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13. ABSTRACT *(Maximum 200 Words)*

We have previously reported that two polyamine antagonists currently undergoing clinical evaluation: the polyamine analog, N^1, N^{11}-diethylnorspermine (DENSPM) and the biosynthetic enzyme inhibitor, 4-amidinoindan-1-one 2'-amidinohydrazone (CGP-48664), appear to exert unusual regulatory effects in prostate carcinoma cell lines relative to other cell lines. We have proposed to develop strategies *in vitro* and *in vivo* for therapeutically exploiting this observation. In pursuit of this goal, we have discovered that CGP-48664 potently induces apoptosis via a p53 / caspase-3 dependent pathway that is unrelated to its intended mode of action as an inhibitor of polyamine biosynthesis. Such an effect has not been previously reported and may have relevance to ongoing clinical trials with the compound. Future studies will investigate the therapeutic significance of this finding *in vivo*. In other developments, we have determined that a polyamine analog related to DENSPM exerts meaningful antitumor activity against DU145 human prostate carcinoma xenografts and are seeking proof-of-principle for a novel polyamine gene-based antiproliferative strategy. The former will be optimized with respect to drug effects on polyamine regulatory responses and on the antitumor activity of the compound. More recently, we have identified a novel targeting strategy involving inducible polyamine catabolism. The overall goal of both research efforts is to identify and develop polyamine-based / prostate-directed therapies as rapidly as possible.

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FOREWORD

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REPORT BODY *(Figures & Figure Legends located in Appendix 1)*

A. *Introduction*:

Polyamines are organic cations found in all cells and known to be essential for the initiation and maintenance of cell growth. Intracellular levels are sensitively maintained by a series of regulatory responses. The relationship of polyamines to the prostate is unique among all tissues since in addition to synthesizing these molecules for cell growth, the gland produces massive quantities for export into semen. It might, therefore, be expected that prostatic tumors could exhibit atypical polyamine-related regulatory responses. Thus, we have observed that in contrast to other normal and tumor cell types, two of three prostate carcinoma lines displayed an altered ability to regulate polyamine transport in responses to polyamine analog or inhibitors (1). More specifically, two lines could not down-regulate uptake in response to analogs and one could not up-regulate it in response to polyamine depletion caused by inhibitors. The findings have obvious therapeutic implications for two polyamine antagonists that have recently undergone clinical evaluation: the polyamine analog, N^1, N^{11}-diethylnorspermine (2, DENSPM; Parke-Davis) and the biosynthetic enzyme inhibitor, 4-amidinoindan-1-one 2'-amidinohydrazone (3-4, CGP-48664, also known as SAM486A Novartis). Thus, it should be possible to identify more effective analogs of these drugs and/or to develop drug treatment schedules capable of optimizing selective delivery of DENSPM (which utilizes the transporter) to prostate tumors and in the case of CGP-48664, to minimize antiproliferative escape via salvage of exogenous polyamines. Herein, we propose to further examine polyamine regulatory responses in prostate carcinoma cell lines treated with analogs and inhibitors; to examine the underlying molecular mechanism(s) responsible for alterations in those responses; and most importantly, to develop *in vitro* and *in vivo* strategies for therapeutically exploiting these fundamental observations.

B. *Originally Proposed Tasks*

- *Task 1.* To further determine the extent to which altered polyamine homeostatic regulatory responses are characteristic of prostate cancer cell lines.

- *Task 2.* To develop effective *in vitro* strategies based on observations made in Task 1 which optimize the anti-proliferative effects of DENSPM, CGP-48664 or related inhibitors and analogs.

- *Task 3.* To investigate mechanisms underlying aberrant regulatory responses identified in Task 1.

- *Task 4.* To extend strategies developed in Task 2 to *in vivo* model systems involving appropriate human prostatic carcinoma lines growing in scid mice.

- *Task 5.* To further optimize the choice of analog and/or inhibitor using compounds available to us from various collaborating laboratories.

Subsequent Task Modifications:

The ordered pursuit of the above proposed tasks has been altered by three significant developments: (i) our observation that CGP-48664 exerts a significant apoptotic effect in LNCaP prostate carcinoma cells and that this response seems to be unrelated to its intended activity as an inhibitor of polyamine biosynthesis; (ii) clinical trials indicated that schedule modification of DENSPM (i.e. splitting a single daily dose to twice or three times daily, as we proposed in Tasks 1 and 2) is not well tolerated in patients and (iii) the current halt in clinical trials of CGP-48664 and DENSPM. In both cases, the decision was based on priority relative to alternative drugs awaiting development in the preclinical pipeline of Novartis and Pfizer (following the take over of Parke Davis). At present, Novartis is seeking to license SAM-486 to a smaller company with expertise in polyamines such as Oridigm (Seattle, OR) or Ilex (San Antonio, TX). Similarly, Pfizer has returned the DENSPM license to Geltex Pharmaceuticals (Waltham, MA, now merged with Genzyme) and clinical trials are on hold. While these issues were being resolved, we placed greater emphasis on Task 5 as it relates to identification of new polyamine analogs having greater antitumor activity and/or reduced host toxicity (in collaboration with (SLIL Pharmaceuticals, Madison, WI). In addition, we have begun the investigation of a novel therapeutic target, the polyamine catabolic enzyme, spermidine/spermine N^1 acetyltransferase (SSAT). This latter strategy was submitted as a Idea Development Award in 2001. Although appeared to be positively reviewed, it was not funded.

C. *Progress:*

1. Mode of CGP-48664 Drug Action in LNCaP Cells:

As originally proposed in Task 1b, we began this project by attempting to confirm variant polyamine regulatory responses to CGP-48664 and DENSPM by examining the effects of other known polyamine inhibitors, and analogs. The goal was to determine that the responses could be generalized to compounds having comparable modes of action as CGP-48664 and DENSPM before moving on to examine the generality of altered polyamine responses in additional prostate carcinoma cell lines. An initial comparison of growth effects between CGP-48664 and MDL-46811, a well known mechanism-based specific inhibitor of S-adenosylmethionine decarboxylase (2, SAMDC), revealed that at 50 µM, the former inhibitor induced an immediate and complete cessation of LNCaP cell growth while at 100 µM, the latter had virtually no effect on the growth these cells (Figure 1). As expected for SAMDC inhibitors (2-4), both drugs similarly increased putrescine and decreased spermidine and spermine pools (data not shown). Our immediate suspicion was that CGP-48664 was exerting unexpected drug effects that were unrelated to inhibition of SAMDC. This possibility was further indicated by the observation that growth inhibition was not preventable with exogenous spermidine.

In the first year report, we indicated that CGP-48664 induces apoptosis in LNCaP cells (subG1 peak and DNA fragmentation) and this cellular response appears to be wholly unrelated to drug effects on SAMDC. Additional findings indicated that the apoptotic effect of CGP-48664 may be related to induction of p53 and more clearly, to activation of caspase-3 dependent apoptosis. The possible protein response interrelationships as they relate to caspase 3-induced apoptosis were defined. A comparison of cytochrome c levels in mitochondrial versus the S-100 fractions of LNCaP treated cells revealed that CGP-48664 caused the mitochondrial protein to be released into the cytosolic fraction; a typical feature of p53/caspase-mediated apoptosis. Indeed, early activation of caspase-3, and caspase-mediated cleavage of poly(ADP-ribose) polymerase (PARP) were seen in treated cells. The individual who performed the original studies has since

accepted a postdoctoral position at San Antonio. We subsequently discovered that the LNCaP cells used in his experiments were contaminated with mycoplasma. In attempting to accommodate the recommendations of reviewers using uncontaminated cells, we were unable to repeat the caspase-3 induction, PARP cleavage or DNA fragmentation reported in the manuscript. We were however, able to demonstrate that CGP-48664 does cause apoptosis by Annexin V staining and that it was not preventable with spermidine. Induction of p53 and p21 are also repeatable. Thus, CGP-48664 induces apoptosis in LNCaP cells via a polyamine unrelated pathway that remains to be defined. This development constitutes a major set-back in progress. Future studies will attempt to identify the pathway responsible for the apoptotic response to CGP-48664.

   2. Antitumor Studies with Recently Available Polyamine Analogs:

       In year 2, we reported CGP-48664 at 50 mg/kg per day i.p. (commonly used dose for this drug) showed absolutely no antitumor activity (data not shown) while DENSPM at 80 mg/kg per day i.p. at the maximally tolerated dose for 5 days yielded a modest antitumor activity which was apparent as a slowing in tumor growth followed by a full growth rate recover (data not shown). During the past two years, we collaborated with SLIL Pharmaceuticals (5) in accessing a novel polyamine analogs that were found by company, to have *in vitro* activity against prostate carcinoma cells lines and *in vivo* activity in a benign prostate hyperplasia model. In previous reports, we indicated that SL-11147, an unsaturated analog of DENSPM having a double bond linking the two center carbons of the molecule, produced antitumor activity nearly identical to DENSPM in the DU145 model. We now report on the antitumor activity of two additional compounds SL-11144 and SL-10093 (Figure 1). The first, SL-11144 (Figure 1), is a diethylated polymer of homospermine units. More specifically, SL-11144 is a terminally N-diethylated molecule with an intra-amine carbon arrangement of [4x(4-4-4)]-2-db-2-[4x(4-4-4)] where db indicates an unsaturated double bond in the middle of a central homospermine moiety. The second, SL-11093 (Figure 1), is an analog of N^1, N^{11}-diethylhomospermine also known as DEHSPM where DE refers to diethyl and where *homo* refers to the intra-amine carbon spacings as 4-4-4 as opposed to 3-4-3 with spermine. The analog is rendered particularly novel by a unique cyclopropyl group connecting the two center carbons of the molecule. This SL-11093 was selected on the basis of its close structural similarity to DEHSPM which has undergone a partial Phase I clinical trial and on its *in vitro* antiproliferative activity against the three human prostate carcinoma cell lines. Against human prostate carcinoma lines, ID_{50} values were in the range of 10-15 µM.

