Heritable Disorders of Connective Tissue and Spontaneous CSF Leaks

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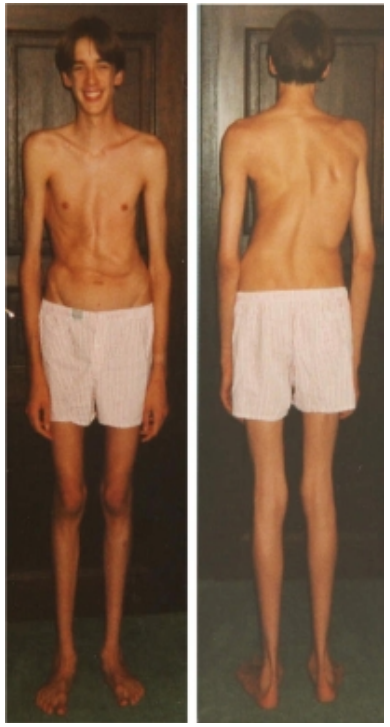
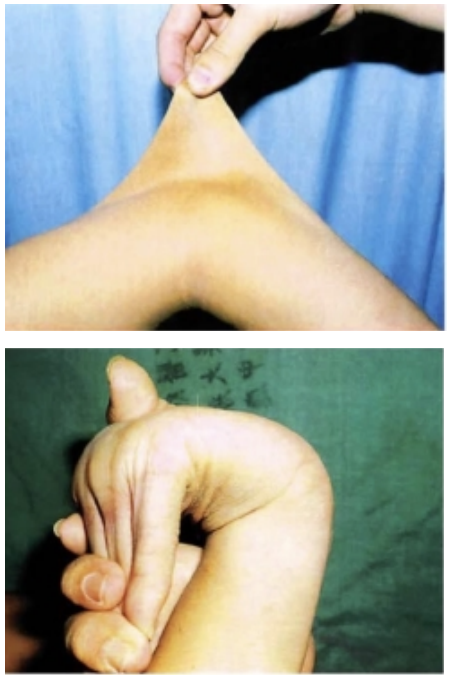
Spontaneous spinal CSF leaks have a strong connective tissue disease association

- Spontaneous Spinal CSF leak patients have connective tissue disease characteristics
  - 20% of patients have nonspecific connective tissue findings
  - 5% have defined connective tissue disorders
- Connective tissue disease patients have higher incidences of leaks
  - Marfan Syndrome
  - Ehlers-Danlos Syndrome
  - Loeys-Dietz Syndrome



Marfan Syndrome

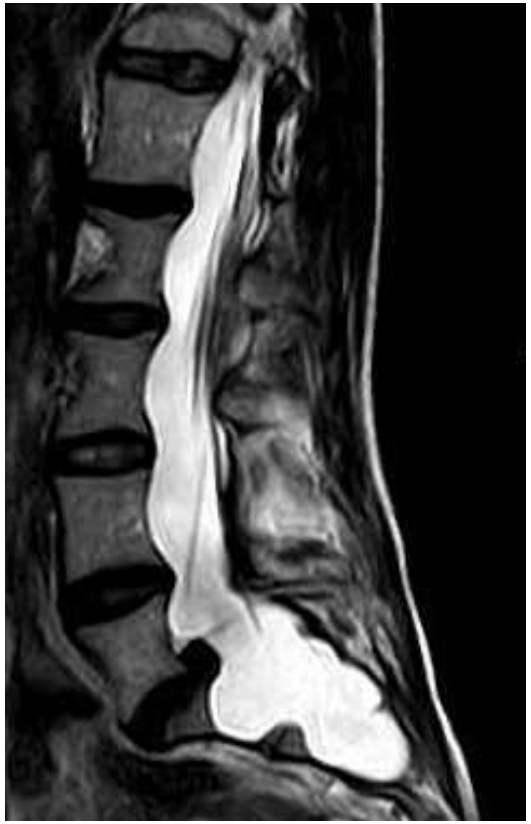


Ehlers-Danlos Syndrome

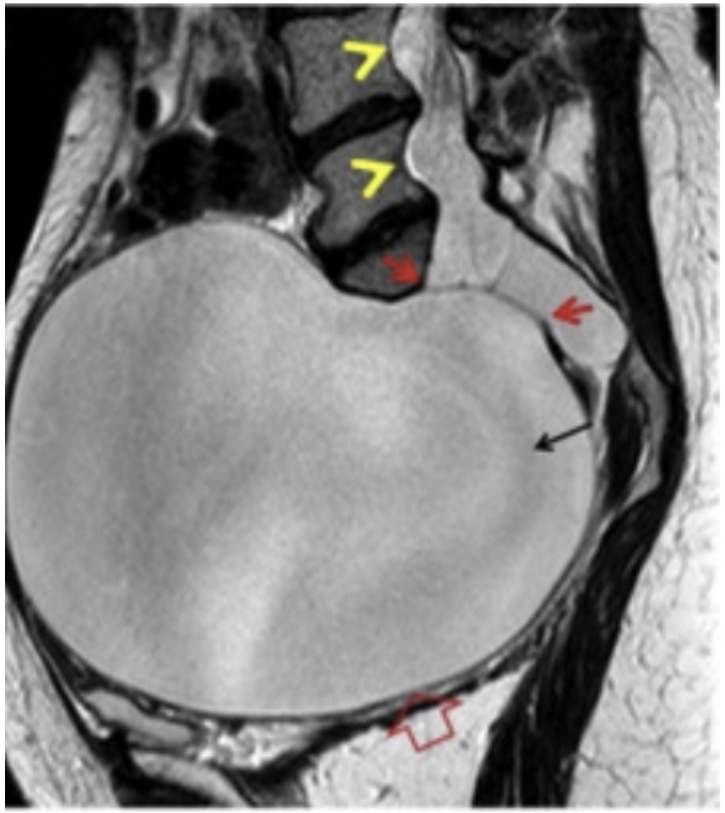
Loeys-Dietz Syndrome

Schrijver I, Schievink WI, Godfrey M, Meyer FB, Francke U. Spontaneous spinal cerebrospinal fluid leaks and minor skeletal features of Marfan syndrome: a microfibrillopathy. J Neurosurg. 2002 Mar;96(3):483-9. doi: 10.3171/jns.2002.96.3.0483. PMID: 11883832.
Puget S, Kondageski C, Wray A, Boddaert N, Roujeau T, Di Rocco F, Zerah M, Sainte-Rose C. Chiari-like tonsillar herniation associated with intracranial hypotension in Marfan syndrome. Case report. J Neurosurg. 2007 Jan;106(1 Suppl):48-52. doi: 10.3171/ped.2007.106.1.48. PMID: 17233313.
Loeys BL, Dietz HC, et. al. A syndrome of altered cardiovascular, craniofacial, neurocognitive and skeletal development caused by mutations in TGFBR1 or TGFBR2. Nat Genet. 2005 Mar;37(3):275-81. doi: 10.1038/ng1511. Epub 2005 Jan 30. PMID: 15731757.

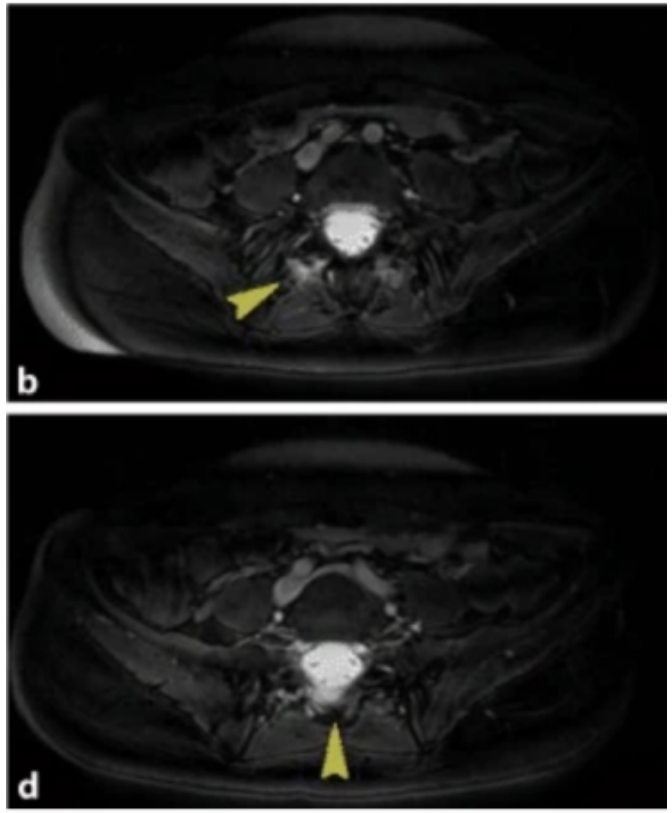
Dural pathologies present in connective tissue diseases



Dural Ectasia



Tarlov Cyst



CSF Leak

1. Hekmatnia A, Dural ectasia in Marfan syndrome. Case study, Radiopaedia.org (Accessed on 26 Jun 2023) https://doi.org/10.53347/rID-73742
2. Paterakis K, Brotis A, Bakopoulou M, Rountas C, Dardiotis E, Hadjigeorgiou GM, Fountas KN, Karantanas A. A Giant Tarlov Cyst Presenting with Hydronephrosis in a Patient with Marfan Syndrome: A Case Report and Review of the Literature. World Neurosurg. 2019 Jun;126:581-587. doi: 10.1016/j.wneu.2019.02.222. Epub 2019 Mar 14. PMID: 30880195.

