

## **Metabolic Labeling Reveals Proteome Dynamics of Mouse Mitochondria**

This online Supplemental Material contains 4 figures and 1 table.

**Supplemental Figure S1. Fractional synthesis of analyzed cardiac mitochondrial proteins as a function of time follows first-order kinetics.** This figure contains the individual kinetic curves for all 314 proteins analyzed in cardiac mitochondria.

**Supplemental Figure S2. Fractional synthesis of analyzed hepatic mitochondrial proteins as a function of time follows first-order kinetics.** This figure contains the individual kinetic curves for all 386 proteins analyzed in hepatic mitochondria.

**Supplemental Figure S3. Correlation between protein turnover rates and biophysical parameters.** This figure plots mitochondrial protein turnover in both organs as functions of four protein biophysical parameters: relative abundance, molecular weight, isoelectric point, and hydrophobicity.

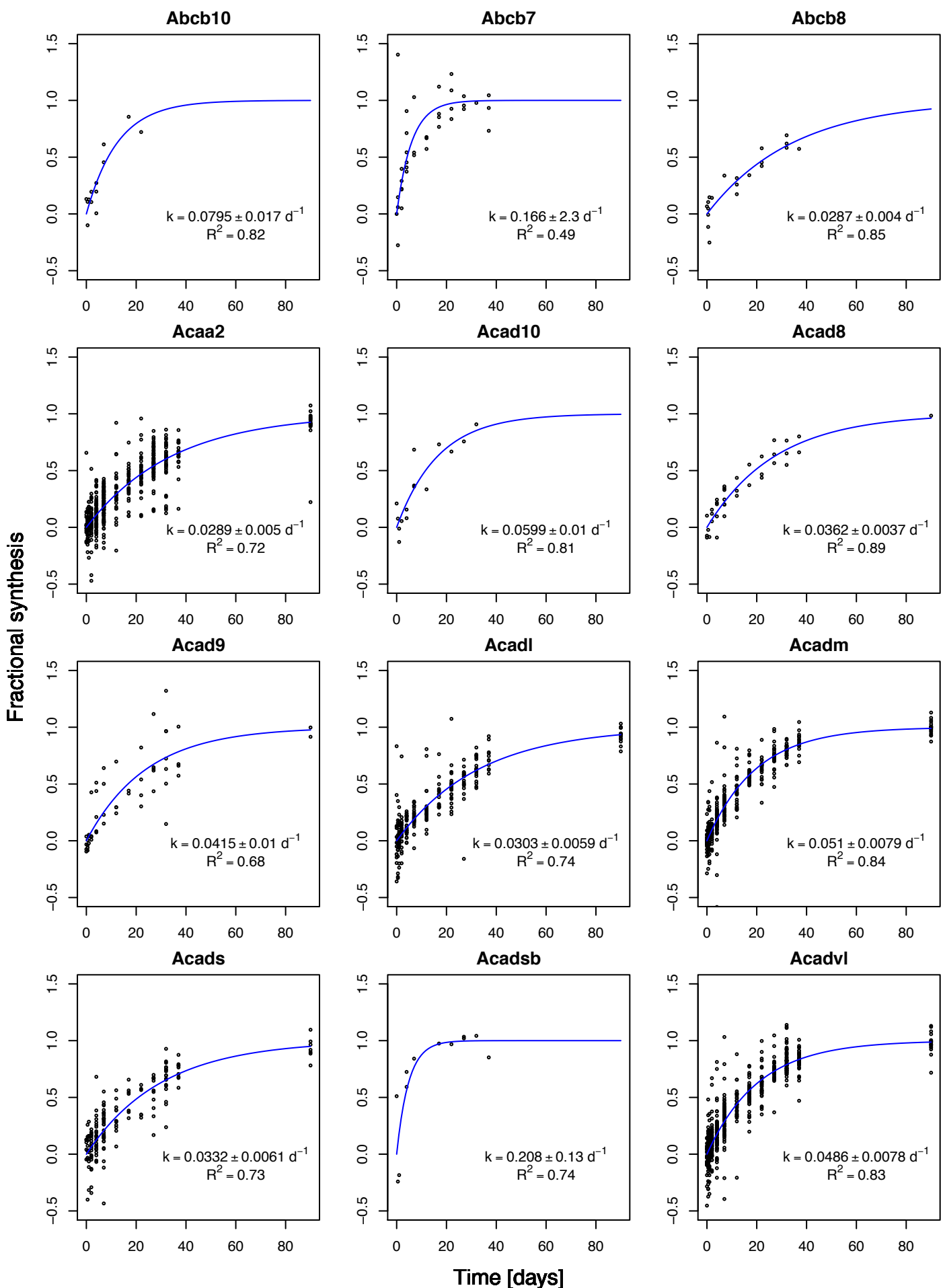
**Supplemental Figure S4. Histogram of the standard errors in the rate constants for cardiac mitochondrial proteins.** This figure portrays a comparison between the errors in rate constants as determined by non-linear curve fitting and the Monte Carlo method.

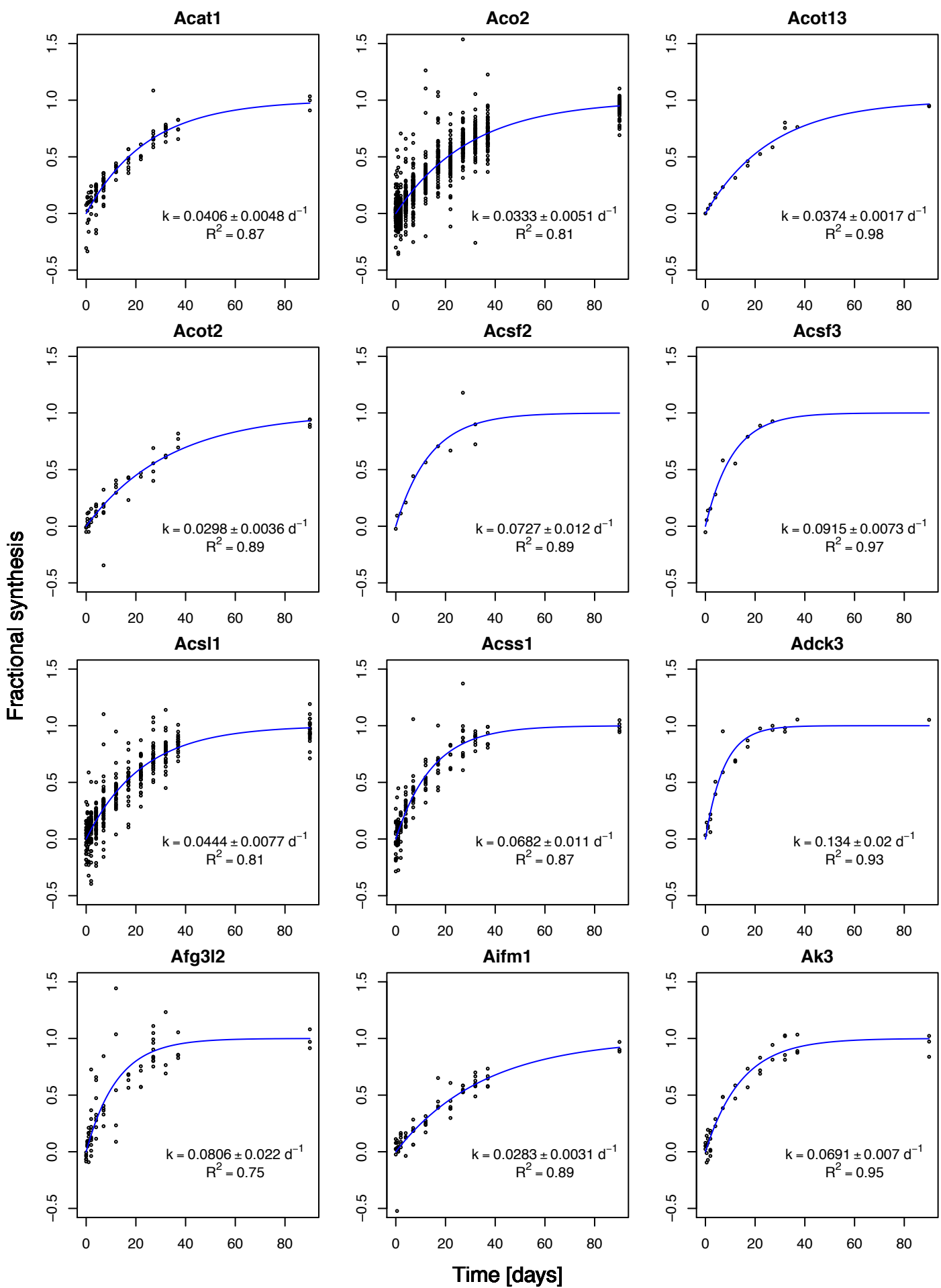
**Supplemental Figure S5. Mitochondrial protein turnover rates in the heart and the liver.** This figure shows the protein turnover rates ( $k$ ) of all analyzed mitochondrial proteins in the heart and the liver on linear, non-logarithmic scale.

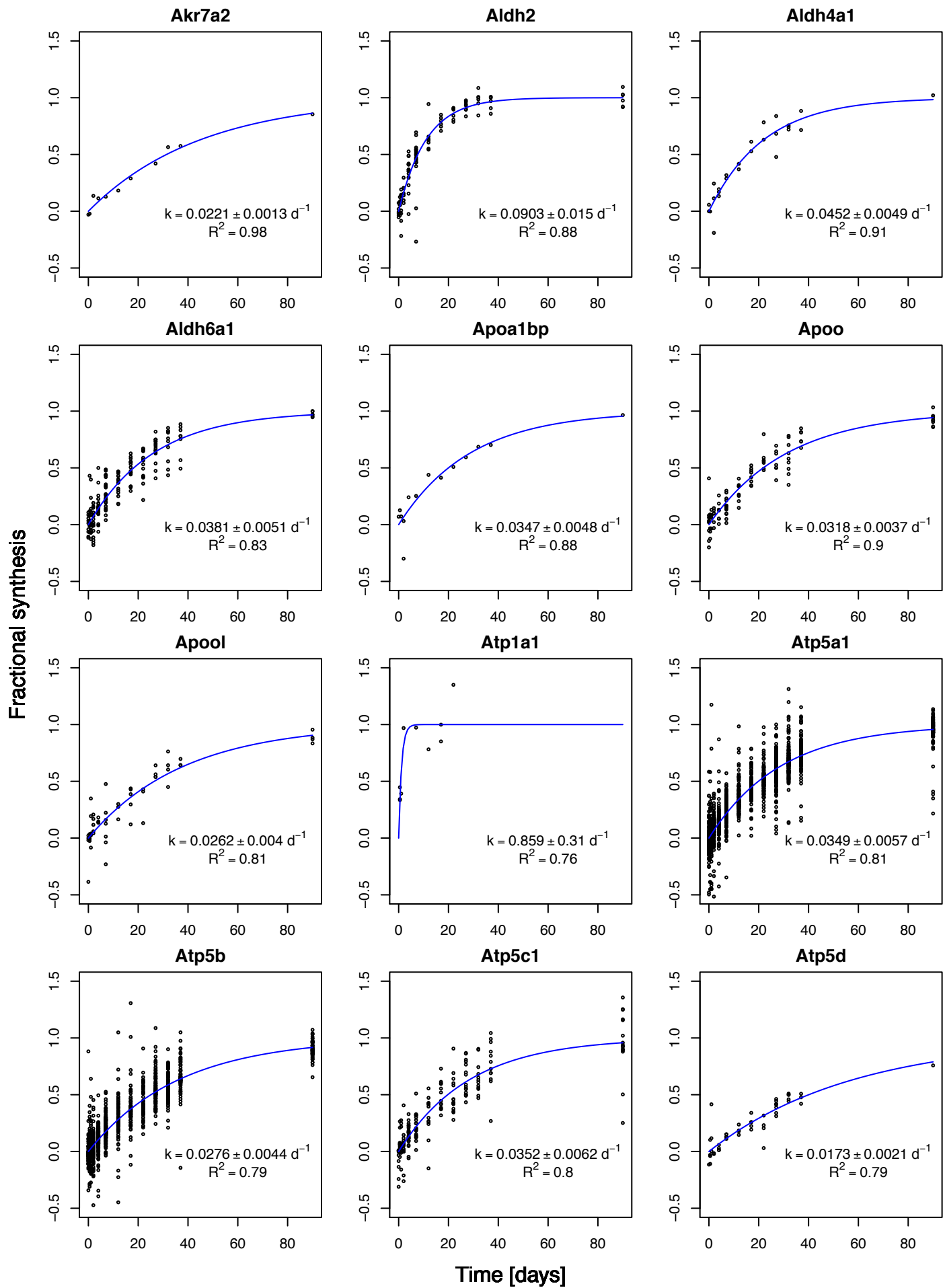
**Supplemental Table S1. Raw kinetic data determined in our mitochondrial protein turnover study using heavy water.** This standalone Excel spreadsheet file contains all the raw kinetic data in this study as well as relevant information including standard errors and protein annotations.

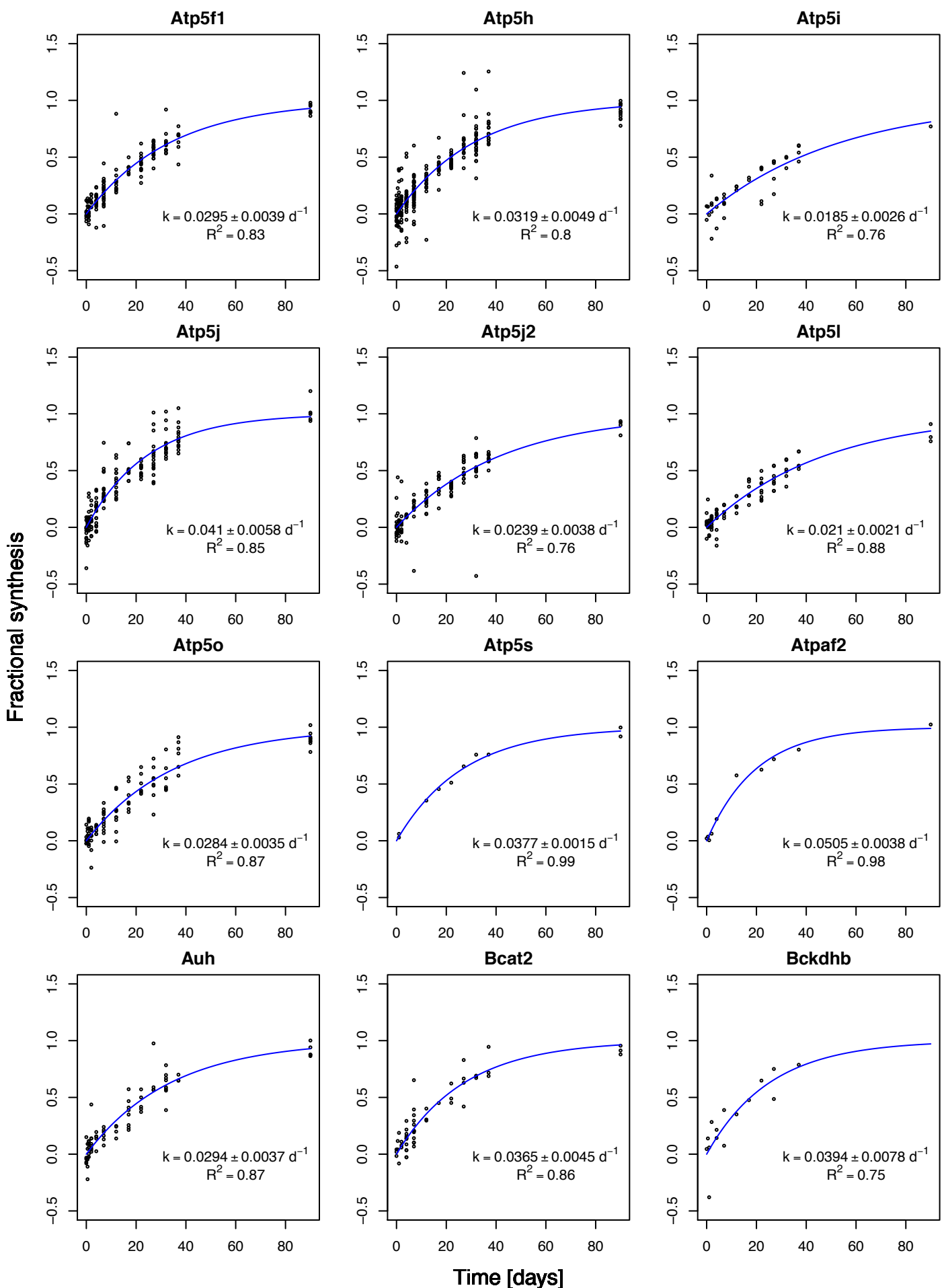
**Supplemental Fig. S1. Fractional synthesis of analyzed cardiac mitochondrial proteins as a function of time follows first-order kinetics.**

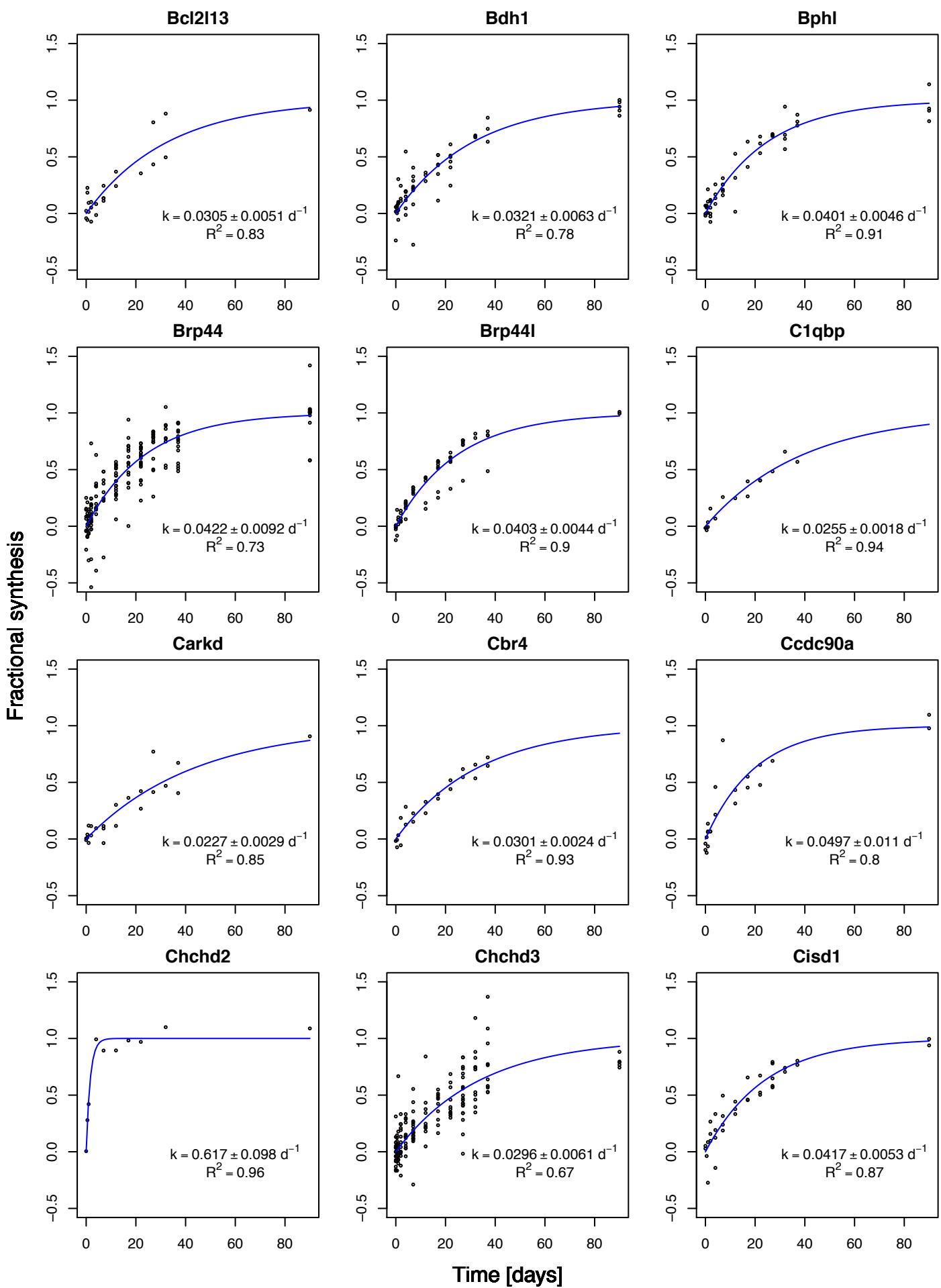
The fractional syntheses of all 314 proteins analyzed in cardiac mitochondria are listed. Each data point represents the experimentally measured relative abundance of a single mass isotopomer belonging to one of the constituent peptides of the protein at a particular time point. A first-order kinetics model was fitted to the data points using non-linear least-squares to derive the rate constant  $k$ . The standard error of  $k$  was calculated stochastically using the Monte Carlo method, by assuming a distribution for the absolute value of the residuals from fitting.

