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Supplementary Materials for

Human IL-22 binding protein isoforms act as a rheostat for IL-22 signaling

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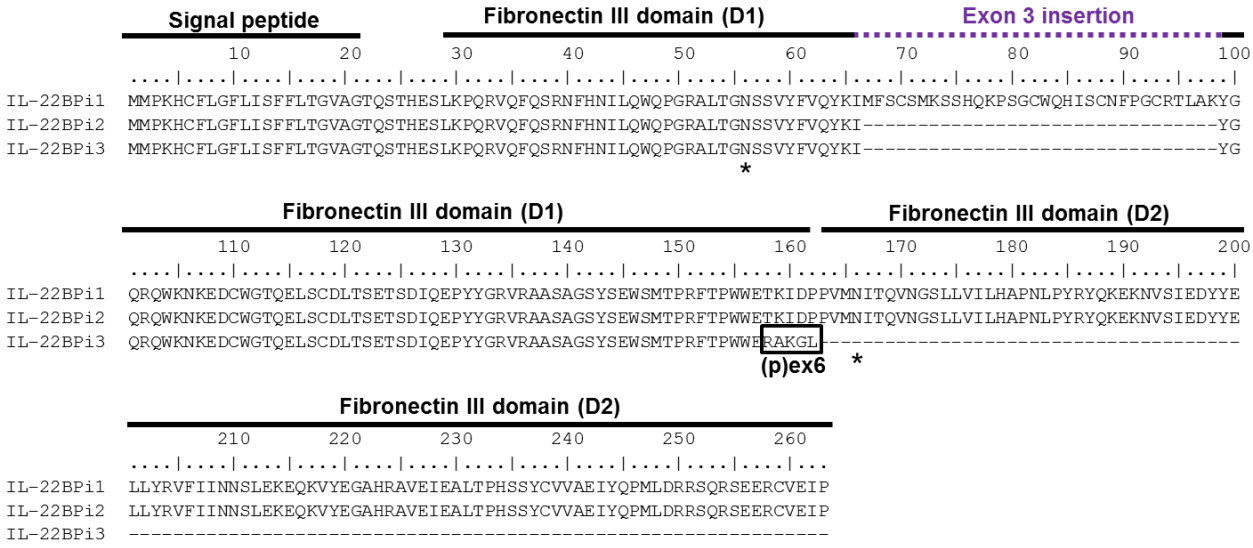

Fig. S1. Alignment of the amino acid sequences of IL-22BPi1, IL-22BPi2, and IL-22BPi3. Alignment of the amino acid sequences of IL-22BPi1, IL-22BPi2, and IL-22BPi3 with CLUSTALW in the BioEdit sequence alignment editor (http://www.mbio.ncsu.edu/bioedit/bioedit.html). Sequences were taken from NCBI (accession numbers NP_443194, NP_851827, and NP_851826, respectively) and Uniprot (accession number Q969J5). Major protein domains are annotated as in Uniprot and based on crystallography data described by de Moura *et al*. (*48*). Asterisks indicate N-glycosylation sites that are predicted by the NetNGlyc 1.0 Server (http://www.cbs.dtu.dk/services/NetNGlyc/).

