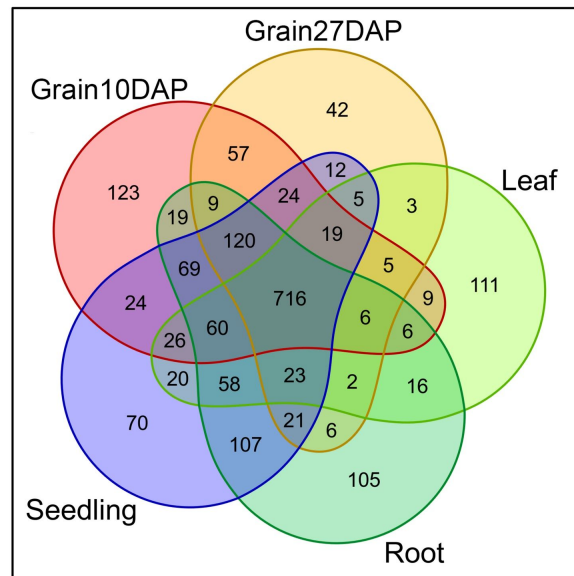


Additional File 2. Supplementary Figures and Tables

A



B

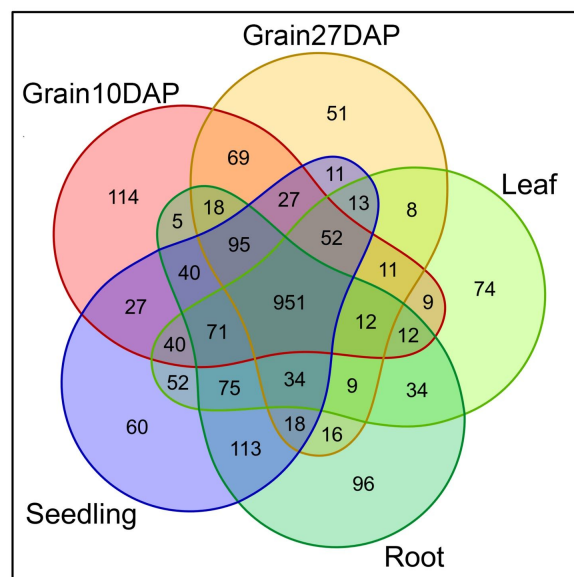
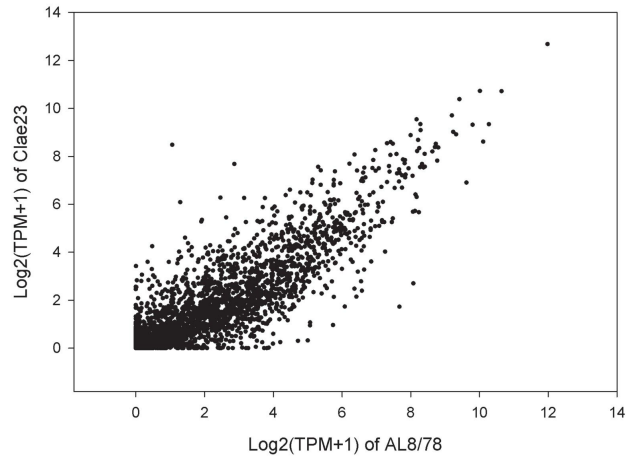


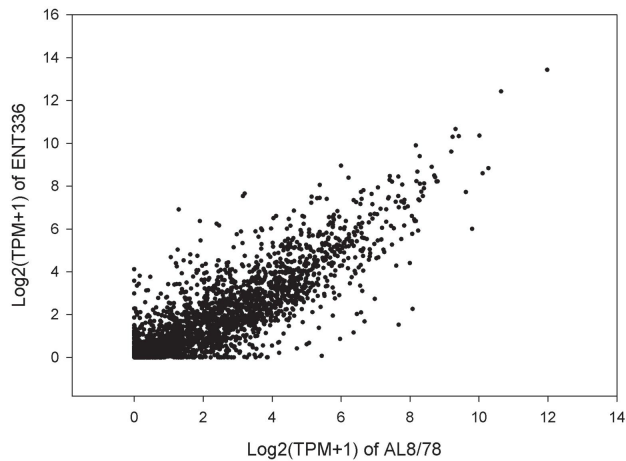
Figure S1 Expression profiles of syntenic genes of *Triticum aestivum* Paragon 3DL and *Aegilops tauschii* AL8/78 3L in five tissues.

