Appendix A

Supplementary Material

Trafficking of the amino acid transporter $B^{0,+}$ (SLC6A14) to the plasma membrane involves an exclusive interaction with SEC24C in its exit from endoplasmic reticulum

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Fig. S1

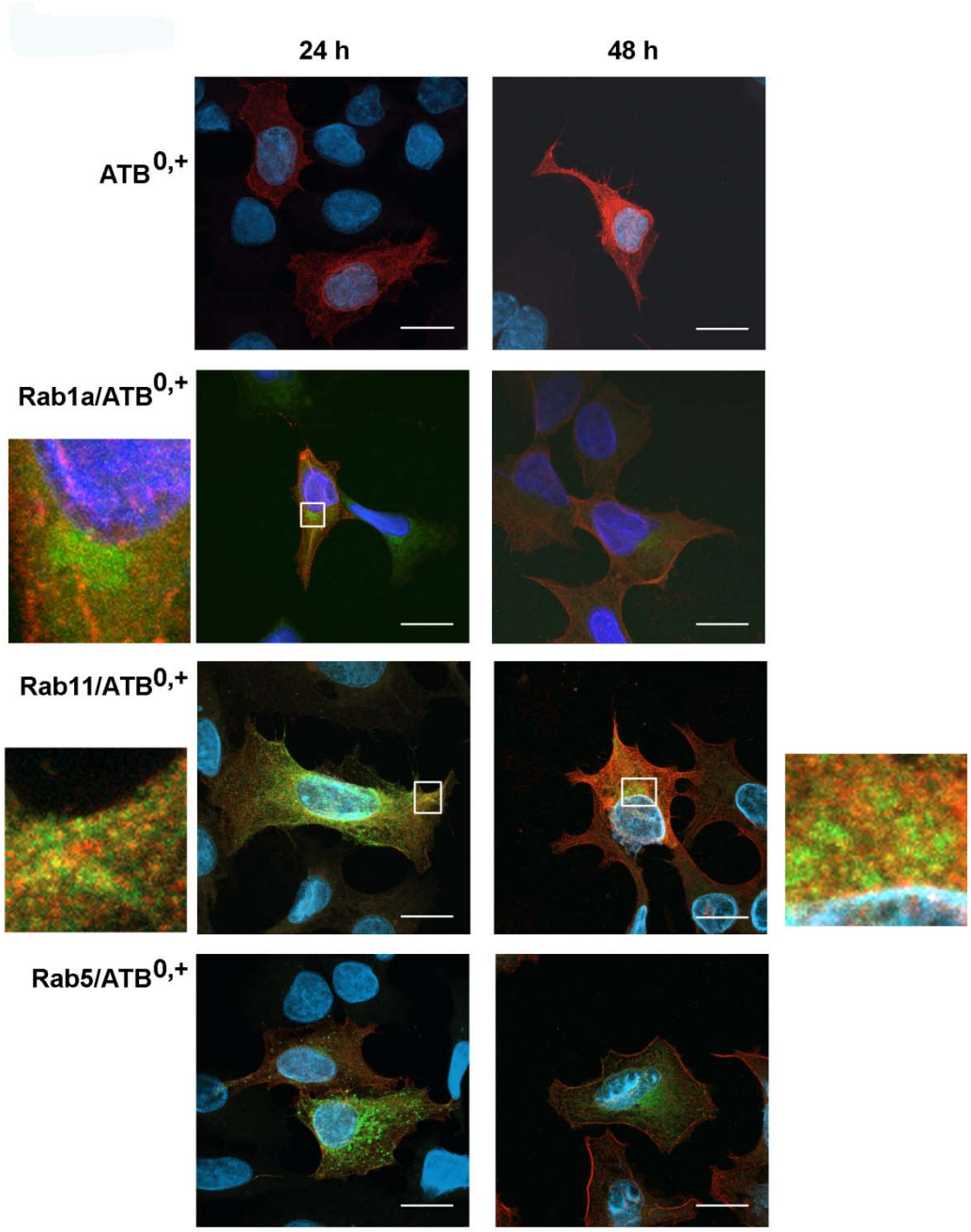

Fig. S1. Localization of $ATB^{0,+}$ and Rab proteins after overexpression in HEK293 cells. Cells were transfected either with a vector coding $ATB^{0,+}$ or together with vectors coding Rab proteins fused with fluorescent proteins: CFP-Rab1a (human), GFP-Rab5 (canine), GFP-Rab11 (canine). The cells were fixed either after 24h or 48h with methanol precooled at -20°C, as given in Materials and methods (section 2.5). $ATB^{0,+}$ is visualized as red with anti-FLAG and anti-mouse antibodies conjugated with Alexa Fluor 568®. Fluorescent proteins are shown in pseudocolors as green, nuclei in blue stained with DAPI, with the exception of cells