

Supplementary Materials

ZEB2 upregulation modulates the polarization of TAMs toward the immunosuppressive state in EGFR-TKI-resistant NSCLC

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Supplementary Table 1. Basic characteristic information of patients recruited in this research

Patient	Gender	Age	Histology	Primary tumor size (mm)	Metastasis	TMN
1	M	50	Adenocarcinoma	34.1	Brain	T3N0M1b
2	M	73	Adenocarcinoma	47.5 23.3	Contralateral lung	T2bN3M1a
3	F	58	Adenocarcinoma	76		T4N2Mx
4	F	54	Adenocarcinoma		Contralateral lung	T4N0M1a
5	F	77	Adenocarcinoma	60.5	Bone Pleura	T4N2M1c
6	F	69	Adenocarcinoma	46		T2N2M0
7	F	68	Adenocarcinoma	61.8 17.6	Contralateral lung Bone	T3N2M1c
8	M	71	Adenocarcinoma		Contralateral lung	T4N2M1a
9	M	41	Adenocarcinoma	42.5	Pleura	T4N4M1a
10	F	49	Adenocarcinoma	73	Rib	T4N3M1c

Supplementary Table 2. Specific primers used in this research

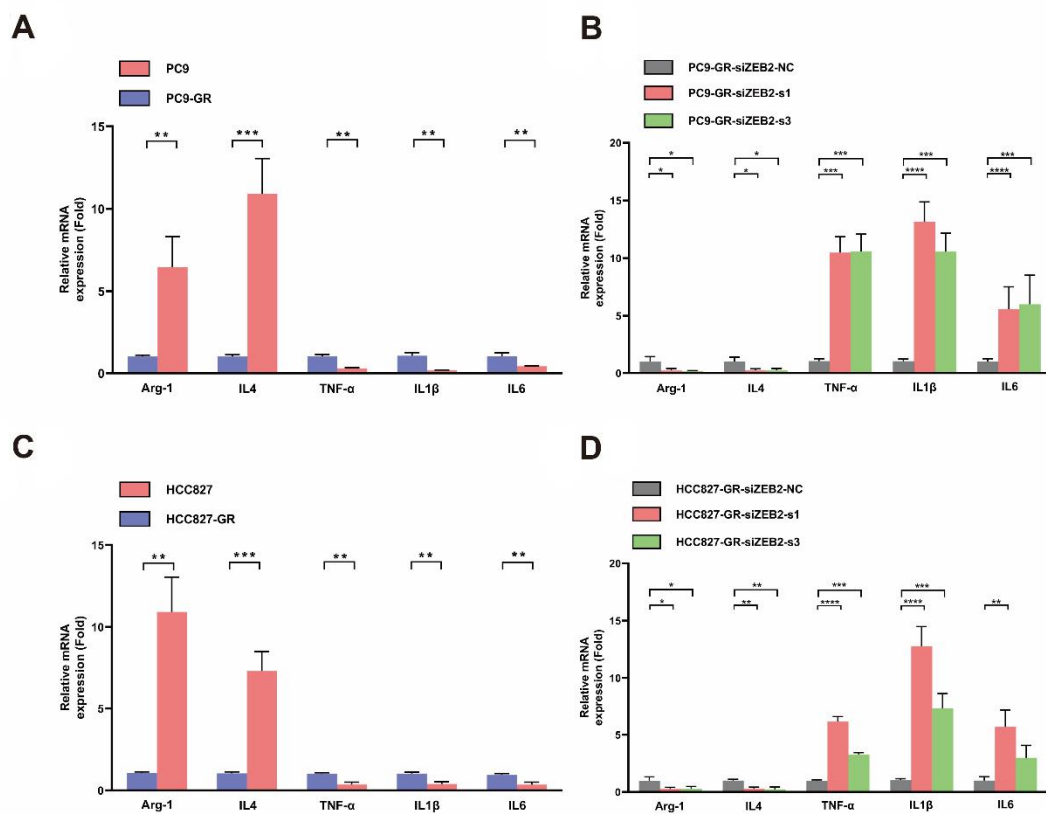
Target genes	Forward	Reverse
<i>IL12</i>	AGGGCCGTCAGCAACATG	TCTTCAGAAGTGCAAGGGTAA AATTC
<i>IL6</i>	AAGCCAGAGCTGTGCAGAT GAGTA	TGTCCTGCAGCCACTGGTTC
<i>IL1β</i>	AGCTACGAATCTCCGACCA C	CGTTATCCCATGTGTCTGAAGA A
<i>TNF-α</i>	CAGAGGGAAGAGTTCCCCA G	CCTGGTCTGGTAGGAGACG
<i>IL4</i>	ATGGGTCTCACCTCCCAAC T	GATGTCTGTTACGGTCAACTC G
<i>Arg-1</i>	CTGGCAAGGTGGCAGAAGT C	ATGGCCAGAGATGCTTCCAA
<i>ZEB2</i>	GCGATGGTCATGCAGTCAG	CAGGTGGCAGGTCATTTTCTT
<i>IL-10</i>	GCTGTCATCGATTTCTTCCC	CTCATGGCTTTGTAGATGCCT
<i>CCL2</i>	CTTCTGTGCCTGCTGCTCAT A	CTTTGGGACACTTGCTGCTG
<i>CCL5</i>	ACCAGTGGCAAGTGCTCCA AC	CAGCCGGGAGTCATACAGGA
<i>CCL8</i>	TGGAGAGCTACACAAGAAT CACC	TGGTCC AGATGCTTCATGGAA
<i>CXCL1</i>	CAAACCGAAGTCATAGCCA CAC	GGATTTGTCACTGTTTCAGCAT CTT
<i>CXCL9</i>	TCTTGCTGGTTCTGATTGGA GTG	TAGTCCCTTGGTTGGTGCTGA T
<i>CXCL10</i>	GGCCATCAAGAATTTACTG AAAGCA	TCTGTGTGGTCCATCCTTGGA A
<i>CXCL11</i>	CCTTGGCTGTGATATTGTGT GCTA	CCTATGCAAAGACAGCGTCCT C
<i>CSF-1</i>	GATGGAGACCTCGTGCCAA ATTA	TGTTATCTCTGAAGCGCATGG TG
<i>CCL20</i>	TGCTGTACCAAGAGTTTGC TC	CGCACACAGACAACTTTTTCT TT
<i>CXCL8</i>	TCTGCTAGCCAGGATCCAC A	TGCTTCCACATGTCCTCACA
<i>GAPDH</i>	GCACCGTCAAGGCTGAGAA C	TGGTGAAGACGCCAGTGGA

