Supplement 3:

Parameterisation and graphical diagnostics of the final model

Multi-compartmental model describing the pharmacokinetics of carvedilol enantiomers and their metabolites hydroxyphenylcarvedilol (OHC) and O-desmethylcarvedilol (DMC).

$$\frac{dA1}{dt} = -Ka \cdot A1$$

$$\frac{dA2}{dt} = Ka \cdot A1 - \frac{\text{residual Cl}}{Vc} \cdot A2 - \frac{Q}{Vc} \cdot A2 + \frac{Q}{Vp} \cdot A3 - \frac{\text{CYP2D6 Cl}}{Vc} \cdot A2 - \frac{\text{CYP2C9 Cl}}{Vc} \cdot A2$$

$$\frac{dA3}{dt} = \frac{Q}{Vc} \cdot A2 - \frac{Q}{Vp} \cdot A3$$

$$\frac{dA4}{dt} = \frac{\text{CYP2D6 Cl}}{Vc} \cdot A2 - \frac{\text{OHC Cl}}{\text{OHC Vc}} \cdot A4$$

$$\frac{dA5}{dt} = \frac{\text{CYP2C9 Cl}}{Vc} \cdot A2 - \frac{\text{DMC Cl}}{\text{DMC Vc}} \cdot A5$$

$$\frac{dA6}{dt} = -Ka \cdot A6$$

$$\frac{dA7}{dt} = Ka \cdot A6 - \frac{\text{residual Cl}}{Vc} \cdot A7 - \frac{Q}{Vc} \cdot A7 + \frac{Q}{Vp} \cdot A8 - \frac{\text{CYP2D6 Cl}}{Vc} \cdot A7 - \frac{\text{CYP2C9 Cl}}{Vc} \cdot A7$$

$$\frac{dA8}{dt} = \frac{Q}{Vc} \cdot A7 - \frac{Q}{Vp} \cdot A8$$

$$\frac{dA9}{dt} = \frac{\text{CYP2D6 Cl}}{Vc} \cdot A7 - \frac{\text{OHC Cl}}{\text{OHC Vc}} \cdot A9$$

$$\frac{dA10}{dt} = \frac{\text{CYP2C9 Cl}}{Vc} \cdot A7 - \frac{\text{DMC Cl}}{\text{DMC Vc}} \cdot A10$$

A1 and **A6**: amount of (S)-(-)- and (R)-(+)-carvedilol in depot.

A2, A7, A4, A9, A5 and **A10**: Amount of (S)-(-)- and (R)-(+)-carvedilol; (S)-(-)- and (R)-(+)-OHC, (S)-(-)- and (R)-(+)-DMC in the central compartment, respectively.

A3 and **A8** amount of (S)-(-)- and (R)-(+)-carvedilol in the peripheral compartment.

Ka: absorption constant

Vc: Central volume of distribution.

Vp: Peripheral volume of distribution.

Q: Inter-compartment clearance

Residual Cl: Residual clearance

CYP2D6 Cl: Clearance by CYP2D6 (formation clearance of OHC)

CYP2C9 Cl: Clearance by CYP2C9 (formation clearance of DMC)

OHC Cl: Clearance of OHC

DMC Cl: Clearance of DMC

Type 2 diabetes mellitus patients on long-term treatment with glibenclamide and metformin (T2DM) and the CYP2D6 extensive (EM) or poor metaboliser (PM) phenotypes were set as categorical variables and their effect was evaluated as discrete changes to the population parameter. The categorical variable FEN = 1 refers to PM phenotype and FEN = 0 to the EM phenotype. The variable DB = 1 refers to T2DM patients and DB = 0 to healthy subjects. The combination of these two variables was parameterised as follows:

$$\begin{aligned}\text{CYP2D6 Cl} = \{&[\text{TV}_{\text{healthy CYP2D6 EM}} \cdot (1-\text{FEN}) \cdot (1-\text{DB})] \\ &+ [\text{TV}_{\text{T2DM CYP2D6 EM}} \cdot \text{DB} \cdot (1-\text{FEN})] \\ &+ [\text{TV}_{\text{T2DM CYP2D6 PM}} \cdot \text{DB} \cdot \text{FEN}]\} \cdot e^{\eta_{\text{CYP2D6}}}\end{aligned}$$

$$\begin{aligned}\text{CYP2C9 Cl} = \{&[\text{TV}_{\text{healthy CYP2D6 EM}} \cdot (1-\text{FEN}) \cdot (1-\text{DB})] \\ &+ [\text{TV}_{\text{T2DM CYP2D6 EM}} \cdot \text{DB} \cdot (1-\text{FEN})] \\ &+ [\text{TV}_{\text{T2DM CYP2D6 PM}} \cdot \text{DB} \cdot \text{FEN}]\} \cdot e^{\eta_{\text{CYP2C9}}}\end{aligned}$$

TV: Typical Value, **T2DM:** Type 2 diabetes *mellitus* patients, **EM:** CYP2D6 extensive metabolisers, **PM:** CYP2D6 poor metabolisers, η_{CYP2D6}**:** inter-individual variability for CYP2D6, η_{CYP2C9}**:** inter-individual variability for CYP2C9.

Figures S6 to S19 presents graphical diagnostics of the final model for carvedilol, hydroxyphenilcarvedilol (OHC) and O-desmethylcarvedilol (DMC) enantiomers.

The goodness of fitting plots (GOF) are presented in figure S6, including population and individual predicted concentrations *vs.* observed concentrations, conditional weighted residual *vs.* observed concentrations and time. Figure S7 shows the visual predictive check (VPC). Figures S8 to S10 present the normalised predictive distribution errors (NPDE), including NPDE histogram, NPDE vs. predicted concentrations and time and the NPDE normal quantile – quantile plot. Figures S11 to S16 summarise the mirror plots and figures S17 to S19 the post predictive checks (PPC) based on $AUC_{0\text{-}24}$.

