

## ***BRaf*<sup>V600E</sup> cooperates with *Pten* silencing to elicit metastatic melanoma (Nature Genetics Supplementary Information)**

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### **Supplementary Table 1. Lethality of *Tyr::Cre*; *BRaf*<sup>CA</sup> mice**

*BRaf*<sup>CA/+</sup> and *BRaf*<sup>CA/CA</sup> mice were crossed to *Tyr::Cre* transgenic mice (*Tec1*). The resulting progeny were analyzed for the presence of the *BRaf*<sup>f</sup> or *BRaf*<sup>CA</sup> alleles and the *Tyr::Cre* transgene by PCR. No *Tyr::Cre*; *BRaf*<sup>CA</sup> mice were obtained in these crosses.

### **Supplementary Table 2. PCR primers used for genotyping *Pten*<sup>lox5</sup> mice**

### **Supplementary Figure 1. Induction of benign melanocytic lesions in *BRaf*<sup>CA/+</sup> mice requires the *Tyr::CreER* transgene**

(a) The ears of neonatal *BRaf*<sup>CA/+</sup> (left) and *Tyr::CreER*; *BRaf*<sup>CA/+</sup> (right) mice were treated with 4-HT and then monitored for 7 weeks for the development of benign melanocytic lesions. The feet of the 4-HT treated *BRaf*<sup>CA/+</sup> (b) and *Tyr::CreER*; *BRaf*<sup>CA/+</sup> (c) mice developed benign melanocytic lesions, most likely due to grooming behavior.

### **Supplementary Figure 2. Melanomas arising in *Tyr::CreER*; *BRaf*<sup>CA/+</sup>; *Pten*<sup>lox4-5/+</sup> mice**

Adult *Tyr::CreER* mice with the indicated genotypes were treated with 4-HT on the right ear, right flank and distal tail and monitored for the development of melanoma. Kaplan-Meier survival analysis of 4-HT treated: 1. *Tyr::CreER*; *BRaf*<sup>CA/+</sup>; *Pten*<sup>+/+</sup> mice (n=22); 2. *Tyr::CreER*; *BRaf*<sup>+/+</sup>; *Pten*<sup>lox4-5/lox4-5</sup> mice (n=5); 3. *Tyr::CreER*; *BRaf*<sup>CA/+</sup>; *Pten*<sup>lox4-5/lox4-5</sup> mice (n=22) and; 4. *Tyr::CreER*; *BRaf*<sup>CA/+</sup>; *Pten*<sup>lox4-5/+</sup> (n=5). Log rank tests of survival plots of the data indicated a statistically significant difference between the following survival curves: *Tyr::CreER*; *BRaf*<sup>CA/+</sup>; *Pten*<sup>n<sup>lox4-5/lox4-5/+</sup></sup> versus *Tyr::CreER*; *BRaf*<sup>CA/+</sup>; *Pten*<sup>lox4-5/lox4-5</sup> (p<0.0001) and *Tyr::CreER*; *BRaf*<sup>+/+</sup>; *Pten*<sup>lox4-5/+</sup> versus *Tyr::CreER*; *BRaf*<sup>CA/+</sup>; *Pten*<sup>+/+</sup> (p<0.0001). Melanomas that arose were solitary in nature and were observed only where 4-HT was administered on the ear (b-d), the tail (e & f) and the right flank (g-i).

### **Supplementary Figure 3. Confluent melanocytic proliferation and localized malignant melanoma induction in *Tyr::CreER*; *BRaf*<sup>CA/+</sup>; *Pten*<sup>lox5/lox5</sup> mice**

Adult *Tyr::CreER*; *BRaf*<sup>CA/+</sup>; *Pten*<sup>lox5/lox5</sup> mice were treated with 5µl of 5nM 4-HT in a solution of 100%

ethanol either on skin of the back (a), the right ear (b) or the distal tail (c). Mice were monitored for weeks prior to photography of the ear and flank melanoma and 6 months for photography of the tail melanoma. The fur of the mouse in (a) was removed using Veet® prior to treatment with 4-HT and again prior to photography.

