

Supplementary Information

***Hydra* Mesoglea Proteome Identifies Thrombospondin as a Conserved Component active in Head Organizer Restriction**

Mark Lommel, Jennifer Strompen, Andrew L. Hellewell, Gnana Prakash Balasubramanian, Elena D. Christofidou, Andrew R. Thomson, Aimee L. Boyle, Derek N. Woolfson, Kane Puglisi, Markus Hartl, Thomas W. Holstein, Josephine C. Adams and Suat Özbek

Supplementary Table S1. Mesoglea proteome of *Hydra magnipapillata*.

| No | Hma identity/ GB Accession No./ Transcript ID | Protein Score | Protein | Domains (from CDD and InterProScan) (/ indicates multiple domain designations for the same region) | Reference |
|----|---|------------------|--------------------------------|---|------------------------------------|
| 1 | Hma2.214226 ABG80452 comp25324_c0_seq1 | 6993 | HCol-6 | Collagen IV non-collagenous domain, Collagen triple-helix, C-type lectin, WAP-type 'four-disulfide core', vWFA | J. Biol. Chem. 282:6792-6802(2007) |
| 2 | Hma2.217448 XP_012558958.1 comp27401_c0_seq1 | 5266 | Laminin, alpha | Laminin N-terminal domain, Laminin EGF, Laminin IV, Concanavalin A-like lectin/Laminin G | |
| 3 | Hma2.229572 XP_002168125.2 comp25312_c0_seq1 | 2971 | Laminin, beta | Laminin N-terminal domain, Laminin EGF, Laminin IV type B | Dev Biol. 1994 Jul;164(1):312-24. |
| 4 | Hma2.222865 AAM77398 comp28519_c0_seq1 | 2332 | HCol-I | Collagen triple-helix, Fibrillar collagen C-terminal domain | J. Biol. Chem. 282:6792-6802(2007) |
| 5 | Hma2.216083 ABG80451 comp18600_c0_seq1 | 2132 | HCol-5 | Collagen triple-helix, Fibrillar collagen C-terminal domain | J. Biol. Chem. 282:6792-6802(2007) |
| 6 | Hma2.232437 ABG80450 comp24511_c0_seq2 | 1683 | HCol-3 | Collagen triple-helix, Fibrillar collagen C-terminal domain, WAP-type 'four-disulfide core', vWFA | J. Biol. Chem. 282:6792-6802(2007) |
| 7 | Hma2.218921 NP_001296598 comp21769_c0_seq1 | 1438 | Collagen type I, alpha chain-1 | Collagen triple-helix, Fibrillar collagen C-terminal domain, WAP-type 'four-disulfide core' | |
| 8 | Hma2.221191 XP_0012557210 comp26030_c0_seq1 | 1081 | Laminin, gamma | Laminin N-terminal domain, Laminin EGF, Laminin IV | |

