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Supporting Information for

Germline *C1GALT1C1* mutation causes a multisystem chaperonopathy

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This PDF file includes:

Supporting Information Text

Additional Case Details

Patient M1

This patient was born small for gestational age after 38 weeks of gestation (birth weight: 2390 g (1st percentile (P1)); length 43 cm (<P1); head circumference 33.5 cm (P24)). The postpartum period was complicated by congenital pneumonia and persistent pulmonary hypertension necessitating high-frequency oscillatory ventilation with NO treatment, neonatal seizures and transient thrombocytopenia. Moreover, hypertrophic cardiomyopathy without signs of cardiac failure and disproportional stature with short limbs were diagnosed.

After birth, the boy showed severe thrombocytopenia which required platelet transfusions. Even thereafter platelet counts remained low, ranging between 100,000 /µL and 200,000 /µL (normal 200,000–400,000 /µL). During his more frequent follow-ups in the last 2.5 years, the patient has shown chronic severe lymphopenia with leading T-lymphopenia. Repeated lymphocyte subtyping analyses showed 30-35% T-lymphocytes ($CD4^+$ 17-20%, $CD8^+$ 10-12%), 42-45% B-lymphocytes and 19-21% NK cells. Additionally, the patient has chronic moderate to severe hypogammaglobulinemia (as low as 150 mg/dL). He receives 500,000 units Penicillin V orally thrice daily.

Whereas hypertrophic cardiomyopathy disappeared spontaneously, the child developed mild aortic valve stenosis and insufficiency related to a dysplastic aortic valve, mild mitral valve stenosis and insufficiency related to a dysplastic mitral valve as well as progressive membranous subaortic stenosis. The latter was resected at age 4 years but became progressive again later-on. There were no signs of cardiac failure.

Persistent and severe growth retardation could not be explained by endocrine abnormalities (IgF-1, IGFBP3 and thyroid hormone levels normal) and was treated with growth hormone therapy from the age of six years.

From early infancy, the boy showed severe psychomotor delay. At age 2 years his speech and motor capabilities were those of a one-year-old child. At the age of three years first steps were possible with the aid of a posterior walker. At the age of 5 years he started to walk without aid and was able to speak about 50 words. Despite normal social functioning, moderate intellectual disability with an IQ value below 55 (SON-R 2-8) was found at the age of 6 years. Due to severe muscular hypotonia and moderate ataxia independent gait was only possible for short distances. A postnatal MRI was unremarkable.

During the neonatal period phenobarbital treatment had been initiated because of generalized seizures. At age 3 months, phenobarbital was switched to levetiracetam which, in turn, was replaced by carbamazepine 3 months later. Carbamazepine was continued until the age of 5 years despite the absence of epileptic seizures as hypersynchronic activity persisted on electroencephalographic recordings.

On examination, the patient showed shortening of extremities, clinodactyly of the fifth finger of both hands and mild syndactyly of the second and third toe with overlapping bended second and fourth toes. He had sparse bright and thin hair and eyebrows. Facial dysmorphisms included mild facial coarsening with a flattened nasal bridge and prominent nasal tip, and an open mouth with downturned corners, prominent lips and a tented upper lip.

At the age of five years the patient developed an atypical hemolytic uremic syndrome with mild complement hyperactivation and renal failure which necessitated peritoneal dialysis. Shigatoxin

and pneumococci were excluded, ADAMTS13 activity was normal. Kidney function normalized after initiation of biweekly Eculizumab therapy which, in turn, was switched to four-weekly Ravulizumab infusions after one year.

Patient M2

At birth in the 41st gestational week, the patient's weight was 2700 g (P2), length 49 cm (P15) and head circumference 34 cm (P22).

At the age of 5 months, the patient was readmitted to the hospital with beginning central pneumonia and bilateral putrid *Haemophilus influenzae* conjunctivitis. During the acute infection, severe neutropenia (360 /µL) developed. Throughout childhood, the patient was repeatedly hospitalized with pneumonia, middle ear infections and gastrointestinal infections, although the frequency and severity of these infections decreased in adolescence. During these hospitalizations until the age of 6 years, moderate to severe thrombocytopenia and mild anemia were repeatedly diagnosed. Further follow-up laboratory parameters starting from the age of 16 years until now show chronic mild thrombocytopenia (100,000-150,000 /µL).

