SUPPLEMENTAL INFORMATION

Supplementary Figures 1-2

Supplementary Methods

Supplementary Reference

Challenges in Targeting a Basic Helix-Loop-Helix Transcription Factor with Hydrocarbon-Stapled Peptides

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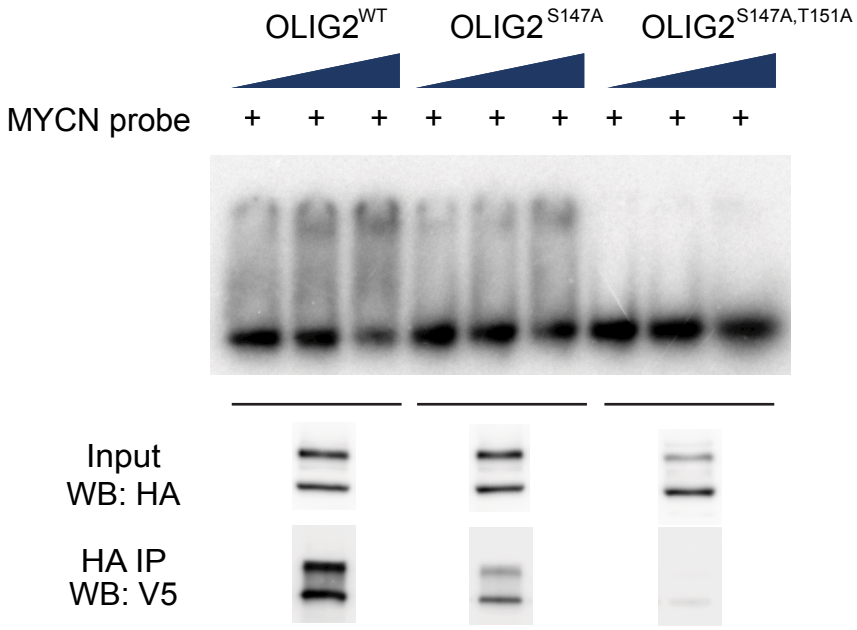

Figure S1. Correlation between OLIG2 homodimerization and DNA binding activity. The indicated single and double mutations in OLIG2 progressively impair DNA binding activity, as evaluated by EMSA assay (top). The extent of the inhibitory effect correlates with the capacity of OLIG2 constructs to self-associate, as monitored by co-immunoprecipitation of HA- and V5-tagged OLIG2 protein from lysates of COS7 cells transiently co-expressing HA- and V5-tagged wild-type OLIG2, or its S147A or S147A,T151A HLH-mutant forms (bottom).

