Genomic sequencing of meningiomas identifies oncogenic *SMO* and *AKT1* mutations

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Supplementary Material

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Supplementary Note: Human Subjects Research

This study was reviewed and approved by the human subjects institutional review boards of the Dana-Farber Cancer Institute, Brigham and Women's Hospital, and the Broad Institute of Harvard and MIT. Written informed consent was obtained from all participants. Representative fresh-frozen blocks with estimated purity of ≥90% were selected from 65 patients with meningiomas. Three study pathologists (SS/DL/ASR) reviewed the histopathologic diagnosis, grade and purity of each tumor. We performed whole-exome sequencing in 6 meningiomas and whole-genome sequencing in 11 meningiomas. To validate mutations identified in the discovery set, we performed focused sequencing on a validation cohort consisting of an additional 30 grade I meningiomas. We also assembled an extrapolation cohort of 15 grade II and 3 grade III meningiomas in order to compare genetic aberrations of low- and high-grade tumors.

We subsequently obtained IRB approval for collection of independent archival paraffin-embedded meningioma samples from the Brigham and Women's Hospital Pathology Department. We identified 95 samples from patients that did not overlap with our cohort of 65 tumors, resected between 2005 and 2012. Forty-nine of these were higher-grade meningiomas. The samples were assayed for SMO and AKT1 mutations using Sequenom's homogeneous Mass-Extend (hME) Genotyping system (Sequenom, Inc. San Diego, CA).

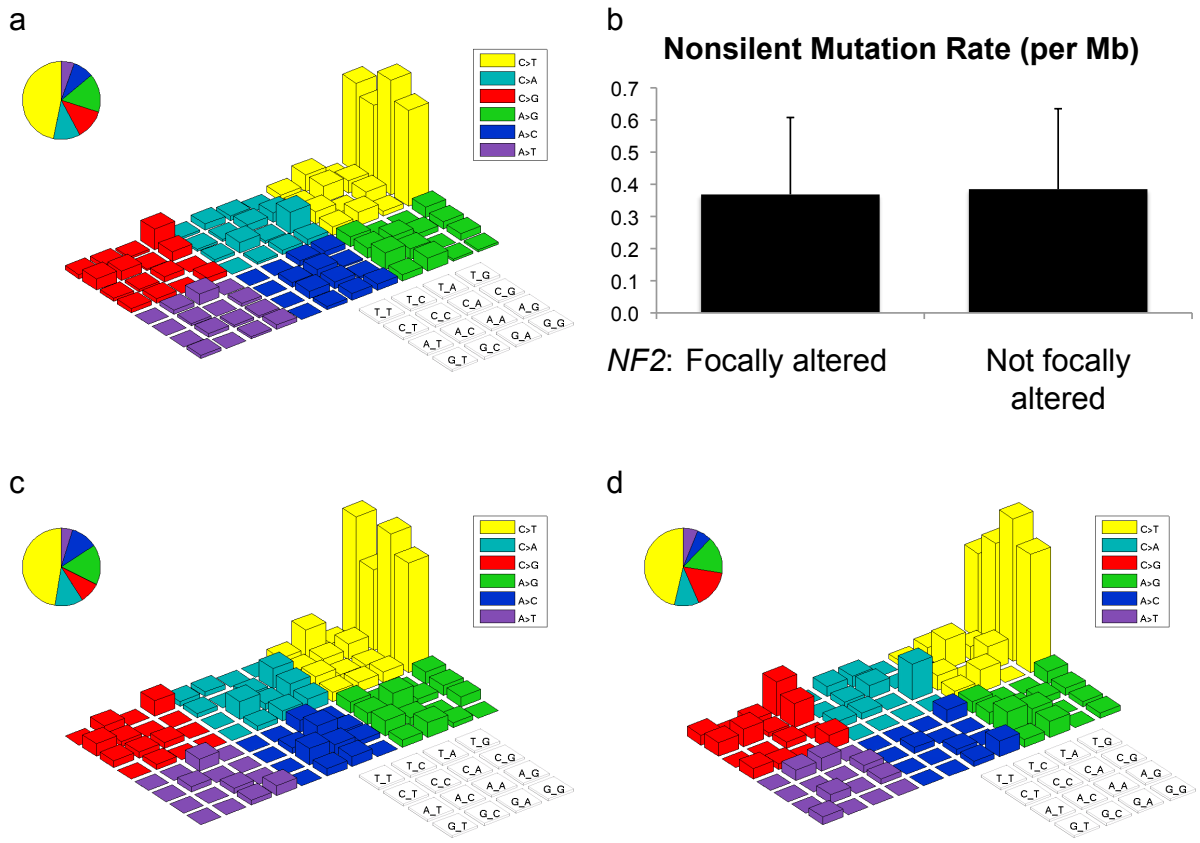
Supplementary Table 1. Demographic and pathologic characteristics of the discovery and validation cohorts.

