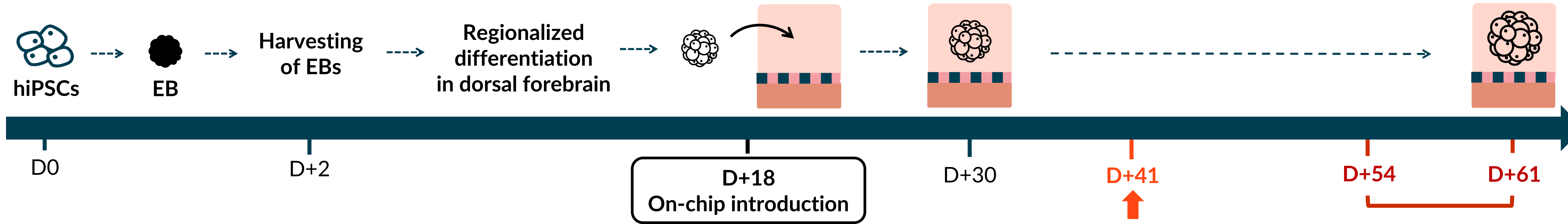


## MATERIALS AND METHODS

### ON-CHIP CULTURE CONDITIONS & COMPOUND EXPOSURES

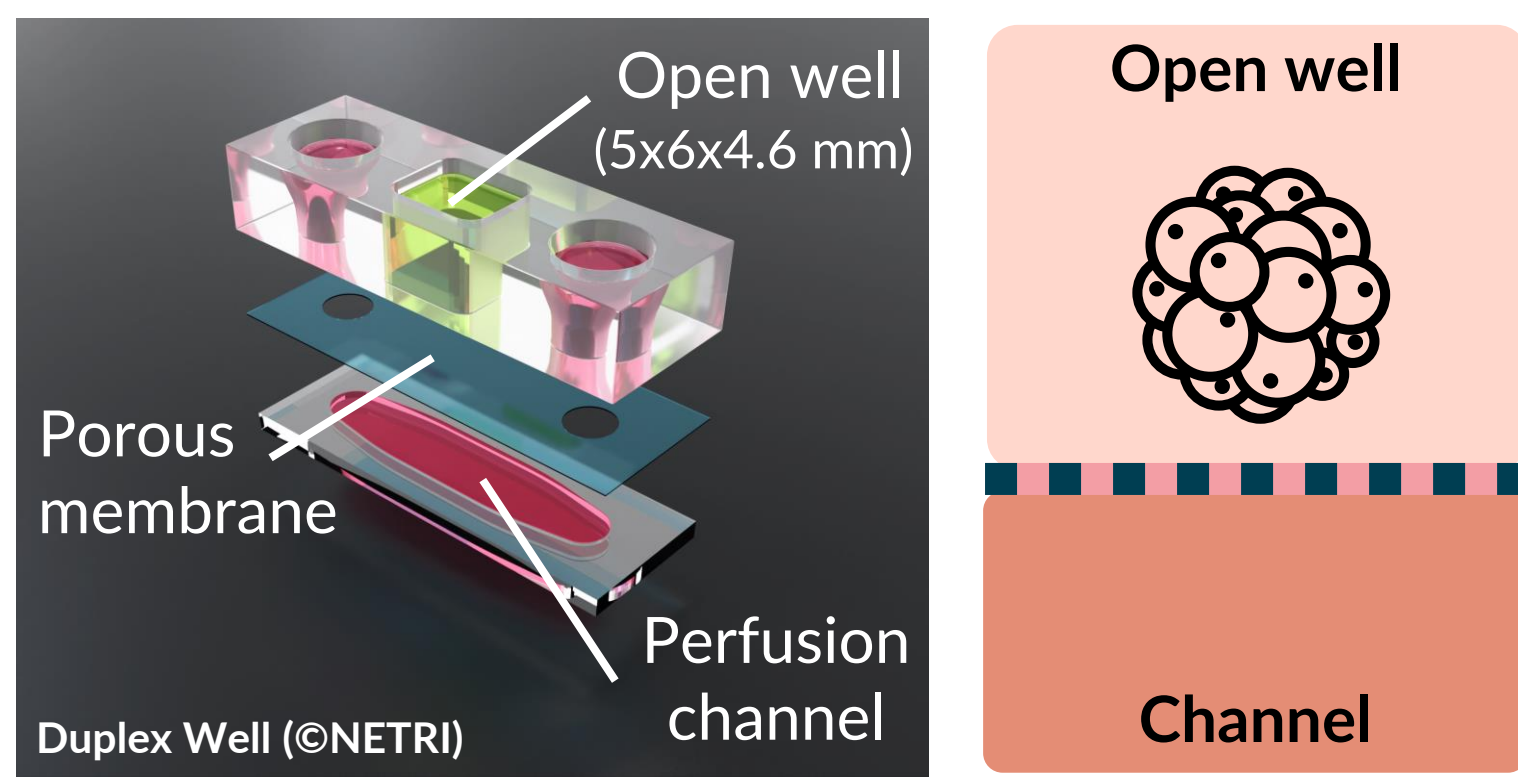


### NETRI's MICROFLUIDIC DEVICE

Adapted to 3D cell culture:  
Two compartments separated by a porous membrane:

- Open well for 3D culture
- Perfusion channel

Adapted to industrial transfer  
Pumpless



**Acute exposure (24h):**  
**Vanillin** (CAS n 121-33-5): 100, 1 000, 10 000 nM  
**Biphenyl-2-ylamine** (CAS n 90-41-5): 20, 200, 2 000  $\mu$ M (meOH)

**Chronic exposure (7 days):**  
**Biphenyl-2-ylamine:** 200  $\mu$ M (meOH)

**Controls:** non-exposed and vehicle-exposed organoids

Timeline of cortical organoid generation and culture protocol (adapted from [2]), on-chip culture conditions, compound exposures, and Duplex Well schematic representations (hiPSCs: human induced pluripotent stem cells, EB: embryoid body).

### QUALITY SCORING

- Cortical organoid characterization at D+60
- Scoring scale: 5 to 0 (from most to least optimal)

### EXPOSURE SCORING

- For compound-exposed cortical organoids (acute & chronic)
- Compared to controls