Patient M2 shows disproportionate short stature (138 cm at 20 years, 8 months), with length and head circumference crossing the third percentile within the first months of life. Arms and legs are short (arm span 128 cm, arm span to height ratio 0.93).

The patient started walking at 13 months, but speech developmental delay soon became apparent. He spoke his first words at 23 months. The ET 6-6 developmental test(1) at the age of 3 years and 7 months showed a delayed global development with test scores on average 2.6 standard deviations below the age-adjusted norm. The patient had a single febrile seizure when he was 3 years old. Early learning support started at age 1 year and 11 months. The patient currently works in a manual job.

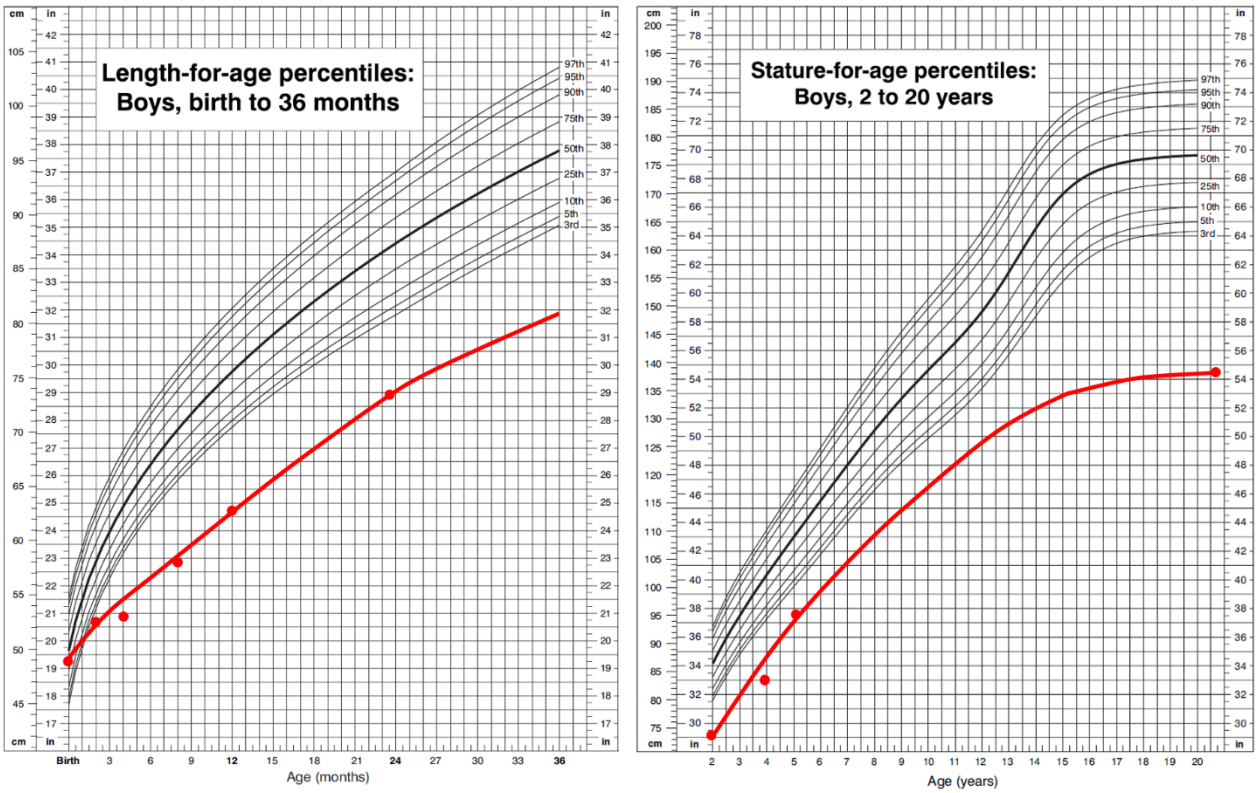
Patient F1

Patient F1 is the mother of patients M1 and M2. She is 39 years old, has short stature (150 cm, P1) and went to a school for children with learning disabilities.

