SUPPORTING INFORMATION

EXPERIMENTAL METHODS

Cloning of IL-1Ra fusion proteins

For periplasmic protein expression, the cDNA encoding human IL-1Ra was amplified from the plasmid IRANP969GO350D6 (RZPD, Berlin, Germany) and cloned on pASK75 (1). A low repetitive PAS#1(600) gene cassette (2) was inserted between the coding regions for the His-tag and the N-terminus of IL-1Ra in a similar manner as previously described (3).

For the construction of a cytoplasmic expression plasmid encoding human IL-1Ra N-terminally fused with the PAS#1(800) polypeptide (without an affinity tag), a synthetic gene for IL-1Ra with optimized codon usage (Thermo Fisher Scientific/Geneart, Regensburg, Germany) was cloned on a pASK40 derivative (4) under control of the lacUV5 promoter and fused with a low repetitive PAS#1(800) gene cassette having high genetic stability (2).

Recombinant protein production and purification

PAS600-IL-1Ra was produced in an 8 L *E. coli* batch fermentation via periplasmic secretion to ensure proper disulfide bond formation (3). The fusion protein was purified from the periplasmic cell fraction to homogeneity via 30% ammonium sulfate precipitation followed by immobilized metal ion affinity chromatography (IMAC) on a Ni-Sepharose High-Performance column (GE Healthcare, Munich, Germany), anion exchange chromatography on a Source™ 15Q column (GE Healthcare), and cation exchange chromatography (CEX) on a Source™ 15S column (GE Healthcare).

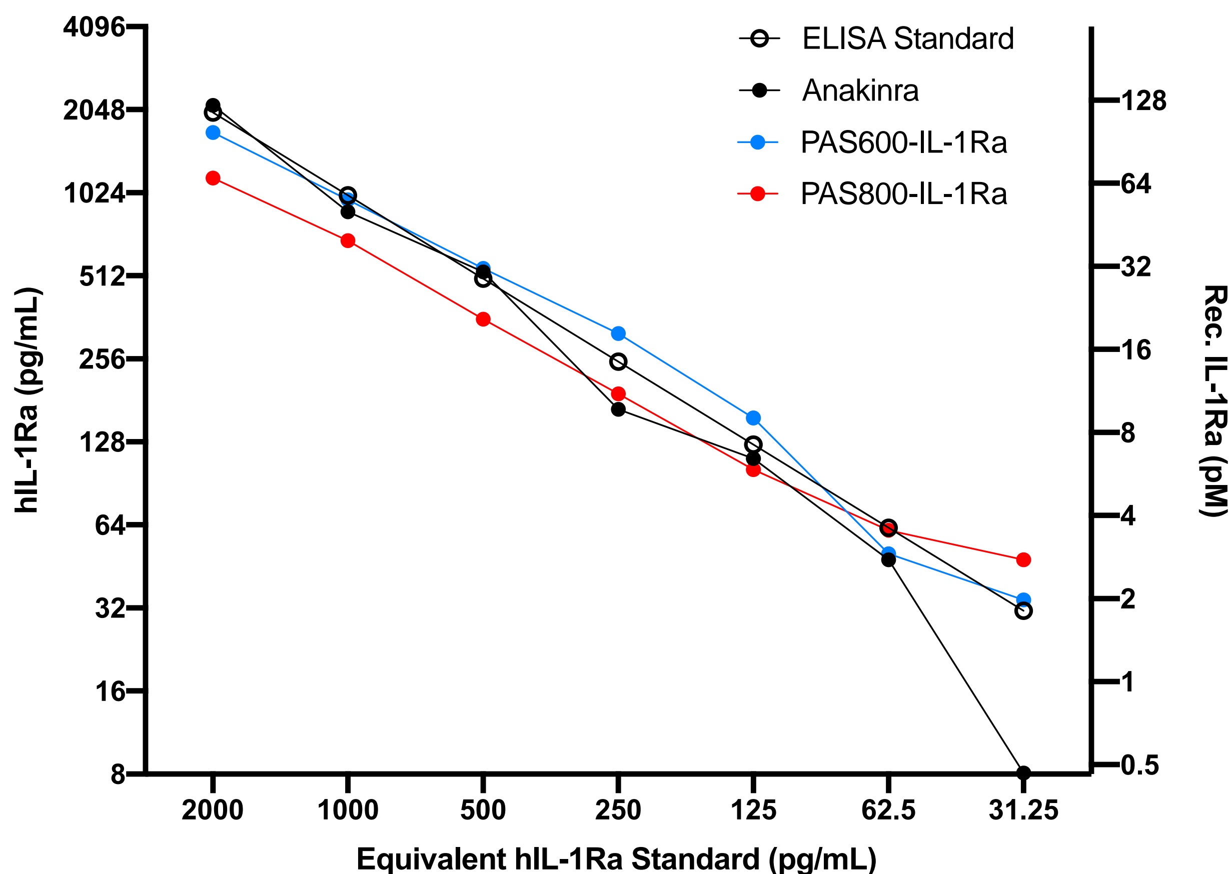
PAS800-IL-1Ra without an affinity tag was produced in the cytoplasm of *E. coli* using 2 L shake flask cultures similarly as a previously described method (5). To ensure efficient disulfide formation of IL-1Ra, the *trx*/*gor* mutant strain *E. coli* Origami™ B (Merck Millipore, Darmstadt, Germany) was chosen as an expression host. This fusion protein was purified via 30% ammonium sulfate precipitation, anion exchange chromatography (AEX) using Fractogel® EMD TMAE (M) resin (Merck Millipore) and preparative size exclusion chromatography (SEC) on a Superdex™ S200 26/60 column (GE Healthcare) as a final polishing step. Residual endotoxin was depleted using a Proteus NoEndo™ HC column (Protein Ark, Sheffield, UK).

