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Supplementary Materials for

Identification of TNFR2 and IL-33 as therapeutic targets in localized fibrosis

David Izadi, Thomas B. Layton, Lynn Williams, Fiona McCann, Marisa Cabrita, Ana I. Espirito Santo, Weilin Xie, Marco Fritzsche, Huw Colin-York, Marc Feldmann, Kim S. Midwood, Jagdeep Nanchahal*

*Corresponding author. Email: jagdeep.nanchahal@kennedy.ox.ac.uk

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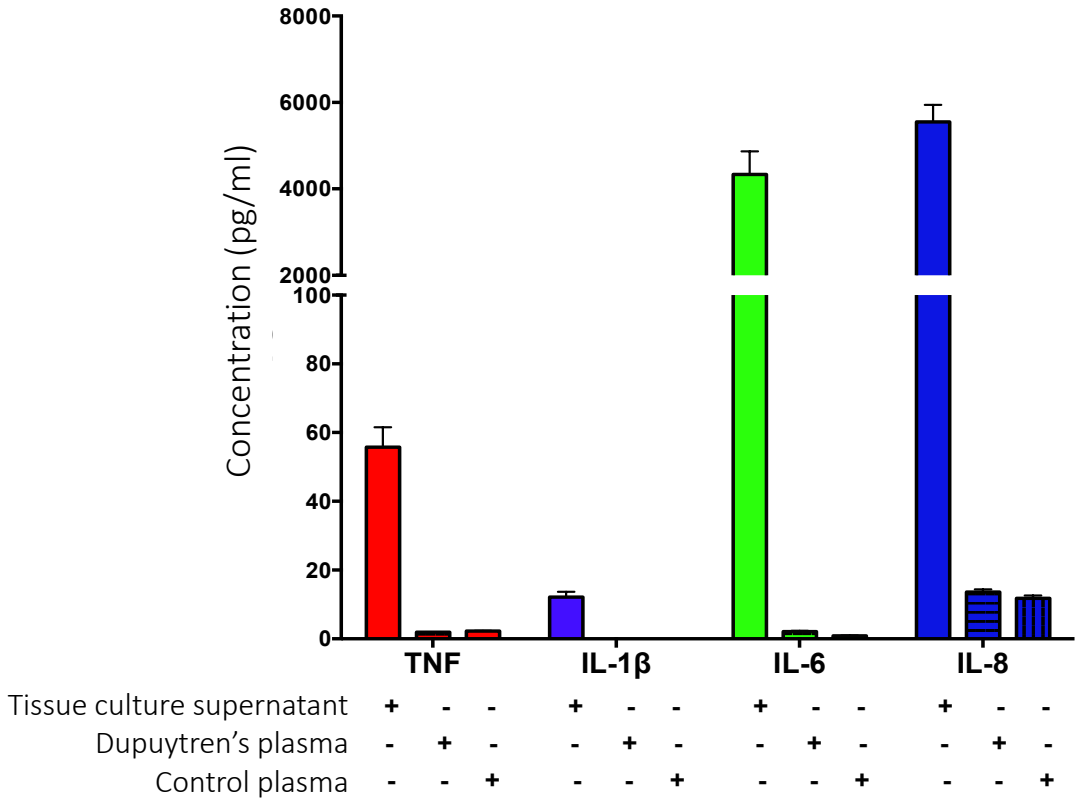


Fig. S1. DD is a localized inflammatory disorder. Bar plot of cytokines in plasma of DD patients compared to levels in freshly disaggregated nodular cells (tissue culture supernatant) and plasma from individuals (control plasma) not affected by DD (n = 35 independent donors).