Syntenic genes expressed with TPM $\geq$ 1 from wheat 3DL (1,893 genes, **A**) and *Ae. tauschii* chr3L (2,217 genes, **B**) among leaf, root, seedling, 10 DAP (Days After Pollination) and 27 DAP developing grain.

**A**

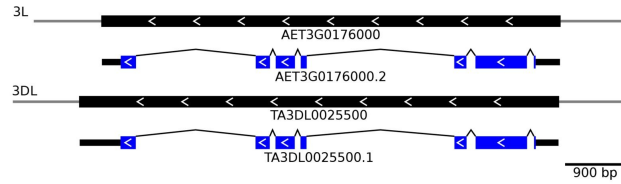
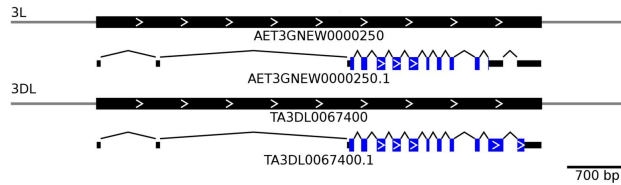
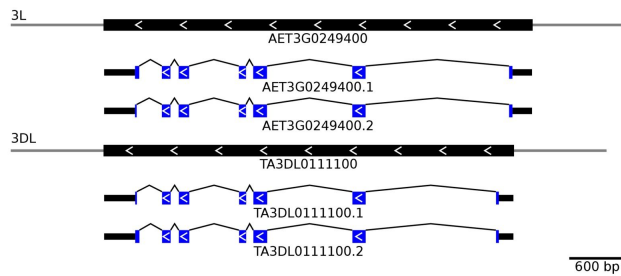
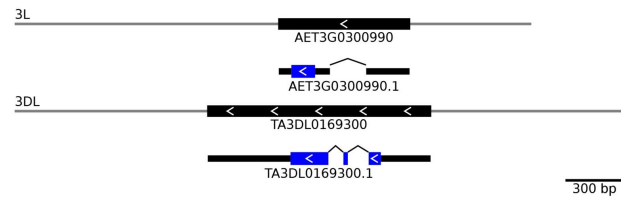
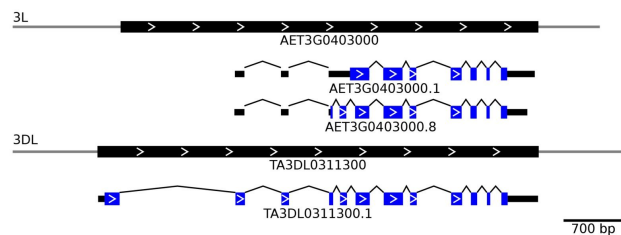


**B**



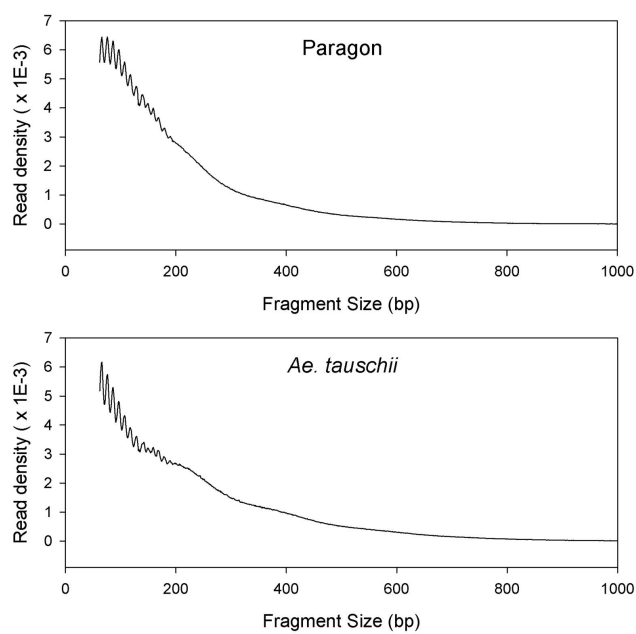
**Figure S2 Comparison of expression levels of between *Aegilops tauschii* varieties AL8/78, Clae23 and ENT336.**

