



Characterization of SNL identified BBB-penetrating moieties (BBB PMs) for delivery of therapeutic cargo to the CNS

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Abstract:

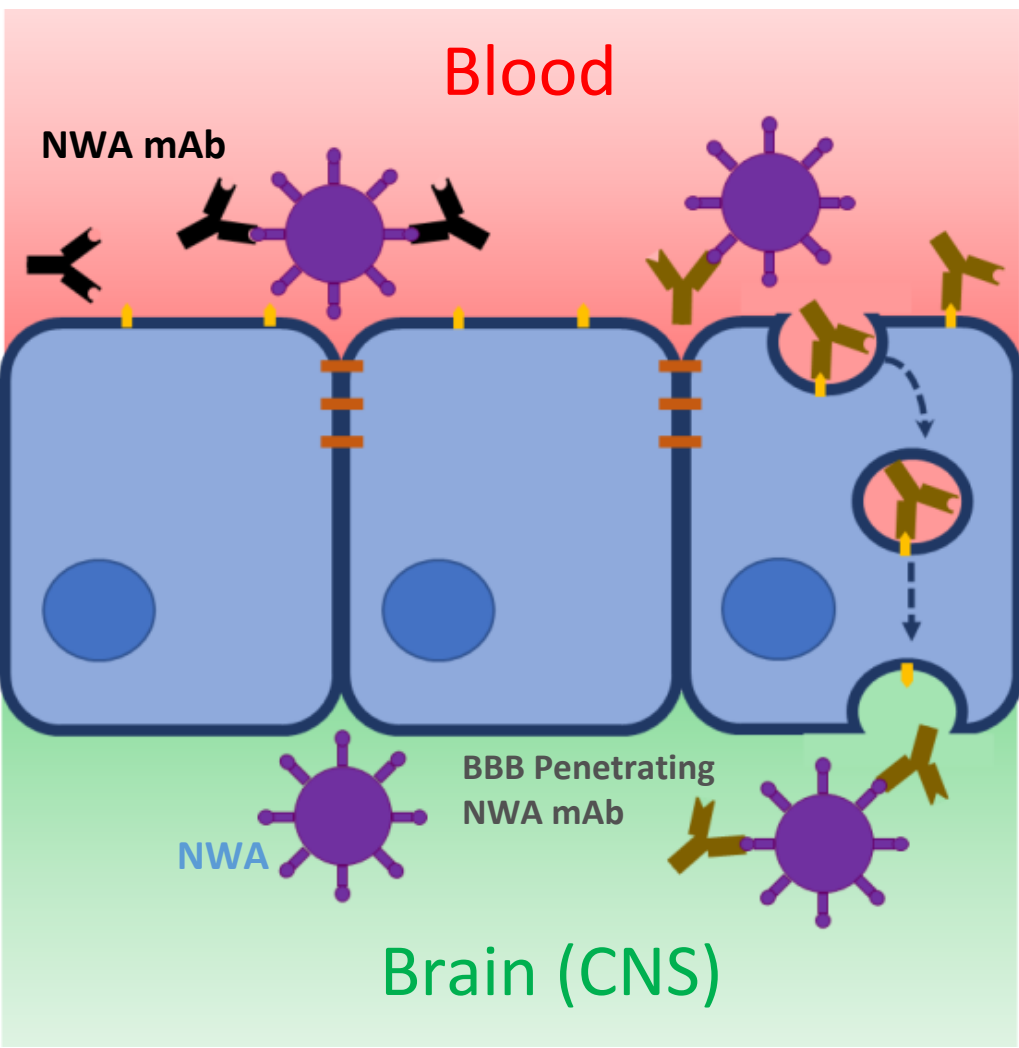
Viral diseases causing encephalic inflammation, including new world alphaviruses (NWA) which can be spread by aerosol transmission, present a significant threat to national security as potential bioterrorism agents. While biologics such as monoclonal antibodies are increasingly popular as rapidly deployable therapeutics, especially targeting emerging viral pathogens, a major challenge complicating the treatment of neurotropic pathogens such as new world alphaviruses is the transport of therapeutic molecules across the blood brain barrier (BBB). Although there are multiple published examples of strongly neutralizing antibodies which show some promise prophylactically against alphaviruses such as Venezuelan Equine Encephalitis virus (VEEV) or Eastern Equine Encephalitis virus (EEEV), there are currently no effective post-exposure treatment strategies available for these pathogens. This is most likely because available therapeutics are excluded from the central nervous system and cannot prevent virus replication in this privileged environment. Receptor mediated transcytosis (RMT) has been identified as a potential method of cargo delivery across the BBB, however approaches targeting previously known BBB RMT pathways, including antibodies binding transferrin receptor and insulin receptor, have demonstrated modest transport efficiency, requiring high peripheral concentration to achieve therapeutic levels within the CNS.

