Supplementary Material

"Association of estrogen receptor alpha expression with survival in
oropharyngeal cancer following chemoradiation therapy"

Supplementary Tables

Supplementary Table 1. Genes with mRNA expression evaluated for activation of nuclear ERα signaling*

Entrez Gene ID	Gene Symbol	Gene Description
21	ABCA3	ATP-binding cassette, subfamily A member 3
8125	ANP32A	acidic nuclear phosphoprotein 32 family member A
162	AP1B1	adaptor-related protein complex 1, beta 1 subunit
322	APBB1	amyloid beta precursor protein-binding family B member 1
522	ATP5J	ATP synthase, H+ transporting, mitochondrial, F0 complex, subunit F
8313	AXIN2	axin 2
672	BRCA1	breast cancer 1, early onset
718	C3	complement component 3
57658	CALCOCO1	calcium binding and coiled-coil domain 1
595	CCND1	cyclin D1
3732	CD82	CD82 molecule
1051	CEBPB	CCAAT/enhancer binding protein (C/EBP), beta
1147	CHUK	conserved helix-loop-helix ubiquitous kinase
80781	COL18A1	collagen, type XVIII, alpha 1
1509	CTSD	cathepsin D
10521	DDX17	DEAD (Asp-Glu-Ala-Asp) box polypeptide 17
79039	DDX54	DEAD (Asp-Glu-Ala-Asp) box polypeptide 54
1826	DSCAM	Down syndrome cell adhesion molecule
9166	EBAG9	estrogen receptor binding site associated, antigen, 9
2033	EP300	E1A binding protein p300
2100	ESR2	estrogen receptor 2 (ER beta)
9687	GREB1	growth regulating estrogen receptor binding 1
3065	HDAC1	histone deacetylase 1
9759	HDAC4	histone deacetylase 4
3298	HSF2	heat shock transcription factor 2
3725	JUN	jun proto-oncogene
3823	KLRC3	killer cell lectin like receptor C3
84458	LCOR	ligand dependent nuclear receptor corepressor
8543	LMO4	LIM domain only 4
5469	MED1	mediator complex subunit 1
4350	MPG	N-methylpurine-DNA glycosylase
9112	MTA1	metastasis associated 1

4609	*MYC*	MYC proto-oncogene, bHLH transcription factor
8648	*NCOA1*	nuclear receptor coactivator 1
10499	*NCOA2*	nuclear receptor coactivator 2
8202	*NCOA3*	nuclear receptor coactivator 3
135112	*NCOA7*	nuclear receptor coactivator 7
9611	*NCOR1*	nuclear receptor corepressor 1
9612	*NCOR2*	nuclear receptor corepressor 2
4731	*NDUFV3*	NADH:ubiquinone oxidoreductase subunit V3
4738	*NEDD8*	neural precursor cell expressed, developmentally down-regulated 8
190	*NR0B1*	nuclear receptor subfamily 0, group B, member 1
8431	*NR0B2*	nuclear receptor subfamily 0, group B, member 2
8204	*NRIP1*	nuclear receptor interacting protein 1
5111	*PCNA*	proliferating cell nuclear antigen
64714	*PDIA2*	protein disulfide isomerase family A, member 2
5241	*PGR*	progesterone receptor
11331	*PHB2*	prohibitin 2
5457	*POU4F1*	POU class 4 homeobox 1
5458	*POU4F2*	POU class 4 homeobox 2
63977	*PRDM15*	PR domain containing 15
5617	*PRL*	prolactin
6294	*SAFB*	scaffold attachment factor B
6418	*SET*	SET nuclear oncogene
4089	*SMAD4*	SMAD family member 4
6647	*SOD1*	superoxide dismutase 1, soluble
10011	*SRA1*	steroid receptor RNA activator 1
6776	*STAT5A*	signal transducer and activator of transcription 5A
7031	*TFF1*	trefoil factor 1
286827	*TRIM59*	tripartite motif containing 59
9039	*UBA3*	ubiquitin-like modifier activating enzyme 3.
9040	*UBE2M*	ubiquitin-conjugating enzyme E2M
7494	*XBP1*	X-box binding protein 1

*Based on Protein Interaction Database ERα nuclear receptor signaling gene set, with *ESR1* omitted, from: http://software.broadinstitute.org/gsea/msigdb/cards/PID_ERA_GENOMIC_PATHWAY.html
See Schaefer CF, Anthony K, Krupa S, et al. PID: the Pathway Interaction Database. Nucleic Acids Res. Jan 2009;37(Database issue):D674-679. ERα = estrogen receptor alpha.

