Supplementary Figure 1. Phenotype of the HI strain.

(A) Phenotype of the HI and wild type plant after flowering (~1month). Wild type plant is tall with well elongated inflorescence. All four HI plants are phenotypically distinct from WT, showing stunted inflorescence.

(B) Uniform appearance of HI plants raised from a self pollinated HI parent. Circles indicate two aneuploid plants. Aneuploids exhibit various phenotypes depending on their genomic composition. Some are as robust as WT or HI plants but are still easily distinguishable from their euploid counterparts.
Supplementary Figure 2. Phenotypic and cytological identification of haploids.

(A) F1 progeny obtained from HI x Ler cross. Haploid plants (white arrow) are similar to diploid Ler except for reduced size and can be easily distinguished from aneuploid plants, which show distinct phenotypes. All unmarked progeny are aneuploids.

(B) GFP positive root tissue expressing GFP-tailswap protein. Green dots correspond to the centromeres of the cells in the root tissue labeled by GFP. Its corresponding bright field image of the root is shown in (C). Scale bar - 50µm.

(D) A GFP negative root from WT showing no GFP dots at the centromeres. Its corresponding bright image image is shown in (E). Scale bar - 50µm.

(F) DAPI stained meiotic cell from an anther tissue at meiosis II showing 5 univalent chromosomes of a haploid plant. Scale bar - 0.5µm.

(G) DAPI stained interphase mitotic nuclei from a haploid plant showing the 5 chromocenters corresponding to 5 centromeres of haploid *A. thaliana*. The DAPI bright signal corresponds to the highly heterochromatic centromeric and pericentromeric chromatin. Scale bar - 0.5µm.
**Supplementary Figure 3.** Identification of different ploidy classes from F1 seedlings derived from SeedGFP-HI X WT.

(A) Seeds from untagged HI visualized in the GFP channel under a fluorescence stereo dissection microscope

(B) SeedGFP-HI selfed seeds visualized in the GFP channel. Compare the bright and uniform GFP fluorescence seen here to the autofluorescent signal in (A).

(C) Hand-sorted F1 seeds from SeedGFP-HI x WT cross showing a range of mottled GFP fluorescence.

(D-E) Dissected WT Col-0 seeds observed under fluorescence stereo microscope (D) and bright field (E) to reveal seed coat (sc), embryo (em) and endosperm (en). Embryo and endosperm exhibit auto fluorescence while the seed coat does not.
(F-G) Dissected SeedGFP-HI seeds showing strong GFP fluorescence in both embryo and endosperm. Seed coat is devoid of GFP fluorescence.

(H) A mottled GFP seed resulting from SeedGFP-HI x WT cross.

(I-J) Mottled GFP seed (showed in H) dissected and viewed under fluorescence stereo microscope (I) and bright field (J) respectively. GFP fluorescence is restricted to the endosperm layer whereas seed coat and potential haploid embryo are devoid of GFP fluorescence.

(K-R) GFP fluorescence pattern in F1 seeds (from SeedGFP-HI X WT) and their respective seedlings (by pairs). K,O: Uniform GFP fluorescence in the seed (K) and residual GFP in seedling (O). This seed gave rise to SeedGFP-HI plant likely resulting from accidental self-pollination. L,P: Seed showing mottled GFP fluorescence and its corresponding seedling (P) displaying red autofluorescence without any trace of GFP. Note that residual GFP from the endosperm is visible within the seed coat (*). This seedling gave rise to haploid plant. M,N: Seeds with uniform GFP fluorescence collected from SeedGFP-HI x WT cross. Seedling shown in (Q) developed as diploid (hybrid) plant whereas (R) developed into an aneuploid plant. The aneuploid seedling (R) has 3 cotyledons. (*) marks leftover seed coat and endosperm after germination. Scale bar in all panels is 200 μm.
**Supplementary Figure 4.** Crossing scheme showing number of generations required to swap the nuclear and cytoplasmic genomes. The cytoplasmic genomes of the parents are as indicated (C) - Col-0 cytoplasm and (L) - Ler cytoplasm.
Supplementary Figure 5. Strategy to maintain Wa-1 cytoplasm in Wa-1 ploidy series.

(A) Crossing strategy to transfer Wa-1 (W) cytoplasm from a tetraploid parent to a synthetic diploid Wa-1 harboring Col-0 (C) cytoplasm. The diploid Wa-1 (C) was made by crossing a tetraploid Wa-1 (W) as the pollen donor onto HI female parent. Hence the resulting diploid Wa-1 contains HI cytoplasm (C) instead of Wa-1 (W) cytoplasm. Subsequent interploidy crosses allowed us to bring the Wa-1 cytoplasm (W) in the diploid Wa-1 background.

