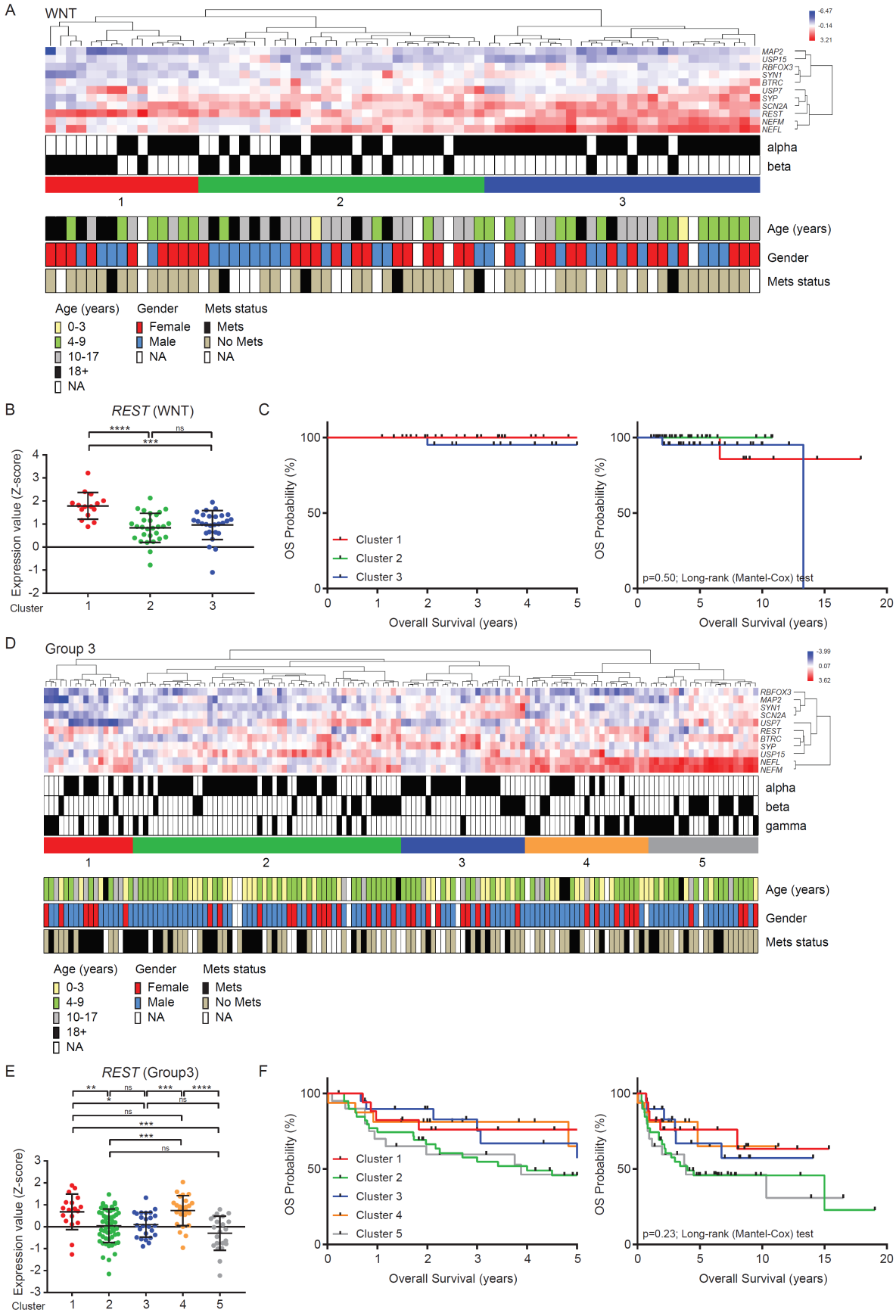
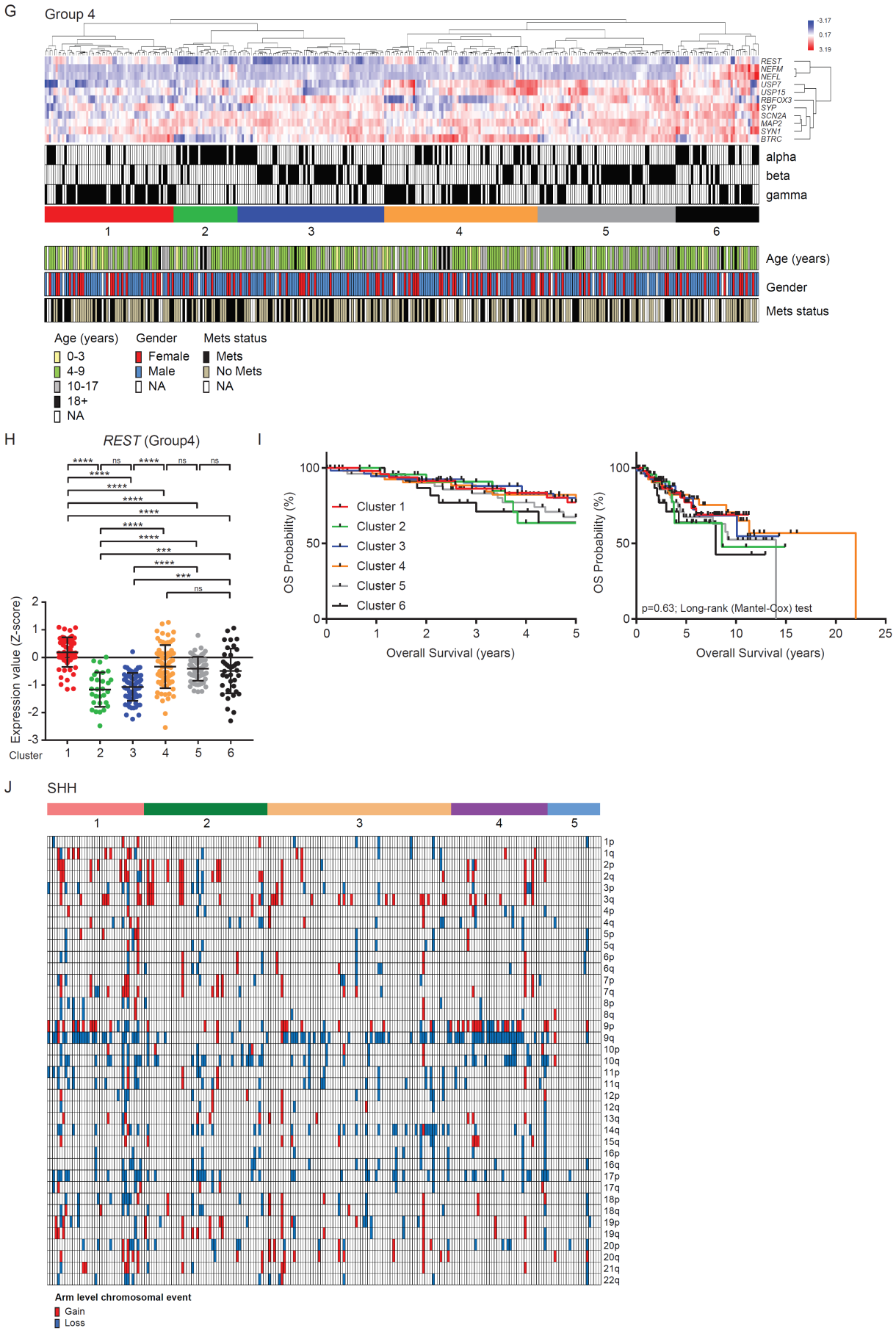
Supplementary Materials

