Carus Promotionskolleg: Ausschreibung 2026

Projektskizze

x Neues Promotionsprojekt (12 Monate Förderdauer)

☐ Bereits begonnenes Promotionsprojekt (6 Monate Förderdauer)

Verantwortliche/r Betreuer/in*: Prof. Dr. Felix Müller-Planitz

Anleitung täglicher Arbeit: Dr. Karina Kettner

Vorschlag für 2. und 3. Betreuer/in*: Dr. Mark Boltengagen

Projekttitel

Studying the mechanistic basis of pathogenic variants of the human nucleosome remodelling enzyme INO80

Hypothese/Fragestellung (bitte präzise formulieren, Ausschlusskriterium!)

Deletion of the mammalian chromatin remodeler INO80 is associated with embryonic lethality (Min *et al*., 2013), while amplifications and mutations of INO80 have been reported in various diseases including tumors (Zhang *et al*., 2017). The mechanistic basis and pathology of these INO80 variants has remained unclear. Here, we propose to study the mechanistic basis of human INO80 variants in the genetically tractable organism *Saccharomyces cerevisiae* to learn how they might contribute to human diseases. We predict that mutations mapping to the conserved ATPase domain tend to cause loss-of-

* Hochschullehrer:in oder habilitationsäquivalent qualifizierte:r Wissenschaftler:in der TU Dresden. Betreuer:innen dürfen nicht der gleichen Einrichtung angehören

function phenotypes whereas mutations mapping to regulatory regions would alter INO80's preference where it positions the nucleosomes in the genome.

Stand der Forschung/Vorarbeiten (ca. ½ DIN A4 Seite)

The evolutionarily conserved, ATP-dependent chromatin remodeler complex INO80, originally discovered in yeast, has been shown to play a role in various cellular functions, such as transcriptional regulation, DNA replication, and DNA repair. Mutations in the human INO80 complex have been reported in various cancers, including diffuse large B-cell lymphoma (DLBCL), hepatosplenic T-cell lymphoma (HSTL), and some solid tumors (Dominguez-Sola *et al*., 2025). However, the role of INO80 in cancer development is not yet understood.

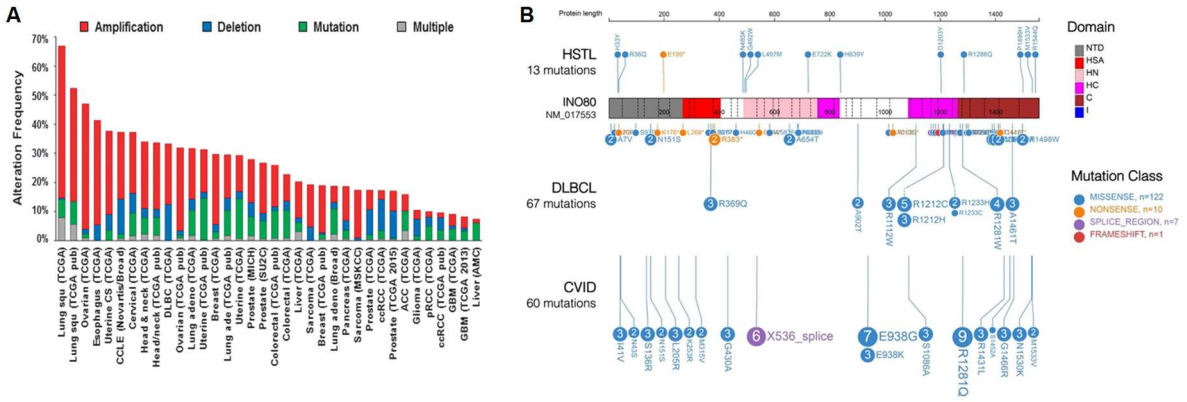


Figure 1: Amplifications and mutations of *INO80* are hallmarks in various diseases.
(A) Alteration frequency of Ino80 in various cancer types (Zhang *et al*., 2017). (B) *INO80* mutations in Hepatosphenic T-Cell Lymphoma (HSTL), Diffuse Large B-Cell Lymphoma (DLBCL) and, Common Variable Immunodeficiency (CVID) (Dominguez-Sola *et al*., 2025).

Studying the function of INO80 *in vivo* has only recently become possible with the advent of a genetic system that eradicates functional redundancies with related chromatin remodelling enzymes. In the genetically tractable organism *S. cerervisisae*, we created a **t**riple **k**nock**o**ut yeast strain TKO (*Δisw1, Δisw2, Δchd1*) in which the redundant factors are deleted so that the function of Ino80 can be isolated. Depletion of Ino80 in this strain (denoted TKOaa_INO80^{-}) results in a severe growth defect (Fig. 2A,B) and hypersensitivity towards several stressors such as elevated temperatures (Fig. 2C,D).

