

**Supplementary Material for:**

**Human lung epithelial cells progressed to malignancy through specific oncogenic  
manipulations**

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## Supplementary Methods

### *Microarray analysis*

RNA was isolated using RNeasy mini kit (QIAGEN, Valencia, CA) and quality was analyzed using an Agilent 2100 Bioanalyzer (Agilent Technologies Inc., Santa Clara, CA). Samples were labeled and hybridized to Illumina HumanHT-12 v4 Expression BeadChips (Illumina Inc., San Diego, CA) (containing 47,231 probes (28,688 unique genes)) following the manufacturer's guidelines. Microarray analysis was performed using in-house Visual Basic software MATRIX V1.483. Functional analysis of differentially expressed genes was performed using Ingenuity Pathway Analysis (IPA) (Ingenuity Systems, Inc. Redwood City, CA). The data discussed in this publication have been made available in the National Center for Biotechnology Information's Gene Expression Omnibus (GEO) public repository (<http://www.ncbi.nlm.nih.gov/geo/>) and are accessible through GEO Series accession number GSE40828.

The predictive ability of the soft agar gene signature was tested using two independent mRNA microarray lung tumor datasets; 209 primary lung adenocarcinomas and squamous cell carcinomas (SPORE dataset) (GSE41271) and 442 primary lung adenocarcinomas (Director's Challenge Consortium dataset) (18). Microarrays for the SPORE dataset were performed as described above using the Illumina HumanWG6 v3 Expression BeadChips (Illumina Inc., San Diego, CA) (containing 48,803 probes (29,346 unique genes)) following the manufacturer's guidelines. Raw cel data from the Director's Challenge Consortium dataset(18) was download from the NCI caArray Database (<https://array.nci.nih.gov/caarray/home.action>) and preprocessed by the Robust Multiarray Average (RMA) algorithm (19) and quantile-quantile normalization.

All gene expression values were  $\log_2$  transformed. The prognostic value of the signature was evaluated only in patients who had not received adjuvant chemotherapy, if information was available. The prediction models were constructed using the signature expression values, and tested against the SPORE dataset. A supervised principal component analysis (SuperPC) (20), a widely used classification model in biomedical research, was applied for building the prediction model. The first three principle components (PCs) from genes scored  $> 0.2$  by SuperPC were submitted to the Cox regression model for overall survival time (calculated from the date of surgery until death or the last follow-up contact). The median of the estimated risk scores was used as the cut off to partition the patients into high-risk and low-risk groups. Kaplan-Meier survival curves for the high and low risk groups were compared with log-rank tests. In order to test the robustness of our signature, we firstly tested the signature by training in the 442 patient Consortium dataset and testing in the SPORE 209 mRNA dataset, then switched the datasets to train in the SPORE dataset and test in the Consortium dataset. Specificity and sensitivity of the predictive model were evaluated by a ROC curve.

**Supplementary Table S1.** Primary antibodies used for immunoblotting.

Antibody	Catalogue	Manufacturer
anti-p53	P6874	Sigma-Aldrich
anti-K-RAS	sc-30	Santa Cruz Biotechnology, Inc.
anti-c-MYC	sc-40	Santa Cruz Biotechnology, Inc.
anti-E-cadherin	610181	BD Bioscience
anti-N-cadherin	610920	BD Bioscience
anti-vimentin	550513	BD Bioscience
anti-p16 <sup>INK4A</sup>	sc-468	Santa Cruz Biotechnology, Inc.
anti-Caspase3	9662	Cell Signaling Technology, Inc.
anti-actin	A4700	Sigma-Aldrich
anti-HSP90	sc-13119	Santa Cruz Biotechnology, Inc.

**Supplementary Table S2.** In vitro and in vivo characterization of soft agar clones derived from manipulated HBECs.

		<u>Genetic manipulation</u>			<u>Protein expression</u>			<u>Serum dependent</u>	<u>In vitro clonogenicity (%)</u>		<u>In vivo tumorigenicity</u>		
		sh-p53	KRAS <sup>V12</sup>	c-MYC	p53	KRAS	c-MYC		Anchorage-dependent	Anchorage-independent	Tumor Formation Rate <sup>b</sup>	Latency <sup>c</sup>	Histology
<b>HBEC3<sup>p53,KRAS</sup></b>	Clone1	+	+	-	-	++	-	No	41.0	20.1	8/8 (100%)	38	Large cell/giant cell carcinoma (7) Squamous cell carcinoma (1)
	Clone5	+	+	-	-	++	-	No	29.5	25.3	7/8 (88%)	57	Large cell/giant cell carcinoma (5) Papillary adenocarcinoma (1) Poorly differentiated carcinoma (1)
	Clone11	+	+	-	-	+	-	Yes	39.5	39.3	6/8 (75%)	117	Large cell/giant cell carcinoma (3) Poorly differentiated carcinoma (2) Adenocarcinoma (1)
	Clone6	+	+	-	-	-	-	Yes	20.5	6.0	0/6 (0%)	-	N/A
	Clone7	+	+	-	-	-	-	No	17.5	7.4	0/7 (0%)	-	N/A
	Clone8	+	+	-	-	+	-	Yes	42.0	5.7	0/8 (0%)	-	N/A
	Clone9	+	+	-	-	+	-	No	35.5	5.0	0/9 (0%)	-	N/A
<b>HBEC3<sup>p53,KRAS,MYC</sup></b>	Clone1	+	+	+	-	+	++	No	n.d.	n.d.	9/9 (100%)	119	Large cell/giant cell carcinoma (6) Adenocarcinoma (2) Giant cell carcinoma (1)
	Clone2	+	+	+	-	+	++	No	n.d.	n.d.	3/8 (38%)	90	Squamous cell carcinoma (2) Large cell/giant cell carcinoma (1)
	Clone7	+	+	+	-	++	++	No	n.d.	n.d.	10/10 (100%)	37	Large cell/giant cell carcinoma (7) Squamous cell carcinoma (3)
	Clone8	+	+	+	-	-	++	Yes	n.d.	n.d.	1/6 (17%)	78	Large cell/giant cell carcinoma (1)

<sup>a</sup> As determined by ability of cells to grow in defined media (KSFM)

<sup>b</sup> Number of subcutaneous tumors/number of injections (percentage)

<sup>c</sup> Median time (days) for s.c. xenografts to reach 250mm<sup>3</sup>

n.d., not determined

**Supplementary Table S3:** Drug response phenotypes of isogenic clones of oncogenically manipulated HBECs.

Cell line		In vivo tumorigenicity	ED <sub>50</sub>		
			Pac + Carb (nM)	Gem + Cis (nM)	Pem + Cis (μM)
HBEC3		No	2 ± 0.1	28 ± 8.2	13 ± 1.0
HBEC3 <sup>p53, KRAS</sup>		Yes	2 ± 0.6	5 ± 0.4	1 ± 0.7
HBEC3 <sup>p53, KRAS</sup>	Clone 1	Yes	3 ± 0.4	4 ± 0.4	0.6 ± 0.3
HBEC3 <sup>p53, KRAS</sup>	Clone 5	Yes	4 ± 0.3	9 ± 4.0	4 ± 3.2
HBEC3 <sup>p53, KRAS</sup>	Clone 6	No	2 ± 0.5	4 ± 0.4	1 ± 0.2
HBEC3 <sup>p53, KRAS</sup>	Clone 7	No	3 ± 0.3	10 ± 7.9	4 ± 1.3
HBEC3 <sup>p53, KRAS</sup>	Clone 8	No	3 ± 0.3	4 ± 1.1	2 ± 2.1
HBEC3 <sup>p53, KRAS</sup>	Clone 9	No	2 ± 0.3	4 ± 0.4	3 ± 1.0
HBEC3 <sup>p53, KRAS</sup>	Clone 11	Yes	3 ± 1.2	5 ± 3.0	3 ± 2.6

Gem, gemcitabine; Cis, cisplatin; Pac, paclitaxel; Carb, carboplatin; Pem, pemetrexed.

