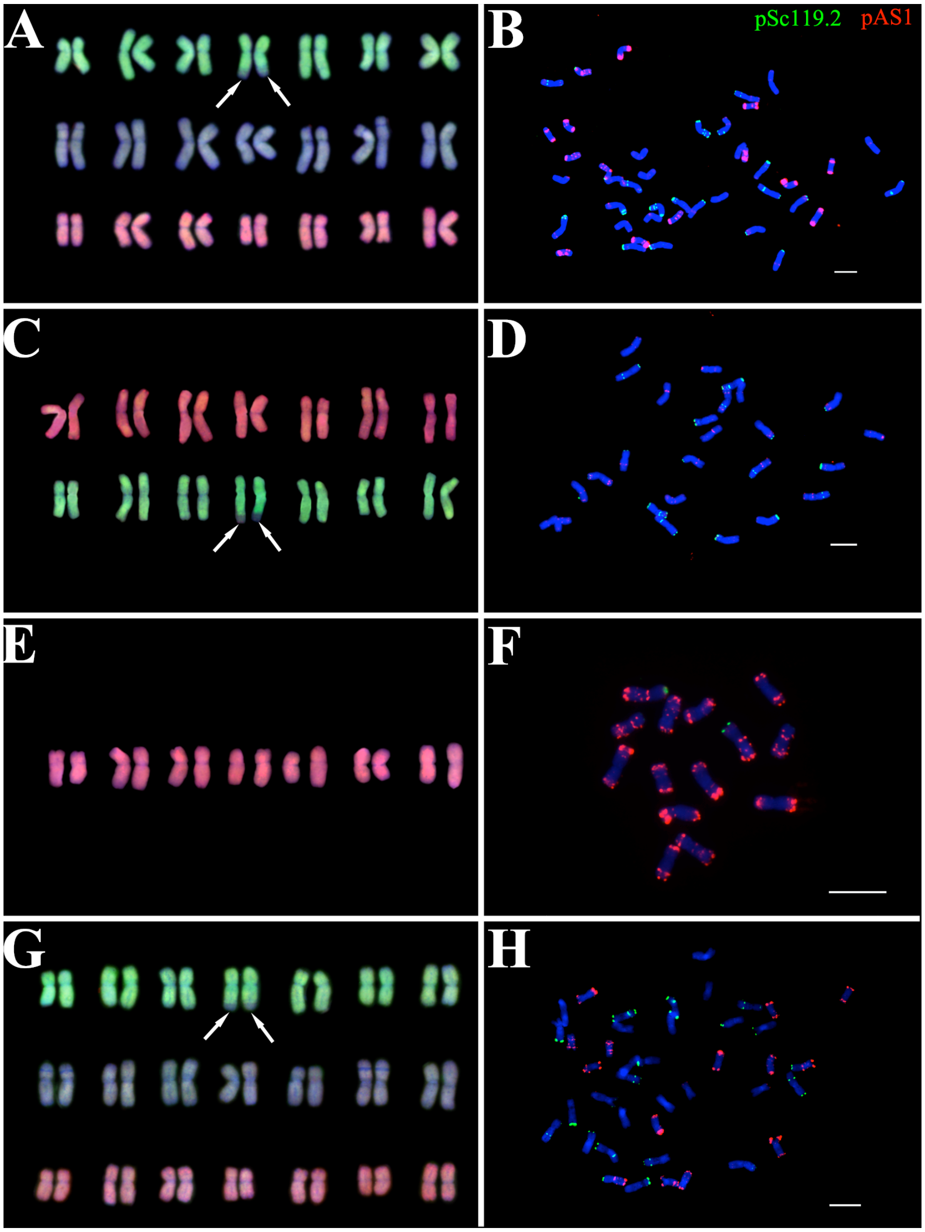
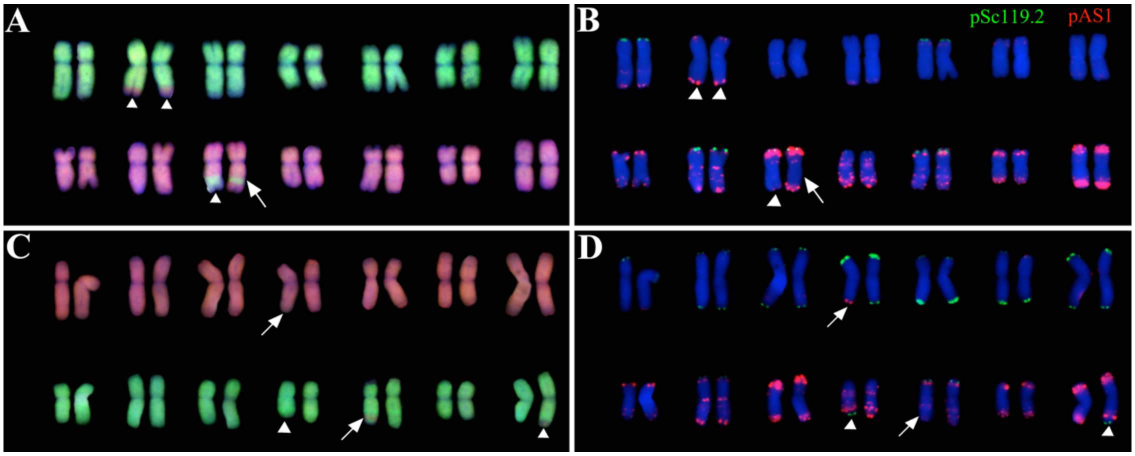