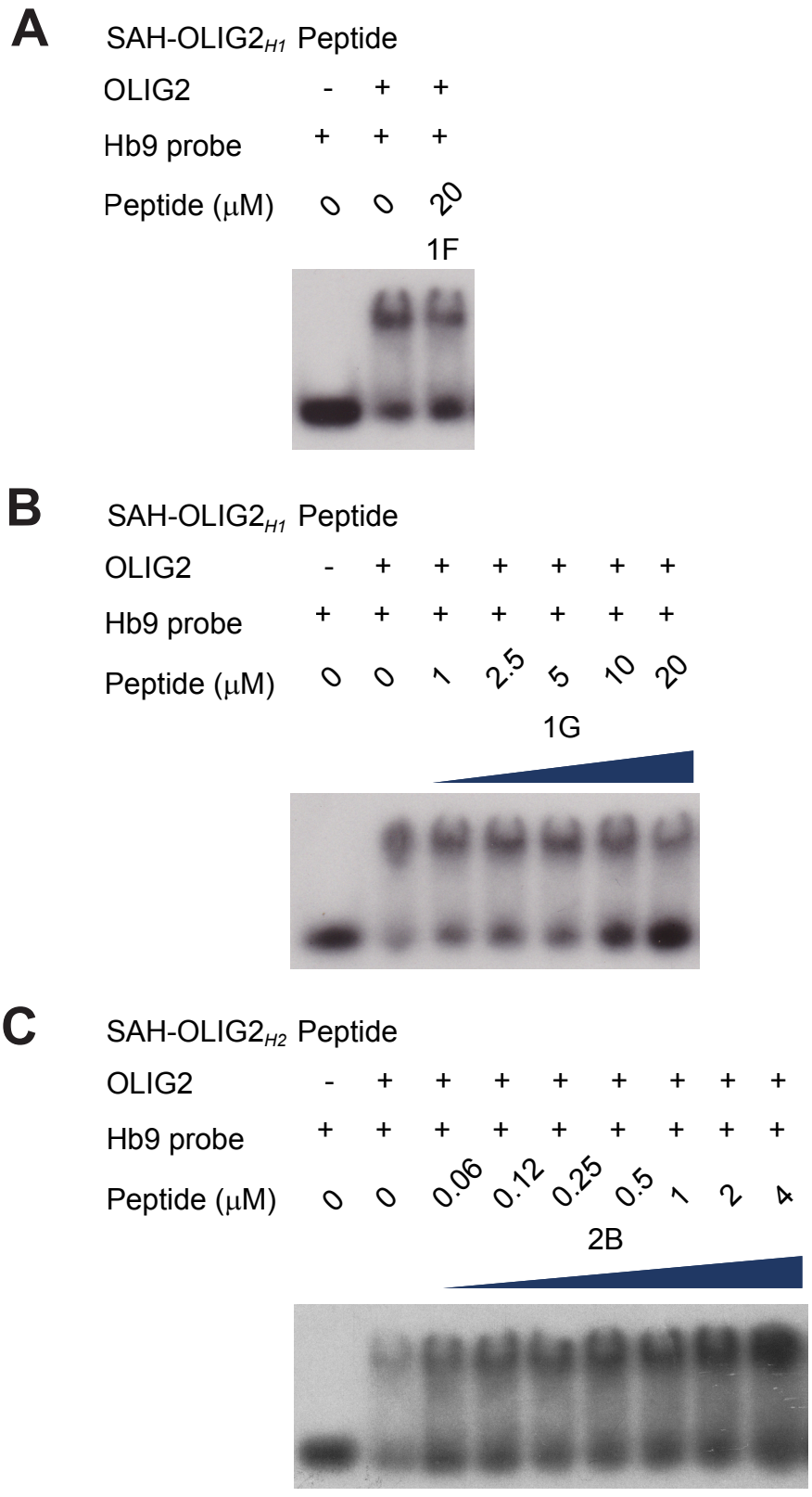

Figure S2. Biochemical activity of SAH-OLIG2 peptides in EMSA assays. (A-C) The effects of SAH-OLIG2$_{H1F}$ (A), SAH-OLIG2$_{H1G}$ (B), and SAH-OLIG2$_{H2B}$ (C) on OLIG2-DNA complex formation, as assessed by EMSA assay. Peptides SAH-OLIG2$_{H1F}$ and SAH-OLIG2$_{H1G}$ were re-evaluated because on the initial screen (**Figure 2A**) the radioactive signal for the protein-DNA complex was too low to draw definitive conclusions. For SAH-OLIG2$_{H2B}$, the initial screen suggested inhibitory activity (**Figure 2B**), but upon expanded dose-responsive evaluation, no inhibitory effect on the OLIG2-DNA complex was observed.

SUPPLEMENTARY METHODS

Cellular Expression and Immunoprecipitation of OLIG2 Constructs. pcDNA3.1 vectors encoding wild-type (WT) or S147A- or S147A,T151A-mutant V5-HIS-tagged OLIG2 were transiently transfected into COS-7 cells along with HA-tagged WT OLIG2. Cells were lysed in hypotonic lysis buffer (50 mM Tris-HCl pH 7.5, 0.2% [v/v] NP-40, 5 mM EDTA) and then high salt buffer (10 mM HEPES, 420 mM NaCl, 0.2 mM EDTA). Cell lysates were incubated with anti-HA agarose beads (Sigma) overnight and the beads washed three times with low salt buffer (50 mM Tris-HCl pH7.5, 100 mM KCl, 0.1% [v/v] NP-40, 10% [v/v] glycerol) followed by a high salt buffer wash (low salt buffer supplemented with 300 mM KCl). All buffers contained protease inhibitors (Roche), 1 mM $NaVO_4$, 5 mM NaF, 1 mM $Na_2P_2O_7$, 25 mM beta-glycerophosphate and 0.5 mM PMSF. Anti-HA precipitated proteins were subjected to 10% [v/v] SDS-PAGE and transferred to PVDF membranes (Millipore), which were then blocked with 5% [w/v] milk for 1 hour at RT and incubated overnight with V5 (Thermo Fisher) or HA (Sigma) antibodies. The membranes were washed in tris-buffered saline and Tween-20 (TBST), incubated with HRP-conjugated antibody (Biorad) for 1 hour, and chemiluminescence signal detected using the VersaDoc system (Biorad).

Electrophoretic Mobility Shift Assays. EMSAs were performed as described in the Methods section except for use of, where indicated, the following, previously-reported Mycn E-box promotor DNA sequence[1]: 5' AGCTAGAAGACAGCTGTTGGAAG 3' and 3' AGCTCTTCCAACAGCTGTCTTCT 5'

SUPPLEMENTARY REFERENCE

1. Meijer, D. H., Sun, Y., Liu, T., Kane, M. F., Alberta, J. A., Adelmant, G., Kupp, R., Marto, J. A., Rowitch, D. H., Nakatani, Y., Stiles, C. D., and Mehta, S. (2014) An amino terminal phosphorylation motif regulates intranuclear compartmentalization of Olig2 in neural progenitor cells, *J Neurosci 34*, 8507-8518.