Multidimensional characterization of parts enhances modeling accuracy in genetic circuits
SUPPLEMENTARY INFORMATION

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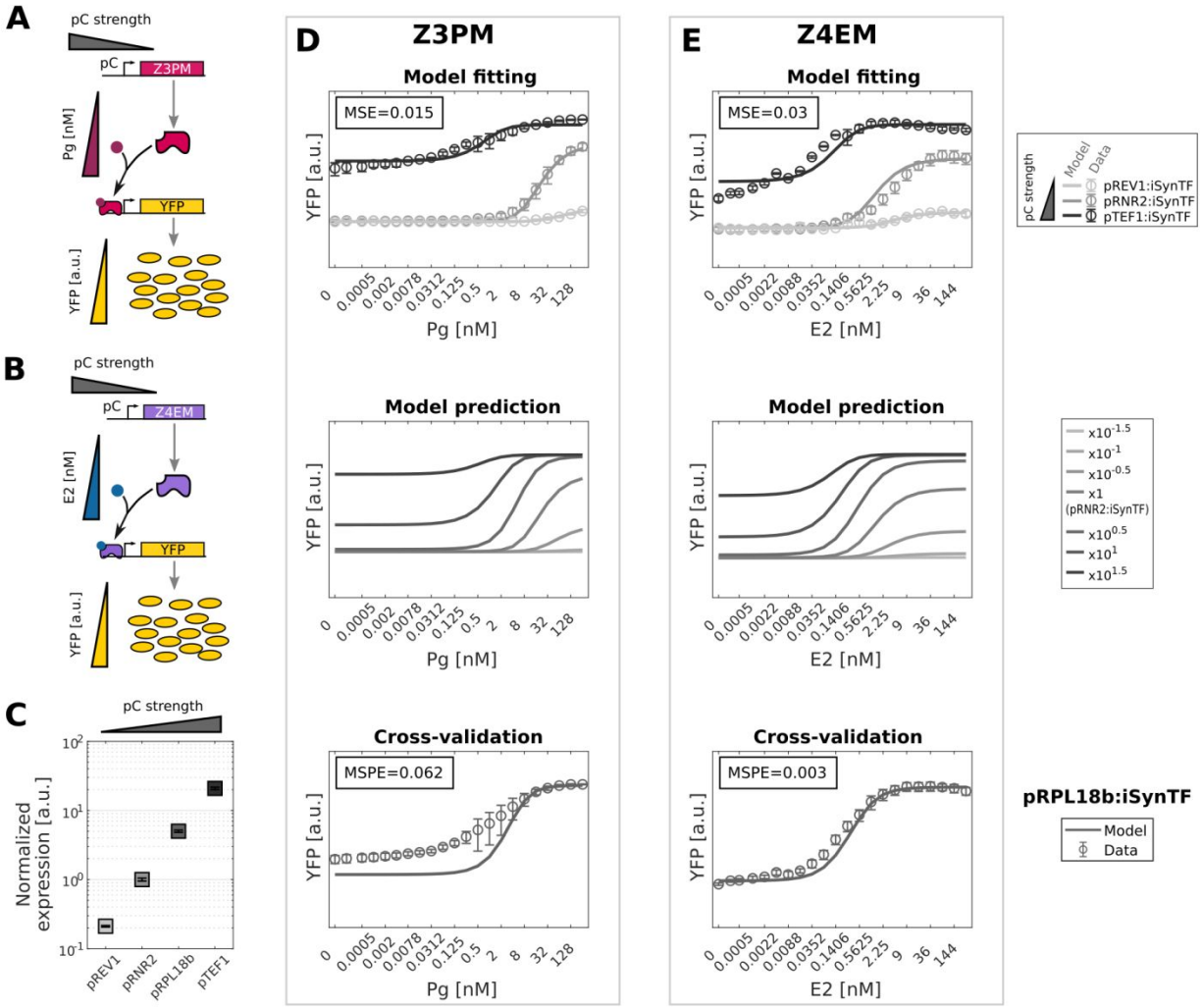
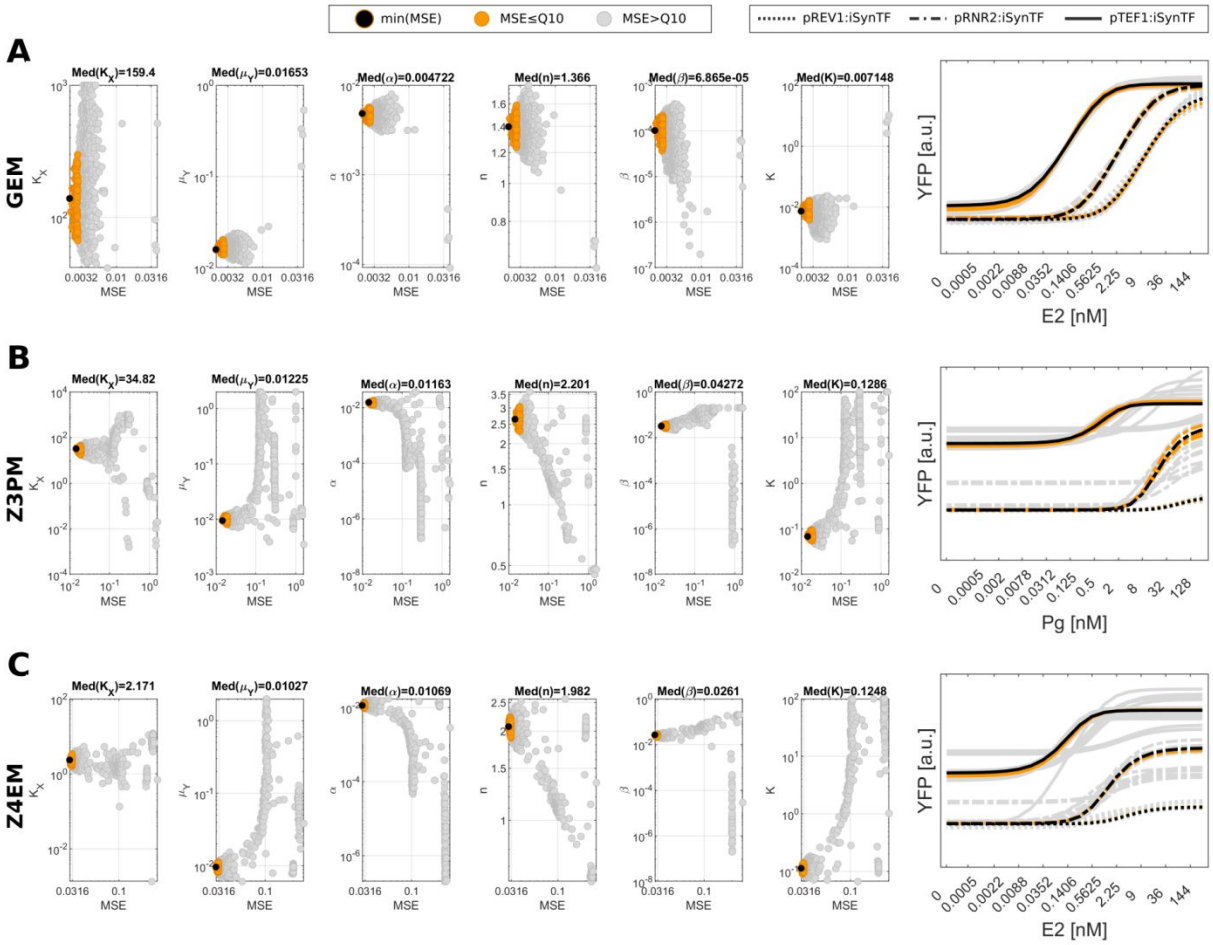
Supplemental Figures

