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14. ABSTRACT The Crk adaptor proteins (CrkI, CrkII and CrkL) play an important role during cellular signalling by mediating the formation of protein complexes. We examined how the loss of Crk would affect tumor progression using RNA interference, as well as examining the consequences of Crk over-expression. For each cancer cell line tested, loss of Crk expression corresponded with a significant decrease in cell migration, invasion, and adhesion, demonstrating that Crk adaptor proteins play an important role in integrating signals of highly malignant cancer cell lines. To formally test this in vivo, Crk was down-regulated using shRNA in breast cancer cells that have a high propensity to form bone metastases. Loss of Crk was associated with a significant decrease in the formation of bone metastases, as well as growth at both the primary and secondary sites. Loss of Crk was also associated with decreases in Rac and Cdc42 activation. These data demonstrate that Crk adaptor proteins play a role in breast cancer progression, however whether Crk proteins can lead to breast cancer development has not been addressed. To test this, transgenic mice over-expressing Crk in the mammary epithelium were established. Transgenic CrkI and CrkII mice undergoing puberty were found to have delayed ductal outgrowth. In post-pubertal CrkII mice, precocious ductal branching was observed, which was associated with enhanced proliferation. Focal mammary tumors or hyperplasias appeared in 18% of CrkII transgenic animals, with an average latency of 14 months. Thus, the present study demonstrates that the Crk adaptor proteins play an important role in integrating signals for mammary gland development and breast cancer progression.					
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Role of Crk adaptor proteins in cellular migration & invasion in human breast cancer

Student: Kelly E. Fathers

BC051116

INTRODUCTION

The Crk protein was originally identified as the oncogene fusion product of the CT10 chicken retrovirus (v-Crk) (1). Cellular homologues of v-Crk, include c-Crk, which encodes two alternatively spliced proteins (c-CrkI and c-CrkII), and c-CrkL. Crk proteins are composed of one Src homology 2 (SH2), and one or two Src homology 3 (SH3) domains (2). The Crk adaptor proteins (CrkI, CrkII and CrkL) play an important role during cellular signalling (of processes such as migration, invasion, & adhesion) by mediating the formation of protein complexes. Previous work in our lab has shown that the over-expression of Crk proteins promotes an invasive phenotype, regardless of upstream signalling implicating a role for Crk proteins in cancer (3, 4). Moreover, Crk proteins are over-expressed in multiple types of cancer, including lung carcinoma, glioblastoma, and breast cancer (5, 6, 7). Although many studies have focused on the over-expression of Crk being important for many cellular processes, their significance in inherently motile cancer cells still remains elusive. Furthermore, much of what has been proposed about Crk proteins in epithelial cells is based upon *in vitro* over-expression studies or the use of dominant interfering mutants of CrkI/II or CrkL. These approaches, while informative, are limited by the challenges intrinsic to over-expression systems and these *in vitro* assays do not truly reflect the complexity of an *in vivo* response. Thus, the goal of our study was to examine if Crk adaptor proteins are truly responsible for mediating signals required for migration and invasion by validating how loss of Crk would affect these processes. Through the use of short interfering RNA (siRNA) silencing techniques and *in vivo* model systems, I will establish experimental models to formally test the hypothesis that *Crk proteins play an important role in cellular migration, invasion and metastasis, through their ability to recruit specific protein-protein complexes*. Our study on Crk adaptor proteins has the potential to identify some of the molecular events, which can occur during metastasis. Furthermore, since Crk proteins are over-expressed in human breast cancer, I will establish mouse models to test the hypothesis that *over-expression of Crk can promote breast tumorigenesis*. This may provide information, which could be used to develop effective treatments for breast cancer.

BODY:

(1) Role of Crk in cellular migration and invasion in various types of cancer

Several studies have suggested that Crk mediates cellular migration and invasion downstream of various stimuli. *I tested the hypothesis that Crk functions as a central adaptor protein that integrates upstream signals for cell migration and invasion by examining how the loss of Crk expression affects these processes in highly invasive cancer cell lines.* Using RNAi, I targeted CrkI, CrkII and CrkL in highly malignant breast cancer cell lines (MDA-435s and MDA-231) and found that the loss of Crk was associated with a decrease in cellular invasion and migration (7). These results demonstrate that Crk adaptor proteins play an important role in the promotion of signals for cell migration and invasion in human breast cancer cell lines irrespective of the upstream regulatory events that promote the invasive response. These results are now published in Molecular Cancer Research (7).

(2) Role of Crk adaptor proteins in metastasis

We previously demonstrated in the first objective that loss of CrkI/II results in decreased cellular migration, invasion and adhesion of various cancer cell lines, demonstrating that CrkI/II proteins are key integrators for these processes. This information identifies a potential role for Crk in metastasis since metastatic cells must possess all the necessary capabilities to efficiently complete the metastatic cascade, including: migratory, invasive, proteolytic, pro-survival, pro-coagulant, and pro-angiogenic properties. The conclusion that Crk adaptor proteins regulate migration and invasion in highly aggressive cancer cells *in vitro*, coupled with the knowledge that elevated levels of CrkI/II proteins are observed in human breast cancer, highlights the fact that the precise role of Crk in breast cancer metastasis still needs to be addressed. *I hypothesize that Crk proteins play a critical role in the metastatic spread of human breast tumors.* To test this hypothesis, I utilized both cell-based studies and animal models to better understand the role of Crk in the metastatic cascade.

Breast cancer metastasizes preferentially to bone, liver, brain and lung. Of the breast cancer patients who die from metastatic disease, 90% of them have bone metastases. Kang and colleagues derived a subpopulation of breast cancer cells (MDA-231 1833TR) with a higher affinity to form bone metastases to identify the molecular mechanisms required for metastasis (8). Since we identified Crk adaptor proteins as being important regulators of processes

necessary for successful metastasis in the parental population of these breast cancer cells *in vitro*, in this next objective, we tested the hypothesis that Crk plays a role in the metastatic process *in vivo*. To test this, we examined whether loss of Crk proteins could decrease the formation of bone metastases in this highly metastatic breast cancer cell line.

We generated stable knockdown of Crk (CrkI/CrkII/CrkL) using a shRNA mediated approach in MDA-231 1833TR breast cancer cell lines. Loss of Crk did not significantly impact cellular proliferation or anchorage independent growth in the presence of serum, albeit loss of Crk did impair the ability of breast cancer cells to grow in soft agar under quiescent conditions. In addition, Crk knockdown decreased the migratory and invasive capabilities of these highly metastatic cells as well as their ability to form invadopodia, which are actin-rich structures believed to be important in cellular invasion. Crk knockdown was associated with a decrease in the formation of bone metastases, with both the number and size of osteolytic lesions decreased in the Crk knockdown cells compared to the control cells. Moreover, loss of Crk inhibited growth at both the primary and secondary site. Crk knockdown was associated with decreased phosphorylation of p130Cas, and decreases in Cdc42 and Rac1 activation. Thus, the Crk adaptor proteins act as key signaling integrators for breast cancer metastasis to bone, facilitating our understanding of this process *in vivo*. The data is provided in Appendix 1 which contains all figures and figure legends.

(3) To examine Crk dependent signaling pathways involved in cancer cell migration and invasion

In this study, I utilized microarray analysis to gain insight into the Crk dependent molecular mechanisms involved in breast cancer progression. Over-expression of Crk can lead to the activation of various transcriptional pathways (AP-1, SRE-1) and Crk is a known activator of JNK and Rac, which can induce transcription (9, 10). Microarray analysis allowed me to identify the extent to which Crk is required for the regulation of genes involved in cancer promoting pathways and hence, determine the specific signals mediated by Crk. Breast cancer cell lines whose cellular migration and invasion are Crk dependent (i.e. MDA-231TR) were transiently transfected with non-targeting siRNA, Crk siRNA, CrkL siRNA, or combinations of the two (Crk, CrkL) and subjected to array analysis. A major advantage of this system, which distinguishes it from previous knockout approaches, is that the cells do not have to grow for long

periods without Crk, and will thus, not have the opportunity to accumulate mutations or adjust expression levels of genes that might compensate for the loss of Crk. Multiple siRNAs will be used in this experiment to control for potential off-targeting effects caused by siRNA duplexes (11). In addition, the gene lists from this experiment were compared to microarray analysis carried out on the stable Crk knockdown MDA-231 1833TR cell lines (described above), thus, providing a smaller gene list that is consistent between two different array experiments.

Several common genes were found when a comparison was made between MDA-231 1833TR Crk shRNA expression profiles and MDA-231TR Crk siRNA expression profiles. Interestingly, the expression of DOCK11, PAK6, ARHGEF4 and ARHGEF30 were all down-regulated upon loss of Crk. DOCK11 is a member of the DOCK180 family of guanine nucleotide exchange factors and is an activator of Cdc42 (12). Although a role for DOCK11 has not been established in breast cancer, DOCK11 is a candidate prognostic marker to identify carcinoma in situ (CIS), a common precursor of testicular germ cell tumours (13). PAK6, which is an effector protein for Cdc42, is highly expressed in primary and metastatic prostate cancers relative to normal prostate tissue (14, 15). Although the precise characterization of ARHGEF4 and ARHGEF30 have not been described, their identification as guanine nucleotide exchange factors for Rho GTPases, suggest that they activate Rho GTPase proteins by exchanging bound GDP for GTP (16).

The above mentioned genes are of interest as Cdc42 and Rac are thought to be important for breast cancer progression. For example, high Cdc42 and Rac1 are observed in human breast cancer (16). Moreover, Rac1 is required for Ras-induced malignant transformation, and is highly expressed in malignant versus benign breast tissue (16). Thus, the potential down-regulation of DOCK11, PAK6, ARHGEF4 and ARHGEF30, coupled with the decreases in Cdc42 and Rac activation observed in Crk knockdown cells and the significant *in vivo* tumour inhibition we observe, implicates both Rac and Cdc42 as being important mediators of breast cancer progression.

Finally, expression profiles from both MDA-231 TR and MDA-231 1833TR data sets were entered into the KEGG pathway analysis program, in which genes have been categorized based on their molecular function and biological processes. Using KEGG, we found that several potential biological processes were altered upon loss of Crk adaptor proteins. Categories significantly affected by loss of Crk proteins included various metabolic pathways, cytokine-

cytokine receptor interactions, focal adhesions, adheren junctions, cell adhesion molecules and extracellular-matrix interactions. An additional pathway analysis program (GO) revealed similar molecular processes affected in Crk knockdown cells, such as actin binding and cytoskeletal protein binding. This data is consistent with the finding that there were alterations in several genes whose molecular functions are involved in potential Crk-dependent pathways, such as Abl, ezrin, radixin and several regulators of Rho GTPase activation, including DOCK2, DOCK10, DOCK11, ELMO1, as well as the Rho guanine nucleotide exchange factors ARHGEF3, ARHGEF4, ARHGEF5, and ARHGEF12. Knockdown of CrkI/II and CrkL mRNA were confirmed through real-time PCR and validation of additional genes is ongoing. Our data implies that loss of Crk adaptor proteins may affect breast cancer development and metastasis in a multi-factorial manner. Thus, in addition to altering cellular signalling (Objective 2), loss of Crk affects the expression of genes whose functions may be required for breast cancer progression. The data is provided in Appendix 1 which contains all figures and figure legends.

(4) Role of Crk adaptor proteins in the development of breast cancer using transgenic mice

To examine *the importance of Crk adaptor proteins in tumor progression*, I used a transgenic mouse model, in which Crk proteins are over-expressed in the mammary epithelium. I have created constructs for CrkI, CrkII and CrkL, which allows the over-expression of these proteins through the hormonally responsive MMTV promoter. At the present time, MMTV-CrkII transgenic mice have been characterized and a manuscript is in preparation. As these results have previously been described in the 2008 final report, a quick summary is provided. Transgenic mice undergoing puberty were found to have delayed ductal outgrowth, compared to their wildtype littermates. This indicated a delayed development of the mammary gland and was characterized by increased collagen surrounding the terminal end bud. The fat pad was eventually filled and in older mice, there was precocious ductal branching associated with increased proliferation. Focal mammary tumours appeared in a subset of animals examined with a latency of approximately 15 months. MMTV-CrkII tumours showed high levels of Crk protein as well as various cytokeratin markers characteristic of these tumour pathologies. This study provides the first demonstration of a potential role for the CrkII adaptor protein in integrating signals for mammary gland development and breast cancer progression *in vivo*, which has important implications for elevated CrkII observed in human cancer.

The MMTV-CrkI and MMTV-CrkLV5 mice have been generated and their characterization is ongoing. For the MMTV-CrkI transgenic mice, the virgin and multiparous cohorts have been established and I am currently examining how over-expression of CrkI affects puberty, pregnancy, lactation and involution. At the present time, we have found that over-expression of CrkI delays ductal outgrowth, similar to over-expression of CrkII. Crosses with ErbB2 transgenic mice have also been established and the study is ongoing. For the MMTV/CrkLV5 transgenic mouse model, the founder lines have been established and the virgin and multiparous cohorts are being established. No delay in ductal outgrowth is evident in these transgenic animals. See Appendix 2 for a summary of the various MMTV-Crk mouse models.

In conclusion, we demonstrate that over-expression of various Crk adaptor proteins is associated with altered mammary gland development and accelerated tumour development. Although the low incidence of tumours in our transgenic mouse model highlights the fact that Crk proteins are not strong oncogenes, the ability of CrkII to induce a branching phenotype during a normally quiescent state suggests that it plays an active role in proliferation and epithelial remodeling. This precocious development, combined with the fact that Crk proteins are downstream of several signaling proteins involved in breast cancer development, highlights the potential consequence of increased Crk proteins in the human disease.

KEY RESEARCH ACCOMPLISHMENTS

- (1) Discovered that Crk adaptor proteins are required for the efficient metastasis of breast cancers to bone.
- (2) Established that Crk adaptor proteins are required for the development of breast cancer through shRNA based studies and mammary fat pad injections.
- (3) Identified Crk adaptor proteins are being important regulators for Cdc42 activation.

- (4) Demonstrated that loss of Crk expression results in the decreases in gene expression of several genes, whose functions are required for various aspects of tumour progression.
- (5) Established the role of Crk adaptor proteins in promoting tumour development. The creation of MMTV-based mouse models over-expressing the Crk adaptor proteins in the mouse mammary gland confirmed that these oncogenes are not very strong, but rather collaborate with additional genetic events to promote tumorigenesis.
- (6) Over-expression of Crk adaptor proteins alter the delicate balance of mammary gland development, leading to delayed ductal outgrowth (MMTV/CrkI, MMTV/CrkII) and enhanced branching (MMTV/CrkII).

REPORTABLE OUTCOMES

Manuscripts

Fathers, KE, Rajadurai, CV, Monast, A, Zhao, H, Pepin, F, Mourskaia, A, Zuo, D, Siegel, P, Park, M. 2009. Crk adaptor proteins act as key signaling integrators for breast cancer metastasis. (Manuscript in preparation for May 2009 submission).

