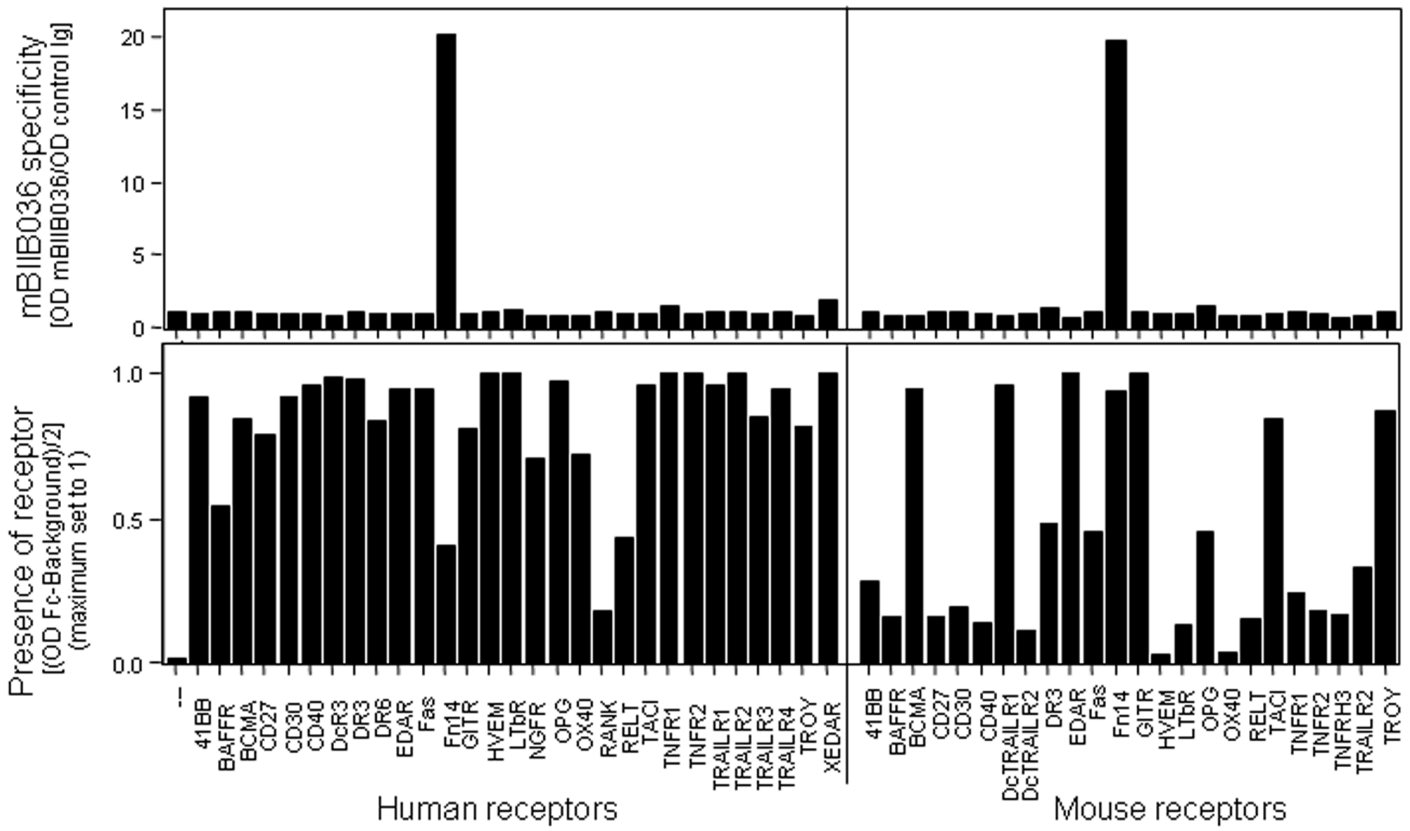
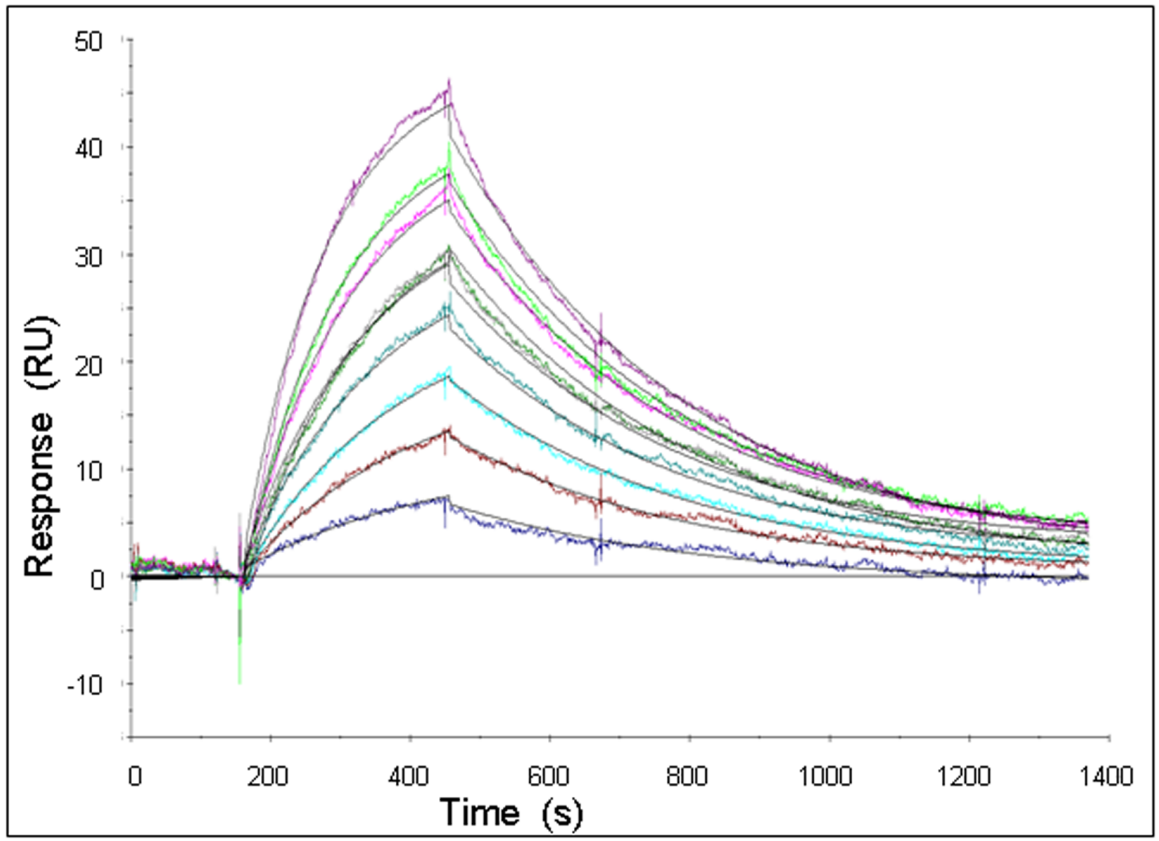


