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► **To cite this version:**

Léa Sta, Guillaume Voisinne, Jesse Cotari, Michael F Adamer, Carmen Molina-París, et al.. Tuning of cytokine signaling through imbalanced abundances of receptors and kinases. 2022. hal-03844964

HAL Id: hal-03844964

<https://hal.science/hal-03844964v1>

Preprint submitted on 9 Nov 2022

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Tuning of cytokine signaling through imbalanced abundances of receptors and kinases

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This manuscript was compiled on October 7, 2022.

1 **Cells use surface receptors to detect extra-cellular ligands (e.g., growth factors or cytokines) and engender signaling cascades. We explore**
2 **the effect of cell-to-cell variability in receptor subunit copy number for cells responding to γ_c cytokines (e.g., IL-2, IL-7 and IL-15). We find that**
3 **primary T cells expressing higher levels of the common γ_c receptor chain have weaker responses to IL-7, both in terms of lowered STAT5**
4 **phosphorylation amplitude and higher EC₅₀. A mathematical model that accounts for abundance imbalance (e.g., insufficient expression of**
5 **JAK kinases compared to the number of receptors, or γ_c competition for other receptor subunit chains) predicts the formation of non-signalling**
6 **complexes, consistent with the observed cellular behaviour. This type of built-in limit on signaling responses illustrates how phenotypic**
7 **heterogeneity generates biological functional diversity.**

Systems biology | Cytokines | Dose-response | Phenotypic heterogeneity | Mathematical modeling

1 Introduction

2 Biological systems have developed a variety of mechanisms for converting external information into cellular responses, including
3 ion channels, pressure sensors, G-protein coupled receptors, receptor tyrosine kinases (RTKs), and receptors requiring associated
4 kinases, such as the Janus kinase (JAK) family of cytokines (1, 2). The latter two families of receptors are similar in many
5 regards. Both RTKs and JAK-associated receptors are activated by ligand-induced dimerization, which leads to phosphorylation
6 of the receptor intra-cellular chains. This phosphorylation event results in the activation of downstream kinases, such as
7 phosphatidylinositol-3-kinase (PI3K) and Ras, as well as the activation of proximal signal transducers and activators of
8 transcription (STATs) (3).

9 In spite of their similarities, these two receptor families have one notable difference in the mechanisms by which they initiate
10 signaling: while RTKs have intrinsic kinase activity, cytokine receptors co-opt JAK family kinases to gain signaling ability.
11 Though, at a first glance, the difference between a kinase that is intrinsic to the receptor and one that is tightly bound to
12 the receptor may seem negligible in terms of biochemistry, our prior studies have shown that cytokine receptor signaling can
13 be significantly altered by the precise abundances of its molecular signaling components (4). Hence, we hypothesized that
14 cytokine receptors and their co-optation of soluble kinases could elicit large non-genetic variability of ligand detection within a

15 population of cells (4, 5). We, thus, combined single-cell experimental measurements and mathematical modeling to explore
16 the impact of the relative abundance of signaling components on the sensitivity and amplitude in response to extra-cellular
17 cytokines (6, 7).

18 We focus on the common gamma (γ_c) family of cytokine receptors, comprising the receptors for IL-2,4,7,9,15, and 21 (8).
19 This family shares the eponymous γ_c chain, a ligand-binding chain without intrinsic kinase activity. The γ_c chain signals
20 instead through the action of an associated JAK kinase, JAK3. All cytokine receptors in this family complete the signaling core
21 of the receptor with a second chain associated with JAK1: IL- x ($x=4,7,9$ or 21) makes use of IL- x R α . IL-2 and IL-15 share
22 the JAK1-associated IL-2R β chain, and include a third chain for specificity and enhanced sensitivity (IL-2R α and IL-15R α ,
23 respectively). Our previous work demonstrated that sharing the γ_c chain led to correlated cellular sensitivity in the γ_c family
24 cytokines: increased abundance of the IL-2R α subunit chain decreased a cell's sensitivity to IL-7 (4). Our computational
25 models and subsequent experiments indicated that IL-2R α decreased sensitivity to IL-7 by sequestering the γ_c chain into
26 pre-formed IL-2 receptor complexes (4).

27 Here we explore a conjecture derived from our initial finding of γ_c chain competition; namely, that increases in γ_c chain
28 abundance would increase the number of fully-formed receptors and the sensitivity to the γ_c family cytokines. Furthermore,
29 by analogy to the well-established role of RTK upregulation in cancer (9, 10), a natural expectation would be that γ_c chain
30 upregulation induces greater maximal signaling upon ligand binding (1). This work, however, stems from follow-up experiments

Significance Statement

Cells rely on cytokines to coordinate their activation, differentiation, proliferation and survival. In particular, γ_c cytokines (interleukins IL-2, 4, 7, 9, 15, and 21) regulate the fate of leukocytes. The signaling cascade induced by these cytokines is relatively simple, and involves the phosphorylation of receptor-associated Janus-like kinases (JAK). Here, we explore the cell-to-cell variability of cytokine responses in primary mouse T cells, and find a paradoxical and quantitative imprint of receptor expression levels and other signaling components. For instance, high abundance of the common γ_c chain reduces cytokine responses (both in terms of signaling amplitudes and characteristic cytokine concentrations triggering 50% of the response). We develop mathematical models to quantify how limited abundances of signaling components (e.g., JAK or other cytokine receptor subunit chains) may explain our experimental observations. We conclude by generalizing this observation of cell-to-cell signaling variability to other ligand-receptor-kinase systems.

All contributed to research design. L.S., G.V. and M.A. performed theoretical modeling and computer simulations; G.V. and J.C. performed experimental measurements and data processing. All contributed to writing the paper.

The authors have no competing interest to report.