Fathers, KE, Monast, A, Rodrigues, SP, Zuo, D, Zhao, H, Chughtai, N, Vasudeva Murthy, I, Cardiff, R, Park, M. 2009. CrkII transgene leads to atypical mammary gland development and tumorigenesis. (Manuscript in preparation for April 2009 submission).

Rodrigues, SP[^], Fathers, KE[^], Chan, G, Zuo, D, Halwani, F, Meterissian, S, Park, M. 2005. CrkI/II function as key signalling nodes for migration and invasion of cancer cells. Molecular Cancer Research. 3 (4):183-94

[^] - Both authors contributed equally to this work.

Abstracts

Fathers KE, Monast A, Rodrigues S, Park M. CrkII transgene leads to abnormal mammary gland development and tumorigenesis. Era of Hope – Department of Defense Breast Cancer Research Program, June 25-28, 2008. Baltimore, Maryland, USA.

Fathers KE, Monast A, Rodrigues S, Park M. CrkII transgene leads to abnormal mammary gland development and tumorigenesis. Gordon Research Conference – Mammary Gland Biology, June 1-5, 2008. Il Ciocco, Barga, Italy.

Fathers KE, Monast A, Rodrigues S, Park M. Over-expression of CrkII leads to abnormal mammary gland development and a basal cancer phenotype. Mechanisms and Models of Cancer. August 8-12th, 2007. La Jolla, California, USA

Fathers KE, Monast A, Rodrigues S, Park M. MMTV-CrkII mice display a defective ductal outgrowth phenotype in peripubertal mice. Gordon Research Conference - Mammary Gland Biology. May 28-June 2, 2006. Il Ciocco, Barga Italy.

Fathers, KE, Rodrigues S, Park M. Crk adaptor proteins play a key role in cellular migration and invasion in human cancer. Canadian Breast Cancer Research Alliance - Reasons for Hope. Fourth Scientific Conference. Montreal, Quebec May 5-8, 2006

Fathers, KE, Rodrigues S, Park M. Crk adaptor proteins play a key role in cellular migration and invasion in human cancer. American Society of Cellular Biology - Systems Integration in Directed Cell Motility. University of Washington, Seattle, WA. July 27-30, 2005

Rodrigues, SP, Fathers, KE, Chan, G, Park, M. Crk adaptor proteins play an essential role in cellular migration and invasion in multiple human cancer cell lines. Twentieth Annual Meeting on Oncogenes, 2004. Frederick, Maryland, USA.

Presentations

Fathers, KE. Crk adaptor proteins act as key signaling integrators in breast cancer development and metastasis. Metastasis and Angiogenesis Research Meeting. McGill University, Montreal, Canada.

Fathers, KE. Crk adaptor proteins act as key signaling integrators in breast cancer development and metastasis. Invited speaker – Dec 10th, 2008. University of Toronto, Toronto, Canada.

Fathers, KE. Crk adaptor proteins act as key signaling integrators in human breast cancer. Molecular Oncology Group Seminar Series. McGill University, Montreal, Canada.

Animal Models

MMTV-CrkLV5

MMTV-CrkII

MMTV-CrkI

Cell Lines

MDA-231 breast cancer cell lines stably expressing shRNA targeting CrkI/II and CrkL

CONCLUSION

Our data demonstrates that Crk adaptor proteins play an important role in integrating signals for migration and invasion of highly malignant human cancer cell lines, regardless of the upstream signals promoting these cellular processes. These results are important, as progression of cancer to an invasive, metastatic lesion is dependent, in part, on the deregulation of signaling pathways involved in migration and invasion. We have shown the significance of Crk adaptor proteins during tumour progression as well as metastasis, which is the rate-limiting factor in cancer treatment. This has important consequences, as Crk proteins are over-expressed in many types of cancer, including breast cancer. In addition, our work has identified several molecular mechanisms by which Crk regulates breast cancer progression. This may provide information, which could be a valuable tool for the design and implementation of a therapeutic drug for breast cancer and its metastases, which is both highly specific and efficient.

“SO WHAT”

Metastasis is a major cause of morbidity and mortality in human malignancies, and is the driving force behind the incessant pursuit of “anti-metastatic” and adjuvant therapies. For instance, patients with metastatic breast cancer have a median survival of only 2 to 3 years and

twenty percent of the patients who present with bone metastases have only a five year survival from the time of diagnosis (17). As a result, current therapies for metastatic breast cancer are aimed at improving palliative care rather than complete remission (17). Further progress in this field of research may be achieved through a better understanding of the various molecular processes defining the complexity and multi-step nature of tumor cell dissemination, otherwise known as the metastatic cascade. My research has shown that Crk adaptor proteins are key integrators of breast cancer development and metastasis.

In addition to identifying a novel role for Crk adaptor proteins in breast cancer metastasis, I have shown that CrkII is linked to the formation of hyperplasias and breast cancer. The low penetrance and low latency suggests that over-expression of CrkII may not drive tumor development, although we know Crk expression is required for tumor progression based on our shRNA experiments. Combining my recent data with the previous data from our lab, which showed that Crk can convert a non-invasive response to an invasive response, suggests that Crk may not necessarily need to be elevated to be important for tumor development. In reality, Crk may synergize with several upstream signals to drive tumor progression rather than driving tumor progression itself. For instance, I have shown that loss of Crk inhibits migration and invasion of several “basal” breast cancer cell lines, signifying that Crk may be a key signaling node for multiple tumors, regardless of their inherent mutations. As these “basal” breast tumors are triple negative (Her2, ER, PR negative), treatment options are limited. Thus, the data generated from this study has the potential to identify Crk adaptor proteins as valuable targets for these types of breast tumors.

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Appendix 1

Crk adaptor proteins act as key signaling nodes for breast cancer metastasis

Kelly E. Fathers, Charles V. Rajadurai, Anie Monast, Hong Zhao, Francois Pepin, Anna Mourskaia, Dongmei Zuo, Peter Siegel, and Morag Park

Figure 1 – Knockdown of Crk alters cell morphology, anchorage independent growth and cellular migration and invasion. Western blot analysis of whole cell lysates (MDA-231 1833TR) with an anti-CrkI/II or anti-CrkL sera revealed that the CrkI/II and CrkL shRNA constructs efficiently and specifically target its respective RNA. Actin protein levels were used as a loading control (A). Alamar blue assay of control and Crk shRNA cells demonstrated no differences in cellular proliferation over time (B). Soft agar assays under low serum conditions (0.5% FBS) revealed differences in the ability of Crk shRNA cells to grow under anchorage independent conditions, relative to control MDA-231 1833TR cells. Images were taken using Lumenera Infinity Analyze software (C). Control cells and Crk shRNA cells plated onto

fibronectin coated coverslips revealed gross differences in cell morphology. Cells were co-stained with α -Paxillin/Alexa Fluor 555 goat anti-mouse and Phalloidin-488 for the F-actin staining. Images were acquired using a Zeiss confocal microscope. Actin and paxillin images were acquired using a 63x objective. Scale bars represent 10 μ m (D). Both control cells and Crk shRNA cells were analysed for their migration and invasion capacity in the presence of serum, with the Crk shRNA showing decreased migration and invasion relative to control cells. Cells were seeded into modified boyden chambers in the presence or absence of matrigel and assayed over a 24 hour period. Cells remaining on bottom of the porous membrane were fixed in formalin phosphate, stained with 0.2% crystal violet, washed, and left to dry overnight. Bottom layers of each transwell were imaged in 5 separate fields for each cell line using a 10x objective in phase contrast. Image analysis of these assays was carried out using ImageScope software. A minimum of three experiments was performed. Error bars represent the standard error of the three experiments (E, F).

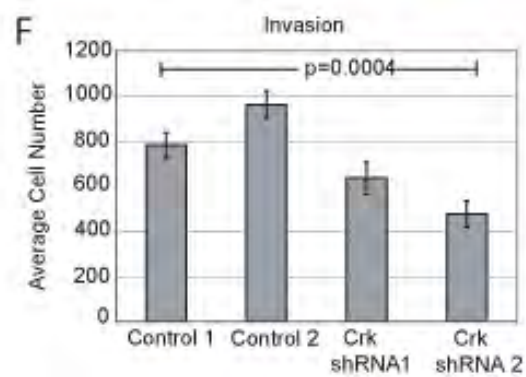
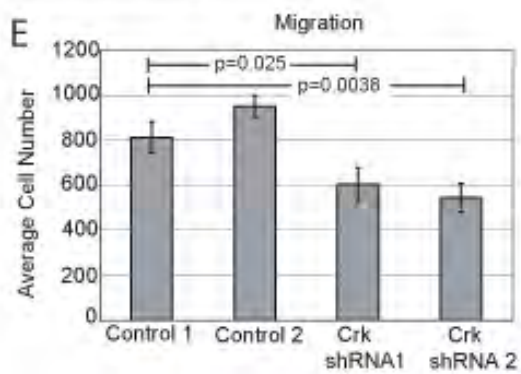
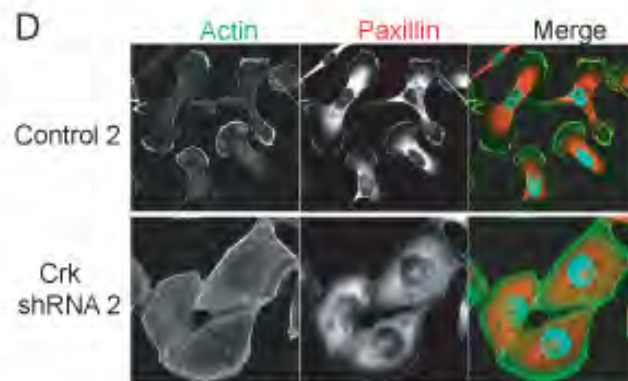
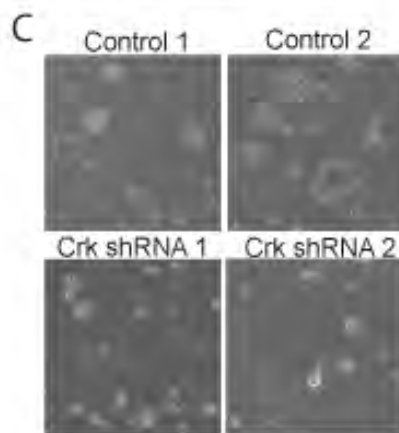
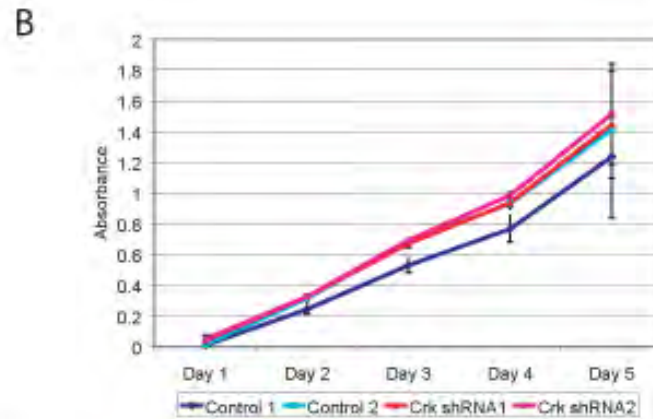
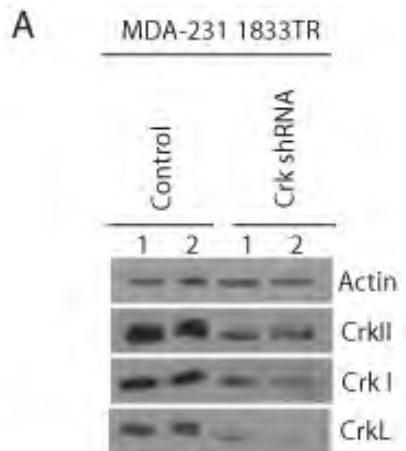


Figure 2 - Crk adaptor proteins are required for efficient metastasis of breast cancer cells to bone. MDA-231 1833TR control cells or those expressing Crk shRNA were injected into the left cardiac ventricle of female nude mice. Breast cancer cell outgrowth in bone was measured by bioluminescence imaging, which revealed less bioluminescence activity in Crk shRNA expressing cells compared to controls. A representative luciferase image at 40 days is shown, which closely resembles the mean bioluminescence values from each group (A). The whole body bioluminescence was quantified as the normalized photon flux (p/sec/cm²/sr) or relative luciferase units (RLU) over time. Averages from each cell line were plotted along with standard error (B). The bioluminescence derived from positive forelimbs and hindlimbs, which are the most common metastatic sites, were quantified in a similar manner to the whole body luminescence. Averages from each cell line were plotted along with standard error (C).

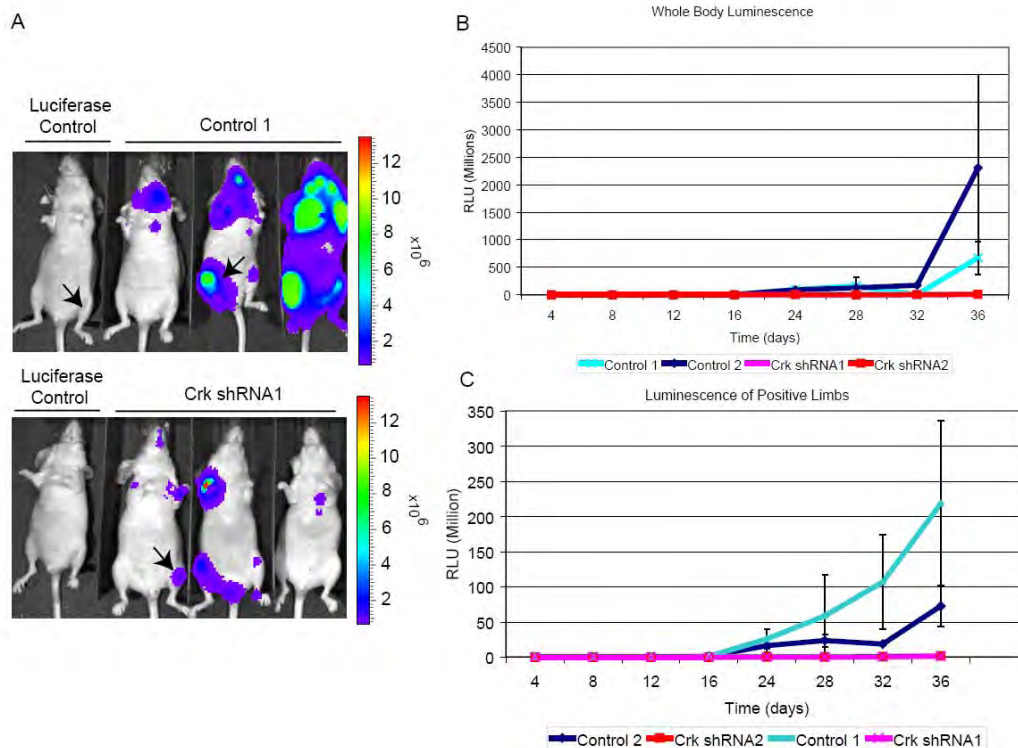


Figure 3 - Crk adaptor proteins are required for the efficient osteolysis of bone. MDA-231 1833TR control cells or those expressing Crk shRNA were injected into the left cardiac ventricle of female nude mice. Representative X-ray imaging of negative control mice, 1833TR control cells and Crk shRNA cells reveals a decrease in the number and size of osteolytic lesions in the Crk shRNA derived metastatic lesions (A). H&E stained tibial (control) and jaw (Crk shRNA) sections show similar pathology, however the intensity of CrkI/II and CrkL staining in Crk shRNA derived tumours is lower compared to control tumours. Images taken at 20x. Scale bar represents 50 μ m (B). X-ray images of all the animals were first blinded and then scored for the number of osteolytic lesions for each condition. The average number of osteolytic lesions is decreased in the Crk shRNA derived metastases (C). X-ray images of all the animals were blinded and then scored for the average size of osteolytic lesions for each condition using ImageJ software. The average size of osteolytic lesions is decreased in the Crk shRNA derived metastases (D).

