Supplementary Information

The neuropathy-linked protein TECPR2 is a Rab5 effector that regulates cargo recycling from early endosomes

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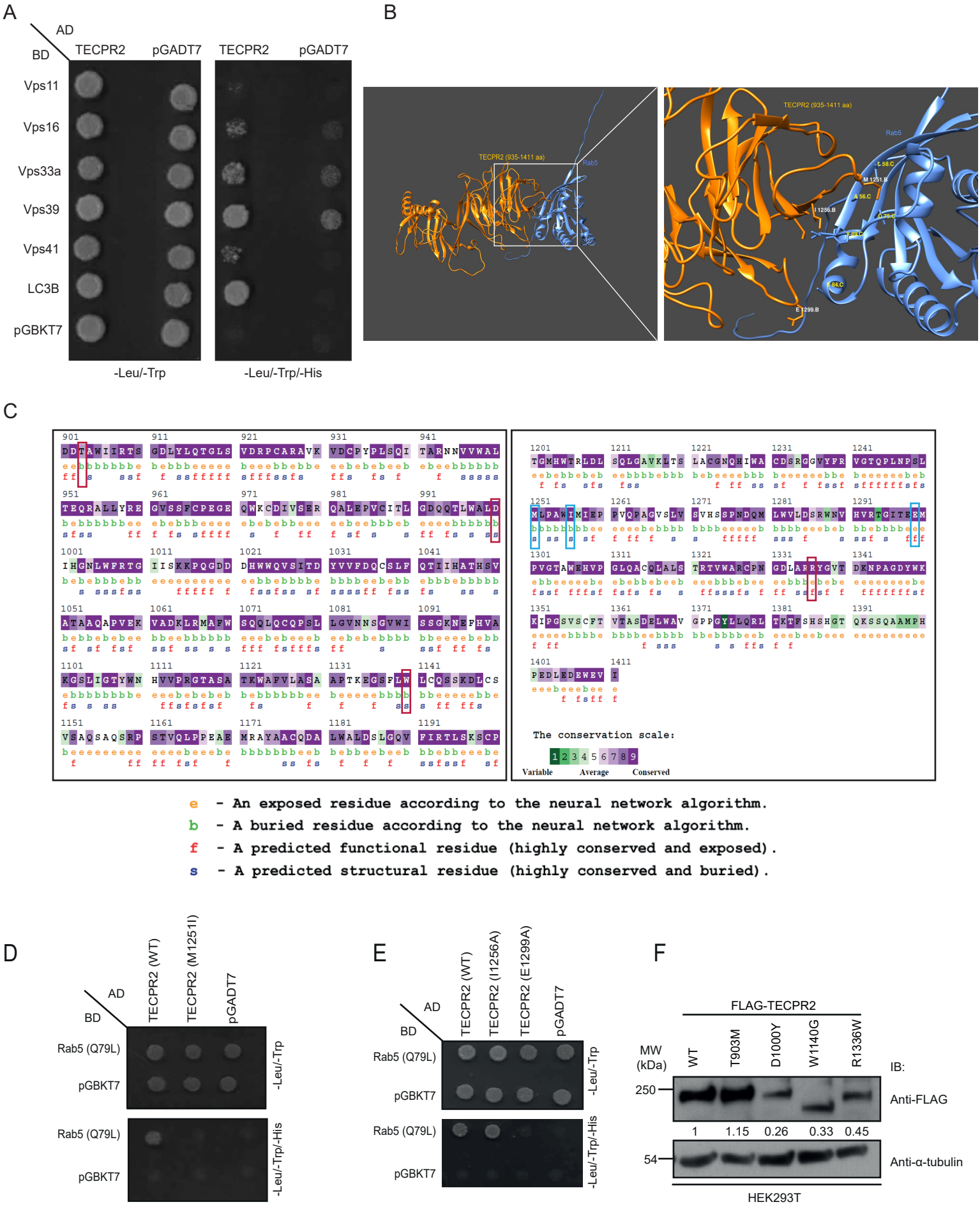
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Running Title: Rab5 effector TECPR2 controls cargo recycling

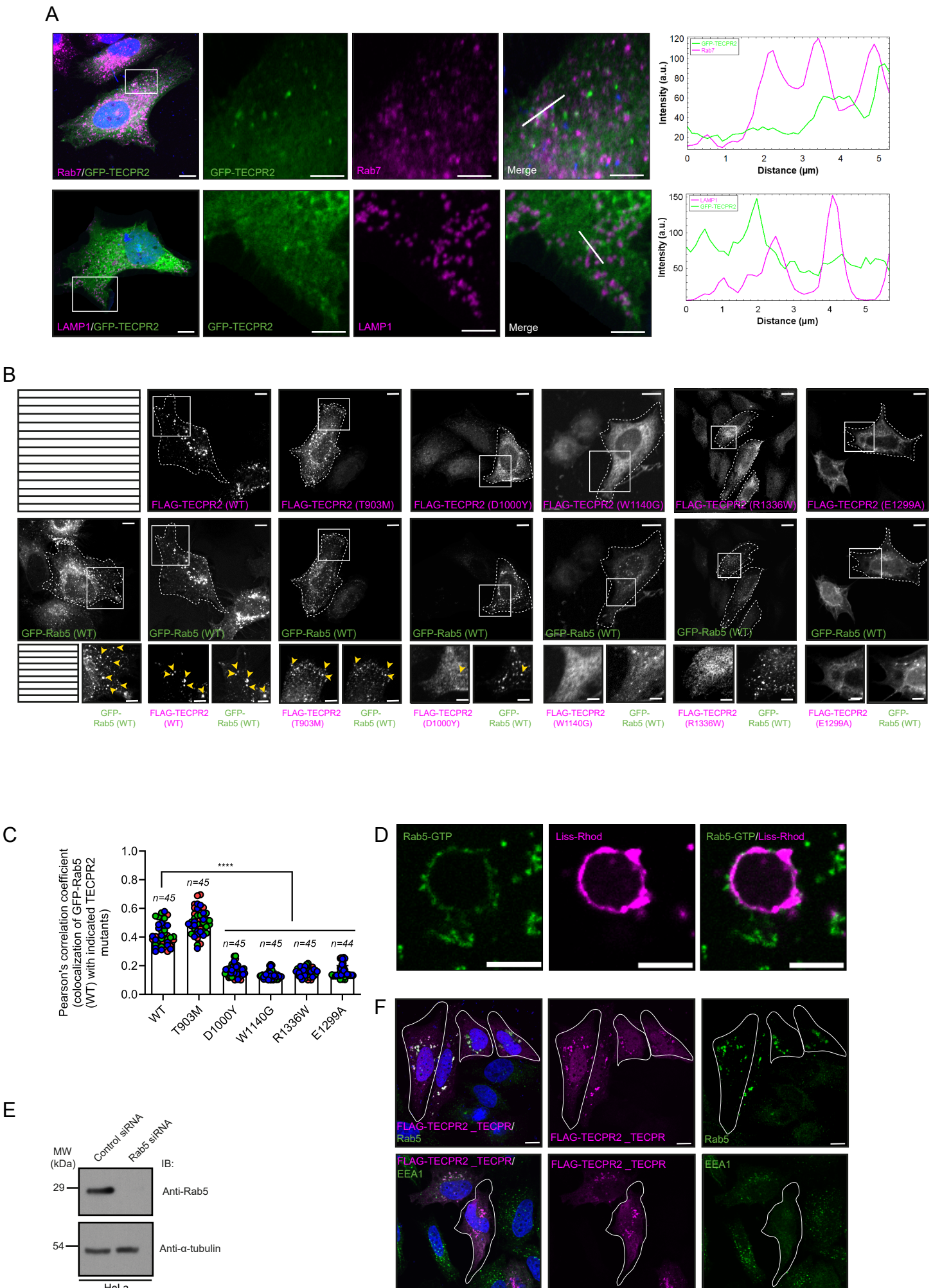
Keywords: TECPR2, Rab5, Actin, Early endosome, Endocytic recycling, Integrin, Trafficking

Supplementary Figure S1



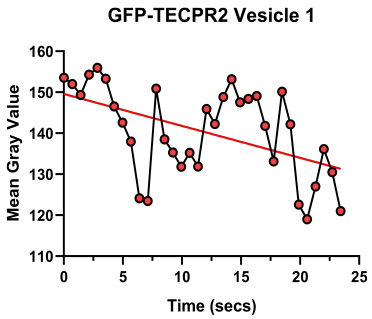
Supplementary Figure S1: TECPR2 interaction analysis with its known binding partners (HOPS complex and LC3B) and identification of residues required for binding to Rab5. **A)** Yeast two-hybrid assay of TECPR2 with HOPS complex subunits and LC3B. Co-transformants were spotted on -Leu/-Trp and -Leu/-Trp/-His media to confirm viability and detect interactions, respectively. **B)** Intramolecular interactions between Rab5 and the C-terminal region of TECPR2 from 935 to 1411 a.a. were modeled using the AlphaFold2-ColabFold tool and visualized using Chimera software. The chain B in orange denotes the TECPR2_TECPR fragment (935-1411 a.a.), and chain C in blue indicates Rab5. **C)** The Consurf tool (https://consurf.tau.ac.il/consurf_index.php) was used to analyze conserved residues in the 901-1411 a.a. fragment of human TECPR2. The amino acid residues highlighted in the red boxes represent a subset of HSAN9-associated variants, and the blue boxes indicate residues identified from the AlphaFold2 analysis described in (**B**). **D-E)** Yeast two-hybrid assay of TECPR2 (WT) and its putative Rab5-binding defective mutants (M1251I, I1256A, and E1299A) with Rab5 (Q79L). Co-transformants were spotted on -Leu/-Trp and -Leu/-Trp/-His to confirm viability and detect interactions, respectively. **F)** Cell lysates of HEK293T cells transfected with the indicated FLAG-tagged TECPR2 (WT) and mutants, and immunoblotted (IB) with anti-FLAG and anti-α-tubulin antibodies. The values represent the densitometric analysis of the FLAG-TECPR2 band intensity normalized to the α-tubulin band intensity. Source data are provided as a Source Data file.

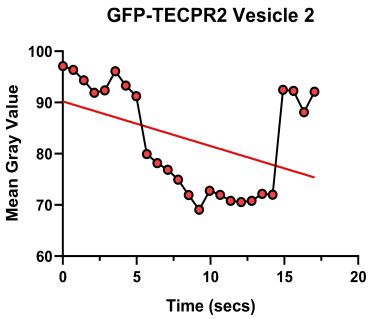
Supplementary Figure S2

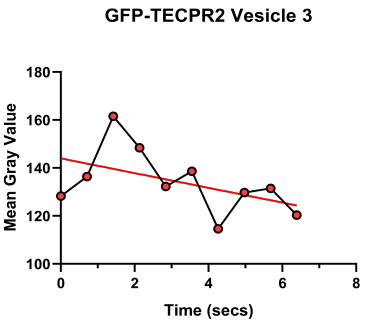


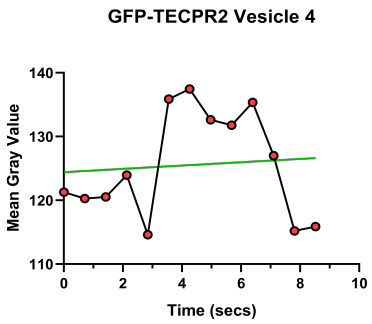
Supplementary Figure S2: TECRP2 does not localize to late endosomes and lysosomes, and HSAN9-associated TECPR2 variants, except T903M, are defective in Rab5-dependent membrane recruitment. A) Representative confocal micrographs of HeLa cells expressing GFP-TECPR2 and immunostained with anti-Rab7 or anti-LAMP1 antibodies. The line-scan analysis shows the fluorescence intensity of both channels along the white line as shown in the merge image. Scale bars: 10 µm (main); 5 µm (inset). **B)** Representative confocal micrographs of HeLa cells transfected with GFP-Rab5 (WT) alone or co-transfected with FLAG-TECPR2 (WT) and the HSAN9-associated variants. Cells were fixed and immunostained using anti-FLAG antibodies. In the micrographs, transfected cells are highlighted with a white dotted line, and the arrowheads in the insets denote the colocalized puncta in both channels. Scale bars: 10 µm (main); 5 µm (inset). **C)** Quantification of the Pearson's colocalization coefficient for GFP-Rab5 (WT) with FLAG-TECPR2 (WT) or the indicated mutant is shown. *n* denotes the total number of cells analyzed, and the error bar represents the mean ± SD from three independent experiments**.** Experiments are color-coded, and each dot represents the individual data points ($^{****}p<0.0001$; unpaired two-tailed Student's *t*-test). **D)** Representative confocal micrographs of Liss-Rhodamine-labeled GUVs incubated with purified GTP-loaded Rab5 and immunostained with anti-Rab5 antibodies. Scale bar: 10 µm. **E)** Lysates of HeLa cells treated with control siRNA and Rab5 siRNA and immunoblotted (IB) with anti-Rab5 and anti-α-tubulin antibodies. **F)** Representative confocal micrograph of HeLa cells expressing FLAG-TECPR2_TECPR and immunostained for the indicated proteins. In the micrographs, transfected cells are highlighted with a white line. Scale bar: 10 µm. Source data are provided as a Source Data file.