       In vivo studies were preceded by *in vitro* growth inhibition studies involving DU-145 cells, SL-11144 was much more cytotoxic than SL-11093 with respect to both dose (Figure 2) and time (Figure 3). Whereas SL-11144 affected growth kinetics in a manner entirely consistent with high cytotoxicity, SL-11093 was weakly antiproliferative and cytostatic at best. For example, the dose-response curves shown in Figure 2 show that growth falls off rapidly with increasing doses of SL-11144, this does not occur with SL-11144 where following an intitial fall-off in growth, cells seem to be unaffected by increasing concentrations of analog (i.e. 1 to 100 µM). As further evidence for the cytotoxicity caused by SL-11144, we note that by annexin staining, 48% of DU-145 cells treated for 48 h with the analog were in late apoptosis while by comparison, only 3.6% of cells treated with SL-11093 showed signs of apoptotic staining.

Antitumor studies began with the treatment of established DU-145 prostate carcinoma tumors (~200 mm^3) at analog doses approaching the maximally tolerated dose as determined by Dr. Cyrus Bacchi (Pace University, NYC). More specifically, SL11144 was administered intraperitoneally at 5-10 mg/kg/d for 5 days per dosing round and SL-11093 was administered at 25-50 mg/kg/d for 5 days per dosing round. Control animals were injected intraperitoneally with saline on the identical schedule. Owing to simplicity of structure, analog availability and lower potential for host toxicity, initial studies were carried out with SL-11093.

A pilot study was conducted with a single dosing round of SL-11093 at 25 and 50 mg/kg/d for 5 days. As indicated in Figure 4, tumor growth was modestly affected in a dose-dependent fashion. Because there was minimal host toxicity and because the analog seemed to be most effective during treatment, we evaluated SL-11093 in a follow-up experiment where the dosing schedule was repeated three times with a 9 day rest period in between dosing rounds. As shown in Figure 5, the growth of DU-145 tumors in treated mice was markedly suppressed (Figure 5B) relative to the saline treated mice (Figure 5A). The drug effect seemed tumoristatic since tumor growth remained relatively flat and there was no indication of tumor regressions. For uncertain reasons, one tumor seemed to escape the antitumor effects of the drug (Figure 5B). When the median values for both groupings are compared in Figure 5C, it is apparent that tumor growth suppression by SL-11093 was highest during treatment and that tumors rapidly recovered growth shortly after treatment ceased. As indicated in Figure 5D, there was a minor loss in body mass during treatment after which mice attained weights similar to those of saline-treated mice. These finding were independently fully confirmed by SLIL-sponsored contractual studies with Southern Research Institute.

We then examined the antitumor activity of SL-11144 (at 5 mg/kg/d) under the schedule of multiple dosing rounds used with SL-11093. As shown in Figure 6, the analog initially suppressed tumor growth during the first two rounds of treatment but then lost effectiveness during the third round. Animal weights indicated minimal host toxicity during the first three rounds of treatment after which there appeared to be a latent weight loss effect.

The multiple (5) dosing experiment with SL-11093 (at 50 mg/kg/d) was then repeated and compared with SL11144 (at 5 mg/kg/d). Antitumor effects during and shortly following treatment with either analog (days 20-98) are shown in Figure 7. Once again, the growth of DU-145 tumors in SL-11093-treated mice was effectively suppressed (Figure 7B) relative to the saline-treated mice (Figure 7A). By comparison, SL-11144 seemed less active in suppressing tumor growth since several tumors escaped its effects (Figure 7C) and those tumors that responded were less affected than those treated with SL-11093. Although there was was virtually no decrease in body weight during treatment with either analog (Figure 7D), saline mice gained weight during the course of the experiment while those treated with analog did not. As with the initial study (Figure 4), the second experiment strongly suggested that the drug effect was tumoristatic. This is most apparent in Figure 8 which shows tumor volumes (within the same experiment) beyond the treatment period shown in Figure 7. Except for two anecdotal regressions in saline (Figure 8A) and SL-11144 (Figure 8C) treated mice, there were no tumor regressions that could be reliably attributed to drug treatment. The strongest indication for the tumoristatic nature of SL-11093 derives from the finding that after being suppressed for ~70 days, tumor growth sharply resumed about 20 days following the last round of treatment with the analog. Taken together, this experiment confirms earlier indication that multiple rounds of dosings with SL-11093 produce meaningful antitumor responses with minimal host toxicity. The antitumor effect is tumoristatic and SL-11093 is more effective than SL-11144 against the

DU-145 prostate carcinoma.

In the most recent experiment, we examined the antitumor activity of SL-11093 against Panc-1 pancreatic carcinoma xenografts. The rationale was based on the clinical need for effective therapies against this disease and the fact that the exocrine pancreas typically contains high levels of polyamines, perhaps because it is heavily involved in protein synthesis. As shown in Figure 9, the experiment was complicated by heterogeneous growth characteristics of the untreated tumor (Figure 9A). Despite beginning at similar starting volumes, tumors tended to either grow rapidly or slowly. As shown in Figure 9B, this same pattern of tumor growth was also seen in the SL-11093 treated tumors. Due to this tumor scatter, the tumors were not subjected to multiple dosing rounds. However, when the saline and analog-treated groups were represented within the same plot (Figure 9C), it appears that a single round of therapy is virtually without effect on tumor growth. Although inconclusive, the data suggest that SL-11093 is much less active against the Panc-1 pancreatic carcinoma than DU-145 prostate carcinoma (Figure 4).

Overall, we believe that the data with SL-11093 are especially encouraging. Both we and S'LIL Pharmaceuticals have interest in developing this particular analog this toward clinical trial. As a result of these experiments, SL-11093 is being developed towards clinical trials for prostate cancer. More specifically, the analogue is currently undergoing Phase I clinical trials by collaborator, Dr. Peter Kanter at Roswell Park Cancer Institute with funding from SLIL Pharmaceuticals (Madison, WI). Following IND approval, the SL-11109 will undergo Phase I clinical trial against human prostate carcinoma.

3. Antiproliferative potential of a polyamine catabolic enzyme:

In the last report, we alluded to studies in which conditional expression transfection strategies (tetracycline repression) were used to unequivocally demonstrate that (a) overexpression of the polyamine catabolic enzyme, spermidine/spermine N^1-acetyltransferase (SSAT) leads to polyamine pool depletion and inhibition of cell growth (Figure 10) and (b) basal SSAT activity levels determine sensitivity to DENSPM (Figure 11), (c) overexpression of the enzyme greatly increases sensitivity to DENSPM (Figure 12) and (d) modest expression of SSAT prevents uptake of polyamines (data not shown) and hence, circumvention of growth inhibition by polyamine inhibitors. The findings are reported in reference 10. The implications of these findings are multifold. At a minimum, regulated expression of SSAT can influence sensitivity to certain analogs, inhibit cell growth and/or augment antitumor activity of polyamine inhibitors by preventing salvage of exogenous polyamines.

By way of convenience, these experiments were conducted in MCF-7 breast carcinoma cells (cells already contained the tetracycline-repressible trans-activator system). Given the demonstrated novelty of polyamine homeostasis in prostate carcinoma (1), we feel that it would be highly worthwhile to use the same technology (conditional regulation of SSAT) to make similar determinations in a prostate carcinoma cell line. Anticipated findings would be used as the basis for a possible gene therapy strategy to enhance the therapeutic potency and selectivity DENSPM towards prostate cancer (this effort is not covered by any other funding mechanism). Towards this goal, we have undertaken the following approaches: (a) to prepare adenovirus vectors engineered to express SSAT under a CMV and PSA promoter and (b) to conditionally express SSAT using a tetracycline expression system similar to that used in our earlier studies. Both studies are underway. With regard to the latter, a LNCaP subline containing the tetracycline-repressible trans-activator system has been obtained from Dr. Thomas Powell

(Cleveland Clinic, 9). Human SSAT cDNA cloned into pTRE expression vector containing a tetracycline responsive element (TRE) has been transfected into the above mentioned LNCaP subline. Three high conditional SSAT overexpression clone, SSAT/LNGK9-17, were isolated and found to have express 28-, 34- and 35-fold more SSAT activity in the absence of Doxycyline than in the presence of the antibiotic (Figure 13). Biological characterization of these cells with respect to growth, polyamine pool effects and sensitivity to the polyamine analog DENSPM is underway.

In anticipation of adenoviral gene therapy type studies, we have cloned human SSAT cDNA into a replication-deficient adenoviral AdCMV5-IRES-GFP vector with an internal ribosomal entry site (IRES) for green florescent protein (GFP), both under the control of a CMV5 promoter for maximum expression (Q-Biogene, Montreal, Canada). Recently, we selected an adenoviral clone which expresses the SSAT and GFP dicistronic message (Figure 14). The selected clone has undergone three rounds of plaque purification and is ready for use in viral transduction of SSAT into various prostate carcinoma cell lines. The expectation based on studies by Vujcic et al. is that SSAT overexpression will inhibit cell growth and render cells especially sensitive to DENSPM treatment.

