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Data in Brief

Transcriptional profiling of apoptosis-deficient *Drosophila* mutants

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ABSTRACT

Apoptosis is a fundamental way to remove damaged or unwanted cells during both developmental and post-developmental stages. Apoptosis deficiency leads to various diseases including cancer. To know the physiological changes in apoptosis-deficient mutants, we conducted non-biased transcriptomic analysis of *Drosophila dark*^{cd4} mutants. As recently reported, combined with metabolome and genetic analysis, we identified systemic immune response, energy wasting, as well as alteration in S-adenosyl-methionine metabolism in response to necrotic cells [1]. Here, we describe in detail how we obtained validated microarray dataset deposited in Gene Expression Omnibus (GSE47853). Our data provide a resource for searching transcriptional alterations in *Drosophila* apoptosis-deficient mutants.

Specifications	
Organism/cell line/tissue	*Drosophila melanogaster*
Sex	Male
Sequencer or array type	Agilent Technologies, DNA microarray system
Data format	Raw data
Experimental factors	Apoptosis-deficient (*dark*^{cd4} homozygous mutant) vs wild type control
Experimental features	Day-five adult flies, whole body homogenates
Consent	n/a
Sample source location	n/a

Direct link to deposited data.

http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE47853.

Experimental design, materials and methods

Drosophila preparation

Flies were maintained on a standard diet containing 4% yeast, 4% cornmeal, 10% glucose and propionic acid. All flies were kept in 25 °C with 60% humidity in the alternate 12 h light and dark cycle. As a model of apoptosis-deficient mutant, we utilized a hypomorphic allele of *Drosophila apaf1* ortholog, *dark*^{cd4} mutants, in which both developmental and stress-induced apoptosis were remarkably diminished [2,3]. For the precise control of genetic background, we have backcrossed *dark*^{cd4} mutants six generations into *w*^{1118} control strains.

RNA extraction, purification, and quality verification

All flies were collected within one day after adult eclosion and incubated for five days for adult maturation with free access to food and mating. Five male flies were collected in one sampling tube and immediately frozen in liquid nitrogen. Flies were homogenized in TRIzol reagent (Invitrogen) by Multi-Beads Shocker (Yasui Kikai) set to 1500 rpm, 15 s × 3 cycles, and total RNA was extracted as reported [4]. Total RNA was then purified using RNeasy Plus Micro Kit (Qiagen) according to the manufacturer's instruction. After checking RNA concentration and purity by NanoDrop 2000c (Thermo Fisher Scientific), RNA quality and quantity were further validated by an Agilent 2100 Bioanalyzer and the Agilent RNA 6000 Nano Kit (Agilent Technologies). Four independent RNA samples of high quality, which had two sharp peaks of 18S and 28S ribosomal RNA [5] were subjected to microarray analysis (Fig. 1).

Experimental procedures for microarray analysis

Cyanine-3 (Cy3)-labeled cRNA was prepared from 50 ng of total RNA by Low Input Quick Amp Labeling Kit, One-Color (Agilent Technologies) according to the manufacturer's instruction. cRNA was purified by RNeasy Mini Kit (Qiagen), and cRNA yield (more than 0.825 μg) and labeling efficiency (6 pmol/μg) were validated by NanoDrop 2000c spectrophotometer (Thermo Fisher Scientific). 600 ng of Cy3-labeled cRNA was then fragmented in a 30-minute incubation at 60 °C

