Fig. S1: Phenotypic effect of antisense injection. Gastrula embryos are shown. TBP-AS was injected at 0.8 ng/embryo, TLF-AS at 0.7 ng/embryo and TBP2-AS at 1 ng/embryo (cf.Veenstra et al., 2000, Jallow et al., 2004).

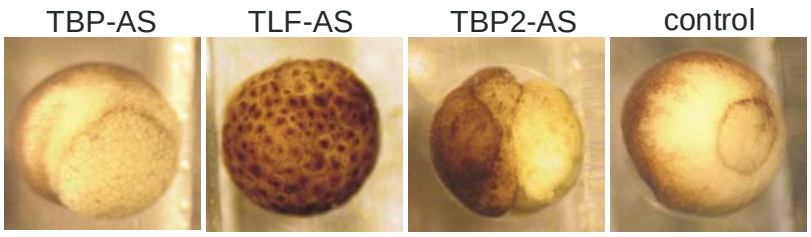

Fig. S2: **Analysis of microarray data. A-C.** Comparison of different scaling methods. A widely used method to normalize or scale Affymetrix microarray experiments is based on the total signal of all features on the microarray. However, conditions that have a global effect on transcription and influence the overall signal intensity could create a normalization bias (Radonjic et al., 2005). This problem can be circumvented by using for example spiked-in controls for normalization. Since we depleted general transcription factors from embryos and expected global effects on transcription we compared different scaling methods with the results obtained by quantitative reverse transcription PCR (qRT-PCR).
The graphs depict qRT-PCR analysis results of 16 genes compared to the hybridization results of these genes for all microarray experiments (biological replicate of 5 different conditions = 10 hybridizations) on a ^{2}log scale (fold change of antisense injected or stage 7 embryos versus stage 10½ control embryos). The correlation coefficient R and the correlation equation are given in the left corner of the graphs. The different scaling methods showed only moderate differences, presumably due to the fact that most transcripts in early embryos are maternally derived (Fig. 2A) and mask global effects on newly synthesized transcripts. Housekeeping genes scaling was chosen as the y-intercept of the correlation equation was closest to zero. **D-F.** qRT-PCR analysis of the most decreased transcripts with TBP-AS (D), TLF-AS (E) and TBP2-AS (F). Down regulation is shown as change on a ^{2}log scale, GAPDH is included as unchanged control.

