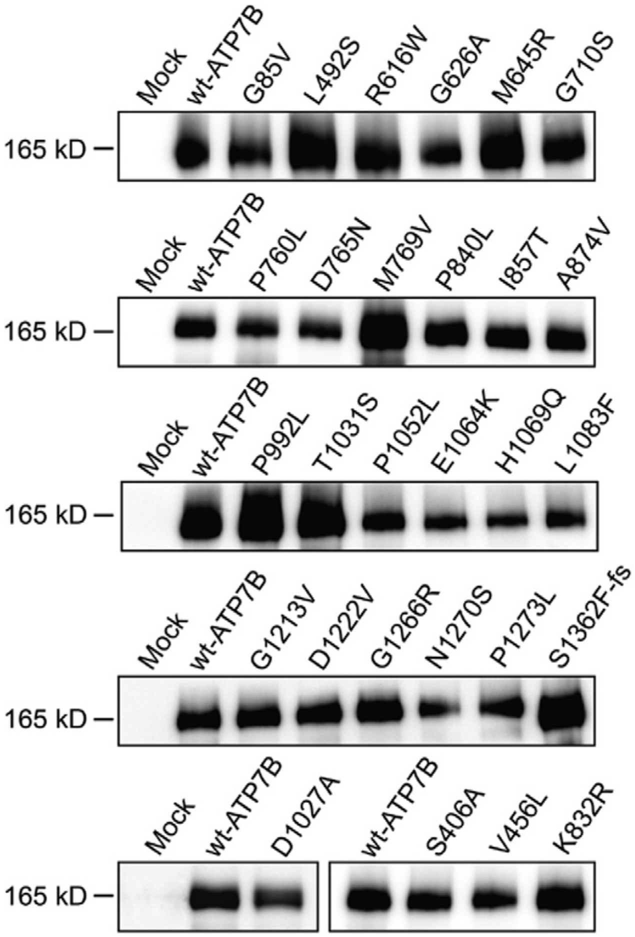
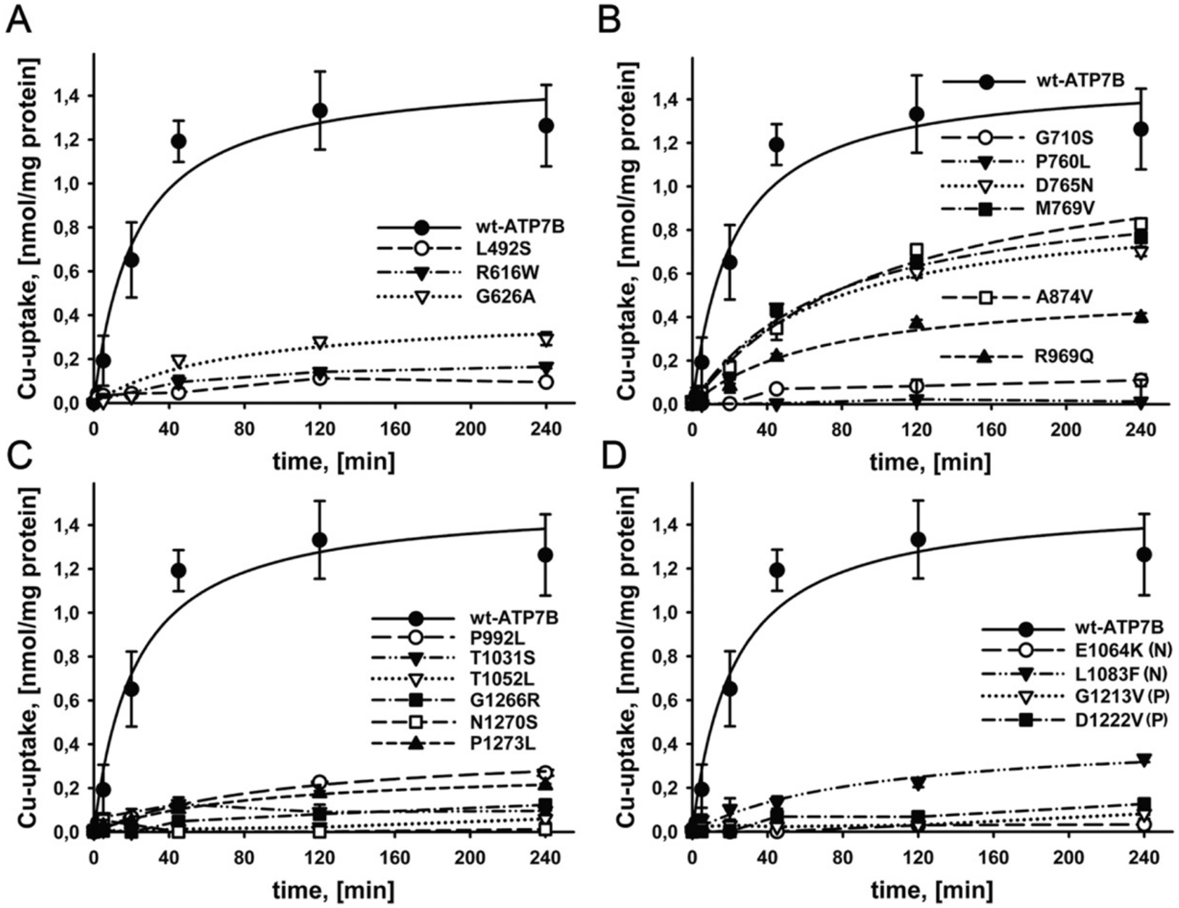
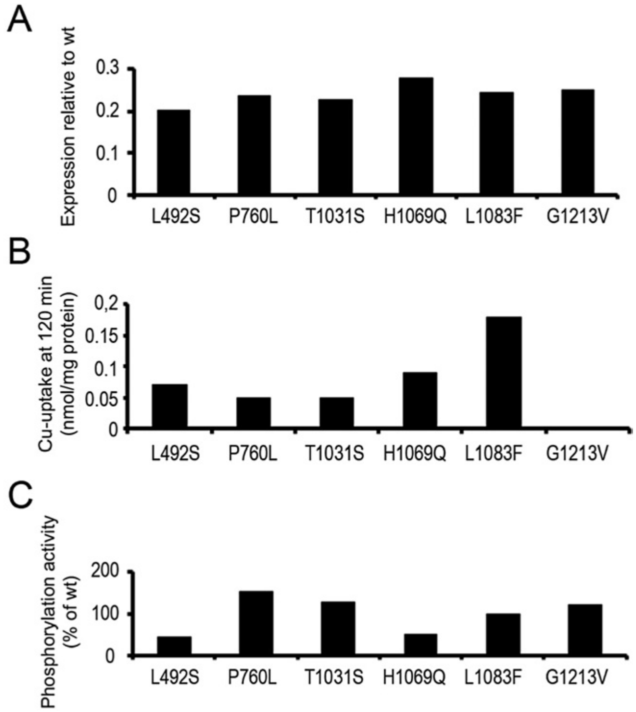


