

Supplementary Figure 1. Effects of S100A9 overexpression on the malignancy of A549 and H1975 cells.

Groups of A549 and H1975 cells: The Vector group and S100A9-OE group were used in the following experiments.

(A-B) Results of the CCK8 assay showing the OD values at 450 nm of A549 or H1975 cells at 0 h, 24 h, 48 h, and 72 h; ****P < 0.0001; N=3 independent biological replicates. The data are the mean \pm SD. **(C-F)** Single-colony formation assay results showing the number of single clones formed by A549 or H1975 cells; ****P < 0.0001; N=3 independent biological replicates. The data are the mean \pm SD. **(G-J)** Results of the EdU assay showing the proliferation rates of A549 and H1975 cells; ****P < 0.0001; N=3 independent biological replicates. The data are the mean \pm SD. **(K-N)** Transwell assay results showing the number of migrating A549 or H1975 cells. ****P < 0.0001; N=3 independent biological replicates. The data are the mean \pm SD. Statistical significance was determined using Student's t test and two-way ANOVA followed by the Bonferroni post hoc correction.

Supplementary Figure 2. Effects of S100A9 overexpression on the malignancy of A549 and H1975 cells.

Groups of A549 and H1975 cells: The Vector group and S100A9-OE group were used in the following experiments.

(A-D) Transwell assay results showing the number of invasive A549 or H1975 cells; ****P < 0.0001; N=3 independent biological replicates. The data are the mean \pm SD. **(E-H)** Scratch assay results showing the widths of the migration distances of A549 and H1975 cells at 0 h and 24 h; ***P < 0.001; ****P < 0.0001; N=3 independent biological replicates. The data are the mean \pm SD. **(I-L)** TUNEL assay results showing the percentages of apoptotic of A549 and H1975 cells. ****P < 0.0001; N=3 independent biological replicates. The data are the mean \pm SD. Statistical significance was determined using Student's t test followed by the Bonferroni post hoc correction.

Supplementary Figure 3. Effects of S100A9 knockout on the malignancy of PC-9 cells.

Groups (A–M): The sg-NC group, sg-S100A9-1 group, and sg-S100A9-2 group were used in the following experiments.

(A) The results of the CCK8 assay showing the OD values at 450 nm of PC-9 cells at 0 h, 24 h, 48 h, and 72 h; ****P < 0.0001; N=3 independent biological replicates. The data are the mean ± SD. **(B–C)** The results of the EdU assay showing the cell proliferation rates of PC-9 cells; ****P < 0.0001; N=3 independent biological replicates. The data are the mean ± SD. **(D–E)** The single-colony formation assay results showing the number of single colonies formed by PC-9 cells; ****P < 0.0001; N=3 independent biological replicates. The data are the mean ± SD. **(F–G)** Scratch assay results showing the widths of the migration distances of PC-9 cells at 0 h and 24 h. **(H–K)** Numbers of migrating cells and invasive PC-9 cells in the Transwell assay; ****P < 0.0001; N=3 independent biological replicates. The data are the mean ± SD. **(L–M)** TUNEL assay results showing the percentage of apoptotic PC-9 cells. ****P < 0.0001; N=3 independent biological replicates. The data are the mean ± SD. Statistical significance was determined using one-way ANOVA followed by Tukey's post hoc test.

Supplementary Figure 4: H1975 cells: The Vector group and S100A9-OE group were used in the following experiments.

(A) The results of the CCK8 assay showing the OD values at 450 nm of HBMECs after 0 h, 24 h, 48 h, and 72 h of coculture with H1975 cells. ****P < 0.0001; N=3 independent biological replicates. The data are the mean ± SD. **(B)** The sodium ion permeability assay showing the sodium ion permeability of HBMECs cocultured with H1975 cells. **P < 0.01; N=3 independent biological replicates. The data are the mean ± SD. **(C–F)** Western blot assay results showing the expression levels of the osmotic pressure-maintaining proteins ZO-1, Occludin, and Claudin-5 in HBMECs cocultured with H1975 cells. **P<0.01; ****P<0.0001; N=3 independent biological replicates. The data are the mean ± SD. **(G–I)** Tube formation assay results showing the number of branches and the total lengths of the tubes formed by HBMECs cocultured with H1975 cells. *P<0.05; **P<0.01; N=3 independent biological replicates. The data are the mean ± SD. Statistical significance was determined using one-way ANOVA followed by

Tukey's post hoc test. **(J-K)** Cell viability of A549 and H1975 cells was assessed via the CCK-8 assay across the Vector group, S100A9-OE group, and S100A9-OE+ITGB1 inhibitor group. ****P<0.01; N=3 independent biological replicates. The data are the mean \pm SD. **(L-Q)** Transwell assays were utilized to quantify the migratory and invasive capacities of A549 and H1975 cells in the Vector group, S100A9-OE group, and S100A9-OE+ITGB1 inhibitor group. ****P<0.01; N=3 independent biological replicates. The data are the mean \pm SD.

Supplementary Figure 5:

Groups (A-Q): The following groups of cells were used in the experiments: Vector group H1975 cells cocultured with HBMECs; S100A9-OE group H1975 cells cocultured with HBMECs; S100A9-OE+si-NC group H1975 cells cocultured with HBMECs; and S100A9-OE+si-Vimentin group H1975 cells cocultured with HBMECs.

