luciferase reporter cells stimulated with recombinant mouse Sonic HedgeHog protein (+ mShh), as compared to in the absence of the peptide shuttle agent ("- FSD250D").

Fig. 4 shows the successful *in vivo* transduction of small molecule inhibitors of HedgeHog signalling (Gant61 and Itraconazole) in skin cells of shaved mice by the peptide shuttle agent FSD250D. Depilation of mouse skin induces hair growth associated with a strong induction of the HedgeHog pathway. This experiment consisted of activating the HedgeHog pathway in mice by depilation, and then measuring the delay in hair regrowth by delivering in the skin cells small molecule HedgeHog pathway inhibitors (Gant61 or Itraconazole) that bind to intracellular targets. The results show that mice treated with the small molecule HedgeHog inhibitors Gant61 or Itraconazole in the presence of FSD250D ("FSD250D+Gant61 100 μM" and "FSD250D+Itraconazole 100 μM") showed delayed hair regrowth at 10 days post-treatment (*), as compared to in the absence of FSD250D ("Gant61 100 μM" and "Itraconazole 100 μM"), or in the presence of the shuttle peptide alone ("FSD250D").

Fig. 5A-5C shows representative patch-clamp electrophysiology whole-cell current traces of HEK293 cells stably expressing the sodium channel Nav1.7 upon exposure to the membrane impermeable sodium channel inhibitor QX-314 with or without FSD194. Reduction of the current amplitude was observed when cells were transiently exposed to QX-314 and GFP-NLS in the presence of FSD194 (i.e., 1 mM QX-314 + 15 μM GFP-NLS + 5 μM FSD194), consistent with the presence of QX-314 inside the cells (**Fig. 5C**). This same current amplitude reduction was not observed in the absence of QX-314 (i.e., 15 μM GFP-NLS + 5 μM FSD194 +; **Fig. 5A**) or in the absence of FSD194 (i.e., 2.5 mM QX-314 + 15 μM GFP-NLS; **Fig. 5B**). Furthermore, GFP-NLS-positive cells were identified in the QX-314 + GFP-NLS + FSD194 and in the FSD194 + GFP-NLS conditions, but not in the QX-314 + GFP-NLS conditions, indicating that GFP-NLS was indeed co-transduced along with the QX-314 by the peptide shuttle agent.

Fig. 6 and **Fig. 7** show the results of a large-scale screening of over 300 candidate peptide shuttle agents for PI and GFP-NLS transduction activity. **Fig. 6** shows results of all candidate peptide shuttle agents screened that had a mean PI transduction efficiency of 10% or higher, sorted based on their level of mean PI transduction efficiency. **Fig. 7** shows results of all candidate peptide shuttle agents screened that had a mean PI transduction efficiency of under 10% and a mean GFP-NLS transduction efficiency of at least 7%, sorted based on their level of mean GFP-NLS transduction efficiency.

SEQUENCE LISTING

This application contains a Sequence Listing in computer readable form created April 15, 2020 having a size of about 122 kb. The computer readable form is incorporated herein by reference.