Search for genetic variants associated with Spontaneous CSF leaks – the genetic basis

- Low effect size
- Low penetrance
- Overall: Fairly common variant, but with enrichment in CSF leak patients

Search for genetic variants associated with Spontaneous CSF leaks – A Case-Control Study

Whole-exome sequencing
49 probands
>5000 controls

Minor Allele
Frequency < 0.01

Missense
Stopgain
Indels
Splice variants

Genes where 10% of probands have rare variants

Gene association with connective tissue phenotypes

Variant list

Analysis conducted by the Mendel project, Sobreira group (Elizabeth Wohler, Dr. Renan Martin)

20% of patients with Type 1b CSF leaks have rare *FBN2* missense variants

- 10/49 probands harbored rare missense *FBN2* variants
- I2394T occurs in 1/2350 people in the general population, but in 8% of unrelated probands with CSF leak

FBN2 variant	No. of patients	Frequency in the general population
p.G475S	1	4.60E-05
p.I2394T	4	2.35E-03
p.A1059T	1	n/a
p.D1588V	1	1.03E-04
p.R347H	1	4.30E-03
p.R195H	1	3.98E-06
p.P37Q	1	3.62E-04

Analysis conducted by Elizabeth Wohler

Burden analysis shows enrichment of rare *FBN2* variants in CSF leak cases vs controls

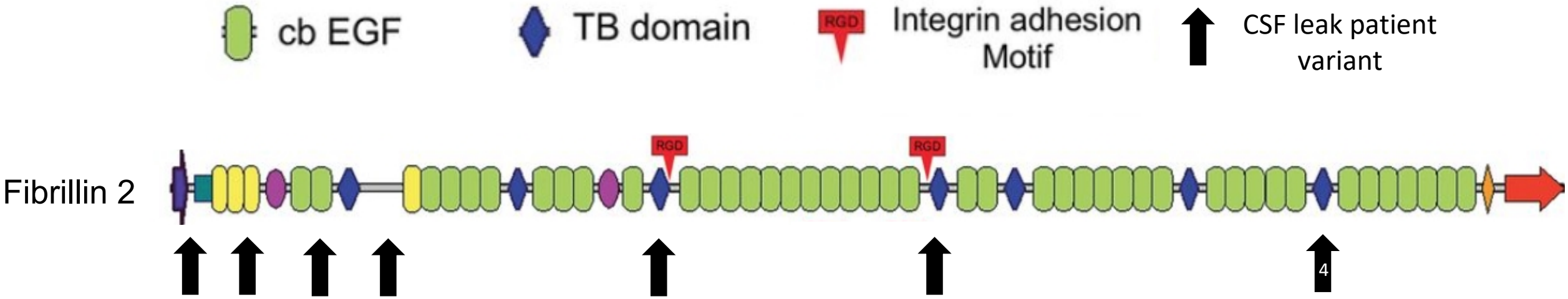
- Variant burden tests compare frequency of rare variants between cases (CSF leak patients) and controls (unaffected individuals)

Control Cohort Name	Number of patients in control cohort	p value (enrichment of rare FBN2 variants in CSF leak cases)
Mendel Initiative (USA)	2244	**0.039**
Belgian Whole-exome cohort	1428	**0.001**
Belgian TAAD cohort	1826	**0.0002**

Analyses by Drs. Silke Peeters and Renan Martin

FBN2 variants in CSF leak patients show nonrandom distribution in the fibrillin-2 protein

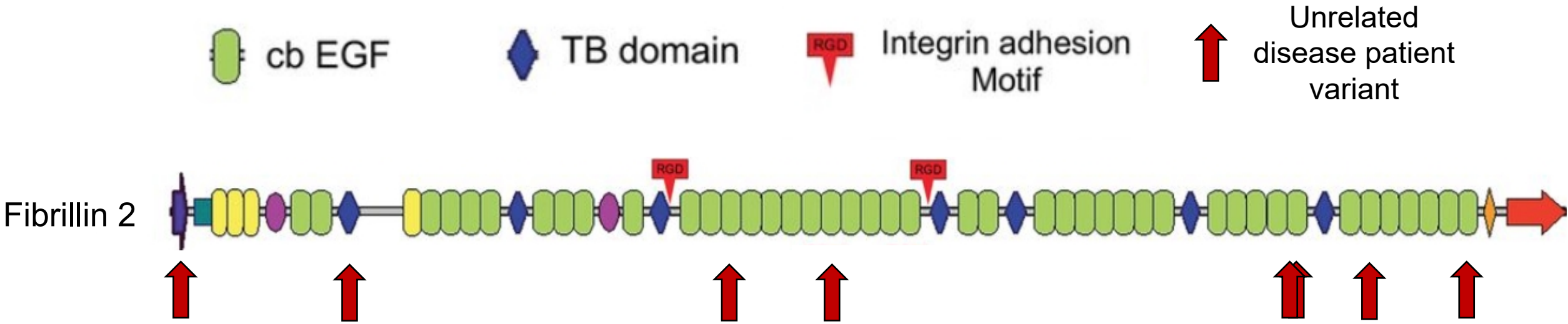
- ~70% of fibrillin 2 is comprised of cbEGF domains
- 90% of variants localize to non-cbEGF regions
- 60% of variants are within 8-cysteine (TB) domains
- 20% of variants lie in or around the 2 integrin-binding domains (RGD)



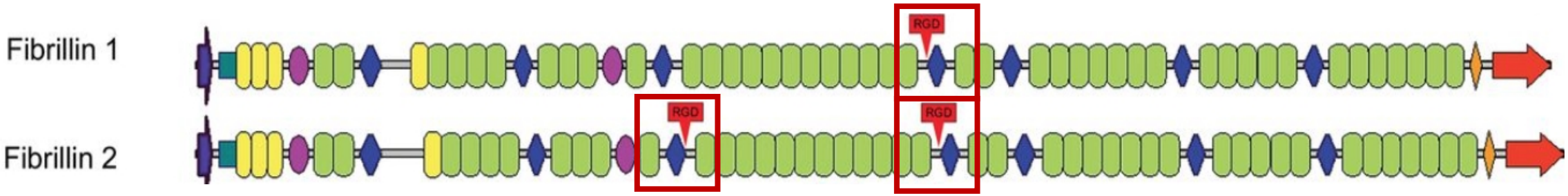
Robertson I, Jensen S, Handford P. TB domain proteins: evolutionary insights into the multifaceted roles offibrillins and LTBPs. Biochem J. 2011 Jan 15;433(2):263-76. doi: 10.1042/BJ20101320. PMID:

FBN2 variants found in a disease unrelated to *FBN2* show the expected random distribution

- ~70% of FBN2 is comprised of cbEGF domains
- 75% of unrelated disease variants localize in cbEGF domains
- 12% of unrelated disease variants localizes in an 8-cysteine (TB) domain
- 0% of unrelated disease variants occur at or near RGD domains



Two CSF leak patient variants localise in and around RGD domains



Extracellular Matrix

Integrin

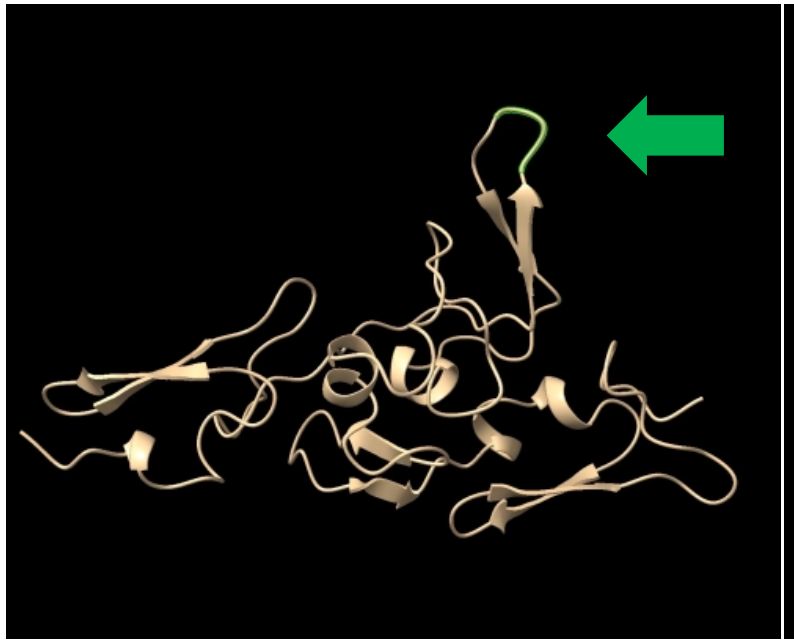
Cell

D1588V

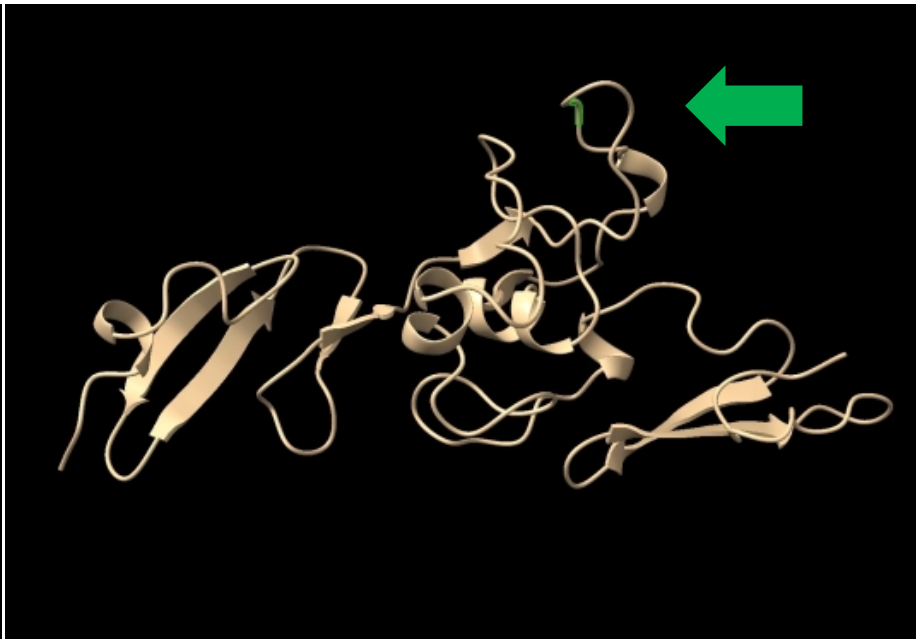
Direct disruption of RGD sequence in TB4
(RGD to RGV) leading to an obligate loss of integrin binding

AI prediction of fibrillin-2 protein structure shows both RGD sequences occur within an exposed loop that is permissive for protein-protein interactions. The recurrent variant in TB7 also occurs in an exposed loop, raising the possibility of a non-RGD integrin binding sequence

TB3



TB4



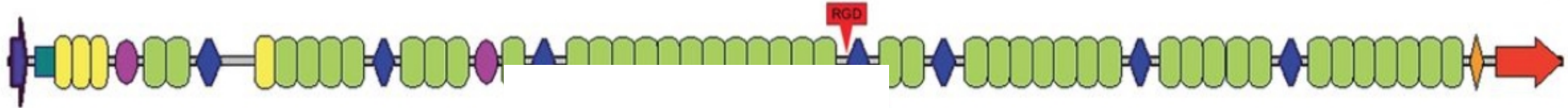
TB7

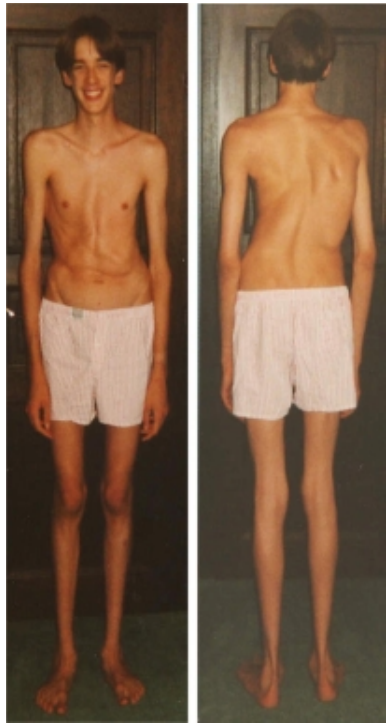
Jumper, J et al. Highly accurate protein structure prediction with AlphaFold. Nature (2021).

Is there precedent for RGD mutations within proteins previously associated with a disease to cause an unexpected (unrelated) condition?

