Supplemental Information

Comprehensive Molecular Profiling Identifies

FOXM1 as a Key Transcription Factor

for Meningioma Proliferation

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SUPPLEMENTAL EXPERIMENTAL PROCEDURES

RNA-seq analysis

Library preparation was performed using the TruSeq RNA Library Prep Kit v2 (RS-122-2001, Illumina, San Diego, CA) and sequenced on an Illumina HiSeq 2500 to at least 30 million unique reads at the Center for Advanced Technology at the University of California San Francisco. Quality control of FASTQ files was performed with FASTQC (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/) and after trimming of adapter sequences, reads were further filtered to remove bases that did not have an average quality score of 20 within a sliding window across 4 bases. Reads were subsequently mapped to the human reference genome hg19 using HISAT2 with default parameters (Kim et al., 2015), and transcript FPKM value estimates were obtained from Cufflinks (Trapnell et al., 2010, 2012). Unsupervised hierarchical clustering using FPKM values for the top 2,000 most variable genes was performed in R using the clusterCons package (Simpson et al., 2010). For differential expression analysis, we extracted exon level count data from the mapped HISAT2 output and then used DESeq2 to identify differentially expressed genes at a significance level of q<0.1 (Love et al., 2014). Gene ontology analysis was performed in DAVID (Huang et al., 2009), and Chromatin Enrichment Analysis (Lachmann et al., 2010) was used to compare gene lists to published ChIP-seq data.

Tissue processing and immunohistochemistry

Tissue microarrays (TMAs) were constructed using a semiautomatic tissue microarrayer (TMArryer, Pathology Devices, Westminster, MD). In brief, recipient blocks were made using 5% agarose (Sigma Aldrich, St Louis, MO) in disposable base molds (37 x 24 x 5 mm, 62352-37, VWR, Radnor, PA). Agarose wafers were processed overnight in a Leica ASP tissue processor (Leica Biosystems, Buffalo Grove, IL) and subsequently embedded using conventional methods. Tissue from donor paraffin-embedded blocks from meningioma cases was sampled using a 1.5 mm punch (02110006, Pathology Devices, Westminster, MD). Triplicates were prepared, with each block contained randomly selected control tissues. Recipient blocks were re-embedded and sectioned at 5 μm thickness for immunohistochemistry.

Immunohistochemistry (IHC) was performed using whole slide sections (FOXM1 and MIB1) or TMAs (β-Catenin) in the Brain Tumor SPORE Biospecimen and Pathology Core, and in the Department of Pathology Histology Laboratory, at the University of California San
Francisco. The following antibodies were used: MIB-1 anti-Ki67 rabbit polyclonal (30-9, Ventana Medical Systems, Tucson, AZ); FOXM1 rabbit monoclonal (EPR17379) and SSTR2A (UMB1) (Abcam, Cambridge, MA); and β-catenin mouse monoclonal (14/β-catenin, BD Biosciences, San Jose, CA). Double immunohistochemistry was performed according to standard methods with FOXM1 antibody incubation and detection followed by denaturation at 90°C for 12 min and MIB-1 antibody incubation and detection. All immunohistochemistry assays were performed on the Ventana Medical Systems Discovery Ultra (Oro Valley, AZ) except β-catenin, which was performed on the Leica BOND III Platform (Buffalo Grove, IL). Digital images were captured using a microscope (Olympus, Model BX41TF) and digital camera (Olympus, Model DP70). FOXM1 IHC was quantified as the average number of positive nuclei from 5 distinct regions within each meningioma using ImageJ to account for intra-meningioma heterogeneity. The presence of any β-catenin nuclear staining was considered nuclear localization.

**Whole Exome Sequencing**

Library preparation, exome capture and sequencing were performed at the Institute for Human Genetics at the University of California San Francisco. Sequencing libraries were prepared using the Kapa Hyper Prep Kit and exome capture was performed with the Nimblegen SeqCap EZ Human Exome Kit v. 3.0. Paired end sequencing with read length 100 base pairs was performed on the Illumina HiSeq4000. All subsequent data analysis was performed with the bcbio pipeline with default parameters [https://github.com/chapmanb/bcbio-nextgen]. Reads were aligned with the Burrows-Wheeler aligner (Li and Durbin, 2009) to the reference human genome (build hg19). Only uniquely aligned reads were included for further processing with the Picard suite (http://broadinstitute.github.io/picard/) and the Genome Analysis Toolkit (DePristo et al., 2011) for de-duplication, local realignment and base quality score recalibration. Alignment quality metrics were calculated with the Picard suite. Somatic variants (point mutations, small indels) were identified from matched tumor-normal samples using Varscan2 (Koboldt et al., 2012), Freebayes (Garrison and Marth, 2012) and Vardict (Lai et al., 2016) at a significance threshold \( P<0.05 \), and results from each algorithm were merged into a single Ensemble callset including only those significant hits identified in at least 2 callers. Hits were further filtered based on quality metrics using default parameters including (i) mean base quality >25, (ii) >5
reads in tumor and normal sample, (iii) >10% variant reads in tumor, (iv) >90% reference reads in normal, and (v) strand bias. The full list of parameters and filters used for each variant caller can be found in the headers of the submitted VCF files. Variants were annotated using Snpeff v4.3 (Cingolani et al., 2012) and were further filtered to only include those marked as high, moderate or low priority and occurring in protein coding or splice site locations. Large scale copy number alterations were called using CNVkit (Talevich et al., 2016) and visualized with custom code in R, with large scale copy number alterations defined as those changes encompassing greater than one third of a chromosomal arm.