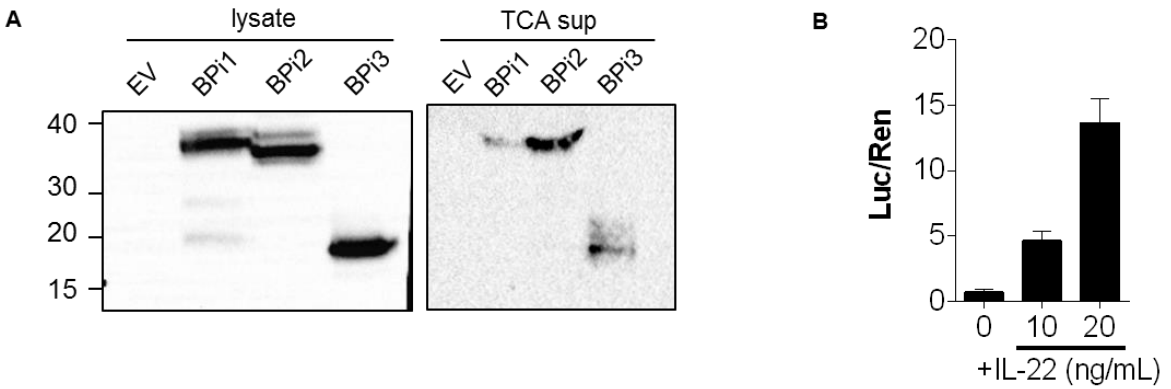

Fig. S2. Analysis of IL-22BP isoform abundance in pSTAT3 Western blotting assays and optimization of the IL-22 concentration used for STAT3 luciferase reporter assays. (**A**) Huh7 cells were transfected with empty vector (EV) or with plasmids encoding IL-22BPi1, IL-22BPi2, or IL-22BPi3, as indicated. Twenty-four hours later, cell lysates (left) and cell culture medium concentrated with TCA (right) were analyzed by Western blotting with an antibody specific for IL-22BP. These lysates and supernatants were obtained from the representative experiment shown in Fig. 1D and demonstrate successful overexpression and reproducibility of IL-22BP isoform secretion. (**B**) Optimization of the IL-22 concentration used for STAT3 luciferase reporter assays. HepG2 cells coexpressing a Firefly luciferase reporter with a STAT3-responsive promoter (pGL4.47, Promega) and a constitutively-produced pRL-TK Renilla reporter (Promega) were serum-starved for 4 to 6 hours and then either left untreated or stimulated with the indicated concentrations of IL-22. Six hours later, the luciferase activity of the STAT3 reporter was assayed. Firefly luciferase activity was normalized against *Renilla* luciferase activity. Data are representative of two experiments and shown as means ± SEM.

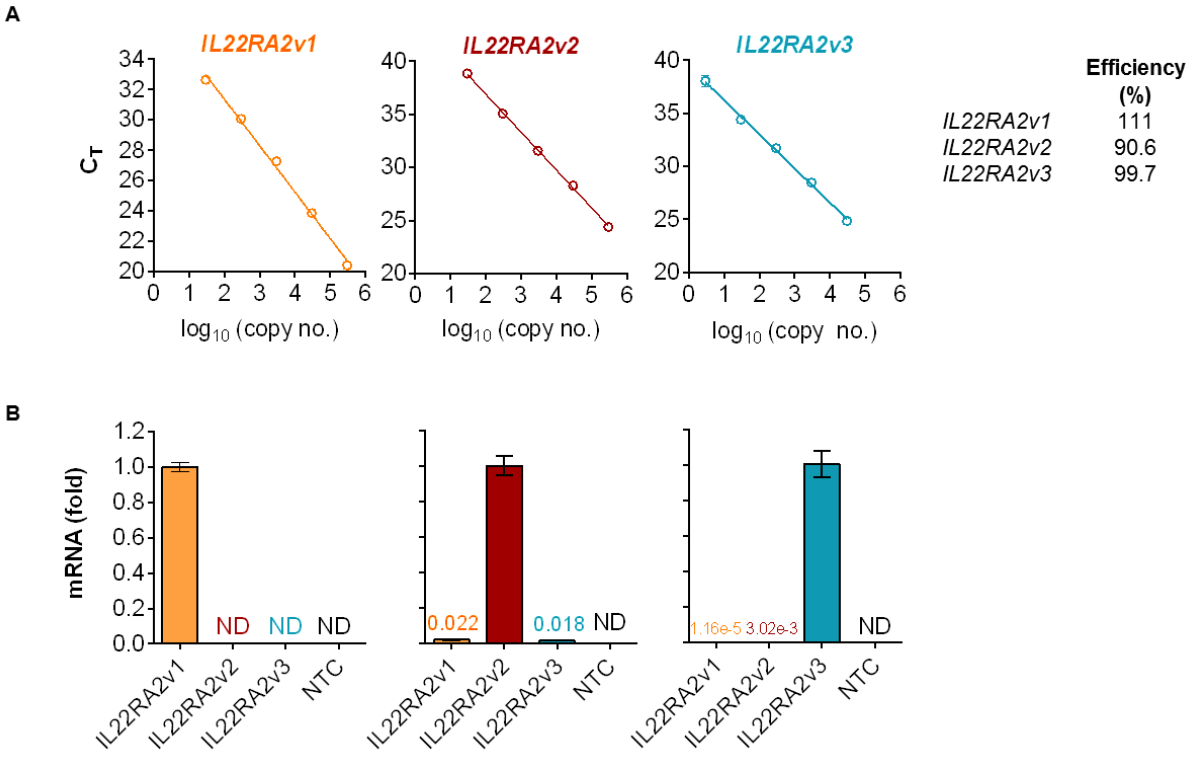

Fig. S3. Analysis of the efficiency and specificity of the primers and probes used for the qPCR-based analysis of *IL22RA2* isoforms. (**A**) Standard curves were obtained by amplifying known copy numbers of plasmids expressing each of the indicated isoforms of *IL22RA2* by qPCR. The efficiency of each probe is provided in the accompanying table (right). (**B**) 3 x 10^5 copies of the plasmids encoding *IL22RA2v1*, *IL22RA2v2*, and *IL22RA2v3* were each amplified with all three sets of qPCR primers and probes to verify their specificity for each isoform. Data are representative of two experiments. The numbers labelled on the bar graphs are mean values of mRNA abundance relative to the abundance of the indicated *IL22RA2* transcript detected with its isoform-specific probes; therefore, they indicate the relative sensitivity of each *IL22RA2* qPCR probe for all three transcripts. Data are means ± SEM ND, not detected.

