

Table S1. Primer Sequences used for RT-qPCR

Gene	Forward Sequence	Reverse Sequence	Reference
<i>FOXG1</i>	AGAAGAACGGCAAGTACGAGA	TGTTGAGGGACAGATTGTGGC	Bissonnette et al., 2011
<i>EN1</i>	CCCTGGTTTCTCTGGGACTT	GCAGTCTGTGGGGTCGTATT	Yan et al., 2005
<i>HOXC6</i>	CACCGCCTATGATCCAGTGAGGCA	GCTGGAACTGAACACGACATTCTC	Moon et al., 2012
<i>PAX6</i>	ACCACACCGGTTTCTCCTTCACA	TTGCCATGGTGAAGCTGGGCAT	Imaizumi et al., 2015
<i>PAX6</i>	ACAGATCTACGCCAGCGACT	CATGGTGTTCGTAGTGGATGC	Liu et al., 2013
<i>NKX2.1</i>	CGCATCCAATCTCAAGGAAT	CAGAGTGTGCCAGAGTGAA	Liu et al., 2013
<i>LHX8</i>	CCAAAACCAGCAAAAAGAGC	TGGCGTGTCTACAATTCTG	Liu et al., 2013
<i>LHX6</i>	ACAGATCTACGCCAGCGACT	CATGGTGTTCGTAGTGGATGC	Liu et al., 2013
<i>GSX2</i>	AGGAGAGAAGGGGACTCAGC	CGGACACTGACATCACCAAC	Vergano-Vera et al., 2009
<i>MEIS2</i>	GACCACGATGATGCAACC	CCTGTGTCTTGCCTAACTG	Zha Y et al., 2014
<i>SOX2</i>	ACACCAATCCCATCCACT	GCAAACCTCCTGCAAAGCTC	Sundberg et al., 2011
<i>MAP2</i>	AATAGACCTAAGCCATGTGACATCC	AGAACCAACTTTAGCTTGGGCC	Muratore et al., 2014
<i>SHH</i>	GAG TCT CTG CAC TAC GAG GG	CGA GTT CTC TGC TTT CAC CG	

Table S2. Anti-human antibodies used for immunocytochemistry

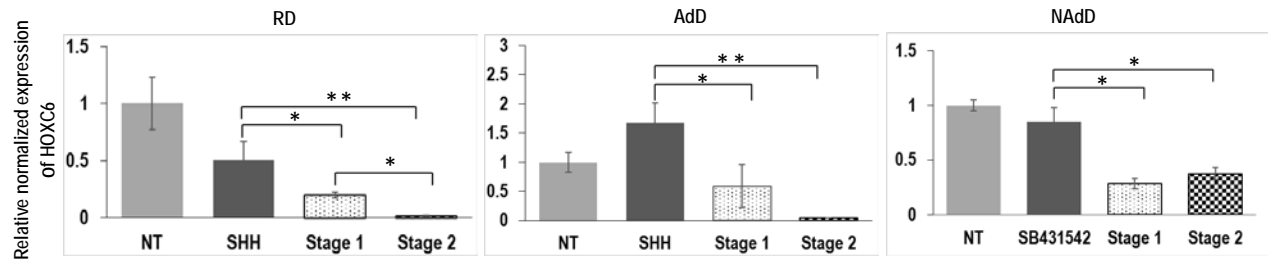
Antibody	Species	Supplier	Dilution
α - SOX2	Rabbit	Cell Signaling Technology	1:400
α - NESTIN	Mouse	Millipore	1:1000
α - FOXG1	Rabbit	Abcam	1:1000
α - MAP2	Mouse	R&D Systems	1:250
α - NKX2.1	Rabbit	Abcam	1:250
α - PAX6	Sheep	R&D Systems	1:200

Table S3. Comparison of the relative gene expression (Δ CT) in MGE generated in the RD, AdD, and the NAdD strategies