DNA Methylation Arrays

Methylation analysis of meningiomas on the Illumina Methylation EPIC Beadchip was performed according to the manufacturer’s instructions at the University of Southern California Molecular Genomics Core. All downstream analysis were performed in R using the minfi Bioconductor package (Aryee et al., 2014; Fortin et al., 2016). Only probes with detection P<0.05 in all samples were included for further analysis. Data were normalized using functional normalization (Fortin et al., 2014). Probes were filtered based on the following criteria: (i) removal of probes targeting the X and Y chromosomes (n=11,551), (ii) removal of probes containing a common single nucleotide polymorphism (SNP) within the targeted CpG site or on an adjacent basepair (n=24,536), and (iii) removal of probes not mapping uniquely to the hg19 human reference genome (n=9,993). A total of 783,236 probes were kept for further analysis. Multidimensional scaling (Euclidean distance) and unsupervised hierarchical clustering (Euclidean distance, average linkage) was performed using the top 2,000 most variable probes across all datasets. For probe-level differential methylation analysis, the limma Bioconductor package was used to fit a linear model accounting for the paired nature of the data with a FDR<0.001 considered significant (Ritchie et al., 2015). β values were used for visualization of methylation levels (β=methylated/[methylated+unmethylated]) and M values were used for statistical analysis (M=log2[methylated/unmethylated]) (Du et al., 2010).

NanoString targeted gene expression profiling

Total RNA was isolated from tumor cores (2-3 mm cores) from FFPE blocks containing >75% tumor cells as determined by H&E staining, according to the manufacture’s
protocol. Concentrations were determined using a spectrophotometer and RNA integrity was assessed using a bioanalyzer (Agilent, San Francisco, CA). Probes for the GX Human Cancer Reference Nanostring panel codeset, as well as 30 additional meningioma related genes, for a total of 266 targets, were synthesized by NanoString technologies (Seattle, WA). RNA (200 ng per meningioma) was analyzed with the NanoString nCounter Analysis System at NanoString Technologies, according to the manufacturer’s protocol.

SUPPLEMENTAL FIGURE LEGENDS

Figure S1. Unsupervised clustering of meningioma RNA-seq data reveals distinct transcriptomic clusters, related to Figure 2.

(A) Unsupervised hierarchical clustering segregates meningiomas into 2 clusters.

(B-D) Meningioma transcriptomic clusters have distinct associations with patient sex, meningioma location and grade (Skull base, SB).

(E, F) Meningioma transcriptomic clusters have no associations with patient age or prior cranial radiation (Cluster 1, C1; cluster 2, C2; radiotherapy, RT).

(G) KEGG pathway analysis shows enrichment of focal adhesion genes in the cluster of meningiomas that are more likely to be low grade and located at the skull base in female patients.

(H) KEGG pathway analysis shows enrichment of metabolism and oxidative phosphorylation genes in the cluster of meningiomas that are more likely to be high grade in male patients.

(I, J) OS and LRFS are not significantly different between meningioma transcriptomic clusters.

(K) FOXM1 targets, as identified from published ChIP-seq data, are enriched in WHO Grade III meningiomas (N=78, 11%) relative to WHO Grade I meningiomas (N=26, 3%) by RNA-seq.

(L) FOXM1 expression by RNA-seq is equivalent across meningioma transcriptomic clusters.

(M) FOXM1 targets, as identified from published ChIP-seq data, are not significantly different between meningioma transcriptomic clusters (Cluster 1, C1, N=103, 5%; cluster 2, C2, N=61, 6%).
Figure S2. Meningioma NF2 mutation is associated with genomic instability, related to Figure 4.
Analysis of the number of large scale chromosomal alterations per meningioma, defined as comprising greater than one third of a chromosomal arm, identifies significantly more events in NF2 mutant tumors, including 1p loss, 6q loss, 10q loss, 18q loss and 22q loss.

