Supplementary Information

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A. Supplementary Tables

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Table S3. Raw proteomics data of Kif5C complexes immunoprecipitated from mouse hippocampus. This list was generated using Scaffold software.

Table S4. Conservative estimate of Kif5C protein cargos in the mouse hippocampus based on 1.5 fold enrichment in IPs. Known synaptic proteins based on NCBI, Uniprot, Genecards and OMIM database searches are highlighted in green.

Table S5. Raw proteomics data of Kif3A complexes immunoprecipitated from mouse hippocampus. This list was generated using Scaffold software.

Table S6. Conservative estimate of Kif3A protein cargos in mouse hippocampus based on 1.5 fold enrichment in IPs. Known synaptic proteins based on NCBI, Uniprot, Genecards and OMIM database searches are highlighted in green.

Table S7. Raw proteomics data of Kif5C complexes immunoprecipitated from mouse PFC. This list was generated using Scaffold software.

Table S8. Conservative estimate of Kif5C protein cargos in the mouse PFC based on 1.5 fold enrichment in IPs. Known synaptic proteins based on NCBI, Uniprot, Genecards and OMIM database searches are highlighted in green.

Table S9. Biological function analysis of Kif5C-IP enriched proteins in mouse hippocampus. Peptides with 95% confidence or greater were analyzed using the Peptide Prophet algorithm and proteins assigned as Kif5C-IP specific were studied using Ingenuity Pathway Analysis (IPA) for mapping to biological functions as listed here.

Table S10. Top 20 biological function categories excluding “Cancer” identified by IPA analysis of Kif5C-IP specific proteins in mouse hippocampus.

Table S11. Biological function analysis of Kif3A-IP enriched proteins in mouse hippocampus. Peptides with 95% confidence or greater were analyzed using the Peptide Prophet algorithm and proteins assigned as Kif3A-IP specific were studied using the IPA for mapping of biological functions as listed here.

Table S12. Top 20 biological function categories excluding “Cancer” identified by IPA analysis of Kif3A-IP specific proteins in mouse hippocampus.

Table S13. Biological function analysis of Kif5C-IP enriched proteins in mouse PFC. Peptides with 95% confidence or greater were analyzed using the Peptide

Prophet algorithm and proteins assigned as Kif5C-IP specific were studied using the IPA for mapping of biological functions as listed here.

Table S14. Top 20 biological function categories excluding “Cancer” identified from IPA analysis of Kif5C-IP specific proteins in mouse PFC.

Table S15. Top 10 categories of diseases and molecular/cellular function analysis of enriched proteins identified in the three kinesin complexes. Following IPA analysis of Kif5C/Kif3A enriched proteins, protein cargos were further classified and sorted based on the number of molecules involved in diseases and molecular/cellular functions.

Table S16. Top 5 networks of protein cargos identified from the three kinesin complexes using IPA. The biological networks were identified by IPA analysis and evaluated by scores (p-score = -log10 (p-value)), which represent the relevance of networks to the input proteins. The top 5 networks for protein cargos of these three kinesin complexes and the scores are listed.

B. Supplementary Figure Legends

Figure S1. Characterization of Kif5C expression. (A) qRT-PCR analysis of expression of mRNAs of 31 kinesins in mouse hippocampus. RNAs were isolated from mouse hippocampus and expression of 31 kinesins were determined by qRT-PCR using specific primers. Data were normalized using GAPDH levels. Error bars represent SEM. (B) RNA *in situ* hybridization analysis of Kif5C expression in mouse hippocampus and CA1 region of the hippocampus using

fluorescently labeled sense and antisense ribo probes. Shown are confocal projection images of Kif5C mRNA (green) co-stained with Hoechst (blue). (C) Immunohistochemical analysis of Kif5C protein expression in mouse hippocampus, showing CA1, CA3 and dentate gyrus (DG). Adult mouse brain slices were stained with Kif5C (red) and α-tubulin (green) antibodies and Hoechst (blue). Shown are confocal projection images of representative examples in different regions of the hippocampus. (D) Representative Western blotting analyses of Kif5C and Kif3A protein expression in mouse PFC, hippocampus and cerebellum. α-tubulin was used as loading control. (E) Identification of Kif5C (NP_032475.2) by mass spectrometry. Peptides identified from mass spectrometry are shown in red. (F) qRT-PCR analysis of mRNA expression of seven kinesins in primary cultures of mouse hippocampal neurons. RNAs were isolated from cultured neurons and same procedure was followed, as described in Figure S1A, to quantify gene expression. (Red scale bar: 200 μm; white scale bar: 20 μm.)

Figure S2. Protein expression characterization of candidate cargos of Kif5C in hippocampus. Adult mouse brain slices were co-stained with specific antibodies that recognize PURα (green) and β-tubulin (red) (A), DIC1 (green) and α-tubulin (red) (B), GluR1 (green) and β-tubulin (red) (C). Nuclei were stained with Hoechst (blue). Confocal projection images show staining in different sub regions (CA1, CA3 and dentate gyrus (DG)) of hippocampus. (Scale bar: 20 μm.)

Figure S3. Knockdown of Kif5C in primary cultured hippocampal neurons. (A & B) siRNA oligonucleotide transfections were performed on DIV3-5 cultured mouse hippocampal neurons to knockdown Kif5C. siRNA transfection efficiency of knockdown (KD) was studied at 48 hrs and 72 hrs after transfection by Western blot analyses. (A). Quantitation of Western data is shown in B. Control (CON, no treatment) and scrambled negative control siRNA (NC) were also used as controls. A two-way ANOVA (time by transfection) revealed a significant knockdown effect of Kif5C siRNA transfection ($F_{(2, 63)} = 16.866$, $p < 0.0001$) and a significant knockdown at 72 hrs of transfection ($F_{(2, 63)} = 12.622$, $p < 0.0001$). This experiment was repeated 8 times (n = 8). Protein level of GluR1 was used as a non-specific knockdown control and α-tubulin was used as a loading control. (C) Kif5C knockdown at 72 hrs after siRNA transfection was characterized by immunocytochemistry analyses of Kif5C (red), α-tubulin (green) and Hoechst (blue) in primary cultures of mouse hippocampal neurons. (D) Quantitative analysis of Kif5C knockdown data shown in "C". A one-way ANOVA followed by Tukey's *post hoc* test revealed the significant knockdown effect of Kif5C siRNA transfection, $F(2, 30) = 81.035$, ***$p < 0.001$. Error bars represent SEM. (White scale bar (for fluorescent images): 20 μm; red scale bar (for DIC images): 8 μm.)

Figure S4. Characterization of Kif3A in mouse hippocampus. (A) Immunocytochemistry analyses of Kif5C and Kif3A distribution in primary hippocampal neurons. Shown are confocal projection images of co-immunostaining of Kif5C (red) and α-tubulin (green), Kif3A (green) and β-tubulin

(red), as well as Kif5C (red) and Kif3A (green). (B) Immunohistochemistry analyses of expression and distribution of Kif3A in mouse hippocampus and hippocampal sub regions CA1, CA3 and DG. Adult mouse brain slices were stained with Kif3A (green) and β-tubulin (red) antibodies and Hoechst (blue). (Red scale bar: 200 μm; white scale bar: 20 μm.)

Figure S5. Identification of Kif3A and distribution of Kif3A cargos in CA1 region of hippocampus. (A) Identification of Kif3A (NM_008443) by mass spectrometry. Peptides identified from mass spectrometry are shaded in yellow.
(B) Protein expression analysis of selected cargos of Kif3A in CA1 region of mouse hippocampus. Adult mouse brain slices were stained with RyR2 (green), SAP97 (green) and SLK (green) antibodies, also co-stained with β-tubulin (red) antibody and Hoechst (blue). Shown are confocal projection images of representative examples. (Scale bar: 20 μm.)

Figure S6. Characterization of Kif3A cargos by knocking down Kif3A in the hippocampal neurons. (A) Western blot analyses of Kif3A knockdown in hippocampal neurons. Proteins were isolated from shRNA (control and Kif3A shRNAs) transfected cultured mouse hippocampal neurons (DIV 3-5). Efficiency of knockdown (KD) at 48 hrs and 72 hrs after shRNA transfection were studied by Western blot analyses using an anti Kif3A antibody. (B) Quantitation of Western data. control (CON, no treatment) and scrambled negative control shRNA (NC). Kif5C was used as a non-specific knockdown control and α-tubulin

was used as a loading control. A two-way ANOVA (time by transfection) revealed a significant effect of Kif3A shRNA transfection ($F_{(2, 36)}$ = 5.432, *P* = 0.0087) and a significant knockdown effect at 48 hrs of transfection ($F_{(2, 36)}$ = 9.671, *P* = 0.0004). This experiment was repeated five times (n = 5; ***P < 0.001, ** P < 0.01). Error bars represent SEM. (C) Immunocytochemistry characterization of Kif5C and Kif3A after Kif5C or Kif3A knockdown in cultured hippocampal neurons. The same Kif5C or Kif3A knockdown procedure was performed on primary cultured hippocampal neurons as described in Figure 4B, followed by immunocytochemistry analyses of Kif5C (green) and α-tubulin (red), as well as Kif3A (green) and β-tubulin (red) in KD neurons compared with CON neurons and NC neurons. Shown are representative confocal projection images. (Scale bar: 20 μm.)

Figure S7. Characterization of Kif5C in mouse PFC. (A) qRT-PCR analysis of mRNA expression of 31 kinesins in mouse PFC. RNAs were isolated from mouse PFC and expression levels of 31 kinesins were determined by qRT-PCR. Data was normalized to GAPDH levels. Error bars represent SEM. (B) Localization of Kif5C mRNA in mouse PFC. Adult mouse brains were sliced and then performed *in situ* hybridization and immunohistochemistry using a DIG labeled Kif5C-antisense RNA probe (green), co-stained with Ctip2 (red) and CUX1 (white) antibodies and Hoechst (blue, only shown in the merged image). Shown are representative confocal projection images. Merged images show distribution of Kif5C RNA and Ctip2 (a marker of deep layer subcortical projection) or CUX1 (a

marker of upper layer subcortical projection). (Scale bar: 200 μm.) (C) IP of Kif5C from mouse PFC. PFC from 7-11 week old mice were used in Kif5C IPs (Figure 1A). Silver stain (upper) and Western blotting analysis (lower) of Kif5C complexes are shown. CREB antibody IP and beads alone IP served as controls for IP.

