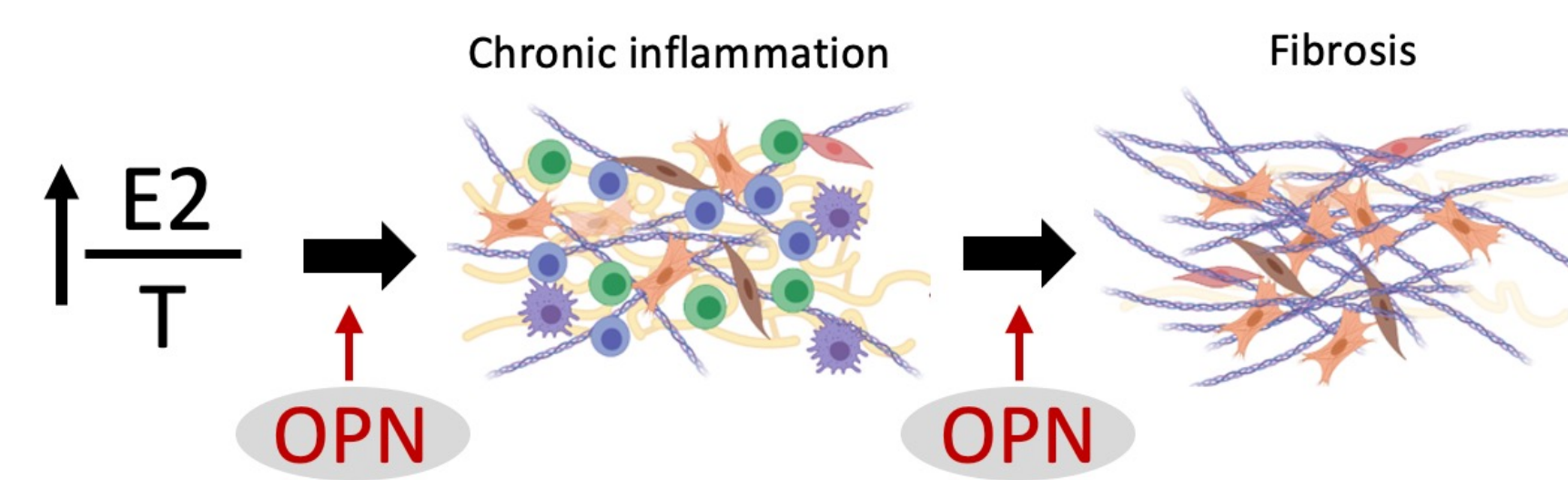


Loss of Osteopontin function attenuates immune cell infiltration and collagen accumulation in a steroid hormone-induced lower urinary tract dysfunction model