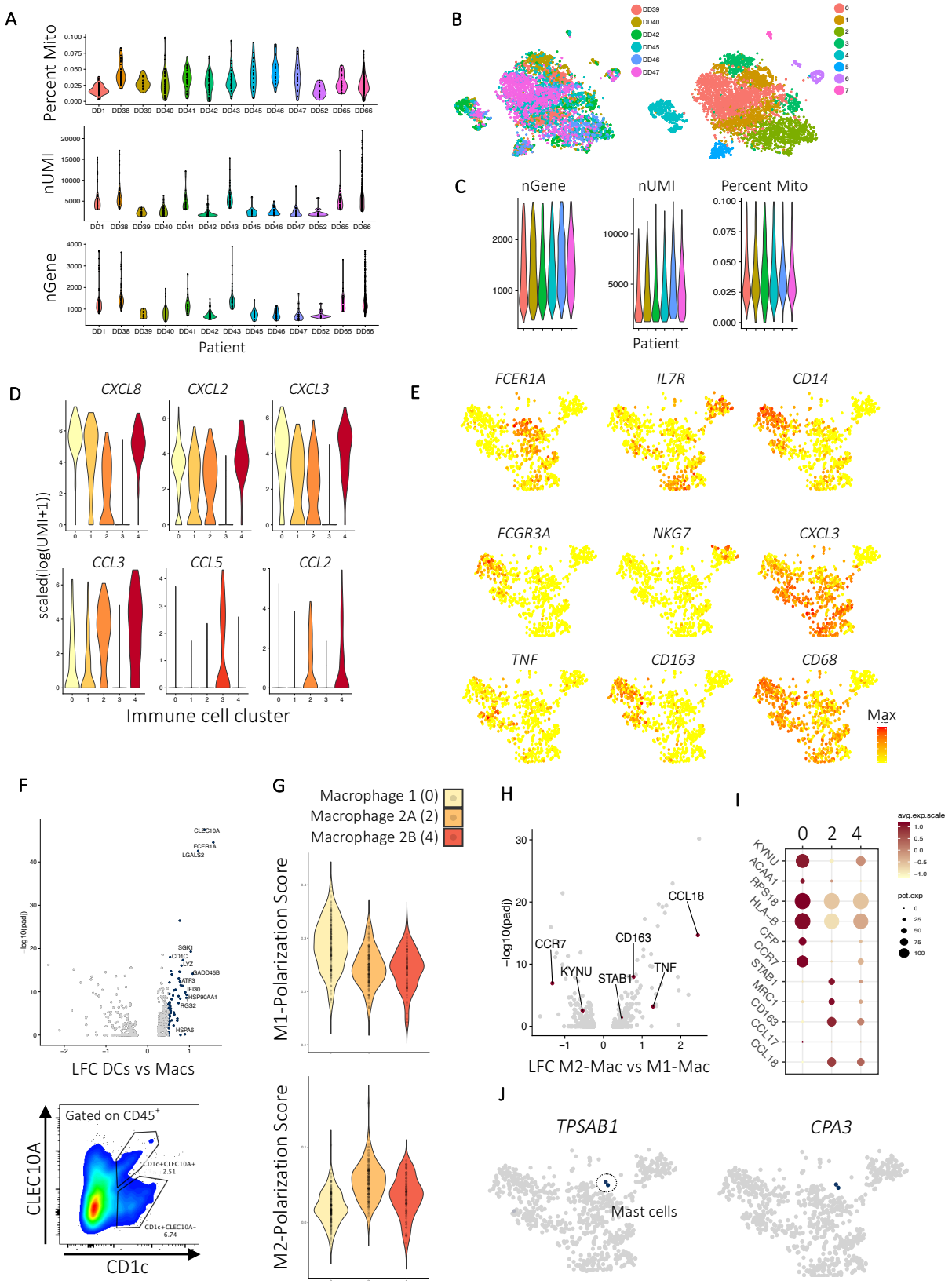

A
Percent Mito
nUMI
nGene
Patient
B
C
nGene
nUMI
Percent Mito
Patient
D
CXCL8
CXCL2
CXCL3
CCL3
CCL5
CCL2
scaled(log(UMI+1))
Immune cell cluster
E
FCER1A
IL7R
CD14
FCGR3A
NKG7
CXCL3
TNF
CD163
CD68
Max
F
LFC DCs vs Macs
Gated on CD45+
CLEC10A
CD1c
G
Macrophage 1 (0)
Macrophage 2A (2)
Macrophage 2B (4)
M1-Polarization Score
M2-Polarization Score
H
CCL18
CCR7
KYNU
CD163
STAB1
TNF
LFC M2-Mac vs M1-Mac
I
avg.exp.scale
pct.exp
KYNU
ACAA1
RPS18
HLA-B
CFP
CCR7
STAB1
MRC1
CD163
CCL17
CCL18
J
TPSAB1
CPA3
Mast cells

