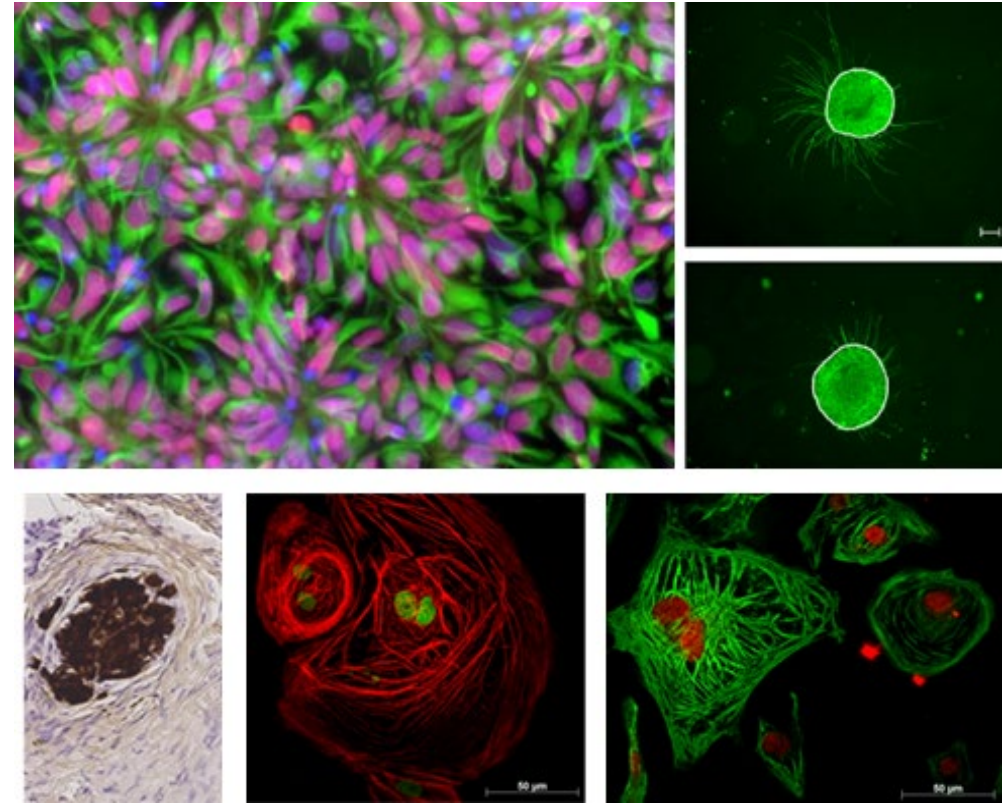# The establishment and characterization of iPSC derived 3D neurosphere cultures from a Kleefstra patient

Andrea Balogh

Kleefstra Syndrome Scientific Conference 2023

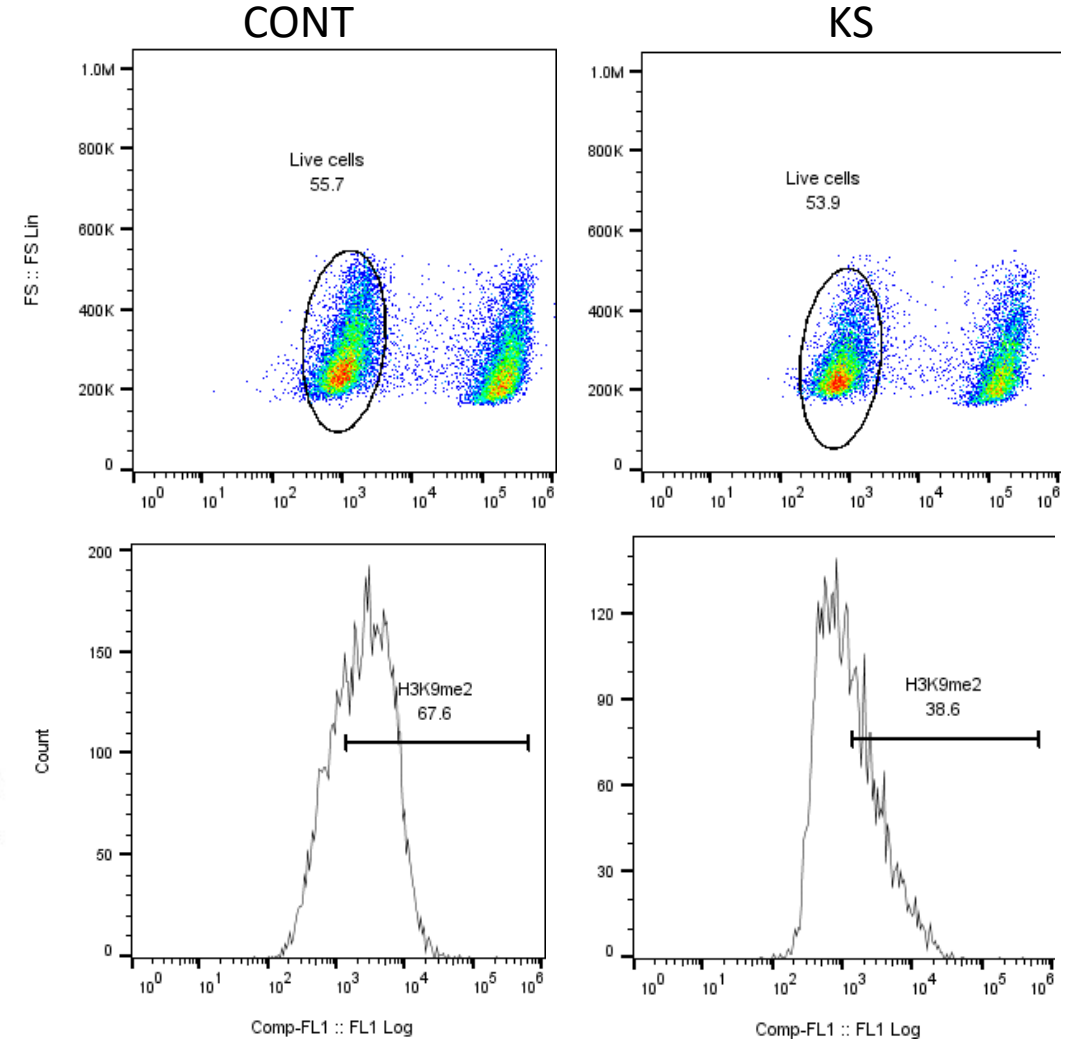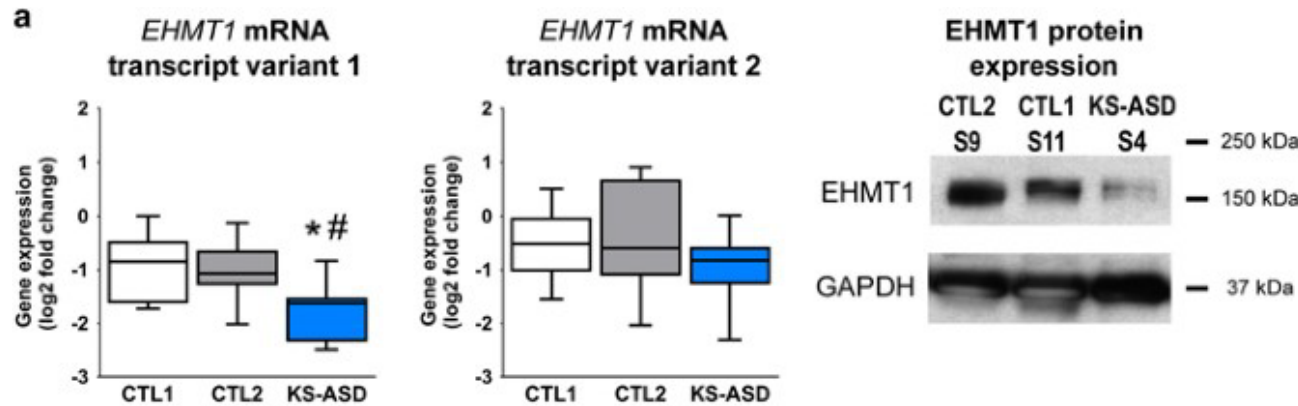# EHMT1 mutation identified in a Kleefstra syndrome patient

Targeted next generation sequencing of a panel of autism-related genes identifies an EHMT1 mutation in a **Kleefstra** syndrome patient with autism and normal intellectual performance.

Bock I, Németh K, Pentelényi K, Balicza P, Balázs A, Molnár MJ, Román V, Nagy J, Lévay G, Kobolák J, **Dinnyés A.**

EHMT1 Trp1138Ter

Neuronal progenitor cells (NPCs) derived from iPSC generated from a Kleefstra syndrome (KS) patient show lower H3K9me2 level compared to the control (CONT) cell line analysed with flow cytometry.

Previously estabilished

Establishment of EHMT1 mutant induced pluripotent stem cell (iPSC) line from a 11-year-old **Kleefstra** syndrome (KS) patient with autism and normal intellectual performance.