Figure S3. Meningioma somatic mutation burden is associated with clinical parameters, related to Figure 4.
(A-C) The number of somatic mutations per meningioma is significantly increased in high grade meningiomas, convexity meningiomas, and meningiomas from patients with a history of prior cranial radiation (*P=0.0217, **P=0.0180, and ***P=0.0168; convexity, CV; radiotherapy, RT).
(D) High meningioma somatic mutation burden is associated with poor LRFS relative to low meningioma somatic mutation burden (N=24).

Figure S4. Meningioma DNA methylation pattern is associated with clinical parameters, related to Figure 5.
(A) Unsupervised hierarchical clustering based on the top 2,000 most variable probes segregates meningiomas into 3 clusters based on high, low and medium DNA methylation with significant differences in β values (β=methylated/[methylated+unmethylated]) (N=26, *P<0.0001).
(B) High meningioma DNA methylation is associated with high nonsynonymous mutation burden relative to low and medium meningioma methylation clusters (*P<0.0001).
(C-E) High and medium meningioma DNA methylation are associated with high grade, convexity location and NF2 mutation relative to low meningioma methylation (Convexity, CV; mutant, M; wild type, WT).
(F) LRFS is not significantly different between high, medium and low meningioma DNA methylation clusters.

Figure S5. Differentially methylated probe analysis reveals unique epigenomic signatures associated with meningioma grade, related to Figure 5.
(A) Differential analysis between meningioma DNA methylation clusters identifies a total of 3,380 hypermethylated probes in the high methylation cluster, and 860 hypermethylated probes
in the medium methylation cluster, compared to the low methylation cluster. Consistently, only 405 hypomethylated sites are identified in the high methylation cluster, and 225 hypomethylated sites are identified in the medium methylation cluster, compared to the low methylation cluster.

(B) GREAT INTERPRO analysis for hypermethylated sites in the high meningioma DNA methylation cluster shows enrichment for homeobox genes.

(C) GREAT molecular signatures database analysis for WHO Grade II hypermethylated sites shows enrichment of focal adhesion genes relative to WHO Grade III meningiomas.

(D) GREAT gene ontology analysis for biological processes for WHO Grade II hypermethylated sites shows enrichment of cell polarity and growth genes relative to WHO Grade III meningiomas.

(E-G) Meningiomas with high FOXM1 mRNA expression by RNA-seq exhibit decreased expression of PRC target genes ATF3, HOXA13 and HOXC5 (*p<0.05).

**Figure S6.** FOXM1 expression is associated with cell proliferation gene expression in aggressive meningioma, related to Figure 6.

(A,B) High grade and recurrent meningiomas are enriched in FOXM1 protein by IHC (N=52). Red denotes FOXM1 high tumors, and blue denotes FOXM1 low tumors.

(C) High FOXM1 mRNA expression in WHO Grade II meningiomas by NanoString targeted gene expression profiling is associated with poor LRFS and OS relative to WHO grade II meningiomas with low FOXM1 mRNA expression (N=63).

(D-F) High expression of FOXM1 cell proliferation target genes by NanoString targeted gene expression profiling, such as TOP2A, CCNA2 and CKS2, is associated with poor LRFS relative to meningiomas with low FOXM1 cell proliferation target gene expression (N=96).

**SUPPLEMENTAL ITEMS**

| Table S1. Clinical data, related to Figures 1-6 |
|-----------------------------------------------|
| **All patients, related to Figure 1**         |
| **Patients**                                 | 261                           |
| **Median age at diagnosis (range)**          | 59 years (14-87 years)        |
| **Male:Female**                              | 97:164 (1:1.7)                |
| **Neurofibromatosis**                        | 8 (3%)                        |
| **History of radiation exposure**            | 13 (5%)                       |
| **Multiple meningiomas**                    | 63 (24%)                      |
| **Race/Ethnicity**                           |                               |
| Ethnicity               | Count (Percentage) |
|------------------------|--------------------|
| African American       | 14 (5%)            |
| Asian                  | 25 (10%)           |
| Caucasian              | 167 (64%)          |
| Hispanic               | 32 (12%)           |
| Native American        | 1 (1%)             |
| Pacific Islander       | 14 (5%)            |
| Other                  | 8 (3%)             |

**All meningiomas, related to Figure 1**

| Meningiomas        | Count (Percentage) |
|--------------------|--------------------|
| Meningiomas        | 280                |
| WHO Grade I        | 143 (51%)          |
| WHO Grade II       | 109 (39%)          |
| WHO Grade III      | 28 (10%)           |
| Median size (range)| 33.4 cm³ (0.3-335 cm³) |
| Recurrent          | 78 (28%)           |
| Prior surgery      | 70 (25%)           |
| Prior radiotherapy | 70 (25%)           |
| Preoperative embolization | 93 (33%) |

