SUPPLEMENTAL RESULTS

A

Alignments for THBS1-like domains

```
3r6b      ------INGGWGPWSPWDIC------------------SVTCGG---GVQKRSRLCNNPTPQFGGK----------------DCVGDVT---------ENQICNKQ-DCP
d1        QGEAEAPTLYWKT-GPWGRCMG----------------DECGPG--GIQTRAVWCAHVEGWTTLH---------------TNCKQAER-------PNNQQNCFK--VCD
d2        -------LYDWRL-GPWNQCQPVISKSLEKP--------LECIKGEEGIQVREIACIQKDKDIPAE--------------DIICEYFEP------KPLLEQACLI--PCQ
d5        --------YGWRT-TEWTECRVDPLLSQQDKRRG--NQTALCGG---GIQTREVYCVQANENLLSQLSTHKNKEASKPMDLKLC-TGPI-------PNTTQLCHI--PCP
d7        ---------DWKA-VRLGNCEPDNG--------------KECGP---GTQVQEVVCINSDGEEVDR---------------QLCRDAIF-------PIPVA-CDA--PCP
d9        -------VYHWQT-GPWGQCIEDTSVSSFNTTTTWNGE-ASCSV---GMQTRKVICVRVNVGQVGP---------------KKCPESLR-------PETVRPCLL--PCK
d11       -------SYRWKT-HKWRRCQLVPWSVQQDSPGAQ----EGCGP---GRQARAITCRKQDGGQAGI---------------HECLQYAG-----PVPALTQACQI--PCQ
d13       -------KYNAQPVGNWSDCILPEGKVEVLLGMKVQGDIKECGQ---GYRYQAMACYDQNGRLVET---------------SRCNSHGY---------IEEACII--PCP
d15       -------QYLWVT-EPWSICKVTFVNM-----------RENCGE---GVQTRKVRCMQNTADGPSE-----------HVEDYLCDPEEM-------PLGSRVCKL--PCP
d17       -------HYDYNV-TDWSTCQLSEK--------------AVCGN---GIKTRMLDCVRSDGKSVDL---------------KYCEALGL----EKNWQMNTSCMV--ECP
d19       ---------RWQY-GQWSPCQVQE---------------AQCGE---GTRTRNISCVVSDGSADDF---------SKVVDEEFCADIELIIDGNKNMVLEESCSQ--PCP
d21       --EYKWMASAWKGSSRTVWCQRSDGINVT----------GGCLVM--SQPDADRSCNP-------------------------PCSQP------------HSYCSETKTCH
```

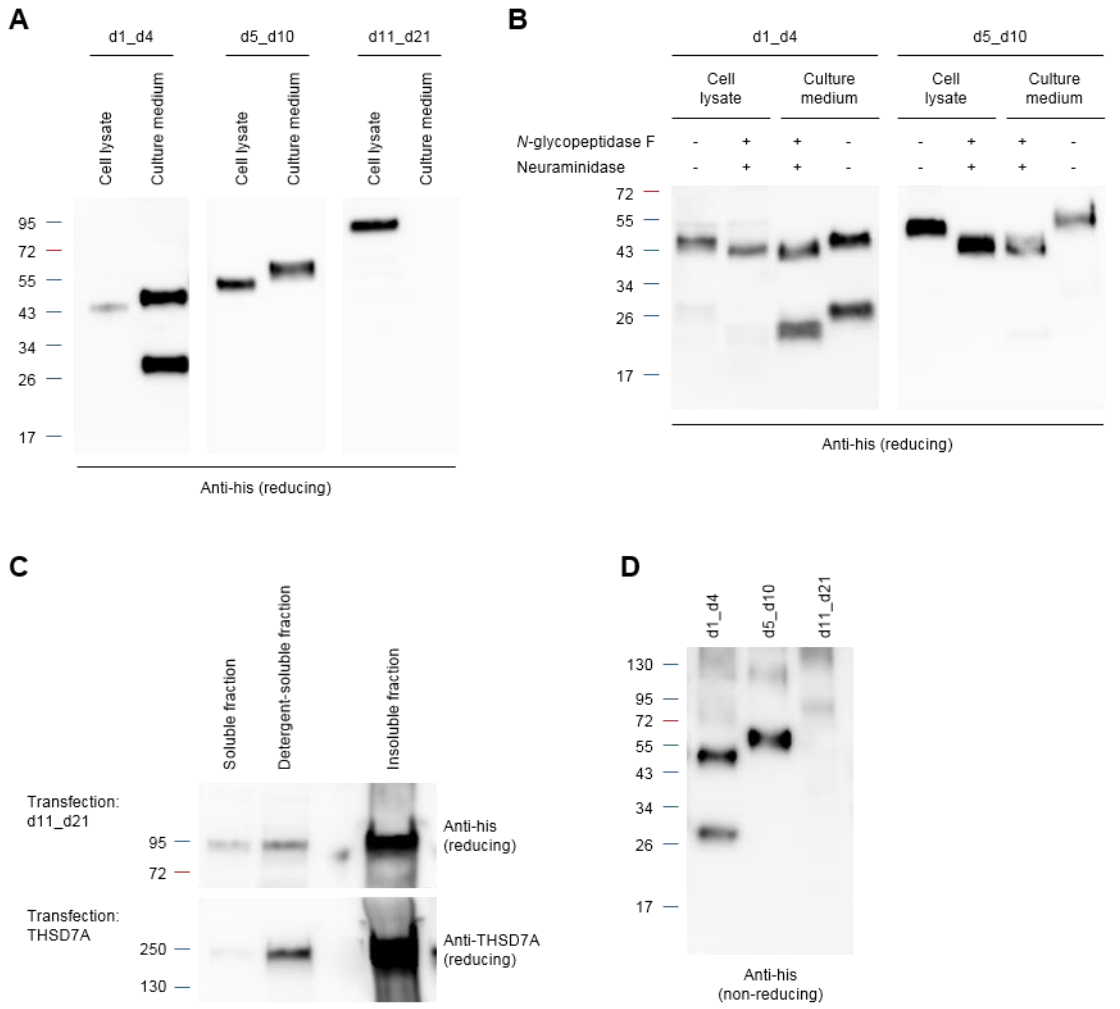
Alignments for C6-like domains

```
3t5o1     --CFCDHYAWTQWTSCSK-TCN--------SGTQSRHRQIVVDKYYQE---NFCEQICSKQ-----ETRECNWQ-----RCP----
3t502     --INCLLGDFGPWSDCD--PCI---------EKQSKVRSVLRPSQFG---GQPCTAP-------LVAFQPCIPSK----LCKIE--
1szl      GSETCIYSNWSPWSACSSSTC-------EKGKRMRQRMLKAQ--LDLSV--PCPDT--------QDFQPC-----MGPGCSDEDG
d3        --QDCIVSEFSAWSECS-KTC-------GSGLQHRTRHVVAPP-QFGGS--GCPNL--------TEFQVC-----QSSPCE----
d4        --KECQVSEWSEWSPCS-KTCHDMV--SPAGTRVRTRTIRQFP-IGSEK--ECPEF--------EEKEPCLSQGDGVVPCA----
d6        --TECEVSPWSAWGPCTYENCNDQQ--GKKGFKLRKRITNEPTGGSGVTGNCPHL--------LEAIPC-----EEPACY----
d8        --KDCVLSTWSTWSSCS-HTC-SGK--TTEGKQIRARSILAYAGEEGGI--RCPNSSA-----LQEVRSC-----NEHPCT----
d10       --KDCIVTPYSDWTSCP-SSCKEGD--SSIRKQSRHRVIIQLP-ANGGR--DCTDP-------LYEEKAC----EAPQACQ----
d12       --DDCQLTSWSKFSSCN-GDC--------GAVRTRKRTLVGKS--KKKE--KCKNSHLYP---LIETQYC--------PCD----
d14       --SDCKLSEWSNWSRCS-KSC-------GSGVKVRSKWLREKP-YNGGR--PCPKLDHVNQAQVYEVVPC------HSDCN----
d16       --EDCVISEWGPWTQCV-LPC------NQSSFRQRSADPIRQP-ADEGR--SCPNA--------VEKEPC----NLNKNCY----
d18       --VNCQLSDWSSWSQCS-QTC------GLTGKMIKRTVTQPF-QGDGR--PCPSLM-------DQSKPC-----PVKPCY----
d20       --GDCYLKDWSSWSLCQ-LTCVNGEDLGFGGIQVRSRPVIIQE-LENQH--LCPEQM-------LETKSC-----YDGQCY----
```