Biochemical and biophysical characterization of PAS-IL-1Ra fusion proteins

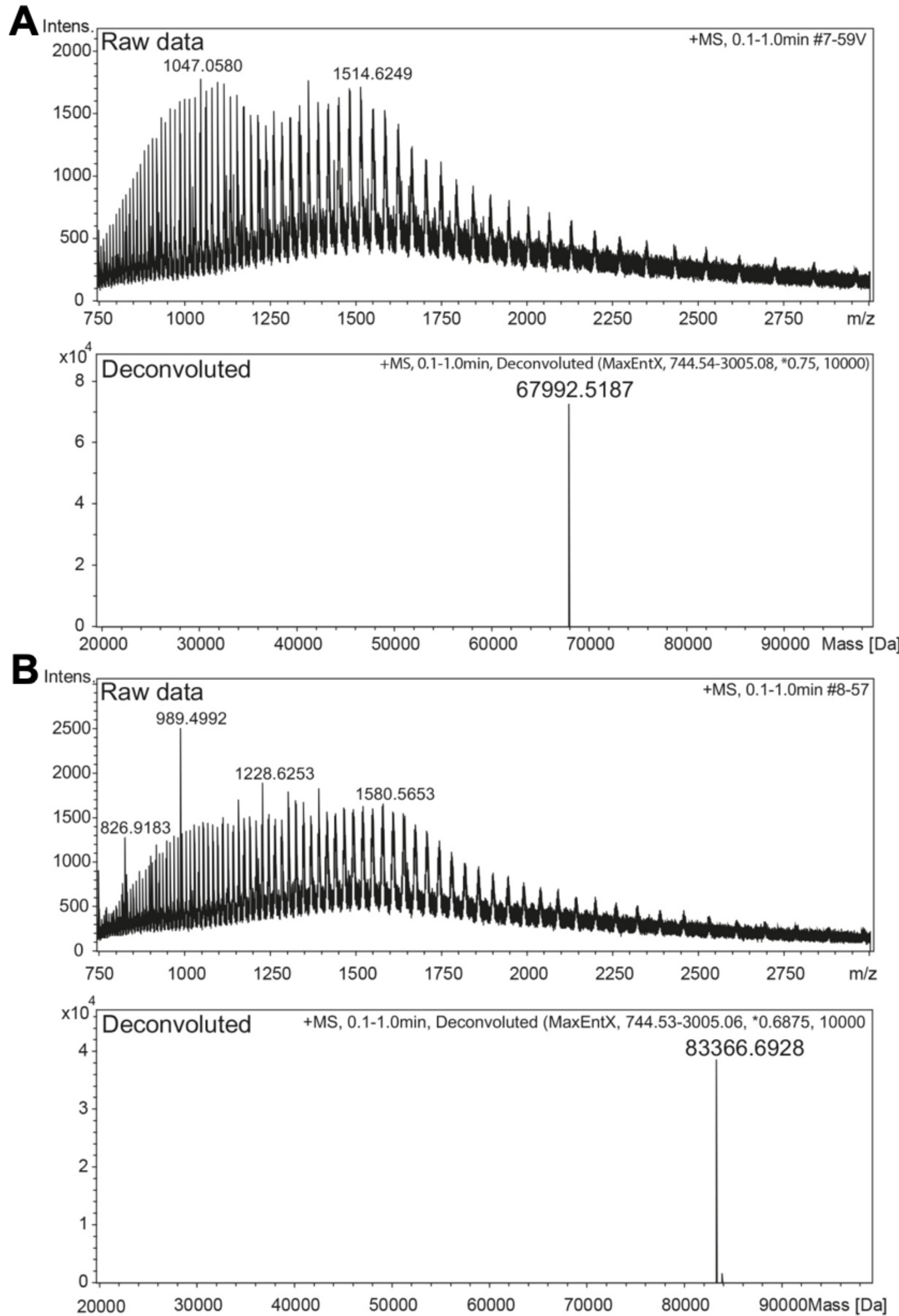
PASylated IL-1Ra proteins were analyzed on 10% SDS-PAGE gels and using Coomassie staining. Molecular masses and integrity of the PAS fusions were analyzed by ESI-MS on a maXis Q-TOF instrument (Bruker Daltonics, Bremen, Germany) operated in the positive ion mode. Raw data was deconvoluted with the MaxEntX algorithm (Bruker Daltonics). Analytical SEC was performed on a Superose™ 6 10/300 GL column (GE Healthcare) using an Äkta™ Purifier 10 system (GE Healthcare) with PBS (4 mM KH_2PO_4, 16 mM Na_2HPO_4, 115 mM NaCl, pH 7.4) as running buffer at a flow rate of 0.5 mL/min.

