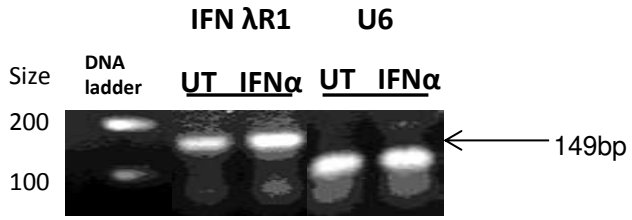

Article:
Alase, AA, El-Sherbiny, Y orcid.org/0000-0003-4791-3475, Vital, EM orcid.org/0000-0003-1637-4755 et al. (3 more authors) (2015) IFNλ Stimulates MxA Production in Human Dermal Fibroblasts via a MAPK-Dependent STAT1-Independent Mechanism. Journal of Investigative Dermatology, 135 (12). pp. 2935-2943. ISSN 0022-202X

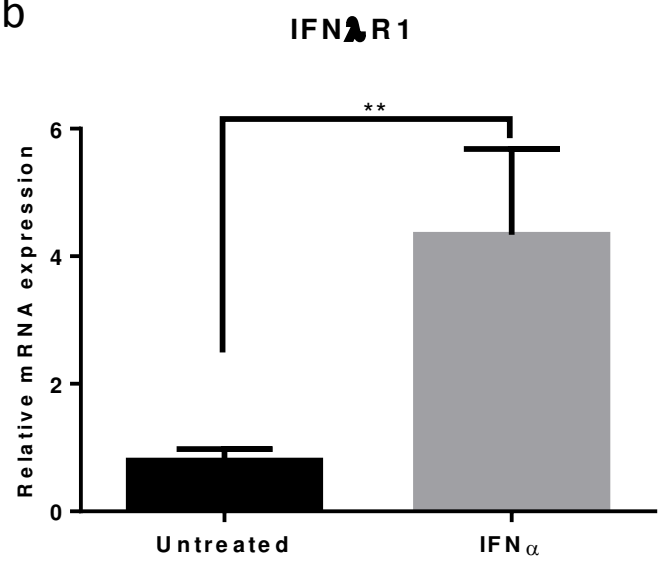
https://doi.org/10.1038/jid.2015.317

IFNλR1 is present in human dermal fibroblasts

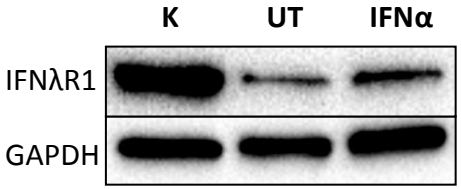
a


IFN λR1
U6
Size
DNA ladder
UT
IFNα
UT
IFNα
200
100
149bp

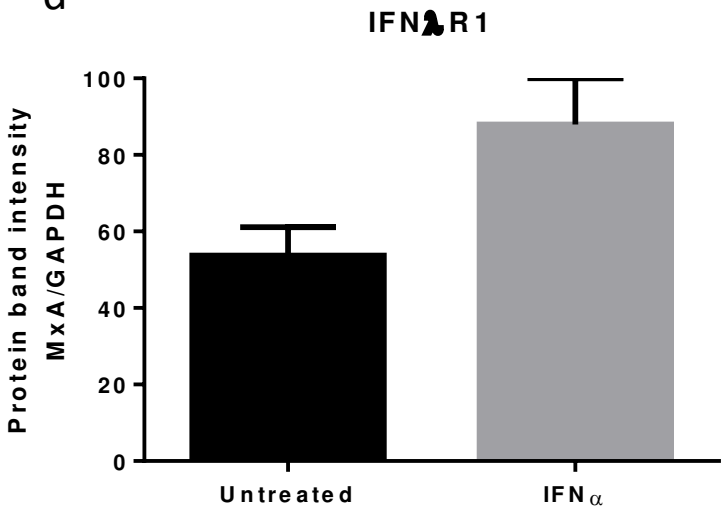
b


IFNλR1
**
Relative mRNA expression
6
4
2
0
Untreated
IFNα

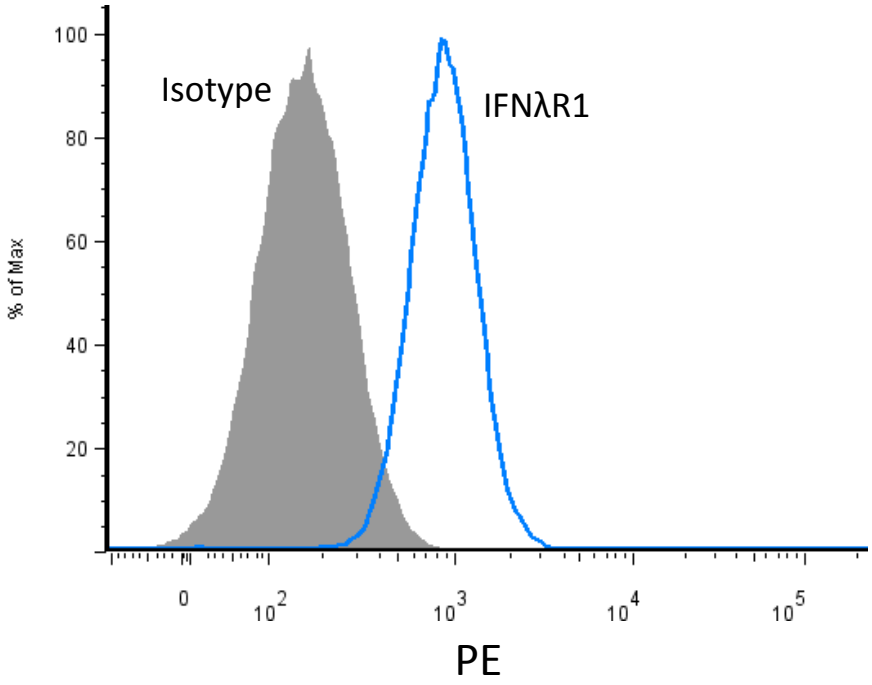
c


K
UT
IFNα
IFNλR1
GAPDH

d


IFNλR1
Protein band intensity
MxA/GAPDH
100
80
60
40
20
0
Untreated
IFNα

e



IFNλ1 Induces the expression of MxA but not other ISGs in dermal fibroblasts

