CHARLES UNIVERSITY
FACULTY OF PHARMACY IN HRADEC KRALOVE

Department of Pharmacology and Toxicology

Study program: Pharmacy

Opinion of the Opponent of the Diploma Thesis

Year of the defense: 2023

Student:	**David Brychta**
Thesis Tutor:	PharmDr. Eduard Jirkovský, Ph.D.
Consultant:	M.Chem. Nika M. Lovšin, Ph.D.
Opponent:	PharmDr. Rona Karahoda, Ph.D.
Thesis title:	**Analysis of the molecular pathways and interactome of FUBP3**

Scope of work, number of 72 pages, 20 figures, 6 tables, 86 citations

Evaluation of the work:

a)	Processing of the theoretical part:	Excellent
b)	The complexity of the methods used:	Excellent
c)	Preparation of the methodological part (clarity, comprehensibility):	Excellent
d)	The quality of the experimental data obtained:	Excellent
e)	Processing of results (clarity):	Excellent
f)	Evaluation of results, including statistical analysis:	Very good
g)	Discussion of results:	Very good
h)	Clarity, conciseness, and adequacy of conclusions:	Excellent
i)	Meeting the objectives of the work:	Very good
j)	Quantity and up to date of references:	Excellent
k)	Language level (stylistic and grammatical level):	Very good
l)	Formal level of the work (text structure, graphic design):	Excellent

I recommend the thesis for recognition as a rigorous thesis ☒

Comments on the evaluation:

The diploma thesis of David Brychta focuses on deciphering the role of FUBP3 protein in osteoporosis via a detailed analysis of its interactome and localization to stress granules. This work was conducted at the University of Ljubljana under the supervision of M.Chem. Nika Lovšin, Ph.D. The thesis is written in very good English (with minor stylistic and grammatical errors), contains all the formal prerequisites, and is properly formatted.

In detail, the section of theoretical background discusses aspects of bone biology and genetics of osteoporosis with special focus on cellular stress as a risk factor for development of osteoporosis. The methodological part gives detailed information on all the bioinformatic analysis and experimental methods such as cell culturing, immunostaining, and gene expression analysis; as such the thesis comprises relatively rich and interesting work performed by the student. The results are well described and the discussion is clear, acknowledging the limitations of the work. Overall, I rate the thesis as excellent and recommend it for defense.

Questions and comments to student:

1) Section 5.6.3. How did you test that the expression of the housekeeping gene (RPLP0) was not affected by the treatment?

2) Table 5 - Primer sequence for TMEM64 and TMEM64 (2) is the same, although in Section 6.3 it is mentioned that a second set of primers was aquired? Moreover, the fold-change results for these two primer sets are substantially different (1.89 vs 3.16). How do you explain these differences? How did the second set of primers differ from the first one (eg. were there any differences in exon-intron boundaries or other)?

3) Similarly, in Section 6.3 it is not clear the part concerning FGF, MPP7 (2), and MPP7 (3), since according to the previous paragraph these results were not taken into account based on melting temperature results. However, FGF results are shown in Figure 20. As for MPP7 (2) and MPP7 (3) again there is no information how do they differ between the MPP7 sequence presented in Table 5.

4) The thesis aims to decipher the role of FUBP3 in relation to osteoporosis. What was the rationale of the visualization of FUBP3 in cell lines such as HOS, A549, BM-MSC, derived from cells not present in bone tissue?

5) Is there anything known on the cell type through which FUBP3 affects bone?

6) In PPI studies what is the the reliability of literature curation and how does it compare to high-throughput assays?

Evaluation of the thesis: Excellent		**For the defense:** **Recommend**
In Hradec Králové	29. května 2023	signature of the opponent