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The flowering gene *SINGLE FLOWER TRUSS* drives heterosis for yield in tomato

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Intercrossing different varieties of plants frequently produces hybrid offspring with superior vigor and increased yields, in a poorly understood phenomenon known as heterosis[1,2]. One classical unproven model for heterosis is overdominance, which posits in its simplest form that improved vigor can result from a single heterozygous gene[3–8]. Here we report that heterozygosity for tomato loss-of-function alleles of *SINGLE FLOWER TRUSS* (*SFT*), which is the genetic originator of the flowering hormone florigen, increases yield by up to 60%. Yield overdominance from *SFT* heterozygosity is robust, occurring in distinct genetic backgrounds and environments. We show that several traits integrate pleiotropically to drive heterosis in a multiplicative manner[9], and these effects derive from a suppression of growth termination mediated by *SELF PRUNING* (*SP*), an antagonist of *SFT*. Our findings provide the first example of a single overdominant gene for yield and suggest that single heterozygous mutations may improve productivity in other agricultural organisms.

Elucidating why hybrid organisms show greater vigor than their inbred parents (heterosis) has intrigued biologists since Darwin first sought to explain why outcrossing is prevalent in nature[2,10]. Although heterosis is used extensively in agriculture[11], a lack of knowledge of the mechanism(s) responsible for heterosis has precluded boosting heterotic effects beyond present values. Three classical models have served as the foundation for dissecting heterosis into genetic and molecular components[1,3,12,13]. The dominance model proposes that distinct sets of deleterious recessive alleles undergo genome-wide complementation in hybrids. Conversely, the overdominance model states that intralocus allelic interactions at one or more heterozygous genes leads to increased vigor. Genes showing overdominance are the most noteworthy and sought after from both a fundamental and applied perspective, because with overdominance theoretically only a single heterozygous gene is needed to achieve heterosis. Although many overdominant quantitative trait loci (QTL) have been identified through genetic mapping experiments, further studies have revealed several examples supporting the third model, pseudo-overdominance, which is dominance that mimics overdominance because the mutations involved are linked. Consequently, there is little support for single-gene overdominance[1,14]. Nevertheless, notable classical reports in non-crop plants[15] and animals[4,5] have suggested, amid controversy[6,7], that heterozygosity for single gene mutations can cause overdominance.

Working under the hypothesis that mutations have the potential to be overdominant, we searched for genes that cause heterosis in tomato (*Solanum lycopersicum*)[16]. To identify overdominant mutations for yield, we crossed 33 diverse fertile mutants with the matching non-mutagenized parent known as 'M82' to create isogenic mutant heterozygotes and compared their yields (**Supplementary Fig. 1**, **Supplementary Table 1** and Online Methods)[17]. Six mutant heterozygotes showed heterosis, with the individual effects ranging from 36% to 88% (**Supplementary Fig. 1a**). From these six lines, two mutants (e0137 and e4537) showed reproducible heterosis as heterozygotes (**Supplementary Fig. 1b**), and, notably, these effects matched overdominant QTL from a wild-species introgression-line population[16].

The gene responsible for mutant e0137 has not yet been identified; however, e4537, which displayed the strongest heterosis, was shown previously to carry a missense mutation in the classical gene *SINGLE FLOWER TRUSS* (*SFT*)[18] (Online Methods). *SFT* encodes the ortholog of *Arabidopsis thaliana* FLOWERING LOCUS T (encoded by *FT*) and is the genetic originator of the flowering hormone florigen[19]. Similar to *Arabidopsis FT*$^{-/-}$ (*ft/ft*) mutants, tomato plants with *SFT* loss-of-function alleles flower later than normal. Additionally, in tomato *SFT*$^{-/-}$ (*sft/sft*) plants, only a few inflorescences develop before reverting to indeterminate vegetative branches that infrequently produce single fertile flowers. Because canonical multiflowered inflorescences almost never form, *sft/sft* plants are large and produce the fewest inflorescences, flowers and fruits of all genotypes (**Fig. 1**).