To identify conserved differentially expressed genes (DEGs) between *Triticum aestivum* Paragon and *Aegilops tauschii* AL8/78, RNAseq data was generated from another two *Ae. tauschii* accessions, Clae23 (**A**) and ENT336 (**B**). TPM values showed a strong correlation between the pairs of accessions. ( $R^2 = 0.7964$  for Clae23 and  $0.7932$  for ENT336)

**A****B****C****D****E**

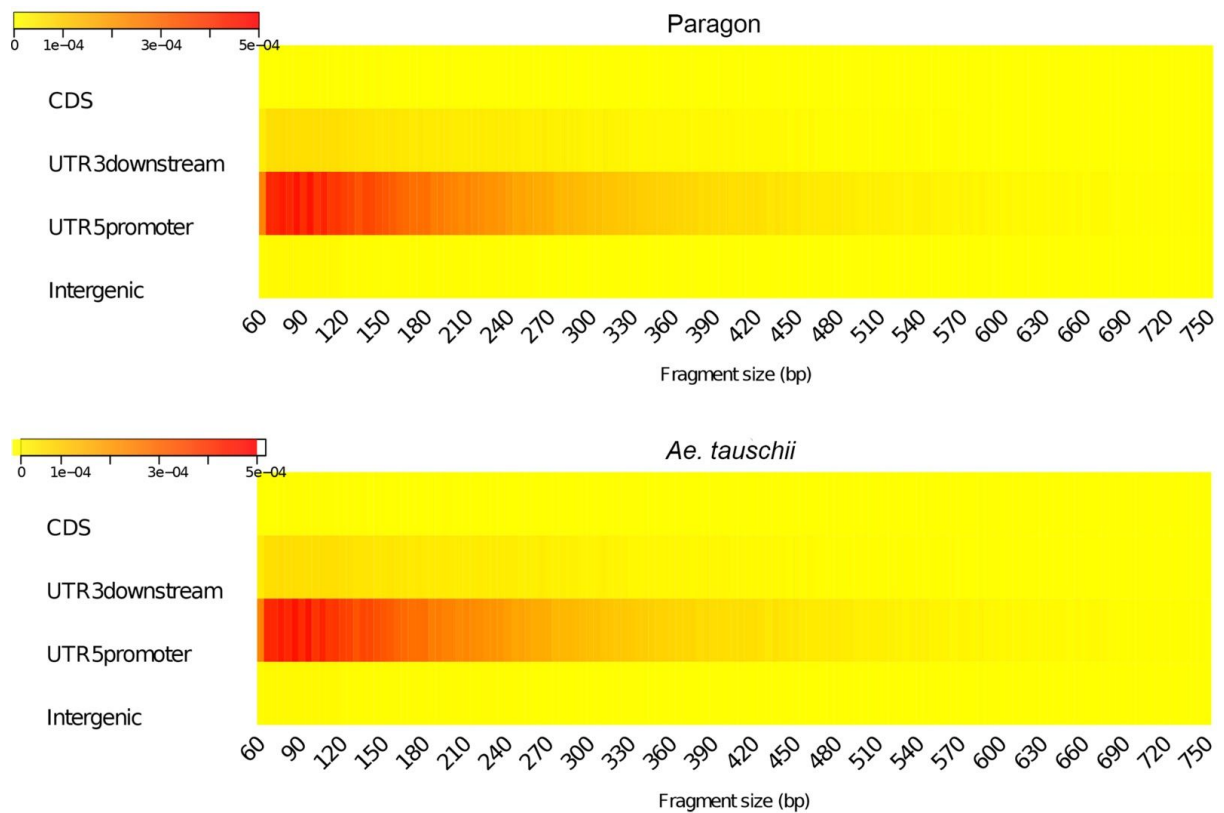
**Figure S3 Illustration of gene structure differences among 106 conserved DEGs between wheat and *Ae. tauschii*.**