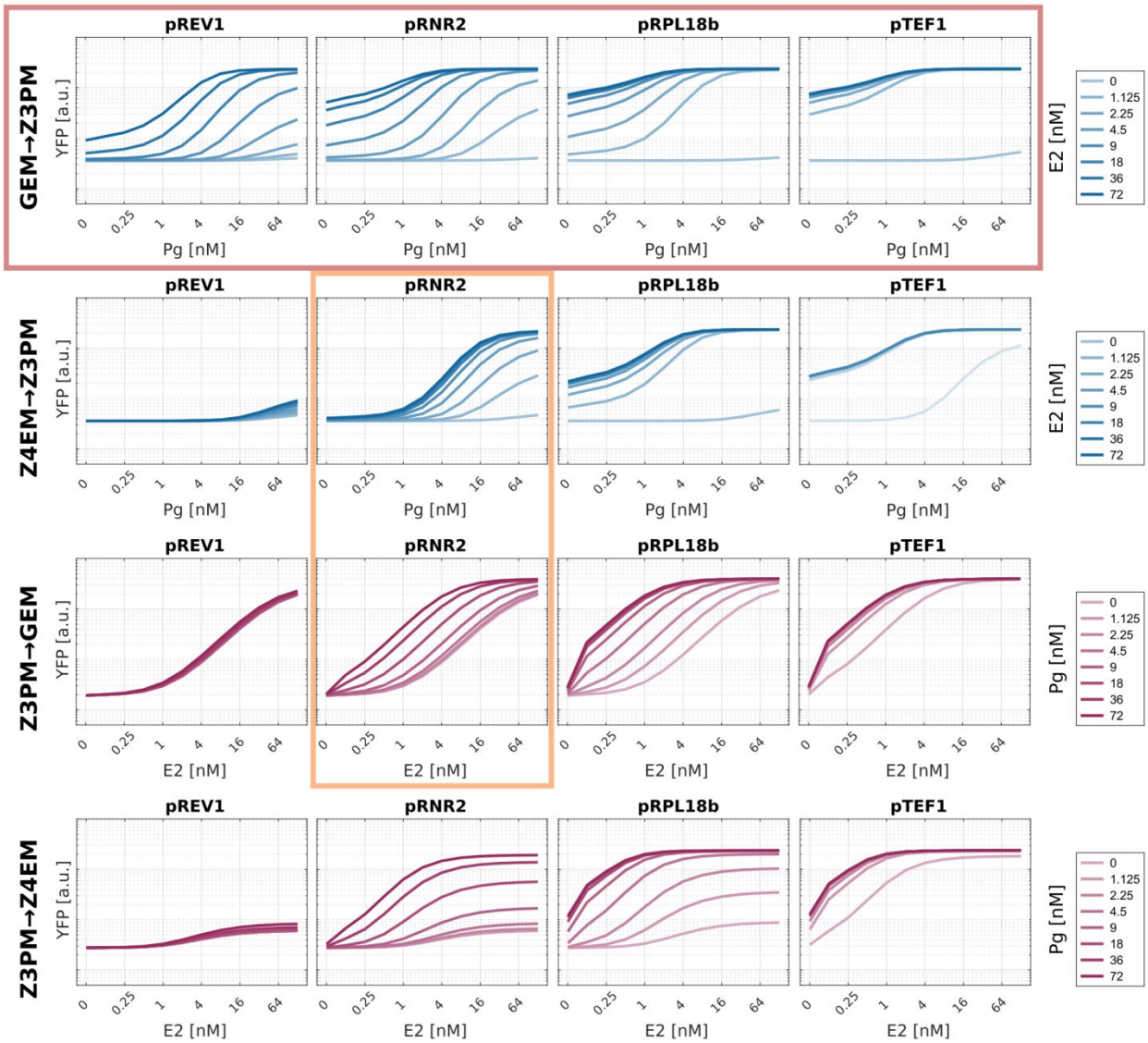


Supplementary Figure 1: Multidimensional characterization and expanded Hill model fit for the Z3PM and Z4EM iSynTFs. (A-B) A constitutively expressed (pC, constitutive promoter) inducible synthetic transcription factor (iSynTF; **(A)** Z3PM, **(B)** Z4EM) is bound by its hormone inducer (**(A)** progesterone, Pg, **(B)** estradiol, E2) and activates transcription of a downstream YFP reporter. **(C)** Measurement of constitutive promoter expression levels using a pC:YFP fusion, where pC represents one of pREV1, pRNR2, pRPL18B, or pTEF1. **(D-E)** Top, Inducer dose response of **(D)** Z3PM and **(E)** Z4EM at three expression levels (pRNR2, pRNR2, and pTEF1 constitutive promoters). The expanded Hill model (described in Fig. 2A) was fit to the observed data (Mean Squared Error, MSE, is shown for each case as an inset box). Middle, Expanded Hill model prediction of inducer dose responses for different expression levels of **(D)** Z3PM and **(E)** Z4EM (see legend for fold-change values). Bottom, Comparison of model prediction and experimental data for **(D)** pRPL18B:Z3PM and **(E)** pRPL18B:Z4EM inducer dose response as cross-validation (Mean Squared Prediction Error, MSPE, is shown for each case as an inset box). Solid lines represent model predictions, open circles and filled squares represent experimental