Figure S1




G
Group 4
REST
NEFM
NEFL
USP7
USP15
RBFOX3
SYP
SCN2A
MAP2
SYN1
BTRC
alpha
beta
gamma
1
2
3
4
5
6
Age (years)
Gender
Mets status
Age (years)
0-3
4-9
10-17
18+
NA
Gender
Female
Male
NA
Mets status
Mets
No Mets
NA
H
REST (Group4)
Expression value (Z-score)
Cluster
I
OS Probability (%)
Cluster 1
Cluster 2
Cluster 3
Cluster 4
Cluster 5
Cluster 6
Overall Survival (years)
p=0.63; Long-rank (Mantel-Cox) test
J
SHH
Arm level chromosomal event
Gain
Loss

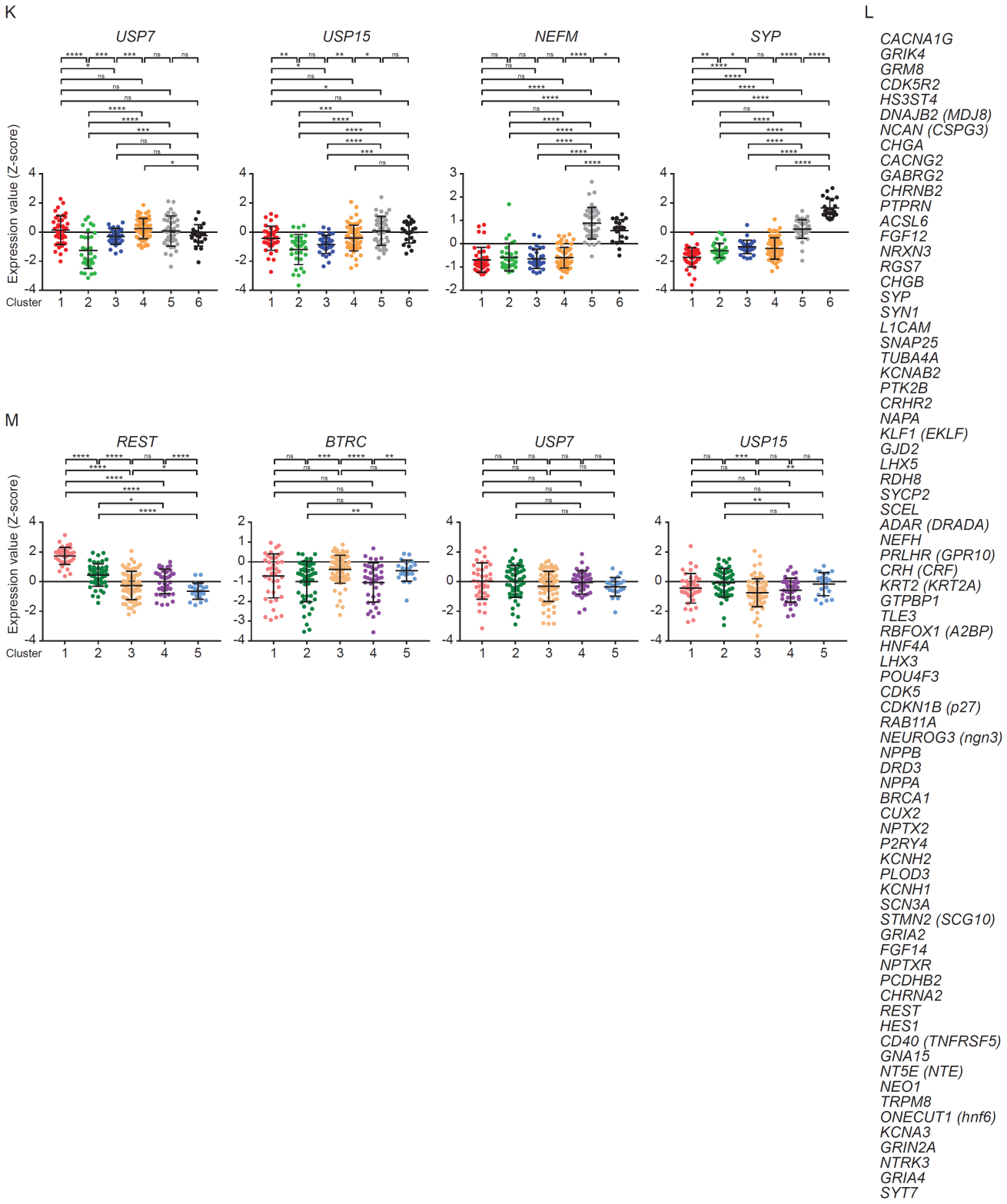


fig S1: Clinical characteristics. (A-C) Clinical characteristics based on clustering analysis of WNT MB patient samples. (D-F) Clinical characteristics based on clustering analysis of Group 3 MB patient samples. (G-I) Clinical characteristics based on clustering analysis of Group 4 MB patient

samples. (J) Arm-level chromosomal copy number change in SHH patients. Red and blue colored bars correspond to the gain and loss in the given chromosomal region, respectively. (K) Gene expression profiles measured by microarray. The patient samples were divided into six distinct clusters based on clustering assay. Each dot corresponds to one individual patient. Bars represent mean with standard deviations. *; $p<0.05$, **; $p<0.01$, ***; $p<0.001$, ****; $p<0.0001$, ns; non-significant. (L) Listed genes used for clustering analysis in the order of top-down pictured in Figure 1.

