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Integrative Genomics of Gene and Metabolic Regulation by Estrogen Receptors α and β and Coregulators

Zeynep Madak-Erdogan, Tze-Howe Charn, Yan Jiang, Edison T Liu, John A. Katzenellenbogen, Benita Katzenellenbogen

Corresponding author: Benita Katzenellenbogen, University of Illinois at Urbana-Champaign

Transaction Report:

(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. The original formatting of letters and referee reports may not be reflected in this compilation.)

1st Editorial Decision   18 December 2012

Thank you again for submitting your work to Molecular Systems Biology. We have now heard back from the three referees who agreed to evaluate your manuscript. As you will see from the reports below, the referees find the topic of your study of potential interest. They raise, however, several concerns on your work, which should be convincingly addressed with additional analysis and experimentation in a major revision of the work.

The comments refer to the need to provide deeper insights into the biological significance of the patterns of binding observed for the co-regulators analyzed in this study, in particular in view of the fact that RIP140 is both a co-activator and a co-repressor. The reviewers point also to the need of adding important additional controls and clarifications.

As a minor detail, we would be grateful if you could group all the accession numbers under a separate sub-section entitled "Data availability" within the Material & Methods section.

If you feel you can satisfactorily deal with these points and those listed by the referees, you may wish to submit a revised version of your manuscript. Please attach a covering letter giving details of the way in which you have handled each of the points raised by the referees. A revised manuscript will be once again subject to review and you probably understand that we can give you no guarantee at this stage that the eventual outcome will be favorable.

REFEREE REPORTS:

Reviewer #1 (Remarks to the Author):

The paper submitted by the Katzenellenbogen lab describes genome wide binding sites for ERa and ERb together with two cofactors, SRC3 and RIP140, in MCF-7 breast cancer cells. There have been a number of previous reports describing ERa and SRC3 binding in breast cancer cells and this study extends those observations by defining ERb binding and RIP140 binding. The experiments have been very well performed and data exhaustively analysed to gain insight into potential mechanisms by which these two receptors regulate cell cycle and cell proliferation. On the other hand, the data are somewhat overwhelming and the contribution and significance of SRC3 and RIP140 binding in the vicinity of ER binding is minimally addressed. Given that the identification of these binding events are a major part of the paper and intended to complement the ER binding analysis it is surprising that the authors have not investigated their significance in more detail.

The authors have determined the binding site repertoire for SRC3 and RIP140 and find that they are enriched in the vicinity of ER binding sites but they do little to address their biological significance. For example, (1) given the cofactors bind to a small fraction of ER binding sites are there sequence motifs that determine this selectivity?(2) Fig 2 suggests that sites that bind RIP140 also bind SRC3. Given that that RIP140 is reported to function as a corepressor, at least at many target genes, while SRC3 function as a coactivator this result would not be unexpected for the majority of sites. This observation should be confirmed for a number of specific genes in ChIP experiments and the effects of SRC3/RIP140 depletion on binding examined; (3) The function of RIP140 as a coactivator for certain genes and as a corepressor for other genes is intriguing and it would be extremely interesting if the authors could distinguish binding sites that underpinned this differential activity. In this regard the authors quote a Cavailles paper published in 1995 but its function as a repressor of specific metabolic genes was only reported in 2004-07 and its function as a coactivator of inflammation and ovarian function in 2008-10.

Reviewer #2 (Remarks to the Author):

In this manuscript, Madak-Erdogan et al, describe ER and ER cistrome under three different conditions, which would allow these receptors to bind as homodimers or heterodimers. Authors then correlate ER binding pattern with E2-dependent SRC3 and RIP140 binding and E2 regulated expression of genes.

