

SUPPLEMENTARY MATERIAL

corresponding to:

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

NEHA GUPTA and SHWETA SARAN

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Dd_EI24
Dd_EI24 ..... 1
At_EI24 ..... M
Ce_EPG4 .MVKFQIIARDPFYHGFIKDSFKGITFVRRIRREFAKEVKV.....EPPKPVBERTVLM
Hs_EI24 MADSVKTFLODLARGIKDSIWGICTISKLDARIQQKREBQR.....
Dm_TANK .MAAIKNITLGLIYGLWDSIRGMTLVLHIDDEVNRQNABQBHRQQLRRTDKDRYERARRSPSPVPSAAA

Dd_EI24
          η1  α1
          10  20  30  40  50  60  70
Dd_EI24 ETFKEYVTKRIDNTIPVVKEMFKLIWLGVADSMKLGKAIIRTIKSEVLRKKNFIHCIFLNGLIFLGLTYLI
At_EI24 . . . . .MRSKSKQVL.LLWLEGFREACSLHRVVICLRSRKLRLRTGQCFLNGLIFLGLSLGV
Ce_EPG4 MR. . . . .RQKQGI.FKR. . . . .P.P. . . . .EPPKKKDSFLKKLWQIYAMNIGFLVLW. . .
Hs_EI24 . . . . .RRRASSVL.AQR. . . . .R.AQSI. . . . .ERKQESBPRIVSRIFQCCAWNGGVWFWSLL
Dm_TANK MIRBEYAKQABBNADEKLTLEKI.LGK. . . . .K.PAAQ. . . . .DQHPQGEKKIAKKLKFCMLNGGFTWLSIVL

Dd_EI24
          η2  α3
          80  90  100  110  120
Dd_EI24 YLYWVSPMLNYLLNHFP. . . . .TL. . . . .SNMFTIIVFSLWVYFVYIFSIHANSKWTETIAKE
At_EI24 FKWFIPLSLQWILPDQCSPLTSQEFCSYGGFYAPL.RGGLLQLFYVFWFYPLYMLSFILSNIWNIDIAKH
Ce_EPG4 . . . . .QVCILI. . . . .LGLFFSFFDRTDLGHNTGYLIIPIFFASRIIQALWFSIDISGA
Hs_EI24 FYRVFIPVLQSVTARII. . . . .GDP.SLHGDVWSWL.EFFLTSIFSALWVLPFLFVLSKVVNAIWFQDIADL
Dm_TANK FENALLPPTLKFCLTI FY. . . . .GAHSETLPVVGWL.HPILSLLFGMMWVLPFIMLSKIVVSSIWFADIANA

Dd_EI24
          α4  α5  α6
          90  100  110  120
Dd_EI24 SFFVISGRITTFAN. . . . .STNGILSSFVDETYRNLLFGVILVMSAIIAF.IIPY
At_EI24 GFBAIEISDLNSAEALRQGEALASLNMANAERFSSGLGGVMIGIGEQVYSIILLTFPFLVVCVVG.VIIPY
Ce_EPG4 CMRALKLL. . . . .PEPPVVPFSSMLAGTLISALHQIFFLIQGMLSQYLPIPL
Hs_EI24 AFEVSGR. . . . .KEHPFSPVSKIIADMFLNLLQALFLIQGMFVSLFPIHL
Dm_TANK AVRVRKG. . . . .RQLIPGISKLVADFLFSMIVVQMLFLVQSMMLVNLVVPVKY

Dd_EI24
          α7
          130  140  150  160
Dd_EI24 . . . . .TNFINTFVITTWLYSFWCFDYKWIILRQKWNLLQRIOYFBTHWAWYFQYGLTFTTCSFF.FPMLTGNATIFS
At_EI24 IGKILNLLLSSWYAYCYEYKWNFSG.ISLKRLDFQSNWAFFAFGSPCVLAIFF.LSPLVSGALMA
Ce_EPG4 ITPVIVYLLHMLNSMYCFDYFDGYN.LSFLRKKDIFESHWPYFGLGTPLALACISSNMVNSVIFA
Hs_EI24 VGQLVSLHMSLLYSLYCFEYRWFNKG.IBMHQRLSNIERNWPYFGLGLPLAFLTAMQSYIIISGCLFS
Dm_TANK VGSSLCFVHCLLCLYSLYSFEYKWFNMG.WELHRLTYLEKNWYFGLGLPLTLVLTNLSSSVIVSSCLFS