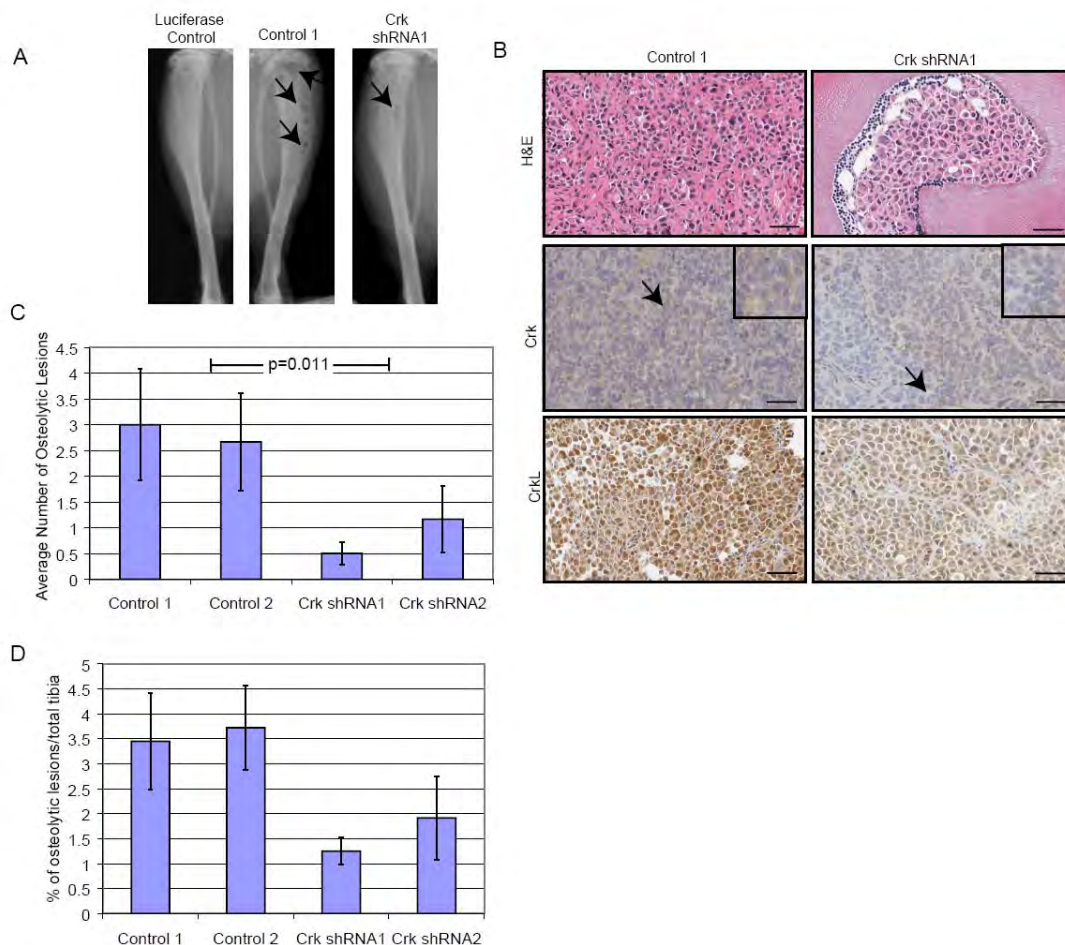


Figure 4 - Crk knockdown affects the outgrowth of breast cancer cells within the bone.

MDA-231 1833TR control cells or those expressing Crk shRNA were injected directly into the tibia of female nude mice. Breast cancer cell outgrowth in the tibia was measured by bioluminescence imaging, which revealed less bioluminescence activity in the Crk shRNA expressing lesions compared to the controls. A representative luciferase image at 32 days is shown, which closely resembles the mean bioluminescence values from each group (A). The tibial outgrowth was quantified using only limbs positive for bioluminescent activity. It was measured as the normalized photon flux (p/sec/cm²/sr) over time, or relative luciferase units (RLU). Averages from each cell line were plotted along with standard error (B). Representative X-ray imaging of negative control mice, 1833TR control cells and Crk shRNA cells reveals a decrease in the number and size of osteolytic lesions in the Crk shRNA derived tibial outgrowths (C). X-ray images of all the animals were first blinded and then scored for the number of osteolytic lesions for each condition. The average number of osteolytic lesions is decreased in the Crk shRNA derived metastases (D). Blinded X-ray images of all the animals were also scored for the size of osteolytic lesions. The average size of osteolytic lesions is decreased in the Crk shRNA derived lesions (E). H&E stained sections show similar pathology. Paraffin embedded sections of tibial lesions derived from 1833TR control and Crk shRNA cells were stained for Ki67 and CrkL. Preliminary staining patterns revealed significant differences between the control and Crk knockdown tumours for Ki67 and CrkL staining intensity. All images taken at 20x where the scale bar represents 50 μ m. Arrows represent positive staining (F).

Figure 4

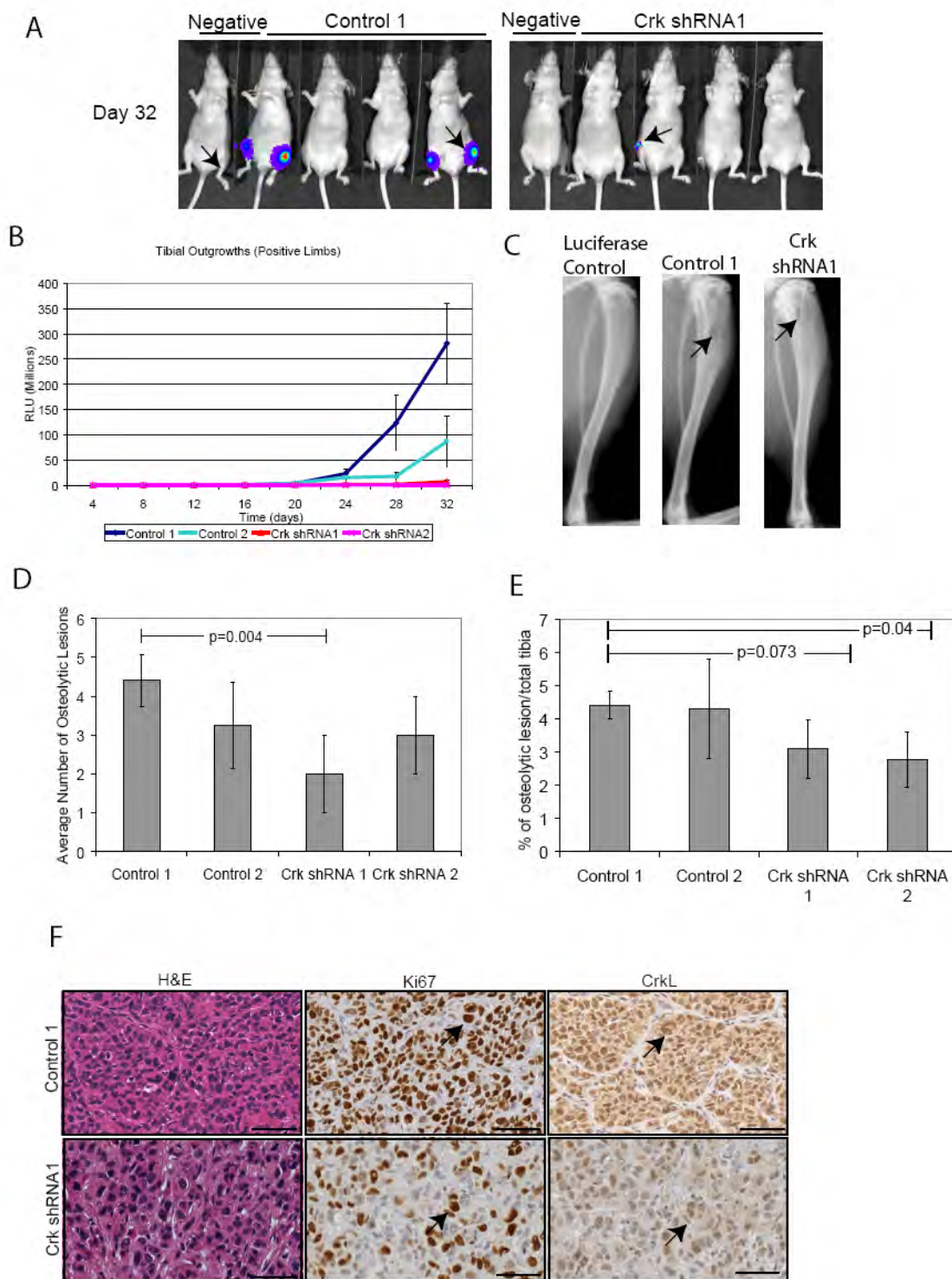


Figure 5 – Crk adaptor proteins are essential for outgrowth in the primary site. One million cells of either control 1833TR cells or Crk shRNA cells were injected into the fourth abdominal mammary fat pad of nude mice. Mammary tumour outgrowth was measured bi-weekly using caliper measurements, which revealed a longer latency of Crk shRNA derived tumours compared to the control cells. The average volume of control (n=10 per condition) and Crk shRNA tumours (n=8 per condition) were plotted over time along with standard error (A). H&E stained sections show similar pathology. Images taken at 20x. Scale bar represents 50 μ m (B). Western blot analysis of Crk protein expression levels from cell lines prior to injection as well as tumour endpoint (C). Paraffin embedded sections of mammary tumours derived from 1833TR control and Crk shRNA cells were stained for Ki67 and TUNEL. Preliminary staining patterns revealed no significant differences between the control and Crk knockdown tumours for Ki67 and TUNEL. All images taken at 20x where the scale bar represents 50 μ m. Arrows represent area of insert (D).

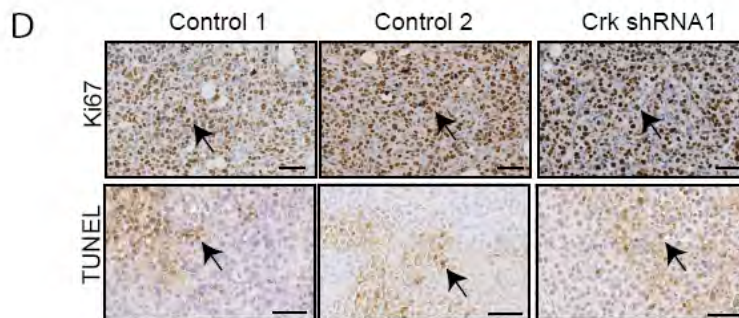
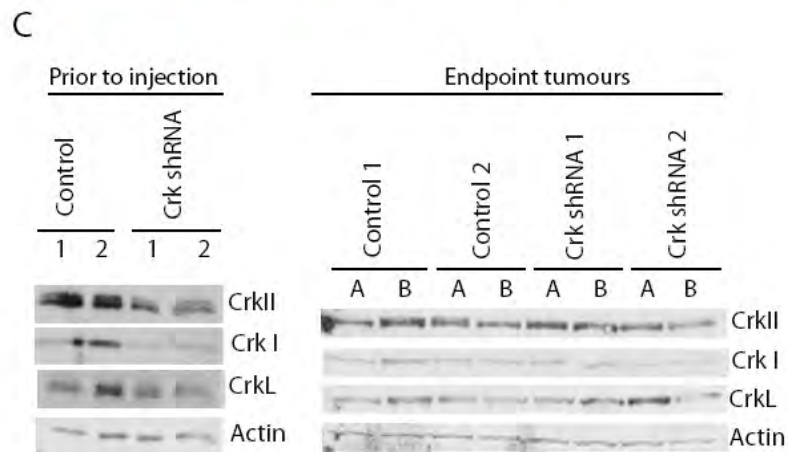
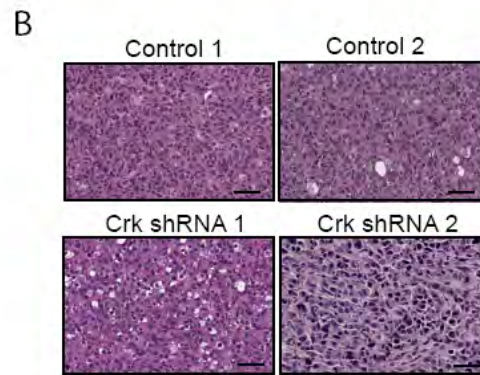
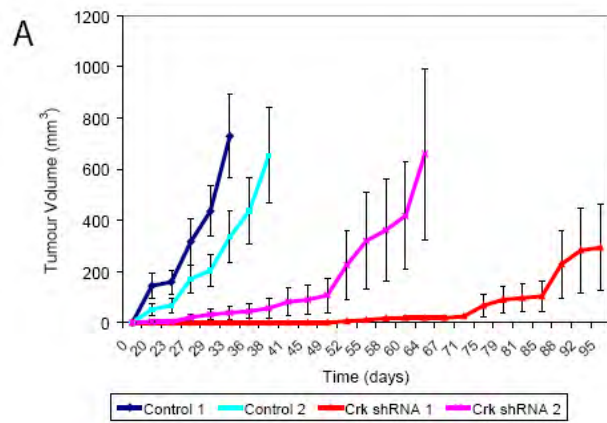


Figure 6 – Crk knockdown alters Cdc42 and Rac activation. Western blot analysis of whole cell lysates (MDA-231 1833TR) with an anti-CrkI/II or anti-CrkL sera revealed that the CrkI/II and CrkL shRNA constructs efficiently and specifically target its respective RNA. Phosphorylation of p130Cas was decreased in Crk knockdown cells relative to the control cells, as determined by immunoblotting using whole cell lysates. No differences were seen in the phosphorylation levels of MAPK. Actin protein levels were used as a loading control (A). The GTP bound form of Rac was precipitated by GST-Pak-PBD and probed for anti-Rac1 sera. Total levels of Rac were visualized by immunoblotting (B). Activated levels of Rac1 were quantified by Image J analysis and compared to the total Rac1 levels and the ratio (activated/total Rac) was graphed. Three experiments were carried out and the average was plotted. The GTP bound form of Cdc42 was precipitated by GST-WASP and probed for anti-Cdc42 sera. Total levels of Cdc42 were visualized by immunoblotting (C). Activated levels of Cdc42 were quantified by Image J analysis as described above for Rac1.

