Supplemental Figure Legends

Supplemental Figure 1.

WT LANApi and K14p adhere to first-order Hill kinetics (Michaelis-Menten kinetics; MM).

A.
Shown are the global mean of raw data points (shown as diamonds, here is an example for the WT K14p in bidirectional configuration) as obtained from individually $n \geq 9$ biological replicates. On the left panel these were fit to a logistic equation with the parameters T and n (the Hill coefficient) to be determined. On the right panel the data are fit to a equation with a fixed n = 1, which has the form of the Michaelis Menten equation.

B.
Based on the logistic fit, the Hill coefficient was determined for various WT input configurations (Bidir. Red; bidirectional promoter reversed in which LANApi directs expression of red luciferase isoform) of the LANA pi. It did not differ significantly from 1, regardless of context or RTA mutant, i.e. RTA activation of the K14p shows no evidence of cooperativity.

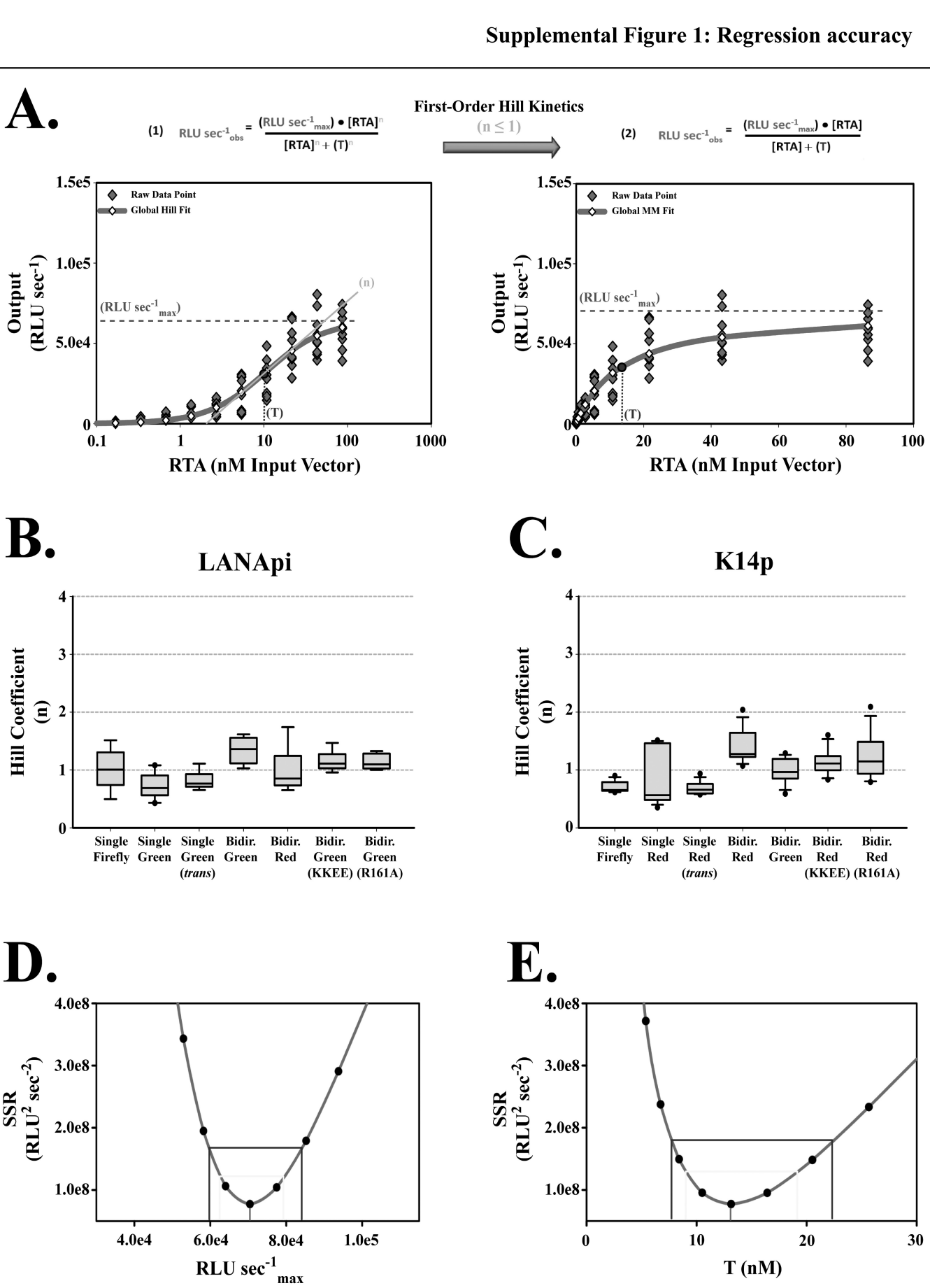
C.
Based on the logistic fit, the Hill coefficient was determined for various WT input configurations (Bidir. Green; bidirectional promoter reversed in which K14p directs expression from green luciferase isoform). It did not differ significantly from 1, regardless of context or RTA mutant, i.e. RTA activation of the K14p shows no evidence of cooperativity.