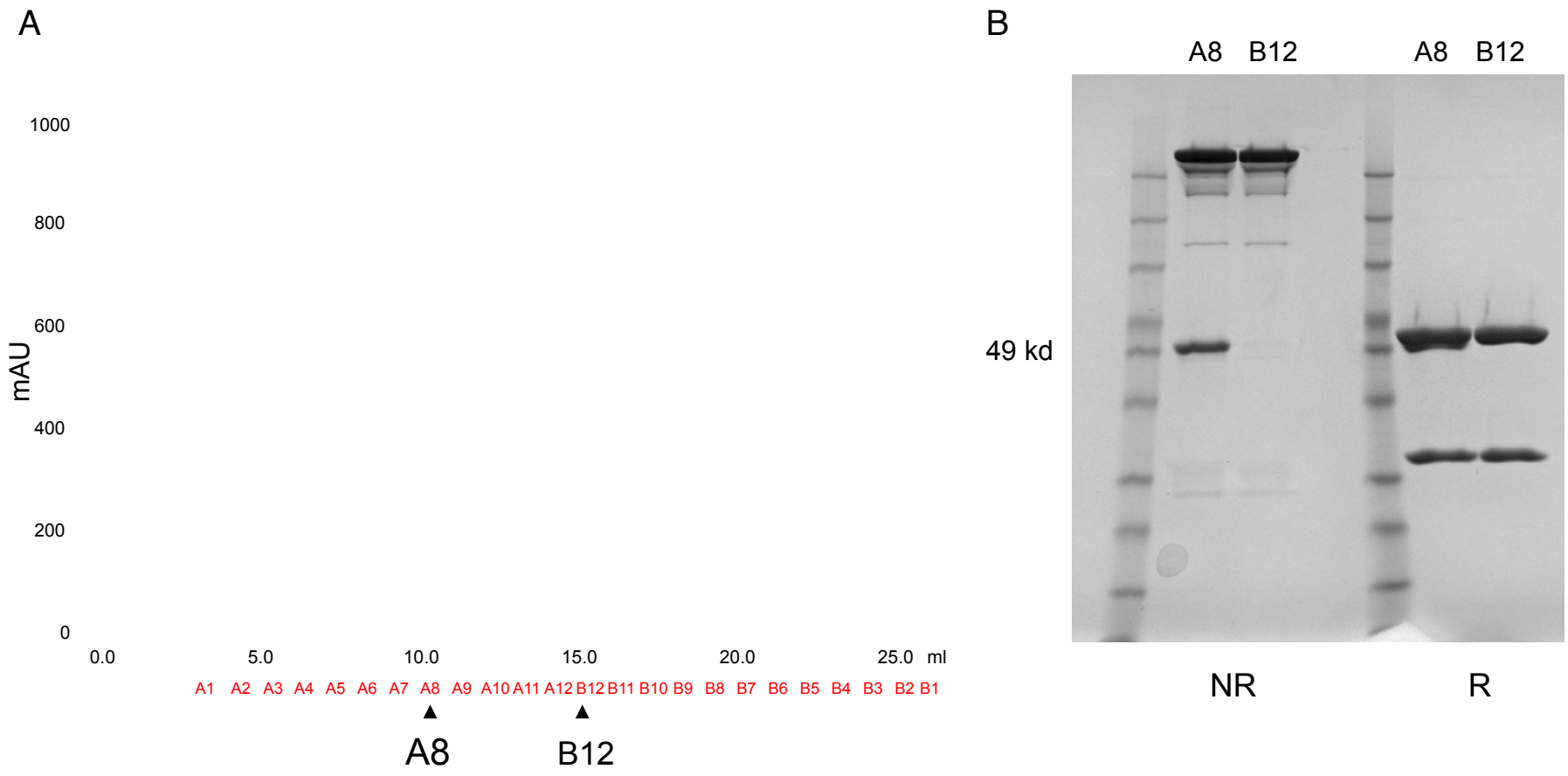
Supplementary Figure 1. *Specificity of BIIB036 for Fn14*
(A) The specificity of mBIIB036 for Fn14 is shown. A panel of receptor-Fc fusion proteins with human and murine versions of TNF family member receptors was expressed. The top panel represents binding of mBIIB036, as compared to control mouse Ig, to the various receptor-Fc proteins, as measured by ELISA. The lower panel shows expression levels of the various receptors.

