SUPPLEMENTAL RESULTS

MODEL SIMPLIFICATION

At sufficiently low cell densities initial conditions 12 imply, that all terms proportional to the concentration of free extracellular ligand can be safely neglected in Eqs. 2-5 [1] to obtain

$$dL_o / dt \approx k_r (n/N_A) C_s , \tag{S1}$$

$$dC_s / dt \approx -(k_r + k_t) C_s + k_x C_i , \tag{S2}$$

$$dC_i / dt \approx k_t C_s + k_f^{'} (R_{i0} - C_i) L_i - (k_r^{'} + k_x + k_{hr}) C_i , \tag{S3}$$

$$(V_e N_A) dL_i / dt \approx -k_f^{'} (R_{i0} - C_i) L_i + k_r^{'} C_i - k_{hl} (V_e N_A) L_i . \tag{S4}$$

Numerical tests using the base line parameters listed in Tables 1-2 illustrate that this neglection is justified for cell densities up to 10^{10} cells/l (not shown). Furthermore, initial conditions 12 imply that $k_t C_s << k_x C_i$, at sufficiently short times. Neglecting the term $k_t C_s$ in Eq. S3 and introducing the *total* number of intracellular ligand molecules per cell, ℓ_i, we obtain the autonomous nonlinear system

$$dC_i / dt \approx k_1 (C_i - C_-[\ell_i])(C_i - C_+[\ell_i]) , \tag{S5}$$

$$d\ell_i / dt \approx -(k_x + k_{hr}) C_i - k_{hl} (\ell_i - C_i) \tag{S6}$$

where

$$k_1 \equiv k_f^{'} / (N_A V_e) , \tag{S7a}$$

$$K_M \equiv (k_r^{'} + k_x + k_{hr}) / k_1 \tag{S7b}$$

and

$$C_+[\ell_i] \equiv \frac{(R_{i0} + K_M + \ell_i) + ((R_{i0} + K_M + \ell_i)^2 - 4R_{i0}\ell_i)^{1/2}}{2} , \tag{S8a}$$

$$C_-[\ell_i] \equiv \frac{(R_{i0} + K_M + \ell_i) - ((R_{i0} + K_M + \ell_i)^2 - 4R_{i0}\ell_i)^{1/2}}{2} \tag{S8b}$$

are the roots of the quadratic equation

$C_i^2 - (R_{i0} + K_M + \ell_i) C_i + R_{i0}\ell_i = 0$.

Eqs. S5-S6 are similar to the equations of enzyme kinetics [2] and can be similarly analyzed using the total quasi-steady state approximation [2,3]. Indeed, Eqs. S5 and S8a,b are formally the same as the equations for the enzyme-substrate complex in irreversible Michaelis-Menten kinetics, with R_{i0} and ℓ_i playing the respective roles of total enzyme and substrate concentrations [2].

THE TOTAL QUASI-STEADY-STATE APPROXIMATION

Initial conditions 12 imply that during the initial transient we can substitute $\ell_i = \ell_{i*}$ into Eq. S5 to obtain

$$dC_i / dt \approx k_1 (C_i - C_-[\ell_{i*}])(C_i - C_+[\ell_{i*}]) . \tag{S9}$$

The solution of this Riccati equation is

$$C_{i,\mathrm{ITA}} = \frac{C_-[\ell_{i*}]\left(C_+[\ell_{i*}] - C_{i*}\right) + C_+[\ell_{i*}]\left(C_{i*} - C_-[\ell_{i*}]\right)e^{-t/t_C}}{\left(C_+[\ell_{i*}] - C_{i*}\right) + \left(C_{i*} - C_-[\ell_{i*}]\right)e^{-t/t_C}}, \tag{S10}$$

where

$$t_C^{-1} \equiv k_1\left(C_+[\ell_{i*}] - C_-[\ell_{i*}]\right) = k_1\left((R_{i0} + K_M + \ell_{i*})^2 - 4R_{i0}\ell_{i*}\right)^{1/2} . \tag{S11}$$

