Supplementary Figure Legends

Supplementary Figure 1. A. RT-PCR analysis of the TSC2-RGΔ transgene using two representative wild-type and TSC2-RGΔ pooled CGNPs. B. External granule layer cell number thickness based on number of cell layers were analyzed and quantified in mice of each genotype at post-natal day 7, showing significant differences between the wild-type and TSC2-RGΔ mice (five mice each)(* P value = 0.0004). Two-tailed t-tests were used to test significance. C. Immunofluorescence analysis of S6 phosphorylation (red) in wild type and TSC2-RGΔ cerebella. Phospho-S6 staining is localized in the mitotic region of EGL, Purkinje neurons, and IGL in post-natal day 7 cerebella. TSC2-RGΔ mice have increased intensity of phospho-S6 staining (right panel) compared to wild-type (left panel). Phospho-S6 (red) was co-stained with DAPI (blue). 40x power magnification was used. Bars: 16 μm.

Supplementary Figure 2. TSC2 and p27 are rapidly degraded in the cytoplasm of Pzp53 medulloblastoma cells (left panel). Pzp53med cells treated with the proteasome inhibitor lactacystin clearly accumulates TSC2 (red)(middle panel), and is associated with increased p27Kip1 (green)(right panel) and TSC2 localization to the nucleus (indicated by arrows). 40x power magnification was used.

Supplementary Figure 3. Human medulloblastomas have TSC1 inactivation

A. Recurrent deletion of TSC1 in primary human medulloblastoma. Integral view of recurrent losses on chromosome 9q in a non-overlapping series of primary human medulloblastomas analyzed on 100K and 500K SNP arrays. Hemizygous losses are
shown in green below the ideogram for each dataset. One medulloblastoma in the 500K dataset harbors a focal homozygous deletion (red box) on 9q34. The minimal common region of loss (boundary determined by the homozygous deletion) is shown as an output from the UCSC Genome Browser (NCBI Build 35) with the TSCI gene highlighted.

B. Deletions targeting TSCI are statistically significant. GISTIC output for chromosome 9 copy number data (500K SNP array; n=123 samples), showing chromosome 9q is a significant region of loss in medulloblastoma. The sharp peak at 9q34.13 encompasses the TSCI locus and pinpoints a minimal region of highly significant loss on 9q in the dataset (q-value=10^{-9.6}; G-score=0.16).

C. Downregulation of TSCI in medulloblastomas with TSCI deletion. qRT-PCR analysis of TSCI expression in a series of medulloblastomas with either normal diploid TSCI status (red bars) or TSCI deletion (blue bars). Normal fetal (5 donors) and adult (5 donors) human cerebellum samples were included as controls. TSCI expression was lower in tumor samples harboring TSCI deletions compared to those retaining two copies of the gene (two-sample Wilcoxon test, p<0.05). Notably, no expression is observed in MB-183, which has a homozygous deletion of TSCI.

Supplementary Figure 4. Down-regulation of TSC2 and CDKN1B in medulloblastoma subgroups. Log2-transformed expression values obtained for TSC2 (A) and CDKN1B (p27Kip1) (B) in normal human cerebellum (fetal: n=9; adult: n=5) and primary medulloblastomas (n=103) are shown as boxplots. Medulloblastomas were classified into four distinct molecular subgroups (WNT, SHH, Group C, and Group D) based on their gene expression signatures. TSC2 exhibits reduced expression in all
medulloblastoma subgroups when compared to normal cerebellum controls. In contrast, CDKN1B (p27\textsuperscript{Kip1}) appears to be specifically down-regulated in WNT and, to a lesser extent, SHH medulloblastomas.