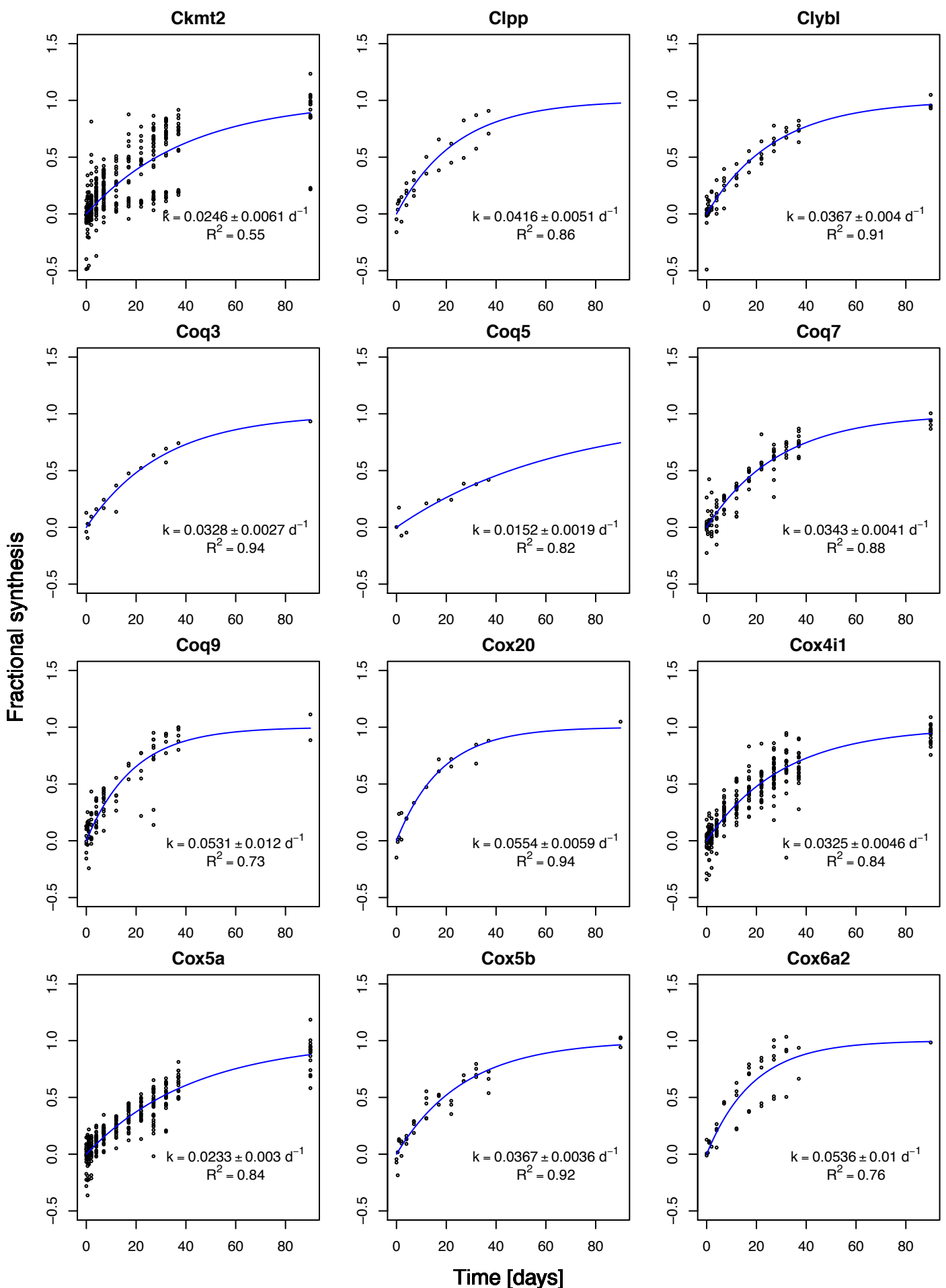




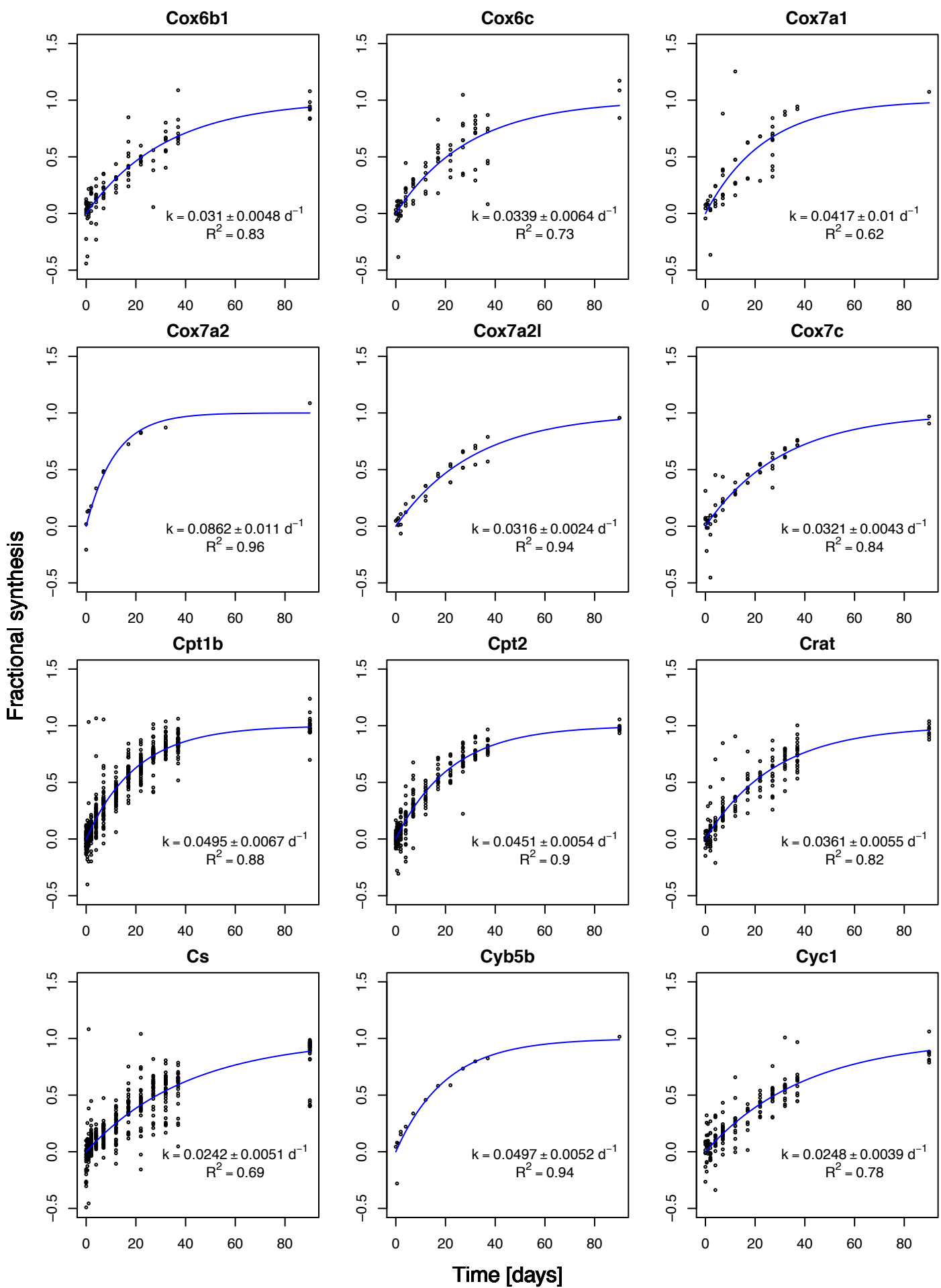


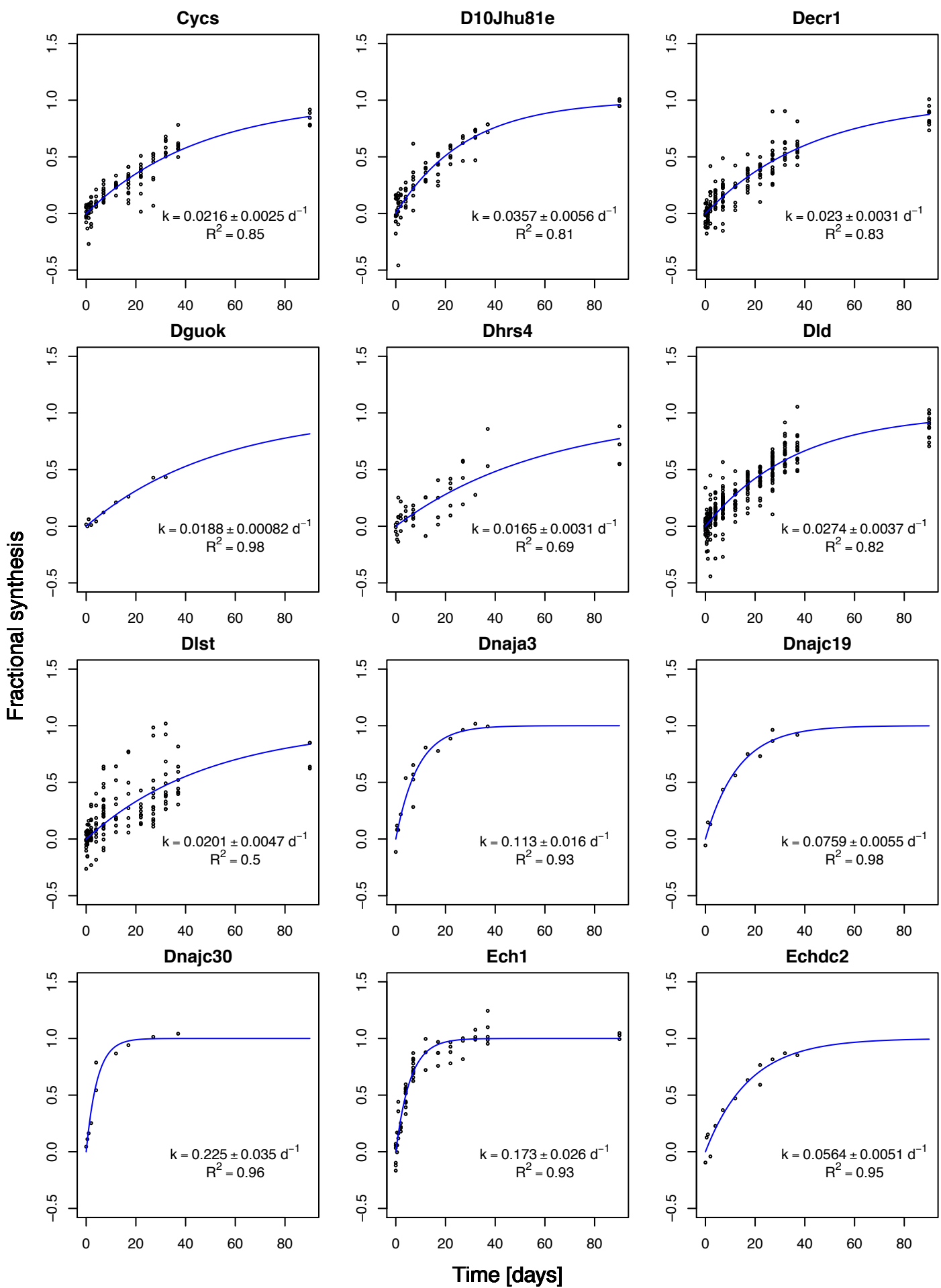


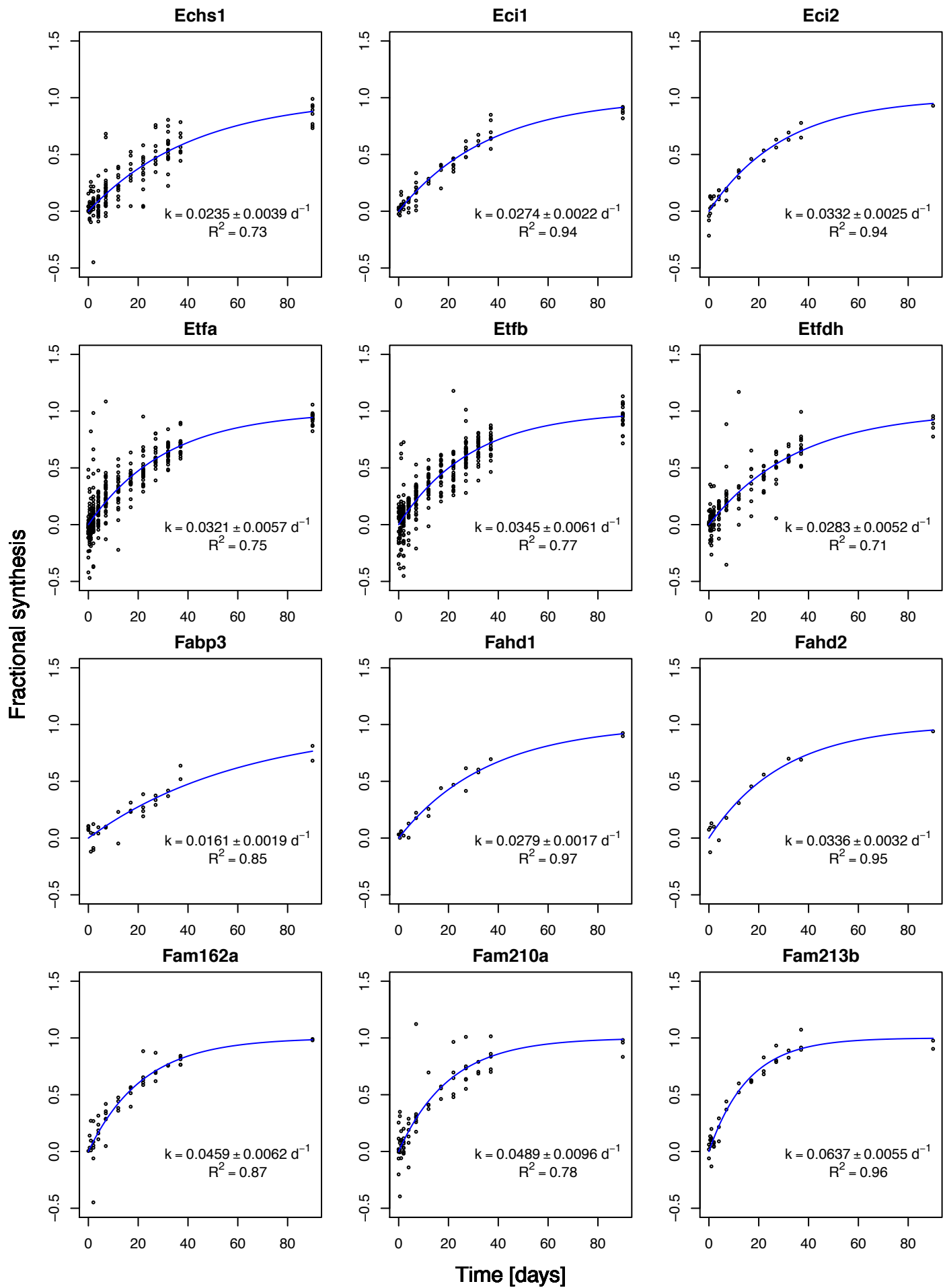


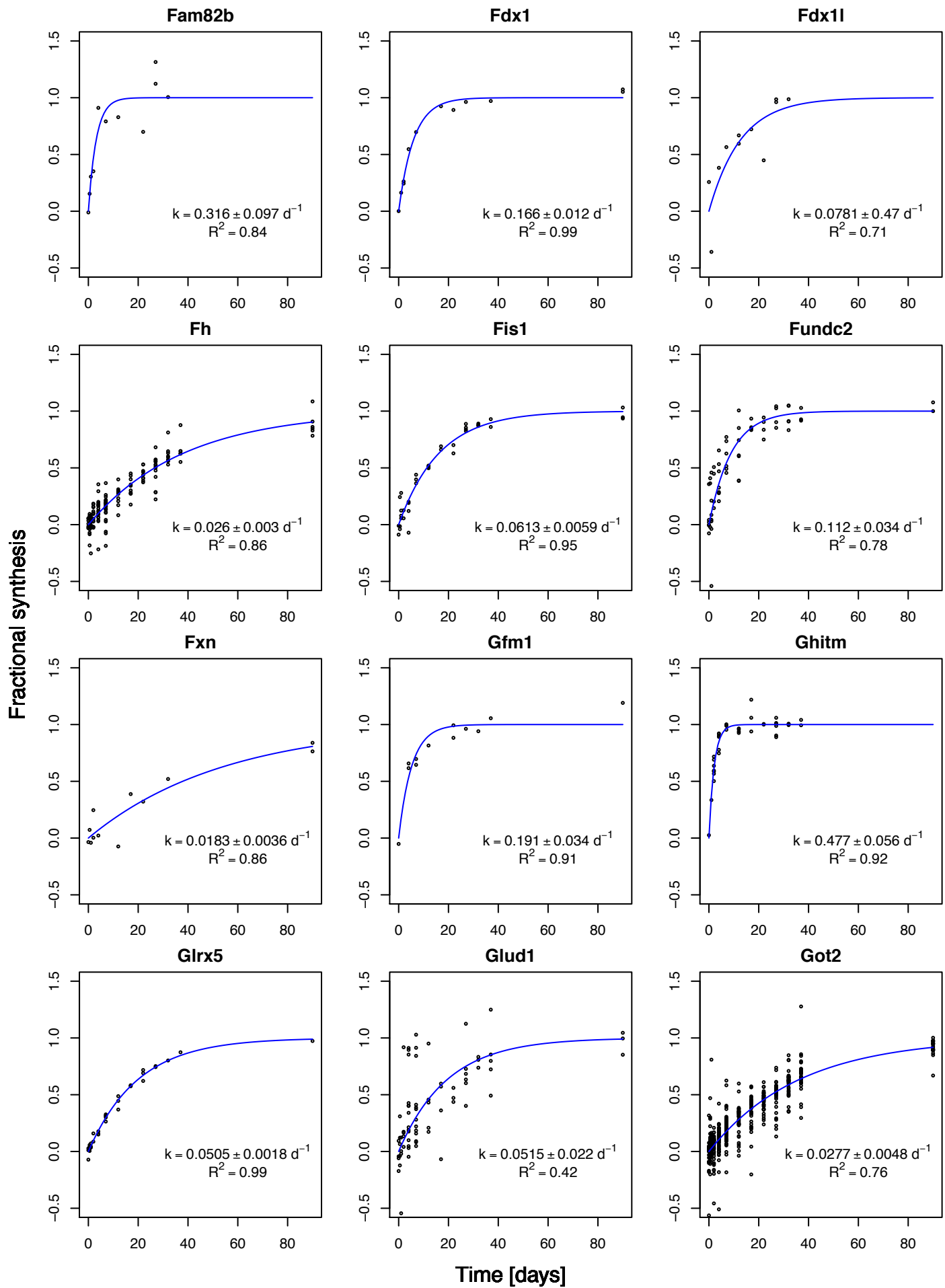


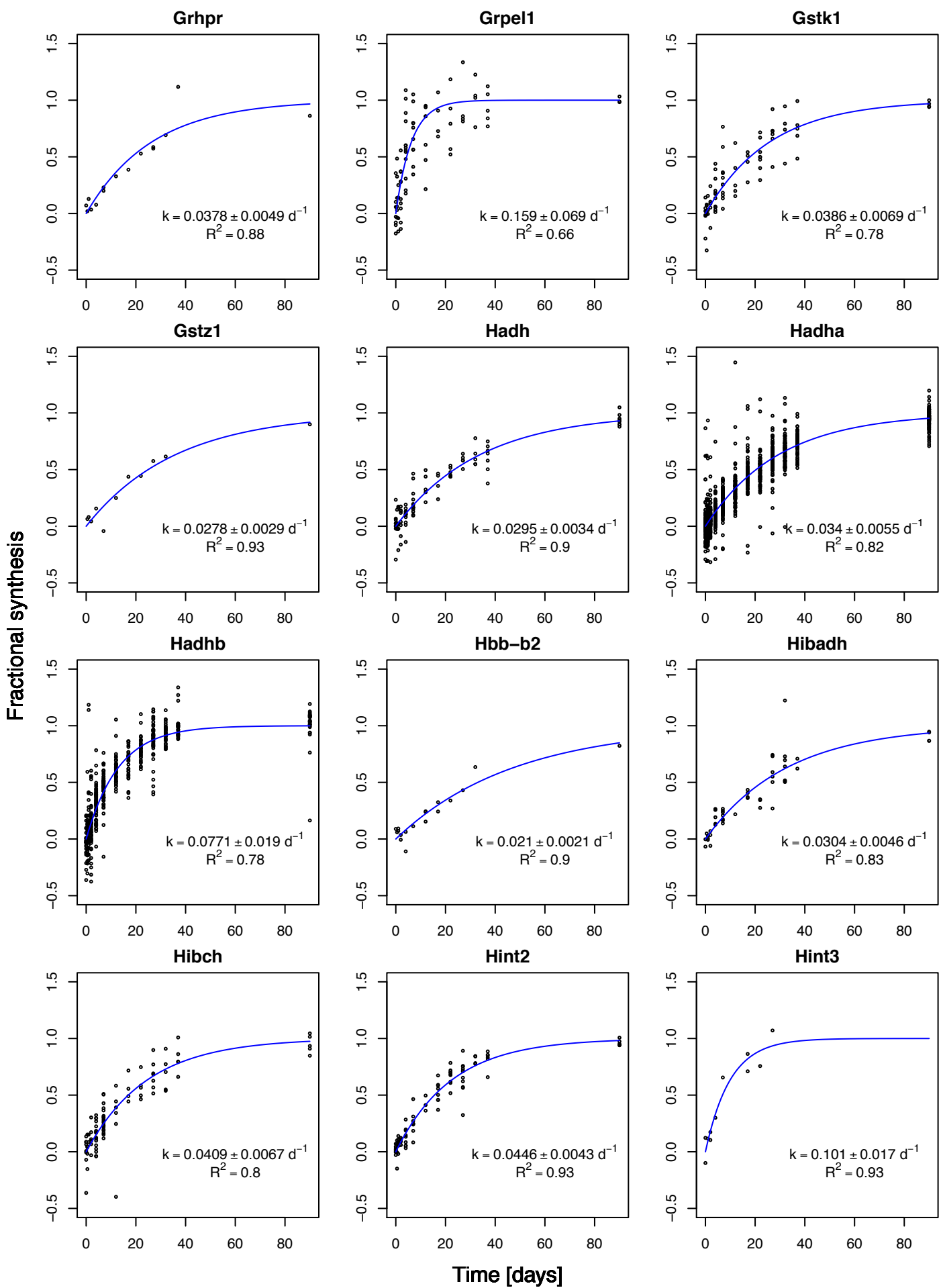


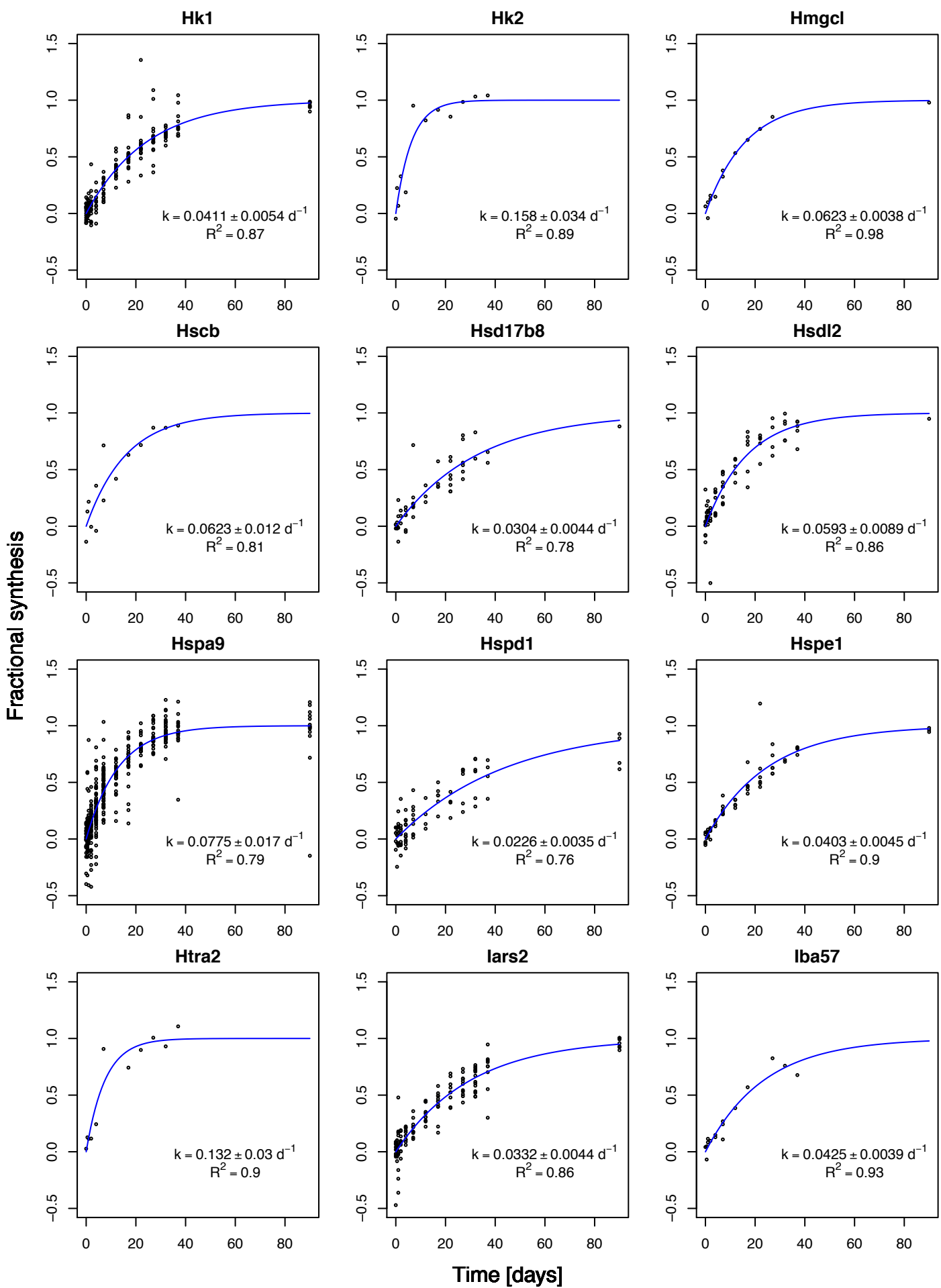


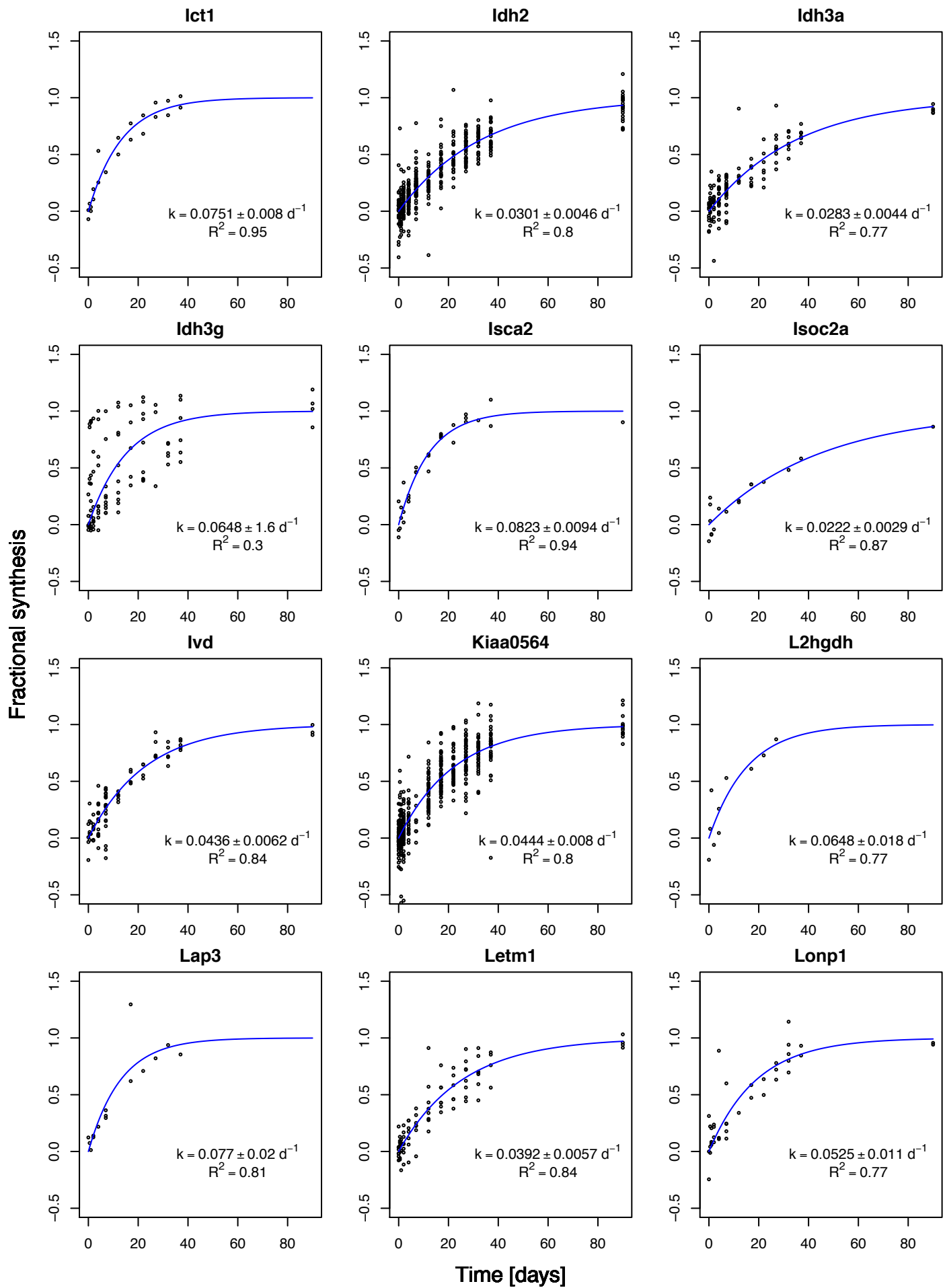


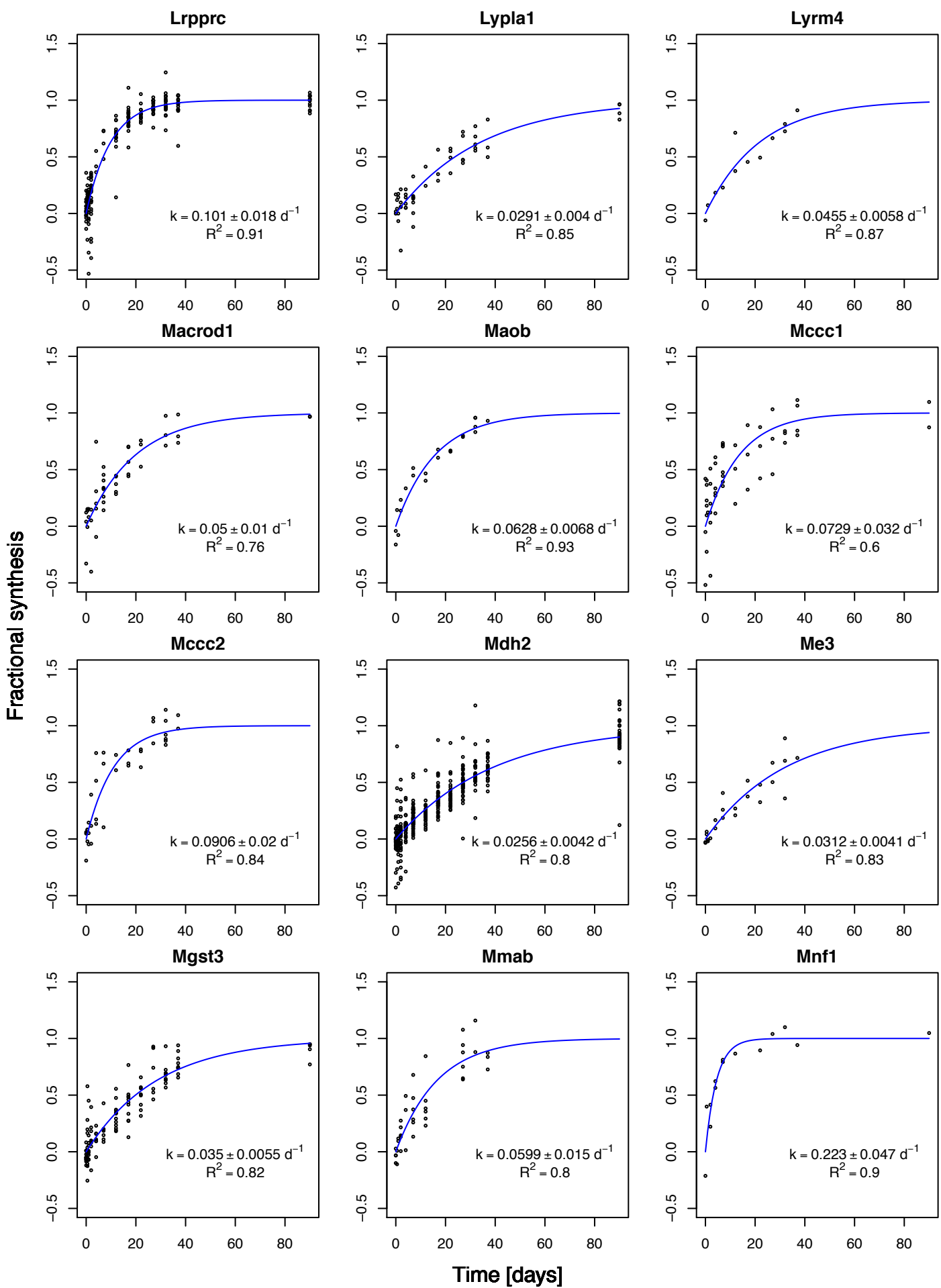




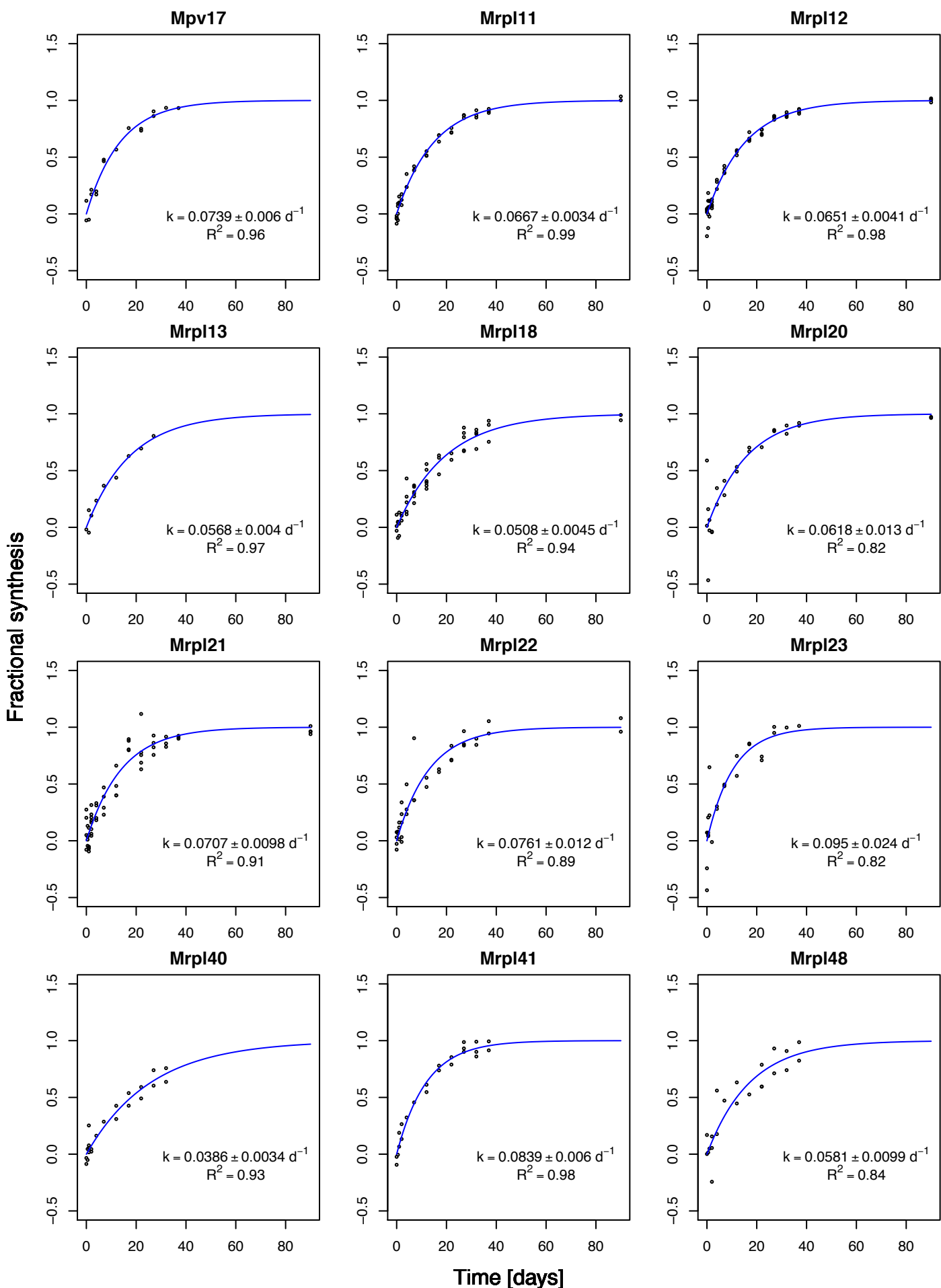


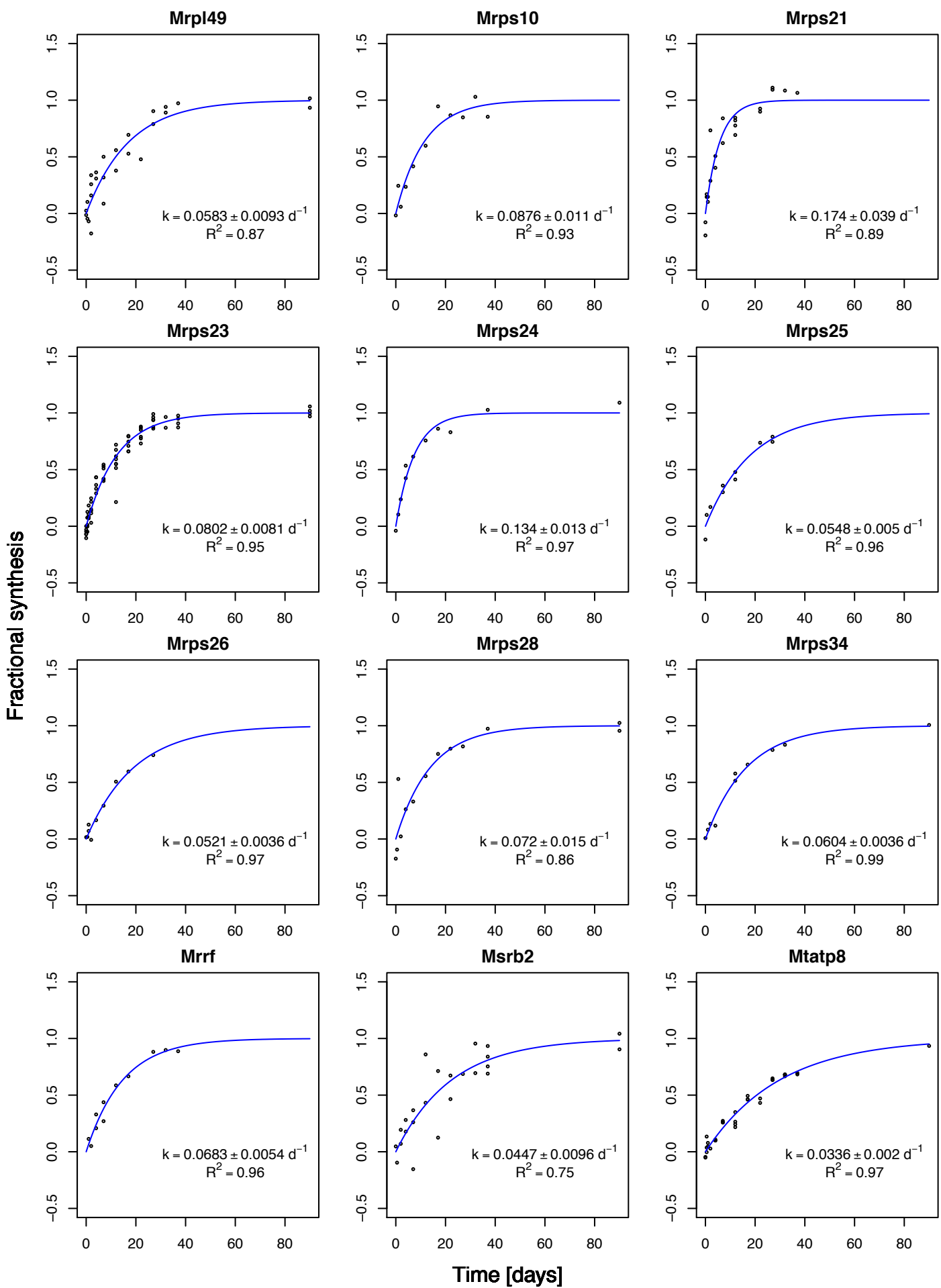


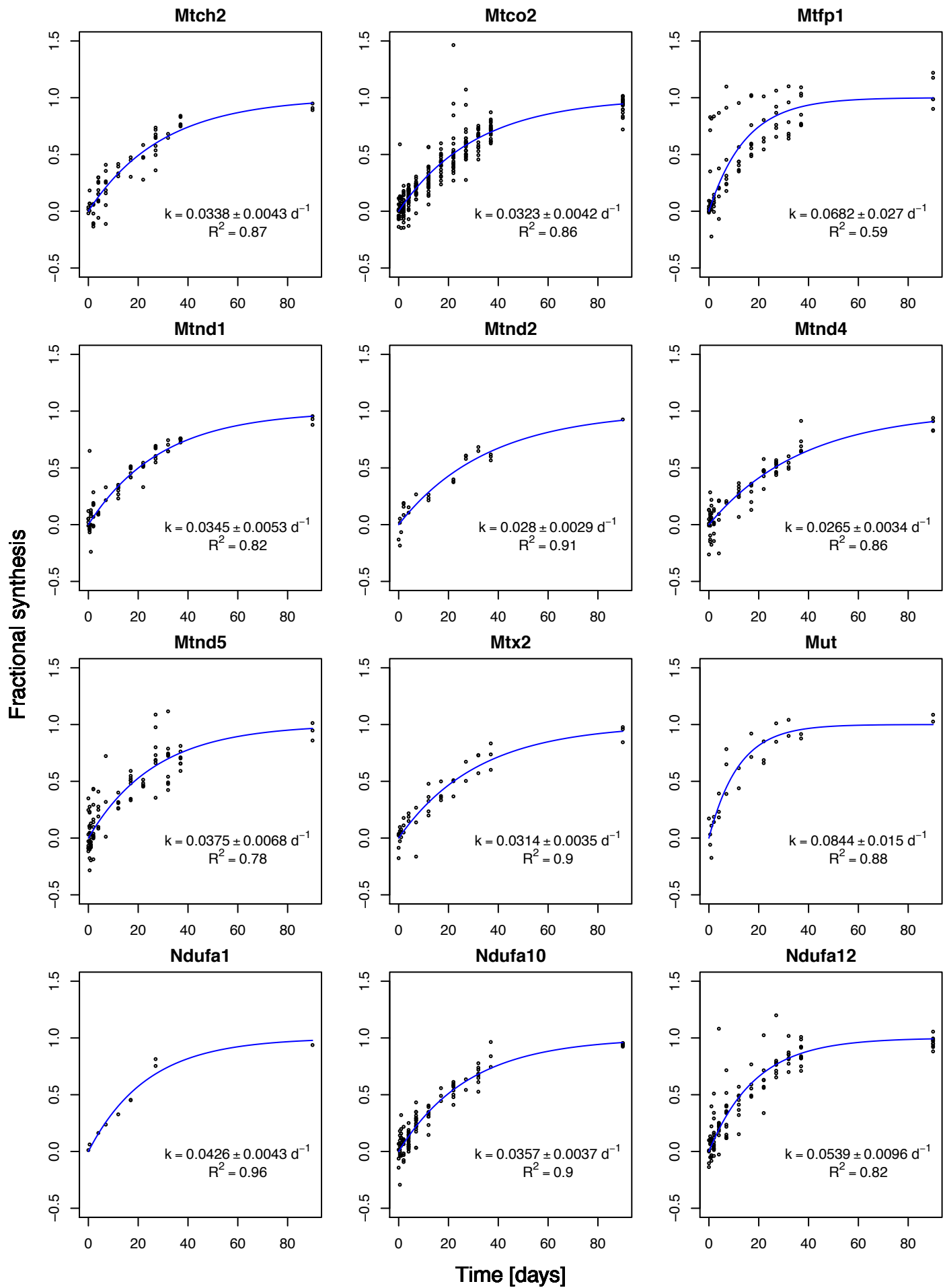


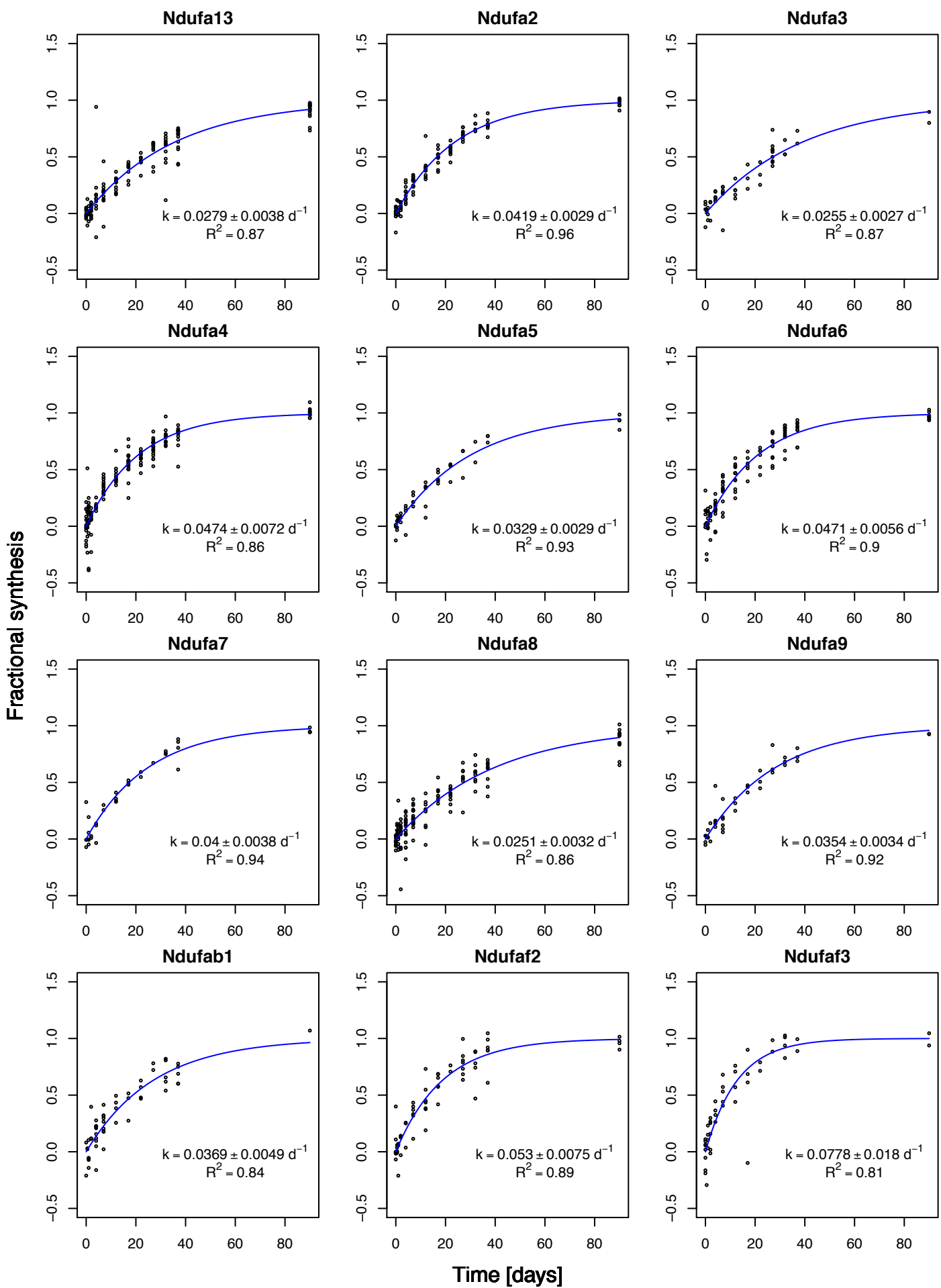


