ABD-derived protein blockers of human IL-17 receptor A as non-IgG alternatives for modulation of IL-17-dependent pro-inflammatory axis

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Supplementary Data

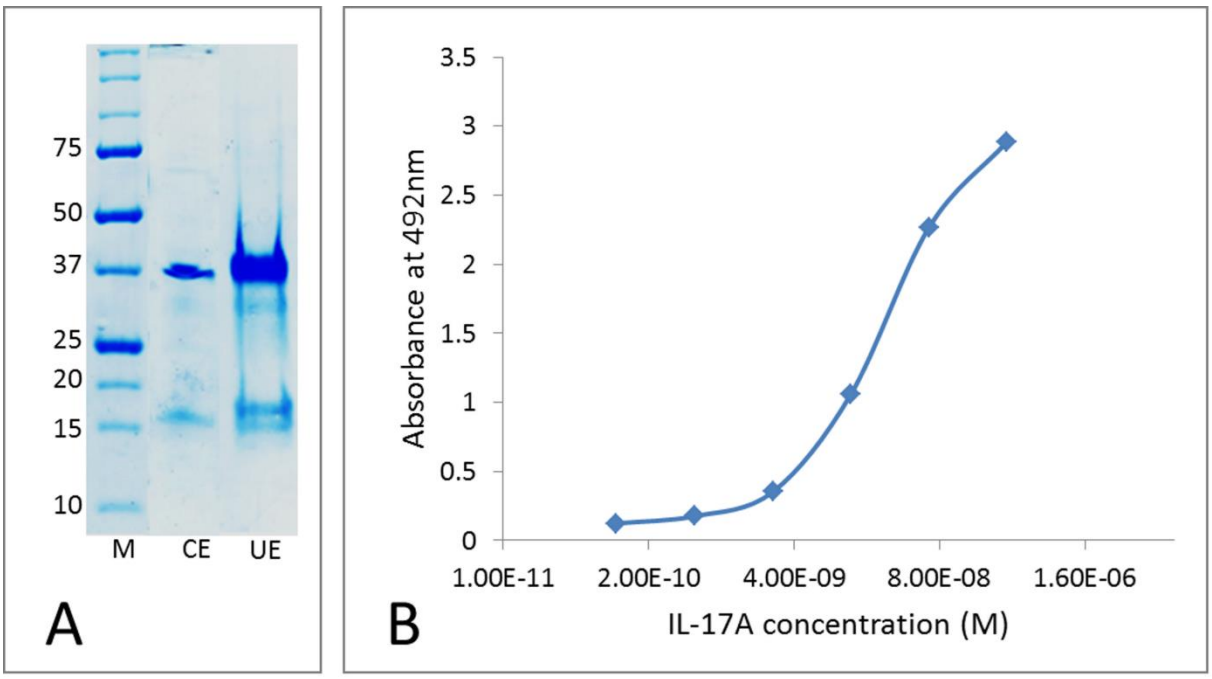

Figure S1. Production and functional verification of the recombinant IL-17RA receptor. (A) The recombinant IL-17RA protein was produced in *E. coli* SHuffle strain, purified on Ni-NTA-agarose chromatography and the purity of the protein was verified on SDS-PAGE (CE indicates a soluble protein purified from a cytosolic fraction, UE indicates protein purified from an urea extract). Recombinant product of 40 kDa was visualized on polyacrylamide gel. (B) Binding activity of recombinant IL-17RA produced in *E. coli* tested in ELISA. The protein purified on Ni-NTA agarose, eluted in 4 M urea and diluted in a coating buffer was immobilized in 96-well plate overnight. The human IL-17A protein was added in series of dilutions in phosphate buffer and its binding was detected using anti-human IL-17A rabbit polyclonal antibody followed by anti-rabbit IgG-HRP conjugate.