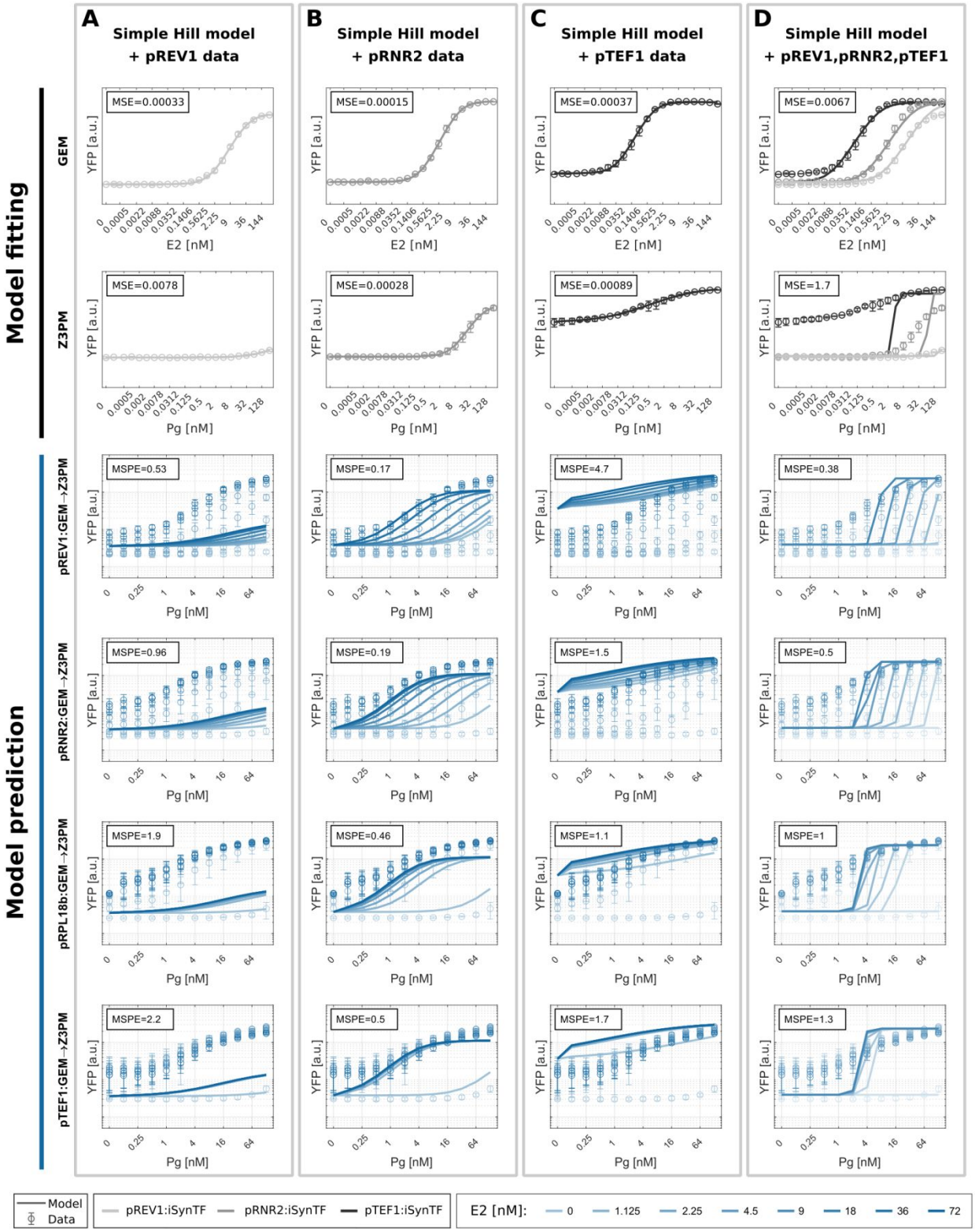
mean, and error bars represent s.d. of three biological replicates. See Supplementary Table 4 for used parameter values.



Supplementary Figure 2: Results of parameter fitting of the expanded Hill model to iSynTF dose-response data. The top parameter sets in terms of their fitting error (lowest Mean Squared Error, MSE) are shown for 1000 fitting chains (see Methods) for **(A)** GEM (Fig. 2A), **(B)** Z3PM (Fig. S1D), and **(C)** Z4EM (Fig. S1E)**.** The plot titles show the median value of each fitted parameter**.** The parameter sets in quantile ten (Q10, lowest 10% MSE of the 1000 chains) are shown in orange, and the case with the lowest observed error (min(MSE)) in black, while all other cases in gray (see legend). The expanded Hill model predictions for 20 random examples and the best case (i.e. lowest MSE) of the 1000 fitting chains are shown at the right (see legend on the top), colored according to their MSE value (min(MSE), black; MSE≤Q10, orange; MSE>Q10, gray).