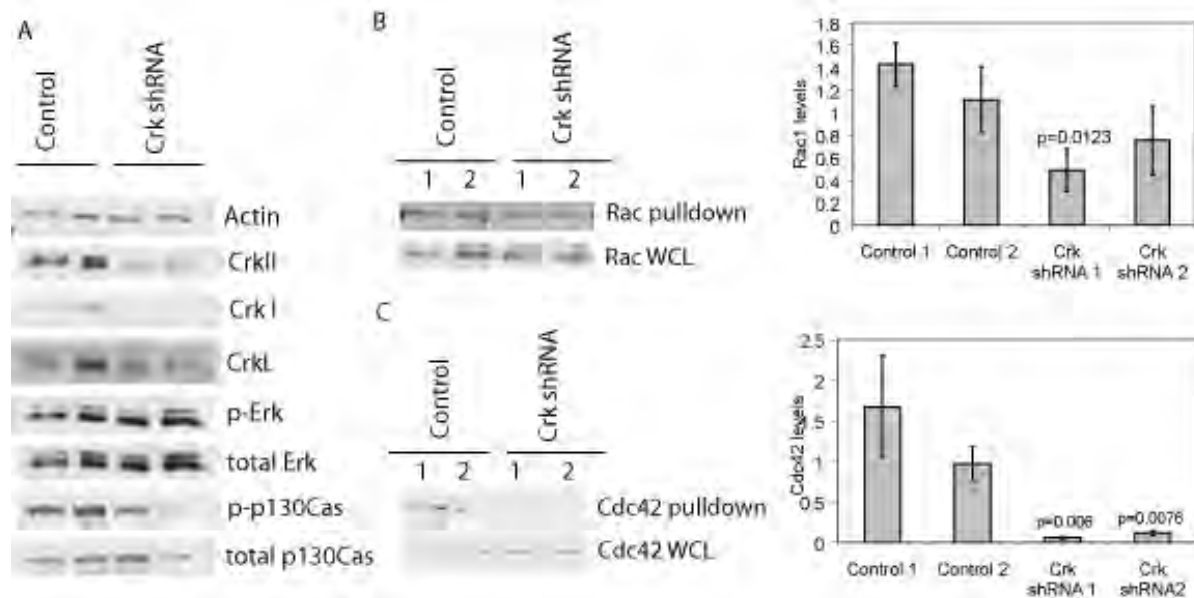
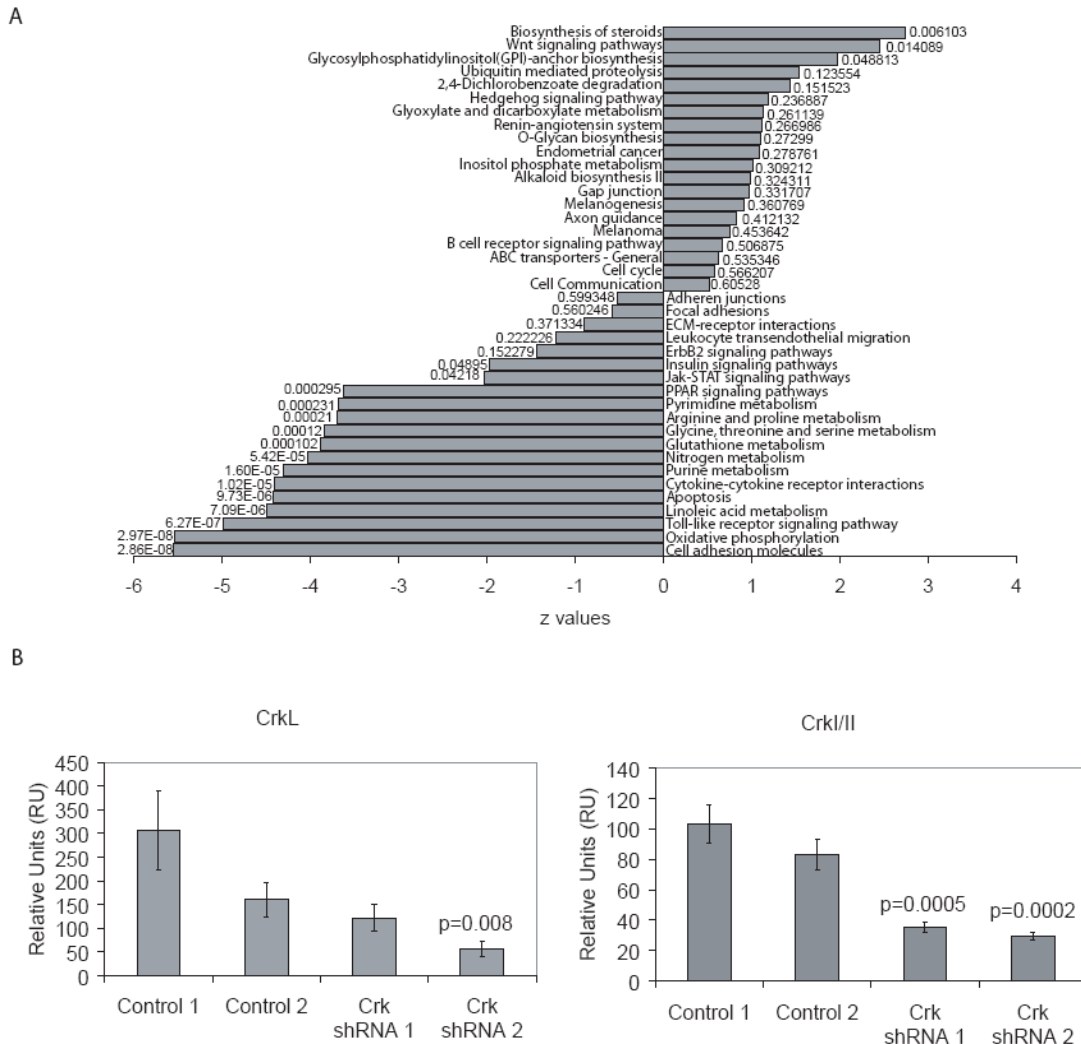
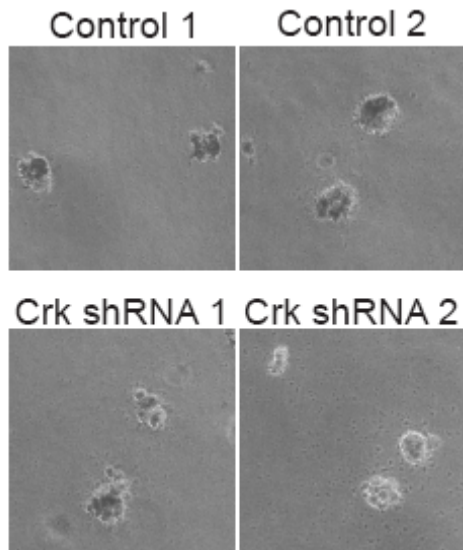


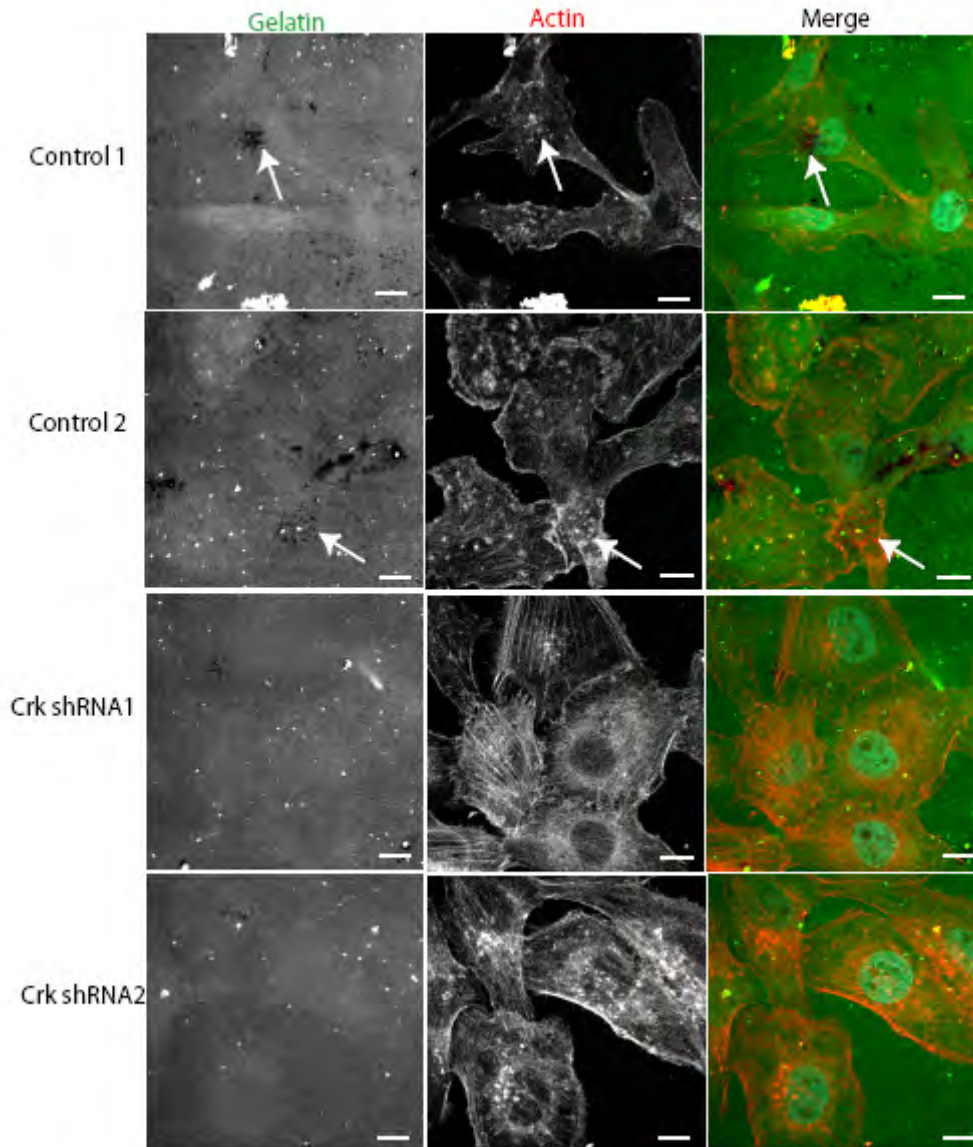
Figure 8 – Crk knockdown results in changes in gene expression of genes whose functions are important for tumour progression. KEGG pathway analysis demonstrates several different pathways up-regulated or down-regulated in Crk knockdown cells relative to control cell lines (A). Quantitative real-time PCR analysis identifies loss of CrkI/II and CrkL in Crk knockdown cells relative to control cells (B).



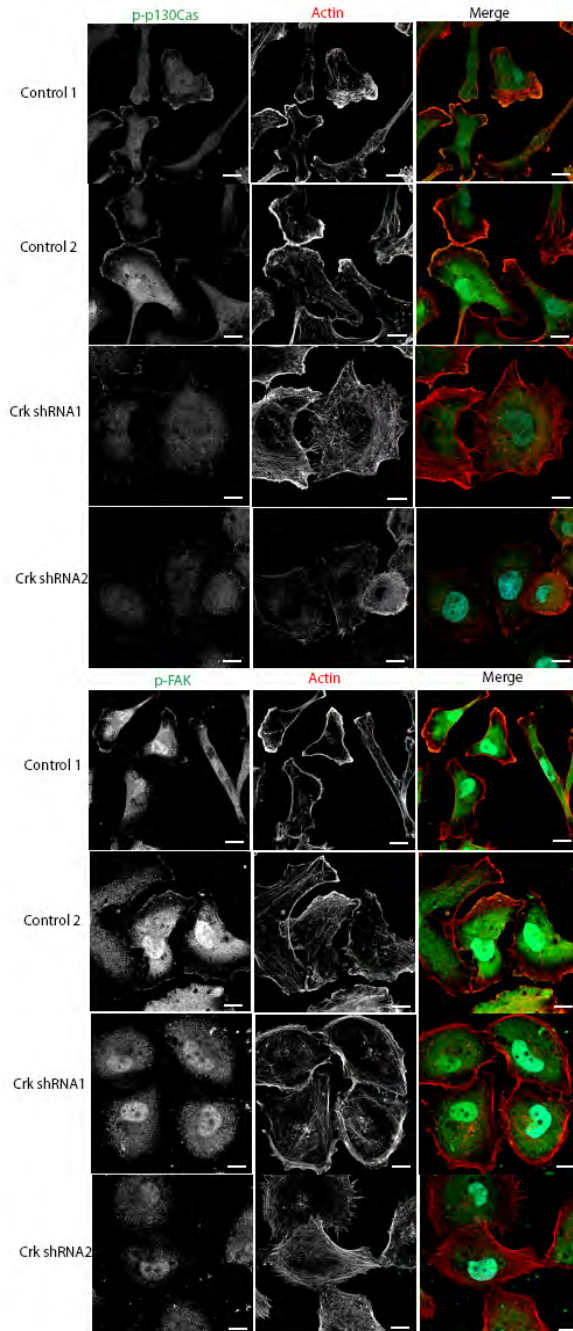
Supplemental Figure 1 - Knockdown of Crk adaptor proteins does not affect anchorage independent growth when grown in serum. Soft agar assays under normal, 10% serum revealed no differences in the ability of Crk shRNA cells to grow under anchorage independent conditions, relative to the control MDA-231 1833TR cells. Images were taken using the Axiovision software.



Supplemental Figure 2 – Crk adaptor proteins are important regulators of invadopodia activity within 1833TR cells. Control 1833TR cells and Crk shRNA cells were plated on FITC-labeled gelatin and the cells were examined for their ability to form actin-rich structures as well as degrade gelatin. The control cells formed prominent actin-rich structures believed to be invadopodia, and had the ability to degrade gelatin. The Crk shRNA cells still formed prominent actin rich structures, but had a significant impairment in their ability to degrade gelatin.