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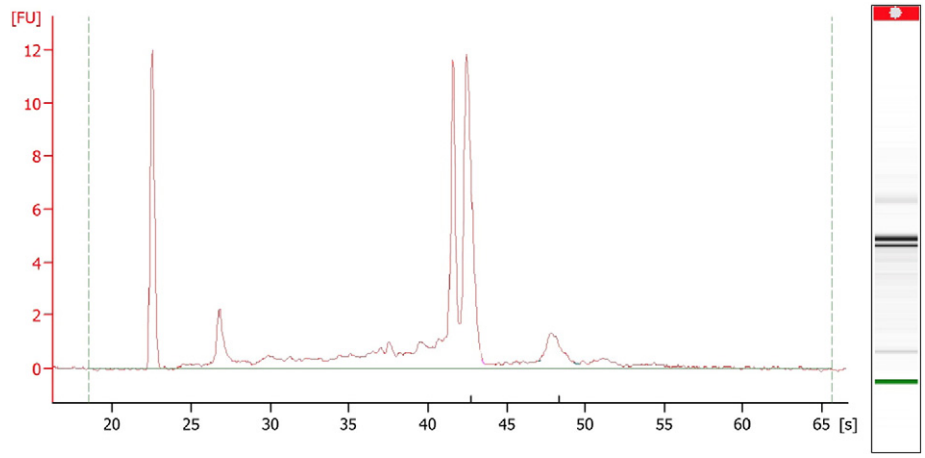

Fig. 1. An example of RNA quality validation by Agilent 2100 Bioanalyzer. *Drosophila* total RNA contains two main peaks, 18S and 28S ribosomal RNA.

Table 1
List of differentially expressed entities in $dark^{cd4}$ (up-regulated, fold change > 1.5, $p < 0.05$).

Probe name	Gene symbol	p-Value	Corrected p-value	Fold change	Entrez gene ID
A_09_P137560	CG6484	1.21E−07	7.59E−04	826.2603	36994
A_09_P074111	CG3397	3.84E−07	0.001204653	543.77155	41454
A_09_P057606	CG30091	3.29E−06	0.005706159	259.83295	246449
A_09_P022946	CG6639	7.32E−06	0.009384108	156.37743	35049
A_09_P012361	Dro	9.64E−06	0.010052347	84.814896	36635
A_09_P164825	CG3397	5.14E−05	0.020792374	54.593582	41454
A_09_P109300	CecC	2.58E−04	0.044110067	50.98296	43599
A_09_P205050	AttA	3.38E−05	0.017613623	45.11442	36636
A_09_P022951	CG18563	1.37E−04	0.036148455	44.82299	35050
A_09_P177575	AttA	5.63E−05	0.022063293	42.283108	36636
A_09_P029506	AttA	2.88E−05	0.016047167	37.23442	36636
A_09_P076441	Lip3	1.72E−04	0.039214883	25.980417	41643
A_09_P042191	Eh	2.37E−06	0.004951735	24.375402	42101
A_09_P017606	CG33459	1.48E−04	0.037204254	22.659021	2768847
A_09_P064286	AttB	1.77E−04	0.039214883	17.377863	36637
A_09_P070721	CG13822	1.98E−05	0.013739567	8.938663	42787
A_09_P022706	pburs	2.07E−04	0.042714648	8.529773	34845
A_09_P074106	CG12224	2.45E−05	0.015018947	7.8313622	41453
A_09_P075311	Spn88Eb	3.79E−05	0.017992863	7.8204455	41829
A_09_P191620	hbs	3.69E−04	0.049772616	7.671836	44129
A_09_P070756	CG10232	3.85E−05	0.017992863	7.1368327	42800
A_09_P199616	Amy-d	3.83E−06	0.006001143	5.9436913	36932
A_09_P142340	Strn-Mlck	1.49E−04	0.037204254	5.568332	36753
A_09_P205465	Amy-p	1.76E−05	0.013231299	5.1067333	47764
A_09_P022091	CG14935	3.30E−04	0.04758134	5.0741186	34598
A_09_P210570	Ast-CC	1.16E−05	0.010380089	4.611063	34538
A_09_P032031	Khc-73	9.36E−05	0.030085392	4.4820924	36718
A_09_P030336	Cp7Fa	5.23E−05	0.0208358	4.3110275	3885650
A_09_P128140	PGRP-SA	3.08E−04	0.045933884	4.280494	32099
A_09_P041241	Amy-d	7.92E−06	0.009384108	4.2164664	36932
A_09_P023551	CG13077	2.78E−04	0.044967778	4.1103	35252
A_09_P013241	elk	2.40E−05	0.015018947	4.0061827	37047
A_09_P029906	Cyp6a9	2.27E−05	0.014955865	3.9369826	36663
A_09_P132150	Cyp6a9	3.44E−05	0.017613623	3.8976274	36663
A_09_P028966	CG14963	2.40E−04	0.043355178	3.778797	38383
A_09_P213450	TepII	9.73E−05	0.030148381	3.7582188	34044
A_09_P043731	MtnA	2.14E−04	0.042714648	3.753855	41202
A_09_P133185	MtnA	2.27E−04	0.042714648	3.7424712	41202
A_09_P008251	Tsf3	2.43E−04	0.043355178	3.7416558	36800
A_09_P212340	CG6283	2.37E−05	0.015018947	3.6835904	43250
A_09_P072171	CG6283	3.06E−05	0.016309947	3.4509046	43250
A_09_P064071	TepII	8.46E−05	0.028292092	3.150775	34044
A_09_P070726	CG10157	1.79E−04	0.039407473	2.9077814	42788
A_09_P030331	Cng	6.87E−05	0.02535672	2.8066733	36806
A_09_P007626	CG8613	6.21E−09	1.56E−04	2.802572	36593
A_09_P057376	CG30026	1.64E−04	0.03885829	2.7476745	246399
A_09_P074211	CG17404	3.38E−04	0.0476427	2.7276456	41482
A_09_P021871	CG16743	1.20E−04	0.033890933	2.6894252	34517
A_09_P032876	Thor	3.51E−04	0.048971787	2.6882927	33569
A_09_P007811	CG10205	2.72E−05	0.015888466	2.3559587	36650
A_09_P003151	CG34195	1.40E−04	0.036542486	2.2845542	37018

