

An approach to capture perturbations of the CREB pathway as an example of MoA driven in vitro neurotoxicity

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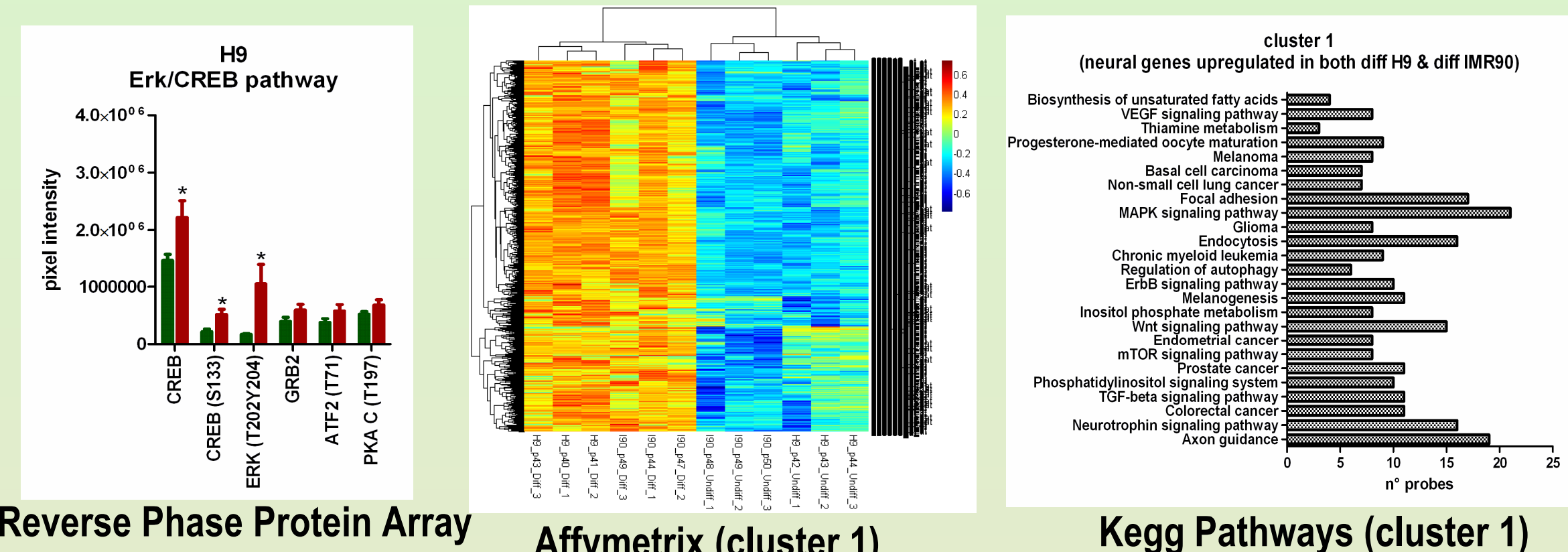
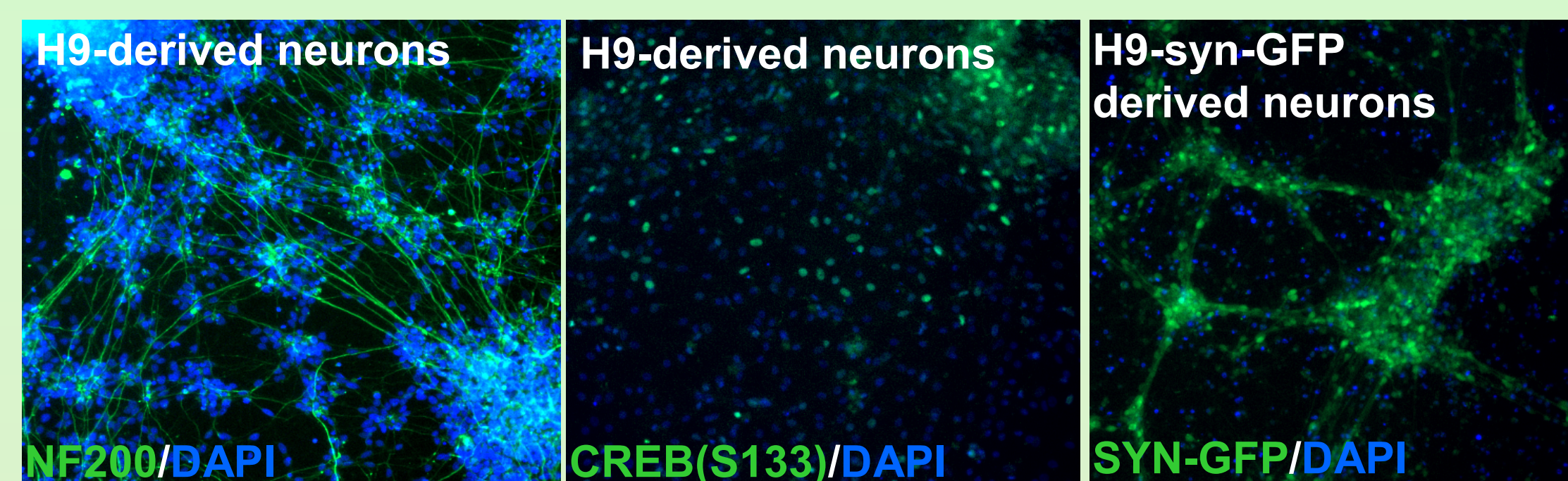
1 Selection of pathway and the definition of endpoints that should be measured by selected readouts

