SUPPLEMENTARY INFORMATION:

Redirecting cell-type specific cytokine responses with engineered interleukin-4 superkines

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SUPPLEMENTARY METHODS:

Yeast Display of IL-4

General yeast display methodologies are modified from previously described protocols[1-3]. Human IL-4 DNA was cloned into yeast display vector pCT302. *S. cerevisiae* strain EBY100 was transformed with the pCT302_IL-4 vector and grown for 3 days at 30 °C on SDCAA plates. Individual colonies of IL-4 yeast were grown overnight at 30 °C in SDCAA liquid media (pH 4.5), followed by induction in SGCAA liquid media (pH 7.0) for 2 days at 20 ºC. The yeast were stained with biotinylated IL-4Rα (b-IL-4Rα), tetramerized biotinylated γc (b-γc), or b-γc in the presence of b-IL-4Rα. Highest concentration γc tetramers were formed by incubating 2 μM b-γc with 470 nM SAV-PE (streptavidin-phycoerythrin conjugate, Invitrogen) for 15 min on ice. Analysis was performed on an Accuri C6 flow cytometer.

Assembly PCR of IL-4 Site Directed Library Construction and Selection

Assembly PCR was done using 11 overlapping primers one of which contained the randomized codon sequence NNK (aa 117, 118, 121, 122, 124, 125, 128, 129). The PCR product was further amplified using the primers:

5'CTAGTGGTGGTGGTGGTTCTGGTGGTGGTGGTTCTGGTGGTGGTGGTTCTGC

TAGCCACAAGTGCGATATCACCTTAC 3'

5'CAGATCTCGAGCAAGTCTTCTTCGGAGATAAGCTTTTGTTCGCCACCAGAG

GATCC 3'

These primers also contained the necessary vector sequence homology for homologous recombination. Insert DNA was combined with linearized vector backbone pCT302 and electrocompetent *S. cerevisiae* EBY100, electroporated, and rescued, as previously described.[27] The electroporations yielded a library of $2x10^8$ transformants. Selections were performed using magnetic activated cell sorting (MACS, Miltenyi). The first round of selection was performed with $2x10^9$ cells from the yeast library, approximately 10-fold coverage of the number of transformants. Subsequent rounds of selection used $1x10^7$ yeast cells. IL-4 libraries were grown fresh overnight at 30°C in SDCAA liquid media (pH 4.5), followed by induction in SGCAA liquid media (pH 7.0) for 2 days at 20 ºC. The yeast were stained with biotinylated IL-4Rα (b-IL-4Rα), tetramerized biotinylated γc (b-γc), or b-γc in the presence of b-IL-4Rγ with a buffer solution of 1xPBS and 0.5% BSA for 2 hrs at 4 C. Tetramers of γc were formed by incubating 2 μM b-γc with 470 nM SAV-PE (streptavidin-phycoerythrin conjugate, Invitrogen) for 15 min on ice. Monomeric selection with γc was done through sequential binding of γc, 2μg/mL SA-PE, and

50μl Miltenyi anti-PE microbeads/1x10^8 yeast cells. Analysis was performed on an Accuri C6 flow cytometer.

Protein Expression and Purification

Human IL-4 variants (amino acids 1-129), the IL-4Rα ectodomain (amino acids 1-202), IL-13Rα1 (amino acids 1-310), and γc (amino acids 34-232) were cloned into the pAcGP67A vector (BD Biosciences) in frame with an *N*-terminal gp67 signal sequence and *C*-terminal hexahistidine tag and produced using the baculovirus expression system as described in [4]. Baculovirus stocks were prepared by transfection and amplification in *Spodoptera frugiperda* (Sf9) cells grown in SF900II media (Invitrogen), and protein expression was carried out in suspension *Trichoplusia ni* (HiFive) cells grown in InsectXpress media (Lonza). Proteins were expressed and captured from HiFive supernatants after 48-60 h by nickel agarose (Qiagen), concentrated and purified by size exclusion chromatography on a Superdex 200 column (GE Healthcare), equilibrated in 10 mM HEPES (pH 7.2) and 150 mM NaCl. The recombinant cytokines were purified to greater than 98% homogeneity. IL-4 variants used in cell based assays were expressed fully glycoslylated as seen by comassie gel. The high affinity binary complexes (IL-4Rα with IL-4 variants) used in SPR measurements were coexpressed and fully glycosylated. For biotinylated receptor expression, IL-4Rα, IL-13Rα1, and γc were cloned into the pAcGP67-A vector with a C-terminal *biotin* acceptor peptide *(BAP)*-LNDIFEAQKIEWHE and hexahistidine tag. Receptor proteins were coexpressed with BirA ligase with excess biotin (100 μM). For crystallization, IL-4 RGA was co-expressed with γc with the N-linked glycosylation site Asn53 mutated to Gln. After nickel purification, the crystallization proteins were treated overnight with carboxypeptidase-A at a

ratio of 1:100, followed by size exclusion. Protein was concentrated to 8–20 mg/mL for crystallization.

Surface Plasmon Resonance

SPR experiments were conducted on a Biacore T100 instrument. Protein concentrations were quantified by UV spectroscopy at 280 nm using a Nanodrop2000 spectrometer (Thermo Scientific). All data was analyzed using the Biacore T100 evaluation software version 2.0 with a 1:1 Langmuir binding model. Experiments used a Biacore SA sensor chip (GE Healthcare). Biotinylated receptors were captured at a low density (100–200 RU) and kinetic runs were conducted at 40 μL/min. An unrelated biotinylated protein was immobilized as a reference surface for the SA sensor chip with matching RU to the experimental surface. All measurements were made using 3-fold serial dilution of IL-4 variants in the running buffer (1xHBS-P (GE Healthcare), 0.01% BSA). The γc surface was regenerated for cytokines IL-4 RQ and IL-4 RGA with 7 mM glycine (pH 3.0) and 250 mM NaCl. Kinetic data was determined using 120 s to 190 s of IL-4 variant association and 20 s to 900 s disassociation.

Crystallization and Data Collection

IL-4 RGA/ γc crystals were grown in sitting drops at 25 °C by mixing 0.1 μL protein [20 mg/mL in 10 mM HEPES-NaOH (pH 7.2) and 150 mM NaCl] with an equal volume of 50 mM HEPES (pH 7.2), 200 mM NaCl, and 30% PEG-4000. Crystals grew to a maximum size of 200 × 40 × 40 μm in 5–10 days. Crystals were flash frozen in liquid nitrogen using mother liquor containing 25% Ethylene Glycol as a cryoprotectant. A 3.3Å data set was collected at beamline 8-2 at the

Advanced Light Source (0.98Å wavelength). Diffraction data were processed using HKL2000.[5] Data processing statistics can be found in Supplementary Table 2.

Structure Determination and Refinement

The IL-4 RGA binary crystal structure (pdb code 3QB7) was solved by molecular replacement with the program PHASER[6] using the coordinates of IL-4 and γc separately (pdb code 3BPL). After the all domains were placed, the WT sequence was converted to IL-4 RGA and iterative rounds of refinement with PHENIX [7] and model adjustment with COOT[8] were used to refine the structures. Ramachandran analysis was done using MolProbity (http://molprobity.biochem.duke.edu). The Ramachandran statistics are 98.0/0.17/1.83 (%), corresponding to favored/allowed/outliers. Buried surface area values were calculated using the Protein Interfaces, Surfaces, and Assemblies (PISA) server (http://www.ebi.ac.uk/msd-srv/prot_int/pistart.html). IL-4 RGA consisted of 2 binary complexes. All structural figures and overlays were prepared using the PyMOL[8].

RT-PCR

RNA was isolated from starved cells with RNeasy Kit (Qiagen). RNA was reverse-transcribed to cDNA using SuperScript II First-Strand Synthesis System for RT-PCR (Invitrogen). Quantitative PCR reactions were performed using a 7900HT sequence detection system (Applied Biosystems). The primer/probe sets to detect IL-4Rα, IL-13Rα1 and γc (FAM-MGB probe), and TaqMan Ribosomal RNA Control Reagents for detecting the 18S ribosomal RNA (VIC-MGB probe) were from Applied Biosystems. The mRNA levels were normalized to 18S ribosomal RNA.

Microarray Analysis of Gene Expression in Monocytes

$CD14^+$ monocytes were isolated from 5 healthy donors as described above and stimulated for 6 hours with 50 ng/ml GM-CSF alone or with 20 ng/ml of IL-4, Super-4 or KFR. Cells were washed in PBS and lyzed in 1 ml TRIzol reagent (Sigma). Total RNA was isolated using a combined TRIzol / RNeasy Micro (Qiagen) protocol. RNA quality was confirmed with a 2100 Bioanalyzer (Agilent). cDNA was generated and labeled using the Two-color Low Input Quick Amp Labeling kit (Agilent), with single treatment samples (GM-CSF alone) labeled with Cy3 and dual treatment samples labeled with Cy5. For each donor, sample from each Cy5-labeled dual treatment (GM-CSF with either IL-4, Super-4 or KFR) was co-hybridized with the corresponding Cy3-labeled single treatment with GM-CSF onto 8x60K SurePrint G3 Gene expression microarrays (Agilent) and processed as per manufacturer's instructions. Raw data were normalized using Feature Extraction software (Agilent). Gene expression analysis was performed on GeneSpring GX11.5 and Excel. Entities only detected in less than 6 arrays, quality control probes and probes for long intergenic non-coding RNAs were excluded. Entities for which there was a significant change (paired *t*-test) in at least one dual treatment group compared to GM-CSF alone (Cy5 vs Cy3) were retained. Fold-change statistical analysis between two given cytokines were determined by paired *t*-test. Microarray data have been submitted to the Gene Expression Omnibus (GEO) Database at NCBI (GEO series accession number: GSE40200).