**Supplementary Table S4:** Significantly differentially expressed genes between tumorigenic (Clones 1, 5 and 11) and non-tumorigenic (Clones 6, 7, 8 and 9) soft agar clones of HBEC3<sup>p53,KRAS</sup>.

Symbol	Illumina Probe	Accession	Tumori- genic clones <sup>a,b</sup>	Non- Tumorigenic clones <sup>a,b</sup>	Tumor- igenic/Non- Tumorigenic <sup>b</sup>	SAM P value (FDR =5%)
HLA-DRB1	ILMN_1697499	NM_002125	9.45	4.01	5.44	0.000
TNFRSF11B	ILMN_1676663	NM_002546	9.14	3.93	5.21	0.000
VIPR1	ILMN_2199389	NM_004624	10.93	5.80	5.13	0.000
COLEC12	ILMN_1689088	NM_130386	9.37	4.87	4.50	0.000
RTN1	ILMN_1756928	NM_021136	8.10	3.77	4.33	0.000
HLA-DRB1	ILMN_1715169	NM_002124	9.44	5.17	4.27	0.000
CD74	ILMN_2379644	NM_004355	7.58	3.41	4.17	0.000
KIAA1199	ILMN_1813704	NM_018689	11.51	7.35	4.16	0.000
RFX8	ILMN_1783840	NM_001145664	7.34	3.18	4.16	0.000
HLA-DRA	ILMN_2157441	NM_019111	9.11	5.03	4.08	0.000
HLA-DPA1	ILMN_1772218	NM_033554	8.30	4.25	4.05	0.000
TNFRSF1B	ILMN_1764788	NM_001066	7.86	3.92	3.93	0.000
SCG2	ILMN_1703178	NM_003469	11.29	7.38	3.92	0.000
CD74	ILMN_1736567	NM_001025159	9.15	5.30	3.85	0.000
NR4A2	ILMN_2339955	NM_006186	8.45	4.72	3.74	0.000
HOXB6	ILMN_1768101	NM_018952	7.77	4.07	3.70	0.000
UCHL1	ILMN_1757387	NM_004181	10.73	7.06	3.67	0.001
HLA-DRB6	ILMN_2066066	L76565	8.39	4.73	3.66	0.000
HLA-DRB1	ILMN_1752592	NM_021983	9.49	5.94	3.54	0.000
IFITM1	ILMN_1801246	NM_003641	10.03	6.50	3.53	0.000
HOXB5	ILMN_1674908	NM_002147	8.85	5.38	3.47	0.000
SLC7A14	ILMN_1676602	NM_020949	8.52	5.16	3.36	0.001
	ILMN_2066060	NR_001298	7.28	4.01	3.27	0.000
PARM1	ILMN_1656560	NM_015393	7.05	3.78	3.27	0.000
SLC14A1	ILMN_1805561	NM_015865	7.83	4.57	3.26	0.000
ARMCX2	ILMN_1785170	NM_177949	8.04	4.89	3.15	0.001
COL22A1	ILMN_1784532	NM_152888	8.39	5.26	3.13	0.000
SLC22A18	ILMN_2382505	NM_002555	8.10	5.00	3.10	0.000
CCL20	ILMN_1657234	NM_004591	11.27	8.21	3.06	0.000
PRRX1	ILMN_1739496	NM_006902	10.29	7.25	3.04	0.000
C11orf96	ILMN_1677402	NM_001145033	9.23	6.21	3.02	0.000
PDE4B	ILMN_2340259	NM_002600	6.91	3.90	3.01	0.000
CFB	ILMN_1774287	NM_001710	11.59	8.59	3.00	0.000
PLA2G7	ILMN_1701195	NM_005084	7.89	4.90	2.99	0.000
NR4A2	ILMN_1782305	NM_006186	10.02	7.05	2.97	0.000
COL6A2	ILMN_1809928	NM_001849	6.85	3.90	2.95	0.000
TOX2	ILMN_2082209	NM_032883	12.20	9.25	2.95	0.001
OSCAR	ILMN_2367418	NM_133169	6.40	3.51	2.89	0.000
IZUMO4	ILMN_1734346	NM_001039846	7.75	4.89	2.86	0.000

