SUPPLEMENTARY MATERIALS:

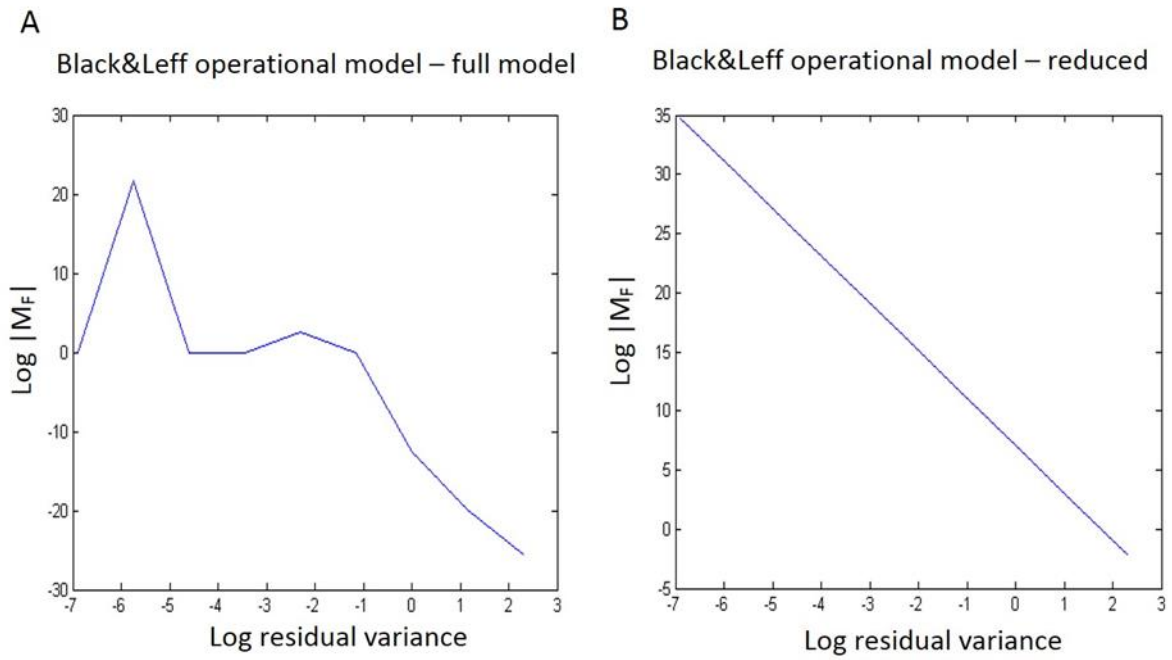

Figure S1 log|$\mathbf{M_F}$| vs. log residual variance for operational model. A) all parameters estimated from operational model (Eq.1) with data from functional assay; B) reduced operational model (Eq.5) with data from functional assay. |M_F|: the determinant of the Fisher Information Matrix. The criteria for claiming identifiability of a model: (i) log|M_F| should have a continuous linear log-log relationship with the log of the random noise and (ii) |M_F| should approach infinity as residual variance approaches zero.

