Supplementary Figure legends

Supplementary 1. (A) The analysis performed on R2 platform in different molecular subgroup of medulloblastoma and in the normal cerebellum samples shows that KCTD1 is significantly downregulated in SHh and WNT subgroup. (**p< 0.01, normal cerebellum versus SHh; ****p<0.0001, normal cerebellum versus WNT and G3). (**B-C**) KCTD1 expression does not affect Gli2 and Gli3 protein levels. HEK293T cells were transfected with Flag tagged Gli2 (**B**) and Flag tagged Gli3 (**C**) alone or with KCTD1. After 24h Protein levels of KCTD1, KCASH, Gli2 and Gli3 were analysed by WB using Gli1 and KCTD1 antibodies. β-Actin protein was used as a normalizer.

Supplementary 2. Circular dichroism analysis of KCASH2. Far-UV spectra of KCASH2 (10 μM) (**A**). Thermal denaturation curves of KCASH2 recorded at 220 nm (**B**).

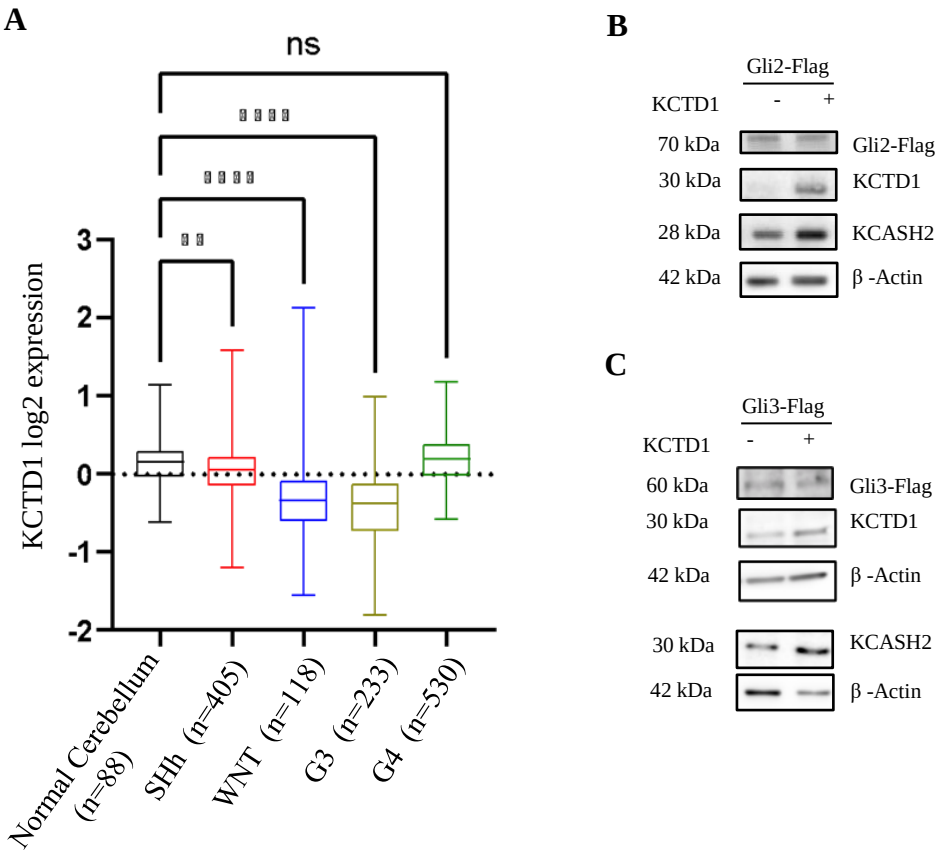
Supplementary 3. (**A**) MST fluorescence signal (fraction bound) of BTB/PZ15 plotted against increasing concentrations of KCASH2. (**B**) MST experiment of the interaction of the KCASH2 with POZ/BTB15. MST traces of triplicate experiments are reported. (**C**) MST fluorescence signal (fraction bound) of BTB/POZ15 plotted against increasing concentrations of BTB/POZ1 KCASH1.

