

# Utilization of the ExVive Human Kidney Tissue Model of the Proximal Tubule to Assess Nephrotoxicity Across a Diverse Set of Chemical Classes

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## Abstract

Due to its exposure to high concentrations of xenobiotics, the kidney proximal tubule (PT) is the primary site of nephrotoxicity which results in late-stage attrition during drug development. We have developed a 3D bioprinted, fully human *in vitro* model of the proximal tubulointerstitial interface to enable more accurate prediction of tissue-level clinical outcomes<sup>1</sup>.

We challenged this model with a diverse set of known renal toxicants and assessed their impact using biochemical and histologic endpoints. Treatment of 3D PT tissues with the chemotherapeutic agent, Cisplatin, induced dose- and time-dependent loss of tissue viability and epithelial function over the 14 day time-course evaluated. Temporal evaluation of the soluble biomarkers N-acetyl-β-D-glucosaminidase (NAG) and lactate dehydrogenase (LDH) demonstrated maximum NAG release earlier than maximum LDH release. Histologic assessment of the tissues showed primarily epithelial cell-specific effects at the 2.5 μM and 5 μM doses, while additional toxicity in the interstitium was observed at the 10 μM dose. Co-treatment of the tissues with 5μM Cisplatin and the OCT2 inhibitor Cimetidine (1mM) attenuated the Cisplatin-induced toxicity.

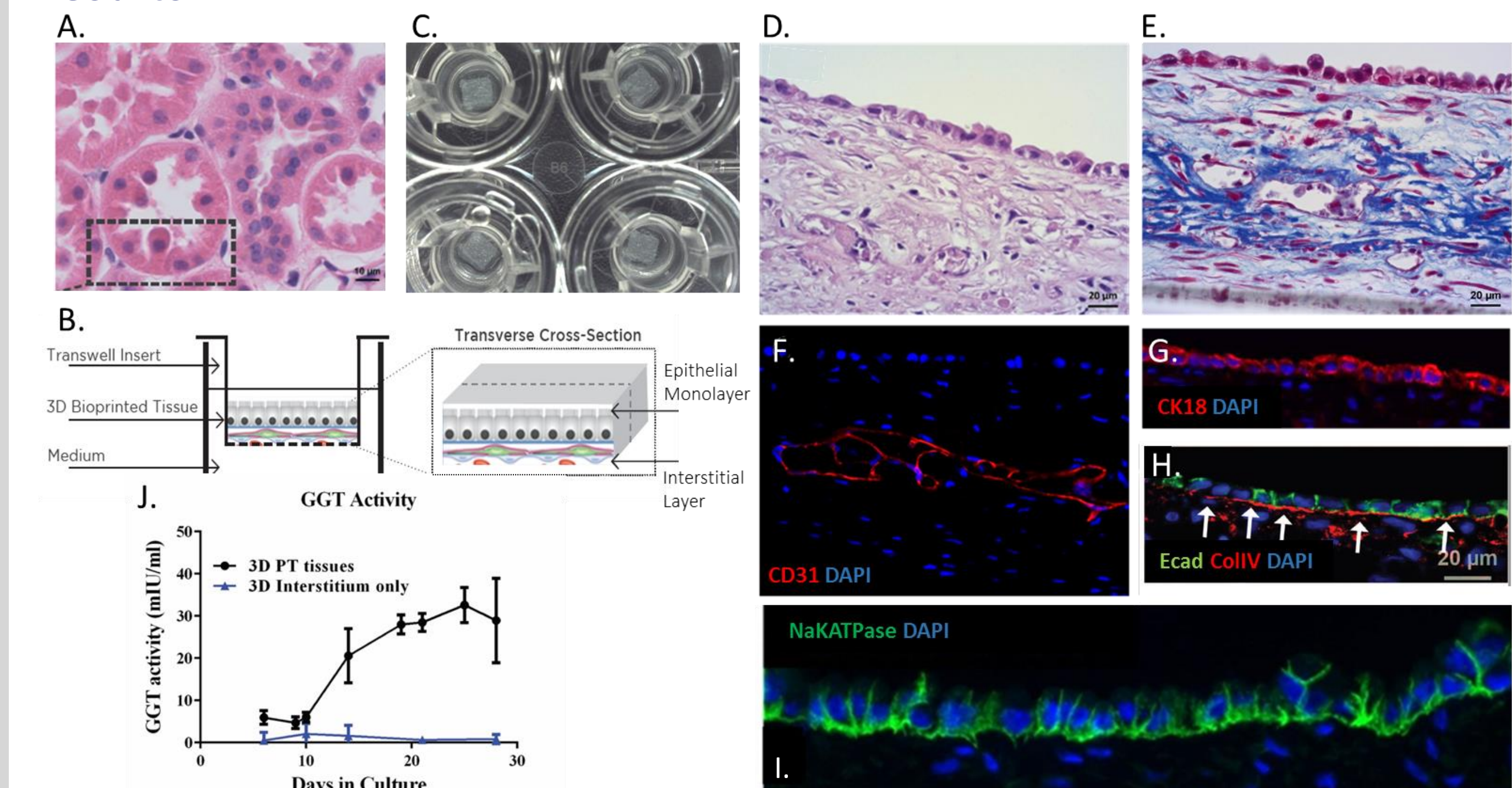
To assess another class of nephrotoxicants, 3D PT tissues were treated with the aminoglycoside antibiotics, Amphotericin B and Gentamicin. Amphotericin B induced a dose-dependent decrease in epithelial function without a corresponding decrease in overall tissue metabolic activity, while Gentamicin treatment resulted in decreases in both epithelial function and tissue metabolic activity. Examination of the soluble biomarkers NAG and LDH showed a rapid response of the tissues to even low concentrations of Amphotericin B early in the treatment period. Consistent with the biochemical findings, histologic assessment of the Amphotericin B-treated tissues demonstrated a flattening of the epithelial cells with no observable impact on the other cells within the tissue.