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31 examining the impact of γ_c chain abundance on T cell responses to IL-7, which led us to the paradoxical observation that
32 increased abundance of the γ_c chain, in fact, decreased both the sensitivity and the amplitude. Making use of mathematical
33 modeling and quantitative flow cytometry data, we demonstrate that part of this counter-intuitive behavior is an intrinsic
34 feature of a receptor requiring an associated kinase. Specifically, the composition of the IL-7 receptor signaling core, comprising
35 two distinct subunit receptor chains and an associated JAK kinase (JAK3), induces a subtle balance between the different
36 molecular components of the receptor-ligand system, required for optimal signaling. Furthermore, we also show that unbalanced
37 expression (upregulation) of any one component is generally insufficient to increase the IL-7 response. To put this observation
38 in perspective, we mathematically investigate the effect of chain copy number variability for a wider class of receptor types:
39 heterodimeric or homodimeric receptors with intrinsic kinase activity, or requiring JAK kinases, and monomeric receptors.
40 This analysis illustrates that the composition of the γ_c family of receptors provides functional diversity in the face of variability
41 in molecular abundances, and demonstrates how the exact biochemical architecture of a receptor may have implications for the
42 oncogenic potential of receptor over-expression.

43 Results and discussion

44 **Effect of γ_c chain abundance on IL-7 response.** To investigate the impact of the abundance of the γ_c cytokine receptor on
45 cell signaling, we analyzed the response of single primary T cells to cytokine stimulation. We first assessed cellular responses
46 to the IL-7 cytokine by flow cytometry using STAT5 phosphorylation. To probe cellular responses over a broad range of γ_c
47 abundances, we transfected primary T cells with plasmids expressing γ_c or GFP. As compared to control cells, γ_c transfected
48 cells showed elevated abundance of the receptor (Fig. S1A). Control and γ_c transfected cells with a similar γ_c abundance
49 exhibited similar responses to IL-7 stimulation (Fig. S1B), suggesting that the transfection did not alter other components of
50 the signaling machinery. Interestingly, cells with a high γ_c abundance showed reduced STAT5 phosphorylation levels, suggesting
51 that this cytokine receptor could have a negative impact on IL-7R signaling.

52 To further characterize how IL-7R signaling is regulated by the abundance of its signaling components, we quantified
53 simultaneously the abundances of γ_c , IL-7R α and JAK3 (Fig. S1C-D). Analysis of single-cell responses under a high dose of
54 IL-7 (10 nM) demonstrated heterogeneous degrees of STAT5 phosphorylation that correlated monotonically with the expression
55 levels of IL-7R α and JAK3 (Fig. 1A). In contrast, and consistently with our previous observation, greater abundance of γ_c
56 subunit chains was associated with reduced levels of phosphorylated STAT5 (pSTAT5).

57 To determine the relative contribution of each protein, we performed cell-to-cell variability analysis (CCVA), a method
58 that systematically parses the effect of protein abundance on downstream responses to a stimulus (4, 11). In this case, CCVA
59 was used to quantify the effects of γ_c , IL-7R α and JAK3 abundance on T cell responses to IL-7. Cells were grouped into
60 bins according to their abundance of γ_c , IL-7R α and JAK3 (Fig. 1B). For each subpopulation, the average response to IL-7

61 stimulation, as measured by pSTAT5, was fitted with a Hill function and characterized in terms of its amplitude (the STAT5
62 phosphorylation level corresponding to the maximum IL-7 dose) and its EC_{50} (the dose of IL-7 that elicits half of the amplitude)
63 (Fig. 1C). The output of this process is a set of maps of the relative effect of IL-7R α , γ_c and JAK3 abundance on both
64 amplitude and EC_{50} (11) (Fig. 1D).

65 The resulting maps revealed a strikingly complex relationship between γ_c levels and IL-7 response. The EC_{50} map (Fig. 1D,
66 bottom) indicated that a greater abundance of γ_c increased EC_{50} values. The amplitude map exposed an even more complex
67 relationship between γ_c expression levels and STAT5 phosphorylation in response to IL-7 stimulation (Fig. 1D, top). A clear
68 trend could be observed for any abundance of IL-7R α and JAK3, in which increasing the abundance of the γ_c chain first
69 increased and then decreased the amplitude. This showed that the reduced pSTAT5 levels observed at a high IL-7 dose (10
70 nM) in Fig. 1A and Fig. 1C can be associated with elevated γ_c abundance, independently of that of IL-7R α and JAK3.

71 **A simple model of imbalance between cytokine receptor and kinase abundances (model I).** Our experimental results present a
72 paradox: high abundance of the γ_c chain, an essential IL-7 receptor component, is found to reduce the responsiveness to this
73 cytokine, reflected both in a smaller amplitude and a greater EC_{50} of the pSTAT dose-response curve. To resolve this, we
74 considered the fact that γ_c does not have intrinsic kinase ability, but rather depends on the kinase activity of receptor-associated
75 JAK3 to activate downstream signaling (2). As such, we hypothesized that increased abundance of the γ_c chain alone could
76 exert a dominant negative effect via the formation of signaling-deficient receptors (or "dummy" complexes in this paper) deprived
77 of the JAK3 kinase (Fig. 2A). Such a negative effect of over-abundance has been previously reported in the case of the CD8
78 co-receptor, which requires an associated downstream kinase (Lck, the lymphocyte specific protein tyrosine kinase) (12). To
79 test this hypothesis, we modified our mathematical model of IL-7 signaling presented in (4) to account for the binding of JAK3
80 to the γ_c chain. The model, depicted in Fig. 2B and which we will refer to as model I, considers affinity constants and molecular
81 abundances (Fig. 2C) which had been previously determined (4) making use of experimentally-validated approximations
82 (Supplementary Table S1). Our theoretical analysis of model I, presented in (13), showed that the amplitude (the number of
83 ligand-bound signaling receptors for a large IL-7 concentration) is equal to the total copy number of the trans-membrane chain
84 (IL-7R α or γ_c) with the lowest availability (limiting chemical reactant), modulated by a ratio that takes a value between 0
85 and 1. This ratio only depends on the total number of JAK3 and γ_c molecules and their affinity constant, K_1 ; thus, clearly
86 demonstrating that the abundance of JAK3 does have an influence on the amplitude, in accordance with our experimental
87 results. Moreover, our analytic study showed that the EC_{50} value is independent of JAK3 abundance (13), in agreement with
88 the experimental data.

