Type-1 cytokines regulate matrix metalloprotease-9 production and E-cadherin disruption to promote melanocyte loss in vitiligo

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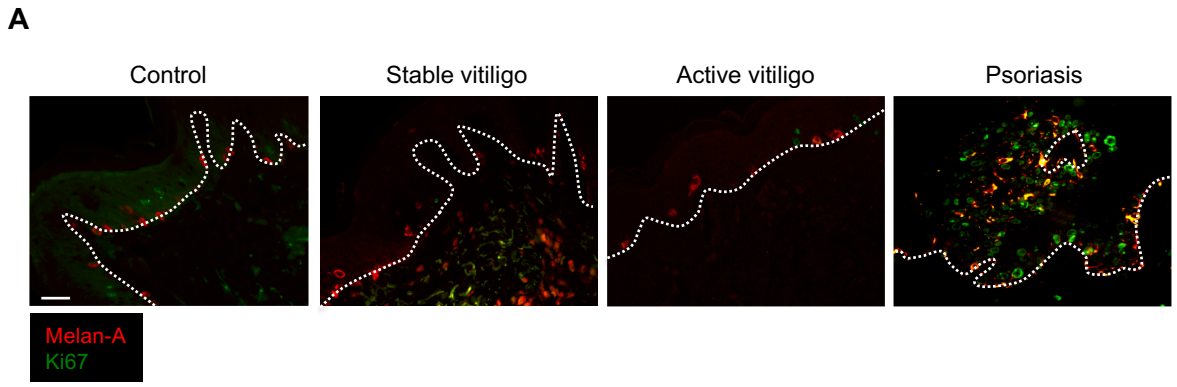
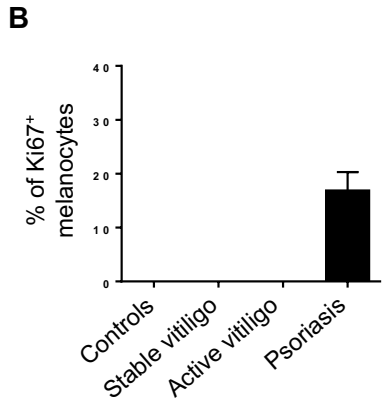
Supplemental Table 1. Distribution of individual features of 135 vitiligo patients depending on their spreading score

Feature	Stable vitiligo (n=69)	Active vitiligo (n=66)	Total (N=135)
Sex, No. (%)			
Female	41(59.42)	45(68.18)	86(63.70)
Male	28(40.58)	21(31.82)	49(36.29)
Age at inclusion (years)			
Mean (±SD)	43.43(±12.98)	43.64(±14.32)	43.53(±13.65)
Range	16-76	14-80	14-80
Age at vitiligo onset (years)			
Mean (±SD)	28.76(±14.93)	29.28(±16.87)	29.02(±15.9)
Range	3-57	4-77	3-77
Disease duration (years)			
Mean (±SD)	14.75(±12.65)	14.79(±11.93)	14.77 (±12.29)
Type of vitiligo, No. (%)			
Acrofacial	32(46.37)	3(4.54)	35(25.93)
Generalized	31(44.92)	47(71.21)	78(57.77)
Universal	2(2.90)	3(4.54)	5(3.71)
Mixed	3(4.35)	12(18.18)	15(11.11)
Unknown	1(1.44)	1(1.51)	2(1.48)
Personal history of autoimmune or autoinflammatory disease, No. (%)			
Yes, Autoimmune thyroiditis	6(8.69)	14(21.21)	20(14.81)
Yes, Other chronic inflammatory diseases	8(11.59)	6(9.09)	14(10.37)
Unknown	0	2(3.03)	2(1.48)
Koebner phenomenon, No. (%)			
Yes, Any type	45(65.21)	59(89.39)	104(77.04)
Koebner phenomenon type 1	8(11.59)	7(10.60)	15(11.11)
Koebner phenomenon type 2a	41(59.42)	59(89.39)	100(74.07)
Koebner phenomenon type 2b	11(15.94)	18(27.27)	29(21.48)
No	24(34.78)	5(7.58)	29(21.48)
Unknown	0	2(3.03)	2(1.48)
Affected body surface area (BSA) (%), mean (±SD)	12(±17.72)	24.18(±16.82)	18.10(±17.27)
Spreading, mean (±SD)	0.39(±0.49)	4.41(±0.80)	2.4 (±0.65)
Spreading, No. (%)			
0	42(60.87)	0	42(31.11)
(+1)	27(39.13)	0	27(20)
(+3)	0	13(19.69)	13(9.62))
(+4)	0	12(18.18)	12(8.88)
(+5)	0	41(62.12)	41(30.37)

