**Supplementary methods:**

**SFTPC long PCR**

DNA was extracted from 200 μL of EDTA-blood with Qiamp blood minikit (Qiagen, Milano, Italy).

Long PCR assay was performed with a 50 μL reaction volume containing 5 μL of distilled water, 100 ng of DNA, 5 μL of 10x PCR Tuning Buffer with Mg2+ (2 mM), 2.5 μL of dNTPs (10 mM each), 400 nM of each primers (SFTPCfor 5’-*CCAGTGGGGACAGAGTTTCC*-3’ and SFTPCrev 5’-*TAGGGAAATGAGCTCGCTGG*-3’), and 2 U of PCR extender polymerase (5 U/μL, PCR Extender System) (5PRIME, Hilden Deutschland). The PCR product size was 3978 bp.

The amplification conditions for the PCR reaction were as follow 1cycle of 93°C for 3 minute, 10 cycles of 93°C 15 seconds, 62°C for 30 seconds and 68°C for 8 minutes, 8 cycles of 93°C 15 seconds, 62°C 30 seconds and 68°C 8 min + 20 seconds every cycles.

**SFTPA and SFTPB Sequencing.**

We sequenced all the 5 exons of SFTPA gene and all the 10 exons of SFTPB gene. Amplicons were directly sequenced in an automatic genetic analyzer (CEQ 8800 Genetic Analyzer; Beckman-Coulter). Primers used are shown in table S1 and S2.

|  |  |  |
| --- | --- | --- |
| **SFTPA Exon** | **Primer** | **Product size** |
| **1** | ACACTATGCCCATTTCCTGC | 219 |
| GCTGGTCCTCTCTGCCTG |
| **2-3** | TGACAGAGCACAGTGGGG | 759 |
| TGTAACTGACTTCAGGGTCGC |
| **4** | GCAGATGGCAAAACACCTG | 218 |
| AGAATGAGGGGAATTTGTGG |
| **5\_1** | TCTGGTAGCAGAGACCCCAG | 688 |
| GGTGCAGTGCTGGGAGAG |
| **5\_2** | ACTTCATTCCTCTGATGGGC | 670 |
| AGAAAGCAGAGCCAGTGGTG |
| **5\_3** | GCCTAGGCCTCTAGGGTGAC | 733 |
| GGCTCAGAGTCAGAGTTCATTTG |

**Table S1.** Primer used for SFTPA gene sequencing.

|  |  |  |
| --- | --- | --- |
| **SFTPB Exon** | **Primer** | **Product size** |
| 1 | CTGCCTAGGAGAGGGGAGGCT | 792 |
| CTATGCCCCAGCCCCTACCCTG |
| 2 | GAGCCCACCCAGCACCCTTC | 451 |
| AGCACTGCTTTGTGCTAGGCAT |
| 3-4 | CAGGCAGGAGGTGAGCTTGCAG | 639 |
| AGCCTCCCCCACTCATGTGTCC |
| 5-6 | GGTATGCGTGTGCTCCTGGGC | 618 |
| GGCCGGCCTGAATAGGGGTG |
| 7-8 | GCCTTGAACGGGCCCTGACC | 624 |
| AGCTGGGTGCTGGGCAGAGA |
| 9-10 | GGGAGCAGAAGGGCCTCCCAT | 802 |
| AGGCCAGGACCACACGACAGGA |

**Table S2.** Primer used for SFTPB gene sequencing.

**Plasmids**

The pEYFP-*hABCA3-WT* vector with YFP fused to the C-terminus of ABCA3 was kindly provided by Prof. A. Holzinger. The ABCA3-G964D point mutation was introduced in the pEYFP-*hABCA3-WT* vector using the QuickChange Site-directed mutagenesis kit (Stratagene) with the following primers: G964D-for 5’-*GCGAGTACGACAGAACCGTCGTG*-3’ and G964D-rev 5’-*CACGACGGTTCTGTCGTACTCGC*-3’.

