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Organochlorine mediated potentiation of the general coactivator p300 through p38 mitogen-activated protein kinase *

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ABSTRACT

The activity of nuclear transcription factors is often regulated by specific kinase signaling pathways. We have previously shown the organochlorine pesticide DDT [dichlorodiphenyltrichloroethane] stimulates AP-1 [activator protein-1] activity through the p38 MAPK [mitogen-activated protein kinase]. Here, we show DDT and its metabolites also stimulate the transcriptional activity of CREB [cAMP response element binding protein] and Elk1 and potentiate gene expression through cAMP and hypoxia response elements. Because DDT stimulates gene expression through various transcription factors and hence, multiple response elements, we hypothesized p38 signaling targets a common shared transcriptional activator. Here, we demonstrate using both pharmacological and molecular techniques, the general coactivator p300 is phosphorylated and potentiated by the p38 MAPK signaling cascade. We further show that p38 directly phosphorylates p300 in its N-terminus. These results, together with our previous work, suggest that p38 stimulates downstream transcription factors in part by targeting the general coactivator p300.

INTRODUCTION

Nuclear transcription factors such as activator protein-1 (AP-1) [activator protein-1] and the estrogen receptor (ER) bind specific DNA response elements located in the promoter regions of target genes, driving transcription. Initiation of transcription requires the recruitment and binding of coactivators to specific regions located within the activation domains of the nuclear factors. For example, when stimulated, the AP-1 components c-Jun [Jun oncogene] and c-Fos [FBJ osteosarcoma oncogene] recruit the p300/CBP [p300/cAMP response element binding protein (CREB)-binding protein] class of general coactivators to their N- and C-terminal activation domains, respectively [1,2]. A C-terminal glutamine-rich region in p300/CBP binds the activation domain 1 [AD1] of the p160 class of coactivators, creating a large coactivator complex that helps diverse nuclear transcription factors transcribe particular genes [3].

p300, as a general transcription factor, is essential in growth, proliferation, differentiation, and cell death, and loss of p300 results in an embryonic lethal phenotype[3,4]. p300 binds a diverse array of transcriptional activators, including CREB [cyclic AMP response element binding protein), nuclear steroid receptors, c-Jun, c-Fos, p53, MyoD [myogenic differentiation 1], HIF-1 [hypoxia inducible factor-1], nuclear factor κB [NFκB], and STAT1/2 [signal transducer and activator of transcription 1 and 2] [4]. Recruitment of p300 promotes gene expression through two major mechanisms. First, p300 contains intrinsic HAT [histone acetyl transferase] activity that has been implicated in the unraveling of target gene promoters through the acetylation of the N-terminal tail of histones. Second, p300 recruits and binds components of the core RNA polymerase machinery, suggesting a role as a transcriptional integrator or adaptor [5,6]. While much is known about how coactivators such as p300 function to enhance transcription, relatively little is known about the regulation of these proteins.

p300 was demonstrated to acetylate SRC-3 [nuclear receptor coactivator 3], a p300 interacting protein [7]. This acetylation causes the dissociation of the nuclear receptor transcriptional complex and thus, stops gene expression. Phosphorylation of p300 at serine 1834 by AKT has been shown to modulate p300's HAT activity [8]. PKC [protein kinase C] phosphorylates and represses the transcriptional activity of p300 [9]. Additionally, members of the MAPK signaling pathways, including ERK [extracellular-signal regulated kinase] and MEKK1 [MAPK/ERK kinase kinase 1] have been demonstrated to phosphorylate and potentiate p300 [10-13]. All of these studies suggest that phosphorylation of p300 plays a large role in its regulation and activity.

p38 MAPK potentiates, in a ligand-inducible manner, nuclear transcription factors such as TR [thyroid hormone receptor], ERα, and ERβ [14-17]. Here, we demonstrate that DDT induced signaling, which we have previously shown to function through the p38 MAPK cascade [18], stimulates both multiple promoter response elements and various nuclear transcription factors. The diversity in p38 signaling prompted us to look for a potential common signaling target. Thus, we hypothesize that p38 MAPK phosphorylates and potentiates the p300 coactivator. To test this hypothesis, we used DDT as a pharmacological tool to test the ability of the p38 MAPK signaling cascade to potentiate p300. Our results obtained from molecular and pharmacological inhibitors in addition to *in vitro* kinase assays strongly suggest that p38 phosphorylates the N-terminus of p300, leading to its potentiation as a general transcription factor.

MATERIALS AND METHODS

Chemicals--o,p'DDT, p,p'DDT, o,p'-dichlorodiphenyldichloroethane (DDD), and p,p'-dichlorodiphenyl acetic acid (DDA) were purchased from AccuStandard (New Haven, CT). p,p'-dichlorodiphenyl ethanol (DDOH) was purchased from Sigma (St. Louis, MO). All DDT metabolites were dissolved in dimethyl sulfoxide (DMSO). Tetradecanoyl-13-phorbol acetate (PMA) was purchased from Sigma (St. Louis, MO) and dissolved in Dulbecco's modified Eagle's medium (DMEM). UO126 (MAPK/ERK kinase (MEK)1/2 inhibitor) was purchased from Promega (Madison, WI). SP600125 (JNK inhibitor) was purchased from BIOMOL Research Laboratories Inc. (Plymouth Meeting, PA). SB203580 (p38 inhibitor) was purchased from Calbiochem (San Diego, CA). All pharmacological inhibitors were dissolved in DMSO.

Plasmids-- cAMP response element-luciferase (pCRE-luc) was purchased from Clonetech (Palo Alto, CA). Hypoxia response element-luciferase (pHRE-luc) was generously donated by Barbara S. Beckman (Tulane University). pFR-Luc, pFA2-CREB (GAL4-CREB), pFA2-Elk1 (GAL4-Elk1), and pFC-MEK1 (CA-MKK1; constitutive active MAPK kinase (MKK) 1) were purchased from Stratagene (La Jolla, CA). CMV-GAL4 (negative control), GAL4-p300 full length, GAL4-p300 (aa: 1-243), GAL4-p300 (aa: 1-743) were generous gifts from Erik Flemington (Tulane University). GAL4-p300 (aa: 1-596), GAL4-p300 (aa: 744-1571), GAL4-p300 (aa: 1572-2414), pGEX-p300N (aa: 1-596), pGEX-p300M (aa: 744-1571), and pGEX-p300C (aa: 1572-2414) were kind gifts from Yang Shi (Harvard University). pcDNA3.1 expression vector was purchased from Invitrogen (Carlsbad, CA). pcDNA3-CA-MKK5 (constitutive active) and dominant negative ERK2 (DN-ERK2) were gifts from Jiing-Dwan Lee (Scripps Research Institute). pcDNA3-CA-MKK6 (constitutive active) and pcDNA3-CA-MKK7 (constitutive active) were kind gifts from Jiahuai Han (Scripps Research Institute).