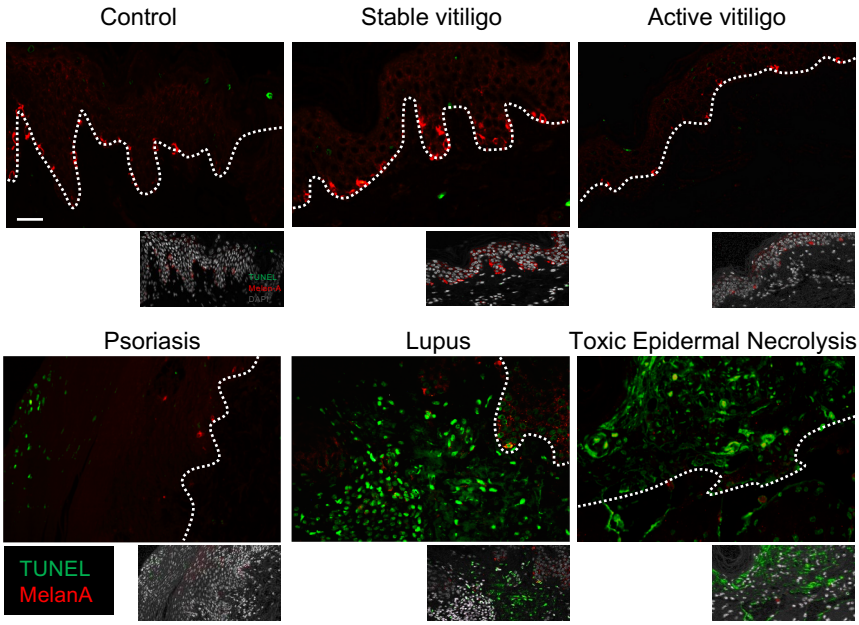
Supplemental Table 2. Distribution of individual features of 37 psoriasis patients

Feature	Psoriasis (n=37)
Sex, No. (%)	
Female	15(40.54)
Male	22(59.45)
Age at inclusion (years)	
Mean (±SD)	49.62(±20.91)
Range	19-94
Age at v onset (years)	
Mean (±SD)	34.11(±20.72)
Range	5-83
Disease duration (years)	
Mean (±SD)	15.51(±17.80)
PASI score	
Mean (±SD)	17.88(±13.63)
Body mass index (BMI) (kg/cm^2)	
Mean (±SD)	25.46(±5.15)

