Supplementary data

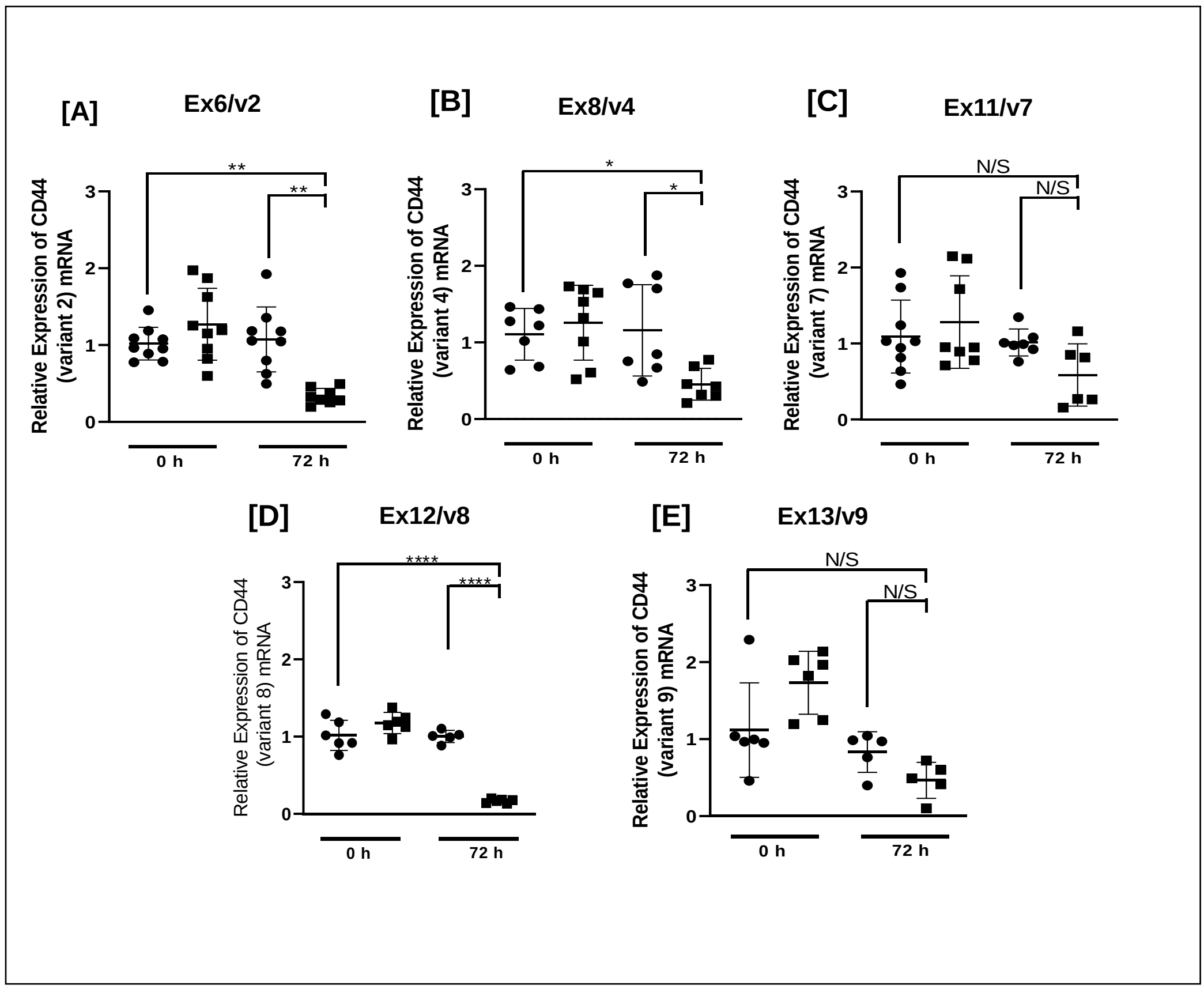

Figure 1- The Expression of CD44 Variants Containing a Single Exon Insert by Fibroblasts and Myofibroblasts
The relative expression of CD44 variants was assessed using qPCR. The expression of each CD44 variant in fibroblasts (black circles) was compared to myofibroblasts (black squares) at 0 h and 72 h timepoints. [A-E] represent; CD44v2 [A], CD44v4 [B], CD44v7 [C], CD44v8 [D] and CD44v9 [E]. Data is shown as mean ± S.D. from three experimental repeats with statistical significance represented by; *p≤0.05, **p≤0.01, ***p≤0.001 and ****p≤0.0001.