Although there are several recent publications on ER cistrome, this manuscript provides unique insight into ER binding pattern in the presence of ER and demonstrates dominant role of ER in controlling ER :E2 induced proliferation. The majority of experiments are well performed and controlled. However, experiments suggested below, mostly related to mechanistic aspects, would certainly enhance the impact.
1) Based on cistrome analysis, ER enhances chromatin binding of RIP140. Since RIP140 has both co-activator or co-repressor function and its expression in breast cancer in some studies is linked to better outcome, it would be appropriate to verify whether RIP140 is essential growth suppressive effects of ER , particularly in G2/M accumulation. Analysis of public primary breast cancer databases for ER , ER , and RIP140 expression under various combinations would be helpful.
2) Authors argue throughout the manuscript that part of the growth suppressive effects of ER is related to its effects on FOXM1. It is important to verify the effect of ER on FOXM1 expression under various conditions as previous studies have demonstrated opposing effects of ER and ER on FOXM1 expression (AJP 179:1148-56). These effects of ER may partly be RIP140 dependent.
3) ER appears to enhance G2/M phase accumulation independent of ligand (Figure 6B) and E2 treatment partially reversed this effect. Therefore, the effect of ER on cell cycle is independent of binding to genome. Reanalyzing gene expression array results for G2/M- associated genes under unliganded and liganded condition may provide insights. Authors need to address this issue as interpretation as presented is incorrect.
4) Figure 4F needs additional control. Similar analysis of a gene that binds to ER but not RIP140 is essential. Data fail to demonstrate any specificity in RIP140 binding to ER -specific gene. PgR, although induced by ER alone, still binds to RIP140.
5) Essential controls in Figure 7A are missing. Control lane without E2 treatment is essential to

show E2-inducibility of ERK activation. Furthermore, E2-induced ERK activation in all three cell lines is essential to demonstrate whether growth inhibition in ER overexpressing cells is due to impaired ERK pathway, which is causing sub G0 cells.

6) Please provide explanation for rows labelled "merged" in supplementary tables

Reviewer #3 (Remarks to the Author):

Review comments to the manuscript MSB-12-4171, entitled "Integrative Genomics of Gene and Metabolic Regulation by Estrogen Receptors and and Coregulators" by Dr Katzenellenbogen and colleagues.

In this manuscript, Madak-Erdogan et al. tried to decipher how the closely related transcription factors - estrogen receptor (ER) and , exert divergent responses to estrogen in breast cancer by integrative analysis of cistrome and transcriptome as well as delicate clustering approach on the basis of engineered ER-expressing cell model.

Overall this manuscript provides a comprehensive and valuable dataset with comparable cistrome and expression data relating to both ER and ER at the same time in a well-designed modified MCF-7 cell model, which is quite helpful to further investigate and compare the actions of ER and ER . The use of cluster algorithm enables the authors to parse the intricate transcriptional regulation of both ERs and their coregulators SRC3 and RIP140. Although some of data are descriptive, the findings that RIP140 is involved as a key coregulator of ER , compared to ER , in mediating ER chromatin binding and transcriptional response to estrogen is interesting.

Major concerns:

1. Since ER and ER are structurally similar and the cell model was constructed by exogenous overexpression of ER in ER only MCF7 cells (as ERa/ cells) or siER MCF7 cells (as ER only cells), it is better to show that the antibodies for ER and ER employed in this study do not cross-react with each other although they are commercially purchased. This is especially important for chromatin binding data quality as well as the following cluster analysis when the purpose is to discriminate the actions of both ERs.

2. Figure 3A and B, the percentage numbers in the venn diagram are a bit different to read and understand considering different cell backgrounds there. Why not change into real overlapped gene numbers?

3. Figure 6A, for the cell proliferation assay, it is better to include the vehicle condition groups.

4. Figure 7B and C, how are the ERs and coregulators (SRC3 and RIP140) binding status for RIP140, DDIT3, FOXO1A and MEF2A genes in different ER background cell models?

5. To rule out the phenotypic difference and transcriptional regulation of two ERs observed here is not due to the overexpression or knockdown pressure on the engineered MCF7 cells, it is better that data in Fig. 6A, 6B and Fig. 7B, 7C could be reproduced by engineering another breast cancer cells like T47D etc.

6. The authors should discuss more about how the findings (e.g. RIP140 part) in this study may implicate targeted treatment of ER positive breast cancers, especially of those ER only breast cancers.

1st Revision - authors' response   15 March 2013

Please find enclosed our revised manuscript (**MSB 12-4171**). Based on the reviewers' comments, we have revised our manuscript accordingly and addressed all issues that were raised.

