

***ERO1L* is a novel and potential biomarker in lung adenocarcinoma and shapes the immune-suppressive tumor microenvironment**

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Supplementary Material

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Table S1. Characteristics of included data sets from GEO database

Project	Histology	Stage	Tumor sample	Normal sample	Experiment type	Platforms
GSE7670	LUAD	I-IV	27	27	Affymetrix microarrays	GPL96
GSE31210	LUAD	I-III	226	20	Affymetrix microarrays	GPL570
GSE32863	LUAD	I-IV	58	58	Affymetrix microarrays	PGL6884
GSE19188	LUAD	I-IV	65	45	Affymetrix microarrays	GPL570

Table S2. Protein expressions of *ERO1L* in HPA database

Patient ID	Gender	Age	Histology	Antibody staining	Intensity	Quantity	Location	Antibody used
1847	Male	64	LUAD	Medium	Moderate	>75%	Cytoplasmic/ membranous	CAB034294
1907	Male	73	LUAD	Medium	Moderate	75%-25%	Cytoplasmic/ membranous	HPA026653/ HPA030053
1932	Female	57	LUAD	Medium	Strong	<25%	Cytoplasmic/ membranous	HPA026653/ HPA030053
2003	Female	61	LUAD	High	Strong	75%-25%	Cytoplasmic/ membranous	HPA026653/ HPA030053
2041	Female	51	LUAD	Medium	Moderate	>75%	Cytoplasmic/ membranous	CAB034294
2222	Male	59	LUAD	Medium	Moderate	>75%	Cytoplasmic/ membranous	CAB034294
2403	Female	65	LUAD	Medium	Moderate	>75%	Cytoplasmic/ membranous	CAB034294
3003	Male	49	LUAD	Low	Weak	>75%	Cytoplasmic/ membranous	CAB034294
3391	Female	70	LUAD	Medium	Moderate	>75%	Cytoplasmic/ membranous	CAB034294
4208	Male	75	LUAD	Low	Weak	75%-25%	Cytoplasmic/ membranous	HPA026653/ HPA030053
4923	Male	57	LUAD	High	Strong	75%-25%	Cytoplasmic/ membranous	HPA026653/ HPA030053

Table S3. Characteristics of included cohorts in PrognScan database

Dataset	Cancer type	Cohort	Endpoint	Patients	Probe ID	HR	95% CIs	<i>p</i>
Jacob-00182-CANDF	LUAD	CAN/DF	OS	82	218498_s_at	0.94	0.46-1.92	0.87
Jacob-00182-HLM	LUAD	HLM	OS	79	218498_s_at	2.31	1.25-4.26	**
Jacob-00182-MSK	LUAD	MSK	OS	104	218498_s_at	1.73	0.82-3.66	0.15
Jacob-00182-UM	LUAD	UM	OS	178	218498_s_at	1.91	1.36-2.68	***
GSE13213	LUAD	Nagoya	OS	117	A_23_P106145	1.30	1.04-1.62	*
GSE13213	LUAD	Nagoya	OS	117	A_24_P407311	1.37	1.06-1.78	*
GSE31210	LUAD	NCCRI	OS	204	222646_s_at	2.42	1.48-3.98	***
GSE31210	LUAD	NCCRI	OS	204	218498_s_at	2.61	1.48-4.58	***
GSE31210	LUAD	NCCRI	PFS	204	222646_s_at	2.35	1.63-3.40	***
GSE31210	LUAD	NCCRI	PFS	204	218498_s_at	2.70	1.78-4.11	***

LUAD, lung adenocarcinoma; OS, overall survival; PFS, progression-free survival; HR, hazard ratio; 95% CI, 95% confidence interval. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

Table S4. Methylation sites mapped to the *ERO1L* gene

cg02221143	cg04318602	cg05380145	cg05837075	cg08081708
cg08136587	cg08554114	cg10224939	cg10872521	cg13827458
cg15393565	cg18380353	cg18561899	cg21157519	cg23580410
cg26575110	cg05837075	cg08554114		

