toward the anterior (left) in *Pofut2* mutants. Number of mutant embryos represented by panels: *Nodal* (6), *Lefty2* (3), *BMP4* (4), *Wnt3* (6), *Bat-gal* (9), *Fgf8* (5), *Flk1* (10), *Gsc* (7), *Foxa2* (8), *Cer1* (3). Anterior is to the left; posterior is to the right. Proximal is up; distal is down. Scale bar indicates 100 μm.

Figure 7. Mesoderm in *Pofut2* mutants preferentially differentiates into vascular endothelial cells.

(A-Di) Whole mount and (B and D) transverse hematoxylin and eosin Y stained sections of E 8.5 wildtype (A-Bi) and *Pofut2* mutant **(**C-Di) embryos. (E-Fi) Immunofluorescence staining with PECAM antibody (green – indicated by white arrowhead) and DAPI (blue) in E 8.5 control (E-Ei) and *RST434* mutant littermates (F-Fi). Approximate plane of sectioning for panels (B, D-G) are indicated by black lines in A and C. Bi and Di represent enlargements of boxed regions in panel B and D. (G, H) O-dianisidine staining of E 8.5 wild type (G) and mutant (H) embryos. Number of mutant embryos represented by panels: C (9), D (7), PECAM (9), O-dianisidine (6). For panels A-D and G and H anterior is left; posterior is right. Proximal is up; distal is down. Abbreviations: bl, primitive erythrocytes; ve, visceral endoderm; ven, vascular endothelial cells; *, condensed mesoderm. Scale bar sizes indicated in μm.

Figure 8. The inability of *Pofut2* mutant embryos to form differentiated tissues derived from all three germ layers was rescued in teratomas.

(A-F) Teratomas (n=6) derived from E 7.5 *Pofut2* mutant embryos were sectioned and stained with hematoxylin and eosin Y. Tumors were comprised of tissues derived from all three germ layers. Endoderm derived tissues include mucin-producing columnar epithelial cells (mu), and ciliated respiratory epithelial-type columnar cells (ce). Ectoderm derived tissues include neural epithelium (ne), keratinized squamous cells (ke), and melanocytes (mc). Smooth muscle (sc) and lymphoid tissue (ly) are derived from mesoderm.

Supplementary Figure 1: *Pofut2* expression in adult tissues and *RST434* insertion analysis

(A) (Above) Semi-quantitative RT-PCR comparison of *Pofut2* and *Gapdh* expression in tissues obtained from 5-week-old males. (Below) *Pofut2* bands were quantified and normalized to *Gapdh*. –RT indicates control PCR using total RNA as template. (B) The BayGenomics 5' RACE data for *RST434* demonstrated that exon 5 of *Pofut2* is spliced to β-geo. Using PCR of *RST434* heterozygous genomic DNA followed by sequencing, we determined the *RST434* allele resulted from insertion of the gene-trap vector pGT0TMpfs within exon 6. The 5' flanking sequences were amplified using primers located within exon 5 (RST434-forward, 5'-GAGGCCGGGAGTACTGGGAT-3') and within the 5' part of the *Sal* I linearized gene trap vector pGT0TMpfs (RST434-down2, 5'-GGTTGCCAGAACCAGCAAACTGAA-3'). The 3' flanking sequences were amplified using primers located at the 3' end of linearized pGT0TMpfs (RSTrap-up3, 5'-TCATGTTCGACGACGCCATTGAGA-3') and a primer that recognizes intron 6 of *Pofut2* (Pofut2-gen-down1, 5'-AAGGAAACCCAGCTTTGAGGAGGA-3'). The gene trap insertion was accompanied by deletion of 38 nucleotides that included the 3' end of exon 6 as well as the 5' donor site of intron 6. *Pofut2* exon 6 is shown in uppercase and introns 5 and 6 are shown in lowercase. Arrowheads indicate the insertion site of the gene trap vector. Sequences located between the arrowheads were deleted during insertion. The RST434-reverse1 primer (primer b) matches the underlined sequence.

Supplementary Figure 2. Disruption of *Pofut2* in the *699Lex* strain resulted in a phenotype comparable to *RST434*.