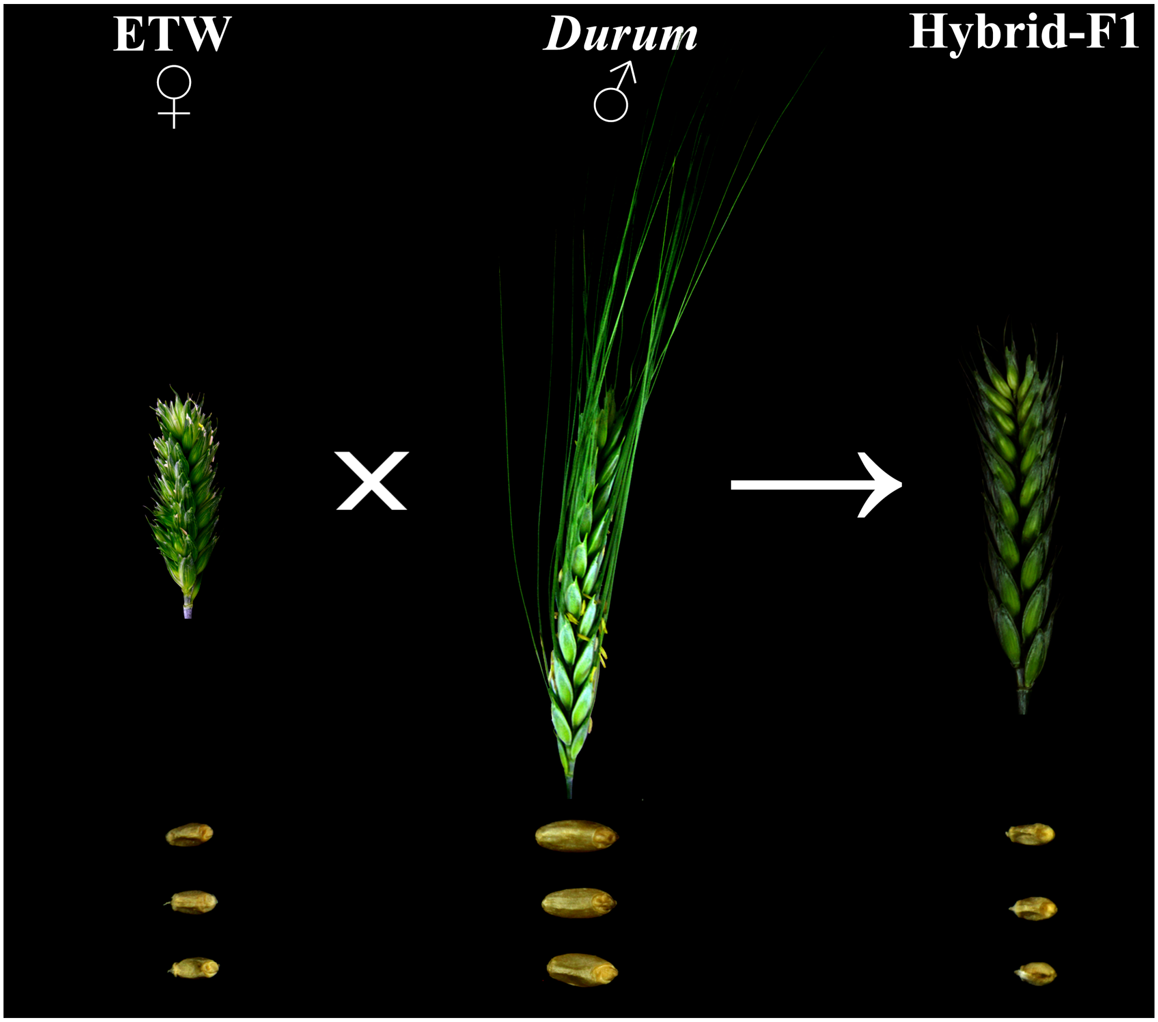


