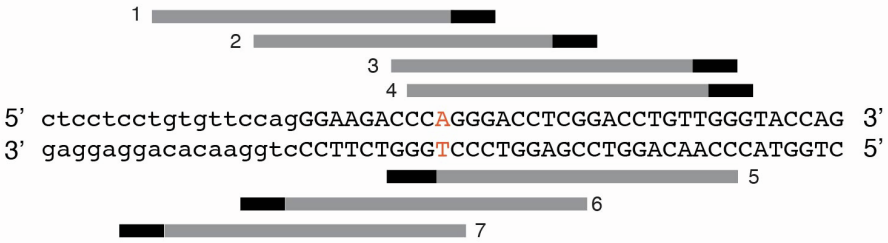
OMTN, Volume *35*

Supplemental information

Allele-specific CRISPR-Cas9 editing inactivates a single nucleotide variant associated with collagen VI muscular dystrophy

Véronique Bolduc, Katherine Sizov, Astrid Brull, Eric Esposito, Grace S. Chen, Prech Uapinyoying, Apurva Sarathy, Kory R. Johnson, and Carsten G. Bönnemann

A



B

ID	Target / PAM	Strand	Broad efficacy score	Broad combined rank	MIT specificity score (0-100)	Number Off-targets (0-1-2 mismatches)	Location of variant vs PAM
5*	CCAACAGGTCCGAGGTCCCT/GGG	(-)	-0.03	3	63	0-1-0	proximal
4	CCAGGGACCTCGGACCTGTT/GGG	(+)	-0.26	7	47	0-1-0	distal
3	CCCAGGGACCTCGGACCTGT/TGG	(+)	-0.21	4	44	0-1-1	distal
1**	tgtgttccagGGAAGACCCA/GGG	(+)	0.86	1	43	0-2-6	proximal
6	CGAGGTCCCTGGGTCTTCCc/tgg	(-)	-0.03	5	39	0-2-0	distal
7	CTGGGTCTTCCctggaacac/agg	(-)	0.11	6	35	0-1-10	distal
2	cagGGAAGACCCAGGGACCT/CGG	(+)	0.13	2	32	0-2-6	proximal

*gRNA-B **gRNA-A

C

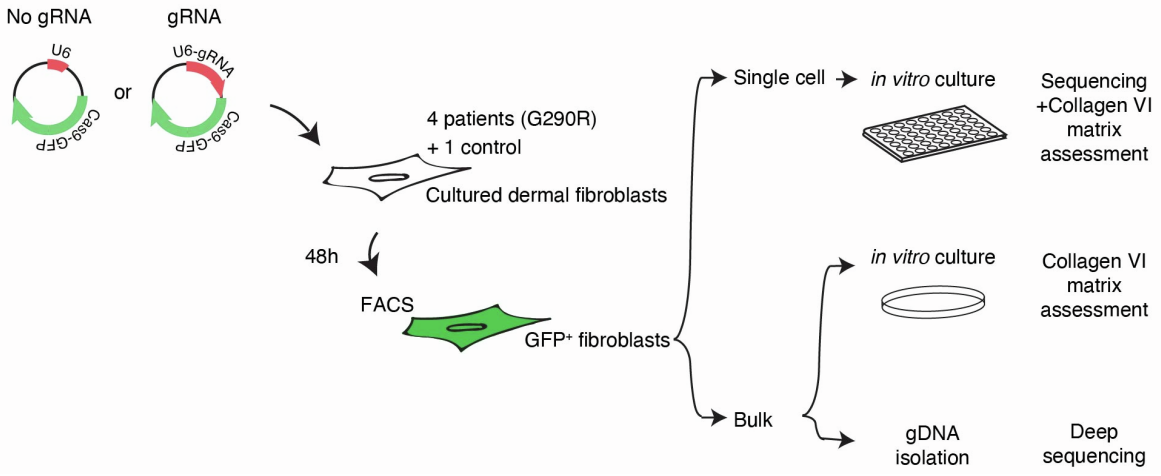


Figure S1. Strategy for allele-specific gene editing of the *COL6A1* c.868G>A (G290R) variant.