**Immunefluorescence**

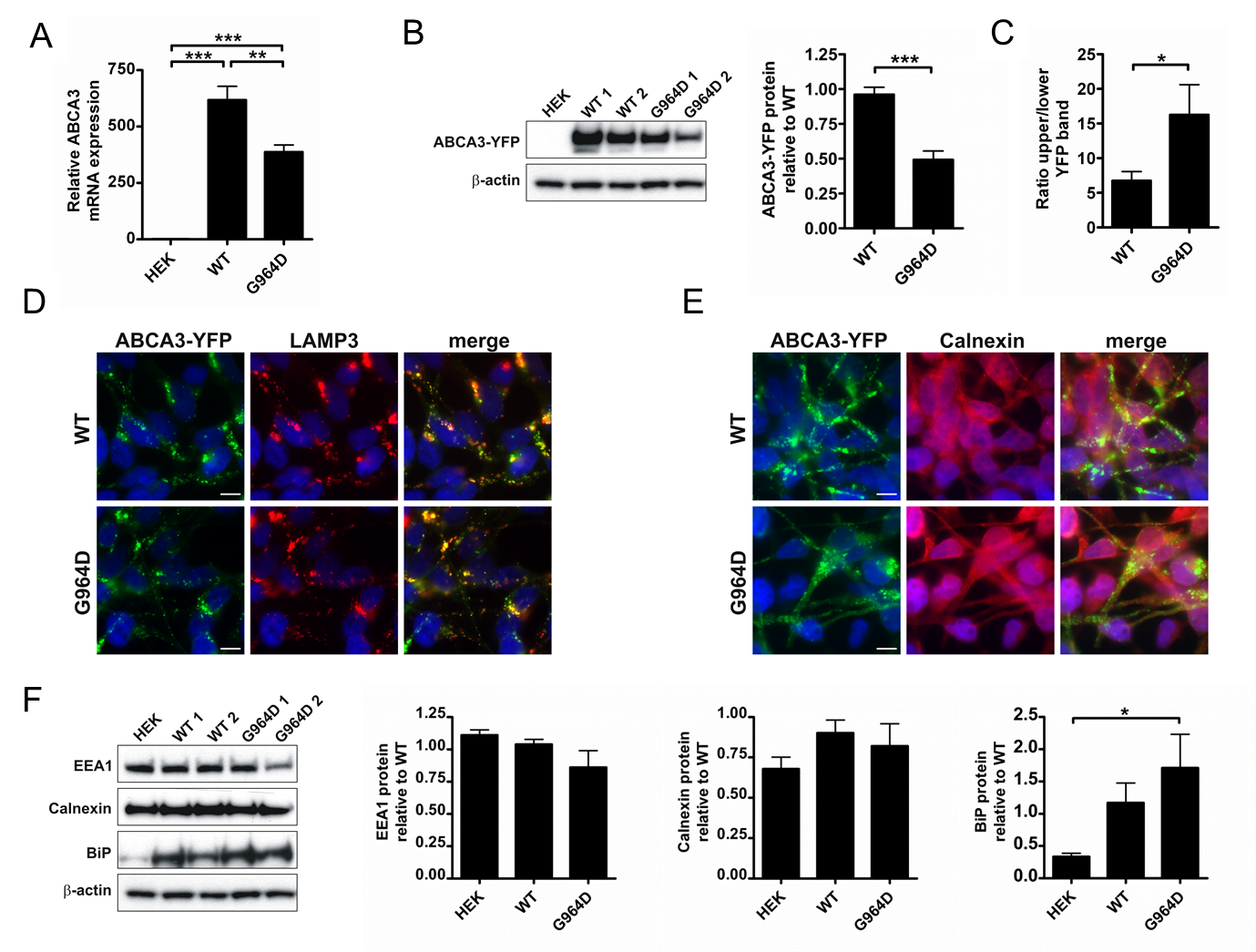
YFP is a yellow fluorescent protein which does not need to be stained. Therefore, only LAMP3 and calnexin were stained according to the material and methods.

