

Figure S1. Okanin and cisplatin inhibit the viability of OSCC Cells.

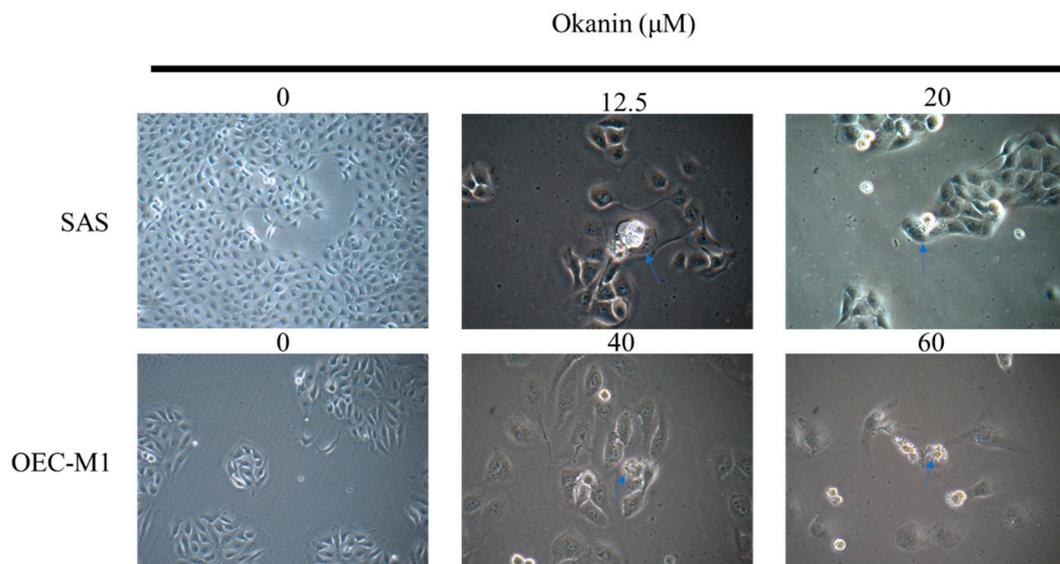


Figure S2. Treatment of oral squamous cell carcinoma (OSCC) with okanin induces morphological characteristics typical of pyroptosis, such as cell swelling, membrane rupture, and the formation of pyroptotic bodies.

Biochemical Markers: We evaluated the activation of pyroptosis-specific markers, including caspase-1 and gasdermin D cleavage. Western blot analysis, and qPCR analysis were performed. These observations have been added to the revised manuscript in the Supplementary Figures S2-S4.

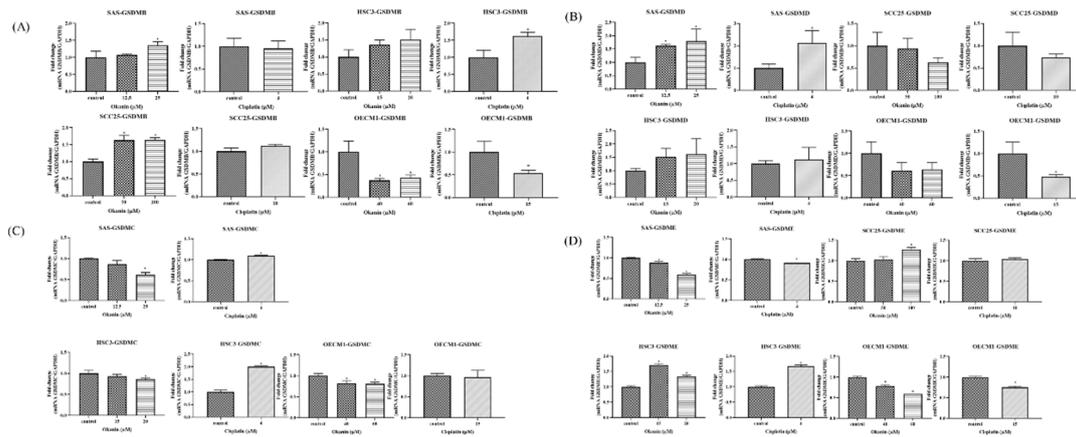


Figure S3. Treating oral squamous cell carcinoma (OSCC) with okanin induces GSDM families' gene expression changes. (A) GSDMB mRNA expression fold change. (B) GSDMD mRNA expression fold change (C) GSDMC mRNA expression fold change. (D) GSDME mRNA expression fold change in oral cancer cell lines.