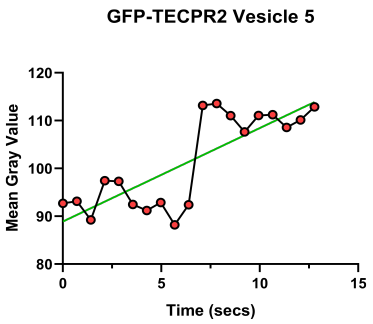
Supplementary Figure S3

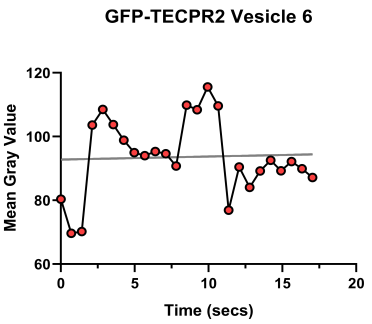


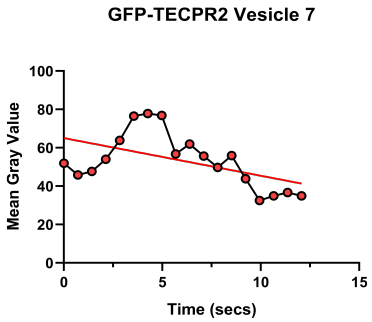


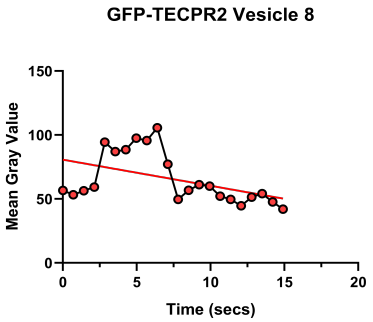


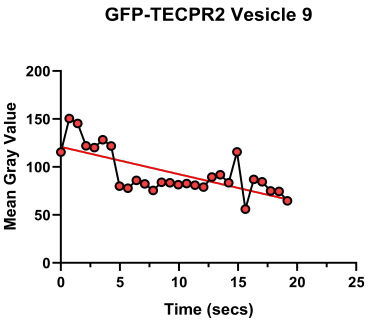


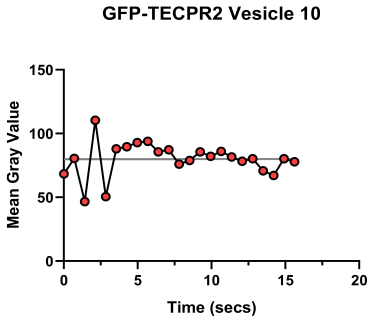


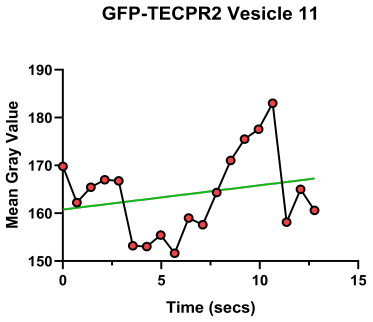


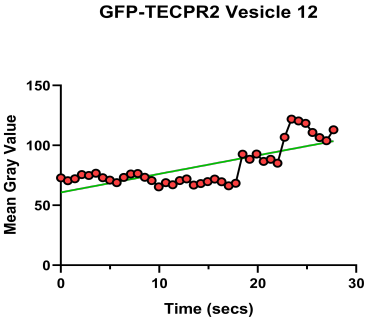


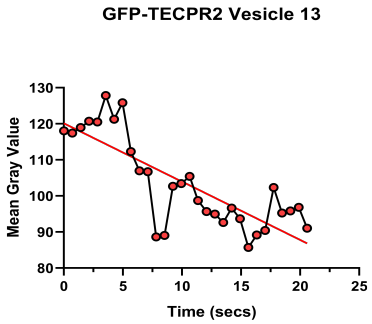


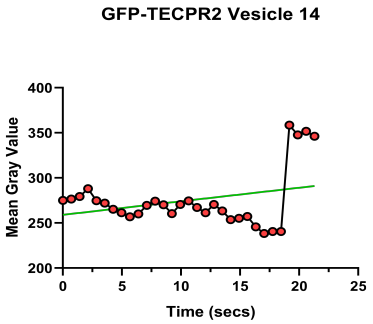


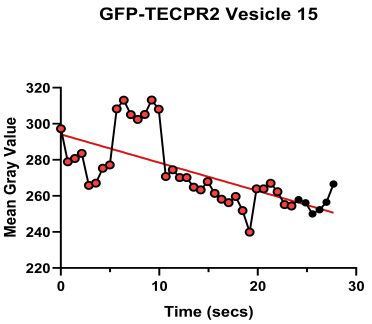






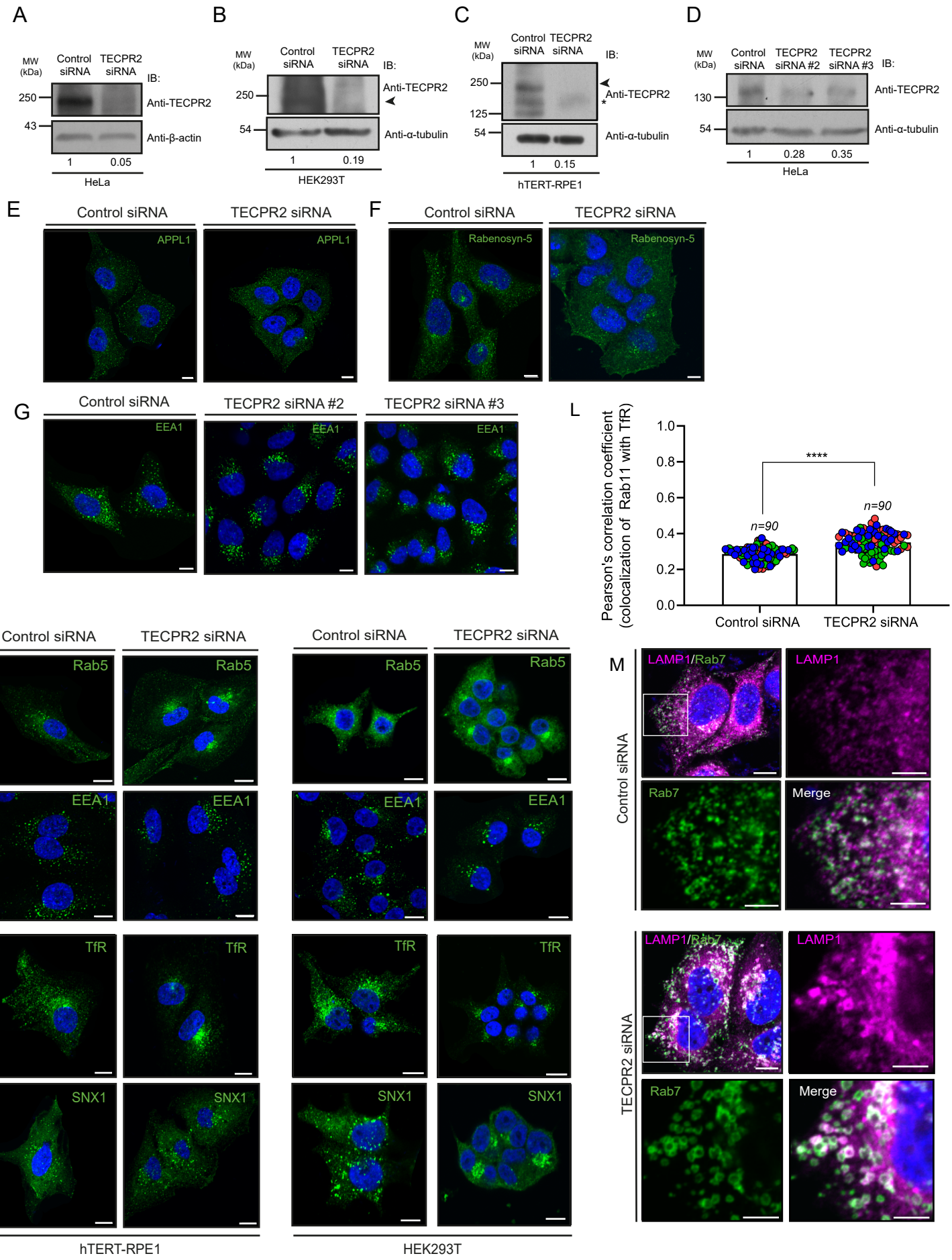






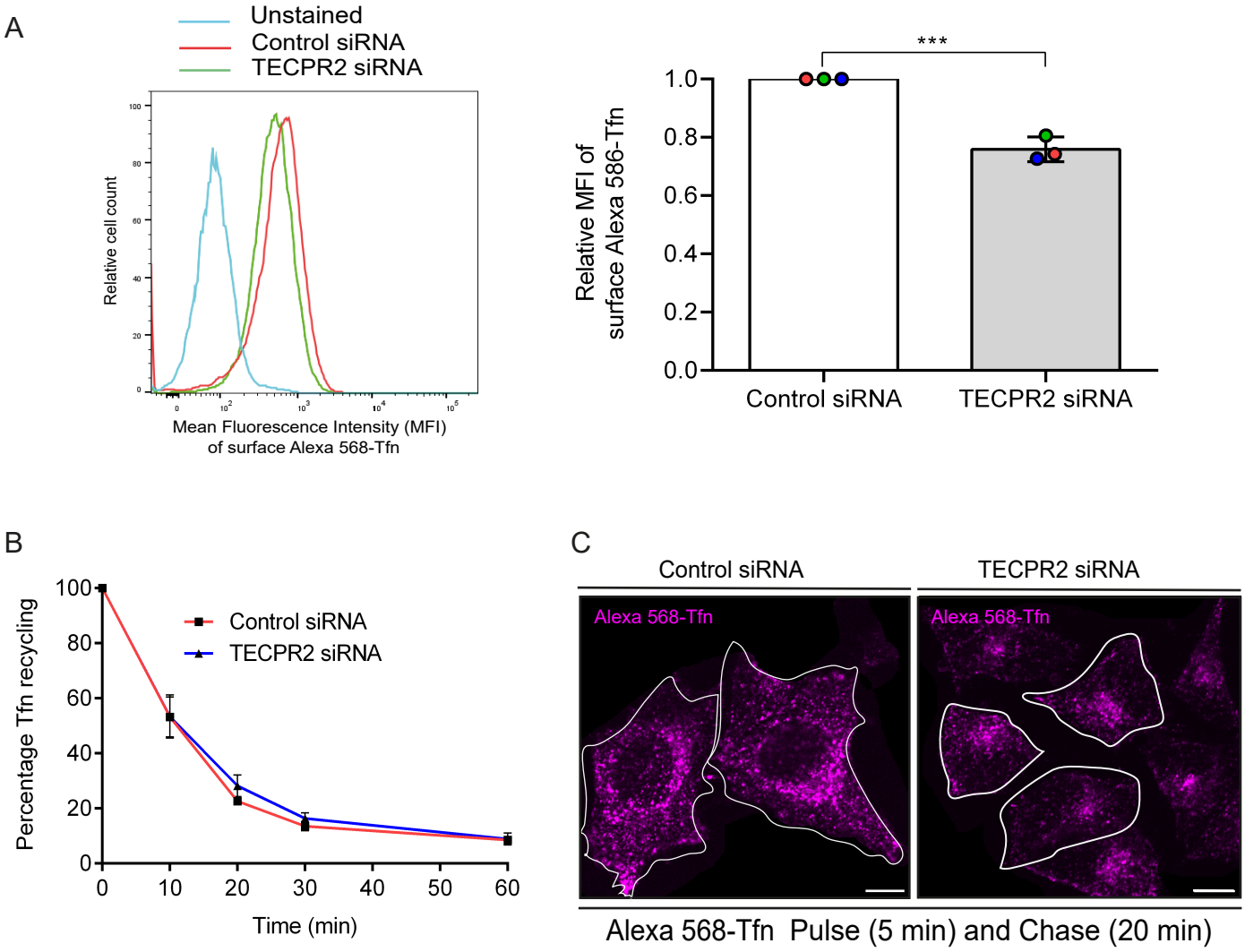
Supplementary Figure S3: GFP-TECPR2 vesicle intensity profile over time showing fission and fusion events. The graph illustrates the fluorescence intensity of individual GFP-TECPR2-positive vesicles over time during fission and fusion processes, as depicted in **Fig. 3A-C** and **Supplementary Movie 3**. Linear regressions are shown for a total of 15 vesicles from three independent experiments. Slightly positive and negative slopes are marked in green and red, respectively, and the stationary events are indicated in grey. Source data of this figure is provided as part of source data for Fig. 3C.

Supplementary Figure S4



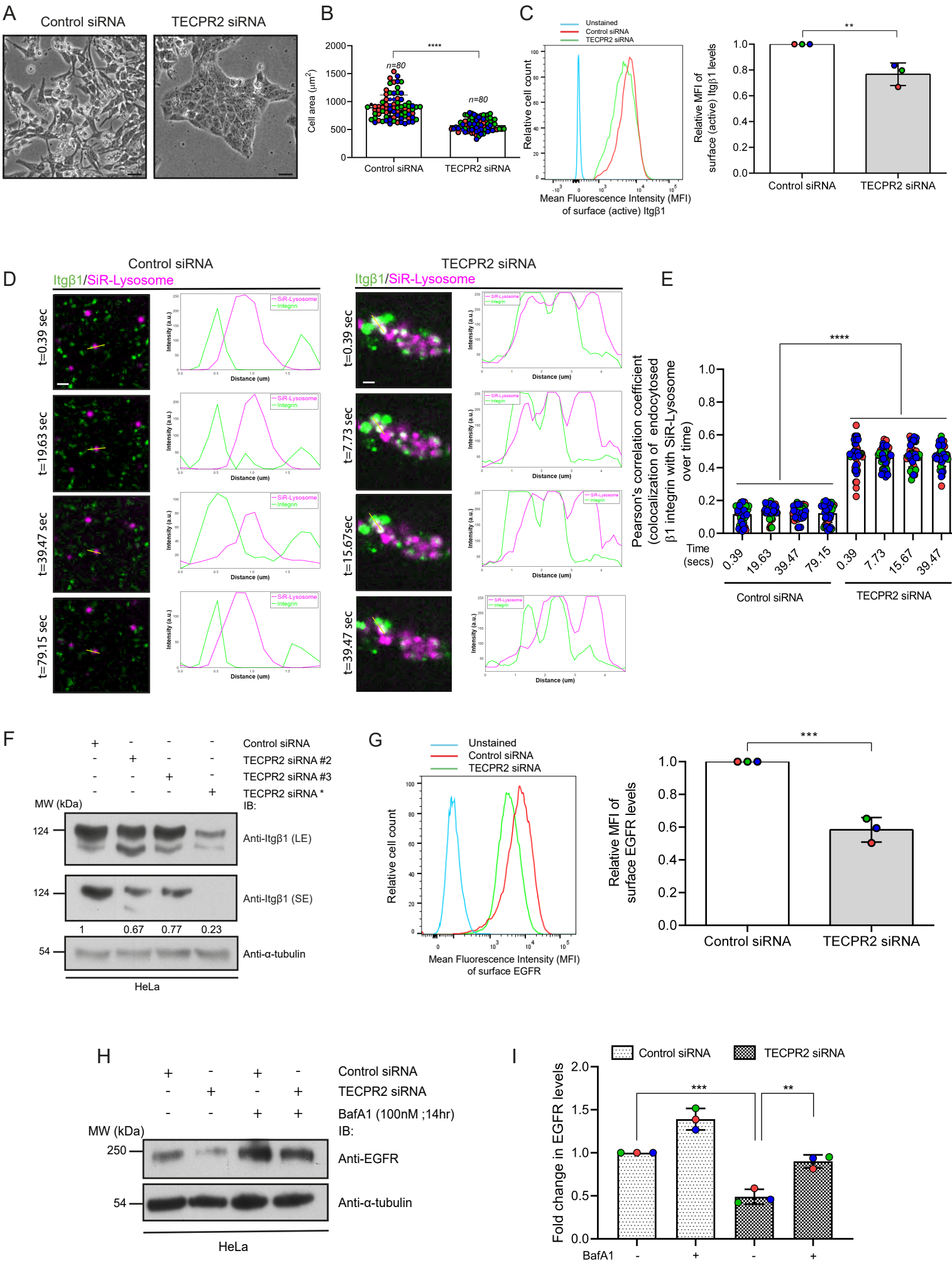
Supplementary Figure S4: TECPR2 depletion results in a collapse of early and recycling endosomes in the perinuclear region and an increase in the size of the late endocytic compartments. A-D) Lysates from indicated human cell lines treated with control siRNA and TECPR2 siRNA and immunoblotted (IB) with the indicated antibodies. β-actin and α-tubulin were used as a loading control. The band of endogenous TECPR2 is marked with an arrowhead, and the asterisk (*) indicates a non-specific band detected by the anti-TECPR2 antibody. The values represent a densitometric analysis of the TECPR2 signal intensity normalized to the loading controls. **E-F)** Representative confocal micrographs of HeLa cells treated with the indicated siRNA, followed by immunostaining with anti-APPL1 (**E**) or anti-Rabenosyn-5 (**F**) antibodies. Scale bar: 10 µm. **G)** Representative confocal micrographs of HeLa cells treated with the indicated siRNA, followed by immunostaining with anti-EEA1 antibody. Scale bar: 10 µm. **H-I)** Representative confocal micrographs of hTERT-RPE1 and HEK293T cells treated with control siRNA and TECPR2 siRNA, followed by immunostaining with the anti-Rab5 (**H)** and anti-EEA1 (**I)** antibodies. Scale bar: 10 µm. **J-K**) Representative confocal micrographs of hTERT-RPE1 and HEK293T cells treated with control siRNA and TECPR2 siRNA, followed by immunostaining with anti-TfR (**J**) and anti-SNX1 (**K**) antibodies. Scale bar: 10 µm. **L)** Quantification of the Pearson's colocalization coefficient of Rab11 with TfR in HeLa cells treated with indicated siRNA. *n* denotes the total number of cells analyzed, and the error bar represents the mean ± SD from three independent experiments**.** Experiments are color-coded, and each dot represents the individual data points ($^{****}p<0.0001$; unpaired two-tailed Student's *t*-test). **M)** Representative confocal micrographs of HeLa cells treated with the indicated siRNA, followed by immunostaining with anti-LAMP1 and anti-Rab7 antibodies. Scale bars: 10 µm (main); 5 µm (inset). Source data are provided as a Source Data file.