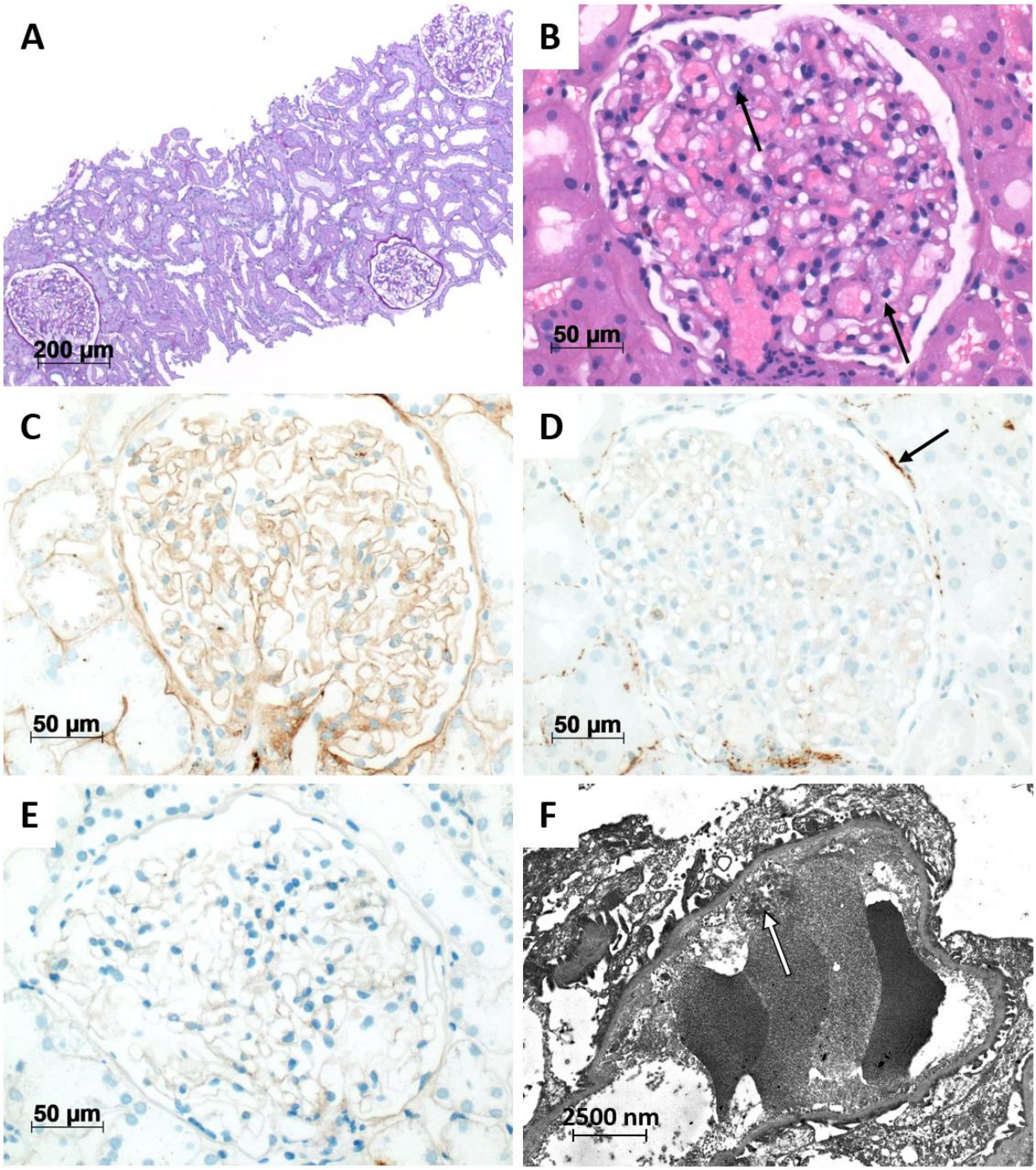
Two other sons were born to a third father. One died two days after birth in the 30th gestational week due to kidney and multiorgan failure of unknown cause. The other is clinically unaffected and in foster care.