Supplemental Figure 1. Sequential FISH and GISH-based karyotypes of common wheat (line TAA10), ETW, *Aegilops tauschii* (line TQ18) and resynthesized allohexaploid wheat XX329.
(A) and **(B)**, **(C)** and **(D)**, **(E)** and **(F)**, and **(G)** and **(H)** are representative FISH and GISH images of TAA10, Extracted tetraploid wheat (ETW), *Ae. tauschii* and XX329, respectively. The FISH/GISH results indicated that intergenomic gross structural rearrangements did not occur in ETW and XX329. Arrows denote the species-specific cyclic translocation of 4A-5A-7B in emmer and bread wheats, which has been preserved in ETW during the extraction process, and also in the resynthesized hexaploid wheat, XX329.The two repetitive DNA probes, pSc119.2 (green) and pAS1 (red) used to diagnose each homologous chromosome pairs are indicated. Scale bars = 10µm.

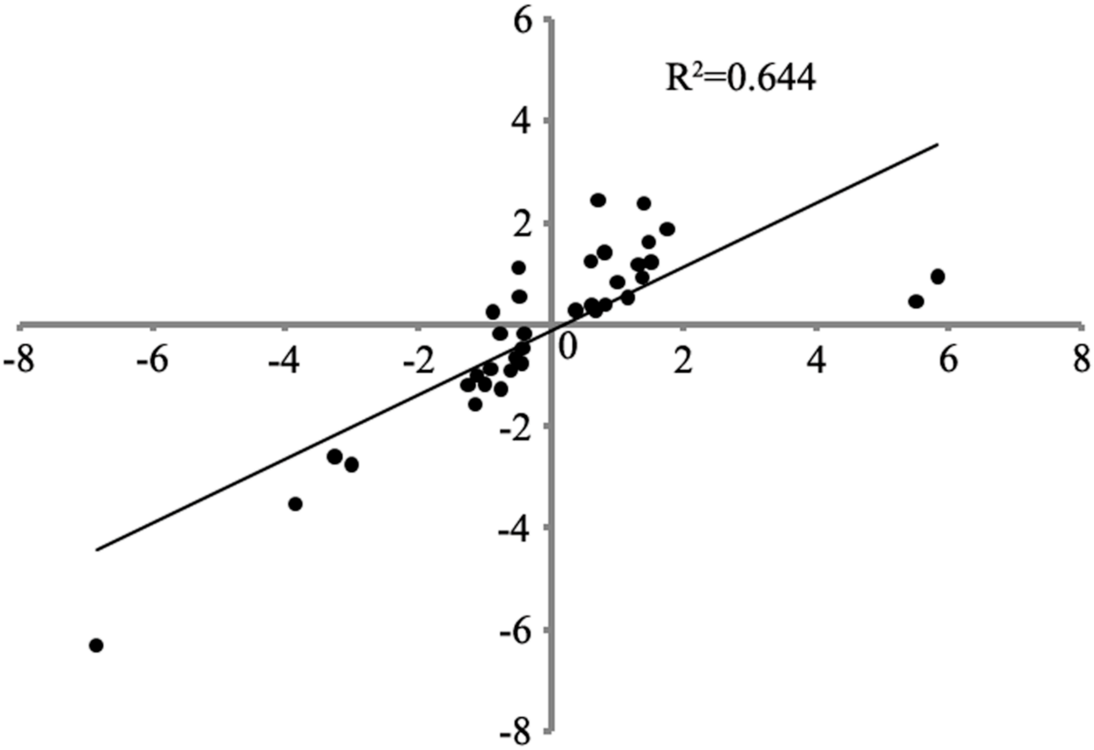


Supplemental Figure 2. Examples of inter-subgenome rearrangements revealed by FISH/GISH-based karyotyping of two newly synthesized allotetraploid wheat lines with genome combinations AADD (*T. urartu* x *Ae. tauschii*) and SSDD (*Ae. bicornis* x *Ae. tauschii*), respectively.
(A) / **(B)** and **(C)** / **(D)** are representative FISH/GISH images of AADD and SSDD, respectively. Triangles and arrows denote terminal and interstitial intergenomic rearrangements, respectively, which can be either reciprocal translocation or unidirectional homeologous segment transfer. The two repetitive DNA probes, pSc119.2 (green) and pAS1 (red) used to diagnose each homologous chromosome pairs are indicated.

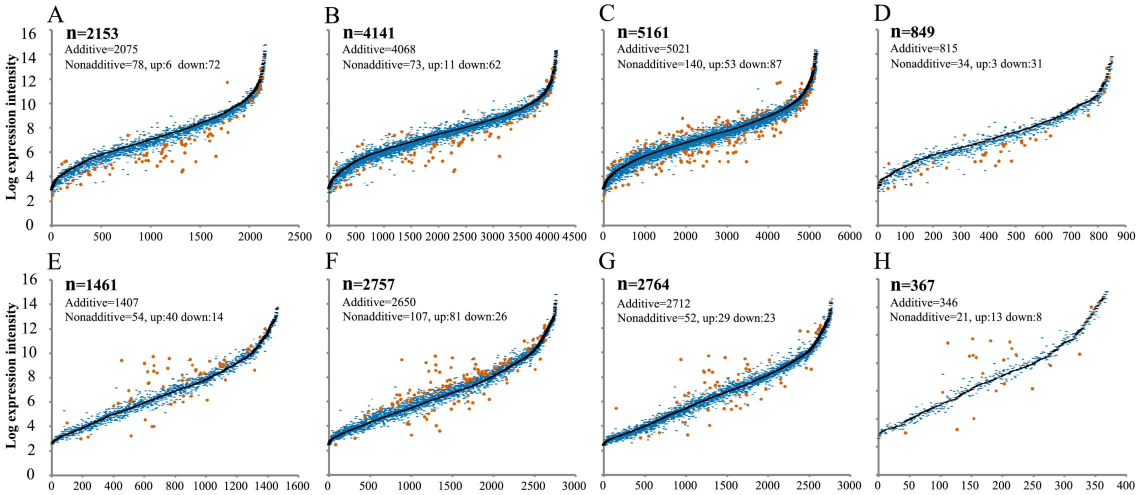


Supplemental Figure 3. Typical spike morphology of ETW, *T. turgidum*, ssp. *durum* (cv. TTR13) and their F1 hybrid.
Spikes of ETW are compacted, highly sterile and awn-less. Spikes of *durum* have long awns. Spikes of the F1 hybrid have short awns, are significantly larger and fully fertile.

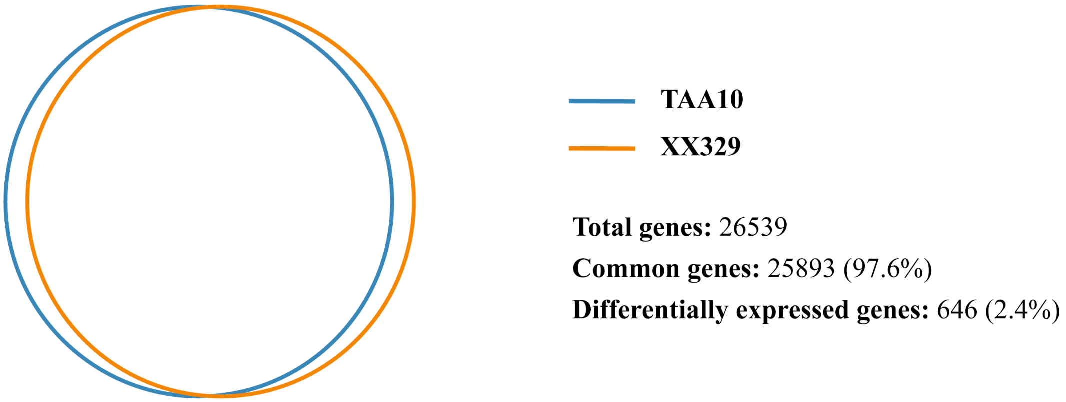
Supplemental Figure 4. Representative segregated spike-shapes in F2 progenies of a cross between ETW and *T. turgidum*, ssp. *durum* (cv. TTR13).