Self consistency requires that the fractional decrease of $\ell_i(t)$ during the initial transient should be small [2]

$$\varepsilon \equiv \left(\ell_{i*} - \ell_i(t_C)\right)/\ell_{i*} << 1 . \tag{S12}$$

Using Eq. S6 to effect a first order McLaurin expansion of $\ell_i(t_C)$ and noting that $0 \le C_{i*} / \ell_{i*} \le 1$ we find

$$\varepsilon \approx [(k_x + k_{hr})C_{i*} / \ell_{i*} + k_{hl}(1 - C_{i*} / \ell_{i*})]t_C \le (k_x + k_{hr} + k_{hl})t_C . \tag{S13}$$

This entails that the validity of the initial transient approximation is guaranteed by the condition

$$(k_x + k_{hr} + k_{hl})t_C << 1 . \tag{S14}$$

It is noteworthy that the baseline estimates listed in Tables 1 and 2 satisfy inequality S14. A more detailed analysis of the validity of inequality S14 is given in a subsequent section.

Eq. S10 implies that $C_i(t)$ decreases and in a time of order t_C approaches the minimal asymptotic value implied by the initial conditions, $C_-[\ell_{i*}]$, which signals the onset of a quasi-steady state (QSS) such that

$$C \approx C_-[\ell_i], \quad t > t_C \tag{S15}$$

$$d\ell_i / dt \approx -(k_x + k_{hr})C_-[\ell_i] - k_{hl}(\ell_i - C_-[\ell_i]) \quad t \geq t_c . \tag{S16}$$

Inequality S12 warrants that Eq. S16 can be solved subject to the initial condition

$$\ell_i = \ell_{i*}, \quad t = t_C . \tag{S17}$$

Equations S16-S17 imply that the QSS time scale is [2]

$$t_\ell \approx \frac{\ell_{i*}}{(k_x + k_{hr})C_-[\ell_{i*}] + k_{hl}(\ell_{i*} - C_-[\ell_{i*}])}, \tag{S18}$$

so that the ratio of time scale of the induction period prior to the QSS, t_C, to the QSS time scale is approximately

$$t_C / t_\ell \approx [(k_x + k_{hr})(C_-[\ell_{i*}]/\ell_{i*}) + k_{hl}(1 - C_-[\ell_{i*}]/\ell_{i*})]t_C \approx \varepsilon . \tag{S19}$$

Inequality S14 therefore guarantees the validity of Eq. S16 even prior to steady state, $t \leq t_C$, thereby justifying the solution of this equation subject to the true initial conditions (Eq. 14).

Moreover, these results can be used to obtain corresponding estimates for free extracellular ligand (Eq. S1) and surface bound ligand (Eq. S2). Since the transient phase ends before significant sorting occurs, only the steady-state value of the internalized complex enters the estimates of surface complex

$$C_s \approx k_x e^{-(k_r+k_t)t} \int_0^t C_i(s)\, e^{(k_r+k_t)s} ds \approx k_x e^{-(k_r+k_t)t} \int_0^t C_-[\ell_i(s)]\, e^{(k_r+k_t)s} ds , \tag{S20}$$

and degraded ligand in the medium

$$dL_{\deg} / dt = (n / N_A)\big(k_{hr} C_i + k_{hl}(\ell_i - C_i)\big) \approx (n / N_A)\big(k_{hr} C_-[\ell_i] + k_{hl}(\ell_i - C_-[\ell_i])\big). \tag{S21}$$

VALIDITY OF THE TOTAL QUASI-STEADY STATE APPROXIMATION

Rewriting S11 in the expanded form

$$t_C^{-1} = k_1 (K_M^2 + 2K_M(R_{i0} + \ell_{i*}) + (R_{i0} - \ell_{i*})^2)^{1/2} \tag{S22}$$

illustrates that t_C is a bounded function in the (ℓ_{i*}, R_{i0}) plane and since it is continuous has a maximum in any closed set R×R. The partial derivatives are

$$\frac{\partial t_C}{\partial R_{i0}} = -\frac{K_M + (R_{i0} - \ell_{i*})}{k_1\left(K_M^2 + 2K_M(R_{i0} + \ell_{i*}) + (R_{i0} - \ell_{i*})^2\right)^{3/2}} \tag{S23}$$

and

$$\frac{\partial t_C}{\partial \ell_{i*}} = -\frac{K_M - (R_{i0} - \ell_{i*})}{k_1\left(K_M^2 + 2K_M(R_{i0} + \ell_{i*}) + (R_{i0} - \ell_{i*})^2\right)^{3/2}}. \tag{S24}$$

