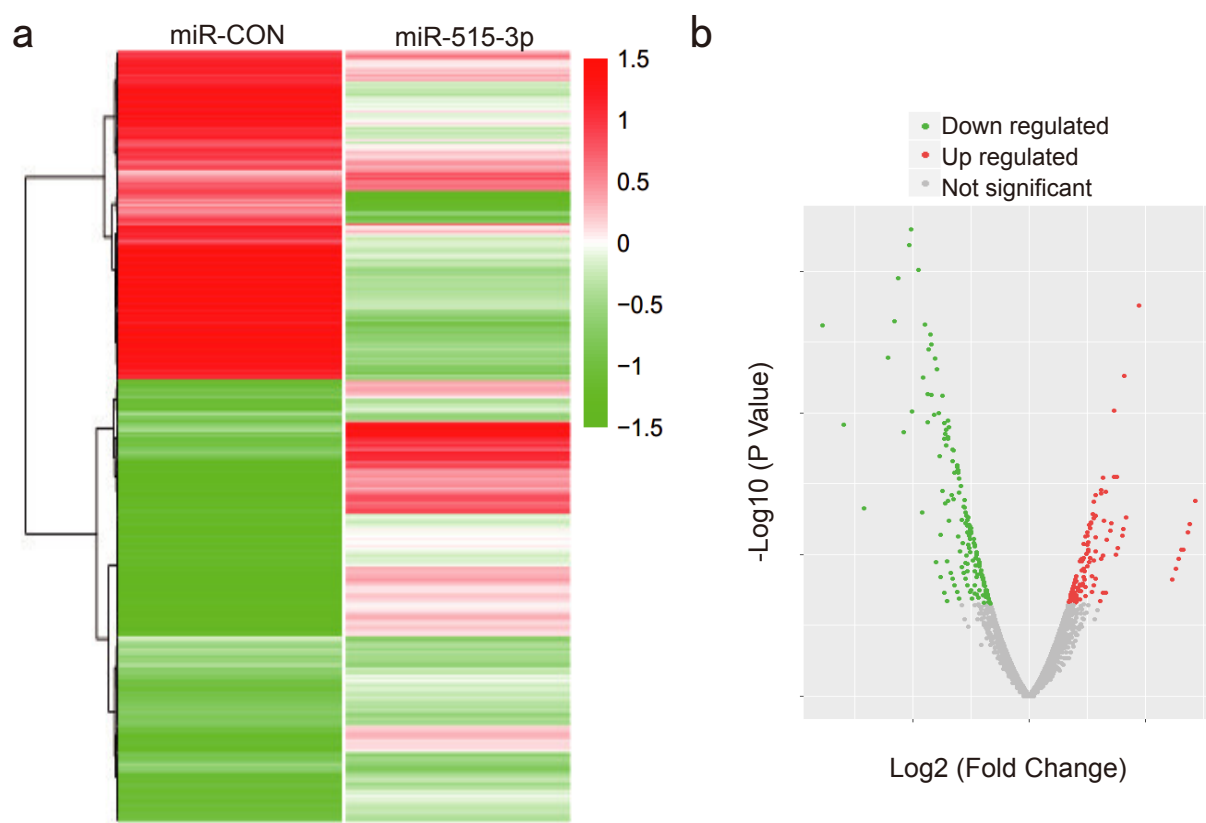
Supplementary Figure S2. Screening of functional miRNAs involved in cancer invasion. (a) Successful establishment of the stable cell lines overexpressing the 6 miRNA candidates indicated, respectively, in KYSE150-Luc-LM3 cells. (b) Effect of the miRNA candidates on invasive potential of ESCC cells was determined using chamber invasion assay. The quantification data indicated miR-515-3p markedly suppressed invasive ability of KYSE150-Luc-LM3 cells. (c) Correlation between miR-515-3p expression and survival of ESCC patients from OncomiR Cancer Database. Multivariate Cox analysis evaluates the combined effect of the miRNA and clinical parameters patient survival duration. A strong degree of significance indicates the likelihood that the miRNA affects survival independently of the given clinical parameter.



