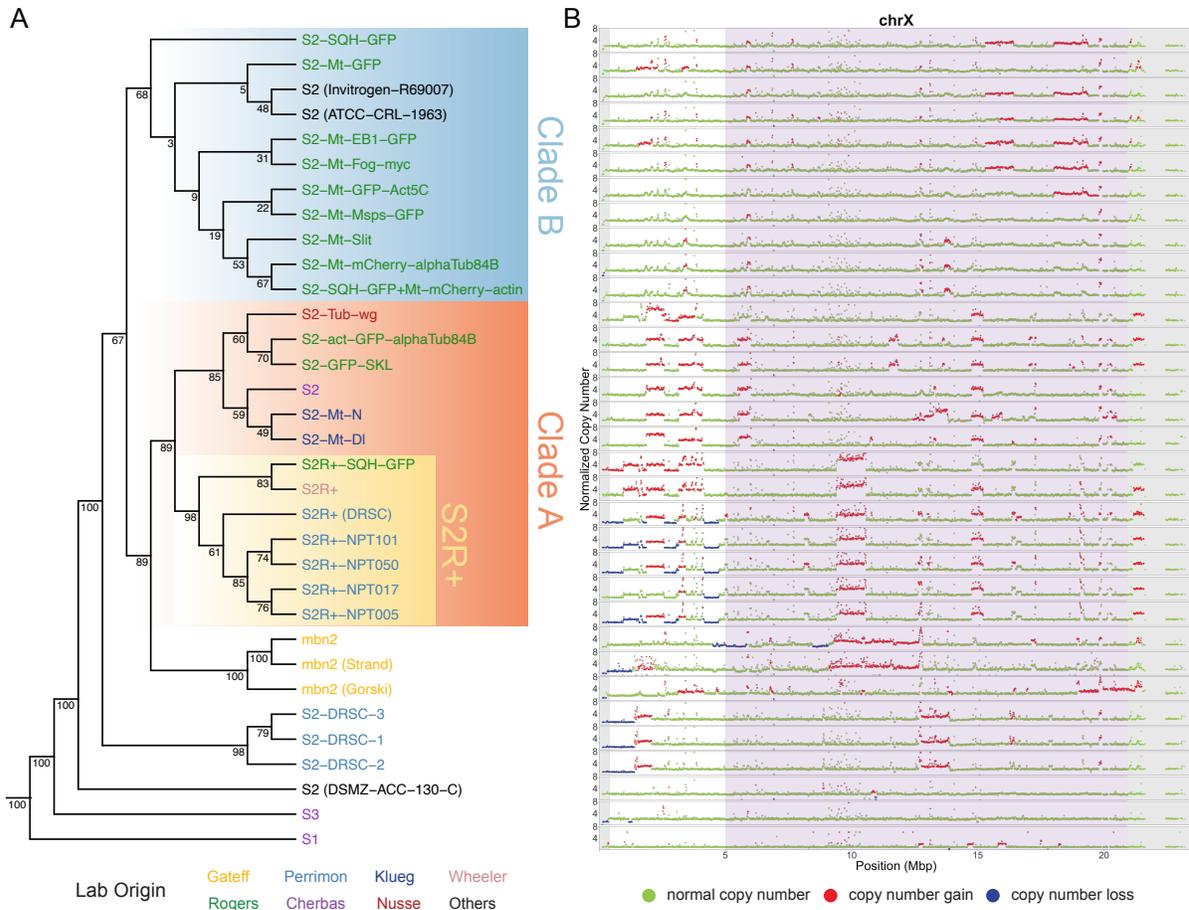
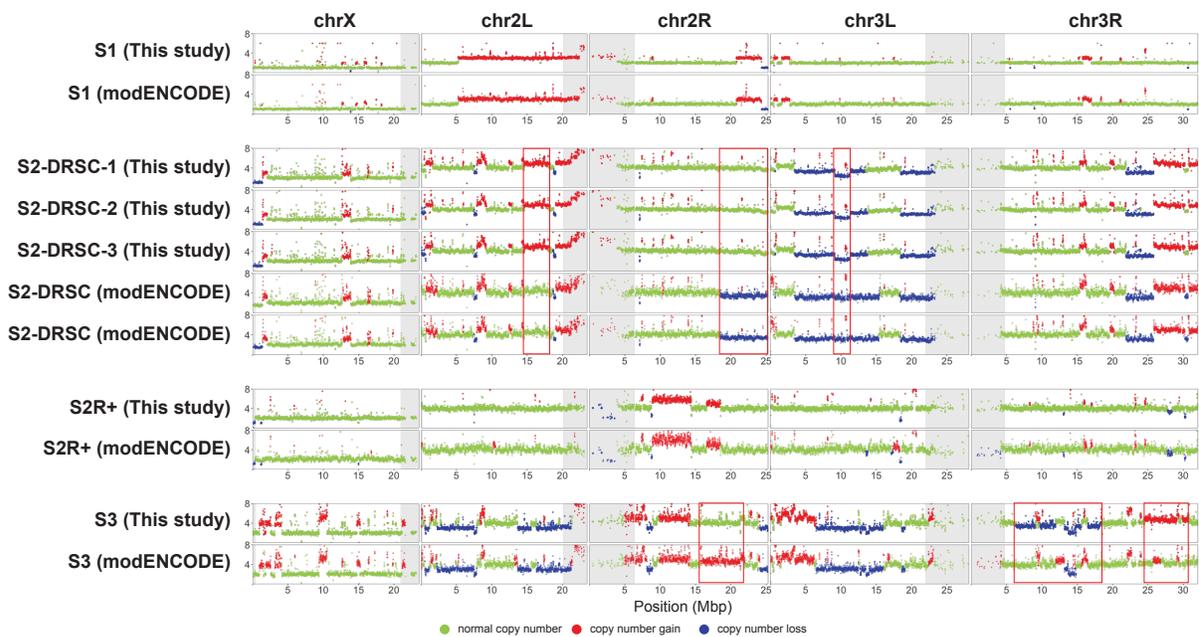