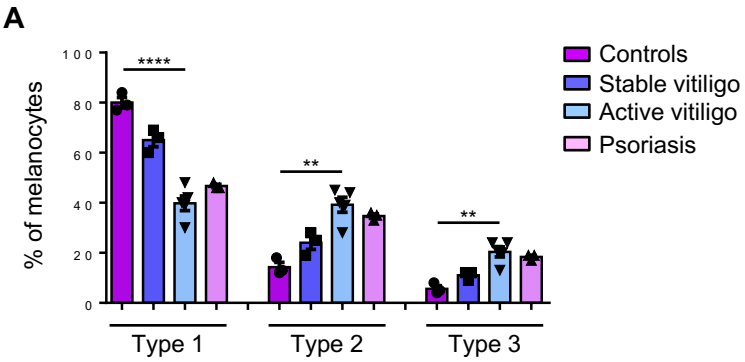


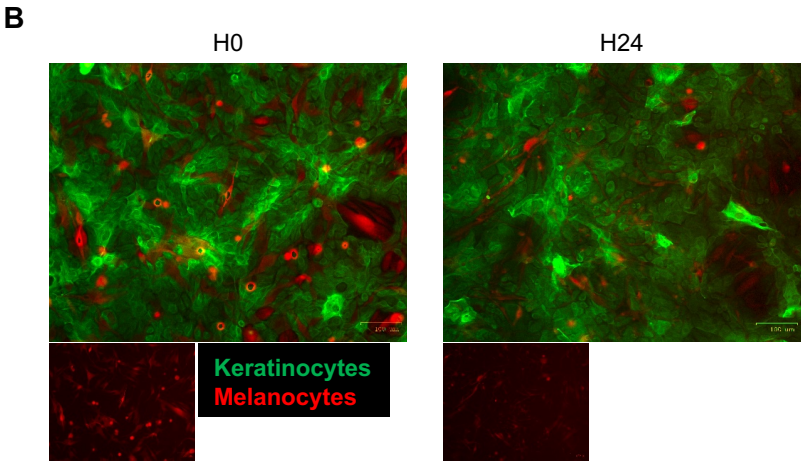


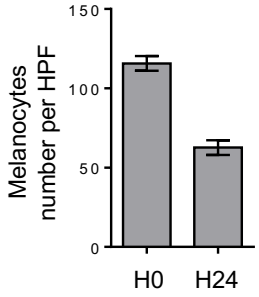
Supplemental Figure 1. Melanocyte proliferation in vitiligo and psoriasis patients. (A) Representative immunofluorescence analysis of Ki67 (green) and melan-A (red) staining in control healthy skin, stable or active vitiligo perilesional skin, and psoriasis lesional skin. Dashed lines represent the dermo-epidermal layer. Scale bar represents 40μm. (**B**) Proportion of Ki67$^+$ melanocytes in control healthy skin (n=3), stable (n=5) or active vitiligo perilesional skin (n=5) and psoriasis lesional skin (n=4). Data show mean + SEM.

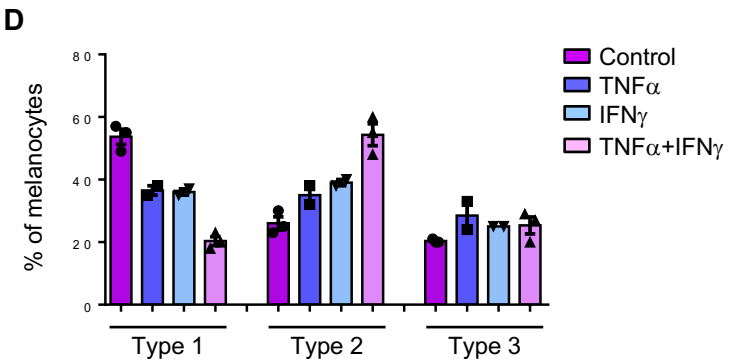


Supplemental Figure 2. Analysis of melanocyte apoptosis in vitiligo and psoriasis skin. Representative analysis of epidermal cell death using a TUNEL assay (green). Melanocytes were stained with anti-melan-A antibody (red), in control healthy skin, stable and active vitiligo perilesional skin, or lesional skin from psoriasis, cutaneous lupus erythematous, and toxic epidermal necrolysis. Dashed lines represent dermo-epidermal layer. Scale bar represents 50μm. Data are representative of five independent experiments.









