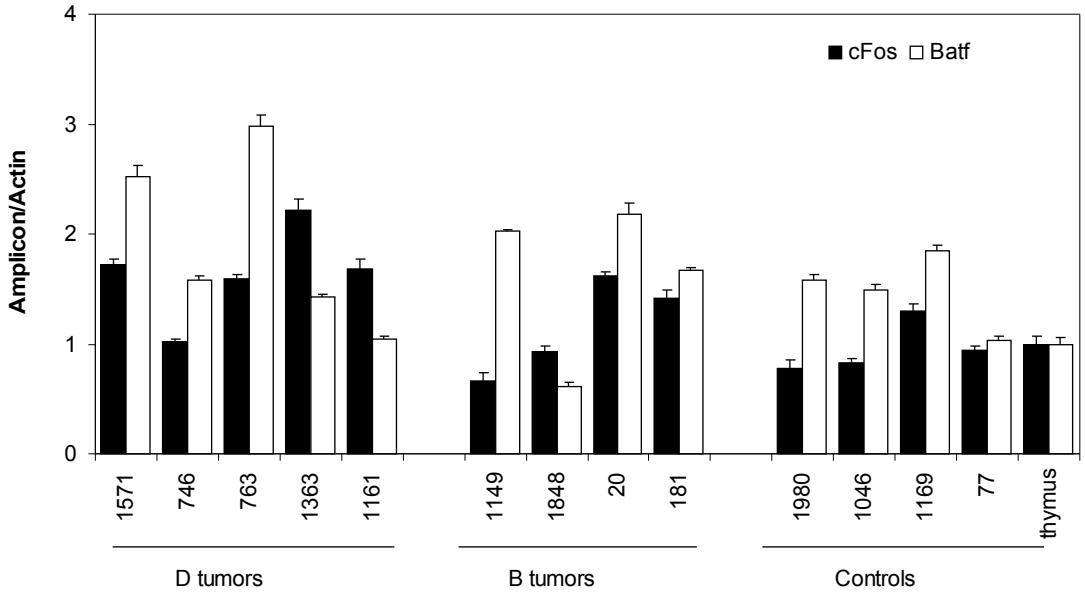

Figure S1. *cFos* and *Batf* are not deregulated as a consequence of MLV insertion in the *Jdp2* locus. The mRNA levels of the *Jdp2*-neighboring genes *cFos* (black bars) and *Batf* (white bars) were quantified by quantitative real-time PCR on a subset of D tumors (MLV integration in *Jdp2* intron 2) and B tumors (MLV integration upstream of *Jdp2*) and compared to that of tumors without integration in the locus as found by retroviral tagging. Signal was normalized to *Actb* (β-Actin) and is shown as fold difference to uninfected thymus (set to 1). Error bars indicate s.e.m. of a triplicated experiment.