D. Key Research Accomplishments:

- Characterization of the antitumor activity of new and novel polyamine analogs against human DU145 prostate carcinoma xenografts. In particular, SL-11093 displays antitumor activity on a multi-treatment schedule. *This drug is currently undergoing preclinical toxicology by Dr. Peter Kanter at Roswell Park Cancer Institute with funding from SLIL Pharmaceuticals (Madison, WI). Following anticipated IND approval, this compound will undergo Phase I clinical trials against human prostate cancer.*

- Experimental evidence that the polyamine catabolic enzyme SSAT is a determinant of drug action for polyamine analogs and when it is over-expressed in MCF-7 human breast carcinoma cells, the enzyme depletes polyamine pools, inhibits cell growth and prevent uptake of exogenous polyamines.

- Consistent with the previous finding and our interest in prostate carcinoma, we have developed a LNCaP prostate carcinoma in which SSAT is conditionally expressed under a teteracycline promoter and for the purpose of gene therapy modeling, we are well-along in developing adenovirus particles capable of transducing SSAT.

E. Reportable Outcomes:

- Mi, Z., Vredenberg, M.R., Kramer, D.L., Diegelman, P. and Porter, C.W. Induction of caspase-3 dependent apoptosis by the S-adenosylmethionine decarboxylase inhibitor CGP-48664 in human prostate carcinoma cells. Cancer Res. (Provisional acceptance; see letter in Appendix)

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polyamine biosynthesis inhibitor, administered on a daily schedule to patients with solid tumors. J. Clinical Oncol. (in press).

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- 2001 DOD Prostate Cancer Research Program, Idea Development Award Proposal, PC010164, "Validation of Induced Polyamine Catabolism as a Strategy for Treating and/or Preventing Human Prostate Cancer", Carl W. Porter, Principal Investigator. Scored 1.5, 79th percentile, not funded.

F. Conclusions (& Future Directions):

Conclusions of the studies to date are two-fold. Firstly, certain new polyamine analogs express meaningful antitumor activity in a human prostate carcinoma xenografts system. In collaboration with S'LIL Pharmaceuticals, we propose to pursue further preclinical evaluation and development towards clinical trial. We also propose to examine the basis for this activity *in vitro* and *in vivo* and to continue to evaluate the antitumor activity of new analogs rationally designed and synthesized by S'LIL on the basis of findings to date. Secondly, the polyamine catabolic enzyme SSAT has potential as a gene modulator of polyamine analog antiproliferative activity and as a direct inhibitor of cell growth. We propose to examine whether these properties offer novel potential for achieving pharmacological selectivity and potency in prostate model systems.

G. Personnel Receiving Salary from Research Effort.

Gregory Gan T	Technician
Kristen Kee	Predoctoral Student
Nicholas Kisiel	Technician
Debora Kramer, Ph.D.	Research Associate
Michelle Troch	Masters Student
Slavoljub Vujcic	Technician

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APPENDIX 1

FIGURE LEGENDS & FIGURES

Figure 1. Structure of the S-adenosylmethionine decarboxylase inhibitor, CGP-48664, and the polyamine analogs, DENSPM, SL-11093 and SL-11144.

Figure 2. Dose-response determination of IC_{50} values for SL-11144 and SL-11093. Note that SL-0093 reaches a maximum inhibition at 1 μM.

Figure 3. Time-dependence of growth inhibition by Sl-11144 and SL-11093. Note that the former analog is much more toxic than SL-11093 which appears to be wholly cytotoxic at the doses used.

Figure 4. Initial experiment showing the effect of single round of SL-11093 treatment on the growth of DU-145 human prostate carcinoma xenografts. The analog was administered intraperitoneally at 25 and 50 mg/kg/d x 5 days (minimally toxic dose). Thin lines represent individual tumor volumes and thick lines represent median tumor volumes.

Figure 5. Experiment showing the effect of multiple (3) round of SL-11093 treatment on the growth of DU-145 human prostate carcinoma xenografts. The analog was administered intraperitoneally at 50 mg/kg/d x 5 days (minimally toxic dose) with a 9 day respite between treatment rounds. Thin lines represent individual tumor volumes and thick lines represent median tumor volumes. Panel D shows animal weight loss during and following treatment.

Figure 6. Experiment showing the effect of multiple (3) round of SL-11144 treatment on the growth of DU-145 human prostate carcinoma xenografts. The analog was administered intraperitoneally at 5 mg/kg/d x 5 days (minimally toxic dose). Thin lines represent individual tumor volumes and thick lines represent median tumor volumes. Panel D shows animal weight loss during and following treatment.

Figure 7. Repeat experiment showing the effect of multiple (3) round of SL-11093 or SL-11144 treatment on the growth of DU-145 human prostate carcinoma xenografts. The analog was administered intraperitoneally at 50 or 5 mg/kg/d x 5 days (respectively) with a 9 day respite between treatment rounds. Thin lines represent individual tumor volumes and thick lines represent median tumor volumes. Panel D shows animal weight loss during and following treatment. *This Figure emphasized Days 20-98.*

Figure 8. Same experiment as in Figure 7 showing data during Days 20-175.

Figure 9. Experiment showing the effect of a single round of SL-11093 treatment on the growth of PANC-1 human pancreatic carcinoma xenografts. The analog was administered intraperitoneally at 50 mg/kg/d x 5 days (minimally toxic dose). Thin lines represent individual tumor volumes and thick lines represent median tumor volumes. Panel D shows animal weight loss during and following treatment.

Figure 10. DENSPM dose-response for H-10 and M-3 cells grown ±Dox . Cells were grown ±Dox for 2 days and then exposed to increasing concentrations of DENSPM for an additional 2 days. Data points represent mean values ± standard deviation.

Figure 11. Effects of conditional overexpression of SSAT on DENSPM-induced growth inhibition of H-10 cells (left panel) and M-3 cells (right panel). Dox was removed at –2 days and 10 μM DENSPM (Rx) treatment began at day 0 for both transfectants. These experiments were performed simultaneously with those shown in Figure 8 where the growth effects of –Dox alone are presented. Data points represent mean values ± standard deviation.

Figure 12. Effects of conditional overexpression of SSAT on clone H-10 (left panel) and clone M-3 (right panel) cell growth. Data points represent mean values ± standard deviation.

Figure 13. Northern blot analysis of SSAT mRNA expression in LNGK9 clones transfected with a Tetracycline regulatable human SSAT cDNA. Note that overexpression ranges from 5- to 35-fold in the absence of Doxycycline.

Figure 14. Northern blot analysis of SSAT-IRES-GFP mRNA expression in 293 cells transduced with SSAT/AdCMV5-IRES-GFP adenoviral clones. Note that clone 1 alone transduces high levels of SSAT-IRES-GFP mRNA expression.

Figure 1. Structures of Polyamine Analogs & Inhibitors
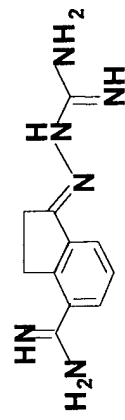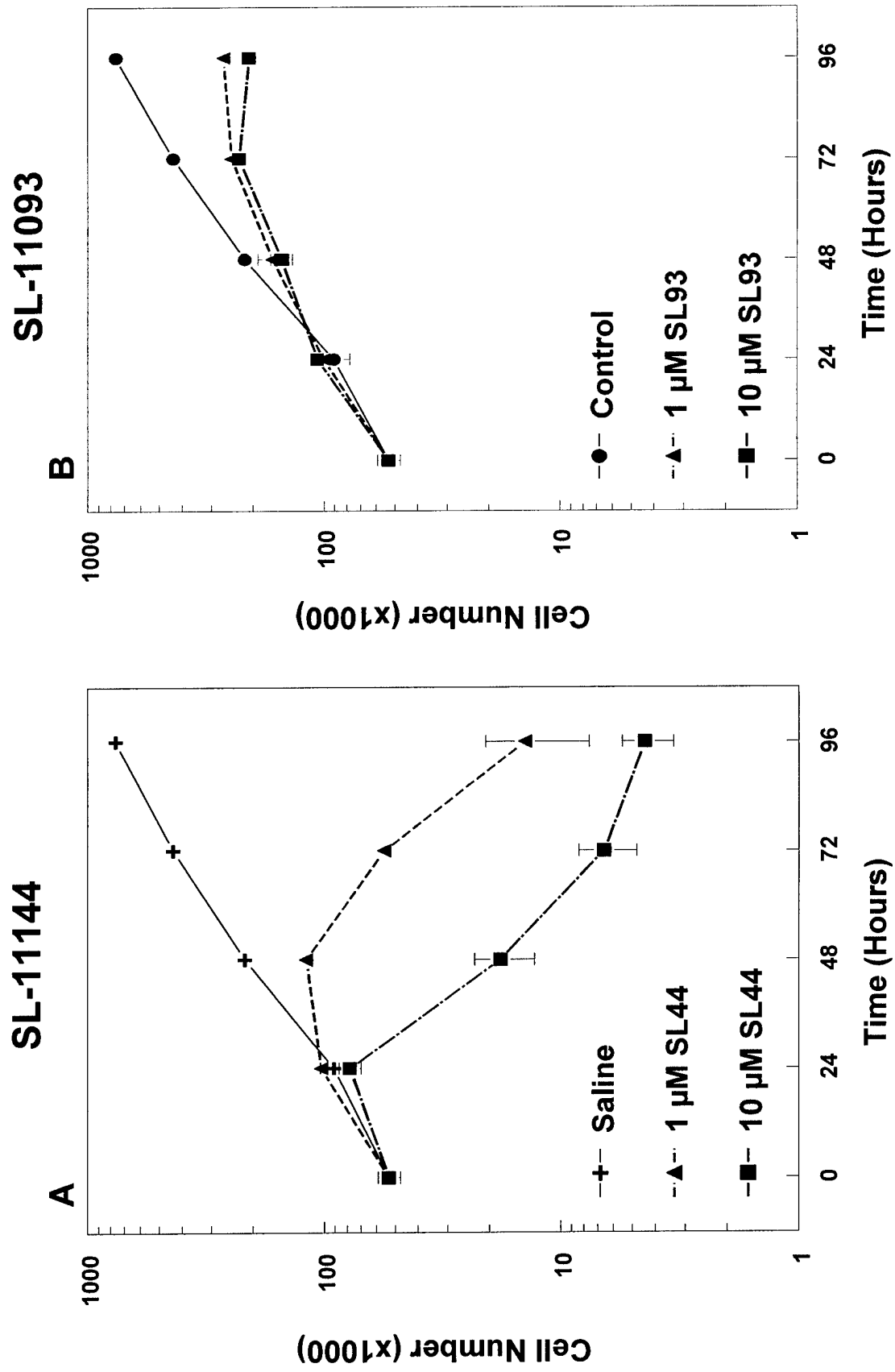
CGP-48664

