

Model-driven Engineering of Gene Expression from RNA Replicons

Supplementary Material

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Contents

1	Exceptions to Replicate Numbers	1
2	Modeling Replication	1
3	Full Details of Predicted vs. Observed Results	2
4	Replicon Sequences	12

1 Exceptions to Replicate Numbers

The following replicates failed, and were omitted from analysis:

- Dose-response time series: Replicate #2 for at hour 3 of lowest dosage.
- Dual transfection experiment: nothing omitted.
- Three-color experiment: Replicate #2 for Mixture #5.

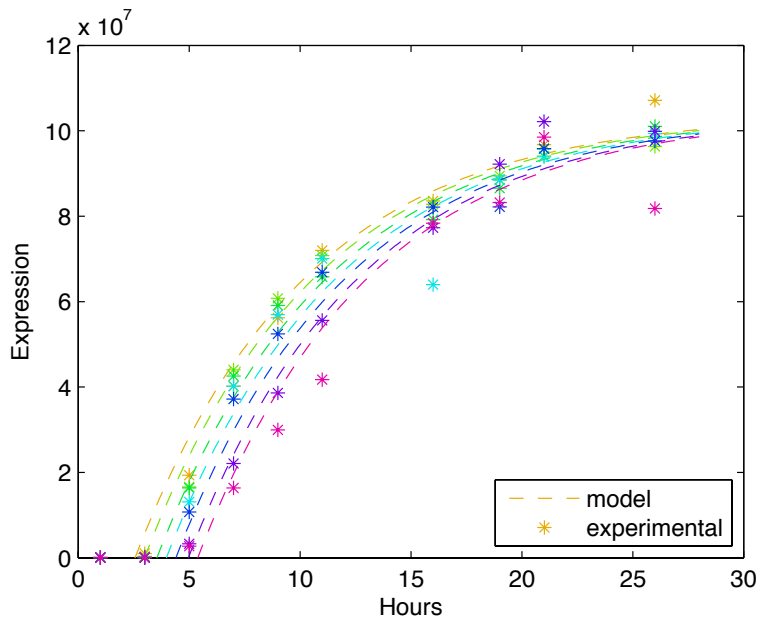
2 Modeling Replication

The RNA replication process is expected to increase the number of copies of RNA exponentially, then saturate at some converged level in response to additional constraints, such as resource scarcity, self-regulation of the NSP complex, cell internal immune response, etc.

This process can be modeled at varying levels of detail. The choice of modeling detail depends critically, however, on the degree to which a model can be constrained by its observable effects, in this case on fluorescent expression.

In the main text, we have chosen the simplest possible approximation, of a fixed delay before saturation. This model is clearly inadequate in general, as indicated by the fact that the lowest two dosages climb toward saturated fluorescent expression noticeably more slowly. Do we have good enough data, however, to support a more complex model? Supplementary Figure 1 shows a least-squares best fit to a model that adds one more degree of freedom, making the lag to saturation logarithmically dependent on the initial dose. As can be seen, however, adding this degree of freedom does not improve the model, as the errors are similar in size to the entire range of systematic variation predicted by the model.

If the fine structure of the initial transient is deemed important, future investigation could improve this model by using measurements with increased precision, gathering data at finer increments of conditions, or using protocols that can measure replication dynamics directly, such as qRT-PCR.



Supplementary Figure 1: When dose-dependence is included in the model of replication, the observed experimental data has errors on the same scale as the prediction variations enabled by dose-dependence, indicating that such a model enhancement cannot be well-supported by the data in this manuscript.

3 Full Details of Predicted vs. Observed Results

Supplementary Figure 2 presents the fold-error for all predictions vs. time, showing that there is no time that dominates the prediction error.

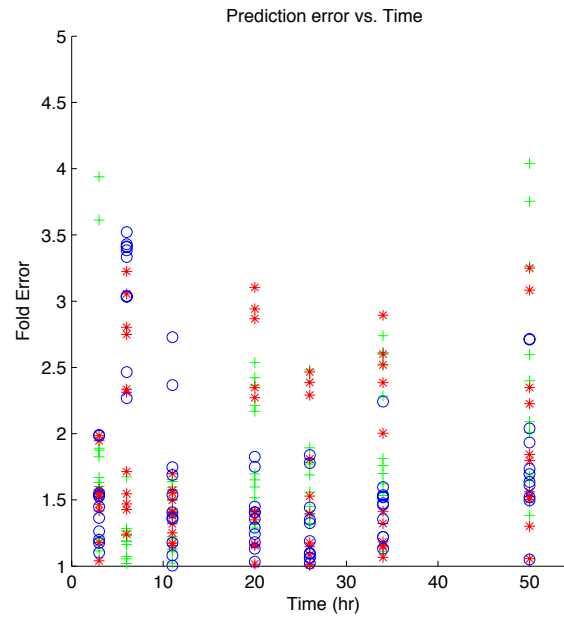
Supplementary Figure 3 compares predicted and experimentally observed geometric mean for all time points and all three-color mixtures. Dosages used for three-color mixtures are listed in the main text and repeated for convenience in Supplementary Table 1.