**Supplementary Figure 4. Cre-mediated silencing of Pten expression in tumors derived from Tyr::CreER; BRaf<sup>CA/+</sup>; Pten<sup>lox5/lox5</sup> mice**

Malignant melanoma was initiated in Tyr::CreER; BRaf<sup>CA/+</sup>; Pten<sup>lox5/lox5</sup> mice by treatment with 4-HT. Melanoma-bearing mice were euthanized and tumor lysates were prepared for analysis of Pten expression by Western blotting. Extracts of NIH 3T3 cells were used as a positive control for Pten expression and Actin was used as a loading control for all lanes.

**Supplementary Figure 5. Cre-mediated recombination of BRaf<sup>CA</sup> and Pten<sup>lox5</sup> in 2697T cells**

DNA was isolated from 2697T cells and the toe of the mouse from which the cell line was derived. PCR analysis was used to confirm the rearrangement of the Pten<sup>lox5</sup> (homozygous) and BRaf<sup>CA</sup> (heterozygous) alleles.

**Supplementary Figure 6. Induction of pyknotic nuclei in Tyr::CreER; BRaf<sup>V600E</sup>; Pten<sup>null</sup> melanoma by treatment with PD325901, Rapamycin or the combination of both agents.**

Tyr::CreER; BRaf<sup>CA</sup>; Pten<sup>lox5/lox5</sup> mice with readily measurable melanoma lesions were randomly divided into 4 groups and administered either solvent control (Vehicle), PD325901 (PD), Rapamycin (Rapa) or the combination of both PD325901 and Rapamycin (PD+Rapa) for 3 weeks as indicated in the legend to Figure 6. Five separate fields of H&E stained paraffin tumor sections were quantified for the presence of pyknotic nuclei per square millimeter by visual inspection. Results are presented as a bar graph indicating the average number of pyknotic nuclei ± SEM with p values where significance was defined by unpaired t tests (Prism Graph).

**Supplementary Table 1. Lethality of *Tyr::Cre; BRaf<sup>CA</sup>* mice**

*BRaf<sup>CA/+</sup>* and *BRaf<sup>CA/CA</sup>* mice were crossed to *Tyr::Cre* transgenic mice (*Tec1*). The resulting progeny were analyzed for the presence of the *BRaf<sup>f</sup>* or *BRaf<sup>CA</sup>* alleles and the *Tyr::Cre* transgene by PCR. No *Tyr::Cre; BRaf<sup>CA</sup>* mice were obtained in these crosses.

Mouse Crosses	Offspring				p-value
	<i>BRaf<sup>CA/+</sup></i> ; -/-	<i>BRaf<sup>CA/+</sup></i> ; <i>Tec1</i> -/-	<i>BRaf<sup>f/+</sup></i> ; -/-	<i>BRaf<sup>f/+</sup></i> ; <i>Tec1</i> -/-	
<i>BRaf<sup>CA/+</sup></i> ; -/- X +/+ ; <i>Tec1</i> -/-	31/25	0/25	36/25	34/25	1.8E-7
<i>BRaf<sup>CA/CA</sup></i> ; -/- X +/+ ; <i>Tec1</i> -/-	21/10	0/10	-	-	4.6E-6
<i>BRaf<sup>CA/+</sup></i> ; -/- X +/+ ; <i>Tec1</i> / <i>Tec1</i>	-	0/11	-	22/11	2.7E-6