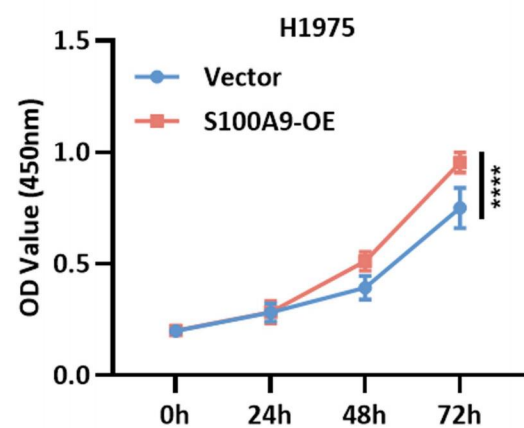
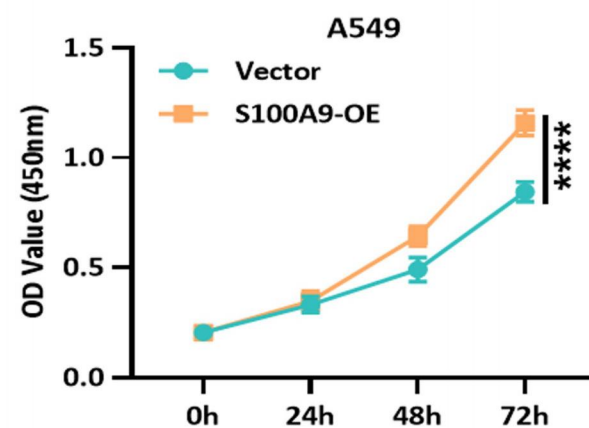
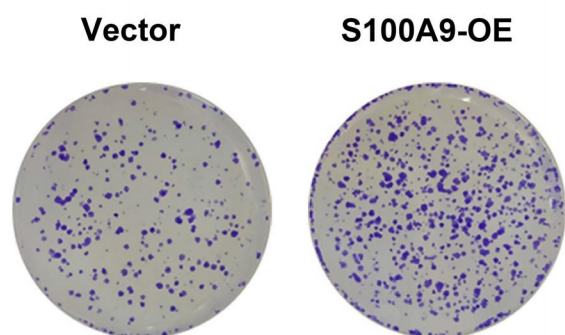
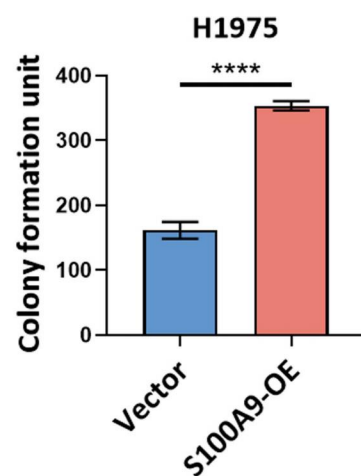
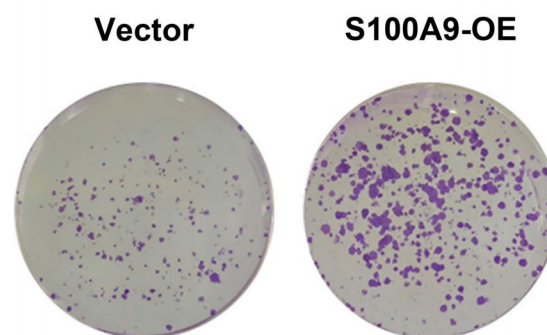
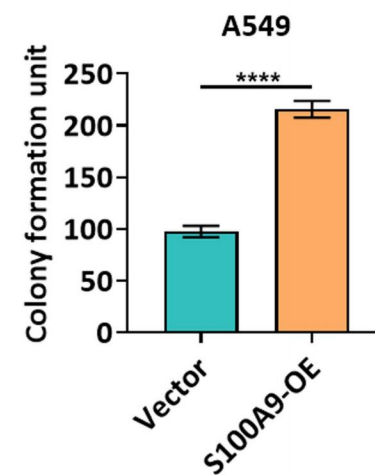
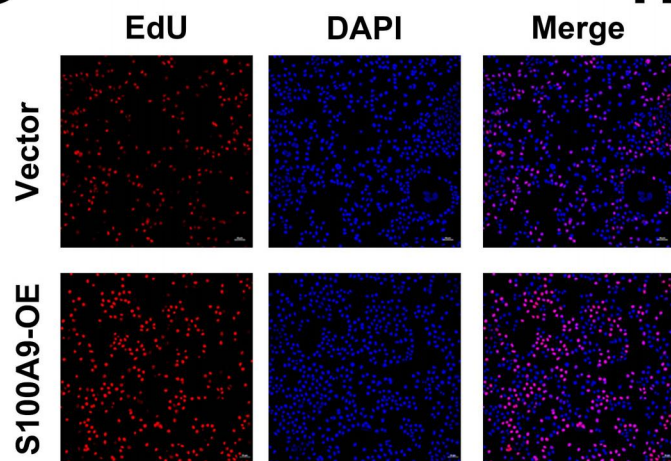
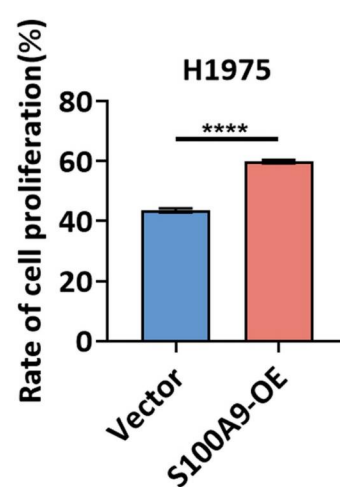
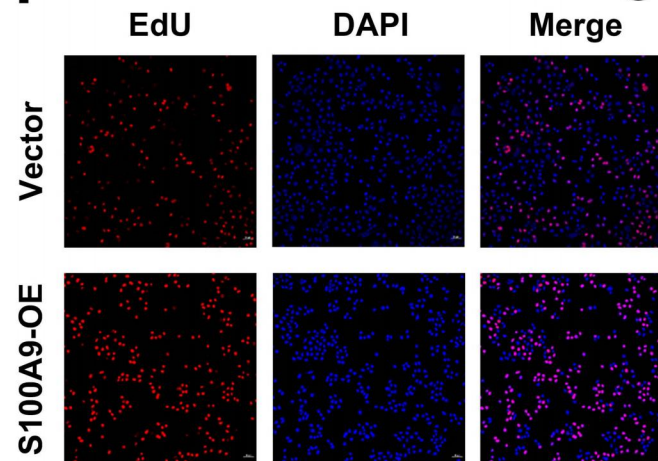
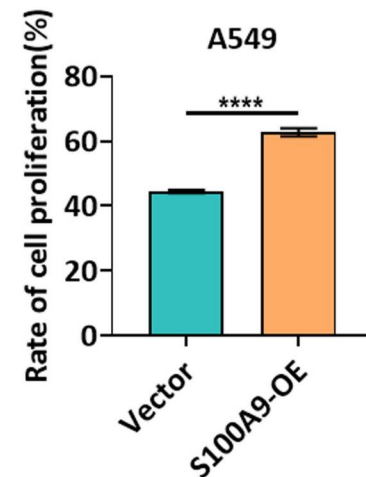
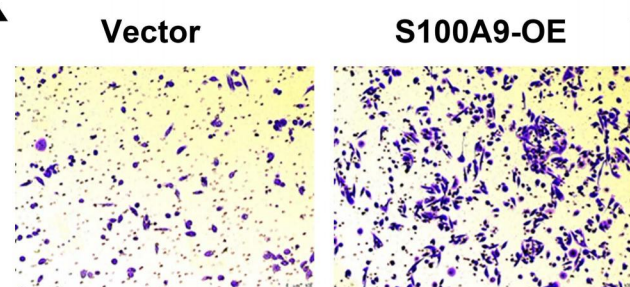
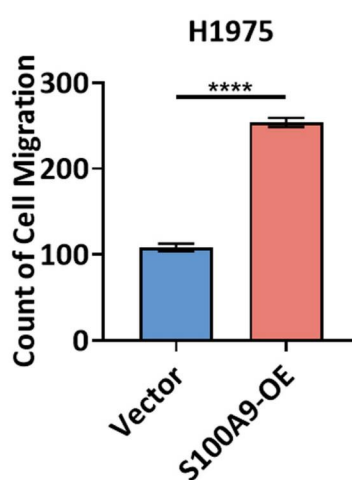
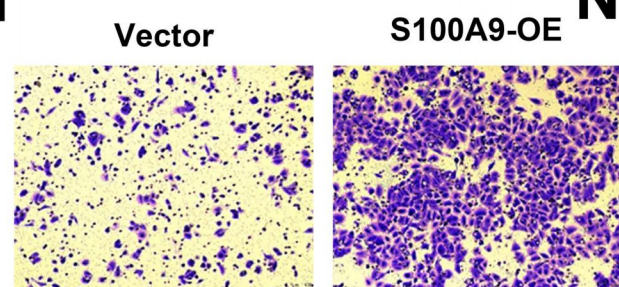
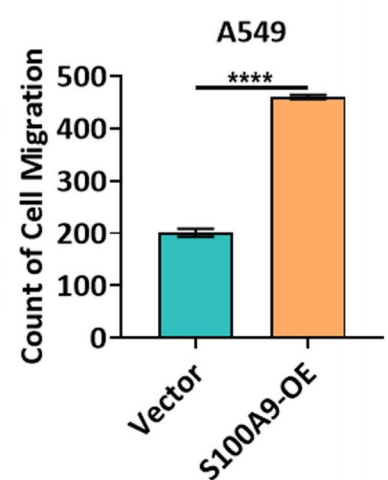
(A-C) RT-qPCR and Western blot results showing the RNA and protein expression levels, respectively, of Vimentin in H1975 cells transfected with si-1, si-2, and si-3. **P<0.01; ***P<0.001; ****P<0.0001; N=3 independent biological replicates. The data are the mean \pm SD. **(D)** The results of the sodium ion permeability experiment showing the sodium ion permeability of HBMECs cocultured with H1975 cells. **P<0.01; ****P<0.0001; N=3 independent biological replicates. The data are the mean \pm SD. **(E)** Immunofluorescence staining results showing the fluorescence intensities of ZO-1, Claudin-5, and Occludin in HBMECs cocultured with H1975 cells. N=3 independent biological replicates. The data are the mean \pm SD. **(F-I)** Western blot experiment results showing the protein expression levels of ZO-1, Claudin-5, and Occludin in HBMECs cocultured with H1975 cells. ***P<0.001; ****P<0.0001; N=3 independent biological replicates. The data are the mean \pm SD. **(J-L)** Tube formation experiment results showing the number of branch points and tube lengths of HBMECs cocultured with H1975 cells. ***P<0.001; ****P<0.0001; N=3 independent biological replicates. The data are the mean \pm SD. **(M)** Reactive oxygen species (ROS) experiment results showing the reactive oxygen species content in HBMECs cocultured with H1975 cells. N=3 independent biological replicates. The data are the mean \pm SD. **(N-**