Fig. S2. Single-cell RNA-seq of immune cells from freshly isolated Dupuytren's nodules. (**A**) Violin plots showing quality control metrics for single-cell RNA-seq of immune cells in DD nodules (n = 12 DD patients, k = 1,033 cells). (**B**) tSNE embeddings of single cell RNA-seq of nodular cells coloured by patient donor (DD39-DD47) and Louvain cluster (0-7). (**C**) Violin plots showing quality control metrics for each DD patient (n = 6 DD patients, k = 7,332 cells). (**D**) Violin plots showing chemokine gene expression in scaled(log(UMI+1)) in immune clusters. (**E**) tSNE embeddings of immune cells in DD nodules showing cell marker gene expression in scaled(log(UMI+1)). (**F**) Top panel: Volcano plot showing differentially expressed genes (Wilcoxon Rank Sum test) between dendritic cell cluster and myeloid clusters. FDR-corrected (BH correction) p-value. Bottom panel; Representative density plots of flow cytometry analysis showing $CD45^{+}CD1C^{+}CLEC10A^{+/-}$ immune cells in freshly disaggregated DD nodules. (**G**) Violin plot for 'M1 and M2-macrophage polarization score' in three macrophage clusters. This score represents the scaled average expression of the test gene sets (n = 198 and n = 462 genes). (**H**) Volcano plot showing differentially expressed genes (Wilcoxon Rank Sum test) between M2-macrophage (2A-B) clusters and M1-macrophage cluster. FDR-corrected (BH correction) p-value. (**I**) Dot plot showing gene expression in macrophage clusters in scaled(log(UMI+1)). Pct.exp = percentage of cells expressing gene. (**J**) tSNE embeddings of immune cells showing expression of mast cell marker genes in scaled(log(UMI+1)). Two mast cells highlighted.