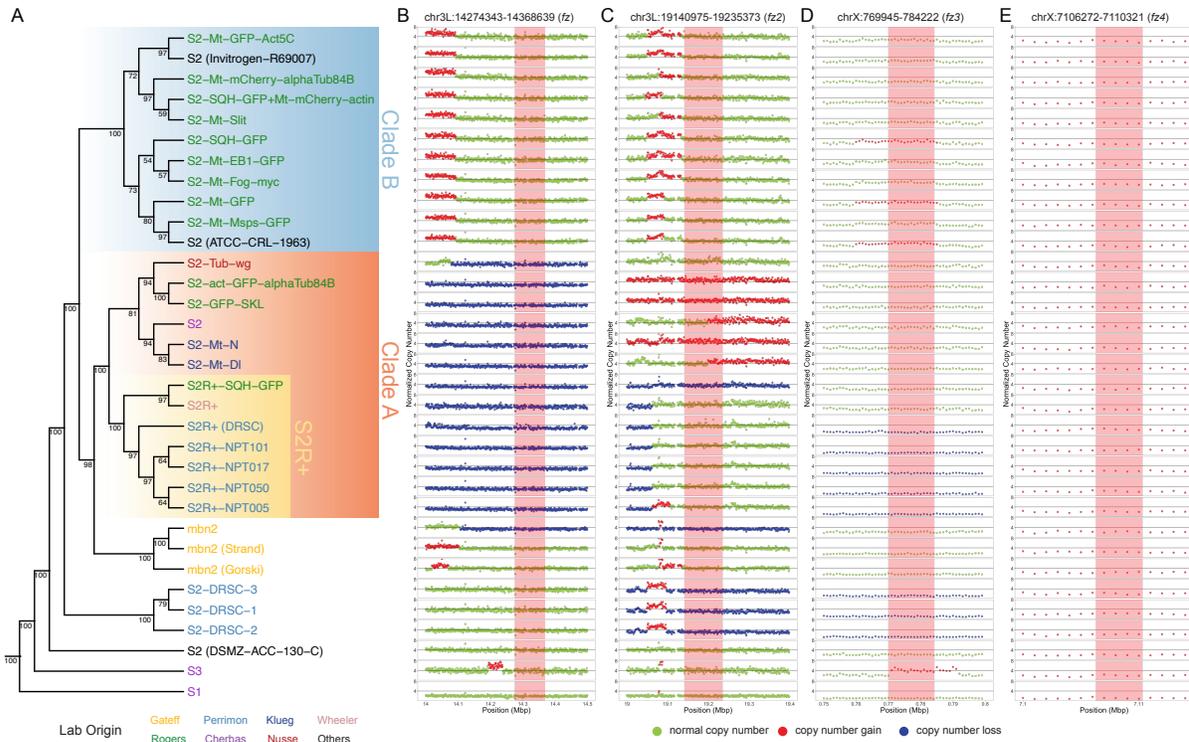
**Figure S1. Genome-wide profiles of intra-sample allele frequency based on SNP variants for Schneider cell lines samples.** B-allele frequency (BAF) was determined as the coverage of reads supporting the non-reference allele divided by total coverage at that variant positions. The majority of SNPs detected in these samples are homozygous (BAF  $\sim$ 1.0). A small patch of heterozygosity at the base of chromosome arm 2L is found in all S2 sub-lines with the exception of the most basal sample S2 (DSMZ-ACC-130-C), which supports the monophyly of the S2 clade based on TE insertions.



