



**Figure S3** Median net F2 hybrid misregulation under varying mutation rates occurring at *cis*-regulatory sites of separate traits in the pleiotropic (clear boxes) and two-domain (gray boxes) models, with a motif length of 24 bits that results in a lower mutation effect size. The 12-bit case is shown in text Figure 4. Results are broadly similar, differing as noted below. Mutation rates are expressed as multiples (0.125–8) of the baseline mutation rate of  $8.333 \times 10^{-5}$  per bit. (A) Median net F2 misregulation of the positively selected trait as a function of varying the overall mutation rate. Misregulation was constrained to lower values in the pleiotropic model relative to the two-domain model and the extent of constraint increased with mutation rate. The constraint is less at high mutation rates for the 24-bit case relative to the 12-bit case. (B) As (A), instead varying the mutation rate only at the *cis*-regulatory locus of the conserved trait. Misregulation was again constrained in the pleiotropic model relative to the two-locus model, but was not sensitive to mutation rate. The constraint at low mutation rates is marginally stronger than in the 12-bit case. (C) Misregulation of the conserved trait as a function of the mutation rate at the *cis*-regulatory locus of the positively selected trait. In the pleiotropy model, misregulation was high at low mutation rates, dropping to zero as mutation rate increased; this constraint was slightly stronger in the 12-bit case of text Figure 4. No misregulation occurred in the two-domain model. Box plots show medians, quartiles and full ranges. Simulation conditions: directional selection was applied at the rate of  $\Delta P_{opt} = 1/4000 \text{ gen}^{-1}$  over the course of 4000 generations; population size = 400.