To:

Dr. Alisha DSouza

Senior Review Editor of JoVE

Yokohama, August 4, 2018

We hereby submit the revised manuscript entitled “Cell based assays of SINEUP non-coding RNAs that can specifically enhance mRNA translation” by Hazuki Takahashi, Harshita Sharma and Piero Carninci to be considered for publication as original methods article of JoVE produced Video.

In summary, we have identified a novel class of antisense RNAs, which have the function of positively regulate protein translation (Carrieri et al, Nature, 2012, doi: 10.1038/nature11508), likely be enhancing the interaction of the sense RNA with the translational machinery mediated by a SINEB2 element embedded in the transcript.

We named these antisense RNAs “SINEUPs”, since they use a SINE element to UP-regulate translation.

Following the initial discovery, in this manuscript we show the cell based assay methods of the SINEUPs translation-enhancing protein activity by high throughput micro well image cytometer, to produce synthetic SINEUPs against a broad range of targets. Synthetic SINEUPs are being used in many studies to specifically increase protein translations, as a counterpart of siRNAs. Here, we explore the design of the antisense regions (called “binding domain”) to produce a first milestone work towards optimization of the design of these RNAs, which is needed to broadly expand applications for many users to enhance protein translation.

The revised version of the manuscript address all of the comments raised by the editor and reviewers, as outlined in the rebuttal letter.

In addition to this, we would like to disclose all the potential conflict of interest around this study. In particular, Piero Carninci (PC) is inventors in a patent (US9353370B2 and patents applications related to this patent, as EU and Japanese patents) owned by our main employers, our academic research institutions (RIKEN). PC funded TransSINE Technologies, Inc., a company located in Japan with the mission to develop and commercialize products based on of the US9353370B2 and related patents. This includes the commercialization of SINEUPs in plasmid expression vectors, including basic vectors and custom made constructs (see details from the UK representative web site: <http://www.cellgs.com/services/sineup.html>). We believe that association with this company to provide reagents worldwide will help to further enable colleagues to use the technology.

TransSINE Technologies has not provided actual funding to this research, nor direct salaries or any direct benefits to any of the members participating in this study. TransSINE Technologies has only provided information that was necessary to the design and the execution of the project. PC, who is also affiliated to TransSINE Technologies, has a key role in the design of the study. TransSINE Technologies has not influenced any conclusions of the study. Affiliation of PC to TransSINE Technologies does not alter our adherence to JoVE policies on sharing data and materials.

We believe that the revised manuscript satisfies all the requirements for the publication in JoVE. We are looking forward to hearing from you.

Sincerely,

Dr. Piero Carninci