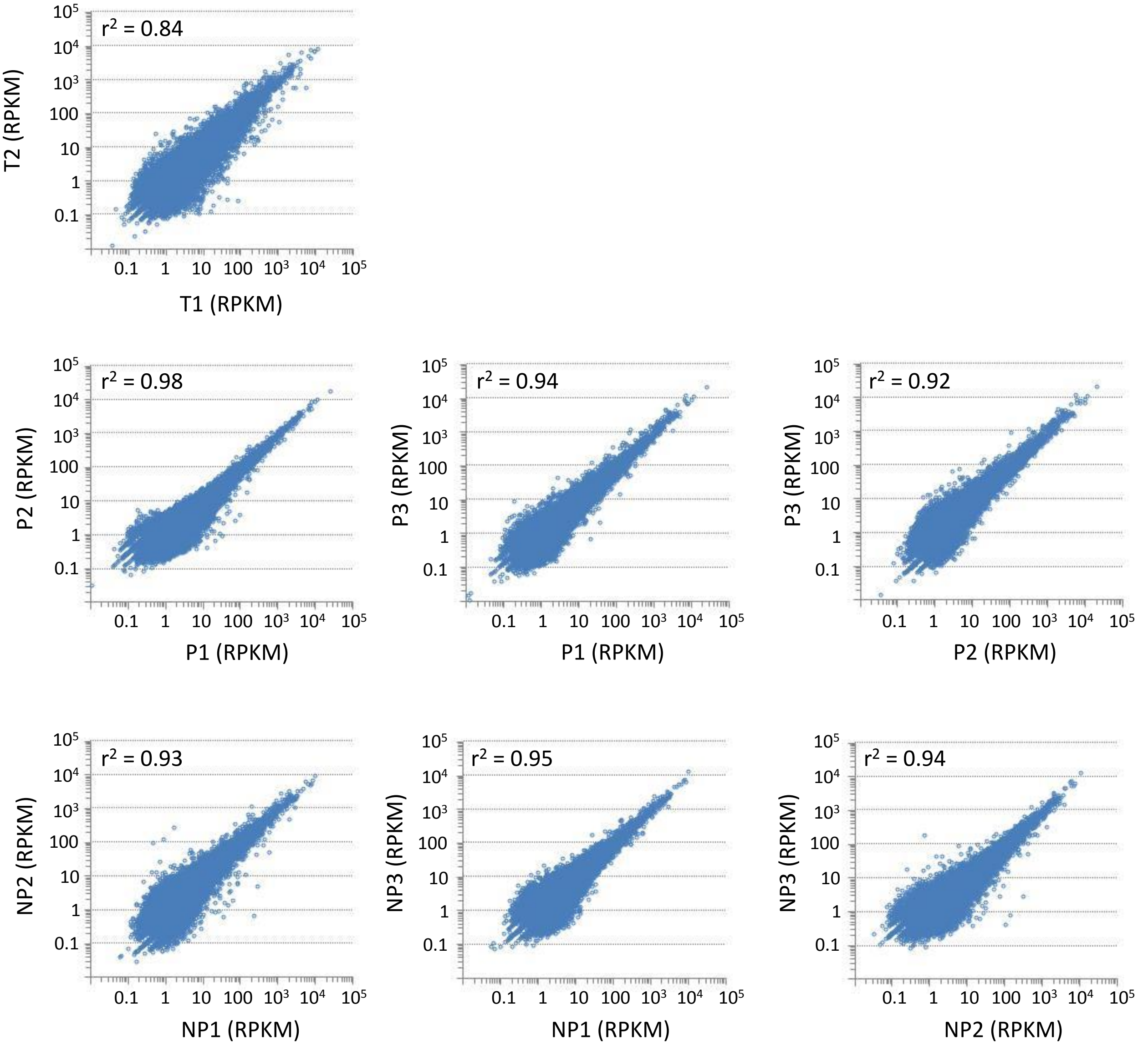
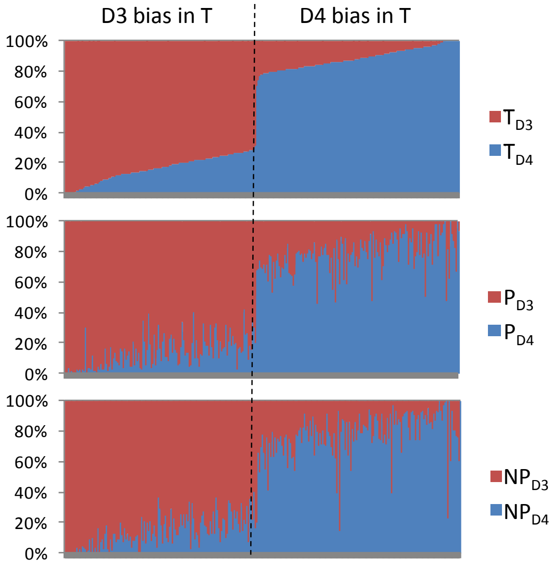
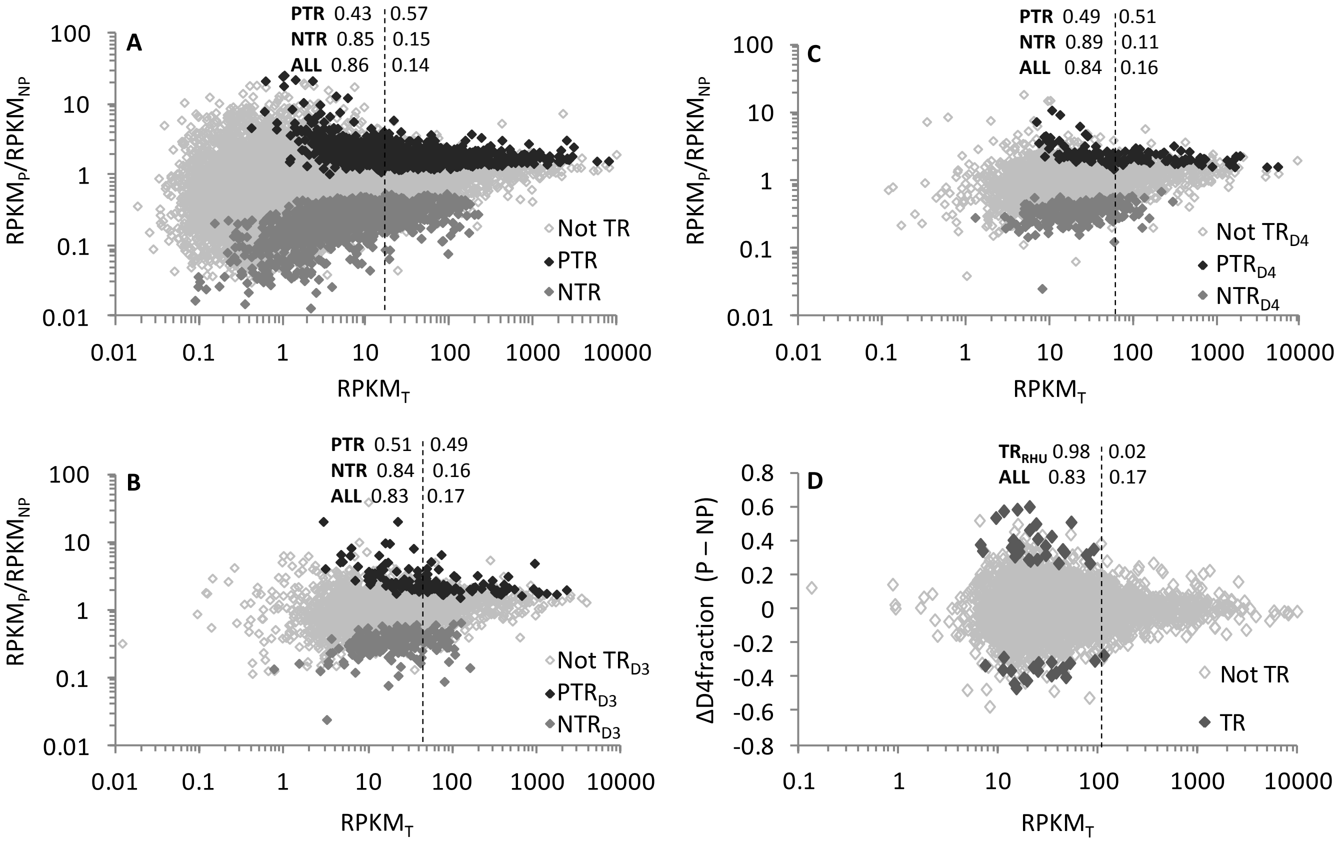


