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Article

Human Keratinocytes Inhibit CD4+ T-Cell Proliferation through TGFB1 Secretion and Surface Expression of HLA-G1 and PD-L1 Immune Checkpoints

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Supplementary Materials

Figure S1. Maps of HLA-G1 and control vectors.

Figure S2. Fibroblast supernatant and PBMC proliferation inhibition.

Figure S3. HLA-G isoform expressed in keratinocytes.

Figure S4. Influence of EGF on PD-L1 expression in keratinocytes.

Figure S5. Impact of induced HLA-G1 overexpression on PD-L1 and TGFB1 expression in keratinocytes.

Figure S6. Cell-surface expression of the HLA-G receptors ILT2 and ILT4 in keratinocytes.

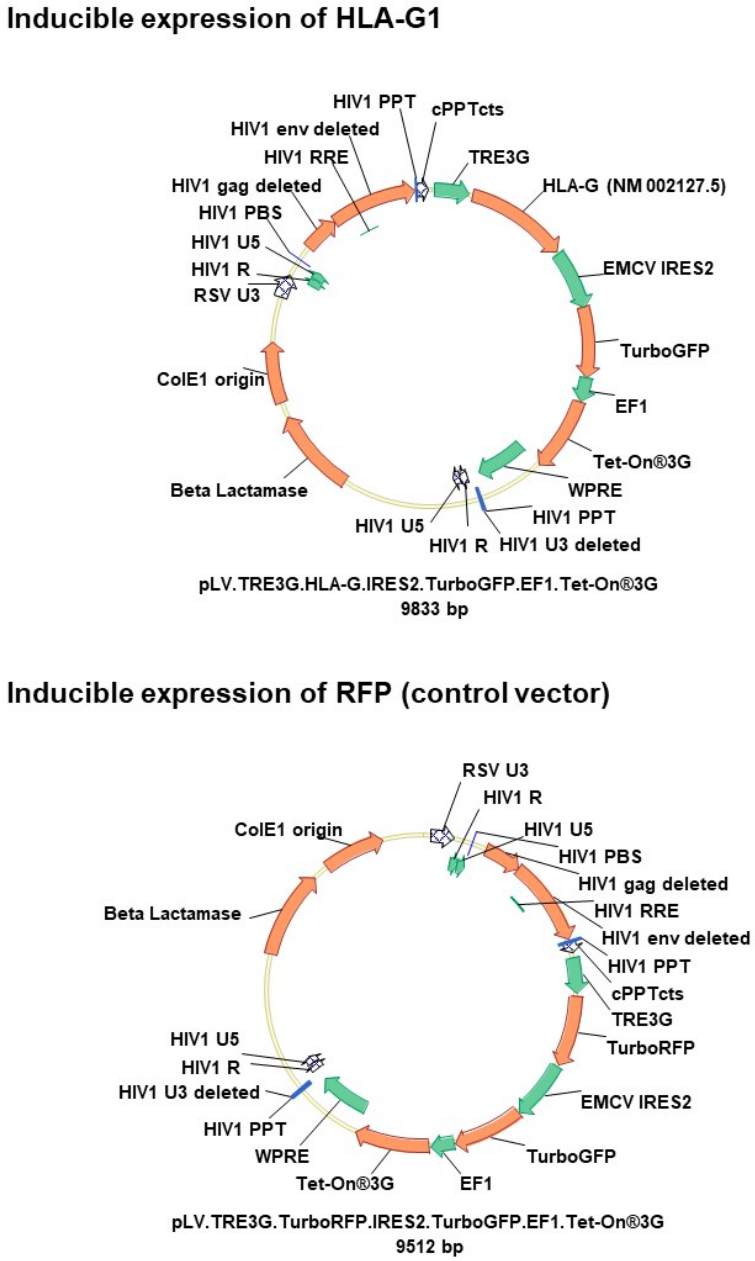

Figure S1. Maps of HLA-G1 and control lentiviral vectors.