**A** An example of wheat TA3DL0025500 with the same structure as its syntenic gene pair in *Ae. tauschii*, and 4 gene pairs with different gene structures: **B** TA3DL0067400 (alternate start codon); **C** TA3DL0111100 (alternate first exon); **D** TA3DL0169300 (exon 2 in *Ae. tauschii* missing); and **E** TA3DL0311300 (*Ae. tauschii* has premature stop codon).



**Figure S4 Size distribution of ATAC-seq fragment lengths between *Triticum aestivum* Paragon and *Aegilops tauschii* AL8/78.**

The 10.5bp DNA pitch reflects the periodicity of right-handed helix in B-DNA. A trace of single and double nucleosome spacing can be seen in the 3L ATAC peaks, at approximately 200bp and 400bp.



**Figure S5 Normalised read enrichments for four classes of chromosome states in *Triticum aestivum* Paragon and *Aegilops tauschii* AL8/78.**

The normalised density plots of ATAC peaks showed that 5'UTR + promoter (2kb) regions had the highest read densities, followed by 3' UTR + downstream (2kb) regions, CDS + intron regions and intergenic regions.

**Table S1 Summary of repetitive elements of *Triticum aestivum* Chinese Spring 3DL and *Aegilops tauschii* AL8/78 3L**

Class	Subclass	<i>Triticum aestivum</i> chr3DL		<i>Aegilops tauschii</i> chr3L	
		Length (bp)	Percentage	Length (bp)	Percentage
<b>DNA transposon</b>		45,694,659	12.29%	47,299,305	12.54%
	CMC-ENSPM	41,305,883	11.11%	42,925,528	11.38%
<b>LINE</b>		1,670,347	0.45%	1,651,400	0.44%
<b>LTR retrotransposon</b>		191,094,703	51.40%	198,840,503	52.70%
	LTR-Copia	56,109,154	15.09%	57,910,706	15.35%
	LTR-Gypsy	103,525,917	27.85%	108,703,865	28.81%
<b>MobileElement</b>		956	0.00%	847	0.00%
<b>RC</b>		58,706	0.02%	73,186	0.02%
<b>rRNA</b>		19,149	0.01%	17,408	0.00%
<b>Simple_repeat</b>		1,493,362	0.40%	1,599,416	0.42%
<b>Low_complexity</b>		336,380	0.09%	345,552	0.09%
<b>Other</b>		20,280,094	5.46%	21,132,447	5.60%
<b>SUM</b>		<b>257806071</b>	<b>69.35%</b>	<b>267937996</b>	<b>71.01%</b>

**Table S2 Number of RNAseq read pairs after trimming in 5 sampled tissues of *Triticum aestivum* Paragon and *Aegilops tauschii* AL8/78**

<b>Tissues</b>	<b>Replicates</b>	<b>Paragon</b>	<b>AL8/78</b>
Leaves	Replicate 1	56,816,727	50,677,603
	Replicate 2	56,452,015	55,377,053
	Replicate 3	38,670,428	57,634,414
Roots	Replicate 1	38,735,310	52,677,010
	Replicate 2	51,869,501	55,016,084
	Replicate 3	50,896,607	53,365,111
Grains (10dd)	Replicate 1	59,835,166	80,624,534
	Replicate 2	66,214,312	77,159,911
	Replicate 3	57,335,248	68,751,597
Grains (27dd)	Replicate 1	55,984,207	88,885,636
	Replicate 2	51,331,203	75,465,851
	Replicate 3	74,165,822	80,256,130
Grains (Pooled)	Replicate 1	63,778,882	60,647,054
Seedling (4dd)	Replicate 1	63,782,697	74,531,986
	Replicate 2	77,936,789	71,175,660
	Replicate 3	75,481,500	57,043,451



**Table S3 Number of differential expressed genes in 5 tissues between *Triticum aestivum* Paragon and *Aegilops tauschii* AL8/78**