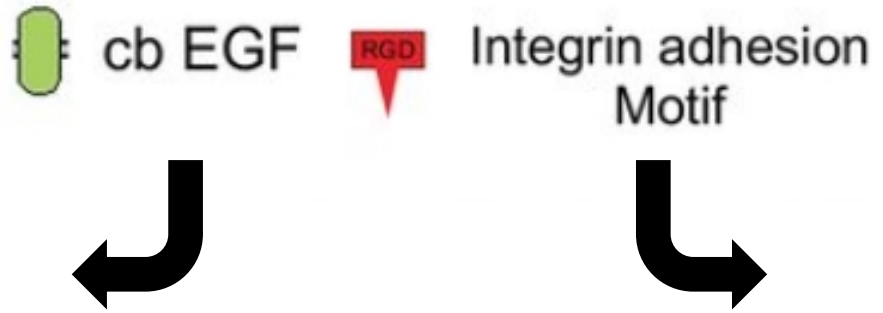
Mutations that disrupt integrin-binding to fibrillin-1 cause a disease that is very different from Marfan syndrome

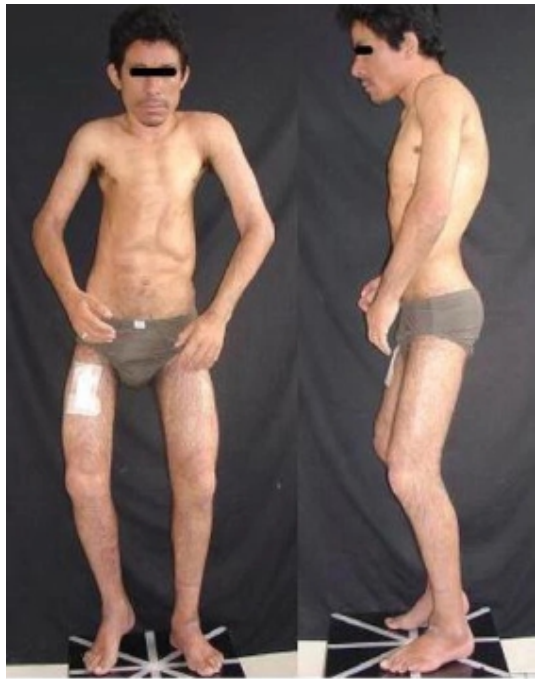
Fibrillin 1





Marfan's Syndrome

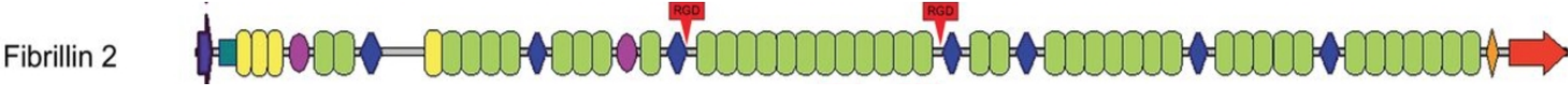


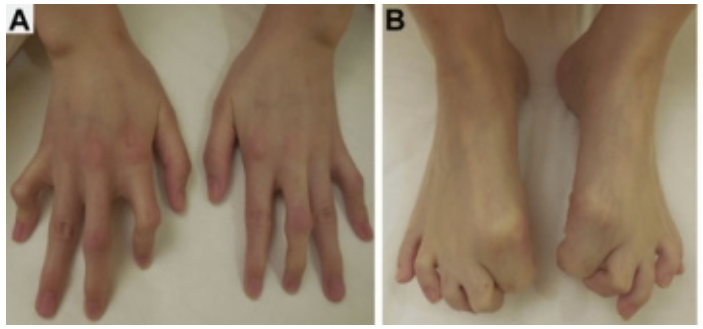


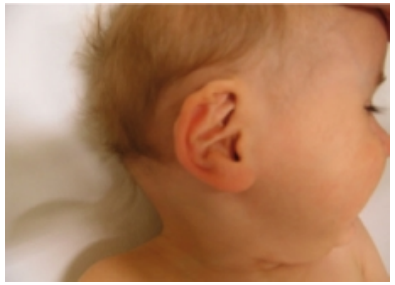
Stiff Skin Syndrome (SSS)

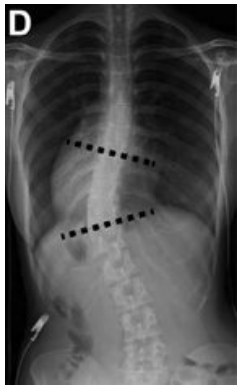
Azevedo, V.F., Serafini, S.Z., Werner, B. *et al.* Stiff skin syndrome versus scleroderma: a report of two cases. *Clin Rheumatol* **28,** 1107–1111 (2009). https://doi-org.proxy1.library.jhu.edu/10.1007/s10067-009-1178-z
Ramirez F., De Backer J. (2019) Marfan Syndrome. In: Oohashi T., Tsukahara H., Ramirez F., Barber C., Otsuka F. (eds) Human Pathobiochemistry. Springer, Singapore. https://doi-org.proxy1.library.jhu.edu/10.1007/

Mutations in cbEGF domains of fibrillin-2 cause a connective tissue disorder called Congenial Contractural Arachnodactyly



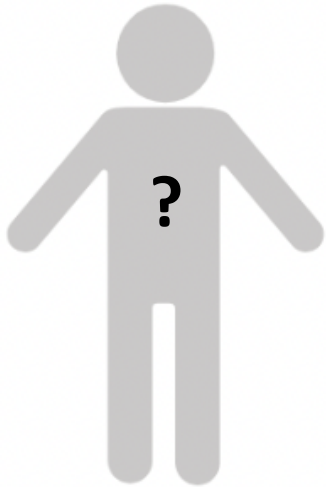






Congenital Contractural Arachnodactyly (CCA)

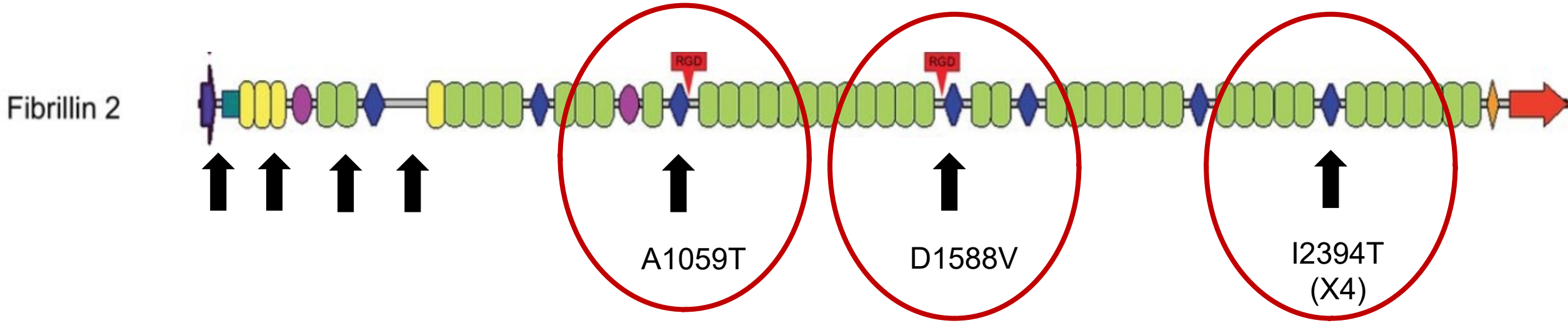
Integrin adhesion Motif

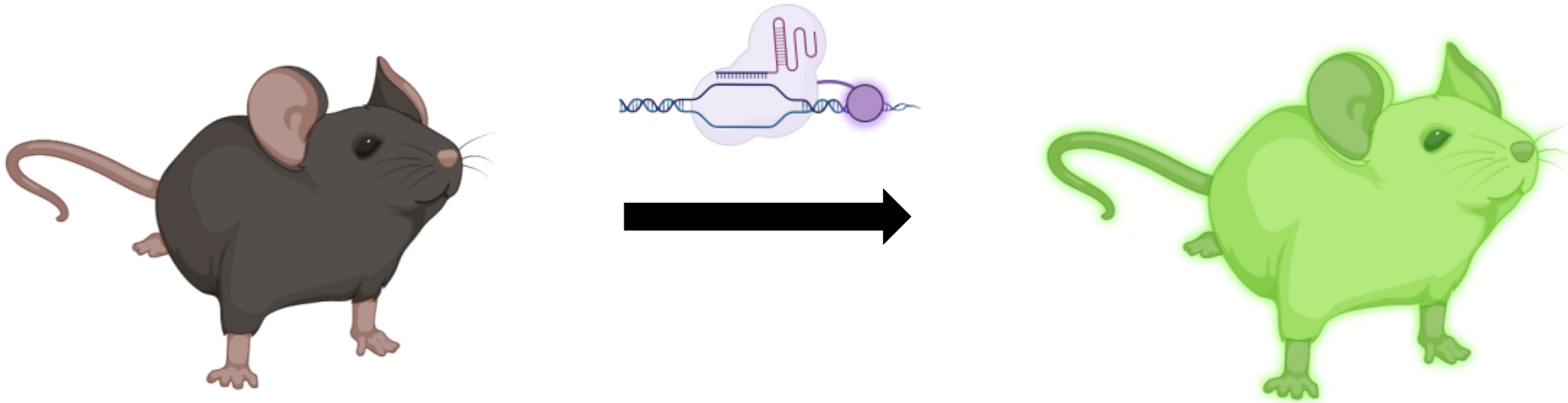


Yagi, Hiroki & Hatano, Masaru & Takeda, Norifumi & Harada, Saori & Suzuki, Yukari & Taniguchi, Yuki & Shintani, Yukako & Morita, Hiroyuki & Kanamori, Norio & Aoyama, Takeshi & Watanabe, Masafumi & Manabe, Ichiro & Akazawa, Hiroshi & Kinugawa, Koichiro & Komuro, Issei. (2015). Congenital Contractural Arachnodactyly without FBN1 or FBN2 Gene Mutations Complicated by Dilated Cardiomyopathy. Internal medicine (Tokyo, Japan). 54. 1237-41. 10.2169/internalmedicine.54.4280.

