Analysis PMN migration

Michael Fauler

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Data in long format is stored in the Excel-file data_long_JE_MiF.xlsx.

2 Raw Data

3 Measurement model

Fluorescence intensity is assumed to be proportional to cell number. The change of fluorescence ΔF is thus related to the number of cells that migrated to the lower compartment. Converting from raw to the change of signals removes background contributions

$$F_{calcein} = \alpha \cdot n_{PMN} + \hat{F}_{calcein} \tag{1}$$

$$\Delta F_{calcein} = \alpha \cdot (n_{PMN}|_t - n_{PMN}|_{t=0}) \tag{2}$$

The fMLP evoked effect is largest in the fMLP control group. This maximum effect size varies quite substantially between independent experiments (see figure 3).

From these results it is clear that the variance between experiments is larger than the variance within an experiment, that is, most of the variance is due to differences between donors or results from the isolation procedure. The origin of these deviations is unknown, and could therefore be considered as random effects in the analysis - or must be removed by an appropriate normalization procedure. Since statistics of hierarchical models are methodologically difficult to handle we choose the latter approach.

Assume the difference between isolations were due to a fraction of low or non-responsive cells. Then we get for fluorescence signal at the *top* detector

$$F_{calcein} = \alpha \cdot \left(n_{PMN}\big|_t + \mathring{n}_{PMN}\right) + \hat{F}_{calcein} \tag{3}$$

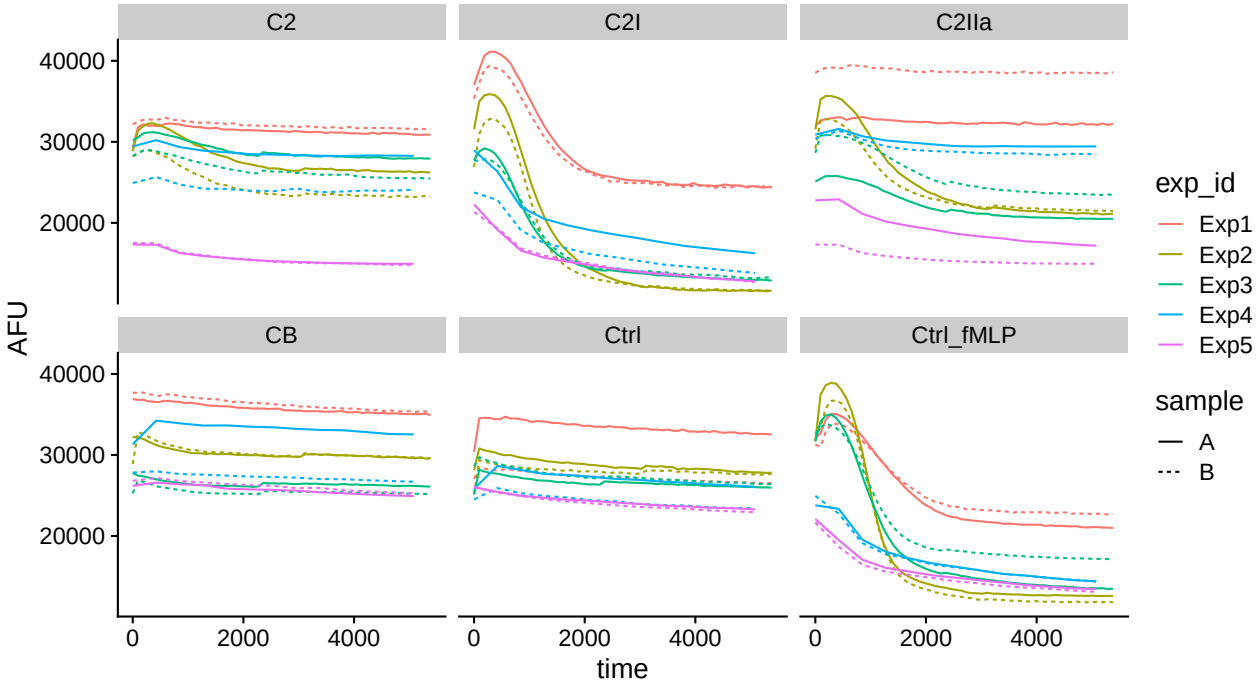

Figure 1: Raw Data. Calcein-fluorescence intensity measured from the top of inserts.

in which $n_{PMN}|_t$ is the number of responsive *PMN* in the upper compartment at time t, while $\mathring{n}_{PMN}$ is the number of non-responsive cells. Since $\mathring{n}_{PMN}$ is constant throughout the timecourse of an experiment, the term $\alpha\mathring{n}_{PMN}$ would add to the background signal and be removed by computing the change of fluorescence. Therefore eq. 2 still holds, but the effective amount of cells $n_{PMN}|_t$ will be smaller.