Matlab Script for Calculation of Number of Assembled IL-4 Receptor Complexes

A Matlab script was utilized to estimate the number of functional IL-4R complexes. In this calculation, we fixed number of IL-4Rα´s to 1500 (line 20, of the script) and varied the ligand concentration from 10 to 1000 pg/ml (lines 45 and 47) and number of γc´s per cell between 500

and 4500 (line 14). Finally we varied the affinity of the ligand/IL-4Rα complex for γc to form functional receptor complexes by using two-dimensional equilibrium binding constants (K_a2) ranging from 0.01 to 1.0 μm^2 (line 33). Note that these K_a2 constants for the reactions within the membrane may actually be concentration dependent and strongly diffusion limited.

Matlab Script:

```
% after a change enter Ctrl-S to save and run script again.
% Kd for IL4 binding to its receptor; don't change
kdIL4 = 1e-10;
% Kd for IL13 binding to its receptor; don't change
kdIL13 = 3e-8;
% number of IL13Ra receptors
% ************ MAY BE CHANGED ***********
numrecsIL13 = 0;
% ***************************************
% 2D concentration of IL13Ra receptors - cell has 275 square microns surface
c_recsIL13 = numrecsIL13/275;
% number of cg (and/or IL13Ra) receptors
% ************ MAY BE CHANGED ***********
numrecsCG_or_IL13Ra = 500;
% ***************************************
% 2D concentration of gc (and/or IL13Ra) receptors
c_recsCG_or_IL13Ra = numrecsCG_or_IL13Ra/275;
% number of IL4 receptors
% ************ MAY BE CHANGED ***********
numrecsIL4 = 1500;
% ***************************************
% 2D concentration of IL4 receptors
c_recsIL4 = numrecsIL4/275;
% two-dim Ka for binding of IL4Ra to cg or IL13Ra or IL13Ra to IL4Ra
% lower limit (weak binder) is 0.01 high limit is 1
% KEEP IN MIND THAT REACTIONS BETWEEN MEMBRANE RECEPTORS ARE
% USUALLY DIFFUSION LIMITED AND WE DON'T KNOW WHETHER THEY ARE
% EQUALLY DISTRIBUTED OR CLUSTERED OR WHETHER THE SIGNAL INDUCES
% CHANGES IN THE DIFFUSIVE BEHAVIOR.
% ************ MAY BE CHANGED ***********
kA2d_IL4Ra_cg = 1.0;
kA2d_IL13Ra_IL4Ra = 0.3;
% ***************************************
% two dim kDs:
k2d_IL4Ra_cg = 1.0/kA2d_IL4Ra_cg;
k2d_IL13Ra_IL4Ra = 1.0/kA2d_IL13Ra_IL4Ra;
```

```
% ************ MAY BE CHANGED ***********
% start for plot range of concentration of IL13 in ng/ml
nIL13_L = 10;
% end for plot range of concentration of IL13 in ng/ml
nIL13_H = 200000;
% start for plot range of concentration of IL4 in ng/ml
nIL4_L = 0.01;
% end for plot range of concentration of IL4 in ng/ml
nIL4_H = 1000;
% ***************************************
nIL13 = nIL13_L:nIL13_L/5:nIL13_H;
nIL4 = nIL4_L:nIL4_L/5:nIL4_H;
% set the following to 'true' to plot for IL4
IL4 = true;
if(IL4)
Conc2D_recs = c_recsIL4;
Conc2D_recs2 = c_recsCG_or_IL13Ra;
KD_IL = kdIL4;
K2D_rec_rec2 = k2d_IL4Ra_cg;
Fraction_bound = ones(length(nIL4), 1);
Num_bound = ones(length(nIL4), 1);
for n=1:length(nIL4),
Conc_IL = nIL4(n)*7.1e-11;
A = Conc2D_recs2 + Conc2D_recs*Conc_IL/(Conc_IL+KD_IL) + K2D_rec_rec2;
Fraction_bound(n) = (A - sqrt((A*A - 4*Conc2D_recs2*Conc2D_recs*Conc_IL/(Conc_IL
+KD_IL))))/(2*Conc2D_recs);
Num_bound(n) = Fraction_bound(n)*Conc2D_recs*275;
end
plot(nIL4, Num_bound);
ylabel('number of IL4 receptors bound to IL4 and cg/IL13Ra');
xlabel('concentration of IL4 in ng/ml');
else
Conc2D_recs = c_recsIL13;
Conc2D_recs2 = c_recsIL4;
KD_IL = kdIL13;
K2D_rec_rec2 = k2d_IL13Ra_IL4Ra;
Fraction_bound = ones(length(nIL13), 1);
Num_bound = ones(length(nIL13), 1);
for n=1:length(nIL13),
Conc_IL = nIL13(n)*8.8e-11;
A = Conc2D_recs2 + Conc2D_recs*Conc_IL/(Conc_IL+KD_IL) + K2D_rec_rec2;
Fraction_bound(n) = (A - sqrt((A*A - 4*Conc2D_recs2*Conc2D_recs*Conc_IL/(Conc_IL
+KD_IL))))/(2*Conc2D_recs);
Num_bound(n) = Fraction_bound(n)*Conc2D_recs*275;
end
plot(nIL13, Num_bound);
```

```
ylabel('number of IL13 receptors bound to IL13 and IL4Ra');
xlabel('concentration of IL13 in ng/ml');
end
% uncomment the following line to have log scala for concentrations:
set(gca, 'xscale', 'log');
grid on;
```

SUPPLEMENTARY REFERENCES:

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3. Levin, A.M. et al. Exploiting a natural conformational switch to engineer an interleukin-2 'superkine'. *Nature* **484**, 529-533 (2012).
4. LaPorte, S.L. et al. Molecular and structural basis of cytokine receptor pleiotropy in the interleukin-4/13 system. *Cell* **132**, 259-272 (2008).
5. Otwinowski, Z. & Minor, W. Processing of X-ray diffraction data collected in oscillation mode. *Methods Enzymol.* **276**, 307-326 (1997).
6. McCoy, A.J. et al. Phaser crystallographic software. *Journal of Applied Crystallography* **40**, 658-674 (2007).
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SUPPLEMENTARY RESULTS:

Supplementary Table 1 - Binding affinities of superkines for γc and IL-13Rα1.

	Cytokine residue								Affinity for γc			Affinity for IL-13Rα1		
	117	118	121	122	124	125	128	129	K_d(nM)	k_{on}(1/ms)	k_{off}(1/s)	K_d(nM)	k_{on}(1/ms)	k_{off}(1/s)
IL-4	**Lys**	**Thr**	**Arg**	**Glu**	**Tyr**	**Ser**	**Ser**	**Ser**	3300	$9.5x10^{4}$	0.31	4200	$4.1x10^{4}$	0.17
RQ			Gln						91	$1.9x10^{5}$	$1.7x10^{-2}$	29000	-	-
RGA	Arg	Val	Gln	Ser	Trp	Phe	Gly	Ala	0.89	$6.6x10^{5}$	$5.9x10^{-4}$	21000	-	-
KF			Lys		Phe				330	$1.1x10^{5}$	$3.6x10^{-2}$	250	$7.3x10^{4}$	$1.8x10^{-2}$
KFR			Lys		Phe	Arg			6400	$5.6x10^{4}$	0.36	9.6	$2.5x10^{5}$	$2.4x10^{-3}$
IL-13	**Leu**	**Leu**	**Lys**	**Lys**	**Phe**	**Arg**	**Gln**	**Phe**	U			30		

*U = unmeasureably weak

Supplementary Table 2 - Data collection and refinement statistics (molecular replacement)

	Super-4/γc
Data collection	
Space group	$P2_12_12_1$
Cell dimensions	
a, *b*, *c* (Å)	75.969, 105.879, 121.093
α, β, γ (°)	90, 90, 90
Resolution (Å)	50.00-3.25(3.37-3.25)
R_{merge}	16.1(99.0)
$I / \sigma I$	13.25(2.07)
Completeness (%)	99.9(99.9)
Redundancy	7.9 (7.9)
Refinement	
Resolution (Å)	50.00-3.25
No. reflections work/test	15980/799
R_{work} / R_{free}	23.1/29.2
No. atoms	5341
Protein	5341
Ligand/ion	
Water	
B-factors	95.1
Protein	95.1
Ligand/ion	
Water	
R.m.s. deviations	
Bond lengths (Å)	0.003
Bond angles (°)	1.324

Supplementary Table 3 – Ligand-receptor contact table comparing interactions of γc with super-4 versus IL-4. Contacts represent van der Waals interactions unless indicated in bold type, which are within H-bonding distance.