89 Median JAK3 abundance was measured using flow cytometry to be 8×10^3 proteins per cell (see Supplementary section H).
90 The median copy number of IL-7R α was reported to be 10^3 proteins per cell (14). We use these two values as default when
91 studying model I. We analysed model I numerically to explore how γ_c abundance impacts the cytokine response (Fig. 2D,

92 E and F, Supplementary section C, and section G). As conjectured, model I demonstrated that the over-abundance of γ_c
93 chains could indeed inhibit the IL-7 signaling response and thus, reduce its amplitude (Fig. 2D). Namely, both the number of
94 signaling and non-signaling (or "dummy") complexes grow linearly as γ_c chain copy number increases (Fig. 2E). When the
95 number of γ_c chains equals the number of IL-7R α chains, the amplitude of the IL-7 response reaches its maximum. As the
96 abundance of γ_c exceeds that of IL-7R α , the number of signaling complexes decreases and that of "dummy" complexes, in turn,
97 increases. In our analytic study (13), we showed that, at high cytokine (IL-7) concentration, the sum of the "dummy" and
98 signaling complexes is always equal to the total copy number of the limiting component (γ_c during the linear growth, and
99 IL-7R α after the maximum amplitude has been reached). This implies that, once the number of γ_c chains exceeds that of
100 IL-7R α , all IL-7R α chains are ligand- and γ_c -bound to form either a signaling or a "dummy" complex. When the number of γ_c
101 chains exceeds that of JAK3 molecules, the number of signaling complexes goes back to zero rather quickly (proportionally to
102 the inverse of the total number of γ_c chains, as derived from the analytic expression of the amplitude in the limit when the
103 copy number of γ_c tends to $+\infty$ (13)), and the IL-7R α chains are used to form "dummy" complexes, without signaling ability.
104 This theoretical result suggests that when γ_c is in excess (compared to the other subunit chains of the receptor complex), it
105 could indeed be inhibitory to the IL-7 signaling amplitude. After fitting the model to the experimental data, we found that
106 model I could reproduce the pSTAT5 response in individual cells with accuracy (Fig. 2G).

107 However, if model I could account for the observed dependency of the maximal pSTAT5 on the abundance of IL-7R α ,
108 JAK3 and γ_c , it could not reproduce the experimental increase of EC₅₀ with increasing expression of γ_c chains (Fig. 2F and
109 H). Including the previously estimated affinity constants K_3 and K_4 in the set of fitted model parameters increased model
110 performance (Supplementary Fig. S7 and Supplementary file Supp_table_1), but still failed to account for the observed EC₅₀
111 variations with respect to γ_c . This suggested that the structure of model I needed to be modified if it was to reproduce these
112 experimental EC₅₀ variations. To this end, we explored how an hypothetical allosteric change induced by JAK3 binding to γ_c
113 could limit binding of IL-7R α to γ_c and the formation of the signaling IL-7 receptor complex (see Supplementary section C.2
114 and Fig. S8). We found that this could result in an increase of the IL-7 EC₅₀ for over-abundant γ_c . As a result, model
115 performance, reproducing the experimental EC₅₀, increased (Supplementary file Supp_table_1 and Supplementary Fig. S8),
116 but the observed variation of EC₅₀ for low and intermediate γ_c abundances could not be reproduced for any set of parameters.
117 Hence, allostery cannot account for the measured change of EC₅₀ with respect to γ_c expression.

118 To further explore the imprint of γ_c abundance on cytokine responses, we repeated our experimental measurements and
119 analysis for the STAT5 phosphorylation responses triggered by IL-2 and IL-15 stimulation. In both cases, the effect of γ_c on
120 the amplitude of response to the respective cytokine was nearly identical to that seen following IL-7 treatment (Fig. S4 in
121 Supplementary). In light of this experimental result, the trimeric receptor model (SRLK with $n = 3$) proposed in Ref. (13),
122 with similar amplitude to that of model I, seems to be a good candidate to characterize the IL-2/IL-2R and IL-15/IL-15R

123 systems. Most interestingly, γ_c abundance did not significantly affect EC_{50} values for either IL-2 or IL-15. Thus, while the
124 behavior of the amplitude seems to be similar across several cytokine-receptor systems, the sensitivity of the cellular response,
125 as measured by EC_{50} , requires further cytokine-specific experimental and theoretical analysis.

126 **Specific competition for the formation of the complete IL-7 receptor can account for the EC_{50} increase in the IL-7 response**

127 **(model II).** Our previous hypothesis of allostery, the existence of differences in binding caused by interactions between the
128 components of the IL-7 receptor complex, did not lead to a mathematical model which could reproduce the experimental
129 data. Thus, we considered the addition of external components to the model (model II). We reasoned that, since the γ_c chain
130 is a component of other cytokine receptors (8), these receptors could limit the amount of γ_c available to form IL-7 receptor
131 complexes, and in turn, decrease IL-7 signaling. To explore this hypothesis we included a putative "decoy" subunit chain (called
132 x), that could bind the γ_c chain (and the γ_c :JAK3 complex), and sequester it away from IL-7R α (see Fig. 3A-C); for instance,
133 x could be other surface receptors (such as IL-2R β or IL-4R α). Further work will be required to identify a suitable candidate
134 for x (see section E for an extended discussion). Model II behaves similarly to model I when considering the IL-7 response
135 amplitude (Fig. 3D-E), as expected from the algebraic analysis conducted in Ref. (13), which shows that the amplitude of
136 both models is the same. This guarantees that model II reproduces the experimental amplitude as accurately as model I. As
137 expected, the number of signaling and "dummy" complexes, as well as the number of decoy complexes (with or without JAK3)
138 are limited by their less abundant component (see Fig. 2E). Also, receptor complexes containing the decoy subunit chain x are
139 formed only when the number of γ_c exceeds the number of IL-7R α chains (*i.e.*, when the number of γ_c is no longer limiting).

