Supplementary Materials and Methods

Complete list of chemical equations used in the model

Note: L refers to ligand, R refers to unphosphorylated receptor, pR refers to phosphorylated receptor, colons indicate bound proteins, and parameter names are given next to reaction arrows. Subscripts on proteins indicate which compartment that protein resides in (Fig. 1). For reference:
C1: Extracellular Space
C2: Cytoplasm (no proteins of interest are modeled in the cytoplasm)
C3: Endocytic vesicles (VC)
C4: Early Endosomes (EE)
C5: Recycling Endosomes (RE)
C6: Late Endosomes (LE)
C7: Lysosomes (LS)
C8: Nucleus (N)

Ligand-receptor interactions: Extracellular Space

\[
\begin{align*}
L_{C1} + R_{C1} & \underset{k_{onC1}}{\overset{k_{offC1}}{\rightleftharpoons}} L_{C1}:R_{C1} \\
L_{C1}:R_{C1} & \underset{k_{p}}{\overset{k_{d}}{\rightarrow}} L_{C1}:pR_{C1} \\
L_{C1}:pR_{C1} & \underset{k_{offC1}}{\rightarrow} L_{C1} + R_{C1}
\end{align*}
\]

Ligand-receptor interactions: Endocytic Vesicles

\[
\begin{align*}
L_{C3} + R_{C3} & \underset{k_{onC3}}{\overset{k_{offC3}}{\rightleftharpoons}} L_{C3}:R_{C3} \\
L_{C3}:R_{C3} & \underset{k_{p}}{\overset{k_{d}}{\rightarrow}} L_{C3}:pR_{C3} \\
L_{C3}:pR_{C3} & \underset{k_{offC3}}{\rightarrow} L_{C3} + R_{C3}
\end{align*}
\]

Ligand-receptor interactions: Early Endosomes

\[
\begin{align*}
L_{C4} + R_{C4} & \underset{k_{onC4}}{\overset{k_{offC4}}{\rightleftharpoons}} L_{C4}:R_{C4} \\
L_{C4}:R_{C4} & \underset{k_{p}}{\overset{k_{d}}{\rightarrow}} L_{C4}:pR_{C4} \\
L_{C4}:pR_{C4} & \underset{k_{offC4}}{\rightarrow} L_{C4} + R_{C4}
\end{align*}
\]

Ligand-receptor interactions: Recycling Endosomes

\[
\begin{align*}
L_{C5} + R_{C5} & \underset{k_{onC5}}{\overset{k_{offC5}}{\rightleftharpoons}} L_{C5}:R_{C5}
\end{align*}
\]
Ligand-receptor interactions: Late Endosomes

\[ \frac{L_{C5} + R_{C5}}{L_{C5}:R_{C5}} \xrightarrow{k_{off}} \frac{L_{C5} + R_{C5}}{L_{C5}:pR_{C5}} \xrightarrow{k_{on}} \]

\[ \frac{L_{C5} + pR_{C5}}{L_{C5}:pR_{C5}} \xrightarrow{k_{off}} \frac{L_{C5} + R_{C5}}{L_{C5}:pR_{C5}} \xrightarrow{k_{on}} \]

Ligand-receptor interactions: Lysosomes

\[ \frac{L_{C6} + R_{C6}}{L_{C6}:R_{C6}} \xrightarrow{k_{off}} \frac{L_{C6} + R_{C6}}{L_{C6}:pR_{C6}} \xrightarrow{k_{on}} \]

\[ \frac{L_{C6} + pR_{C6}}{L_{C6}:pR_{C6}} \xrightarrow{k_{off}} \frac{L_{C6} + R_{C6}}{L_{C6}:pR_{C6}} \xrightarrow{k_{on}} \]

Ligand-receptor interactions: Nucleus

\[ \frac{L_{C7} + R_{C7}}{L_{C7}:R_{C7}} \xrightarrow{k_{off}} \frac{L_{C7} + R_{C7}}{L_{C7}:pR_{C7}} \xrightarrow{k_{on}} \]

\[ \frac{L_{C7} + pR_{C7}}{L_{C7}:pR_{C7}} \xrightarrow{k_{off}} \frac{L_{C7} + R_{C7}}{L_{C7}:pR_{C7}} \xrightarrow{k_{on}} \]

