nature cell biology

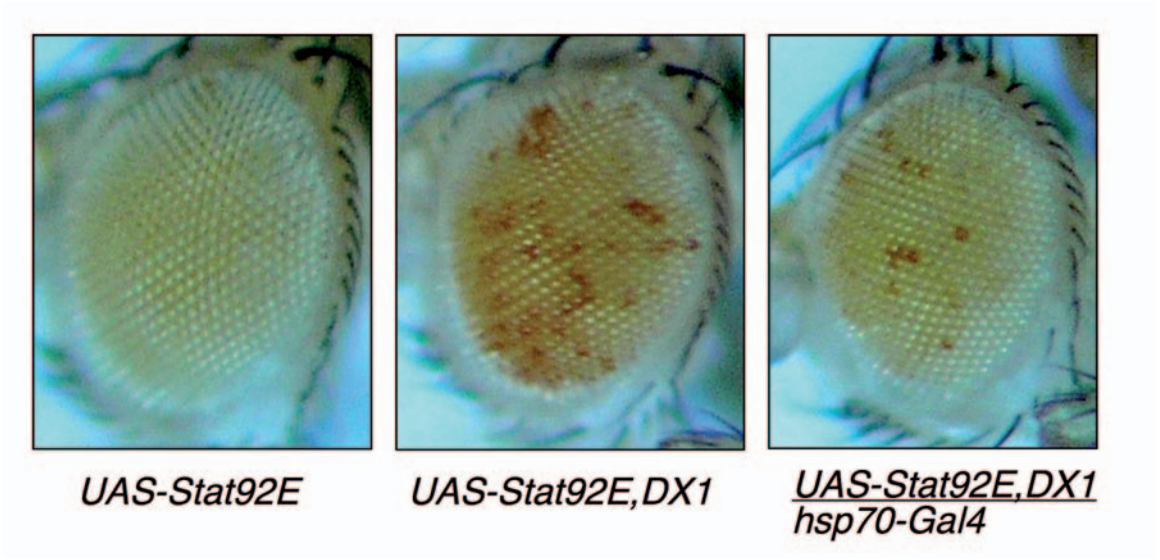

Figure S1 STAT92E enhances repeat-mediated variegation. Eye pigmentation phenotypes of adult flies of indicated genotypes raised at 25ºC. The light orange background was due to the presence of *mini-w*$^{+}$ in the *UAS-Stat92E*$^{+}$ transgene. Note the deceased pigmentation when *UAS-Stat92E*$^{+}$ was expressed by the basal level of *hsp70-Gal4* transgene (which does not carry *mini-w*$^{+}$).

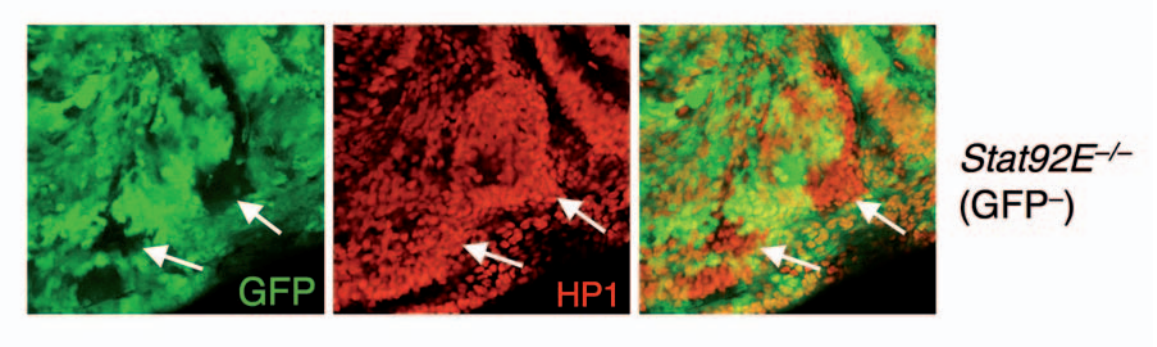

Figure S2 Loss of Stat92E^{+} does not affect HP1 expression in imaginal discs. A 3rd instar wing imaginal disc bearing *Stat92E*$^{-/-}$ cells (marked by lack of GFP) is shown for HP1 levels (stained by anti-HP1, red).

