Using single-chain variable fragments (scFv) to map the β3-subunit binding site on the pain-sensing sodium channel Nav1.7

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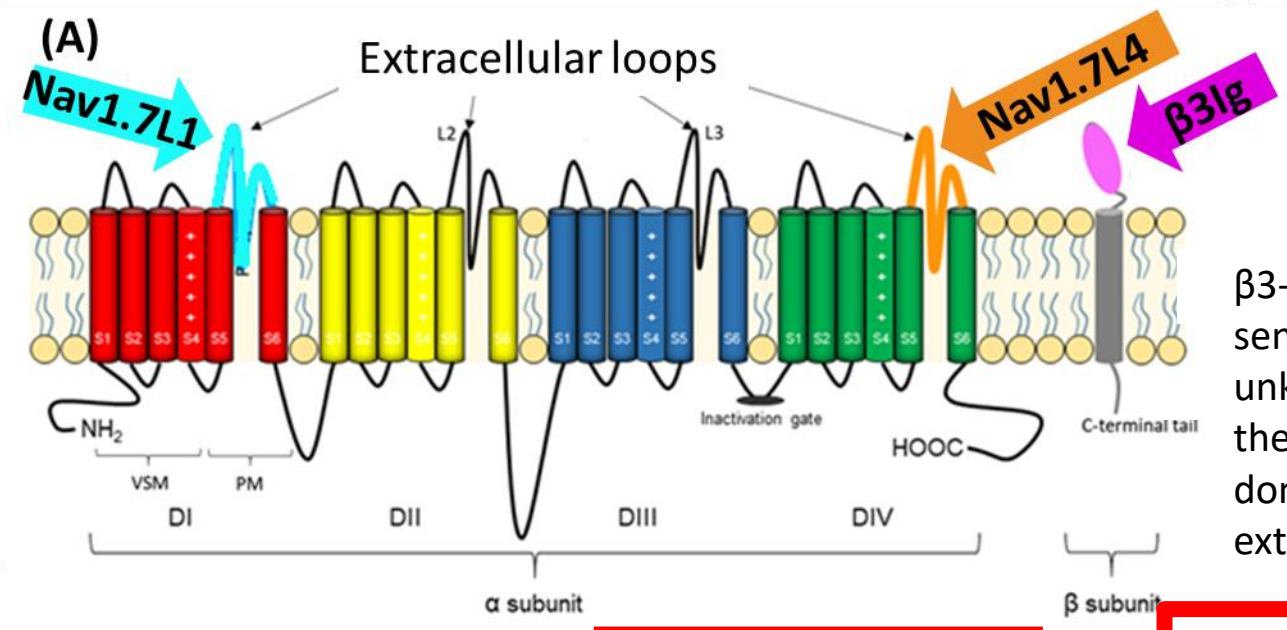
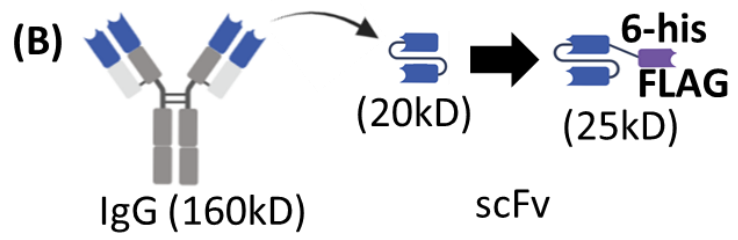
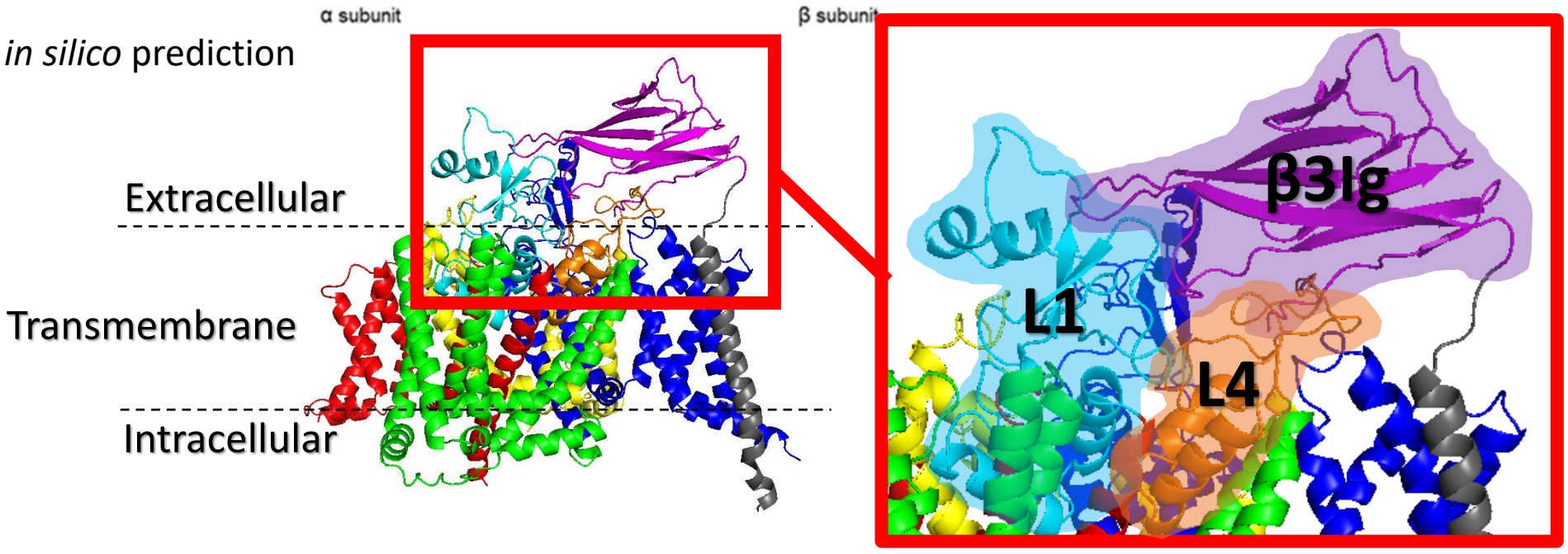
Introduction