γc	IL-4 wt	Super-4
45(VAL)		125(PHE)
102(LEU)	122(GLU)	125(PHE)
103(TYR)	118(THR) 121(ARG) 122(GLU) 125(SER)	115(ARG) 118(VAL) **121(GLN)** 122(SER) 125(PHE)
125(LYS)	114(GLU) 118(THR)	**114(GLU)**
127(GLN)	118(THR) 121(ARG)	121(GLN)
128(ASN)	121(ARG)	
159(HIS)	124(TYR) 125(SER) 127(CYS) 128(SER)	**124(TRP)** **125(PHE)** 127(CYS)
160(CYS)	125(SER)	125(PHE)
182(TYR)	8(GLN) 124(TYR)	**8(GLN)** 124(TRP)
207(PRO)	11(ILE) 15(ASN) 121(ARG)	11(ILE) **15(ASN)** **121(GLN)**
208(LEU)	8(GLN) 11(ILE) 12(LYS) 121(ARG) 124(TYR)	8(GN) 11(ILE) 121(GLN) 124(TRP)
209(CYS)	121(ARG) 124(TYR) 125(SER)	121(GLN) 124(TRP) 125(PHE)
210(GLY)	121(ARG)	121(GLN)
211(SER)	121(ARG)	15(ASN) **117(ARG)** 121(GLN)

Bold:potential H-bonds (3.5Å cut off) or salt bridge from γc to Super-4
Italics: potential H-bonds (3.5Å cut off) or salt bridge from γc to IL-4 wt. Blue: amino acid substitution in super-4.

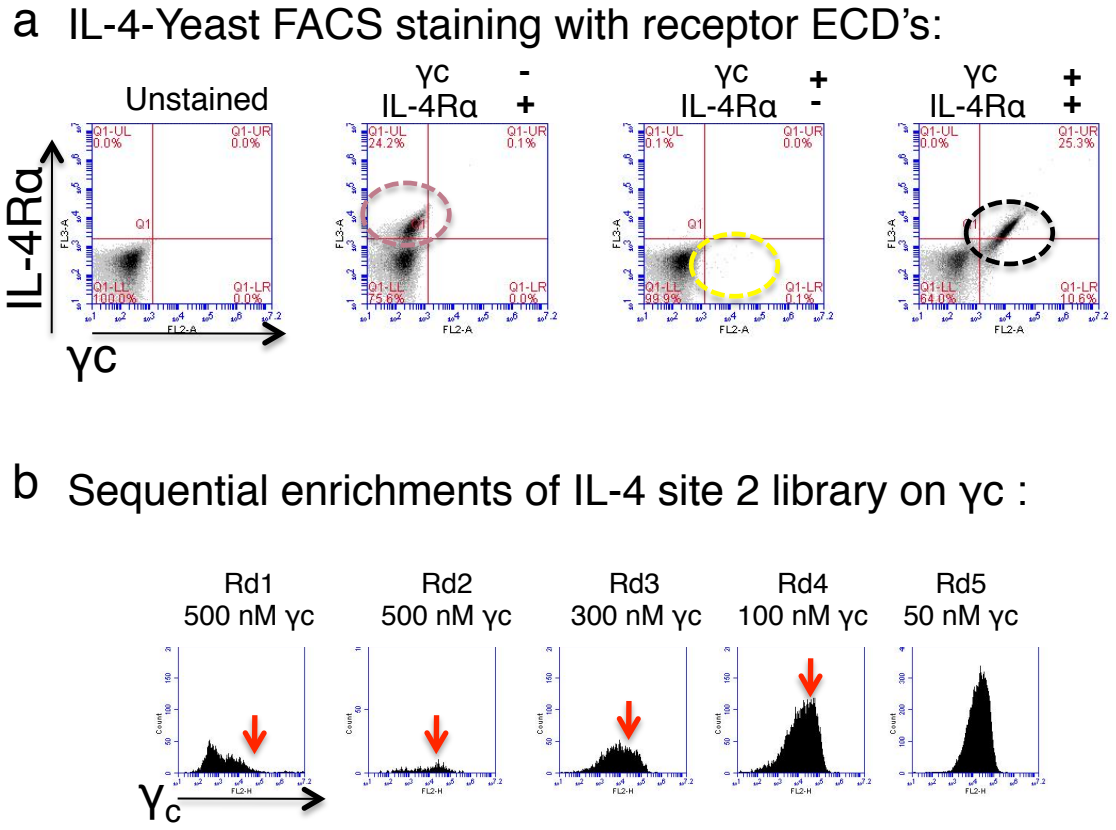
Supplementary Table 4 – Fold induction (mean, first three columns of data) and p values (paired *t*-test, last three columns of data) of superkine-selective genes

Gene ID	IL-4 mean	Super-4 mean	KFR mean	PPT(W-S)	PTT(W-K)	PPT(S-K)
GPR183	1.361	1.544	1.225	0.069	0.009	0.003
ASB1	-0.639	-0.832	-0.585	0.235	0.001	0.007
SLA	4.143	4.329	4.023	0.236	0.030	0.040
BIRC2	-0.312	-0.258	-0.351	0.280	0.024	0.010
TMEM216	-0.302	-0.423	-0.257	0.307	0.006	0.003
NMD3	0.795	0.984	0.742	0.399	0.019	0.039
FUNDC2	0.162	0.232	0.126	0.473	0.012	0.037
GLT1D1	-0.848	-1.013	-0.856	0.910	0.029	0.024
ANKRD23	-0.024	-0.693	-0.264	0.176	0.037	0.016
SP110	-0.386	-0.821	-0.520	0.282	0.036	0.047
LIX1L	-0.103	-0.395	-0.171	0.669	0.049	0.018
POMZP3	-0.078	0.223	-0.067	0.939	0.011	0.014
POLDIP3	0.051	0.227	0.143	0.138	0.032	0.003
CNST	-0.359	-0.932	-0.670	0.233	0.005	0.042
FBLN5	1.714	2.249	1.846	0.503	0.017	0.017
UTRN	1.096	1.223	0.955	0.279	0.035	0.039
GPR68	-2.802	-2.817	-2.479	0.001	0.000	0.597
EMP1	-2.299	-2.310	-1.986	0.001	0.001	0.790
C13orf31	-1.467	-1.414	-1.166	0.001	0.004	0.442
ALDH2	0.944	0.948	0.728	0.002	0.031	0.963
BRD7	-0.480	-0.438	-0.339	0.003	0.035	0.068
CUTC	0.631	0.714	0.564	0.003	0.040	0.201
TOR3A	1.440	1.484	1.218	0.004	0.022	0.408
APBB1IP	1.782	1.845	1.609	0.004	0.008	0.331
CCL2	-4.117	-4.266	-3.431	0.005	0.007	0.419
KANK1	-2.617	-2.467	-2.059	0.006	0.043	0.376
DPYSL2	1.093	1.111	0.936	0.006	0.018	0.643
APOL6	1.646	1.808	1.386	0.006	0.006	0.085
BCL6	-1.346	-1.322	-1.179	0.006	0.012	0.445
RPL18A	0.600	0.535	0.387	0.009	0.023	0.285
NDUFV1	0.706	0.701	0.534	0.010	0.002	0.868
HLA-DPA1	0.518	0.419	0.293	0.010	0.049	0.133
HEBP1	-1.656	-1.581	-1.383	0.011	0.030	0.485
FRY	-1.784	-1.592	-1.416	0.011	0.020	0.115
C13orf18	-1.647	-1.580	-1.243	0.013	0.038	0.571
CAT	1.444	1.387	1.212	0.014	0.001	0.313
BAI1	-1.312	-1.242	-1.024	0.014	0.018	0.272
MED30	-1.048	-0.919	-0.727	0.014	0.036	0.244
TLR1	-1.409	-1.392	-1.170	0.015	0.013	0.681
TRIB3	-2.562	-2.576	-2.318	0.016	0.001	0.857
PTGS2	-4.304	-4.276	-3.757	0.017	0.030	0.843

