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#RESEARCHNEVERSTOPS

Cellular Assays and Stem Cell Research for HD

FOR FURTHER INFORMATION:

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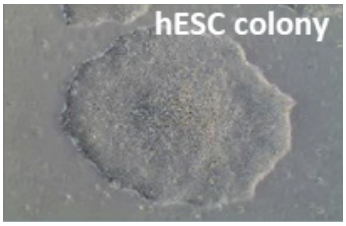
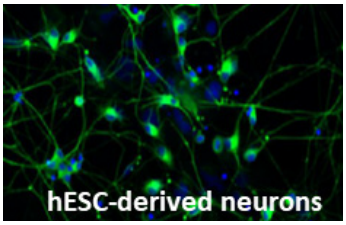
(STEM) CELL RESEARCH AT EVOTEC

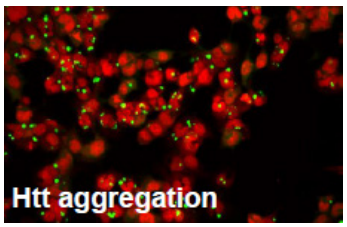
- Long-standing experience in stem-cell-based reseach. From human ES cells to fully differentiated (motor/cortex) neurons, astrocytes and microglia. Protocol for generation of midbrain dopaminergic neurons under development.
- Access to iPSC lines, expansion, banking and differentiation. Access to several cell lines and ability to generate primary neurons and knockdown or overexpress targets of choice.
- Assay development and scale-up for phenotypic screening possible. Ability to perform various assays such as phenotypic screens, target-directed expression (or knockdown) by AAV, Mechanism of Action studies, electrophysiology, -omics approaches, high-content imaging and screening, and expression profiling.

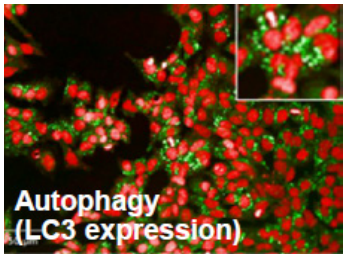




HIGH-CONTENT SCREENING/IMAGING AND BIOCHEMICAL ASSAYS

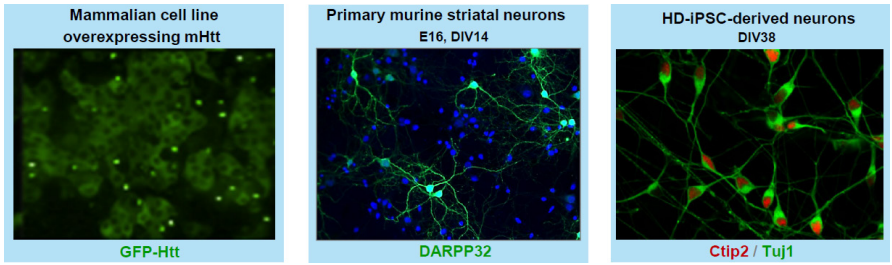
- Using imaging and IHC to access complex read-outs such as subcellular events of protein aggregation and localization, post-translational modification, and protein levels. Enabled to look at cellular morphology (neurite outgrowth, spine morphology) and cell survival and autophagy (example images of HTT aggregation and LC3 expression shown).
- Can be used for, in example, siRNA screening, CRISPR, AAV knockdown and overexpression, and assessing the effect of compound treatment.
- Multiple technologies available and established in different cellular system for HD: Phenix Opera system, HCI, MSD and Singulex assays, TR-FRET assays for Htt measurements, autophagy assays, IHC options, and so on.





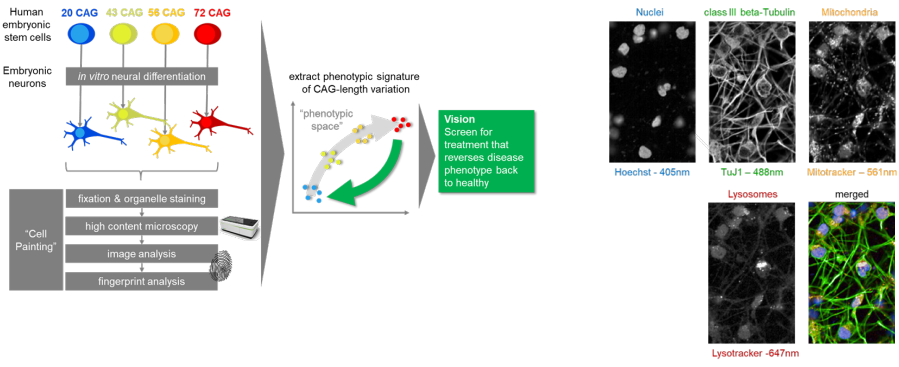
MULTIPLE *IN VITRO* PLATFORMS ESTABLISHED TO RESEARCH HD

- Different cell lines available overexpressing mHtt with Htt aggregate formation
- Primary cortical/striatal neuronal cultures from HD transgenic mice and rats
- iPSC-derived neurons from healthy controls and HD patients
- Protocol for generation of Medium Spiny neurons from human control and HD fibroblast lines under development.



CASE STUDY HUNTINGTON DISEASE – USING CELL PAINTING TO EXAMINE A HD PHENOTYPE

- Cell Painting Method: Use of different cell lines, i.e. human ES cell lines genetically modified for expression of mutant HTT with different CAG repeat length to generate cortical- and striatal-like neurons, and comparing them by different staining methods to identify a unique fingerprint.
- Can be used to either find specific "disease" phenotypes or help with deconvolution of the mechanism of action of compounds (by comparing to known Mechanism of Action).
- One hypothesis that is being investigated is whether mHTT can induce morphometric changes in early cortical neurons of HD vs WT genotype. See poster at Conference: Cavaleri et al, 2019



CASE STUDY 2 HUNTINGTON DISEASE – PRIMARY NEURONS AND TARGET VALIDATION

- Primary cerebellar neurons: Overexpression of target of interest and follow-on ICC analysis to confirm expression.
- Electrophysiological properties of the cells and confirm the impact of the target on such parameters.