**Figure S2. Evolutionary relationship among S2 sub-lines inferred using TE profiles in regions without major shared copy number losses.** (A) Dollo parsimony tree including a panel of 26 *Drosophila* S2 sub-lines constructed using non-reference TE insertions predicted by TEMP (Zhuang *et al.*, 2014) in regions of chromosome X without major shared copy number losses (chrX:500000-20928973) indicated by purple shading. Samples from S1, S3, and mbn2 cell lines and replicate samples for S2-DRSC were also included. Percent bootstrap support was annotated below each node. *Drosophila* Genomics Resource Center (DGRC) cell line names are used as taxa labels. Samples obtained from other sources are labeled in the format of “cell line name (source name)”. Taxa labels were colorized based on donor labs in which cell sub-lines were developed. (B) Copy number profiles of chromosome X for samples included in panel A. Each data point represents normalized copy number (ratio\*ploidy) for a given 10 kb window estimated by Control-FREEC (Boeva *et al.*, 2012). Data points for each window are colorized by CNV status (red: CNV gain; green: no CNV; blue: CNV loss), which are based on the comparison between normalized copy number estimated by Control-FREEC and baseline ploidy estimated by Lee *et al.* (2014). Regions without major shared copy number losses among S2 sub-lines are shaded in purple. Low recombination regions are shaded in grey.



**Figure S3. Copy number profiles of four Schneider cell sub-line samples using data collected from this study and the modENCODE project.** Each data point represents normalized copy number (ratio\*ploidy) for a given 10 kb window estimated by Control-FREEC (Boeva *et al.*, 2012). Data points for each window are colorized by CNV status (red: CNV gain; green: no CNV; blue: CNV loss), which are based on the comparison between normalized copy number estimated by Control-FREEC and baseline ploidy estimated by Lee *et al.* (2014). For each cell line sample, regions that exhibit differences in major CNV patterns between this study and the modENCODE project are marked in red boxes. Low recombination regions are shaded in grey.



**Figure S4. Copy number profiles of four *frizzled* genes for Schneider cell sub-line samples.** (A) Dollo parsimony tree including a panel of 26 *Drosophila* S2 sub-lines constructed using non-reference TE predictions made by TEMP (Zhuang *et al.*, 2014). Samples from S1, S3, and *mbn2* cell lines and replicate samples for S2-DRSC were also included. Percent bootstrap support was annotated below each node. *Drosophila* Genomics Resource Center (DGRC) cell line names are used as taxa labels. Samples obtained from other sources are labeled in the format of “cell line name (source name)”. Taxa labels were colored based on donor labs in which cell sub-lines were developed. (B-E) Copy number profiles of *fz*, *fz2*, *fz3*, and *fz4* gene loci for samples included in panel A. Each data point represents normalized copy number (ratio\*ploidy) for a given 1 kb window estimated by Control-FREEC (Boeva *et al.*, 2012). Data points for each window are colored by CNV status (red: CNV gain; green: no CNV; blue: CNV loss), which are based on the comparison between normalized copy number estimated by Control-FREEC and baseline ploidy estimated by Lee *et al.* (2014). Red shading indicates *frizzled* gene regions.

