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SUPPLEMENTARY MATERIAL

corresponding to:

Deletion of etoposide-induced 2.4 kb transcript (ei24) reduced cell proliferation and aggregate-size in *Dictyostelium discoideum*

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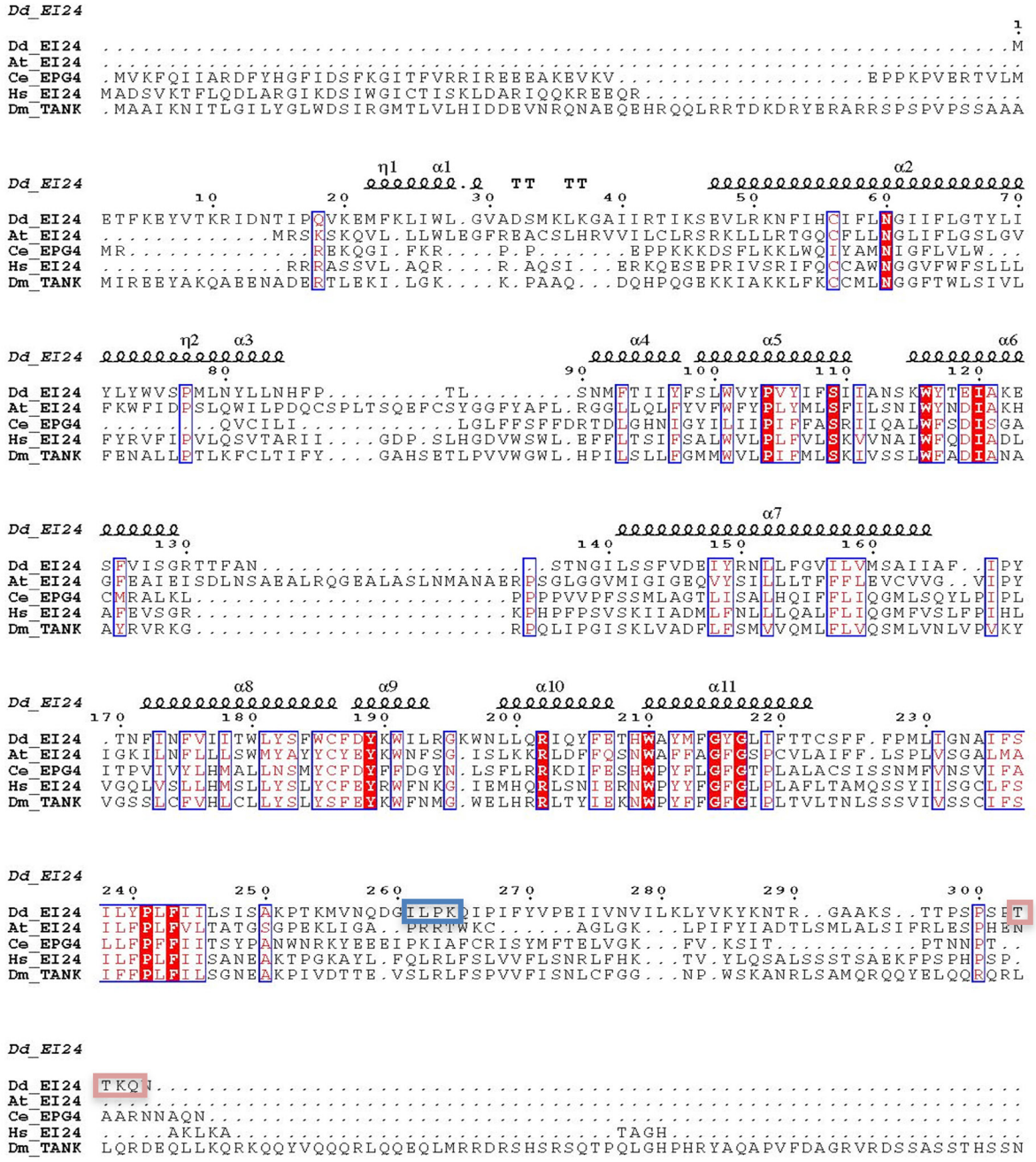
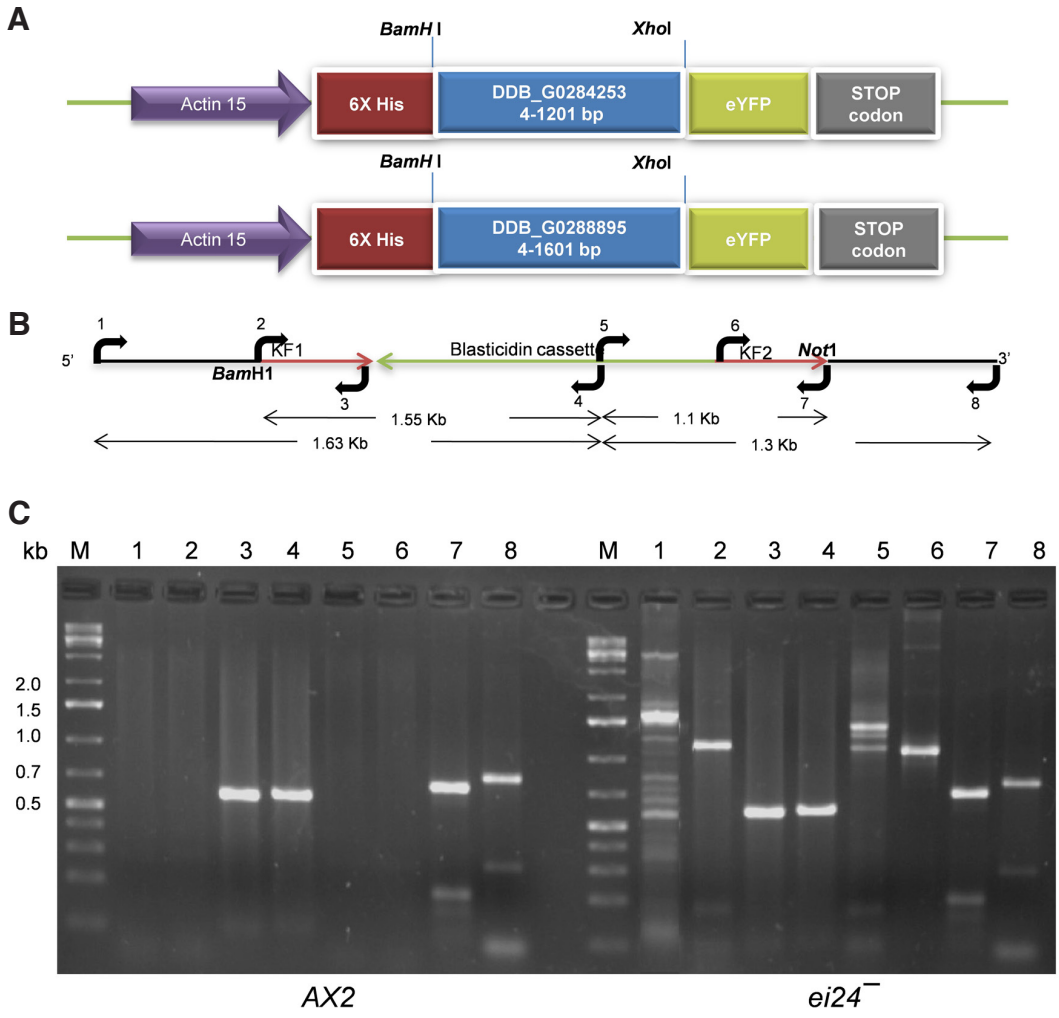
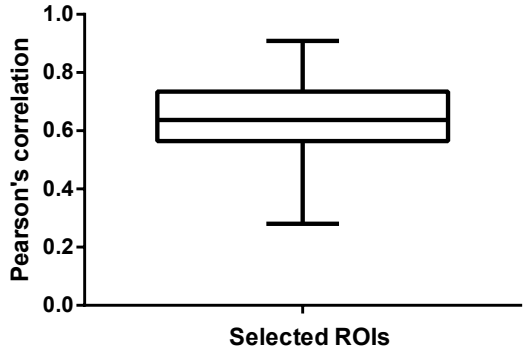