(A) The *Pofut2* exon and intron structure is diagrammed (Above). The exons are numbered and the location of the ERE motif required for POFUT2 activity is indicated above exon 9. The structure of the linearized VCTR 48 Omnibank gene trap vector is diagrammed below the *Pofut2* gene. A splice acceptor (SA) site is located upstream of the neomycin phosphotransferase coding sequence (NEO). The arrowheads indicate the location of genotyping primers: 1197-upper (a), 1197-3' (b), and LTR-rev2 (c) (refer to Materials and Methods). (Below) Partial sequence of intron 1 is shown. The arrow indicates the insertion site of the gene trap vector. LTR: viral long terminal repeat. PolyA: polyadenylation signal. (B) The Lexicon Genetics *699Lex* data suggested VICTR 48 Omnibank Vector is inserted into *Pofut2* intron 1. To determine the sequence of the

fusion transcript, RT-PCR was carried out with the total RNA of *699Lex* heterozygous mouse. The forward primer (TTACATTAAAGCTTATGGCGGCGCTCAGCGTCGTCTG) matches the exon 1 of *Pofut2* gene, and the reverse primer (ATGTTTCGCTTGGTGGTCGAATGG) matches the coding sequence of neomycin phosphotransferase of vector. The PCR product was sequenced after gel purification. Partial sequence of the *Pofut2-VICTOR48* fusion transcript is shown. *Pofut2* exon1 sequence is indicated in uppercase, and the VICTR48 Omnibank gene trap sequence in lowercase. Two strong Kozak sequences are boxed. Two in frame stop codons are shaded in grey. The translated amino acid sequence is shown under the cDNA sequence. If translated, the *699Lex* fusion transcript was predicted to encode a 23 amino acid POFUT2 peptide. In addition, if the second Kozak sequence is utilized the fusion transcript will encode neomycin phosphotransferase (underlined). (C) Protein *O*-fucosyltransferase assays were carried out with tissue extracts from 5-week-old *wildtype* (n=2, open bars) and heterozygous *699Lex* (n=2, filled bars) mice. (Left panel) POFUT2 activity was reduced in heterozygotes when compared with wildtype littermates. (Right panel) POFUT1 activity, examined as control, was not affected in heterozygous tissues. Protein was obtained from kidney, k; lung, l; and brain, b. (D-F) Whole mount wild type, heterozygous and homozygous *699Lex* embryos at E7.5 are shown with the genotyping data (Di-Fi). (G) Transverse section through the embryonic ectoderm is shown after hematoxylin and eosin Y staining. Number of mutant embryos represented by panels: F (8), G (3), The putative primitive streak region is located on the right side. Scale bar = 100μm.

Supplementary Figure 3: Real time PCR confirmed elevated gene expression. Real time PCR detected significant elevation of *Nodal, Fgf8, Wnt3* and *BMP4* expression and significant down regulation of *T, Cer1, and Lefty2* expression ($P<0.01$ for all genes). The expression in wildtype embryos (empty bar) is presented as one and the expression level in homozygous mutant embryos (filled bar) is presented as folds when compared with wildtype embryos.

Supplementary Figure 4: PECAM expression is absent in distal sections of *Pofut2* mutant embryos.

(A) E 8.5 Whole mount *Pofut2* mutant with approximate distal plane of sectioning for panels B and Bi is indicated. (B) Immunofluorescence staining with PECAM1 antibody (green) and (Bi) PECAM1 plus DAPI (blue) in transverse sectioned *RST434* mutant. More proximal sections are shown in Figure 7. Number of embryos represented by panels: A (9) and B (9). Proximal is up; Distal is down.