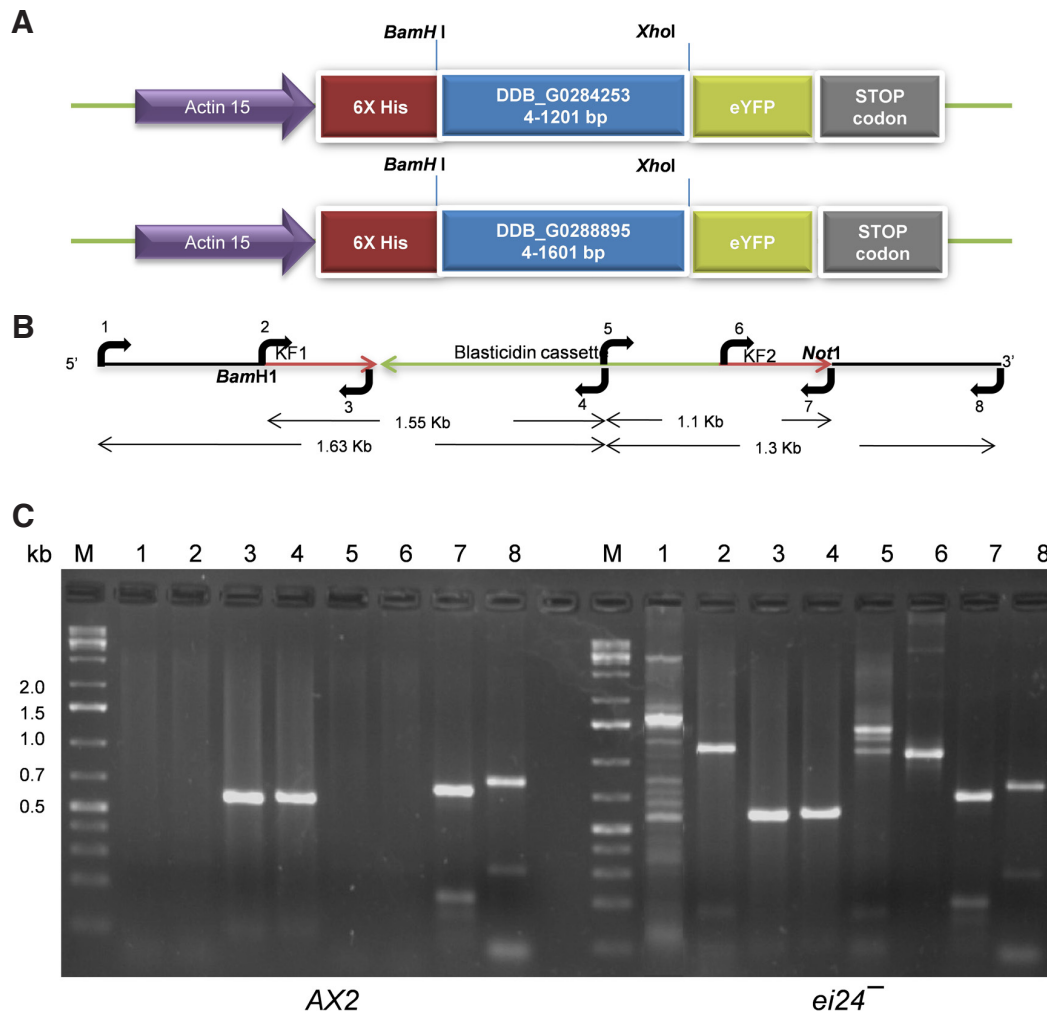
Dd_EI24
          α8  α9  α10  α11
          170  180  190  200  210  220  230
Dd_EI24 . . . . .ILYPLFVILSISAKPTKMNVDGILPKDIPFYVPEIIVNVILKLYVKYKNTR.GAAKS.TTSPSET
At_EI24 ILFPLFVLTATGSGPEKLIQA.PRRTWKC. . . . .AGLGK.LPIFYIADTSLMLALSIFRLESPHEN
Ce_EPG4 LLFPFFIITSYPANWNRKYEEEIPKIAPCRISYMFTELVGK.FV.KSIT. . . . .PTNPT. .
Hs_EI24 ILFPLFIISANEAKTPGKAYL.FQLRFLSLVFLSNRFLFK.TV.YLQSAISSSTSAEKFPSPHPS
Dm_TANK IFFPLFIIISGNEAKPIVDTTE.VSLRFLSPVVFISNLCPGG.NP.WSKANRLSAMQRQQYELQQRRL