CREB-pathway

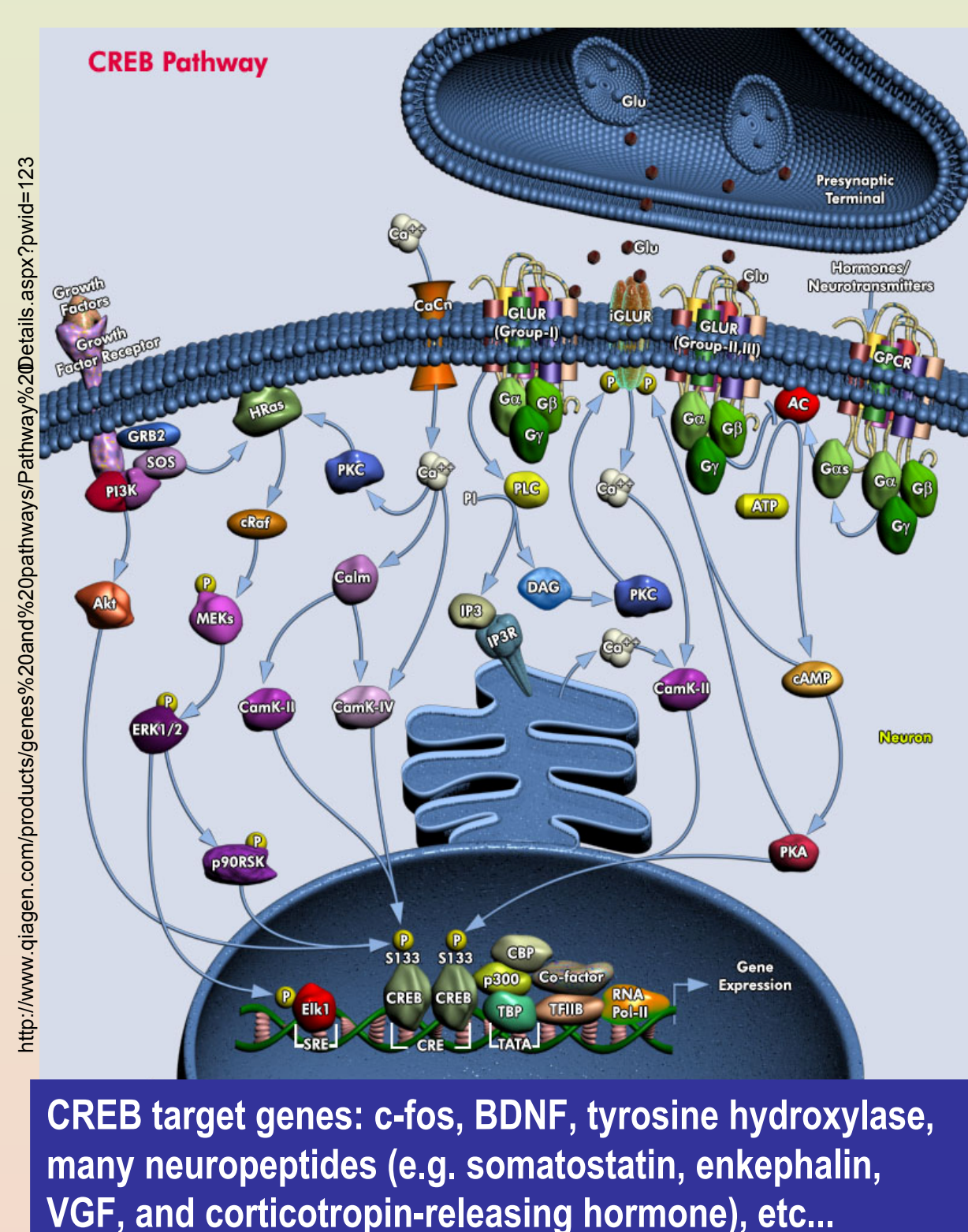
- neuronal survival
- long-term synaptic plasticity
- neuronal excitability
- neurite outgrowth
- adult SVZ neurogenesis
- long-term potentiation
- drug addiction
- affected by: organophosphates, dieldrin, Ni²⁺, tributyltin, trimethyltin
- downregulated by: ROS & β -Amyloids