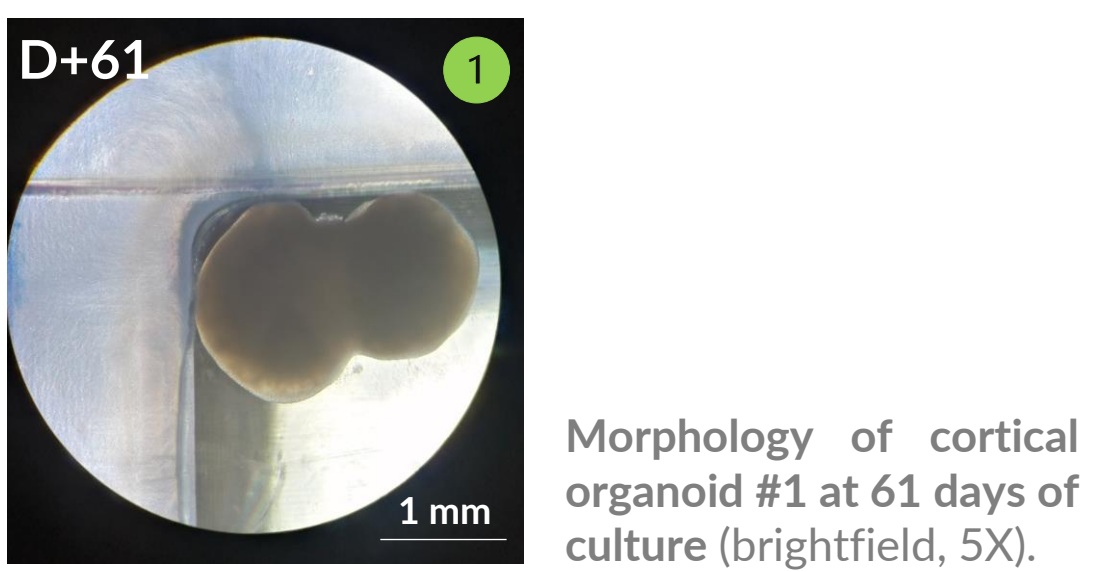
### PREDICTION ALGORITHM

- For compound classification into 3 neurotoxicological categories

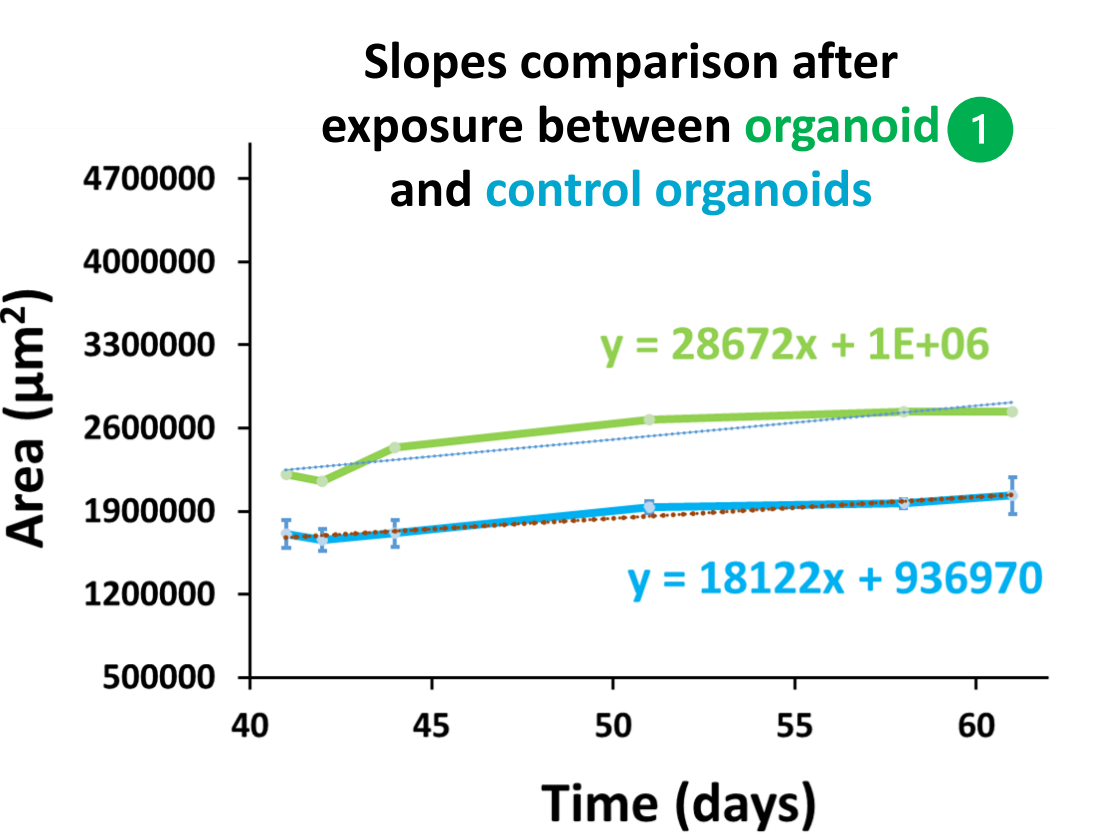
## RESULTS COMPOUND CLASSIFICATION USING THE PREDICTION ALGORITHM

Example 1: acute exposure with 10 000 nM vanillin