$n_{PMN}|_t$ could be expressed from the fraction of cells that have not yet translocated from the upper to the lower compartment at each timepoint t

$$n_{PMN}\big|_t = n_{PMN}\big|_{t=0} \cdot f_{PMN}\big|_t \tag{4}$$

or for $\Delta F_{calcein}$

$$\Delta F_{calcein}\big|_t = \alpha \cdot \left(n_{PMN}\big|_t - n_{PMN}\big|_{t=0}\right) = \alpha n_{PMN}\big|_{t=0} \cdot \left(f_{PMN}\big|_t - 1\right) \tag{5}$$

Herein, $f_{PMN}|_t$ were - per definition - independent from the number of cells, and would thus allow for comparison between experiments. Since, $f_{PMN}|_{t\to\infty} = 0$ the normalization of $\Delta F_{calcein}|_t$ by the maximum effect removes $\alpha n_{PMN}|_{t=0}$

$$\begin{aligned} \frac{\Delta F_{calcein}\big|_t}{\Delta F_{max}} &= \frac{\alpha n_{PMN}|_{t=0} \cdot (f_{PMN}|_t - 1)}{\alpha n_{PMN}|_{t=0} \cdot (0-1)} \\ &= 1 - f_{PMN}\big|_t \end{aligned} \tag{6}$$

4 Maximum change of fluorescence signal

The time-dependent change of the measured signal is an surrogate for the rate of migration. Of interest is the maximum (unsigned) disapearance of cells from the upper compartment when they transmigrate through the filter inserts. This might be measured as the smallest value of the first time derivative of the fluorescence signal. Additive (background) contributions in the signal are irrelevant in the derivative. The number of

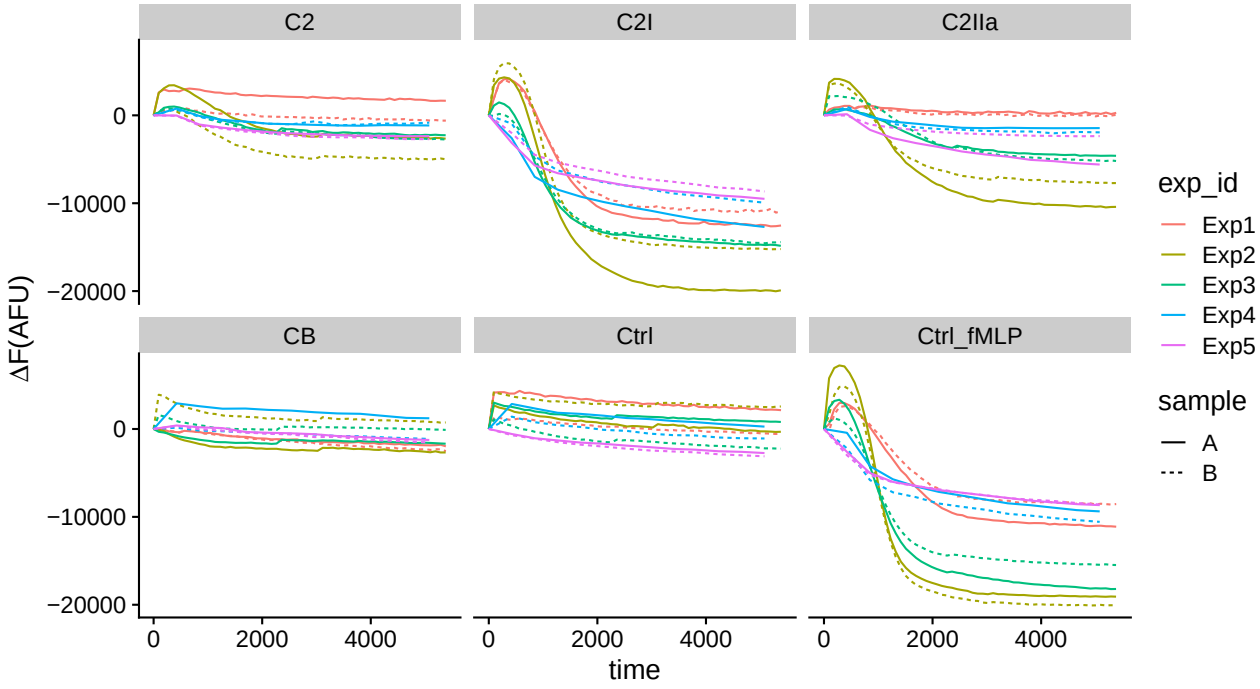

Figure 2: Absolute effect sizes.

