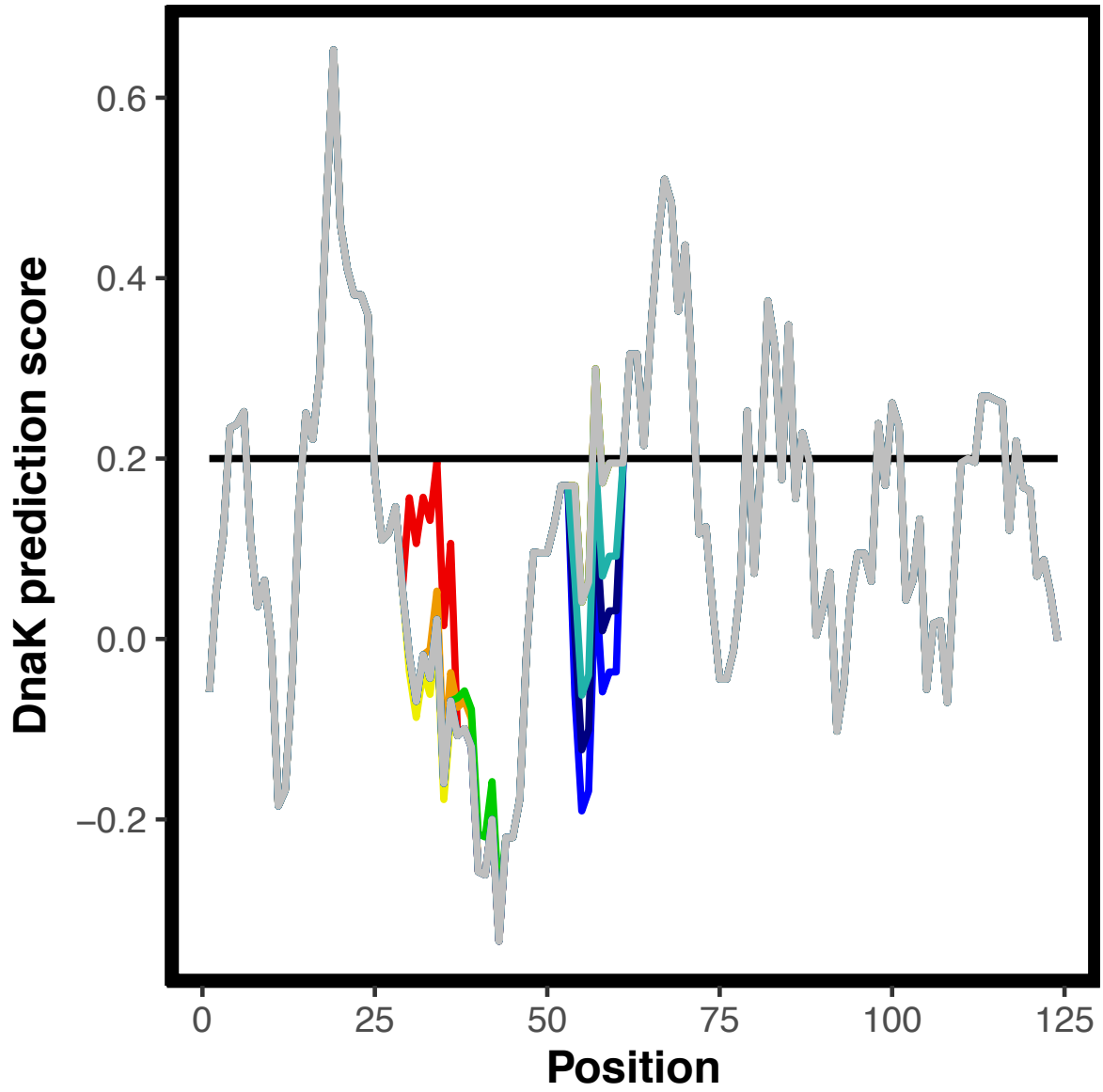
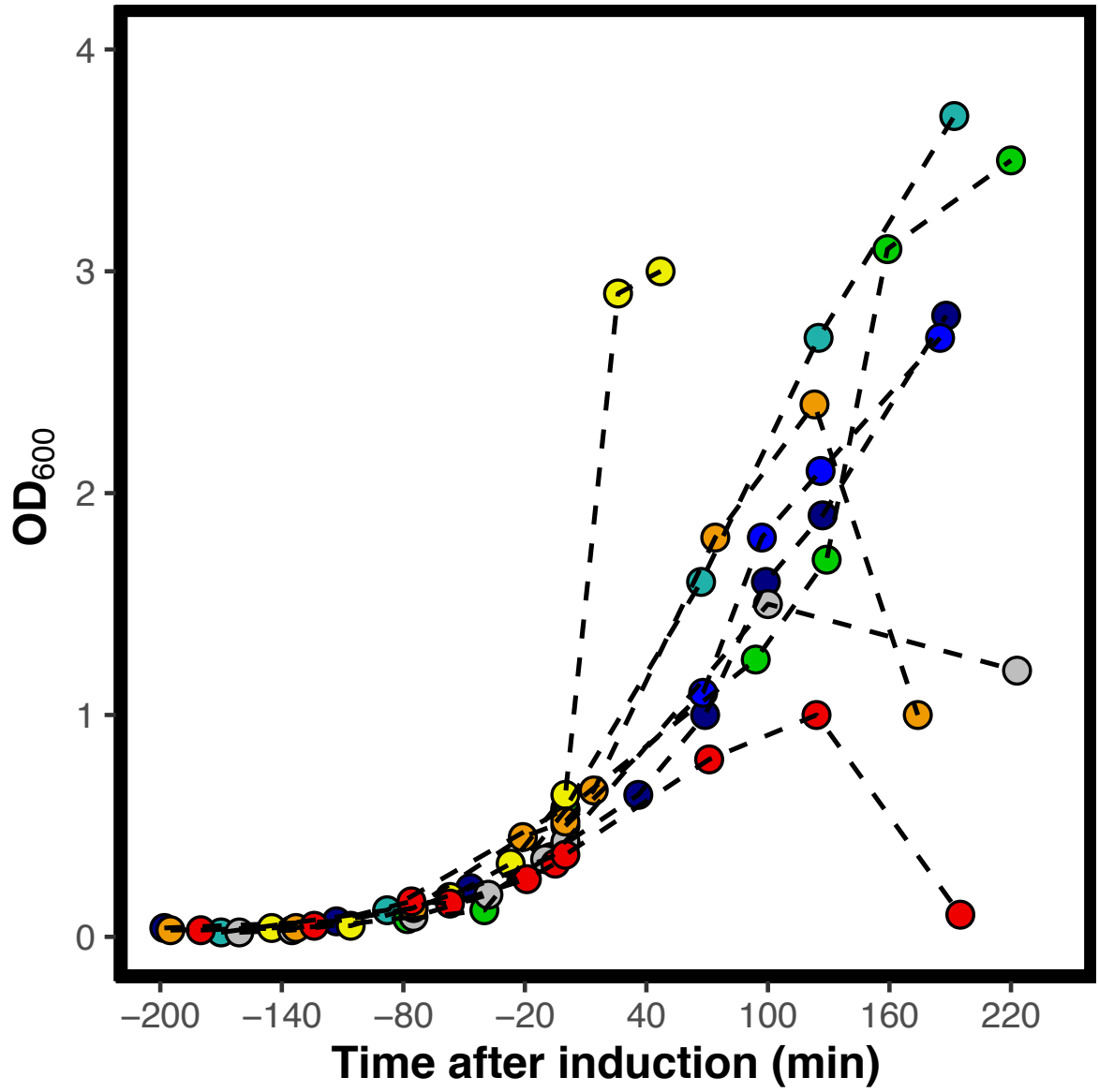