Supplemental Figure 3. Adhesion defect of melanocytes in vitiligo patients skin and *in vitro* in response to TNFα and IFNγ. (**A**) Proportion of melanocytes displaying type 1, type 2, or type 3 E-cadherin labeling in healthy control skin (n=3), perilesional skin of patients with stable (n=3) or active (n=5) vitiligo or psoriasis lesional skin (n=3). (**B-C**) Impact of TNFα and IFNγ on melanocyte adhesion in cocultures of primary human keratinocytes (green) and melanocytes (red). (B) Representative images before (H0) and after 24h of treatment with 10ng/ml of TNFα and IFNγ (H24). (C) The number of melanocytes was evaluated before and after treatment. (**D**) Proportion of melanocytes displaying type 1, type 2, or type 3 labeling (n=3) in RHPE treated for 24h in the presence or absence of 10ng/ml of TNFα and/or IFNγ. Type 1: Homogeneous E-cadherin staining; type 2: Heterogeneous E-cadherin staining; and type 3: Absence of E-cadherin staining. Data show mean ± SEM. **P* < 0.05, calculated with Kruskall-Wallis test.

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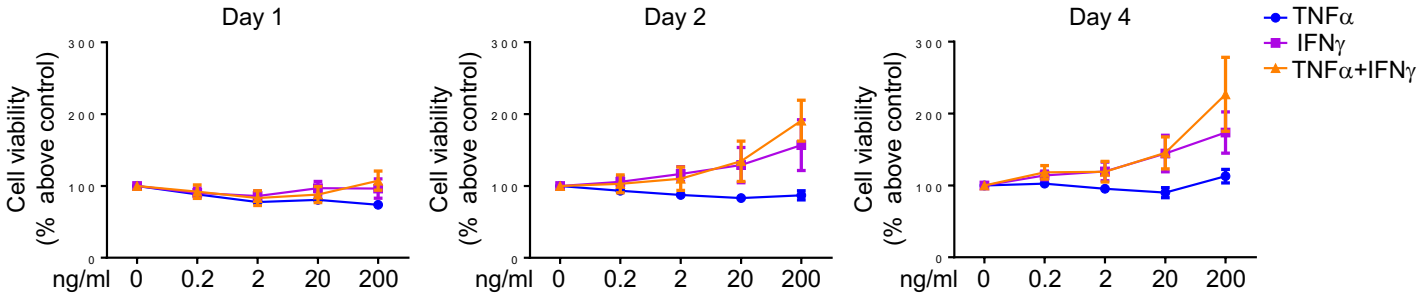

Supplemental Figure 4. TNFα and IFNγ impact on melanocyte viability. NHEM viability was assessed in response to increasing concentrations of TNFα and/or IFNγ for the indicated time. Results are shown as % of viable cells in comparison to the control culture. Data show mean ± SEM of 6 independent experiments.