Because our initial screen focused on first-generation (F_1) heterozygotes, DNA changes other than the missense mutation at *SFT* could have caused the heterosis. We therefore confirmed that overdominance co-segregated with heterozygosity for the *sft* mutant e4537 (hereafter referred to as *sft-4537*) (**Supplementary Fig. 2** and Online Methods). However, we could not rule out the possibility that molecular changes linked to *SFT* were involved in heterosis. Furthermore, because *sft-4537* results in only one amino acid change and is a weak allele (Online Methods)[18], we could not determine if overdominance resulted from an intralocus allelic interaction between the functional and mutated alleles of *SFT* or if this was a case of mutant overdominance, in which having only one normal copy of *SFT* drives the effect. To resolve both issues, we confirmed that two independent strong

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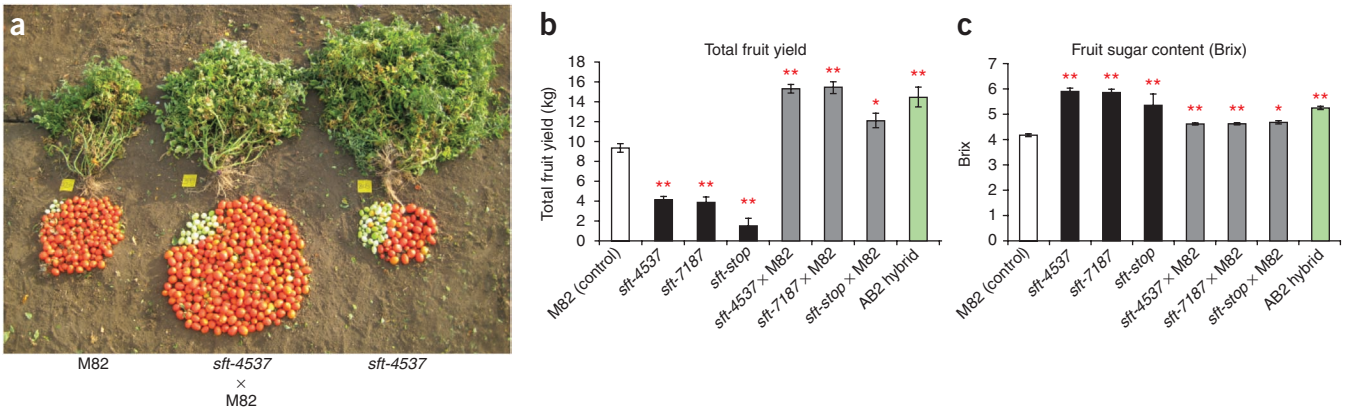

Figure 1 Heterozygosity for loss-of-function mutations in *SFT* drives heterosis in tomato. (**a**) Representative plant and total fruit yield from a high-yielding M82 inbred control plant (left), a low-yielding homozygous loss-of-function mutant allele of *SFT* (*sft-4537*, right; Online Methods) and a highly heterotic *sft-4537*/+ heterozygote (middle). All genotypes are isogenic in the M82 background. (**b**) Statistical comparison of mean values (± s.e.m.) for total fruit yields between three independently derived *sft*/*sft* homozygous mutants (carrying the *sft-4537* weak allele, *sft-7187* strong allele and *sft-stop* strong allele, respectively; Online Methods), the inbred M82 control and the F_1 *sft*/+ hybrids of the *sft*/*sft* mutants with M82. Total fruit yields from all three *sft*/+ heterozygotes were heterotic over M82 controls, and *sft-4537*/+ and *sft-7187*/+ heterozygotes achieved the same yields as AB2, which is a leading commercial processing-tomato hybrid. (**c**) Statistical comparison of mean values (± s.e.m.) for fruit sugar content (Brix value) showing an intermediate effect for *sft*/+ heterozygotes relative to M82 controls (low sugar) and *sft*/*sft* homozygotes (high sugar). Lines marked with asterisks are significantly different from the M82 control according to the 'compare with control' (Dunnett's) method: $*P < 0.05$, $**P < 0.01$. Similar results were obtained using multiple comparison analysis (Tukey-Kramer test; $**P < 0.05$) for total fruit yield, which revealed a significant difference between AB2 and *sft*/+ heterozygotes compared to M82 plants and *sft*/*sft* homozygotes. For Brix values, all four groups of genotypes were significantly different from each other (Tukey-Kramer test; $**P < 0.05$).

mutant alleles of *SFT* (*sft-7187* and *sft-stop*) showed significant heterosis (**Fig. 1b** and Online Methods). These results unequivocally link *sft*/+ heterozygosity with heterosis and provide the first example of a single overdominant gene for yield. Furthermore, overdominance can be achieved by having only a single functional allele of a gene, as opposed to a synergistic interaction between two alleles.

