

## Supplementary material

Amino acid sequence of the recombinant p40 protein (rt-p40) from *L. rhamnosus* GR1

	Signal peptide		
Recombinant_p40	MDTSASIASNKSETNDLLKQIEAANTEVINLNKQIDAKNGEISDATAKISATDAKIASLSGEITAAQKNVAAR	73	
<i>L. rhamnosus</i> GR1	MKFNKAMMTLVAAVTLAGSVSAVTPVFAADTSASIASNKSETNDLLKQIEAANTEVINLNKQIDAKNGEISDATAKISATDAKIASLSGEITAAQKNVAAR	100	
<i>L. rhamnosus</i> GG	MKFNKAMMTLVAAVTLAGSVSAVTPVFAADTSASIASNKSETNDLLKQIEAANTEVINLNKQIDAKNGEISDATAKISATDAKIASLSGEITAAQKNVAAR	100	
Recombinant_p40	KNNLKDQLISLQKKAGSSVSGNVYIDFVLNSQSLDLIARTMTVGKLSQASKDALDAVTAKDKLAALKSEQETARQTLVSTKASLETQKSQLETLQKTA	173	
<i>L. rhamnosus</i> GR1	KNNLKDQLISLQKKAGSSVSGNVYIDFVLNSQSLDLIARTMTVGKLSQASKDALDAVTAKDKLAALKSEQETARQTLVSTKASLETQKSQLETLQKTA	200	
<i>L. rhamnosus</i> GG	KNNLKDQLISLQKKAGSSVSGNVYIDFVLNSQSLDLIARTMTVGKLSQASKDALDAVTAKDKLAALKSEQETARQTLVSTKASLETQKSQLETLQKTA	200	
Recombinant_p40	SDKQDALNKEIADHKDELVALQSQFAQESEAATKATQAALKTAAASTASSSTSSTSNKSANSSVLTGTSTNTSSNGASSTVISNTASGSGSHADYS	273	
<i>L. rhamnosus</i> GR1	SDKQDALNKEIADHKDELVALQSQFAQESEAATKATQAALKTAAASTASSSTSSTSNKSANSSVLTGTSTNTSSNGASSTVISNTASGSGSHADYS	300	
<i>L. rhamnosus</i> GG	SDKQDALNKEIADHKDELVALQSQFAQESEAATKATQAALKTAAASTASSSTSSTSNKSANSSVLTGTSTNTSSNGASSTVISNTASGSGSHADYS	300	
Recombinant_p40	GSGNTYPWQCTWYVKSVAWAGNGWNGAEWGASAAAAGFTVNHTPAAGSIIVFAAGQSVGGQWTADGSYGHVAYVQSVSGDSVTITQGGMGFDSPTGP	373	
<i>L. rhamnosus</i> GR1	GSGNTYPWQCTWYVKSVAWAGNGWNGAEWGASAAAAGFTVNHTPAAGSIIVFAAGQSVGGQWTADGSYGHVAYVQSVSGDSVTITQGGMGFSPTGP	400	
<i>L. rhamnosus</i> GG	GSGNTYPWQCTWYVKSVAWAGNGWNGAEWGASAAAAGFTVNHTPAAGSIIVFAAGQSVGGQWTADGSYGHVAYVQSVSGDSVTITQGGMGFSPTGP	400	
Recombinant_p40	NTQTISGASSYVYIHR	389	
<i>L. rhamnosus</i> GR1	NTQTISGASSYVYIHR	416	
<i>L. rhamnosus</i> GG	NTQTISGASSYVYIHR	416	