Patient F2

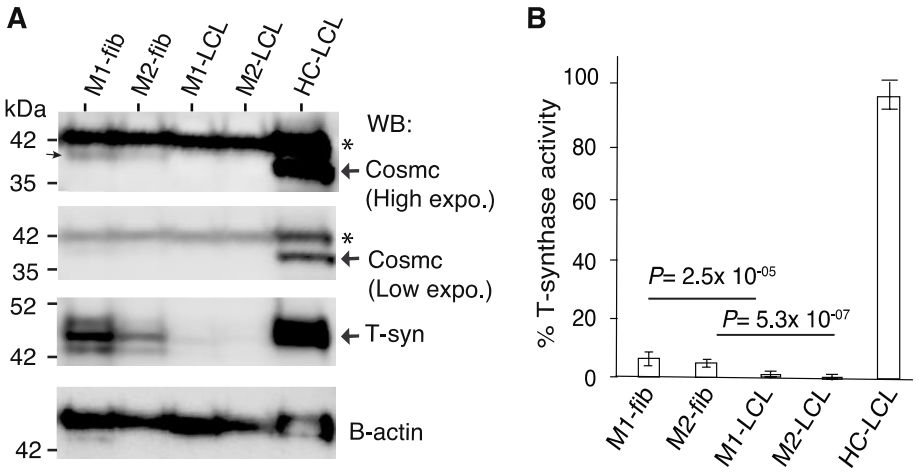
Patient F2 is the mother of patient F1, i.e. the maternal grandmother of patients M1 and M2. She is 58 years old, has low-normal stature (157 cm, P9) and went to a school for children with learning disabilities. Her facial features are similar to those of patient F1. We found slight thrombocytopenia (144,000 /µL), but otherwise normal blood results including differential blood counts.



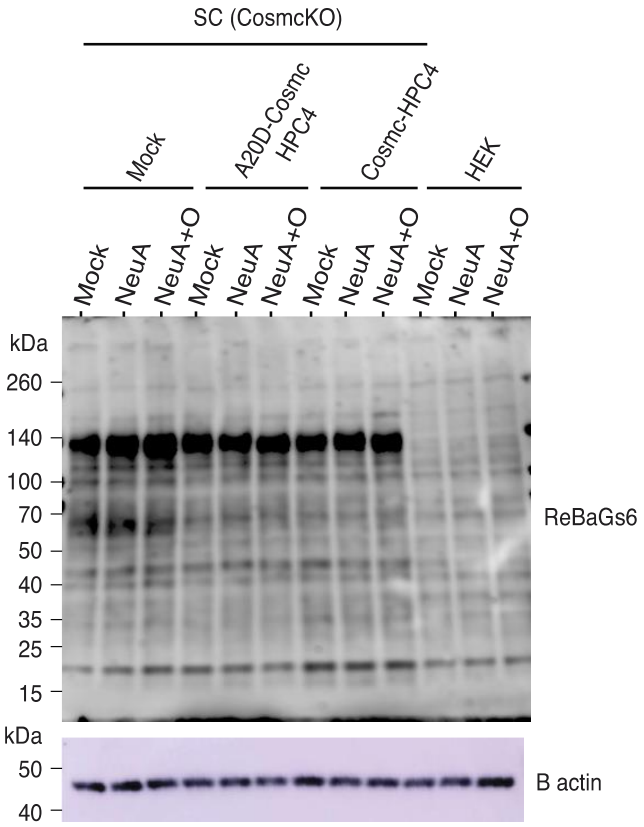
Supplementary Figure S1: Growth development of patient M2 from birth to the age of 20 years and 8 months. Growth between measured values has been estimated. Reference percentile curves were taken from the US Centers for Disease Control and Prevention reference growth charts.