| | | | | | |
|----|--|-----|--|---|--|
| 9 | Hma2.202536 XP_012565750 comp20380_c0_seq1 | 882 | Collagen type IV | Collagen IV non-collagenous domain, C-type lectin | J Biol Chem. 2000 Dec 15;275(50):39589-99 |
| 10 | Hma2.213656 XP_012560707.1 comp29438_c0_seq1 | 467 | Collagen type VI-like | vWFA | |
| 11 | Hma2.218942 XP_012558303.1 comp24178_c0_seq1 | 422 | HmTSP | Concanavalin A-like lectin domain/Laminin G domain, EGF-like, TSP type-3 repeat, TSP C-terminal domain | |
| 12 | Hma2.208898 XP_002154462 | 171 | Actin, non muscle | Actin | Gene. 1989 Dec 7;84(1):55-64 |
| 13 | Hma2.206846 XP_002165741 comp7940_c0_seq1 | 125 | Selenoprotein P-like | Selenoprotein P N-terminal domain, Thioredoxin-like fold | |
| 14 | Hma2.200137 XP_012560867.1 comp16204_c0_seq1 | 117 | Novel | WSC superfamily | |
| 15 | Hma2.205448 XP_012564433.1 comp1273_c0_seq1 | 93 | SecA-like | Protein translocase subunit SecA, SecA motor DEAD/P-loop containing nucleoside triphosphate hydrolase, Helicase C-terminal domain, Tetratricopeptide repeat | |
| 16 | Hma2.226267 NP_001274304 comp11507_c0_seq1 | 90 | Nematocyst outer wall antigen (NOWA) | CAP domain, C-type lectin, Minicollagen cysteine-rich | J Cell Sci. 2002 Oct 15;115(Pt 20):3923-34 |
| 17 | Hma2.216559 XP_002169906.2 comp18662_c0_seq1 | 81 | Cnidarian proline-rich protein-1 (CPP-1) | Minicollagen cysteine-rich domain | J Biol Chem. 2012 Mar 23;287(13):9672-81 |
| 18 | Hma2.203602 XP_004212336.2 comp27961_c0_seq7 | 75 | E3 ubiquitin-protein ligase RNF213 | RNF213 protein, P-loop containing nucleoside triphosphate hydrolase | |
| 19 | Hma2.227385 XP_012554267 comp17112_c0_seq1 | 74 | Hemicentin-related | Immunoglobulin (Ig), TLDC | |
| 20 | Hma2.220431 XP_012557586.1 comp26730_c0_seq3 | 73 | Centromere protein F-like | Coiled-coil | |
| 21 | Hma2.213111 XP_012560975.1 comp28013_c0_seq1 | 73 | Kazal-type serine proteinase inhibitor | Thyroglobulin type I repeats, Kazal | |
| 22 | Hma2.222286 XP_012556696.1 comp28009_c0_seq5 | 61 | Uncharacterized zoneadhesin-like protein | Growth factor receptor cysteine-rich, Chitin binding, TM helix | |

| | | | | |
|-----------|--|----|---|---|
| 23 | Hma2.221223 XP_012557188.1 comp27373_c0_seq1 | 58 | Centromere protein F-like | Coiled-coil |
| 24 | Hma2.227701 XP_012554110.1 comp27109_c0_seq1 | 57 | Novel | Coiled-coil |
| 25 | Hma2.200623 XP_012556036.1 | 54 | Novel | DUF4116 |
| 26 | Hma2.223488 XP_002166597.1 comp28907_c0_seq1 | 52 | Novel, cnidarian-restricted | |
| 27 | Hma2.209361 XP_012562660.1 comp28021_c0_seq1 | 51 | Golgin subfamily B member 1-like | Coiled-coil |
| 28 | Hma2.225007 XP_012555480.1 comp20469_c0_seq1 | 51 | NHL repeat-containing protein 2-like | Thioredoxin-like, TolB-like/NHL repeat |
| 29 | Hma2.205944 XP_002170701 comp712702_c0_seq1 | 50 | Novel | Zinc finger C2CH-type domain, harbinger transposase-derived Nuclease domain/THAP domain |
| 30 | Hma2.213602 XP_012563309.1 comp24822_c0_seq3 | 42 | Dystrophin | Spectrin repeats, PLAT/LH2, WW, EF hand, EF hand type 2/Zinc finger ZZ type |
| 31 | Hma2.232620 XP_012555788.1 comp25533_c0_seq4 | 42 | Collagen type IV, alpha chain-2 | Collagen triple-helix |
| 32 | Hma2.229657 XP_012566950.1 | 41 | Novel | TM helix |
| 33 | Hma2.216831 XP_012559234.1 | 41 | E3 ubiquitin-protein ligase RNF213-like | |
| 34 | Hma2.229673 XP_012555349.1 comp12798_c0_seq2 | 41 | Novel | Alpha-L-arabinofuranosidase B/ fascin superfamily β -trefoil |
| 35 | Hma2.208777 XP_012563382.1 | 41 | Fibrillin 1-like (partial) | |
| 36 | Hma2.231712 XP_012565417.1 | 40 | Voltage-dependent calcium channel type A, subunit alpha-1 | Ion transport domain, Voltage-dependent channel, four-helix bundle domain |
| 37 | Hma2.224063 XP_012555935.1 | 40 | Sec1-like protein | Sec1-like, sec1-like domain 2 |

