

Figure S1. Validation of cyclin D1 expression after cyclin D1 intervention by western blot analysis. Underexpression of cyclin D1 in (A) HB96 and (B) CAL27 cells after transfection with siRNA against cyclin D1. Overexpression of cyclin D1 in (C) HN30 and (D) CAL27 cells after cyclin D1 gene transfection.

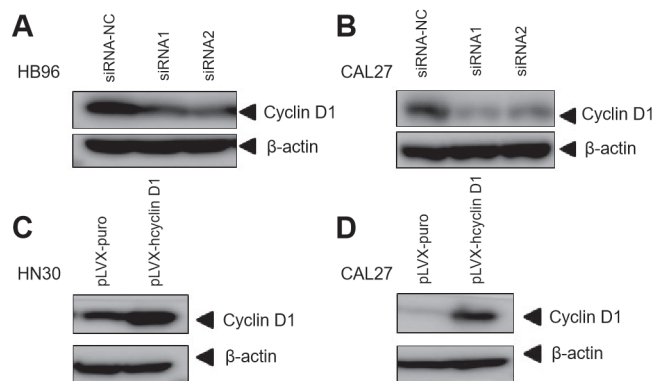


Figure S2. (A) CAL27 and (B) HB96 cells were treated with indicated concentrations of docetaxel, cisplatin and 5-FU altogether following cyclin D1 knockdown, before cell viability was measured, The concentrations of each agent applied, namely docetaxel 0.5 nM, cisplatin 5 μ M, 5-fluorouracil 5 μ M were deduced based on the IC₅₀ from single-agent experiments, followed by 2X dilution. 5-FU, 5-fluorouracil.

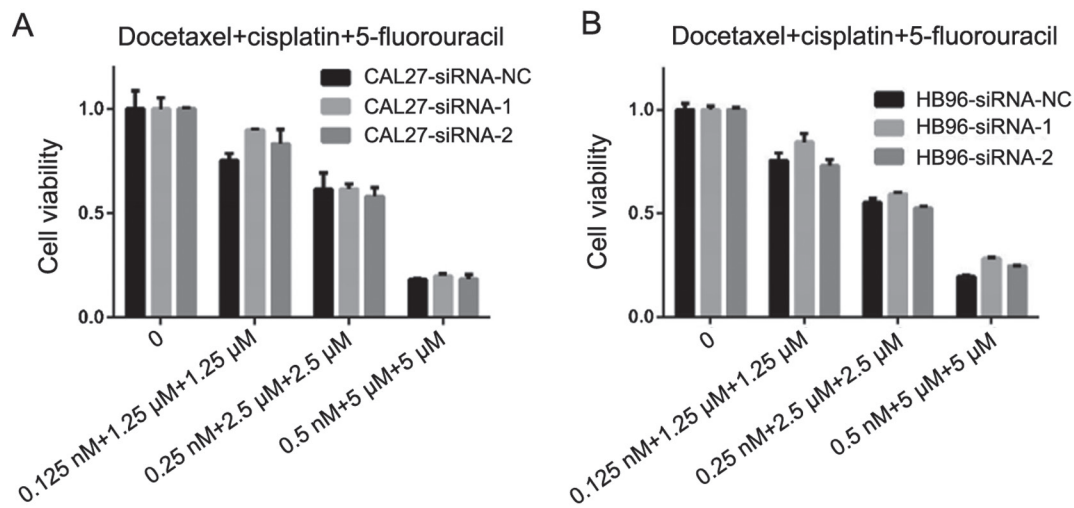


Table SI. Baseline characteristics and cyclin D1 expression in the 232 patients with oral squamous cell carcinoma.