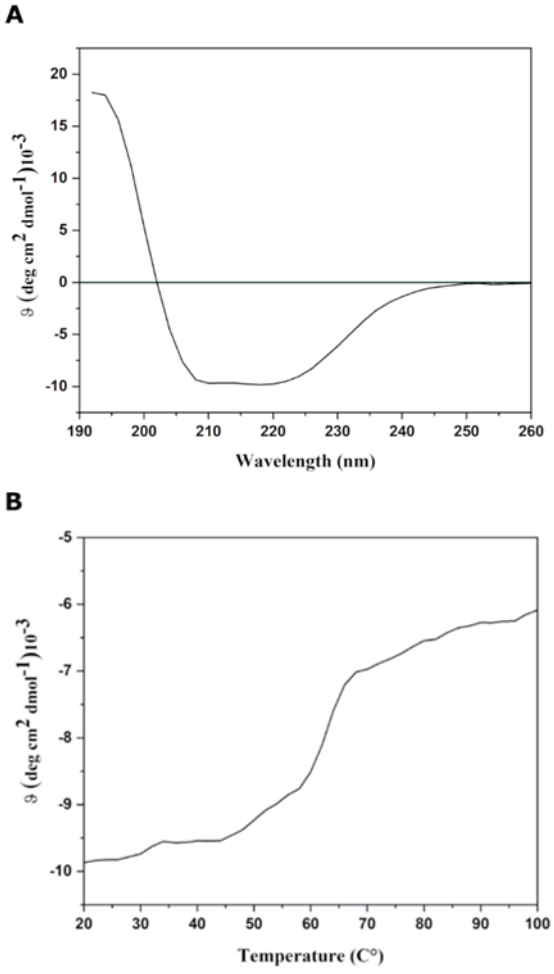
Supplementary 4. MST experiment of the interaction of BTB/POZ11 (**A**) and KCASH2 (**B**) with BTB/POZ1. MST traces of triplicate experiments are reported.

Supplementary 5. (**A**) BTB/POZ15 binds KCTD1-Flag and BTB/POZ1. MST fluorescence signal (fraction bound of BTB/POZ15 plotted against increasing concentration of full length KCTD1 (flKCTD1) (red curve) and BTB/POZ1 (orange curve). (**B**) MST experiments of interaction of the KCTD1(**B**) and its BTB/POZ domain (**C**) with BTB/POZ15. MST traces of triplicated experiments are reported.

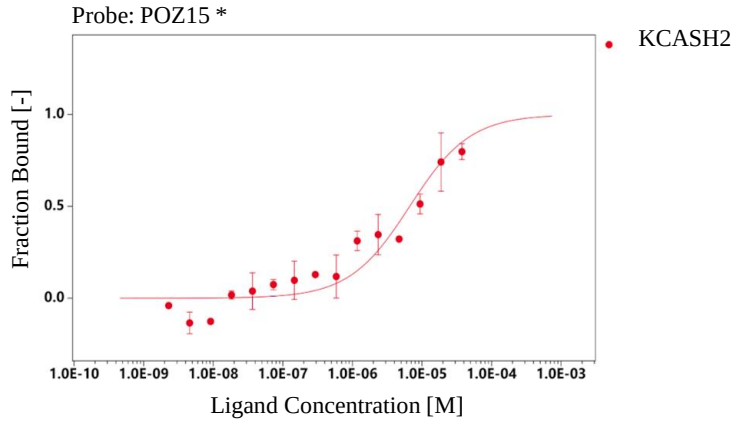
Supplementary 6. Validation of CRISPR/Cas9 HEK293T cell line KCASH2 KNOCK OUT. WB analysis of KCASH2 protein expression in parental or genome-edited cell line with deletion of KCASH2. Protein lysates were analysed using KCASH2 antibodies. β-Actin protein was used as a normalizer.

Supplementary 7. In medulloblastoma cell line the proliferative cells detected by Ki-67 signals were evidently de- creased after the KCTD1 overexpression. D283 Med cells were transfected with the control plasmid and KCTD1-Flag, then 24h the cells were fixed and stained with the anti-Ki67 (red) and nuclei were counterstained with Hoechst 33342 (blue). Number of Ki67 positive cells was calculated over total cells and expressed as percentage vs total cells. KCTD1 2μg vs CTR (*p< 0.05. Results are expressed as the mean ± SD of three independent experiments, Student's t-test).