Supplementary Table 1. Primers used for expression analysis of putative POFUT2 target proteins, MMP-2, and MMP-9 in mouse E7.5 embryos.*

genes		Primer sequence	Expression at E 7.5
ADAMTS1	forward	5-ATAACAATGCTGCTATGTGCGCGG-3	Yes
	reverse	5-TTATGCTTCCTGGTCCTGATGGCT-3	
ADAMTS2	forward	5-TTGGTGTCCCACGTGGTGTCTTT-3	Yes
	reverse	5-AGGAGGCTTTAGGTAAGTCTGCCA-3	
ADAMTS3	forward	5-AAGGTGAAGTGGAAAGGACCGACA-3	Yes
	reverse	5-TGAAGCACTTCCGTGTAGATGGCT-3	
ADAMTS4	forward	5-TCCACAGTTAGGGTGTGGAGCATT-3	Yes
	reverse	5-TAACCGTCAGCAGGTAGCGCTTTA-3	
ADAMTS5	forward	5-TATGACAAGTGTGGAGTGTGCGGA-3	Yes
	reverse	5-GGCATGACTTTCTGTGCTTTGGGT-3	
ADAMTS6	forward	5-TGATCCAAAGTGCTGGACACGGTA-3	Yes
	reverse	5-TGCTTCAGTCTCTGCCATGCCTTA-3	
ADAMTS7	forward	5-ACCTGCCATTTGCTTGGTGATGTC-3	Yes
	reverse	5-TTTCTGCTGCTGCTGTTCCCAATG-3	
ADAMTS8	forward	5-GCAAGGAAAGAGCAACCACCAACA-3	Yes
	reverse	5-TCATAAGCAAGGTCCAGTCCACCA-3	
ADAMTS9	forward	5-TGAAGGCACCAAATGTGATGCTGG-3	Yes
	reverse	5-AGCCGGAATCAAGTTTCCCTGAGA-3	
ADAMTS10	forward	5-TCAAGGTCACGCATGCCTTCAGAT-3	Yes
	reverse	5-TGACGCAGAGAGGAACGCTTGTAT-3	
ADAMTS12	forward	5-TCAAGTCCCTGCCAGAATACCACA-3	Yes
	reverse	5-TCCCGCTGTAGCTCTTGTTTCACA-3	
ADAMTS13	forward	5-TGGGACCCTAAGCCTCTGTTTGTT-3	No
	reverse	5-TTGAGCTTGATCCTGGCAGCTGTA-3	
ADAMTS14	forward	5-ACGACTATGTTGTGACGGTGCCTT-3	No
	reverse	5-TTGTGAAGGTCACCATGAGGCTCT-3	
ADAMTS15	forward	5-AATGGCTACAACCACAGCACCAAC-3	Yes
	reverse	5-ACGCTGACGGATATCAATGCTGGA-3	
ADAMTS16	forward	5-ATGACATCATGCACTACCAGCGGA-3	Yes
	reverse	5-GCGTCTTCCACAGAAATGCTGCTT-3	
ADAMTS17	forward	5-TCACCAACGAACACACAGTGGAGA-3	Yes
	reverse	5-AGCAATTCCAACAGTGTCGCAAGG-3	
ADAMTS18	forward	5-TGCTCAGAGCCTGTAACACCAACT-3	Yes
	reverse	5-TAAATCGTCCATGTGACCCTGCCT-3	
ADAMTS19	forward	5-AAGATCTCTGCCAAAGGTCCCACA-3	No
	reverse	5-TCGCCACACGTAGCATTTGGAGTA-3	
ADAMTS20	forward	5-AGAACGGTTGGCCCAGTGACTTAT-3	No
	reverse	5-ATCTGCCGTAGACTTTGGTTCCGT-3	
ADAMTSL1	forward	5-TGCTGCTCACAGATGTGTCCTTCT-3	No
	reverse	5-TGCTCTGATGGTAAGGTGATGCCA-3	
ADAMTSL2	forward	5-AGAGGCCTTGCTTCAAGTGGTACA-3	Yes
	reverse	5-AGAAATGGGCAGGTACTGCTTCCT-3	
ADAMTSL3	forward	5-TGTTCAGCTACCTGTGGTGTTGGA-3	No
	reverse	5-TTCGTTCCCAGTTTCGCTTTGCTG-3	