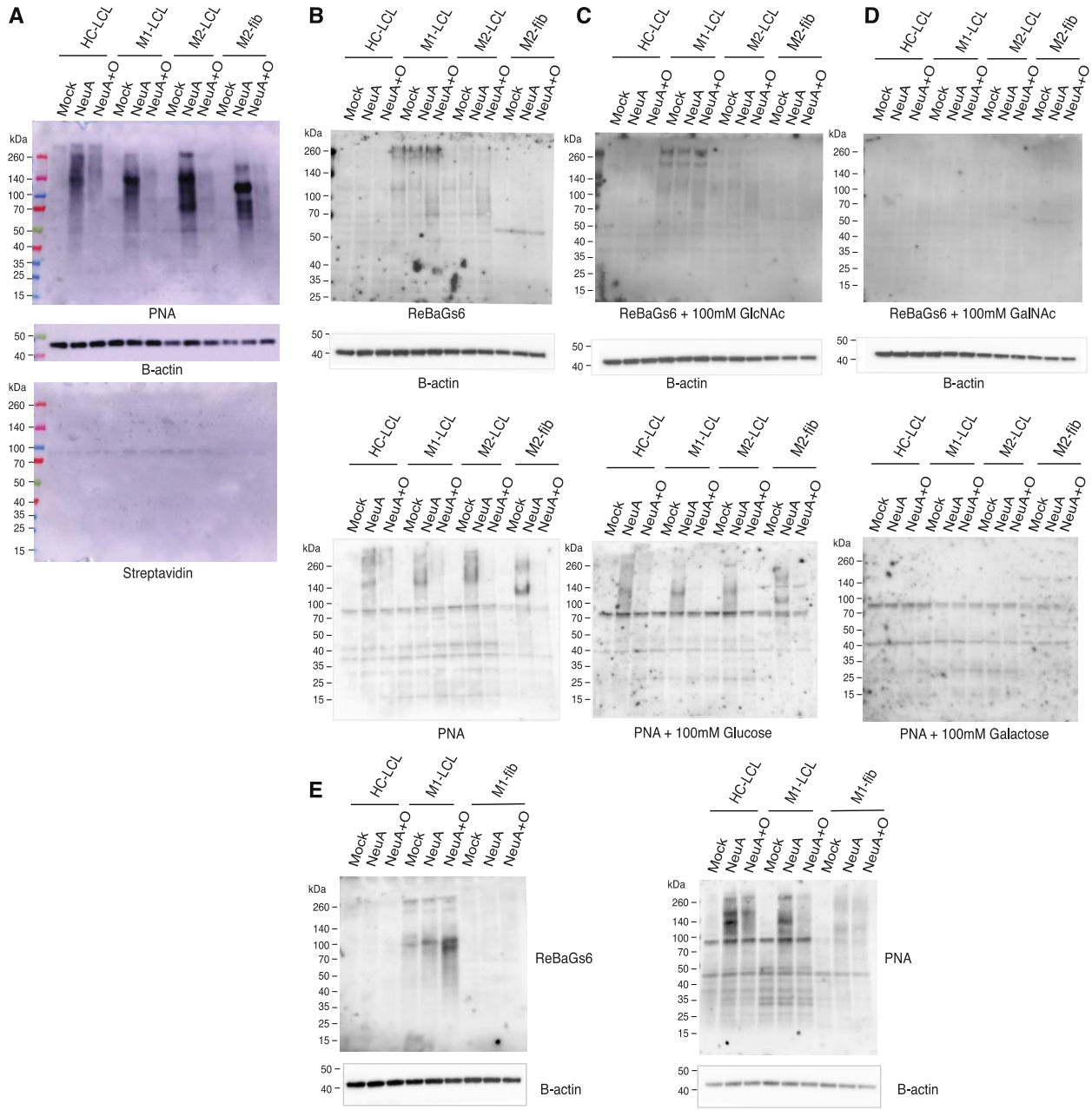
Supplementary Figure S2: Renal histology from patient M2 six months after clinical diagnosis of thrombotic microangiopathy and four months after discontinuation of Eculizumab treatment. **A**, Renal biopsy with unremarkable glomerular and tubulointerstitial compartment (PAS, 100x). **B**, At higher magnification, glomerulus with only mild signs of endothelial activation (arrows) with enlarged endothelial cell nuclei (H&E, 400x). **C-E**, Lack of glomerular deposits of C3c (**C**), C5b-9 (**D**) and IgA (**E**) with only traces of C5b-9 deposited at the Bowman´s capsule in the C5b-9 staining (arrow), all 400x original magnification. **F**, Electron microscopy showing only mild irregularities in small segments of the inner layer of the glomerular basement membrane and associated mild loss of fenestration of the endothelial cell (ultrathin section, 5000x).