Varga E, Nemes C, Táncos Z, Bock I, Berzsenyi S, Lévay G, Román V, Kobolák J, **Dinnyés A.**
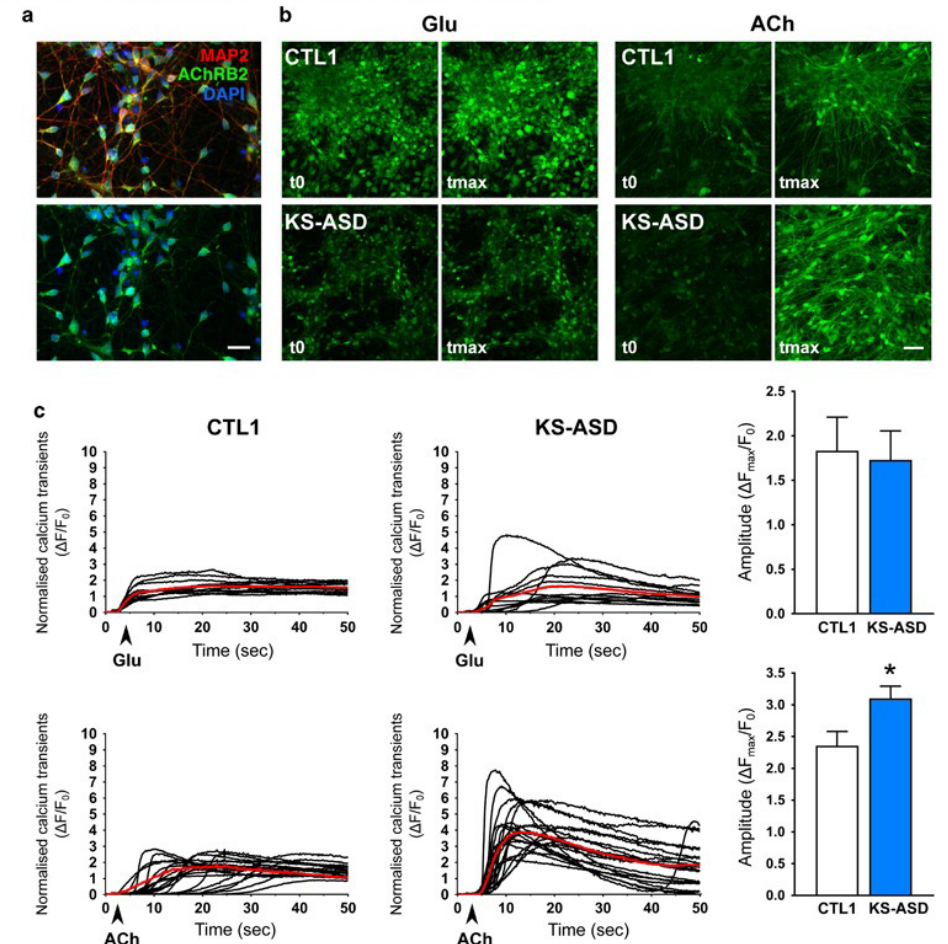
Altered neurite morphology and cholinergic function of induced pluripotent stem cell-derived neurons from a patient with **Kleefstra** syndrome and autism.

