1 **LncRNA Miat and Metadhedrin maintain a treatment resistant stem-like niche of** 2 **medulloblastoma cells**

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4 Kai-Lin Peng¹, Harish N. Vasudevan^{1,2}, Dennis T. Lockney¹, Rachel Baum¹, Ronald C.
5 Hendrickson³, David R. Raleigh^{2*}, and Adam M. Schmitt^{1*}

6

7 ¹Division of Translational Oncology, Department of Radiation Oncology, Memorial
8 Sloan Kettering Cancer Center, New York, NY, USA

9 ²Departments of Radiation Oncology and Neurological Surgery, University of
10 California San Francisco, San Francisco, CA, USA

11 ³Microchemistry and Proteomics, Memorial Sloan Kettering Cancer Center, New York,
12 NY, USA

13

14 *Corresponding authors: David R. Raleigh, Adam M. Schmitt

15 Emails: david.raleigh@ucsf.edu, schmitta@mskcc.org

16

17 **Abstract**

18 Medulloblastoma is a pediatric brain tumor arising from the cerebellum and brainstem
19 that is curable with surgery and aggressive cytotoxic therapy including craniospinal
20 irradiation and chemotherapy. Preclinical models indicate that a small pool of de-
21 differentiated, stem cell-like medulloblastoma cells are resistant to cytotoxic treatment
22 and contribute to medulloblastoma relapse after aggressive therapy. Here, we identify
23 *Miat* as a Shh and Myc regulated long noncoding RNA (lncRNA) that is required for
24 maintenance of a treatment-resistant medulloblastoma stem-like phenotype. Loss of
25 *Miat* delays medulloblastoma formation in genetically defined mouse models of Shh

1 medulloblastoma and enforces differentiation of tumorigenic stem-like
2 medulloblastoma cells into a non-tumorigenic cell state. Miat facilitates treatment
3 resistance in part by downregulating p53 signaling and impairing radiation induced cell
4 death in stem-like MB cells, which can be reversed by therapeutic inhibition of Miat
5 with antisense oligonucleotides. The RNA binding protein Metadhedrin (Mtdh), which
6 is associated with resistance to cytotoxic therapy in numerous types of cancer, co-
7 localizes with Miat in stem-like medulloblastoma cells. Further, loss of Mtdh activates
8 p53 signaling and reduces tumorigenicity in stem-like medulloblastoma cells. Taken
9 together, these data reveal a critical role for the lncRNA Miat in sustaining a treatment
10 resistant pool of tumorigenic stem-like medulloblastoma cells.

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1 Introduction

2 Medulloblastoma (MB) is the most common malignant brain tumor in children (1).
 3 Treatment with high dose chemotherapy and craniospinal irradiation can cure
 4 medulloblastoma, but frequently at the expense of neurocognitive toxicities (2). While
 5 tumor classifications based on molecular profiling have revealed pathogenic drivers of
 6 the disease and differentiate prognostic groups, attempts to dose de-escalate
 7 craniospinal irradiation has been hindered by increased risks of treatment failure, even
 8 in the Wnt group of MB patients who generally have the best prognosis (3, 4). Stem-
 9 like cancer cells have been postulated to be a reservoir of treatment resistance in
 10 medulloblastoma and other brain tumors (5-8). Identifying and targeting mechanisms
 11 that maintain treatment resistant stem-like medulloblastoma cells could provide an
 12 opportunity to reduce the risk of treatment failure and ultimately support strategies to
 13 de-escalate the curative cytotoxic regimen.

14 The Sonic Hedgehog (Shh) group of medulloblastoma accounts for the largest
 15 proportion of patients with the disease. Outcomes in Shh medulloblastoma are highly
 16 variable and include favorable and unfavorable subtypes. Indeed, Shh
 17 medulloblastomas with *TP53* mutations account for some of the worst prognoses,
 18 perhaps due to deficiencies in p53-dependent cell death following cytotoxic therapy (9,
 19 10). p53 signaling also plays an important role in preventing cell dedifferentiation and
 20 downregulation of p53 is a requirement for the generation of induced pluripotent stem
 21 cells and stem-like cancer cells (11, 12). Furthermore, subpopulations of stem-like
 22 medulloblastoma cells have been associated with treatment resistance (7, 8, 13).
 23 Therefore, understanding the mechanisms that facilitated the maintenance of stem-like
 24 medulloblastoma and their treatment resistance could improve treatment outcomes in
 25 the disease.

