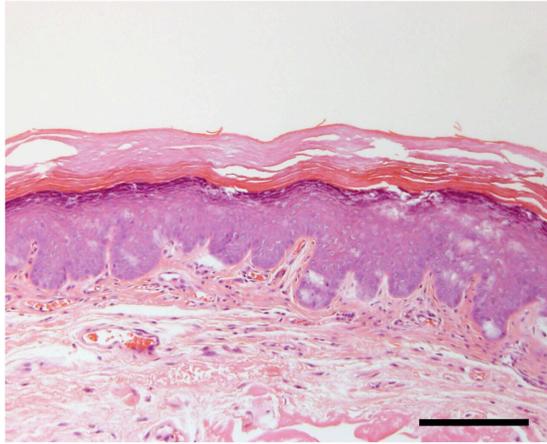
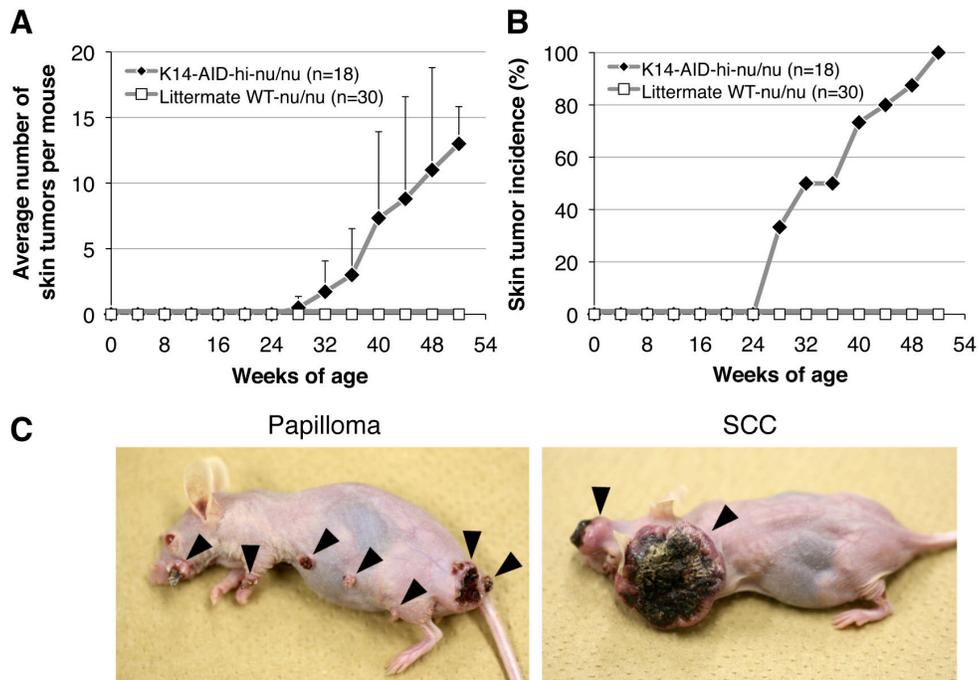


