

**Stem Cell Reports, Volume 2**

**Supplemental Information**

## **Human Neural Precursor Cells Promote Neurologic**

## **Recovery in a Viral Model of Multiple Sclerosis**

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## **Supplemental Experimental Procedures**

### **Characterization of hNPCs by gene expression array**

For hNPC samples and other control cell samples, one well of a 6-well plate containing approximately  $10^6$  cells was collected and pelleted by centrifugation. Each pellet was flash frozen and stored at  $-80^{\circ}\text{C}$ . RNA was purified using the mirVana miRNA Isolation Kit (Life Technologies). Collected RNA was quantified using the Qubit Fluorometer and RNA BR assay kit (Life Technologies). The RNA quality of each sample was determined using the 2100 Bioanalyzer (Agilent Technologies) to obtain an RNA integrity Number (RIN). Samples with acceptable RINs (8.2-10.0) were amplified, labeled and hybridized onto Illumina HumanHT-12 v4 Expression Beadchips according to the manufacturer's instructions. Hybridized chips were scanned using the iScan system (Illumina). Raw data extraction was performed with Genome Studio (Illumina) and probes without a detection p-value (a measure of confidence that the signal observed is above background fluorescence) of less than 0.01 in at least 1 sample were removed. The remaining probes were then quantile-normalized to correct for between-sample variation. Normalized data were then analyzed using Qlucore Omics Explorer (Qlucore).

### **Generation of LUC<sup>+</sup> H9 cells**

Stbl3 cells containing the plasmid pLenti CMV Puro LUC (w168-1) were obtained (Plasmid 17477; Addgene) and purified plasmid was transfected into a 6-well plate of confluent 293T cells for virus generation. The supernatant containing virus was collected 48 h post transfection and filtered through a  $0.45\mu\text{m}$  filter. STEMPRO® hESC SFM

(Life Technologies) supplemented with 10 $\mu$ g/ml polybrene was added to a 6-well plate of feeder free adapted WA09 cells that were less than 50% confluent. 150 $\mu$ l of virus-containing supernatant per well was added and the plates were spininfected at 1800RPM for 1 hour at 37°C. After 48 hours, 5 $\mu$ g/ml puromycin was added to select for virally transduced cells. Medium was changed daily and puromycin selection was maintained. Firefly luciferase (FLUC) production was verified by adding luciferin to culture wells and imaging them using a Bio-Rad Gel Doc system (Bio-Rad).

### **Viral RNA Quantification**

For quantitative RT-PCR experiments to determine viral RNA levels in spinal cords, primers specific for the JHMV spike protein (forward:GGCTGTTGACGCATGTGATT; reverse: GAGCACGCGTCTGAATGAAC) were designed using PrimerQuest software (Integrated DNA Technologies, Inc.). SYBR Green Real-Time PCR Master Mix (Life Technologies) was used according to manufacturer's specifications and RT-PCR was performed using the Applied Biosystems ViiA 7 Real-Time PCR System.  $C_t$  values of S protein transcripts were normalized to  $\beta$ -actin  $C_t$  values (forward: GGCCAGAGCAAGAGAGGTATCC; reverse: ACGCACGATTTCCCTCTCAGC) and compared using the  $\Delta\Delta C_t$  method.

### **Table S1. Gene Expression Array Data from Figure 1B**

(Table begins on following page.)

