



To whom it may concern

Reviewer report: project proposed by E.V. Kravchenko (JINR, Dubna, Moscow)

Title: Study of the radioprotective properties of the Damage Suppressor (Dsup) protein on a model organism *D. melanogaster* and human cell culture HEK293T

The proposal submitted by prof. E.V. Kravchenko (leader) and prof. A.V. Rzyanina (deputy leader) involves the JINR (DLNP), the Institute of Developmental Biology RAS (Moscow), and the Liminological Institute SB RAS, and focuses on the examination of radioprotective properties of a new Damage suppressor (Dsup) protein on a model object *D. melanogaster* and on human cell cultures by studying the mechanism of action of the protein at molecular level. The objective is the increase of radio-resistance against different types of ionizing radiation will allow to understand the beneficial effect of Dsup at the molecular level and at the level of the whole organism.

– **scientific merits and intellectual contribution;**

the project is a continuation of studies relative to the role played by Dsup in the the mechanisms of radio-resistance to ionizing radiation initially reported for tardigrades. The objectives of the proposal are innovative and well defined, they encompass, among others, the following topics: i) the evaluation of the effect of Dsup protein under normal conditions and after exposure to various types of ionizing radiation on the functioning of a multicellular organism (*D. melanogaster*) at the level of longevity and radio-resistance; and ii) the effect of Dsup protein in Human cells at the transcriptome level as a response to changes in the radio-resistance of a cell culture expressing Dsup after exposure to protons and heavy ions. The scientific merits and intellectual contribution of the project are of high level and well defined.

– **technical feasibility of the project within the proposed timescale;**

The technical feasibility for the project is accurately described reporting a detailed description of the different Tasks of the project, including: i) Optimization and synthesis of DNA sequence encoding a Dsup protein; ii) Generation of the *D. melanogaster* line stably expressing Dsup; iii) Evaluation of the radio-resistance of *D. melanogaster* strains stably expressing Dsup; iv) Generation of a stable cell line HEK293T expressing fusion protein GFP-Dsup and assessment of the radio-resistance of this cell line to various types of ionizing radiation; v) Transcriptome analysis of *D. melanogaster* and the HEK293T cell line expressing Dsup protein under normal conditions and after exposure to ionizing radiation; vi) Study of GFP-Dsup fusion protein distribution on *D. melanogaster* polytene chromosomes; vii) Study of the life span of *D. melanogaster* lines expressing Dsup; and viii) Creation of an expression vector for production of Dsup protein in *E. coli* cells, extraction and purification of Dsup protein for preliminary crystallization experiments. Overall, the technical feasibility of the project appears to be adequate for achieving all the proposed objectives within the indicated timescale.

– **compliance of the requested financial resources with the project objectives;**

The GAANT of the project (TimeTable schedule) is described in detail. The requested financial source (160 K USD) is completely adequate for the suggested work plan, it includes materials, equipment and travel resources.

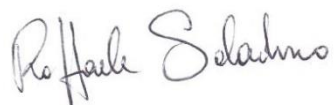
– availability of adequate human resources at JINR and in the collaborating institutions."

The human resources at JINR and in the collaborating institutions are full adequate for the feasibility of the project.

Overall report: Highly recommended.

Best regards

Prof. Raffaele Saladino

A handwritten signature in black ink that reads "Raffaele Saladino". The signature is written in a cursive style with a large initial 'R'.