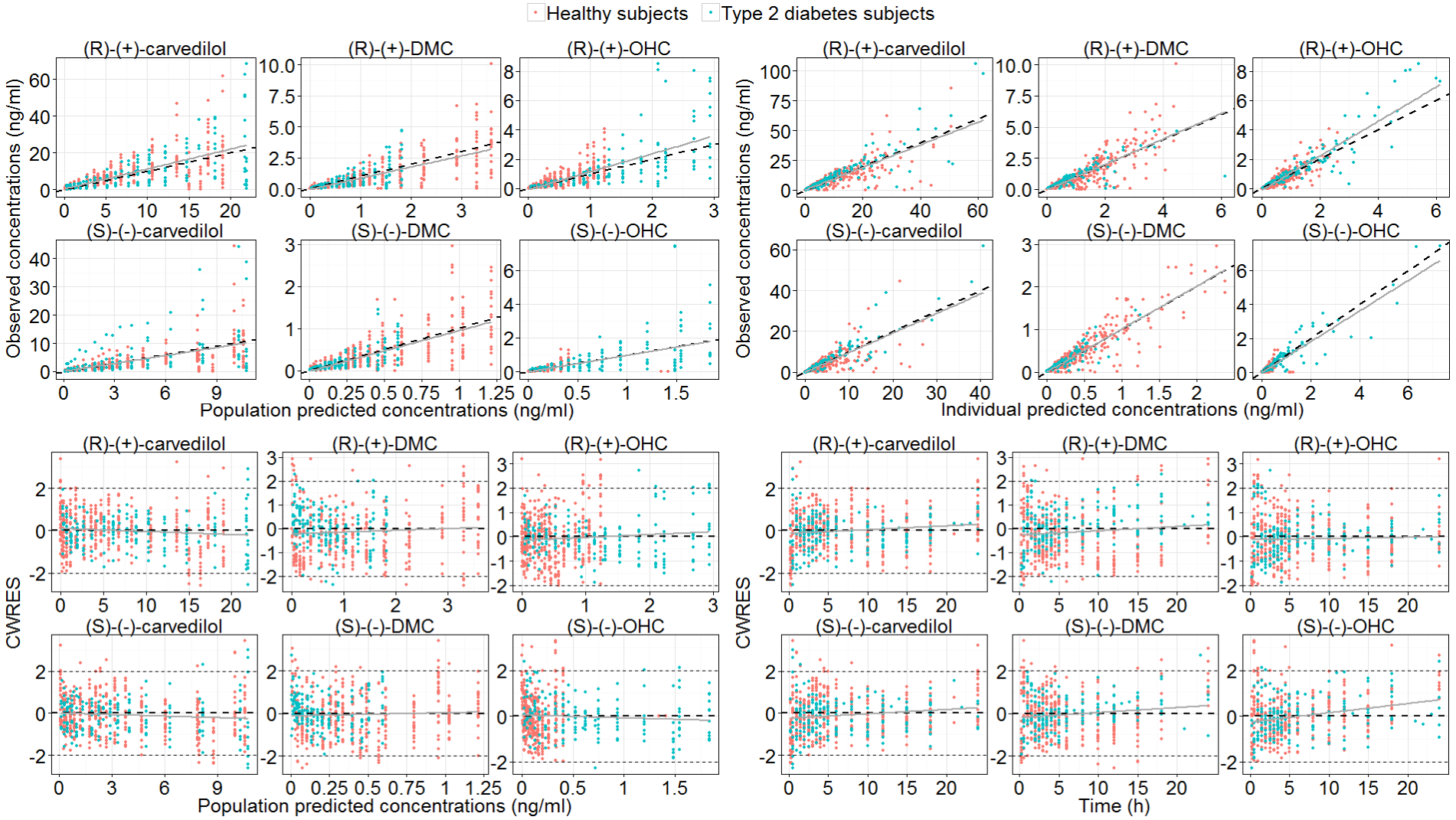

Figure S6: Goodness-of-fit (GOF) plots for the final model describing the pharmacokinetics of the enantiomers of carvedilol, O-desmethylcarvedilol (DMC) and hydroxyphenylcarvedilol (OHC). Individual observed concentrations *vs.* population predicted and individual predicted concentrations. Conditional weighed residuals (CWRES) *vs.* population predicted concentrations and time. The points represent the data. The solid gray line in each plot is the line of identity. The dashed lines are trend lines describing eventual patterns in the data.

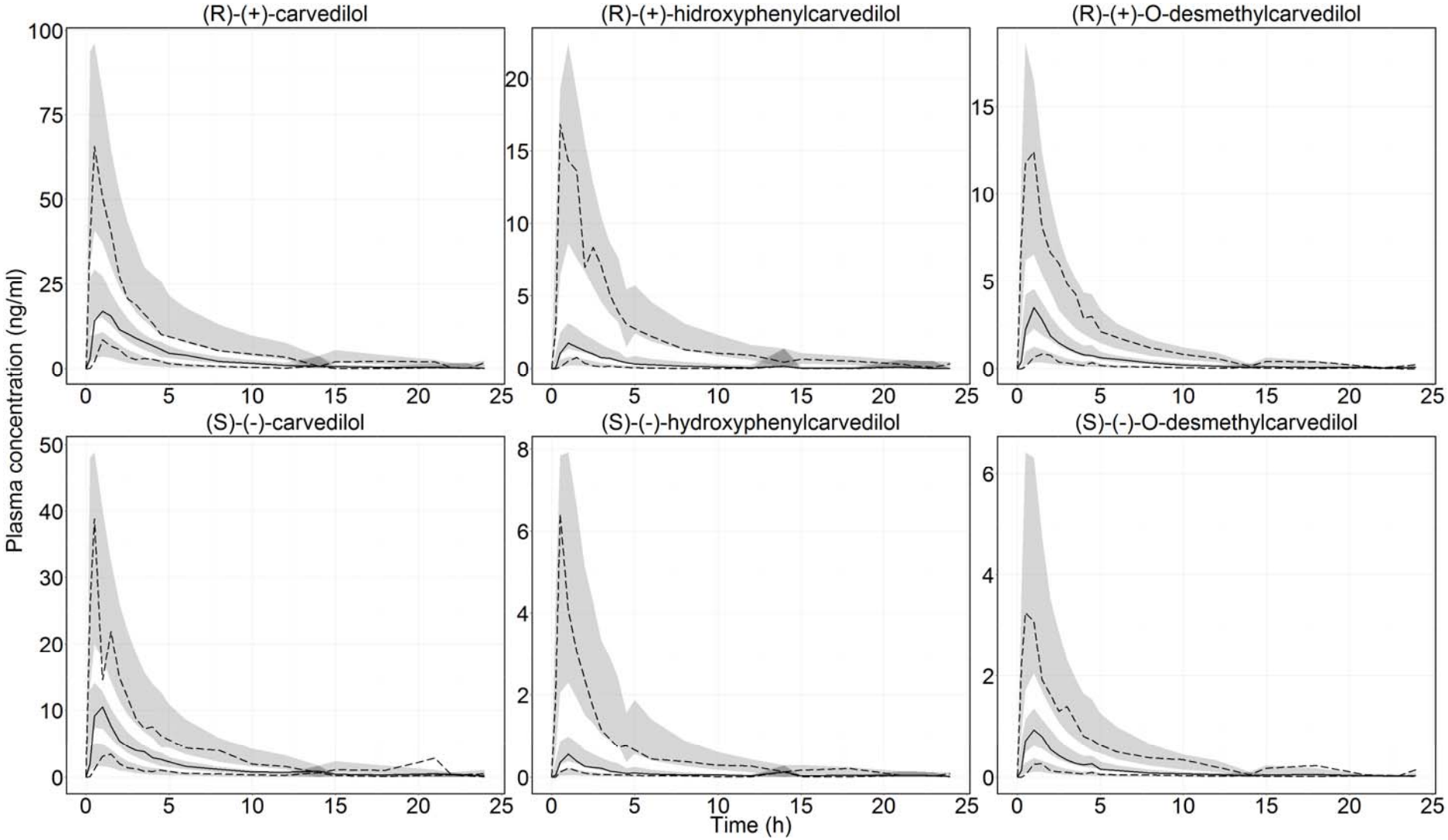

Figure S7: Visual predictive check (VPC) for the final model describing the pharmacokinetics of the enantiomers of carvedilol, O-desmethylcarvedilol (DMC) and hydroxyphenylcarvedilol (OHC). Lines describe the 5^{th}, 50^{th} and 95^{th} percentiles of observed plasma concentrations over time. Shaded areas depict the 95% confidence intervals of the 5^{th} , 50^{th} and 95^{th} percentiles obtained from 1000 simulations.

