

TN-301, a Highly Specific HDAC6 Inhibitor, Improves Muscle Function and Molecular Pathology in *mdx* Mice and Corrects Human DMD iPSC-Cardiomyocyte Phenotypes

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HDAC6 inhibition by TN-301 demonstrates superiority over pan-HDAC inhibition in preclinical models of DMD

OVERVIEW

TN-301 is a potent and highly selective HDAC6 inhibitor that reverses measures of cardiomyopathy and diastolic dysfunction in mouse models of genetic cardiomyopathy and heart failure with preserved ejection fraction. To test the hypothesis that TN-301 may delay or reverse both skeletal muscle pathology and cardiomyopathy in DMD, researchers conducted studies comparing TN-301 with givinostat, a pan-HDAC inhibitor approved for the treatment of DMD.

In *mdx* mice, TN-301 treatment at doses as low as 3 mg/kg improved grip strength to wild-type levels within five weeks, whereas *mdx* mice treated with givinostat (10 mg/kg, approximating clinical exposures) failed to reach wild-type performance. TN-301 mediated functional improvements were accompanied by reductions in circulating creatine kinase and favorable changes in gene expression and histology.

In cardiomyocytes derived from human DMD-induced pluripotent stem cells, TN-301 corrected calcium handling abnormalities and mitochondrial dysfunction, while givinostat exacerbated these established drivers of DMD cardiomyopathy.

Together, these data support advancement of TN-301 as a potential DMD therapy with benefits for both skeletal and cardiac muscle without thrombocytopenia and QT prolongation risks reported with pan-HDAC inhibitors.

BACKGROUND

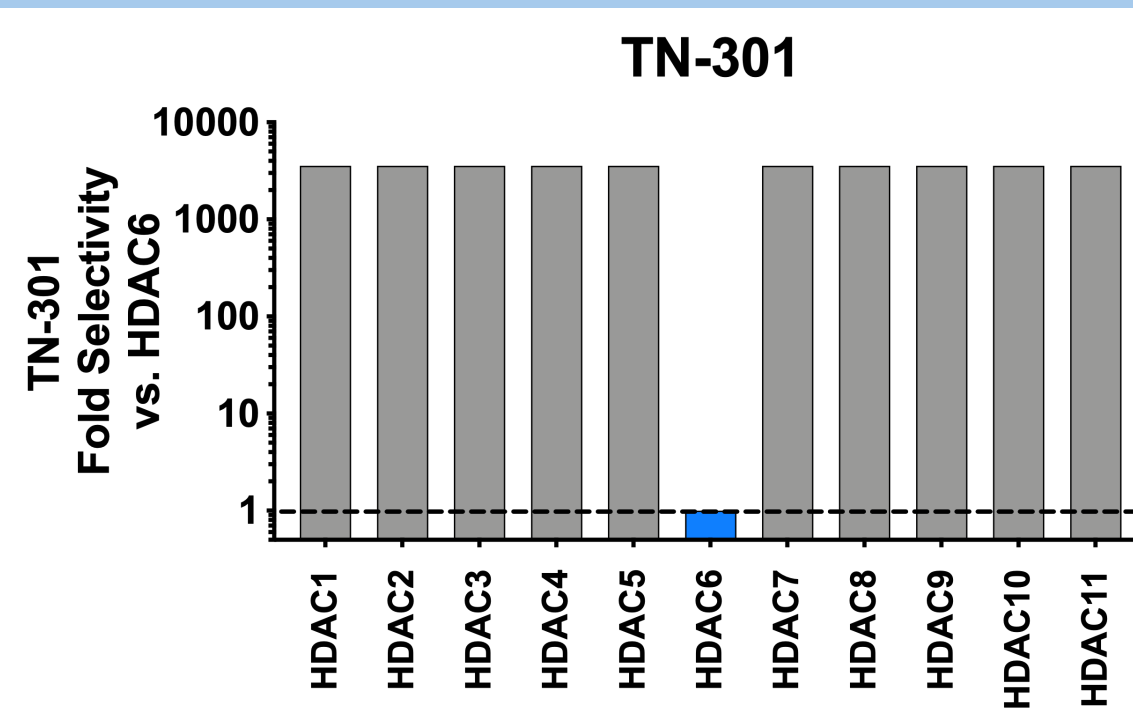
As DMD progresses, degeneration extends beyond skeletal muscle to involve the heart, leading to dilated cardiomyopathy, a major contributor to morbidity and mortality in DMD.