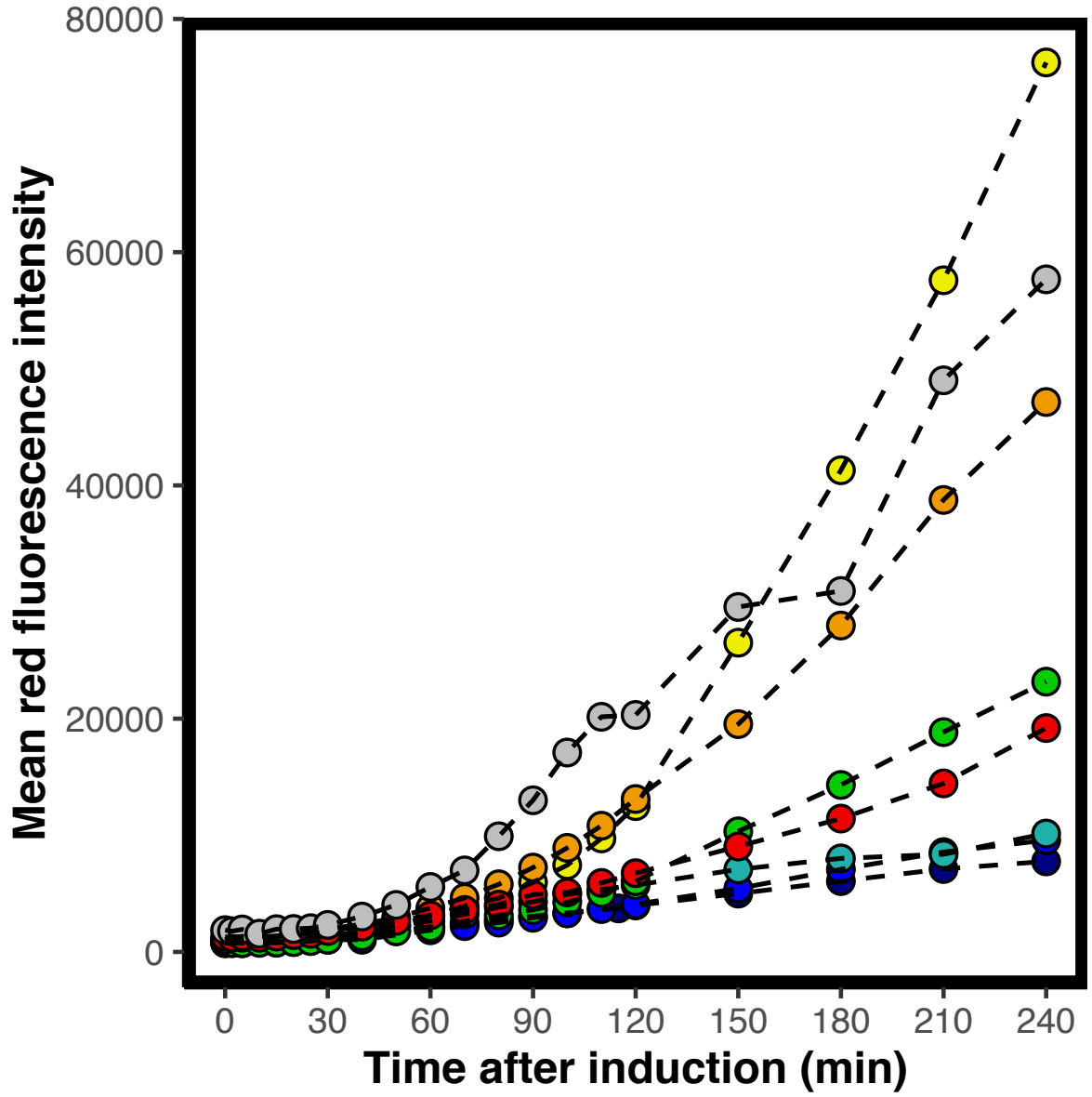


