Figure S1. Protein-protein interaction predicted by STRING of confirmed differentially expressed genes, KRAS and AKT.

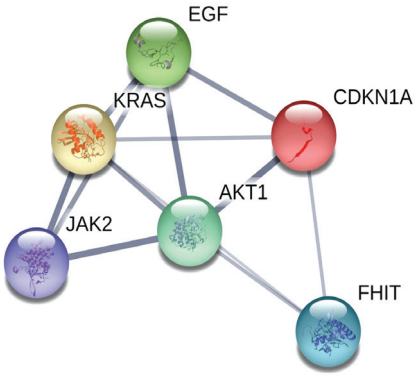

Figure S2. CDKN1A, ERCC1 and TS expression analysis by western blot analysis. Protein expression was evaluated in the whole lysates of untreated and treated RAL cells and in the MRC-5 (RRID: CVCL_0440) normal lung fibroblast cell line. MRC-5 cells were cultured in Eagle's minimum essential medium (cat. no. 30-2003; ATCC), supplemented with 10% FBS, according to ATCC product sheet. post wo, post-treatment washout; TS, thymidylate synthase; ERCC1, DNA excision repair cross-complementation group 1.

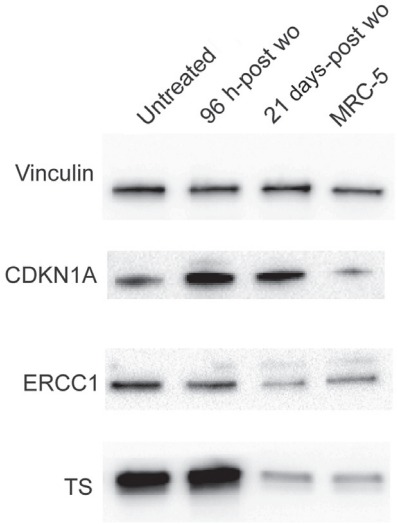

Figure S3. Time course of *CDKN1A* silencing in RAL cells. Gene expression analysis of *CDKN1A* gene in control cells (control) and cells transfected with scramble RNA (scr), as silencing negative control, or *CDKN1A* siRNA, at 24-48-120-144 h post-transfection. (A) *CDKN1A* mRNA level quantified by qPCR. Mean values and SEM of two independent experiments are reported. (B) Representative western blots. Vinculin was used as a loading control.

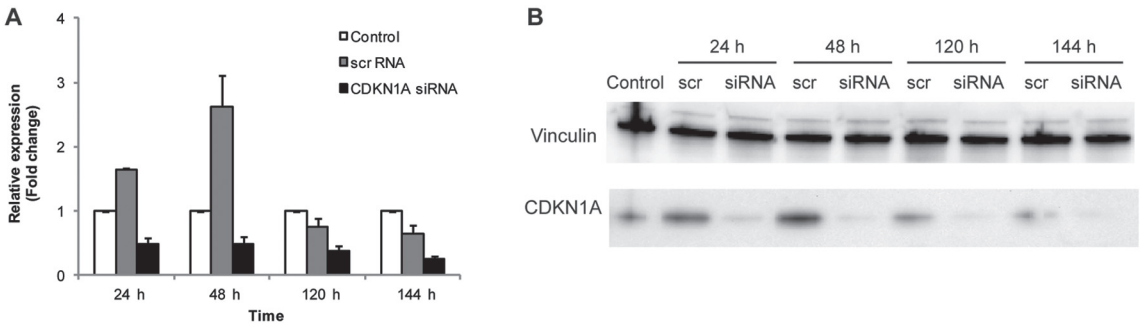

Figure S4. Representative images of the apoptosis analysis by flow cytometry. Plots for TUNEL and Annexin V assays, as well as for cell cycle analysis are presented for the scramble control and *CDKN1A*-silenced cells, either in the untreated and 96 h post-wo conditions. post wo, post-treatment washout.