We now provide additional analysis of the coregulator function and biological significance of RIP140 and also validation of our findings from MCF-7 cells in another breast cancer model. We have addressed both of these issues from the reviewers and provide detailed responses below, but in brief, we have generated 6 additional cell lines, where we knocked down RIP140 or SRC3 in the three different cell backgrounds (ERα cells, ERα/β cells and ERβ cells). Then we performed gene expression analysis and ChIP assays for coregulator recruitments to delineate the role of each coregulator (SRC3 or RIP140) in the different cell backgrounds. Also, we extended cell cycle analysis and adipogenesis studies beyond MCF-7 cells to another ERα-positive breast cancer cell line, T47D, into which we introduced ERβ. We have also included more analysis using breast cancer clinical datasets. This new information has been added as new panels E, F, and G of Figure 7 and new Supplementary Figures 3-10.

Below, please find our detailed responses to the comments of the reviewers. We thank the reviewers for their thoughtful comments and questions, and hope that with the additional data and incorporated discussion of the findings, our manuscript will now be acceptable for publication in *Molecular Systems Biology*.

Reviewer #1 (Remarks to the Author):

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The authors have determined the binding site repertoire for SRC3 and RIP140 and find that they are enriched in the vicinity of ER binding sites but they do little to address their biological significance. For example,

 (1) Given the cofactors bind to a small fraction of ER binding sites are there sequence motifs that determine this selectivity?

Author Response

We thank the Reviewer for highlighting this issue. We conducted CEAS (Supplementary Table 1) and Seqpos (Supplementary Table 4) analysis of these binding sites. These binding sites did not show any major differences in the composition of transcription factor binding motifs or strength of EREs detected. Therefore, we believe the selectivity might be due to some epigenetic marks or other factors (like FOXA1, GATA3) that render these sites accessible for ER binding with SRC3 and/or RIP140 (Supplementary Figure 2), and we have now made mention of this on page 9 (top paragraph) in the Results section.

(2) Fig 2 suggests that sites that bind RIP140 also bind SRC3. Given that that RIP140 is reported to function as a corepressor, at least at many target genes, while SRC3 function as a coactivator this result would not be unexpected for the majority of sites. This observation should be confirmed for a number of specific genes in ChIP experiments and the effects of SRC3/RIP140 depletion on binding examined;

Author Response

This is a very valid point. As the reviewer suggested, we knocked down RIP140 or SRC3 in the different cell backgrounds and performed gene expression analysis and ChIP experiments to monitor recruitment of SRC3 or RIP140, which we have now added as Supplementary Figure 7. These experiments revealed that SRC3 acted as a coactivator, increasing E2 mediated expression for all genes monitored, in all three receptor cell backgrounds. RIP140 function as a coactivator or corepressor was more gene and cell background dependent, as shown in new Supplementary Figure 7. One notable finding from the ChIP experiments was increased recruitment of SRC3 at gene regulatory sites where we saw an increase in gene expression in cells with knock-down of RIP140 , suggesting that RIP140 is acting as a true corepressor, so that its removal increases mRNA output of the gene. We now present these findings on page 15 (middle).

(3) The function of RIP140 as a coactivator for certain genes and as a corepressor for other genes is intriguing and it would be extremely interesting if the authors could distinguish binding sites that underpinned this differential activity. In this regard the authors quote a Cavailles paper published in 1995 but its function as a repressor of specific metabolic genes was only reported in 2004-07 and its function as a coactivator of inflammation and ovarian function in 2008-10.

Author Response

This is a very interesting question and we did some further analysis. We monitored occupancy of RIP140 binding sites by other factors (ERK2, FOXA1, GATA3, histone marks) and found that about 50% of RIP140 binding sites are also occupied by ERK2, and that ERK2 might be a determinant of RIP140 function in a cell background dependent manner. By contrast, the other factors did not show a correlation. In our previous studies (Madak-Erdogan and Katzenellenbogen, Toxicological Sci. 2012), we showed that when ERα was present together with AhR and RIP140, RIP140 had a corepressor role; however, when AhR and RIP140 were present alone, RIP140 acted as a coactivator. Based on this data, we suggested in that publication that an enzyme activity associated with ERα might function to convert RIP140 to a corepressor. We also showed in another study (Madak-Erdogan et.al, Mol Cell Biol, 2011) that 70% of the ERK2 binding sites overlapped with ERα binding sites in ERα only MCF-7 cells. Therefore, it is possible that ERK2 may be a determinant of RIP140 coregulator function, but this hypothesis needs a great deal of further experimentation and validation that is well beyond the scope of this paper.