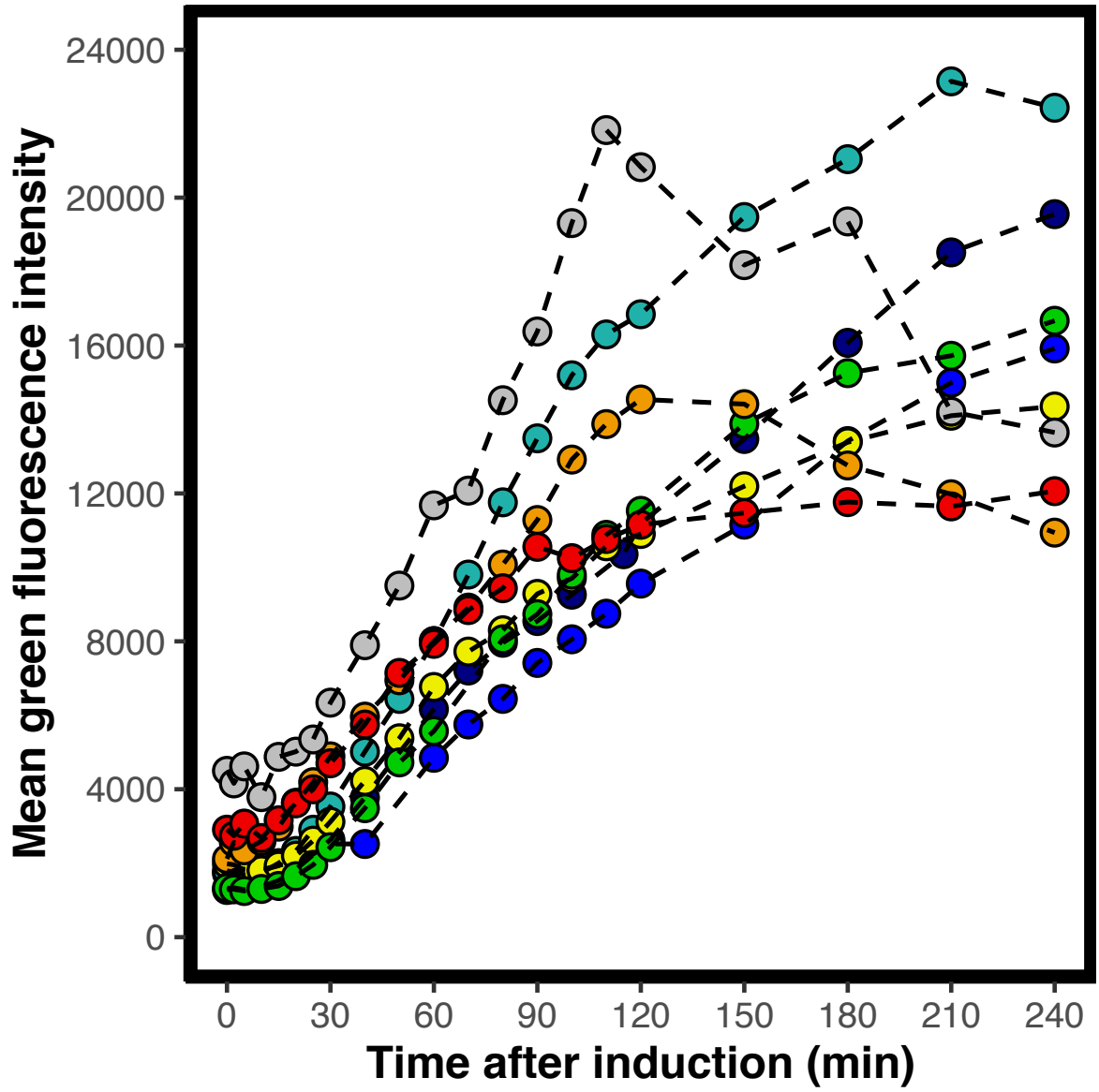

S 1 **Prediction of DnaK binding sites in N102LT variants**. DnaK binding sites predicted by the qualitative ChaperISM algorithm {Guiterres, 2020} for variants: L57G (dark blue), L57C (blue), L57P (cyan), M40A (green), Q33S (yellow), V36I (orange), Q33Y (red) and wt (grey). Where only grey is visible the score is identical for all variants. Cut-off for DnaK binding is 0.2 (black line).