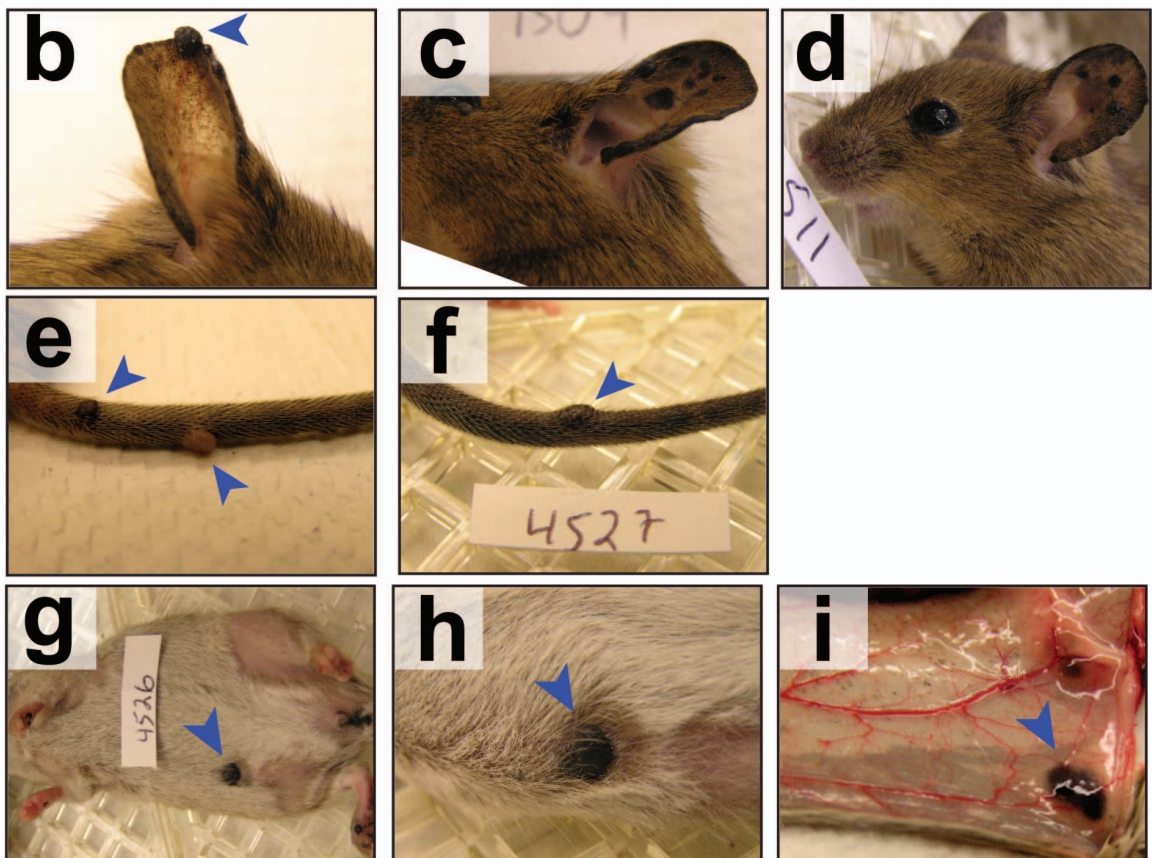
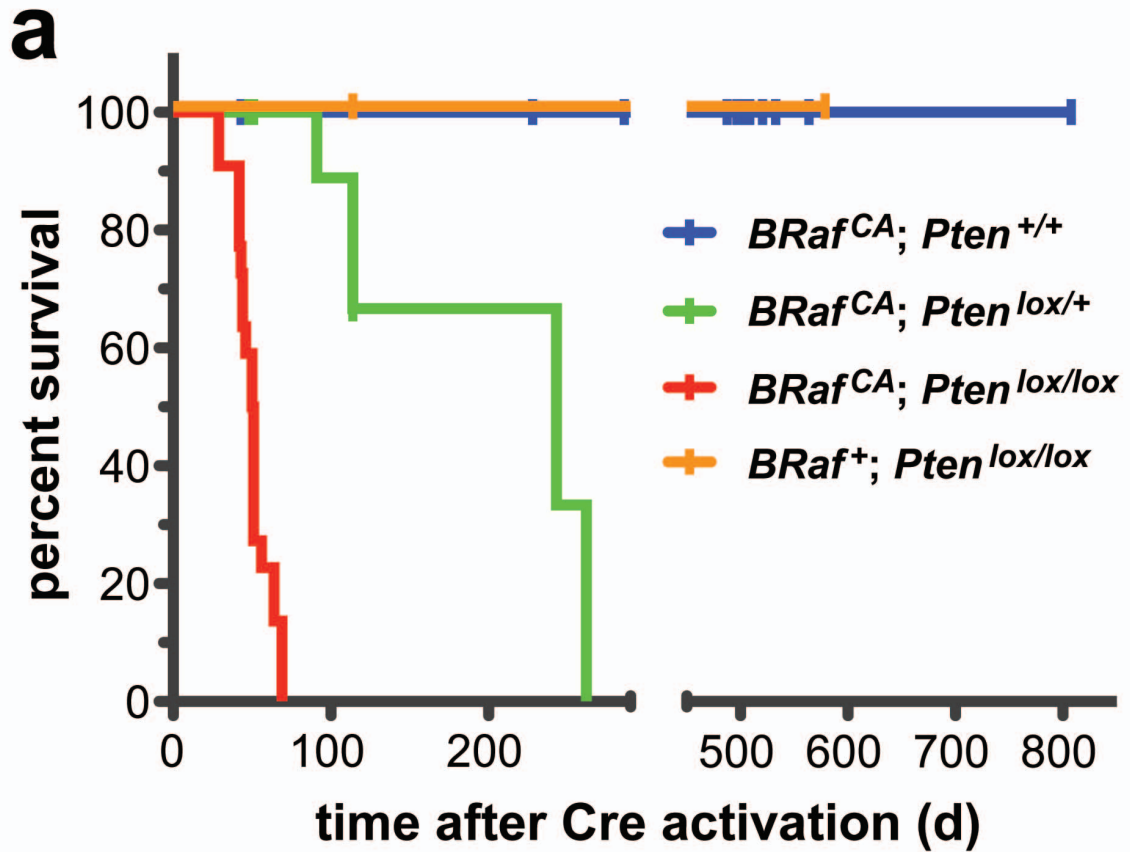
The indicated crosses were carried out. The potential genotypes of the offspring are indicated with shaded regions representing genotypes not possible in these matings. The observed /expected number of offspring of the indicated genotypes are depicted as is the Chi-square derived p-value.