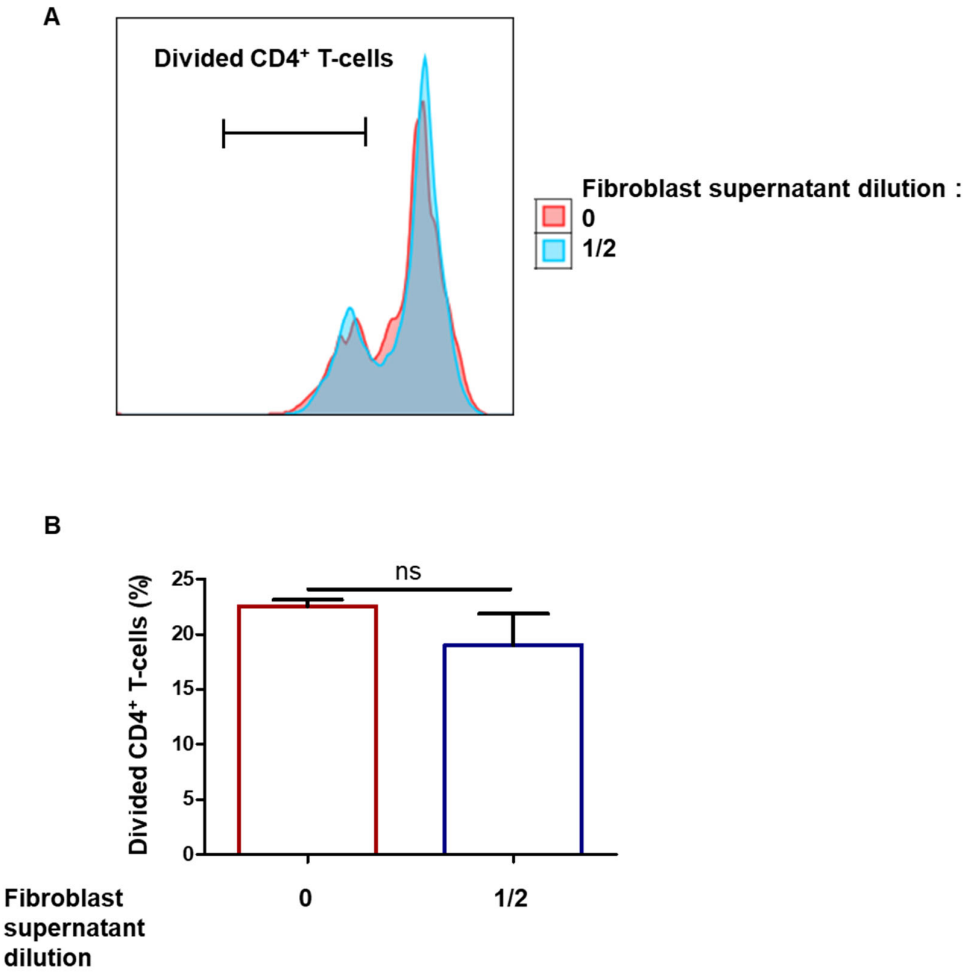

Figure S2. Fibroblast supernatant and PBMC proliferation inhibition. Fibroblast supernatants from one representative donor were incubated with 100,000 PBMCs for 7 days. PBMCs were pre-marked with a dye and activated using $CD3^+$ $CD28^+$ beads. PBMC proliferation was quantified by dye decrease at day 7. (**A**) Representative flow cytometry profiles at day 7. (**B**) $CD4^+$ T-cell proliferation depending on presence of fibroblast supernatant (mean+SEM, $p < 0.05$, $n = 3$). Exact p-values were determined on t-test.