(continued on next page)

Table 1 *(continued)*

Probe name	Gene symbol	p-Value	Corrected p-value	Fold change	Entrez gene ID
A_09_P052476	ple	1.51E−04	0.037204254	2.1827874	38746
A_09_P074666	CG8449	2.99E−04	0.045802735	2.1643517	41628
A_09_P030576	Fer1HCH	3.29E−04	0.04758134	2.0881882	46415
A_09_P017531	Sp212	2.59E−04	0.044110067	2.0289657	2768666
A_09_P013336	Pms2	1.37E−04	0.036148455	1.9307616	36705
A_09_P128510	pot	1.75E−04	0.039214883	1.8138448	32154
A_09_P074856	CG9312	1.43E−05	0.011565035	1.7268412	41686
A_09_P112520	CG31664	3.71E−04	0.049772616	1.7204942	33359
A_09_P064416	yellow-c	3.59E−04	0.049245566	1.7027588	34879
A_09_P079531	CG1927	4.83E−05	0.020355958	1.700692	38262
A_09_P171400	CG9760	1.11E−05	0.010331039	1.7006437	39388
A_09_P030571	Fer2LCH	4.83E−05	0.020355958	1.6878232	44965
A_09_P078611	Idgf4	1.69E−04	0.039214883	1.6784037	31926
A_09_P121595	Wnt2	2.12E−04	0.042714648	1.6761436	35975
A_09_P079936	CG14787	1.32E−04	0.036148455	1.6502483	31096
A_09_P145165	CG9284	7.03E−05	0.025569357	1.6346469	50130
A_09_P055421	CG10646	7.58E−05	0.026382491	1.6279341	39426
A_09_P032636	Spred	1.96E−04	0.041651685	1.5928276	36643
A_09_P154000	Idgf4	3.09E−04	0.045933884	1.5830332	31926
A_09_P020526	spz3	2.86E−04	0.045345914	1.5688794	34077
A_09_P217505	CG9449	3.00E−04	0.045802735	1.5068132	40117
A_09_P125405	Fer2LCH	2.94E−04	0.045802735	1.5040354	44965

Table 2
List of differentially expressed entities in *dark*^{cd4} (down-regulated, fold change > 1.5, $p < 0.05$).