DENSPM
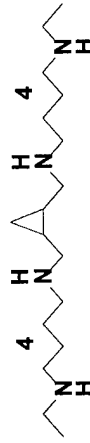
SL-11093
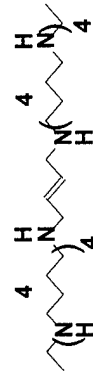
SL-11144

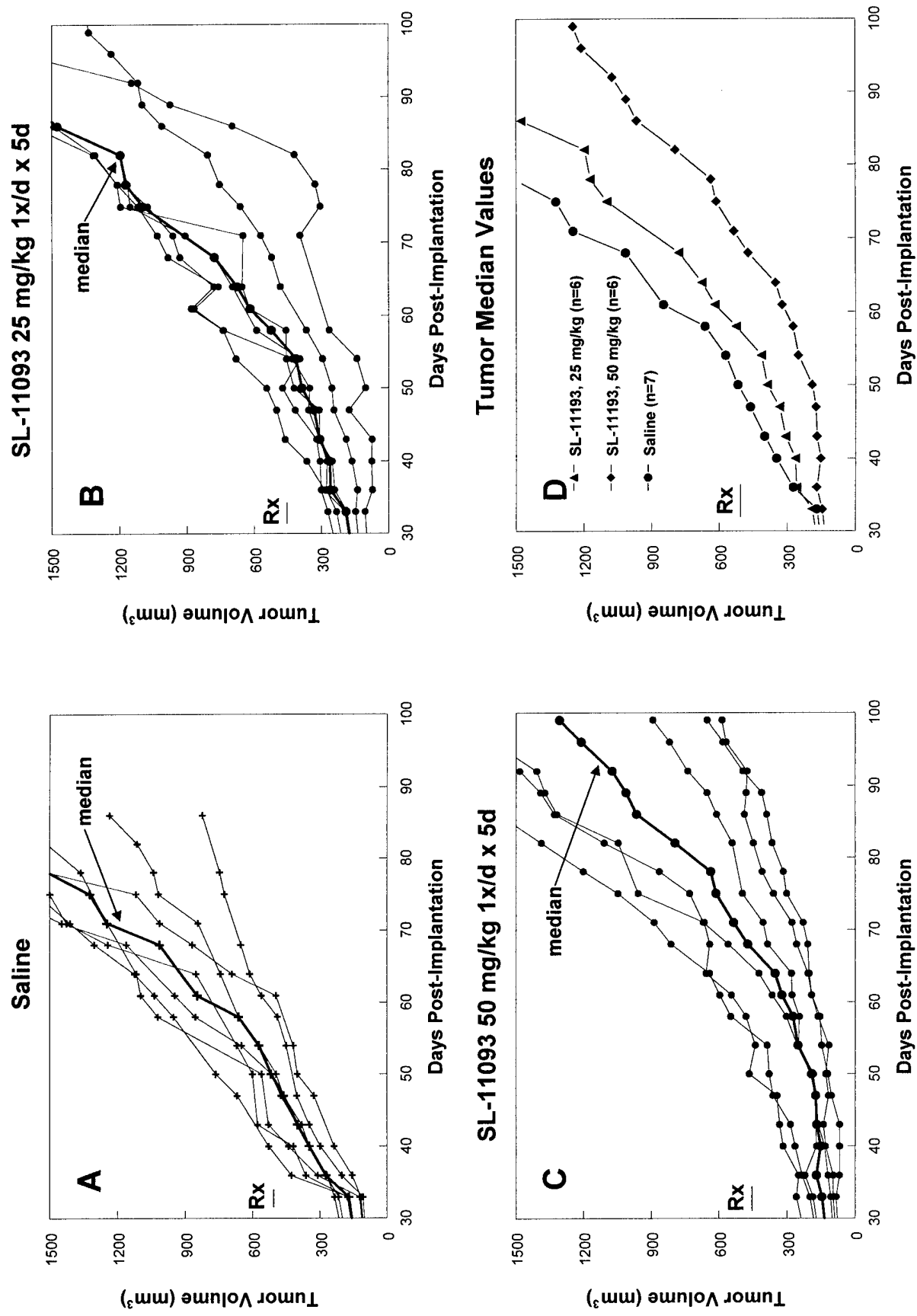*Figure 2. Dose-Response Determination in DU-145 Prostate Carcinoma Cells*
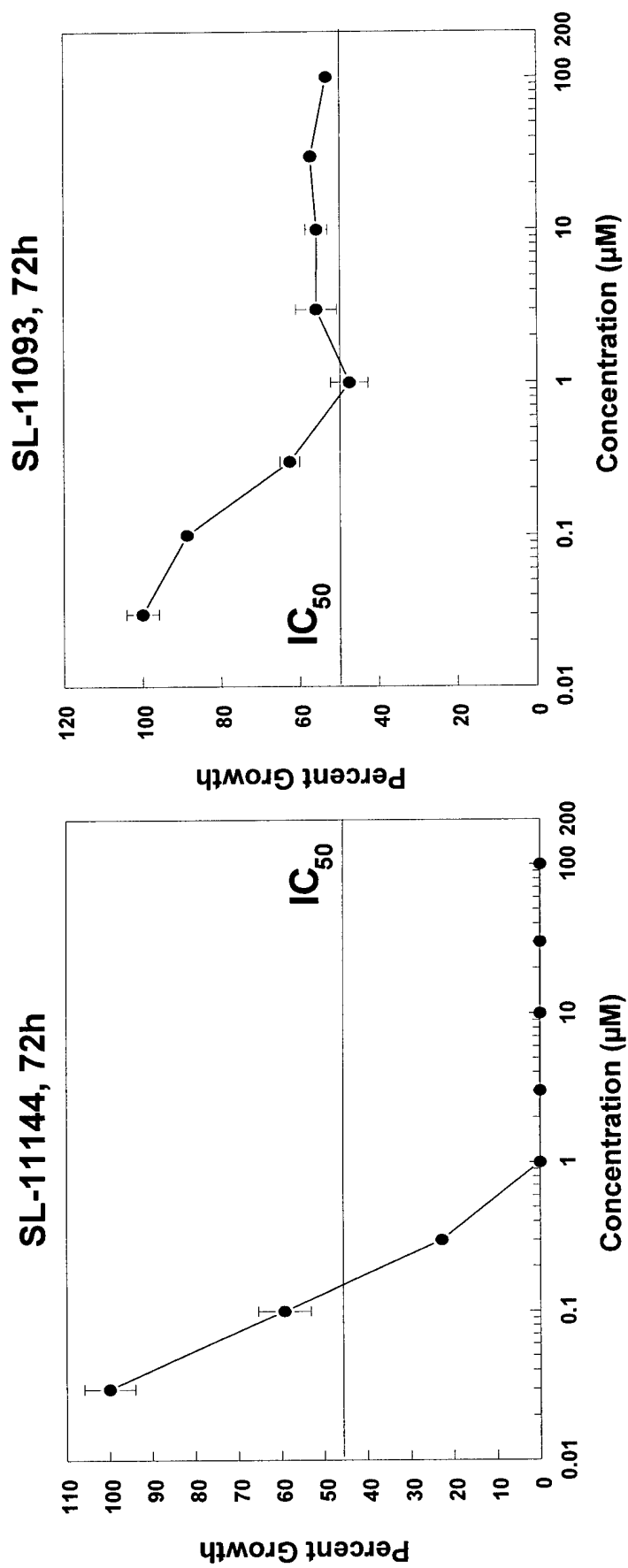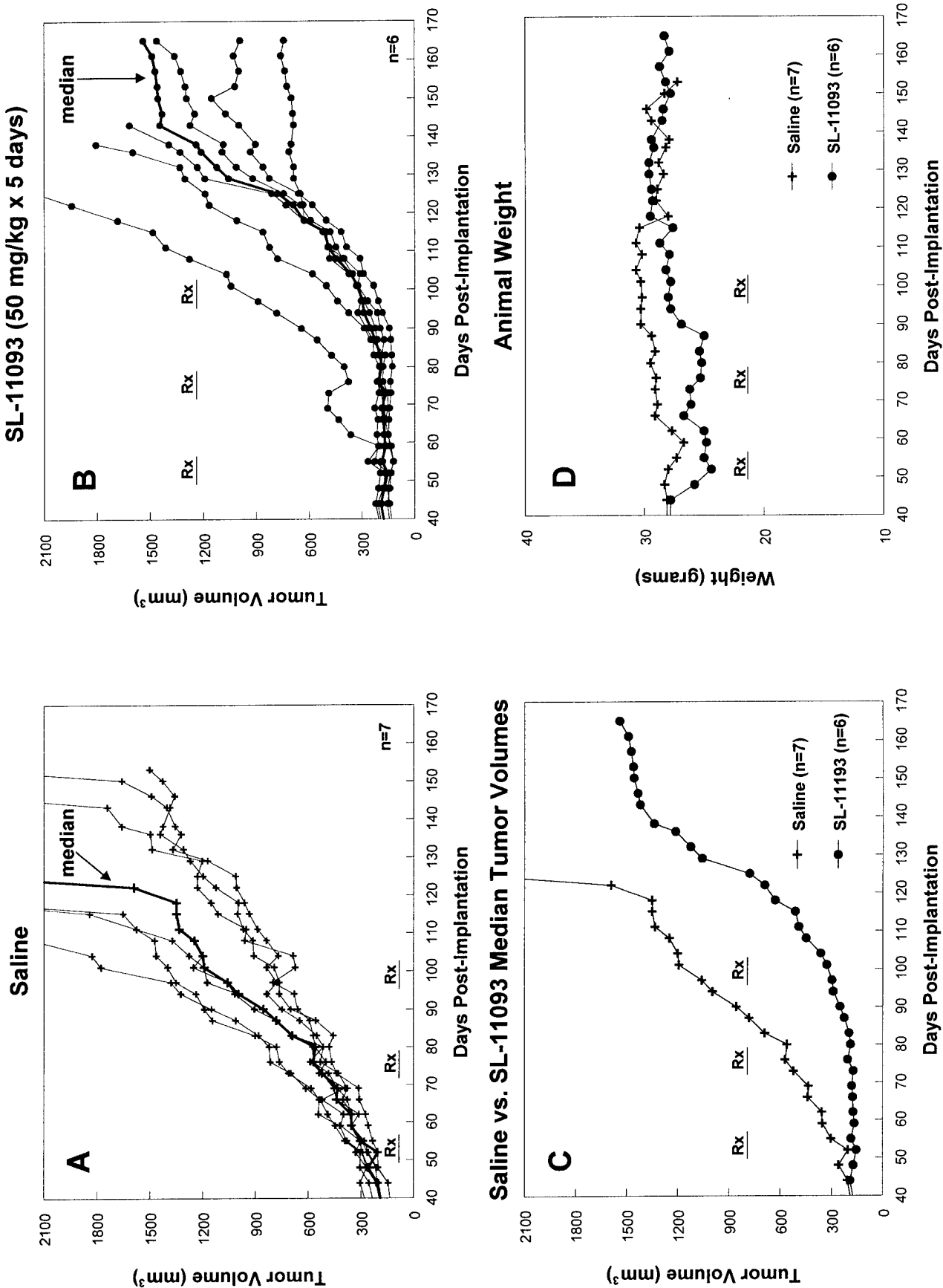
Figure 3. Growth-Response Determination in DU-145 Prostate Carcinoma Cells