Collectively, these results suggest that bioprinted kidney tissues are well-suited to assess multiple mechanisms of nephrotoxicity including fibrosis *in vitro*, following biologically-relevant dosing regimens, and that biochemical, transcriptional and histologic endpoints provide a comprehensive means of examining the progression of tissue injury on a mechanistic basis.

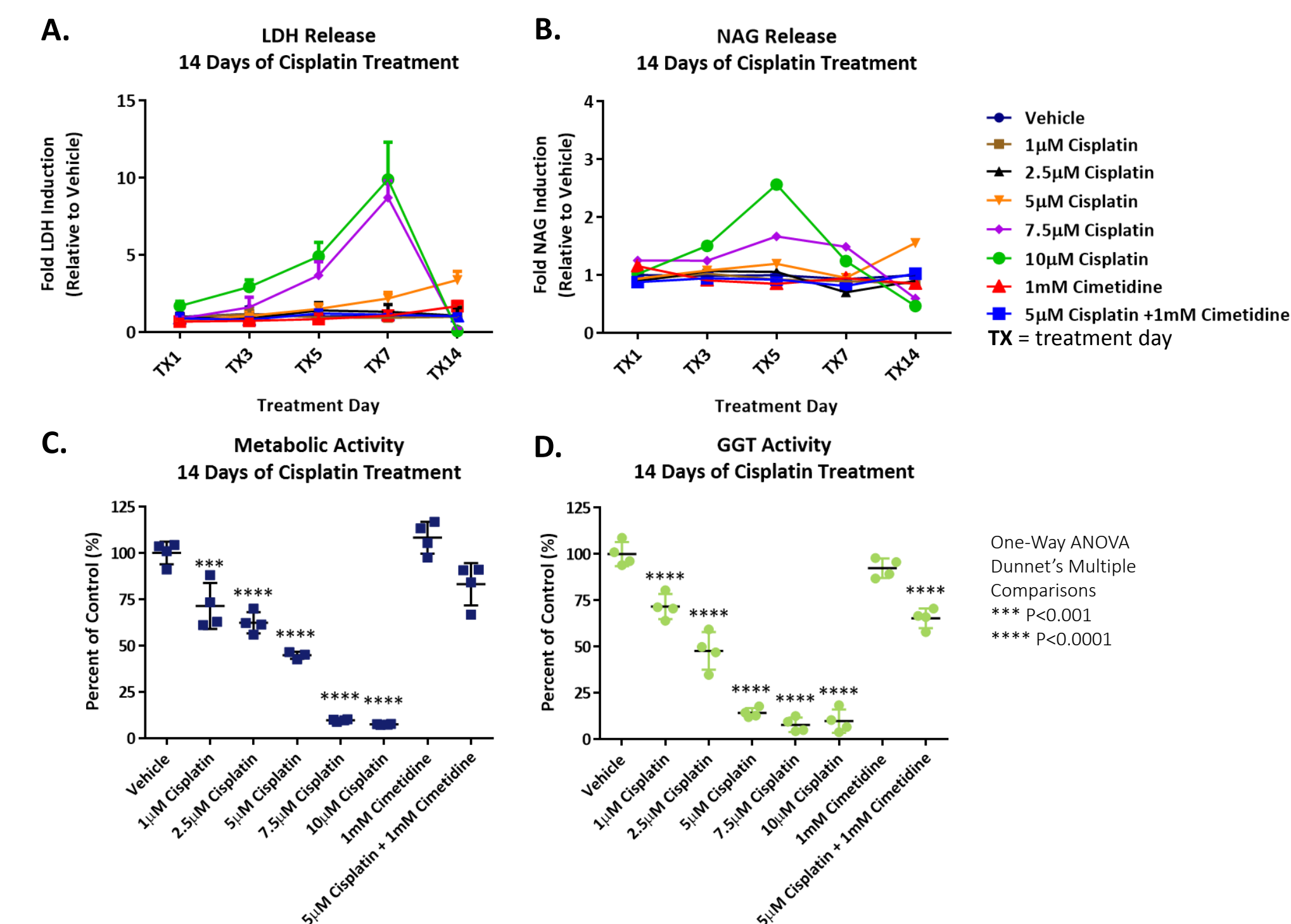
## Methods

Using Organovo's proprietary bioprinting platform, we have developed a human *in vitro* 3D tissue model of the proximal tubule to enable more accurate prediction of clinical outcomes. The tissue is composed of a renal interstitium of primary human renal fibroblasts and endothelial cells, providing a basement membrane and support for a polarized monolayer of primary human PT epithelial cells. Test articles were dosed daily.