	Discovery	Validation	Extrapolation	All Samples
Total	17	30	18	65
Grade I	17	30	0	72.3%
Grade II	0	0	15	23.1%
Grade III	0	0	3	4.6%
MIB-1 (%)	3.1%	4.1%	Grade II: 9.8% Grade III: 26.6%	6.2%
Histopathological Subtype				
Fibroblastic	8 (47.1%)	11 (36.7%)	N/A	
Transitional	5 (29.4%)	8 (26.7%)	N/A	
Meningothelial	2 (11.8%)	10 (33.3%)	N/A	
Angiomatous	2 (11.8%)	1 (3.3%)	N/A	
Location				
Convexity	11 (64.7%)	15 (50%)	7 (38.9%)	31 (47.7%)
Falco-Tentorial	3 (17.6%)	7 (23.3%)	7 (38.9%)	17 (26.2%)
Skull Base	3 (17.6%)	8 (26.7%)	3 (16.7%)	14 (21.5%)
Intraventricular	0 (0%)	0 (0%)	1 (5.6%)	1 (1.5%)
Age (at resection)	51 (28-71)	58 (28-86)	60.5 (40-85)	58 (28-86)
Gender				
Female	16 (94.1%)	24 (80%)	13 (72%)	53 (81.5%)
Male	1 (5.9%)	6 (20%)	5 (27.8%)	12 (18.4%)
Remote radiation exposure	1 (5.9%)	3 (10%)	3 (21.4%)	7 (10.7%)
Recent radiation treatment	0 (0%)	1 (2.9%)	3 (21.4%)	4 (6.2%)