Supplementary Figure S5



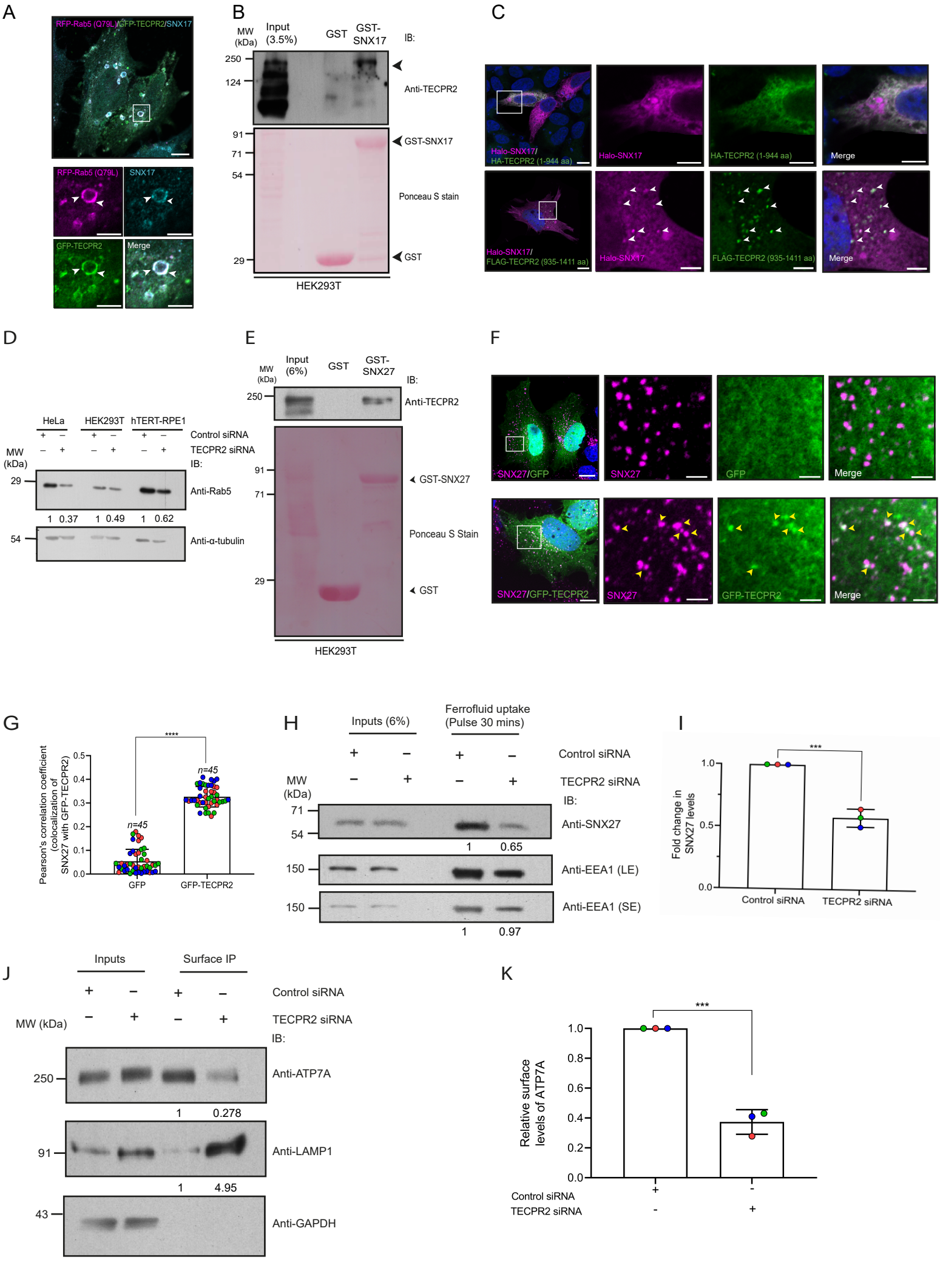
Supplementary Figure S5: **TECPR2-depleted cells show only a modest delay in transferrin receptor recycling. A)** HeLa cells treated with indicated siRNA were serum-starved for 30 min, followed by incubation with Alexa Fluor 568-labeled Transferrin (Tfn) for 1 hr on ice and analyzed using flow cytometry. A total of 30,000 cells were analyzed for each condition. The histogram shows the mean fluorescence intensity (MFI) of the surface-bound Alexa 568-Tfn signal, and the graph shows the fold-change in MFI normalized to the control siRNA treatment. The data shown is mean ± SD from three independent experiments. Experiments are color-coded, where each dot represents the mean value ($^{***}p$=0.0006; unpaired two-tailed Student's *t*-test). **B)** Tfn recycling assay. HeLa cells treated with indicated siRNA were serum starved for 30 min, followed by incubation with Alexa Fluor 568-Tfn for 5 min (pulse only) and analyzed by flow cytometry or chased in complete media at 37°C for the indicated time points before analysis by flow cytometry. A total of 30,000 cells were analyzed for each condition. The percentage of non-recycled Tfn remaining in the cell as a function of the pulse-only signal is plotted. The data shown is mean ± SD from three independent experiments. **C)** Representative confocal micrographs of HeLa cells treated with indicated siRNA and serum starved for 30 min, followed by a pulse of Alexa 568-Tfn for 5 min and chase in complete media for 20 min at 37°C prior to fixation. The cell boundary is highlighted with a white line. Scale bar: 10 μm. Source data are provided as a Source Data file.

Supplementary Figure S6



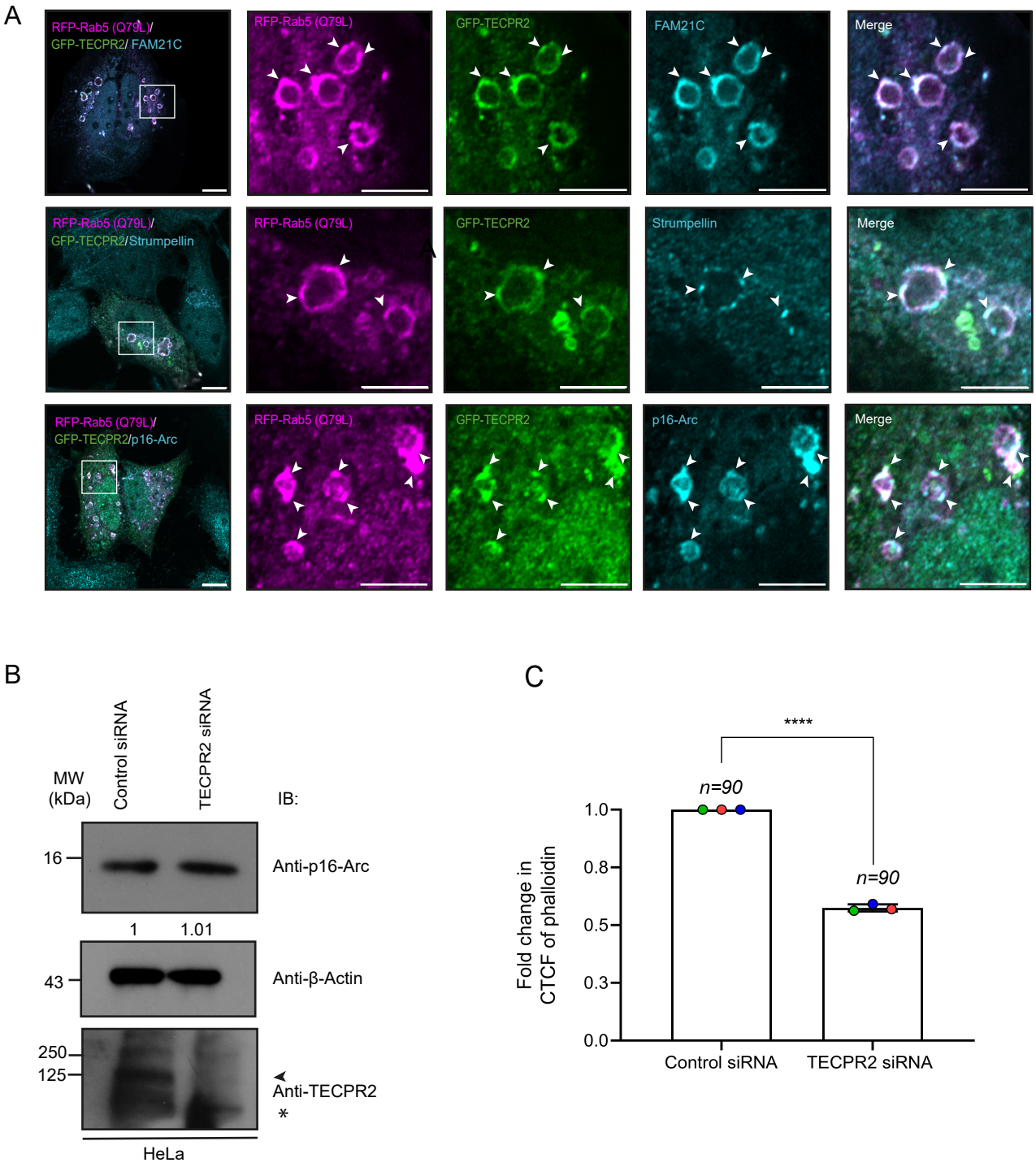
Supplementary Figure S6: **TECPR2 depletion leads to impaired retrieval of constitutive recycling cargo receptors, including Itgβ1 and EGFR, from lysosomal degradation. A)** Representative phase-contrast microscopy images of HeLa cells treated with control and TECPR2 siRNA. Scale bar: 100 µm. **B)** Quantification of cell area in control- and TECPR2 siRNA-treated HeLa cells. *n* denotes the total number of cells analyzed, and the error bar represents the mean ± SD from three independent experiments. Experiments are color-coded, and each dot represents the individual data points ($^{****}p<0.0001$; unpaired two-tailed Student's *t*-test). **C)** A histogram showing mean fluorescence intensity (MFI) of the surface Itgβ1 (active; 12G10) levels in HeLa cells treated with the indicated siRNA and analyzed by flow cytometry. A total of 30,000 cells were analyzed for each condition. The graph shows the fold-change in MFI of surface Itgβ1 normalized to the control siRNA-treated sample. The data shown is mean ± SD from three independent experiments. Experiments are color-coded, where each dot represents the mean value ($^{**}p=0.01$; unpaired two-tailed Student's *t*-test). **D)** Live-cell imaging of HeLa cells treated with indicated siRNA and incubated with anti-Itgβ1 (12G10) (green) and SiR-Lysosome (magenta) (see **Supplementary Movie 7**). Scale bar: 5 µm. The line-scan analysis of the fluorescence intensity of Itgβ1 and SiR-Lysosome across the indicated endosome is shown. **E)** Pearson's colocalization coefficient of Itgβ1 with SiR-Lysosome over time such as that shown in panel (**D**). A total of 15 cells for each time point were analyzed per experiment. The error bar represents the mean ± SD from three independent experiments. Experiments are color-coded, and each dot represents the individual data points ($^{****}p<0.0001$; unpaired two-tailed Student's *t*-test). **F)** Lysates of HeLa cells treated with indicated siRNA and immunoblotted (IB) with anti-Itgβ1 (clone 9EG7) and anti-α-tubulin antibodies. The values represent the densitometric analysis of Itgβ1 normalized to α-tubulin. The TECPR2 siRNA marked with an asterisk (*) indicates the siRNA oligo predominantly used across our experiments. LE=Long Exposure; SE=Short Exposure. **G)** A histogram showing MFI of surface EGFR levels in control siRNA and TECPR2 siRNA-treated HeLa cells, as analyzed by flow cytometry. A total of 30,000 cells were analyzed for each condition. The graph shows the fold-change in MFI of surface EGFR normalized to the control siRNA-treated samples. The data shown is mean ± SD from three independent experiments. Experiments are color-coded, where each dot represents the mean value ($^{***}p=0.0006$; unpaired two-tailed Student's *t*-test). **H)** Lysates of HeLa cells transfected with indicated siRNA and treated with or without bafilomycin A1 (BafA1), followed by immunoblotting with anti-EGFR and anti-α-tubulin antibodies. **I)** Densitometric analysis of EGFR levels normalized to α-tubulin, such as those shown in panel (**H**) is plotted. The fold-change in EGFR levels with respect to the control siRNA treatment is shown in the graph. The data shown is mean ± SD from three independent experiments. Experiments are color-coded, where each dot represents the mean value ($^{***}p=0.0005$ for control and TECPR2 without BafA1 treatment; $^{**}p=0.0035$ for TECPR2 siRNA with and without BafA1 treatment; unpaired two-tailed Student's *t*-test). Source data are provided as a Source Data file.

Supplementary Figure S7



Supplementary Figure S7: TECPR2 interacts with SNX17 and SNX27 and regulates recycling of SNX27-dependent cargoes. A) Representative Airyscan super-resolution image of HeLa cells co-transfected with GFP-TECPR2 and RFP-Rab5 (Q79L) and immunostained with anti-SNX17 antibody. Arrowheads denote the presence of the three proteins on the same subdomain of the enlarged early endosome. Scale bars: 10 µm (main); 5 µm (inset). **B)** GST and GST-SNX17 were used for pulldown with HEK293T cell lysates. The precipitates were immunoblotted (IB) with anti-TECPR2 antibody, and GST proteins were visualized using Ponceau S staining. **C)** Representative confocal micrographs of HeLa cells co-transfected with plasmids expressing Halo-SNX17 with HA-TECPR2 (1-944 a.a.) or with FLAG-TECPR2 (935-1411 a.a.) and immunostained with anti-HA or anti-FLAG antibodies. Arrowheads denote colocalized pixels of Halo-SNX17 and FLAG-TECPR2 (935-1411 a.a.). Scale bars: 10 µm (main); 5 µm (inset). **D)** Lysates of indicated cell types treated with control siRNA and TECPR2 siRNA were IB with the indicated antibodies. The values represent the densitometric analysis of the Rab5 signal intensity normalized to that of α-tubulin (used as a loading control). **E)** GST and GST-SNX27 were used for pulldown with HEK293T cell lysates. The precipitates were IB with anti-TECPR2 antibody, and GST proteins were visualized using Ponceau S staining. **F)** Representative confocal images of HeLa cells transfected with GFP or GFP-TECPR2 and immunostained with anti-SNX27 antibody. Arrowheads in the insets denote colocalized pixels in both channels. Scale bars: 10 µm (main); 5 µm (inset). **G)** Quantification of the Pearson's colocalization coefficient of SNX27 with GFP or GFP-TECPR2, such as those shown in panel (**F**) is plotted. *n* denotes the total number of cells analyzed and the error bar represents the mean ± SD from three independent experiments. Experiments are color-coded, and each dot represents the individual data points ($^{****}p<0.0001$; unpaired two-tailed Student's *t*-test). **H)** HeLa cells were treated with indicated siRNA and incubated with ferrofluid (FF) for 30 min at 37°C. The FF-containing compartments were purified and IB for the presence of indicated proteins. Densitometric analysis of SNX27 and EEA1, normalized to their inputs and control siRNA pulldown signal, is shown. LE=Long Exposure; SE=Short Exposure. **I)** Densitometric analysis of SNX27 upon TECPR2 depletion, normalized to the input and control siRNA treatment such as those shown in panel (**H**) is plotted. The data shown is the mean ± SD from three independent experiments. Experiments are color-coded, where each dot represents the mean value ($^{***}p=0.0006$; unpaired two-tailed Student's *t*-test). **J)** Biotinylation of cell surface proteins in HeLa cells treated with indicated siRNA. Biotinylated proteins were pulled down using streptavidin-conjugated beads and IB for the presence of indicated proteins. Immunoblotting for GAPDH was performed to confirm that intracellular proteins were not biotinylated. Densitometric analysis for ATP7A and LAMP1, normalized to their inputs and control siRNA pulldown signal, is shown. **K)** Densitometric analysis of surface-biotinylated ATP7A upon TECPR2 depletion, normalized to the input and control siRNA treatment such as those shown in panel (**J**) is plotted. The data shown is the mean ± SD from three independent experiments. Experiments are color-coded, where each dot represents the mean value ($^{***}p=0.0002$; unpaired two-tailed Student's *t*-test). Source data are provided as a Source Data file.