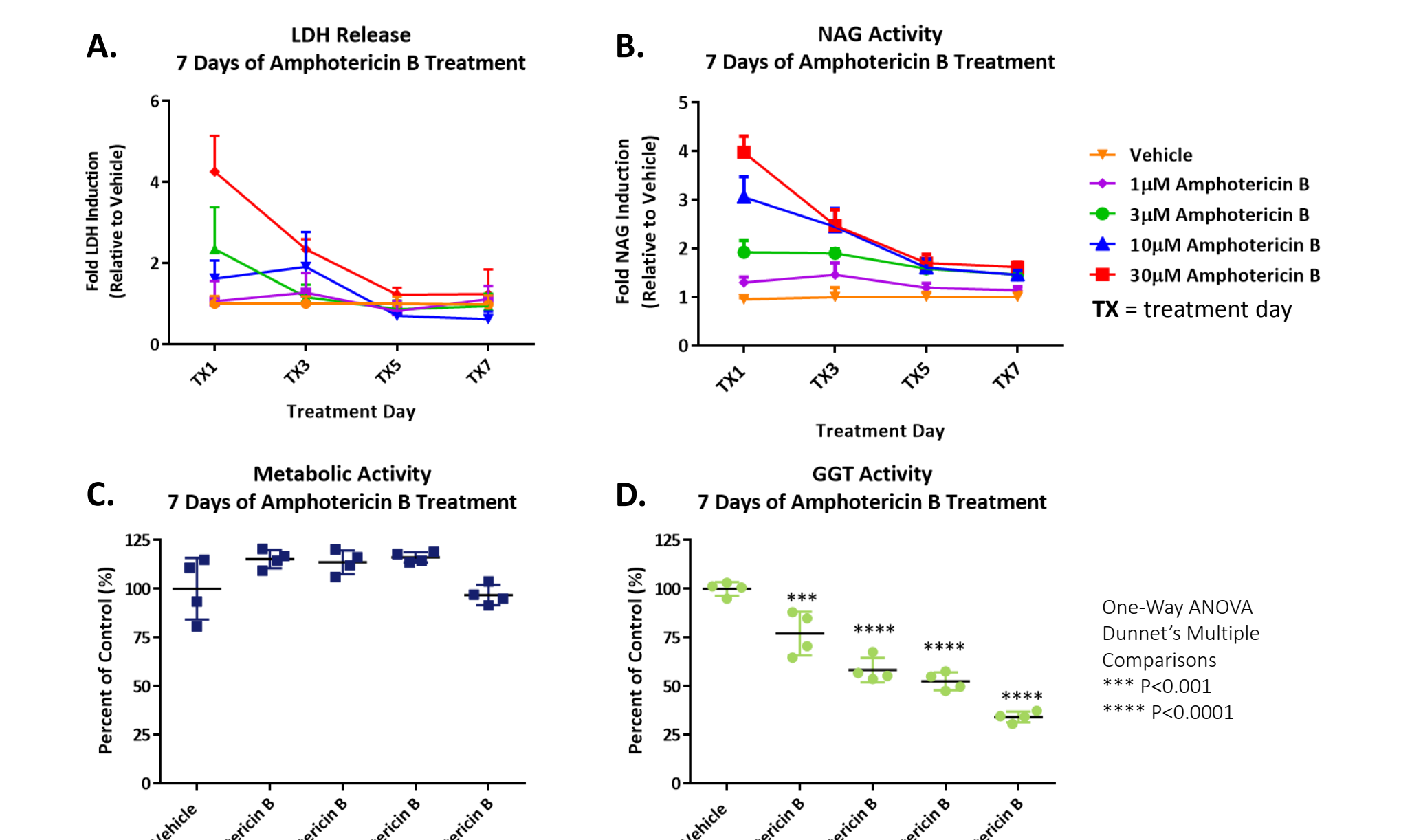
## Results



**Figure 1. Design and Characterization of ExVive™ Human Kidney Tissue.** A, H&E cross-section of native human kidney proximal tubules. B, Schematic of laminar model of the proximal tubule, comprising an interstitial layer of renal fibroblasts and endothelial cells supporting a polarized epithelial monolayer in standard Transwell format. C, Bioprinted tissues are highly reproducible from well to well. D, H&E. E, Masson's Trichrome stain for collagen (blue). F, CD31 (red) staining for endothelial cell networks. G, Cytokeratin 18 (red) staining for epithelial cells. H, E-cadherin staining (green) for junctions between epithelial cells, Collagen IV staining (red) for basement membrane. I, Basolateral localization of Na<sup>+</sup>K<sup>+</sup>ATPase. DAPI (blue) staining for nuclei. J, Gamma-glutamyl transferase (GGT) activity as a measure of renal proximal tubule epithelial cell-specific function over time.



**Figure 2. Biochemical evaluation of Cisplatin-induced toxicity and rescue with inhibition of OCT2.** 3D PT kidney tissues were dosed daily for 14 days and assessed for biochemical endpoints of toxicity. Cimetidine was co-administered as indicated to inhibit OCT2-mediated uptake of Cisplatin. **A and B**, Release of LDH or NAG as biomarkers of toxicity. **C**, Concentration-dependent reduction of overall metabolic activity as measured by alamarBlue®. **D**, Reduction in PT epithelial cell function as measured by GGT activity.