Unique protein hits resulting from the Orbitrap mass spectrometry analysis are listed in descending order according to their MASCOT protein score. References are given for proteins previously characterized at the protein or mRNA level. Domain annotations were performed as described in the Methods. Transcript IDs were obtained by BLAST searches of the hydra head regeneration transcriptome assemblies on <https://research.nhgri.nih.gov/hydra>.

Supplementary Table S2. Sequences of siRNAs, qPCR and ChIP primers.

| siRNA | nucleotide sequence (5' to 3') |
|---------------------|---------------------------------------|
| siGFP | TAGATGGTGATGTTAATGGGC |
| Scrambled siGFP | AACUCAUCGAUUCACACCGGUUUU |
| siTSP1 | AAGATATGGTGCCTATCAGTT |
| siTSP2 | CAGATTACTTATAAGTTTTAA |
| si β -Catenin | AAGGUUAUGAUGAUGAUGAAGUU |
| siWnt3 | AAAGAGGCUAUAACGUUAAUAUU |

| Gene | qPCR Primer Sequences (5' to 3') |
|------------------------------|---|
| Hm β -Catenin fw | CCTGCTATTGTTGAGGCTGGA |
| Hm β -Catenin rv | TCTGCAAGAGTCCTTCTAAACCA |
| Hm Wnt3 fw | ATTACAACAGCCAGCAGAGAAAG |
| Hm Wnt3 rv | TTATCGCAACGACAGTGGAC |
| HmTSP fw | ACCAAATGATCAAGCTGGATCCT |
| HmTSP rev | ACCACCAAACCTTTCAACTCCA |
| γ -Tubulin subunit fw | CTGCTTGTGTAGCATACTTTGAAAT |
| γ -Tubulin subunit rv | TCATG TTCAGCTACAAGAAATTCAC |

| TCF binding sites | ChIP PCR primer sequences (5' to 3') |
|-------------------------------|---|
| 2 juxtaposed distal sites fw | CCCGACTGTTGAAACTGC |
| 2 juxtaposed distal sites rev | TGTTCTCAAGGCTCACGC |
| Proximal site fw | TTGAAGGCATTTAACAACTTGC |
| Proximal site rev | TGCCCAAATGTAAAGTTCTGTG |
| Intronic site fw | GACAAAACCAAAGAAAAAATG |
| Intronic site rev | TCCCTTGTAATCAGGTTAG |
| Control fw | ACTTTCATATAAGGCCGCTTC |
| Control rev | CCCAGCGATATAGAGACTGC |