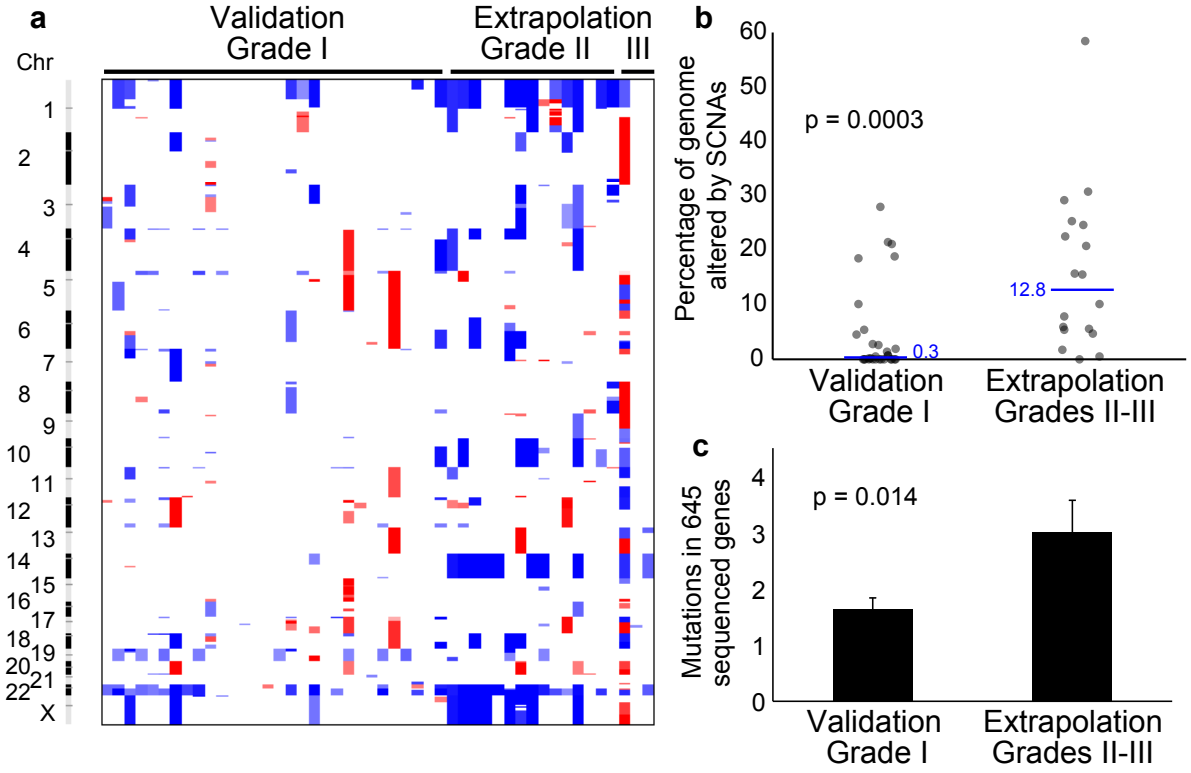
Convexity: Frontal, Frontoparietal, Parietal, Frontotemporal, Temporal, Occipital
Falcotentorial: Parasagittal, Falcine, Parafalcine, Torcular, and Tentorial
Skull Base: Olfactory Groove, Sphenoid Wing, Clinoidal, Planum Sphenoidale, Cavernous Sinus, Petrous Apex, CP Angle, Other Posterior Fossa, Foramen Magnum, and Cervical (C1-C2)
Intraventricular: Right Lateral Ventricular

Supplementary Table 6. Mutations in AKT1 (E17K) and SMO (L412F or W535L) in 46 additional grade I and 49 grade II/III tumors. F: forward, R: reverse probe