140 The EC_{50} of model II, as is the case in model I, does not depend on JAK3 abundance (as demonstrated in (13)). However
141 it depends on the abundances of γ_c and x . More specifically, for a fixed abundance of IL-7R α , the EC_{50} increases with the
142 abundance of the x chain, and this effect vanishes as γ_c abundance increases (see Supplementary Fig. S5). Hence the presence
143 of the x subunit chain provides a way to modulate the EC_{50} . Still, a constant amount of x , or an amount proportional to the
144 γ_c abundance is not sufficient to reproduce the experimental EC_{50} dynamics. However, when we conjecture that the abundance
145 of the unknown species x (N_x) increases with that of γ_c (N_γ) according to a power-law relation, such that $N_x = N_\gamma^p$, $p > 1$,
146 the EC_{50} of model II increases with γ_c (see Supplementary Fig. S6). Moreover, this increase is observed even for low and
147 intermediate γ_c levels, in agreement with our experimental observations.

148 In this last setting, model II could reproduce both the experimental EC_{50} and the maximum pSTAT5 across subpopulations
149 with different IL-7R α , JAK3 and γ_c abundances (Fig. 3H). As a result, a better correlation between theoretical and experimental
150 pSTAT5 levels across all cytokine concentrations was achieved (Fig. 3G). Importantly, the performance of model II was only
151 slightly reduced when the parameters K_3 and K_4 were held fixed at, or constrained to be close to their previously estimated
152 values (see Supp_table_1 and Supplementary Fig. S9), suggesting a consistency between this model and our previous work.

153 **General insight on the variability and robustness of receptor-ligand signaling systems.** Our study demonstrates that the
154 increased expression of a given IL-7 receptor component may paradoxically reduce the amplitude and increase the EC_{50} of
155 the response to IL-7. In this section, we comprehensively explore the impact of varied abundances of receptor onto signal
156 transduction. We generalize models I and II for five different configurations: hetero- or homo-dimeric receptors, each either
157 requiring a downstream kinase or possessing intrinsic kinase activity, and monomeric receptors (Fig. 4A). We note that
158 the hetero-dimeric receptor model with downstream kinase is the configuration of model I. For every receptor system we
159 considered the up/downregulation of a “primary” receptor chain, equivalent to the γ_c subunit chain in our experiments. For
160 the homo-dimeric or monomeric receptors, this is the only receptor chain. For a hetero-dimeric receptor, we set the median
161 abundance of the secondary receptor chain to 10^3 proteins per cell, by analogy with IL-7R α in our system. If a downstream
162 kinase is required, we assumed there is only one kinase (*e.g.*, JAK3 in the IL-7 receptor system), and set its level to 10^4 proteins
163 per cell. Finally, we took the (no allostery) affinity constant values described in Fig. 2 (see Supplementary Table S3 and
164 section F): \mathcal{K}_1 , binding of downstream kinase to primary receptor chain, \mathcal{K}_2 , binding of primary to secondary (or primary to
165 primary, in the case of homo-dimeric receptors) receptor chain, with or without downstream kinase, and \mathcal{K}_3 , binding of ligand
166 to the receptor complex, with or without downstream kinase. We used the analytic method presented in Ref. (13) to derive the
167 amplitude and EC_{50} for each of these models when possible. The results are summarized in Supplementary Table S4. We also
168 analysed the global sensitivity of the amplitude and EC_{50} using numerical and machine-learning methods (see Supplementary
169 section G). Our theoretical results and numerical exploration indicate a striking difference for the effect of primary receptor
170 abundance depending on the receptor arrangement:

- 171 1. When the primary chain is the only one in the receptor (monomeric or homo-dimeric RTK), increasing the abundance of
172 that chain proportionally increases the amplitude, as expected from Supplementary Table S4. Thus, a 10^2 -fold increase
173 in expression of the primary chain leads to a 10^2 -fold rise in amplitude for the monomeric and a 50-fold rise for the
174 homo-dimeric RTK. This results in a large potential for enhanced signaling (Fig. 4B).
- 175 2. The less abundant chain always determines the theoretical maximum of the amplitude. However, this maximum is not
176 attained for receptors with downstream kinase due to the formation of “dummy” complexes (Fig. 4B).
- 177 3. Receptors with downstream kinase present a loss of signaling when the number of primary chains increases, thus, rendering
178 the tuning of the response by the primary chain abundance more complex than RTKs (Fig. 4B).
- 179 4. The affinity constants of the biochemical reactions only play a minor role in the value of the amplitude. However, they
180 have greater relevance in the EC_{50} (Fig. S10 in Supplementary).

181 We then explored the functional implications of the five receptor configurations aforementioned, in terms of signal robustness
182 and variability. Indeed, our experimental studies highlighted the large variability in the abundance of signaling components

183 (receptors, kinases, phosphatases, etc.), even within isogenic population of cells (4, 5). In fact, this variability drives some
184 phenotypic heterogeneity (4, 5, 15, 16). We simulated cellular populations for each of the arrangements, with the distribution
185 of each protein described by a lognormal distribution with a CV of 0.25 (Fig. 4D). The abundance of the secondary receptor
186 chain (if present) was set to have a median of 10^3 and downstream kinase a median of $10^{3.5}$, respectively. We then simulated
187 populations with a 10-fold up/downregulation of the primary chain, with a median of 10^4 for the wild type.