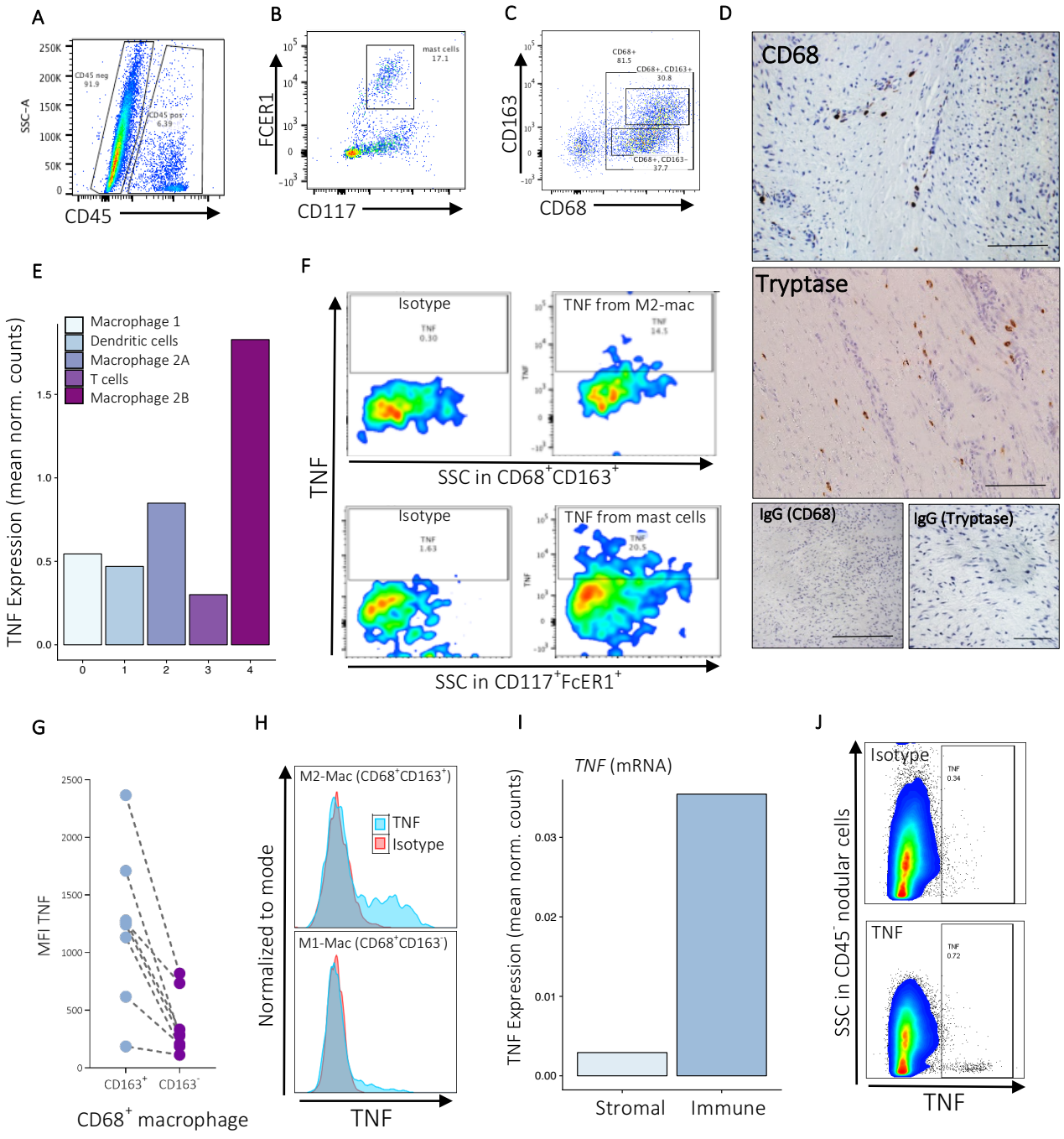

A
SSC-A
CD45
CD45 neg
91.9
CD45 pos
6.39
B
FCER1
CD117
mast cells
17.1
C
CD163
CD68
CD68+
81.5
CD68+, CD163+
30.8
CD68+, CD163-
37.7
D
CD68
Tryptase
IgG (CD68)
IgG (Tryptase)
E
TNF Expression (mean norm. counts)
Macrophage 1
Dendritic cells
Macrophage 2A
T cells
Macrophage 2B
F
TNF
Isotype
TNF from M2-mac
SSC in CD68+CD163+
Isotype
TNF from mast cells
SSC in CD117+FcER1+
G
MFI TNF
CD163+
CD163-
CD68+ macrophage
H
Normalized to mode
M2-Mac (CD68+CD163+)
TNF
Isotype
M1-Mac (CD68+CD163-)
TNF
I
TNF (mRNA)
TNF Expression (mean norm. counts)
Stromal
Immune
J
Isotype
TNF
SSC in CD45- nodular cells
TNF

Fig. S3. Mast cells and M2 macrophages are the predominant sources of TNF in DD. (**A**) Scatter plot of flow cytometry gating strategy on $CD45^+$ in freshly isolated nodular cells (*n* = 5 DD patients). (**B**) Scatter plot of flow cytometry gating strategy on $CD45^+FCER1^+CD117^+$ mast cells in DD nodules. (**C**) Scatter plot of flow cytometry gating strategy on $CD45^+CD68^+CD163^{+/-}$ macrophages in freshly isolated nodular cells. (**D**) Representative images of immunohistochemistry for $tryptase^+$ mast cells and $CD68^+$ macrophages in DD nodules, with isotype control. Scale bar = 40 μm (top two panels) and 20 μm (bottom two panels). (**E**) Bar plot of *TNF* gene expression in immune cell clusters in single cell RNA-seq. Expression in average normalized counts per cell cluster. (**F**) Representative density plots of flow cytometry analysis showing TNF expression in mast cells and macrophages (*n* = 5 DD patients). (**G**) Line plot of flow cytometry analysis showing TNF expression (MFI) in $CD163^+$ and $CD163^-$ macrophages (*n* = 7 DD patients). (**H**) Representative histograms of flow cytometry analysis for TNF expression in $CD163^-$ and $CD163^+$ macrophages in freshly disaggregated nodules with isotype control (*n* = 5 DD patients). (**I**) Bar plot of *TNF* gene expression in stromal cells and immune cells in single cell RNA-seq. Expression in average normalized counts per cluster. (**J**) Representative density plots of flow cytometry analysis showing TNF expression in $CD45^-$ stromal cells in freshly disaggregated nodules with isotype control (*n* = 6 DD patients).