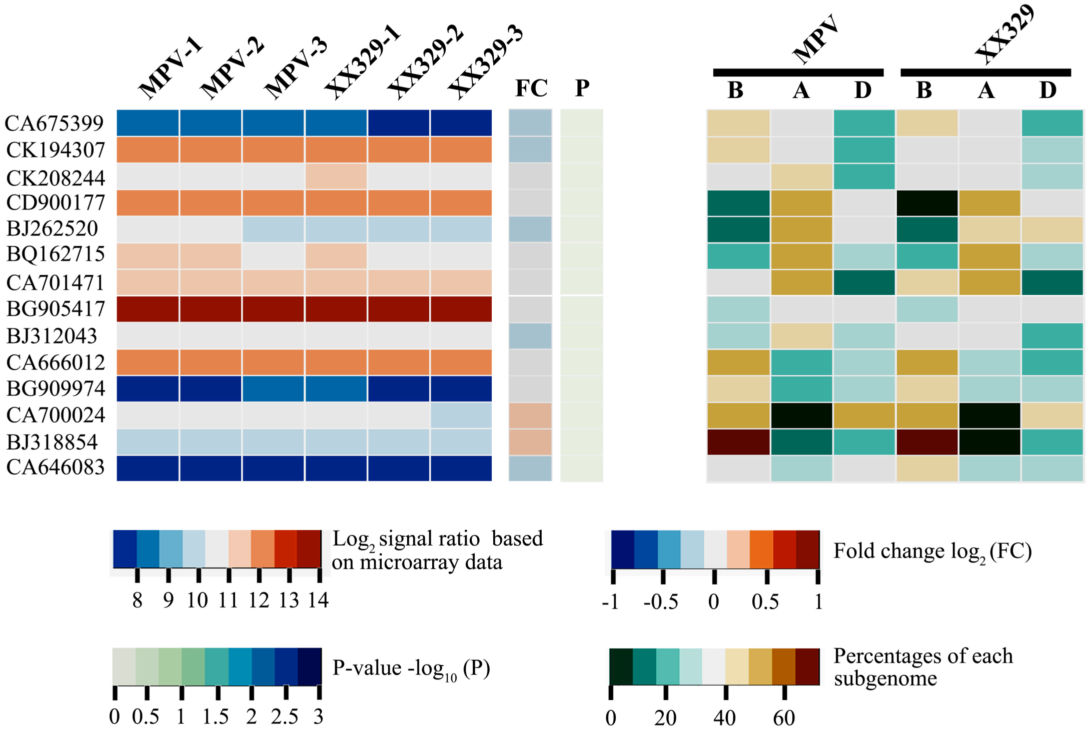
Supplemental Figure 5. Validation of the microarray data by qRT-PCR. The level of differential expression (log2 scale) for a set of 36 comparisons (25 selected genes, **Supplemental Dataset 1 online**) in ETW relative to at least one of the three subspecies (*durum*, *carthlicum* and *dicoccoides*) of *T. turgidum* was calculated according to the qRT-PCR data and plotted against that calculated based on the microarray data.



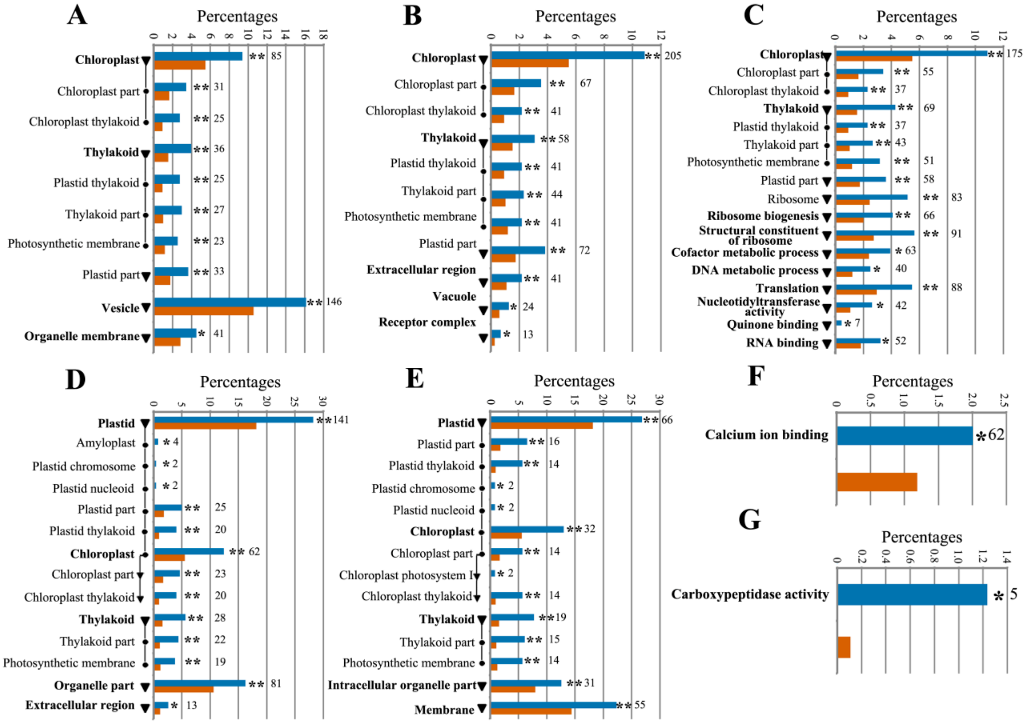
Supplemental Figure 6. Graphical distribution of gene expression patterns in the resynthesized allohexaploid wheat XX329 relative to their corresponding mid-parent values (MPVs) for ETW *vs.* natural allotetraploid up- and down-regulated genes.
Gene expression levels (probe hybridization intensity values of the microarray data) are presented as log-ratios of normalized data, obtained for each transcript in the leaf tissue of XX329 (blue) and their corresponding MPVs (black). Genes that showed nonadditive expression in XX329 were presented as vermilion dots. Genes were ordered by their normalized expression levels in MPVs (black curves). The expression patterns in XX329 for ETW *vs.* each or all three subspecies of *T. turgidum* up- or down regulated genes are presented. **(A)** ETW vs. *durum* up-regulated genes in XX329; **(B)** ETW *vs. carthlicum* up-regulated genes; **(C)** ETW *vs. dicoccoides* up-regulated genes; **(D)** ETW *vs. durum*, *carthlicum* and *dicoccoides* up-regulated genes; **(E)** ETW *vs. durum* down-regulated genes; **(F)** ETW *vs. carthlicum* down-regulated genes; **(G)** ETW *vs. dicoccoides* down-regulated genes; and **(H)** ETW *vs. durum*, *carthlicum* and *dicoccoides* down-regulated genes.