MDK	ILMN_2261876	NM_001012334	6.60	3.78	2.82	0.000
COL6A2	ILMN_1783909	NM_001849	7.60	4.78	2.82	0.001
AFF3	ILMN_1775235	NM_001025108	6.71	3.93	2.78	0.000
SNTB1	ILMN_1793410	NM_021021	7.35	4.62	2.73	0.001
GFPT2	ILMN_1709674	NM_005110	11.49	8.79	2.70	0.000
C4orf31	ILMN_1689176	NM_024574	6.27	3.83	2.44	0.000
PDLIM3	ILMN_2230025	NM_014476	7.37	5.01	2.36	0.000
TNFSF15	ILMN_1759501	NM_005118	6.17	3.81	2.36	0.000
METRNL	ILMN_2342066	NM_001004431	7.17	4.81	2.35	0.000
C1R	ILMN_1764109	NM_001733	9.48	7.14	2.34	0.000
MICA	ILMN_1803945	NM_006674	9.71	7.38	2.33	0.000
MRPS6	ILMN_1787813	NM_006933	8.24	5.94	2.30	0.000
HSD17B14	ILMN_1809483	NM_016246	5.70	3.41	2.29	0.000
PKIB	ILMN_2337263	NM_032471	7.25	4.97	2.28	0.000
FRMD4A	ILMN_1678961	NM_018027	9.18	6.92	2.26	0.000
IL1RAPL1	ILMN_2160428	AJ243874	7.80	5.54	2.25	0.000
BCAT1	ILMN_1766169	NM_005504	8.88	6.64	2.24	0.001
SLC22A18	ILMN_1724902	NM_183233	5.78	3.54	2.24	0.000
SOD2	ILMN_1792922	NM_000636	5.99	3.77	2.22	0.000
MDK	ILMN_2349393	NM_001012334	10.73	8.52	2.21	0.000
DDIT4L	ILMN_1696537	NM_145244	7.95	5.74	2.21	0.000
HLA-DRB1	ILMN_1815895	HM067851	6.61	4.40	2.21	0.000
PDLIM3	ILMN_1695299	NM_014476	6.50	4.30	2.20	0.000
DDR2	ILMN_2410523	NM_006182	5.99	3.80	2.19	0.000
KCNG1	ILMN_1673769	NM_002237	9.62	7.43	2.19	0.000
ST8SIA1	ILMN_1664859	NM_003034	5.97	3.78	2.18	0.000
	ILMN_1862684	0	6.51	4.35	2.16	0.000
JAM2	ILMN_1672350	NM_021219	6.75	4.61	2.14	0.000
NAMPT	ILMN_1653871	NM_005746	10.78	8.67	2.11	0.000
C13orf15	ILMN_1658494	NM_014059	9.97	7.92	2.05	0.001
VWA5A	ILMN_1764769	NM_014622	7.68	5.63	2.05	0.001
FLJ42351	ILMN_1885771	AK124342	6.32	4.30	2.02	0.000
KCNAB1	ILMN_1744968	NM_003471	6.42	4.40	2.02	0.000
CD302	ILMN_1716797	NM_014880	5.91	3.90	2.01	0.001
SIRT4	ILMN_1657868	NM_012240	6.14	4.13	2.01	0.000
HLA-B	ILMN_1778401	NM_005514	11.85	9.84	2.01	0.000
APCDD1L	ILMN_1689431	NM_153360	11.09	9.11	1.98	0.000
ARRDC3	ILMN_2198516	NM_020801	6.63	4.65	1.98	0.001
TRIB2	ILMN_1714700	NM_021643	8.51	6.64	1.88	0.000
SIRPG	ILMN_1771801	NM_001039508	5.54	3.66	1.88	0.000
PYROXD2	ILMN_1684497	NM_032709	7.90	6.03	1.87	0.000
RPS6KA2	ILMN_1702501	NM_001006932	9.50	7.63	1.87	0.000
CA11	ILMN_1743219	NM_001217	6.60	4.76	1.84	0.000
MAFB	ILMN_1764709	NM_005461	9.05	7.25	1.80	0.001
TTLL3	ILMN_1682818	NM_001025930	6.74	4.94	1.80	0.001
SLC2A3	ILMN_1775708	M20681	10.89	9.11	1.78	0.000
THBS4	ILMN_1736078	NM_003248	5.41	3.68	1.74	0.000
	ILMN_1773819	NG_021318	6.35	4.64	1.71	0.000
EGR2	ILMN_1743199	J04076	7.16	5.45	1.71	0.001
STEAP2	ILMN_2344298	NM_152999	7.36	5.66	1.70	0.001



H6PD	ILMN_1721136	NM_004285	6.94	5.28	1.66	0.000
	ILMN_2186806	0	10.93	9.28	1.65	0.001
MAN1A1	ILMN_1742187	NM_005907	5.80	4.17	1.63	0.000
CHGB	ILMN_1765966	NM_001819	5.33	3.70	1.63	0.000
KCNMB4	ILMN_1727466	NM_014505	7.46	5.84	1.62	0.000
PRRX1	ILMN_1811851	NM_006902	5.68	4.09	1.59	0.000
REPS2	ILMN_1656934	NM_004726	7.97	6.38	1.59	0.000
ATHL1	ILMN_1794707	NM_025092	6.43	4.85	1.58	0.000
ISG20	ILMN_1659913	NM_002201	10.51	8.95	1.56	0.000
DBP	ILMN_1715555	NM_001352	7.42	5.86	1.56	0.000
C3orf17	ILMN_2331658	NM_015412	6.43	4.89	1.54	0.001
VEGFA	ILMN_1803882	NM_001025367	8.95	7.41	1.54	0.001
ZFP36	ILMN_1720829	NM_003407	11.00	9.47	1.53	0.001
	ILMN_2130441	M32106	12.35	10.82	1.53	0.000
TMTC1	ILMN_1811426	NM_175861	6.17	4.69	1.48	0.001
ABCC3	ILMN_1677814	AF085692	8.81	7.34	1.47	0.000
WIPI1	ILMN_1781386	NM_017983	9.48	8.01	1.47	0.000
SERPINE2	ILMN_1655595	NM_006216	14.63	13.16	1.47	0.000
CRAT	ILMN_1737992	NM_000755	6.64	5.17	1.47	0.001
MIER2	ILMN_1694878	AB033019	7.33	5.88	1.45	0.001
	ILMN_1699058	0	7.67	6.22	1.45	0.000
FAM196A	ILMN_2071682	NM_001039762	5.04	3.63	1.41	0.000
TMEM159	ILMN_1655876	NM_020422	8.50	7.14	1.36	0.001
OSBPL5	ILMN_1802151	NM_020896	7.75	6.39	1.36	0.001
TPP1	ILMN_1729234	NM_000391	11.00	9.67	1.33	0.001
CEBPD	ILMN_1782050	NM_005195	11.64	10.32	1.32	0.001
TEF	ILMN_1706511	NM_003216	6.07	4.76	1.31	0.001
IZUMO4	ILMN_2400546	NM_001039846	4.90	3.59	1.31	0.000
	ILMN_1670410	NR_028326	5.99	4.69	1.30	0.000
RGS17	ILMN_1725485	NM_012419	9.05	7.77	1.28	0.001
LETMD1	ILMN_2264681	NM_015416	8.34	7.06	1.28	0.000
FOXO4	ILMN_1712095	NM_005938	8.51	7.28	1.23	0.001
MTHFR	ILMN_1734830	NM_005957	5.79	4.58	1.21	0.001
SPRY2	ILMN_2089329	NM_005842	10.87	9.66	1.21	0.000
TRIB1	ILMN_1803811	NM_025195	10.71	9.54	1.17	0.000
FAM108C1	ILMN_1788416	NM_021214	10.94	9.77	1.17	0.001
PTPRA	ILMN_1695461	NM_080840	5.52	4.36	1.16	0.001
TSC22D1	ILMN_1692177	NM_006022	12.02	10.89	1.13	0.000
DMRTB1	ILMN_1754187	NM_033067	4.40	3.30	1.10	0.001
HLA-A	ILMN_1671054	NM_002116	11.54	10.44	1.10	0.001
SLC27A1	ILMN_1787718	NM_198580	8.21	7.12	1.09	0.001
KLF9	ILMN_1778523	NM_001206	10.70	9.61	1.09	0.001
	ILMN_1761478	AK130343	4.31	3.22	1.09	0.000
FMNL2	ILMN_1730491	NM_052905	8.21	7.13	1.08	0.001
BCL6	ILMN_1737314	NM_001706	10.36	9.28	1.08	0.000
AMY2A	ILMN_1801303	NM_000699	4.87	3.83	1.04	0.000
TRAF5	ILMN_1740493	NM_004619	5.14	4.11	1.03	0.001
GALNT10	ILMN_1662556	NM_198321	5.23	6.25	-1.02	0.000
CHTF18	ILMN_1756705	NM_022092	5.97	7.00	-1.02	0.001
MT1A	ILMN_1691156	NM_005946	13.56	14.61	-1.05	0.000

