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Supplementary Materials for

Multiple approaches converge on three biological subtypes of meningioma and extract new insights from published studies

James C. Bayley,V Caroline C. Hadley, Arif O. Harmanci, Akdes S. Harmanci*, Tiemo J. Klisch*, Akash J. Patel*

*Corresponding author. Email: akash.patel@bcm.edu (A.J.P.); klisch@bcm.edu (T.J.K.); akdes.serinharmanci@bcm.edu (A.S.H.)

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The PDF file includes:

Other Supplementary Material for this manuscript includes the following:

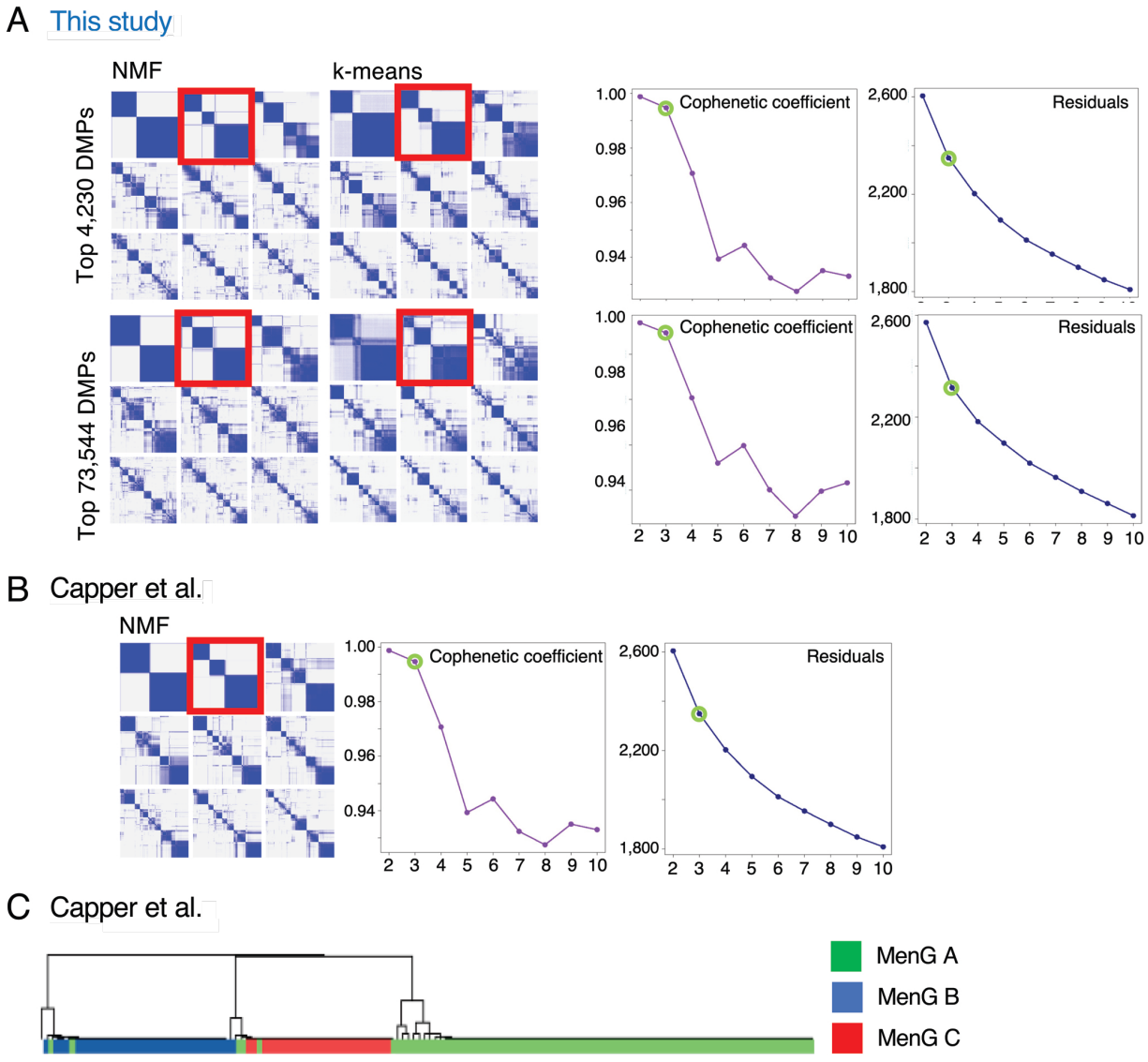

Fig. S1. Identification and validation of meningioma methylation clusters. (**A**) Consensus plots from the four initial DNA methylation analyses utilizing two algorithms (NMF and k-means clustering) across two sets of probes (the top 4,230 and top 73,544 most variably methylated probes) demonstrate that three clusters are optimal. The cophenetic coefficients and residuals from the two NMF analyses corroborate three clusters as optimal. (**B**) NMF of the meningiomas from the Heidelberg CNS tumor classifier (Capper et al.) *(22)* demonstrates three clusters to be optimal per consensus maps, cophenetic coefficients, and residuals. (**C**) These clusters derived from NMF of the Heidelberg samples across our signature probes shows high concordance to assignments made by a random forest classifier trained on data from our cohort and then applied to the Heidelberg meningiomas, demonstrating that our methylation clusters arise independently from this external dataset over our signature probes.