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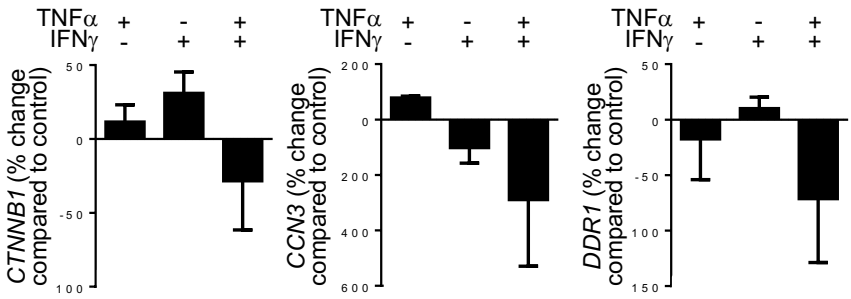
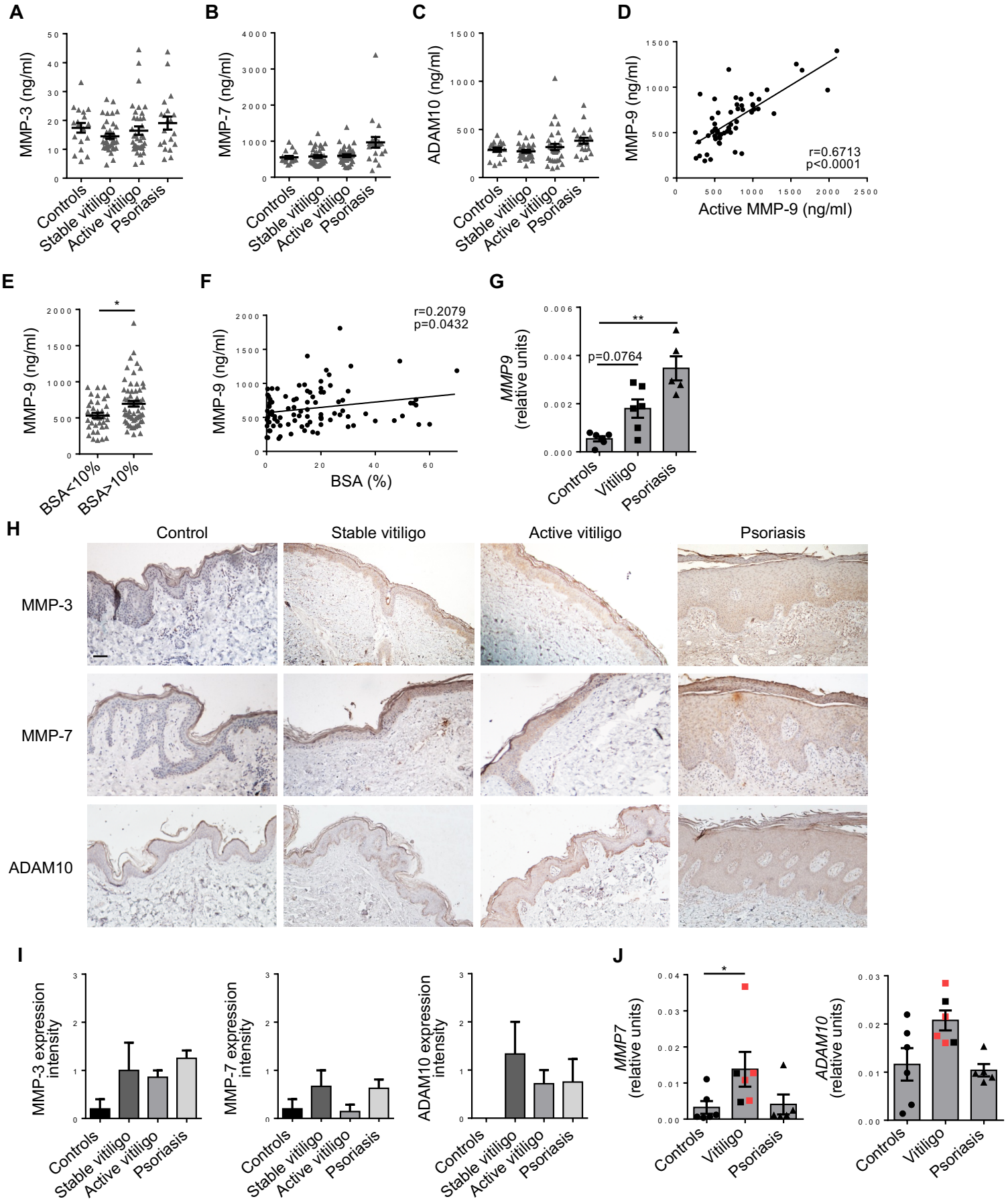
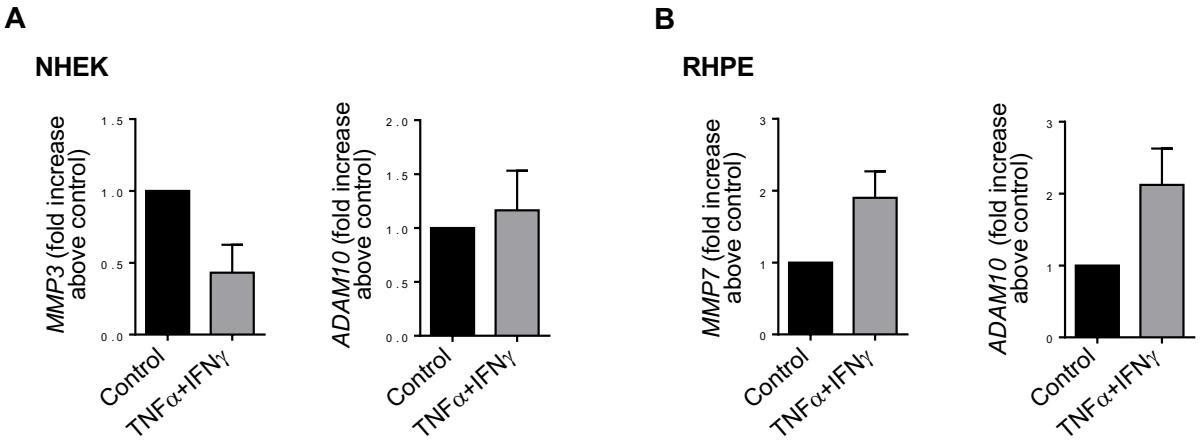