Supplementary Figures 4 through 10 compare predicted and experimentally observed distributions of fluorescence for every time point and every three-color mixture. Note that the raggedness of the experimental data at the lowest levels comes from quantization of very low flow cytometry values.

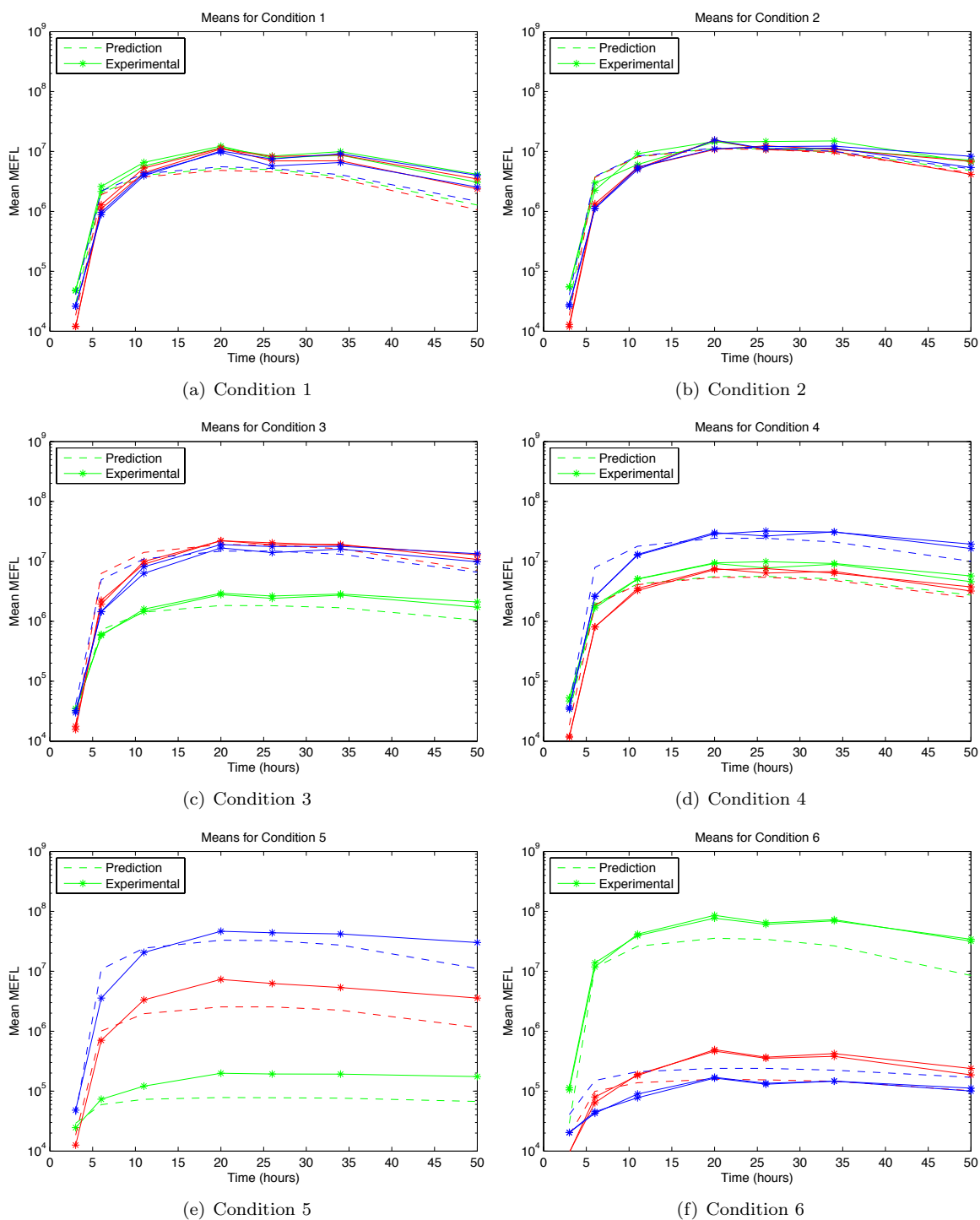
Predictions for Conditions 1 through 4 relate to the observed values in a fairly straightforward manner: differences are generally caused by small differences in the location of the peaks and the relative weight of the transfected and untransfected populations. For Conditions 5 and 6, in which some replicons have very low dosage, the observed behavior is significantly less bimodal than the predictions, with a distribution that “smears” more evenly between low expression and untransfected cells. This suggests that additional sources of variability not currently included in our model may be having a significant impact, and may be worth studying for future inclusion in order to improve accuracy.