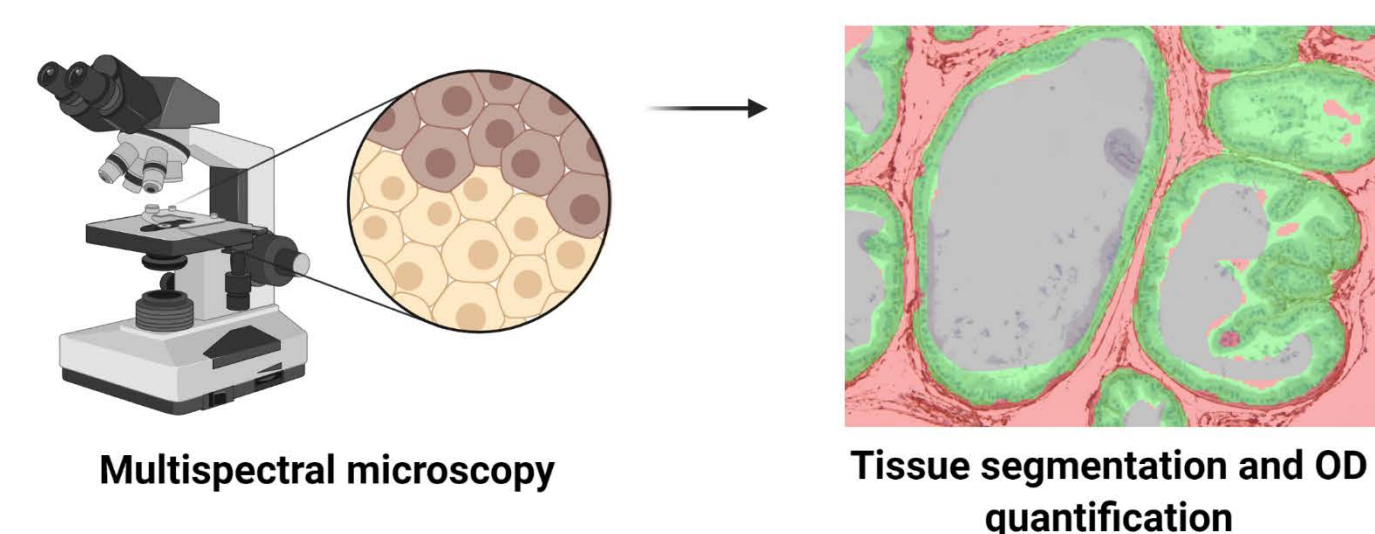
Background

- Lower urinary tract symptoms (LUTS) deteriorate the quality of life of a significant proportion of the male population, particularly those >50 years of age.
- Current therapeutics target smooth muscle dysfunction and prostatic enlargement, but resistance may develop. Prostatic inflammation has also been linked to LUTS, but its molecular process is not well understood.
- The age-related increase in estradiol:testosterone ratio leading to hormonal imbalance is likely a key factor in prostatic disease by triggering inflammation in the prostate, but this has not been shown previously.¹
- Osteopontin (OPN) is a pro-inflammatory cytokine which we found to be associated with LUTS and demonstrated its role in aggravating prostatic inflammation and fibrosis in mice.^{2,3}
- We hypothesize that osteopontin facilitates the infiltration of immune cells and the development of fibrosis in the prostate triggered by steroid hormone imbalance. We believe these processes contribute to prostatic disease and the development of LUTS.



Materials and Methods

- Male C57BL/6J (WT) and *Spp1^{tm1Blh}/J* (OPN-KO) mice were surgically implanted with subcutaneous T+E2 pellets composed of 25 mg testosterone (T) and 2.5 mg estradiol (E2). Tissues were collected 2, 6, or 12 weeks later.
- Immunohistochemistry (IHC) was performed on ventral prostate (VP) and dorsal prostate (DP) tissues. Type 1 collagen was identified with a Col1a1 antibody (1:500). Immune cells counts were identified with common immune cell marker CD45 (1:2000).
- IHC staining was imaged with Mantra multispectral imaging platform and analyzed with inForm software.



- Multiplex fluorescent hybridization chain reaction (HCR) was performed to target genes for macrophages/monocytes (*Cd68*), B-cells (*Cd19*), and T-cells (*Cd3e*).

Results

Loss of OPN function results in a hindered inflammatory response induced by steroid hormone imbalance