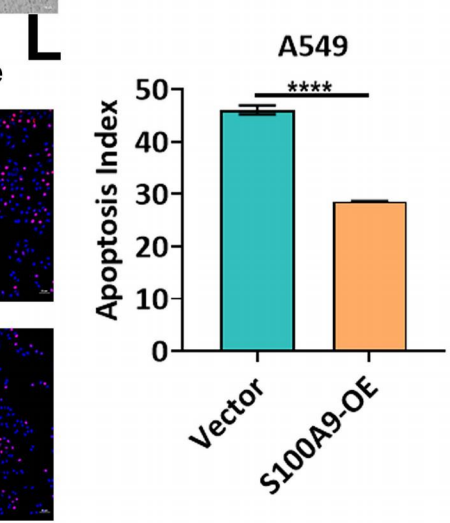
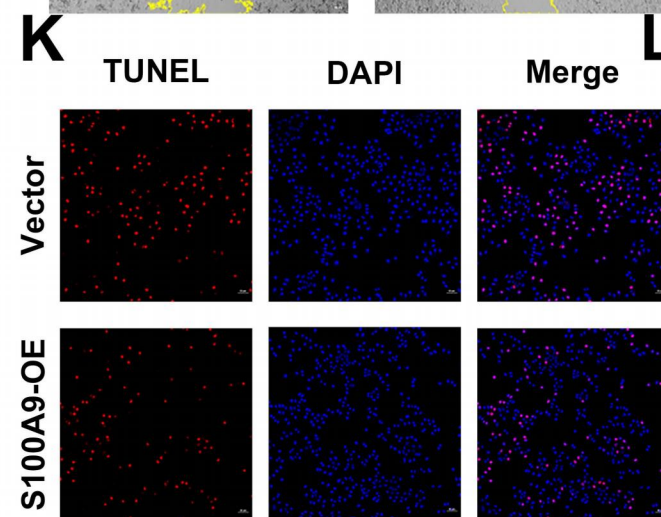
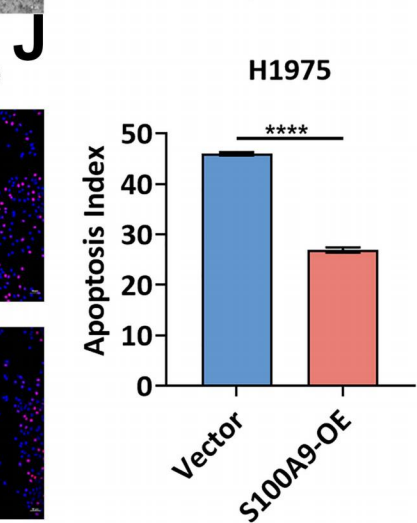
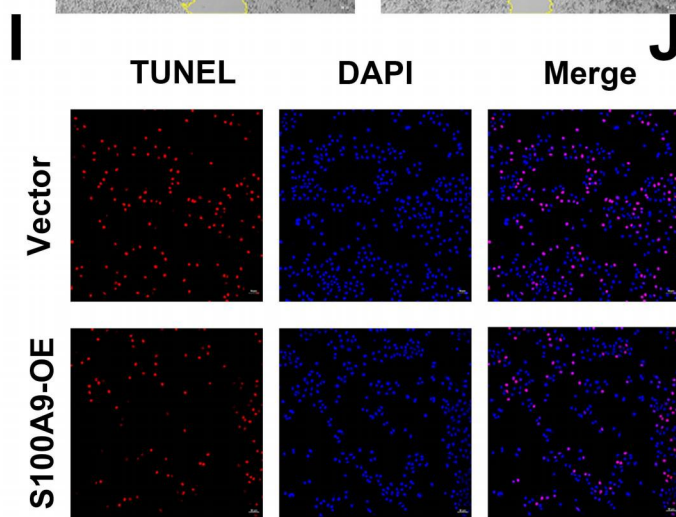
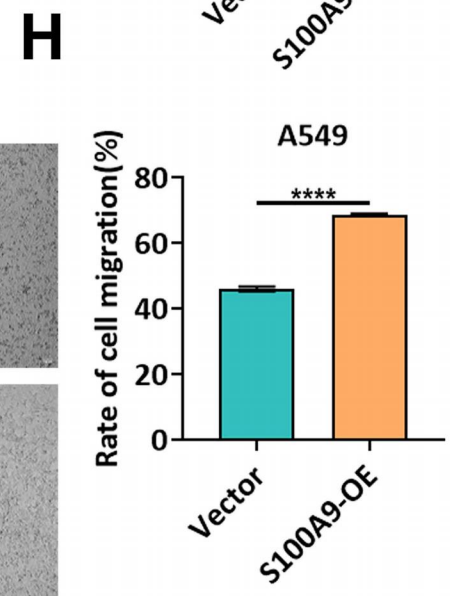
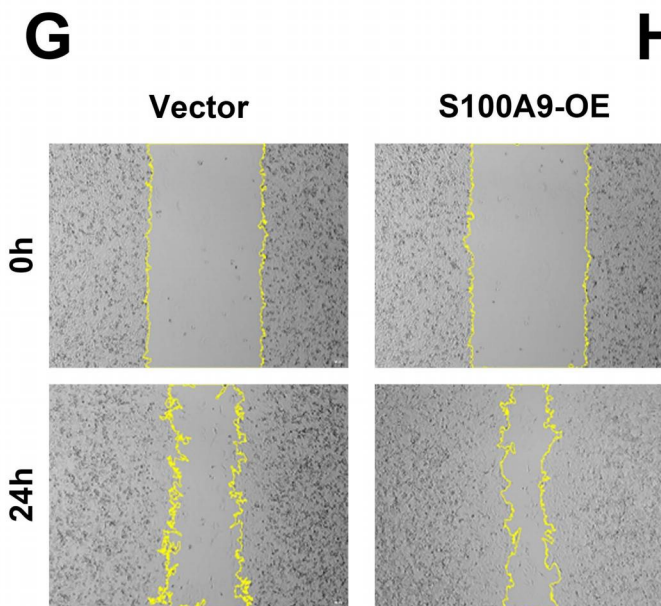
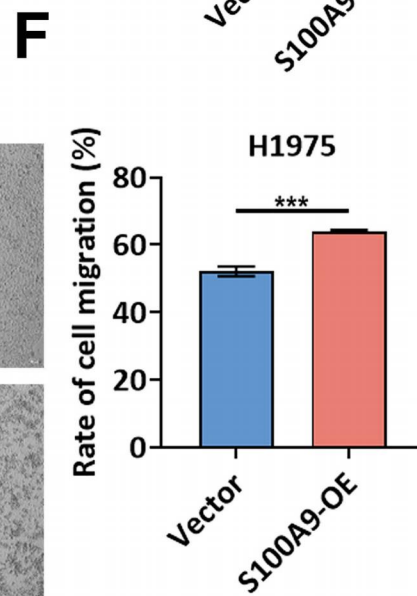
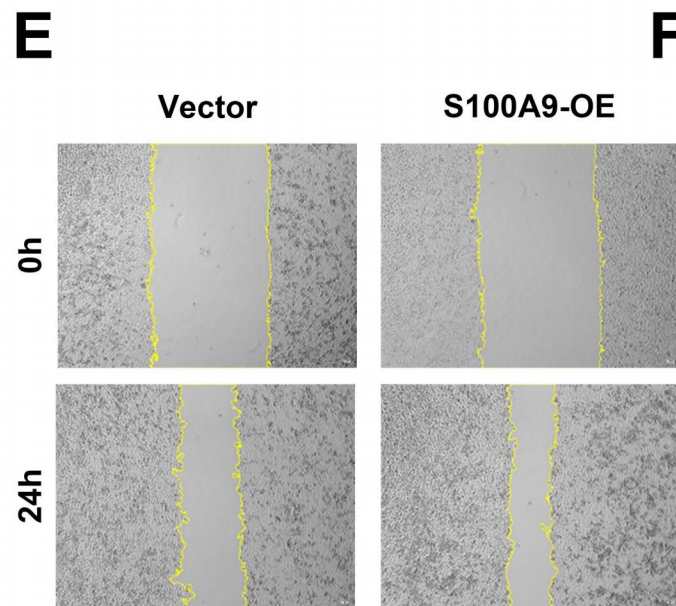
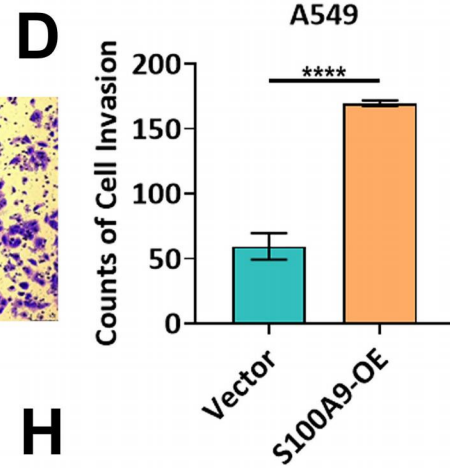
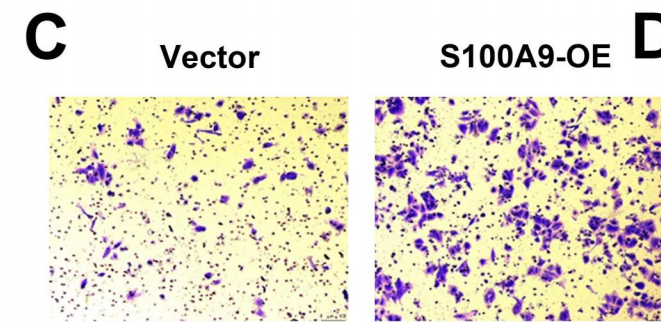
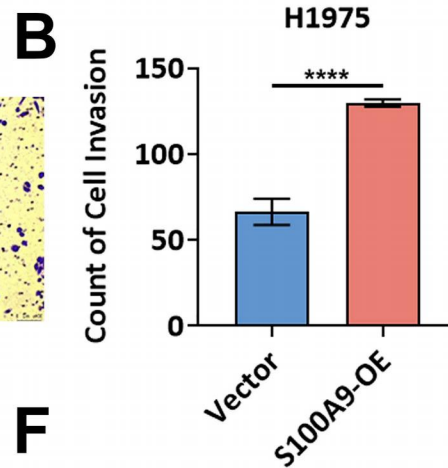
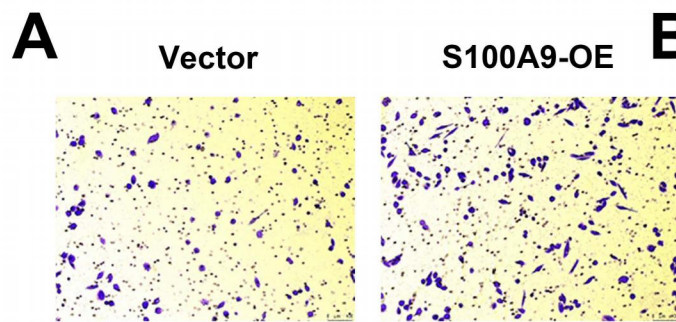
Q) Western blot showing the protein expression levels of VE-Cadherin, N-Cadherin, and α -SMA in HBMECs cocultured with H1975 cells. ***P<0.001; ****P<0.0001; N=3 independent biological replicates. The data are the mean \pm SD. Statistical analysis was performed using one-way analysis of variance (ANOVA).