Trafficking: Extracellular space to endocytic vesicles

\[ \frac{L_{C1} + k_{int}(R)}{L_{C1}} \xrightarrow{k_{int}(R)} \frac{R_{C1} + k_{int}(R)}{R_{C1}} \xrightarrow{k_{int}(pR)} \frac{L_{C1} + pR_{C1}}{L_{C1} + pR_{C1}} \xrightarrow{k_{int}(pR)} \frac{L_{C1} + pR_{C1}}{L_{C1} + pR_{C1}} \xrightarrow{k_{int}(pR)} \]

Trafficking: Endocytic vesicles to early endosomes

\[ \frac{L_{C3} + k_{VCToEE}(R)}{L_{C3}} \xrightarrow{k_{VCToEE}(R)} \frac{R_{C3} + k_{VCToEE}(R)}{R_{C3}} \xrightarrow{k_{VCToEE}(pR)} \frac{L_{C3} + pR_{C3}}{L_{C3} + pR_{C3}} \xrightarrow{k_{VCToEE}(pR)} \]
Trafficking: Early endosome recycling

\[ L_{C4}^{krecEE (R)} \rightarrow L_{C1} \]
\[ R_{C4}^{krecEE (R)} \rightarrow R_{C1} \]
\[ L_{C4}^{pR_{C4}}^{krecEE (pR)} \rightarrow L_{C1}^{R_{C1}} \]

Trafficking: Early endosomes to recycling endosomes

\[ L_{C4}^{kEEtoRE (R)} \rightarrow L_{C5} \]
\[ R_{C4}^{kEEtoRE (R)} \rightarrow R_{C5} \]
\[ L_{C4}^{pR_{C4}}^{kEEtoRE (pR)} \rightarrow L_{C5}^{R_{C5}} \]

Trafficking: Recycling endosome recycling

\[ L_{C5}^{krecRE (R)} \rightarrow L_{C1} \]
\[ R_{C5}^{krecRE (R)} \rightarrow R_{C1} \]
\[ L_{C5}^{pR_{C5}}^{krecRE (pR)} \rightarrow L_{C1}^{R_{C1}} \]

Trafficking: Early endosomes to late endosomes

\[ L_{C4}^{kEEtoLE (R)} \rightarrow L_{C6} \]
\[ R_{C4}^{kEEtoLE (R)} \rightarrow R_{C6} \]
\[ L_{C4}^{pR_{C4}}^{kEEtoLE (pR)} \rightarrow L_{C6}^{R_{C6}} \]

Trafficking: Early endosomes to nucleus

\[ L_{C4}^{kEEtoN (R)} \rightarrow L_{C8} \]
\[ R_{C4}^{kEEtoN (R)} \rightarrow R_{C8} \]
\[ L_{C4}^{pR_{C4}}^{kEEtoN (pR)} \rightarrow L_{C8}^{R_{C8}} \]

Trafficking: Late endosomes to lysosomes

\[ L_{C6}^{kLEtoLS (R)} \rightarrow L_{C7} \]
\[ R_{C6}^{kLEtoLS (R)} \rightarrow R_{C7} \]
\[ L_{C6}^{pR_{C6}}^{kLEtoLS (pR)} \rightarrow L_{C7}^{R_{C7}} \]

Trafficking: Late endosomes to nucleus
Degradation

\[
\begin{align*}
\text{L}_{C6} & \xrightarrow{k\text{EtoN} (R)} \text{L}_{C8} \\
\text{R}_{C6} & \xrightarrow{k\text{EtoN} (R)} \text{R}_{C8} \\
\text{L}_{C6} \cdot \text{pR}_{C6} & \xrightarrow{k\text{EtoN} (pR)} \text{L}_{C8} \cdot \text{R}_{C8}
\end{align*}
\]

\[
\begin{align*}
\text{L}_{C7} & \xrightarrow{k\text{deg} (R)} \emptyset \\
\text{R}_{C7} & \xrightarrow{k\text{deg} (R)} \emptyset \\
\text{L}_{C7} \cdot \text{pR}_{C7} & \xrightarrow{k\text{deg} (pR)} \emptyset
\end{align*}
\]
Supplementary Figures and Tables