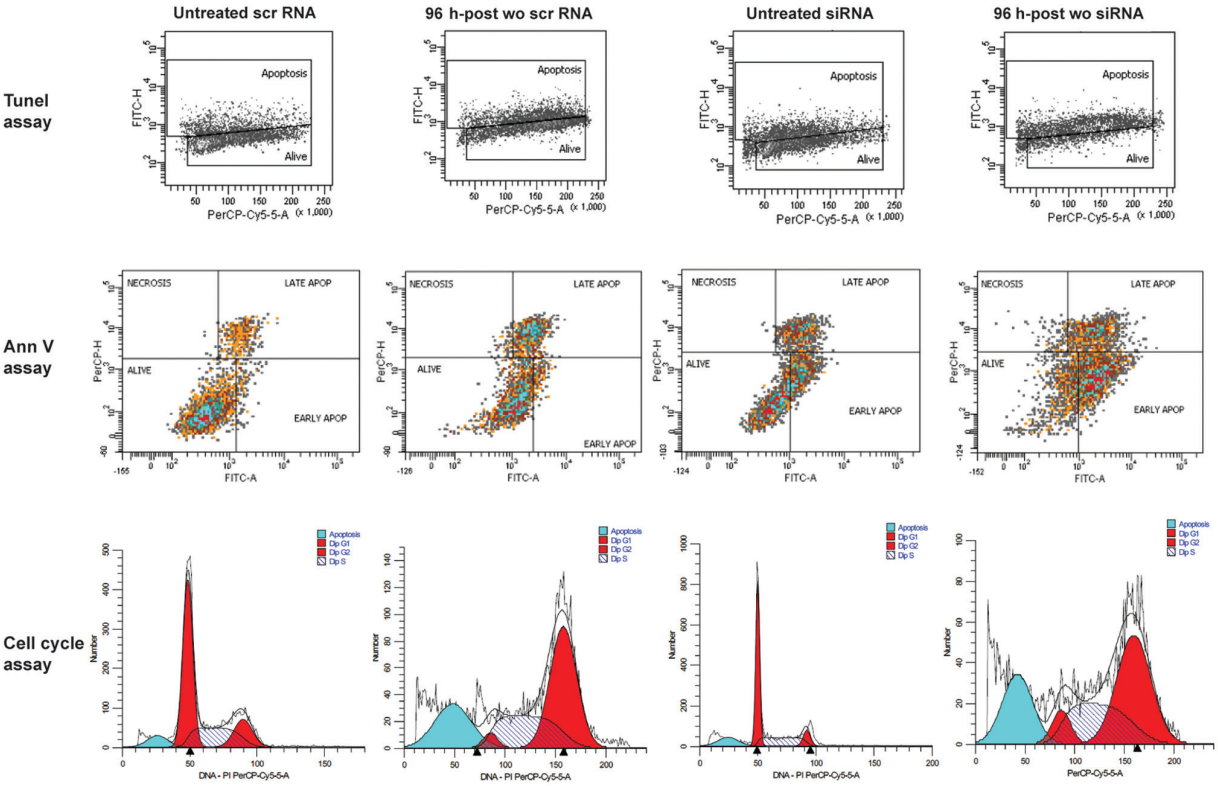

Table SI. Complete list of the human oncogenes and tumor suppressor genes in the RT2 Profiler™ PCR array.