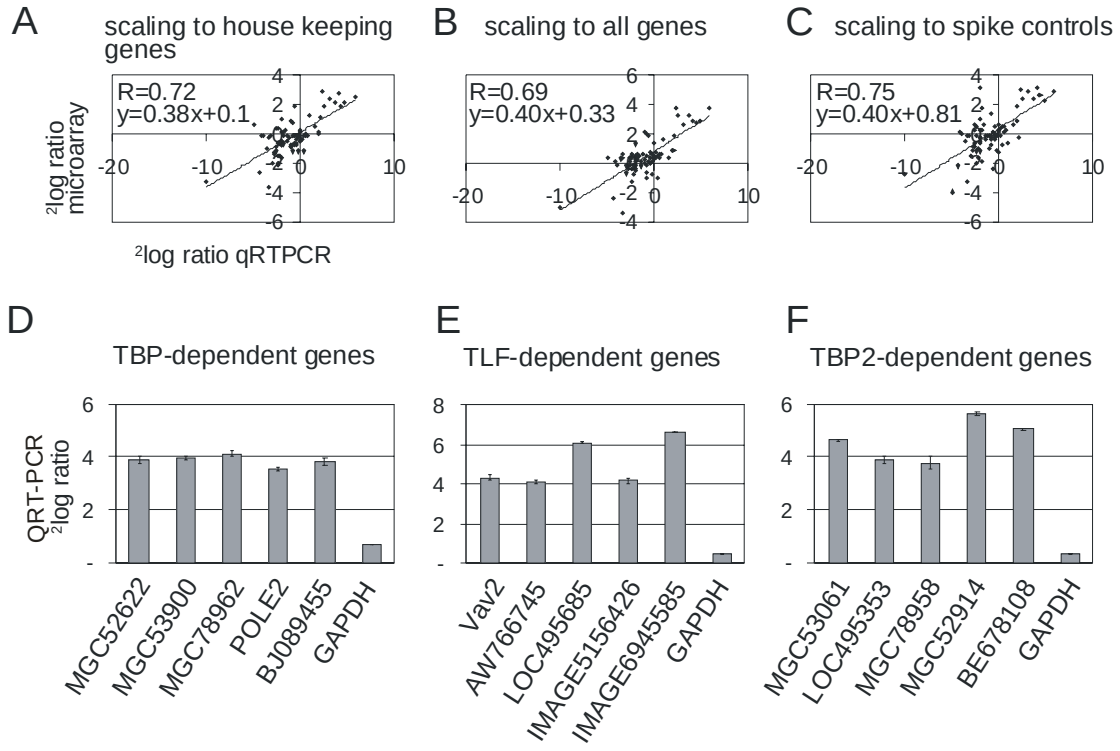

Fig. S3: Reproducibility of differential expression in biological replicates as detected by Affymetrix microarrays. The scatter plots show the ^{2}log ratio of experimental condition (A=st.7 control, B=TLF-AS, C=TBP-AS, D=TBP2-AS) versus st.10½ control embryos, on the x-axis experiment 2, on the y-axis the first replicate. E. scatter plot of signal vs. signal of stage 10½ control embryos.