Heterosis in agriculture is predominantly based on full-genome hybridization between different inbred plants, and the heterotic response can vary widely depending on environment and genetic background[1]. The processing-tomato industry seeks varieties with both high total fruit yield and high sugar content (Brix value), but total yield is the trait that is primarily sought[20]. We tested the strength and consistency of *sft*/+ overdominance in diverse planting conditions, environments and genetic backgrounds. We observed that both *sft-4537*/+ and *sft-7187*/+ heterozygotes achieved the same yields as a leading commercial tomato hybrid (**Fig. 1b** and Online Methods). Furthermore, despite the typical inverse relationship between fruit yield and sugar content[21], the Brix values increased in *sft*/+ heterozygotes relative to the M82 tomatoes, although the Brix effect was intermediate (additive: $d/[a]$ ratios of −0.49; see Online Methods) between M82 and *sft* mutant plants (**Fig. 1c** and Online Methods), similar to plant weight (**Supplementary Fig. 3**). Furthermore, in seven different field-based experiments, we consistently detected significant heterosis in *sft*/+ heterozygotes (**Supplementary Table 2**). Total fruit yield was significantly ($P < 0.05$) overdominant in all but three instances, which involved either dense spacing or nonirrigated conditions, but the yields of *sft*/+ heterozygotes still exceeded those of M82 plants and their sugar production was higher, resulting in heterosis for the multiplicative phenotype of Brix-yield. Finally, we also observed strong *sft*/+ overdominance in crosses with two distinct genetic backgrounds (**Fig. 2**, **Supplementary Table 3** and Online Methods). Thus, *SFT*-dependent heterosis is extremely robust and shows substantial potential for broad agricultural application.

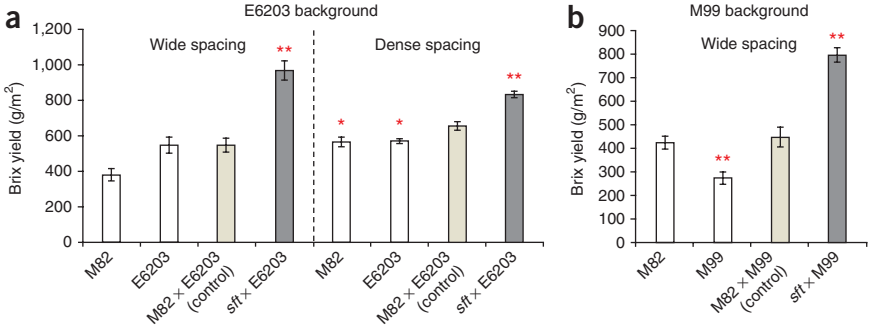

Figure 2 *sft*/+ heterozygosity causes heterosis in distinct genetic backgrounds and growth conditions. In the tomato industry, genotypes with high yield and Brix value (that is, high values of Brix-yield, the multiplied output of Brix and total fruit yield measured in g/m^2) are the most efficient for the production of various tomato concentrates. (**a**) Statistical comparison of Brix-yield between *sft*/+ heterozygotes in the background of a full-genome hybrid between M82 and the processing-tomato line E6203 (dark gray) (Online Methods), the homozygous inbred lines M82 and E6203 (white) and the hybrid (M82 × E6203) control (light gray). Experiments were performed in both wide- and dense-spacing conditions (Online Methods). (**b**) Statistical comparison of Brix-yield between *sft*/+ heterozygotes in the background of the large-fruited fresh market tomato line M99 (dark gray) (Online Methods), the homozygous inbred lines M82 and M99 (white) and the hybrid controls (M82 × M99) (light gray). The mean values (± s.e.m.) for each genotype marked by asterisks reflect a significant difference from the control hybrids according to the 'compare with control' (Dunnett's) method: $*P < 0.05$, $**P < 0.01$. Similar results were obtained using multiple-range means comparison (Tukey-Kramer test; $**P < 0.05$), which revealed a significant difference between *sft*/+ heterozygotes and their corresponding controls.