Probe name	Gene symbol	p-Value	Corrected p-value	Fold change	Entrez gene ID
A_09_P057096	CG9284	1.46E−08	1.83E−04	−8526.699	50130
A_09_P056531	Obp57a	2.21E−07	9.46E−04	−460.8703	246670
A_09_P112980	CG6602	1.04E−05	0.010052347	−228.13199	38691
A_09_P058096	CG30325	5.27E−08	4.41E−04	−117.28926	246541
A_09_P017491	CG33306	1.56E−06	0.003922256	−89.36473	2768915
A_09_P025106	CG14759	1.51E−04	0.037204254	−55.705208	35803
A_09_P022106	CG16964	2.44E−04	0.043355178	−16.280725	34605
A_09_P053136	Jon65Aii	1.51E−04	0.037204254	−13.278319	38684
A_09_P025336	CG8235	2.75E−04	0.044967778	−8.24512	35892
A_09_P147930	RpS23	6.10E−05	0.022846662	−7.943826	36576
A_09_P009466	CG10051	1.29E−04	0.035887856	−6.1546483	37222
A_09_P103745	Ark	3.21E−06	0.005706159	−6.0242305	36914
A_09_P010541	LysB	1.15E−04	0.033018567	−5.5206275	38125
A_09_P048621	se	2.03E−05	0.013739567	−4.5446115	38973
A_09_P076786	Ark	6.00E−05	0.022796065	−4.5321417	36914
A_09_P051166	Pkn	2.25E−04	0.042714648	−3.7968385	35950
A_09_P008396	Syn2	7.89E−06	0.009384108	−3.762351	36848
A_09_P058576	CG30471	7.76E−05	0.026382491	−3.7158039	246633
A_09_P076781	Ark	2.34E−04	0.042868607	−3.460493	36914
A_09_P023806	CG9317	8.23E−06	0.009384108	−3.2488701	35334
A_09_P023916	CG9270	3.87E−05	0.017992863	−3.2225685	35366
A_09_P008036	CG8204	2.19E−04	0.042714648	−3.194339	36728
A_09_P007056	CG12374	3.38E−04	0.0476427	−3.0680518	36410
A_09_P007696	Oaz	3.26E−04	0.04751293	−2.807796	36609
A_09_P061211	CG7912	2.88E−04	0.045445487	−2.6248696	43561
A_09_P054936	CG12289	1.02E−04	0.030490918	−2.5969746	39279
A_09_P022701	CG16885	3.33E−04	0.0476427	−2.499752	34811
A_09_P061331	CG9682	3.36E−04	0.0476427	−2.149636	43602
A_09_P004556	CG34457	2.29E−04	0.042714648	−2.1067915	5740661
A_09_P008606	CG15605	1.79E−05	0.013231299	−2.0780075	36933
A_09_P024716	CG11112	1.33E−05	0.0111519	−2.0632255	35658
A_09_P007636	Arc1	4.58E−05	0.020355958	−2.0212839	36595
A_09_P054941	CG7551	7.68E−05	0.026382491	−1.9311175	39280
A_09_P008236	mrj	1.91E−04	0.041254394	−1.926147	36797
A_09_P134485	Aplip1	1.76E−04	0.039214883	−1.8730602	53472
A_09_P057636	CG30099	3.87E−05	0.017992863	−1.7842374	36818
A_09_P023122	Ntf-2r	3.18E−04	0.046977073	−1.7637537	35101
A_09_P196550	Rh6	4.87E−05	0.020355958	−1.732648	41889
A_09_P224825	Os-C	1.11E−04	0.03227867	−1.6809722	40942
A_09_P055141	CG7264	2.32E−04	0.042849243	−1.6779261	39339
A_09_P023376	CG17572	2.22E−04	0.042714648	−1.6729306	35182
A_09_P008841	CG6410	3.47E−04	0.048562963	−1.6300013	37015
A_09_P202455	Rh6	3.03E−04	0.045802735	−1.6175661	41889
A_09_P020711	CG14277	2.21E−04	0.042714648	−1.5980519	34129
A_09_P031996	Rh6	2.76E−04	0.044967778	−1.5790434	41889
A_09_P112005	CG10357	1.49E−05	0.011698553	−1.5706613	38435
A_09_P049331	Cbp53E	1.95E−04	0.041651685	−1.5593123	36905
A_09_P008671	Lhr	1.60E−04	0.038315967	−1.501875	36957