**Location**

| Location          | Count (Percentage) |
|-------------------|--------------------|
| Frontal           | 45 (14%)           |
| Temporal          | 9 (3%)             |
| Parietal          | 20 (7%)            |
| Occipital         | 5 (2%)             |
| Parasagittal      | 72 (26%)           |
| Falx              | 9 (3%)             |
| Olfactory         | 18 (6%)            |
| Orbit             | 4 (1%)             |
| Cavernous sinus   | 7 (3%)             |
| Sphenoid          | 36 (13%)           |
| Middle cranial fossa | 11 (4%)     |
| Petrous face      | 7 (3%)             |
| Petroclival       | 11 (4%)            |
| Tentorium         | 19 (7%)            |
| Cerebellum        | 1 (1%)             |
| Ventrical         | 5 (2%)             |
| Spine             | 1 (1%)             |
| Skull base        | 124 (44%)          |
| Anterior cranial fossa | 60 (21%)    |
| Middle cranial fossa | 67 (24%)   |
| Posterior cranial fossa | 38 (14%) |
| Convexity         | 172 (61%)          |
| Midline           | 130 (46%)          |

**Radiographic and Histopathologic characteristics**

| Characteristic     | Count (Percentage) |
|--------------------|--------------------|
| Bone invasion      | 94 (34%)           |
| Brain invasion     | 48 (17%)           |
| Edema              | 154 (55%)          |
| Necrosis           | 78 (28%)           |
| Median MIB1 labeling index (range) | 7% (1-72%) |
|-----------------------------------|------------|

**All treatments, related to Figure 1**

| Extent of resection                  |            |
|--------------------------------------|------------|
| Gross total                          | 158 (56%)  |
| Subtotal                             | 119 (43%)  |
| Unknown                              | 3 (1%)     |

| Adjuvant radiotherapy                |            |
|--------------------------------------|------------|
| External beam radiation              | 27 (10%)   |
| Radiosurgery                         | 12 (4%)    |
| Brachytherapy                         | 13 (5%)    |
| Unknown                              | 19 (7%)    |

**All outcomes, related to Figure 1**

| Median follow-up (range)             | 4.2 years (0-25 years) |
|--------------------------------------|------------------------|
| Local recurrence                     | 107 (36%)              |
| WHO Grade I                          | 32 (22%)               |
| WHO Grade II                         | 54 (50%)               |
| WHO Grade III                        | 17 (61%)               |

| Median local recurrence free survival| 7.4 years |
|--------------------------------------|-----------|
| WHO Grade I                          | 11.3 years|
| WHO Grade II                         | 4.8 years |
| WHO Grade III                        | 1.0 years |

| Death                                | 72 (26%)  |
| WHO Grade I                          | 19 (13%)  |
| WHO Grade II                         | 39 (36%)  |
| WHO Grade III                        | 14 (50%)  |

| Median overall survival              | 11.9 years |
| WHO Grade I                          | 15.4 years |
| WHO Grade II                         | 9.8 years  |
| WHO Grade III                        | 4.1 years  |

| Cause of death                       |            |
|--------------------------------------|------------|
| Progressive disease                  | 36 (14%)   |
| Adverse effect of treatment          | 10 (4%)    |
| Other                                | 10 (4%)    |
| Unknown                              | 16 (6%)    |

**RNA-seq samples, related to Figure 2**

| Patients                             | 42         |
|--------------------------------------|------------|
| Median age at diagnosis (range)      | 55 years (14-79 years) |
| Male:Female                          | 18:24 (1:1.3) |

| Meningiomas                          | 42         |
| WHO Grade I                          | 14 (33%)   |
| WHO Grade II                         | 23 (55%)   |
| WHO Grade III                        | 5 (12%)    |

| Recurrent                            | 24 (57%)   |
| Skull base                           | 22 (52%)   |
| Convexity                            | 27 (64%)   |

**Extent of resection**
|                          |                  |      |
|--------------------------|------------------|------|
| **Gross total**          |                  | 19 (45%) |
| **Subtotal**             |                  | 23 (55%) |
| **Unknown**              |                  | 0 (0%) |
| **Adjuvant radiotherapy**|                  | 13 (31%) |
| **Median MIB1 labeling index (range)** |              | 5% (1-25%) |
| **Median follow-up (range)** |               | 3.3 years (0-25 years) |
| **Local recurrence**     |                  | 23 (55%) |
| **Median local recurrence free survival** |             | 5.8 years |
| **Death**                |                  | 15 (46%) |
| **Median overall survival** |              | 11.2 years |

