**Supplementary figure legends**

*Supplementary figure S1: mRNA expressionlevels of MELK in central nervous system (CNS) tissues during embryonic development, derived from the Brainspan atlas (http://brainspan.org) and analyzed using R2.*

*Supplementary figure S2: Dose-response curves of JHH-DIPG-01 cells stably transduced with shRNA constructs targeting MELK, treated for 96 hours with OTSSP167. Data are presented as percentage of cells compared to untreated controls +/- SEM (n=5).*

*Supplementary figure S3: Ingenuity pathway analysis (IPA) of upstream factors capable of regulating differentially expressed genes in HSJD-DIPG-12, HSJD-DIPG-07, SF7761 and JHH-DIPG-01 cells treated for 24 hours with 20 nM OTSSP167.*

*Supplementary figure S4: Western blot showing expression levels of VUMC-DIPG-F neurospheres in comparison with JHH-DIPG-01 neurospheres. Red arrow indicates 74 kDa band corresponding to full-length MELK. Actin was used as a loading control.*

**Supplementary table legends**

*Supplementary table S1: shRNA sequences of constructs used in this study.*

*Supplementary table S2: List of off-target effects of OTSSP167 occurring at concentrations lower than 50nM, derived from the publicly available phosphoproteomics database at* [*http://proteomicsdb.org*](http://proteomicsdb.org)*.*