Figure S2

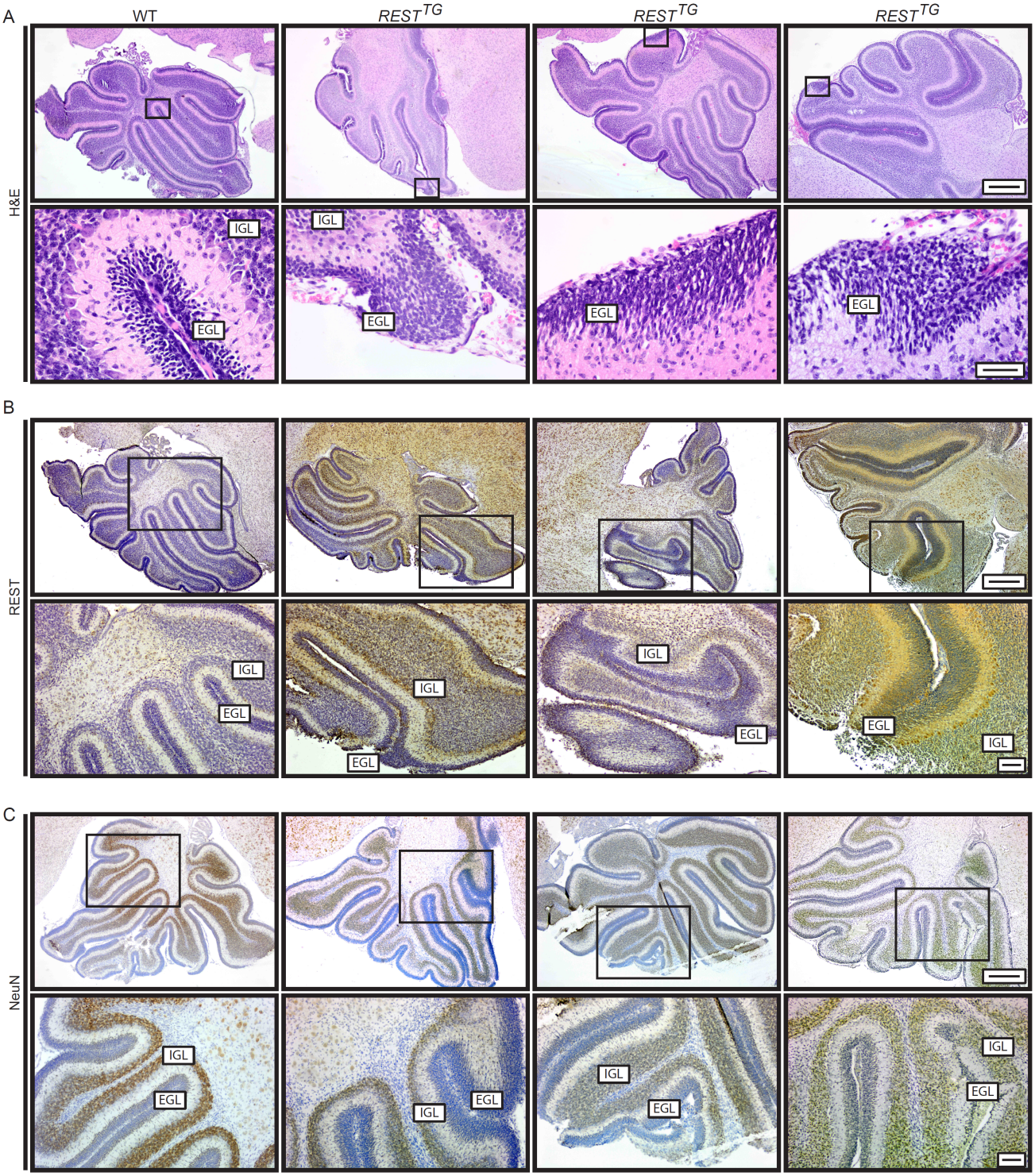


fig S2: Cerebellar architecture and protein expression in age-matched *REST*^{TG} and WT mice. (A) WT or *REST*^{TG} p8 brain sections stained with H&E. Sections were analyzed by IHC for (B) REST and (C) NeuN protein expression in CGNPs using specific antibodies. Scale bars = 200 μm (top panel -4x) and 20 μm (bottom panel - 40X).