Supplementary Table 3. LogFC and adjusted P value of 17 common most highly upregulating DEGs in HCC827 before and after erlotinib resistance

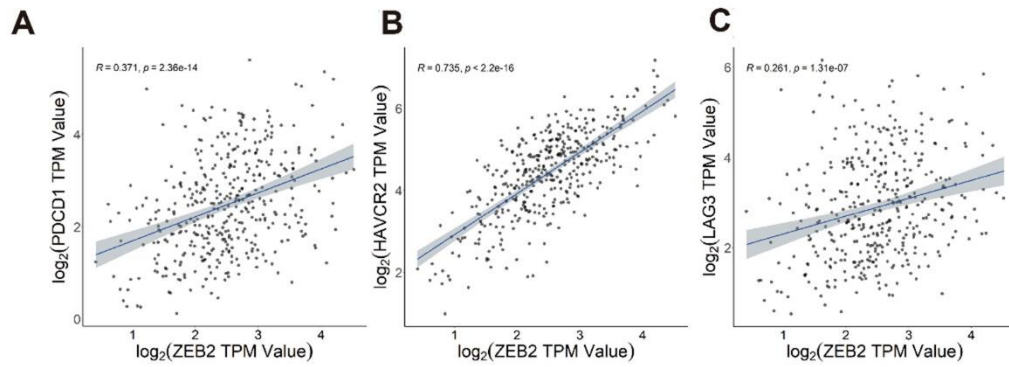
Gene Name	Log FC	Adjusted P value
<i>CSDC2</i>	10.74173	9.39E-14
<i>ZEB2</i>	9.0393	4.21E-18
<i>SERPINE1</i>	8.979462	6.72E-10
<i>AK5</i>	8.799701	6.28E-18
<i>TENM2</i>	8.667625	0.000223
<i>ANO2</i>	8.437445	2.61E-05
<i>APCDD1L</i>	8.279027	0.000576
<i>RNF182</i>	8.251074	2.29E-10
<i>TWIST1</i>	8.047293	0.002829
<i>CHST9</i>	7.926356	5.80E-17
<i>RGS4</i>	7.884844	3.46E-08
<i>ACSM5</i>	7.797203	1.53E-09
<i>CDK15</i>	7.72232	0.001702
<i>FAM43B</i>	7.542032	2.15E-07
<i>MMP16</i>	7.259201	3.84E-06
<i>CNN1</i>	7.150792	3.60E-05
<i>ALPK2</i>	7.145911	6.44E-13