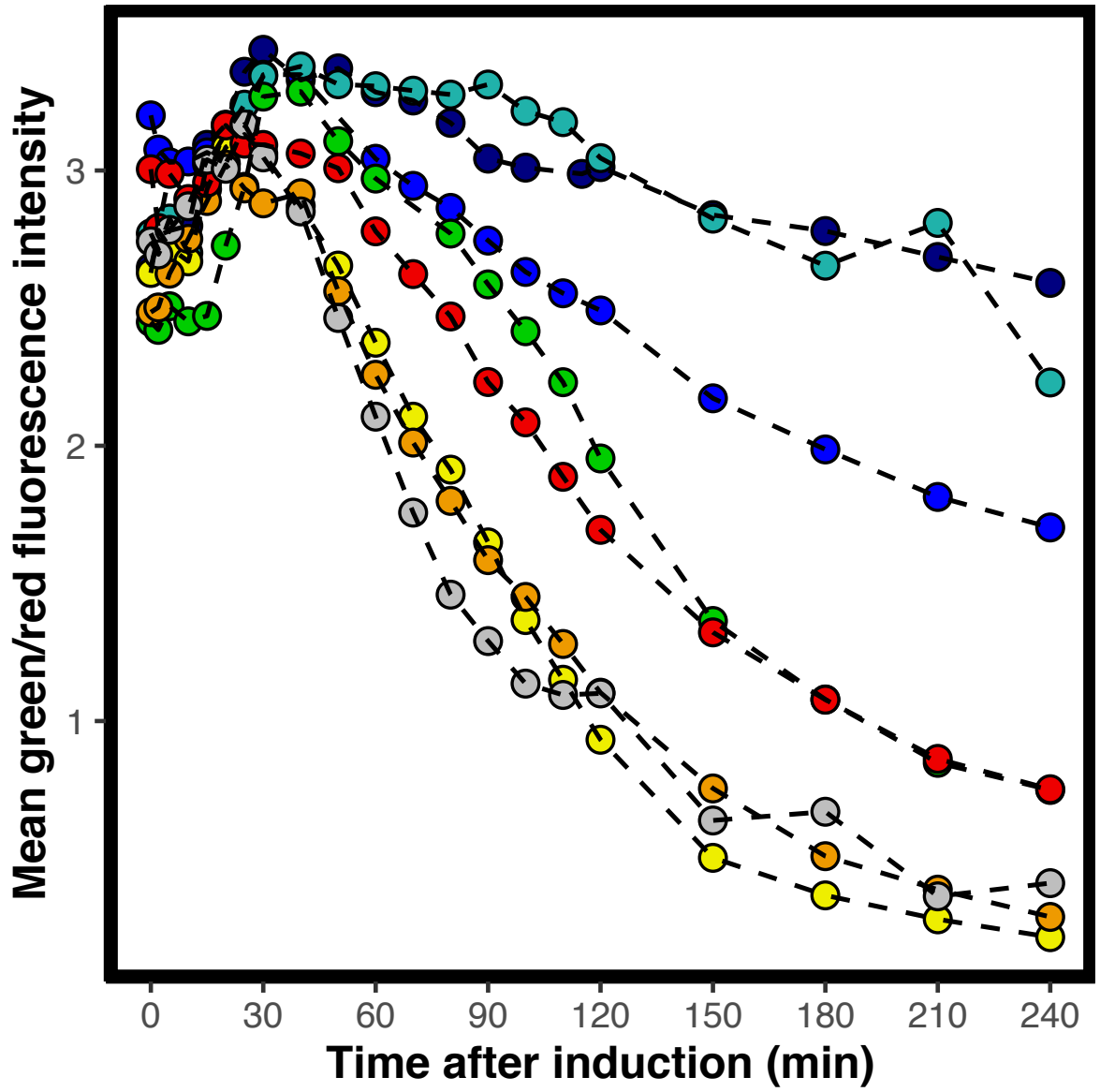

S 2 **Growth of cells expressing N102LT variants.** $OD_{600}$ as a function of time after induction of protein synthesis for cells expressing the variants: L57G (dark blue), L57C (blue), L57P (cyan), M40A (green), Q33S (yellow), V36I (orange), Q33Y (red) and wt (grey). One of two replicates are shown for each variant.



