Supplementary Material

A Glycoengineered Interferon-β Mutein (R27T) Generates Prolonged Signaling by an Altered Receptor-binding Kinetics

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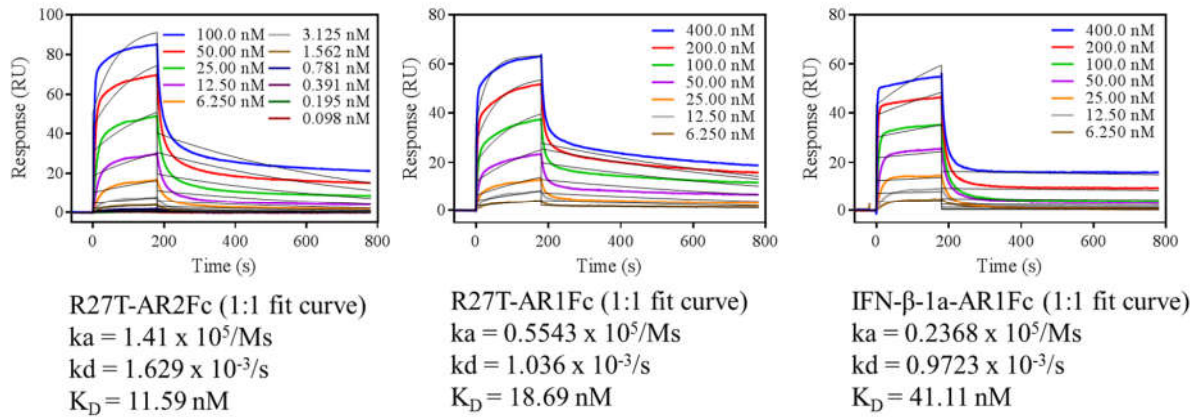
1 Supplementary Materials and Methods

Flow cytometry analysis Daudi, Jurkat, Ramos, THP-1, and MDA-MB-231 cells were used for flow cytometry analysis. Harvested cells were washed with DPBS (Hyclone Laboratories Inc., Logan, UT, USA) and placed in separate tubes at a cell density of 2.0×10^5 cells. Antibodies (anti-human IFNAR1, 85228, Thermo Fisher; anti-human IFNAR2, 10359-MM07-A, Sino Biological Inc.) were then used to determine the level of expression of type I IFN receptors in cells, and analyzed using a BD FACSCalibur instrument (BD Biosciences, Franklin Lakes, NJ, USA).

SPR analysis, western blot, real-time PCR, and competitive binding assays All experimental methods were performed as described in the Materials and Methods section.
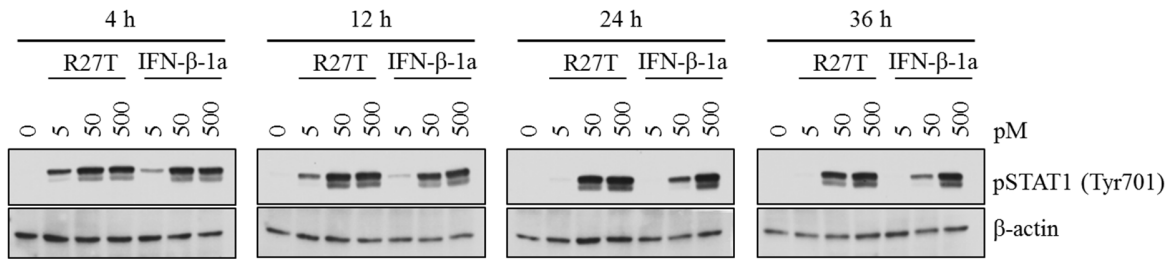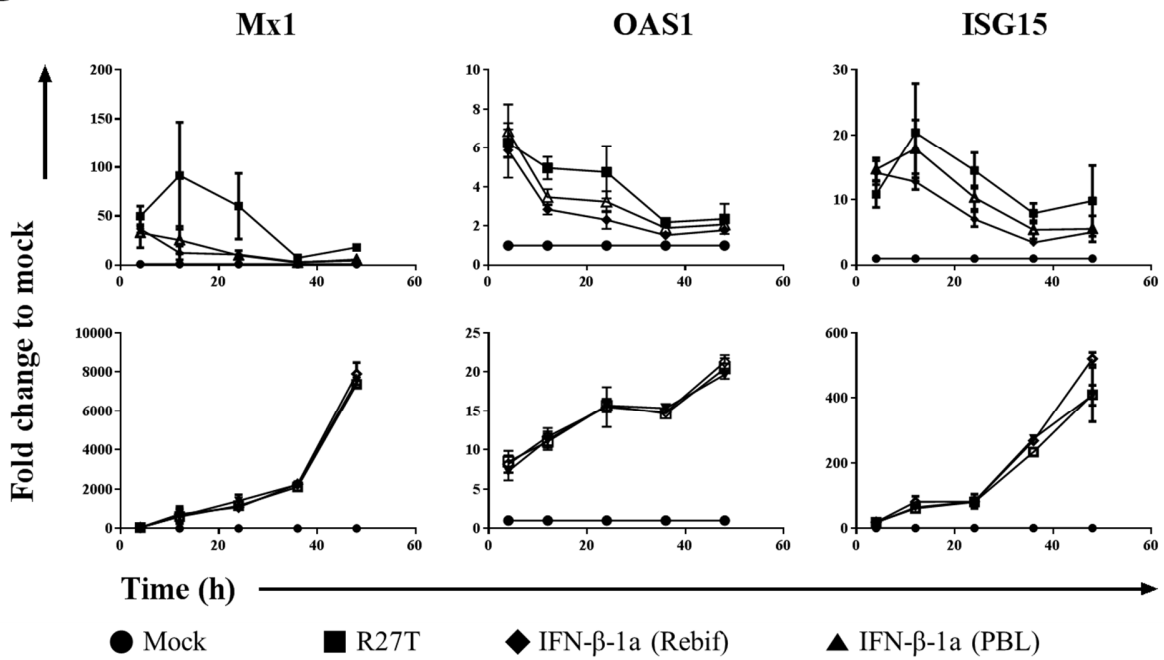
2   Supplementary Figures and Tables