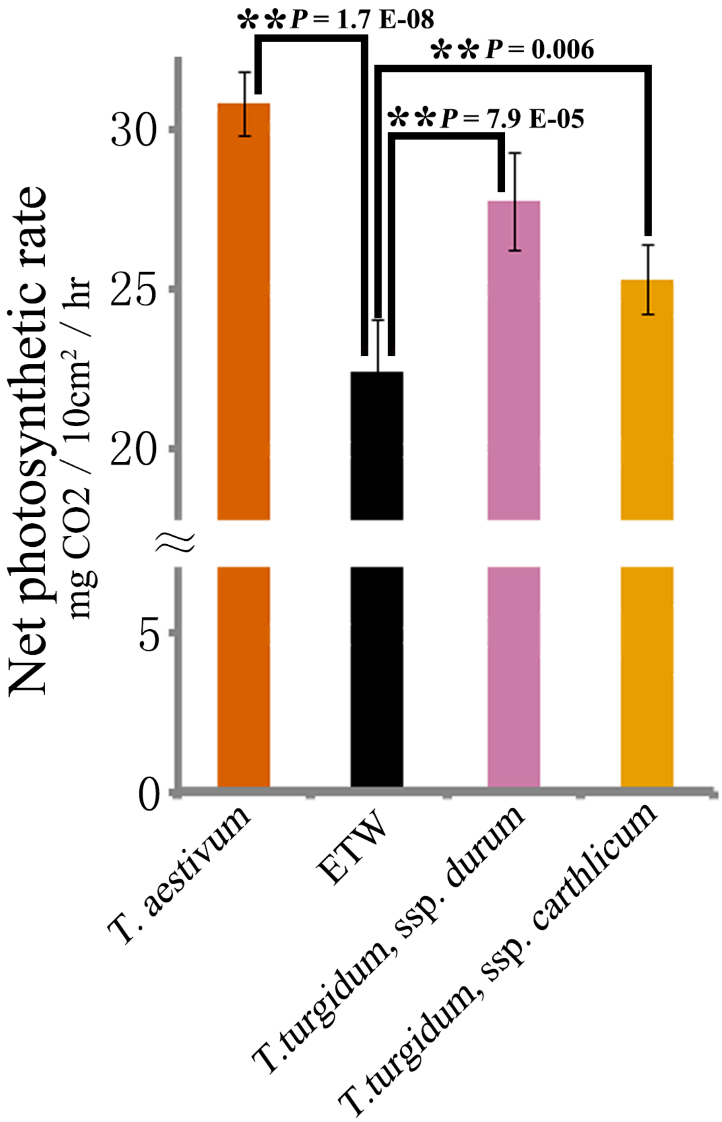
Supplemental Figure 7. Overall gene expression similarity between TAA10 (the bread wheat donor to ETW) and the resynthesized hexaploid wheat XX329 (parented by ETW) based on the Affymetrix GeneChip Wheat Genome Array with three biological replicates for each genotype.



Supplemental Figure 8. Dissecting subgenome contribution to each of 14 selected genes (Supplemental Dataset 2 online) that showed additive expression in the resynthesized allohexaploid wheat XX329 (equal expression levels between XX329 and its parental cDNA mixes: MPVs, based on the microarray data) by gene-specific cDNA-pyrosequencing. Collective transcript levels for each gene based on the microarray data of all three biological replications of XX329 and three independent MPVs (ETW: *Ae. tauschii* = 2:1) are shown (*left panel*). Additive expression for each gene was reflected by their insignificant (FDR, $P > 0.05$) fold changes (FC) between XX329 and its MPVs. The relative transcript contribution by the B, A and D subgenomes for each gene was calculated based on mean ratios of pyrosequencing data of three biological replications (*right panel*) using the same cDNAs as for microarray analysis. Possible altered transcript ratios among the B, A and D subgenomes for each gene in XX329 *vs.* MPVs was determined by t test, and no alteration was detected ($P < 0.05$). The color keys are indicated.