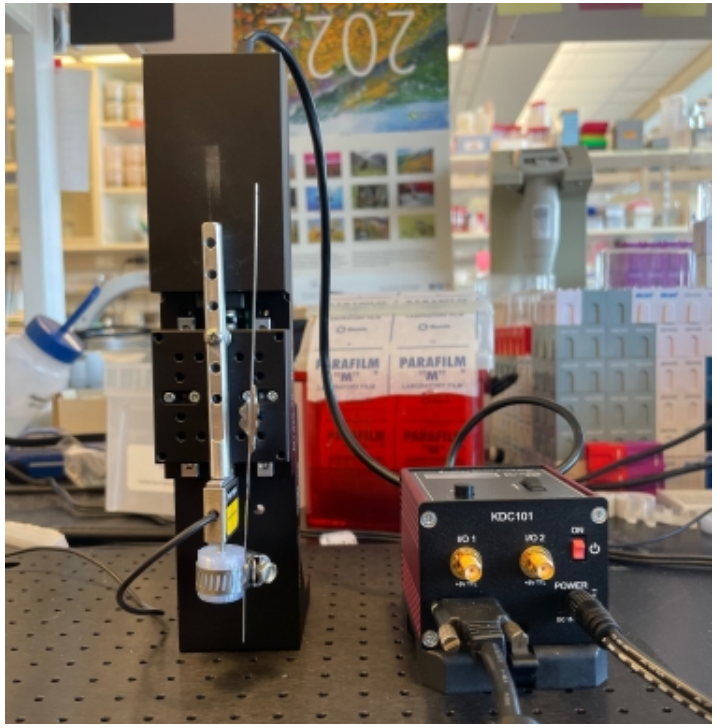
Central hypothesis: Mutations in functionally important domains of *FBN2* lead to altered tissue homeostasis, predisposing patients to dural tears and spontaneous CSF leaks

CRISPR mouse lines created with equivalent patient mutations



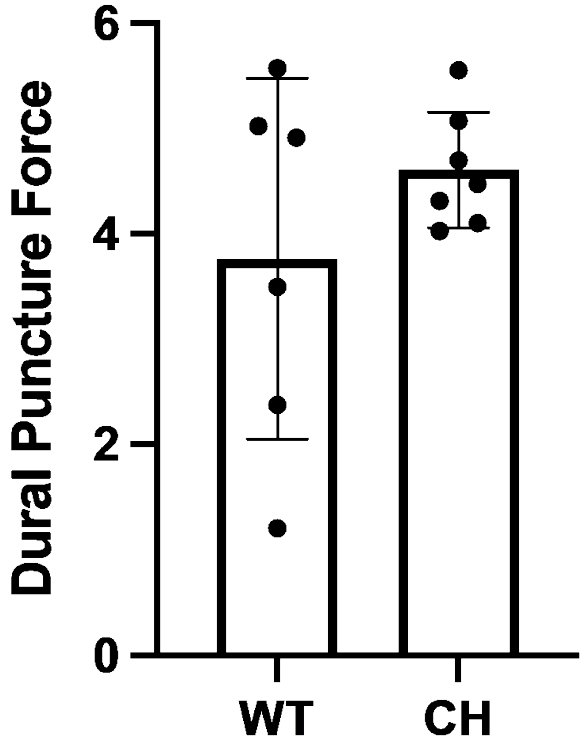


No meaningful difference in cranial dural strength of MFS or *Fbn2* mutant mice by puncture

