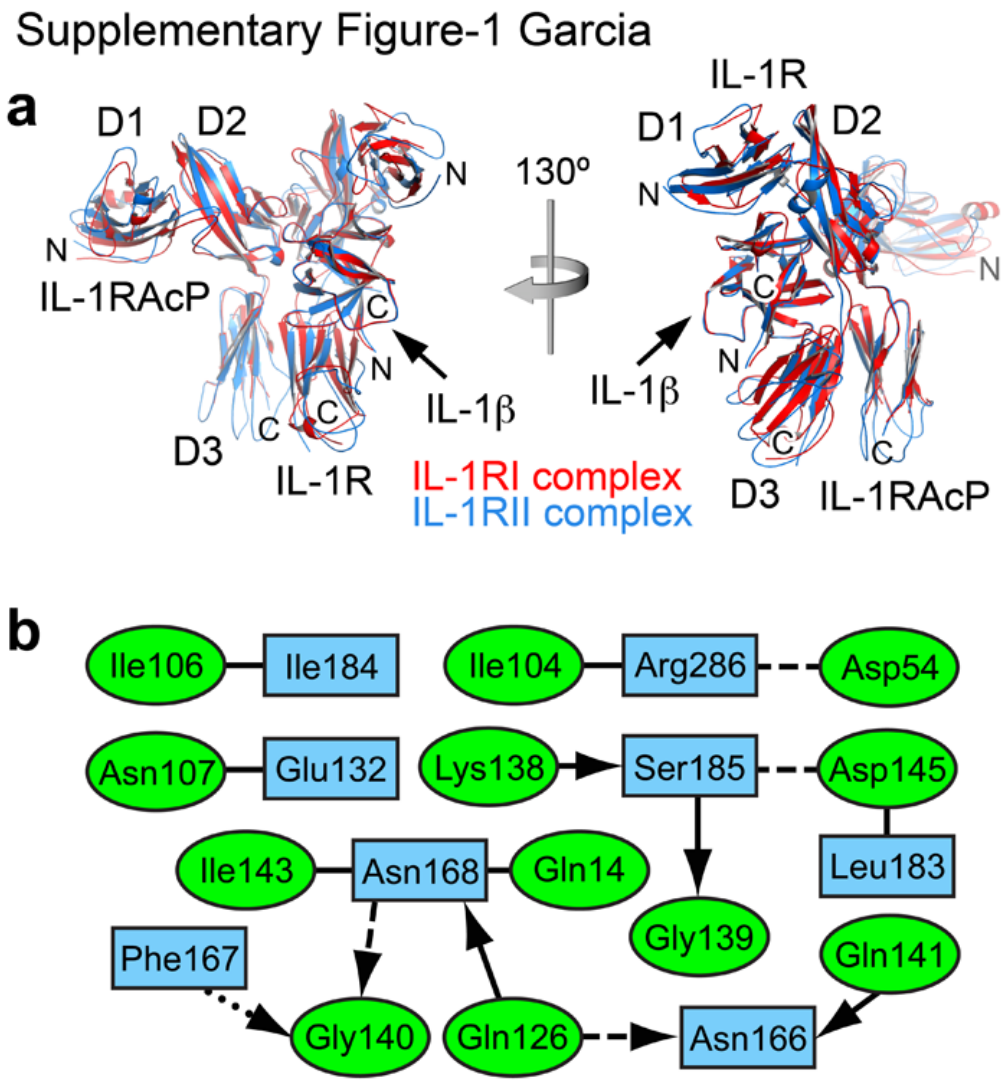
