Supplementary Information

Germline *BAP1* mutations predispose to malignant mesothelioma

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Supplementary Table 1. Summary of the genetic and demographic data of the patients studied. L, Louisiana mesothelioma family; W, Wisconsin mesothelioma family; SP, sporadic mesotheliomas; MM, malignant mesothelioma. Mesotheliomas show variable histologies, the most common variants being epithelial (E), which represents ~65% of cases, sarcomatoid (10%) and biphasic (B), seen in ~25%; the latter show both epithelial and spindle cell morphologies. All familial mesotheliomas studied here were of the epithelial type; however, the significance of this finding could not be established due to the relatively small number of familial mesothelioma cases.

Supplementary Table 2. Primers used for the amplification of genomic DNA for sequencing. Shown are pairs of primers used to amplify various regions of the *BAP1* gene as well as the expected PCR product sizes.

Supplementary Figure 1. Joint familial mesothelioma linkage results for L and W families. Parametric multipoint LOD score (y axis), calculated in ALLEGRO assuming a high-penetrance autosomal dominant model with low disease susceptibility allele frequency (see Online Methods), is plotted against chromosomal location (x axis), with vertical dotted lines separating the individual chromosomes. Linkage analyses conducted assuming that only those with mesothelioma were affected did yield other regions with similar evidence for linkage in both the W and L families. Exome sequencing (conducted in parallel, and completed after the *BAP1* mutations had been identified by conventional Sanger sequencing) verified the *BAP1* mutations and allowed us to rule out the possibility that missense or nonsense variants in genes mapping to other regions contributed to risk of mesothelioma in the W and L families.

Supplementary Figure 2. DNA analysis of two mesothelioma families and splicing assay of mutation in family W. (a) Electropherogram depicting heterozygous germline *BAP1* splice site

mutation in family W. (b) Mini-gene expression construct used for splicing assay *(upper left)*. RT-PCR revealed two *BAP1* bands in 293T cells transfected with wild-type construct but only the smaller band with mutant construct *(upper right)*. Sequencing revealed that the larger band contained correctly spliced exons 6-8, while smaller band contained only exons 6 and 8 *(bottom)*. (c) Electropherogram of 25-bp deletion within exon 4 of *BAP1* of tumor W-III-04T. Deletion results in frameshift and premature termination of BAP1. (d) Electropherogram depicting *BAP1* nonsense mutation (g. chr3:52,436,624 C>T) observed in germline DNA of affected family L members. Resulting CAG>TAG stop codon causes premature truncation leading to loss of BAP1 nuclear localization signal. In family L, *BAP1* mutations were not detected in two individuals with prostate cancer.

Supplementary Figure 3. Asbestos in family W home. (**a**) SEM images of exfoliated vermiculite mineral used in attic insulation in the house, and (**b**) SEM images of amphibole asbestos (tremolite or winchite with small amounts of richterite) found within "sheets" of the vermiculite layers. "Zonolite" was the commercial name of this product. We found some chrysotile asbestos in the basement of the house as insulation (wrappings), but it was not deteriorated and, thus, an unlikely source of exposure. Abbreviations: see Online Methods.

Supplementary Figure 4. Asbestos in one of the L family homes. (**a**) SEM image of bulk sample showing asbestos fibers (chrysotile); (**b**) powder XRD spectra of bulk sample showing chrysotile type asbestos, with spectra matching that of JCPDF standard number 00-25-0645 chrysotile; (**c**) electron diffraction pattern of typical chrysotile; and (**d**) energy dispersive spectra of Mg and Si, indicating chrysotile type asbestos. Thus, SEM and powder XRD of bulk samples, and TEM, EDS and ED for individual asbestos fibers, positively identified chrysotile asbestos. Abbreviations: see Online Methods.

Supplementary Figure 5. Expression analysis of BAP1 in mesothelioma (MM) cell lines and effect of re-expression of BAP1 on clonogenic growth. (**a**) Immunoblot analysis demonstrating loss of detectable BAP1 expression in 4/9 cell lines shown here (7/12 lines tested overall). LP9, hTERT-immortalized normal mesothelial cells, were used as a control. GAPDH, loading control. (**b**) Re-expression of BAP1 in two mesothelioma cell lines lacking detectable endogenous expression of BAP1 resulted in decreased colony-forming ability.