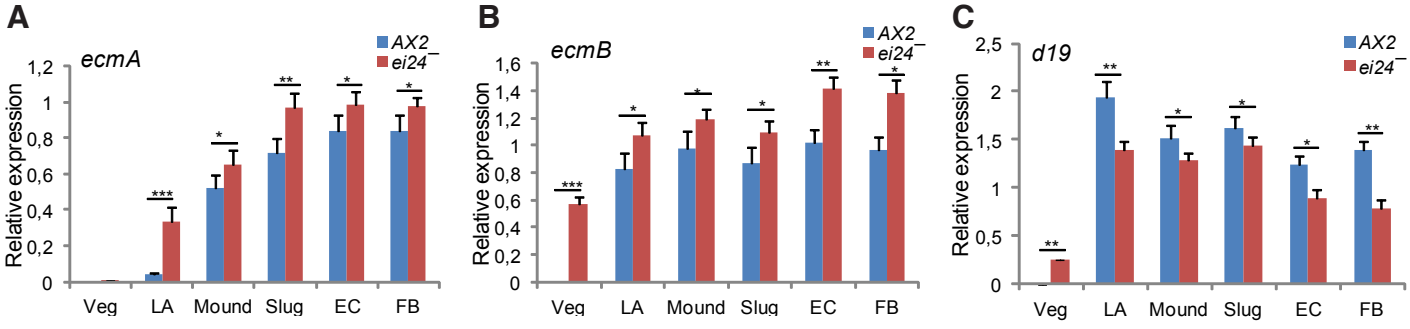
Supplementary Fig. S1. CLUSTALX alignment of the DdEI24 with other organisms. *Conserved amino acids are marked with a red box. Peptide sequence of DdEI24 was analyzed using the PSORT II program to detect the potential topology motifs. ER membrane retention signals (KKXX-like motif) are represented in a pink box (TTKQ) and a possible vacuolar targeting motif is represented in a blue box (ILPK). Abbreviations: At, Arabidopsis thaliana; Ce, Caenorhabditis elegans; Dd, Dictyostelium discoideum; Dm, Drosophila melanogaster; Hs, Homo sapiens.*