Figure S8. IPA analysis of proteins that are enriched in Kinesin complexes. Biological pathway analyses of cargo proteins that are selectively associated with the three kinesin complexes (shown in Figure 6) using IPA (Ingenuity Pathway Analysis). Top ten diseases/disorders and cellular functions identified from IPA analysis (***P < 0.001) are shown. Top ten diseases/disorders and molecular/cellular functions of Kif5C protein cargos in hippocampus (A), Kif3A protein cargos in hippocampus (B) and Kif5C protein cargos in PFC (C). Each bar graph shows the number of proteins assigned to particular disorder or cellular function.

Figure S9. Top 3 networks for protein cargos of Kif5C in mouse hippocampus identified from IPA analysis. The biological networks were identified by Ingenuity Pathway Analysis (IPA) of proteins enriched in Kif5C-IP from mouse hippocampus and ranked by scores (p-score = -log10(p-value)), the top three networks (A, B and C) are shown. Node (protein) and edge (protein relationship) symbols were described in bottom right.

Figure S10. Top 3 networks for protein cargos of Kif3A in mouse hippocampus identified from IPA analysis. The IPA analysis was performed on proteins enriched in Kif3A-IP from mouse hippocampus and the ranked top three networks were identified (A, B and C). Node (protein) and edge (protein relationship) symbols were described in bottom right.

Figure S11. Top 3 networks for protein cargos of Kif5C in mouse PFC identified from IPA analysis. The IPA analysis was performed on proteins enriched in Kif5C-IP from mouse PFC and the ranked top three networks were identified (A, B and C). Node (protein) and edge (protein relationship) symbols were described in bottom right.

Figure S12. Identification of components of retrograde transport motor dynein in Kif5C and Kif3A IPs from hippocampus, and Kif5C IP from PFC. Bar graphs show average fold enrichment (n = 4) identified from the proteomics data. Fold enrichment of dynein heavy chain 1 (UniProtKB: Q9JHU4) (A), dynein light chain 2 (UniProtKB: Q9D0M5) (B), dynein intermediate chain 1 (UniProtKB: Q8R1Q8) (C), dynein intermediate chain 2 (UniProtKB: O88487) (D), and dynactin subunit 1 (UniProtKB: O08788) (E) from the three kinesin IPs are shown.

C. Supplementary Methods

Animals, Reagents, Antibodies: 7-11 week old male mice were obtained from The Jackson Laboratory (Bar Harbor, ME), and bred in a temperature-controlled vivarium in the Animal Resource Center at Scripps Florida. All procedures were conducted and all experimental mice were terminated by cervical dislocation in adherence with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of The Scripps Research Institute.

Immunoprecipitation of Kif5C/Kif3A and Western blotting: Mouse hippocampus or prefrontal cortex (PFC) were manually dissected and homogenates were prepared in Nonidet P-40 lysis buffer (0.50 % Nonidet P-40, 50 mM Tris-HCl pH 7.5, 125 mM NaCl, 1 mM EDTA pH 8.0, 1 mM DTT, 1 protease inhibitor cocktail tablet (Roche), 100 µl of phosphatase inhibitor cocktail 1 and 2 each (Sigma), 100 µl BSA) and fractionated by centrifugation (10,000 X g, 15 min. at 4 °C) to obtain tissue lysates. For a single co-immunoprecipitation of endogenous cargos with Kif5C or Kif3A, tissue lysates from hippocampus or PFC isolated from three animals were incubated with 30 µl of anti-KHC (Millipore) or Kif3A antibody (Abcam) or CREB antibody (Cell Signaling). All the incubations were kept on a rotator for overnight at 4°C, then we added 200 µl of a 50% (w/v) slurry of protein A/G beads (Thermo Scientific) and incubated for 2 hrs at 4°C. Kif Ab IPs, control Ab IPs and beads alone control IPs were then briefly centrifuged

(100 g, for 60 seconds) and the slurry was washed three times with Tris Buffer A (20 mM Tris-HCl pH 7.5, 250 mM NaCl, 0.5 % NP40, 0.5 mM DTT, protease and phosphatase inhibitors). The resulting protein complexes were eluted with SDS sample buffer and boiled for 10 minutes and separated by 8% SDS-PAGE.

For Western analyses, proteins were isolated from prefrontal cortex or hippocampus or primary cultured neurons using IP buffer or urea SDS lysis buffer (8M urea, 2% SDS, 150 mM Tris). Efficiencies of different IPs were calculated by analysis of Western blots. Briefly using NIH IMAGEJ software, we compared the amount of specific kinesin band in total, IP and post IP supernatant. We found that our IP conditions immunoprecipitated more than 70% of specific kinesins from the total extracts (Kif5C, hippocampus 72.3 ± 9.5%; Kif5C, PFC 73.4 ± 7.5%; Kif3A, hippocampus, 97.13 ± 1.3 %). For the Western analysis of cargos, protein concentration was determined using a BCA kit (Pierce, Rockford, IL). 10-25 µg of protein were used for Western analysis. Antibodies used are listed in supplemental Table 2. The target proteins were detected using anti-rabbit or anti-mouse secondary antibodies (Cell Signaling Technology, Inc., Danvers, MA, USA) at 1:5000 dilution, then visualized by chemiluminescence (Amersham Biosciences, Piscataway, NJ). The autoradiograms were analyzed by IMAGEJ.

Proteomics analysis: For protein identification, each IP sample was fractionated by SDS-PAGE approximately 1 cm into a standard mini-gel, and then sectioned

into six gel slices (2 mm x 7 mm). Each slice was washed, reduced with DTT, alkylated and digested in-gel with trypsin overnight. Following digestion, peptides were analyzed with nano-UPLC MS/MS (Easy NanoLC1000 interfaced with a Q-Exactive, Thermo Electron, San Jose, CA). Each sample was vent loaded onto a 2 cm x 75 µm C_{18} trap column (Acclaim PepMap100, 3 µM, 100 Å Nanoviper, Thermo Scientific) at a flow rate of 3-µL min^{-1}. Peptides were then eluted across a 15 cm x 50 µM analytical column with a gradient of 5 % CH3CN, 0.1 % FA increasing to 40 % CH4CN over a period of 45 min. and electrosprayed directly into the mass spectrometer. Data dependent MS/MS spectra were acquired with a standard top-10 method. For protein identification and spectral counting, all MS/MS data were searched against the Uniprot SwissProt database (version 11-2012, species *Mus Musculus*, including decoy search) and loaded into Scaffold v 3.4.5 (Proteome Software, OR). Data was first analyzed by Scaffold software and identified proteins that are present only in IPs and not in controls. Subsequently data were analyzed to identify proteins that are enriched at least 1.5 fold in the IP and were considered to be specifically associated with each kinesin complexes.

Super resolution SIM microscopy: Structured Illumination Microscopy (SIM) was performed with an ELYRA PS.1 microscope from Carl Zeiss Microscopy. Images were observed through a 63x/1.4NA objective and recorded using an Andor iXon 885 EMCCD (1024 x 1024 pixels, 8 x 8 µm pixel size, 65% QE), for a maximum field of view of 80 x 80 µm of the sample. Structured Illumination raw

datasets were acquired by projecting grids onto the sample generated from the interference of the 0 th and ±1 st diffraction orders from a phase grating. For the 405, 488, 561 and 642 nm excitation, phase gratings of spacing 23, 28, 34 and 34 µm (respectively) were used to generate illumination grids for maximum resolution improvement of each color. Each super-resolved image required five grid shifts (phases) and three grid rotations for a total of 15 images per super-resolved z-plane per color. The ELYRA PS.1 system's maximum laser output was 50, 200, 200, 150 mW (respectively), with a dedicated ND filter wheel for each laser for fine power control. During acquisition, laser power, camera exposure time and camera gain was adjusted so that high contrast images (50% camera dynamic range, 16 bit) were acquired. For most images, a camera exposure time of 50 ms was used. For 3D images, z-stacks were acquired using a z-piezo stage insert by PI (PI-737). Images were reconstructed through a proprietary Zeiss Fourier-based algorithm. Maximal projection SR-SIM images were processed using ZEN 2011 software.

Primary neuronal culture and siRNA/shRNA transfection, cargo analysis: Primary hippocampal cultures were prepared[1] from the brains of embryonic day 18-21 mice. Cells were plated at a density of 5×10^5 on poly-D-lysine-coated (700 µg/ml) dishes and glass coverslips. Cultures were plated in Neurobasal medium (Invitrogen) supplemented with 10% fetal bovine serum and penicillin/streptomycin mix and grown in Neurobasal medium supplemented with 2% B27 (Invitrogen), 0.5 mM glutamine, and penicillin/streptomycin mix at 37°C

in 5% CO_2. siRNAs against Kif5C (catalog# SR302564B), shRNAs against Kif3A (catalog# GI590642), a scrambled siRNA control (catalog# SR30004) as well as a scrambled shRNA control (catalog# TR30013) were obtained from ORIGENE (Kim and Seed, 2010), and introduced to primary hippocampal neurons (3-5 days *in vitro* (DIV)) using Lipofectamine RNAiMAX or Lipofectamine 2000 (Invitrogen) according to manufacturer's guidelines. One day before transfection, fresh culture medium was prepared and mixed evenly with the old medium. One-half of the mixed mediums were left with the cells for transfection, and the other one-half was saved for medium replacement after transfection. Briefly, for the transfection of cells growing in the single well of a 24-well format dish, 6 pmol of RNAi duplex was mixed with 1 µl of Lipofectamine RNAiMAX in 100 µl of Neurobasal medium and incubated for 20 minutes. The complexes of RNAi and Lipofectamine RNAiMAX were added to the cells for 6 hr incubation at 37°C in 5% CO_2. Cells were then returned to the saved culture medium. shRNA transfection to knockdown Kif3A was performed following the similar protocol as described for Kif5C siRNA transfection. α/β-tubulin immuno histochemistry was used for visualizing any changes in microtubule cytoskeleton due to siRNA/shRNA transfection of primary cultured neurons.