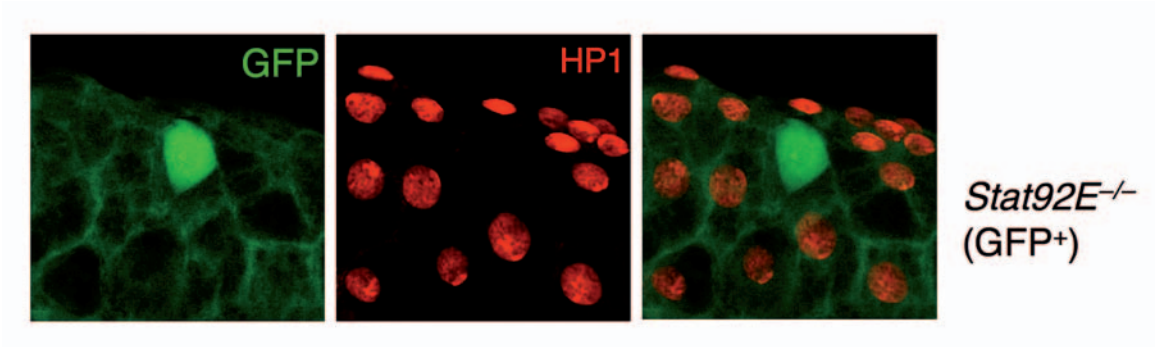

Figure S3 Long-term loss of STAT92E reduced HP1. A *Stat92E* homozygous loss of function cell (marked by GFP) was induced during embryogenesis and examined for HP1 (red) in late 3rd instar larval salivary gland.