JNK1 and p38α MAPK dominant negative mutants (DN-JNK1, DN-p38α) were kindly provided by Roger Davis (University of Massachusetts Medical School). Empty GST expression vector was purchased from Amersham Biosciences (Piscataway, NJ).

Cell culture and transient transfection--Human embryonic kidney (HEK) 293 cells were grown as previously described [18-20]. These cells are ER negative and easily transfectable. Therefore, we can use them to study ER mutants without a wild-type background. Cultures of cells were transferred to phenol red-free DMEM supplemented with 5% dextran-coated charcoal-treated fetal bovine serum (DCC-FBS), BME amino acids, MEM non-essential amino acids, sodium pyruvate, and penicillin-streptomycin for 24 h prior to plating. Cells were plated at a density of 5 x 10^5 cells/well in 24-well plates (approximately 80% confluency) and maintained for an additional 24 h in DMEM with 5% DCC-FBS.

For real-time PCR analysis, MCF-7 breast cancer cells were plated in 10 cm^2 plates at ~ 25% confluency. Cells were grown for 48 hours in phenol red-free media containing 5% charcoal-stripped FBS. Cells were then incubated for 18 hrs. in the presence of either DMSO (vehicle), 50 μM o',p'-DDT, 10 μM RWJ 67657, or both DDT and RWJ. Cells were harvested, and total RNA was extracted using the RNAeasy mini kit from Qiagen. cDNA was prepared from 1 μg of total RNA using BioRad's cDNA synthesis kit. RT-PCR assays were assembled in 96 well plates using 5 uL of a 1:10 dilution of the synthesized cDNA, 0.1 ug of a 1:1 mixture of forward and reverse primers, and 1x SYBR-Green solution (BioRad).

For response element assays, HEK 293 cells were then transfected for 5 h with 100 ng of response element-luciferase using FuGENE 6^{TM} lipofection reagent (Roche, Indianapolis, IN) according to the manufacturer's protocol.

For GAL4 one-hybrid assays, 50 ng of pFR-Luc was transfected with FuGENE 6™ lipofection reagent according to the manufacturer's protocol for 6 h in combination with 25 ng of GAL4-fusion protein construct, with or without 100 ng empty expression vector or CA-MKK construct. Cytomegalovirus (CMV) promoters drove all expression vectors.

For dominant negative experiments, HEK293 cells were transfected with FuGENE 6™ lipofection reagent according to the manufacturer's protocol for 24 h using 50 ng of pFR-Luc and 10 ng of GAL4-p300 full length in conjunction with 0, 50, 100, or 150 ng of dominant negative mutant plasmid. Total DNA volume was brought up, if necessary, using empty pcDNA3.1 expression vector. CMV promoters drove all dominant negative mutant expression vectors.

Reporter gene assay--For all luciferase assays, transfected HEK 293 cells were incubated for 18-24 h in DMEM with 5% DCC-FBS in the presence of vehicle or various chemicals as previously described [18,20]. Where indicated, kinase inhibitors were added 1 h prior to the addition of DMSO, PMA, or DDT metabolites and maintained during the remainder of the incubation period. Kinase inhibitor concentrations were chosen based on non-toxic levels, published IC_{50} values from manufacturers, previous experiments demonstrating inhibition of known MAPK signaling pathways [21], and unpublished data. PMA was used as a positive control as our lab has previously shown 20 ng/ml PMA gives significant activation of PKC, downstream MAPKs, and AP-1 activation [22]. In our results we have shown the data from treatments using 10-50 μM DDT and its metabolites, which gave significant AP-1 activity as previously demonstrated [18,20]. Finally, cells were harvested and luciferase activity was measured using 30 μl of cell extract and 100 μl of Luciferase Assay Substrate (Promega,

Madison, WI) in a Berthold AutoLumat Plus luminometer. The data shown are an average of at least three independent experiments with two replicates.

GST fusion protein purification--The glutathione-S-transferase (GST) and GST-coactivator fusion proteins were generated using pGEX expression vectors transformed into BL21 star cells (Invitrogen, Carlsbad, CA). Bacteria were grown overnight in LB supplemented with 50 μg/ml ampicillin at 37°C with shaking. The following morning, bacteria was diluted 1:100 in fresh LB supplemented media and grown at 37°C to an A_{600} = .5-2. Protein expression was then induced for 3 h with 0.1M isopropyl-β-D-thiogalactopyranoside (IPTG). After induction, cells were collected by centrifugation at 7500 x g for 10 min at 4°C. The supernatant was discarded; the pellet was resuspended in cold 1X phosphate buffered saline (PBS) supplemented with .1 mM phenylmethylsulfonyl fluoride (PMSF) and 10 μl protease inhibitor cocktail for use with bacterial cell lysates (Sigma, St. Louis, MO). Resuspensions were frozen overnight at –80°C. The following morning, suspensions were thawed and then sonicated mildly twice for 45 sec. 20% Triton X-100 was added to the suspensions to a final concentration of 1% and mixed gently for 30 min to help solubilize the fusion proteins. The suspensions were then centrifuged at 12,000 x g for 10 min at 4°C. The supernatants were transferred to fresh tubes and GST fusion proteins were purified using the Bulk GST Purification Module (Amersham Biosciences, Piscataway, NJ) according to the manufacturer's protocol.

In vitro kinase assay--Roughly 3-5 μg of eluted purified GST fusion protein or 200 ng of purified MAPK activated protein kinase-2 (MAPKAPK-2) (Upstate Biotechnology, Lake Placid, NY) was then incubated for 30 min at 30°C with shaking with 0.06U of activated p38α (Upstate Biotechnology, Lake Placid, NY) in the presence of Magnesium/ATP cocktail containing [γ-^{32}P] (Upstate Biotechnology, Lake Placid, NY) according to the manufacturer's protocol. Reactions

were stopped by the addition of 20 μl 2X SDS sample buffer containing .1M PMSF, protease inhibitor cocktail, phosphatase inhibitor cocktail (Sigma, St. Louis, MO), and β-mercaptoethanol and boiling samples for 5 min. Samples were analyzed by 4-12% SDS-PAGE (Invitrogen, Carlsbad, CA), stained with coomassie blue to monitor expression, and subjected to autoradiography.