Selected endpoints	Readouts
Synaptogenesis	HCI
Neurite outgrowth	HCI
Electrical activity	MEA
% of neural/neuronal cells	HCI
Pathway activation	qPCR/RPPA
Transcriptional regulation	qPCR

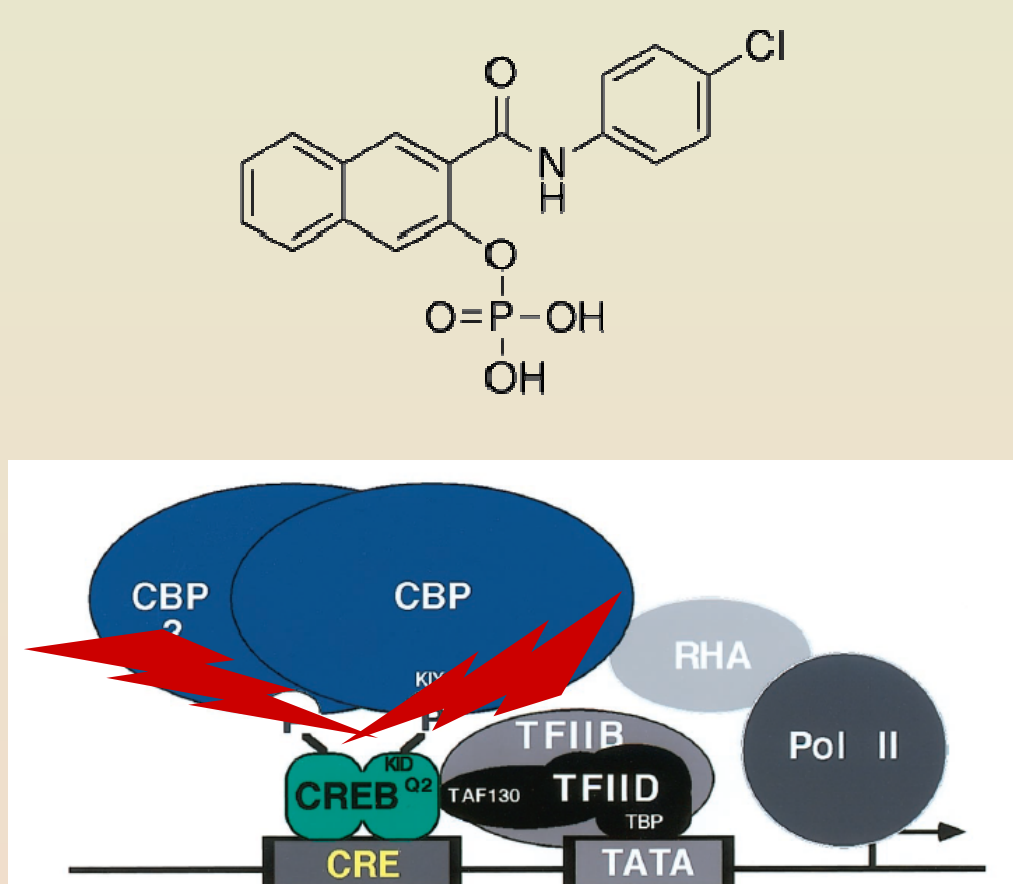
2 Selection of cellular model expressing the physiological pathway