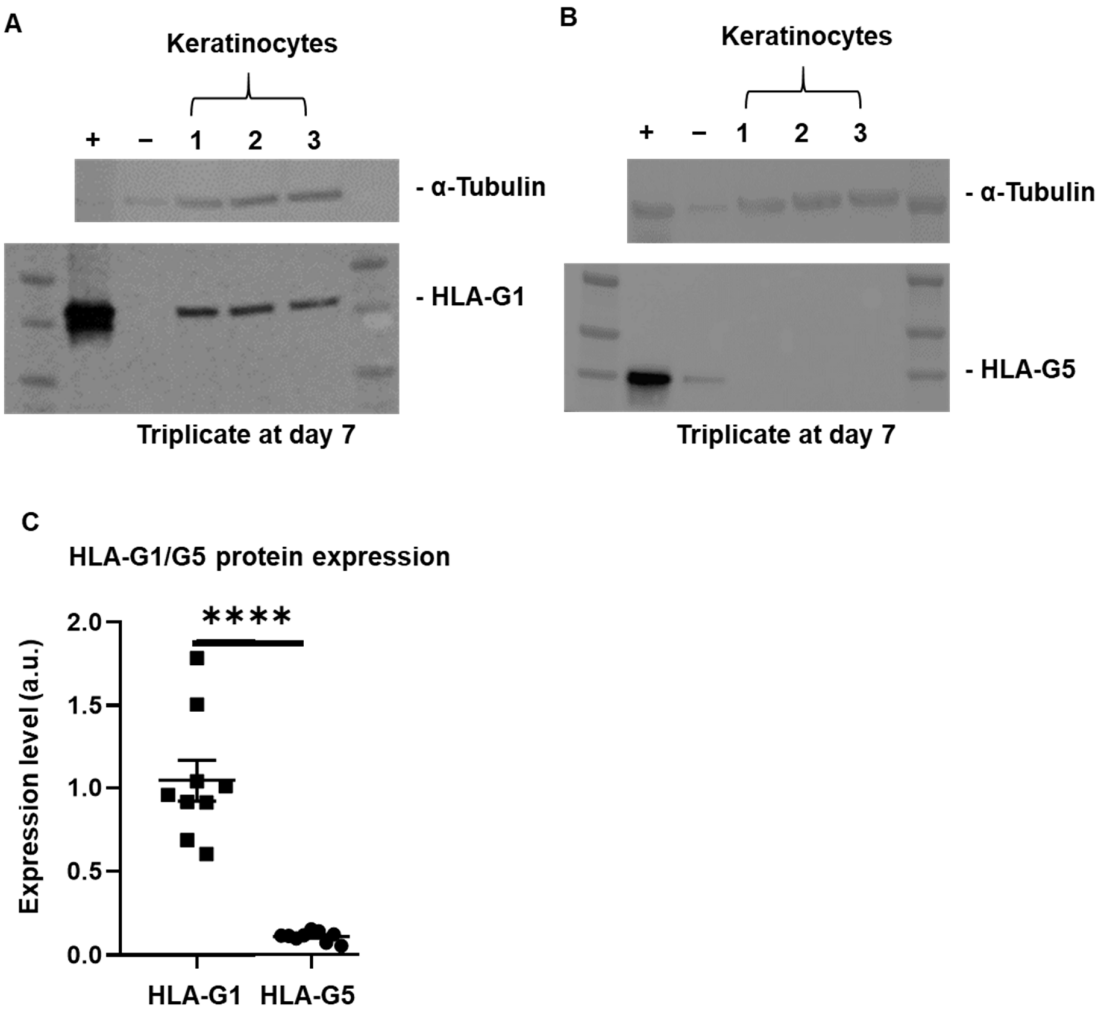

Figure S3. HLA-G isoform expressed in keratinocytes. Cells from one representative donor were cultivated for 7 days after removal in an undefined medium with serum and a layer of feeder cells. Analysis by Western blotting. (**A**) Typical gel photograph corresponding to 3 different cultures, with α-tubulin detection as loading control. K562 cells were used as negative control and K562 cells transduced with HLA-G1 were used as positive control with 4H84 antibody. (**B**) Typical gel photograph corresponding to 3 different cultures, with α-tubulin detection as loading control. M8 cells were used as negative control and M8 cells transduced with HLA-G5 were used as positive control with 5A6G7 antibody. (**C**) Scatter plot of quantification (mean+SEM, $p < 0.0001$, $n = 9$). Exact p-values were determined using the Mann-Whitney U-test. ****: $p<0.0001$.

