

1 **Structure of the IL-27 quaternary receptor signaling complex**

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12 **Abstract**

13 Interleukin 27 (IL-27) is a heterodimeric cytokine that functions to constrain T cell-mediated
14 inflammation and plays an important role in immune homeostasis. Binding of IL-27 to cell
15 surface receptors IL-27Ra and gp130 results in activation of receptor-associated Janus
16 Kinases and nuclear translocation of STAT1 and STAT3 transcription factors. Despite the
17 emerging therapeutic importance of this cytokine axis in cancer and autoimmunity, a molecular
18 blueprint of the IL-27 receptor signaling complex, and its relation to other gp130/IL-12 family
19 cytokines, is currently unclear. We used cryogenic-electron microscopy (cryo-EM) to
20 determine the quaternary structure of IL-27 (p28/Ebi3) bound to receptor subunits, IL-27Ra
21 and gp130. The resulting 3.47 Å resolution structure revealed a three-site assembly
22 mechanism nucleated by the central p28 subunit of the cytokine. The overall topology and
23 molecular details of this binding are reminiscent of IL-6 but distinct from related heterodimeric
24 cytokines IL-12 and IL-23. These results indicate distinct receptor assembly mechanisms used
25 by heterodimeric cytokines with important consequences for targeted agonism and
26 antagonism of IL-27 signaling.

27 **Introduction**

28 Cytokines are secreted factors that mediate cell-cell communication in the immune system
29 (Spangler et al., 2015). Binding of cytokines to cell surface receptors leads to activation of
30 receptor-associated Janus Kinase (JAK) proteins which phosphorylate each other as well as
31 downstream Signal Transducer and Activator of Transcription (STAT) proteins, triggering
32 nuclear translocation and regulation of gene expression. Cytokines can be classified by their
33 use of shared receptors which transduce signals for multiple cytokines within a family.
34 Interleukin-6 signal transducer (IL-6ST), also known as glycoprotein 130 (gp130), is a shared
35 receptor that mediates signaling of multiple cytokines including IL-6, IL-11, and IL-27. Unlike
36 other gp130 family cytokines, IL-27 is a heterodimeric cytokine consisting of a four-helix
37 bundle, IL-27p28 (p28), with similarity to IL-6, complexed with a secreted binding protein,
38 Epstein-Barr Virus-Induced 3 (Ebi3), with homology to type I cytokine receptors (Pflanz et al.,
39 2002). IL-27 signals through a receptor complex consisting of IL-27Ra (TCCR/WSX-1) and
40 gp130 expressed predominantly on T cells and NK cells (Pflanz et al., 2002, 2004) (Figure
41 1A). Binding of IL-27 to its receptor subunits triggers the activation of receptor-associated
42 Janus Kinase 1 (JAK1) and JAK2 leading to phosphorylation of STAT1 and STAT3 (Lucas et
43 al., 2003; Owaki et al., 2008; Wilmes et al., 2021). Functionally, IL-27 signaling serves to
44 constrain inflammation by antagonizing differentiation of pro-inflammatory Th17 cells
45 (Stumhofer et al., 2006), stimulating T-bet expression in regulatory T cells (Tregs) (Hall et al.,
46 47 48

49 2012), and inducing production of the anti-inflammatory cytokine IL-10 (Stumhofer et al.,
50 2007). The important role of IL-27 in constraining inflammation has prompted the development
51 of antagonist antibodies, one of which is currently being developed as a cancer
52 immunotherapy (Patnaik et al., 2021).

53
54 Although IL-27 is a member of the gp130 family, its heterodimeric composition is similar to IL-
55 12 and IL-23 which are cytokines that share a p40 subunit similar to Ebi3. In this system, the
56 p40 subunit of IL-12 and IL-23 directly engages the shared receptor IL-12R β 1 in a manner
57 distinct from IL-6 and related cytokines (Glassman et al., 2021a). Given the diverse receptor
58 assembly mechanisms used by cytokine receptors, we sought to determine the structure of
59 IL-27 in complex with its signaling receptors, gp130 and IL-27R α , by cryogenic-electron
60 microscopy (cryo-EM).