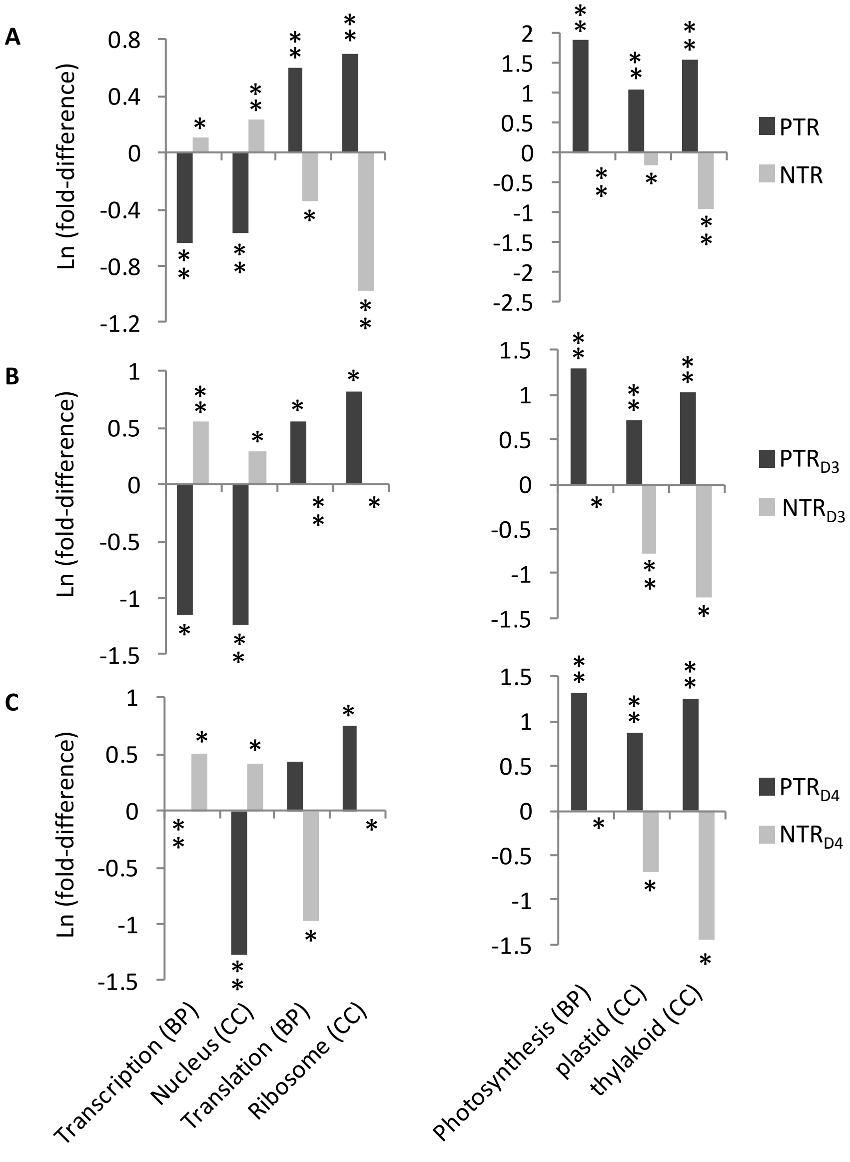
Supplemental Figure 1. Correlations of combined expression estimates among biological replicates within each RNA fraction. Total, T; Polysomal, P; Nonpolysomal; NP.



