SUPPLEMENTAL MATERIALS AND METHODS

Immunohistochemistry of Tissue Sections. It was essentially performed as previously described by us (9). A Tissue microarray (TMA) of paraffin embedded human melanoma tissue samples was obtained from US Biomax (Mel207; 69 cases/207 cores). Slides were de-paraffinized in xylene, dehydrated through a graded series of ethanol washes, and placed in PBS. After performing antigen retrieval by heating the sections in 10 mM Citrate Buffer (pH 6.0) in a pressure cooker, tissue sections were incubated for 15 min in 3% H_2O_2 to quench endogenous peroxidase. After rinsing samples with PBS, sections were blocked with 10% goat serum in PBS for 30 minutes at RT. Samples were then incubated with primary antibodies diluted in 1% BSA in PBS overnight at 4°C. Primary antibodies were used at the following dilutions: anti-Cav1 pAb (1:500; N-20 Santa Cruz). Binding of primary antibody was visualized using an IHC detection kit from Vector Labs. A Nova Red Kit (Vector Labs) was used as the HRP substrate. Images were acquired using an Olympus (BX 51) inverted microscope.

Semi-quantitative Analysis of Immunoreactivity. After performing CAV1 immunostaining (see above), individual tissue melanoma cores were scored for intensity of CAV1 immunoreactivity. An expert dermatopathologist (JBL) blindly scored all the samples giving a score of 0 to absence of CAV1 staining, 1 for weak CAV1 staining, 2 for moderate CAV1 staining, and 3 for strong CAV1 staining. CAV1 immunoeactivity scores for primary melanoma and metastatic melanoma lesions were then grouped for analysis. Statistical significance between categories (immunoreactivity scores) was determined by χ^2 test for independence.

Orthotopic Injections in Mice. Injctions were performed essentially as previously described (16). 10^6 B16F10 melanoma cells were suspended in 100 µl of PBS and intradermally injected in 3-4 mo old C57Bl/6J female mice. Tumor growth was monitored weekly by measuring the length

(*L*) and width (W) of the tumor using a caliper. Tumor volume was determined by the following formula: V= 0.52 LW^2. Body weight was also recorded at the beginning and at the end of the experiment. 18 days after cell injections, tumors were harvested, weighted, and processed for IHC.

Experimental Metastasis Assay. Metastatic ability was assessed essentially as previously described (17). 10^5 B16F10 melanoma cells were suspended in 100µl of PBS and intravenously injected in 3-4 month old C57Bl/6 female mice. 18 days after injections, lungs were removed, insufflated with 10% buffered formalin and the number of visible pulmonary metastases on the surface of the lungs was counted. Lungs were imaged using a low power stereomicroscope (Nikon SMZ-1500).