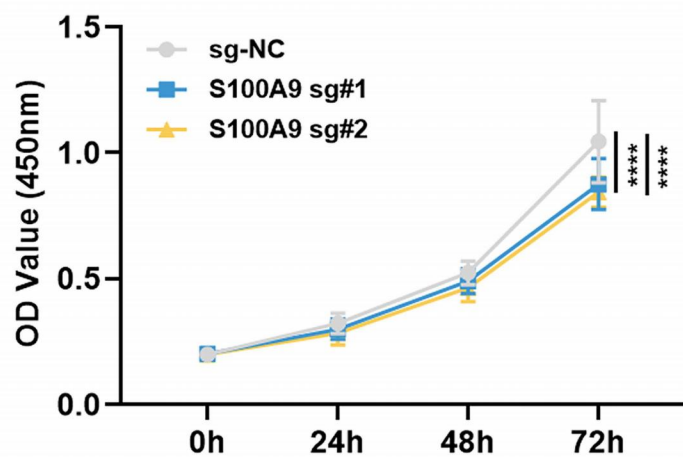
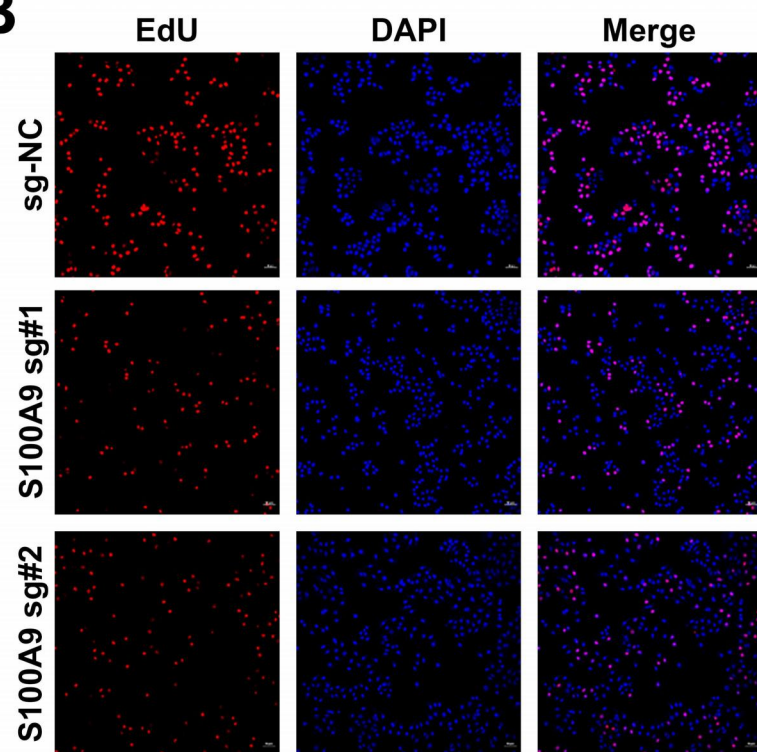
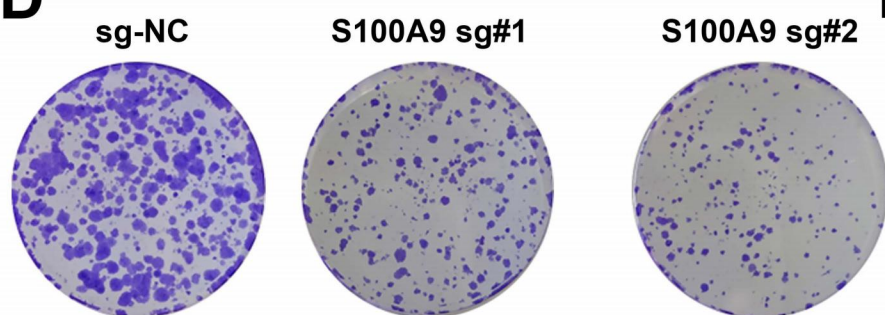
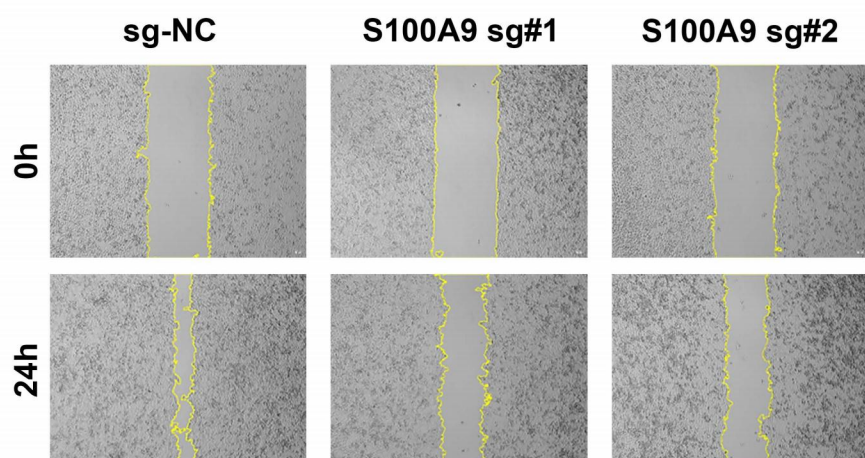
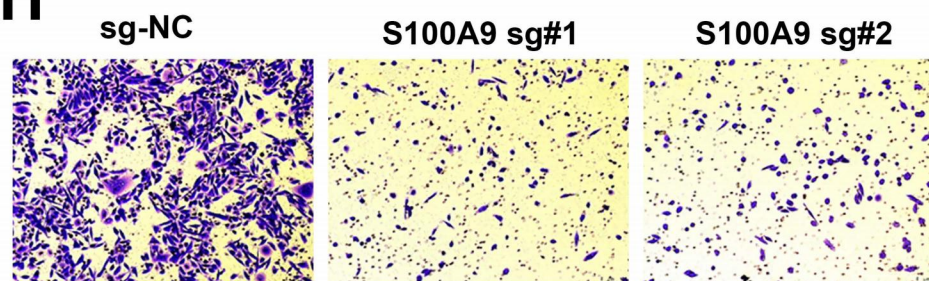
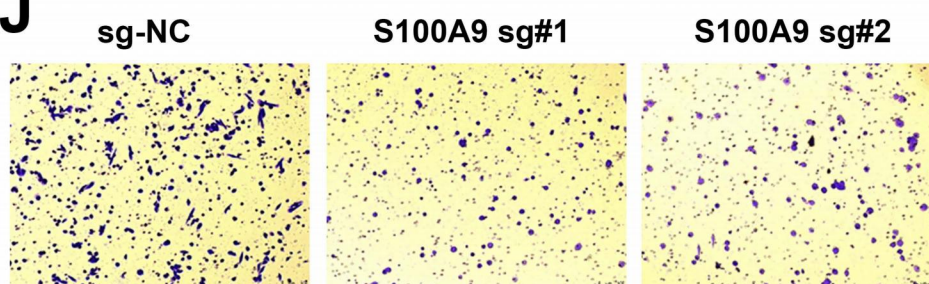
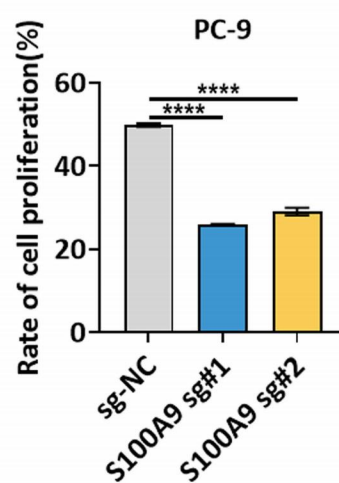
