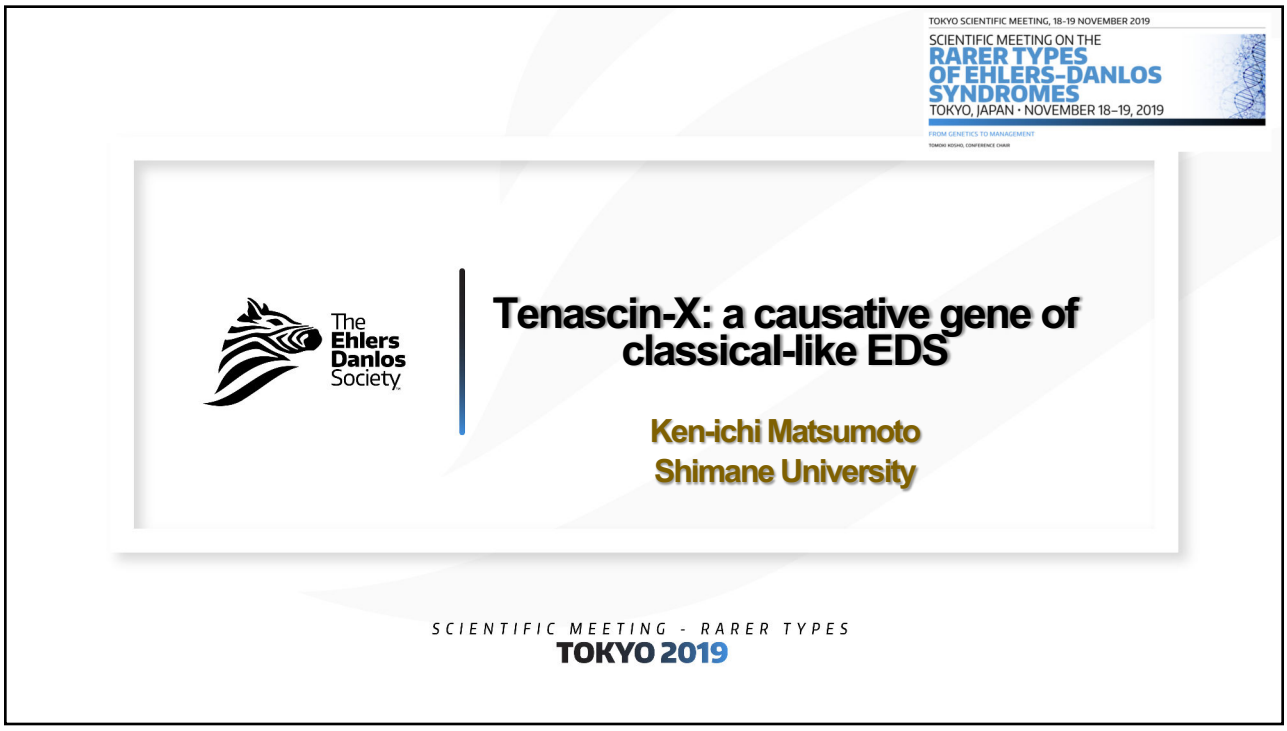

Classification of Ehlers-Danlos syndrome

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EDS subtype		Abbreviation	Prevalence	Inheritance pattern	Protein	Gene
* 1	Classical	cEDS	1/20 000	AD	Type V collagen	*COL5A1/COL5A2*
* 2	**Classical-like**	**clEDS**	**24**	**AR**	**Tenascin-X (Complete deficiency)**	***TNXB***
* 3	Cardiac-valvular	cvEDS	4	AR	Type I collagen	*COL1A1*
* 4	Vascular	vEDS	1/50 000-250 000	AD	Type III collagen	*COL3A1*
* 5	Hypermobile	hEDS	1/5000-20 000	AD	Unknown	*Unknown*
* 6	Arthrochalasia	aEDS	49	AD	Type I collagen	*COL1A1/COL1A2*
* 7	Dermatosparaxis	dEDS	8	AR	ADAMTS-2	*ADAMTS2*
* 8	Kyphoscolitotic	kEDS	1/100 000	AR	Lysyl hydroxylase-1, FKBP14	*PLOD1, FKBP22*
* 9	Brittle Cornea syndrome	BCS	11	AR	ZNF469, PRDM5	*ZNF469, PRDM5*
*10	Spondylodysplastic	spEDS	8	AR	β4GalT7, β3GalT6	*B4GALT7, B3GALT6*
*11	Musculocontractural	mcEDS	31	AR	CHST14, DSE	*CHST14, DSE*
*12	Myopathic	mEDS		AD or AR	Type XII collagen	*COL12A1*
*13	Periodontal	pEDS		AD	C1r, C1s	*C1R, C1S*

Malfait F. *et al.*, *Am. J. Med. Genet. Part C* 175C, 8-26, 2017.
Kosho T. *et al.*, *Pediatr. Int.* 58, 88-99, 2016.

Clinical feature of TNX-deficient type EDS (clEDS)

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Typical clinical feature

- Generalized joint hypermobility (with or without (sub)luxations)
- Skin hyperextensibility
- Easy bruising

Other common clinical feature

- Foot and hand deformities
  - Piezogenic papules
  - Pes planus
  - Hallux valgus
  - Broad forefeet
  - Brchydactyly
  - Acrogeric skin of hands
- Neurological symptoms
  - Muscle weakness
  - Chronic back pain, myalgias, arthralgias ⇨ *Prof. Emiko Ashitaka*
  - Severe fatigue
- Cardiovascular abnormality (valvular problems)
- Gastrointestinal manifestation ⇨ *Dr. Hiroaki Hanafusa*

In contrast to the classical type EDS (cEDS)

- Inheritance pattern: Autosomal recessive
- Without atrophic scarring

Demirdas *et al.*, *Clinical Genet. 91*, 411-425, 2017.
Malfait *et al.*, Am. J. Med. Genet. Part C Semin. Med. Genet. 175C, 8-26, 2017.