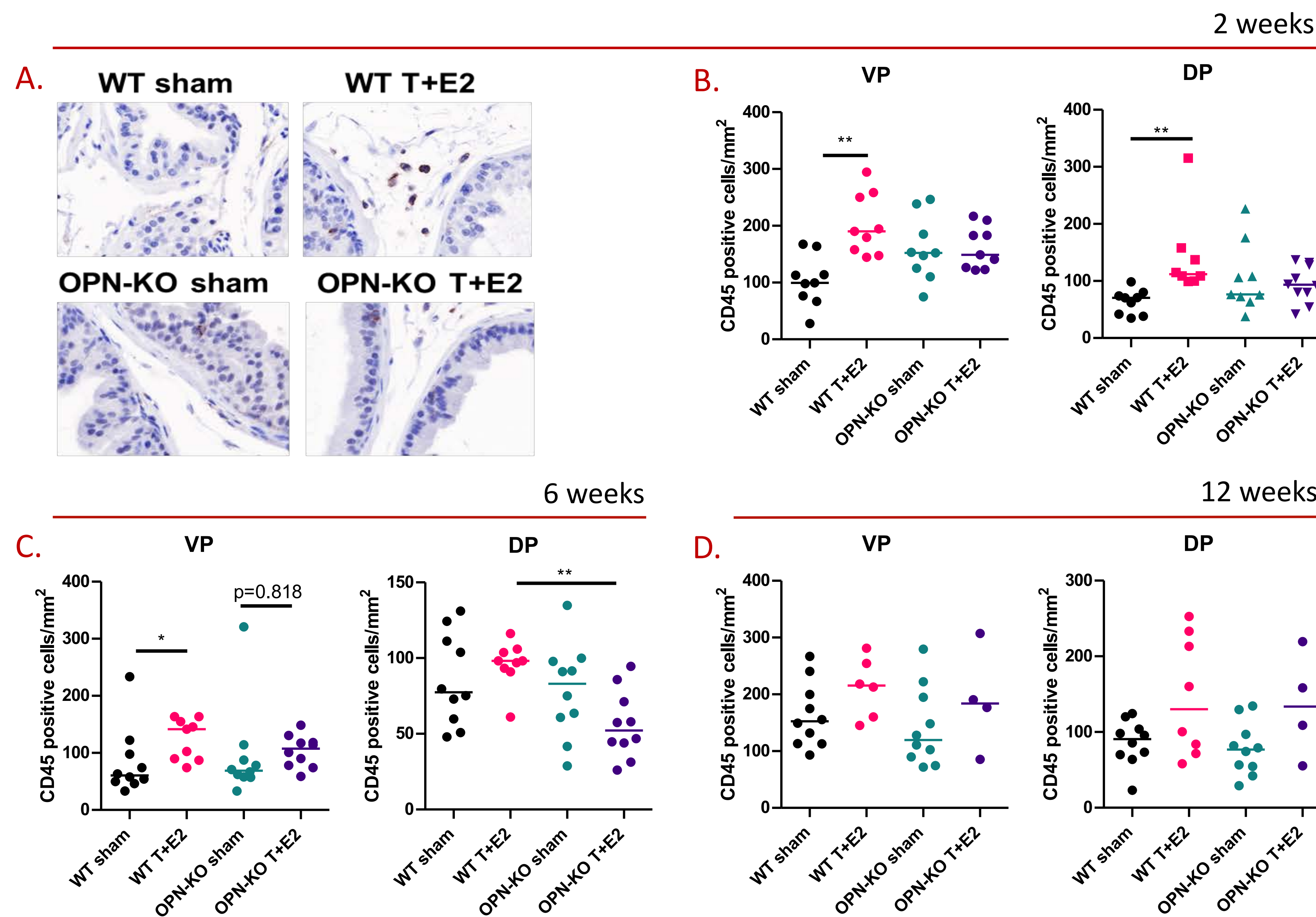


Figure 1. Immune cell number significantly increases in response to early (2 week) T+E2 treatment in WT but not in OPN-KO mice. Panel A: Representative images of VP tissue from mice after 2 weeks of T+E2 treatment. **Panels B, C, and D:** CD45-positive cell counts from the VP and DP after 2, 6, or 12 weeks of T+E2 treatment, respectively. Immunohistochemistry was performed on VP and DP tissues with CD45 to identify immune cells. Images of VP and DP tissue (6/tissue) were taken at 40x magnification. CD45-positive cells were counted manually and was expressed as CD45 cells/mm². Tissue from prostates with incidental high-grade inflammation (>400 cells/mm²) were excluded from the dataset for analysis. Significance was determined with Kruskal-Wallis test. *: p<0.05 **: p<0.01

Loss of OPN function results in lower prostatic levels of collagen I in chronic steroid hormone imbalance