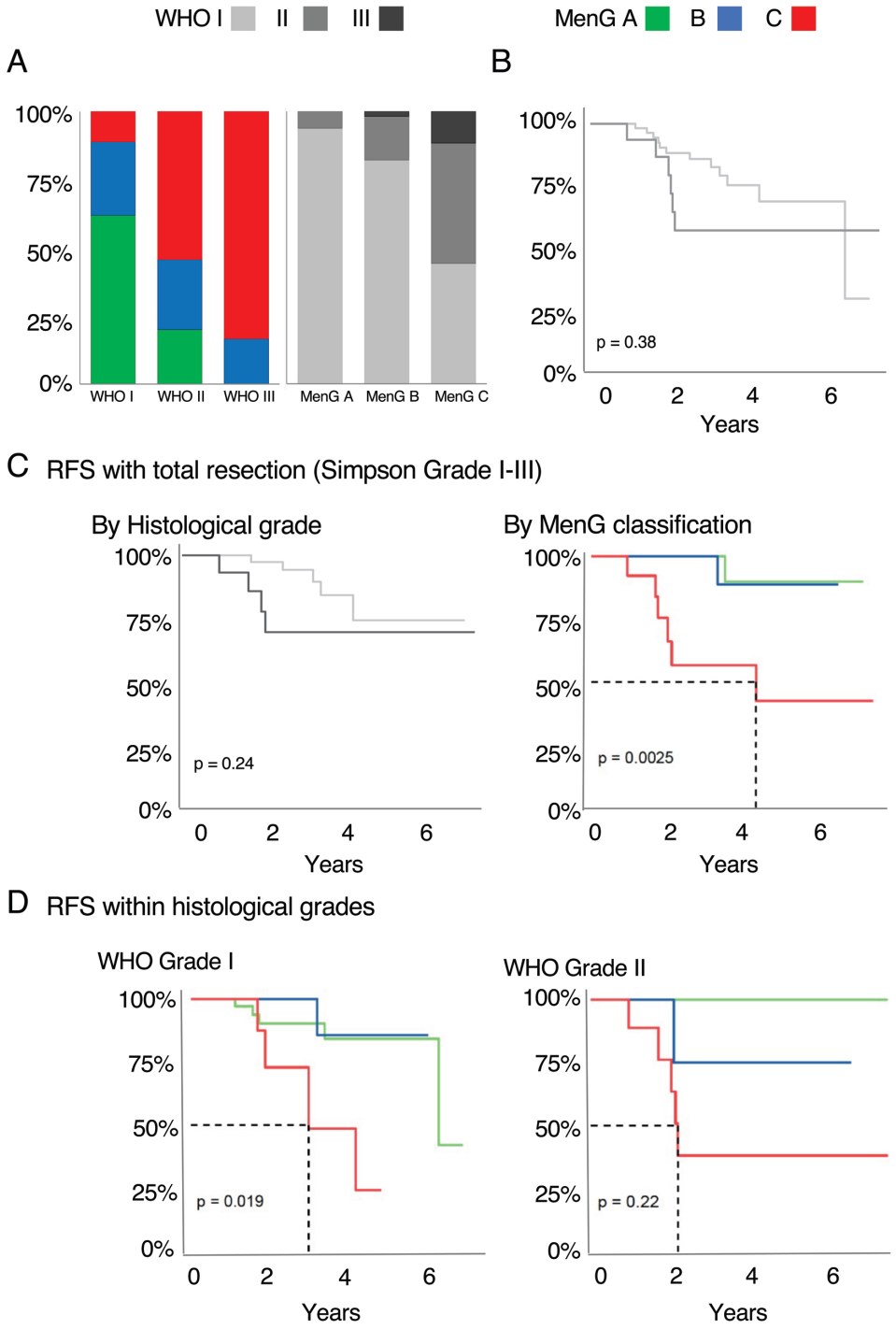

Fig. S2. Clinical outcomes by histologic grade and meningioma molecular groups. (**A**) Proportions of each WHO grade that match to a given MenG, and vice versa. (**B**) Recurrence-free survival (RFS) for WHO I and WHO II meningiomas in our cohort. (**C**) RFS for those tumors that underwent gross total resection, by grade and by MenG. (**D**) RFS shown by MenG, separating our tumors by WHO grade.