Figure S1. Model trafficking parameters determined by fitting experimental data. Trafficking parameters were fit by comparing generalized receptor model results to experimental data\textsuperscript{173,174,181–183}. Receptor types used for fitting are: ICAM-1 in Muro (2003)\textsuperscript{181} and Muro (2004)\textsuperscript{174}, heparin sulfate and integrins in Greene (2012)\textsuperscript{173}, EGFR in Danglot (2010)\textsuperscript{183}, and VEGFR2 in Lampugnani (2006)\textsuperscript{182}. Shown is fitting using Ang2-Tie2 parameters (ligand concentration, receptor concentration, ligand-receptor interaction kinetics). To obtain possible range of trafficking parameters (Table 2), this fitting is performed for all eight RTKs and best fit parameters were compiled as mean ± standard deviation. Experimental data include (A) percent total receptor internalized, (B) percent total receptor localized to the nucleus, (C) percent total receptor co-localization with early endosomes, (D) receptor co-localization with early endosomes over time, (E) percent total receptor co-localization with late endosomes, and (F) receptor co-localization with late endosomes over time.
Figure S2. EGFR phosphorylation compartmentalization following ligand stimulus. The percent of phosphorylated EGFR relative to total cell EGFR are given on (A) the cell membrane, (B) endocytic vesicles, (C) early endosomes, (D) recycling endosomes, (E) late endosomes, (F) lysosomes, and (G) in the nucleus. The activation time constant ($\tau_a$, time from ligand stimulus to 63.2% max phosphorylation) and decay time constant ($\tau_d$, time from max phosphorylation to 36.8% max phosphorylation) in minutes are also given for each compartment. Data is represented as mean ± standard deviation from 10,000 Monte Carlo simulations, randomly seeded across the possible trafficking kinetics (Table 2). (H) Mean phosphorylated receptor localization relative to total cell receptors at 5, 60, 120, 180 and 240 minutes after ligand stimulus.
Figure S3. FGFR1 phosphorylation compartmentalization following ligand stimulus. The percent of phosphorylated FGFR1 relative to total cell FGFR1 are given on (A) the cell membrane, (B) endocytic vesicles, (C) early endosomes, (D) recycling endosomes, (E) late endosomes, (F) lysosomes, and (G) in the nucleus. The activation time constant ($\tau_a$, time from ligand stimulus to 63.2% max phosphorylation) and decay time constant ($\tau_d$, time from max phosphorylation to 36.8% max phosphorylation) in minutes are also given for each compartment. Data is represented as mean ± standard deviation from 10,000 Monte Carlo simulations, randomly seeded across the possible trafficking kinetics (Table 2). (H) Mean phosphorylated receptor localization relative to total cell receptors at 5, 60, 120, 180 and 240 minutes after ligand stimulus.
**Figure S4. IGFR1 phosphorylation compartmentalization following ligand stimulus.** The percent of phosphorylated IGFR1 relative to total cell IGFR1 are given on (A) the cell membrane, (B) endocytic vesicles, (C) early endosomes, (D) recycling endosomes, (E) late endosomes, (F) lysosomes, and (G) in the nucleus. The activation time constant ($\tau_a$, time from ligand stimulus to 63.2% max phosphorylation) and decay time constant ($\tau_d$, time from max phosphorylation to 36.8% max phosphorylation) in minutes are also given for each compartment. Data is represented as mean ± standard deviation from 10,000 Monte Carlo simulations, randomly seeded across the possible trafficking kinetics (Table 2). (H) Mean phosphorylated receptor localization relative to total cell receptors at 5, 60, 120, 180 and 240 minutes after ligand stimulus.
Figure S5. PDGFRα phosphorylation compartmentalization following ligand stimulus. The percent of phosphorylated PDGFRα relative to total cell PDGFRα are given on (A) the cell membrane, (B) endocytic vesicles, (C) early endosomes, (D) recycling endosomes, (E) late endosomes, (F) lysosomes, and (G) in the nucleus. The activation time constant ($\tau_a$, time from ligand stimulus to 63.2% max phosphorylation) and decay time constant ($\tau_d$, time from max phosphorylation to 36.8% max phosphorylation) in minutes are also given for each compartment. Data is represented as mean ± standard deviation from 10,000 Monte Carlo simulations, randomly seeded across the possible trafficking kinetics (Table 2). (H) Mean phosphorylated receptor localization relative to total cell receptors at 5, 60, 120, 180 and 240 minutes after ligand stimulus.
Figure S6. PDGFRβ phosphorylation compartmentalization following ligand stimulus. The percent of phosphorylated PDGFRβ relative to total cell PDGFRβ are given on (A) the cell membrane, (B) endocytic vesicles, (C) early endosomes, (D) recycling endosomes, (E) late endosomes, (F) lysosomes, and (G) in the nucleus. The activation time constant (τ_a, time from ligand stimulus to 63.2% max phosphorylation) and decay time constant (τ_d, time from max phosphorylation to 36.8% max phosphorylation) in minutes are also given for each compartment. Data is represented as mean ± standard deviation from 10,000 Monte Carlo simulations, randomly seeded across the possible trafficking kinetics (Table 2). (H) Mean phosphorylated receptor localization relative to total cell receptors at 5, 60, 120, 180 and 240 minutes after ligand stimulus.