61
62 Here we report the 3.47 Å resolution structure of the complete IL-27 receptor complex. In this
63 structure, the central IL-27 cytokine engages IL-27R α through a composite interface consisting
64 of p28 and the D2 domain of Ebi3. A conserved tryptophan residue at the tip of p28 then
65 engages the D1 domain of gp130 to facilitate quaternary complex assembly. This receptor
66 assembly mechanism bears striking resemblance to that of IL-6 but is distinct from IL-12 and
67 IL-23, indicating divergent receptor binding modes for heterodimeric cytokines.

68 69 **Results**

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71 **CryoEM structure of the IL-27 quaternary complex**

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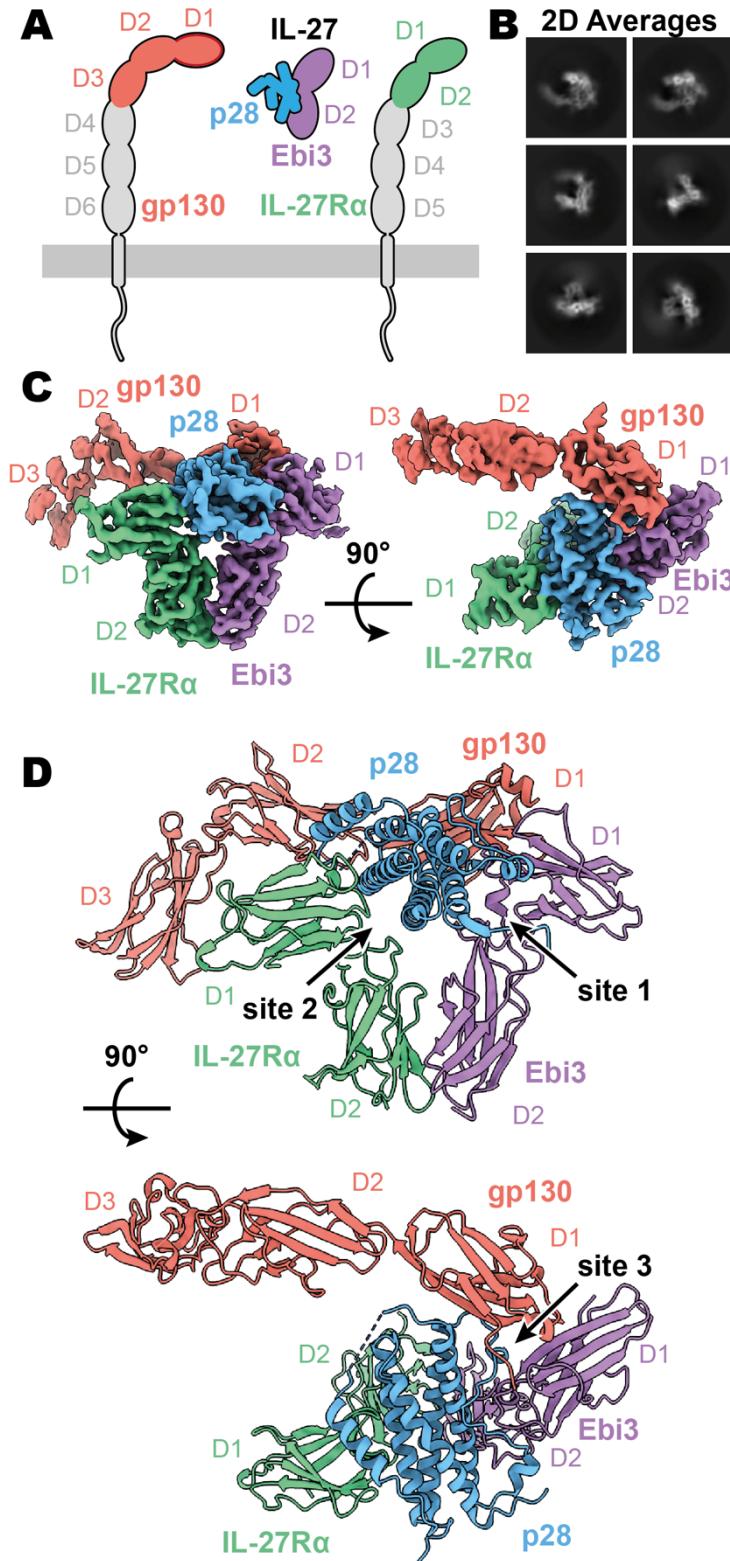
73 IL-27 (p28/Ebi3) signals through a receptor complex composed of IL-27R α and the shared
74 receptor gp130 (Figure 1A). The extracellular domain (ECD) of IL-27R α consists of five
75 fibronectin type III (FNIII) domains (D1-5) of which the N-terminal D1-D2 constitute a cytokine-
76 binding homology region (CHR). gp130 has a similar domain architecture but with the addition
77 of an N-terminal immunoglobulin (Ig) domain (D1). Initial attempts at reconstituting a soluble
78 receptor complex through mixing of IL-27, IL-27R α D1-D2, and gp130 D1-D3 resulted in
79 dissociation on size-exclusion chromatography due to the low affinity of gp130. To stabilize
80 the complex, we expressed p28 fused to gp130 through a long (20 a.a.) flexible linker which
81 enabled us to purify the complete IL-27 receptor complex by size exclusion chromatography
82 (SEC). Importantly, the long linker did not constrain the binding mode but rather raised the
83 effective concentration of gp130. The complex was vitrified and subject to single-particle
84 cryoEM analysis. The quaternary complex was determined to a resolution of 3.47 Å (Table
85 S1, Supplementary Figure 1). All domains of IL-27 and IL-27R α , as well as D1 of gp130 had
86 well resolved cryoEM density (Figure 1B-C, Supplemental Figure 1E), with localised resolution
87 estimates near and exceeding 3 Å in this region (Supplemental Figure 1B). The localised
88 resolution for the D2 density of gp130 is lower, yet able to be built with confidence, while D3
89 electron density was interpretable for domain placement (Figure 1C, Supplementary Figure
90 1B).