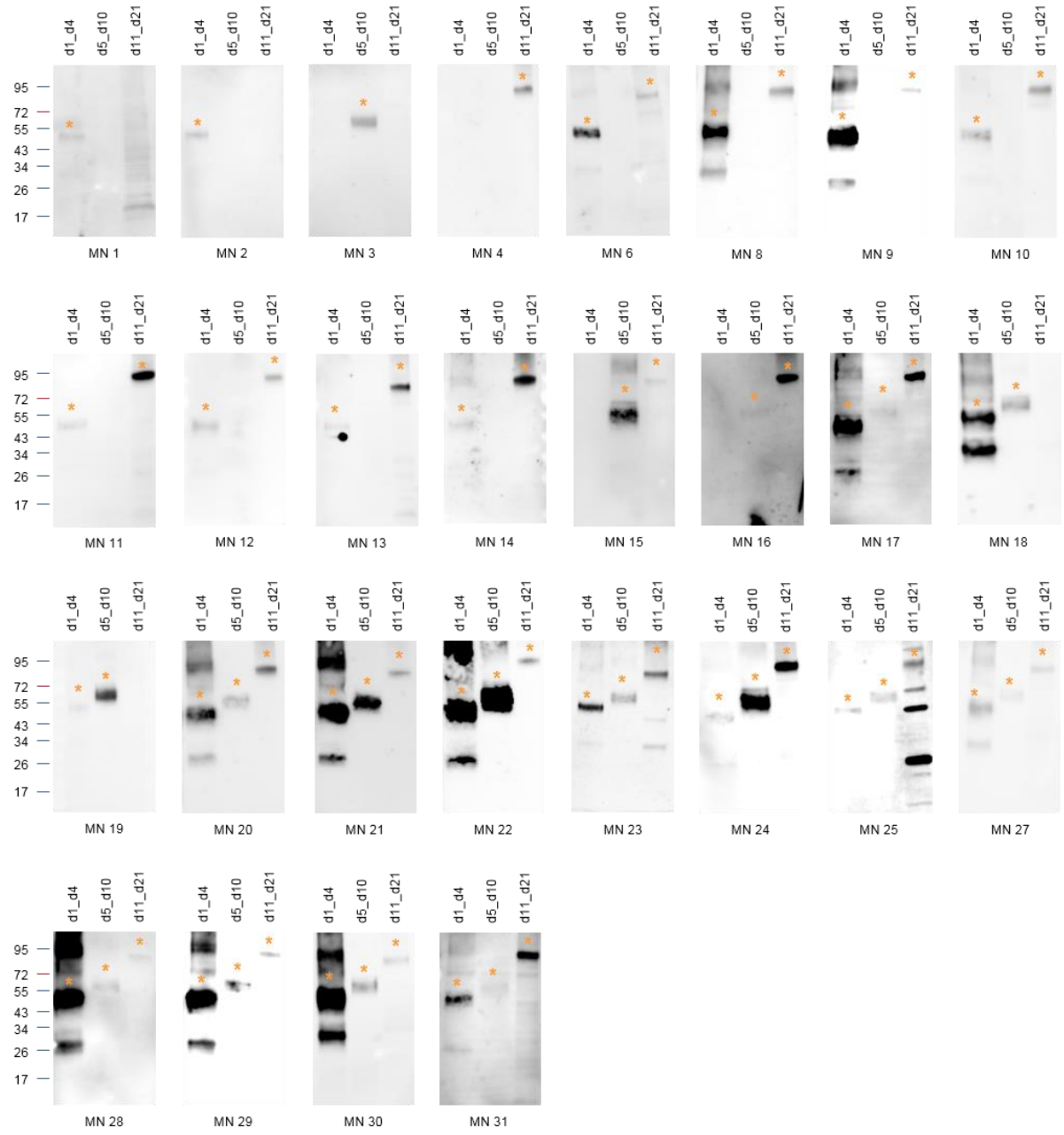
B

```
leader    MGLQARRWASGSRGAAGPRRGVLQLLPLPLPLLLLLLLRPGAGRA
d1        AAQGEAEAPTLYLWKTGPWGRCMGDECGPGGIQTRAVWCAHVEGWTTLHTNCKQAERPNNQQNCFKVCD
d2        WHKELYDWRLGPWNQCQPVISKSLEKPLECIKGEEGIQVREIACIQKDKDIPAEDIICEYFEPKPLLEQACLIPCQ
d3        QDCIVSEFSAWSECSKTCGSGLQHRTRHVVAPPQFGGSGCPNLTEFQVCQSSPCE
cc        AEELRYSLHVGPWSTCSMPHSRQVRQARRRGKNKEREKDRSKGVKDPEARELIKKKRNRNRQNRQENKYWDIQIGYQTREVMCINKTGKAADLSFCQQEKLPMTFQSCVIT
d4        KECQVSEWSEWSPCSKTCHDMVSPAGTRVRTRTIRQFPIGSEKECPEFEEKEPCLSQGDGVVPCA
d5        TYGWRTTEWTECRVDPLLSQQDKRRGNQTALCGGGIQTREVYCVQANENLLSQLSTHKNKEASKPMDLKLCTGPIPNTTQLCHIPCP
d6        TECEVSPWSAWGPCTYENCNDQQGKKGFKLRKRRITNEPTGGSGVTGNCPHLLEAIPCEEPACY
d7        DWKAVRLGNCEPDNGKECGPGTQVQEVVCINSDGEEVDRQLCRDAIFPIPVACDAPCP
d8        KDCVLSTWSTWSSCSHTCSGKTTEGKQIRARSILAYAGEEGGIRCPNSSALQEVRSCNEHPCT
d9        VYHWQTGPWGQCIEDTSVSSFNTTTTWNGEASCSVGMQTRKVICVRVNVGQVGPKKCPESLRPETVRPCLLPCK
d10       KDCIVTPYSDWTSCPSSCKEGDSSIRKQSRHRVIIQLPANGGRDCTDPLYEEKACEAPQACQ
d11       SYRWKTHKWRRCQLVPWSVQQDSPGAQEGCGPGRQARAITCRKQDGGQAGIHECLQYAGPVPALTQACQIPCQ
d12       DDCQLTSWSKFSSCNGDCGAVRTRKRTLVGKSKKKEKCKNSHLYPLIETQYCPCD
d13       KYNAQPVGNWSDCILPEGKVEVLLGMKVQGDIKECGQGYRYQAMACYDQNGRLVETSRCNSHGYIEEACIIPCP
d14       SDCKLSEWSNWSRCSKSCGSGVKVRSKWLREKPYNGGRPCPKLDHVNQFQVYEVVPCHSDCN
d15       QYLWVTEPWSICKVTFVNMRENCGEGVQTRKVRCMQNTADGPSEHVEDYLCDPEEMPLGSRVCKLPCP
d16       EDCVISEWGPWTQCVLPCNQSSFRQRSADPIRQPADEGRSCPNAVEKEPCNLNKNCY
d17       HYDYNVTDWSTCQLSEKAVCGNGIKTRMLDCVRSDGKSVDLKYCEALGLEKNWQMNTSCMVECP
d18       VNCQLSDWSPWSECSQTCGLTGKMIRRRTVTQPFQGDGRPCPSLMDQSKPCPVKPCY
d19       RWQYGQWSPCQVQEAQCGEGTRTRNISCVVSDGSADDFSKVVDEEFCADIELIIDGNKNMVLEESCSQPCP
d20       GDCYLKDWSSWSLCQLTCVNGEDLGFGGIQVRSRPVIIQELENQHLCPEQMLETKSCYDGQCY
d21       EYKWMASAWKGSSRTVWCQRSDGINVTGGCLVMSQPDADRSCNPPCSQPHSYCSETKTCH
stalk/tm  CEEGYTEVMSSNSTLEQCTLIPVVVLPTMEDKRGDVKTSRAVHPTQPSSNPAGRGRTWFLQPFGPDGRLKTW
cytosol   VYGVAAGAFVLLIFIVSMIYLACKKPKKPQRRQNNRLKPLTLAYDGDADM
```

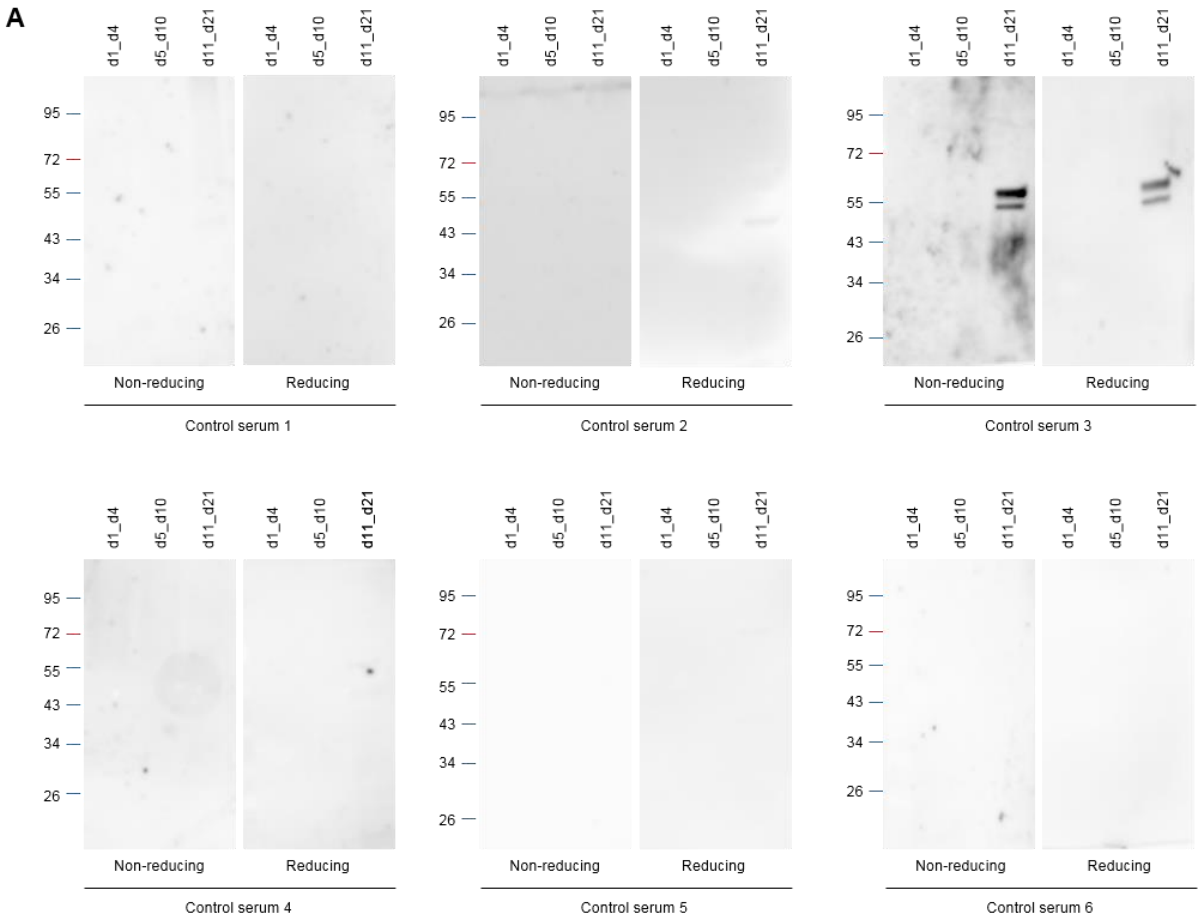
Supplemental Figure 1. Identification of TSP-1 domains and structure of human THSD7A. (**A**) Reference sequences for TSP-1 domains as in THBS1 (protein data bank [pdb] code 3r6b[1], upper panel) and for TSP-1 domains as in C6 (pdb code 3t5o[2]) or F-spondin (pdb code 1szl[3]) and alignment with the 21 TSP-1 domains of human THSD7A. Yellow, cysteine; magenta, tryptophane; cyan, arginine. (**B**) Amino acid sequence of full-length human THSD7A with colored tagging of all parts. Purple, leader peptide; red, THBS1-like TSP-1 domain; green, C6-like TSP-1 domain; blue, coiled coil (cc) domain; brown, extracellular stalk region, transmembrane (tm) region and cytosolic tail.

