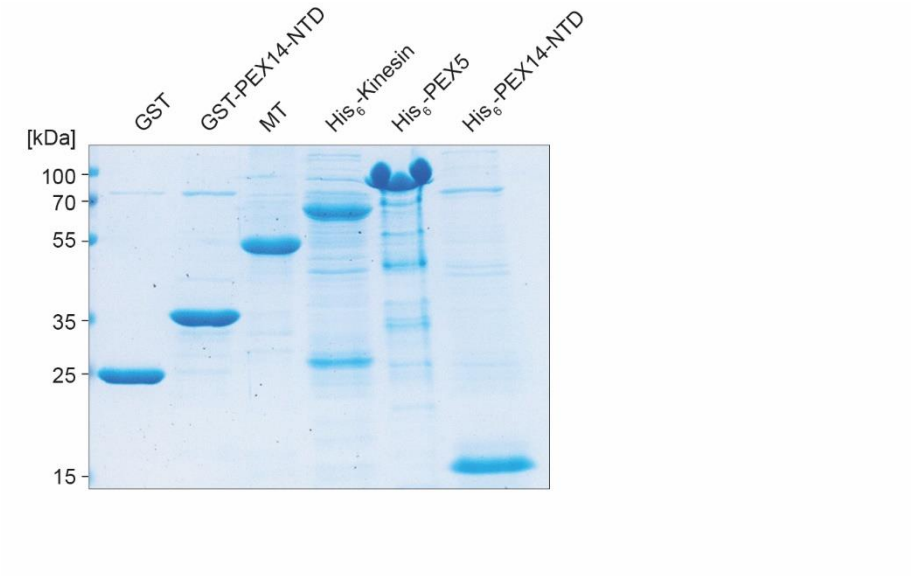
Supplemental Material

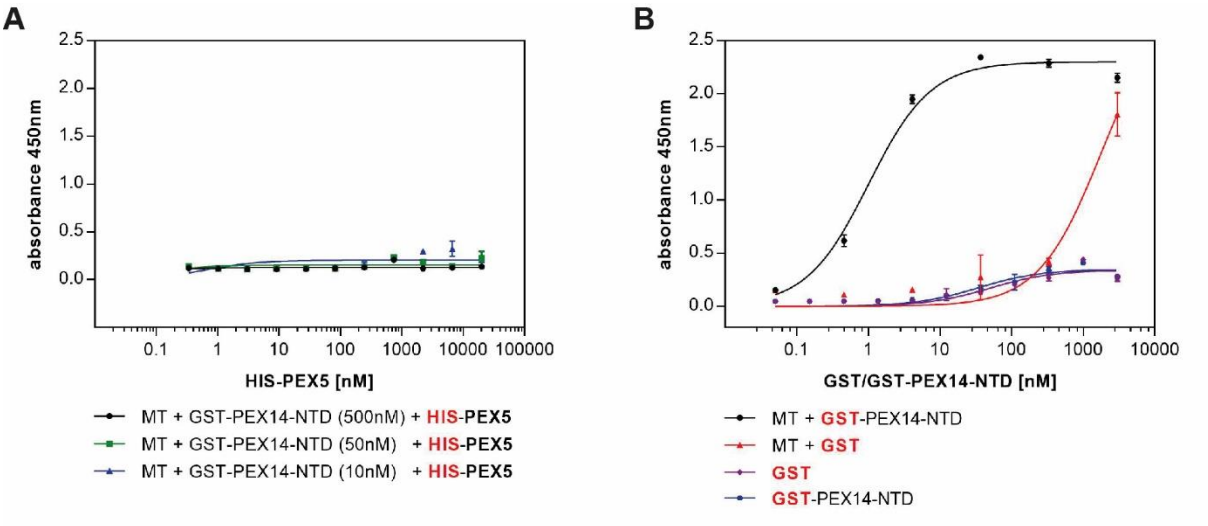
Supplementary Table 1. Oligonucleotides used in this study

