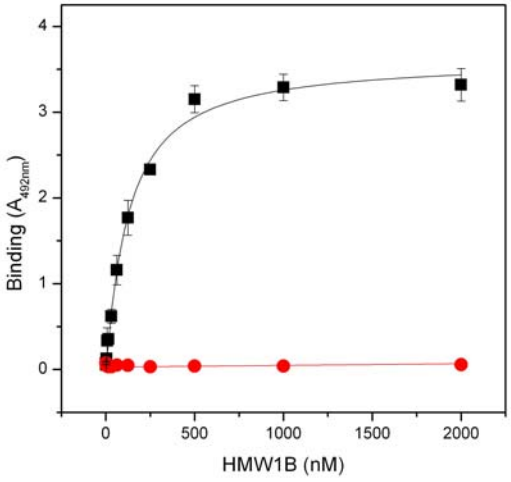
Supplemental Figure 2. A. Interaction between HMW1B and GST::HMW1-PP. The binding of HMW1B to GST::HMW1-PP (black) or to GST control (red) was measured with an ELISA-based protein binding assay as described in Material and Methods. The data (n = 4) was fitted to a saturation binding curve using a nonlinear regression fitting routine of the Origin 6.1 software to the following equation: $Y = B_{max} \cdot X^n / (K_d^n + X^n)$, where B_{max} is the maximal absorbance at 492 nm in the binding assay, X is the concentration of HMW1B (nM), n is the curve slope factor, and K_d is the dissociation constants. This fit yielded the following parameters: $B_{max} = 3.61 \pm 0.14$, n = 1.1, and $K_d = 125.8 \pm 14.4$ (nM). **B. Inhibition of HMW1B binding to GST::HMW1-PP by HMW1-PP.** Mixtures of a constant concentration of HMW1B (0.1 μM) and varying concentrations of GST-free HMW1-PP were incubated with GST::HMW1-PP immobilized microplates, and the binding affinity was measured by an ELISA-based protein binding assay as described in Material and Methods. The data (n = 4) was fitted to a competitive binding curve using a nonlinear regression fitting routine of the Origin 6.1 software to the following logistic equation: $Y = A_2 + (A_1-A_2) / (1 + (X / IC_{50})^p)$, where X is the concentration of HMW1-PP (nM), A_1 is the maximal binding of HMW1B and GST::HMW1-PP in the absence of HMW1-PP, A_2 is the binding of HMW1B to GST::HMW1-PP in the presence of a saturating concentration of HMW1-PP, p is the curve slope factor, and IC_{50} is the concentration of HMW1-PP that blocks 50% of the binding of HMW1B to GST::HMW1-PP. HMW1B binding to GST::HMW1-PP in the absence of HMW1-PP was set to 100%. The fit yielded an IC_{50} value of 125.5 ± 12.5 nM.

A.



B.