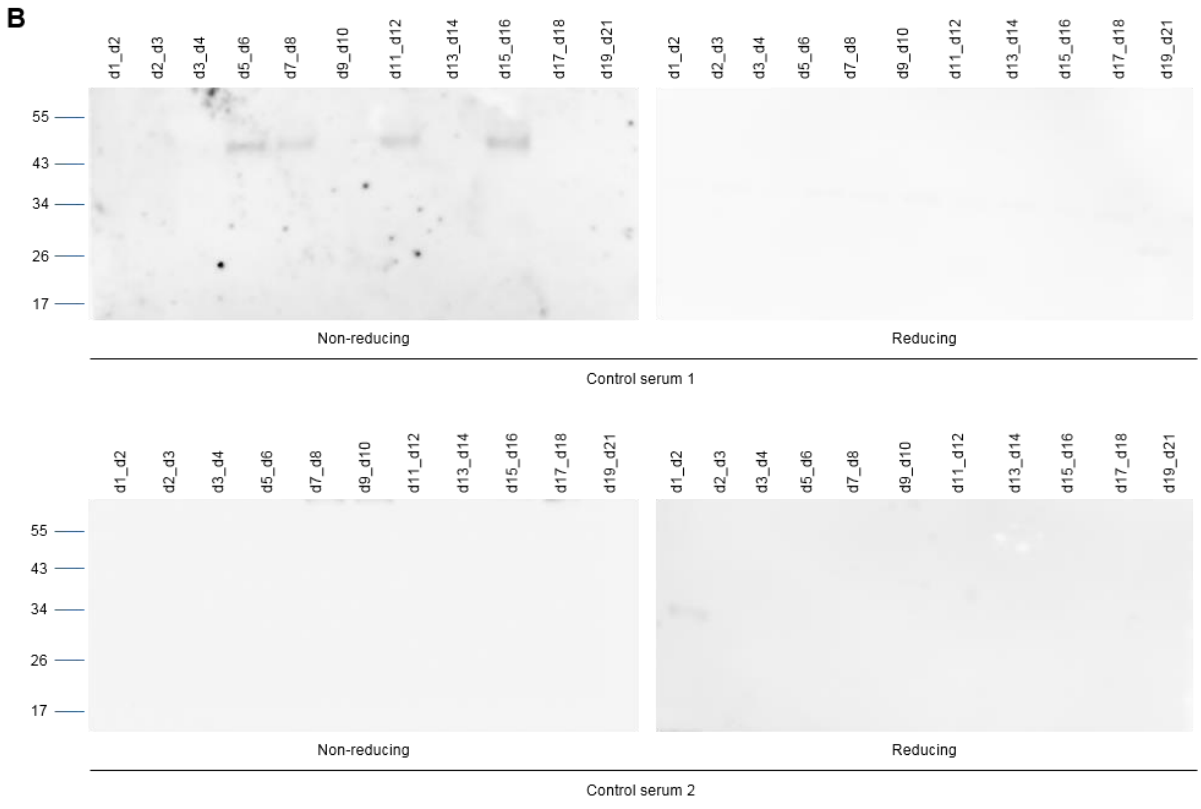


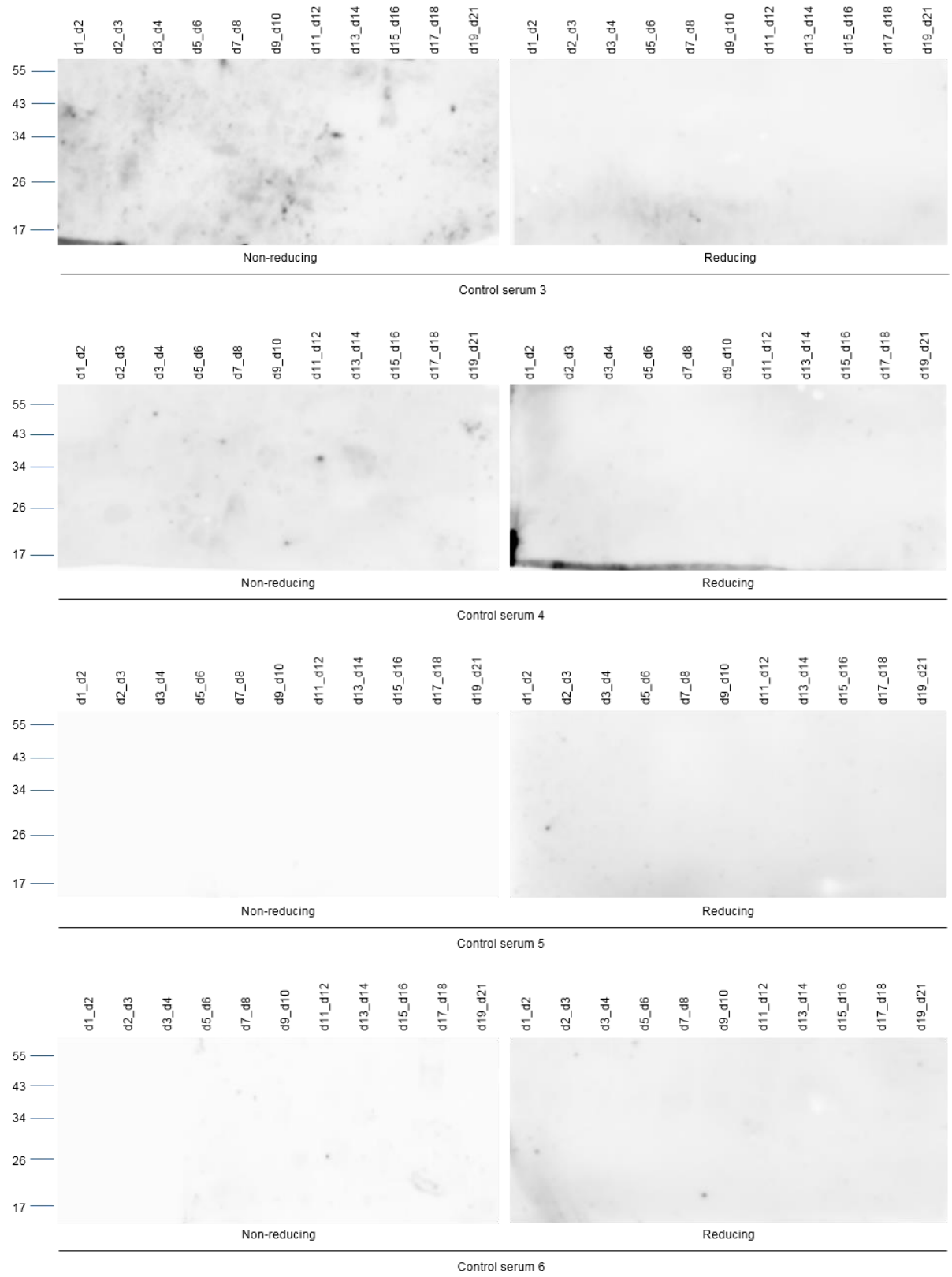
Supplemental Figure 2. Expression of three THSD7A fragments in HEK293 cells. (**A**) Western blot of cell lysates and culture media of transfected HEK293 cells with an anti-his antibody. Constructs d1_d4 and d5_d10, but not d11_d21, were secreted to the culture media. Secreted proteins showed a slightly higher molecular mass compared with the proteins in the cellular fraction. (**B**) Western blot of cell lysate and culture media fractions of d1_d4 and d5_d10 before and after enzymatic deglycosylation with *N*-glycopeptidase F and neuraminidase. Deglycosylated fragments migrated to the same molecular mass, demonstrating that the difference in size was due to incomplete glycosylation of the protein in the cellular fraction. (**C**) Western blot of different subcellular fractions of d11_d21-transfected HEK293 cells with an anti-his antibody. The d11_d21 construct was found in both the soluble and the detergent-soluble fraction (upper panel). As an experimental control, HEK293 cells transfected with full-length THSD7A were treated identically (lower panel). Both the d11_d21 construct and full-length THSD7A were also present in the insoluble fraction. (**D**) Western blot analysis of the three THSD7A fragments (culture media for d1_d4 and d5_d10 and cell lysate for d11_d21) with an anti-his antibody under non-reducing conditions.



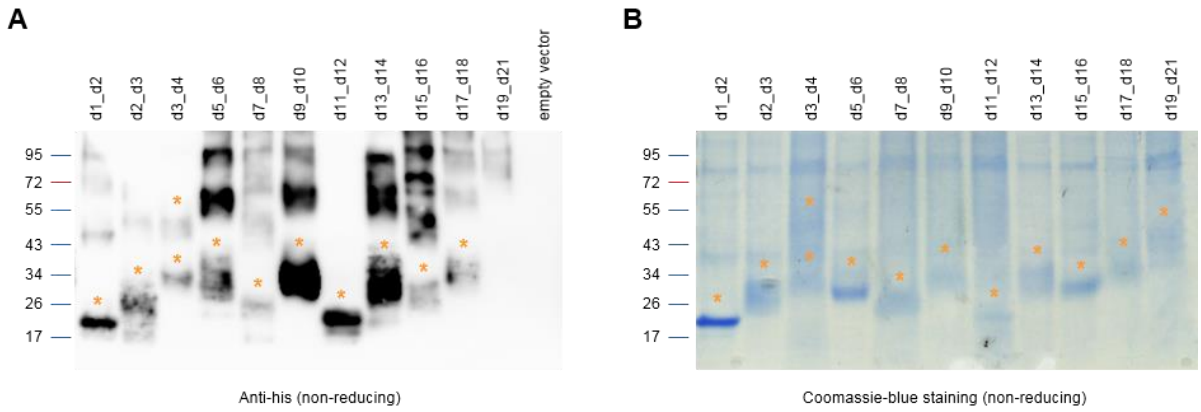
Supplemental Figure 3. Western blot analysis of the three THSD7A fragments d1_d4, d5_d10 and d11_d21 with sera from 28 patients with THSD7A-associated MN under non-reducing conditions (results of the remaining three patients are depicted in **Figure 2A** in the main body).


