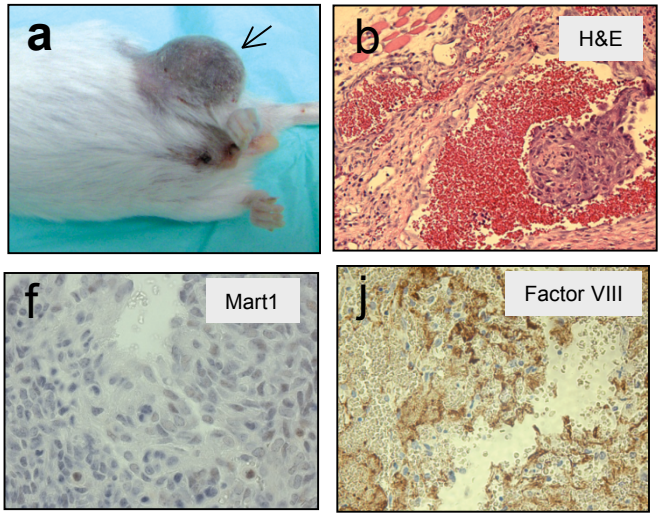
Figure S1

A



B

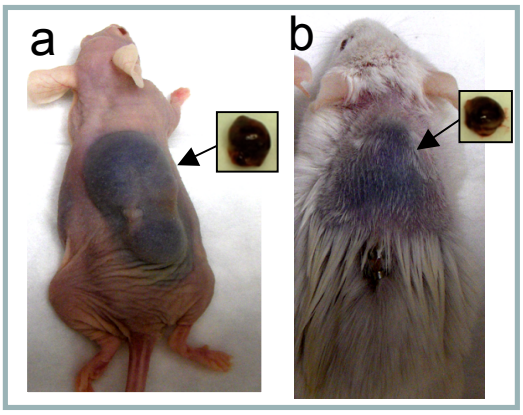


Figure S1

C

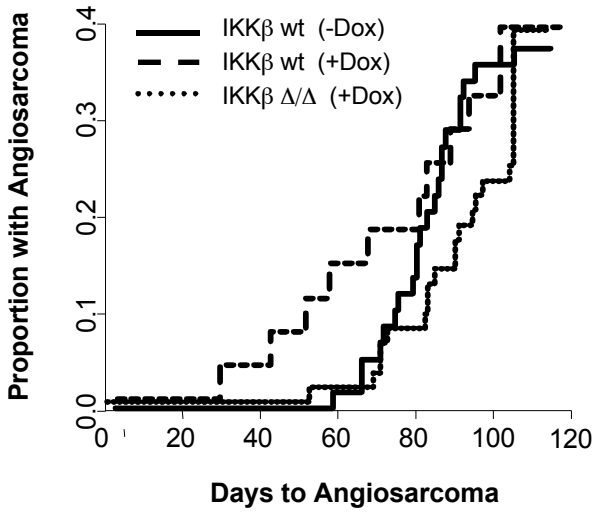
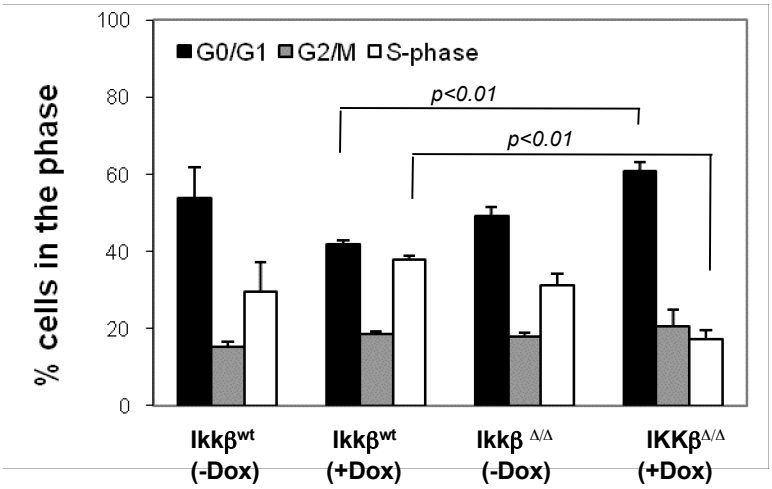


Table S1. *Ikkβ* **signature of angiosarcoma lesions**

Genotype			Cohort Size	Angiosarcoma		
Ikkβ	HRas^{V12}	Ink4a/Arf	n	n	Incidence (%)	Latency (Days)
+/+	-	-/-	60	22	36	85 ± 120
+/+	**+**	**-/-**	60	21	32	105 ± 21
-/-	**+**	**-/-**	65	21	35	77 ± 20