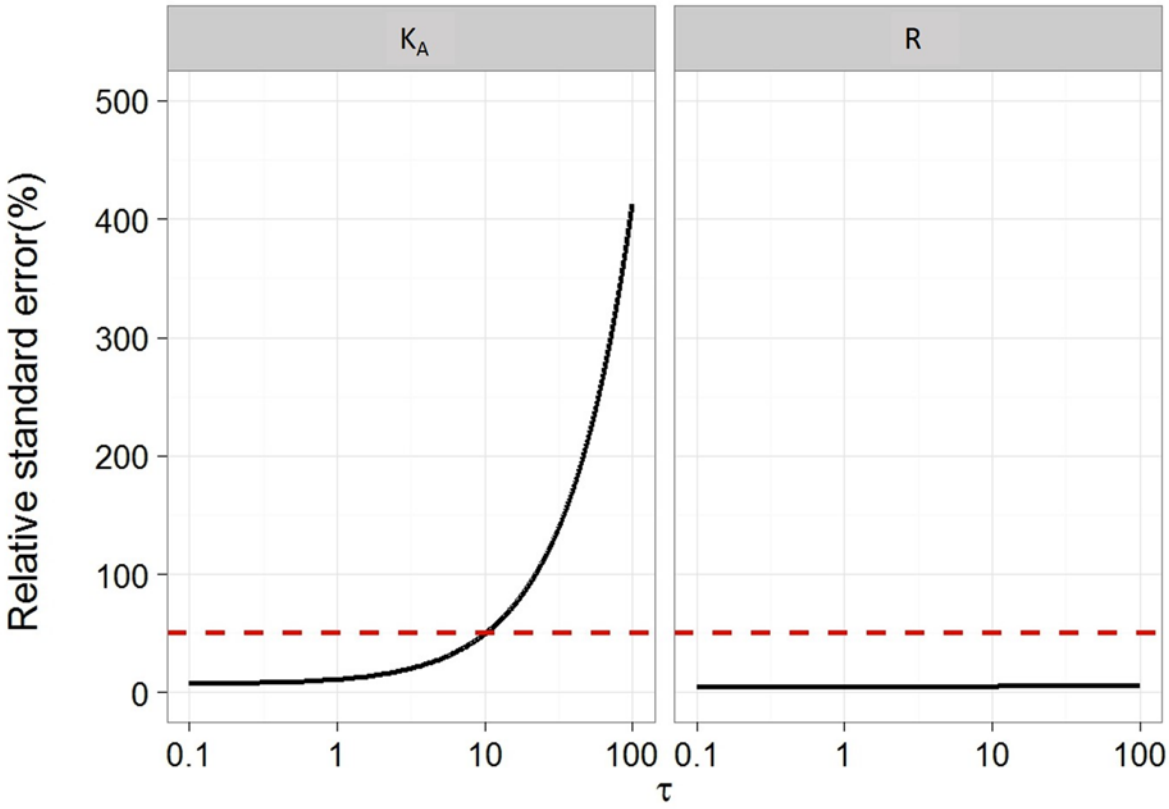

Figure S2 The relative standard error of estimated parameters vs. τ for Eq.8. In this study, a generic study design with sampling concentrations $log(A)$ from -13 to -4, increment of 1, was adopted. A proportional measurement error with 10% coefficient of variation were assumed. K_A was set to an arbitrary value, 10^{-8} mol L^{-1}. R was defined as the ratio of τ and K_A. The deterministic identifiability was assessed for each value of τ, ranging from 0.1 to 100. The left panel is for K_A and the right panel is for R. The red dashed line indicates the 50% relative standard error, considered as the threshold for precise estimation.