Supplementary Figure 6. Zoomed-in image of array-CGH analysis profiles of tumors L-III-18T and W-III-06T. Red profile shows a focal ~218-kb homozygous deletion encompassing *BAP1* within a larger 3p deletion (tumor L-III-18T); blue profile shows the start of an amplicon immediately proximal to *BAP1* (tumor W-III-06T). Expanded diagram at *right* depicts $\log_2$ ratios of two probes within the *BAP1* locus (chr3:52,435,027-52,444,009), i.e., A_16_P00704764 (chr3:52438014-52438066) and A_14_P128339 (chr3:52443209-52443268) in tumor W-III-06T. The $\log_2$ ratios for these two *BAP1* probes were -0.03 and -0.28, respectively, whereas the nearest two centromeric probes, A_16_P16224907 (chr3:52448321-52448380) and A_14_P200097 (chr3:52452536-52452595), located within the *PHF7* gene, showed $\log_2$ ratios of 1.08 and 0.91, respectively, indicative of a transition to the higher copy number of the amplicon. Expanded view at *left* depicts $\log_2$ ratios (-0.89, -1.18, -1.60 and -1.21, respectively) of same probes in tumor L-III-18T. Region shown represents a portion of a homozygous deletion in 3p21.1 encompassing *BAP1*, *PHF7*, and several other genes (*DNAH1*, *SEMA3G*, *TNNC1*, *NISCH*, *STAB1*, *NT5DC2*, and *PBRM1* – not shown). pter, distal end of short arm of chromosome 3; cen, centromere.

Supplementary Table 1 Summary of genetic and demographic data of cases in this study

Sample ID	Age[a]	Gender	MM	MM Histology	Uveal Melanoma	Other Cancers	Germline *BAP1* Mutation	Mutations Identified in Mesothelioma Specimens
L-II-05	82a	F	No		No	Squamous cell ca. (skin)	Exon 16 (52,436,624 C>T-nonsense)	
L-II-12	68a	F	No		No	Basal cell ca.	Exon 16 (52,436,624 C>T-nonsense)	
L-II-18	54d	F	No		**Yes**	Metastasis to liver	(no DNA available)	
L-II-09	65d	F	**Yes**	N.A.	None	None	(no DNA available)	
L-II-14	57d	M	**Yes**	N.A.	No	None	Exon 16 (52,436,624 C>T-nonsense)[b]	
L-II-03	73d	F	No		No	Pancreatic ca.	Exon 16 (52,436,624 C>T-nonsense)[b]	
L-II-07	70d	F	**Yes**	N.A.	No	None	Exon 16 (52,436,624 C>T-nonsense)[b]	
L-III-18	59	F	**Yes**	E	**Yes**	None	Exon 16 (52,436,624 C>T-nonsense)	Exon 16 (52,436,624 C>T-nonsense) [c]
L-III-22	63	F	**Yes**	E	No	None	Exon 16 (52,436,624 C>T-nonsense)	N.D.
L-III-31	50	M	**Yes**	E	No	None	Exon 16 (52,436,624 C>T-nonsense)	N.D.
L-II-02	86a	M	No		No	Prostate ca.	None	
L-III-15	81a	F	**Yes**	N.A.	No	None	Exon 16 (52,436,624 C>T-nonsense)[b]	
L-III-20	59	M	No		No	Prostate ca	None	
W-III-04	58	M	**Yes**	E	No	None	Intron 6 (52,441,334 A>G-splice site)	Intron 6 (52,441,334 A>G-splice site); Exon 4 (52,442,507-531 ATTGATGATGATATTGTGAATAACA del)
W-III-06	50	F	**Yes**	E	No	None	Intron 6 (52,441,334 A>G-splice site)	Intron 6 (52,441,334 A>G-splice site) [d]
W-III-08	58	F	**Yes**	E	No	None	Intron 6 (52,441,334 A>G-splice site)	Intron 6 (52,441,334 A>G-splice site) [e]
W-IV-21	44	F	**Yes**	E	No	None	Intron 6 (52,441,334 A>G-splice site)	N.D.
W-IV-17	37	F	No		No	Breast ca.	Intron 6 (52,441,334 A>G-splice site)	
W-III-09	57	F	No		No	Clear cell renal cell ca.	Intron 6 (52,441,334 A>G-splice site)	
W-II-01	92d	M	No		No	None	None	
W-II-02	36	F	**Yes**	N.A.	No	None	Intron 6 (52,441,334 A>G-splice site)[b]	
W-III-01	57a	M	No		No	None	None	
W-III-03	59a	F	No		No	None	None	
W-III-10	59	F	No		No	Ovarian ca.	Intron 6 (52,441,334 A>G-splice site)[b]	
SP-002	55	F	**Yes**	E	**Yes**	Leiomyosarcoma	Exon 13 (52,437,444 C del)	N.D.
SP-008	63	M	**Yes**	E	**Yes**	None	Exon 14 (52,437,159-162 TCAC del)	N.D.
SP-007	55	F	**Yes**	E	No	Basal cell ca.	None	N.D.
SP-011	63	M	**Yes**	B	No	Basal cell ca.	None	None
SP-015	82	M	**Yes**	E	No	Basal cell ca.	None	Exon 9 (52,440,352 G del) Exon 13 (52,437,664 C del)
SP-026	66	M	**Yes**	B	No	Basal cell ca.	None	None
SP-020	75	M	**Yes**	E	No	Basal cell ca.; Meningioma	None	None
SP-025	52	M	**Yes**	E	No	Basal cell ca.; Squamous cell ca. (skin)	None	None
SP-005	34	F	**Yes**	E	No	Breast ca.; Leiomyosarcoma	None	N.D.
SP-010	69	F	**Yes**	E	No	Breast ca.; Bronchioalveolar ca.; Pancreatic ca.	None	N.D.
SP-019	71	M	**Yes**	B	No	Colon ca.	None	None
SP-016	74	M	**Yes**	E	No	Colon ca.; Prostate ca.	None	None
SP-004	62	F	**Yes**	B	No	Hairy cell leukemia	None	N.D.
SP-003	64	M	**Yes**	E	No	Melanoma (skin)	None	N.D.
SP-017	74	M	**Yes**	E	No	Melanoma (skin)	None	None
SP-018	70	M	**Yes**	E	No	Prostate ca.	None	Exon 17 (52,436,398-399 CG del)
SP-013	70	M	**Yes**	B	No	Prostate ca.	None	Exon 16 (52,436,599-627 GCTCAGGAAGGTGAGGGGATGCGCTGCTG del)
SP-021	61	M	**Yes**	E	No	Prostate ca.	None	None
SP-012	58	F	**Yes**	E	No	Squamous cell ca. (skin)	None	None
SP-001	63	M	**Yes**	E	No	None	None	Exon 11 (52,439,219 C del)
SP-006	60	M	**Yes**	E	No	None	None	N.D.
SP-009	55	M	**Yes**	E	No	None	None	None
SP-014	60	M	**Yes**	E	No	None	None	None
SP-022	56	M	**Yes**	E	No	None	None	None
SP-023	53	F	**Yes**	B	No	None	None	None
SP-024	78	M	**Yes**	B	No	None	None	None