ADAMTSL4	forward	5-TATCAATGGGAACTGGGCTGTGGA-3	Yes
	reverse	5-TGACTCACGGGAAATGCAGAGGAA-3	
ADAMTSL5	forward	5-AATTCACCCTGACCCTGCTCTTCA-3	Yes
	reverse	5-TTCAGCTAGGCAGTTGAGGTCACA-3	
ADAMTSL6	forward	5-GAACCTTTCAATGGGCAGCTGGAA-3	Yes
	reverse	5-GTCATTTGGCCGGAGCTTCATGTT-3	
BAI1	forward	5-TGCAGGGAAAGTTCTTCGGCTACT-3	Yes
	reverse	5-GTAAGCAAGTGCGGGTTCGAGTTT-3	
BAI2	forward	5-ACATGGATACGGCAGAGTGAACCA-3	Yes
	reverse	5-TCCGAGTGTGCATCACCTTCTCAA-3	
BAI3	forward	5-ACGTGTGCCTGTTCTGGTGTCTTA-3	Yes
	reverse	5-ATTCCACAAGACTCGGTTCTCCCA-3	
C6	forward	5-GCTGATGGGCACTGGGTTTCATTT-3	Yes
	reverse	5-GCAAGGGTCAAACTTGGCTGCATA-3	
CCN1	forward	5-AGACCAGAACTGTGAAGATGCGGT-3	Yes
	reverse	5-CTGTCAAAGGACAAGCAGGGCTTT-3	
CCN2	forward	5-AAGCCAGGAAGTAAGGGACACGAA-3	Yes
	reverse	5-ACTTGCCACAAGCTGTCCAGTCTA-3	
CCN3	forward	5-TCTCCGCACCAAGAAATCCCTGAA-3	Yes
	reverse	5-ACAGCACAGGAAAGGAAGTCACCT-3	
CCN4	forward	5-TGTGATGATGACGCAAGGAGACCA-3	Yes
	reverse	5-TGGAACTTTACCCTGAGCCACACA-3	
CCN5	forward	5-TCTGGCCATTTCCTTCCTCTGCAT-3	No
	reverse	5-ACACTGAATCCACCCAGGACAGTT-3	
CCN6	forward	5-TGGAGCAGCATTGGAGGTGTATCA-3	Yes
	reverse	5-CACTTGGTTGCTTGCACGAGACAT-3	
Cfp (Properdin)	forward	5-TCACATGCTCCAAAGGAACCCAGA-3	Yes
	reverse	5-ATGACCGTTTCTCTTCCACCACCA-3	
CILP2	forward	5-AGCCACAGTCACCATCATCCTTGA-3	No
	reverse	5-AGCTTGATCAGGTGTTCCTGAGGT-3	
HMCN1	forward	5-AGTCGCAGACAACAGTAACGGTGA-3	Yes
	reverse	5-AACGCGGGTCTCTGTGATCTTCAT-3	
ISM2	forward	5-CAACACAGACTTGAGCGCACCAAA-3	Yes
	reverse	5-ATATCCGTTGCATTGTGGGCAAGG-3	
Papilin	forward	5-GCATTCGGAGAAGGCAGGTTGTTT-3	No
	reverse	5-TGCTCCGATGCAAAGTTATTGGCG-3	
SEMA 5a	forward	5-AGAGAGCCGGCCTTGTGTATTTGA-3	Yes
	reverse	5-TGAGGAGCAGCGAGTTTACAACGA-3	
SEMA 5b	forward	5-TGACCTTGGCAGTGTACCTGTCTT-3	Yes
	reverse	5-AACCAAAGTGGTTCTACGGTCCCA-3	
SPON1 (f-Spondin)	forward	5-TTGACTGTGAACTCAGCGAGTGGT-3	Yes
	reverse	5-TGCACGCTCTGATCTCCTTCTTGT-3	
SSPO (Sco-spondin)	forward	5-ATCGCTGTGACCTTCAGGTGAACT-3	No
	reverse	5-CACAGCTCAGCATTCACATGCACA-3	