transfected with Rab1a, in case of which nuclei were stained with TO-PRO®-3. Wherever the co-localization signal could be detected, the magnified images of the boxed sections were shown either to the left (24 h) or to the right (48 h) of the main image. Bar 20 μm.

Fig. S2

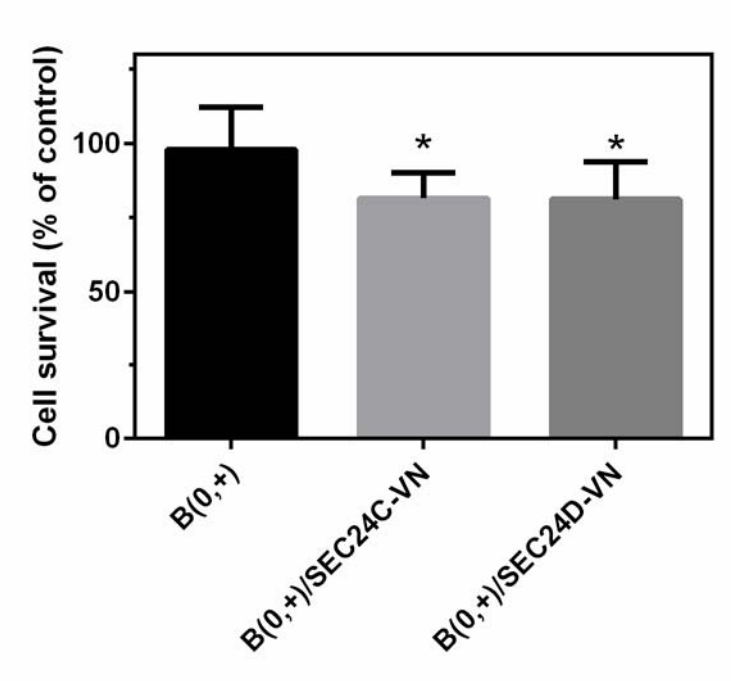

Fig. S2. Effect of SEC24 dominant negative mutants on viability of HEK293 cells. Cells were transfected either with a vector coding $ATB^{0,+}$ or together with a 9-fold excess of vector coding either SEC24C-VN or SEC24D-VN. Cell viability was assayed after 48 h using 0.4% trypan blue staining and a hemocytometer chamber. The statistical analysis was performed using GraphPad Prism program using ANOVA with Tuckey's multiple comparison test. The statistical significance was set at $P<0.05$. Asterisk indicates the significant change towards control, there was no significant difference between SEC24C-VN and SEC24D-VN transfected cells.

