

Supplementary information

Table S1. Analysis of thyroid tumors developed in mice of the indicated genotypes

Genotype	AE (months)	N	MD	CEA (%)	Ki67 (%)
<i>Rb</i> ^{+/+} ; <i>ATM</i> ^{+/+}	12.8 ± 1.2	7	ND	1.5 ± 0.5	1.5 ± 0.3
<i>Rb</i> ^{+/+} ; <i>ATM</i> ^{-/-}	9.4 ± 1.4	8	ND	1.2 ± 0.4	1.2 ± 0.4
<i>Rb</i> ^{+/-} ; <i>ATM</i> ^{+/+}	12.5 ± 1.5	12	1.5 ± 0.3	1.7 ± 0.5	3.5 ± 0.2
<i>Rb</i> ^{+/-} ; <i>ATM</i> ^{+/-}	7.1 ± 1.3	15	9.3 ± 0.5	93.6 ± 1.2	61.1 ± 1.2
<i>Rb</i> ^{+/-} ; <i>ATM</i> ^{-/-}	5.4 ± 1.3	6	7.8 ± 0.6	96.3 ± 1.4	52.3 ± 1.3

Data shown include average age at examination (AE), number of thyroids examined (N), mean diameter of tumors (MD), and percentage of CEA-, and Ki67-positive cells included in the samples. Data are shown as average ± SEM. ND: not detected.

Table S2. Sequence of the primers used for bisulfite PCR of the *Ink4a* promoter

Primer	Sequence
Unmethylation specific	
Forward	5' CGATTGGGCGGGTATTGAATTTTCGC-
Reverse	3' -CACGTCATACACACGACCCTAAACCG-
Methylation-specific	
Forward	5' GTGATTGGGTGGGTATTGAATTTTGTG-
Reverse	3' -CACACATCATACACACAACCCTAAACCA-3'

Table S3. Sequence of the primers used for RT-PCR

Gene	Sequence
<i>Ink4a</i>	
Forward	5' -TCTGGAGCAGCATGGAGTCC-3'
Reverse	5' -TCGCAGTTCGAATCTGCACC-3'

DNMT1

Forward	5' -GAGGAAGGCTACCTGGCTAA-3'
Reverse	5' -AGTGAGAGTGTGTGTTCCGT-3'

Table S4. Sequence of the primers used for bisulfite sequencing

Gene	Sequence
<i>Ink4a</i>	
Outer PCR	
Forward	5' -GTTGTGTATAGAATTTTAGTATTG-3'
Reverse	5' -AAATAAAACACTCCTTACCTAC-3'
Inner PCR	
Forward	5' -TTTTAATATTTGGGTGTTGTATTG-3'
Reverse	5' -CCACCCTAACCAATCTATCTACAAC-3'
<i>Shc2</i>	
Outer PCR	
Forward	5' -GGGTAGGATATGATTGAACGTG-3'
Reverse	5' -CGAAAAACCCCGACTAAACC-3'
Inner PCR	
Forward	5' -GGTAGGATGTTGTTGGAATAGT-3'
Reverse	5' -AAAACACTACTCCTAAAACCCC-3'
<i>Noggin</i>	
Outer PCR	
Forward	5' -GAAAATGTTTTATGTATATGGAGAA-3'
Reverse	5' -CCACTTCTCTCTACCTACTCCGAAC-3'
Inner PCR	
Forward	5' -TGGTTTTAGCGTCGTTACGAGT-3'
Reverse	5' -CTACTCCGAACAACGCAACC-3'
<i>FoxO6</i>	
Outer PCR	
Forward	5' -TATTTCGACGGTCGGGGTTTA-3'
Reverse	5' -CCCCTACCGAACTCGAAAA-3'
Inner PCR	
Forward	5' -GTATAGAGAATAGGTAGGGTTGTGG-3'
Reverse	5' -TTCAAAAATTCTCCCAA-3'

Table S5. Sequence of the primers used for ChIP assay

Gene	Sequence
<i>Ink4a</i>	
ChIP	
Outer PCR	
OUTF1	5' -GATGACACAATTTTCTGCTAAGATG-3'
OUTR2	5' -AAAGAGTTCGGGGCGTTG-3'
Inner PCR	
Proximal region	
PRXF3	5' -AAGGAAGGAGGGACCCACT-3'
PRXR4	5' -CCTGGCCAGTCTGTCTGC-3'
Middle region	
MDLF5	5' -TCCTGAACCCTGCATCTCTT-3'
MDLR6	5' -CACACTCTGCTCCTGACCTG-3'
Distal region	
DSTF7	5' -TTCTAATACCTGGGTGTTGCAC-3'
DSTR8	5' -GAAGAGATGCAGGGTTCAGG-3'
qChIP	
AS109F	5' -AAGGAAGGAGGGACCCACT-3'
AS109R	5' -GACTCCATGCTGCTCCAGAT-3'

Table S6. List of the commonly upregulated genes in 2 independent microarray experiments

Gene Symbol	Full name from nomenclature authority	Genbank Accession
1100001G20Rik	RIKEN cDNA 1100001G20 gene	NM_183249
1700003E16Rik	RIKEN cDNA 1700003E16 gene	NM_027948
2210011C24Rik	RIKEN cDNA 2210011C24 gene	XM_985599
2310040G24Rik	RIKEN cDNA 2310040G24 gene	XM_001479091
2410075B13Rik	RIKEN cDNA 2410075B13 gene	NM_001163518
2610019F03Rik	RIKEN cDNA 2610019F03 gene	NM_173744
3830403N18Rik	RIKEN cDNA 3830403N18 gene	NM_027510
4930522L14Rik	RIKEN cDNA 4930522L14 gene	XR_035683
4930583H14Rik	RIKEN cDNA 4930583H14 gene	NM_026358
4932425I24Rik	RIKEN cDNA 4932425I24 gene	NM_001081025

4933400F03Rik		AK016591
4933439C10Rik	RIKEN cDNA 4933439C10 gene	NR_015585
5430435G22Rik	RIKEN cDNA 5430435G22 gene	NM_145509
6720401G13Rik	RIKEN cDNA 6720401G13 gene	NR_026976
9830001H06Rik	RIKEN cDNA 9830001H06 gene	NM_001164663
Actg2	actin, gamma 2, smooth muscle, enteric	NM_009610
Acy3	aspartoacylase (aminoacylase) 3	NM_027857
Adam23	a disintegrin and metallopeptidase domain 23	NM_011780
Adamts15	a disintegrin-like and metallopeptidase (reprolysin type) with thrombospondin type 1 motif, 15	NM_001024139
Adamts17	a disintegrin-like and metallopeptidase (reprolysin type) with thrombospondin type 1 motif, 17	NM_001033877
Agap2	ArfGAP with GTPase domain, ankyrin repeat and PH domain 2	NM_001033263
AI467606	expressed sequence AI467606	NM_178901
Akr1c13	aldo-keto reductase family 1, member C13	NM_013778
Alox5	arachidonate 5-lipoxygenase	NM_009662
Amigo1	adhesion molecule with Ig like domain 1	NM_146137
Amn	Amnionless	NM_033603
Amz1	archaelysin family metallopeptidase 1	NM_173405
Ankrd2	ankyrin repeat domain 2 (stretch responsive muscle)	NM_020033
Ankrd37	ankyrin repeat domain 37	NM_001039562
Aoc3	amine oxidase, copper containing 3	NM_009675
Apobr	apolipoprotein B receptor	NM_138310
Apod	apolipoprotein D	NM_007470
Apol10b	apolipoprotein L 10b	NM_177820
Arhgap9	Rho GTPase activating protein 9	NM_146011
Atp10d	ATPase, class V, type 10D	NM_153389
Atp1b2	ATPase, Na ⁺ /K ⁺ transporting, beta 2 polypeptide	NM_013415
Atp2a3	ATPase, Ca ⁺⁺ transporting, ubiquitous	NM_001163337
Atp6ap11	ATPase, H ⁺ transporting, lysosomal accessory protein 1-like	NM_001145879
B3gnt8	UDP-GlcNAc:betaGal beta-1,3-N-acetylglucosaminyltransferase 8	NM_146184
Batf2	basic leucine zipper transcription factor, ATF-like 2	NM_028967
BC026762	cDNA sequence BC026762	XM_001480912
BC039966	cDNA sequence BC039966	XM_001474172
BC061194	cDNA sequence BC061194	NM_001001334
Bglap-rs1	bone gamma-carboxylglutamate protein, related sequence 1	NM_031368
Birc7	baculoviral IAP repeat-containing 7 (livin)	NM_001163247
Bnip3	BCL2/adenovirus E1B interacting protein 3	NM_009760
Bok	BCL2-related ovarian killer protein	NM_016778
C130050018Rik	RIKEN cDNA C130050018 gene	NM_177000
C1qI3	C1q-like 3	NM_153155
C530028021Rik	RIKEN cDNA C530028021 gene	NM_175696
Cacng7	calcium channel, voltage-dependent, gamma subunit 7	NM_133189
Cadm4	cell adhesion molecule 4	NM_153112
Calr4	calreticulin 4	NM_001033226