TN-301 was discovered by Tenaya Therapeutics and exhibits a multi-modal mechanism of action, including reducing inflammation, fibrosis, and mitochondrial dysregulation and improving autophagy. In a Phase 1 study in healthy adults, TN-301 did not demonstrate serious adverse events or dose-limiting toxicities over a wide dose range. HDAC6 specific inhibition *in vivo* was demonstrated in peripheral blood mononuclear cells by increases in acetylated tubulin, with no changes in histone acetylation. Plasma half-life supports once daily oral dosing.

The pan HDAC inhibitor givinostat has been shown to increase muscle tissue, reduce fatty infiltration, and slow the decline in motor function, in DMD patients; however, its use is limited by side effects including thrombocytopenia, requiring dose reduction, and by QT prolongation risk – liabilities not observed clinically in the Phase 1 study of TN-301. Moreover, whether givinostat benefits DMD cardiomyopathy is not yet known.

TN-301 Is Selective for the Cytoplasmic HDAC, HDAC6, Avoiding Nuclear HDACs & Supporting Beneficial Effects

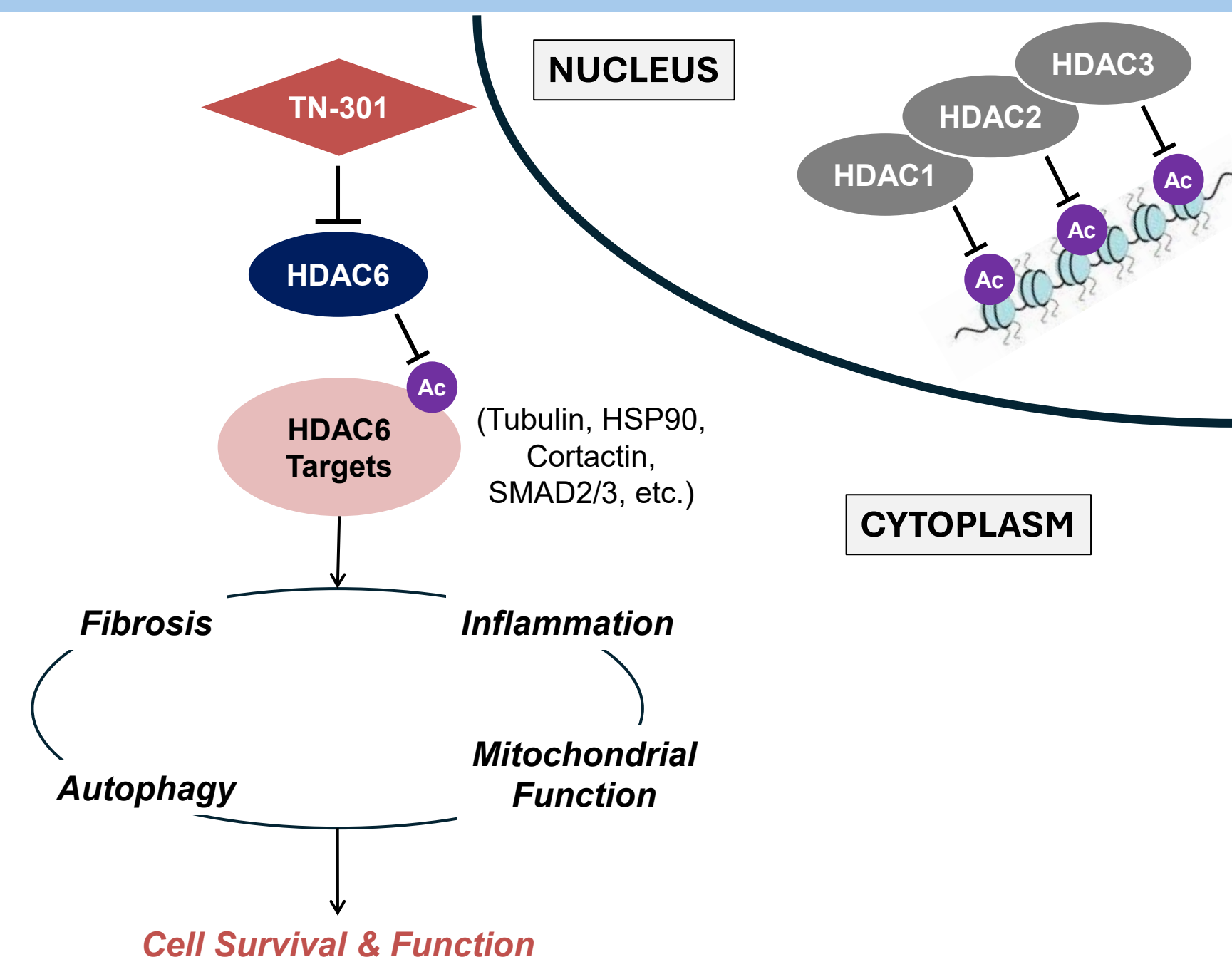
TN-301 Has $\geq 3500\times$ Biochemical Selectivity for HDAC6 vs. Other HDACs