We also thank the reviewer for pointing out the references, and we also cite other papers which support our findings for RIP140 dual function.

Reviewer #2 (Remarks to the Author):

In this manuscript, Madak-Erdogan et al, describe ERα and ERβ cistrome under three different conditions, which would allow these receptors to bind as homodimers or heterodimers. Authors then correlate ER binding pattern with E2-dependent SRC3 and RIP140 binding and E2 regulated expression of genes.

Although there are several recent publications on ERα cistrome, this manuscript provides unique insight into ERα binding pattern in the presence of ERβ and demonstrates dominant role of ERβ in controlling ERα:E2 induced proliferation. The majority of experiments are well performed and controlled. However, experiments suggested below, mostly related to mechanistic aspects, would certainly enhance the impact.

1) Based on cistrome analysis, ERβ enhances chromatin binding of RIP140. Since RIP140 has both co-activator or co-repressor function and its expression in breast cancer in some studies is linked to better outcome, it would be appropriate to verify whether RIP140 is essential growth suppressive effects of ERβ, particularly in G2/M accumulation. Analysis of public primary breast cancer databases for ERα, ERβ, and RIP140 expression under various combinations would be helpful.

Author Response

We have done additional cell cycle analysis based on the reviewer's suggestion. We include this data as Supplementary Figure 5 and discuss this on p. 13 (bottom) – p. 14 (middle). We found that RIP140 was indeed essential for G2/M accumulation because knock-down of RIP140 completely abrogated G2/M transition in all of the receptor backgrounds studied. We've also examined ERα, ERβ and RIP140 expression in various clinical breast cancer databases. The clinical data supports our observations in the cell models and reveals that RIP140 is crucial for the growth suppressive effects of ERβ. These findings have been added on page 16. We also generated an ERβ and RIP140 associated signature of 20 genes using our gene expression and ChIP-seq data. Analysis of various tumor datasets showed that patients with tumors over-expressing these genes had a better prognosis and disease-free and overall survival, consistent with a tumor suppressive role for ERβ and RIP140 (Figure 7 E,F and G and new Supplementary Figure 10 and mentioned in the text on page 16).

2) Authors argue throughout the manuscript that part of the growth suppressive effects of ERβ is related to its effects on FOXM1. It is important to verify the effect of ERβ on FOXM1 expression under various conditions as previous studies have demonstrated opposing effects of ERα and ERβ on FOXM1 expression (AJP 179:1148-56). These effects of ERβ may partly be RIP140 dependent.

Author Response

Studies from our group have previously shown that ERβ did indeed decrease the levels of FOXM1 in breast cancer cells (Chang et al., Mol Endo, 2006). We also monitored the effect of RIP140, and RIP140 seems to be required for the E2 mediated increase in FOXM1 expression in ERα cells (Supplementary Figure 4C). We did not see any further increase in FOXM1 mRNA with E2 treatment in ERα/ERβ cells or ERβ only cells, and coregulator knockdowns did not have any significant effects on FOXM1 in these cells. We mention these findings in the text (page 14).

3) ERβ appears to enhance G2/M phase accumulation independent of ligand (Figure 6B) and E2 treatment partially reversed this effect. Therefore, the effect of ERβ on cell cycle is independent of binding to genome. Reanalyzing gene expression array results for G2/M- associated genes under unliganded and liganded condition may provide insights. Authors need to address this issue as interpretation as presented is incorrect.

Author Response

We repeated cell cycle analysis experiments and reexamined the gene expression data as the reviewer suggested (Supplementary Figure 4). In ERβ-containing cells, the % cells in G2/M phase never reaches the level that is observed in ERα cells with E2. [So, even though there is some basal increase in G2/M accumulation, there is no further effect of E2.] Likewise, in ERβ-containing cells, when we reanalyzed gene expression in unliganded and liganded conditions, we always observed a decrease in the basal expression levels of G2/M activators and an increase in the basal levels of G2/M inhibitors along with the loss of hormone regulation. Thus, we think that our interpretation is correct and we have now included this new analysis as Supplementary Figures 4 and 5 and discuss the findings on page 13-14.

4) Figure 4F needs additional control. Similar analysis of a gene that binds to ERα but not RIP140 is essential. Data fail to demonstrate any specificity in RIP140 binding to ERβ-specific gene. PgR, although induced by ERα alone, still binds to RIP140.