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92 The IL-27 quaternary complex exhibits a molecular architecture containing “sites 1-3”, as seen
93 in other gp130 family cytokines (Boulanger et al., 2003) (Figure 1D). In this structure, IL-27
94 bridges IL-27R α and gp130 to initiate downstream signaling through JAK1 and JAK2 (Ferrao
95 et al., 2016; Pradhan et al., 2010). Within IL-27, the four-helical bundle of p28 packs against
96 the hinge between Ebi3 D1 and D2 in a site 1 interaction. Opposite Ebi3, IL-27R α D1-D2

97 engages the helical face of p28 in an interaction stabilized by stem contact between the D2
98 domains of IL-27R α and Ebi3 to form a site 2 interaction. On the posterior face of IL-27, the
99 D1 domain of gp130 binds to a conserved tryptophan at the tip of p28 to make a classical site
100 3 interaction (Boulanger et al., 2003).

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Figure 1. Composition and cryoEM structure of human IL-27 quaternary complex. (A) Cartoon representation of the components of the IL-27 quaternary signaling complex. gp130 (red),

105 p28 (blue), Ebi3 (purple), IL-27Ra (green), with domains excluded from the imaged constructs in
106 grey. The D1 Ig domain of gp130 is represented by red, with an additional dark red outline to
107 distinguish it from FNIII domains in gp130, Ebi3, and IL-27Ra. (B) Reference-free 2D averages
108 from cryoEM of the IL-27 quaternary complex. (C) Refined and sharpened cryoEM density maps
109 of IL-27 quaternary complex, colored as in A. (D) Ribbon representation of the atomistic modelling
110 of IL-27 quaternary complex, colored as in A.

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112 Site 1-3 interface architecture of IL-27 quaternary complex