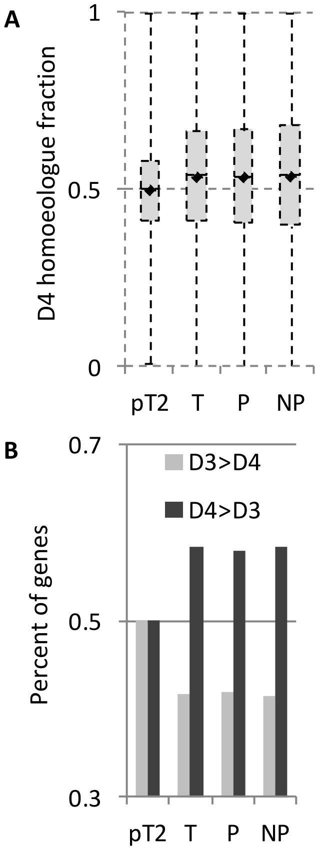
Supplemental Figure 2. Strong transcriptional biases in homoeologue usage are preserved in the translatome. Relative homoeologue usage in the total transcriptome (T), and in the polysomal (P) and non-polysomal (NP) fractions, for genes with biased homoeologue expression in T. The upper chart shows relative homoeologue expression in T for 632 genes with significant homoeologue bias, ranked in order of increasing D4 homoeologue fraction. The second and third charts show relative homoeologue expression in P and NP, respectively, for the same genes, in the same order as in T.