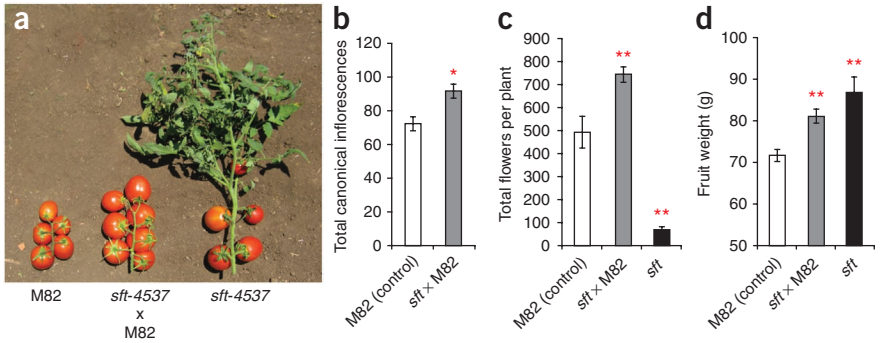

Figure 3 *SFT*-dependent heterosis arises from multiple phenotypic changes on component traits that integrate to improve yield. (**a**) Representative inflorescences from M82 plants (left), *sft*/*sft* homozygous mutants (right) and *sft*/+ heterozygotes (middle). The *sft*/*sft* homozygotes produce only a few inflorescences before reverting to indeterminate vegetative branches that infrequently produce single fertile flowers, which were counted. Because canonical multiflowered inflorescences almost never form, *sft*/*sft* mutant plants have the fewest inflorescences, flowers and fruits of any genotype. (**b**–**d**) Quantification and statistical comparison of three component traits for yield. (**b**) *sft*/+ heterozygotes (dark gray) produce more inflorescences compared to M82 plants. As canonical inflorescences almost never form in *sft*/*sft* homozygous mutants, no data was collected for this genotype. (**c**) *sft*/+ heterozygotes produce the most flowers per plant of all genotypes (are overdominant) and show an additive effect for fruit weight (**d**), with a *d*/[*a*] value of 0.25. Mean values (± s.e.m.) were compared to the M82 isogenic line (white) using the 'compare with control' (Dunnett's) method when three genotypes were present, and a *t*-test analysis was performed when two genotypes were present (total inflorescence). Significant differences compared to M82 plants are represented by asterisks: $*P < 0.05$, $**P < 0.01$.

Classical tomato heterosis is driven predominantly by QTL that control reproductive traits that integrate multiplicatively[16,22]. To determine the basis of *sft*/+ heterosis, we examined the contributions of two major component traits of yield: total number of flowers per plant (total inflorescences multiplied by flowers per inflorescence) and fruit weight (**Fig. 3**). Consistent with previous experiments, total fruit yield was overdominant and Brix and plant weight showed intermediate effects (data not shown). Due to the small number of non-canonical vegetative inflorescences that form in plants with the *sft*/*sft* mutation, only individual flowers scattered throughout the plant were counted (**Figs. 1a** and **3a**). We found that both total inflorescences (**Fig. 3b**) and flowers per inflorescence (M82, 6.5 ± 0.32; *sft*/+ heterozygotes, 7.5 ± 0.21; $P < 0.05$) were higher in *sft*/+ heterozygotes compared to M82 plants, indicating that each of these traits contributes to overdominance of total flower number (**Fig. 3c**). Although plants with the *sft*/*sft* mutation produced the fewest flowers, they were completely fertile, indicating that low fruit yield results from low flower production. Fruit weight had an intermediate effect and was therefore also a partial contributor to *sft*/+ heterosis as a nonreproductive trait (**Fig. 3d**). Thus, similar to classical heterosis, *sft*/+ heterosis is based on the integration of multiple component traits, the majority of which are overdominant.

Due to its prominent role in flower number and total fruit yield, increased inflorescence production was the most likely impetus behind *sft*/+ heterosis. To understand how increased numbers of inflorescences form, we quantified their accumulation over time throughout

