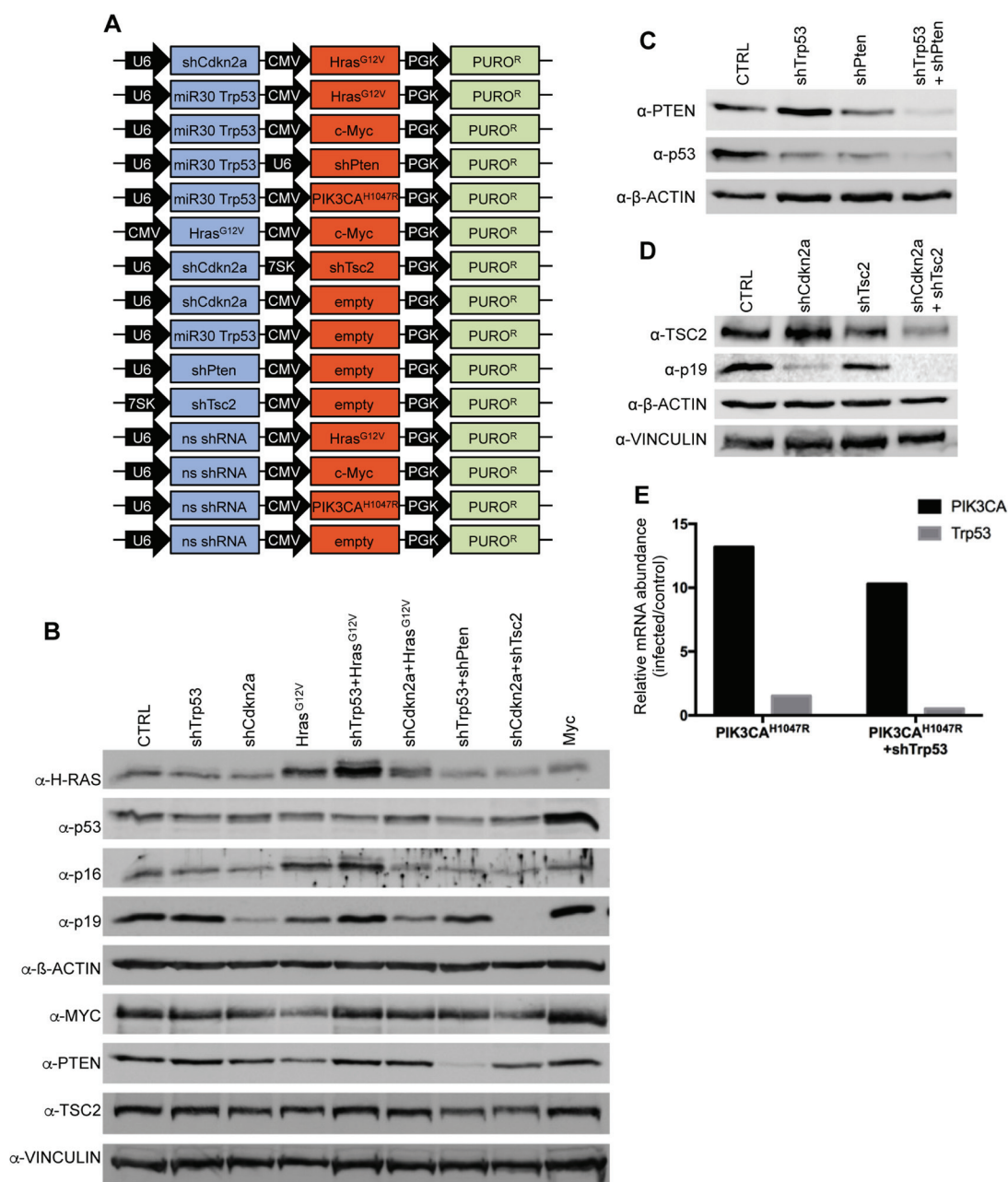
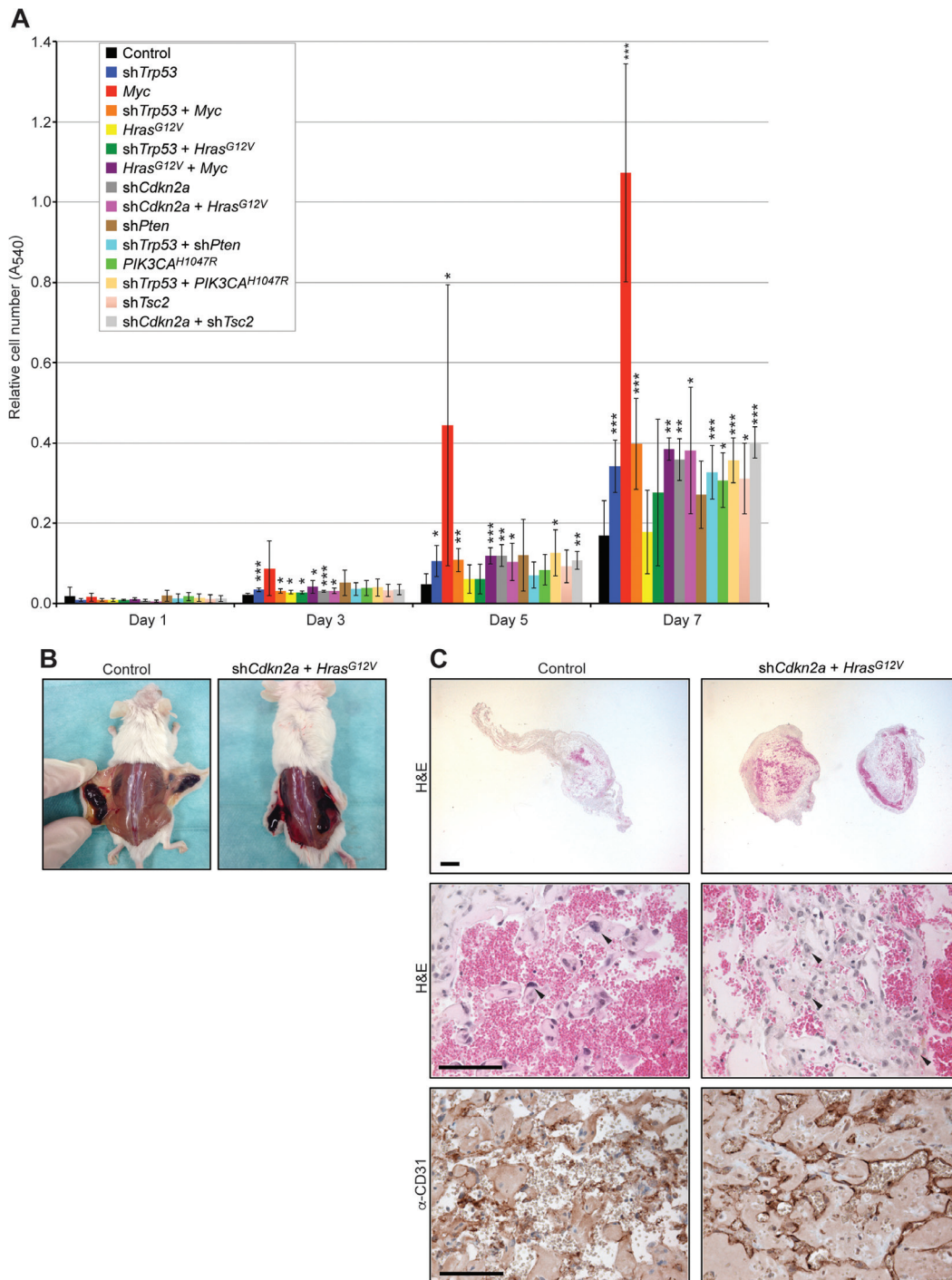


## Mouse genetic background influences whether *Hras*<sup>G12V</sup> expression plus *Cdkn2a* knockdown causes angiosarcoma or undifferentiated pleomorphic sarcoma

### SUPPLEMENTARY MATERIALS

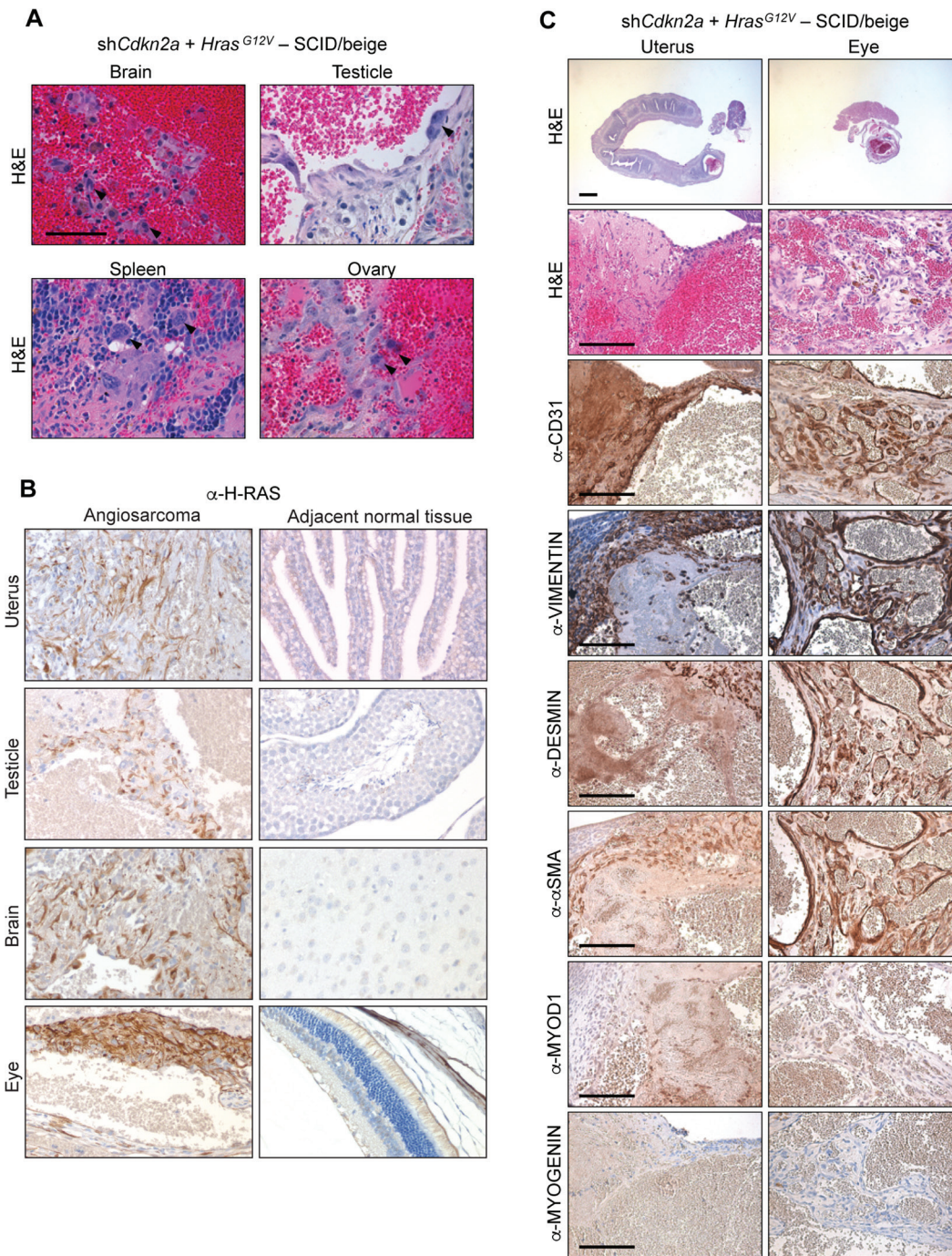


**Supplementary Figure 1: Validation of MuLE vectors by western blot and real time PCR analysis.** (A) Schematic of MuLE vectors simultaneously expressing combinations of shRNAs against *Cdkn2a*, *Trp53*, *Pten* and *Tsc2* with or without expression of *Hras*<sup>G12V</sup>, *Myc* or *PIK3CA*<sup>H1047R</sup> and puromycin resistance. (B–D) Western blots of primary endothelial cells from the spleen (pMSECs) transduced with the indicated lentiviruses. Control (CTRL) refers to the final vector depicted in A expressing a non-silencing control shRNA and lacking a cDNA behind the CMV promoter. (E) Relative gene expression of *PIK3CA* and *Trp53* in pMSECs transduced with the indicated lentiviruses.