NAME	DESCRIPTION	NPC1	NPC2	NPC3	NPC4	NPC5	iPSC1	iPSC2	ESC1	ESC2	Fibro
ILMN_1769694	<i>ACCN2</i>	1358.4	817.4	1531.3	1512.0	521.3	232.6	289.1	215.3	208.5	431.2
ILMN_1658660	<i>ACTC1</i>	14512.5	20589.1	13632.2	18397.5	15337.5	692.4	794.8	775.4	747.3	1202.9
ILMN_1713751	<i>ADAM19</i>	7057.0	10109.4	8829.5	10117.4	8227.5	1293.3	953.0	1250.7	1353.7	1019.3
ILMN_1716264	<i>ANKRD1</i>	1297.4	1226.1	1808.1	921.1	1233.1	145.3	125.7	131.1	179.5	373.7
ILMN_1678143	<i>ARHGD1B</i>	4263.2	5150.6	7285.0	8835.9	7360.0	193.7	168.6	190.6	175.8	671.3
ILMN_1786041	<i>ASB9</i>	1309.6	882.2	544.6	1000.9	316.9	164.9	142.6	132.2	203.2	169.1
ILMN_1752899	<i>BCL11A</i>	159.6	154.0	141.1	173.6	171.0	929.4	1052.4	1067.2	1940.4	291.7
ILMN_2255133	<i>BCL11A</i>	119.3	130.4	111.3	113.1	166.6	936.0	1125.8	1062.1	1967.4	220.9
ILMN_1740900	<i>BMP4</i>	5466.2	2667.2	6016.8	1994.9	1281.1	253.6	251.1	270.6	551.9	197.7
ILMN_3239861	<i>C10orf140</i>	3941.0	3362.2	3217.2	2215.4	2086.7	865.4	695.7	516.9	709.1	331.3
ILMN_1656369	<i>C8orf4</i>	624.7	931.3	526.7	693.0	2482.7	104.7	106.8	113.2	118.7	119.0
ILMN_2195482	<i>CACNB3</i>	2381.5	2212.1	2836.3	2804.0	1972.1	402.0	647.1	362.1	617.7	1173.4
ILMN_1719236	<i>CDH5</i>	808.3	914.5	1093.0	2974.0	2259.2	123.3	124.6	111.4	125.9	119.9
ILMN_1731237	<i>CLSTN2</i>	713.4	1236.4	1371.9	1629.0	1636.0	127.0	120.6	124.6	142.6	131.0
ILMN_1810054	<i>CNN1</i>	1723.1	2343.2	1023.6	2715.2	1637.4	273.8	294.9	219.5	214.8	324.8
ILMN_1789507	<i>COL11A1</i>	3393.6	4130.0	4188.6	3354.4	1391.1	284.0	265.9	258.8	404.7	431.1
ILMN_2392803	<i>COL11A1</i>	715.9	1098.8	680.0	971.6	617.4	166.8	148.0	146.3	177.4	179.1
ILMN_1653028	<i>COL4A1</i>	13750.1	17375.0	14224.1	17508.3	19251.6	2446.1	3306.3	2672.5	3392.3	1799.8
ILMN_1724994	<i>COL4A2</i>	1686.5	2072.7	1832.2	2722.0	1244.1	357.7	450.1	260.8	346.0	422.7
ILMN_1809496	<i>COPG2</i>	261.4	287.6	278.2	209.1	188.6	1333.5	1246.3	1002.0	657.7	406.4
ILMN_1784294	<i>CPA4</i>	675.6	781.9	1503.7	1420.7	2667.3	133.2	122.5	119.1	116.7	273.6
ILMN_1731374	<i>CPE</i>	5804.3	7861.2	7046.8	6933.3	7280.0	554.1	598.6	486.3	455.6	959.3
ILMN_1654072	<i>CX3CL1</i>	857.1	787.7	785.4	1036.0	349.2	174.1	243.3	199.3	219.4	117.0
ILMN_1748323	<i>CXCL14</i>	931.6	1206.2	443.9	883.3	280.5	165.5	146.9	165.7	159.6	160.3
ILMN_1758128	<i>CYGB</i>	775.4	713.8	824.8	1879.5	870.0	173.2	193.8	157.4	155.3	400.5
ILMN_1807291	<i>CYP11A1</i>	219.2	505.0	503.9	1042.1	348.9	127.1	123.2	115.1	123.5	124.6
ILMN_1741603	<i>DBC1</i>	141.1	161.0	146.6	200.5	168.3	1163.0	1184.5	1098.7	1418.0	559.1
ILMN_1666503	<i>DENND2A</i>	2341.0	2600.8	2225.9	3975.8	1805.3	702.7	665.5	600.9	528.3	476.7
ILMN_1682775	<i>EDN1</i>	2648.4	2435.0	1552.5	2855.5	3508.7	156.1	123.5	120.0	116.1	437.2
ILMN_1796629	<i>EDNRA</i>	1475.2	1415.3	2101.6	848.4	1033.3	191.1	234.5	220.3	218.3	165.1
ILMN_1703852	<i>EFNB2</i>	3049.8	5270.9	3514.2	3750.1	7589.1	880.1	688.9	453.3	1249.9	807.8
ILMN_1779147	<i>ENC1</i>	1246.6	1384.7	1749.8	1453.9	2303.2	316.9	261.4	416.7	520.3	356.0
ILMN_1668092	<i>ESAM</i>	368.8	1103.5	744.9	1272.5	807.4	132.6	120.0	121.6	138.4	114.8
ILMN_2105919	<i>FGF2</i>	228.4	293.9	243.9	175.3	595.5	1390.6	1939.4	675.3	1501.1	2453.0
ILMN_1665738	<i>FLI1</i>	521.6	538.9	576.1	902.2	859.3	131.2	134.4	143.7	143.6	331.2
ILMN_1697491	<i>FLJ14213</i>	651.6	748.0	791.1	1069.9	1078.8	259.4	283.0	161.8	169.8	227.9
ILMN_1805665	<i>FLRT3</i>	1367.0	1459.8	3317.6	756.4	1925.9	159.1	122.6	130.5	438.1	168.0
ILMN_2406656	<i>GATA3</i>	472.8	427.5	875.0	556.0	355.0	114.5	125.7	120.9	160.8	133.0
ILMN_1660549	<i>GPR177</i>	1489.8	1670.1	1795.2	963.2	753.7	136.6	154.8	144.5	186.0	341.2
ILMN_1671260	<i>GPR177</i>	2753.1	2612.7	2836.6	1414.1	1251.2	167.0	137.3	167.6	230.2	443.9
ILMN_1753913	<i>GPR177</i>	871.8	840.4	744.7	469.9	373.2	126.9	120.6	118.4	156.6	163.8
ILMN_2283325	<i>GPR177</i>	8195.7	6197.0	6948.9	3702.4	3383.9	310.9	288.1	243.1	633.9	832.4
ILMN_2399769	<i>GPR177</i>	1374.6	1677.3	1348.3	1003.2	856.7	153.9	136.9	135.3	221.6	268.4
ILMN_2121408	<i>HBEGF</i>	1810.1	3336.5	2582.6	3729.9	5887.9	243.5	259.1	247.3	294.4	220.5