Figure S3

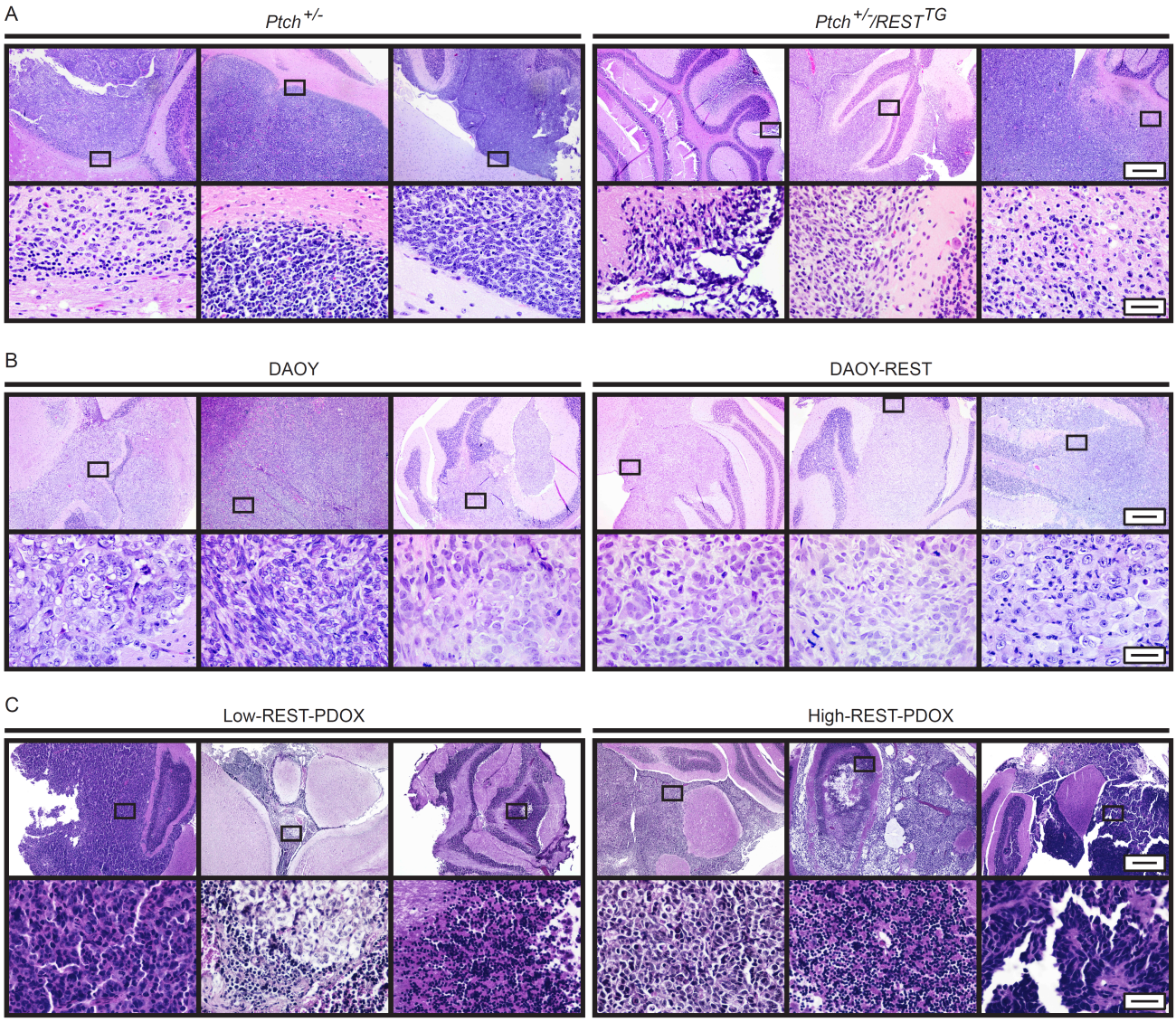
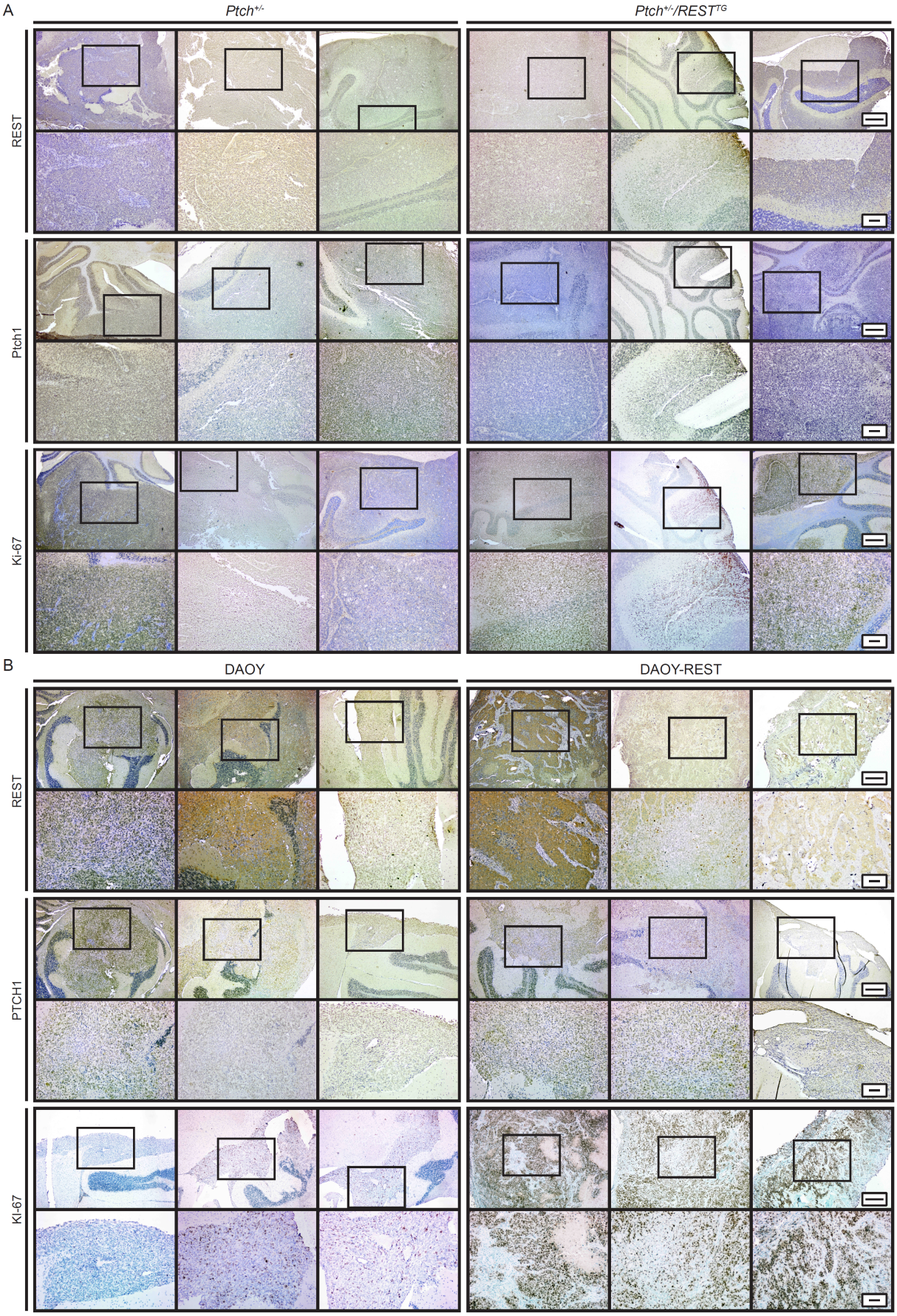