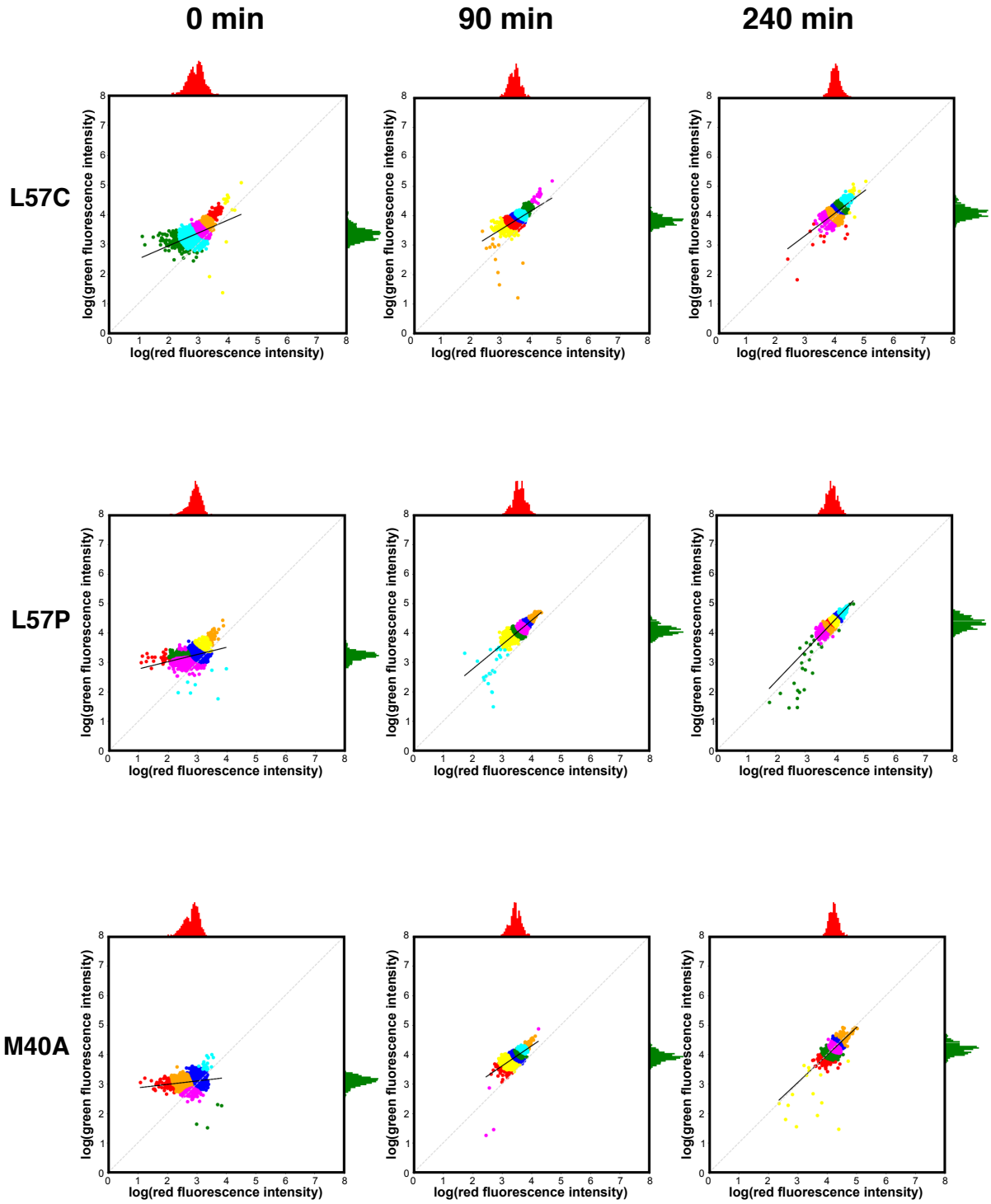

S 3 **Abundance of tagRFP fusion proteins monitored by red fluorescence.** Mean red fluorescence intensity of cells expressing variants: L57G (dark blue), L57C (blue), L57P (cyan), M40A (green), Q33S (yellow), V36I (orange), Q33Y (red) and wt (grey). One of two replicates are shown for each variant.