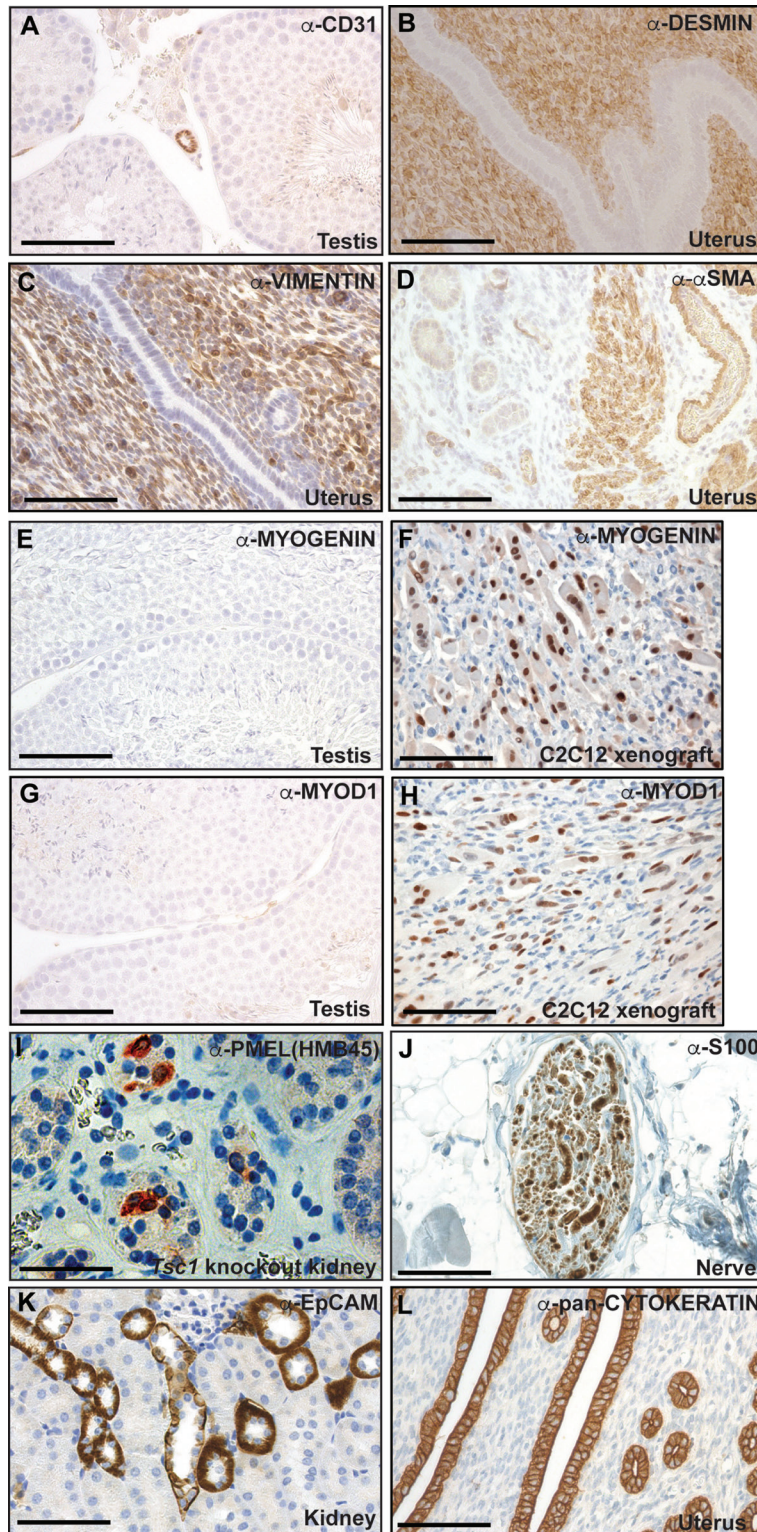
**Supplementary Figure 2: Analyses of functions of oncogenes and tumour suppressors in pMSECs.** (A) Proliferation assays of pMSECs generated in Supplementary Figure 1A using SRB assays. Graph depicts mean  $\pm$  s.d. derived from experimental triplicates from each of three independent experiments. Statistical comparisons were made to pMSECs infected with an empty MuLE virus (Control) using Student's *t* test. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ . (B) Images of blood-filled lesions formed 4 weeks after subcutaneous injection of control pMSECs or pMSECs infected with MuLE lentiviruses expressing a shRNA against *Cdkn2a* together with *Hras<sup>G12V</sup>*. (C) H&E and anti-CD31 immunohistochemical staining of the lesions described in B. Arrowheads highlight aberrant nuclei.