2.1   Supplementary Figures

R27T-AR2Fc (1:1 fit curve)
$ka = 1.41 \times 10^5/Ms$
$kd = 1.629 \times 10^{-3}/s$
$K_D = 11.59\ nM$

R27T-AR1Fc (1:1 fit curve)
$ka = 0.5543 \times 10^5/Ms$
$kd = 1.036 \times 10^{-3}/s$
$K_D = 18.69\ nM$

IFN-β-1a-AR1Fc (1:1 fit curve)
$ka = 0.2368 \times 10^5/Ms$
$kd = 0.9723 \times 10^{-3}/s$
$K_D = 41.11\ nM$

Supplementary Figure S1. Fitting of SPR data using the 1:1 Langmuir model. Kinetic analysis of R27T binding to AR2Fc (A) and AR1Fc (B), and IFN-β-1a binding to AR1Fc (C). Fusion proteins were evaluated using a BIAcore T200 instrument at 11 (A, 0.098 to 100 nM) and seven (B and C, 6.25 to 400 nM) different concentrations with a CM5 gold chip containing captured anti-human Fc IgG.
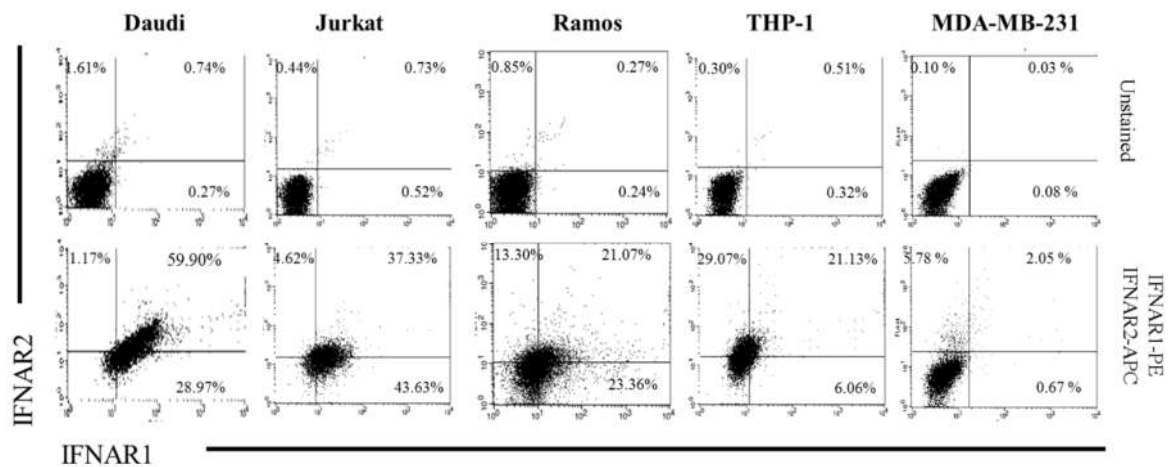
Supplementary Figure S2. Screening of the expression of type I IFN receptors on the cell surface in various cell lines by flow cytometry analysis.