Figure S2

A


100
80
60
40
20
0
% cells in the phase
G0/G1
G2/M
S-phase
p<0.01
p<0.01
Ikkβwt (-Dox)
Ikkβwt (+Dox)
Ikkβ Δ/Δ (-Dox)
IKKβΔ/Δ (+Dox)

B

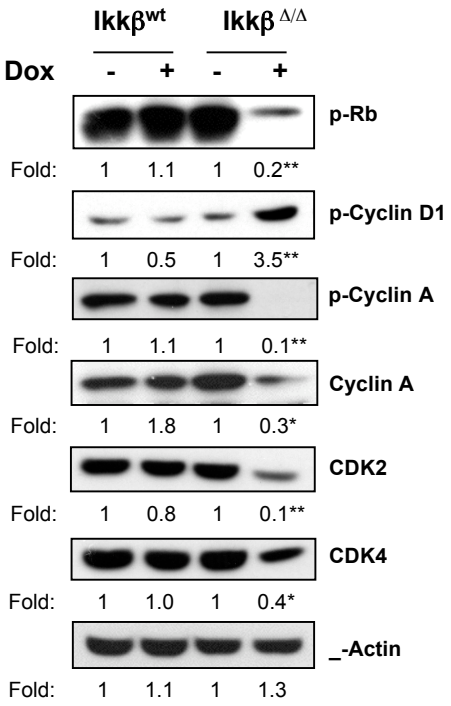

Ikkβwt
Ikkβ Δ/Δ
Dox - + - +
p-Rb
Fold: 1 1.1 1 0.2**
p-Cyclin D1
Fold: 1 0.5 1 3.5**
p-Cyclin A
Fold: 1 1.1 1 0.1**
Cyclin A
Fold: 1 1.8 1 0.3*
CDK2
Fold: 1 0.8 1 0.1**
CDK4
Fold: 1 1.0 1 0.4*
_-Actin
Fold: 1 1.1 1 1.3

Figure S3

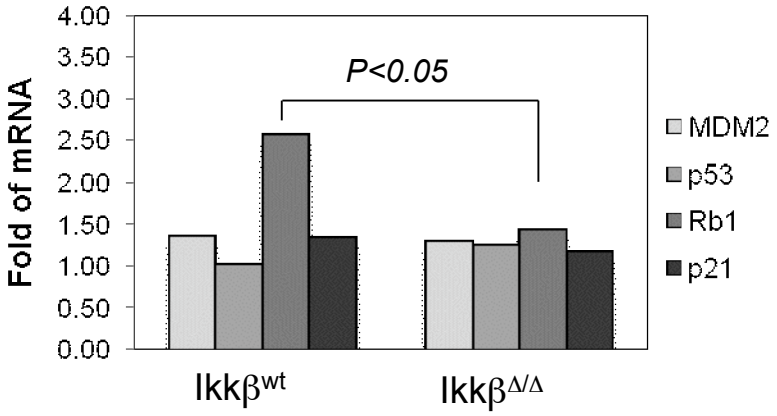
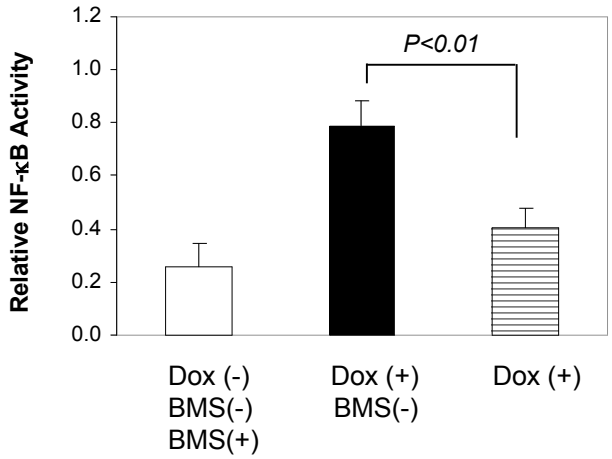