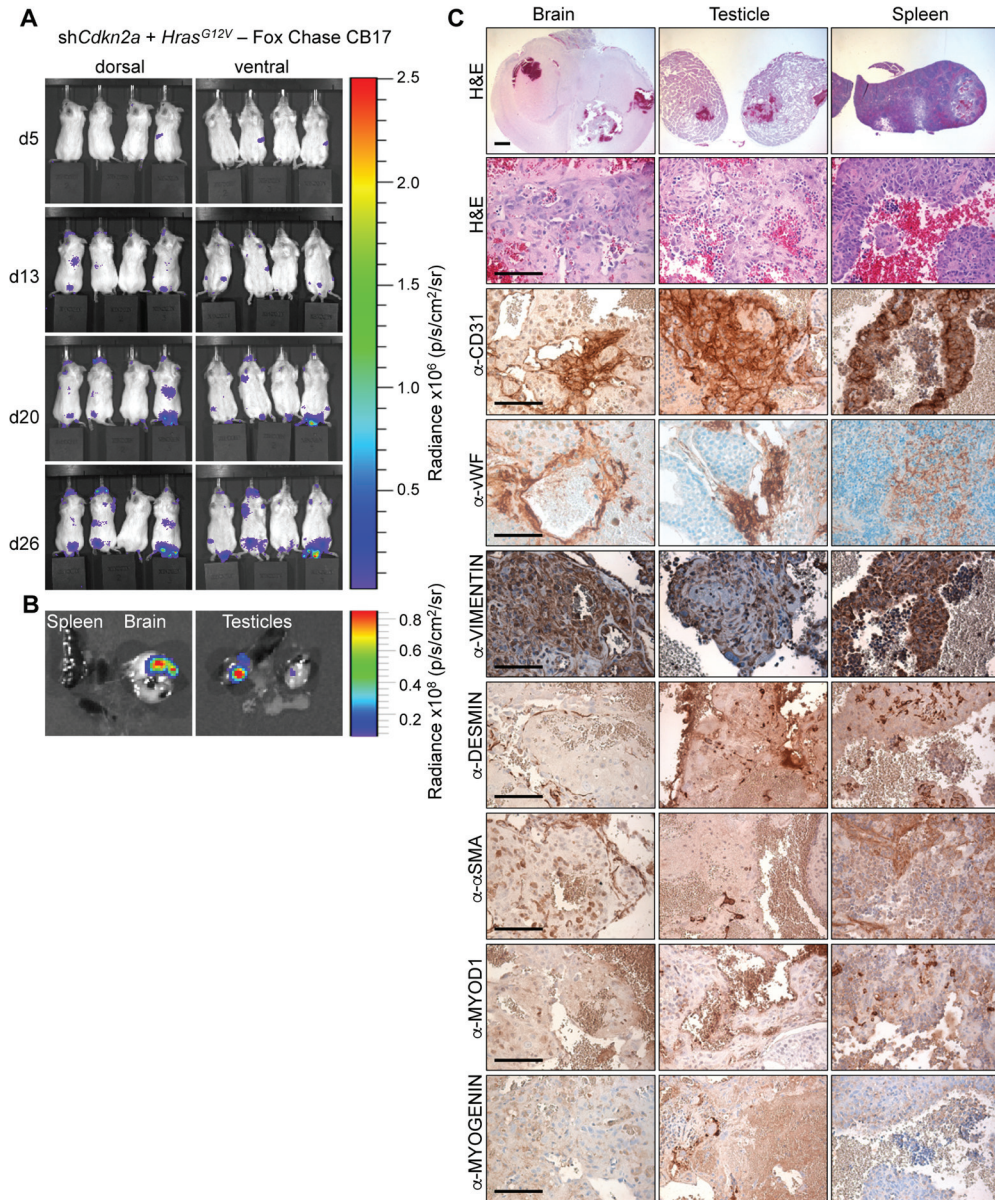
**Supplementary Figure 3: Additional information related to Figure 1 and Figure 2.** (A) High magnification images of H&E stained tumour sections derived from the systemic injection of *shCdkn2a* plus *Hras<sup>G12V</sup>* viruses in SCID/beige mice. Arrowheads indicate atypical nuclei. Scale bar: 100  $\mu$ m. (B) Immunohistochemical stainings using an anti-H-RAS antibody of *shCdkn2a* plus *Hras<sup>G12V</sup>* angiosarcomas and adjacent normal tissue. (C) H&E and immunohistochemical stainings of additional tumours derived from the systemic injection of *shCdkn2a* plus *Hras<sup>G12V</sup>* viruses. Scale bars: 100  $\mu$ m.



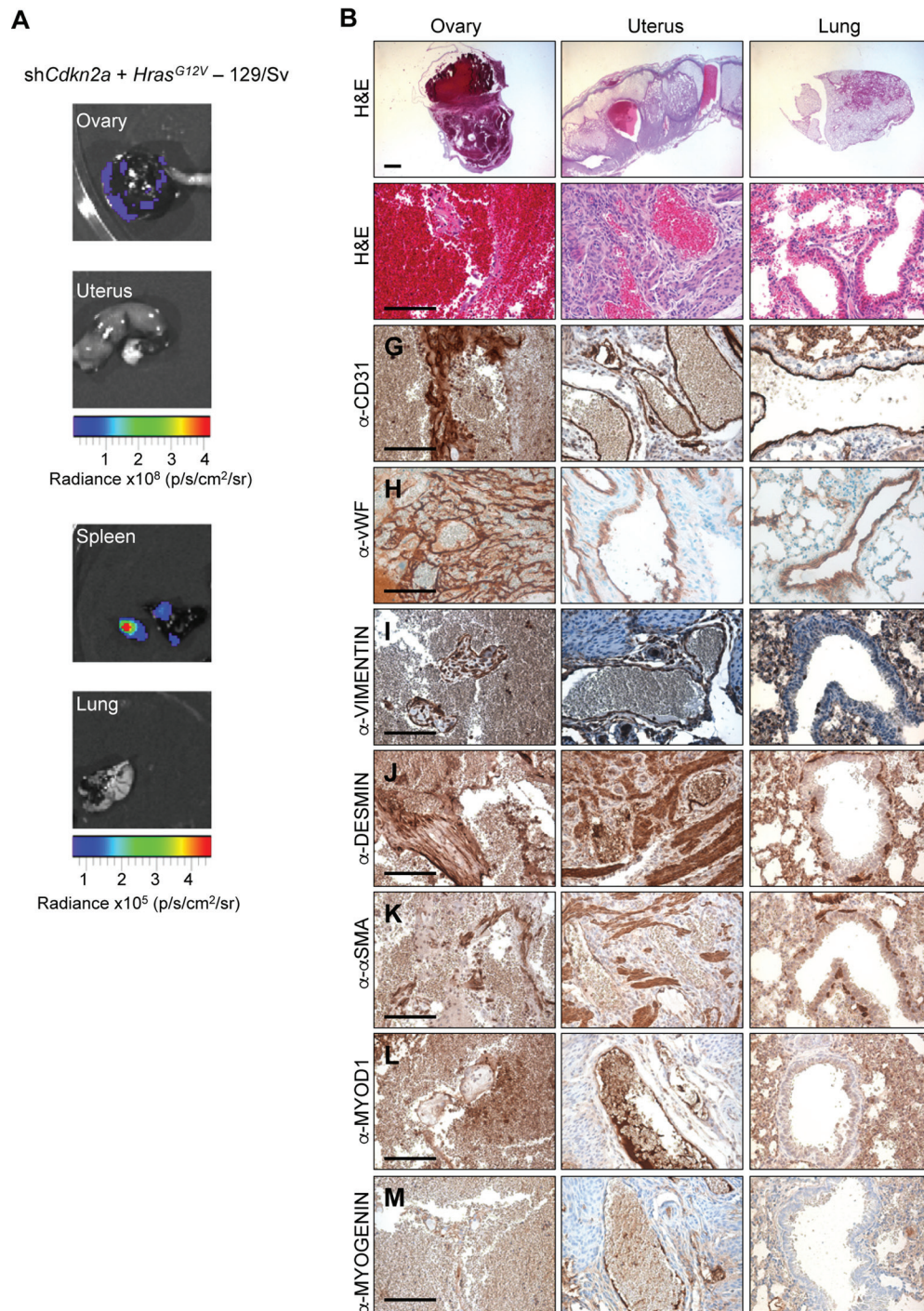


**Supplementary Figure 4: Positive and negative immunohistochemical staining controls of antibodies used in this study.** (A) Mouse testis showing CD31 positivity in a blood vessel and negative staining in tubules. (B) Mouse uterus showing DESMIN positivity in the stroma and negative staining in epithelia. (C) Mouse uterus showing VIMENTIN positivity in the stroma and negative staining in epithelia. (D) Mouse uterus showing alpha-SMA positivity in myometrium and negative staining in epithelial cells. (E) Negative staining for MYOGENIN in testis and (F) positive staining in xenograft tumour of the C2C12 myoblast cell line. (G) Negative staining for MYOD1 in testis and (H) positive staining in xenograft tumour of the C2C12 myoblast cell line. (I) Positive staining for PMEL(HMB45) in a *Tsc1* mutant kidney, a mouse model of renal angiomyolipoma (from Gonçalves et al. Nature Communications, 2017, DOI: 10.1038/s41467-017-01514-3). (J) Positive staining for S100 in an intramuscular nerve fibre. (K) Positive and negative immunoreactivity for EpCAM in renal epithelial tubules. (L) Mouse uterus showing positive staining for pan-CYTOKERATIN in epithelia and absence of staining in stroma. Scale bars: 100  $\mu$ m.





