Supporting Information Online

Table S1. IL-1β-stimulated phosphorylation of ERK and JNK

Phosphorylation of downstream signal transduction mediators demonstrates that XOMA 052-reduces, but does not eliminate downstream signaling at high levels of IL-1β. Ranges in parentheses are 95% confidence intervals.

Cell Line	Signal molecule	EC_{50}, IL-1β (pM)	EC_{50}, IL-1β + XOMA 052 (pM)	Fold inhibition
Hep G2	p-ERK	13.4 (9.9-18.2)	1724 (1420-2094)	129
	p-JNK	13.0 (9.5-17.3)	1471 (1021-2117)	113
A549	p-ERK	14.6 (9.8-21.0)	994 (519-1903)	68
	p-JNK	9.8 (6.5-15.0)	767 (547-1074)	78

Fig. S1 Derivation of Antibody interacting with Receptor and Ligand

Let:

R=total receptor on cell

L=total ligand concentration

A=total kinetic modifying antibody concentration

r=concentration of free receptor

l=concentration of free ligand

a=concentration of free antibody

x=concentration of RL complex (active)

y=concentration of AR complex

z=concentration of AL complex

w=con concentration of RLA complex (active)

Allow the antibody to bind to R, L, or RL complex. Let RL (*x*) and RLA (*w*) both cause active cell signaling. The following relationships hold, with their respective affinities:

$$rl = K_{RL}x \tag{0.1}$$

$$al = K_{AL}z \tag{0.2}$$

$$ar = K_{AR}y \tag{0.3}$$

$$xa = K_{RLA}w \tag{0.4}$$

$$yl = K_{ARL}w \tag{0.5}$$

$$zr = K_{ALR} w \tag{0.6}$$

There are six equations, but four unknown concentrations *x*,*y*,*z*, and *w*. It turns out that the remaining equations relate two of the affinities to the other four. Noting that:

$$K_{RL} = rl / x \tag{0.7}$$

$$K_{AL} = al / z \tag{0.8}$$

$$K_{AR} = ar / y \tag{0.9}$$

$$K_{RLA} = xa / w \tag{0.10}$$

$$K_{ARL} = yl / w \tag{0.11}$$

$$K_{ALR} = zr / w \tag{0.12}$$

Then

$$K_{RL} K_{RLA} = arl / w \tag{0.13}$$

$$K_{AR} K_{ARL} = arl / w \tag{0.14}$$

$$K_{AL} K_{ALR} = arl / w \tag{0.15}$$

so that

$$K_{RL} K_{RLA} = K_{AL} K_{ALR} = K_{AR} K_{ARL} \tag{0.16}$$

To simplify the additional derivations, let

$$A >> L >> R \tag{0.17}$$

Then:

$$a \approx A \tag{0.18}$$

$$l \approx L - z \tag{0.19}$$

$$r = R - x - y - w \tag{0.20}$$

So:

$$(R-x-y-w)(L-z) \approx K_{RL}x \tag{0.21}$$

$$A(L-z) \approx K_{AL}z \tag{0.22}$$

$$A(R-x-y-w) \approx K_{AR}y \tag{0.23}$$

$$xA \approx K_{RLA}w \tag{0.24}$$

$$y(L-z) \approx K_{ARL}w \tag{0.25}$$

$$z(R-x-y-w) \approx K_{ALR}w \tag{0.26}$$

We note first that *z* is the easiest to derive from equation (0.22):

$$z = \frac{AL}{K_{AL}+A} \tag{0.27}$$

Likewise,

$$L-z = \frac{K_{AL}L}{K_{AL}+A} = \frac{L}{\left(1+\frac{A}{K_{AL}}\right)} \tag{0.28}$$

We next relate *w* and *y* to *x*. From equation (0.24):

$$w = x\left(\frac{A}{K_{RLA}}\right) \tag{0.29}$$

and from equations (0.23), (0.21):

$$y = \left(K_{RL}\frac{A/K_{AR}}{L-z}\right)x \tag{0.30}$$

As we are most interested in $u=x+w$, so we transpose the above equations as follows:

$$u = x + w = x\left(\frac{A}{K_{RLA}} + 1\right) \tag{0.31}$$

and

$$x = \frac{1}{\left(\frac{A}{K_{RLA}} + 1\right)}u \tag{0.32}$$

$$w = \frac{A}{K_{RLA}}\frac{1}{\left(1 + \frac{A}{K_{RLA}}\right)}u \tag{0.33}$$

$$y = \frac{K_{RL}}{\left(\frac{A}{K_{RLA}} + 1\right)}\frac{A}{K_{AR}}\frac{1}{(L-z)}u = \frac{b}{(L-z)}u \tag{0.34}$$

where we conveniently define

$$b = \frac{K_{RL}}{\left(\frac{A}{K_{RLA}} + 1\right)}\frac{A}{K_{AR}} \tag{0.35}$$