Genetic analysis of TNX-deficient patients

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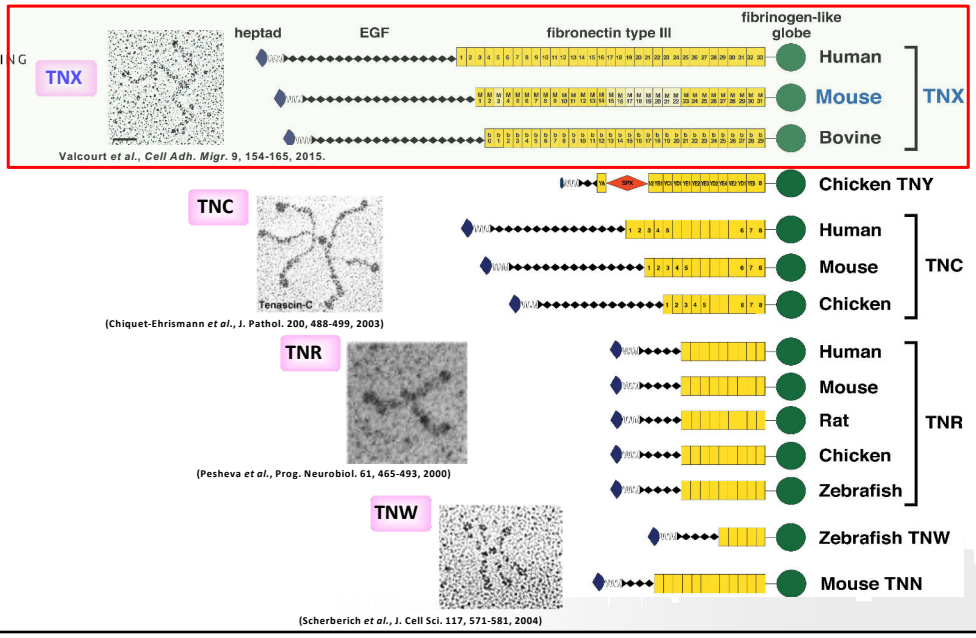
- A 2 bp deletion (c.3290_3291del)
- Two different 30 kb deletions both generating a TNXB/TNXA fusion gene, and pseudogene-derived missense variant [c.12174C>G p.(Cys4058Trp)]
- Premature stop codon [c.903del p.(Tyr301*)], [c.12553C>T p.(Arg4185*)], [c.2461C>T p.(Arg821*)], [c.2590C>T p.(Gln864*)]
- Splice site mutation [c.7826-1G>C p.(?)], [c.12464-1G>A p.(?)]
- A small deletion/insertion [c.107_108delinsA p.(Ala36Aspfs*68]
- A pseudogene-derived 120 bp deletion (c.11435_11524+30del)

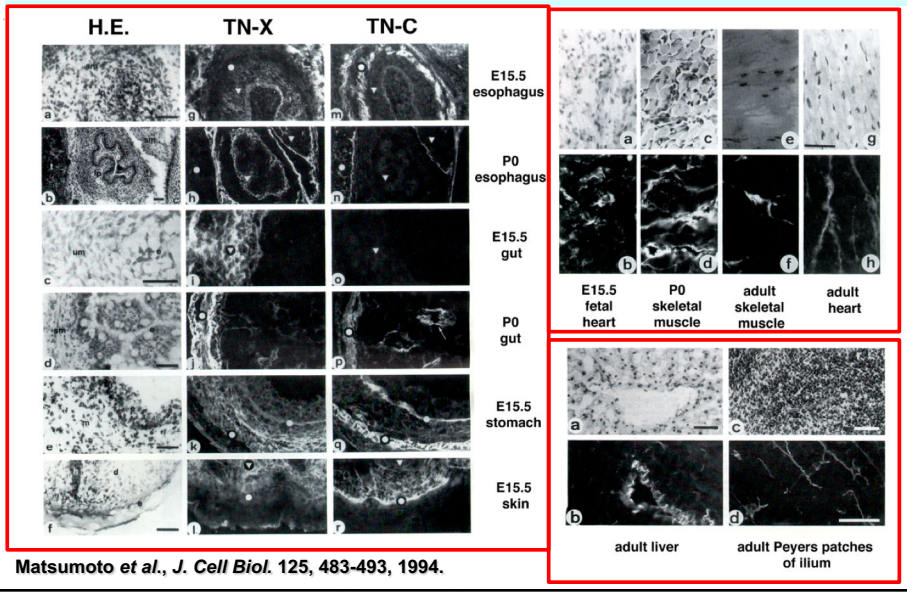
Demirdas *et al.*, *Clinical Genet. 91*, 411-425, 2017

Tenascin family



Expression of TNX

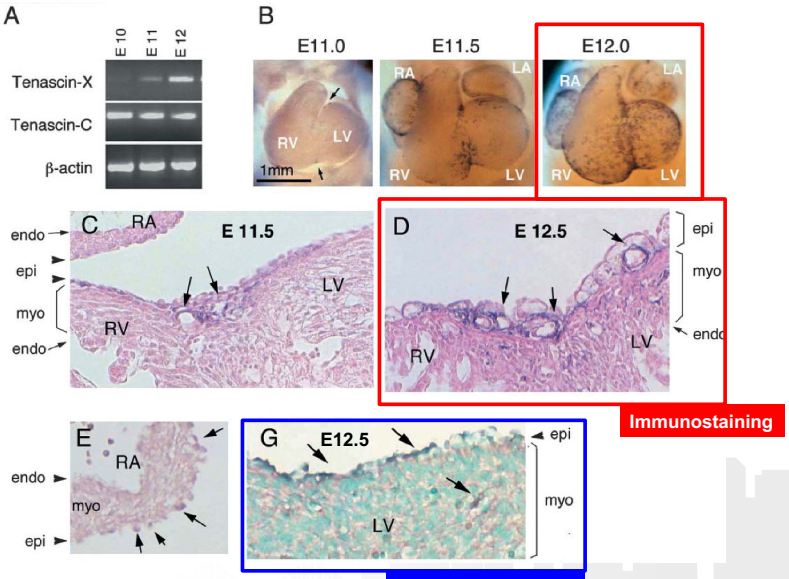
Reciprocal expression of TNX to TNC



Matsumoto *et al.*, *J. Cell Biol.* 125, 483-493, 1994.