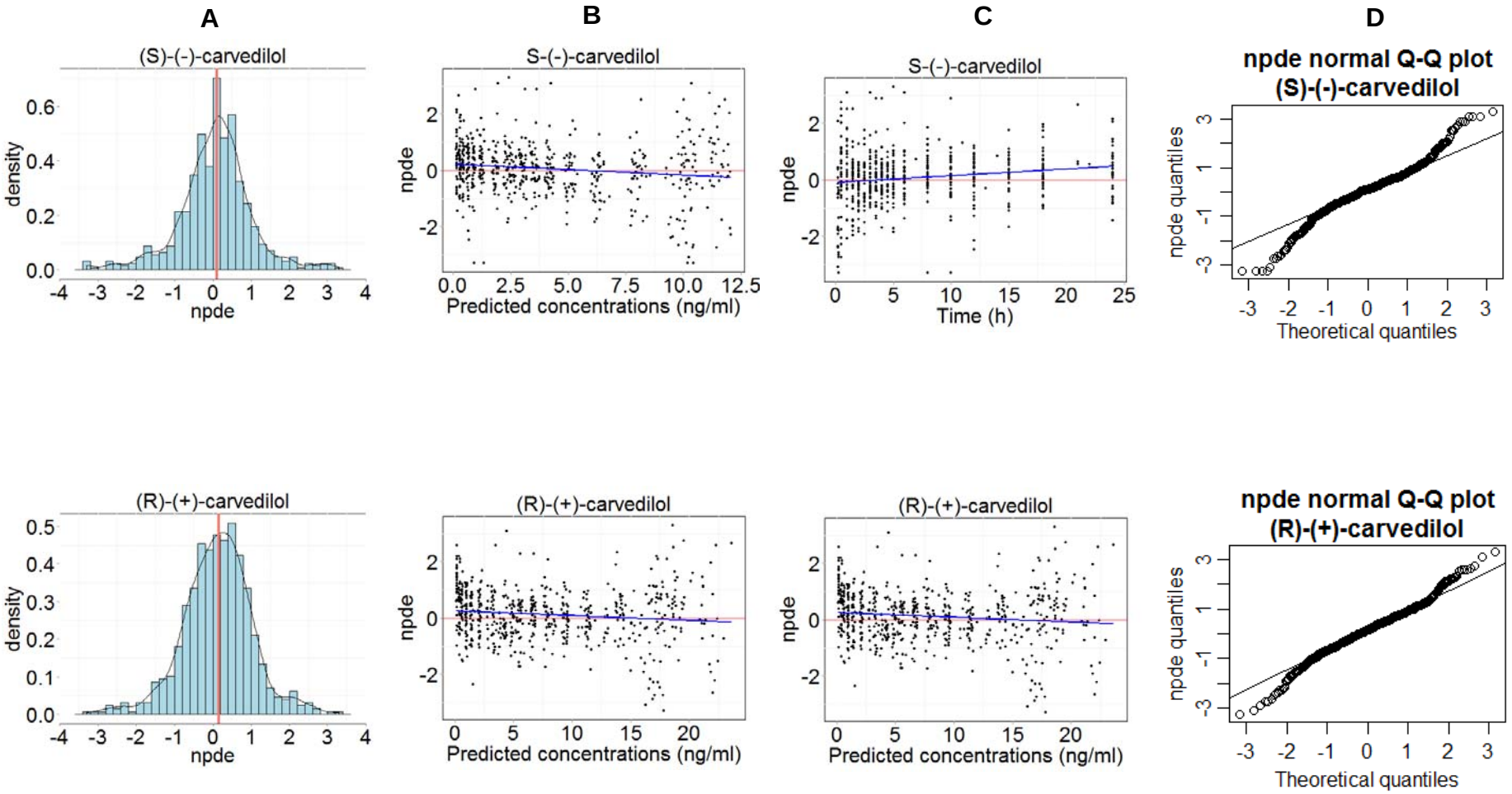

Figure S8: Normalised predictive distribution errors (NPDE) of the final model for carvedilol enantiomers. NPDE histogram (**A**); NPDE vs. predicted concentrations (**B**) and time (**C**); NPDE normal quantile – quantile plot (**D**).

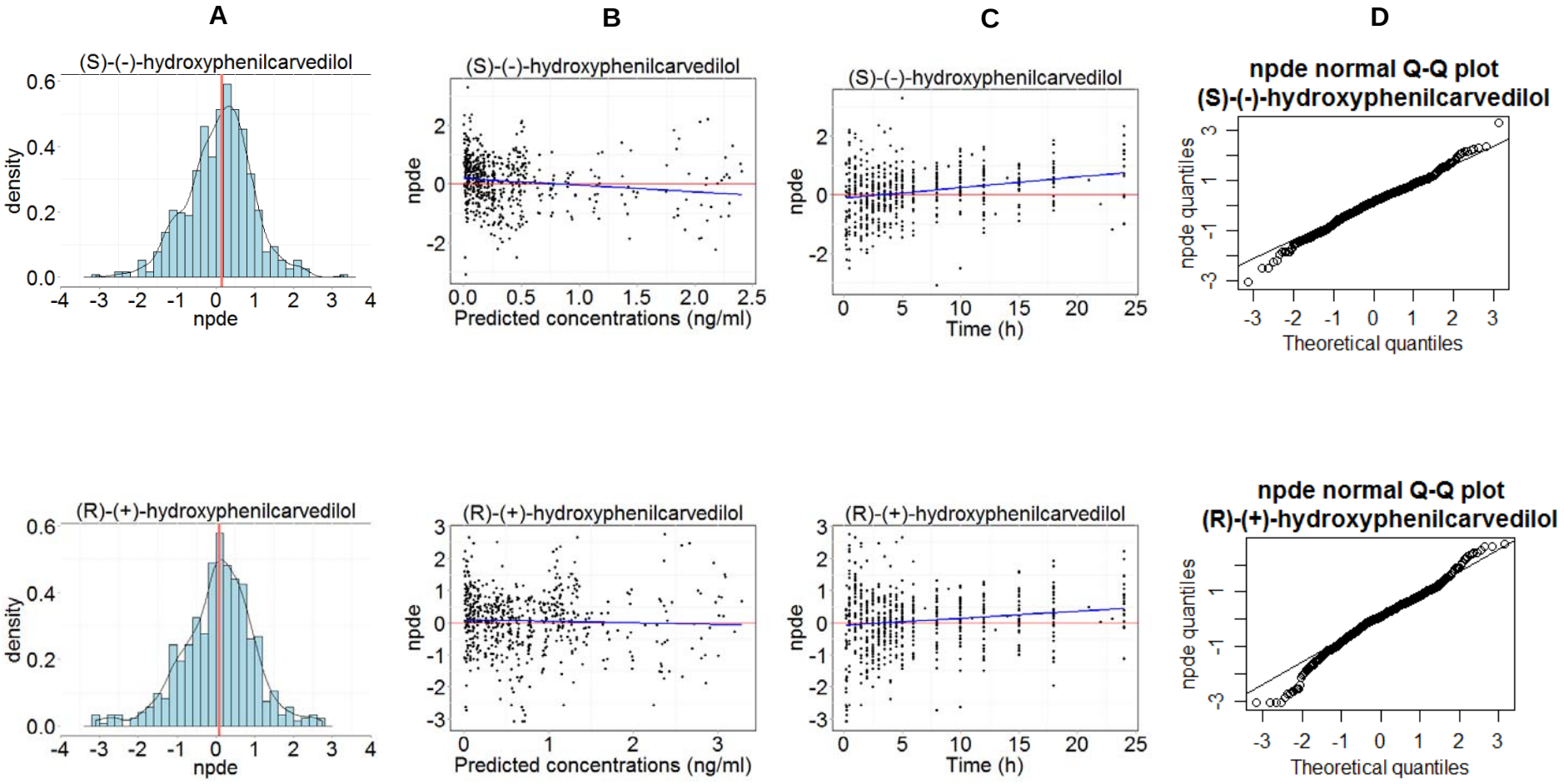

Figure S9: Normalised predictive distribution errors (NPDE) of the final model for the enantiomers of hydroxyphenilcarvedilol. NPDE histogram (**A**); NPDE vs. predicted concentrations (**B**) and time (**C**); NPDE normal quantile – quantile plot (**D**).