DNA constructs	Primer pair sequences (5'-3')	Expression vector
GST-PEX14-NTD K56A	(s)CAGGAGAGCATTTCTAAAGAAGGCAGGGCTGACAGATGAAG (as)CTTCATCTGTCAGCCCTGCCTTCTTTAGAAATGCTCTCCTG	pGEX-4T
GST-PEX14-NTD F52A	(s)CCCTTTCTTCTTTAGAGCTGCTCTCCTGGTTGCAAGTGG (as)CCCACTTGCAACCAGGAGAGCAGCTCTAAAGAAGAAAGGGC	pGEX-4T
GST-PEX14-NTD K54A	(s)CAACCAGGAGAGCATTTCTAGCGAAGAAAGGGCTGACAGATG (as)CATCTGTCAGCCCTTTCTTCGCTAGAAATGCTCTCCTGGTTG	pGEX-4T
GST-PEX14-NTD F52AK56A	(s)CCCTTTCTTCTTTAGAGCTGCTCTCCTGGTTGCAAGTGG (as)CCCACTTGCAACCAGGAGAGCAGCTCTAAAGAAGAAAGGGC	pGEX-4T
*Hs*PEX26	(s)GATCTGTACAAGTCCGGACTCAGATCTAAG (as)CTTCATCTGTCAGCCCTGCC TTCTTTAGAAATGCTCTCCTG	pIRES2-*Hs*PEX5L-EGFP
ß-tubulin(388-444)	(s)AATTCCATGGATCCA ATGTTCCGCCGGAAGGCC (as)TTTTCTCGAGAATTCTTAGGCCTCCTCTTCGGCCTCC	pPROEX HTa

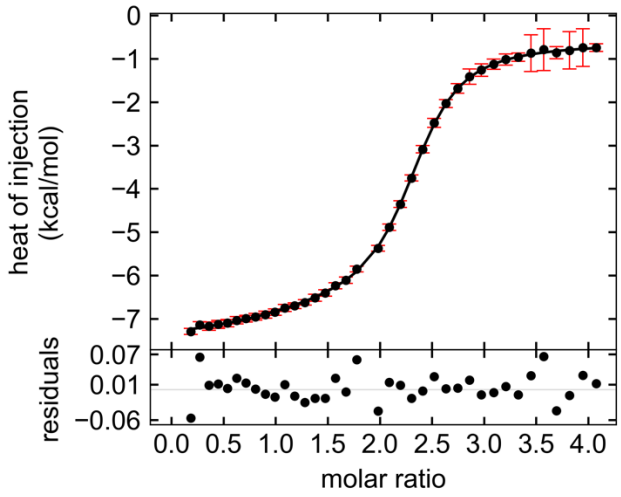
Supplementary Table 1. Abbreviations used: Oligonucleotides (s), sense; (as), antisense.



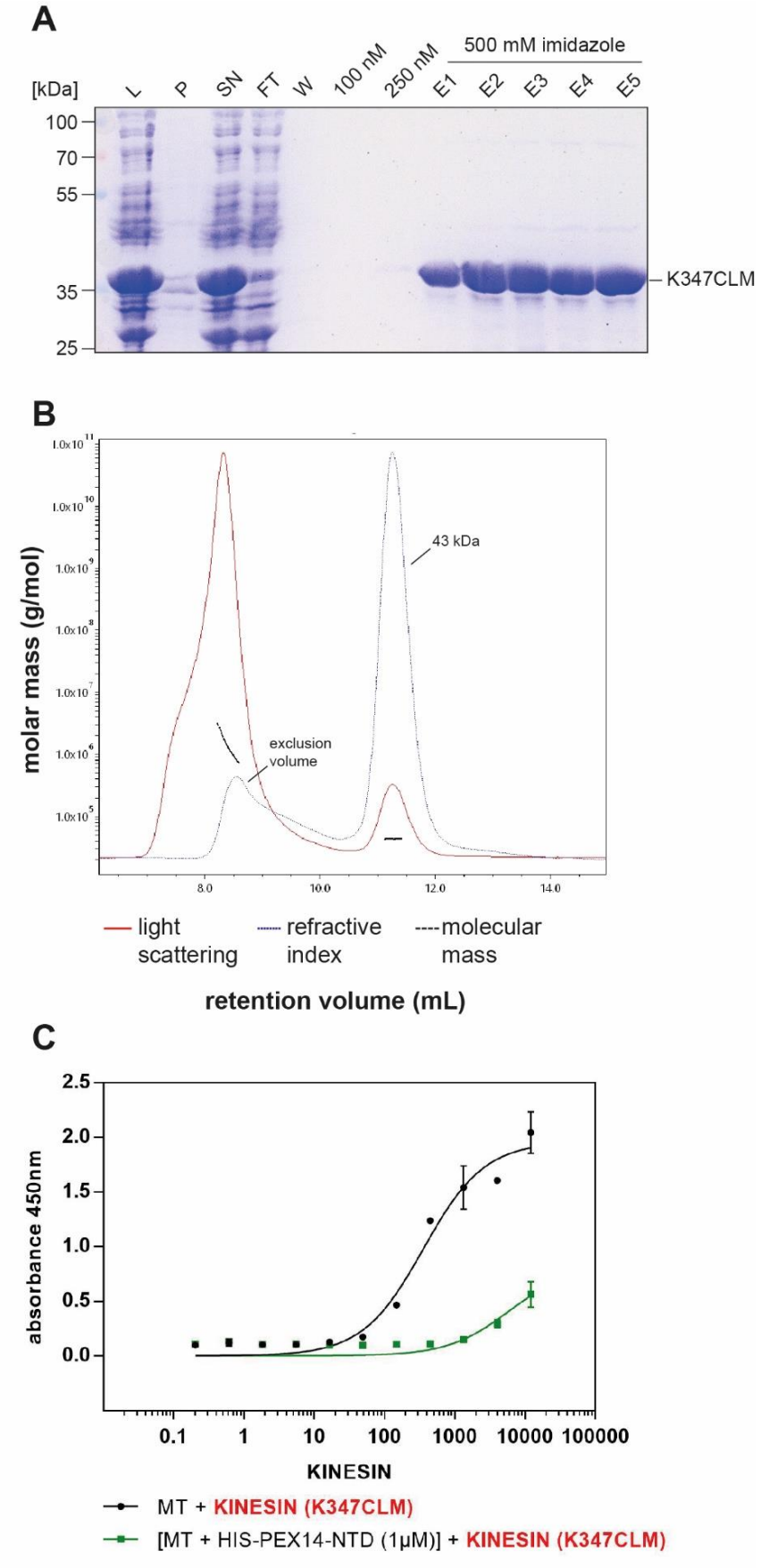
Supplementary Figure 1. Purification of proteins used in the ELISA MT-binding assays. GST, GST-PEX14-NTD, His_6-tagged kinesin GFP (Rphk339), PEX5 and PEX14-NTD were expressed in *E.coli* and affinity-purified. MT were isolated from porcine brain homogenates using polymerizing/depolymerizing cycles and centrifugation steps. Enriched protein fractions containing 5 nmol protein each were analyzed by SDS-PAGE and Coomassie staining.



