

Table S1 Search strategy

Database	Strategy
PubMed	((lung cancer[Title/Abstract]) OR (lung adenocarcinoma[Title/Abstract]) OR (adenocarcinoma of lung[Title/Abstract]) OR (NSCLC[Title/Abstract]) OR (lung neoplasms[Title/Abstract]) OR (lung tumor[Title/Abstract])) AND ((estrogen receptor[Title/Abstract]) OR (oestrogen receptor[Title/Abstract]) OR (oestrogen[Title/Abstract]) OR (estrogen receptor β [Title/Abstract]) OR (ER β [Title/Abstract]) OR (estrogen[Title/Abstract]) OR (ERbeta[Title/Abstract]))
Web of Science	(TS=(lung cancer) OR TS=(lung adenocarcinoma) OR TS=(adenocarcinoma of lung) OR TS=(NSCLC) OR TS=(lung neoplasms) OR TS=(Non Small Cell Lung Carcinoma) OR TS=(lung tumor)) AND (TS=(estrogen) OR TS=(estrogen receptor) OR TS=(estrogen receptor β) OR TS=(oestrogen receptor) OR TS=(oestrogen) OR TS=(ERbeta) OR TS=(ER β)) AND (ALL=(survival) OR ALL=(overall survival) OR ALL=(outcome) OR ALL=(prognosis) OR ALL=(prognostic))
Embase	((estrogen receptor beta) or (ERbeta) or (estrogen receptor) or (estrogen) or (oestrogen receptor) or (oestrogen)).ab. AND ((lung cancer) or (lung adenocarcinoma) or (adenocarcinoma of lung) or (lung neoplasms) or (lung tumor) or (NSCLC)).ab.

Table S2 Summary of primers used in this study

Gene description	Species	Primer bank ID	NCBI gene ID	Sequence (5'-3')	Length	Tm	Location
ESR2	Human	333609292c1	2100	Forward primer: AGCACGGCTCCATATACATACC	22	61.4	77–98
				Reverse primer: TGGACCACTAAAGGAGAAAGGT	22	60.4	275–254
GAPDH	Human	378404907c1	2597	Forward primer: GGAGCGAGATCCCTCCAAAAT	21	61.6	108–128
				Reverse primer: GGCTGTTGTCATACTTCTCATGG	23	60.9	304–282

ESR2, ER β , estrogen receptor beta; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