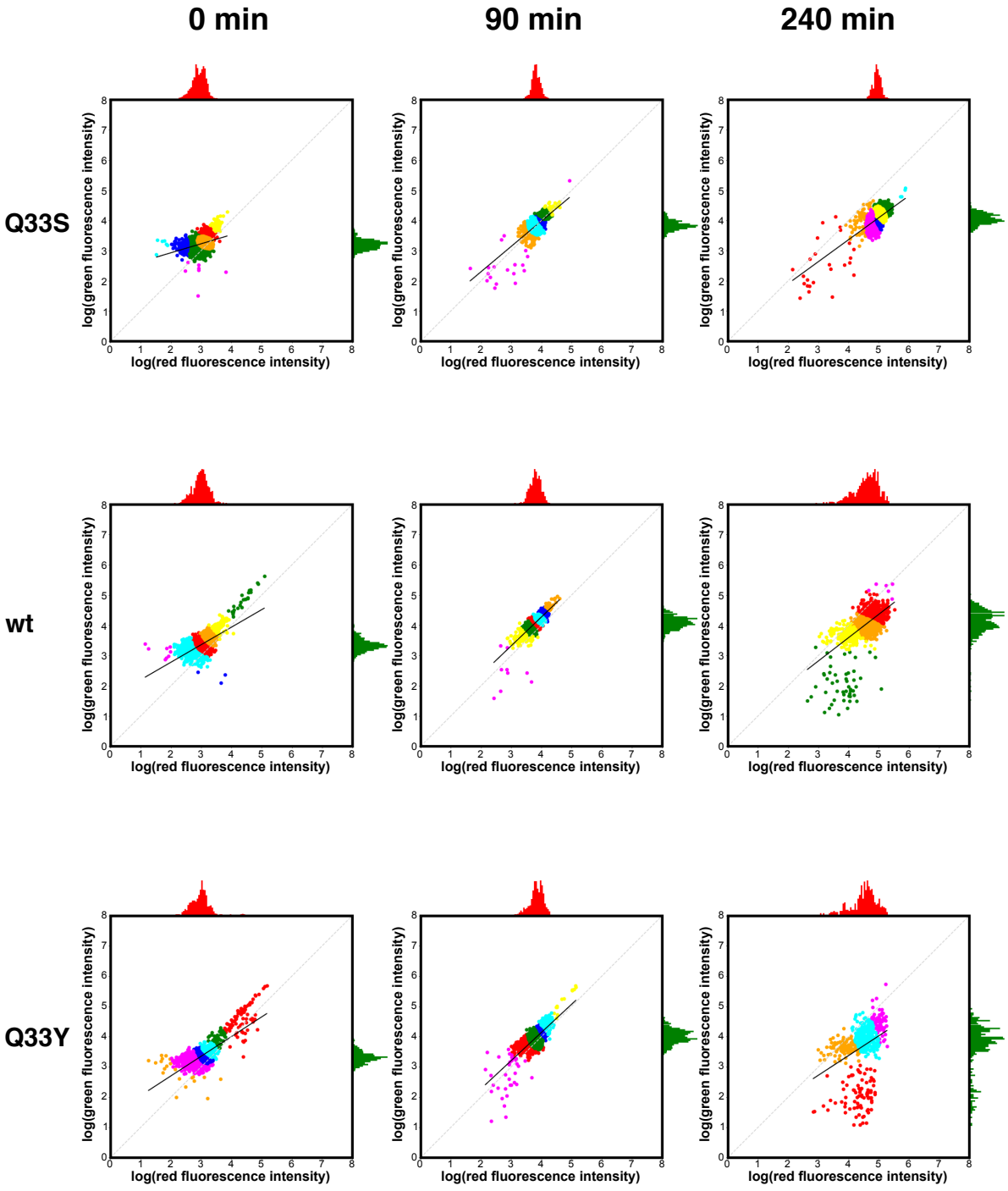

S 4 **Transcription from the DnaK promoter monitored by green fluorescence.** Mean green fluorescence intensity of cells expressing variants: L57G (dark blue), L57C (blue), L57P (cyan), M40A (green), Q33S (yellow), V36I (orange), Q33Y (red) and wt (grey). One of two replicates are shown for each variant.