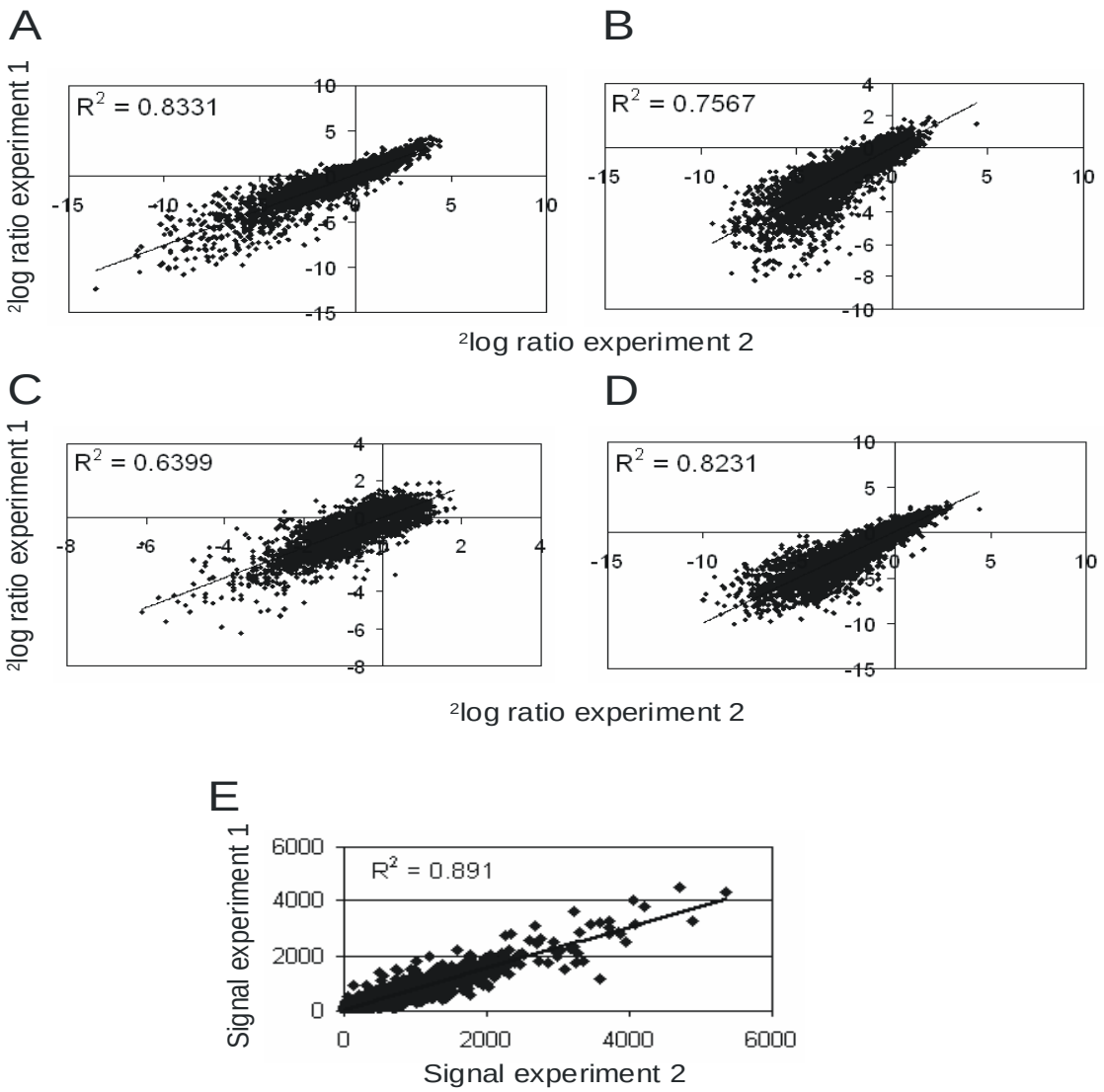

Fig. S4: K-means clustering of ^{2}log ratios of both replicates, k=8. Transcripts are clustered as dependent on different TBP family members and either maternal-embryonic or embryonic. 1 = TBP2, 2 = embryonic, TBP2 and TLF, 3 = maternal-embryonic, unaffected 4 = maternal-embryonic TBP2 and TLF, 5 = embryonic and maternal-embryonic, TBP, TLF and TBP2, 6 = TLF.

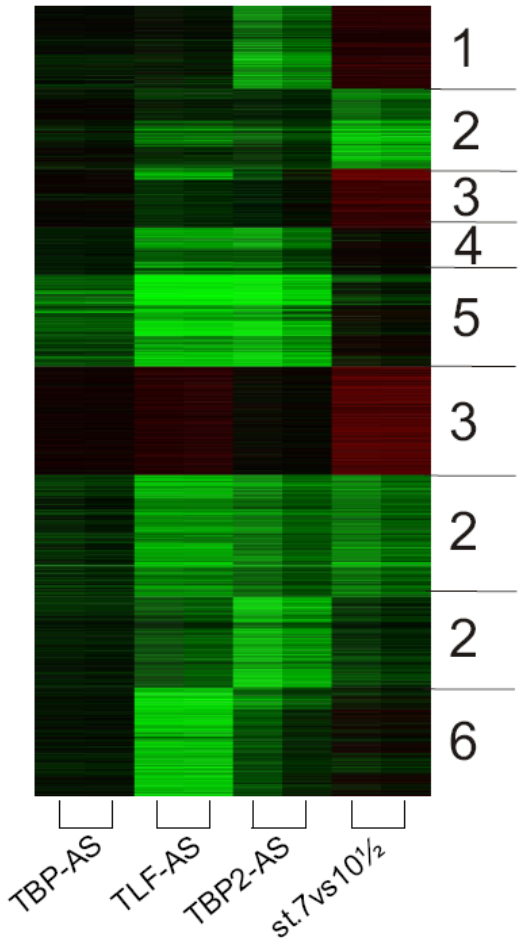

Table S1: Absolute and relative numbers of different categories of dependent genes corresponding with Venn diagrams of figure 2.

dependent on	Affymetrix change call, cf. Fig. 2D	decreased >2.8-fold (^{2}log-1.5), cf. Fig. 2E	maternal-embryonic, decreased >2.8-fold (^{2}log-1.5), cf. Fig. 2F	embryonic, decreased >2.8-fold (^{2}log-1.5), cf. Fig. 2G
	Number (%)	Number (%)	Number (%)	Number (%)
TBP	298 (5.6)	74 (2.1)	57 (3.2)	3 (0.5)
TBP and TBP2	147 (2.7)	31 (0.9)	22 (1.2)	3 (0.5)
TBP, TLF and TBP2	857 (16)	284 (8.1)	102 (5.7)	68 (11.8)
TBP and TLF	345 (6.5)	90 (2.6)	48 (2.6)	20 (3.5)
TBP2	899 (17)	696 (19.7)	447 (25)	50 (8.7)
TBP2 and TLF	1258 (23.7)	963 (27.3)	386 (21.7)	178 (31)
TLF	1514 (28.5)	1387 (39.3)	718 (40.3)	254 (44.1)
total	5318	3525	1780	576

Fig. S5: Rescue experiments. Embryos were injected with either TBP-AS, TLF-AS or TBP2-AS and co-injected with different amounts of TBP mRNA, TLF mRNA or TBP2 mRNA (rescue). Expression was analyzed by RT-PCR and calculated relative to control embryos. Titration of RNA dose is important to achieve optimal results in rescue experiments.

