

Supporting Information

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SI Materials and Methods

Histology, Immunohistochemistry, and Immunofluorescence. The following primary antibodies were used: Rac1, phospho-Histone H2A.X (Upstate), smooth muscle α -actin (Abcam), cytokeratin, vWF (Chemicon), CD31, CD34 (BD PharMingen), p53 (Santa Cruz), and 8-oxodG antibody (Japan Institute for the Control of Aging). Protocols provided by the manufacturers were followed. Antibody concentration was optimized individually. For immunohistochemistry, color development was achieved using diaminobenzidine staining kits from Santa Cruz Biotechnologies. Slides were counterstained with hematoxylin (Santa Cruz Biotechnologies), dehydrated, and mounted with Permount (Biomed). For immunofluorescence microscopy, imaging was achieved using a Zeiss LSM-510 Confocal Laser Scanning Microscope. Substitution of the primary antibody with irrelevant IgG isotopes served as negative controls.

Western Blot. Mouse HIF-1 α antibody was obtained from Sigma. A Western blot assay was performed according to the manufacturer's instructions.

Chromosomal Aberration Analysis. For the evaluation of structural chromosomal aberrations, mitotic arrest was initiated 2 h before harvest by addition of colcemid (0.2 μ g/mL), and cells were then trypsinized. After centrifugation, cells were resuspended in a prewarmed hypotonic solution (75 mM KCl) for 30 min and fixed in methanol/acetic acid (3:1). Chromosome spreads were covered with Wright's stain for 1 min and washed. Three hundred metaphases were examined for the occurrence of different types of structural aberrations. Both chromatid-type and chromosome-type aberrations were scored. Gaps were excluded in the calculation of chromosome breakage frequencies. Chromatid exchange configurations and dicentric and ring chromosomes were scored as 2 breaks. Metaphases containing more than 5 aberrations were considered as cells with multiple aberrations.

Soft Agar Assay. The 5% (weight/vol) agar stock was made in PBS and sterilized in the autoclave. To prepare the bottom agar layers, 90 mL of culture medium was mixed with 20 mL of 5% agar at 45 °C; 5 mL per 60-mm dish was used and allowed to solidify. To make the cell layer, a single cell suspension was prepared at 1×10^4 cells/mL, 1×10^5 cells/mL, and 1×10^6 cells/mL. The cells were briefly warmed to 4 °C and mixed gently with 2 mL of the above 0.5% agar/medium, and the mixture was transferred to the hardened 0.5% agar base layer. The plates were cultured for 3 weeks. Cells were fed twice a week by drop-wise addition of the growth media. Five plates for each cell line were used. The colonies (≥ 30 cells) that each cell line produced were counted.

Flow Cytometric Analysis. Two hundred microliters of peripheral blood collected from each mouse was centrifuged at $200 \times g$ for 10 min, and plasma was removed. Red blood cells were lysed with 3 mL of erythrocyte lysis buffer (Buffer EC; QIAGEN). Cell phenotyping was performed by flow cytometric multiparameter analysis using a FACSCalibur Flow Cytometer (BD Biosciences) according to the standard procedure. Briefly, cells were stained

with the following fluorescence-conjugated antibodies for 30 min at 4 °C: fluorescein isothiocyanate (FITC)-Gr1, allophycocyanin (APC)-CD11c (BD PharMingen), phycoerythrin (PE)-CD11b, phycoerythrin-Texas Red (PETR)-CD4, PE-NK 1.1, PETR-CD8, and FITC-B220 (Caltag). Antibody concentration was individually optimized. Cells stained with corresponding fluorescence-conjugated B20 antibody were used as a positive control. At least 100,000 events per sample were routinely examined. Data analysis was done using FlowJo software, MAC version 6.4 (Tree Star). Dead cells were excluded from analysis by propidium iodide staining.

RT-PCR. Total RNA was isolated from various mouse tissues using QIAGEN RNeasy kit (Qiagen). Target mRNA was amplified using an AccuRT RNA PCR Kit (Applied Biosystems) following the manufacturer's instructions.

Nested PCR. Nested PCR for detection of human herpesvirus-8 was performed as described previously (1).

Primer Sequences and PCR Conditions.