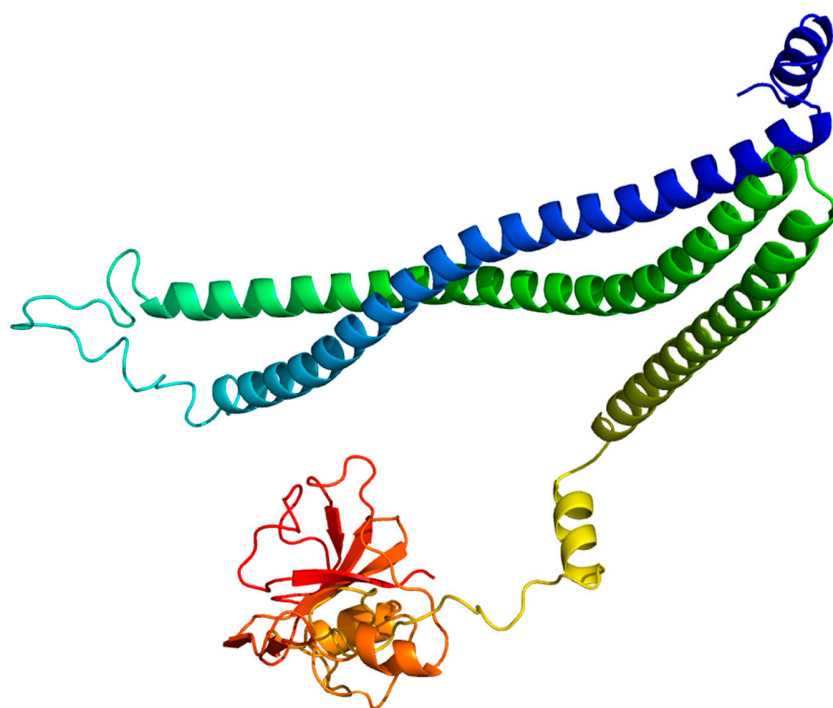
**Figure S1.** Amino acid sequence alignment of the sequences of our rt-p40, p40 from *L. rhamnosus* GR1 and p40 from LGG.

Comparison of the amino acid sequences between our recombinant p40 protein and p40 from LGG.

Recombinant_p40	MDTSASIASNKSEETNDLLKQIEAANTEVINLNKQIDAKNGEISDATAKISATDAKIASLS	60
p40_LGG	ADTSASIASNKSEETNDLLKQIEAANTEVINLNKQIDAKNGEISDATAKISATDAKIASLS	60
Recombinant_p40	GEITAAQKNVAARKNNLKDQLISLQKKAGSSVSGNVYIDFVLNSQSLDLIARTMTVGKL	120
p40_LGG	GEITAAQKNVAARKNNLKDQLISLQKKAGSSVSGNVYIDFVLNSQSLDLIARTMTVGKL	120
Recombinant_p40	SQASKDALDAVTAKDKLAALKSEQETARQTLVSTKASLETQKSQLETLQKTASDKQDAL	180
p40_LGG	SQASKDALDAVTAKDKLAALKSEQETARQTLVSTKASLETQKSQLETLQKTASDKQDAL	180
Recombinant_p40	NKEIADHKDELVALQSQFAQESEAATKATQAALKTAAASTASSSTSSTSNKSANSSVLTST	240
p40_LGG	NKEIADHKDELVALQSQFAQESEAATKATQAALKTAAASTASSSTSSTSNKSANSSVLTST	240
Recombinant_p40	GTSSSTNTSSNSGASSTVISNTASGSGSHADYSGSGNTYPWQCTWYVKSVAWAGNGWG	300
p40_LGG	GTSSSTNTSSNSGASSTVISNTASGSGSHADYSGSGNTYPWQCTWYVKSVAWAGNGWG	300
Recombinant_p40	NGAEWGASAAAAGFTVNHTPAAGSIIVFAAGQSVGGQWTADGSYGHVAYVQSVSGDSVTI	360
p40_LGG	NGAEWGASAAAAGFTVNHTPAAGSIIVFAAGQSVGGQWTADGSYGHVAYVQSVSGDSVTI	360
Recombinant_p40	TQGGMGFDSPTGPNTQTISGASSYVYIHR	389
p40_LGG	TQGGMGFSSPTGPNTQTISGASSYVYIHR	389