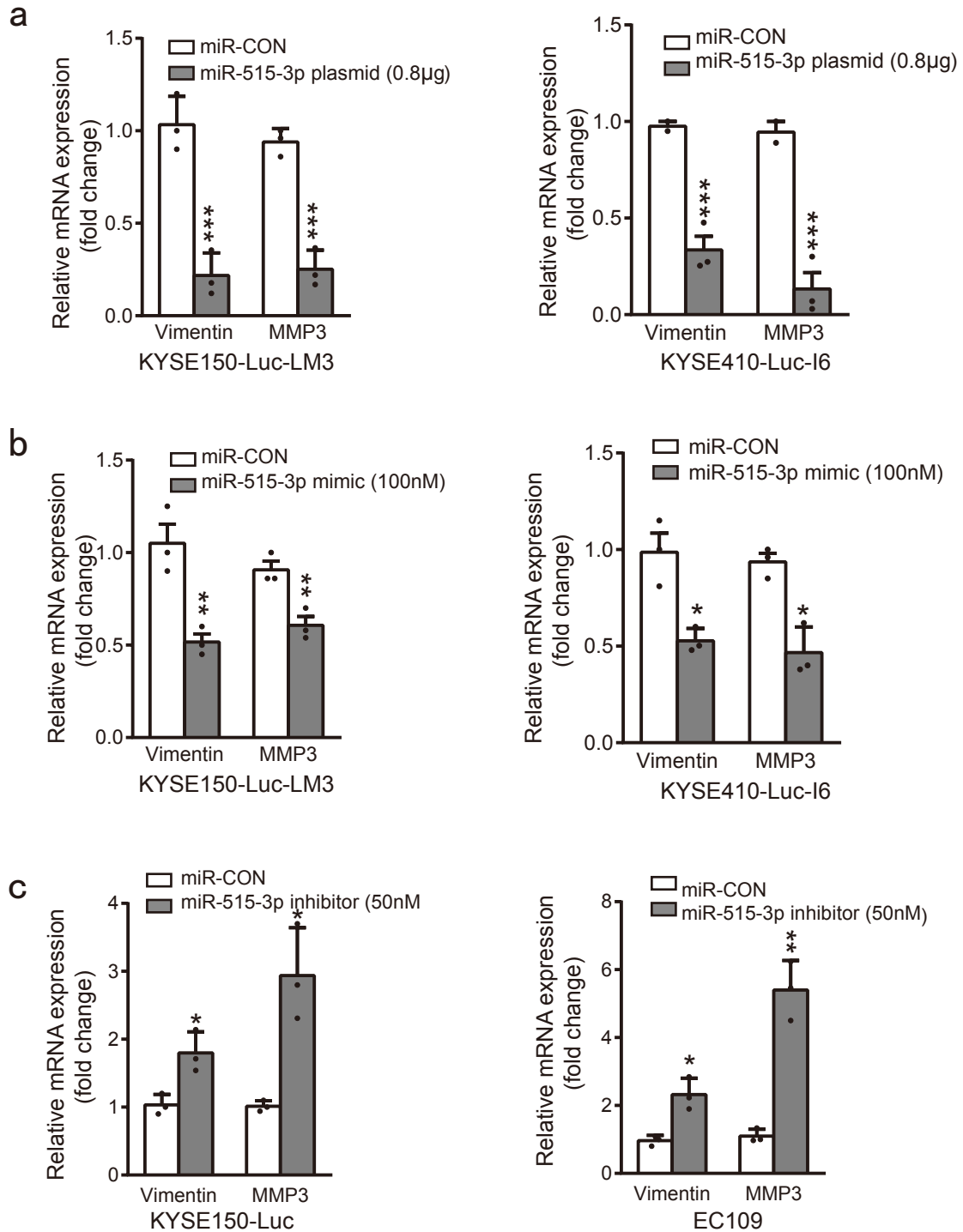
Supplementary Figure S3. miR-515-3p inhibits ESCC migration and adhesion. (a) Comparison of the migration and adhesion potential of miR-515-3p-overexpressing cells and control cells by migration assay, wound healing assay and cell adhesion assay. (b) Comparison of migration and adhesion potential of miR-515-3p-knockdown cells and control cells.