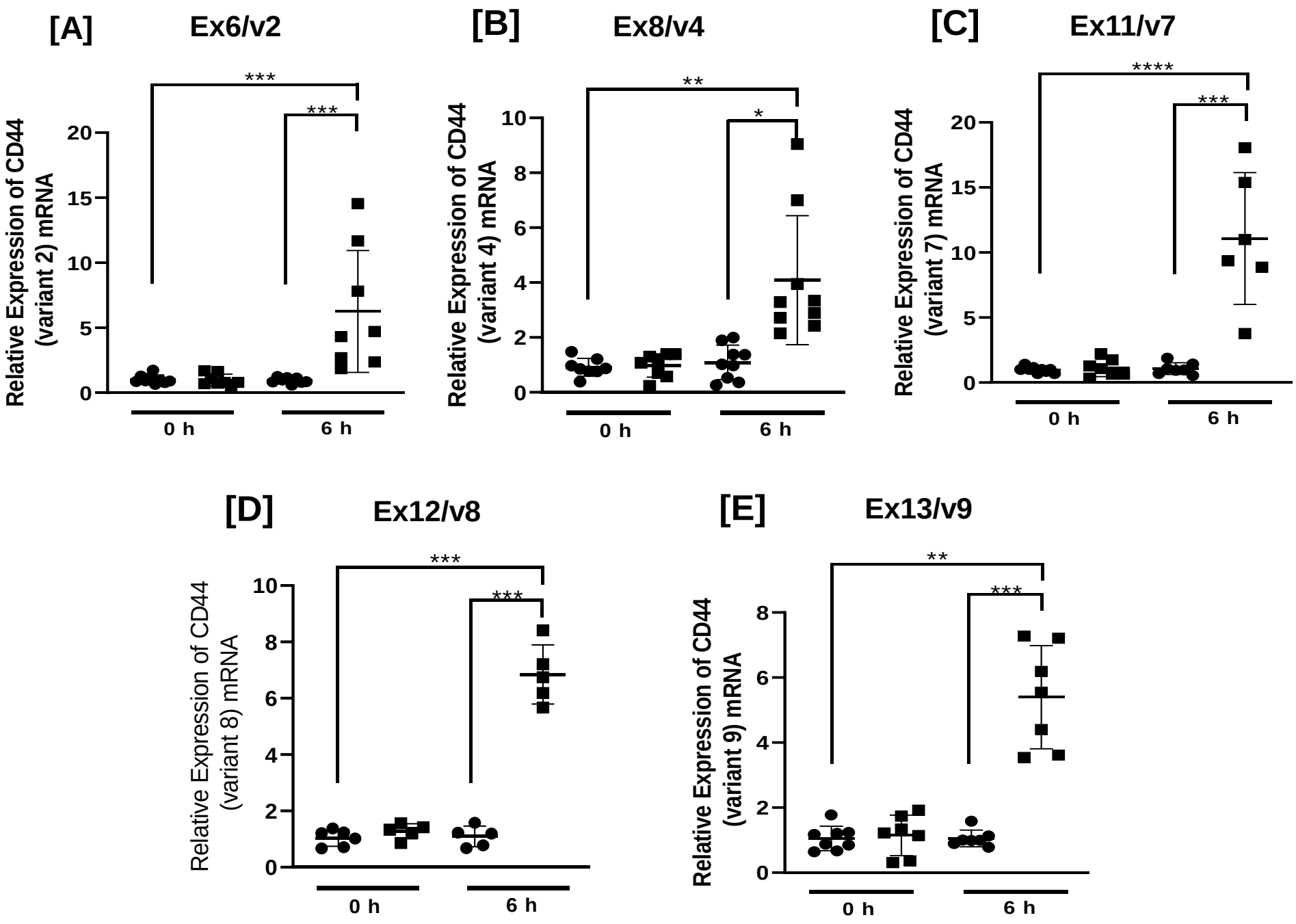

Figure 2 - The expression of CD44 variants containing a single exon insert by IL-1β treated fibroblasts.
The relative expression of CD44 variants was assessed using qPCR. The expression of each CD44 variant in fibroblasts (black circles) was compared to 1.0 ng/mL IL-1β stimulated fibroblasts (black squares) at 0 h and 6 h timepoints. **[A-E]** represent; CD44v2 **[A]**, CD44v4 **[B]**, CD44v7 **[C]**, CD44v8 **[D]** and CD44v9 **[E]**. Data is shown as ± S.D. from three experimental repeats with statistical significance represented by; *p≤0.05, **p≤0.01, ***p≤0.001 and ****p≤0.0001

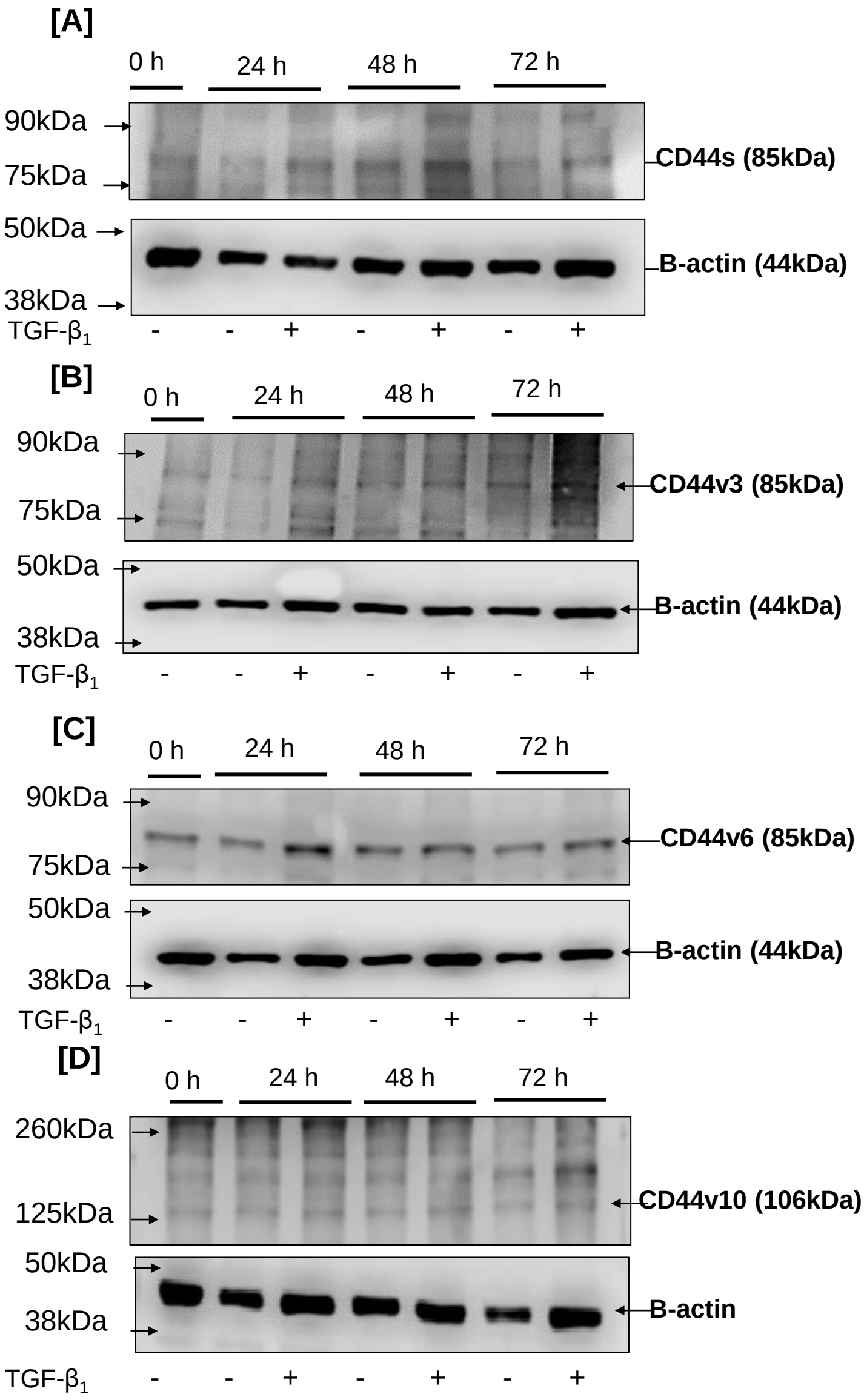

Figure 3- Protein expression of CD44 variants by fibroblasts and myofibroblasts.

Western blot and densitometry analysis were used to investigate the protein expression of CD44 variants. Proteins were extracted using RIPA buffer at 24 h, 48 h or 72 h following treatment with TGF-β_1 (10ng/ml) or with serum free media alone. A 0 h timepoint was used to assess basal protein expression levels of CD44 variants. Western blots show the protein expression of CD44s **[A]**, CD44v3 **[B]**, CD44v6 **[C]** and CD44v10 **[D]**. B-actin was used as a loading control. Densitometry analysis was used to semi-quantify protein expression of the CD44 variants **[E-H]** from three separate western blots. . Data is shown as mean ± S.D. from three experimental repeats where N/S is (not significant).