	Gene symbol	UniGene ID	GeneBank ID	Name	Description
1	*ABL1*	Hs.431048	NM_005157	C-abl oncogene 1, non-receptor tyrosine kinase	Transcription factor
2	*AKT1*	Hs.525622	NM_005163	V-akt murine thymoma viral oncogene homolog 1	Angiogenesis
3	*APC*	Hs.158932	NM_000038	Adenomatous polyposis coli	Cell adhesion
4	*ATM*	Hs.367437	NM_000051	Ataxia telangiectasia mutated	Tumor suppressor gene, cell cycle
5	*BAX*	Hs.624291	NM_004324	BCL2-associated X protein	Oncogene, apoptosis
6	*BCL2*	Hs.150749	NM_000633	B-cell CLL/lymphoma 2	Apoptosis
7	*BCL2L1*	Hs.516966	NM_138578	BCL2-like 1	Oncogene, apoptosis
8	*BCR*	Hs.517461	NM_004327	Breakpoint cluster region	Both oncogenic and tumor suppressor properties
9	*BRCA1*	Hs.194143	NM_007294	Breast cancer 1, early onset	Tumor suppressor gene, transcription factor
10	*BRCA2*	Hs.34012	NM_000059	Breast cancer 2, early onset	Tumor suppressor gene, transcription factor, epithelial-to-mesenchymal transition
11	*CASP8*	Hs.599762	NM_001228	Caspase 8, apoptosis-related cysteine peptidase	Oncogene
12	*CCND1*	Hs.523852	NM_053056	Cyclin D1	Cell cycle
13	*CDH1*	Hs.461086	NM_004360	Cadherin 1, type 1, E-cadherin (epithelial)	Tumor suppressor gene, cell adhesion
14	*CDK4*	Hs.95577	NM_000075	Cyclin-dependent kinase 4	Oncogene, cell cycle
15	*CDKN1A*	Hs.370771	NM_000389	Cyclin-dependent kinase inhibitor 1A (p21, Cip1)	Cell cycle
16	*CDKN2A*	Hs.512599	NM_000077	Cyclin-dependent kinase inhibitor 2A (melanoma, p16, inhibits CDK4)	Transcription factor, cell adhesion, cell cycle
17	*CDKN2B*	Hs.72901	NM_004936	Cyclin-dependent kinase inhibitor 2B (p15, inhibits CDK4)	Tumor suppressor gene, epithelial-to-mesenchymal transition, cell cycle
18	*CDKN3*	Hs.84113	NM_005192	Cyclin-dependent kinase inhibitor 3	Tumor suppressor gene
19	*CTNNB1*	Hs.712929	NM_001904	Catenin (cadherin-associated protein), beta 1, 88 kDa	Transcription factor, epithelial-to-mesenchymal transition, cell adhesion
20	*E2F1*	Hs.654393	NM_005225	E2F transcription factor 1	Tumor suppressor gene, transcription factor
21	*EGF*	Hs.419815	NM_001963	Epidermal growth factor	Both oncogenic and tumor suppressor properties, angiogenesis
22	*ELK1*	Hs.181128	NM_005229	ELK1, member of ETS oncogene family	Oncogene, transcription factor
23	*ERBB2*	Hs.446352	NM_004448	V-erb-b2 erythroblastic leukemia viral oncogene homolog 2, neuro/glioblastoma derived oncogene homolog (avian)	Both oncogenic and tumor suppressor properties, epithelial-to-mesenchymal transition, angiogenesis
24	*ESR1*	Hs.744830	NM_000125	Estrogen receptor 1	Both oncogenic and tumor suppressor properties, transcription factor
25	*ETS1*	Hs.369438	NM_005238	V-ets erythroblastosis virus E26 oncogene homolog 1 (avian)	Oncogene, transcription factor
26	*FHIT*	Hs.655995	NM_002012	Fragile histidine triad gene	Tumor suppressor gene
27	*FOS*	Hs.25647	NM_005252	FBJ murine osteosarcoma viral oncogene homolog	Both oncogenic and tumor suppressor properties, transcription factor
28	*FOXD3*	Hs.546573	NM_012183	Forkhead box D3	Tumor suppressor gene, transcription factor
29	*HGF*	Hs.396530	NM_000601	Hepatocyte growth factor (hepapoietin A; scatter factor)	Oncogene, cell cycle

Table SI. Continued.