Supplementary Table 2. Chemotherapy regimens for the OPSC-CR cohort

Regimen	No. of cases (%)
Cisplatin only	31 (14.4)
Carboplatin only	5 (2.3)
Carboplatin + paclitaxel	83 (38.6)
Cetuximab	32 (14.9)
Carboplatin +/- paclitaxel, cetuximab or panitumumab	9 (4.2)
Paclitaxel alone	5 (2.3)
Carboplatin + paclitaxel + cetuximab	4 (1.9)
Carboplatin + paclitaxel + panitumumab (Protocol 05-401)	5 (2.3)
Carboplatin + other (data unavailable)	6 (2.8)
Cisplatin + fluorouracil	1 (0.5)
Cisplatin + paclitaxel	1 (0.5)
Unspecified carboplatin or cisplatin	1 (0.5)
Unspecified	32(14.9)
Total	215 (100.0)

Supplementary Table 3. Five-year survival versus ERα status, OPSC-CR cohort

Survival type	Five-year survival, % (95% CI)		P-value*
	ERα-positive	ERα-negative	
Overall survival	90.0 (84.1-95.9)	64.0 (54.2-73.8)	< 0.001
Disease-specific survival	91.0 (85.1-96.9)	66.5 (56.7-76.3)	< 0.001
Progression-free survival	86.2 (80.3-92.1)	69.5 (59.7-79.3)	0.004
Relapse-free survival	90.4 (84.5-96.3)	76.7 (66.9-86.5)	0.01

* *P* values were calculated using a two-sided *z*-test. ER = estrogen receptor; CI = confidence interval.

Supplementary Table 4. Clinical characteristics of the HPV-positive subset of the OPSC-CR cohort and their relations to ERα status

Clinical characteristic	No. (%)			*P*-value
	Total (n=177)	ERα- (n=74)	ERα+ (n=103)	
Sex				
Male	145 (81.9)	64 (86.5)	81 (78.6)	0.24*
Female	32 (18.1)	10 (13.5)	22 (21.4)	
Age, y				
≤ 60	123 (69.5)	52 (70.2)	71 (68.9)	0.87*
> 60	54 (30.5)	22 (29.8)	32 (31.1)	
Race				
Caucasian	161 (91.0)	64 (94.1)	97 (97.0)	0.44*
Non-Caucasian	7 (3.9)	4 (5.9)	3 (3.0)	
Unknown	9 (5.1)	—	—	
Site				
Tonsil	83 (46.9)	33 (44.6)	50 (48.5)	0.11‡
BOT	88 (49.7)	36 (48.6)	52 (50.5)	
Other	6 (3.4)	5 (6.8)	1 (1.0)	
Alcohol Use				
No Abuse	143 (80.8)	61 (85.9)	82 (88.2)	0.81*
Abuse	21 (11.9)	10 (14.1)	11 (11.8)	
Unknown	13 (7.3)	—	—	
Smoking Status				
Never	70 (39.5)	27 (36.5)	43 (41.2)	0.53*
Ever	106 (59.9)	47 (63.5)	59 (57.8)	
Unknown	1 (0.6)	—	—	
Tumor Classification				
T0-2	130 (73.5)	52 (73.2)	78 (77.2)	0.59*
T3-4	42 (23.7)	19 (26.7)	23 (22.8)	
Unknown	5 (2.8)	—	—	
Nodal Classification				
N0-N2a	70 (39.5)	34 (46.6)	36 (35.6)	0.16*
N2b-N3	104 (58.8)	39 (53.4)	65 (64.4)	
Unknown	3 (1.7)	—	—	
Metastasis at presentation				
M0	167 (94.4)	70 (95.9)	97 (98.0)	0.50*
M1	5 (2.8)	3 (4.1)	2 (2.0)	
Unknown	5 (2.8)	—	—	
Tumor Keratinization				
No	146 (82.5)	57 (77.0)	89 (86.4)	0.11*
Yes	31 (17.5)	17 (23.0)	14 (13.6)	

* *P* values were calculated using a two-sided Fisher exact test. BOT = base of tongue; HPV = human papillomavirus.