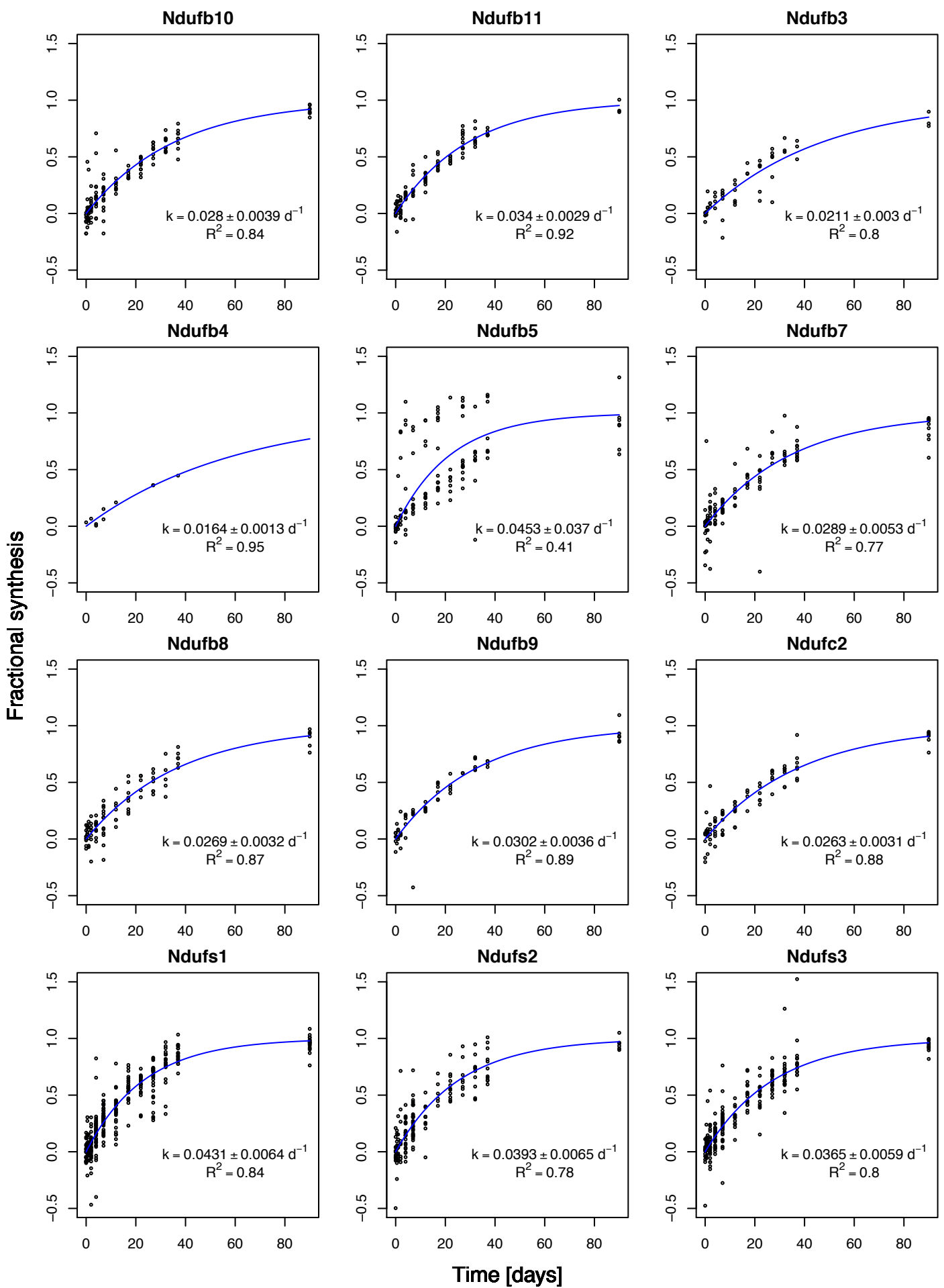




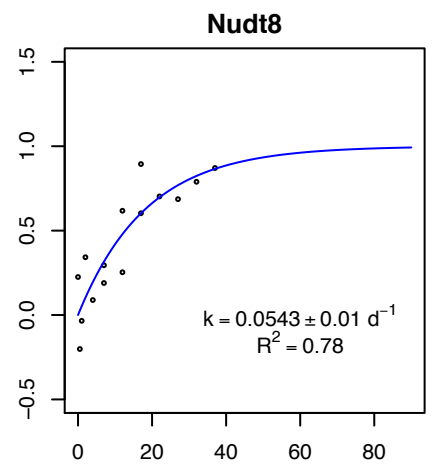
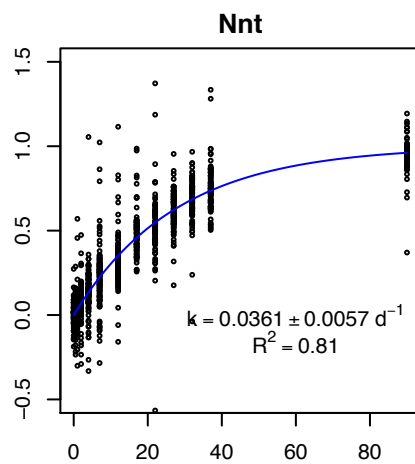
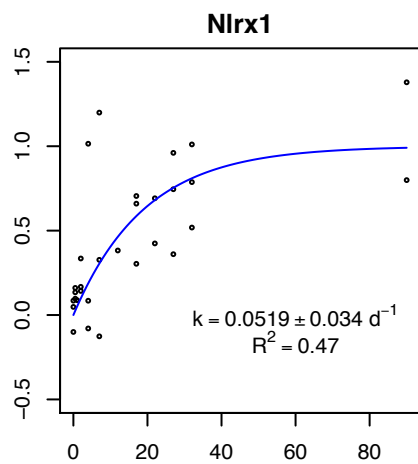
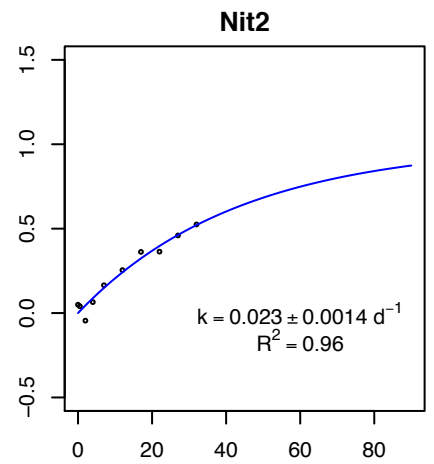
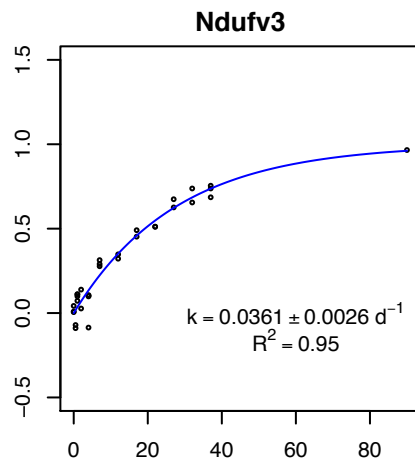
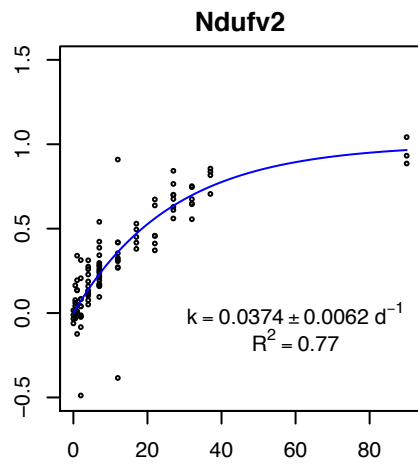
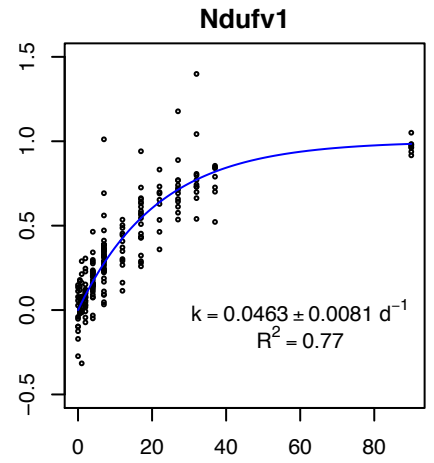
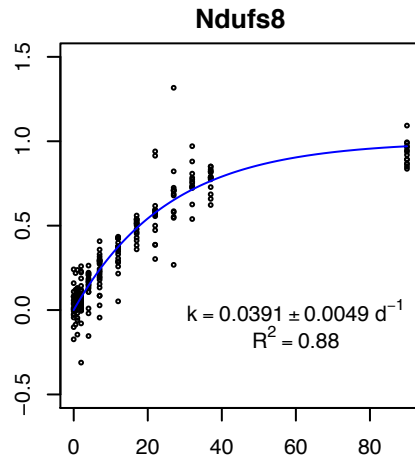
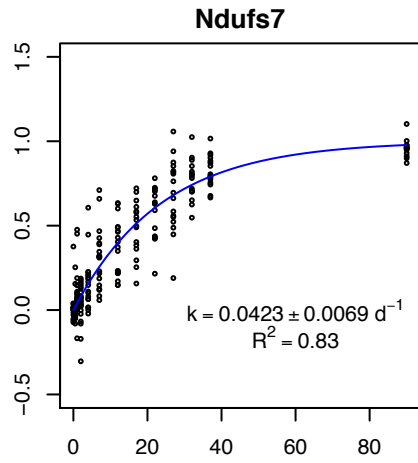
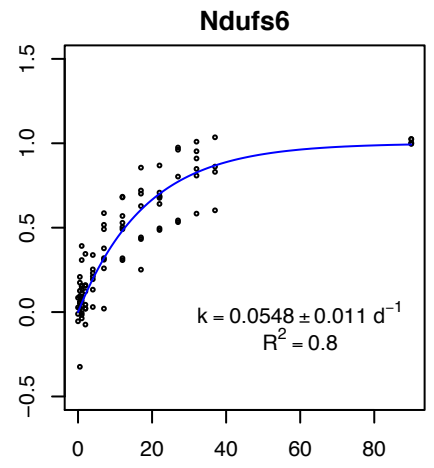
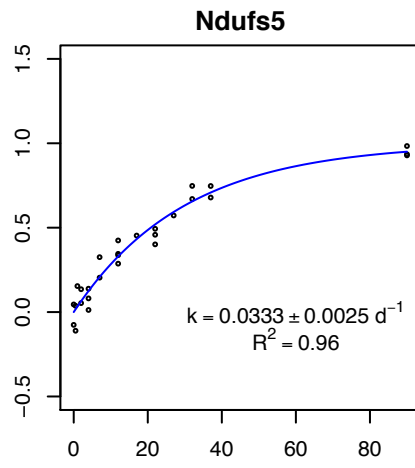
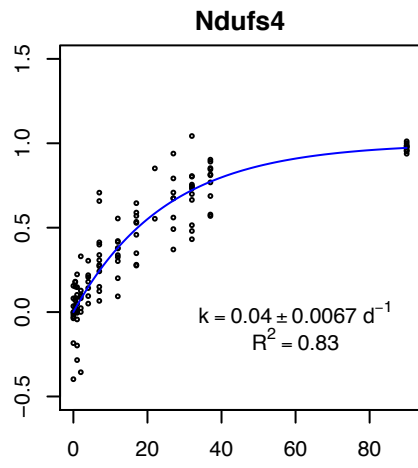




