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Dear Thesis Committee,

I have been asked to write an evaluation of Nikol Dibus thesis work entitled: "Functional Characterization of SCFFBXO38 Ubiquitin Ligase-dependent Protein Degradation".

My name is Vincenzo D'Angiolella and I work as a Principal Investigator at the "University of Oxford", where I direct my laboratory focused on the study of cell cycle and ubiquitin mediated proteolysis. In the past I studied the role of many Cullin Ring ubiquitin Ligases in cell cycle and cancer biology, thus, I have the necessary expertise for the evaluation of this proposal. I don't have any conflict of interest deriving from studying complementary themes.

Nikol Dibus reports two important discoveries on the role of Fbxo38. Fbxo38 was studied before and connected to the regulation of PD-1 levels in human cancers. However, Nikol Dibus presents very strong evidence that Fbxo38 is an exclusively nuclear protein with a different function. By conducting proteomic analyses of the interacting partners of Fbxo38, she identifies ZXDA, ZXDB and ZXDC as interactors of Fbxo38. Further studies confirm that ZXDA and ZXDB are substrates of Fbxo38. While the function of ZXDA/B/C factor has been poorly studied, Nikol Doibus identifies a new function for these factors in regulating the organisation of DNA at centromeric regions in chromatin. The Fbxo38- ZXDA/B axis balances the organisation of centromeric chromatin. Nikol Dibus studies did not stop at the functional characterization of Fbxo38 from a biochemical point of view, but she also investigated the genetic phenotypes associated to Fbxo38 depletion in mouse models. The mice develop at lower mendelian ratio than expected and are overall smaller than the Wild-type counterpart. Given the pattern of expression of Fbxo38 in Sertoli cells and the striking phenotype, the author focuses on investigation the defects in spermatogenesis. The phenotype is reconducted to defective ribosomal RNA biogenesis and corresponds to problems in maturation of sperm progenitors.

The introduction covered well the themes being discussed in the results section. There was a notable attention to details and the capacity to explore the literature in a comprehensive and critical manner. Nikol Dibus discussed evolutionary aspects of the proteins she is studying, which is critical, but I have seen rarely in a thesis. The results report experiments which are well controlled overall. There is a logical progression from one experiment to the next. Figures are well presented with attention to experimental details. Indeed, the results are reported in two manuscripts which have been published in international journals. In the discussion I could see many ideas being presented, showing critical thinking. Hypotheses on the mouse phenotype are well presented to establish connections with the role of Fbxo38 in regulating centromeric chromatin. The scientific English used is great and results are presented in a crystal-clear manner.

Overall, this is a truly comprehensive work which shows that the student was able to master both basic biochemical methods and genetic methods in mouse models. The techniques presented cover a wide range of methods which are complex to execute. However, all experiments were well controlled showing maturity in experimental design and ideation. I would like to commend Nikol for the quality and quantity of work she could complete in such a short period of time. I could gather a curiosity and passion from the work which is an important trait in scientists, and I am confident Nikol Dibus will continue to do important discoveries.

Thus, without hesitation I confirm that the student can defend the work.

Sincerely,

I am outlining few questions below to further assess critical thinking and independence

1. Can you speculate on the signals impacting on Fbxo38 activity?
2. Can you clarify what are the cancer mutations present in ZXDA//B/C and how they can impact on Fbxo38 binding and, separately on ZXDA, B, C function?
3. Is the reduced weight of the Fbxo38 K/O mouse associated to muscle disease?
4. You mention in the thesis stronger interaction between Fbxo38 and ZXDA/B. Do you know of more quantitative methods to measure interaction strength?