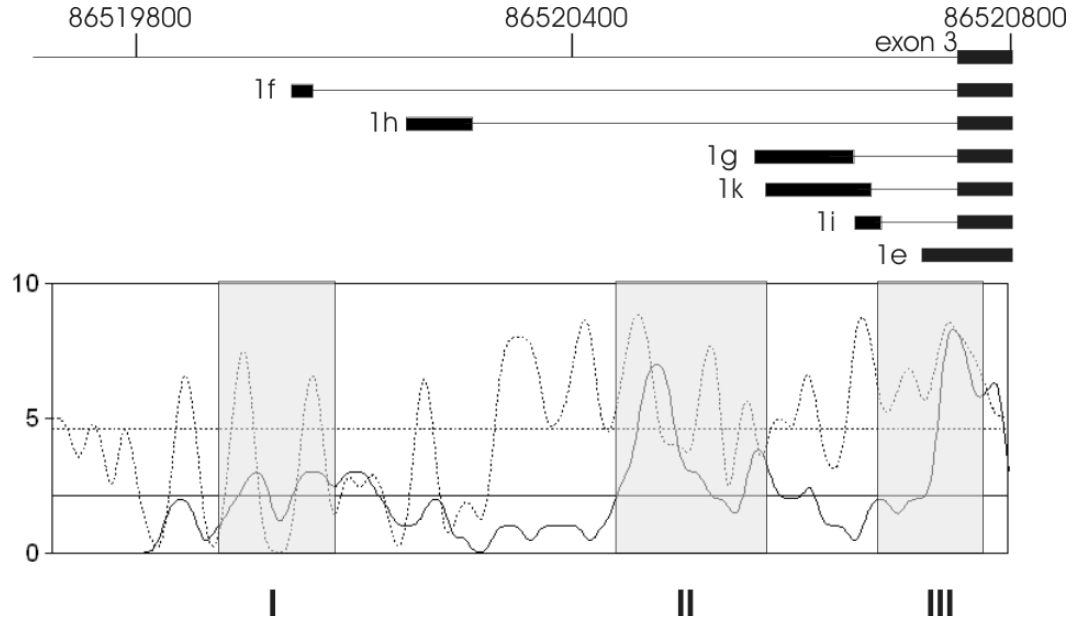

Figure S2. Conservation of the 3' end of *Jdp2* intron 2.
The various alternative exon 1-exon 3 structures are shown schematically as black boxes with chromosomal positions above (UCSC mm8 Feb 2006) (*top*). Relative similarity score for Dialign alignment (see Materials and Methods) between orthologous regions of mouse, rat, human, chimpanzee, macaque, dog and cow (black line) and mouse and rat (stippled line) with horizontal lines marking the average score across the region (chr12: 86519676-86520803, UCSC mm8 Feb 2006) (*bottom*). Larger regions with no homology to mouse were removed from further analysis. Three selected sub-regions (I-III) with high relative conservation score are grey-shaded and analyzed for conserved *cis*-regulatory motifs in Fig S3-5.