Camk2b	calcium/calmodulin-dependent protein kinase II, beta	NM_001174053
Capn3	calpain 3	NM_007601
Car6	carbonic anhydrase 6	NM_009802
Casp1	caspase 1	NM_009807
Ccr11	chemokine (C-C motif) receptor-like 1	AY072938
Cdhr1	cadherin-related family member 1	NM_130878
Cdsn	Corneodesmosin	NM_001008424
Chst15	carbohydrate (N-acetylgalactosamine 4-sulfate 6-O) sulfotransferase 15	NM_029935
Cited1	Cbp/p300-interacting transactivator with Glu/Asp-rich carboxy-terminal domain 1	NM_007709
Clca6	chloride channel calcium activated 6	NM_207208
Clstn3	calsyntenin 3	NM_153508
Col15a1	collagen, type XV, alpha 1	NM_009928
Col5a3	collagen, type V, alpha 3	NM_016919
Col6a4	collagen, type VI, alpha 4	NM_026763
Col9a3	collagen, type IX, alpha 3	NM_009936
Cox6a2	cytochrome c oxidase, subunit VI a, polypeptide 2	NM_009943
Cp	ceruloplasmin	NM_001042611
Crb2	crumbs homolog 2 (Drosophila)	NM_001163566
Creb3l3	cAMP responsive element binding protein 3-like 3	NM_145365
Crispld2	cysteine-rich secretory protein LCCL domain containing 2	NM_030209
Csdc2	cold shock domain containing C2, RNA binding	NM_145473
Ctsk	cathepsin K	NM_007802
Cyfp2	cytoplasmic FMR1 interacting protein 2	NM_133769
Cyp2c55	cytochrome P450, family 2, subfamily c, polypeptide 55	NM_028089
Cys1	cystin 1	NM_138686
D430040L24Rik	RIKEN cDNA D430040L24 gene	AK085126
Des	desmin	NM_010043
Dlx4	distal-less homeobox 4	NM_007867
Dmkn	dermokine	NM_001166173
Dmpk	dystrophia myotonica-protein kinase	NM_032418
Dnajc22	DnaJ (Hsp40) homolog, subfamily C, member 22	NM_176835
Dusp13	dual specificity phosphatase 13	NM_001007268
Ebf4	early B-cell factor 4	NM_001110513
Edn1	endothelin 1	NM_010104
Edn2	endothelin 2	NM_007902
EG666699		XM_989887
Enpp1	ectonucleotide pyrophosphatase/phosphodiesterase 1	AK014707
Enpp1	ectonucleotide pyrophosphatase/phosphodiesterase 1	NM_008813
Epb4.9	erythrocyte protein band 4.9	NM_013514
Esrrb	estrogen related receptor, beta	NM_001159500
F8	coagulation factor VIII	NM_007977
Fam180a	family with sequence similarity 180, member A	NM_173375
Fam20a	family with sequence similarity 20, member A	NM_153782
Fam81a	family with sequence similarity 81, member A	NM_029784
Fate1	fetal and adult testis expressed 1	NR_003243

Fcgr4	Fc receptor, IgG, low affinity IV	NM_144559
Foxa1	forkhead box A1	NM_008259
Foxf2	forkhead box F2	NM_010225
Foxo6	forkhead box O6	NM_194060
Foxs1	forkhead box S1	NM_010226
Fyb	FYN binding protein	NM_011815
Gas6	growth arrest specific 6	NM_019521
Gdf7	growth differentiation factor 7	NM_013527
Gfra2	glial cell line derived neurotrophic factor family receptor alpha 2	NM_008115
Glipr1	GLI pathogenesis-related 1 (glioma)	NM_028608
Gm10220	predicted gene 10220	NM_001134299
Gm10487	predicted gene 10487	NM_001100609
Gm10639	predicted gene 10639	NM_001122660
Gm16430	predicted gene 16430	NM_001166601
Gm2012	predicted gene 2012	NM_001104946
Gm2066	predicted pseudogene 2066	XM_001472346
Gm2397	predicted gene 2397	XM_001473420
Gm2659	predicted gene 2659	XM_001474258
Gm3137	predicted gene 3137	XM_001477000
Gm3586		XM_001477872
Gm3697	predicted gene 3697	XM_001478221
Gm3792	predicted gene 3792	XM_001478043
Gm3916	predicted gene 3916	XM_001478609
Gm41	predicted pseudogene 41	XM_111935
Gm5168	predicted gene 5168	NM_001025607
Gm5532	predicted gene 5532	AK147021
Gm5604	predicted gene 5604	XR_004919
Gm5622	predicted gene 5622	NM_001013816
Gm5935	predicted gene 5935	NM_001081657
Gm6664	predicted gene 6664	XR_035618
Gm7676	predicted gene 7676	XM_977607
Gm7967	predicted gene 7967	XM_991070
Gm7999	predicted gene 7999	XM_982515
Gm9732	predicted gene 9732	XM_001475911
Gngt2	guanine nucleotide binding protein (G protein), gamma transducing activity polypeptide 2	NM_001038664
Gpha2	glycoprotein hormone alpha 2	NM_130453
Gpsm3	G-protein signalling modulator 3 (AGS3-like, <i>C. elegans</i>)	NM_134116
Grem1	gremlin 1	NM_011824
Grk4	G protein-coupled receptor kinase 4	NM_019497
Grtp1	GH regulated TBC protein 1	NM_025768
Gsdmc	gasdermin C	NM_031378
Gsdmd	gasdermin D	NM_026960
Gsta2	glutathione S-transferase, alpha 2 (Yc2)	NM_008182
Gsta4	glutathione S-transferase, alpha 4	NM_010357
Gstm6	glutathione S-transferase, mu 6	NM_008184
H19	H19 fetal liver mRNA	NR_001592
H2-DMb1	histocompatibility 2, class II, locus Mb1	NM_010387
H2-K1	histocompatibility 2, K1, K region	NM_001001892