Author Response

We have now included data for several genes as Supplementary Figure 4 and discuss the findings on page 13-14. We show analysis of the Ki-67 gene which recruits ERα but not RIP140 in ERα cells. Analysis of OTUB2, an ERβ preferential gene, demonstrates specificity in RIP140 binding. Examination of two other genes, DDIT3 and FOXO1A, which are associated with adipogenesis shown in Supplementary Figure 8, demonstrate preferential RIP140 binding in cells containing ERβ.

5) Essential controls in Figure 7A are missing. Control lane without E2 treatment is essential to show E2-inducibility of ERK activation. Furthermore, E2-induced ERK activation in all three cell lines is essential to demonstrate whether growth inhibition in ERβ overexpressing cells is due to impaired ERK pathway, which is causing sub G0 cells.

Author Response

We have now added the controls to Figure 7A. This makes clear that the stimulatory effect of estradiol on ERK2 activation is lost when ERβ is present in cells.

6) Please provide explanation for rows labelled "merged" in supplementary tables

Author Response

These "merged" rows come directly from the output of Seqpos software. The software computes transcription factor binding motifs close to the center of binding site peaks, and it uses motif databases for human, mouse and other organisms. These merged rows seem to be the motifs obtained when a reference organism for the motif databases is not specified. However, because these "merged" rows do not change any conclusions from the data, and may be confusing, we now use only human data, and therefore, the merged rows have been removed from the Supplementary Tables.

Reviewer #3 (Remarks to the Author):

Review comments to the manuscript MSB-12-4171, entitled "Integrative Genomics of Gene and Metabolic Regulation by Estrogen Receptors α and β and Coregulators" by Dr Katzenellenbogen and colleagues.

In this manuscript, Madak-Erdogan et al. tried to decipher how the closely related transcription factors - estrogen receptor (ER) α and β, exert divergent responses to estrogen in breast cancer by integrative analysis of cistrome and transcriptome as well as delicate clustering approach on the basis of engineered ER-expressing cell model.

Overall this manuscript provides a comprehensive and valuable dataset with comparable cistrome and expression data relating to both ERα and ERβ at the same time in a well-designed modified MCF-7 cell model, which is quite helpful to further investigate and compare the actions of ERα and ERβ. The use of cluster algorithm enables the authors to parse the intricate transcriptional regulation of both ERs and their coregulators SRC3 and RIP140. Although some of data are descriptive, the findings that RIP140 is involved as a key coregulator of ERβ, compared to ERα, in mediating ERβ chromatin binding and transcriptional response to estrogen is interesting.

Major concerns:

1. Since ERα and ERβ are structurally similar and the cell model was constructed by exogenous overexpression of ERβ in ERα only MCF7 cells (as ERa/β cells) or siERα MCF7 cells (as ERβ only cells), it is better to show that the antibodies for ERα and ERβ employed in this study do not cross-react with each other although they are commercially purchased. This is especially important for chromatin binding data quality as well as the following cluster analysis when the purpose is to discriminate the actions of both ERs.

Author Response

This is an important point. These controls are already shown in our data in Fig. 4F, 4I and 4L where we pull down ERα or ERβ and monitor recruitment of the receptors to various binding sites in different backgrounds. We do not observe any recruitment of ERβ in ERα cells or any recruitment of ERα to chromatin sites in ERβ cells, suggesting that our antibodies are specific for the receptor we are monitoring.

2. Figure 3A and B, the percentage numbers in the venn diagram are a bit different to read and understand considering different cell backgrounds there. Why not change into real overlapped gene numbers?

Author Response

We have changed the percentages to numbers, as suggested.

3. Figure 6A, for the cell proliferation assay, it is better to include the vehicle condition groups.

Author Response

We have now included the data from the Vehicle treated groups in Figure 6A.

4. Figure 7B and C, how are the ERs and coregulators (SRC3 and RIP140) binding status for RIP140, DDIT3, FOXO1A and MEF2A genes in different ER background cell models?

Author Response

We now show the binding site data for ERs and coregulators in RIP140, FOXO1A, MEF2A and DDIT3 genes from our ChIP-Seq analysis as Supplementary Figure 6 and mention the binding site information in the text on page 15.