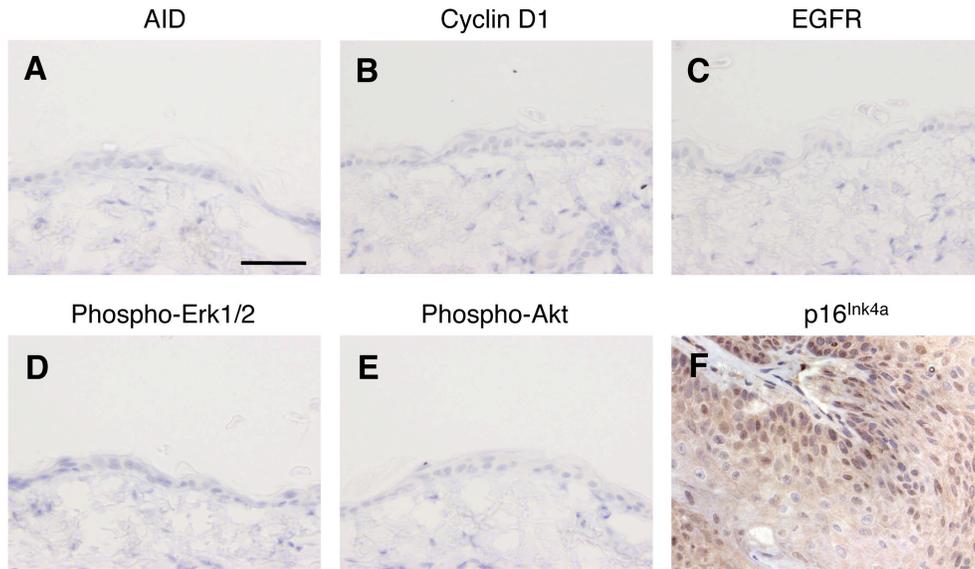
Supplemental data



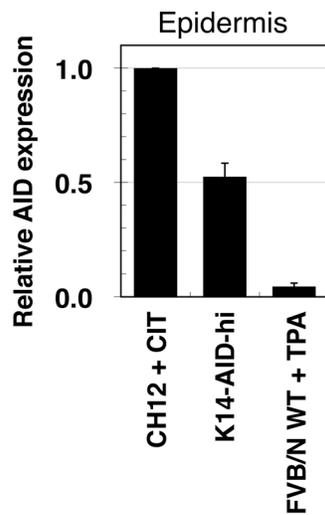
Supplemental Figure 1. Hematoxylin and eosin (HE) staining of a psoriasis-like skin lesion in a TPA-treated CAG-AID Tg-nu/nu mouse. Here 40 nmol of TPA was applied to the dorsal skin of a CAG-AID Tg-nu/nu mouse twice weekly for 8 weeks. Scale bar, 200 μ m.



Supplemental Figure 2. Spontaneous skin carcinogenesis in K14-AID transgenic mice on a nude background. (A) The average numbers of skin tumors that developed spontaneously in K14-AID-hi-nu/nu mice and littermate WT-nu/nu mice (mean \pm SD). All mouse groups bear homozygous Ptch1 T1267N alleles. nu/nu, mice with homozygous nude alleles. (B) Incidence of skin tumors that developed spontaneously in K14-AID-hi-nu/nu mice. (C) Representative photographs of skin tumors that developed spontaneously in K14-AID-hi-nu/nu mice. Arrowheads indicate skin tumors. SCC, squamous cell carcinoma.



Supplemental Figure 3. Immunohistochemistry of negative and positive control samples for Figure 4I. (A) Normal FVB/N WT murine epidermis was stained for AID. (B–E) Normal K14-AID Tg murine epidermis was stained for Cyclin D1, EGFR, Phospho-Erk1/2, and Phospho-Akt. (F) A spontaneously developing papilloma from a K14-AID Tg mouse was stained for p16^{Ink4a}. Scale bar, 50 μm.



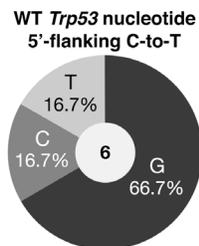
Supplemental Figure 4. Relative AID mRNA expression in TPA-treated epidermis from FVB/N WT mice. A single 16-nmol TPA dose was applied to the dorsal skin of FVB/N WT mice. Epidermis was harvested 24 h after treatment and the AID mRNA expression was analyzed by qRT-PCR (mean ± SD, n = 3). K14-AID-hi indicates a mouse with high K14-AID transgene expression. CH12, mouse B lymphoma cell line; CIT, combination of CD40 ligand, IL-4, and TGFβ.

A

Gene	Mouse	Tissue	Mutation		Human-equivalent codon	Domain	Protein function †	
			Nucleotide	Codon			Structure-based	Transactivation
<i>Trp53</i>	FVB/N WT	Epidermis (TPA alone)	C243T	Synonymous	A84A		N/A	N/A
			C330G	F110L	F113L	DNA binding	non-functional	non-functional
			C477T	Synonymous	I162I	DNA binding	N/A	N/A
			G492A	Synonymous	Q167Q	DNA binding	N/A	N/A
			G492A	Synonymous	Q167Q	DNA binding	N/A	N/A
			G725A	G242D	G245D	DNA binding	non-functional	non-functional
			T825A	Synonymous	P278P	DNA binding	N/A	N/A
			C861T	Synonymous	R290R		N/A	N/A
			C943 deletion	Q315 frameshift	None		N/A	N/A
			A976G	T326A	T329A		N/A	functional
AID-/- FVB/N	Epidermis (TPA alone)	A171 deletion	S58 frameshift	None		N/A	N/A	
		T1053A	H351Q	None		N/A	N/A	
		T1056 deletion	A352 frameshift	A355 frameshift		N/A	N/A	

