

Abstract

Inflammation is a natural and beneficial response to injury and pathogen invasion. However, chronic inflammation is linked to the progression of various neurodegenerative diseases. Although the exact etiology is unknown, Alzheimer's disease is associated with the overactivation of the NF- κ B inflammatory pathway. NF- κ B is a transcription factor that, in an unstimulated cell, is sequestered in the cytoplasm as a complex with its inhibitor, I κ B α . When the pathway is activated by an external signal, I κ B α is phosphorylated and subsequently degraded in the proteasome. Liberated NF- κ B translocates to the nucleus, where it acts as a transcription factor for pro-inflammatory genes, highlighting its potential as a therapeutic target. Our research investigates the exact point of interference of novel anti-inflammatory drugs (provided by P2D Biosciences) with the NF- κ B pathway through Western blotting and immunofluorescence.

The NF- κ B Pathway

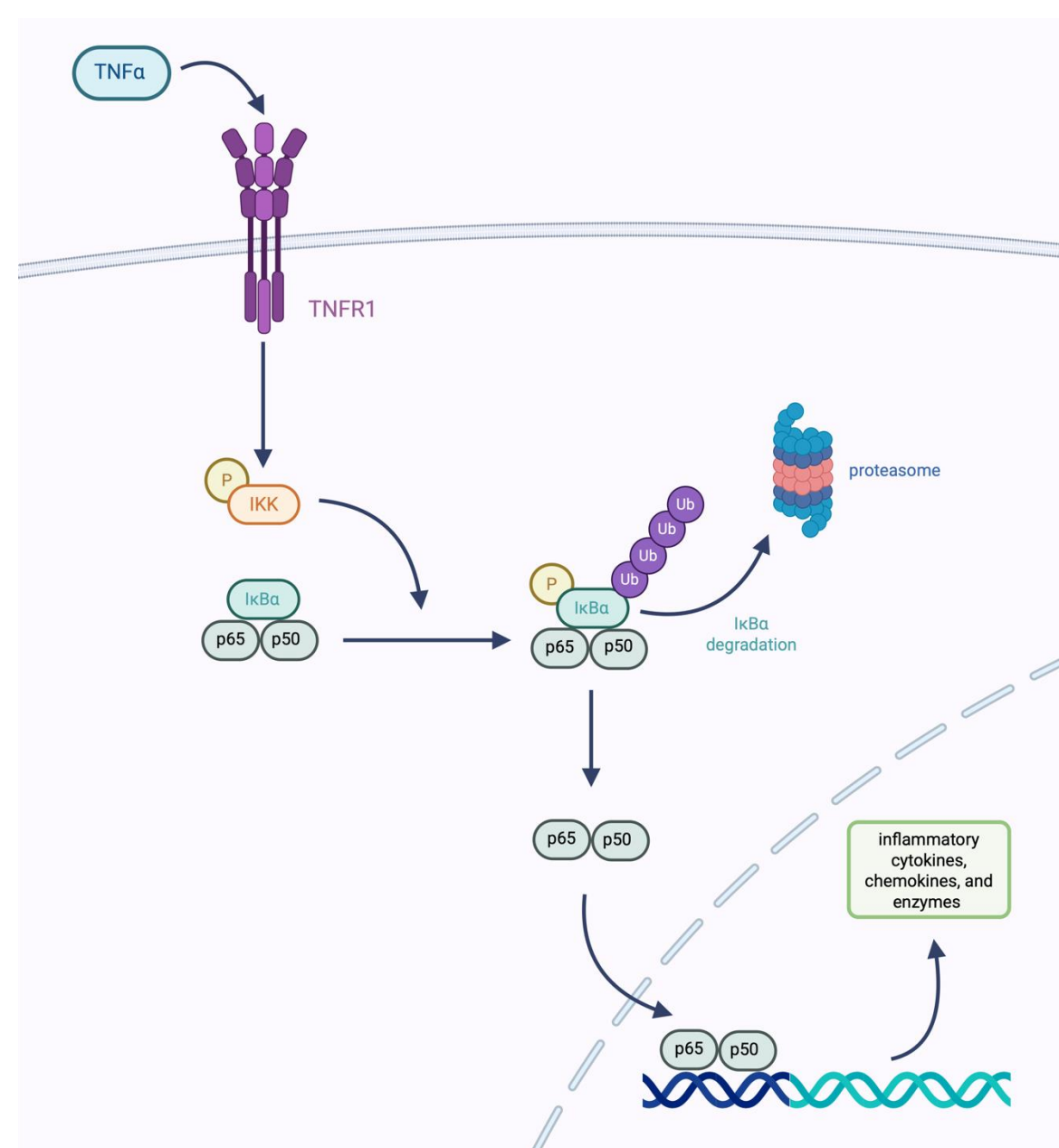


Figure 1. NF- κ B signaling pathway.

Mechanism of action of PD2244 might be through inhibition of NF- κ B translocation to the nucleus