5. To rule out the phenotypic difference and transcriptional regulation of two ERs observed here is not due to the overexpression or knockdown pressure on the engineered MCF7 cells, it is better that data in Fig. 6A, 6B and Fig. 7B, 7C could be reproduced by engineering another breast cancer cells like T47D etc.

Author Response

We agree this is worthwhile. Therefore, we have engineered T47D cells to contain the 3 complements of ERs as we did in MCF-7 cells. We show (new Supplementary Figure 8) that similar trends in proliferation (panel A), adipogenesis (panel B) and regulation of adipogenesis gene expression (panel C) are seen for these breast cancer cells as were observed in MCF-7 cells. Therefore, the findings are observed in other breast cancer cells and rule out phenotypic differences and transcriptional regulatory effects being unique to the MCF-7 cells.

6. The authors should discuss more about how the findings (e.g. RIP140 part) in this study may implicate targeted treatment of ER positive breast cancers, especially of those ERβ only breast cancers.

Author Response

We have expanded our discussion (p. 18 top) about this important issue. As Gruvberger-Saal SK, Clinical Cancer Research 2007 showed, ERβ is a predictor of tamoxifen response and favorable prognosis in ERα negative tumors. Therefore, utilization of ERβ selective agonists and activation of ERβ gene programs in ERβ-containing breast cancers, or increasing levels of RIP140 which could have a growth suppressive activity through ERβ might provide a benefit for patients with breast tumors containing ERβ.

2nd Editorial Decision   18 April 2013

Thank you again for submitting your revised work to Molecular Systems Biology. The reviewers are now supportive and I am pleased to inform you that we will be able to accept your paper for publication in Molecular Systems Biology, pending the following minor points:

- Reviewer #2 has some remaining points that we would kindly ask you to address with suitable amendments in the text.

Thank you for submitting this paper to Molecular Systems Biology.

REFREREE REPORTS:

Reviewer #2 (Remarks to the Author):

1) Could not find the response letter indicating changes.

2) Please clarify discrepancy in page 7 and 8. Page 7 bottom, Clusters 2 and 3 contained Er binding sites. In page 8, "two coregulators were virtually absent at binding sites in clusters 1-3 and 10-11, which represent ERalpha and ERbeta binding sites, respectively". ER binding sites in page 7 should be ERalpha binding sites.

3) Page 10, it may be worth doing a statistical analysis of uniquely regulated genes at 4 hours. It appears that at 4 hours Erbeta has significantly higher number of uniquely regulated genes than ERalpha (303 out of 352 in Erbeta for induced genes, whereas it was 128 out of 361 for Eralpha, figure 3a).

4) Supplementary figure 10 is poorly explained. Better legend is required (red and yellow circles)

2nd Revision - authors' response   22 April 2013

Author Response to Reviewer 2 Minor Comments

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We have made this change that the reviewer suggested.

3) Page 10, it may be worth doing a statistical analysis of uniquely regulated genes at 4 hours. It appears that at 4 hours Erbeta has significantly higher number of uniquely regulated genes than ERalpha (303 out of 352 in Erbeta for induced genes, whereas it was 128 out of 361 for Eralpha, figure 3a).

As we described in Materials and Methods section, the gene list was obtained after statistical analysis of gene expression data.

"CEL files were processed using GeneSpring GX 11.0 software (Agilent) to obtain fold-change and p-value with Benjamini and Hochberg multiple test correction (Hochberg & Benjamini, 1990) for each gene for each treatment relative to the vehicle control. We considered genes with fold-change > 1.8 and p-value < 0.05 as statistically significant, differentially expressed. "

This data suggests that, in the absence of ERα, ERβ induces a gene program that is unique to ERβ only cells. ERα and ERα and ERβ containing cells present a similar transcriptome, whereas lack of ERα and presence of ERβ induces a novel set of genes that shows poor overlap with the ones observed in the other two cell lines.

4) Supplementary figure 10 is poorly explained. Better legend is required (red and yellow circles)

We have added the following to the Supplementary Figure 10 legend:

Using Oncomine concept maps, the ERβ/RIP140 gene signature was found to be significantly associated with outcome -metastasis, survival and recurrence- (yellow circles) in several data sets (red circles). Cytoscape software was used to generate the association map. Association *P values* were denoted on the edges for each data set and concept.