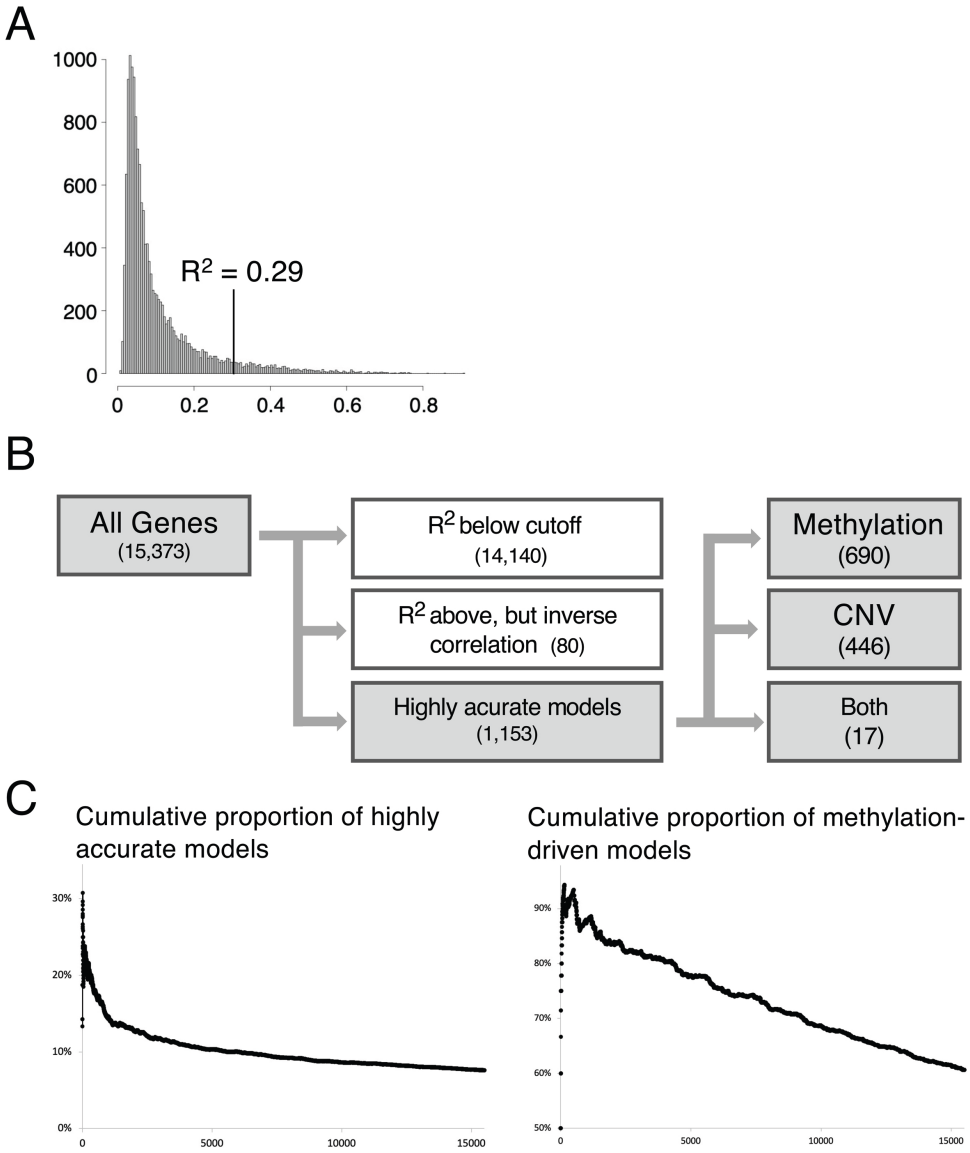

Fig. S3. Computational modeling to determine the strength of association between promoter methylation, copy number variability, and transcriptional changes in meningioma. (**A**) Partial least square (PLS) models were generated for all available genes. We used a cutoff of two standard deviations above the median to define 'highly accurate models,' which were then classified as being most strongly correlated with CNV, methylation, or both. (**B**) Flowchart of the classification of genes in the PLS model. (**C**) Genes were sorted in descending order of promoter methylation variance to explore the relationship between promoter methylation and model characteristics. As promoter variance decreased, fewer models were highly accurate and the proportion of models driven by methylation decreased.