**Cause of death**

- Progressive disease: 11 (73%)
- Adverse effect of treatment: 1 (7%)
- Other: 2 (13%)
- Unknown: 1 (7%)

**FOXM1 IHC samples, related to Figure 3**

| Patients | 52 |
|----------|----|
| Median age at diagnosis (range) | 61 years (23-87 years) |
| Male:Female | 20:32 (1:1.6) |
| Meningiomas | 52 |
| WHO Grade I | 20 (38%) |
| WHO Grade II | 26 (50%) |
| WHO Grade III | 6 (12%) |
| Recurrent | 16 (31%) |
| Skull base | 23 (44%) |
| Convexity | 29 (56%) |

**Extent of resection**

|                  |                  |      |
|------------------|------------------|------|
| **Gross total**  |                  | 30 (58%) |
| **Subtotal**     |                  | 22 (42%) |
| **Unknown**      |                  | 0 (0%) |
| **Adjuvant radiotherapy** |            | 17 (33%) |
| **Median MIB1 labeling index (range)** |        | 9.5% (1-60%) |
| **Median follow-up (range)** |            | 5.2 years (1-17 years) |
| **Local recurrence** |              | 24 (46%) |
| **Median local recurrence free survival** |         | 6.2 years |
| **Death**        |                  | 14 (27%) |
| **Median overall survival** |         | 10.8 years |

**Cause of death**

- Progressive disease: 9 (57%)
- Adverse effect of treatment: 1 (8%)
- Other: 2 (14%)
- Unknown: 3 (21%)

**WES samples, related to Figure 4**

| Patients | 24 |
|----------|----|
| Median age at diagnosis (range) | 52 years (21-77 years) |
| Male:Female | 15:9 (1:0.6) |
### Meningiomas

| WHO Grade | Count (Percentage) |
|-----------|-------------------|
| I         | 8 (33%)           |
| II        | 11 (46%)          |
| III       | 5 (21%)           |

| Recurrence | Count (Percentage) |
|------------|--------------------|
| RECURRENT  | 14 (58%)           |

| Location  | Count (Percentage) |
|-----------|--------------------|
| Skull base| 8 (33%)            |
| Convexity | 17 (71%)           |

### Extent of resection

| Type         | Count (Percentage) |
|--------------|--------------------|
| Gross total  | 9 (38%)            |
| Subtotal     | 15 (62%)           |
| Unknown      | 0 (0%)             |

| Adjunct radiotherapy | Count (Percentage) |
|----------------------|--------------------|
| 12 (50%)             |

| Median MIB1 labeling index (range) | 6.5% (1-25%) |
| Median follow-up (range)          | 6.2 years (9-20 years) |
| Local recurrence                  | 19 (79%) |
| Median local recurrence free survival | 2.1 years |
| Death                              | 9 (38%) |
| Median overall survival            | 14.5 years |

### Cause of death

- Progressive disease: 8 (88%)
- Adverse effect of treatment: 1 (12%)
- Other: 0 (0%)
- Unknown: 0 (0%)

### DNA methylation profiling samples, related to Figure 5

| Patients | Count |
|----------|-------|
| Median age at diagnosis (range) | 52 years (21-77 years) |
| Male:Female | 16:10 (1:0.6) |

| Meningiomas | Count (Percentage) |
|-------------|--------------------|
| WHO Grade I | 8 (31%)            |
| WHO Grade II| 13 (50%)           |
| WHO Grade III| 5 (19%)          |

| Recurrence | Count (Percentage) |
|------------|--------------------|
| RECURRENT  | 16 (62%)           |

| Location  | Count (Percentage) |
|-----------|--------------------|
| Skull base| 10 (38%)           |
| Convexity | 18 (69%)           |

### Extent of resection

| Type         | Count (Percentage) |
|--------------|--------------------|
| Gross total  | 9 (35%)            |
| Subtotal     | 17 (65%)           |
| Unknown      | 0 (0%)             |

| Adjunct radiotherapy | Count (Percentage) |
|----------------------|--------------------|
| 12 (46%)             |

| Median MIB1 labeling index (range) | 6.5% (1-25%) |
| Median follow-up (range)          | 4 years (0-20 years) |
| Local recurrence                  | 20 (77%) |
| Median local recurrence free survival | 1.9 years |
| Death                              | 9 (35%) |
| Median overall survival            | 10.8 years |

### Cause of death
| Category                                           | Value |
|----------------------------------------------------|-------|
| Progressive disease                                | 9 (89%) |
| Adverse effect of treatment                         | 1 (11%) |
| Other                                              | 0 (0%) |
| Unknown                                            | 0 (0%) |