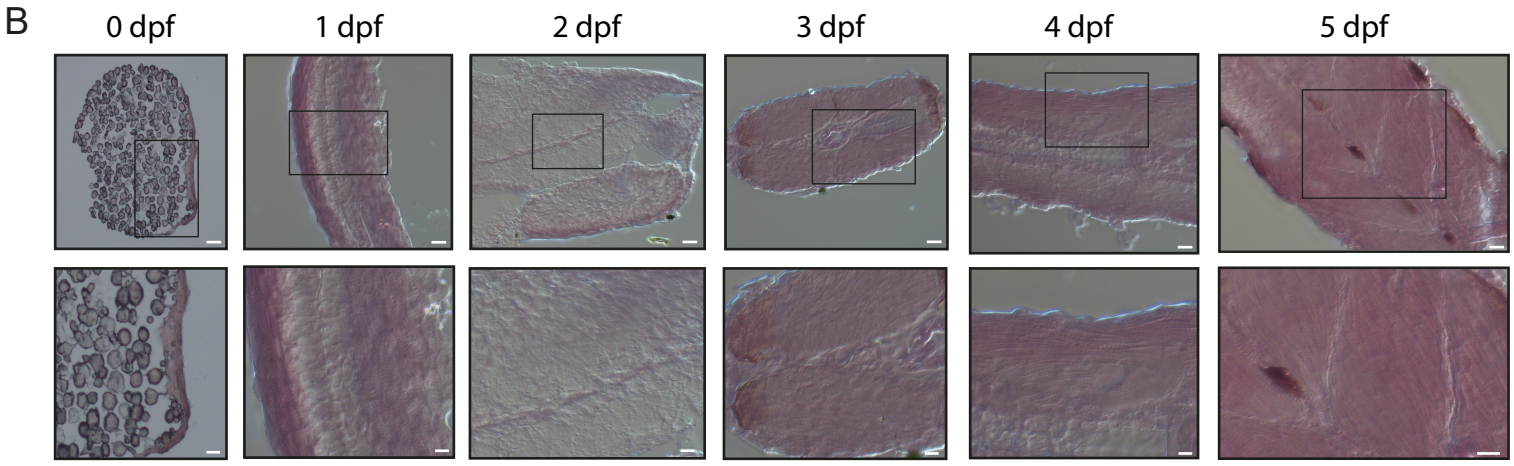
Supplementary Figure S8

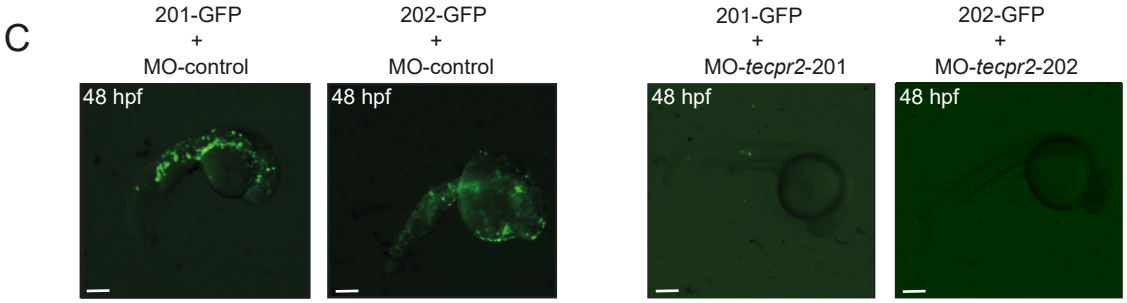


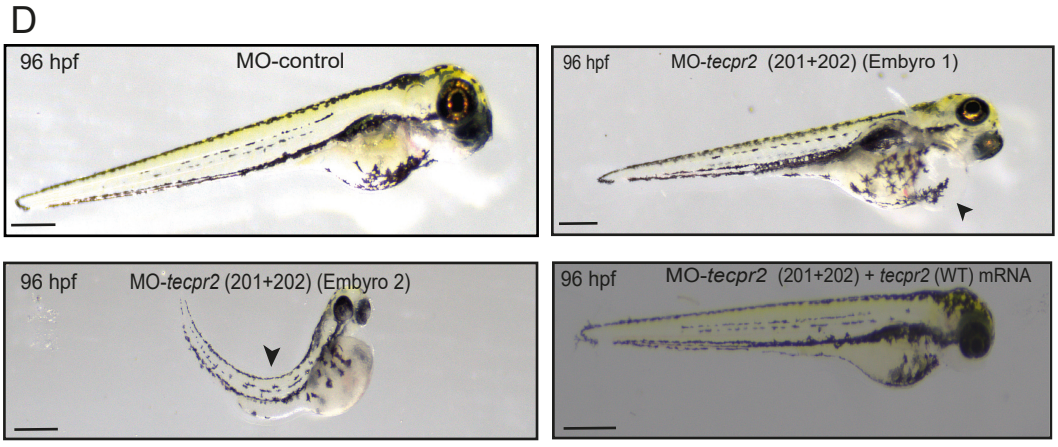
Supplementary Figure S8: TECPR2 colocalizes with WASH subunits on membrane subdomains of enlarged early endosomes. A) Representative Airyscan super-resolution image of HeLa cells co-transfected with GFP-TECPR2 and RFP-Rab5 (Q79L) and immunostained with antibodies against FAM21C, Strumpellin, and p16-Arc. Arrowheads denote the presence of the three proteins on the same subdomain of the enlarged early endosome. Scale bars: 10 µm (main); 5 µm (inset). **B)** Lysates of HeLa cells treated with control or TECPR2 siRNA were immunoblotted (IB) with the indicated antibodies. The band of endogenous TECPR2 is marked with an arrowhead, and the asterisk (*) indicates a non-specific band detected by the anti-TECPR2 antibody. The values represent the densitometric analysis of the p16-Arc band intensity normalized to the β-actin band intensity. **C)** The fold change in the corrected total cell fluorescence (CTCF) of the phalloidin signal in HeLa cells upon treatment with the indicated siRNA is shown. A total of 30 cells were analyzed per experiment for each sample. The data shown is the mean ± SD from three independent experiments. Experiments are color-coded, where each dot represents the mean value ($^{****}p<0.0001$; unpaired two-tailed Student's *t*-test). Source data are provided as a Source Data file.

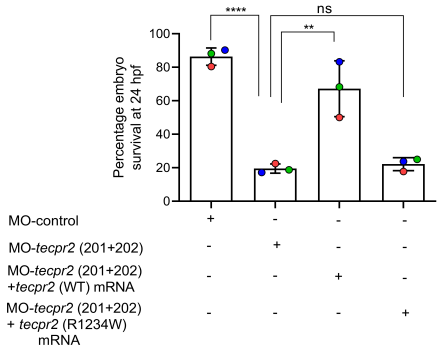
Suppplementary Figure S9

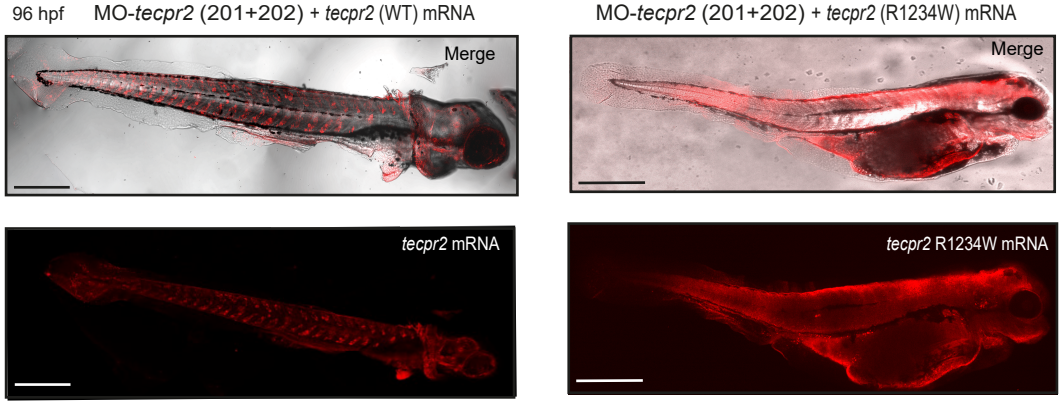












Supplementary Figure S9: Analysis of morpholino-based knockdown of Tecpr2 isoforms in zebrafish embryos and localization of Tecpr2 WT and R1234W mutant. A) Sequence alignment of TECPR2 orthologs across different vertebrate species. The blue box indicates that the HSAN-associated residue analyzed in this study (R1336 in the case of human TECPR2) is conserved across different vertebrate species. **B)** Representative brightfield images show zebrafish embryo sections probed for *tecpr2* expression by *in situ* hybridization at different days post fertilization (dpf). Scale bar: 200 µm. **C)** Representative images of zebrafish embryos acquired at 48 hours post fertilization (hpf) after co-injection of MO-control, MO-*tecpr2*-201 or MO-*tecpr2*-202 along with a GFP-expressing reporter construct (either containing or lacking the *tecpr2* MO-target sequences (201 or 202) upstream of the GFP tag). Scale bar: 200 µm. **D)** Lateral view of zebrafish embryos at 96 hpf upon injection with MO-control (upper left), MO-*tecpr2* (201+202) (upper right and lower left), and MO-*tecpr2* (201+202) + *tecpr2* mRNA (rescue) (lower right). Scale bar: 200 µm. **E)** The graph represents the percentage survival of zebrafish embryos at 24 hpf upon injection with MO-control, MO-*tecpr2* (201+202), MO-*tecpr2* (201+202) + *tecpr2* (WT)-mRNA (rescue), and MO-*tecpr2* (201+202) + *tecpr2* (R1234W)-mRNA. The total number of embryos analyzed was 106 for MO-control, 92 for MO-*tecpr2* (201+202), 107 for MO-*tecpr2* (201+202) + *tecpr2* (WT)-mRNA (rescue), and 127 for MO-*tecpr2* (201+202) + *tecpr2* (R1234W)-mRNA. The error bar represents the mean ± SD from three independent experiments, where each dot indicates the mean value ($^{****}p<0.0001$; $^{**}p=0.0082$; ns=0.3852; unpaired two-tailed Student's *t*-test). **F)** Representative image of whole-mount immunostaining of zebrafish embryo acquired at 96 hpf and immunostained with anti-HA antibody in embryos injected with MO-*tecpr2* (201+202) along with *tecpr2* (WT)-mRNA (rescue) and MO-*tecpr2* (201+202) + *tecpr2* (R1234W)-mRNA. Scale bar: 200 µm. Source data are provided as a Source Data file.

Supplementary Table I: AlphaMissense score and pathogenicity prediction for TECPR2 clinical variants.

TECPR2 variants	AlphaMissense Score	Pathogenicity Prediction
p.Thr903Met	0.13	Likely_benign
p.Asp1000Tyr	0.958	Likely_pathogenic
p.Trp1140Gly	0.903	Likely_pathogenic
p.Arg1336Trp	0.968	Likely_pathogenic

Supplementary Table II: List of DNA constructs used in this study.

Plasmid Name	Description	Source
Mammalian expression constructs:		
pcDNA3.1(-) vector	Mammalian expression vector	Available in the lab
pcDNA3.1(-)-FLAG-TECPR2 (WT)	Full-length human TECPR2 (WT) with N-terminal FLAG tag cloned in pcDNA3.1(-) vector	This study
pcDNA3.1(-)-FLAG-TECPR2 (T903M)	Full-length human TECPR2 with T903M mutation and N-terminal FLAG tag cloned in pcDNA3.1(-) vector	This study
pcDNA3.1(-)-FLAG-TECPR2 (D1000Y)	Full-length human TECPR2 with D1000Y mutation and N-terminal FLAG tag cloned in pcDNA3.1(-) vector	This study
pcDNA3.1(-)-FLAG-TECPR2 (W1140G)	Full-length human TECPR2 with W1140G mutation and N-terminal FLAG tag cloned in pcDNA3.1(-) vector	This study
pcDNA3.1(-)-FLAG-TECPR2 (I1256A)	Full-length human TECPR2 with I1256A mutation and N-terminal FLAG tag cloned in pcDNA3.1(-) vector	This study
pcDNA3.1(-)-FLAG-TECPR2 (E1299A)	Full-length human TECPR2 with E1299A mutation and N-terminal FLAG tag cloned in pcDNA3.1(-) vector	This study
pcDNA3.1(-)-FLAG-TECPR2 (R1336W)	Full-length human TECPR2 with R1336W mutation and N-terminal FLAG tag cloned in pcDNA3.1(-) vector	This study
pcDNA3.1(-)-FLAG-TECPR2 (935-1411 aa)	N-terminal FLAG-tagged human TECPR2 from 935-1411 amino acid cloned in pcDNA3.1(-) vector	This study
pcDNA3.1(-)-HA-TECPR2 (1-944 aa)	N-terminal HA-tagged human TECPR2 from 1-944 amino acid cloned in pcDNA3.1(-) vector	This study
pcDNA3.1(-)-FLAG-TECPR2 (Rescue)	Full-length human TECPR2 rescue construct designed against TECPR2 siRNA using silent mutations and with N-terminal FLAG tag cloned in pcDNA3.1(-) vector	This study
pcDNA3.1(+)-Untagged-Rab5a (WT)	Full-length human Rab5 (WT) cloned in pcDNA3.1(+) vector	This study
pEGFP-C1 vector	Mammalian GFP expression vector	Gift from Prof. Peter Cullen (University of Bristol, UK)

pEGFP-C1-TECPR2 (WT)	Full length human TECPR2 (WT) with N-terminal GFP tag cloned in pEGFPC1 vector	This study
pEGFP-C1-TECPR2 (R1336W)	N-terminal GFP-tagged human TECPR2 with R1336W mutation and cloned in pEGFP-C1 vector	This study
pEGFP-C1-TECPR2 (Rescue)	Full-length human TECPR2 rescue construct designed against TECPR2 siRNA using silent mutations and with N-terminal GFP tag cloned in pEGFP-C1 vector	This study
pEGFP-C1-Rab5a (WT)	Full-length Rab5a (WT) with N-terminal GFP-tag cloned in pEGFP-C1 vector	Gift from Prof. Steve Caplan (University of Nebraska Medical Center, USA)
pEGFP-C1-Rab5a (Q79L)	Full-length Rab5 with Q79L mutation (GTP-locked) and cloned in pEGFP-C1 vector	Gift from Prof. Steve Caplan (University of Nebraska Medical Center, USA)
pEGFP-C1-Rab5a (S34N)	Full-length Rab5 with S34N mutation (GDP-locked) and cloned in pEGFP-C1 vector	Gift from Prof. Steve Caplan (University of Nebraska Medical Center, USA)
pMRFP-C3-Rab5a (WT)	Full-length Rab5a (WT) with N-terminal RFP tag cloned in pmRFP-C3 vector	Gift from Prof. Ari Helenius (Addgene plasmid # 14437)
pMRFP-C3-Rab5a (Q79L)	Full-length Rab5a (Q79L) with N-terminal RFP tag cloned in pmRFP-C3 vector	This study
pMRFP-C3-Rab5a (S34N)	Full-length Rab5a (S34N) with N-terminal RFP tag cloned in pmRFP-C3 vector	This study
pcDNA3.3-Rab5A (Q79L)-BirA*-HA-MAO	Rab5a lacking last three amino acids (Δcys form) with Q79L mutation cloned in JB28 vector	Gift from Prof. Sean Munro (Addgene plasmid # 128900)
pcDNA3.3-Rab5A (S34N)-BirA*-HA-MAO	Rab5a lacking last three amino acids (Δcys form) with S34N mutation cloned in JB28 vector	This study
pEGFP-N1-SNX17	Full-length SNX17 cloned in pEGFP-N1 vector	Gift from Prof. Lawrence Banks (ICGEB, Italy)
pmCherry-C1-VAPB	Full-length VAPB cloned in pmCherry-C1 vector	Gift from Prof. Gerald Rv Hammond (University of Pittsburgh School of Medicine, USA)
pHTN Halo Tag CMV-neo vector	Mammalian expression plasmid	Available in the lab
pHTN Halo Tag CMV-neo-SNX17	Full-length SNX17 with N-terminal Halo tag cloned in pHTN Halo Tag CMV-neo vector	This study