	Gene symbol	UniGene ID	GeneBank ID	Name	Description
30	*HIC1*	Hs.72956	NM_006497	Hypermethylated in cancer 1	Tumor suppressor gene, transcription factor
31	*HRAS*	Hs.37003	NM_005343	V-Ha-ras Harvey rat sarcoma viral oncogene homolog	Both oncogenic and tumor suppressor properties
32	*IGF2R*	Hs.487062	NM_000876	Insulin-like growth factor 2 receptor	Tumor suppressor gene
33	*JAK2*	Hs.656213	NM_004972	Janus kinase 2	Oncogene, epithelial-to-mesenchymal transition
34	*JUN*	Hs.696684	NM_002228	Jun proto-oncogene	Both oncogenic and tumor suppressor properties, transcription factor
35	*JUNB*	Hs.25292	NM_002229	Jun B proto-oncogene	Oncogene, transcription factor
36	*JUND*	Hs.2780	NM_005354	Jun D proto-oncogene	Oncogene, transcription factor
37	*KIT*	Hs.479754	NM_000222	V-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog	Oncogene, epithelial-to-mesenchymal transition
38	*KITLG*	Hs.661108	NM_003994	KIT ligand	Oncogene, cell adhesion
39	*KRAS*	Hs.505033	NM_004985	V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog	Both oncogenic and tumor suppressor properties
40	*MCL1*	Hs.632486	NM_021960	Myeloid cell leukemia sequence 1 (BCL2-related)	Oncogene, apoptosis
41	*MDM2*	Hs.484551	NM_002392	Mdm2 p53 binding protein homolog (mouse)	Both oncogenic and tumor suppressor properties, transcription factor
42	*MEN1*	Hs.423348	NM_000244	Multiple endocrine neoplasia I	Tumor suppressor gene, transcription factor, cell cycle
43	*MET*	Hs.132966	NM_000245	Met proto-oncogene (hepatocyte growth factor receptor)	Oncogene
44	*MGMT*	Hs.501522	NM_002412	O-6-methylguanine-DNA methyltransferase	Tumor suppressor gene, apoptosis
45	*MLH1*	Hs.195364	NM_000249	MutL homolog 1, colon cancer, nonpolyposis type 2 (E. coli)	Tumor suppressor gene
46	*MOS*	Hs.533432	NM_005372	V-mos Moloney murine sarcoma viral oncogene homolog	Oncogene
47	*MYB*	Hs.626299	NM_005375	V-myb myeloblastosis viral oncogene homolog (avian)	Oncogene, Transcription factor
48	*MYC*	Hs.202453	NM_002467	V-myc myelocytomatosis viral oncogene homolog (avian)	Both oncogenic and tumor suppressor properties, transcription factor
49	*MYCN*	Hs.25960	NM_005378	V-myc myelocytomatosis viral related oncogene, neuroblastoma derived (avian)	Both oncogenic and tumor suppressor properties, transcription factor
50	*NF1*	Hs.113577	NM_000267	Neurofibromin 1	Tumor suppressor genes, transcription factor, epithelial-to-mesenchymal transition, angiogenesis, cell adhesion
51	*NF2*	Hs.187898	NM_000268	Neurofibromin 2 (merlin)	Tumor suppressor genes, cell adhesion
52	*NFKB1*	Hs.618430	NM_003998	Nuclear factor of kappa light polypeptide gene enhancer in B-cells 1	Both oncogenic and tumor suppressor properties, transcription factor
53	*NFKBIA*	Hs.81328	NM_020529	Nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha	Oncogene
54	*NRAS*	Hs.486502	NM_002524	Neuroblastoma RAS viral (v-ras) oncogene homolog	Oncogene
55	*PIK3C2A*	Hs.736397	NM_002645	Phosphoinositide-3-kinase, class 2, alpha polypeptide	Both oncogenic and tumor suppressor properties
56	*PIK3CA*	Hs.553498	NM_006218	Phosphoinositide-3-kinase, catalytic, alpha polypeptide	Oncogene
57	*PML*	Hs.654583	NM_033238	Promyelocytic leukemia	Oncogene, transcription factor, angiogenesis

Table SI. Continued.