**NanoString gene expression profiling samples, related to Figure 6**

| Category                                           | Value |
|----------------------------------------------------|-------|
| Patients                                           | 83    |
| Median age at diagnosis (range)                    | 60 years (19-87 years) |
| Male:Female                                        | 34:49 (1:1.4) |
| Meningiomas                                        | 96    |
| WHO Grade I                                        | 13 (14%) |
| WHO Grade II                                       | 64 (66%) |
| WHO Grade III                                      | 19 (20%) |
| Recurrent                                          | 33 (34%) |
| Skull base                                         | 38 (43%) |
| Convexity                                          | 66 (69%) |

**Extent of resection**

| Category                                           | Value |
|----------------------------------------------------|-------|
| Gross total                                        | 45 (47%) |
| Subtotal                                           | 51 (53%) |
| Unknown                                            | 0 (0%) |

**Adjuvant radiotherapy**

| Category                                           | Value |
|----------------------------------------------------|-------|
| Adjuvant radiotherapy                              | 42 (44%) |
| Median MIB1 labeling index (range)                 | 10% (2-60%) |
| Median follow-up (range)                           | 5.4 years (0-15 years) |
| Local recurrence                                   | 49 (51%) |
| Median local recurrence free survival              | 6.5 years |
| Death                                              | 35 (42%) |
| Median overall survival                             | 11.9 years |

**Cause of death**

| Category                                           | Value |
|----------------------------------------------------|-------|
| Progressive disease                                | 17 (49%) |
| Adverse effect of treatment                         | 5 (14%) |
| Other                                              | 2 (6%) |
| Unknown                                            | 11 (31%) |

**β-catenin TMA samples, related to Figure 6**

| Category                                           | Value |
|----------------------------------------------------|-------|
| Patients                                           | 218   |
| Median age at diagnosis (range)                    | 60 years (19-87 years) |
| Male:Female                                        | 77:141 (1:1.8) |
| Meningiomas                                        | 232   |
| WHO Grade I                                        | 127 (55%) |
| WHO Grade II                                       | 83 (36%) |
| WHO Grade III                                      | 22 (9%) |
| Recurrent                                          | 47 (20%) |
| Skull base                                         | 100 (43%) |
| Convexity                                          | 140 (60%) |

**Extent of resection**

| Category                                           | Value |
|----------------------------------------------------|-------|
| Gross total                                        | 138 (59%) |
| Subtotal                                           | 91 (39%) |
| Unknown                                            | 3 (2%) |
### Adjuvant radiotherapy
- 53 (23%)

### Median MIB1 labeling index (range)
- 7% (1-72%)

### Median follow-up (range)
- 4.3 years (0-15 years)

### Local recurrence
- 73 (31%)

### Median local recurrence free survival
- 9.4 years

### Death
- 53 (23%)

### Median overall survival
- 11.9 years

### Cause of death
- Progressive disease: 21 (40%)
- Adverse effect of treatment: 9 (17%)
- Other: 8 (15%)
- Unknown: 15 (28%)

### Table S7. Cox hazard analyses, related to Figure 6

#### LRFS univariate Cox hazard analysis: FOXM1 NanoString samples

| Variable                | HR  | RSE | 95% Confidence interval | P-value |
|-------------------------|-----|-----|-------------------------|---------|
| Recurrent tumor         | 2.62| 0.78| 1.47 – 4.68             | 0.001   |
| Subtotal resection      | 1.56| 0.44| 0.89 – 2.71             | 0.117   |
| No adjuvant radiation   | 0.91| 0.26| 0.52 – 1.60             | 0.743   |
| High grade              | 1.78| 0.82| 0.72 – 4.37             | 0.210   |
| High FOXM1              | 2.53| 0.77| 1.40 – 5.59             | 0.002   |

#### LRFS multivariate Cox hazard analysis: FOXM1 NanoString samples

| Variable                | HR  | RSE | 95% Confidence interval | P-value |
|-------------------------|-----|-----|-------------------------|---------|
| Recurrent tumor         | 1.84| 0.58| 0.99 – 3.41             | 0.053   |
| Subtotal resection      | 1.50| 0.49| 0.80 – 2.84             | 0.208   |
| No adjuvant radiation   | 1.29| 0.40| 0.70 – 2.37             | 0.422   |
| High grade              | 1.38| 0.62| 0.57 – 3.31             | 0.472   |
| High FOXM1              | 2.06| 0.64| 1.12 – 3.80             | 0.020   |

