



Supplemental Figure S1. The reporter backbone is neutral for transcription. To estimate the frequency of promoter trapping (cases where the reporter is transcribed from an endogenous *Drosophila* promoter), we used a promoterless reporter. **(a)** RT-PCR shows that the integrated promoterless construct (0) produces substantially less transcripts than integrated reporter I. Mock electroporation without Sleeping Beauty (no SB) shows that some active reporters are integrated in a transposition-independent manner. Mock electroporation without barcoded library (no lib.), control without reverse transcription (no RT) or without template (no DNA) show that the signal is specific. The size of the amplicon is 219 bp. The order of the lanes has been changed but they come from the same original acquisition of the gel. **(b)** To assert that the construct can report both high and low expression, we checked that the promoter of *Hsp70b* is fully heat-inducible when cloned in this backbone. Each panel is a FACS analysis where the \log_{10} GFP fluorescence detected in FL1 (530/30) is plotted against the signal detected in FL2 (585/42) to exclude autofluorescence. 1) Mock transfection. 2) Transient

electroporation of *Hsp70b* without heat shock. 3) Integrated and barcoded *Hsp70b* reporter without heat shock. 4) Integrated promoter I reporter library without heat shock. 5) Transient electroporation of *Hsp70b* with 1 hour heat shock at 37 °C. 6) Integrated *Hsp70b* reporter library with 1 hour heat shock at 37 °C. In both cases, FACS analysis was performed 24 hours after heat shock.