

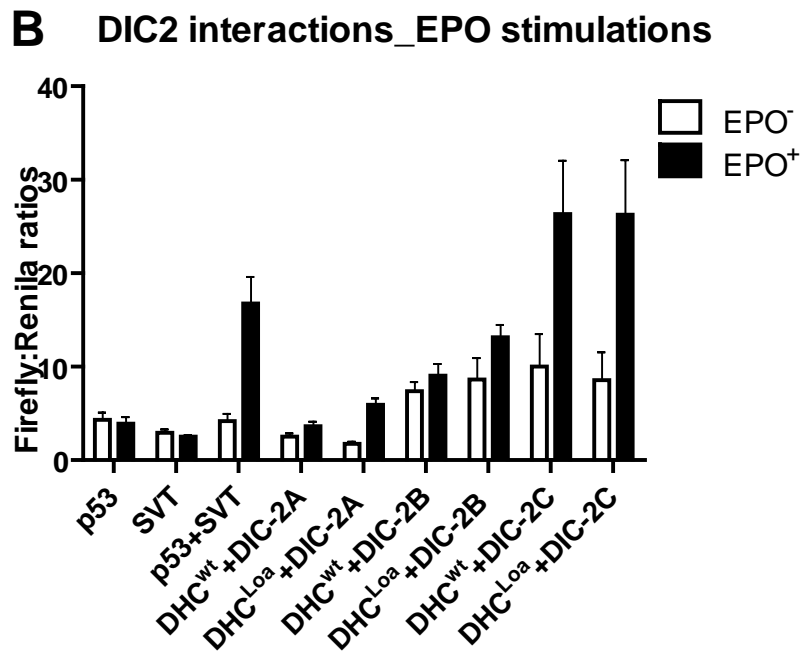
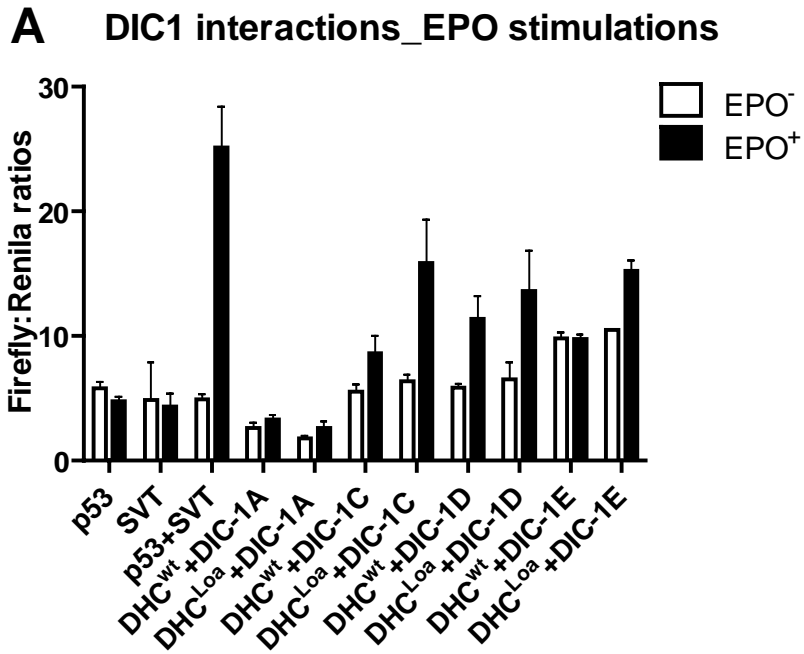
## Supplementary Data

Supplementary Figure 1: DHC fragments spanning the mutation site in *Loa* interact with DIC isoforms to induce Firefly luciferase expression. HEK293T cells were co-transfected with Renilla luciferase (for measuring transfection efficiency) and Firefly luciferase (protein interaction reporter gene) in combination with p53 bait (negative control), SV40 large T antigen prey (SVT, negative control), p53 bait and SVT prey (positive control), wildtype or mutant DHC baits and individual DIC isoform preys (A), or wildtype and mutant DHC baits and individual DIC2 isoform preys (B) with or without erythropoietin (EPO) stimulation. Firefly and Renilla luciferase activities were measured using LARII and Stop and Glo (Promega), respectively, as priming agents on a Lucy Luminometer (Labteck). The results were analyzed using the Stingray software. Firefly:Renilla luciferase ratios indicated protein-protein interactions up on stimulation with EPO.