A
d1_d4
d5_d10
d11_d21
95
72
55
43
34
26
Non-reducing
Reducing
Control serum 1
Control serum 2
Control serum 3
Control serum 4
Control serum 5
Control serum 6


B
d1_d2
d2_d3
d3_d4
d5_d6
d7_d8
d9_d10
d11_d12
d13_d14
d15_d16
d17_d18
d19_d21
55
43
34
26
17
Non-reducing
Reducing
Control serum 1
Control serum 2

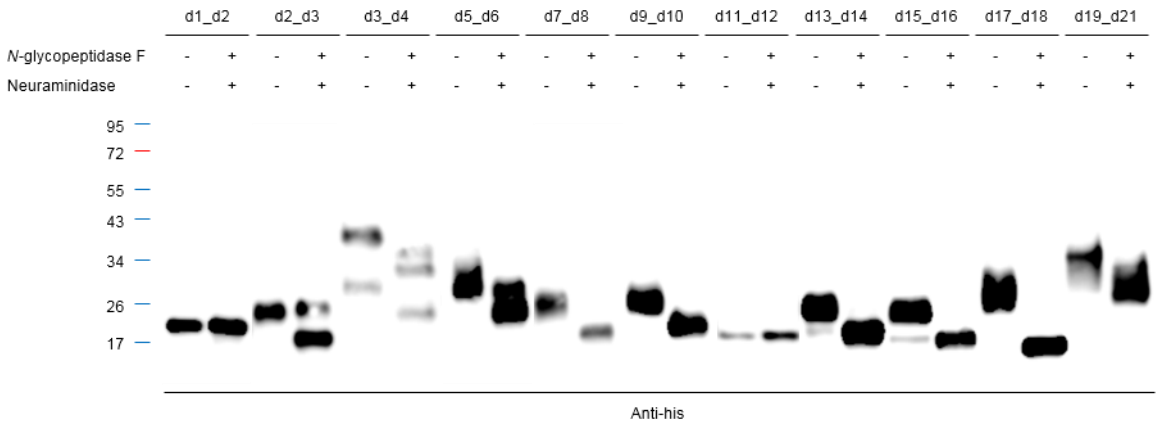


Supplemental Figure 4. Baseline negative controls. (**A, B**) Images depict results of Western blot analyses of (**A**) the three larger THSD7A fragments and (**B**) the small TSP-1 domain constructs with serum from six healthy individuals (1:100) under non-reducing and reducing conditions.



Supplemental Figure 5. (**A**) Western blot analysis under non-reducing conditions of cell culture supernatants of HEK293 cells expressing the soluble TSP-1 domain constructs. With the exception of d19_d21, all constructs were well recognized by the anti-his antibody despite good recognition of d11_d21 under reducing conditions (**Figure 3A** in the main body), suggesting that the 6x his-epitope in the d19_d21 construct lacks antibody accessibility under non-reducing conditions. Some of the constructs cross-link and build multimers, which were well recognized by the anti-his antibody. Asterisks mark the recognized TSP-1 domain constructs. (**B**) Coomassie-blue staining of cell culture supernatants of HEK293 cells expressing the soluble TSP-1 domain constructs. All eleven TSP-1 domain constructs can be detected using this method.

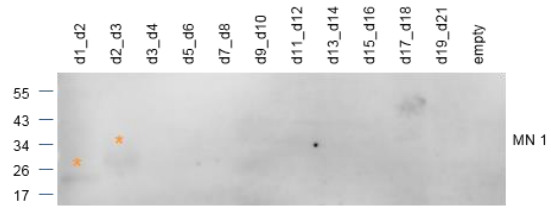
A

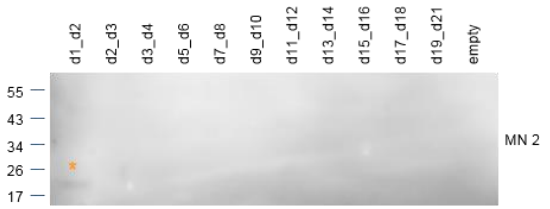


B

TSP-1 domain construct	Expected molecular mass	Molecular mass after deglycosylation
d1_d2	18	20
d2_d3	16	16
d3_d4	27	32
d5_d6	18	26
d7_d8	14	18
d9_d10	16	20
d11_d12	15	18
d13_d14	16	18
d15_d16	15	17
d17_d18	15	15
d19_d21	23	29

Supplemental figure 6. (**A**) Glycosylation of individual TSP-1 domain constructs. The TSP-1 domain constructs were enzymatically deglycosylated using *N*-glycopeptidase F and neuraminidase. Shifts in protein mass were evaluated by immunodetection with an anti-his antibody in Western blot analysis under reducing conditions. (**B**) Predicted molecular mass according to www.sciencegateway.org/tools/proteinmw.htm and molecular mass as estimated from migration in gel electrophoresis after enzymatic deglycosylation. The fragments corresponded well to their expected molecular masses or run slightly above.


d1_d2
d2_d3
d3_d4
d5_d6
d7_d8
d9_d10
d11_d12
d13_d14
d15_d16
d17_d18
d19_d21
empty
55
43
34
26
17
MN 1


d1_d2
d2_d3
d3_d4
d5_d6
d7_d8
d9_d10
d11_d12
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d15_d16
d17_d18
d19_d21
empty
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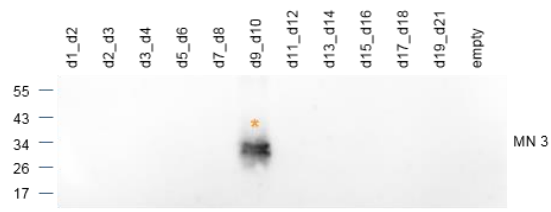
43
34
26
17
MN 2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d17_d18
d19_d21
empty
55
43
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26
17
MN 3