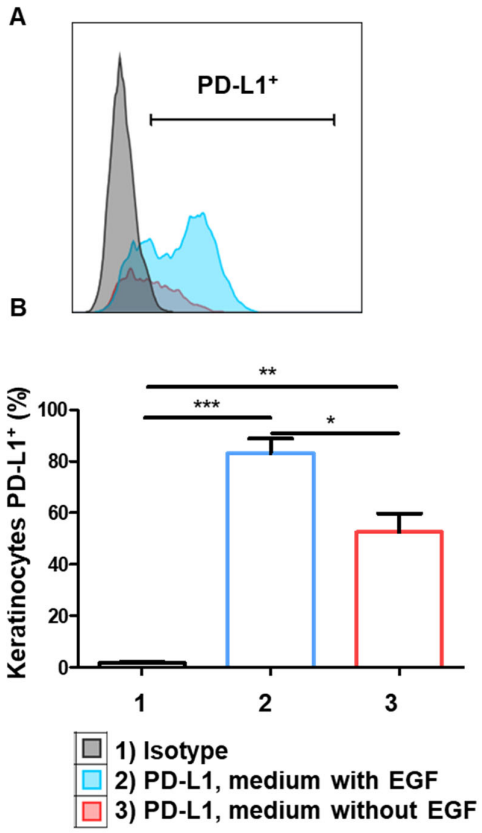

Figure S4. Influence of EGF on PD-L1 expression in keratinocytes. Amplified keratinocytes expressing PD-L1 were reamplified with medium with or without EGF. (**A**) Representative cytometry profiles after 7 days. (**B**) PD-L1 expression according to presence of EGF in the medium (mean+SEM, $p < 0,05$, n=3). Exact p-values were determined on t-test. *: $p < 0.05$, **: $p < 0.01$, and ***: $p < 0.001$.

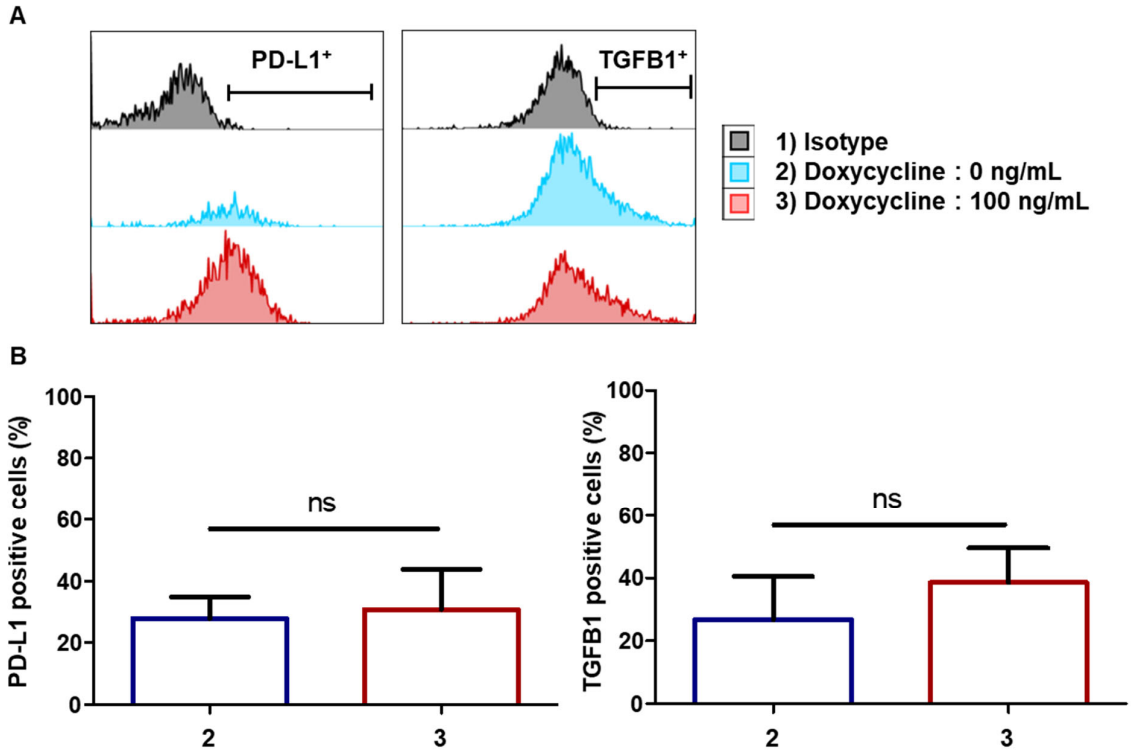

Figure S5. Impact of induced HLA-G1 overexpression on PD-L1 and TGFB1 expression in keratinocytes. Keratinocytes cultures of transduced with the lentiviral vector allowing doxycycline-inducible expression of HLA-G1 were maintained for 7 days in induced and non-induced conditions. Expression of PD-L1 and TGFB1 proteins was analyzed in both control and HLA-G1-overexpressing keratinocytes. (**A**) Representative profiles of PD-L1 and TGFB1 expression are shown. (**B**) Absence of PD-L1 and TGFB1 protein expression modulation in response to HLA-G1 overexpression induced by doxycycline treatment (mean+SEM, $p<0.05$, $n=3$). Exact p-values were determined using the t-test.