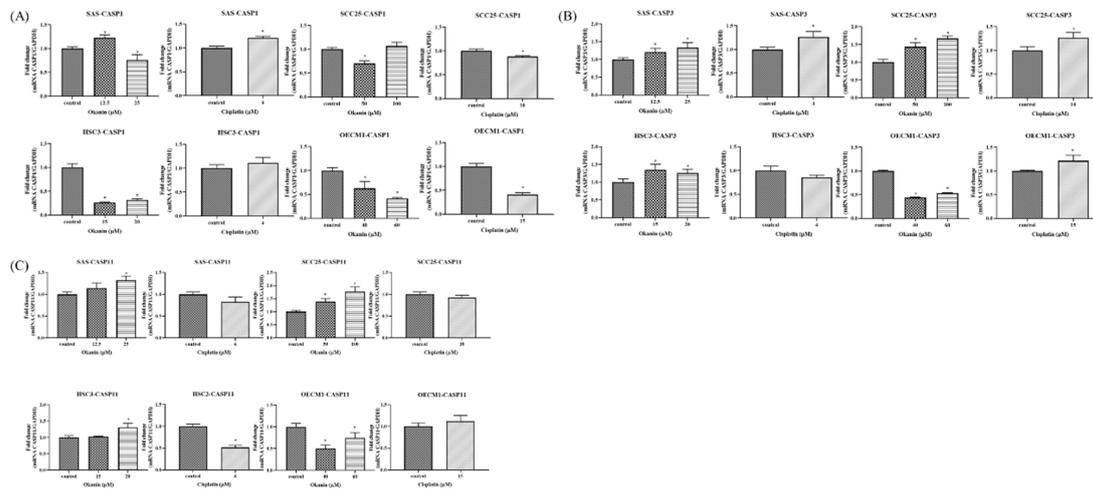


Figure S4. Treating oral squamous cell carcinoma (OSCC) with okanin induces GSDM families' gene expression changes. (A) CASP1 mRNA expression fold change. (B) CASP3 mRNA expression fold change (C) CASP11 mRNA expression fold change in oral cancer cell lines.