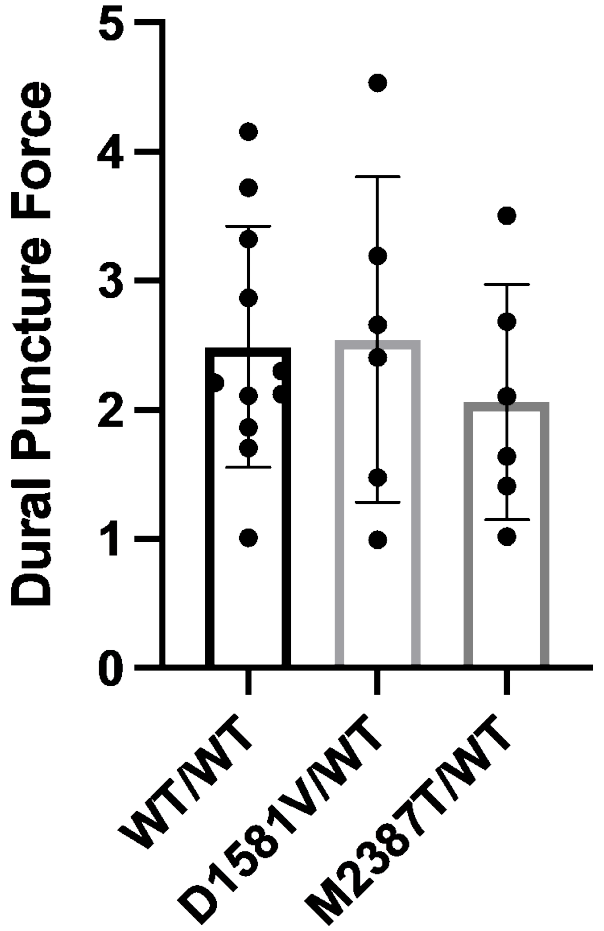


Lacrimal probe attached to force sensor and linear actuator

1 year-old Marfan Mice



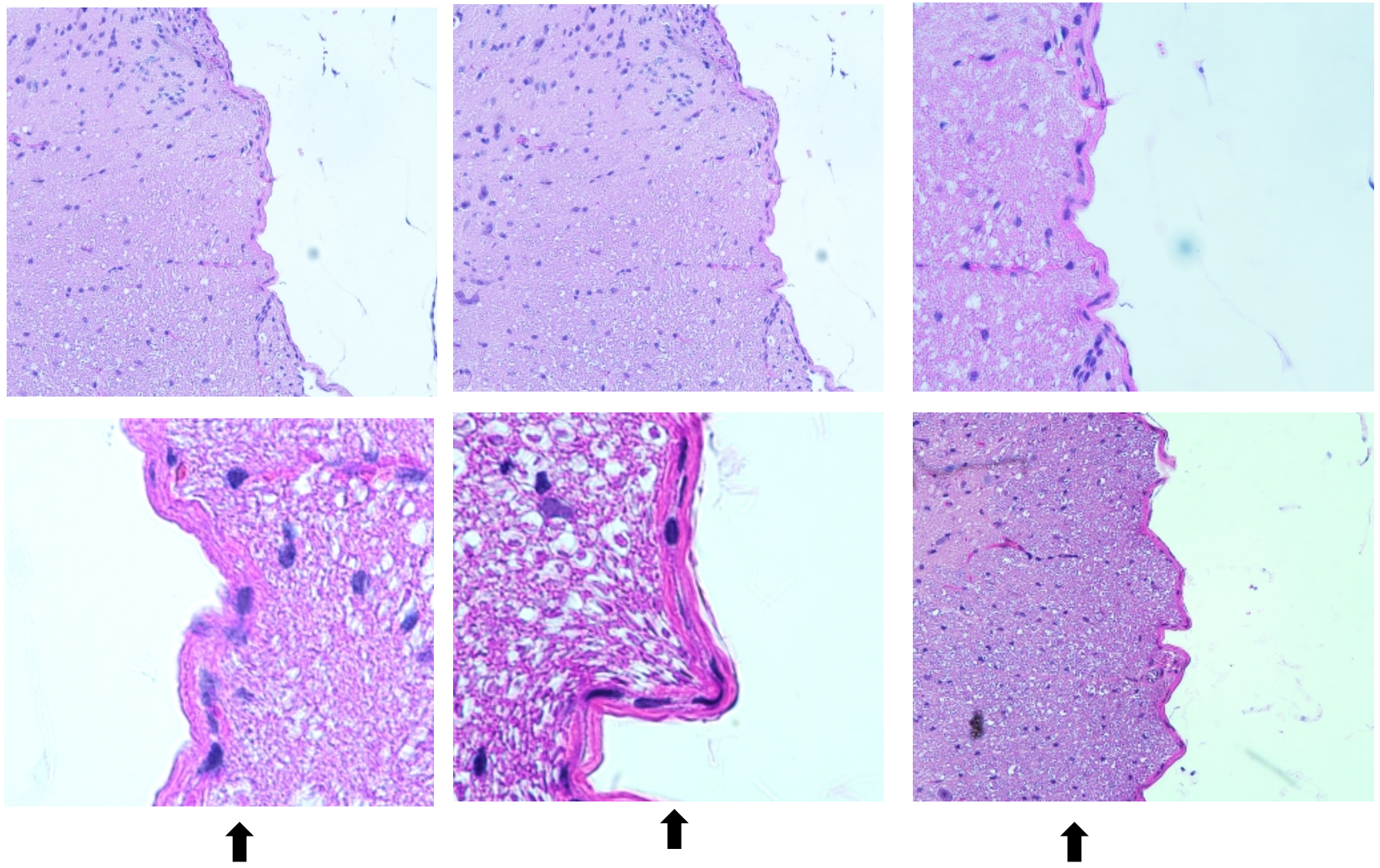
25 week-old *Fbn2* Mutant Mice



H&E shows no difference between WT and FBN2 mutants at various magnifications

WT

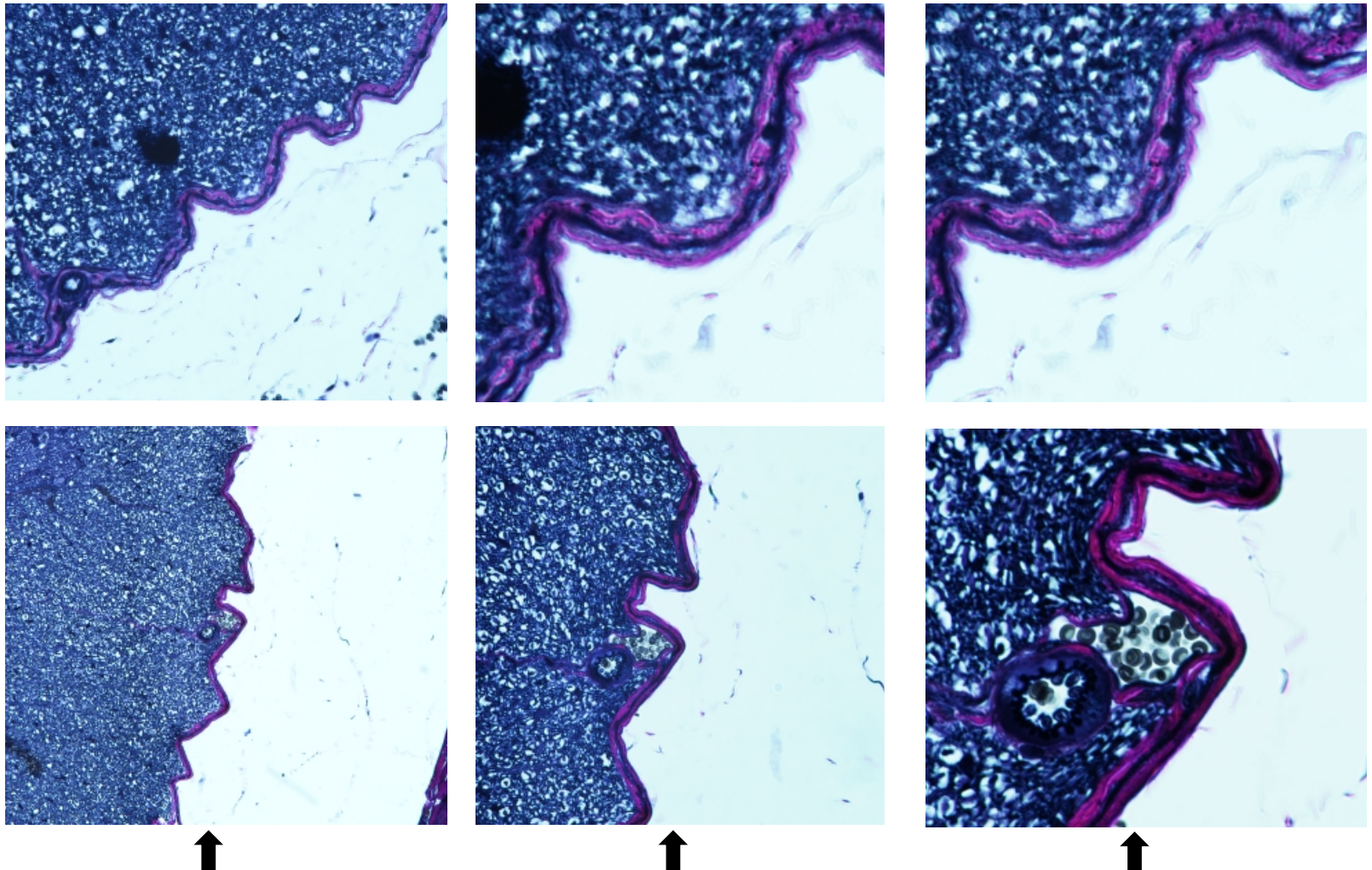
Fbn2$^{D1581V/D1581V}$



VVG shows no difference between WT and FBN2 mutants at various magnifications

WT

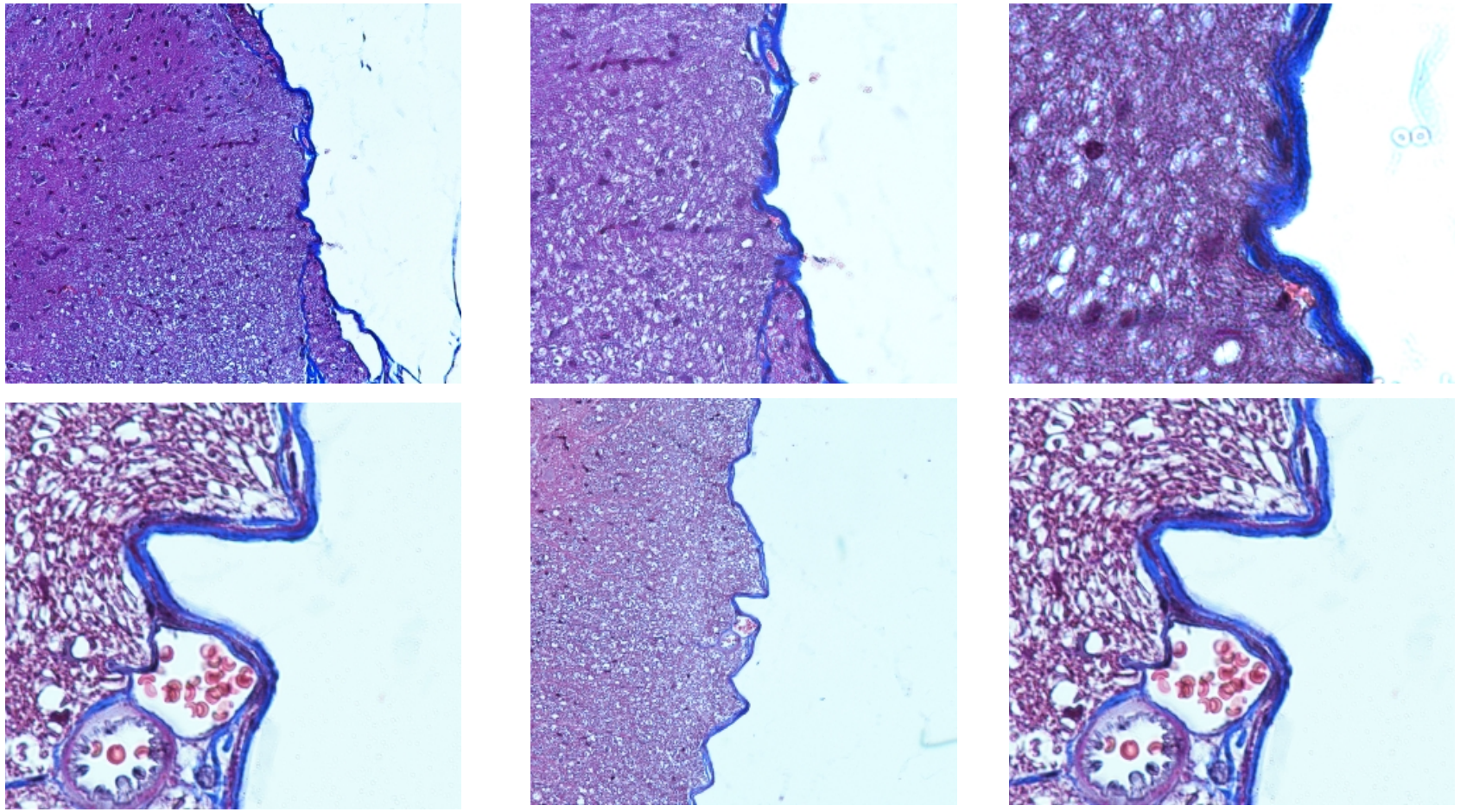
Fbn2$^{D1581V/D1581V}$



Trichrome shows no difference between WT and FBN2 mutants at various magnifications

WT

Fbn2$^{D1581V/D1581V}$



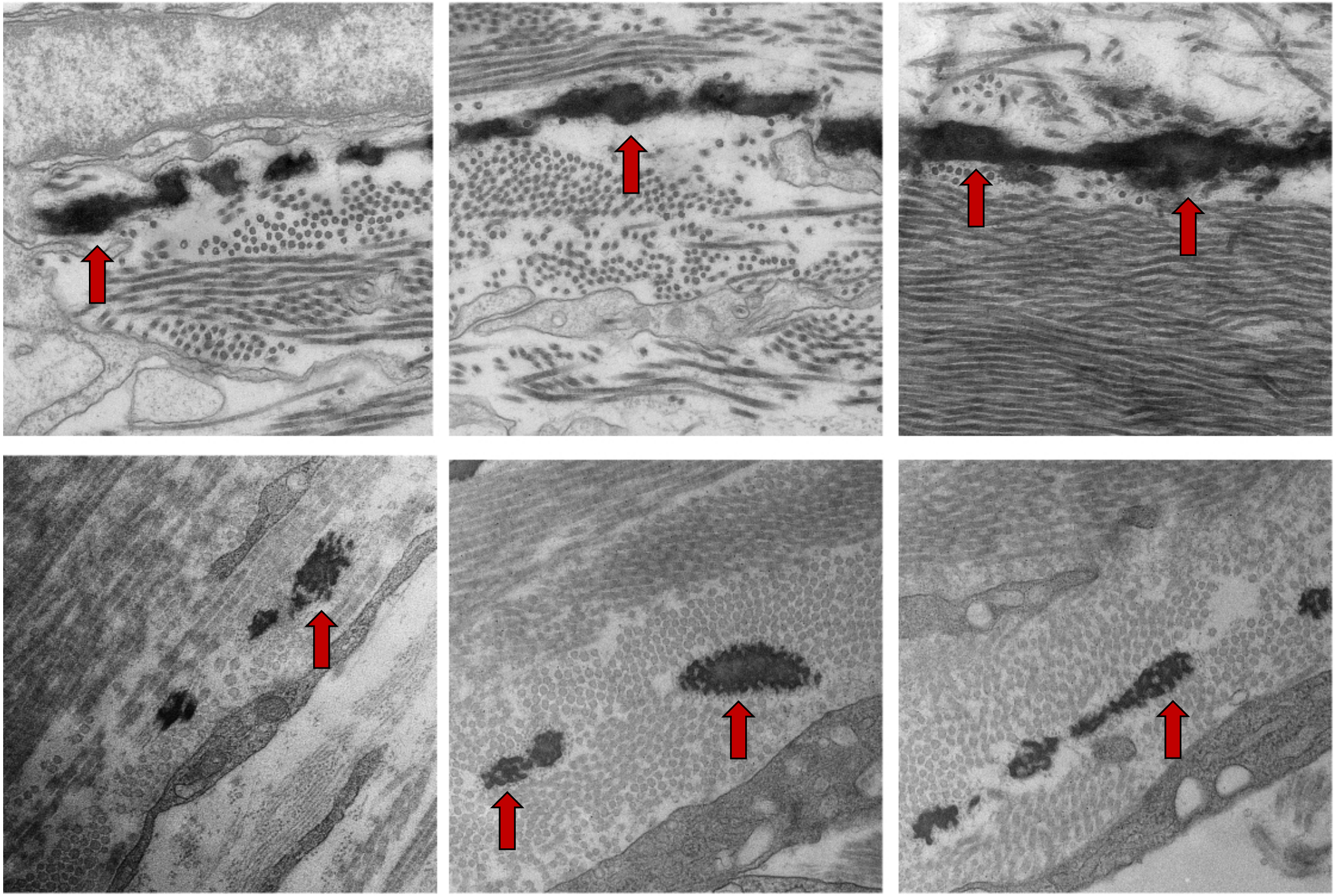
Reinstein E, Pariani M, Bannykh S, Rimoin DL, Schievink WI. Connective tissue spectrum abnormalities associated with spontaneous cerebrospinal fluid leaks: a prospective study.Eur J Hum Genet. 2013 Apr;21(4):386-90. doi: 10.1038/ejhg.2012.191. Epub 2012 Aug 29. PMID: 22929030; PMCID: PMC3598315.

