

1 **The human IL-17A/F heterodimer: a two-faced**
2 **cytokine with unique receptor recognition**
3 **properties**

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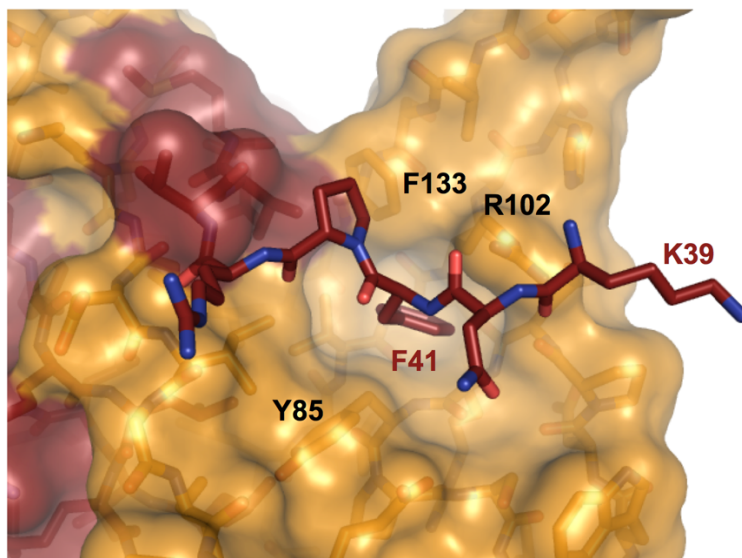
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27 **Supplementary information**

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30 **Supplementary Figure 1** Blockade of Site 1F of free IL-17A/F by Phe41 from the A-subunit. Close-up view
31 showing a surface representation of the free IL-17A/F heterodimer. The F- and A-subunits are in orange and
32 carmine, respectively. The N-terminal residues Lys39 to Arg43 of the A-subunit are shown in stick
33 representation and not included in the surface representation of the cytokine for clarity. Note the binding of
34 the side-chain of Phe41 into a pocket lined by Tyr85, Phe133 and Arg102 from the F-subunit. This pocket
35 corresponds to the binding site for Trp62 of IL-17RA.