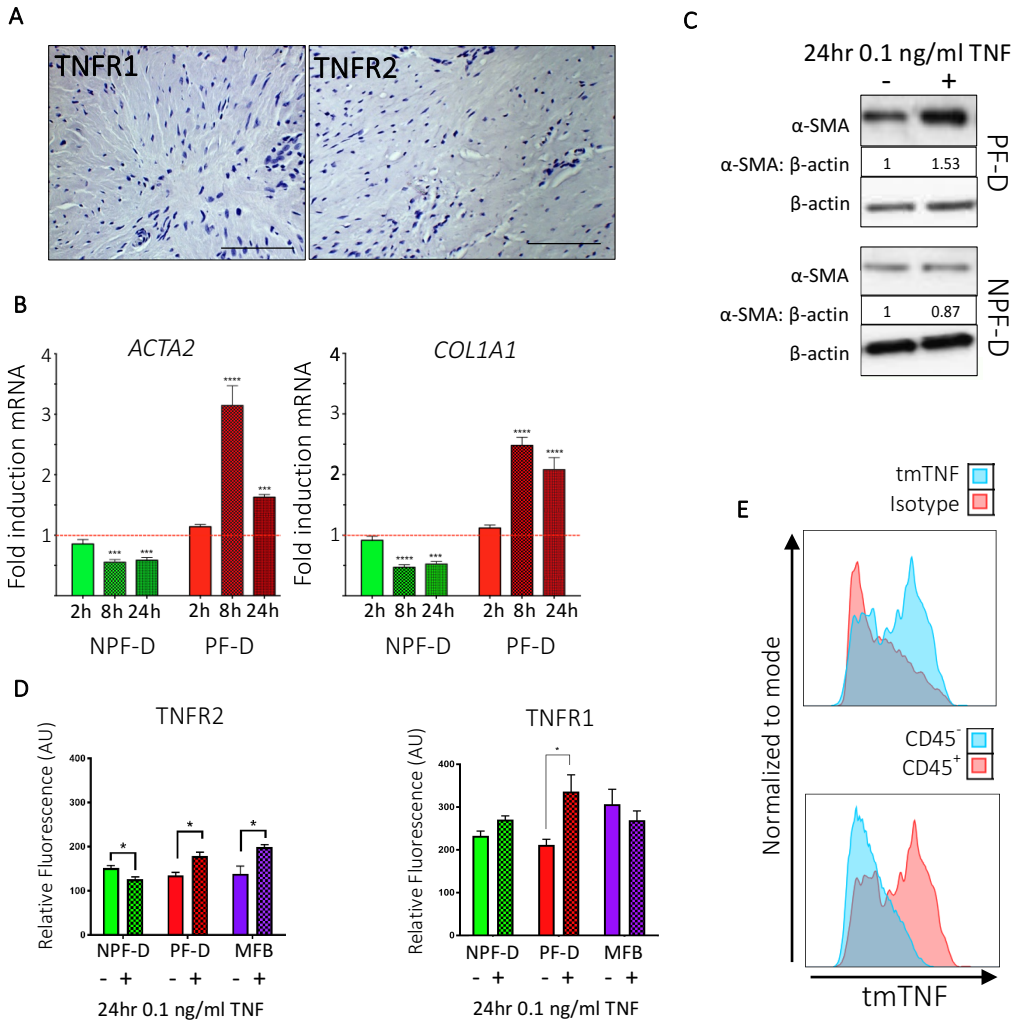
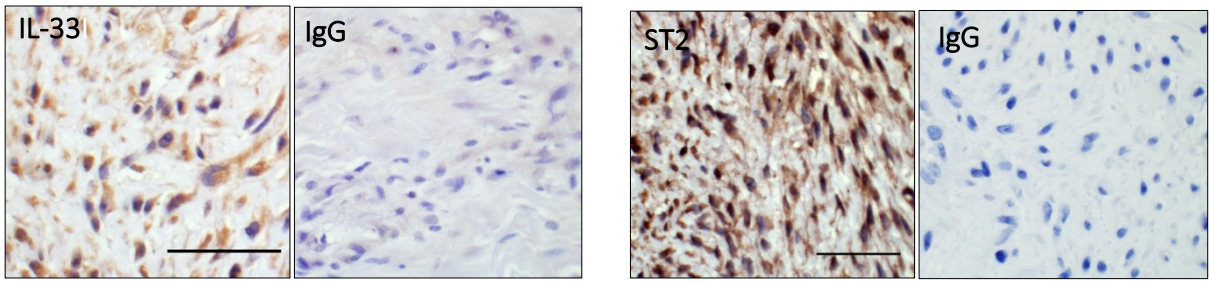
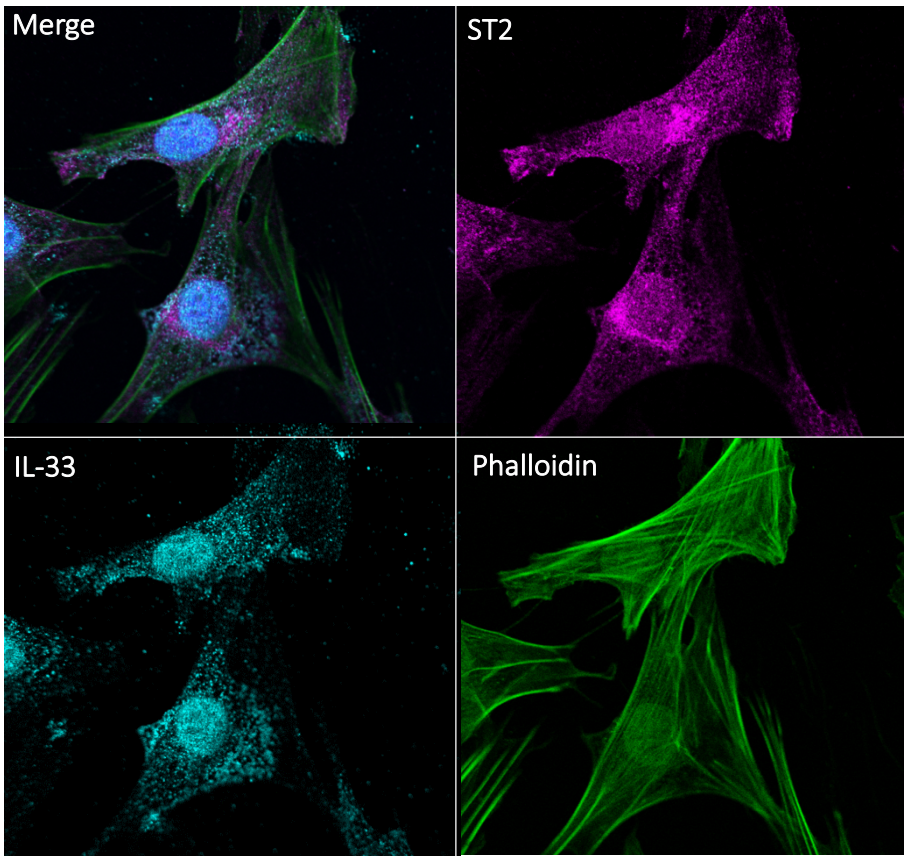