Western blot--Western blots were performed as previously described using approximately 50 μg of crude bacterial lysate or 10 μg of purified GST fusion proteins analyzed by 4-12% SDS-PAGE. Proteins were transferred to nitrocellulose membranes and probed using a 1:1000 dilution of goat anti-GST antibody (Amersham Biosciences, Piscataway, NJ) in blocking solution followed by rabbit anti-goat peroxidase labeled antibody (1:2500 dilution in blocking solution) (Kirkegaard and Perry Laboratories, Gaithersburg, MD).

Statistical analysis--Data was analyzed using one-way ANOVA and post hoc Tukey's multiple comparisons with GraphPad Prism, Version 3.02 (GraphPad Software, Inc.). Statistically significant changes were determined at the $p < 0.05$, $p < 0.01$, or $p < 0.001$ level as indicated for each figure or table.

RESULTS

DDT stimulates p38-mediated gene transcription. We have previously shown that DDT stimulates p38 phosphorylation and activation [18]. To show that this activation culminates in specific p38-mediated gene transcription, we utilized real-time PCR analysis of the Fra-1 gene in MCF-7 cells after DDT incubation. Fra-1 is a heterodimeric partner of the AP-1 transcription complex, and its expression has been shown to be upregulated by p38 MAP kinase [23,24].

DDT increased the transcription of Fra-1 by 5 fold over vehicle alone (Figure 1). The selective p38 inhibitor RWJ67657 greatly reduced DDT-stimulated Fra-1 expression (Figure 1). These data provide a direct link between DDT-stimulated p38 activation and p38-mediated gene transcription.

DDT and its metabolites stimulate multiple promoter response elements and nuclear transcription factors. Because DDT-induced p38 activity stimulates the AP-1 complex posttranslationally [18], we hypothesized DDT targets a more general transcriptional mechanism. To determine if DDT stimulation of AP-1 was a selective process, we tested whether DDT treatment could stimulate other promoter response elements and transcription factors. Using luciferase reporter gene assays, we demonstrate in HEK 293 cells that, similar to AP-1 sites, DDT and its metabolites also target CRE and HRE sites (Figures 2A and B). In addition, GAL4 one-hybrid analysis showed DDT and its metabolites potentiate the nuclear transcription factors CREB and Elk1 (Figures 2C and D). In all cases, the active metabolites (o,p'DDT, p,p'DDT, o,p'DDD) stimulated luciferase expression, while the inactive metabolites (p,p'DDA and p,p'DDOH) had no effect. These experiments indicate DDT signaling targets a pathway shared by multiple transcription factors/response elements.

The transcriptional coactivator p300 is stimulated after DDT metabolite treatment--p300 has been shown to interact with multiple nuclear transcription factors, such as AP-1, CREB, HIF-1, and Elk1, as well as members of the core RNA transcriptional machinery, suggesting a role as a transcriptional integrator or adaptor. After determining DDT signals these same transcription factors potentially through a more general mechanism, we examined whether DDT and its metabolites could stimulate the coactivator p300. HEK 293 cells exposed to o,p'DDT, p,p'DDT, or o,p'DDD showed a potentiation of GAL4 fusion protein of full length p300, but not

the empty GAL4 expression vector (Figure 3A). Therefore, we can conclude that p300 contains a DDT-regulated transcriptional activation function.

The p38 MAPK signaling cascade activates p300--Since DDT-induced AP-1 activity is mediated through p38 MAPK, we tested if p38 MAPK affected p300 activity. Constitutive active MKK mutants transfected into HEK 293 cells in conjunction with the GAL4-coactivator construct showed that CA-MKK6, which selectively activates p38, was the only MKK that significantly stimulated p300 (Figure 3B).

To demonstrate DDT-induced coactivator activity is mediated through p38, we blocked o,p'DDT-stimulated coactivator activity with both molecular and pharmacological inhibitors of the various MAPK pathways. HEK 293 cells were transfected with increasing amounts of DN-[dominant negative] ERK2, JNK1 [c-Jun N-terminal kinase 1], or p38α along with a GAL4 reporter plasmid and a full length GAL4-p300 chimera followed by treatment with vehicle (DMSO) (Figure 4A) or 50 μM o,p'DDT (Figure 4B). DN-p38α, but not DN-ERK2 or DN-JNK1, inhibited DDT-induced p300 activity (Figure 4B). All inhibitory effects were significantly greater ($p < 0.05$) in o,p'DDT treated cells versus vehicle treated cells. DN-p38α significantly inhibited ($p < 0.001$) o,p'DDT-induced p300 activity (70-80% inhibition). Additionally, DN-ERK2 also inhibited p300, albeit to a lesser degree than DN-p38α.

To confirm our molecular inhibitory findings, we blocked DDT-induced coactivator activity with pharmacological inhibitors of the MAPK pathways. GAL4 empty expression vector or GAL4-coactivator fusions were transfected into HEK 293 cells and treated with vehicle or different MAPK inhibitors for 1 h, followed by treatment with vehicle or 50 μM o,p'DDT. The p38 pharmacological inhibitor SB203580 significantly blocked ($p < 0.01$) o,p'DDT induction of p300, whereas there was no effect on cells transfected with empty GAL4 expression

plasmid (Figure 4C). The ERK inhibitor, UO126, significantly inhibited ($p < 0.05$) p300. The p300 inhibition by UO126 was not as potent as the effects caused by SB203580. Collectively, these data confirm that the transcriptional coactivator p300 is activated by DDT via the p38 MAPK pathway.

p38 phosphorylates p300--Recent reports have demonstrated various kinases, including the MAPKs ERK and JNK, can potentiate p300 through phosphorylation [11-13,25-28]. We hypothesized p38 MAPK phosphorylates p300, leading to its potentiation. To test this, we bacterially expressed recombinant p300 using GST purification. Western blots using antibodies against GST revealed GST-fusion proteins broke down into smaller fragments (data not shown), consistent with what others have seen [13]. A map of the GST-fusion proteins tested is shown (Figure 5A). Purified fusion proteins were then subjected to an *in vitro* kinase assay in the presence of ^{32}P and activated p38 MAPK. The p300 N-terminal fragment containing amino acids 1-596 was the only p300 fragment phosphorylated (Figure 5B). This data demonstrates for the first time that p38 MAPK directly phosphorylates and activates p300.

