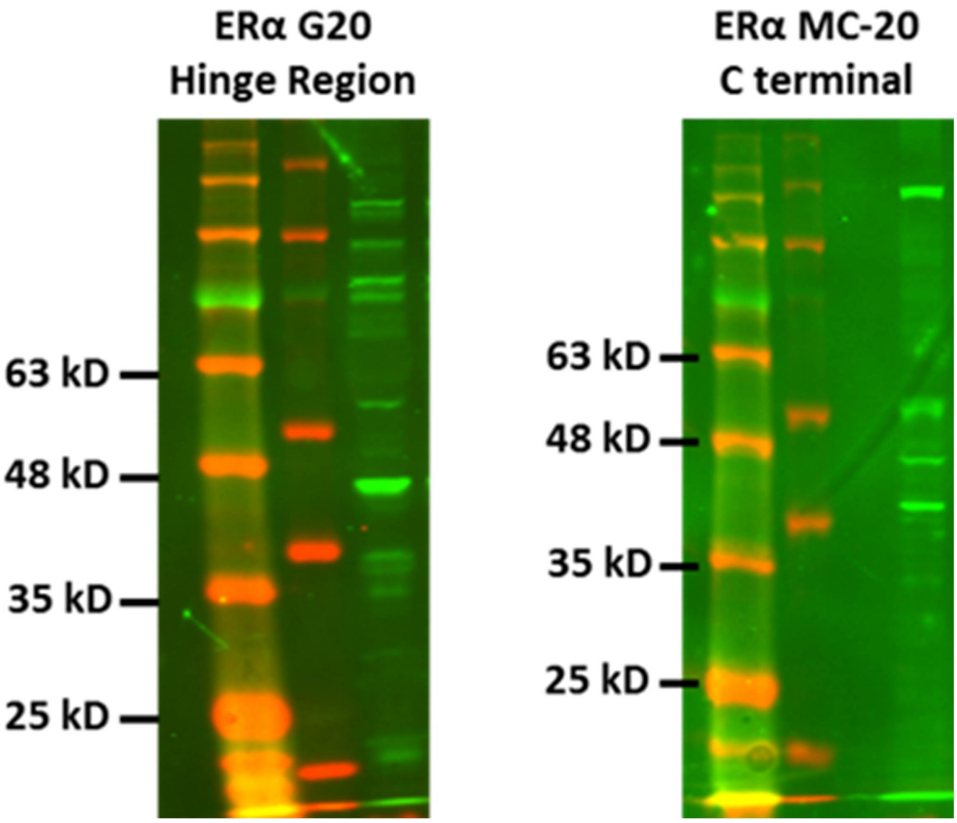

Supplemental Figure 1. Two commercially available ERα antibodies were used to perform western blots on cell lysate from Ishikawa cells. All antibodies show numerous nonspecific bands, precluding their use in assays utilizing immunofluorescent labeling. For each antibody, the targeted receptor epitope is listed.

PROPOSED MODEL FOR ESTROGEN-INITIATED SIGNALING IN THE HUMAN ENDOMETRIUM

We created a mathematical representation of the signaling diagram in Figure 3A assuming Michaelis-Menten binding and mass-action dynamics. We assume that a ligand binds ERα66, ERβ, ERα46, and GPER according to Michaelis-Menten kinetics. The K_m and V_{max} related to each of these receptors is specific to the chemical properties of the ligand. Here, we distinguish between the concentration of a receptor (e.g., [ERα66]), and the concentration of its activated form (e.g., [ERα66*]). We assume receptor concentrations [ERα66], [ERβ], and [ERα46] are constant ([ERα66] = [ERβ] = [ERα46] = 1).

ERα66 activity is modulated by ligand binding,

$$[\text{ER}\alpha66^*] = \frac{V_{max}^{\alpha66}[\text{ligand}][\text{ER}\alpha66]}{[\text{ligand}] + K_m^{\alpha66}}, \tag{1}$$

where $V_{max}^{\alpha66}$ and $K_m^{\alpha66}$ are the typical Michaelis-Menten constants (gain and the half-maximum activation concentration, respectively). Other receptors share similar activation kinetics:

$$[\text{ER}\beta^*] = \frac{V_{max}^{\beta}[\text{ligand}][\text{ER}\beta]}{[\text{ligand}] + K_m^{\beta}}, \tag{2}$$

$$[\text{ER}\alpha46^*] = \frac{V_{max}^{\alpha46}[\text{ligand}][\text{ER}\alpha46]}{[\text{ligand}] + K_m^{\alpha46}}, \text{ and} \tag{3}$$

$$[\text{GPER}^*] = \frac{V_{max}^{GPER}[\text{ligand}][\text{GPER}]}{[\text{ligand}] + K_m^{GPER}}. \tag{4}$$

The dynamics of the receptor activation, transcription, and proliferation are governed by a system of coupled ordinary differential equations. GPER concentrations are driven transcriptionally by ERα66 activation. This process is balanced by first-order turnover of the GPER protein,

$$\frac{d}{dt}[\text{GPER}] = k^{GPER}\left([\text{ER}\alpha66^*] - [\text{GPER}]\right). \tag{5}$$

Activation of ERα36 is dynamic and is modulated by [GPER*],

$$\frac{d}{dt}[\text{ER}\alpha36^*] = k^{\alpha36}\left([\text{GPER}^*] - [\text{ER}\alpha36^*]\right). \tag{6}$$