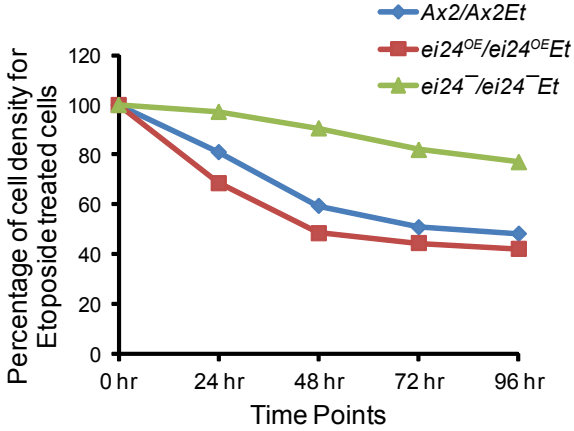
Supplementary Fig. S2. Characterization of the *ei24* mutants. (A) *Schematic representation of overexpressing construct of* ei24 (ei24OE) *and* p53 (p53OE). *Both the constructs were prepared with* eYFP *reporter gene construct at its C-terminal and were driven under actin15 promoter.* **(B)** *Schematic representation of the knockout construct of* ei24. *The primers and the expected amplicon sizes are marked.* **(C)** *Validation of knockout strain by PCR amplifications. The primer pair used and the size of the amplicon are as: Lane 1= #1 and 4; size=1.63 kb; Lane 2= #5 and 8; size=1.2 kb; Lane 3= #2 and 3; size=0.58 kb; Lane 4= #6 and 7; size=0.61 kb; Lane 5= #2 and 4; size=1.55 kb; Lane 6= #5 and 7; size=1.1 kb; Lane 7= # 1 and 3; size=0.68 kb; Lane 8= # 6 and 8; size=0.71 kb. In Ax2 strain lanes 3, 4, 7 and 8 are positive, In* ei24¯ *all lanes 1-8 are positive for the amplified DNA fragment (M denotes the 1 Kb plus DNA ladder from Fermentas, KF1-knockout fragment 1; KF2-knockout fragment 2).*



Supplementary Fig. S3. Pearson's correlation analysis. ei24-eYFP *cells were merged with ER tracker red for colocalization analysis. Different ROI were selected from ~50 cells and Pearson's correlation coefficient was calculated.*



Supplementary Fig. S4. *ei24* null mutant altered the mRNA expression of *Dictyostelium* cell-type-specific genes. *RT-PCR analyses of specific genes* **(A)** ecmA, **(B)** ecmB *and* **(C)** d19 (pspA) *during developmental stages of* Ax2 *and* ei24 *-after normalization to* ig7 *are shown. [Veg- vegetative; LA-loose aggregate; EC-early culminant; FB-fruiting body; n=3; Student t-test, p-value ≤0.05, ≤0.01 and ≤0.001 has been represented as *, ** and ***, respectively].*