TN-301 Was Well Tolerated with Expected PK & PD in Single- and Multiple-Ascending Dose Phase 1 Study



HDAC6 is a Cytoplasmic Enzyme that Deacetylates Non-histone Proteins, Regulating Cytoskeletal Structure, Protein Quality Control & Stress Responses



TN-301 Supports Greater Grip Strength Improvements than Givinostat in *mdx* Mice without Reducing Platelets

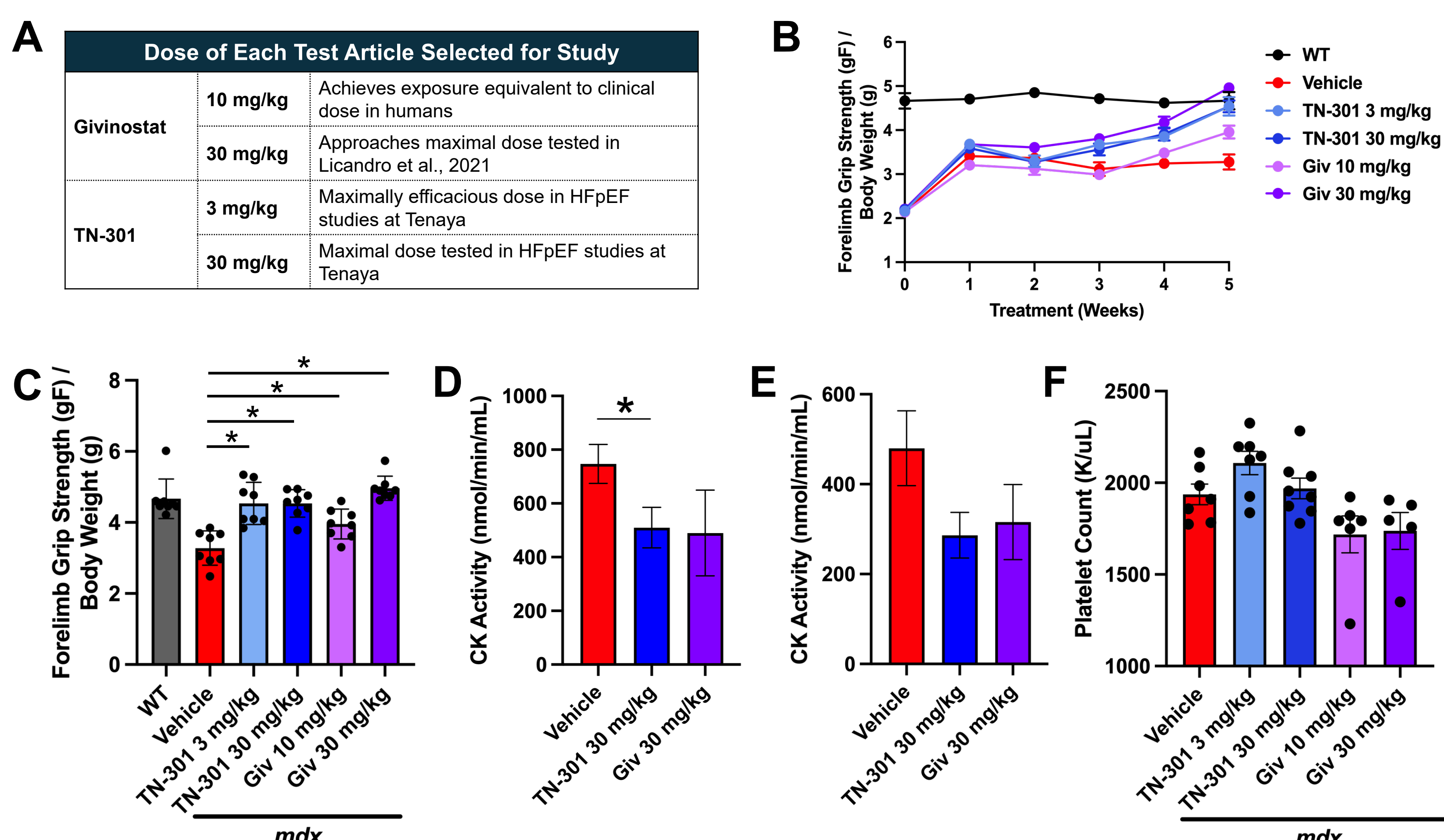


Figure 1. TN-301 Supports Greater Grip Strength Improvements than Givinostat in *mdx* Mice at Clinically Relevant Doses. **A)** Rationale for dose levels used in study. Male WT or *mdx* mice received drug or vehicle via daily oral dosing for 5 weeks starting at 7 weeks of age. **B)** Weekly and **(C)** 5-week forelimb grip strength measurements show *mdx* groups receiving either dose level of TN-301 reached WT grip strength levels at 5 weeks of dosing, as did those receiving the high dose, not low dose, of givinostat. **D)** Creatine kinase (CK) activity in serum – a clinically-relevant marker of skeletal muscle damage – collected after 4- or **(E)** 6-weeks of dosing is reduced with treatment consistent with improved grip strength (3 dilutions were tested per animal and data are aggregated). **F)** Platelet count after 5 weeks of treatment showed trending reductions in response to givinostat, but no reductions at either dose of TN-301. *, $p \leq 0.01$; WT, wild-type; Giv, givinostat

Gene Sets Underlying Key Drivers of DMD, Including Apoptosis, Are Disrupted in *mdx* Mice & Improved by TN-301