Hba-a2	hemoglobin alpha, adult chain 2	NM_001083955
Heyl	hairly/enhancer-of-split related with YRPW motif-like	NM_013905
Hmcn1	hemicentin 1	NM_001024720
Hoxa7	homeobox A7	NM_010455
Hpse	heparanase	NM_152803
Hyal1	hyaluronoglucosaminidase 1	NM_008317
Ifi2711	interferon, alpha-inducible protein 27 like 1	NM_026790
Ifi2712a	interferon, alpha-inducible protein 27 like 2A	NM_029803
Ifitm1	interferon induced transmembrane protein 1	NM_001112715
Ifitm6	interferon induced transmembrane protein 6	NM_001033632
Inadl	InaD-like (Drosophila)	NM_172696
Inadl	InaD-like (Drosophila)	NM_001005787
Ins1	insulin I	NM_008386
Irf6	interferon regulatory factor 6	NM_016851
Itga10	integrin, alpha 10	NM_001081053
Itga9	integrin alpha 9	NM_133721
Itgb4	integrin beta 4	NM_001005608
Kcnab1	potassium voltage-gated channel, shaker-related subfamily, beta member 1	NM_010597
Kcnmb4	potassium large conductance calcium-activated channel, subfamily M, beta member 4	NM_021452
Kcnn4	potassium intermediate/small conductance calcium-activated channel, subfamily N, member 4	NM_008433
Klk8	kallikrein related-peptidase 8	NM_008940
Krt14	keratin 14	NM_016958
Krt7	keratin 7	NM_033073
db3	LIM domain binding 3	NM_011918
Lgals2	lectin, galactose-binding, soluble 2	NM_025622
Lhx2	LIM homeobox protein 2	NM_010710
Lims2	LIM and senescent cell antigen like domains 2	NM_144862
Lmod1	leiomodien 1 (smooth muscle)	NM_053106
LOC100041034		XM_001475680
LOC100041580		XM_001475945
LOC100044874		XM_001473540
LOC100047159		XM_001477549
LOC100047598		XM_001478476
LOC625953		XM_001480391
LOC631151		XM_904876
Lrrc15	leucine rich repeat containing 15	NM_028973
Lrrc17	leucine rich repeat containing 17	NM_028977
Lrrn4cl	LRRN4 C-terminal like	NM_001013019
Marco	macrophage receptor with collagenous structure	NM_010766
Matn4	matrilin 4	NM_013592
Mc1r	melanocortin 1 receptor	NM_008559
Me3	malic enzyme 3, NADP(+)-dependent, mitochondrial	NM_181407
Mmp17	matrix metalloproteinase 17	NM_011846
Mr1	major histocompatibility complex, class I-related	NM_008209
Mreg	melanoregulin	NM_001005423
Murc	muscle-related coiled-coil protein	NM_026509
Myh6	myosin, heavy polypeptide 6, cardiac muscle,	NM_001164171

	alpha	
Myom2	myomesin 2	NM_008664
Naip2	NLR family, apoptosis inhibitory protein 2	NM_010872
Naip6	NLR family, apoptosis inhibitory protein 6	NM_010871
Ncrna00085	non-protein coding RNA 85	NM_001162909
Ndrp1	N-myc downstream regulated gene 1	NM_008681
Ndufa412	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 4-like 2	NM_001098789
Neur1a	neuralized homolog 1A (Drosophila)	NM_021360
Nkain3	Na ⁺ /K ⁺ transporting ATPase interacting 3	NM_172987
Nog	noggin	NM_008711
Nppb	natriuretic peptide type B	NM_008726
Olf435	olfactory receptor 435	NM_146653
Olf694	olfactory receptor 694	NM_146452
Onecut2	one cut domain, family member 2	NM_194268
Ophn1	oligophrenin 1	NM_052976
P2rx5	purinergic receptor P2X, ligand-gated ion channel, 5	NM_033321
Pax3	paired box gene 3	NM_008781
Pcp411	Purkinje cell protein 4-like 1	NM_025557
Pde2a	phosphodiesterase 2A, cGMP-stimulated	NM_001143848
Pgm211	phosphoglucomutase 2-like 1	NM_027629
Phex	phosphate regulating gene with homologies to endopeptidases on the X chromosome (hypophosphatemia, vitamin D resistant rickets)	NM_011077
Phkg1	phosphorylase kinase gamma 1	NM_011079
Phldb3	pleckstrin homology-like domain, family B, member 3	NM_001102613
Pla1a	phospholipase A1 member A	NM_134102
Pla2g2e	phospholipase A2, group IIE	NM_012044
Plac1	placental specific protein 1	NM_019538
Plac8	placenta-specific 8	NM_139198
Plb1	phospholipase B1	NM_001081407
Plch2	phospholipase C, eta 2	NM_175556
Plin4	perilipin 4	NM_020568
Plxna4	plexin A4	NM_175750
Plxnb3	plexin B3	NM_019587
Podn1	podocan-like 1	NM_001013384
Postn	periostin, osteoblast specific factor	NM_015784
Ppp1r14a	protein phosphatase 1, regulatory (inhibitor) subunit 14A	NM_026731
Ppp4r4	protein phosphatase 4, regulatory subunit 4	NM_028980
Prm3	protamine 3	NM_013638
Prokr1	prokineticin receptor 1	NM_021381
Prrt4	proline-rich transmembrane protein 4	NM_001101443
Prune2	prune homolog 2 (Drosophila)	NM_181348
Psd	pleckstrin and Sec7 domain containing	NM_028627
Pthlh	parathyroid hormone-like peptide	NM_008970
Ptprb	protein tyrosine phosphatase, receptor type, B	NM_029928
Rab26	RAB26, member RAS oncogene family	NM_177375
Ralgps2	Ral GEF with PH domain and SH3 binding motif 2	NM_023884

Rasgef1a	RasGEF domain family, member 1A	NM_027526
Rbmyl1a1	RNA binding motif protein, Y chromosome, family 1, member A1	NM_011253
Rdh19	retinol dehydrogenase 19	NM_147222
Reep6	receptor accessory protein 6	NM_139292
Rgs10	regulator of G-protein signalling 10	NM_026418
Rgs4	regulator of G-protein signaling 4	NM_009062
Rgs5	regulator of G-protein signaling 5	NM_009063
Rtn2	reticulon 2 (Z-band associated protein)	NM_013648
Rtn4r	reticulon 4 receptor	NM_022982
Rwdd2a	RWD domain containing 2A	NM_027100
S100b	S100 protein, beta polypeptide, neural	NM_009115
S1pr3	sphingosine-1-phosphate receptor 3	NM_010101
Samd10	sterile alpha motif domain containing 10	NM_172676
Scd4	stearoyl-coenzyme A desaturase 4	NM_183216
Sct	secretin	NM_011328
Sdr39u1	short chain dehydrogenase/reductase family 39U, member 1	NM_001082975
Sdsl	serine dehydratase-like	NM_133902
Sema3a	sema domain, immunoglobulin domain (Ig), short basic domain, secreted, (semaphorin) 3A	NM_009152
Sema3b	sema domain, immunoglobulin domain (Ig), short basic domain, secreted, (semaphorin) 3B	NM_001042779
Sema3c	sema domain, immunoglobulin domain (Ig), short basic domain, secreted, (semaphorin) 3C	NM_013657
Sema3g	sema domain, immunoglobulin domain (Ig), short basic domain, secreted, (semaphorin) 3G	AK129018
Serpinb10-ps	serine (or cysteine) peptidase inhibitor, clade B (ovalbumin), member 10, pseudogene	NM_198028
Serpinf1	serine (or cysteine) peptidase inhibitor, clade F, member 1	NM_011340
Shc2	SHC (Src homology 2 domain containing) transforming protein 2	NM_001024539
Sigirr	single immunoglobulin and toll-interleukin 1 receptor (TIR) domain	NM_023059
Sla2	Src-like-adaptor 2	NM_029983
Slamf9	SLAM family member 9	NM_029612
Slc11a1	solute carrier family 11 (proton-coupled divalent metal ion transporters), member 1	NM_013612
Slc12a5	solute carrier family 12, member 5	NM_020333
Slc13a5	solute carrier family 13 (sodium-dependent citrate transporter), member 5	NM_001004148
Slc25a45	solute carrier family 25, member 45	NM_134154
Slc2a5	solute carrier family 2 (facilitated glucose transporter), member 5	NM_019741
Slc46a3	solute carrier family 46, member 3	NM_027872
Slc4a11	solute carrier family 4, sodium bicarbonate transporter-like, member 11	NM_001081162
Slx	Sycp3 like X-linked	NM_001136476
Smpd3	sphingomyelin phosphodiesterase 3, neutral	NM_021491
Sncg	synuclein, gamma	NM_011430
Soat2	sterol O-acyltransferase 2	NM_146064
Sostdc1	sclerostin domain containing 1	NM_025312