188 Homo-dimeric receptors with associated kinases (RTKs) or monomeric receptors had the greatest variability of amplitude,
189 matching the distribution of the receptor exactly. Homo-dimeric receptors requiring downstream kinases and hetero-dimeric
190 receptors with associated kinases (RTKs) had the least variability, maintaining amplitude at a moderately high level. Hetero-
191 dimeric receptors requiring downstream kinases had larger variability, but had lower EC_{50} when upregulating or downregulating
192 the primary chain. These results indicated that the composition of a receptor's signaling core could have strong effects on the
193 variability of cellular responses to extra-cellular ligands. The γ_c family's arrangement tends to allow for large variability, but
194 leads to lower amplitudes. Hetero-dimeric receptors with intrinsic kinase ability, such as the receptor for insulin, IGF-1, or
195 homo-dimeric receptors with downstream kinases, such as the IL-6 receptor, tend to have intermediate but consistent amplitude.
196 Homo-dimeric receptors with associated kinase activity, such as EGFR, are highly variable in EC_{50} and their amplitude does
197 not saturate (17, 18) (Figs. 4B and C).

198 We also computed the EC_{50} for each of the receptor configurations (Fig. 4C). We observed that the EC_{50} of the hetero-dimeric
199 models are the same (see Table S4 in Supplementary), and the EC_{50} of the homo-dimeric models are similar (Fig. 4C). This
200 led us to conjecture that the variability of the EC_{50} with the abundance of the primary chain depends on the manner the
201 extra-cellular receptor is built and is independent of its intra-cellular activity. Further experimental work will be required
202 to identify a molecular mechanism to test such conjecture. We note that the EC_{50} of the model in Fig 3 which, this time,
203 captures the experimental behavior, is also independent of the kinase activity of the receptor. We also observe that the EC_{50}
204 for all receptor configurations tends to a constant for large values of γ_c abundance. This value is the dissociation constant of
205 the ligand to the receptor $\frac{1}{K_3}$ (see Supplementary Table S4).

206 Conclusion

207 We conjecture that the requirement for abundance balance between kinases and receptor subunit chains provides built-in
208 protection against aberrant activation, as observed during oncogenic transformation (17). Robust input/output response does
209 limit functional variability but also protects against enhancement of receptor signaling and its dysregulated accumulation of
210 downstream survival signals, such as Bcl-2. Of course, many additional molecules (or proteins) and biochemical reactions are
211 required to proceed from upregulation of a receptor chain to upregulation of cellular survival signals, not least of which is
212 ligand availability. Nonetheless, the examination of gene expression data from 4,087 tumors sequenced as part of The Cancer

213 Genome Atlas (TCGA) partially validated our conjecture. EGFR was upregulated in 28% of analyzed tumors (compared to
214 healthy counterparts), whereas γ_c was upregulated in only 11%. Furthermore, of the 11% of cases in which γ_c was upregulated,
215 78% of those also included upregulation of a secondary γ_c family signaling chain, such as IL-4R α or IL-2R β . Interestingly,
216 IL-7R α was upregulated for 24% of the analyzed tumors compared to normal cells. This could reflect different factors, such
217 as the fact that IL-7R α was the chain of limiting abundance in our system and/or that IL-7 is ubiquitously available at low
218 levels, from secretion by endothelial cells (19). While increased immune infiltrates could potentially be responsible for the
219 upregulation of lymphocyte-associated receptors in the TCGA tumor samples, the frequent expression in non-haematopoietic
220 cancer cell lines (20), and confirmed *in situ* expression of IL-2R α , IL-2R β and γ_c proteins in breast tumor epithelial cells (21),
221 suggests that expression of cytokine receptors may play a role in non-lymphoid tumorigenesis. We also note that simply
222 quantifying upregulation does not account for the possibility of activating mutations, such as the I241-V253 reported in the
223 IL-7R α trans-membrane domain, which allows for ligand-independent activation (22). Hence, a broader analysis tracking
224 receptor upregulation in T or B cell lymphomas over time will provide much insight and validation of the impact of signaling
225 core composition on oncogenicity. Our study, demonstrating that the quantitative effects of receptor chain upregulation can be
226 vastly different, depending on the elements of a receptor's signaling core, provides a theoretical and quantitative framework with
227 which to interpret the potential functional significance of receptor up/downregulation during lymphocyte differentiation (23, 24),
228 oncogenesis (1) or drug treatment (25).

229 **Materials and Methods**

230 See Supplementary Information.

231 **ACKNOWLEDGMENTS.** This project has received funding from the European Union's Horizon 2020 research and innovation programme
232 under the Marie Skłodowska-Curie grant agreement number 764698 (LS and CMP). This work was partially supported by the intramural
233 research program of the NCI (GAB).