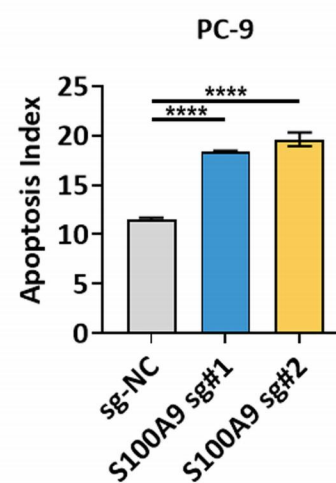
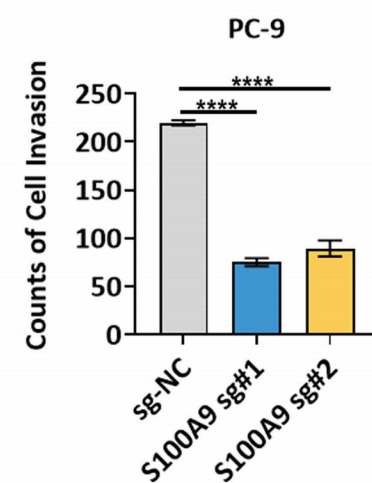
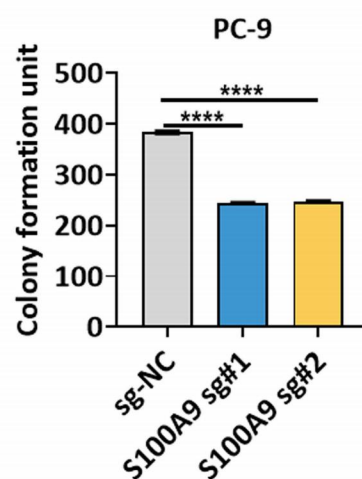
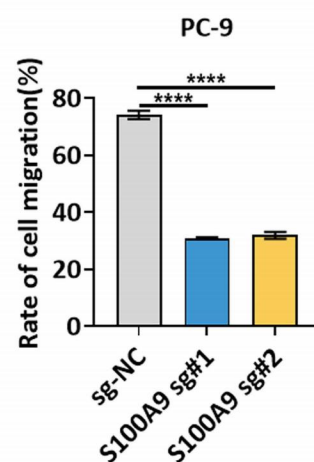
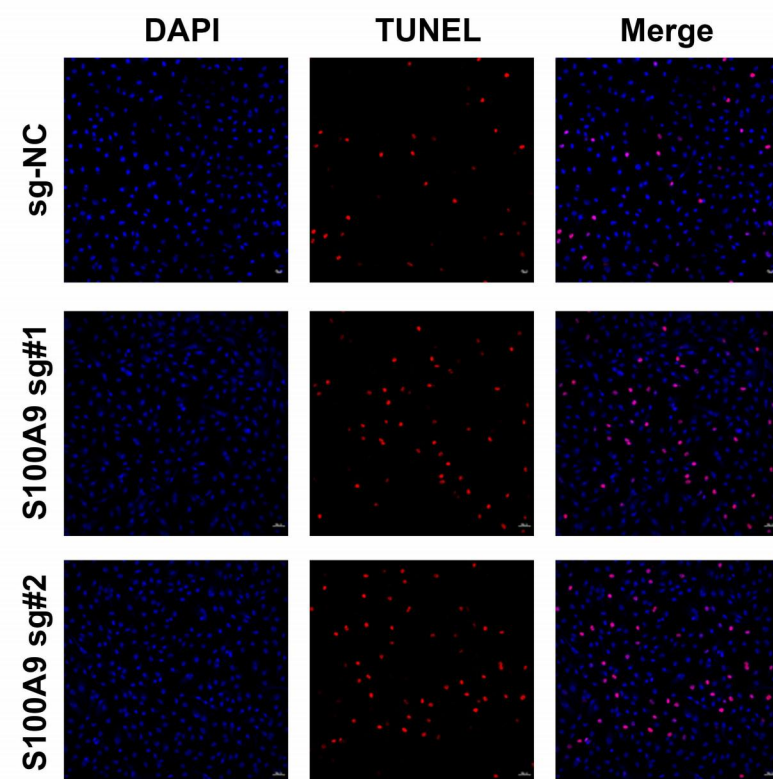
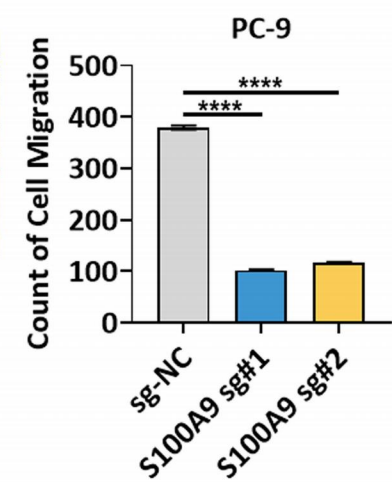
Supplementary Figure 6:

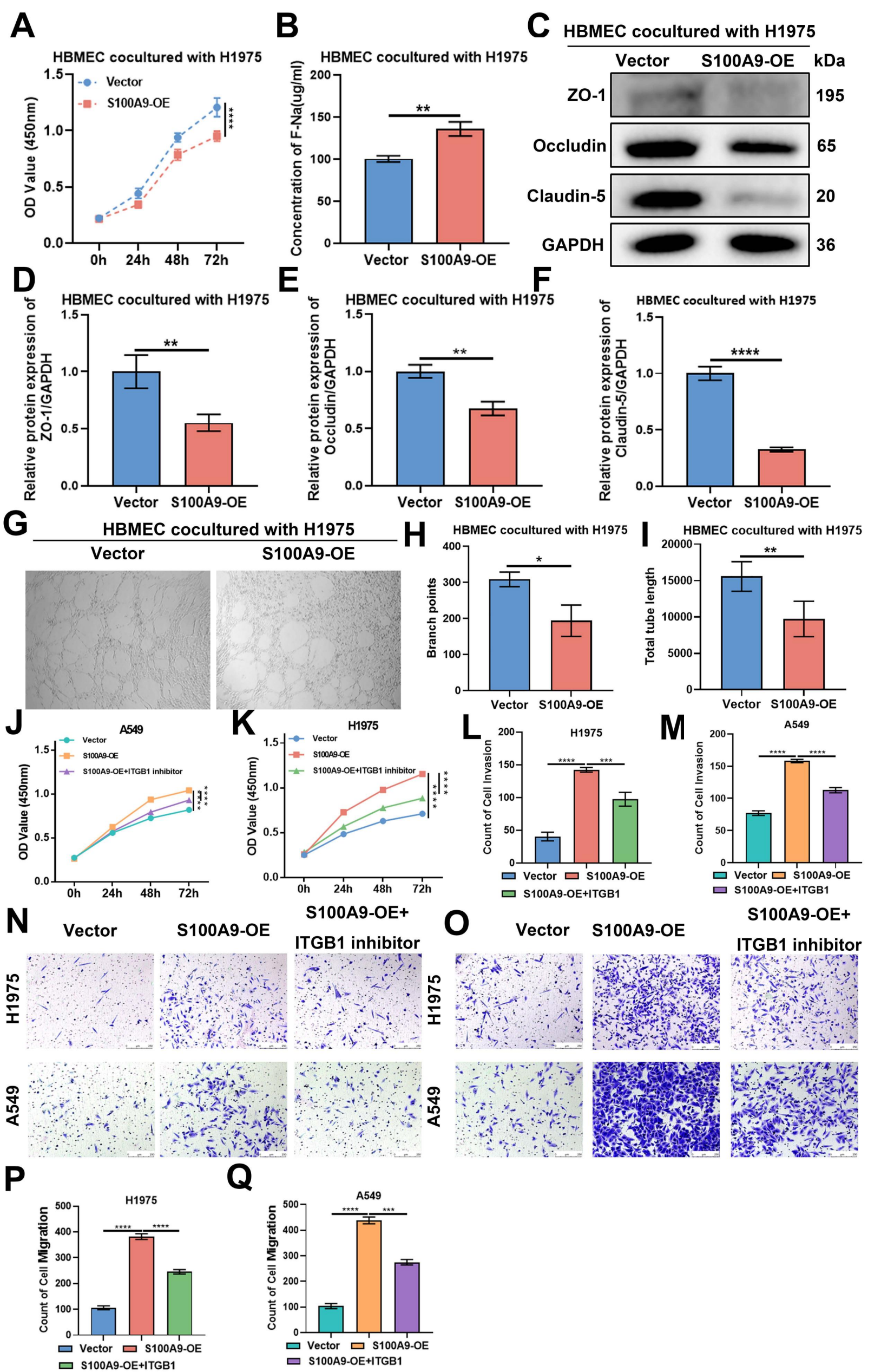
Groups (A–N): The following groups of cells were used in the experiments: Vector group H1975 cells cocultured with HBMECs; S100A9-OE group H1975 cells cocultured with HBMECs; S100A9-OE+si-NC group H1975 cells cocultured with HBMECs; and S100A9-OE+si-Vimentin group H1975 cells cocultured with HBMECs.