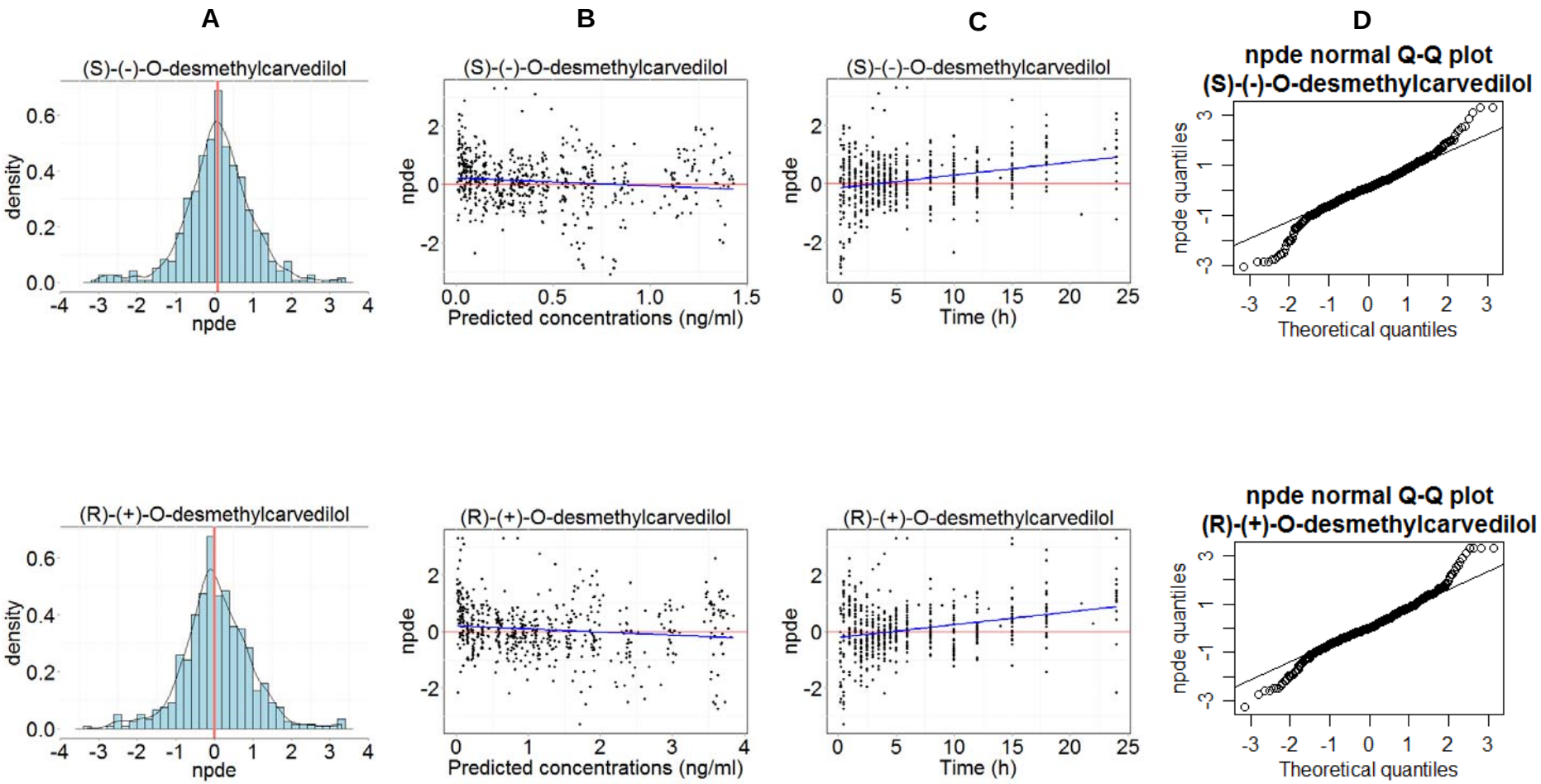

Figure S10: Normalised predictive distribution errors (NPDE) of the final model for the enantiomers of O-desmethylcarvedilol. NPDE histogram (**A**); NPDE vs. predicted concentrations (**B**) and time (**C**); NPDE normal quantile – quantile plot (**D**).

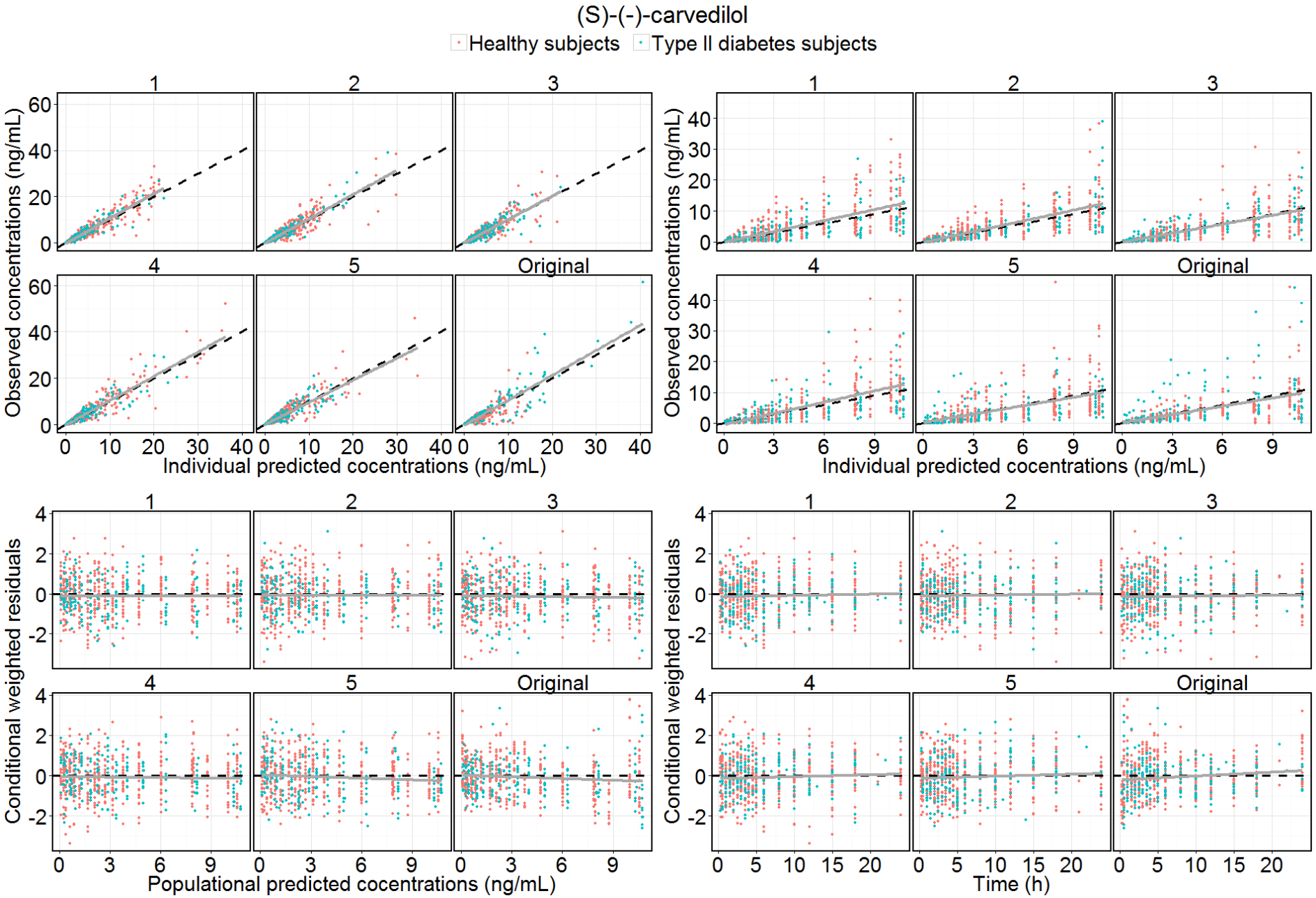

Figure S11: Mirror plots of the final model for (S)-(-)-carvedilol. Individual observed concentrations *vs*. population predicted and individual predicted concentrations. Conditional weighed residuals (CWRES) *vs.* population predicted concentrations and time. The solid gray line in each plot is the line of identity. The dashed lines are trend lines describing eventual patterns in the data.