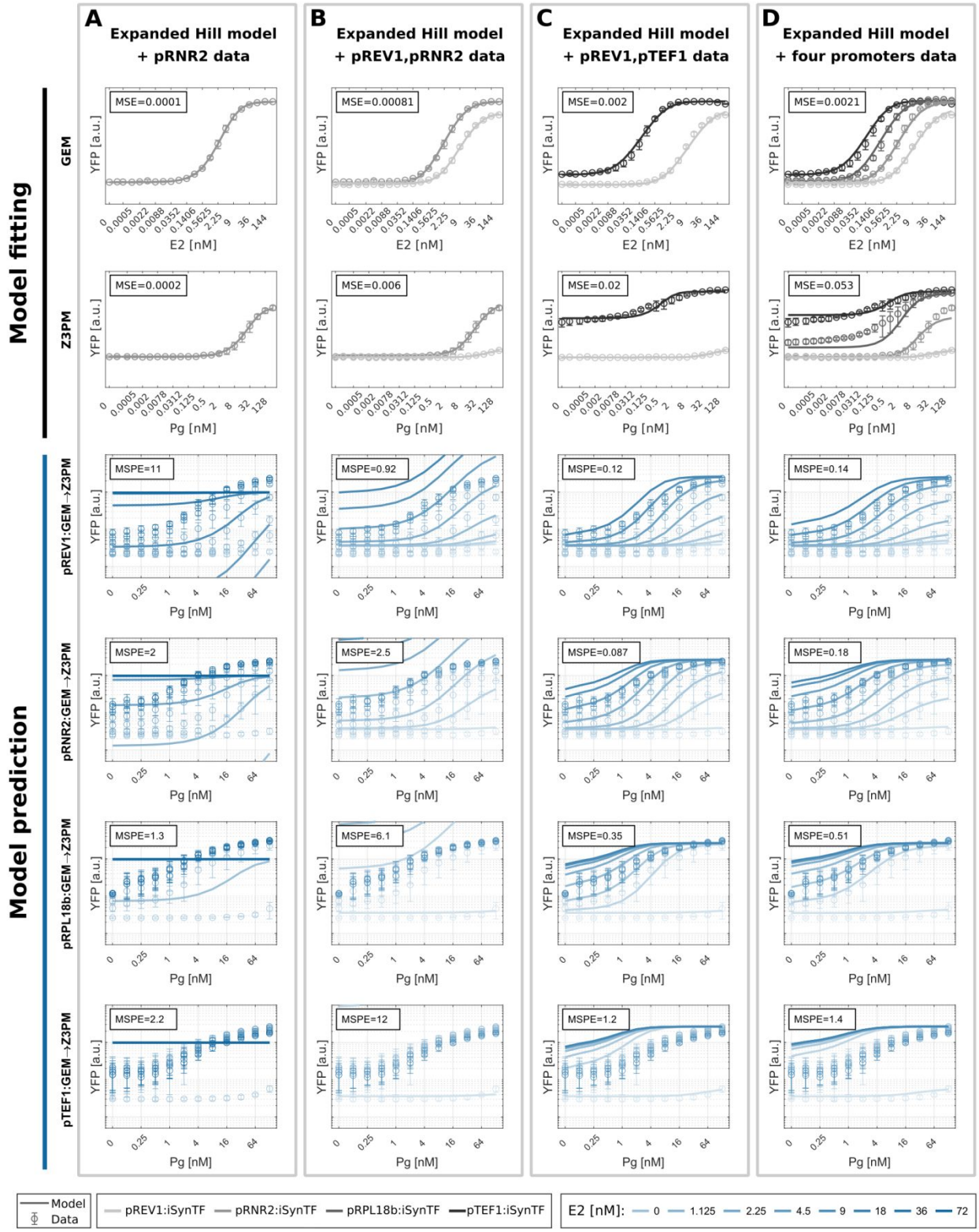


Supplementary Figure 3: Using refined models to explore circuit designs. For the proposed circuit in Fig. 3A, expanded Hill model predictions for 2nd TF inducer dose responses (x-axis) for four possible arrangements varying the chosen iSynTFs (see row titles), at four different expression levels of the 1st TF expression level (see column titles), and 1st TF inducer concentrations (see legend). We experimentally verified six of these predictions, highlighted by the red (Fig.3B) and orange (Fig.3C-D) boxes. See Supplementary Table 4 for used parameter values.



Supplementary Figure 4: Simple Hill model fails to predict circuit behavior when fit either to diverse single hormone dose response data or three hormone dose response data. We compare the *simple Hill model* (see Fig. 1A and Methods) fit either using **(A-C)** only the inducer dose response data for GEM and Z3PM expressed from one promoter (**(A)** pREV1, **(B)** pRNR2,

(C) pTEF1), or **(D)** the inducer dose response data for GEM and Z3PM expressed from three promoters (pREV1, pRNR2, pTEF1). The top plots show each model fit to the respective data (Mean Squared Error, MSE, as inset box), and bottom plots show the predicted response (lines) compared to the observed (experimental) response (circles) for the pC:GEM→Z3PM cascade circuit (Fig. 3A) to changes in progesterone concentration (Pg, x-axis) as the constitutive promoter strength (see plot titles) and estradiol concentration (E2, legend) vary. See Supplementary Table 4 for used parameter values.



Supplementary Figure 5: Expanded Hill model needs to be fit to multiple hormone dose data capturing the range of response to predict circuit behavior. We compare the *expanded Hill model* (see Fig. 2A and Methods) fit either using **(A)** only the inducer dose response data for GEM and Z3PM expressed from one promoter (pRNR2), **(B-C)** the inducer

dose response data for GEM and Z3PM expressed from two promoters (**(B)** pREV1, pRNR2, **(C)** pREV1, pTEF1), or **(D)** the inducer dose response data for GEM and Z3PM expressed from four promoters (pREV1, pRNR2, pRPL18b, pTEF1). The top plots show each model fit to the respective data (Mean Squared Error, MSE, as inset box), and bottom plots show the predicted response (lines) compared to the observed (experimental) response (circles) for the pC:GEM→Z3PM cascade circuit (Fig. 3A) to changes in progesterone concentration (Pg, x-axis) as the constitutive promoter strength (see plot titles) and estradiol concentration (E2, legend) vary. See Supplementary Table 4 for used parameter values.

