

# FBXO47 Is Involved in Centromere Pairing for Pachynema Progression and Spindle Formation at Prometaphase in Mouse Spermatocytes

Ani Ma<sup>1,3,\*</sup>, Yali Yang<sup>1</sup>, Lianbao Cao<sup>2</sup>, Lijun Chen<sup>3</sup>, Jian V. Zhang<sup>1</sup>

<sup>1</sup>Center for Energy Metabolism and Reproduction, Shenzhen Institute of Advanced Technology, Chinese Academy of Sciences, Shenzhen, China

<sup>2</sup>Department of Gynecological Oncology, Shandong Cancer Hospital and Institute, Shandong First Medical University and Shandong Academy of Medical Sciences, Jinan, China

<sup>3</sup>Shenzhen Key Laboratory of Fertility Regulation, Center of Assisted Reproduction and Embryology, The University of Hong Kong-Shenzhen Hospital, Shenzhen, China

## Email address:

maani0431@126.com (Ani Ma), jian.zhang@siat.ac.cn (Jian V. Zhang)

\*Corresponding author

## Abstract

The study explores the role of the Skp1-Cullin1-F-box protein (SCF) E3 ubiquitin ligase complex, with a particular focus on F-box protein 47 (FBXO47). In mice, we observed that spermatocytes deficient in centromere-expressed FBXO47 face significant challenges in double-strand break (DSB) repair, leading to a meiotic arrest at a pachytene-like stage. This arrest is accompanied by unstable centromere pairing, which results in the disruption of the synaptonemal complex (SC) structure and a breakdown in the telomere-nuclear envelope (NE) attachment system during pachytene. Immunoblotting analysis revealed that the absence of FBXO47 impairs the expression of centromere protein C (CENP-C) and SC components starting at the pachytene-like stage. Additionally, FBXO47 deletion leads to the depletion of its partner, SKP1, at centromeres and chromosomes in *Fbxo47*<sup>-/-</sup> spermatocytes. Notably, the phenotypic patterns observed in FBXO47-deficient mice—such as the recruitment of HORMAD1 to chromosomes and the reduced levels of CENP-C—parallel those seen in SKP1-deficient mice. Co-immunoprecipitation (Co-IP) analysis further supports that FBXO47 interacts with SKP1 and plays a critical role in stabilizing SKP1 by reducing its ubiquitination in HEK293 cells. These findings suggest that the SCF complex, formed at centromeres, is crucial for stabilizing centromere pairing, potentially through regulation of HORMAD1 during meiosis. Moreover, FBXO47 was found to localize between the centromeres and spindle microtubules during prometaphase in mouse spermatocytes. Knocking down FBXO47 with shRNA led to distorted spindle structures and defects in proper chromosome-microtubule attachment. These results highlight the pivotal role of FBXO47 in coordinating and regulating spindle formation during meiosis. In conclusion, our study underscores the essential function of FBXO47 within the SCF complex, specifically in stabilizing centromere pairing and regulating meiotic spindle formation, thereby contributing to the overall integrity of meiotic progression.

## Keywords

FBXO47, Meiosis, Synapsis, Centromere Pairing, Spindle, Microtubule