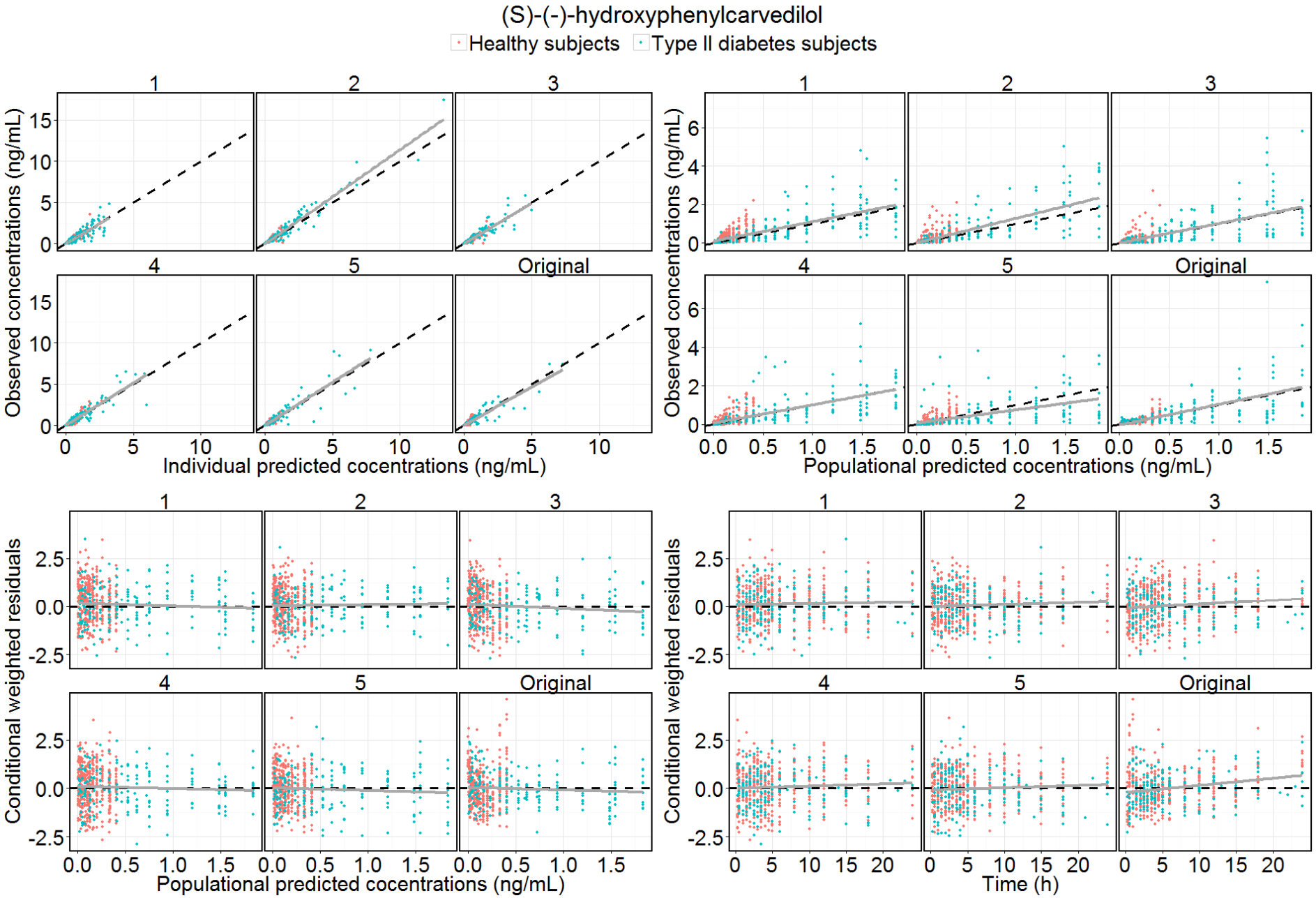

Figure S12: Mirror plots of the final model for (S)-(-)-hydroxyphenylcarvedilol. Individual observed concentrations *vs.* population predicted and individual predicted concentrations. Conditional weighed residuals (CWRES) *vs.* population predicted concentrations and time. The solid gray line in each plot is the line of identity. The dashed lines are trend lines describing eventual patterns in the data.

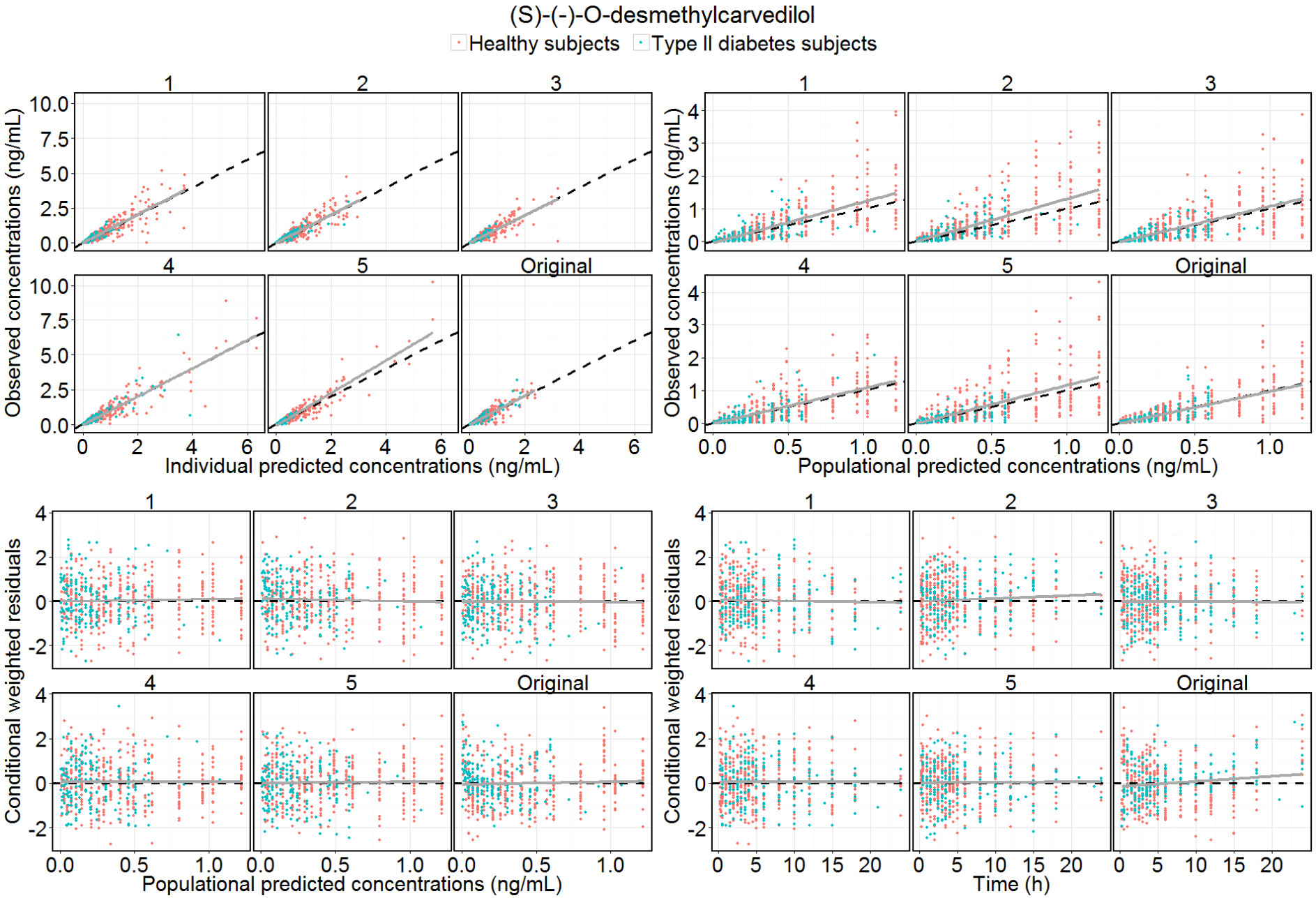

Figure S13: Mirror plots of the final model for (S)-(-)-O-desmethylcarvedilol. Individual observed concentrations *vs.* population predicted and individual predicted concentrations. Conditional weighed residuals (CWRES) *vs.* population predicted concentrations and time. The solid gray line in each plot is the line of identity. The dashed lines are trend lines describing eventual patterns in the data.