#### OS univariate Cox hazard analysis: FOXM1 NanoString samples

| Variable                | HR  | RSE | 95% Confidence interval | P-value |
|-------------------------|-----|-----|-------------------------|---------|
| Age                     | 1.04| 0.02| 1.01 – 1.08             | 0.019   |
| Recurrent tumor         | 3.85| 1.47| 1.82 – 8.13             | <0.001  |
| Subtotal resection      | 1.91| 0.67| 0.96 – 3.81             | 0.066   |
| No adjuvant radiation   | 0.81| 0.28| 0.41 – 1.60             | 0.544   |
| High grade              | 3.50| 2.65| 0.79 – 15.5             | 0.098   |
| High FOXM1              | 2.91| 1.12| 1.37 – 6.18             | 0.005   |

#### OS multivariate Cox hazard analysis: FOXM1 NanoString samples

| Variable                | HR  | RSE | 95% Confidence interval | P-value |
|-------------------------|-----|-----|-------------------------|---------|
| Age                     | 1.05| 0.02| 1.02 – 1.10             | 0.004   |
| Recurrent tumor         | 2.28| 1.07| 0.91 – 5.71             | 0.078   |
| Subtotal resection      | 2.69| 1.03| 1.27 – 5.73             | 0.010   |
| No adjuvant radiation   | 1.84| 0.69| 0.88 – 3.85             | 0.106   |
| High grade              | 2.04| 1.80| 0.36 – 11.5             | 0.421   |
| High FOXM1              | 3.13| 1.39| 1.31 – 7.47             | 0.007   |

#### LRFS univariate Cox hazard analysis: Nuclear β-catenin TMA samples

| Variable                | HR  | RSE | 95% Confidence interval | P-value |
|-------------------------|-----|-----|-------------------------|---------|
| Variable                      | HR   | RSE  | 95% Confidence interval | P-value |
|-------------------------------|------|------|-------------------------|---------|
| Recurrent tumor               | 3.33 | 0.92 | 1.94 – 5.72             | <0.001  |
| Subtotal resection            | 2.14 | 0.58 | 1.27 – 3.63             | 0.005   |
| No adjuvant radiation         | 1.42 | 0.42 | 0.80 – 2.55             | 0.234   |
| High grade                    | 2.17 | 0.58 | 1.28 – 3.66             | 0.004   |
| Nuclear β-Catenin             | 2.69 | 0.74 | 1.57 – 4.60             | <0.001  |

LRFS multivariate Cox hazard analysis: Nuclear β-catenin TMA samples

| Variable                      | HR   | RSE  | 95% Confidence interval | P-value |
|-------------------------------|------|------|-------------------------|---------|
| Recurrent tumor               | 4.34 | 1.12 | 2.61 – 7.20             | <0.001  |
| Subtotal resection            | 2.65 | 0.63 | 1.67 – 4.21             | <0.001  |
| No adjuvant radiation         | 0.53 | 0.13 | 0.33 – 0.86             | 0.011   |
| High grade                    | 2.99 | 0.76 | 1.82 – 4.91             | <0.001  |
| Nuclear β-Catenin             | 2.67 | 0.82 | 1.46 – 4.88             | 0.001   |

Table S8. Primary meningioma cells and molecular reagents, related to Figure 7

| Cell line | Grade | Setting   | Location        | NF2 diagnosis |
|-----------|-------|-----------|-----------------|---------------|
| M3        | II    | Primary   | Sphenoid        | -             |
| M6        | III   | Recurrent | Olfactory groove| -             |
| M8        | II    | Primary   | Frontal convexity| -             |
| M10       | III   | Recurrent | Parasagittal    | Yes           |
| M12       | II    | Recurrent | Parasagittal    | -             |

| Gene       | qRT-PCR sense primer | qRT-PCR antisense primer |
|------------|----------------------|--------------------------|
| CCNA2      | CTGCATTTTGCTGTGAACCTAC | ACAAACCTCTGCTACTTCTGGA    |
| CCNB2      | CCTCCCTTTTCAGTCCGC   | CTCCTGTGTCAATATTCTCCAATCTC |
| FOXM1      | ACTTTAAGCACATTGCAAAGC | CGTGCAAGGAAGGGTGT         |
| GAPDH      | TGCCCCCATGTTTGATGAG  | TGTGGTATGAGCCCTTCC        |

FOXM1 shRNA

| KD1        | GCAAGAAGAAATCTCGGTAA |
| KD2        | GCCAATCGGTCTCTGACAGA |
| KD3        | GCCCAACAGGAGTCTAATCAA |
| KD4        | GCACATACCAACATAGCTAT |
Figure S1. Unsupervised clustering of meningioma RNA-seq data reveals distinct transcriptomic clusters, related to Figure 2.