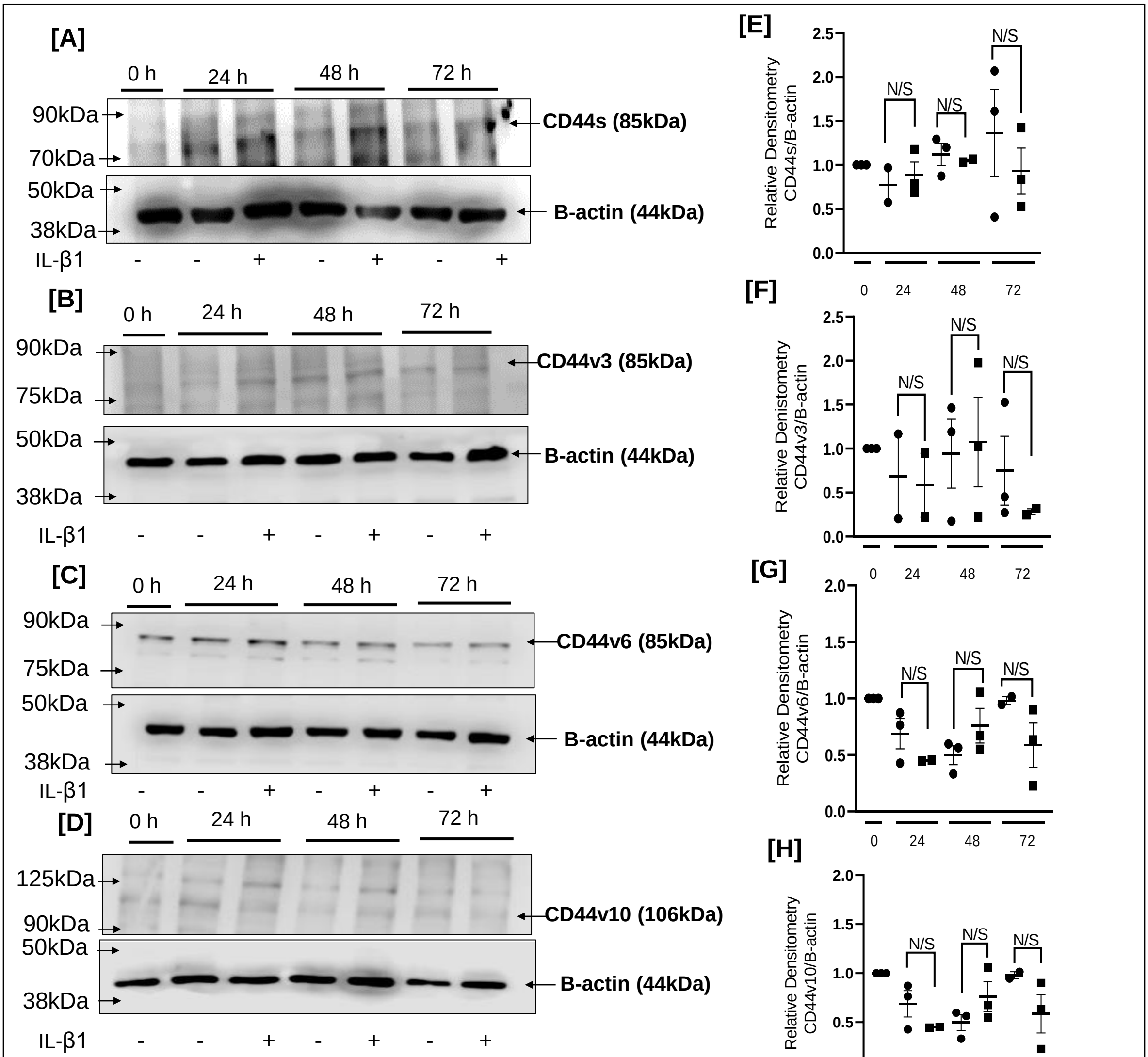

Figure 4 - Protein expression of CD44 variants by fibroblasts and IL-1β stimulated fibroblasts.

Western blot and densitometry analysis were used to investigate the protein expression of CD44 variants. Protein from HLFs was extracted using RIPA buffer at 24 h, 48 h or 72 h following treatment with IL1β (1ng/ml) or with serum free media alone. A 0 h timepoint was used to assess basal protein expression levels of CD44 variants. Western blots show the protein expression of CD44s [A], CD44v3 [B], CD44v6 [C] and CD44v10 [D]. B-actin was used as a loading control. Densitometry analysis was used to semi-quantify protein expression of the CD44 variants [E-H] from three separate western blots. Data is shown as mean ± S.D. from three experimental repeats where N/S is (not significant).