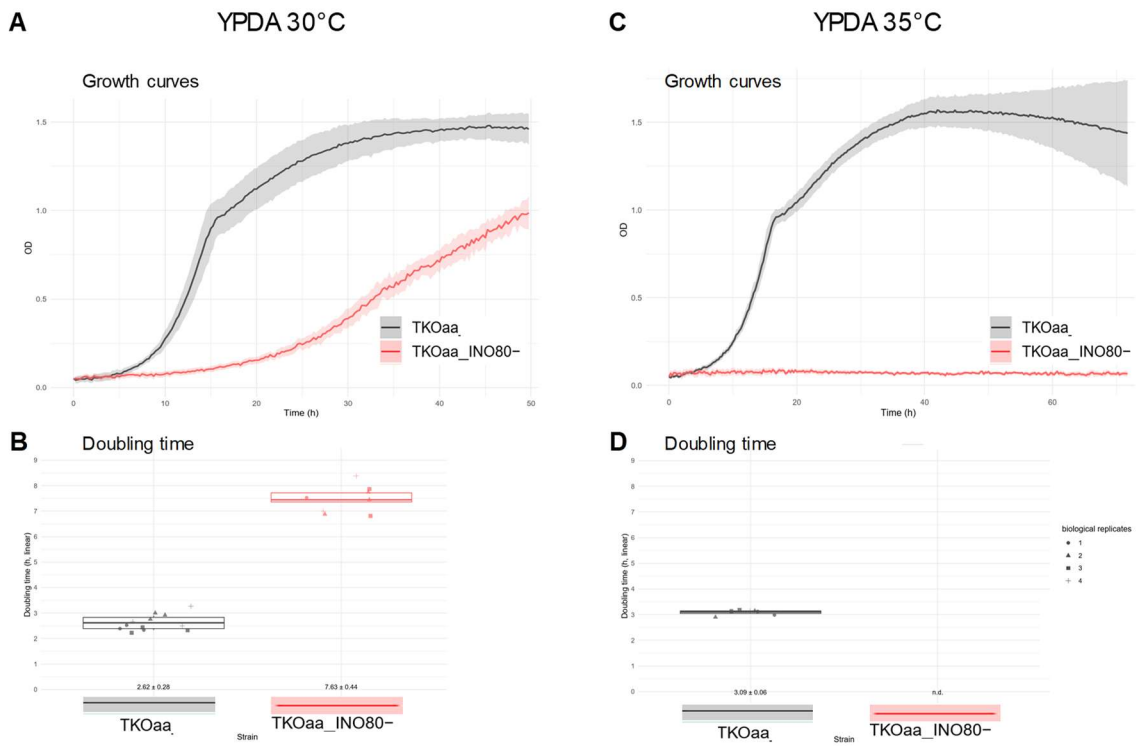
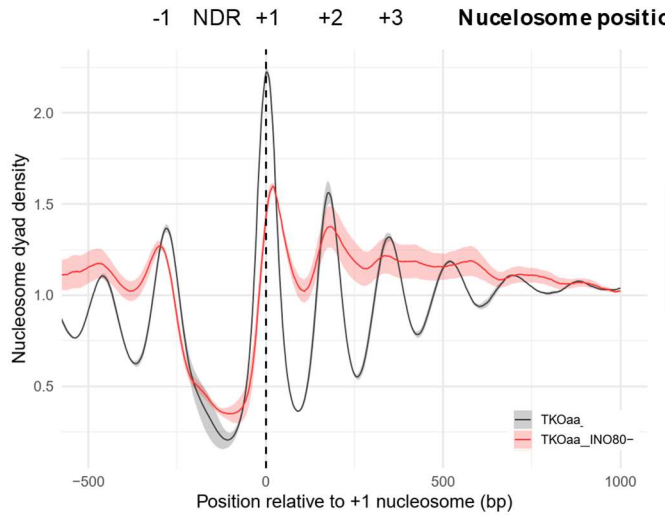


Figure 2: Ino80 depleted yeast strains show a severe growth defect.
The TKO triple knockout strain (Δisw1, Δisw2, Δchd1) and the Ino80 depleted strain TKOaa-INO80^{-} were grown on full medium (YPDA) at 30°C (A, B) and under temperature stress condition (35°C; C, D). Doubling times (B, D) were calculated using the exponential growth phases of the respective strains.

The underlying effects of remodeler defects on chromatin architecture, analyzed by MNase-Sequencing (Singh *et al.,* 2021), revealed strong changes in nucleosomal landscape (Fig. 3). For instance, the absence of Ino80 caused a shift of the +1 and the -1 nucleosome, increased the nucleosome depleted region over gene promoters and produced irregular nucleosome arrays over gene bodies.
These results demonstrate that the combination of growth analysis and MNase-Seq can robustly distinguish between different remodeling states and is therefore ideally suited to mapping functional consequences of INO80 mutations.
All key methods for the proposed project including CRISPR-based targeted mutagenesis, MNase-Sequencing, and tetrad analysis have been successfully established in our lab (Singh *et al.,* 2021; Singh & Mueller-Planitz, 2026). The relevant strains have been generated and functionally characterized. Therefore, the planned analyses are realistically feasible within the funding period.



	-1	+1	+2	+3
TKOaa	-280	3	178	348
TKOaa_INO80-	-292	24	186	344

Figure 3: Ino80 depleted TKO cells show a decreased nucleosome array regularity.
MNase-Seq analysis of nucleosome organization in TKO cells prior to (black) and upon Ino80 depletion (2 h, red). Nucleosome positions are given in the table.