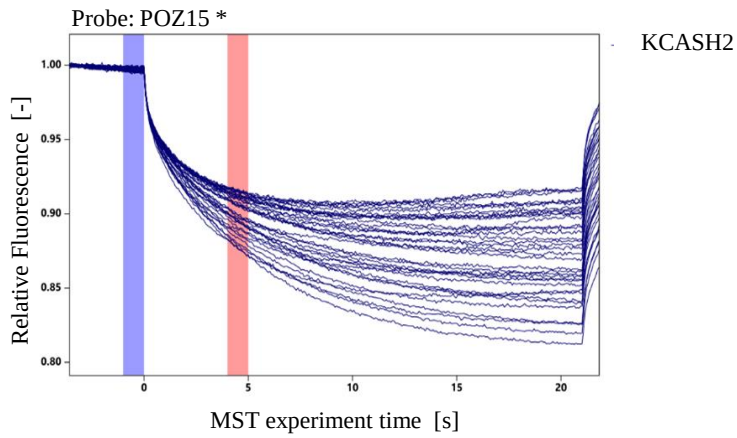

A
ns
KCTD1 log2 expression
3
2
1
0
-1
-2
Normal Cerebellum (n=88)
SHh (n=405)
WNT (n=118)
G3 (n=233)
G4 (n=530)
B
Gli2-Flag
KCTD1
-
+
70 kDa
Gli2-Flag
30 kDa
KCTD1
28 kDa
KCASH2
42 kDa
β -Actin
C
Gli3-Flag
KCTD1
-
+
60 kDa
Gli3-Flag
30 kDa
KCTD1
42 kDa
β -Actin
30 kDa
KCASH2
42 kDa
β -Actin


A
20
15
10
5
0
-5
-10
190
200
210
220
230
240
250
260
Wavelength (nm)
B
-5
-6
-7
-8
-9
-10
20
30
40
50
60
70
80
90
100
Temperature (C°)

