Supplemental Material

Patient	SB Villus Structure	SB EL/ED	SB Apoptotic activity	SB CLPI	SB ALPI	LB. Ab Arch	LB EL/ED	LB Apoptotic Activity	LB CLPI	LB ALPI
P1 - pre	TVA	+++	++	+	+	+++	++	+++	+	-
P1 - M6	PVA	++	++	+	+	+++	+++	++	++	-
P1 - M18	PVA	-	++	++	+	++	+	-	++	-
P1 - latest (10 years post HSCT)	Patchy VA	++	+	+	-	No LB	No LB	No LB	No LB	No LB
P2 - pre	TVA	+++	++	+++	++	++	+	++	++	+
P2 - M6	TVA	+++	++	+	++	++	++	+	+++	+
P2 - M18	PVA	+	+	++	+	++	++	-	++	-
P3 - pre	TVA	+++	++	++	+	No LB	No LB	No LB	No LB	No LB
P3 - M6	Patchy VA	++	++	++	-	++	++	++	++	-
P3 - M18	TVA	+	+	++	+	++	+	+	+	+
P4 - pre	TVA	+++	n/a	+++	-	n/a	n/a	n/a	n/a	n/a
P4 - M6	TVA	+++	n/a	+++	-	n/a	n/a	n/a	n/a	n/a
P4 - M18	PVA	++	++	++	+	n/a	n/a	n/a	n/a	n/a

Table S1. **Small bowel histology pre- 6-months and long term post HSCT** (SB = small bowel, LB = large bowel, A/CLPI = acute/chronic lamina propria infiltration, TVA = Total Villus Atrophy, PVA = partial villus atrophy, EL/ED = Epithelial Loss/Detachment + = mild, ++ = moderate, +++ = severe loss/destruction or infiltration, pre = Assessment biopsies within 1months prior to HSCT, M6 = 6 months post HSCT, M18 = 18 months post HSCT).

Genetics (coding sequence/ genomic position)	Phenotype	Immunology	Donor	Age at HSCT [months]	Conditioning	Chimerism	Outcome	PN Dependence post HSCT	Citation
c.2033C>A c.2134C>T	MIA	T-B-NK-	MUD 10/10	10	Cyclophosphamide Thiotepa	Not available	Died day 55 post HSCT from CMV pneumonitis	Too early	[6]
c.829C>T c.829C>T	MIA	T-B-NK-	UCB 5/6	8	Not available	Not available	Died 1 month post HSCT from sepsis	Too early	[8]
2:47221652_47221655delAAGT c.2468T>C	MIA	T-	UCB 6/6	6.5	Not available	Not available	Died 1 year post HSCT from disease progression	Deterioration	[4]
c.211G>A c.211G>A	AIE	T-NK-B+	N/A	N/A	Not available	Too early	Died early post HSCT from unknown causes	No change	[7]
c.211G>A c.211G>A	AIE	T+B-	N/A	N/A	Not available	100% donor	Died 9 months post HSCT from infection	No change	[7]

Table S2. **Clinical characteristics for the five patients reported in the literature who underwent allogeneic HSCT for TTC7A deficiency.** Data extracted from references: [4 6-8] (MIA = multiple intestinal atresias, AIE = autoimmune enterocolitis, MSD = matched sibling donor, MUD = matched unrelated donor, UCB = unrelated cord blood)

Pt	(Pt age)	CD4+ x10^9/L	CD8+ x10^9/L	CD19+ x10^9/L	CD16+ CD56+ x10^9/L	CD4 TRECs /10^6 T cells	Naïve CD4 %	Naïve CD8 %
1	Pre SCT (7 m)	1.2	2.3	0.7	0.10	NA	NA	NA
	Post SCT (6 y)	2.0	1.2	0.3	0.04	NA	50	81
2	Pre SCT (3 m)	1.9	1.1	0.5	0.08	42000	78	94
	Post SCT (20 m)	1.0	1.1	1.0	0.15	NA	50	19
3	Pre SCT (3 m)	0.2	0.2	0.5	0.01	10832	75	83
	Post SCT (18 m)	0.7	0.4	0.2	0.07	NA	41	67
4	Pre SCT (2.5m)	0.05	0.02	0.5	0.12	<252	25	77
	Post SCT (4y)	0.7	0.8	0.1	0.11	NA	44	50

Pt	(Pt age)	VBeta spectratyping	PHA	IgA g/L	IgM g/L	IgG g/L
1	Pre SCT (7 m)	NA	absent	0.6	2.1	0.9
	Post SCT (6 y)	normal	normal	3.2	2.7	6.6
2	Pre SCT (3 m)	normal	impaired	0.3	0.2	0.9
	Post SCT (20 m)	normal	normal	2.9	1.0	7.8
3	Pre SCT (3 m)	normal	impaired	<0.07	<0.07	8.76
	Post SCT (18 m)	normal	normal	0.3	0.4	2.6
4	Pre SCT (2.5m)	normal	absent	<0.07	0.05	<0.75
	Post SCT (4y)	normal	normal	2.65	1.59	5.64

Table S3. Immune function of the 4 described cases before and after stem cell transplantation. NA: Not available.TRECs: T cell receptor excision circles, PHA: phytohaemagglutinin test.