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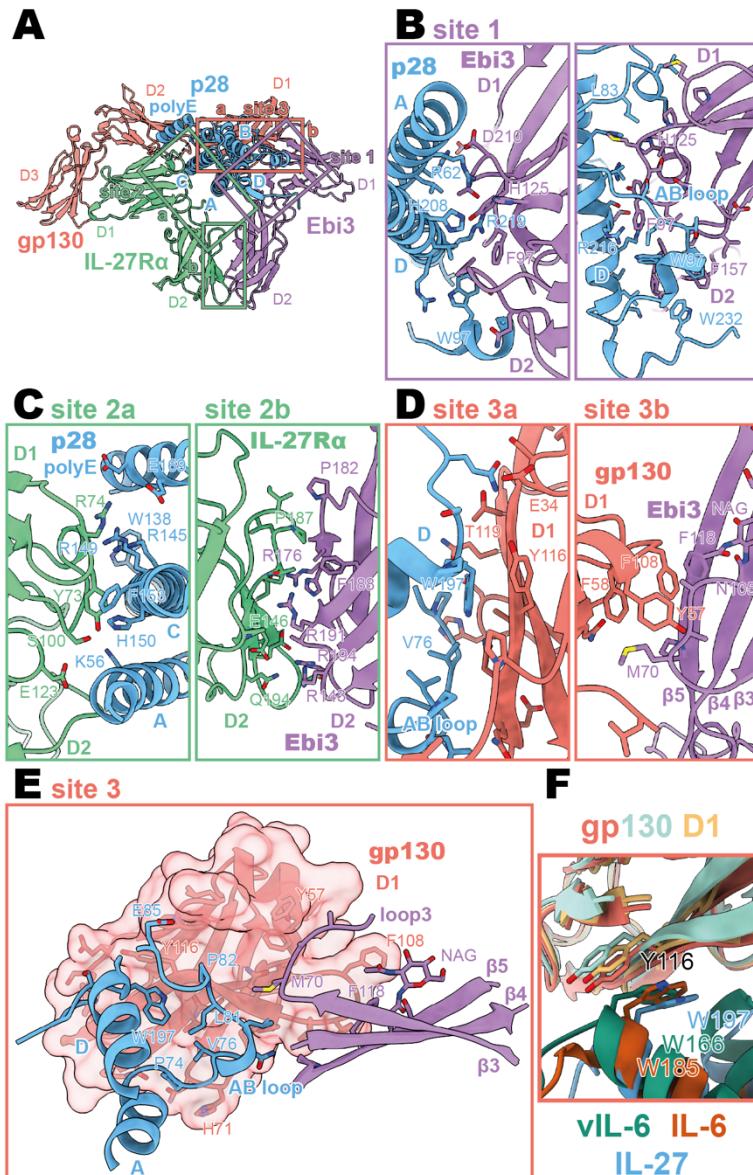
114 A site 1 interface between p28 and Ebi3 is used to form the heterodimeric cytokine IL-27. In
115 this interaction, the alpha helices A and D, the AB loop, and the C-terminus of p28 pack tightly
116 against the hinge region between D1 and D2 of Ebi3 (Figure 2A-B). This interaction is
117 mediated by a variety of hydrogen bonds between p28 and the hinge region of Ebi3,
118 particularly along alpha helices A and D of p28. At the center of this interface is p28 R219
119 which is coordinated by D207 and T209 in Ebi3. Extending from this region, a hydrophobic
120 patch of residues on the C-terminus and AB loop of p28 (F94, W97, L223, W232) packs
121 against residues in the Ebi3 hinge (F97, F157, I160). The extensive and hydrophobic nature
122 of this interface is consistent with the observation that co-expression of p28 and Ebi3 is
123 required for efficient cytokine secretion (Pflanz et al., 2002).

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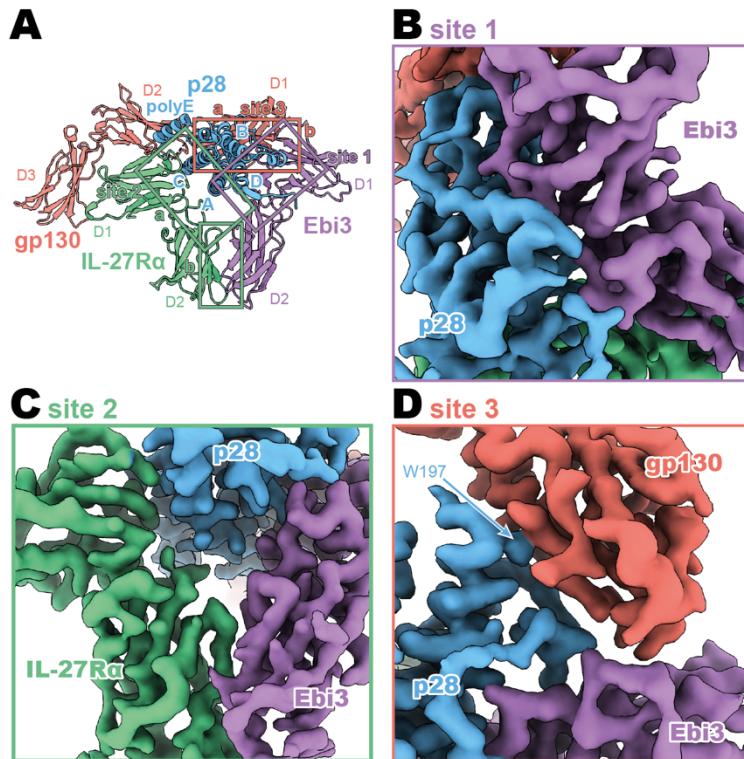
125 Opposing the Ebi3 binding site, p28 engages the hinge region of IL-27Ra (Figure 2C, site 2a).
126 This interaction is stabilized by stem-stem contacts between the D2 domains of IL-27Ra and
127 Ebi3 (Figure 2C, site 2b). Alpha helices A and C of p28 form the bulk of the site 2a interaction.
128 In contrast to the site 1 interaction which forms the holo-cytokine, the site 2a interaction is
129 more limited both in terms of hydrophobicity and buried surface area (Site 1: 1339.4 Å², Site
130 2a: 810.4 Å²) (Krissinel and Henrick, 2007). One notable feature of this interface is the
131 contribution of a 13 amino acid polyglutamic acid region in p28 which forms an alpha helix
132 (polyE helix) that contacts R74 and K77 in the IL-27Ra D1 domain. Cytokine binding is
133 stabilized by “stem-stem” interactions between D2 FNIII domains of IL-27Ra and Ebi3. This
134 site 2b interaction is characterized by a high degree of shape and charge complementarity
135 with an arginine-rich patch of Ebi3 (R143, R171, R194) packing against the positively charged
136 base of IL-27Ra D2 domain (D138, D142, E146). The composite nature of IL-27Ra binding
137 explains in part the inability of p28 to mediate IL-27 signaling in the absence of Ebi3
138 (Stumhofer et al., 2010).