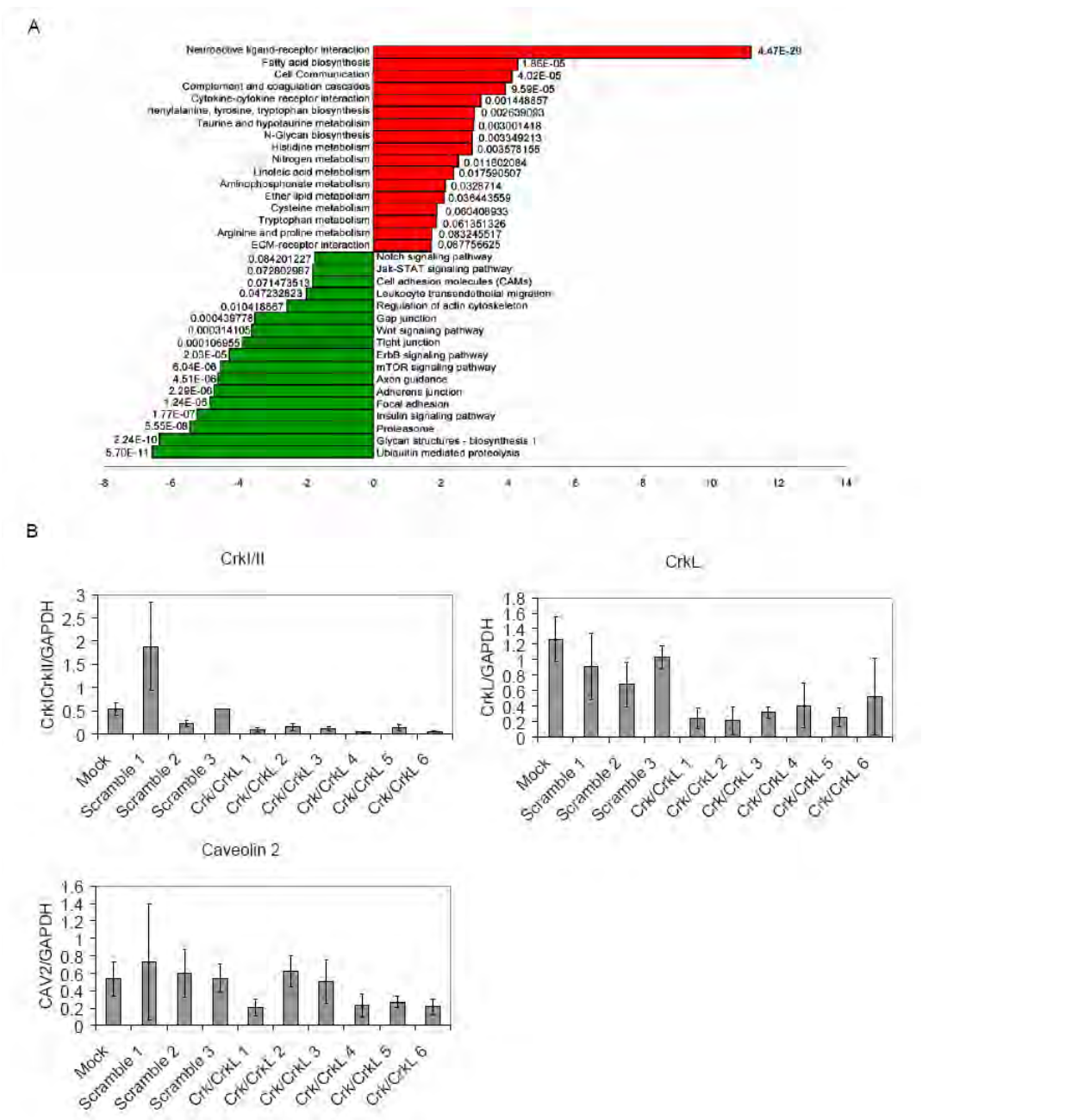


Supplemental Figure 3 – Altered localization of phosphorylated Fak and p130Cas is observed in Crk shRNA cells, relative to control 1833TR cells. Both control 1833TR and Crk shRNA cells were plated on collagen for 24 hours before fixation and staining for phosphorylated FAK and p130Cas. The localization of FAK and p130Cas is prominent at sites of membrane ruffling in control 1833TR cells, whereas in Crk shRNA cells, phosphorylated FAK and p130Cas is mainly in the cytosol.



Supplemental Figure 4 – Crk knockdown in MDA231TR parental cells results in changes in gene expression. Western blot analysis of whole cell lysates (MDA-231TR) with an anti-CrkI/II or anti-CrkL sera revealed that the CrkI/II and CrkL siRNA transfected cells efficiently and specifically target their respective RNA. Actin protein levels were used as a loading control (A). Hierarchical clustering of all transfected samples reveal the scrambled siRNAs are more closely related to another than to the Crk knockdown samples and vice versa (B). Heatmap analysis demonstrates global changes in gene expression between the scrambled controls and the Crk knockdown samples (C).

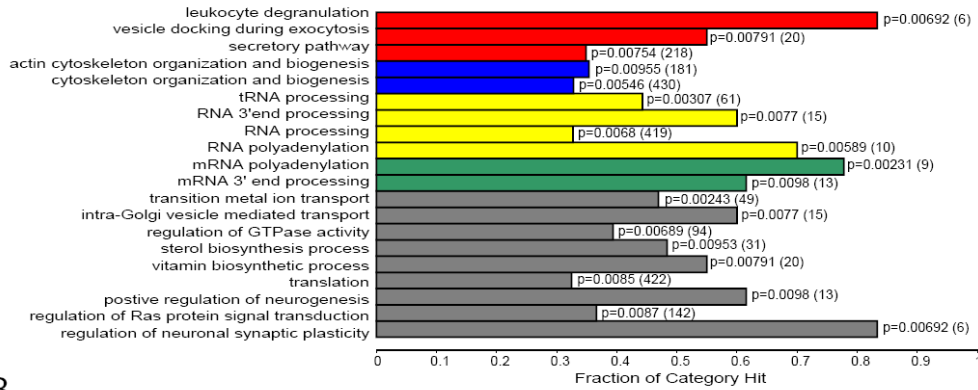
in gene expression of genes whose functions are important for tumour progression. KEGG pathway analysis demonstrates several different pathways up-regulated or down-regulated in the Crk knockdown cells relative to the scrambled controls (A). Quantitative real-time PCR analysis identifies loss of Crk, CrkL and caveolin 2 in the Crk knockdown cells relative to the scrambled cells (B).



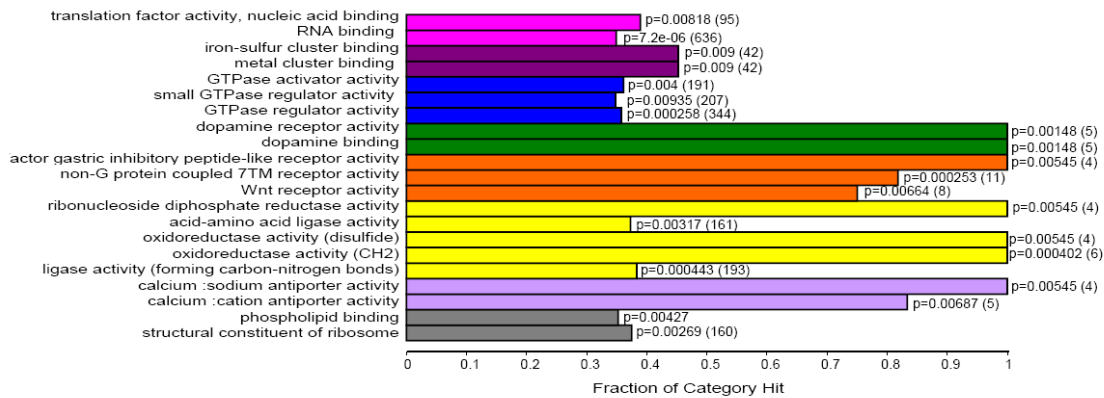
Supplemental Figure 6 - GO pathway analysis. Summary of GO biological processes (A) and

molecular functions (B) altered in the Crk knockdown cells relative to the controls.

A



B



Supplemental Table 2 - Common genes from the microarray data derived from the 1833TR

cell lines and the transient siRNA microarray data derived from the parental MDA-231 TR cell lines. The fold change is the comparison between the Crk knockdown samples relative to the controls (ie. IL13RA2 is 13.7 fold lower in the Crk shRNA cells relative to the control cells.)

Gene Name	1833TR Fold Change	1833TR p value	TR Fold Change	TR p value	Full gene name
IL13RA2	-13.6808939	0.0002347	-1.98646	0.01921	interleukin 13 receptor, alpha 2
VNN1	-6.95535264	1.78E-05	-1.63787	0.0068	vanin 1
LOC254848	-6.13293017	7.25E-06	-1.35131	0.03108	hypothetical protein LOC254848
FAM133A	-5.65761095	0.0055752	-1.85332	0.0248	family with sequence similarity 133, member A
TMEM46	-4.78836403	0.0083533	-1.63827	0.04869	transmembrane protein 46
BM921567	-4.40264123	0.0038649	-1.78387	0.01359	NA
DGAT2	-4.30853428	0.0159685	-1.66367	0.04765	diacylglycerol O-acyltransferase homolog 2 (mouse)
SAA4	-4.22720126	0.0257297	-1.6798	0.04394	serum amyloid A4, constitutive
DGAT2	-4.1959465	0.009523	-1.69376	0.02921	diacylglycerol O-acyltransferase homolog 2 (mouse)
LMO2	-4.13032901	1.65E-05	-1.56137	0.00273	LIM domain only 2 (rhombotin-like 1)
FAM133A	-4.02132751	0.0085004	-2.49403	0.00172	family with sequence similarity 133, member A
A_24_P745883	-4.00791219	1.79E-09	-1.22379	0.00191	NA
TNIP3	-3.76144159	0.0182833	-1.6925	0.03146	TNFAIP3 interacting protein 3
ADAMTS9	-3.66556454	1.26E-05	-1.49397	0.00202	ADAM metalloproteinase with thrombospondin type 1 motif, 9
DIAPH2	-3.64715144	4.86E-05	-1.58329	0.00201	diaphanous homolog 2 (Drosophila)
ATP8A2	-3.35423604	6.27E-08	-1.23264	0.00402	ATPase, aminophospholipid transporter-like, Class I, type 8A, member 2
ABI3BP	-3.34420874	5.97E-06	-1.27316	0.01327	ABI gene family, member 3 (NESH) binding protein
BCL2A1	-3.32913346	0.0245678	-1.55912	0.04941	BCL2-related protein A1
RNF125	-3.07302977	1.30E-08	-1.1706	0.00609	ring finger protein 125

AKR1C1	-2.91509637	0.0003373	-1.31558	0.02483	aldo-keto reductase family 1, member C1 (dihydrodiol dehydrogenase 1; 20-alpha (3-alpha)-hydroxysteroid dehydrogenase)
SLC16A2	-2.89319015	0.0020676	-1.67603	0.00336	solute carrier family 16, member 2 (monocarboxylic acid transporter 8)
CD8A	-2.8900828	1.57E-05	-1.20112	0.03501	CD8a molecule
FAT3	-2.80630465	3.19E-07	-1.14631	0.02593	FAT tumour suppressor homolog 3 (Drosophila)
GALNT14	-2.78894489	0.0026633	-1.4924	0.01104	UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 14 (GalNAc-T14)
MIG7	-2.78457371	0.0097971	-1.78427	0.00429	mig-7
PAGE2	-2.73056308	0.032266	-1.67383	0.01602	P antigen family, member 2 (prostate associated)
BX537688	-2.65258648	0.0257571	-2.06338	0.00209	hypothetical LOC654433
CORO2B	-2.61349029	9.61E-08	-1.1058	0.04292	coronin, actin binding protein, 2B
BIN1	-2.5963029	0.0061543	-1.46505	0.01595	bridging integrator 1
HRASLS	-2.50614313	0.0008531	-1.47169	0.00419	HRAS-like suppressor
RAGE	-2.50100421	5.84E-06	-1.18823	0.01685	renal tumour antigen
HLA-DPB1	-2.49480976	0.016417	-1.37659	0.04783	major histocompatibility complex, class II, DP beta 1
FHOD3	-2.47463527	0.0335784	-1.67929	0.01016	formin homology 2 domain containing 3
NSBP1	-2.41706444	7.76E-05	-1.37744	0.00899	nucleosomal binding protein 1
LAPTM5	-2.41549233	0.0481303	-1.45093	0.04642	lysosomal associated multispinning membrane protein 5
RBM41	-2.40438669	3.29E-07	-1.11133	0.03818	RNA binding motif protein 41
NEO1	-2.36062657	0.0014895	-1.35963	0.01199	neogenin homolog 1 (chicken)
HLA-DMB	-2.33012835	0.0008432	-1.2728	0.02423	major histocompatibility complex, class II, DM beta
CABYR	-2.27244577	0.0404844	-1.61087	0.01094	calcium binding tyrosine-(Y)-phosphorylation regulated (fibrousheathin 2)
CTSW	-2.23358201	0.0495172	-1.50863	0.02239	cathepsin W

BC034319	-2.14380172	0.0371169	-1.48338	0.01704	NA
SSPN	-2.12287155	0.0003169	-1.31315	0.00517	sarcospan (Kras oncogene-associated gene)
MCFD2	-2.1194618	0.0340585	-1.50201	0.01323	multiple coagulation factor deficiency 2
SLC26A7	-2.1162635	0.0007071	-1.29376	0.00995	solute carrier family 26, member 7
BE064950	-2.11454639	9.38E-08	-1.16256	0.00201	NA
CRADD	-2.08512481	0.0039307	-1.45898	0.00373	CASP2 and RIPK1 domain containing adaptor with death domain
SEMA7A	-2.08122765	0.0001422	-1.18935	0.02388	semaphorin 7A, GPI membrane anchor (John Milton Hagen blood group)
BCL2A1	-2.07305055	0.0035873	-1.27631	0.02505	BCL2-related protein A1
ARHGAP30	-2.05222232	0.0003482	-1.21537	0.01989	Rho GTPase activating protein 30
APXL	-2.02576126	0.0072438	-1.25537	0.04206	shroom family member 2
A_24_P24786	-1.9856981	0.0425859	-1.52914	0.00846	NA
OR2A9P	-1.96786149	0.0131544	-1.37095	0.01313	olfactory receptor, family 2, subfamily A, member 9 pseudogene
AK023472	-1.96523986	0.0017398	-1.28065	0.01178	NA
A_24_P341078	-1.95929581	0.0409962	-1.35986	0.03062	NA
LIMD2	-1.94477418	0.0129492	-1.49951	0.00368	LIM domain containing 2
C1orf21	-1.94339396	0.0243355	-1.42508	0.01097	chromosome 1 open reading frame 21
DLG3	-1.9379978	0.0196697	-1.38303	0.01378	discs, large homolog 3 (neuroendocrine-dlg, Drosophila)
SATB1	-1.93641722	4.22E-05	-1.17597	0.01194	SATB homeobox 1
MGC17624	-1.93329959	5.25E-05	-1.18872	0.00989	chromosome 16 open reading frame 74
ALDH2	-1.93030659	0.0424135	-1.52736	0.00698	aldehyde dehydrogenase 2 family (mitochondrial)
ARHGEF4	-1.92492665	0.005376	-1.35665	0.00732	Rho guanine nucleotide exchange factor (GEF) 4
ENST00000331306	-1.91166031	0.0156162	-1.256	0.0455	NA
AHNAK	-1.91105841	0.0033596	-1.30005	0.01053	AHNAK nucleoprotein
CSTF2	-1.9105459	0.0005065	-1.22561	0.01234	cleavage stimulation factor, 3' pre-RNA, subunit 2, 64kDa
CYB5R2	-1.90835	2.86E-05	-1.32547	0.00039	NA
PLXDC2	-1.89938929	0.0176651	-1.44437	0.00622	plexin domain containing 2
ENOX1	-1.88587167	0.0166233	-1.97803	0.00011	ecto-NOX disulfide-thiol exchanger 1