(B) Plastid specific dCAPs marker (see methods) based genotyping of the cytoplasm transfer as indicated in the panel.
Supplementary Figure 6. Chromosome dosage analysis of SOG1/sog1 diploids using low coverage high throughput sequencing.

Three putative diploids with sog1 mutations obtained from a cross of HI onto tetraploid TILLING lines were sequenced on Illumina platform to around 2x genome coverage. Mapped reads were binned in 25kb windows, counted, compared to a diploid control and plotted to identify copy number variation, as previously described. All individuals characterized show euploid copy numbers on all chromosomes. None of the individuals characterized show any dosage variation between chromosomes (aneuploidy or insertions or deletions).
Supplementary Figure 7. Chromosome dosage analysis of A. suecica haploids using low coverage high throughput sequencing.

A. suecica is an allopolyploid resulting from an ancient hybridization event between ancestral A. thaliana and A. arenosa individuals. In the absence of the A. arenosa reference genome, reads were aligned to a theoretical genome that combined the reference genome from A. thaliana and A. lyrata. The mapped reads were pooled by consecutive, non-overlapping bins of 100,000 bp. Number of reads per bin were normalized to a wild type A. suecica control, using the method described by Henry et al. 36. The results confirm the euploid status of the two haploid individuals and the presence of all 13 A. suecica chromosomes. Ploidy was determined using the flow cytometric measurement of nuclear DNA content (Fig. 1E).
**Supplementary Figure 8.** M1 haploid forward genetics screen.

(A) Bar graph showing the percentage of haploid, diploid and aneuploid progeny as a result of haploid induction using unirradiated pollen (0 Gy), 100 Gy irradiated pollen and 200 Gy irradiated pollen.

(B) Wild type *Ler gl1* haploid.

(C) M1_01 mutant haploid showing mottled, variegated leaf phenotype.

(D) M1_02 mutant haploid showing pale yellow leaf phenotype.

(E) M1_03 mutant with late-flowering phenotype (left) shown next to wild type *Ler gl1* haploid.

(F) To search for the potential presence of large scale insertions or deletions, M1_01 and M1_02 were subjected to whole-genome Illumina sequencing. Sequencing reads were mapped to the Arabidopsis reference genome. The mapped reads were grouped into non-overlapping 25kb bins, counted and compared to a euploid *Ler gl1* haploid control. Because the M1 haploids are derived from Col-0 (HI) x *Ler gl1* cross, single nucleotide polymorphisms (SNPs) unique to *Ler gl1* were used to confirm that the resultant haploids are 100% *Ler gl1* (data not shown). The plot from all five chromosomes show M1_01 and M1_02 have balanced chromosome numbers after mutagenesis using gamma irradiation, as no abnormal chromosomal structural changes (insertion, deletion or altered chromosome dosage) could be detected.
Supplementary Figure 9. Phenotypes of dme-2 and mea-1 haploids.

(A) dme-2 haploid similar in phenotype to WT Ler haploid.
(B) dme-2 haploid showing incompletely penetrant floral abnormalities as zoomed in on panel.
(C) Inflorescence from the plant in panel B zoomed to show floral development abnormalities. Flower with only 2 petals (light green arrow) and 4 irregularly shaped petals (yellow arrow) are visible.
(D) Phenotype of mea-1 and MEA haploids. Both sporophytes look similar without any obvious phenotypes.
Supplementary Table 1  Ploidy analysis of F₁ seeds obtained from SeedGFP-HI x WT cross.

**Uniform GFP fluorescence seeds**

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<thead>
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<th>Ploidy category</th>
<th>Number of seeds</th>
<th>%</th>
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<tbody>
<tr>
<td>Aneuploids</td>
<td>16</td>
<td>32</td>
</tr>
<tr>
<td>Self-pollinated</td>
<td>26</td>
<td>52</td>
</tr>
<tr>
<td>Diploid</td>
<td>8</td>
<td>16</td>
</tr>
<tr>
<td><strong>Total</strong></td>
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<td><strong>100</strong></td>
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</table>

**Mottled GFP fluorescence seeds**

<table>
<thead>
<tr>
<th>Ploidy category</th>
<th>Number of seeds</th>
<th>% excluding dead seeds (only viable progeny)</th>
<th>% including dead seeds</th>
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</thead>
<tbody>
<tr>
<td>Haploids</td>
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<td>91.19</td>
<td>71.54</td>
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<tr>
<td>Aneuploids</td>
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<td>Diploids</td>
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<td>0.00</td>
<td>0.00</td>
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<tr>
<td><strong>Dead/did not</strong></td>
<td><strong>53</strong></td>
<td><strong>0.00</strong></td>
<td><strong>21.54</strong></td>
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<tr>
<td><strong>Total</strong></td>
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<td><strong>100.00</strong></td>
<td><strong>100.00</strong></td>
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<tr>
<td><strong>Total viable</strong></td>
<td><strong>193</strong></td>
<td></td>
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