Immunohistochemistry: (a) Brain Slices: Perfused brain from 7-11 week old mice were removed from the skull and post-fixed in the same fixative overnight. Immunohistochemical staining for protein cargos were performed on free-floating brain sections. 30 µM Cryo-sections were washed three times for 10 minutes in

0.01M phosphate buffered sodium (PBS; pH 7.4) and pretreated with 0.5%Trion-X100-PBS for 20 min at room temperature to rupture membrane. Subsequently, sections were incubated in 10% normal goat serum in PBS for 30 min followed by incubation with primary antibodies (listed in Supplementary Table 2) overnight at 4°C. After four washes with PBS with 0.1% Triton, the immunoreactivity was probed using Rhodamine Red-X- or Oregon Green 488/568-conjugated secondary antibodies (1:1000; Molecular Probes) for 1 hr at room temperature. Both primary and secondary antibodies were diluted in PBST containing 1% BSA. For counterstaining of nuclei, the sections were stained using Hoechst. The images were collected using a Zeiss LSM 780 confocal microscope system.

(b) Neuronal cultures: The primary cultured neurons on glass coverslips are processed for immunocytochemistry, 3 days after si-RNA transfection. Neuronal culture medium was carefully removed and after two rinses in PBS, the cells were fixed in a freshly prepared solution of 4% paraformaldehyde for 15 minutes. After two more rinses in PBS, the cells were permeabilized in 0.5% Triton X-100 in PBS for 15 min. The cells were then incubated in 10% BSA in PBS for 30 minutes and incubated with primary antibody at 4ºC overnight. The immunoreactivity was probed using Rhodamine Red-X- or Oregon Green 488/568-conjugated secondary antibodies (1:1000; Molecular Probes) for 1 hr at room temperature. For the triple labeling studies, DAPI/HOECHST-containing fluoro gel ‖ was used for mounting. The images were taken with a Leica DM5500 microscope.

mRNA *in situ* hybridization: The *in situ* hybridization using digoxigenin (DIG) labeled ribo probes was followed as described[2]. 15 µM Cryo-sections were fixed in a freshly prepared solution of 4% paraformaldehyde for 10 min at room temperature, washed three times for 5 min in 0.01M PBS_{DEPC} and performed acetylation in freshly prepared TEA solution for 10 min and prehybridization at 68ºC overnight. After hybridization, the sense and antisense RNA probes were visualized using a Fluorescent Antibody Enhancer kit (Roche, Basel, Switzerland) for DIG detection. The immunohistochemical analyses of α-tubulin and cortex layer marker Ctip2 and CUX1 were performed as described for immunohistochemical analysis. Texas Red conjugated anti-rabbit secondary antibodies (Invitrogen) were used for visualization. Images were acquired by using Zeiss LSM 780 confocal microscope system with 10X/63X objective. Only projection images are shown.

Quantitative Real-Time PCR (qRT-PCR): One µg of RNA was used in 40 µl of reaction performed with qScript™ cDNA SuperMix from Quanta BioSciences according to the manufacturer's protocol. All amplifications were primed by pairs of chemically synthesized 18- to 24- mer oligonucleotides designed using freely available primer design software (Primer-3,) to generate target amplicons of 70–110 bp. Sequence information on the forward and reverse primers used are listed in Supplementary Table 1. The qRT-PCR mixture had a total volume of 10 µl containing 2 µl of H_2O, 2 µl of cDNA, 5 µl of 2X Master Mix, 1.0 µl of 10 µM

(each) forward and reverse primer. The reaction was carried out in a 7900HTFast Real-Time PCR System (Applied Biosystems) under the following conditions: 95°C for 10 min followed by 40 cycles of 95°C for 15 sec., 60°C for 1 min. There were four biological replicates and four technical replicates for each biological replicate. Quantification of the target transcripts was normalized to the Mouse GAPDH reference gene using the Pfaffl method[3,4].

Bioinformatics analysis: Identifications associated with keratins and antibodies were removed, and the remaining was analyzed with the Peptide Prophet algorithm to assign identification confidence probability. Peptides with 95% confidence or greater were preserved and the parent protein names were assembled into three categories Kif5C-Hip, Kif3A-Hip and Kif5C-PFC. Ingenuity Pathway Analysis (Ingenuity Systems, Inc. Redwood City, CA) was used with these identifications to run network/pathways analysis. For each group, the resulting biological pathways were ordered according to the number of molecules that map to a biological function, pathway, or network, and the top 20 were preserved.

Statistical analysis: Data were analyzed using SigmaPlot 12.5 statistical software or Prism 5 (GraphPad Software). The two-way ANOVA procedure followed by the Tukey's *post hoc* tests or one-way ANOVA followed by Dunnett's *post hoc* test, or two-tailed Student's T test was applied to determine the

statistical significance of differences between groups. P value of < 0.05 was considered to be significant. Each experiment was repeated at least four times.

D. Supplementary References

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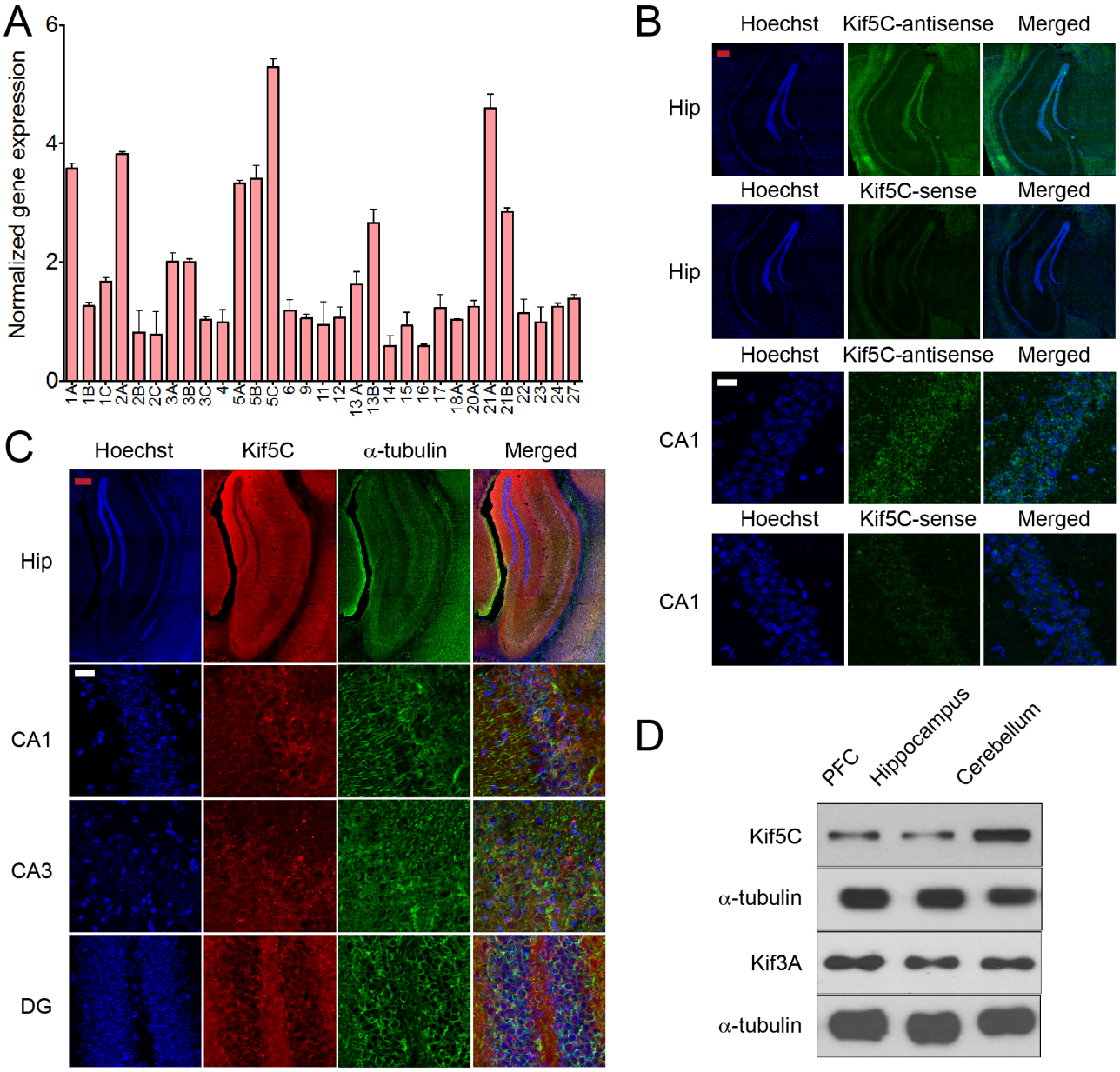
4. Pfaffl MW, Horgan GW, Dempfle L (2002) Relative expression software tool (REST) for group-wise comparison and statistical analysis of relative expression results in real-time PCR. Nucleic Acids Res 30(9):e36.