Expression of TNX

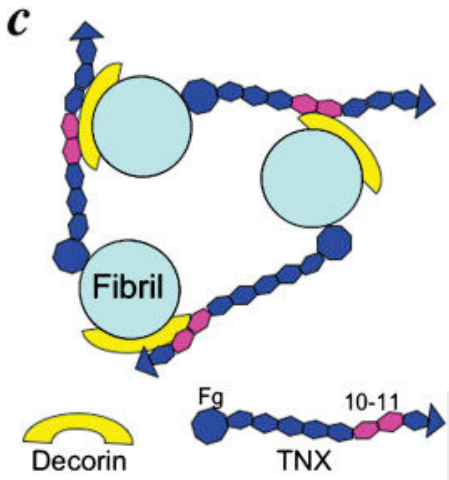
Expression of TNX in E10-12.5 hearts



Imanaka-Yoshida *et al*., *Differentiation* 71, 291-298, 2003.

Property of TNX

Modulation of collagen fibril architecture and tissue integrity

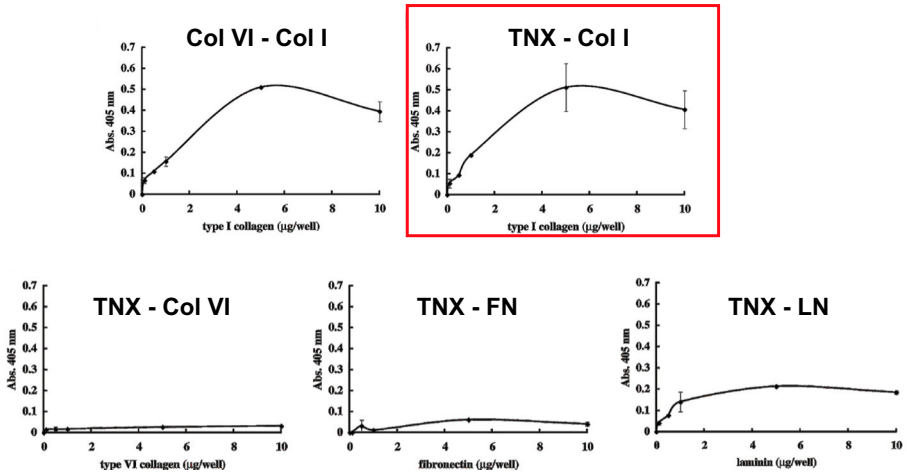


Bristow J., *Am. J. Med. Genet. C Semin. Med. Genet.* 139, 24-30, 2005.

Property of TNX

Interaction of TNX with type I collagen

Solid-phase binding assay

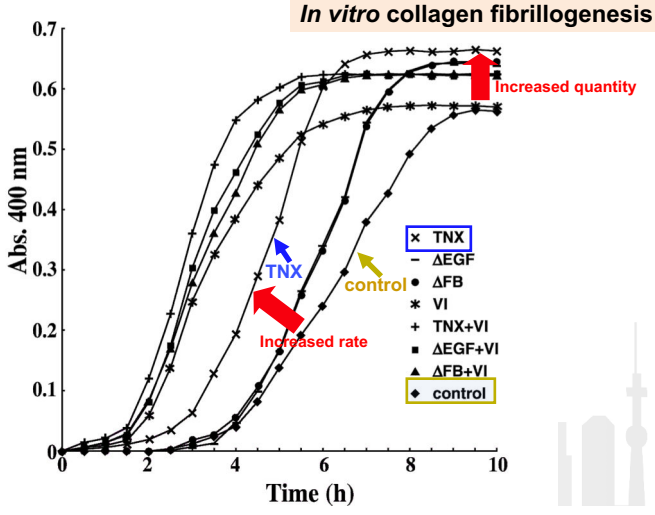


Minamitani *et al.*, *Exp. Cell Res.* 298, 305-315, 2004.

Property of TNX

TNX increases the rate and quantity of collagen fibril formation

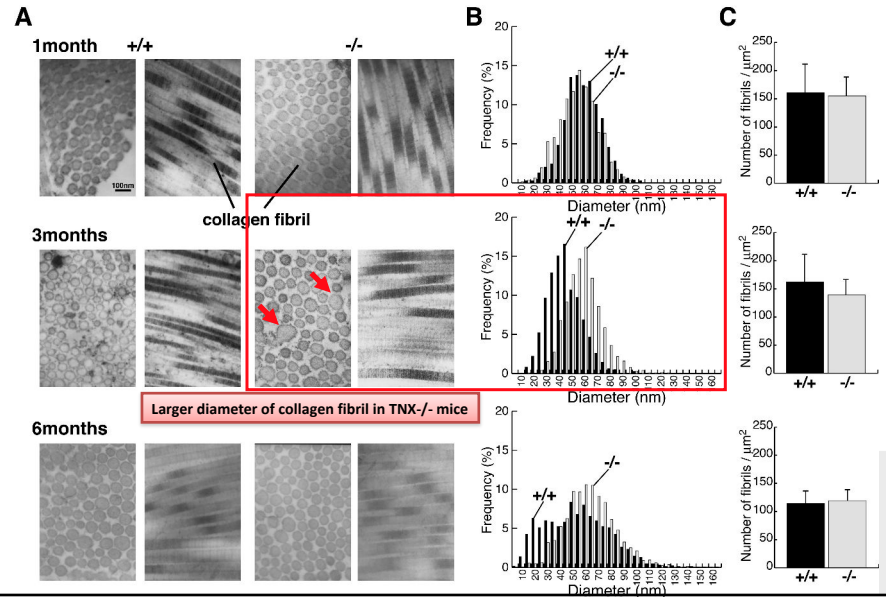
In vitro collagen fibrillogenesis assay



Minamitani *et al.*, *Exp. Cell Res.* 298, 305-315, 2004.

Property of TNX

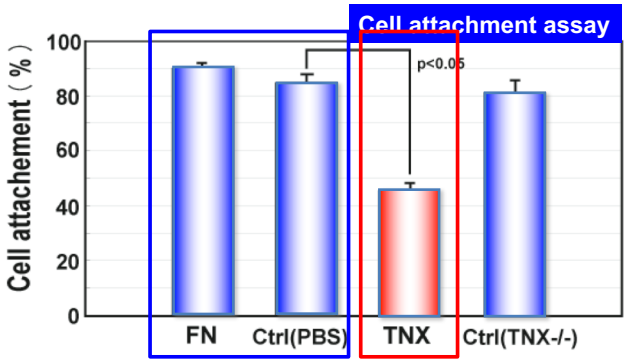
Modulation of collagen fibrillogenesis by TNX



Minamitani *et al.*, *Exp. Cell Res.* 298, 305-315, 2004.