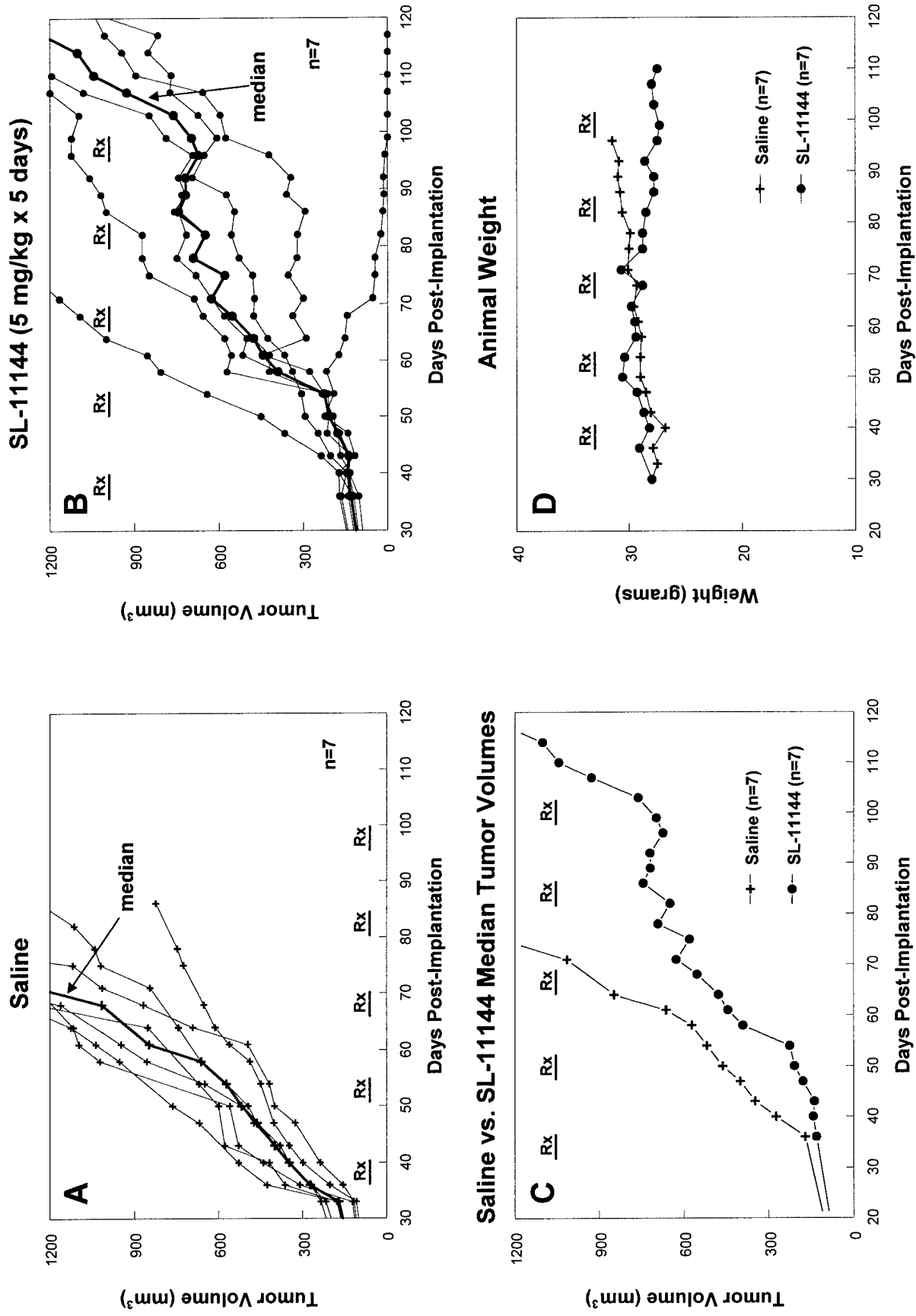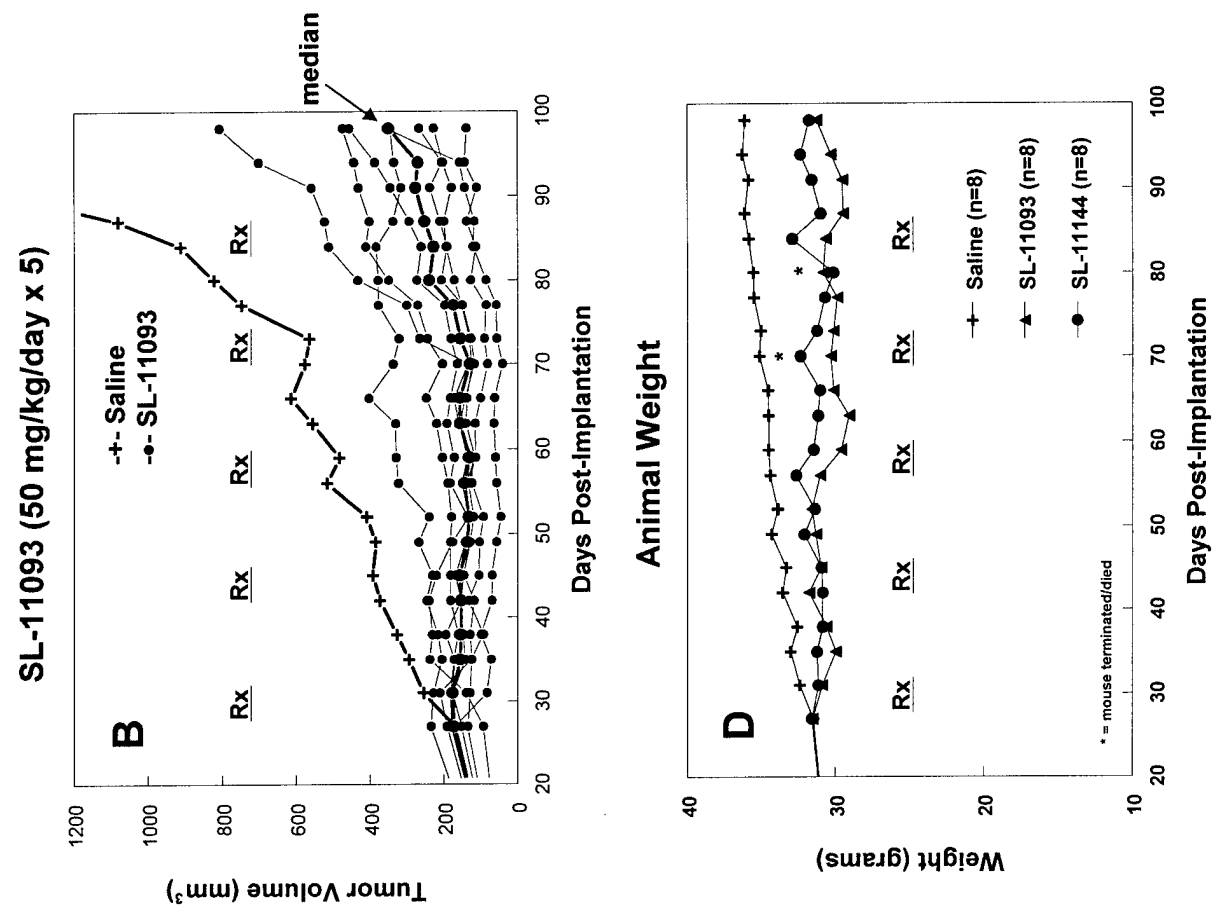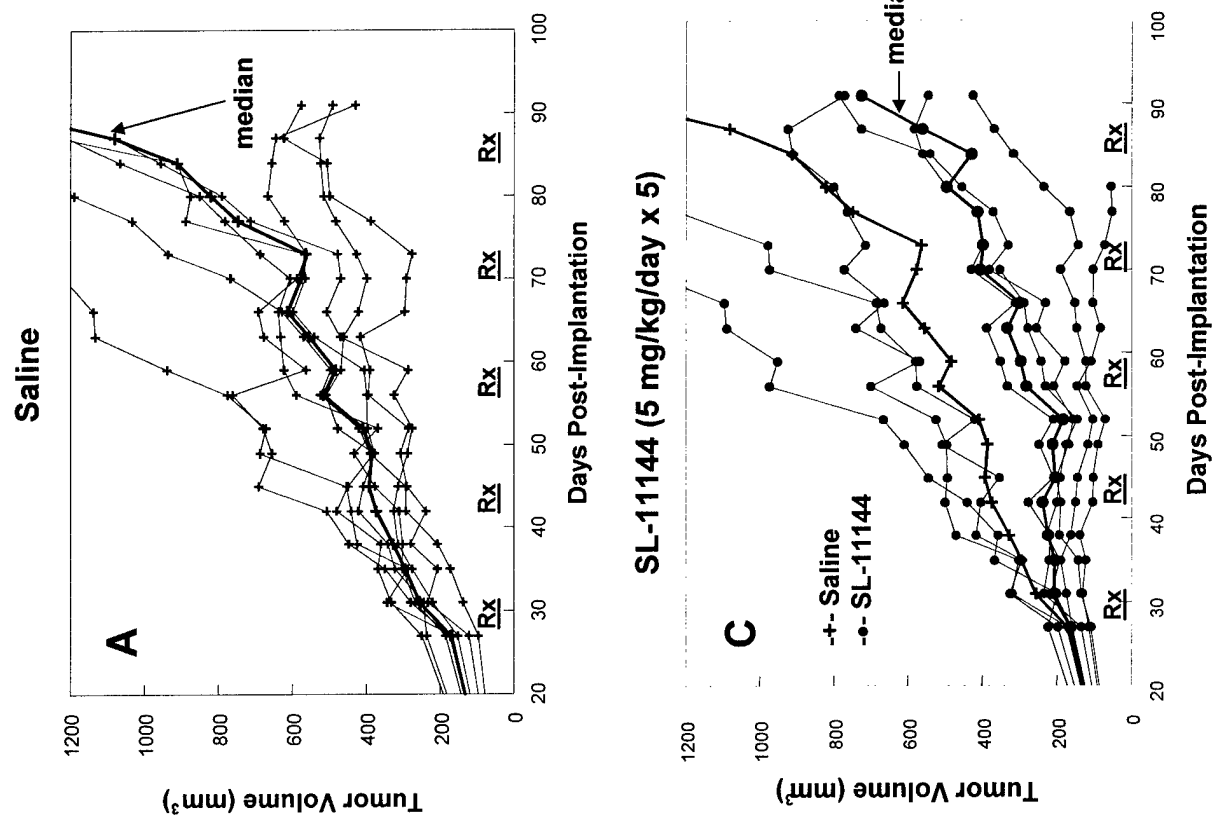Figure 4. DU-145 Prostate Carcinoma Xenografts, Rx SL-11093