Sp110	Sp110 nuclear body protein	NM_175397
Sp140	Sp140 nuclear body protein	NM_001013817
Srpx	sushi-repeat-containing protein	NM_016911
Synm	synemin, intermediate filament protein	NM_201639
Synm	synemin, intermediate filament protein	NM_183312
Tbxa2r	thromboxane A2 receptor	NM_009325
Tbxas1	thromboxane A synthase 1, platelet	NM_011539
Tesc	tescalcin	NM_021344
Tg	thyroglobulin	NM_009375
Tle4	transducin-like enhancer of split 4, homolog of Drosophila E(spl)	NM_011600
Tmcc3	transmembrane and coiled coil domains 3	NM_172051
Tmem184a	transmembrane protein 184a	NM_001161548
Tmem37	transmembrane protein 37	NM_019432
Tmem47	transmembrane protein 47	NM_138751
Tmprss6	transmembrane serine protease 6	NM_027902
Tnfaip2	tumor necrosis factor, alpha-induced protein 2	NM_009396
Tnnt3	troponin T3, skeletal, fast	NM_011620
Tnnt3	troponin T3, skeletal, fast	NM_001163664
Trem2	triggering receptor expressed on myeloid cells 2	NM_031254
Trpv2	transient receptor potential cation channel, subfamily V, member 2	NM_011706
Tspan32	tetraspanin 32	NM_020286
Tspan32	tetraspanin 32	NM_001128080
Tspan8	tetraspanin 8	NM_146010
Vipr1	vasoactive intestinal peptide receptor 1	NM_011703
Vit	vitrin	NM_028813
Wnt10b	wingless related MMTV integration site 10b	NM_011718
Wnt5b	wingless-related MMTV integration site 5B	NM_009525
Xlr	X-linked lymphocyte-regulated complex	NM_011725
		AK134871
		AK078204
		AK076101
		AK087744
		BC028765
		AK034180
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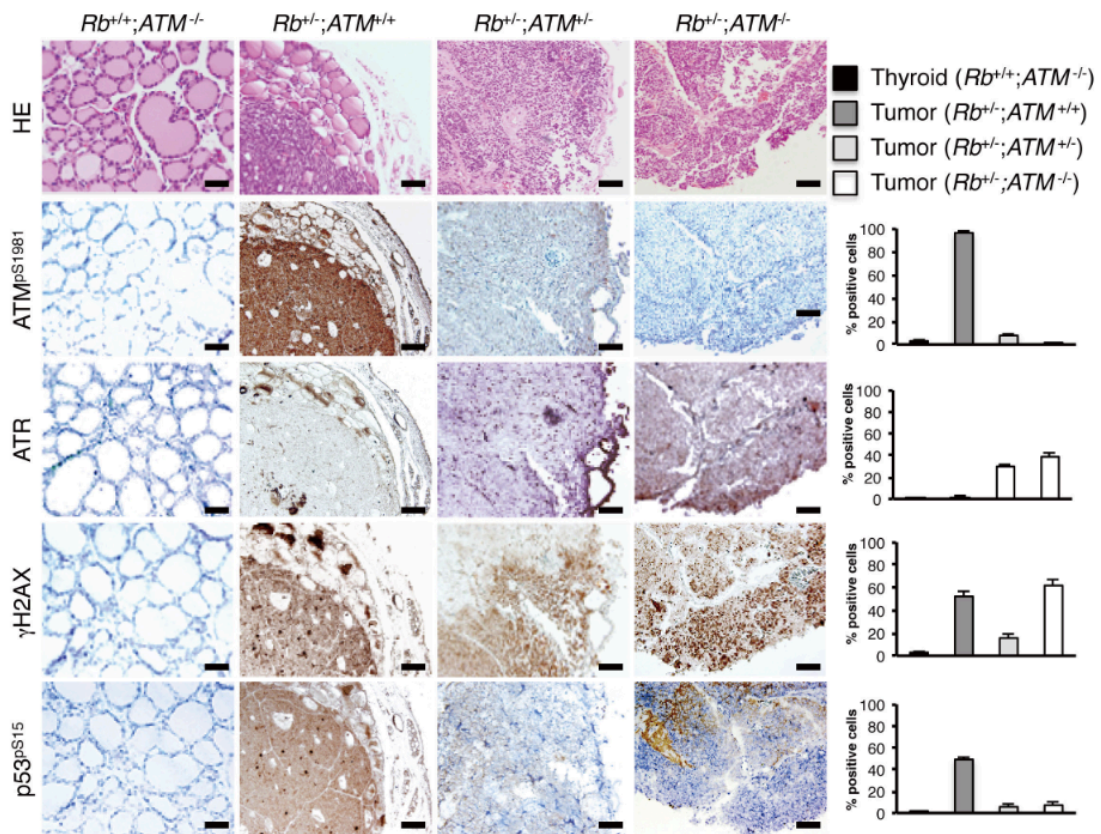


Fig. S1. Analysis of *Rb*-deficient C cell tumors of various *ATM* genotypes. Serial sections of thyroid tumors developed in the indicated genotypes of mice were analyzed by hematoxylin-eosin (HE) staining and immunostained with antibodies to the indicated proteins. Frequency of immunopositive cells per 200 normal thyroid or tumor cells was quantified. Columns are mean + SEM. Scale bars: 100 μ m.