Characteristics	Total patients, n (%)	Cyclin D1 expression level		P-value ^a
		Low (n=155) n (%)	High (n=77) n (%)	
Gender				
Male	160 (69.9)	103 (66.5)	57 (74.0)	0.240
Female	72 (30.1)	52 (33.5)	20 (26.0)	
Age, years				
<60	157 (65.6)	105 (67.7)	52 (74.0)	0.974
≥60	75 (34.4)	50 (32.3)	25 (32.5)	
Site				
Tongue	98 (44.1)	62 (40.0)	36 (46.8)	0.366
Buccal	43 (17.6)	31 (20.0)	12 (15.6)	
Gingiva	38 (15.6)	28 (18.1)	10 (13.0)	
Floor of mouth	29 (11.7)	18 (11.6)	11 (14.3)	
Palate	14 (7.0)	10 (6.5)	4 (5.2)	
Retromolar trigone	10 (3.9)	4 (2.6)	6 (7.8)	
Clinical T stage				
T1/T2	61 (25.8)	42 (27.1)	19 (24.7)	0.693
T3/T4	171 (74.2)	113 (72.9)	58 (75.3)	
Clinical N stage				
N0	99 (43.0)	65 (41.9)	34 (44.2)	0.905
N1	86 (36.7)	59 (38.1)	27 (35.1)	
N2	47 (20.3)	31 (20.0)	16 (20.8)	
Clinical stage				
III	160 (69.1)	109 (70.3)	51 (66.2)	0.526
IVA	72 (30.9)	46 (29.7)	26 (33.8)	
Pathological differentiation				
Well	75 (31.2)	49 (31.6)	16 (20.8)	0.178
Moderately	156 (64.5)	98 (63.2)	58 (75.3)	
Poorly	11 (4.3)	8 (5.2)	3 (3.9)	
Smoking status ^b				
Current/former	110 (49.2)	69 (44.5)	41 (53.2)	0.210
Never	122 (50.8)	86 (55.5)	36 (46.8)	
Alcohol consumption ^c				
Positive	88 (40.5)	54 (34.8)	34 (44.2)	0.168
Negative	144 (59.4)	101 (65.2)	43 (55.8)	

^a χ^2 -test used to compare the differences in the baseline characteristics between the low and high cyclin D1 expression groups. ^bFormer/current smokers defined as ≥1 pack-year history of smoking. ^cPositive alcohol consumption was defined as current alcohol consumption of >1 drink per day for 1 year (12 ounces of beer with 5% alcohol, 5 ounces of wine with 12-15% alcohol or one ounce of liquor with 45-60% alcohol). All other patients were classified as negative alcohol consumption.

Table SII. Calculated 5-year survival rate of the patients with low and high Cyclin D1 expression^a.

Calculated 5-year survival rate	Patients with low cyclin D1 expression, %	Patients with high cyclin D1 expression, %
Overall survival rate	69.0	48.5
Disease-free survival rate	60.5	41.1
Locoregional recurrence-free survival rate	61.1	44.8
Distant metastasis-free survival rate	68.3	47.6

^aThe cyclin D1 expression index was determined on the basis of the proportion of stained cells using a semi-quantitative scale: i) Negative, $\leq 10\%$ of stained cells; ii) weakly positive, $< 50\%$ of stained cells; and iii) strong positive, $\geq 50\%$ of stained cells. Low cyclin D1 expression was defined as negative and weakly positive cyclin D1 expression, whilst high cyclin D1 expression was defined as strong positive cyclin D1 expression.

Table SIII. IC₅₀ values of docetaxel, cisplatin or 5-FU for each cell line with cyclin D1 intervention.

Cells	Docetaxel, nM	Cisplatin, μ g/ml	5-FU, μ g/ml
HB96-siRNA-NC	1.47	0.40	0.20
HB96-siRNA1	7.62	1.04	0.98
HB96-siRNA2	4.92	0.90	0.83
CAL27-siRNA-NC	5.60	0.58	0.85
CAL27-siRNA1	7.78	1.20	1.12
CAL27-siRNA2	20.13	1.00	0.99
CAL27-pLVX-puro	0.64	0.98	3.76
CAL27-pLVX-hcyclin D1	0.30	0.69	2.34
HN30-pLVX-puro	1.98	2.67	0.10
HN30-pLVX-hcyclin D1	0.47	0.70	0.07

5-FU, 5-fluorouracil; siRNA, small interfering RNA; NC, negative control.