PHA	Pt n1	Control for Pt n1	Pt n2	Control for Pt n2	Pt n3	Control for Pt n3
0 ug/ml	150	125	62	62	59	51
1.0 ug/l	173	63718	251	15560	102	51759
2.0 ug/ml	318	58775	892	20974	2160	58790
4.0 ug/ml	225	52212	7404	15735	3276	58263
8.0 ug/ml	769	45386	6941	10980	2934	66843

Table S4. Phytohemagglutinin response (PHA) at diagnosis for Patients n.1, 2 and 3. Response is expressed in mean counts per minute (CPM)

Extended Patient history

Patient one (P1) was the first female born to non-consanguineous parents of Mediterranean origin. At birth, she presented with acute intestinal obstruction, which required emergency surgical exploration on day three of life revealing pyloric stenosis and a microcolon. She developed steroid unresponsive profuse diarrhoea and intestinal failure, for which she was initiated on total parenteral nutrition (TPN). Histology of gut biopsies revealed grossly abnormal epithelial morphology, abundance of apoptotic debris and a mixed chronic and acute inflammatory infiltrate. At nine month of age she underwent defunctioning ileostomy and was trialled on steroid and monoclonal antibodies. This resulted in alleviation of her gut symptoms but did not improve enteral tolerance or reduction of intestinal inflammation. Her early clinical course was complicated by several septic events requiring multiple admissions to the paediatric intensive care unit (PICU). Interestingly, the patient had no clear signs of immunodeficiency at birth. At six months of age, her lymphocytes subsets were normal and her immunoglobulin (Ig) M and IgA levels were adequate with low IgG levels (0.9 g/L) possibly secondary to gut losses. Given the inability to improve enteral tolerance, control intestinal inflammation and the evidence of immunedysregulation resulting in life-threatening septic episodes she finally underwent HSCT. The patient received a conditioning regimen consisting of total body irradiation (2Gy), cyclophosphamide, fludarabine and antithymocyte globulin (ATG). She was transplanted at 17 month of age with cells from an unrelated cord blood donor (HLA 8/10; 1A and 1B allele mismatches). HSCT was relatively well tolerated,

with no major toxicities. Five years post transplantation she required subtotal colectomy and ileostomy formation. At the time of writing she was nine years and seven months post transplantation. The patient has never achieved full donor chimerism (stable donor engraftment at 51% in the CD3-, and 15% in the CD15 line). Her immune reconstitution shows normal T and NK cells and low B cells, with minor reduction in IgG, normal IgA/IgM and normal PHA. Ten years post SCT she developed thrombocytopenia of unknown origin. No anti PLT antibodies could be documented and a bone marrow aspirate and trephine documented a trilineage hypocellular marrow with negative cytogenetics. The child has not developed further intestinal strictures and manages small quantities of liquid and solid enteral nutrition, though she still requires PN (50% of TCR) to meet her calorie target. She has also remained on immunosuppression (weekly methotrexate, monthly TNFα blockade and low dose steroids). She developed hypothyroidism 60 months post transplantation with positive thyroid peroxidase antibodies and severe sensorineural hearing loss for which she is wearing earing aids. The genetic diagnosis was finally established through our NGS gene discovery pipeline and confirmed through Sanger sequencing. While writing this paper she was diagnosed with bridging fibrosis on liver biopsies and she is currently evaluated for a possible combined liver and small bowel transplant.