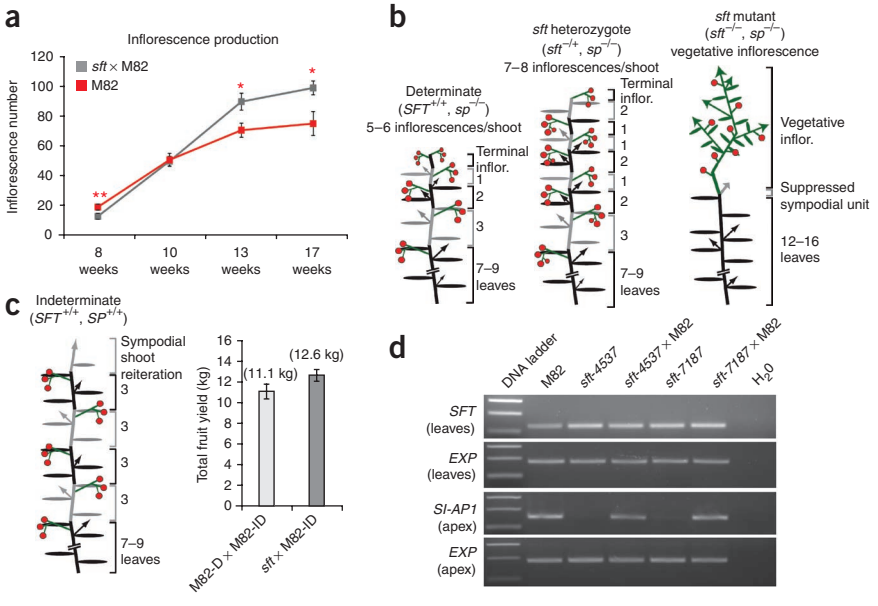

Figure 4 Overdominance for inflorescence production is based on a dosage-dependent suppression of growth termination mediated by *SP*. (**a**) Temporal accumulation of inflorescences in M82 plants (red) and *sft*/+ heterozygotes (gray) throughout growth. Significant differences between mean values were calculated in each time point using *t*-test analyses and are represented by asterisks: $*P < 0.05$, $**P < 0.01$. (**b**) Diagrams showing the reiteration of modular sympodial units along individual shoots from M82-determinate (left), *sft*/*sft* homozygote (right) and *sft*/+ heterozygote plants (middle). Numbers indicate total number of leaves in each sympodial unit, which are indicated by brackets. Flattened gray and black ovals indicate sets of leaves in alternating sympodial units. Black and gray arrows represent axillary shoots preceding the inflorescence in each sympodial unit. Note that there are more sympodial units, inflorescences and leaves along each shoot of *sft*/+ heterozygotes compared to M82-determinate and *sft*/*sft* mutant plants (**Supplementary Fig. 4**). (**c**) Diagram of a shoot from an M82-indeterminate plant (left) and a yield comparison between *sft*/+ heterozygotes in an indeterminate background (*sft*/+, *sp*/+) and control M82-indeterminate plants (*sft*/+, *sp*/+). Total fruit yields of M82-determinate × M82-indeterminate (light gray) and *sft*/*sft* × M82-indeterminate (dark gray) do not differ significantly from each other, and both are lower yielding than the heterotic *sft*/+ M82-determinate plants, which are the highest yielding of all genotypes (**Supplementary Fig. 5**). (**d**) RT-PCR analysis of *SFT* and *SI-AP1* expression levels in young expanding leaves and shoot apices, respectively, showing no qualitative differences in transcript accumulation between M82 plants and heterozygote genotypes for either gene. *EXP* (*EXPRESSED*, SGN-U346908) is a published gene showing stable expression across diverse tissues, used here as real-time PCR control (Online Methods).

a growing season (**Fig. 4a**). Whereas M82 plants had slightly more inflorescences than *sft*/+ heterozygotes at 8 weeks, their inflorescence production slowed by 13 weeks and plateaued at 17 weeks, a few weeks before harvesting. In contrast, *sft*/+ heterozygote inflorescences accumulated linearly up through 13 weeks and production only began to plateau at 17 weeks, indicating that *sft*/+ heterozygotes develop more inflorescences in a shorter time.

Given that the normal function of *SFT* is to promote flowering, we reasoned that *sft*/+ heterozygote heterosis might be based on a shift in the balance of vegetative to reproductive growth. Tomato plants form inflorescences and flowers within a compound sympodial shoot system, which is defined by the continuous cycling of growth termination and renewal[23]. In a typical plant, all growth arises from the reiteration of modular sympodial units that each produce three leaves and a multiflowered inflorescence[23,24] (**Fig. 4**). All field-grown varieties of tomato, including M82, are determinate (D) plants whose shoots produce an average of six sympodial units, each harboring a single inflorescence, within which leaf number gradually decreases before a precocious termination of growth owing to a mutation in *SP*—an ortholog of *TERMINAL FLOWER1* (*TFL1*) and a member of the *SFT*/*FT* gene family[23] (**Fig. 4b**). In *sft*/+ heterozygotes, we found a greater number of inflorescences and leaves on all shoots than in M82 plants (**Supplementary Fig. 4**). Further, more sympodial units developed on the *sft*/+ heterozygotes, and each sympodial unit harbored a variable number of leaves ranging from 1 to 3 (**Fig. 4b**). Thus, *sft*/+ heterozygosity transforms the way sympodial units are reiterated and structured in determinate plants. These observations suggest that *sft*/+ heterosis might be based on having only one fully functional copy of *SFT*, causing a dosage-dependent suppression of growth termination imposed by *SP*. We tested this hypothesis by crossing plants with the *sft* mutation to M82-indeterminate (ID) plants carrying a functional *SP* gene, and in all cases, heterosis was eliminated (**Fig. 4c** and **Supplementary Fig. 5**). The relationship of *sft*/+ heterozygosity to an *SP* background also highlights a role for epistasis in heterosis.