TEM

22,000x

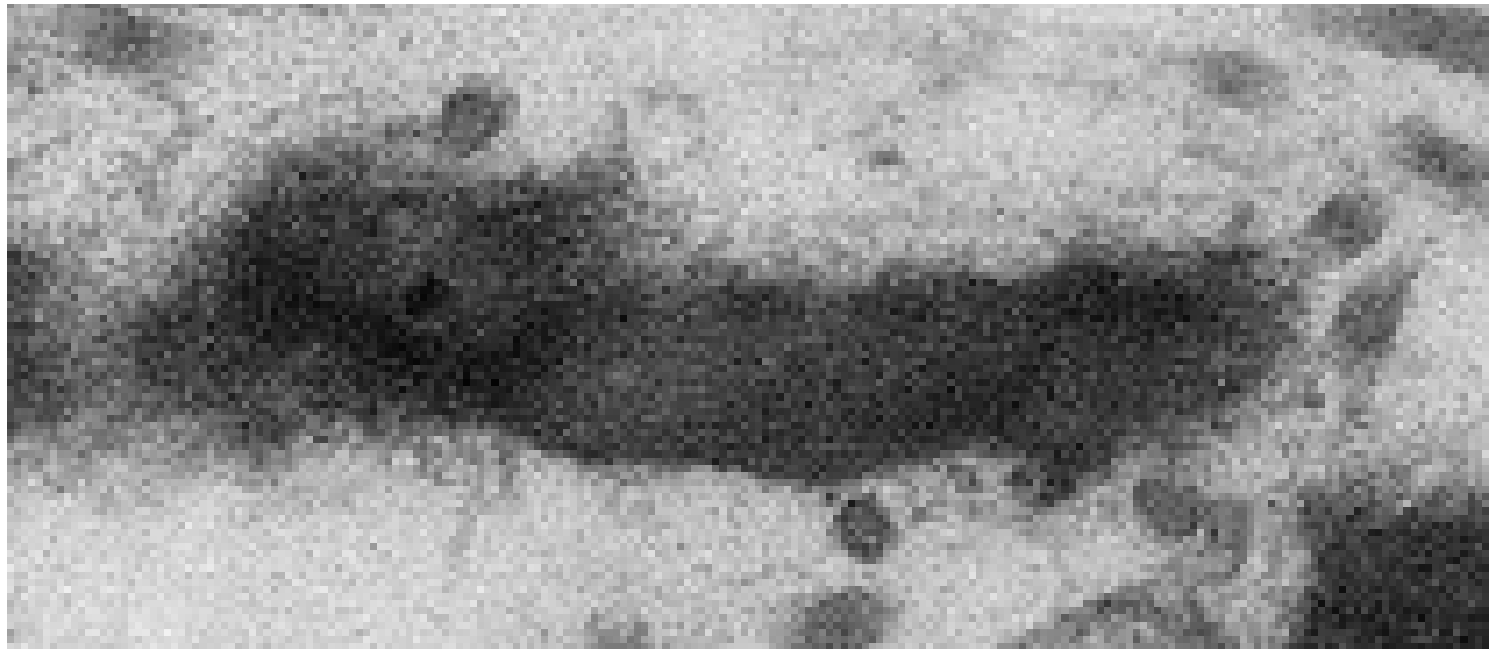
WT

Fbn2$^{A1052T/A1052T}$

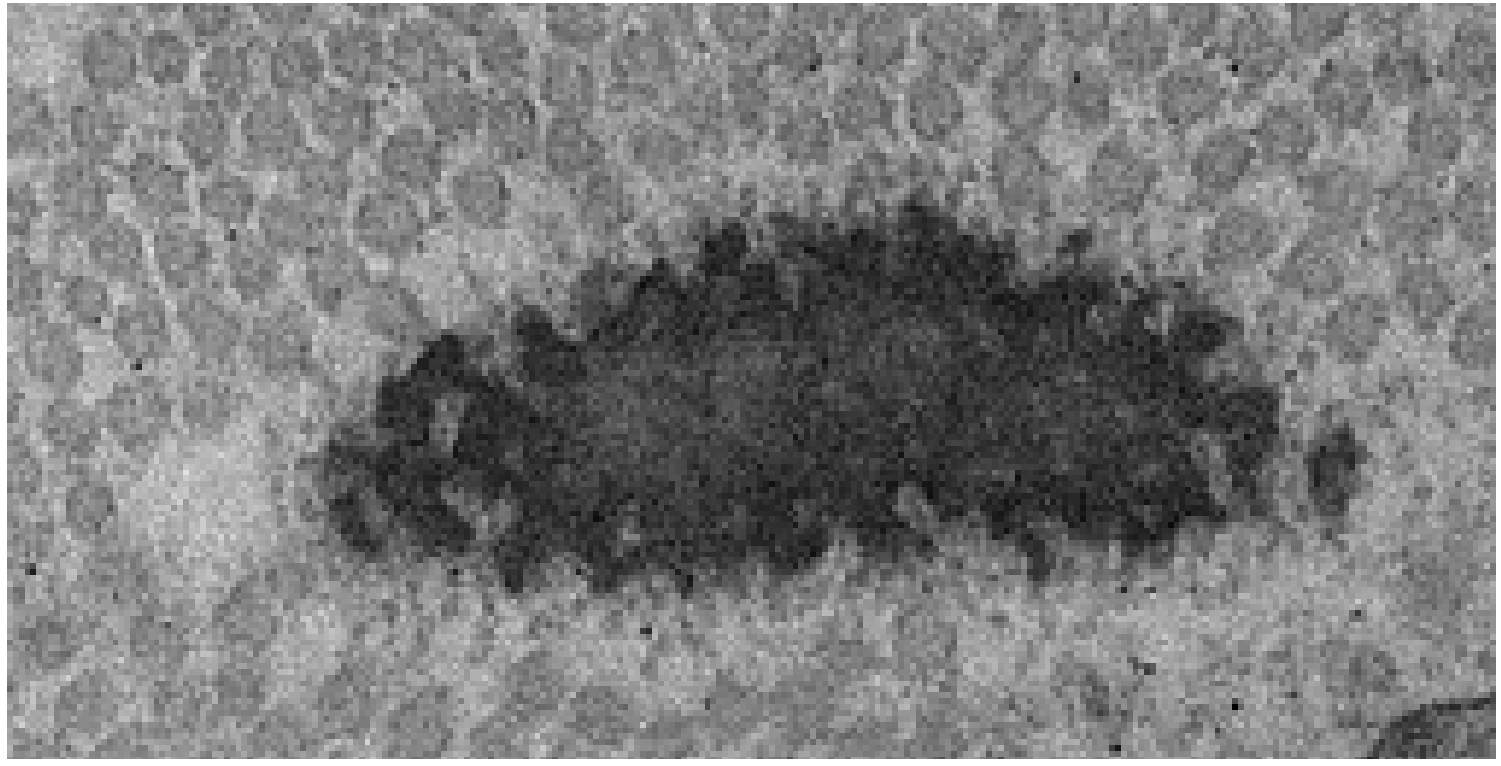


TEM

WT

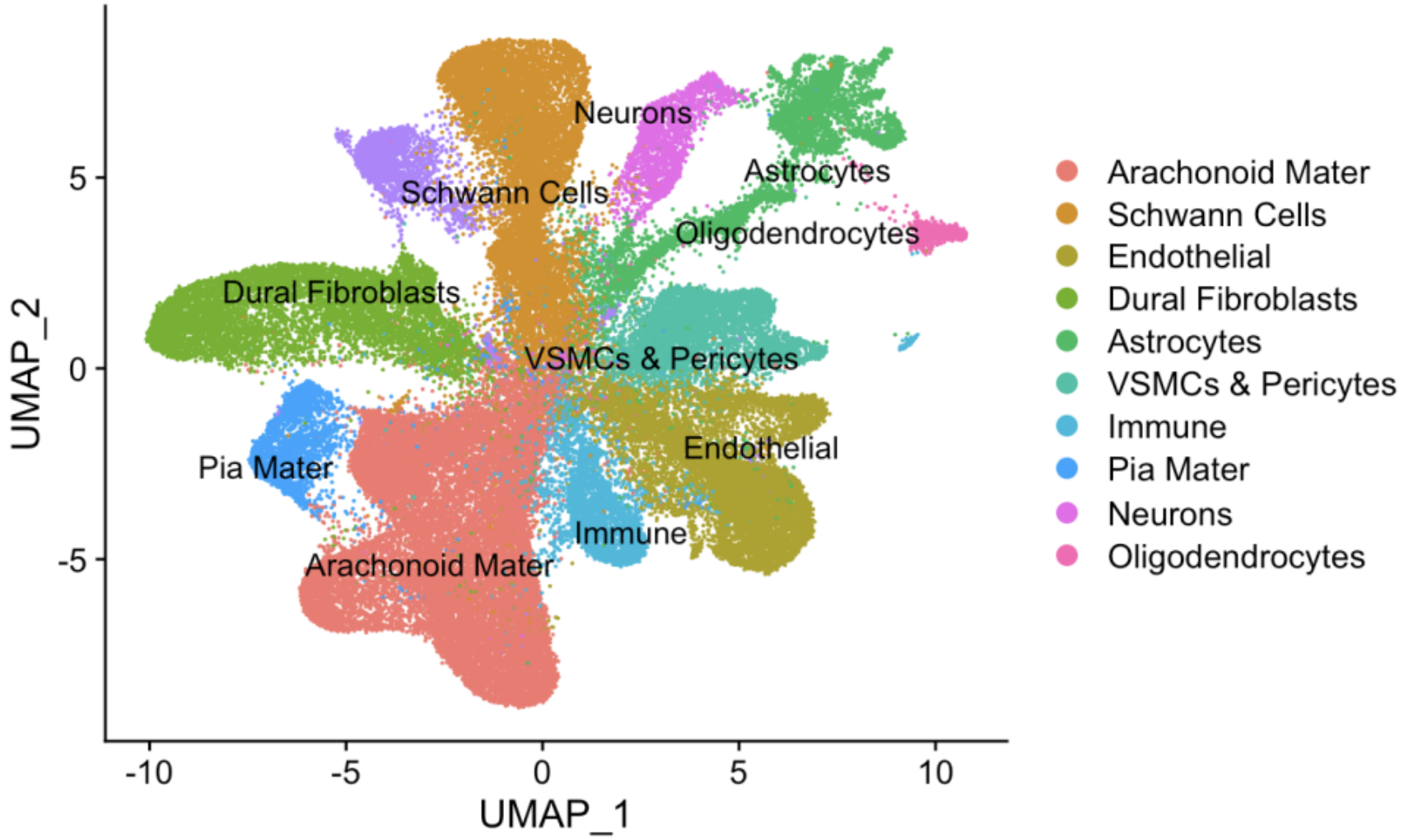


Fbn2$^{A1052T/A1052T}$

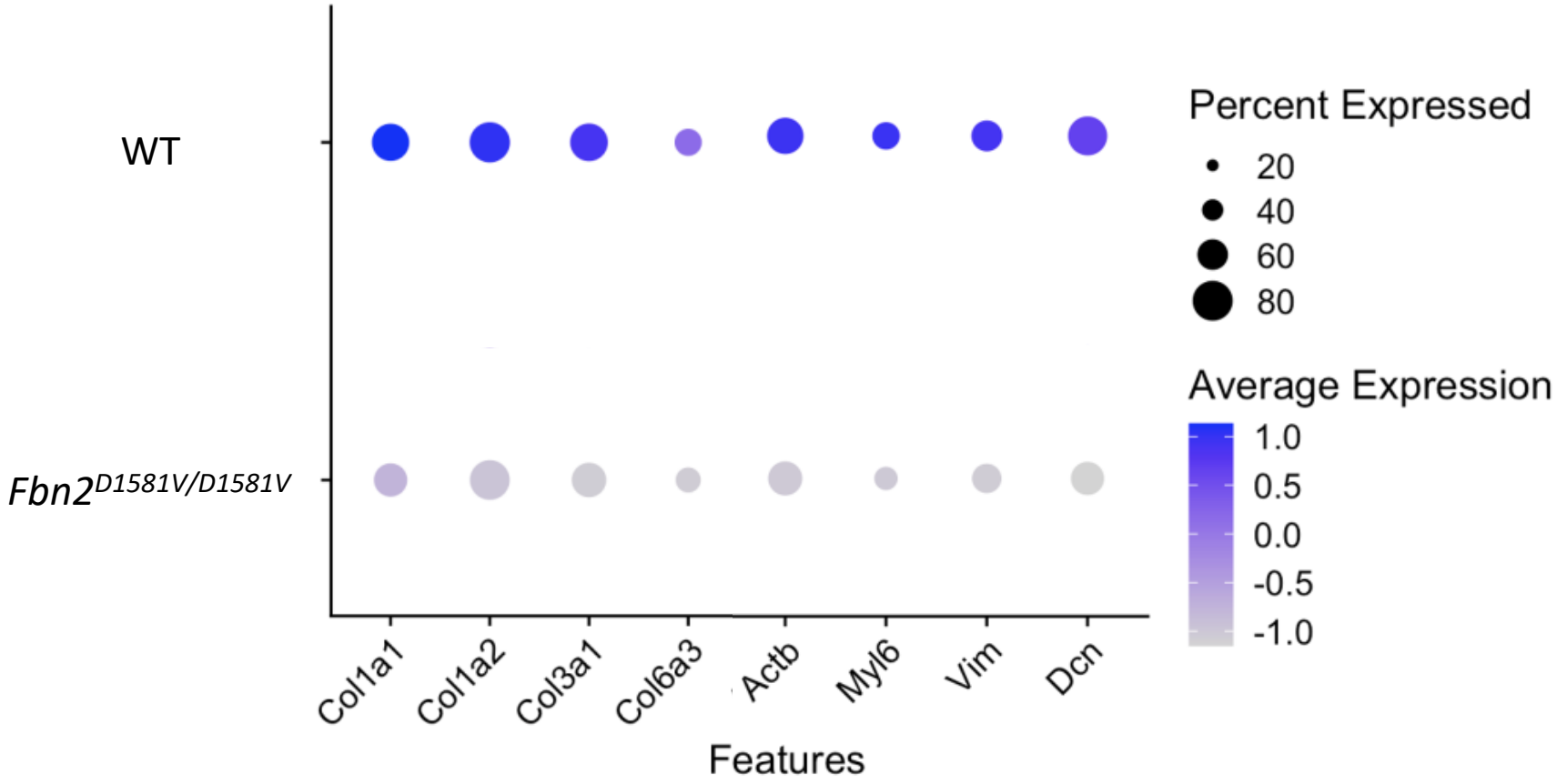


Single-nuclear RNA-sequencing

Single-nuclear RNAseq of mouse spinal dura identifies all expected cell types



Differential expression of dural fibroblasts in WT vs *Fbn2* mutant suggests decreased ECM synthesis