Supplementary Table 1 - Integration plasmids used in this study (all KanR)

Name	Content	Yeast Auxotrophic Marker
pAN144	pTEF1-GEM-tADH1	ura3
pAN145	pRPL18b-GEM-tADH1	ura3
pAN146	pRNR2-GEM-tADH1	ura3
pAN147	pREV1-GEM-tADH1	ura3
pAN160	pGal1-Venus-tPGK1	leu2
pAN248	pTEF1-Z4EM-tADH1	ura3
pAN249	pRPL18b-Z4EM-tADH1	ura3
pAN250	pRNR2-Z4EM-tADH1	ura3
pAN251	pREV1-Z4EM-tADH1	ura3
pAN360	pTEF1-Venus-tPGK1	leu2
pAN361	pRPL18b-Venus-tPGK1	leu2
pAN362	pRNR2-Venus-tPGK1	leu2
pAN363	pREV1-Venus-tPGK1	leu2
pGD240	pZ4 (-Gal4 site)-Venus-tPGK1	leu2
pGD470	pTEF1-GEM-tADH1 -- pGal1-Z3PM(fixed)-tPGK1	ura3
pGD471	pRPL18b-GEM-tADH1 -- pGal1-Z3PM(fixed)-tPGK1	ura3
pGD472	pRNR2-GEM-tADH1 -- pGal1-Z3PM(fixed)-tPGK1	ura3
pGD473	pREV1-GEM-tADH1 -- pGal1-Z3PM(fixed)-tPGK1	ura3
pGD484	pZ3-Venus-tSSA1	leu2
pGD485	pZ3-Venus-tSSA1 -- pGal1-mScarlet-linker-tENO1	leu2
pGD488	pTEF1-Z3PM(fixed)-tPGK1	ura3
pGD489	pRPL18b-Z3PM(fixed)-tPGK1	ura3
pGD490	pRNR2-Z3PM(fixed)-tPGK1	ura3
pGD491	pREV1-Z3PM(fixed)-tPGK1	ura3
pGD532	pGal1-Venus-tPGK1 -- pZ3-mScarlet-Linker-tENO1	leu2
pGD533	pRNR2-Z3PM(fixed)-tPGK1 -- pZ3-GEM-tADH1	ura3
pGD898	pRNR2-Z4EM-tADH1 -- pZ4 (-Gal4 site)-Z3PM(fixed)-tPGK1	ura3

Supplementary Table 2 - Oligos used in this study

Name	Sequence	Description
oAN019	ACGCGGCCTTTTTACGGTTC	Dueber GG sequencing F
oAN074	CTCCTGTTGATAGATCCAGT	Dueber GG sequencing R
g01	GCATCGTCTCATCGGTCTCATATGAAGCTACTGTCT TCTATCGAACAAGCATGCGATATTTGCCGACTTAAA AAGCTCAAGTGCTCCAAAGAAAAACCGAAGTGCGCC AAGTGTCTGAAGAACAACTGGGAGTGTCGCTACTCT CCCAAAACCAAAAGaTCTCCGCTGACTAGGGCACAT CTGACAGAAGTGGAATCAAGGCTAGAAAGACTGGAA CAGCTATTTCTACTGATTTTTCCTCGAGAAGACCTT GACATGATTTTGAAAATGGATTCTTTACAGGATATA AAAGCATTGTTAACAGGATTATTTGTACAAGATAAT GTGAATAAAGATGCCGTCACAGATAGATTGGCTTCA GTGGAGACTGATATGCCTCTAACATTGAGACAGCAT AGAATAAGTGCGACATCATCATCGGAAGAGAGTAGT AACAAAGGTCAAAGACAGTTGACTGTATCGATTGAC TCGGCAGGATCCGGTGACGGTGCTGGTTTAATTAAC tctgctggagacatgagagctgccaacctttggcca agcccgctcatgatcaaacgctctaagaagaacagc ctggccttgtccctgacggccgaccagatggtcagt gccttgttggatgctgagcccccatactctattcc gagtatgatcctaccagacccttcagtgaagcttcg atgatgggcttactgaccaacctggcagacagggag ctggttcacatgatcaactgggcgaagagggtgcca ggctttgtggatttgaccctccatgatcaggtccac cttctagaatgtgcctggctaTGAGACGGCAT	GG3_GEM_gBlock_1