**Figure S7. VEGFR1 phosphorylation compartmentalization following ligand stimulus.** The percent of phosphorylated VEGFR1 relative to total cell VEGFR1 are given on (A) the cell membrane, (B) endocytic vesicles, (C) early endosomes, (D) recycling endosomes, (E) late endosomes, (F) lysosomes, and (G) in the nucleus. The activation time constant ($\tau_a$, time from ligand stimulus to 63.2% max phosphorylation) and decay time constant ($\tau_d$, time from max phosphorylation to 36.8% max phosphorylation) in minutes are also given for each compartment. Data is represented as mean ± standard deviation from 10,000 Monte Carlo simulations, randomly seeded across the possible trafficking kinetics (Table 2). (H) Mean phosphorylated receptor localization relative to total cell receptors at 5, 60, 120, 180 and 240 minutes after ligand stimulus.
Figure S8. VEGFR2 phosphorylation compartmentalization following ligand stimulus. The percent of phosphorylated VEGFR2 relative to total cell VEGFR2 are given on (A) the cell membrane, (B) endocytic vesicles, (C) early endosomes, (D) recycling endosomes, (E) late endosomes, (F) lysosomes, and (G) in the nucleus. The activation time constant ($\tau_a$, time from ligand stimulus to 63.2% max phosphorylation) and decay time constant ($\tau_d$, time from max phosphorylation to 36.8% max phosphorylation) in minutes are also given for each compartment. Data is represented as mean ± standard deviation from 10,000 Monte Carlo simulations, randomly seeded across the possible trafficking kinetics (Table 2). (H) Mean phosphorylated receptor localization relative to total cell receptors at 5, 60, 120, 180 and 240 minutes after ligand stimulus.
Figure S9. EGFR signaling compartmentalization with altered parameters. The percent of total EGFR signaling associated with each compartment were quantified with altered EGF-EGFR parameters. Recycling endosome- and lysosome-based receptor signaling are not included as they account for < 0.01% total receptor signaling. The four parameters changed are (A) ligand-receptor off-rate, (B) ligand-receptor on-rate, (C) ligand concentration, and (D) receptor concentration. Data is represented as mean ± standard deviation from 10,000 Monte Carlo simulations, randomly seeded across the possible trafficking kinetics (Table 2).
Figure S10. FGFR1 signaling compartmentalization with altered parameters. The percent of total FGFR1 signaling associated with each compartment were quantified with altered FGF-FGFR1 parameters. Recycling endosome- and lysosome-based receptor signaling are not included as they account for < 0.01% total receptor signaling. The four parameters changed are (A) ligand-receptor off-rate, (B) ligand-receptor on-rate, (C) ligand concentration, and (D) receptor concentration. Data is represented as mean ± standard deviation from 10,000 Monte Carlo simulations, randomly seeded across the possible trafficking kinetics (Table 2).
Figure S11. IGFR1 signaling compartmentalization with altered parameters. The percent of total IGFR1 signaling associated with each compartment were quantified with altered IGF-IGFR1 parameters. Recycling endosome- and lysosome-based receptor signaling are not included as they account for < 0.01% total receptor signaling. The four parameters changed are (A) ligand-receptor off-rate, (B) ligand-receptor on-rate, (C) ligand concentration, and (D) receptor concentration. Data is represented as mean ± standard deviation from 10,000 Monte Carlo simulations, randomly seeded across the possible trafficking kinetics (Table 2).
Figure S12. PDGFRα signaling compartmentalization with altered parameters. The percent of total PDGFRα signaling associated with each compartment were quantified with altered PDGFB-PDGFRα parameters. Recycling endosome- and lysosome-based receptor signaling are not included as they account for < 0.01% total receptor signaling. The four parameters changed are (A) ligand-receptor off-rate, (B) ligand-receptor on-rate, (C) ligand concentration, and (D) receptor concentration. Data is represented as mean ± standard deviation from 10,000 Monte Carlo simulations, randomly seeded across the possible trafficking kinetics (Table 2).
Figure S13. PDGFRβ signaling compartmentalization with altered parameters. The percent of total PDGFRβ signaling associated with each compartment were quantified with altered PDGFAA-PDGFRβ parameters. Recycling endosome- and lysosome-based receptor signaling are not included as they account for < 0.01% total receptor signaling. The four parameters changed are (A) ligand-receptor off-rate, (B) ligand-receptor on-rate, (C) ligand concentration, and (D) receptor concentration. Data is represented as mean ± standard deviation from 10,000 Monte Carlo simulations, randomly seeded across the possible trafficking kinetics (Table 2).
Figure S14. VEGFR1 signaling compartmentalization with altered parameters. The percent of total VEGFR1 signaling associated with each compartment were quantified with altered VEGFA-VEGFR1 parameters. Recycling endosome- and lysosome-based receptor signaling are not included as they account for < 0.01% total receptor signaling. The four parameters changed are (A) ligand-receptor off-rate, (B) ligand-receptor on-rate, (C) ligand concentration, and (D) receptor concentration. Data is represented as mean ± standard deviation from 10,000 Monte Carlo simulations, randomly seeded across the possible trafficking kinetics (Table 2).
Figure S15. VEGFR2 signaling compartmentalization with altered parameters. The percent of total VEGFR2 signaling associated with each compartment were quantified with altered VEGFA-VEGFR2 parameters. Recycling endosome- and lysosome-based receptor signaling are not included as they account for < 0.01% total receptor signaling. The four parameters changed are (A) ligand-receptor off-rate, (B) ligand-receptor on-rate, (C) ligand concentration, and (D) receptor concentration. Data is represented as mean ± standard deviation from 10,000 Monte Carlo simulations, randomly seeded across the possible trafficking kinetics (Table 2).
Membrane Signaling