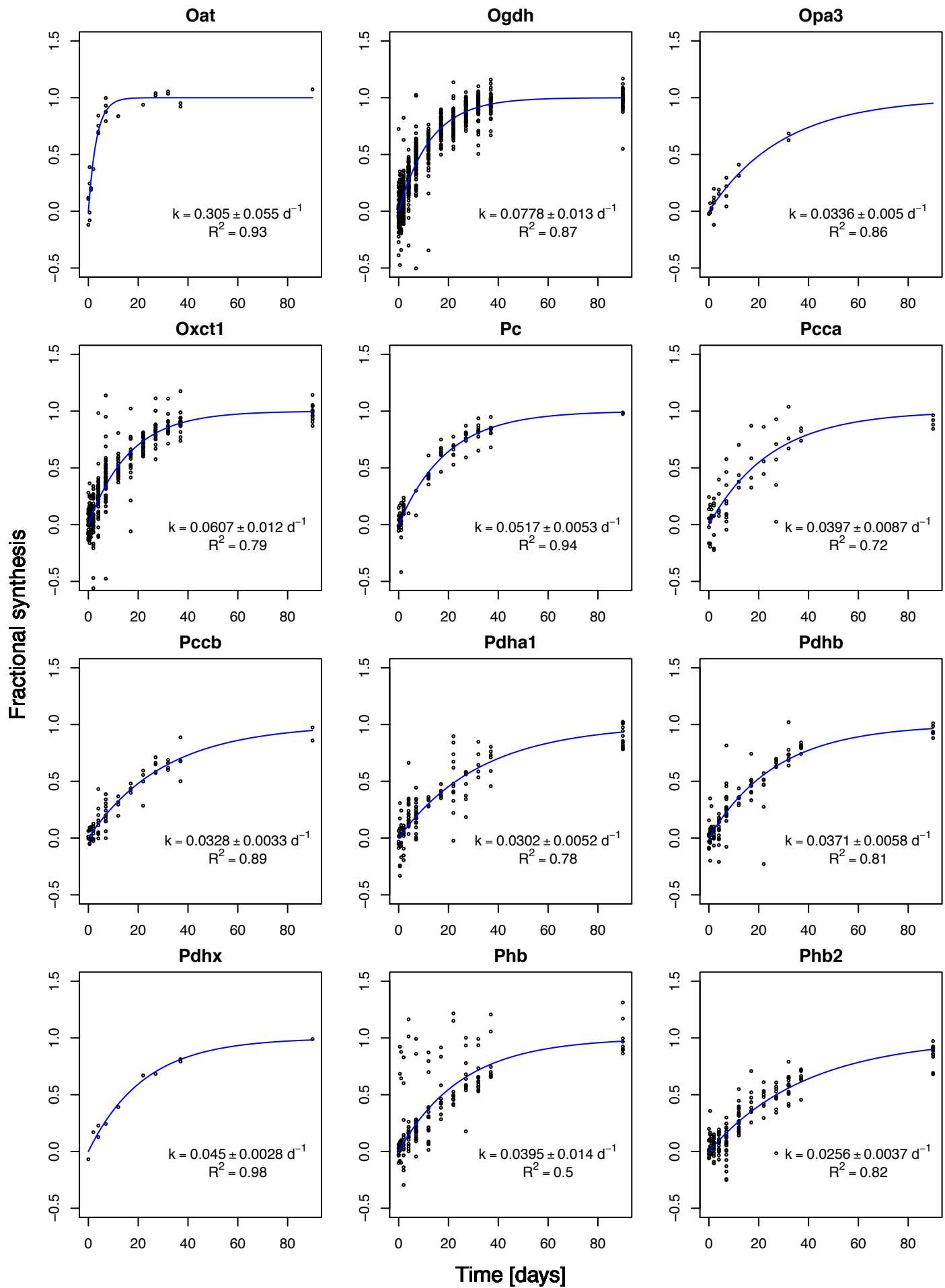


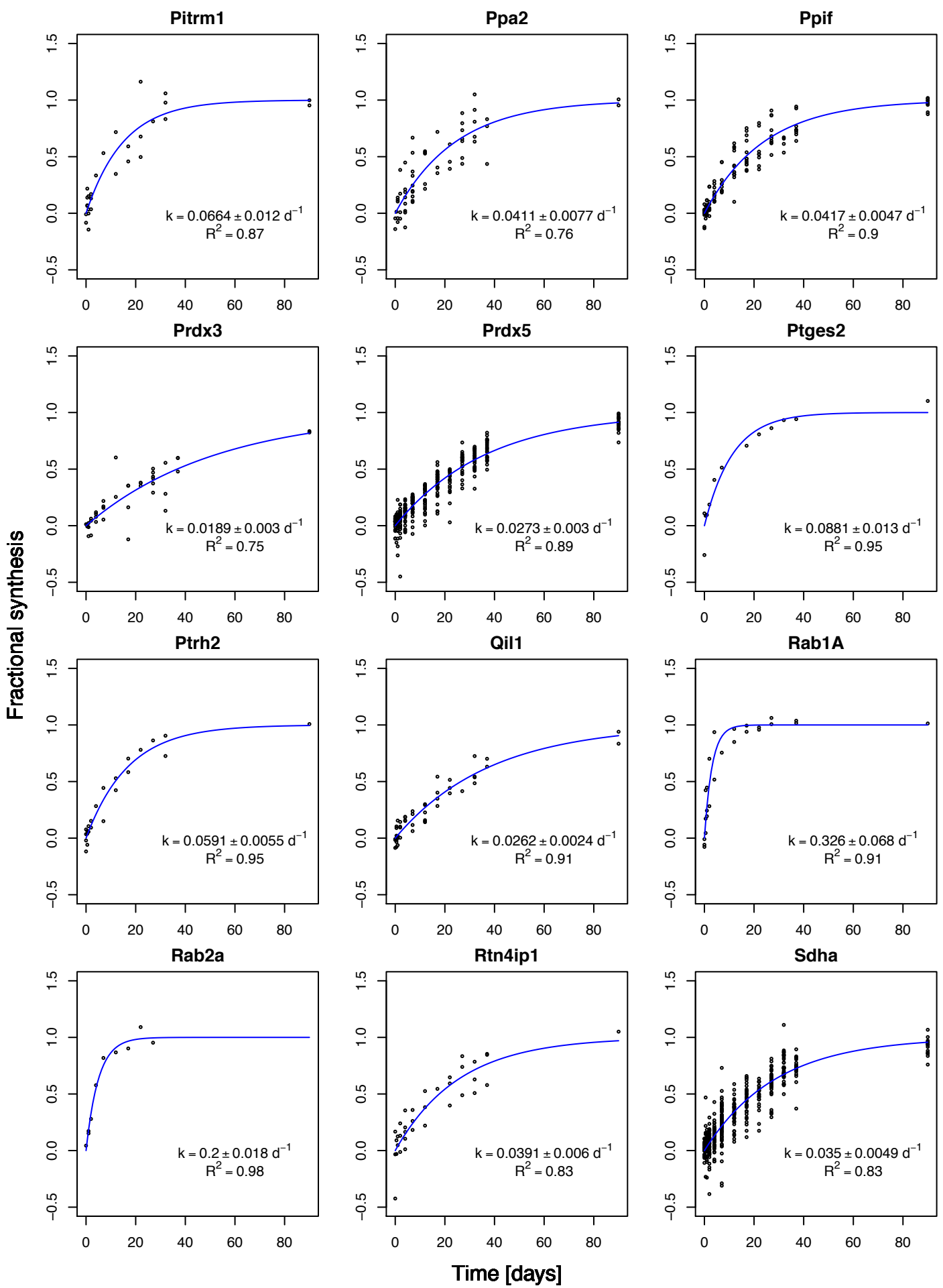


Fractional synthesis

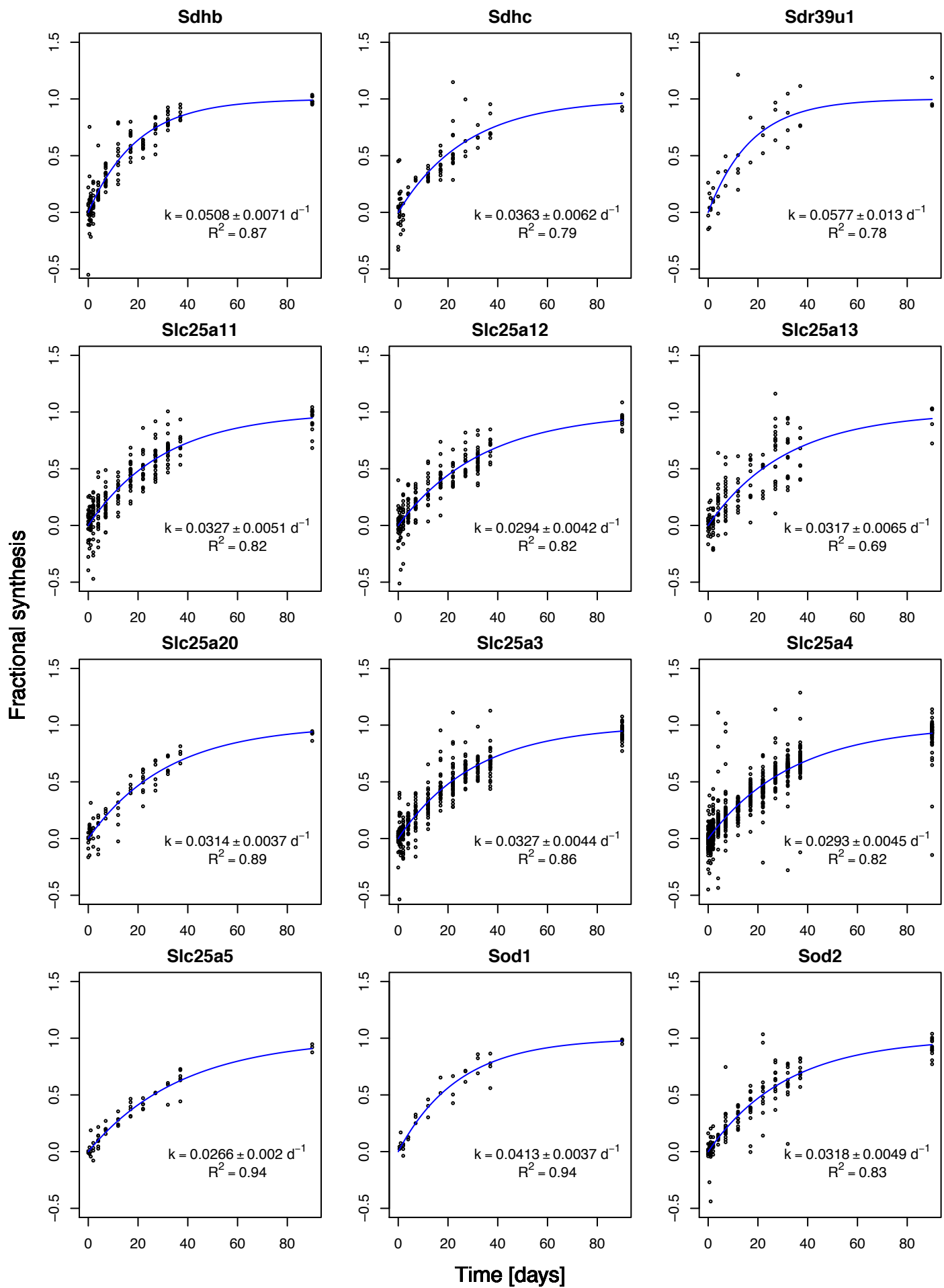


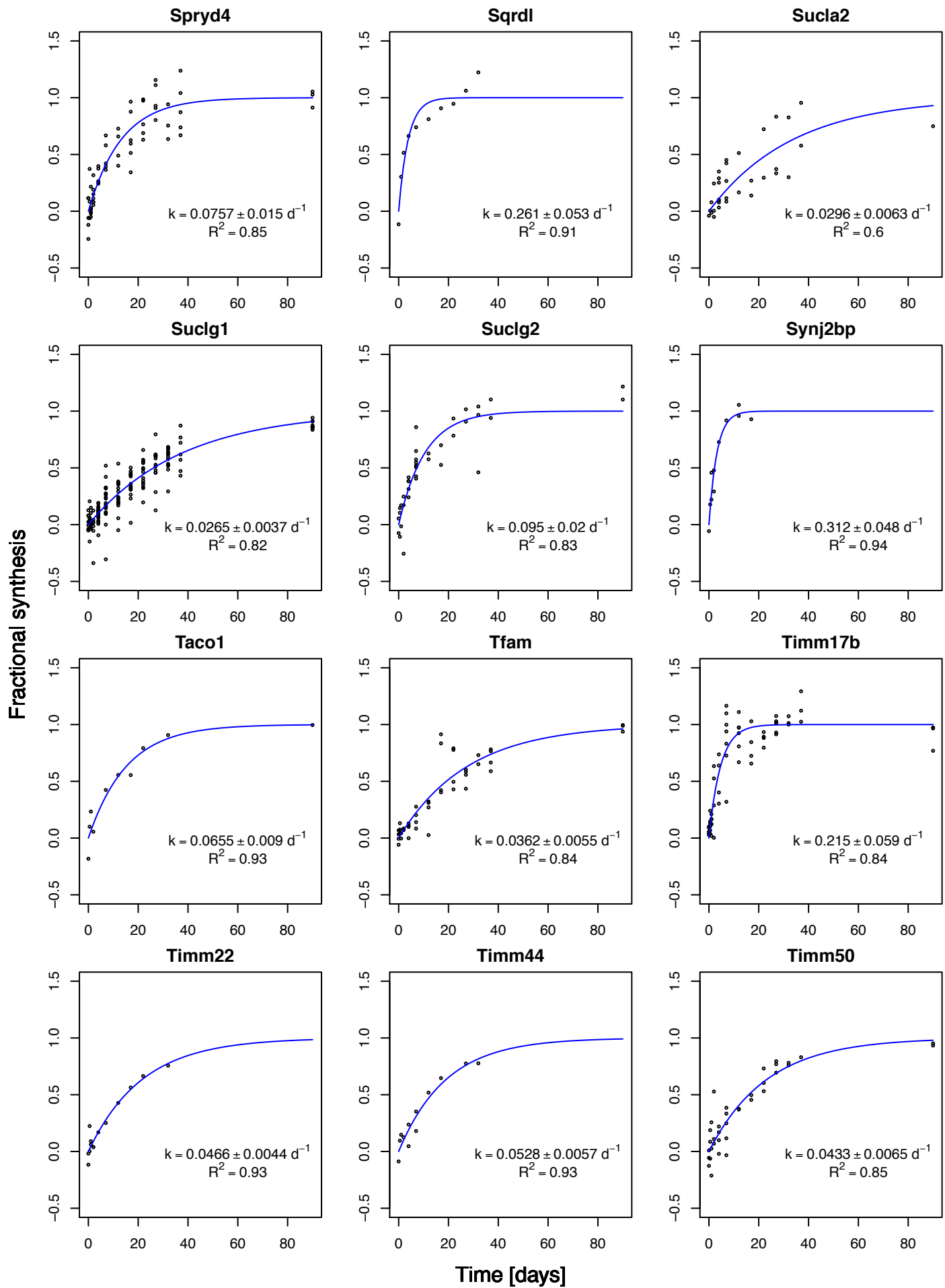
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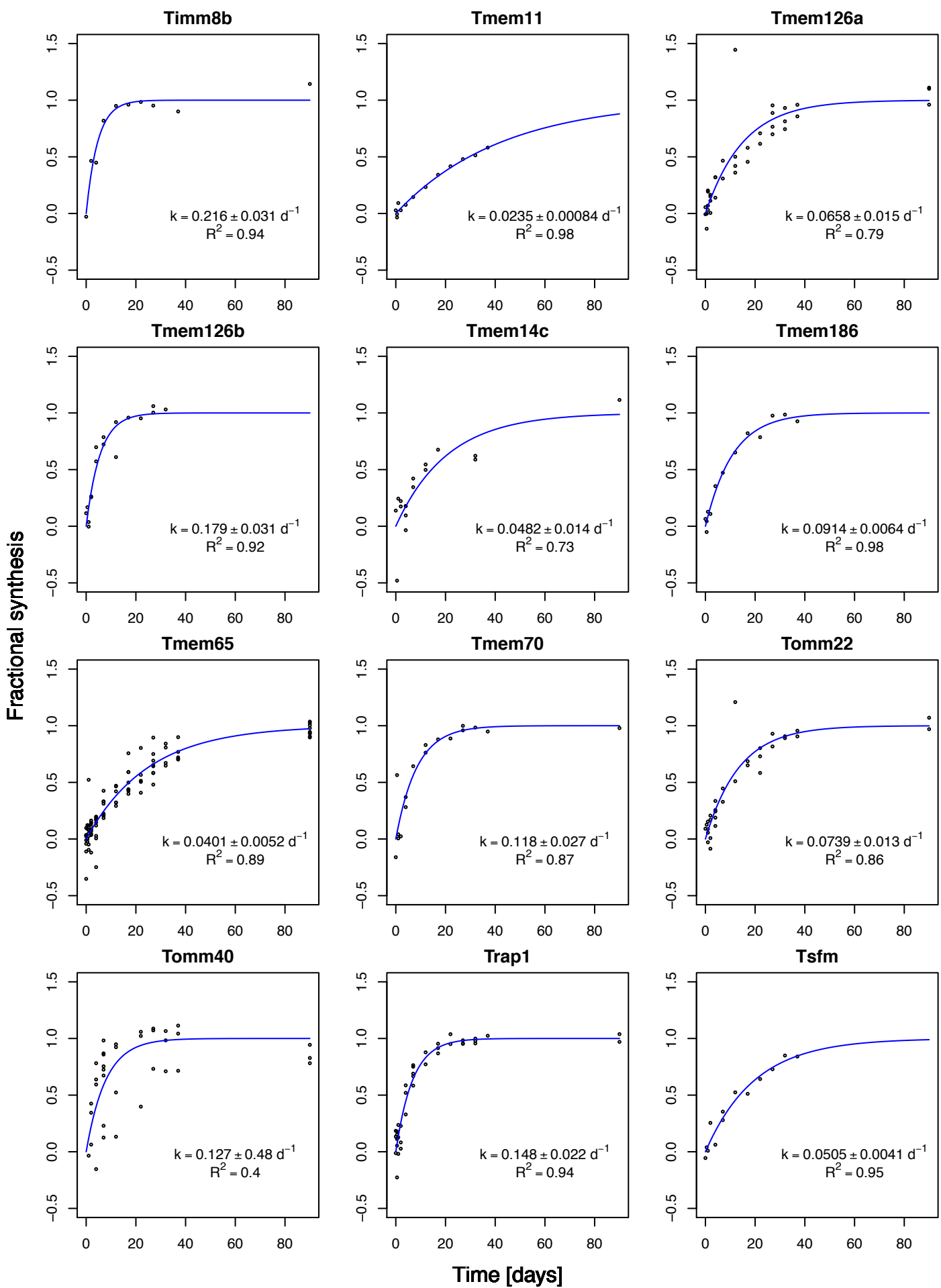


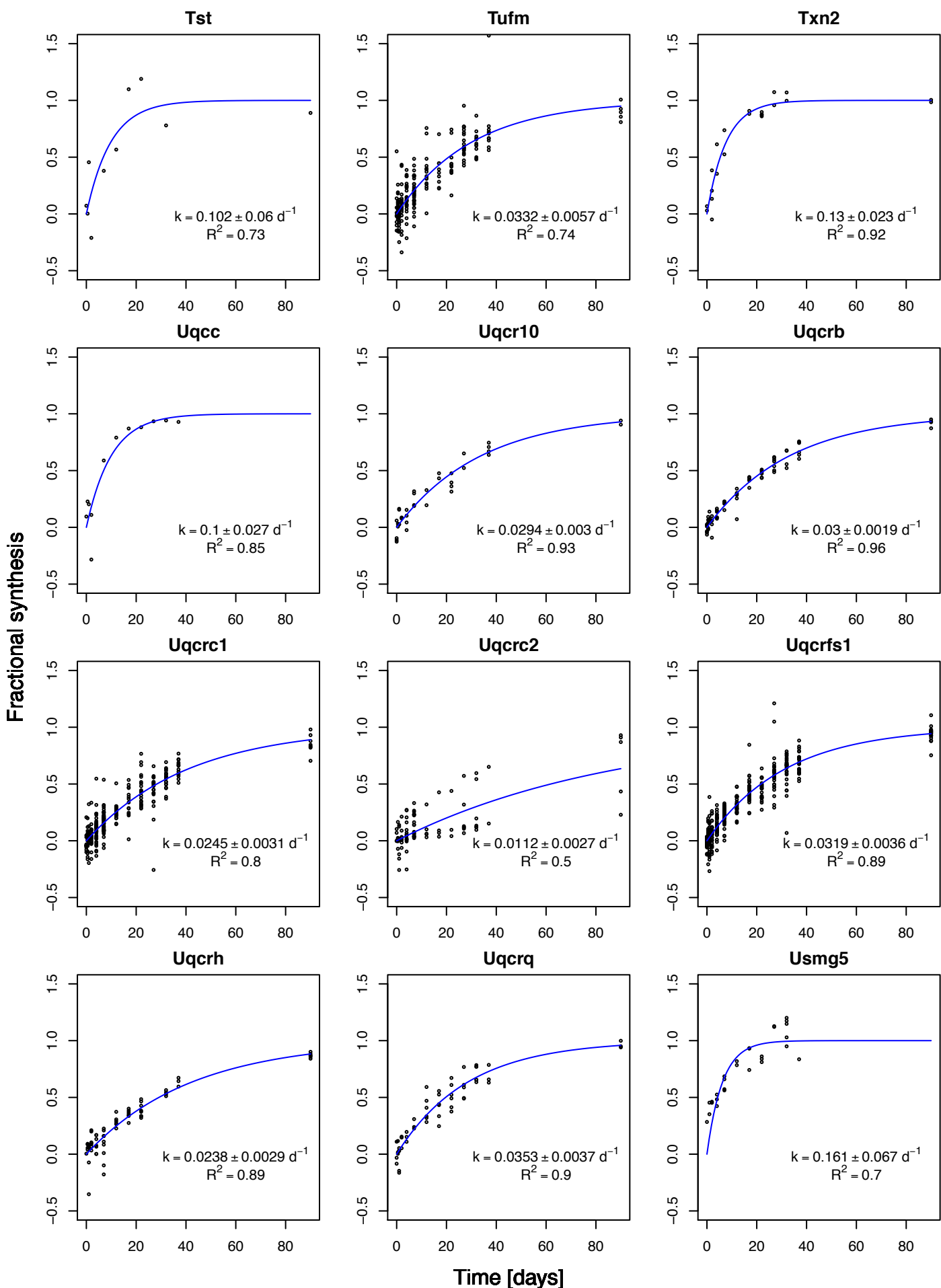




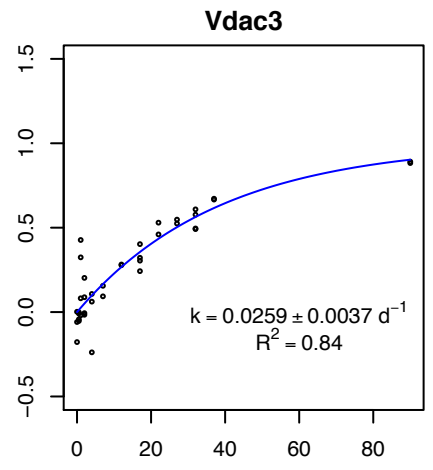
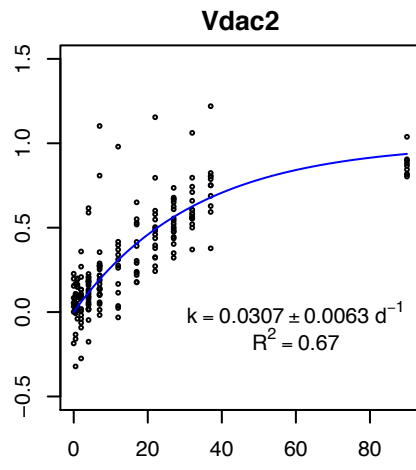
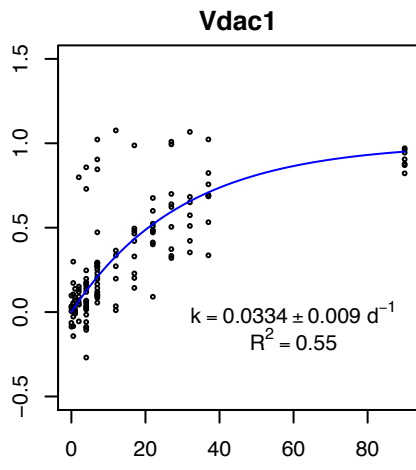








Fractional synthesis

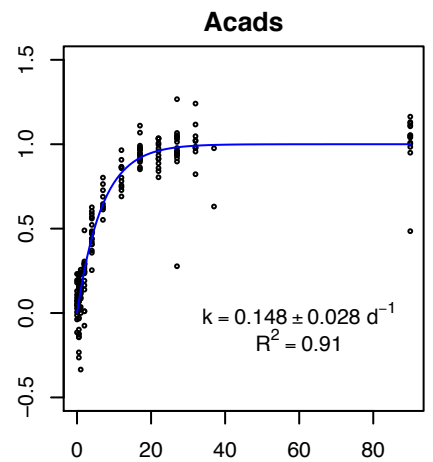
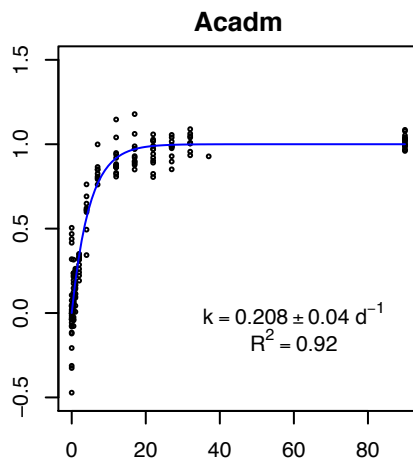
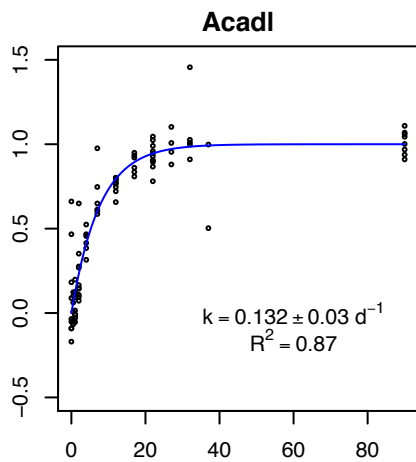
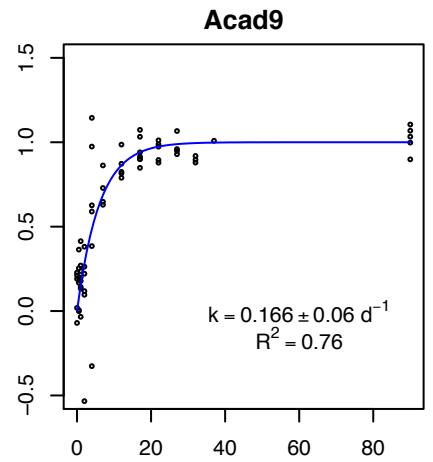
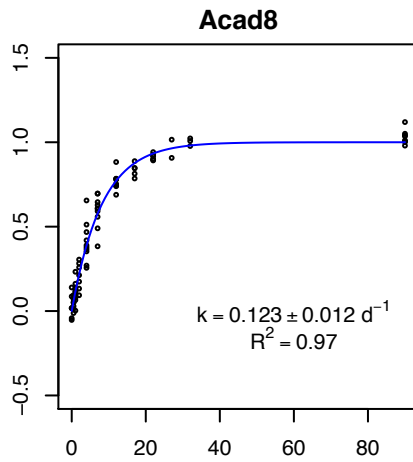
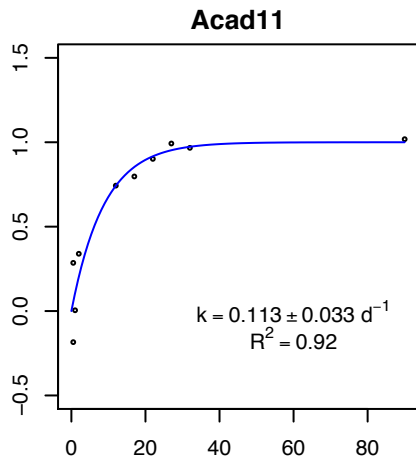
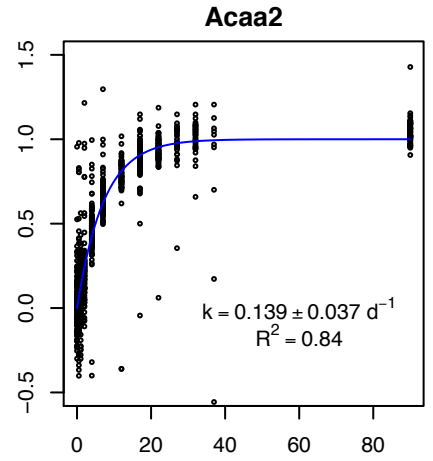
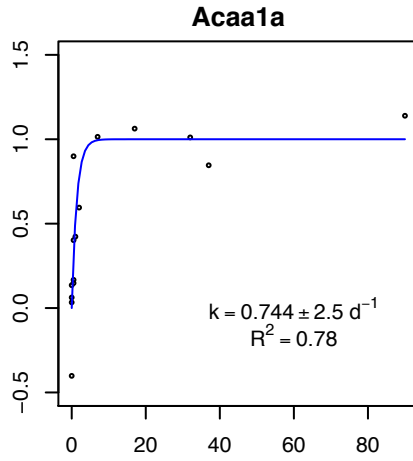
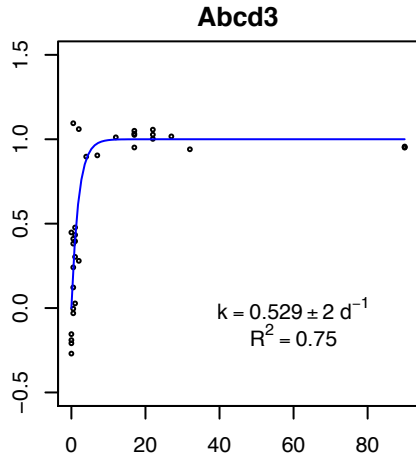
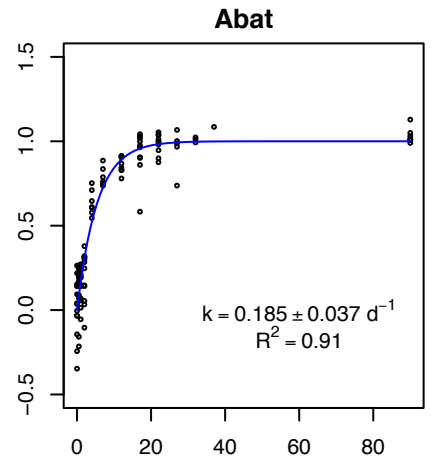
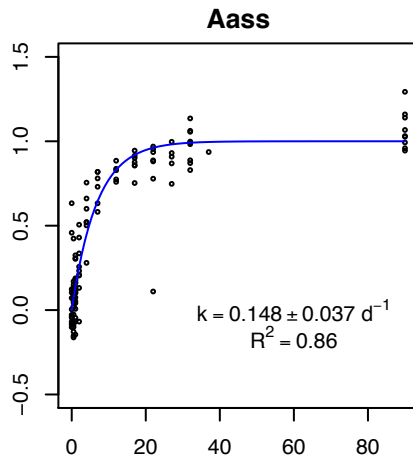
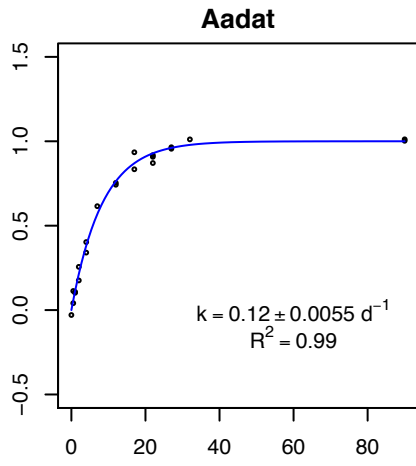


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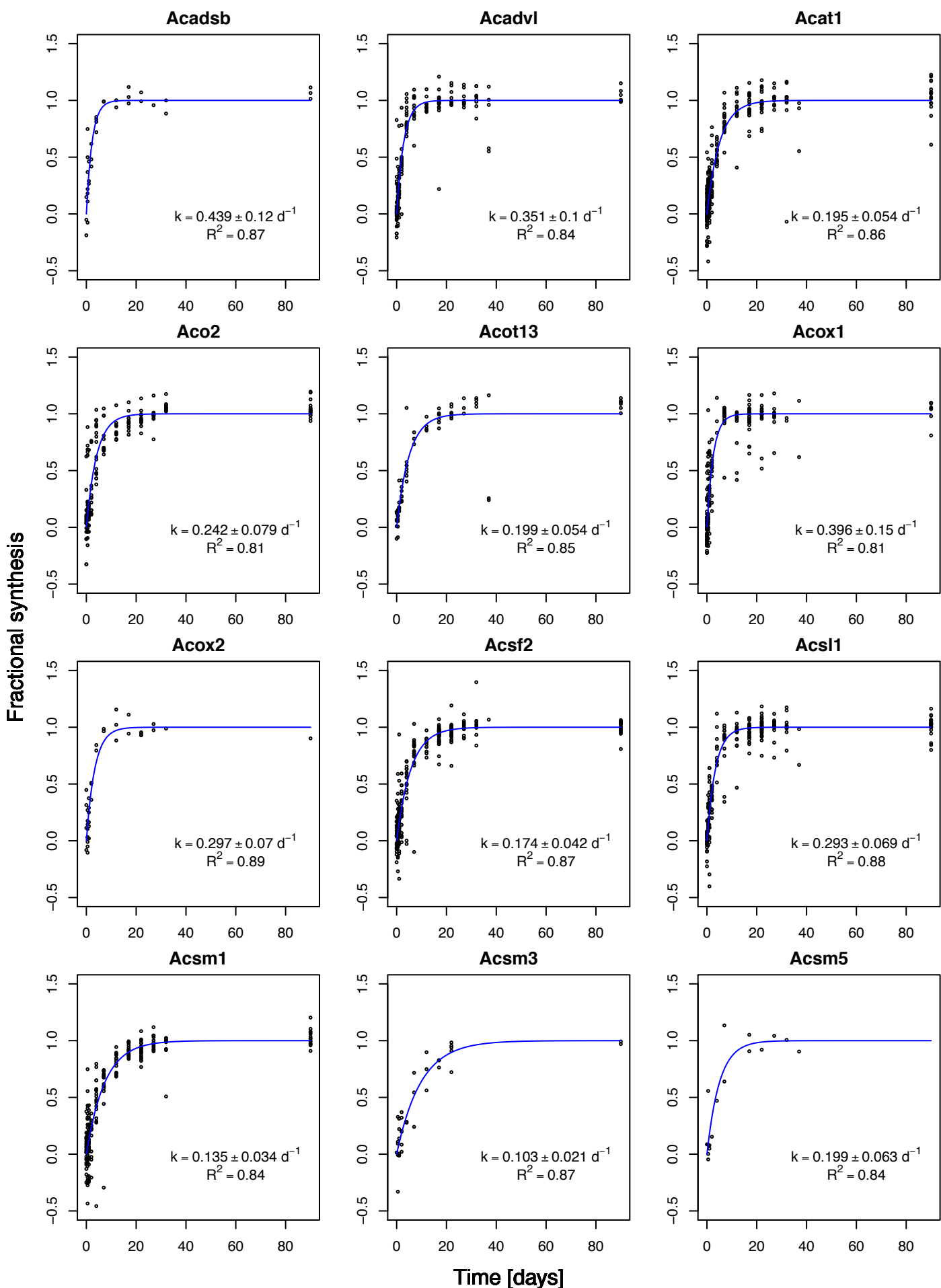
**Supplemental Fig. S2. Fractional synthesis of analyzed hepatic mitochondrial proteins as a function of time follows first-order kinetics.**

The fractional syntheses of all 386 proteins analyzed in hepatic mitochondria are listed. Each data point represents the experimentally measured relative abundance of a single mass isotopomer belonging to one of the constituent peptides of the protein at a particular time point. A first-order kinetics model was fitted to the data points using non-linear least-squares to derive the rate constant  $k$ . The standard error of  $k$  was calculated stochastically using the Monte Carlo method, by assuming a distribution for the absolute value of the residuals from fitting.