Table S3 Summary of included studies and characteristics of patients

Author (year)	Time of patients diagnosed	Study design	Female (%)	EGFR mutation/ expression (%)	Aromatase+ (%)	Adenocarcinoma (%)	ERβ antibody	ERβ positive cut-off definition	Covariate adjustment
Kawai 2005	1995–1997	Retrospective cohort study	42.4	NR	NR	77.3	H-150, Santa Cruz Biotechnology, 1:100 dilution in PBS	The proportion and intensity scores for total score, score 2–8	NR
Schwarz 2005	1990–2004	Retrospective cohort study	NR	NR	NR	100	Mouse anti-ERβ-1 monoclonal antibody-MCA1974S (Serotec, Oxford, United Kingdom)	Samples with at least weak (1+) staining in ≥10% of tumor cells	Sex, race, age at diagnosis, stage at diagnosis, smoking status, pack-years, history of tuberculosis, and histology
Wu 2005	1990–2001	Retrospective cohort study	42.2	NR	NR	64.5	BioGenex, 1:100	Moderate-to-strong nuclear staining of more than 50% of the neoplastic cells	Stage of tumor, differentiation, smoking status, state of vascular invasion
Skov 2008	1989–1992	Retrospective cohort study	31.7	NR	NR	38.5	Oestrogen Receptor Clone PPG5/10, Code M7292, Dako Cytomation, Denmark	At least weak staining in more than 10% tumor cells	Gender, age, stage at diagnosis and histology
Toh 2010	1999–2002	Retrospective cohort study	40.6	39/61	NR	100	Oestrogen Receptor Clone PPG5/10, Dako Cytomation, Denmark 1:100	At least one + staining in ≥10% of tumor cells	NR
Mauro 2010	1997–2004	Retrospective cohort study	31.6	36/58*	NR	31.6	Chicken polyclonal antibody	≥5% tumor cells positive	NR
Nose 2011	2004–2009	Retrospective cohort study	46.5	30/13	NR	100	H-150 (Biotechnology, Santa Cruz, CA) diluted 1:10	5–8 score	NR
Mah 2011	NR	Retrospective cohort study	NR/NR	NR	60/0; 190/0	NR	Mouse anti-ERβ-1 monoclonal antibody (clone PPG5/10, product #MCA1974ST, AbDSerotec, Raleigh, NC)	$[(3x) + (2y) + (1z)] / 100$ where x, y, and z are % staining at intensity 3, 2, and 1, respectively; 57th percentile for overall ERβ; higher than median levels for cytoplasmic ERβ	(Cytoplasmic ERβ) stage, age, and grade
Stabile 2011	1992–2006	Cohort study	49.7	55/120*	56/123	59	Mouse anti-ERβ-1 monoclonal antibody MCA1974ST, AbD Serotec, Raleigh, NC	Score >7 for cytoplasmic ERβ and total ERβ	Age, tumor stage, sex, smoking status
Monica 2012	2008–2010	Retrospective cohort study	25.5	NR	NR	53.8	mouse anti-ERβ (clone PPG5/10, Dako), dilution, 1:50	8–12 score	NR
Navaratnam 2012	1999/2000–2002	Retrospective cohort study/ retrospective cohort study	59.5/NR	NR	NR	NR	monoclonal, 14C8, Genetex, TX, USA	≥ median IHC score	NR
Verma(1)2012	NR	Retrospective cohort study	39.5	NR	140/22	74.1	clone 14C8; GeneTex Inc., San Antonio, TX, 1:50	≥10% tumour cells positive	NR
Verma(2)2012	1993–2003	Retrospective cohort study	39.1	NR	146/23	76.3	clone 14C8; GeneTex Inc., San Antonio, TX, 1:50	≥10% positive results	NR
He 2015	2010–2012	Retrospective cohort study	32.6	NR	NR	71.7	from Beijing Bioss Biosynthesis Biotechnology Co., Ltd., (Beijing, China)	NR	NR
Tanaka 2016	2004–2008	Retrospective cohort study	48.7	0/78	35/43	100	clone14C8 GeneTex, CA, USA, 1:200	Score 1+/2+/3+	NR
Gao 2017	2004–2009	Retrospective cohort study	NR	27/35	NR	NR	ERβ (B-1) Santa Cruz sc-390243 1:500	≥median value of score	NR
Ding 2018	2011–2016	Retrospective cohort study	55.6	64/62	NR	100	mouse monoclonal antibody 14C8 (cat.no.ab288; Abcam, Cambridge, UK) 1:100	>10% of tumor cells exhibited specific, positive staining in the nucleus or cytoplasm with at least 1+ staining	NR
Yu 2018	2011–2013	Retrospective cohort study	100	NR	NR	100	Abcam 288#14C8	NR	NR
Cheng 2018	2005–2011, Cohort	Retrospective cohort study	55.4	NR	NR	57.2	PPG5/10 (ERβ-1 isoform specific) AbD Serotec. MCA1974ST	Quartile 4 vs. 1 of formula $1 \times (\% \text{ cells } 1+) + 2 \times (\% \text{ cells } 2+) + 3 \times (\% \text{ cells } 3+)$ with the weighted average of percent positivity values	Age, race, sex, smoking status, histology, and tumor stage
He 2019	2012–2014	Retrospective cohort study	72.1	122/79	NR	100	Mouse monoclonal anti-human ERβ1 antibody PPG5/10 (cat.no. M7292; Dako) 1:50	Total ERβ: score >9 nuclear ERβ: score >6	NR
Lee 2020	2010–2012	Retrospective cohort study	48.8	81/3*	NR	100	Clone 14C8, Abcam, Cambridge, UK 1:100	Score 3–8	NR
Enwere	2003–2006	Retrospective cohort study	49.8	NR	NR	54.2	mouse monoclonal, clone PPG5/10, 1:500, Abcam, Cambridge, MA, USA)	HALO score	Stage, age, histology, gender and smoking status

*, high expression/ low expression. ERβ, estrogen receptor beta; NR, not reported; EGFR, epidermal growth factor receptor.