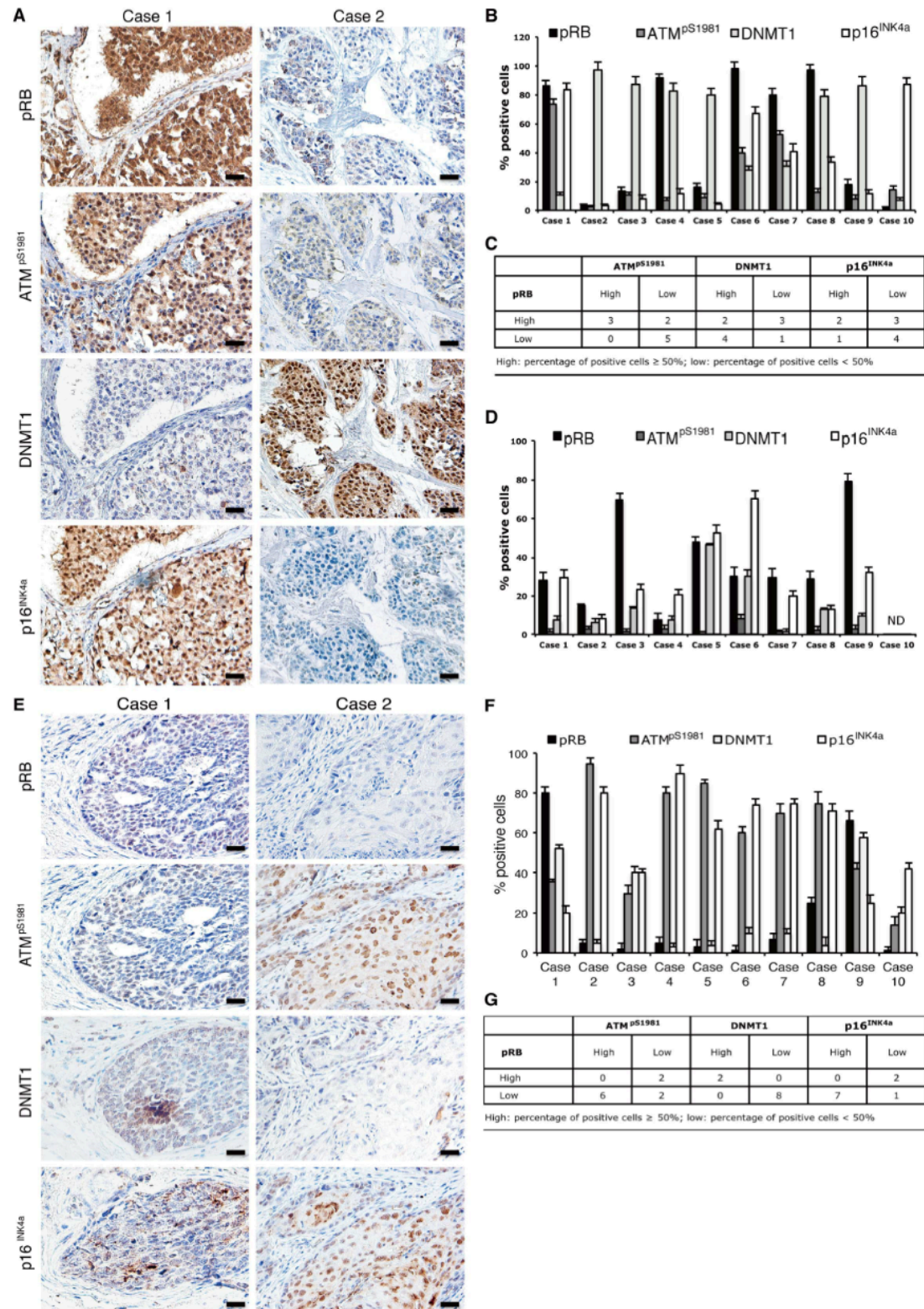


Fig. S2. Analysis of human sporadic medullary thyroid carcinomas and esophageal squamous cell carcinomas. (A) Representative micrographs of serial sections of 10 human sporadic medullary thyroid carcinomas (MTCs) immunostained with antibodies to the indicated proteins. (B) Frequency of immunopositive cells per 200

tumor cells in all the cases. **(C)** Classification of cases according to the frequency of immunopositive cells for the indicated proteins. **(D)** Frequency of immunopositive cells per 200 tumor cells in normal thyroid tissues included in the samples were quantified as in B. **(E)** Representative micrographs of serial sections of 10 human esophageal squamous cell carcinomas (SCCs) immunostained with antibodies to the indicated proteins. **(F)** The frequency of immunopositive cells in all the cases. **(G)** Classification of cases according to the frequency of immunopositive cells for the indicated proteins. The cut off value: $\geq 50\%$; high and $< 50\%$; low. Columns are mean + SEM. Scale bars: 100 μm .

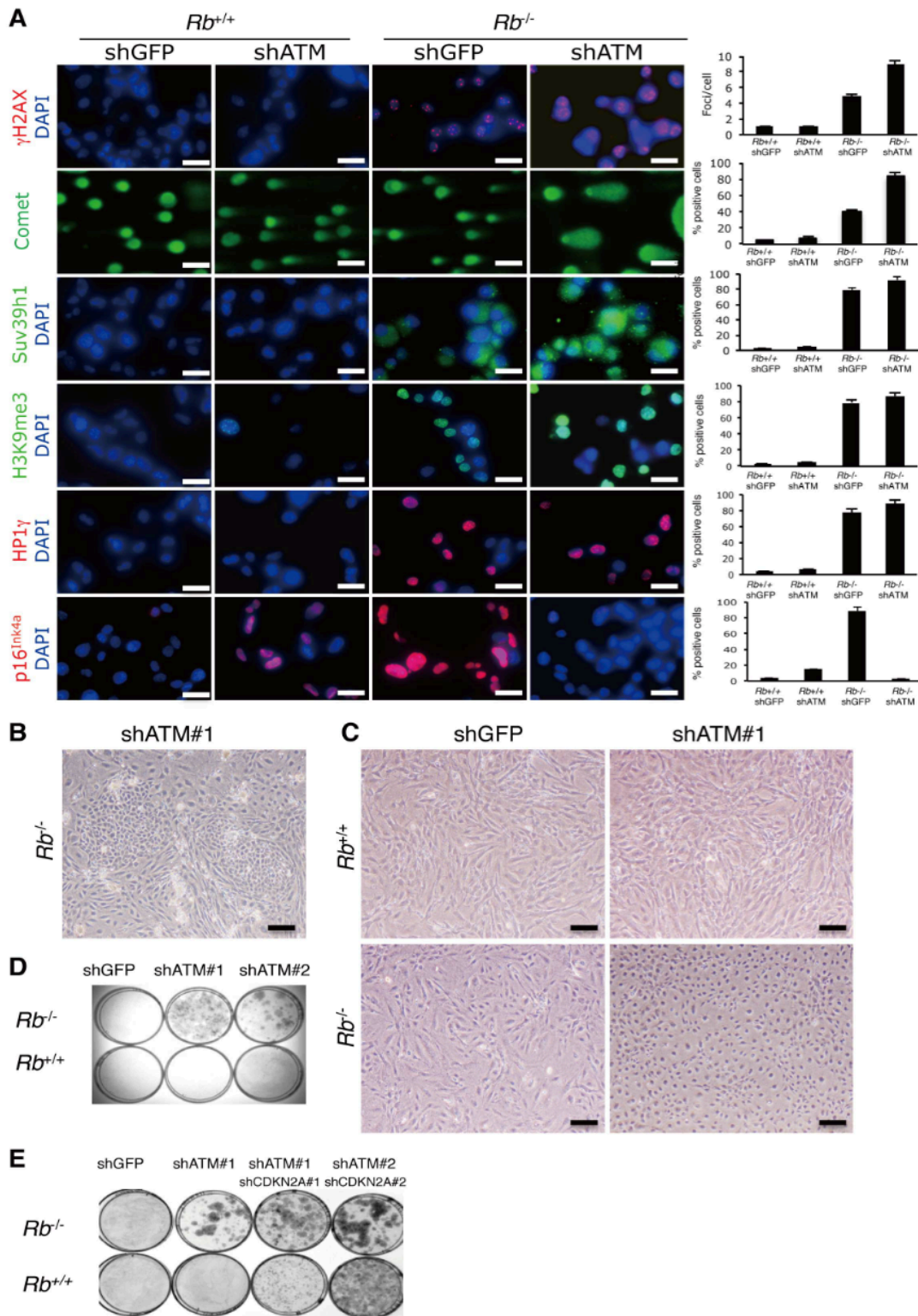


Fig. S3. IF analysis and phenotypic changes induced by ATM depletion from *Rb*-deficient MEFs. (A) MEFs were infected with lentiviruses expressing shRNAs that target the indicated genes, selected with 4 μ g/ml puromycin for 5 days, cultivated under normal conditions for additional 12 days, and immunostained with antibodies

to the indicated proteins. The frequency of immunopositive cells per 200 cells was quantified. Columns are mean + SEM. Scale bars: 100 μ m. **(B)** Micrographs of *Rb*^{-/-} MEFs infected with lentivirus expressing shRNA that targets ATM, and cultured for 5 days without drug selection. Note that cells with different morphology appeared in culture. **(C)** Micrographs of MEFs of the indicated genotypes that were infected with lentiviruses expressing shRNA targeting the indicated genes and selected with 4 μ g/ml puromycin for 5 days. **(D and E)**, Colony formation assay, MEFs of the indicated genotypes were infected with lentiviruses expressing shRNAs targeting the indicated genes and selected with 4 μ g/ml puromycin for 5 days and 1.0x10³ cells were plated onto 60 mm dish, and cultured for 15 days. Scale bars: 100 μ m.

