Supplemental Information

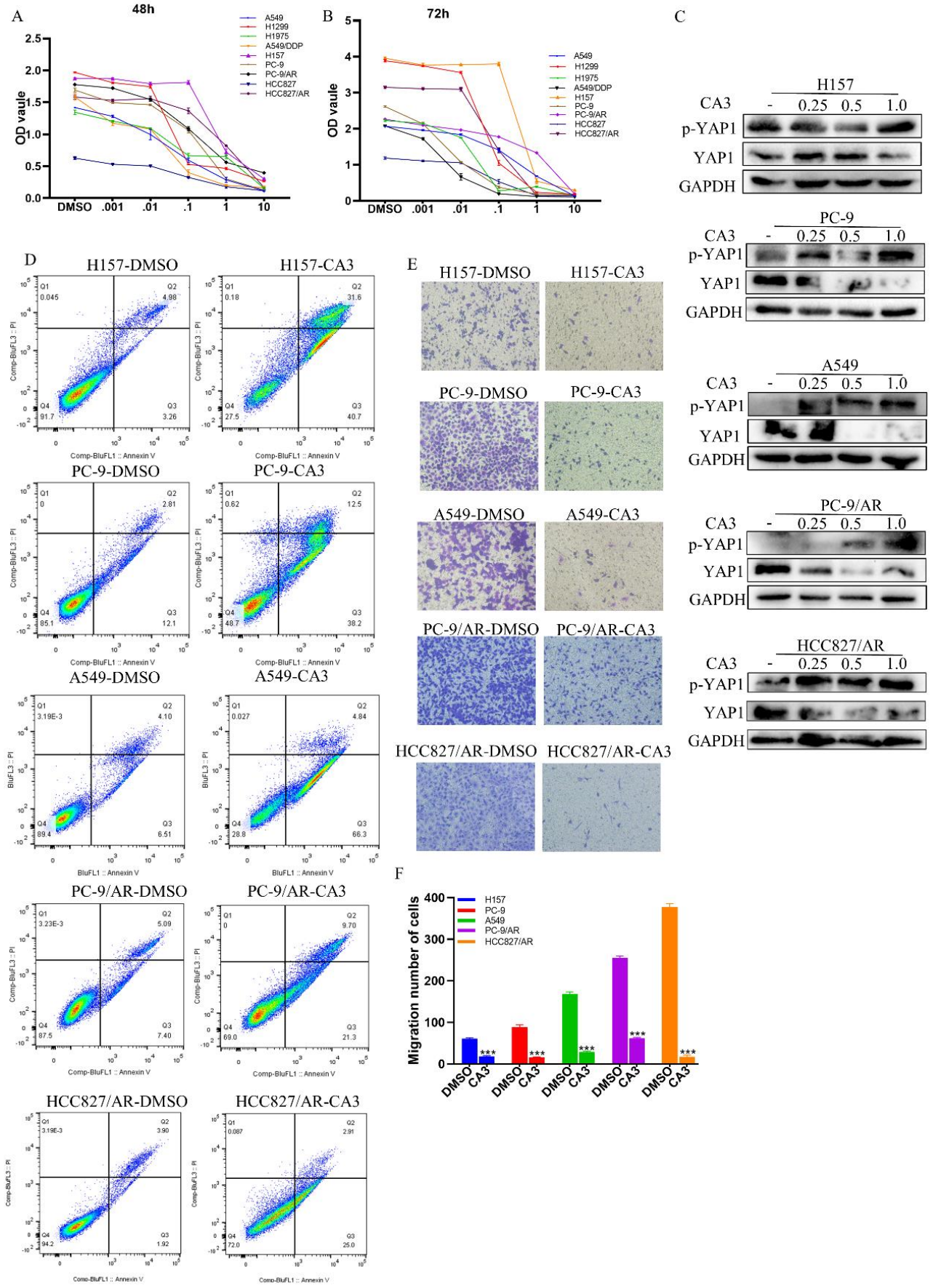

Figure S1. CA3 inhibits proliferation and metastasis and promotes apoptosis of NSCLC cell lines. (A-B) A549, A549/DDP, H157, PC-9, H1299, H1975, HCC827, PC-9/AR and HCC827/AR were administrated with CA3 at different concentrations for 48h and 72h, and OD value was measured by CCK8. (C) Immunoblot detection