Supplementary References

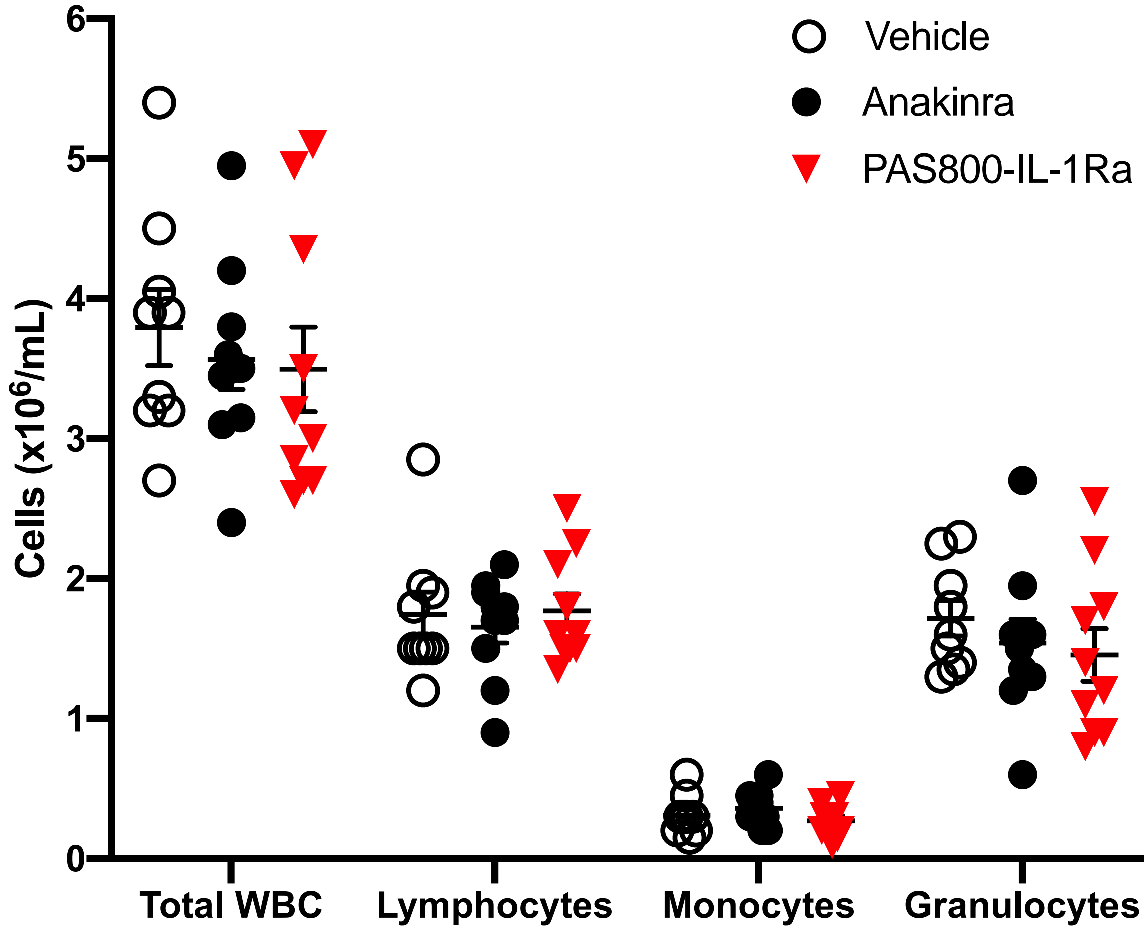
1. Skerra, A. (1994) Use of the tetracycline promoter for the tightly regulated production of a murine antibody fragment in *Escherichia coli*. *Gene* **151**, 131-135
2. Binder, U., and Skerra, A. (2017) PASylation®: A versatile technology to extend drug delivery. *Current Opinion in Colloid & Interface Science* **31**, 10-17
3. Schlapschy, M., Binder, U., Börger, C., Theobald, I., Wachinger, K., Kisling, S., Haller, D., and Skerra, A. (2013) PASylation: a biological alternative to PEGylation for extending the plasma half-life of pharmaceutically active proteins. *Protein Engineering Design and Selection* **26**, 489-501
4. Skerra, A., Pfitzinger, I., and Plückthun, A. (1991) The functional expression of antibody Fv fragments in *Escherichia coli*: improved vectors and a generally applicable purification technique. *Nature Biotechnology* **9**, 273-278
5. Breibeck, J., and Skerra, A. (2018) The polypeptide biophysics of proline/alanine-rich sequences (PAS): Recombinant biopolymers with PEG-like properties. *Biopolymers* **109**, e23069