E

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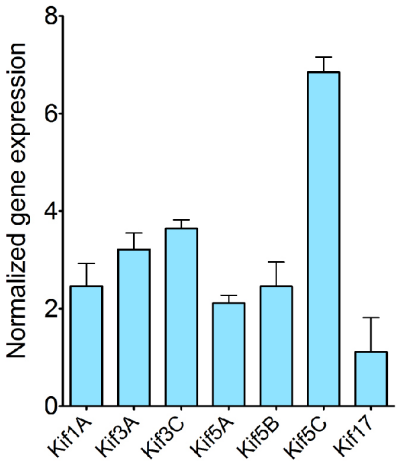

Figure S1

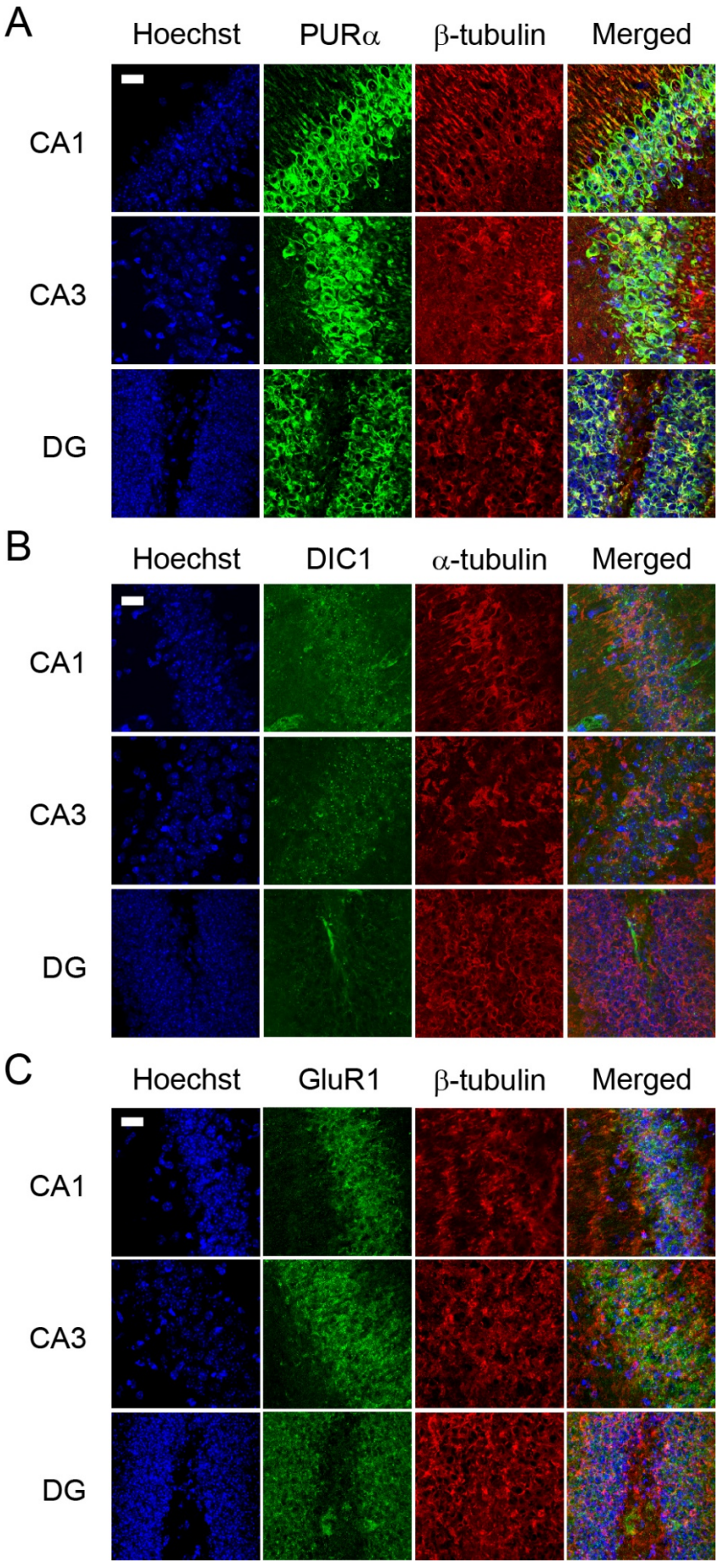
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Figure S2

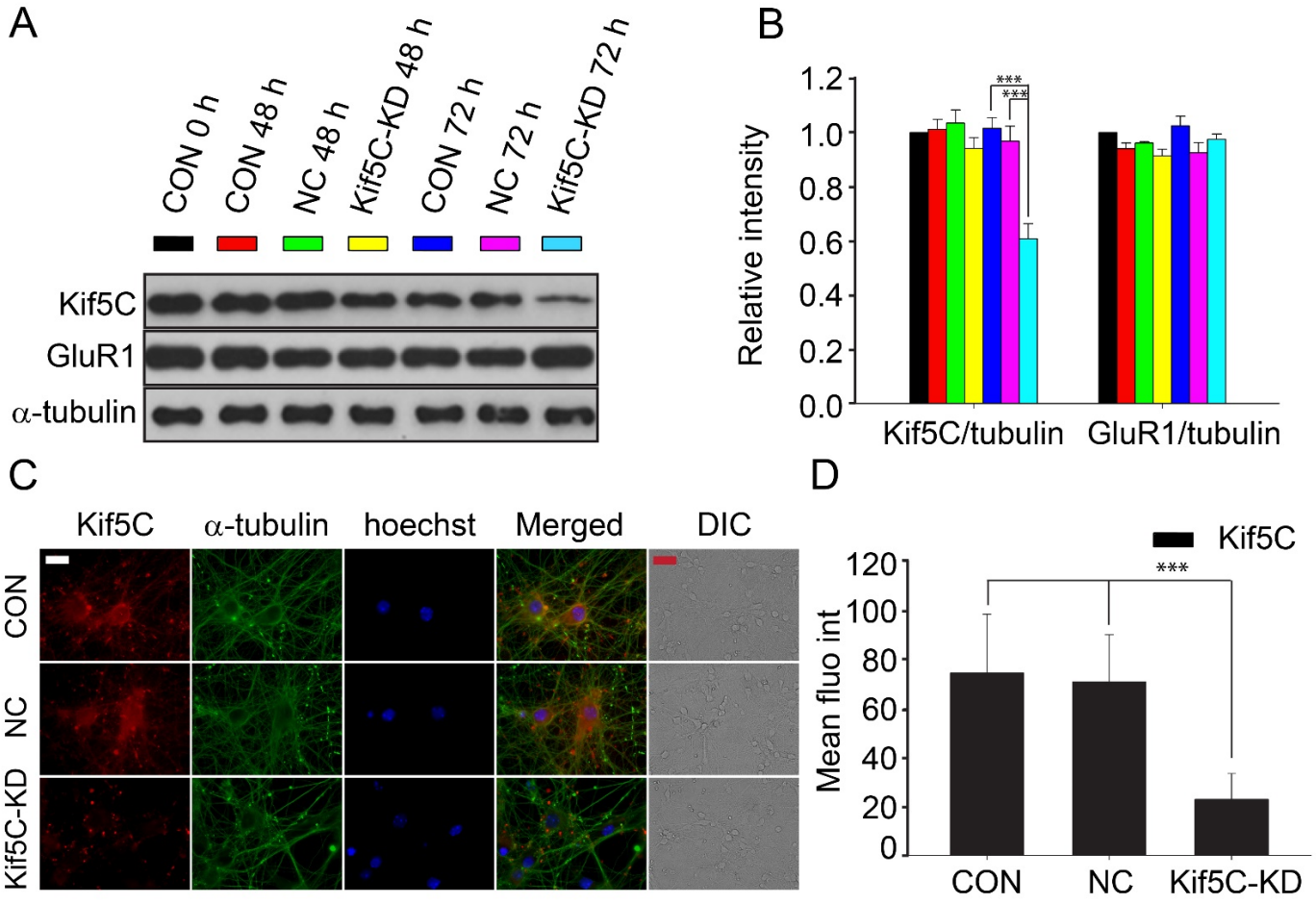
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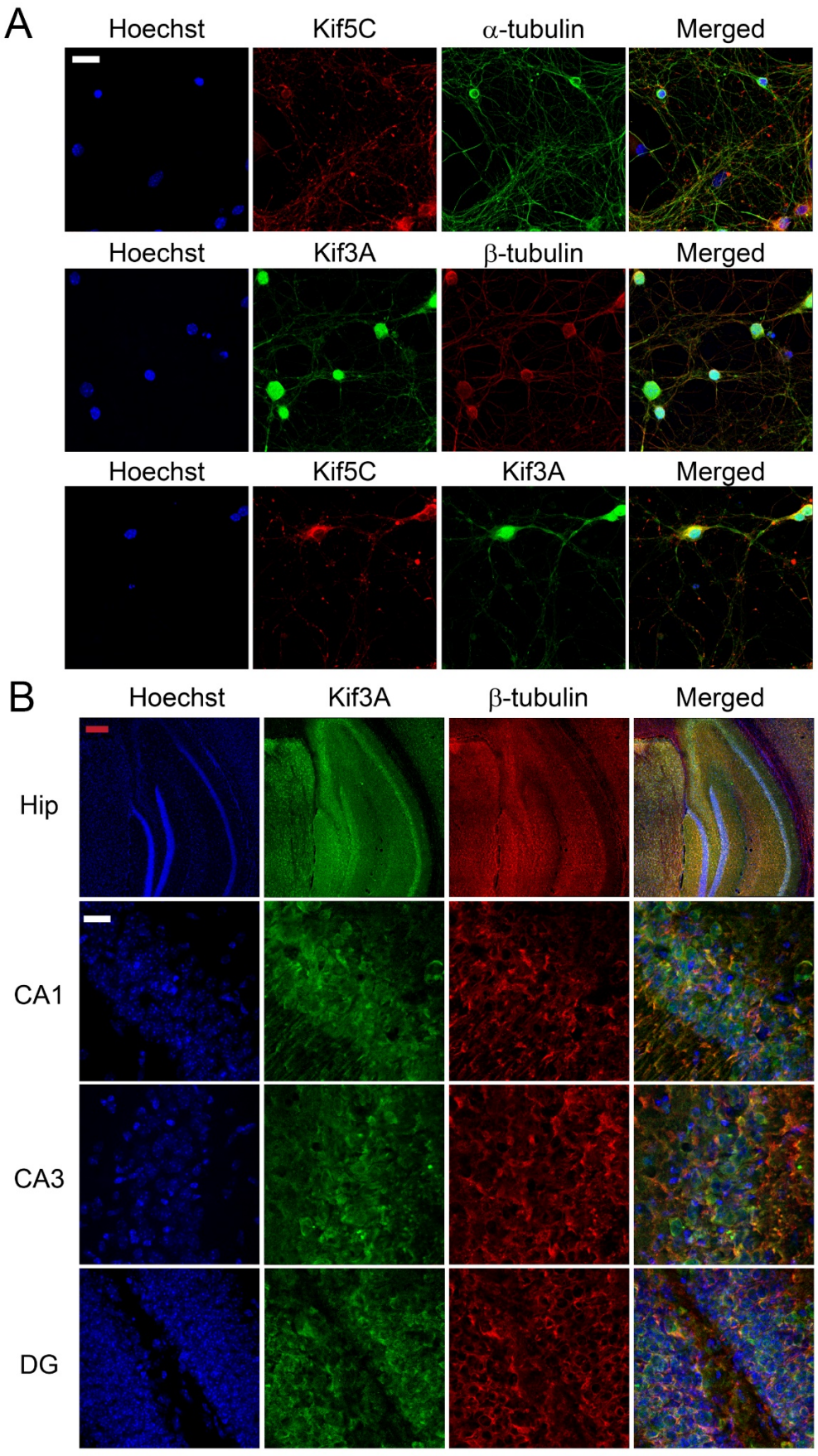
Figure S3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β-tubulin
Merged
Hip
CA1
CA3
DG