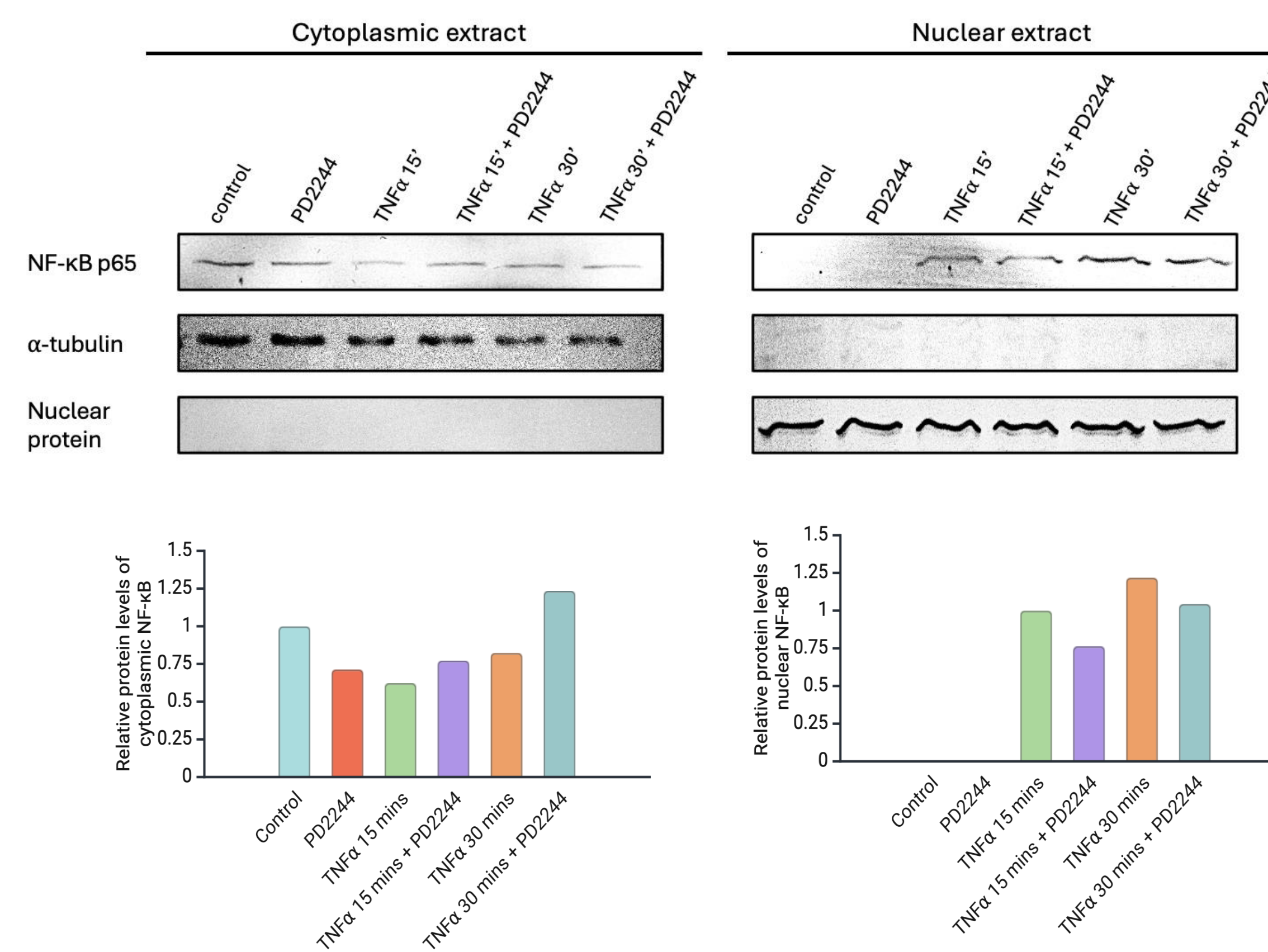


Figure 2. PD2244 inhibited NF- κ B activation in HeLa cells (a) Western blot of cytoplasmic and nuclear protein extract of HeLa cells measuring NF- κ B translocation to the nucleus induced by TNF α . (b) The densitometric analysis of NF- κ B data, normalized to α -tubulin (cytoplasm) or a nuclear protein (nucleus).

Methods

- HeLa and 293HEK cell culture
- Drug treatment followed by TNF α stimulant
- Sample preparation followed by Western blotting
- Immunofluorescence and confocal microscopy

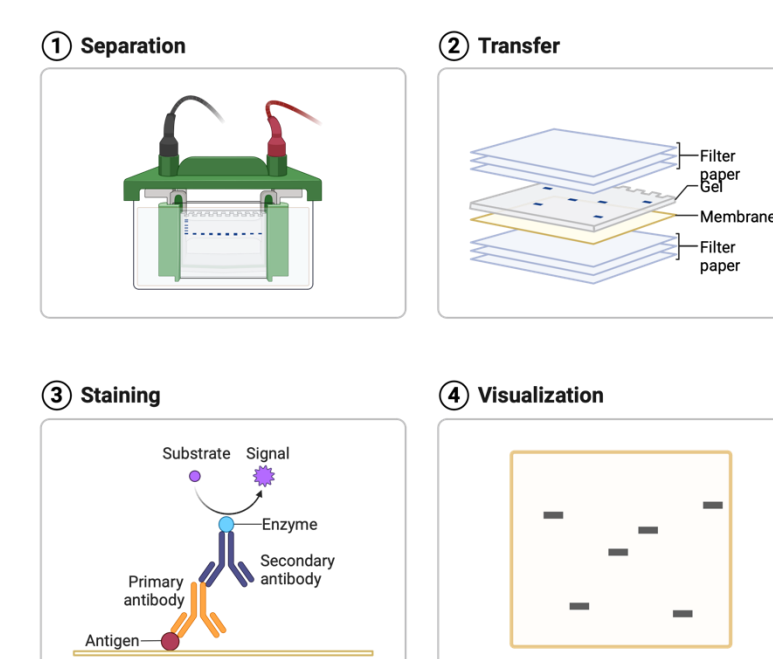


Figure 3. Western blotting protocol

b)