Figure S16. Correlation analysis between RTK parameters and membrane signaling. Membrane signaling among the eight RTKs was fit to the following RTK parameters: (A) receptor level, (B) extracellular ligand concentration, (C) ligand-receptor dissociation constant, and (D) complex level: defined as the product of extracellular ligand concentration and membrane receptor level divided by the ligand-receptor dissociation constant. The $R^2$ goodness of fit, using a lognormal fit assumption, is given for each RTK parameter.
Figure S17. Correlation analysis between RTK parameters and endocytic vesicle signaling. Endocytic vesicle signaling among the eight RTKs was fit to the following RTK parameters: (A) receptor level, (B) extracellular ligand concentration, (C) ligand-receptor dissociation constant, and (D) complex level: defined as the product of extracellular ligand concentration and membrane receptor level divided by the ligand-receptor dissociation constant. The $R^2$ goodness of fit, using a lognormal fit assumption, is given for each RTK parameter.
Early Endosome Signaling

Figure S18. Correlation analysis between RTK parameters and early endosome signaling. Early endosome signaling among the eight RTKs was fit to the following RTK parameters: (A) receptor level, (B) extracellular ligand concentration, (C) ligand-receptor dissociation constant, and (D) complex level: defined as the product of extracellular ligand concentration and membrane receptor level divided by the ligand-receptor dissociation constant. The $R^2$ goodness of fit, using a lognormal fit assumption, is given for each RTK parameter.
Figure S19. Correlation analysis between RTK parameters and late endosome signaling. Late endosome signaling among the eight RTKs was fit to the RTK parameters: (A) receptor level, (B) extracellular ligand concentration, (C) ligand-receptor dissociation constant, and (D) complex level: defined as the product of extracellular ligand concentration and membrane receptor level divided by the ligand-receptor dissociation constant. The $R^2$ goodness of fit, using a lognormal fit assumption, is given for each RTK parameter.