

RESULTS

CpdA inhibits NF- κ B activity in C2C12 muscle cells

The stable C2C12 skeletal muscle mouse cell line expressing an NF- κ B luciferase reporter gene was used to assay the anti-NF- κ B activity in tissue culture containing CpdA and PNSL at eleven $\frac{1}{2}$ log concentrations (Figure S1). We found that CpdA, at concentrations from 1 μ M, blocked the TNF-induced increase in NF- κ B activity in both myoblasts and myotubes and the concentration required to inhibit NF- κ B activity by 50% (IC_{50}) was 2.5 μ M and 1.9 μ M respectively (Figure S1A). Marked reduction of cell viability, assessed by metabolic activity using the MTT assay, occurred at concentrations of CpdA from 5 μ M in both myoblasts and myotubes (Figure S1C). At the maximum non-toxic CpdA concentration of 4 μ M, NF- κ B activity was reduced by 76% in myoblasts and 80% in myotubes. PNSL at a concentration of 10 μ M, reduced NF- κ B activity by 52% in myoblasts and 48% in myotubes and had the advantage of being biologically active at concentrations as low as 0.1 nM (Figure S1B). PNSL had minimal effect on cell viability and this was only observed at concentrations approaching 10 μ M (Figure S1D).

CpdA reduces TNF-induced p65 nuclear translocation in H-2K^b-tsA58 (H-2K) mdx muscle cells.

A qualitative assessment of the effect of CpdA and PNSL on TNF-induced p65 nuclear translocation using immunofluorescence, demonstrated predominantly nuclear staining of p65 following 10 ng/mL TNF in both myoblasts and myotubes, but visually more cytoplasmic staining following 1 ng/mL TNF stimulation (Figure S2).

CpdA does not improve ex vivo force measurements in the Extensor Digitorum Longus (EDL) muscle of mdx mice.

Muscles were removed from mice at 8 weeks of age and studied *ex vivo* to assess muscle function. The EDL muscles from VEH-treated WT mice demonstrated significantly higher specific forces (SF, mN/mm²) (+39%) and were more resistant to lengthening-induced declines in measured force than VEH-treated *mdx* (Figure S3B & C). There was no significant difference in maximal isometric force (MIF, mN) between VEH-treated WT and VEH-treated *mdx* mice. MIF in *mdx* mice following CpdA 3.75mg/kg/day, CpdA 7.5mg/kg/day and PNSL 5.0mg/kg/day treatments were not significantly different to VEH-treated *mdx* mice (Figure S3A) but SF was significantly higher in the PNSL 5.0mg/kg/day (+12%) treatment group. Forces measured at the end of the lengthening-contraction protocol (percent of original force) were not improved by CpdA 3.75mg/kg/day, CpdA 7.5mg/kg/day or PNSL 5.0mg/kg/day treatments (Figure S3C) in WT or *mdx* mice.