Nagy J, Kobolák J, Berzsenyi S, Ábrahám Z, Avci HX, Bock I, Bekes Z, Hodoscsek B, Chandrasekaran A, Téglási A, Dezső P, Koványi B, Vörös ET, Fodor L, Szél T, Németh K, Balázs A, **Dinnyés A**, Lendvai B, Lévay G, Román V.
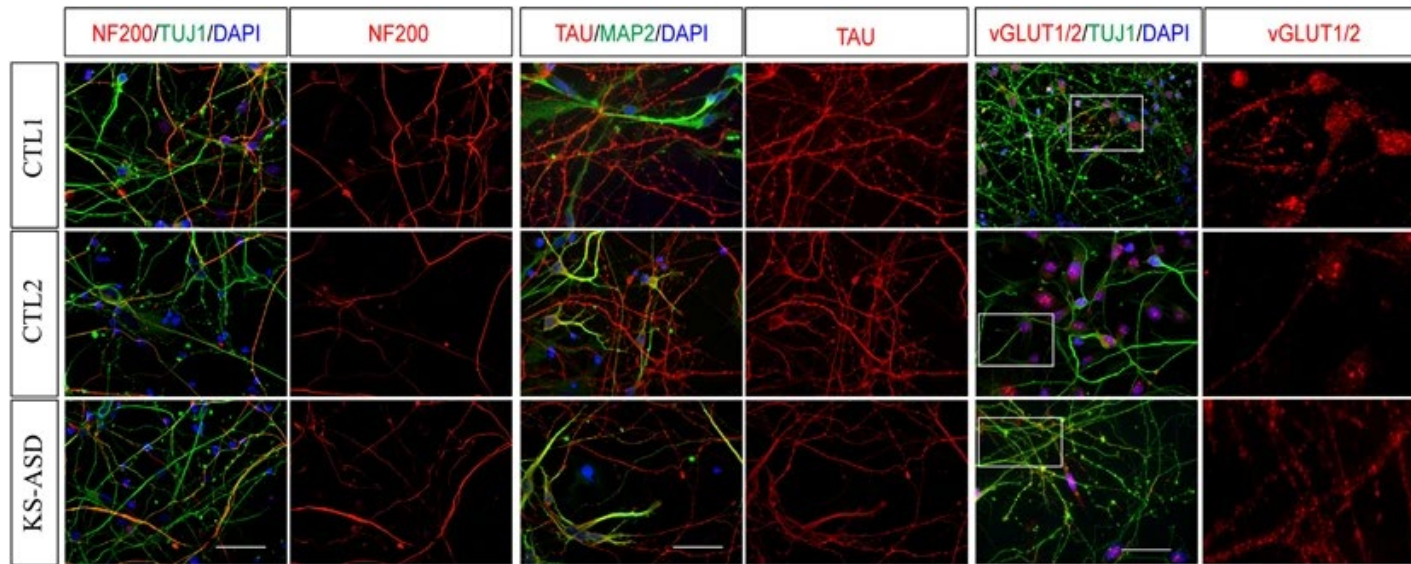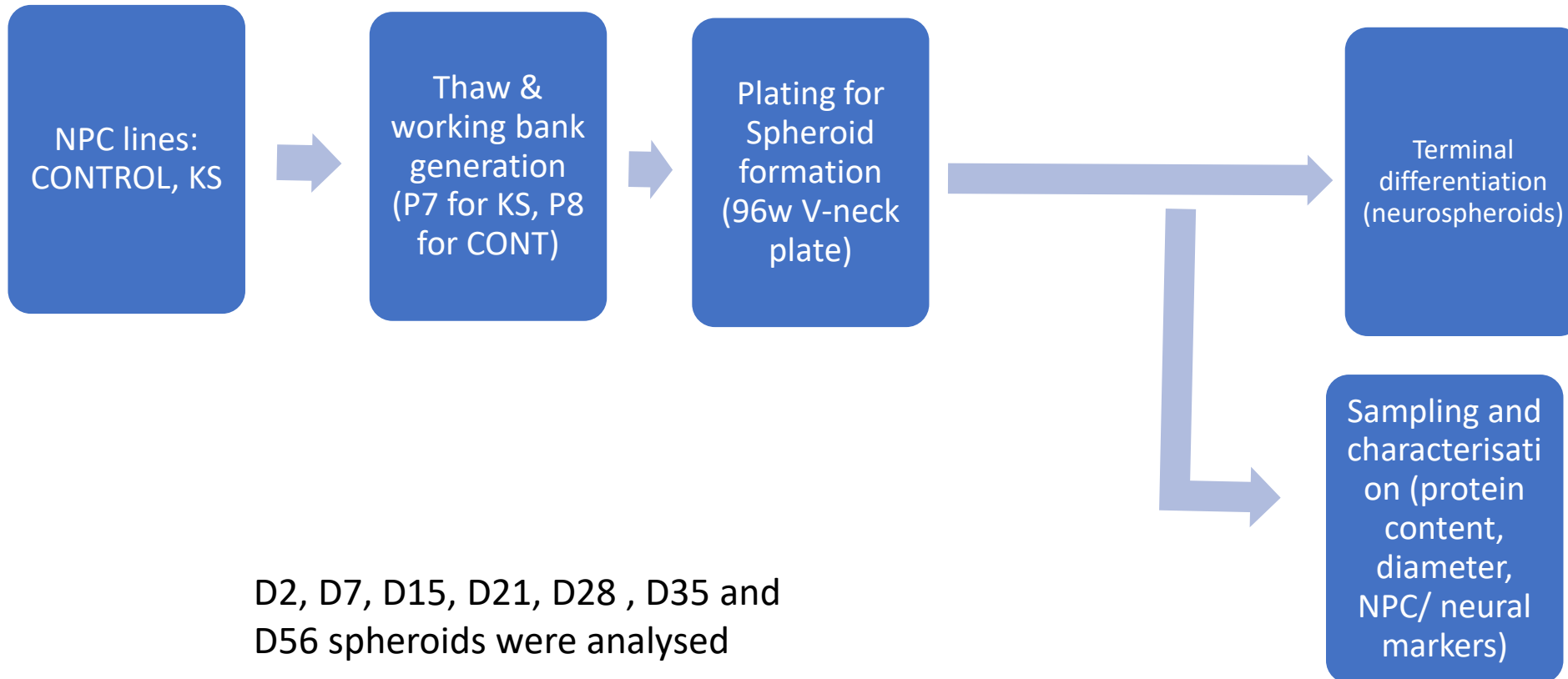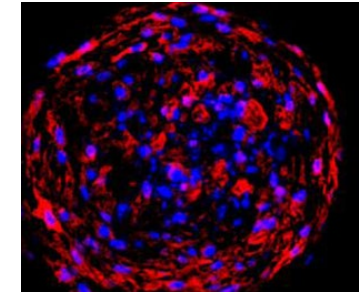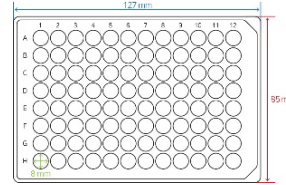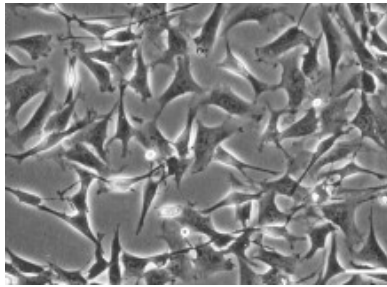
Neurons differentiated from human induced pluripotent stem cell (hiPSC)-derived neuronal precursor cells (NPCs). Representative images of CTL1, CTL2 and KS-ASD cells expressing the neuronal markers class III beta-tubulin (TUJ), Neurofilament 200 (NF200), and microtubule-associated proteins TAU and MAP2 on day 35 of terminal differentiation. The neurons are also positive for the glutamatergic subtype-specific marker vesicular neurotransmitter transporter 1/2 (vGLUT1/2).
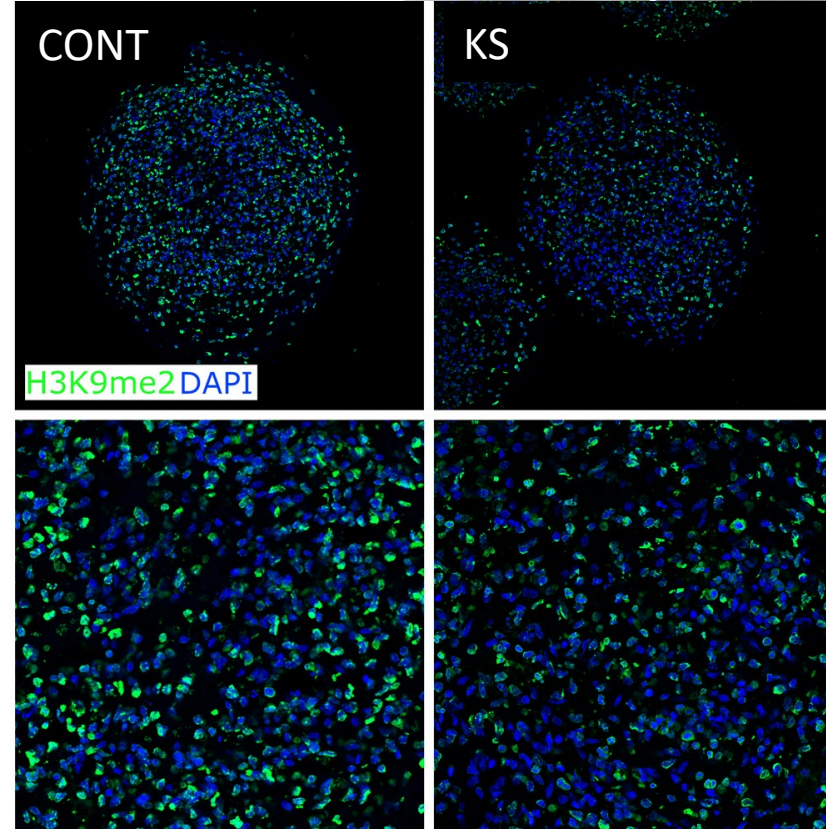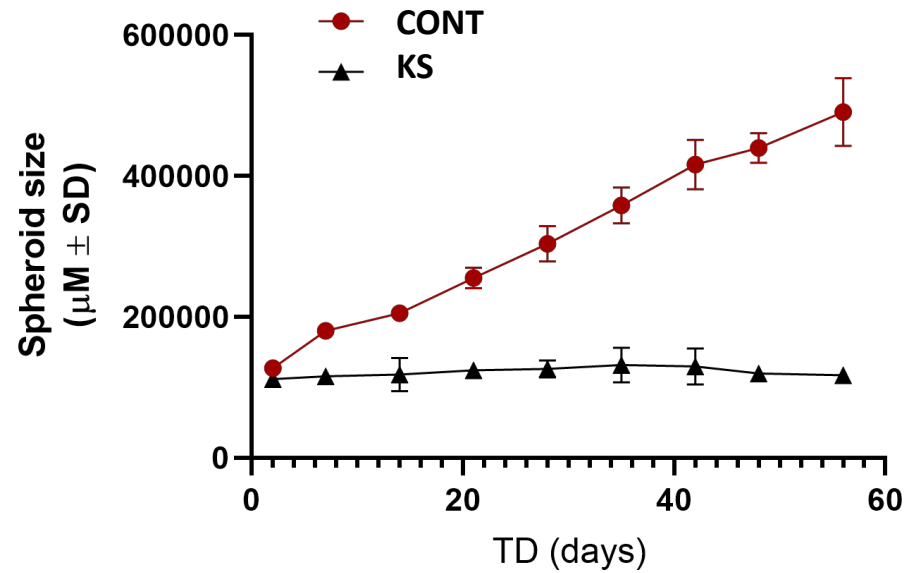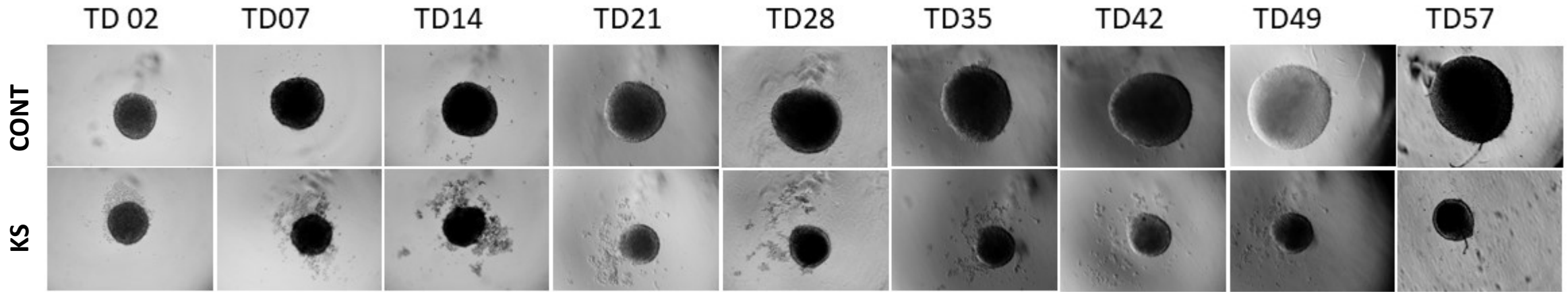
Experimental workflow