GBP5	-1.85630895	0.0366746	-1.48781	0.00631	guanylate binding protein 5
BHLHB9	-1.85050455	0.0109531	-1.43521	0.00388	basic helix-loop-helix domain containing, class B, 9
ENST00000338548	-1.83627694	0.0002889	-1.14424	0.04113	NA
MCTS1	-1.83589089	0.0349407	-1.27297	0.04685	malignant T cell amplified sequence 1
MGC39900	-1.83584725	0.0002486	-1.16783	0.02199	hypothetical protein MGC39900
CXorf39	-1.81869383	0.0322462	-1.40517	0.01011	chromosome X open reading frame 39
KCNAB2	-1.80434013	3.25E-05	-1.13777	0.01763	potassium voltage-gated channel, shaker-related subfamily, beta member 2
OSBPL5	-1.79831249	0.0112158	-1.23266	0.03602	oxysterol binding protein-like 5
ARMCX1	-1.79718876	0.0340051	-1.27046	0.04181	armadillo repeat containing, X-linked 1
C4orf26	-1.79068675	0.0045879	-1.34706	0.00426	chromosome 4 open reading frame 26
PAK6	-1.78572697	0.0041881	-1.31004	0.00631	p21(CDKN1A)-activated kinase 6
Mar-01	-1.78047918	0.0215903	-1.34214	0.01215	membrane-associated ring finger (C3HC4) 1
Mar-01	-1.75814375	0.0002526	-1.21366	0.006	membrane-associated ring finger (C3HC4) 1
CASP1	-1.756368	0.0065444	-1.41851	0.00192	caspase 1, apoptosis-related cysteine peptidase (interleukin 1, beta, convertase)
REPS2	-1.74630806	0.0174255	-1.30299	0.01462	RALBP1 associated Eps domain containing 2
ENST00000309379	-1.73297253	0.0160837	-1.2615	0.02259	NA
RPL13	-1.71966264	0.0036394	-1.1648	0.04898	ribosomal protein L13
A_24_P349489	-1.71662999	0.0057871	-1.33491	0.004	NA
BC031314	-1.70815481	0.0003549	-1.15433	0.02123	NA
PIK3CD	-1.70365979	0.0471282	-1.26888	0.03696	phosphoinositide-3-kinase, catalytic, delta polypeptide
CTSS	-1.69905997	0.011178	-1.26429	0.01475	cathepsin S
BHLHB3	-1.69671795	0.0265229	-1.28629	0.01926	basic helix-loop-helix domain containing, class B, 3
DPF3	-1.68457885	0.0015336	-1.30568	0.00201	D4, zinc and double PHD fingers, family 3
THC2265983	-1.66678939	0.0115145	-1.81302	3.88E-05	NA
A_24_P144383	-1.64540667	0.0429059	-1.38443	0.00657	NA
THC2343435	-1.64277481	0.0077242	-1.23129	0.01496	NA

BC071811	-1.63352208	0.0468525	-1.29528	0.01894	similar to R28379_1
A_32_P153361	-1.62613524	0.0277247	-1.30059	0.01133	NA
THC2334681	-1.62335138	0.0006568	-1.20085	0.00619	NA
IL13RA1	-1.61817573	0.0234823	-1.29791	0.0099	interleukin 13 receptor, alpha 1
LOC649839	-1.60859252	0.0498451	-1.24279	0.03543	similar to large subunit ribosomal protein L36a
EMR1	-1.59692192	0.0017219	-1.15911	0.02238	egf-like module containing, mucin-like, hormone receptor-like 1
SLC25A5	-1.58342009	0.0202321	-1.20432	0.03115	solute carrier family 25 (mitochondrial carrier; adenine nucleotide translocator), member 5
ENST00000078131	-1.58182253	0.0135808	-1.17496	0.04223	NA
A_24_P298228	-1.5809011	6.92E-05	-1.17477	0.00257	NA
RPL30	-1.58047881	0.0121669	-1.21556	0.01829	ribosomal protein L30
DVL1	-1.55435247	0.0386317	-1.23429	0.0246	dishevelled, dsh homolog 1 (Drosophila)
DDIT4L	-1.53187783	0.0005879	-1.12246	0.02534	DNA-damage-inducible transcript 4-like
PKIA	-1.5290402	0.0115972	-1.49297	0.00021	protein kinase (cAMP-dependent, catalytic) inhibitor alpha
ICAM2	-1.52668924	0.0220377	-1.18344	0.03503	intercellular adhesion molecule 2
LOC286044	-1.52610283	0.0335411	-1.19792	0.03494	hypothetical protein LOC286044
PDZK11	-1.51044488	0.0211305	-1.24692	0.0097	PDZ domain containing 11
APBB1	-1.49900018	0.0001349	-1.12767	0.00857	amyloid beta (A4) precursor protein-binding, family B, member 1 (Fe65)
SLC25A5	-1.49640387	0.0351928	-1.2687	0.00904	solute carrier family 25 (mitochondrial carrier; adenine nucleotide translocator), member 5
CYP26B1	-1.45880527	0.019056	-1.20533	0.01287	cytochrome P450, family 26, subfamily B, polypeptide 1
MRPL30	-1.45076981	0.0455216	-1.1879	0.03143	mitochondrial ribosomal protein L30
ENST00000295249	-1.44815529	0.0149138	-1.16683	0.02271	NA
DOCK11	-1.44364184	0.0399542	-1.63673	6.43E-05	dedicator of cytokinesis 11
A_32_P9924	-1.43763982	0.0219257	-1.25438	0.00478	NA
PIK3CD	-1.43006431	0.0266865	-1.2928	0.00262	phosphoinositide-3-kinase, catalytic, delta polypeptide

FDFT1	-1.41977931	0.0220704	-1.29378	0.00199	farnesyl-diphosphate farnesyltransferase 1
F8	-1.41313945	0.0030824	-1.13668	0.01507	coagulation factor VIII, procoagulant component (hemophilia A)
PMVK	-1.40771948	0.0466899	-1.16201	0.03936	phosphomevalonate kinase
A_24_P233560	-1.3895796	0.0032768	-1.11894	0.02192	NA
MCC	-1.38745841	0.0073252	-1.19397	0.0044	mutated in colorectal cancers
THC2400010	-1.37939888	0.0410653	-1.19337	0.01422	NA
A_24_P32930	-1.37652361	0.0050943	-1.14299	0.01166	NA
ENST00000332097	-1.37433853	0.0040694	-1.13106	0.0145	NA
TYW3	-1.36917036	0.0073275	-1.16343	0.00766	tRNA-yW synthesizing protein 3 homolog (S. cerevisiae)
THAP3	-1.35872666	0.0235096	-1.12474	0.04205	THAP domain containing, apoptosis associated protein 3
POLK	-1.35817485	0.0233707	-1.14594	0.02314	polymerase (DNA directed) kappa
DNAJC7	-1.32654408	0.0389278	-1.24147	0.00262	DnaJ (Hsp40) homolog, subfamily C, member 7
PITPNC1	-1.31351127	0.0168194	-1.19915	0.00265	phosphatidylinositol transfer protein, cytoplasmic 1
ENST00000319163	-1.30846371	0.0092078	-1.13425	0.01007	NA
A_24_P533142	-1.30242141	0.0114302	-1.21543	0.00107	NA
SMAP-1	-1.29243741	0.0191719	-1.10985	0.02963	unc-45 homolog A (C. elegans)
ADAMTSL1	-1.28376428	0.0026453	-1.07378	0.04068	ADAMTS-like 1
OPHN1	-1.2780599	0.0080402	-1.27105	0.00011	oligophrenin 1
A_24_P255836	-1.27390676	0.0274754	-1.10337	0.0371	NA
C10orf118	-1.26235548	0.0293211	-1.12609	0.01545	chromosome 10 open reading frame 118
A_24_P928467	-1.26108443	0.0471743	-1.14314	0.01287	NA
(+)E1A_r60_a97	-1.25821705	0.0058342	-1.09551	0.04635	NA
(-)3xSLv1	-1.18000607	0.0406061	-1.29009	0.01646	NA
SPRR2C	1.219489706	0.0487437	1.086247	0.04897	small proline-rich protein 2C
THC2318474	1.222897877	0.0493686	1.107441	0.02304	NA
ZMAT2	1.226000727	0.0393321	1.093396	0.03503	zinc finger, matrin type 2
WDR40A	1.235613892	0.0114054	1.110969	0.00879	WD repeat domain 40A
PMS1	1.237871205	0.0099509	1.093621	0.01722	PMS1 postmeiotic segregation increased 1 (S. cerevisiae)
ZMAT2	1.246395112	0.0234823	1.144395	0.00563	zinc finger, matrin type 2
THC2361927	1.263915582	0.0376765	1.141063	0.01178	NA

KCNQ3	1.279251548	0.0325198	1.249684	0.00081	potassium voltage-gated channel, KQT-like subfamily, member 3
ZC3H13	1.280570554	0.0335266	1.144007	0.01271	zinc finger CCCH-type containing 13
THC2309461	1.293526414	0.0458248	1.115233	0.04428	NA
GSTCD	1.312390676	0.0484466	1.149364	0.02192	glutathione S-transferase, C-terminal domain containing
ZXDC	1.321275756	0.049164	1.137192	0.03376	ZXD family zinc finger C
HERC4	1.325519563	0.0222685	1.134899	0.02172	hect domain and RLD 4
AF111705	1.331480211	0.0274521	1.13484	0.02662	NA
THC2342574	1.338652977	0.0308973	1.127563	0.03814	NA
NKTR	1.339587076	0.0428055	1.177782	0.01363	natural killer-tumour recognition sequence
THC2386560	1.345479717	0.0050399	1.090865	0.04934	NA
SEMA4G	1.352499411	0.033931	1.126055	0.04784	sema domain, immunoglobulin domain (Ig), transmembrane domain (TM) and short cytoplasmic domain, (semaphorin) 4G
TIGD3	1.355746275	0.0094348	1.121904	0.02589	tigger transposable element derived 3
BX478538	1.356794685	0.0309512	1.126867	0.04603	NA
AK026811	1.358912355	0.01575	1.151382	0.01564	NA
A_24_P369172	1.366799981	0.0462777	1.146144	0.03991	NA
PLEKHA4	1.376067722	0.0112942	1.173613	0.00839	pleckstrin homology domain containing, family A (phosphoinositide binding specific) member 4
GATA2	1.377594277	0.0102142	1.183247	0.00632	GATA binding protein 2
PRKY	1.392638164	0.0057871	1.161639	0.00884	protein kinase, Y-linked
Lin10	1.396976034	0.0340051	1.168896	0.0248	chromosome 16 open reading frame 70
IL17R	1.398236048	0.0197677	1.158559	0.02203	interleukin 17 receptor A
Lin10	1.398995844	0.0312931	1.168418	0.02399	chromosome 16 open reading frame 70
THC2364247	1.402130087	0.0042427	1.128762	0.02068	NA
AK022252	1.402182188	0.0112942	1.120385	0.04503	NA
B4GALT1	1.404297585	0.0335736	1.149205	0.04102	UDP-Gal:betaGlcNAc beta 1,4-galactosyltransferase, polypeptide 1
EP400	1.411526519	0.0061574	1.122992	0.03261	E1A binding protein p400
A_24_P885910	1.413251713	0.0066684	1.155221	0.01408	NA
A_23_P320728	1.414359697	0.0219257	1.138862	0.04375	NA
A_24_P936393	1.41917375	0.035701	1.21983	0.01102	NA

TM7SF2	1.423834506	0.0037721	1.129243	0.02276	transmembrane 7 superfamily member 2
FTO	1.428792063	0.0133908	1.133118	0.04257	fat mass and obesity associated
LOC400987	1.431640246	0.0287546	1.198525	0.01584	similar to ankyrin repeat domain 36
TNRC6A	1.434344329	0.0268836	1.183427	0.02113	trinucleotide repeat containing 6A
TGFBRAP1	1.436105739	0.0024666	1.10281	0.04778	transforming growth factor, beta receptor associated protein 1
SNX3	1.438187731	0.0042312	1.132019	0.02526	NA
ELL3	1.442620535	0.0209259	1.144277	0.04566	elongation factor RNA polymerase II-like 3
REPIN1	1.445029763	0.0115972	1.196375	0.00985	replication initiator 1
AF483645	1.448911276	0.0115544	1.166189	0.01995	NA
CAMK2D	1.466360314	0.0025657	1.16141	0.01077	calcium/calmodulin-dependent protein kinase (CaM kinase) II delta
KIAA1666	1.479486005	0.0065444	1.160845	0.02058	KIAA1666 protein
LSM14B	1.480842423	0.0177632	1.157328	0.03982	LSM14B, SCD6 homolog B (S. cerevisiae)
AF009267	1.483479042	0.0003361	1.118458	0.01609	NA
ATP8B2	1.490301354	0.0148723	1.159069	0.03671	ATPase, Class I, type 8B, member 2
MGC22265	1.499412572	0.011371	1.256494	0.00482	similar to Beta-glucuronidase precursor
NFATC3	1.49963418	0.0091896	1.308531	0.00172	nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 3
THC2310998	1.526697637	0.0005706	1.113093	0.03261	NA
AK095300	1.536588057	0.0002436	1.099395	0.03902	NA
HDAC4	1.539648506	0.0001252	1.102479	0.02719	histone deacetylase 4
KRT86	1.544341163	0.0008404	1.127539	0.0276	keratin 86
C5orf32	1.54954483	0.0121804	1.208345	0.01722	chromosome 5 open reading frame 32
ZNF808	1.552180992	0.042414	1.223658	0.03093	zinc finger protein 808
ZNF700	1.563268406	0.0082012	1.206755	0.01523	zinc finger protein 700
BC042520	1.564094686	0.0051239	1.168521	0.02573	NA
PCLO	1.566486976	0.0217355	1.2039	0.02992	piccolo (presynaptic cytomatrix protein)
GPRC5B	1.571682087	0.0008431	1.216915	0.00358	G protein-coupled receptor, family C, group 5, member B
C14orf113	1.580195458	4.30E-05	1.108672	0.01745	chromosome 14 open reading frame 113
MBNL1	1.58315443	0.0291186	1.194668	0.04623	muscleblind-like (Drosophila)
MYO1B	1.585567569	0.0093922	1.173294	0.03639	myosin IB