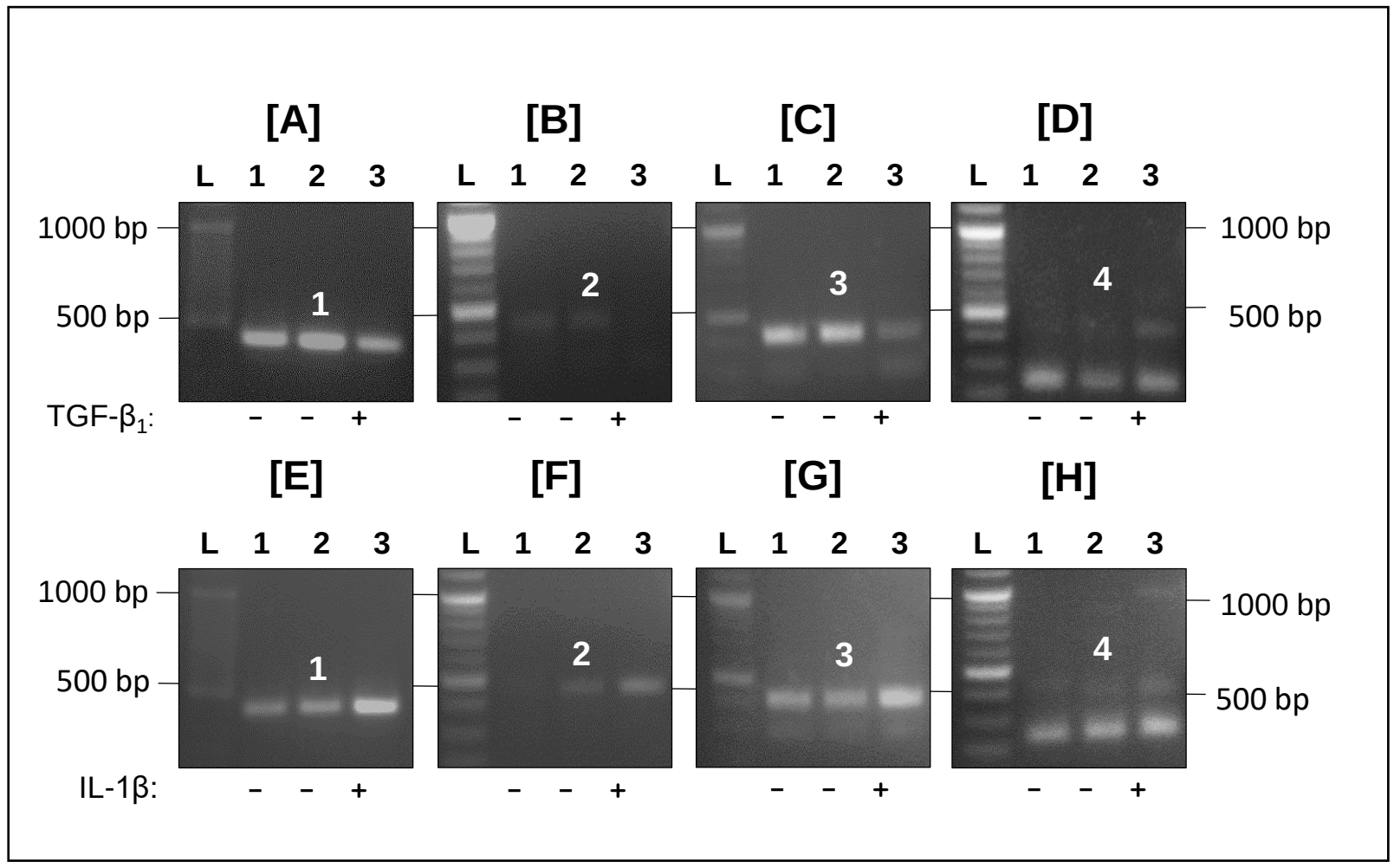

Figure. 5 – Spliced Variant Expression by fibroblasts Stimulated with TGF-β_1 or IL-1β.

Fibroblasts were grown to 80% confluence, then growth-arrested in serum free medium for 48h [A-H]. Cells were then treated with TGF-β_1 (10 ng/ml) or IL-1β (1 ng/ml) or serum free media alone (control samples). A further control group was extracted at 0 h, prior to the addition of medium. RNA was extracted at the time points shown and analysed by Touchdown RT-PCR for standard CD44 and the other variants (here identified by band number). The agarose gels were visualised by UV illumination and the bands excised for sequencing (QIAquick Gel Extraction Kit, Qiagen) and identification. Lane 1: control extracted 48 h after growth arrest; Lane 2 control treated with serum free medium; Lane 3 TGF-β_1 stimulated fibroblasts (72 h) or IL-1β stimulated fibroblasts (6 h). Gels shown are representative of those from three independent experiments. The bands were identified as CD44s (band 1 [A&E]), CD44v2 (band 2 [B&F]), CD44v3 (band 3 [C&G]) and CD44v4 (band 4 [D&H)