g02	GCATCGTCTCAgctagagatcctgatgattggActc gtctggcgctccatggagcacccagggaagctactg tttgctcctaacttgctcttggacaggaaccaggga aaatgtgtagagggcatggtggagatcttcgacatg ctgctggctacatcatctcggttccgcatgatgaat ctgcagggagaggagtttgtgtgcctcaaatctatt attttgcttaattctggagtgtacacatttctgtcc agcaccctgaagtctctggaagagaaggaccatatc caccgagtcctggacaagatcacagacactttgatc cacctgatggccaaggcaggcctgaccctgcaGCAG CAGCACCAGCGGCTGGCCCAGCTCCTCCTCATCCTC TCCCACATCAGGCACATGAGTAACAAAGGCATGGAG CATCTGTACAGCATGAAGTGCAAGAACGTGGTGCCC CTCTATGACCTGCTGCTGGAGATGCTGGACGCCCAC CGCCTACATGCGCCCACTAGCCGTGGAGGGGCATCC GTGGAGGAaACGGACCAAAGCCACTTGGCCACTGCG GGCTCTACTTCATCGCATTCCTTGCAAAAGTATTAC ATCACGGGGGAGGCAGAGGGTTTCCCTGCCACAGTC GCGGCtGCAGGTGACGGTGCTGGTTTAATTAACATG ACGGTCGACCATGATTTCAATAGCGAAGATATTTTA TTCCCCATAGAAAGCATGAGTAGTATACAATACGTG GAGAATAATAACCCAAATAATATTAACAACGATGTT ATCCCGTATTCTCTAGATATCAATGAGACGGCAT	GG3_GEM_gBlock_2
g03	GCATCGTCTCATCAAAAACACTGTCTTAGATAGTGC GGATCTCAATGACATTCAAAATCAAGAAACTTCACT GAATTTGGGGCTTCCTCCACTATCTTTCGACTCTCC ACTGCCCGTAACGGAAACGATACCATCCACTACCGA TAACAGCTTGCATTTGAAAGCTGATAGCAACAAAAA TCGCGATGCAAGAACTATTGAAAATGATAGTGAAAT TAAGAGTACTAATAATGCTAGTGGCTCTGGGGCAAA TCAATACACAACTCTTACTTCACCTTATCCTATGAA CGACATTTTGTACAACATGAACAATCCGTTACAATC ACCGTCACCTTCATCGGTACCTCAAAATCCGACTAT AAATCCTCCCATAAATACAGCAAGTAACGAAACTAA TTTATCGCCTCAAACTTCAAATGGTAATGAAACTCT TATATCTCCTCGAGCCCAACAACATACGTCCATTAA AGATAATCGTCTGTCCTTACCTAATGGTGCTAATTC GAATCTTTTCATTGACACTAACCCAAACAATTTGAA CGAAAAACTAAGAAATCAATTGAACTCAGATACAAA TTCATATTCTAACTCCATTTCTAATTCAAACTCCAA TTCTACGGGTAATTTAAATTCCAGTTATTTTAATTC ACTGAACATAGACTCCATGCTAGATGATTACGTTTC TAGTGATCTCTTATTGAATGATGATGATGATGACAC TAATTTATCACGCCGAAGATTTAGCGACGTTATAAC AAACCAATTTCCGTCAATGACAAATTCGAGGAATGA GCTCGGATCCTGAGACCTGAGACGGCAT	GG3_GEM_gBlock_3
oAN030	GCATCGTCTCATCGGTCTCATATGGGTACCCGCCCA TATG	GG3_Z3PM/Z4EM_PCR_1_F

oAN145	ATGCCGTCTCACTGCAGCCGCCTTTTTATGAAAGAG	GG3_Z3PM_PCR_1_R
oAN146	GCATCGTCTCAGCAGGTGACGGTGCTGGTTTAAT	GG3_Z3PM_PCR_2_F
oAN147	ATGCCGTCTCAGGTCTCAGGATCCGAGCTCTTGGTTTGTTATAACG	GG3_Z3PM(fixed)_PCR_2_R
oAN027	ATGCCGTCTCACAATCATCAGGATCTCTAGCCAG	GG3_Z4EM_PCR_1_R
oAN028	GCATCGTCTCAATTGGACTCGTCTGGCGCTCC	GG3_Z4EM_PCR_2_F
oAN031	ATGCCGTCTCAGGTCTCAGGATCCGAGCTCATTCCTCGAATTTG	GG3_Z4EM_PCR_2_R
oAN022	GCATCGTCTCATCGGTCTCAAACGTTATATTGAATTTTCAAAAATTCTTACTTTTTTTTT	GG2_pZ3/4_PCR_1_F
oAN025	ATGCCGTCTCAGGTCTCACATAGATCTTATAGTTTTTTCTCCTTGACGTTAAAG	GG2_pZ3_PCR_1_R
oGD122	ATGCCGTCTCAGCAattTCTAGACTCCTCCGCC	GG2_pZ4_(-Gal4_site)_PCR_1_R
oGD123	GCATCGTCTCAtTGCGTCCTCGTCTTCACCG	GG2_pZ4_(-Gal4_site)_PCR_2_F
oGD124	ATGCCGTCTCAGGTCTCACATAGATCTGATCTTATAGTTTTTTCTCCTTGAC	GG2_pZ4_(-Gal4_site)_PCR_2_R

Supplementary Table 3 - Yeast strains used in this study

Strain	Genotype	Background Strain	Plasmid(s)
yWCD230	BY4741, HIS3 repaired (MATa, his3Δ1, leu2Δ0, met15Δ0, ura3Δ0)		
yAHN061	pGal1-Venus-tPGK1::leu2; BY4741, HIS3 repaired	yWCD230	pAN160
yAHN066	pTEF1-GEM-tADH1::ura3; pGal1-Venus-tPGK1::leu2; BY4741, HIS3 repaired	yAHN061	
yAHN067	pRPL18B-GEM-tADH1::ura3; pGal1-Venus-tPGK1::leu2; BY4741, HIS3 repaired	yAHN061	
yAHN068	pRNR2-GEM-tADH1::ura3; pGal1-Venus-tPGK1::leu2; BY4741, HIS3 repaired	yAHN061	
yAHN069	pREV1-GEM-tADH1::ura3; pGal1-Venus-tPGK1::leu2; BY4741, HIS3 repaired	yAHN061	
yGD074*	pTEF1-mTagBFP2-tENO2::HO, HIS3; pTEF1-mScarlet-tADH1::ura3; pTEF1-Venus-tPGK1::leu2; BY4741, HIS3 repaired	yWCD230	pGD151; pAN548; pAN360
yGD075*	pRPL18b-mTagBFP2-tENO2::HO, HIS3; pRPL18b-mScarlet-tADH1::ura3; pRPL18b-Venus-tPGK1::leu2; BY4741, HIS3 repaired	yWCD230	pGD152; pAN434; pAN361
yGD076*	pRNR2-mTagBFP2-tENO2::HO, HIS3; pRNR2-mScarlet-tADH1::ura3; pRNR2-Venus-tPGK1::leu2; BY4741, HIS3 repaired	yWCD230	pGD153; pAN549; pAN362
yGD077*	pREV1-mTagBFP2-tENO2::HO, HIS3; pREV1-mScarlet-tADH1::ura3; pREV1-Venus-tPGK1::leu2; BY4741, HIS3 repaired	yWCD230	pGD154; pAN550; pAN363
yGD175	pZ4 (-Gal4 site)-Venus-tPGK1::leu2; BY4741, HIS3 repaired	yWCD230	pGD240
yGD177	pTEF1-Z4EM-tADH1::ura3; pZ4 (-Gal4 site)-Venus-tPGK1::leu2; BY4741, HIS3 repaired	yGD175	pAN248
yGD178	pRPL18B-Z4EM-tADH1::ura3; pZ4 (-Gal4 site)-Venus-tPGK1::leu2; BY4741, HIS3 repaired	yGD175	pAN249
yGD179	pRNR2-Z4EM-tADH1::ura3; pZ4 (-Gal4 site)-Venus-tPGK1::leu2; BY4741, HIS3 repaired	yGD175	pAN250
yGD180	pREV1-Z4EM-tADH1::ura3; pZ4 (-Gal4 site)-Venus-tPGK1::leu2; BY4741, HIS3 repaired	yGD175	pAN251
yGD307	pZ3-Venus-tSSA1::leu2; BY4741, HIS3 repaired	yWCD230	pGD484
yGD308	pZ3-Venus-tSSA1 -- pGal1-mScarlet-Linker-tENO1::leu2; BY4741, HIS3 repaired	yWCD230	pGD485

