Title: Translation elongation factor eEF1A2 is a potential oncogene that is overexpressed in two-thirds of breast tumors.

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Author's response to reviews: see over
Cover Letter: point by point description of changes made

Reviewer: Charlotte Knudsen

**Minor revision 1:** This has been added to the start of the results section (p8); the antibodies are being described in a paper in preparation, but we have included details requested by both reviewers.

**Minor revision 2:** This is incorrect, concentrations were already given.

**Minor revision 3:** The wording has been changed to “eEF1A is unlikely to be rate-limiting” (p12)

**Minor revision 4:** This has been corrected.

**Minor revision 5:** This is absolutely right. I have changed the title to say oncoprotein rather than oncogene, and changed the wording about the p53 sites to clarify that I am talking about the genes encoding these proteins and not the proteins themselves (p11).

We are in complete agreement with the referee that the relationship to ER positivity is an interesting one, but feel that any comment made at this stage would be too speculative to add any real value to the paper.

Reviewer: Anil Sood

**Major revision 1:** The wording has been changed to reflect this valid criticism (p2). We would like to note, though, that Anand et al have shown eEF1A2 to be capable of transforming cells in general, so the oncogenicity of this protein when inappropriately expressed is unlikely to be confined to ovarian tumours.

**Major revision 2:** This has been addressed as in minor revision 1, above.

**Major revision 3:** Information about percentage tumour versus stromal content is available for some but not all the tumours analysed by RT-PCR. It varies between 30 and 90%, with most tumours falling in the range 40-60%. However, as the difference between ER+ve and ER-ve tumours was confirmed in an independent set of samples by immunohistochemistry we think it unlikely that our conclusions were affected by the variation in stromal content.

**Major revision 4:** This has been done and the figures renumbered accordingly (described on p8).

**Major revision 5:** Some of this information is available but not all. We have no data available on response to adjuvant therapy; whilst this would have been useful, it does not affect our conclusions. We also have no data on survival as many of these samples were obtained from women who were only diagnosed within the last few years. Information on tumour stage and lymph node positivity is available for samples studied by RT-PCR and IHC, and information on grade is available for the samples studied by RT-PCR. There is no correlation between eEF1A2 expression and tumour stage or lymph node status and a sentence to this effect has been added (p 9).
completely agree that all these potential correlations need be investigated but this will require a much larger sample set for which as much background information as possible has been obtained.

**Major revision 6:** We reported staining intensity rather than percentage of tumour cells staining because in almost all cases examined the staining was near-uniform amongst tumour cells. Stroma was negative in every case. We have added a sentence to this effect (p9).

The tissue microarrays were commercial samples, and two cuts per tumour were analysed. Each cut was analysed with a different eEF1A2-specific antibody (ie. raised against a separate peptide) providing an additional level of confidence in the results. There was almost perfect correlation between the two levels in terms of staining intensity. Scoring was carried out by two independent researchers (VT and CA). This detail has been added to the methods section (p7).

**Major revision 7:** The p53 status was assessed only by immunohistochemistry; this was not done by us but was information received with the commercial TMAs. We have no way of carrying out mutation analysis on these samples, so if the reviewer thinks it necessary we will remove this section of the manuscript.

**Major revision 8:** There were no associations seen in these cell lines- for example, HepG2 cells have wild-type p53 and no eEF1A2, whereas MDAMB231 are reported to have mutant p53 but are overexpressing eEF1A2. The situation in cell lines with respect to eEF1A2 clearly doesn’t reflect the behaviour of specific primary tumours, so I am not sure that this lack of correlation negates the preliminary finding of a possible association in the tumours, but again we are happy to remove the section about p53 if the reviewer wishes us to.

**Minor revision 1:** I don’t think that giving the tumour numbers will add anything to this figure- they are simply internal code numbers which will be meaningless to the reader. Each area on the x axis bounded by short lines represents a different tumour sample, and I have added a sentence to the figure legend to clarify this (p 16).