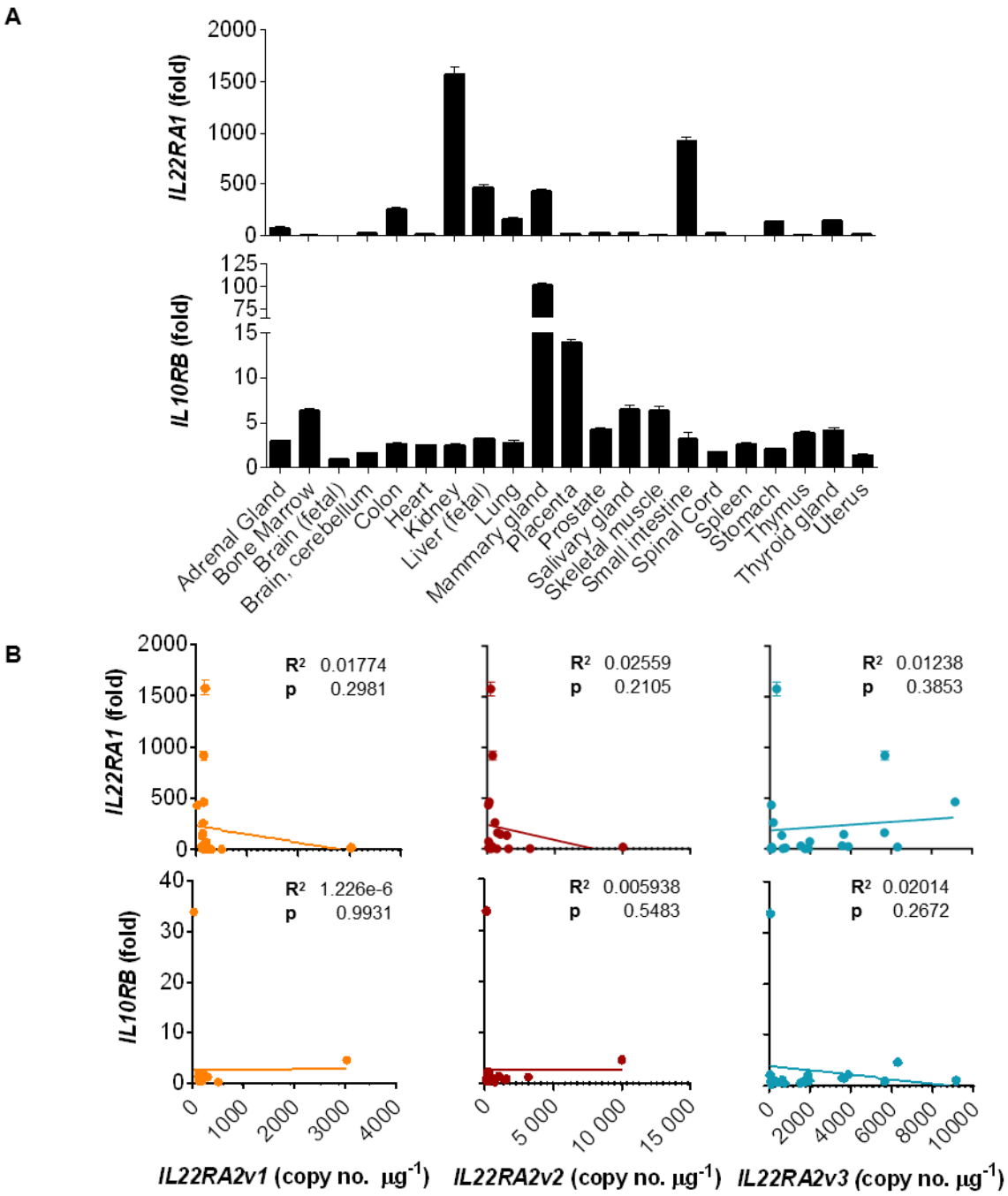

Fig. S4. Correlation between the expression of genes encoding membrane-bound IL-22R subunits and that of the *IL22RA2* isoforms. (**A**) The relative abundances of *IL22RA1* mRNA (top) and *IL10RB* mRNA (bottom) were measured by qPCR analysis of 21 human tissues and are plotted as the fold-difference compared to the mRNA abundances in fetal brain. *ACTB* was used as an endogenous control. Data are means ± SEM of three technical replicates from a RNA tissue panel containing pooled samples. (**B**) Linear regression analysis was performed to assess the correlation between the *IL22RA2* isoform copy number and the expression of *IL22RA1* or *IL10RB*. R^2 and *P* (slope ≠ 0) values are inset for each graph. ND, not detected. NTC, no template control. Analysis was performed based on the values obtained in (A) and data are means ± SEM of three technical replicates.