responsive cells is maintained as a simple factor when derivatives are computed. Thus, the analysis is done on the derived signal of the fraction of responsive cells in the upper compartment $f_{PMN}|_t$.

The first time derivative is computed as the finite difference

$$\frac{df_{PMN}}{dt} = \frac{\Delta f_{PMN}}{\Delta t} \tag{7}$$

Since high frequency noise is known to deteriorate results, signals are first low-pass filtered by applying a standard second order Savitzky-Golay smoothing filter.

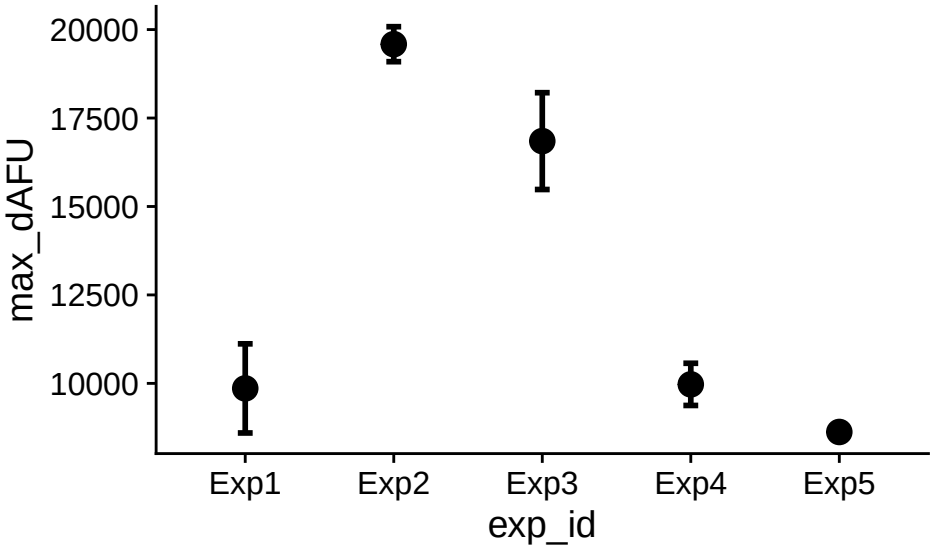

Figure 3: Maximum effect sizes. Control data from *fMLP* treatment.