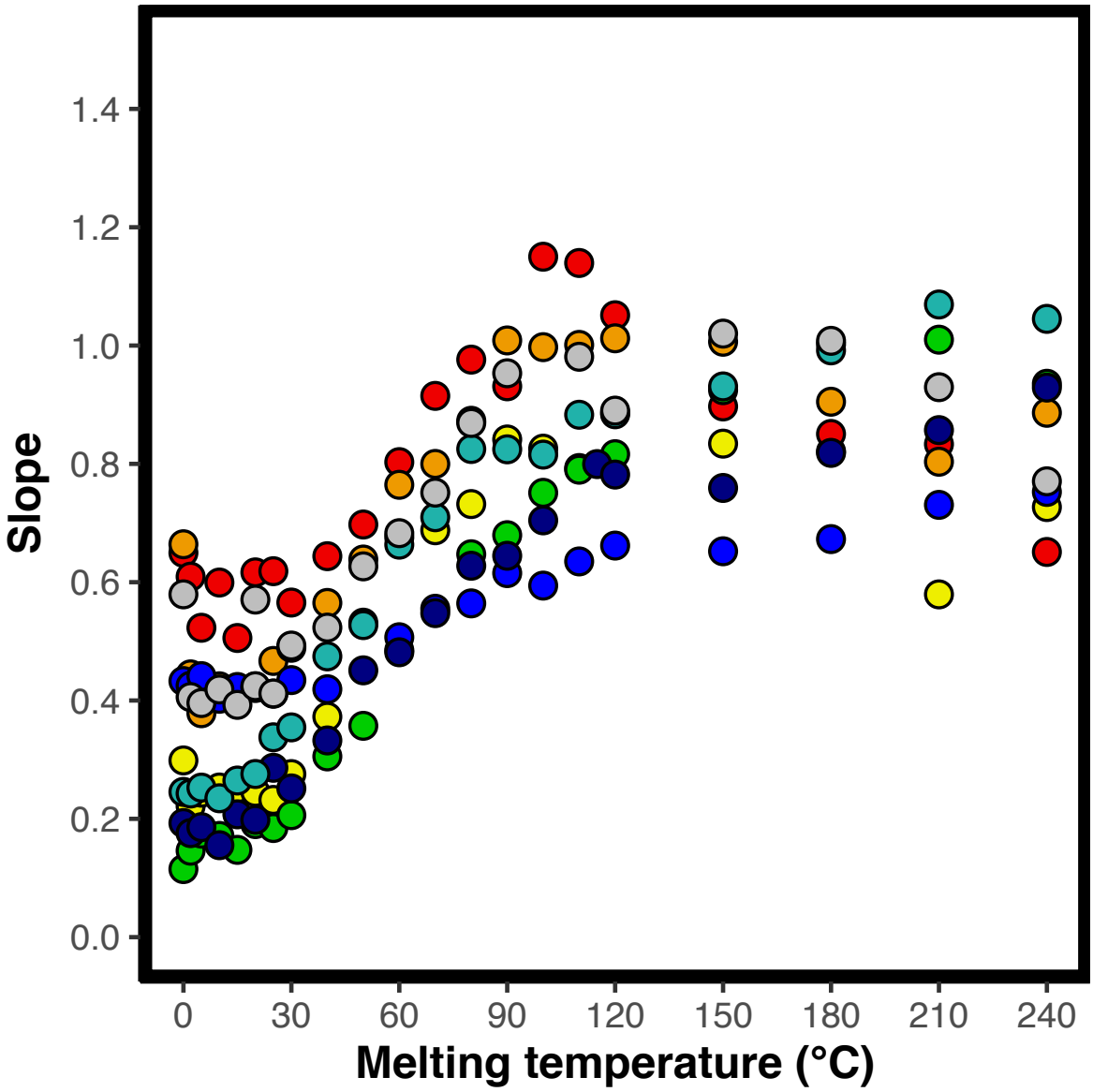

**S 5 Accumulated transcription from the DnaK promoter normalised to protein abundance** Mean green fluorescence intensity divided by red fluorescence intensity of cells expressing variants: L57G (dark blue), L57C (blue), L57P (cyan), M40A (green), Q33S (yellow), V36I (orange), Q33Y (red) and wt (grey). One of two replicates are shown for each variant.



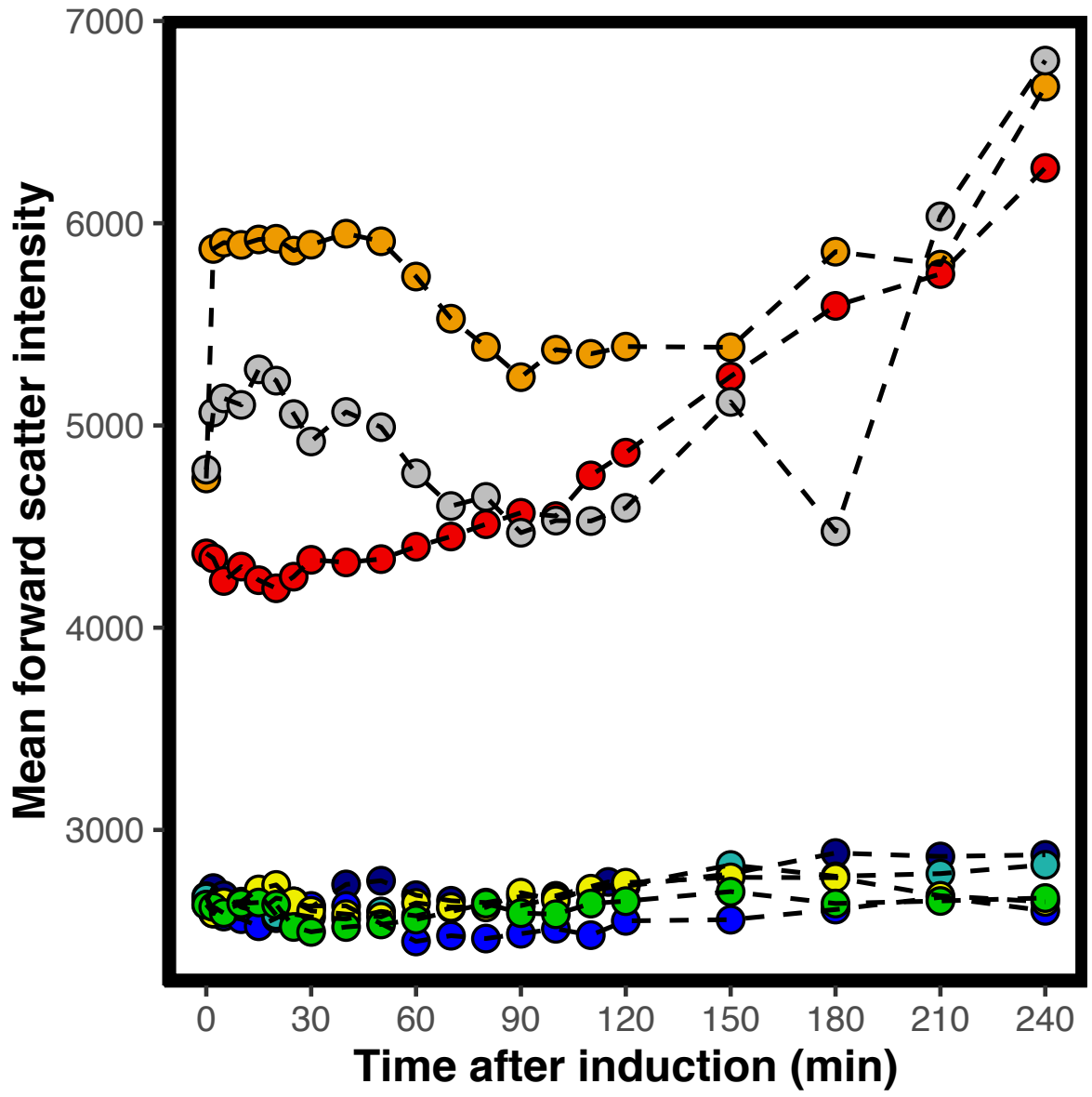

S 6 **Red and green fluorescence of single cells expressing L57C, L57P and M40A.**The red and green fluorescence for each individual cell in a population expressing L57C (top), L57P (middle) and M40A (bottom) at the time of induction (left) and 240 min after induction with IPTG (right). Colouring is based on a Bayesian Gaussian mixture model for cluster assignment. The x-axis histogram (red) shows the distribution of red fluorescence, and the y-axis histogram (green) shows the distribution of green fluorescence within the population.