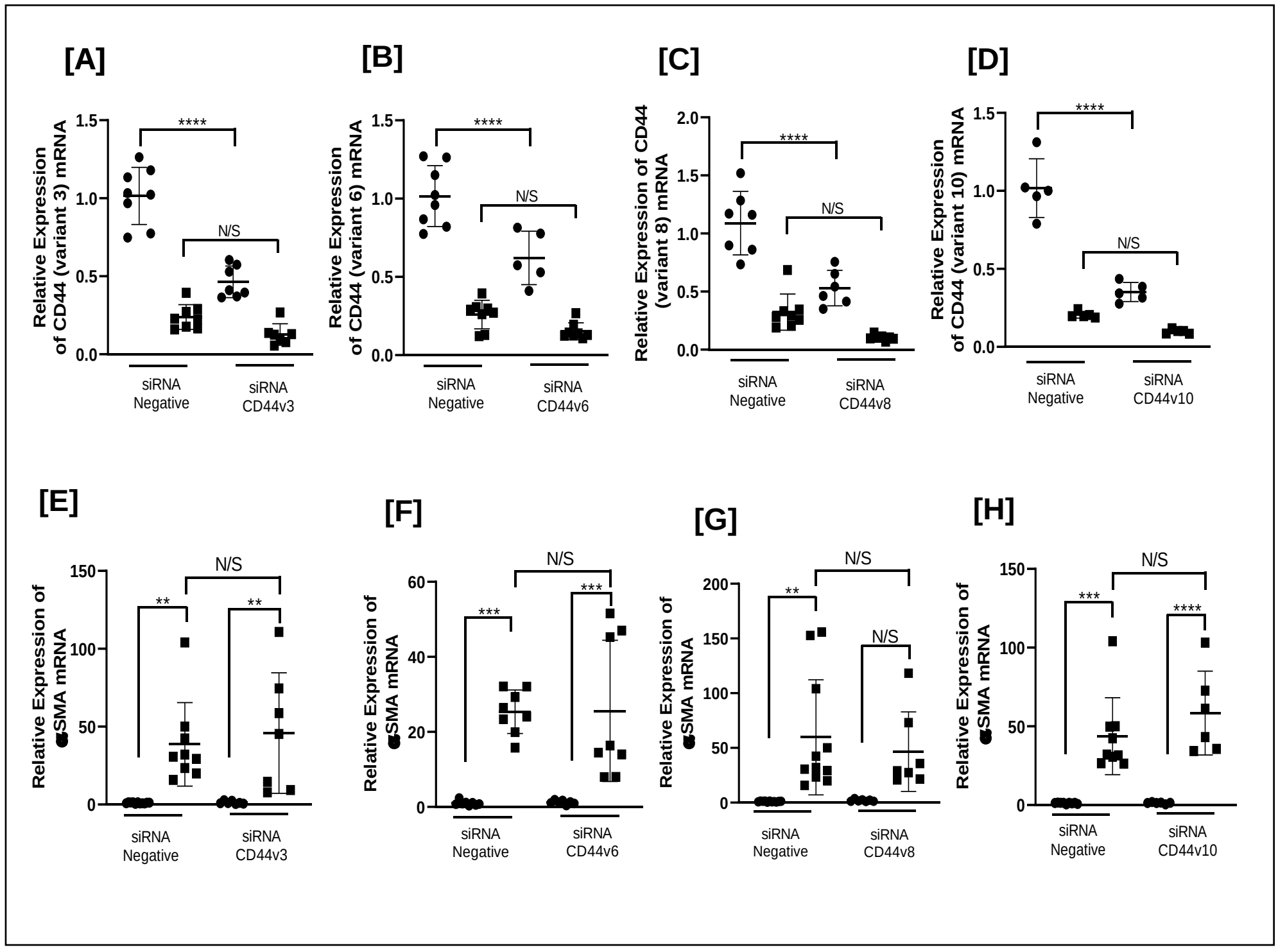

Figure 6 – CD44 variants do not affect αSMA expression by Myofibroblast

Fibroblasts were cultured in 6 well plates until 50-60% confluent. Cells were transiently transfected with siRNA prior to treatment with TGF-β_1 10 ng/ml for 72 h. RTqPCR was used to assess the efficiency of CD44 variant knockdown (CD44v3, CD44v6, CD44v8 and CD44v10) [A-D] and αSMA expression [E-H]. Fibroblasts (black circles) were compared to myofibroblasts (black squares). A negative siRNA was used as a control. Data is shown as mean ± S.D. from three experimental repeats with statistical significance represented by; *p≤0.05, **p≤0.01, ***p≤0.001 and ****p≤0.0001

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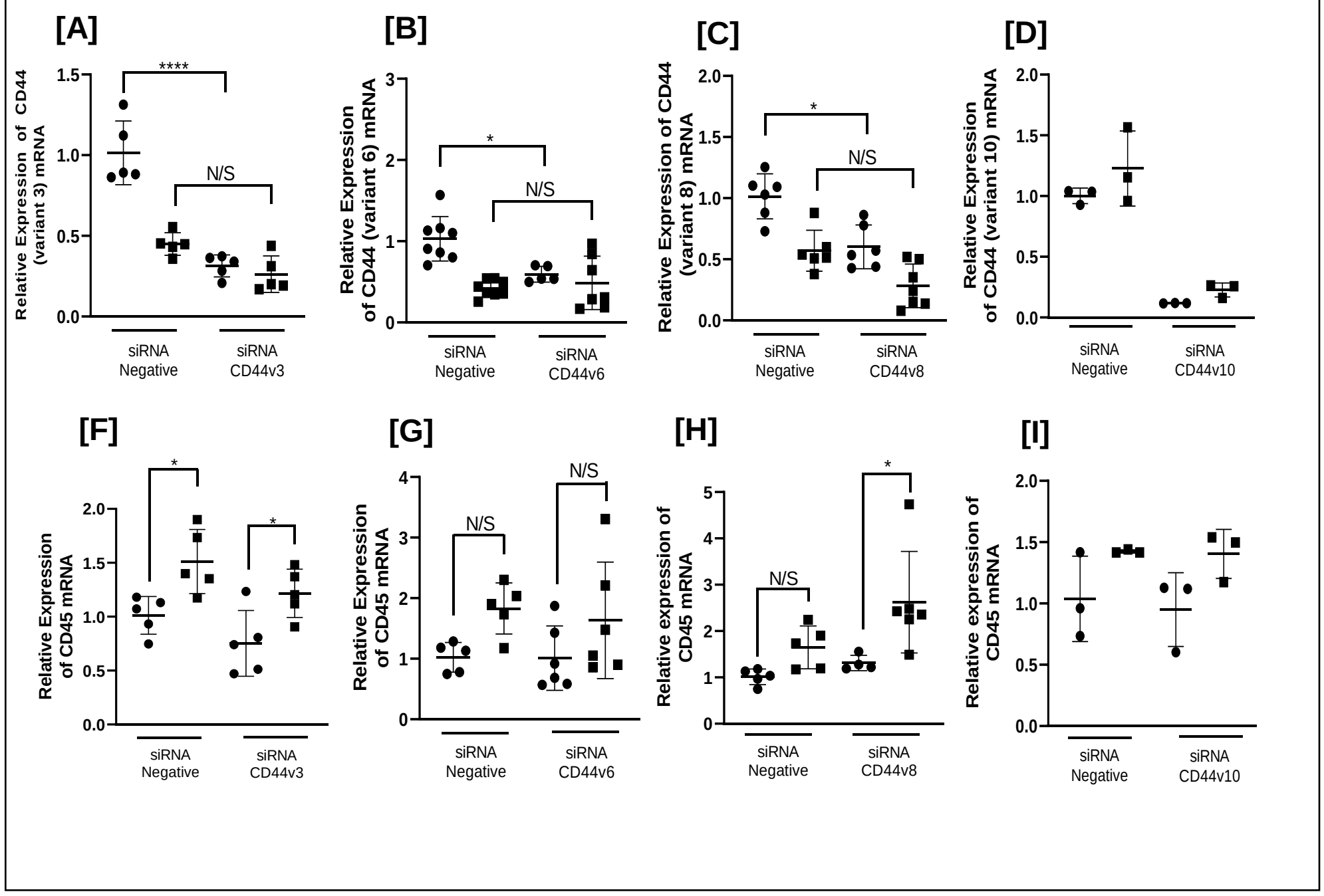


Figure 7– CD44 variants do not affect Monocyte Binding

Fibroblasts were cultured in 6 well plates until approx. 50-60% confluent. Cells were transiently transfected prior to treatment with IL-1β 1nng/ml for 6 h. RT-qPCR was used to assess the efficiency of CD44 variant knockdown (CD44v3, CD44v6, CD44v8, CD44v7/8 and CD44v10) [A-E] and CD45 expression, as a measure of monocyte binding. [F-J]. Fibroblasts (black circles) were compared to fibroblasts stimulated with IL-1β (black squares). A negative siRNA was used as a control. Data is shown as mean ± S.D. from three experimental repeats with statistical significance represented by; *p≤0.05, **p≤0.01, ***p≤0.001 and ****p≤0.0001

