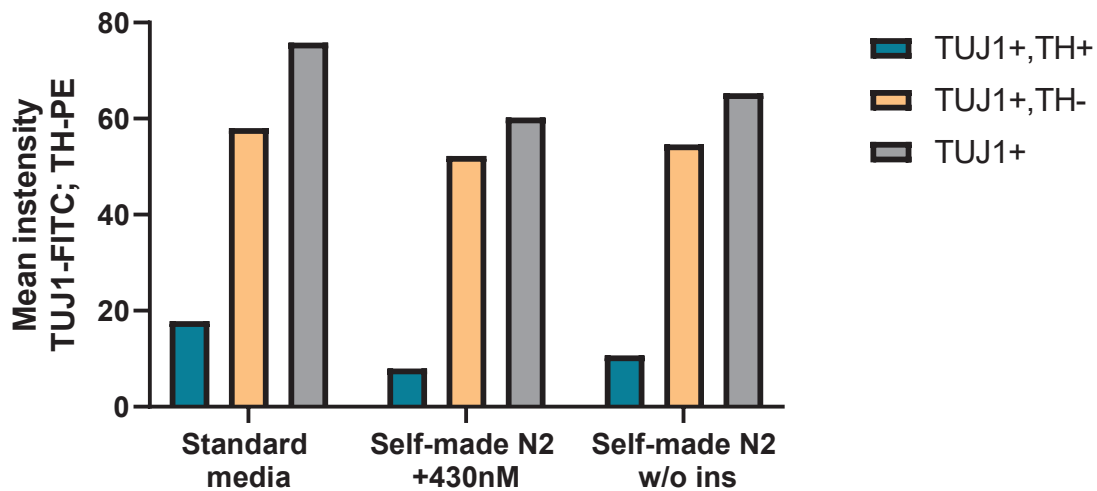
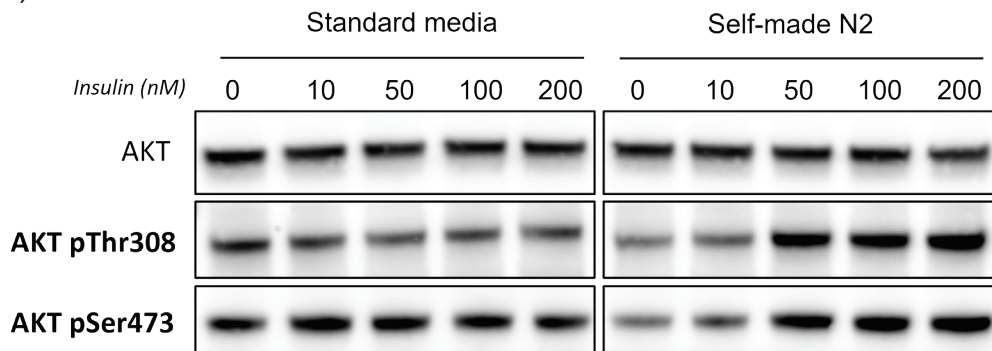


(a)



(b)



**Figure S4. Self-made N2 supplement validation in 2D iPSC-derived dopaminergic neurons.**

Dopaminergic neurons were cultured in Standard media, self-made N2 media + 430nM insulin (to mimic theoretical insulin concentration in standard media), and self-made N2 media w/o insulin until day 30 of differentiation. (a) Percentage of cells quantified by FACS, expressing the indicated neuronal differentiation markers at day 30 of differentiation. (b) Activation of AKT signaling pathway was evaluated after 30 days of NESC differentiation in the two media (standard vs self-made N2), upon acute stimulation with increasing insulin doses (from 10nM to 200nM) for 30 minutes. AKT phosphorylation at both Thr308 and Ser473 residues was assessed by immunoblotting and normalized against total AKT levels.