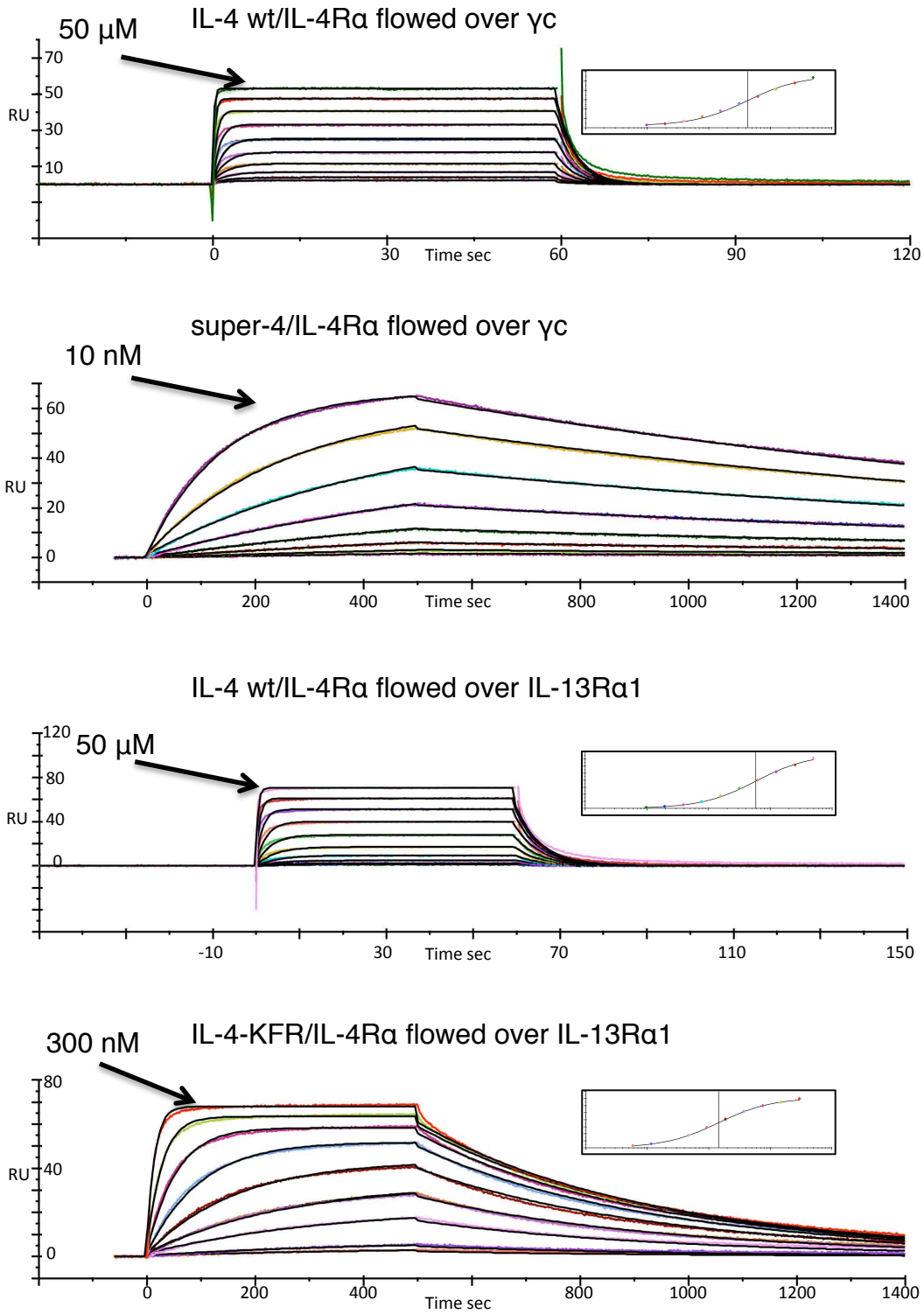
Gene ID	IL-4 mean	Super-4 mean	KFR mean	PPT(W-S)	PTT(W-K)	PPT(S-K)
RGS1	-3.528	-3.737	-3.027	0.017	0.011	0.414
MR1	0.501	0.544	0.397	0.018	0.001	0.368
HOMER3	-1.025	-1.016	-0.877	0.019	0.032	0.772
TCHH	-2.325	-2.623	-1.958	0.019	0.034	0.328
PEX13	-0.876	-0.766	-0.635	0.019	0.013	0.186
PLSCR1	-1.664	-1.499	-1.253	0.019	0.022	0.153
FAU	0.315	0.273	0.197	0.019	0.041	0.321
AK3L1	-1.313	-1.439	-1.089	0.020	0.037	0.460
NUMB	-1.239	-1.225	-1.041	0.020	0.013	0.823
CXCL1	-3.749	-3.567	-2.932	0.021	0.019	0.594
SAV1	-1.783	-1.872	-1.584	0.023	0.047	0.539
TrEMBL	-0.715	-0.632	-0.445	0.025	0.039	0.245
ZAK	-0.791	-0.898	-0.628	0.027	0.037	0.347
TCEB2	0.430	0.427	0.328	0.028	0.038	0.930
CXCL3	-3.772	-3.851	-3.136	0.028	0.009	0.688
MTF1	-1.430	-1.282	-1.073	0.029	0.048	0.133
MECP2	-0.804	-0.700	-0.574	0.032	0.012	0.099
GMFG	-0.722	-0.676	-0.607	0.033	0.039	0.459
LRRC25	-1.256	-1.309	-0.992	0.034	0.031	0.731
MLH1	-0.950	-0.888	-0.737	0.035	0.032	0.364
PSMA1	-0.315	-0.265	-0.212	0.035	0.037	0.242
PAM	-0.947	-1.033	-0.677	0.035	0.004	0.312
LOC283050	-1.567	-1.523	-1.351	0.036	0.009	0.644
LOC100292409	-0.740	-0.703	-0.306	0.039	0.026	0.855
C2CD2	1.050	1.058	0.750	0.039	0.006	0.899
TAP1	0.516	0.483	0.389	0.041	0.030	0.515
ADAM10	-1.495	-1.481	-1.229	0.041	0.035	0.928
SNCA	-1.466	-1.362	-1.148	0.041	0.014	0.513
CKAP4	-2.725	-2.800	-2.324	0.042	0.003	0.597
DOK3	-1.694	-1.692	-1.462	0.042	0.006	0.984
CISH	1.088	1.030	0.887	0.042	0.020	0.438
ARMC8	-1.117	-1.089	-0.769	0.043	0.048	0.882
BEST1	-1.281	-1.268	-1.082	0.044	0.036	0.807
CARD6	-1.550	-1.474	-1.204	0.045	0.001	0.505
ZNF366	3.182	3.166	2.895	0.046	0.035	0.885
SORT1	1.074	1.098	0.913	0.046	0.011	0.737
RNF149	-1.619	-1.631	-1.333	0.047	0.004	0.878
KCNE1L	-1.687	-1.353	-1.110	0.048	0.032	0.175
C21orf7	-3.142	-2.878	-2.454	0.049	0.040	0.084
TBL1XR1	0.759	0.813	0.575	0.049	0.043	0.204
AMOTL1	0.261	-0.026	-0.758	0.015	0.029	0.208
ADCY4	-0.017	-0.066	0.137	0.017	0.009	0.498
LOC100128348	0.166	0.295	0.522	0.033	0.002	0.307
PRB3	0.276	0.275	-0.480	0.044	0.013	0.998
TRIM59	-0.293	-0.329	-0.660	0.045	0.017	0.776
TSR2	-0.129	-0.204	-0.302	0.008	0.002	0.122

Gene ID	IL-4 mean	Super-4 mean	KFR mean	PPT(W-S)	PTT(W-K)	PPT(S-K)
FARS2	0.868	0.573	0.585	0.001	0.804	0.004
MEI1	-1.908	-1.762	-1.715	0.003	0.392	0.050
AK1	0.852	0.674	0.627	0.008	0.149	0.008
ZNF512B	1.508	1.282	1.289	0.011	0.897	0.013
SPSB1	1.433	1.065	1.169	0.011	0.368	0.015
ATP6V0C	-0.269	-0.195	-0.178	0.013	0.653	0.013
ZFYVE16	-1.122	-0.958	-0.904	0.015	0.455	0.013
RP11-94I2.2	-0.732	-0.541	-0.522	0.015	0.851	0.033
P4HA1	-0.527	-0.374	-0.372	0.008	0.971	0.043
CAB39	-1.100	-0.911	-0.820	0.023	0.384	0.007
KCTD11	-0.434	-0.597	-0.554	0.023	0.620	0.042
BTN3A2	0.751	0.578	0.571	0.025	0.868	0.047
C1orf56	-1.137	-0.974	-0.910	0.027	0.261	0.009
SPIRE1	-0.933	-0.740	-0.653	0.028	0.380	0.007
PRDM8	-2.687	-2.278	-2.293	0.028	0.941	0.024
ZNF655	-0.429	-0.294	-0.251	0.030	0.391	0.047
TCEB3	0.356	0.515	0.499	0.031	0.806	0.031
SAMM50	0.599	0.479	0.471	0.032	0.780	0.048
COX7A2	-0.644	-0.538	-0.482	0.035	0.400	0.040
ABCF3	0.769	0.645	0.616	0.036	0.587	0.041
ATP5G2	0.597	0.489	0.460	0.036	0.396	0.037
H2A.2	0.270	0.385	0.382	0.037	0.910	0.017
AP1S2	-2.491	-1.801	-1.582	0.042	0.441	0.002
YIPF5	-0.546	-0.756	-0.610	0.046	0.102	0.034
IGBP1	0.514	0.430	0.376	0.046	0.237	0.006
DAZAP1	-0.546	-0.335	-0.249	0.049	0.356	0.032
PSD4	0.702	0.468	0.232	0.010	0.015	0.010
PSPN	-0.754	0.225	-0.027	0.016	0.040	0.003
STX10	0.622	0.089	0.136	0.004	0.731	0.009
CNP	0.206	0.048	0.064	0.008	0.739	0.029
TAFI63	-1.184	-0.209	0.032	0.009	0.217	0.012
ASB6	0.321	0.233	0.096	0.010	0.061	0.030
LY6E	1.431	1.167	1.180	0.018	0.490	0.008
LRRC37A3	0.345	0.066	0.048	0.024	0.829	0.007
ADPRH	-0.309	-0.194	-0.059	0.027	0.187	0.019
GPR150	-0.532	-0.437	-0.326	0.027	0.124	0.042
CCPG1	-0.633	-0.201	-0.197	0.028	0.963	0.014
PDLIM5	-0.345	-0.099	-0.181	0.032	0.352	0.029
REPIN1	0.864	0.662	0.603	0.039	0.407	0.042
MKRN1	-0.164	-0.103	0.019	0.040	0.145	0.011
PRDM13	-0.555	0.042	0.004	0.041	0.694	0.017
TBCA	-0.459	-0.282	-0.238	0.042	0.573	0.009
COPS7B	1.131	0.512	0.199	0.049	0.407	0.002
NOTCH2NL	-0.906	-0.480	-0.386	0.009	0.245	0.038
CD44	-0.580	-0.417	-0.377	0.018	0.436	0.006
GSTK1	0.545	0.407	0.207	0.044	0.129	0.002
SLC41A3	-0.430	-0.255	-0.220	0.007	0.540	0.003