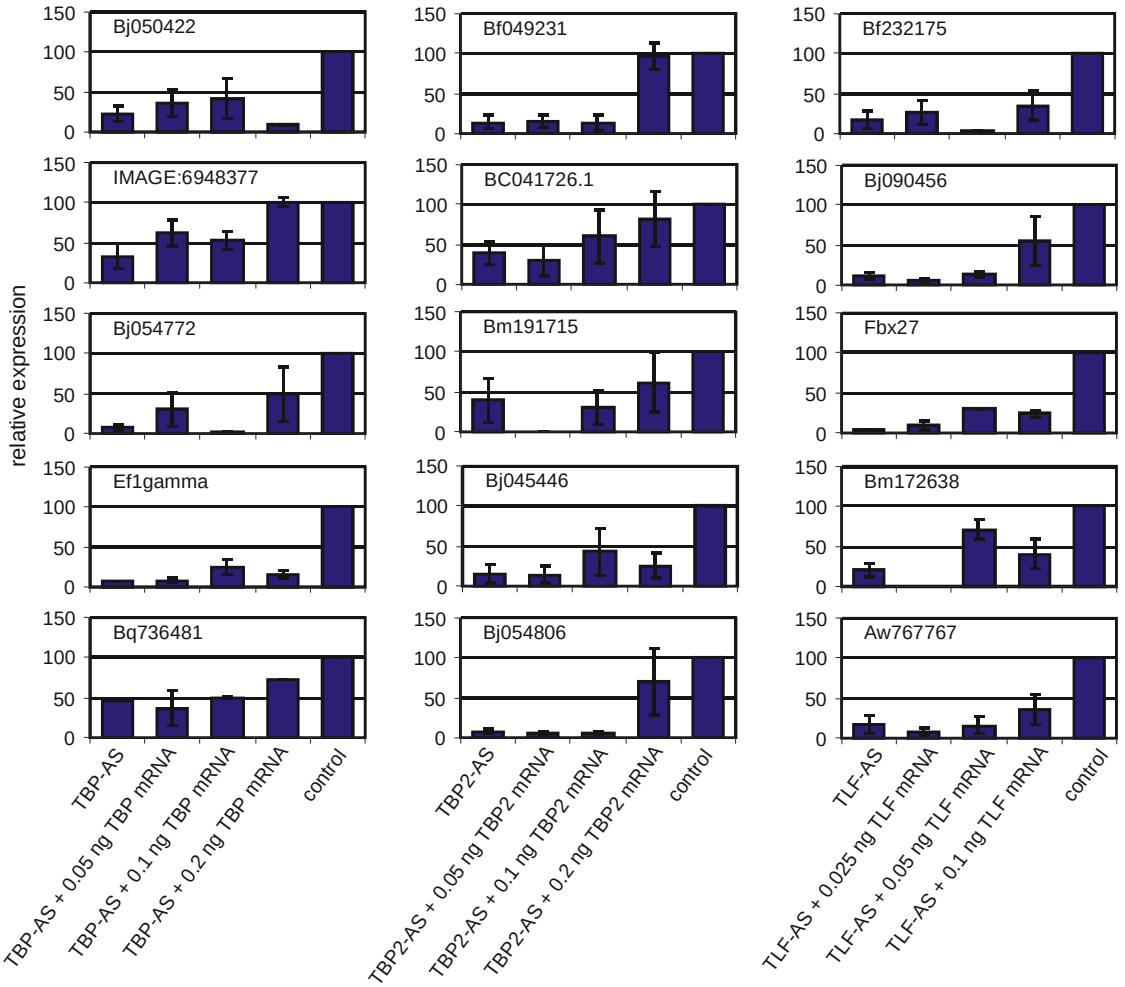

Fig. S6: Factor selectivity in single and triple knockdown experiments. A. RT-PCR analysis of expression of single knockdown embryos relative to un-injected control embryos. The average transcript level was calculated for randomly selected transcripts identified in the microarray data as exclusively TBP-, TLF- or TBP2-dependent. **B.** RT-PCR analysis of expression of triple knockdown embryos and embryos co-injected with different amounts of TBP mRNA, TLF mRNA or TBP2 mRNA. The average transcript level of the indicated number of transcripts was calculated. The same transcripts were analyzed in both types of experiments.