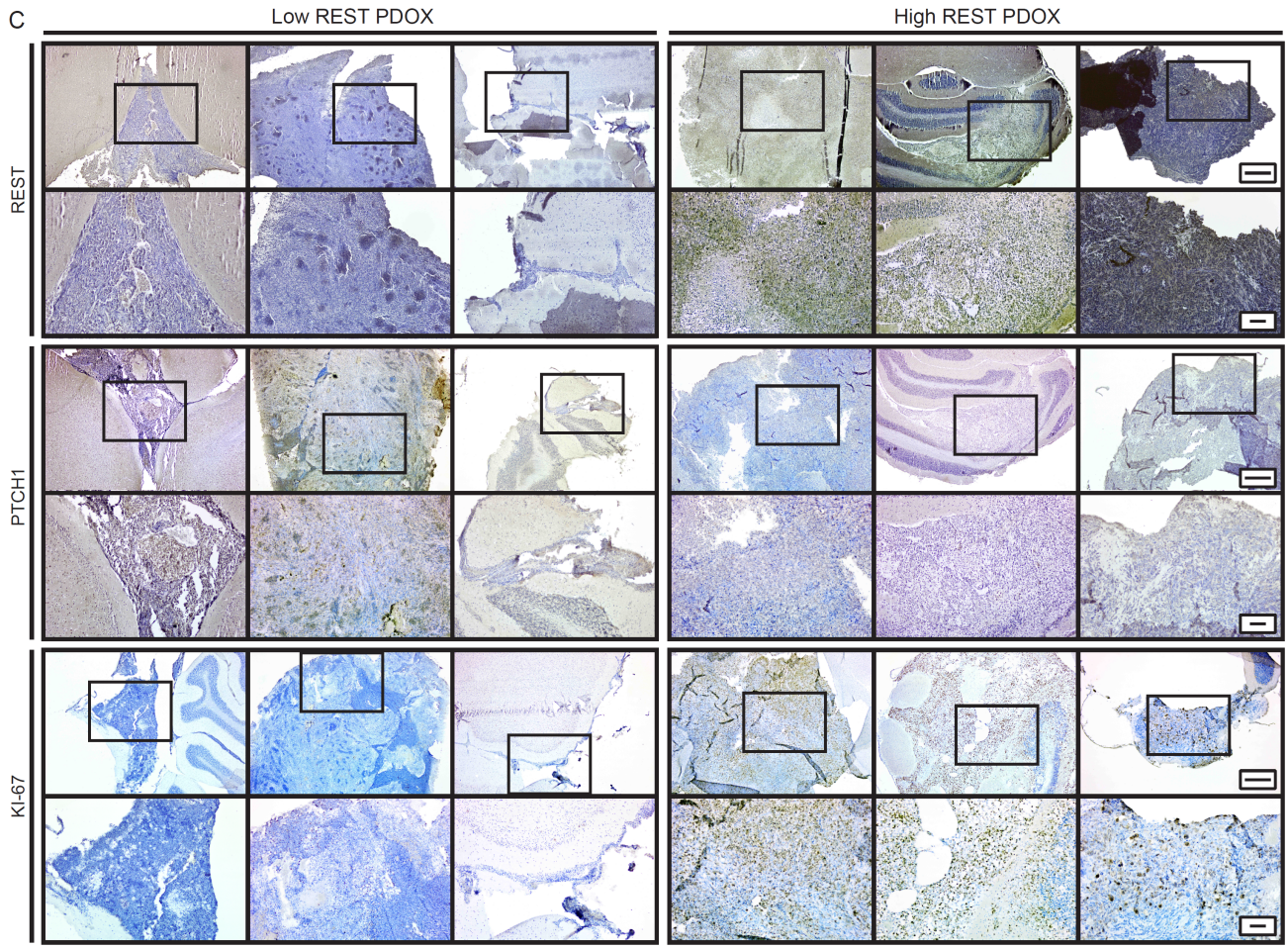
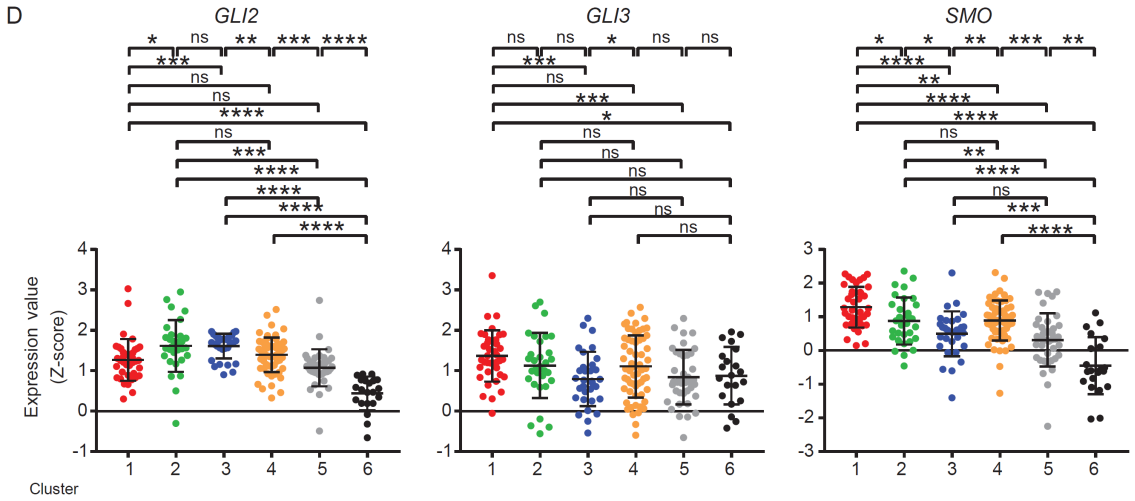