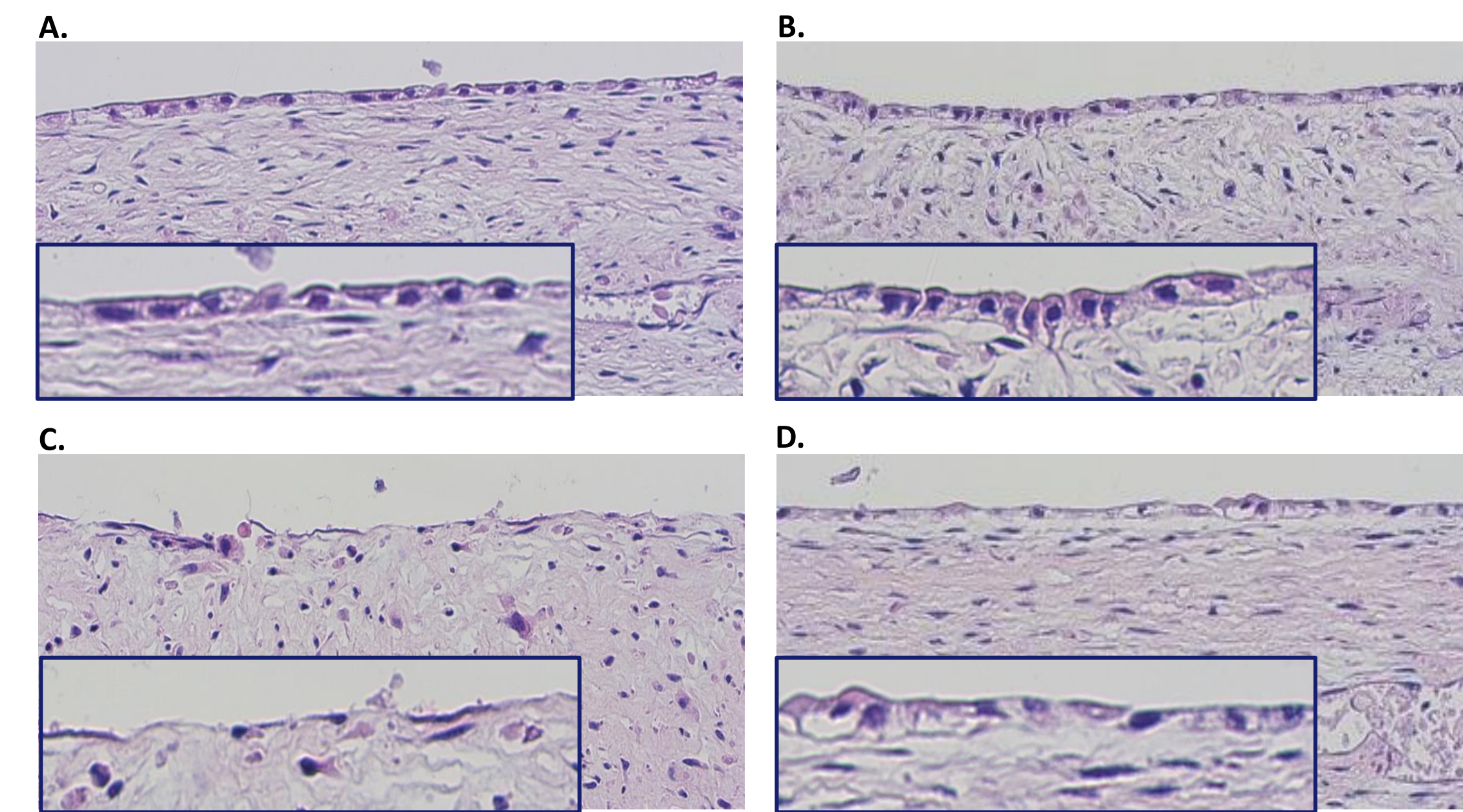


**Figure 4: Biochemical evaluation of Amphotericin B-induced toxicity.** 3D PT kidney tissues were dosed daily for 7 days and assessed for biochemical endpoints of toxicity. **A and B**, Release of LDH or NAG as biomarkers of toxicity. **C**, Concentration-dependent reduction of overall metabolic activity as measured by alamarBlue®. **D**, Reduction in PT epithelial cell function as measured by GGT activity.

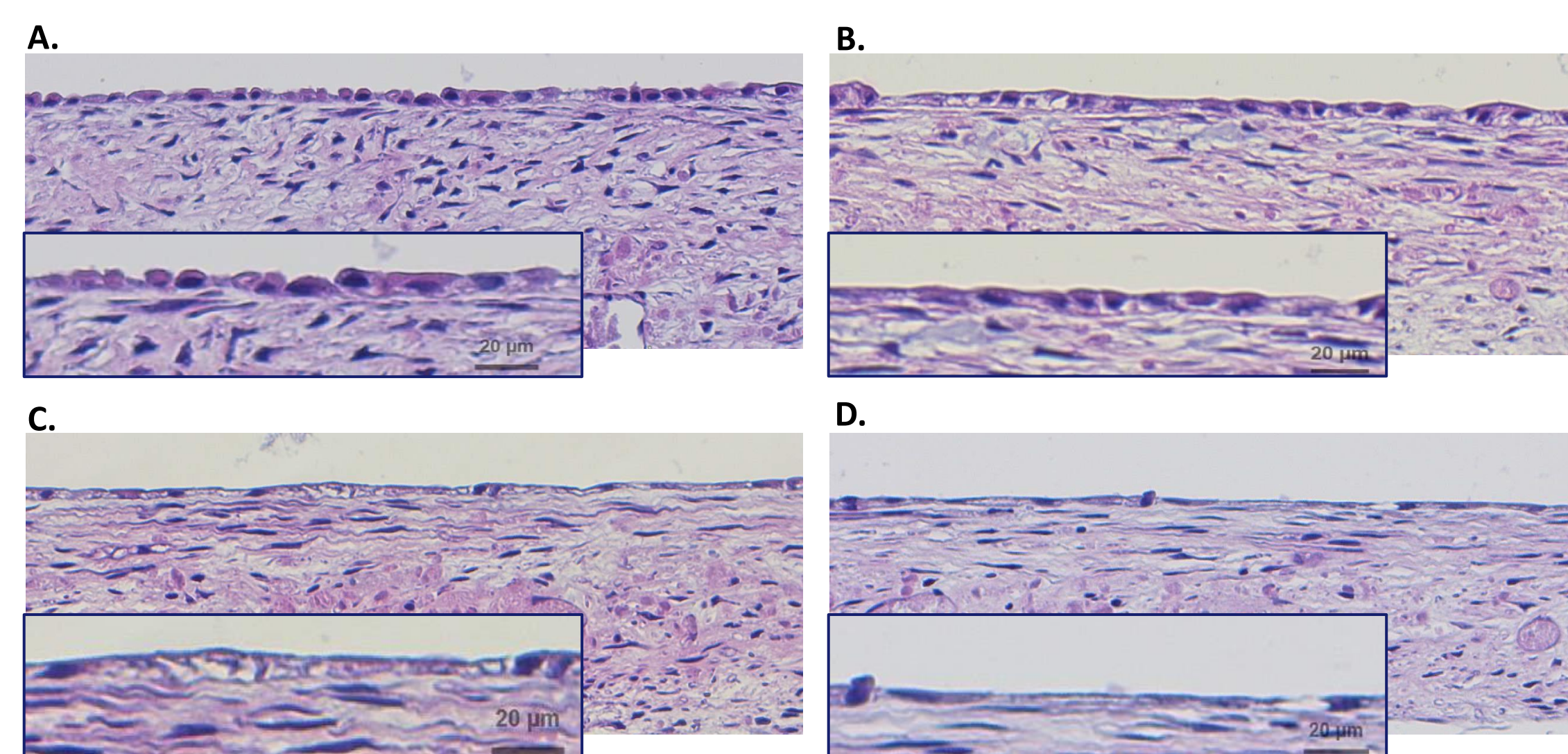
**Figure 6. Biochemical evaluation of Gentamicin-induced toxicity.** 3D PT kidney tissues were dosed daily for 14 days and assessed for biochemical endpoints of toxicity. **A**, Release of LDH as a biomarker of toxicity. **TX**, treatment day. **B**, Concentration-dependent reduction of overall metabolic activity as measured by alamarBlue®. **C**, Concentration-dependent reduction in PT epithelial cell function as measured by GGT activity.