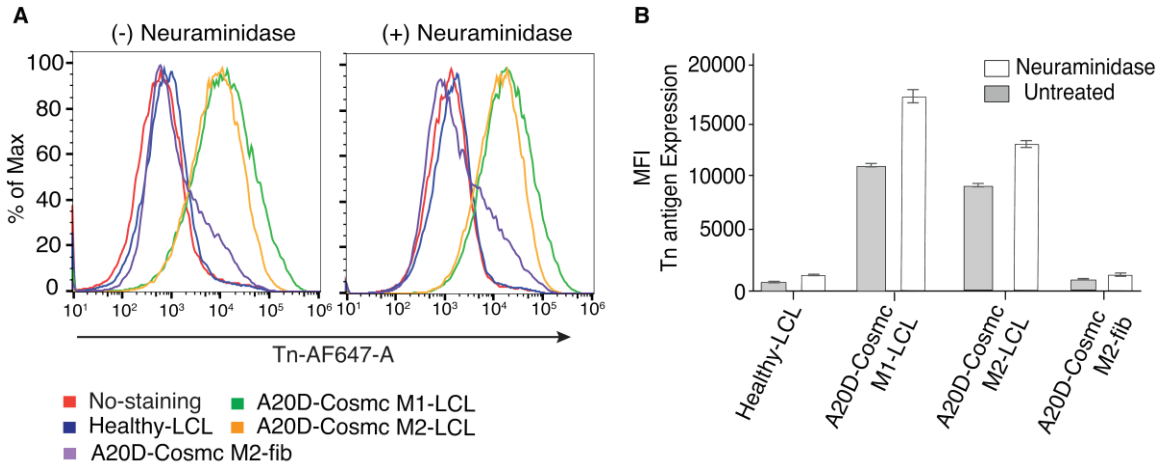
Supplementary Figure S3. Endogenous A20D-Cosmc expression and T-synthase activity in M1 patient fibroblasts. A, Whole-cell lysates from cultured M1 fibroblasts (M1-fib), M2-fib, and other lymphoblastoid cell lines (LCL) healthy cells (HC), and patients (M1 and M2) were resolved in SDS-PAGE WB and probed as indicated, arrow indicates protein of interest. Thin arrow shows A20D-Cosmc. *: Non-specific bands. Representative example of 3 independent experiments. **B**, T-synthase activity measurements. Whole-cell lysates of parallel T-synthase activity (n=9, three independent experiments of n=3).



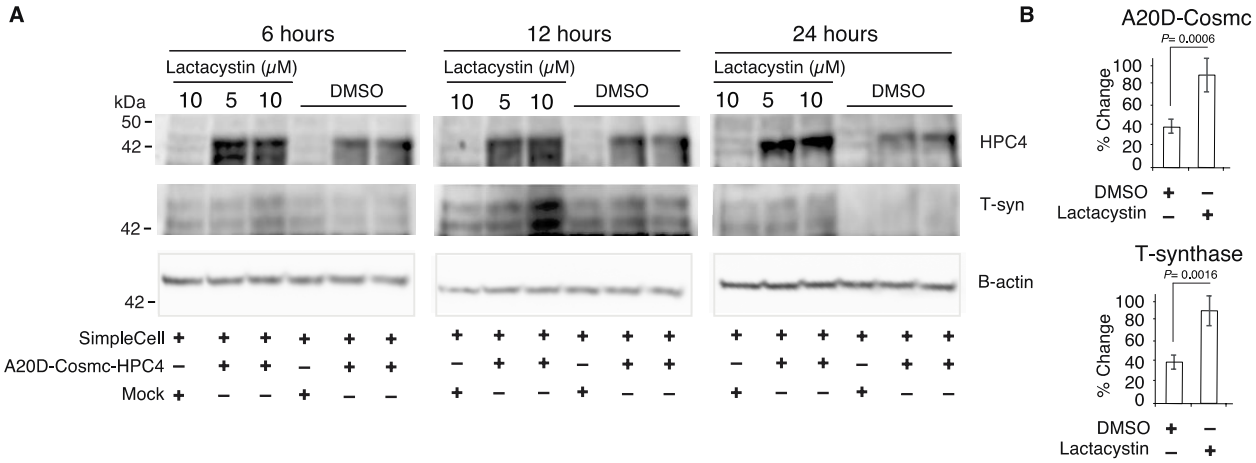
Supplementary Figure S4. Transient transfection of A20D-Cosmc to SimpleCells can partly reduce the expression of Tn-antigen. Whole-cell lysates, prepared as in **Figure 4F**, were analyzed for Tn-antigen expression using SDS-PAGE-WB probed with ReBaGs6. Representative example of two independent experiments. SC: SimpleCell, NeuA: neuraminidase, O: O-glycosidase.