DAF	1.599681812	0.0175588	1.197804	0.03502	CD55 molecule, decay accelerating factor for complement (Cromer blood group)
ELAVL4	1.617268115	0.0051611	1.159669	0.04173	ELAV (embryonic lethal, abnormal vision, Drosophila)-like 4 (Hu antigen D)
PDPR	1.631266685	0.0057166	1.276974	0.00561	pyruvate dehydrogenase phosphatase regulatory subunit
NARG1	1.641568196	0.0019952	1.172107	0.02246	NMDA receptor regulated 1
KRT14	1.673710756	0.0110764	1.212565	0.02957	keratin 14 (epidermolysis bullosa simplex, Dowling-Meara, Koebner)
TITF1	1.675090723	0.0057166	1.170094	0.0459	NK2 homeobox 1
MALAT1	1.682138387	0.0150424	1.752568	0.00011	metastasis associated lung adenocarcinoma transcript 1 (non-coding RNA)
ZNF398	1.685333585	0.0044106	1.220895	0.01596	zinc finger protein 398
FLJ12331	1.688133188	0.000933	1.174067	0.01884	ribosomal protein S2 pseudogene
VAC14	1.701789971	0.0001128	1.11784	0.03546	Vac14 homolog (S. cerevisiae)
ZNF275	1.725122885	0.0005797	1.198125	0.01048	zinc finger protein 275
THC2304438	1.738456688	0.000431	1.206513	0.008	NA
PLCB1	1.748595091	0.0002075	1.196321	0.00728	phospholipase C, beta 1 (phosphoinositide-specific)
RDX	1.753628709	0.0074925	1.196797	0.0439	Radixin
MBOAT2	1.766398682	0.0012781	1.153815	0.04719	membrane bound O-acyltransferase domain containing 2
AP1G1	1.773995172	0.0006447	1.143065	0.04687	adaptor-related protein complex 1, gamma 1 subunit
THC2281660	1.783818476	5.85E-06	1.106532	0.02288	NA
SP100	1.794795216	0.0006117	1.178668	0.02211	SP100 nuclear antigen
AK025323	1.803520438	0.0017967	1.211531	0.02011	NA
MGC12982	1.811383568	0.0326676	1.280607	0.03786	hypothetical protein MGC12982
THC2376828	1.820496184	4.12E-05	1.129697	0.02621	NA
BX108121	1.835692673	0.028526	1.267084	0.04453	NA
ZNF66	1.854446016	0.00621	1.289894	0.01419	zinc finger protein 66
THC2378865	1.874059961	3.87E-05	1.139857	0.02305	NA
ZNF708	1.939440159	0.0084596	1.267963	0.03024	zinc finger protein 708

HOM-TES-103	1.940294124	0.009876	1.35502	0.01164	hypothetical protein LOC25900
CCRK	1.945342967	0.003511	1.283548	0.01527	cell cycle related kinase
AF086536	1.962903505	0.008618	1.238184	0.04862	NA
AK094623	1.963538562	8.98E-05	1.195756	0.01198	NA
ZNF117	1.970594804	0.0051779	1.310292	0.01471	zinc finger protein 117
MOCOS	1.972006941	0.0020982	1.188489	0.0459	molybdenum cofactor sulfurase
IFIT2	1.985845558	0.0174709	1.290949	0.03939	interferon-induced protein with tetratricopeptide repeats 2
C5orf25	2.03473902	0.0006908	1.200851	0.03337	chromosome 5 open reading frame 25
THC2290974	2.040564558	0.028923	1.382786	0.02482	NA
AA359500	2.054466601	0.0006861	1.267755	0.01178	similar to nuclear pore membrane protein 121
NANOS1	2.104863635	0.0002487	1.372429	0.00186	nanos homolog 1 (Drosophila)
ZNF331	2.109759529	0.0011548	1.214267	0.04001	zinc finger protein 331
A_32_P205792	2.13152956	0.0051591	1.267883	0.03905	NA
ZNF492	2.134487428	0.0009421	1.233215	0.02896	zinc finger protein 492
MLL	2.142005066	1.92E-06	1.130949	0.02177	myeloid/lymphoid or mixed-lineage leukemia (trithorax homolog, Drosophila)
ZNF100	2.165516612	0.0019355	1.269231	0.0258	zinc finger protein 100
THC2438118	2.172248115	0.0115544	1.34209	0.02878	NA
APC2	2.181823113	6.91E-05	1.211391	0.01493	adenomatosis polyposis coli 2
FBXL7	2.182990385	3.29E-05	1.204467	0.01216	F-box and leucine-rich repeat protein 7
CH25H	2.196168413	0.0001349	1.280209	0.00657	cholesterol 25-hydroxylase
ZNF539	2.225650467	0.0042638	1.295457	0.03133	zinc finger protein 254
PRSS7	2.233104326	0.0239284	1.353533	0.04528	protease, serine, 7 (enterokinase)
HIST1H2AC	2.247090772	1.78E-06	1.128667	0.02865	histone cluster 1, H2ac
ZNF430	2.248476407	0.0018432	1.235121	0.04832	zinc finger protein 430
THC2319755	2.282384896	0.0006908	1.218382	0.04421	NA
FN1	2.390944617	0.0338647	1.542253	0.01904	fibronectin 1
ZNF682	2.395440728	0.0052608	1.372545	0.02219	zinc finger protein 682
ZNF708	2.398776945	0.0140002	1.397422	0.03113	zinc finger protein 708
THC2381319	2.701962825	4.59E-06	1.15481	0.04423	NA
DKFZp779O175	2.768368159	0.008618	1.489181	0.0214	hypothetical protein DKFZp779O175
SHOX	2.907257973	0.0002012	1.285548	0.02816	short stature homeobox
THC2290002	3.169503218	0.0010781	1.329106	0.04714	NA
PDE9A	3.648639728	0.0111694	1.804233	0.01291	phosphodiesterase 9A

Supplemental Table 3 - Full list of KEGG pathway analysis results from stable MDA-231 1833TR microarray experiment.

z	Pvalue	Description
-3.96361	7.38E-05	Proteasome
-0.58248	0.560246	Focal adhesion
-1.87466	0.060839	Dorso-ventral axis formation
-0.89398	0.371334	ECM-receptor interaction
-2.46339	0.013763	SNARE interactions in vesicular transport
-1.65327	0.098275	T cell receptor signaling pathway
-5.54983	2.86E-08	Cell adhesion molecules (CAMs)
2.742175	0.006103	Biosynthesis of steroids
0.663711	0.506875	B cell receptor signaling pathway
1.123703	0.261139	Glyoxylate and dicarboxylate metabolism
-0.48893	0.624889	Colorectal cancer
-2.58352	0.00978	Aminophosphonate metabolism
-3.08488	0.002036	Fc epsilon RI signaling pathway
-2.56157	0.01042	Benzoate degradation via CoA ligation
-1.3157	0.188276	Aminoacyl-tRNA biosynthesis
-2.59418	0.009482	Renal cell carcinoma
-0.84958	0.395561	Pancreatic cancer
-3.08386	0.002043	Parkinson's disease
1.083106	0.278761	Endometrial cancer
-0.84826	0.396292	Glutamate metabolism
0.545702	0.585271	Glioma
-1.22965	0.218828	Alanine and aspartate metabolism
-4.97149	6.64E-07	Arachidonic acid metabolism
-2.03197	0.042157	Prostate cancer
-1.07858	0.280775	Fatty acid biosynthesis
-4.49087	7.09E-06	Linoleic acid metabolism
-0.85436	0.392904	Thyroid cancer
-1.01346	0.31084	Circadian rhythm
-2.44172	0.014617	Fatty acid elongation in mitochondria
0.123149	0.901989	Basal cell carcinoma
0.749357	0.453642	Melanoma
-0.03814	0.969577	Protein export
-0.52534	0.599348	Adherens junction
-0.5471	0.584307	Bladder cancer
-0.90197	0.367073	Notch signaling pathway
-1.22063	0.222226	Leukocyte transendothelial migration
-1.65482	0.09796	Regulation of autophagy
-1.50514	0.132287	Propanoate metabolism

-2.5434	0.010978	Chronic myeloid leukemia
-2.29952	0.021476	Selenoamino acid metabolism
-0.53142	0.595129	Ethylbenzene degradation
-5.25917	1.45E-07	Metabolism of xenobiotics by cytochrome P450
-1.54474	0.122408	Acute myeloid leukemia
-0.33267	0.739383	Small cell lung cancer
-1.22109	0.222053	Amyotrophic lateral sclerosis (ALS)
-0.43687	0.662203	Folate biosynthesis
-3.84639	0.00012	Glycine, serine and threonine metabolism
0.20136	0.840417	Non-small cell lung cancer
-2.46348	0.013759	Atrazine degradation
-1.96903	0.04895	Insulin signaling pathway
-3.06174	0.002201	Fatty acid metabolism
0.317991	0.750492	Long-term potentiation
0.260549	0.79444	Synthesis and degradation of ketone bodies
-0.75069	0.452837	GnRH signaling pathway
-0.19253	0.84733	Tight junction
1.182804	0.236887	Hedgehog signaling pathway
0.913901	0.360769	Melanogenesis
-0.84633	0.397366	Starch and sucrose metabolism
-3.1167	0.001829	Lysine degradation
-1.45212	0.146467	mTOR signaling pathway
-2.16528	0.030367	Bile acid biosynthesis
-2.2957	0.021693	Butanoate metabolism
-0.63472	0.525612	Cyanoamino acid metabolism
-2.71907	0.006547	Huntington's disease
-0.00461	0.996322	Methionine metabolism
-2.66502	0.007698	Adipocytokine signaling pathway
-0.65473	0.512643	Cysteine metabolism
-1.57877	0.11439	Long-term depression
-2.3118	0.020789	MAPK signaling pathway
0.97068	0.331707	Gap junction
-1.43153	0.152279	ErbB signaling pathway
0.116053	0.90761	TGF-beta signaling pathway
-0.75321	0.451326	N-Glycan biosynthesis
-0.21041	0.83335	N-Glycan degradation
1.096202	0.27299	O-Glycan biosynthesis
-2.36798	0.017886	Ubiquinone biosynthesis
-3.61939	0.000295	PPAR signaling pathway
0.533276	0.593843	Dentatorubropallidoluysian atrophy (DRPLA)
-2.98285	0.002856	Valine, leucine and isoleucine degradation

-0.19095	0.848562	Type II diabetes mellitus
-4.42305	9.73E-06	Apoptosis
-0.7706	0.440943	Terpenoid biosynthesis
-0.13431	0.89316	Olfactory transduction
0.267204	0.789312	Calcium signaling pathway
-1.1777	0.238917	Carbon fixation
1.786254	0.074058	Taste transduction
-3.22723	0.00125	Limonene and pinene degradation
0.820147	0.412132	Axon guidance
-0.80897	0.418533	Nucleotide sugars metabolism
-2.01237	0.044181	Streptomycin biosynthesis
-3.7063	0.00021	Arginine and proline metabolism
-2.10479	0.035309	Porphyrin and chlorophyll metabolism
-1.08438	0.278198	One carbon pool by folate
-0.91249	0.36151	C21-Steroid hormone metabolism
-3.88556	0.000102	Glutathione metabolism
1.030907	0.302584	Prion disease
0.000381	0.999696	Valine, leucine and isoleucine biosynthesis
-4.46397	8.05E-06	Type I diabetes mellitus
-1.15754	0.247052	Glycan structures - biosynthesis 1
0.294995	0.767998	Glycan structures - biosynthesis 2
-0.26994	0.787207	Glycan structures – degradation
-4.03666	5.42E-05	Nitrogen metabolism
0.288851	0.772695	Reductive carboxylate cycle (CO ₂ fixation)
-1.24292	0.213897	Aminosugars metabolism
-0.28086	0.778817	VEGF signaling pathway
-0.62584	0.531422	Glycosaminoglycan degradation
-1.11754	0.263762	Cholera – Infection
-3.29953	0.000968	Histidine metabolism
-1.36388	0.172605	Chondroitin sulfate biosynthesis
-1.93953	0.052437	Keratan sulfate biosynthesis
-0.04901	0.960909	Methane metabolism
-0.53952	0.589527	Heparan sulfate biosynthesis
-2.9081	0.003636	Androgen and estrogen metabolism
-1.93864	0.052545	Complement and coagulation cascades
-1.75724	0.078877	Maturity onset diabetes of the young
-4.75687	1.97E-06	Antigen processing and presentation
1.110029	0.266986	Renin-angiotensin system
-0.1878	0.851036	Sulfur metabolism
-1.2424	0.214087	Thiamine metabolism
-3.0097	0.002615	Glycolysis / Gluconeogenesis

-2.49072	0.012748	Epithelial cell signaling in Helicobacter pylori infection
-3.13259	0.001733	Tyrosine metabolism
-4.56084	5.10E-06	Ribosome
0.266872	0.789568	Regulation of actin cytoskeleton
0.516822	0.60528	Cell Communication
-4.98281	6.27E-07	Toll-like receptor signaling pathway
-3.51463	0.00044	Caprolactam degradation
-0.99768	0.318433	Phenylalanine, tyrosine and tryptophan biosynthesis
-2.07878	0.037638	Riboflavin metabolism
-3.26587	0.001091	Citrate cycle (TCA cycle)
-2.08135	0.037401	Pathogenic Escherichia coli infection – EHEC
-1.38214	0.166928	Phenylalanine metabolism
-2.08135	0.037401	Pathogenic Escherichia coli infection – EPEC
-0.48134	0.630271	gamma-Hexachlorocyclohexane degradation
-1.93953	0.052436	RNA polymerase
0.388889	0.697358	Benzoate degradation via hydroxylation
-1.06429	0.287198	Bisphenol A degradation
-1.1039	0.269635	Basal transcription factors
-2.03174	0.04218	Jak-STAT signaling pathway
-2.04295	0.041057	Sphingolipid metabolism
-0.37537	0.707384	Glycosphingolipid biosynthesis – lactoseries
0.177007	0.859503	Glycosphingolipid biosynthesis - neo-lactoseries
-0.40727	0.68381	Phenylpropanoid biosynthesis
-1.74998	0.080121	beta-Alanine metabolism
-1.72628	0.084297	Glycosphingolipid biosynthesis – globoseries
-2.22135	0.026327	Glycosphingolipid biosynthesis – ganglioseries
-3.52427	0.000425	Urea cycle and metabolism of amino groups
-4.41297	1.02E-05	Cytokine-cytokine receptor interaction
-2.55843	0.010515	Vitamin B6 metabolism
-4.54259	5.56E-06	Pentose phosphate pathway
-3.17318	0.001508	Glycerolipid metabolism
1.016877	0.309212	Inositol phosphate metabolism
1.970217	0.048813	Glycosylphosphatidylinositol(GPI)-anchor biosynthesis
-2.32375	0.020139	Glycerophospholipid metabolism
-0.59303	0.553164	DNA polymerase
-2.42503	0.015307	Ether lipid metabolism
0.573646	0.566207	Cell cycle
-3.29181	0.000995	Hematopoietic cell lineage
-0.11686	0.906969	Alkaloid biosynthesis I
-0.71305	0.475816	p53 signaling pathway
-4.31468	1.60E-05	Purine metabolism

0.424351	0.67131	Phosphatidylinositol signaling system
-5.59757	2.17E-08	Nicotinate and nicotinamide metabolism
-0.95021	0.342005	Pentose and glucuronate interconversions
-3.08843	0.002012	Tryptophan metabolism
-5.54344	2.97E-08	Oxidative phosphorylation
2.454983	0.014089	Wnt signaling pathway
1.540028	0.123554	Ubiquitin mediated proteolysis
-1.90172	0.057208	Natural killer cell mediated cytotoxicity
0.619866	0.535346	ABC transporters – General
-2.02786	0.042574	Pyruvate metabolism
-1.2663	0.205404	Taurine and hypotaurine metabolism
0.985638	0.324311	Alkaloid biosynthesis II
1.434173	0.151523	2,4-Dichlorobenzoate degradation
-3.19351	0.001406	Alzheimer's disease
-1.18045	0.237823	Neuroactive ligand-receptor interaction
-2.67561	0.007459	Pantothenate and CoA biosynthesis
-0.49189	0.622795	1- and 2-Methylnaphthalene degradation
-3.68216	0.000231	Pyrimidine metabolism
-1.65858	0.097201	Tetrachloroethene degradation
-1.77187	0.076416	Neurodegenerative Disorders
-2.79865	0.005132	Naphthalene and anthracene degradation
-2.68845	0.007178	Fructose and mannose metabolism
-3.27395	0.001061	Galactose metabolism
-2.43123	0.015048	Ascorbate and aldarate metabolism

Supplemental Table 4 - Full list of GO terms (Biological Processes and Molecular Functions) from stable MDA-231 1833TR knockdown, where t equals the total number of genes in that particular category, r represents the number of genes affected in the Control versus Crk knockdown comparison and p represents the statistically significant p-value.