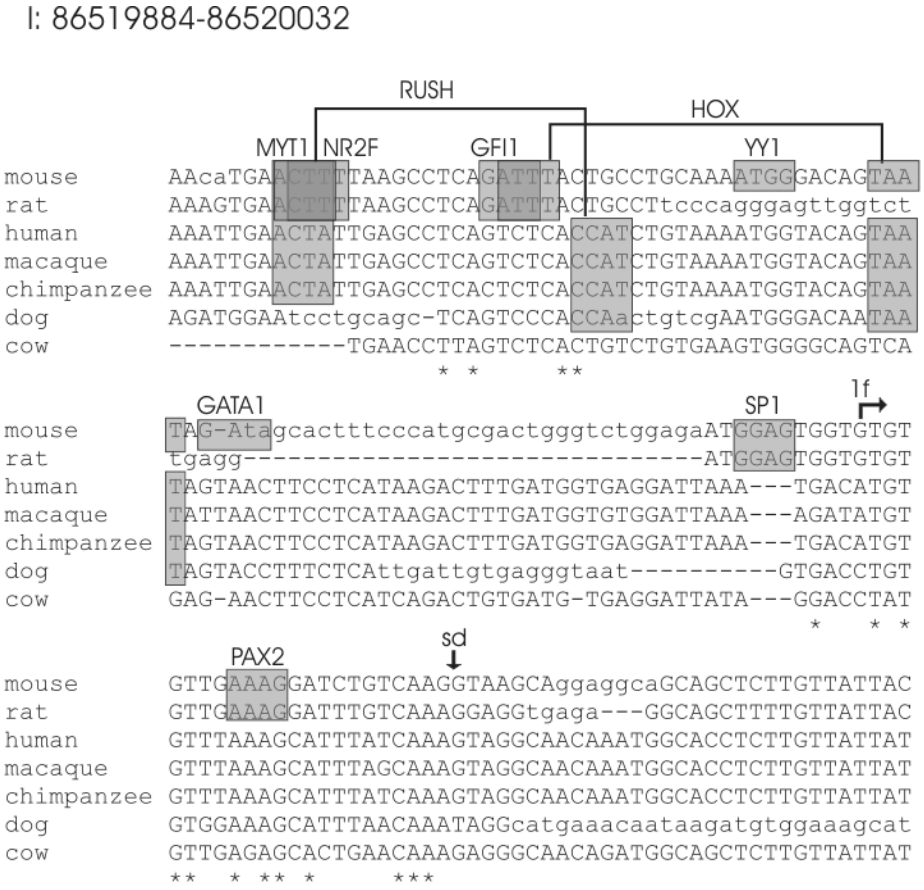

Figure S3. *cis*-regulatory motifs in sub-region I.
7-species alignment of sub-region I (chr12: 86519884-86520032 according to the mouse genome mm8 assembly, see Fig. S2) with potential binding sites for transcription factors/modulators as found using the MatInspector suite. The names of the matrix family is given above the 4 bp 'core' binding motifs (grey boxes). For clarity, 'core' binding motifs for non-mouse species are only shown if also found in the mouse sequence. Binding site motifs in exon 3 are omitted. A star (*) indicates maximum conservation for the individual base. Capital letters denote bases considered homologous by Dialign while lower-case letters are not considered aligned. Transcription start site of exon 1f (1f) and the exon 1f splice donor site (sd) are indicated.

II: 86520343-86520514

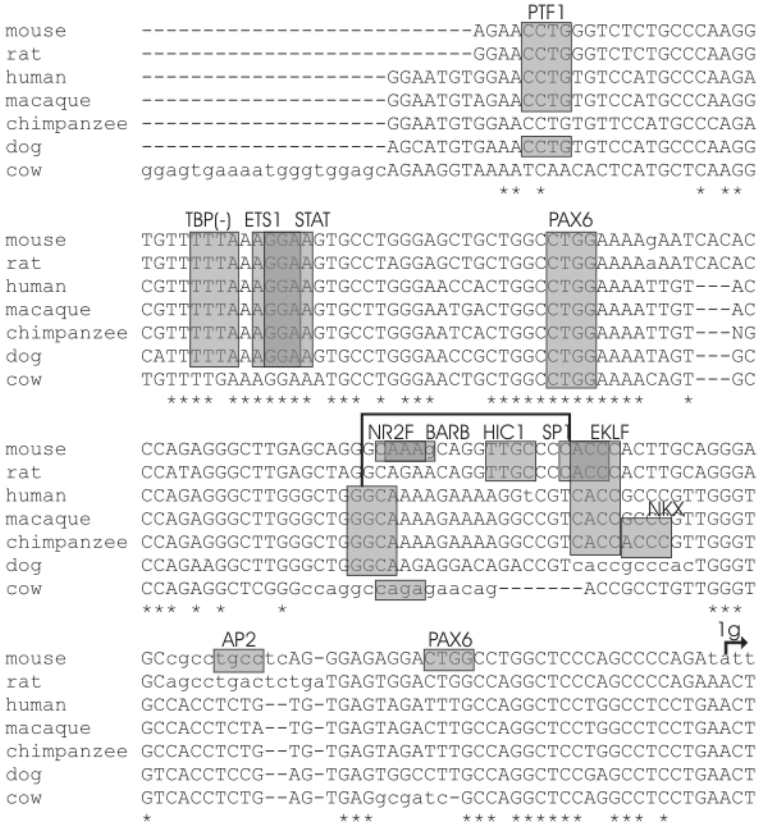

Figure S4. *cis*-regulatory motifs in sub-region II.
7-species alignment of sub-region I (chr12: 86520343-86520514 according to the mouse genome mm8 assembly, see Fig. S2). The transcription start site of exon 1g (1g) is indicated. Legend as in Fig. S2.