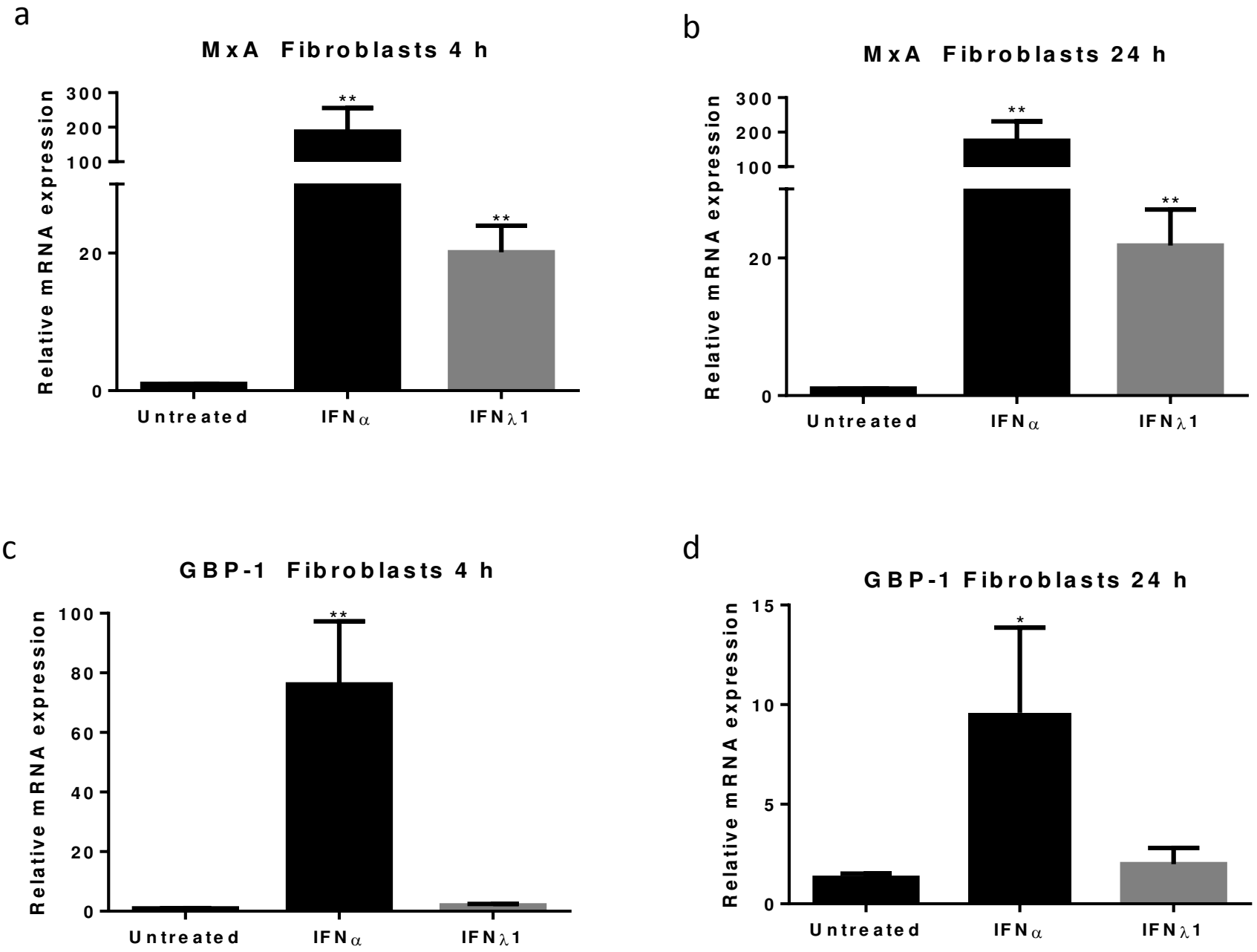

a
MxA Fibroblasts 4 h
Relative mRNA expression
300
200
100
20
0
**
**
Untreated
IFNα
IFNλ1
b
MxA Fibroblasts 24 h
Relative mRNA expression
300
200
100
20
0
**
**
Untreated
IFNα
IFNλ1
c
GBP-1 Fibroblasts 4 h
Relative mRNA expression
100
80
60
40
20
0
**
Untreated
IFNα
IFNλ1
d
GBP-1 Fibroblasts 24 h
Relative mRNA expression
15
10
5
0
*
Untreated
IFNα
IFNλ1

Figure 2

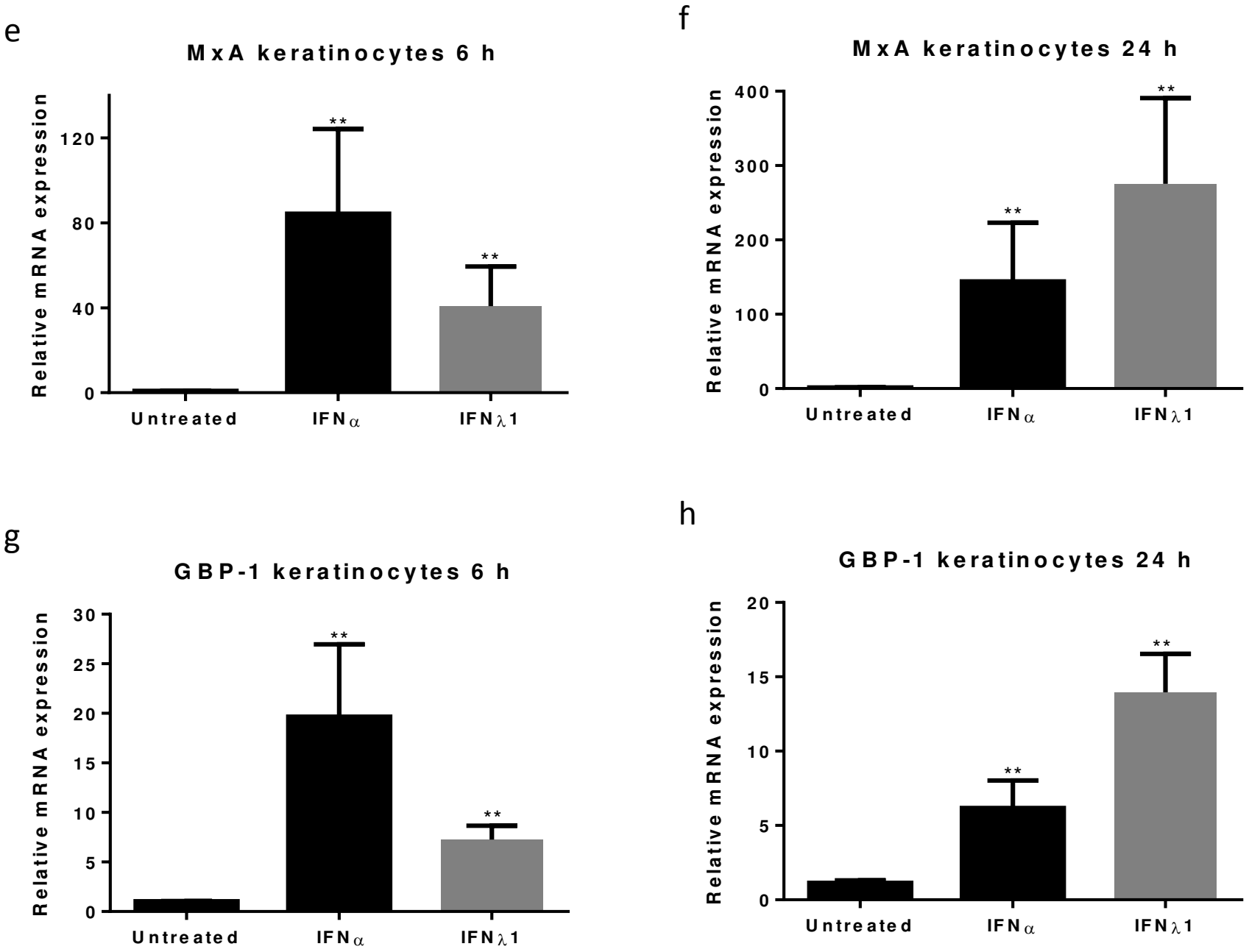

e
MxA keratinocytes 6 h
Relative mRNA expression
0
40
80
120
**
**
Untreated
IFNα
IFNλ1
f
MxA keratinocytes 24 h
Relative mRNA expression
0
100
200
300
400
**
**
Untreated
IFNα
IFNλ1
g
GBP-1 keratinocytes 6 h
Relative mRNA expression
0
5
10
15
20
25
30
**
**
Untreated
IFNα
IFNλ1
h
GBP-1 keratinocytes 24 h
Relative mRNA expression
0
5
10
15
20
**
**
Untreated
IFNα
IFNλ1

Figure 2

IFNλ1 induces the phosphorylation of p38 MAP kinase but not STAT1 (Tyr701) in dermal fibroblasts