Condition	mVenus (ng)	mKate (ng)	EBFP2 (ng)
1	180	180	180
2	540	540	540
3	180	900	720
4	360	360	1080
5	18	180	900
6	720	36	36

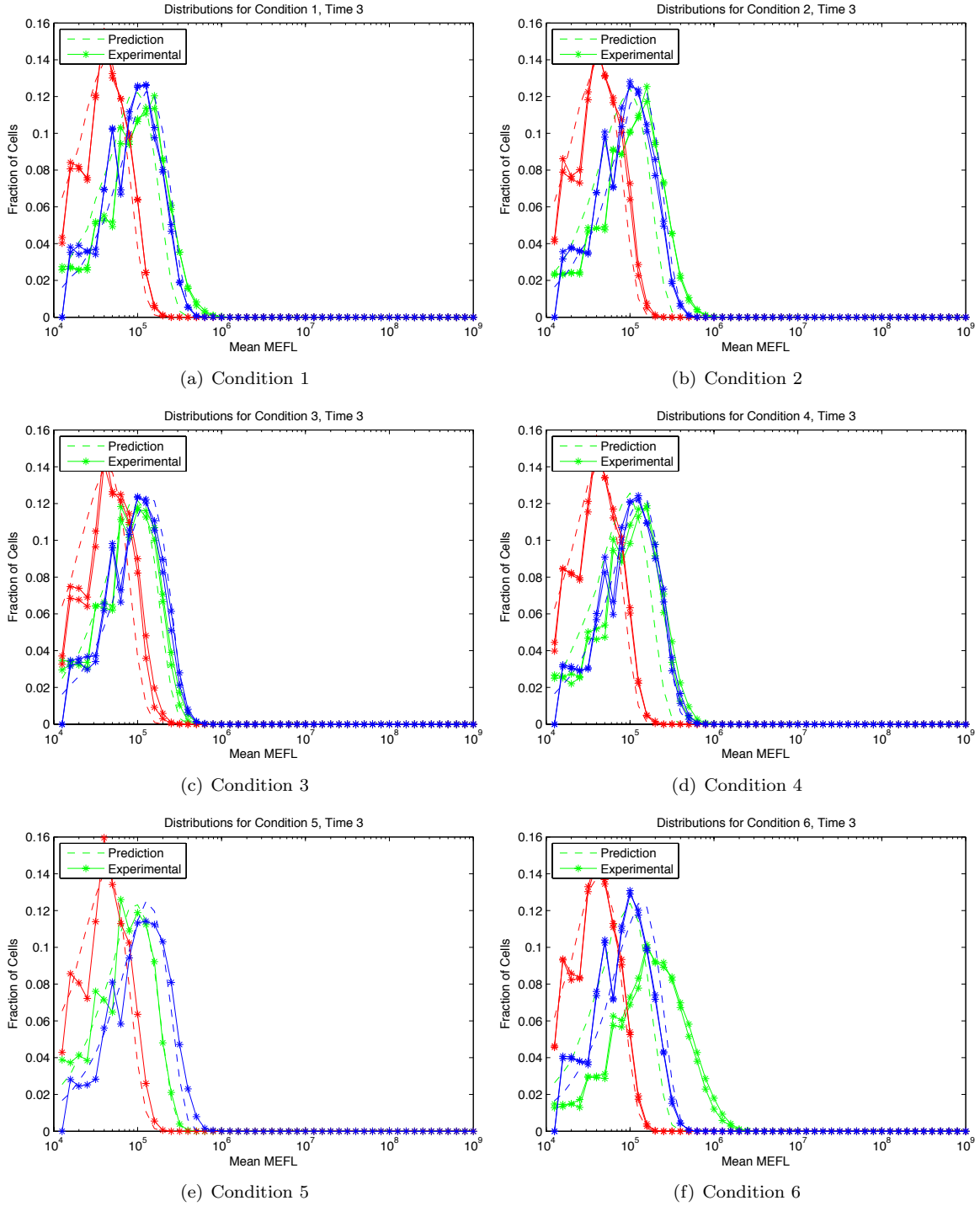
Supplementary Table 1: Dosages used for three-replicon mixtures.