pEGFP-C1-WASH	Full-length murine WASH with N-terminal GFP tag cloned in pEGFP-C1 vector	Gift from Prof. Robert Insall (University of Glasgow, UK)
pLenti-CapZβ-GFP	CapZβ with C-terminal GFP tag cloned in pLenti vector	Gift from Prof. Jianbo Yue (City University of Hong Kong Chengdu Research Institute, China)
pEGPN1-zebrafish *tecpr2*-201	Zebrafish *tecpr2*-201 morpholino binding site cloned upstream of GFP tag in pEGFPN1 vector	This study
pEGPN1-zebrafish *tecpr2*-202	Zebrafish *tecpr2*-202 morpholino binding site cloned upstream of GFP tag in pEGFPN1 vector	This study
Bacterial expression constructs:		
pGEX-4T3 vector	Bacterial protein expression vector for making N-terminal GST proteins	Available in the lab
pGEX6P1-Rab5a (WT)	Full-length human Rab5a (WT) with N-terminal GST tag cloned in pGEX6P1 vector	Gift from Prof. Sunando Datta (IISER Bhopal, India)
pGEX6P1-Rab5a (Q79L)	Full-length human Rab5a with Q79L mutation and cloned in pGEX6P1 vector	This study
pGEX6P1-Rab5a (S34N)	Full-length human Rab5a with S34N mutation and cloned in pGEX6P1 vector	This study
pMAL-C2X vector	Bacterial protein expression vector for making N-terminal MBP-tagged proteins	Available in the lab
pMAL-C2X-TECPR2_TECPR (935-1411 aa)	Human TECPR2 from 935-1411 amino acid with N-terminal MBP tag cloned in pMAL-C2X vector	This study
pMAL-C2X-TECPR2_TECPR (935-1411 aa; R1336W)	Human TECPR2 from 935-1411 amino acid with R1336W mutation and cloned in pMAL-C2X vector	This study
pGEX6P1-GFP-Nanobody	Expresses GST-tagged anti-GFP Nanobody in *E. coli* cells	Gift from Prof. Kazuhisa Nakayama (Addgene plasmid # 61838)
Rab5a-Q79L-1-212-(C19S, C63S)	Bacterial protein expression vector for making N-terminal His- and SUMO-tagged Rab5a (1-212 aa) with Q79L, C19S, and C63S mutations	Gift from Prof. Roger L. Williams (MRC Laboratory of Molecular Biology, UK)
pGEX6P2-SNX17	Full-length human SNX17 with N-terminal GST tag cloned in pGEX6P2 vector	This study

pGEX6P2-SNX27	Full-length human SNX27 with N-terminal GST tag cloned in pGEX6P2 vector	This study
Yeast two-hybrid constructs:		
pGBKT7 vector	GAL4-DNA binding domain yeast two-hybrid vector	Available in the lab
pGBKT7-Rab1a (Q70L)	Rab1a with Q70L mutation cloned in pGBKT7 vector	Gift from Prof. Marci Scidmore (Cornell University, USA)
pGBKT7-Rab2a (Q65L)	Rab2a with Q65L mutation cloned in pGBKT7 vector	Gift from Prof. Coenraad Kuijl (Amsterdam UMC, Amsterdam)
pGBKT7-Rab3b (Q81L)	Rab3b with Q81L mutation cloned in pGBKT7 vector	Gift from Prof. Coenraad Kuijl (Amsterdam UMC, Amsterdam)
pGBKT7-Rab4a (Q70L)	Rab4a with Q70L mutation cloned in pGBKT7 vector	Gift from Prof. Marci Scidmore (Cornell University, USA)
pGBDC1-Rab5a (WT)	Rab5a (WT) cloned in pGBDC1 vector	Gift from Prof. Tamotsu Yoshimori (Osaka University, Japan)
pGBDC1-Rab5a (Q79L)	Rab5 with Q79L mutation cloned in pGBDC1 vector	Gift from Prof. Tamotsu Yoshimori (Osaka University, Japan)
pGBDC1-Rab5a (S34N)	Rab5 with S34N mutation cloned in pGBDC1 vector	Gift from Prof. Tamotsu Yoshimori (Osaka University, Japan)
pGBKT7-Rab6a (Q72L)	Rab6a with Q72L mutation cloned in pGBKT7 vector	Gift from Prof. Marci Scidmore (Cornell University, USA)
pGBDC1-Rab7a (Q67L)	Rab7a with Q67L mutation cloned in pGBD-C1 vector	Gift from Prof. Tamotsu Yoshimori (Osaka University, Japan)
pGBKT7-Rab8a (Q67L)	Rab8a with Q67L mutation cloned in pGBKT7 vector	Gift from Prof. Coenraad Kuijl (Amsterdam UMC, Amsterdam)
pGBDC1-Rab9a (Q67L)	Rab9a with Q67L mutation cloned in pGBD-C1 vector	Gift from Prof. Tamotsu Yoshimori (Osaka University, Japan)
pGBKT7-Rab10 (Q48L)	Rab10 with Q48L mutation cloned in pGBKT7 vector	Gift from Prof. Marci Scidmore (Cornell University, USA)
pGBKT7-Rab11a (Q70L)	Rab11a with Q70L mutation cloned in pGBKT7 vector	Gift from Prof. Marci Scidmore (Cornell University, USA)
pGBKT7-Rab14 (Q70L)	Rab14 with Q70L mutation cloned in pGBKT7 vector	Gift from Prof. Coenraad Kuijl (Amsterdam UMC, Amsterdam)

pGBKT7-Rab18 (Q67L)	Rab18 with Q67L mutation cloned in pGBKT7 vector	Gift from Prof. Coenraad Kuijl (Amsterdam UMC, Amsterdam)
pGBKT7-Rab20 (Q67L)	Rab20 with Q67L mutant cloned in pGBKT7 vector	Gift from Prof. Coenraad Kuijl (Amsterdam UMC, Amsterdam)
pGBKT7-Rab23 (Q67L)	Rab23 with Q67L mutant cloned in pGBKT7 vector	Gift from Prof. Coenraad Kuijl (Amsterdam UMC, Amsterdam)
pGBKT7-Rab24 (S67L)	Rab24 with S67L mutation cloned in pGBKT7 vector	Gift from Prof. Coenraad Kuijl (Amsterdam UMC, Amsterdam)
pGBKT7-Rab29 (Q67L)	Rab29 with Q67L mutation cloned in pGBKT7 vector	This study
pGBKT7-Rab30 (Q68L)	Rab30 with Q68L mutation cloned in pGBKT7 vector	This study
pGBKT7-Rab33a (Q95L)	Rab33a with Q95L mutation cloned in pGBKT7 vector	This study
pGBKT7-Rab39 (Q72L)	Rab39 with Q72L mutation cloned in pGBKT7 vector	This study
pGBKT7-Vps11	Vps11 cloned into pGBKT7 vector	Available in the lab
pGBKT7-Vps16	Vps16 cloned into pGBKT7 vector	Available in the lab
pGBKT7-Vps33a	Vps33a cloned into pGBKT7 vector	Available in the lab
pGBKT7-Vps39	Vps39 cloned into pGBKT7 vector	Available in the lab
pGBKT7-Vps41	Vps41 cloned into pGBKT7 vector	Available in the lab
pGBKT7-LC3b	LC3b cloned into pGBKT7 vector	Available in the lab
pGBKT7-zRab5	Zebrafish *Rab5* cloned into pGBKT7 vector	This study
pGADT7 vector	GAL4-activation domain yeast two-hybrid vector	Available in the lab
pGADT7-TECPR2 (WT)	Full-length human TECPR2 wild-type cloned into pGADT7 vector	This study
pGADT7-TECPR2 (1-944 aa)	Human TECPR2 from 1-944 amino acid cloned into pGADT7 vector	This study
pGADT7-TECPR2 (935-1411 aa)	Human TECPR2 from 935-1411 amino acid cloned into pGADT7 vector	This study
pGADT7-TECPR2 (I1256A)	Full-length human TECPR2 with I1256A mutation cloned into pGADT7 vector	This study
pGADT7-TECPR2 (E1299A)	Full-length human TECPR2 with E1299A mutation cloned into pGADT7 vector	This study
pGADT7-TECPR2 (T903M)	Full-length human TECPR2 with T903M mutation cloned into pGADT7 vector	This study

pGADT7-TECPR2 (D1000Y)	Full-length human TECPR2 with D1000Y mutation cloned into pGADT7 vector	This study
pGADT7-TECPR2 (W1140G)	Full-length human TECPR2 with W1140G mutation cloned into pGADT7 vector	This study
pGADT7-TECPR2 (M1251I)	Full-length human TECPR2 with M1251I mutation cloned into pGADT7 vector	This study
pGADT7-TECPR2 (R1336W)	Full-length human TECPR2 with R1336W mutation cloned into pGADT7 vector	This study
pGADT7-zTECPR2	Zebrafish *TECPR2* (201 transcript; NCBI NP_001038644.1) cloned into pGADT7 vector	This study
pGADT7-zTECPR2 (R1234W)	Zebrafish *TECPR2* (201 transcript; NCBI NP_001038644.1) with R1234W mutation cloned into pGADT7 vector	This study

Supplementary Table III: List of antibodies used in this study.

(IF: Immunofluorescence; WB: Western Blotting; IP: Immunoprecipitation; FC: Flow Cytometry)

Antibody	**Source**	**Catalog #**
Rabbit-TECPR2 (WB-1:1000)	Sigma-Aldrich	HPA000658
Rabbit-TECPR2 (IF-1:500) (Used in GUVs Assay)	Abcam	ab121109
Mouse anti-Rab5 (IF-1:300; WB-1:1000; IP-2 µg)	BD Biosciences	610724
Rabbit anti-Rab5 (IF-1:300)	Cell Signaling Technology	3547
Mouse anti-Rab7 (IF-1:25)	Santa Cruz Biotechnology	sc-376362
Rabbit anti-Rab11 (IF-1:200; WB-1:5000)	Abcam	ab128913
Mouse anti-EEA1 (IF-1:300; WB-1:1000)	BD Biosciences	610457
Rabbit anti-EEA1 (IF-1:200)	Cell Signaling Technology	3288
Rabbit anti-APPL1 (IF-1:500)	Cell Signaling Technology	3858T
Rabbit anti-Rabenosyn-5 (IF-1:500)	Proteintech	22218-1-AP
Mouse anti-SNX1 (IF-1:500)	BD Biosciences	611482
Rabbit anti-SNX17 (IF-1:200; WB-1:1000)	Atlas Antibodies	HPA043867
Mouse anti-SNX27 (IF-1:500; WB-1:1000)	Abcam	ab77799
Rabbit anti-HRS (IF-1:500)	Cell Signaling Technology	15087
Rabbit anti-FAM21C (IF-1:200; WB-1:1000)	Invitrogen	PA5-35883
Rabbit anti-Strumpellin (IF-1:300; WB-1:1000)	Bethyl Laboratories	A304-809A
Rabbit anti-WASHC1 (IF-1:500; WB-1:1000)	Atlas Antibodies	HPA002689
Rabbit anti-KIAA1033/SWIP (WB-1:1000)	Bethyl Laboratories	A304-919A
Rabbit anti-ARPC5/p16^{Arc} (IF-1:300; WB-1:1000)	Abcam	ab51243
Rabbit anti-C16orf62 (WB-1:1000)	Invitrogen	PA5-28553
Mouse anti-SV2 (IF-1:200)	Developmental Studies Hybridoma Bank	SV2
Mouse anti-Paxillin (IF-1:500)	BD Biosciences	610051
Mouse anti-LAMP1 (IF-1:1000; WB-1:1000)	BD Biosciences	555798
Rabbit anti-LAMP1	Abcam	ab24170

(IF-1:2500)		
Rabbit anti-TGN46 (WB-1:1000)	Abcam	ab50595
Mouse anti-Itgβ1 (12G10) (IF-1:500)	Abcam	ab30394
Mouse anti-Itgβ1 (CD29) (WB-1:1000)	BD Biosciences	610647
Rabbit anti-Itgα5 (IF-1:300)	Abcam	ab150361
Rabbit anti-TfnR (IF-1:300)	Abcam	ab84036
Mouse anti-EGFR (FC-1:500)	Thermo Fisher Scientific	AHR5062
Rabbit anti-EGFR (WB-1:3000)	Cell Signaling Technology	4267
Mouse anti-ATP7A (WB-1:1000)	Santa Cruz Biotechnology	sc-376467
Mouse anti-α-tubulin (WB-1:5000)	Sigma-Aldrich	T9026
Mouse anti-β-actin (WB-1:5000)	Santa Cruz Biotechnology	sc-47778
Mouse anti-GAPDH (WB-1:3000)	Santa Cruz Biotechnology	sc-166574
Mouse anti-GFP (WB-1:5000)	Santa Cruz Biotechnology	sc-9996
Mouse anti-FLAG tag (IF-1:500)	Sigma-Aldrich	F1804
Rabbit anti-FLAG tag (WB-1:2000)	Invitrogen	PA1-984B
Rat anti-FLAG tag resin (IP: 10-15 μL slurry)	BioLegend	651503
Mouse anti-HA tag (IF-1:500)	Biolegend	901503
Rabbit anti-HA tag (IF-1:500)	Sigma-Aldrich	H6908
HRP-conjugated Anti-MBP (WB-1:5000)	New England Biosciences	E8038S
HRP-conjugated goat anti-rabbit IgG (WB-1:5000)	Jackson ImmunoResearch	111-035-144
HRP-conjugated goat anti-mouse IgG (WB-1:5000)	Jackson ImmunoResearch	115-035-166
Alexa-Fluor 488-conjugated α-Bungarotoxin	Thermo Fisher Scientific	B13422
Alexa-Fluor 647-conjugated Phalloidin	Thermo Fisher Scientific	A22287
Alexa-Fluor 488-conjugated goat anti-rabbit IgG (IF-1:500)	Thermo Fisher Scientific	A11034
Alexa-Fluor 568-conjugated goat anti-rabbit IgG (IF-1:500)	Thermo Fisher Scientific	A11036
Alexa-Fluor 488-conjugated goat anti-mouse IgG (IF-1:500)	Thermo Fisher Scientific	A11029

Alexa-Fluor 568-conjugated goat anti-mouse IgG (IF-1:500)	Thermo Fisher Scientific	A11031
Alexa-Fluor 647-conjugated goat anti-mouse IgG (IF-1:500)	Thermo Fisher Scientific	A21235

Supplementary Table IV: Multiple sequence alignment of TECPR2 and Rab5 across different vertebrate species. The HSAN variants of TECPR2 used in this study are highlighted by a green box.