With such pronounced phenotypic consequences, it might be expected that *sft*/+ overdominance results from large transcriptional changes at *SFT* or its primary floral targets; however, a subtle change in gene dosage predicts relatively minor transcriptional changes of no greater than 2–3-fold[25]. After examining *SFT* expression, we observed no qualitative changes (**Fig. 4d**, **Supplementary Table 4** and Online Methods), and we detected a minor quantitative increase (twofold or less) for all *SFT* homozygote and heterozygote genotypes relative to M82 plants (**Supplementary Fig. 6**). This weak change is consistent with a dosage-dependent mechanism. We also examined expression in inflorescence shoot apices for the tomato homolog of *APETALA1* (*Sl-AP1*), which is a direct target of *SFT*/*FT*[26]. Consistent with the *SFT* phenotype (**Figs. 1a** and **3a**), *Sl-AP1* expression was strongly reduced in plants homozygous for the *sft* mutation but not in those heterozygous for it. Thus, *sft*/+ heterosis is not caused by substantial transcriptional changes of *SFT* or its primary target.

It has been suggested that heterosis might result from the tuned activities of dosage-dependent regulatory systems controlling signaling cascades and transcriptional networks[27]. Our results provide a notable example of single-gene overdominance for tomato yield and offer empirical evidence that, rather than being caused by allelic interactions, subtle changes in gene dosage can lead to overdominance. In our case, overdominance depends on opposing flowering signals from *SFT* and *SP*, a relationship that has been proposed to be critical for overall plant growth[19]. We hypothesize that *sft*/+ heterosis is based on the altered dosage of functional SFT protein within each modular sympodial unit. Due to the dynamic nature of the reproductive transition in the stem cell populations, where opposing activities of SFT and SP operate, a shift in the reproductive transition due to *sft*/+ heterozygosity would be transient and difficult to capture molecularly. This is because as one sympodial unit transitions, a new SFT-SP balance is established in the subsequent unit. We predict that sympodial units are particularly sensitive to the altered dosage of SFT in plants homozygous for the *sp* mutation, which would explain the dramatic pleiotropic changes for the entire plant if *sft*/+ heterozygosity quantitatively suppresses multiple *sp*-induced termination events, thereby allowing more units to develop. Such a mechanism could explain heterosis in other field tomatoes, which are all in the homozygous *sp* mutant background. It is also conceivable that the impact of *sft*/+ heterozygosity on determinate growth might exemplify a more widespread mechanism in which altering the dosage of any genes controlling growth determinacy through mutant heterozygosity can explain or lead to heterosis in other species.

Notably, our data suggest that any case of heterosis involving deleterious recessive mutations can be based on altered gene dosage. In this regard, it is worth noting that all crops underwent selection for multiple mutations during domestication[28]. Indeed, although there has been renewed support for the idea that dominance complementation explains classical heterosis in maize[29] and there is evidence that epistasis plays a role in rice heterosis[30], overdominance from mutant heterozygosity involving many loci with small effects on component traits could also be a principal mechanism[31]. This is consistent with the observation that copy number variation, often in the form of insertions and/or deletions (indels) that span genes, is widespread among inbred maize lines[32]. By extension, new mutations arising in nature, which are inevitably heterozygous, could show overdominance and be relevant in the short term for natural selection and over a longer time frame for population genetics[2]. Finally, our findings reveal a new and simple methodology to identify genes with the potential to cause agricultural heterosis. That our original screen revealed a noteworthy percentage of putative overdominant mutants suggests that heterosis through mutant heterozygosity might be a general phenomenon for plants and other organisms[33,34]. Screening large sets of heterozygous mutants in other model and crop species could yield additional examples of single-gene heterosis and provide innovative germplasm for plant breeding.

METHODS

Methods and any associated references are available in the online version of the paper at http://www.nature.com/naturegenetics/.

Accession codes. The sequences of *SFT* and *SP* have been deposited in the NCBI nucleotide database under accession codes AY186735 and LEU84140, respectively.

Note: Supplementary information is available on the Nature Genetics website.

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AUTHOR CONTRIBUTIONS

U.K., Z.B.L. and D.Z. planned and carried out all experiments, collected the data, performed the statistical analyses and wrote the paper.

COMPETING FINANCIAL INTERESTS
The authors declare competing financial interests: details accompany the full-text HTML version of the paper at http://www.nature.com/naturegenetics/.