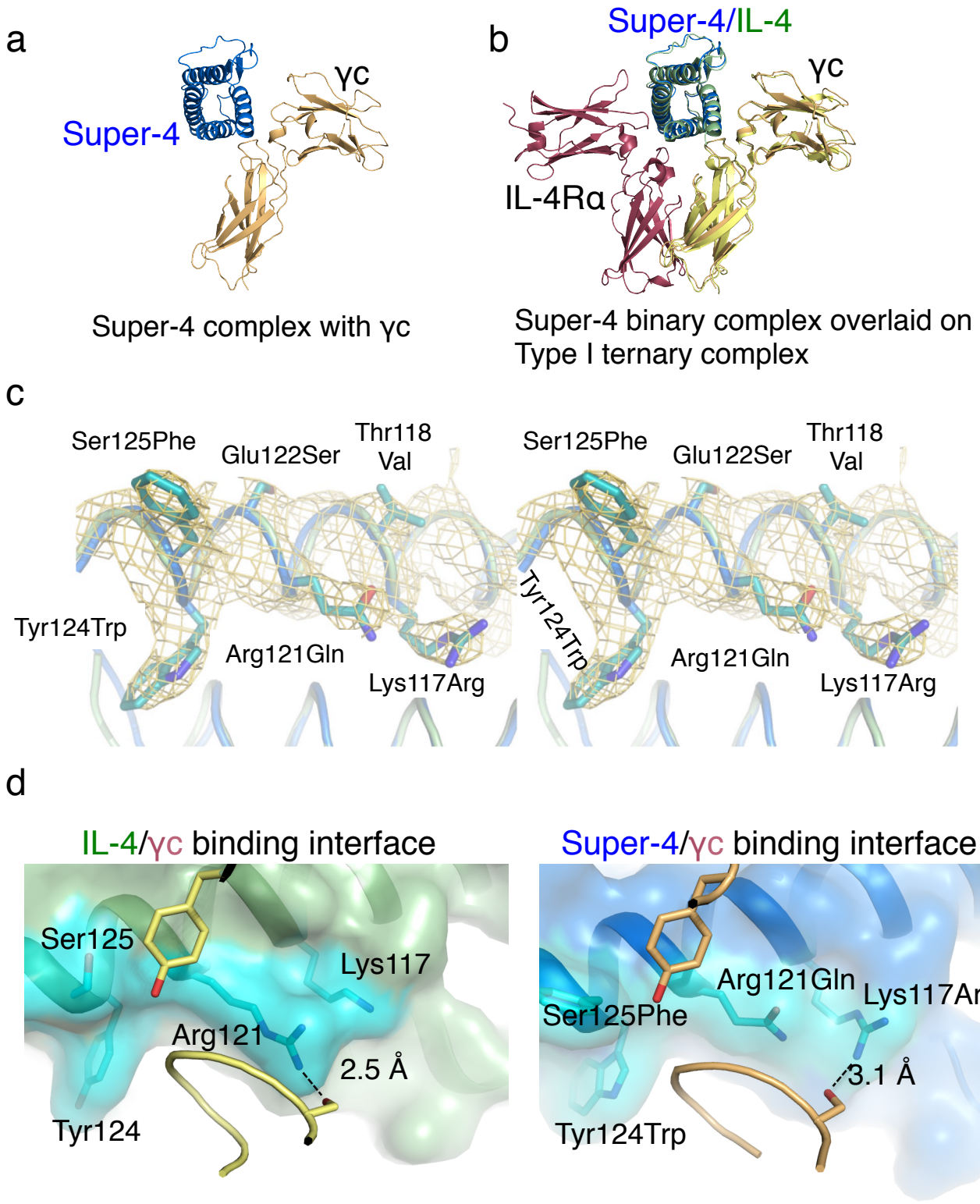
SUPPLEMENTARY FIGURES:



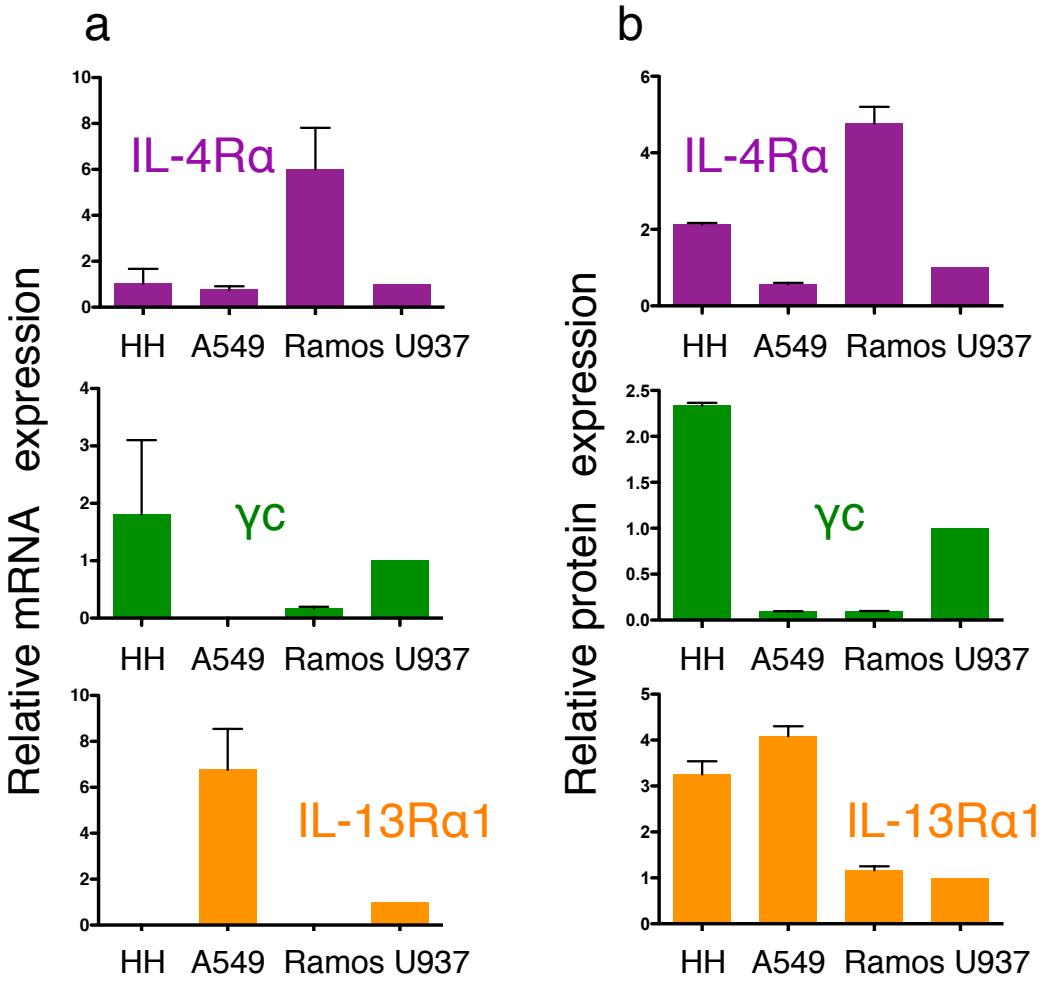
Supplementary Figure 1 – (a) Cooperative assembly of the IL-4/IL-4Rα/γc ternary ectodomain complex on yeast displaying IL-4. (b) Step-wise enrichment of the IL-4 site 2 library shown in Figure 1 by selection on γc tetramers. The library was complexed with IL-4Rα in the first step of selection, subsequent rounds did not include IL-4Rα. A final sixth round of selection was on γc monomer at 1μm (not shown).