For TECPR2:

MSA *The multiple sequence alignment result as produced by T-coffee.*

```
T-COFFEE, Version_11.00 (Version_11.00)
Cedric Notredame
SCORE=970
*
 BAD AVG GOOD
*
Homo       :  96
Canis      :  96
Mus        :  96
Bos        :  96
Rattus     :  97
Macaca     :  97
Pan        :  97
Gallus     :  96
Xenopus    :  95
Danio      :  94
cons       :  97

Homo       -----MASISEPVTFREFCPLYYLLNAIPTKIQKGFRSIVVYLTALDTNGDYIAVGSSIGMLYLYCRHLNQM
Canis      -----MASVSEPITFREFCPLYYLLNAIPTKVQKGFRSIVVYLTALDTNGDYIAVGSSIGMLYLYCRHFNQM
Mus        -----MASVSDPVTFREFCPLYYLLNAIPTKIQRGFRSIVVYLTALDTNGDYIAVGSSIGMLYLYCRHLNQM
Bos        -----MASVSEPVTFREFCPLYYLLNSIPTKIQKGFRSLVVYLTALDTNGDYIAVGSSIGMLYLYCRHHDQM
Rattus     -----MASVSEPVTFREFCPLYYLLNAIPTKIQRGFRSIVVYLTALDTNGDYIAVGSSIGMLYLYCRHLNQM
Macaca     -----MASISEPVTFREFCPLYYLLNAIPTKIQKGFRSIVVYLTALDTNGDYIAVGSSIGMLYLYCRHLNQM
Pan        -----MASISEPVTFREFCPLYYLLNAIPTKIQKGFRSIVVYLTALDTNGDYIAVGSSIGMLYLYCRHLNQM
Gallus     MPVLAMASVASPVLFKEFCPLYYLLNAIPTKIQKGFRSIVVYLTALDTNGDYIAVGSSIGMLYLYCRHLNQM
Xenopus    -----MAAQ-RVGTFKEFCPLYSILNAIPAKVQKGFRSILVYLTALDANSDFIAVGSSIGMLYLYCRHTSQM
Danio      -----MATNPAALPLREFCPLYYLLNAIPAKVQKGFRSVLVYLTALDSNSDYIAIGSSIGMLYLYCRRVSQM

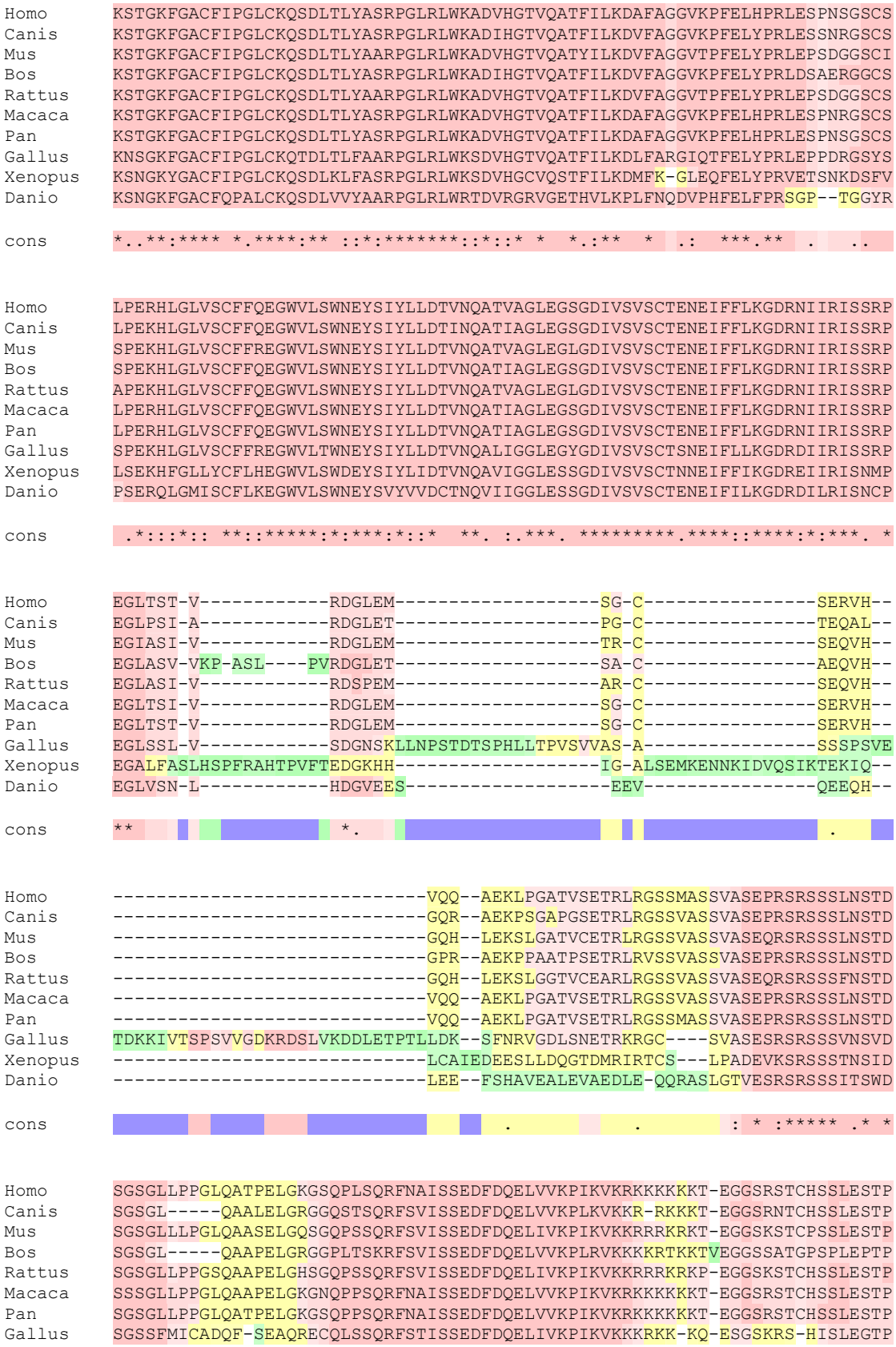
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Homo       RKYNFEGKTESITVVKLLSCFDDLVAAGTASGRVAVFQLVSSLPGRNKQLRRFDVTGIHKNSITALAWSPNG
Canis      KKYNFEGKTEPITVVKLLSCFDDLVAAGTASGRVAVFQLVSSLPGRNKQLRRFDVTGIHKNSITALAWSPNG
Mus        KKYNVDGKMESITVVKLLSCFDDLVAAGTASGRVAVFQLVSSLPGRNKQLRRFDVTGVHKTSITALAWSPNG
Bos        KKYNFEGKTESITVVKLLSCFDDLVAAGTASGRVAVFQLVSSLPGRNKQLRRFDVTGIHKNSITALAWSPNG
Rattus     KKYNFEGKTESITVVKLLSCFDDLVAVGTASGRVAVFQLVSSLPGRNKQLRRFDVTGVHKTSITALAWSPNG
Macaca     RKYNFEGKTESITVVKLLSCFDDLVAAGTASGRVAVFQLVSSLPGRNKQLRRFDVTGIHKNSITALAWSPNG
Pan        RKYNFEGKTESITVVKLLSCFDDLVAAGTASGRVAVFQLVSSLPGRNKQLRRFDVTGIHKNSITALAWSPNG
Gallus     KKYNFEGKCESITFVKLLSCFDDLVAVGTASGRVAVFQLVSSLPGRNKQLRRFDVAGIHKSSITALAWSPNG
Xenopus    KKYSLEGKTESISVVKLLSCFDDLVAVGTVSGRVAVFQLVSPLPGRNKQLRRFDVLGIHKSNITALAWSPNG
Danio      NKYNLEGKCEAITAVKLLSCFDDLVAVGTASGRVAFFQLVSPLPGRNKQLRRFDVVGLHKSTITALAWSANG

cons       .**..:** *.*: ************.**.*****.*****.************* *:**..*******.**


Homo       MKLFSGDDKGKIVYSSLDLDQGLCNSQLVLEEPSSIVQLDYSQKVLLVSTLQRSLLFYTEEKSVRQIGTQPR
Canis      MKLFSGDDKGKIVYSSLDLDQGVCNSHLVLEEPSSIVQLDYSQKVLLVSTLQRSLLFYTEEKSVKQIGTQPR
Mus        MKLFSGDDKGKIVYSSLDLDQGLCSSHLVLEEPSSIVQLDYSQKVLLVSTLQRSLLFYTEEKAVKQIGSQPR
Bos        MKLFSGDDKGKIVYSSLDLDQGVCQSHLVLEEPASIVQLDYSQKVLLVSTLQRSLLFYTEERSVQQVGTQPR
Rattus     MKLFSGDDKGKIVYSSLDLDQGICNSHLVLEEPSSIVQLDYSQKVLLVSTLQRSLLFYTEEKAVKQIGSQPR
Macaca     MKLFSGDDKGKIVYSSLDLDQGLCNSQLVLEEPSSIVQLDYSQKVLLVSTLQRSLLFYTEEKSVRQIGTQPR
Pan        MKLFSGDDKGKIVYSSLDLDQGLCNSQLVLEEPSSIVQLDYSQKVLLVSTLQRSLLFYTEEKSVRQIGTQPR
Gallus     MKLFSGDDRGKIVYSALDLDHGVCNSSLVLEEPSSIVQLDYNQKVLLVSTLQRTILFYTEEKSVKQVGTQPR
Xenopus    MKLFSGDDRGKIIQSDLDFDKGLCNPSLILEELSSVVQLDYSQKVLLVSTTQRCLLFYTEEKAVKQVGTQPR
Danio      MKLFSGDDKGKVVYSAVDLDQGVCNPVVLFEEPSAIVQLEYSQKVLLVSSYQRSMLFYTQEQSHQQLGSKPR

cons       ********:**:: * :*:*:*:*.. :::** :::***:*.*******: ** :****:*:: :*:*::**
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Homo KSTGKFGACFIPGLCKQSDLTLYASRPGLRLWKADVHGTVQATFILKDAFAGGVKPFELHPRLESPNSGSCS
Canis KSTGKFGACFIPGLCKQSDLTLYASRPGLRLWKADIHGTVQATFILKDVFAGGVKPFELYPRLESSNRGSCS
Mus KSTGKFGACFIPGLCKQSDLTLYAARPGLRLWKADVHGTVQATYILKDVFAGGVTPFELYPRLEPSDGGSCI
Bos KSTGKFGACFIPGLCKQSDLTLYASRPGLRLWKADIHGTVQATFILKDVFAGGVKPFELYPRLDSAERGGCS
Rattus KSTGKFGACFIPGLCKQSDLTLYAARPGLRLWKADVHGTVQATFILKDVFAGGVTPFELYPRLEPSDGGSCS
Macaca KSTGKFGACFIPGLCKQSDLTLYASRPGLRLWKADVHGTVQATFILKDAFAGGVKPFELHPRLESPNRGSCS
Pan KSTGKFGACFIPGLCKQSDLTLYASRPGLRLWKADVHGTVQATFILKDAFAGGVKPFELHPRLESPNSGSCS
Gallus KNSGKFGACFIPGLCKQTDLTLFAARPGLRLWKSDVHGTVQATFILKDLFARGIQTFELYPRLEPPDRGSYS
Xenopus KSNGKYGACFIPGLCKQSDLKLFASRPGLRLWKSDVHGCVQSTFILKDMFK-GLEQFELYPRVETSNKDSFV
Danio KSNGKFGACFQPALCKQSDLVVYAARPGLRLWRTDVRGRVGETHVLKPLFNQDVPHFELFPRSGP--TGGYR
cons *..**:**** *.****:** ::*:*******::*::* * *.:** * .: ***.** . ..
Homo LPERHLGLVSCFFQEGWVLSWNEYSIYLLDTVNQATVAGLEGSGDIVSVSCTENEIFFLKGDRNIIRISSRP
Canis LPEKHLGLVSCFFQEGWVLSWNEYSIYLLDTINQATIAGLEGSGDIVSVSCTENEIFFLKGDRNIIRISSRP
Mus SPEKHLGLVSCFFREGWVLSWNEYSIYLLDTVNQATVAGLEGLGDIVSVSCTENEIFFLKGDRNIIRISSRP
Bos SPEKHLGLVSCFFQEGWVLSWNEYSIYLLDTVNQATIAGLEGSGDIVSVSCTENEIFFLKGDRNIIRISSRP
Rattus APEKHLGLVSCFFQEGWVLSWNEYSIYLLDTVNQATVAGLEGLGDIVSVSCTENEIFFLKGDRNIIRISSRP
Macaca LPERHLGLVSCFFQEGWVLSWNEYSIYLLDTVNQATIAGLEGSGDIVSVSCTENEIFFLKGDRNIIRISSRP
Pan LPERHLGLVSCFFQEGWVLSWNEYSIYLLDTVNQATIAGLEGSGDIVSVSCTENEIFFLKGDRNIIRISSRP
Gallus SPEKHLGLVSCFFREGWVLTWNEYSIYLLDTVNQALIGGLEGYGDIVSVSCTSNEIFLLKGDRDIIRISSRP
Xenopus LSEKHFGLLYCFLHEGWVLSWDEYSIYLIDTVNQAVIGGLESSGDIVSVSCTNNEIFFIKGDREIIRISNMP
Danio PSERQLGMISCFLKEGWVLSWNEYSVYVVDCTNQVIIGGLESSGDIVSVSCTENEIFILKGDRDILRISNCP
cons .*:::*:: **::*****:*:***:*::* **. :.***. *********.****::****:*:***. *
Homo EGLTST-V----------RDGLEM-----------------SG-C----------------SERVH--
Canis EGLPSI-A----------RDGLET-----------------PG-C----------------TEQAL--
Mus EGIASI-V----------RDGLEM-----------------TR-C----------------SEQVH--
Bos EGLASV-VKP-ASL----PVRDGLET-----------------SA-C----------------AEQVH--
Rattus EGLASI-V----------RDSPEM-----------------AR-C----------------SEQVH--
Macaca EGLTSI-V----------RDGLEM-----------------SG-C----------------SERVH--
Pan EGLTST-V----------RDGLEM-----------------SG-C----------------SERVH--
Gallus EGLSSL-V----------SDGNSKLLNPSTDTSPHLLTPVSVVAS-A----------------SSSPSVE
Xenopus EGALFASLHSPFRAHTPVFTEDGKHH-----------------IG-ALSEMKENNKIDVQSIKTEKIQ--
Danio EGLVSN-L----------HDGVEES-----------------EEV----------------QEEQH--
cons ** *. .
Homo ----------VQQ--AEKLPGATVSETRLRGSSMASSVASEPRSRSSSLNSTD
Canis ----------GQR--AEKPSGAPGSETRLRGSSVASSVASEPRSRSSSLNSTD
Mus ----------GQH--LEKSLGATVCETRLRGSSVASSVASEQRSRSSSLNSTD
Bos ----------GPR--AEKPPAATPSETRLRVSSVASSVASEPRSRSSSLNSTD
Rattus ----------GQH--LEKSLGGTVCEARLRGSSVASSVASEQRSRSSSFNSTD
Macaca ----------VQQ--AEKLPGATVSETRLRGSSVASSVASEPRSRSSSLNSTD
Pan ----------VQQ--AEKLPGATVSETRLRGSSMASSVASEPRSRSSSLNSTD
Gallus TDKKIVTSPSVVGDKRDSLVKDDLETPTLLDK--SFNRVGDLSNETRKRGC----SVASESRSRSSSVNSVD
Xenopus ----------LCAIEDEESLLDQGTDMRIRTCS---LPADEVKSRSSSTNSID
Danio ----------LEE--FSHAVEALEVAEDLE-QQRASLGTVESRSRSSSITSWD
cons . . : * :***** .* *
Homo SGSGLLPPGLQATPELGKGSQPLSQRFNAISSEDFDQELVVKPIKVKRKKKKKKT-EGGSRSTCHSSLESTP
Canis SGSGL-----QAALELGRGGQSTSQRFSVISSEDFDQELVVKPLKVKKR-RKKKT-EGGSRNTCHSSLESTP
Mus SGSGLLLPGLQAASELGQSGQPSSQRFSVISSEDFDQELIVKPIKVKKRRRKRKT-EGGSKSTCPSSLESTP
Bos SGSGL-----QAAPELGRGGPLTSKRFSVISSEDFDQELVVKPLRVKKKKRTKKTVEGGSSATGPSPLEPTP
Rattus SGSGLLPPGSQAAPELGHSGQPSSQRFSVISSEDFDQELIVKPIKVKKRRRKRKP-EGGSKSTCHSSLESTP
Macaca SSSGLLPPGLQAAPELGKGNQPPSQRFNAISSEDFDQELVVKPIKVKRKKKKKKT-EGGSRSTCHSSLESTP
Pan SGSGLLPPGLQATPELGKGSQPPSQRFNAISSEDFDQELVVKPIKVKRKKKKKKT-EGGSRSTCHSSLESTP
Gallus SGSSFMICADQF-SEAQRECQLSSQRFSTISSEDFDQELIVKPIKVKKKRKK-KQ-ESGSKRS-HISLEGTP

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Xenopus   SGFSLTVTAEPGSPN--NGQRPTSQRFSIISNEDFDQELVVKSMKVKKKKKKRYV-ENGLKNQDACSRESTP
Danio     SAQGMFTTTEAS------ASELSSSRYSTITQEDFQQELVVKAIKLKKKGKRRRQ-ESGNRMNERSSWSESI

cons      *. .:            *.*:. *:.***:***:**.:::*:: :    *.*     . . :


Homo      CSE--FPGDSPQSLNTDLL---SMTSSVL-GSSVDQLSAESPDQESSFNGEVNGVPQENTDPETFNVLEVSG
Canis     CYE--FPGDSPQSLNTDLL---SMTSS-L-GSIVDRSSTESPDQDSNLSGEVNGVLQENLDPEAFSVLEVPA
Mus       CSE--LPADSPQSLNTDLL---SMTSSAL-GSSMDQLSTESPEQESNLGGEVNGISQENSGPEAFHVLELPG
Bos       GRE--CPGDSPQSVSTDLP---SAASS-L-GSIVDRTSTGSPDQESSLSREASGVLLEYSDPEAFSVLEVPL
Rattus    CSE--LPADSPQSLSTDLV---SLASSVLSGSSVDQLSTEPPDQEISPSGELDGIIQDNNGPQTLHDLEVPG
Macaca    CSE--FPGDSPQSLNTDLL---SMTSSVL-GSSMDQLSAESPDLESGFSGEVNGVPQENTDPETFNVLEVPG
Pan       CSE--FPGGSPQSLNTDLL---SMTSSVL-GSSVDQLSAESPDQESGFNGEVNGVPQENTDPETFNVLEVSG
Gallus    NYERQLSGDSPHSVNADSF---SMTSSLM-SGSIDHLSTGSPDQESMFSVESNTVLQEDNGSETFSILQSPE
Xenopus   ST------DSPLTLNSDFF---SATEICP-VSSIDHLSS----QESLFSGKNQGVLDNSI--DVFSAVQPTE
Danio     FSQ--DG-AGSDVLNTPMSEPPSDHTSLL-CSSLDL-DGEYHS-SDAFPIQSPSEPLLNQEPCLVAVNQAAQ

cons      .. :.:  *  . :*  .  .  :  .  : .


Homo      SMPDSL----AEEDDI-RTEMPH---CHHAHGRELLNGA---------REDVG-GSDV-TGLGDEPCPADDG
Canis     LAPDVL----NEGNGD-RPGILP---CNRAGEIGPHSGVWTSLL--ELREAME-GSDV-TELREESCPTDDG
Mus       PAPGPASTPTDEENCT-GKEASQ---CSHTQDTDLLSGVLTLPL--QPEEDVG-GADIISGLDEQPGPAADA
Bos       PAPDLL----NGGAEG-RTESPL---GNGEGGTEPPCGAGSSSW--HPTEFAEDTEDI-VELEEEPQPADSG
Rattus    STPSPV----DEENSP-RKEASQ---CDHTQDTDLLKGVLTLPL--QPGEDEG-DADVTSELEEKPGPAADA
Macaca    SAPDFL----AVEDDI-RTEMPH---CHHAYGQELLNGV---------RKDVR-GSDV-TGLEDEPCPADDG
Pan       SVPDSL----AEEDDI-RTEMPH---CHHAHGRELLNGA---------REDVG-GSDV-TGLEDEPCPADDG
Gallus    SATVLI----NEENGD-TADLQKLPNSDSGMGTSTIIDALTSVSPPILTEDLT-STVV-TENNHCVFSTSSE
Xenopus   TFPTIL----GNGSNPPAKQTE----------EL--------------------LDVNSDNNADPLPPA--
Danio     TS------------------------------------------------------------PPCPASLT

cons      .. .


Homo      PNSTQLPFQEQDSSPGAHDG---EDIQPIGPQSTFCEVPL-LNSLTVPSSLSWAPSAEQ-WLPGT-------
Canis     PSSGQSPCQEQDSSAEAREV---GDMEPDDSQSTFSEAPF-LNSPPVPSSLSWASHAEQ-RLLAT-------
Mus       AVHTEFCLRGQNSSAEEQEA---DTMEFSDPQSTFSEAPL-LDSSMLPSSLSWPPGAEQ-WLPGI-------
Bos       PGS----------TAEARGD---GAVQPNGPPGAFSEACL-LDSAPEPSHLSWAPSAEQ-RLPGA-------
Rattus    AIHTELCLQEQNSSAEQQEV---DTMEFSDPQSTFSEAPL-LDSSMLPASLSWPPGAEQ-WLPEI-------
Macaca    PHSTQIPFQEQHSSPGVQNG---EDVQPIGPQSTFCEAPL-LNSLTVPSSLSLAPSAGQ-WLPGT-------
Pan       PNSTQLPFQEQDSSPGAHDG---EDVQPIGPQSTFCEVPL-LNSLTVPSSLSWAPSAEQ-WLPGT-------
Gallus    CCLGKLGQEEQDFSRLCKINEDLDKMKLQDGEKCFEEP-D-Q--TLLEC--SSVLGVQT-SLTVN-------
Xenopus   ---------ECDSISA----------------VNLLMTDPLELNVLMVKNNAAFLPEKDPDYIDCCAVDKCPT
Danio     -LDMEC-------------------------------QLYS-ENVFAENEQIAATPDVDM-LLECT-------

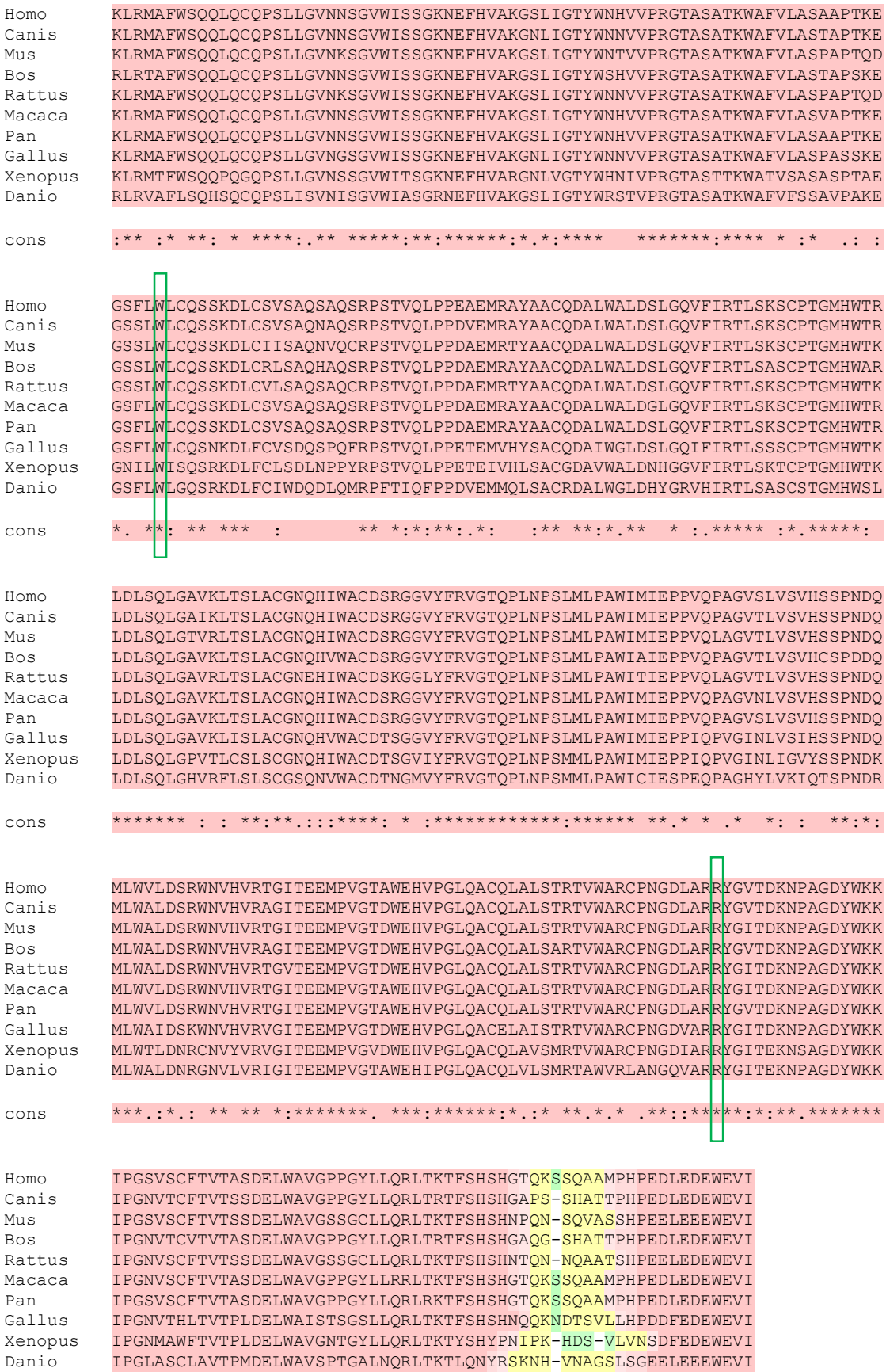
cons      :  :  :


Homo      --------------RADEGSPVEPSQEQDILTSMEASG-------------HLSTNLWHAVTDDDTGQKEIP
Canis     --------------SADEGSAEEPSKEQGFLMHMGGPG-------------HLGSTPWHAVTDSDTGLKEVI
Mus       --------------GVYDITTETASPEADILTHVAVPS-------------HLSSNPWHLLTDSNTGQKETP
Bos       --------------TADEGRAEKPPEEQGFLTHMGAPH-------------NLSPSPQRTAPDADTGQTVVV
Rattus    --------------EGYEVSTEEASPEADILTQAEVPS-------------HLSSNPWHLLTDS---QKEAP
Macaca    --------------GADEGSPVEPSQEQDVLTSMEASG-------------HLSTNLWHVGTDDDTGQKETP
Pan       --------------RADEGSPVEPSQEQDVLTSMEASG-------------HLSTNLWHAVTDDDTGQKEIP
Gallus    --------------ESDETRREDQQQNSLTVTALSVPSVHHFDICEGVDSEHSSISGWNFE-----------
Xenopus   VLETLSSKNCNIAYGLEEEKPLESNKDEILILDEDQQF-------------QEKSRTWQVSSS---------
Danio     --------------F----SYMQASDEQDIMKKYV-KE-------------HK-----DVLEDSEEHFKQSL

cons      :  :  :


Homo      ISERVLGSVGGQLTPV-SAL------AASTHKPWLEQPPRDQTLTSSDEEDIYAHGLPSSSSETSVTELGPS
Canis     APECDLGTVGGRLTPPVSAL------AVCPREPRHEQPSRDQVLTSSDEEDIYAHGLPSSSSETSVTELGGS
Mus       TFQCDMGNVEGQVTPLASAS------VASTHAHWLDQPFQDQAVTSSDEEDIYAHGLPSSSSETSVTELGAG
Bos       TSKRDLGG-GGRLPPRTSA-------AS-----SQEQPPRDQVLTSSDEEDIYGHGLPSSSSETSVTELGGG
```