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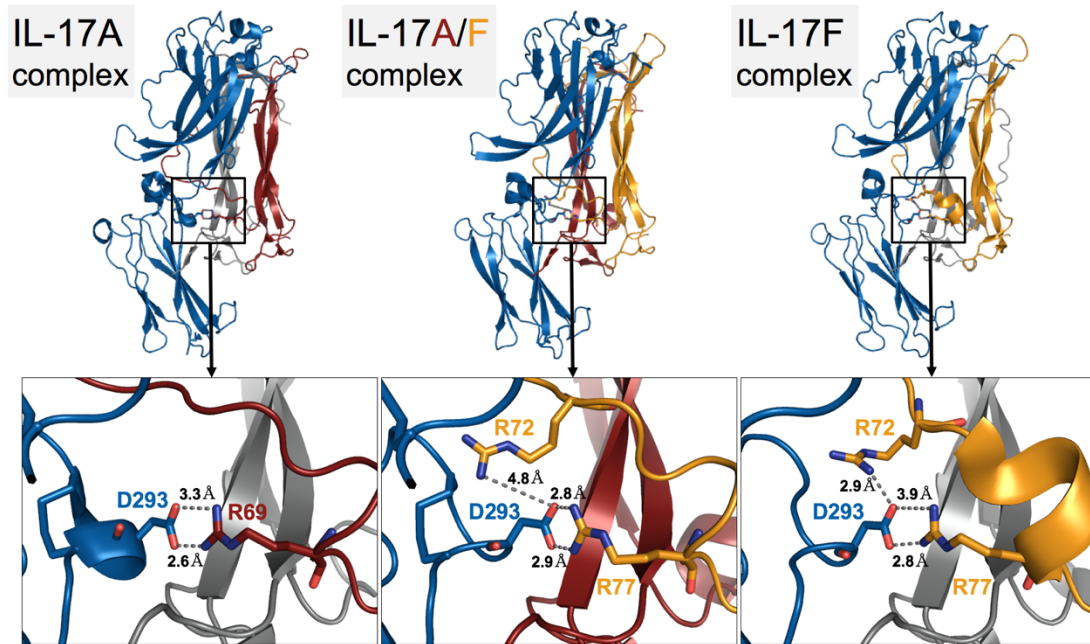
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IL-17	Chain	Site 1		Site 2		Site 3	
A	A	L49, I51, E80, E83, Y85, L122, R124 , F133		N55, S63 , D65 , Y67, R78 , V88, I89, P86, W90		K61, S64, Y66, R69	
	A'	R43, L135		Q116, Q117 , E118 , K137, L139		L97, Y108, H109 , N111 , V113, V147, P149, I150, V151, H153, V154	
F	F	I59, I61, W88, P90, N91, Y93 , S95, V130, R132, H134 , V139, F141		Q66, R67 , S71, N73 , E75 , Y84, V86, P94, E96, V97, V98, V148		M70, R77 , R72	
	F'	S54, M55		Q124, Q125 , E126 , K145, L147		L105, I116, S117, N119 , S120, V121, V155, P157, V158, I159, H160, H161, V162	
A/F		Site 1A	Site 1F	Site 2A	Site 2F	Site 3A	Site 3F
	A	L49, I51, E80, E83, Y85, L122, R124, F133	V45	N55, S63, D65, Y67, R78, V88, I89, P86, W90	Q117, E118, K137, L139	K61, S64, Y66, R69	L97, Y108, H109, N111, V113, V147, P149, V151, H152, H153, V154
F	G53	I59, I61, W88, P90, N91, Y93 , S95, V130, R132, H134 , V139, F141	Q94, Q95, E96, K115, L117	Q66, R67 , S71, N73 , E75 , Y84 , V86, P94, E96 , V97, V98, V148	L105, I116, S117, N119, S120, V121, V155, P157, V158, I159, H160, H161, V162	M70, I74, R72 , R77	

53 **Supplementary Table 1** Conserved binding sites in IL-17A, IL17F and IL-17A/F. Residues lining the IL-
54 17RA binding sites 1-3 in IL-17A (4hsa.pdb), IL-17F (3jvf.pdb) and IL-17A/F (this work) are listed. While
55 the two sides of the homodimeric cytokines are identical by symmetry, the "A-face" differs from the "F-face"
56 in the heterodimeric IL-17A/F, hence Sites 1-3 are sub-divided into Sites 1A-3A and Sites 1F-3F. Residues
57 lining Sites 1F-3F were directly identified from the crystal structure of the IL-17A/F complex with IL-17RA.
58 Residues lining the "A-face" were listed based on the assumption that the binding of IL-17RA to the "A-face"
59 of IL-17A/F is similar to its binding to IL-17A. Residues in bold case are engaged in H-bonded interactions
60 with IL-17RA, while underlined residues form salt-bridges with the receptor.

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65 **Supplementary Figure 2** Key salt-bridge interaction between IL-17RA (blue ribbon) and IL-17A (left view;
 66 the two IL-17A subunits are shown as a carmine and gray ribbon, respectively; figure based on 4hsa.pdb),
 67 IL-17A/F (center; carmine ribbon: A- subunit; orange ribbon: F-subunit; this work), and IL-17F (right view;
 68 the two IL-17F subunits are shown as an orange and gray ribbon, respectively; based on 3jvf.pdb). The top
 69 panel shows an overall view of the complex, the lower panel a close-up view of the salt-bridge interaction
 70 with the side-chains involved depicted in stick representation.