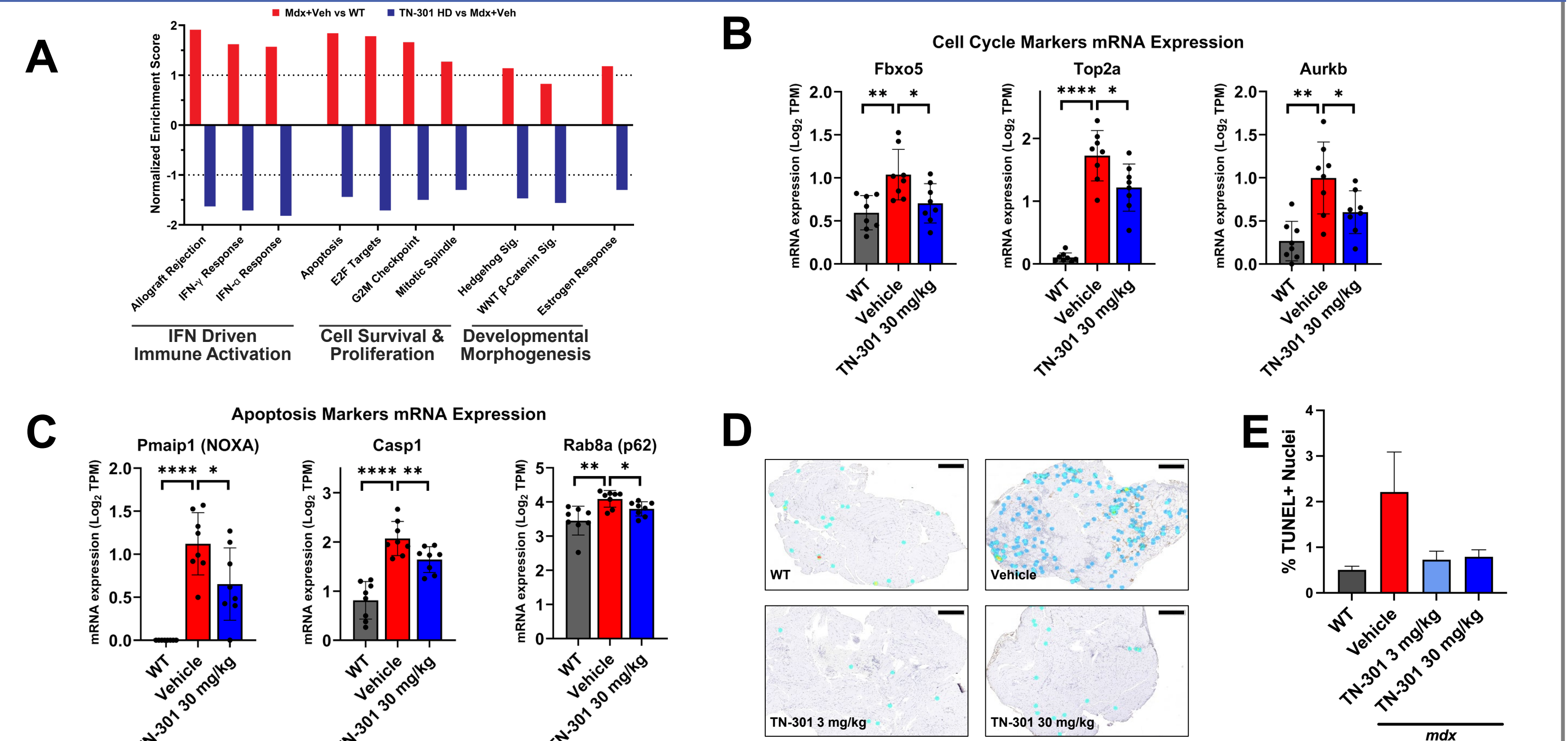


Figure 2. Gene Sets Underlying Key Drivers of Muscular Dystrophy Are Dysregulated in *mdx* Mice and Improved by TN-301. **A)** Gene Set Enrichment Analysis (GSEA) highlights key cellular functions associated with response to TN-301 treatment. **B)** Key regulators of cell cycle progression are normalized following TN-301 treatment. **C)** TN-301 restores key apoptotic markers toward control levels in skeletal muscle. **D)** Representative TUNEL staining images from soleus muscle sections of WT or *mdx* mice treated with vehicle or TN-301. **E)** Quantification of TUNEL staining from images in D. WT, wildtype; Error bars, SEM; Scale bars, 400 μm

TN-301 Improves Calcium Handling in Engineered Heart Tissues from Human DMD iPSCs