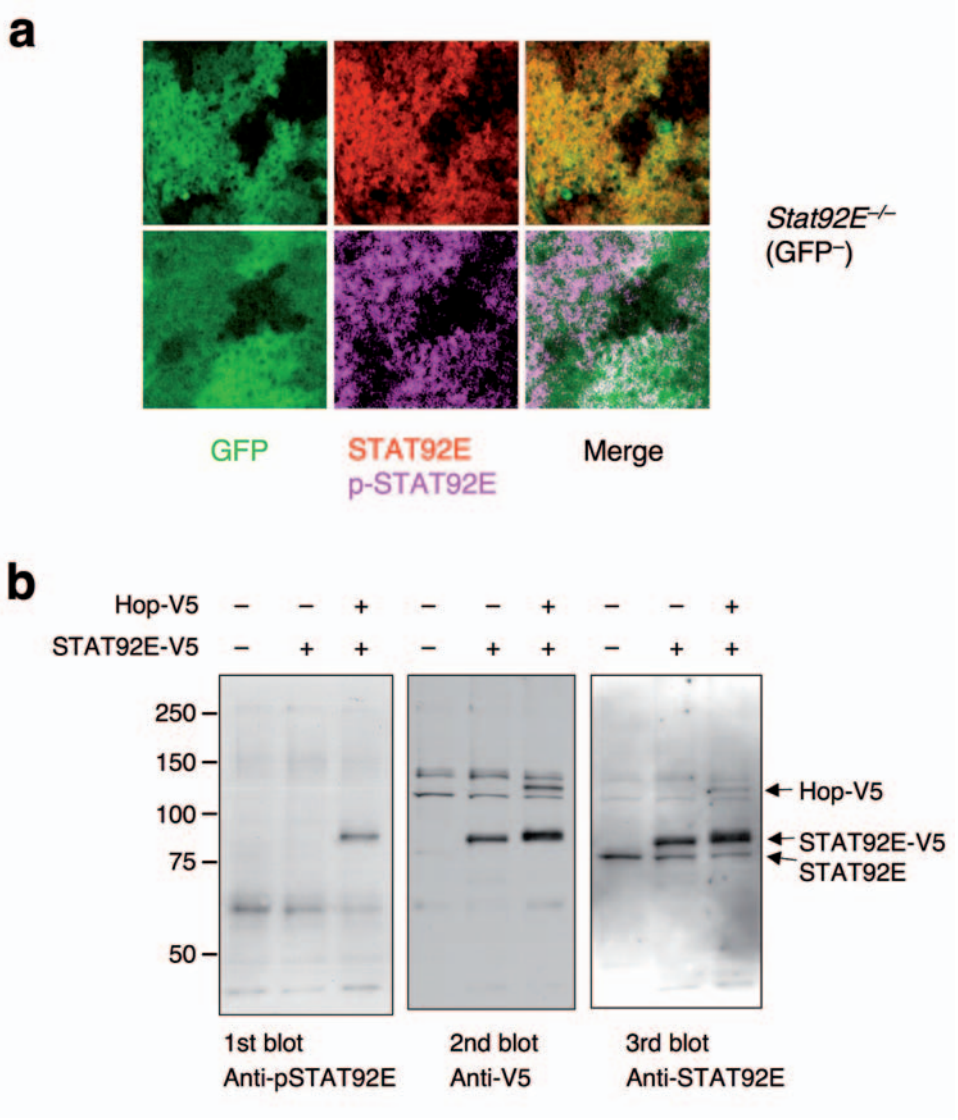

Figure S4 Specificity of anti-STAT92E and anti-pSTAT92E. **(a)** A 3rd instar wing imaginal disc bearing *Stat92E*$^{-/-}$ cells (marked by lack of GFP) was stained by anti-STAT92E (red). Note that the red signals closely matches STAT92E protein levels, and that no signal is detected in *Stat92E*$^{-/-}$ cells. **(b)** S2 cells were transfected with Hop-V5 and/or STAT92E-V5. Total cell lysates were subjected to SDS-PAGE and transferred to nitrocellulose membrane and immunoblotted sequentially with anti-pSTAT92E, anti-V5, and anti-STAT92E, respectively. Note that anti-pSTAT92E does not cross-react with non-phospho-STAT92E (lack of signals in lane 1, 2, left panel), and that anti-STAT92E does not detect other proteins (right panel). Extra bands on the right panel were due to incomplete stripping. Anit-V5 detected non-specific bands (bands across all 3 lanes in middle panel).