	Gene symbol	UniGene ID	GeneBank ID	Name	Description
58	*PRKCA*	Hs.531704	NM_002737	Protein kinase C, alpha	Oncogene
59	*RAF1*	Hs.159130	NM_002880	V-raf-1 murine leukemia viral oncogene homolog 1	Oncogene
60	*RARA*	Hs.654583	NM_000964	Retinoic acid receptor, alpha	Oncogene, transcription factor
61	*RASSF1*	Hs.476270	NM_007182	Ras association (RalGDS/AF-6) domain family member 1	Tumor suppressor gene
62	*RB1*	Hs.408528	NM_000321	Retinoblastoma 1	Both oncogenic and tumor suppressor properties, transcription factor
63	*REL*	Hs.633256	NM_002908	V-rel reticuloendotheliosis viral oncogene homolog (avian)	Oncogene, transcription factor
64	*RET*	Hs.350321	NM_020630	Ret proto-oncogene	Both oncogenic and tumor suppressor properties
65	*ROS1*	Hs.1041	NM_002944	C-ros oncogene 1, receptor tyrosine kinase	Oncogene
66	*RUNX1*	Hs.149261	NM_001754	Runt-related transcription factor 1	Oncogene, transcription factor, angiogenesis
67	*RUNX3*	Hs.170019	NM_004350	Runt-related transcription factor 3	Tumor suppressor gene, transcription factor, epithelial-to-mesenchymal transition
68	*S100A4*	Hs.654444	NM_002961	S100 calcium binding protein A4	Tumor suppressor gene, epithelial-to-mesenchymal transition
69	*SERPINB5*	Hs.55279	NM_002639	Serpin peptidase inhibitor, clade B (ovalbumin), member 5	Tumor suppressor gene
70	*SH3PXD2A*	Hs.678727	NM_014631	SH3 and PX domains 2A	Both oncogenic and tumor suppressor properties
71	*SMAD4*	Hs.75862	NM_005359	SMAD family member 4	Tumor suppressor gene, transcription factor, epithelial-to-mesenchymal transition
72	*SRC*	Hs.195659	NM_005417	V-src sarcoma (Schmidt-Ruppin A-2) viral oncogene homolog (avian)	Oncogene
73	*STAT3*	Hs.463059	NM_003150	Signal transducer and activator of transcription 3 (acute-phase response factor)	Oncogene, transcription factor
74	*STK11*	Hs.515005	NM_000455	Serine/threonine kinase 11	Tumor suppressor gene, cell cycle
75	*TGFB1*	Hs.645227	NM_000660	Transforming growth factor, beta 1	Both oncogenic and tumor suppressor properties, transcription factor, angiogenesis
76	*TNF*	Hs.241570	NM_000594	Tumor necrosis factor	Both oncogenic and tumor suppressor properties, transcription factor, apoptosis
77	*TP53*	Hs.437460	NM_000546	Tumor protein p53	Both oncogenic and tumor suppressor properties, transcription factors, cell cycle
78	*TP73*	Hs.192132	NM_005427	Tumor protein p73	Tumor suppressor genes, transcription factor
79	*TSC1*	Hs.370854	NM_000368	Tuberous sclerosis 1	Tumor suppressor gene, transcription factor
80	*VHL*	Hs.517792	NM_000551	Von Hippel-Lindau tumor suppressor	Tumor suppressor gene, transcription factor, epithelial-to-mesenchymal transition, apoptosis
81	*WT1*	Hs.591980	NM_000378	Wilms tumor 1	Tumor suppressor gene, transcription factor
82	*WWOX*	Hs.461453	NM_016373	WW domain containing oxidoreductase	Tumor suppressor gene
83	*XRCC1*	Hs.98493	NM_006297	X-ray repair complementing defective repair in Chinese hamster cells 1	Tumor suppressor gene
84	*ZHX2*	Hs.377090	NM_014943	Zinc fingers and homeoboxes 2	Oncogene, transcription factor

Table SII. Assays list for genes validated by qPCR and primers used for bisulfite sequencing and ChIP.

A, list genes validated for qPCR

Gene symbol	RT^2 catalog
RASSF1	PPH08711A
FHIT	PPH05710F
MYC	PPH00100B
CDKN3	PPH10297A
CDKN1A	PPH00211E
RB1	PPH00228F
EGF	PPH00137B
JAK2	PPH00758C

B, list or primers used for bisulfite sequencing and ChIP

Primer name	Sequence 5'-3'
Bisulfite sequencing	
CDKN1A forward	AGGGAAGTGTTTTTTTGTAGT
CDKN1A reverse	TAACCAAAAATTCCTATACTTA
RASSF1 forward	AGTGGGTAGGTTAAGTGTGTTGT
RASSF1 reverse	AACRAAACCACAAAACRAACCC
ChIP	
CDKN1A forward	AGGCACTCAGAGGAGGTGAGA
CDKN1A reverse	CAGAAACACCTGTGAACGCA
RASSF1 forward	TGGGGTGTGAGGAGGGGACGA
RASSF1 reverse	AGAGCCGCGCAATGGA