d1_d2
d2_d3
d3_d4
d5_d6
d7_d8
d9_d10
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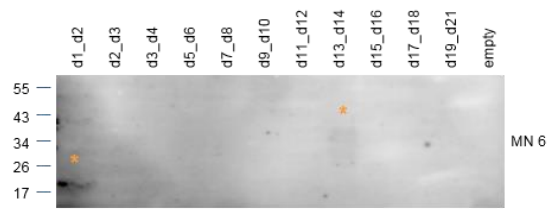
MN 4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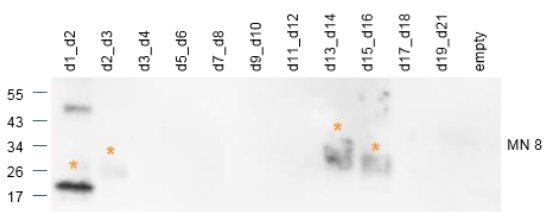
17
MN 6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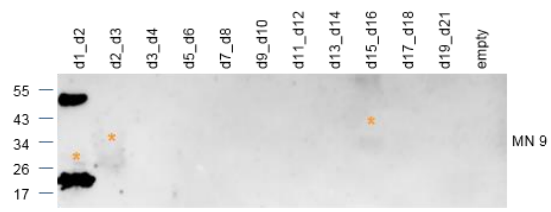
17
MN 8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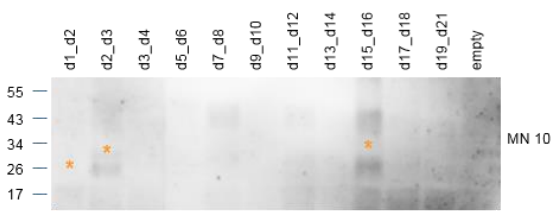
MN 9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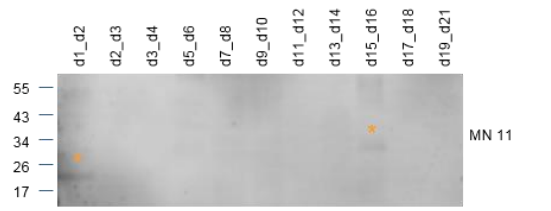
MN 10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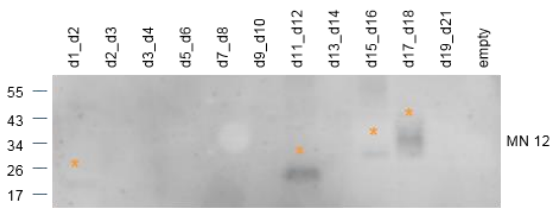
MN 11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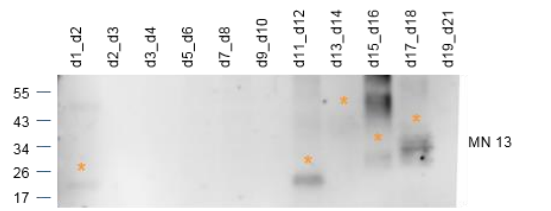
MN 12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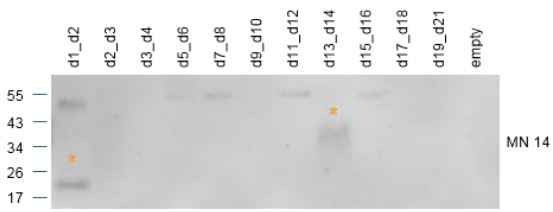
MN 13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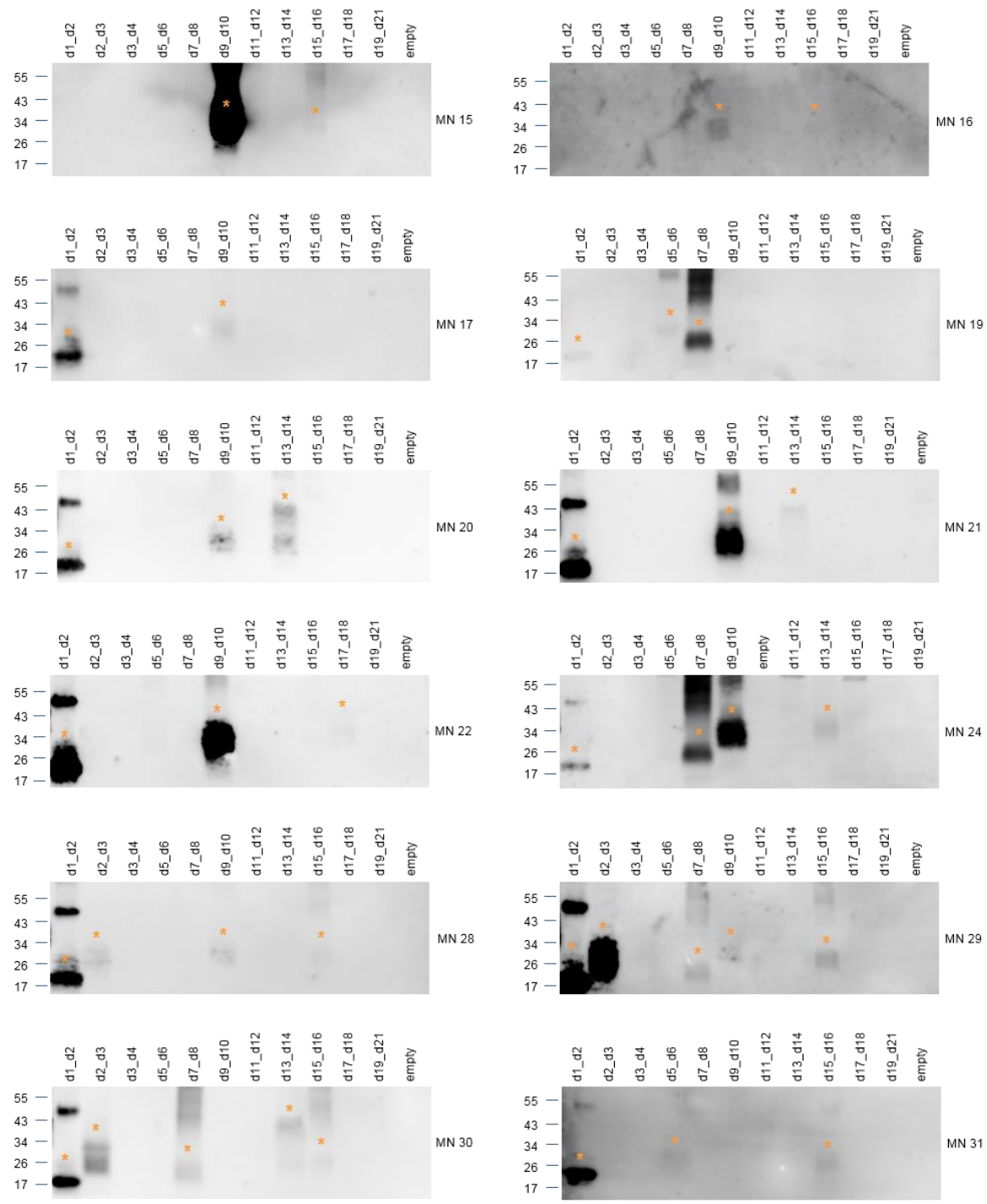
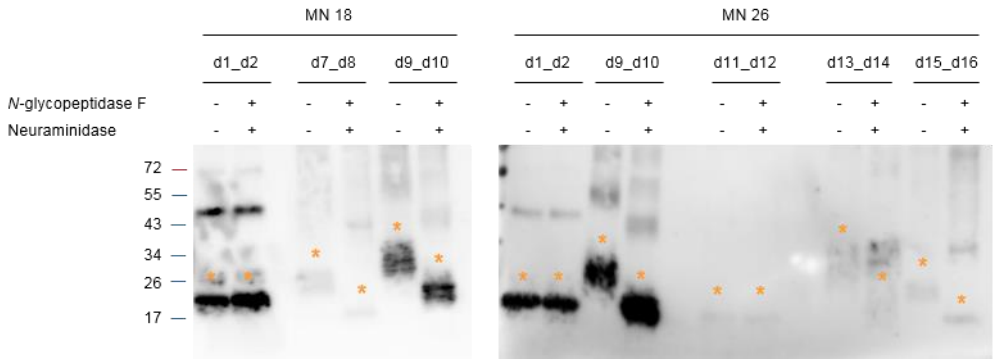
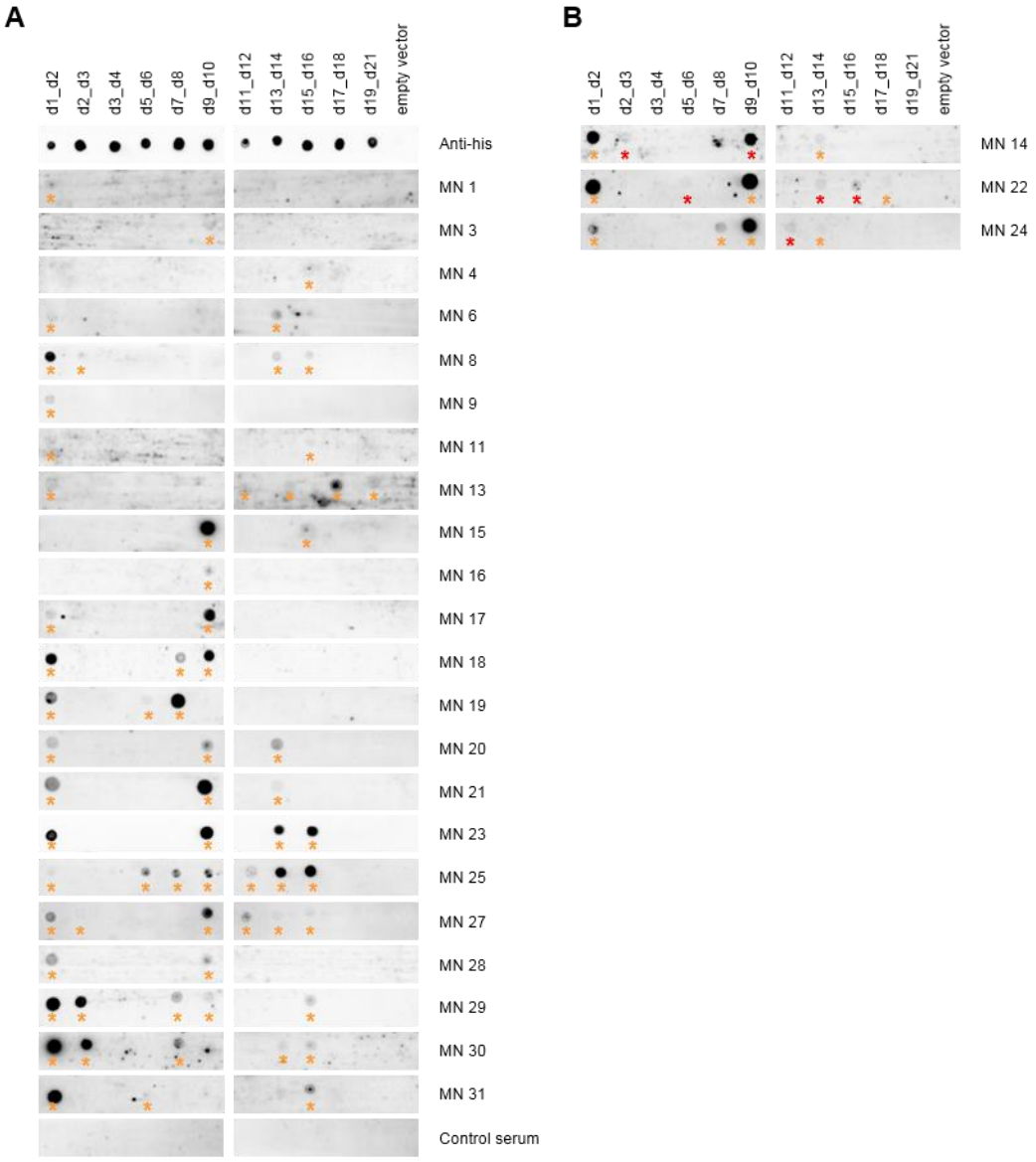
MN 14



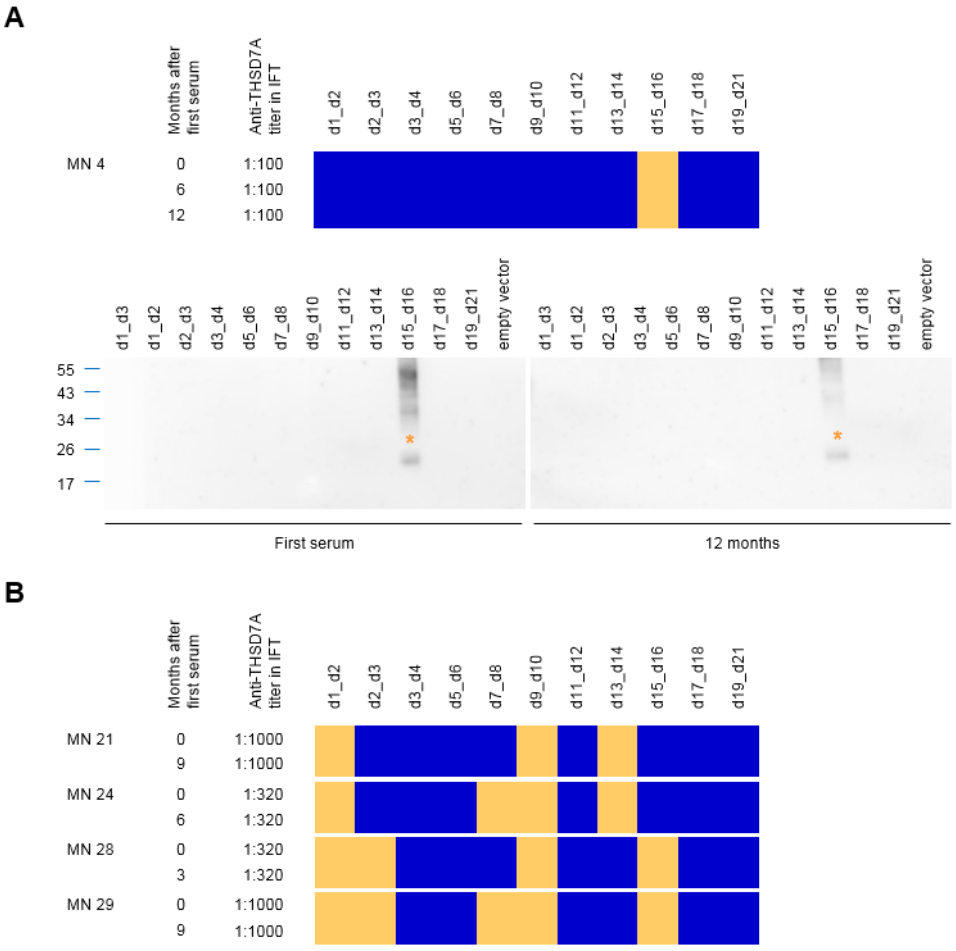
Supplemental Figure 7. Western blot analysis of the TSP-1 domain constructs with sera from the remaining 24 patients that are not depicted in the results section (**Figures 3B**, **4** and **5**) with THSD7A-associated MN under non-reducing conditions (serum dilution 1:100). Asterisks mark the recognized TSP-1 domain constructs.