Supplementary Figure 1



Supplementary Figure 2. *Binding kinetics of soluble human Fn14 to BIIB036*
0.3 - 3 nM soluble human Fn14 was run over immobilized BIIB036 (colored lines). Data were processed and fitted to a 1:1 kinetic binding model (black lines) using Biaevaluation software. Kinetic parameters determined from three independent experiments were: ka = 1.8 ± 0.3 x 10^{6} $M^{-1}s^{-1}$; kd = 3.0 ± 0.1 x 10^{-3} s-1. KD = 1.7 ± 0.3 nM was calculated from the ratio of the rate constants.

Supplementary Figure 2



Supplementary Figure 3. *Biochemical analysis of BIIB036-multimer*
(A). The first peak (fraction A8) in the size exclusion chromotography profile represents BIIB036-multimer, whereas the second peak (fraction B12) is BIIB036 alone. (B) The SDS-PAGE gel confirms the identity of these fractions. BIIB036-multimer (fraction A8) shows protein A at MW 49 KD, whereas this is not present in the BIIB036 alone fraction (B12). The protein A is evident under non-reducing conditions, but is overlaid with the heavy chain of BIIB036 under reducing conditions.

Supplementary Figure 3

SUPPLEMENTARY METHODS

Cross-reactivity studies

Receptor-Fc fusion proteins were cloned in a previously described expression vector [28]. These vectors contained the sequence coding for the extracellular domain of various TNF receptors with their own signal peptide or, when indicated, preceded by the hemagglutinin or immunoglobulin G1 heavy chain signal peptides (HA signal and Ig signal, respectively): h4-1BB-(1-186), m4-1BB-(1-181), hBAFF-R-(2-71) (HA signal), mBAFF-R-(2-70) (HA signal), hBCMA-(2-54) (Ig signal), mBCMA-(1-46) (Ig signal), hCD27-(1-191), mCD27-(1-182), hCD30-(1-380), mCD30-(1-285), hCD40-(1-193), mCD40-(1-193), hDcR3-(1-300), hDR3-(25-199) (Ig signal), mDR3-(1-194), hDR6-(1-351), mDcTRAILR1-(1-158), mDcTRAILR2-(40-171) (HA signal), hEDAR-(1-183), mEDAR-(1-183), hFas-(1-170), mFas-(1-169), hFn14-(1-75), mFn14-(1-75), hGITR-(26-161) (HAsignal), mGITR-(1-153), hHVEM-(1-200), mHVEM-(1-206), hLTbR-(1-220), mLTbR-(1-217), hNGFR-(1-250), hOPG-(1-202), mOPG-(1-214), hOX40-(1-214), mOX40-(1-209), hRANK-(29-213) (HA signal), hRELT-(1-125), mRELT-(1-165), hTACI-(2-160) (HA signal), mTACI-(2-78) (HA signal), hTNFR1-(1-211), mTNFR1-(1-210), hTNFR2-(1-257), mTNFR2-(1-257), mTNFRH3-(1-162), hTRAIL-R1-(1-239), hTRAIL-R2-(1-212), mTRAIL-R-2-(1-166), hTRAIL-R3-(1-236), hTRAIL-R4-(1-211), hTROY-(1-168), mTROY-(1-168), and hXEDAR-(1-134) (Ig signal).