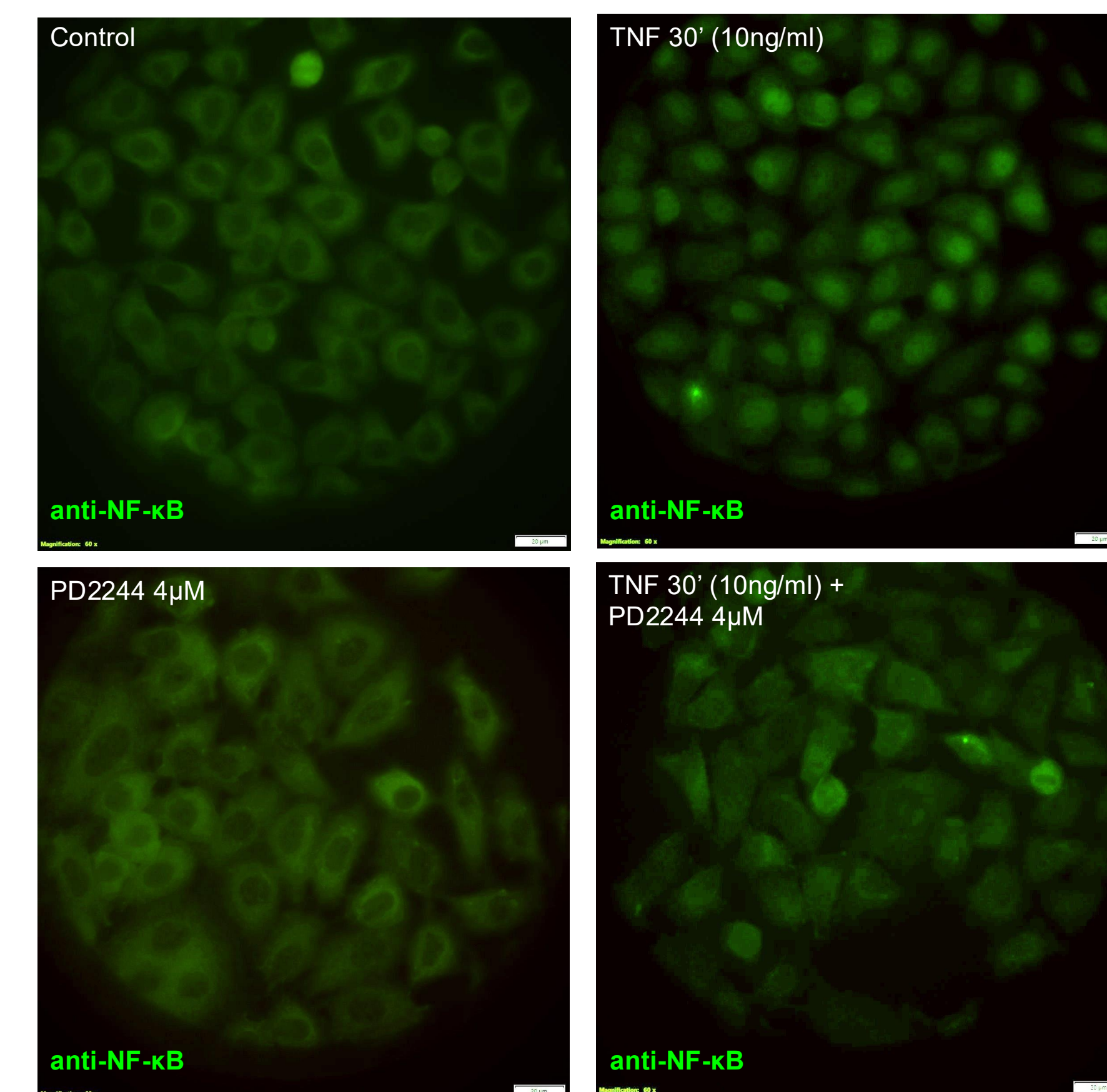


Figure 4. Confocal microscopy after immunofluorescence staining with anti-NF- κ B p65 antibody

Conclusion

- PD2244 inhibits NF- κ B translocation to the nucleus as suggested by Western blotting and immunofluorescence.
- Additional experimental replications are needed to assess statistical significance and reproducibility.

References

Peng, Chao, et al. "The NF- κ B Signaling Pathway, the Microbiota, and Gastrointestinal Tumorigenesis: Recent Advances." *Frontiers in Immunology*, vol. 11, June 2020, <https://doi.org/10.3389/fimmu.2020.01387>.

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