Dd_EI24
          240  250  260  270  280  290  300
Dd_EI24 ILYPLFVILSISAKPTKMNVDGILPKDIPFYVPEIIVNVILKLYVKYKNTR.GAAKS.TTSPSET
At_EI24 ILFPLFVLTATGSGPEKLIQA.PRRTWKC. . . . .AGLGK.LPIFYIADTSLMLALSIFRLESPHEN
Ce_EPG4 LLFPFFIITSYPANWNRKYEEEIPKIAPCRISYMFTELVGK.FV.KSIT. . . . .PTNPT. .
Hs_EI24 ILFPLFIISANEAKTPGKAYL.FQLRFLSLVFLSNRFLFK.TV.YLQSAISSSTSAEKFPSPHPS
Dm_TANK IFFPLFIIISGNEAKPIVDTTE.VSLRFLSPVVFISNLCPGG.NP.WSKANRLSAMQRQQYELQQRRL

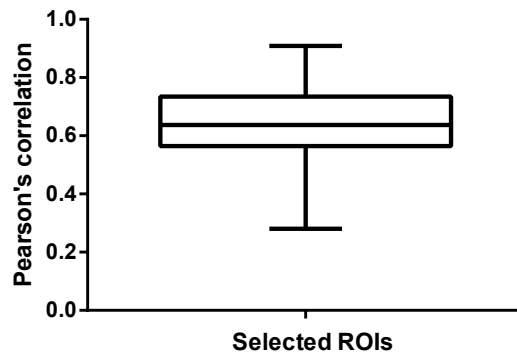
Dd_EI24
Dd_EI24 TKQ
At_EI24
Ce_EPG4 AARNNAQN
Hs_EI24 AKLKA
Dm_TANK LQRDEQLLKQRKQYVQQRLQQEQLMRDRSRSRSTPQLGHPHRYAQAPVFDAGRVRDSASSTHSSN

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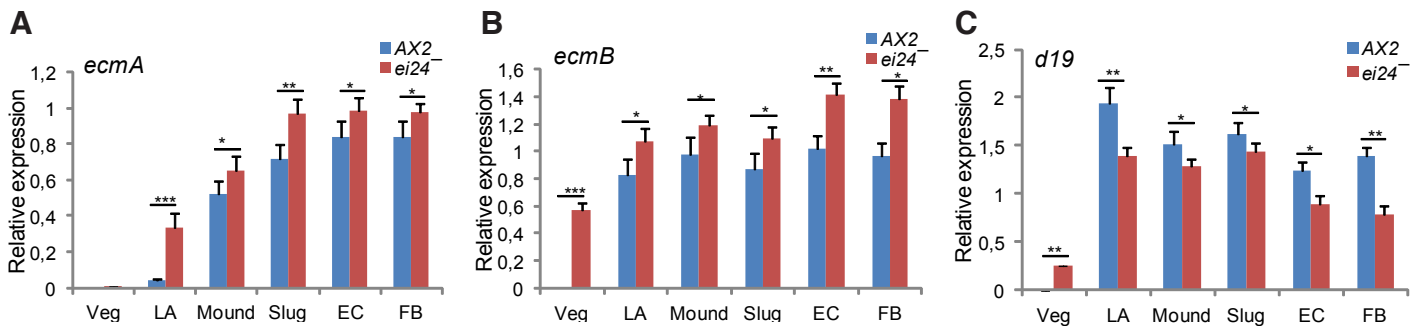
Supplementary Fig. S1. CLUSTALX alignment of the DdEI24 with other organisms. Conserved amino acids are marked with a red box. Peptide sequence of DdEI24 was analyzed using the PSORT II program to detect the potential topology motifs. ER membrane retention signals (KKXX-like motif) are represented in 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*.