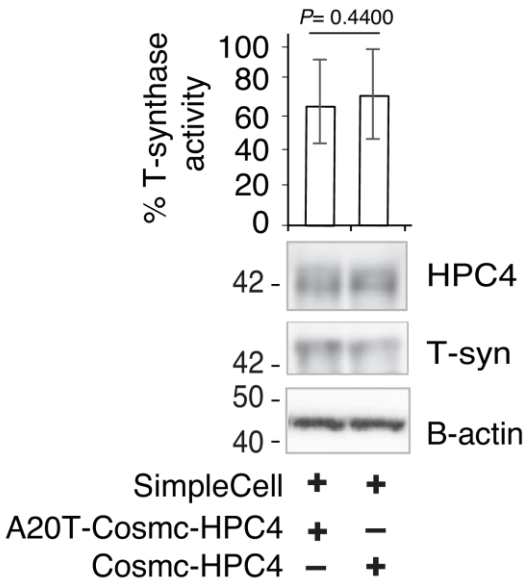
Supplementary Figure S5. A20D-Cosmc in patient fibroblasts generates remarkably normal O-glycans, and the cells contain no detectable level of Tn-antigen as opposed to lymphoblastoid cells. **A**, Normal O-glycan profile on the different cell types of patient M2. Whole-cell lysates prepared from patient fibroblasts (M2-fib) compared to other cultured cell lines (LCL) from the same patient and M1 (similar to **Figure 4G**), were further treated with Neuraminidase and O-glycanase as indicated and analyzed using SDS-PAGE-LB probed with PNA, B-actin (loading control), and streptavidin (signal from secondary reagent alone). **B-D**, Similar to **A**, the lysates were analyzed for Tn-antigen expression using ReBaGs6 (**B**), ReBaGs6 in the presence of GlcNAc (**C**), and ReBaGs6 in the presence of GalNAc (**D**). Stripped membranes from **B-D**, probed for B-actin (loading control); middle panel stripped again and re-probed for PNA as indicated. **E**, Similar to **B**, M1 lysates were prepared, processed and probed as indicated. B-actin serves as loading control. All experiments were repeated two times. NeuA: neuraminidase, O: O-glycosidase.



Supplementary Figure S6. A20D-Cosmc in patient fibroblasts shows normal cell surface O-glycans compared to lymphoblastoid cells. **A**, Lymphoblastoid cultured cell lines from healthy and both male patients (M2-LCL and M1-LCL) as well as fibroblast cells (M2-fib), were analyzed using flow cytometry for their surface glycan expression (Tn-antigen) as indicated using ReBaGs6. **B**, Quantification of **A**, error bars represent ±1 SD of three independent experiments (n=3). *p* value=0.0466 (without Neuraminidase treatment for Healthy-LCL vs. M2-fib), and *p* value=0.6355 (with neuraminidase treatment for Healthy-LCL vs. M2-fib). Other relevant *p* values: for neuraminidase untreated samples $p<0.0001$ (Healthy-LCL vs. M1-LCL), $p=0.0003$ (Healthy-LCL vs. M2-LCL), $p=0.0004$ (M2-LCL vs. M2-fib); for neuraminidase treated samples $p=0.0003$ (Healthy-LCL vs. M1-LCL), $p<0.0001$ (Healthy-LCL vs. M2-LCL), $p=0.0001$ (M2-LCL vs. M2-fib). MFI: Mean Fluorescence Intensity.



Supplementary Figure S7. Proteasomal degradation of A20D-Cosmc. **A**. Lysates from A20D-Cosmc-HPC4 or Mock transfected cells that were treated with Lactacystin (as shown) or parallel equal volume with DMSO were resolved on SDS-PAGE. Immunoblots were probed for the proteins as indicated to the right of each blot. **B**. Quantification of labeled HPC4 and T-synthase bands from A. All values obtained from each time point were converted to percentages and grouped into treated vs. untreated (6 cases each). A20D-Cosmc (top) and T-synthase (bottom). Error bars represent ±1SD.