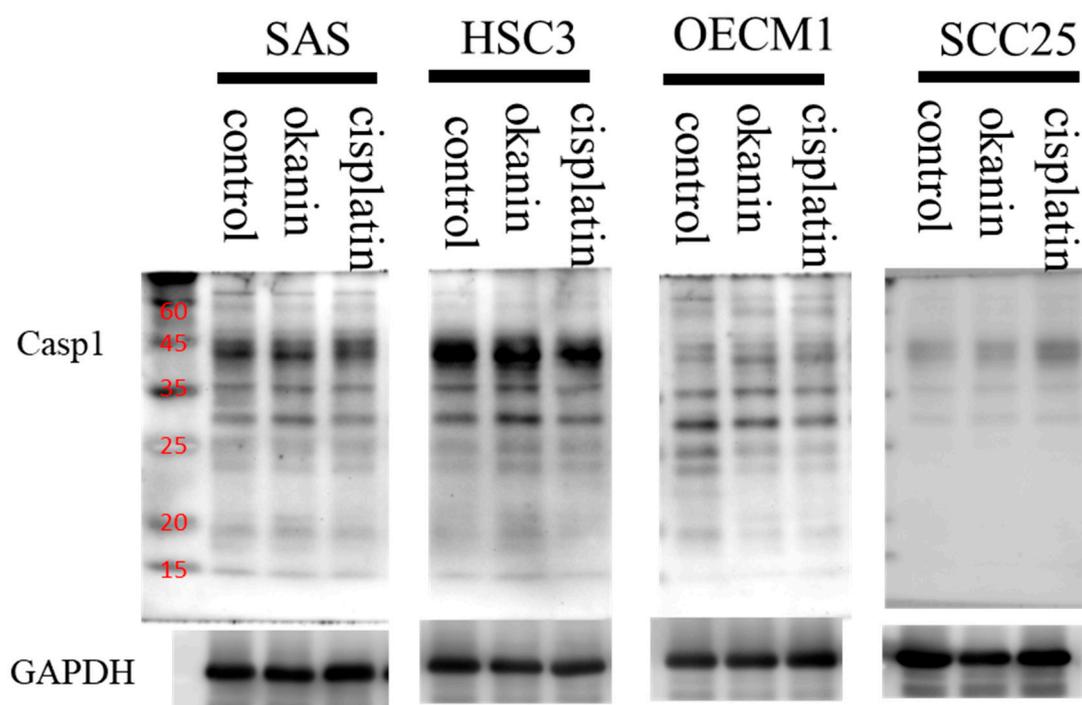
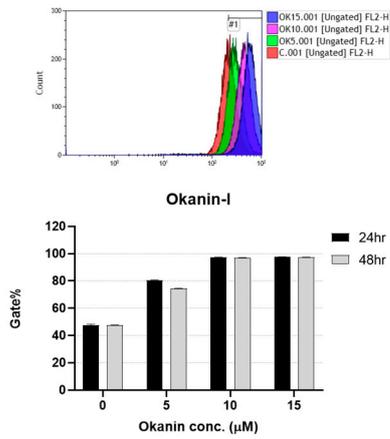


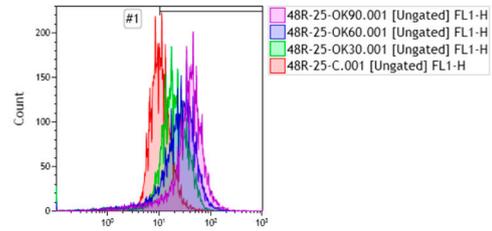
Figure S5. Treating oral squamous cell carcinoma (OSCC) with okanin induces caspase1 (CASP1) expression changes in oral cancer cell lines.

ROS-SAS



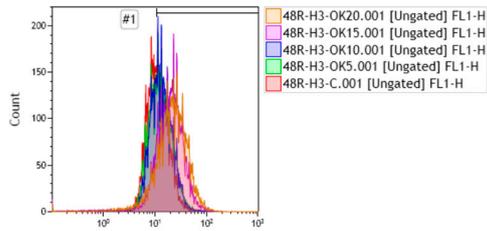
ROS-SCC25

ROS-48hr Okanin(μM)	SCC25	
	Gate%	X-Med
0	50.44±0.29	13.71±0.06
30	88.38±0.19	21.17±0.17
60	88.63±0.34	29.90±0.17
90	92.03±0.23	42.15±0.20



ROS-HSC3

ROS-24hr		HSC3		ROS-48hr		HSC3	
Okanin(μM)	Gate%	X-Med	Okanin(μM)	Gate%	X-Med		
0	49.53±3.21	16.88±0.16	0	51.72±0.18	15.10±0.08		
5	50.60±0.26	16.77±0.02	5	63.89±2.49	16.55±0.14		
10	69.76±0.39	18.42±0.05	10	68.81±2.39	16.46±0.30		
15	85.99±0.41	23.17±0.13	15	85.49±0.14	22.10±0.15		
20	81.34±0.57	23.68±0.12	20	85.34±0.31	24.89±0.20		



ROS-OECM1

ROS-48hr Okanin(μM)	OECM1	
	Gate%	X-Med
0	51.78±0.85	13.76±0.07
30	75.47±0.48	17.42±0.12
60	83.48±0.26	30.98±0.25
90	80.22±5.99	37.62±0.05