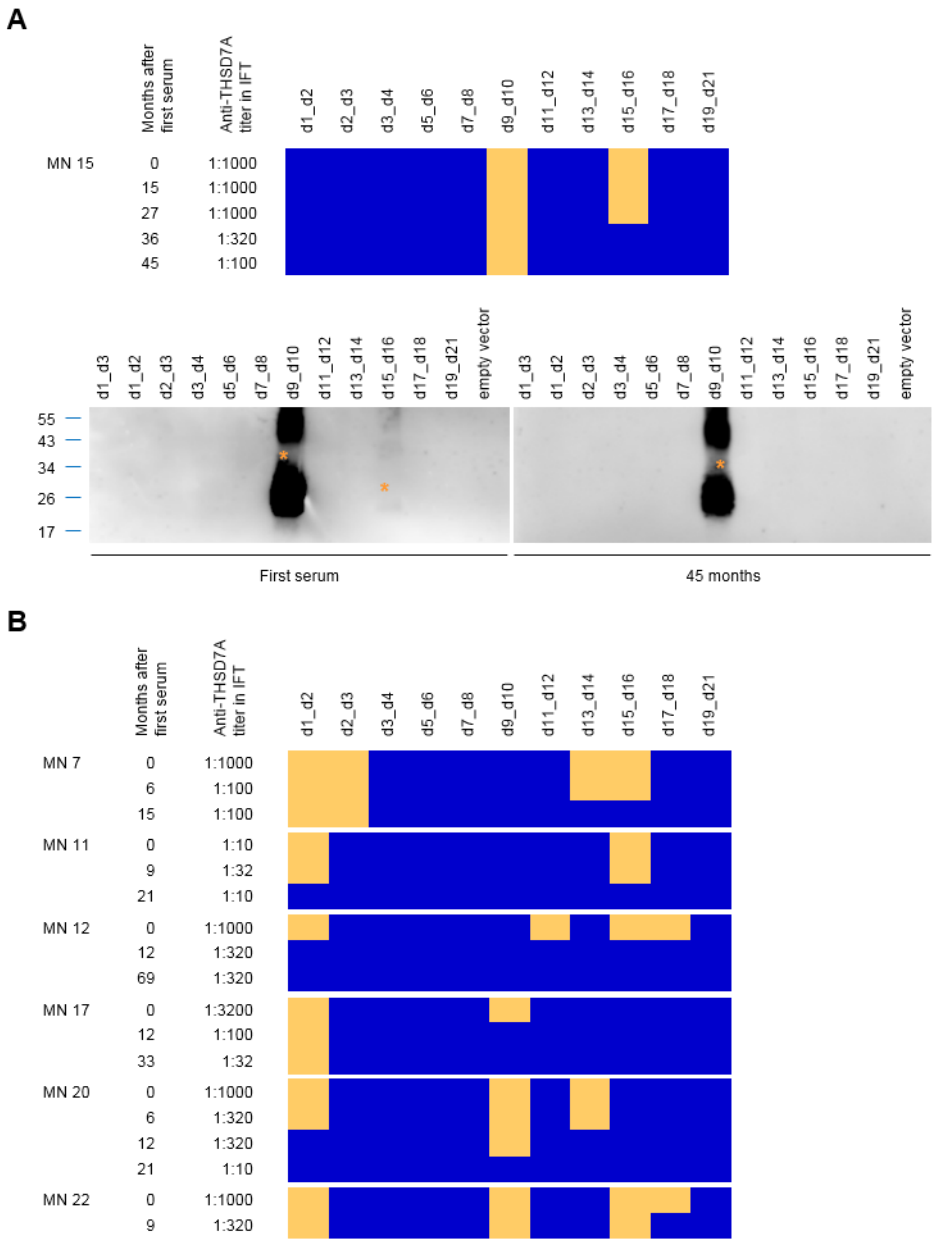
Supplemental Figure 8. Serum recognition of deglycosylated TSP-1 domain constructs. The TSP-1 domain constructs were enzymatically deglycosylated using *N*-glycopeptidase F and neuraminidase. Sera recognized both the glycosylated and deglycosylated forms in Western blot analysis under non-reducing conditions. Asterisks mark the recognized TSP-1 domain constructs.



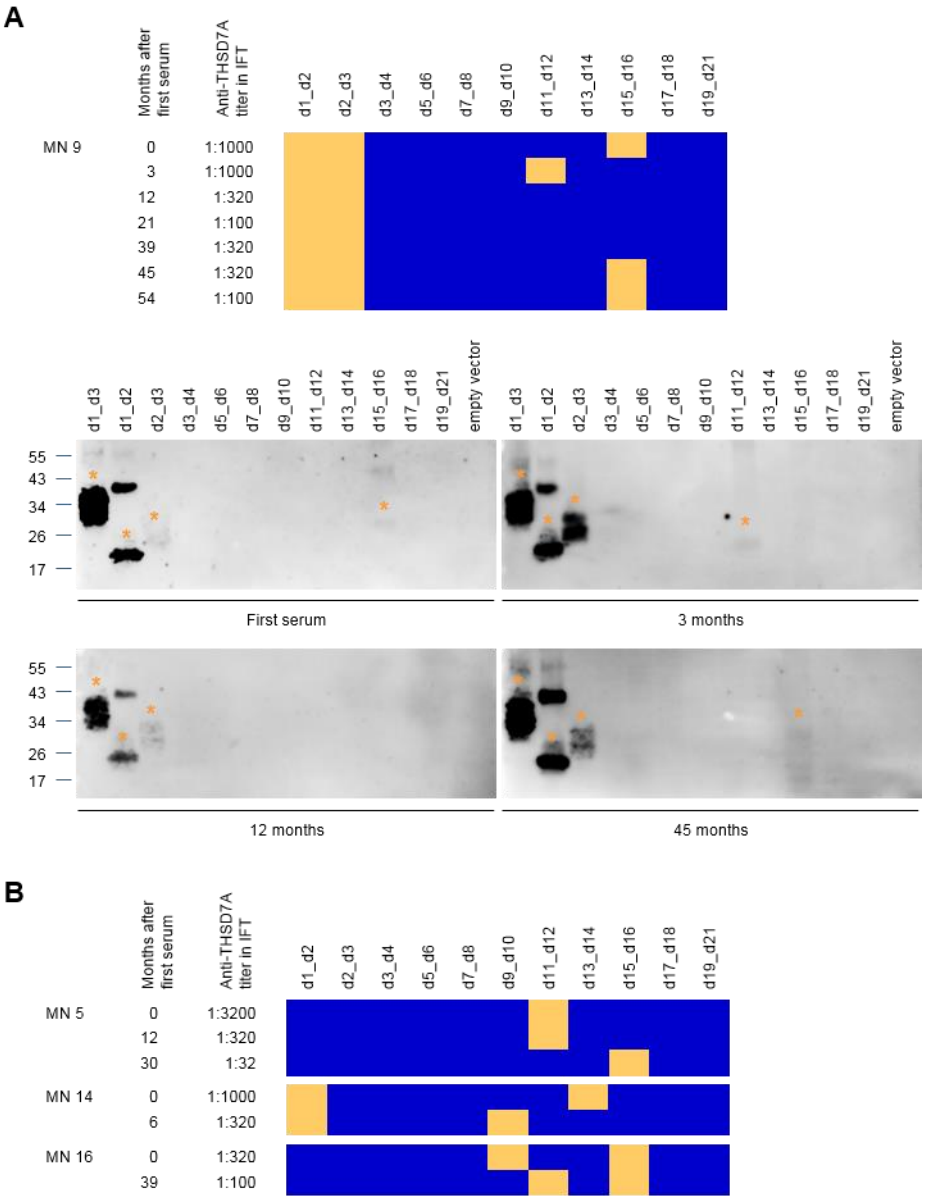
Supplemental Figure 9. Native blotting (dot blot analysis) of purified TSP-1 domain constructs with sera from patients with THSD7A-associated MN. (**A**, **B**) Dot blot analysis of TSP-1 domain constructs with sera that (**A**) recognized the same or less TSP-1 domain constructs or (**B**) showed additional reactivity with TSP-1 domain constructs when compared with Western blotting. MN 9, MN 16 and MN 28 failed to recognize a TSP-1 domain construct that was previously recognized in Western blotting and MN 2 and MN 12 failed to recognize any TSP-1 domain construct in native blotting (data not shown). Yellow asterisks mark the recognized TSP-1 domain constructs that were also appreciated in Western blotting while red asterisks mark additional TSP-1 domain recognition.



Supplemental Figure 10. Anti-THSD7A antibody levels and epitope profiles during follow-up in patients with steady TSP-1 domain recognition. (**A**) In patient MN 4, epitope profiling showed serum recognition of the d15_d16 construct, which remained unchanged during the observation period. IFT titer also remained stable and proteinuria was constantly in the nephrotic range. The patient did not receive immunosuppressive therapy. Lower panels depict the Western blot analyses of the TSP-1 domain constructs with the first and last available serum from this patient. Asterisks mark the recognized TSP-1 domain constructs. (**B**) Anti-THSD7A antibody levels and epitope profiles of four more patients with stable TSP-1 domain recognition and persistent active disease. MN 21 and MN 28 received immunosuppressive therapy during follow-up.



Supplemental Figure 11. Anti-THSD7A antibody levels and epitope profiles during follow-up in patients with a loss of TSP-1 domain recognition. (**A**) In patient MN 15, epitope profiling showed serum recognition of the d9_10 and d15_d16 constructs. During follow-up, d15_d16 reactivity was lost, the anti-THSD7A antibody level decreased, and the patient had a partial remission of proteinuria. The patient did not receive immunosuppressive therapy. Lower panels depict the Western blot analyses of the TSP-1 domain constructs with the first and last available serum from this patient. Asterisks mark the recognized TSP-1 domain constructs. (**B**) Anti-THSD7A antibody levels and epitope profiles of six more patients with a loss of TSP-1 domain recognition. MN 7, MN 12, MN 16, and MN 22 received immunosuppressive therapy during follow-up.



Supplemental Figure 12. Anti-THSD7A antibody levels and epitope profiles during follow-up in patients with a change in TSP_1 domain recognition. (**A**) In patient MN 9, epitope analysis showed serum reactivity with the d1_d2, d2_d3, and d15_d16 constructs. While reactivity with d15_d16 was lost three months later, the serum additionally recognized d11_d12. The patient was treated with prednisone and cyclosporine A, had a partial remission of proteinuria associated with a decrease of anti-THSD7A antibody levels and a loss of reactivity with d11_d12. Proteinuria relapsed after 45 months, which was accompanied by renewed serum reactivity with d15_d16 in the presence of a persistent anti-THSD7A antibody titer of 1:320 in IFT. Lower panels depict the Western blot analyses of the TSP_1 domain constructs with several follow-up sera from this patient. (**B**) Anti-THSD7A antibody levels and epitope profiles of three more patients with a change in TSP_1 domain recognition. One patient (MN 5) achieved a partial remission during follow-up. No data on proteinuria during follow-up where available for the other two patients.

