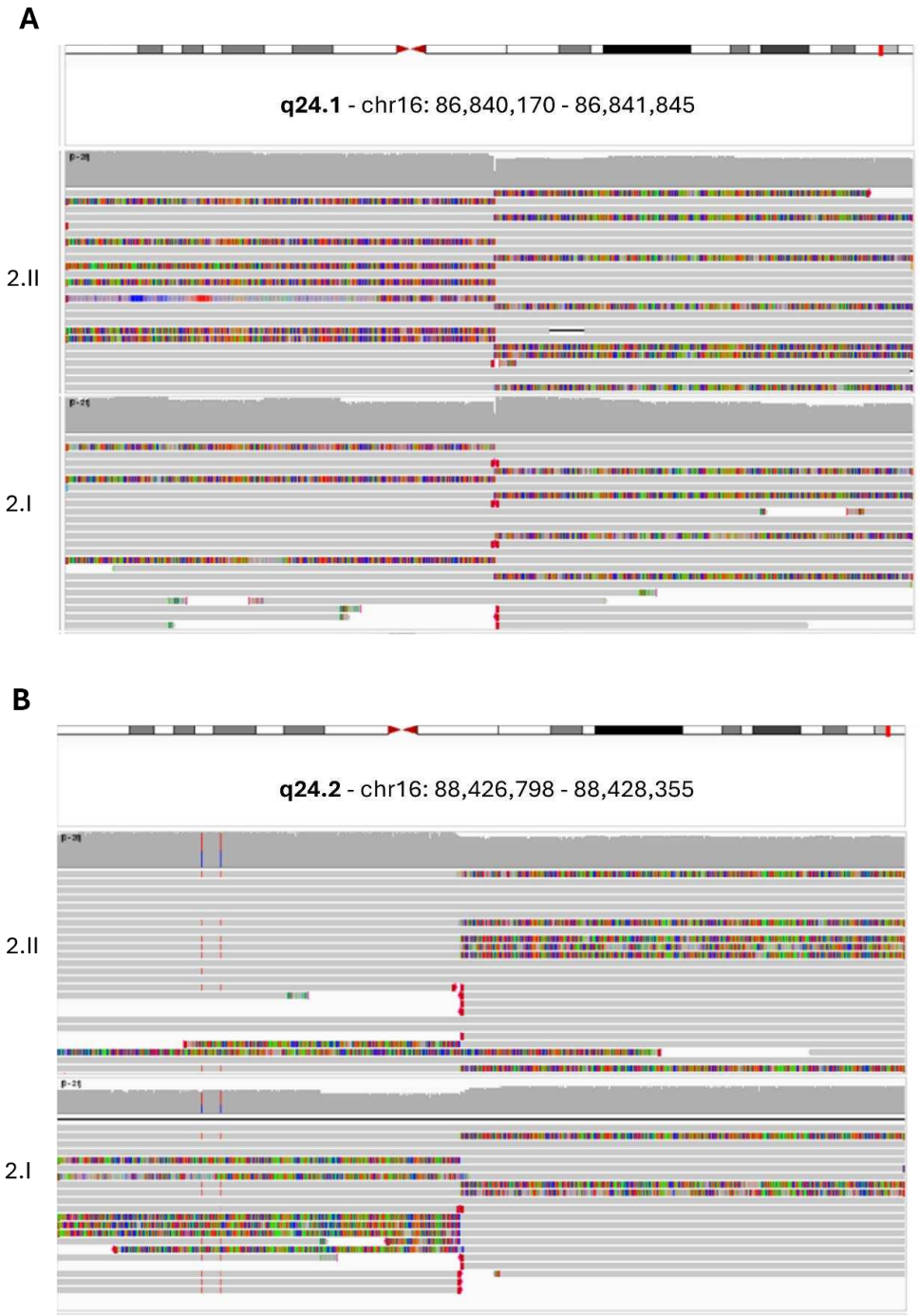
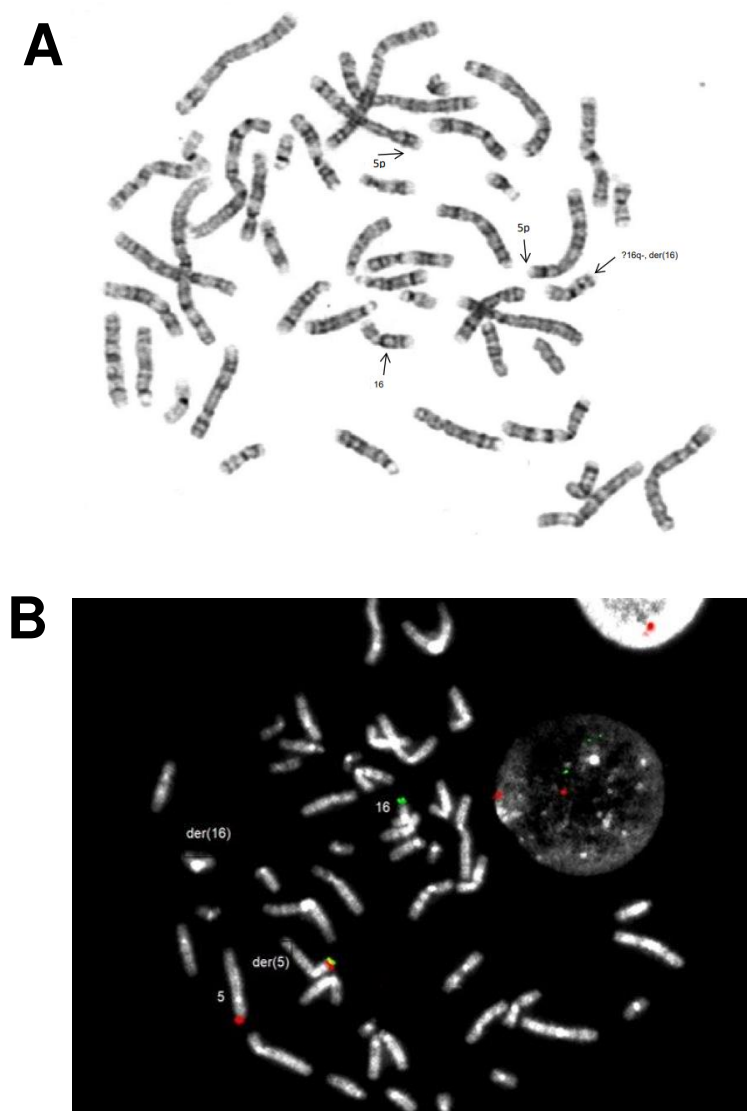
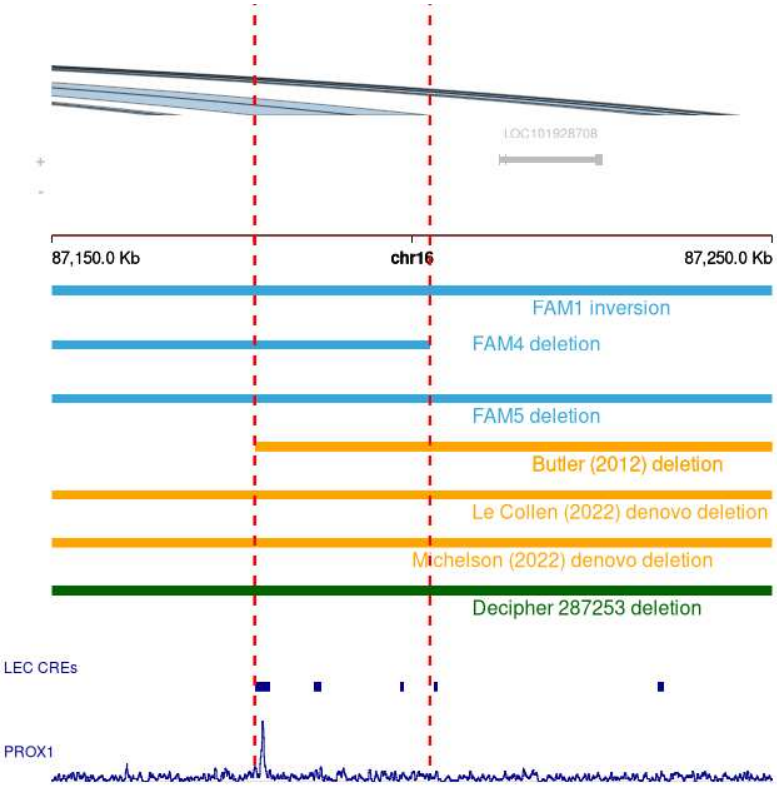