At Sandia, we have utilized a phage-display bio-panning approach to identify several novel nanobody candidates which can cross the BBB and are enriched in the brain after IP injection. These novel nanobodies, or BBB penetrating moieties (BBB PMs), present the opportunity to develop a brain delivery platform for biologic therapy. Given that our BBB PM candidates were selected agnostic of previously identified RMT pathways in a mouse *in vivo* model system, we are currently working to 1) validate that these BBB PMs are also functional in human endothelial systems and 2) characterize the cellular transport pathways utilized by top candidates to cross the BBB endothelial layer. Our findings will advance our understanding of the mechanisms utilized by these novel nanobodies to cross the BBB and will inform their development as shuttles for therapeutic antibodies targeting encephalitic new world alphaviruses as well as a platform which can be adapted to transport therapeutics targeting other diseases affecting the CNS.

Introduction:

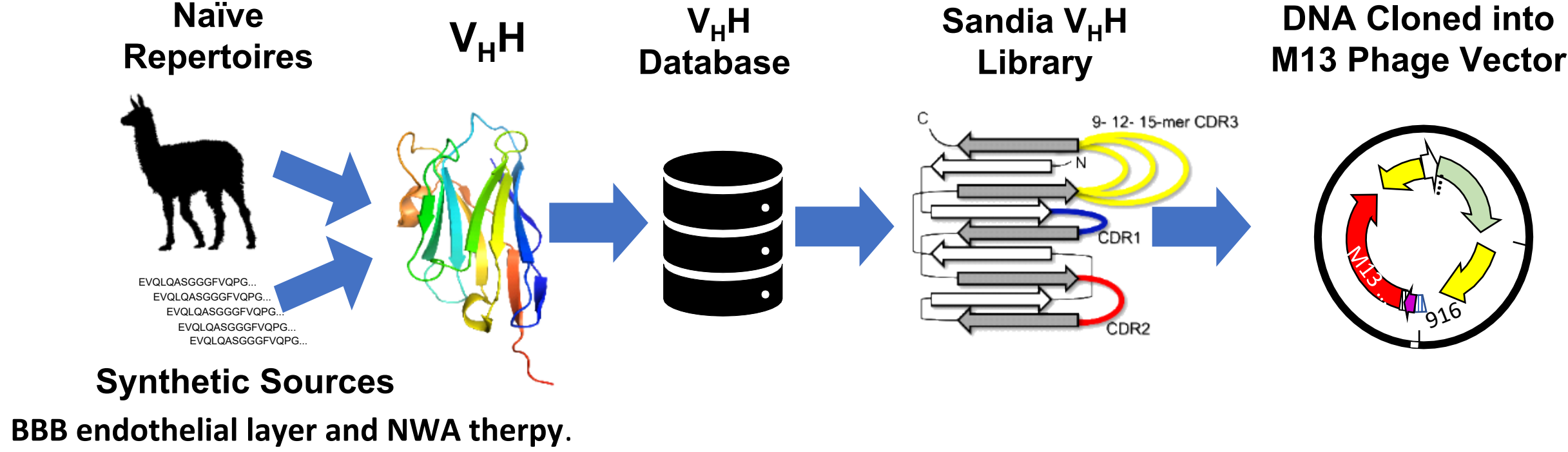
A key component of the blood brain barrier (BBB) is an endothelial layer which tightly controls the passage of ions, molecules, cells, and pathogens into the central nervous system. Similarly, the BBB restricts the entry of many drug products into the brain after systemic administration, including biologics such as monoclonal antibodies. Many metabolites are transported to the brain by receptor mediated transcytosis (RMT), and antibodies targeting these receptors have previously been demonstrated co-opt these pathways to cross the BBB.