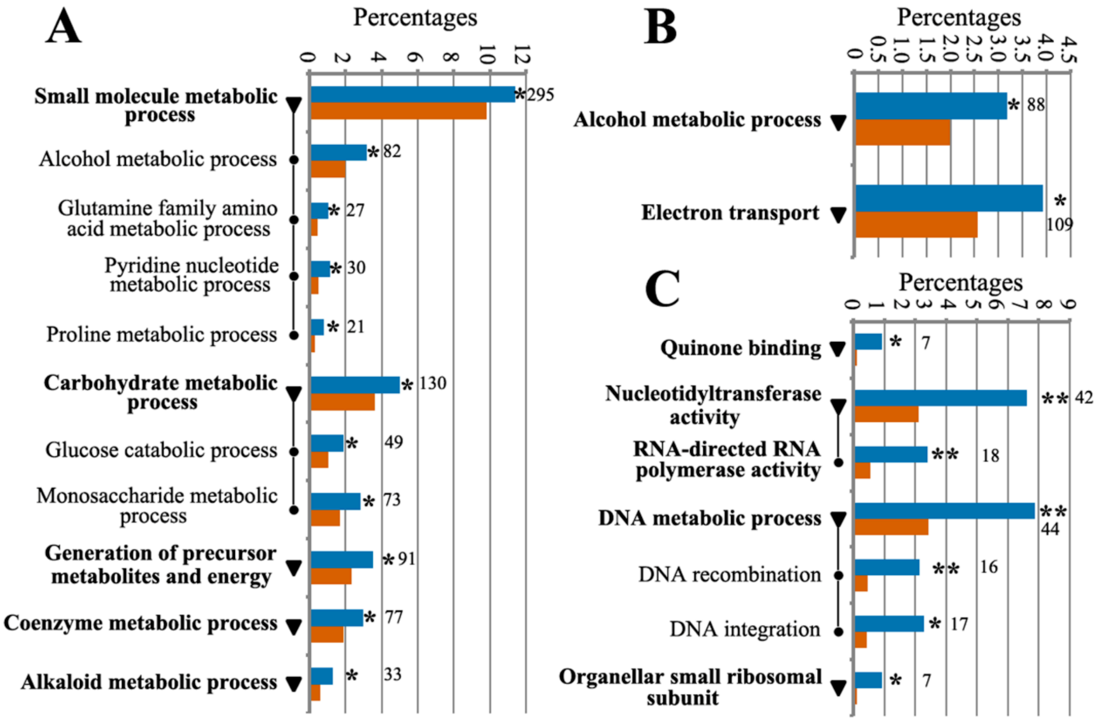


Supplemental Figure 9. Gene ontology (GO) enrichment (> 50%) for genes that are differentially expressed between ETW and the three natural subspecies of *T. turgidum*, *durum* (cv. TTR13), *carthlicum* (cv. Blackbird) and *dicoccoides* (line TD265) (blue bars). The *x*-axis is the GO category terms, and the *y*-axis is the percentages of genes mapped by the GO category terms, which are calculated by the number of genes mapped to a given GO category divided by the number of all mapped genes (Vermilion bars denote the percentages of each GO category in all expressed genes, 26,539 in total). Values above the blue bars indicate the numbers of annotated genes in the specific GO categories. * and ** denote statistical significance of FDR P < 0.05 and P < 0.01, respectively. **(A)**, **(B)**, **(C)**, **(D)**, **(E)**, **(F)** and **(G)** are respectively ETW *vs. durum* down-regulated genes, ETW *vs. carthlicum* down-regulated genes, ETW *vs. dicoccoides* down-regulated genes, ETW *vs.* both *durum* and *carthlicum* down-regulated genes, ETW *vs.* all three natural subspecies of *T. turgidum* down-regulated genes, ETW *vs. dicoccoides* up-regulated genes, and ETW *vs.* all three natural subspecies of *T. turgidum* up-regulated genes. Note that no enrichment was observed for the following categories of genes: ETW *vs. durum* up-regulated genes and ETW *vs. carthlicum* up-regulated genes.



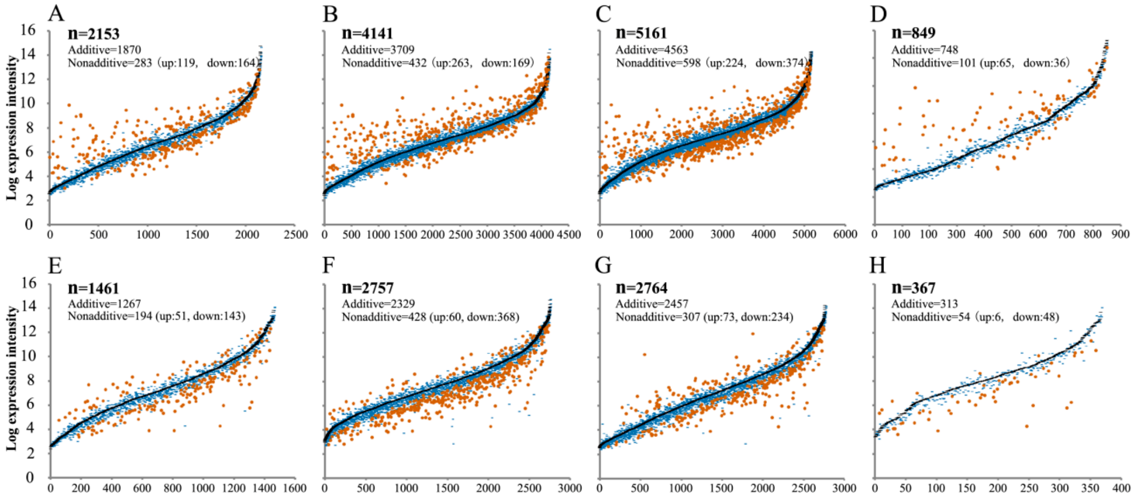
Supplemental Figure 10. Net photosynthetic rate in ETW relative to its bread wheat donor (line TAA10), and the two domesticated natural subspecies of *T. turgidum*, *durum* (cv. TTR13) and *carthlicum* (cv. Blackbird).

Error bars, mean ± s.e. of more than five individual plants measurements. ** denote statistical significance by t test ($P < 0.01$).

