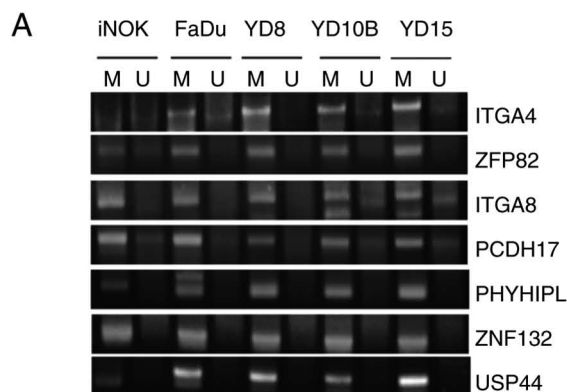


Figure S2. Methylation status of hypermethylated candidate genes in oral cancer cell lines by MSP. (A) PCR products of unmethylated (U) and methylated (M) ITGA4, ZFP82, ITGA8, PCDH17, PHYHIPL, ZNF132 and USP44 from sodium bisulfite-treated genomic DNA from cell lines were visualized by ethidium bromide staining. (B) Methylation state of ITGA4, ZFP82, ITGA8, PCDH17, PHYHIPL, ZNF132, and USP44 analyzed by MSP in a total of oral cancer cell lines. U unmethylated, M methylated, P, partially methylated, U/M unmethylated and methylated. MSP, methylation-specific PCR; ITGA4, integrin subunit α 4; ZFP82, zinc finger protein 82; PCDH17, protocadherin 17; PHYHIPL, phytanoyl-CoA 2-Hydroxylase Interacting Protein Like; ZNF132, zinc Finger Protein 132; USP44, ubiquitin specific peptidase 44; iNOK, immortalized human normal oral keratinocyte.



B

Cell line	Origin of cell	ITGA4	ZFP82	ITGA8	PCDH17	PHYHIPL	ZNF132	USP44
iNOK	Immortalized normal oral keratinocyte	U	M	M	U/M	M	M	P/M
FaDu	Hypopharyngeal squamous cell carcinoma	U/M	M	M	M	M	M	M
YD8	Tongue squamous cell carcinoma	M	M	M	M	M	M	M
YD10B		M	M	U/M	U/M	M	M	M
YD15		M	M	U/M	U/M	M	M	M

Figure S3. ITGA4 inhibits cell proliferation and induces apoptosis in YD8 and YD10B cells. The cells were transfected with the vector or pcDNA4-ITGA4 plasmid for 48 h. (A) Cell proliferation was examined by MTT assays at the indicated time points. The data are shown as the mean \pm standard deviation of three separate experiments. ***P<0.001 vs. the control. (B) Apoptotic cells were evaluated by dual staining with Annexin V/PI and counted via flow cytometry. The graph shows the relative numbers of apoptotic cells among the ITGA4-treated cells. ITGA4, integrin subunit α 4.