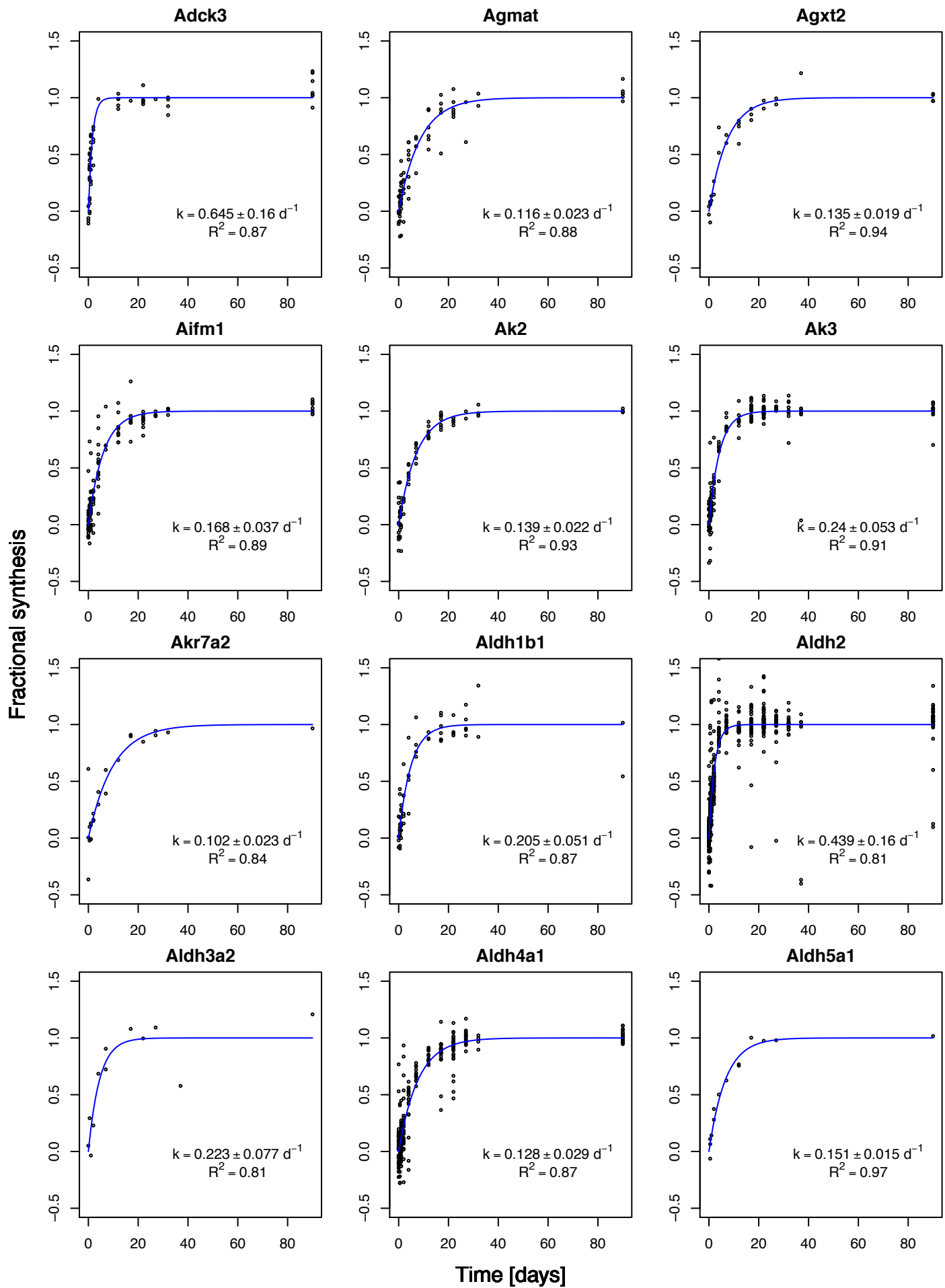
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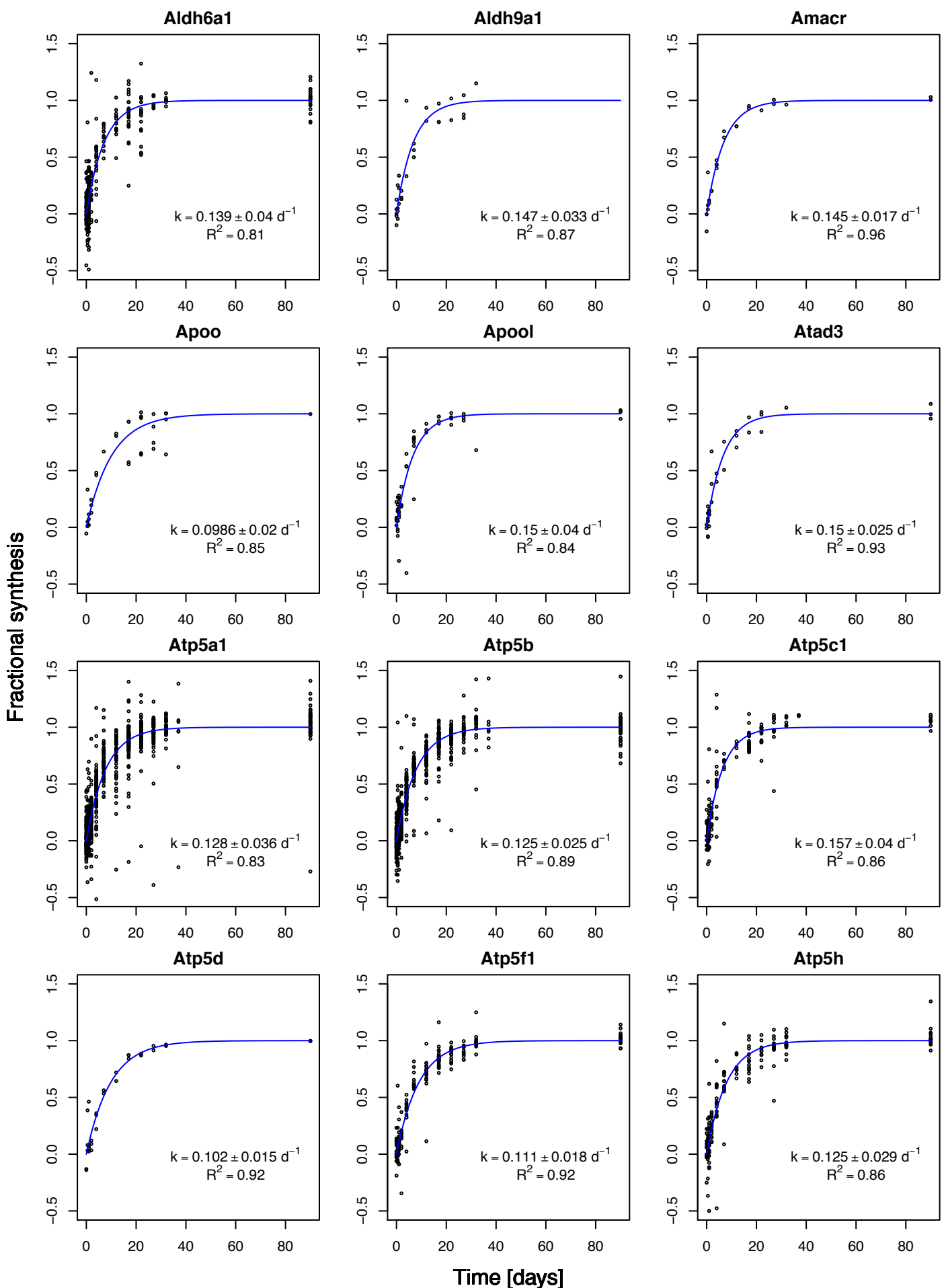


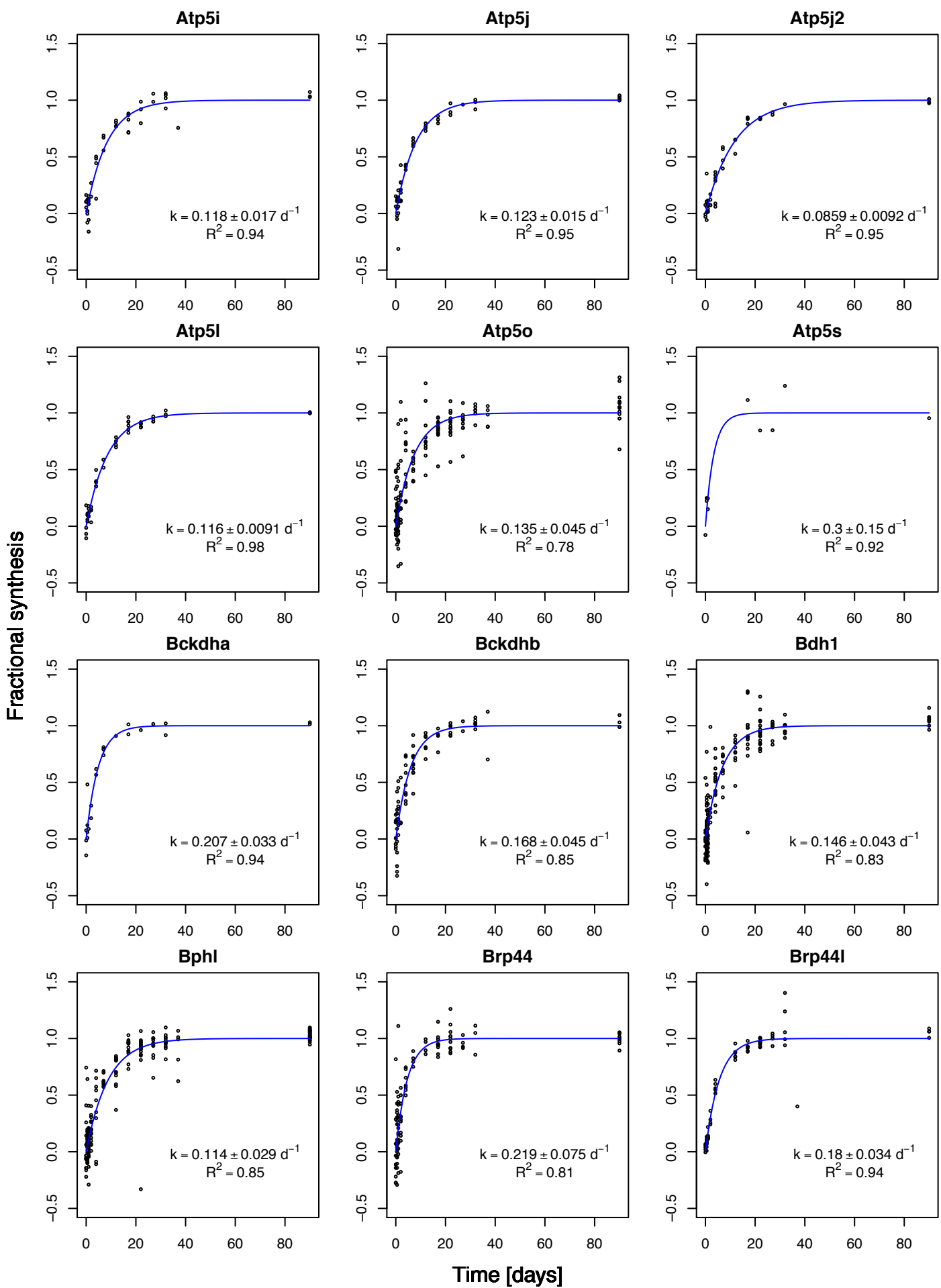
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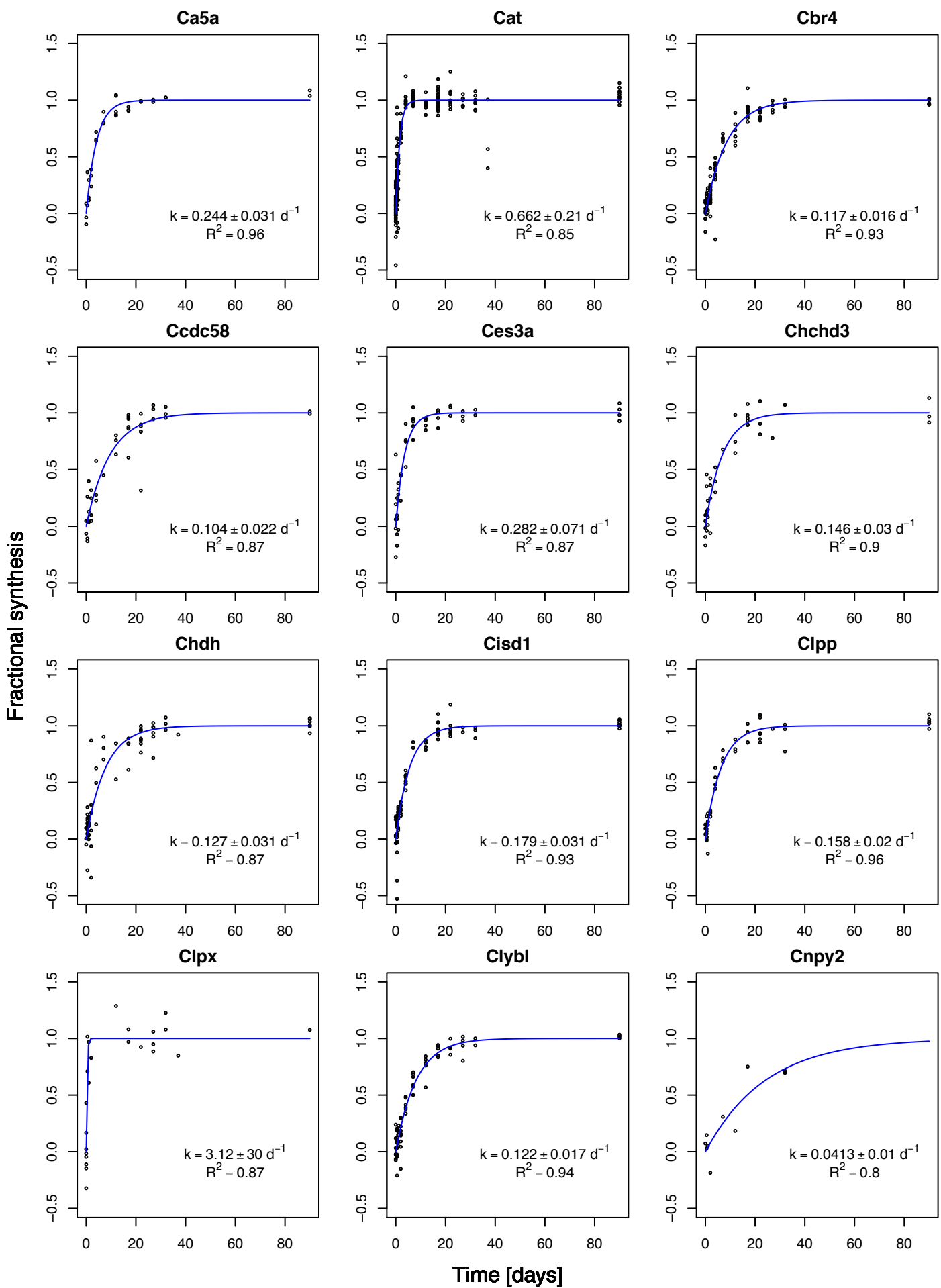