NPC lines: CONTROL, KS → Thaw & working bank generation (P7 for KS, P8 for CONT) → Plating for Spheroid formation (96w V-neck plate) → Terminal differentiation (neurospheroids) / Sampling and characterisation (protein content, diameter, NPC/ neural markers)
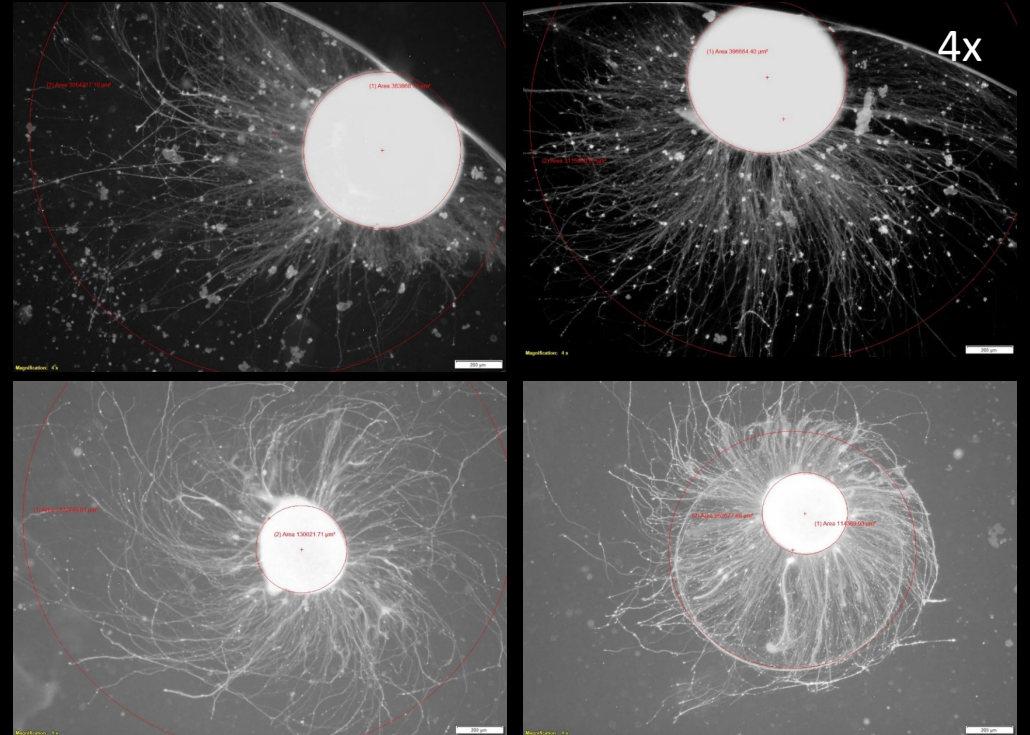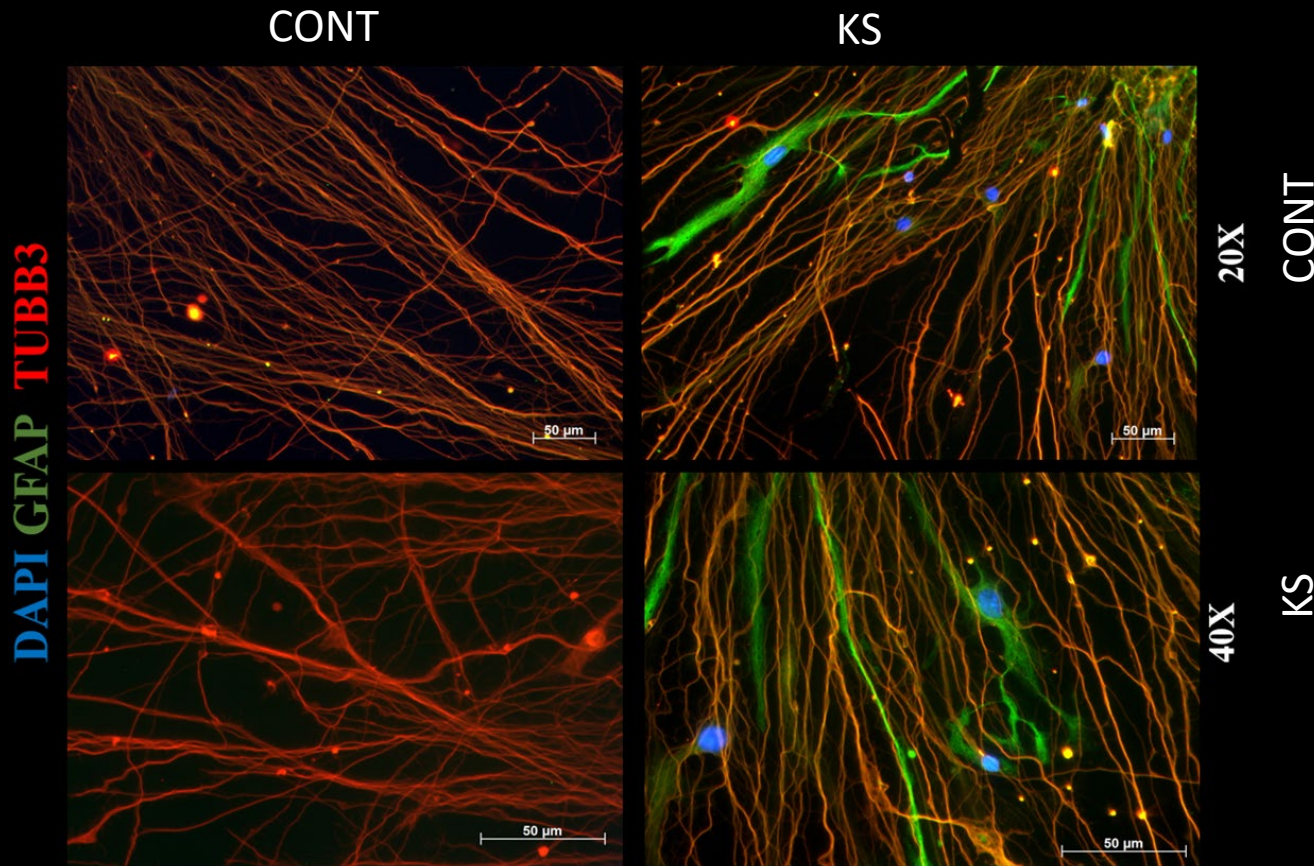
D2, D7, D15, D21, D28 , D35 and
D56 spheroids were analysed

Spheroid formation and growth over time


H3K9me2 expression is lower in KS cells

Neurite outgrowth

CONT

KS

DAPI GFAP TUBB3

20X

40X

Spheroids at TD21 were placed on laminin coated plate and immunostaining was performed for neuronal (TUBB3) and astorcytoc (GFAP) markers.

CONT

KS

4x

Neurite outgrowth of TD56 spheroids placed on laminin coated plate.

# Proliferation and growth of NPCs in control and Kleefstra cell lines
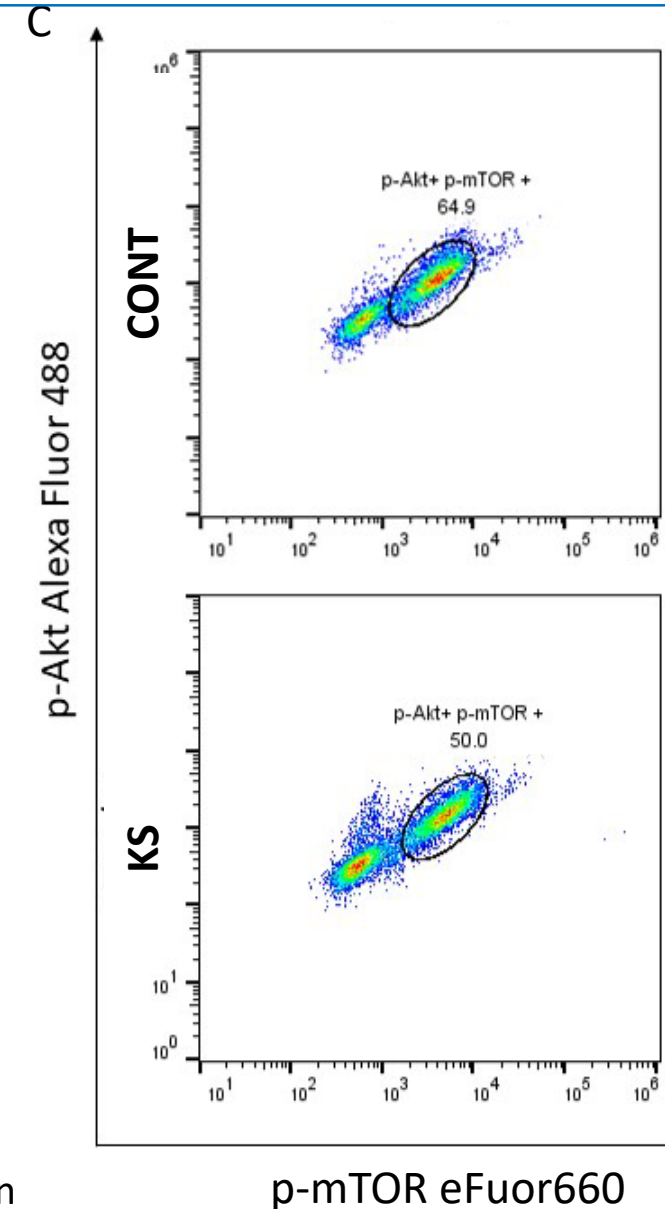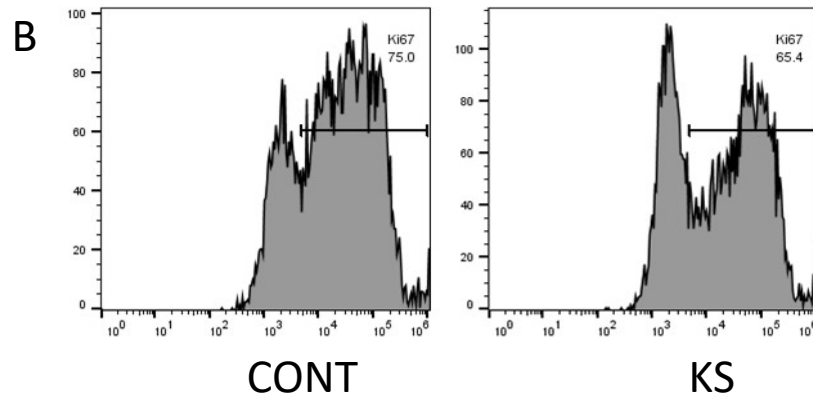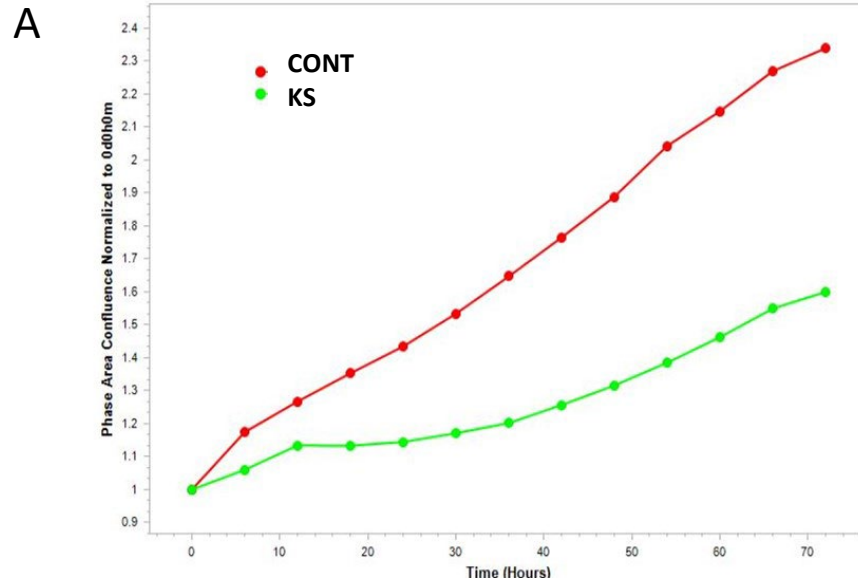
Reduced cell proliferation rate of KS cell line was detected during NPC stage.
A. Change in cell confluence over 72 hrs monitored with Incucyte (Sartorius).
B Ki67 detection using flow cytometry. C. p-Akt and p-mTOR expression in proliferating NPC cells using flow cytometry