### Forward Looking Statement

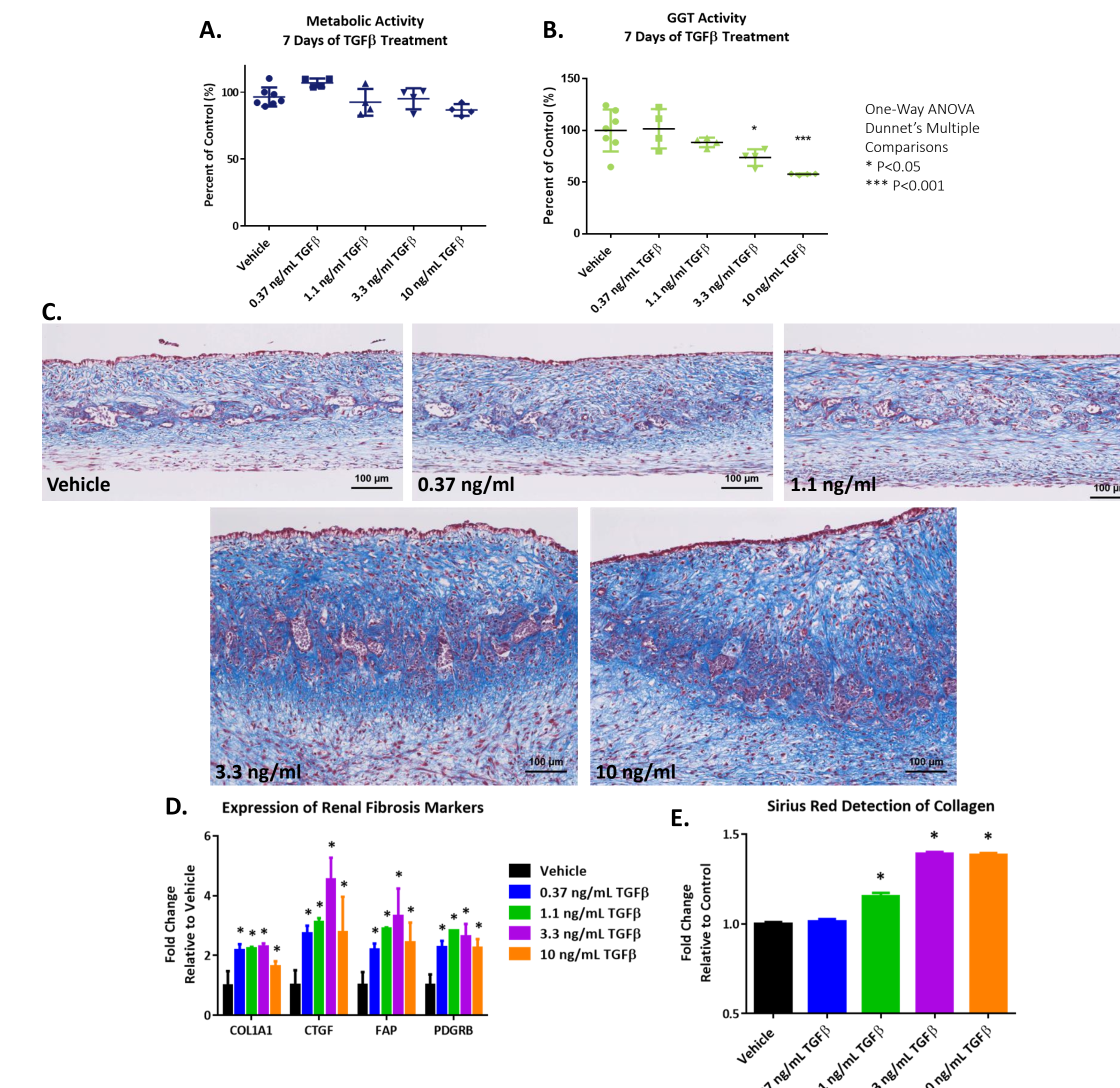
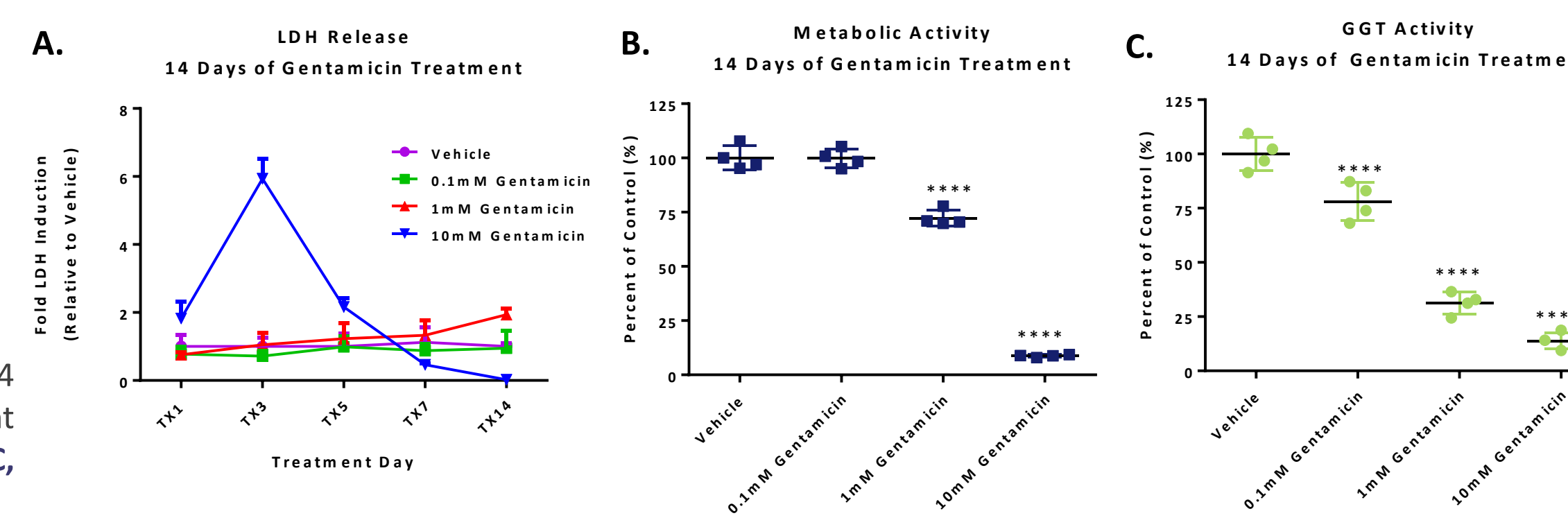
This presentation contains statements about future events and expectations known as "forward-looking statements" within the meaning of Section 27A of the Securities Act of 1933, as amended (the "Securities Act"), and Section 21E of the Securities Exchange Act of 1934, as amended (the "Exchange Act"). The Company has based these forward-looking statements on its current expectations and the information currently available to it, but any forward-looking statements are subject to a number of risks and uncertainties. The factors that could cause the Company's actual future results to differ materially from its current expectations, or from the results implied by any forward-looking statements, include, but are not limited to, risks and uncertainties relating to the Company's ability to develop, market and sell products and services based on its technology; the expected benefits and efficacy of the Company's products, services and technology; the Company's ability to successfully complete studies and provide the technical information required to support market acceptance of its products, services and technology, on a timely basis or at all; the Company's ability to generate revenue and control its operating losses; the validity of the Company's intellectual property rights and the ability to protect those rights; the Company's ability to implement the strategy, research, product development, regulatory approval, marketing and distribution plans and strategies; the Company's ability to secure additional contracted collaborative relationships; and the Company's ability to meet its fiscal year 2017 outlook and/or its long-range outlook. These and other factors are identified and described in more detail in the Company's filings with the Securities and Exchange Commission ("the SEC"), including those factors listed under the caption "Risk Factors" in the Company's Form 10-K for the year ended March 31, 2016, filed with the SEC on June 9, 2016, as well as other filings Organovo makes with the SEC from time to time. Readers are cautioned not to place undue reliance on forward-looking statements, which speak only as of the date of this presentation. Except as required by applicable law, we do not intend to update any of the forward-looking statements to conform these statements to reflect actual results, later events or circumstances or to reflect the occurrence of unanticipated events.