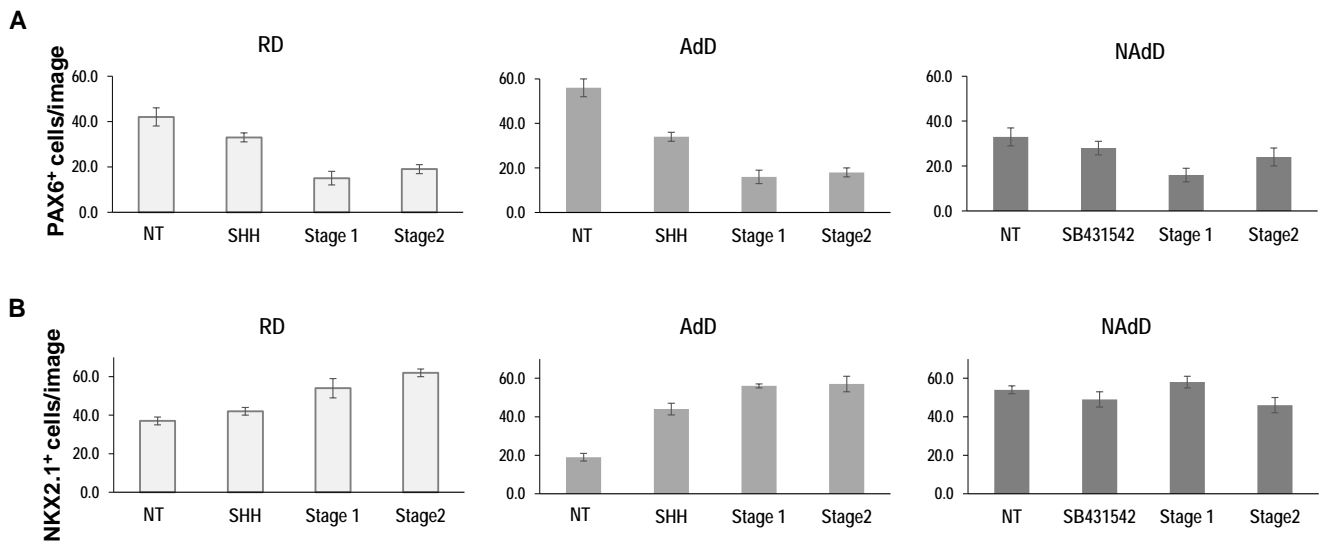
Differentiation protocol	RD		AdD		NAdD		Gene
Treatment	Relative quantity	SE	Relative quantity	SE	Relative quantity	SE	
NT	0.898	0.036	0.674	0.026	0.661	0.036	GAPDH
Baseline *	0.757	0.097	0.816	0.056	0.814	0.248	
Stage 1	0.498	0.049	0.502	0.044	0.461	0.133	
Stage 2	0.642	0.043	0.888	0.047	0.798	0.053	
NT	0.960	0.132	0.161	0.070	0.040	0.003	FOXP1
Baseline	0.561	0.193	0.276	0.085	0.050	0.005	
Stage 1	0.496	0.038	0.361	0.013	0.925	0.087	
Stage 2	0.579	0.018	0.409	0.120	0.482	0.093	
NT	0.108	0.002	0.115	0.016	0.114	0.002	EN1
Baseline	0.029	0.004	0.249	0.043	0.139	0.009	
Stage 1	0.010	0.001	0.373	0.061	0.230	0.010	
Stage 2	0.029	0.022	0.063	0.008	0.054	0.006	
NT	0.016	0.004	0.021	0.002	0.081	0.003	NKX2.1
Baseline	0.200	0.012	0.232	0.051	0.322	0.005	
Stage 1	0.960	0.084	0.202	0.013	0.667	0.047	
Stage 2	0.304	0.033	0.340	0.023	0.306	0.023	
NT	0.009	0.004	0.037	0.004	0.017	0.013	LHX8
Baseline	0.046	0.012	0.236	0.044	0.051	0.004	
Stage 1	0.913	0.084	0.044	0.013	0.915	0.092	
Stage 2	0.228	0.033	0.333	0.023	0.374	0.013	
NT	0.033	0.016	0.031	0.004	0.165	0.023	LHX6
Baseline	0.276	0.008	0.249	0.044	0.051	0.004	
Stage 1	0.014	0.006	0.214	0.033	0.038	0.009	
Stage 2	0.278	0.025	0.681	0.073	0.244	0.023	
NT	0.403	0.009	0.308	0.048	0.382	0.063	PAX6
Baseline	0.311	0.043	0.256	0.003	0.266	0.040	
Stage 1	0.068	0.018	0.217	0.013	0.138	0.010	
Stage 2	0.085	0.030	0.139	0.013	0.209	0.034	
NT	0.092	0.018	0.257	0.066	0.496	0.043	GSX2
Baseline	0.518	0.061	0.486	0.034	0.026	0.005	
Stage 1	0.013	0.014	0.093	0.011	0.256	0.015	
Stage 2	0.202	0.025	0.183	0.009	0.163	0.024	
NT	0.113	0.002	0.104	0.023	0.029	0.002	MEIS2
Baseline	0.016	0.001	0.162	0.005	0.109	0.003	
Stage 1	0.007	0.001	0.278	0.025	0.021	0.001	
Stage 2	0.092	0.018	0.126	0.019	0.086	0.003	
NT	0.903	0.094	0.685	0.101	0.504	0.015	MAP2
Baseline	0.794	0.058	0.765	0.051	0.664	0.056	
Stage 1	0.558	0.120	0.604	0.033	0.314	0.026	
Stage 2	0.574	0.022	0.808	0.040	0.502	0.027	
NT	0.683	0.016	0.685	0.101	0.660	0.139	SOX2
Baseline	0.794	0.080	0.705	0.096	0.891	0.014	
Stage 1	0.498	0.062	0.534	0.006	0.416	0.024	
Stage 2	0.574	0.041	0.728	0.040	0.753	0.116	