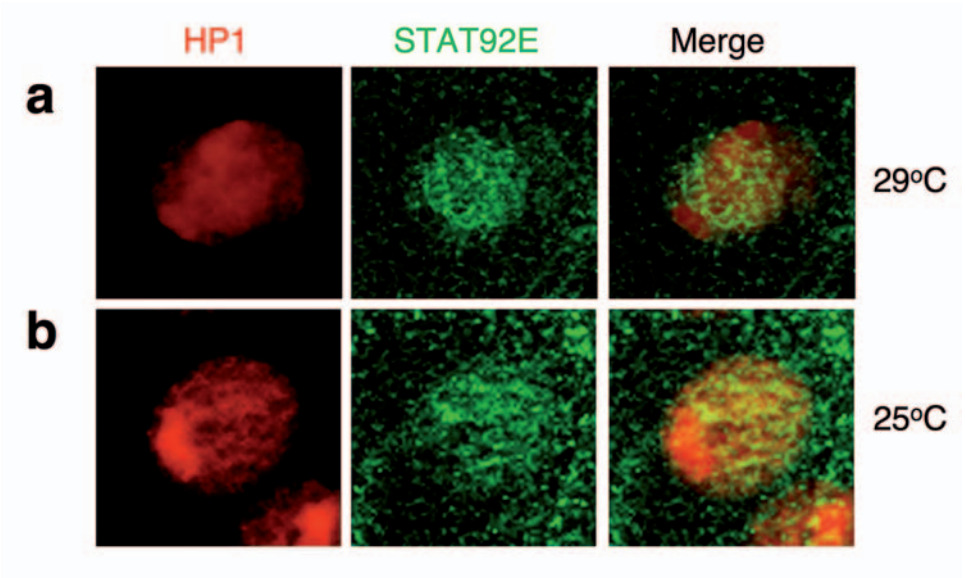

Figure S5 HP1 and STAT92E distribution in *hop*^{GOF} mutant larvae**.** Salivary glands isolated from *hop*$^{GOF/+}$ (*hop*$^{Tum\text{-}l/+}$) larvae were stained with anti-HP1 (red) and anti-STAT92E (green). (**a**) In glands isolated from *hop*$^{Tum\text{-}l/+}$ larvae raised at 29ºC, both HP1 and STAT92E appear diffused, lacking prominent heterochromatin foci. (**b**) In *hop*$^{Tum\text{-}l/+}$ larvae raised at 25ºC, a moderately nonpermissive temperature[1], a fraction of the nuclei (32%; n=64/198 nuclei) exhibited nearly normal patterns of HP1 foci (left panel), whereas in nearly all of these nuclei (95%; n=61/64 nuclei), STAT92E appeared dispersed and did not colocalize with HP1 (middle panel). These results suggest that STAT92E disperses in response to Hop$^{Tum\text{-}l}$ phosphorylation, and this process precedes changes in HP1 subnuclear localization.

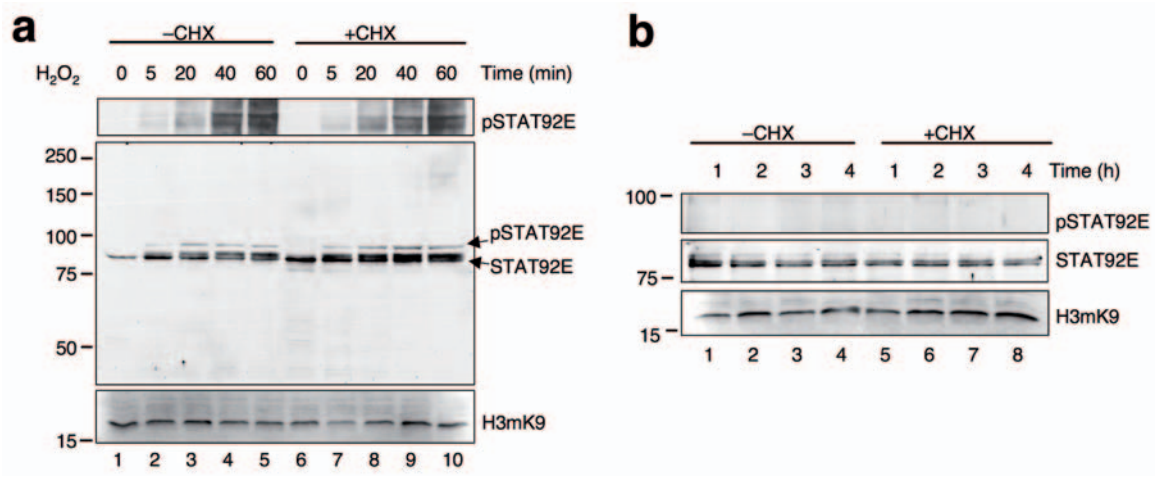

Figure S6 Effects of H2O2/vanadate and CHX on STAT92E phosphorylation and histone H3 K9 methylation. S2 cells were cultured with or without CHX for 1 h, and then stimulated with H_2O_2/vanadate for indicated times **(a)**, or incubated in the presence of CHX for indicated times **(b)**. Cell extracts were blotted sequentially with anti-pSTAT92E and anti-STAT92E, or with anti-H3mK9, respectively. Membrane was stripped between blots.