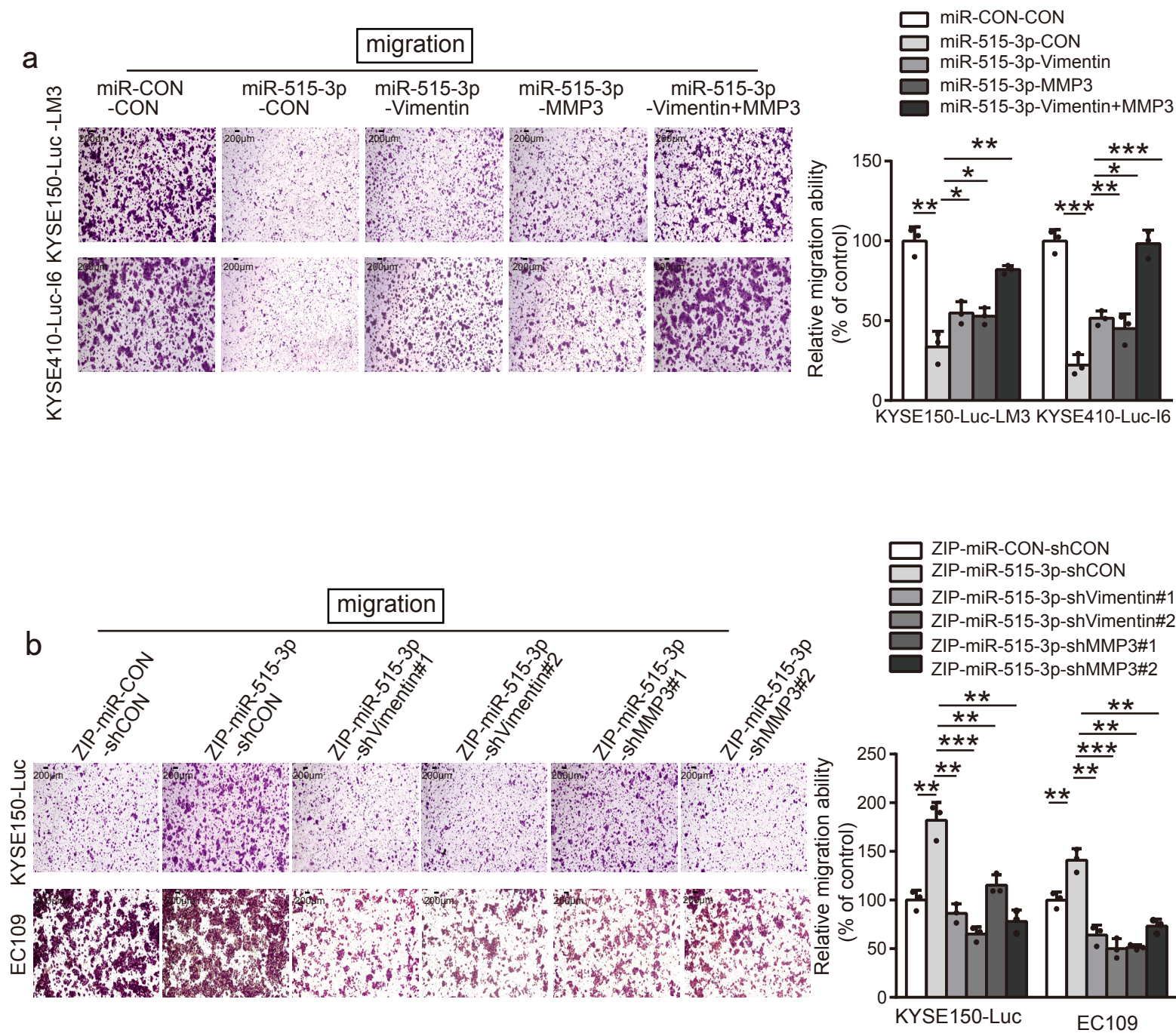
Supplementary Figure S4. miR-515-3p did not exert effect on ESCC proliferation and apoptosis. (a, b) miR-515-3p did not affect ESCC cell proliferation (a) and apoptosis (b) within 24 h, indicated by WST-1 and Annexin V-FITC/PI double staining assay.