Property of TNX

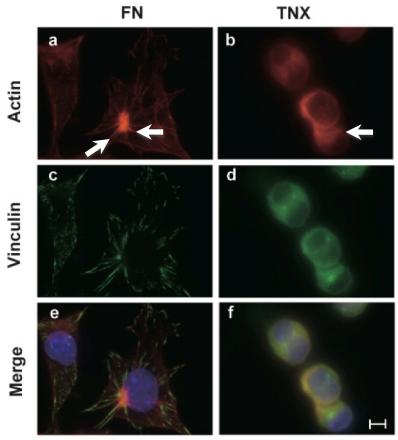
Compromised cell adhesion on TNX substrate



Fujie *et al., Biol. Pharm. Bull.* 32, 1795-1799, 2009.

Property of TNX

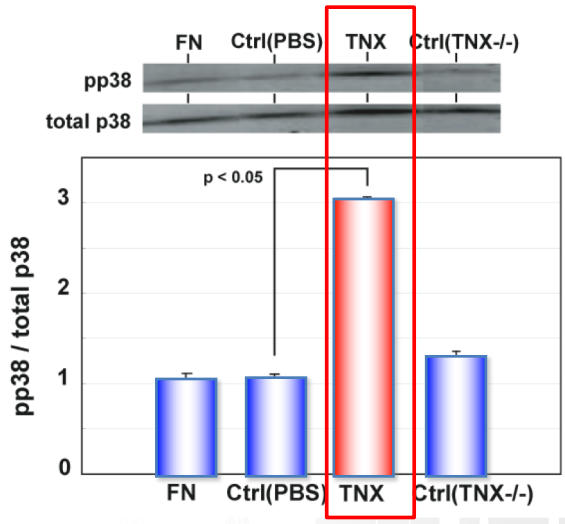
Compromised cell adhesion on TNX substrate



Fujie *et al., Biol. Pharm. Bull.* 32, 1795-1799, 2009.

Property of TNX

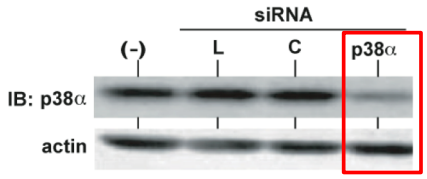
TNX induces p38 MAP kinase activity

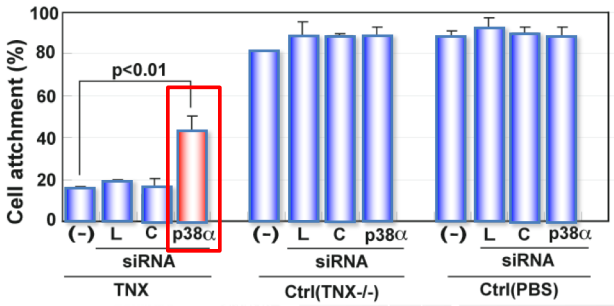


Fujie *et al., Biol. Pharm. Bull.* 32, 1795-1799, 2009.

Property of TNX

Suppression of TNX-Induced cell detachment by knocking down p38α MAP kinase expression

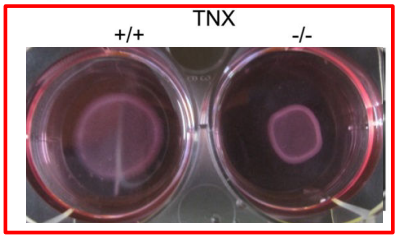


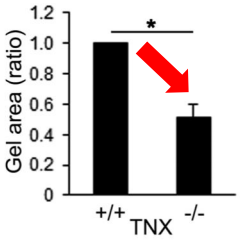


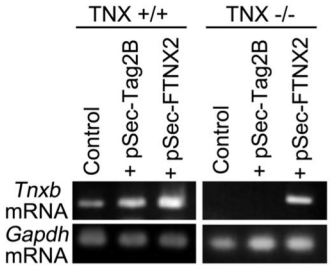
Fujie *et al.*, *Biol. Pharm. Bull.* 32, 1795-1799, 2009.

Collagen gel contraction containing TNX-deficient MEFs

Property of TNX







Hashimoto *et al.*, *Exp. Cell Res.* 363, 102-113, 2018.

Effect of MMP inhibitors on collagen gel contraction

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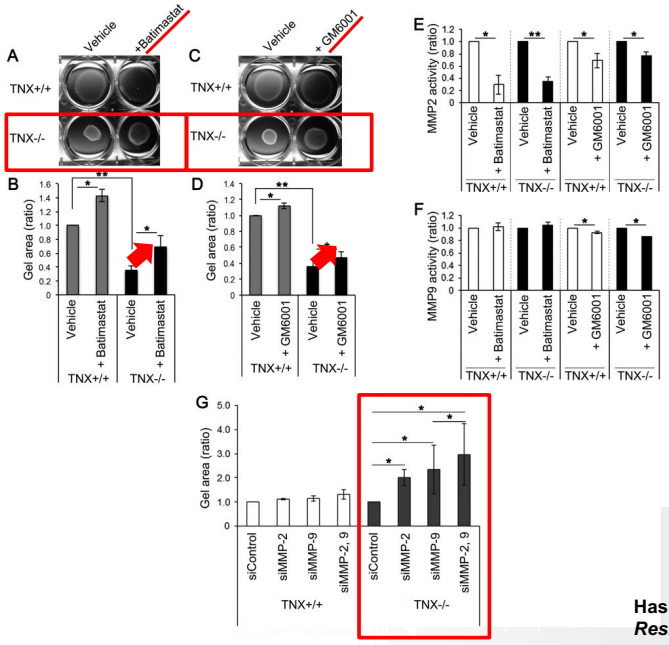
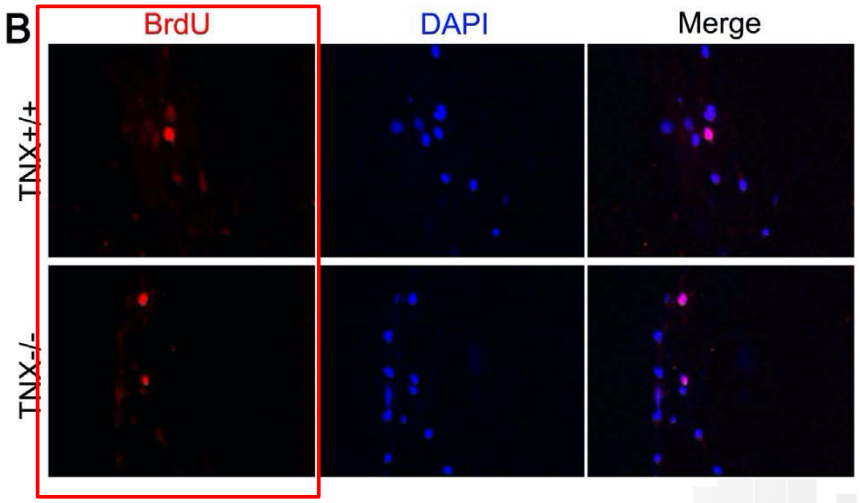
Property of TNX