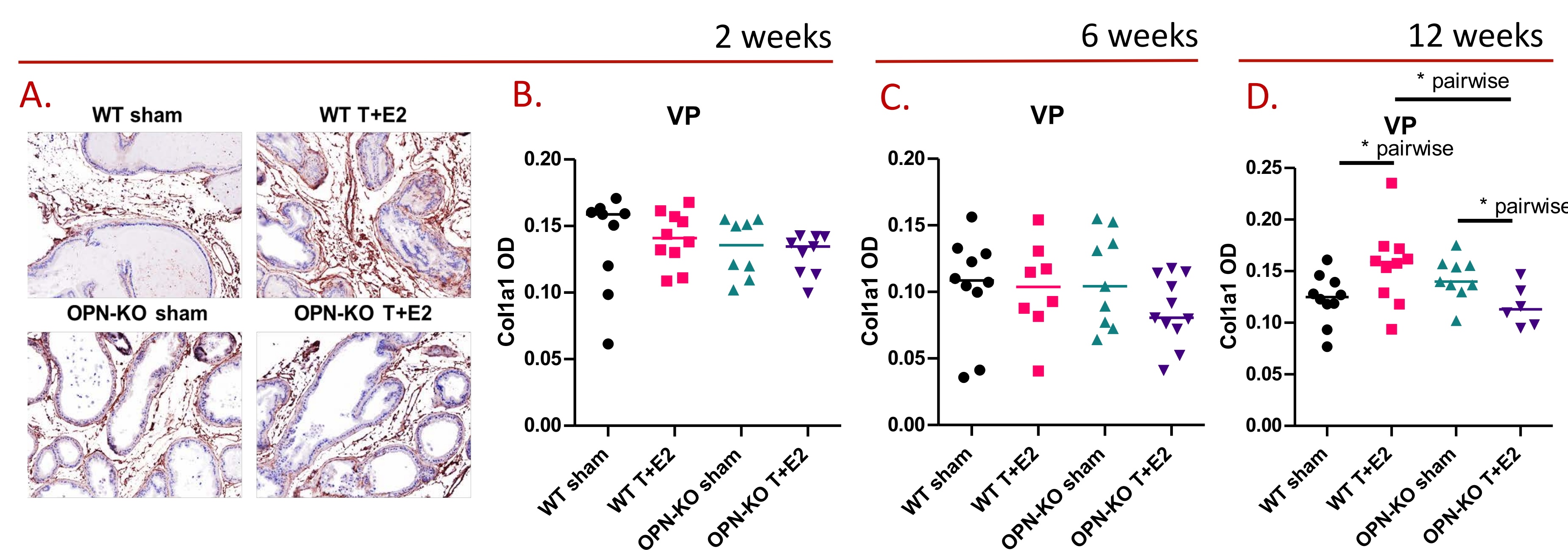


Figure 2. Collagen density significantly increases in response to long-term (12 week) T+E2 treatment in WT but not in OPN-KO mice. Panel A: Representative images of VP tissue from mice after 2 weeks of T+E2 treatment. **Panels B, C, and D:** Data from Col1a1 optical density (OD) quantification in the stroma of VP tissue after 2, 6, or 12 weeks of T+E2 treatment, respectively. Immunohistochemistry was performed on VP and DP tissues with Col1a1 antibody to identify type 1 collagen. Images of VP and DP tissue (3/tissue) were taken at 20x magnification. Col1a1 OD was calculated via tissue segmentation to differentiate stroma from epithelium. Significance was tested via pair-wise comparison with Mann-Whitney test. *: p<0.05

Results (cont.)

Loss of OPN function prevents macrophage/monocyte increase in steroid hormone imbalance