III: 86520633-86520780

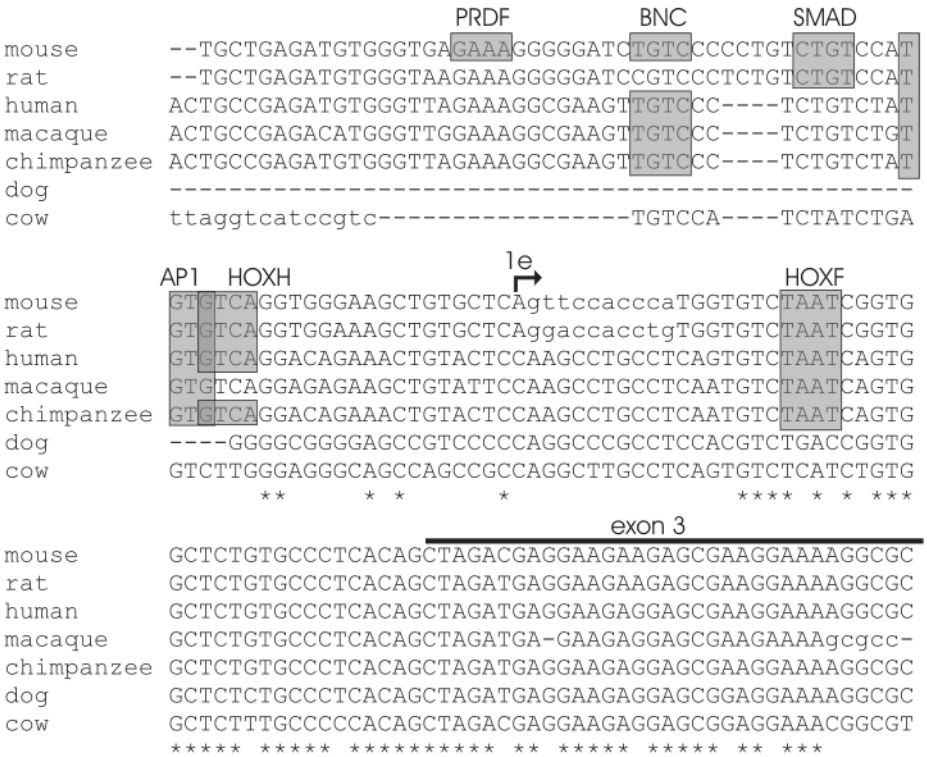

Figure S5. *cis*-regulatory motifs in sub-region III.
7-species alignment of sub-region I (chr12: 86520663-86520780 according to the mouse genome mm8 assembly, see Fig. S2). The transcription start site of exon 1e-1 (1e) as well as exon 3 sequence is indicated. Legend as in Fig. S2.

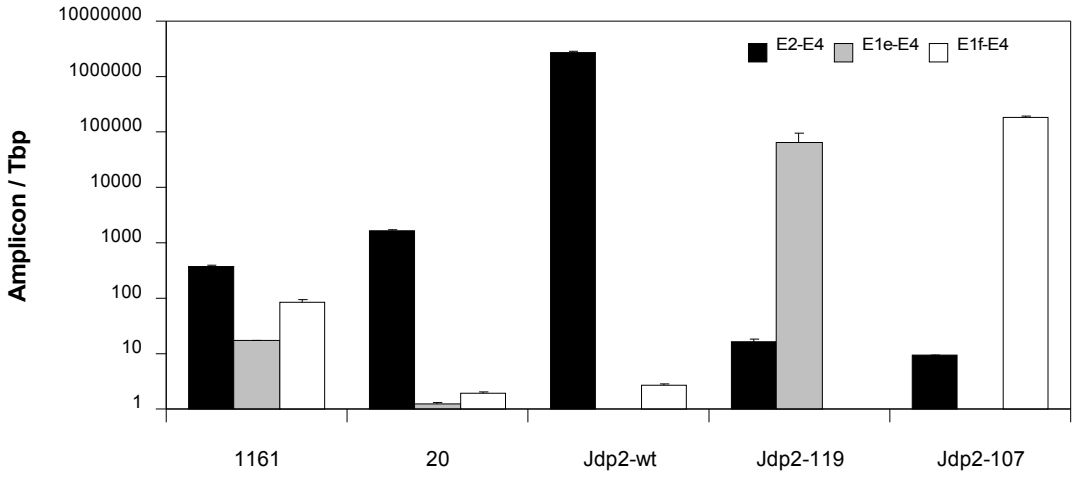

Figure S6. Ectopic expression of *Jdp2* and *Jdp2* isoforms in fibroblasts exceed the MLV-induced expression in murine T-cell lympomas.
Expression levels of *Jdp2* wild type (amplicon E2-E4, black bars), *Jdp2* exon 1e (amplicon E1e-E4, grey bars) or *Jdp2* exon 1f (amplicon E1f-E4, white bars) were assessed by quantitative real-time PCR on cDNA from tumors 1161 and 20 expressing high levels of canonic and alternative *Jdp2*, and NIH 3T3 cells transiently transfected with either pFLAG-Jdp2-wt, pFLAG-Jdp2-119 or pFLAG-Jdp2-107. Signal was normalized to *Tbp* levels and is shown as fold difference to cell transfected with empty vector (set to 1). Standard bars indicate s.e.m. of an experiment run in triplicate. Note the ten fold upregulation of canonic *Jdp2* mRNA upon over-expression of Jdp2-119 and Jdp-107.