Supplementary Fig. S1

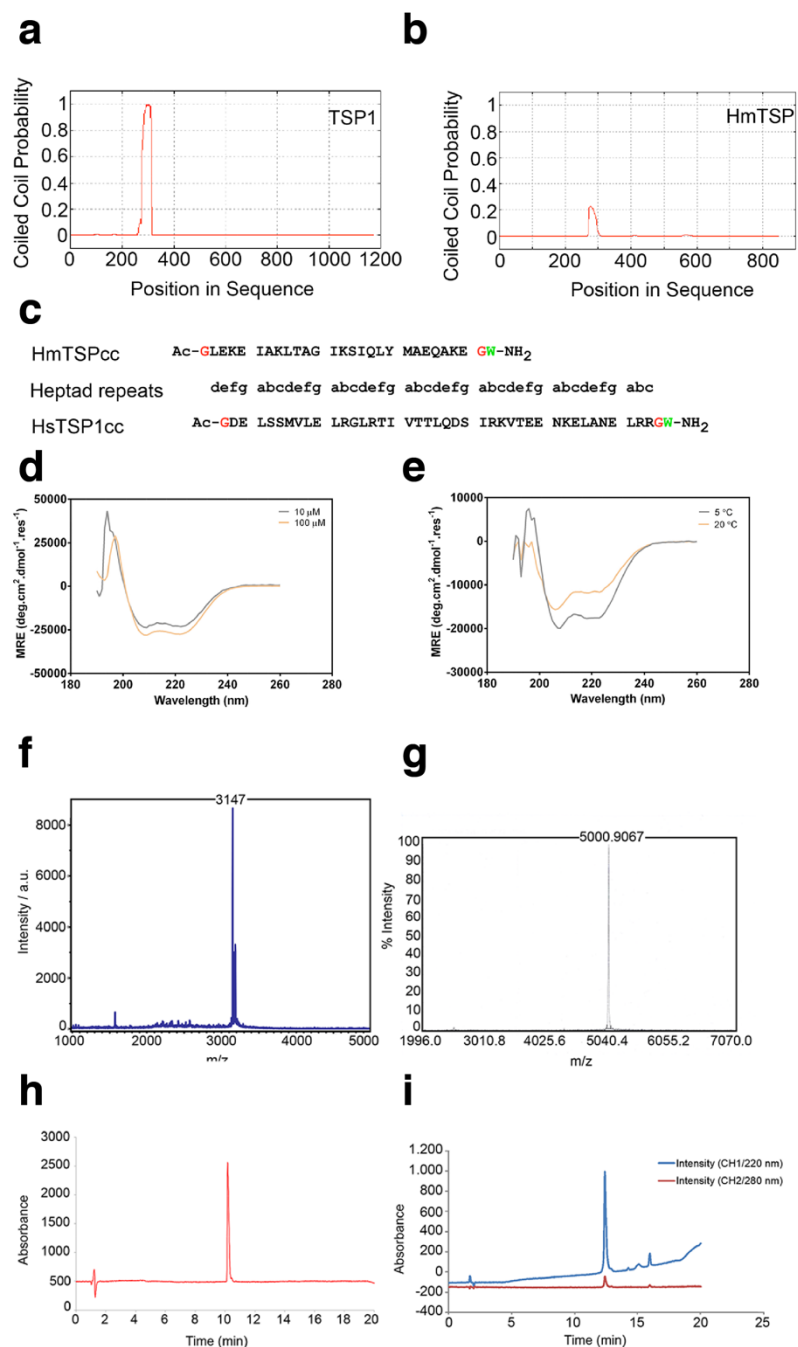


Fig. S1. Preparation of synthetic peptides from the oligomerization regions of TSP1 and *Hydra* TSP. **a, b.** Coiled-coil region probabilities in human TSP1 (**a**) or *Hydra* TSP (**b**), as predicted in MARCOIL. **c.** Protein sequences of the synthetic peptides corresponding to the predicted oligomerization regions of *Hydra* TSP (HmTSPcc) and human TSP1 (HsTSP1cc). In each peptide, the native sequence is given in black, added glycines in red and added tryptophan in green. The heptad repeat phase is predicted from MARCOIL. **d, e.** Circular dichroism spectra for HsTSP1cc (**d**) or HmTSPcc (**e**) peptides. HsTSP1cc was analyzed at two concentrations at 5 °C. HmTSPcc was analyzed at 100 μM at 5 °C and 20 °C. MRE = mean residue ellipticity. **f, i.** Quality control for the synthetic peptides. Spectra from MALDI-TOF mass spectrometry for HmTSPcc (**f**) and HsTSP1cc (**g**) peptides. Each identifies a single peak consistent with the predicted mass for each peptide. **h, i.** Traces from RP-HPLC of HmTSPcc (**h**) and HsTSP1cc (**i**) peptides, demonstrating the purity of the preparations.