Hashimoto *et al., Exp. Cell Res.* 363, 102-113, 2018.

Property of TNX

Effects of TNX absence on proliferation of MEFs in collagen gels



Hashimoto *et al., Exp. Cell Res.* 363, 102-113, 2018.

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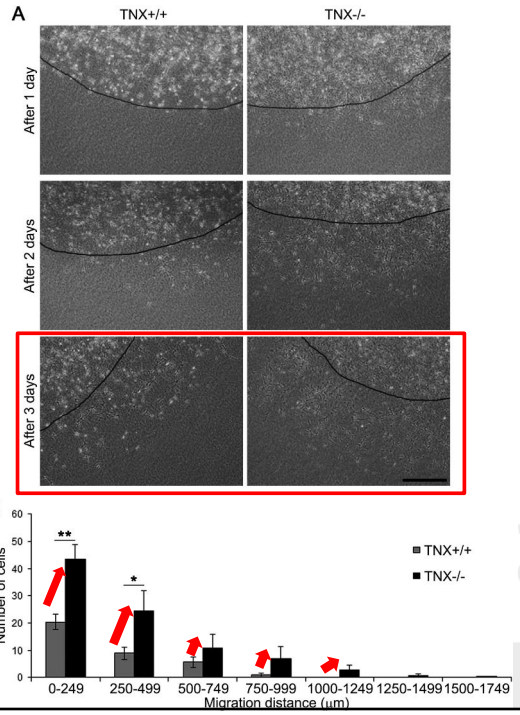
Property of TNX

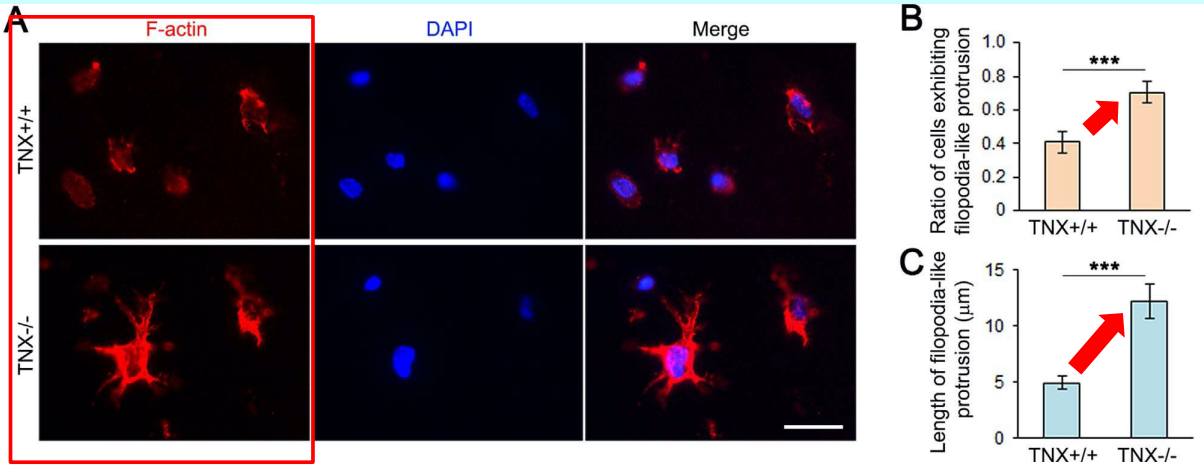
Effects of TNX absence on migration of MEFs in collagen gels



Hashimoto *et al., Exp. Cell Res.* 363, 102-113, 2018.

Property of TNX

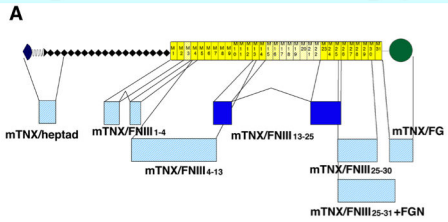
Effect of TNX absence on the formation of filopodia-like protrusions of MEFs cultured in contracted collagen gels

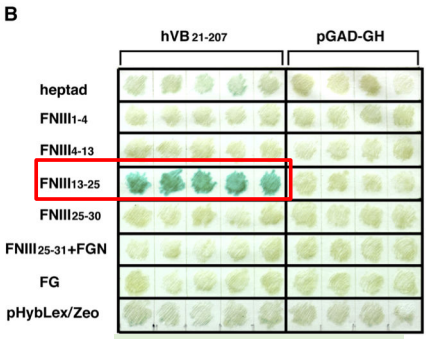


Hashimoto *et al., Exp. Cell Res.* 363, 102-113, 2018.

Property of TNX

Screening of TNX-binding proteins



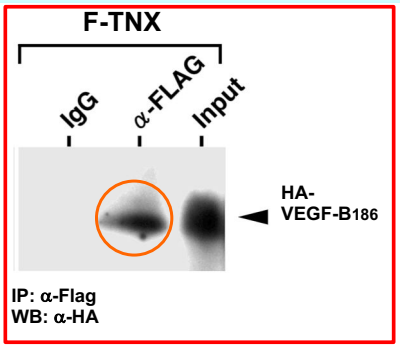


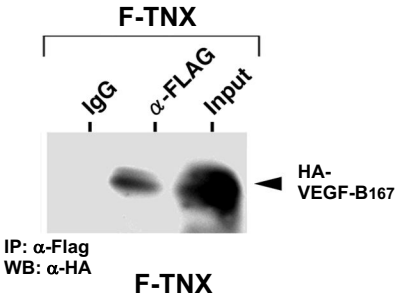
Yeast two-hybrid assay

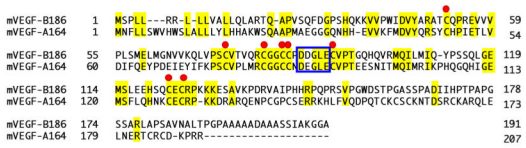
Ikuta *et al*., *Genes Cells* 5, 913-928, 2000.