DEM1	ILMN_1783996	NM_022774	6.16	7.23	-1.07	0.000
	ILMN_1761281	0	11.04	12.18	-1.14	0.000
LAMC2	ILMN_1653824	NM_005562	10.33	11.51	-1.18	0.000
NHLRC3	ILMN_1698365	NM_001012754	3.93	5.12	-1.19	0.001
CDC45	ILMN_1670238	NM_003504	9.37	10.60	-1.23	0.000
ZBTB8A	ILMN_2264205	NM_001040441	4.46	5.69	-1.23	0.000
MOSPD1	ILMN_1727798	NM_019556	4.56	5.81	-1.25	0.000
FGF5	ILMN_2399100	NM_033143	4.44	5.72	-1.28	0.000
TMEM27	ILMN_1772894	NM_020665	4.50	5.78	-1.28	0.000
BLCAP	ILMN_1675612	NM_006698	9.36	10.68	-1.32	0.000
HMMR	ILMN_1781942	NM_012485	9.55	10.89	-1.33	0.000
LAMC2	ILMN_1706519	NM_018891	6.76	8.10	-1.34	0.000
GCLM	ILMN_2225974	NM_002061	5.81	7.15	-1.34	0.001
BUB3	ILMN_2386100	NM_001007793	8.62	9.96	-1.34	0.000
DOCK5	ILMN_1752455	NM_024940	6.43	7.78	-1.35	0.000
BLM	ILMN_1709484	NM_000057	4.95	6.31	-1.36	0.001
DTL	ILMN_1779711	NM_016448	6.20	7.55	-1.36	0.000
BIRC5	ILMN_1803124	NM_001012271	5.72	7.10	-1.39	0.000
FAM41C	ILMN_1728059	BC047940	7.45	8.85	-1.40	0.000
HMMR	ILMN_2409220	NM_012484	8.79	10.19	-1.40	0.000
DNAJC19	ILMN_1742109	NM_145261	5.22	6.64	-1.42	0.000
ARHGAP11A	ILMN_1744830	NM_014783	4.01	5.47	-1.45	0.000
DST	ILMN_1675992	NM_001723	5.33	6.84	-1.51	0.000
NUP62CL	ILMN_1756550	NM_017681	5.37	6.88	-1.51	0.000
CENPM	ILMN_2368721	NM_001002876	6.67	8.22	-1.55	0.001
EDEM2	ILMN_1662640	BC133653	8.38	9.94	-1.57	0.000
MT1IP	ILMN_2136089	AF348997	9.52	11.13	-1.61	0.000
SLC29A1	ILMN_1723971	NM_001078174	6.24	7.93	-1.69	0.000
FEZ1	ILMN_1794106	NM_022549	6.82	8.53	-1.71	0.000
P2RY2	ILMN_2372915	NM_002564	5.57	7.30	-1.73	0.000
MT1G	ILMN_1715401	NM_005950	7.88	9.63	-1.75	0.000
SERPINB7	ILMN_2395139	NM_003784	7.41	9.21	-1.80	0.000
SERPINB7	ILMN_1767685	NM_003784	8.59	10.41	-1.82	0.000
MCM10	ILMN_2413899	NM_018518	4.61	6.44	-1.83	0.000
FAIM	ILMN_2351548	NM_001033032	3.55	5.40	-1.85	0.001
KRT7	ILMN_2163723	NM_005556	10.66	12.54	-1.88	0.000
MT1E	ILMN_2173611	NM_175617	11.31	13.20	-1.89	0.000
TCF7	ILMN_2367141	NM_201632	4.33	6.30	-1.97	0.000
FERMT1	ILMN_1696585	NM_017671	6.99	9.07	-2.08	0.000
CLN6	ILMN_1776267	NM_017882	3.63	5.75	-2.12	0.000
SLC29A3	ILMN_1717326	AY358686	3.85	6.02	-2.17	0.000
TTLL11	ILMN_1784356	NM_001139442	5.40	7.57	-2.17	0.000
NXN	ILMN_1791226	NM_022463	7.92	10.14	-2.22	0.000
CADM1	ILMN_1680132	NM_014333	5.85	8.18	-2.33	0.000
DSC3	ILMN_1765363	NM_024423	6.20	8.60	-2.40	0.000
CDH3	ILMN_1704294	NM_001793	6.61	9.02	-2.41	0.000
LOC730755	ILMN_1660067	NM_001165252	5.61	8.04	-2.43	0.000
SERPINB5	ILMN_1793888	U04313	8.92	11.41	-2.49	0.000
JAG2	ILMN_1764729	NM_002226	6.10	8.81	-2.71	0.000
GJB3	ILMN_2334531	NM_001005752	3.21	6.00	-2.79	0.000

JAG2	ILMN_2399523	NM_145159	4.47	7.27	-2.80	0.000
ANXA8L1	ILMN_1799098	NM_001098845	7.68	10.54	-2.86	0.000
PLXNA2	ILMN_1844408	NM_025179	4.90	7.79	-2.89	0.000
ANXA8L2	ILMN_1778087	BC008813	8.78	11.67	-2.89	0.000
SCARA3	ILMN_1723358	NM_016240	5.03	7.96	-2.93	0.000
S100A2	ILMN_1725852	NM_005978	8.43	11.42	-2.99	0.000
ANXA8	ILMN_2095610	NM_001040084	6.95	10.12	-3.17	0.000
TP63	ILMN_2138801	NM_003722	5.82	9.06	-3.24	0.000
ANXA8L2	ILMN_2186983	NM_001630	7.13	10.40	-3.27	0.000
RNASE7	ILMN_1712849	AY170392	3.65	6.98	-3.33	0.000
KBTBD11	ILMN_1784630	NM_014867	3.25	6.65	-3.40	0.001
KRT81	ILMN_1801442	NM_002281	4.13	7.63	-3.50	0.000
LOC728449	ILMN_1661628	XM_001128973	5.67	9.36	-3.69	0.000
ZBED2	ILMN_1651365	NM_024508	4.07	8.21	-4.14	0.000

<sup>a</sup>Mean expression across tumorigenic (Clones 1, 5, and 11) and non-tumorigenic (Clones 6, 7, 8, and 9) soft agar clones

<sup>b</sup>Log<sub>2</sub> transformed

**Supplementary Figure S1:** **A)** Anchorage-dependent colony formation assays comparing tumorigenic clones (Clone1, Clone5, and Clone11) and non-tumorigenic clones (Clone6, Clone7, Clone8 and Clone9). **B)** Phase contrast photomicrographs of HBEC3 infected with LacZ control vector (*left*), high level KRAS<sup>V12</sup> (*middle*) lentivirus, or moderate level KRAS<sup>V12</sup> (*right*) retrovirus showing high level KRAS<sup>V12</sup>-induced morphologic changes: enlarged, flattened cells with multiple vacuoles, suggestive of senescence (arrows) (20X magnification). **C)** Senescence-associated  $\beta$ -galactosidase (SA- $\beta$ -gal) staining of confluent cultures for HBEC3 with wildtype p53 (*left*) or p53 knockdown (*right*) infected with high level KRAS<sup>V12</sup> lentivirus showing the flattened, vacuole-rich cells are positive for SA- $\beta$ -gal staining (arrows) (10X magnification). **D-E)** Anchorage-dependent colony formation assay to compare acute KRAS<sup>V12</sup>-induced toxicity in HBEC3 (**D**) and HBEC4 (**E**) with wildtype p53 or p53 knockdown. **F)** Anchorage-dependent colony formation assay to compare acute KRAS<sup>C12</sup>- and KRAS<sup>D12</sup>-induced toxicity in HBEC3 with wildtype p53 or p53 knockdown. **G)** Phase contrast images of HBEC3 taken two weeks after transduction with either pLenti6-KRAS<sup>WT</sup>, -KRAS<sup>C12</sup>, or -KRAS<sup>D12</sup> lentivirus and blasticidin selection. **H)** Cell cycle analysis of HBEC3 with wildtype p53 or p53 knockdown harvested three days after infection with either KRAS<sup>V12</sup> or LacZ lentivirus.