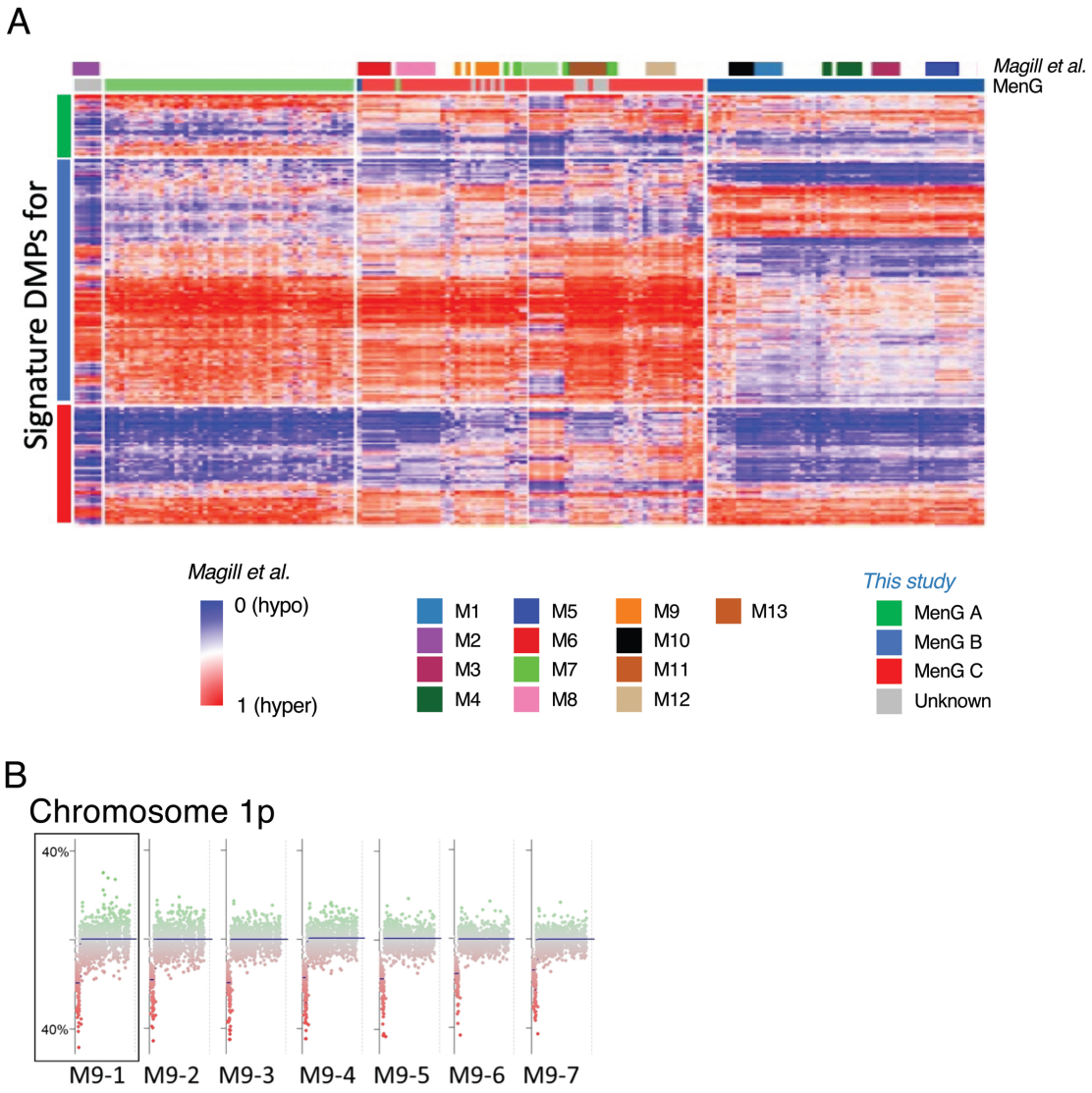

Fig. S4. MenG classification of tumor samples from Magill et al. (**A**) Heatmap of methylation signature probes for our cohort combined with 86 spatially distinct samples from 13 meningiomas (Magill et al.) *(33)*. Samples were organized using k-means clustering (k = 5). These unsupervised clusters align with our integrated MenG assignments. Note that the addition of the Magill samples, which included recurrences, reveals two subclasses within the MenG C grouping. Note, too, that M2 features a substantially different methylation profile and forms its own cluster. Finally, while some samples were assigned MenG A by our methylation classifier, albeit marginally, none of Magill's samples are within the MenG A cluster which is consistent with our MenG assignments. (**B**) Copy number variation calculated from DNA methylation data for tumor M9 demonstrates deletion of the telomeric end of chromosome 1p that was consistent across all samples. This small deletion of 1p did not meet our criterion for a large-scale deletion (at least one-third of a chromosomal arm), so this tumor was cytogenetically classified as '22q loss' (MenG B). However, RNA-Seq, methylation, and NF2/CIN all indicated MenG C and so this tumor was classified as MenG C overall. We propose this small deletion includes the biologically relevant segment of chromosome 1p that drives malignancy.

Supplementary Tables

Only **Table S2** is in Word (below); the other tables are in separate Microsoft Excel files, but their full legends are provided below.

Table S1. Subclassification and Meningioma Group (MenG) assignments of our meningioma samples.

Table S2. **Methylation signature and promoter patterns.** Yellow cells highlight the dominant finding. for a given category.

(A) Signature probe composition			
	Total	Promoters	Non-promoters
Meth 1	16% (1179)	14% (162)	16% (1017)
Meth 2	57% (4308)	38% (432)	60% (3876)