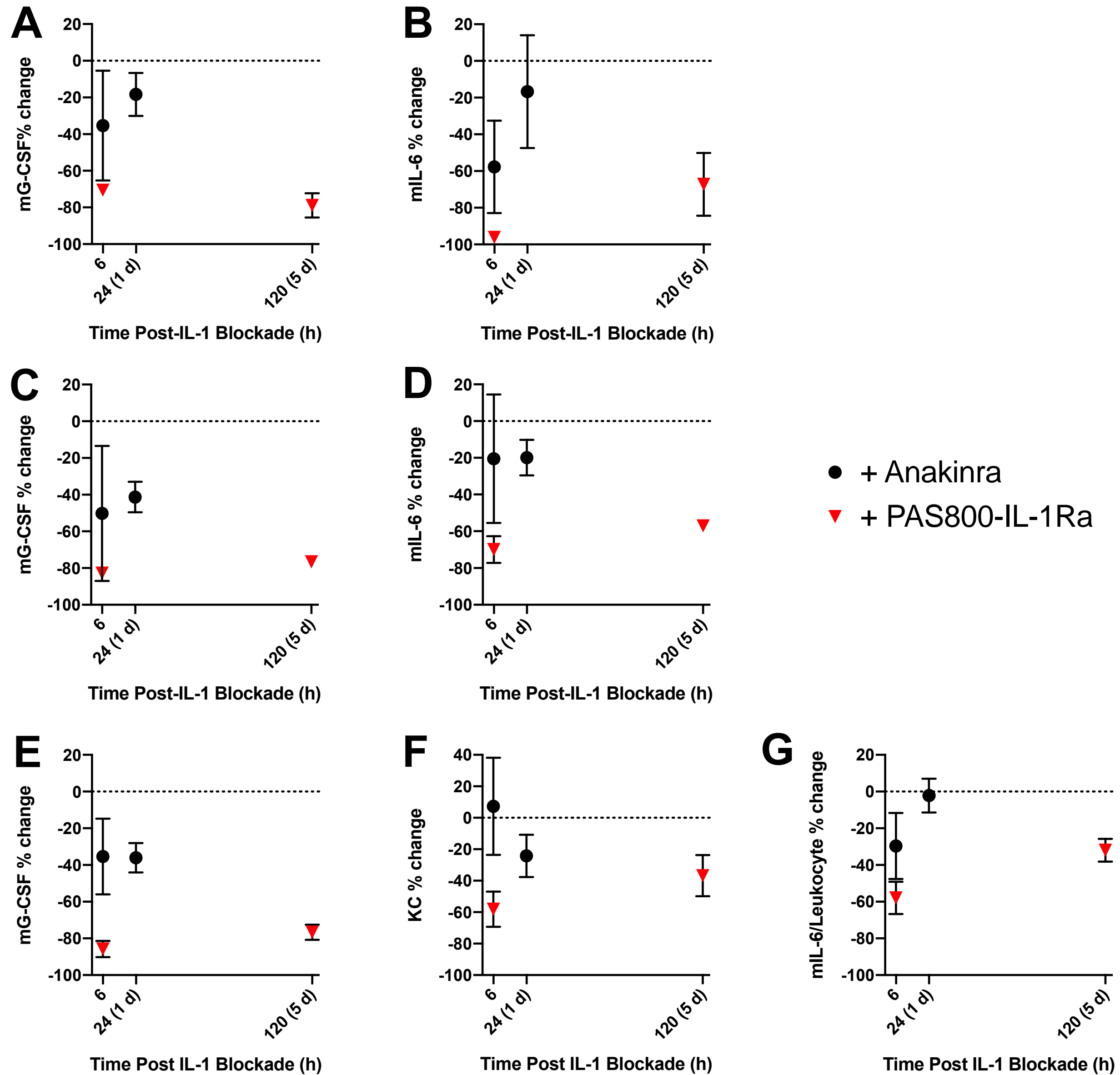
Supplementary Figure 1. Anakinra, PAS600-IL-1Ra, PAS800-IL-1Ra detected by ELISA at equimolar concentrations. Anakinra, PAS600-IL-1Ra, and PAS800-IL-1Ra are detected at similar concentrations as compared to human IL-1Ra standard (Human IL-1Ra/IL-1F3 DuoSet ELISA, R&D Systems, Minneapolis, MN, USA). Linear range 31.25-2000 pg/mL, experimental n = 2.