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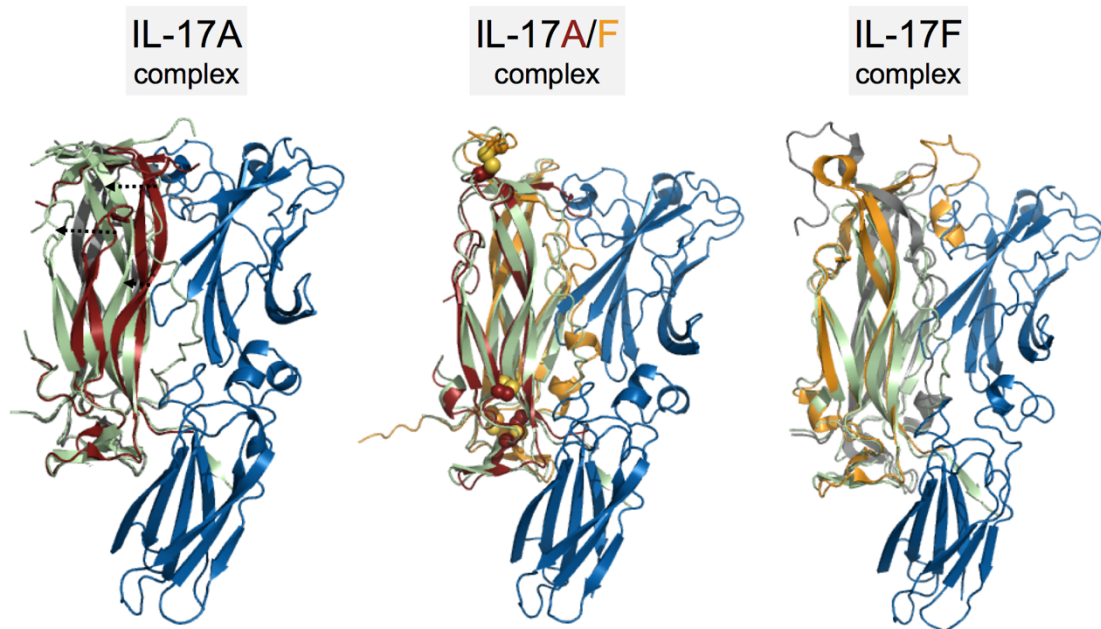
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Cytokine	Variant	IL-17RA K _d (nM)	IL-17RC K _d (nM)
IL-17A	wt	0.7	0.4
	R69A	297	1500
IL-17F	wt	136	4
	R77A	726	905
IL-17A/F	wt	17.5	0.6
	A(R69A)	42	15
	F(R77A)	30	5
	A(R69A), F(R77A)	202	1100

87 **Supplementary Table 2** Summary of equilibrium dissociation constants obtained from SPR analysis of
88 wild-type and variant IL-17A, IL-17F and IL-17A/F binding to the IL-17 receptors IL-17RA or IL-17RC (see
89 also Fig. 5). The K_d values shown are the mean from at least five independent experiments.