To expand the catalogue of BBB crossing shuttles, and to improve on previously characterized low-efficiency transport systems, we have isolated and characterized nanobody sequences which penetrate the BBB *in-vivo* for further development as BBB penetrating shuttles. These nanobodies, which are much smaller than Fab fragments, can be used to functionalize neutralizing antibodies such as those against encephalitic New World Alphaviruses (NWA) to allow them to cross the BBB, and improve their utility against established NWA infection.



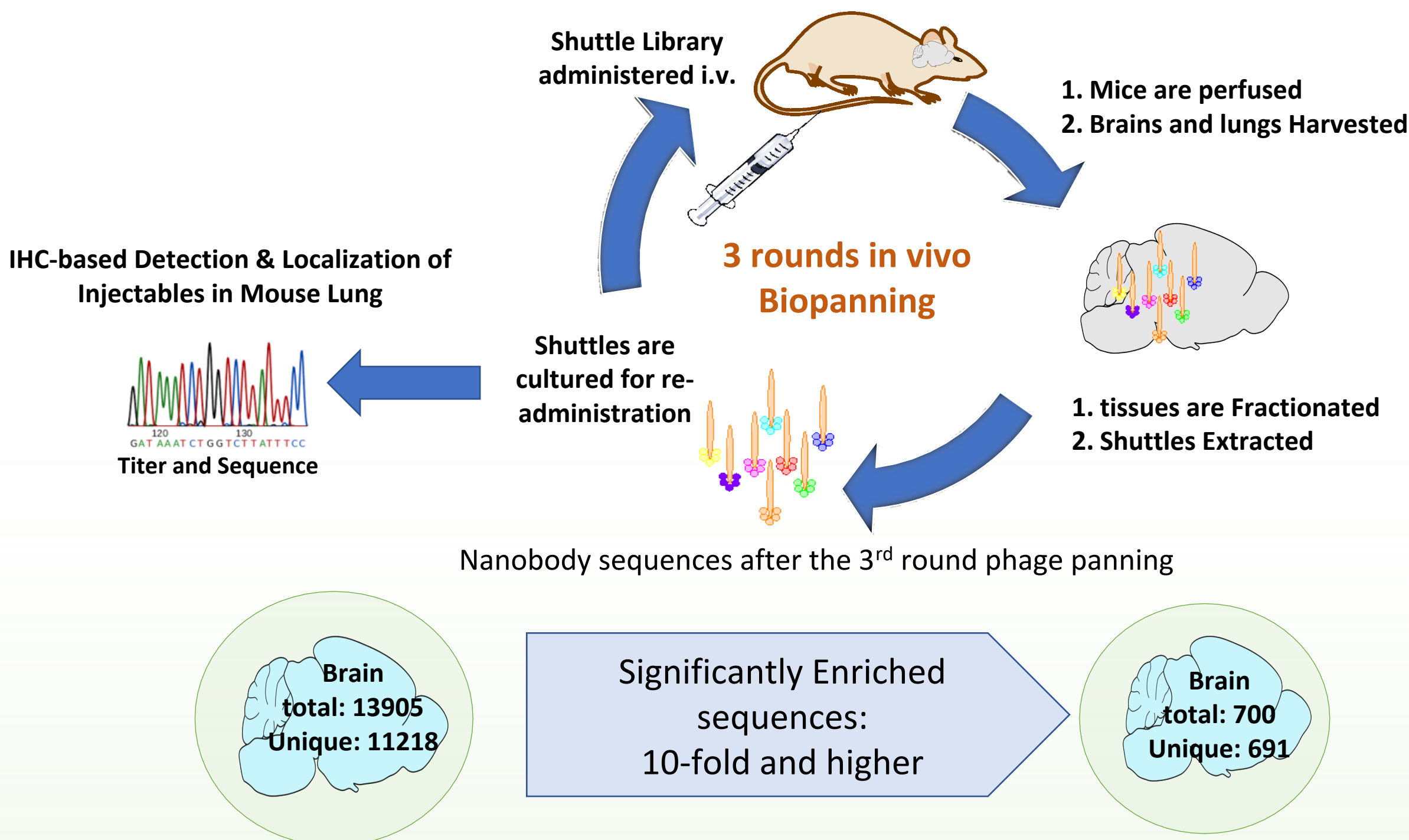
BBB endothelial layer and NWA therapy. NWA mAbs (Black) are excluded from the CNS, and can only bind peripheral viral particles. Bi-specific antibodies targeting both NWA and a BBB RMT pathway (Gold) can cross the BBB to neutralize virus both peripherally and in the CNS.