Patient two (P2) was the third male to a consanguineous family of Middle Eastern origin. A previous sibling had died in infancy because of undefined gastrointestinal symptoms. The patient was diagnosed at birth with multiple intestinal atresias involving the duodenum, the ascending and transverse colon for which multiple surgical resections were required in the neonatal period. Histology from bowel resections and intestinal biopsies revealed shedding of the intestinal epithelium marked mixed inflammatory infiltrate and florid apoptotic activity. Parenteral nutrition was started in the first months, and before reaching one year of age he was on a combination of Infliximab, Basiliximab, steroids and cyclosporine in the attempt to control his symptoms. At 11 months he was presented with a skin rash, which was biopsied and considered to be consistent with Langerhans cell histocytosis. Further staging revealed small lytic lesions on his middle cranial fossa and orbital bones with no evidence of gut involvement. From the immunological point of view, the patient showed normal T, B, NK and regulatory T cells, normal naïve CD4 and CD8 proportions and mildly reduced T-cell receptor excision circles (TRECs) levels. His Vbeta spectra typing was normal. Lymphocytes had a normal mitogen response. Similarly to patient one, IgG levels were low, in the presence of normal IgA and IgM. At 14 months of age, having failed conventional immunosuppressant strategies, he underwent treosulfan and cyclophosphamide conditioned allogeneic bone marrow transplantation from his HLA 10/10 matched sibling donor. Post transplantation, he developed grade I skin graft vs. host disease (GvHD), which was successfully treated with topical steroids. He reactivated Epstein Barr virus (EBV) and adenovirus (ADV) but never showed clinical signs of virus related disease. He also had recurrent E. Coli sepsis, which never required intensive care support. Two years post transplantation he developed thrombocytopenia of unknown origin (absent anti platelets antibodies, negative virology) while remaining 100% donor engrafted and off immunosuppression. At that stage he still required PN, returned to the Middle East and was lost to follow up. Additional clinical information had become available from a specialist centre in the United States where he was subsequently managed. According to clinical reports, he continued to require PN four years after transplantation was malnourished, severely anaemic and developed further intestinal strictures in multiple sites requiring two bowel reconstruction surgeries (pyloric atresia, duodenal stenosis and jejunum obstruction with 3 intestinal webs). He had advanced liver disease with bridging fibrosis. At the time of writing and six years after transplantation, his enteral tolerance is improving albeit still requiring daily PN. He developed an intestinal fistula and intestinal biopsies continue to show marked chronic inflammation. Similarly to patient 1, the genetic diagnosis was established through NGS and confirmed via Sanger sequencing.

Patient three (P3) was the first child to a non-consanguineous couple of British origin. He had an antenatal diagnosis with exomphalos at 35 weeks of gestation. At birth, he underwent a laparotomy, which revealed ileal atresia requiring distal ileal resection. Histology from intestinal biopsies showed a structurally abnormal epithelium, apoptotic debris and increased inflammatory activity. A second laparotomy was required at 19 days of life revealing pyloric atresia and a stenosis at the previous site of end-to-end anastomosis. Pyloroplasty and gastro-duodenal anastomosis were performed. At two months of age he was trialled on steroids as he showed clinical signs of pancolitis and could not establish feeds. Immunophenotyping revealed low T and NK cells, with normal B cells and low IgG level. He had a normal proportion of naïve cells but his TRECS were lower than normal for age. His Vbeta repertoire was normal. His mitogen test was impaired. By then the clinical phenotype of TTC7A deficiency has been reported in the literature and due to the specific phenotype, candidate gene sequencing for TTC7A was performed externally and confirmed at our centre. Given the bleak outcome of the sparse data available on the disease and his abnormal immunophenotype experimental HSCT was considered. At nine months of age he underwent conditioning with treosulfan, fludarabin and alemtuzumab and received peripheral blood stem cells from a HLA matched (12/12), unrelated donor. Post transplantation, he suffered one central line sepsis, which responded adequately with standard antimicrobial protocol. He required a single administration of Rituximab for EBV reactivation with no signs of lymphoproliferative disorder. He had no GvHD. Immunosuppression was stopped 7 months post-transplantation, but later re-started upon evidence of inflammation in his gut biopsies. At the time of writing the child was one year and nine months post-transplantation. He has not required further abdominal surgeries, remains on immunosuppression to control intestinal symptoms and requires parenteral nutrition but tolerates bolus feeding via gastrostomy. He has a high level of donor engraftment in his CD3 line (96% donor cells) but has progressively lost his chimerism in both the CD15 and the CD19 lines (7% and 0% donor engraftment respectively). His immune reconstitution is adequate; IVIG supplementation has been required following Rituximab administration until 11 months post transplantation.

Patient four (P4) has been previously reported[6]. He was the second child born to non-consanguineous parents of French-Canadian and mixed European descent. He was suspected antenatally to have bowel obstruction and underwent multiple surgeries for intestinal atresia. Histology from resected bowel showed large areas lacking mucosa, epithelial disarray and chronic inflammation. He was found to have near absent TRECs by universal state-wide newborn screening ad diagnosed with SCID based on severe T cell lymphopenia, very low proliferation to phytohemagglutinin and hypogammaglobulinemia. His

brother was found to be a HLA 9/10 rejection directly only match. At the age of 3 months, he received unmanipulated bone marrow infusion after 3 days of equine anti-thymocyte globulin. No GVHD prophylaxis was given and a skin rash that was not thought to be GVHD responded to topical treatment alone. Post-transplantation he has had no opportunistic infections but has had multiple admissions for central line infections. He never required immunosuppressive treatment. He remains dependent on parenteral nutrition and has developed progressive liver disease. T cell are donor-derived and myeloid cells show minimal donor contribution. His immune reconstitution is adequate, he is off of IVIG and has had appropriate responses to vaccines. Longer post HSCT follow up documented the appearance of a flaky skin phenotype together with lung dysfunction of unknown origin.