β3-subunit is co-expressed with Nav1.7 in pain-sensing neurons, but its binding site remains unknown. We used in silico modelling to predict the β3 binding site. We used scFvs against Nav1.7 domain I extracellular loop (L1) and domain IV extracellular loop (L4) to test this model.

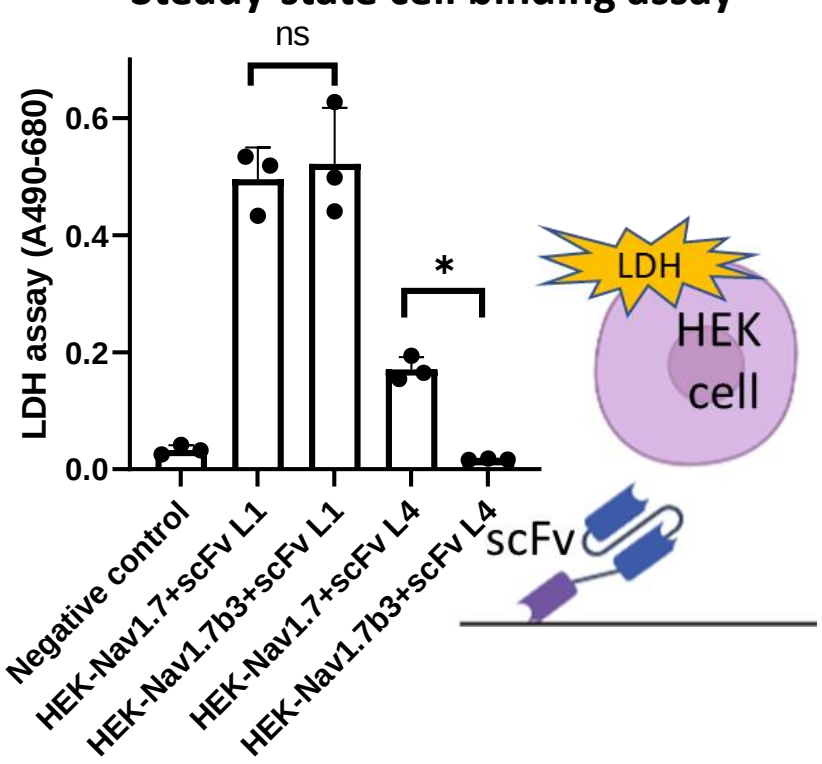
(C) *in silico* prediction



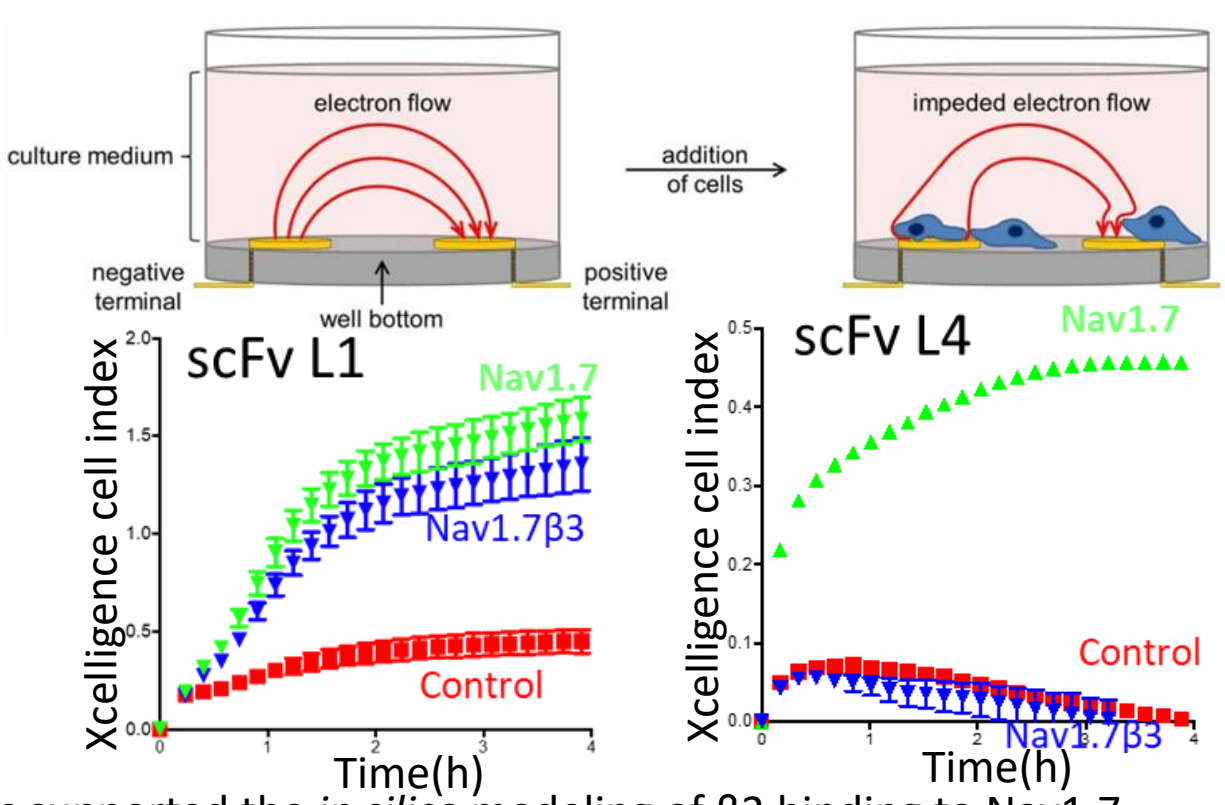
Hypothesis: L1 scFv should bind in the presence of β3 Ig domain, but L4 scFv should not.