**Table S1. Summary of 33 Schneider cell line samples analyzed in this study.** *Drosophila* Genomics Resource Center (DGRC) cell line names are given for all cell line samples except for samples obtained from other sources, which are labeled in the format of “cell line name (source name)”. The “Lab Origin” represents the lab that originally created the sub-line. “Inferred ploidy” and “Inferred sex” represent the ploidy and sex of the cell line, respectively, estimated by Lee *et al.* (2014). “Read pairs” represents the number of paired-end reads for a given cell sub-line sample. “Coverage” represents the average mapped depth of coverage after quality and adaptor trimming. N.A. indicates that this information is not available.

Cell line	DGRC ID	Lab origin	Inferred ploidy	Inferred sex	SRA	Read length	Read pairs	Coverage
mbn2	DGRC-147	Gateff	4	male	SRR13360020	151	55531647	109.61
mbn2 (Gorski)	N.A.	Gateff	4	male	SRR13360019	151	63738692	126.11
mbn2 (Strand)	N.A.	Gateff	4	male	SRR13360018	151	68440069	132.00
S1	DGRC-9	Cherbas	2	male	SRR10981795	101	34904345	35.79
S2	DGRC-6	Cherbas	4	male	SRR10981796	101	28189507	31.67
S2 (ATCC-CRL-1963)	N.A.	Others	4	male	SRR10981814	101	50088154	47.26
S2 (DSMZ-ACC-130-C)	N.A.	Others	4	male	SRR10981794	101	51683568	48.34
S2 (Invitrogen-R69007)	N.A.	Others	4	male	SRR10981793	101	43038240	42.18
S2-act-GFP-alphaTub84B	DGRC-170	Rogers	4	male	SRR10981789	101	37705915	37.57
S2-DRSC-1	DGRC-181	Perrimon	4	male	SRR10981786	101	31515040	34.76
S2-DRSC-2	DGRC-181	Perrimon	4	male	SRR10981812	101	49916928	51.87
S2-DRSC-3	DGRC-181	Perrimon	4	male	SRR10981811	101	50084326	49.18
S2-GFP-SKL	DGRC-197	Rogers	4	male	SRR10981805	101	47302631	48.96
S2-Mt-DI	DGRC-152	Klueg	4	male	SRR10981802	101	37297961	38.88
S2-Mt-EB1-GFP	DGRC-171	Rogers	4	male	SRR10981788	101	34454428	39.07
S2-Mt-Fog-myc	DGRC-218	Rogers	4	male	SRR10981803	101	38085006	40.59
S2-Mt-GFP	DGRC-194	Rogers	4	male	SRR10981808	101	41882171	43.14
S2-Mt-GFP-Act5C	DGRC-169	Rogers	4	male	SRR10981790	101	44383445	46.85
S2-Mt-mCherry-alphaTub84B	DGRC-195	Rogers	4	male	SRR10981807	101	44960162	44.06
S2-Mt-Msps-GFP	DGRC-206	Rogers	4	male	SRR10981804	101	47561052	48.99
S2-Mt-N	DGRC-154	Klueg	4	male	SRR10981792	101	40733228	43.91
S2-Mt-Slit	DGRC-192	Rogers	4	male	SRR10981810	101	41033908	41.86
S2-SQH-GFP	DGRC-172	Rogers	4	male	SRR10981787	101	26690953	29.73
S2-SQH-GFP+Mt-mCherry-actin	DGRC-193	Rogers	4	male	SRR10981809	101	42525580	41.74
S2-Tub-wg	DGRC-165	Nusse	4	male	SRR10981791	101	41826501	43.40
S2R+	DGRC-150	Wheeler	4	male	SRR10981813	101	20056094	23.23
S2R+ (DRSC)	N.A.	Perrimon	4	male	SRR11000336	151	47640215	82.65
S2R+-NPT005	DGRC-229	Perrimon	4	male	SRR10981801	101	45413880	45.17
S2R+-NPT017	DGRC-230	Perrimon	4	male	SRR10981800	101	34459982	35.27
S2R+-NPT050	DGRC-231	Perrimon	4	male	SRR10981799	101	29162882	28.27
S2R+-NPT101	DGRC-232	Perrimon	4	male	SRR10981798	101	43114190	43.26
S2R+-SQH-GFP	DGRC-196	Rogers	4	male	SRR10981806	101	49206117	47.08
S3	DGRC-5	Cherbas	4	male	SRR10981797	101	23764412	27.52

