ONLINE METHODS

Patient Materials. Patients with mesothelioma, uveal melanoma and other cancer types were diagnosed at the treating hospitals. Mesothelioma diagnoses were independently reviewed by M.Ca., a board-certified pathologist and expert in mesothelioma diagnosis. Informed consent was obtained from all participants (affected and unaffected family members) according to institutional guidelines of the University of Hawaii. DNA was extracted from peripheral blood using standard methods. There was no relationship between the two sporadic mesothelioma cases with *BAP1* mutations and the mesothelioma families. The uveal melanomas were treated by laser or radiation therapy; therefore, no biopsies were available.

Genetic Linkage Studies. Germline DNA samples from all available family members were genotyped using Affymetrix Genome-Wide Human SNP Array 6.0. Allele calls were performed by plate using the BIRDSEED version 2 algorithm resulting in 906,600 SNPs for quality control analyses[23]. We used PLINK[24] to remove SNPs with a minor allele frequency below 5% in HapMap[25] CEU samples (ethnicity matched using EIGENSTRAT[26], data not shown), SNPs monomorphic in our data, and SNPs with less than perfect call rates. We also used PLINK to verify relationships in the pedigrees by generating estimates for the proportion of SNPs inherited identical by descent among family members. For this analysis, HapMap CEUs were included in the sample to generate reference allele frequencies. Genetically corrected pedigrees were created using CRANEFOOT[27]. Parametric linkage analyses utilized an ~0.2 cM SNP map (high minor allele frequency SNPs were selected to improve information content), assumed a rare dominant model, and were conducted using ALLEGRO[28]. We utilized linkage analyses to test for haplotypic sharing of the region identified in the studies of affected mesothelioma patients as

harboring the mutation, in family members with non-mesothelioma cancers. These analyses demonstrated the cosegregation of the mutation with alternate cancers in the families, and were further confirmed through sequencing. Analyses to confirm presence or absence of the mutation in additional cancer cases was inferred based on an ~0.05 cM SNP haplotype map enriched with 609 additional SNPs within 2 Mb of *BAP1* to help resolve the boundaries of recombination events.

Cloning and Sequence Analysis. Cloning of genomic PCR products and DNA sequencing (independently performed at Fox Chase and University of Hawaii Cancer Centers to verify reproducibility) were carried out using standard procedures. Nine PCR products encompassing the entire *BAP1* coding exons, adjacent intron sequences, and 5' and 3' untranslated regions were PCR amplified for sequencing. PCR primers used to amplify the *BAP1* gene for sequencing are shown in **Supplementary Table 2**. Advantage2 DNA polymerase (Clontech, Palo Alto, CA) was used with each pair of primers under the following conditions: denaturation at 95°C for 2 min; then 5 cycles of the following: 95°C for 1 min and 68°C for 1 min; afterwards 35 cycles of 95°C for 30sec, 63°C for 30sec, and 68°C for 30 sec, and concluding with 68°C for 5 min. PCR products were gel purified and Sanger sequenced. To investigate the splice acceptor site mutation seen in family W, a PCR-based strategy was used to clone genomic *BAP1* sequences encompassing exons 6-8 and intervening introns, including the intron 6 splice mutation. Primers incorporated a *Xho*I restriction site at the 5' end and an *Eco*RI restriction site at the 3'end of the PCR product. Gel-purified PCR products were cloned into pcDNA 3.1(-) plasmid (Invitrogen, Carlsbad, CA) using the two restriction sites. Individual clones were sequenced verified. Numbering of locations of mutations is based on the February 2009 human reference sequence

(GRCh37/hg19).

Exome Sequencing. DNA libraries were prepared from 2 µg genomic DNA using a modification of Illumina's Genomic PE Sample Prep Kit protocol (Illumina, San Diego, CA) in which all DNA purification steps were performed with AMPure SPRI bead purification (Beckman Coulter Genomics, Danvers, MA). Coding sequences were captured using the Agilent SureSelect Target Enrichment System with the Human All Exon Kit targeting 50 Mb of sequence. The captured DNA libraries were PCR amplified using the manufacturer's paired-end PCR primers and sequenced in one lane of an Illumina Genome Analyzer IIx generating 2x120 bp reads. For data processing and analysis, sequence reads were mapped to the reference genome (hg18) using the SamTools package and BWA. Duplicated reads were removed with Picard. Recalibration of base quality and indel realignment was performed with the GATK package. Single nucleotide variants and indel variants were identified using the Unified Genotyper caller of the GATK package consisting of 44 samples, including samples from other projects. Mutations were annotated with SeattleSeq Annotation (http://gvs.gs.washington.edu/SeattleSeqAnnotation/).