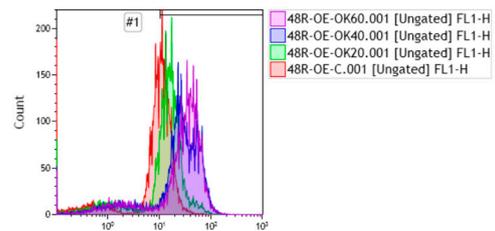
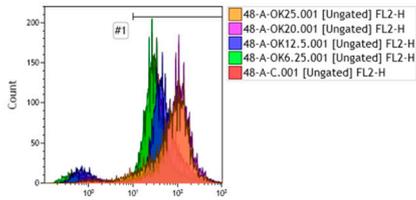


Figure S6. ROS assay for okanin treatment.

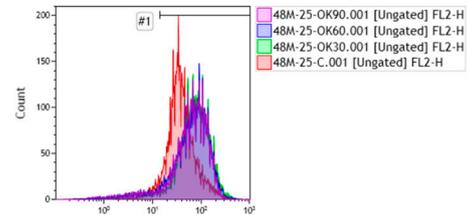
Mitochondrial membrane potential-SAS

MP-24hr		SAS		MP-48hr		SAS	
Okaniin(μM)	Gate%	X-Med		Okaniin(μM)	Gate%	X-Med	
0	82.51±0.29	70.25±0.36		0	91.03±0.38	32.52±0.30	
6.25	85.46±0.24	53.23±0.03		6.25	91.03±0.04	29.83±0.10	
12.5	87.46±0.14	58.72±0.25		12.5	87.87±0.61	48.10±0.25	
20	93.31±0.31	53.97±0.25		20	94.03±0.14	105.07±0.41	
25	88.78±5.44	44.86±0.27		25	93.47±0.21	88.22±0.56	



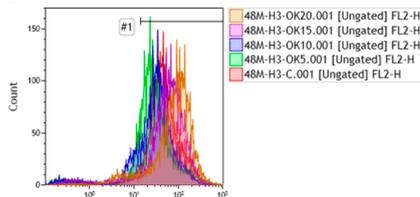
Mitochondrial membrane potential-SCC25

MP-48hr		SCC25	
Okaniin(μM)	Gate%	X-Med	
0	93.34±0.19	37.86±0.13	
30	94.14±0.25	78.47±0.65	
60	91.62±0.17	74.92±0.48	
90	91.33±0.17	73.42±0.86	



Mitochondrial membrane potential-HSC3

MP-24hr		HSC3		MP-48hr		HSC3	
Okaniin(μM)	Gate%	X-Med		Okaniin(μM)	Gate%	X-Med	
0	91.06±0.18	58.34±0.18		0	92.91±0.16	47.05±0.32	
5	89.88±0.16	45.76±0.14		5	83.99±0.31	29.75±0.33	
10	90.72±0.13	47.92±0.14		10	81.55±0.40	37.03±0.09	
15	92.15±0.34	63.06±0.4+6		15	90.11±0.08	67.70±0.52	
20	94.09±0.08	60.69±0.14		20	90.71±0.23	98.55±1.36	



Mitochondrial membrane potential-OECM1

MP-24hr		OECM1		MP-48hr		OECM1	
Okaniin(μM)	Gate%	X-Med		Okaniin(μM)	Gate%	X-Med	
0	92.70±0.28	58.34±0.19		0	84.64±0.63	36.83±0.26	
20	95.68±0.22	77.55±0.01		20	92.09±0.17	64.68±0.30	
40	95.45±0.16	81.60±0.54		40	91.98±0.06	85.80±0.50	
60	93.95±0.17	85.09±0.22		60	88.07±0.24	107.69±0.94	

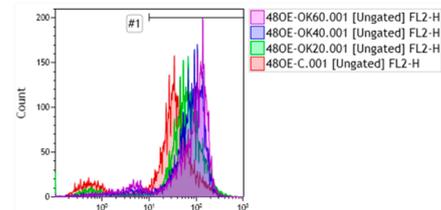


Figure S7. Mitochondrial membrane potential assay for okaniin treatment.