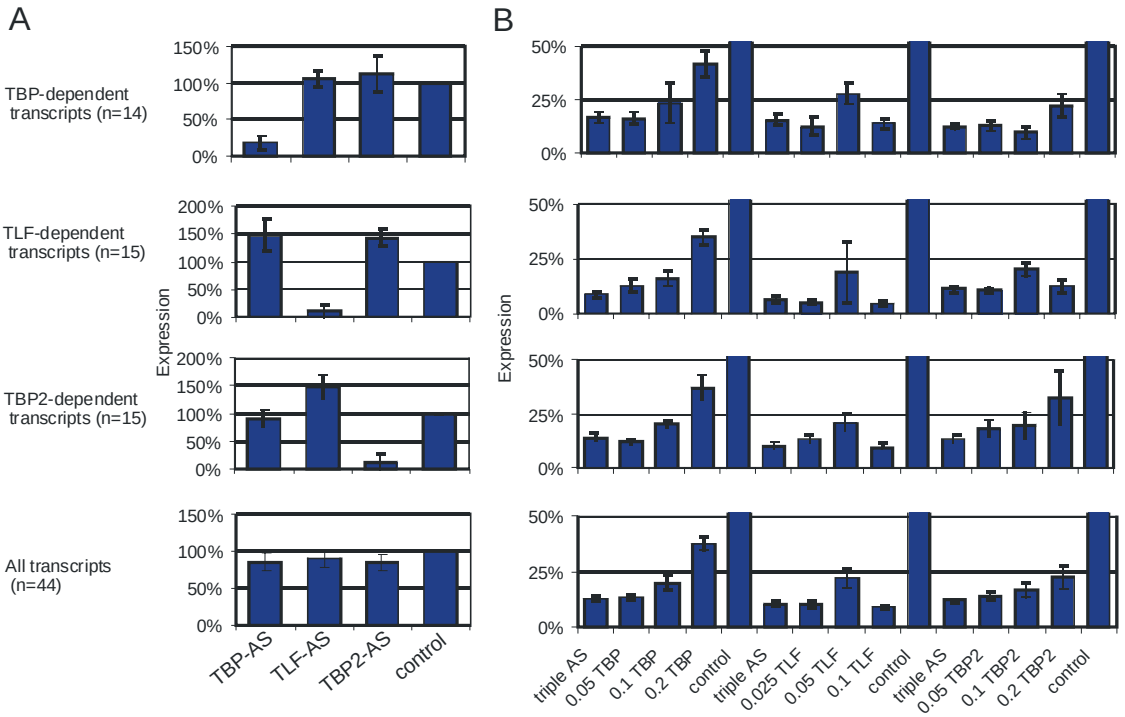

Fig. S7: Normalized enrichment of TBP and TBP2 on TBP-dependent and TBP2-dependent promoters. Enrichment (fold over background) was normalized to the average enrichment in all promoters. Normalized enrichment values were then averaged for TBP- and TBP2-dependent subgroups.