B

WT Epidermis <i>Trp53</i>	From	To					total
		A	T	G	C	del	
From	A	0	1	0	0	1	1
	T	1	0	0	0	0	1
	G	3	0	0	0	0	3
	C	0	3	1	0	1	5
total		4	3	2	0	1	10

**C**

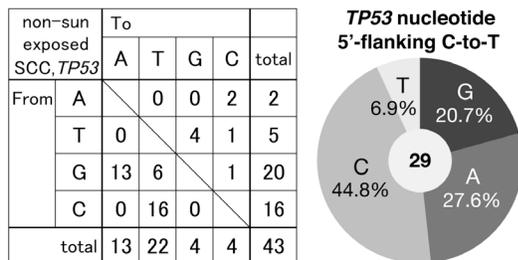
AID-/- Epidermis <i>Trp53</i>	From	To					total
		A	T	G	C	del	
From	A	0	0	0	0	1	1
	T	1	0	0	0	1	2
	G	0	0	0	0	0	0
	C	0	0	0	0	0	0
total		1	0	0	0	2	3

Supplemental Figure 5. *Trp53* mutations identified in TPA-alone-treated epidermis from AID-deficient mice and littermate FVB/N WT mice. Dorsal skin was treated with 16 nmol of TPA twice weekly for 21 weeks, and the epidermis was subjected to *Trp53* mutation analysis. *Trp53* transcripts were amplified, and PCR products were cloned into a vector and sequenced. (A) Summary of *Trp53* mutations in TPA-alone-treated epidermis. †, Predicted functions of p53 mutants were determined by comparison with human-equivalent mutants according to the IARC TP53 database. -/-, deficient mice. (B, C) Base substitution patterns and the nucleotide 5'-flanking C-to-T transition observed in *Trp53* in the TPA-alone-treated epidermis of FVB/N WT mice (B) and AID-deficient FVB/N mice (C).