Morphology: optimal

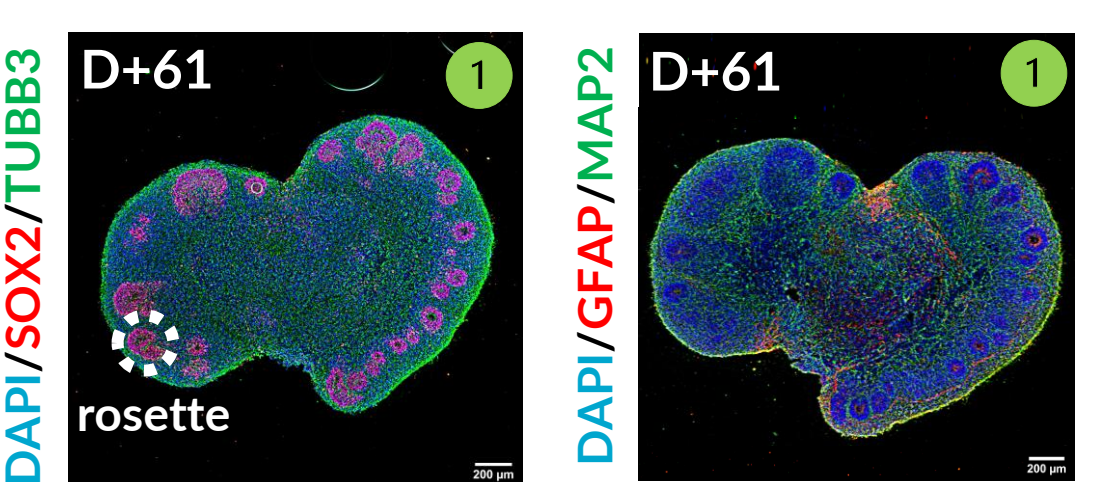


Growth profile: similar compared to controls

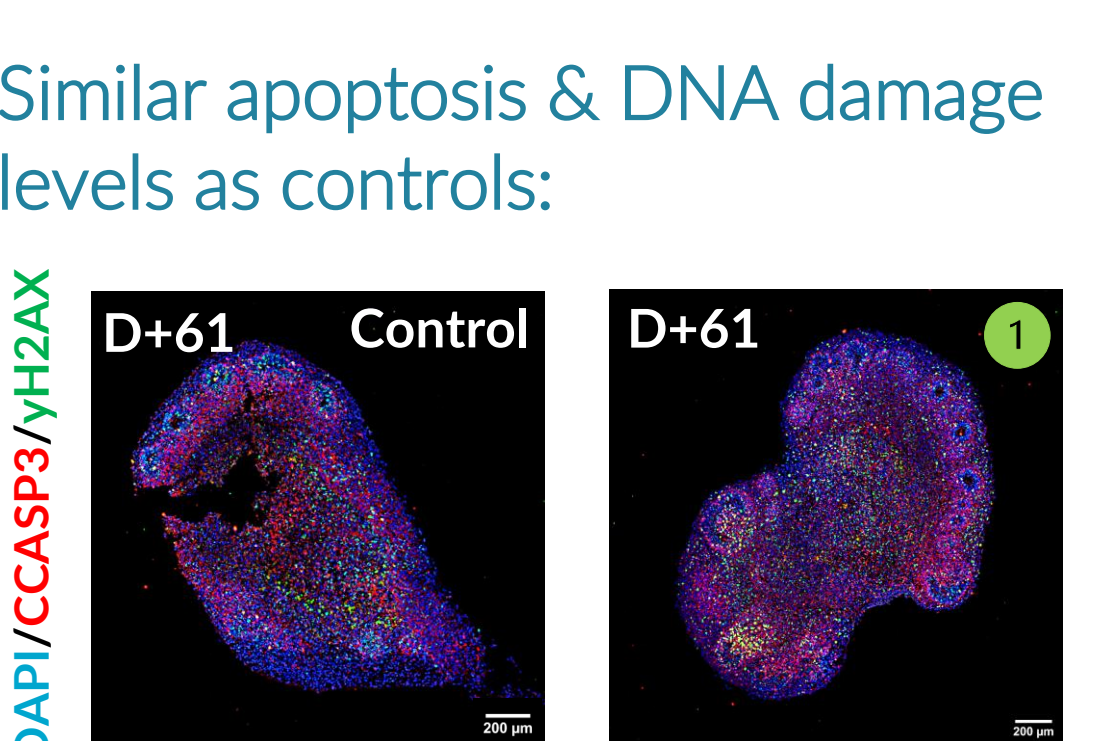


Cortical organoids growth curves and slopes between exposure (D+41) and end of culture (D+61) (for controls: mean  $\pm$ SEM, n=4).