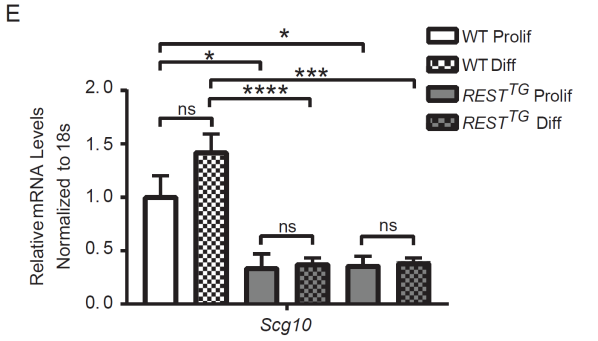
fig S3: REST elevation contributes to infiltrative Shh-driven MB development. Comparison of H&E stained brain sections from (A) *Ptch*$^{+/-}$ and *Ptch*$^{+/-}$/*REST*^{TG} animals with localized and infiltrative tumors, respectively (n=3), (B) DAOY and DAOY-REST xenografts (n=3), and (C) Low REST and High REST PDOXs (n=3). Scale bars = 200 µm (top panel -4x) and 20 µm (bottom panel - 40X).

Figure S4


A
Ptch+/-
Ptch+/-/RESTTG
REST
Ptch1
Ki-67
B
DAOY
DAOY-REST
REST
PTCH1
KI-67


C
Low REST PDOX
High REST PDOX
REST
PTCH1
KI-67


D
GLI2
GLI3
SMO
Expression value (Z-score)
Cluster


E
WT Prolif
WT Diff
REST^TG Prolif
REST^TG Diff
Relative mRNA Levels Normalized to 18s
Scg10

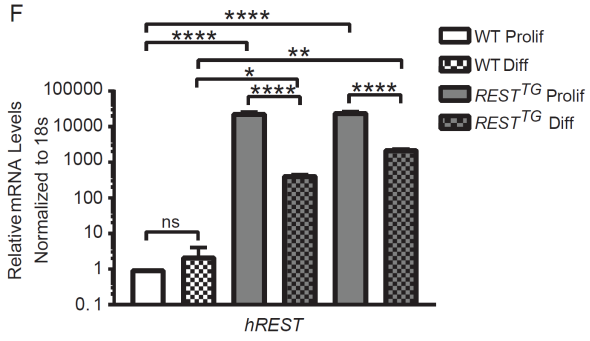

F
WT Prolif
WT Diff
REST^TG Prolif
REST^TG Diff
Relative mRNA Levels Normalized to 18s
hREST

fig S4: Differential regulation of PTCH1 expression by REST in MB tumors and CGNPs. Cerebellar sections of (A) tumor-bearing *Ptch*$^{+/-}$ and *Ptch*$^{+/-}$/*REST*^{TG} mice (n=3) and (B) DAOY and DAOY-REST xenografts (n=3) and (C) human SHH- subgroup PDOXs (n=3) were analyzed by IHC for REST, PTCH1, and KI-67 expression using specific antibodies. (D) *GLI2, GLI3,* and *SMO* mRNA expression profile measured by microarray. The patient samples were divided into six distinct clusters based on clustering assay. Each dot corresponds to one individual patient. (E) *hREST* and (F) *Scg10* mRNA expression was measured in WT and *REST*^{TG} GNPs after culturing with proliferation or differentiation media. WT data represents the mean ± SD (of fold change) of triplicate samples (n=3), *REST*^{TG} data represents 2 individual pups (n=2). For (A, B, and C) scale bars = 200 μm (top panel - 4X) and 50 μm (bottom panel -10X). For (E and F), p values were calculated by one-tailed t test of dCT values: *; $p<0.05$, **; $p<0.01$, ***; $p<0.001$, ****; $p<0.0001$, ns; non-significant.

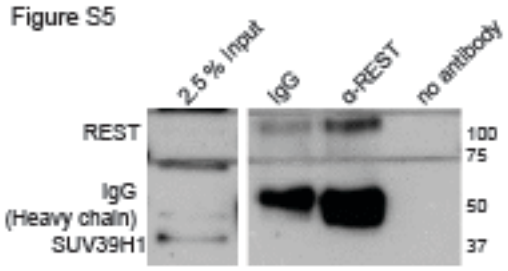
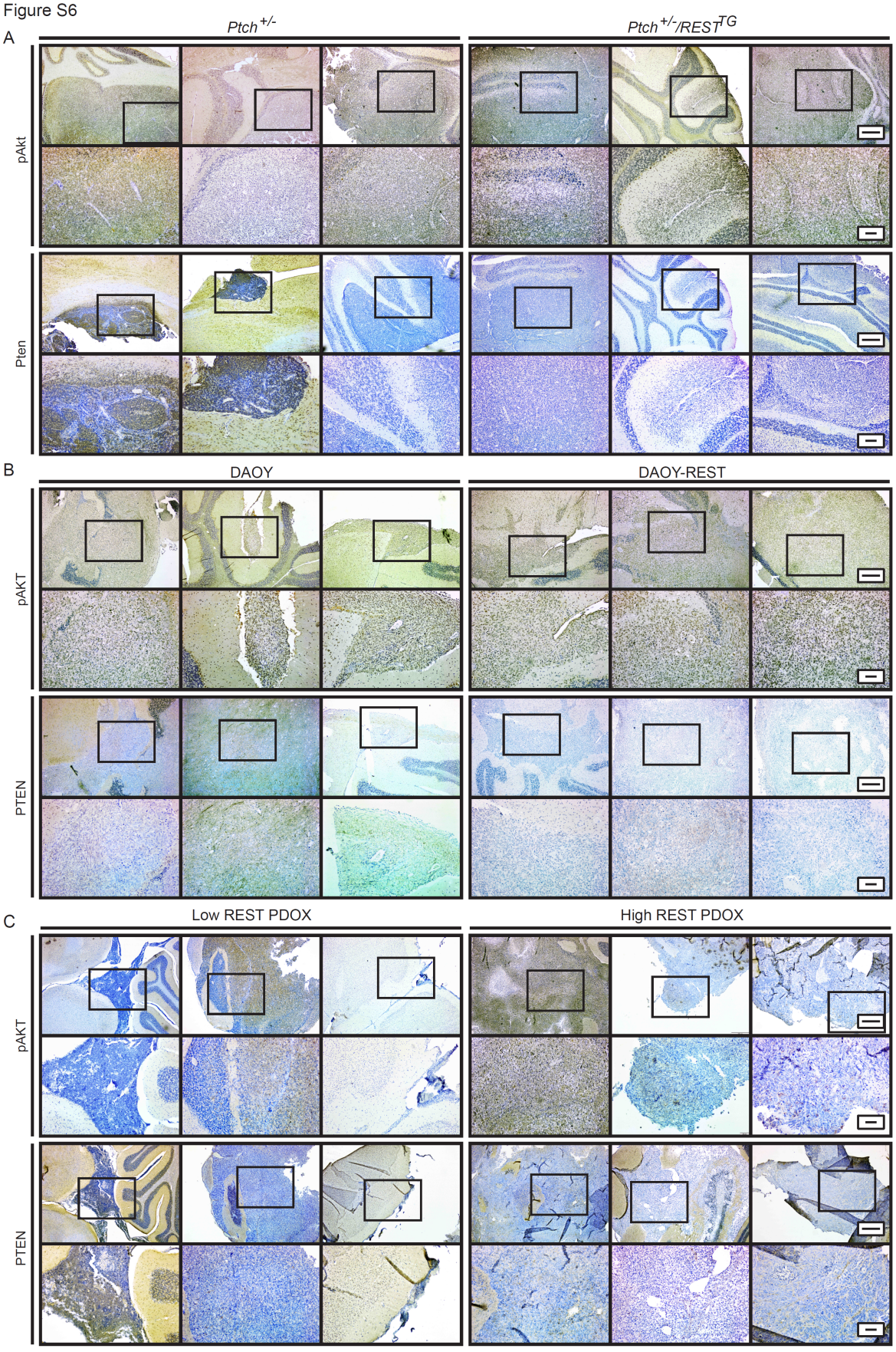
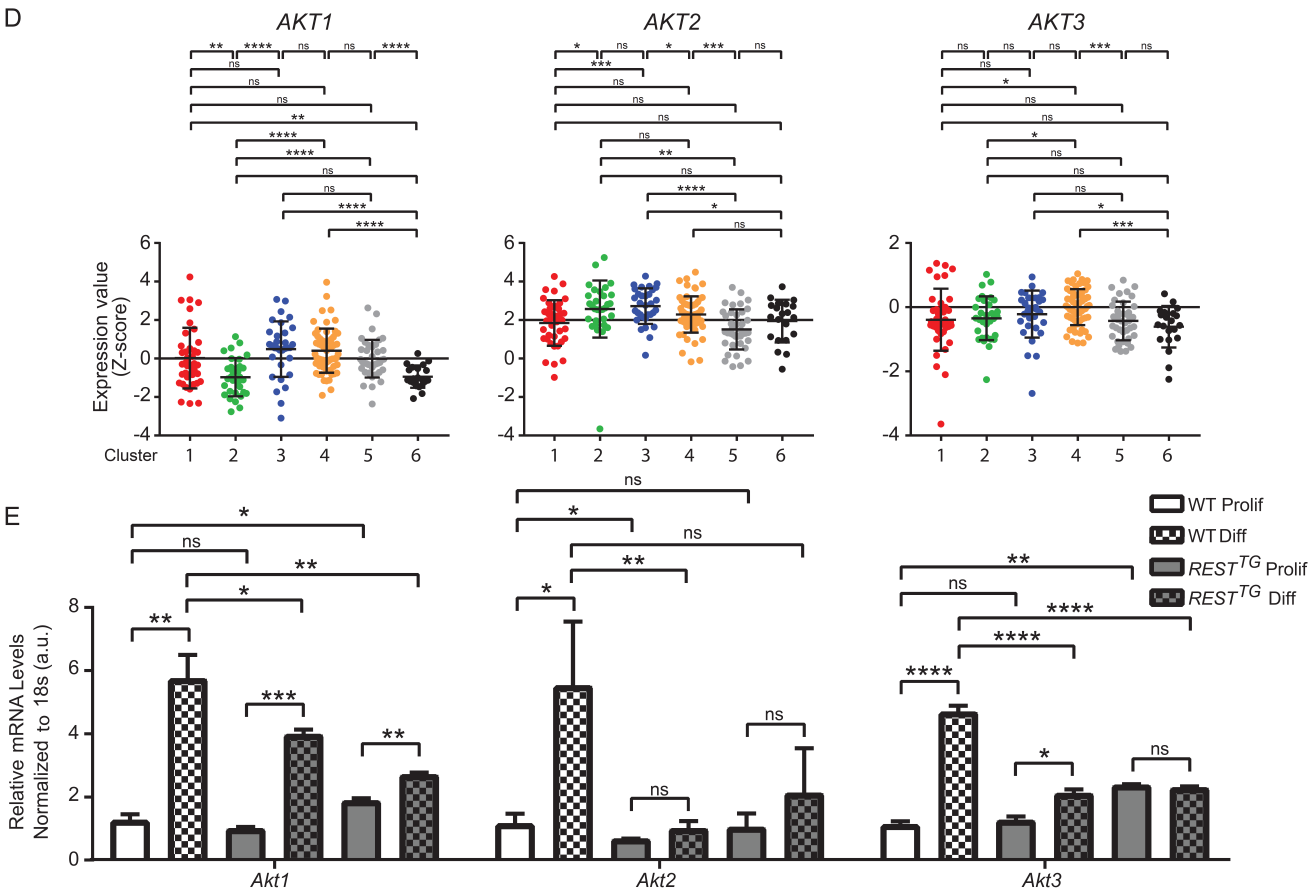