Supplementary Figure 2. Control experiments for the ELISA MT-binding assay. (A) Microtiter plates were coated with MT and incubated with three different concentrations of GST-tagged PEX14-NTD (10, 50 and 500 nM) and varying concentrations of His-tagged PEX5. Neither His-tagged PEX5 nor PEX5/GST-PEX14-NTD complex interact with immobilized MT. (B) Microtiter plate wells which were coated with MT and milk powder as blocking reagent or incubated with blocking solution alone were tested with varying concentrations of GST-PEX14 and GST alone. GST alone showed unspecific binding to MT at protein concentrations in the μM range. Bound proteins were immunodetected by specific antibodies against His-tag (A) or GST (B). The experimental data were fitted to a standard one-site model using non-linear regression and shown with standard errors (n=2).



Supplementary Figure 3. ITC data of ß-tubulin(388–444) and PEX14-NTD interaction. PEX14-NTD in the syringe was injected into the ITC cell containing recombinant ß-tubulin(388–444). The data were best fitted to a non-symmetric two binding-sites model. The bottom panel shows the residuals of the fit. The 1:2 stoichiometry of the interaction is also evident from the molar ratio that corresponds to the mid-point of the binding curve.


A
[kDa]
L
P
SN
FT
W
100 nM
250 nM
500 mM imidazole
E1
E2
E3
E4
E5
100
70
55
35
25
K347CLM
B
molar mass (g/mol)
43 kDa
exclusion volume
light scattering
refractive index
molecular mass
retention volume (mL)
C
absorbance 450nm
KINESIN
MT + KINESIN (K347CLM)
[MT + HIS-PEX14-NTD (1µM)] + KINESIN (K347CLM)

Supplementary Figure 4. Analysis of MT interaction and oligomerization grade of the kinesin fragment K347CLM. (A) The His-tagged Kinesin motor domain was expressed in *E. coli* and bound to Ni-NTA affinity chromatography column and eluted with 500 nM imidazole. The purification steps were analyzed by SDS-PAGE and Coomassie staining (*E. coli* lysate, L; Pellet , P; Supernatant, S; Wash fraction with buffer, W; 100 nM imidazole; 250 nM imidazole; eluate fractions, E1 to E5 with 500 nM imidazole). **(B)** The purified kinesin motor domain was analyzed by SEC/MALS using Superdex 75 size exclusion column. The chromatogram displays light scattering at 90° angle and refractive index curves together with the molecular mass of the peak calculated by MALS. **(C)** The binding affinity of the His-tagged kinesin motor domain K347CLM for MT was evaluated by ELISA plate assay using primary anti-His antibodies. A standard one-site-model was fitted to the experimental data using non-linear regression and shown with standard errors (N=2). Binding of monomeric kinesin with MT was impaired when MT were preincubated with 1 mM His_6-tagged PEX14-NTD for 1 hour.