**Supplemental Material SDC2**

**Supplemental Material and Methods and Supplemental Figure Legends**

**Supplemental Material**

**Laser Capture Micro-dissection**

Laser capture micro-dissection was performed to isolate enriched tumor DNA and RNA from frozen tissue samples. Seven µm frozen tissue sections were cut and placed on MMI membrane slides (MMI 50103) and stored in -80°C. Samples were removed from the freezer, allowed to thaw for 30 seconds, and then fixed by rinsing slides in 70% isopropanol for 30 seconds followed by hydration in deionized H2O (dH2O) for another 30 seconds. Slides were then placed on a staining tray and 50-100µl of hematoxylin was directly pipetted onto each sample, incubated for 15 seconds and rinsed in dH2O for 30 seconds. Samples were then gradually dehydrated by rinsing in 70% isopropanol for 30 seconds and finally rinsed with 100% isopropanol for 2 minutes. Tumor tissue was then micro-dissected using a MMI CellCut Plus (Molecular Machines & Industries, MI, USA). Micro-dissected specimens were collected on MMI IsolationCaps (MMI 50202).

**Multiplex Ligation-Dependent Probe Amplification**

The MLPA assay was performed according to the manufacturer’s protocol (SALSA MLPA probemix P417-B1 BAP1, MRC-Holland, Amsterdam, The Netherlands).

MLPA was performed on micro-dissected tumor tissues to ensure tumor purity. All micro-dissected tissue sections consisted of at least 60-70% tumor cells, which represent the sensitivity threshold of MLPA (34). Tumor purity of micro-dissected tissues was confirmed by calretinin staining (tumor cells stain positive) performed on serial sections, sequential to those used for micro-dissection. The MLPA assay was performed in duplicate for each sample. BAP1 exon dosage was detected using BAP1-specific probes spanning all 17 exons and 23 reference probes were used to detect levels of housekeeping genes. BAP1 exon levels were first normalized against the reference probes within each sample (intra-normalization; Supplemental Digital Content 3A). Then, relative BAP1 exon levels in tumor samples were normalized to the relative BAP1 exon levels detected in standard controls (Normalized Dosage Quotient, NDQ). Standard controls were represented by a pool of 10 germline DNA samples. (DNA samples HG00250, HG00255, and NA19655 obtained from the 1000 genome projects; DNA extracted from 3 healthy human mesothelial (HM) cell cultures; germline WT BAP1 DNA obtained from 4 healthy individuals. MLPA was also performed on germline DNA isolated from blood leukocytes of each MM patients to evaluate inter-individual variation in germline BAP1 exon levels (all patients included in this study carried wild type germline BAP1 gene). MLPA analysis indicates that all patients exhibited BAP1 exon levels within the range of the healthy standard controls. NDQ levels relative to standard controls of a representative germline DNA sample is shown in Supplemental Digital Content 3B. Based on these results NDQ values between 0.7 and 1.3 were considered to be normal exon dosage, as also reported elsewhere (34). NDQ thresholds for detection of heterozygous BAP1 alterations were validated in samples with known BAP1 alterations. A heterozygous 4-bp deletion in BAP1 exon 14, initially identified by Sanger sequencing in the BAP1 gene ([5](#_ENREF_5)), was accurately detected by MLPA as exon 14 dosage decrease, with an NDQ of 0.53 (Supplemental Digital Content 3C). A second sample (patient NYU937, previously SP-015, tumor DNA) was used to further validate the MLPA threshold for the detection of heterozygous alterations of BAP1. A heterozygous single nucleotide deletion (G) previously identified by Sanger sequencing in exon 9 (p.Val234fsX15) ([5](#_ENREF_5)) was detected by MLPA with an NDQ value of 0.7 (Supplemental Digital Content 3D). Based on our screening and validation results, we set an NDQ value 0.70 as our upper threshold limit for MLPA scoring of heterozygous alterations as also reported elsewhere (34). An NDQ lower than 0.4 was scored as homozygous deletion, while values above 1.3 indicated exon copy number increase.