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ONLINE METHODS

Plant material and genotyping. Thirty-three homozygous mutants were selected from a mutant library in the processing-tomato cultivar M82, which is determinate due to homozygosity for a mutation in the *SP* gene (see URLs)[17] (**Supplementary Table 1**). Screening focused on fertile mutants that had various defects in developmental traits and overall growth, which were tested by progeny test for single-gene segregation. Individual mutant descriptions were based on a large phenotypic screen for mutants within ~16,000 independent M_2-generation families categorized into primary and subphenotypic categories[17]. These phenotypic categories were general for the purpose of the database, and more detailed study on some of the mutants has resulted in more precise phenotypic descriptions. For example, the descriptor 'partial sterility' is used to describe the *sft* mutation, but this refers to the observation that few flowers and fruits are produced in plants homozygous for this mutation, as opposed to flower sterility or fruit set. Seven mutants of known loci were chosen (**Supplementary Table 1**), and for two of them, two alleles were selected and plants bearing the different alleles were counted as independent lines (*LATERAL SUPPRESSOR* (*LAS*), e0064m2 and e9148m2; *ENTIRE* (*EN*), e2986m1 and e3335m1). This is because it could not be assumed that all mutant alleles for a particular gene would result in the same phenotypic response. All mutants were crossed as males to M82 plants. The alleles *sft-4537* and *sft-7187* tested in 2009 derive from the original M82 mutant library and have been described as weak (*sft-4537*) and strong (*sft-7187*) late-flowering mutants due to DNA mutations in the flowering gene *SFT*[18]. The mutation in *sft-4537* is a single-base-pair change at nucleotide position 194 (C to T) that results in a single-amino-acid change from threonine to isoleucine. The mutation in *sft-7187* is a two-base-pair deletion at positions 466 through 467 that causes a frameshift and a presumed truncated C terminus of the SFT protein. The *sft-stop* allele was discovered separately in the M82 background and verified to be a new mutant allele in *SFT* by complementation tests with both *sft-4537* and *sft-7187*. Subsequent sequencing revealed a single-base-pair change at position 148 (C to T) that introduces a nonsense mutation (stop codon) and truncates the last two-thirds of the SFT protein. Both *sft-7187* and *sft-stop* are considered strong alleles relative to *sft-4537* based on their late-flowering effects and highly vegetative inflorescences, and *sft-stop* is likely a null allele. Homozygous *sft-4537* and *sft-7187* mutations backcrossed at least three times into the M82 background were gifts from Y. Eshed (Weizmann Institute of Science, Rehovot, Israel). Progeny tests and PCR-based genotyping markers were used to identify plants heterozygous for *sft-stop* after backcrossing to M82 at least twice to eliminate other mutations. The F2-generation segregating population of *sft-4537* was obtained by self-fertilization of heterozygous plants. To simplify our phenotypic analysis and experimental design, PCR-based genotyping markers were developed for all *SFT* mutant alleles (**Supplementary Table 4**). Based on their similar heterozygous phenotypic effects, only *sft-4537* and *sft-7187* alleles were used as inbred parents to generate full-genome heterozygotes with the inbred genetic backgrounds E6203 and M99. Like M82, E6203 is an inbred processing-tomato variety that serves as a common experimental inbred line and has been used extensively in research[35]. M99 was developed in our lab and is a large-fruited fresh market tomato inbred line. AB2 is a leading processing-tomato commercial hybrid from the AB Seed Company. Indeterminate lines containing two functional alleles of the *SP* gene were M82ID, M83, rin and Alisa Craig, and crosses with these were performed with *sft-4537* and *sft-7187* serving as male parents.

Field trials. Field trials were conducted during the years 2008 and 2009. Experiments were performed at the Western Galilee experimental station in Akko, Israel; in Kfar Masaryk, Israel; and at the Cornell horticultural experiment station in Riverhead, New York. Seedlings were grown in a commercial nursery for 35–40 d and transplanted to the field at the beginning of April. The Akko experiments were conducted under wide (1 plant per m^2) and dense (2.5 plants per m^2) spacing using two irrigation regimes. In the nonirrigated treatments, 60 m^3 of water per 1,000 m^2 were applied immediately after transplanting, whereas 300 m^3 of water per 1,000 m^2 were applied in the irrigated treatments during the season according to drip irrigation protocols standard for the area. Given their low fertility and extreme plant size, *sft*/*sft* homozygous mutants for all three alleles were tested in isolation in a block neighboring other experiments. To test for consistency of *sft*/+ heterosis, selected experiments were repeated using both replicated single plant experiments or replicated individual plots of ten plants in 4 m^2 under commercial field and diverse agricultural conditions at Kfar Masaryk, Israel, and in the United States. Each genotype in the single-plant experiments was represented by a minimum of 15 replicates, and in some experiments 60 replicates were used. Twelve replicates for each genotype were used in the plot experiments. As few as five replicates were used to represent homozygous *sft*/*sft* mutants due to their consistently low yields and large plant stature. All plants were transplanted in a completely randomized design. Experiments involving indeterminate lines were transplanted both to the field and to greenhouses and treated according to commercial cultivation protocols.