Identification of domains of p300 involved in p38 responsiveness--To determine the specific regions p38 potentiates p300 and to compliment the *in vitro* kinase data, HEK 293 cells were transfected as before with either empty expression vector or CA-MKK6 for 6 h in conjunction with GAL4-coactivator fragment constructs. A map of the GAL4-protein fusions is shown (Figure 6A). Cells were then treated with vehicle or o,p'DDT (50 μM) overnight. Hence, p38 was stimulated both by molecular (CA-MKK6) and pharmacological (o,p'DDT) means. Consistent with our *in vitro* kinase data, both CA-MKK6 and o,p'DDT potentiated the N-terminus of p300 (Figure 6B). Specifically o,p'DDT significantly increased ($p < 0.001$) both fragments containing amino acids 1-596 and 1-743. CA-MKK6, however, only significantly

increased ($p < 0.05$) the p300 fragment containing amino acids 1-743, indicating that the major p38 target site is located in a region we were unable to detect in our *in vitro* kinase assay as the N-terminal GST-p300 fusion protein was missing amino acids 597-743. Thus, our *in vitro* kinase data strongly correlates with the GAL4 studies and shows that p38 targets the N-terminus of p300.

DISCUSSION

Coactivators are critical components involved in the process of transcriptional initiation and elongation. The general coactivator p300 is essential to basically all cellular functions, including growth, differentiation, and apoptosis [4]. Thus, determining the regulation of this coactivator is central to understanding how signaling mechanisms control gene expression and ultimately biological function. Here, we demonstrate the p38 MAPK phosphorylates and potentiates the nuclear coactivator p300.

The pesticide DDT was previously demonstrated by us to signal p38, leading to increased AP-1-mediated gene expression [18,19]. The increased AP-1 activity was due to 1) increased expression of *c-jun* and 2) posttranslational activation of c-Jun and c-Fos. We show here that p38 activation leads to increased transcription of Fra-1, a component of the AP-1 complex. Therefore, the DDT stimulated AP-1 activity could be due to a third factor, a p38-induced increase in Fra-1 expression. The data presented here indicate DDT-induced activity also increases the transcriptional activities of other transcription factors such as CREB and Elk1. In support of this, we show DDT increases gene expression through cAMP and hypoxia responsive elements (Figures 2A and B). While p38 has been shown to target Elk1, CREB, HIF-1, and the *c-jun* promoter [1,28,29], how it potentiates c-Jun, c-Fos is unclear [18,30]. All of these

transcription factors recruit common coactivators, indicating that perhaps p38 signaling to the transcription factors could be accomplished by an indirect potentiation, one favoring the shared coactivators. Mammalian one-hybrid assays using different GAL4-coactivator constructs demonstrate o,p'DDT, p,p'DDT, and o,p'DDT, which were previously shown to stimulate AP-1 activity [19], stimulated p300 (Figure 3A). Another DDT metabolite found in humans, p,p'DDA, which does not potentiate AP-1, likewise had no effect on p300. While DDT was used primarily as a pharmacological tool to stimulate p38 activity, these data do also represent a novel mechanism by which environmental compounds can co-opt transcriptional regulation.

Since DDT induces AP-1 activity through p38, we sought to determine the effect of MAPK signaling on p300 using both pharmacological and molecular regulators of the ERK, JNK, and p38 signaling pathways. Constitutive active MKK6 significantly increased p300 transcriptional activity (Figure 3B). Conversely, both a dominant negative mutant of p38α and SB203580, a pharmacological inhibitor of p38, blocked DDT-induced coactivator signaling (Figure 4). This potentiation also appeared to be due, in part, to the ERK signaling cascade, although to a lower degree. ERK has previously been demonstrated to potentiate p300 [10-12]. However, it must be noted that CA-MKK1, did not stimulate p300 in HEK 293 cells in our hands, contrasting the UO126 and dominant negative ERK data as well as the effects seen by other labs using HeLa cells and cardiac myocytes. Obviously, additional work is required to understand the exact role of the ERK signaling cascade in p300 potentiation in HEK 293 cells before conclusions can be drawn. Our results shown here mirror the effect DDT has on AP-1-mediated transcription, indicating those results were most likely not due to targeting of AP-1 itself, but rather a targeting of the AP-1 associated proteins.

Sequence analysis indicates several possible p38 MAPK sites in p300, and we demonstrate *in vitro* that activated p38 phosphorylates p300 (Figure 5B). Consistent with what other labs have shown for the ERK MAPK signaling cascade, p38 phosphorylates the N-terminal region of p300 [10-12]. However, Sang et al did demonstrate ERK phosphorylates the C-terminus of p300 in HeLa cells [12], demonstrating the cell specific effects kinase signaling pathways can have on protein substrates. GAL4 one-hybrid assays confirm the same regions we demonstrated to be phosphorylated *in vitro* are targeted *in vivo* (Figure 6B). While p38 phosphorylated a p300 fragment containing amino acids 1-596, this phosphorylation did not appear very strong, nor did constitutive active MKK6 significantly potentiate the corresponding GAL4 fusion. The potentiation seen with the pharmacological stimulant may have been caused by another kinase stimulated by o,p'DDT, such as ERK, as sequence analysis suggests S133 and T524 are potential proline-directed kinase sites. However, no one has identified if these sites are targeted. Constitutive active MKK6 did, however, significantly stimulate the GAL4-p300 fragment containing amino acids 1-743, indicating that an unidentified p38 responsive site lies between amino acids 596 and 743 (Figure 6). Unfortunately, our *in vitro* kinase assay did not address this small but apparently important region. This region contains part of the KIX domain, which binds certain transcription factors such as CREB, c-Jun, ATF-1/2, and c-myb as well as the TATA-binding protein [3]. It would be interesting to see if targeting of this section of p300 alters binding to transcription factors or even components of the basal transcription machinery. It must be noted, however, that the C-terminal p300 fragment (amino acids 1572-2414) was difficult to purify in high concentrations as seen by the light coomassie blue stained bands in Fig. 6B. It still is possible that with greater amounts of this protein fragment present, there may be some phosphorylation. However, our results here detecting no signal, combined with p38's

inability to potentiate this region (Figure 6B) in the mammalian one-hybrid assay suggest this is not the case.

Our results demonstrate a novel mechanism for p38-induced transcription. Transcription factors such as Elk1, ATF-2 [activating transcription factor 2], and ER are all convergent points for p38 signaling pathways. Some transcription factors such as Elk1 and ATF2 are directly phosphorylated by p38. Here, we show another level of p38-transcriptional regulation, p38 targeting of the recruited coactivator p300. This added requirement may help p38 selectively activate target genes.