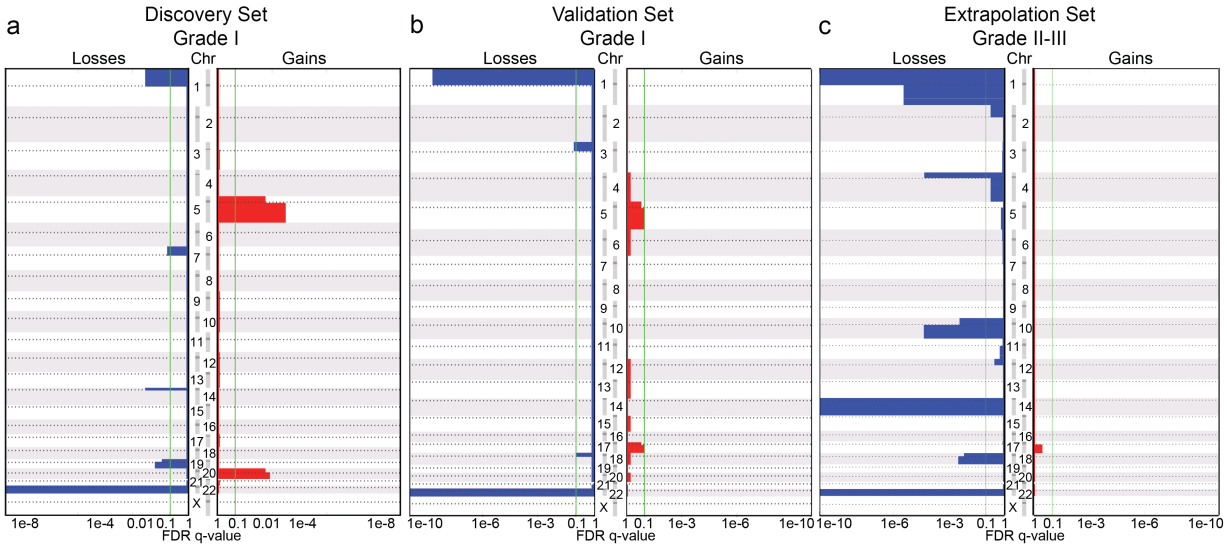
Assay	Mutation observed	Grade	Histopathology	Location	Location Category	Other
AKT1_E17K_chr14:105246551C>T_hg19_h_F	high confidence	III	Anaplastic	Falcine	Falco-tentorial	Recurrent
AKT1_E17K_chr14:105246551C>T_hg19_h_R	high confidence					
AKT1_E17K_chr14:105246551C>T_hg19_h_F	high confidence	I	Meningothelial	Suprasellar/Left optic canal	Skull Base	
AKT1_E17K_chr14:105246551C>T_hg19_h_R	high confidence					
SMO_L412F_chr7:128846398C>T_hg19_h_F	high confidence	I	Transitional	Olfactory groove/Planum sphenoidale	Skull Base	
SMO_L412F_chr7:128846398C>T_hg19_h_R	high confidence					
AKT1_E17K_chr14:105246551C>T_hg19_h_F	high confidence	I	Meningothelial	Right sphenoid wing	Skull Base	
AKT1_E17K_chr14:105246551C>T_hg19_h_R	high confidence					