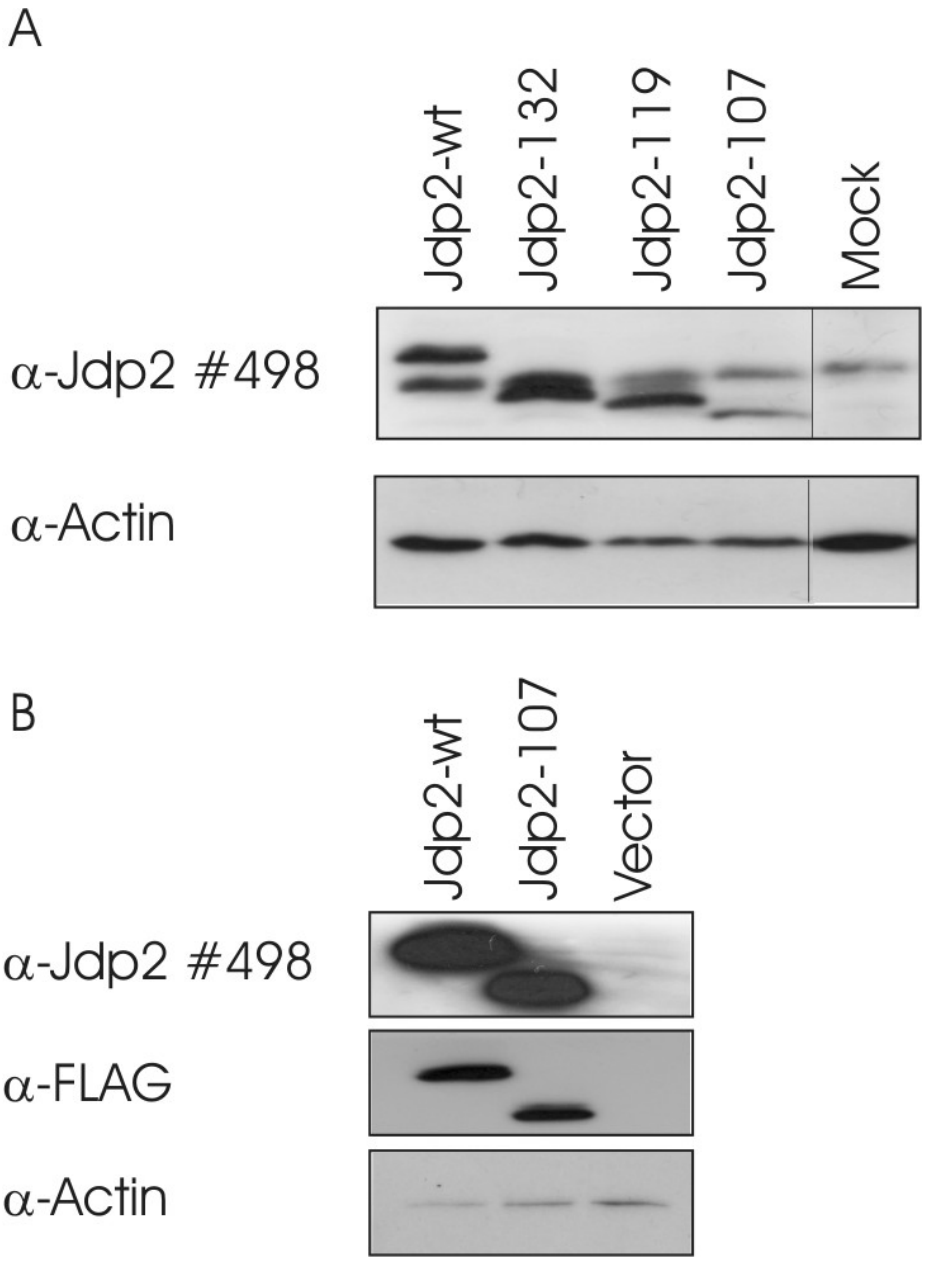

Figure S7. Stable retroviral/IRES-driven expression of Jdp2 results in lower ectopic protein levels compared to plasmid-derived transient expression.
(A) Whole cell extracts from NIH 3T3 murine fibroblasts transduced at a multiplicity of infection = 1 with AKV-*neo*-IRES expressing FLAG-tagged Jdp2-wt, Jdp2-132, Jdp2-119 or Jdp2-107 were subjected to immunoblotting with polyclonal anti-Jdp2 antibody. Mock is an AvrII-fragment deletion of AKV-*neo*-IRES lacking part of the IRES. Vertical lines indicate repositioned lanes. (B) Whole cell extracts from NIH 3T3 murine fibroblast transiently transfected with pFLAG vector expressing either full length Jdp2 or Jdp2-107 isoform were subjected to immunoblotting with the same polyclonal anti-Jdp2 antibody as in (A), and subsequently with an anti-FLAG antibody. After extended exposure endogenous Jdp2 in cells transfected with the empty vector (Vector) could be distinguished (not shown). Approximately 15 times less protein was used in (B) than in (A). Immunblotting with an anti-β-Actin antibody was done as loading control.

MATERIAL AND METHODS

Bioinformatic analyses. An 1129 bp region from 300 bp upstream of the transcription start site (TSS) of exon 1f to 100 base pair downstream of the TSS of exon 1e was extracted from the mm8 Feb mouse assembly (http://genome.ucsc.edu/). Orthologous sequence chains from rat (rn4), human (hg18), chimpanzee (panTro2), macaque (rheMac2), dog (canFam2), cow (bosTau3), and chicken (galGal3) were obtained. Due to almost complete lack of sequence similarity outside exon 3 chicken was excluded from further analysis. All species-multiple alignment and pair-wise rat-mouse alignment were done with Dialign (http://dialign.gobics.de/), which creates a global multiple alignment by assembling of local gap-free alignments (1). The relative