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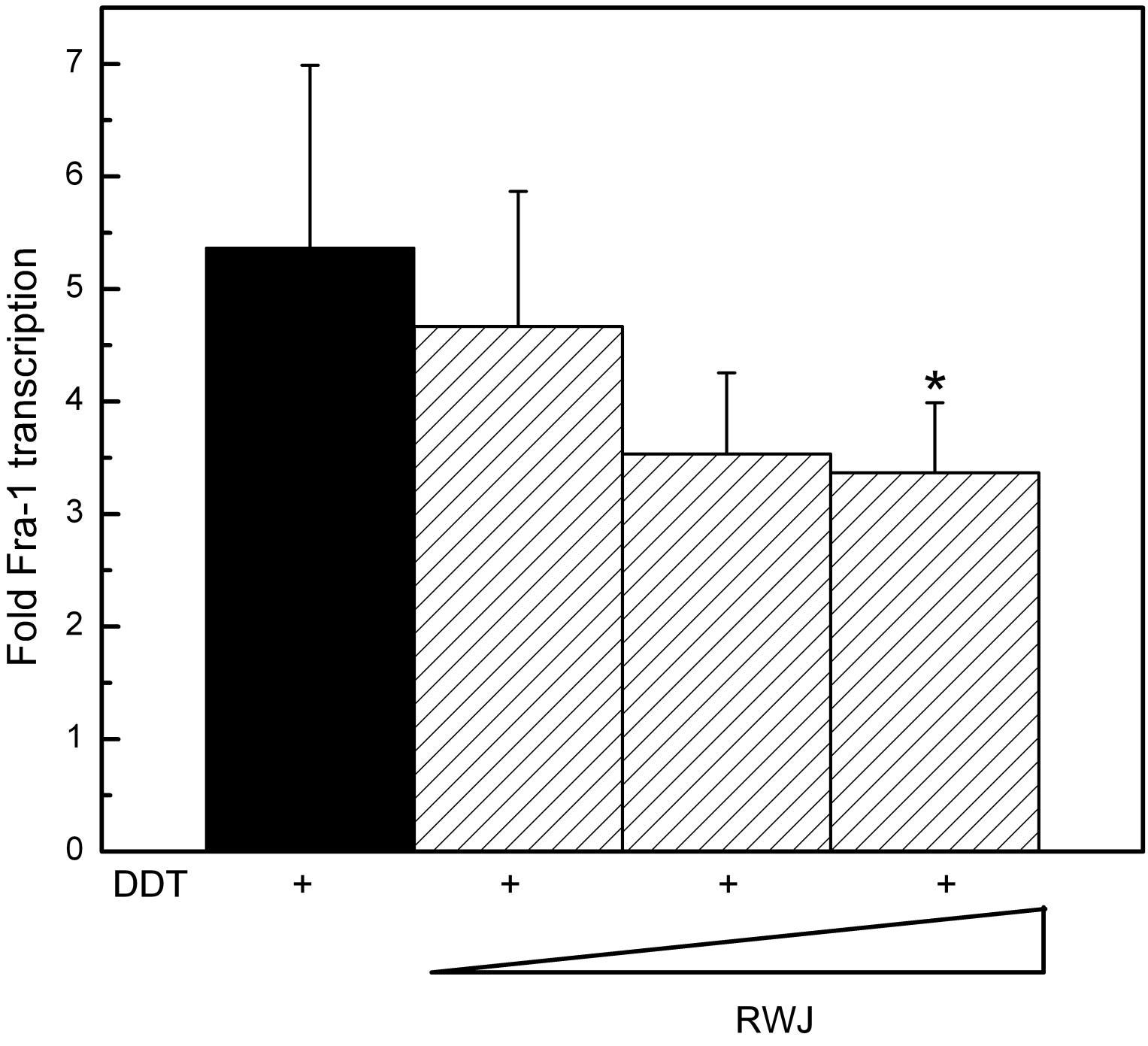
