**Supplemental Materials and Methods**

**RNAseq analysis**

RNA libraries were prepared for sequencing and data analysis was performed as previously described 1. In brief, we used the NEBNext Ultra RNA Library Prep Kit for Illumina (Cat# E7530L, New England BioLabs, Ipswich, MA) which included the removal of large and small RNA, synthesis of cDNA, and construction of cDNA libraries. Libraries were barcoded using NEBNext Multiplex Oligos for Illumina (New England BioLabs, Ipswich, MA). Libraries were sequenced using Illumina HiSeq 2500, with paired-end 100bp reads. Paired-end reads were aligned to UCSC human transcriptome 19 (hg19) using Tophat (Bowtie v2.0.9). Alignment quality and read distribution was assessed via Samtools (v0.1.19). Transcript assembly was conducted using Cufflinks (v2.2.1). Normalized expression data was generated from aligned BAM files using Cuffnorm and Cuffdiff. Transcripts with zero values for FPKM across all samples were removed. Differential splicing analysis for LEPR transcript isoforms was conducted using JuncBASE (Junction-Based Analysis of Splicing Events) to identify and classify alternative splicing events, relying on *de novo* transcript assembly from cufflinks.

**Microarray data analysis**

Microarray expression profiling data from publicly available datasets cataloged in GEO (Gene Expression Omnibus) was normalized as previously described 2,3. Data was from the Affymetrix Human Genome U133 Plus 2.0 Array Platform [HG-U133\_Plus\_2]. The following datasets were used: GSE5675 (pilocytic astrocytoma), GSE16155 (ependymoma), GSE16581 (meningioma), GSE19404 (primitive neuroectodermal tumor), GSE34771 (CNS lymphoma), GSE35493 (atypical teratoid rhabdoid tumors/ATRT; medulloblastoma), GSE34824 (pediatric glioblastoma), GSE36245 (adult glioblastoma). Heat maps were generated using Genepattern tools (Broad Institute, <http://www.broadinstitute.org/cancer/software/genepattern/>).

We first identified the genes that were highly differentially expressed in meningioma compared to other brain tumors using publicly available expression profiling data in GEO (Gene Expression Omnibus) that included 68 meningioma and 282 other brain tumor/normal samples including pilocytic astrocytoma (PA), ependymoma (EP), primitive neuroectodermal tumor (PNET), primary CNS lymphoma (PCNSL), medulloblastoma (MB), atypical teratoid rhabdoid tumor (AT/RT), and glioblastoma (GBM) as well as normal brain (NB). We ranked this gene list of all differentially expressed genes between meningioma and other brain tumor samples (**Table S9**) according to an enrichment score calculated using the module of GSEA in GenePattern platform from Broad Institute (<http://software.broadinstitute.org/cancer/software/genepattern/modules/docs/GSEA/14>). We analyzed the top 140 ranked genes with enrichment scores >0.95 using a Transcription Factor Checkpoint tool (http://norstore-trd-bio0.hpc.ntnu.no/ ), and found the 13 TFs that were highly differentially expressed in meningioma (1. KLF5, ranked 13; 2. MEOX2, ranked 16; 3. SIX1, ranked 32; 4. BNC2, ranked 35; 5. FOXC2, ranked 52; 6. OSR1, ranked 59; 7. SIX2, ranked 66; 8. FOXC1, ranked 69; 9. TBX15, ranked 76; 10. FOXD2, ranked 83; 11. HLF, ranked 88; 12. SNAI2, ranked 125; 13. FOXD1, ranked 136) labeled as red in new Table S9. We also performed an additional statistical differential expression analysis (now presented in new Table S10) using the module of Comparative Marker Selection in GenePattern from the Broad Institute (http://software.broadinstitute.org/cancer/software/genepattern/modules/docs/ComparativeMarkerSelection/10 ) and we analyzed the top 225 most significant upregulated genes (t-test score > 22 and p < 0.0002) using a Transcription Factor Checkpoint tool (http://norstore-trd-bio0.hpc.ntnu.no/ ), and found 18 TFs that were highly differentially expressed in meningioma (1. FOXC1, ranked 3; 2. KLF5, ranked 4; 3. MEOX2, ranked 12; 4. BNC2, ranked 16; 5. SIX1, ranked 18; 6. TBX15, ranked 27; 7. FOXD1, ranked 38; 8. FOXC2, ranked 80; 9. FOXD2, ranked 89; 10. FOXP1, ranked 101;11. HLF, ranked 108; 12. SNAI2, ranked 141; 13. KLF2, ranked 151; 14. STAT6, ranked 171; 15. SIX4, ranked 176; 16. CREB3L2, ranked 192; 17. SIX2, ranked 194; 18. FOXO1, ranked 222;) labeled as red in new Table S10. Based on this analysis and the enrichment score analysis described above, we focused our subsequent work on the eight most highly ranked TFs (SIX1, SIX2, FOXC1, FOXC2, FOXD1, BNC2, MEOX2 and KLF5) that were identified using both analyses methods.