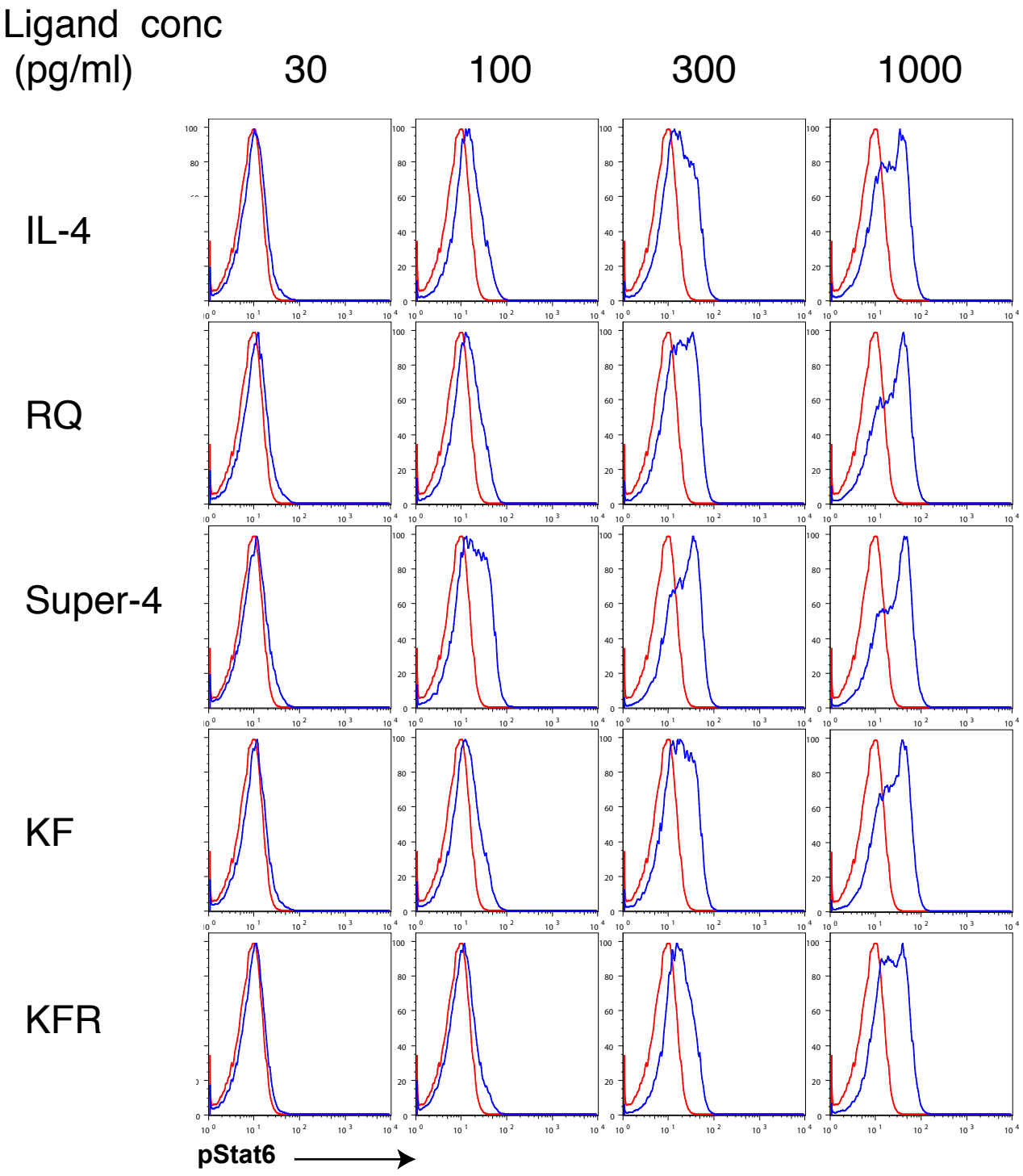
Supplementary Figure 2 – Surface plasmon resonance analysis of IL-4 and superkine binding to γc immobilized on surface through C-terminal biotinylation. Inset shows curve fits, data is shown in Supplementary Table 1.



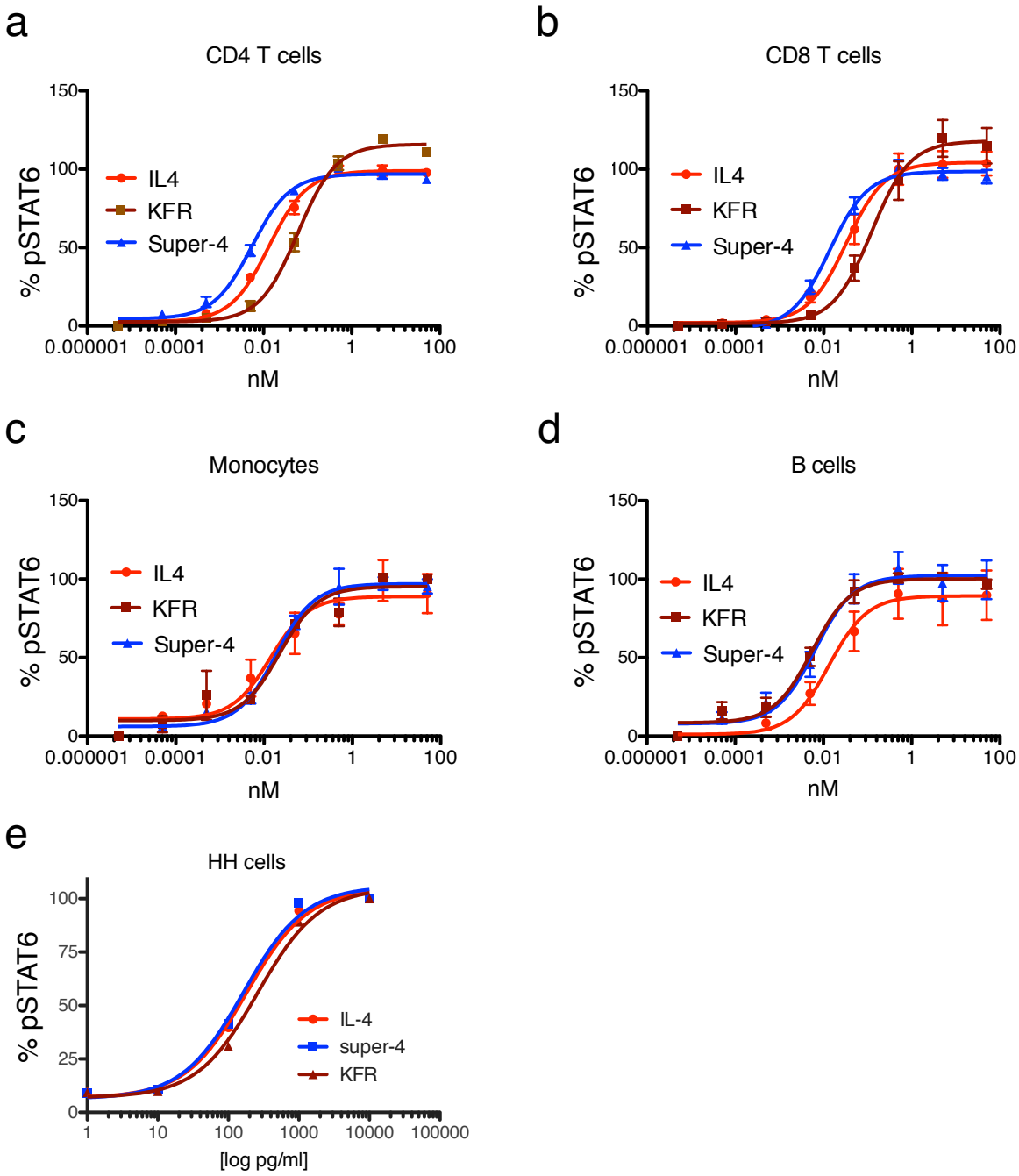
Supplementary Figure 3 – (a) Crystal structure of the super-4/γc binary complex at 3.25Å. (b) Structural superposition of the super-4/γc binary complex with the IL-4/IL-4Rα/γc ternary complex based on alignment of the cytokines (C-a residues 3-103, 106-127 align with an RMSD of 0.5Å). The docking mode of super-4 with γc is essentially identical to that of IL-4. (c) SigmaA-weighted 2Fo-Fc electron density of super-4 Helix D shown in stereo view. IL-4 wild type helix backbone overlaid in light green. (d) Remodeled hydrogen bonding interactions in the super-4/γc interface. Hydrogen bond lost in Arg121Gln, and new h-bond formed by Lys117Arg.



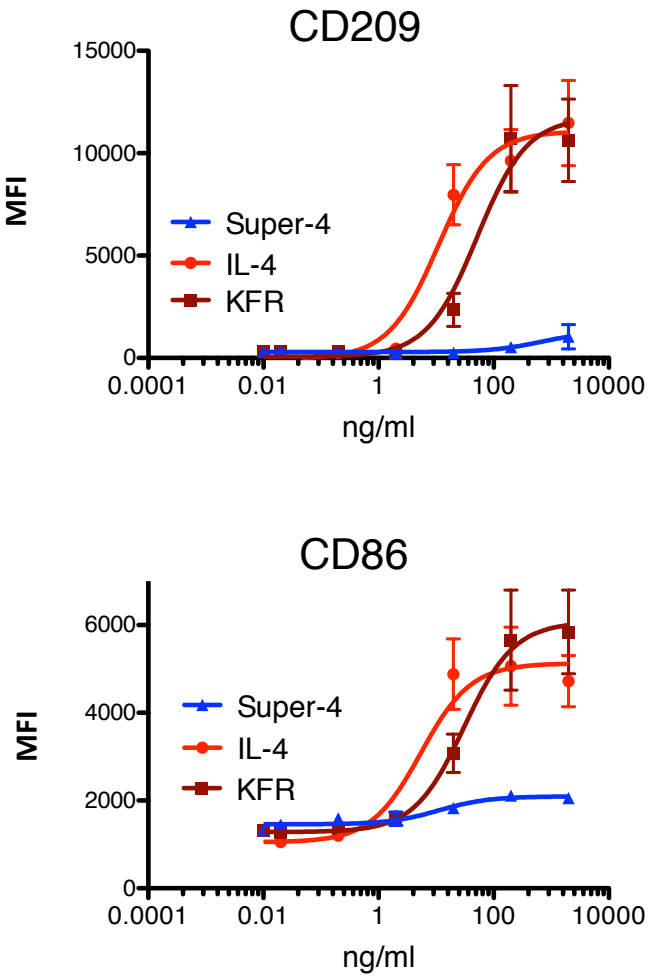
Supplementary Figure 4 – (a) IL-4 receptor chains mRNA levels and (b) IL-4 receptor chains protein levels on different IL-4 responsive cells