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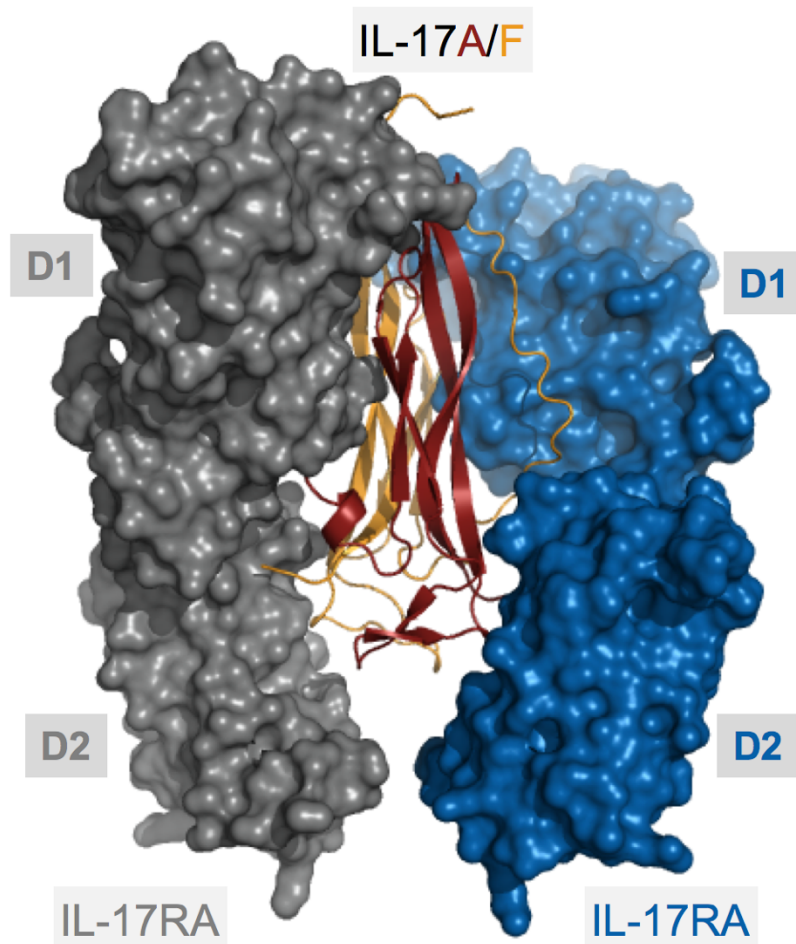
93 **Supplementary Figure 3** IL-17RA (blue ribbon) induced allosteric changes in IL-17A (left view, figure
94 based on 4hr9.pdb and 4hsa.pdb), IL-17A/F (center, this work) and IL-17F (right view; based on 1jpy.pdb
95 and 3jvf.pdb). The receptor-bound cytokines are depicted as a green ribbon, while the free cytokines are
96 shown in carmine/grey (IL-17A), carmine/orange (IL-17A/F A- and F-chain, respectively) and orange/grey
97 (IL-17F). Structure-based alignments were computed with Lsqman⁴⁶ using a maximum matching distances
98 of 1.2Å. Note the large allosteric changes observed with IL-17A, indicated by dotted arrows.

Maximum matching distance		IL-17A	IL-17A/F	IL-17F
3.50Å	rms deviation (Å)	1.52	1.08	1.14
	matched residues	132	192	192
1.2Å	rms deviation (Å)	0.56	0.72	0.69
	matched residues	81	141	133

100 **Supplementary Table 3** Least square superpositions of the free- and IL-17RA-bound forms of IL-17A, IL-
 101 17A/F and IL-17F, calculated with Lsqman⁴⁶.

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105 **Supplementary Figure 4** Structural model of IL-17A/F (A-chain:carmine/F-chain:orange ribbon) bound to
 106 two copies of the IL-17RA ECD (blue and grey surface representations). The blue IL-17RA is based on the
 107 crystal structure of the IL-17A/F complex with IL-17RA bound to the “F-face” of the cytokine. The grey IL-
 108 17RA model was placed by applying the transformation that brings the F-chain onto the A-chain of the
 109 heterodimer to the blue IL-17RA model. Note that there is no steric clash between the two receptor chains.

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Model	Resolution limit (Å)	R_{work}	R_{free}	R_{free}-R_{work}
Model refined against 3.30Å data	3.30	0.1856	0.2431	0.0575
	3.57	0.1851	0.2426	0.0575
	3.62	0.1839	0.2411	0.0572
Model refined against 3.57Å data	3.57	0.1951	0.2547	0.0596
Model refined against 3.62Å data	3.62	0.1922	0.2532	0.0610

112 **Supplementary Table 4** Paired refinement tests showing that the model refined against 3.30Å data fits the
113 diffraction data to 3.57Å or 3.62Å resolution better (lower R_{free}) than the corresponding models refined
114 against 3.57Å or 3.62Å resolution data.