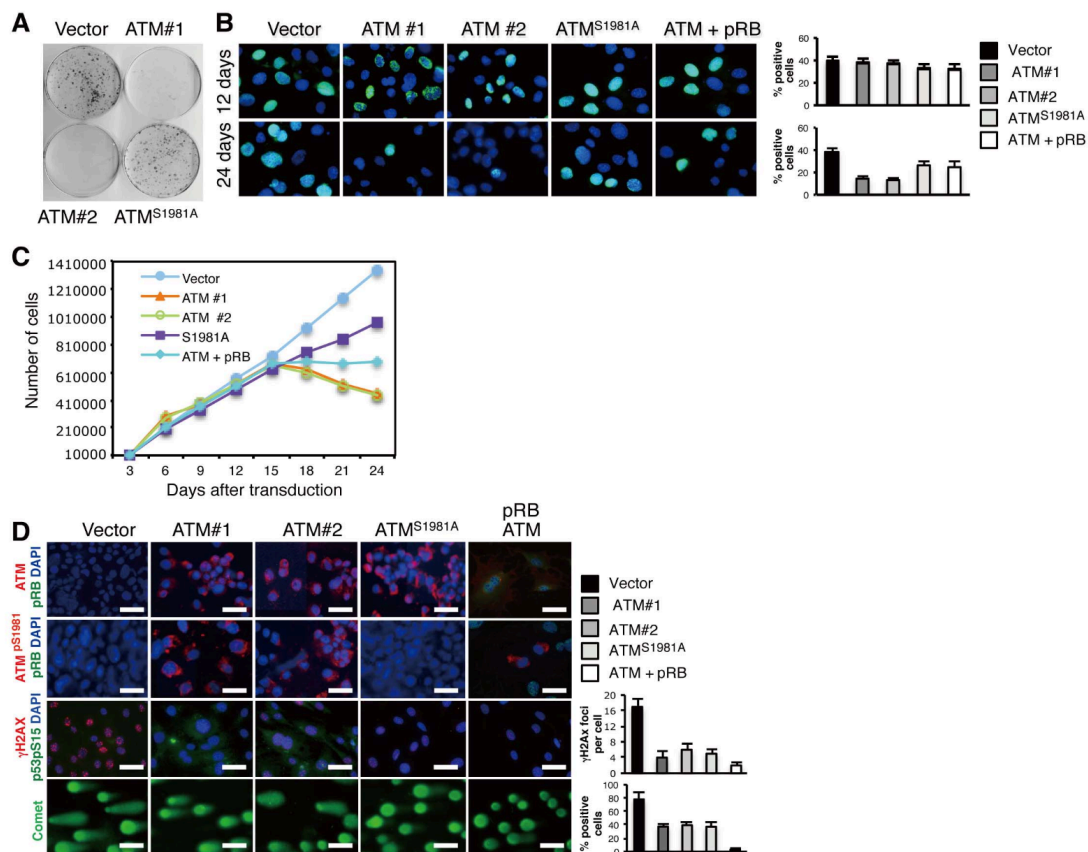


Fig. S4. Phenotypic changes in *Rb*^{-/-}; *ATM*^{-/-} MEFs induced by reintroduction of ATM and pRb. MEFs were nucleofected with vectors expressing the indicated proteins, selected with 4 μ g/ml puromycin and/or 8 μ g/ml blasticidin S for 5 days, cloned and analyzed. **(A)** Colony formation, 1.0 x10³ cells onto 60 mm dish and cultured for 15

days. (B) BrdU incorporation, cells were cultured under normal culture conditions and analyzed at the indicated days after nucleofection. (C) Proliferation of *Rb*^{-/-}; *ATM*^{-/-} MEFs nucleofected with vectors expressing the indicated proteins. (D) IF analysis with antibodies to the indicated proteins, Commet assay of representative clones are indicated. The frequency of positive cells per 200 cells was quantified. Columns are mean + SEM. Scale bars: 100 μ m.

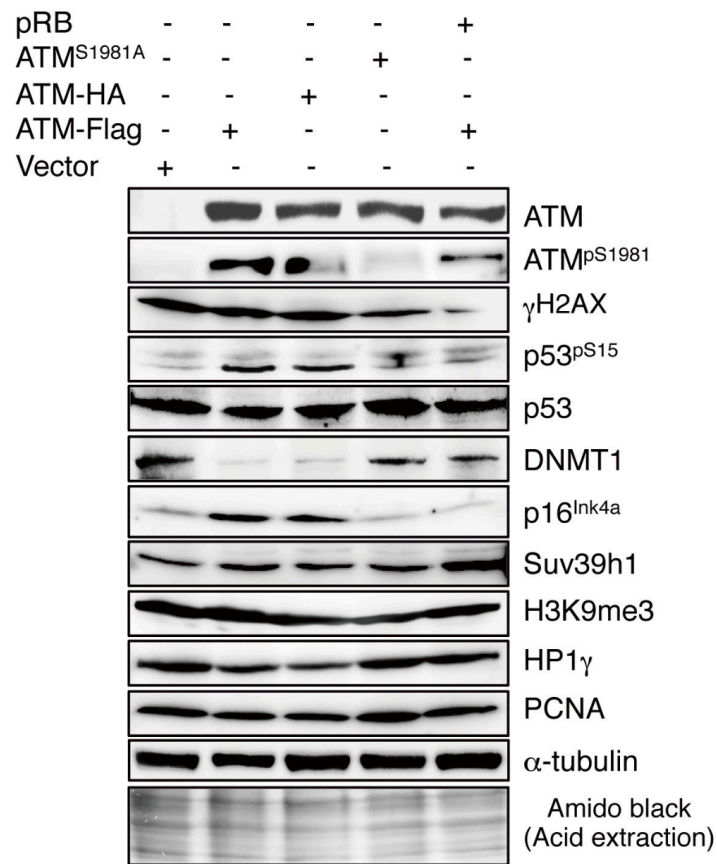


Fig. S5. Immunoblotting analysis of *Rb*-deficient *ATM*^{-/-} C cell adenocarcinoma cells. Cells were nucleofected with vectors expressing the indicated proteins, selected with 4 μ g/ml puromycin and 8 μ g/ml blasticidin S for 5 days, and analyzed with antibodies to the indicated proteins.

