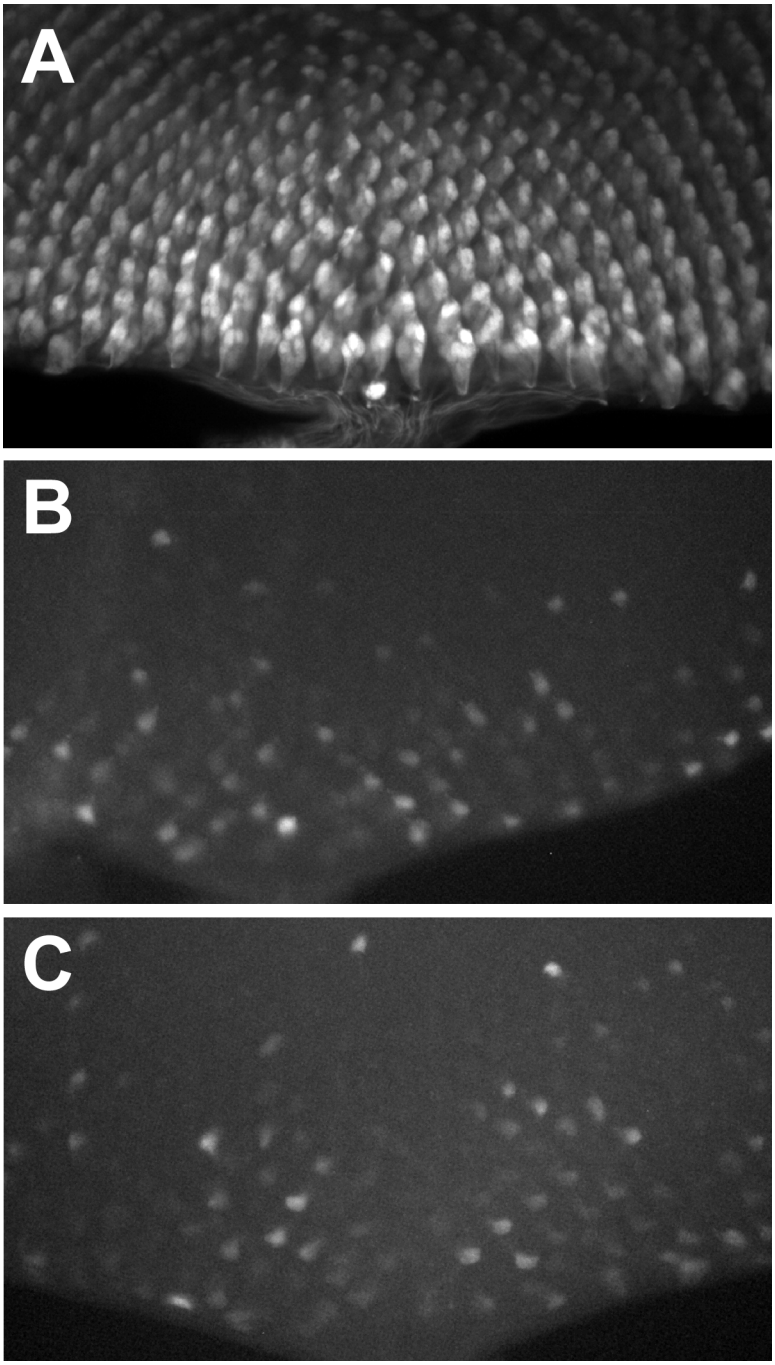

Figure S1 GMR acts in *trans* at polytene position 38F. GFP fluorescence is shown for discs where GMR acts in *cis* (A) or in *trans* (B-C). The constructs carried are (A), Complete^{gfp} alone; (B), Enhancerless construct paired with an insertion of a precursor to the Promoterless construct with a compromised promoter (see below); (C), Enhancerless paired with Complete^{lacZ}, which carries an intact promoter. In (B), the precursor is identical to the Promoterless construct except that the hsp70 promoter flanked by loxP sites has not been excised (See Materials and Methods). Quantitative RT-PCR shows that the precursor construct expresses *lacZ* in *cis* to a level of ~5% of Complete^{lacZ} (data not shown), indicating that the loxP sites compromise the function of the promoter. We therefore consider the expression in (B) to be analogous to that of Enhancerless paired with Promoterless.