yGD310	pTEF1-Z3PM(fixed)-tPGK1::ura3; pZ3-Venus-tSSA1::leu2; BY4741, HIS3 repaired	yGD307	pGD488
yGD311	pRPL18B-Z3PM(fixed)-tPGK1::ura3; pZ3-Venus-tSSA1::leu2; BY4741, HIS3 repaired	yGD307	pGD489
yGD312	pRNR2-Z3PM(fixed)-tPGK1::ura3; pZ3-Venus-tSSA1::leu2; BY4741, HIS3 repaired	yGD307	pGD490
yGD313	pREV1-Z3PM(fixed)-tPGK1::ura3; pZ3-Venus-tSSA1::leu2; BY4741, HIS3 repaired	yGD307	pGD491
yGD325	pTEF1-GEM-tADH1 -- pGal1-Z3PM(fixed)-tPGK1::ura3; pZ3-Venus-tSSA1 -- pGal1-mScarlet-Linker-tENO1::leu2; BY4741, HIS3 repaired	yGD308	pGD470
yGD326	pRPL18B-GEM-tADH1 -- pGal1-Z3PM(fixed)-tPGK1::ura3; pZ3-Venus-tSSA1 -- pGal1-mScarlet-Linker-tENO1::leu2; BY4741, HIS3 repaired	yGD308	pGD471
yGD327	pRNR2-GEM-tADH1 -- pGal1-Z3PM(fixed)-tPGK1::ura3; pZ3-Venus-tSSA1 -- pGal1-mScarlet-Linker-tENO1::leu2; BY4741, HIS3 repaired	yGD308	pGD472
yGD328	pREV1-GEM-tADH1 -- pGal1-Z3PM(fixed)-tPGK1::ura3; pZ3-Venus-tSSA1 -- pGal1-mScarlet-Linker-tENO1::leu2; BY4741, HIS3 repaired	yGD308	pGD473
yGD354	pGal1-Venus-tENO2 -- pZ3-mScarlet-Linker-tENO1::leu2; BY4741, HIS3 repaired	yWCD230	pGD532
yGD356	pRNR2-Z3PM(fixed)-tPGK1 -- pZ3-GEM-tADH1::ura3; pGal1-Venus-tENO2 -- pZ3-mScarlet-Linker-tENO1::leu2; BY4741, HIS3 repaired	yGD354	pGD533
yGD588	pRNR2-Z4EM-tADH1 -- pZ4 (-Gal4 site)-Z3PM(fixed)-tPGK1::ura3; pZ3-Venus-tSSA1::leu2; BY4741, HIS3 repaired	yGD307	pGD898

* Plasmids represent BFP, RFP, and YFP cassettes. Each was integrated separately in the reverse order. Only the YFP plasmid is listed in Supplementary Table 1 as it was the only measurement taken.

Supplementary Table 4 - Model Parameter Values

Figures	Model	Part	Parameter values
1	Simple Hill	GEM	μ_Y = 0.0167 nM/min; α = 0.00513; n = 1.53; K = 0.828 nM; γ = 0.01 min^{-1}
2, 3, S3	Expanded	GEM	K_X = 139 nM; β = 0.000102; n = 1.4; K = 0.00731 nM; μ_Y = 0.0159 nM/min, α = 0.00483; γ = 0.01 min^{-1}
3, S1, S3	Expanded	Z3PM	K_X = 32.5 nM; β = 0.0317; n = 2.64; K = 0.0683 nM; μ_Y = 0.00944 nM/min; α = 0.0153; γ = 0.01 min^{-1}
3, S1, S3	Expanded	Z4EM	K_X = 2.35 nM; β = 0.0263; n = 2.07; K = 0.113 nM; μ_Y = 0.00966 nM/min; α = 0.0115; γ = 0.01 min^{-1}
S4	Simple Hill + pREV1	GEM	μ_Y = 0.00741 nM/min; α = 0.00996; n = 1.44; K = 0.52 nM; γ = 0.01 min^{-1}
S4	Simple Hill + pREV1	Z3PM	μ_Y = 0.000717 nM/min; α = 0.2; n = 0.753; K = 32.1 nM; γ = 0.01 min^{-1}
S4	Simple Hill + pRNR2	GEM	μ_Y = 0.0167 nM/min; α = 0.00513; n = 1.53; K = 0.828 nM; γ = 0.01 min^{-1}
S4	Simple Hill + pRNR2	Z3PM	μ_Y = 0.00448 nM/min; α = 0.0339; n = 1.4; K = 4.8 nM; γ = 0.01 min^{-1}
S4	Simple Hill + pTEF1	GEM	μ_Y = 0.0158 nM/min; α = 0.00954; n = 1.5; K = 1.11 nM; γ = 0.01 min^{-1}
S4	Simple Hill + pTEF1	Z3PM	μ_Y = 0.0141 nM/min; α = 0.103; n = 0.481; K = 7.31 nM; γ = 0.01 min^{-1}
S4	Simple Hill + 3 promoters	GEM	μ_Y = 0.0155 nM/min; α = 0.00546; n = 1.26; K = 1.29 nM; γ = 0.01 min^{-1}
S4	Simple Hill + 3 promoters	Z3PM	μ_Y = 0.00935 nM/min; α = 0.0169; n = 10; K = 5.24 nM; γ = 0.01 min^{-1}
S5	Expanded + pRNR2	GEM	K_X = 186 nM; β = 0.00241; n = 1.84; K = 0.00384 nM; μ_Y = 0.0162 nM/min, α = 0.00275; γ = 0.01 min^{-1}