of the YAP1, p-YAP1 expression of A549, H157, PC-9, PC-9/AR and HCC827/AR treated with different concentrations of CA3 for 24h. (D) CA3 at IC50 value was treated with A549, H157, PC-9, PC-9/AR and HCC827/AR, and the apoptosis cells dyed with Annexin V/PI were detected by Flow Cytometry. (E-F) CA3 at IC50 value was treated with A549, H157, PC-9, PC-9/AR and HCC827/AR. The Transwell chamber was used to examine the migration of NSCLC cells.

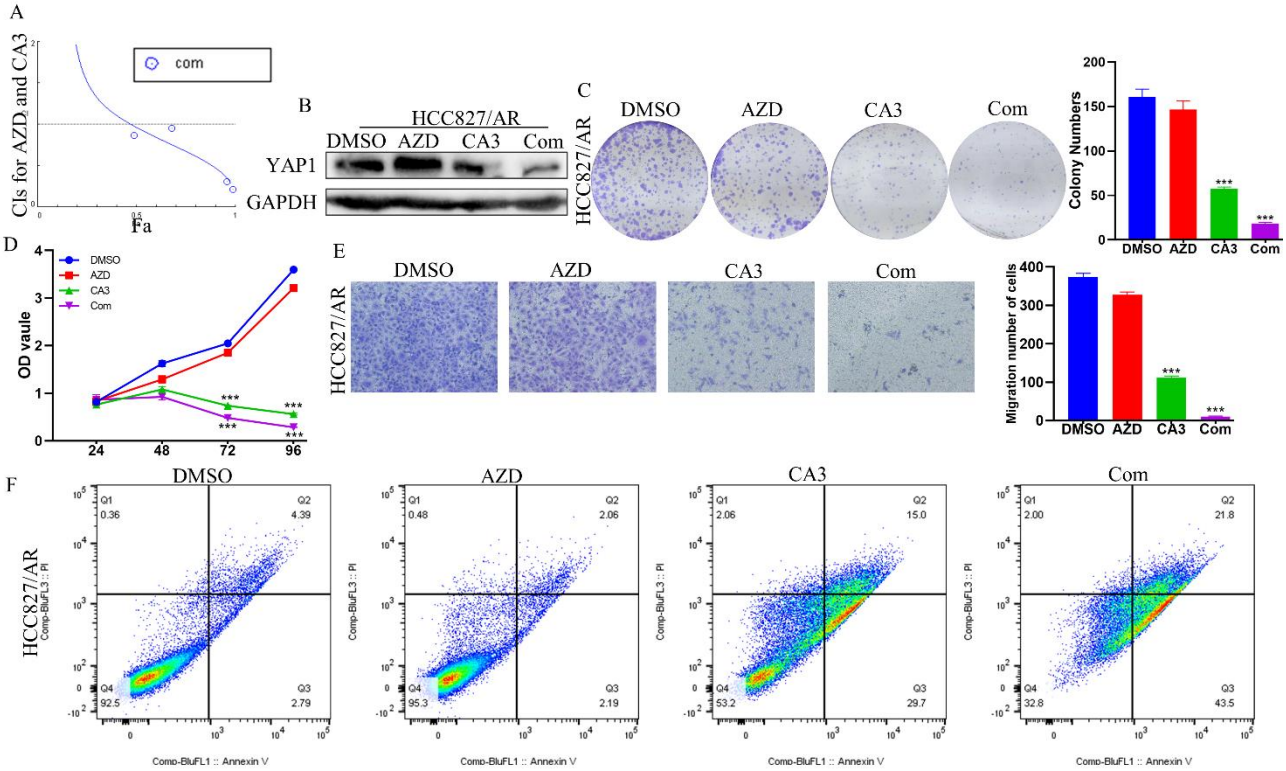

Figure S2. Knockdown YAP1 could overcome osimertinib resistance in HCC827/AR. (A) HCC827/AR cells were incubated with osimertinib and CA3 at different ratios, and the CI value was calculated by the medium dose analysis. CI value <1 is considered synergism. (B) The effect of YAP1 inhibition in HCC827/AR treated with osimertinib, CA3, or CA3 combined osimertinib at 48h. (C-D) CCK8 and Colony formation analysis for HCC827/AR cells treated with 0.5μmol/L CA3 and 1μmol/L osimertinib alone or combined. (E) Transwell analysis of HCC827/AR cells administrated with 0.5μmol/L CA3 and 1μmol/L osimertinib alone or combined for 16h. (F) Apoptosis cells were examined by Flow Cytometry in the PC-9/AR treated with 0.25μmol/L CA3 or 0.5μmol/L osimertinib.