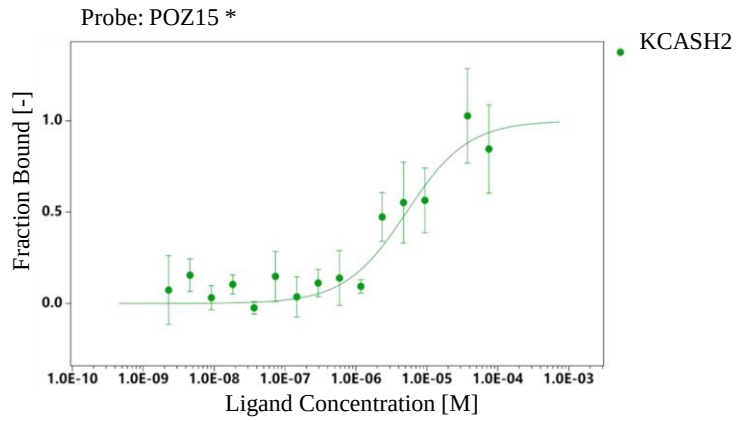
A

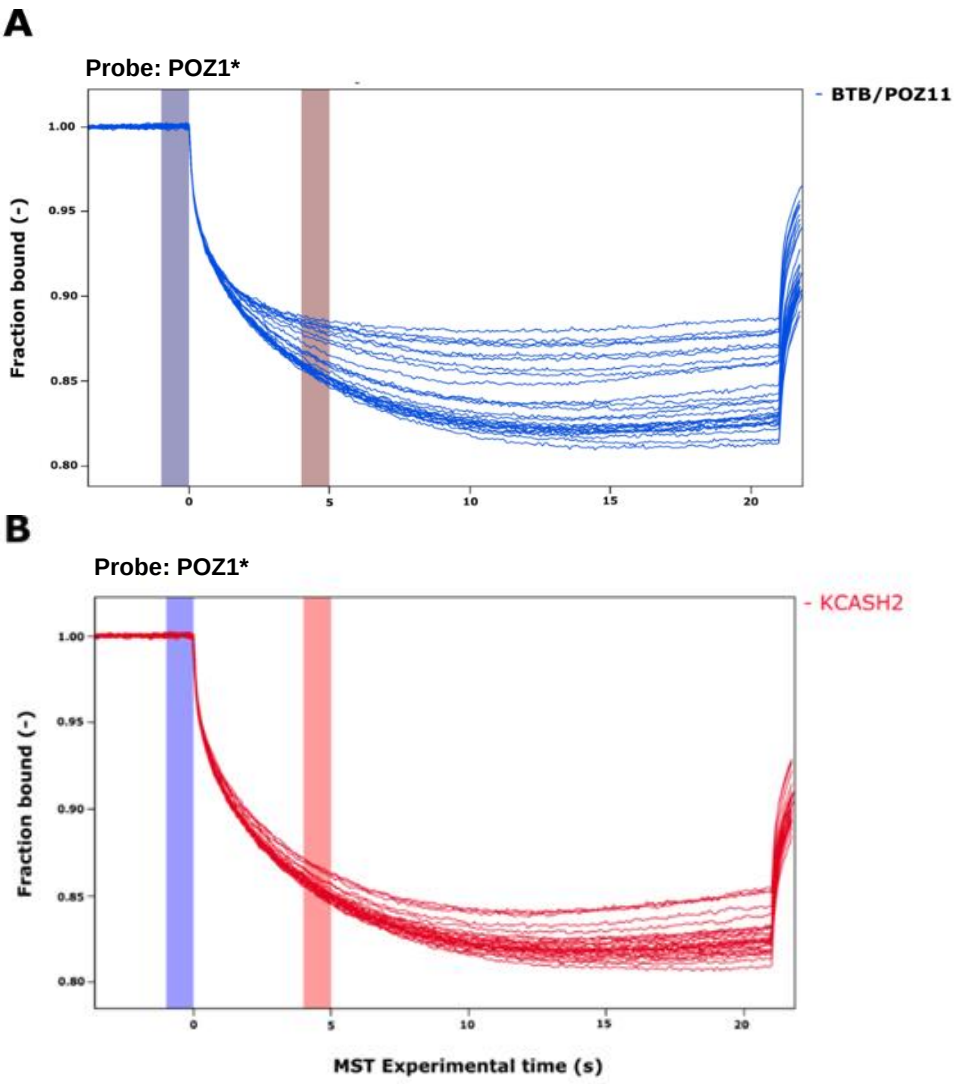

Probe: POZ15 *
KCASH2
Fraction Bound [-]
1.0
0.5
0.0
1.0E-10
1.0E-09
1.0E-08
1.0E-07
1.0E-06
1.0E-05
1.0E-04
1.0E-03
Ligand Concentration [M]

B

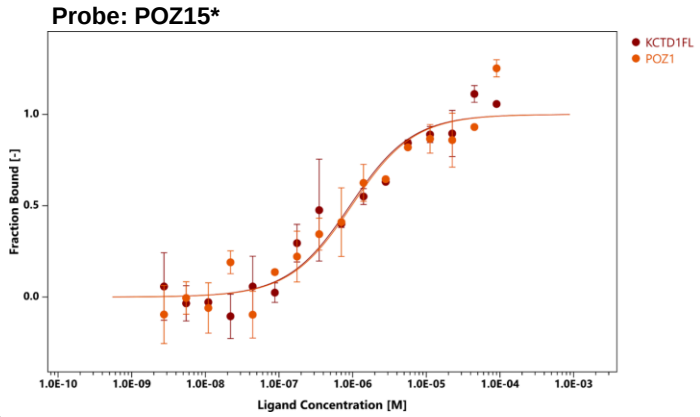

Probe: POZ15 *
KCASH2
Relative Fluorescence [-]
1.00
0.95
0.90
0.85
0.80
0
5
10
15
20
MST experiment time [s]