Human dermal fibroblasts

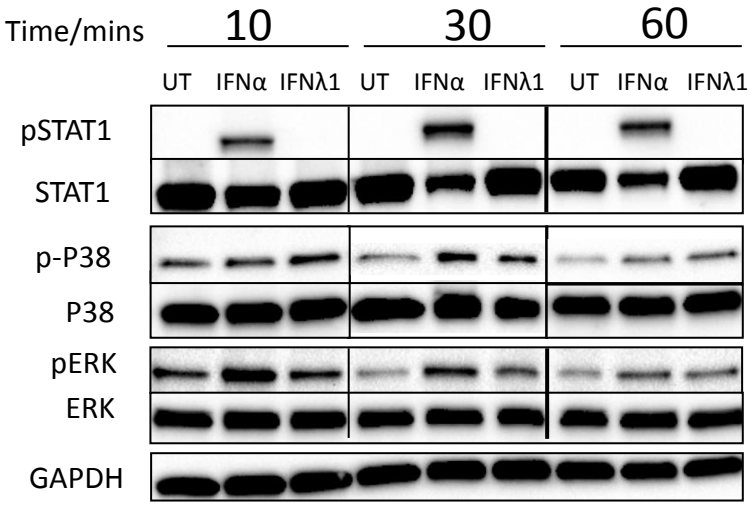


Figure 3a

Human primary keratinocytes

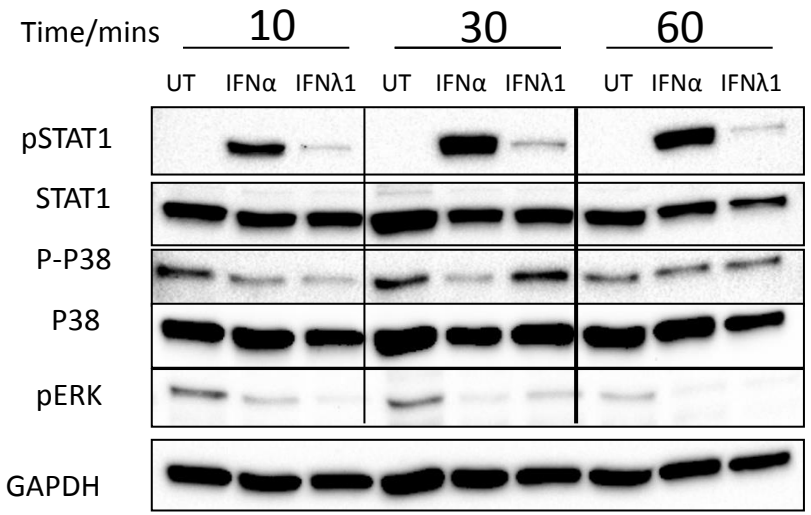
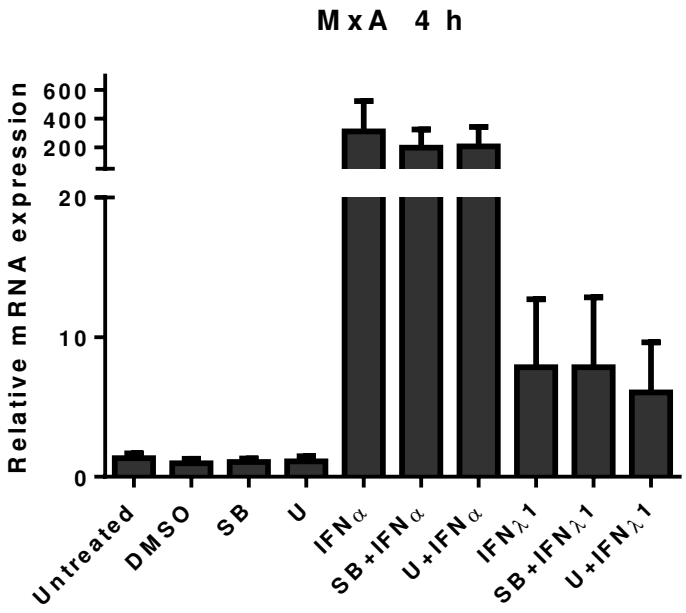


Figure 3b

Inhibition of p38 phosphorylation abrogated IFNλ1-induced MxA protein production by dermal fibroblasts

a


MxA 4 h
Relative mRNA expression
600
400
200
20
10
0
Untreated
DMSO
SB
U
IFNα
SB+IFNα
U+IFNα
IFNλ1
SB+IFNλ1
U+IFNλ1

b

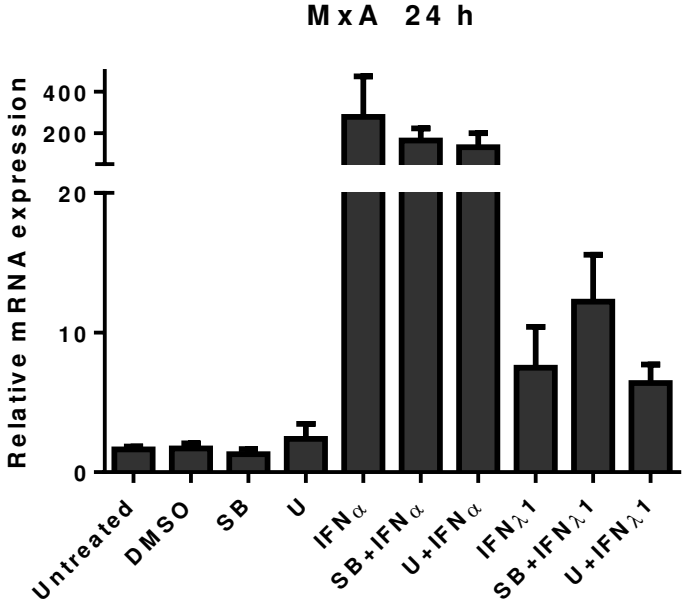
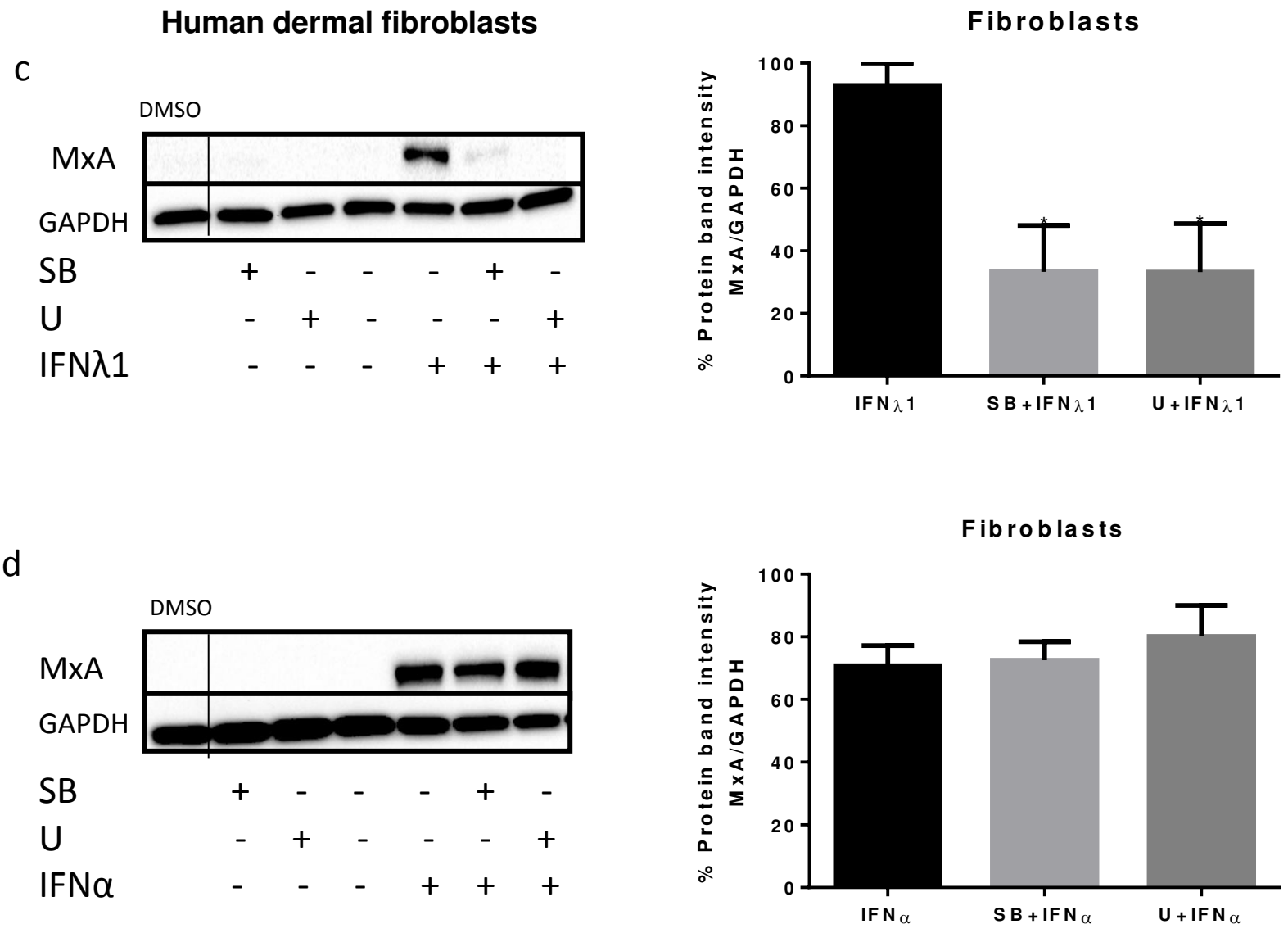

MxA 24 h
Relative mRNA expression
400
200
20
10
0
Untreated
DMSO
SB
U
IFNα
SB+IFNα
U+IFNα
IFNλ1
SB+IFNλ1
U+IFNλ1