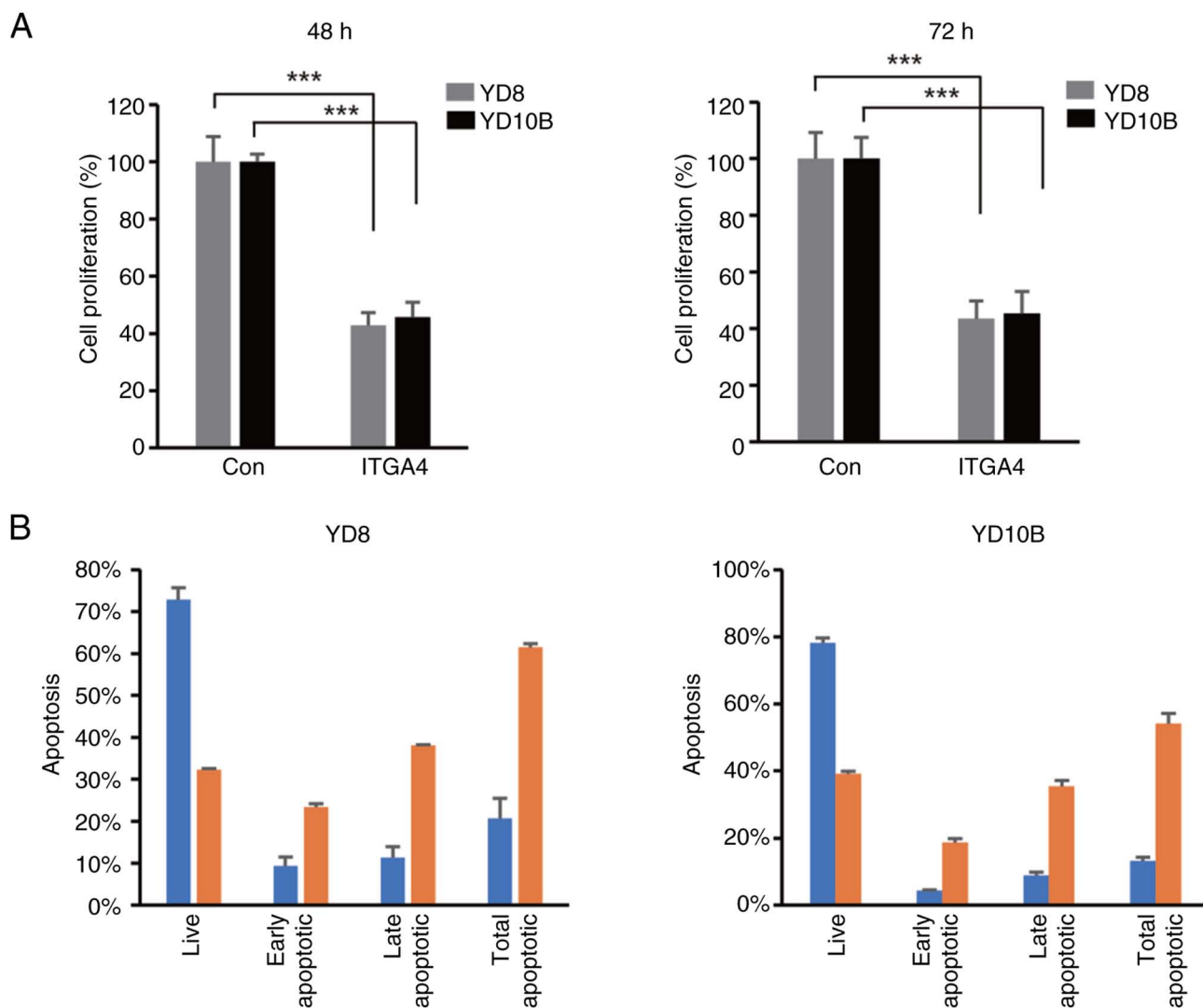


Figure S4. ITGA4 inhibits the motility of YD-8 oral cancer cells. A wound healing assay was performed to identify the effects of ITGA4 on YD-8 cell motility at 24 h and 48 h. Con, control.

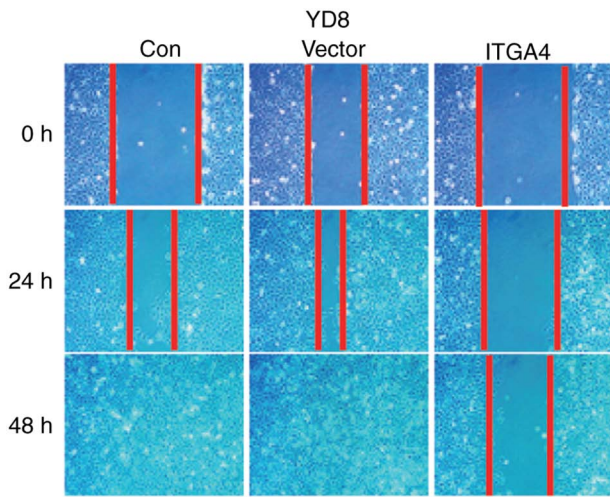


Figure S5. Proteomic profile of ITGA4-overexpressing oral cancer cells. (A) Heatmap of the proteomic profile of oral cancer cells transfected with/without the ITGA4 plasmid. (B) Volcano plot illustrating the significantly differentially expressed proteins. The red box represents the upregulated proteins, and the green box represents the downregulated proteins in the comparative analysis (cutoff fold change >1.5). (C) Functional enrichment analysis of the proteins identified from comparative proteomic analysis of ITGA4-overexpressing oral cancer cells (FaDu and YD-15 cells) via KEGG pathway enrichment analysis. (D) Venn diagram showing the differentially expressed proteins from the comparative proteomic analysis of the control (untreated) and ITGA4-transfected groups. The first 16 significantly upregulated and downregulated genes are labeled. ITGA4, integrin subunit α 4; KEGG, Kyoto Encyclopedia of Genes and Genomes.