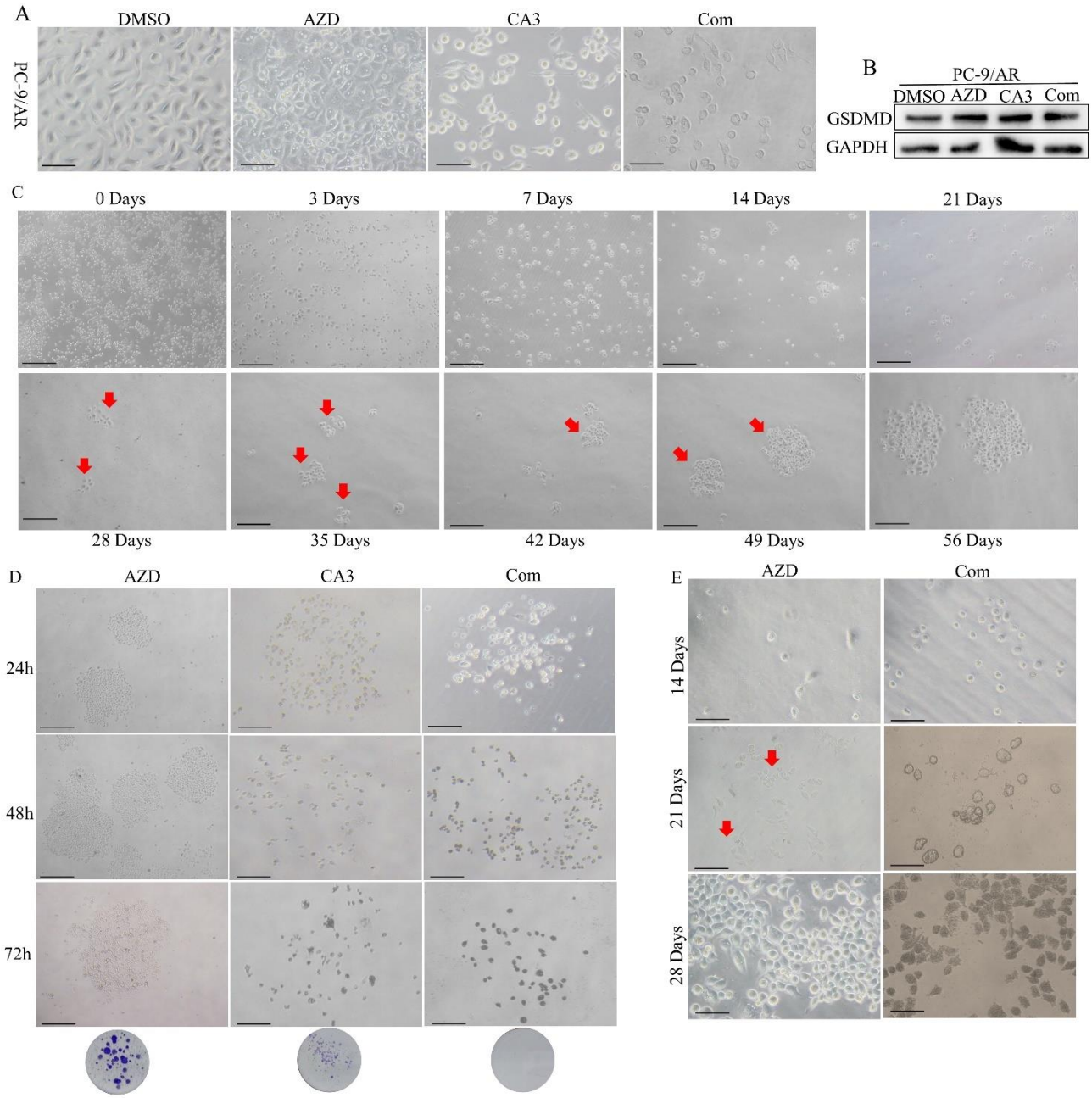

Figure S3. Combined CA3 and osimertinib eliminated a senescence-like dormant state and delayed the acquired resistance of osimertinib. (A-B) PC-9/AR cells were treated with 0.25μmol/L CA3, osimertinib 0.5μmol/L for 24h. The pyroptotic body cannot see in the light microscope. Western Blot analysis indicated GSDMD had no change.__Scar Bar 25μm. (C) Images of cells dormant PC-9 cells treated with 1μmol/L osimertinib at different time points.__Scar Bar 25μm. (D) The dormant PC-9 cells at 56 days were administrated with 1μmol/L osimertinib and 0.5μmol/L CA3 alone or combined for 24h, 48h, and 72h. __ Scar Bar 25μm.(E) PC-9 cells were planted in 24-well plates and exposed to 1 μmol/L osimertinib, 0.25μmol/L CA3 plus 0.5μmol/L osimertinib plus CA3, respectively. The cells were pictured every 7 days. The red bar indicated the recolonized cells.__Scar Bar 10μm.