A

Gene	Tumor type	Tumor sites	HPV	Mutation		Domain	Protein function †		PubMed ID	IARC
				Nucleotide	Codon		Structure-based	Transactivation		
TP53	SCC	Penis	negative	C423 insertion	C141 frameshift	DNA binding	N/A	N/A	18705766	28333
		Penis	negative	G524A	R175H	DNA binding	non-functional	non-functional	18705766	28332
		Penis	negative	G743T	R248L	DNA binding	non-functional	non-functional	18705766	28330
		Penis	negative	G746C	R249T	DNA binding	non-functional	non-functional	18705766	28331
		Vulva	negative	T428C	V143A	DNA binding	non-functional	non-functional	10802655	12993
		Vulva	negative	G437A	W146*	DNA binding	N/A	N/A	10486784	11418
		Vulva	negative	C451T	P151S	DNA binding	non-functional	non-functional	10802655	12998
		Vulva	negative	C455T	P152L	DNA binding	non-functional	non-functional	10802655	12983
		Vulva	negative	C455T	P152L	DNA binding	non-functional	non-functional	8617483	5140
		Vulva	negative	G467A	R156H	DNA binding	non-functional	partially functional	10486784	11417
		Vulva	negative	G469A	V157I	DNA binding	non-functional	partially functional	17539962	26138
		Vulva	negative	G469A	V157I	DNA binding	non-functional	partially functional	17539962	26139
		Vulva	negative	G469A	V157I	DNA binding	non-functional	partially functional	17539962	26140
		Vulva	negative	C482T	A161V	DNA binding	functional	partially functional	10802655	12991
		Vulva	negative	C502T	H168Y	DNA binding	functional	functional	10802655	12997
		Vulva	negative	A503C	H168P	DNA binding	functional	non-functional	8183560	2674
		Vulva	negative	G517T	V173L	DNA binding	non-functional	non-functional	10802655	12982
		Vulva	negative	G517T	V173L	DNA binding	non-functional	non-functional	10802655	12996
		Vulva	negative	G517T	V173L	DNA binding	non-functional	non-functional	10802655	12985
		Vulva	negative	G517T	V173L	DNA binding	non-functional	non-functional	8183560	2673
		Vulva	negative	G524A	R175H	DNA binding	non-functional	non-functional	10802655	12986
		Vulva	negative	G524A	R175H	DNA binding	non-functional	non-functional	10802655	12988
		Vulva	negative	C532T	H178Y	DNA binding	non-functional	partially functional	10802655	12989
		Vulva	negative	C535T	H179Y	DNA binding	non-functional	partially functional	10802655	12995
		Vulva	negative	T590G	V197G	DNA binding	non-functional	non-functional	17539962	26141
		Vulva	negative	T590G	V197G	DNA binding	non-functional	non-functional	17539962	26142
		Vulva	negative	T590G	V197G	DNA binding	non-functional	non-functional	17539962	26143
		Vulva	negative	T590G	V197G	DNA binding	non-functional	non-functional	17539962	26144
		Vulva	negative	A703 insertion	N235 frameshift	DNA binding	N/A	N/A	17539962	26148
		Vulva	negative	G733A	G245S	DNA binding	non-functional	non-functional	8617483	5137
		Vulva	negative	C742T	R248W	DNA binding	non-functional	non-functional	10802655	12981
		Vulva	negative	G743A	R248Q	DNA binding	non-functional	non-functional	10802655	12990
		Vulva	negative	G743A	R248Q	DNA binding	non-functional	non-functional	17539962	26145
		Vulva	negative	C817T	R273C	DNA binding	non-functional	non-functional	10802655	12999
		Vulva	negative	C817T	R273C	DNA binding	non-functional	non-functional	9887230	10058
		Vulva	negative	G818A	R273H	DNA binding	non-functional	non-functional	3894933	23202
		Vulva	negative	C832T	P278S	DNA binding	non-functional	non-functional	10486784	11419
		Vulva	negative	C843T	Synonymous	DNA binding	N/A	N/A	10802655	12987
		Vulva	negative	C844T	R282W	DNA binding	non-functional	non-functional	10802655	12984
		Vulva	negative	C844T	R282W	DNA binding	non-functional	non-functional	17539962	26146
		Vulva	negative	C844T	R282W	DNA binding	non-functional	non-functional	17539962	26147
		Vulva	negative	C844T	R282W	DNA binding	non-functional	non-functional	8617483	5139
		Vulva	negative	G853A	E285K	DNA binding	non-functional	non-functional	8183560	2671
Vulva	negative	G880T	E294*		N/A	N/A	8617483	5138		
Vulva	negative	A890C	H297P		N/A	functional	8183560	2672		

B



Supplemental Figure 6. TP53 mutations identified in human cutaneous SCC in the non-UV exposed regions. Cases of cutaneous SCC that developed in the sun-protected areas were collected from a dataset of TP53 somatic mutations in the IARC TP53 database; the selection included SCC of the penis and vulva in the category of external genitalia but excluded anal, vaginal, and urethral cancers. Radiation-induced, germline-mutated, and HPV-positive cancers were also excluded. (A) A summary of TP53 mutations identified in the non-UV exposed cutaneous SCC. †, Functions of p53 mutants were determined according to the IARC TP53 database. (B) Base substitution patterns and a nucleotide 5'-flanking C-to-T transition seen in TP53 gene in the non-UV exposed cutaneous SCC.

% of spontaneous skin tumor at head and neck area	
Mice	(No. of skin tumor at head and neck area / Total No. of skin tumor)
K14-AID-hi (n=13) †	41.67 (5/12)
K14-AID-hi-nu/nu (n=18) ‡	35.96 (32/89)

Supplemental Table 1. Frequency of spontaneous skin tumors in the head and neck area of K14-AID-hi Tg mice. † Mice were observed at 85 weeks of age. ‡ Mice were observed at 38 weeks of age. No., number.