A

Alignments for THBS1-like domains

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3r6b     -------INGGWGPWSPWDICSV------------------TCGG-GVQKRSRLCNNPTPQF----------------GGKDCVG---------DVTENQICNK-QDCP
d1       QGDTEVPTLYLWK-TGPWGRCMG-----------------DDCGPGGIQTRAVWCAHVEGWTT----------LHTNCKQAV-------RPSNQQNCF--KVCD
d2       ----WHKELYDWR-LGTWDRCQPVISKSL-----EKSRECVKGEE-GIQVREIMCIQKDKDIPA--------------EDIICEYFEP------KPLLEQACL--IPCQ
d5       --------TYGWR-TTEWTECHVDPLLSQQD--KRRANQTALCGG-GVQTREIYCIQTNDNMLSHGNTQKDKEASKPVDSKLCTGP--------VPNTTQLCH--VPCP
d7       ----------DWK-SVRLGDCEPD-------------NGKSCGP-GTQVQEVVCINSDGEEV----------------DRQLCRDA--------IFPIPVACD--APCP
d9       --------VYHWQ-TGPWGQCIEDTSVSSFN-TTTTWNGEASCSV-GMQTRKVICVRVNVGQV----------------GPKKCPESL-------RPETVRPCL--LPCR
d11      --------SYRWK-THKWRRCQLVPWSIQQD----VPGAQEGCGP-GRQARAITCRKQDGGQA----------------SIQECLQYA-----GPVPALTQACQ--IPCQ
d13      --------KYNAQPVGNWSDCILPEGKAEVLLGMKVQGDSKECGQ-GYRYQAMACYDQNGRLV----------------ETSRCNS---------HGYIEEACI--IPCP
d15      --------QYIWV-TEPWSVCKVTFV-----------DMRDNCGE-GVQTRKVRCMQNTADGPSEHV-----------EDYLCDPE-------DMPLGSRECK--LPCP
d17      --------HYDYN-VTDWSTCQLS--------------EKAVCGN-GIKTRMLDCVRSDGKSV----------------DLKYCEELG----LEKNWPMNTSCT--VECP
d19      ----------RWQ-YGQWSPCQVQ----------------EAQCGE-GTRTRNISCVVSDGSAEDFSKVV---------DEEFCANTELIIDGNKQIVLEETCT--QPCP
d21      ---EYKWVASAWKGSSRTVWCQRSDGINV----------TGGCLV-VQPDTDRSCNP------------------------PCSQ------------PHSYCSEMKTCR
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Alignments for C6-like domains

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3t5o1    --CFCDHYAWTQWTSCSK-TCN-------SGTQSRHRQIVVDKYYQE---NFCEQICSKQ-----ETRECNWQ-----RCP----
3t5o2    --INCLLGDFGPWSDCD--PCI--------EKQSKVRSVLRPSQFG---GQPCTAP-------LVAFQPCIPSK----LCKIE--
1szl     GSETCIYSNWSPWSACSSSTCE-------KGKRMRQRMLKAQLDL----SVPCPDT--------QDFQPCMGP-----GCSDEDG
d3       --KDCIVSEFSPWSECSR-TCG-------SGLQHRTRHVVAPPQYG---GSGCPNL--------TEFQVCQSN-----PCE----
d4       --KECQVSEWLEWSPCSK-TCHDVT--SPTGTRVRTRTITQFPIGS---EKECPAL--------EEKEPCVSQGDGAVLCA----
d6       --IECEVSPWSAWGPCTYENCNDQQ--GKKGFKLRKRRITNEPTGGSGATGNCPHL--------LEAIPCEEP-----SCY----
d8       --KDCVLSAWSSWSSCSH-TCSGKT---TEGKQTRARSILAYAGEEG--GIRCPNI-----SALQEVRSCNEH-----PCT----
d10      --KDCVVTPYSDWTPCPS-SCREGD--SGARKQSRQRVIIQLPANG---GKECSDP-------LYEEKACEAPP----TCH----
d12      --DDCQFTSWSKFSSCNG-DCG--------AVRTRKRAIVGKSKK----KEKCKNS---HLYPLIETQYC---------PCD----
d14      --SDCKLSEWSNWSRCSK-SCG-------SGVKVRSKWLREKPYNG---GRPCPKLDHVNQAQVYEVVPCHS------DCN
d16      --EDCVISEWGPWTQCAL-PCNP------SGSRQRSADPIRQPADE---GRACPDA--------VEKEPCSLNK----NCY----
d18      --VNCQLSDWSSWSQCSQ-TCGL------TGKMIRKRTVTQPFQGD---GRPCPSL-------MEQSKPCPVK-----PCY----
d20      --GDCYLNDWSSWSLCQL-TCVNGEDLGFGGIQVRSRAVIIQELEN---QHLCPEQ-------MLETKSCDDG-----QCY----
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B

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leader       MGLRAGRLASPSRGVLQLLRLPLLLLLLLSSGARGA
d1           AAQGDTEVPTLYLWKTGPWGRCMGDDCGPGGIQTRAVWCAHVEGWTTLHTNCKQAVRPSNQQNCFKVCD
d2           WHKELYDWRLGTWDRCQPVISKSLEKSRECVKGEEGIQVREIMCIQKDKDIPAEDIICEYFEPKPLLEQACLIPCQ
d3           KDCIVSEFSPWSECSRTCGSGLQHRTRHVVAPPQYGGGSGCPNLTEFQVCQSNPCE
cc           EDESLYSLQVGPWSACSVPHTRQARQARRRGKNKEREKERGKAVKDPEARELIKKKRNRNRQNRQENRYWDIQIGYQTRDVTCLNRTGKSADLSFCQQERLPMTFQSCVIT
d4           KECQVSEWLEWSPCSKTCHDVTSPTGTRVRTRTITQFPIGSEKECPALEEKEPCVSQGDGAVLCA
d5           TYGWRTTEWTECHVDPLLSQQDKRRANQTALCGGGVQTREIYCIQTNDNMLSHGNTQKDKEASKPVDSKLCTGPVPNTTQLCHVPCP
d6           IECEVSPWSAWGPCTYENCNDQQGKKGFKLRKRRITNEPTGGSGATGNCPHLLEAIPCEEPSCY
d7           DWKSVRLGDCEPDNGKSCGPGTQVQEVVCINSDGEEVDRQLCRDAIFPIPVACDAPCP
d8           KDCVLSAWSSWSSCSHTCSGKTTEGKQTRARSILAYAGEEGGIRCPNISALQEVRSCNEHPCT
d9           VYHWQTGPWGQCIEDTSVSSFNTTTTWNGEASCSVGMQTRKVICVRVNVGQVGPKKCPESLRPETVRPCLLPCR
d10          KDCVVTPYSDWTPCPSSCREGDSGARKQSRQRVIIQLPANGGKECSDPLYEEKACEAPPTCH
d11          SYRWKTHKWRRCQLVPWSIQQDVPGAQEGCGPGRQARAITCRKQDGGQASIQECLQYAGPVPALTQACQIPCQ
d12          DDCQFTSWSKFSSCNGDCGAVRTRKRAIVGKSKKKEKCKNSHLYPLIETQYCPCD
d13          KYNAQPVGNWSDCILPEGKAEVLLGMKVQGDSKECGQGYRYQAMACYDQNGRLVETSRCNSHGYIEEACIIPCP
d14          SDCKLSEWSNWSRCSKSCGSGVKVRSKWLREKPYNGGRPCPKLDHVNQAQVYEVVPCHSDCN
d15          QYIWVTEPWSVCKVTFVDMRDNCGEGVQTRKVRCMQNTADGPSEHVEDYLCDPEDMPLGSRECKLPCP
d16          EDCVISEWGPWTCALPCNPSGSRQRSADPIRQPADEGRACPDAVEKEPCSLNKNCY
d17          HYDYNVTDWSTCQLSEKAVCGNGIKTRMLDCVRSDGKSVDLKYCEELGLEKNWPMNTSCTVECP
d18          VNCQLSDWSSWSQCSQTCGLTGKMIRKRTVTQPFQGDGRPCPSLMEQSKPCPVKPCY
d19          RWQYGQWSPCQVQEAQCGEGTRTRNISCVVSDGSAEDFSKVVDEEFCANTELIIDGNKQIVLEETCTQPCP
d20          GDCYLNDWSSWSLCQLTCVNGEDLGFGGIQVRSRAVIIQELENQHLCPEQMLETKSCDDGQCY
d21          EYKWVASAWKGSSRTVWCQRSDGINVTGGCLVVSQPDTDRSCNPPCSQPHSYCSEMKTCR
stalk/tm     CEEGYTEVMSSNSTLEQCTLIPVVVIPTVEDKRGDVKTSRAVHPTQPSINPAGRGRTWFLQPFGPDGRLKTW
cytosol      VYGVAAGAFVLLVFIVSMIYLACKKPKKPQRRQNNRLKPLTLAYDGDADM
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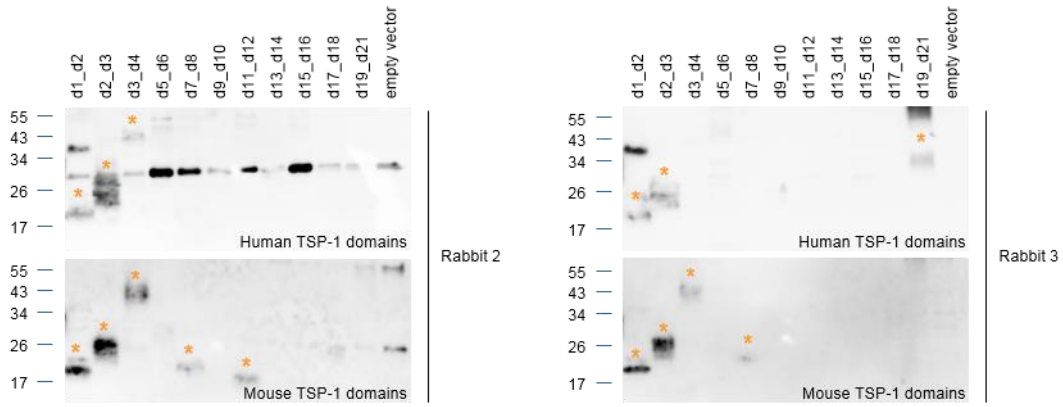
Supplemental Figure 13. Identification of TSP-1 domains and structure of mouse THSD7A. (**A**) Reference sequences for TSP-1 domains as in THBS1 (protein data bank [pdb] code 3r6b[1], upper panel) and for TSP-1 domains as in C6 (pdb code 3t5o[2]) or F-spondin (pdb code 1szl[3]) and alignment with the 21 TSP-1 domains of mouse THSD7A. Yellow, cysteine; magenta, tryptophane; cyan, arginine. (**B**) Amino acid sequence of full-length mouse THSD7A with colored tagging of all parts. Purple, leader peptide; red, THBS1-like TSP-1 domain; green, C6-like TSP-1 domain; blue, coiled coil (cc) domain; brown, extracellular stalk region, transmembrane (tm) region and cytosolic tail.