# Proliferation and growth of 3D_TD56 in control and Kleefstra cell lines

Flow cytometry analysis of Ki67, p-AKT and p-mTOR
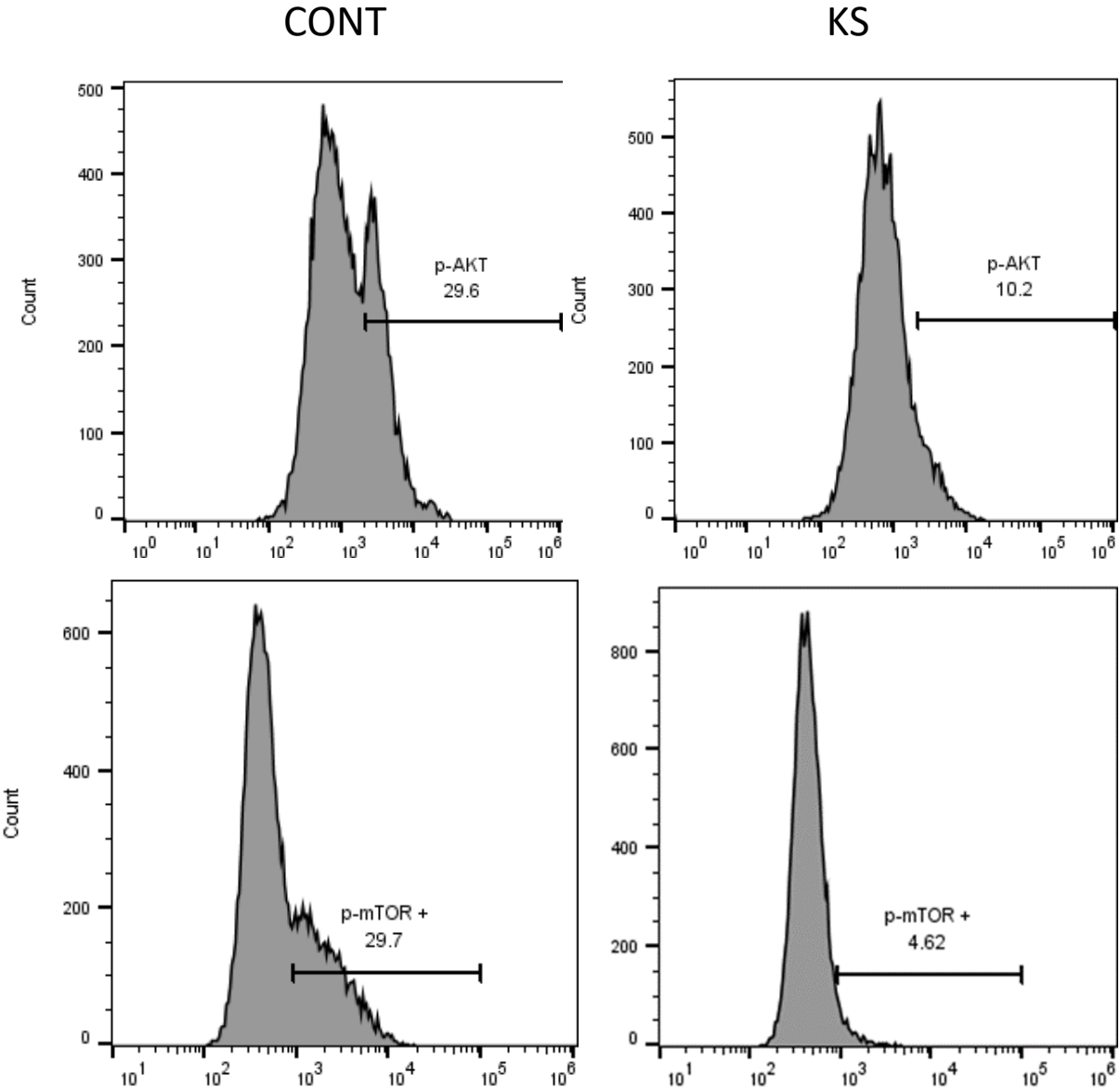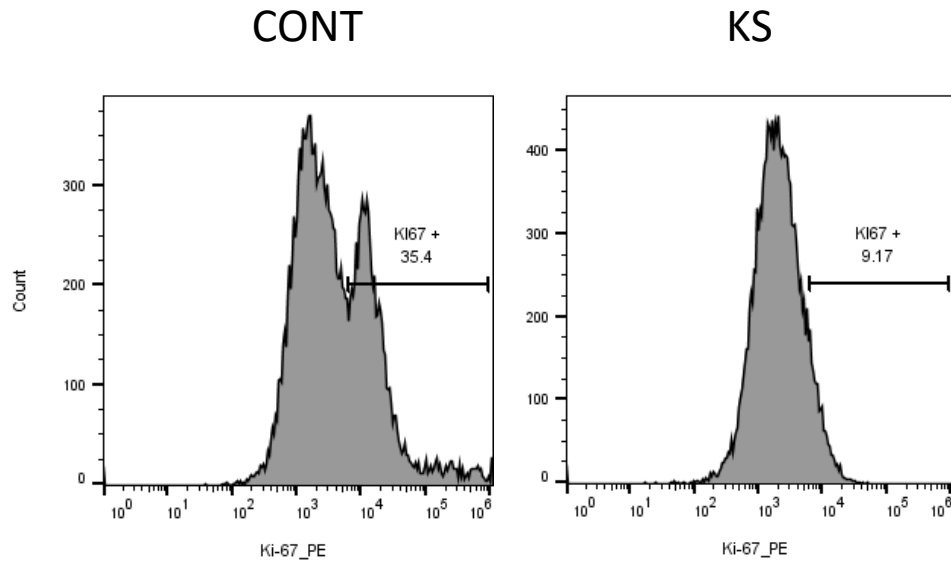
qPCR analysis reveals diffrences in neuronal subtypes of the KS 3D_TD56 spheroids
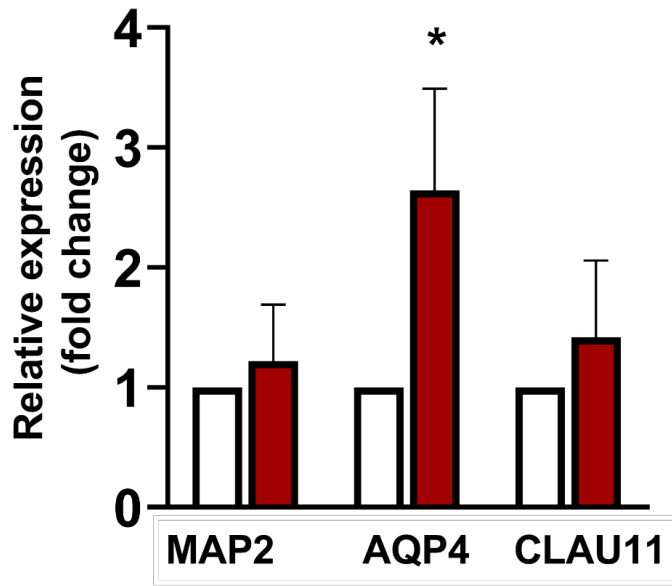