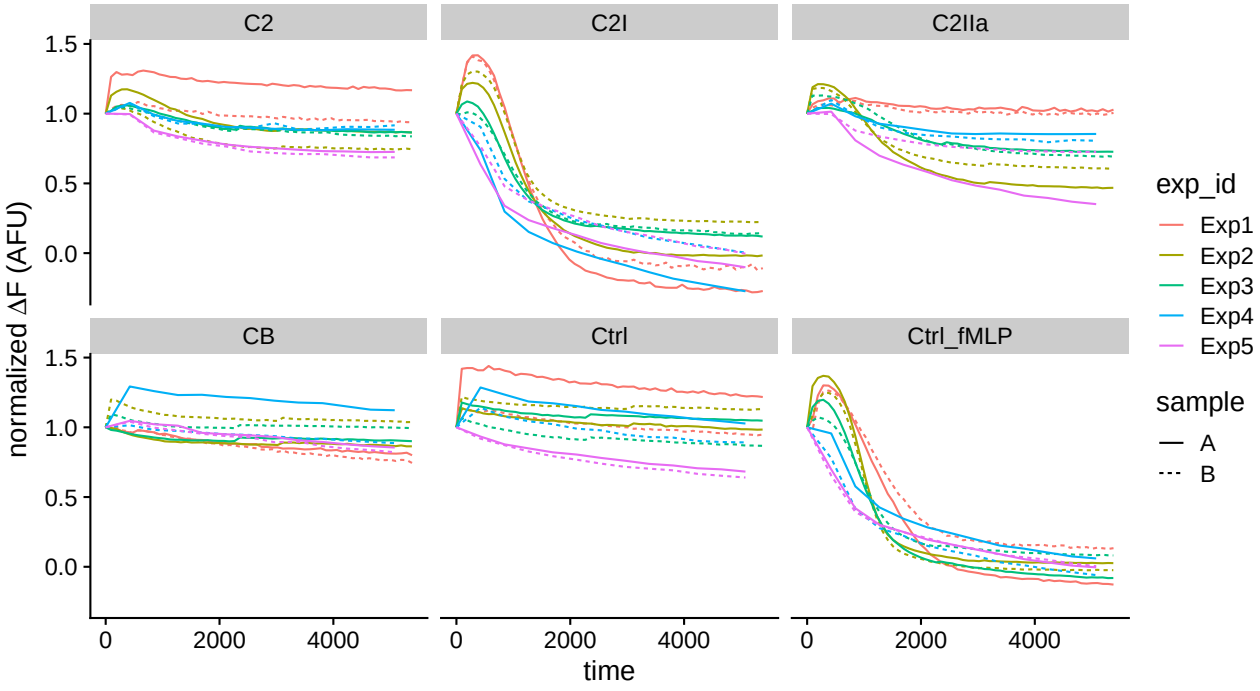

Figure 4: Normalized data.

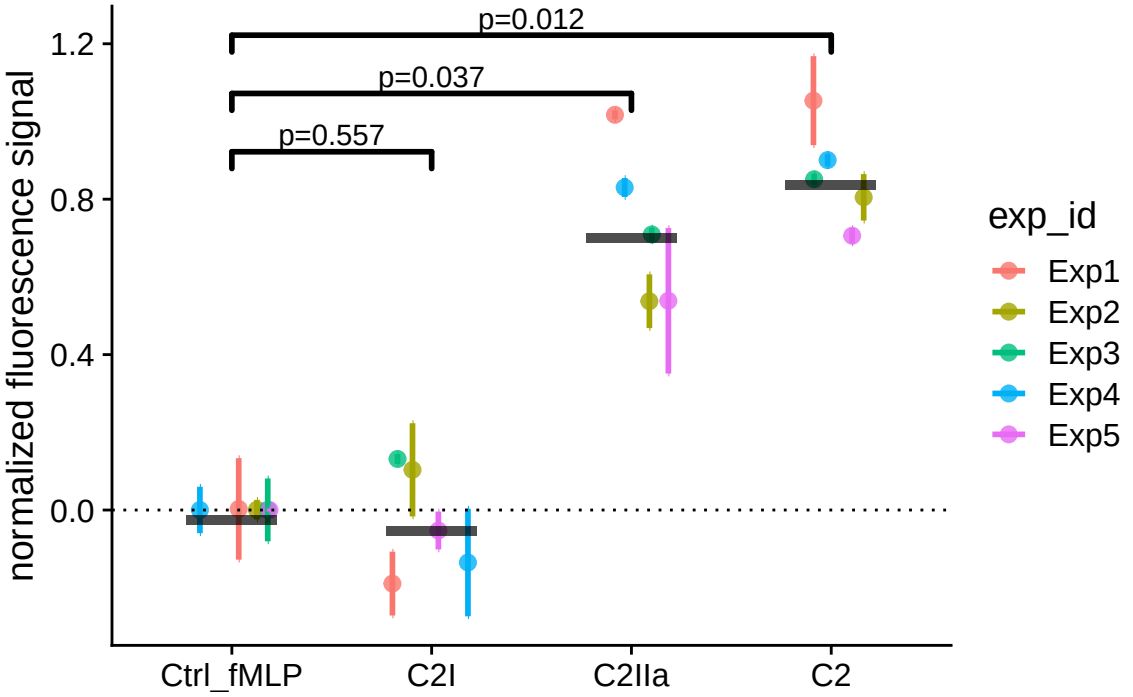

Figure 5: Normalized data at steady state at the end of the observation interval. Whiskers represent the values of two technical repeats. p-values are from Dunn-Tests on the ranks of the means of these technical repeats. Usually deviations between technical repeats are small, so that this test procedure is adequate. But especially for the C2IIa-group the deviation in experiment 5 (Exp5) is large. Maybe there is an invalid measurement, and one of the repeats should be declared an outlier. The result is still significant, even if the smaller values from the C2IIa-group are compared to the larger values of the Ctrl-group. Alternatively we could bootstrap the test result on resamples from the distributions on the experiment level.