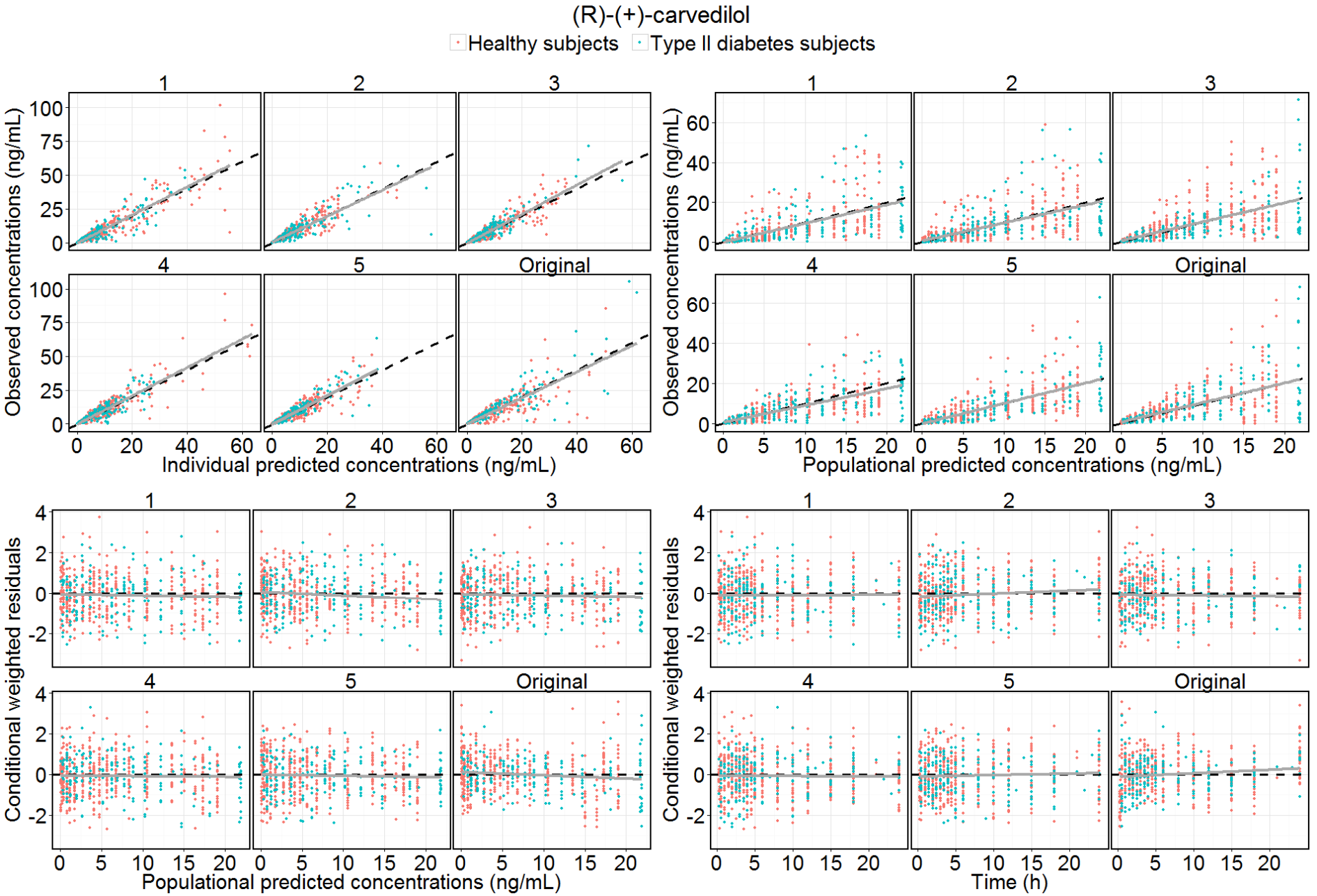

Figure S14: Mirror plots of the final model for (R)-(+)-carvedilol. Individual observed concentrations *vs.*. population predicted and individual predicted concentrations. Conditional weighed residuals (CWRES) *vs.* population predicted concentrations and time. The solid gray line in each plot is the line of identity. The dashed lines are trend lines describing eventual patterns in the data.

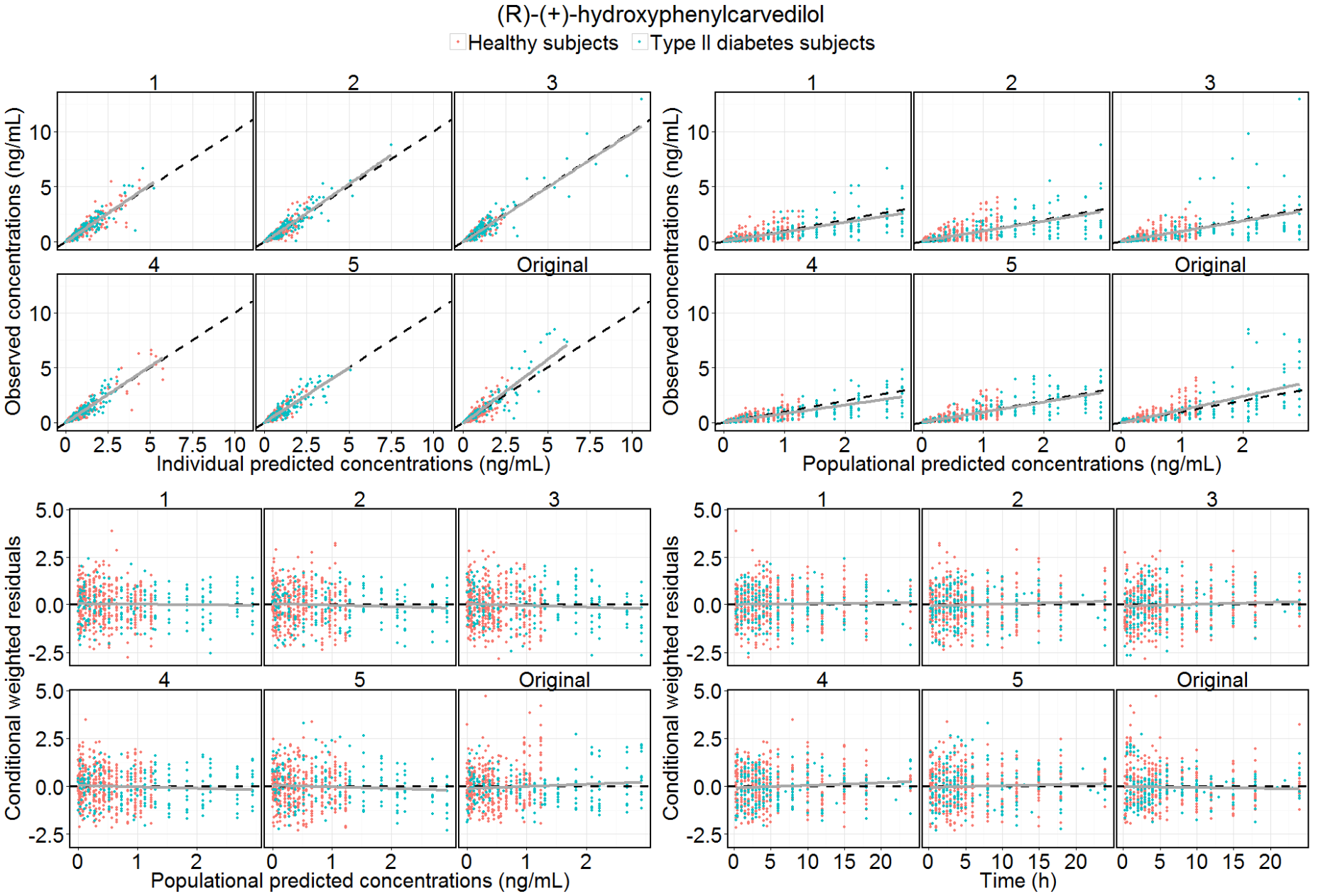

Figure S15: Mirror plots for the final model for (R)-(+)-hydroxyphenylcarvedilol. Individual observed concentrations *vs.* population predicted and individual predicted concentrations. Conditional weighed residuals (CWRES) *vs.* population predicted concentrations and time. The solid gray line in each plot is the line of identity. The dashed lines are trend lines describing eventual patterns in the data.