**Supplementary Figure S2:** **A)** Representative adenocarcinoma histology from formalin-fixed paraffin-embedded sections of subcutaneous xenografts of oncogenically manipulated HBECs at lower (*upper panel*) and higher (*lower panel*) magnification stained with adenocarcinoma (Napsin A and cytokeratin 7 (CK7)) and squamous cell (CK5) markers. **B)** Transformation of HBEC17 with sh-p53, KRAS<sup>V12</sup>, and c-MYC as measured by anchorage-independent growth in soft agar. \*\*P < 0.01 (*t*-test).

**Supplementary Figure S3:** **A)** Cell proliferation assay of HBEC3 and HBEC3<sup>p53,KRAS</sup> in KSFM (with EGF/BPE) or RPMI1640 with or without FBS. **B)** qRT-PCR analysis of differentiation and CSC markers in HBEC3, HBEC3<sup>p53,KRAS</sup> and HBEC3<sup>p53,KRAS,MYC</sup>. **C)** qRT-PCR analysis of differentiation and CSC markers in HBEC3, HBEC3<sup>p53,KRAS</sup> and HBEC3<sup>p53,KRAS,MYC</sup> following growth in KSFM or KSFM+5%FBS for 96 hr. **D)** Phase contrast images of HBEC3, HBEC3<sup>p53,KRAS</sup> and HBEC3<sup>p53,KRAS,MYC</sup> following growth in KSFM or KSFM+5%FBS for 96 hr. **E)** TUNEL staining of subcutaneous tumor xenografts derived from HBEC3<sup>p53,KRAS</sup> and HBEC3<sup>p53,KRAS,MYC</sup> showing the presence of nicks in the DNA identified by terminal deoxynucleotidyl transferase (TdT) labeled with FITC. Nuclei stained with DAPI. GCC, giant cell carcinoma; LCC, large cell carcinoma; SCC, squamous cell carcinoma; AC, adenocarcinoma; P diff, poorly differentiated; M-W diff, moderate to well differentiated.

**Supplementary Figure S4:** **A)** Southern blotting for parental population and clones of HBEC3<sup>p53,KRAS</sup> and HBEC3<sup>p53,KRAS,MYC</sup> derived from very large soft agar colonies. DNA was probed with a biotin-labeled probe amplified from the *blasticidin* mammalian resistance gene present in the pLenti-KRAS<sup>V12</sup> vector. Parental, uncloned population grown in R10; MM, Molecular Marker; Clone5\*, cell line re-established from a tumor produced by Clone 5. **B)** Anchorage-independent growth of isogenic derivatives of HBEC3 in soft agar assays in KSFM with and without 20% FBS.

**Supplementary Figure S5: Representative dose-response curves of HBEC3 and HBEC3<sup>p53,KRAS</sup> treated with standard lung cancer chemotherapy doublets.**

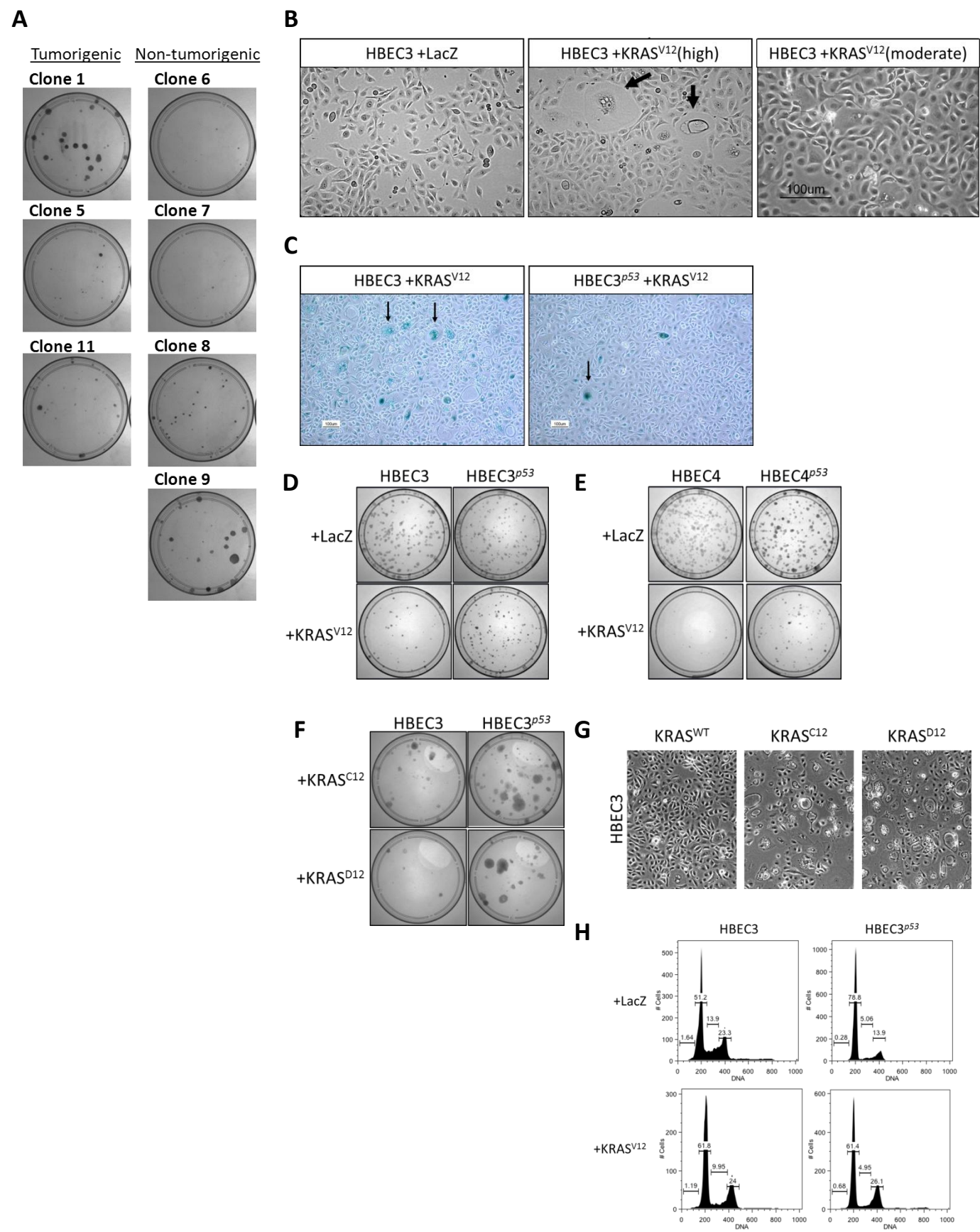


**Supplementary Figure S6: Cell cycle analysis of soft agar clones of HBEC3<sup>p53, KRAS</sup>.** Cell cycle analysis comparing tumorigenic clones (Clone1, Clone5, and Clone11) and non-tumorigenic clones (Clone6, Clone7, Clone8 and Clone9).