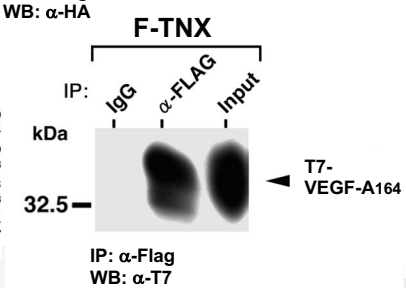
Property of TNX

Interaction of TNX with VEGF-B









Co-immunoprecipitaion assay

Ikuta *et al*., *Genes Cells* 5, 913-928, 2000.

Property of TNX

Endothelial cell proliferation by VEGF-B in combination with TNX

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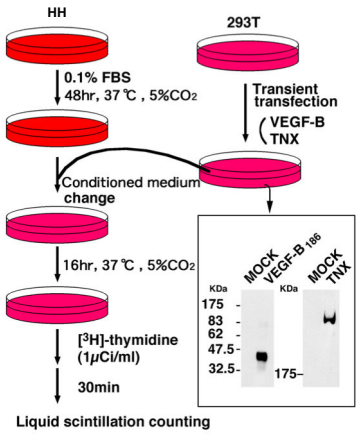

Ikuta *et al.*, *Genes Cells* 5, 913-928, 2000.

Property of TNX

Stimulation of the autophosphorylation in VEGFR-1 by VEGF-B with TNX

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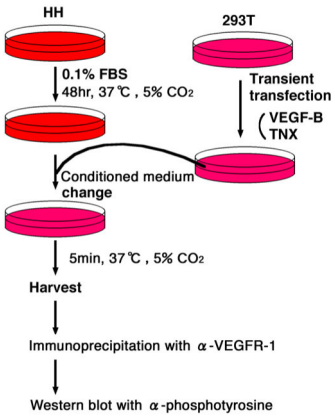
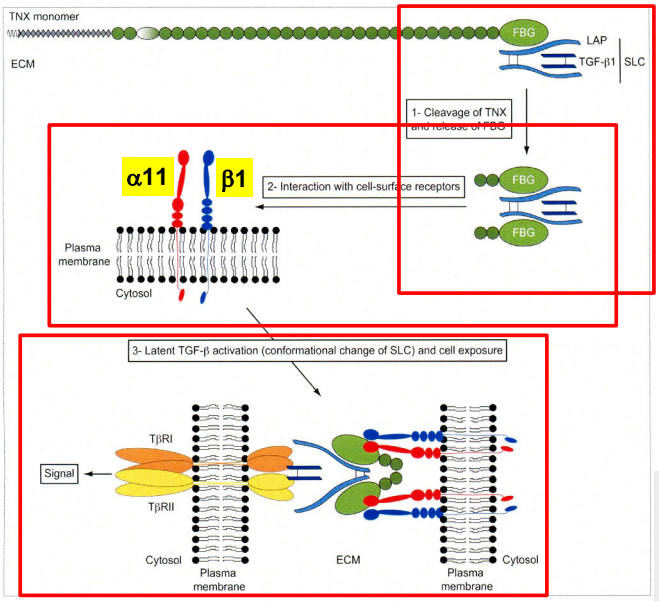


Ikuta *et al.*, *Genes Cells* 5, 913-928, 2000.

TGF-β activation by the FBG-like domain of TNX

Property of TNX



Alcaraz *et al.*, *J. Cell Biol.* 205, 409-428, 2014.

Valcourt *et al.*, *Cell Adh. Migr.* 9, 154-165, 2015.

Involvement of tenascin-X in osteoclast maturation

Osteoblast and osteoclast lineages for bone metabolism

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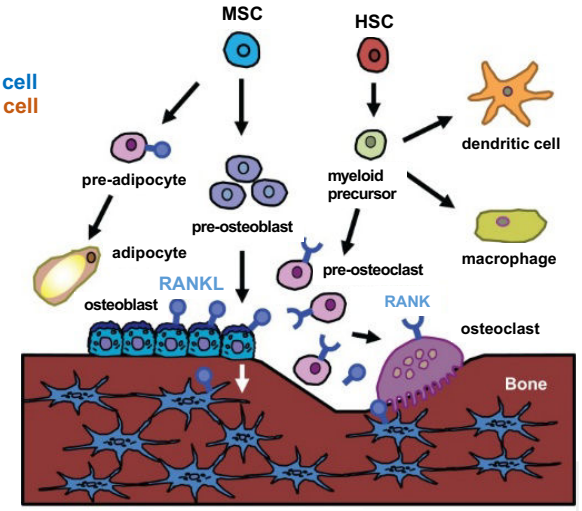
MSC: mesenchymal stem cell
HSC: hematopoietic stem cell



Ikeda K. *et al. J Biochem*: 159, 1-8, 2016.

Histological analysis of femur

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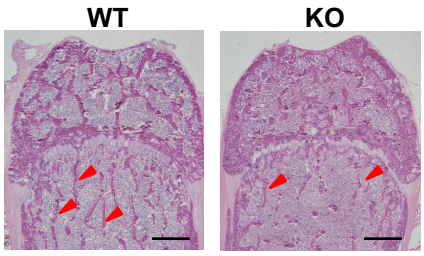
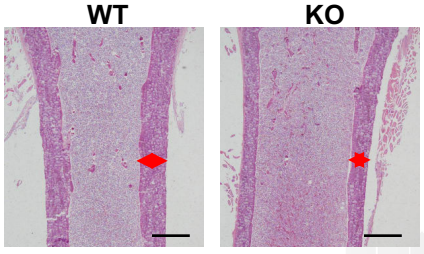
Femoral distal end