Rattus AFECDMGNVKGQVTPLTSAL------VANTHAHWLDQPFQDQAVTSSDEEDIYAHRLPSSSSETSVTELGAG
Macaca FSERDLGSVGGQLTPV-SAL------AASTPKAWLEQPPRDQALTSSDEEDIYAHGLPSSSSEASVTELGPS
Pan ISERVLGSVGGQLTLV-SAL------AASTHKPWPEQPPRDQTLTSSDEEDIYAHGLPSSSSETSVTELGPS
Gallus ------SVIKGKSSTSTAEW------VTNTH-----ESKAEEESASSDEEDIYGHGLPYSSSETSVPEVGAE
Xenopus -------F--IEDSPLNSTYCTSQSPSNDSEEQRIEGGNQDPMLASSDEEDIYGHRFPHSTSGSSVADVAEM
Danio HEDFNLDLS---YDPI-RP---------------LGYSPEPEPSPSSDEEDIYAHGVPSSASLGDGLN---A
cons .******** .* :*
Homo CSQQD--LSRLGAEDAGLLKPDQFAESWMGYSGPGYGILSLVVSEKYIWCLDYKGGLFCSALPGAGLRWQKF
Canis RSLQD--LSQPGTDDTGLLKSDQFAESWMGYSGPGYGILSLAVSEKYIWCLDYKGGLFCSALPGAGLRWQKF
Mus RSLQD--LSQPGAEETTLLKADQFAESWMGYSAPGYGILSLAVSEKFLWCLDYKGGLFCSPLPGAGLRWQRF
Bos RSLQD--LRQLGTDDPGLLKADQFAESWMGYSGPGHGILSLAVSEKYLWCLDYKGGLFCSALPGAGLRWQKF
Rattus RSLQD--LSQPGAEETSLLKADQFAESWMGYSGPGYGILSLAVSEKFIWCLDYKGGLFCSALPGAGLRWQRF
Macaca RSQQD--LSRLGADDAGLLKPDQFAESWMGYSGPGYGILSLVVSEKYIWCLDYKGSLFCSALPGAGLRWQKF
Pan CSQQD--LSQLGAEDAGLLKPDQFAESWMGYSGPGYGILSLVVSEKYIWCLDYKGGLFCSALPGAGLRWQKF
Gallus PGSQD--MTRISLDEMVLLKSDQFAESWMGYSGPGYGILSLAVSEKYIWCLDYRGSLYCSALPAAGLRWQKF
Xenopus TASQD--MACSTQEVSGLLKSEQLAESWMVYSGPPNGILSLVVSEKYIWCLDYKGSLYCSALPGAGLRWQKF
Danio LSLQSSSAEQKEDEETQLLKADQLAESWMGYSGPGCGILSLVVTDRYIWCLDFKGGLFCSGLSNGGLNWQKF
cons . *. : ***.:*:***** **.* *****.*:::::****::*.*:** *. .**.**:*
Homo EDAVQQVAVSPSGALLWKIEQKSNRAFACGKVTIKGKRHWYEALPQAVFVALSDDTAWIIRTSGDLYLQTGL
Canis EDAVQQVAVSPSGALLWKIEQKSNRAFACGKVTIKGKRHWYEALPQAVFVALSDDTAWIIRTNGDLYLQTGL
Mus EDAVQQMAVSPSGALLWKIEQKSNRAFACGKVTIKGKRHWYEALPQAVFVALSDDTAWIIRTNGDLYLQTGL
Bos EDAVQQVAVSPSGALLWKIEQKSNRAFACGKVTIKGKRHWYEALPQAVFVALSDDTAWIIRTNGDLYLQTGL
Rattus EDAVQQMAVSPSGALLWKIEQKSNRAFACGKVTIKGKRHWYEALPQAVFVTLSDDTAWIIRTNGDLYLQTGL
Macaca EDAVQQVAVSPSGALLWKIEQKSNRAFACGKVTIKGKRHWYEALPQAVFVALSDDTAWIIRTSGDLYLQTGL
Pan EDAVQQVAVSPSGALLWKIEQKSNRAFACGKVTIKGKRHWYEALPQAVFVALSDDTAWIIRTSGDLYLQTGL
Gallus EDGVQQVAVSPSGALLWKIEQKTNKAFACGKVTIKGKRHWYEALPQAVFVALSDDTAWIIRTNGDLYLQTGL
Xenopus EDGVQQVAVSPSGALLWKVEQRSDKAFACGKVTIKGKRHWYEALPQAAFVALSDDTAWIIRTNGDLYLQTGL
Danio EENVHQVALSPSGSLLWKVEQKTMTAYACGKVTIKGKRHWYKALDDTAFVALGDDTAWIIRTNGDLYIQTGL
cons *: *:*:*:****:****:**:: *:*************** ::.**:*.*********.****:****
Homo SVDRPCARAVKVDCPYPLSQITARNNVVWALTEQRALLYREGVSSFCPEGEQWKCDIVSERQALEPVCITLG
Canis SVDRPCARAVKVDCPYPLSQVTARNSVVWALTEQRALLYREGVSSFCPEGEQWKCDIVSEKQALEPVCITLG
Mus SVDRPCARAVKVDCPYPLSQIAARNSVVWALTEQRALLYREGVSSFCPEGEQWKCDIVSERQTLEPVCITLG
Bos SVDRPCARAVKVDCPFPLSQVTARNSVVWALTEQRALLYREGVSSFCPEGELWKCDIVSERQALEPVCVTLG
Rattus SVDRPCARAVKVDCPYPLSQITARNSVVWALTEQRALLYREGVSSFCPEGEQWKCDIVSERQALEPVCITLG
Macaca SVDRPCARAVKVDCPYPLSQITARNNVVWALTEQRALLYREGVSSFCPEGEQWKCDIVSERQALEPVCITLG
Pan SVDRPCARAVKVDCPYPLSQITAQNNVVWALTEQRALLYREGVSSFCPEGEQWKCDIVSERQALEPVCITLG
Gallus SVDRPCARAVKVDCPYPLSQVTSRNNVVWALSEQRALLYREGVRSFCPEGEQWKSDIVSEMQALEPVCITLG
Xenopus SVDRPCARAVKVDCPCPLSQITARNHVVWALTEQRGLLFREGVNSFSPEGEAWKCDLISERQALEPVCVTLG
Danio SADRPCARSVKVDCPCPMAQMAARGGVVWALSEQRGVFYREGLNSYCAEGEHWKYDTVSESQGLEPICIALG
cons *.******:****** *::*::::. *****:***.:::***: *:..*** ** * :** * ***:*::**
Homo DQQTLWALDIHGNLWFRTGIISKKPQGDDDHWWQVSITDYVVFDQCSLFQTIIHATHSVATAAQAPVEKVAD
Canis DQQTLWALDIRGNLWFRTGIVSKKPQGDDDHWWQVSITDYVVFDQCSLFQTIIHATHSVATAAQVPVEKVAD
Mus DQHTLWALDIHGKLWFRTGVVPKKPQGDDDHWWQVSITDYVVFDQCSLFQTIMHATHSVATAAQAPVEKVAD
Bos DQQSLWALDVRGNLWFRTGIVSKKPQGDDDHWWQVSVTDYVVFDQCSLFQTVIRATHSVATVAQAPVEKVAD
Rattus DQHTLWALDIHGKLWFRTGVIPKKPQGDDDHWWQVSITDYVVFDQCSLFQTIMHATHSVATAAQAPVEKVAD
Macaca DQQTLWALDIHGNLWFRTGIVSKTPQGDDDHWWQVSITDYVVFDQCSLFQTIIHATHSVATAAQAPVEKVAD
Pan DQQTLWALDIHGNLWFRTGIISKKPQGDDDHWWQVSITDYVVFDQCSLFQTIIHATHSVATAAQAPVEKVAD
Gallus DQQTLWALDIHGNLWFRTGIVSKKPQGDDNHWWQVSITDYVVFDQCSLFQTIIQATHSVATAAQAPVEKVAD
Xenopus DQDTVWILDASGNLWFRSGITAKNPQGEDDHWWQVNITDYVVFDHYTLFQTLLQATQTVATVAQAPVEKVAD
Danio ENNTVWALDTSGNLWFRTGVTAKKPQGEDDHWWQVSITDYVVFDQGSLFQTLIQATQTVATVTRAPVERVAE
cons ::.::* ** *:****:*: .*.***:*:*****.:*******: :****:::**::***.::.***:**:


Homo KLRMAFWSQQLQCQPSLLGVNNSGVWISSGKNEFHVAKGSLIGTYWNHVVPRGTASATKWAFVLASAAPTKE
Canis KLRMAFWSQQLQCQPSLLGVNNSGVWISSGKNEFHVAKGNLIGTYWNNVVPRGTASATKWAFVLASTAPTKE
Mus KLRMAFWSQQLQCQPSLLGVNKSGVWISSGKNEFHVAKGSLIGTYWNTVVPRGTASATKWAFVLASPAPTQD
Bos RLRTAFWSQQLQCQPSLLGVNNSGVWISSGKNEFHVARGSLIGTYWSHVVPRGTASATKWAFVLASTAPSKE
Rattus KLRMAFWSQQLQCQPSLLGVNKSGVWISSGKNEFHVAKGSLIGTYWNNVVPRGTASATKWAFVLASPAPTQD
Macaca KLRMAFWSQQLQCQPSLLGVNNSGVWISSGKNEFHVAKGSLIGTYWNHVVPRGTASATKWAFVLASVAPTKE
Pan KLRMAFWSQQLQCQPSLLGVNNSGVWISSGKNEFHVAKGSLIGTYWNHVVPRGTASATKWAFVLASAAPTKE
Gallus KLRMAFWSQQLQCQPSLLGVNGSGVWISSGKNEFHVAKGNLIGTYWNNVVPRGTASATKWAFVLASPASSKE
Xenopus KLRMTFWSQQPQGQPSLLGVNSSGVWITSGKNEFHVARGNLVGTYWHNIVPRGTASTTKWATVSASASPTAE
Danio RLRVAFLSQHSQCQPSLISVNISGVWIASGRNEFHVAKGSLIGTYWRSTVPRGTASATKWAFVFSSAVPAKE
cons :** :* **: * ****:.** *****:**:******:*.*:**** *******:**** * :* .: :
Homo GSFLWLCQSSKDLCSVSAQSAQSRPSTVQLPPEAEMRAYAACQDALWALDSLGQVFIRTLSKSCPTGMHWTR
Canis GSSLWLCQSSKDLCSVSAQNAQSRPSTVQLPPDVEMRAYAACQDALWALDSLGQVFIRTLSKSCPTGMHWTR
Mus GSSLWLCQSSKDLCIISAQNVQCRPSTVQLPPDAEMRTYAACQDALWALDSLGQVFIRTLSKSCPTGMHWTK
Bos GSSLWLCQSSKDLCRLSAQHAQSRPSTVQLPPDAEMRAYAACQDALWALDSLGQVFIRTLSASCPTGMHWAR
Rattus GSSLWLCQSSKDLCVLSAQSAQCRPSTVQLPPDAEMRTYAACQDALWALDSLGQVFIRTLSKSCPTGMHWTK
Macaca GSFLWLCQSSKDLCSVSAQSAQSRPSTVQLPPDAEMRAYAACQDALWALDGLGQVFIRTLSKSCPTGMHWTR
Pan GSFLWLCQSSKDLCSVSAQSAQSRPSTVQLPPDAEMRAYAACQDALWALDSLGQVFIRTLSKSCPTGMHWTR
Gallus GSFLWLCQSNKDLFCVSDQSPQFRPSTVQLPPETEMVHYSACQDAIWGLDSLGQIFIRTLSSSCPTGMHWTK
Xenopus GNILWISQSRKDLFCLSDLNPPYRPSTVQLPPETEIVHLSACGDAVWALDNHGGVFIRTLSKTCPTGMHWTK
Danio GSFLWLGQSRKDLFCIWDQDLQMRPFTIQFPPDVEMMQLSACRDALWGLDHYGRVHIRTLSASCSTGMHWSL
cons *. **: ** *** : ** *:*:**:.*: :** **:*.** * :.***** :*.*****:
Homo LDLSQLGAVKLTSLACGNQHIWACDSRGGVYFRVGTQPLNPSLMLPAWIMIEPPVQPAGVSLVSVHSSPNDQ
Canis LDLSQLGAIKLTSLACGNQHIWACDSRGGVYFRVGTQPLNPSLMLPAWIMIEPPVQPAGVTLVSVHSSPNDQ
Mus LDLSQLGTVRLTSLACGNQHIWACDSRGGVYFRVGTQPLNPSLMLPAWIMIEPPVQLAGVTLVSVHSSPNDQ
Bos LDLSQLGAVKLTSLACGNQHVWACDSRGGVYFRVGTQPLNPSLMLPAWIAIEPPVQPAGVTLVSVHCSPDDQ
Rattus LDLSQLGAVRLTSLACGNEHIWACDSKGGLYFRVGTQPLNPSLMLPAWITIEPPVQLAGVTLVSVHSSPNDQ
Macaca LDLSQLGAVKLTSLACGNQHIWACDSRGGVYFRVGTQPLNPSLMLPAWIMIEPPVQPAGVNLVSVHSSPNDQ
Pan LDLSQLGAVKLTSLACGNQHIWACDSRGGVYFRVGTQPLNPSLMLPAWIMIEPPVQPAGVSLVSVHSSPNDQ
Gallus LDLSQLGAVKLISLACGNQHVWACDTSGGVYFRVGTQPLNPSLMLPAWIMIEPPIQPVGINLVSIHSSPNDQ
Xenopus LDLSQLGPVTLCSLSCGNQHIWACDTSGVIYFRVGTQPLNPSMMLPAWIMIEPPIQPVGINLIGVYSSPNDK
Danio LDLSQLGHVRFLSLSCGSQNVWACDTNGMVYFRVGTQPLNPSMMLPAWICIESPEQPAGHYLVKIQTSPNDR
cons ******* : : **:**.:::****: * :************:****** **.* * .* *: : **:*:
Homo MLWVLDSRWNVHVRTGITEEMPVGTAWEHVPGLQACQLALSTRTVWARCPNGDLARRYGVTDKNPAGDYWKK
Canis MLWALDSRWNVHVRAGITEEMPVGTDWEHVPGLQACQLALSTRTVWARCPNGDLARRYGVTDKNPAGDYWKK
Mus MLWALDSRWNVHVRTGITEEMPVGTDWEHVPGLQACQLALSTRTVWARCPNGDLARRYGITDKNPAGDYWKK
Bos MLWALDSRWNVHVRAGITEEMPVGTDWEHVPGLQACQLALSARTVWARCPNGDLARRYGVTDKNPAGDYWKK
Rattus MLWALDSRWNVHVRTGVTEEMPVGTDWEHVPGLQACQLALSTRTVWARCPNGDLARRYGITDKNPAGDYWKK
Macaca MLWVLDSRWNVHVRTGITEEMPVGTAWEHVPGLQACQLALSTRTVWARCPNGDLARRYGITDKNPAGDYWKK
Pan MLWVLDSRWNVHVRTGITEEMPVGTAWEHVPGLQACQLALSTRTVWARCPNGDLARRYGVTDKNPAGDYWKK
Gallus MLWAIDSKWNVHVRVGITEEMPVGTDWEHVPGLQACELAISTRTVWARCPNGDVARRYGITDKNPAGDYWKK
Xenopus MLWTLDNRCNVYVRVGITEEMPVGVDWEHVPGLQACQLAVSMRTVWARCPNGDIARRYGITEKNSAGDYWKK
Danio MLWALDNRGNVLVRIGITEEMPVGTAWEHIPGLQACQLVLSMRTAWVRLANGQVARRYGITEKNPAGDYWKK
cons ***.:*.: ** ** *:*******. ***:******:*.:* **.*.* .**::*****:*:**.*******
Homo IPGSVSCFTVTASDELWAVGPPGYLLQRLTKTFSHSHGTQKSSQAAMPHPEDLEDEWEVI
Canis IPGNVTCFTVTSSDELWAVGPPGYLLQRLTRTFSHSHGAPS-SHATTPHPEDLEDEWEVI
Mus IPGSVSCFTVTSSDELWAVGSSGCLLQRLTKTFSHSHNPQN-SQVASSHPEELEEEWEVI
Bos IPGNVTCVTVTASDELWAVGPPGYLLQRLTRTFSHSHGAQG-SHATTPHPEDLEDEWEVI
Rattus IPGNVSCFTVTSSDELWAVGSSGCLLQRLTKTFSHSHNTQN-NQAATSHPEELEDEWEVI
Macaca IPGNVSCFTVTASDELWAVGPPGYLLRRLTKTFSHSHGTQKSSQAAMPHPEDLEDEWEVI
Pan IPGSVSCFTVTASDELWAVGPPGYLLQRLRKTFSHSHGTQKSSQAAMPHPEDLEDEWEVI
Gallus IPGNVTHLTVTPLDELWAISTSGSLLQRLTKTFSHSHNQQKNDTSVLLHPDDFEDEWEVI
Xenopus IPGNMAWFTVTPLDELWAVGNTGYLLQRLTKTYSHYPNIPK-HDS-VLVNSDFEDEWEVI
Danio IPGLASCLAVTPMDELWAVSPTGALNQRLTKTLQNYRSKNH-VNAGSLSGEELEEEWEVI

```
cons        ***  : .:**. *****:. .* * :** :* .:  .          .::*:*****
```

For Rab5:

MSA*The multiple sequence alignment result as produced by T-coffee.*

```
T-COFFEE, Version_11.00 (Version_11.00)
Cedric Notredame
SCORE=999
*
 BAD AVG GOOD
*
Homo        :  99
Canis       :  99
Mus         :  99
Bos         :  99
Macaca      :  99
Gallus      : 100
Xenopus     : 100
Danio       :  99
cons        :  99

Homo        MASRG-ATRPNGPNTGNKICQFKLVLLGESAVGKSSLVLRFVKGQFHEFQESTIGAAFLTQTVCLDDTTVKF
Canis       MANRG-ATRPNGPNTGNKICQFKLVLLGESAVGKSSLVLRFVKGQFHEFQESTIGAAFLTQTVCLDDTTVKF
Mus         MANRG-ATRPNGPNTGNKICQFKLVLLGESAVGKSSLVLRFVKGQFHEFQESTIGAAFLTQTVCLDDTTVKF
Bos         MANRG-ATRPNGPNTGNKICQFKLVLLGESAVGKSSLVLRFVKGQFHEFQESTIGAAFLTQTVCLDDTTVKF
Macaca      MANRG-ATRPNGPNTGNKICQFKLVLLGESAVGKSSLVLRFVKGQFHEFQESTIGAAFLTQTVCLDDTTVKF
Gallus      ------------------------------------------------------AFLTQTVCLDDTTVKF
Xenopus     ----------------------------------------------------ESTIGAAFLTQTLCLDDTTVKF
Danio       MANRGGATRPNGSNAGNKICQFKLVLLGESAVGKSSLVLRFVKGQFHEFQESTIGAAFLTQTLCLDDTTVKF

cons                                                          ******:*********


Homo        EIWDTAGQERYHSLAPMYYRGAQAAIVVYDITNEESFARAKNWVKELQRQASPNIVIALSGNKADLANKRAV
Canis       EIWDTAGQERYHSLAPMYYRGAQAAIVVYDITNEESFARAKNWVKELQRQASPNIVIALSGNKADLANKRAV
Mus         EIWDTAGQERYHSLAPMYYRGAQAAIVVYDITNEESFARAKNWVKELQRQASPNIVIALSGNKADLANKRAV
Bos         EIWDTAGQERYHSLAPMYYRGAQAAIVVYDITNEESFARAKNWVKELQRQASPNIVIALSGNKADLANKRAV
Macaca      EIWDTAGQERYHSLAPMYYRGAQAAIVVYDITNEESFARAKNWVKELQRQASPNIVIALSGNKADLANKRAV
Gallus      EIWDTAGQERYHSLAPMYYRGAQAAIVVYDITNEESFARAKNWVKELQRQASPNIVIALAGNKADLANKRAV
Xenopus     EIWDTAGQERYHSLAPMYYRGAQAAIVVYDITNTETFARAKNWVKELQRQASPNIVIALSGNKADLSSKRAV
Danio       EIWDTAGQERYHSLAPMYYRGAQAAIVVYDITNEESFARAKNWVKELQRQASPNIVIALSGNKADLANKRAV

cons        ******************************** *:**********************:******:.****


Homo        DFQEAQSYADDNSLLFMETSAKTSMNVNEIFMAIAKKLPKNEPQNPGANSARGRGVDLTEPTQPTRNQCCSN
Canis       DFQEAQSYADDNSLLFMETSAKTSMNVNEIFMAIAKKLPKNEPQNPGANSARGRGVDLTEPTQPTRSQCCSN
Mus         DFQEAQSYADDNSLLFMETSAKTSMNVNEIFMAIAKKLPKNEPQNPGANSARGRGVDLTEPAQPARSQCCSN
Bos         DFQEAQSYADDNSLLFMETSAKTSMNVNEIFMAIAKKLPKNEPQNPGANSTRGRGVDLTEPTQPTRSQCCSN
Macaca      DFQEAQSYADDNSLLFMETSAKTSMNVNEIFMAIAKKLPKNEPQNPGANSARGRGVDLTEPTQPTRSQCCSN
Gallus      DFQEAQAYADDNSLLFMETSAKTSMNVNEIFMAIAKKLPKNEPQNTGASSARGRGVDLTEPTQPPKSQCCSN
Xenopus     DFQEAQAYADDNSLLFMETSAKTAMNVNEIFMAIAKKLPKNEPQNAQGNTGRNRGVDLQENNPPSRSQCCSN
Danio       DFQDAQSYADDNSLLFMETSAKTSMNVNEIFMAIAKKLPKSEPQAAGANSGRSRGVDLTETAQPTKAPCCSN

cons        ***:**:****************:***************.*** . ..: *.***** *   *.:  ****
```