in a reaction mixture containing 1× Agilent fragmentation buffer and 2× Agilent GE blocking agent. After fragmentation, 2× Agilent GE hybridization buffer HI-RPM was added to the sample and then hybridized to SurePrint G3 custom microarray 8 × 60K (G4102A#040871) for 17 h at 65 °C in a rotating Agilent hybridization oven (Agilent Technologies). Slides were scanned after washing on the SureScan Microarray Scanner using AgilentG3_GX_1Color_HighSensitivity (Agilent Technologies). Feature Extraction Software 10.7.3.1 (Agilent Technologies) was used with default parameters (protocol GE1_107_Sep09 and Grid: 040871_D_F_20120511) to obtain background-subtracted and spatially-detrended Processed Signal intensities. Data quality was evaluated by Evaluation Metrics for GE1_QCMT_Sep09 in the QC Report.

Data processing and analysis

Extracted text data were processed using GeneSpring GX12.1 (Agilent Technologies). Non-uniform or saturated probes as well as population outliers were compromised and quantile normalization was applied to each data set as the following setting: Threshold raw signal 1.0, Algorithm, Percentile Shift, Percentile Target, 75. Baseline was corrected by the median of all samples. Probes from all samples with intensity less than 20% were filtered out, resulting in 25,083 validated entities. These data from four independent samples for wild type and $dark^{cd4}$ flies were subjected to statistical analysis by unpaired student's *t*-test with Benjamini Hochberg FDR correction. We obtained differentially expressed 188 ($p < 0.05$) or 481 entities ($p < 0.1$), and subsequent cut-off by fold change > 1.5 yielded 149 (Tables 1, 2) or 321 entities, respectively. GO analysis of these entities clearly demonstrated that immune-related genes were drastically elevated in $dark^{cd4}$ mutants, while no GO term was enriched significantly for down-regulated genes. dFoxO target genes such as *thor* or *lip3* were also significantly induced in $dark^{cd4}$ mutants (Table 1). Reduction in *dark* expression was confirmed as three entities corresponding to *dark* were downregulated 6.0-, 4.5- and 3.5-fold compared to control (Table 2). *Drosophila gnmt*, the gene of our interest from metabolome analysis [1], was also included in the list of upregulated genes ($p < 0.1$), as two probes indicated 3.1- and 3.2-fold increase in $dark^{cd4}$ mutants.

Discussion

Here we described a transcriptomic profiling of *Drosophila* apoptosis-deficient mutants, $dark^{cd4}$. As reported recently, necrotic wing cells triggered spontaneous immune response in apoptosis-deficient mutants at this stage. Our well-controlled microarray data delineated the phenotypes observed in $dark^{cd4}$ mutants and helped us clarify the systemic responses against necrotic cells. As far as we know, this is the first microarray analysis to describe transcriptional changes in apoptosis-deficient mutants in *Drosophila*. It is interesting that many other genes are also down- or up-regulated in these mutants, and this dataset may be useful for revealing novel and unexpected phenotypes triggered in response to necrosis or other functions of Dark/caspase.

Conflict of interest

The authors declare no conflicts of interest.

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