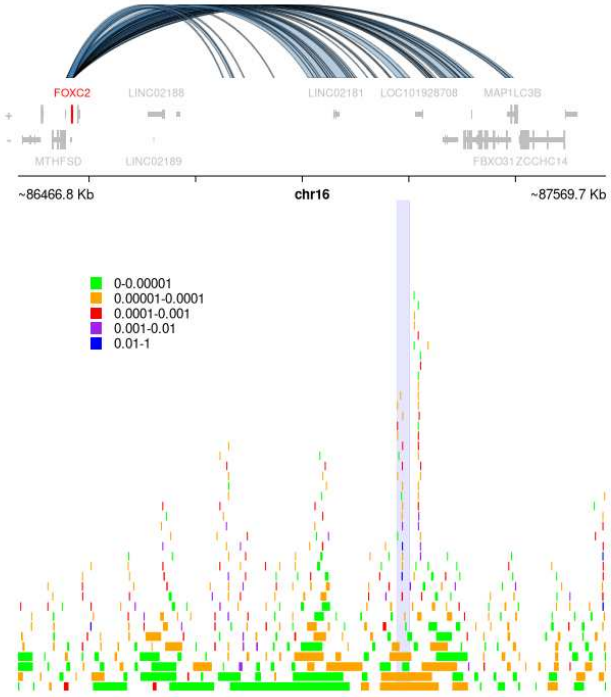
SUPPLEMTARY FIGURE 1. Nanopore sequencing confirmation of inversion in Family 1 showing the two breakpoints on chromosome 16q24.1 and 16q24.2. (A) Predicted breakpoint at chr16:86841018-86841022 for affected individual (2.II) (top panel) and affected individual (2.I) (lower panel). (B) Predicted breakpoint at chr16:88427541 for affected individual (2.II) (top panel) and affected individual (2.I) (lower panel).