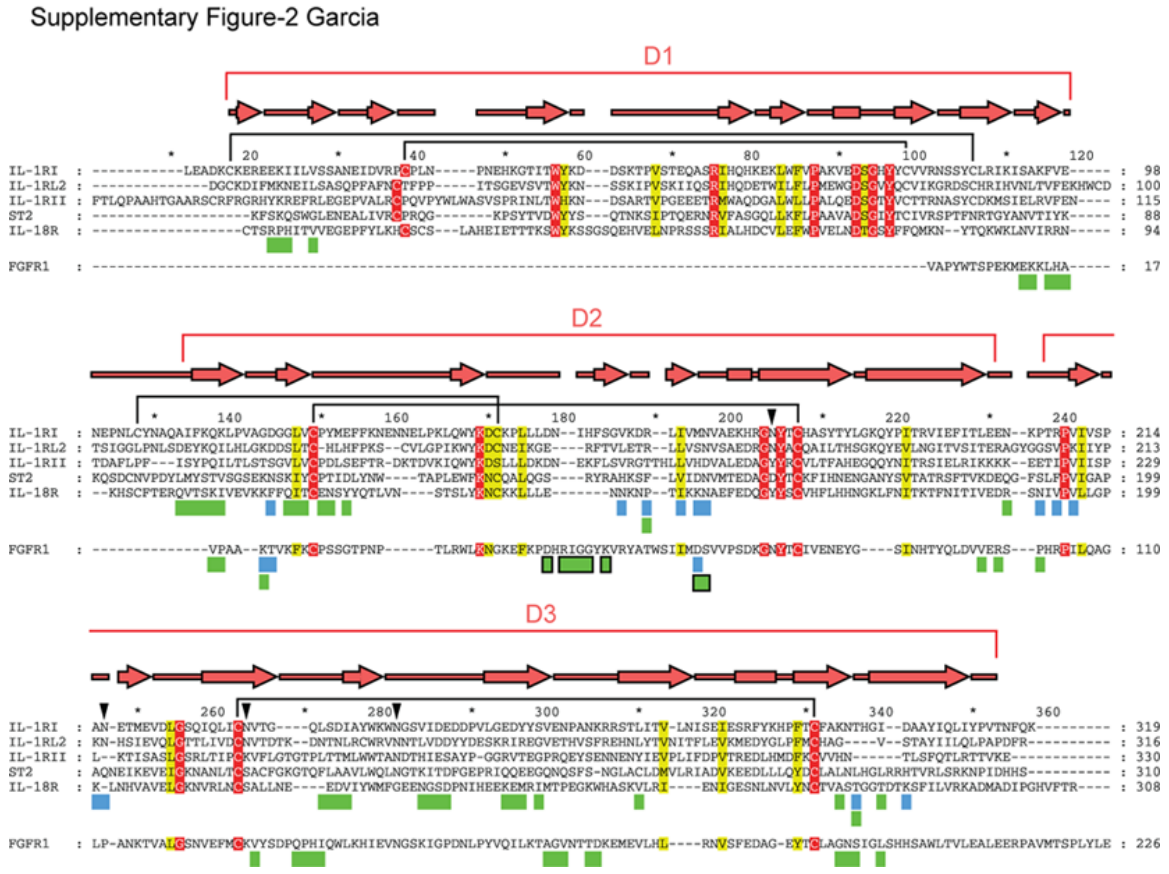
Supplementary Figures