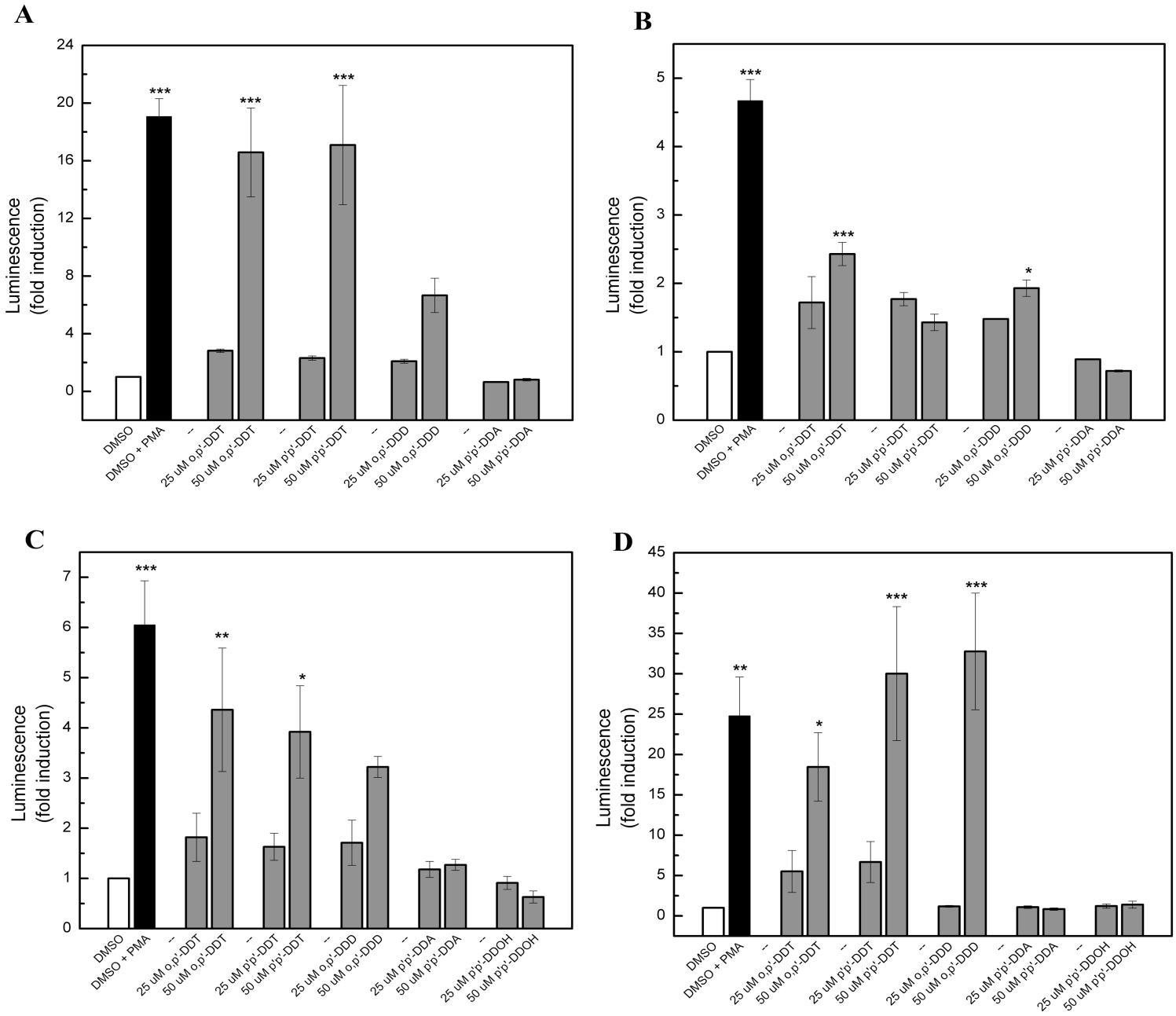
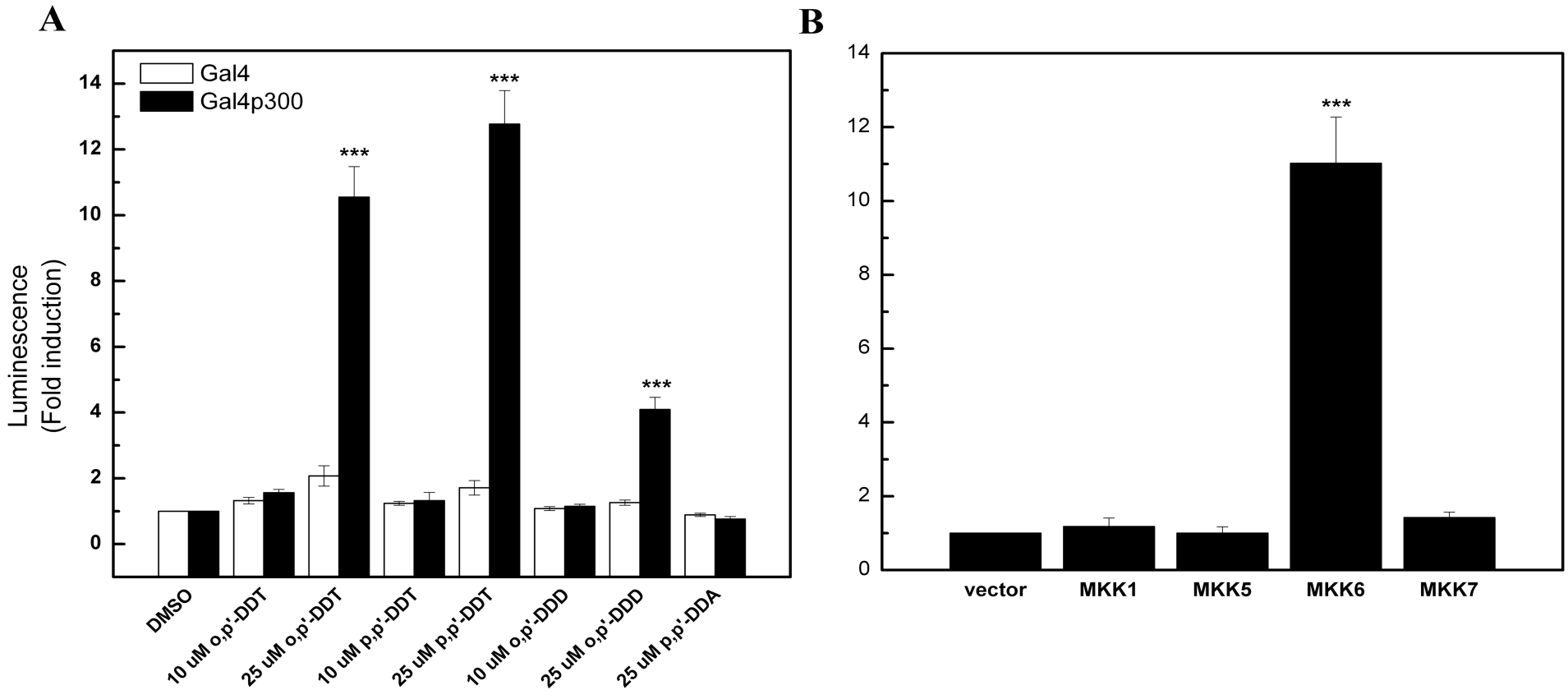