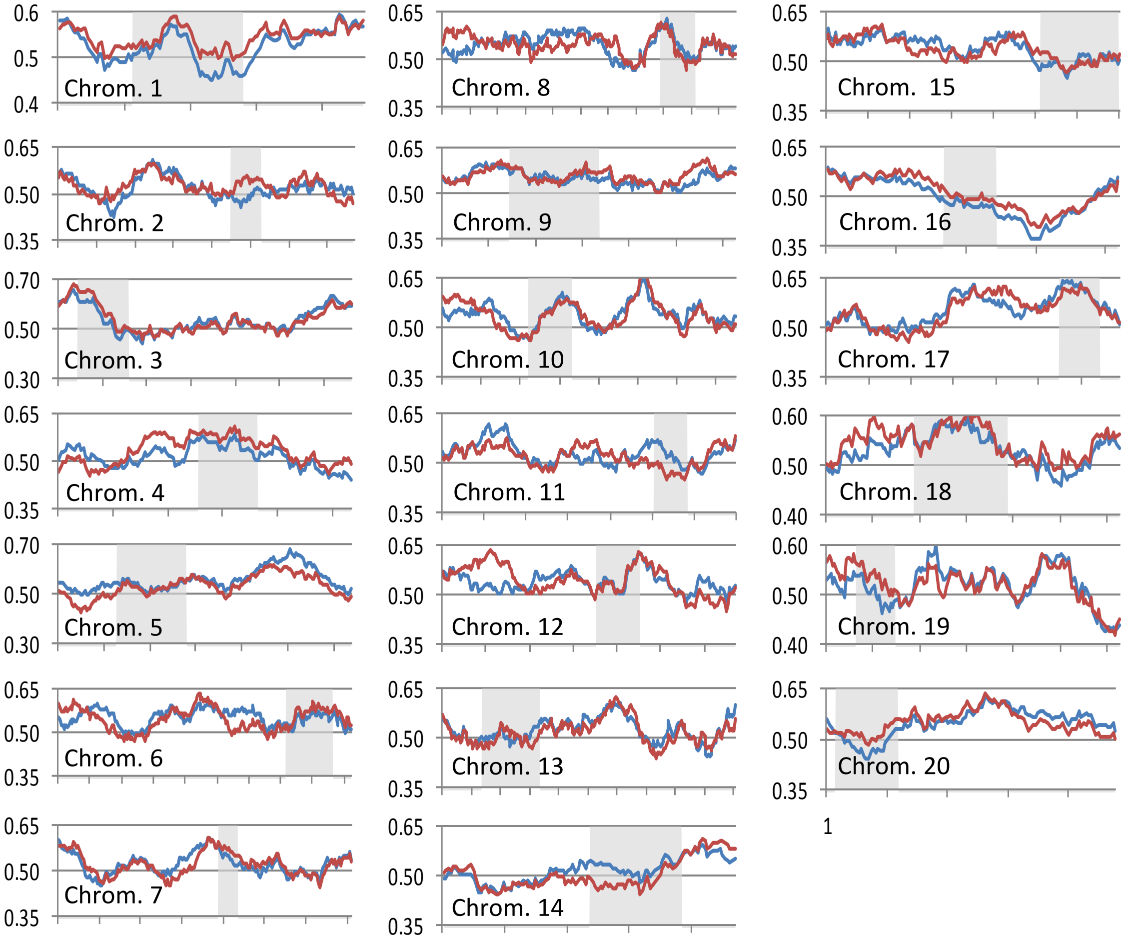
Supplemental Figure 3. Distribution of transcript levels (Transcriptome RPKM, $RPKM_T$; x-axis) for genes that are translationally regulated (black and dark grey diamonds), and those that are not (light grey diamonds). (*A*) Combined expression for all genes expressed in T. (*B*) D3 homoeologue expression for all genes in the homoeologue gene set. (*C*) D4 homoeologue expression for all genes in the homoeologue gene set. (*D*) Relative homoeologue usage for all genes in the homoeologue gene set. In order to separate genes that are PTR from those that are NTR, the ratio of $RPKM_P/RPKM_{NP}$ is plotted against $RPKM_T$ in panels A-C ($RPKM_P/RPKM_{NP}$ is greater than 1 for PTR and less than 1 for NTR). In Panel D, the shift in the D4 fraction of combined expression (P – NP) is plotted against $RPKM_T$ (values greater than 0 indicate translational regulation favoring D4, and values less than 0 indicate translational regulation favoring D3). PTR, positive translational regulation; NTR, negative translational regulation; Not TR, not translationally regulated; TR_{RHU}, translation regulation of relative homoeologue usage. In each panel, the dashed line indicates the mean RPKM in T for all genes displayed, and numbers for PTR, NTR, TR_{RHU} and ALL indicate what fraction of each has RPKM greater or less than this mean.