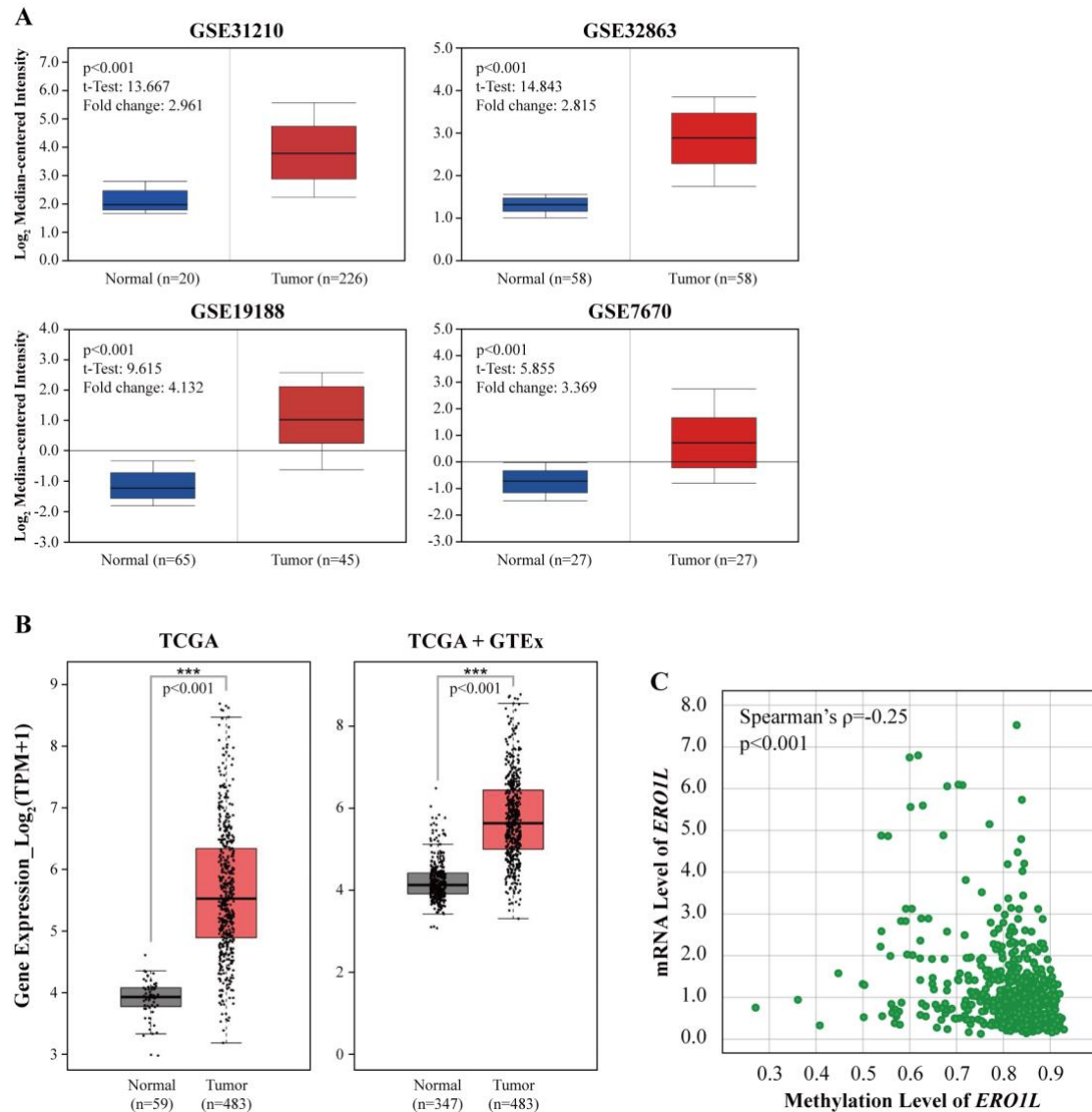


Figure S1. Expression and methylation profiles of *ERO1L* in LUAD. (A, B) Expression levels of *ERO1L* mRNA in LUAD, compared with normal tissues in the Oncomine database (A) and the TCGA database (B). (C) Correlation analysis between mRNA levels and methylation levels of *ERO1L*. GTEx, Genotype-Tissue Expression. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