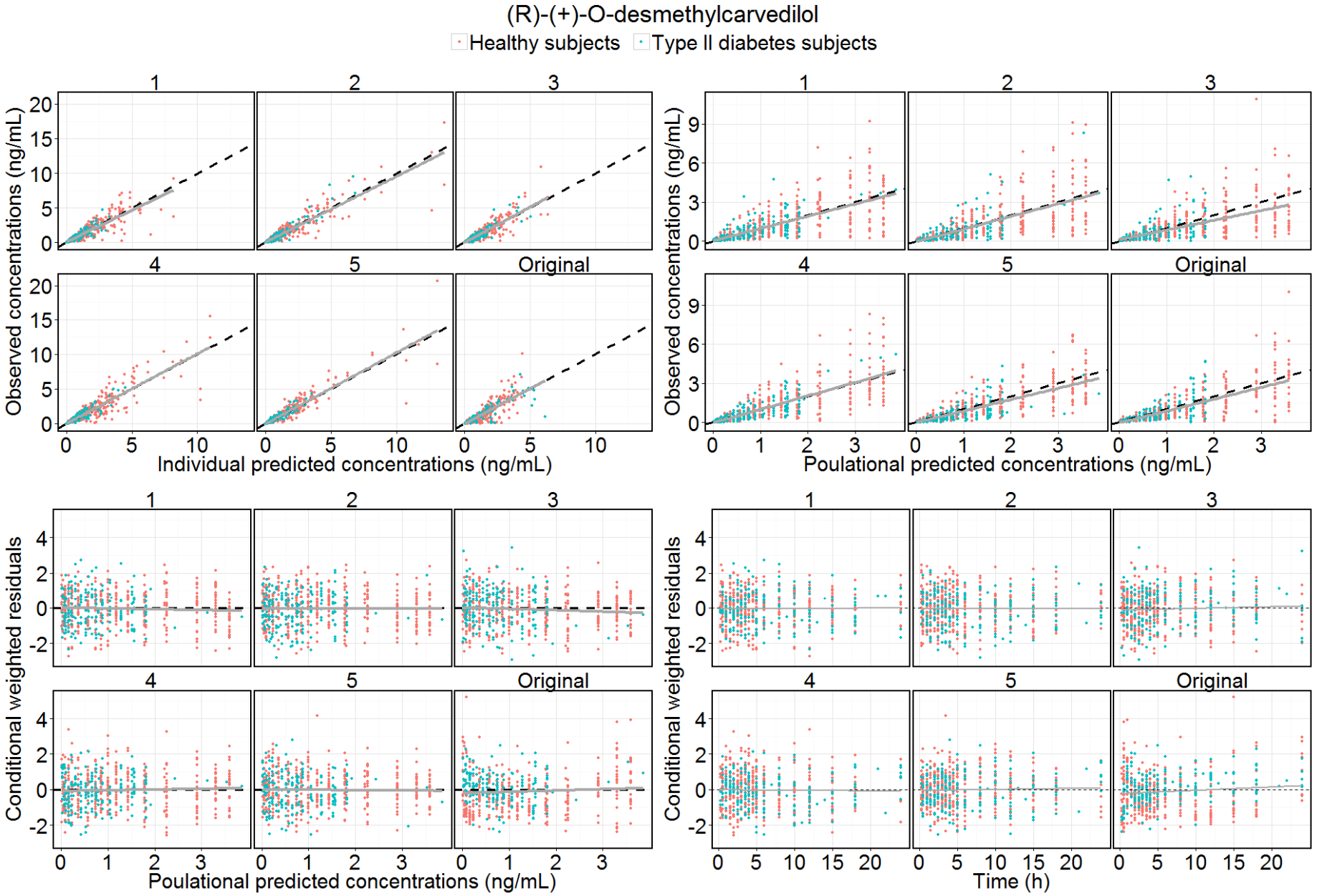

Figure S16: Mirror plots for the final model of (R)-(+)-O-desmethylcarvedilol. Individual observed concentrations *vs.* population predicted and individual predicted concentrations. Conditional weighed residuals (CWRES) *vs.* population predicted concentrations and time. The solid gray line in each plot is the line of identity. The dashed lines are trend lines describing eventual patterns in the data.

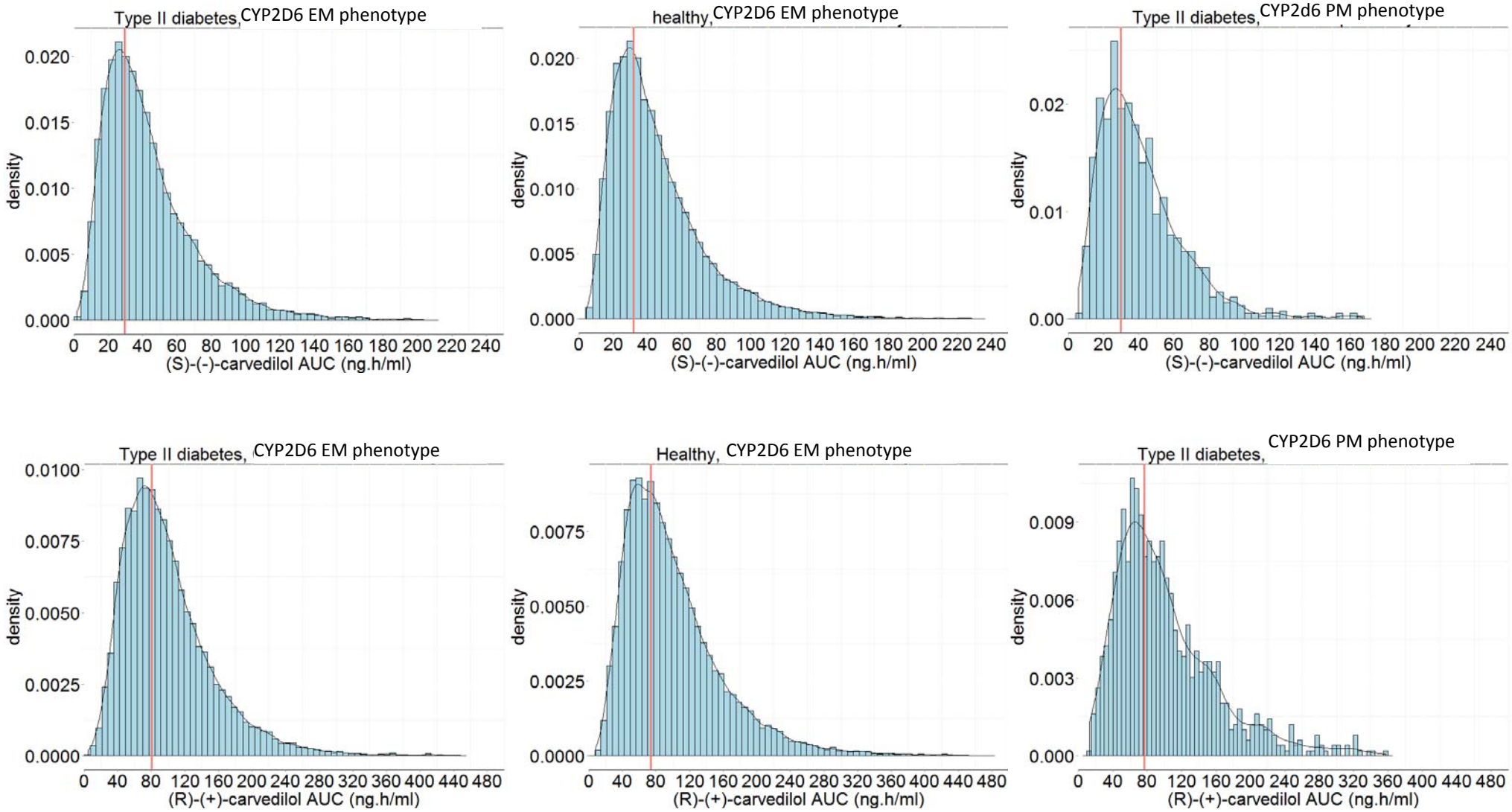

Figure S17: Post predictive check (PPC) of the final model for carvedilol enantiomers. Frequency histograms of simulated $AUC_{0\text{-}24}$ (n=1000). Solid vertical line depicts the geometric mean of observed $AUC_{0\text{-}24}$.