Figure 5. DU-145 Prostate Carcinoma Xenografts, Rx SL-11093

Figure 6. DU-145 Prostate Carcinoma Xenografts, Rx SL-11144

Panel A — Saline; Tumor Volume (mm³) vs Days Post-Implantation; median; n=7; Rx

Panel B — SL-11144 (5 mg/kg x 5 days); Tumor Volume (mm³) vs Days Post-Implantation; median; n=7; Rx

Panel C — Saline vs. SL-11144 Median Tumor Volumes; Tumor Volume (mm³) vs Days Post-Implantation; Saline (n=7); SL-11144 (n=7); Rx

Panel D — Animal Weight; Weight (grams) vs Days Post-Implantation; Saline (n=7); SL-11144 (n=7); Rx

Figure 7. DU-145 Prostate Carcinoma Xenografts (Days 20 to 98)

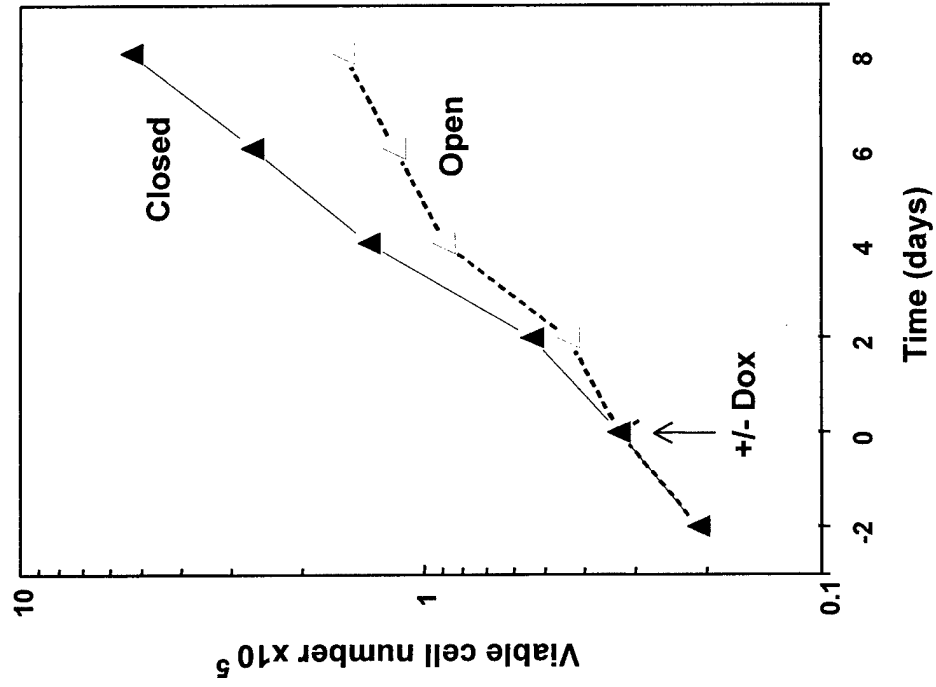*Figure 8. DU-145 Prostate Carcinoma Xenografts (Days 20 to 175)*
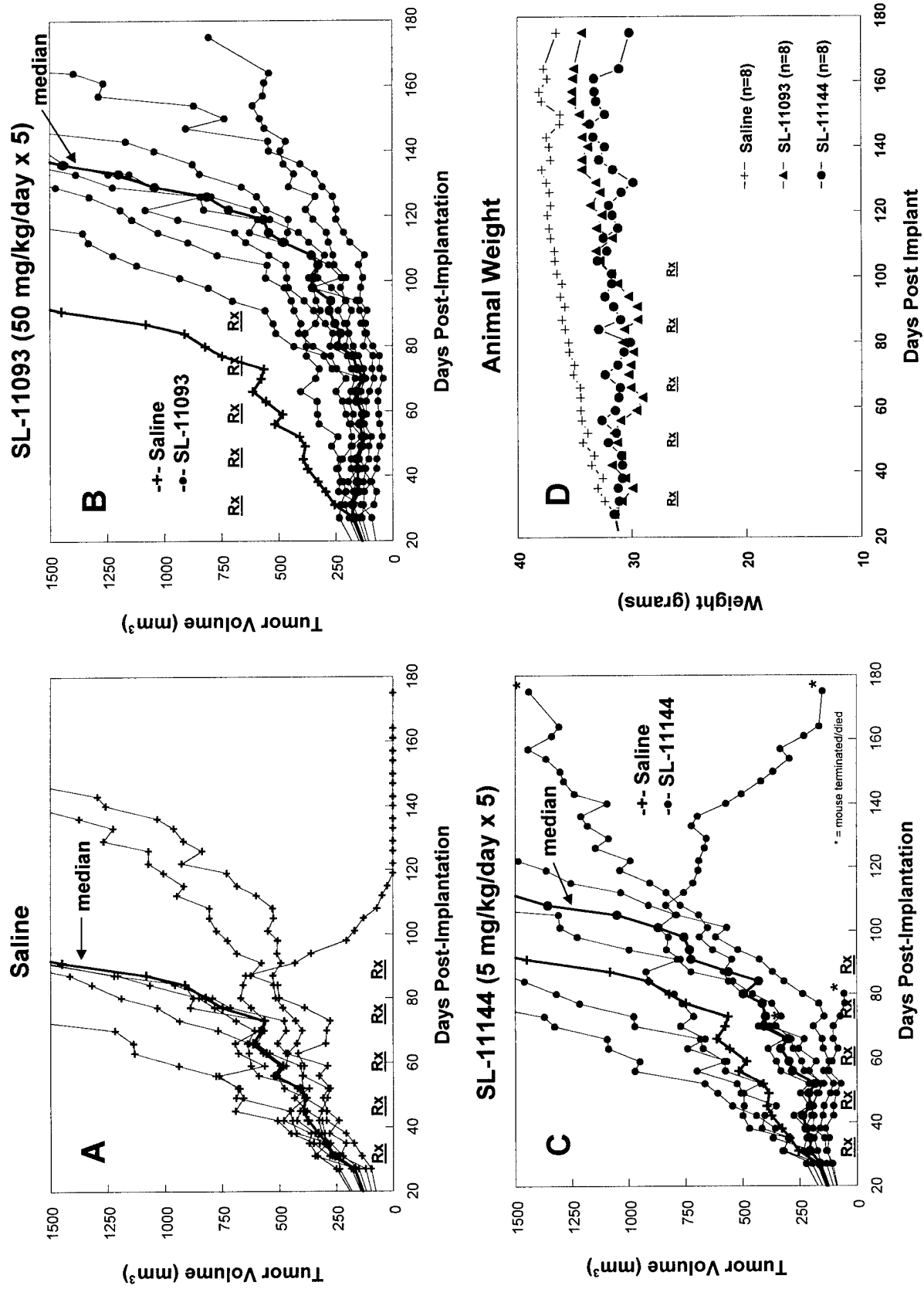
Figure 9. Panc-1 Pancreatic Carcinoma Xenografts, Rx SL-11093
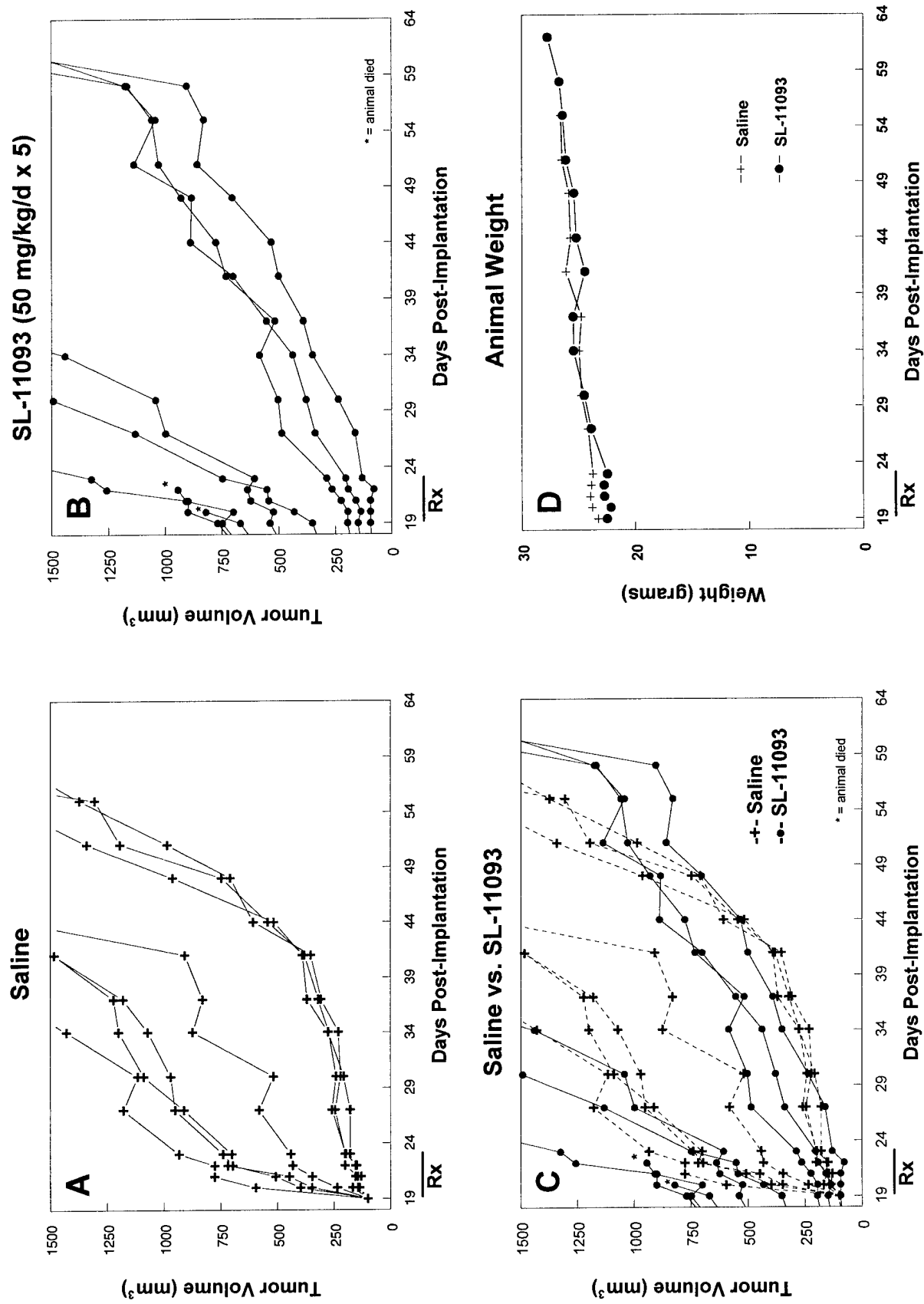
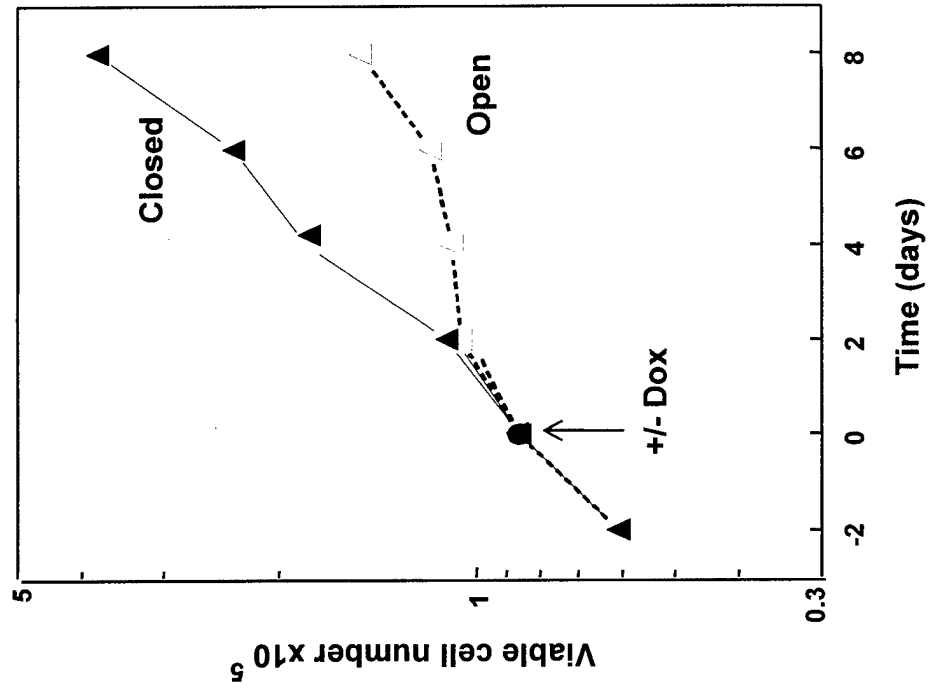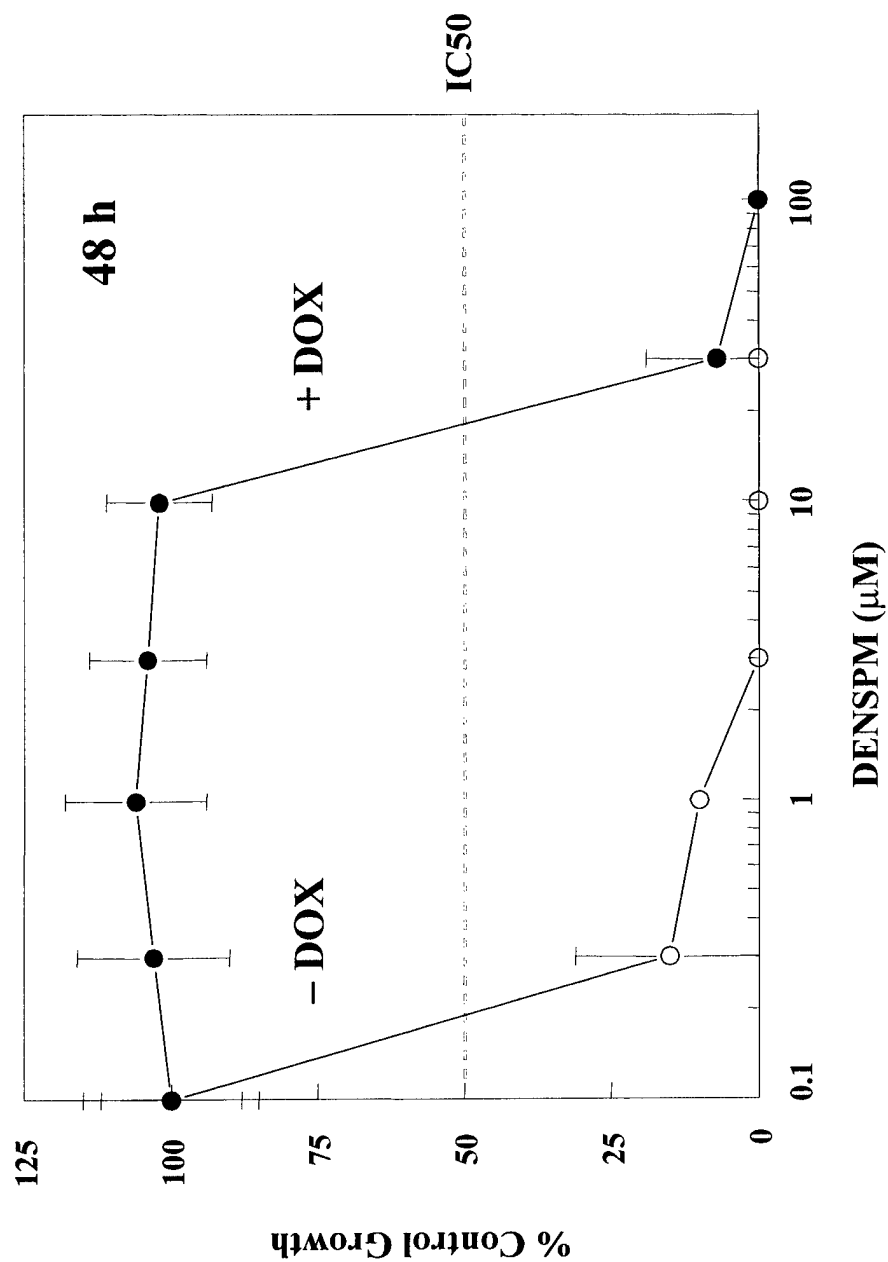*Figure 10.* SSAT Inhibition of Cell Growth
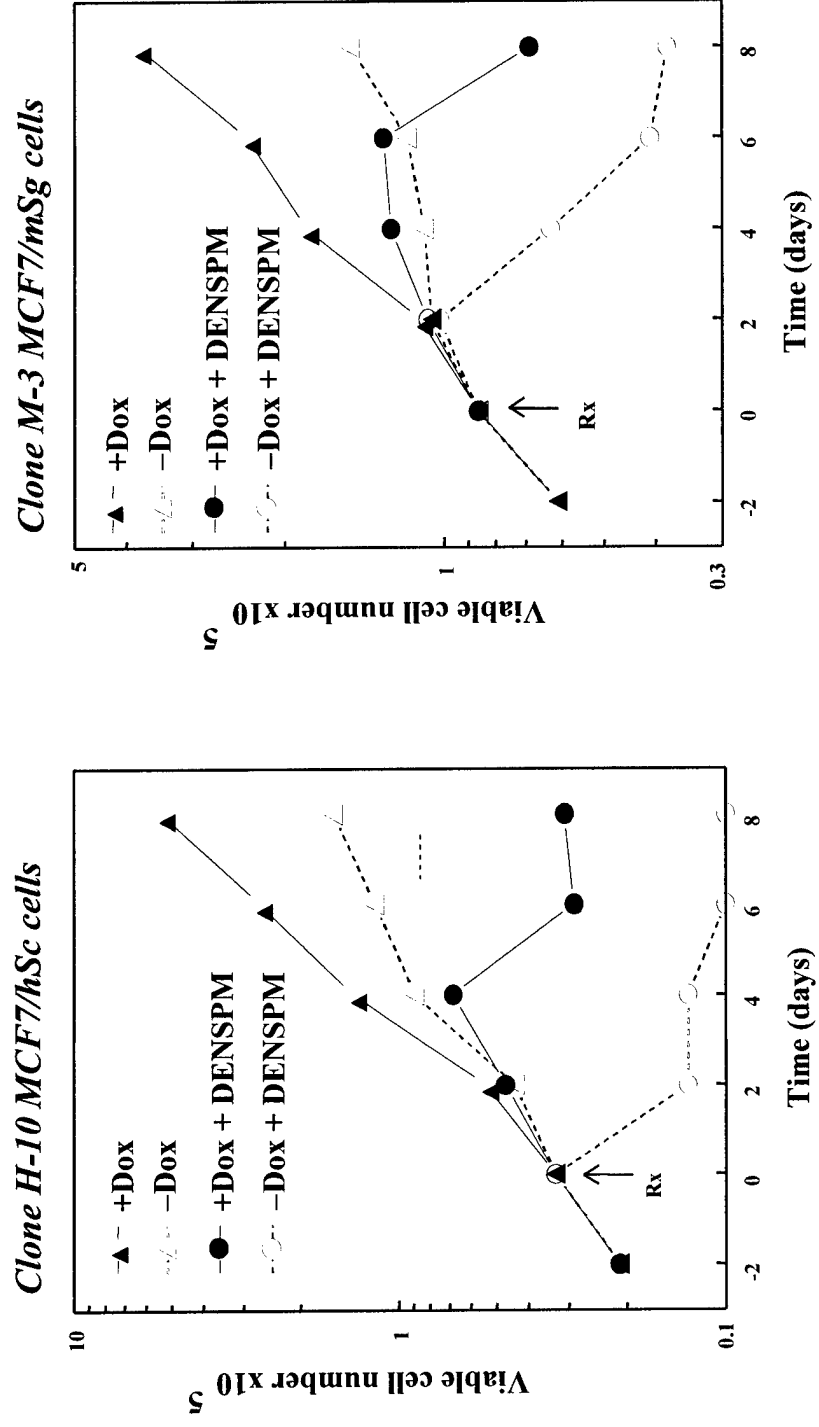
Figure 11. Dose-Dependent Effects of SSAT Expression on DENSPM Cell Growth Inhibition