Transcription has a base rate (zero-order) that is modulated by first-order turnover. Transcription rate is positively impacted by ERα66, and negatively impacted by ERα36 and ERα46,

$$\frac{d}{dt}[\text{Transcription}] = k^{trans}\left(1 - [\text{Transcription}] + [\text{ER}\alpha66^*] - [\text{ER}\alpha46^*] - \phi_{\alpha36}^{trans}[\text{ER}\alpha36^*]\right). \tag{7}$$

Proliferation (P) depends on transcription, ERα36, and ERβ,

$$P = k^P\left([\text{ER}\alpha36^*] + [\text{Transcription}] - [\text{ER}\beta^*]\right). \tag{8}$$

The rate constants k^{GPER}, $k^{\alpha36}$, k^{trans}, and k^P are set at 1 for simplicity. $\phi_{\alpha36}^{trans}$ describes the relative contributions of ER$\alpha36^*$ acting (a) through the kinase cascade (positive) and (b) through transcription (negative). To avoid degenerate coupling of these two mechanisms, it is important that $\phi_{\alpha36}^{trans} \neq 1$. Here, we assume $\phi_{\alpha36}^{trans} = 0.1$.

Equations 5–8 were evaluated in python using the `scipy.integrate` library with initial conditions as defined in Supplemental Table I. Five different ligands—each with distinct estrogen receptor binding behavior—were simulated to generate Figure 3B. ODEs were evaluated to their steady-state solutions ($t = 2000$), which were used

for Figure 3B. Parameters defining these ligands were chosen to emulate the properties of known endocrine-active compounds Supplemental Table II.

Here, constant ligand concentrations were used and we have assumed no background level of receptor activation from endogenous estrogens. Neither of these assumptions are critical, and the model framework could be evaluated for more complicated inputs. For example, it may be appropriate to use a time-dependent (decaying) concentration of ligand for a system for which metabolism or clearance are known to be important. The effect of repeat dosing could be simulated by selecting an appropriate mathematical representation for [ligand]. Similarly, the impact of endogenous ligands on system dynamics could easily be considered.

When ligand concentration is constant (as is assumed in Fig. 3B), a steady-state solution to Eq. 8 can be determined analytically. The steady state value of GPER concentration, $[\text{GPER}]^{ss}$, follows from Eq. 5 as,

$$\begin{aligned} 0 &= k^{GPER}\left([\text{ER}\alpha 66^*] - [\text{GPER}]^{ss}\right) \\ [\text{GPER}]^{ss} &= [\text{ER}\alpha 66^*]\,. \end{aligned} \tag{9}$$

The steady-state concentration of activated GPER, $[\text{GPER}^*]^{ss}$, follows from Eq. 4,

$$[\text{GPER}^*]^{ss} = \frac{V_{max}^{GPER}[\text{ligand}][\text{ER}\alpha 66^*]}{[\text{ligand}] + K_m^{GPER}}\,. \tag{10}$$

Similarly, from Eq. 6,

$$\begin{aligned} 0 &= k^{\alpha 36}\left([\text{GPER}^*]^{ss} - [\text{ER}\alpha 36^*]^{ss}\right) \\ [\text{ER}\alpha 36^*]^{ss} &= [\text{GPER}^*]^{ss} \\ [\text{ER}\alpha 36^*]^{ss} &= \frac{V_{max}^{GPER}[\text{ligand}][\text{ER}\alpha 66^*]}{[\text{ligand}] + K_m^{GPER}}\,. \end{aligned} \tag{11}$$

And from Eq. 7,

$$\begin{aligned} 0 &= k^{trans}\left(1 - [\text{Transcription}]^{ss} + [\text{ER}\alpha 66^*] - [\text{ER}\alpha 46^*] - \phi_{\alpha 36}^{trans}[\text{ER}\alpha 36^*]^{ss}\right) \\ [\text{Transcription}]^{ss} &= 1 + [\text{ER}\alpha 66^*] - [\text{ER}\alpha 46^*] - \phi_{\alpha 36}^{trans}[\text{ER}\alpha 36^*]^{ss}\,. \end{aligned} \tag{12}$$

Proliferation at steady state becomes,

$$\begin{aligned} P^{ss} &= k^P\left(1 + (1 - \phi_{\alpha 36}^{trans})[\text{ER}\alpha 36^*]^{ss} + [\text{ER}\alpha 66^*] - [\text{ER}\alpha 46^*] - [\text{ER}\beta^*]\right) \\ &= k^P\left(1 + (1 - \phi_{\alpha 36}^{trans})\frac{V_{max}^{GPER}[\text{ligand}][\text{ER}\alpha 66^*]}{[\text{ligand}] + K_m^{GPER}} + [\text{ER}\alpha 66^*] - [\text{ER}\alpha 46^*] - [\text{ER}\beta^*]\right)\,, \end{aligned} \tag{13}$$

which depends on ligand concentration, but not time.

Supplemental Table I: Initial conditions for simulation in Figure 3B.

Variable	Initial State
[GPER]	1
[ERα36]	1
[Transcription]	1

Supplemental Table II: Parameter sets used for simulation in Figure 3B.

	ERα66		ERβ		ERα46		GPER	
	K_m	V_{max}	K_m	V_{max}	K_m	V_{max}	K_m	V_{max}
ERα66-selective	1.0	1.0	1.0	0.2	1.0	0.1	0.5	0.5
nonspecific	1.0	1.0	0.1	0.3	1.0	0.1	0.5	0.5
ERβ-selective	1.0	0.1	0.1	0.3	1.0	0.1	0.5	0.5
ERα46-selective	1.0	0.1	1.0	0.2	0.1	0.1	0.5	0.5
GPER-selective	1.0	1.0	1.0	0.2	1.0	0.1	0.01	0.5