A
TNFR1
TNFR2
B
ACTA2
COL1A1
Fold induction mRNA
2h 8h 24h
NPF-D
PF-D
C
24hr 0.1 ng/ml TNF
- +
α-SMA
α-SMA: β-actin
1 1.53
β-actin
PF-D
α-SMA
α-SMA: β-actin
1 0.87
β-actin
NPF-D
D
TNFR2
TNFR1
Relative Fluorescence (AU)
NPF-D PF-D MFB
24hr 0.1 ng/ml TNF
E
tmTNF
Isotype
Normalized to mode
CD45-
CD45+
tmTNF

Fig. S4. TNF induced protein expression and TNF receptor expression in Dupuytren's cord. (**A**) Representative images of immunohistochemistry for TNFR1 and TNFR2 expression in Dupuytren's cord. Scale bar = 20 μm. (**B**) Bar plots of *ACTA2* and *COL1A1* gene expression in palmar (PF-D) and non-palmar (NPF-D) dermal fibroblasts from DD patients treated with 0.1 ng/ml rTNF at 2, 8 and 24 hours (h). (*n* = 5 DD patients, mean ± SEM). (**C**) Western blot analysis showing α-SMA protein expression in NPF-D and PF-D on treatment with 0.1 ng/ml rTNF. (**D**) Bar plots of quantitative immunofluorescence for TNFR1 and TNFR2 protein expression in non-palmar (NPFD) and palmar (PDF) dermal fibroblasts and myofibroblasts from DD patients on treatment with 0.1 ng/ml rTNF (*n* = 5 DD patients, mean ± SEM). (**E**) Representative histogram plots of flow cytometry analysis for transmembrane TNF expression in freshly disaggregated DD nodular cells (*n* = 4 DD patients). *P < 0.05, **P< 0.01, ***P < 0.001, ****P<0.0001.

A


IL-33
IgG
ST2
IgG

B


Merge
ST2
IL-33
Phalloidin

C

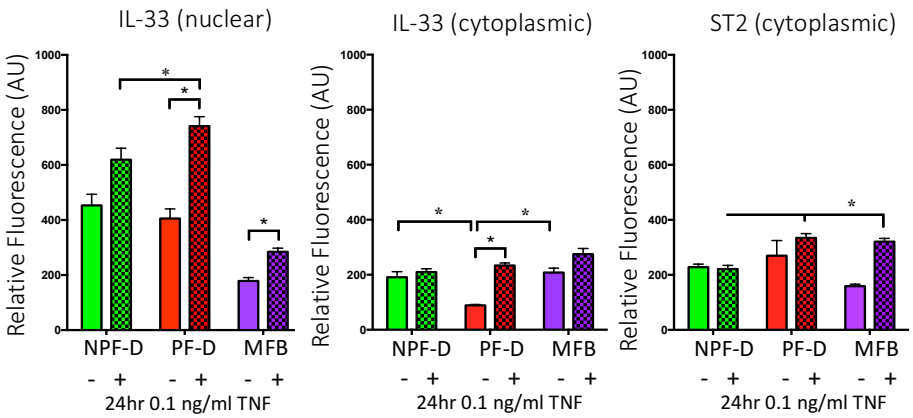

IL-33 (nuclear)
IL-33 (cytoplasmic)
ST2 (cytoplasmic)
Relative Fluorescence (AU)
1000
800
600
400
200
0
NPF-D
PF-D
MFB
- + - + - +
24hr 0.1 ng/ml TNF
*