Nanobody Library Construction:



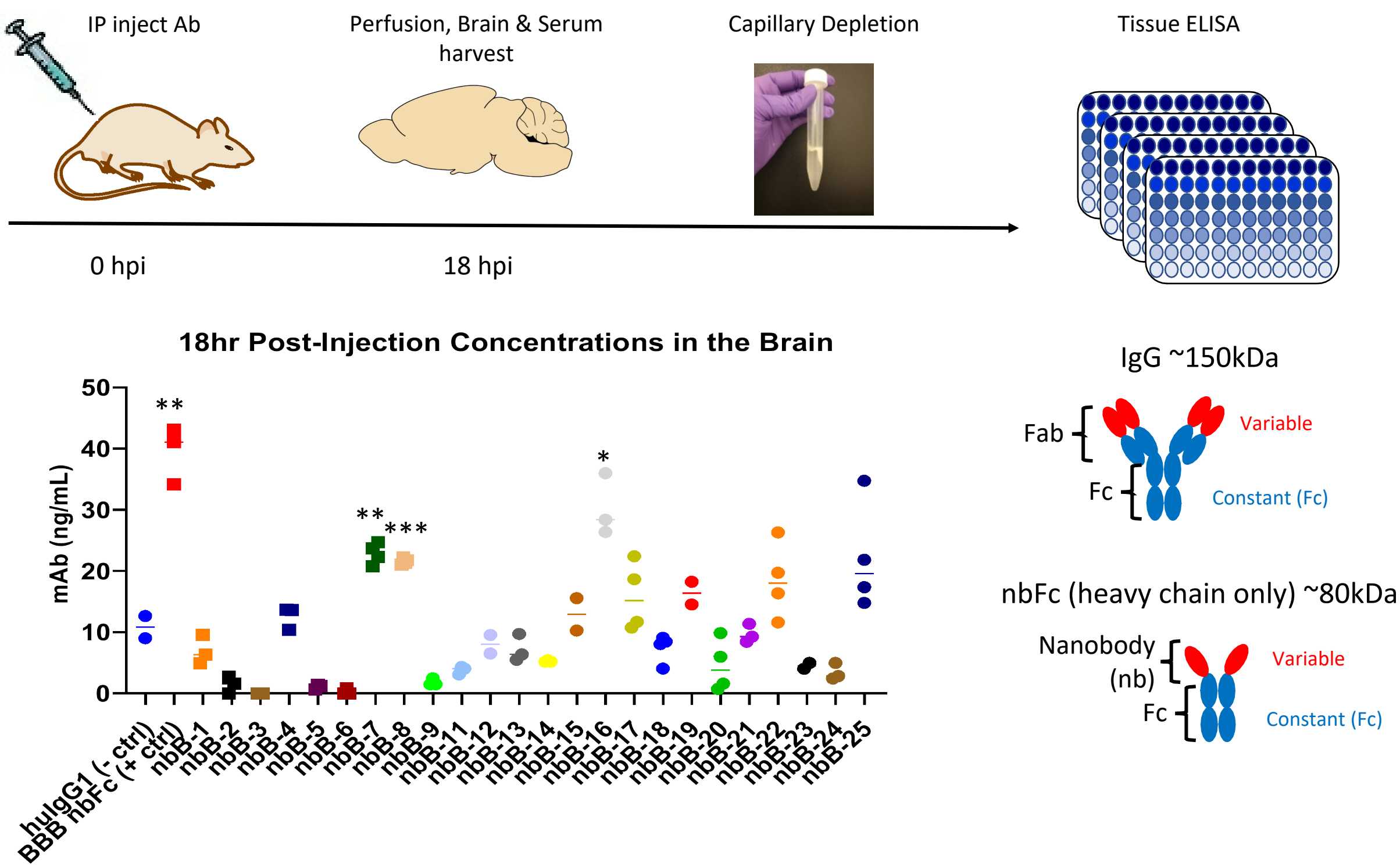
- Nanobody phage library profiled by next generation sequencing
 - High diversity library - 3.24x10¹⁰ sequences
 - Distribution of CDR3 length