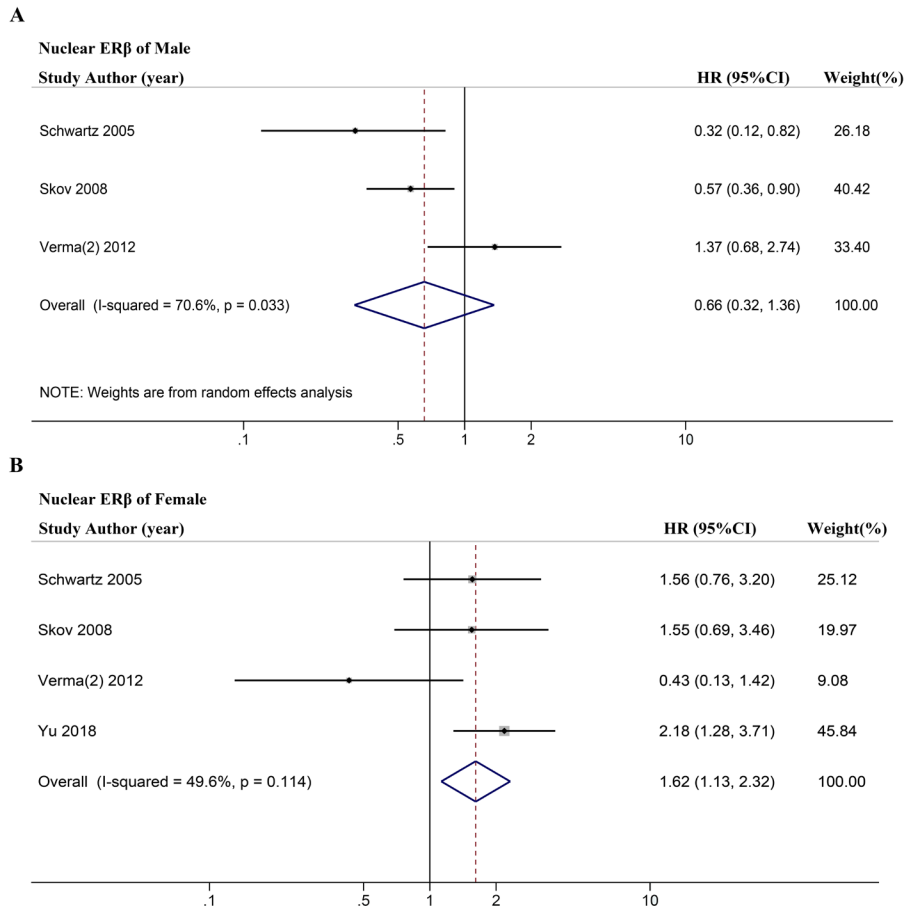


Figure S1 Forest plots of associations between nuclear ERβ protein expression and OS by sex. (A) Effect of nuclear ERβ on OS of NSCLC in male. (B) Effect of nuclear ERβ on OS of NSCLC in female. HR: hazard ratio; CI: confidence interval; ERβ: estrogen receptor beta. The size of the blocks or diamonds represents the weight and the length of the straight line represents the width of 95% CI.

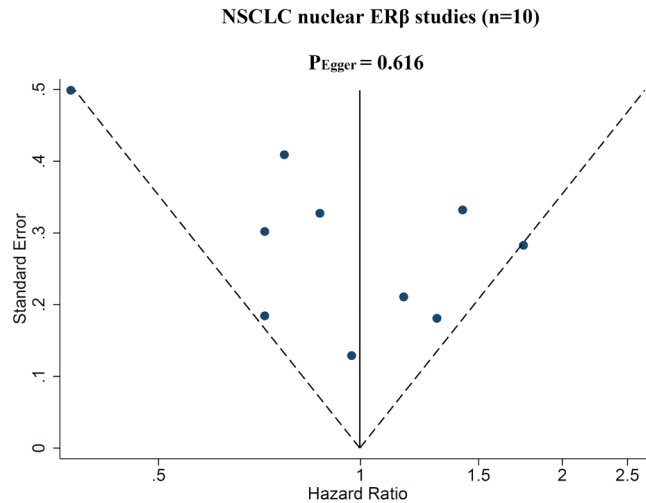


Figure S2 Funnel plot of Publication bias using Egger's test for NSCLC (excluded lung adenocarcinoma-specific studies) nuclear ER group. ERβ, estrogen receptor beta; NSCLC, non-small cell lung cancer. Each dot represents a single study