Set #1 from ORF26 (minor capsid protein vp23) Product: 233bp

Outer primers.

HV1-1 (CAC CCA GCT AGC AGT GCT ACC C)

HV1-2 (CGT GAG CAG ACG GAG ACA CCC)

Cycler settings: 94 °C for 1 min, 65 °C for 1 min and 72 °C for 1 min over 40 cycles.

Inner primers

HV1-3 (AGC CGA AAG GAT TCC ACC AT)

HV1-4 (TCC GTG TTG TCT ACG TCC AG)

Cycler settings: 94 °C for 1 min, 58 °C for 1 min and 72 °C for 1 min over 30 cycles.

Set #2: from ORF25 (major capsid protein, MCP) Product: 213 bp

Outer primers

HV2-1 (AGG CAA CGT CAG ATG TGA C)

HV2-2 (GAA ATT ACC CAC GAG ATC GC)

Cycler settings: 94 °C for 30 sec, 60 °C for 1 min and 72 °C for 1 min over 30 cycles.

Inner primers

HV2-3 (CAT GGG AGT ACA TTG TCA GGA CCT C)

HV2-4 (GGA ATT ATC TCG CAG GTT GCC)

Cycler settings: 94 °C for 30 sec, 60 °C for 1 min and 72 °C for 1 min over 20 cycles.

Set #3 also from ORF25 Product: 115 bp

Outer primers

HV3-1 (GGC GAC ATT CAT CAA CCT CAG G)

HV3-2 (ATA TCA TCC TGT GCG TTC ACG AC)

Cycler settings: 94 °C for 30 sec, 60 °C for 1 min and 72 °C for 1 min over 30 cycles.

Inner primers

HV3-3 (CGC ATG GAG GAC CTA GTC AAT AAC)

HV3-4 (GGT TGT AGT CAT TCT CGT CCA GGG)

Cycler settings: 94 °C for 30 sec, 60 °C for 1 min, and 72 °C for 1 min over 20 cycles

1. Albrecht D, Meyer T, Lorenzen T, et al. (2004) Epidemiology of HHV-8 infection in HIV-positive patients with and without Kaposi sarcoma: Diagnostic relevance of serology and PCR. *J Clin Virol* 30:145–149.