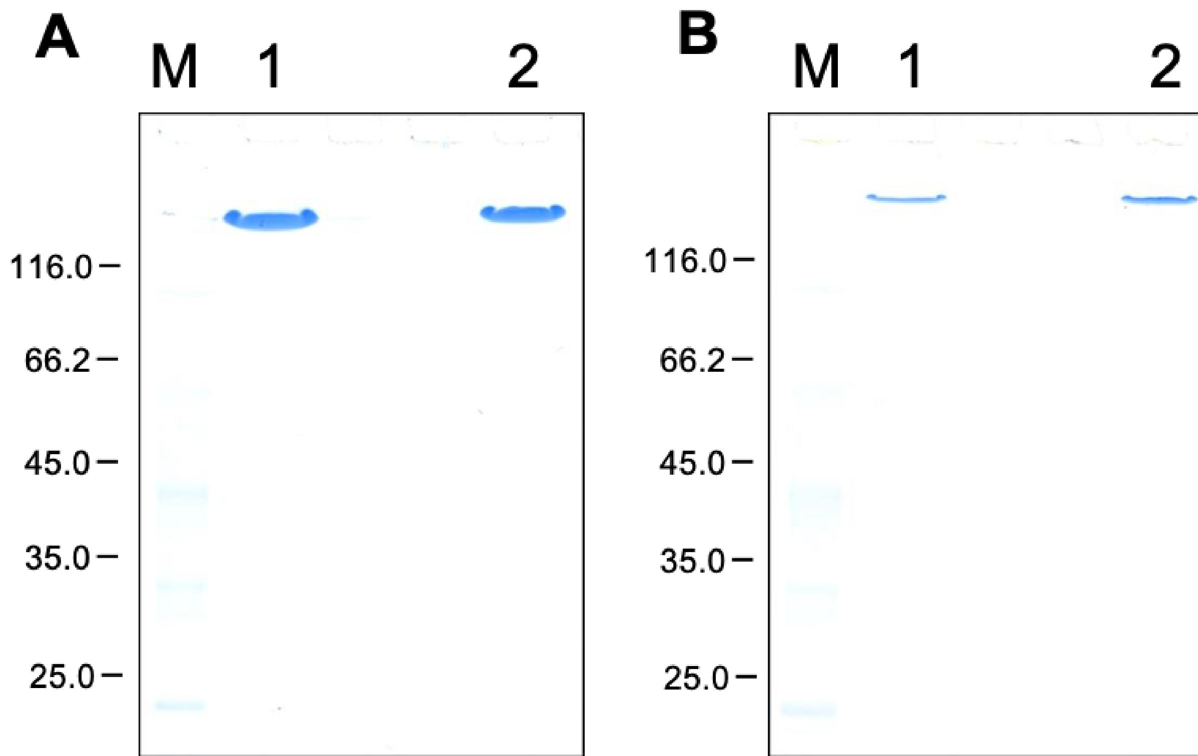
Supplementary Figure 2. ESI-MS analysis of PASylated IL-1Ra. Electrospray ionization mass spectrometry (ESI-MS) (raw and deconvoluted spectra) of PAS600-IL-1Ra (*A*) and PAS800-IL-1Ra (*B*). Both measured masses perfectly match the calculated masses of PAS600-IL-1Ra (67992.9 Da) and PAS800IL-1Ra (83366.2 Da). The single peak in each deconvoluted spectrum confirms the monodisperse composition of both PASylated IL-1Ra versions and the absence of product-related impurities or truncation products. Furthermore, this analysis reveals the efficient intracellular cleavage of the signal peptide and the start methionine residue, respectively, for both expression constructs. This confirms the utility of the cytoplasmic production route in *E. coli* as a particularly viable strategy to manufacture PAS800-IL-1Ra at a larger scale.