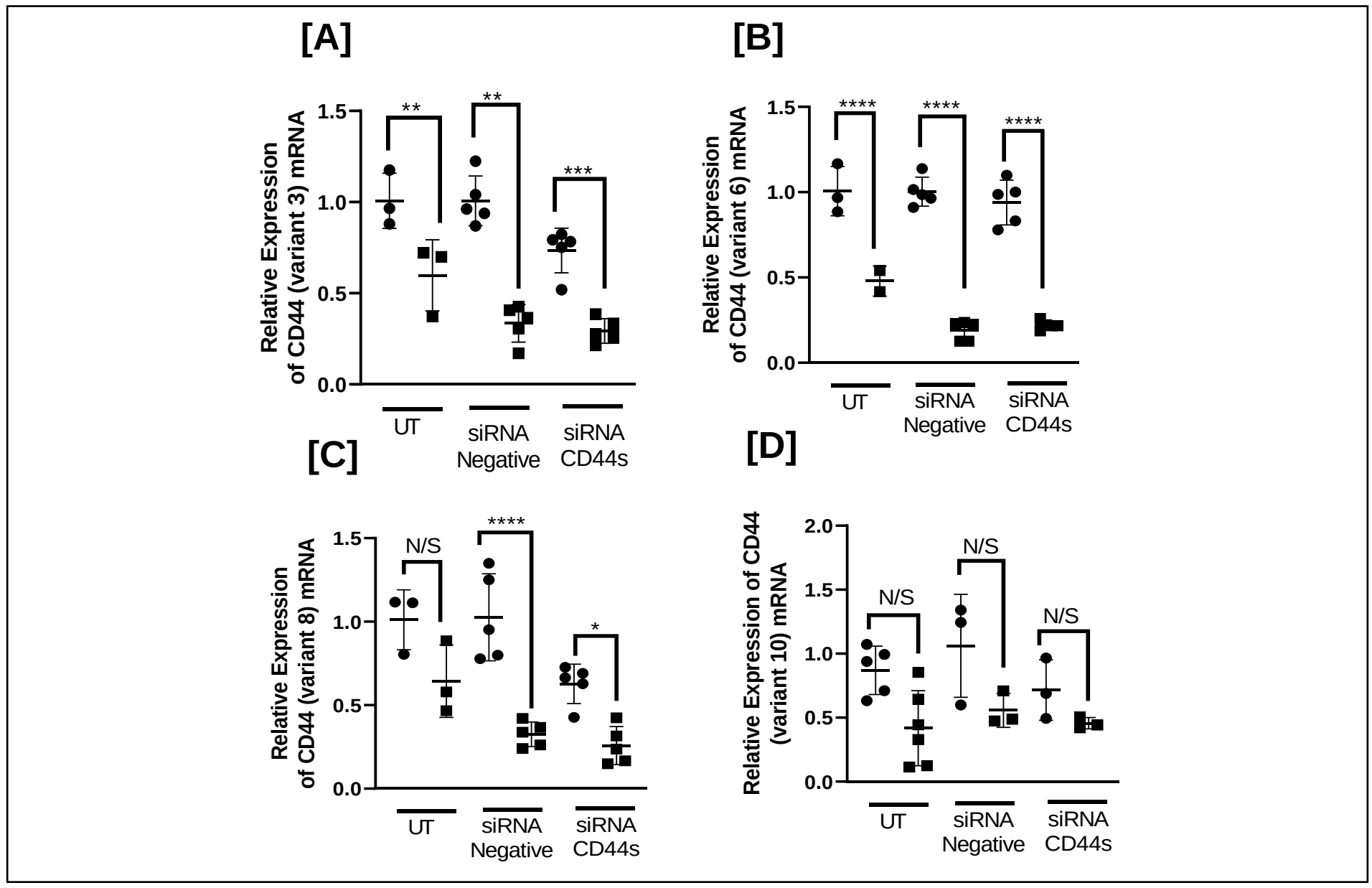


Figure 8– Silencing CD44 standard and the effects on CD44 variants

Fibroblasts were cultured in 6 well plates until approx. 50-60% confluent before being growth arrested and transiently transfected with an siRNA to CD44s or a negative siRNA control. An untransfected control was used to verify specific effects. Following transfection fibroblasts were treatment with TGF-β_1 10ng/ml for 72 h.

RT-qPCR was used to assess the effect of silencing CD44s on CD44v3 [A], CD44v6 [B], CD44v8 [C], and CD44v10 [D] on fibroblasts (black circles) and myofibroblasts (black squares). Data is shown as mean ± S.D. from three experimental repeats with statistical significance represented by; *p≤0.05, **p≤0.01, ***p≤0.001 and ****p≤0.0001