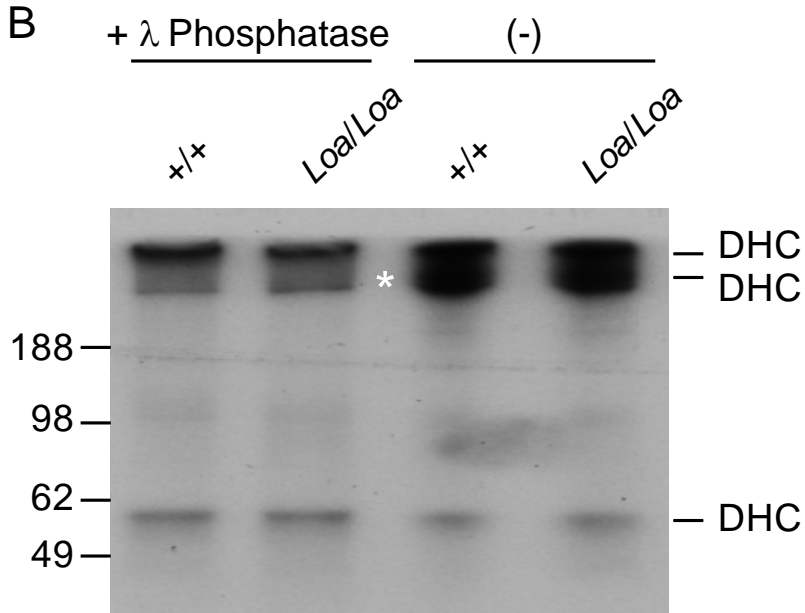
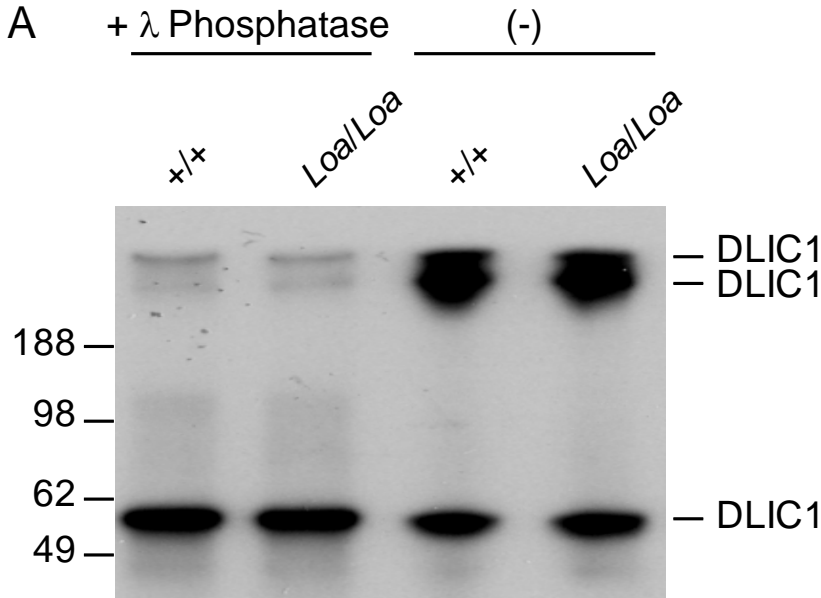
Supplementary Figure 2: A population of DHC and DLIC1 polypeptides form phosphorylation-dependent supercomplexes. Dynein was purified by immunoprecipitation with anti-DIC antibody from newborn mouse brain homogenates. The blot was probed with antibodies against DLIC1 (A) and DHC (B). (A) DLIC1 cannot be separated completely from DHC on 4-12% SDS-PAGE before treatment with  $\lambda$ -protein phosphatase; it was separated completely from DHC after  $\lambda$ -protein phosphatase. (B) The DHC signal in the lower band shifted upwards (asterisk) after dephosphorylation with  $\lambda$ -protein phosphatase.

Supplementary Figure 3: A DHC polypeptide, encompassing residues 268-992, forms aggregates if overexpressed in HEK293T cells. DHC<sub>wt</sub> prey (A) or DHC<sub>Loa</sub> prey (B) was transfected into HEK293T cells. The cells were fixed and permeabilized with 0.5 % glutaraldehyde and 0.1% Triton X-100, rinsed twice and incubated for 15 minutes at room temperature with 1% glutaraldehyde in warmed up CB buffer (see materials and methods). The cells were rinsed twice in warmed CB buffer followed by 5 minute incubation in 0.5 mgml<sup>-1</sup> sodium borohydride at room temperature with period agitation. The DHC<sub>wt</sub> or DHC<sub>Loa</sub> were visualized using anti-FLAG mouse primary antibody (1/400) (Sigma) followed by Alexa Fluor 456 goat anti-mouse IgG secondary antibody (1/200) (Molecular probes), followed by microscopy using a Delta Vision microscope. The wild type and mutant FLAG-tagged DHCs are shown in red. Nuclei are shown in blue (DAPI). Arrows point to areas of aggregation. Scale bars represent 30 $\mu$ m.

Supplementary Figure 1



Supplementary Figure 2



Supplementary Figure 3