**Supplementary Table 2. PCR primers for genotyping for the presence of the *Pten<sup>lox5</sup>* allele**

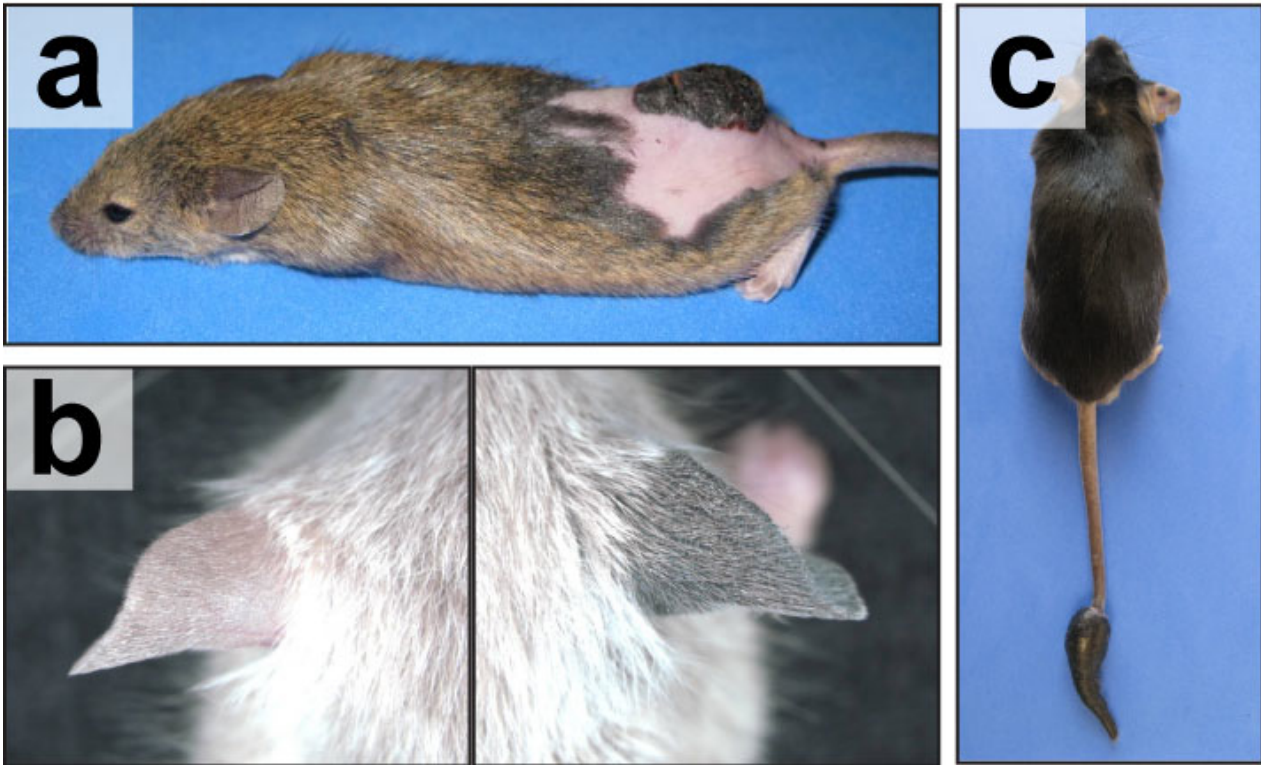
PCR primers for genotyping for the presence of the <i>Pten<sup>lox5</sup></i> allele	
<i>Pten<sup>lox5</sup></i> primer 1	5'-CTTCGGAGCATGTCTGGCAATGC-3'
<i>Pten<sup>lox5</sup></i> primer 2	5'-CTTCGGAGCATGTCTGGCAATGC-3'
<i>Pten<sup>lox5</sup></i> primer 2	5'-CTTCGGAGCATGTCTGGCAATGC-3'