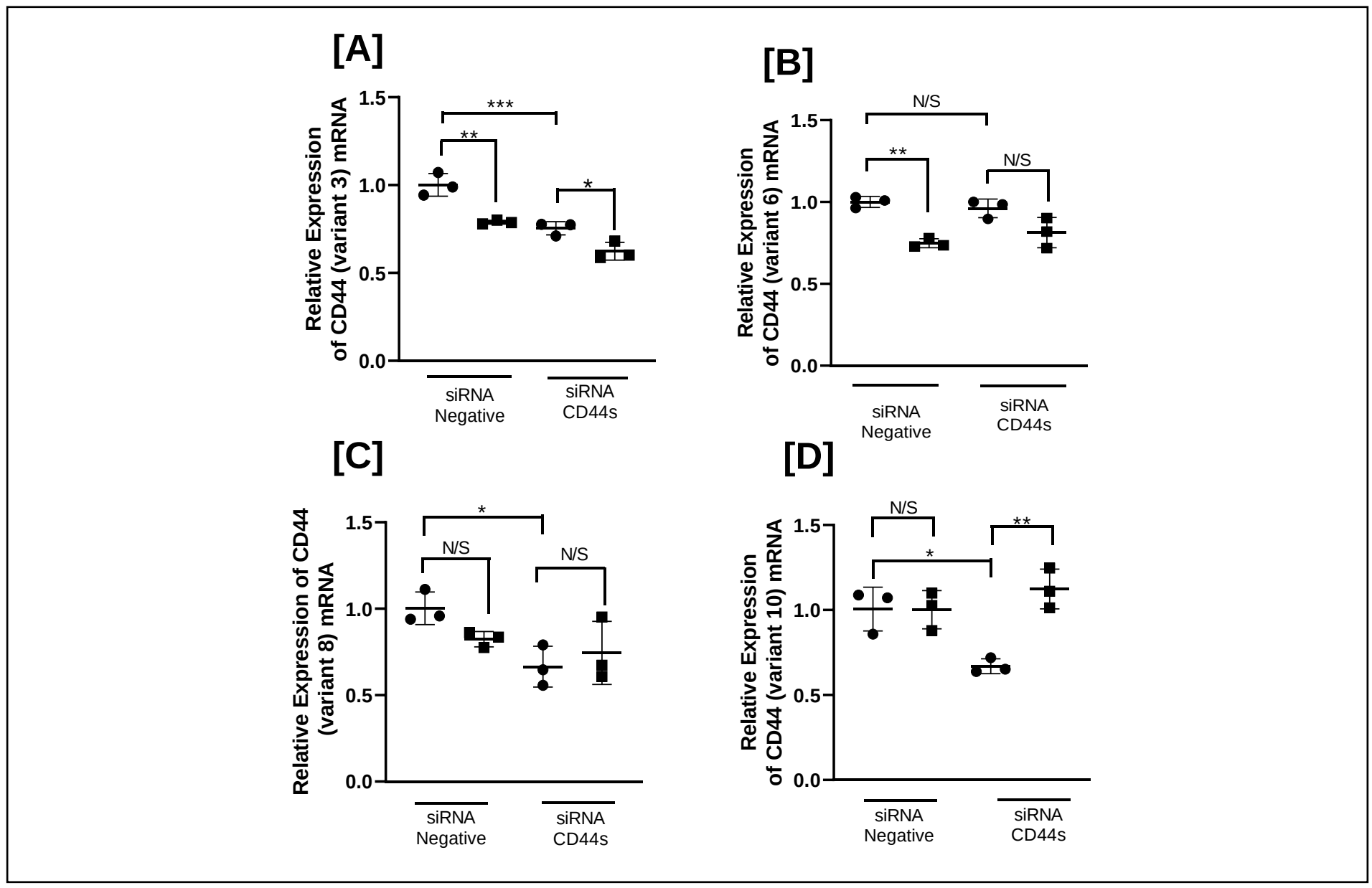


Figure 9 – Silencing CD44 standard and the effects on CD44 variants

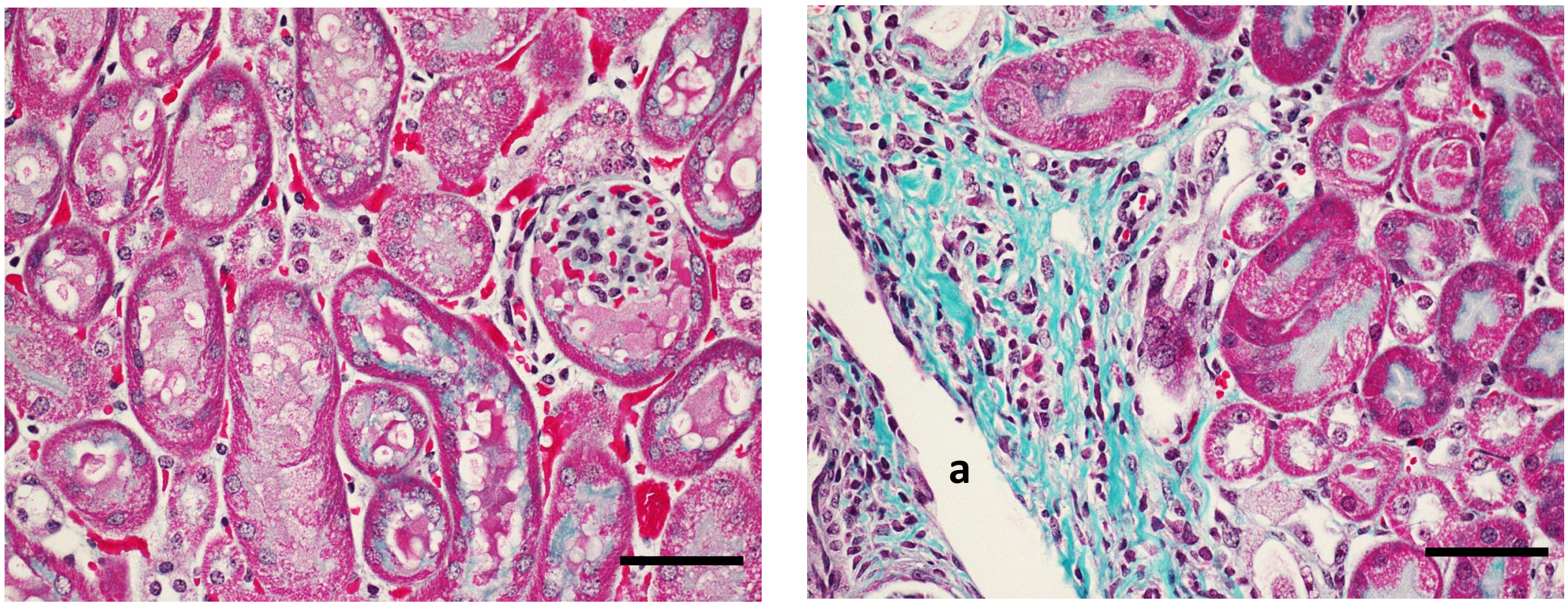
Fibroblasts were cultured in 6 well plates until approx. 50-60% confluent before being growth arrested and transiently transfected with an siRNA to CD44s or a negative siRNA control. Following transfection fibroblasts were treatment with IL-β 1 ng/ml for 72 h. RT-qPCR was used to assess the effect of silencing CD44s on CD44v3 [A], CD44v6 [B], CD44v8 [C], and CD44v10 [D] on fibroblasts (black circles) and myofibroblasts (black squares). Data is shown as mean ± S.D. from three experimental repeats with statistical significance represented by; *p≤0.05, **p≤0.01, ***p≤0.001 and ****p≤0.0001

A

healthy mouse kidney

chronic fibrosis (AAN)

Masson's Trichrome



B

healthy mouse kidney

chronic fibrosis (AAN)