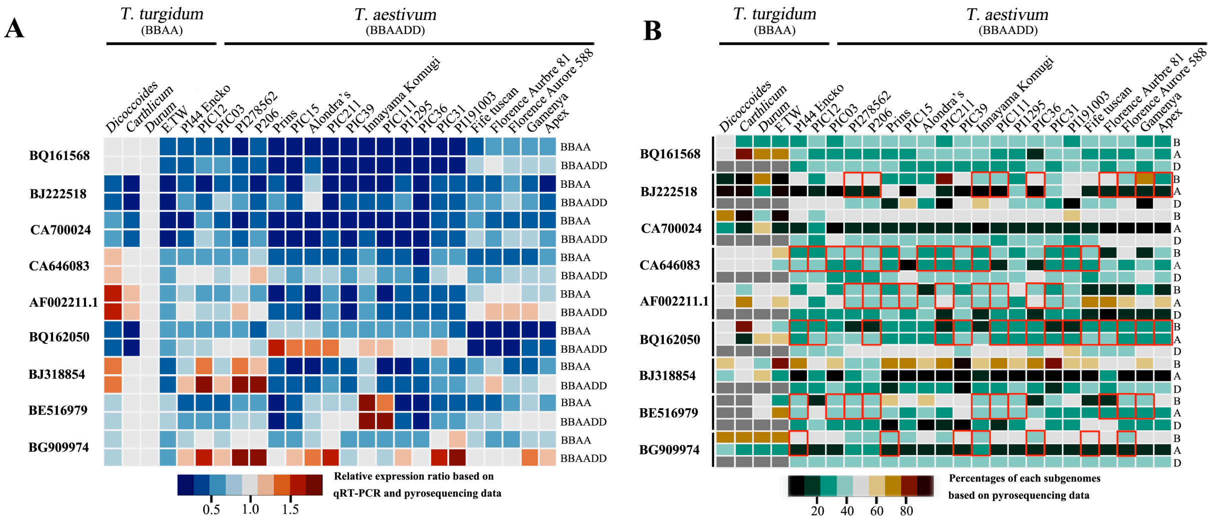


Supplemental Figure 11. Gene ontology (GO) enrichment (>50%) analysis for genes that are differentially expressed between any two of the three natural subspecies of *T. turgidum*, *durum* (cv. TTR13), *carthlicum* (cv. Blackbird) and *dicoccoides* (line TD265) (blue bars).

The *x*-axis is the GO category terms, and the *y*-axis is the percentages of genes mapped by the GO category terms, which are calculated by the number of genes mapped to a given GO category divided by the number of all mapped genes. Vermilion bars denote the percentages of each GO category in all expressed genes (26,539 in total). Values above the blue bars indicate the numbers of annotated genes in the specific GO categories. * and ** denote statistical significance of FDR $P < 0.05$ and $P < 0.01$, respectively. **(A)**, **(B)** and **(C)** are respectively *durum vs. dicoccoides* up-regulated genes, *carthlicum vs. dicoccoides* up-regulated genes, and *durum vs. dicoccoides* down-regulated genes. No enrichment was found for the following categories of genes: *carthlicum vs. durum* up-regulated genes, *carthlicum vs. durum* down-regulated genes, and *carthlicum vs. dicoccoides* down-regulated genes.



Supplemental Figure 12. Graphical distribution of gene expression patterns in the newly synthesized allohexaploid wheat Allo960 relative to their corresponding mid-parent values (MPVs) for the ETW *vs.* natural allotetraploid wheat up- and down-regulated genes.
Gene expression levels (probe hybridization intensity values of the microarray data) are presented as log-ratio of normalized data, obtained for each transcript in the leaf tissue of Allo960 (blue) and their corresponding MPVs (black). Genes that showed nonadditive expression in Allo960 were presented as vermilion dots. Genes were ordered by their normalized expression levels in MPVs (black curves). The expression patterns in Allo960 for ETW *vs.* each or all three subspecies of *T. turgidum* up- or down regulated genes are presented. **(A)** ETW vs. *durum* up-regulated genes; **(B)** ETW *vs. carthlicum* up-regulated genes; **(C)** ETW *vs. dicoccoides* up-regulated genes; **(D)** ETW *vs. durum*, *carthlicum* and *dicoccoides* up-regulated genes; **(E)** ETW *vs. durum* down-regulated genes; **(F)** ETW *vs. carthlicum* down-regulated genes; **(G)** ETW *vs. dicoccoides* down-regulated genes; and **(H)** ETW *vs. durum*, *carthlicum* and *dicoccoides* down-regulated genes.



Supplemental Figure 13. Clustering of expression of nine ETW vs. *durum* (cv. TTR13) down-regulated genes in 21 bread wheat cultivars of diverse origins (Supplemental Dataset 2 online) as collective transcripts for each gene based on qRT-PCR.
(A) and relative transcript contribution by the B, A and D subgenomes for each gene based on cDNA pyrosequencing data **(B)**. Expression of these genes in the other two tetraploid subspecies, *dicoccoides* (line TD265) and *carthlicum* (cv. Blackbird) were also included for reference. For each gene the collective expression of only the BBAA subgenome (proportion calculated based on pyrosequencing, **B**) and all three subgenomes in each bread wheat cultivar were shown **(A)**. Equal or differential expression between the BBAA subgenome of each bread wheat cultivar and *durum* (cv. TTR13) for each of the ten analyzed genes were determined by t test, and most of them were significantly down-regulated in 21 bread wheat cultivars. The relative transcript contribution by the B, A and D subgenomes for each gene is calculated based on mean ratios of pyrosequencing data using the same cDNAs as for qRT-PCR analysis. Concordant or independent changes to the B and A subgenome transcripts were determined according to statistical insignificant (t test, $P > 0.05$) or significant (t test, $P < 0.05$) changes of the ratios of relative expression by the B and A homeologues between each bread wheat cultivar and *durum* (cv. TTR13), and concordant changes are marked by red rectangles. The color keys are indicated.

Supplemental Table 1. Differentially expressed genes between ETW and each or all the three subspecies, *durum* (cv. TTR13), *carthlicum* (cv. Blackbird) and *dicoccoides* (line TD265), of natural allotetraploid wheat *Triticum turgidum*, based on the Affymetrix Wheat Genome Array data.