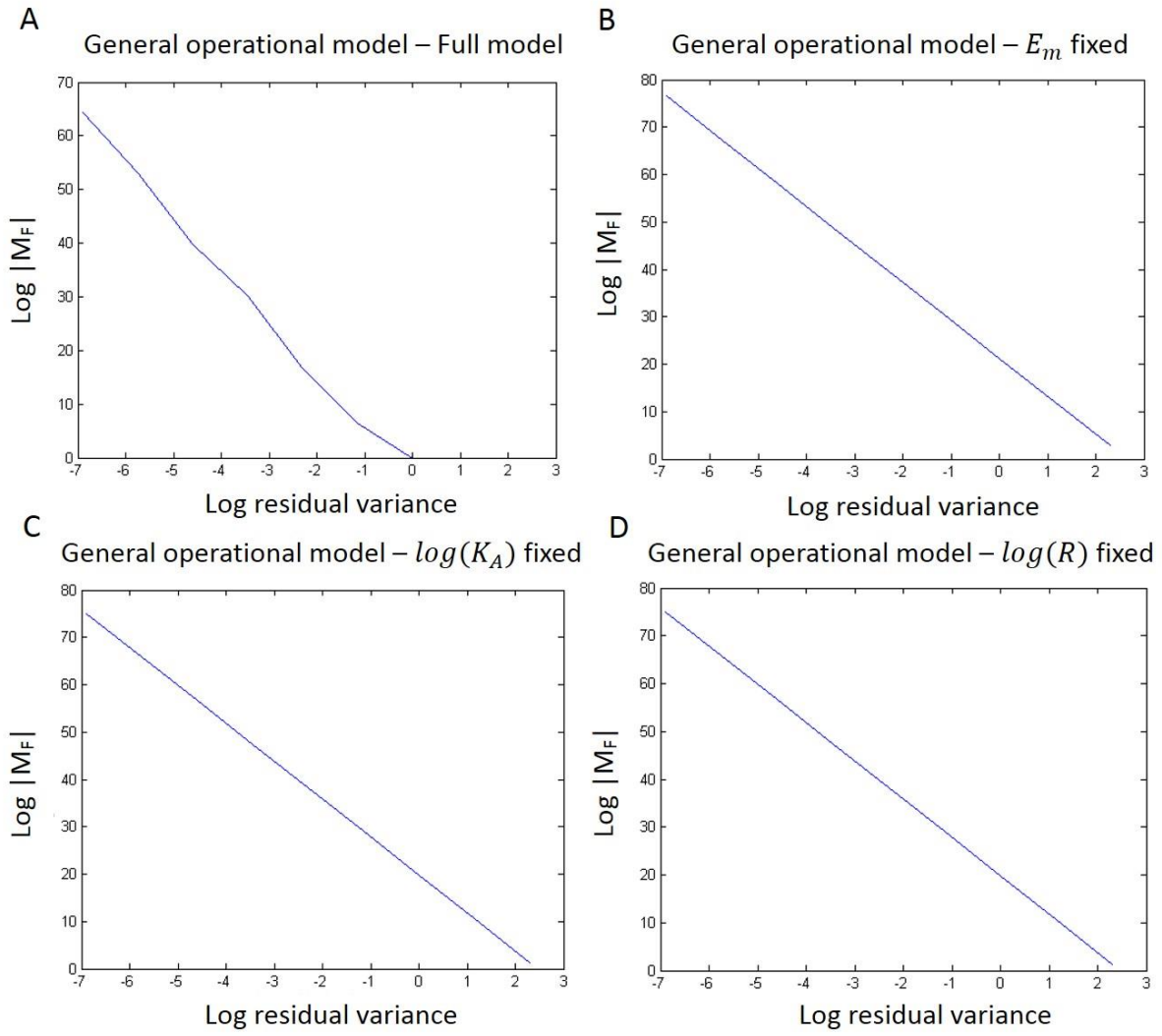

Figure S3 log|$\mathbf{M_F}$| vs. log residual variance for general operational model (Eq.2). A) all parameters estimated (E_m, $Basal$, n,$log(K_A)$, $log(R)$); B) E_m fixed; C) $log(K_A)$ fixed; D) $log(R)$ fixed.

An illustrating example for Method IV with step-by-step analysis processes

Here, we conducted a new analysis and applied method IV to cAMP concentration–response curves obtained from six CB1 agonists in pplss-3HA-hCB$_1$ HEK cell line, following >16 h pretreatment in the presence of PTX (Figure 2B in Finlay et.al., 2017). We use this example to illustrate the analysis processes of Method IV.

1. Fit the classical E_{max} model (Eq.3) to the data from every ligand

The estimation method for step 1 is implemented in GraphPad Prism v7.0 as below:

Definition:

Y=Basal + (Emax-Basal)/(1+10^((LogEC50-X)*n))

Description:

Here, Basal and n are system parameters shared among different ligands. Emax and LogEC50 are ligand specific parameters.

2. Select the ligand with maximal Emax from the current study and set as if it were a full agonist (termed pseudo full agonist). Classify all the remaining ligands as partial agonists.

Here, WIN55212-2 has the largest Emax value. Hence, it is selected as a pseudo full agonist. All the other ligand are considered as partial agonists. The data of WIN55212-2 is moved to the first column of the dataset and all the others start from second column onwards.

3. For partial agonists, the concentration-response curve is directly fitted with the general operational model, whereas for the pseudo full agonist, the value of K_A is set to 1 mol L^{-1}.

The estimation method for steps 3 is implemented in GraphPad Prism v7.0 as below:

Definition:

A=10^X

operate1=((1+A)/((10^LogR)*A))^n

operate2=((1+A/(10^LogKA))/((10^LogR)*A))^n

Y1=basal+ (Em-basal)/(1+operate1)

Y2=basal+ (Em-basal)/(1+operate2)

<A:A>Y=Y1

<~A:A>Y=Y2

Description:

Put the ligand with maximal observed response in the first column, and all the others start from second column onwards. For each pathway, the values of n, Basal and Em are shared for all the ligands and there is no constraint on the values of LogR and LogKA.