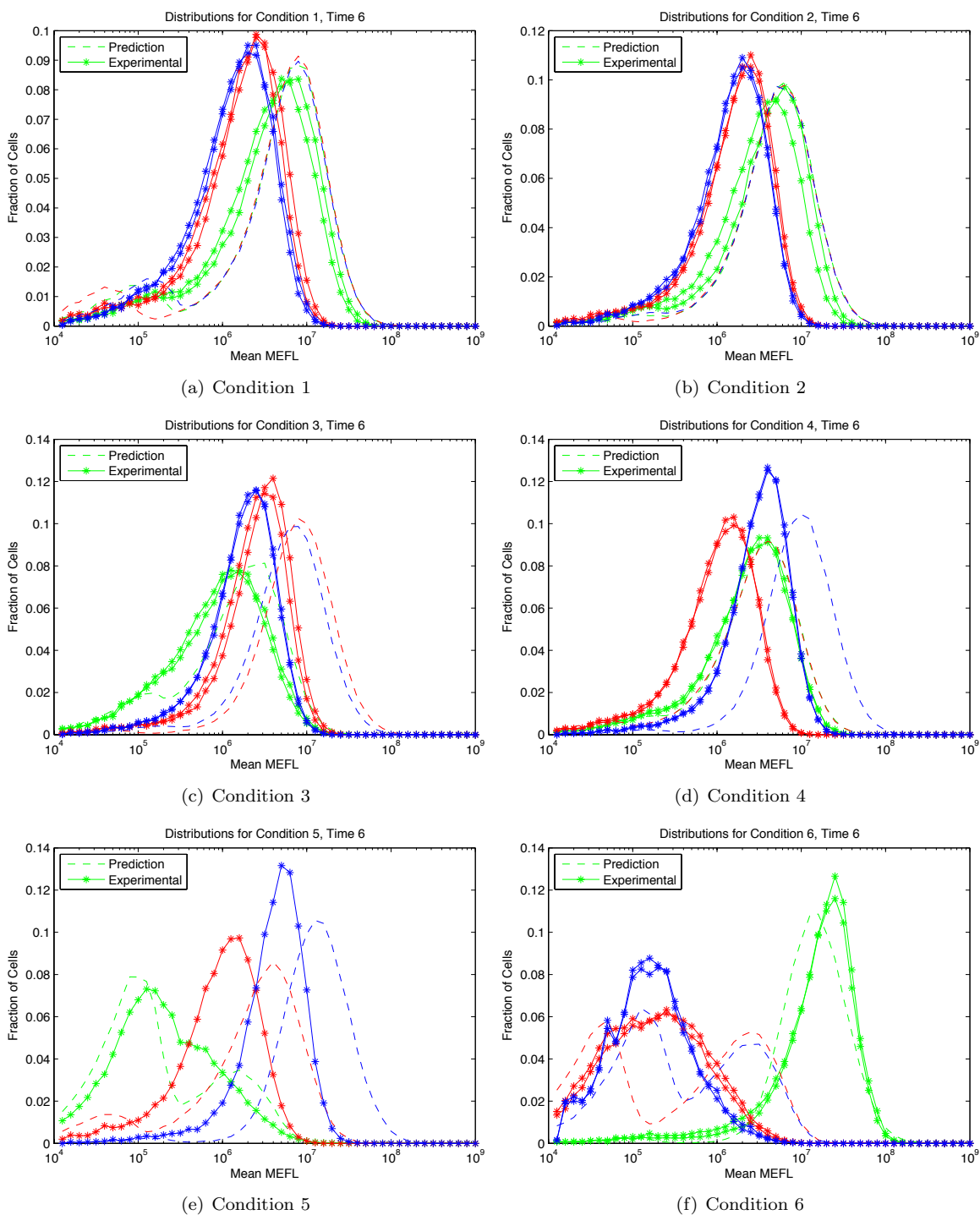
Supplementary Figure 2: The error of predictions is not dominated by samples at any particular time.