Fig. S5. IL-33 and its receptor ST2 are expressed on Dupuytren's myofibroblasts. (**A**) Representative images of immunohistochemistry for IL-33 and the receptor ST2 expression with corresponding isotype controls in DD nodules. Scale bar = 20 μm. (**B**) Representative confocal images of immunofluorescence for ST2 and IL-33 in freshly isolated myofibroblasts from DD nodules. Scale bar = 10 μm. (**C**) Bar plots of quantitative immunofluorescence showing cytoplasmic and nuclear IL-33 staining and ST2 cytoplasmic staining in non-palmar (NPDF) and palmar (PDF) dermal fibroblasts and myofibroblasts from DD patients on treatment with 0.1 ng/ml rTNF (n = 5 DD patients, mean ± SEM).

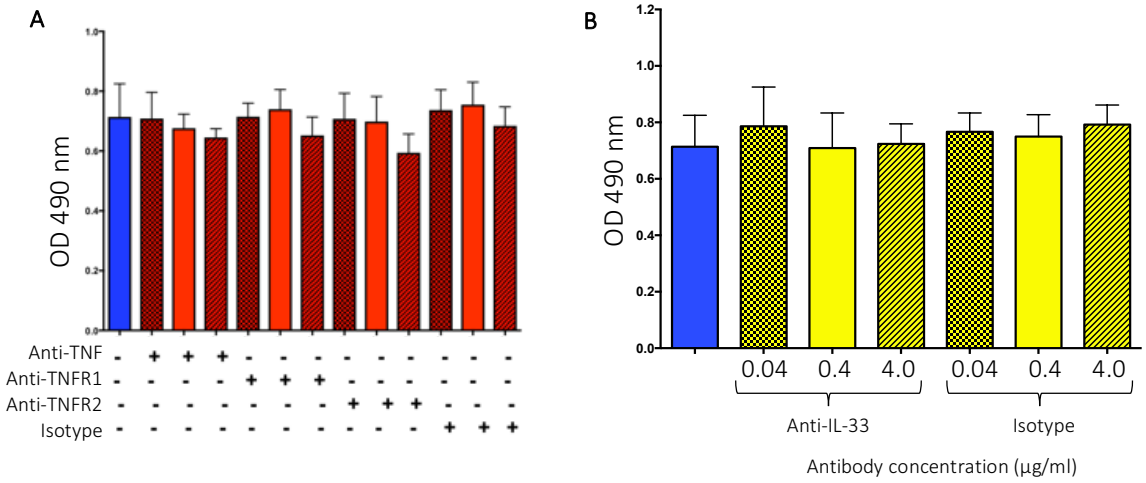


Fig. S6. No reduction in cell viability following treatment with neutralizing antibodies. (**A**) Bar plot of MTS cell viability assay of myofibroblasts treated over 24 hours with 1, 10 and 100 µg/ml of neutralizing anti-TNF, anti-TNFR1, anti-TNFR2 antibodies and isotype control. (*n* = 3 DD patients, in triplicate, mean ± SEM). (**B**) Bar plot of MTS cell viability assay of myofibroblasts treated over 24 hours with 0.04, 0.4 and 4 µg/ml of neutralizing anti-IL-33 and isotype control. (*n* = 3 DD patients, in triplicate, mean ± SEM). OD = Optical density.

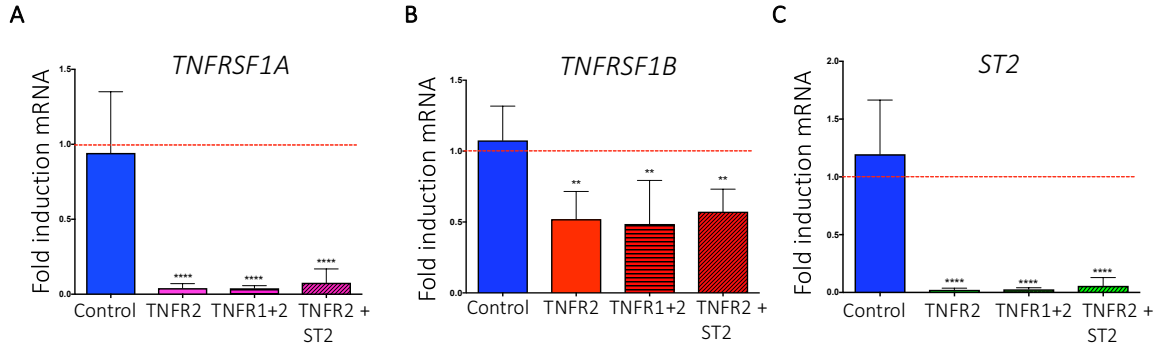


Fig. S7. siRNA knockdown validation for *TNFRSF1A, TNFRSF1A,* and *ST2*. (**A-C**) Bar plots of qPCR analysis showing gene expression of labelled genes following siRNA mediated knockdowns. (*n* = 3 DD patients, in triplicate, mean ± SEM). P < 0.05, **P< 0.01, ***P < 0.001, ****P<0.0001.