Long noncoding RNAs (lncRNAs) are increasingly recognized as central actors in and cancer phenotypes (14). Prior work in our lab and others have demonstrated that lncRNAs regulate p53 signaling and cellular differentiation (15, 16). Since many lncRNAs mediate mechanisms that are highly tissue selective or context specific, they are attractive targets for drug development since toxicity and off-target effects are anticipated to be minimal (17, 18). Furthermore, advances in antisense drug development have allowed for efficient targeting and degradation of RNAs, including lncRNAs, by systemic or CNS restricted drug delivery (19, 20). Identification of therapeutically targetable lncRNAs involved in the maintenance of medulloblastoma cells with a stem-like phenotype could augment conventional therapy, improve tumor control, and potentially allow for de-escalation of cytotoxic therapy to reduce neurocognitive toxicity.

Results and Discussion

To identify lncRNA candidates that could participate in Shh medulloblastoma differentiation, we annotated lncRNAs that were differentially expressed in Shh medulloblastoma from *Math1-Cre*, *SmoM2^c* mice, which are frequently used to model the disease. Here, the expression of a constitutively active, oncogenic point mutation of *Smo* (*SmoM2^c*) is restricted to cerebellar granule neuron progenitors (CGNP) using the conditional transgene *Math1-Cre*. RNA sequencing of *Math1-Cre*; *SmoM2^c* MB revealed that 642 annotated lncRNAs were differentially expressed in tumors compared to age matched normal cerebella (**Figure 1A, Supplemental Figure 1A**)(21). We examined whether lncRNAs differentially expressed in medulloblastoma were regulated during the differentiation of cerebellar granule neuron progenitors (CGNPs), the likely cell of origin of Shh medulloblastoma(22-24). Of the 642 lncRNAs differentially expressed in medulloblastoma, we identified 113 lncRNAs that changed

1 dynamically during CGNP differentiation and cerebellar development, 44 of which
2 with increased expression in medulloblastoma; and 70 with reduced expression in
3 medulloblastoma compared to normal developing cerebellum (**Supplemental Figure**
4 **1B**).

5 To further enrich for lncRNAs that contribute to Shh medulloblastoma, we
6 filtered lncRNAs differentially expressed in the Shh group of human medulloblastoma
7 compared to other groups. Using expression data from previously published human MB
8 datasets(25, 26), we identified 10 medulloblastoma-associated lncRNAs that change
9 dynamically during CGNP differentiation and for which expression differed in the Shh
10 group MB compared to other groups (Figure 1A). Among these lncRNAs, *MIAT* was
11 of particular interest since *MIAT* expression was enriched in human Shh MB (**Figure**
12 **1B-C**). Moreover, a Shh-specific enhancer element was noted 12kb upstream of the
13 *MIAT* transcription start site (**Supplemental Figure 1C**), *MIAT* is expressed most
14 highly in the Shh- β subgroup of high risk infant Shh medulloblastoma (**Figure 1D**) (27,
15 28), and enriched during early cerebellum development at the earliest stages of CGNP
16 neurogenesis and repressed at later stages of development following CGN
17 differentiation (**Figure 1E**). To determine whether *Miat* contributes to Shh MB
18 development, we crossed *Math1-Cre; SmoM2^c* with *Miat^{-/-}* mice(29) in both *p53^{+/+}* and
19 *p53^{-/-}* backgrounds since p53 mutations are strongly associated with aggressive Shh MB
20 subtype with an exceptionally poor prognosis (30). Homozygous genetic loss of *Miat*
21 prolonged median survival in both *p53^{+/+}* and *p53^{-/-}* MB animals, indicating *Miat*
22 supports MB development in multiple contexts of Shh MB (**Figure 1F-G**).
23 Homozygous genetic deletion of *Miat* also reduced the proliferative fraction of cells as
24 measured by Ki67 staining and partially restored cerebellar architecture (**Figure 1H**),
25 indicating *Miat* supports the growth of Shh MB.

