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Supplemental Information

Astrocytic *trans*-Differentiation Completes a Multicellular Paracrine Feedback Loop Required for Medulloblastoma Tumor Growth

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Supplemental Table 1. Related to STAR Methods section "EXPERIMENTAL MODEL AND SUBJECT DETAILS, Mice", and to the first section in "RESULTS, Establishment of MADM-based and other genetic models for medulloblastoma". Medulloblastoma models used in this paper.

Model#	Genotype	Genetic components and their roles	Uniqueness of the model and its usage in this paper
A	**Ptch1 KO/wildtype; MADM-p53KO; Math1-Cre**	**Ptch1 KO**: Driver mutation for Shh-subtype medulloblastoma **MADM-p53KO**: Increase the tumor penetrance to 100% upon Cre-mediated knockout **Math1-Cre**: Induce inter-chromosomal recombination to generate sporadic p53-null GFP+ GNPs	This model enables single-cell resolution analysis of the entire course of tumorigenesis, and was used in Figures 1E-R, 2B, and Supplemental Figures 1F, 2A.
B	**Ptch1 KO/wildtype; p53flox/p53flox; Math1-Cre; ROSA26-LSL-tdTomato**	**Ptch1 KO**: Driver mutation for Shh-subtype medulloblastoma **p53flox/p53flox**: Increase the tumor penetrance to 100% upon Cre-mediated knockout **Math1-Cre**: Induce loxP-mediated recombination to knockout p53 and turn on Cre reporter tdTomato in all GNPs **ROSA26-LSL-tdTomato**: Cre reporter used for lineage tracing of all cells derived from Math1-Cre+ cells	This model enables lineage tracing from tumor GNPs even after they differentiate into other cells types, and was used in Figure 7J-K, Supplemental Figures 1G, 2E-H, 3E-G, 7G-H.
C	**Ptch1 KO/wildtype; p53flox/p53flox; Math1-Cre; Math1-GFP**	**Ptch1 KO**: Driver mutation for Shh-subtype medulloblastoma **p53flox/p53flox**: Increase the tumor penetrance to 100% upon Cre-mediated knockout **Math1-Cre**: Induce loxP-mediated recombination to knockout p53 in all GNPs **Math1-GFP**: Transgenic reporter for wildtype and tumor GNPs, which turns off after they differentiate	This model enables one to distinguish GFP+ tumor from GFP- TME cells, and was used in Figures 4F-G, 6J-K, 7C-I, and Supplemental Figures 3A-D, 4A-C, 7C-F.
D	**Ptch1 KO/wildtype; p53KO/p53KO; Math1-GFP**	Ptch1 KO: Driver mutation for Shh-subtype medulloblastoma p53KO/p53KO: Increase the tumor penetrance to 100% in the absence of Cre Math1-GFP: Transgenic reporter for wildtype and tumor GNPs, which turns off after they differentiate	This model frees up Cre for other purposes, such as to label TuAstrocytes with GLAST-CreER and Cre reporter in Supplemental Figure 2B-D and Figure 3, to knock out IGF1R after tumor formation with Math1-CreER in Figure 4H-J and Supplemental Figure 4E-I, to knock out IGF1 in TAMs with CSF1R-Cre in Figure 5H-J, and to trace the lineage of TAMs with CX3CR1-CreER in Figure 6A-E and Supplemental Figure 6.

Supplemental Table 2. Related to Figure 7. Luminex profiling of cytokines and chemokines. Tumor tissues were dissected out based on Math1-GFP expression and lysed in Tissue Extraction Reagent I (Invitrogen) supplemented with protease inhibitors. Normal cerebelli from age-matched mice were processed in the same fashion as control samples. Protein concentration of the lysates was determined by BCA protein quantification method. All lysates were carefully adjusted to 1.5mg/ml with lysis buffer, and 25µl each was applied to mouse 32-plex cytokine/chemokine luminex panel. The absolute quantity of cytokine/chemokine present in cell lysate was expressed as picogram per 10ug of tissue lysate. Four tumors and three normal cerebella were used for this study.

