



Figure S2. Suggested crossing scheme for generating Dam-fusion lines using FlyORF-TaDa. (A) Homozygous FlyORF flies (note the compatible FlyORF library types listed) are crossed to FlyORF-TaDa line containing hsFLP. (B) F1 progeny are heat shocked during larval stages and adults crossed to third chromosome balancer. NB: Any *w*- line can be substituted at this stage if it is not necessary to balance immediately (e.g. if isogenising is intended). (C) Resulting progeny with the correct marker combination (white eye + dsRed2) over the same balancer chromosome are crossed to obtain balanced progeny for the generation of a stable stock.

Notes: (1) It is expected that resulting FlyORF-TaDa fusions will be viable as homozygotes, so will not require the presence of the balancer for long-term viability. (2) Compatible FlyORF variants contain the transgene in the ZH-86Fb landing site and an FRT5 recombination site (i.e. all lines that are listed as ORF-3xHA, ORF-VN or ORF-VNshort).