	Expressed genes	DEGs	Pseudogenes
Developing grain (10dd)	1,716	277 (up 147, down 130)	44
Developing grain (27dd)	1,516	173 (up 86, down 87)	38
Leaf	1,564	262 (up 112, down 150)	42
Root	1,784	327 (up 176, down 151)	44
Seedling	1,829	251 (up 144, down 107)	47
Sum	2,375	674	66

**Table S4 Mapping statistics after alignment of bisulfite treated *Triticum aestivum* Paragon and *Ae. tauschii* AL8/78 samples**

	<b>Paragon</b>	<b><i>Ae. tauschii</i></b>
Number of genes mapped	2,810	3,997
C sites mapped on genes (min 10X)	846,450	458,347
C sites mapped on 3DL promoters (min 10X)	418,818	289,521
C's methylated overall		
CpG	89.9%	87.1%
CHG	59.4%	53.4%
CHH	3.8%	3.5%
C's methylated genes		
CpG	66.7%	41.2%
CHG	13.2%	18.9%
CHH	1.0%	3.8%
% converted	98.7	98.6

**Table S5** Number of regions for methylation analysis in *Triticum aestivum* Paragon 3DL and *Aegilops tauschii* AL8/78 3L

	<b>Paragon</b>	<b>AL8/78</b>	<b>Comparable regions</b>
Gene CpG	2,378	2,664	1,533
Gene CHG	2,467	2,643	1,586
Gene CHH	2,706	2,932	1,913
Promoter CpG	1,703	2,308	891
Promoter CHG	1,791	2,229	901
Promoter CHH	2,174	2,703	1,353

**Table S6 Summary of ATAC peaks and covered genes in different chromosome states of Paragon wheat and *Ae. tauschii* AL8/78**

	Number of Peaks (Genes covered*)	
	Paragon	AL8/78
5'UTR+promoter	1,266 (1,098 genes)	1,739 (869 genes)
CDS+Intron	53 (42 genes)	171 (130 genes)
3'UTR+downstream	226 (211 genes)	480 (350 genes)
Intergenic	1,425	2,570
Total peaks	2,970 (1,187 genes)	4,960 (936 genes)

\*All genes including non-syntenic genes

**Table S7 Number of syntenic genes covered by ATAC-seq peaks**

	<b>Paragon</b>	<b>AL8/78</b>
Overall gene pairs	930 genes (159 DEGs)	
Total	774 (128 DEGs)	362(88 DEGs)
CDS	25(1 DEGs)	46(13 DEGs)
UTR3downstream	157(27 DEGs)	132(35 DEGs)
UTR5promoter	727(125 DEGs)	345(85 DEGs)
Gene pairs with differential peaks	816 genes (133 DEGs)	
CDS	69 (14 DEGs)	
UTR3downstream	251 (52 DEGs)	
UTR5promoter	684 (98 DEGs)	

**Table S8 Protein sequences used to evidence the exons in wheat chromosome 3DL annotation**

<b>Species</b>	<b>Version</b>	<b>Number</b>	<b>Sources</b>
<i>Aegilops tauschii</i>	GCA_000347335.1	33,928	Ensembl-25
<i>Arabidopsis thaliana</i>	TAIR10	35,386	Ensembl-25
<i>Brachypodium distachyon</i>	v1.0	31,029	Ensembl-25
<i>Oryza sativa</i>	IRGSP-1.0	42,132	Ensembl-25
<i>Sorghium bicolor</i>	Sorbi1	36,338	Ensembl-25
<i>Triticum urartu</i>	GCA_000347455.1	33,483	Ensembl-25
<i>Hordeum vulgare</i>	082214v1	62,311	Ensembl-25
<i>Triticum aestivum</i>	IWGSC2	99,354	Ensembl-25
<i>Triticum aestivum</i>	TriFLDB	43,150	<a href="http://trifldb.psc.riken.jp/v3">http://trifldb.psc.riken.jp/v3</a>
<i>Zea mays</i>	AGPv3	63,235	Ensembl-25