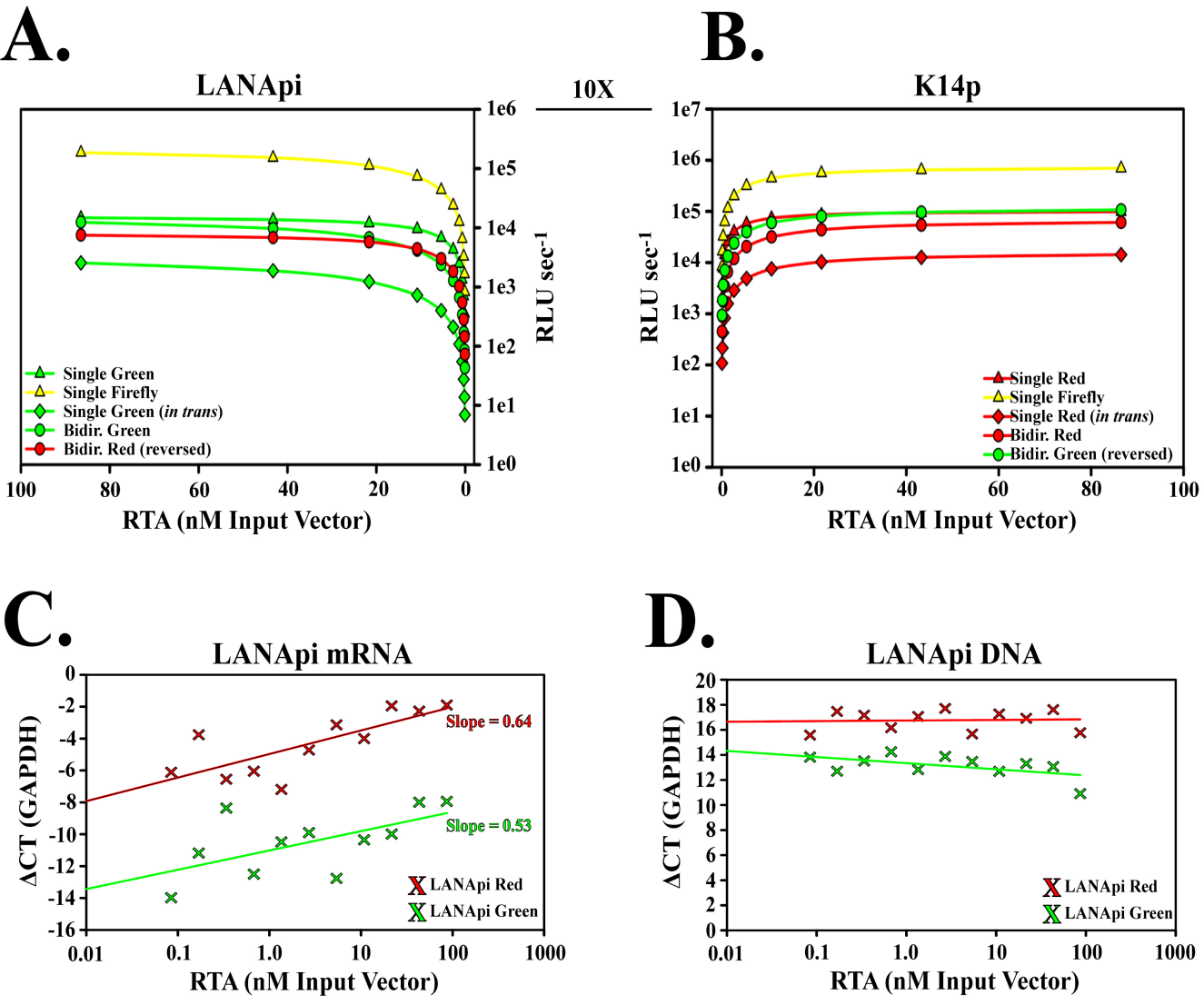
D.
To address the accuracy of our analysis, we generated a plot of the sum of squared residuals (SSR) against extracted parameters $RLUsec^{-1}_{max}$ fitted via MM kinetics (shown for the WT K14p bidirectional configuration).