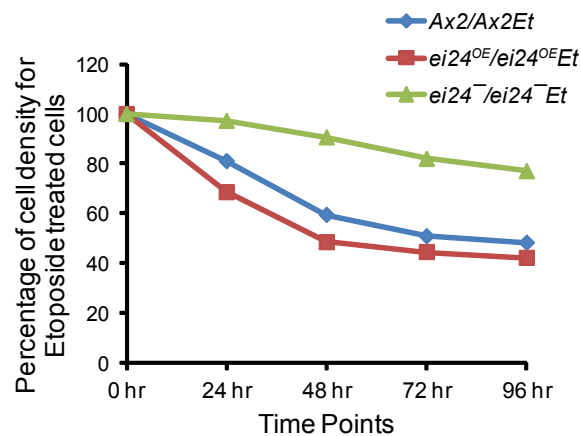
Supplementary Fig. S2. Characterization of the *ei24* mutants. (A) Schematic representation of overexpressing construct of *ei24* (*ei24OE*) and *p53* (*p53OE*). Both the constructs were prepared with *eYFP* reporter gene construct at its C-terminal and were driven under *actin15* promoter. **(B)** Schematic 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-fruited 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	<i>ei24^{OE}</i>	CCAAGGATCCGAGACATTTAAGAATATG	AACCCTCGAGAATTTGTTTTGTAGTTGGT
2	<i>ei24</i> RT	TTCCAAATGGTATACAGAGA	AATGCCATCTTGATTAACC
3	<i>p53^{OE}</i>	CCAAGGATCCTCAAAGAAAAAACATCTTGGGGT	AACCCTCGAGAACCACCTTGATGATTACATGGAAC
4	<i>p53</i> RT	TCGATCCATCATTTGCATGTT	ACCACTTGATGATTACATGG
5	<i>ei24 in situ</i>	TTGGCTCGAGTCATTATGGGTTTATCCAGTT	GGTTAAGCTTTGAAACACCAAAATGAATA
6	<i>ei24</i> KF1	TTCCGGATCCGAGACATTTAAGAATATGTA	AAGGAAGCTTAGACATTACCAAAATTACACC
7	<i>ei24</i> KF2	GAAGTCTAGAGCAATCATTGCATTCATTCCA	TGTGCGGCCGATTTGTTTTGTAGTTGGTGA
8	<i>Bsr</i>	TTTGTCCATTCGAAACTGCA	TGCAGTTTCGAATGGACAAA
9	<i>ei24</i>	AACAACACACAAAAGGAAAT	ATGATCGTTACAAGTGAAAATATG
10	<i>ig7</i> RT	TGAATTGAAGTCTGAGTAAACGG	TAGATAGGGACCAAACTGTCTCAC
11	<i>acaA</i> RT	AGTACACCACATAATAAATCAT	CTCTGGAATTACAATATCTCTCTT
12	<i>carA-1</i> RT	TGTATGGCAGTGTGATTGGT	ATGGTGATGGATTTGTTATTGT
13	<i>pdsA</i> RT	ATGGCATTAAATAAAAAATT	TAAATACAAATTTGGATCACC
14	<i>gbfA</i> RT	CCATTACCATTACCATCTATA	TGATGGTGATGGTGTATTACT
15	<i>cadA</i> RT	TCTGTTGATGCAAATAAAGTAAAA	ATAGTCATATGGTGTATGTGTTTG
16	<i>csaA</i> RT	GTGAACGACTCTATTAACCTGCT	AGTTGGAGTGTCTGGAATTGTATA
17	<i>ctnA</i> RT	ATTTTAGCTTTATTCTTGCAAC	GTGTAAGCAATTGAGAGGGTGAAT
18	<i>cotA</i> RT	TAATAAGCTTGAAAGATAATTGTGGAGAAGGTGGTGATG	TTATCTCGAGGGAAGAGCTTGATGATGCAGATGAAG
19	<i>cotB</i> RT	GGTCAAGCTTAGAGATAGTAACGATTGTCTTGCTAG	TTACCTCGAGATAGTTGATGGATTGATACAGATTGG
20	<i>rad51</i> RT	TGTCATACATTATGTGTA	TTGTTCTTTATAATCGG
21	<i>rad52</i> RT	CAAGAGGATGTTGGTTAT	TTGGAATTGAGGGATGGC
22	<i>rad54</i> RT	AGAAGTTTAGCACCAAGT	TTTGAAAAGACGTAAGTGC
23	<i>ku70</i> RT	GATGGTGATGATTGGGAT	CAGAGAATTTGGAACCTTG
24	<i>ku80</i> RT	ATGACAACACTACAATACCA	ACGAGTCATTACTGATTG
25	<i>dnapks</i> RT	GAGATGACACAACCTGTTT	ACTTGTCAATATGATCT

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

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