**Immunohistochemistry and scoring**

The following primary antibodies were used in this study: SIX1 (1:100 dilution, Cat# HPA001893, Sigma-Aldrich), FOXC1 (1:500 dilution, Cat# ab5079, Abcam), FOXC2 (1:100 dilution, Cat# ab65141, Abcam), BNC2 (1:50 dilution, Cat# ab84845, Abcam), MEOX2 (1:1500, Cat# WH0004223M3, Sigma-Aldrich), KLF5 (1:250, Cat# PIPA5-27876  Thermo Fisher), EMA (1:200, Cat# M0613, Dako) , LEPR (1:75, Cat# HPA030899, Sigma-Aldrich) and Tryptase (1:3000, Cat# M7052, Dako). Antigen retrieval was performed using a pressure cooker in citrate buffer, except for EMA which required no pre-treatment. Slides were incubated with primary antibody for 45 to 60 minutes at room temperature. Secondary antibody was applied for 30 minutes at room temperature followed by visualization with 3,3'-Diaminobenzidine (DAB) treatment for 5 minutes. We substituted non-immune goat or rabbit serum for the primary antibodies for negative controls.

Staining on whole sections and TMA slides was semi-quantitatively estimated by visual microscopic review while blinded to clinical and pathologic information. The IHC score was a composite obtained by multiplying the values of staining intensity and relative abundance of positive cells (frequency) as previously described 2,4. For whole sections, the intensity was grades as 0 (no staining), 1 (weak staining), 2 (weak-moderate staining), 3 (moderate staining), 4 (moderate-strong staining) or 5 (strong staining). The abundance of positive cells was graded from 0 to 5 (0, <5% positive cells; 1, 5–20%; 2, >20–40%; 3, >40–60%; 4, >60-80%); 5, >80-100%). The scoring range for whole sections was 0 to 25. The IHC scoring for TMAs was simplified due to the complexity of visually scoring large numbers of cores and a range of 0 to 12 was used: the intensity of staining was scored from 0 to 3 (0, no staining; 1, weak staining, 2, moderate staining; 3, strong staining) and the abundance of positive cells was scored from 0 to 4 (0, <5% positive cells; 1, 5–25%; 2, >25–50%; 3, >50–75%; 4, >75%). A composite IHC score for each case was calculated as the mean value from available replicate cores (1 to 3) (**Table S2**), and a composite IHC score greater than the median value for all cases was designated as ‘high expression’ and less than or equal to the median value was designated as ‘low expression’. Tryptase-positive mast cells were counted per core and the average number of infiltrated mast cells was calculated for each case (using all cores). An average number greater than the median value for all cases was considered “high infiltration” and less than or equal to the median value was considered “low infiltration”.

For the analysis of pulmonary meningothelial nodules, the archives of Brigham and Women’s Hospital were searched from 2004 to 2013 for cases in which meningothelial nodules were described in the pathology report of pulmonary wedge resections. Forty-six cases were identified. A group of 46 patients that had undergone pulmonary resections from the same time-period and with the same gender distribution (female>male) were selected as a control group. The last weight recorded in the electronic medical record for these patients prior to the surgery was ascertained and used to calculate the patient’s BMI.

**Immunofluorescence assay**

Meningioma PDCLs were seed in chamber slides and cultured overnight. Primary antibodies for SIX1 (1:500 dilution, Cat# HPA001893, Sigma-Aldrich), FOXC1 (1:500 dilution, Cat# ab5079, Abcam) and FOXC2 (1:500 dilution, Cat# ab65141, Abcam) were used.