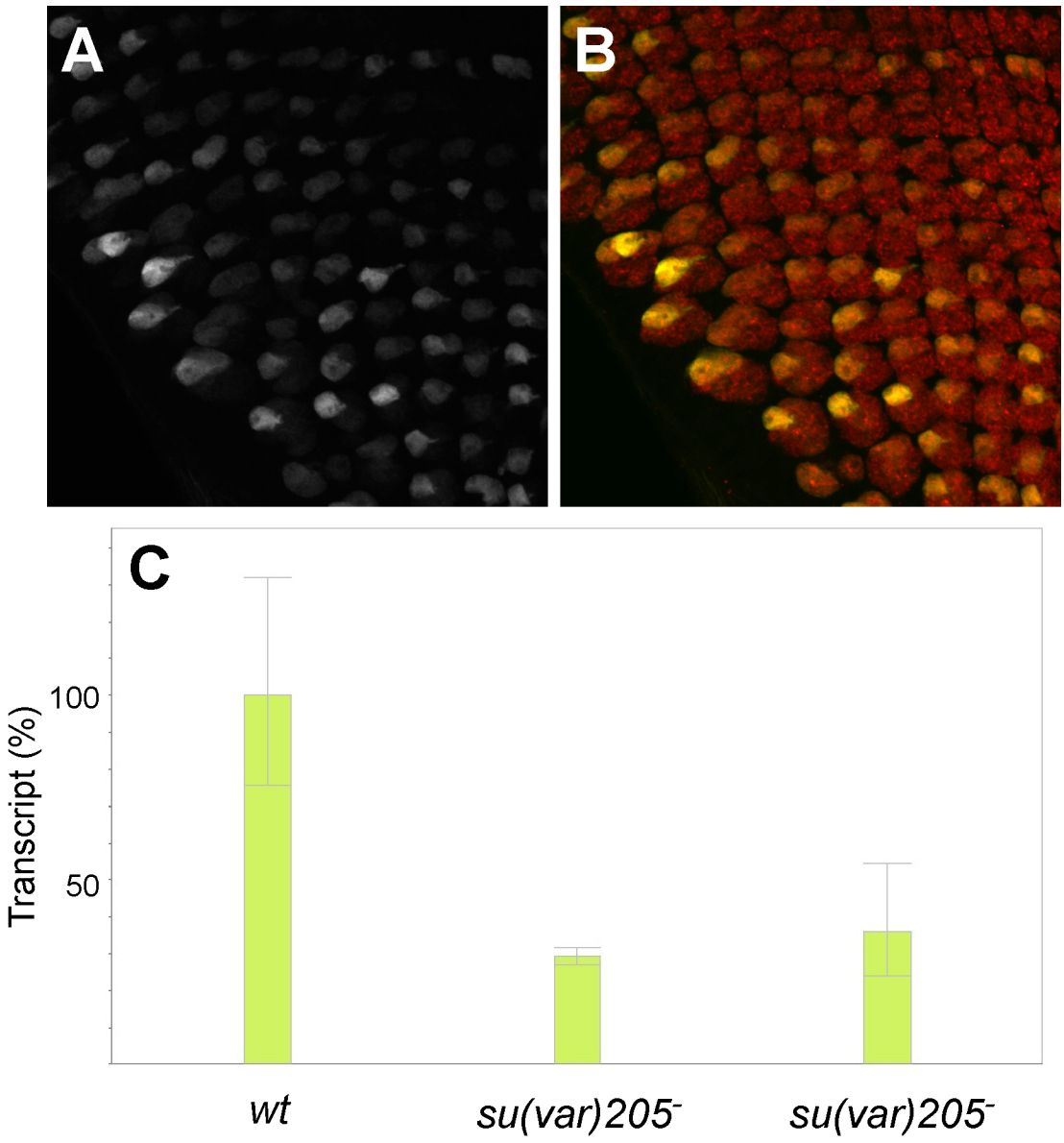

Figure S2 Variegation of GMR action in *trans* is not sensitive to reduction in HP1. (A, B) Max-projected confocal z-stacks showing discs carrying Enhancerless and Promoterless constructs at 53F in a background where *Su(var)205* (encoding HP1) expression is reduced in the eye disc via the expression of a hairpin under the control of *ey-GAL4*. (A) anti-GFP staining only; (B) merged image showing GFP in green and anti-Elav staining in red. Flies carrying the hairpin P[TRiP.HMS00278] targeting *Su(var)205* were obtained from the Bloomington Drosophila Stock Center. (C) Quantitative RT-PCR on cDNA prepared from eye-antennal discs (see Materials and Methods) using primers suvar205_RT_2F (5'-CTGCTGGCGCGTCCTTGAGT-3') and suvar205_RT_2R (5'-CAGCAGTACGAGGCGAGCCG-3') targeting *Su(var)205* exonic sequences. "*wt*", genotype *Enhancerless/Promoterless* (defined as 100%); "*Su(var)205*$^{-}$" genotype *Enhancerless/Promoterless; ey-GAL4/P[TRiP.HMS00278]*. Two independent *Su(var)205*$^{-}$ cDNA preparations derived from separate crosses show a reduction in *Su(var)205* mRNA levels to 29.2% (95% CI, 27.0-31.7%) and 36.1% (95% CI, 23.8-54.6%) relative to *wt*; note that this includes mRNA from the antennal portion of the eye-antennal disc where the hairpin is not expressed, and therefore likely represents an overestimate of transcript abundance in the eye disc. Although this represents a sizeable reduction in *Su(var)205* mRNA, we cannot exclude the possibility that HP1 protein levels are less significantly impacted by expression of the hairpin.