Decrease in trabecular bone in TNX-KO mice

Femoral shaft



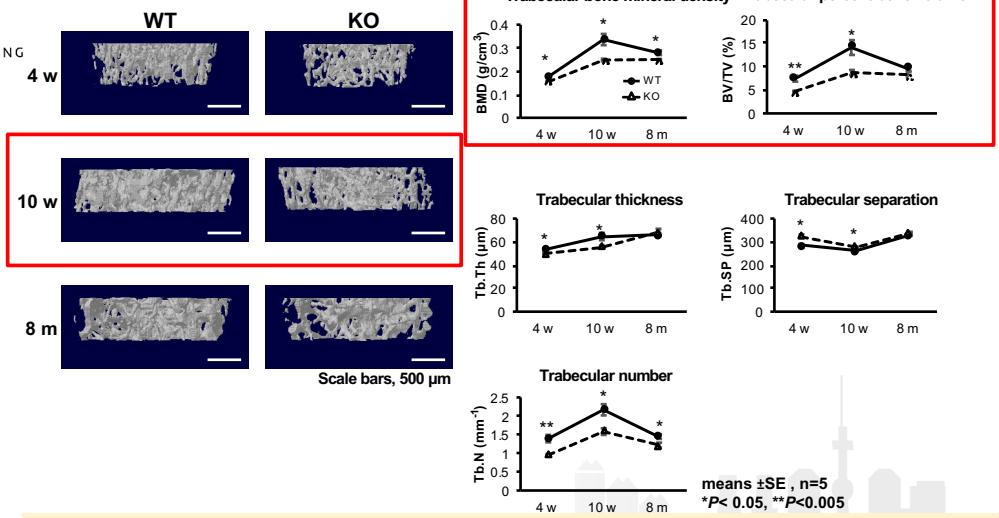
Decrease in cortical bone thickness in TNX-KO mice.

Scale bars, 500 μm

TNX-KO mice exhibit lower bone mass.

Kajitani *et al., Biochem. Biophys. Res. Commun.* 512, 659-664, 2019.

Bone morphology analysis of trabecular bone

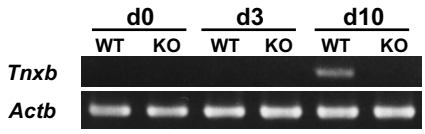


The bone mass and bone mineral density (BMD) of trabecular bone are significantly lower in *Tnxb*-KO mice than those of age-matched WT mice.

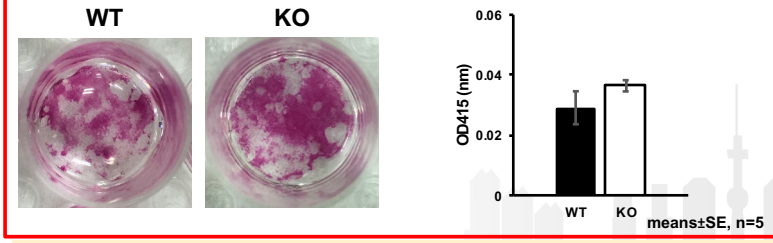
Kajitani *et al., Biochem. Biophys. Res. Commun.* 512, 659-664, 2019.

Analysis of osteoblast differentiation

***Tnxb* expression during osteogenic differentiation**



Alizarin red S staining



Osteoblast differentiation is not affected by TNX deficiency.

Kajitani *et al., Biochem. Biophys. Res. Commun.* 512, 659-664, 2019.

Analysis of osteoclast differentiation

The Ehlers Danlos Society

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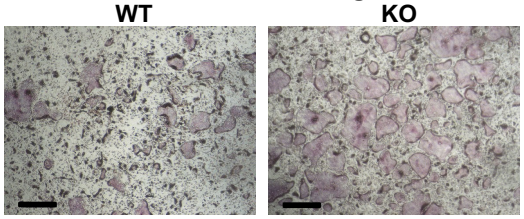
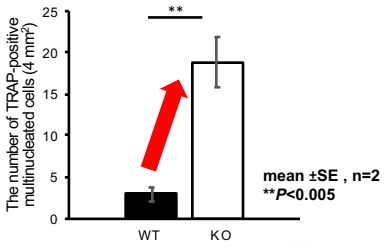
TRAP staining



The number of multinucleated cells



The number of TRAP-positive multinucleated cells were significantly increased in *Tnxb*-KO cells compared with WT.

Osteoclast differentiation is enhanced in *Tnxb*-KO mice.

Kajitani *et al., Biochem. Biophys. Res. Commun.* 512, 659-664, 2019.

Expression of osteoclast markers

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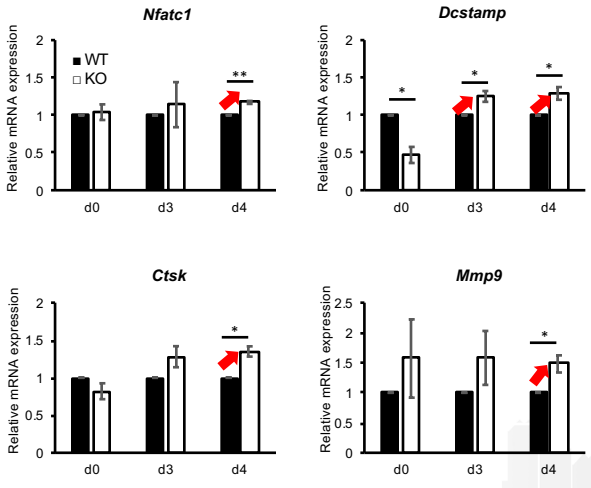


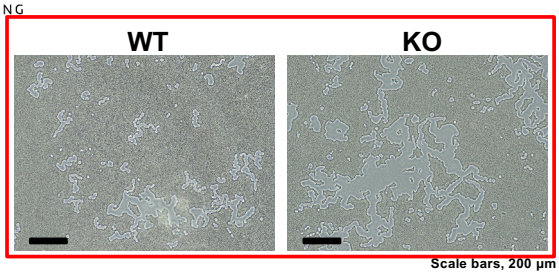
The expression of osteoclast markers was higher in *Tnxb*-KO cells at day 4 than in WT.