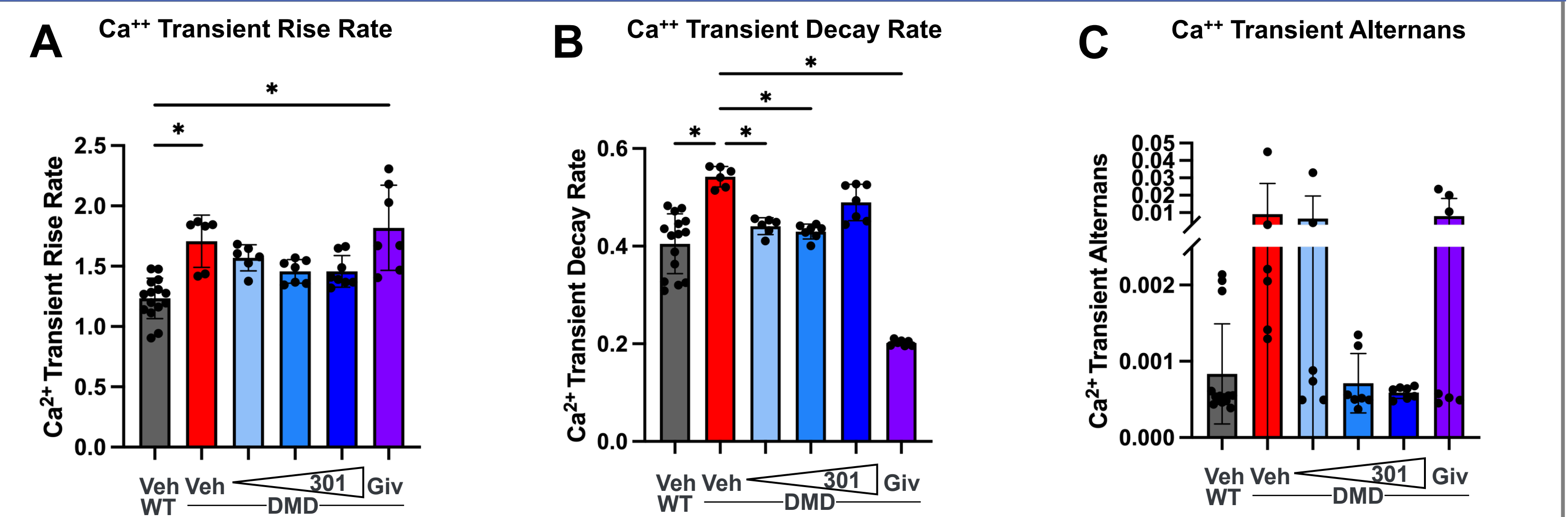


Figure 3. TN-301 Improves Calcium Handling in Engineered Heart Tissues (EHTs) from Human DMD iPSCs. EHTs were cast from male human WT or DMD patient-derived iPSCs for 25 days and then treated with vehicle; 0.3, 0.1, or 3 μM TN-301; or 3 μM givinostat for two days followed by Ca^{2+} imaging with 1Hz pacing. **A)** Ca^{2+} transient rise and **(B)** decay rates, and **(C)** Ca^{2+} transient alternans – central drivers of cardiomyopathy in human DMD – were measured. There was a trend toward improved Ca^{2+} transient rise rate with TN-301. Both decay rate and Ca^{2+} transient alternans, the beat-to-beat fluctuation in Ca^{2+} transient amplitude, were corrected by TN-301. Givinostat did not improve Ca^{2+} transient rise rate or alternans. *, $p \leq 0.01$; WT, wild-type; Veh, vehicle; 301, TN-301; Giv, givinostat

TN-301 Improves Mitochondrial Oxygen Consumption in Human DMD iPSC-derived Cardiomyocytes