**Detection of Promoter Methylation**

Tumor DNA was modified with sodium bisulfide by using EZ DNA methylation kit (ZYMO research, Orange, CA) according to the manufacturer’s protocol. Briefly, bisulfite treated DNA was amplified by using the Human BAP1 methylation assay [(ADS1756, EpigenDx, Hopkington, MA and and PyroMark PCR kit (QIAGEN)]. PCR conditions were set at 95 °C 15 min and 45 cycles of 95°C 30 seconds, 58°C 30s, 72°C 30s. The sequencing was processed by PyroMark Q24 instrument (QIAGEN) at the Genomics Shared Resource, University of Hawai'i Cancer Center. Results were analyzed using the PyroMarkQ24 software (QIAGEN). Human low methylation genomic DNA control (80-8062-HGUM5), human high methylation genomic DNA control (80-8061-HGHM5) (EpigenDx, Hopkington, MA), and normal DNA from patients’ blood were used as controls.

**Detection of whole gene methylation**

Using the Methyl Primer Express® Software v1.0 (Applied Biosystems), two CpG islands were identified as being associated with the BAP1 gene (RefSeq: NM\_004656.3), a large island located in the promoter region and extending into exon 1, and a shorter one in exon 13 (also see Table, Supplemental Digital Content 8). The Cancer Genome Atlas portal database (https://tcga-data.nci.nih.gov/tcga/) was use to examine methylation status at BAP1 gene, which was covered by nineteen probes (data generated using the Infinium HumanMethylation450 BeadChip). Data from the following tumors and corresponding non malignant tissues, if available, were examined: bladder, breast, cervix, colon and rectum, diffuse large B cell lymphoma, esophageal cancer, glioblastoma multiforme, head and neck squamous, hepatocellular, clear cell kidney, chromophobe renal cell, lower grade glioma, lung adenocarcinoma, lung squamous cell, mesothelioma, pancreatic ductal cell, prostate adenocarcinoma, sarcomas, cutaneous melanoma, stomach adenocarcinoma, papillary thyroid carcinoma, and endometrial carcinoma.

Note: Please refer to Reference List included in the main manuscript.

**Supplemental Figure Legends**

**Supplemental Figure, SDC3.** Determination and validation of MLPA threshold NDQ values for the detection of heterozygous alterations of BAP1. Intra-normalization: BAP1 copy number relative to reference gene copy number of a representative healthy DNA control sample carrying wild type BAP1 is shown (A). MLPA analysis of a representative BAP1 wild type germline DNA sample shows an average NDQ of 1.05 (B). Detection of a known heterozygous BAP1 germline mutation by MLPA assay is shown (C). MLPA normalized DQ values show a dosage decrease in exon 14 (NDQ of 0.53) (upper panel) and the heterozygous deletion of 4bp in BAP1 exon 14 (TCAC) was confirmed by Sanger sequencing (bottom panel). Detection of a known heterozygous BAP1 somatic mutation by MLPA assay is shown (D). The MLPA assay detected a loss of exon 9 with an NDQ of 0.7 (top panel) in sample NYU937 and Sanger sequencing confirmed the presence of a heterozygous G deletion in exon 9 (bottom panel). The DNA sequence below shows the location of the deleted G within the exon 9 MLPA probe. Green: exon sequence; red: deleted sequence; yellow highlight: MLPA probe region.

**Supplemental Figure, SDC4.** Detection of BAP1 alterations in sample NYU207. Sequencing results from RNA (top panel) and genomic DNA (bottom panel) show the deletion of exon 7 to exon 13.

**Supplemental Figure, SDC5.** Detection of BAP1 alterations in sample NYU1306. The relative fold increase of many exons as detected by MLPA was confirmed by TaqMan copy number assay.

**Supplemental Figure, SDC6.** Detection of allele deletions in NYU tumor biopsies 866 and 1024 by MLPA. (A) Samples NYU524 (B) NYU1024. Both samples exhibit decrease in exon dosage by the MLPA assay.

**Supplemental Figure SDC7.** Target DNA sequence for BAP1 promoter pyrosequencing (A). Yellow highlight: assay target sequence; blue highlights: CpG sites; green nuleotides: exon 1 (ATG start codon underlined). The fourth CG corresponds to the transcriptional start site. Pyrosequencing on the high methylation genomic DNA control (B), and the low methylation genomic DNA controls (C) is shown. Boxes indicate the percentage of methylation at each site.

**Supplemental Figure, SDC10.** Kaplan-Meier survival curve in MM relative to somatic *BAP1* status analyzed by IHC on combined NYU and NMVN cohorts (BAP1 pos: positive BAP1 nuclear staining; BAP1 neg: BAP1 negative nuclear staining).

**Supplemental Figure SDC11.** Flow Chart representing an overview of the study design, methodology and results.