Supplemental Figure 14. (**A**) Western blot analysis of reactivity of two different rabbit antisera with the human and mouse TSP-1 domain constructs under non-reducing conditions. Asterisks mark the recognized TSP-1 domain constructs.

	Number of patients with epitope recognition	
	At study start	**At the end of follow-up**
d1_d2	6	3
d2_d3	0	0
d3_d4	0	0
d5_d6	0	0
d7_d8	4	2
d9_d10	1	0
d11_d12	2	0
d13_d14	5	1
d15_d16	2	0
d17_d18	0	0
d19_d21	6	3

Supplemental Table 1. Epitope pattern during follow-up in seven patients, in whom recognition of one or more epitopes was lost.

	Amino acid sequence homology (%)		
	Rabbit versus human	**Rabbit versus mouse**	**Mouse versus human**
Full-length THSD7A	92	90	91
d1_d2	92	88	91
d2_d3	91	85	90
d3_d4	87	84	85
d5_d6	91	88	86
d7_d8	95	93	94
d9_d10	90	89	90
d11_d12	98	96	95
d13_d14	99	97	98
d15_d16	94	91	89
d17_d18	94	92	94
d19_d21	92	90	92

Supplemental Table 2. Amino acid sequence homology in percent between rabbit, human and mouse TSP-1 domain orthologs.

SUPPLEMENTAL METHODS

Cell culture, cell transfection and recombinant protein expression

Two lines of human embryonic kidney cells (HEK) were kept in culture for recombinant expression of THSD7A fragments. HEK293-6E is a cell line that can be cultivated in the absence of serum and is especially suitable for the expression of secreted proteins.[4,5] HEK293T is a standard cell line for recombinant protein expression.[6] HEK293-6E cells were cultured in cell culture bottles with 10 ml of serum-free medium (Freestyle 293, Gibco by Life Technologies, Grand Island, USA). HEK293T cells were cultured in 10 cm dishes with 10 ml Dulbecco's Modified Eagle Medium (DMEM, Life Technologies, Grand Island, USA) with addition of 10% fetal bovine serum (Thermo Scientific, Cramlington, United Kingdom) and 1% Penicillin/Streptomycin (Life Technologies, Grand Island, USA).

All protein fragments were transfected to both cell types and expression efficiency was evaluated. The d11_d21 construct was the only one not to be secreted into the culture medium and expression was higher in HEK293T cells. Thus, we chose to express d11_d21 in HEK293T cells for further experiments. All other constructs were well secreted into the culture medium when expressed in HEK293-6E cells.

For transfection of HEK293-6E cells, a polythylenimine (PEI)-based method (Polyscience Inc., Warrington, USA) was used. For each approach, 126 µL (40 µg) of PEI was mixed with 124 µL water and 250 µL NaCl (300 mM) was added. 10 µg Plasmid DNA in 250 µL water was mixed with the same amount of NaCl in a separate tube. Subsequently, solutions were slowly mixed, vortexed and incubated for 30 min. The solution was then added to the cells. After 24 h the cells were fed with 250 µL feeding medium (Freestyle 293 medium supplemented with 20% tryptone). Five days later, cells were collected using centrifugation at 300 *g* and the supernatant was centrifuged again at 14,000 *g* for 30 min.

For transfection of HEK293T cells, a calcium phosphate-based method was used.[6] Ten µg of plasmid DNA was mixed with 36 µL of 2 M $CaCl_2$ and diluted with sterile water up to a volume of 300 µL. This solution was gently mixed with an equal volume of 2x HEPES buffered saline (HBS, 275 mM NaCl, 55 mM HEPES, pH 7.0) and incubated 30 min at room temperature. The resulting solution was added drop wise to the cells. Medium was changed 24 h after transfection. After 48 h, cells were scraped, centrifuged at 1,500 rpm for 5 minutes, washed with PBS, and centrifuged again at 1,500 rpm for 5 minutes. Cells were then lysed in 50 mM Tris pH 7.4, 1 mM EDTA, 150 mM NaCl, 1% Triton after addition of a protease inhibitor cocktail (Roche Diagnostics, Indianapolis, USA), sonicated, and centrifuged at 14,000 *g* and 4 °C for 1 h. The supernatant was saved and protein concentration was determined using the Pierce BCA protein assay kit according to the manufacturer's instructions (Thermo Fisher, Waltham, USA). All expressions (medium and cell lysate) were validated by Western blot and

immunological detection using an anti-his antibody (1:1,000, Thermo Scientific, Cramlington, United Kingdom). For analyses with patient sera, the culture media of d1_d4- and d5_d10-transfected cells and the cell lysate of d11_d21-transfected cells were used.

Protein fractionation and deglycosylation

To determine the subcellular location of the d11_d21 construct, cells were first lyzed in 50 mM Tris pH 7.4, 0.32 M sucrose and 10 mM EDTA with a protease inhibitor cocktail (as above), sonicated, rotated for 30 min at 4 °C, and centrifuged at 100,000 *g* for 1 h at 4 °C. The supernatant was taken and labeled as the soluble (cytoplasmatic) fraction. The pellet was then resuspended in 50 mM Tris pH 7.4, 150 mM NaCl, 0.1% SDS, 1% NP40 and 0.5% Na-DOC with a protease inhibitor cocktail (as above), sonicated, rotated for 30 min at 4 °C, and centrifuged at 100,000 *g* for 1 h at 4 °C. The supernatant was labeled as the detergent-soluble (membrane) fraction. The cell pellet was then resuspended in 50 mM Tris pH 7.4, 10 mM DTT and 8 M urea, sonicated, and labeled as the insoluble fraction. As a control experiment, HEK293 cells transfected with full-length THSD7A were treated identically.

Deglycosylation experiments were performed using a combination of *N*-glycopeptidase F and neuraminidase (both Roche Diagnsotics, Mannheim, Germany). Protein samples were incubated overnight at 37 °C with deglycosylating enzymes or an equal volume of PBS (1 µl per 100 µg of total protein).

Western blot and immunological detection

Protein samples were prepared for Western blot analysis by addition of 5x Laemmli buffer (1.5 M Tris-HCl pH 6.8, 50% glycerol, 10% SDS, 1% bromophenol blue) and subsequent heating to 95 °C for 10 minutes. If reducing conditions were desired, 20% β-mercaptoethanol was added to the 5x loading buffer. Proteins were separated by electrophoresis in 4-15% gradient gels (Bio-Rad, Hercules, USA) in an electrophoresis chamber (Bio-Rad, Hercules, USA) in the presence of a migration buffer (25 mM Tris, 192 mM glycine, 0.1% SDS; Amresco, Solon, USA). Subsequently, proteins were transferred to methanol-soaked PVDF membranes (Millipore, Billerica, USA) under semi-dry conditions in the presence of 25 mM Tris pH 8.5, 192 mM glycine, ethanol 20% using the Transblot Turbo system (Bio-Rad, Hercules, USA) at 25 V constant for 35 min. Membranes were blocked in 5% dry milk with PBS plus Tween 0.05% (PBS-T) for 2 h at room temperature followed by the incubation with the primary antibody (anti-his antibody, 1:1,000, Thermo Scientific; anti-THSD7A antibody, 1:1,000, Atlas, #000923), human serum, rabbit serum or mouse serum (all 1:100) in 0,5% dry milk in PBS-T. The next day, membranes were washed three times with PBS-T the next day and incubated with the secondary antibody in 5% dry milk in PBS-T for 2 h. HRP-conjugated mouse anti-human IgG4 and HRP-conjugated mouse anti-human IgG (both 1:20,000, SouthernBiotech, Birmingham,

USA), were used to analyze serum reactivity with the various THSD7A constructs. HRP-conjugated goat anti-mouse IgG was used as secondary antibody for his detection and HRP-conjugated goat anti-rabbit for THSD7A detection (both 1:20,000, SouthernBiotech, Birmingham, USA). To visualize the protein bands, membranes were incubated in a chemiluminescent substrate (SuperSignal West Pico and SuperSignal West Femto, Thermo Scientific, Rockford, USA, used in a 4:1 ratio) followed by incremental luminescence detection for up to 12 minutes with a Fujifilm LAS imager 3000. For testing human serum, protein loading was standardized based on anti-his reactivity under reducing conditions and all sera were used in a 1:100 dilution at all times.

Native blotting (dot blot analysis) and immunological detection

His-tagged TSP-1 domain constructs (d1_d2 to d19_d21) were purified under native conditions using a Ni-NTA resin (His-Pur, Thermo Scientific, Rockford USA, #88221) applying the batch method according to the manufacturer's instructions. Equilibration, wash, and elution buffers were PBS-based (pH 7.4) and supplemented with 10 mM imidazol, 25 mM imidazole, and 250 mM imidazole, respectively. Protein purification was validated by Coomassie-blue staining of electrophoresed proteins. Purified proteins were dotted on nitrocellulose membranes (Protran, Whatman, Dassel, Germany) and allowed to dry for 10 minutes at room temperature. Membranes were then blocked in 4% dry milk in PBS plus Tween 0.08% (PBS-T) for 3 hours, followed by incubation with human serum (1:100) or anti-his antibody (1:1000) in PBS-T with 0.5% dry milk. A mixture of HRP-conjugated anti-human IgG and anti-human IgG4 or anti-mouse IgG (all 1:20,000, SouthernBiotech, Birmingham, USA) were used as secondary antibodies and visualization of the protein signal was performed as described above.

Immunohistochemical analyses

1 µM paraffin sections of mouse kidneys were deparaffinized and rehydrated. Antigen retrieval was obtained for rabbit IgG by digestion with protease XXIV (5 µg/mL, Sigma) for 15 min at 37°C. Nonspecific binding was blocked with 5% horse serum (Vector, Burlingame, USA) with 0.05% Triton X-100 (Sigma) in PBS for 30 min at room temperature prior to incubation at 4 °C overnight with biotinylated anti-rabbit IgG (1:400) in blocking buffer. Staining was visualized with the ZytochemPlus AP Polymer kit (Zytomed Systems, Berlin, Germany) according to the manufacturer's instruction with neufuchsin (Merck) as a color substrate. Nuclei were counterstained with hemalaun (Merck) and sections were mounted with gum Arabic (Sigma, St. Louis, USA). Negative controls were performed by omitting primary antibodies. Stainings were evaluated with an Axioskop using the Axiovision software (all Zeiss).

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