Figure 4


Human dermal fibroblasts
c
DMSO
MxA
GAPDH
SB + - - - + -
U - + - - - +
IFNλ1 - - - + + +
Fibroblasts
% Protein band intensity MxA/GAPDH
100
80
60
40
20
0
IFNλ1
SB+IFNλ1
U+IFNλ1
d
DMSO
MxA
GAPDH
SB + - - - + -
U - + - - - +
IFNα - - - + + +
Fibroblasts
% Protein band intensity MxA/GAPDH
100
80
60
40
20
0
IFNα
SB+IFNα
U+IFNα

e

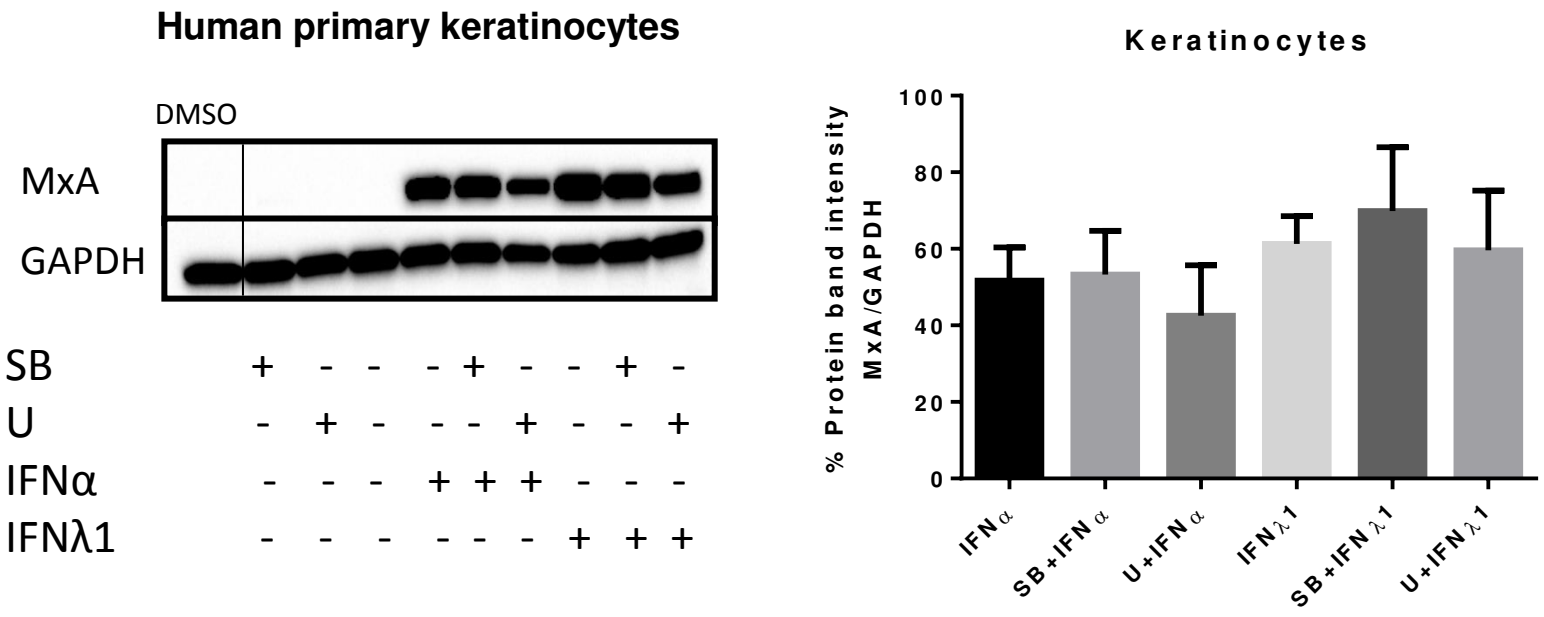

Human primary keratinocytes
DMSO
MxA
GAPDH
SB + - - - + - - + -
U - + - - - + - - +
IFNα - - - + + + - - -
IFNλ1 - - - - - - + + +
Keratinocytes
% Protein band intensity MxA/GAPDH
100
80
60
40
20
0
IFNα
SB+IFNα
U+IFNα
IFNλ1
SB+IFNλ1
U+IFNλ1

Figure 4

IFNλ1 synergise with TGFβ1 to enhance collagen mRNA in dermal fibroblasts

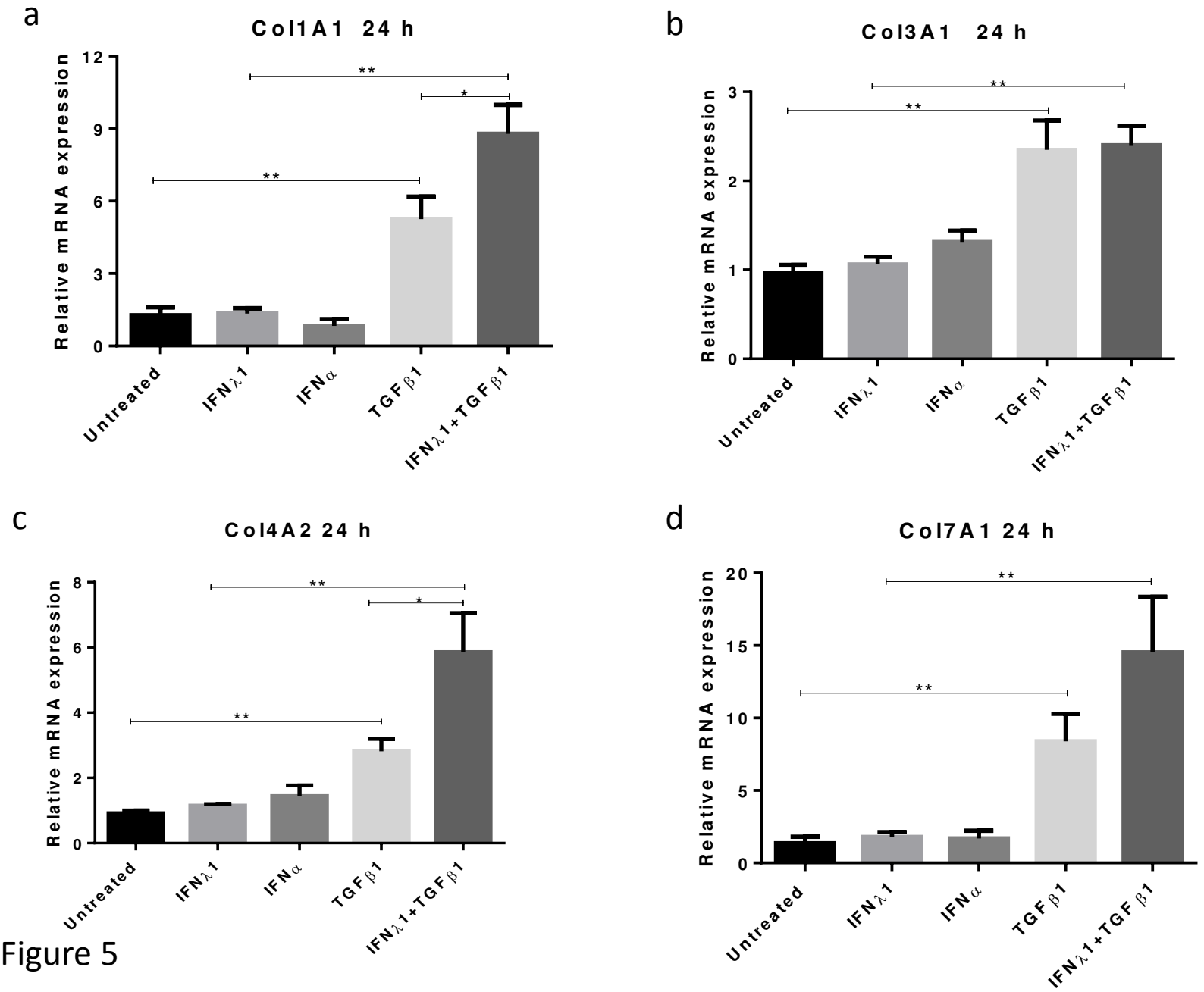

a
Col1A1 24 h
Relative mRNA expression
12
9
6
3
0
**
**
*
Untreated
IFNλ1
IFNα
TGFβ1
IFNλ1+TGFβ1
b
Col3A1 24 h
Relative mRNA expression
3
2
1
0
**
**
Untreated
IFNλ1
IFNα
TGFβ1
IFNλ1+TGFβ1
c
Col4A2 24 h
Relative mRNA expression
8
6
4
2
0
**
**
*
Untreated
IFNλ1
IFNα
TGFβ1
IFNλ1+TGFβ1
d
Col7A1 24 h
Relative mRNA expression
20
15
10
5
0
**
**
Untreated
IFNλ1
IFNα
TGFβ1
IFNλ1+TGFβ1