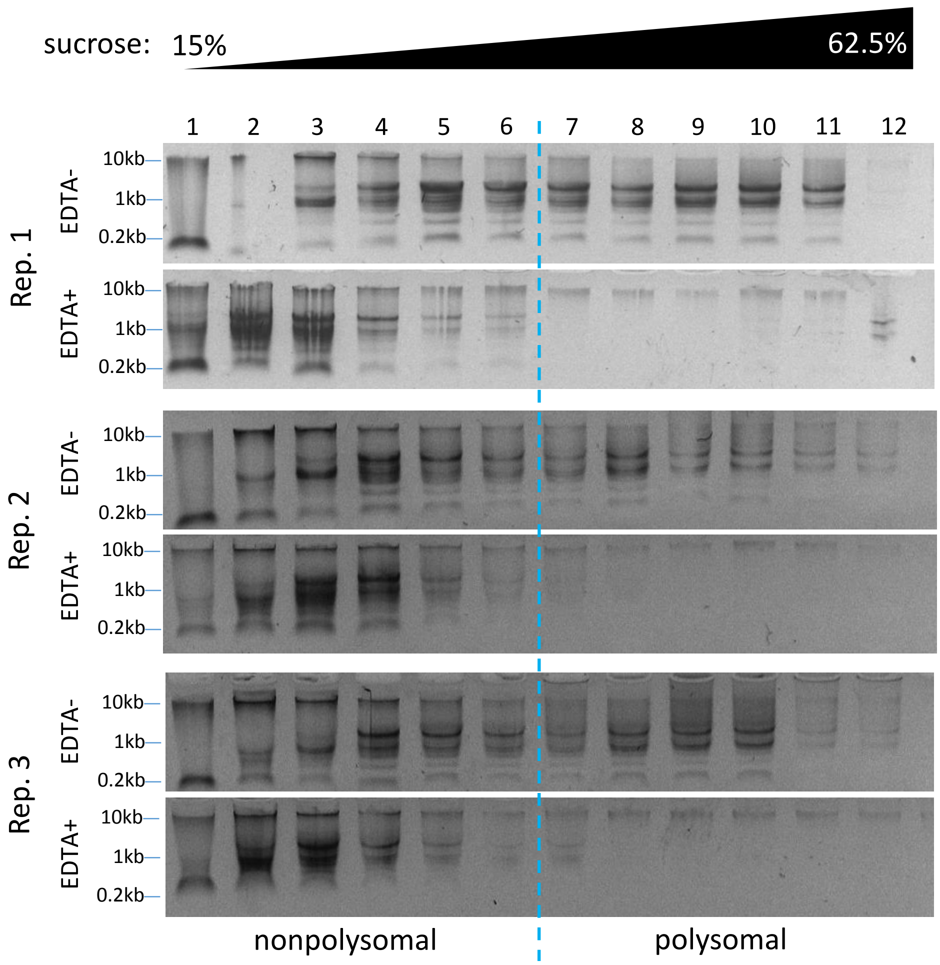
Supplemental Figure 4. Relative enrichment or depletion of Plant GOSlim terms among genes that are translationally regulated. (*A*) Combined expression. (*B*) D3 homoeologue. (*C*) D4 homoeologue. Fold-difference, the fraction of genes mapped to the specified Plant GOSlim term in PTR or NTR divided by the fraction in the reference gene set. For combined expression, the reference set is all genes expressed in T and in P and/or NP (N = 35655). For homoeologue-specific expression, the reference set is the homoeologue gene set (N = 3874). BP, biological process GO term; CC, cellular component GO term. Single asterisks indicate enrichment with an unadjusted p-value < 0.05; double asterisks indicate significant enrichment with FDR < 0.05. Where no bars are shown, no genes were assigned to the corresponding GO term.