Modifying equation (0.21) further:

$$(R - u - \frac{b}{L-z}u)(L-z) = (R-u)(L-z) - bu = \tag{0.36}$$

$$(R-u)\left(\frac{L}{\left(1 + \frac{A}{K_{AL}}\right)}\right) - bu = \left(\frac{K_{RL}}{\left(1 + \frac{A}{K_{RLA}}\right)}\right)u \tag{0.37}$$

Further:

$$(R-u)L=\left(\frac{K_{RL}}{\left(1+\frac{A}{K_{RLA}}\right)}+b\right)\left(1+\frac{A}{K_{AL}}\right)u=K_{RL}\frac{\left(1+\frac{A}{K_{AR}}\right)\left(1+\frac{A}{K_{AL}}\right)}{\left(1+\frac{A}{K_{RLA}}\right)}u= \tag{0.38}$$

$$=eu \tag{0.39}$$

where we conveniently define

$$e=K_{RL}\frac{\left(1+\frac{A}{K_{AR}}\right)\left(1+\frac{A}{K_{AL}}\right)}{\left(1+\frac{A}{K_{RLA}}\right)} \tag{0.40}$$

Rearranging yields:

$$u=\frac{RL}{e+L} \tag{0.41}$$

which makes

$$e=K'_{RL}=K_{RL}\frac{\left(1+\frac{A}{K_{AR}}\right)\left(1+\frac{A}{K_{AL}}\right)}{\left(1+\frac{A}{K_{RLA}}\right)} \tag{0.42}$$

the effective affinity.

Fig. S2 XOMA 052 does not bind IL-1α or IL-1ra. (a) IL-1β, IL-1α, and IL-1ra at 100 nM injected over XOMA 052 immobilized on an SPR sensor surface demonstrate specificity of XOMA 052. (**b**) Injections over immobilized IL-1 sRI verify activity of the cytokines.

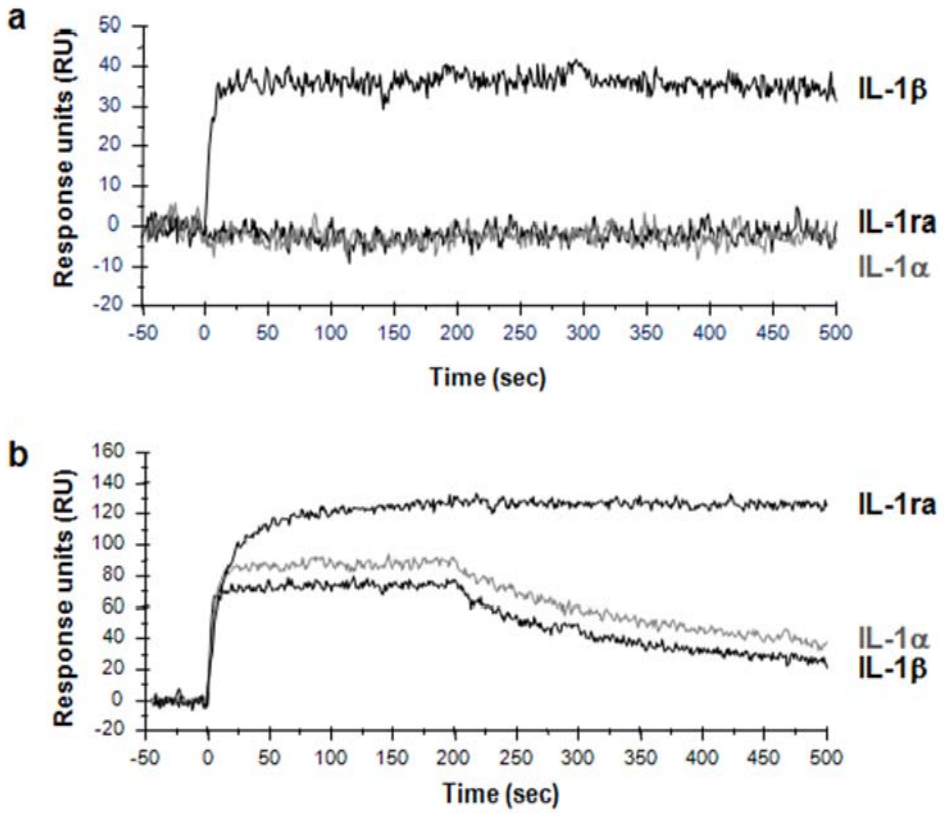


Fig. S3 XOMA 052 neutralizes IL-1β activity with 10-fold greater potency than IL-1ra in MRC-5 cells challenged with 100 pg/mL IL-1β. Error bars are standard deviation of duplicate samples. This experiment was repeated 2 times.