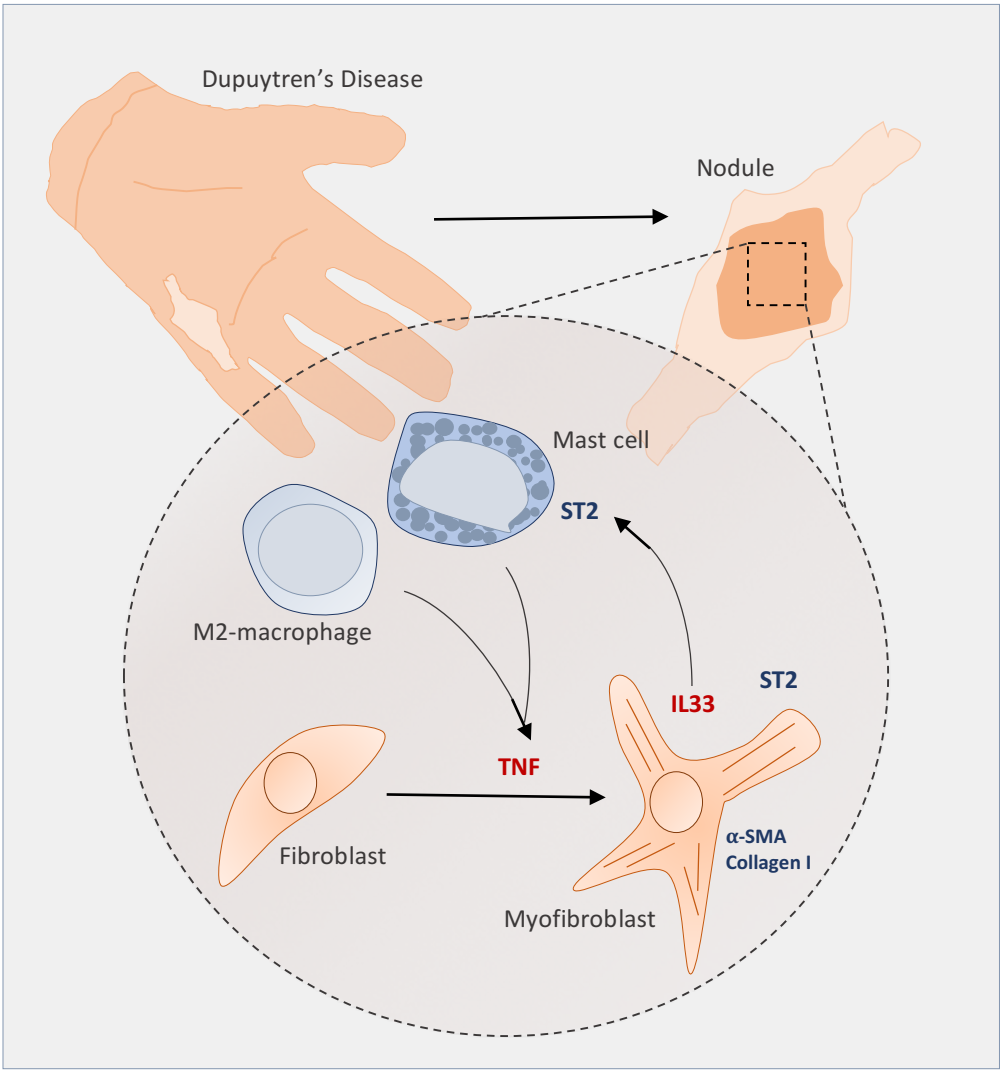

Dupuytren's Disease
Nodule
Mast cell
ST2
M2-macrophage
ST2
IL33
TNF
Fibroblast
α-SMA
Collagen I
Myofibroblast

Fig. S8. IL-33 and TNF stromal–immune cell cross-talk in DD. Schematic illustrating that immune cells (mast cells and M2-macrophages) are the predominant source of TNF in DD that stimulates the development of the myofibroblast phenotype. Myofibroblasts are a significant source of IL-33 which further activates TNF secretion from immune cells.