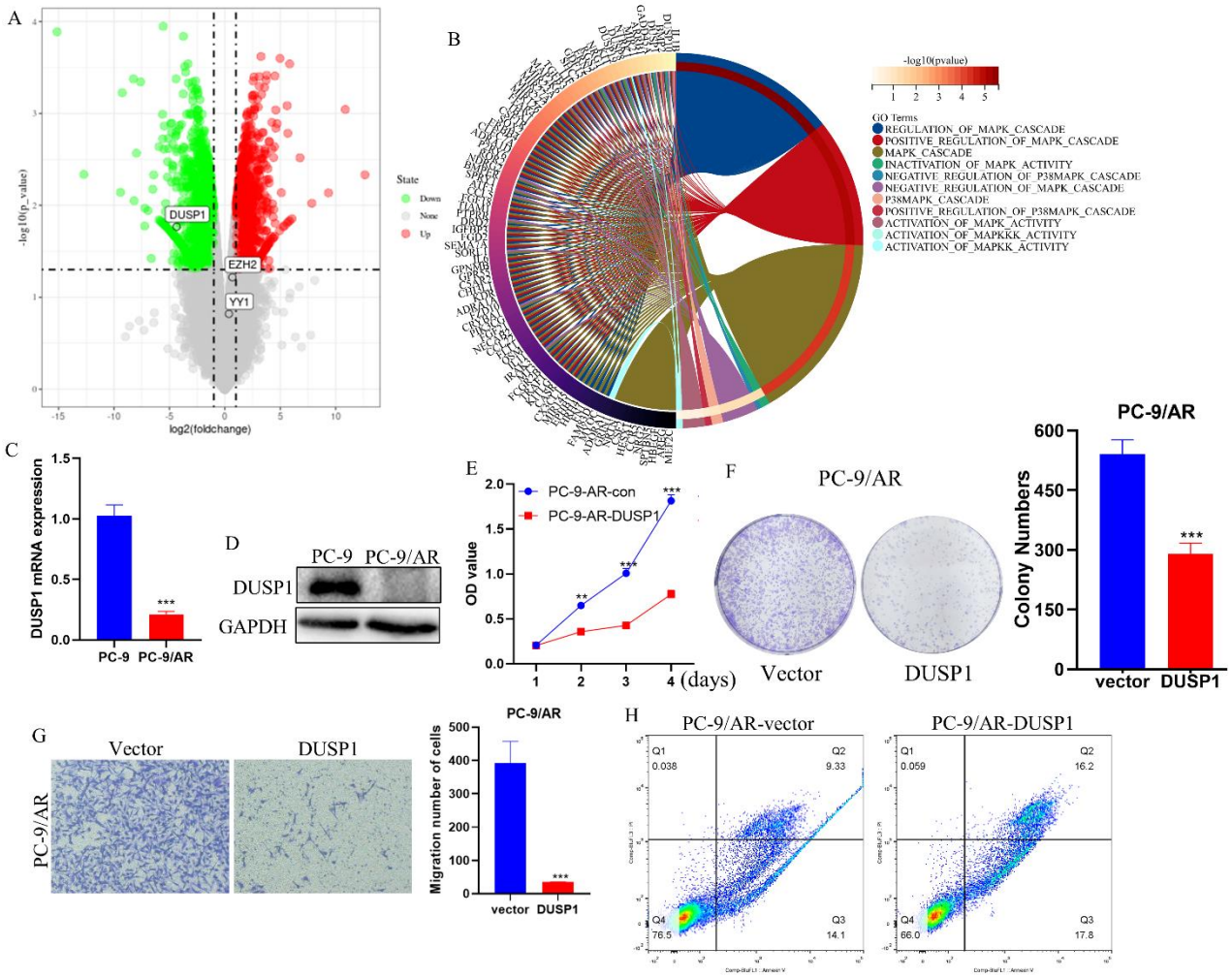

Figure S4. Overexpression of DUSP1 inhibits proliferation, migration and promotes apoptosis of osimertinib NSCLC cells. (A) Volcano plots pictured the transcriptomic data, with the x-axis representing Log2FoldChange (sample/control) values and the y-axis representing the -Log10 (p value). Green, red, and gray circles respectively represent genes that were downregulated, upregulated, and not differentially regulated. (B) The circle diagram of enriched genes of MAPK pathway upon CA3 treatment. (C-D) Differential expression of DUSP1 in EGFR-mutant PC-9 and osimertinib resistant PC-9/AR cells was tested by qPCR and Western blot analysis. (E-F) PC-9/AR cells were transfected with vector or DUSP1 plasmids, and CCK8 and Colony formation measured the proliferation. (G) Transwell measured the migration ability of DUSP1 overexpression PC-9/AR cells. (H) Flow Cytometry examined the apoptosis percentage of DUSP1 overexpression PC-9/AR cells.

Table.S1 Patient mutation information before and after osimertinib treatment

