

Figure W1. (A) Sequence alignment of human KCTD proteins. Matching amino acids are colored. The BTB domain is underlined on the sequences. DFY is included in a black frame. A gene tree alignment of the three genes (with all the homolog from the different species) is available in the Ensemble Web site at the following link: http://www.ensembl.org/Homo_sapiens/Gene/Comparative_Tree?collapse=1698431%2C1698526%2C1698557%2C1698502%2C1698474%2C1698581%2C1698491%2C1698481%2C1698496%2C1698492%2C1698476;db=core;g=ENSG00000213859;r=17:7255208-7258258;t=ENST00000333751. (B) D283 cells transfected with siCTR and siCASH2, in the presence or absence of Flag-tagged KCASH2, were immunoblotted with anti-KCASH2 and antiactin antibodies. Different amounts of total lysates were analyzed to compare exogenous and endogenous signal (1:3 ratio, respectively). Unless otherwise indicated, all experiments were performed in triplicate, and mean \pm SD is shown.

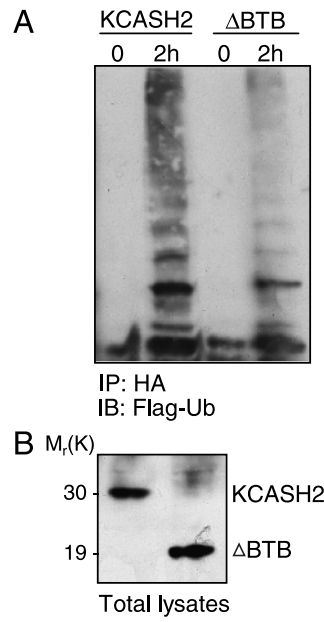


Figure W2. (A) KCASH2 shows E3 ubiquitin ligase activity. HEK293T cells, transfected with an HA-tagged KCASH2 or Δ BTB KCASH2 (Δ BTB) vectors, were treated with MG132 (50 μ M) 6 hours before lysis. Immunoprecipitation of cell lysates was carried out with an anti-HA antibody, and immunocomplexes were subjected to an *in vitro* ubiquitination assay in the presence of Flag-ubiquitin (Flag-Ub) and immunoblotted with an anti-Flag antibody to detect proteins ubiquitinated by KCASH2. (B) KCASH2 and Δ BTB-KCASH2 levels in total cell lysates.

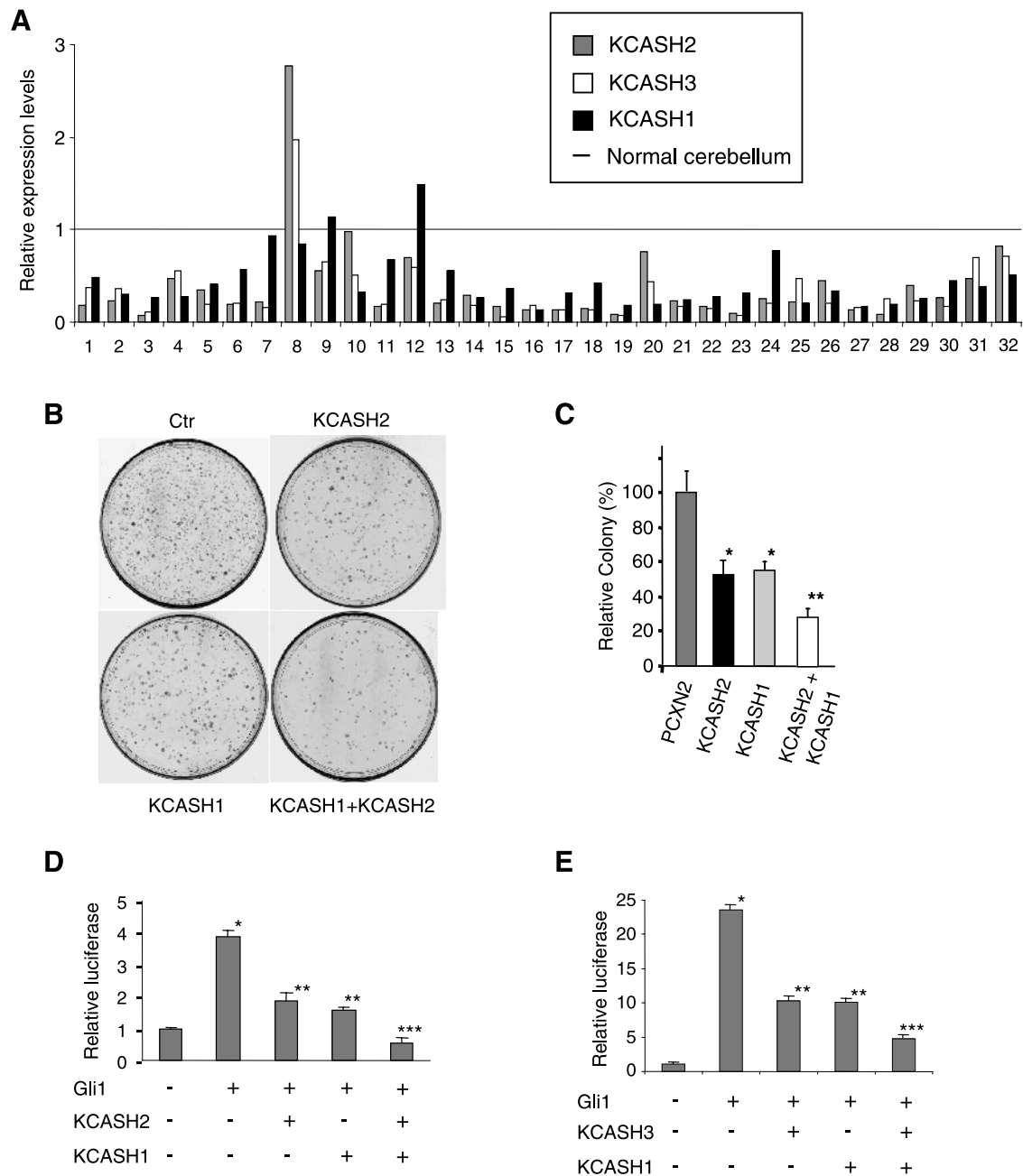


Figure W3. (A) Expression levels of KCASH1-3 in MB samples. Expression levels are shown relative to the average value of normal cerebellum (value set to 1). (B, C) Colony formation assay: Daoy cells were transfected with indicated vectors. (B) Representative images from experiments are shown. (C) Relative colony numbers are indicated. $*P < .05$ KCASH2 and KCASH1 versus control vector; $**P < .05$ KCASH1 + KCASH2 versus KCASH1 and KCASH2. Unless otherwise indicated, all experiments were performed in triplicate, and mean \pm SD is shown. (D, E) Relative luciferase activity in HEK293T cells transfected with 12 \times Gli-Luc and pRL-TK Renilla and the indicated plasmids. Luciferase data are indicated as mean ratios with respect to pRL-TK Renilla Luciferase control (Ctr). $*P < .05$ Gli1 versus control. $**P < .05$ KCASH1, KCASH2 or KCASH3 + Gli1 versus Gli1. $***P < .05$ KCASH2 + KCASH1 (or KCASH3 + KCASH1) + Gli1 versus KCASH1, -2, or -3 + Gli1.