# Sirtuin 6 is critical for maintaining intervertebral disc homeostasis during spine aging

Pranay Ramteke[1], Victoria Tran[1], Bahiyah Watson[1], Mallory Toci[1], Shira Johnston[1], Maria Tsingas[1], John Collins[1], Richard Loeser[2], Makarand Risbud[1]

1. Thomas Jefferson University, Philadelphia, Pennsylvania, USA.
2. University of North Carolina, Chapel Hill, North Carolina, USA.

**Introduction**: Intervertebral disc degeneration (IVDD) is one of the main contributors for low back pain viz. the leading cause of disability worldwide[1]. Although environmental and genetic factors are the known etiological causes for disc degeneration, age is still the most significant risk factor. Recent findings report that senescence plays a major role in ageing as well as disc degeneration in human and mouse models[2]. Interestingly, Sirtuin 6 (SIRT6) has been shown to decelerate ageing across different species as well as have an anti-senescence effect[3]. However, its role in intervertebral disc degeneration is largely unknown. Therefore, the aim of this study is to explore the role of SIRT6 in intervertebral disc degeneration.

**Methods**: We studied the spinal phenotype of mice exhibiting conditional deletion of SIRT6 (cKO) in Acan-expressing cells ($Acan^{CreERT2}SIRT6^{fl/fl}$) at healthy and aged time points i.e., 12, 18 and 24 months. uCT analysis was performed to check effects of SIRT6 KO on physiological parameters of the disc and vertebral bones. SaF-O staining was performed on intervertebral discs sections and evaluated by at least six blind graders to generate Modified Thompson grading scores to characterize and measure degenerative phenotype. Picrosirius red staining was performed to check changes in collagen type. FTIR analysis was performed to determine aggrecan, collagen and proteoglycan content. Immunohistochemistry analysis and Lipofuscin staining was performed to measure senescence in the disc.

**Results**: uCT analyses of SIRT6 cKO mice showed significant changes in disc height (DH), vertebral height (VH) and disc height index (DHI) as compared to WT control mice. SaF-O staining and Modified Thompson grading of SaFO stained discs showed that SIRT6 cKo exhibit significantly higher grades of degeneration as compared to their respective age matched controls, especially in the AF (annulus fibrosus). Surprisingly, Picrosirius red staining showed no significant differences in the collagen content in SIRT6 cKO mice. FTIR analysis showed no significant changes in aggrecan, collagen or proteoglycan content of the cKO mice. Interestingly, immunohistochemistry analysis showed an increase in p21, a bonafide marker of senescence in SIRT6 cKO discs as compared to their respective controls. Lipofuscin staining and IHC analysis shows changes in DNA damage and autophagy.

**Discussion**: Higher modified Thompson grading scores for disc degeneration in SIRT6cKO mice suggest its fundamental role in disc homeostasis. Surprisingly, Picrosirius red staining showed no significant differences in the collagen fibrils, although AF tissue was the most affected in the disc compartment. Increased p21 in SIRT6 cKO mice suggests that senescence- the major etiological factor for disc degeneration, is regulated by SIRT6. Therefore, SIRT6 cKO accelerates disc degeneration via senescence.

**Significance**: In summary, our work provides new insights into $SIRT6^{cKO}$ mediated disc degeneration at the pathological, cellular, and molecular levels, thereby defining the significance of epigenetic landscape in this unique tissue. Further exploration of these findings may lead to development of therapeutic targeting of SIRT6 to mitigate disc degeneration.

**References**:

1. Hartvigsen et al., Lancet Low Back Pain Series Working Group. What low back pain is and why we need to pay attention. Lancet. 2018 Jun 9;391(10137):2356-2367. doi: 10.1016/S0140-6736(18)30480-X. Epub 2018 Mar 21. PMID: 29573870.
2. Micco et al., Cellular senescence in ageing: from mechanisms to therapeutic opportunities. Nat Rev Mol Cell Biol. 2021 Feb;22(2):75-95. doi: 10.1038/s41580-020-00314-w. Epub 2020 Dec 16. PMID: 33328614; PMCID: PMC8344376.
3. Roichman et al., Restoration of energy homeostasis by SIRT6 extends healthy lifespan. Nat Commun. 2021 May 28;12(1):3208. doi: 10.1038/s41467-021-23545-7. PMID: 34050173; PMCID: PMC8163764.