fig S5: REST and SUV39H1 do not co-immunoprecipitate in DAOY cells. Co-IP was performed to determine interaction between REST and SUV39H1. Whole cell extracts from DAOY were immunoprecipitated with IgG (control), REST, or no antibody, run on an SDS-PAGE and probed for REST and SUV39H1 protein. Blot shown is representative of 3 experiments.

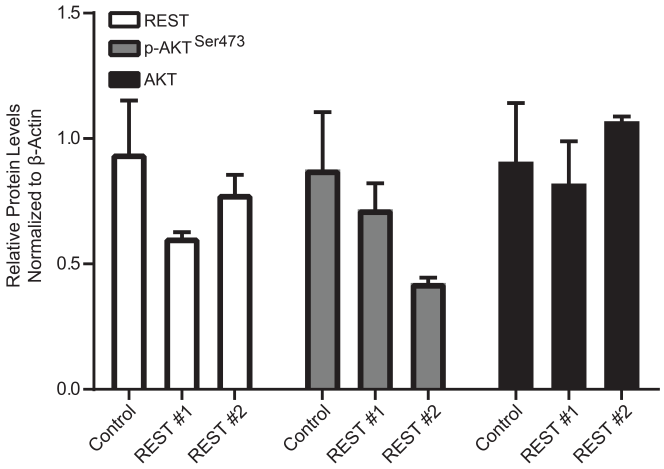
Figure S6


A
Ptch+/-
Ptch+/-/RESTTG
pAkt
Pten
B
DAOY
DAOY-REST
pAKT
PTEN
C
Low REST PDOX
High REST PDOX
pAKT
PTEN



SF6: REST elevation increases AKTSer473 phosphorylation. IHC was performed with specific antibodies targeting p-AKT^{S473}, AKT, or PTEN protein in (A) *Ptch*$^{+/-}$ and *Ptch*$^{+/-}$/*REST*^{TG} tumors (n=3), (B) DAOY and DAOY-REST xenografts (n=3) and (C) human SHH- subgroup PDOXs (n=3). (D) *AKT1, AKT2,* and *AKT3* mRNA expression profile measured by microarray. The patient samples were divided into six distinct clusters based on clustering assay. Each dot corresponds to one individual patient. (E) *Akt1, Akt2,* and *Akt3* mRNA expression was measured in WT and *REST*^{TG} GNPs after culturing with proliferation or differentiation media. WT data represents the mean ± SD (of fold change) of triplicate samples (n=3), *REST*^{TG} data represents 2 individual pups (n=2). For (A, B, and C) scale bars = 200 µm (top panel - 4X) and 50 µm (bottom panel -10X). For (E), p values were calculated by one-tailed t test of dCT values: *; p<0.05, **; p<0.01, ***; p<0.001, ****; p<0.0001, ns; non-significant.