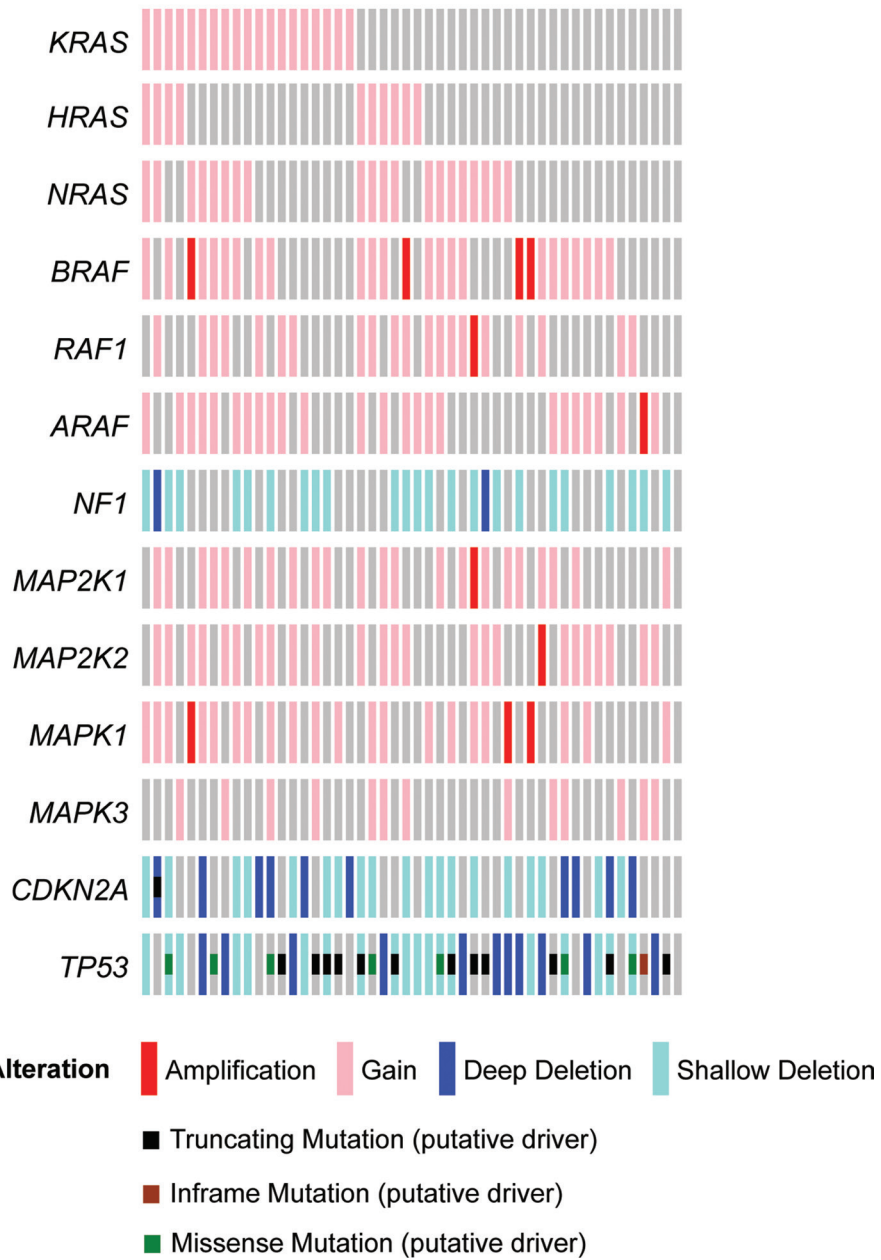
**Supplementary Figure 5: *Hras<sup>G12V</sup>* expression plus knockdown of *Cdkn2a* causes angiosarcomas in Fox Chase CB17 mice.** (A) Bioluminescence imaging 5, 13, 20 and 26 days after the injection of MuLE lentiviruses expressing a shRNA against *Cdkn2a* together with *Hras<sup>G12V</sup>* into the tail vein of 4-6-week-old Fox Chase CB17 mice. (B) Bioluminescence imaging showing examples of tumour-bearing organs. (C) H&E and immunohistochemical stainings using the indicated antibodies. Low magnification scale bar: 1000  $\mu$ m and high magnification scale bar: 100  $\mu$ m.



**Supplementary Figure 6: Additional information related to Figure 3.** (A) Bioluminescence imaging showing examples of tumour-bearing organs. (B) H&E and immunohistochemical stainings using the indicated antibodies. Low magnification scale bar: 1000  $\mu$ m and high magnification scale bar: 100  $\mu$ m.



Undifferentiated Pleomorphic Sarcoma/  
Malignant Fibrous Histiocytoma/  
High-Grade Spindle Cell Sarcoma (n=48)



**Supplementary Figure 7: Genetic landscape of human UPS tumours.** Oncoprint analysis from cBioPortal of the chromosomal copy number status and mutation status of the indicated genes of the RAS-RAF-MEK-MAPK pathway and of *CDKN2A* and *TP53* in 48 cases of human UPS from the TCGA Sarcoma provisional dataset.