Results:

As shown in Figure S4, the fitting result from Method IV is desirable. From Table S1, it is noted that the estimation of $log(R)$ is precise for all the ligands.

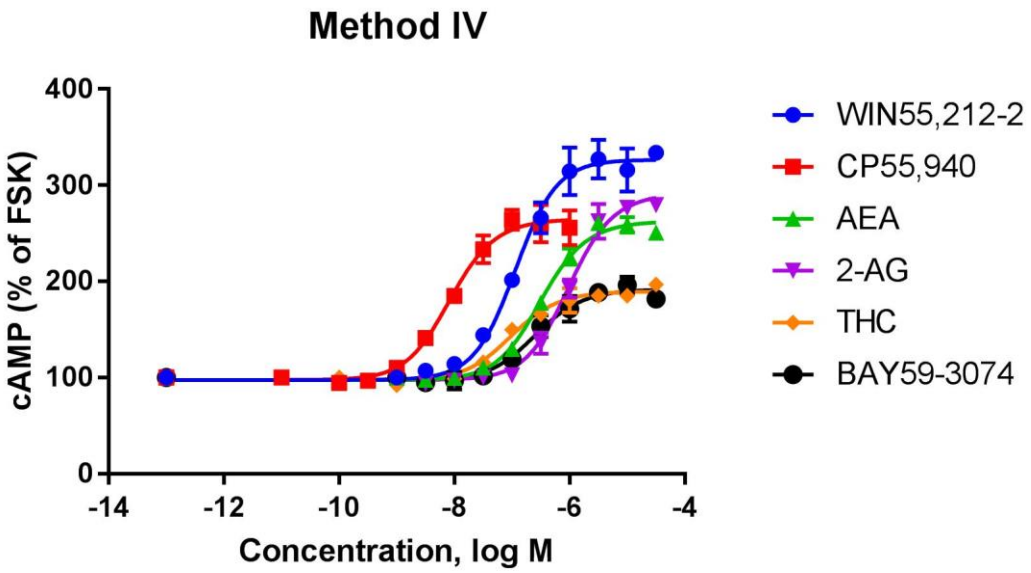

Figure S4 Concentration–response curves for cAMP formation showing pplss-3HA-hCB1 HEK signalling, on stimulation with 2.5 μM FSK and a panel of six agonists, following >16 h pretreatment in the presence of PTX. The data is fitted by Method IV.

Table S1 The estimation of transduction coefficient ($log(R)$) via Method IV

Ligand	$log(R) \pm SE$	$\Delta log(R) \pm SE$
WIN55,212-2	6.938 ± 0.0307	-
CP55,940	8.016 ± 0.0523	1.078 ± 0.0606
AEA	6.436 ± 0.0535	-0.502 ± 0.0617
2-AG	5.99 ± 0.0459	-0.948 ± 0.0552
THC	6.768 ± 0.0909	-0.17 ± 0.0959
BAY59-3074	6.353 ± 0.0907	-0.585 ± 0.0958

The exact numerical evaluation becomes unfeasible for the case where the slope factor is different from unity

From Eq.S1,

$$E = Basal + \frac{(E_m - Basal) \cdot \tau^n \cdot A^n}{(K_A + A)^n + \tau^n \cdot A^n} \quad (S.1)$$

the basal, location of mid-point, asymptote of maximal response and mid-point gradient are defined (Black et al., 1985):

$$Basal_{i_{obs}} = Basal \quad (S.2)$$

$$E_{max,i_{obs}} = Basal + \frac{(E_m - Basal) \cdot \tau_i^n}{\tau_i^n + 1} \quad (S.3)$$

$$EC_{50,i_{obs}} = \frac{K_{A_i}}{\sqrt[n]{\tau_i^n + 2} - 1} \quad (S.4)$$

$$G_{i_{obs}} = \frac{0.576 \cdot n \cdot (\tau_i^n + 2) \cdot (\sqrt[n]{\tau_i^n + 2} - 1)}{(\tau_i^n + 1) \cdot \sqrt[n]{\tau_i^n + 2}} \quad (S.5)$$

Here, $Basal$, E_m and n were the true system parameter values shared by all ligands and τ_i and K_{A_i}were the true parameter values for i[th] ligand.