SUPPLEMTARY FIGURE 2 – Experimental investigations of Family 2 (A) Historical karyotype of proband from Family 2 in metaphase with inconclusive identification of translocation. (B) Fluorescence in situ hybridization was carried out on metaphase chromosomes prepared from a blood sample from II.3, using probes specific for the short arm subtelomere region of chromosome 5 (Cytocell, TexasRed (5ptel48, 5p15.33) and the long arm subtelomere region of chromosome 16 (Cytocell, FITC (16qtel48, 16q24.3). The image shows a single segment translocation, with transfer of a terminal segment of material from the long arm of chromosome 16 to the terminal region of the chromosome 5 short arm, with no reciprocal transfer of material from the short arm of chromosome 5 to the long arm of chromosome 16.



SUPPLEMENTARY FIGURE 3. Genomic plot of 16q24 focusing on regions shared by reported SVs. Zoom in of Fig. 3. Red vertical dashed lines demark chr16: 87178000 - 87202000. The uppermost track depicts promoter capture Hi-C interactions between the *FOXC2* promoter and the downstream region in endothelial precursors(1). Locations of SVs reported in this paper and literature are shown. Bottom tracks depict regulatory annotations including predicted cis-regulatory regions (CREs) and PROX1 transcription factor binding, both in lymphatic endothelial cells (LECs).