S5	**Expanded + pRNR2**	**Z3PM**	**K_X = 232 nM; β = 0.0959; n = 3.03; K = 0.0169 nM; μ_Y = 0.00394 nM/min; α = 2.65 × 10^{-7}; γ = 0.01 min^{-1}**
S5	**Expanded + pREV1,pRNR2**	**GEM**	**K_X = 58.2 nM; β = 3.87 × 10^{-7} ; n = 1.54; K = 0.0136 nM; μ_Y = 0.0182 nM/min, α = 0.0043; γ = 0.01 min^{-1}**
S5	**Expanded + pREV1,pRNR2**	**Z3PM**	**K_X = 44.8 nM; β = 0.104; n = 2.49; K = 0.367 nM; μ_Y = 0.499 nM/min; α = 2.89 × 10^{-6}; γ = 0.01 min^{-1}**
S5	**Expanded + pREV1,pTEF1**	**GEM**	**K_X = 171 nM; β = 8.63 × 10^{-5} ; n = 1.28; K = 0.00817 nM; μ_Y = 0.0166 nM/min, α = 0.00423; γ = 0.01 min^{-1}**
S5	**Expanded + pREV1,pTEF1**	**Z3PM**	**K_X = 31 nM; β = 0.0312; n = 2.6; K = 0.074 nM; μ_Y = 0.0106 nM/min; α = 0.0135; γ = 0.01 min^{-1}**
S5	**Expanded + 4 promoters**	**GEM**	**K_X = 114 nM; β = 0.000126; n = 1.43; K = 0.00894 nM; μ_Y = 0.0171 nM/min, α = 0.00471; γ = 0.01 min^{-1}**
S5	**Expanded + 4 promoters**	**Z3PM**	**K_X = 17.9 nM; β = 0.0511; n = 2.1; K = 0.124 nM; μ_Y = 0.0107 nM/min; α = 0.0132; γ = 0.01 min^{-1}**

*** Note:** We assume the measured fluorescence arbitrary units ([a.u.]) are proportional to the molecule concentration ([nM]), with 1 a.u. = 0.4 nM. Then, for each constitutive promoter the measured expression (before normalization on Fig. 1C, 2C, & S1C) is: (1) 0.0128 nM for pREV1; (2) 0.0607 nM for pRNR2; (3) 0.3042 nM for pRPL18b; and (4) 1.2669 nM for pTEF1.
**** Note:** The found parameter values are in similar range of values as previously reported for yeast kinetic models; e.g. Chen *et al.* (2000; *Molecular biology of the cell*) and Kofahl & Klipp (2004; *Yeast*): regulated (first-order) synthesis rate, [0.02,0.1] min^{-1}, dilution (first-order) rate, [0.01,0.24] min^{-1} , Michaelis-Menten constants, [0.01,100] nM.

pZ4 (-Gal4 site) sequence

TTATATTGAATTTTCAAAAATTCTTACTTTTTTTTTGGATGGACGCAAAGAAGTTTAATAATCA
TATTACATGGCATTACCA***CCATATACATAT***CCATATACATATCCATATCTAATCTTACTTATAT
GTTGTGGAAATGTAAAGAGCCCCATTATCTTAGCCTAAAAAAACCTTCTCTTTGGAACTTTCAG
TAATACGCTTAACTGCTCATTGCTATATTGAAGTGTGGCC**GCGGCGGAGGAG**T**GCGGCGGAGGA**
GGA**GCGGCGGAGGAG**T**GCGGCGGAGGAG**GA**GCGGCGGAGGAG**T**GCGGCGGAGGAG**TCTAGA**<u>AAT</u>**
TGCGTCCTCGTCTTCACCGGTCGCGTTCCTGAAACGCAGATGTGCCTAATGCCGCACTGCTCCG
AACAATAAAGATTCTACAATACTAGCTTTTATGGTTATGAAGAGGAAAAATTGGCAGTAACCTG
GCCCCACAAACCTTCAAATTAACGAATCAAATTAACAACCATAGGATGATAATGCGATTAGTTT
TTTAGCCTTATTTCTGGGGTAATTAATCAGCGAAGCGATGATTTTTGATCTATTAACAGATATA
TAAATGGAAAAGCTGCATAACCACTTTAACTAATACTTTCAACATTTTCAGTTTGTATTACTTC
TTATTCAAATGTCATAAAAGTATCAACAAAAAATTGTTAATATACCTCTATACTTTAACGTCAA
GGAGAAAAAACTATA***AGATC***

Bold: Z4 operator sequence
<u>**Bold and underline**</u>: `CCG` mutated to `AAT` to break Gal4 consensus site
Bold and Italicized: insertions from cloning. Upstream insertion sequence seems to be a duplication from amplification. Terminal insertion sequence was a copy error due to implementing YTK part standards.