(A) The localization of potential allele-specific gRNAs for the c.868G>A variant is illustrated along the *COL6A1* genomic sequence. gRNAs hybridizing to the negative (-) strand are shown below, while gRNAs hybridizing to the positive (+) strand are shown above the sequence. **(B)** The sequences of all potential gRNAs are listed, together with the sequences of the adjacent PAM sites.

The presence of the mutation within a 10-nt distance of the PAM site was considered proximal. ID 1 was chosen as gRNA-A and ID 5 was chosen as gRNA-B. **(C)** Schematics of the experimental procedures followed in the study. Note that all experiments were conducted on GFP^{+}-sorted cells (enriching for cells transfected with the gene editing constructs).

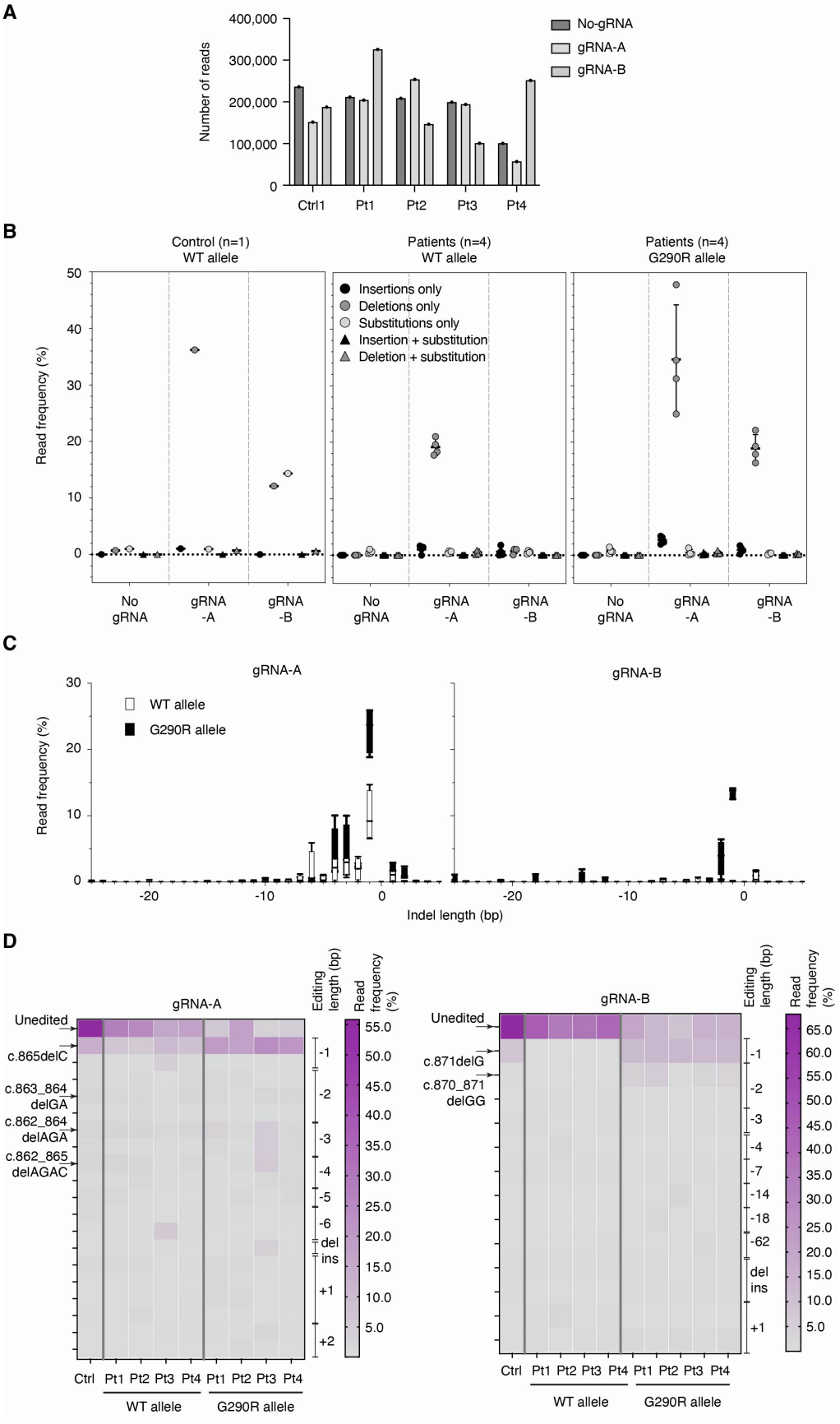

A
Number of reads
400,000
300,000
200,000
100,000
0
No-gRNA
gRNA-A
gRNA-B
Ctrl1
Pt1
Pt2
Pt3
Pt4
B
Control (n=1)
WT allele
Patients (n=4)
WT allele
Patients (n=4)
G290R allele
Read frequency (%)
Insertions only
Deletions only
Substitutions only
Insertion + substitution
Deletion + substitution
No gRNA
gRNA -A
gRNA -B
C
gRNA-A
gRNA-B
WT allele
G290R allele
Read frequency (%)
Indel length (bp)
D
gRNA-A
gRNA-B
Editing length (bp)
Read frequency (%)
Unedited
c.865delC
c.863_864 delGA
c.862_864 delAGA
c.862_865 delAGAC
c.871delG
c.870_871 delGG
del
ins
Ctrl
Pt1 Pt2 Pt3 Pt4
WT allele
G290R allele

Figure S2. Targeted re-sequencing analysis of the repair outcomes after gene editing with gRNA-A or gRNA-B.
Analysis of Illumina Mi-Seq sequencing reads for four patient and one control primary cells co-transfected with Cas9-GFP and with either gRNA-A, gRNA-B, or without gRNA (No gRNA). **(A)** Total number of sequencing reads, per sample. The total number of reads was subsequently used as the denominator to calculate the read frequency in each sample. **(B)** Total read frequencies per indel/edit type (deletions, insertions, substitutions, or combination thereof) and per allele type (WT or G290R). Bars represent average ± standard deviation. **(C)** Total read frequencies per indel length, and per allele type, reported as box and whisker plots, for gRNA-A (left) and gRNA-B (right). On the x axes, the negative scale represents length of deletions, while the positive site represents length of insertions. **(D)** Heatmaps of the top 20 motifs for their total read frequencies, identified at the G290R allele in patient samples, and displayed by individual.