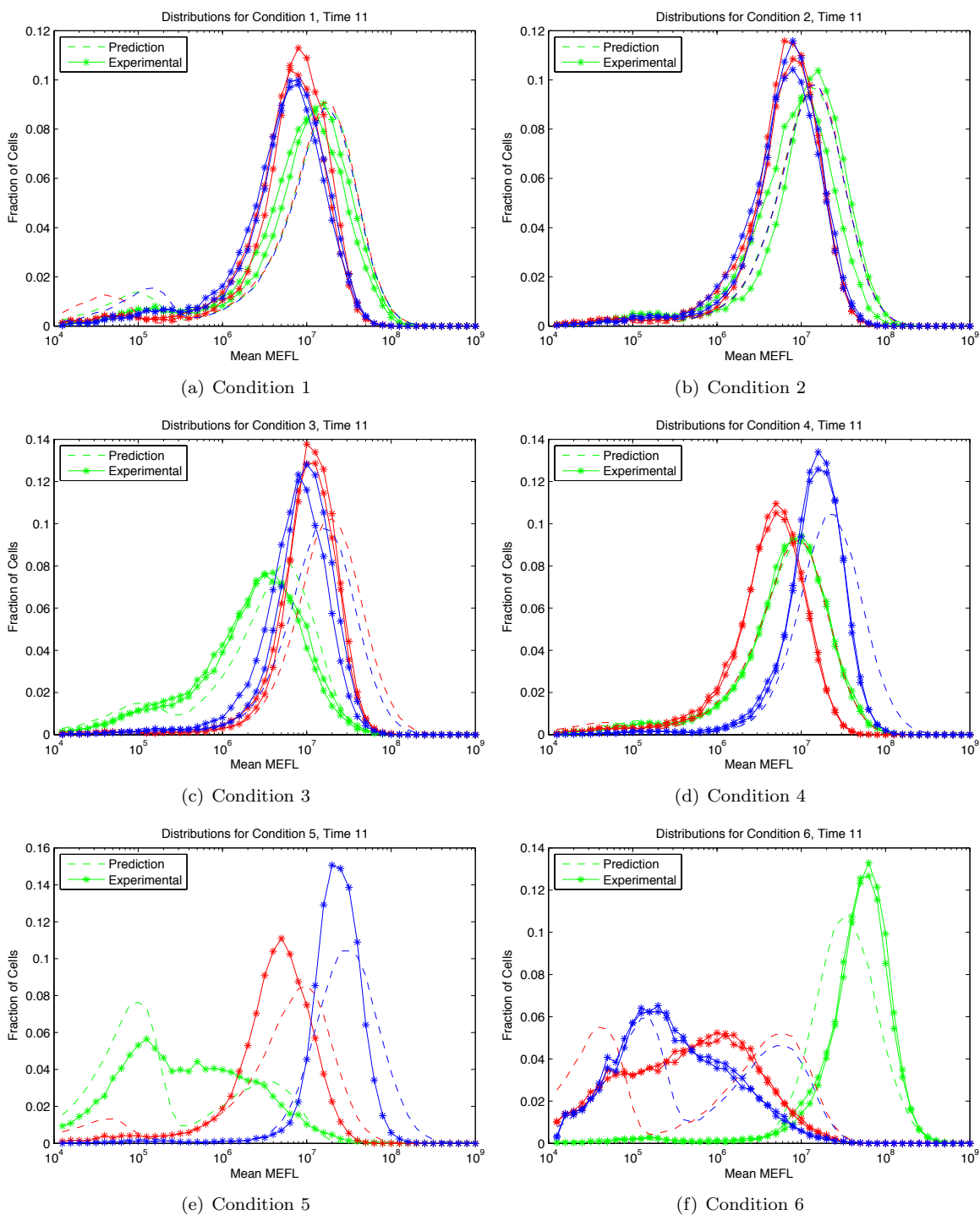
Supplementary Figure 3: Predicted (dashed) vs. experimentally observed (solid) geometric mean fluorescence from mVenus (green), mKate (red), and EBFP2 (blue) for all conditions and time points.



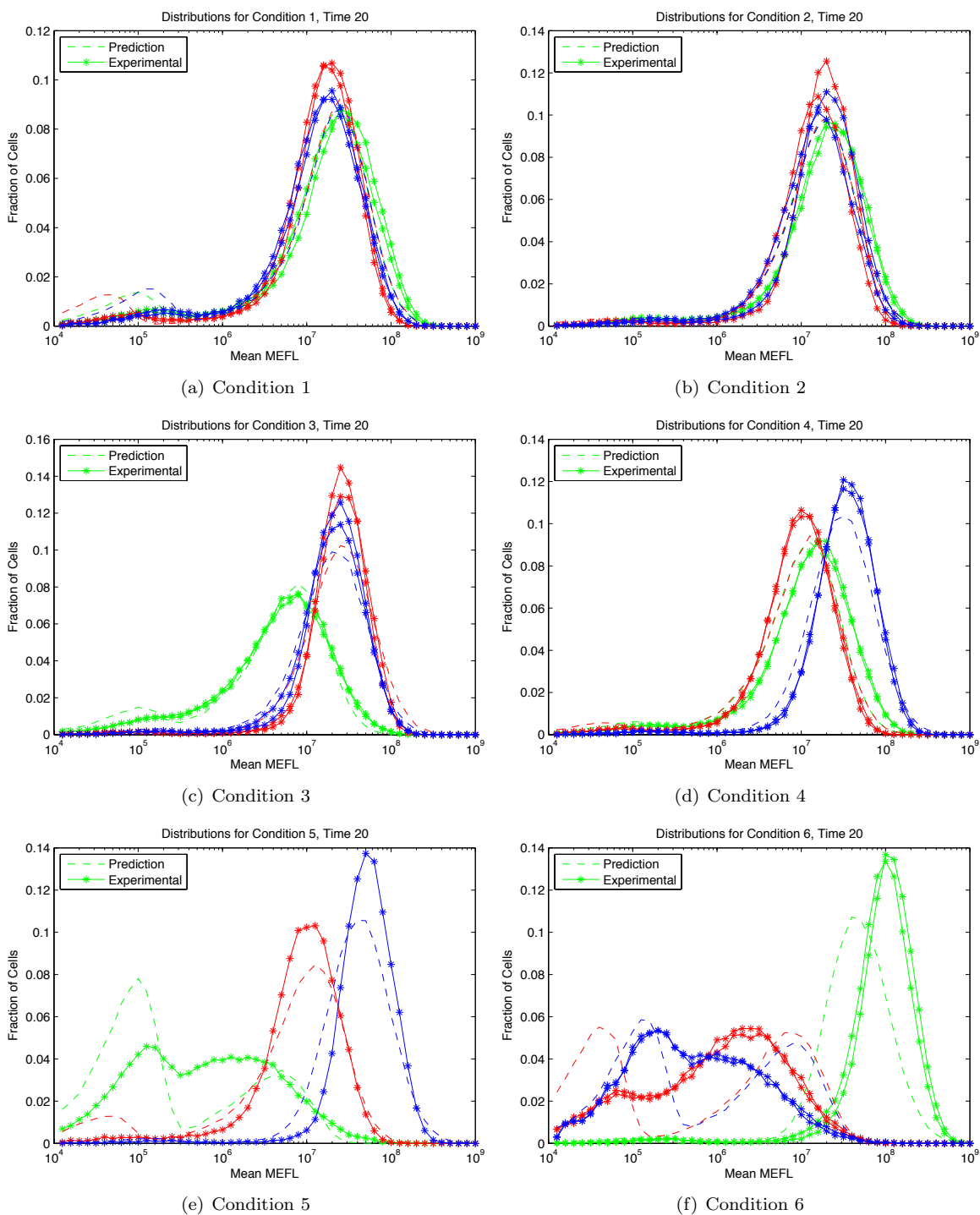
Supplementary Figure 4: Predicted (dashed) vs. experimentally observed (solid) distributions of fluorescence from mVenus (green), mKate (red), and EBFP2 (blue) for all conditions, 3 hours post-transfection.