The conditions for extrema are therefore

$$K_M + (R_{i0} - \ell_{i*}) = 0 \tag{S25}$$

and

$$K_M - (R_{i0} - \ell_{i*}) = 0. \tag{S26}$$

This has no solution, implying that the maximum is on the boundary. It is easy to verify that the maximum is attained at $R_{i0} = \ell_{i*} = 0$. Thus,

$$t_C(\ell_{i*}, R_{i0}) \le t_C(0,0) = (k_r^{'} + k_x + k_{hr})^{-1} \tag{S27}$$

and inequality S12 is guaranteed by the purely kinetic criterion

$$\mu \equiv (k_x + k_{hr} + k_{hl})\, t_C(0,0) = \frac{k_x + k_{hr} + k_{hl}}{k_r^{'} + k_x + k_{hr}} << 1, \tag{S28}$$

which is valid whenever dissociation of endosomal complex is much faster than the elimination of internalized ligand by degradation and recycling. Moreover, inequality S14 can also be satisfied even when $\mu = O(1)$, provided that $\tau \equiv t_C(\ell_{i*}, R_{i0}) / t_C(0,0) << 1$. The later inequality is valid for a wide range of initial ligand loading and endosomal receptor numbers (Figure S1).

APPROXIMATE LIGAND TIME-COURSE CURVES

The complexity of Eq. S8b does not allow for an explicit closed form solution of the total quasi-steady state rate equation (Eq.S16). To that end we now proceed to simplify the quasi-steady state concentration of endosomal complex (Eq. S8b) in zones I-III ad defined by inequalities 20-23 (Figure 4).

Zone I

Inequality 20 implies that [2]

$$C_{-}[\ell_i] \approx \frac{R_{i0}\ell_i}{K_M + \ell_i} << \ell_i \,, \tag{S29}$$

$$t_C^{-1} \approx k_1(\ell_{i^*} + K_M) \,. \tag{S30}$$

Substituting S29 into S16 yields

$$d\ell_i / dt \approx -(k_x + k_{hr})C_{-}[\ell_i] - k_{hl}\ell_i = -\frac{k_\ell \ell_i + k_{hl}\ell_i^2}{\ell_i + K_M} \,, \tag{S31}$$

where

$$k_\ell \equiv (k_x + k_{hr})R_{i0} + k_{hl}K_M \,. \tag{S32}$$

When $\ell_{i^*} << K_M$, inequality 20 reduces to the $K_M >> R_{i0}$ limit of inequality 21, studied below. When $\ell_{i^*} >> K_M + R_{i0}$, inequality 20 reduces to inequality 23, also studied below. The unique limit of inequality 20 is defined by $\ell_{i^*} \approx K_M >> R_{i0}$, in which case S31 cannot be integrated explicitly. However, we can approximate the initial apparent dynamics by linearizing the tQSSA results around the initial condition $\ell_i = \ell_{i^*}$. Linearizing S31

$$d\ell_i / dt \approx -\ell_i / t_I \,, \quad t_I \equiv \frac{\ell_{i^*} + K_M}{k_\ell + k_{hl}\ell_{i^*}} \,, \tag{S33}$$

and integrating we find

$$\ell_i \approx \ell_{i^*} e^{-t/t_I} \,. \tag{S34}$$

Linearization of S29 yields

$$C_{-}[\ell_i] \approx \frac{R_{i0}\ell_i}{K_M + \ell_{i^*}} \approx \left(\frac{R_{i0}\ell_{i^*}}{K_M + \ell_{i^*}}\right) e^{-t/t_I} \,. \tag{S35}$$