Supplementary Fig. S2

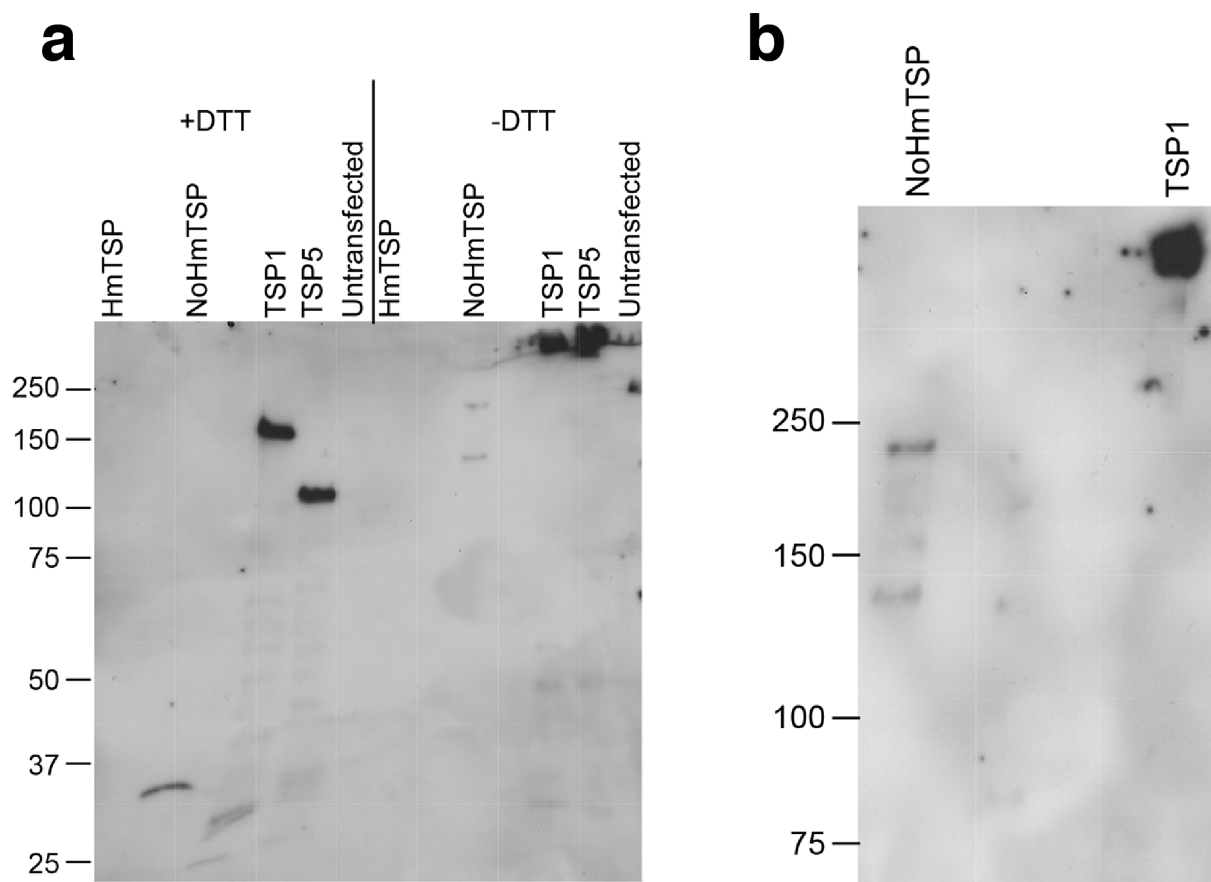
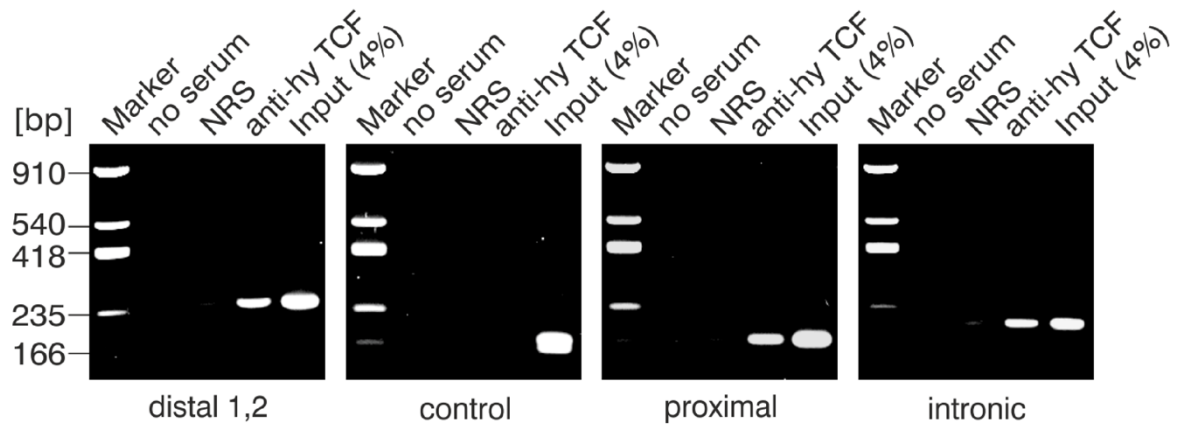