qPCR measurement of main cell types from 3D_TD56 spheroids. Graphs represent normalized relative expression values usind the ddCt method. Mean values and ± SEM of four biological replicates (n=4).
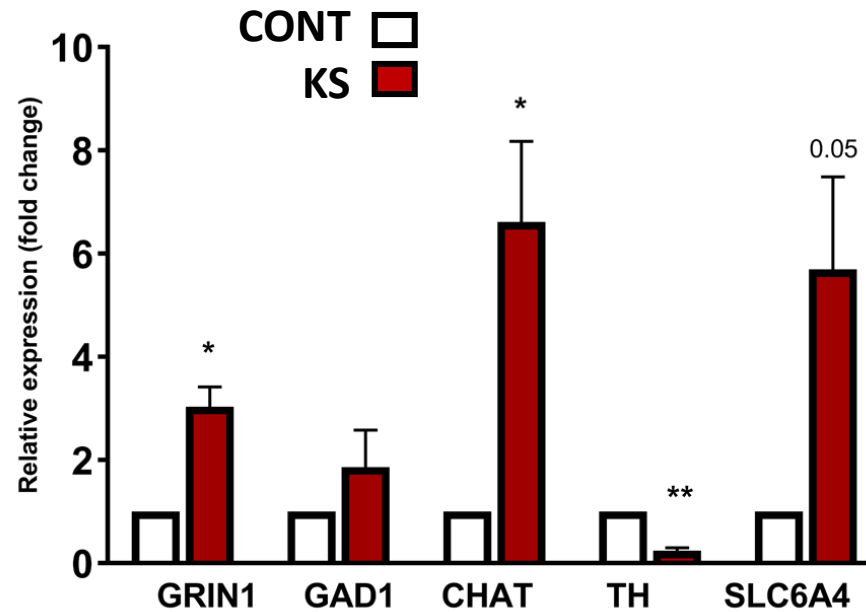
qPCR measurement of neuronal subtypes from 3D_TD56 spheroids. Graphs represent normalized relative expression values usind the ddCt method.
Mean values and ± SEM of biological replicates (*GAD1*: n=3; *GRIN1*, *TH* n=4; *CHAT*, *SLC6A4* n=5). Significance was determined by unpaired *t* test with Welch's correction.

GRIN1:Glutamate Ionotropic Receptor NMDA Type Subunit 1;
GAD1: Glutamate Decarboxylase 1;
CHAT: Choline O-Acetyltransferase;
TH: Tyrosine Hydroxylase;
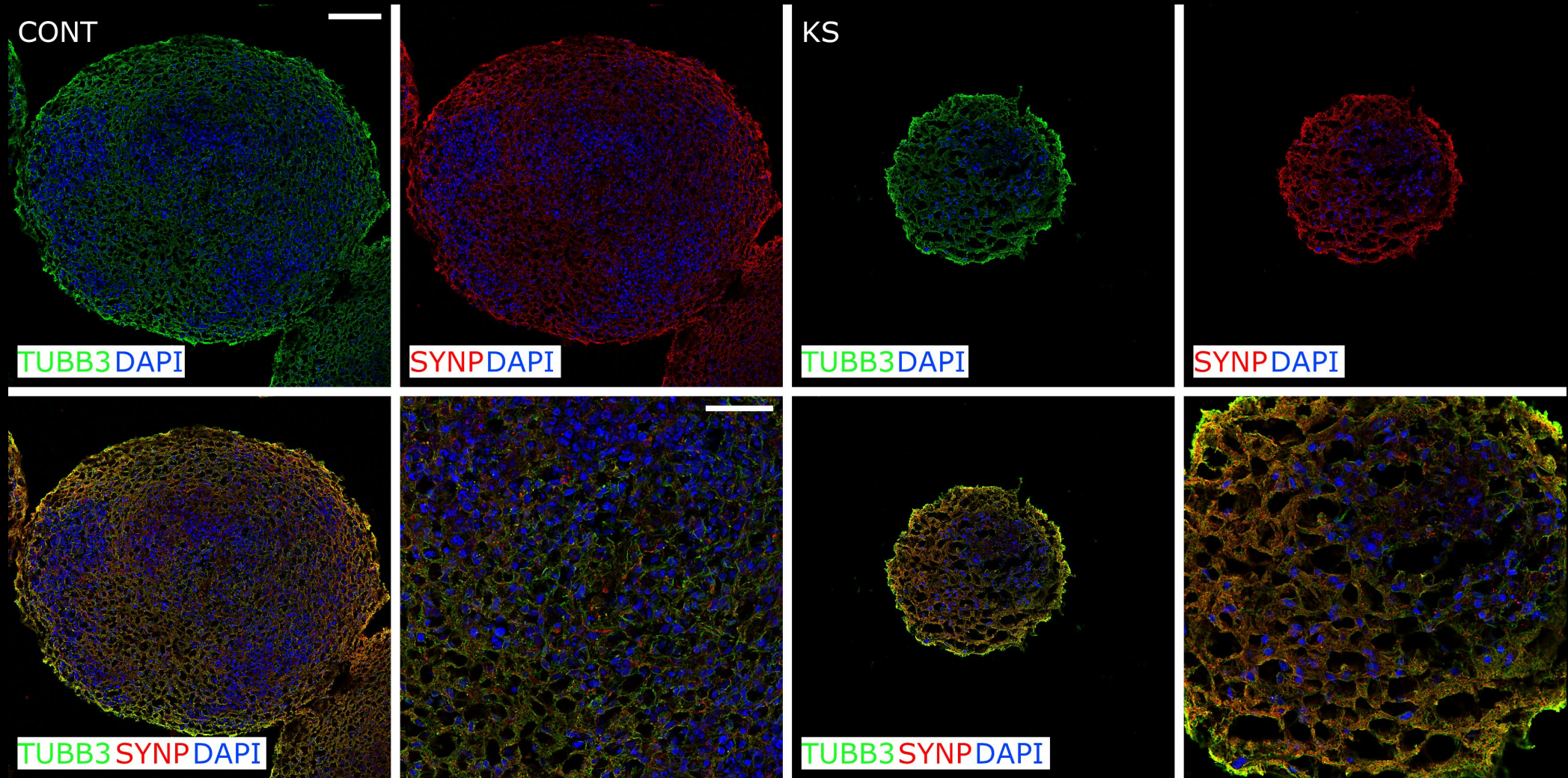*SLC6A4*: Solute Carrier Family 6 Member 4
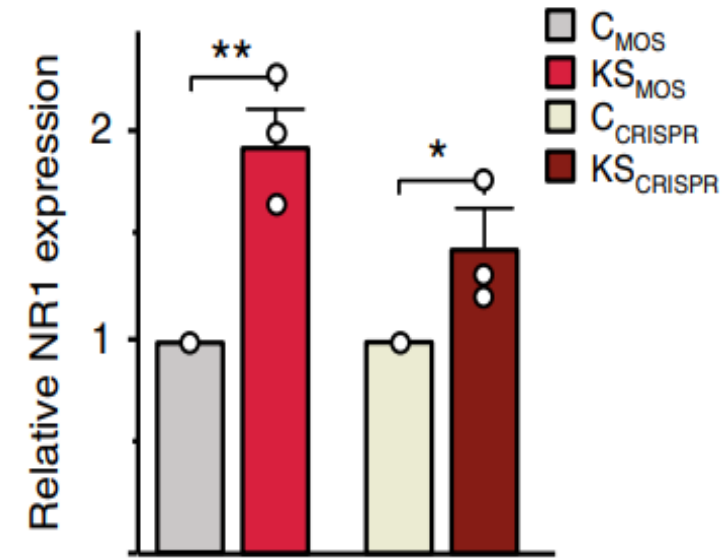
Neuronal network dysfunction in a model for Kleefstra syndrome mediated by enhanced NMDAR signalling
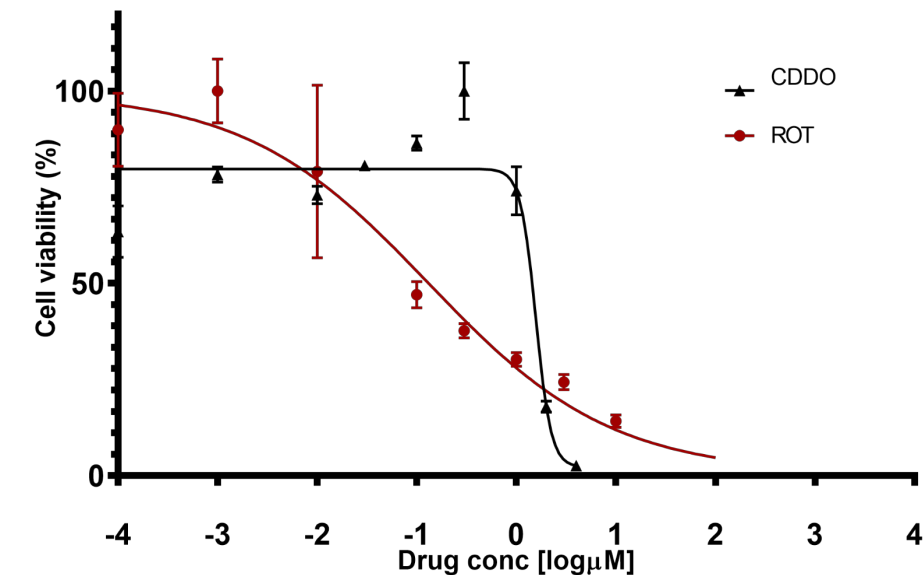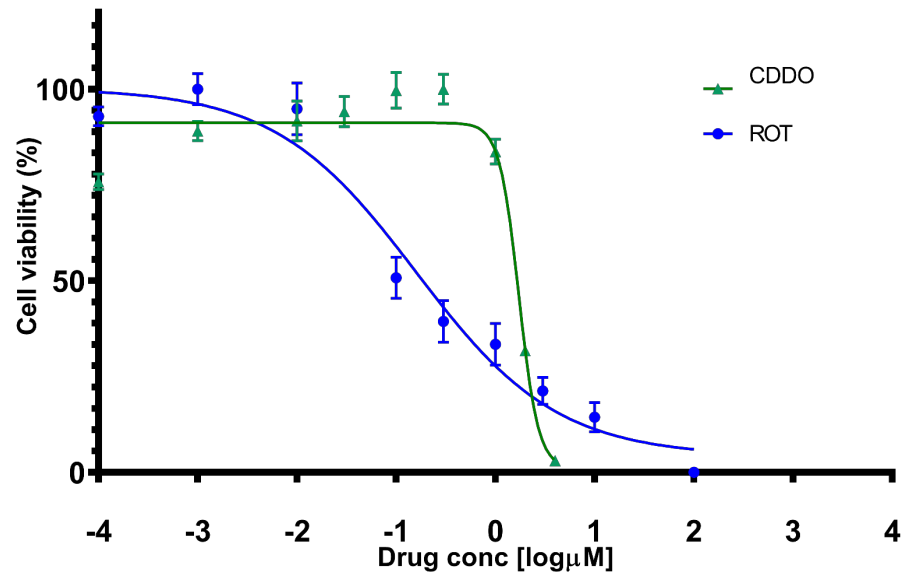
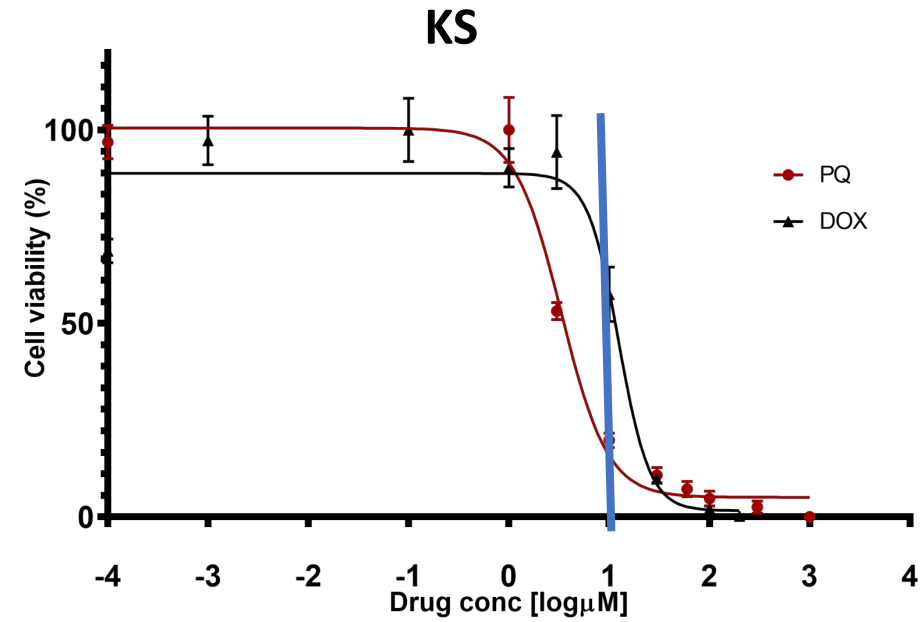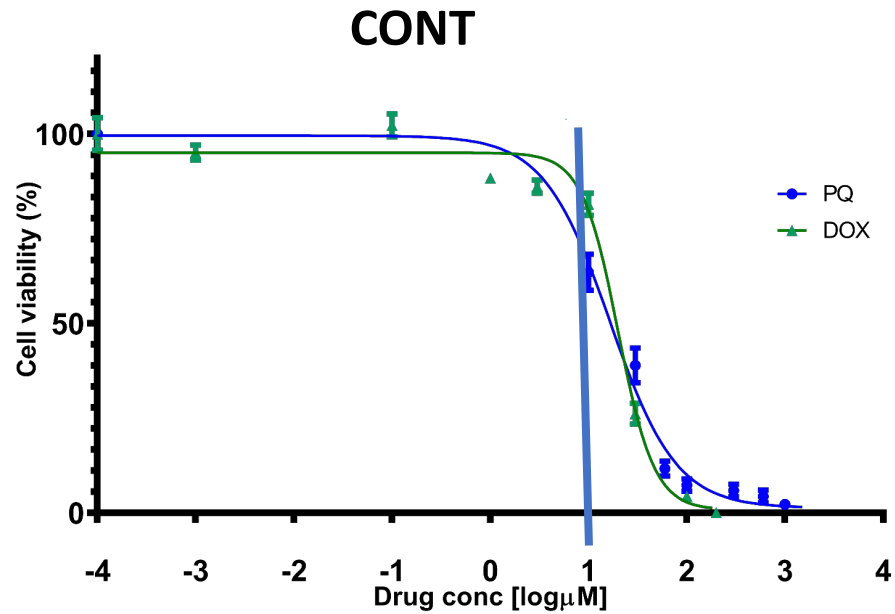*Nature Communications, Frega et al 2019*