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140 On the posterior face of the cytokine, a classical site 3 interface is formed through the
141 interaction of the D1 Ig domain of gp130 associating with both p28 (site 3a) and Ebi3 (site 3b)
142 (Figure 2D,E). Similar to IL-12 family members, IL-23 and IL-12 (Glassman et al., 2021a), and
143 the gp130 family members IL-6 (Boulanger et al., 2003) and viral IL-6 (Chow et al., 2001), this
144 interaction is anchored by the conserved W197 on p28 which packs tightly against the base
145 of the D1 Ig domain of gp130 (Figure 2F). The site 3 interaction is extended by the AB loop of
146 p28 which makes contact with both gp130 and Ebi3. The tip of gp130 D1 packs against the
147 top of Ebi3 D1 in a limited interface centered around Ebi3 F118.



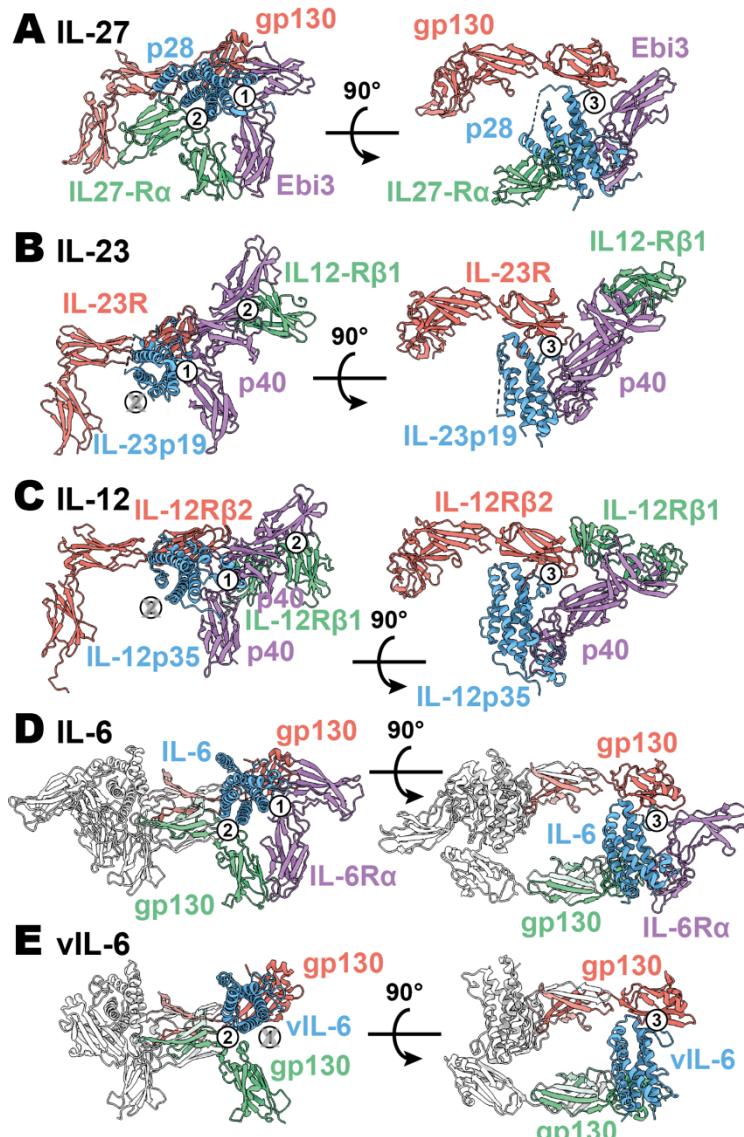
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149 **Figure 2. Binding interfaces of the human IL-27 quaternary complex.** (A) Ribbon
150 representation of the IL-27 quaternary signaling complex, containing gp130 (red), p28 (blue), Ebi3
151 (purple), and IL-27Ra (green). Regions containing sites 1, 2, and 3 are boxed in purple, green, and
152 red respectively. (B) Two views of the site 1 interface, colored as in A. (C) The site 2 interface,
153 comprised of a site 2a, p28 to IL-27Ra, interaction, and a site 2b, Ebi3 to IL-27Ra, interaction. All
154 proteins are colored as in A. (D,E) The site 3 interface, comprised of a site 3a, p28 to gp130,
155 interaction, and a site 3b, Ebi3 to IL-27Ra, interaction. All proteins are colored as in A. (F) Structural
156 overlay of site 3 interacting domains from IL-27 complex, IL-6 complex (PDB 1P9M), and viral IL-
157 6 (vIL-6) complex (PDB 1L1R). IL-27 quaternary complex colored as in A, IL-6 ternary complex in
158 orange, and vIL-6 binary complex in teal.