1 Since *Miat* is highly expressed in the earliest stages of CGNP development, we
2 next examined whether *Miat* regulates the differentiation of MB cells. To study the role
3 of *Miat* in the differentiation of Shh MB, we isolated stem-like MB cells from *Math1-
4 Cre; SmoM2^c* MB by *in vitro* propagation in defined serum-free neural stem cell
5 medium (31, 32). A stem-like phenotype in these cells was confirmed by differentiation
6 (**Supplemental Figure 2A**) and confirmation of tumorigenic potential upon
7 engraftment in the posterior fossa of mice (data not shown). In stem-like MB cells,
8 treatment with smoothened agonist (SAG) induced the expression of *Miat* while Shh
9 signaling antagonist cyclopamine downregulated *Miat* expression, indicating the Shh
10 signaling regulates the expression of *Miat* (**Supplemental Figure 2B**). To understand
11 how *Miat* is regulated in response to Shh signaling, we performed knockdown of Gli2
12 and n-Myc, transcription factors in the Shh pathway, as well as c-Myc which is required
13 for pluripotency and maintenance of stem-like cancer cells and examined the effect on
14 *Miat* expression. Knockdown of Gli2 modestly decreased the expression of *Miat*, while
15 knockdown of n-Myc or c-Myc substantially downregulated *Miat* expression
16 (**Supplemental Figure 2C-D**). In human DAOY cells, a Shh MB cell line, anti-myc
17 ChIP recovered a region of the *MIAT* promoter that is also associated with histone
18 H3K27ac defined enhancer element upstream of *MIAT* that is specific to Shh group
19 medulloblastoma(33), indicating that the Shh group specific enhancer is a myc bound
20 enhancer (**Supplemental Figures 1C, 2E**). These data indicate that *Miat* is a myc-
21 regulated lncRNA in the Shh pathway.

22 To elucidate the function of *Miat*, we generated stem-like MB cells with *Miat*
23 knockdown using CRISPR inhibition (CRISPR-i). qRT-PCR confirmed efficient
24 knockdown of *Miat* by multiple different sgRNAs targeting the *Miat* TSS (**Figure 2A**).
25 RNA-sequencing revealed that *Miat* knockdown resulted in substantial changes in

1 genes involved in stem cell differentiation, apoptosis, neural development, cell-
2 substrate adhesion, and regulation of smoothened signaling (**Figure 2B**) and notably
3 with significant enrichment of canonical p53-dependent genes amongst genes more
4 highly expressed in the *Miat* knockdown samples (FDR adjusted $P < 10^{-4}$ by KEGG
5 pathway analysis). As stem-like cancer cells and impair p53-dependent induced cell
6 death have been linked to resistance to cytotoxic therapies, we were interested to
7 examine the implications of p53 dysregulation by *Miat* on MB responses to cytotoxic
8 therapy. qRT-PCR confirmed that knockdown of *Miat* was associated with increased
9 expression of p53-regulated genes involved in apoptosis and cell cycle arrest, *Puma*,
10 *Bax*, and *Cdkn1a*, which are necessary for efficient killing of medulloblastoma cells by
11 cytotoxic therapies such as radiation (**Figure 2C**), suggesting that *Miat* expressing
12 stem-like MB cells may be relatively resistant to cell death by cytotoxic therapy.
13 Consistent with a role for *Miat* in resistance to cytotoxic therapy, knockdown of *Miat*
14 in stem-like MB cells significantly increased the fraction of dead stem-like MB cells
15 following radiation therapy and lowered the threshold for cytotoxicity in stem-like MB
16 cells following radiation therapy (**Figures 2D-E, Supplemental Figure 2F**). In contrast,
17 knockdown of *Miat* in Med1 cells, a differentiated mouse Shh MB cell line that
18 expressed *Miat*, had no effect on sensitivity to radiation, suggesting that *Miat*'s role in
19 promoting tumor initiation and treatment resistance is limited to the stem-like MB cells.
20 (**Supplemental Figures 2G-H**). Thus, *Miat* downregulates the expression of p53
21 pathway effector genes in stem-like MB cells and maintains a treatment resistant stem-
22 like MB cell phenotype.

23 Nuclear lncRNAs can be efficiently targeted for therapeutic inhibition and *Miat*
24 ^{-/-} mice are healthy and fertile, making *Miat* an attractive therapeutic target. To examine
25 whether *Miat* can be therapeutically targeted, we generated custom Antisense LNA

1 GapmeRs to knockdown *Miat* in stem-like MB cells. Two GapmeRs achieved efficient
2 knockdown *in vitro*, and induced p53-dependent genes *Puma*, *Bax*, and *Cdkn1a* similar
3 to knockdown of *Miat* using CRISPRi (**Figure 2F-G**). A single treatment of stem-like
4 MB cells with *Miat* GapmeRs increased cell death at baseline and following radiation
5 therapy and reduced colony formation *in vitro* (**Figure 2H-I**). Hence, *Miat* is a
6 candidate therapeutic target in Shh medulloblastoma to reduce the tumorigenicity and
7 treatment resistance of stem-like MB cells.