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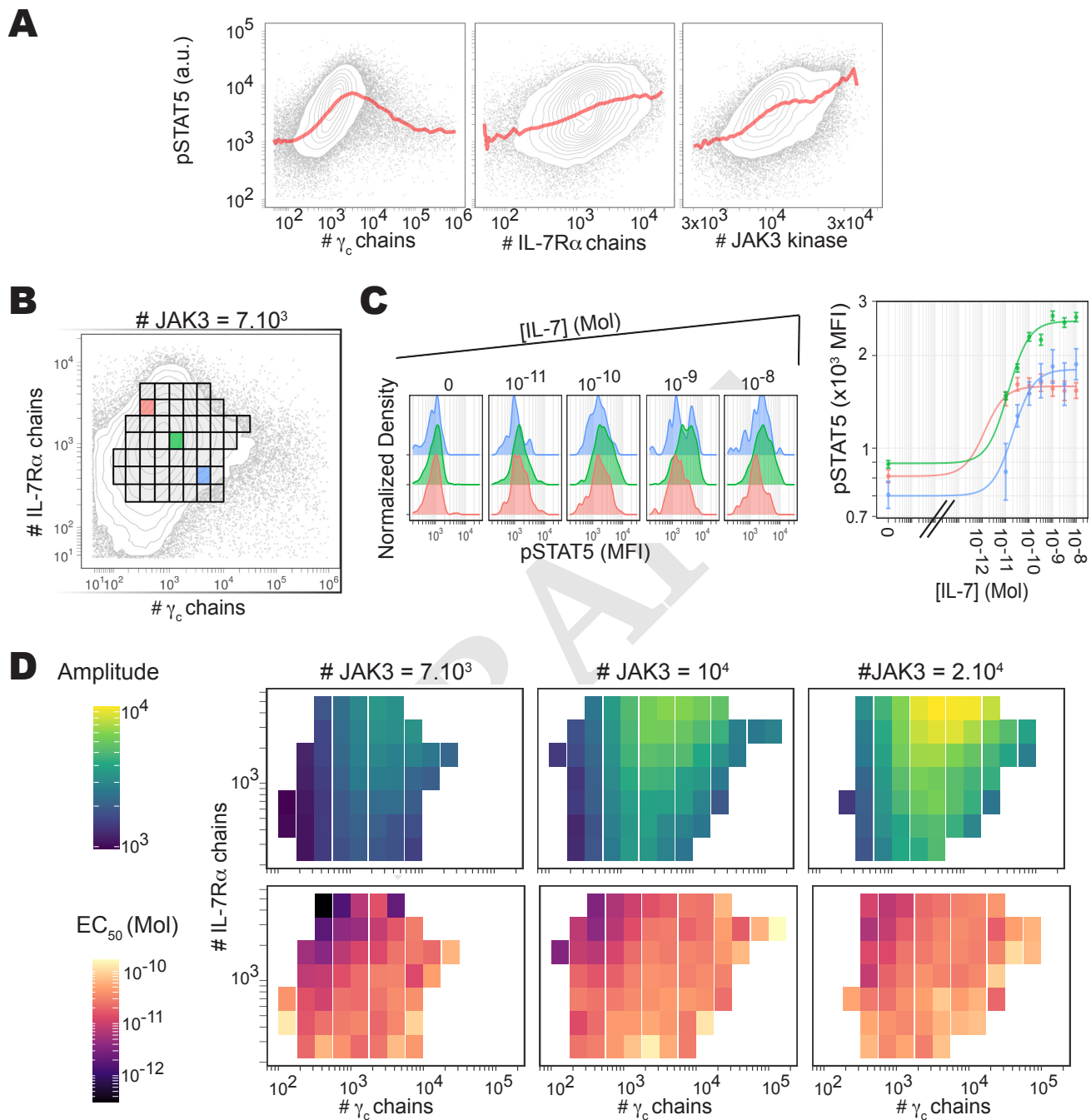


Fig. 1. Cell-to-cell variability analysis of the pSTAT5 response to IL-7. A) Distribution of STAT5 phosphorylation (pSTAT5) in response to 10 nM of IL-7, for different abundances of IL-7R α , JAK3, and γ_c (red line represents the geometric mean of pSTAT5 for each level of signaling component). B) Definition of three emblem subpopulations based on surface abundance of IL-7R α and γ_c subunit chains. C) pSTAT5 distribution for increasing concentrations of IL-7 for the three emblem subpopulations. D) Complete maps of pSTAT5 amplitude (top) and EC₅₀ (bottom) for different expression levels of the signaling components: IL-7R α , JAK3, and γ_c .