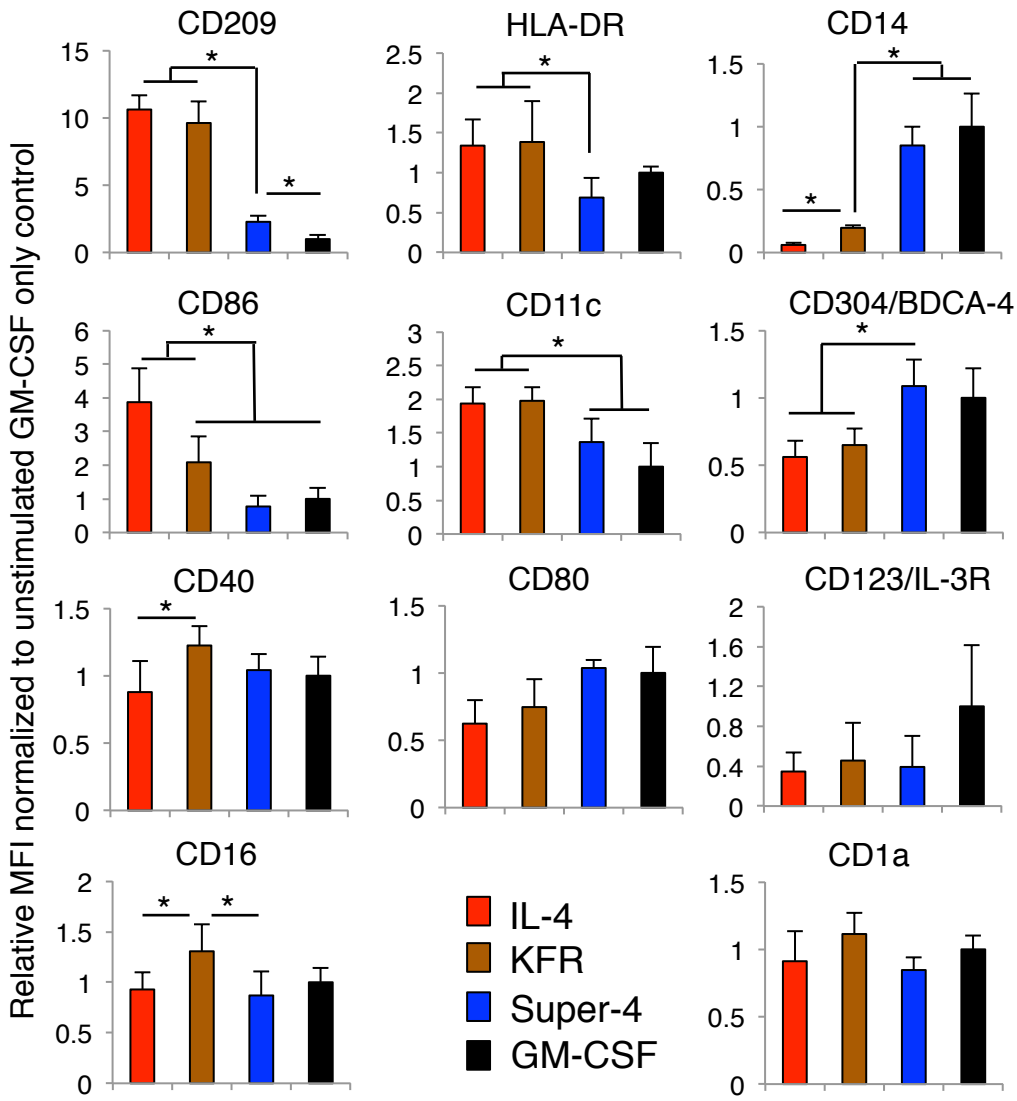
Supplementary Figure 5 – Representative experiment on superkine-induced STAT6 Tyr641 Phosphorylation in Ramos cells, for details see main Figure 2.



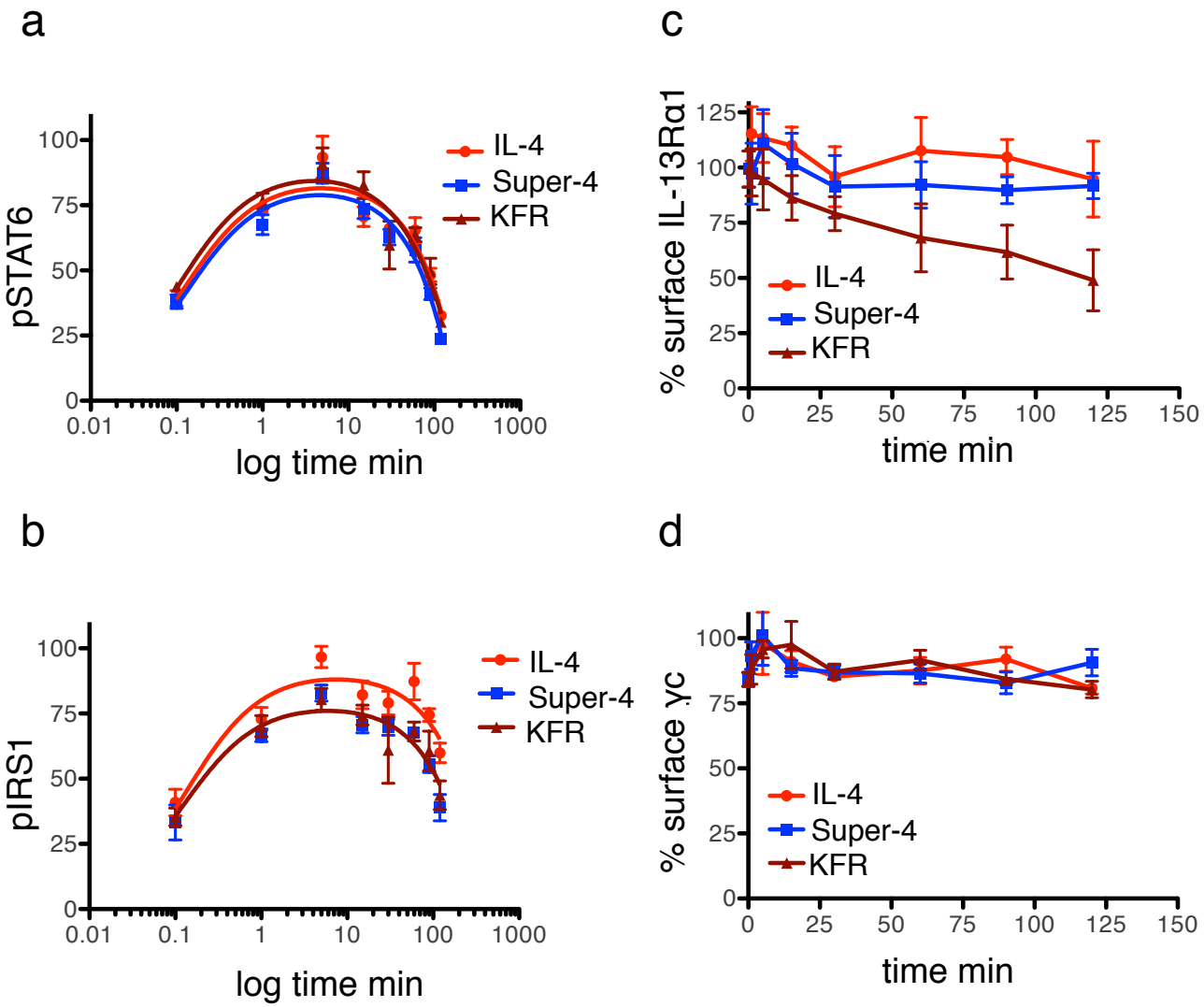
Supplementary Figure 6 -- STAT6 phosphorylation induced by IL-4 and the two superkines. Cells were stimulated with the indicated doses of IL-4 and the two superkines and the levels of STAT6 phosphorylation were detected in (a) CD4 T cells, (b) CD8 T cells, (c) Monocytes, (d) B cells and (e) HH cells using phospho-STAT6 specific antibodies fluorescently labeled and flow cytometry. Mean and SEM were obtained from three different experiments.