C

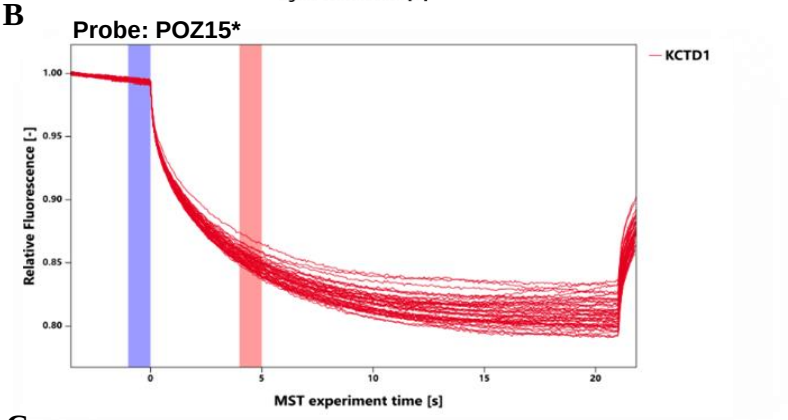

Probe: POZ15 *
KCASH2
Fraction Bound [-]
1.0
0.5
0.0
1.0E-10
1.0E-09
1.0E-08
1.0E-07
1.0E-06
1.0E-05
1.0E-04
1.0E-03
Ligand Concentration [M]


A
Probe: POZ1*
- BTB/POZ11
Fraction bound (-)
1.00
0.95
0.90
0.85
0.80
0
5
10
15
20
B
Probe: POZ1*
- KCASH2
Fraction bound (-)
1.00
0.95
0.90
0.85
0.80
0
5
10
15
20
MST Experimental time (s)

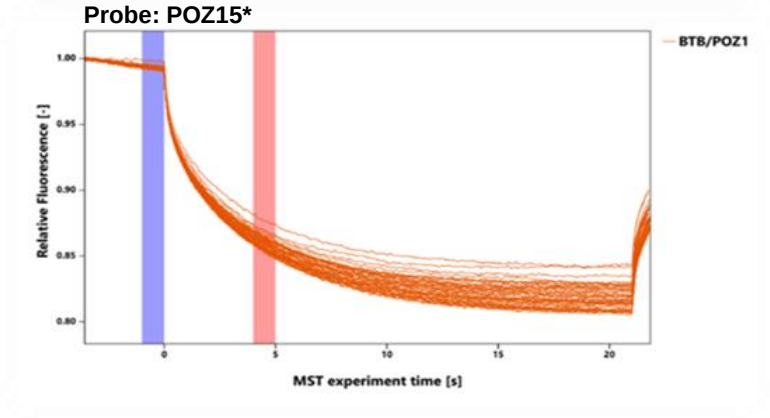
A

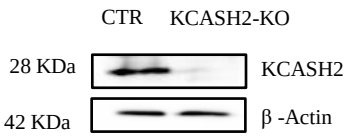

Probe: POZ15*
KCTD1FL
POZ1
Fraction Bound [-]
1.0
0.5
0.0
1.0E-10
1.0E-09
1.0E-08
1.0E-07
1.0E-06
1.0E-05
1.0E-04
1.0E-03
Ligand Concentration [M]

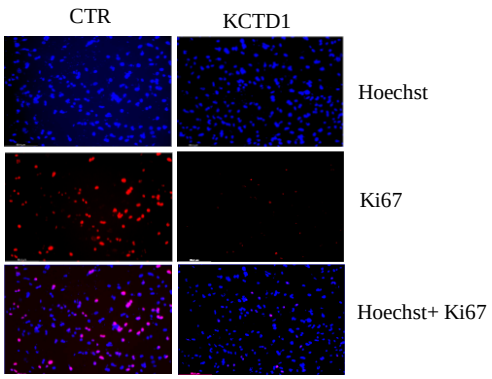
B

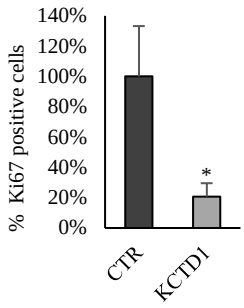

Probe: POZ15*
KCTD1
Relative Fluorescence [-]
1.00
0.95
0.90
0.85
0.80
0
5
10
15
20
MST experiment time [s]

C


Probe: POZ15*
BTB/POZ1
Relative Fluorescence [-]
1.00
0.95
0.90
0.85
0.80
0
5
10
15
20
MST experiment time [s]


CTR
KCASH2-KO
28 KDa
KCASH2
42 KDa
β -Actin


CTR
KCTD1
Hoechst
Ki67
Hoechst+ Ki67


140%
120%
100%
80%
60%
40%
20%
0%
% Ki67 positive cells
*
CTR
KCTD1