Expected cell types and optimal cytoarchitectural organization:



Similar apoptosis & DNA damage levels as controls:



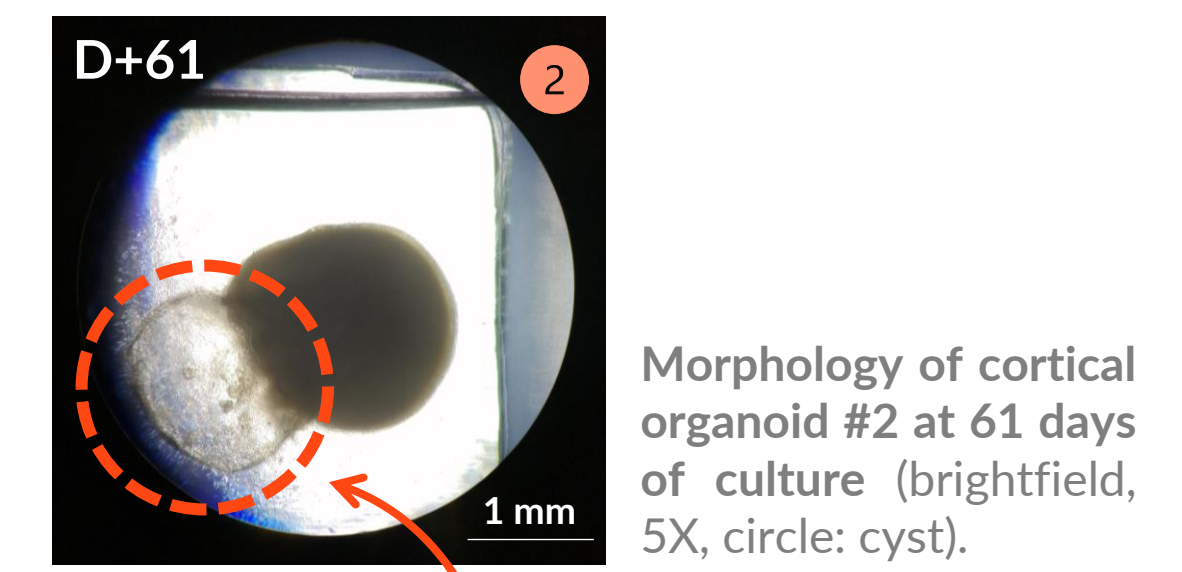
Immunofluorescence staining of apoptosis (CCASP3) and DNA damage (yH2AX) (Thunder microscope, Leica, objective 20X).