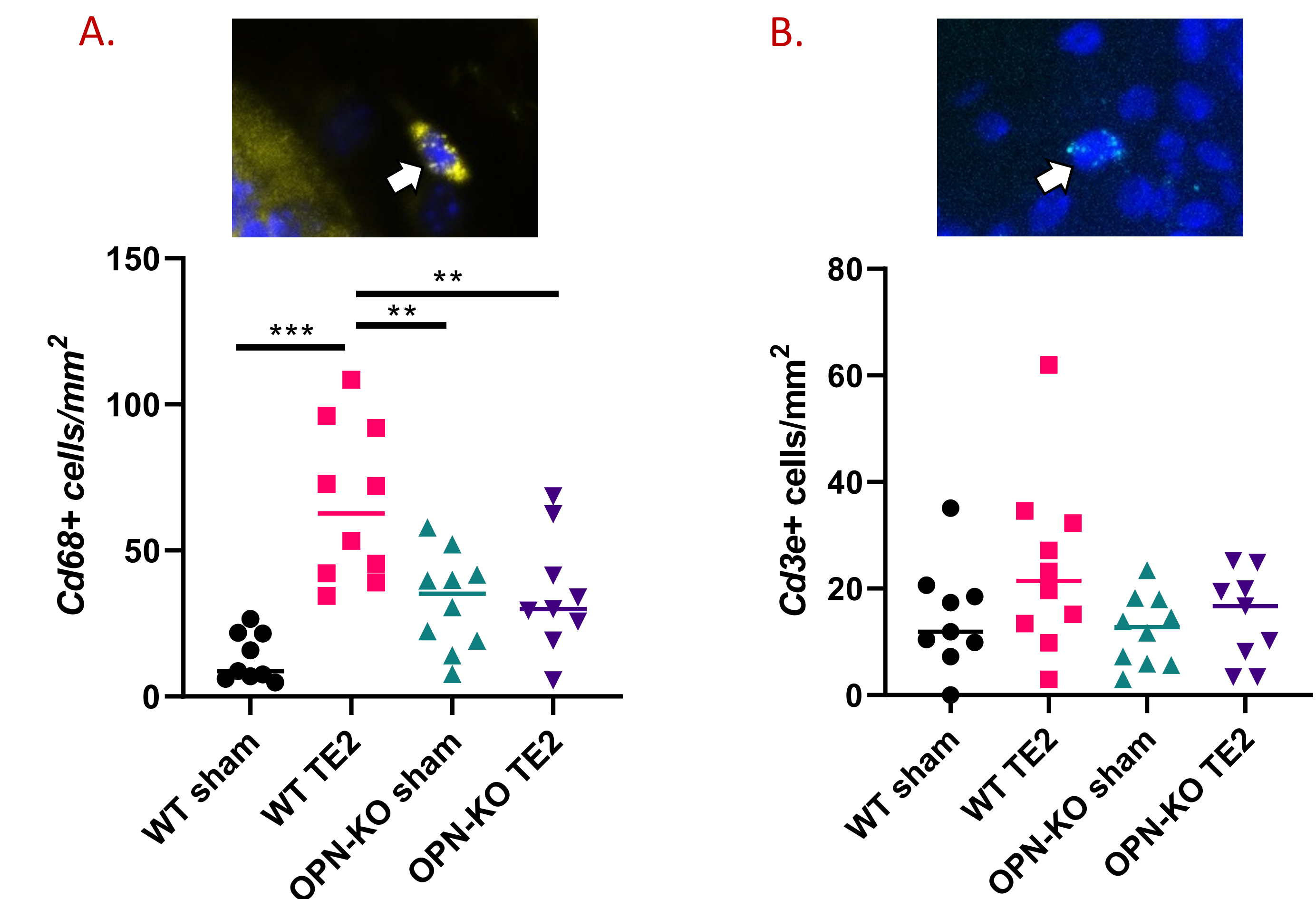


Figure 3. Macrophage/monocyte presence increases in response to early (2 week) T+E2 treatment in WT but not in OPN-KO mice. Panel A: Representative image of a macrophage/monocyte and *Cd68*-positive cell counts after 2 weeks of T+E2 treatment. **Panel B:** Representative image of a T-cell and *Cd3e*-positive cell counts after 2 weeks of T+E2 treatment. Multiplex fluorescent HCR was performed on VP tissue targeting *Cd68* and *Cd3e* to identify macrophages/monocytes and T-cells, respectively. Images of VP tissues (6/tissue) were taken at 40x magnification. Positive cells were counted manually and expressed as cells/mm². Significance was determined with one-way ANOVA. **: p<0.01 ***: p<0.001

Discussion

- Loss of osteopontin function in our steroid hormone imbalance model results in:
 - a hindered inflammatory response
 - lower levels of type 1 collagen, indicative of less fibrotic development
 - decreased presence of macrophages/monocytes
 - unaffected presence of T-cells
 Note: B-cells were not detected in the prostate in our model
- OPN plays a role in aggravating inflammation and fibrosis associated with steroid hormone imbalance.
- Future research: Extend HCR protocol to further understand presence of immune cell subsets.

Acknowledgements

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