Gene name	**Normal cerebellum (Means±SD, pg/10 µg)**	**Tumor mass (Means±SD, pg/10µg)**	**p value**
G-CSF	4.47±1.10	6.49±2.46	
GM-CSF	14.98±1.80	20.02±3.57	
M-CSF	4.32±0.96	5.21±1.84	
IFNg	3.13±0.16	3.20±2.0	
IL1a	31.04±7.45	20.53±9.28	
IL1b	92.74±13.23	77.68±58.19	
IL2	**90.09±4.0**	**13.81±8.31**	$p<0.001$
IL3	Below detection	Below detection	
IL4	**5.97±1.37**	**9.66±1.49**	$p<0.05$
IL5	1.98±0.12	1.83±0.61	
IL6	8.76±2.97	6.44±0.54	
IL7	2.02±0.86	1.68±0.29	
IL9	**868.44±68.9**	**546.4±68.19**	$p<0.01$
IL10	13.47±0.69	30.08±20.36	
IL12	4.06±0.32	4.06±0.50	
IL13	635.6±49.22	857.7±571.1	
IL15	**60.50±12.03**	**33.50±7.74**	$p<0.05$
IL17A	**4.92±0.61**	**1.01±0.41**	$p<0.01$
LIF	Below detection	1.33±0.52	
LIX	61.3±5.77	49.74±44.2	
CXCL10	**17.01±2.21**	**102.87±25.70**	$p<0.01$
KC	15.69±2.57	106.60±128.97	
MCP1	28.94±4.97	39.15±22.62	
MIP-1a	**26.79±2.85**	**81.56±33.99**	$p<0.05$
MIP-1b	**62.24±15.45**	**24.08±4.31**	$p<0.05$
MIP-2	17.21±0.55	19.18±3.30	
MIG	**12.40±2.94**	**29.11±5.66**	$p<0.05$
RANTES	2.45±0.12	3.72±0.96	
Eotaxin	**8.98±1.85**	**88.17±38.09**	$p<0.05$
TNFa	2.25±0.25	2.01±0.88	

Supplemental Table 3. Related to "Key Resources Table, Oligonucleotides section". Oligonucleotides used in this paper. This is a list of oligonucleotides that were used for mouse genotyping, qRT-PCR, and probes for in situ hybridization.

Mouse genotyping	Source	Reference
TG11ML, GT11ML mouse genotyping: Chr11_CS1: 5'-TGGAGGAGGACAAACTGGTCAC-3' Rosa4: 5'-TCAATGGGCGGGGGTCGTT-3' Chr11_CS2: 5'- TTCCCTTTCTGCTTCATCTTGC-3'	This paper	N/A
IGF1R flox mouse genotyping: 10451: 5'-CTTCCCAGCTTGCTACTCTAGG-3' 10452: 5'-CAGGCTTGCAATGAGACATGGG-3' IGF1R-rec-F: 5'-TGAGACGTAGCGAGATTGCTGTA-3'	This paper	N/A
IGF1 flox mouse genotyping: 10663 5'-CACTAAGGAGTCTGTATTTGGACC-3' 10664 5'-AAACCACACTGCTCGACATTG-3' 11736 5'-GGCAAATGGAAATCCTATGTCT-3'	This paper	N/A
CSF1R-iCre mouse genotyping: Cre-F: 5'-CACCCTGTTACGTATAGCCG-3' Cre-R: 5'-GAGTCATCCTTAGCGCCGTA-3'	This paper	N/A
CX3CR1-CreER^{T2} mouse genotyping: Cre-F: 5'-CACCCTGTTACGTATAGCCG-3' Cre-R: 5'-GAGTCATCCTTAGCGCCGTA-3'	This paper	N/A
Ptch1-KO mouse genotyping: Ptc-Neo-F3: 5'-TGGGGTGGGATTAGATAAATGCC-3' Ptc-R3: 5'-TGTCTGTGTGTGCTCCTGAATCAC-3' Ptc-WT3: 5'-CTGCGGCAAGTTTTTGGTTG-3' Ptc-WT4: 5'-AGGGCTTCTCGTTGGCTACAAG-3'	This paper	N/A
p53-KO mouse genotyping: neo tail: 5'-ACCGCTATCAGGACATAGCGTTGG-3' p53 TJW5: 5'-CACAGCGTGGTGGTACCTTATG-3' p53 TJW3: 5'- GGTATACTCAGAGCCGGCCTG-3'	This paper	N/A
p53-flox mouse genotyping: T008: 5'-CACAAAAACAGGTTAAACCCAG-3' T009: 5'-AGCACATAGGAGGCAGAGAC-3'	This paper	N/A
Math1-Cre mouse genotyping: Cre-F: 5'-CACCCTGTTACGTATAGCCG-3' Cre-R: 5'-GAGTCATCCTTAGCGCCGTA-3'	This paper	N/A
Math1-CreER^{T2} mouse genotyping: Cre-F: 5'-CACCCTGTTACGTATAGCCG-3' Cre-R: 5'-GAGTCATCCTTAGCGCCGTA-3'	This paper	N/A
Math1-GFP mouse genotyping: FACS G5': 5'-CTTCAAGTCCGCCATGCCCGA-3' GFP2-Hermie: 5'-TCCAGCAGGACCATGTGATCGC-3' IMR0015 (IC): 5'-CAAATGTTGCTTGTCTGGTG-3' IMR0016 (IC): 5'-GTCAGTCGAGTGCACAGTTT-3'	This paper	N/A
IL4-GFP reporter mouse genotyping: FACS G5': 5'-CTTCAAGTCCGCCATGCCCGA-3' GFP2-Hermie: 5'-TCCAGCAGGACCATGTGATCGC-3' IMR0015 (IC): 5'-CAAATGTTGCTTGTCTGGTG-3' IMR0016 (IC): 5'-GTCAGTCGAGTGCACAGTTT-3'	This paper	N/A