Substituting S35 into S20 and S21 we find, respectively

$$C_s \approx \frac{k_x C_{-}[\ell_{i^*}](e^{-t/t_I} - e^{-(k_r + k_t)t})}{k_r + k_t - t_I^{-1}} \tag{S36}$$

and

$$\frac{dL_{\deg} / dt}{(n / N_A)} \approx \left(\frac{k_{hr}R_{i0} + k_{hl}(\ell_{i^*} + K_M)}{\ell_{i^*} + K_M}\right) \ell_{i^*} e^{-t/t_I} \approx k_{hl}\ell_{i^*} e^{-t/t_I}$$

with the solution

$$\frac{L_{\deg}}{(n/N_A)\ell_{i*}} \approx k_{hl} t_I (1 - e^{-t/t_I}) . \tag{S37}$$

Substituting S36 into S1 yields

$$\frac{L_o}{(n/N_A)\ell_{i*}} \approx \frac{k_r k_x R_{i0}}{(\ell_{i*} + K_M)(k_r + k_t - t_I^{-1})} \left(\frac{1 - e^{-t/t_I}}{t_I^{-1}} - \frac{1 - e^{-(k_r + k_t)t}}{k_r + k_t} \right). \tag{S38}$$

Zone II

Inequality 21 implies that [2]

$$C_-[\ell_i] \approx \frac{R_{i0}\ell_i}{K_M + R_{i0}}, \tag{S39}$$

$$t_C^{-1} \approx k_1 (R_{i0} + K_M) . \tag{S40}$$

Substituting S39 into S16 yields

$$d\ell_i / dt \approx -t_R^{-1} \ell_i , \quad t_R \equiv (K_M + R_{i0})/k_\ell \tag{S41}$$

with the solution

$$\ell_i \approx \ell_{i*} e^{-t/t_R} . \tag{S42}$$

Substituting S39 and S42into Eqs. S20-S21 yields, respectively

$$C_s \approx \frac{k_x C_-[\ell_{i*}](e^{-t/t_R} - e^{-(k_r + k_t)t})}{k_r + k_t - t_R^{-1}} \tag{S43}$$

and

$$dL_{\deg} / dt \approx (n/N_A) \left(\frac{k_{hr} R_{i0} + k_{hl} K_M}{R_{i0} + K_M} \right) \ell_{i*} e^{-t/t_R}$$

with the solution

$$\frac{L_{\deg}}{(n/N_A)\ell_{i*}} \approx \left(\frac{k_{hr} R_{i0} + k_{hl} K_M}{R_{i0} + K_M} \right) t_R (1 - e^{-t/t_R}) . \tag{S44}$$

Substituting S43 into S1 yields

$$\frac{L_o}{(n/N_A)\ell_{i*}} \approx \frac{k_r k_x R_{i0}}{(R_{i0} + K_M)(k_r + k_t - t_R^{-1})} \left(\frac{1 - e^{-t/t_R}}{t_R^{-1}} - \frac{1 - e^{-(k_r + k_t)t}}{k_r + k_t} \right). \tag{S45}$$

Zone III

Inequality 22 implies that [3]

$$C_{-}[\ell_i] \approx \ell_i \ , \tag{S46}$$

$$t_C^{-1} \approx k_1(R_{i0} - \ell_{i*}) \ . \tag{S47}$$

Substituting S46 into S16 yields

$$d\ell_i / dt \approx -(k_x + k_{hr})\ell_i$$

with the solution

$$\ell_i \approx \ell_{i*} e^{-(k_x + k_{hr})t} \ . \tag{S48}$$

Substituting S46 and S48 into Eqs. S20-S21 yields, respectively

$$C_s \approx \frac{k_x \ell_{i*}(e^{-(k_x+k_{hr})t} - e^{-(k_r+k_t)t})}{k_r + k_t - (k_x + k_{hr})} \tag{S49}$$

and

$$dL_{\deg} / dt \approx (n / N_A) k_{hr} \ell_i \approx (n / N_A) k_{hr} \ell_{i*} e^{-(k_x+k_{hr})t}$$

with the solution

$$\frac{L_{\deg}}{(n / N_A)\ell_{i*}} \approx \frac{k_{hr}}{k_x + k_{hr}}(1 - e^{-(k_x+k_{hr})t}) \ . \tag{S50}$$