Pairwise comparison	Total differentially expressed genes	Up-regulated	Down-regulated	P-value
ETW *vs. durum*	3614 (13.6%[a])	2153 (59.6%[b])	1461 (40.4%[b])	9.36 e-31
ETW *vs. carthlicum*	6898 (26.0% [a])	4141 (60.0%[b])	2757 (40.0 %[b])	1.14 e -62
ETW *vs. dicoccoides*	7925 (29.9%[a])	5161 (65.1%[b])	2764 (34.9%[b])	4.82 e -162
ETW *vs. durum* & *carthlicum*	1941 (7.3% [a])	1201 (61.9%[b])	740 (38.1%[b])	9.55 e -26
ETW *vs. durum* & *dicoccoides*	1838 (6.9% [a])	1284 (69.9%[b])	554 (30.1%[b])	1.33 e -66
ETW *vs. carthlicum* & *dicoccoides*	3016 (11.4% [a])	2166 (71.8%[b])	850 (28.2%[b])	5.51 e -131
ETW *vs. durum*, *carthlicum* & *dicoccoides*	1216 (4.6% [a])	849 (69.8%[b])	367 (30.2%[b])	1.97 e -44
durum vs. carthlicum	4696 (17.7%[a])	2424 (51.6%[b])	2272 (48.4%[b])	0.0275
durum vs. dicoccoides	6823 (25.7%[a])	4096 (60.0%[b])	2727 (40.0%[b])	5.20 e -62
carthlicum vs. dicoccoides	7206 (27.2%[a])	4190 (58.1%[b])	3016 (41.9%[b])	1.29 e -43
durum & *carthlicum vs. dicoccoides*	3908 (14.7%[a])	2376 (60.8%[b])	1532 (39.2%[b])	9.35 e -42

[a] percentages of all expressed genes; [b] percentages of total differentially expressed genes.

p-value: Binomial test p-value of the up and down regulated genes of the pairwise comparisons.

Supplemental Table 2. Expression pattern of the up- and down-regulated genes between ETW and each of the three subspecies, *durum* (cv. TTR13), *carthlicum* (cv. Blackbird) and *dicoccoides* (line TD265), as well as between any two of the three natural tetraploid wheat species, in a resynthesized allohexaploid wheat XX329.

Pairwise comparison	Total analyzed genes	Additive	Nonadditive (Total)	Nonadditive (up-regulated)	Nonadditive (down-regulated)
ETW *vs. durum* up-regulated genes	2153 (8.1% [a])	2075 (96.4% [b])	78 (3.6% [b])	6 (7.7% [c])	72 (92.3% [c])
ETW *vs. carthlicum* up-regulated genes	4141 (15.6% [a])	4068 (98.2% [b])	73 (1.8% [b])	11 (15.1% [c])	62 (84.9% [c])
ETW *vs. dicoccoides* up-regulated genes	5161 (19.4% [a])	5021 (97.3% [b])	140 (2.7% [b])	53 (37.9% [c])	87 (62.1% [c])
ETW *vs. durum* down-regulated genes	1461 (5.5% [a])	1407 (96.3% [b])	54 (3.7% [b])	40 (74.1% [c])	14 (25.9% [c])
ETW *vs. carthlicum* down-regulated genes	2757 (10.4% [a])	2650 (96.1% [b])	107 (3.9% [b])	81 (75.7% [c])	26 (24.3% [c])
ETW *vs. dicoccoides* down-regulated genes	2764 (10.4% [a])	2712 (98.1% [b])	52 (1.9% [b])	29 (55.8% [c])	23 (44.2% [c])
ETW *vs. durum, carthlicum* & *dicoccoides* up-regulated genes	849 (3.2% [a])	815 (96.0% [b])	34 (4.0% [b])	3 (8.8% [c])	31 (91.2% [c])
ETW *vs. durum, carthlicum* & *dicoccoides* down- regulated genes	367 (1.4% [a])	346 (94.3% [b])	21 (5.7% [b])	13 (61.9% [c])	8 (38.1% [c])

[a] percentages of all expressed genes; [b] percentages of all specially up- or down-regulated genes; [c] percentages of all nonadditive genes.

Supplemental Table 3. Expression pattern of the up- and down-regulated genes between ETW and each of the three subspecies, *durum* (cv. TTR13), *carthlicum* (cv. Blackbird) and *dicoccoides* (line TD265), as well as between any two of the three natural tetraploid wheat species, in a newly synthesized allohexaploid line Allo960.

Pairwise comparison	Total analyzed genes	Additive	Nonadditive (Total)	Nonadditive (up-regulated)	Nonadditive (down-regulated)
ETW *vs.* *durum* up-regulated genes	2153 (8.1% [a])	1870 (86.9% [b])	283 (13.1% [b])	119 (42.9% [c])	164 (58.0% [c])
ETW *vs.* *carthlicum* up-regulated genes	4141 (15.6% [a])	3709 (89.6% [b])	432 (10.4%)	263 (60.9% [c])	169 (39.1% [c])
ETW *vs.* *dicoccoides* up-regulated genes	5161 (19.4% [a])	4563 (88.4% [b])	598 (11.6% [b])	224 (37.5% [c])	374 (62.5% [c])
ETW *vs.* *durum* down-regulated genes	1461 (5.5% [a])	1267 (86.7% [b])	194 (13.3% [b])	51 (26.3% [c])	143 (73.7% [c])
ETW *vs.* *carthlicum* down-regulated genes	2757 (10.4% [a])	2329 (84.5% [b])	428 (15.5% [b])	60 (14.0% [c])	368 (86.0% [c])
ETW *vs.* *dicoccoides* down-regulated genes	2764 (10.4% [a])	2457 (88.9% [b])	307 (11.1% [b])	73 (23.8% [c])	234 (76.2% [c])
ETW *vs.* *durum*, *carthlicum* & *dicoccoides* up-regulated genes	849 (3.2% [a])	748 (88.1% [b])	101 (11.9% [b])	65 (64.4% [c])	36 (35.6% [c])
ETW *vs.* *durum*, *carthlicum* & *dicoccoides* down regulated genes	367 (1.4% [a])	313 (85.3% [b])	54 (14.7% [b])	6 (11.1% [c])	48 (88.9% [c])
Allo960 *vs*. MPV	26539	24040 (90.6% [d])	2499 (9.4% [d])	828 (33.1% [c])	1671 (66.9% [c])

[a] percentages of all expressed genes; [b] percentages of all specially up- or down-regulated genes; [c] percentages of all nonadditive genes; percentages of all expressed genes.