Case	Gender	Age	Drugs	TNM Stage	Before Therapy	After Therapy
1	Female	58	Gefinitinib →Osimertinib	T1N3M1, IV	EGFR Exon 19 Del	Unknown
2	Male	52	Gefinitinib →Osimertinib	T2aN0M1, IVB	EGFR L858R	Unknown
3	Male	60	Icotinib →Osimertinib	T1N2M1	EGFR L858R	Unknown
4	Male	62	Gefinitinib →Osimertinib	T3N1M1, IVA	EGFR Exon 19 Del/T790M	Exon 19 Del/T790M
5	Female	46	Endostar, Osimertinib	T3N0M0, IIB	EGFR Exon 19; TP53	EGFR Exon 19, TP53
6	Female	53	Bevacizumab, Osimertinib, Camrelizumab, Docetaxel, Tislelizumab, Anlotinib	T4N2M1, IV	EGFR Exon 20	EGFR Exon 20; FGFR3; TP53
7	Female	55	Icotinib, Osimertinib	T3N2M1, IV	EGFR Exon 19; TP53	EGFR Exon 19; TP53
8	Female	51	Osimertinib + Bevacizumab	T2N2M0, IIIA	EGFR Exon 19; TP53	Unknown
9	Male	46	Gefinitinib →Osimertinib	TxN1M1c, IVB	EGFR Exon 19; TP53	EGFR; TP53
10	Female	55	Icotinib →Osimertinib	T3N3Mx	EGFR Exon19-Del	Unknown

Table.S2 Association between the expression of YAP1 protein and the clinical parameters of LUAD patients with EGFR mutation information