Methods & Results

Steady-state cell binding assay

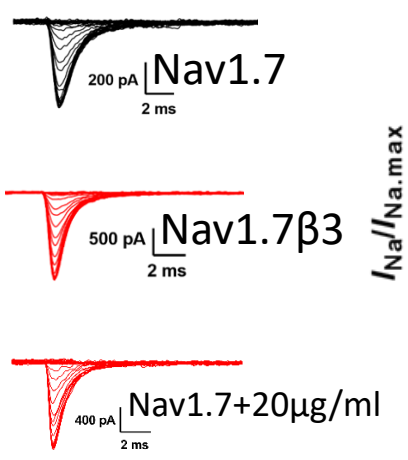


Real-time cell binding assay

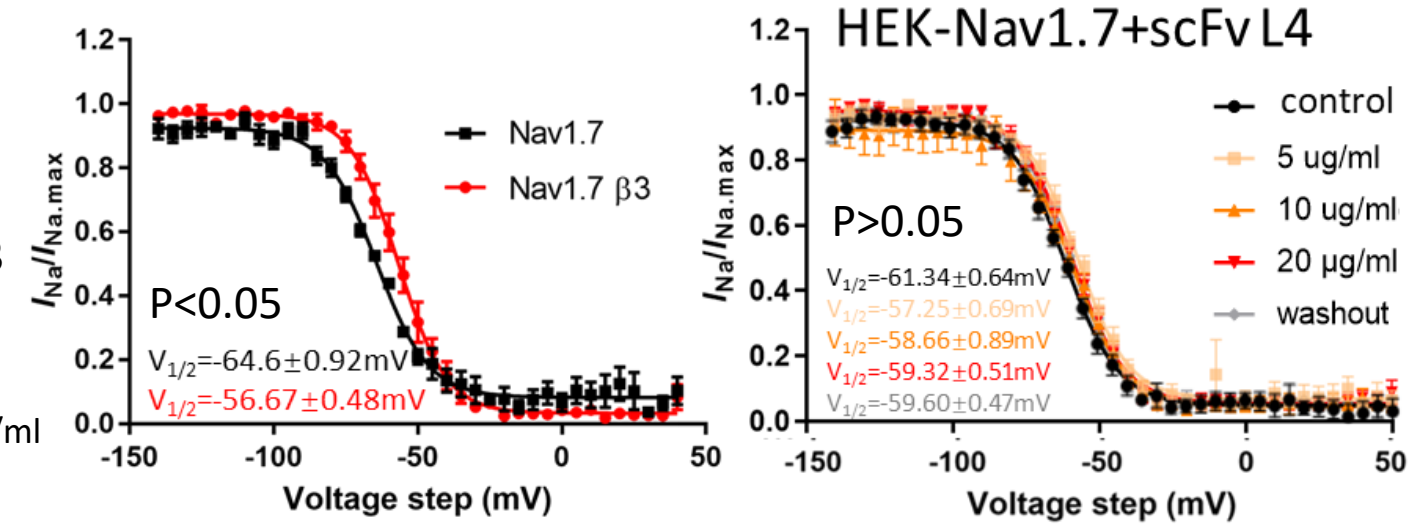


Conclusion: These results supported the *in silico* modeling of β3 binding to Nav1.7.

Nanion Patchliner automated patch-clamp



Effect of β3 or scFv on Nav1.7 inactivation



Conclusion: Unlike β3, the L4 scFv had no effect on Nav1.7 inactivation.