**Figure 3. Histological evaluation of Cisplatin-induced toxicity and rescue with inhibition of OCT2.** 3D PT kidney tissues were dosed daily for 14 days and assessed for histological phenotype. Cimetidine co-administered as indicated to inhibit OCT2-mediated uptake of Cisplatin and protect PT epithelial cells from toxicity. Panels display H&E stains for: **A**, Vehicle. **B**, 1mM Cimetidine. **C**, 5μM Cisplatin. **D**, 5μM Cisplatin + 1mM Cimetidine. Insets show magnified view of PT epithelial cell morphology.



**Figure 5. Histological evaluation of Amphotericin B-induced epithelial toxicity.** 3D PT kidney tissues were dosed daily for 7 days and assessed for histological phenotype. Panels display H&E stains for: **A**, Vehicle. **B**, 1μM Amphotericin B. **C**, 10μM Amphotericin B. **D**, 30μM Amphotericin B. Insets show magnified view of PT epithelial cell morphology.



**Figure 7. TGFβ-induced fibrosis.** 3D PT kidney tissues were dosed daily for 7 days with Vehicle, 0.37 ng/ml, 1.1ng/ml, 3.3ng/ml, or 10ng/ml TGFβ. **A**, Increasing doses of TGFβ do not impact overall tissue metabolic activity as measured by alamarBlue®. **B**, Reduction in PT epithelial cell function at higher doses as measured by GGT activity. **C**, Increased tissue thickening and ECM deposition as assessed by Gomori's Trichrome stain. **D**, Dose-dependent induction of fibrotic markers, collagen I (COL1A1), connective tissue growth factor (CTGF), fibroblast-activating protein (FAP), or platelet-derived growth factor receptor β (PDGFRB) as assessed by quantitative RT-PCR. \*p<0.0001. **E**, Quantification of collagen in Sirius red-stained tissue sections. \*p<0.05.

## Summary

- Conventional preclinical renal assays such as *in vitro* cell culture and animal models are insufficient in modeling the complexity of drug-induced organ toxicity due to limited functionality or species-specific variation.
- ExVive™ Human Kidney Tissue is a 3D multicellular model of the renal tubulointerstitial interface, comprised of a polarized monolayer of primary human proximal tubule epithelial cells supported by an interstitium of human renal fibroblasts and endothelial cells.
- Drug-induced, dose-dependent renal injury following exposure to classical nephrotoxicants including the chemotherapeutic agent, Cisplatin, and aminoglycoside antibiotics, Amphotericin B and Gentamicin, can be detected at the biochemical and histological levels.
- TGFβ exposure demonstrates extended capability of ExVive™ Human Kidney Tissue to mount measurable responses at the biochemical, transcriptional, and histological levels consistent with renal injury and fibrosis, a disease phenotype not achievable in traditional systems of epithelial cell monolayer culture with limited longevity.
- Together, these results support the utility of this novel 3D tissue model of the proximal tubule for assessment of human renal toxicity over extended time in culture.

### References

- King, S.M., et al. (2017). 3D Proximal Tubule Tissues Recapitulate Key Aspects of Renal Physiology to Enable Nephrotoxicity Testing. *Frontiers in Physiology*. In Press.
- Gentamicin studies were done in partnership with Dr. Kristine de Dios and Dr. Andrew Seacat at La Jolla Pharmaceuticals.