Screening for Functional Tissue Targeting Nanobodies *in vivo*:

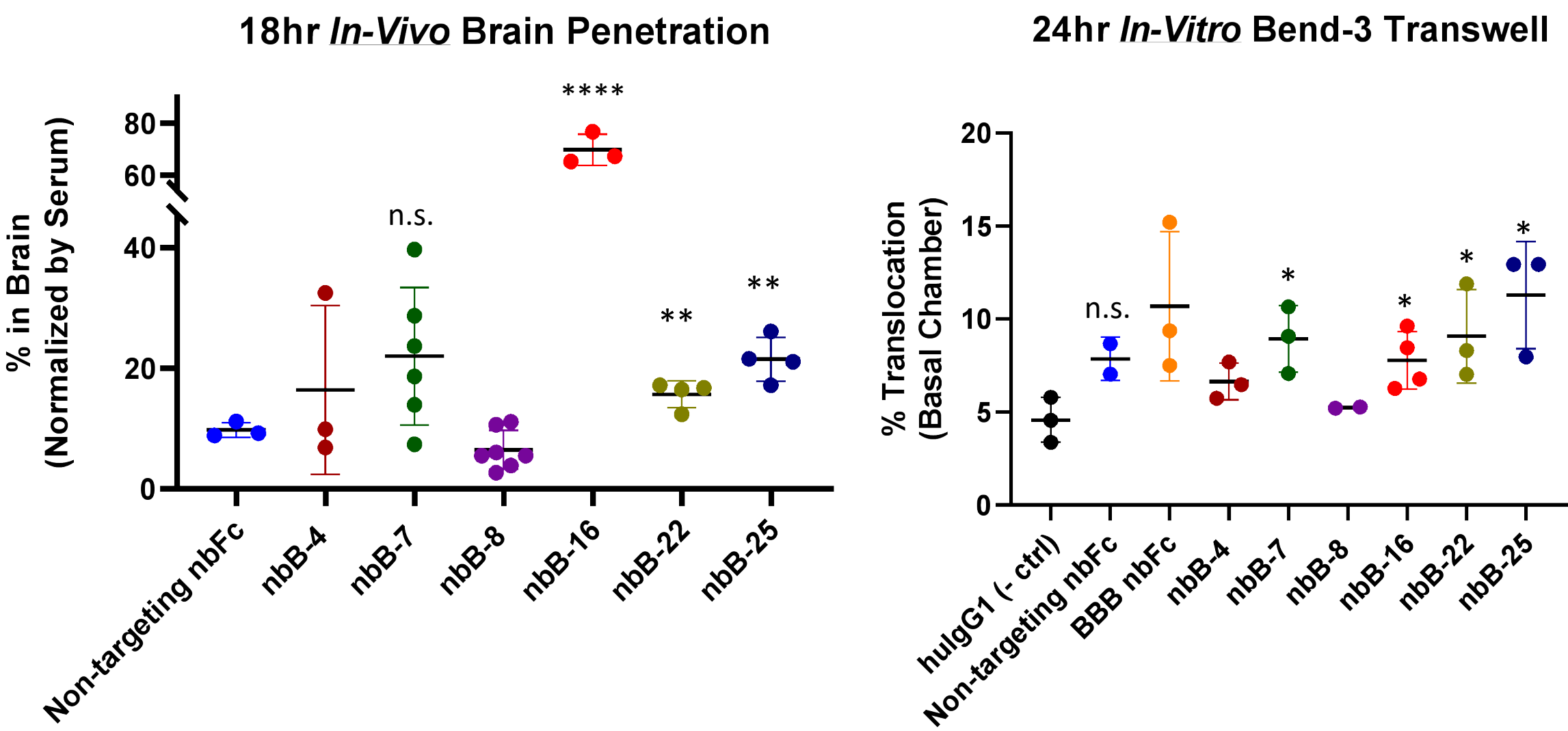


- Of sequences enriched in the brain, top 25 nanobodies expressed and purified as heavy chain only antibody with