THBS-1 (TSP-1)	forward	5-TGGCTTCCTTGAGGCAGATGAAGA-3	Yes
	reverse	5-ATGGTAACCGAGTTCTGGCAGTGA-3	
THBS-2 (TSP-2)	forward	5-ACAGTGTCACCCTGGAAGAACCAT-3	Yes
	reverse	5-TGCAGAGTGTGAACAGGATGGACA-3	
THSD7a	forward	5-TGCCAGCTTTCTGACTGGTCTTCT-3	Yes
	reverse	5-TGGGAATCACCACCACGGGAATAA-3	
THSD7b	forward	5-GCAACACGATCCACTTTGGCATGA-3	Yes
	reverse	5-AGGTCTTACAGTTTCTGGCCGTGT-3	
UNC5a	forward	5-CATCAAGCCCAGCAAAGCAGACAA-3	Yes
	reverse	5-TTCTGGCTTGTGCAGAGTGAGGTA-3	
XM_283765	forward	5-TGTGGAAATGGCAACCAGAAACGG-3	Yes
	reverse	5-AACTCGGTGCTGATGAGATTGGGA-3	
MMP-2	forward	5-TGTTTACCATGGGTGGCAATGCAG-3	Yes
	reverse	5-TGTTTGCAGATCTCCGGAGTGACA-3	
MMP-9	forward	5-TCTCTGGACGTCAAATGTGGGTGT-3	Yes
	reverse	5-ACTGCACGGTTGAAGCAAAGAAGG-3	

* Forty putative POFUT2 targets were expressed at E7.5 by RT-PCR with E 7.5 embryo total RNA (not shown). The 10 putative targets that were not expressed include: ADAMTS13, ADAMTS14, ADAMTS19, ADAMTS20, ADAMTSL1, ADMTSL3, CCN5, CILP2, Papilin, and Sco-spondin. Primers were designed based on the mRNA and genomic DNA sequences. Each pair of primer spans at least one intron to avoid the amplification of genomic DNA.

Supplementary Table 2. Primers used for real time PCR.

Gene		Primer sequence
GAPDH	forward	5’-GATTGTCAGCAATGCATCCTGC-3’
	reverse	5’-GTTCAGCTCTGGGATGACCTT-3’
Fgf8	forward	5’-TGAGCTGCCTGCTGTTGCACTT-3’
	reverse	5’-ATGTCGCTGTGTGACTTTAGGCAGGA-3’
Bmp4	forward	5’-GCTTCTGCAGGAACCAATGGA-3’
	reverse	5’-TCCCGGTCTCAGGTATCAAACTAG-3’
Wnt3	forward	5’-GGAGAAACGGAAGGAGAAATGCC-3’
	reverse	5’-CAGGTCGTTTATCACATGCAGC-3’
Nodal	forward	5’-GTGGACTTCAACCTGATTGGCT-3’
	reverse	5’-CATGCTCAGTGGCTTGGTCTT-3’
T	forward	5’-GTACCCAGCTCTAAGGAACCAC-3’
	reverse	5’-CAGAGACTGGGATACTGGCTAGA-3’
Lefty2	forward	5’-CGTGCAATGTGCAGAAGCAGAA-3’
	reverse	5’-AATGACATGGGCAAAGCTGCCA-3’
Cer1	forward	5’-TCAAAAGCCACGAAGTACACTGG-3’
	reverse	5’-TGCATCACCATCTTGACCACG-3’

Supplementary Table 3. Primers used for cloning *in situ* template plasmids.

gene	Primer name	Primer sequence
Pofut2	Pofut2-up1	5-TTACATTAAAGCTTATGGCGGCGCTCAGCGTCGTCTG-3
	Pofut2-down1	5-AACATGATCTCGAGTCAGTACGCAATCTTCCAGTGTGTG-3
Foxa2	Foxa2-forward	5-GGATCCAATTAACCCTCACTAAAGGGCGGCCGCAATGGACCT CAAGGCCTACGAACA-3
	Foxa2-reverse	5-GGATCCTAATACGACTCACTATAGGGCTCGAGATCAGACCCG AGACCTGGATTTCA-3
Sox2	Sox2-forward	5-GGATCCTAATACGACTCACTATAGGCTCGAGTACAGCATGTC CTACTCGCAGCA-3
	Sox2-reverse	5-GGATCCAATTAACCCTCACTAAAGGCGGCCGCTACATGGATT CTCGGCAGCCTGAT-3
Gsc	GSC-forward	5-GGATCCGCGGCCGCATCTTCACCGATGAGCAGCTCGAA-3
	GSC-reverse	5-GGATCCCTCGAGAGCAGTCCTGGGCCTGTACATTAT-3