Supplementary Figure legends

Figure S1, related to Figure 1.

DHX9 is a binding partner of KIF1Bβ apoptotic domain.

(A) Corresponding anti-Flag immunoblot analysis (Figure 1C and 1D) of cells that were transfected with plasmid encoding Flag-KIF1Bβ or Flag-KIF1Bβ-deletions as indicated. **(B)** Immunoblot analysis of CHP212 cells that were infected with lentivirus encoding short hairpin targeting EGLN3 (shEGLN3) or non-targeting control virus (shSCR).

Figure S2, related to Figure 2.

DHX9 is required for KIF1Bβ-induced apoptosis.

(A) Crystal violet staining of NB1 cells after infection with lentivirus encoding three independent shRNAs targeting DHX9 (shDHX9) or control virus (shSCR) and subsequently transfected with Flag-KIF1Bβ600-1400 or empty plasmid. Cells were selected with G418 (500µg/ml) for several weeks. Right panel: corresponding immunoblot analysis. **(B)** NB1 cells were transfected with plasmid encoding GFP-histone along with plasmid encoding RFP-KIF1Bβ600-1400 or WT-DHX9 (wild type) alone; or RFP-KIF1Bβ600-1400 in combination with WT-DHX9 or mutant DHX9 (ΔNTD-DHX9 or TD-DHX9). Shown is the percentage of GFP-positive nuclei exhibiting apoptotic changes 72h after transfection. (mean ± SD; n=3; *p-value<0.05). **(C)** Schematic diagram of wild-type DHX9 (WT-DHX9), nuclear transport-deleted DHX9 (ΔNTD-DHX9) and minimal transactivation mutant DHX9 (TD-DHX9). **(D)** Fluorescence images of cells in (2F) 48h after transfection.

Figure S3, related to Figure 3.

KIF1Bβ promotes DHX9 nuclear localization.

(A) Immunofluorescence staining of NB1 cells transfected with plasmid encoding RFP-KIF1Bβ-WT (wild type), RFP-KIF1Bβ600-1400 or empty backbone (control). Cells were stained against DHX9 (green), RFP-KIF1Bβ (red) and Hoechst (blue). Right panel: graphical representation of images showing percentage of transfected cells with either nuclear or cytoplasmic DHX9 localization (mean ± SD; n=3; **p-value<0.01). **(B)** Immunofluorescence images of SK-N-SH cells that were engineered to induce Flag-KIF1Bβ upon tetracycline treatment. Cells were stained against DHX9 (green) and Fibrillarin (red) and Hoechst to visualize nuclei (blue). Right panel: corresponding anti-Flag immunoblot analysis. **(C)** Left panel: anti-DHX9, anti-KIF1Bβ and anti-EGLN3 immunoblot analysis of SK-N-SH cells stably expressing short hairpins (sh) targeting KIF1Bβ, EGLN3 or non targeting control (shSCR). GAPDH serves as loading control. Right panel: Corresponding immunofluorescence images of the cells stained against DHX9 (green) and counterstained with Hoechst to visualize nuclei (blue). **(D)** Immunofluorescence images (graphical representation shown in Figure 3E) of NB1 cells transfected with Flag-KIF1Bβ mutants as indicated. **(E)** Anti-DHX9 immunoblot analysis of NB1 cells transfected to produce Flag-KIF1Bβ variants and immunoprecipitated with anti-Flag antibody.

Figure S4, related to Figure 4.

Quantification of Exportin-2 protein level.

Densitometric ratio Exportin-2/Tubulin of XPO2 immunoblot analysis shown in Figure 4B of cells transduced with shRNA lentivirus targeting XPO2.

Figure S5, related to Figure 6.

Induction of KIF1Bβ during NGF withdrawal increases nuclear DHX9 and XAF1 expression.

(A) Quantification of KIF1Bβ and XAF1 protein level of differentiated PC12 cells subjected to NGF withdrawal. Corresponding immunoblot is shown in Figure 6A. **(B)** XAF1 mRNA levels in PC12 cells that were subjected to NGF withdrawal 24h or 48h as indicated. mRNA was determined by real-time RT-PCR using SYBR-Green. **(C)** Immunoblot analysis of differentiated PC12 cells before (+) and after (-) NGF withdrawal as indicated. Prior to NGF withdrawal, differentiated cells were infected with lentivirus encoding short hairpins targeting DHX9 (shDHX9), or non-targeting control virus (shSCR).

Figure S6, **related to Figure 7.**

DHX9 is localized to the nucleus in mouse sympathetic neurons but not in human neuroblastoma tumors deficient in KIF1Bβ.

(A) Immunofluorescence images of mouse SCG at P1 showing DHX9 localization (red) in neurons expressing Tyrosine Hydroxylase (green). **(B)** and **(C)** Immunohistochemistry for DHX9 on paraffin-embedded tissue sections of human neuroblastoma tumors counter-stained with hematoxylin. K9 tumor is KIF1Bβ-negative and DHX9-positive and K11 tumor is negative for both, KIF1Bβ and DHX9. K11 serves as negative control for the anti-DHX9 immunohistochemistry performed in (7F). Low magnification images were acquired using a 20x objective while high magnification images of selected regions were acquired using a 100x objective. **(D)** Kaplan-Meier overall survival curve for individuals with high and low expression of *KIF1Bβ* using the 'Tumor Neuroblastoma public - Versteeg - 88 - MAS5.0 - u133p2' dataset from R2: microarray analysis and visualization platform. The KIF1B reporter 209234_at is specific for the KIF1Bβ splice variant and not cross-reactive to the KIF1Bα splice variant (Affymetrix Target Sequence: HG-U133_PLUS_2:209234_AT). The

graph depicts the best *p*-value corrected for multiple testing (Bonferroni correction). The numbers in parentheses indicate the number of individuals in each group.

Figure S7, **related to Figure 7G and discussion.**

Model proposing mechanism of KIF1Bβ mediated apoptosis in a developing neuroblast (top) competing for limiting trophic factor NGF. KIF1Bβ mediates nuclear localization of DHX9 in cooperation with XPO2. Subsequently, nuclear DHX9 activates XAF1 to facilitate apoptosis. Bottom, a cancerous neuroblast (1p36 deleted) lacking KIF1Bβ protein expression gained ability to survive in the absence of NGF. Loss of KIF1Bβ prevents DHX9 to localize to the nucleus and subsequently fails to activate pro-apoptotic factor XAF1.