MM, malignant mesothelioma; ca., carcinoma; N.A., not available; N.D., not determined; E, epithelial MM histology; del, deletion; B, biphasic MM histology.

[a] Age at diagnosis. When this information was not available, either current age of patient who is still alive (e.g., 82a) or age at death (e.g., 92d) are indicated.

[b] Presence of mutation inferred based on the results of linkage analysis; all others were determined by DNA sequencing.

[c] An aCGH analysis revealed a focal homozygous deletion (~218 kb in size) encompassing the entire *BAP1* locus, indicating that at least a subset of tumor cells have loss of both mutant and wild-type *BAP1* alleles.

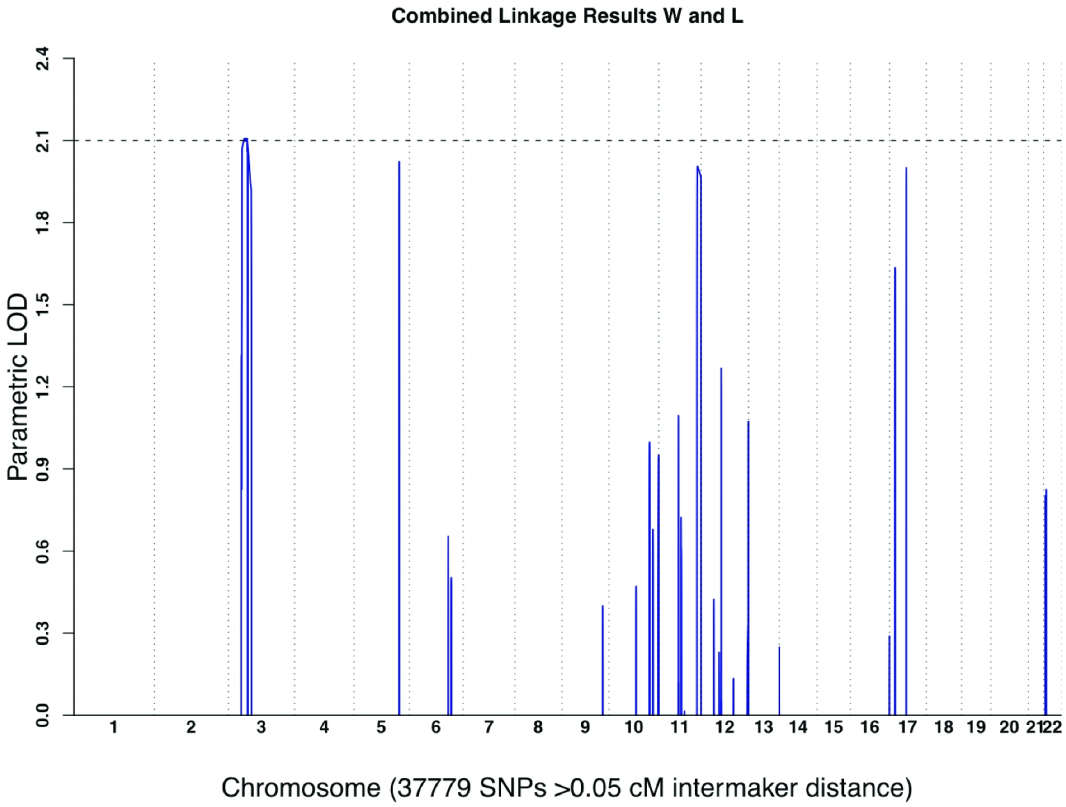
[d] aCGH analysis showed amplicon within 4 kb of *BAP1* locus.