human Fc region (nbB-1 to nbB-25)

Brain Nanobody *in vivo* Screen Identifies Novel, BBB-Penetrating Hits



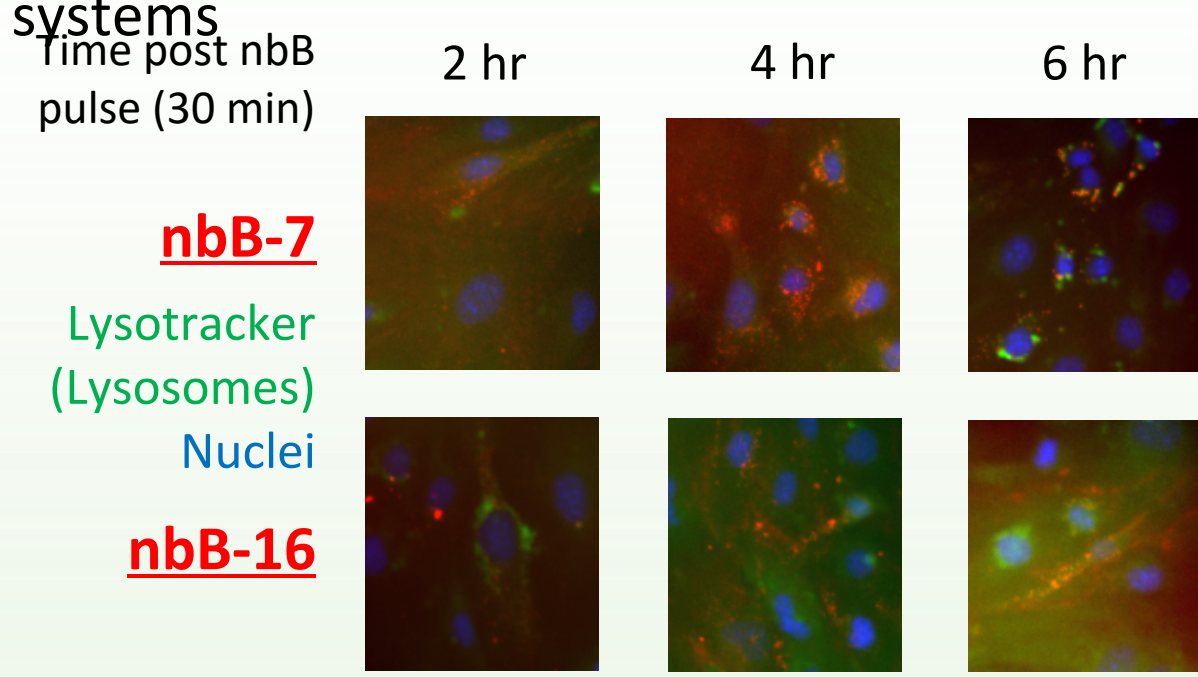
Validation of top BBB penetrating candidates *In-Vivo* and *In-Vitro*