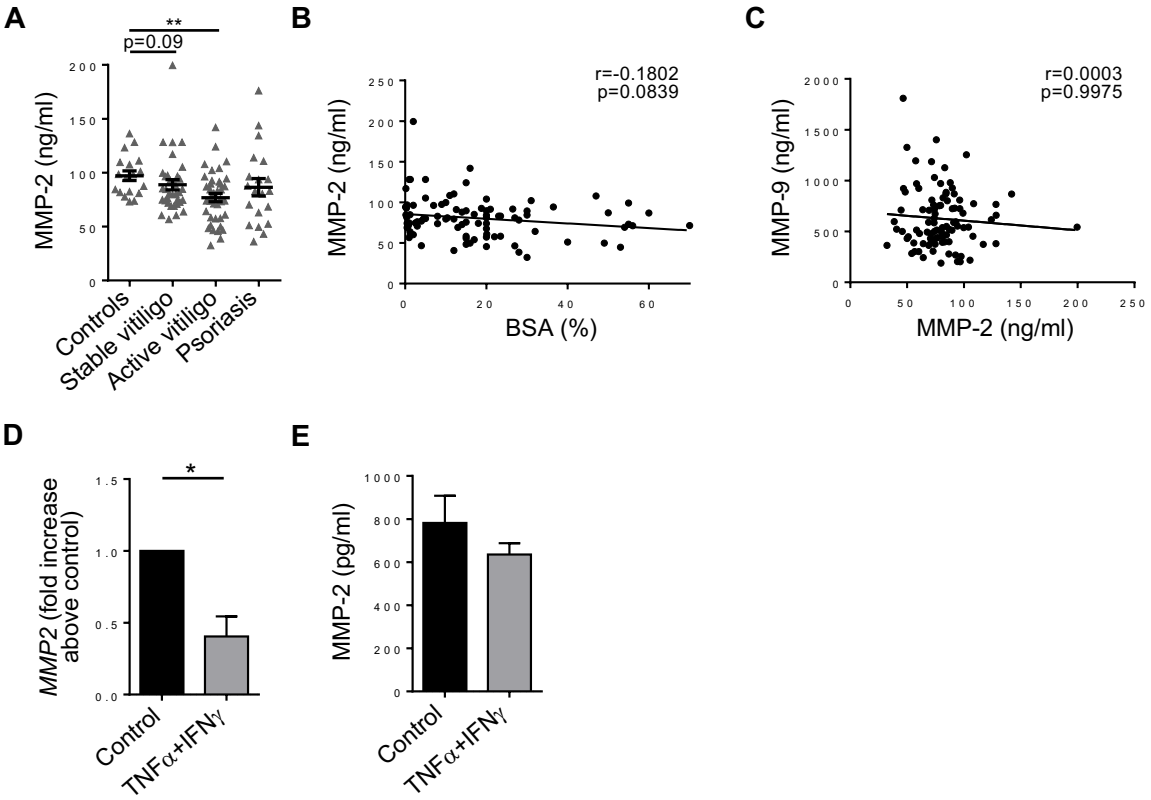
Supplemental Figure 5. Biological activity of type-1 cytokines IFNγ and TNFα on melanocyte adhesion. Primary cultures of NHEM were stimulated in the presence or absence of 20ng/ml of IFNγ and/or TNFα. *CTNNB1*, *CCN3*, and *DDR1* gene expression was analyzed by real-time PCR. Results are shown as the % of change compared to the control culture. *GAPDH* was used as housekeeping gene. Data show mean ± SEM of 4 to 7 independent experiments.