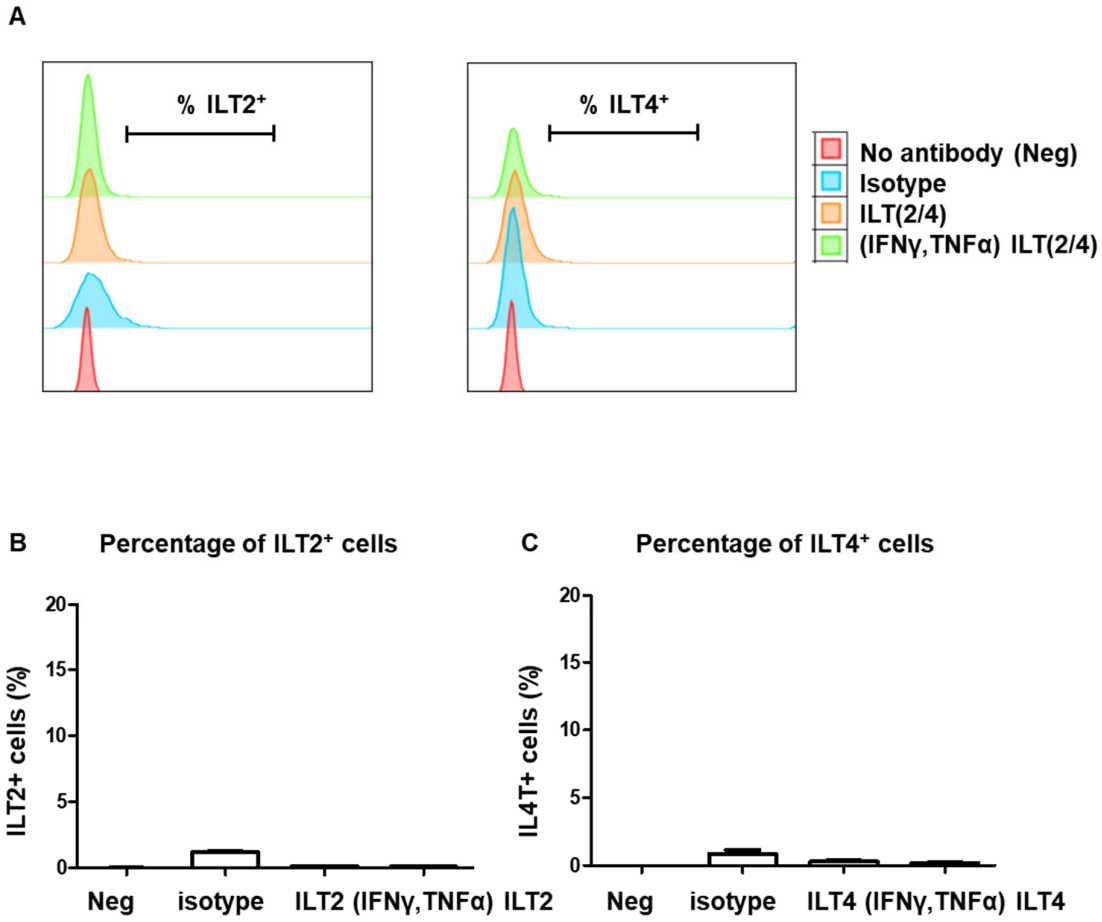

Figure S6. Cell-surface expression of the HLA-G receptors ILT2 and ILT4 in keratinocytes. Cell cultures were performed during 2 days in the absence or presence of 10 ng/mL of IFNγ and 10 ng/mL TNFα to mimic an inflammatory context, and keratinocytes were analyzed for ILT2 and ILT4 cell-surface expression by flow cytometry. (**A**) Representative profiles are shown. Absence keratinocytes positive for both ILT2 (**B**) and ILT4 (**C**) cell-surface expression.