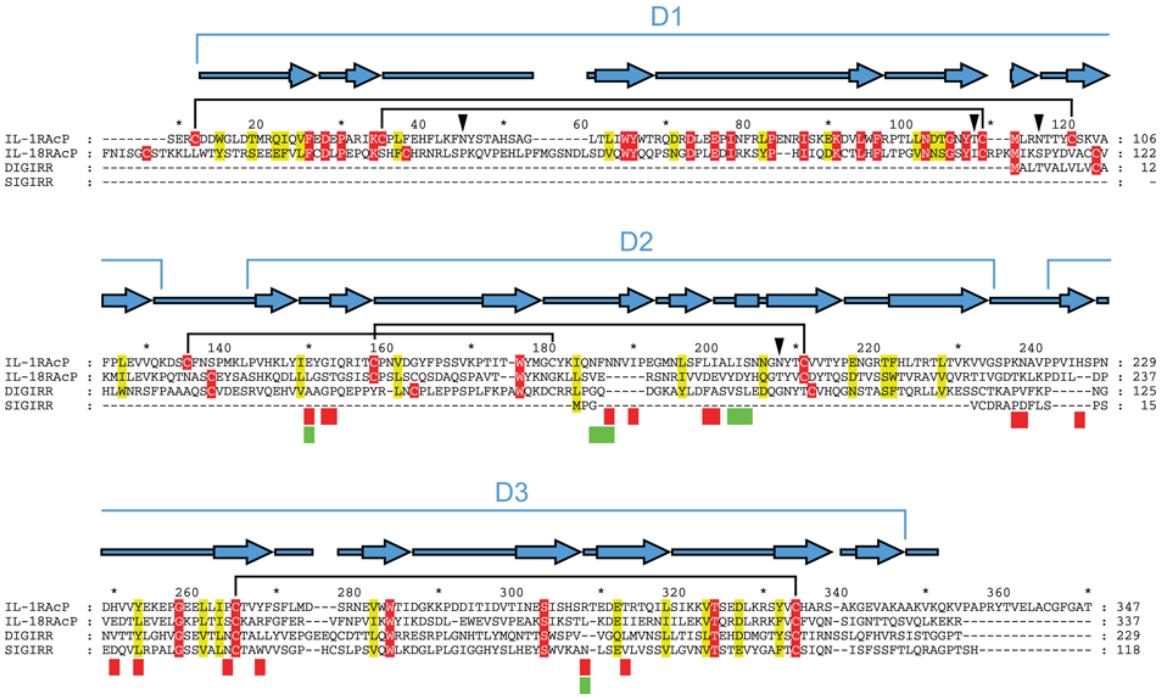


Supplementary Figure 1: Features shared between IL-1β decoy and signaling complex. (a) Superposition of the IL-1β signaling complex with the IL-1β decoy receptor complex (IL-1β ligands were superimposed), showing two different views, related to each other by a 130º rotation around the vertical axis. **(b)** Two-dimensional interaction map of the IL-1β–IL-1RAcP interface in the signaling complex.



Supplementary Figure 2: Sequence alignment of the human primary receptors in the IL-1 family and of FGFR1. Physico-chemically conserved amino acids are colored yellow; residues that are invariant are shown in red. Residues involved in interfaces are denoted by rectangles below the alignment, colored according to Figures 1 and 2, and Supplementary Figure 1b (green: IL-1β and FGF interface, respectively; blue: interface with IL-1RAcP and second FGFR1 molecule, respectively). Rectangles outlined in black mark residues of FGFR1 contacting the second FGF molecule. The secondary structural elements of IL-1RI are depicted on top of the alignment. Cysteine residues that form disulfide bridges are connected by black lines. Glycosylation sites are marked by arrows.

Supplementary Figure-3 Garcia



Supplementary Figure 3: Sequence alignment of the human accessory receptors in the IL-1 family, human SIGIRR, and DIGIRR from *Takifugu rubripes*. Physico-chemically conserved amino acids are colored yellow; residues that are invariant are shown in red. Interacting residues are denoted by rectangles below the alignment, colored according to Figures 1 and 2, and Supplementary Figure 1b (green: IL-1β interface; red: interface with IL-1RI). The secondary structural elements of IL-1RAcP are depicted on top of the alignment. Cysteine residues that form disulfide bridges are connected by black lines. Glycosylation sites are marked by arrows.

Supplementary Table 1 Data collection and refinement statistics

	IL-1β signaling complex
Data collection	
Space group	$P2_1$
Cell dimensions	
a, *b*, *c* (Å)	105.99, 65.90, 163.40
α, β, γ (°)	90, 90.35, 90
Resolution (Å)	19.7-3.1 (3.2-3.1) *
R_{sym}	6.8 (36.1)
$I / \sigma I$	10.5 (2.2)
Completeness (%)	96.3 (98.7)
Redundancy	1.7 (1.7)
Refinement	
Resolution (Å)	19.71-3.10
No. reflections	41079
R_{work} / R_{free}	21.0/27.7
No. atoms	
Protein	11476
Carbohydrate	196
Water	7
B-factors	
Protein	54.0
Carbohydrate	68.5
Water	29.2
R.m.s. deviations	
Bond lengths (Å)	0.017
Bond angles (°)	1.52

*Values in parentheses are for highest-resolution shell.

Supplementary Discussion

The first functional group of IL-1 cytokines includes IL-1α, IL-1-β and IL-1Ra that all interact with either functional IL-1RI or decoy IL-1RII receptors; IL-1RAcP serves as the common accessory chain for IL-1α and IL-1β complexes with either primary receptor[1,2]. The second functional group revolves around the IL-18 cytokine that binds IL-18R, and together cluster with IL-18RAcP[1,3]. Two decoy proteins for this system include the single Ig chain soluble receptor IL-18BP, and more speculatively, a more recently discovered cytokine called IL-37 that is reported to block IL-18 actions[4,5]. The third functional group centers on the IL-33 cytokine that binds to its primary ST2 receptor, and then shares the accessory IL-1RAcP chain[6-8]. Both the IL-33 and IL-1α/β primary receptor assemblies are inhibited by SIGIRR, a single Ig domain orphan receptor chain that is more closely related to other secondary receptors in the family[9]; this suggests that its mechanism of action may be through binding competition with the common IL-1RAcP chain[10-12]. SIGIRR likely arose through the piecemeal loss of Ig domains D1 and D2 from an IL-1RAcP-like ancestor, and intermediates in its evolution (that have lost only D1) have been captured in teleost fish[13]; SIGIRR receptor orthologs have a damaged or mutated intracellular TIR domain that does not propagate signals[11,14].