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167 The heterodimeric nature of IL-27 has led some to classify it as an IL-12 family cytokine
168 (Trinchieri et al., 2003). However, comparison of the IL-27 receptor complex with that of IL-23
169 and IL-12 reveals striking differences (Figure 3A-C). In IL-27, the central p28 subunit engages
170 all receptor components. In contrast, each subunit of IL-23 and IL-12 engages a different
171 receptor in a modular interaction mechanism (Glassman et al., 2021a). The assembly of IL-
172 27 more closely resembles that of IL-6, in which the central four-helix bundle encodes binding
173 sites for all receptor components (Figure 3D). However, in the case of IL-6, this motif is
174 duplicated through a C2 symmetry axis due to a dual role of gp130 at site 2 and site 3. The
175 similarity between IL-27 and IL-6 is observed not only in overall architecture but also in
176 molecular detail, where IL-6, vIL-6, and IL-27 all engage gp130 using a highly convergent
177 interface in which a tryptophan from helix D of the cytokine (W197 in p28, W185 in IL-6, W166
178 in vIL-6) forms an aromatic anchor that is capped by Y116 of gp130 (Figure 2F, 3D-E).
179 Alignment of gp130 D1-(v)IL-6 pairs to the IL-27 structure reveals a tight correlation in gp130
180 binding pose with C α RMSDs of 1.039 Å (IL-6) and 1.015 Å (vIL-6) across 149 and 124
181 trimmed residue pairs. This striking convergence of gp130 binding modes helps to explain the
182 ability of p28 to antagonize IL-6 signaling (Stumhofer et al., 2010) and contextualises its place
183 in respect to both gp130 and IL-12 family cytokines.

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Figure 3. Comparison of IL-27 to IL-12 and IL-6 family complexes. (A) Ribbon representation of the IL-27 signaling complex, containing gp130 (red), p28 (blue), Ebi3 (purple), and IL-27Ra (green). Sites 1, 2, and 3 are noted with a numbered circle. (B) Ribbon representation of the IL-23 signaling complex (IL-12 family), containing IL-23R (red), IL-23p19 (blue), p40 (purple), and IL-12R β 1 (green) (PDB 6WDQ). Sites 1 and 3 are noted as in A, with the unoccupied site 2 marked with a grey 'X' and the distinct IL-12/IL-23 site 2 marked with a circled number 2. (C) Ribbon representation of a model of the IL-12 signaling complex (IL-12 family), containing IL-12R β 2 (red), IL-12p35 (blue), p40 (purple), and IL-12R β 1 (green) (Baek et al., 2021; Glassman et al., 2021a). Sites 1 and 3 are noted as in A, with the unoccupied site 2 marked with a grey 'X' and the distinct IL-12/IL-23 site 2 marked with a circled number 2. (D) Ribbon representation of the IL-6 signaling complex (IL-6 family), containing gp130 (red and green), IL-6 (blue), and IL-6Ra (purple) (PDB 1P9M). Secondary copies of each protein in the complex are colored in white. Sites 1, 2, and 3 are noted as in A. (E) Ribbon representation of the viral IL-6 signaling complex (IL-6 family), containing gp130 (red and green) and (blue) (PDB 1I1R). Secondary copies of each protein in the complex are colored in white. Sites 2 and 3 are noted as in A, with the unoccupied site 1 marked with a grey 'X'.