Substituting result S49 into S1 yields

$$\frac{L_o}{\ell_{i*}(n / N_A)} \approx \frac{k_r k_x}{k_r + k_t - (k_x + k_{hr})}\left(\frac{1 - e^{-(k_x+k_{hr})t}}{k_x + k_{hr}} - \frac{1 - e^{-(k_r+k_t)t}}{k_r + k_t}\right). \tag{S51}$$

Zone IV

Inequality 23 implies that [3]

$$C_{-}[\ell_{i*}] \approx R_{i0} \ , \tag{S52}$$

$$t_C^{-1} \approx k_1(\ell_{i*} - R_{i0}) \ . \tag{S53}$$

Substituting S52 into S16 yields

$$\dot{\ell}_i \approx (k_{hl} - (k_x + k_{hr}))R_{i0} - k_{hl}\ell_i$$

with the solution

$$\ell_i \approx \left(\ell_{i*} + \left(1 - \frac{k_x + k_{hr}}{k_{hl}}\right)R_{i0}\right)e^{-k_{hl}t} + \left(1 - \frac{k_x + k_{hr}}{k_{hl}}\right)R_{i0} \ . \tag{S54}$$

This equation is only valid while ℓ_i satisfies inequality 23 and $C_i \approx R_{i0}$. Eventually $\ell_i \approx K_M + R_{i0}$ and the approximation breaks down.

ON THE RATIO R_{i0}/K_M

It is straightforward to verify that Eq. 16 is equivalent to the hyperbolic relationship

$$C_i = \frac{R_{i0}(\ell_i - C_i)}{K_M + (\ell_i - C_i)} = \frac{R_{i0} N_A V_e L_i}{K_M + N_A V_e L_i}. \quad \text{(S55)}$$

Eq. S55 implies that K_M corresponds to the free ligand number for which half of the binding sites are saturated, $C_i = R_{i0}/2$ and $C_i/\ell_i = R_{i0}/(R_{i0} + 2K_M)$. Thus, the fraction of ligand that is bound at half saturation increases with the ratio R_{i0}/K_M. For a general ligand concentration $(N_A V_e)L_i = \alpha K_M$ and $C_i/\ell_i = R_{i0}/(R_{i0} + (\alpha+1)K_M)$, directly illustrating that the fraction of bound ligand always increases with the ratio R_{i0}/K_M and tends to unity as $R_{i0}/K_M \to \infty$.

INTERNALIZATION KINETICS IMPACT ENDOSOMAL STABILITY

Following [4] we replace the dynamical model of surface kinetics and internalization by a constant prescribed flux of growth factor-receptor complex,

$$C_s \approx R_{s0} L_0/(K_d + L_0). \quad \text{(S56)}$$

This reduces our model to

$$\dot{C}_i = k_e C_s + k_f' R_i L_i - k_r' C_i - (k_{hr} + k_x) C_i, \quad \text{(S57)}$$

$$(V_e N_{av})\dot{L}_i = -k_f' R_i L_i + k_r' C_i - k_{hl}(V_e N_{av}) L_i. \quad \text{(S58)}$$

$$\dot{R}_i = -k_f' R_i L_i + k_r' C_i - (k_{hr} + k_x) R_i. \quad \text{(S59)}$$

Note that this reduced model neglects the internalization of free receptors, and therefore underestimates the number of endosomal receptors. As predicted by our analysis the reduced model underestimation the stability of endosomal ligand (Figure S.2).