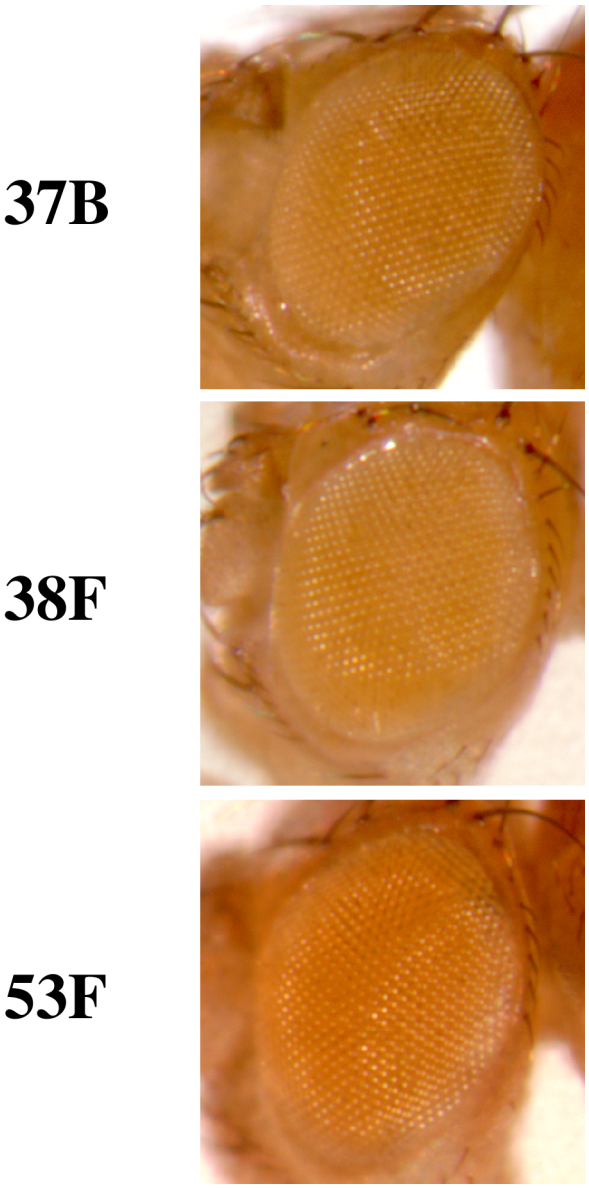

Figure S3 Lack of variegation of *mini-white* expression from RMCE targets at 53F, 37B, or 38F. Each image represents a fly carrying a single copy of the P[attP.w+.attP] target cassette, which is marked with *mini-white*. No variegation is evident.

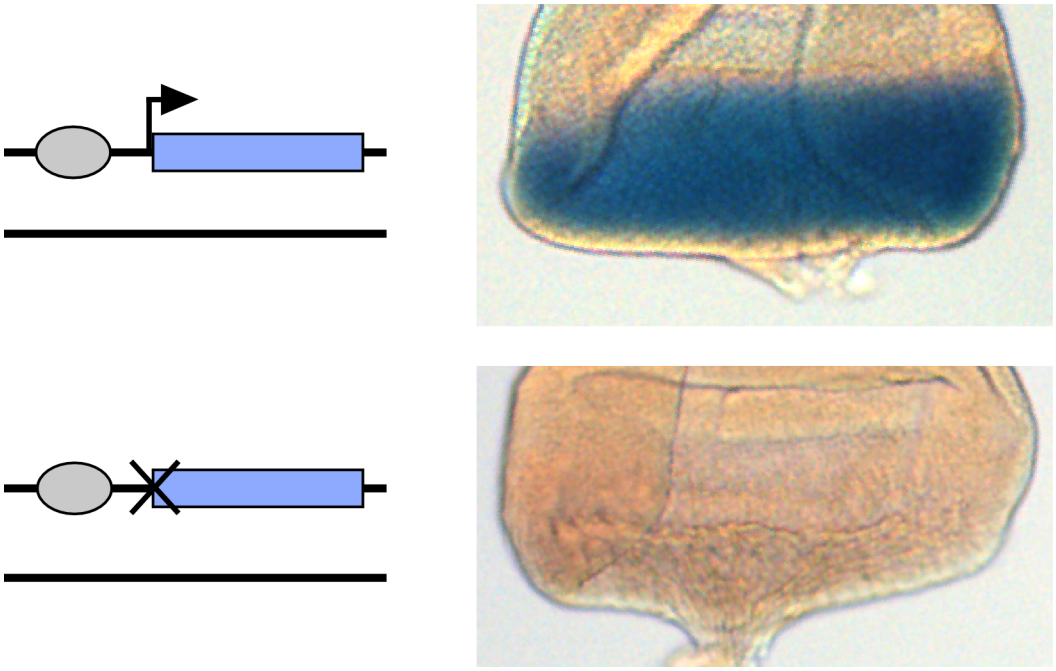

Figure S4 Expression of *lacZ* in *cis*. X-gal stained discs carrying constructs Complete^{lacZ} (top) or Promoterless (bottom) alone at 53F. In the top disc, the staining reaction was stopped after 20 minutes, whereas for the bottom disc, staining was carried out overnight. Quantitative RT-PCR indicates that expression from the Promoterless construct is <1% that of Complete^{lacZ} (data not shown).