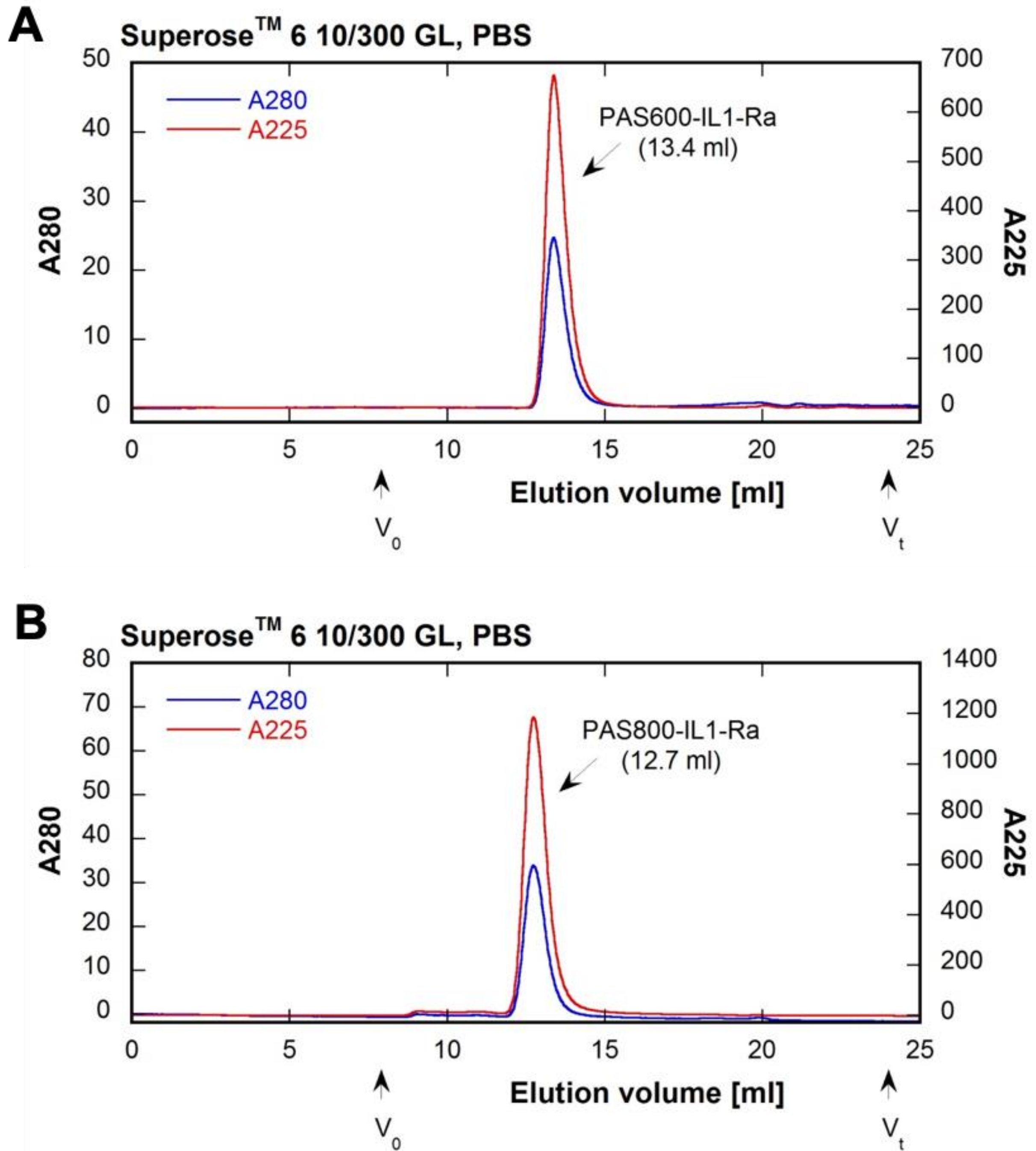
Supplementary Figure 3. Peripheral Blood Leukocyte populations in MSU crystal peritonitis after vehicle, anakinra, and PAS800-IL-1Ra treatments. Circulating leukocyte populations were assessed in blood using an automated cell counter. Experiments 1 (5 mice) and 2 (5 mice) were combined, one point represents one mouse. Data presented as mean ± SEM. No population was significantly different (minimum significance $p < 0.27$) between treatment groups (two-tailed Student's t-test).



Supplementary Figure 4. Comparison of Anakinra and PAS800-IL-1Ra efficacy in MSU crystal-induced peritonitis. Selected representation of results from Figures 4, 5, and 6, which represent two different experiments (Experiment 1: Figures 4 and 5, n = 10, points at 28 (1 d) and 124 h (5 d);; Experiment 2: Figure 6, n = 5, points at 10 h). (*A*-*B*) Comparison of detected IP fluid mouse G-CSF (*A*) and mouse IL-6 (*B*). (*C*-*D*) Comparison of detected plasma mouse G-CSF (*C*) and mouse IL-6 (*D*). (*E*-*F*) Comparison of detected blood lysate mouse G-CSF (*E*) and KC (*F*). Spontaneous mouse IL-6 production in whole-blood culture, diluted one part in five, was also compared (*G*). Data are expressed as percent change, with the vehicle group mean of each experiment set at 100%. Error bars, mean ± SEM.



Supplementary Figure 5. SDS-PAGE analysis of PASylated IL-1Ra. SDS-PAGE analysis of PAS600-IL-1Ra (*A*) and PAS800-IL-1Ra (*B*). M: Molecular size marker, Lane 1: PASylated IL-1Ra reduced, Lane 2: PASylated IL-1Ra non-reduced. Both PASylated IL-1Ra fusion proteins show a high purity. Due to the poor SDS binding of the PAS moiety, PASylated proteins run at an apparently much higher molecular weight than expected from their true masses. Also, bands appear much fainter than expected due to the PAS polypeptide being poorly stained by Coomassie brilliant blue.



Supplementary Figure 6. Analytical SEC analysis of PASylated IL-1Ra. Analytical SEC of PAS600-IL-1Ra (*A*) and PAS800-IL1Ra (*B*) on a Superose™ 10/300 GL column (GE Healthcare) using PBS as running buffer. V_0 indicates the void volume and V_t the bed volume (24 ml) of the column. For each PAS-IL-1Ra fusion, only a single peak appears, corresponding to the monomeric PASylated protein without signs of aggregation.