[e] DNA sequencing revealed absence of wild-type *BAP1* allele

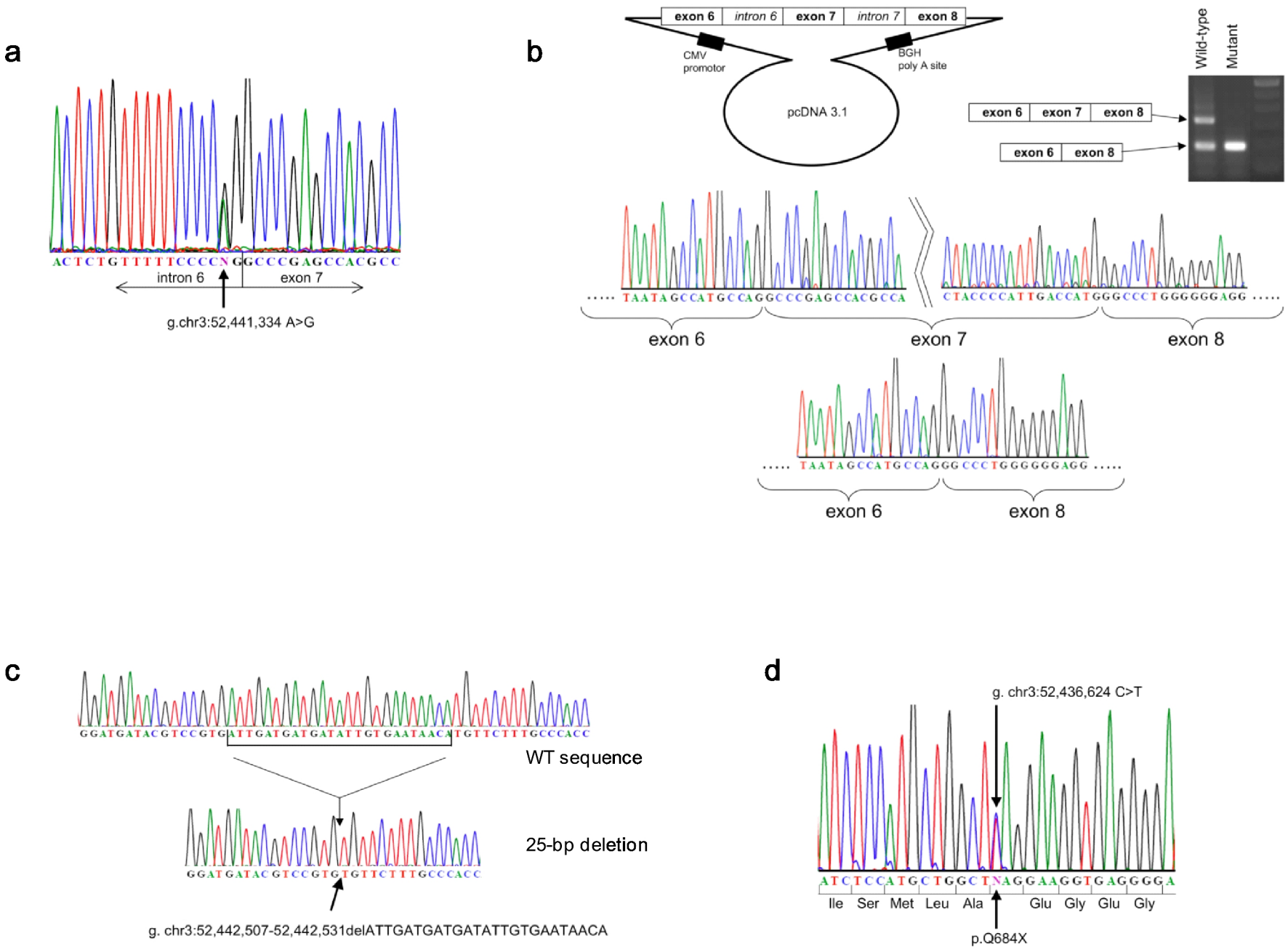
Supplementary Table 2 Primers used for the amplification of genomic DNA for sequencing