SUPPLEMENTARY FIGURE 4. GnomAD structural variants (deletions) in 16q region. A regional plot of 16q24 details the genomic location of genes including *FOXC2* (highlighted in red). The uppermost track depicts promoter capture Hi-C interactions (arcs) between the *FOXC2* promoter and the downstream region in endothelial precursors(1). 401 deletions identified from gnomAD are shown, coloured by frequency in gnomAD. The majority of the deletions are rare (MAF 0.00001-0.0001; yellow) or extremely rare (MAF 0-0.00001; green). The region shared by our reported SVs (chr16: 87178000 – 87202000) is shaded in blue.

Type	Location (GRCh38)	Size (kb)	Distance to 3' UTR FOXC2 (kb)	Other genes	Detection method	FH	Lymph-oedema	Distichi-asis	Varicose veins	Heart defect	Phenotype	FAM/ REF
INV	chr16:86841016 – 88427540	1587	271	10	GS	Y	BLL	Y	Y	TCV	Bilateral cataracts, extra set of adult teeth, left ptosis‡, bilateral hearing loss, Raynaud's	1
ABT	chr16:86619099 - chr5:1162909	N/A	49		GS	Y	BLL	Y	Y	TOF		2
DEL - mosaic	chr16:86567588 – 86568736	1.1	Overlap 3' end *FOXC2*		Array CGH	N	Y	Y	Y			3
DEL	chr16:86592947 - 87202476	610	23	1	SNP array	Y	Y	Y	Y			4
DEL	chr16:86701917 -88132633	1400	132	9	ES +Array CGH	Y	Y				Mildly delayed psychomotor development, splenomegaly, submucous cleft palate, ptosis, misplaced atrophic left kidney	5
DEL – *de novo*	chr16:87119186 -87812135	693	550	6	Array CGH	N		Y			mild ID, uterine septum, vesicoureteral reflux, obesity	(2)*
DEL – *de novo*	chr16:86568969 -87463421	894	overlaps 3' UTR	8	SNP array	N	Y‡	Y			Bilateral hydronephrosis, global developmental delay	(3)
ABT	chr16:86685151 -86691699; chr22q13.1	N/A	~120		KaryoMap SNP array	Y	Y	Y			Recurrent miscarriages with severe fetal hydrops	(4)
DEL	chr16:87178192 -87443227	265	608	4	Chr. BAC microarray analysis	Y		Y	Y		Microcephaly, bilateral grade IV vesicoureteral reflux, mild ID	(5)
Complex	LOH in region§	N/A	N/A		Gene Chip 250k array + PCR		Y	Y			Scoliosis and strabismus	(6)
ABT	~chr16:86688088; chrYq12	N/A	~120 kb		Chr. painting and FISH		Y‡				Severe hydrops at delivery	(7,8)
DEL	chr16:86727745 -87938533	1210	~158kb	8			Y				ID	** (2)

SUPPLEMENTARY TABLE 1. Details of 16q structural variants in families from this study and those with LDS reported in the literature. Phenotypes listed are not necessarily present in every individual but taken from the family as a whole. FAM, refers

to the four families presented here; REF, refers to cases identified from the literature and are given in ( ). ABT, assumed balanced translocation; BLL, bilateral lower limb lymphoedema; Chr, chromosomal; DEL, deletion; FH, family history; ID, intellectual disability; INV, inversion; N, No; TCV, congenital tricuspid valve abnormality; UTR, untranslated region; Y, Yes; GS, short read whole genome sequencing; ES, short read exome sequencing. ‡congenital; *Decipher ID: 400063; **Decipher ID: 287253; §LOH in region: chr16:86450860-86586585 (includes CN: chr16:86568652-86568791 and DUP 5' : chr16:86556735-86556986). Distance to 3' UTR *FOXC2* defined based on GRCh38 coordinates of *FOXC2* transcript (ENST00000649859.1, including UTRs) from Gencode V46: chr16:86566829-86569728. Overlapping protein coding genes defined by canonical transcripts with gene names identified through Ensembl BioMart Human Genes dataset. Positions are reported in GRCh38 utilising UCSC LiftOver tool.

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