Figure S4

A

B

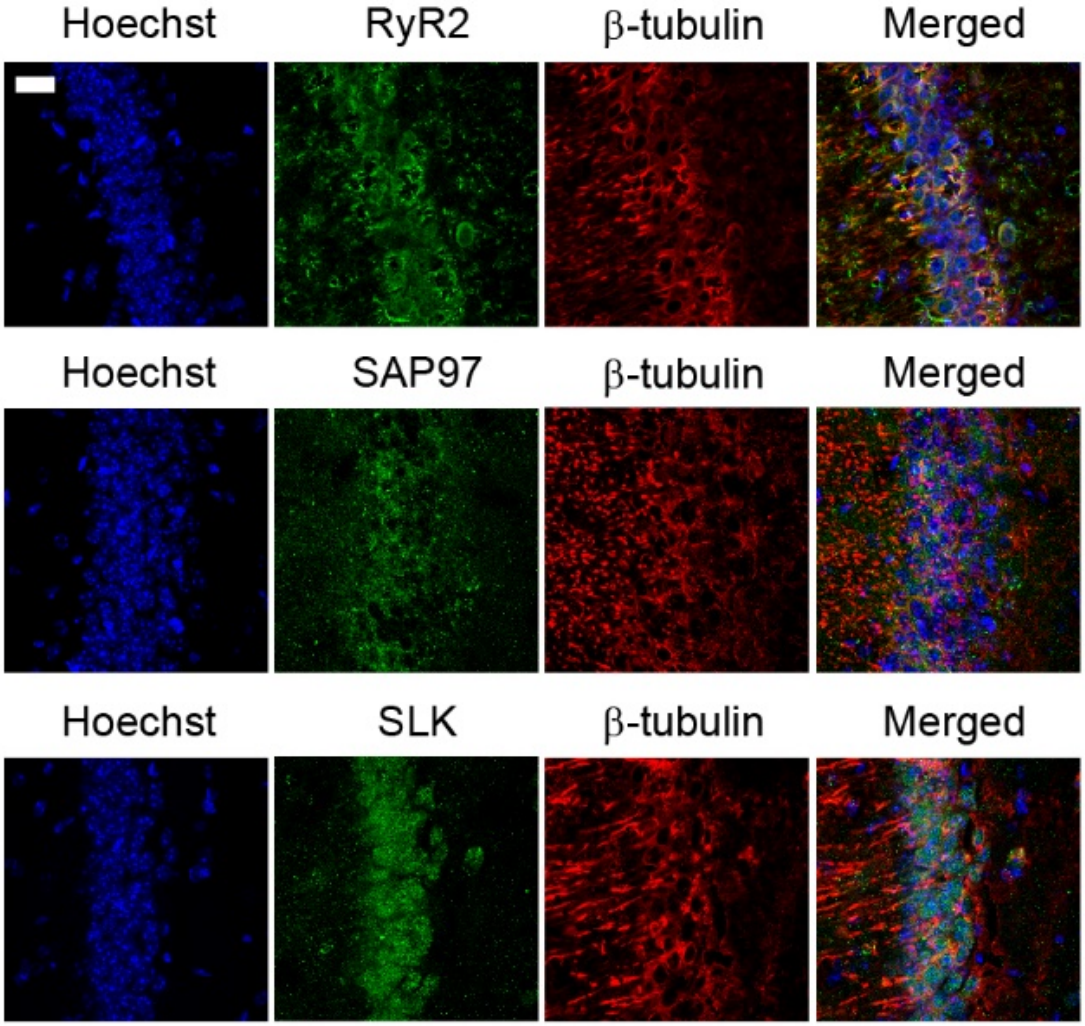
Hoechst
RyR2
β-tubulin
Merged
Hoechst
SAP97
β-tubulin
Merged
Hoechst
SLK
β-tubulin
Merged

Figure S5

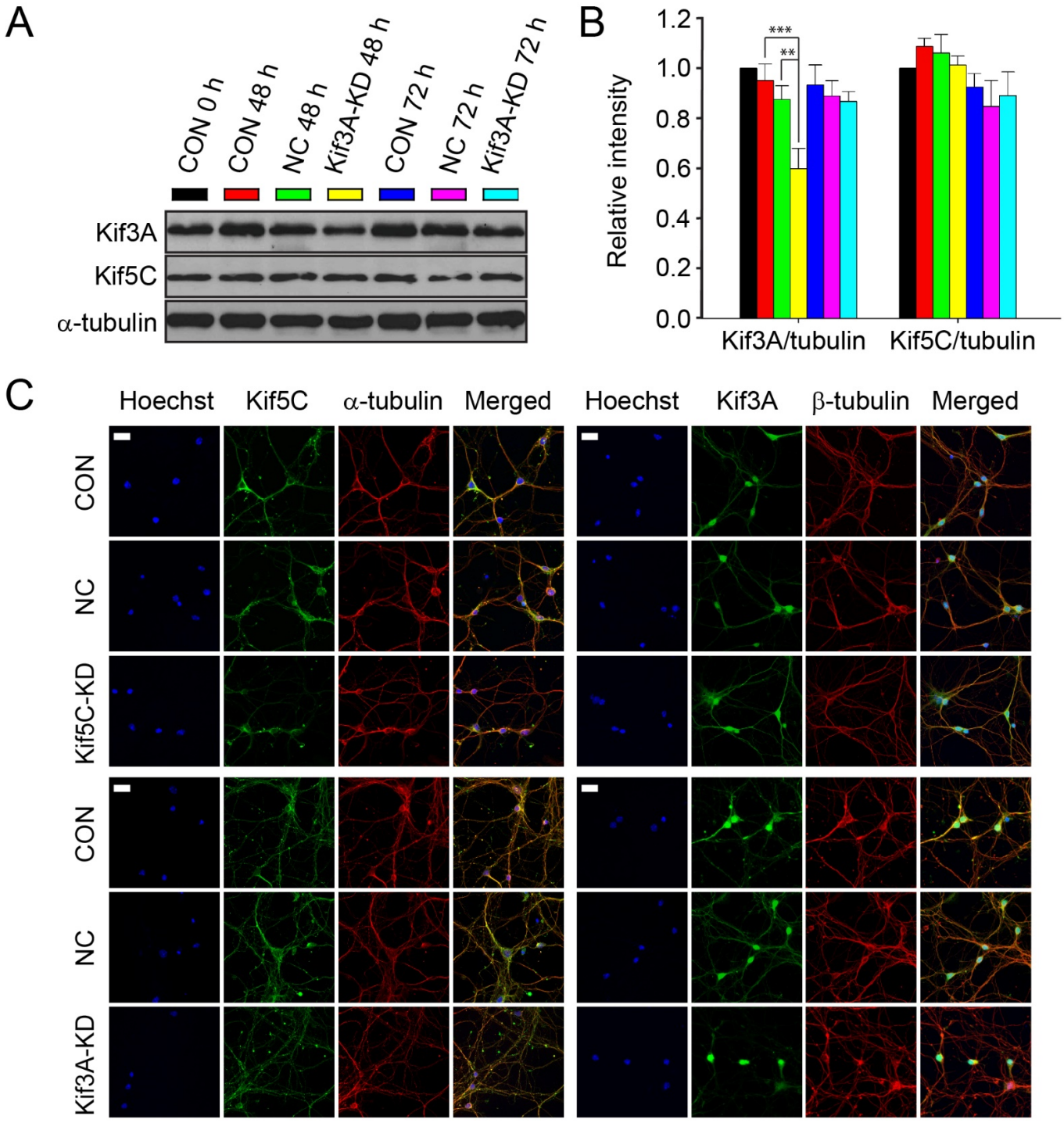
A
CON 0 h
CON 48 h
NC 48 h
Kif3A-KD 48 h
CON 72 h
NC 72 h
Kif3A-KD 72 h
Kif3A
Kif5C
α-tubulin
B
Relative intensity
1.2
1.0
0.8
0.6
0.4
0.2
0.0
***
**
Kif3A/tubulin
Kif5C/tubulin
C
Hoechst
Kif5C
α-tubulin
Merged
Hoechst
Kif3A
β-tubulin
Merged
CON
NC
Kif5C-KD
CON
NC
Kif3A-KD