ICC staining of the spheroid sections confirms the qPCR data on protein level
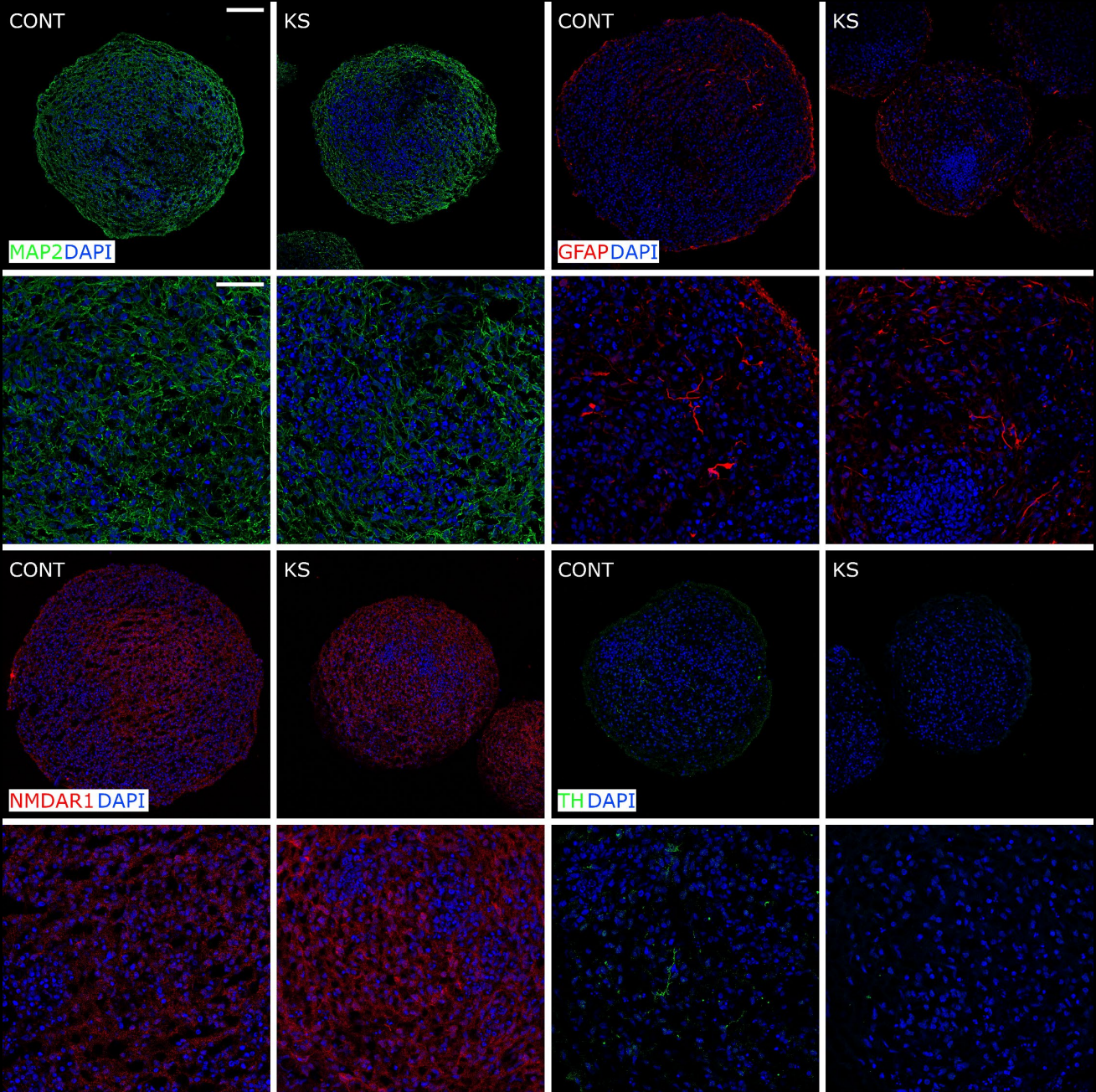
**ICC staining of the spheroid sections confirms the qPCR data on protein level**