**Supplementary Figure S7: Microarray analysis of 203-probe mRNA gene signature differentially expressed between tumorigenic and non-tumorigenic soft agar clones of HBE3<sup>p53, KRAS</sup> against clinical variables. A-B)** Kaplan-Meier log-rank analysis of relapse-free (RF) survival in lung cancer patients predicted to have good or poor outcome using the 203 probe signature identified in the supervised analysis. A supervised principal component analysis was used to train the model in one dataset (SPORE) and test in a second dataset (Consortium) (A) then the datasets were reversed to test for model robustness (B).

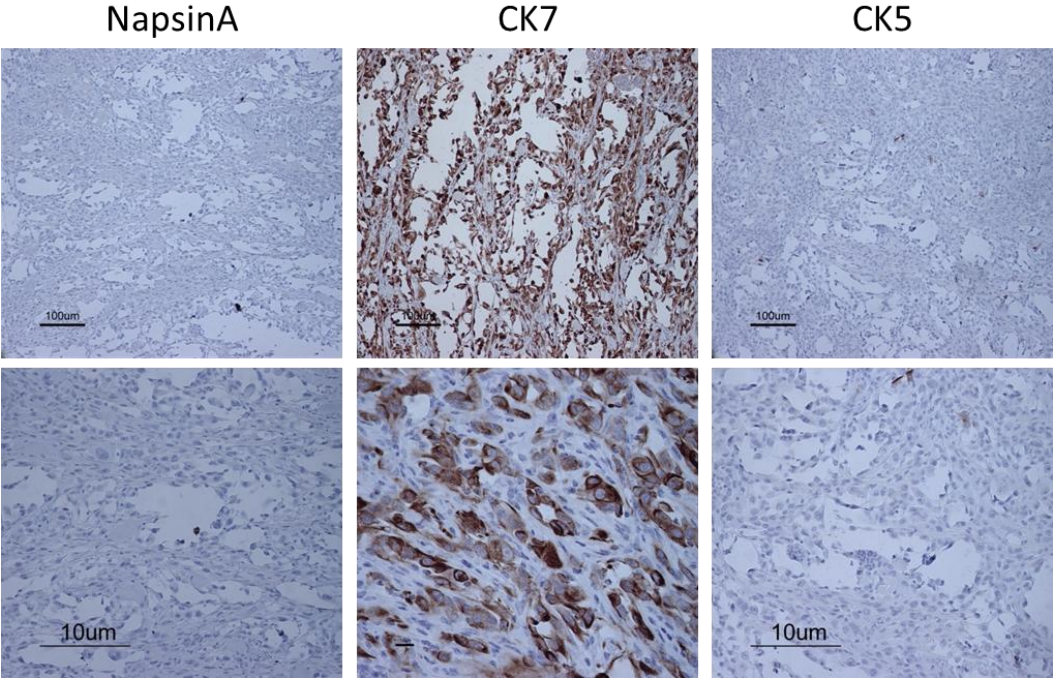
**Supplementary Figure S8: Full, uncut blots of immunoblots presented in main article.**

Supplementary Figure S1

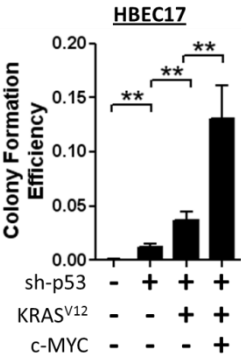


Supplementary Figure S2

A

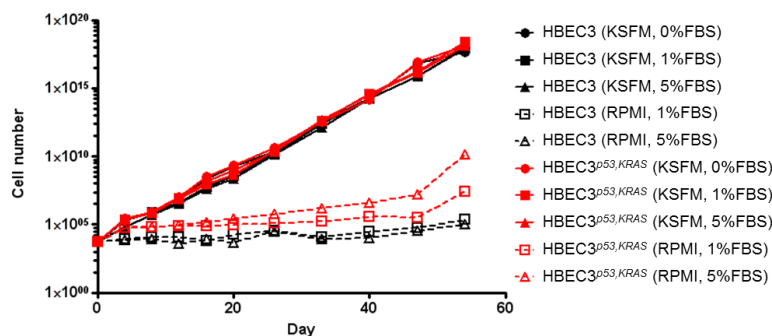


B

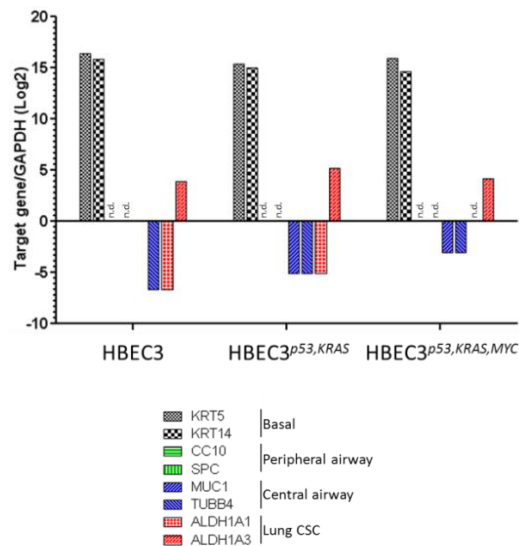


Supplementary Figure S3

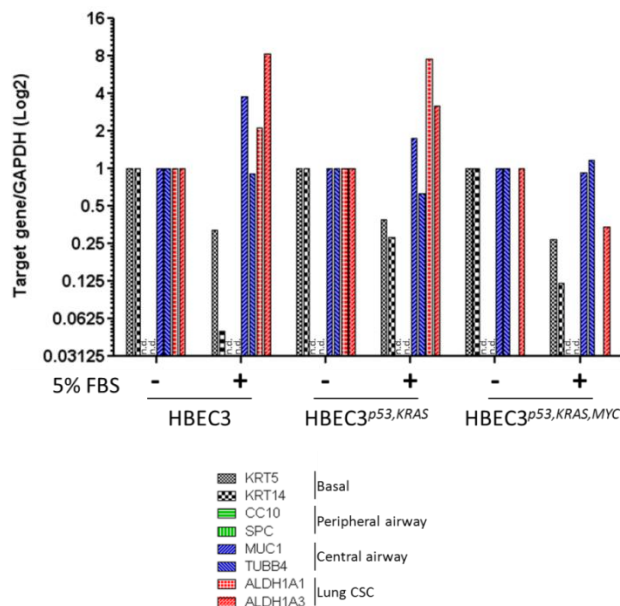
A



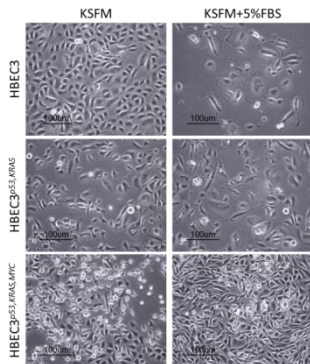
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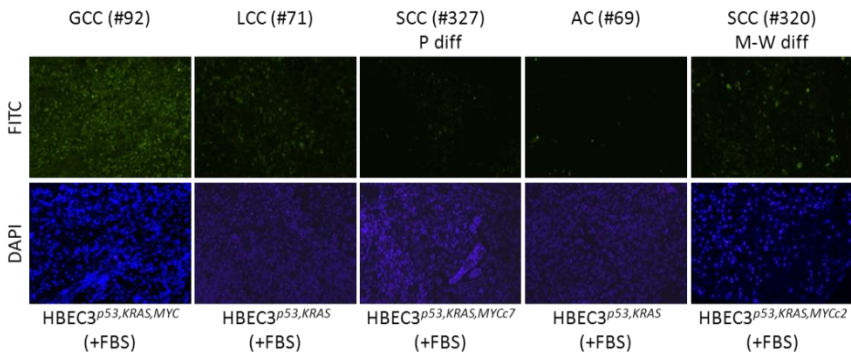
C