Figure S6

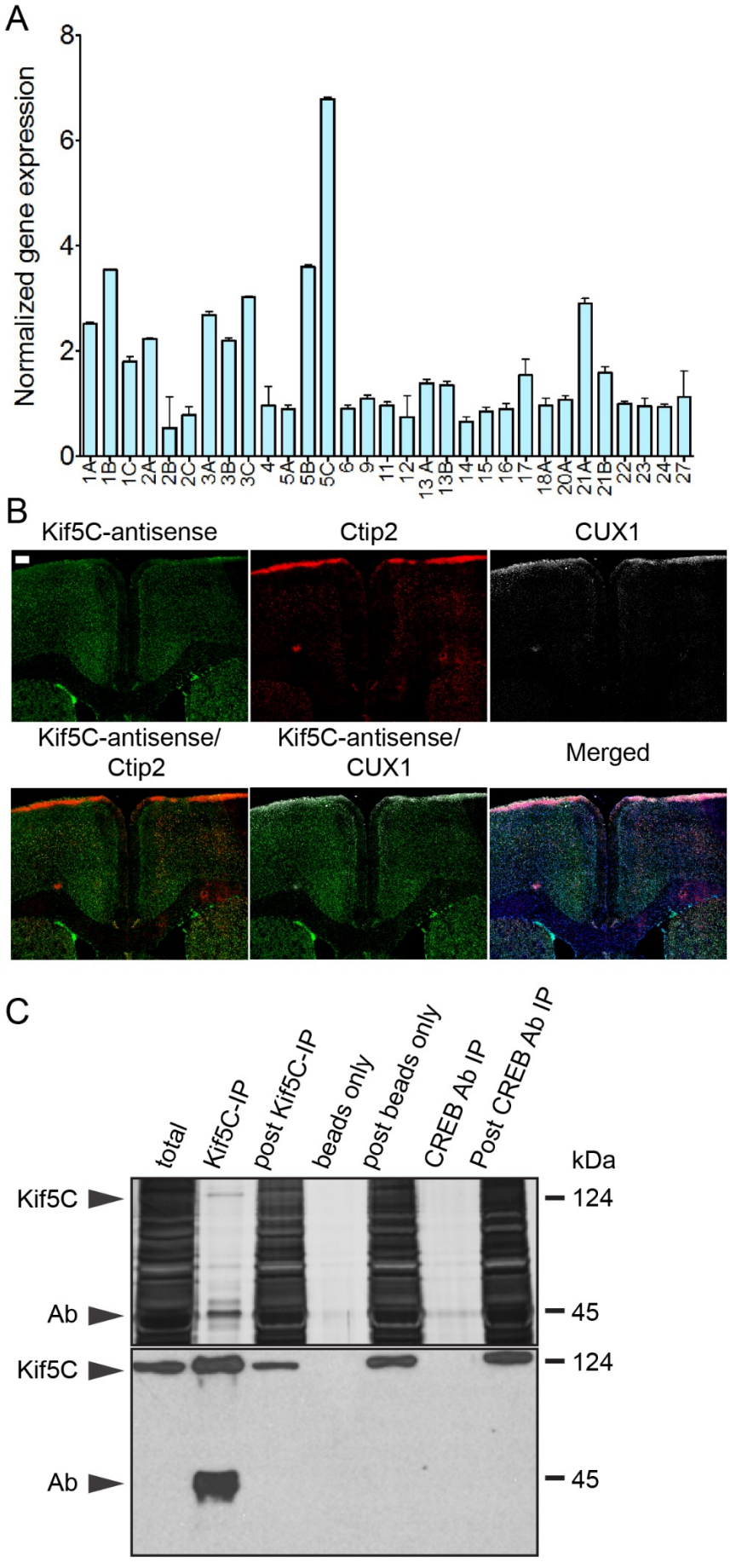
A
Normalized gene expression
0
2
4
6
8
1A
1B
1C
2A
2B
2C
3A
3B
3C
4
5A
5B
5C
6
9
11
12
13 A
13B
14
15
16
17
18A
20A
21A
21B
22
23
24
27
B
Kif5C-antisense
Ctip2
CUX1
Kif5C-antisense/
Ctip2
Kif5C-antisense/
CUX1
Merged
C
total
Kif5C-IP
post Kif5C-IP
beads only
post beads only
CREB Ab IP
Post CREB Ab IP
kDa
Kif5C
Ab
Kif5C
Ab
124
45
124
45

Figure S7

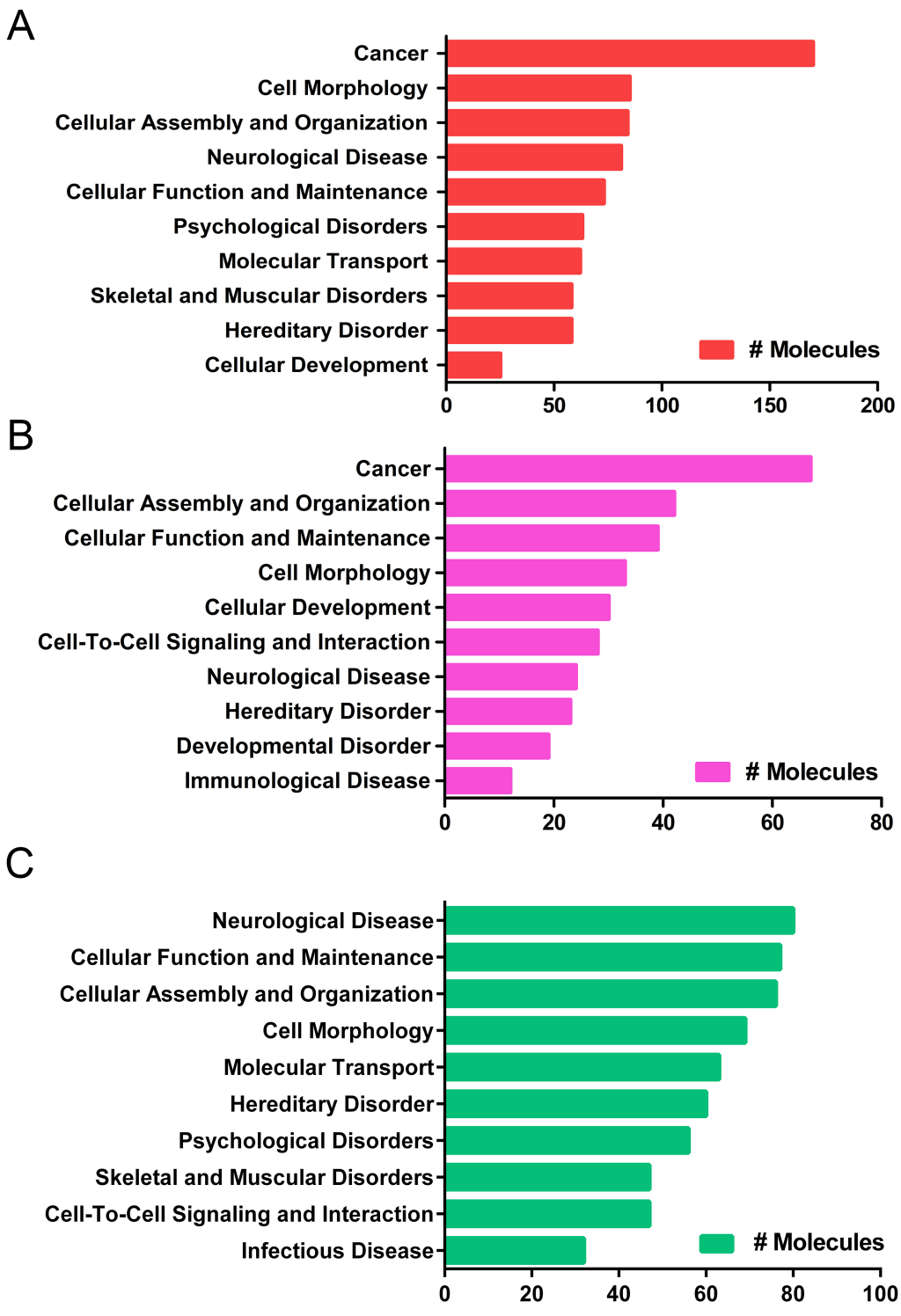
A
Cancer
Cell Morphology
Cellular Assembly and Organization
Neurological Disease
Cellular Function and Maintenance
Psychological Disorders
Molecular Transport
Skeletal and Muscular Disorders
Hereditary Disorder
Cellular Development
Molecules
0
50
100
150
200
B
Cancer
Cellular Assembly and Organization
Cellular Function and Maintenance
Cell Morphology
Cellular Development
Cell-To-Cell Signaling and Interaction
Neurological Disease
Hereditary Disorder
Developmental Disorder
Immunological Disease
Molecules
0
20
40
60
80
C
Neurological Disease
Cellular Function and Maintenance
Cellular Assembly and Organization
Cell Morphology
Molecular Transport
Hereditary Disorder
Psychological Disorders
Skeletal and Muscular Disorders
Cell-To-Cell Signaling and Interaction
Infectious Disease
Molecules
0
20
40
60
80
100