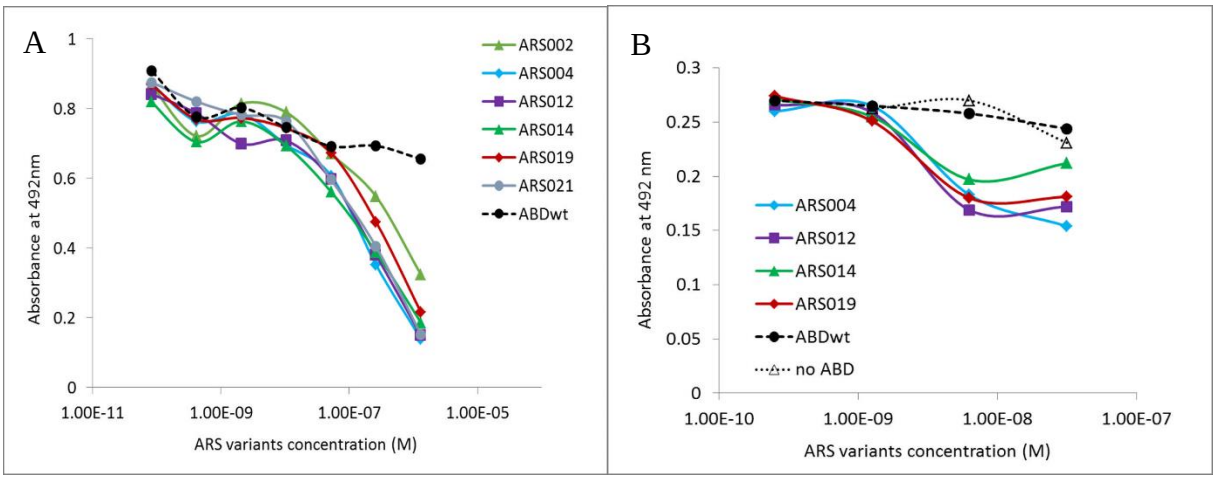

Figure S2. ARS ligands compete with IL-17A cytokine for binding to human recombinant IL-17RA (A) or IL-17RA-IgG chimera (B) in ELISA. IL-17RA produced in *E. coli* SHuffle host cells was immobilized on 96-well Polysorp plate. Serially diluted His_6-ARS-TolA-AVI ligands were used to compete for binding with 10 nM of human IL-17A cytokine. Bound IL-17A was detected with anti-human IL-17A rabbit polyclonal antibody in combination with secondary anti-IgG-HRP conjugate. His_6-ABDwt-TolA-AVI served as a negative control.

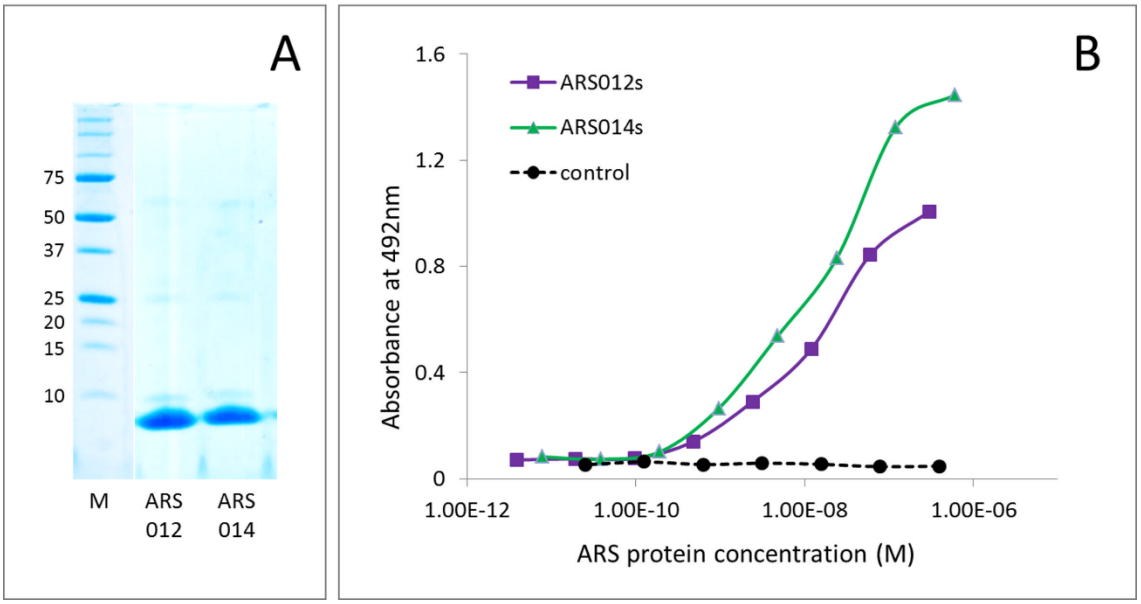

Figure S3. Generation and characterization of short ARS proteins. (A) Production of *in vivo* biotinylated His_6-ARS-AviTag protein variants shown as stained bands of 8.3 kDa Ni-NTA-agarose purified proteins on SDS-PAGE. (B) Binding of biotinylated ARS012s and ARS014s proteins to immobilized recombinant IL-17RA, produced in *E. coli* SHuffle host cells, tested in ELISA using streptavidin-HRP conjugate. As a negative control, coated BSA in the absence of IL-17RA was used.