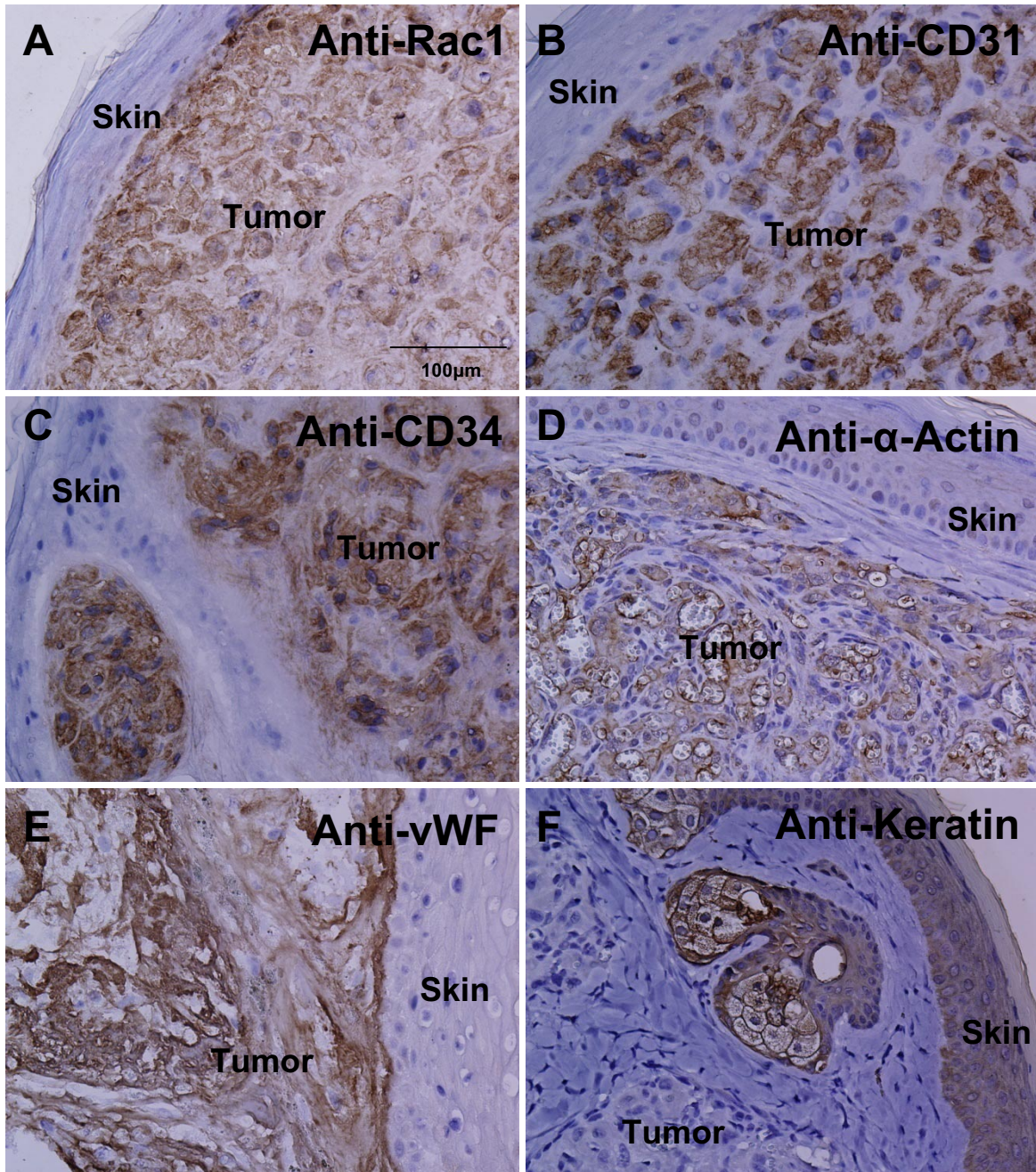


Fig. S2. Immunophenotype of the RacCA tumor tissue. Immunohistochemical determination for Rac1, CD31, CD34, vWF, α -actin, and cytokeratin in RacCA tumor tissue.