Figure 12. Effects of SSAT Expression on DENSPM Cell Growth Inhibition

Clone H-10 MCF7/hSc cells

+Dox
−Dox
+Dox + DENSPM
−Dox + DENSPM

Viable cell number x10^5

Time (days)

Rx

Clone M-3 MCF7/mSg cells

+Dox
−Dox
+Dox + DENSPM
−Dox + DENSPM

Viable cell number x10^5

Time (days)

Rx

Figure 13. SSAT RNA expression in LNGK9 clones transfected with tet-regulatable human SSAT cDNA
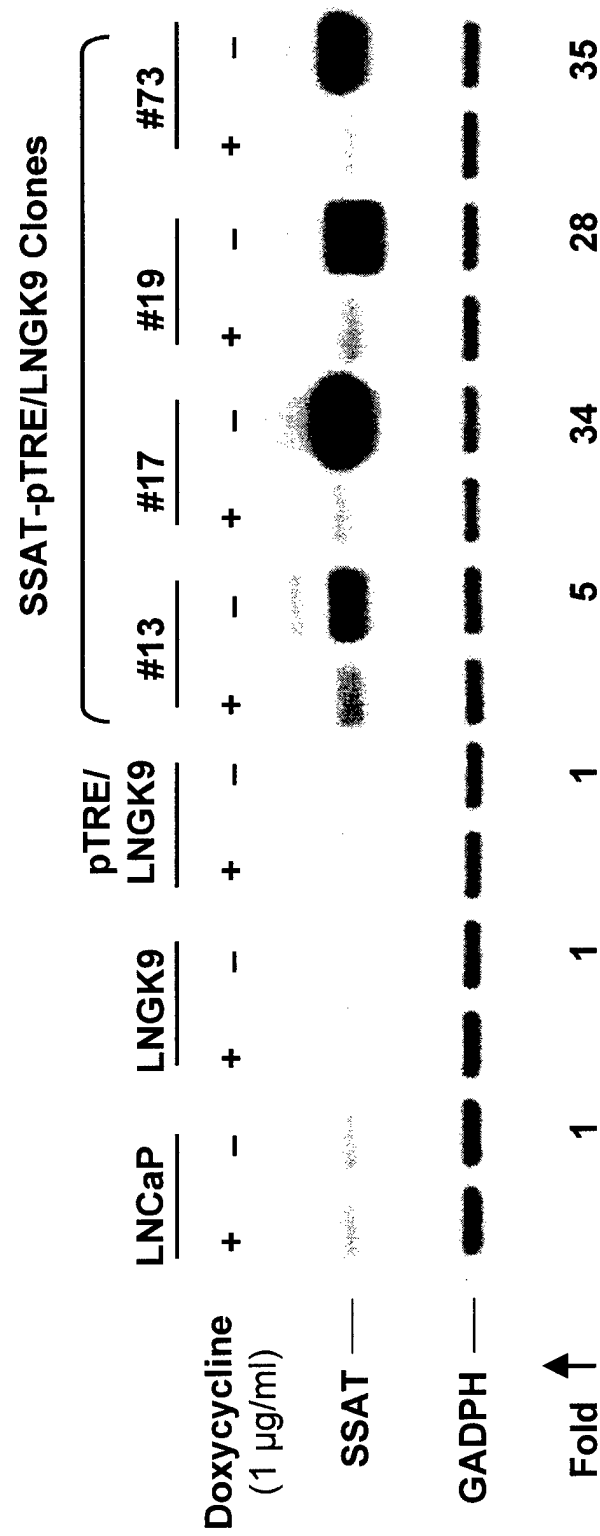
Figure 14. *SSAT-IRES-GFP mRNA in 293 cells transduced with SSAT/AdCMV5-IRES-GFP adenoviral clones*