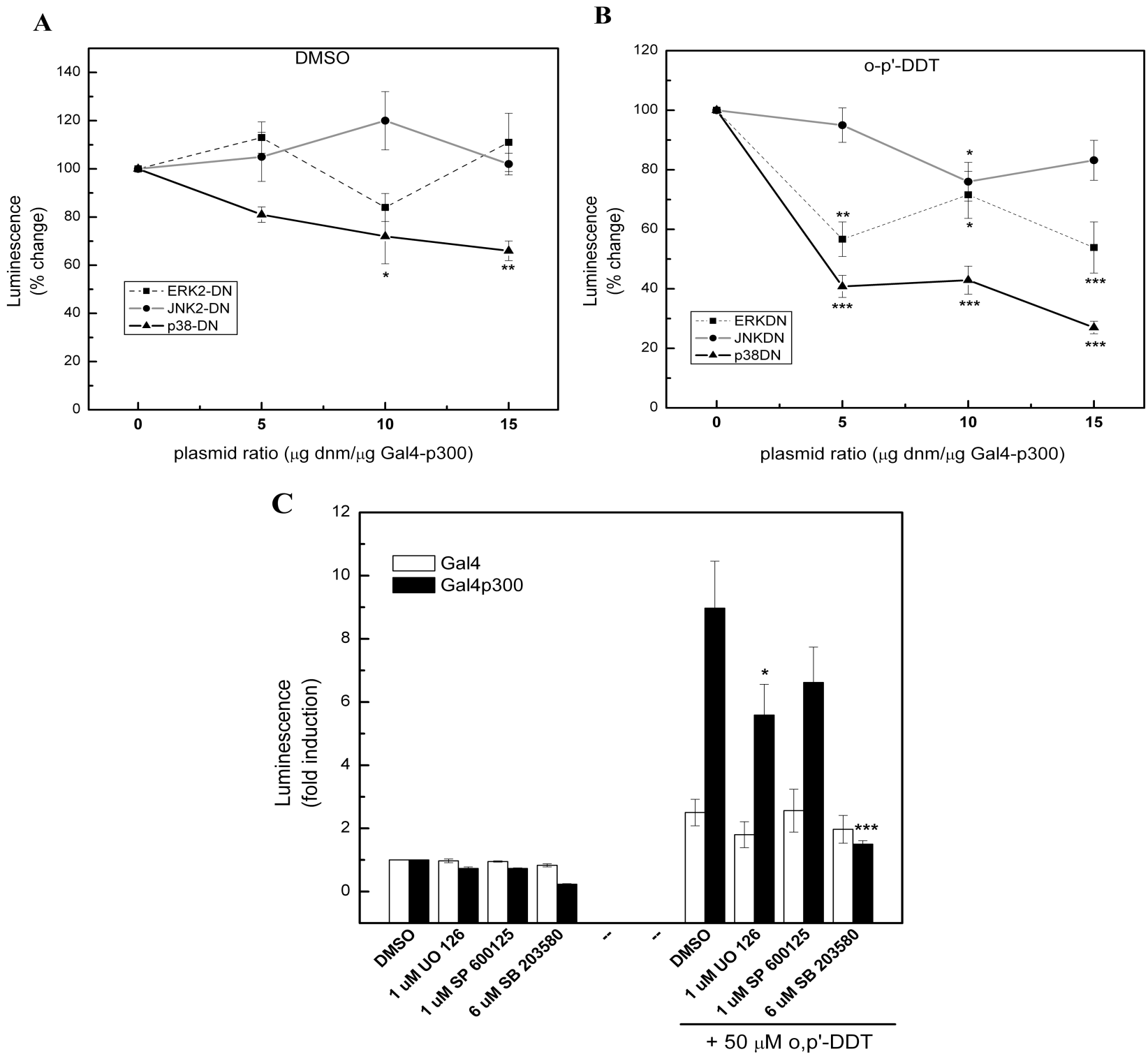
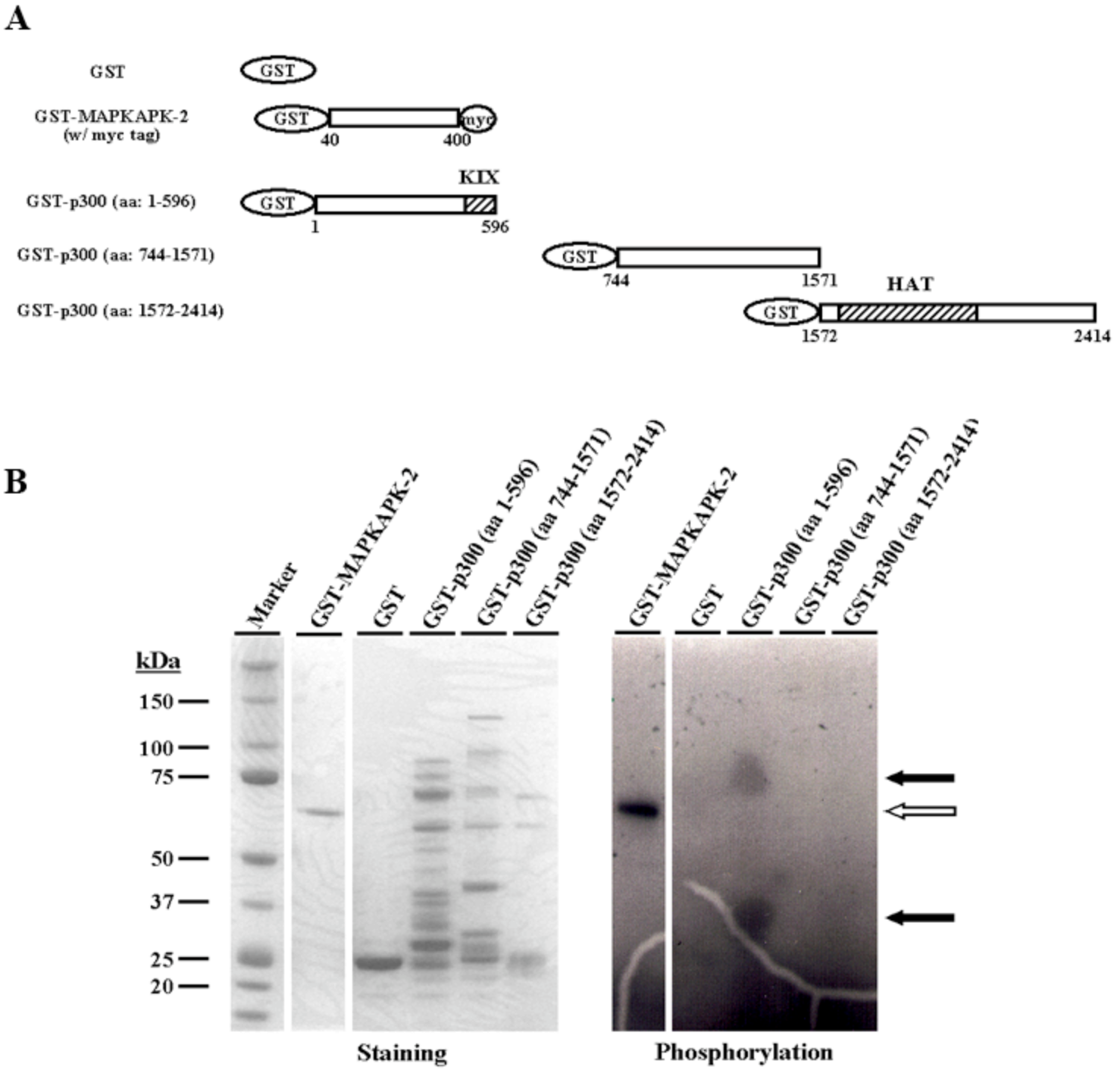