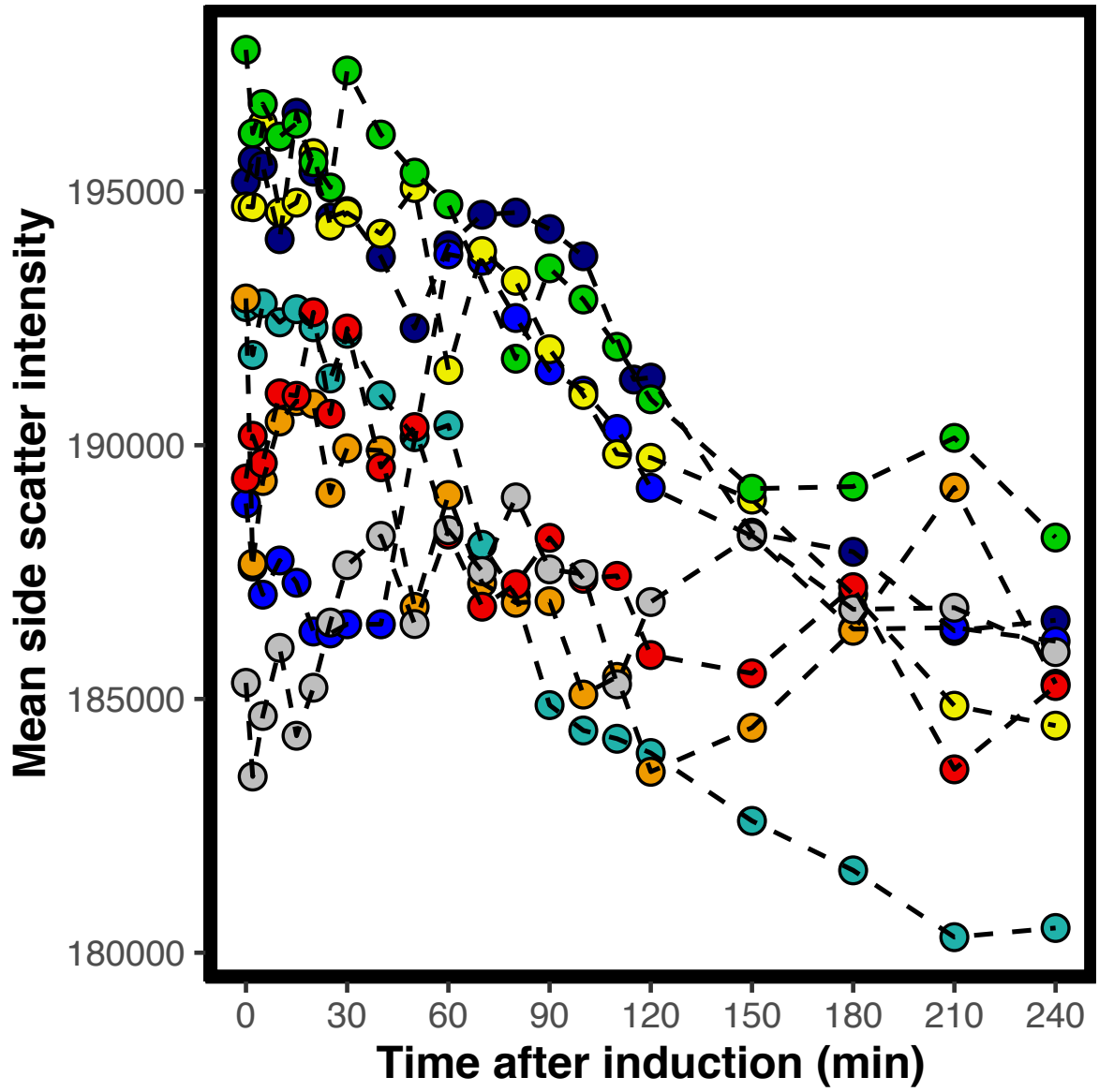

S 7 **Red and green fluorescence of single cells expressing Q33S, wt and Q33Y.** The red and green fluorescence for each individual cell in a population expressing Q33S (top), wt (middle) and Q33Y (bottom) at the time of induction (left) and 240 min after induction with IPTG (right). Colouring is based on a Bayesian Gaussian mixture model for cluster assignment. The x-axis histogram (red) shows the distribution of red fluorescence, and the y-axis histogram (green) shows the distribution of green fluorescence within the population.




S 8 **Slope of fluorescent population over time.** For a population of cells expressing the variants: L57G (dark blue), L57C (blue), L57P (cyan), M40A (green), Q33S (yellow), V36I (orange), Q33Y (red) and wt (grey) the slope of log transformed red fluorescence vs log transformed green fluorescence is calculated. One of two replicates are shown for each variant.




S 9 **Forward scatter of whole populations expressing N102LT variants.** Forward scatter as a function of time for variants: L57G (dark blue), L57C (blue), L57P (cyan), M40A (green), Q33S (yellow), V36I (orange), Q33Y (red) and wt (grey) One of two replicates are shown for each variant.




S 10 **Side scatter of whole populations expressing N102Lt variants.** Side scatter as a function of time for variants: L57G (dark blue), L57C (blue), L57P (cyan), M40A (green), Q33S (yellow), V36I (orange), Q33Y (red) and wt (grey). One of two replicates are shown for each variant.