Discussion

205 A key paradigm in cytokine structural biology is the use of shared receptor components that
206 mediate diverse signaling outputs. IL-27 extends this, given that it shares the gp130 subunit
207 with IL-6 but mediates distinct and often countervailing functional effects. Here we find that IL-
208 27 assembles a receptor complex reminiscent of IL-6 but distinct from other heterodimeric IL-
209 12/23 class cytokines Interestingly, the soluble form of IL-6R α (sIL-6R α) generated through
210 alternative splicing or proteolytic cleavage can complex with IL-6 to potentiate signaling in
211 gp130-expressing cells (Harbour et al., 2020). This IL-6/sIL-6R α complex thus may be
212 functionally analogous to heterodimeric cytokine IL-27.

213
214 As in other types of cytokine signaling, there is context dependent therapeutic potential in both
215 the inhibition and potentiation of IL-27 signaling. The targeting of the heterodimeric cytokines
216 of the IL-12 family has been well explored, with various clinically approved inhibitors, targeting
217 either IL-23 signaling via p19 (Risankizumab, Guselkumab, Tildrakizumab) (Tait Wojno et al.,
218 2019) or IL-12 and IL-23 signaling via the shared p40 (Ustekinumab) (Luo et al., 2010). In
219 these cases, the inhibition of IL-12 family members antagonizes the pro-inflammatory effects
220 of these cytokines, while IL-27 plays an inverse role in the regulation of inflammation. Due to
221 the role of IL-27 in the regulation T cell mediated inflammation (Tait Wojno et al., 2019),
222 inhibition of IL-27 is currently being explored for its use in the inhibition of aberrant IL-27
223 signaling in cancer (Patnaik et al., 2021). The structure of the IL-27 quaternary signaling
224 complex provides ample opportunities for the design of IL-27 inhibitors targeting different steps
225 in receptor assembly to better regulate IL-27 signaling.

226
227 On the other end of the spectrum, IL-27 agonism has been explored for its therapeutic use in
228 inflammatory autoimmune dysregulation such as experimental autoimmune encephalomyelitis
229 (EAE) (Fitzgerald et al., 2013) and colitis (Hanson et al., 2014). Despite the promise of IL-27
230 as a therapeutic, the bipartite nature of IL-27 limits its usefulness in the clinic. Using the
231 interfacial information provided in the structure of the IL-27 quaternary complex, structure-
232 guided protein engineering techniques can now be used to improve the therapeutic potential
233 of IL-27. One such application may be to generate single agent IL-27 agonists by affinity
234 maturing the interactions between p28 and its receptor subunit, as has been done recently to
235 generate IL-6 which do not require IL-6R α binding for efficient signaling (Martinez-Fabregas
236 et al., 2019). Conversely, attenuating the affinity of p28 for IL-27R α might be used to generate
237 cell-type biased partial agonists with selective activity based on differences in IL-27R α
238 expression across cell-type and activation state (Villarino et al., 2005) as has been done for
239 IL-2 (Glassman et al., 2021b), IL-10 (Saxton et al., 2021), and IL-12 (Glassman et al., 2021a).
240

241 Resolution of the complete IL-27 receptor complex reveals how this therapeutically important
242 cytokine engages its receptor subunits. The receptor assembly mechanism bears striking
243 resemblance to that of IL-6 but is distinct from IL-12 and IL-23, indicating divergent receptor
244 binding modes for heterodimeric cytokines. This structural insight further paves the way for
245 the continued development of therapeutics that modulate IL-27 signaling.