**Western immunoblot**

YFP was detected with a mouse-anti-GFP antibody (Clontech) and the Novex WesternBreeze Immunodetection Kit (Invitrogen). All other antibodies and procedures were as described in the materials and methods.

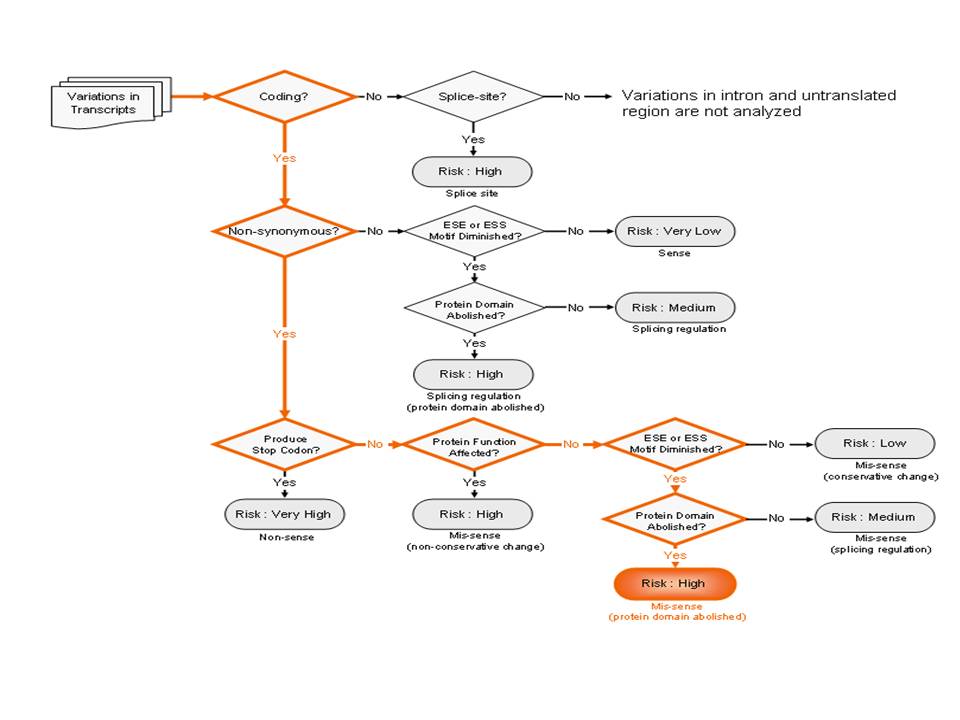
Acknowledgement: The pEYFP-*hABCA3-WT* vector was kindly provided by Prof. A. Holzinger.

**Supplementary Figures**



**Figure S1. Cellular effects of stable ABCA3 expression in HEK cells.**

A. ABCA3 mRNA expression levels analyzed by quantitative real time PCR. B. Western immunoblot analysis of YFP-tagged ABCA3 in total cell lysates. β-actin was used as a loading control. C. Ratio of the upper/lower ABCA3 processing form. D.Fluorescence of YFP-tagged ABCA3 and immunostaining of the lysosomal (lamellar body) marker LAMP3. E. Fluorescence of YFP-tagged ABCA3 and immunostaining of the ER marker calnexin. F. ER (calnexin, BiP) and early endosome markers (EEA1) in cells stably transfected with ABCA3. β-actin was used as a loading control. Scale bars: 7.5 µm, \**P*<0.05, *\*\*P*<0.01, *\*\*\*P*<0.001.

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**Figure S2. Bioinformatic analysis.** Bioinformatic analysis of the ABCA3 G>A transition at nucleotide 2891 by FANS (Functional analysis of novel SNPs and mutations in human and mouse genomes) [12].