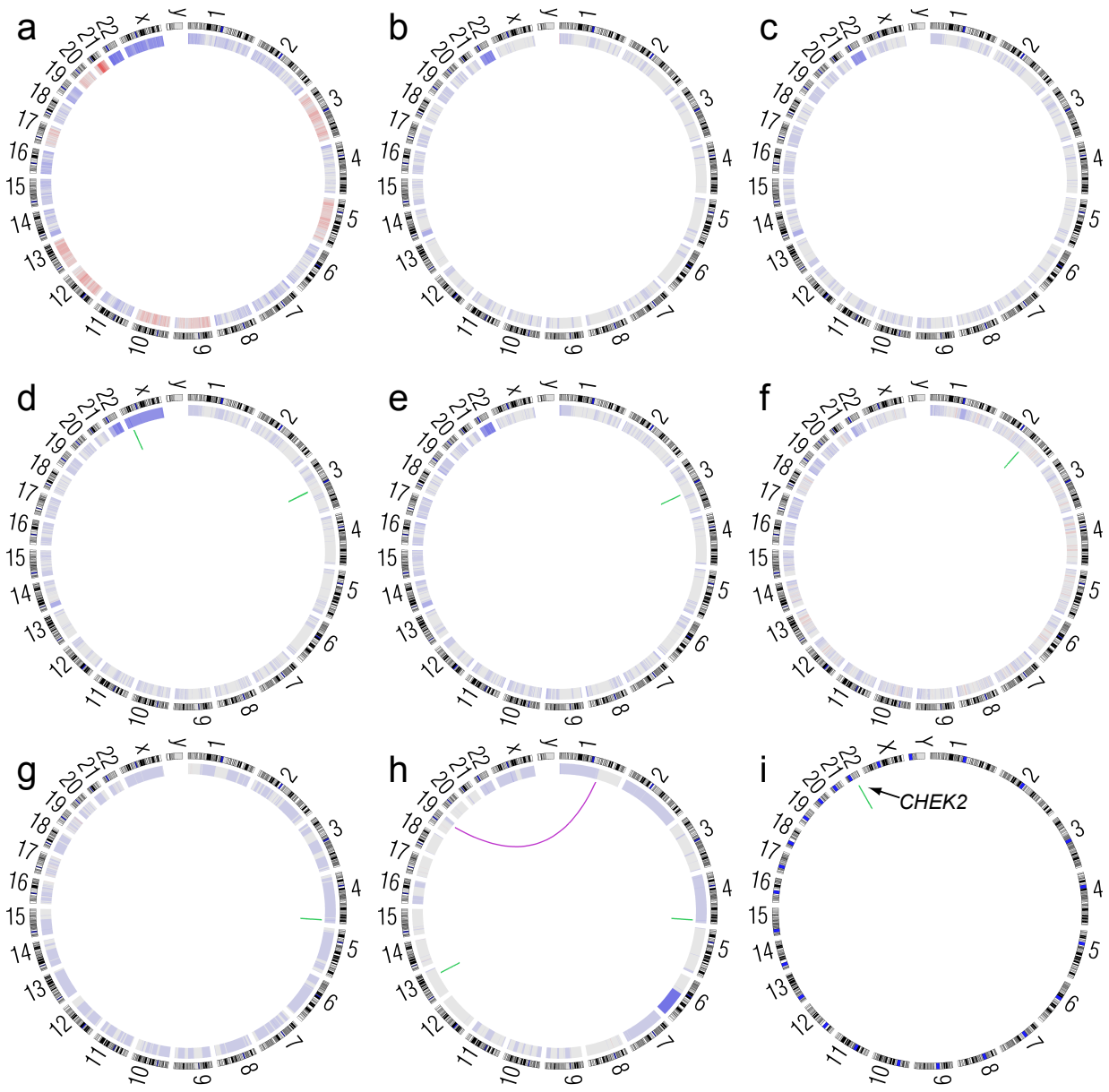
Supplementary Figure 1. Detailed spectrum of coding-region mutation subtypes in meningioma. (a) The entire discovery set (n=17) shows a predominance of spontaneous deaminations in CpG context (yellow, right columns). (b) Mutation rates do not differ significantly between samples with (n=10) or without (n=7) *NF2* focal alterations (mean +/- SEM). (c) Samples with focal *NF2* aberrations and (d) samples without focal *NF2* alterations do not differ appreciably in the spectrum of their mutation subtypes.