(A) Unsupervised hierarchical clustering segregates meningiomas into 2 clusters. 
(B-D) Meningioma transcriptomic clusters have distinct associations with patient sex, meningioma location and grade (Skull base, SB). 
(E, F) Meningioma transcriptomic clusters have no associations with patient age or prior cranial radiation (Cluster 1, C1; cluster 2, C2; radiotherapy, RT). 
(G) KEGG pathway analysis shows enrichment of focal adhesion genes in the cluster of meningiomas that are more likely to be low grade and located at the skull base in female patients. 
(H) KEGG pathway analysis shows enrichment of metabolism and oxidative phosphorylation genes in the cluster of meningiomas that are more likely to be high grade in male patients. 
(I, J) OS and LRFS are not significantly different between meningioma transcriptomic clusters.

(K) FOXM1 targets, as identified from published ChIP-seq data, are enriched in WHO Grade III meningiomas (N=78, 11%) relative to WHO Grade I meningiomas (N=26, 3%) by RNA-seq. 
(L) FOXM1 expression by RNA-seq is equivalent across meningioma transcriptomic clusters. 
(M) FOXM1 targets, as identified from published ChIP-seq data, are not significantly different between meningioma transcriptomic clusters (Cluster 1, C1, N=103, 5%; cluster 2, C2, N=61, 6%).
Figure S2. Meningioma NF2 mutation is associated with genomic instability, related to Figure 4.
Analysis of the number of large scale chromosomal alterations per meningioma, defined as comprising greater than one third of a chromosomal arm, identifies significantly more events in NF2 mutant tumors, including 1p loss, 6q loss, 10q loss, 18q loss and 22q loss.

NF2 wild type (N=10)
NF2 mutant (N=14)
P=0.0015
Figure S3. Meningioma somatic mutation burden is associated with clinical parameters, related to Figure 4.

(A-C) The number of somatic mutations per meningioma is significantly increased in high grade meningiomas, convexity meningiomas, and meningiomas from patients with a history of prior cranial radiation (*P=0.0217, **P=0.0180, and ***P=0.0168; convexity, CV; radiotherapy, RT).

(D) High meningioma somatic mutation burden is associated with poor LRFS relative to low meningioma somatic mutation burden (N=24).
Figure S4. Meningioma DNA methylation pattern is associated with clinical parameters, related to Figure 5.

(A) Unsupervised hierarchical clustering based on the top 2,000 most variable probes segregates meningiomas into 3 clusters based on high, low and medium DNA methylation with significant differences in β values ($\beta$=methylated/[methylated+unmethylated]) (N=26, *P<0.0001).
(B) High meningioma DNA methylation is associated with high nonsynonymous mutation burden relative to low and medium meningioma methylation clusters (*P<0.0001).
(C-E) High and medium meningioma DNA methylation are associated with high grade, convexity location and NF2 mutation relative to low meningioma methylation (Convexity, CV; mutant, M; wild type, WT).
(F) LRFS is not significantly different between high, medium and low meningioma DNA methylation clusters.
Figure S5. Differentially methylated probe analysis reveals unique epigenomic signatures associated with meningioma grade, related to Figure 5.

(A) Differential analysis between meningioma DNA methylation clusters identifies a total of 3,380 hypermethylated probes in the high methylation cluster, and 860 hypermethylated probes in the medium methylation cluster, compared to the low methylation cluster. Consistently, only 405 hypomethylated sites are identified in the high methylation cluster, and 225 hypomethylated sites are identified in the medium methylation cluster, compared to the low methylation cluster.

(B) GREAT INTERPRO analysis for hypermethylated sites in the high meningioma DNA methylation cluster shows enrichment for homeobox genes.

(C) GREAT molecular signatures database analysis for WHO Grade II hypermethylated sites shows enrichment of focal adhesion genes relative to WHO Grade III meningiomas.

(D) GREAT gene ontology analysis for biological processes for WHO Grade II hypermethylated sites shows enrichment of cell polarity and growth genes relative to WHO Grade III meningiomas.

(E-G) Meningiomas with high FOXM1 mRNA expression by RNA-seq exhibit decreased expression of PRC target genes ATF3, HOXA13 and HOXC5 (*P<0.05).
Figure S6. FOXM1 expression is associated with cell proliferation gene expression in aggressive meningioma, related to Figure 6. (A,B) High grade and recurrent meningiomas are enriched in FOXM1 protein by IHC (N=52). Red denotes FOXM1 high tumors, and blue denotes FOXM1 low tumors. (C) High FOXM1 mRNA expression in WHO Grade II meningiomas by NanoString targeted gene expression profiling is associated with poor LRFS and OS relative to WHO grade II meningiomas with low FOXM1 mRNA expression (N=63). (D-F) High expression of FOXM1 cell proliferation target genes by NanoString targeted gene expression profiling, such as TOP2A, CCNA2 and CKS2, is associated with poor LRFS relative to meningiomas with low FOXM1 cell proliferation target gene expression (N=96).
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