Primer Pairs	Primer Sequences	PCR Product Size (bp)
BAP1-2F BAP1-2R	GTGGGTCACGCGGACTATGACCTTC CTCCGCCTCTGGGCTCGTCTTC	591
BAP1-3F BAP1-3R	CTCTTCCCTTCGCCCGCCTCGT AGTAGGGAAGGACAGCCCCTGATGAGT	630
BAP1-4F BAP1-4R	CTGGAGAGCGACCCAGGTGAGGAG AAAAGACATTGTGTGACCGGGGTCTTC	599
BAP1-5F BAP1-5R	CTCTGAGTGCCCGCTCCTGATCAAACT TCCAGGAGTCCACCCAGTCTCCTTATG	586
BAP1-6F BAP1-6R	GTGGGTGTTCATTTGCTTTCCTGACTG CAAACAAAGCACAGAGTCCAGCAGACC	579
BAP1-7F BAP1-7R	CCCTTACTTCCCCCAGCCCTGTATATG AGGCATGAGTTGCACAAGAGTTGGGTA	576
BAP1-8F BAP1-8R	TCCAGTGGGTATTTGGTAGGTGCTTGT GACACTAGGAAGCAACATGGCCTGAGA	592
BAP1-9F BAP1-9R	GCCACTGGGAATGCTACCACATGATATT GGCCTGTGATAGGCACATAGCTGACAA	681
BAP1-10F BAP1-10R	GGGGTGGGAGTAGGGGGAGTATCATTT CAGAGAGTAGAACAGGGCAGGCACAGG	578
BAP1-11F BAP1-11R	GCTCTTCTCTGTCTTCCTTCCCACTCC CCGCCATCAGGTTGAGGCAGATA	572
BAP1-12F BAP1-12R	TTCCAGATAGGCCCCTCATACAGCTTG GGCTCTACCCATTCACTCACAGGGAAA	574
BAP1-13F BAP1-13R	TTCCCCCACAGCATTTGTCTCTGATTC GGGAAGGACTGCTCTCCCTCTACCTTC	578
BAP1-14F BAP1-14R	CCTCTGAGGGCAACCACACAGGTACT GCTTCACCACTAGCTTGGGTTTGTTGG	572
BAP1-15F BAP1-15R	TTCTTCTCTGGGAAGTGCTGGTTCACA GCCCTGAAACACATGCCTTTATTTTGC	595
BAP1-16F BAP1-16R	TGGGTTGCTAGGTTCCTCTGCCTGATA CAGGATGGGATCCGAAGCACCTAGA	588
BAP1-17F BAP1-17R	TCTTTGTCCCAGGAGGAAGAAGACCTG GGTCCAAGCAACTTGAACTAGCCATGC	584
BAP1-18F BAP1-18R	AGGGATGGAGGAGATGTGGGTGGT AGCGCAGTGGCGAGTTGAAAGC	568
BAP1-1819F BAP1-1819R	CCCAGAAGGACCTCTCAATTCCTCTGTC GCTTCCACGACCTCCTTCTCCACTG	571
BAP1-19F BAP1-19R	GGAGGAGGGAAGTGGCCAAGTGAC GCCAGATCAGGCAACTGGAGAAATCAC	624
BAP1-20F BAP1-20R	TCATCCTTGCCTCTAGCTGCCTATTGC GCCTTGTAGGGGCGAGAGCGTTT	656
BAP1-21F BAP1-21R	CCTCTCCTGAGGCTTGAGCAGACCTT ATGATACAAGGACCTGGGCCCACCA	676
BAP1-22F BAP1-22R	GAGTTGGGGCACAGCGAGGTACTG TGGTAATACTGAGGGGCTGGACAGAGG	658
BAP1-23F BAP1-23R	TGTTCTAGCCAGGCTGTTCAAGACTGC CACAGGAGGGTTCATTTCTCAGGAGATTC	583
BAP1-24F BAP1-24R	ATGGCTTTGAAAAAGGTGATCCAAGCA AGTGCACCCTGTCTACAGTCCACCTGA	381