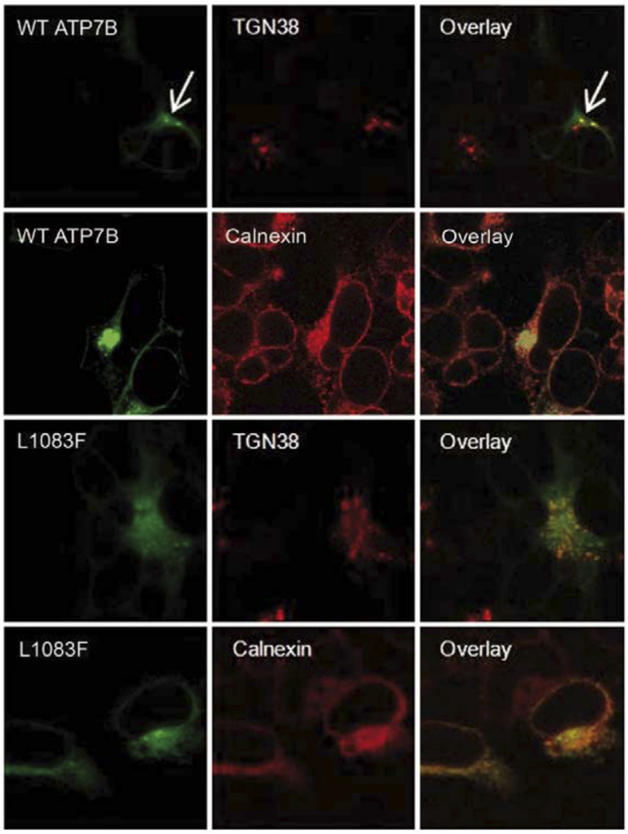
Supplementary Figure 1. Protein expression levels of Sf9 cell vesicles detected by Western blot analysis using polyclonal antibody a-ABD of wt-ATP7B and various mutants. All mutants were expressed and detected in vesicles.



Supplementary Figure 2. The effects of *ATP7B* mutations on Cu transport. (*A*) N-terminal Cu binding unit domain mutants: L492S; R616W; G626A. (*B*) Transmembrane domain and A-domain mutants: G710S; P760L; D765N; M769V; A874V; R969Q. (*C*) ATP-binding-domain: P992L; T1031S; P1052L; G1266R; N1270S; P1273L. (*D*) ATP-binding-domain: E1064K; L1083F; G1213V, D1222V. N, nucleotide binding domain; P, phosphorylation domain.



