Supplementary Materials

KINESIN ADAPTER JLP LINKS PIKfyve TO MICROTUBULE-BASED ENDOSOME-TO-TGN TRAFFIC OF FURIN

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1. Supplementary Experimental Procedures

Construction of PIKfyve-cFab1 chimera resistant to mouse PIKfyve siRNAs

Yeast Fab1 is the functional and structural ortholog of PIKfyve (10, 43). The two enzymes are highly homologous in their evolutionary conserved domains, including the C-terminally positioned PI kinase domain and share similar *in vitro* lipid kinase activities. The related intracellular functions of the two enzymes are underscored by the data demonstrating that ectopically expressed Fab1 rescues defects due to PIKfyve dysfunction in mammalian cells and, *vice versa*, ectopically expressed PIKfyve rescues all defects in yeast strains with *Fab1* deletion (10, 16, 50). Two out of the four siRNA duplexes of the mouse PIKfyve Smart pool (M-0040127-01) target regions within the PIKfyve catalytic domain (at nt 5583 and nt 5764). Therefore, to obtain an siRNA-resistant PIKfyve construct, we have substituted the C-terminal sequence in PIKfyve (residues 1813 - 2052, spanning nucleotides 5572-6297) with the corresponding fragment in Fab1 (residues 2041-2278, spanning nucleotides 6979-7694) to generate a PIKfyve-cFab1 chimera. The 238 aa-fragment in Fab1 and the corresponding 240-aa-fragment in PIKfyve, while highly homologous at the protein levels (see Fig. 2C in Ref. 43) differ quite significantly at the nucleotide level. Concordantly, the two C-terminal mouse PIKfyve siRNA duplexes scored below 30% homology vs. the Fab1 C-terminus nucleotide sequence used in the substitution, as revealed by BLASTn and ClustalW analyses (data not shown). To generate the PIKfyve$^{1-1812}$-Fab1^{Ct} chimera (PIKfyve-cFab1), PCR fragments flanked with Sma1 (sense primer, 5'-GATAACCCGGGGGGGGAAAAGTG, complementary to nucleotides 6979-6991) or Sal1 (antisense primer, 5'- GGCGGTCGACTTAATTTCCTTCCCTATAC, complementary to nucleotides 7677-7694) were used to amplify the nucleotide sequence between nt 6979 and 7694 of Fab1 cDNA using clone pRS416 as a template (kindly provided by Dr. Scott Emr). The Sma1/Sal1 PCR fragment (714 bp) together with the Kpn1/Sma1 piece (2.6 kb) from pBluescript II SK^{+}-PIKfyve^{WT} (43) were subcloned into the Kpn1/Sal1 digest of pGEM4Z. The Kpn1/Sal1 fragment of the new vector was then released with Kpn1 and Sal1 and then used to replace the Kpn1/Sal1 fragment from pCMV5-HA-PIKfyve^{WT}. The new pCMV5-HA-PIKfyve-cFab1 construct was verified by sequencing, restriction endonuclease mapping and western blotting.

2. Supplementary Figure

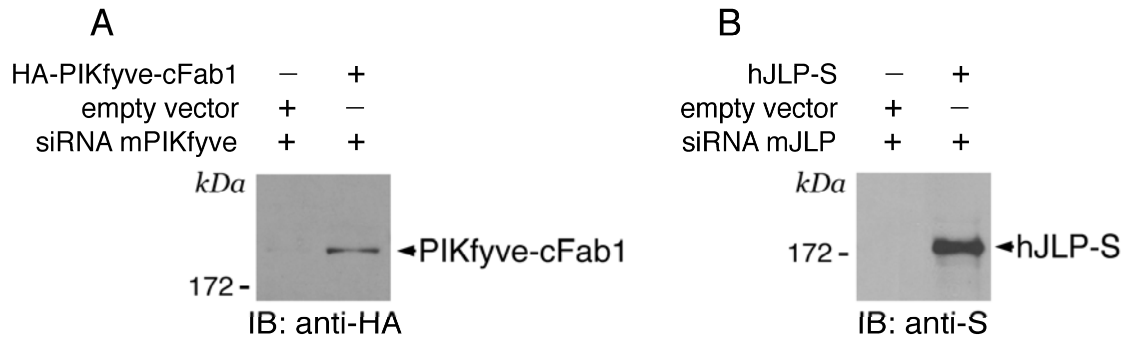

Fig. S1. Expression of HA-PIKfyve-cFab1 chimera and hJLP^{WT}-S under silencing of endogenous PIKfyve and JLP in TRVb1/TTF cells

The CHO-based TRVb1/TTF cells were transfected by Oligofectamine with the mouse-specific siRNA duplexes (100 nM) targeting specific regions in mouse JLP or PIKfyve. Forty-eight hours later, cells were cotransfected with the pCMV5-HA-PIKfyve-cFab1 cDNA or the empty pCMV5 vector (A) or with pcDNA3-hJLP^{WT}-S or the empty pcDNA3 vector (B) as indicated, using Lipofectamine as a transfection reagent. On the next day, cells were lysed and analyzed by SDS-PAGE and immunoblotting with the indicated antibodies. Apparent is expression of HA-PIKfyve-cFab1 or hJLP-S in the background of knock-down of the respective endogenous proteins. Shown are chemiluminescence detections of representative immunoblots out of two independent experiments with similar results.