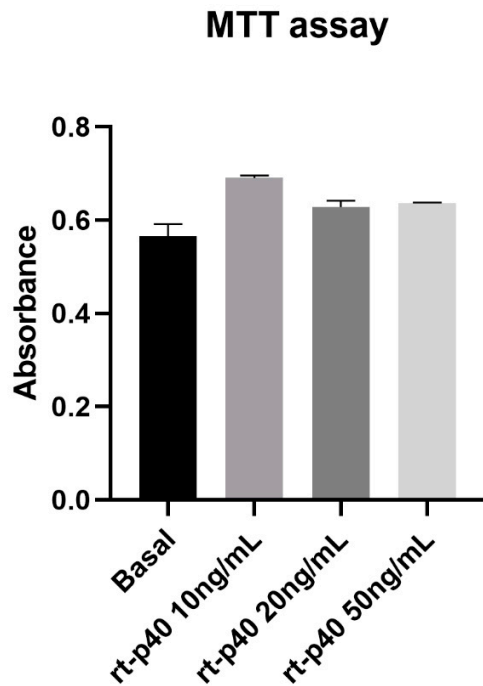
**Figure S2.** Amino acid sequence alignment between our rt-p40 with p40 from LGG, comparing the differences between them according to the physicochemical properties of the amino acids.

Three-dimensional structure of our rt-p40



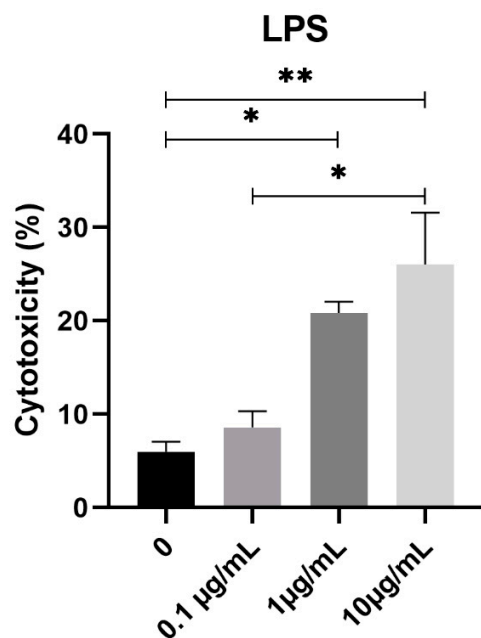
**Figure S3.** Three-dimensional structure of our rt-p40, obtained by the PHYRE2 algorithm.

Viability assay using the CyQUANT™ MTT Cell Proliferation Assay kit.



**Figure S4.** Different concentrations of rt-p40 do not reduce the cell viability of HaCaT keratinocytes.  $1 \times 10^5$  HaCaT cells were seeded and incubated with 10, 20 and 50 ng/mL rt-p40 for 1 hour. Viability was assessed through the conversion of water-soluble MTT to water-insoluble formazan by viable cells. The formazan crystals were solubilized with DMSO to generate a solution whose color is proportional to the concentration. A negative control without cells was used. Data were obtained from the measurement of 3 independent experiments in triplicate (mean  $\pm$  SEM).

Cytotoxicity assay using CyQUANT™ LDH Cytotoxicity Assay Kit



**Figure S5.** HaCaT cells were stimulated with 0.1 – 10 µg/mL LPS to determine cell cytotoxicity.  $1 \times 10^4$  HaCaT cells were seeded and incubated with LPS for 24 hours. Cell cytotoxicity was assessed through LDH release upon damage to the cell membrane. LDH catalyzes the reaction of lactate to pyruvate via NAD<sup>+</sup> reduction. NADH is then oxidized by diaphorase to reduce tetrazolium salt to formazan. Formazan is directly proportional to LDH released. Data were obtained from the measurement of 3 independent experiments in triplicate (mean ± SEM).