Figure S8

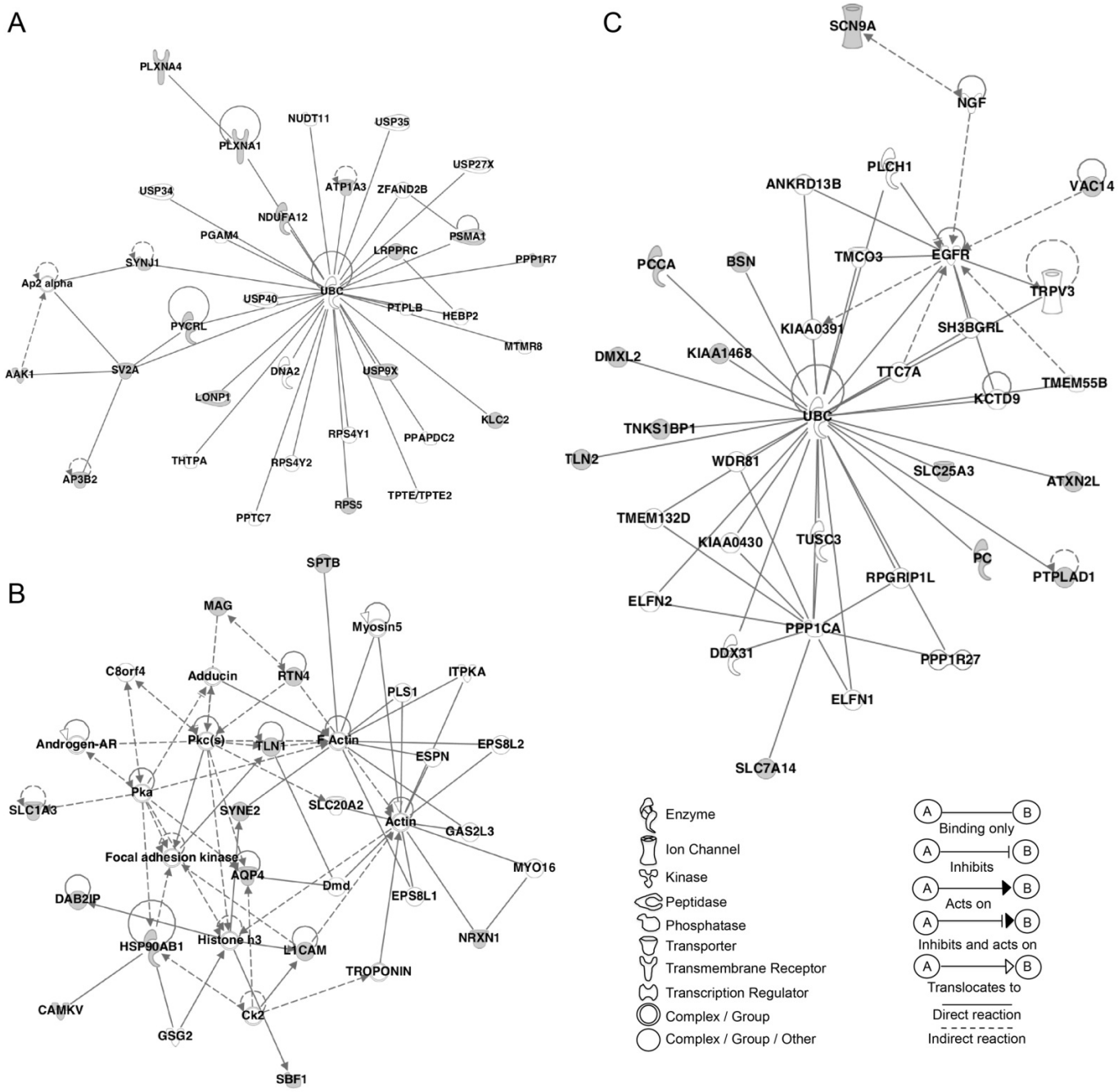
A
PLXNA4
PLXNA1
NUDT11
USP35
USP27X
ATP1A3
ZFAND2B
USP34
NDUFA12
PGAM4
PSMA1
LRPPRC
SYNJ1
PPP1R7
Ap2 alpha
UBC
USP40
PTPLB
HEBP2
PYCRL
MTMR8
AAK1
SV2A
DNA2
USP9X
LONP1
KLC2
RPS4Y1
PPAPDC2
THTPA
RPS4Y2
AP3B2
TPTE/TPTE2
RPS5
PPTC7
B
SPTB
MAG
Myosin5
C8orf4
Adducin
RTN4
ITPKA
PLS1
Androgen-AR
Pkc(s)
TLN1
F-Actin
EPS8L2
ESPN
Pka
SLC1A3
SYNE2
SLC20A2
Actin
GAS2L3
Focal adhesion kinase
MYO16
AQP4
Dmd
DAB2IP
EPS8L1
HSP90AB1
Histone h3
L1CAM
NRXN1
TROPONIN
CAMKV
Ck2
GSG2
SBF1
C
SCN9A
NGF
PLCH1
ANKRD13B
VAC14
PCCA
BSN
TMCO3
EGFR
TRPV3
KIAA0391
SH3BGRL
DMXL2
KIAA1468
TTC7A
TMEM55B
KCTD9
UBC
TNKS1BP1
TLN2
WDR81
SLC25A3
ATXN2L
TMEM132D
KIAA0430
TUSC3
PC
PTPLAD1
RPGRIP1L
ELFN2
PPP1CA
DDX31
PPP1R27
ELFN1
SLC7A14
Enzyme
Ion Channel
Kinase
Peptidase
Phosphatase
Transporter
Transmembrane Receptor
Transcription Regulator
Complex / Group
Complex / Group / Other
A
B
Binding only
Inhibits
Acts on
Inhibits and acts on
Translocates to
Direct reaction
Indirect reaction

Figure S9

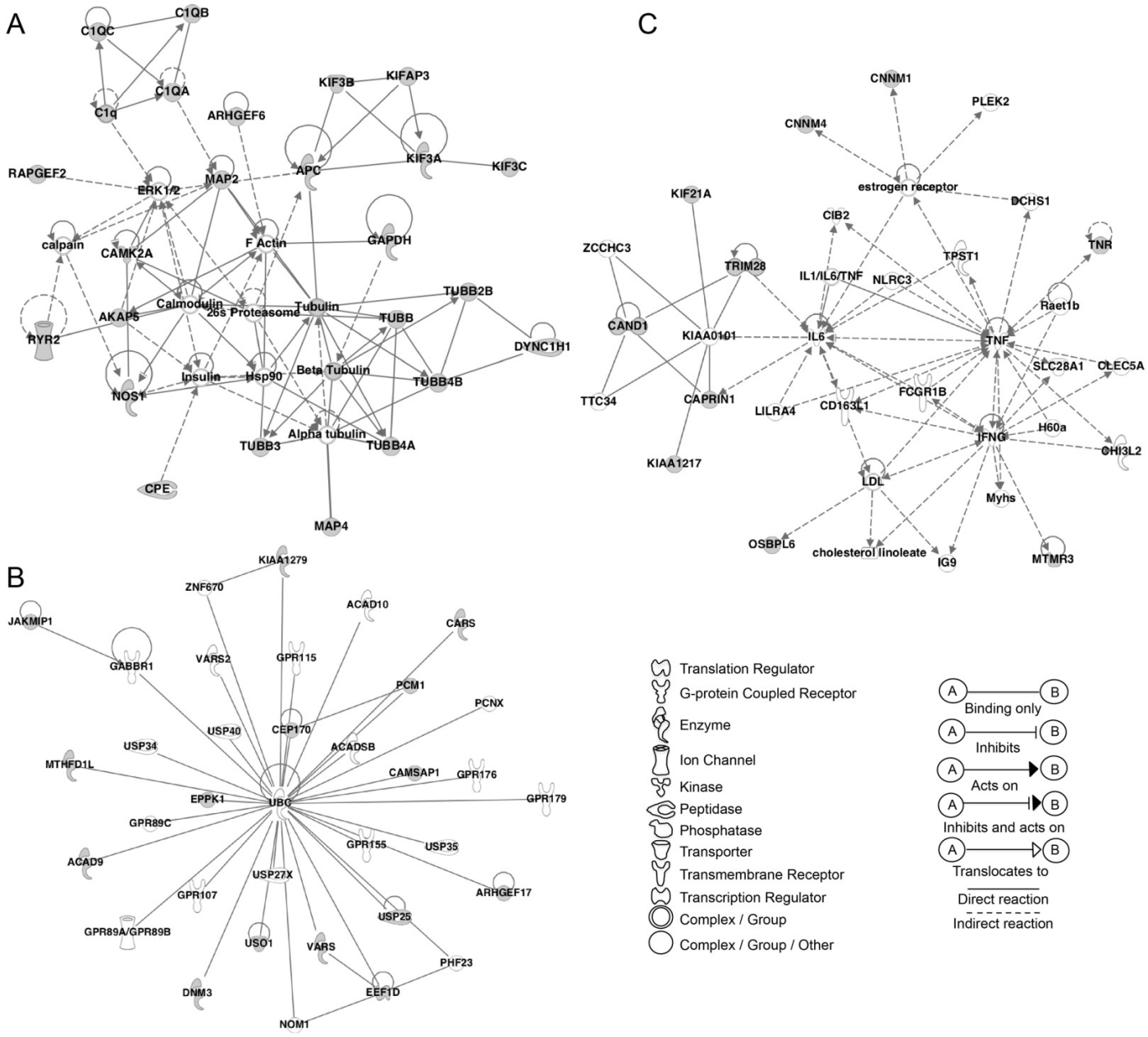
A
C1QB
C1QC
C1QA
C1q
KIF3B
KIFAP3
ARHGEF6
KIF3A
KIF3C
RAPGEF2
ERK1/2
MAP2
APC
calpain
CAMK2A
F Actin
GAPDH
TUBB2B
Calmodulin
26S Proteasome
Tubulin
TUBB
AKAP5
RYR2
DYNC1H1
Insulin
Hsp90
Beta Tubulin
TUBB4B
NOS1
Alpha tubulin
TUBB3
TUBB4A
CPE
MAP4
B
KIAA1279
ZNF670
ACAD10
CARS
JAKMIP1
GABBR1
VARS2
GPR115
PCM1
PCNX
USP40
CEP170
USP34
ACADSB
MTHFD1L
CAMSAP1
GPR176
EPPK1
UBC
GPR179
GPR89C
GPR155
USP35
ACAD9
USP27X
GPR107
ARHGEF17
USP25
GPR89A/GPR89B
USO1
VARS
PHF23
DNM3
EEF1D
NOM1
C
CNNM1
PLEK2
CNNM4
estrogen receptor
DCHS1
KIF21A
CIB2
TNR
ZCCHC3
TRIM28
TPST1
IL1/IL6/TNF
NLRC3
Raet1b
CAND1
KIAA0101
IL6
TNF
SLC28A1
CLEC5A
TTC34
CAPRIN1
FCGR1B
LILRA4
CD163L1
H60a
IFNG
CHI3L2
KIAA1217
LDL
Myhs
OSBPL6
cholesterol linoleate
IG9
MTMR3
Translation Regulator
G-protein Coupled Receptor
Enzyme
Ion Channel
Kinase
Peptidase
Phosphatase
Transporter
Transmembrane Receptor
Transcription Regulator
Complex / Group
Complex / Group / Other
A
B
Binding only
Inhibits
Acts on
Inhibits and acts on
Translocates to
Direct reaction
Indirect reaction