Fig. S3

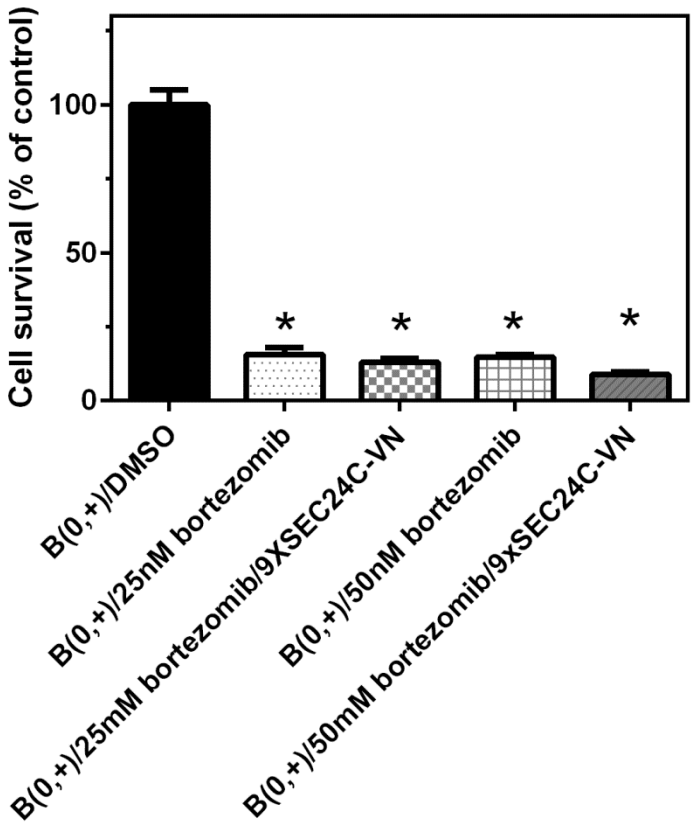

Fig. S3. Effect of bortezomib on viability of HEK393 cells. Cells were transfected either with a vector coding $ATB^{0,+}$ or together with a 9-fold excess of a vector coding SEC24C-VN. Bortezomib, at indicated concentrations, was added together with the vectors. Cell viability was assayed after 48 h using 0.4% trypan blue staining and a hemocytometer chamber. The statistical analysis was performed using GraphPad Prism program using ANOVA with Tuckey's multiple comparison test. The statistical significance was set at $P<0.05$. Asterisk indicates the significant change towards control, there was no significant difference among other experimental groups.

Fig. S4

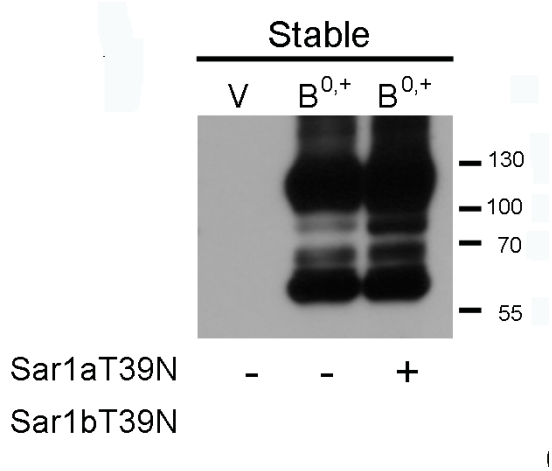

Fig. S4. Effect of Sar1 dominant negative mutants on $ATB^{0,+}$. HEK293 cells were stably transfected with either p3xFLAG-CMV14 (V) or p3xFLAG-CMV14/$B^{0,+}$ ($B^{0,+}$). Vectors coding Sar1aT39N and Sar1bT39N (in a 3-fold excess each) were used for transfection 48 h before lysing the cells. Cells were lysed in 150 mM NaCl, 10 mM EDTA, 1% IGEPAL CA-630 (nonidet P-40), 50 mM Tris, pH 7.4 supplemented with protease inhibitor cocktail and the protein extracts were subjected to Western blot analysis using anti-FLAG antibody. The highest molecular weight species corresponds to fully glycosylated form, the lowest molecular weight species to non-glycosylated transporter. The intermediate bands, whose amount increases upon co-transfection with Sar1 negative mutants correspond to core-glycosylated bands retarded in ER.