8 Our RNA-seq data also demonstrated that knockdown of *Miat* significantly
9 altered the expression of genes associated with cancer stem cells and differentiation.
10 qRT-PCR validated that knockdown of *Miat* reduced the expression of pluripotent
11 cancer stem cell markers such as *Cx3cl1*, *Cxcr4*, and *Itga6* (**Figure 3A**); and in contrast,
12 upregulated markers of differentiation, like *Gsc*, *Pax2*, *Hoxa7*, *Phf19* (**Figure 3B**).
13 Since *Miat* knockdown in stem-like MB cells was associated with increased expression
14 of differentiation markers and reduced expression of genes associated with the stem-
15 like phenotype, we examined the effect of *Miat* knockdown on *in vitro* colony formation
16 and neurosphere formation, an assay of a stem-like cancer cell phenotype. *Miat*
17 knockdown completely impaired the ability of stem-like MB cells to form colonies and
18 neurospheres (**Figure 3C**) but had only a modest effect on *in vitro* cell proliferation
19 (**Figure 3D**). *Miat* knockdown had no effect on colony formation in Med1-MB cells, a
20 differentiated mouse MB cell line derived from a *Ptch1*^{+/-} mouse (**Supplemental**
21 **Figure 3A**) (34, 35). Since *Miat* is required for the maintenance of a stem-like
22 phenotype in MB cells *in vitro*, we next examined whether *Miat* contributes to
23 medulloblastoma tumor initiation from stem-like cells *in vivo*. Control stem-like MB
24 cells readily form luciferase-expressing medulloblastoma tumors after engraftment in
25 the posterior fossa and subsequently developed rapidly progressive neurologic

1 symptoms including severe ataxia within 8 weeks of engraftment (**Figures 3E-F**).
 2 However, in contrast, mice engrafted with *Miat* knockdown stem-like MB cells showed
 3 no evidence of medulloblastoma formation by luciferase and had prolonged survival
 4 compared to mice engrafted with control stem-like MB cells (**Figures 3E-F**). H&E and
 5 Ki67 staining demonstrated that control stem-like MB cells formed tumors in posterior
 6 fossa that were uniformly positive for Ki67, however, no tumor cells were found in
 7 mice engrafted with stem-like MB cells with *Miat* knockdown (**Figure 3E**). In sum,
 8 these data suggest *Miat* is necessary for medulloblastoma stem-like cell maintenance
 9 and tumorigenesis.

10 LncRNA mechanisms are frequently mediated by their interactions with other
 11 cellular macromolecules such as protein, RNA or DNA. To identify the mechanisms by
 12 which *Miat* functions to maintain the stem-like MB phenotype, we next characterized
 13 the proteins that co-localized with *Miat* in stem-like MB cells. sgRNAs targeting *Miat*
 14 guided the APEX2-dCas13 fusion protein to *Miat*-enriched nuclear domains and
 15 proteins were subsequently labeled by proximity biotinylation (36). Mass spectrometry
 16 of recovered biotinylated proteins identified Metadherin (*Mtdh*), also known as LYRIC,
 17 as a highly enriched protein in samples isolated from stem-like MB cells expressing
 18 APEX2-dCas13 and sgRNAs targeting *Miat* (**Supplemental Figure 3B, Supplemental**
 19 **Table 1**). *Mtdh* is a known RNA binding protein previously shown to contribute to
 20 mechanisms of chemoresistance and is required for tumor initiating capacity of cancer
 21 cells (37-39). Transient knockdown of *Mtdh* using siRNAs as well as CRISPR
 22 knockout of *Mtdh* (*Mtdh*^{-/-}) in stem-like MB cells impaired neurosphere formation *in*
 23 *vitro*, similar to knockdown of *Miat* (**Figures 3G-H, Supplemental Figures 3C-F**).
 24 Furthermore, gene expression in *Mtdh*^{-/-} stem-like MB cells suggested changes
 25 associated with differentiation, similar to *Miat* knockdown in stem-like MB cells, with

1 downregulation of stem cell marker *Cxcr4* and upregulation of the differentiation
2 marker *Pax2* (**Figures 3I-J**). *Mtdh*^{-/-} stem-like MB cells, similarly, had increased
3 expression of p53 effector genes *Puma*, *Bax*, and *Cdkn1a* and reduced stem-like MB
4 cell viability following radiation therapy (**Figure 3K, Supplemental Figures 3G-H**).
5 Thus, *Mtdh* is necessary for the maintenance of a treatment resistant stem-like
6 population of medulloblastoma cells, phenotypically similar to loss of *Miat*.