Supplementary Figure S8. A20T-Cosmc variant expression and T-synthase activity. Whole cell lysates prepared from transiently transfected A20T-Cosmc-HPC4 and WT Cosmc-HPC4 were analyzed for T-synthase activity (n=8, two independent experiments of n=4). Additionally, the same preparation of the lysates was resolved on SDS-PAGE and immunoblotted with antibody as indicated on the right (n=3, representative example).

Supplementary Table S1: Complement activity monitoring of patient M1 for 30 months after first presentation.

	Normal range	05/2019	09/2019	12/2019	03/2020	06/2020	08/2020 09/2020	12/2020	04/2021	06/2021	09/2021	12/2021
sC5b-9 (ng/mL)	58-239	286 ↑	127	99	155	215	257 ↑	156	137	179	197	197
LDH (U/L)	120-300	609 ↑	246	211	192	235	316 ↑	216	236	235	229	274
Platelets (/nL)	150-530	54 ↓↓	170	198	185	165	175	215	176	105 ↓	130 ↓	127 ↓
Comment		PT	Ecu start			Ecu stop	TMA flare, Ecu restart	Ravu switch				

Preceeding anti-C5 therapy and again upon treatment discontinuation, there is mild to moderate complement hyperactivation as evidenced by elevated serum terminal complex (sC5b-9) concentrations. PT: acute kidney injury pre-treatment, Ecu: Eculizumab, TMA: thrombotic microangiopathy, Ravu: Ravulizumab.

Supplementary Table S2: Phenotypic features in Tn syndrome and *Cosmc*-CDG

	Clinical feature	Tn syndrome	*Cosmc*-CDG
Hematology	Thrombocytopenia	+	+
	Neutropenia	+	+[1]
	Lymphopenia	+	+[1]
	Hemolytic anemia	+	+[1]
Immunology	Hypogammaglobulinemia	-	+[1]
	Immune deficiency	+	+[1]
	Complement hyperactivation	-	+[1]
Nephrology	Acute kidney failure	-	+[1]
Gastroenterology	Acute pancreatitis	-	+[1]
Neurology	Developmental delay	-	+
	Intellectual/learning disability	-	+
Growth	Short stature	-	+
	Facial dysmorphism	-	+

Comparison of the clinical features in Tn syndrome and *Cosmc*-CDG. [1]: Feature so far not observed in heterozygous females.

Supplementary Dataset 1 Legend:

Raw data and statistical calculations underlying the Main and Supplementary Figures:

Figure 4B: Percent T-Synthase activity of whole cell lysates.

Figure 4C: Percent Hexosaminidase activity of whole cell lysates.

Figure 4D: Percent T-Synthase activity of whole cell lysates.

Figure 4F: T-Synthase activity of whole cell lysates.

Figure 4H: Percent T-Synthase activity of whole cell lysates.

Figure 5A: Mean florescence intensity (MFI) of PBMC expressing Tn antigen

Figure 5B: FACS analysis for cell surface O-glycan structures. Mean florescence intensity (MFI) of healthy and patients lymphoblastoid cells for Tn antigen expression (using ReBaGs6) and for normal core1 structure (using PNA lectin). Data for samples treated with or without Neuraminidase.

Figure 5C: FACS analysis for cell surface N-glycan structures. Mean florescence intensity (MFI) of healthy and patients lymphoblastoid cells were analyzed using ConA lectin. Data for samples treated with or without Neuraminidase.

Figure 5H: FACS analysis for cell surface O-glycan structures. Mean florescence intensity (MFI) of healthy and patients lymphoblastoid cells for Tn antigen expression (using ReBaGs6) and for normal core1 structure (using PNA lectin), after Neuraminidase treatment.

Suppl. Figure 3: Percent T-Synthase activity of whole cell lysates.

Suppl. Figure 6: Quantification of FACS analysis for cell surface O-glycan structures as presented in Suppl. Figure 5A. Mean florescence intensity (MFI) of healthy and patients lymphoblastoid cells for Tn antigen expression (using ReBaGs6). Data for samples treated with or without Neuraminidase.

Suppl. Figure 7B: Quantification of Western blot images of panel A using ImageJ software.

Suppl. Figure 8: Percent T-Synthase activity of whole cell lysates.

SI References

1. T. Macha, F. Petermann, The ET 6–6. A method for developmental assessment for German-speaking countries. *Zeitschrift Für Psychologie / Journal of Psychology* **216**, 154–160 (2008).