Figure 5

Primary keratinocytes

a

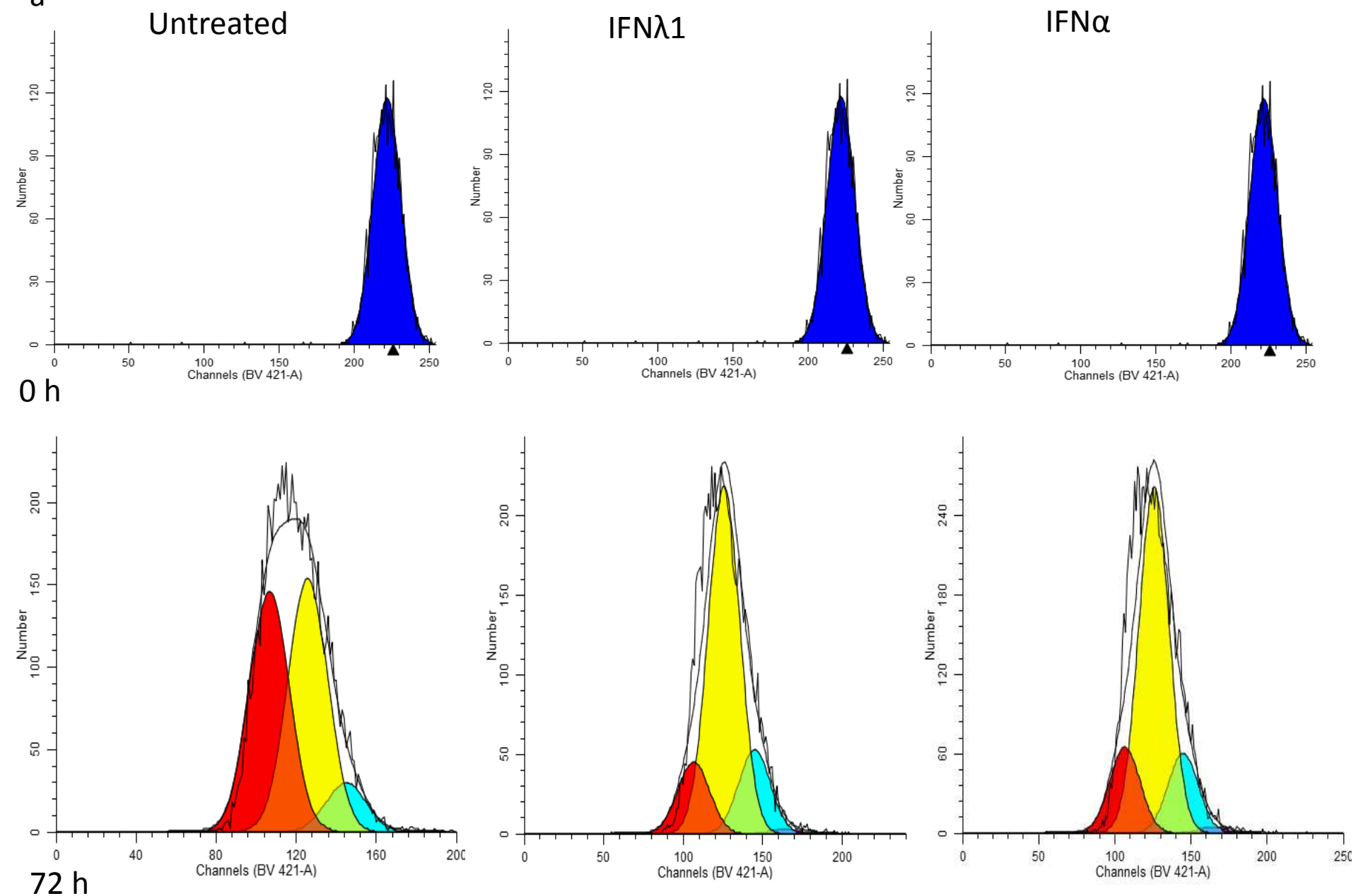


Figure 6

b

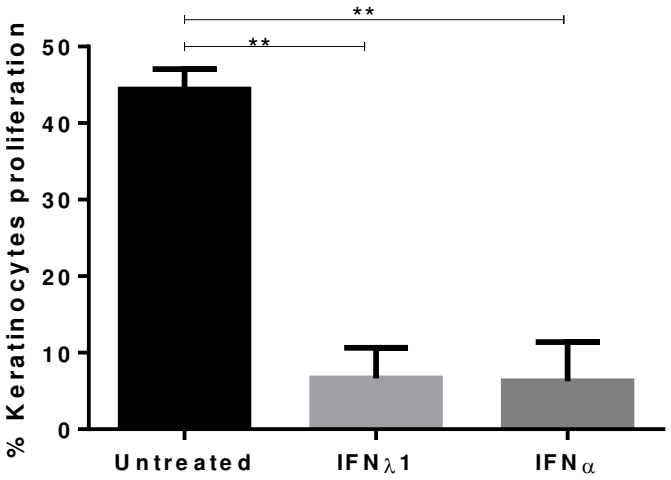

**
**
% Keratinocytes proliferation
50
40
30
20
10
0
Untreated
IFNλ1
IFNα

Figure 6

Dermal fibroblasts

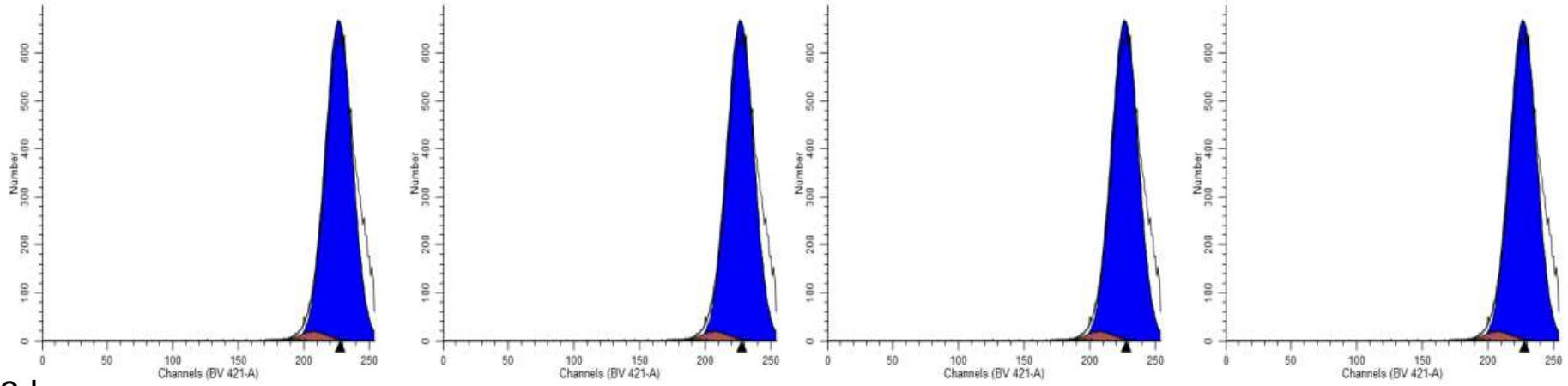
c

Untreated

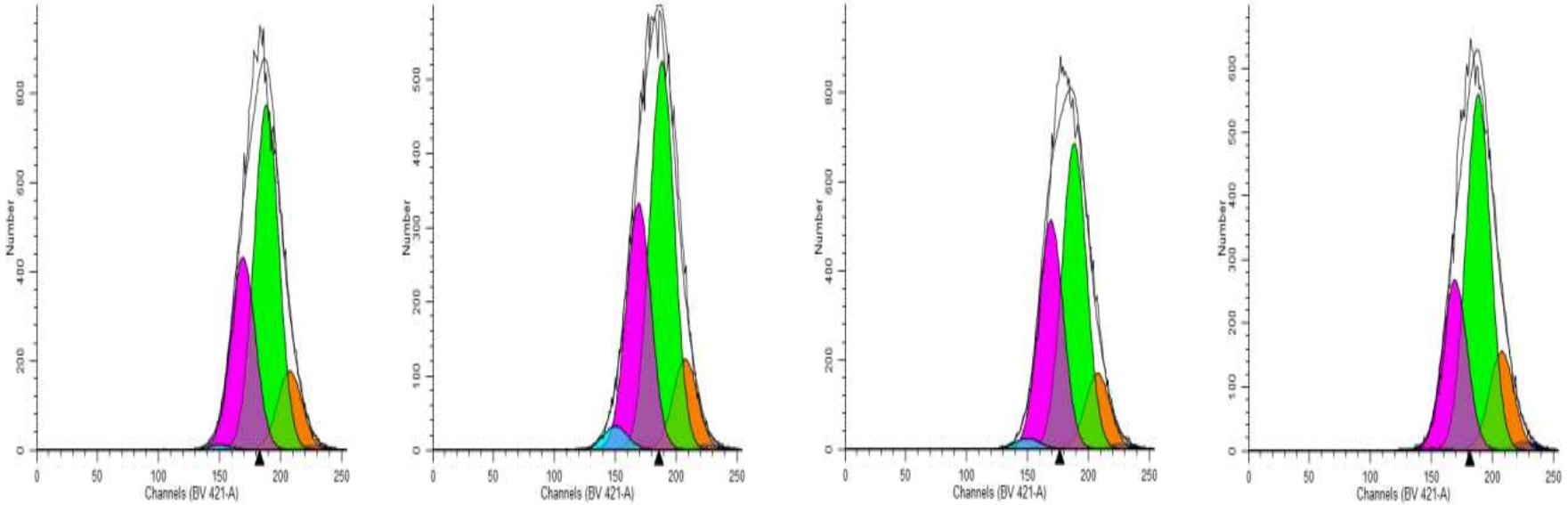
TGFβ1

IFNλ1

IFNα



0 h