Meth 3	27% (2065)	48% (544)	24% (1521)

(B) Signature probes						
	Total (7552)		Promoters (1,138)		Non-promoters (6,414)	
	Hypo	Hyper	Hypo	Hyper	Hypo	Hyper
Meth 1	7% (500)	9% (679)	8% (96)	6% (66)	6% (404)	10% (613)
Meth 2	44% (3,320)	13% (988)	29% (330)	9% (102)	47% (2,990)	14% (886)
Meth 3	8% (622)	19% (1,443)	4% (43)	44% (501)	9% (579)	15% (942)

(C) Promoter islands				
	Signature (401)		Genome (17,316)	
	Hypo	Hyper	Hypo	Hyper
Meth 1	10% (42)	0% (0)	12% (2,086)	8% (1457)
Meth 2	2% (9)	1% (4)	8% (1,445)	5% (952)
Meth 3	0% (2)	86% (344)	6% (1,074)	59% (10,302)

Table S3. Meningioma Group (MenG) classification of Capper et al. samples*(22)*. In the Heidelberg classifier, the criteria to match to a tumor diagnosis is 0.9, while 0.5 permits matching to a tumor family.

Table S4: Details of samples from our cohort that eluded MenG classification. Transcriptional type probability is an output of the random forest model signifying the probability a sample belongs to that transcriptional type. Methylation type silhouette width is a measure of how closely a sample resembles the assigned methylation type, ranging from -1 (no resemblance) to +1 (complete resemblance). GTR, gross total resection; STR, subtotal resection.

Table S5. Clinical and molecular data on our cohort of 110 primary tumors. Note that some tumors recurred, but the data here pertain only to the primary tumor. GTR, gross total resection;

STR, subtotal resection; PLS, partial least squares; N/A not available.

Table S6. Meningioma Group (MenG) classification of Viaene et al. samples *(30)*.

Table S7. Meningioma Group (MenG) classification of Magill et al. samples *(33)*.

Table S8. Partial least squares (PLS) model results and gene classifications. Summary of PLS results for all genes and different gene sets. The detailed results of every gene are included in tab 2. Lists of genes in each subset tested are provided in tab 3 and results can be obtained by copying the list into tab 4.