Figure S1  Verification of Bxb1-mediated DNA integrations attPX-52 host lines and in crb-PX genomic engineering founder lines. A. A portion of sequencing chromatograms of PCR products from four integration samples (Figure 1B), namely, #1 to #4. Sequence were aligned with the expected recombinant sequence (“ref”) in Vector NTI. Bxb1-attL ("attLX") sequence is inverted in the ref sequence (bottom) and highlighted in the box underneath. The common core sequence of Bxb1 attachment sites “gcgggtctc” (GHOsh et al. 2003) is in red. A noisy peak in sample #1 chromatogram created an artificial gap right before attLX in sequence alignment.

B. Verification of crb-PC<sup>GR<sup>[w</sup>]</sup> lines by PCR-1.

C. Verification of crb-G80EY<sup>GR<sup>[w</sup>]</sup> lines by PCR-2.

Exact sizes of PCR-1 or PCR-2 are illustrated in Figure 2.

Red arrowhead: size of the PCR products from integration lines.

Black arrowhead: size of the PCR products from founder lines.

MW: 1kb-plus DNA ladder (from Invitrogen), only bands from 100bp to 1kb are shown.
Figure S2  Scheme for generating crb conditional alleles by successive integrations using fc31 and Bxb1. A. Genomic engineering founder line crb^{GSK24[w1]} is first converted to crb^{FRT-PX^{GSK24[w1]}} via fc31-mediated integration of pGE-attBX-crb^{FRT-FRX} in which a FRT site is inserted into the intron right before the exons encoding the transmembrane and intracellular domains of Crb ("Crb-C", 1.5 kb). The w+ and vector sequences (e.g. Amp R etc) are then removed by Cre to generate a rescued founder line crb^{FRT-PX^{GSK24[w1]}} (i.e. "crb^{FRT-PX}") specifically for making conditional crb alleles. B. crb^{FRT-PX} can be used to integrate the plasmids as pGE-attBX-Crb-FRT-" via Bxb1-mediated DNA integration. pGE-attBX-Crb-FRT-" contains an ubiquitously expressed GFP marker, a FRT and Crb-C sequence with desired modifications ("**"). After removing the w+ by Cre, the resulted crb^{FRT-*(GFP*)} is a conditional allele controlled by FLP and marked by GFP. FLP-mediated recombination will convert crb^{FRT-*(GFP*)} to mutant crb^{*(GFP*)} marked by the loss of GFP. The scheme in A and B can be very flexible by alternately using the fc31 and Bxb1 integrases at different steps. For instance, in B one may instead first integrate into crb^{FRT-PX} a construct containing attBX-GFP-attPC^{full} to generate a crb^{FRT-PC^{full}} founder line. crb^{FRT-PC^{full}} would already contain the GFP marker and carry a full length attPC site for more efficient fc31-mediated integration of conditional mutant constructs. The size of full length attPC is not of concerns here since in FLP-converted mutant alleles any sequences between the two FRT sites will be removed, including attRX, GFP and full length attRC. 5’ and 3’: the 5’ and 3’ flanking genomic DNA of crb.