‡ *P* value was calculated using a two-sided Pearson Chi-square test.

Supplementary Table 5. Concordance and validation of 6-predictor models in the OPSC-CR Cohort.
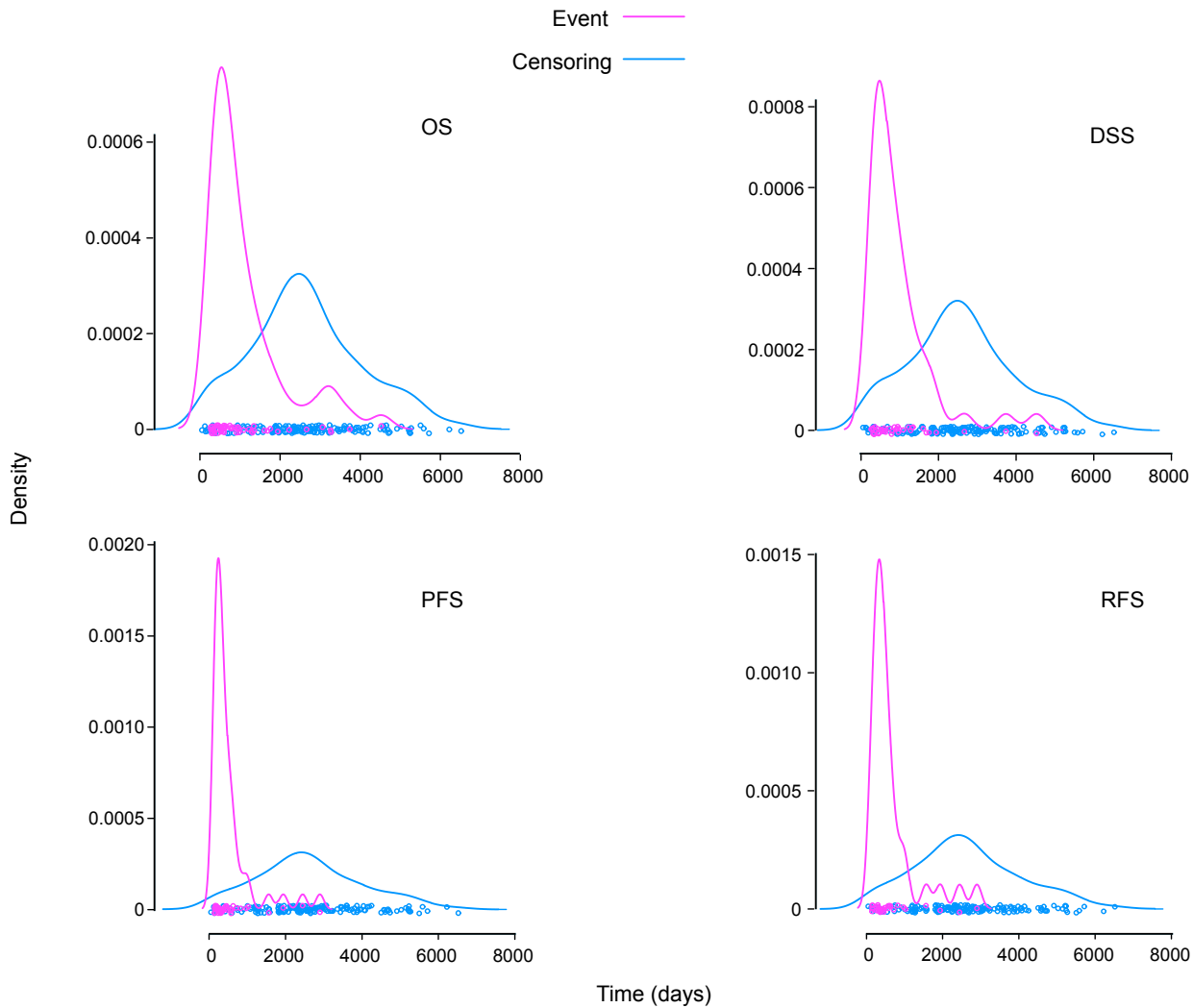
| Model | Concordance | Bootstrap validation/calibration* | | | | Proportional hazard (PH) tests† | | | | |
| | | Adjusted Concordance | Slope optimism | MAE | Coefficients | Model in main text | | Stratified model‡ | | |
						Global PH	Coefficient PH	Global PH	Coefficient PH	HR (95% CI)
OS	0.77	0.75	0.127	0.016	ERα	0.87	0.89	--	--	--
				0.017	HPV		0.82			
				0.042	T > 2		0.39			
					Ever Smoker		0.41			
					Age > 60 y		0.58			
					Sex		0.72			
DSS	0.79	0.75	0.146	0.010	ERα	0.53	0.81	0.98	0.79	0.30 (0.14 - 0.67)
				0.014	HPV		0.46		0.48	0.72 (0.33 - 1.57)
				0.015	T > 2		0.89		0.85	3.32 (1.65 - 6.65)
					Ever Smoker		1.00		0.99	2.04 (0.87 - 4.77)
					Age > 60 y		0.05		Stratified	--
					Sex		0.68		0.63	0.78 (0.27 - 2.28)
PFS	0.75	0.72	0.149	0.030	ERα	0.08	0.98	1.00	0.97	0.50 (0.25 - 0.99)
				0.032	HPV		0.90		0.87	0.78 (0.35 - 1.72)
				0.020	T > 2		0.05		Stratified	--
					Ever Smoker		0.86		0.84	1.92 (0.86 - 4.28)
					Age > 60 y		0.01		Stratified	--
					Sex		0.67		0.82	0.36 (0.11 - 1.20)
RFS	0.74	0.69	0.279	0.018	ERα	0.62	0.20	--	--	--
				0.009	HPV		0.95			
				0.041	T > 2		0.47			
					Ever Smoker		0.80			
					Age > 60 y		0.55			
					Sex		0.28			