**RNA *in situ* hybridization**

RNAscope was performed to detect meningioma TF mRNA in FFPE sections as previously described 2,4,5. Briefly 5 um FFPE sections were deparaffinized, boiled with pretreatment reagent for 15 minutes, and then protease digested at 40ºC for 30 minutes, followed by hybridization for 2 hours at 40ºC with Probe-Hs-SIX1(Custom design, Advanced Cell Diagnostics, USA), Probe-Hs-FOXC2(Cat# 313201, Advanced Cell Diagnostics, USA), Probe-Hs-FOXD1(Custom design, Advanced Cell Diagnostics, USA), Probe-Hs-LEPR-tv1 (LEPR LONG, Cat# 410371, Advanced Cell Diagnostics, USA), Probe-Hs-LEPR alltv (LEPR TOTAL, Cat# 410381, Advanced Cell Diagnostics, USA), Probe-Hs-PTGDR (Custom design, Advanced Cell Diagnostics, USA). The probe was visualized with 3,3'-Diaminobenzidine (DAB) and cell nuclei were stained with Hematoxylin. The Probe-DAPB (Cat# 310043, Advanced Cell Diagnostics, USA) was used as a negative control, and Probe-Hs-PPIB (Cat# 313901, Advanced Cell Diagnostics, USA) was used as a positive control. All the slides were digitally scanned, and then visually evaluated by two pathologists (ZD and SS). We excluded cases if there were staining artifacts, less than 90% tumor remaining in the cores or if there were very low or undetectable levels of PPIB. We developed an analysis pipeline to analyze the number of dots using open-source CellProfiler image analysis software (<http://www.cellprofiler.org/>) 4. We classified all cases with an average number of dots per cell greater than the median for all cases in a cohort as “high expression”, and lower than the median for all cases in a cohort as “low expression”.

The *in situ* hybridization images of meningioma TFs in E14.5 mouse and E15.5 mouse were obtained from GenePaint.org (<http://www.genepaint.org/Frameset.html>) and the Allen Developing Mouse Brain Atlas (<http://developingmouse.brain-map.org/>), respectively, with expert review from one of the developers of the Genepaint web-based gene expression atlas for the mouse (L.G.) 6.

**qRT-PCR assays**

For qRT-PCR assay, we extracted total RNA from frozen meningioma tissues and cell lines using the TRIzol Plus RNA Purification Kit (Cat# 12183-555, Life Technologies) per the manufacturer’s protocols. We used high capacity cDNA Reverse Transcription Kit with RNase Inhibitor (Cat# 4374966, Life Technologies) for reverse transcription. TaqMan assays for SIX1 (Hs00195590\_m1), FOXC1 (Hs00559473\_s1), FOXC2 (Hs00270951\_s1), FOXD1 (Hs00270117\_s1), BNC2 (Hs00417700\_m1), MEOX2 (Hs00232248\_m1), KLF5 (Hs00156145\_m1), LEPR TOTAL (Hs00174497\_m1), LEPR LONG (Hs00174492\_m1), PTGDR (Hs00235003\_m1), EYA1 (Hs00166804\_m1), EYA2 (Hs00193347\_m1), EYA3 (Hs00544914\_m1) and EYA4 (Hs00187965\_m1) were used. We used as an internal control eukaryotic 18S rRNA Endogenous Control (Cat# 4333760T, Life Technologies). The relative expression level was determined as 2−ΔΔCt, and data is presented as the expression level relative to the calibrator (control sample). Moreover, the Ct value of target genes was normalized to 18S rRNA Ct value of 9, and the average normalized Ct value is presented.

**t-SNE and EMGM analysis**

t-distributed stochastic neighbor embedding (t-SNE) analysis was performed with protein (IHC) and mRNA (RNAscope) expression data from meningioma resection samples using the CYT visualization tool. This analysis included data from all 99 cases from HTMA 283 and 285 which had data available for all of the markers assessed including EMA IHC, SIX1 IHC, FOXC1 IHC, FOXC2 IHC, BNC2 IHC, MEOX2 IHC, KLF5 IHC, LEPR IHC, SIX1 RNAscope, FOXC2 RNAscope, FOXD1 RNAscope, LEPR TOTAL RNAscope, LEPR LONG RNAscope, and PTGDR RNAscope. The expression level of each antibody or RNAscope probe was mapped to color. 79 cases were excluded because data from one or more biomarkers was missing. Unsupervised clustering was performed using expectation-maximization Gaussian mixture (EMGM) modeling to define each sample as well-differentiated or de-differentiated and this data was superimposed on the t-SNE plot. The viSNE implementation of t-SNE and EMGM algorithms from the CYT single-cell analysis package were obtained from the Pe’er laboratory at Columbia University 7 , and were run with MATLAB R2017a.