Clinicopathological features (n)	YAP1 protein expression		
	Low (%)	High (%)	p Value
EGFR mutation			
Wild type (57)	26(16.80)	31(37.60)	0.0283*
EGFR mutation (68)	21(23.20)	47(22.40)	
Age (y)			
<60 (60)	22(17.60)	38(30.40)	0.8554
≥60 (65)	25(20.00)	40(32.00)	
Gender			
Female (59)	20(16.00)	39(31.20)	0.4628
Male (66)	27(21.60)	39(31.20)	
Smoking			
No (34)	9(7.20)	25(20.00)	0.1475
Yes (91)	38(30.40)	53(42.40)	
T			
T1-2 (66)	27(21.60)	39(31.20)	0.4628
T3-4 (59)	20(23.20)	39(22.40)	
N			
N0 (96)	37(29.60)	59(47.20)	0.8276
N1-3 (29)	10(8.00%)	19(15.20%)	
Clinical stages			
I - II (63)	27(21.60)	36(28.80)	0.26890
III-IV (62)	20(23.20)	42(33.60)	

Note: χ^2, P <0 .05 (two-tailed)

Table.S3 Chip-seq of the binding sites of YAP1 in DUSP1 DNA sequence

Start	End	Peak Score	Annotation	Detailed Annotation		Distance to TSS	Gene Name
172199002	172200068	445	Intergenic	CpG-21390	Promoter	-1332	DUSP1
172192496	172192894	163	Intergenic	Intergenic		5508	DUSP1
172228215	172228592	114	Intergenic	Intergenic		-30200	DUSP1
172222297	172222619	113	Intergenic	Intergenic		-24255	DUSP1
172194063	172194201	92	TTS	TTS		4071	DUSP1

Table.S4 The prediction of binding sites of TFs in DUSP1 promoter and UTR-3'

PROMO database						JASPAR database				
Gene	Matrix ID	Start	End	Predicted sequence	Score	Matrix ID	Start	End	Predicted sequence	Score
DUSP1-Promoter	YY1 [T00915]	88	91	CCAT	8.20	MA0095.2.YY1	85	96	CAACATGGTGAA	10.31
DUSP1-Promoter	YY1 [T00915]	102	105	ATGG	8.20	MA0095.2.YY1	99	110	CAGGATGGTCTC	8.08
DUSP1-Promoter	YY1 [T00915]	259	262	ATGG	8.20	MA0095.2.YY1	837	848	CACAATGGCCCG	6.19
DUSP1-Promoter	YY1 [T00915]	387	390	CCAT	8.20	MA0809.2.TEAD4	303	314	TAACATTCTATG	8.91
DUSP1-Promoter	YY1 [T00915]	487	490	CCAT	8.20	MA0809.2.TEAD4	1105	1116	GTGGATTCCAGG	8.42
DUSP1-Promoter	YY1 [T00915]	840	843	ATGG	8.20	MA0809.2.TEAD4	481	492	AAGCACTCCATG	5.89
DUSP1-Promoter	YY1 [T00915]	995	998	ATGG	8.20	MA1964.1.SMAD2	1938	1947	GGCCAGACCC	11.62
DUSP1-Promoter	YY1 [T00915]	1246	1249	ATGG	8.20	MA1964.1.SMAD2	1007	1016	CTCCAGACTG	11.57
DUSP1-Promoter	YY1 [T00915]	1902	1905	CCAT	8.20	MA1964.1.SMAD2	1284	1293	TCCCAGACTT	11.33
DUSP1-Promoter						MA1964.1.SMAD2	1760	1769	GGCCGGACAG	8.72
DUSP1-Promoter						MA1964.1.SMAD2	1033	1042	CGCCCGACAC	8.53
DUSP1-Promoter						MA1964.1.SMAD2	2044	2053	CCCCCGACTG	7.67
DUSP1-Promoter						MA1964.1.SMAD2	1729	1738	GACCGGACCC	5.88
DUSP1-Promoter						MA1964.1.SMAD2	104	113	ATCGAGACCA	5.73
DUSP1-Promoter						MA1964.1.SMAD2	386	395	CTCCATACAG	5.58
DUSP1-Promoter						MA1964.1.SMAD2	1839	1848	AGCCAGAGGG	4.54
DUSP1-Promoter						MA1964.1.SMAD2	1854	1863	GGCCAGGCGC	4.42
DUSP1-Promoter						MA1964.1.SMAD2	1097	1106	CGCCAAACCC	4.36
DUSP1-Promoter						MA1964.1.SMAD2	1090	1099	CTCCAAACGC	4.29
DUSP1-Promoter						MA1964.1.SMAD2	1957	1966	CCCCAGAGGC	4.23
DUSP1-Promoter						MA1964.1.SMAD2	513	522	ATCCAGGCAG	4.17
DUSP1-Promoter						MA1964.1.SMAD2	660	669	TTCCTGACTC	4.11
DUSP1-Promoter						MA1964.1.SMAD2	1341	1350	ACCAAGACCG	4.07