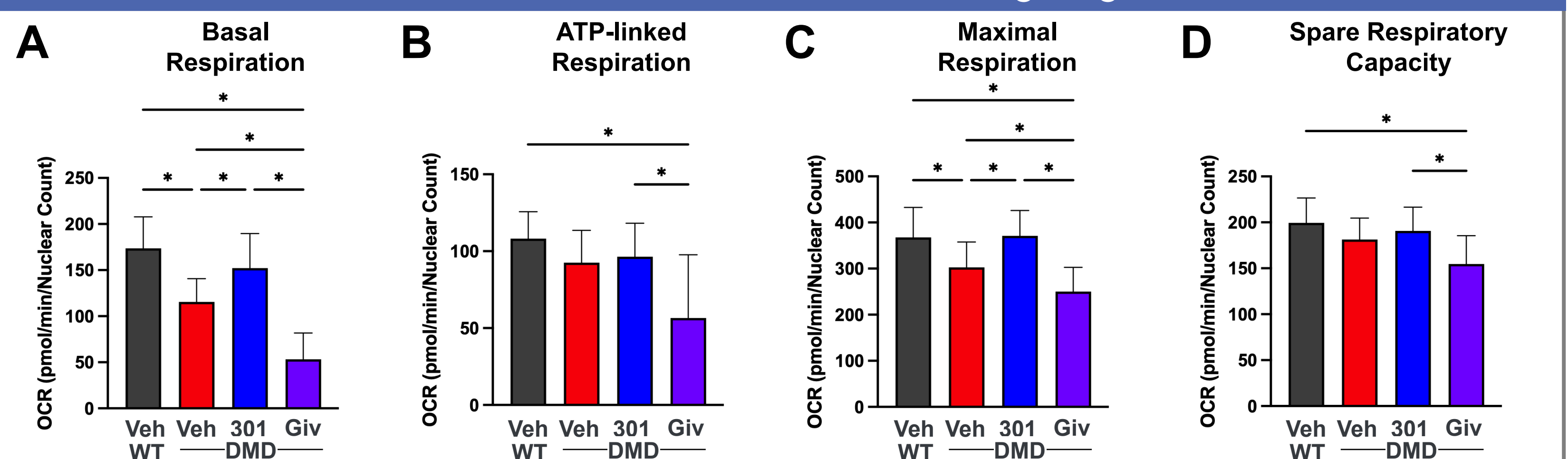


Figure 4. TN-301 Improves Oxygen Consumption in Human DMD iPSC-derived Cardiomyocytes (iPSC-CMs). Male human WT or DMD patient iPSC-CMs were treated with 3 μM TN-301 or 3 μM givinostat for two days followed by a Seahorse mitochondrial stress test measuring oxygen consumption rate (OCR). **A)** Basal respiration, **(B)** ATP-linked respiration, **(C)** maximal respiration, and **(D)** spare respiratory capacity were decreased in DMD iPSC-CMs, consistent with their role as central drivers of cardiomyopathy in human DMD. Basal and maximal respiration were corrected by TN-301 and worsened by givinostat. *, $p \leq 0.05$; WT, wild-type; Veh, vehicle; 301, TN-301; Giv, givinostat

CONCLUSIONS & NEXT STEPS

- TN-301 has the potential to improve skeletal and cardiac muscle deficits in DMD with reduced liabilities compared to pan-HDAC inhibitors
- In head-to-head studies comparing the effects of TN-301 and givinostat in *mdx* mice, TN-301 restored grip strength to WT levels and outperformed givinostat suggesting that HDAC6 inhibition may be driving critical advantages on skeletal muscle function that underlie the benefits observed with pan-HDAC inhibition
- In *mdx* mice, TN-301 reduced serum creatine kinase activity and muscle apoptosis without reducing platelets
- TN-301 improved several clinically relevant drivers of DMD cardiomyopathy, including calcium handling and mitochondrial respiration
- Totally of clinical and preclinical data for TN-301 to date supports clinical study in both ambulatory and non-ambulatory DMD patients with cardiomyopathy as well as other cardiac diseases and cardiac adjacent indications