Supplementary Methods

Cloning procedures. Expression construct wtFOXC2/pAMC was produced by subcloning *FOXC2* cDNA (IMAGE clone 2758434) into the pAMC vector. Regions containing the mutations were amplified by PCR using DNA from the LD patients and subcloned into the wtFOXC2/pAMC. The notation of the mutations was the same as in [1] : mutant A – 14 bp duplication-insertion at the nucleotide 232 of *FOXC2* cDNA (GeneBank accession number NM 005251), termination at amino acid (aa) 100; mutant B-C→T at nucleotide 252, Q→ Term at aa 84; mutant C- deletion of A at nucleotide 505, termination at aa 202; mutant F- insertion of C at nucleotide 609, termination at aa 463; mutant K – 16bp deletion at nucleotide 1238, termination at aa 427. Mutants A, B and C have truncated DNA binding domains (DBDs), whereas mutants F and K contain entire DBDs. The expected protein products were detected by Western blotting of lysates from 293T cells, transiently transfected with the wt, mutant C, K and F constructs. Mutants A and B were expressed at low levels, likely due to instability of the truncated proteins.

Oligonucleotides

5'GATCCTTAAGTAAGTAAACAAACAAGATCCTTAAGTAAGTAAACAAACAA GATC-3' and

5'GATCTTGTTTGTTTACTTACTTAAGGATCTTGTTTGTTTACTTACTTAAGGAT -3' containing two FOXC2 binding sites were annealed and cloned into the *Hind* III site of pTAL-luc (Clontech). The cloning was repeated two more times to produce the plasmid 6xFOXC2/pTAL-luc containing six FOXC2 binding sites.

For the GAL4 heterologous transcriptional activation assay, fragments of *FOXC2* cDNA corresponding to the amino acids 237-501, 338-501, and 414-501 were cloned in frame with GAL4 DBD of pM1 vector (Clontech), to produce C-term 264/pM1, C-term 163/pM1 and C-term 87/pM1 constructs.

1. Finegold, D.N. et al. Truncating mutations in FOXC2 cause multiple lymphedema syndromes. *Hum. Mol. Gen.* **10**, 1185–1189 (2001).