Abbreviations: F, forward; R, reverse; bp, base pairs



Supplementary Figure 1



Supplementary Figure 2

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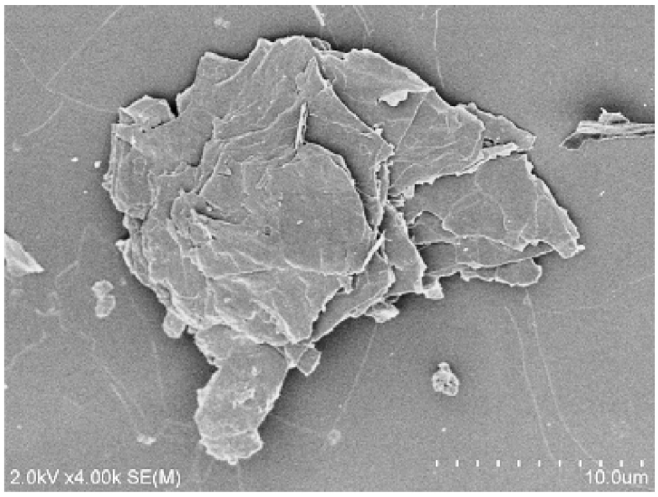

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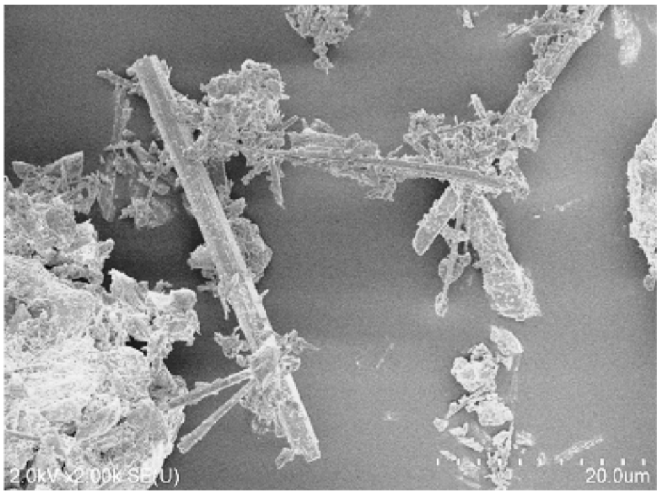
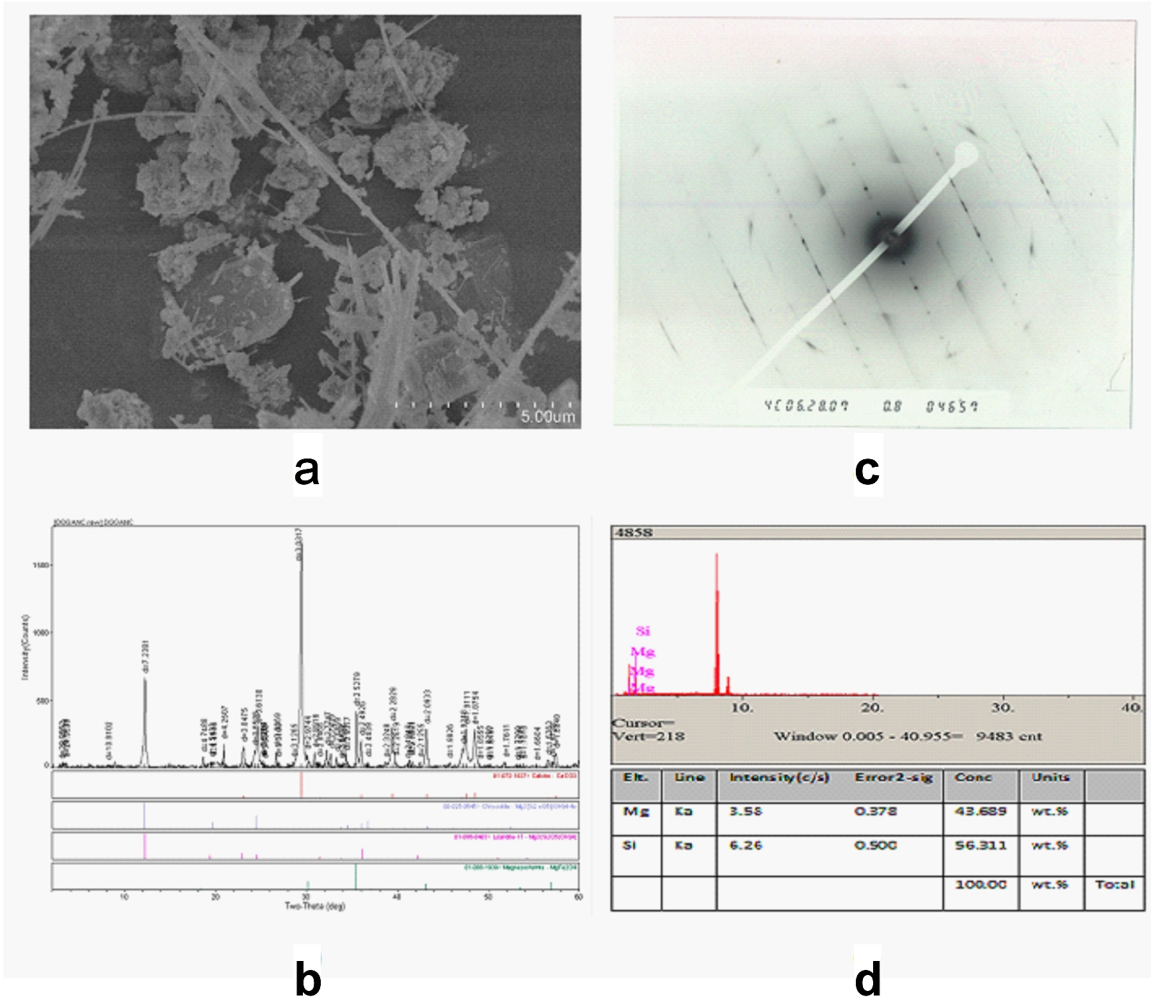