Dankort *et al.* Supplemental Figure 1



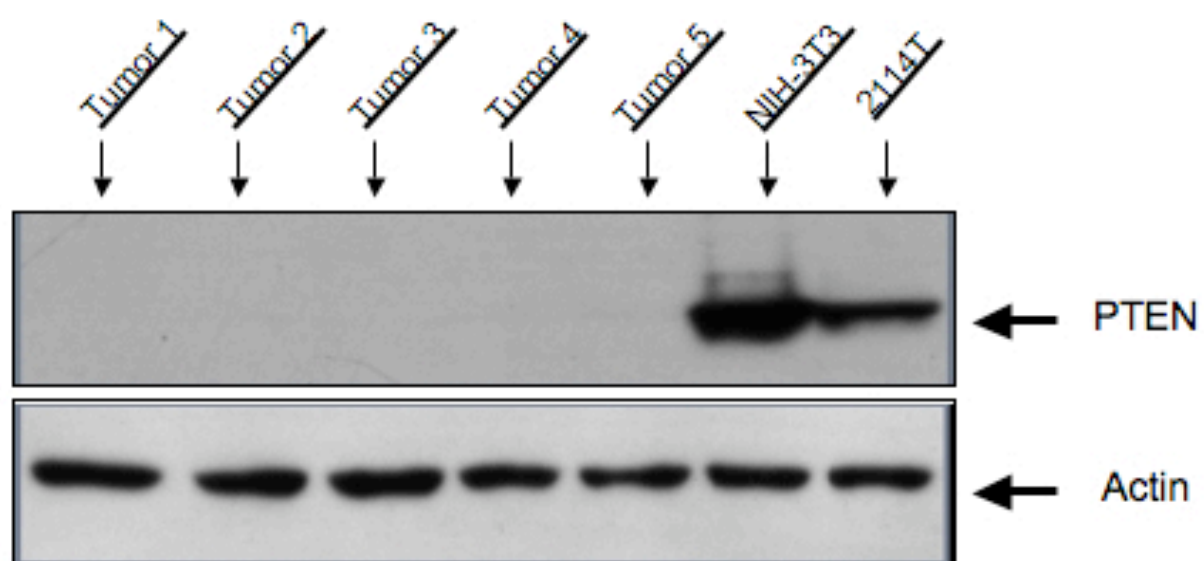


# Dankort *et al.* Supplemental Figure 3

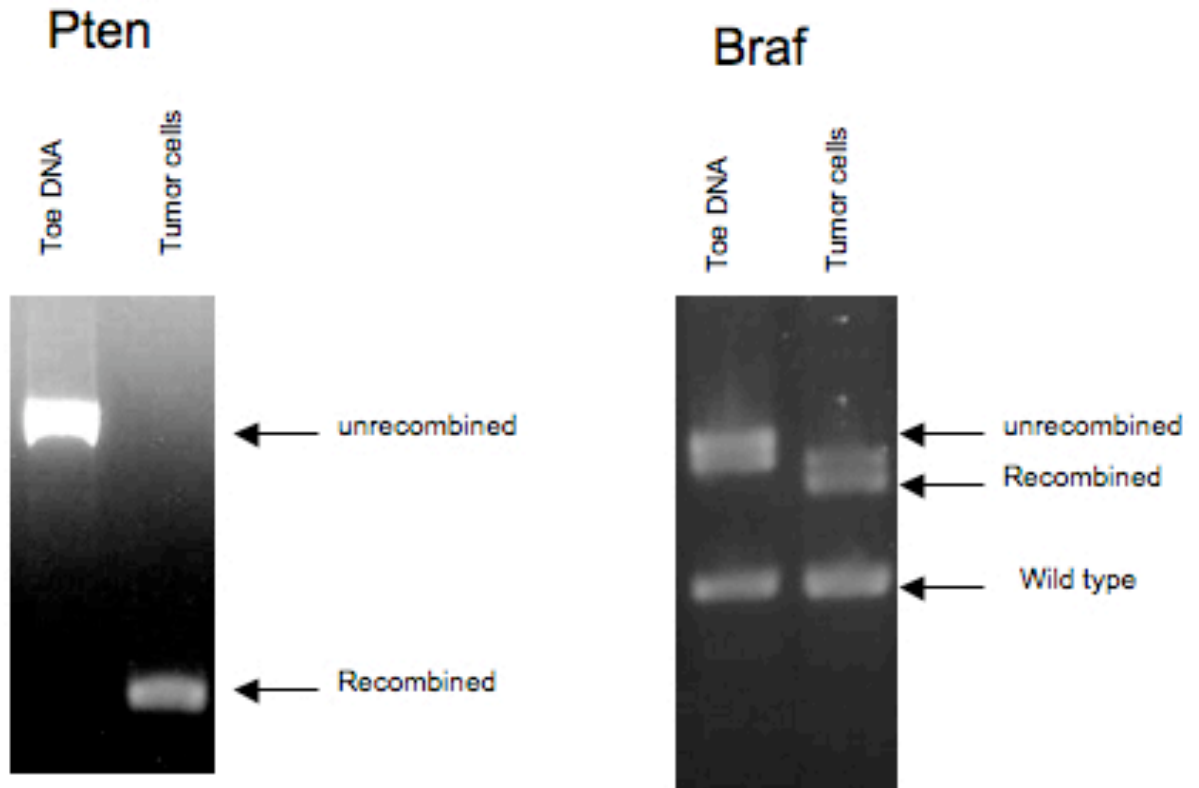




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# Dankort et al. Supplemental Figure 5





# Dankort *et al.* Supplemental Figure 6