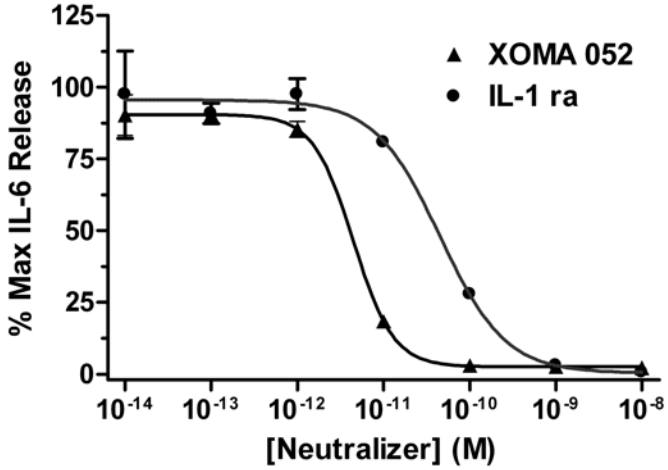
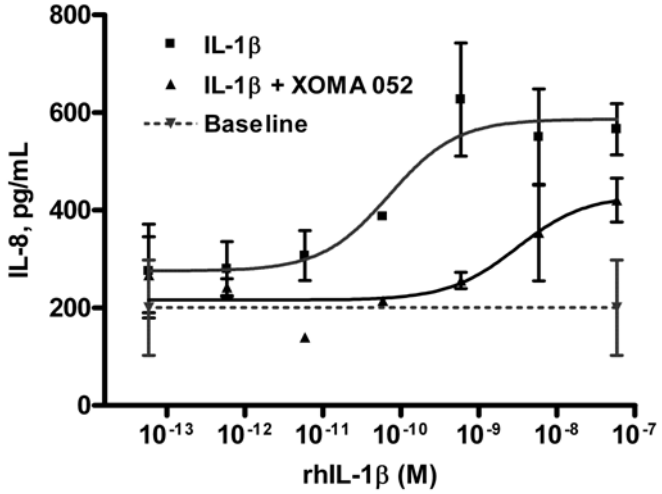


Fig. S4 XOMA 052 attenuates the dose-response of IL-1β in a whole blood assay of IL-8 stimulation. Titration of IL-1β in the presence and absence of a 10-fold molar excess of XOMA 052 shifts the dose response curve to the right and increases the EC_{50}. Error bars are standard deviation of duplicate samples. The results shown here represent a single experiment.