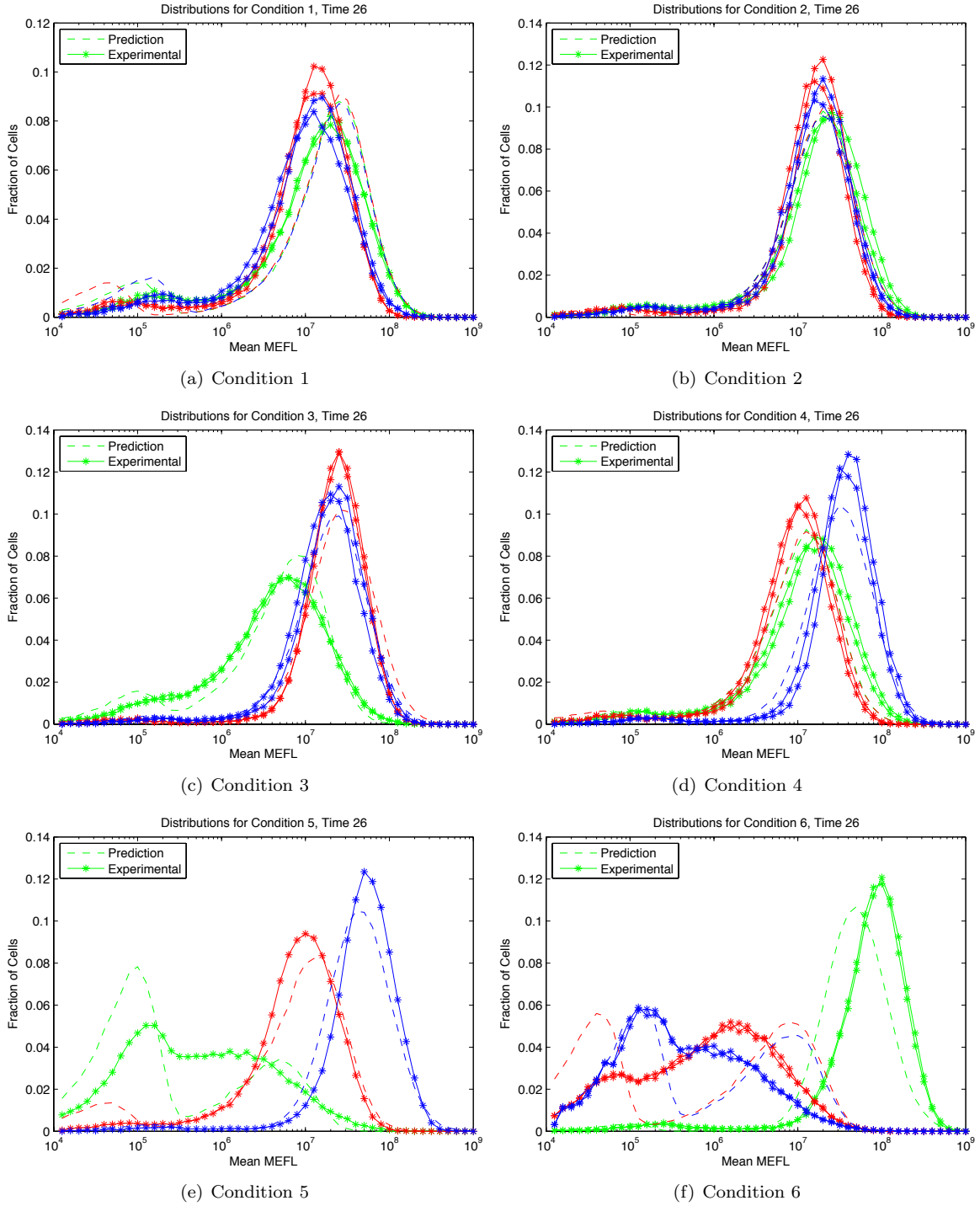
Supplementary Figure 5: Predicted (dashed) vs. experimentally observed (solid) distributions of fluorescence from mVenus (green), mKate (red), and EBFP2 (blue) for all conditions, 6 hours post-transfection.