Since these constraints (*i.e.*, Eq.S2-5) need to be met by all the workable parameter sets, the links between true parameter values and misspecified parameter values were set up as follows:

$$Basal' = Basal \quad (S.6)$$

$$Basal' + \frac{(E_m' - Basal') \cdot {\tau_i'}^{n'}}{{\tau_i'}^{n'} + 1} = Basal + \frac{(E_m - Basal) \cdot \tau_i^n}{\tau_i^n + 1} \quad (S.7)$$

$$\frac{K_{A_i}'}{\sqrt[n']{{\tau_i'}^{n'} + 2} - 1} = \frac{K_{A_i}}{\sqrt[n]{\tau_i^n + 2} - 1} \quad (S.8)$$

$$\frac{0.576 \cdot n' \cdot ({\tau_i'}^{n'} + 2) \cdot (\sqrt[n']{{\tau_i'}^{n'} + 2} - 1)}{({\tau_i'}^{n'} + 1) \cdot \sqrt[n']{{\tau_i'}^{n'} + 2}} = \frac{0.576 \cdot n \cdot (\tau_i^n + 2) \cdot (\sqrt[n]{\tau_i^n + 2} - 1)}{(\tau_i^n + 1) \cdot \sqrt[n]{\tau_i^n + 2}} \quad (S.9)$$

Here, the prime symbol denotes the corresponding misspecified parameter values.

When n is not equal to 1, the expression of mid-point gradient cannot be simplified and τ is still constrained by mid-point gradient. This makes the exact numeric analysis unfeasible for the case that the slope factor is different from unity.

R code example for stochastic simulation

```
#number of replicates
n.rep<-1000

#Different level of n
n<-c(0.5,0.6,0.7,0.8,0.9,1,1.25,1.5,1.75,2)

#Model system parameters
Em <- 500
Basal<-10
prop.err<-0.1
logA<-seq(from=-13,to=-4,by=0.5)

# ligand 1
tau1<-6
KA1<-10^-8
logKA1<-log10(KA1)
logR1<-log10(tau1/KA1)

#ligand 2
tau2<-2
KA2<-10^-7
logKA2<-log10(KA2)
logR2<-log10(tau2/KA2)

#Define general operational model
operationalModel <- function(Em,Basal,n,logA,logKA,logR,prop.err){
  f <- Basal+(Em-Basal)/(1+((10^(logA-logKA)+1)/10^(logA+logR))^n)
  err<-rnorm(length(logA),mean=0,sd=prop.err)
```

```
  E <- f*(1+err)
  return(E)
}

#Generate 1000 replicates for each n value
for (j in 1:length(n)){
  for (i in 1:n.rep){
    eff1<-operationalModel(Em,Basal,n[j],logA,logKA1,logR1,prop.err)
    eff2<-operationalModel(Em,Basal,n[j],logA,logKA2,logR2,prop.err)
    dat<-data.frame(logA=logA,effH=eff1,effL=eff2)
    write.csv(dat,file=paste0("test",j-1,i,".csv"),row.names=F)
  }
}
```

GraphPad Prism script for batch analysis

```
SetPath "the address of the working folder"
OpenOutput S2_FIX_KA.csv
Table CSV
 ForEach test*.csv
 Goto D
 ClearTable
 Import
 WTable "File name"  %F
 GoTo R 1 V1
 WTable "LogR_h", 3, 1
 WTable "LogR_l", 3, 2
 WTable "LogR_h_se", 8, 1
 WTable "LogR_l_se", 8, 2
Next
Beep
```

Reference:

Finlay DB, Cawston EE, Grimsey NL, Hunter MR, Korde A, Vemuri VK, Makriyannis A, Glass M (2017). Gαs signalling of the CB1 receptor and the influence of receptor number. *Br J Pharmacol* 174:2545-2562

Black JW, Leff P, Shankley NP, Wood J (1985). An operational model of pharmacological agonism: the effect of E/[A] curve shape on agonist dissociation constant estimation. *Br J Pharmacol* 84: 561-571.