Phenotypic evaluations and statistical analyses. Harvests were conducted when all plants in a trial had 80% or more red fruit. For each individual plant in the wide-spacing experiments, phenotypic measurements of plant weight and total fruit yield were taken after plants were manually removed from the soil, along with ten random fruits from which to estimate the average fruit weight and total soluble solids content (mainly sugars), the latter referred to as Brix value and measured by a digital Brix refractometer (ATAGO Palette PR-32α). For the dense-spacing experiments, total fruit yield was measured on plots 4 m^2, and 20 random fruits were used to estimate fruit weight and Brix. All statistical calculations were performed using the JMP 7.0.1 software package (SAS Institute). Mean values for each measured yield parameter were analyzed using the "Fit Y by X" function and statistically compared using a Tukey-Kramer multiple comparison test, Dunnett's 'compare with control' test, or *t*-test, whenever appropriate. The degree of dominance for each trait was calculated using the $d/[a]$ equation, where the additive effect (a) was half of the difference between the *SFT* mutant and M82; the dominance deviation (d) was the difference between *sft*/+ heterozygotes and the mid value of its parents; and the degree of dominance for each *SFT* mutant allele ($d/[a]$) was calculated by dividing the mean dominance deviation by the absolute mean additive effect. Measurements of plant structure and yield component traits were taken from four equivalent trials at 8, 10, 13 and 17 weeks after planting to capture all major developmental changes. At each time point, individual replicate plants were dissected for selected component traits for yield (plant weight, total fruit yield, Brix value, fruit weight, inflorescence number and flowers per inflorescence). Inflorescences were considered only after one or more fruits had set on a truss. Total organ number per shoot was evaluated from five plants per genotype, each presented by four or more shoots that contained more than three inflorescences to provide a minimum of 20 replicates for each genotype.

Data availability. All raw data for the heterosis field trials are available to analyze and download from the site for Phenom Networks (see URLs), which is a web-based platform for complex phenotype analysis in plant breeding.

Expression analysis (RT-PCR). Three biological replicates per genotype were extracted from leaves (the third-oldest leaf was used) and apices of 4-week-old seedlings grown in a greenhouse (eight representative plants in each sample). Total RNA was extracted using Trizol (Invitrogen) and treated with DNase (Promega) according to the manufacturer's protocol. Reverse transcription was performed using 1 μg of total RNA with the SuperScript First-Strand Synthesis System for RT-PCR (Invitrogen). Qualitative RT-PCR was performed for *SFT* and *Sl-AP1* (**Supplementary Table 4**), and PCR was carried out at three different total cycle numbers (24, 28 and 32) for each gene using the third uppermost expanding leaf 4–5 cm in length (*SFT*) and inflorescence apices carrying the first flower and first sympodial unit and inflorescence meristem (*AP1*). Agarose gels (3%) were run for each cycle, and analysis was performed using the cycle number that approximated the linear phase of amplification based on the increase in band intensity. To achieve a quantitative estimation of transcript abundance, real-time quantitative RT-PCR reactions were performed using the SuperScript III First-Strand Synthesis SuperMix followed by Power SYBR Green PCR Master Mix (Invitrogen). At least two biological replicates for each genotype and each gene were analyzed in three technical triplicates using a Bio-Rad/MJ Chromo4 real-time PCR machine, and each data point was calculated from the Opticon program and analyzed as the average of the three biological replicates at the beginning of the linear amplification

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phase. Quantification was based on the number of PCR cycles (Ct) required to cross a threshold of fluorescence intensity and was compared to a previously published real-time PCR control gene showing stable expression across diverse tissues[36] (*EXP* (*EXPRESSED*), SGN-U346908) using the $2^{-\Delta\Delta Ct}$ technique.

URLs. Tomato mutant library, http://zamir.sgn.cornell.edu/mutants; Phenom Networks, http://phnserver.phenome-networks.com/icis/. All raw data, and a PowerPoint explanatory demonstration for statistical analysis for the heterosis field trials, are available to analyze and download from Phenom Networks (http://phnserver.phenome-networks.com/icis/), and the data are freely available on the World Wide Web.

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