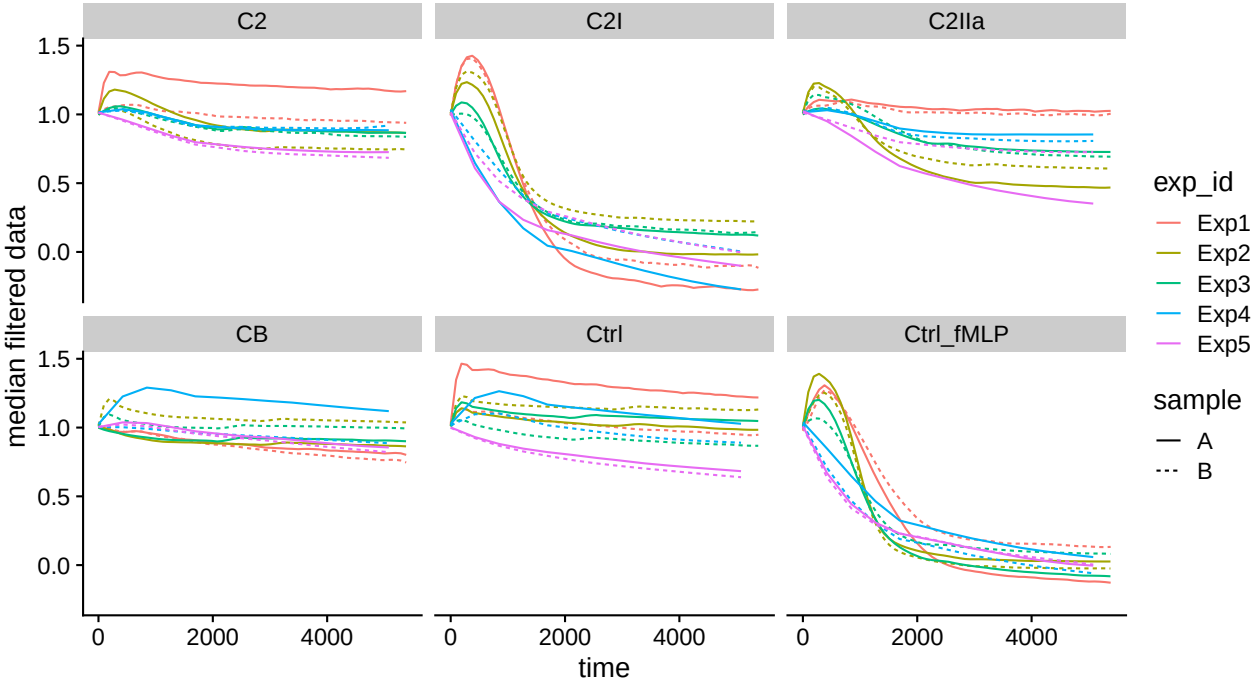

Figure 6: Savitzky-Golay smoothing filter.