D

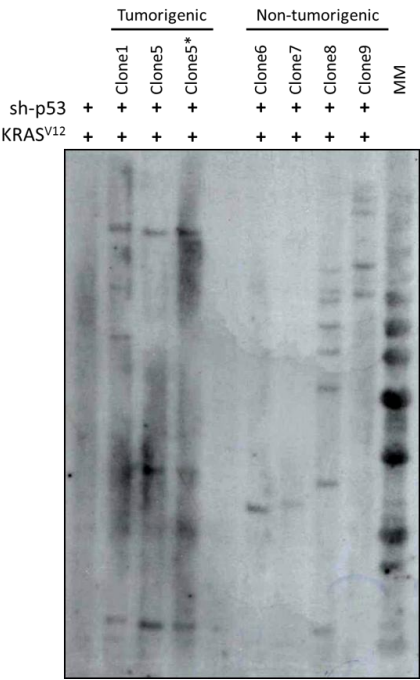


E

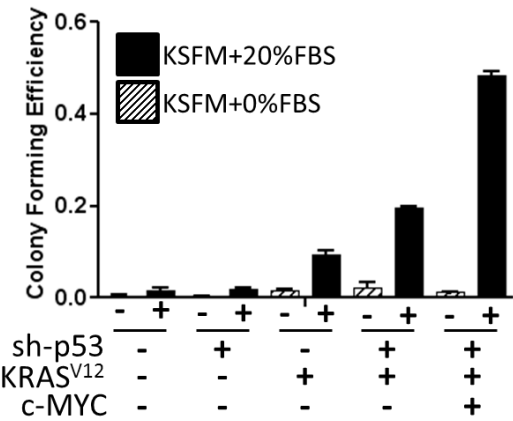


Supplementary Figure S4

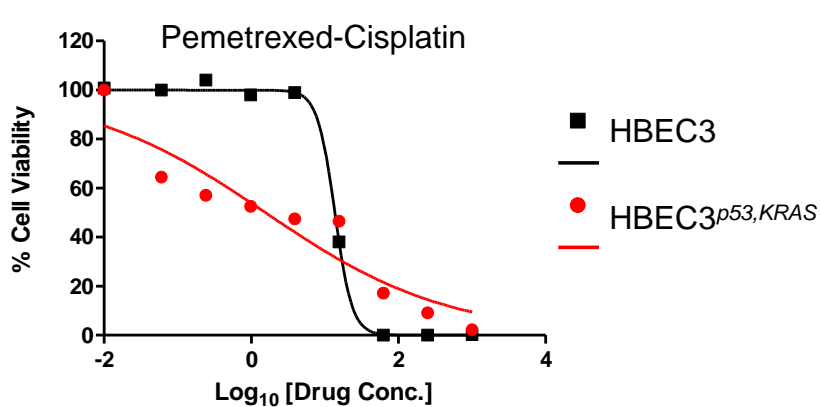
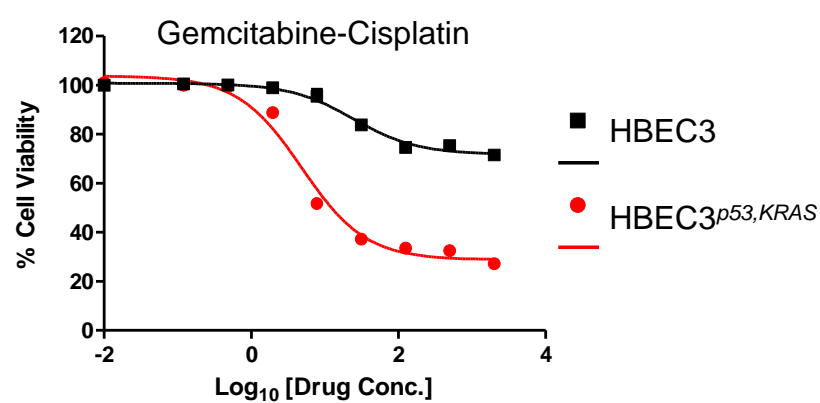
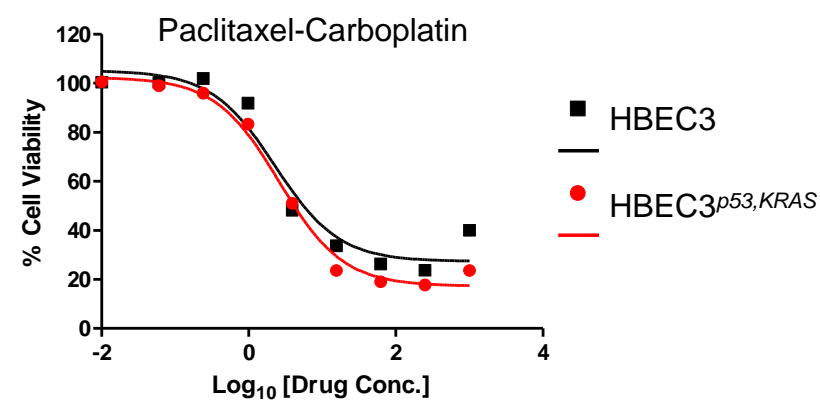
A