Supplementary Figure 3. (*A*) Several ATP7B mutants were expressed in parallel under identical conditions, and protein amounts were quantified by densitometry. Their (*B*) copper uptake and (*C*) catalytic activity were then normalized to the protein expression levels. A significant variation of copper uptake and catalytic activity irrespective of expression level was observed.



Supplementary Figure 4. The localization of the L1083F mutant at 28°C compared to the wt-ATP7B. The wt-ATP7B (*green*) shows predominantly TGN localization as evidenced by co-staining with the TGN marker TGN38 (*red*). In contrast, the L1083F mutant (*green*) localizes in both the ER compartment and TGN as evidenced by partial overlay with both the ER marker Calnexin (*red*) and the TGN marker, TGN38 (*red*).

Supplementary Table 1. Mutagenesis Primers Used for Site-Directed Mutagenesis

Amino acid change	Protein location	Forward primer (5′-3′)	Reverse primer (5′-3′)
D1027A	P-domain	GAC TGT GAT GTT TGC CAA GAC TGG CAC C	GGT GCC AGT CTT GGC AAA CAT CAC AGT C
G85V	NTD	CAG GAT TTC CAA TTT GAA AGT CAT CAT CAG CAT GAA GGT TTC	GAA ACC TTC ATG CTG ATG ATG ACT TTC AAA TTG GAA ATC CTG
L492S	NTD	CCG CAG AAG TGC TTC TCA CAG ATC AAA GGC ATG A	TCA TGC CTT TGA TCT GTG AGA AGC ACT TCT GCG G
R616W	NTD	GAC CCG GAA ATT ATC GGT CCA TGG GAT ATT ATC	GAT AAT ATC CCA TGG ACC GAT AAT TTC CGG GTC
G626A	NTD	TCA AAA TTA TTG AGG AAA TTG CCT TTC ATG CTT CCC TGG C	GCC AGG GAA GCA TGA AAG GCA ATT TCC TCA ATA ATT TTG A
M645R	NTD	CAC TTG GAC CAC AAG AGG GAA ATA AAG CAG TGG A	TCC ACT GCT TTA TTT CCC TCT TGT GGT CCA AGT G
G710S	Loop –3	GTC CAG CTC CTC AGT GGG TGG TAC TTC TAC G	CGT AGA AGT ACC ACC CAC TGA GGA GCT GGA C
P760L	TMD 4	GGC GGA GAG GAG CCT TGT GAC ATT CTT CG	CGA AGA ATG TCA CAA GGC TCC TCT CCG CC
D765N	TMD 4	GCC CTG TGA CAT TCT TCA ACA CGC CCC CCA TGC	GCA TGG GGG GCG TGT TGA AGA ATG TCA CAG GGC
M769V	TMD 4	CGA CAC GCC CCC CGT GCT CTT TGT GTT C	GAA CAC AAA GAG CAC GGG GGG CGT GTC G
P840L	A-domain	CTG GGG GAA AGT TTC TAG TGG ATG GGA AAG TCC T	AGG ACT TTC CCA TCC ACT AGA AAC TTT CCC CCA G
I857T	A-domain	CTG ATG AGT CCC TCA CCA CAG GAG AAG CCA	TGG CTT CTC CTG TGG TGA GGG ACT CAT CAG
A874V	A-domain	CGG AAG CAC TGT AAT TGT GGG GTC TAT AAA TGC ACA	TGT GCA TTT ATA GAC CCC ACA ATT ACA GTG CTT CCG
A874V	(HEK 293)	ACC CGG AAG CAC TGT AAT TGT GAG GTC TAT AAA TGC ACA TGG C	GCC ATG TGC ATT TAT AGA CCC TCA AAT TAC AGT GCT TCC GGG T
R969Q	Loop 5–6	CAG AGG TGA TCA TCC AGT TTG CTT TCC AGA CGT CC	GGA CGT CTG GAA AGC AAA ATG GAT GAT CAC CTC TG
R969Q	(HEK 293)	CCC AGA CAG AGG TGA TCA TCC AGT TTG CTT TCC AGA CGT CCA T	ATG GAC GTC TGG AAA GCA AAC TGG ATG ATC ACC TCT GTC TGG G
P992L	P-domain	GGG GCT GGC CAC GCT CAC GGC TGT CAT GG	CCA TGA CAG CCG TGA GCG TGG CCA GCC CC
T1031S	P-domain	GTT TGA CAA GAC TGG CTC CAT TAC CCA TGG CG	CGC CAT GGG TAA TGG AGC CAG TCT TGT CAA AC
P1052L	P-domain	GTG GCC ACA CTG CTC CTC AGG AAG GTT C	GAA CCT TCC TGA GGA GCA GTG TGG CCA C
E1064K	N-domain	GTG GGG ACT GCG AAG GCC AGC AGT GA	TCA CTG CTG GCC TTC GCA GTC CCC AC
H1069Q	N-domain	GGC CAG CAG TGA ACA ACC CTT GGG CGT G	CAC GCC CAA GGG TTG TTC ACT GCT GGC C
L1083F	N-domain	CCA AAT ACT GTA AAG AGG AAT TTG GAA CAG AGA CCT TGG G	CCC AAG GTC TCT GTT CCA AAT TCC TCT TTA CAG TAT TTG G
L1083F	(HEK 293)	GTC ACC AAA TAC TGT AAA GAG GAA TTT GGA ACA GAG ACC TTG GG	CCC AAG GTC TCT GTT CCA AAT TCC TCT TTA CAG TAT TTG GTG AC
G1213V	P-domain	GCT GCA GAG CAT GGT TGT GGA CGT GGT TCT	AGA ACC ACG TCC ACA ACC ATG CTC TGC AGC
D1222V	P-domain	CTG ATC ACG GGG GTC AAC CGG AAG ACA G	CTG TCT TCC GGT TGA CCC CCG TGA TCA G
G1266R	P-domain	AAG TCG CCA TGG TGA GGG ATG GGG TCA ATG	CAT TGA CCC CAT CCC TCA CCA TGG CGA CTT
N1270S	P-domain	GGG GGA TGG GGT CAG TGA CTC CCC GGC C	GGC CGG GGA GTC ACT GAC CCC ATC CCC C
P1273L	P-domain	GTC AAT GAC TCC CTG GCC TTG GCC CAG G	CCT GGG CCA AGG CCA GGG AGT CAT TGA C
S1362F-fs	TM 8	CGG CCA TGG CAG CCT TCT GTG TCT GTG GTG C	GCA CCA CAG ACA CAG AAG GCT GCC ATG GCC G
S406A	NTD	GCA ACA GTT CTT TAT A AT CCC TCT GTA ATT AGC CCA GAA GAA C	GTT CTT CTG GGC TAA TTA CAG AGG GAT TAT AAA GAA CTG TTG C
V456L	NTD	CTA CAG ATG GTA CAC CTA CAT CTG TGC AGG AAG TGG	CCA CTT CCT GCA CAG ATG TAG GTG TAC CAT CTG TAG
K832R	A-domain	GCG GGG CGA TAT CGT CAG GGT GGT CCC	GGG ACC ACC CTG ACG ATA TCG CCC CGC