Gene	Mouse	Tissue	Mutation		Human-equivalent codon	Domain	Protein function †		
			Nucleotide	Codon			Structure-based	Transactivation	
<i>Hras1</i>	K14-AID Tg	SCC (spontaneous)	G37A, G38T	G13I	G13I	G1	N/A	N/A	
			SCC (TPA alone)	A182T	Q61L	Q61L	adjacent to G3	constitutive active	N/A
				G38T	G13V	G13V	G1	constitutive active	N/A
		Papilloma (TPA alone)	G38T	G13V	G13V	G1	constitutive active	N/A	
			G38T	G13V	G13V	G1	constitutive active	N/A	
			G38T	G13V	G13V	G1	constitutive active	N/A	
		CAG-AID Tg	SCC (TPA alone)	A182T	Q61L	Q61L	adjacent to G3	constitutive active	N/A
			Papilloma (TPA alone)	A182T	Q61L	Q61L	adjacent to G3	constitutive active	N/A
		FVB/N WT	SCC (DMBA+TPA)	A182T	Q61L	Q61L	adjacent to G3	constitutive active	N/A
				A182T	Q61L	Q61L	adjacent to G3	constitutive active	N/A
	A182T			Q61L	Q61L	adjacent to G3	constitutive active	N/A	
	A182T			Q61L	Q61L	adjacent to G3	constitutive active	N/A	
	A182T			Q61L	Q61L	adjacent to G3	constitutive active	N/A	
	Papilloma (DMBA+TPA)		A182T	Q61L	Q61L	adjacent to G3	constitutive active	N/A	
			A182T	Q61L	Q61L	adjacent to G3	constitutive active	N/A	
			A182T	Q61L	Q61L	adjacent to G3	constitutive active	N/A	
			A182T	Q61L	Q61L	adjacent to G3	constitutive active	N/A	
			A182T	Q61L	Q61L	adjacent to G3	constitutive active	N/A	
	<i>Trp53</i>	K14-AID Tg	SCC (spontaneous)	G185A	R62Q	R65Q		N/A	N/A
				C191T	S64L	None		N/A	N/A
A220T				T74S	None		N/A	N/A	
C235G				P79A	P82A		N/A	functional	
C275T				S92F	S95F		N/A	partially functional	
C322T				Synonymous	L111L	DNA binding	N/A	N/A	
G336T				Q112H	None	DNA binding	N/A	N/A	
C338T				S113F	S116F	DNA binding	functional	functional	
C371G				S124C	S127C	DNA binding	non-functional	non-functional	
C379A				L127I	L130I	DNA binding	non-functional	partially functional	
C388T				Synonymous	None	DNA binding	N/A	N/A	
C388A				L130I	None	DNA binding	N/A	N/A	
G403A				A135T	A138T	DNA binding	non-functional	partially functional	
C532A				R178S	R181S	DNA binding	functional	partially functional	
C577T				R193W	R196W	DNA binding	N/A	N/A	
C577T, G578T			R193L	R196L	DNA binding	non-functional	partially functional		
T703C			C235R	C238R	DNA binding	non-functional	non-functional		
C930T			Synonymous	S313S		N/A	N/A		
SCC (TPA alone)			G341A	G114E	G117E	DNA binding	functional	partially functional	
			C353T	S118F	S121F	DNA binding	non-functional	functional	
	C377T	P126L	None	DNA binding	N/A	N/A			
Papilloma (TPA alone)	G724T	G242C	G245C	DNA binding	non-functional	non-functional			
	T246A	Synonymous	P85P		N/A	N/A			
	G248 insertion	A83 frameshift	A86 frameshift		non-functional	N/A			
	C301G	Q101E	Q104E		functional	functional			
	T337G	S113A	S116A	DNA binding	functional	functional			
Epidermis	C943 deletion	Q315 frameshift	None		N/A	N/A			
CAG-AID Tg	SCC (TPA alone)	C245 deletion	P82 frameshift	P85 frameshift		non-functional	N/A		
	Papilloma (TPA alone)	G804A	Synonymous	E271E	DNA binding	N/A	N/A		
		A296 insertion	T99 frameshift	T102 frameshift		non-functional	N/A		

Supplemental Table 2. Summary of *Hras1* and *Trp53* mutations identified in skin tumors of AID transgenic mice. Predicted functions of H-Ras and p53 mutants were determined through comparison with human-equivalent mutants. †, H-Ras functions were predicted on the basis of data reported in the literature; p53 functions were predicted on the basis of the IARC TP53 database.