alignment score on individual base pairs (9 denoting maximum similarity) as computed by Dialign was plotted onto the murine region for rat-mouse and all-species alignments. To search for transcription factor binding motifs sequences were analyzed using the free academic MatInspector license from Genomatix (http://www.genomatix.de/) using default parameters. We only considered transcription factor binding motifs with 'core similarity' higher than 0.85 and 'matrix similarity' above the optimized threshold as described (2).

Real-time quantitative PCR. Quantitative real-time PCR (QRT-PCR) was done in triplicate using Platinum SYBR Green QRT-PCR SuperMix UDG (Invitrogen) following the manufacturer's recommendations. Amplifications were done on a Mx3005 apparatus (Stratagene) with cDNA corresponding to 15 ng total RNA. Relative quantification was done with the Window-of-Linearity-based method applying LinReqPCR software (version 11) using an averaged amplification efficiency per amplicon per run (3). Signal was normalized to the expression level of either the TATA box binding protein (*Tbp*) (Fig S6) or *Actb* (β-Actin) (Fig S1). Primers in *cFos* were: Fos-178, 5'-atgggctctcctgtcaacac-3' and Fos-179, 5'-cacggaggagaccagagt-3'. Primers in *Batf* were: Batf-74, 5'-gaagagccgacagagacagac-3' and Batf-77, 5'-cctcggtgagctgtttgat-3'. Primers in *Tbp* and *Actb* were: Tbp-60, 5'-agagagccacggacaactg-3'; Tbp-61, 5'-actctagcatattttcttgctgct-3'; Actin-165, 5'-acacagtgctgtctggtggt-3'; Actin-166, 5'-ctggaaggtggacagtgagg-3'. PCR conditions consisted of an initial melting step of 95 °C for 10 minutes, then amplification was conducted through 40 repetitions of a 95 °C step for 15 seconds and a combined annealing and extension step at 58 °C for 30 seconds. Lack of unspecific amplification was ensured with a final melting curve analysis from 55 °C to 95 °C.