Supporting Information Methods

Binding analysis with SPR

All SPR experiments were performed on a Biacore 2000 instrument (GE Healthcare) or a multi SPR array system (ProteOn XPR 36™, Bio-Rad) at 25°C using HBS running buffer (0.01 M Hepes pH 7.4, 0.15 M NaCl, and 0.05% Surfactant P20). Kinetic analysis of the interactions between IL-1β and immobilized IL-1 receptors sRI and sRII in the presence or absence of XOMA 052 were determined using two or three methods of immobilization including a standard amine coupling chemistry. Amine coupling of sRI and sRII to the carboxymethyldextran-coated gold sensor chip was conducted in 0.01 M sodium acetate (pH 4.5) on a Biacore CM5 (GE Healthcare, cat# BR-1003-99) or a ProteOn GLM (Bio-Rad, cat#176-5012) sensor chip that was activated with a 1:1 mixture of 0.1 M N-hydroxysuccinimide (NHS) and 0.1 M 3-(N,N dimethylamino)propyl-N-ethylcarbodimide (EDC) and subsequently blocked with 1 M ethanolamine. Reference channels were prepared similarly except that no protein was coupled. Additionally, soluble receptors were immobilized using a capture method by first amine coupling NeutrAvidin (Thermo Scientific cat# 31000) or a mouse anti-human IgG Fc antibody (GE Healthcare cat# BR-1008-39) in 0.01 M sodium acetate (pH 4.5) to an activated sensor chip surface at high density (≥10,000 RU) followed by capture of biotinylated receptors (sRI, sRII) or receptor sRI Fc chimera at densities of 300 to 600 RU. For biotinylation, proteins were reacted with 5 to 10 molar excess of NHS-PEO_{12}-Biotin reagent (Thermo Scientific cat# 21329) according to the vendor's instructions. Excess free biotin was removed by centrifugation of proteins through a desalting column (Thermo Scientific, Zeba™ Desalt Spin Column, cat#89882). Analyte samples of either IL-1β or IL-1β pre-complexed with a 20-fold molar excess of XOMA 052 were serially diluted in running buffer and injected at 20°C at a flow rate of 30 to 50 μL min^{-1} for 2-3 min in random order (Biacore 2000) followed by a 5 minute dissociation in running buffer. Each injection was performed at least twice to ensure reproducibility within each experiment. Regeneration of the SPR surface was achieved by using two or three 20-second pulses of 50 mM sodium acetate pH 4.5 for sRI and 3 M $MgCl_2$ for sRII at 100 μL min^{-1}. Data was double referenced by subtraction of the signal from the reference channel and the signal from a buffer injection over the active channel [28] . The data were fit globally using a simple 1:1 Langmuir interaction model with Scrubber II (BioLogic Software). In the case of sRI, a correction for mass transport was used to calculate the affinities of the interaction (K_D). K_D constants were calculated for each individual experiment, and these values were used to calculate the averages and standard error of the means reported in

Table 1. Kinetic analysis of IL-1 RAcP binding to IL-1β/sRII was determined by SPR using the ProteOn XPR 36™. Biotinylated sRII was captured on GLM sensor chip (400 RU) with NeutrAvidin protein. 100 nM of IL-1β or IL-1β pre-complexed with a 10-fold molar ratio of XOMA 052 were injected over the sRII surface for 2 min at a flow rate of 30 µL/min followed by 3 min dissociation. Because the dissociation of IL-1β or IL-1β/XOMA 052 from captured sRII is negligible (~ 1x 10^{-4} M/s, not shown) compared to that of RAcP from either complex, a simple subtraction was used to correct for this factor. A 1:2 dilution series of RAcP protein starting from 500 nM was injected over the sRII and the reference channels. To isolate binding of RAcP to sRII, resulting sensorgrams were double subtracted from the reference channel and the buffer injection using the Scrubber Analysis program. Data were fit globally to 1:1 interaction model.

MRC-5 IL-6 release assay

MRC-5 human lung fibroblast cells (ATCC, Manassas, VA) were seeded into a sterile 96-well tissue culture plate at 5000 cells per well in MEM complete growth medium (Invitrogen) with 10% fetal bovine serum (FBS, Hyclone). After an overnight incubation at 37°C with 5% CO_2, supernatants were removed and replaced with growth medium containing recombinant human IL-1 β plus either IL-1ra or XOMA 052 at the concentrations indicated. Following a 20-hour incubation at 37°C with 5% CO_2, cell supernatants were removed and diluted according to estimated IL-6 concentration and assayed for human IL-6 by ELISA (Quantikine human IL-6 ELISA, R&D Systems, cat# D6050) according to the manufacturer's instructions. All samples were set up and assayed in duplicate or triplicate.

Whole blood IL-8 induction assay

Normal human blood was collected by venipuncture into collection tubes containing heparin sulfate. For potency assays recombinant human IL-1 β was pre-incubated with indicated amounts of antibody for 1 hour. For the IL-1 β dose-response assay increasing amounts of IL- β were preincubated with a 10-fold molar excess of XOMA 052 for 1 hour at 37°C in RPMI (Invitrogen) with 10% FBS prior to addition to whole blood. Samples were next incubated for 6 hours at 37°C in 96-well round bottom plates (Corning Costar, cat# 3799) then lysed with Triton X-100 at a final concentration of 0.5% for 10 minutes. Lysates were centrifuged for 5 minutes at 2000 rpm to remove debris and transferred to a clean plate. After repeating the centrifugation step, lysates were transferred to a -80°C freezer for overnight storage. The following morning lysates were thawed and tested for human IL-8 by ELISA (Quantikine human IL-8 ELISA, R&D Systems, cat# D8000C) according to manufacturer's instructions. All samples were set up and assayed in duplicate or triplicate.