* Via *validate* and *calibrate* functions in R *rms* package, with default settings. MAE: mean absolute error in optimism-corrected predicted fraction surviving for 18, 36, and 84 months (from top to bottom in each cell). HPV = human papilloma virus; CI = confidence interval; HR = hazard ratio; OS = overall survival; DSS = disease-specific survival; PFS = progression-free survival; RFS = relapse-free survival.
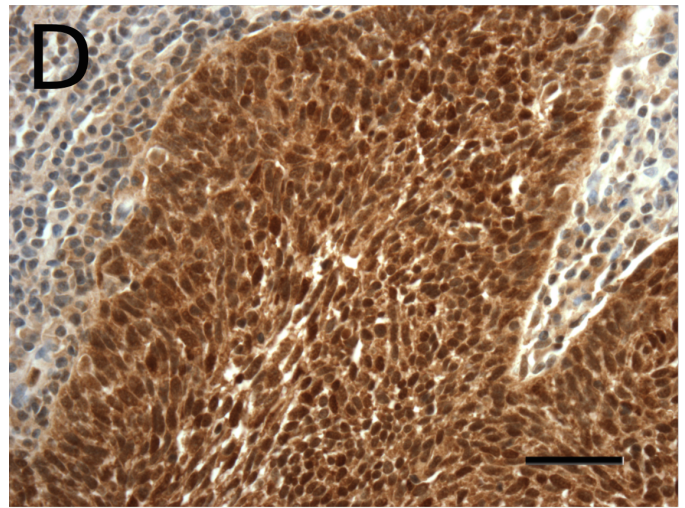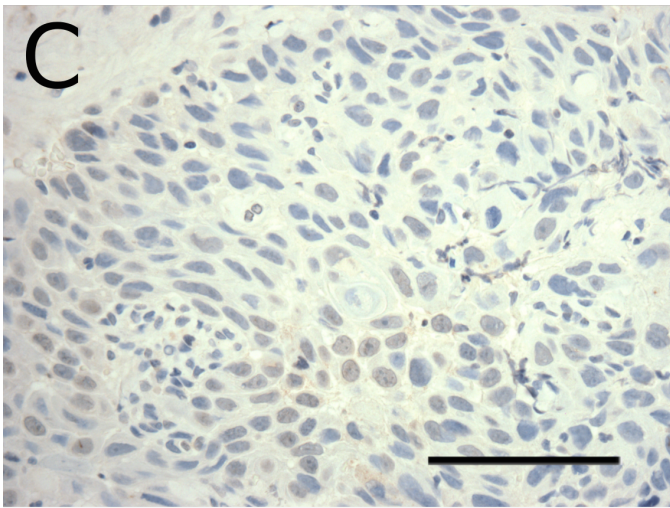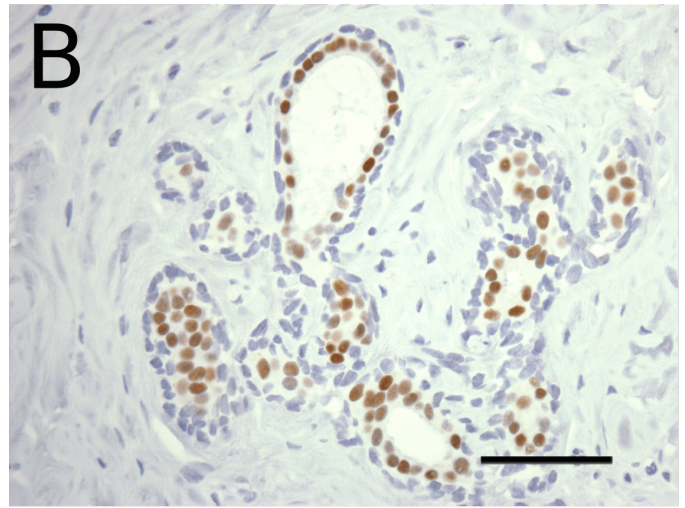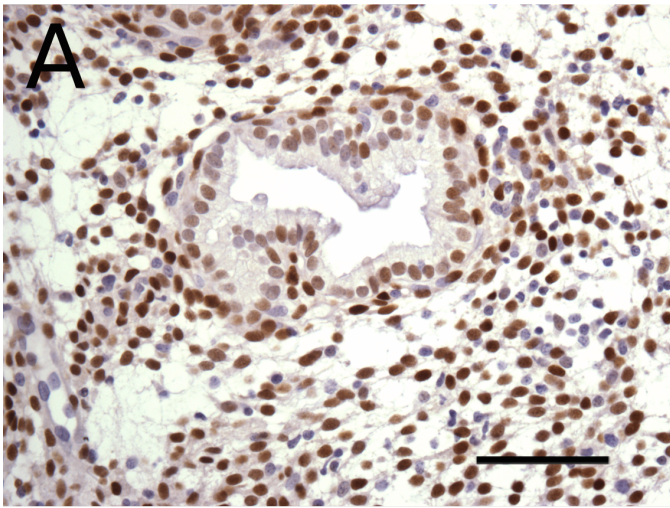
† Test of trend in scaled Schoenfeld residuals with time; via *cox.zph* function in R *survival* package, with default settings.