246
247 **Methods**

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249 **Cloning and protein expression**
250 Human IL-27R α (D1-2, residues 36-231), Ebi3 (residues 21-228), and p28 (residues 29-243)-
251 (GGGGS)₄-gp130 (D1-D3, residues 23-321) were cloned into the expression vector pD649.
252 IL-27R α and Ebi3 were expressed with signal sequence of influenza hemagglutinin and N-

253 terminal 6-His tags. Proteins were co-expressed in Expi293F cells (GIBCO) maintained in
254 Expi293 Expression Media (GIBCO) at 37°C with 5% CO₂ and gentle agitation.

255

256 **Protein purification**

257 IL-27 quaternary complex was purified by Ni-NTA chromatography followed by size exclusion
258 chromatography with a Superdex 200 column (GE Lifesciences). Fractions containing pure IL-
259 27 complex were pooled and stored at 4°C until vitrification.

260

261 **Cryo-electron microscopy**

262 Aliquots of 3 µL of IL-27 quaternary complex were applied to glow-discharged Quantifoil®
263 (1.2/1.3) grids. The grids were blotted for 3 seconds at 100% humidity with an offset of 3 and
264 plunge frozen into liquid ethane using a Vitrobot Mark IV (Thermo Fisher). Grid screening and
265 preliminary dataset collection occurred at Stanford cEMc. Final grids were imaged on a 300
266 kV FEI Titan Krios microscope (Thermo Fisher) located at the HHMI Janelia Research
267 Campus and equipped with a K3 camera and energy filter (Gatan). Movies were collected at
268 a calibrated magnification of ×105,000, corresponding to a 0.839 Å per physical pixel. The
269 dose was set to a total of 50 electrons per Å² over an exposure of 50 frames. Automated data
270 collection was carried out using SerialEM with a nominal defocus range set from -0.8 to -2.0
271 µM. 18,168 movies were collected.

272

273 **Image processing**

274 All processing was performed in cryoSPARC (Punjani et al., 2017) unless otherwise noted
275 (Supplementary Figure 1). The 18,168 movies were motion corrected using patch motion
276 correction and micrographs were binned to 0.839 Å per pixel. The contrast transfer functions
277 (CTFs) of the flattened micrographs were determined using patch CTF and 6,387,370 particles
278 were picked using blob picking and subsequently template picking. A subset of 2,008,987
279 particles were used in reference-free 2D classification. A particle stack containing 109,536 2D
280 cleaned particles was used to generate three *ab initio* models, one of which resembled the IL-
281 27 quaternary complex. This good *ab initio* model was then used against two junk classes in
282 6 rounds of iterative heterogenous refinement to reduce the full particle stack to 548,147
283 particles. These particles were refined using cryoSPARC non-uniform refinement (Punjani et
284 al., 2020) followed by local refinement to achieve a global resolution of 3.47 Å. Resolution was
285 determined at a criterion of 0.143 Fourier shell correlation gold-standard refinement
286 procedure. The final map was sharpened using deepEMhancer (Sanchez-Garcia et al., 2020).

287

288 **Model building and refinement**

289 AlphaFold models (Jumper et al., 2021) of IL-27R α , Ebi3, p28, and gp130 were docked into
290 the map using UCSF Chimera X (Pettersen et al., 2021). The resultant model was then refined
291 using Phenix real space refine (Adams et al., 2010) and manual building in Coot (Emsley and
292 Cowtan, 2004). The final model fit the map well (EMRinger (Barad et al., 2015) score 2.33)
293 and produced a favorable MolProbity score of 1.60 (Chen et al., 2010) with side-chain
294 rotamers occupying 97.37% Ramachandran favoured and 0.00% outliers (Supplementary
295 Table 1).

296

297 **Data Availability**

298 CryoEM maps and atomic coordinates for human IL-27 quaternary complex have been
299 deposited in the EMDB (EMD- 26382) and PDB (7U7N) respectively.

300

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307

308 **Author Contributions**

309 NAC contributed to conceptualization, methodology, investigation, analysis, writing – original
310 draft, review, and editing. CRG contributed to conceptualization, methodology, investigation,
311 analysis, writing – original draft, review, and editing. KMJ contributed to analysis, writing –
312 review, and editing. NT contributed to methodology, investigation, and writing – review. KGC
313 contributed to conceptualization, supervision, writing – review and editing, and funding
314 acquisition.

315

316 **Competing Interests**

317 KCG is the founder of Synthekine.

318

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