* Baseline in the RD and the AdD protocols represents treatment with SHH alone

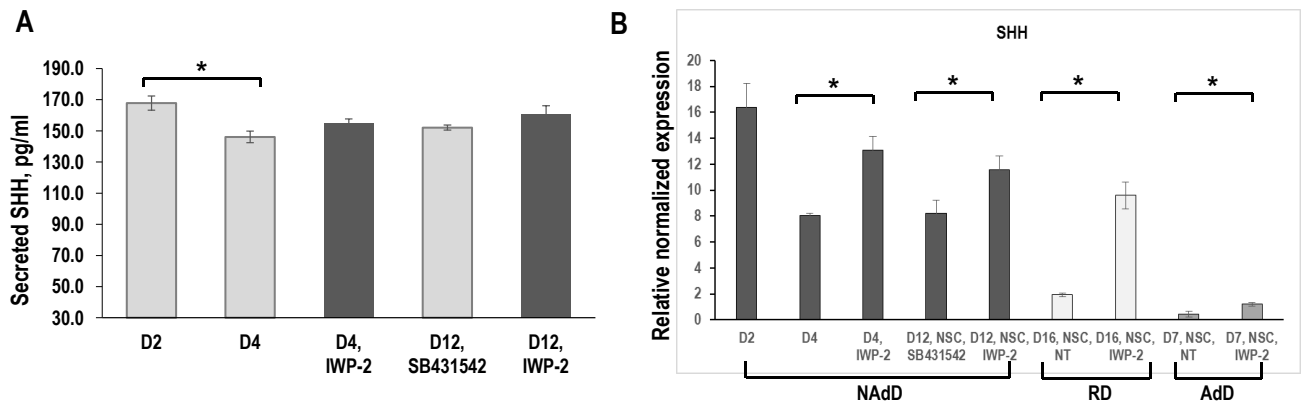
* Baseline in the NAdD protocol represents treatment with SB431542



Supplementary figure S1. Quantitative RT-PCR analysis of a hindbrain marker HOXC6 (expression relative to non-treated cells, NT = 1) in NPC generated in the RD, AdD, and NAdD protocols. Data are presented as mean \pm SEM. * P < 0.05 and ** P < 0.001 difference between treatment with Shh alone (in the RD and AdD protocols) or SB431542 alone, (in the NAdD protocol)] and Wnt inhibition supplemented during NSC stage (Stage 1) or NPC stage (Stage 2).



Supplementary Figure S2. Wnt inhibition changes expression of dorsal (PAX6) and ventral (NKX2.1) forebrain progenitors in the three differentiation protocols. Decrease in number of PAX6⁺ cells (**A**) and increase in number of NKX2.1⁺ cells (**B**) in NPC treated with SHH and/or SB431542 alone and in combination with IWP-2 during NSC (Stage 1) and NPC (Stage 2) stages of neural differentiation. Data are presented as mean \pm SD.



Supplementary Figure S3. The level of SHH during neuronal induction/neuroepithelia generation.

A - The level of SHH in culture media at D2, D4, and D12 secreted by the cells undergoing NAAd differentiation with or without Wnt inhibition. Significant decrease in the level of SHH between D2 and D4 is observed in the samples without IWP-2. Wnt inhibition is needed to sustain SHH level.

B - *SHH* gene expression during neuroepithelia generation and at the stage of NSC in the three differentiation strategies. The highest level of *SHH* is detected in the NAAd protocol. The expression of *SHH* is higher in all protocols in the presence of IWP-2.