

Supplemental Table 1. Quantification of GFP fluorescence in S⁵G^CS³ and G⁵G^CS³-mut2 spermatids and spermatocytes.

Transgene ¹	Spermatid or spermatocyte ²	Pixel intensity, Mean \pm SD ³	Background: cell-free or spermatocyte ²	Pixel Intensity Mean \pm SD ³	Ratio ⁴	P-value ⁵
S ⁵ G ^C S ³ Ln84	meiotic	7.4 \pm 1.3	cell-free	7.5 \pm 1.2	0.98	0.57
S ⁵ G ^C S ³ Ln84	step 11	9.4 \pm 2.3	cell-free, zygotene, diplotene	8.4 \pm 1.9	1.1	0.037
S ⁵ G ^C S ³ Ln84	step 12	21.4 \pm 7.6	zygotene, meiotic figures, secondary	7.6 \pm 1.3	1.47	<0.0001
S ⁵ G ^C S ³ founder Ln 20	meiotic	9.1 \pm 1.7	cell-free	9.3 \pm 1.3	1.02	0.26
S ⁵ G ^C S ³ founder Ln 20	step 11	11.4 \pm 2.7	cell-free, zygotene, diplotene, secondary	9.9 \pm 1.8	1.15	0.16
S ⁵ G ^C S ³ founder Ln 20	step 12	19.9 \pm 2.4	2 nd spermatocytes	7.6 \pm 1.3	2.6	<0.0001
S ⁵ G ^C S ³ Ln37	meiotic	9.9 \pm 2.9	cell-free	9.2 \pm 2.6	1.07	0.3
S ⁵ G ^C S ³ Ln37	step 11	9.7 \pm 2.1	zygotene, diplotene	8.7 \pm 1.4	1.1	0.03
S ⁵ G ^C S ³ Ln37	step 12	44.0 \pm 7.9	zygotene, secondary	9.6 \pm 2.7	4.6	<0.0001
G ⁵ G ^C S ³ -mut2 Ln59 25 dpp	pachytene	21.6 \pm 1.9	cell-free	21.3 \pm 2.3	1.01	0.3
G ⁵ G ^C S ³ -mut2 Ln59 25 dpp	step 1	21.1 \pm 1.7	pachytene	18.8 \pm 1.7	1.12	<0.0004
G ⁵ G ^C S ³ -mut2 Ln59 25dpp	step 3	44.4 \pm 4.8	pachytene	23.4 \pm 1.9	1.89	<0.0001
G ⁵ G ^C S ³ -mut2 Ln78 28 dpp	pachytene	10.6 \pm 1.5	cell-free	11.1 \pm 1.3	0.95	0.29
G ⁵ G ^C S ³ -mut2 Ln78 28 dpp	step 1	13.2 \pm 1.5	pachytene	10.0 \pm 1.2	1.3	<0.0001
G ⁵ G ^C S ³ -mut2 Ln78 28 dpp	step 3	16.2 \pm 2.2	pachytene	8.2 \pm 0.5	1.95	<0.0001
G ⁵ G ^C S ³ -mut2 Ln117 founder	pachytene	11.6 \pm 1.5	cell-free	11.2 \pm 1.5	1.04	0.84
G ⁵ G ^C S ³ -mut2 Ln117 founder	step 1	11.4 \pm 1.6	cell-free	9.9 \pm 0.5	1.15	0.16
G ⁵ G ^C S ³ -mut2 Ln117 founder	step 3	23.2 \pm 1.6	cell-free, pachytene	12.4 \pm 1.3	1.87	<0.0001

Pixel intensities of adjacent spermatids, spermatocytes and cell-free areas were measured with ImageJ as illustrated in Supplemental Figure 1. The pixel intensities of various stages of meiotic cells (zygotene, pachytene, diplotene and secondary spermatocytes) and cell-free areas were virtually identical, and were assumed to be background. ¹Transgene and specific line or founder. All testes were from adult males except for two sexually immature 25 dpp and 28 dpp testes. ²Stage of spermatid or spermatocyte measured. ³Average and standard deviation of pixel intensities of 10 cells or cell free-areas. ⁴Ratio of average pixel intensity in spermatocytes and spermatids in column 2 to that of background in column 5. ⁵P-value calculated using Student's two-sided paired t-test for

samples of 10 adjacent cells and/or cell-free areas.