Splicing Assay. This assay was performed using a mini-gene expression construct. Genomic DNA encompassing exons 6-8 of wild-type or splice site mutant *BAP1* was cloned into pcDNA 3.1(-). Plasmids were transfected into 293T cells.

DNA Copy Number Analysis. Oligonucleotide array-CGH analysis was performed using 244K Human Genome CGH microarrays (G4411B) from Agilent Technologies (Santa Clara, CA).

DNA (2-3 μg) from formalin-fixed, paraffin-embedded mesothelioma specimens was labeled using Agilent's Genomic DNA ULS Labeling Kit. Hybridization and DNA copy number analysis were as described[29].

Western Blot Analysis. Immunoblot analysis was performed as described[29,30]. BAP1 (C-4) and GAPDH antibodies were from Santa Cruz Biotechnology (Santa Cruz, CA). Tumor cell lines used for immunoblotting were established from surgically resected primary human mesothelioma specimens as described elsewhere[31].

Immunohistochemistry. Immunohistochemical analysis was as described[30]. Both BAP1 C-4 (1:100) antibody from Santa Cruz and rabbit polyclonal antibody N-term (1:100) from Abgent (San Diego, CA) were used with similar results.

Clonogenicity Assay. This assay was performed by seeding BAP1-deficient mesothelioma cell lines (2×10^5 cells/well) in 6-well plates and incubating at 37°C overnight. Cells were transfected with 2 μg of wild-type *BAP1* plasmid (OriGene, Rockville, MD) or control vector using Lipofectamine 2000 (Invitrogen). Forty-eight hours after transfection, cells were selected in medium containing 400 μg/mL G418 (Invitrogen). Two weeks after selection, colonies were stained with Diff-Quik stain (Dade Behring, Newark, DE).

Mineralogical Studies. We collected and tested samples from the ceiling, roof, tiles, driveways, and other surfaces of each house where mesothelioma families lived 20 or more years ago, since mesothelioma latency is 30-50 years from initial exposure[1], and we also tested nearby soil.

Samples were analyzed as previously described[11]. SEM (Scanning Electron Microscopy), XRD (X-Ray Diffraction), TEM (Transmission Electron Microscopy), EDS (Energy Dispersive Spectroscopy/X-Ray Microanalysis), ED (Electron Diffraction/Selected Area Electron Diffraction) were performed on all samples collected to detect and correctly identify fibers.

21. Bott, M. et al. The nuclear deubiquitinase BAP1 is commonly inactivated by somatic mutations and 3p21.1 losses in malignant pleural mesothelioma. *Nat. Genet.* **43,** 668-672 (2011).

22. Albert, D.M. & Van Buren, J.J. Intraocular Melanomas. in Cancer, *Principles & Practice of Oncology*, Vol. 1 (eds. De Vita, V.T., Lawrence, T.S. & Rosenberg, S.A.) 2090-2098 (Lippincott Williams & Wilkins, Philadelphia, PA, 2011).

(Online Methods references)

23. Korn, J.M. *et al.* Integrated genotype calling and association analysis of SNPs, common copy number polymorphisms and rare CNVs. *Nat. Genet.* **40**, 1253-1260 (2008).

24. Purcell, S. *et al.* PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am. J. Hum. Genet.* **81**, 559-575 (2007).

25. Consortium, I.H. A second generation human haplotype map of over 3.1 million SNPs. *Nature* **449**, 851-861 (2007).

26. Price, A.L. *et al.* Principal components analysis corrects for stratification in genome-wide association studies. *Nat. Genet.* **38**, 904-909 (2006).

27. Mäkinen, V.P. *et al.* High-throughput pedigree drawing. *Eur. J. Hum. Genet.* **13**, 987-989 (2005).

28. Gudbjartsson, D.F., Thorvaldsson, T., Kong, A., Gunnarsson, G. & Ingolfsdottir, A. Allegro version 2. *Nat. Genet.* **37**, 1015-1016 (2005).

29. Altomare, D.A. *et al.* Activated TNF-alpha/NF-kappaB signaling via down-regulation of Fas-associated factor 1 in asbestos-induced mesotheliomas from Arf knockout mice. *Proc Natl Acad Sci USA* **106**, 3420-5 (2009).

30. Yang, H. *et al.* Programmed necrosis induced by asbestos in human mesothelial cells causes high-mobility group box 1 protein release and resultant inflammation. *Proc. Natl. Acad. Sci. USA* **107**, 12611-12616 (2010).

31. Cheung, M., *et al.* The promyelocytic leukemia zinc finger gene, *PLZF*, is frequently downregulated in malignant mesothelioma cells and contributes to cell survival. *Oncogene* **29**, 1633-1640 (2010).