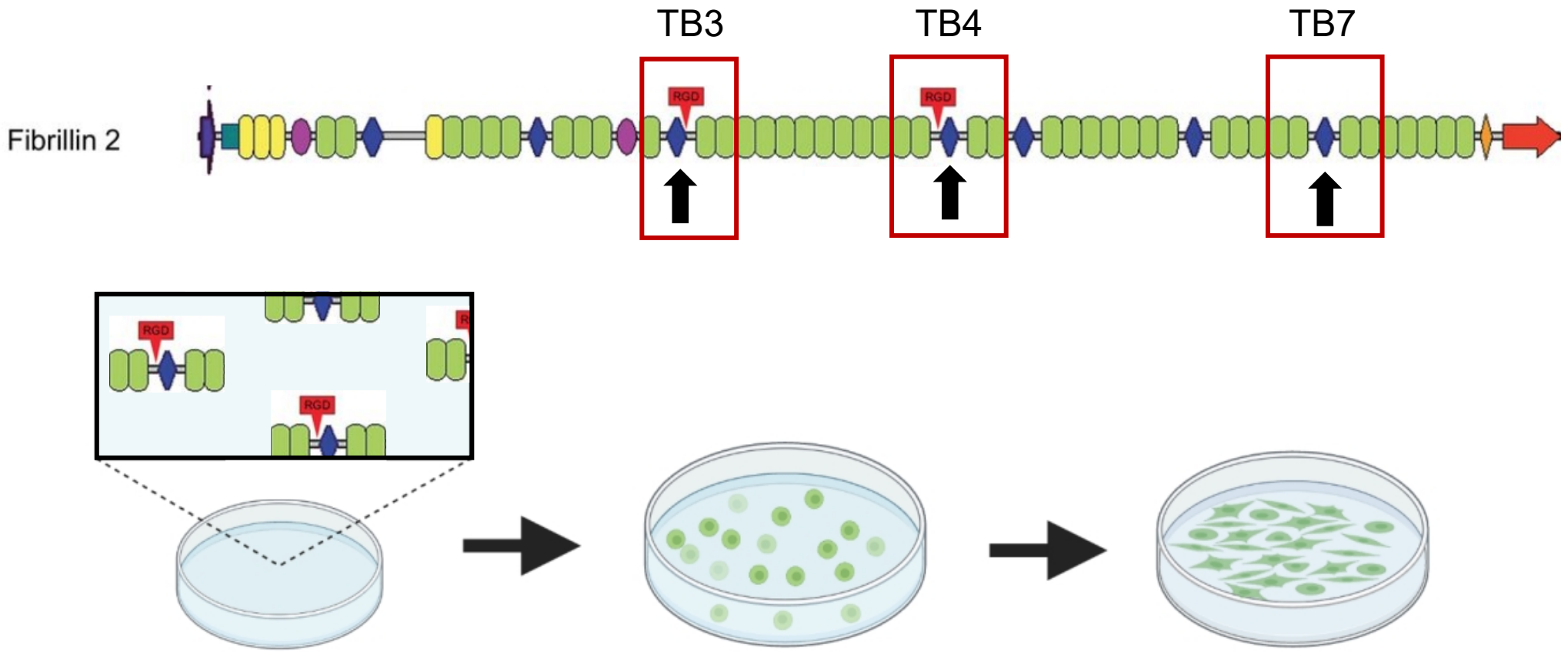


All corrected p<0.05 using MAST DE

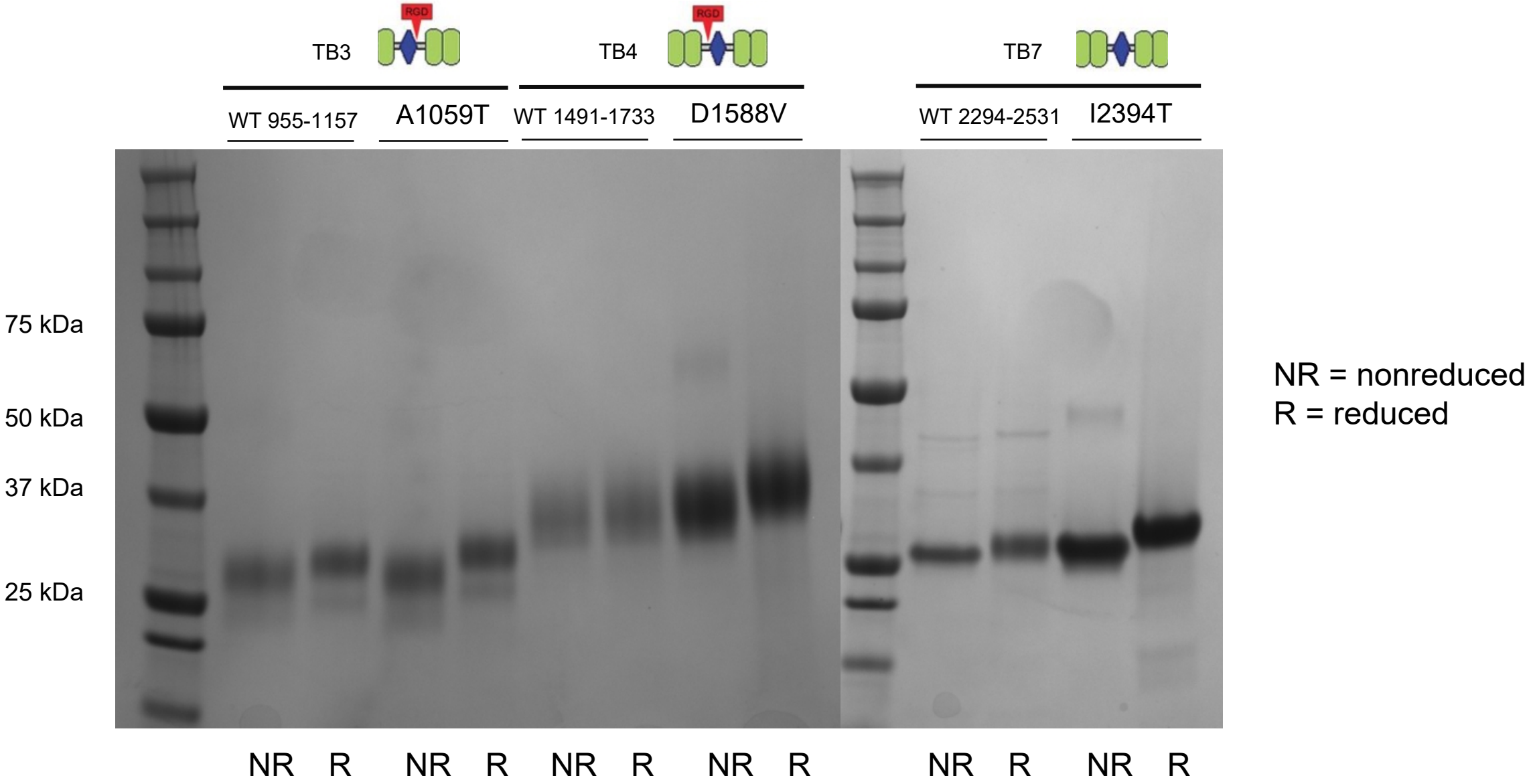
Mechanistic hypothesis: Disruption of integrin-mediated interaction between meningeal cells and fibrillin-2 impairs dural integrity

In vitro assays: dural fibroblast adhesion to fibrillin-2 fragments

In vitro fibrillin-2 binding assays will determine whether fibrillin-2 has novel binding sites altered by CSF leak patient mutations