7 These results have identified a new, therapeutically targetable mechanism that
8 maintains a niche of tumorigenic and treatment resistant stem-like MB cells that
9 requires the lncRNA *Miat* and its interaction with the protein *Mtdh*. Extensive prior
10 work in preclinical models indicated that *Mtdh*-dependent processes would be attractive
11 targets for improving cancer sensitivity to cytotoxic therapy (37, 38), but translation to
12 clinic has been hampered by the fact that *Mtdh* has no ligand binding domain and is
13 considered an undruggable target (40). Further, sterility in *Mtdh*^{-/-} males also limits
14 enthusiasm for drug development target *Mtdh* due to concerns for toxicity. *Miat* is
15 critical for the maintaining a treatment resistant and tumorigenic potential of stem-like
16 MB cells while simultaneously dispensable for health and normal development, making
17 it an especially attractive therapeutic target in MB. More broadly, these data suggest
18 that conserved cancer mechanisms thought to be undruggable may be vulnerable to
19 modulation by targeting lncRNAs in the pathway.

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1 **Methods**

2 Please refer to Supplemental Methods for details.

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4 **Data Availability**

5 All raw sequencing data will be accessible in the NCBI's GEO database.

6

7 **Study Approval**

8 All animal experiments and procedures were approved by MSKCC's Institutional
9 Animal Care and Use Committee.

10

11 Conflict of interest: The authors declare no conflict of interest

12

13 **Author Contributions**

14 HV, DRR and AMS conceived the study. KLP, HNV, DRR, RCH, and AMS designed
15 experiments. DRR and AMS supervised the study. KLP performed all mouse, cellular,
16 and biochemical experiments presented in the figures. HNV and KLP conducted
17 genomics analyses. RCH supervised protein identification by mass spectrometry. DTL
18 and RB performed experiments that contributed importantly to the conception of the
19 study but were not ultimately displayed in the figures. KLP, HV, RCH, DRR, AMS
20 conducted data analyses. The manuscript was prepared by KLP, HNV, DRR, AMS.
21 All authors reviewed the manuscript.

22

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10

Figure Legends

12 **Figure 1. *Miat* is a developmentally regulated, Shh associated lncRNA that**
13 **enhances MB development.** A, Flow chart illustrating criteria to identify candidate
14 lncRNAs involved in Shh MB differentiation. B-C, *MIAT* expression in molecular
15 groups in Robinson et al. (B) and Sturm et al. (C). D, *MIAT* expression by subgroups
16 of Shh MB. E, *Miat* expression in the cerebella of mice of Math1-cre⁺, SmoM2^{fl/fl} (red
17 line) and cre⁻, SmoM2^{fl/fl} mice (black line) from postnatal day 0 through 35. F, Kaplan-
18 Meier survival curves in Math1-cre⁺, SmoM2^{fl/fl} Shh MB mice in the *p53*^{+/+}
19 background, n=12 *Miat*^{+/+} with median survival of 34 days, n=10 *Miat*^{-/-} with median
20 survival of 39.5 days, *: p <0.05, log-rank test. G, Kaplan-Meier survival curves in
21 Math1-cre⁺, SmoM2^C Shh MB mice in the *p53*^{-/-} background, n=7 *Miat*^{+/+} with median
22 survival of 30 days, and n=6 *Miat*^{-/-} mice with a median survival of 38.5 days, ** p<0.01,

1 log-rank test. **H**, Representative H&E and Ki67 stains of midline sagittal sections
2 through the cerebella from of Math1-Cre; SmoM2^{fl/fl} *Miat*^{+/+} and *Miat*^{-/-} mice.