Figure S7



SF7: REST-dependent AKT phosphorylation in cell-lines. (A) Western blot analysis of p-AKT^{Ser473} and AKT protein expression following REST knockdown in DAOY cells (Fig. 6 B, right). Densitometry was obtained using Image Lab software (Bio-Rad). Bars represent mean with standard deviations (n=3).

Tables:

Table S1: Synergy between MS275 and UNC0638. Synergy was calculated DAOY and UW228 at 48H post treatment either with each drug alone or in combination.

Cell Line	Incubation (hrs)	Drug Conc (μM) MS275 and UNC0638	Survival (MS275) γ_1	Survival (UNC0638) γ_2	Predicted survival MS275 + UNC0638 $\gamma_1\gamma_2$	Observed survival MS275 + UNC0638 γ_{12}
DAOY	48	0.625 and 1.0	0.6	0.9	0.54	0.34
		1.25 and 2.5	0.47	0.79	0.37	0.17
		2.5 and 5.0	0.25	0.68	0.17	0.07
UW228	48	0.625 and 1.0	0.8	1.0	0.8	0.84
		1.25 and 2.5	0.87	1.2	1.04	0.85
		2.5 and 5.0	0.11	1.2	0.132	0.11

Synergy: $\gamma_1\gamma_2>\gamma_{12}$

Antagonism: $\gamma_1\gamma_2<\gamma_{12}$

Additive: $\gamma_1\gamma_2=\gamma_{12}$

Table S2: IC50 values for UW228 and DAOY cells treated with MK-2206. Analysis of MTT assays was used to determine IC50 values after UW228 and DAOY cells were treated with MK-2206 at various concentrations as indicated for 24, 48 or 72 hours.

Cell lines	Time (Hours)	IC50 of MK-2206 (μM)
UW228	24	7.75
	48	3.25
	72	0.70
DAOY	24	8.50
	48	5.00
	72	0.95

Table S3: Antibodies for IHC, qChIP, and Western blotting assays.

Antibodies for IHC	Cat. #	Company	Dilution
Phospho-AKT (Ser^{473})	sc-7985-R	Santa Cruz Biotech.	1 : 400
PTCH1	LS-C176173	LSBio	1 : 400
REST	HPA006079	Sigma-Aldrich	1 : 75
KI-67	ab15580	Abcam	1 : 100

PTEN	9188	Cell Signaling Technologies	1 : 100
anti-rabbit-HRP	111-035-003	Jackson ImmunoResearch	1 : 200
anti-mouse-HRP	115-035-003	Jackson ImmunoResearch	1 : 200

Antibodies for qChIP assay	**Catalog Number**	**Company**
REST	07-579	Millipore
REST	17-641	Millipore
Gli1	ab151796	Abcam
Acetyl Histone H3	06-599	Millipore
Trimethyl Histone H3 Lys4	9751	Cell Signaling Technologies
Monomethyl Histone H3 Lys9	14186	Cell Signaling Technologies
Dimethyl Histone H3 Lys9	4658	Cell Signaling Technologies
Trimethyl Histone H3 Lys9	13969	Cell Signaling Technologies

Antibody for Western Blot	**Cat. #**	**Company**	**Dilution**
REST	07-579	EMD Millipore	1: 800
AKT	9272S	Cell Signaling	1: 1000
Phospho-AKT (Ser^{473})	9271S	Cell Signaling	1: 800
Cleaved capsase-3	9661S	Cell Signaling	1: 300
Cleaved PARP	9541S	Cell Signaling	1: 500
Histone H3	PA5-16183	Invitrogen	1: 4000
β-Actin	4967S	Cell Signaling	1: 100000
Anti-Rabbit-HRP	7074	Cell signaling	1: 5000

Table S4: Primers for qChIP and qRT-PCR assays.

Primers for ChIP-qPCR	**Primer Sequence**
Human PTCH RE1-2-Forward	5'-ATGAGCACACTCTCAGGGAAAC-3'
Human PTCH RE1-2-Reverse	5'-GTAGTTCAAGACCTCAGTGATCC-3'
Human PTCH TSS-Forward	5'-GAAATCCAAGCCCAGCGTCC-3'-3'
Human PTCH TSS-Reverse	5'-TGGCTGCACTCACCATAGCC-3'
Mouse PTCH RE1-Forward	5'-AGGAATGTACTACGGCGTGTC-3'
Mouse PTCH RE1-Reverse	5'-ATGCGGACGCACAGGTAAC-3'
Mouse PTCH TSS-Forward	5'-CAGGGCAGACAAAGAGACCC-3'
Mouse PTCH TSS-Reverse	5'-GCTGACCGTGAGGTTCACA-3'

Primers for qRT-PCR	**Primer Sequence**
Human *REST*-Forward	5'-*GGCAGCTGCTGTGATTACCT*-3'
Human *REST*-Reverse	5'-*AGTTGTTATCCCCAACCGGC*-3'
Mouse *Scg10*-Forward	5'-*TGCGTGCACATCCCTACAAT*-3'
Mouse *Scg10*-Reverse	5'-*TGCTTCACCTCCATGTCGTC*-3'
Mouse *Syn1*-Forward	5'-*GGACGGAAGGGATCACATTATT*-3'
Mouse *Syn1* Reverse	5'-*ACCACAAGTTCCACGATGAG*-3'
Mouse *Ptch*-Forward	5'-*GGAAGTTGGTGGACGAGTGAG*-3'
Mouse *Ptch*-Reverse	5'-*GAGTGCTGAGTCCAGGTGTTG*-3'
Mouse *Gli1*-Forward	5'-*AGCTGAAGTCAGAGCTGGATATG*-3'

Mouse *Gli1*-Reverse	5'-*CTCATACACAGACTCAGGCTC*-3'
Mouse *Akt1*-Forward	5'-*TCGTGTGGCAGGATGTGTAT*-3'
Mouse *Akt1*-Reverse	5'-*ACCTGGTGTCAGTCTCAGAGG*-3'
Mouse *Akt*2-Forward	5'-*CAGCTGGGAGACCCAAGA*-3'
Mouse *Akt2*-Reverse	5'-*CACACGCTGTCACCTAGCTT*-3'
Mouse *Akt*3-Forward	5'-*TGGACCACTGTTATAGAGAGAACATTT*-3'
Mouse *Akt3*-Reverse	5'-*TGGATAGCTTCCGTCCACTC*-3'