3 Selection of appropriate specific modulators to assess the dynamics of the pathway



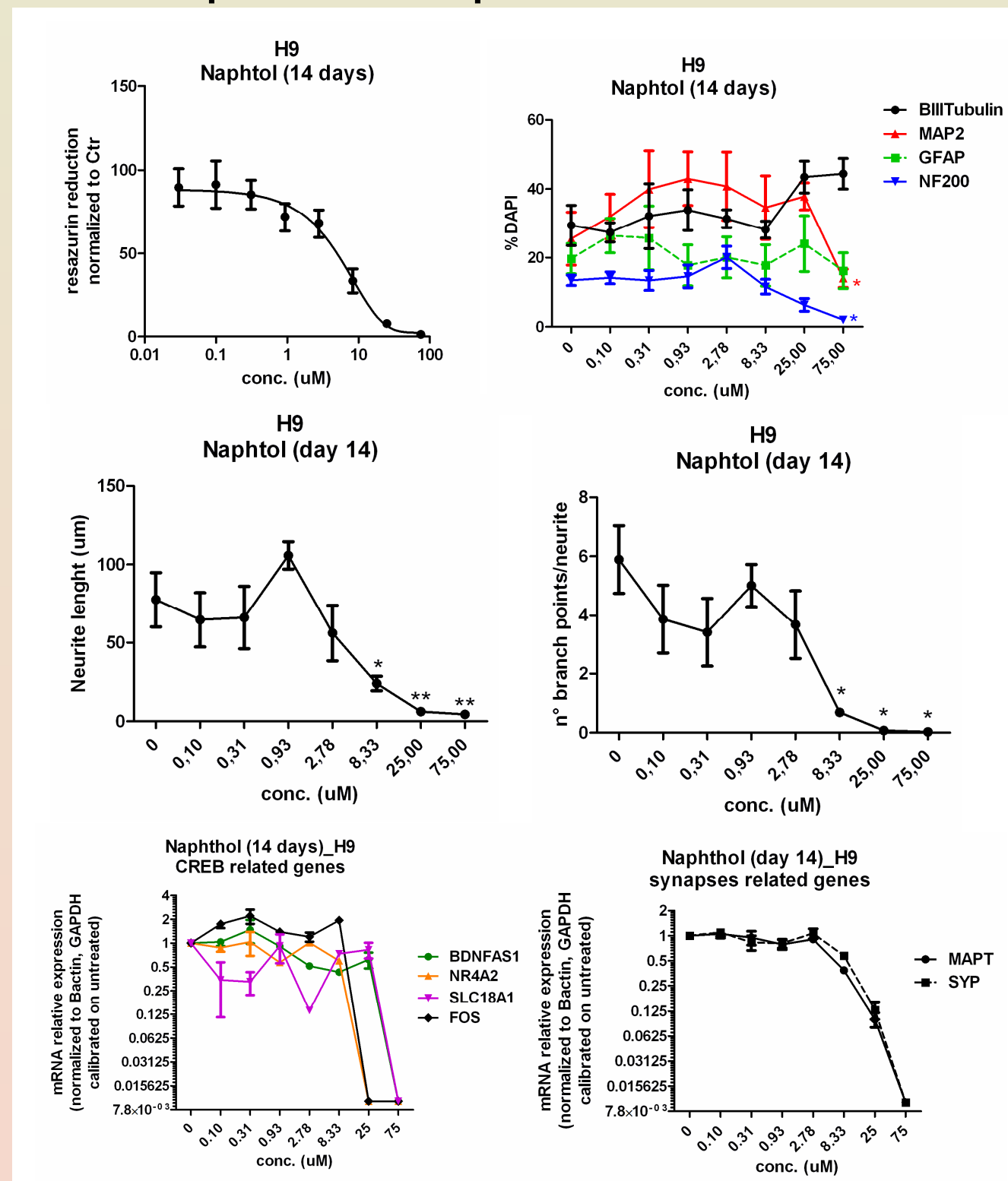
Naphthol AS-E
→ Disruption of CREB:CBP complex
→ attenuated target gene transcription.