E.
To address the accuracy of our analysis, we generated a plot of the sum of squared residuals (SSR) against the induction threshold (T) fitted via MM kinetics (shown for the WT K14p bidirectional configuration). Black lines indicate 95% confidence interval. This depiction shows that if we use the simplified n=1 MM equation we obtain high confidence values for T (within ±10 nM) and for Vmax (within $\pm 2 \times 10^4$ RLU sec^{-1}).

Supplemental Figure 2.

The relative advantage in efficiency of the K14p over LANApi is static irrespective of luciferase isoform. Fitted output curves derived from assay with the LANApi (A.) or the K14p TSS (B., note change in scale) in various indicated WT cloning configurations. C. mRNA production from the WT LANApi cloned into a red-encoding single reporter construct (red X's and red fitted line) or into a green-encoding single reporter construct (green X's and green fitted line); indicating a similar response to RTA irrespective of luciferase isoform as assayed by primer sets specific for each isoform (Red_1430_FOR/Red_1606_REV and Green_712_FOR/Green_836_REV, respectively). D. Input DNA from the same experiment as in 2C assayed contemporaneously indicating results are not reflective of increased input DNA.


A.
First-Order Hill Kinetics
(n ≤ 1)
(1) RLU sec-1obs = (RLU sec-1max) • [RTA]n / [RTA]n + (T)n
(2) RLU sec-1obs = (RLU sec-1max) • [RTA] / [RTA] + (T)
Raw Data Point
Global Hill Fit
Global MM Fit
Output (RLU sec-1)
RTA (nM Input Vector)
(RLU sec-1max)
(n)
(T)
B.
LANApi
C.
K14p
Hill Coefficient (n)
Single Firefly
Single Green
Single Green (trans)
Bidir. Green
Bidir. Red
Bidir. Green (KKEE)
Bidir. Green (R161A)
Single Red
Single Red (trans)
Bidir. Red
Bidir. Green
Bidir. Red (KKEE)
Bidir. Red (R161A)
D.
E.
SSR (RLU2 sec-2)
RLU sec-1 max
T (nM)


A.
LANApi
RLU sec-1
RTA (nM Input Vector)
Single Green
Single Firefly
Single Green (in trans)
Bidir. Green
Bidir. Red (reversed)
10X
B.
K14p
RLU sec-1
RTA (nM Input Vector)
Single Red
Single Firefly
Single Red (in trans)
Bidir. Red
Bidir. Green (reversed)
C.
LANApi mRNA
ΔCT (GAPDH)
RTA (nM Input Vector)
Slope = 0.64
Slope = 0.53
LANApi Red
LANApi Green
D.
LANApi DNA
ΔCT (GAPDH)
RTA (nM Input Vector)
LANApi Red
LANApi Green