72 h

Dermal fibroblasts

d

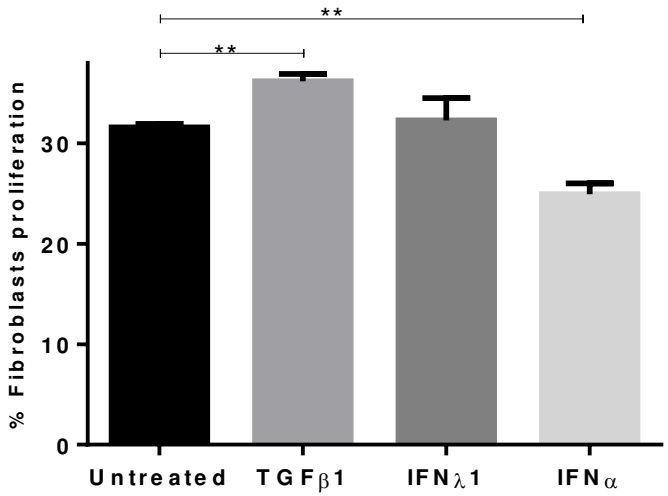
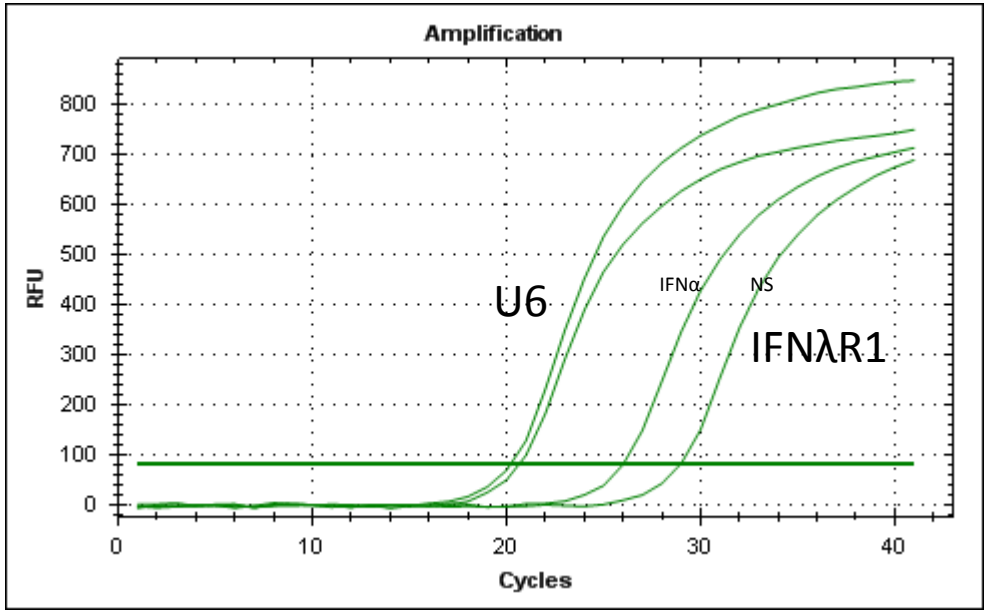


Figure 6

Supplementary figures


Amplification
RFU
800
700
600
500
400
300
200
100
0
0
10
20
30
40
Cycles
U6
IFNα
NS
IFNλR1

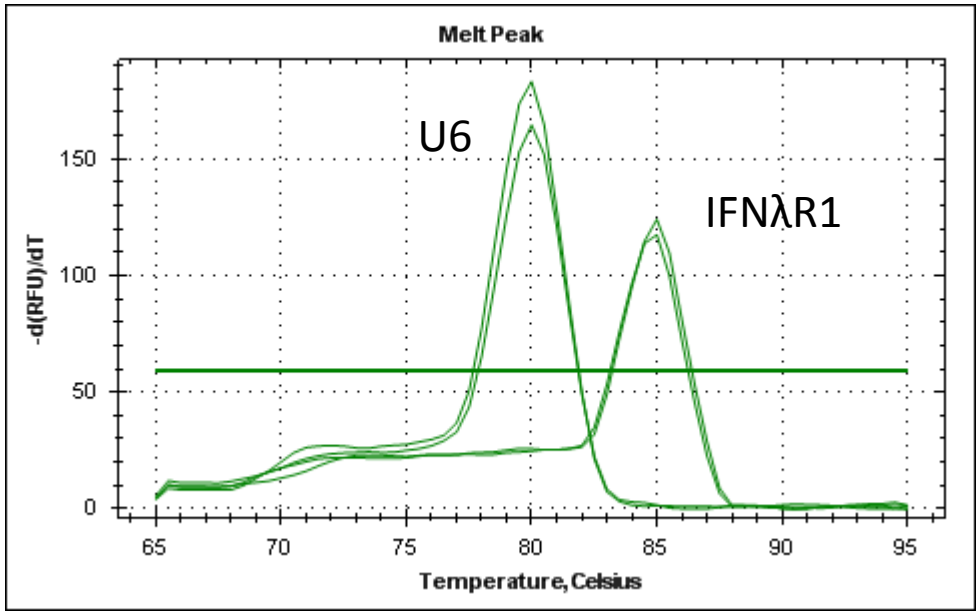

Melt Peak
-d(RFU)/dT
150
100
50
0
65
70
75
80
85
90
95
Temperature, Celsius
U6
IFNλR1