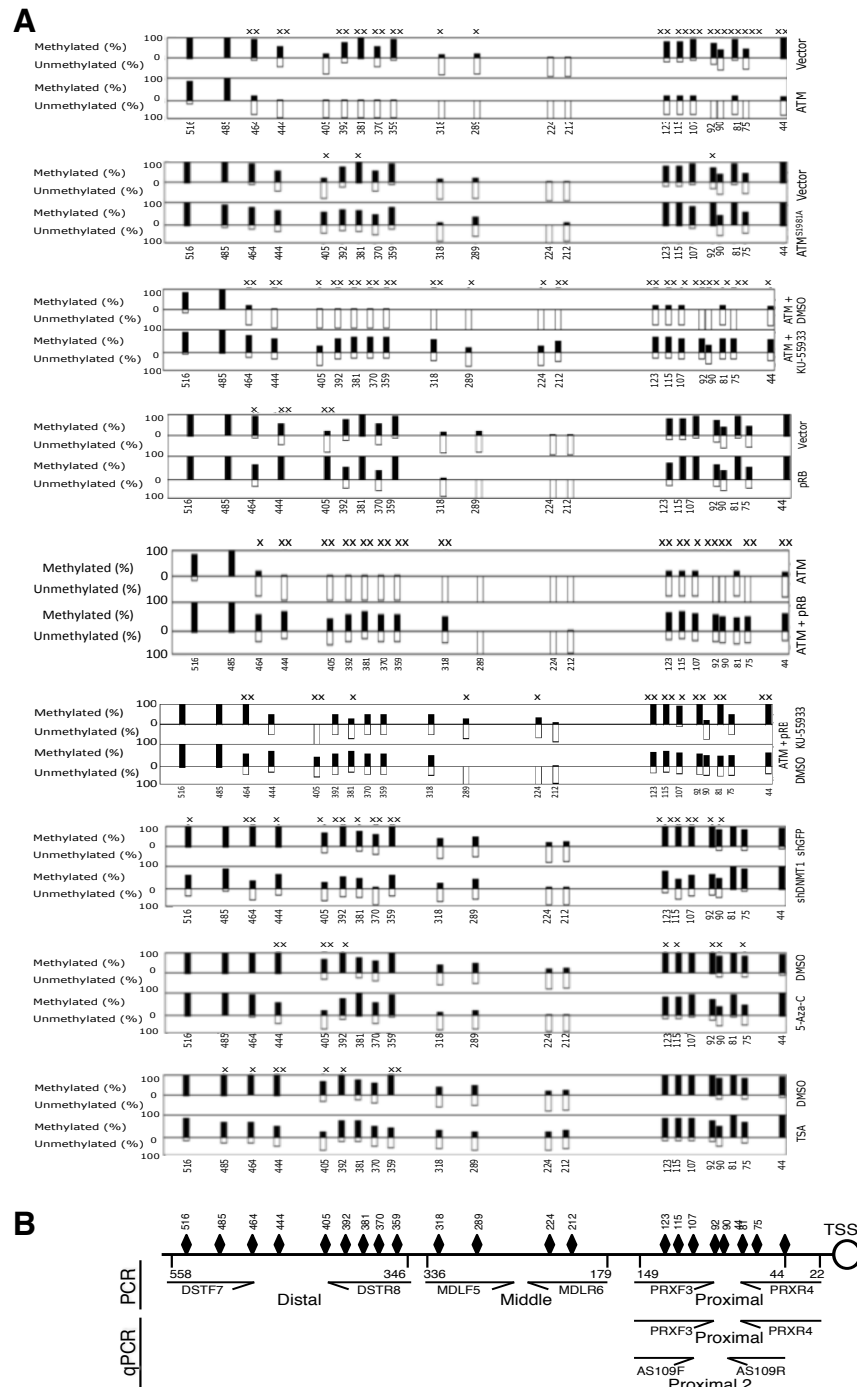


Fig. S6. Analysis of the *Ink4a* promoter. **(A)** Statistical analysis of the frequency of methylated CpG nucleotides, and their location in the *Ink4a* promoter region in *Rb*^{-/-}; *ATM*^{-/-} MEFs under various conditions. Columns indicate the percentage of methylated (filled) and unmethylated (unfilled) CpG nucleotides in the *Ink4a* promoter region (-44 to -516) of cells analyzed in Fig. 3c. Data represents average of more than 20 clones. X: p-value <0.1, XX: p-value <0.01 in each pair of comparison provided by QUMA program. **(B)** A schematic presentation of the structure of mice

Ink4a promoter region (-22 to -558) upstream of the transcription start site (TSS) and location of CpG nucleotides. Shown are the locations of primers (arrows) used in PCR or qPCR amplification of CpG nucleotide-rich regions in ChIP analysis.

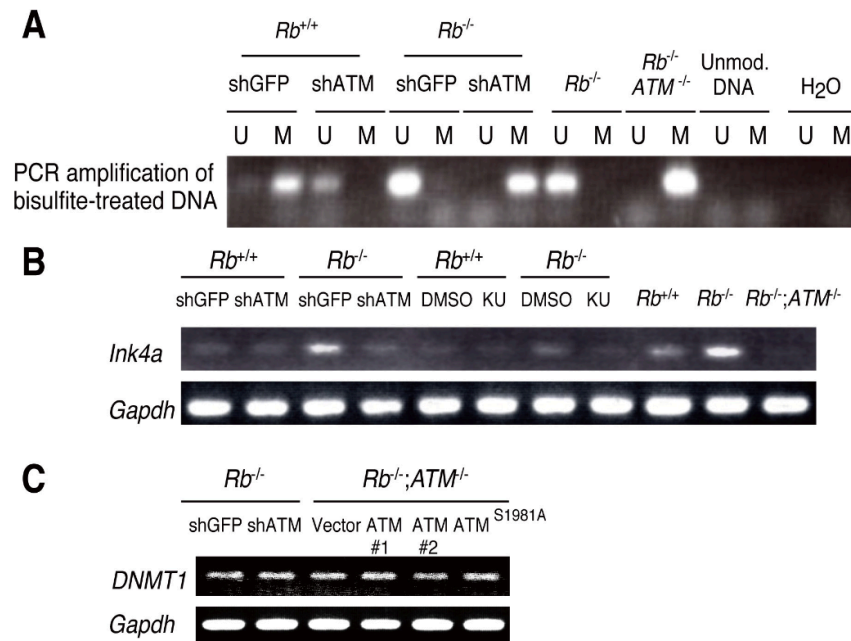


Fig. S7. Effects of the status of pRb and ATM on the transcription of *Ink4a* and *DNMT1*. **(A)** Analysis of the *Ink4a* promoter by bisulfite PCR. MEFs of the indicated genotypes, or those infected with lentiviruses expressing shRNAs that target the indicated genes, and selected with 4 μ g/ml puromycin for 5 days, were cultured for additional 10 days under normal conditions and analyzed. Bisulfite-treated DNA was amplified with the primers specific for unmethylated (U) or methylated (M) allele shown in Table S2. **(B)** MEFs of the indicated genotypes were infected with lentiviruses expressing shRNAs that target the indicated genes, selected with 4 μ g/ml puromycin for 5 days, cultured for additional 10 days under normal conditions; or treated with 10 mM KU-55933 (KU) or DMSO for 6 days. Resultant cells were analyzed by RT-PCR for the indicated genes. **(C)** MEFs of the indicated genotypes were infected with lentiviruses expressing shRNAs that target the indicated genes, or

transfected with vectors expressing the indicated proteins, selected with 4 $\mu\text{g/ml}$ puromycin for 5 days, cultured for additional 10 days under normal conditions, and analyzed by RT-PCR for the indicated genes.