IL4-KO mouse genotyping: oIMR0077 5'-GCACAGAGCTATTGATGG -3' GTCIMR0078 5'-GCTGTGAGGACGTTTGGC-3' oIMR0079 5'-TCAGGACATAGCGTTGGC-3'	This paper	N/A
Rosa26-LSL-tdTomato mouse genotyping: oIMR9020 5'-AAGGGAGCTGCAGTGGAGTA-3' oIMR9021 5'-CCGAAAATCTGTGGGAAGTC-3' oIMR9103 5'-GGCATTAAAGCAGCGTATCC-3' oIMR9105 5'-CTGTTCCTGTACGGCATGG-3'	This paper	N/A
Aldh1L1-GFP reporter mouse genotyping: FACS G5': 5'-CTTCAAGTCCGCCATGCCCGA-3' GFP2-Hermie: 5'-TCCAGCAGGACCATGTGATCGC-3' IMR0015 (IC): 5'-CAAATGTTGCTTGTCTGGTG-3' IMR0016 (IC): 5'-GTCAGTCGAGTGCACAGTTT-3'	This paper	N/A
qRT-PCR		
Mouse IGF1 qPCR Forward: 5'-GGCATTGTGGATGAGTGTTG-3'	This paper	N/A
Mouse IGF1 qPCR Reverse: 5'-TCTCCTTTGCAGCTTCGTTT-3'	This paper	N/A
Mouse CSF1R qPCR Forward: 5'-TCCACCGGGACGTAGCA-3'	This paper	N/A
Mouse CSF1R qPCR Reverse: 5'-CCAGTCCAAAGTCCCCAATCT-3'	This paper	N/A
mouse GAPDH qPCR Forward: 5'-CAGGTTGTCTCCTGCGACTT-3'	This paper	N/A
mouse GAPDH qPCR Reverse: 5'-ATGTAGGCCATGAGGTCCAC-3'	This paper	N/A
mouse PDGFA qPCR Forward: 5'-GAGATACCCCGGGAGTTGAT-3'	This paper	N/A
mouse PDGFA qPCR Reverse: 5'-TCTTGCAAACTGCAGGAATG-3'	This paper	N/A
mouse PDGFB qPCR Forward: 5'-GATCTCTCGGAACCTCATCG-3'	This paper	N/A
mouse PDGFB qPCR Reverse: 5'-GGCTTCTTTCGCACAATCTC-3'	This paper	N/A
mouse HGF qPCR Forward: 5'-TTCCCAGCTGGTCTATGGTC-3'	This paper	N/A
mouse HGF qPCR Reverse: 5'-TGGTGCTGACTGCATTTCTC-3'	This paper	N/A
Mouse FGF2 qPCR Forward: 5'-AGCGGCTCTACTGCAAGAAC-3'	This paper	N/A
Mouse FGF2 qPCR Reverse: 5'-GCCGTCCATCTTCCTTCATA-3'	This paper	N/A
mouse EGF qPCR Forward: 5'-CCCAGGCAACGTATCAAAGT-3'	This paper	N/A
mouse EGF qPCR Reverse: 5'-GGTCATACCCAGGAAAGCAA-3'	This paper	N/A
mouse NT3 qPCR Forward: 5'-GATCCAGGCGGATATCTTGA-3'	This paper	N/A
mouse NT3 qPCR Reverse: 5'-AGCGTCTCTGTTGCCGTAGT-3'	This paper	N/A

mouse KitL qPCR Forward: 5'-TGGTGGCAAATCTTCCAAAT-3'	This paper	N/A
mouse KitL qPCR Reverse: 5'-TTCTTCGGTGCGTTTTCTTC-3'	This paper	N/A
mouse Math1 qPCR Forward: 5'-GCCAATGCAGAGCAACTTTT-3'	This paper	N/A
mouse Math1 qPCR Reverse: 5'-AATGGGTCCAAATACGCAAT-3'	This paper	N/A
mouse Aldh1L1 qPCR Forward: 5'-GCCTTCCAACCTTCTGTTGC-3'	This paper	N/A
mouse Aldh1L1 qPCR Reverse: 5'-CGCCACCGAGGGAACTTAAA-3'	This paper	N/A
Probes for in situ hybridization		
mouse IGF1 in situ Forward: 5'-ACACAAGTAGAGGAAGTGCAGGA-3'	Allen brain	http://mouse.brain-map.org
mouse IGF1 in situ Reverse: 5'-GCAGCCAAAATTCAGAGAGAA-3'	Allen brain	http://mouse.brain-map.org
mouse IL4 in situ Forward: 5'-TCACTGACGGCACAGAGC-3'	Allen brain	http://mouse.brain-map.org
mouse IL4 in situ Reverse: 5'-TGCTCTTTAGGCTTTCCAGG-3'	Allen brain	http://mouse.brain-map.org
Arg1-TaqMan Probe	Mm00475988_m1	N/A
Chil3-TaqMan Probe	Mm00657889_mH	N/A
Retnla-TaqMan Probe	Mm00445109_m1	N/A
IGF1-TaqMan Probe	Mm00439560_m1	N/A
GAPDH-TaqMan Probe	Mm99999915_g1	N/A