Figure S1

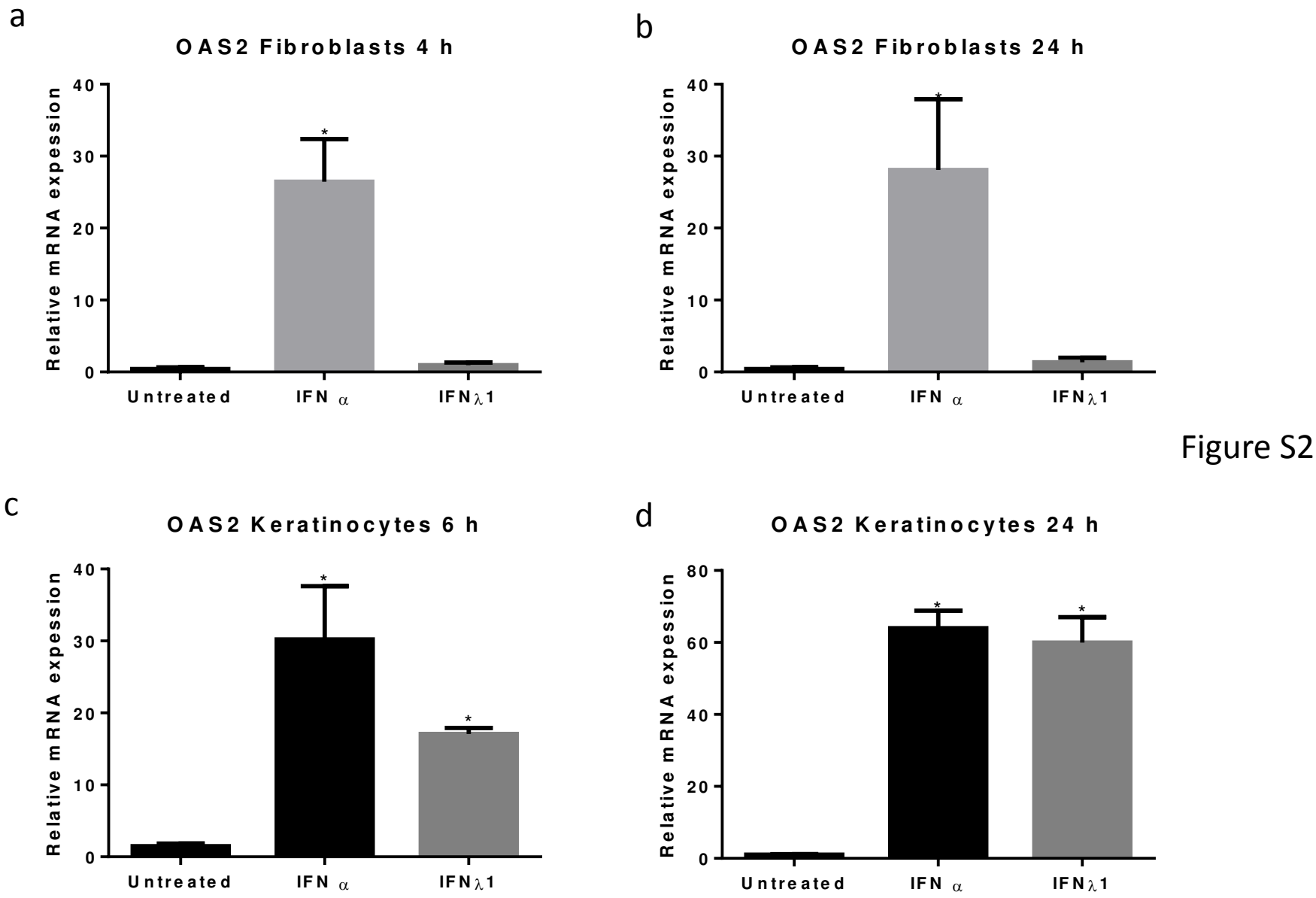

a
OAS2 Fibroblasts 4 h
Relative mRNA expession
40
30
20
10
0
*
Untreated
IFN α
IFNλ1
b
OAS2 Fibroblasts 24 h
Relative mRNA expession
40
30
20
10
0
*
Untreated
IFN α
IFNλ1
c
OAS2 Keratinocytes 6 h
Relative mRNA expession
40
30
20
10
0
*
*
Untreated
IFN α
IFNλ1
d
OAS2 Keratinocytes 24 h
Relative mRNA expession
80
60
40
20
0
*
*
Untreated
IFN α
IFNλ1

Figure S2

Dose Kinetic

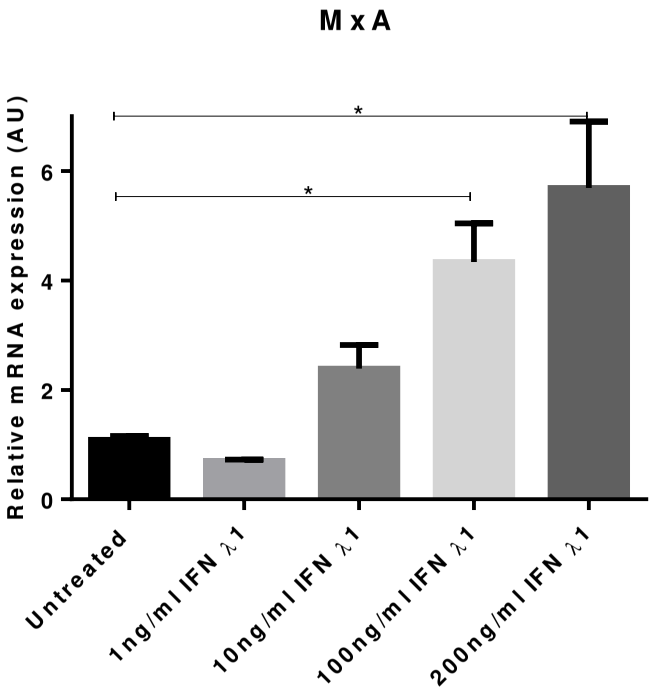


Figure S3

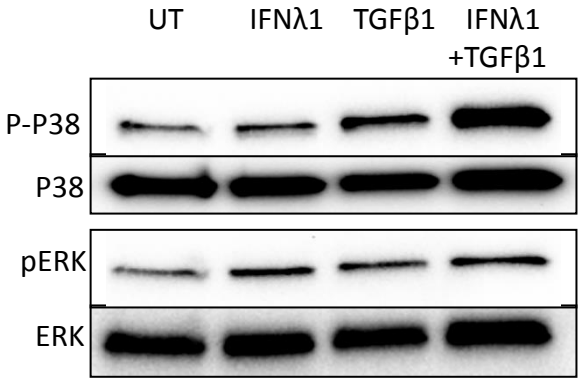
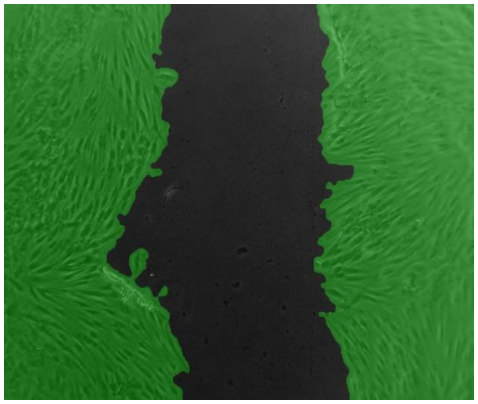
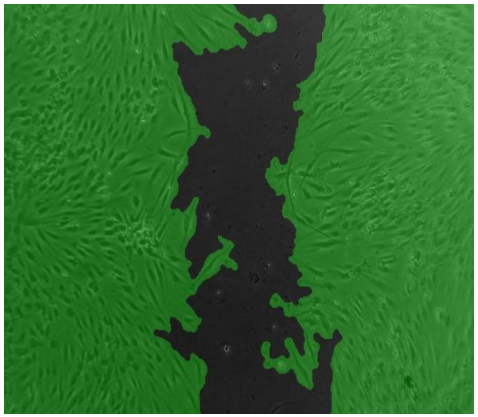
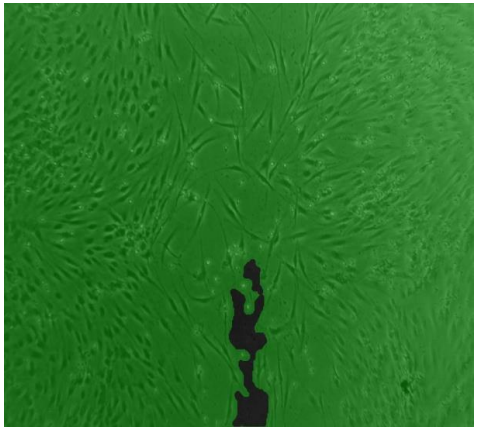
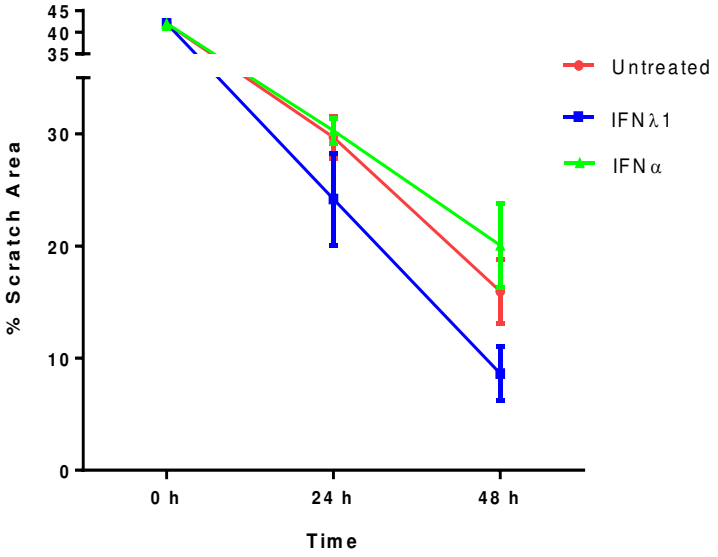
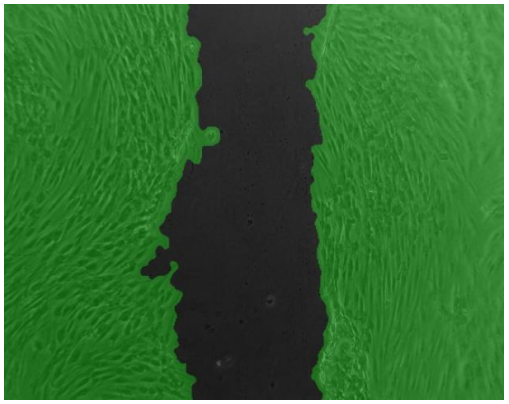
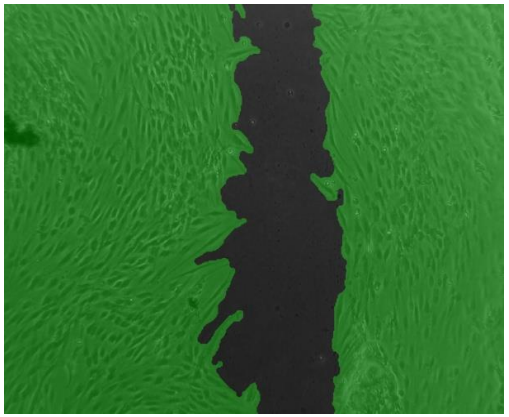