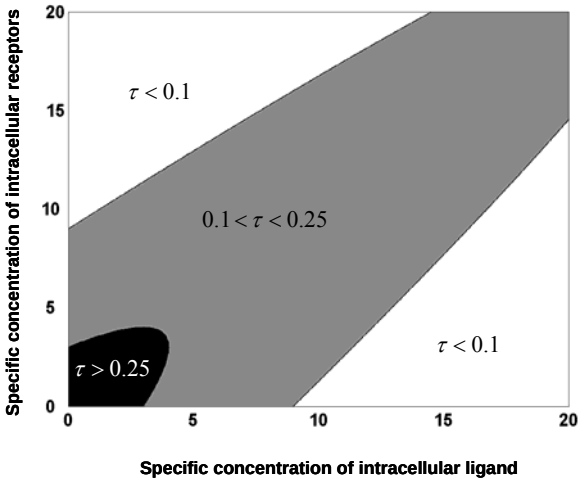

Figure S1 Normalized binding time scale $\tau \equiv t_C(\ell_{i^*}/K_M, R_{i0}/K_M)/t_C(0,0)$ as a function of specific endosomal ligand ℓ_{i^*}/K_M and receptor R_{i0}/K_M concentrations. Color code: (White) $\tau \le 0.1$, (gray) $0.1 \le \tau \le 0.25$ and (black) $0.25 < \tau \le 1$.

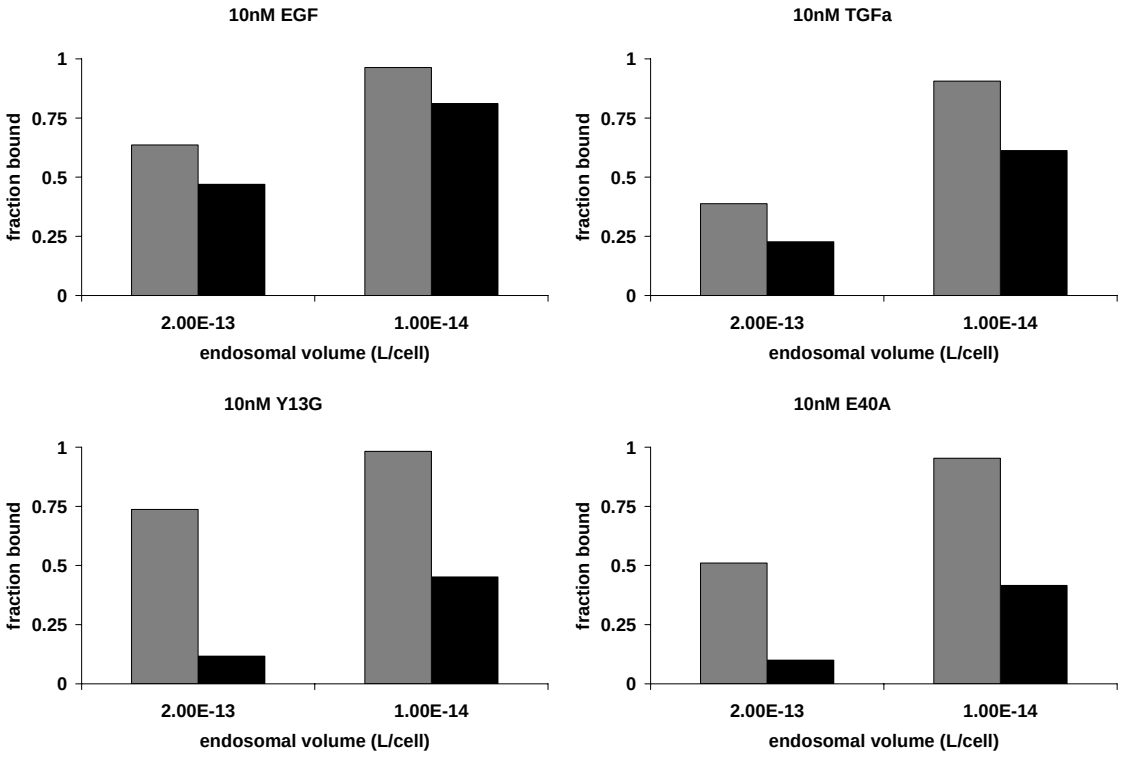

Figure S2 Fraction of bound endosomal ligand at the end of 180 minute incubations with 10nM of EGF, TGFα, Y13G or E40A. (Grey) Eqs. 1-6, (black) Eqs. S56-S59. Both sets of simulations employ the parameter values listed in Tables 1-2.

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