Supplementary Fig. S5. Comparative time dependent effect of etoposide treatment on cell viability. *Cell viability in Ax2,* ei24^{OE} *and* ei24^{-} *cells using MTT assay.*

SUPPLEMENTARY TABLE S1

LIST OF PRIMERS USED IN THIS STUDY

S. No	Oligo Name	5'-3' Sequence (Forward Primer)	5'-3' Sequence (Reverse Primer)
1	*ei24*^{OE}	CCAAGGATCCGAGACATTTAAAGAATATG	AACCCTCGAGAATTTTGTTTTGTAGTTGGT
2	*ei24* RT	TTCCAAATGGTATACAGAGA	AATGCCATCTTGATTAACC
3	*p53*^{OE}	CCAAGGATCCTCAAAAGAAAAAACATCTTGGGGT	AACCCTCGAGAACCACTTGTATGATTACATGGAAC
4	*p53* RT	TCGATCCATCATTTGCATGTT	ACCACTTGTATGATTACATGG
5	*ei24 in situ*	TTGGCTCGAGTCATTATGGGTTTATCCAGTT	GGTTAAGCTTTCGAAACACCAAAATGAATA
6	*ei24*$^{-}$ KF1	TTCCGGATCCGAGACATTTAAAGAATATGTA	AAGGAAGCTTAGACATTACCAAAATTACACC
7	*ei24*$^{-}$ KF2	GAAGTCTAGAGCAATCATTGCATTCATTCCA	TGTGCGGCCGCATTTTGTTTTGTAGTTGGTGA
8	*Bsr*	TTTGTCCATTCGAAACTGCA	TGCAGTTTCGAATGGACAAA
9	*ei24*$^{-}$	AACAACACACAAAAAGGAAAT	ATGATCGTTACAAGTGAAAATATG
10	*ig7* RT	TGAATTGAAGTCTGAGTAAACGG	TAGATAGGGACCAAACTGTCTCAC
11	*acaA* RT	AGTACACCACATAATAATAATCAT	CTCTGGAATTACAATATCTCTCTT
12	*carA-1* RT	TGTATGGCAGTGTTGATTGGT	ATGGTGATGGATTGTTATTGT
13	*pdsA* RT	ATGGCATTAAATAAAAAATT	TTAAATACAAATTGGATCACC
14	*gbfA* RT	CCATTACCATTACCATCTATA	TGATGGTGATGGTGTATTACT
15	*cadA* RT	TCTGTTGATGCAAATAAAGTAAAA	ATAGTCATATGGTGTATGTGTTTG
16	*csaA* RT	GTGAACGACTCTATTAACTCTGCT	AGTTGGAGTGTCTGGAATTGTATA
17	*ctnA* RT	ATTTTAGCTTTATTCCTTGTCAAC	GTGTAAGCAATTGAGAGGGTGAAT
18	*cotA* RT	TAATAAGCTTGAAAGATAATTGTGGAGAAGGTGGTGATG	TTATCTCGAGGGAAGAGCTTGATGATGCAGATGAAG
19	*cotB* RT	GGTCAAGCTTAGAGATAGTAACGATTGTCTTGCTAG	TTACCTCGAGATAGTTGATGGATTGATACAGATTGG
20	*rad51* RT	TGTCATACATTATGTGTA	TTGTTCTTTATAATCGG
21	*rad52* RT	CAAGAGGATGTTGGTTAT	TTGGAATTGAGGGATGGC
22	*rad54* RT	AGAAGTTTAGCACCAAGT	TTTGGAAAGACGTACTGC
23	*ku70* RT	GATGGTGATGATTGGGAT	CAGAGAATTTGGAACTTG
24	*ku80* RT	ATGACAACTACAATACCA	ACGAGTCATTACTGATTG
25	*dnapkcs* RT	GAGATGACACAACTGTTT	ACTTGTTCAATATGATCT

See Supplementary Videos V1-V3 at https://dx.doi.org/ 10.1387/ijdb.170327ss

Supplementary Videos V1-V3 showing cell-migration in response to the cAMP gradient (5 μl, 100 μM). (V1) *Ax2;* **(V2)** ei24^{OE} *and* **(V3)** ei24^{-} *cells.*