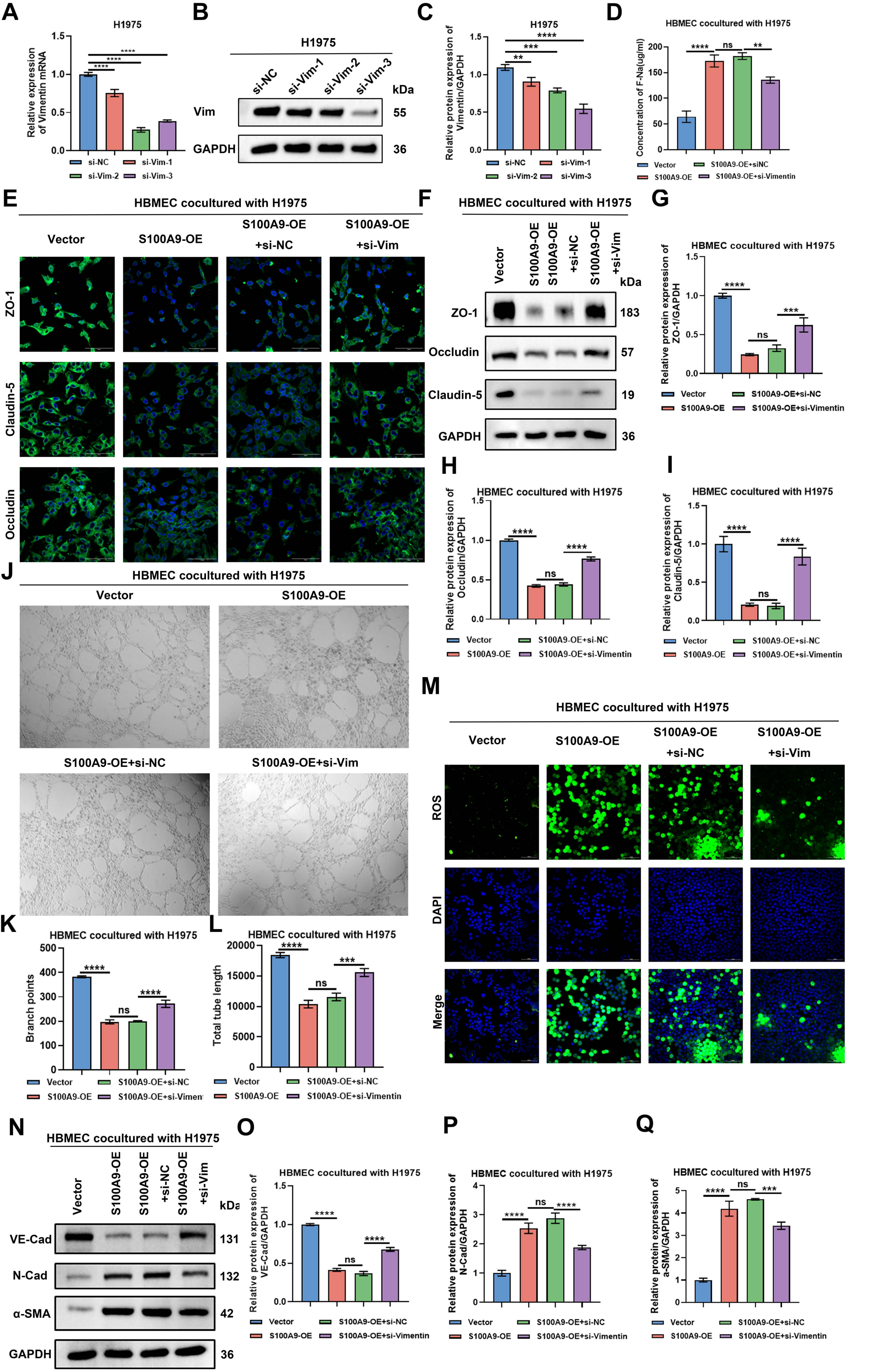
(A) Results of the CCK8 assay showing the OD values at 450 nm of HBMECs after 0 h, 24 h, 48 h, and 72 h of coculture with H1975 cells; ***P<0.001; ****P<0.0001; N=3 independent biological replicates. The data are the mean \pm SD. **(B–C)** Results of the EdU assay showing the proliferation rates of HBMECs cocultured with H1975 cells; ***P<0.001; ****P<0.0001; N=3 independent biological replicates. The data are the mean \pm SD. **(D–E)** Scratch assay results showing the migration distance of HBMECs cocultured with H1975 cells; ***P<0.001; ****P<0.0001; N=3 independent biological replicates. The data are the mean \pm SD. **(F–H)** Results of the JC-1 assay showing the mitochondrial membrane potential in of HBMECs cocultured with H1975 cells; N=3 independent biological replicates. The data are the mean \pm SD. **(I–J)** Immunofluorescence confocal microscopy results showing the pH of lysosomes in HBMECs cocultured with H1975 cells; N=3 independent biological replicates. The data are the mean \pm SD. **(K–N)** Western blot results of the protein expression level of the autophagy-related protein p62 and the p-Beclin 1/Beclin 1 and LC3-II/I ratios in HBMECs cocultured with H1975 cells. *P<0.05; **P<0.01; ****P<0.0001; N=3 independent biological replicates. The data are the mean \pm SD. Statistical analysis was performed using one-way analysis of variance (ANOVA).