The fourth functional group is shaped by the most recently described clan of IL-1 cytokines that were discovered in a genomic cluster on human chromosome 2, proximal to the IL-1α, IL-1β and IL-1Ra genes[15]. Adopting the new IL-36 designation are IL-1F5, F6, F8 and F9, renamed as IL-36Ra (a decoy cytokine), and IL-36α, IL-36β and IL-36γ[16]; IL-1F10 is still not characterized, but is grouped with the IL-36s due to its closer sequence similarity. As mentioned earlier, IL-37 (IL-1F7) is at the moment placed in the IL-18 system[4,5]. The functional IL-36 signaling complex is completed by the addition of the IL-1RAcP chain, an accessory receptor shared with IL-1α/β and IL-33[17-20]. It is not known if SIGIRR also antagonizes the IL-36 ternary complexes, but it is a distinct possibility since these all involve the IL-1RAcP accessory chain.

Two orphan receptors, IL-1RAPL1 and IL-1RAPL2, are depicted in the inner wheel since they are more closely related to accessory chain receptors[21-23]; however, their ligands are unknown, and they are drawn unpaired. The structural integrity of the IL-1RAPL1 and IL-1RAPL2 intracellular TIR domains nonetheless suggests that they are capable of forming active signaling complexes[24]. The striking neuronal expression of these two orphan IL-1 receptors, and genetic links to human cognitive ability[25], and central nervous system disorders like autism, schizophrenia and mental retardation[26], suggests that their natural ligands are also neuronally expressed.

Methods

Protein expression and purification

Mature human IL-1β (residues 1-153) was cloned into the pGEX-6P-1 vector (GE Healthcare) and expressed in *Escherichia coli* strain BL21(DE3). IL-1β containing an N-terminal glutathione S-transferase (GST) tag was purified using Glutathione Sepharose 4 Fast Flow (GE Healthcare) resin, followed by digestion with GST-tagged 3C protease to remove the tag of IL-1β. After buffer exchange, the cleaved tag and 3C protease were removed by glutathione resin, and IL-1β was further purified by gel filtration (Superdex 75, GE Healthcare) in 30 mM HEPES pH 7.5, 150 mM NaCl.

Human IL-1RI (residues 1-330) and human IL-1RAcP (residues 1-350), both containing the native signal peptide, were secreted from suspended HEK293 GnTI$^-$ cells grown in Pro293 medium (Lonza) using the BacMam expression vector pVL-AD6[27]. The proteins were purified by nickel-affinity chromatography, deglycosylated with endoglycosidase H_f (NEB), and digested with 3C protease at 16ºC overnight. A final gel filtration step was carried out using a Superdex 75 (S75) column (GE Healthcare) equilibrated in HEPES-buffered saline (HBS).

The ternary complex was formed by mixing IL-1β, IL-1R, and IL-1RAcP in equimolar ratios. The complex mixture was digested with Carboxypeptidases A and B at 16ºC overnight, and purified by gel filtration (S75, HBS).

Crystallization and Structure Determination

Crystallization experiments were carried out using hanging-drop vapor diffusion at 20ºC. The IL-1β–IL-1RI–IL-1RAcP ternary complex (12 mg/mL) crystallized in 12% PEG 5000 MME, 100 mM MES pH 6.5, 12% 1-propanol. Prior to data collection, crystals were cryo-protected (15% PEG 5000 MME, 100 mM MES pH 6.5, 13% 1-propanol, 1xHBS, 25% glycerol) and frozen in liquid nitrogen. The data set was collected at 1.0000 Å and 100 K at beamline 8.2.1 of the Advanced Light Source (ALS), Berkeley. Data were indexed, integrated and scaled with the XDS package[28]. The structure was solved by molecular replacement using the program Phaser[29] and the previously published structures of the binary IL-1β–IL-1RI complex[30] and of IL-1RAcP[31] as search models. The program Coot[32] was used to build the model and to determine the Ramachandran statistics. Refinement was carried out with Phenix[33]. In the Ramachandran plot, 91.7%, 8.3%, and 0% of residues in the final model are in the preferred, allowed, and disallowed regions, respectively. Interaction maps of interfaces were drawn in Adobe Illustrator using data from the AquaProt server[34]. As the electron density of the second complex in the asymmetric unit was at several places better defined than that of the first complex, the second complex was used for the interface analyses. Molecular graphics images were prepared using PyMOL (Schrödinger).

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