B



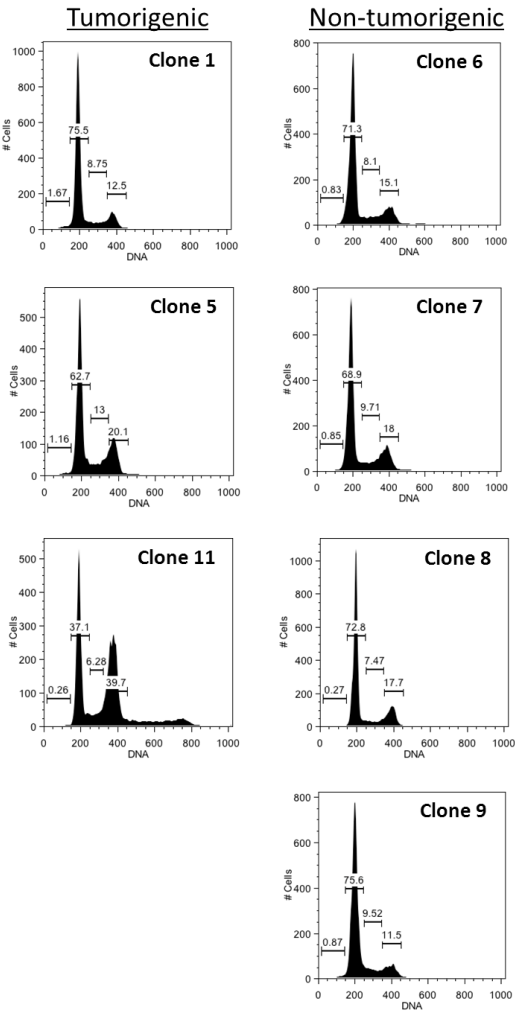
Supplementary Figure S5





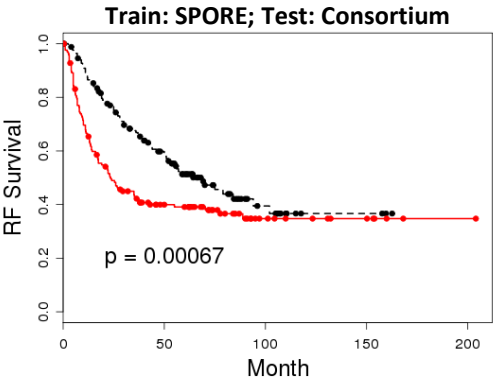
# Supplementary Figure S6

**Basal cell cycle profile of soft agar clones  
of HBEC3<sup>p53, KRAS</sup>**

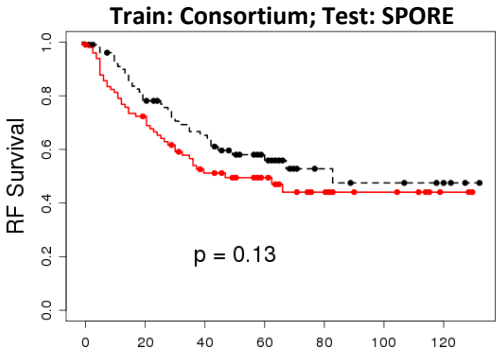


# Supplementary Figure S7

**A**

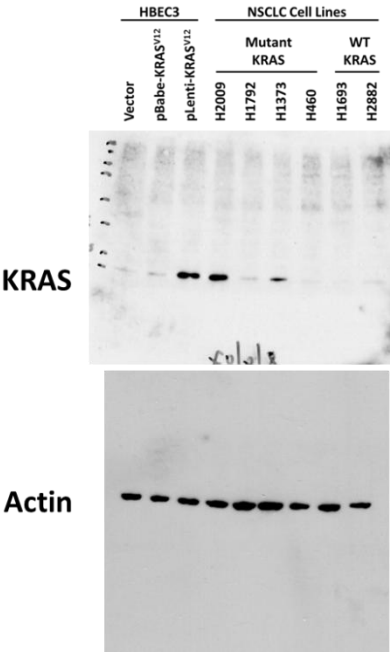


**B**

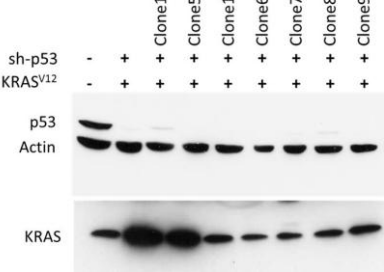


Supplementary Figure S8

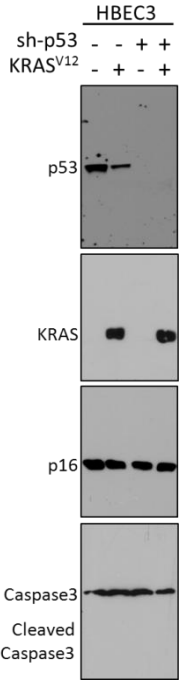
Full-length blot of Figure 1A



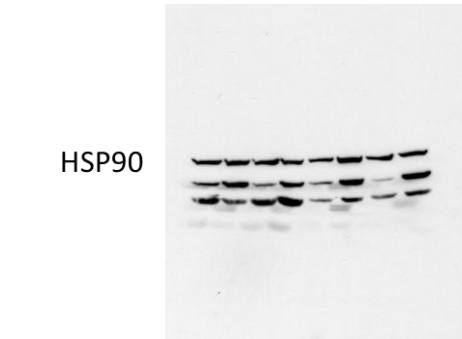
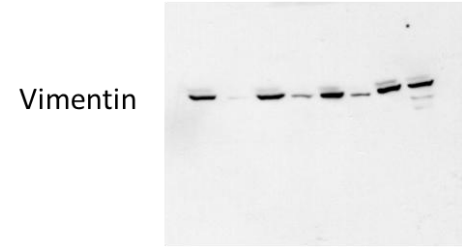
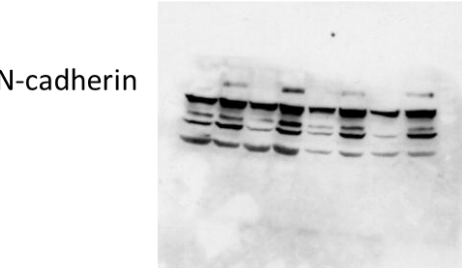
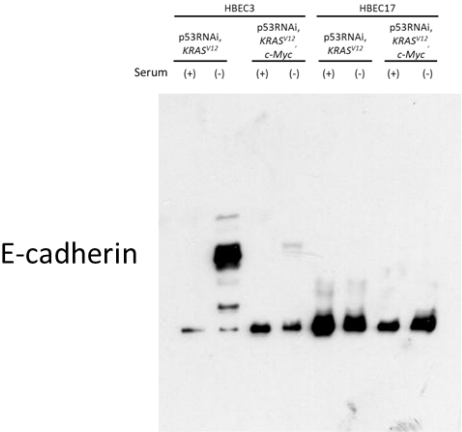
Full-length blot of Figure 1C



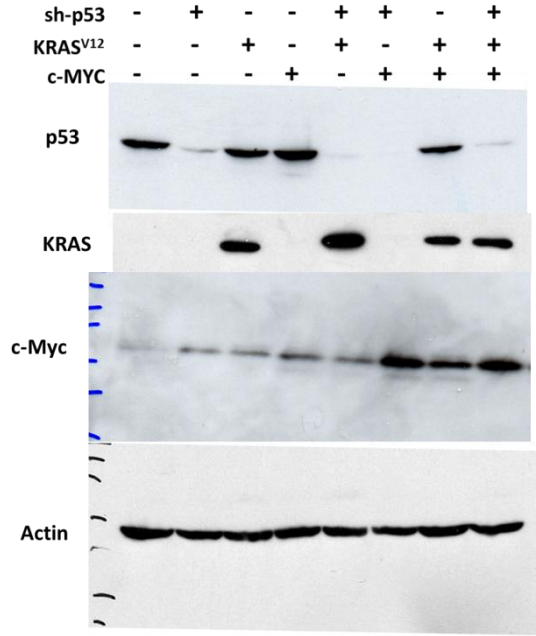
Full-length blot of Figure 1G



Full-length blot of Figure 4B



Full-length blot of Figure 2A



Full-length blot of Figure 5B