Supplementary Table 4. LogFC and adjusted P value of 17 common most highly upregulating DEGs in HCC4006 before and after erlotinib resistance

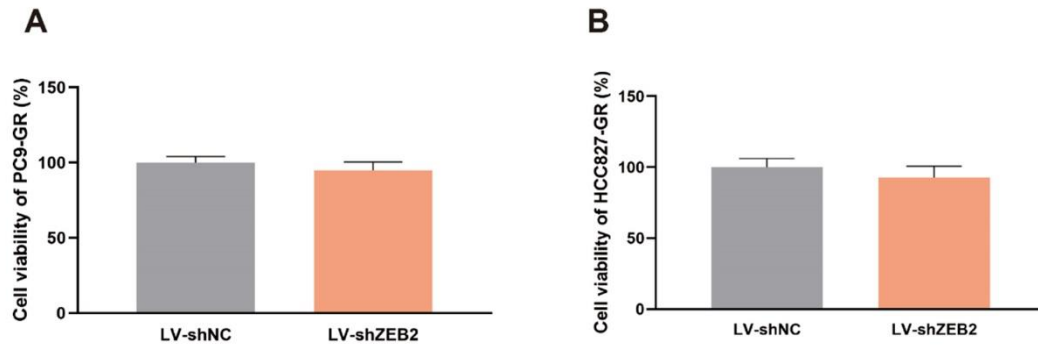
Gene Name	Log FC	Adjusted P value
<i>TENM2</i>	14.63483	8.34E-18
<i>CNN1</i>	14.28409	1.59E-28
<i>APCDD1L</i>	10.50306	8.71E-13
<i>CHST9</i>	10.47656	1.31E-13
<i>RGS4</i>	10.31609	8.82E-14
<i>ALPK2</i>	10.05742	2.06E-12
<i>ACSM5</i>	9.868678	7.87E-13
<i>CDK15</i>	9.746589	1.18E-11
<i>RNF182</i>	9.647935	2.05E-12
<i>FAM43B</i>	8.814982	2.45E-10
<i>ZEB2</i>	8.454783	8.60E-48
<i>CSDC2</i>	8.279258	8.39E-17
<i>ANO2</i>	8.193553	4.69E-09
<i>AK5</i>	8.191282	4.93E-18
<i>MMP16</i>	8.019539	2.67E-14
<i>SERPINE1</i>	7.665034	5.52E-24
<i>TWIST1</i>	7.464798	2.99E-58



Supplementary Figure 1. Effect of ZEB2 in EGFR-TKI resistance on M1/M2 polarization of primary human TAMs in NSCLC. (A) qRT-PCR analysis showing the induction of M1/M2 polarization in human TAMs by conditional media (CM) from EGFR-TKI sensitive and resistant PC9 cells; (B) Effect of ZEB2 knockdown on M1/M2 polarization of human TAMs in PC9-GR; (C) qRT-PCR analysis showing the induction of M1/M2 polarization in human TAMs by CM from EGFR-TKI sensitive and resistant HCC827 cells; (D) Effect of ZEB2 knockdown on M1/M2 polarization of human TAMs in HCC827-GR.



Supplementary Figure 2. The correlations between ZEB2 expression and biomarkers of T cell dysfunction. (A-C) Pearson correlations between the expression of ZEB2 and the expression of PDCD1 ($R = 0.371$), HAVCR2(TIM-3) ($R = 0.735$) and LAG3 ($R = 0.261$) in LUAD cohort from TCGA database. With correlation coefficient > 0.6 , ZEB2 was strongly correlated with HAVCR2(TIM-3), which was an immune checkpoint receptor that played a crucial role in regulating immune responses by promoting T cell exhaustion and limiting immune activation in NSCLC.



Supplementary Figure 3. Effect of ZEB2 knockdown on the viability of PC9-GR and HCC827-GR cells assessed by CCK-8 assay. (A) The effect of ZEB2 knockdown on the cell viability of PC9-GR cells; (B) The effect of ZEB2 knockdown on the cell viability of HCC827-GR cells. The data represented the average results from three independent experiments. The statistical significance was determined by comparing the LV-shZEB2 groups with the LV-shNC groups.