**Results**

**The *broaddisc* enhancer is a model of a developmentally decommissioned regulatory site during *Drosophila* wing development.**

In order to interrogate the mechanisms that control developmental enhancer decommissioning we selected a previously characterized enhancer, *broaddisc* (*brd*), as an experimental model. A FAIRE-seq timecourse spanning larval to late pupal wing development previously identified the *brd* locus as a ~2kb region on the third chromosome that is significantly open in late larvae and prepupae, that is dramatically closed after the prepupal-pupal transition (Fig 1A,B; Uyehara 2017).

One of the challenges when experimenting with a decommissioned enhancer is that measuring a loss of fluorescent from a traditional enhancer reporter design is complicated by the perdurance of fluorophore. This introduces a significant disconnect between changes in enhancer driven transcription and measured fluorescent signal from a reporter. In order to better capture the dynamics of a decommissioned enhancer like *brd* we assembled a novel reporter that we term the “Switch” reporter (Fig 1C). The Switch reporter uses a two fluorophore (fp) design, with the first fp tdTomato contained within an FRT-site flanked cassette that includes two terminator sequences. The second fp, a myristoylated GFP (myrGFP), is downstream of this cassette such that it will not be transcribed as long as the FRT-cassette is present. By inducing the expression of flippase (FLP) the FRT-cassette containing tdTomato can be excised allowing for expression of myrGFP. If the enhancer is inactive at the time of FLP induction no myrGFP should be produced.

In order to better characterize the dynamics of the *brd* enhancer and confirm its usefulness as a model of enhancer decommissioning, we used the Switch reporter to compare GFP in larval vs pupal stage wings (Fig 1D). When a heat-inducible FLP was expressed in wandering larvae with *brd-Switch* we observed membrane localized GFP throughout the wing disc, confirming the enhancer is active in this tissue at this time. In contrast, when we induced FLP at ~24h after pupal formation (APF), well after the stage at which by FAIRE-seq we see a loss of accessibility at the endogenous locus, we observe little or no nascent GFP expression in most of the cells of the pupal wing, indicating the enhancer is largely inactive at this stage.

Decommissioning of the *brd* enhancer is controlled by ecdysone signaling and the ecdysone induced temporal identity factor Eip93F (E93). We have previously shown that during wing development the accessibility and activity of many stage-specific regulatory sites are targets of ecdysone signaling. To confirm our previous observations of *brd* dependence on E93 for decommissioning and test the usefulness of the Switch reporter, we express an E93 RNAi in the posterior of the developing wing using an Engrailed GAL4 (en-GAL4) and checked for nascent GFP expression from the Switch reporter. When we induced fluorophore switching at ~24h APF we observe strong GFP expression in E93-KD cells relative to WT cells of the wing anterior (Fig 1E).

By providing a better measure of enhancer dynamics, the Switch reporter validates our previous observations that the *brd* enhancer loses activity within the developing wing blade as the animal progresses through metamorphosis, correlated with a loss of accessibility, and that this process is directly regulated. Thus, *brd* is a model of targeted developmental enhancer decommissioning, making it a strong candidate to study potential mechanisms of enhancer decommissioning.

**The *Drosophila* Brahma nucleosome remodeling complex is required to repress *brd*.**