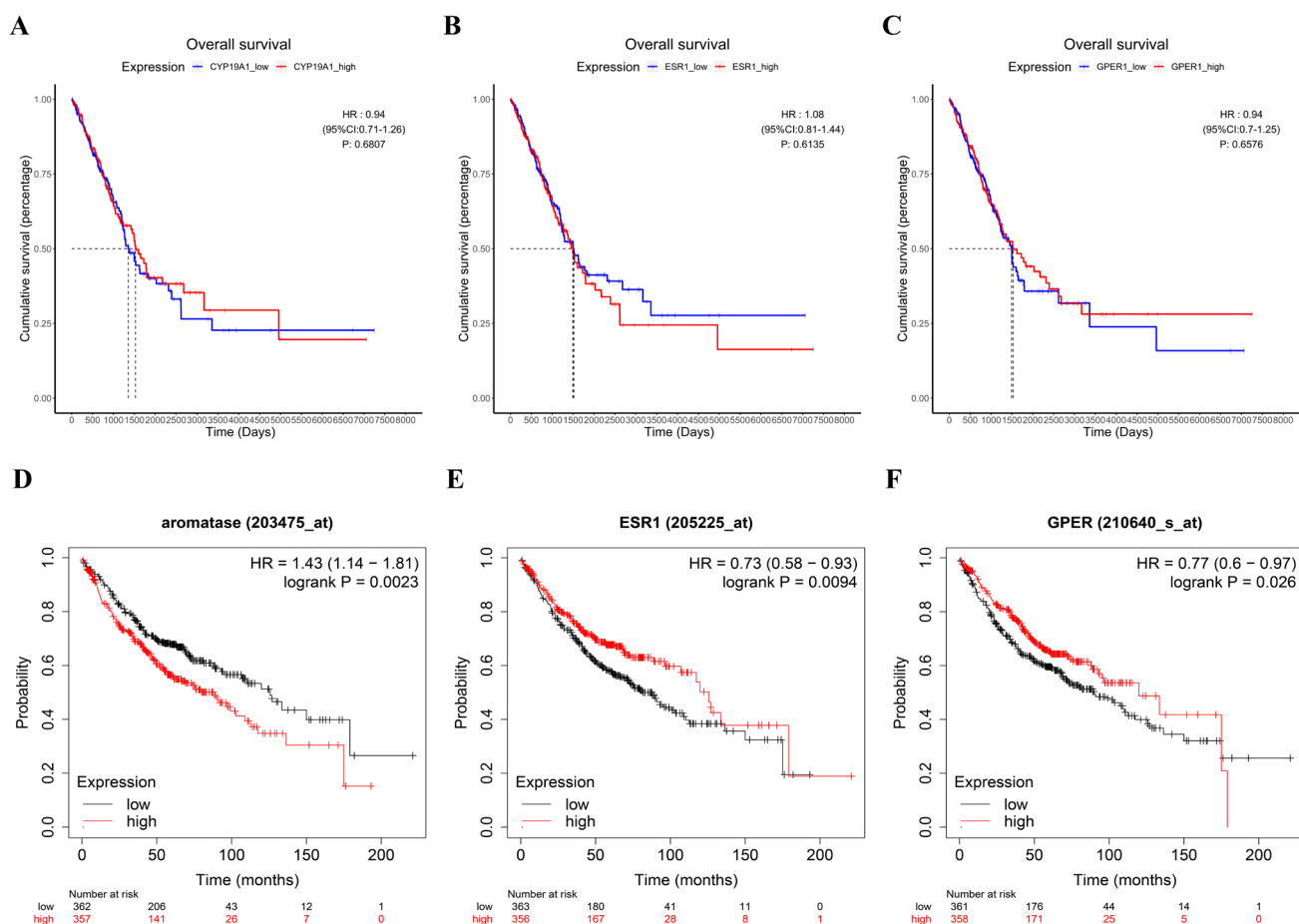


Figure S3 The Kaplan Meier curves showing association between overall survival and mRNA expression of (A) aromatase from TCGA-LUAD (B) ESR1 from TCGA-LUAD (C) GPER1 from TCGA-LUAD (D) aromatase from Kaplan Meier plotter (E) ESR1 from Kaplan Meier plotter (F) GPER1 from Kaplan Meier plotter. CYP19A1, aromatase; ESR1, ER α , estrogen receptor alpha; GPER1, G-protein coupled estrogen receptor 1; HR, hazard ratio; CI, confidence interval; TCGA-LUAD, The Cancer Genome Atlas Lung Adenocarcinoma.

Table S4 The differential mRNA expression of 4 genes in lung adenocarcinoma

Genes	logFC	AveExpr	t	P value	adj. P value	B	Change
<i>GPER1</i>	-2.07839	6.852989	-10.5405	6.02E-24	2.41E-23	43.47529	down
<i>CYP19A1</i>	1.176672	4.3038	5.684142	2.05E-08	4.11E-08	8.350722	up
<i>ESR2</i>	0.403834	5.56775	2.67291	0.007724	0.010299	-3.80118	stable
<i>ESR1</i>	0.289788	8.347502	1.265863	0.206054	0.206054	-6.54536	stable

ESR2, ER β , estrogen receptor beta; ESR1, ER α , estrogen receptor alpha, GPER1, G-protein coupled estrogen receptor 1; CYP19A1, aromatase.

Table S5 Characteristics of 4 GEO datasets in lung adenocarcinoma

GSE	GPL	Tissue	Sample type		Reference	PMID
			Normal	Tumor		
GSE10072	GPL96	LUAD	49	58	Landi, Dracheva <i>et al.</i> 2008	18297132
GSE40791	GPL570	LUAD	100	94	Zhang, Foreman <i>et al.</i> 2012	23187126
GSE32863	GPL6884	LUAD	58	58	Selamat, Chung <i>et al.</i> 2012	22613842
GSE43458	GPL6244	LUAD	30	80	Kabbout, Garcia <i>et al.</i> 2013	23659968

LUAD, lung adenocarcinoma.