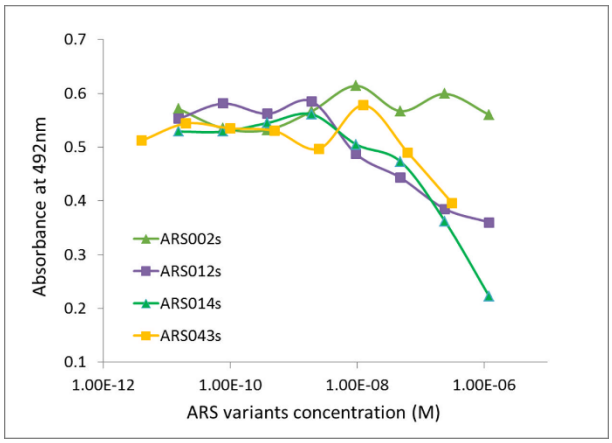

Figure S4. Short versions of ARS ligands inhibit binding of human IL-17A cytokine to recombinant IL-17RA. Serially diluted ARS proteins in PBSTB buffer competed with 10 nM IL-17A for binding to *E. coli*-produced IL-17RA. Bound IL-17A was detected with anti-IL-17A polyclonal antibody in combination with secondary anti-IgG-HRP conjugate.

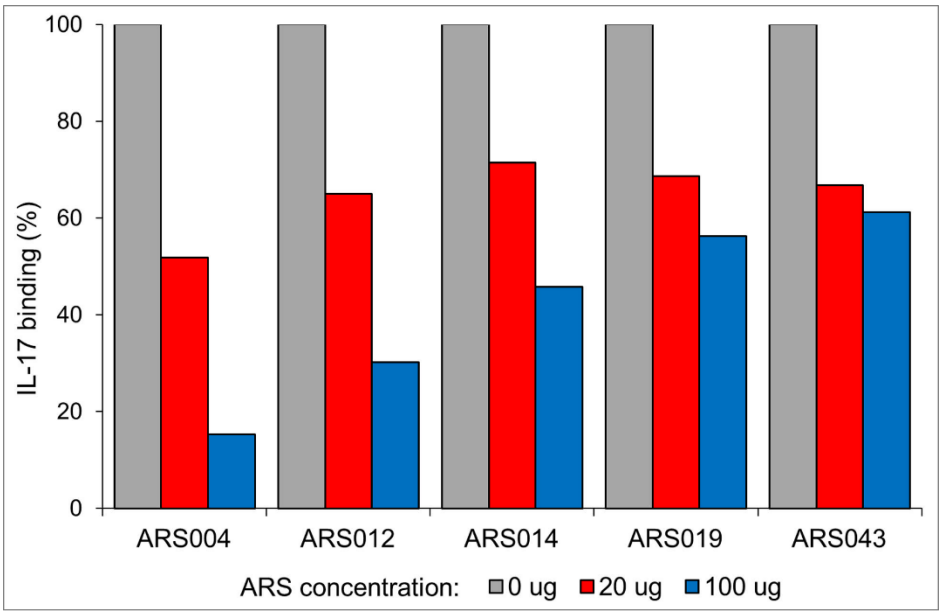

Figure S5. ARS ligands inhibit binding of IL-17 to THP-1 cells. THP-1 cells ($2x10^{5}$) expressing IL-17RA were pre-incubated without or with two different concentrations (20 and 100 µg/ml) of particular ARS proteins (30 min at 4°C) and incubated with 10 µg/ml of IL-17 (30 min at 4°C). After washing, the surface-bound IL-17 was stained with anti-IL-17 rabbit antibody (30 min at 4°), washed and after the incubation with a Cy5-labeled goat anti-rabbit IgG (30 min at 4°C) analyzed by flow cytometry. Binding of IL-17 to ARS-untreated THP-1 cells was taken as 100%.

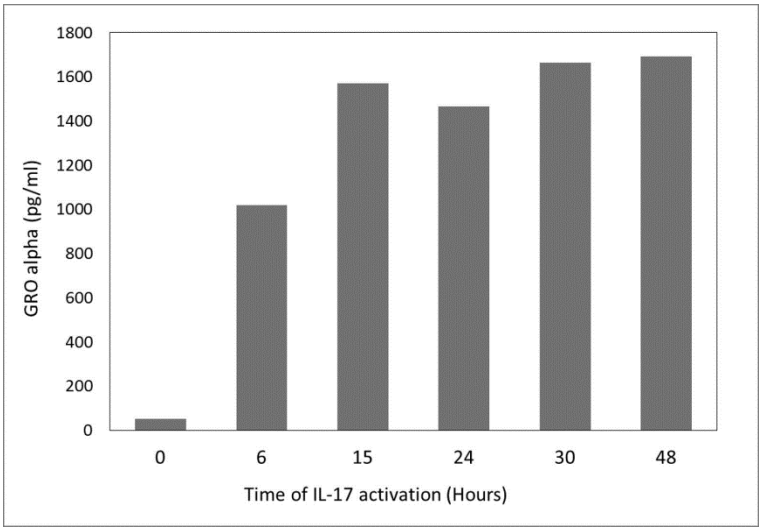

Figure S6. Secretion of Gro-α by CCD-1070Sk skin fibroblasts upon stimulation by IL-17A. Cells were stimulated by 20 ng/ml human IL-17A cytokine and after cultivation for 6, 15, 24, 30 or 48 hours, cell supernatants were collected and the levels of Gro-α were measured by ELISA.