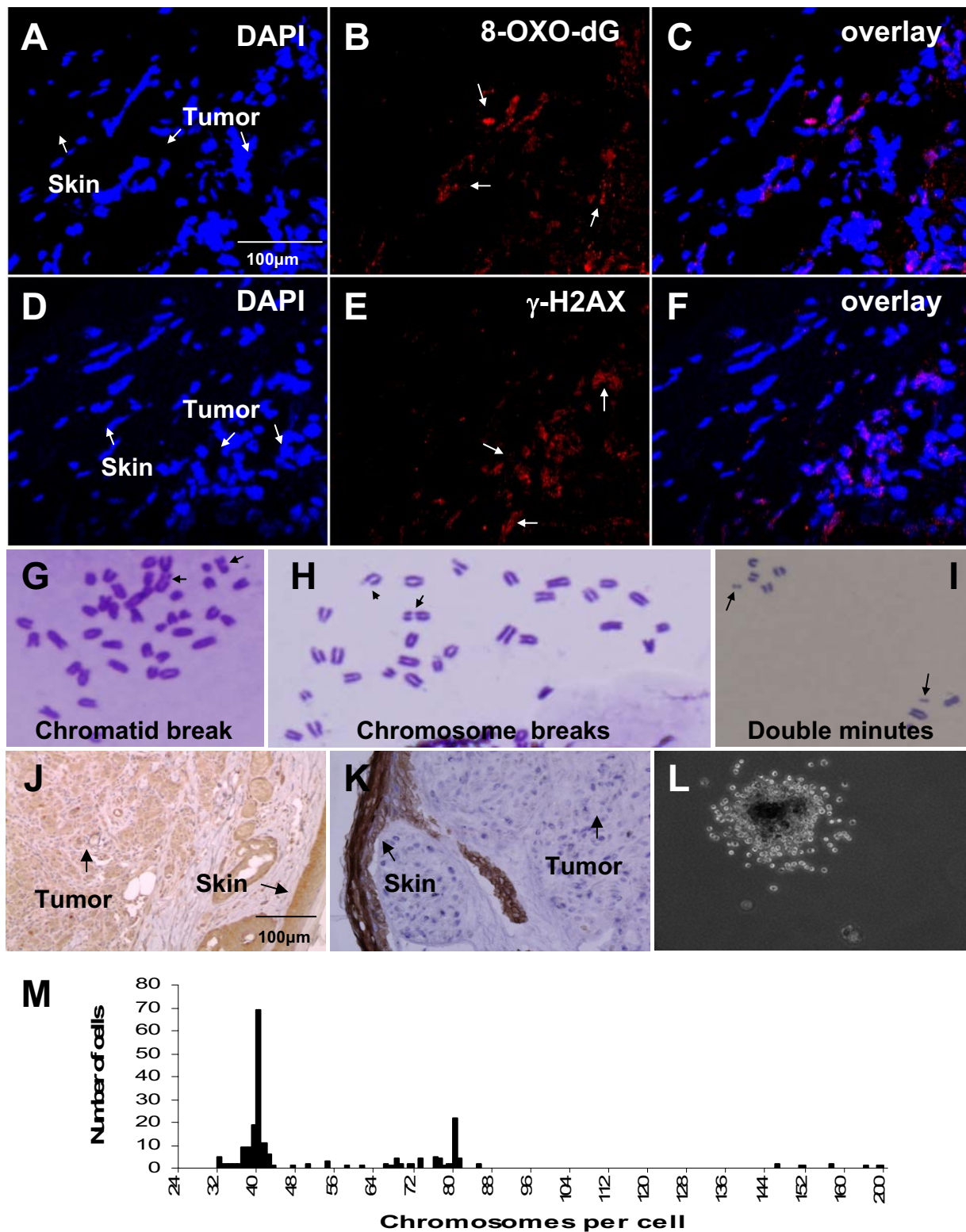


Fig. 53. Genome instability in RacCA tumors. (A–C) DNA oxidative damage measured by nuclear 8-oxodG immunostaining in RacCA tumor tissue compared with neighboring skin tissue. (D–F) Immunohistochemistry staining of phospho-H2AX to detect foci of DNA repair in tumor tissue. (G–I) Presence of chromosome rearrangements in primary cultures of RacCA tumor cells. Loss of p53 expression is a frequent event (67%) in RacCA tumors. Immunohistochemistry detection of p53 in RacCA p53⁺ (J) and p53⁻ tumors showing adjacent p53⁺ skin (K). (L) Colony formation in soft agar of cultured RacCA cells (RacCA efficiency, 4%; SMC, 0%). (M) RacCA tumor cells exhibit aneuploidy as indicated by chromosome numbers from 207 tumor cells at passage 2.

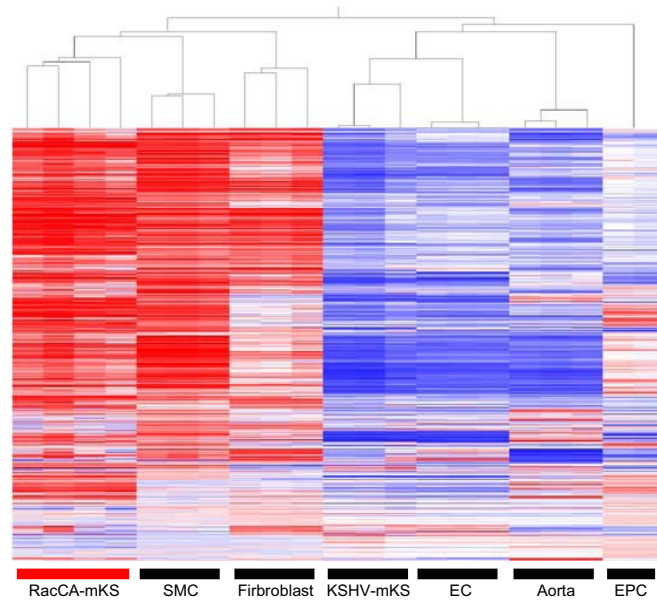


Fig. S4. Lineage analysis. Unsupervised clustering of RacCA-mKS transcriptome placed it between mouse SMCs and mouse fibroblasts.

Other Supporting Information Files

[Table S1](#)

[Table S2](#)

[Table S3](#)