Supplementary Figure S3. (A) STAT1 phosphorylation analysis by western blotting. (B) Comparison of IFN-sensitive ISG levels following stimulation with R27T or IFN-β-1as. Mx1, OAS1, and ISG15 mRNA levels in 5 pM (upper) or 500 pM (lower) treated samples were determined at the indicated time points by qRT-PCR analysis.
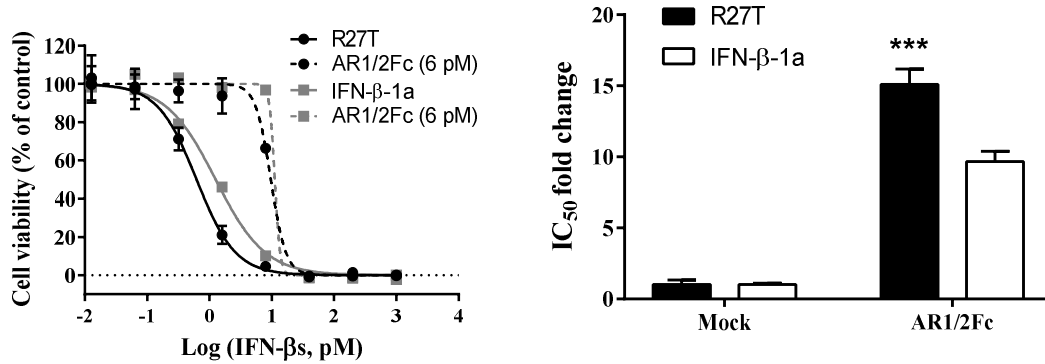
Supplementary Figure S4. Displacement of AR1/2Fc (6 pM) by increasing concentrations of R27T or IFN-β-1a on Daudi cells expressing type I IFN receptors. IC_{50} values with AR1/2Fc and cell values are means ± s.d. of three independent experiments performed in triplicate. ***$p < 0.001$ (two-way ANOVA, Bonferroni's multiple comparison *post hoc* tests).

2.2 Supplementary Table

Supplementary Table 1. Oligonucleotide primers used for real-time PCR

Gene	Primer sequence	Reference
GAPDH	F: 5′-TCCCTGAGCTGAACGGGAAG-3′	(Ahmad et al., 2013)
	R: 5′-GGAGGAGTGGGTGTCGCTGT-3′	
ISG15	F: 5′-TCCTGGTGAGGAATAACAAGGG-3′	(Du et al., 2012)
	R: 5′-GTCAGCCAGAACAGGTCGTC-3′	
Mx1	F: 5′-TCCCACCCTCTATTACTGAATGG-3′	(Du et al., 2012)
	R: 5′-GGGAAGGGCAACTCCTGAC-3′	
OAS1	F: 5′-CATCCGCCTAGTCAAGCACTG-3′	(Kato et al., 2004)
	R: 5′-CCACCACCCAAGTTTCCTGTAG-3′	
CXCL10	F: 5′-TGCTGGGTCTGAGTGGGACT-3′	(Varona et al., 2005)
	R: 5′-CCCTATGGCCCTCATTCTCAC-3′	
CCR1	F: 5′-CTCTTCCTGTTCACGCTTCC-3′	(Martínez-Iglesias et al., 2016)
	R: 5′-GCCTGAAACAGCTTCCACTC-3′	
PLSCR1	F: 5′-GAATGCTTCTCACCCGGAAA -3′	(Song et al., 2011)
	R: 5′-TCCTGGAGGTCCTTGGAATG-3′	

3   Reference

Ahmad, T.a.F.T., Jubri, Z., Rajab, N.F., Rahim, K.A., Yusof, Y.a.M., and Makpol, S. (2013). Gelam honey protects against gamma-irradiation damage to antioxidant enzymes in human diploid fibroblasts. *Molecules* 18**,** 2200-2211.

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