Biological Processes	T	R	P
Regulation of global transcription from RNA polymerase II promoter	4	3	0.00224
Regulation of transcription, DNA dependent	2016	216	5.23E-05
Transcription, DNA dependent	2070	219	0.000102
Regulation of transcription	2141	227	6.33E-05
RNA biosynthetic process	2074	230	8.27E-05
Transcription	2231	230	0.000326
RNA metabolic process	2546	251	2.36E-03
Sterol biosynthetic process	31	8	3.38E-03
DNA packaging	286	48	2.81E-06
DNA metabolic process	675	76	5.25E-03
Nucleosome assembly	76	27	3.61E-11
Protein-DNA complex assembly	135	37	6.18E-11
Chromatin assembly	87	29	4.12E-11
Establishment and/or maintenance of chromatin structure	280	48	1.52E-06
Regulation of nucleobase, nucleoside, nucleotide, and nucleic acid metabolic process	2199	229	1.83E-04
Nucleobase, nucleoside, nucleotide, and nucleic acid metabolic process	3327	332	1.25E-04
NAD biosynthetic process	6	3	9.83E-03
NAD metabolic process	6	3	9.83E-03
Cellular component pathway	531	68	3.03E-04
Chromosome organization and biogenesis	346	52	2.80E-05
Golgi organization and biogenesis	10	4	0.00696
Organelle organization and biogenesis	998	115	1.77E-04
Cellular component organization and biogenesis	2315	225	8.15E-03
Cytoskeletal anchoring	10	4	0.00696
Regulation of cellular metabolic process	2375	246	1.38E-04
Extracellular structure organization and biogenesis	72	14	2.43E-03
Regulation of metabolic process	2462	250	4.34E-04
Negative regulation of smooth muscle cell differentiation	2	2	7.01E-03

Regulation of smooth muscle cell differentiation	2	2	7.01E-03
Negative regulation of muscle cell differentiation	2	2	7.01E-03
Regulation of muscle cell differentiation	2	2	7.01E-03
Synaptogenesis	22	7	1.63E-03
Synapse organization and biogenesis	28	7	7.25E-03
Regulation of cellular process	3709	348	7.37E-03
Regulation of biological process	4009	373	8.90E-03
Negative regulation of nitric oxide synthase activity	2	2	7.01E-03
Negative regulation of oxidoreductase activity	2	2	7.01E-03
Antigen processing and presentation of peptide or polysaccharide antigen via MHC class II	19	6	3.67E-03

Molecular Function	T	R	P
zinc ion binding	2073	226	7.10E-05
transition metal ion binding	2479	265	5.86E-05
DNA binding	2025	238	1.43E-07
nucleic acid binding	2948	326	2.00E-07
metal ion binding	3686	366	8.20E-04
cation binding	3416	338	1.83E-03
ion binding	3763	374	6.41E-04
actin binding	263	36	3.93E-03
cytoskeletal protein binding	381	17	8.33E-03
trans 1,2 dihydrobenzene 1,2 diol dehydrogenase activity	2	2	7.48E-03
polyamine oxidase activity	2	2	7.48E-03
oxidized purine base lesion DNA N-glycosylase activity	4	3	2.42E-03
oxidized base lesion DNA N-glycolase activity	4	3	2.42E-03
cGMP inhibited cyclic nucleotide phosphodiesterase activity	2	2	7.48E-03
macrophage colony stimulating factor receptor activity	2	2	7.48E-03
MHC class II receptor pathway	15	5	6.91E-03
gastric inhibitory peptide receptor activity	2	2	7.48E-03

Supplemental Table 5 - KEGG pathway analysis results from transient MDA-231 TR microarray experiment.

z	p value	Description
-7.94641	1.92E-15	Ribosome
-7.75665	8.72E-15	Oxidative phosphorylation
-7.71088	1.25E-14	Biosynthesis of steroids
-6.58882	4.43E-11	O-Glycan biosynthesis
-6.35578	2.07E-10	Apoptosis
-6.1325	8.65E-10	Glycan structures - biosynthesis 1
-5.74121	9.40E-09	Pyrimidine metabolism
-5.54703	2.91E-08	Ubiquitin mediated proteolysis
-5.20739	1.92E-07	One carbon pool by folate
-4.94375	7.66E-07	Olfactory transduction
-4.80769	1.53E-06	Thiamine metabolism
-4.76663	1.87E-06	Synthesis and degradation of ketone bodies
-4.76496	1.89E-06	Valine, leucine and isoleucine degradation
-4.70202	2.58E-06	Proteasome
-4.46983	7.83E-06	mTOR signaling pathway
-4.32839	1.50E-05	Insulin signaling pathway
-4.29217	1.77E-05	Purine metabolism
-4.29038	1.78E-05	Long-term potentiation
-4.28458	1.83E-05	Glyoxylate and dicarboxylate metabolism
-4.22724	2.37E-05	Type I diabetes mellitus
-4.20377	2.63E-05	Terpenoid biosynthesis
-3.89974	9.63E-05	Adherens junction
-3.76453	0.000167	Butanoate metabolism
-3.60239	0.000315	Axon guidance
-3.49355	0.000477	Wnt signaling pathway
-3.43173	0.0006	ErbB signaling pathway
-3.4108	0.000648	Pyruvate metabolism
-3.37492	0.000738	Chondroitin sulfate biosynthesis
-3.33437	0.000855	Huntington's disease
-3.33033	0.000867	Valine, leucine and isoleucine biosynthesis
-3.27073	0.001073	Renal cell carcinoma
-3.23939	0.001198	Circadian rhythm
-3.23367	0.001222	Neurodegenerative Disorders
-3.13731	0.001705	Benzoate degradation via CoA ligation
-3.08942	0.002006	Sphingolipid metabolism
-2.99498	0.002745	Keratan sulfate biosynthesis
-2.96183	0.003058	Fructose and mannose metabolism
-2.95967	0.00308	Glioma
-2.85721	0.004274	Glycan structures - biosynthesis 2
-2.83847	0.004533	Focal adhesion
-2.80581	0.005019	Dentatorubropallidoluysian atrophy (DRPLA)
-2.7968	0.005161	Nicotinate and nicotinamide metabolism

-2.78528	0.005348	Heparan sulfate biosynthesis
-2.7208	0.006512	Glycan structures – degradation
-2.68748	0.007199	Gap junction
-2.65506	0.00793	Tetrachloroethene degradation
-2.62825	0.008583	Phosphatidylinositol signaling system
-2.5739	0.010056	Alanine and aspartate metabolism
-2.41365	0.015794	Melanoma
-2.38859	0.016913	Colorectal cancer
-2.37759	0.017426	Tight junction
-2.37707	0.017451	Carbon fixation
-2.3687	0.017851	Folate biosynthesis
-2.23754	0.025251	Bisphenol A degradation
-2.16365	0.030491	Methane metabolism
-2.14553	0.03191	Starch and sucrose metabolism
-2.03595	0.041755	Lysine degradation
-2.02022	0.043361	Small cell lung cancer
-1.99311	0.046249	ABC transporters – General
-1.98024	0.047676	Pantothenate and CoA biosynthesis
-1.97359	0.048428	Glycosylphosphatidylinositol(GPI)-anchor biosynthesis
-1.87067	0.061391	Regulation of actin cytoskeleton
-1.85615	0.063432	Ethylbenzene degradation
-1.83891	0.065928	DNA polymerase
-1.83152	0.067023	Notch signaling pathway
-1.80473	0.071117	Bile acid biosynthesis
-1.67647	0.093646	Fatty acid metabolism
-1.63483	0.102085	Riboflavin metabolism
-1.60175	0.109211	Reductive carboxylate cycle (CO ₂ fixation)
-1.56355	0.117923	Pentose phosphate pathway
-1.52965	0.126103	Alzheimer's disease
-1.52408	0.127489	Cell adhesion molecules (CAMs)
-1.48214	0.138304	Chronic myeloid leukemia
-1.46704	0.142365	Antigen processing and presentation
-1.44583	0.148224	Methionine metabolism
-1.41901	0.155895	Limonene and pinene degradation
-1.41508	0.157045	Pancreatic cancer
-1.39323	0.163551	Glycosphingolipid biosynthesis – lactoseries
-1.38429	0.16627	Inositol phosphate metabolism
-1.34804	0.177646	Jak-STAT signaling pathway
-1.27556	0.202113	Cell cycle
-1.27136	0.2036	Galactose metabolism
-1.24997	0.211309	N-Glycan degradation
-1.20635	0.227681	1- and 2-Methylnaphthalene degradation
-1.18187	0.237257	Benzoate degradation via hydroxylation
-1.08485	0.277988	Glycosphingolipid biosynthesis – globoseries
-1.07184	0.283791	Non-small cell lung cancer
-1.06552	0.286643	Propanoate metabolism

-1.06001	0.289141	Glycosaminoglycan degradation
-0.97814	0.328007	Leukocyte transendothelial migration
-0.9713	0.331398	Glutamate metabolism
-0.92937	0.352696	Cyanoamino acid metabolism
-0.86966	0.384487	Glycolysis / Gluconeogenesis
-0.8401	0.400853	Glycerolipid metabolism
-0.81339	0.415994	Glycine, serine and threonine metabolism
-0.74474	0.456429	Pathogenic Escherichia coli infection – EHEC
-0.74474	0.456429	Pathogenic Escherichia coli infection – EPEC
-0.72518	0.46834	Phenylpropanoid biosynthesis
-0.71616	0.473893	Regulation of autophagy
-0.66973	0.503029	Melanogenesis
-0.63361	0.526332	Vitamin B6 metabolism
-0.6023	0.546975	Sulfur metabolism
-0.59391	0.552574	GnRH signaling pathway
-0.55687	0.577614	beta-Alanine metabolism
-0.4257	0.670328	Amyotrophic lateral sclerosis (ALS)
-0.41047	0.681462	Prion disease
-0.36319	0.716463	Glycosphingolipid biosynthesis - neo-lactoseries
-0.35373	0.723543	Protein export
-0.33977	0.734028	Epithelial cell signaling in Helicobacter pylori infection
-0.31038	0.756275	Bladder cancer
-0.30156	0.76299	Dorso-ventral axis formation
-0.30072	0.76363	Toll-like receptor signaling pathway
-0.28402	0.776392	Natural killer cell mediated cytotoxicity
-0.09647	0.923147	Fatty acid elongation in mitochondria
-0.04446	0.964536	Aminoacyl-tRNA biosynthesis
0.004868	0.996116	Nucleotide sugars metabolism
0.042128	0.966397	VEGF signaling pathway
0.099933	0.920398	MAPK signaling pathway
0.118328	0.905808	Aminosugars metabolism
0.151978	0.879204	PPAR signaling pathway
0.173999	0.861867	Acute myeloid leukemia
0.206678	0.836261	C21-Steroid hormone metabolism
0.220249	0.825677	Alkaloid biosynthesis II
0.244632	0.806742	Streptomycin biosynthesis
0.328915	0.74222	Long-term depression
0.356673	0.721337	p53 signaling pathway
0.373629	0.70868	Calcium signaling pathway
0.404926	0.685532	Adipocytokine signaling pathway
0.409938	0.681852	Cholera – Infection
0.440253	0.659754	RNA polymerase
0.457854	0.647057	Basal transcription factors
0.474012	0.635491	gamma-Hexachlorocyclohexane degradation
0.499338	0.617541	Ubiquinone biosynthesis
0.523125	0.600887	B cell receptor signaling pathway

0.534347	0.593101	Endometrial cancer
0.545953	0.585098	Phenylalanine metabolism
0.552545	0.580575	Glycosphingolipid biosynthesis – ganglioseries
0.554004	0.579576	Ascorbate and aldarate metabolism
0.658538	0.510192	Type II diabetes mellitus
0.72325	0.469527	Basal cell carcinoma
0.866201	0.38638	T cell receptor signaling pathway
0.891546	0.372636	Urea cycle and metabolism of amino groups
0.925765	0.354568	Glutathione metabolism
1.009307	0.312827	Fc epsilon RI signaling pathway
1.014032	0.310567	Thyroid cancer
1.035254	0.30055	Selenoamino acid metabolism
1.132198	0.257551	SNARE interactions in vesicular transport
1.144238	0.252525	TGF-beta signaling pathway
1.213972	0.224758	Hematopoietic cell lineage
1.240353	0.214845	Caprolactam degradation
1.276691	0.201711	Prostate cancer
1.413849	0.157406	Hedgehog signaling pathway
1.438455	0.150305	Glycerophospholipid metabolism
1.457693	0.144925	Parkinson's disease
1.459738	0.144362	Cysteine metabolism
1.640367	0.100929	2,4-Dichlorobenzoate degradation
1.78364	0.074482	Linoleic acid metabolism
1.810873	0.070161	Tryptophan metabolism
1.91878	0.055012	Citrate cycle (TCA cycle)
1.92259	0.054532	Arginine and proline metabolism
1.935078	0.052981	Aminophosphonate metabolism
2.114271	0.034492	Nitrogen metabolism
2.114302	0.034489	Naphthalene and anthracene degradation
2.173186	0.029766	Ether lipid metabolism
2.329344	0.019841	Taurine and hypotaurine metabolism
2.349429	0.018802	Renin-angiotensin system
2.353877	0.018579	Alkaloid biosynthesis I
2.436073	0.014848	Pentose and glucuronate interconversions
2.47494	0.013326	ECM-receptor interaction
2.580341	0.00987	N-Glycan biosynthesis
2.608033	0.009106	Histidine metabolism
2.927623	0.003416	Complement and coagulation cascades
3.05579	0.002245	Phenylalanine, tyrosine and tryptophan biosynthesis
3.095907	0.001962	Cytokine-cytokine receptor interaction
3.110163	0.00187	Taste transduction
3.179501	0.001475	Tyrosine metabolism
3.494923	0.000474	Atrazine degradation
3.85381	0.000116	Fatty acid biosynthesis
4.173509	3.00E-05	Arachidonic acid metabolism
4.724546	2.31E-06	Metabolism of xenobiotics by cytochrome P450

4.824256	1.41E-06	Maturity onset diabetes of the young
5.050351	4.41E-07	Cell Communication

Appendix 2 - Ductal outgrowth delay in MMTV/CrkI and MMTV/CrkII transgenic mice, but not in MMTV/CrkLV5 or FVB mice.