Supplementary Figure 3



Supplementary Figure 4

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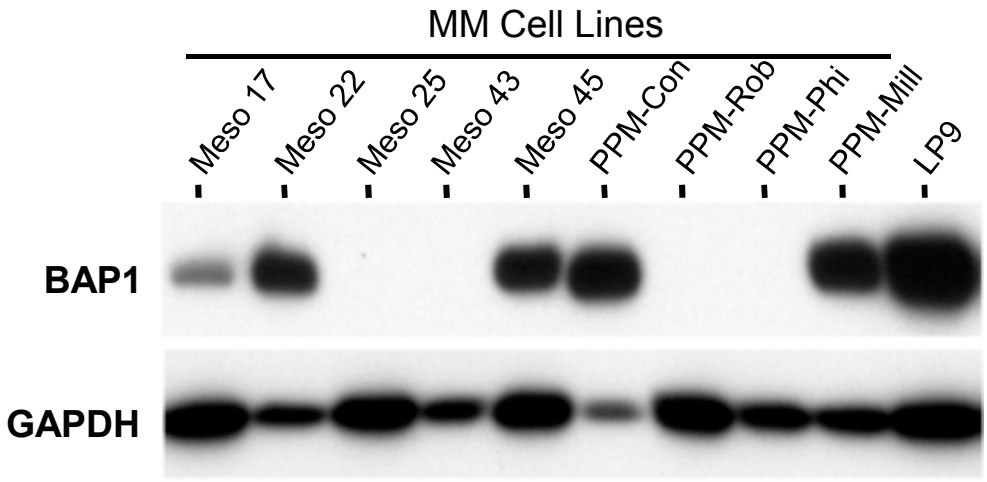