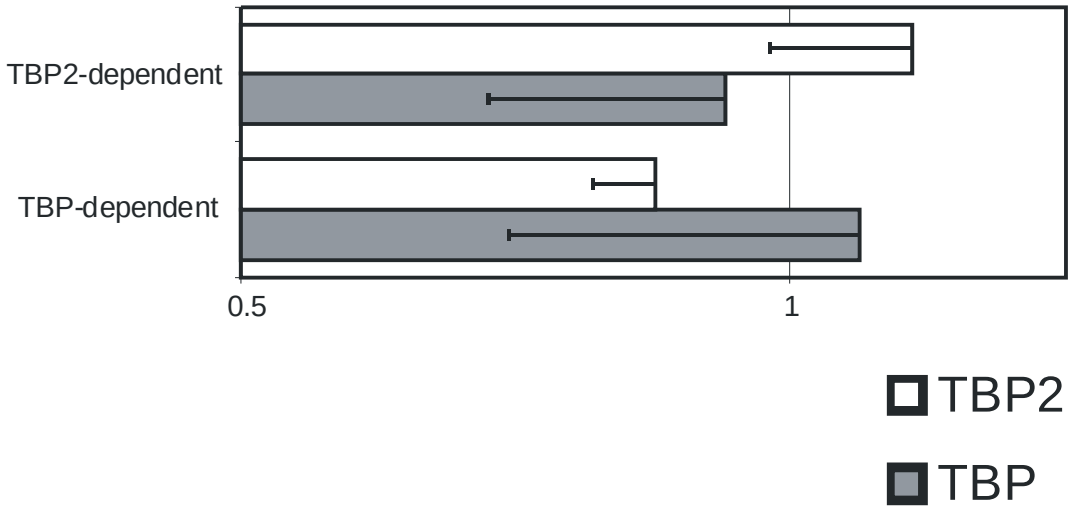

Table S2: **Ortholog analysis of TBP, TBP2 and TLF-dependent genes**. Genes were assigned to groups (Venn groups, cf. Fig. 2) on the basis of decreased transcript levels at gastrula stage 10½ in one or more antisense knockdown experiments. Strict criteria were applied, meaning that the Affymetrix statistical change calls should be consistent in two biological replicates; in addition the ^{2}log(ratio) values should be below the cutoff value of -1.5 for affected transcripts in both biological replicates. Dependencies: TBP-only, transcript affected in TBP-AS but not in TBP2-AS or TLF-AS knockdown embryos; TBP2-only, transcript affected in TBP2-AS but not in TBP-AS or TLF-AS knockdown embryos; TLF-only, transcript affected in TLF-AS but not in TBP-AS or TBP2-AS knockdown embryos. Enrichment (Fold) is based on the genes represented on the microarray (Reference). P values were determined using the hypergeometric distribution. Maternal-embryonic (Mat.-Embr.): Not changed or decreased between stage 7 and 10½ in both replicate experiments; Embryonic (Embr.): Increased between stage 7 and 10½ above cutoff of ^{2}log(ratio)=1.5 in both replicate experiments. Yeast homologs: Number of *X. tropicalis* Ensembl genes that have an ortholog in yeast (*S. cerevisiae*). Metazoan-specific: Number of *X. tropicalis* Ensembl gene that have an ortholog in fly (*Drosophila*) but not in yeast. Vertebrate-specific: Number of *Xenopus tropicalis* Ensembl gene that have an ortholog in human but not fly.

	Reference: All Affy											
	Total	**%**										
X.tropicalis Ensembl genes	5014											
Yeast homologs	1094	21.8										
Metazoan-specific	1496	29.8										
Vertebrate-specific	2161	43.1										
	TBP-only				**Mat.-Embr. TBP-only**				**Embr. TBP-only**			
	Total	**%**	**Fold**	**p-value**	**Total**	**%**	**Fold**	**p-value**	**Total**			
X.tropicalis Ensembl genes	32				29				0			
Yeast homologs	15	46.9	2.15	1.01e-03	14	48.3	2.22	1.04E-03	0			
Metazoan-specific	5	15.6	0.52	3.31e-02	3	10.3	0.35	9.55E-03	0			
Vertebrate-specific	10	31.3	0.73	5.83e-02	10	34.5	0.80	9.86E-02	0			
	TBP2-only				**Mat.-Embr. TBP2-only**				**Embr. TBP2-only**			
	Total	**%**	**Fold**	**p-value**	**Total**	**%**	**Fold**	**p-value**	**Total**	**%**	**Fold**	**p-value**
X.tropicalis Ensembl genes	273				179				22			
Yeast homologs	71	26.0	1.19	1.36e-02	51	28.5	1.31	6.92E-03	1	4.5	0.21	2.71E-02
Metazoan-specific	96	35.2	1.18	7.78e-03	64	35.8	1.20	1.41E-02	4	18.2	0.61	9.83E-02
Vertebrate-specific	101	37.0	0.86	5.56e-03	61	34.1	0.79	2.74E-03	15	68.2	1.58	1.07E-02
	TLF-only				**Mat.-Embr. TLF-only**				**Embr. TLF-only**			
	Total	**%**	**Fold**	**p-value**	**Total**	**%**	**Fold**	**p-value**	**Total**	**%**	**Fold**	**p-value**
X.tropicalis Ensembl genes	548				312				105			
Yeast homologs	148	27.0	1.24	3.97e-04	96	30.8	1.41	3.71E-05	20	19	0.87	7.77E-02
Metazoan-specific	193	35.2	1.18	6.04e-04	116	37.2	1.25	7.84E-04	38	36.2	1.21	3.01E-02
Vertebrate-specific	191	34.9	0.81	6.39e-06	95	30.4	0.71	6.71E-07	43	41	0.95	7.21E-02

Fig. S8: TLF is linked to carbohydrate catabolism. RT-PCR analysis of a number of transcripts, expression was calculated relative to control embryos. Shown are single knockdowns of all three TBP family members and rescue data of TLF-dependent transcripts.