Figure S4



SUPPLEMENTAL RESULTS

Melanocyte-specific deletion of Ikkβ does not prevent formation of angiosarcoma lesions induced by loss of Ink4a/Arf. Although both $p16^{Ink4a}$ and $p19^{Arf}$ are negative regulators of NF-κB and act as inhibitors of cyclin-dependent kinases, loss of the *Ink4a/Arf* gene alone in the FVB mouse strain failed to induce melanoma tumors (Figure 2B). However, angiosarcoma lesions did arise in 32%-36% of the *Ink4a/Arf* null mice independent of $HRas^{V12}$ expression or loss of *Ikkβ* (Table S1). Angiosarcoma lesions were readily distinguished from melanoma. Angiosarcoma lesions formed in subcutaneous muscle tissue (Figure 1sAa), were comprised of blood filled sacs (Figure S1Aa,) and H & E staining of these lesions show that the highly vascular angiosarcoma contained large islands of blood cells (Figure S1Ab); angiosarcoma lesions are negative for the melanoma marker, Mart1 (Figure S1Ac); angiosarcoma lesions exhibited diffuse factor VIII staining throughout the tumor (Figure S1Ad). Factor VIII, synthesized and secreted by endothelial cells, is recognized as an endothelial cell marker. Cells from angiosarcomas that arose from both of $Ikk\beta^{wt}$ and $Ikk\beta^{\Delta/\Delta}$ mice were cultured in DMEM/F-12 medium containing 10%FBS, washed, suspended in saline and inoculated subcutaneously into mice. One month after inoculation, angiosarcoma lesions were observed in nu/nu mice (Figure S1Ba) or FVB mice (Figure S1Bb). The histological features of the angiosarcoma graft are identical to that of primary angiosarcoma (data not shown).

Legends:

Figure S1

Distinct features of angiosarcoma lesions: **(A)** Photographs of angiosarcoma lesions arising on mice (a); Sections of angiosarcoma (b-d) were stained with H&E (b), Mart1 (c) and Factor VIII (d). **(B)** Angiosarcoma xenografts were formed in nude mice (a) and FVB mice (b) by subcutaneous inoculation of early passage angiosarcoma cells (2×10^6) isolated from the angiosarcoma lesions that spontaneously arose on *Ikk*β^{wt} mice without doxycycline induction. **(C)** the cumulated angiosarcoma incidence from *Ikk*$\beta^{\Delta/\Delta}$ mice or *Ikk*β^{wt} mice treated with or without 1 mg/ml of doxycycline in drinking was plotted.

Figure S2

Deletion of *Ikk*β affects cell cycle progression**. (A)** Melanocytes derived from either E19 *Ikk*β^{wt} or E19 *Ikk*$\beta^{\Delta/\Delta}$ without synchronization and cell cycle was analyzed after 4 days of doxycycline induction for expression of $HRas^{V12}$ and/or deletion of *Ikk*β. The percentage of cells in cell cycle phases is indicated. **(B)** Lysates from early passage E19 melanocytes prepared by the indicated doxycycline treatment were analyzed for expression of cell cycle checkpoint proteins by immunoblotting with the indicated antibodies. β-actin was used as a loading control. The protein expression by immunoblotting was quantified and each value represented three independent experiments. The difference for the specific protein expression between doxycycline induced $HRas^{V12}$ expressed *Ikk*β^{wt} cells and the doxycycline-induced *Ikk*β deletion and $HRas^{V12}$ expressed *Ikk*$\beta^{\Delta/\Delta}$ cells was statistically analyzed. $^{**}p<0.01$, $^{*}p<0.05$.

Figure S3

Deletion of *Ikkβ* impacts cell cycle checkpoints transcriptionally. E19 melanocytes derived from Ikkβ^{wt} or Ikk$\beta^{\Delta/\Delta}$ mice had been treated with or without doxycycline (1μg/ml) for inducible expression of HRas^{V12} in both *Ikkβ^{wt}* and *Ikkβ$^{\Delta/\Delta}$* cells and inducible deletion of *Ikkβ* in the *Ikkβ$^{\Delta/\Delta}$* cells. Total RNA was extracted and mRNA was determined by Real Time RT-PCR method for quantitative analysis of *Mdm2, p53, Rb1* and *p21* gene. Results showed that HRas^{V12} induced *Rb* mRNA expression was ablated when *Ikkβ* was deleted (*p<0.05*).

Figure S4

Ikkβ inhibitor reduces intratumoral NF-κB activity. The FVB mice were xenografted with melanoma cells derived from a melanoma tumor excised from the skin of an FVB mouse with a genetic background of *Ikkβ$^{f/f}$::Tyr-rtTA::TetO-HRas^{V12}::Ink4a/Arf*. The subsequent tumor-bearing mice were subjected to treatments with BMS-345541 and/or doxycycline as described in Figure 6C legend. Tumor lysate was prepared to the final protein concentration of 0.5 mg/ml. The intratumoral NF-κB activity was determined using PathScan Phospho-NF-κB/p65 (Ser536) Sandwich ELISA kit (Cell Signaling Technology, Beverly, MA) per the manufacture's protocol. The NF-κB/p65 phosphorylation at Ser536 reflects the NF-κB activity in the tumor tissue. Results show that the elevated NF-κB activity upon the doxycycline-induced HRas^{V12} expression was significantly inhibited by administration of BMS-345541 (*p<0.01*). Each value represents the mean from 5 mice. Dox=doxycycline; BMS=BMS-345541.