Supplemental Figure 5. D4 homoeologue expression dominance in T2. (*A*) Box and whisker plots of the D4 contribution to combined expression in the homoeologue gene set (N = 3,874) for the pseudotetraploid (pT2) transcriptome (D4 diploid / [D3 diploid + D4 diploid]; see text for details), the T2 transcriptome (T), the T2 translatome (P), and the T2 nonpolysomal fraction (NP). Diamonds represent means, and horizontal lines dividing the grey boxes represent medians. The tops and bottoms of the grey boxes represent 3rd and 1st quartiles, respectively. Error bars represent maximum and minimum values. (*B*) Percent of genes showing preferential expression (>50%) of D3 and D4 homoeologues in pT2, T, P, and NP.



Supplemental Figure 6. Average D4 homoeologue fraction of combined expression in the T2 transcriptome (T, blue line) and translatome (P, red line) in 25-gene sliding windows across each soybean reference chromosome. Tick marks on the X-axis indicate 25 gene increments (starting from the 25th gene). Grey shading delineates sliding windows in which the majority of genes are located in pericentromeric regions defined by Du et al. (2012).



Supplemental Figure 7. Nondenaturing gels of RNA samples extracted from sucrose gradient fractions. For each replicate, fractions 1-6 were pooled to form the nonpolysomal (NP) sample, and fractions 7-12 were pooled to forrm the polysomal (P) sample. Rep., Biological replicate.

	T2 vs. D3		T2 vs. D4		T2 vs. D3 and D4	
	$P_{Diff} < T_{Diff}$	$P_{Diff} \geq T_{Diff}$	$P_{Diff} < T_{Diff}$	$P_{Diff} \geq T_{Diff}$	$P_{Diff} < T_{Diff}$	$P_{Diff} \geq T_{Diff}$
All genes	14496	22507	13626	23855	12487	24516
≠ Diploid	898	415	1065	637	141	55
X_2	450		475		127	
p	<0.001		<0.001		<0.001	

T_{Diff}, difference in RPKM between T2 transcriptome (T) and diploid transcriptomes (absolute value [$RPKM_T - RPKM_{Diploid}$]).

P_{Diff}, difference in RPKM between T2 translatome (P) and diploid transcriptome (absolute value [$RPKM_P - RPKM_{diploid}$]).

Supplemental Table 1. Translation reduces transcript-level differences in expression between T2 and its diploid progenitors.
Distribution of genes for which the difference in expression between the T2 translatome and diploid transcriptome is greater than or less than the difference between the T2 transcriptome and diploid transcriptome, for all expressed genes ("All genes") and genes that are differentially transcribed between T2 and the diploid (FDR < 0.05; " ≠ Diploid").