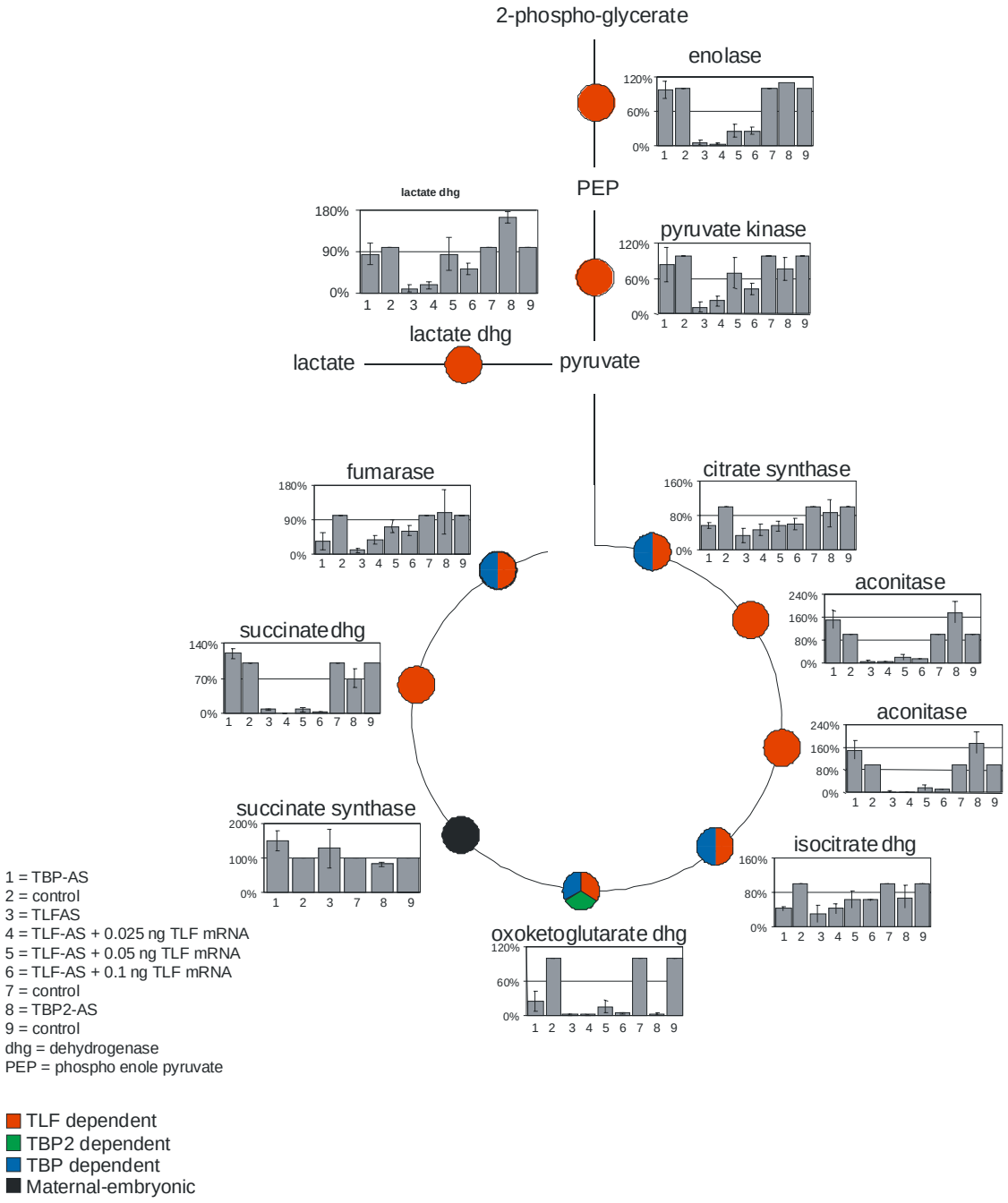

Fig. S9: Ventrally enriched transcripts. Venn diagram shows the dependency of ventrally enriched transcripts on the TBP family members. Ventrally enriched transcripts were defined by Baldessari et al. Among these transcripts 19 out of 30 require TBP2 for their expression, 11 of which exclusively depend on TBP2 rather than TBP or TLF. The p-value was calculated using the hypergeometric distribution.