DUSP1-Promoter						MA1964.1.SMAD2	1988	1997	GGCCAGGCTC	4.05
DUSP1-Promoter						MA1964.1.SMAD2	42	51	CACCACACCC	4.00
DUSP1-Promoter						MA1964.1.SMAD2	1152	1161	CCCCTGACCT	3.73
DUSP1-Promoter						MA1964.1.SMAD2	740	749	CCCTGGACAG	3.56
DUSP1-UTR-3’	YY1 [T00915]	30	33	CCAT	2.60	MA0095.2.YY1	230	241	CAAAATGGGGCA	7.85
DUSP1-UTR-3’	YY1 [T00915]	41	44	CCAT	2.60	MA0095.2.YY1	599	610	CAAAATGTCTTC	6.99
DUSP1-UTR-3’	YY1 [T00915]	146	149	ATGG	2.60	MA0809.2.TEAD4	24	35	TCACATCCCATT	8.75
DUSP1-UTR-3’	YY1 [T00915]	221	224	CCAT	2.60	MA0809.2.TEAD4	315	326	TGACATACCTAC	7.11
DUSP1-UTR-3’	YY1 [T00915]	233	236	ATGG	2.60	MA0809.2.TEAD4	508	519	GAAAATACCAGT	6.69
DUSP1-UTR-3’						MA1964.1.SMAD2	387	396	AGGCAGACAC	7.49
DUSP1-UTR-3’						MA1964.1.SMAD2	335	344	TTCCCGACGA	6.60
DUSP1-UTR-3’						MA1964.1.SMAD2	163	172	GTCAAGACAT	4.14

Table. S5 GRAMM-X prediction of binding site between DUSP1 and YAP1.

YAP1	ASA	BSA	ΔiG	DUSP1	ASA	BSA	ΔiG
A:GLU 100	119.77	18.91 ||	-0.23	B:ARG 54	174.98	35.18 |||	-0.54
A:PRO 101	139.68	44.41 ||||	0.35	B:LYS 57	190.54	27.23 ||	-0.71
A:LYS 102	148.14	61.35 |||||	0.50	B:TYR 169	64.08	27.11 |||||	0.17
A:SER 103	102.10	82.17 ||||||||	0.15	B:ALA 208	54.11	11.14 |||	0.14
A:HIS 104	183.41	18.27 |	-0.14	B:PRO 224	79.55	70.56 ||||||||	0.61
A:SER 105	74.43	30.80 |||||	0.40	B:VAL 225	8.62	7.60 ||||||||	0.12
A:ARG 106	243.07	30.83 ||	0.34	B:GLU 226	76.28	27.65 ||||	0.39
A:GLN 107	157.33	2.62 |	-0.03	B:ASP 227	58.77	25.53 |||||	-0.11
A:THR 110	129.03	3.02 |	0.05	B:ASN 228	59.46	23.86 |||||	-0.17
A:ALA 112	94.58	33.18 ||||	0.52	B:HIS 229	136.01	2.22 |	-0.02
A:GLY 113	71.84	19.18 |||	-0.20	B:LYS 230	169.41	91.91 ||||||	-1.15
A:THR 114	135.44	4.79 |	-0.05	B:ALA 231	16.23	14.41 ||||||||	0.23
A:ALA 115	106.93	9.69 |	-0.11	B:ASP 232	78.82	3.77 |	-0.03
A:GLY 116	81.60	25.46 ||||	-0.12	B:SER 235	74.18	8.19 ||	0.02
A:ALA 117	101.21	72.84 |||||||	0.58	B:TRP 236	67.73	38.33 ||||||	0.35
A:LEU 118	181.49	92.76 ||||||	1.05	B:GLN 259	92.72	24.43 |||	0.39
A:THR 119	129.44	120.22 |||||||||	0.73	B:ALA 260	20.41	15.56 |||||||	0.25
A:PRO 120	133.39	91.44 |||||||	0.84	B:ILE 262	48.72	35.83 |||||||	0.57
A:GLN 121	175.79	132.93 ||||||||	-0.56	B:SER 263	6.15	6.00 |||||||||	0.09
A:HIS 122	186.65	91.68 |||||	0.42	B:ARG 264	21.26	6.12 |||	-0.23
A:VAL 123	130.60	77.40 ||||||	1.10	B:LYS 280	104.40	7.03 |	-0.22
A:ARG 124	223.06	36.74 ||	0.20	B:PHE 285	33.26	6.41 ||	0.10
A:HIS 126	161.13	8.36 |	0.09	B:SER 296	63.96	42.60 |||||||	-0.28
A:SER 400	93.16	8.22 |	-0.09	B:PRO 297	11.72	8.35 |||||||	-0.10