Figure S4




0 hr

24 hrs

48 hrs

Figure S5

IFNλ1

IFNα

Proposed IFNα and IFNλ signalling in fibroblasts

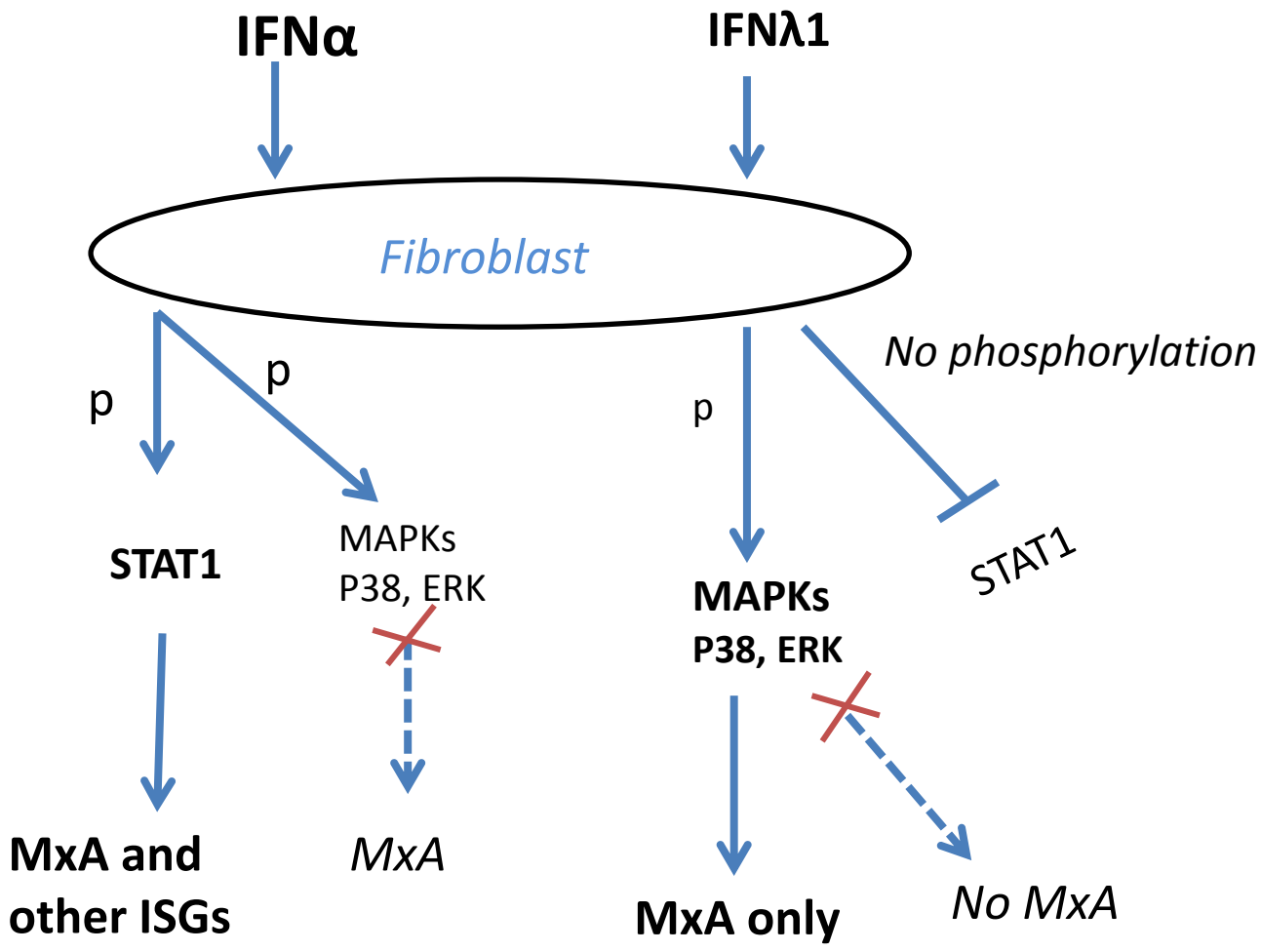
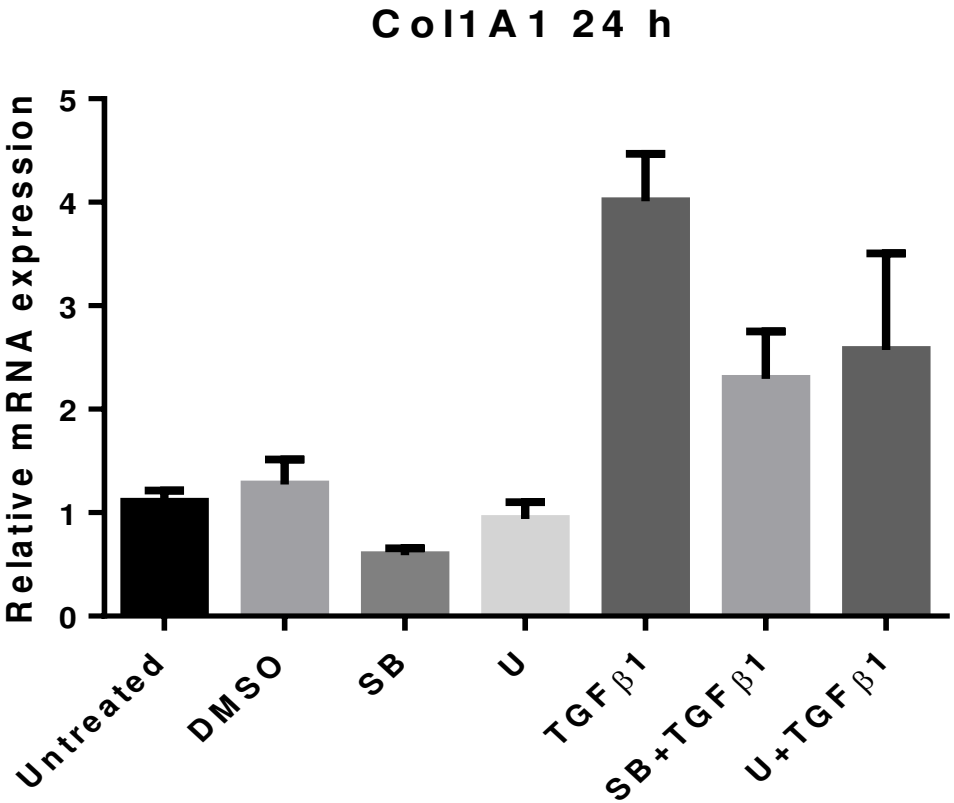


Figure S6


Col1A1 24 h
Relative mRNA expression
0
1
2
3
4
5
Untreated
DMSO
SB
U
TGFβ1
SB+TGFβ1
U+TGFβ1