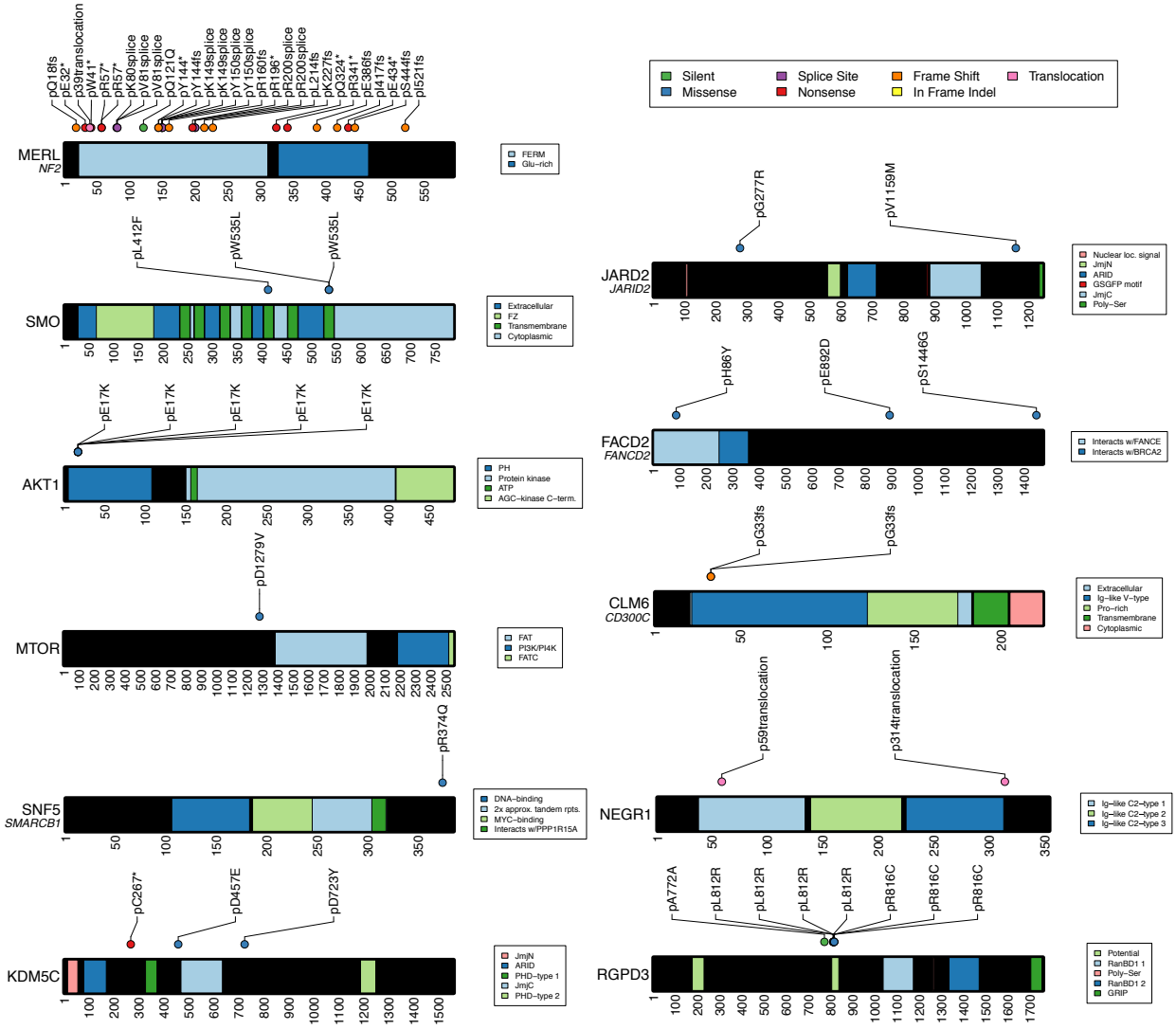
Supplementary Figure 2. Higher-grade meningiomas harbor more genetic alterations than their grade I counterparts. (a) Copy-number heat-map for tumors in the validation cohort (red: gain, blue: loss). Samples are in the same order as in Figure 3. (b) Percentage of the genome affected by somatic copy-number alterations and (c) number of mutations are significantly higher in grade II-III tumors (mean +/- SEM).



Supplementary Figure 3. Significant broad somatic copy-number alterations (SCNAs) in meningiomas. GISTIC 2.0 broad analysis was used with SegSeq copy-number data to determine the significance of recurrent events in (a) the discovery set of 17 grade I meningiomas and (b) the 18 grade II-III tumors of the validation set. Chr22 loss is the most frequent and significant broad SCNA (q=0).



Supplementary Figure 4. Somatic rearrangements in the remaining whole-genome sequenced meningiomas (see Figure 2). Circos plots show the CNAs (inner ring heat-map) and intra-chromosomal (green) and inter-chromosomal (purple) rearrangements in eight whole-genome sequenced meningiomas (a-h). Samples a-e have focal alterations in *NF2*, while samples f-h do not. (i) A rearrangement in a grade III meningioma in the validation set causes fusion of *CHEK2* with a neighboring gene on chr22. There may be additional translocations in this tumor, as only 645 genes were sequenced in this sample.



Supplementary Figure 5. Locations of somatic mutations, insertions/deletions, and rearrangements in *NF2* and other genes.