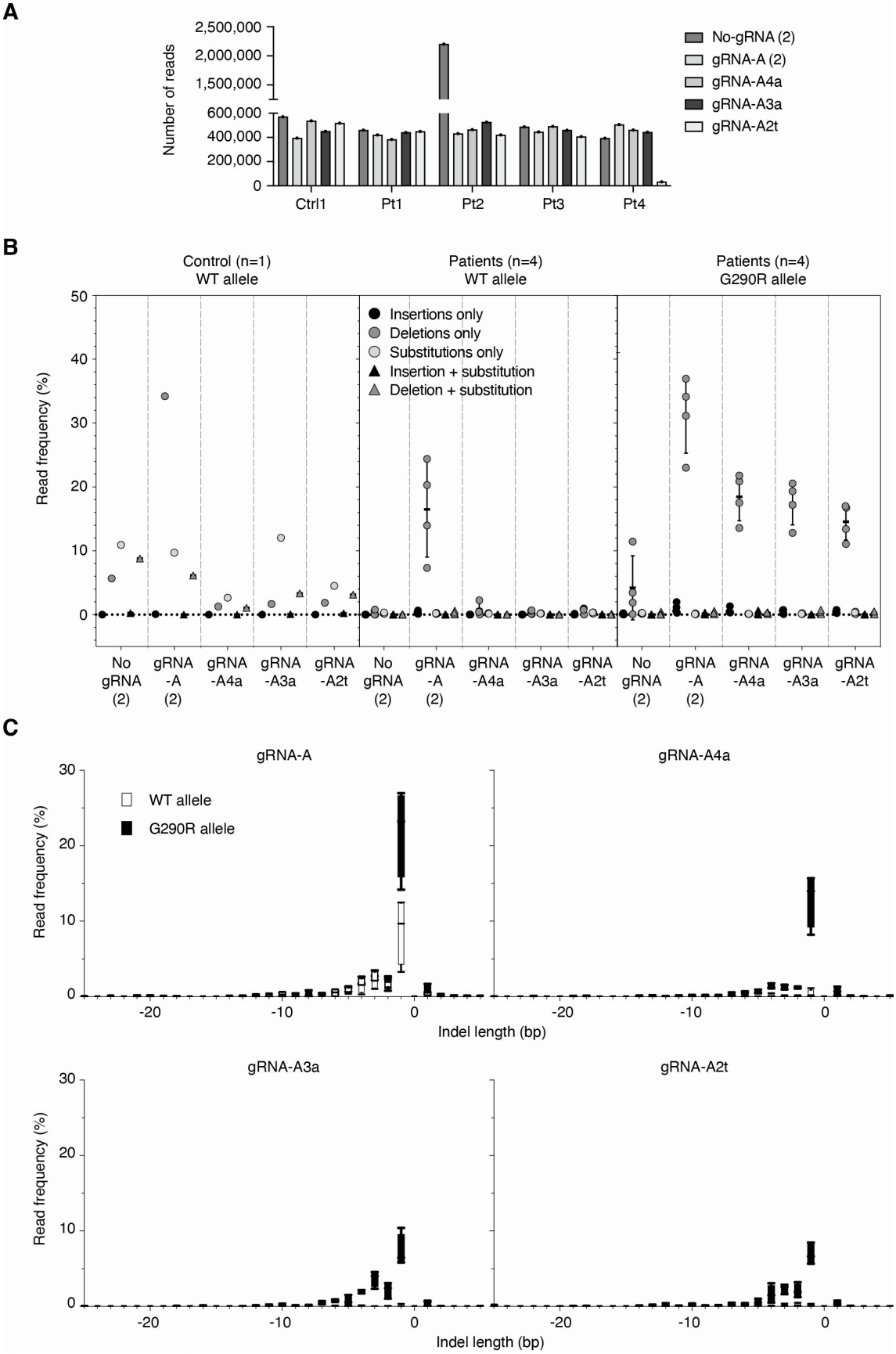

A
Number of reads
2,500,000
2,000,000
1,500,000
600,000
400,000
200,000
0
Ctrl1
Pt1
Pt2
Pt3
Pt4
No-gRNA (2)
gRNA-A (2)
gRNA-A4a
gRNA-A3a
gRNA-A2t
B
Control (n=1)
WT allele
Patients (n=4)
WT allele
Patients (n=4)
G290R allele
Read frequency (%)
Insertions only
Deletions only
Substitutions only
Insertion + substitution
Deletion + substitution
No gRNA (2)
gRNA -A (2)
gRNA -A4a
gRNA -A3a
gRNA -A2t
C
gRNA-A
gRNA-A4a
gRNA-A3a
gRNA-A2t
WT allele
G290R allele
Read frequency (%)
Indel length (bp)

Figure S3. Targeted re-sequencing analysis of the repair outcomes after gene editing with gRNA-A4A, gRNA-A3A or gRNA-A2T.
Analysis of Illumina Mi-Seq sequencing reads for four patient and one control primary cells co-transfected with Cas9-GFP and with either gRNA-A, gRNA-A4a, gRNA-A3a, gRNA-A2t, or without gRNA (No gRNA). Note that gRNA-A and No gRNA in this experiment are replicates from the previous experiment. **(A)** Total number of sequencing reads, per sample. The total number of reads was subsequently used as the denominator to calculate the read frequency in each sample. **(B)** Total read frequencies per indel/edit type (deletions, insertions, substitutions, or combination thereof) and per allele type (WT or G290R). Bars represent average ± standard deviation. **(C)** Total read frequencies per indel length, and per allele type, reported as box and whisker plots, for each of the gRNA. On the x axes, the negative scale represents length of deletions, while the positive site represents length of insertions. **(D)** Heatmaps of the top 20 motifs for their total read frequencies, identified at the G290R allele in patient samples, and displayed by individual.