3

4 **Figure 2. Miat sustains a treatment-resistant stem-like medulloblastoma niche and**
5 **is therapeutically targetable. A**, qRT-PCR of *Miat* expression in stem-like MB cells

6 with 3 sgRNAs targeting CRISPRi to *Miat* or control nontargeting sgRNA (Gal4-4). **B**,

7 Gene ontology (GO) terms associated with genes differentially expressed in *Miat*

8 knockdown stem-like MB cells compared to controls. **C**, Expression of p53-dependent

9 genes *Bax*, *Cdkn1a* and *Puma* in stem-like MB cells with sgRNAs targeting CRISPRi

10 to *Miat* or control nontargeting sgRNA (Gal4-4) (n=3). **D**, Fraction of dead cells as

11 measured by Propidium iodide (PI) staining 24 hours after irradiation (5 Gy), in stem-

12 like MB cells with sgRNAs targeting CRISPRi to *Miat* or control nontargeting sgRNA

13 (Gal4-4) (n=3). **E**, Cell viability as measured by MTS assay 48 hours after increasing

14 doses of irradiation (IR) (0, 1, 2, 3, 4, 6, 8, 10 Gy), in stem-like MB cells with sgRNAs

15 targeting CRISPRi to *Miat* or control nontargeting sgRNA (Gal4-4) (n=4). **F-G**,

16 Expression of *Miat* (**F**) and p53-dependent genes *Bax*, *Cdkn1a* and *Puma* (**G**) in stem-

17 like MB cells treated with ASOs targeting *Miat* or control (n=3). **H**, Fraction of dead

18 cells as measured by Propidium iodide (PI) staining 24 hours after irradiation (5 Gy) in

19 stem-like MB cells treated with ASOs targeting *Miat* or control (n=3). **I**, Colony

20 formation in stem-like MB cells treated with ASOs targeting *Miat* or control, Right:

21 quantification (n=3). *** p-value<0.001; ** p-value<0.01 by Student's T test.

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24 **Figure 3. Miat and protein partner Mtdh maintain a stem-like phenotype crucial**

25 **for tumorigenesis. A-B**, Expression of genes associated with cancer stem cells (**A**) or

1 stem cell differentiation (**B**) in stem-like MB cells with indicated sgRNAs targeting
2 *Miat* for CRISPRi or nontargeting control (Gal4-4) (n=3). **C**, Colony formation and
3 neurosphere formation in stem-like MB cells with indicated sgRNAs targeting *Miat* for
4 CRISPRi or nontargeting control. Knockdown of *Miat* impaired the ability to form
5 colonies (upper panel) and neurospheres (lower panel) (n=3). Below: quantitation of
6 neurosphere formation. **D**, Relative fraction of cells proliferating (EdU+) in stem-like
7 MB cells with indicated sgRNAs targeting *Miat* for CRISPRi or nontargeting control.
8 **E**, Orthotopic tumor formation of stem-like MB cells with indicated sgRNAs targeting
9 *Miat* for CRISPRi or nontargeting control with luciferase imaging 2 weeks after
10 engraftment (upper panel) and H&E and Ki67 histopathology at the experimental
11 endpoint (lower). **F**, Kaplan-Meier survival curves for mice orthotopically engrafted
12 with stem-like MB cells with indicated sgRNAs targeting *Miat* for CRISPRi (n=4) or
13 nontargeting control (n=4). **G**, WB in stem-like MB cells after CRISPR with sgRNAs
14 targeting *Mtdh* or nontargeting (Gal4-4). *Mtdh* targeting sgRNAs efficiently eliminated
15 *Mtdh* protein expression. **H**, Neurosphere formation in stem-like MB cells with
16 *Mtdh*^{+/+} (Gal4-4) and *Mtdh*^{-/-} (*Mtdh*-1 and *Mtdh*-2), right: quantification of neurosphere
17 formation in each condition (n=3). **I-J**, Expression of *Miat*-regulated, pluripotent
18 cancer stem cell marker *Cxcr4* (**I**) and cellular differentiation marker *Pax2* (**J**) in
19 *Mtdh*^{+/+} and *Mtdh*^{-/-} cells stem-like MB cells (n=3). **K**, Cell viability as measured by
20 MTS assay 48 hours after increasing doses of irradiation (IR) (0, 1, 2, 3, 4, 6, 8, 10 Gy
21 in *Mtdh*^{+/+} and *Mtdh*^{-/-} stem-like MB cells (n=4 in each group). *** p-value<0.001; **
22 p-value<0.01; * p-value<0.05 via Student's T test.

Figure 1

A 642 lncRNAs differentially expressed in MB compared to normal cerebellum

113 lncRNAs dynamically expressed during CGNP differentiation and cerebellar development

Robinson et al.

Sturm et al.

Tumor Enriched

Tumor Depleted

Miat
Nav1
Cmde
Xist
Rmst

Meg3
Pclo
Malat1
Fam120aos
Inafm2

B

C

D

F

G

E

H

Figure 2

Figure 3