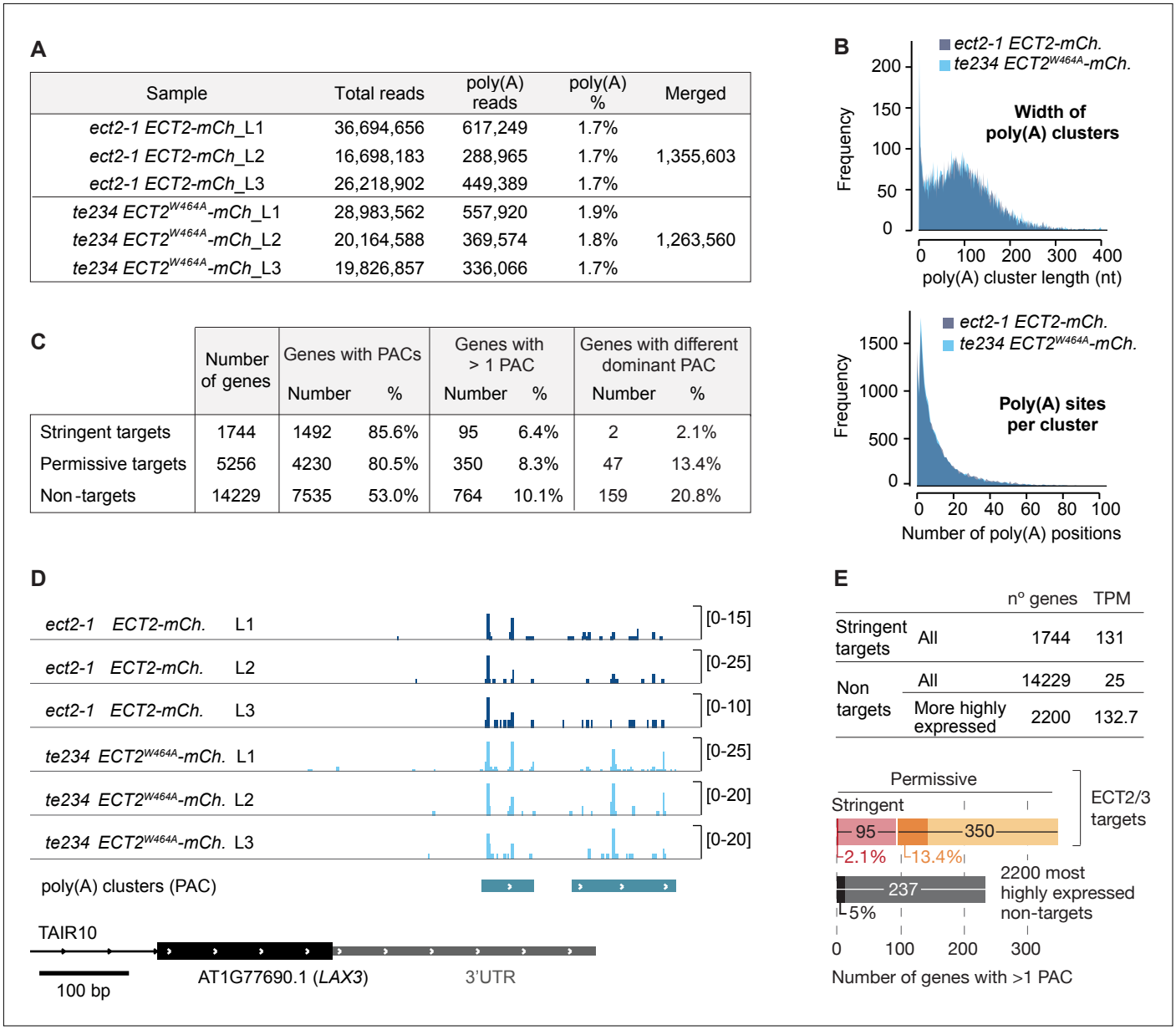

Sample	Total reads	poly(A) reads	poly(A) %	Merged
*ect2-1 ECT2-mCh*_L1	36,694,656	617,249	1.7%	
*ect2-1 ECT2-mCh*_L2	16,698,183	288,965	1.7%	1,355,603
*ect2-1 ECT2-mCh*_L3	26,218,902	449,389	1.7%	
*te234 ECT2^{W464A}-mCh*_L1	28,983,562	557,920	1.9%	
*te234 ECT2^{W464A}-mCh*_L2	20,164,588	369,574	1.8%	1,263,560
*te234 ECT2^{W464A}-mCh*_L3	19,826,857	336,066	1.7%	

	Number of genes	Genes with PACs		Genes with > 1 PAC		Genes with different dominant PAC	
		Number	%	Number	%	Number	%
Stringent targets	1744	1492	85.6%	95	6.4%	2	2.1%
Permissive targets	5256	4230	80.5%	350	8.3%	47	13.4%
Non-targets	14229	7535	53.0%	764	10.1%	159	20.8%

		n° genes	TPM
Stringent targets	All	1744	131
Non targets	All	14229	25
	More highly expressed	2200	132.7

Figure 5—figure supplement 2. Poly(A) sites do not change in ECT2/3 targets upon loss of *ECT2/3/4* function (extended data). (A) Summary of poly(A)-containing reads (i.e. reads with at least 9 untemplated As) after removal of reads mapping upstream of purine-rich sites. These reads were used for cluster identification, yielding 17,028 putative poly(A) site clusters (PACs), from which 14,667 were retained after further filtering of potential false positives (see Methods). **(B)** Features of PACs. (Upper panel) Genomic distance between most upstream and most downstream poly(A) site within each cluster (median length of 105 bp). (Lower panel) Total number of genomic positions within the cluster where at least 1 read with an untemplated poly(A) tail was detected (median of 12 poly(A) sites per cluster). **(C)** PACs sorted by ECT2/3 target status. Percentages of genes with more than 1 PAC refer to the number of genes with PACs. Percentages of genes with a dominant PAC (defined as the cluster with the most reads) that is different between *te234 ECT2^{W464A}-mCherry* and *ect2-1 ECT2-mCh* samples refer to the number of genes with more than 1 PAC. **(D)** Variation in dominant polyadenylation sites for the non-ECT2/3-targeted transcript *LAX3*, an example of gene with different dominant PAC. Independently of the fact that the total amount of poly(A) reads is generally higher in *te234 ECT2^{W464A}-mCherry* compared to *ect2-1 ECT2-mCh* samples (notice that the scales have been adjusted for optimal comparison of PAC usage within samples), the ratio between the number of reads in the upstream and the downstream clusters is different in the 2 genotypes. Transcript annotation is based on TAIR10. **(E)** Mean TPMs (Smart-seq2 data of sorted protoplasts, combining all 6 samples) of genes in the different ECT2/3 targets groups (upper panel). The significantly lower likelihood for ECT2/3 targets to have a different dominant PAC upon loss of ECT2/3/4 function depletion compared to non targets (*Figure 5E*) could be due to differences in transcript abundance between the target and non-target groups. Looking at only the 2200 most highly expressed non-target genes, only 5.5% of these genes have a different dominant PAC in *te234 ECT2^{W464A}-mCherry* than *ect2-1 ECT2-mCh* samples (lower panel, dark shading refers to genes with different dominant PAC as in *Figure 5E*), significantly smaller than the percentage for all non-target genes (20.8%, *Figure 5E*) (p=3.2e-9, Fisher's exact test).