A-domain, actuator domain; NTD, N-terminal Cu-binding domain; N-domain, nucleotide-binding domain; P-domain, phosphorylation domain; TMD, transmembrane domain.

Supplementary Table 2. Copper Transport Rates of wt-, Mutant, and Variant ATP7B

	Protein domain	Cu-uptake rates, *nmol/mg protein/min* (SD)
wt-ATP7B	—	0.038 (0.022)
Artificial mutant of phosphorylation site		
D1027A	P-domain	0.005 (0.002)
Human mutants		
G85V	NTD	0.008 (0.002)
L492S	NTD	0.006 (0.002)
R616W	NTD	0.004 (0.004)
G626A	NTD	0.002 (0.002)
M645R	NTD	0.034 (0.002)
G710S	Loop 2–3	0.000 (0.000)
P760L	TMD 4	0.000 (0.000)
D765N	TMD 4	0.008 (0.002)
M769V	TMD 4	0.012 (0.006)
P840L	A-domain	0.008 (0.002)
I857T	A-domain	0.024 (0.002)
A874V	A-domain	0.010 (0.004)
R969Q	Loop 5–6	0.006 (0.004)
P992L	P-domain	0.002 (0.002)
T1031S	P-domain	0.014 (0.004)
P1052L	P-domain	0.002 (0.002)
E1064K	N-domain	0.000 (0.000)
H1069Q	N-domain	0.017 (0.002)
L1083F	N-domain	0.010 (0.008)
G1213V	P-domain	0.004 (0.004)
D1222V	P-domain	0.006 (0.004)
G1266R	P-domain	0.002 (0.000)
N1270S	P-domain	0.010 (0.002)
P1273L	P-domain	0.006 (0.006)
S1362F-fs	TMD 8	0.004 (0.002)
Human variants		
S406A	NTD	0.020 (0.002)
V456L	NTD	0.008 (0.002)
K832R	A-domain	0.020 (0.002)

NOTE. Mutations in bold are likely to be most severe (markedly affect copper transport) and mutations in italics type are mutations that are likely to be less severe or potentially correctable.

A-domain, actuator domain; NTD, N-terminal Cu-binding unit domain; N-domain, nucleotide (ATP) binding domain; P-domain, phosphorylation domain; TMD, transmembrane domain (see Figure 1B).