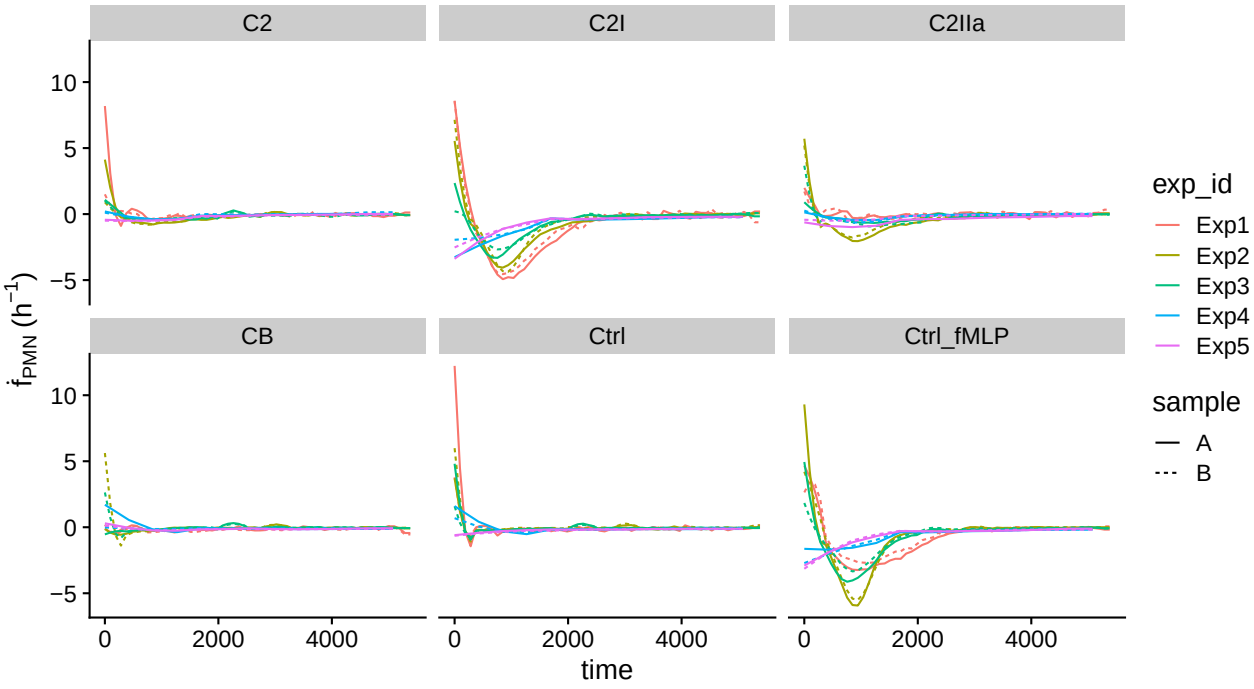

Figure 7: Time derivative of filtered data. Computed by finite-differences.

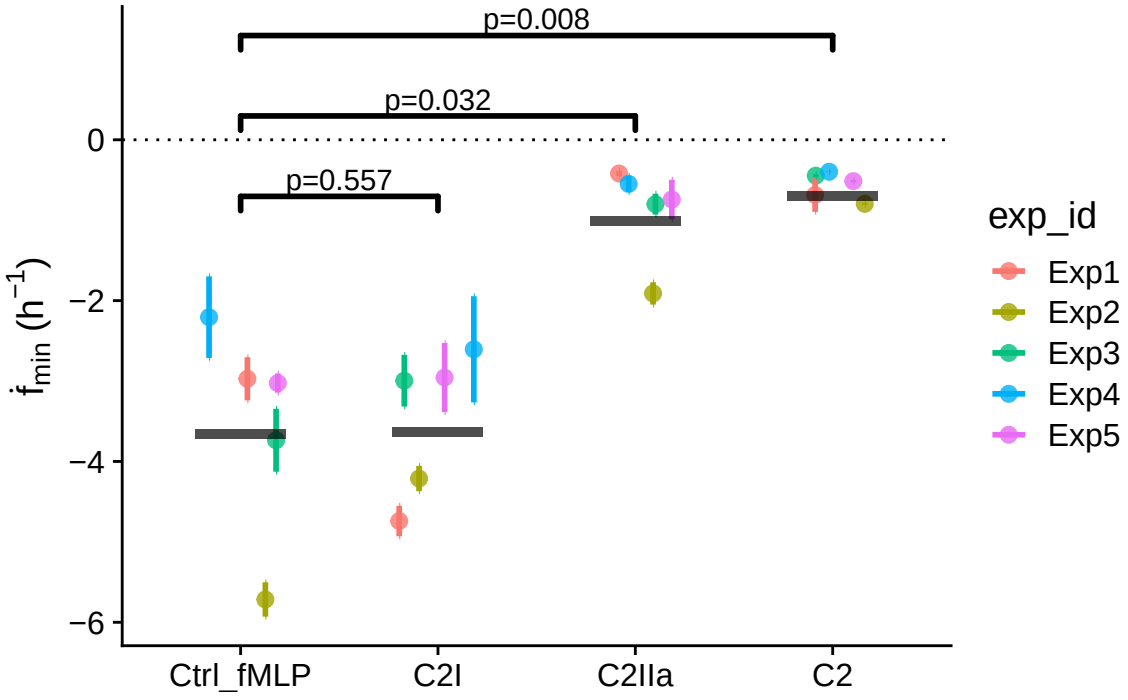

Figure 8: Fastest change of $f(t)$.