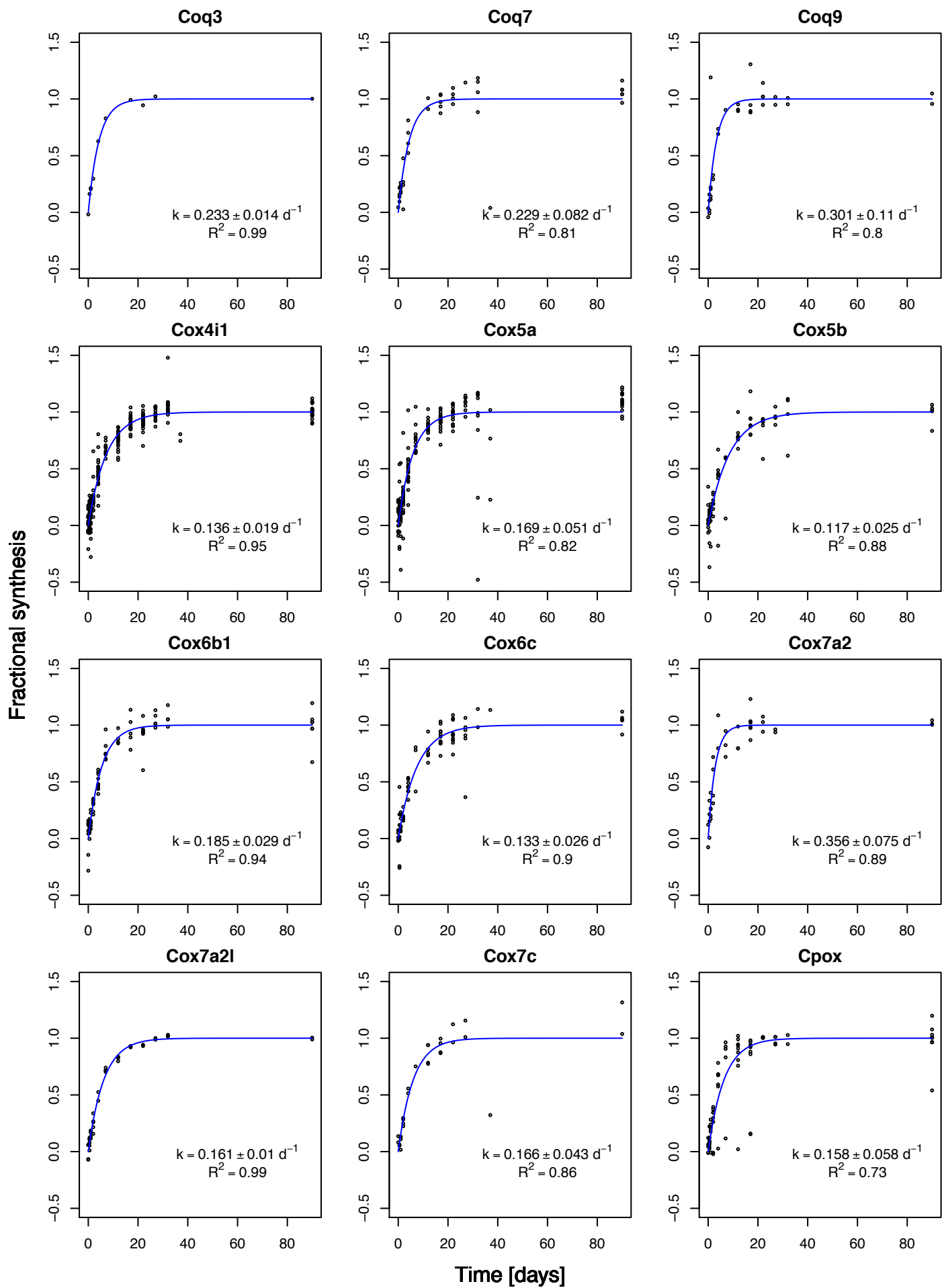


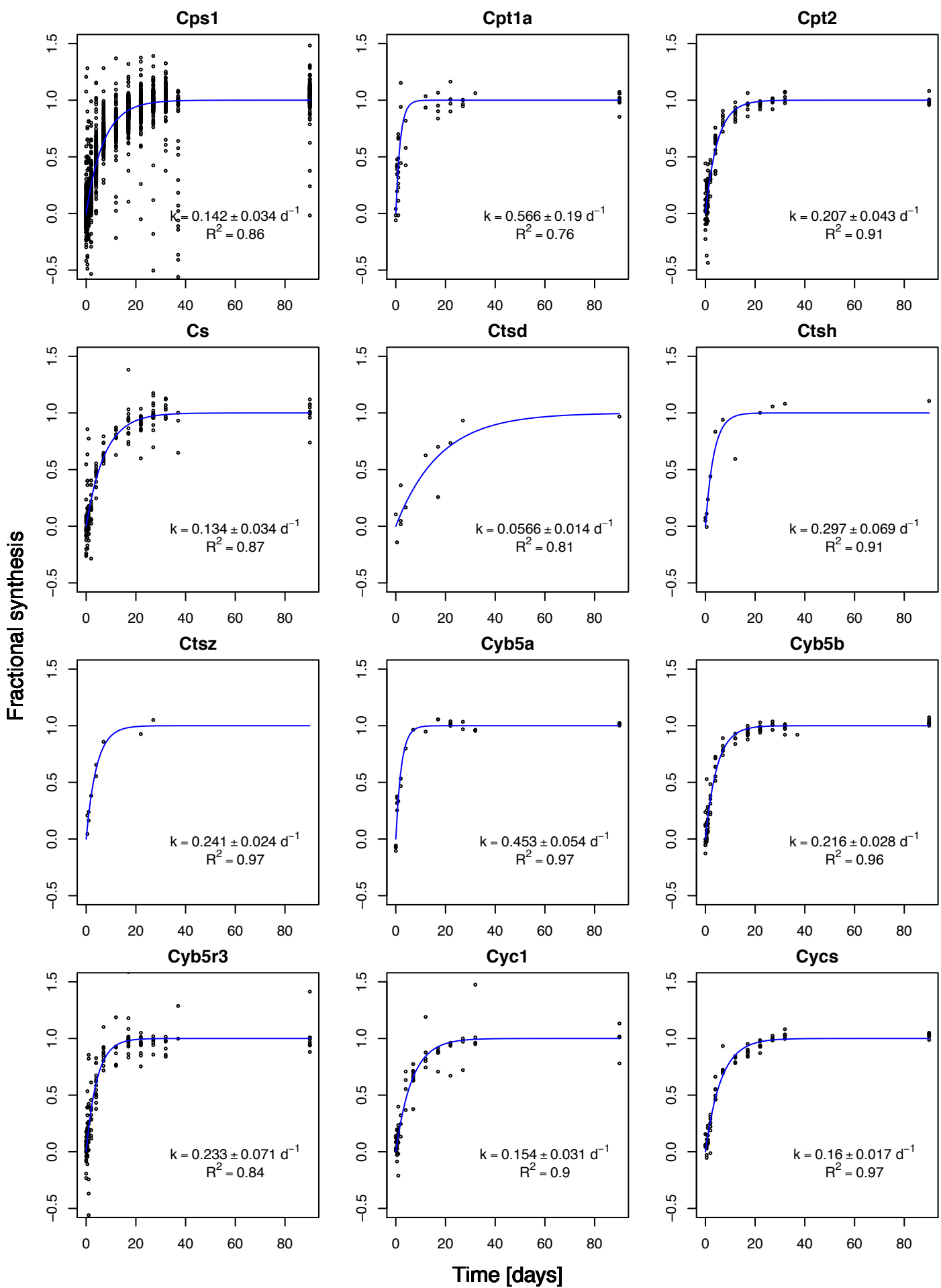


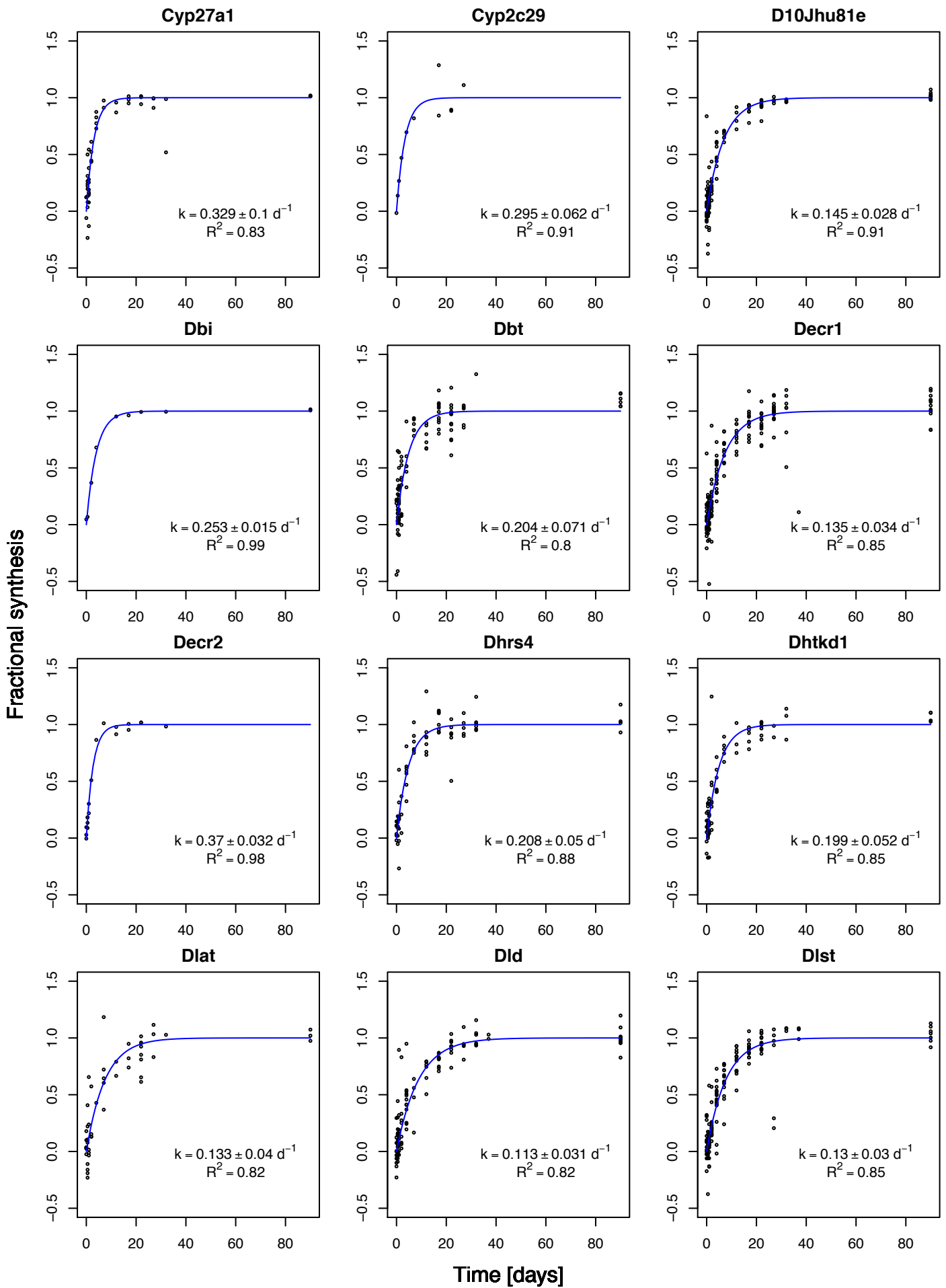


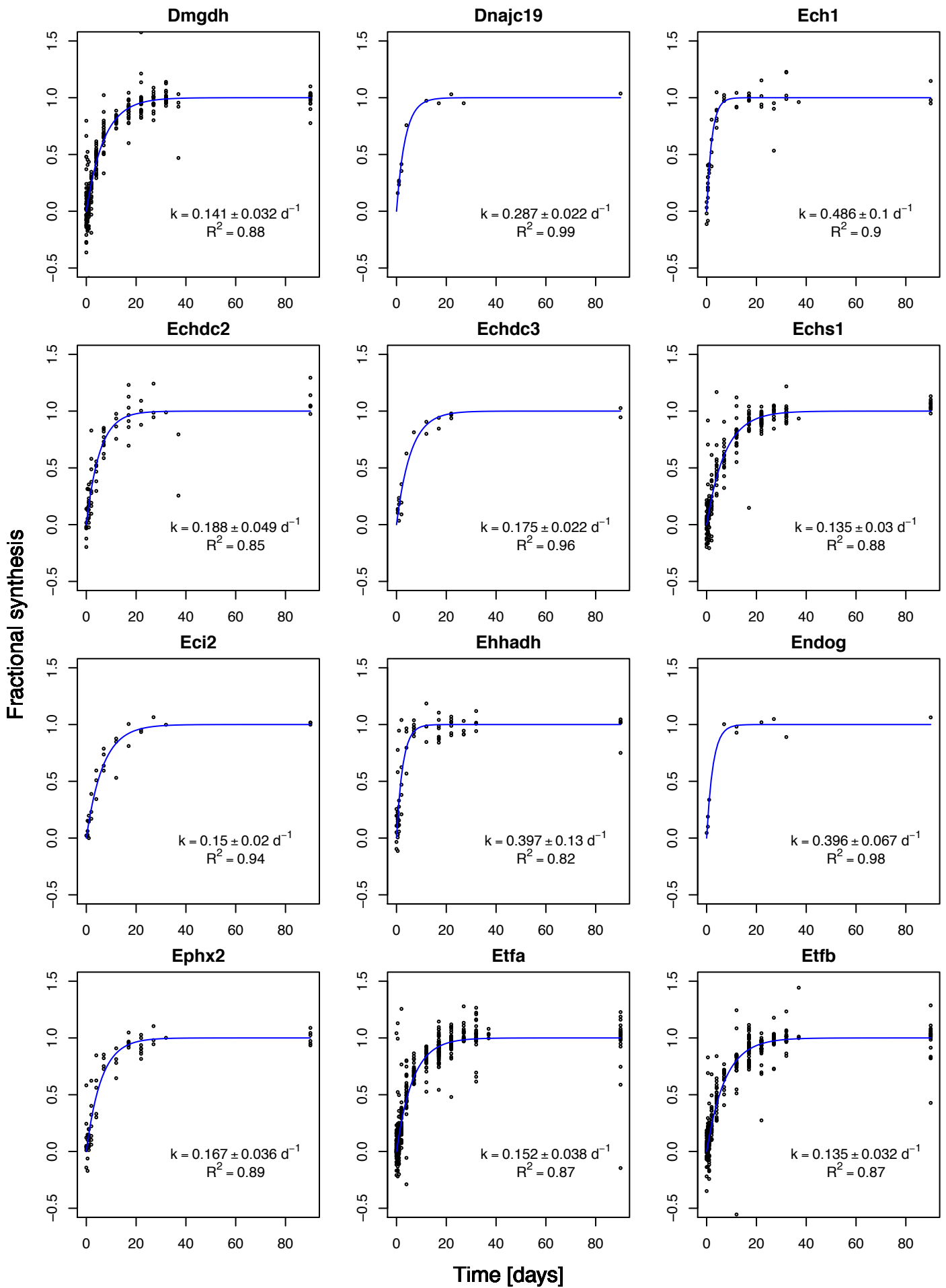




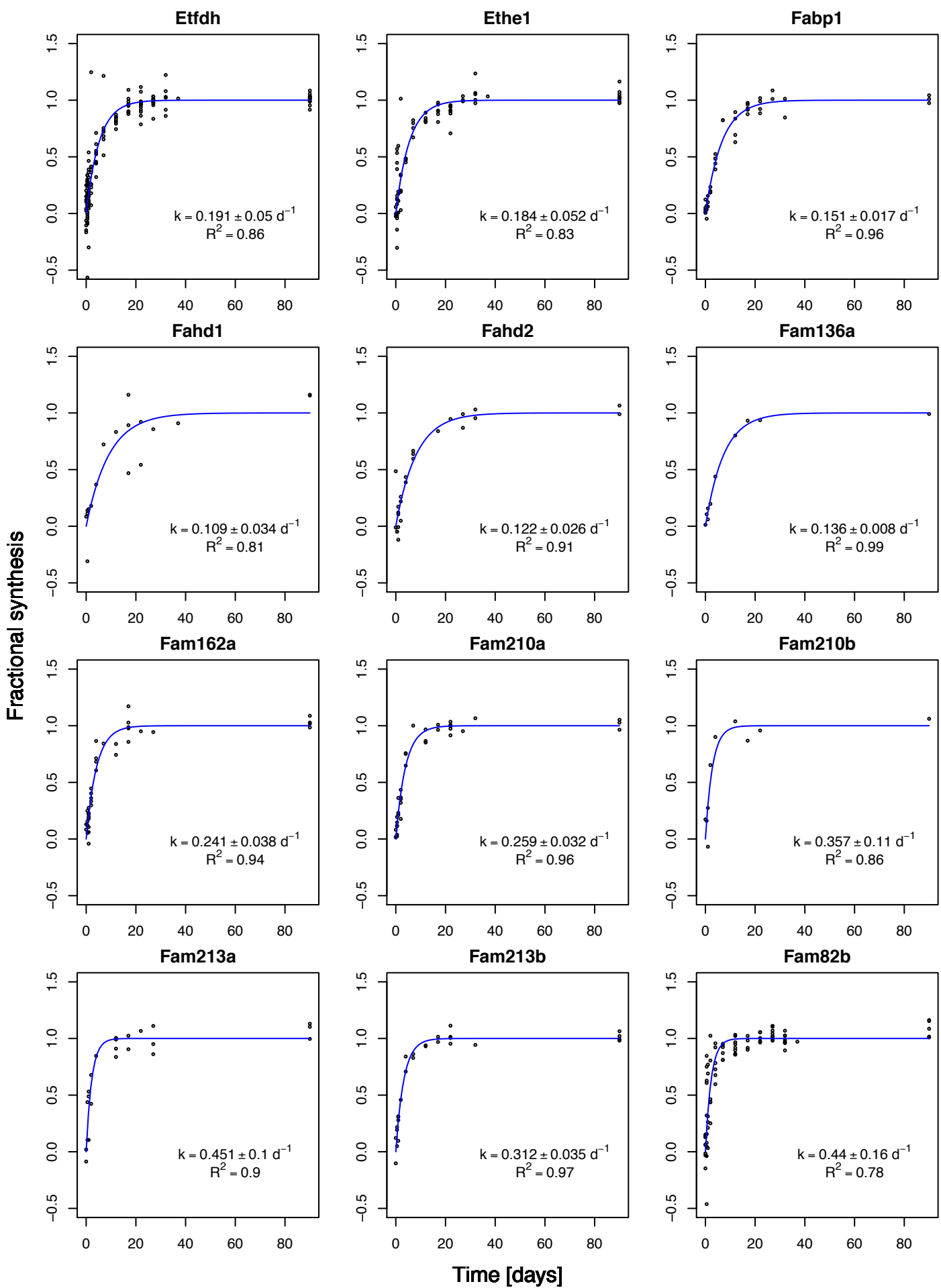


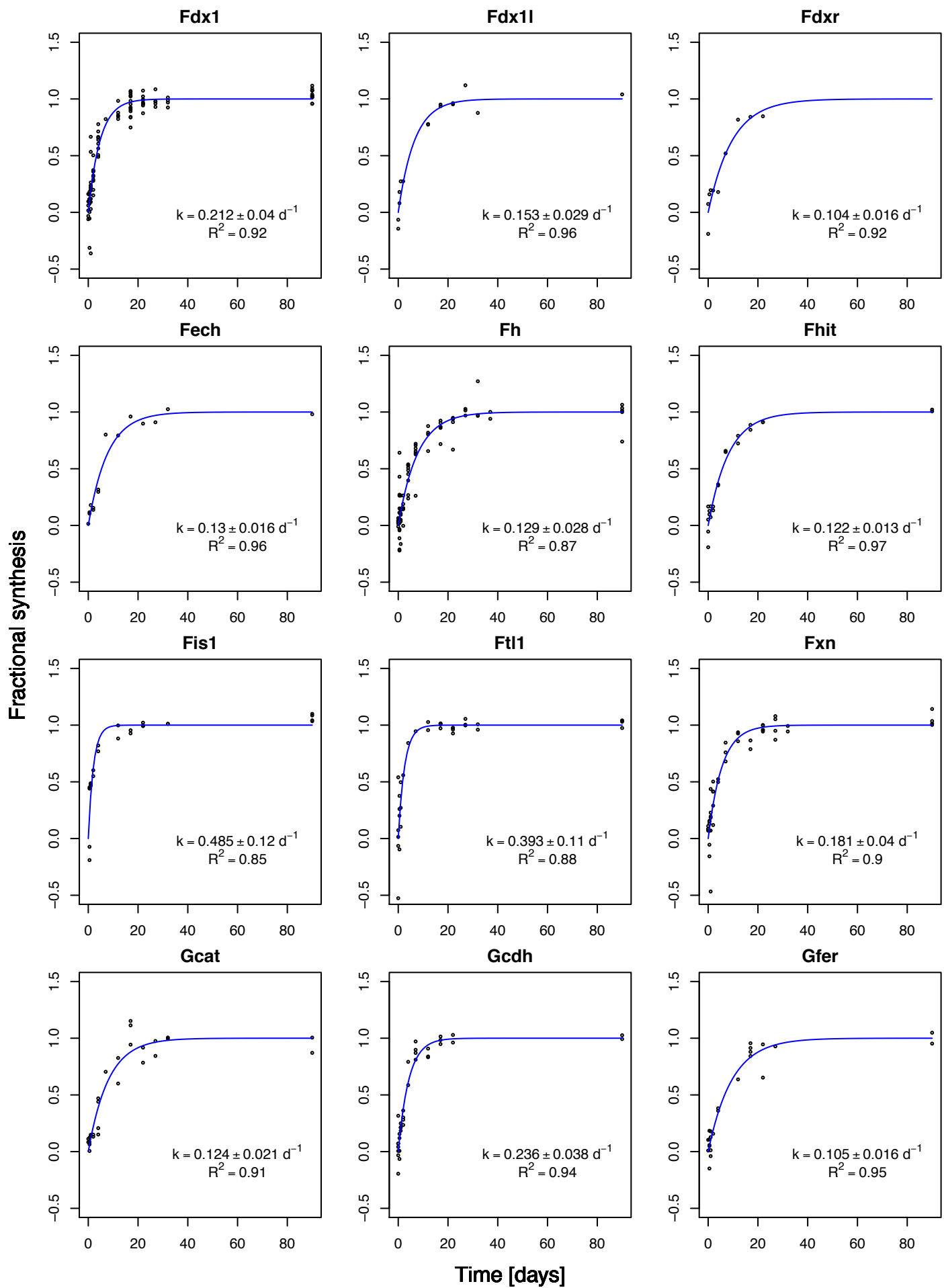


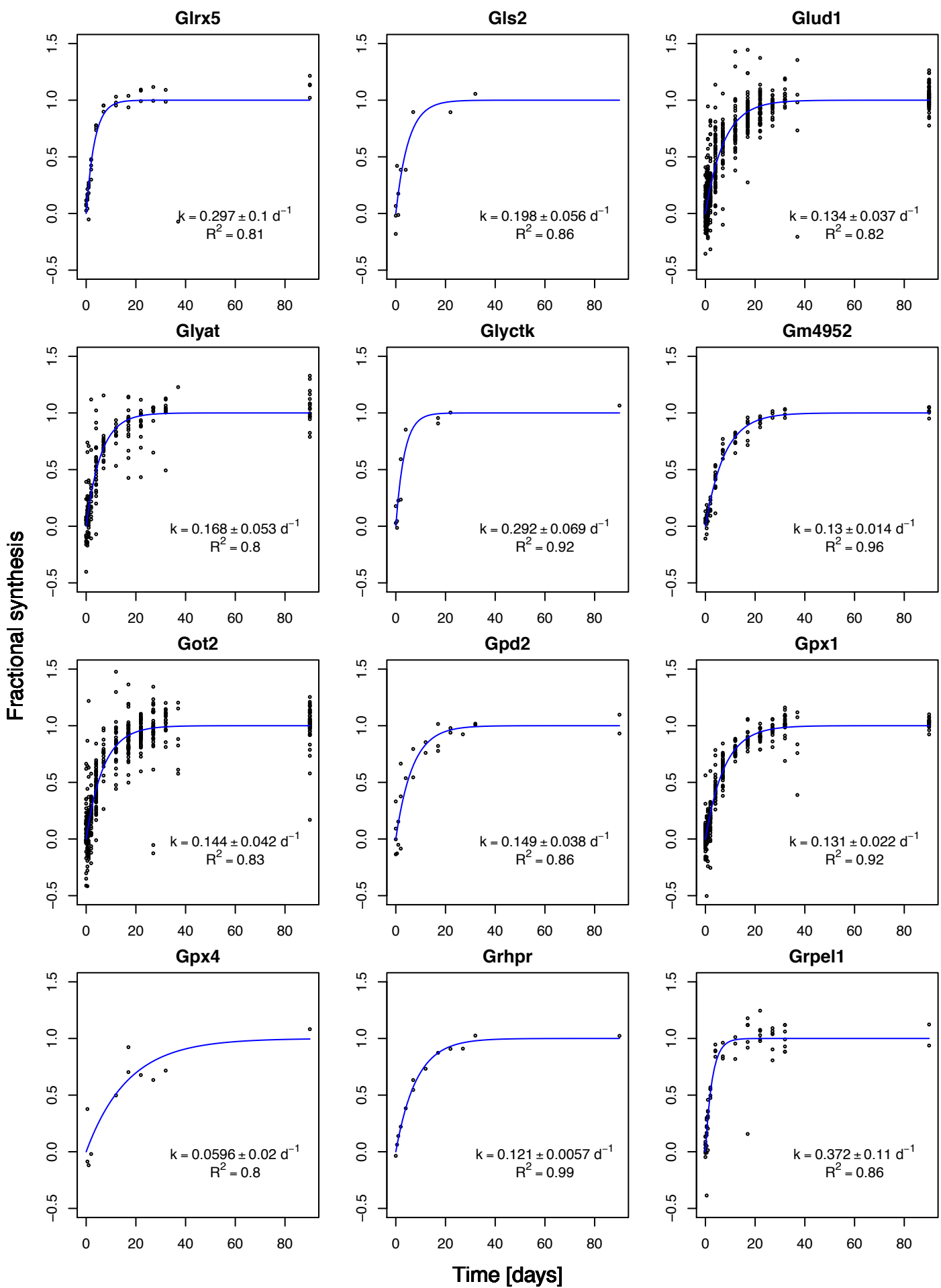


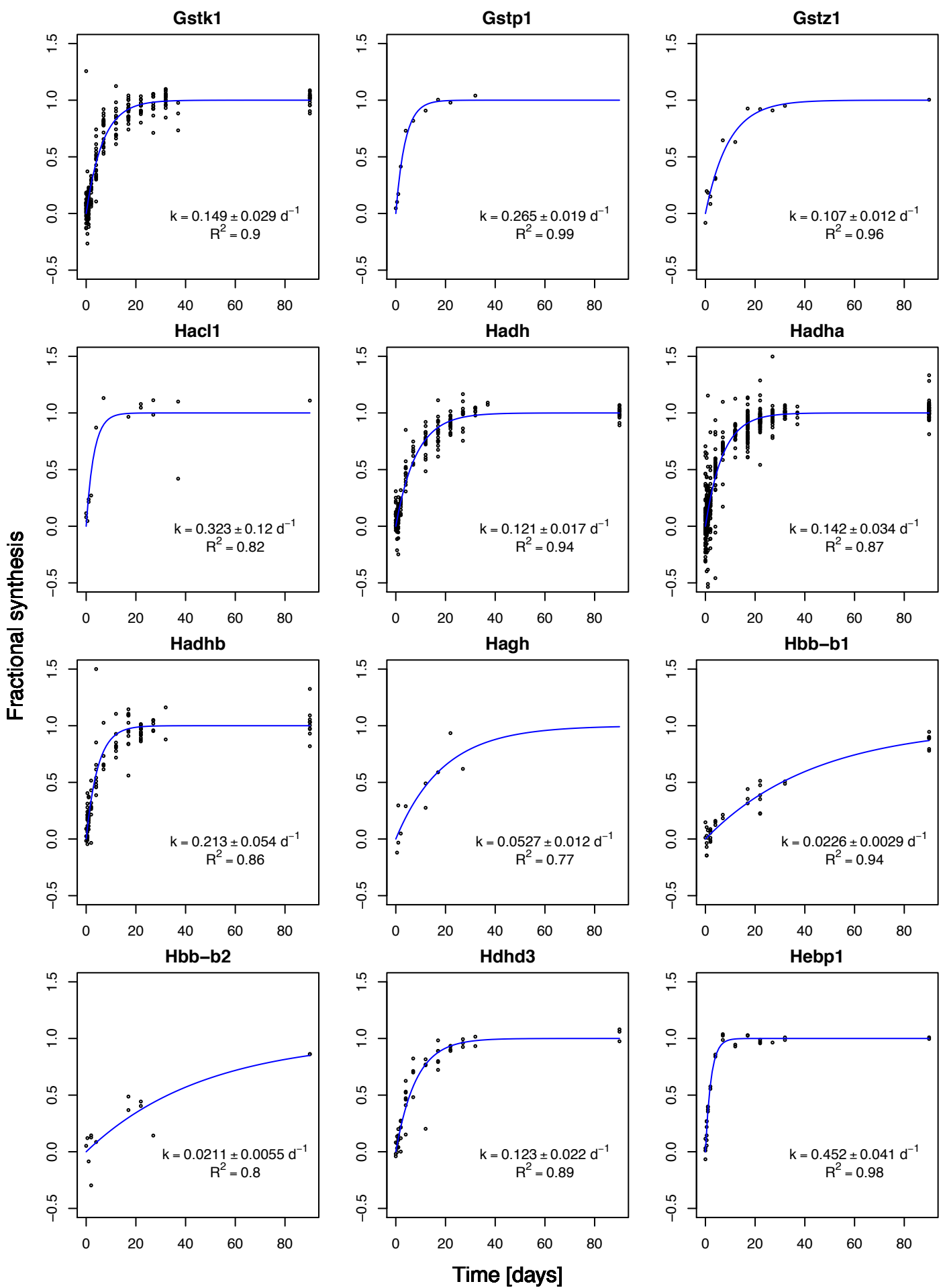


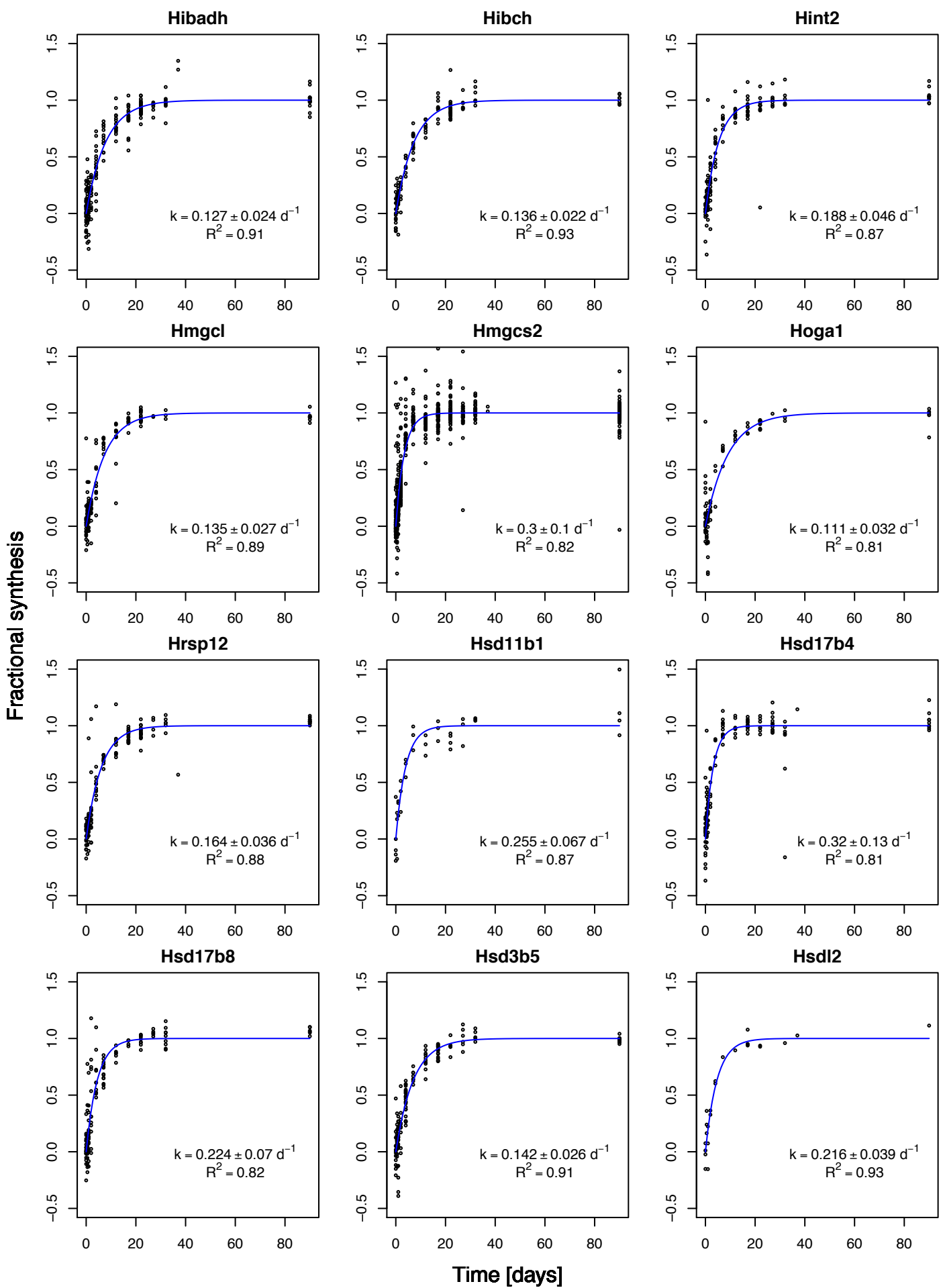


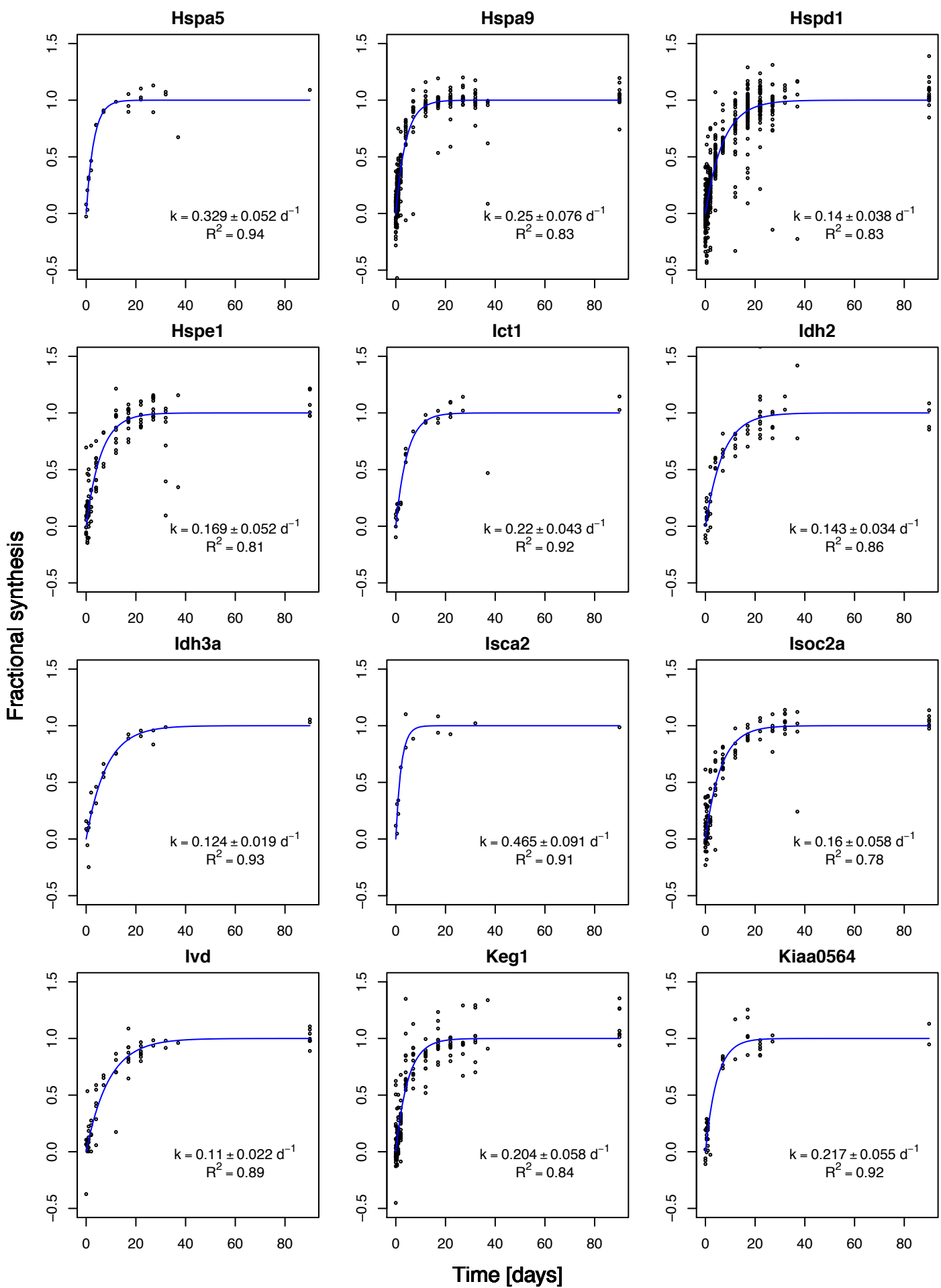


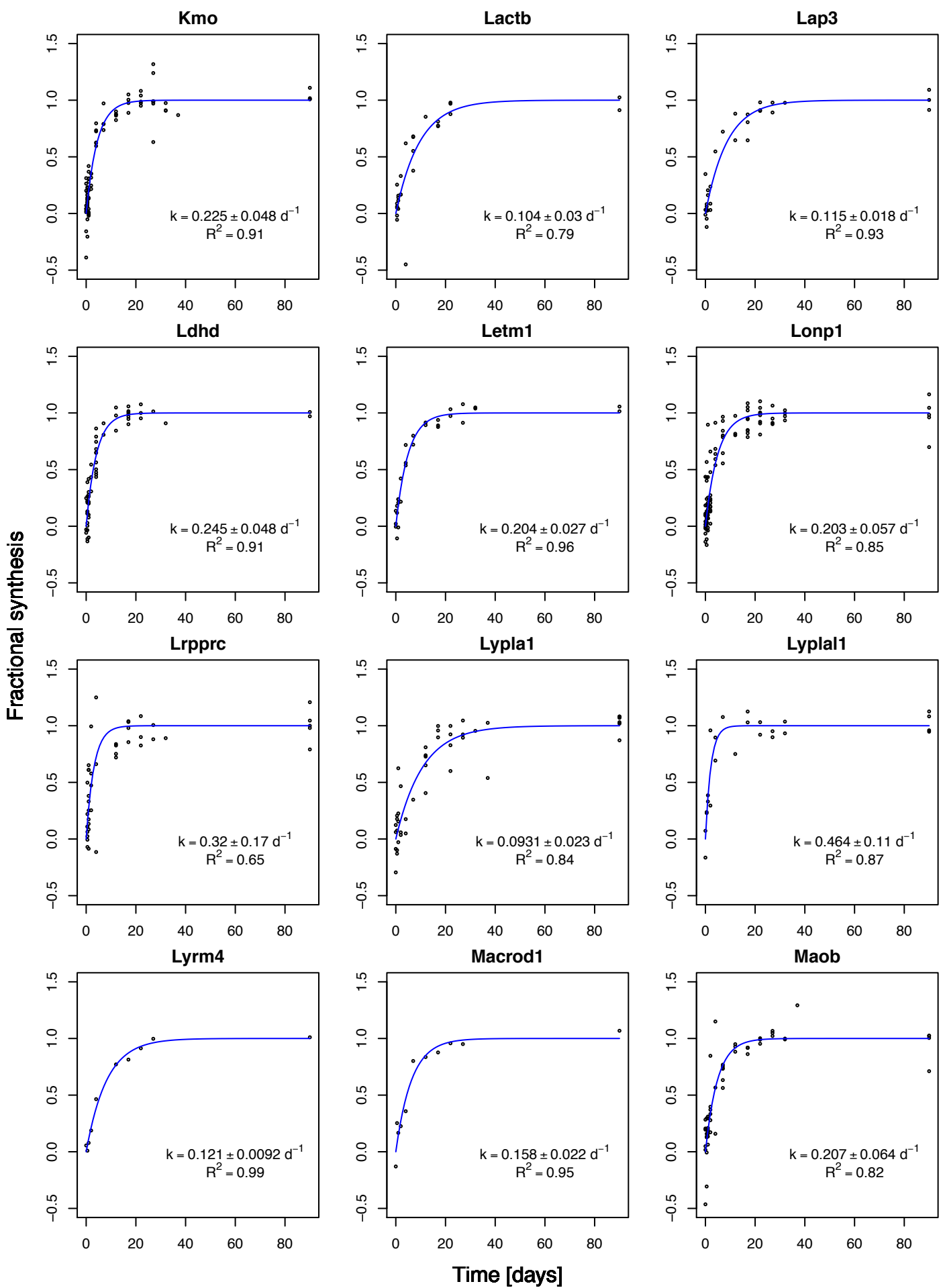


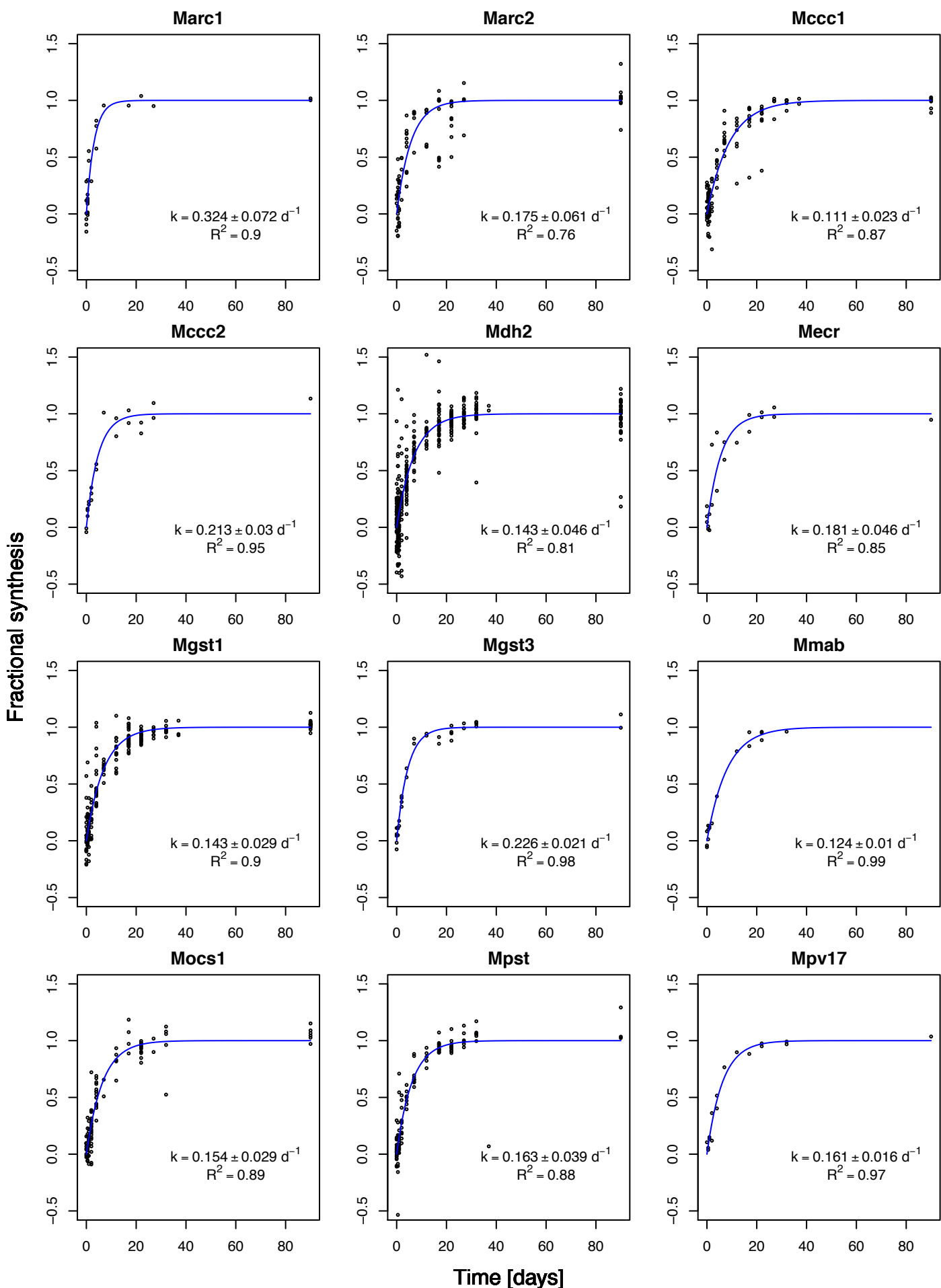




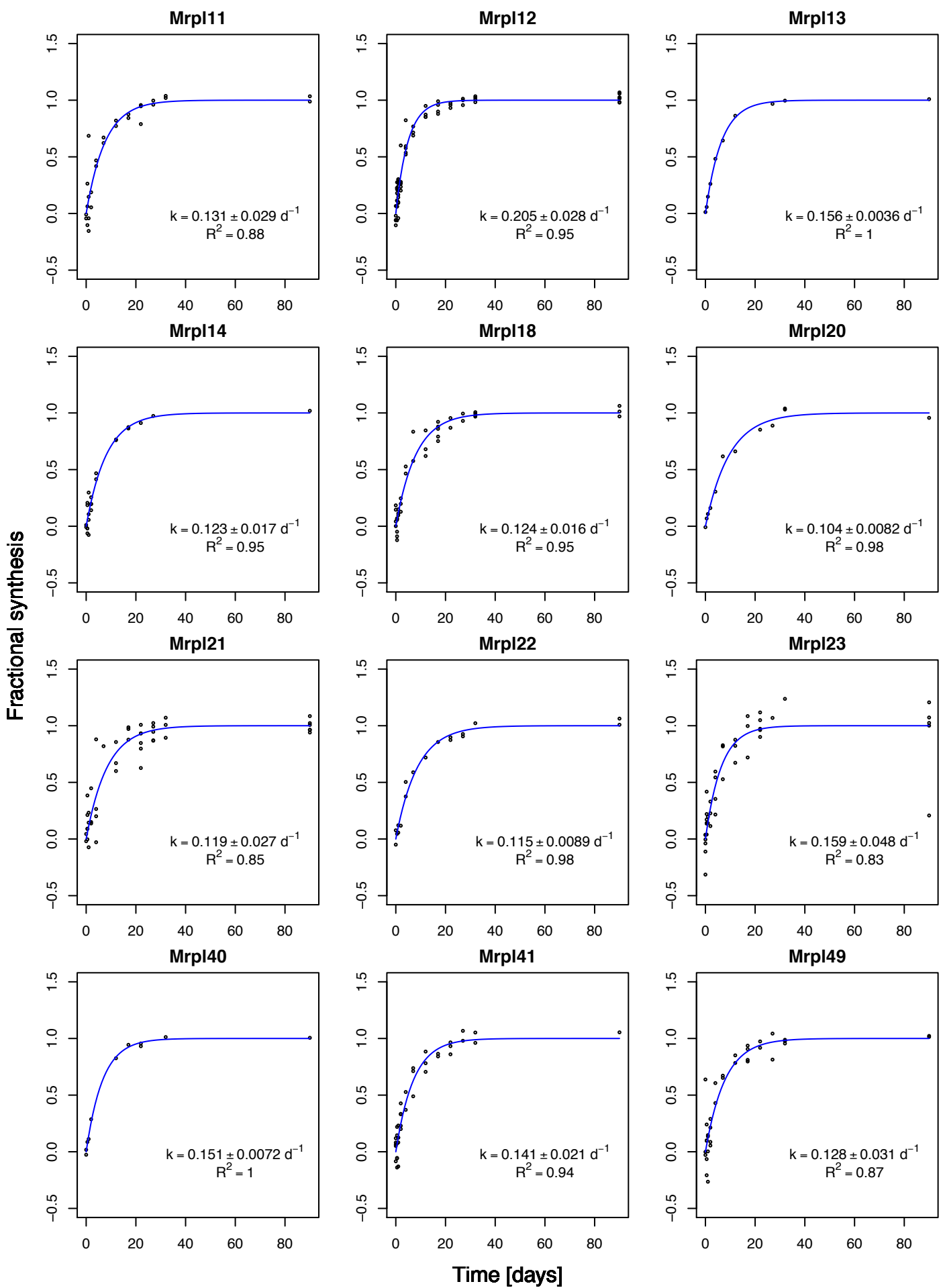


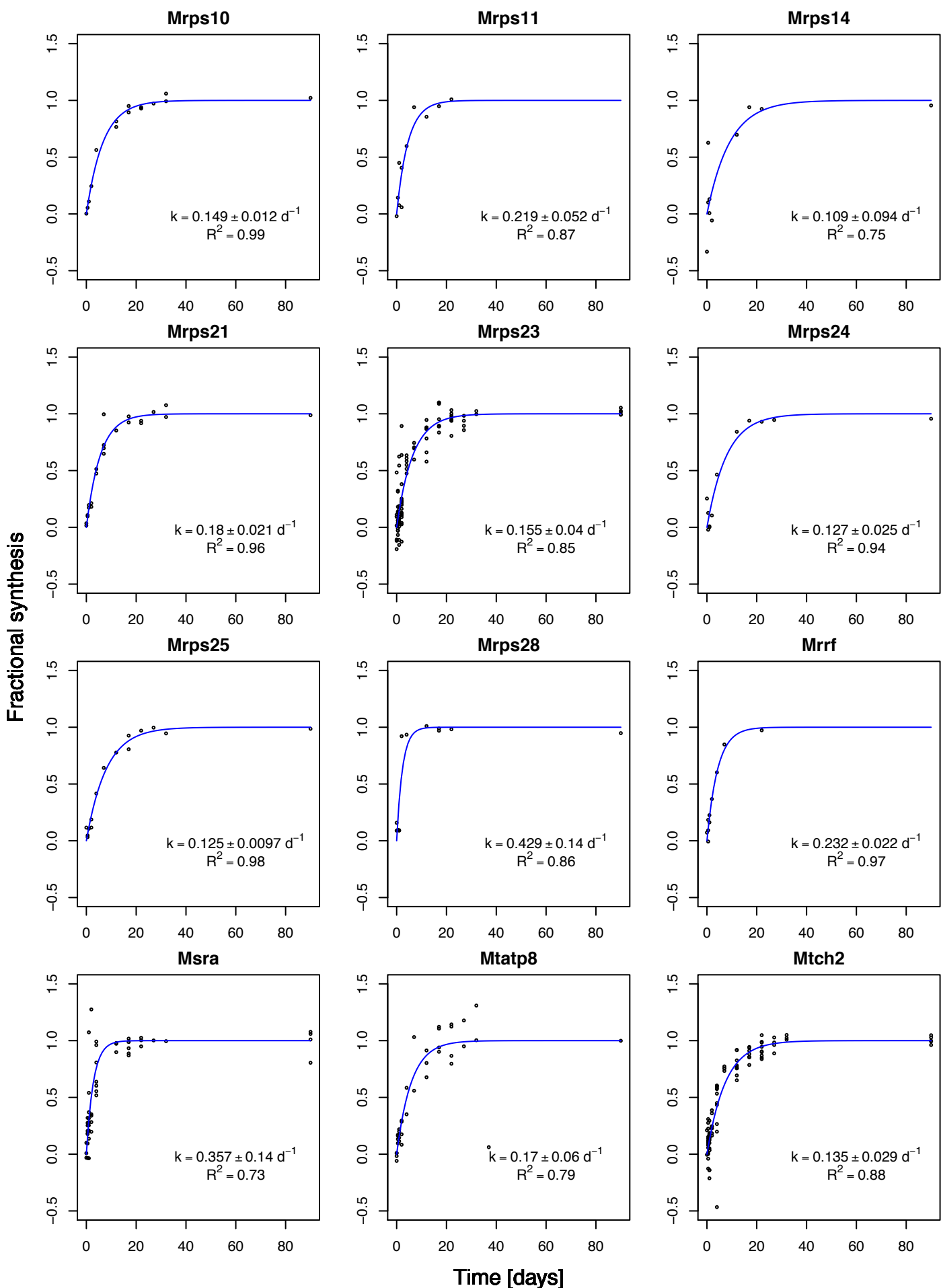


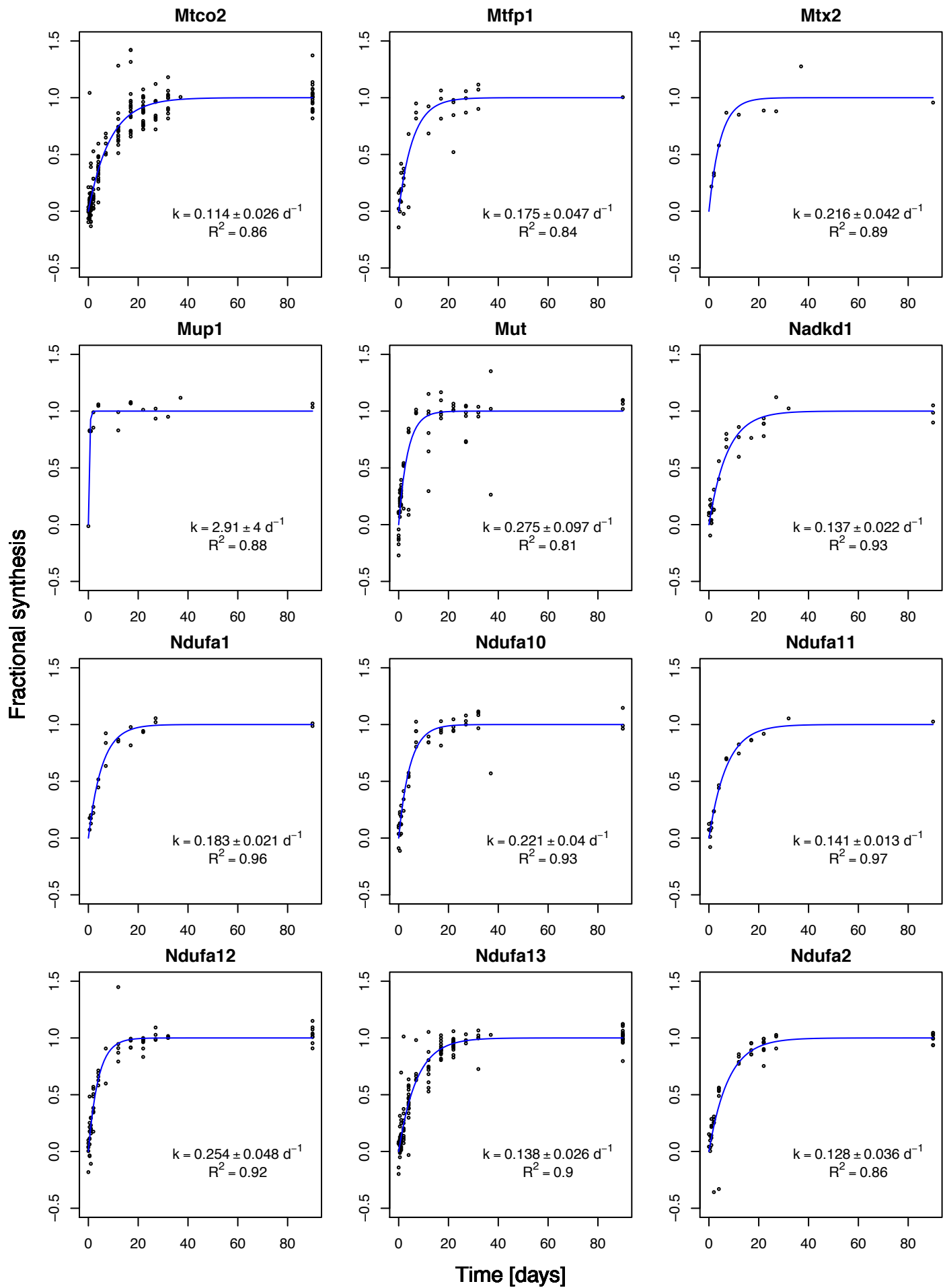


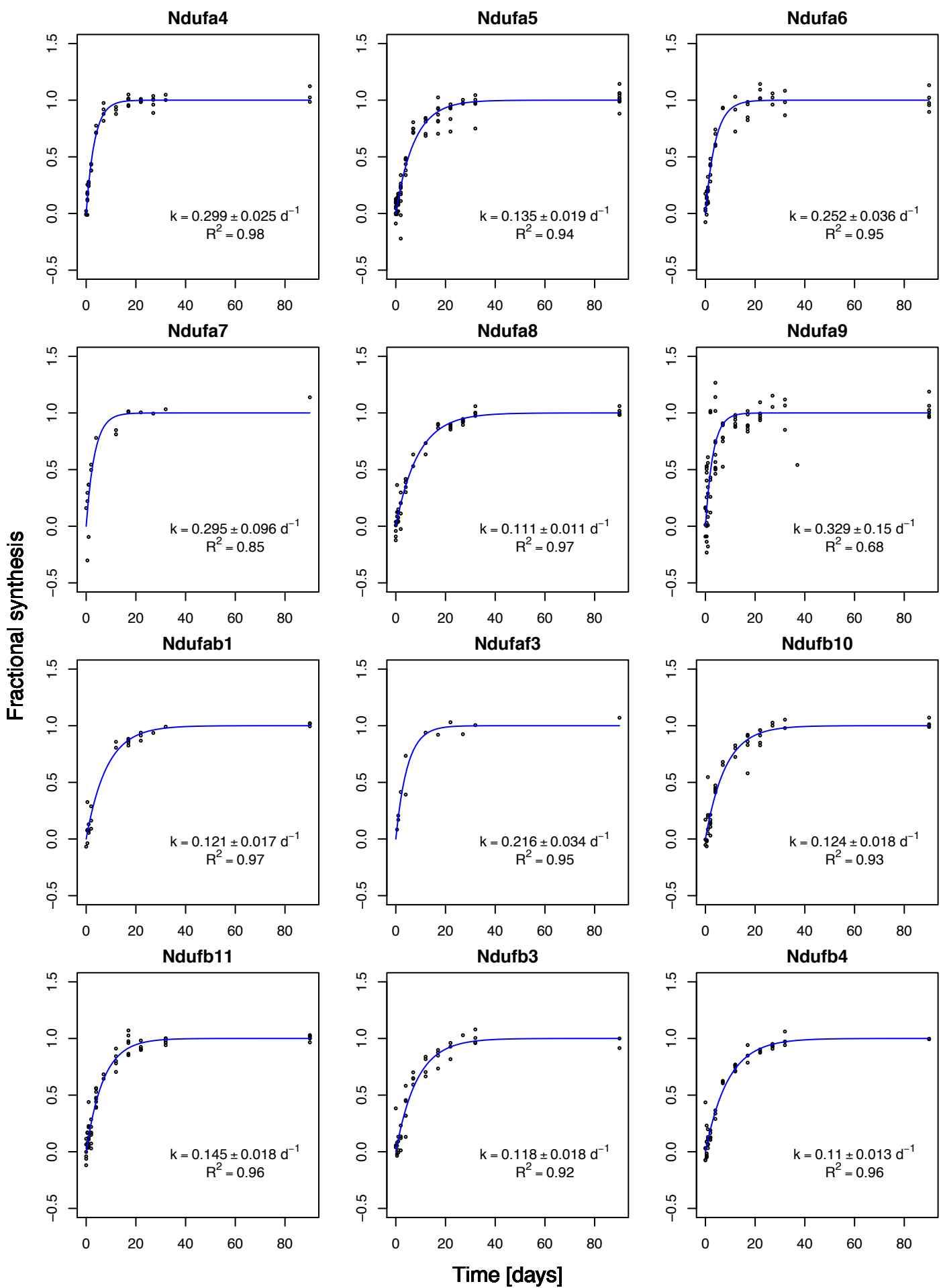


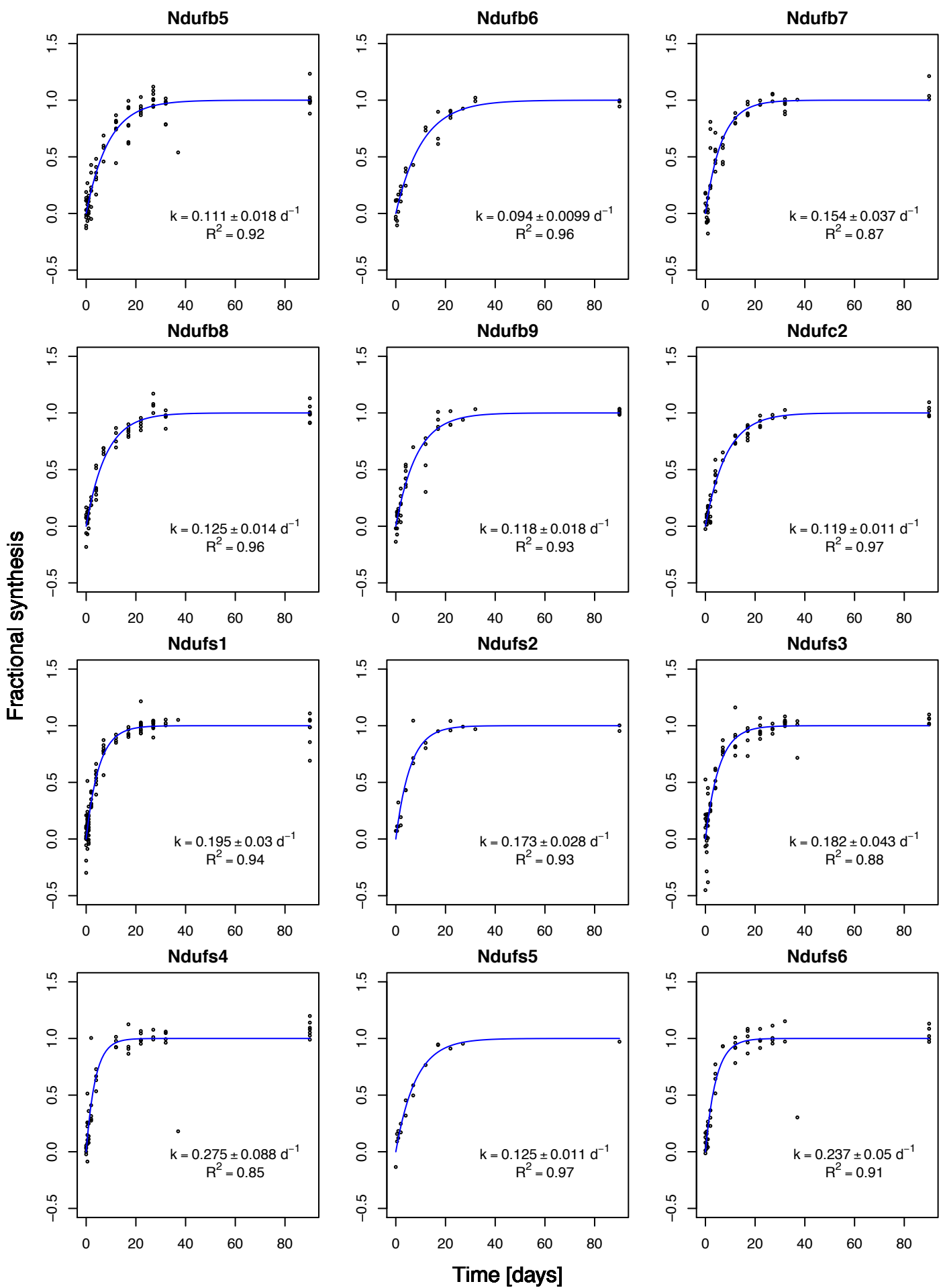


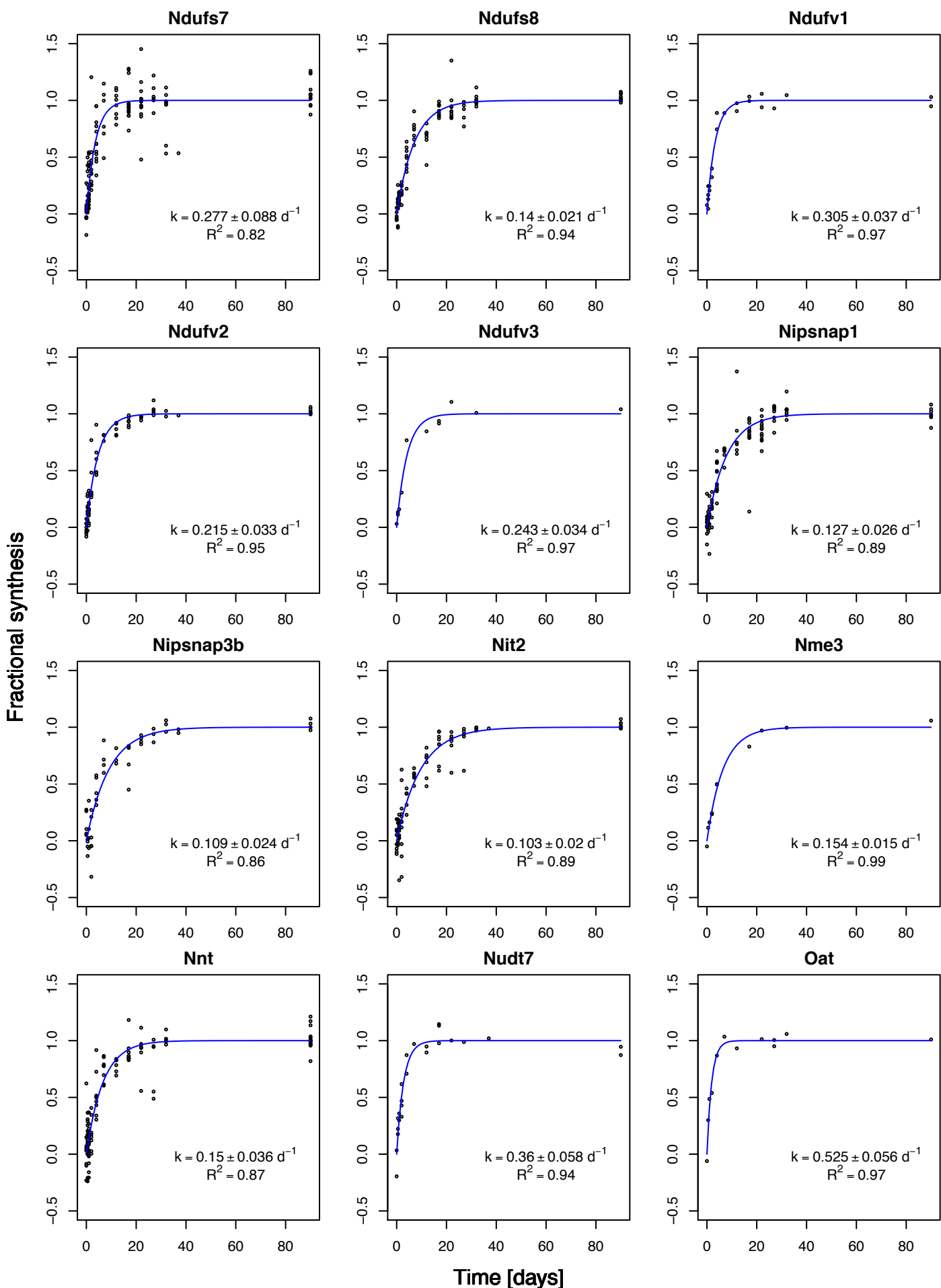


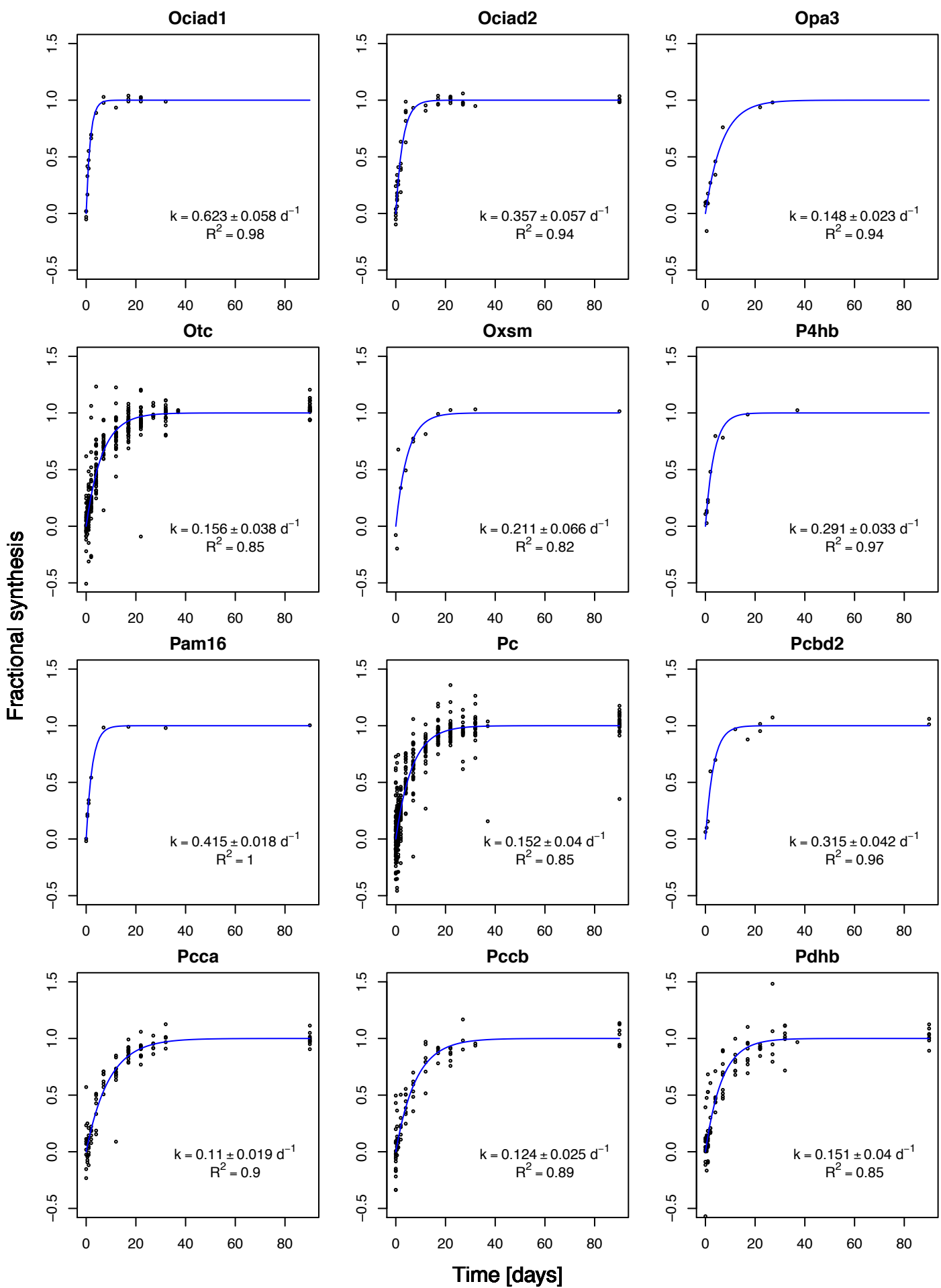


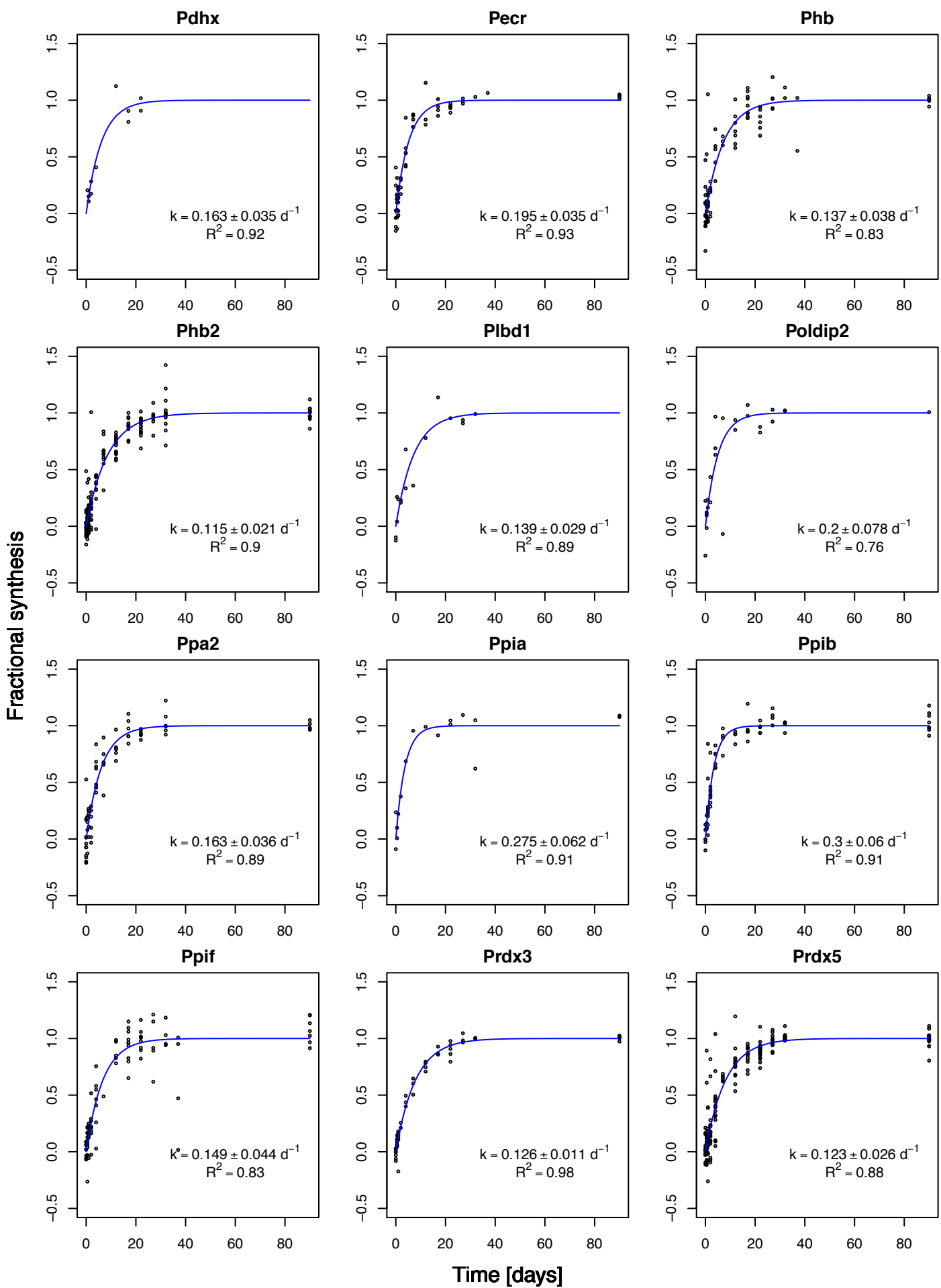




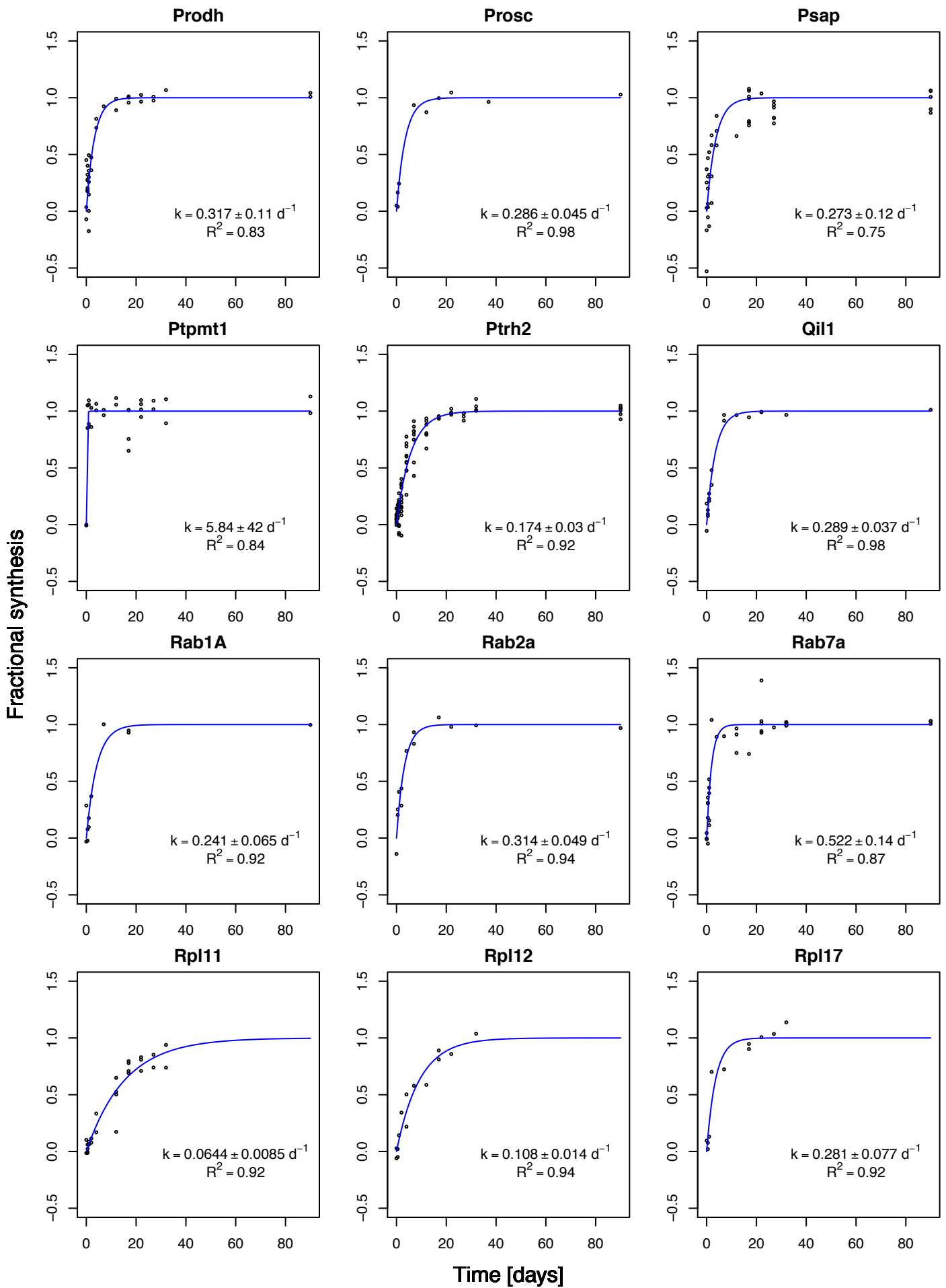


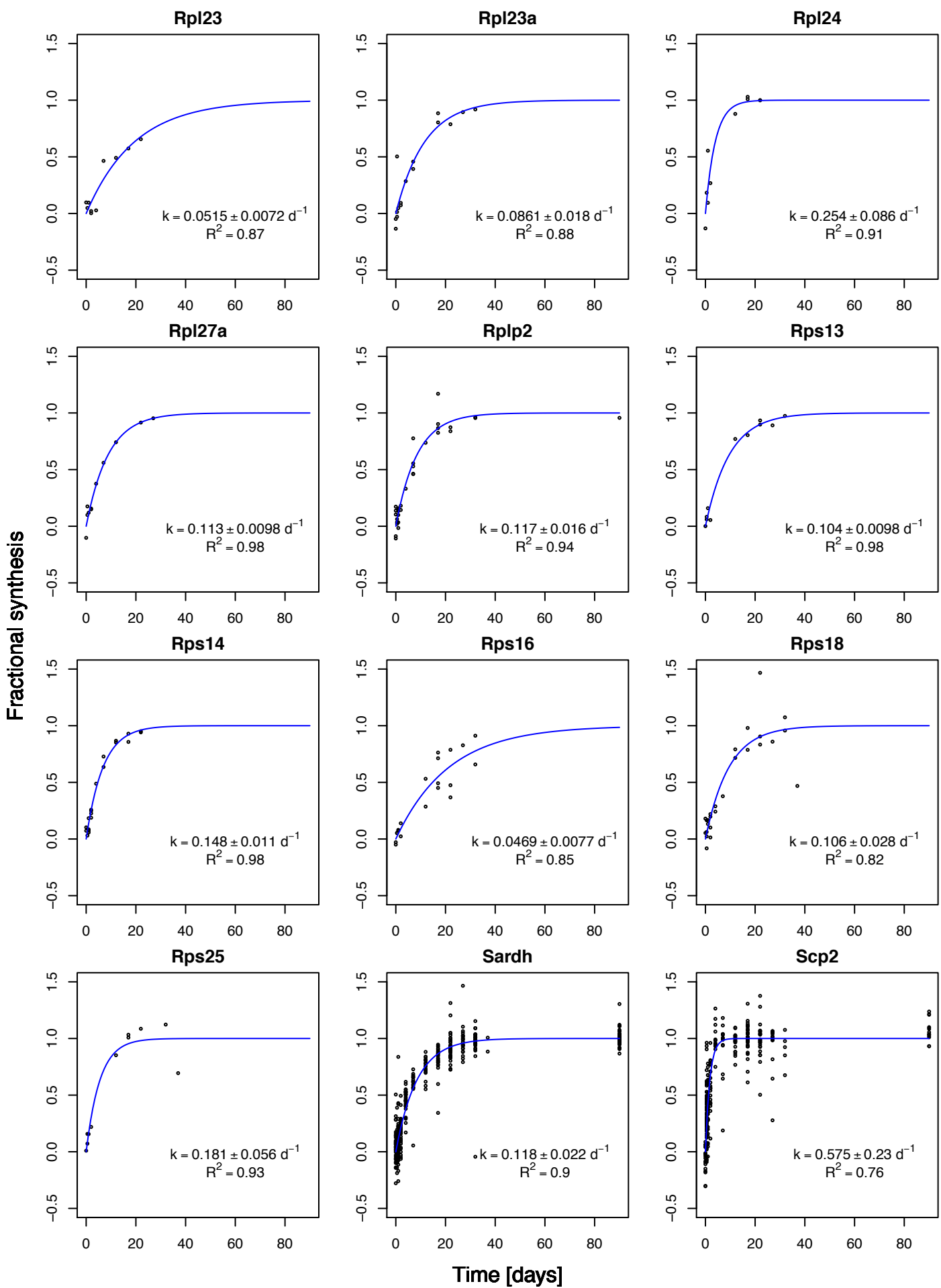


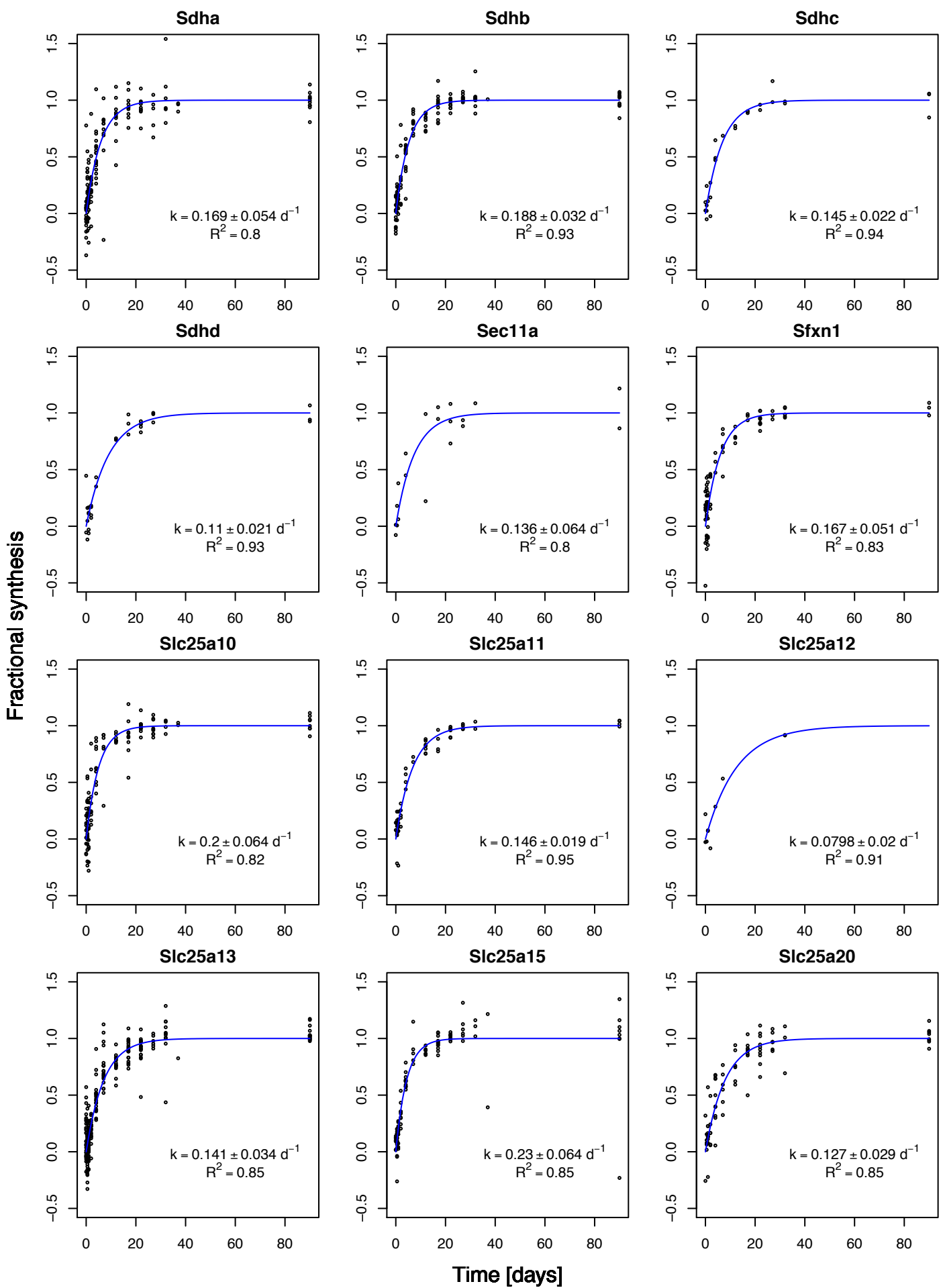


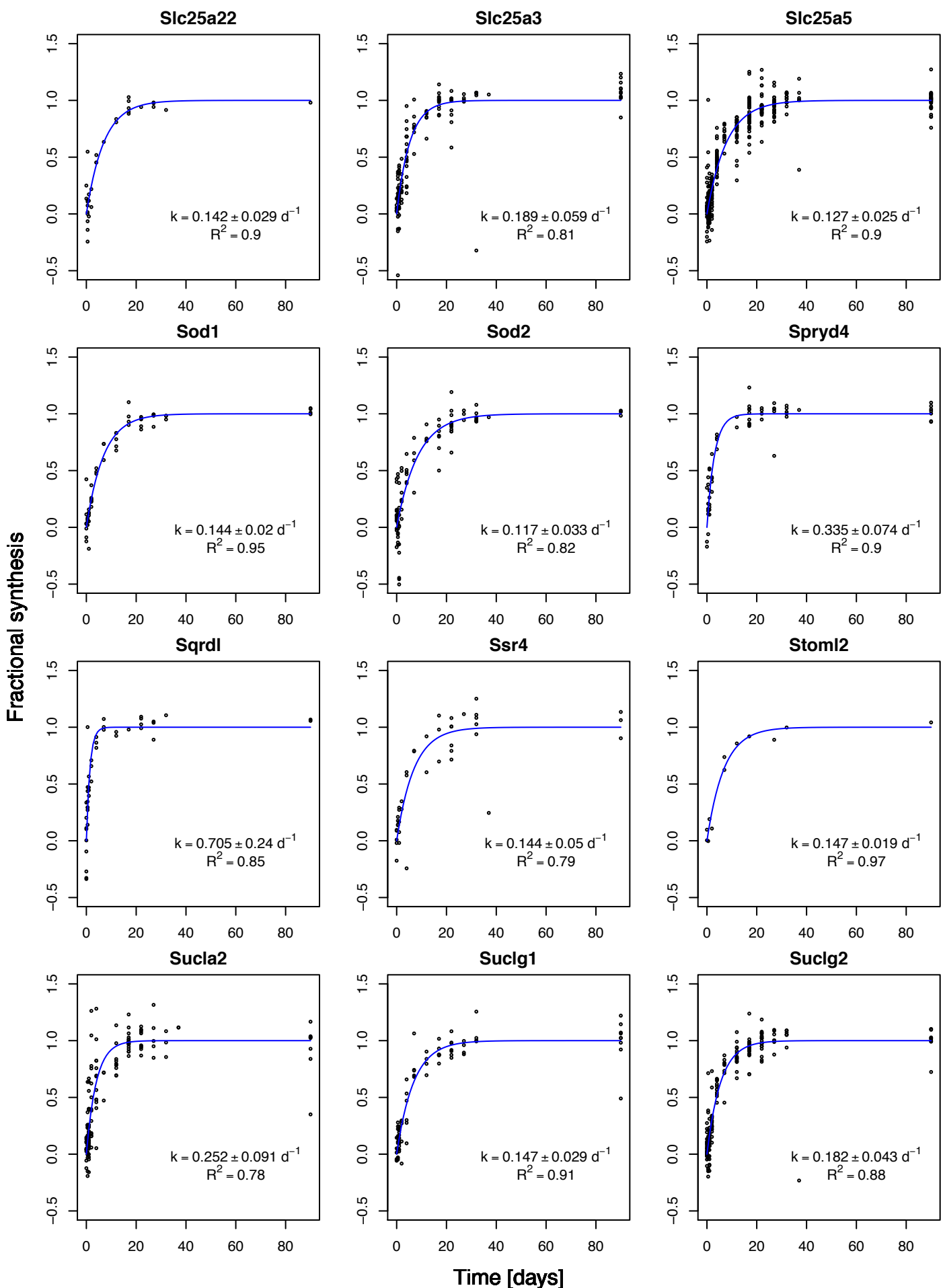


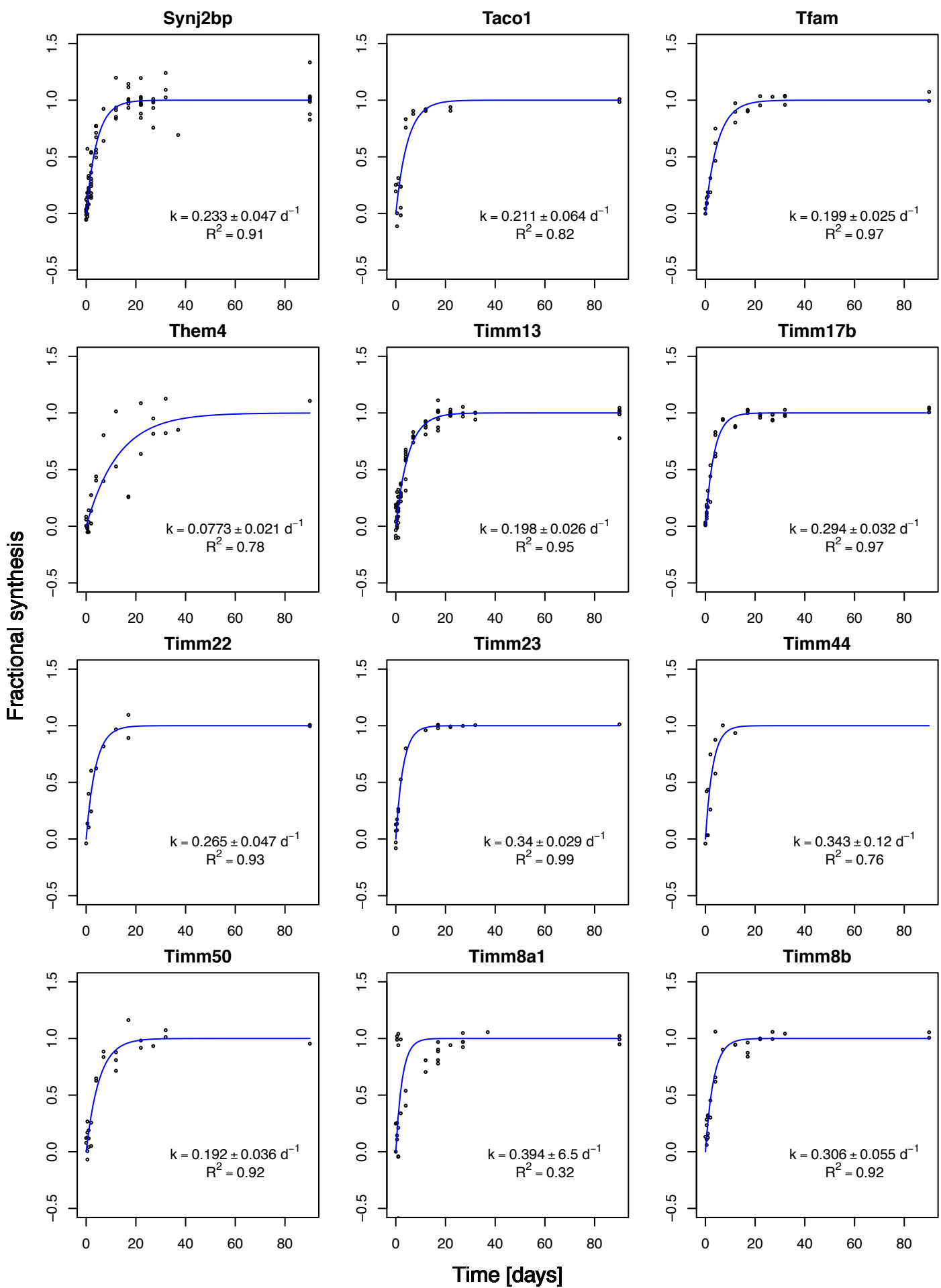


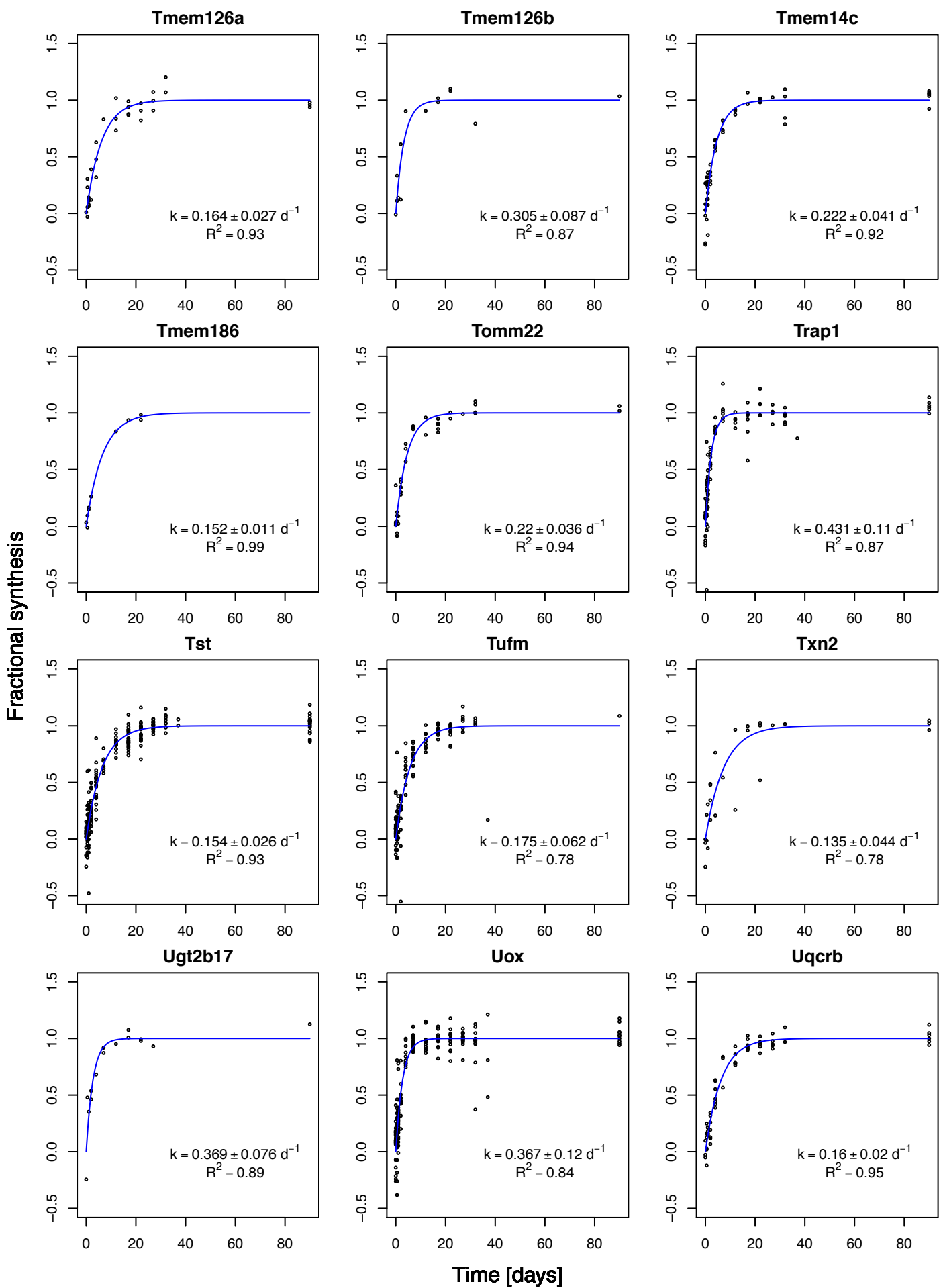


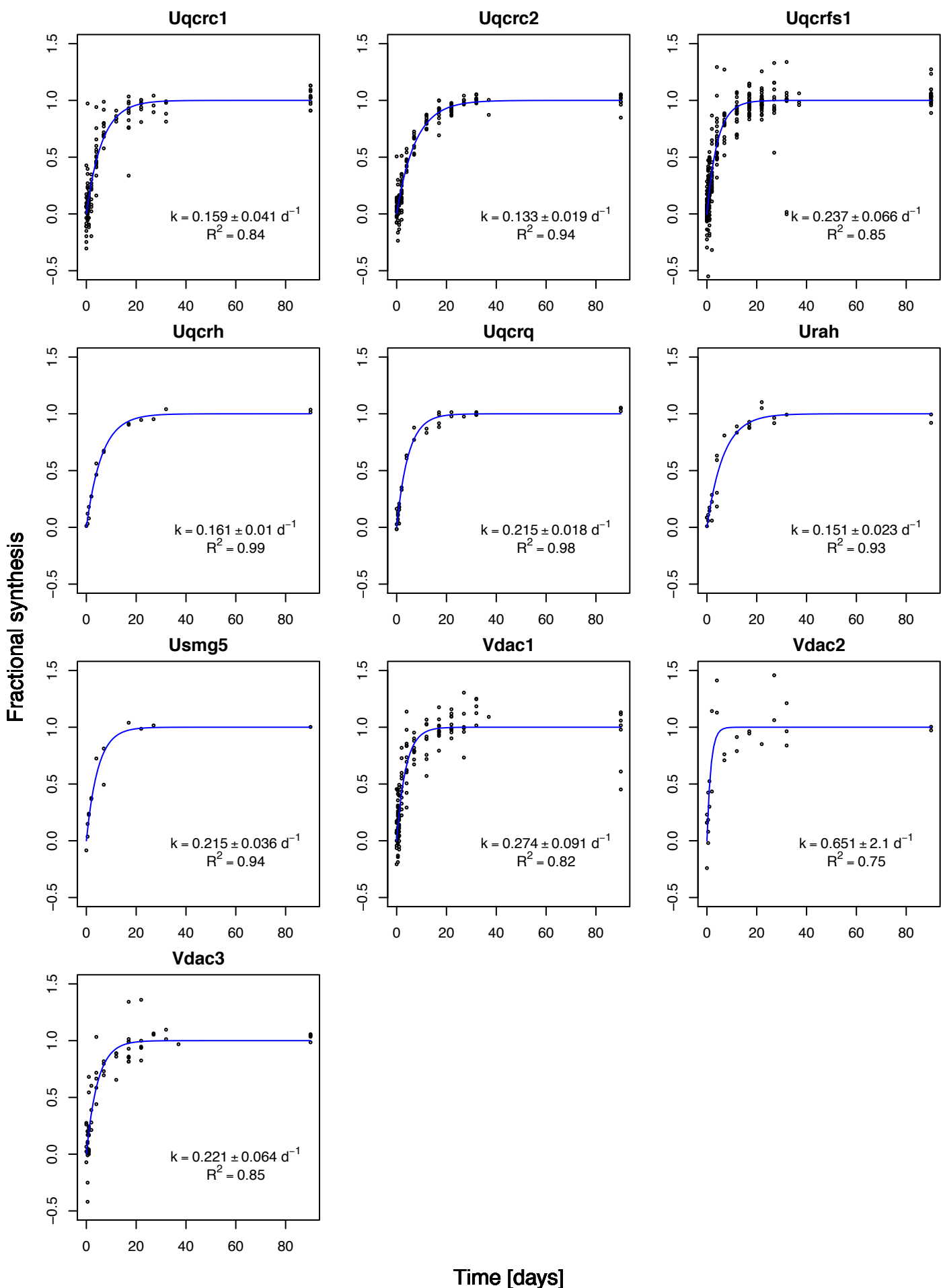








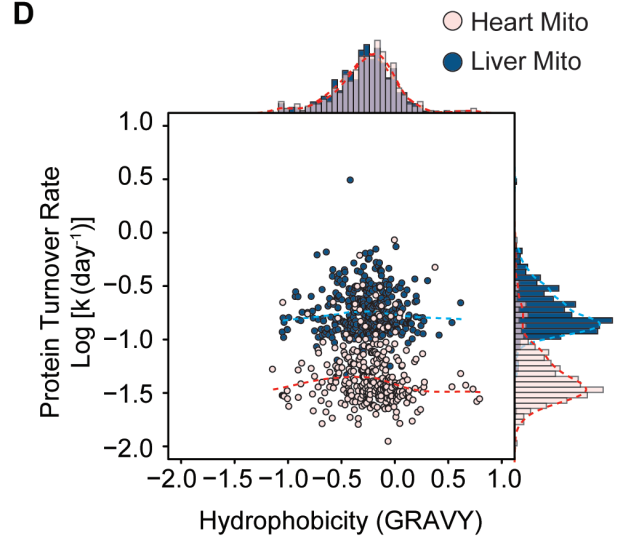