### Morphology

- Overall color and structural density/compactness: 1 and 2 = score 4
- Border integrity: 1 and 2 = score 4
- Presence/absence of cysts: 1 = score 4; 2 = score 2

Example 2: acute exposure with 2 000  $\mu$ M biphenyl-2-ylamine

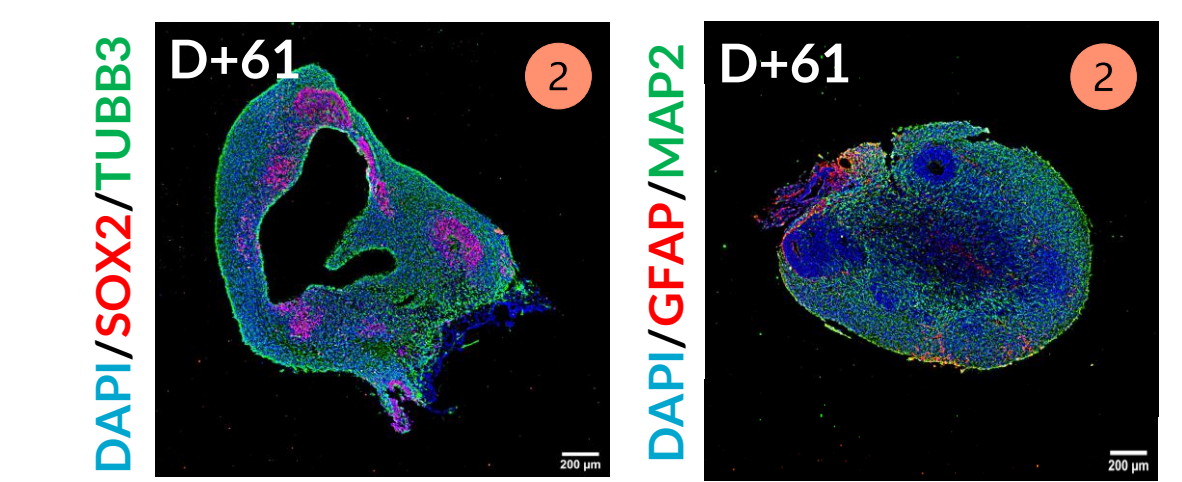
Morphology: altered



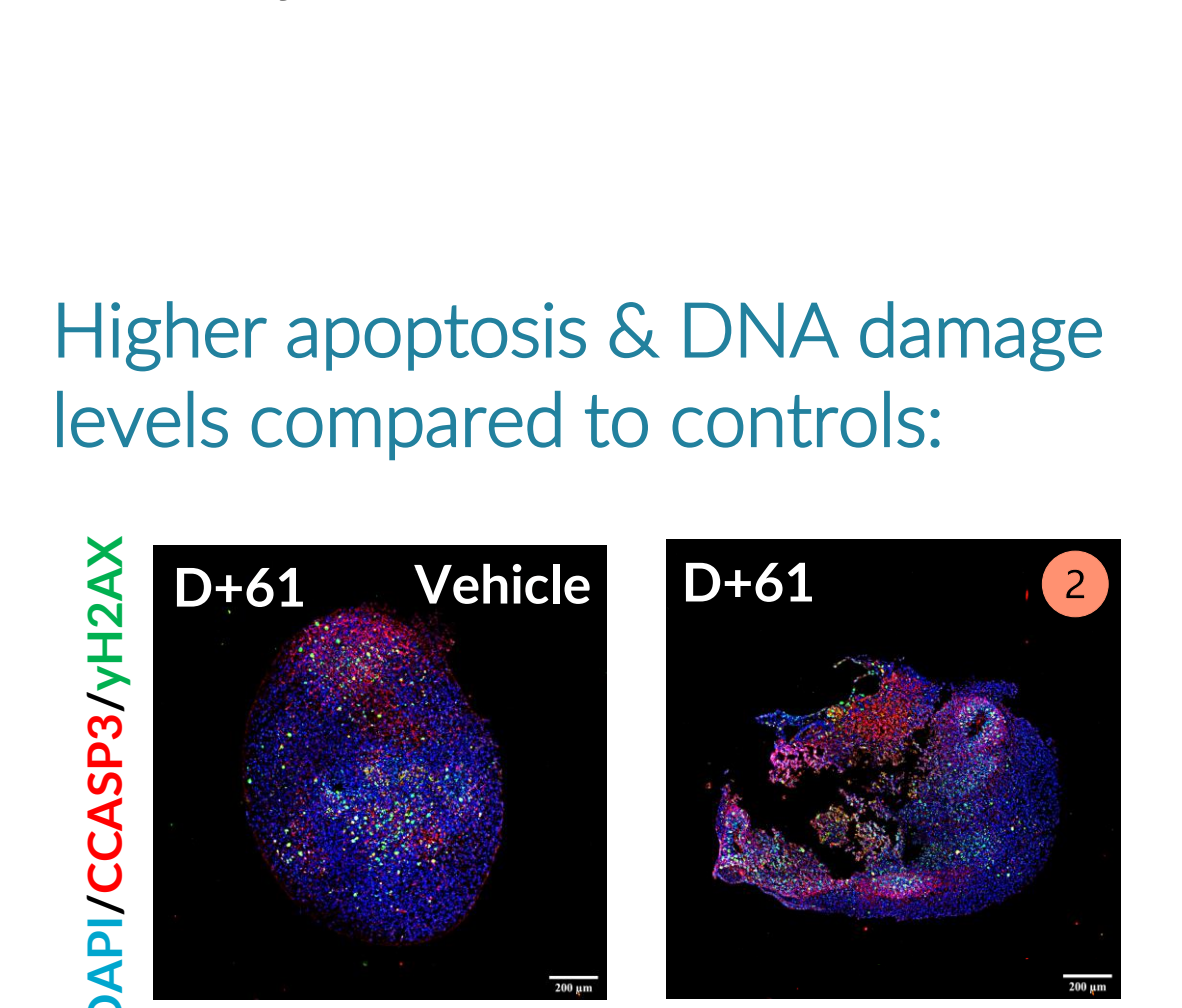
Presence of a large cyst (> 25% of total surface area)

Expected cell types, but disorganized cytoarchitectures:

- Altered pattern of neurogenic areas (rosettes)
- Presence of a cyst and a large zone without cells (necrotic core)



Higher apoptosis & DNA damage levels compared to controls:



Immunofluorescence staining of apoptosis (CCASP3) and DNA damage (yH2AX) (Thunder microscope, Leica, objective 20X).

All scores  $\geq$  4

**Growth profile**  
Slope between exposure and end of culture timepoints: similar to controls ( $\pm$  20 000 units)

**Cellular populations**  
Presence of the three cell types: neural progenitors, neurons & astrocytes

Proportion of each cell type similar to controls

Astrocyte reactivity similar to controls

Cellular density similar to controls

Rosettes at least similar to controls

Cysts and zones without cells at least similar to controls

Apoptosis and DNA damage markers similar to controls

Low Concern Compound

Potential Concern Compound

High Concern Compound

## CONCLUSION

- Brain Organoid-on-Chip platform + Scorings + Prediction Algorithm: adapted to neurotoxicity evaluations
- Vanillin exposures: no discernable impact on morphology, cytoarchitectures & viability  $\rightarrow$  low concern
- Biphenyl-2-ylamine exposures: altered morphology & disorganized cytoarchitectures in a dose-response manner  $\rightarrow$  high concern

## PERSPECTIVES

- Implementation of additional criteria for organoid cytotoxicity characterization
- Paves the way for neurotoxicological studies & drug screening