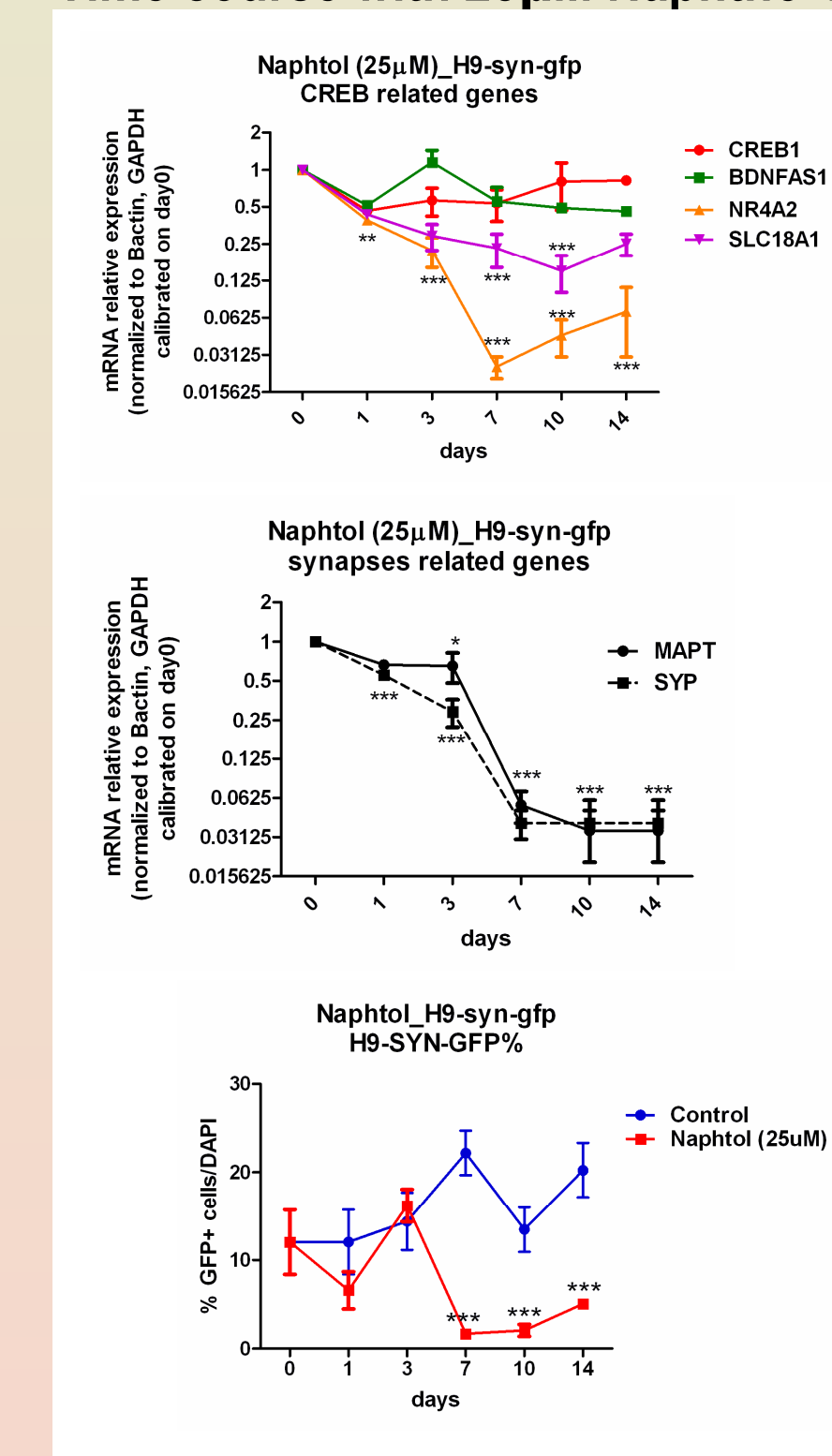
Phosphorylation CREB at Ser-133 triggers gene expression via the recruitment of CREB binding protein (CBP) and others to the promoter.