Cell culture and cell culture experiments. NIH 3T3 cells were grown in Dulbecco's Modified Eagle's Medium supplemented containing Glutamax (Gibco) and supplemented with 10 % new born calf serum (Invitrogen) and 1 % penincillin-streptomycin. Cells were transfected with a pSG5-modified vector pFLAG expressing N-terminally FLAGed Jdp2, Jdp2-132, Jdp2-119 or Jdp2-107 using Lipofectamine 2000 (Invitrogen) according to the manufacturer's recommendations. Transduction with pAKV-*neo*-IRES expressing N-terminally FLAGed Jdp2, Jdp2-132, Jdp2-119 or Jdp2-107 was done in the presence of 6 ug/ml polybrene. Whole cell extracts for Western blot analysis were obtained 48 hours post transfection/transduction using a cell scraper in radioimmunoprecipitation assay buffer (50 mM TRIS-HCl pH 8, 150 mM NaCl, 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS) complemented with 1 mM PMSF and complete Mini, EDTA-free (Roche) protease inhibitor cocktail. Samples were briefly subjected to sonication, incubated on ice for 30 minutes and

protein collected after centrifugation for 30 minutes at 13000 * g. Total protein concentrations were determined according to a bovine serum albumin standard curve using the BCA kit (Pierce) Total RNA was extracted 48 post transfection using TRIZOL reagent (Invitrogen) following the manufacturer's recommendations. The integrity of the isolated RNA was assessed by visual inspection of the intensity of the 28S and 18S ribosomal bands on a non-denaturating ethidium bromide stained agarose gel. RNA quantity was determined spectrophotometrically.

Western blotting. Whole cell extracts separated by 15% SDS-PAGE, and electro-transferred onto a 0.2 μM P^{SQ} polyvinylidene fluoride (PVDF)-membrane (Millipore). Membranes were blocked at room temperature in TBS (20 mM TRIS-HCl, 200 mM NaCl pH 7.6) containing 5% (w/v) fat-free milk for one hour, incubated with primary antibody over-night at 4 °C in TBS-T (TBS with 0.05% Tween-20) containing 5% (w/v) fat-free milk (polyclonal anti-Jdp2, 1:1200 dilution, polyclonal anti-β-Actin (I-19), 1:6666 dilution Santa Cruz Biotechnology, sc-1616, and monoclonal anti-FLAG-HRP, 1:2000 dilution), washed three times in TBS-T, incubated with secondary antibody for forty-five minutes at room temperature in TBS-T with 5% fat-free milk (goat anti-rabbit IgG-HRP, 1:10000 dilution, Santa Cruz Biotechnology sc-2004; rabbit anti-goat IgG-HRP, 1:5000 dilution, Santa Cruz Biotechnology sc-2768) and finally washed three times in TBS-T. Membranes were developed using ECL PLUS Western Blotting Detection System (GE Healthcare) and exposed to medical film (Konica X-Ray AX medical films Santax Medico). Rabbit polyclonal anti-Jdp2 serum was kindly provided by Dr. A. Aronheim, Haifa, Israel (4).

REFERENCES

1. Brudno, M., Chapman, M., Gottgens, B., Batzoglou, S. and Morgenstern, B. (2003) Fast and sensitive multiple alignment of large genomic sequences. *BMC Bioinformatics*, **4**, 66.
2. Cartharius, K., Frech, K., Grote, K., Klocke, B., Haltmeier, M., Klingenhoff, A., Frisch, M., Bayerlein, M. and Werner, T. (2005) MatInspector and beyond: promoter analysis based on transcription factor binding sites. *Bioinformatics*, **21**, 2933-2942.
3. Ruijter, J.M., Ramakers, C., Hoogaars, W.M., Karlen, Y., Bakker, O., van den Hoff, M.J. and Moorman, A.F. (2009) Amplification efficiency: linking baseline and bias in the analysis of quantitative PCR data. *Nucleic Acids Res*.
4. Aronheim, A., Zandi, E., Hennemann, H., Elledge, S.J. and Karin, M. (1997) Isolation of an AP-1 repressor by a novel method for detecting protein-protein interactions. *Mol Cell Biol*, **17**, 3094-3102.