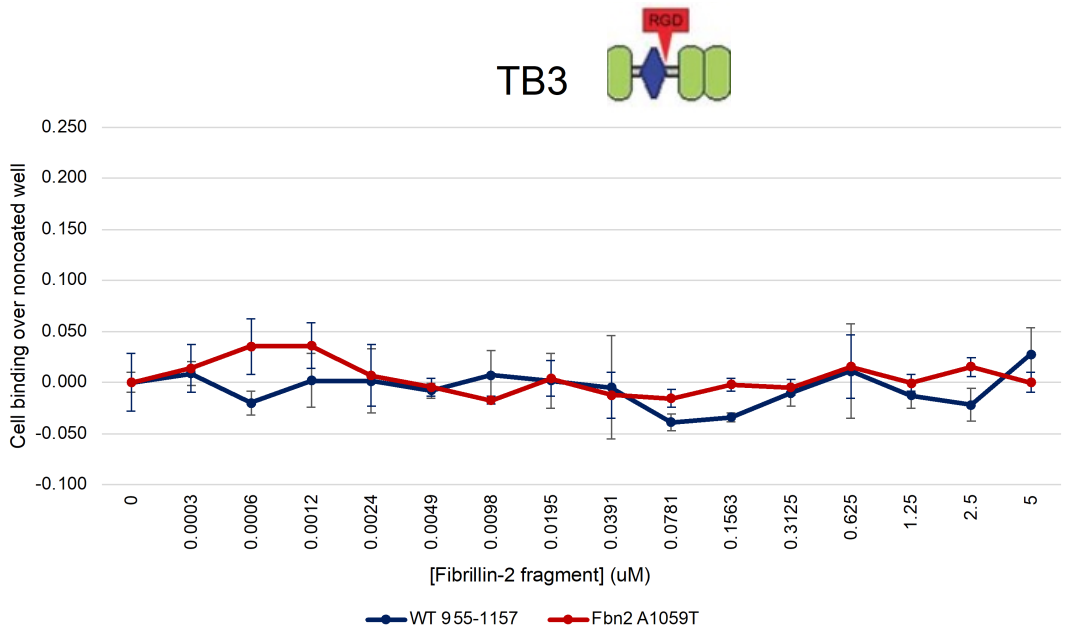


Robust expression of recombinant fibrillin-2 peptides

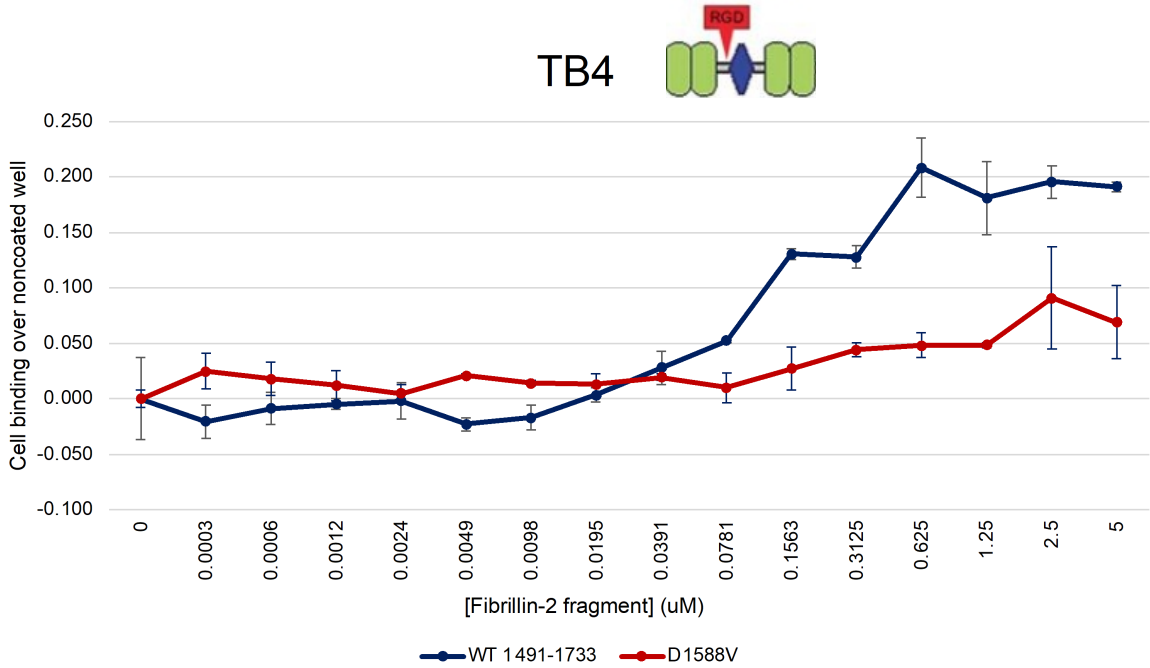


NR = nonreduced
R = reduced

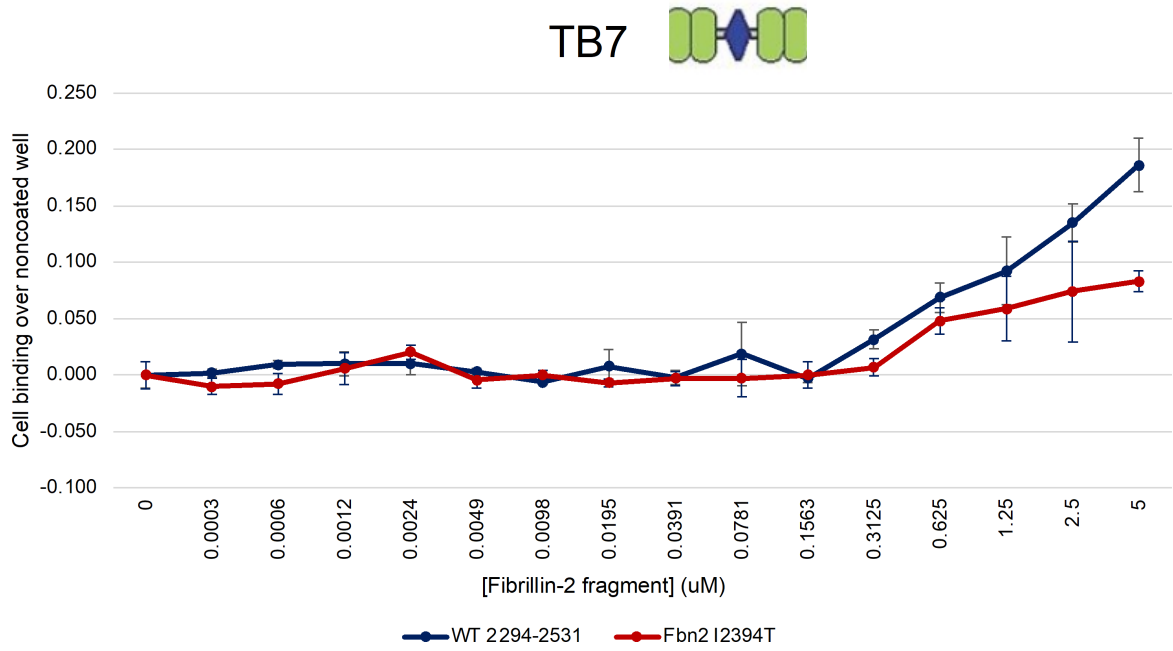
TB3


RGD
0.250
0.200
0.150
0.100
0.050
0.000
-0.050
-0.100
Cell binding over noncoated well
0
0.0003
0.0006
0.0012
0.0024
0.0049
0.0098
0.0195
0.0391
0.0781
0.1563
0.3125
0.625
1.25
2.5
5
[Fibrillin-2 fragment] (uM)
WT 955-1157
Fbn2 A1059T

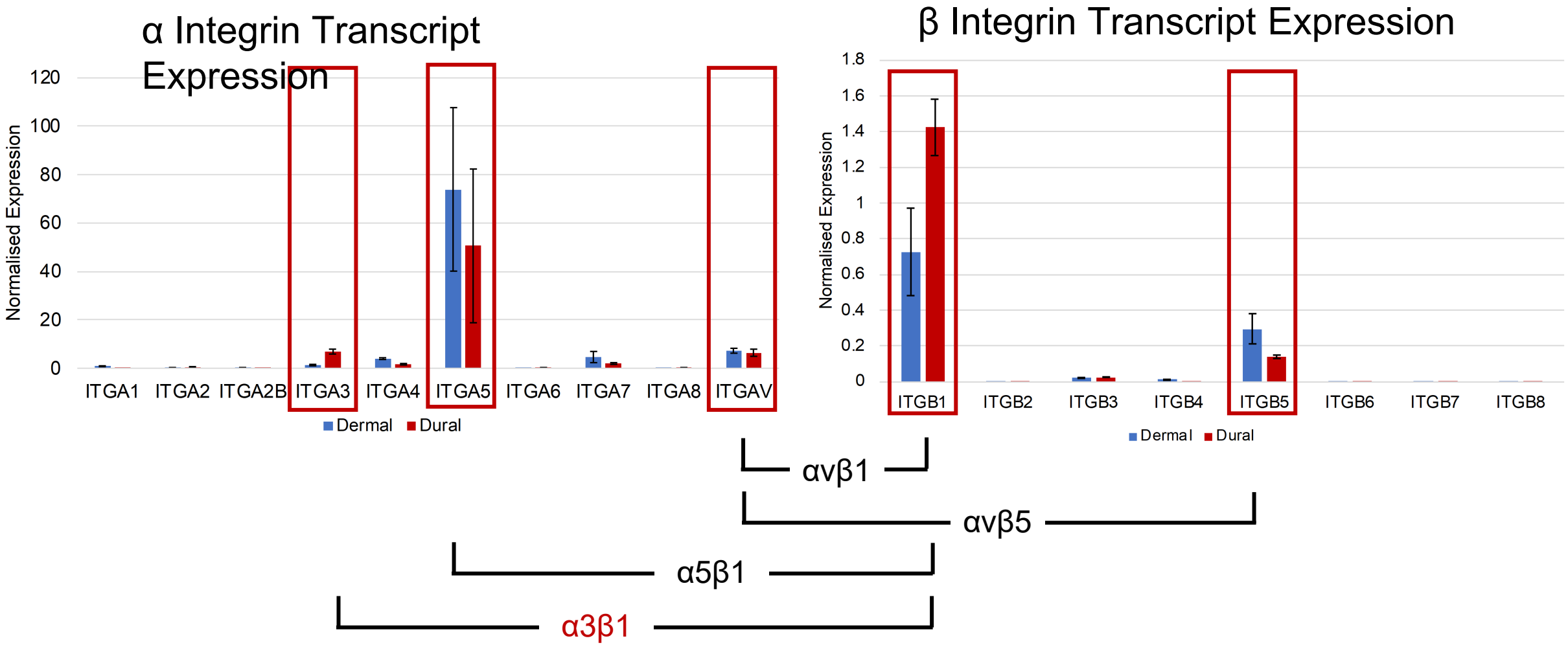
TB4


RGD
0.250
0.200
0.150
0.100
0.050
0.000
-0.050
-0.100
Cell binding over noncoated well
0
0.0003
0.0006
0.0012
0.0024
0.0049
0.0098
0.0195
0.0391
0.0781
0.1563
0.3125
0.625
1.25
2.5
5
[Fibrillin-2 fragment] (uM)
WT 1491-1733
D1588V

TB7


0.250
0.200
0.150
0.100
0.050
0.000
-0.050
-0.100
Cell binding over noncoated well
0
0.0003
0.0006
0.0012
0.0024
0.0049
0.0098
0.0195
0.0391
0.0781
0.1563
0.3125
0.625
1.25
2.5
5
[Fibrillin-2 fragment] (uM)
WT 2294-2531
Fbn2 I2394T

Human Dural Fibroblasts express RGD-binding Integrins



Conclusions

There is enrichment for rare and functionally consequential DNA sequence variants in patients with Type 1b spinal CSF leaks.

The data suggest that loss of cell-fibrillin-2 contacts impairs matrix synthesis and integrity.

Future mechanistic studies may inform therapeutic strategies.

Many Thanks To:

Dietz Lab

Hal Dietz
Emily Juzwiak
Anthony Zeng
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