4 Parameters for in vitro test development

Preliminary exps (H9):
Dose-response with Naphthol AS-E

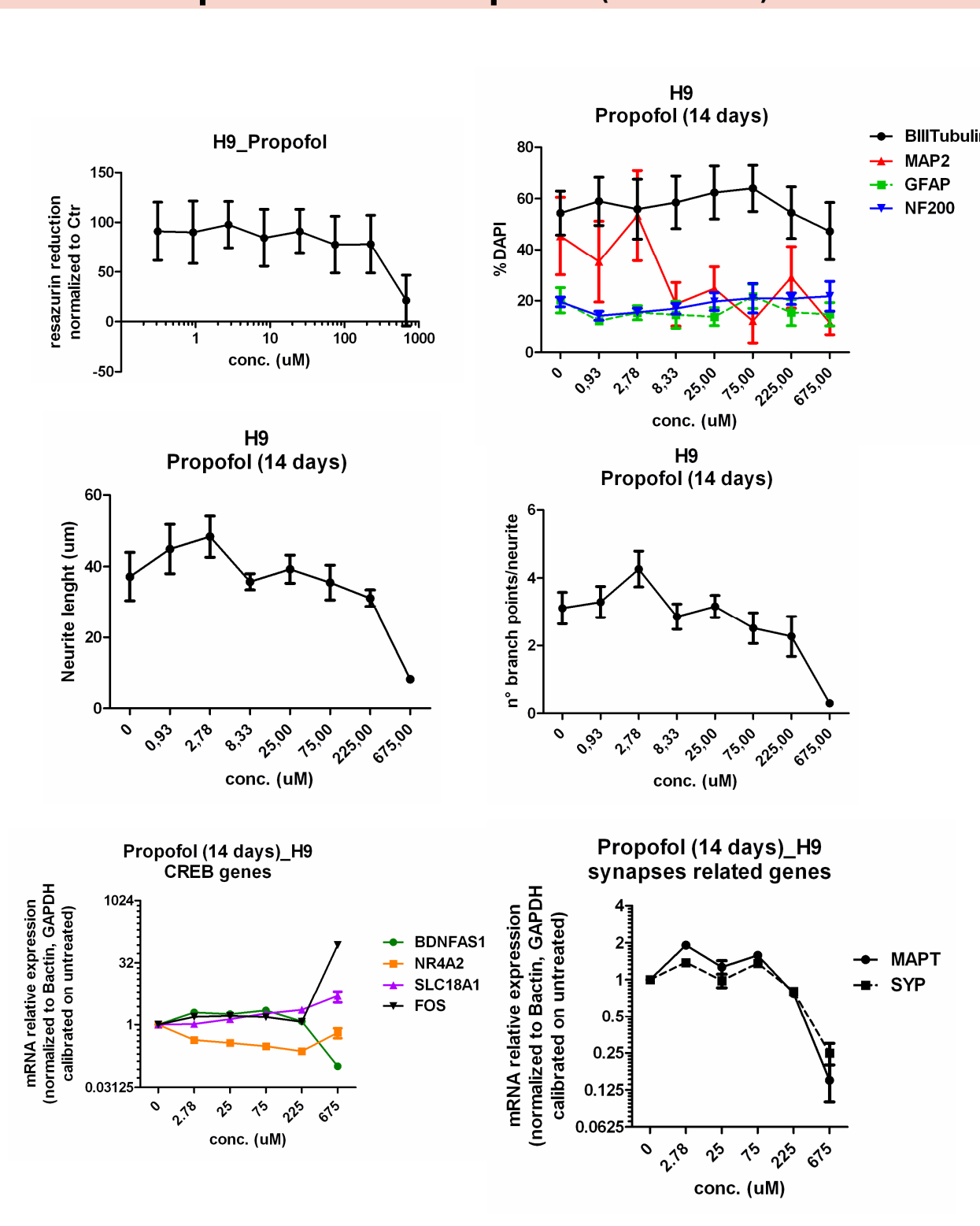


Preliminary exps (H9):
Time course with 25 μ M Naphthol AS-E

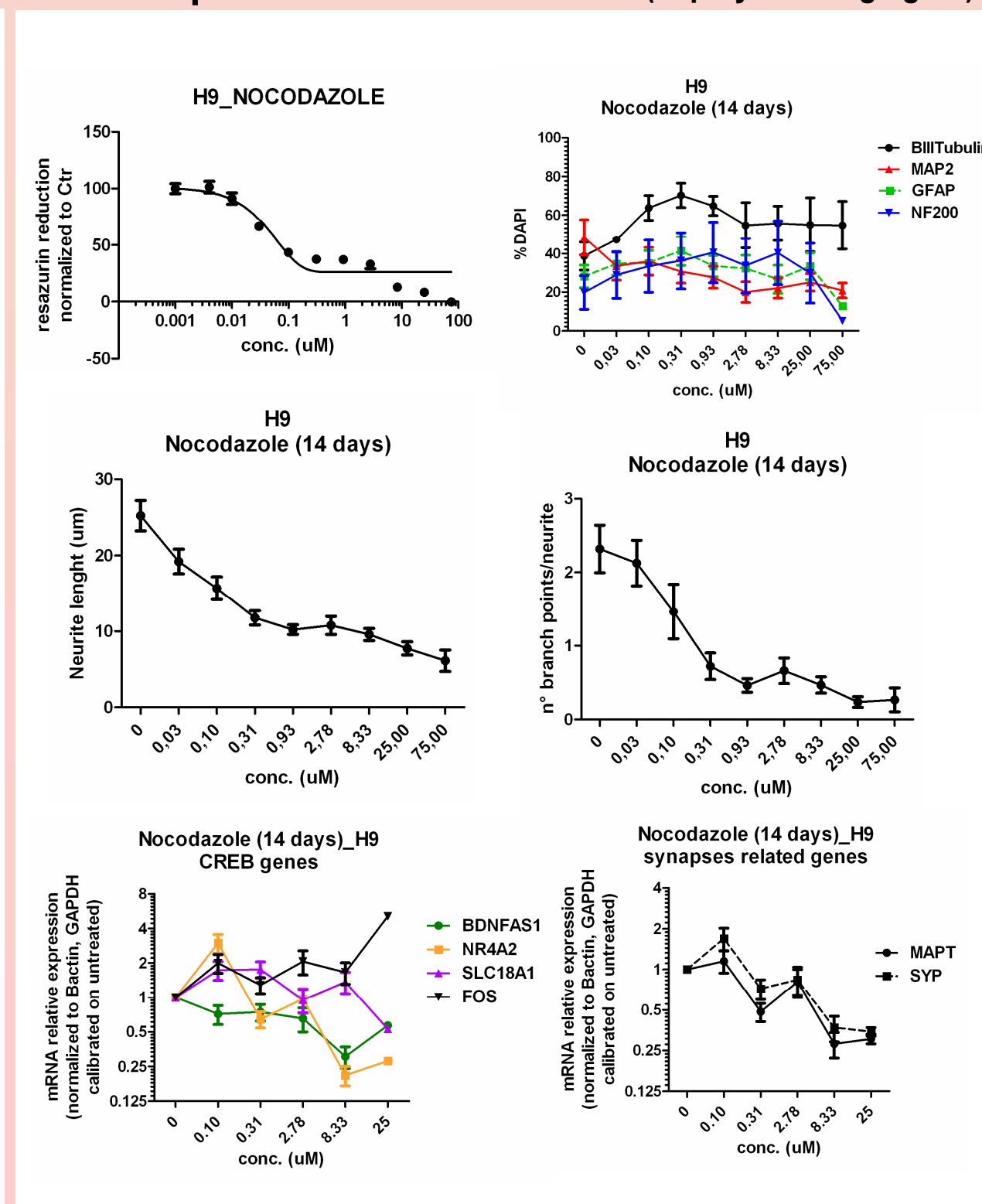


5 Selection of training set of compounds

Preliminary exps (H9):
Dose-response with Propofol (anesthetic)



Preliminary exps (H9):
Dose-response with Nocodazole (depolymerising agent)



6 Development of HTS approaches



HTS facility in Systems Toxicology Unit at the IHCP (JRC)



Abi Prism 7900 (microfluidic qPCR)

Cellomics ArrayScan (HCI)