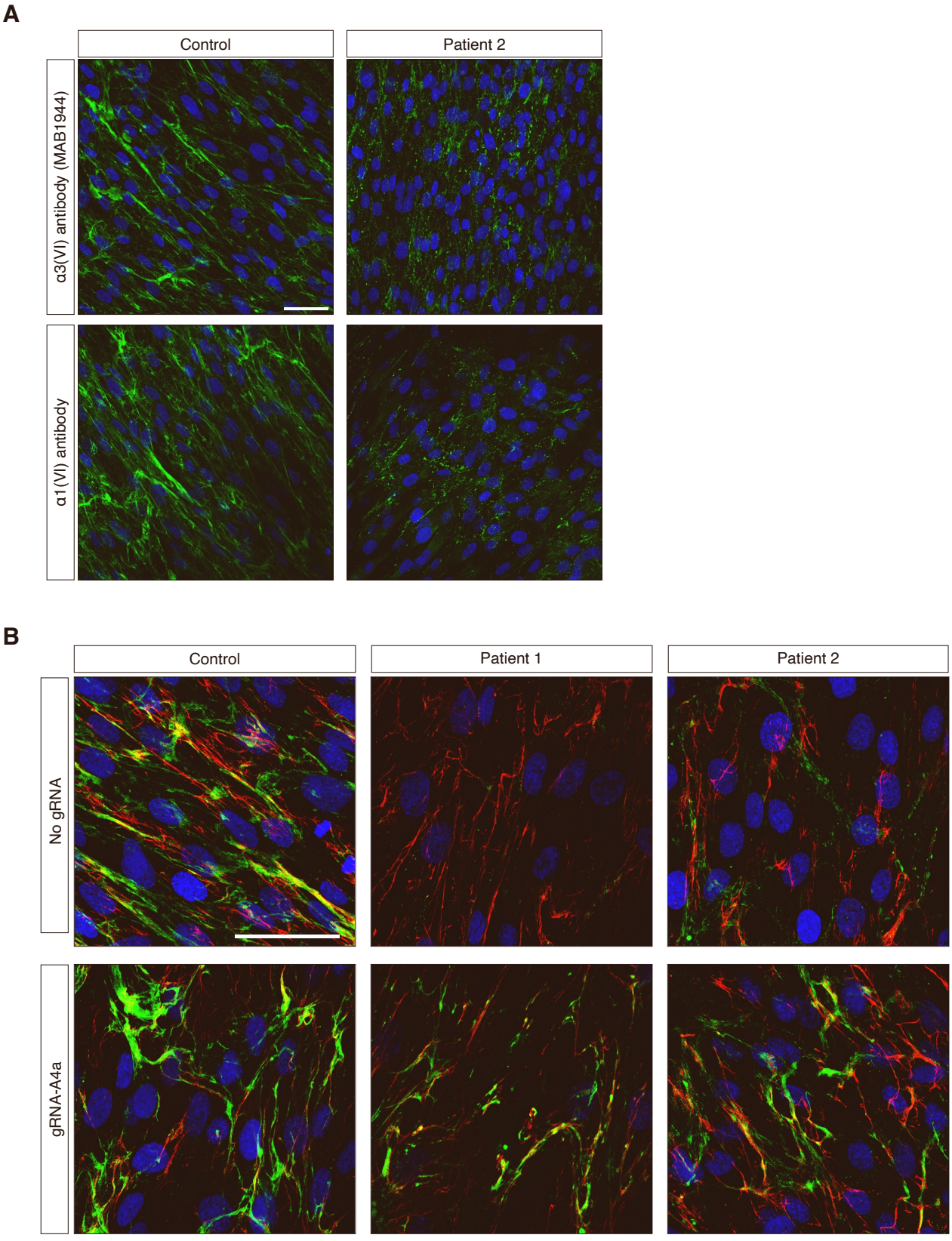

A
Control
Patient 2
α3(VI) antibody (MAB1944)
α1(VI) antibody
B
Control
Patient 1
Patient 2
No gRNA
gRNA-A4a

Figure S4. Assessment of the collagen VI matrix in the extracellular space.
Immunostaining of the collagen VI matrix secreted by patient-derived cultured fibroblasts. **(A)** Comparision of a Collagen α1(VI) antibody (gift of M.L. Chu) and a Collagen α3(VI) antibody (MAB1944) to detect the collagen VI matrix. Green signal is collagen VI, blue signal represents the nuclei. Bar = 50 µm. **(B)** High-resolution confocal microscopy of the collagen VI matrix (green) interaction with fibronectin (red). Nuclei are labeled in blue. Bar = 50 µm.