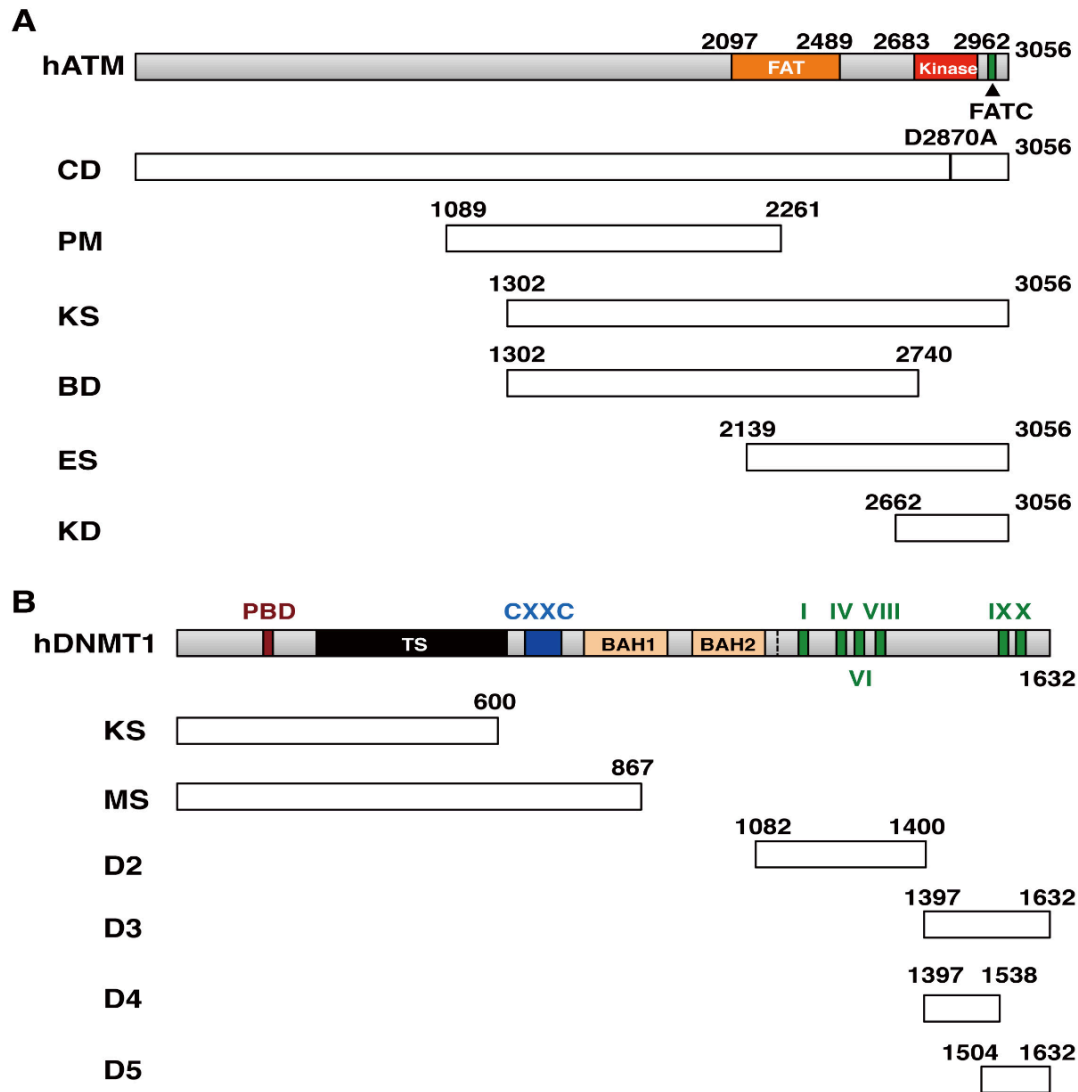


Fig. S8. A schematic presentation of the structure of human ATM (hATM) and DNMT1 (hDNMT1) proteins and their deletion mutants used in this study. **(A)** CD: D2870A conversion leading to reduced kinase activity; PM: containing a region nested by PstI and MscI restriction sites; KS: KpnI-SmaI; BD: KpnI-PstI; ES: EcoRI-SmaI; KD: kinase domain (aa2662-aa3056). **(B)** KS and MS were Flag-tagged, and D2, D3, D4 and D5 were HA-tagged. The DNA fragments coding these truncated proteins were inserted into pEF-BOS vector.

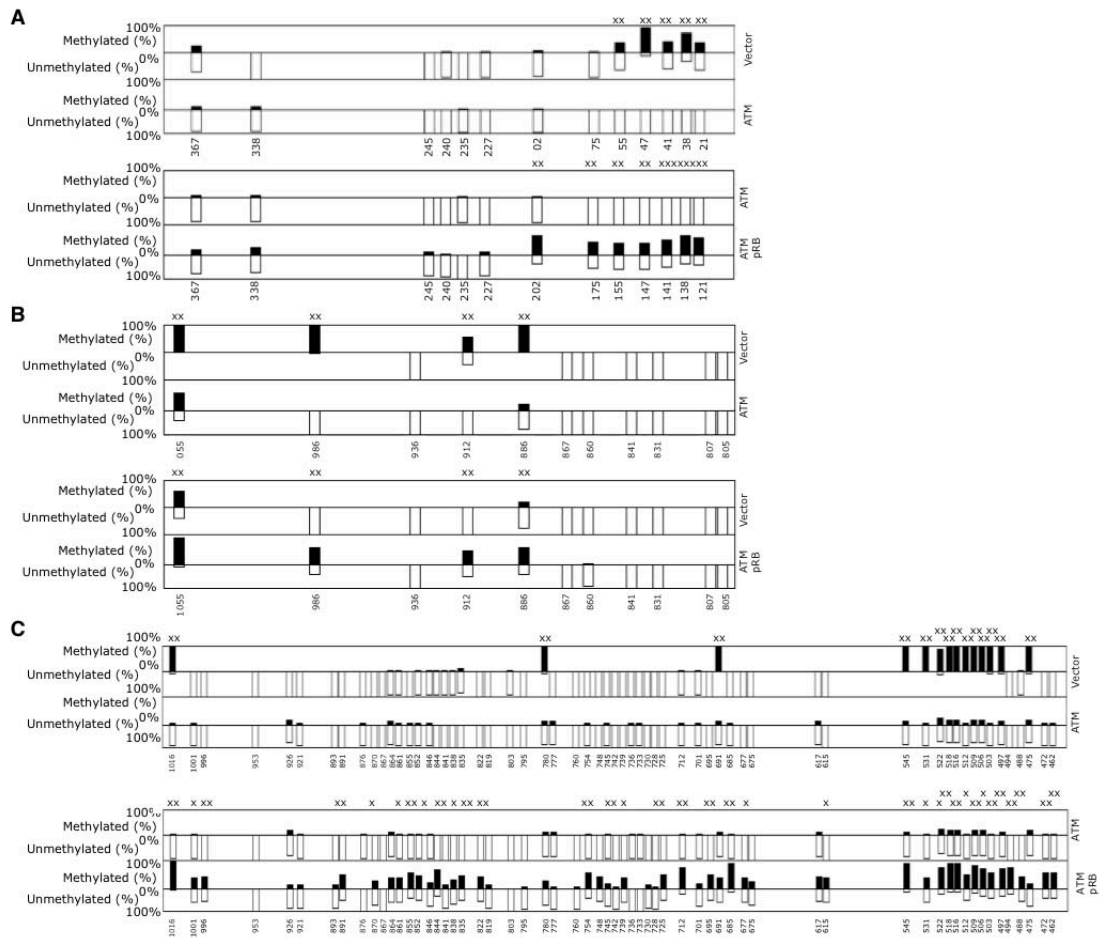


Fig. S9. Statistical analysis of the frequency of methylated CpG nucleotides and their location in the promoters of three representative cancer related genes in *Rb^{-/-};ATM^{-/-}* MEFs with different status of pRB and ATM. **(A)** MEFs were transduced with vectors expressing the indicated proteins, selected with 4 $\mu\text{g/ml}$ puromycin and/or 8 $\mu\text{g/ml}$ blasticidin S for 5 days, and analyzed by bisulfite sequencing after 10 days of culturing under normal conditions. Shown is the percentage of methylated and unmethylated CpG nucleotides in the *Shc2* promoter region (-121 to -367) under different conditions. **(B)** MEFs were prepared and analyzed as in (A) Shown is the percentage of methylated and unmethylated CpG nucleotides in the *FoxO6* promoter region (-805 to -1055) under different conditions. **(C)** MEFs were prepared and analyzed as in (A). Shown is the percentage of methylated and unmethylated CpG nucleotides in the *Noggin* promoter region (-462 to -1016) under different conditions. Columns indicate the percentage of methylated (filled) and unmethylated (unfilled) CpG nucleotides. Data represents average of more than 15 clones. X: p-value <0.1, XX: p-value <0.01 given by QUMA program.