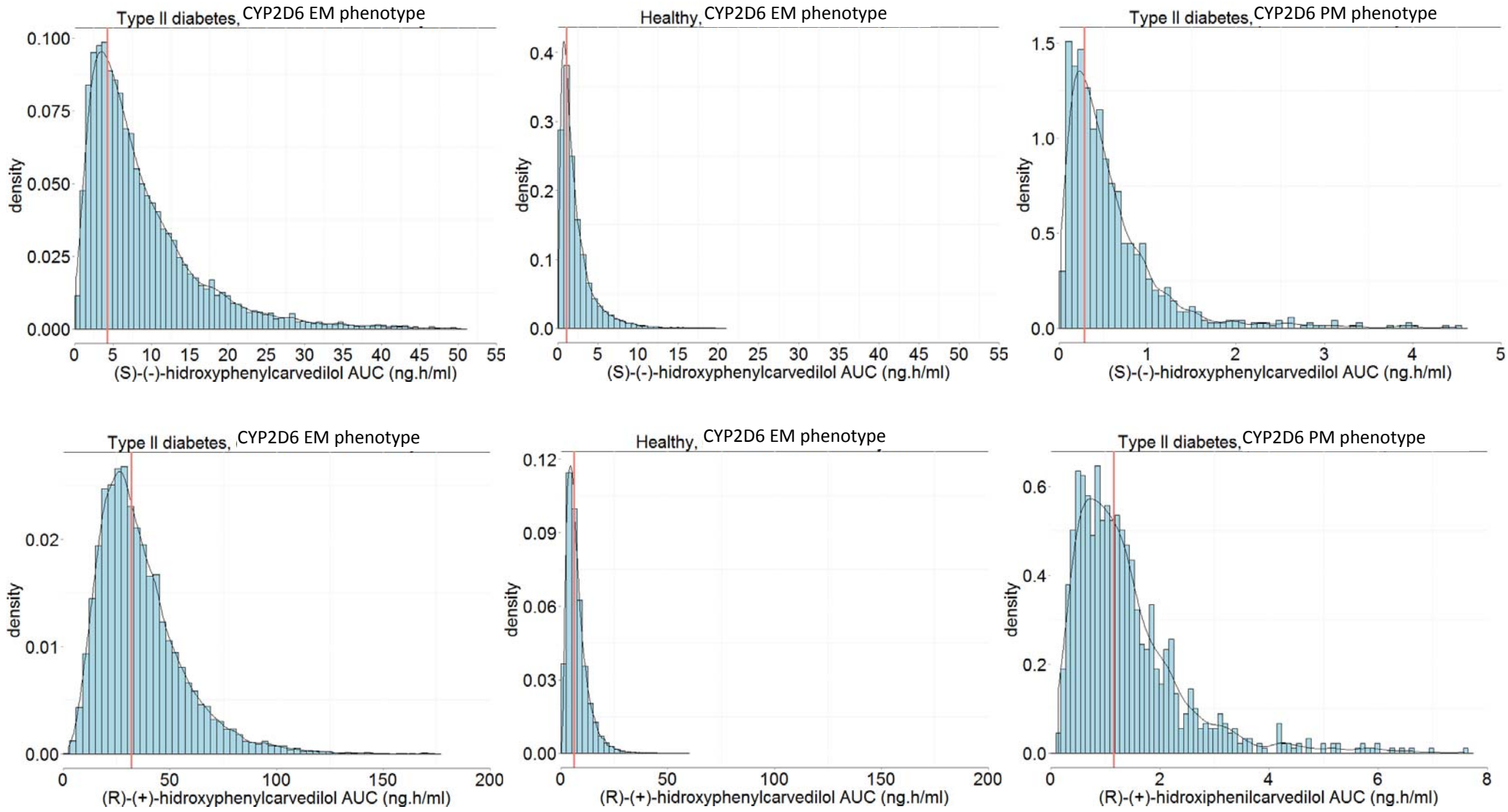

Figure S18: Post predictive check (PPC) of the final model for the enantiomers of hydroxyphenylcarvedilol. Frequency histograms of simulated $AUC_{0\text{-}24}$ (n=1000). Solid vertical line depicts the geometric mean of observed $AUC_{0\text{-}24}$

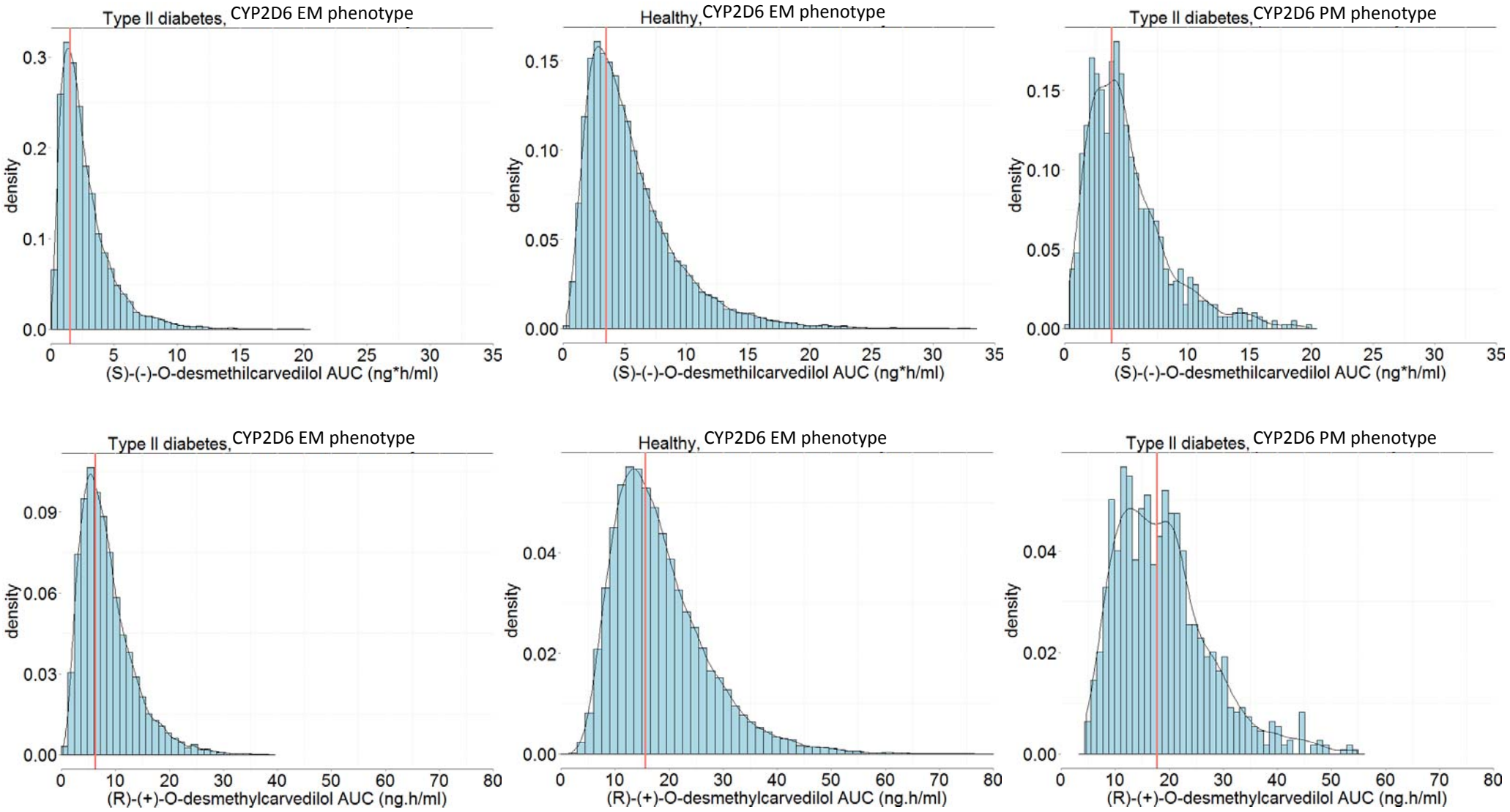

Figure S19: Post predictive check (PPC) of the final model for the enantiomers of O-desmethylcarvedilol. Frequency histograms of simulated $AUC_{0\text{-}24}$ (n=1000). Solid vertical line depicts the geometric mean of observed $AUC_{0\text{-}24}$.