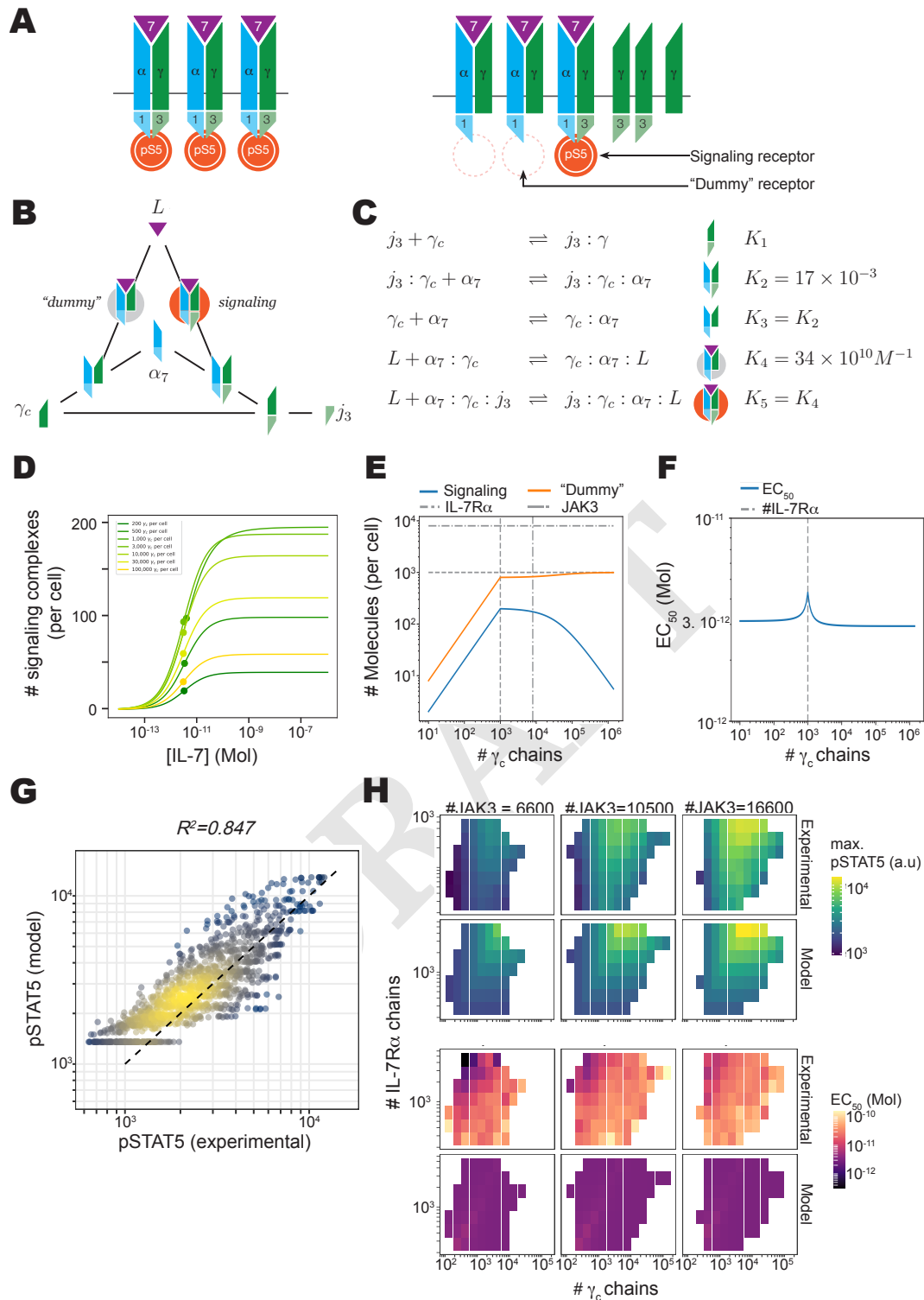


Fig. 2. Model I of stoichiometric imbalance of JAK3 compared to γ_c accounts for the formation of non-signaling (or "dummy") receptor complexes and the loss of pSTAT5 response for high copy numbers of γ_c . **A**) Cartoon of the formation of the IL-7/IL-7 receptor complex. Note that some of the fully engaged receptors will fail to signal due to the absence of JAK3. **B**) Sketch of the biochemical reactions associated with the formation of the IL-7/IL-7R complex. **C**) Chemical equilibrium for the reactions depicted in **B** (j_3 : JAK3, α_7 : IL-7R α , L : IL-7). K_i are affinity constants for $i = 1, \dots, 5$. **D**) Modeled pSTAT5 in response to increasing doses of IL-7 (for different abundance of γ_c chains). **E**) Amplitude of responses: *i.e.*, number of fully signaling IL-7/IL-7R (blue) or "dummy" (orange) complexes for increasing abundance of γ_c at high ligand concentration. **F**) EC₅₀ for pSTAT5 in response to IL-7 for increasing abundance of γ_c . It is essentially constant and fails to reproduce the experimental observations. **G**) model I is broadly accurate in predicting pSTAT5 for a wide range of IL-7 concentrations, across different cellular subpopulations. **H**) Model I can account for the (IL-7R α and γ_c) dependence of pSTAT5 amplitude, but fails to reproduce the dependence on EC₅₀ (theoretical and experimental results to be compared). Theoretical results correspond to simulations of model I with best-fit parameters corresponding to the entry "model_I" in Supplementary file Supp_table_1.