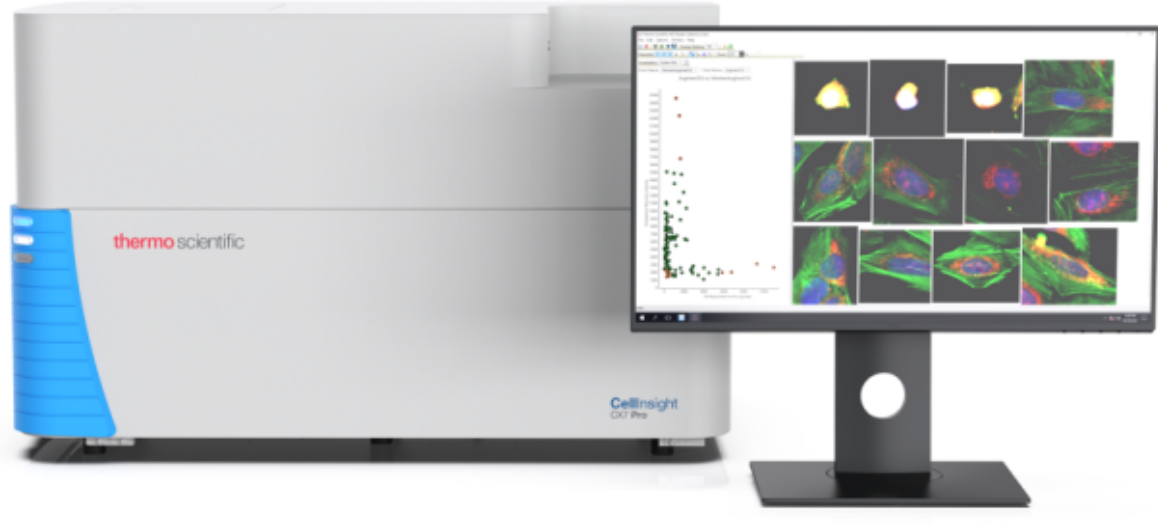
- In vitro* Bend-3 model does not fully replicate *In-Vivo* brain penetration phenotypes

Conclusions and Future Directions

- In-vivo* bio panning used to identify BBB penetrating nanobody sequences enriched in the brain
- Selected nanobody sequences demonstrate BBB penetration in the nbFc format *In vivo*, suggesting specific RMT transport into the brain
 - In-vitro* model must be improved to accurately reflect *In-vivo* BBB penetration phenotypes
- We will utilize the CellInsight™ CX7 platform to conduct live cell imaging of BBB penetrating nbFc intracellular trafficking as well as co-localization with cellular markers in order to characterize the mechanisms of RMT transport in both Bend-3 and human hCMEC-D3 endothelial monolayer systems



Intracellular nbB distribution (Bend-3). Timepoints 2, 4, 6 hours after 30 min incubation with nbB.



CellInsight™ CX7 Pro High Content Screening (HCS) Platform (Thermo Scientific)