☐ Tierversuchsantrag wird benötigt

☐ Tierversuchsantrag ist bereits bewilligt (AZ_____)

x Tierversuchsantrag ist nicht notwendig

☐ Ethikvotum ist bereits beantragt

☐ Ethikvotum liegt vor (AZ_____)

x Ethikvotum ist nicht notwendig

x Gentechnikanmeldung liegt vor (AZ_ 45-8451/91/6 __)

☐ Gentechnikanmeldung ist nicht notwendig

x Ich versichere, dass auch alle weiteren Voraussetzungen erfüllt sind, um das Projekt am 1.10.2026 zu starten

Fehlende oder unvollständige Angaben sind ein Ausschlusskriterium!

Arbeits- und Zeitplan einschließlich des Eigenanteils des/der Kollegiaten/Kollegiatin (ca. 1 DIN A4 Seite)

The project is designed to run for twelve months and follows a clearly structured process that includes the establishment of the model system and the functional analysis of the selected human Ino80 mutations. The work packages build logically upon one another and enable the doctoral candidate to make an independent, clearly defined scientific contribution.

Work package (WP) 1: Construction of humanized yeasts

The human INO80 gene (*Hs*INO80) is amplified and introduced into a diploid TKO strain. Subsequently, it is tested whether the resulting protein, HsIno80, can complement the phenotype induced by loss of *S. cerevisiae ScINO80*. This is done using tetrad analysis and growth analyses under standard and stress conditions. In parallel, we will humanize key conserved domains of INO80 by replacing the fungal domains with the human counterpart (Paret *et al*., 2000).

WP 2: Introduction of disease-associated mutations of *HsINO80*

The establishment of humanized yeast strains in WP 1 serves as the perfect system for analyzing *Hs*Ino80 variants. To do so, we will introduce selected, disease-associated *HsINO80* variants into humanized yeast established in WP 1 using CRISPR-based mutagenesis. Successful mutagenesis and absence of secondary mutations will be validated by sequencing.

Should none of the humanized strains in WP 1 complement the phenotype sufficiently, we will simply directly introduce mutations found in human INO80 into the homologous positions of yeast INO80.

Every strain will be subjected to a basic functional characterization, encompassing growth behaviour, doubling time analyses, and sensitivity to replication stress (Hydroxyurea) and DNA damage stress (Zeocin). This early phenotyping serves to prioritize the mutants for in-depth molecular analyses.

WP 3: Detailed functional analysis of the mutants using MNase-Seq

MNase-Seq captures the effects of the INO80 mutations on nucleosome positioning. The data will be integrated and the mutations classified into functional categories. My lab has considerable experience in studying INO80 function by MNase-Seq (Singh *et al*., 2021; Singh & Mueller-Planitz, 2026), including the necessary bioinformatic analysis, which will be assisted by Dr. Mark Boltengagen, a postdoc in my lab.

Angaben zur Erfahrung in der Betreuung von Promotionen (entfällt bei berufenen Professoren):

Angaben zur Perspektive des Kollegiaten / der Kollegiatin die Promotionsarbeit als Erstautor/in zu veröffentlichen:

The data obtained will be prepared for a first-author publication in reputable scientific journals.

x Wöchentliche Projektbesprechungen mit dem Kollegiaten / der Kollegiatin werden durchgeführt **von: Dr. Karina Kettner und Prof. Dr. Felix Müller-Planitz**

☐ **Für die Kollegiaten/innen kann ich folgenden Kurs / folgendes Seminar anbieten:**

Literatur

Zhang S, Zhou B, Wang L, Li P, Bennett BD, Snyder R, Garantziotis S, Fargo DC, Cox AD, Chen L, Hu G. INO80 is required for oncogenic transcription and tumor growth in non-small cell lung cancer. Oncogene. 2017; 36: 1430-1439. doi: 10.1038/onc.2016.311.

Min JN, Tian Y, Xiao Y, Wu L, Li L, Chang S. 2013 The mINO80 chromatin remodeling complex is required for efficient telomere replication and maintenance of genome stability. Cell Res. 23, 1396– 1413. doi: 10.1038/cr.2013.113.

Dominguez-Sola *et al*. 2025, https://reports.mountsinai.org/article/tisch2025-04-ino80- mutations-and-lymphoma

Singh, A. K., Schauer, T., Pfaller, L., Straub, T., Mueller-Planitz, F. (2021). The biogenesis and function of nucleosome arrays. Nat. Commun., 12: 7011-7026 doi: 10.1038/s41467-021-27285-6.

Singh, A. K., Mueller-Planitz F. (2026) The Ies6 subunit is essential for INO80-mediated nucleosome organization. Sci Rep.; 16: 7466. doi: 10.1038/s41598-026-40504-8.

Paret C, Lode A, Krause-Buchholz U, Rödel G. The P(174)L mutation in the human hSCO1 gene affects the assembly of cytochrome c oxidase. Biochem Biophys Res Commun. 2000; 279(2): 341-7. doi: 10.1006/bbrc.2000.3949.

Unterschrift