

**Table S1 Summary of HDR results**

Genotype of injected embryos	Injected plasmid(s)	DNA concentrationg RNA/donor (ng/ $\mu$ l)	Independent experiments	No. of injected embryos	Embryo survival rate (%)	No. of G <sub>0</sub> males crossed	No. of sterile or dead G <sub>0</sub> males	No. of male founders/ fertile G <sub>0</sub> males screened (%)	No. of RFP-positive F <sub>1</sub> males/ total screened (%)	No. of confirmed knock-ins into wg/ total screened (%) <sup>a</sup>
<i>nos-cas9 CFD2, U6:3-gRNA-wg</i>	<i>pDonor wg-3P3-RFP</i>	500	Expt 1	125	nd	10	2	7/8 (88)	41/180 (23)	35/36 (97)
<i>nos-cas9 CFD2, U6:3-gRNA-wg</i>	<i>pDonor wg-3P3-RFP</i>	100	Expt 1	151	30	13	0	9/13 (69)	46/242 (19)	7/8 (88)
<i>nos-cas9 TH_attP2, U6:3-gRNA-wg</i>	<i>pDonor wg-3P3-RFP</i>	100	Expt 1 Expt 2 <b>Total</b>	131 149 <b>280</b>	18 32 <b>25</b>	9 10 <b>19</b>	2 1 <b>3</b>	7/7 (100) 5/9 (56) <b>12/16 (75)</b>	43/123 (35) 13/97 (13) <b>56/220 (25)</b>	7/8 (88) 5/6 (83) <b>12/14 (86)</b>
<i>nos-cas9 CFD2</i>	<i>pU6:3-gRNA-wg/ pDonor wg-3P3-RFP</i>	500/500	Expt 1	91	nd	10	2	7/8 (88)	19/222 (9)	14/18 (78)
<i>nos-cas9 CFD2</i>	<i>pU6:3-gRNA-wg/ pDonor wg-3P3-RFP</i>	75/100	Expt 1 Expt 2 <b>Total</b>	136 117 <b>253</b>	32 34 <b>33</b>	16 8 <b>24</b>	0 0 <b>0</b>	8/16 (50) 3/8 (38) <b>11/24 (46)</b>	18/232 (8) 9/270 (3) <b>27/502 (5)</b>	5/6 (83) 4/6 (67) <b>9/12 (75)</b>
<i>nos-cas9 TH_attP2</i>	<i>pU6:3-gRNA-wg/ pDonor wg-3P3-RFP</i>	75/100	Expt 1	134	22	11	3	6/8 (75)	33/217 (15)	4/4 (100)
<i>w<sup>1118</sup></i>	<i>pDCC6-gRNA-wg/ pDonor wg-3P3-RFP</i>	75/100	Expt 1 Expt 2 <b>Total</b>	268 123 <b>391</b>	7 52 <b>22</b>	3 26 <b>29</b>	1 5 <b>6</b>	2/2 (100) 2/21 (10) <b>4/23 (17)</b>	6/46 (13) 6/322 (2) <b>12/368 (3)</b>	3/3 (100) 3/3 (100) <b>6/6 (100)</b>

Abbreviations: nd – not determined; Expt – experiment.

a, In total, 87/98 RFP-positive flies were confirmed to have integration at the expected site in the *wg* locus using two diagnostic PCRs (Figure S2). For the other 11 flies, PCRs resulted in either a product for only one of the reactions (six flies; indicating a potential complex insertion event in the *wg* locus), or no products for either reaction (five flies; indicating potential insertion of the RFP cassette at another genomic location)