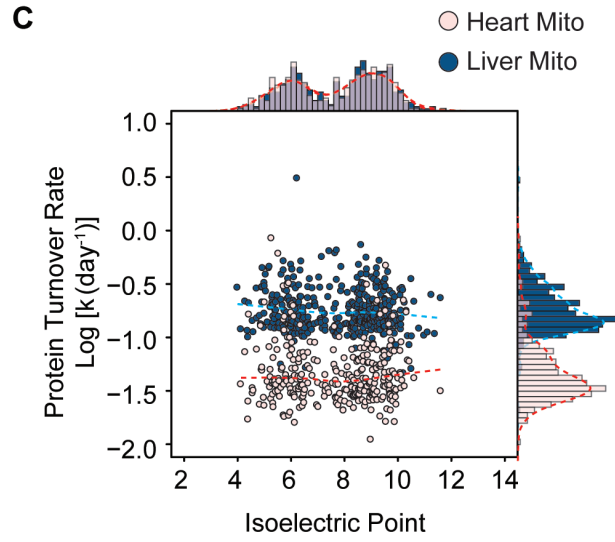
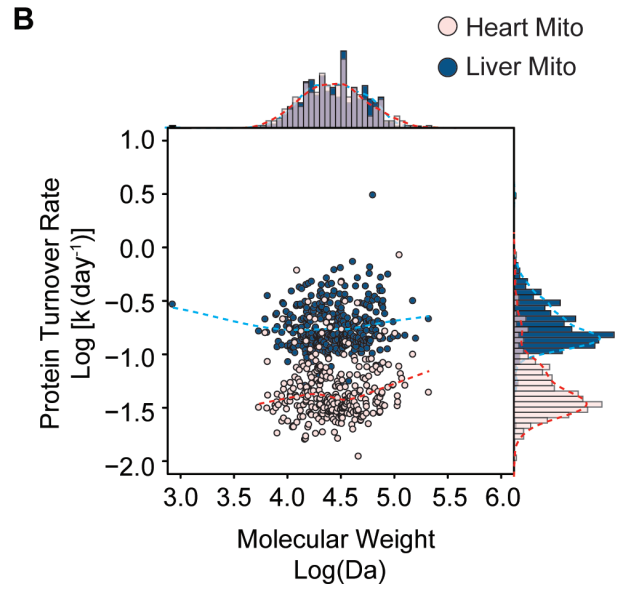
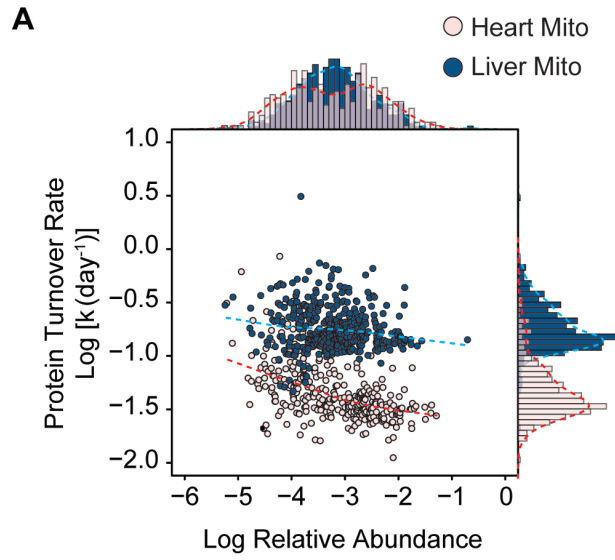




**Supplemental Fig. S3. Correlation between protein turnover rates and biophysical parameters.**

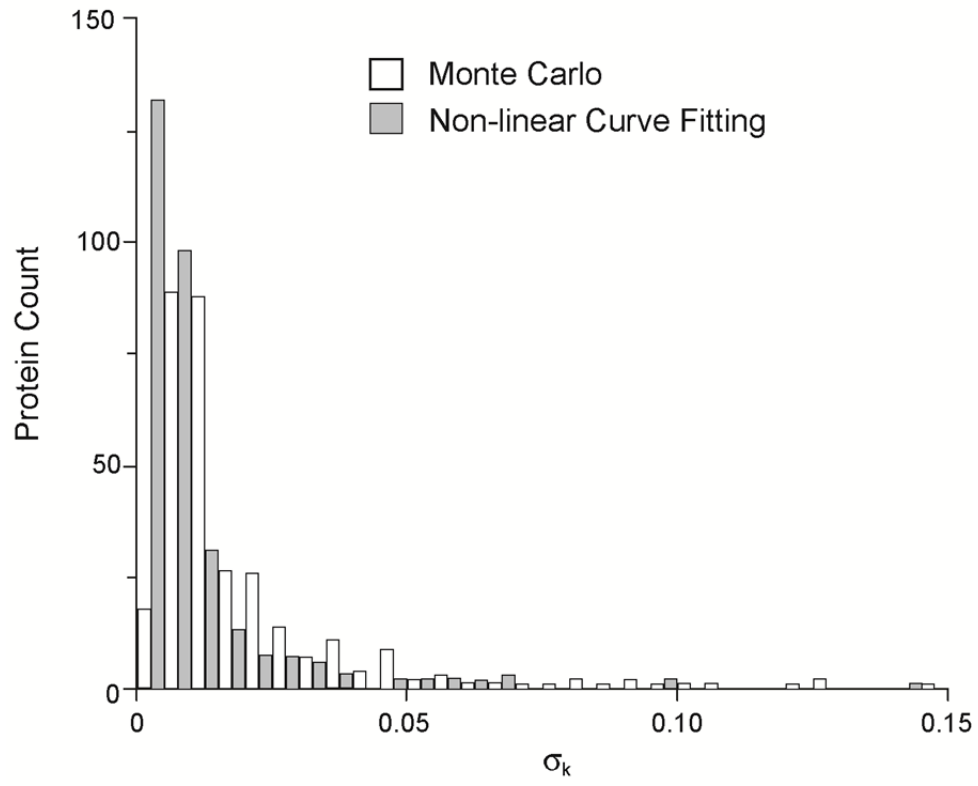
(A) A weak inverse correlation was observed between protein turnover rate and relative protein abundance (heart:  $\rho = -0.46$ ,  $P < 2.2 \times 10^{-16}$  and liver:  $\rho = -0.19$ ,  $P = 7.95 \times 10^{-3}$ ), suggesting abundant proteins are turned over more slowly in general. The relative abundance of a protein was determined by the summation of total chromatographic areas of the constituent peptide ion peaks divided by the areas of all identified peptide ions in the experimental dataset using Progenesis LC-MS (Ver. 4.0.4441.29989, Nonlinear Dynamics). By contrast, we observed no significant correlations in either tissue between protein turnover rates and their molecular weights (B), or their isoelectric points (C), or their hydrophobicities (D).





**Supplemental Fig. S4. Histograms of the standard errors in the rate constants for cardiac mitochondria proteins.