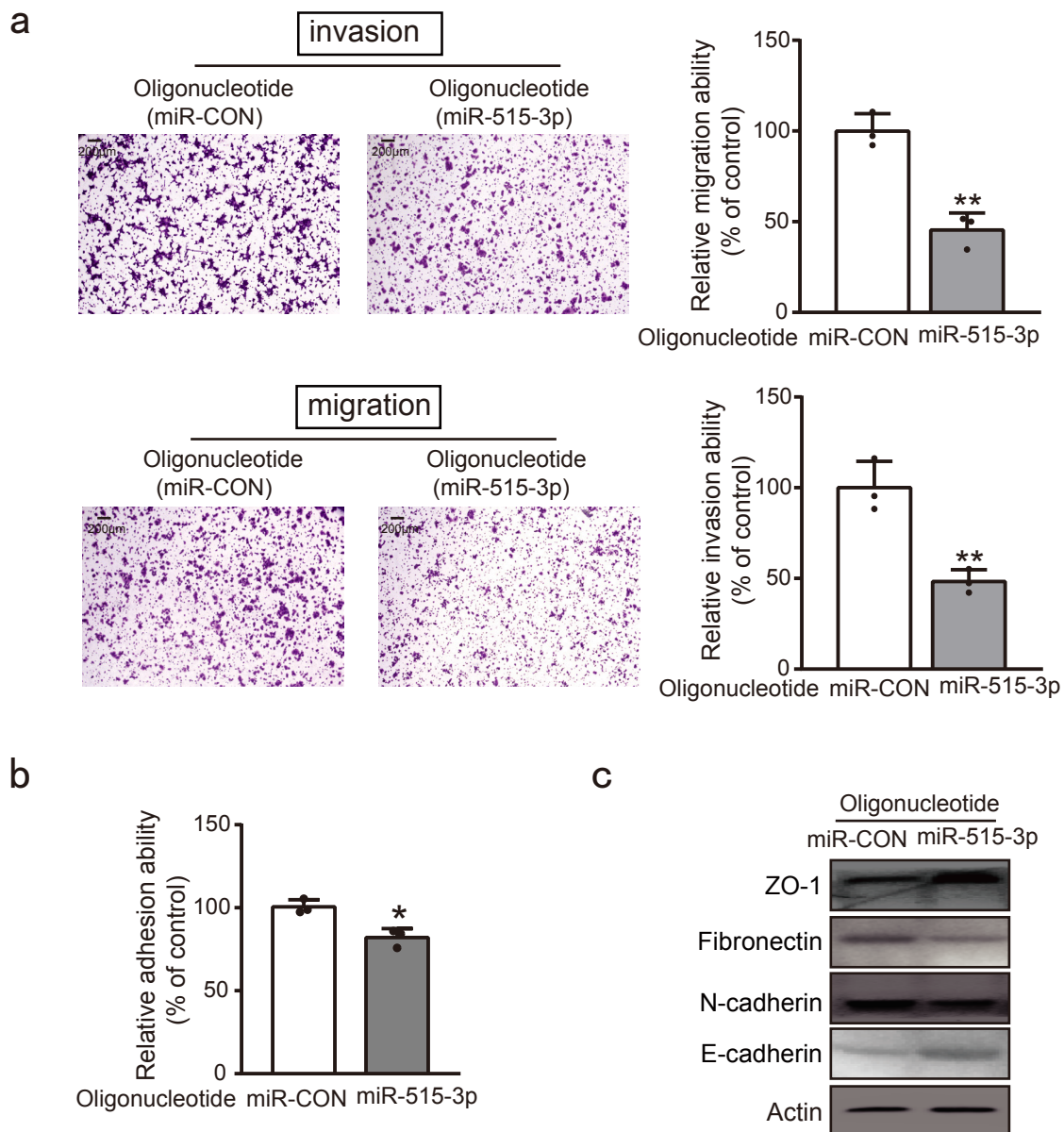
Supplementary Figure S5. Comparison of gene profiles of KYSE150-Luc-LM3-miR-515-3p and KYSE150-Luc-LM3-miR-CON cells. (a) Heatmap of the gene profiles of KYSE150-Luc-LM3-miR-515-3p and KYSE150-Luc-LM3-miR-CON cell lines analyzed by using RNA-sequencing. Red color for high abundance and green color for low abundance. (b) Volcano map of the upregulated and downregulated genes in KYSE150-Luc-LM3-miR-515-3p cells compared with KYSE150-Luc-LM3-miR-CON cells.