A:LEU 402	178.86	15.72 |	-0.18	B:ASN 298	34.89	33.66 ||||||||	0.54
A:MET 404	168.98	15.96 |	-0.18	B:PHE 299	163.14	66.69 |||||	0.89
A:SER 405	95.19	33.64 ||||	-0.15	B:MET 302	50.25	2.51 |	0.04
A:SER 406	85.76	20.82 |||	-0.17	B:SER 310	69.85	16.74 |||	0.27
A:TYR 407	208.89	71.60 ||||	0.27	B:ALA 314	35.43	4.17 ||	0.07
A:SER 408	94.27	40.02 |||||	0.00	B:PRO 315	91.25	52.57 |||||	0.62
A:VAL 409	141.54	7.36 |	-0.07	B:HIS 316	136.69	0.67 |	0.01
A:PRO 410	128.79	44.71 ||||	0.72	B:CYS 317	112.80	86.87 |||||||	2.57
A:ARG 411	195.07	84.42 |||||	-0.44	B:SER 318	107.51	27.34 |||	-0.15
A:THR 412	113.19	42.15 ||||	0.67	B:ALA 319	87.56	13.04 ||	0.21
A:PRO 413	125.91	41.92 ||||	0.51	B:GLU 320	166.41	19.07 ||	-0.20
A:ASP 415	120.13	14.20 ||	-0.15	B:ALA 321	90.01	40.32 |||||	0.65
A:PHE 416	200.82	109.77 ||||||	1.24	B:GLY 322	73.19	23.62 ||||	0.04
A:LEU 417	171.84	33.22 ||	0.53	B:PRO 324	120.66	15.06 ||	0.24
A:ASN 418	155.05	17.35 ||	-0.08	B:ALA 325	94.57	7.70 |	0.12
A:SER 419	104.22	32.67 ||||	0.21	B:PHE 341	165.83	13.92 |	0.22
				B:VAL 343	105.72	29.94 |||	0.48
				B:ILE 345	140.16	45.33 ||||	0.73
				B:PRO 346	107.27	4.53 |	-0.05
				B:VAL 347	141.30	12.37 |	0.20
				B:HIS 348	168.76	67.38 ||||	0.26
				B:SER 349	107.64	7.86 |	-0.01
				B:THR 350	120.03	93.79 |||||||	0.71
				B:ASN 351	160.42	12.87 |	0.05
				B:TYR 356	162.43	19.21 ||	-0.14
				B:LEU 357	131.93	73.74 |||||	0.51

	B:GLN 358	170.60	26.26 ||	0.10
	B:SER 359	97.24	45.80 |||||	0.03
	B:PRO 360	131.79	13.98 ||	0.13
	B:ILE 361	157.91	59.79 ||||	0.94
	B:THR 362	131.34	81.42 |||||||	0.37
	B:SER 364	92.97	40.18 |||||	0.58
	B:PRO 365	135.20	11.94 |	-0.06
	B:SER 366	105.12	2.83 |	-0.02
	B:CYS 367	197.43	65.71 ||||	0.71

Note: ASA (Accessible Surface Area, Å²); BSA (Buried Surface Area, Å²); ΔiG (Solvation energy effect, kcal/mol)

|||| Buried area percentage, one bar per 10%.