Fig. S2. Uncropped Western blot images used in Fig. 3d. a. Recombinant NoHmTSP.V5 and human TSP1.V5 and TSP5.V5 analyzed under reducing and non-reducing conditions, including lanes presented in Fig. 3D, left panel. **b.** Recombinant NoHmTSP and human TSP1 analyzed under non-reducing conditions as presented in Fig. 3d, right panel. Each immunoblot was probed with V5 antibody. To improve resolution of high-molecular weight proteins, some molecular weight markers were run off the bottom of each gel a and b as indicated.

Supplementary Fig. S3

a



b

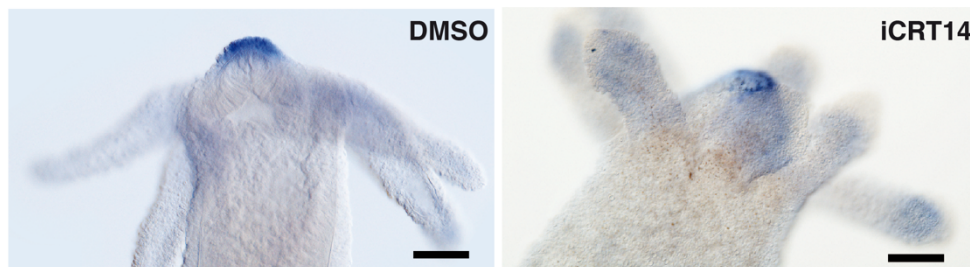


Fig. S3. Regulation of *HmTSP* expression by β -Catenin. **a.** Replication of the ChIP experiment analyzing the *Hydra TSP* promoter region (compare Fig. 7b) using an independent chromatin preparation from whole *Hydra* animals. A polyclonal antibody directed against *Hydra* TCF was used for precipitation, followed by PCR amplification of the indicated fragments from the *TSP* regulatory region including a region without TCF binding site (control). Reactions with normal rabbit serum (NRS) or total chromatin (Input) were used as further controls. PCR products were resolved by agarose gel electrophoresis and visualized by ethidium bromide staining. **b.** Representative *HmTSP* expression in DMSO-treated control animal at 72hrs (left) or animal treated with 50 μ M of β -Catenin inhibitor iCRT14 for 24 h and fixed after 72 hrs. Bars: 500 μ m.