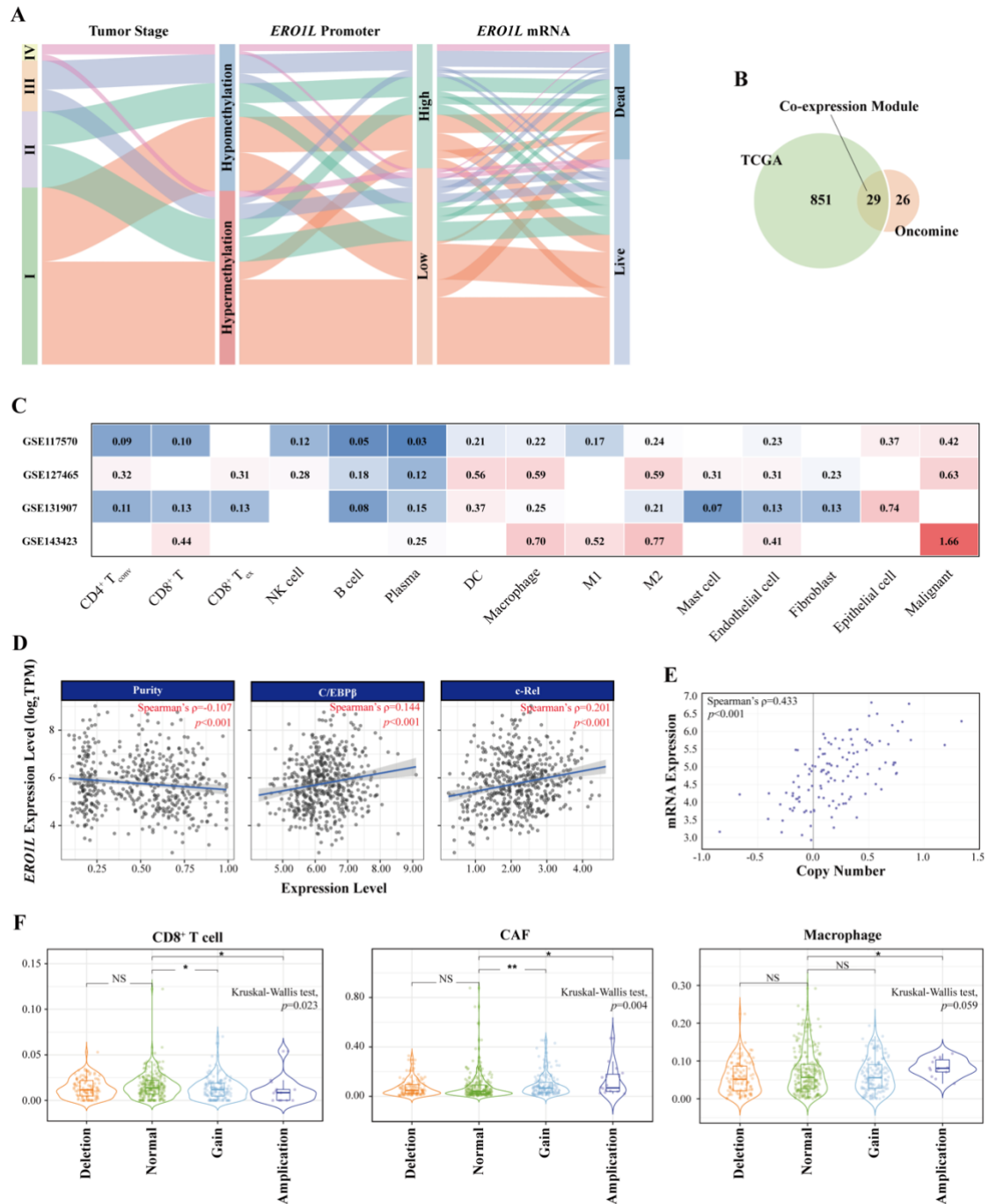


Figure S2. Overexpression of *ERO1L* predicts a poorer prognosis and shapes an immune-suppressive tumor microenvironment. (A) Path analysis described paths and patterns across tumor stages, promoter methylation levels, *ERO1L* mRNA expression, and survival status. (B) Venn plot for co-expression analysis of expression data from the TCGA program and Oncomine database. (C) Correlation analysis between *ERO1L* and infiltrating immune cells from four single-cell sequencing datasets. (D) Correlation analysis between *ERO1L* and *C/EBPβ* as