‡ Stratified by coefficients found to have $P < 0.2$ in PH tests of full model. Hazard ratios for remaining variables are similar to those in the models presented in the main text (**Figures 5 and 6**).

Supplementary Figures

Supplementary Figure 1. Distributions of event and censoring times for the OSPC-CR cohort. Density plots of event times (magenta) and censoring times (cyan) for overall survival (OS), disease-specific survival (DSS), progression-free survival (PFS), and relapse-free survival (RFS), as defined in the text. For all measures of survival, censoring times are typically much greater than event times, indicating that further follow up would not have substantially affected survival analysis. Median time to event in years (1st and 3rd quartiles): OS 2.06(1.27-3.66); DSS, 1.74(1.21-3,18); PFS, 0.89(0.64-1.57); RFS, 1.20(0.85-1.86) Median time to censoring (1st and 3rd quartiles): OS, 6.98 (5.00-9.54); DSS, 6.93(4.97-9.42); PFS, 6.90 (4.98-9.73); RFS,6.90(4.98-9.73).

Supplementary Figure 2. ERα immunohistochemical controls and OPSC p16 staining. A) Normal endometrium positive for ERα. **B**) Breast cancer positive control for ERα. **C**) OPSC negative for p16. **D**) OPSC positive for p16. Scale bars represent 200 micrometers. ERα=estrogen receptor alpha; OPSC = oropharyngeal squamous carcinoma.