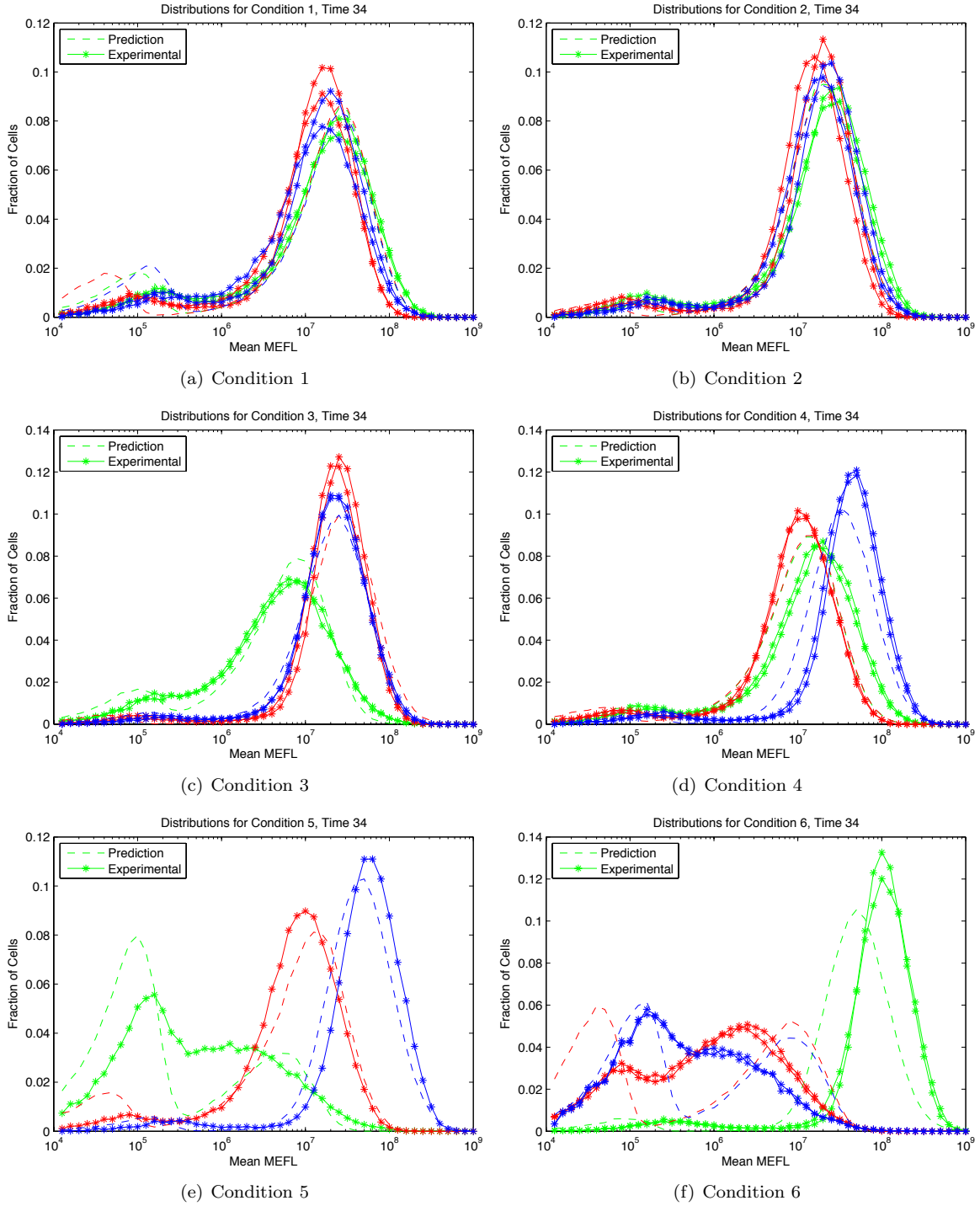
Supplementary Figure 6: Predicted (dashed) vs. experimentally observed (solid) distributions of fluorescence from mVenus (green), mKate (red), and EBFP2 (blue) for all conditions, 11 hours post-transfection.