CD147

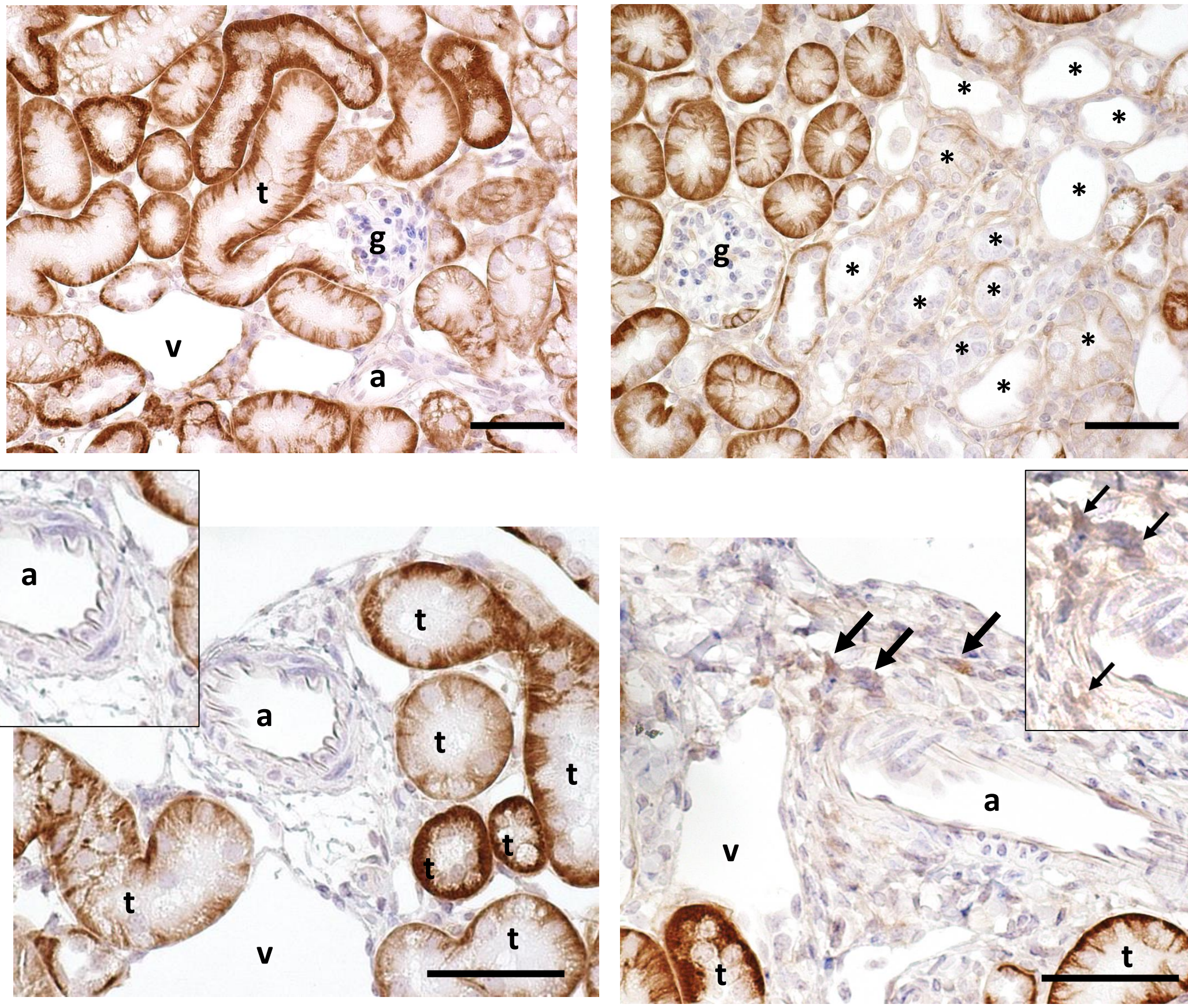


Figure 10. Renal expression of CD147 in health and chronic fibrosis.
A chronic fibrosis mouse model was used to assess the expression of CD147 in renal fibrosis. In this chronic aristolochic acid nephropathy (AAN), at 28 days there is visible fibrosis, including the deposition of collagen by myofibroblasts in the cortical interstitium, as seen in human disease. **A.** Masson's trichrome staining showing absence of collagen deposition in interstitium of healthy mouse kidneys and pathological collagen deposition in the peri-vascular and peri-tubular interstitium of mouse kidneys from the chronic aristolochic acid nephropathy (AAN) model. **B.** Immunohistochemistry (IHC) was used to assess localisation of CD147 expression (*brown*) in the healthy and fibrosed mouse kidneys. CD147 is strongly expressed by tubular epithelium of proximal and distal tubules (t) and is absent from glomeruli (g), vasculature (a – artery, v – vein) and interstitium in healthy kidneys. In chronic fibrosis, loss of CD147 expression is associated with injured tubules (marked by *) and increased CD147 expression is evident in interstitial fibrotic regions with myofibroblast expansion (*arrows*). Scale bar is 50 μm.

Table 1 – custom designed SYBR green primer sequences

Target Gene	Primer Sequence
CD44s	Forward 5’-GCTACCAGAGACCAAGAC-3’ Reverse 5’-GCTCCACCTTCTTGACTCCC-‘3
CD44v2	Forward 5’-CCTGCTACCACTTTGATGAGC-’3 Reverse 5’-GTGTCTTGGTCTCCAGCCAT-‘3
CD44v3	Forward 5’ -TGCTACCAGTACGTCTTCAAAT-‘3 Reverse 5’-GTGTCTTGGTCTCTGGTGCT-‘3
CD44v4	Forward 5’-CTGCTACCATTTCAACCACACC-‘3 Reverse 5’-TGGTCTCAGTCATCCTTGTGG -‘3
CD44v5	Forward 5’-CAGAATCCCTGCTACCAATGT-’3 Reverse 5’-TCTTGGTCTCTTGTGCTTGTAGA-’3
CD44v6	Forward 5’-TGCTACCATCCAGGCAACTC-’3 Reverse 5’-GGAATGTGTCTTGGTCTCCAGC-‘3
CD44v7	Forward 5’-GAATCCCTGCTACCACAGCCTC- ‘3 Reverse 5’-TCTCCCATCCTTCTTCCTGCTT-’3
CD44v8	Forward 5’-ATGTGTCTTGGTCTGGCGTT-’3 Reverse 5’-TCCCTGCTACCAATATGGACTC-‘3
CD44v9	Forward 5’-CAGAATCCCTGCTACCAAGC-‘3 Reverse 5’-ACTGGGGTGGAATGTGTCTT-‘3
CD44v10	Forward 5’-TCCCTGCTACCAATAGGAATGA-‘3 Reverse 5’-TAAGGAACGATTGACATTAGAGTTG-‘3
CD147	Forward 5’- CAGAGTGAAGGCTGTAAGTCG-‘3 Reverse 5’-TCGGAGGAACTCACGAAGAA-’3
GAPDH	Forward 5’-CCTCTGATTCAACAGCGACAG-’3 Reverse 5’-TGTCATACCAGGAAATGAGCTTGA-’3

Table 2 – Taqman Primers

Primer target	Primer Identity Number
PTPRC (CD45) (Applied Biosystems)	HS_00236304
αSMA (ACTA2) (Applied Biosystems)	HS_00426835_gl
18s Ribosomal RNA (Applied Biosystems)	Hs99999901_s1

Table 3 - Custom designed Primers for TD-PCR

Target	Primer sequence
Common forward primer	5’TCAATGCTTCAGCTCCACCT’3
CD44S Reverse	5’CAAAGCCAAGGCCAAGAGGGATGC’3
CD44v2 Reverse	5’CAGCCATTTGTGTTGTTGTGTGAA’3
CD44v3 Reverse	5’CCTTCATCATCATCATCAATGCCTGATCC’3
CD44v4 Reverse	5’TTTGAATGGCTTGGGTTCCACTGG’3
CD44v5 Reverse	5’GCTTGTAGAATGTGGGGTCTCTTC’3
CD44v6 Reverse	5’GAATGGGAGTCTTCTTTGGGTGTT’3
CD44v7 Reverse	5’CCATCCTTCTTCCTGCTTGATGAC’3
CD44v8 Reverse	5’GTCATTGAAAGAGGTCCTGTCCTG’3
CD44v9 Reverse	5’TGTCAGAGTAGAAGTT’3
CD44v10 Reverse	5’TGGAATCTCCAACAGTAACTGCAGT’3