Gene	Mouse	Tissue	Mutation		Human-equivalent codon	Domain	Protein function †	
			Nucleotide	Codon			Structure-based	Transactivation
<i>Trp53</i>	FVB/N WT	SCC (DMBA+TPA)	A349C	K117Q	K120Q	DNA binding	non-functional	non-functional
			G355T	V119F	V122F	DNA binding	N/A	N/A
			A481T	K161*	K164*	DNA binding	N/A	N/A
			A569T	H190L	H193L	DNA binding	non-functional	non-functional
			G673T	E225*	None	DNA binding	N/A	N/A
			A698C	Y233S	Y236S	DNA binding	non-functional	non-functional
			T715G	C239G	C242G	DNA binding	non-functional	non-functional
			G716A	C239Y	C242Y	DNA binding	non-functional	non-functional
			G809A	R270H	R273H	DNA binding	non-functional	non-functional
			A982T	K328*	None		N/A	N/A

Supplemental Table 3. *Trp53* mutations identified in DMBA+TPA-induced SCC in FVB/N WT mice. All *Trp53* mutation data were extracted from whole-exome sequencing (WES) data published by Nassar et al. (25). Briefly, DMBA+TPA-induced mouse skin SCC was treated with collagenase and FACS-isolated tumor cells were subjected to WES. †, Predicted functions of p53 mutants were determined through comparison with human-equivalent mutants according to the IARC TP53 database.

WT SCC DMBA+TPA <i>Trp53</i>		To				total
		A	T	G	C	
From	A	3	0	2	5	
	T	0	1	0	1	
	G	2	2	0	4	
	C	0	0	0	0	
total		2	5	1	2	10

Supplemental Table 4. Base substitution patterns observed in *Trp53* in DMBA+TPA-induced SCC from FVB/N WT mice. The same mutation data (25) were subjected to analysis as those for Supplemental Table 3.

Test	Gene	Sequence
qRT-qPCR	<i>Aicda</i>	Forward : CGTGGTGAAGAGGAGAGATAGTG Reverse : CAGTCTGAGATGTAGCGTAGGAA Probe : 5'-FAM-CACCTCCTGCTCACTGGACTTCGGC-BHQ1-3'
	<i>Hprt</i>	Forward : AATTGACACTGGTAAAACAATGC Reverse : CCTGTATCCAACACTTCGAGAG Probe : 5'-VIC-AGTACAGCCCCAAAATGGTTAAGGTTGC-BHQ1-3'
	<i>K14-AID</i>	Forward : CCCGAGGATCTTGAGAAC Reverse : CTGTCCATGGCGAATTGA Probe : 5'-FAM-CGTATTCGCCCTATAGTGAGTCGT-BHQ1-3'
	<i>Ccnd1</i>	Forward : CGCCCTCCGTATCTTACTTC Reverse : CTTCATCTTAGAGGCCACGAA
	<i>Egfr</i>	Forward : CAACATAGATGACGCATTCT Reverse : GGGCAGTGTTGAGATACTCAG
	<i>Trp53</i>	Forward : GGTGTCACGCTTCTCCGAAGA Reverse : GCATGGGCATCCTTTAACTCT
	<i>Pten</i>	Forward : CTCTCTCCTCCTTTTTCTTCA Reverse : TCATGGTATTTATCCCTCTT
	<i>AICDA</i>	Forward : TATTTTACTGCTGGAATACTTT Reverse : TGACATTCCTGGAAGTTGCTA Probe : 5'-FAM-AAGGGCTGCATGAAAATTCAGTTTCG-BHQ1-3'
	<i>RPLP0</i>	Forward : TGCATCAGTACCCATTCTATCA Reverse : AAGGTGTAATCCGTCTCCACAGA
	<i>CD19</i>	Forward : GGAAAGTATTATTGTCACCGTGGCAACCTG Reverse : CAGTCCTCAGCAGCCAGTGCCATAGTA Probe : 5'-FAM-TGTCATTCCACCTGGAGATCACTGCTCGGC-BHQ1-3'
Sequencing	<i>Hras1</i>	Forward : GATTGGCAGCCGCTGTA Reverse : CCTCTTTCCTTCCGTCG
	<i>Trp53</i>	Forward : AAGTGAAGCCCTCCGAGTGTC Reverse : TAGCATGGGCATCCTTTAACT
SNP genotyping	<i>Ptch1</i>	Forward : CCATCCGGACTCCAGACATC Reverse : CCAGAATGCCCTTCAGTAGAAA Probe (T1267N) : 5'-FAM-TCCCTTGAACCCTC-BHQ1-MGB-3' Probe (T1267T) : 5'-VIC-TCCCTTGACCCCTC-BHQ1-MGB-3'

Supplemental Table 5. Primers and probes used for qRT-PCR, sequencing, and SNP genotyping. MGB, minor groove binder.