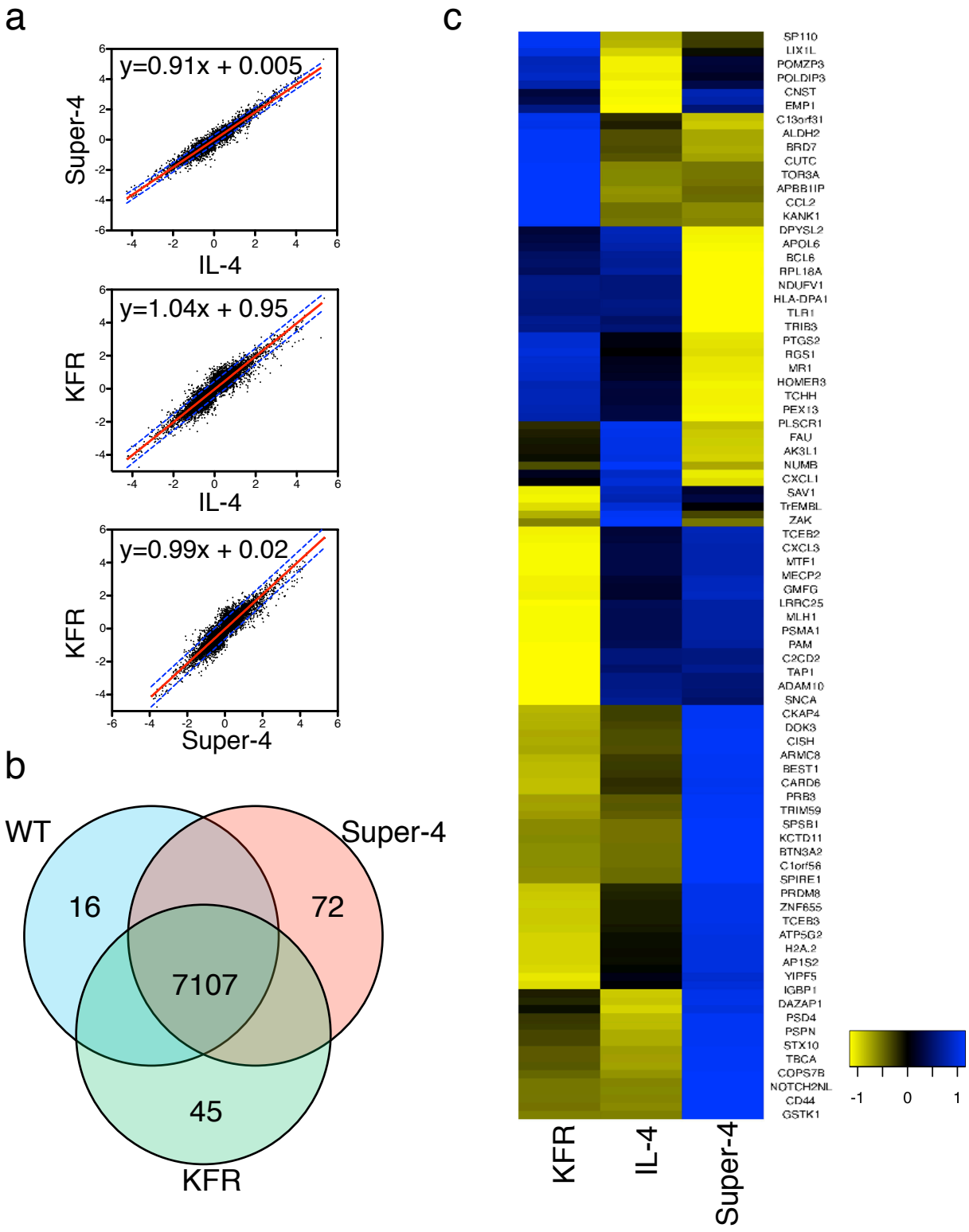
Supplementary Figure 7 – Dendritic cells differentiation potency exhibited by the IL-4 superkines. $CD14^+$ monocytes were isolated (>97% purity) and subsequently cultured with 50 ng/mL GM-CSF alone or with the indicated concentrations of IL-4, KFR or super-4. Cells were processed on day 6-7 and stained with Fluorescently labeled antibodies against CD86, CD209. Dendritic cell differentiation was assessed by flow cytometry with a BD LSRII flow cytometer and the mean fluorescent intensity (MFI) was determined on the FlowJo software (Treestar). Data represent mean ± SEM from 3 healthy donors.



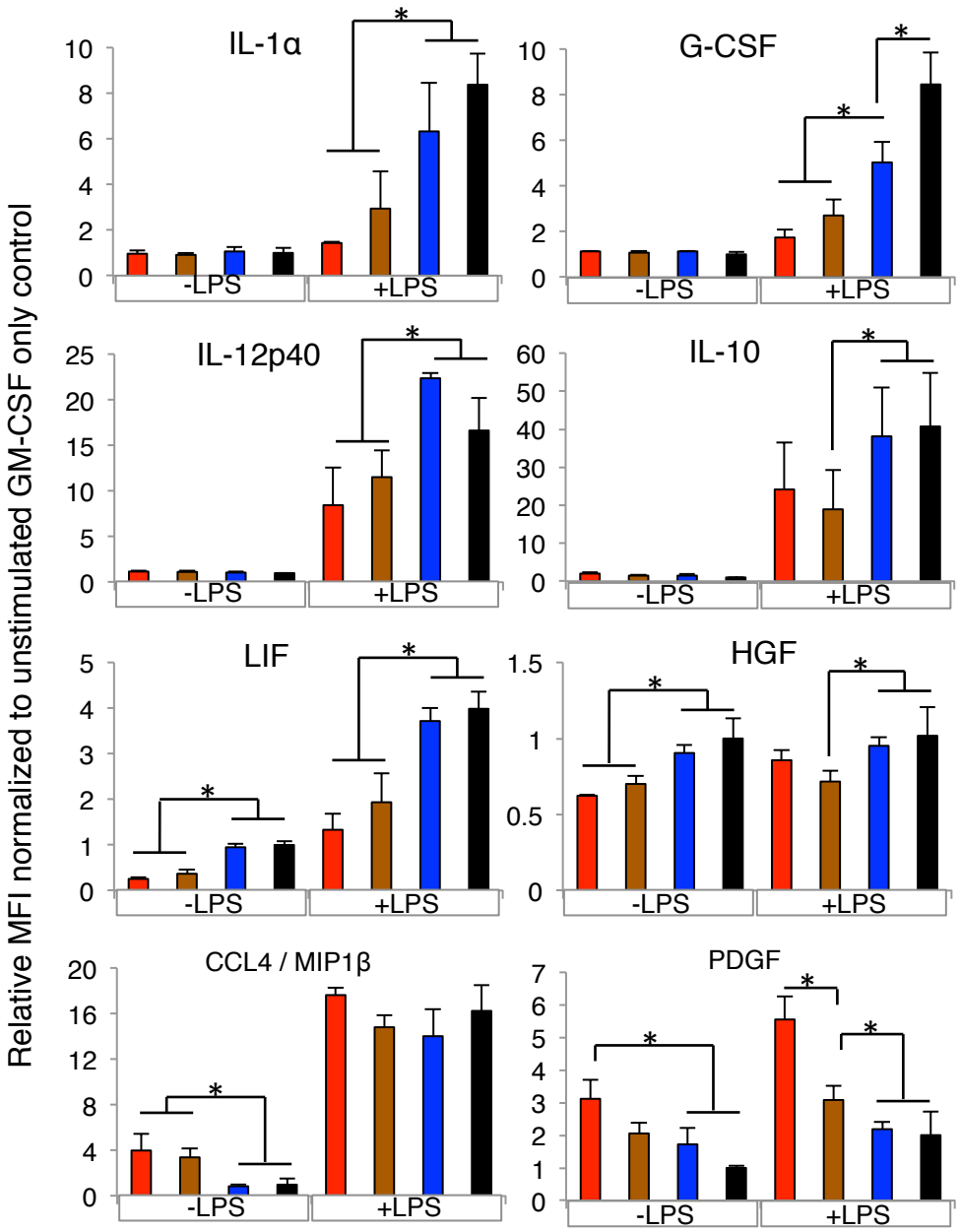
Supplementary Figure 8 – Differential expression of surface markers in the presence of IL-4, KFR or super-4.Monocytes were cultured for 7 days with 50 ng/ml GM-CSF, with or without IL-4, KFR or super-4 (20 ng/ml). Data represent mean ± SD from 3 healthy donors (normalized to GM-CSF alone group). *$p<0.05$ (paired T-test). CD1c (BDCA-1) and CD141 (BDCA-3) were not detected above background levels.



Supplementary Figure 9 – Receptor downregulation and STAT6 and IRS1 phosphorylation kinetics induced by IL-4 and the two superkines in monocytes. Monocytes were stimulated with 50 nM of IL-4 or the two superkines for the indicated times and cells were either (a-b) fixed and permeabilized with 100% methanol for 30 min and stained with phospho-STAT6 and phospho-IRS1 antibodies; or (c-d) were stained with anti-IL-13Rα1 or γc specific antibodies for 30 min at 4°C, to prevent receptor internalization, fixed with 4% PFA and analyzed by flow cytometry. Data (mean and SEM) are from four healthy donors.



Supplementary Figure 10 – Gene expression profiles induced by IL-4 and the two superkines in monocytes**.** Purified monocytes from 5 healthy donors were stimulated for six hours with GM-CSF alone or combined with IL-4 or the two superkines and their RNA was extracted and analyze by two-color Agilent microarrays (60,000 features). (a) Scatter plot correlation comparing the gene induction by the three cytokines are shown. (b) Venn diagram where genes significantly down/up- regulated by the three cytokines, when compared to the GM-CSF control condition, are shown. (c) Heatmap showing a selected list of genes (Supplementary Table 4) significantly regulated by IL-4/super-4 vs KFR, IL-4/KFR vs super-4 or IL-4 vs super-4/KFR ($p<0.05$, paired T-test). Blue color denotes higher expression and yellow color denotes lower expression.



Supplementary Figure 11 – Differential induction of cytokine secretion by IL-4, KFR and super-4.Monocytes were cultured for 7 days with 50 ng/ml GM-CSF, with or without IL-4, KFR or super-4 (20 ng/ml) then stimulated for 24h with LPS (2 mg/ml), data represent mean ± SD from 3 healthy donors (normalized to unstimulated GM-CSF alone group). *p<0.05 (paired T-test).