**

The standard errors ( $\sigma_k$ ) in the rate constants for cardiac mitochondrial protein turnover were calculated using both the Monte Carlo and the Non-linear curve fitting methods. The distributions of the standard errors are not significantly different, although the Monte Carlo method is more conservative in the estimated errors.



**Supplemental Fig. S5. Mitochondrial protein turnover rates in the heart and the liver.**

The protein turnover rates ( $k$ ) of all analyzed murine mitochondrial proteins in the liver and in the heart are displayed on linear, non-logarithmic scale based on protein functional categories. The median turnover rates in the heart and the liver were 0.04 and 0.163 d<sup>-1</sup>, respectively. The number in the parenthesis represents the total number of proteins belonging to a functional category. Cardiac and hepatic mitochondrial proteins are indicated in red and blue dots, respectively.

**Total Distinct Protein (458)**

Heart Mitochondrial Proteins:  
Median of  $k_{deg} = 0.040 \text{ day}^{-1}$   
Interquartile Range 0.030~0.064  $\text{day}^{-1}$

**Heart Protein (314)**

**Liver Protein (386)**

Liver Mitochondrial Proteins:  
Median of  $k_{deg} = 0.163 \text{ day}^{-1}$   
Interquartile Range 0.130~0.241  $\text{day}^{-1}$