Oxidative stress response of control and KS cell lines in 3D_TD56:
72 hours post treatment with doxorubicine, paraquat, bardoxolone and rotenone

Oxidative stress response: EC50 values of control and KS cell lines at NPC and 3Dstage: 72 hours post treatment with doxorubicine, paraquat, bardoxolone and rotenone
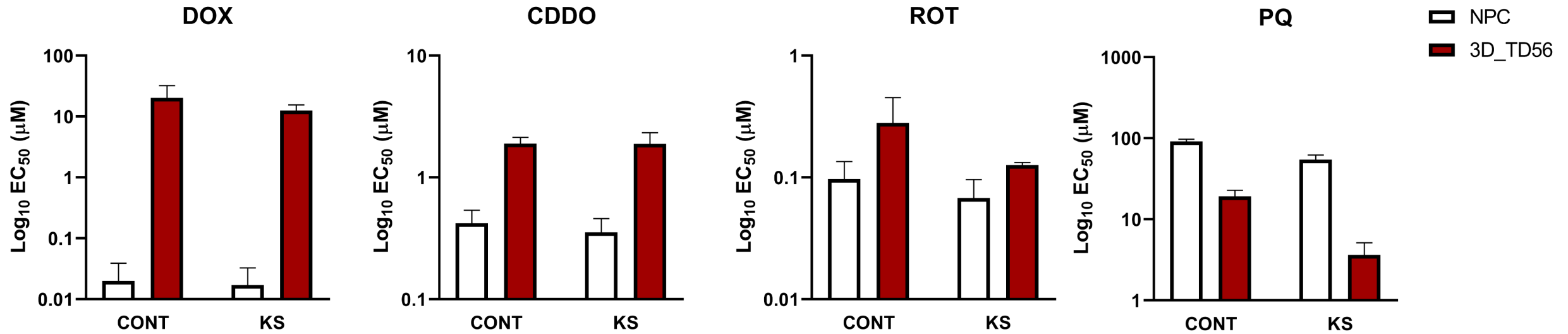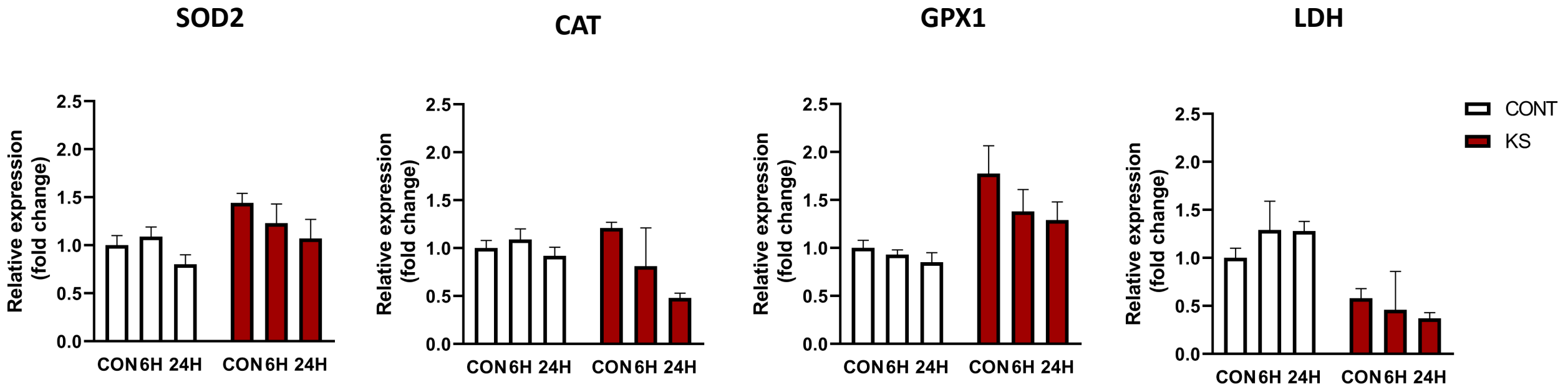

	Doxorubicine		Bardoxolone		Rotenone		Paraquat	
	NPC	3D_T56	NPC	3D_T56	NPC	3D_T56	NPC	3D_T56
CONT	0.02	20.22	0.42	1.89	0.097	0.280	91.59	19.16
KS	0.017	12.54	0.35	1.88	0.067	0.123	54.53	3.63

Fold change in sensitivity to PQ

4.7 x

15 x
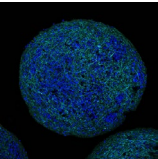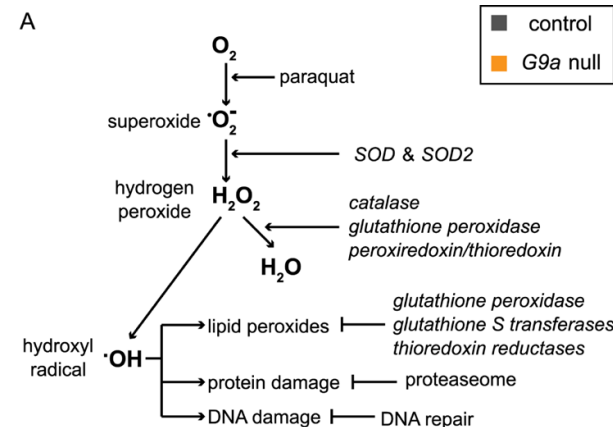
CAT: catalase; GPX1:glutathione peroxidase; SOD2: superoxide dismutase-2; LDH: lactate dehidrogenase

Summary

- The 3D organoid culture is a feasible disease model for drug screening and toxicology assays in KS

- Differences in the subtypes of neurons were assessed

- Altered growth and metabolic pathways identified
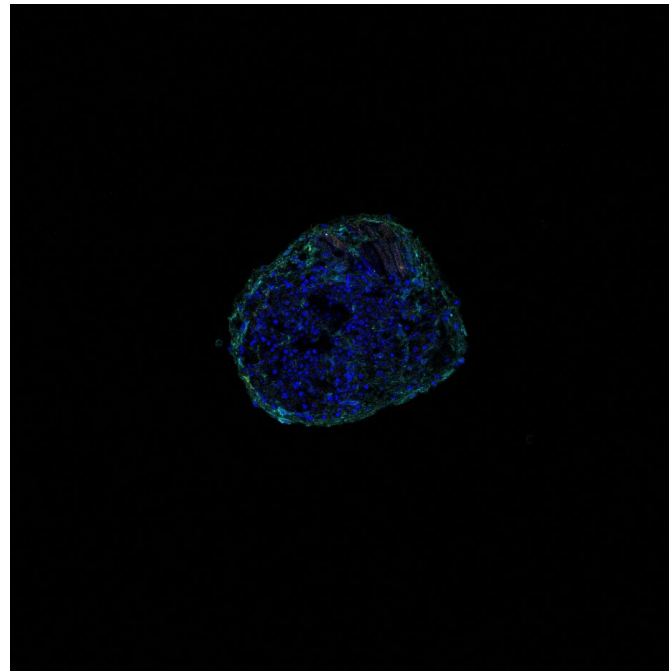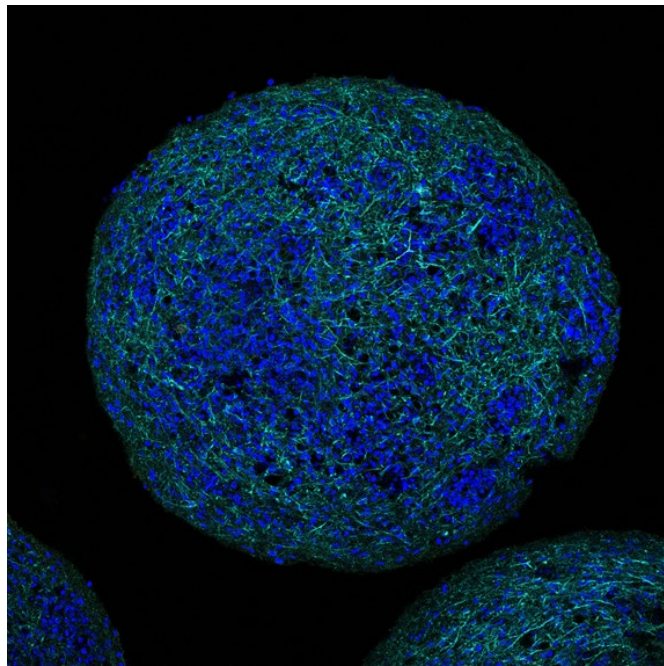
Future experiments:

- Analyse additional cell lines

- Investigate the contribution of astrocytes

and microglia to the diseased phenotype

THANK YOU FOR YOUR ATTENTION

RICHTER GEDEON

Mária Bódi-Jakus, toxicology
Balázs Széky, 3D culture
Anita Fehér, PhD, qPCR
Tamás Bellák, PhD, IHC
Melinda Zana, PhD
András Dinnyés, PhD

www.biotalentum.eu
www.biotalentumconsulting.eu
andras.dinnyes@biotalentum.hu