well as *c-Rel*. (e,f) High expression level of *ERO1L* which were resulted from copy number variations (E) and relationships with infiltrating CD8⁺ T cells, CAF, and macrophages (F). NK, natural killer; DC, dendritic cell; CAF, cancer-associated fibroblast. NS, no significance. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

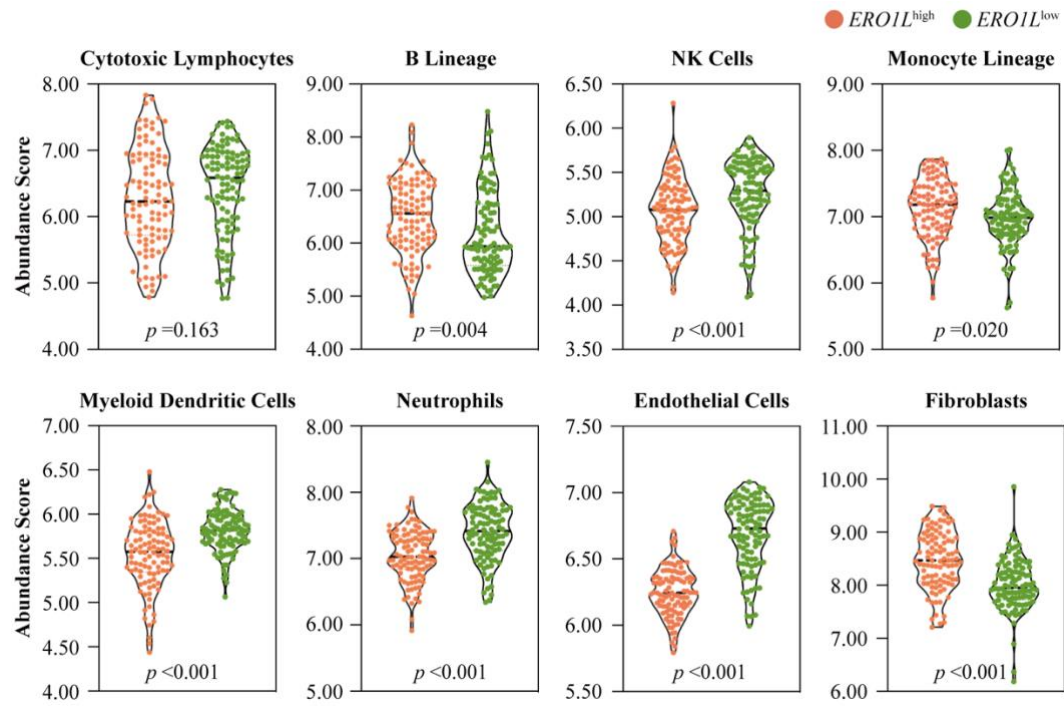


Figure S3. Quantifying the different immune cell populations. Immune cells within the $EROIL^{high}$ and $EROIL^{low}$ groups were calculated using the MCP counter framework. NK cells, natural killer cells.