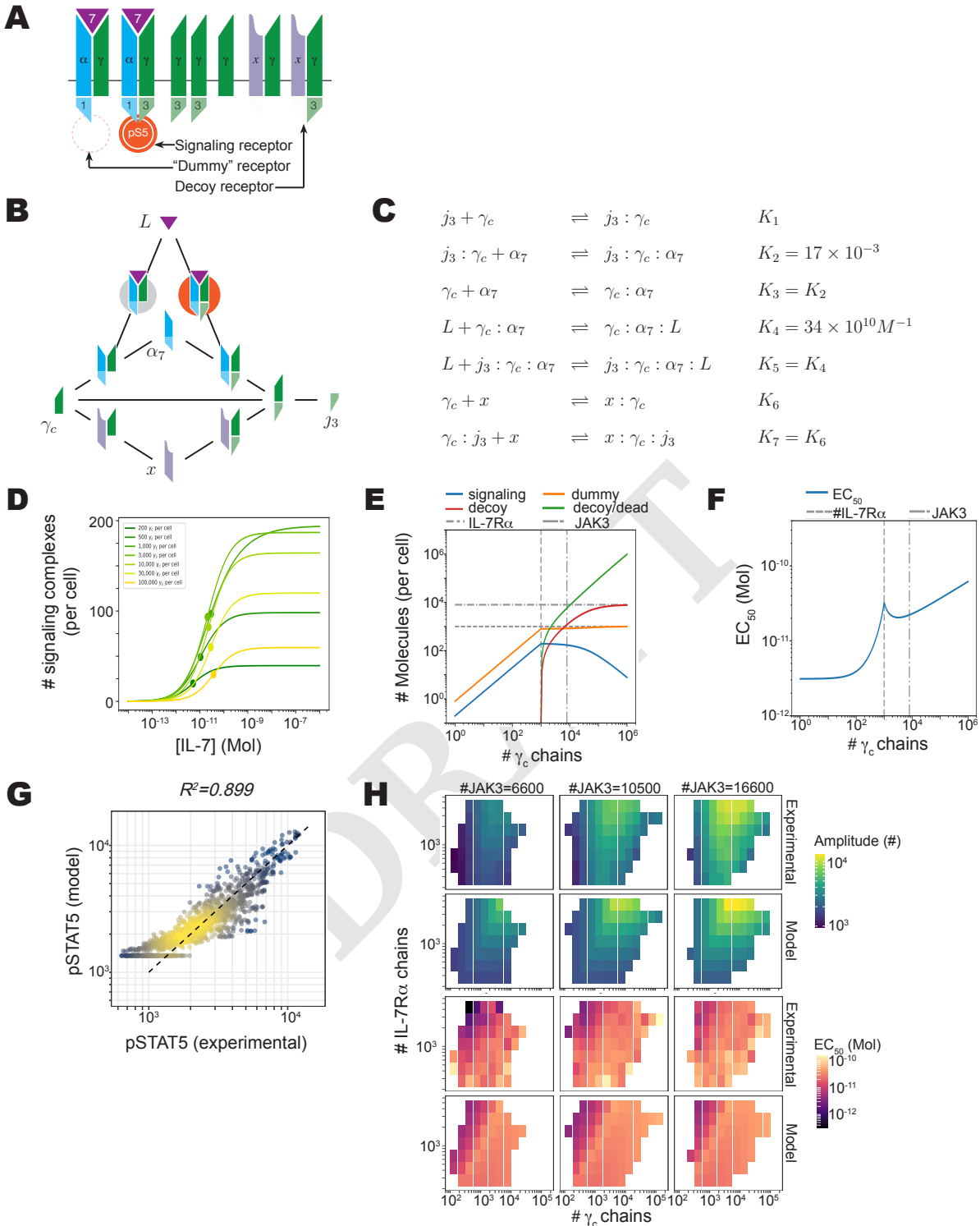


Fig. 3. Model II augments model I with a decoy receptor to account for an increased EC_{50} , as well as the loss of pSTAT5 response at high levels of γ_c . A) Cartoon of the formation of IL-7/IL-7R complex. This model adds a decoy receptor that sequesters γ_c . B) Sketch of the biochemical reactions associated with the formation of the IL-7/IL-7R complex. C) Chemical equilibrium for the reactions depicted in B (j_3 : JAK3, α_7 : IL-7R α , L : IL-7, x : decoy receptor). K_i are affinity constants for $i = 1, \dots, 7$. D) Modeled pSTAT5 in response to increasing doses of IL-7 (for different abundances of γ_c). E) Amplitude, *i.e.*, number of signaling (blue) or "dummy" complexes (orange), for increasing number of γ_c chains and for excess cytokine. F) EC_{50} for pSTAT5 in response to IL-7 for increasing levels of γ_c . Model II agrees with the experimental results when compared to model I. G) Model II improves predictions (compared to model I) for pSTAT5 levels across all IL-7 concentrations, as well as across all cellular subpopulations. H) Model II accounts well for the (IL-7R α , γ_c) dependence of the pSTAT5 amplitude and EC_{50} (theoretical map to be compared to experimental results). Theoretical results correspond to simulations of model II with best-fit parameters corresponding to the entry "model_II" in Supplementary file Supp_table_1.

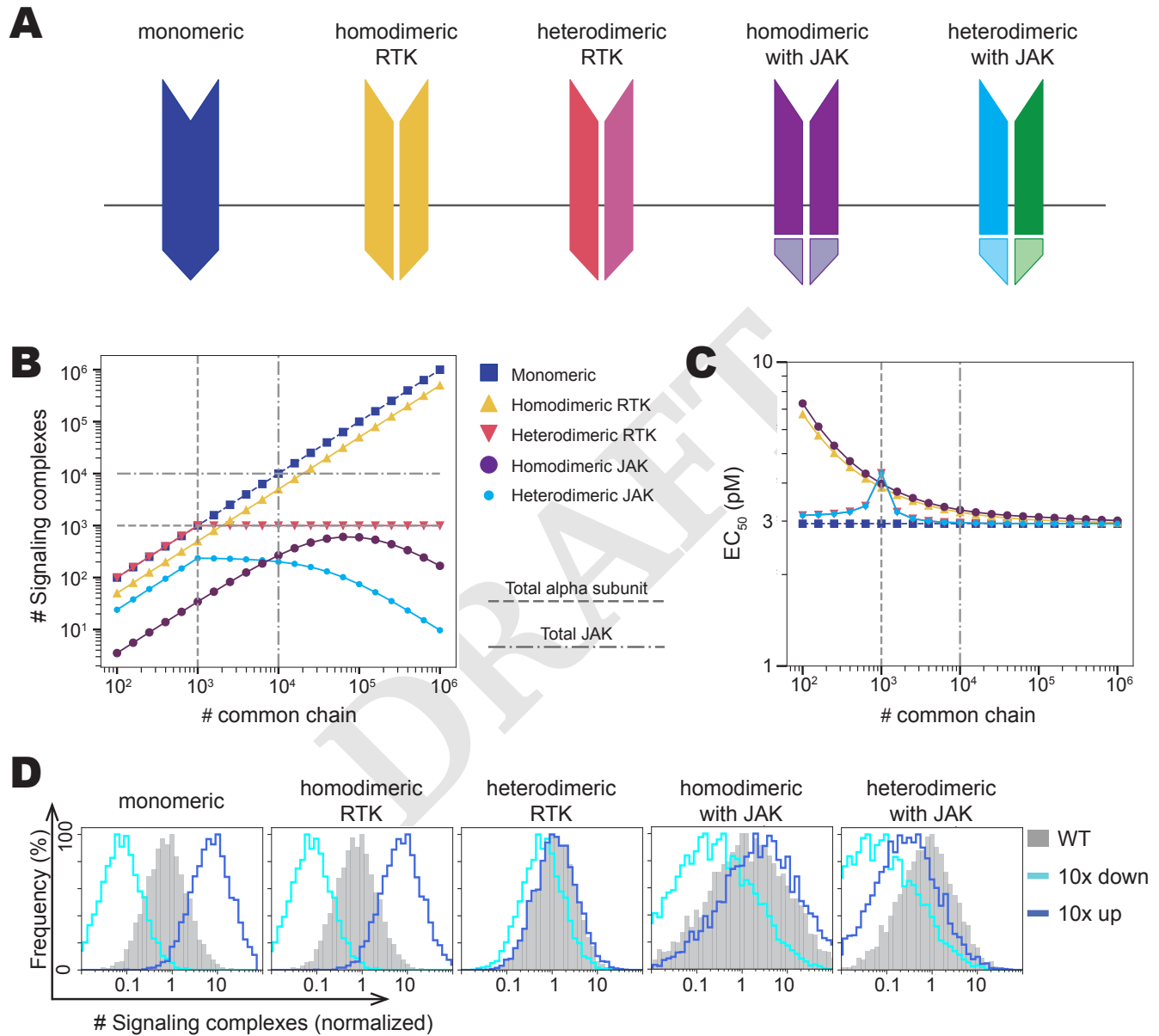


Fig. 4. Different architecture of ligand-receptor systems leads to a range of signaling behaviour. A) Receptors might come in five configurations (monomeric, and homo-dimeric or hetero-dimeric, with/without endogeneous kinase). B) Each configuration results in a different relation between the maximal number of signaling receptors (signaling complexes) and the copy number of the primary chain when ligand is in excess, as well as C) EC₅₀. D) Distribution of signaling complexes for each receptor configuration when one receptor is up/downregulated by 10-fold.