A
MMP-3 (ng/ml)
Controls
Stable vitiligo
Active vitiligo
Psoriasis
B
MMP-7 (ng/ml)
C
ADAM10 (ng/ml)
D
MMP-9 (ng/ml)
r=0.6713
p<0.0001
Active MMP-9 (ng/ml)
E
MMP-9 (ng/ml)
*
BSA<10%
BSA>10%
F
MMP-9 (ng/ml)
r=0.2079
p=0.0432
BSA (%)
G
MMP9 (relative units)
**
p=0.0764
Controls
Vitiligo
Psoriasis
H
Control
Stable vitiligo
Active vitiligo
Psoriasis
MMP-3
MMP-7
ADAM10
I
MMP-3 expression intensity
MMP-7 expression intensity
ADAM10 expression intensity
Controls
Stable vitiligo
Active vitiligo
Psoriasis
J
MMP7 (relative units)
*
ADAM10 (relative units)
Controls
Vitiligo
Psoriasis

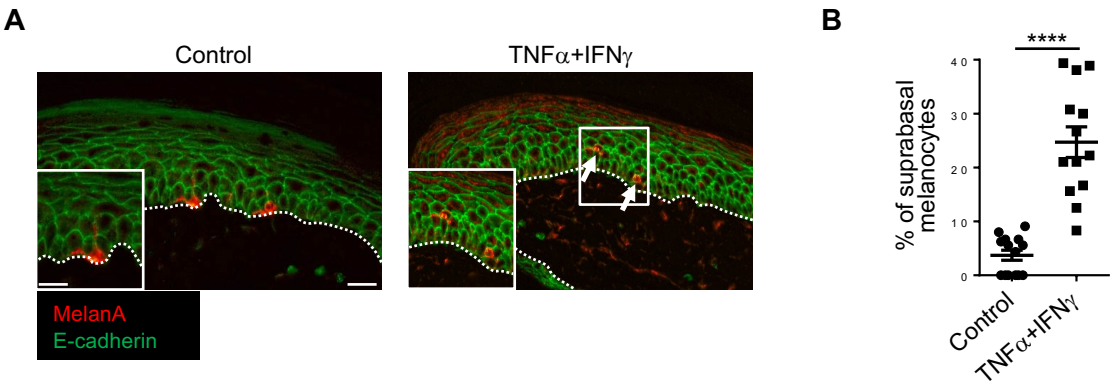
Supplemental Figure 6. Expression of MMPs in blood and skin of vitiligo and psoriasis patients. (**A-C**) Assessment by ELISA of levels of MMP-3 (**A**), MMP-7 (**B**), and ADAM10 (**C**) in sera of healthy controls (n=18), patients with stable (n=37) or active (n=37) vitiligo, or psoriasis (n=20). (**D**) Spearman's rho correlation (two-tailed) between serum total MMP-9 and active MMP-9 levels (n=56). (**E**) MMP-9 serum levels in vitiligo patients according to the BSA involved (BSA<10%, n=36, or BSA>10%, n=59). (**F**) Spearman's rho correlation (two-tailed) between serum total MMP-9 and BSA involved (n=95) in vitiligo patients. (**G**) Real-time PCR analysis of *MMP9* gene expression in control healthy skin (n=6), vitiligo perilesional skin (n=6), and lesional psoriasis skin (n=5). (**H**) Representative IHC staining of MMP-3 (top), MMP-7 (middle), and ADAM10 (bottom) in healthy skin, perilesional skin of stable and active vitiligo, and psoriasis lesional skin. Scale bar represents 100µm. (**I**) Semi-quantitative analysis of MMP-3, MMP-7, and ADAM10 expression in skin from healthy controls (n=3-5), perilesional skin of vitiligo patients with stable (n=2-3) or active (n=7) disease, and lesional psoriasis skin (n=4-8). (**J**) Real-time PCR analysis of *MMP3* and *ADAM10* gene expression in control healthy skin (n=6), vitiligo perilesional skin (n=6; black squares: stable vitiligo, red squares: active vitiligo), and psoriasis lesional skin (n=5). Data in A,B,C,E,G,I,J show mean ± SEM. **P* < 0.05, ***P* < 0.01 ; calculated with Wilcoxon (E) or Kruskal-Wallis (G,J) tests.