Figure S10

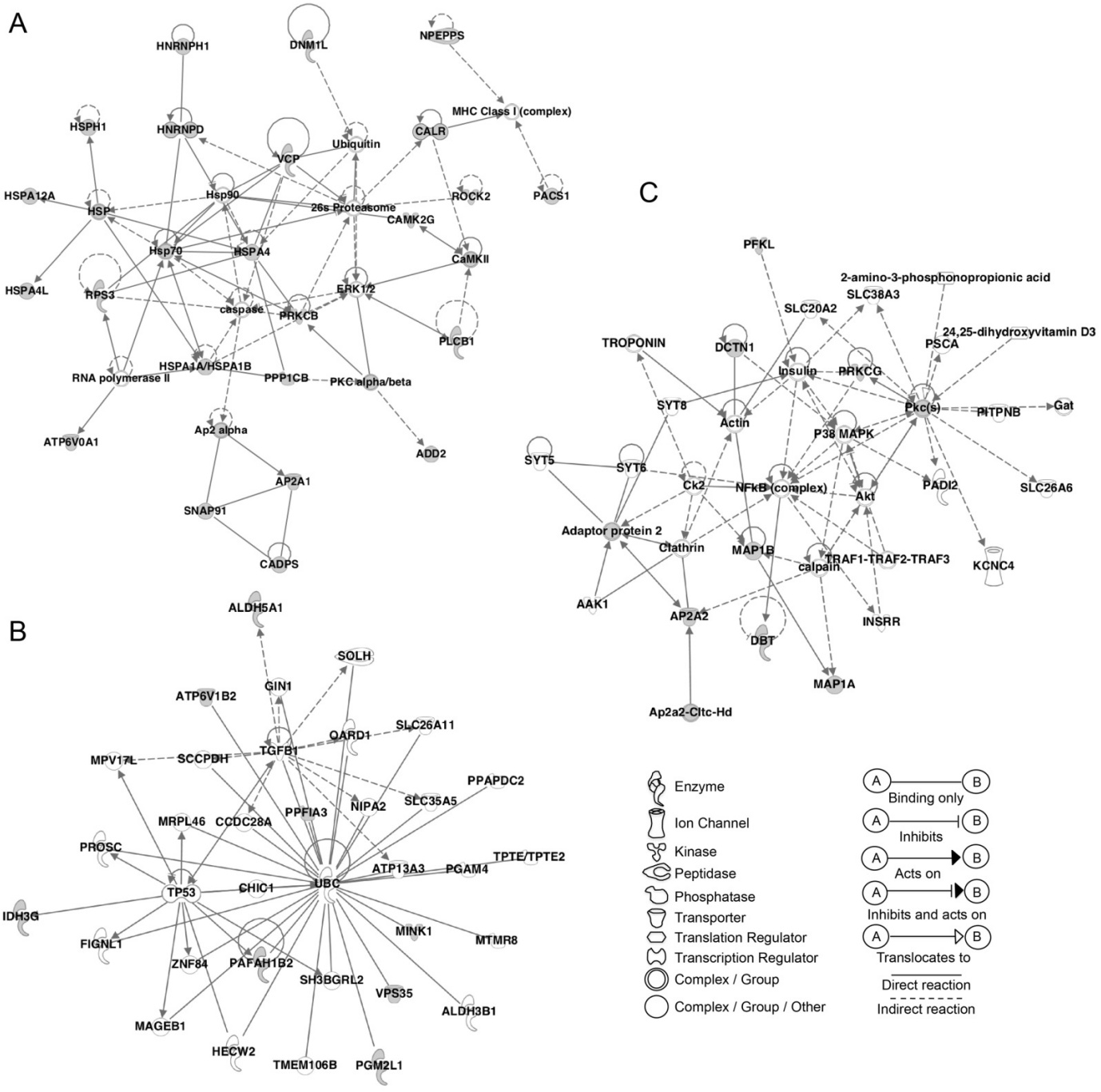
A
HNRNPH1
DNM1L
NPEPPS
MHC Class I (complex)
HSPH1
HNRNPD
CALR
Ubiquitin
VCP
HSPA12A
Hsp90
ROCK2
PACS1
HSP
26s Proteasome
CAMK2G
Hsp70
HSPA4
CaMKII
HSPA4L
RPS3
ERK1/2
caspase
PRKCB
PLCB1
RNA polymerase II
HSPA1A/HSPA1B
PPP1CB
PKC alpha/beta
ATP6V0A1
Ap2 alpha
ADD2
AP2A1
SNAP91
CADPS
B
ALDH5A1
SOLH
GIN1
ATP6V1B2
SLC26A11
QARD1
MPV17L
SCCPDH
TGFB1
PPAPDC2
NIPA2
SLC35A5
PPFIA3
MRPL46
CCDC28A
PROSC
TPTE/TPTE2
ATP13A3
PGAM4
TP53
CHIC1
UBC
IDH3G
MINK1
MTMR8
FIGNL1
ZNF84
PAFAH1B2
SH3BGRL2
VPS35
ALDH3B1
MAGEB1
HECW2
TMEM106B
PGM2L1
C
PFKL
2-amino-3-phosphonopropionic acid
SLC38A3
SLC20A2
24,25-dihydroxyvitamin D3
PSCA
TROPONIN
DCTN1
Insulin
PRKCG
SYT8
Actin
Pkc(s)
PITPNB
Gat
P38 MAPK
SYT5
SYT6
Ck2
NFkB (complex)
Akt
PADI2
SLC26A6
Adaptor protein 2
Clathrin
MAP1B
TRAF1-TRAF2-TRAF3
calpain
KCNC4
AAK1
AP2A2
INSRR
DBT
MAP1A
Ap2a2-Cltc-Hd
Enzyme
Ion Channel
Kinase
Peptidase
Phosphatase
Transporter
Translation Regulator
Transcription Regulator
Complex / Group
Complex / Group / Other
A
B
Binding only
Inhibits
Acts on
Inhibits and acts on
Translocates to
Direct reaction
Indirect reaction

Figure S11

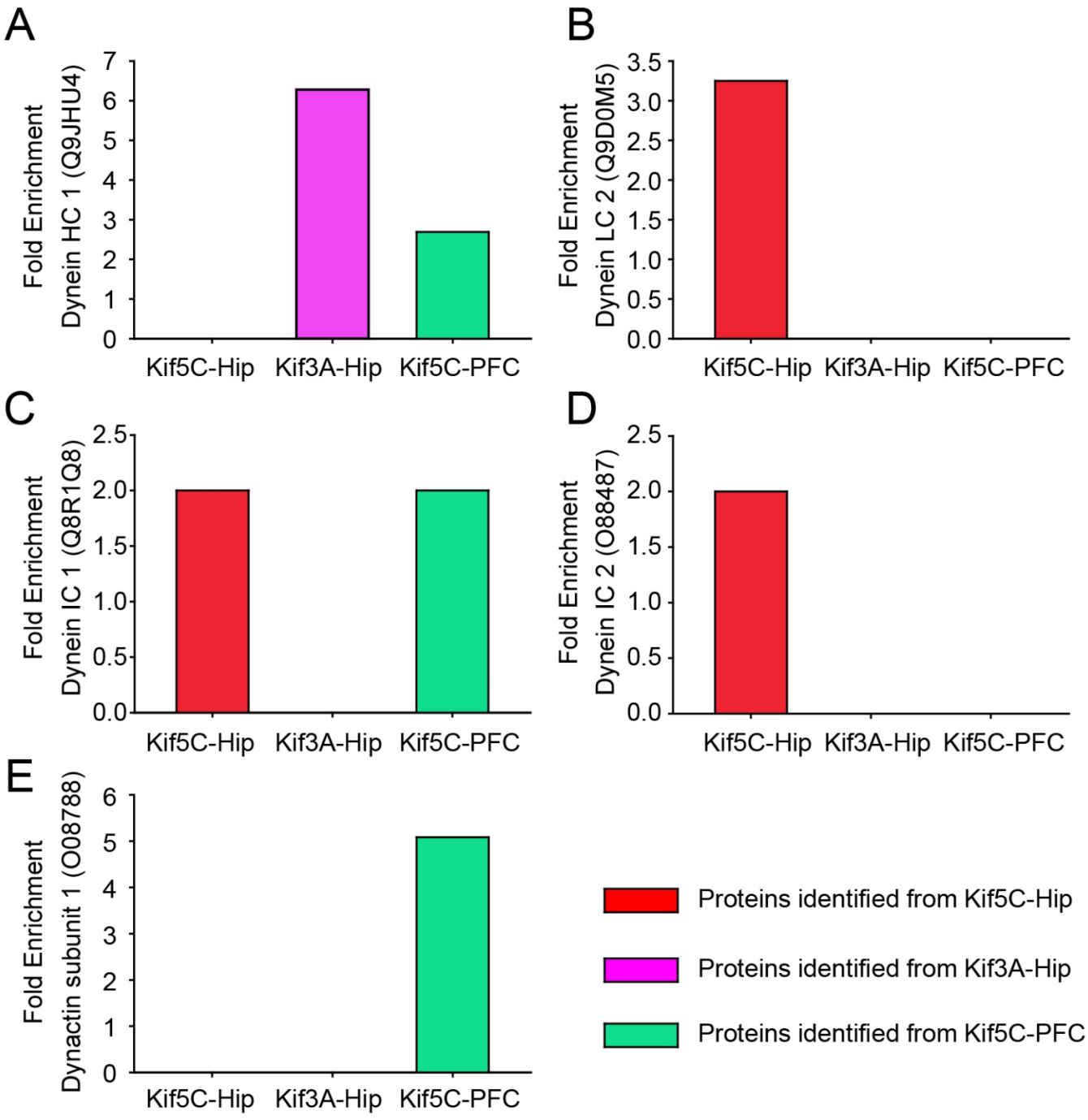
A
Fold Enrichment
Dynein HC 1 (Q9JHU4)
7
6
5
4
3
2
1
0
Kif5C-Hip
Kif3A-Hip
Kif5C-PFC
B
Fold Enrichment
Dynein LC 2 (Q9D0M5)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Kif5C-Hip
Kif3A-Hip
Kif5C-PFC
C
Fold Enrichment
Dynein IC 1 (Q8R1Q8)
2.5
2.0
1.5
1.0
0.5
0.0
Kif5C-Hip
Kif3A-Hip
Kif5C-PFC
D
Fold Enrichment
Dynein IC 2 (O88487)
2.5
2.0
1.5
1.0
0.5
0.0
Kif5C-Hip
Kif3A-Hip
Kif5C-PFC
E
Fold Enrichment
Dynactin subunit 1 (O08788)
6
5
4
3
2
1
0
Kif5C-Hip
Kif3A-Hip
Kif5C-PFC
Proteins identified from Kif5C-Hip
Proteins identified from Kif3A-Hip
Proteins identified from Kif5C-PFC

Figure S12