A**B****C****D****E****F****G****H****I****J****K****L****M****N**



A**B****D****F****H****J****C****M****K****E****G****L****I**





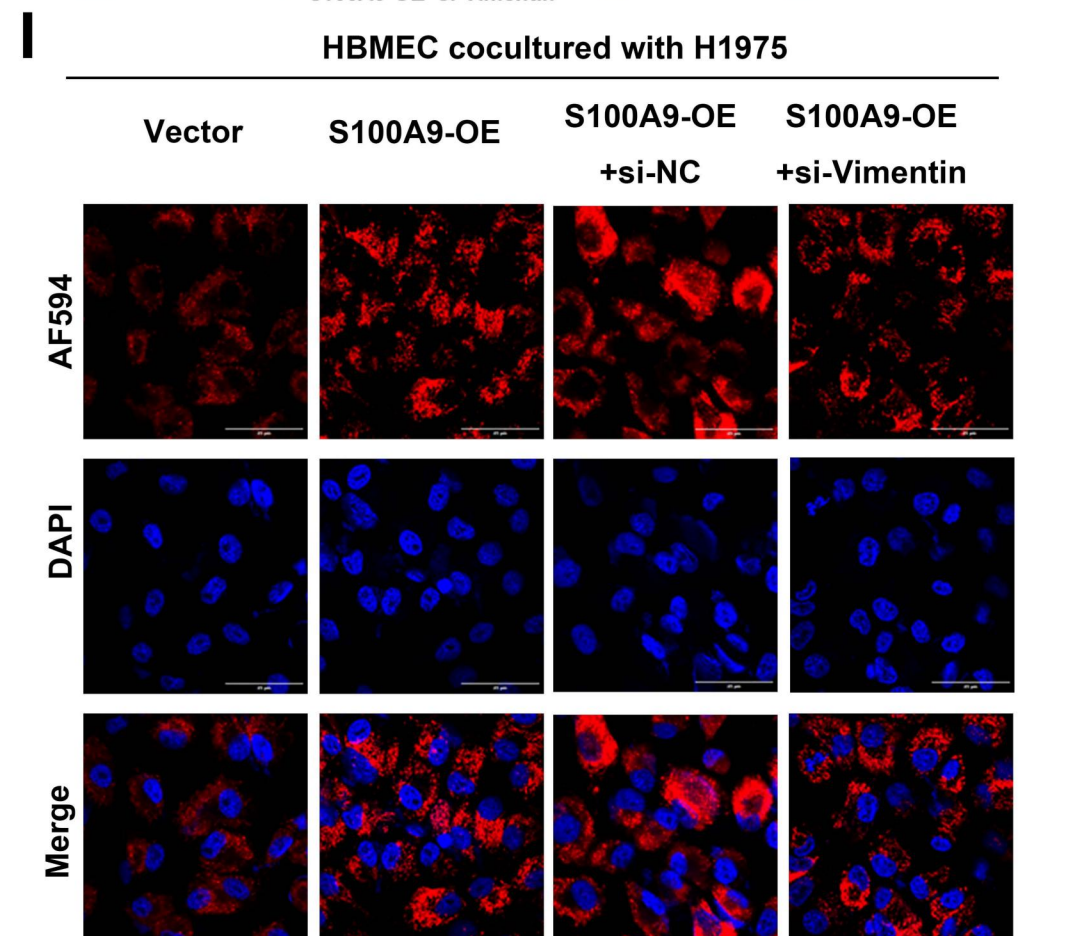


Table 1. Primer for following genes

Gene name	Primer sequence
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S100A9 Reverse	GGCCTGGCTTATGGTGGTG
GAPDH Forward	GGAGCGAGATCCCTCCAAAAT
GAPDH Reverse	GGCTGTTGTCATACTTCTCATGG
Vimentin Forward	AGTCCACTGAGTACCGGAGAC
Vimentin Reverse	CATTTCACGCATCTGGCGTTC