293T cells were transiently transfected with the above constructs by the calcium phosphate procedure and were grown in serum free Opti-MEM I medium for 4-7 days. Supernatants were collected and frozen until use.

ELISA plates were coated with 5 μg/ml mouse anti-human antibody (Jackson) and blocked. 20 μl of receptor-Fc in serum-free media was added in a final volume of 100 μl incubation buffer (PBS, 0.4% powdered skimmed milk, 0.05% Tween 20), and detected with three different protocols: a) 100 μl of biotinylated mBIIB036 at 100 ng/ml, followed by HRP-coupled streptavidin; b) 100 μl of isotype matched control (biotinylated MOPC-21 mouse Ig) at 100 ng/ml, followed by HRP-coupled streptavidin; c) 100 μl of HRP-coupled donkey anti-human antibody (Jackson) at 1/1000 (0.8 μg/ml) in incubation buffer. All incubations were performed for 1 hour at 37°C, and plates were washed 3 times with PBS, 0.05% Tween 20 between each step. Plates were detected with *o*-phenylenediamine reagent (Sigma), stopped with 2 N HCl, and absorbance was read at 492 nm. The presence of receptors-Fc was inferred from the anti-human staining. Specific binding of mBIIB036 to various TNF receptors was expressed as the ratio of signals obtained for test (mBIIB036) versus control (mouse Ig).

Biacore assays

All experiments were performed using a Biacore 3000 instrument (GE Healthcare). BIIB036 was immobilized on a CM5 sensorchip using the Biacore Amine Coupling kit according to manufacturer's instructions. Briefly, BIIB036 was diluted to 50 μg/ml in 10 mM acetate, pH 5.5 and 10 μl was injected over a chip surface that had been activated with a 10 μl injection of 1:1 N-hydroxsuccinimide (NHS): 1-Ethyl-3(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC). Excess free amine groups were then capped with a 50 μl injection of 1 M Ethanolamine. An underivatized surface treated in the same manner without protein injection was used as a background control. Typical immobilization levels were 1000-1500 RU. The running buffer used during

immobilization was 10 mM HEPES pH7.0, 150 mM NaCl, 3 mM EDTA, 0.005% detergent P-20.

Soluble, monomeric human Fn14 was diluted in 10 mM HEPES pH7.0, 150 mM NaCl, 3 mM EDTA, 0.005% detergent P-20, 0.05% BSA to concentrations ranging from 0.3 nM to 3 nM in 0.3 nM steps and injected over the BIIB036 derivatized surface, or an underivatized surface as a background control, at a flow rate of 50 μl/min. In addition a buffer only sample was injected as a further background control. This was followed by a 10 min dissociation in buffer alone. The sensorchip surface was then regenerated with 2 x 15 μl injections of 15 mM NaOH. Any remaining bound ligand was completely removed and the signal returned to baseline. In all cases binding to the blank chip was negligible. Resulting data were processed by first subtracting the response for each concentration sensorgram on the blank surface from the sensorgram on the BIIB036 surface, and then subtracting the buffer only sensorgram on the BIIB036 surface from the Fn14 sensorgrams on the same surface (so called double referencing of the data). Kinetic parameters were determined by global fitting of all double referenced sensorgrams to the kinetic model shown below for simultaneously determining the on and off rates within the BIAevaluation software:

$dAB/dt = (k_a*A*B) – (k_d*AB)$

$dB/dt = –((k_a*A*B) – (k_d*AB))$

A = Concentration

B[0] = Rmax

AB[0] = 0

Affinity was determined from the ratio of the rate constants where $K_D = k_d/k_a$.

Analysis of BIIB036-multimer

Following incubation at 4˚ C on a rotator overnight, the BIIB036-Protein A mixture was run through a Superdex 200 column (GE Healthcare) using a AKTA FPLC (GE Healthcare) in PBS running buffer at a flow rate of 0.5 ml/minute, and 1 ml fractions were collected. The identity of the two peaks from the elution profile was assessed by running a sample on a 4-20% Tris-Glycine Gel (Invitrogen) under reducing and non-reducing conditions and then staining with Simply Blue Safe Stain (Invitrogen).