**Cell viability assay**

Cell viability was assessed in three different settings. For siRNA treatment, 3,000 primary meningioma cells were seeded in 96 well plates a day prior to transfection. The cells were transfected with 100 nM of siRNA pools or 20 nM of individual Silencer Select siRNA and five days later the cell viability was assessed. For recombinant human Leptin treatment, 3,000 primary meningioma cells were seeded in 96-well plates in serum free DMEM media, and on the following day, the cells were treated with the indicated amount of recombinant human leptin at (Cat# 398-LP-01M, R&D systems). Leptin was added to serum free DMEM. Leptin supplemented media was refreshed at day three and cell viability assay was assessed four days later. For the meningioma cells that were stably transduced with lentivirus expressing shRNA, 3,000 cells were seeded in 96-well plates and cultured for 7 days at which point cell viability was assessed.

CellTiter-Glo Luminescent Cell Viability Assay (Cat# G7573, Promega, USA) was used to assess the relative cell number. Briefly, we equilibrated the plate and its contents at room temperature for 30 mins, and then added 20 µl of CellTiter-Glo® Reagent to the 100 µl of cell culture medium present within each well, and mixed contents for 2 minutes to lysis the cells. After 10 minutes, at room temperature, the luminescence was recorded using a plate reader (POLARstar Omega, BMG LABTECH). The relative cell number between control and experimental was calculated.

**siRNA transfection to evaluate effects on mRNA expression**

Before transfection, 2×105cells per well were plated into 6-well plates and the following day, at 40% to 60% confluence, they were transfected with 100 nM of target-specific siRNAs pool (see **Table S6** for details about the sequences of siRNA pools) or 100 nM negative control siRNA (Sigma, USA), using lipofectamine RNAiMAX (Cat# 13778-030, Life Technologies, USA). In the indicated experiments, the cells were transfected with 20 nM of three different individual SIX1 or EYA2 Silencer Select siRNAs (SIX1: Cat# s12874, Cat# s12875, Cat# s12876; EYA2: Cat# s4907, Cat# s4908 and Cat# s4909, Life Technologies Corp., USA) or 20 nM of Silencer Select negative control (Cat# 4390843, Life Technologies Corp., USA). In all cases, the cells were harvested for RNA extraction 72 h following transfection.

**Stable cell line generation using lentivirus shRNA infection**

Before transfection, 2×105cells per well were plated into 6-well plates and the following day, at 40% to 60% confluence, the cells were infected with lentivirus shRNA against SIX1, LEPR and LACZ (see **Table S7** for details about the shRNA sequences) using polybrene (Cat# sc-134220, Santa Cruz, USA). Three days following infection, the cells were selected using 2 µg/ml puromycin (Cat#A11138, Life Technologies Corp. USA).

**Apoptosis detection assay and cell cycle assay**

Annexin V-Cy3 Apoptosis Detection Kit with SYTOX (Cat# ab14144, Abcam, USA) was used. Briefly 1-5 ×105 of MG15 cells infected with lentivirus shRNA against SIX1, LEPR and LACZ without puromycin selection were collected, and stained with 5 ul of Annexin V-Cy3 and 1 ul of SYTOX Green dye for 5 min. Then quantification was measured by flow cytometry using DXP11 Analyzer in BWH Flow Cytometry Core. For cell cycle assay, MUSE Cell Cycle Assay Kit (Cat# MCH100106, Millipore) was used. Briefly 1-5 ×105 of MG15 cells infected with lentivirus shRNA against SIX1, LEPR and LACZ without puromycin selection were collected and fixed by cold 70% ethanol overnight, and then stained with propidium iodide (PI) for 30 min. Then quantification was measured by MUSE Cell Analyzer.

**Statistical analysis**

We used Chi-Square tests to evaluate differences between high and low expressors in meningioma of different grades. Kaplan-Meier (KM) analysis and log-rank tests were used to assess survival rate, and to compare differences in survival curves. We excluded the patients who died from other causes (non-meningioma related) from our overall survival analysis, which is disease-specific survival. We considered p values of < 0.05 as statistically significant. We analyzed data using GraphPad Prism 6 software and SPSS12.0.

Reference

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