Fold Fra-1 transcription
0
1
2
3
4
5
6
7
*
DDT
+
+
+
+
RWJ

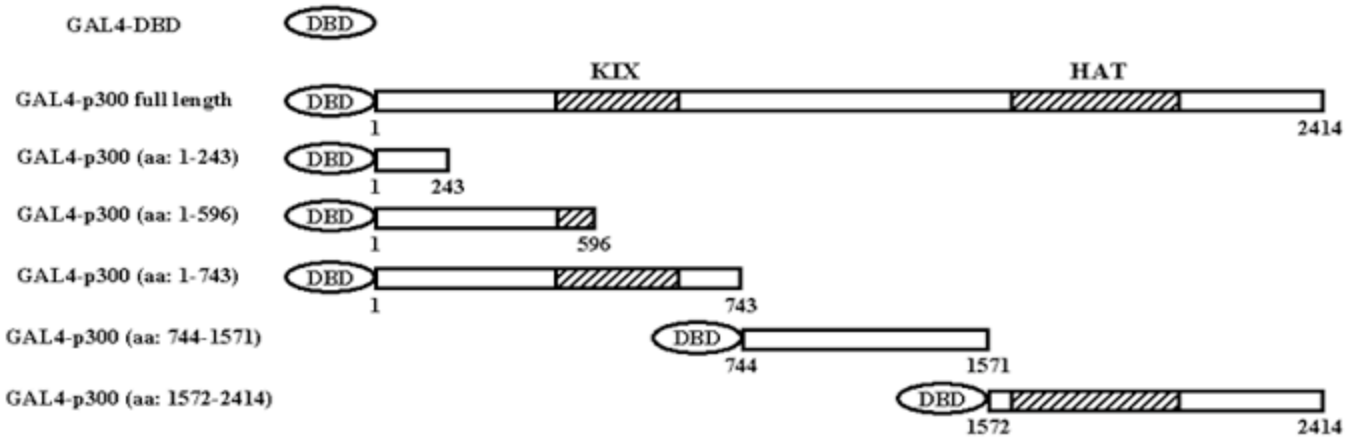

A
Luminescence (fold induction)
DMSO
DMSO + PMA
25 uM o,p'-DDT
50 uM o,p'-DDT
25 uM p'p'-DDT
50 uM p'p'-DDT
25 uM o,p'-DDD
50 uM o,p'-DDD
25 uM p'p'-DDA
50 uM p'p'-DDA
B
Luminescence (fold induction)
DMSO
DMSO + PMA
25 uM o,p'-DDT
50 uM o,p'-DDT
25 uM p'p'-DDT
50 uM p'p'-DDT
25 uM o,p'-DDD
50 uM o,p'-DDD
25 uM p'p'-DDA
50 uM p'p'-DDA
C
Luminescence (fold induction)
DMSO
DMSO + PMA
25 uM o,p'-DDT
50 uM o,p'-DDT
25 uM p'p'-DDT
50 uM p'p'-DDT
25 uM o,p'-DDD
50 uM o,p'-DDD
25 uM p'p'-DDA
50 uM p'p'-DDA
25 uM p'p'-DDOH
50 uM p'p'-DDOH
D
Luminescence (fold induction)
DMSO
DMSO + PMA
25 uM o,p'-DDT
50 uM o,p'-DDT
25 uM p'p'-DDT
50 uM p'p'-DDT
25 uM o,p'-DDD
50 uM o,p'-DDD
25 uM p'p'-DDA
50 uM p'p'-DDA
25 uM p'p'-DDOH
50 uM p'p'-DDOH


A
Gal4
Gal4p300
Luminescence
(Fold induction)
0
2
4
6
8
10
12
14
***
***
***
DMSO
10 uM o,p'-DDT
25 uM o,p'-DDT
10 uM p,p'-DDT
25 uM p,p'-DDT
10 uM o,p'-DDD
25 uM o,p'-DDD
25 uM p,p'-DDA
B
0
2
4
6
8
10
12
14
***
vector
MKK1
MKK5
MKK6
MKK7

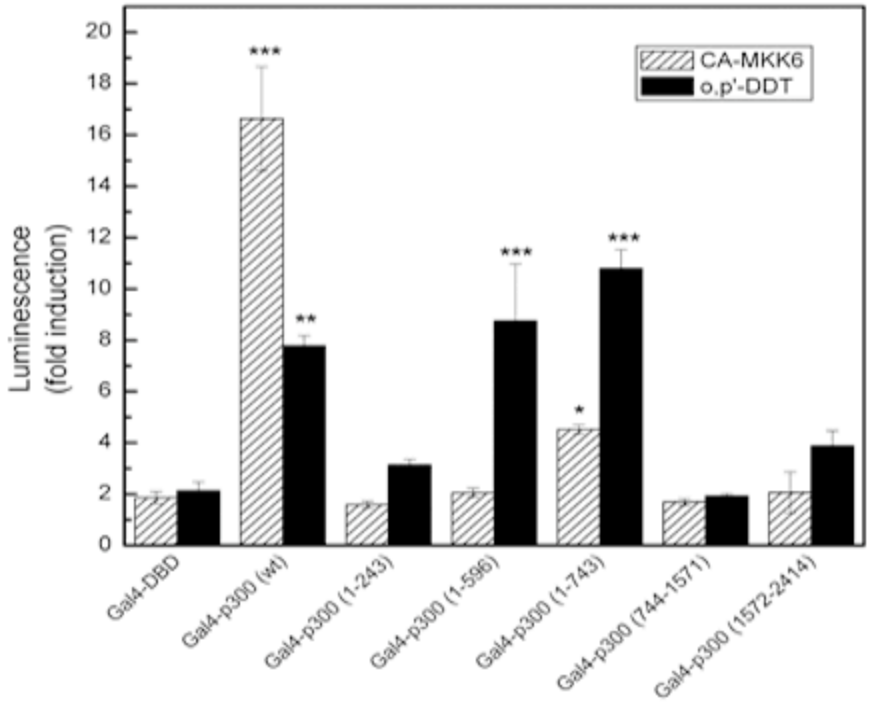

A
DMSO
Luminescence (% change)
plasmid ratio (μg dnm/μg Gal4-p300)
ERK2-DN
JNK2-DN
p38-DN
B
o-p'-DDT
Luminescence (% change)
plasmid ratio (μg dnm/μg Gal4-p300)
ERKDN
JNKDN
p38DN
C
Luminescence (fold induction)
Gal4
Gal4p300
DMSO
1 uM UO 126
1 uM SP 600125
6 uM SB 203580
+ 50 μM o,p'-DDT


A
GST
GST
GST-MAPKAPK-2
(w/ myc tag)
GST
myc
40
400
KIX
GST-p300 (aa: 1-596)
GST
1
596
GST-p300 (aa: 744-1571)
GST
744
1571
HAT
GST-p300 (aa: 1572-2414)
GST
1572
2414
B
Marker
GST-MAPKAPK-2
GST
GST-p300 (aa 1-596)
GST-p300 (aa 744-1571)
GST-p300 (aa 1572-2414)
GST-MAPKAPK-2
GST
GST-p300 (aa 1-596)
GST-p300 (aa 744-1571)
GST-p300 (aa 1572-2414)
kDa
150
100
75
50
37
25
20
Staining
Phosphorylation

A


GAL4-DBD
DBD
KIX
HAT
GAL4-p300 full length
1
2414
GAL4-p300 (aa: 1-243)
243
GAL4-p300 (aa: 1-596)
596
GAL4-p300 (aa: 1-743)
743
GAL4-p300 (aa: 744-1571)
744
1571
GAL4-p300 (aa: 1572-2414)
1572

B


Luminescence (fold induction)
CA-MKK6
o,p'-DDT
Gal4-DBD
Gal4-p300 (wt)
Gal4-p300 (1-243)
Gal4-p300 (1-596)
Gal4-p300 (1-743)
Gal4-p300 (744-1571)
Gal4-p300 (1572-2414)