Supplementary Figure S6. Effect of miR-515-3p on vimentin and MMP3 expression at mRNA level. ESCC cells were transfected with miR-515-3p-expressing plasmid (a), miR-515-3p mimic (b), or miR-515-3p inhibitor (c), respectively, and mRNA expression of vimentin and MMP3 was compared with the control cells by qRT-PCR.



Supplementary Figure S7. Vimentin and MMP3 mediate the effect of miR-515-3p on cancer cell migration. (a) Comparison of the migration potential of the cells co-overexpressing miR-515-3p, vimentin and MMP3 or miR-515-3p and vimentin, or miR-515-3p and MMP3, as well as the control cells by chamber migration assay. (b) The migration potential of the cells were compared by chamber migration assay.



Supplementary Figure S8. miR-515-3p oligonucleotide inhibits ESCC invasion, migration and adhesion in ESCC cells. (a) migration and cell adhesion assays were performed to determine the migration and adhesion potential of cells transfected with miR-515-3p oligonucleotide and miR-CON, respectively. (b) Western blot analysis of ZO-1, fibronectin, N-cadherin and E-cadherin expression.

Supplementary Table 1. Correlation between miR-515-3p expression and survival of cancer patients.

Head and neck squamous cell carcinoma	
Clinical Parameter	Multivariate Log Rank <i>P</i>-value
Clinical M Status	2.97E-04
Clinical N Status	1.49E-04
Clinical Stage	2.65E-04
Clinical T Status	3.36E-04
Histologic Grade	3.15E-04
Pathologic N Status	4.92E-04
Pathologic Stage	4.38E-04
Pathologic T Status	6.40E-04
Kidney chromophobe	
Clinical Parameter	Multivariate Log Rank <i>P</i>-value
Pathologic N Status	1.45E-04
Kidney renal papillary cell carcinoma	
Clinical Parameter	Multivariate Log Rank <i>P</i>-value
Pathologic M Status	0.0068
Pathologic N Status	0.0275

Multivariate Cox analysis evaluates the combined effect of the miRNA and clinical parameters patient survival duration. A strong degree of significance indicates the likelihood that the miRNA affects survival independently of the given clinical parameter.

Supplementary Table 2. Correlation between miR-515-3p expression and clinical parameters of cancer patients.

Bladder urothelial carcinoma	
Clinical Parameter	ANOVA <i>P</i>-value
Clinical T Status	3.68E-02
Pathologic Stage	2.20E-05
Pathologic T Status	2.02E-02
Colon adenocarcinoma	
Clinical Parameter	ANOVA <i>P</i>-value
Pathologic M Status	4.43E-02
Kidney chromophobe	
Clinical Parameter	ANOVA <i>P</i>-value
Pathologic M Status	7.64E-07
Pathologic Stage	2.67E-04
Pathologic T Status	2.84E-07
Kidney renal clear cell carcinoma	
Clinical Parameter	ANOVA <i>P</i>-value
Pathologic T Status	2.51E-10
Kidney renal papillary cell carcinoma	
Clinical Parameter	ANOVA <i>P</i>-value
Pathologic N Status	4.45E-02
Pathologic T Status	4.47E-03
Lung adenocarcinoma	
Clinical Parameter	ANOVA <i>P</i>-value
Pathologic M Status	6.79E-04
Pathologic Stage	3.65E-03
Skin cutaneous melanoma	
Clinical Parameter	ANOVA <i>P</i>-value
Pathologic N Status	1.87E-02
Testicular germ cell tumors	
Clinical Parameter	ANOVA <i>P</i>-value
Clinical T Status	1.11E-03
Pathologic T Status	2.96E-02
Uterine corpus endometrial carcinoma	
Clinical Parameter	ANOVA <i>P</i>-value
Clinical Stage	3.69E-04
Uveal melanoma	
Clinical Parameter	ANOVA <i>P</i>-value
Clinical M Status	2.51E-05
Clinical N Status	3.88E-06

Analysis of variance (ANOVA) compares miRNA expression between the different cohorts within each clinical parameter.