Supplemental Figure 7. Expression of *MMP3*, *MMP7*, and *ADAM10* genes in NHEK and RHPE. (**A**) NHEK and (**B**) RHPE were stimulated for 24h in the absence or presence of TNFα and IFNγ. Real-time PCR analysis of *MMP3, ADAM10,* and *MMP7* gene expression. *GAPDH* was used as a housekeeping gene. Data are shown as fold increase above the control culture (mean + SEM of 7 (A) or 6 (B) independent experiments). *MMP*3 and *MMP7* genes were below the detection limit in NHEK and RHPE respectively.



Supplemental Figure 8. Expression of MMP-2 in vitiligo patients. (**A**) Assessment by ELISA of MMP-2 levels in sera of healthy controls (n=18), patients with stable (n=34) or active (n=39) vitiligo, or psoriasis (n=20). (**B-C**) Spearman's rho correlation (two-tailed) between (**B**) MMP-2 serum levels and BSA involved and (**C**) MMP-2 and MMP-9 serum levels in vitiligo patients (n=94). (**D-E**) NHEK were stimulated for 24h in the absence or presence of 20 ng/ml of TNFα and IFNγ. (**D**) Real-time PCR analysis of *MMP2* gene expression. *GAPDH* was used as housekeeping gene. Data are shown as fold increase above the control culture. (**E**) ELISA analysis of MMP-2 levels in cell-free culture supernatants. Data show mean + SEM of six independent experiments. **P* < 0.05, ***P* < 0.01; calculated with Kruskall-Wallis (A) or Wilcoxon (D) tests.



Supplemental Figure 9. *In vivo* impact of TNFα and IFNγ on melanocyte detachment. C57BL/6 mouse tails were treated by intradermal injection of saline solution (control) or 1µg TNFα and IFNγ according to the same protocol described in Figure 4F. (**A**) Representative immunofluorescence analysis of melan-A (red) and E-cadherin (green) staining in the different groups. Dashed lines represent the dermo-epidermal layer. Arrows show suprabasal melanocytes. Scale bars represent 20µm. (**B**) Proportion of suprabasal melanocytes was assessed in the different groups (n=7-9). Data shown mean ± SEM ****$P < 0.0001$, calculated with two-tailed Mann–Whitney test.