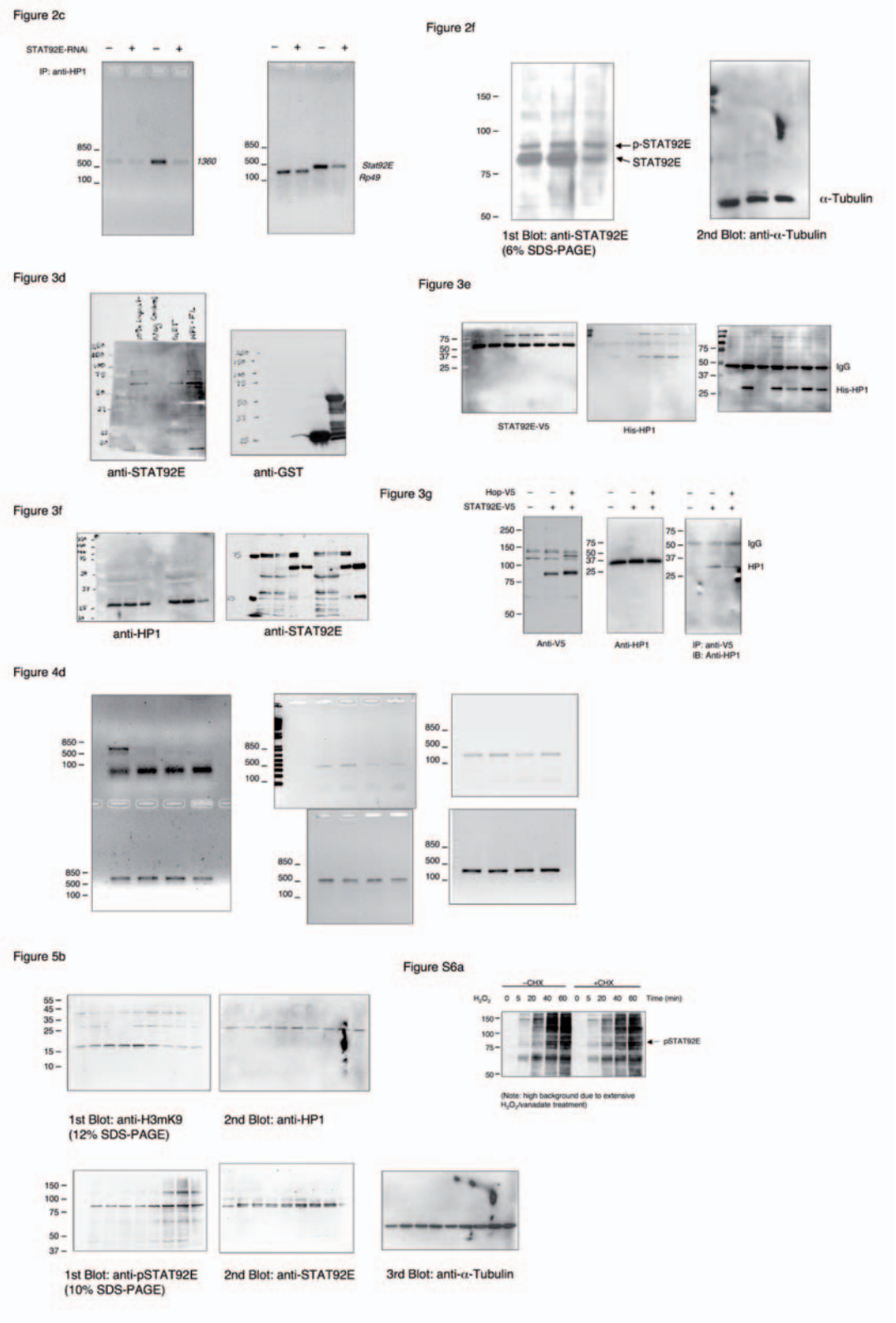

Figure S7 Full-length protein gels. Note that Fig. 2f used a lower percentage gel and longer run, which resulted in a greater separation phospho-STAT92E from STAT92E bands (compared with Fig. 5b).

References:
1. Hanratty, W. P. & Dearolf, C. R. The Drosophila Tumorous-lethal hematopoietic oncogene is a dominant mutation in the hopscotch locus. Mol Gen Genet 238, 33-7 (1993).