**Table S2. Number of non-reference TE predictions made by TEMP for 31 Schneider sub-lineage samples.** Numbers of non-reference TE insertion predictions made by TEMP are based on default McClintock (Nelson *et al.*, 2017) settings. *INE-1* and non-reference TE insertion predictions in low recombination regions were excluded. N.A. indicates that this information is not available.

Cell line	DGRC ID	SRA	# of TEs
mbn2	DGRC-147	SRR13360020	1934
mbn2 (Gorski)	N.A.	SRR13360019	2195
mbn2 (Strand)	N.A.	SRR13360018	1980
S1	DGRC-9	SRR10981795	743
S2	DGRC-6	SRR10981796	1268
S2 (ATCC-CRL-1963)	N.A.	SRR10981814	847
S2 (DSMZ-ACC-130-C)	N.A.	SRR10981794	655
S2 (Invitrogen-R69007)	N.A.	SRR10981793	845
S2-act-GFP-alphaTub84B	DGRC-170	SRR10981789	975
S2-DRSC-1	DGRC-181	SRR10981786	1281
S2-DRSC-2	DGRC-181	SRR10981812	1083
S2-DRSC-3	DGRC-181	SRR10981811	1057
S2-GFP-SKL	DGRC-197	SRR10981805	866
S2-Mt-DI	DGRC-152	SRR10981802	1263
S2-Mt-EB1-GFP	DGRC-171	SRR10981788	1230
S2-Mt-Fog-myc	DGRC-218	SRR10981803	1293
S2-Mt-GFP	DGRC-194	SRR10981808	1323
S2-Mt-GFP-Act5C	DGRC-169	SRR10981790	804
S2-Mt-mCherry-alphaTub84B	DGRC-195	SRR10981807	1032
S2-Mt-Msps-GFP	DGRC-206	SRR10981804	1053
S2-Mt-N	DGRC-154	SRR10981792	1134
S2-Mt-Slit	DGRC-192	SRR10981810	1038
S2-SQH-GFP	DGRC-172	SRR10981787	1533
S2-SQH-GFP+Mt-mCherry-actin	DGRC-193	SRR10981809	994
S2-Tub-wg	DGRC-165	SRR10981791	1210
S2R+	DGRC-150	SRR10981813	1820
S2R+ (DRSC)	N.A.	SRR11000336	2924
S2R+-NPT005	DGRC-229	SRR10981801	1435
S2R+-NPT017	DGRC-230	SRR10981800	1607
S2R+-NPT050	DGRC-231	SRR10981799	1281
S2R+-NPT101	DGRC-232	SRR10981798	1494
S2R+-SQH-GFP	DGRC-196	SRR10981806	1300
S3	DGRC-5	SRR10981797	1204

## 18 **Supplemental References**

- 19 Boeva, V., T. Popova, K. Bleakley, P. Chiche, J. Cappelletti, *et al.*, 2012 Control-FREEC: a tool for assessing copy  
20 number and allelic content using next-generation sequencing data. *Bioinformatics* **28**: 423–425.
- 21 Lee, H., C. J. McManus, D.-Y. Cho, M. Eaton, F. Renda, *et al.*, 2014 DNA copy number evolution in *Drosophila*  
22 cell lines. *Genome Biol* **15**: R70.
- 23 Nelson, M. G., R. S. Linheiro, and C. M. Bergman, 2017 McClintock: an integrated pipeline for detecting trans-  
24 posable element insertions in whole-genome shotgun sequencing data. *G3* **7**: 2749–2762.
- 25 Zhuang, J., J. Wang, W. Theurkauf, and Z. Weng, 2014 TEMP: a computational method for analyzing transposable  
26 element polymorphism in populations. *Nucleic Acids Res* **42**: 6826–6838.