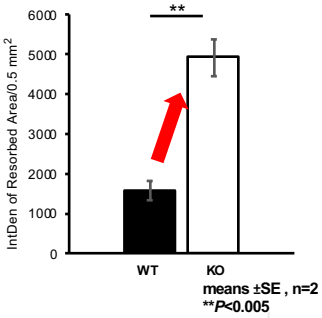
means ±SE , n=3
*P< 0.05, **P<0.005

Osteoclast differentiation is enhanced in *Tnxb*-KO mice.

Kajitani *et al., Biochem. Biophys. Res. Commun.* 512, 659-664, 2019.

Bone resorption activity assay





The bone resorption pits were significantly increased in *Tnxb*-KO osteoclasts compared with WT.

Osteoclast function is enhanced in *Tnxb*-KO mice.

Kajitani *et al.*, *Biochem. Biophys. Res. Commun.* 512, 659-664, 2019.

Measurement of serum tenascin-X in joint hypermobility syndrome (JHS)/ EDS (hEDS) Patients

Hypermobility EDS (hEDS)

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* Generalized joint hypermobility

* Two or more among the following features
  - A. Generalized connective tissue disorder
    - e.g.,
      - Unusually soft or velvety skin
      - Mild skin hyperextensibility
      - Unexplained striae
      - Bilateral piezogenic papules of heel
      - Recurrent or multiple abdominal hernia
  - B. Positive family history
  - C. Musculoskeletal complications

* All the following prerequisites must be met
  - e.g.
    - Absence of unusual skin fragility
    - Exclusion other heritable connective tissue disorders

Beighton *et al.*, *Am. J. Med. Genet.* 77, 31-37,1998.

Malfait *et al.*, Am. J. Med. Genet. Part C Semin. Med. Genet. 175C, 8-26, 2017.

In 2003, Zweers was reported that there is a small subset of patients with JHS/hEDS who have haploinsufficiency of tenascin-X (TNX).

However, the relationship between TNXB and JHS/hEDS has not been reported at all afterwards.

At present, the causative gene of hEDS remained to be identified.

Zweers *et al.*, *Am. J. Hum. Genet.* 73, 214-217, 2003.

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Clinical profiles of patients with JHS/hEDS in this study

		Number (%) (n=18)
Family history of JHS	9	(50%)
Joint dislocations (any)	18	(100%)
Shoulders	16	(89%)
Fingers	15	(83%)
Knees	10	(56%)
Wrists	7	(39%)
Elbows	6	(33%)
Ankles	6	(33%)
Pain (any)	18	(100%)
Joint pain	17	(94%)
Back pain	16	(89%)
Muscle pain	14	(78%)
Skin manifestation (any)	7	(39%)
Skin hyperextensibility	7	(39%)
Fragile skin	3	(17%)
Chronic fatigue	18	(100%)
Easy bruising	12	(67%)
Postural hypotension	18	(100%)
Headache	14	(78%)
Temporomandibular disorder	13	(72%)
Recurrent caries	9	(50%)
Gum fragility	11	(61%)
Temporomandibular joint hypermobility	8	(44%)
Gastritis	7	(39%)
Abdominal pain	5	(28%)
Chronic diarrhea	7	(39%)

Watanabe *et al., Int. J. Mol. Med.* 37, 461-467, 2016

sTNX concentrations in sera from patients with hEDS

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sTNX concentration
50 Healthy individuals: 110 ng/ml
17 JHS/hEDS patients: 90 ng/ml

JHS/hEDS patient no.	1	3	4	7	10	12	14	22	29	31	34	40	45	51	53	68	73
JHS/hEDS family no.	7	1	5									14	13	15	16	18	19
Pedigree of proband	Proband	Affected mother of JHS/hEDS 2	Proband	Proband	Proband	Proband	Proband	Proband	Proband	Mother of JHS/hEDS 14	Mother of JHS/hEDS 7	Proband	Proband	Proband	Proband	Proband	Proband
Age	22	42	34	27	16	19	32	28	25	64	57	31	26	25	22	42	33
sTNX concentration (ng/mL) Mean±SE	95±10	72±5	76±6	71±7	131±1	87±5	75±11	138±12	100±8	80±5	76±8	97±10	97±6	80±1	87±3	83±6	83±8
Student's t-test	n. s.	##	##	#	#	n. s.	#	n. s.	n. s.	##	#	n. s.	n. s.	##	#	#	n. s.
Ratio	0.86	0.66	0.7	0.65	1.19	0.8	0.68	1.26	0.92	0.73	0.69	0.88	0.88	0.73	0.8	0.76	0.75
MLPA	-	N. P.	-	-	-	-	-	-	-	N. P.	N. P.	-	-	-	-	-	-
WES	-	-	-	-	-	+a)	-	-	-	-	-	-	-	-	-	-	-

The mean sTNX concentration in sera from healthy individuals was 110 ± 6 (ng/mL, Mean ± SE, n=3).

Heterozygous nonsense mutation [NM_019105.7:c.4957>T (p.Arg1653*)] was inherited from unaffected mother.

MLPA, Multiplex ligation-dependent probe amplification; N. P.; not performed; WES, Whole exome sequencing; +, positive; -, negative.

Decrease in sTNX concentration could be used as a risk factor for hEDS.

Yamada *et al., Bio. Pharm. Bull.* 42, 1596-1599, 2019.

Proteins with differential levels in patients with JHS/hEDS and in healthy control individuals

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Unused ProtScore[a]	%Coverage[b]	Peptides[c] (95%)	UniProt number	Gene Symbol	Protein name	iTRAQ ratio[d] Average ± SE	p value[e]	Molecular function
3.4	12.8	3	P00736	C1R	* Complement C1r subcomponent	1.40 ± 0.11	0.0100	Complement
211.3	42.8	133	P04114	APOB	Apolipoprotein B-100	1.26 ± 0.05	0.0195	Ligand for LDL receptor
19.8	28.7	12	P04004	VTN	* Vitronectin	1.23 ± 0.06	0.0344	Cell adhesion molecule
6.0	16.3	4	P02748	C9	* Complement component C9	1.15 ± 0.12	0.0402	Complement
10.6	22.8	6	P04003	C4BPA	* C4b-binding protein α chain	1.10 ± 0.09	0.0372	Complement
6.2	52.4	4	P02766	TTR	Transthyretin	1.06 ± 0.10	0.0171	Thyroxine and retinol-binding protein

Watanabe *et al., Int. J. Mol. Med.* 37, 461-467, 2016

Collaborators

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Expression of TNX

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