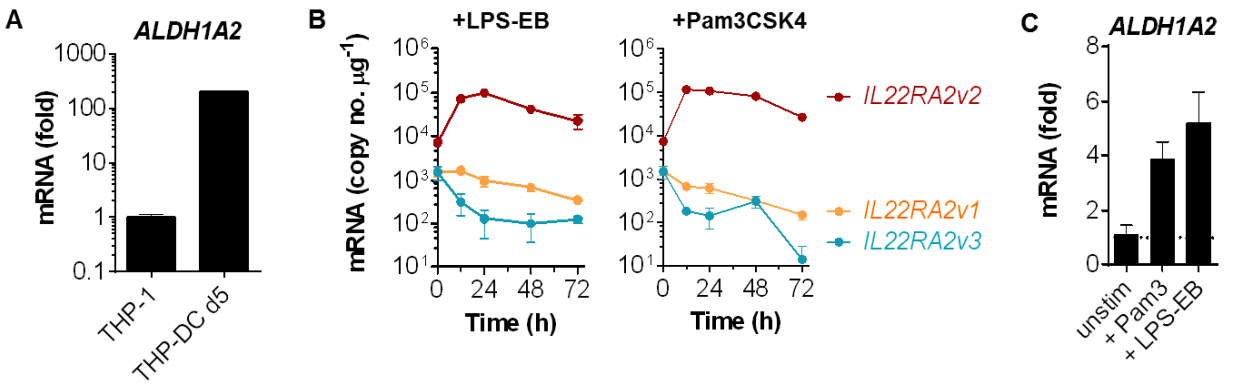

Fig. S5. Analysis of the expression of *ALDH1A2* in THP-1 cells and THP-1–derived DCs and of *IL22RA2v2* expression in HL-60–derived neutrophils stimulated with Pam3CSK4 or LPS-EB. (**A**) Undifferentiated THP-1 cells and THP-1–derived DCs after 5 days of differentiation were analyzed by qPCR to compare the relative abundance of *ALDH1A2* mRNA in undifferentiated THP-1 cells and DC-like differentiated THP-1 cells Data are representative of three experiments and are shown as means ± SEM. (**B** and **C**) HL-60 cells cultured with DMSO for 5 days to differentiate into neutrophil-like cells were stimulated with Pam3CSK4 and LPS-EB for 72 hours. (B) The relative abundances of the indicated *IL22RA2* isoform mRNAs were measured at the indicated times. (C) The relative abundance of *ALDH1A2* mRNA in the indicated cells was measured 12 hours after stimulation. Data in (B) and (C) are representative of two experiments and are shown as means ± SEM.

Table S1. Concentrations of the rhIL-22BP isoforms used to give 25-, 50-, and 100-fold molar excess compared to IL-22.

IL-22BP:IL-22 ratio	IL-22 / ng ml^{-1} (16.5 kD monomer)	rhIL-22BPi2-Fc / ng ml^{-1} (51.3 kD)	rhIL-22BPi3-Histag / ng ml^{-1} (14.5 kD)
25:1	10.0	777.3	219.7
50:1	10.0	1554.6	439.5
100:1	10.0	3109.1	878.9
25:1	20.0	1554.6	439.5
50:1	20.0	3109.1	878.9
100:1	20.0	6218.2	1757.9

Table S2. Sequences of the probes and primers used to quantify the expression of the *IL22RA2* isoforms by qPCR analysis.

Transcript	**Primer**	**5′ - Sequence - 3′**
IL22RA2v1	Forward	ATTTTGCAATGGCAGCCTG
	Probe	TCTCACCAGAAGCCAAGTGGATGC
	Reverse	GCCTGGGAAGTTACAAGAAATG
IL22RA2v2	Forward	GTGCAGTACAAAATATATGGACAGA
	Probe	CTCGGCTGGGAGCTACTCAGAAT
	Reverse	GAGGATCTATTTTTGTTTCCCACC
IL22RA2v3	Forward	GTGCAGTACAAAATATATGGACAGA
	Probe	CTCGGCTGGGAGCTACTCAGAAT
	Reverse	CTTTTGCTCTTTCCCACCAG