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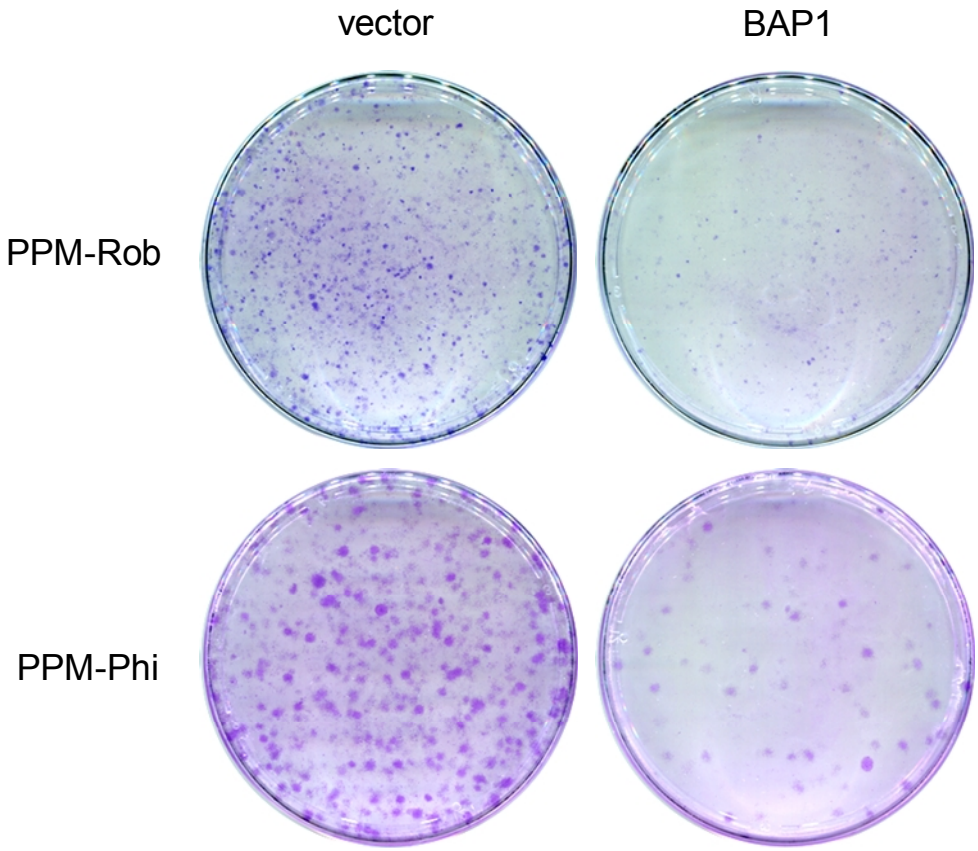
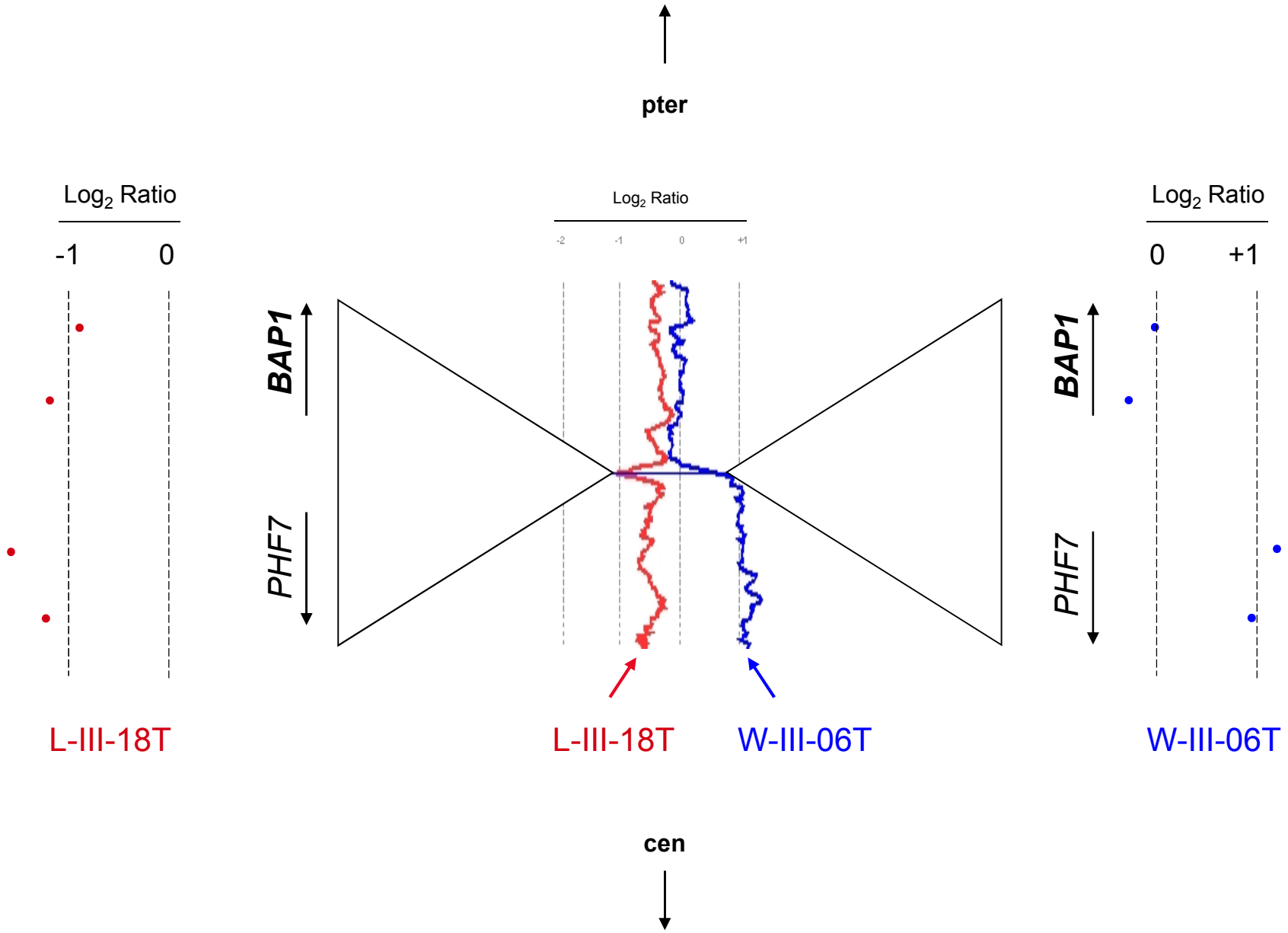


Supplementary Figure 5



Supplementary Figure 6