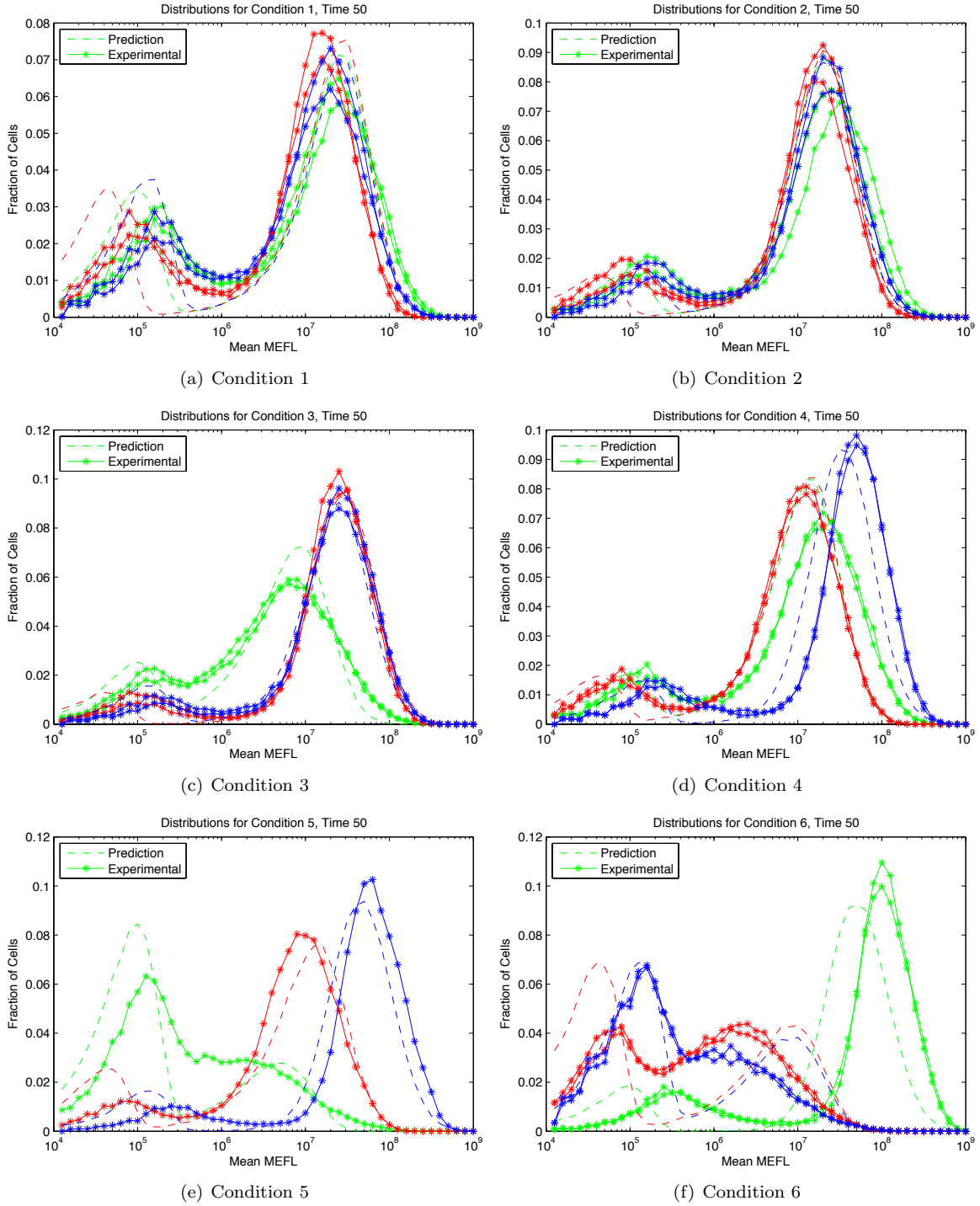
Supplementary Figure 7: Predicted (dashed) vs. experimentally observed (solid) distributions of fluorescence from mVenus (green), mKate (red), and EBFP2 (blue) for all conditions, 20 hours post-transfection.



Supplementary Figure 8: Predicted (dashed) vs. experimentally observed (solid) distributions of fluorescence from mVenus (green), mKate (red), and EBFP2 (blue) for all conditions, 26 hours post-transfection.



Supplementary Figure 9: Predicted (dashed) vs. experimentally observed (solid) distributions of fluorescence from mVenus (green), mKate (red), and EBFP2 (blue) for all conditions, 34 hours post-transfection.



Supplementary Figure 10: Predicted (dashed) vs. experimentally observed (solid) distributions of fluorescence from mVenus (green), mKate (red), and EBFP2 (blue) for all conditions, 50 hours post-transfection.

4 Replicon Sequences

LOCUS pTK035 10544 bp DNA circular UNA 20-JUN-2013
 DEFINITION SP6 SIN P1234 (P726S ncp) SGP mKate.
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 VERSION urn.local...1371481879831.1250
 KEYWORDS .
 SOURCE Sindbis
 ORGANISM Sindbis

FEATURES

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KEYWORDS .

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ORGANISM Sindbis

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