ILMN_1653466	<i>HES4</i>	9815.7	13300.6	12883.0	12383.2	5076.5	502.4	961.3	510.4	821.5	536.1
ILMN_1788203	<i>HEY1</i>	1128.3	984.6	1006.1	1577.7	1785.5	215.1	155.7	167.1	227.2	120.0
ILMN_3236367	<i>IFFO2</i>	1126.1	942.1	1091.1	1598.3	1082.1	212.2	240.1	182.4	243.9	293.5
ILMN_1778010	<i>IL32</i>	893.1	612.1	429.6	1332.9	605.4	128.4	122.1	129.3	118.8	152.3
ILMN_2368530	<i>IL32</i>	1793.0	1168.8	1029.4	2640.2	1553.5	144.5	132.1	128.6	186.1	196.9
ILMN_1685397	<i>ITGA3</i>	1565.2	1234.1	2656.8	3190.2	2023.0	423.2	414.2	373.5	452.9	550.0
ILMN_1800697	<i>LDB2</i>	595.3	417.7	532.0	390.8	334.1	3864.7	2985.2	3052.1	4158.7	2191.4
ILMN_2213136	<i>LEF1</i>	747.3	852.4	822.6	1013.4	647.5	155.0	146.2	117.7	186.5	129.6
ILMN_1657373	<i>LEPREL1</i>	3482.0	5302.0	6330.5	4782.0	8674.7	1296.6	1367.0	799.9	2546.2	575.8
ILMN_3242105	<i>LOC100134073</i>	1118.2	1645.1	1908.6	1344.0	2018.4	352.7	272.7	295.4	293.0	135.0
ILMN_1813131	<i>LOC643431</i>	1003.5	487.1	2180.1	947.3	1268.1	256.3	245.3	320.0	252.4	165.4
ILMN_1773002	<i>LOC730417</i>	2074.1	1600.1	1845.2	1127.1	680.6	494.5	389.5	320.6	418.2	264.4
ILMN_1773650	<i>LRRN3</i>	825.5	1664.2	1051.0	858.6	3947.3	148.3	134.6	125.3	172.2	189.3
ILMN_2048591	<i>LRRN3</i>	554.8	1080.9	903.6	824.0	2438.6	123.3	131.2	124.2	151.5	125.4
ILMN_2376205	<i>LTB</i>	1011.7	694.1	975.9	943.5	258.8	134.0	139.3	145.0	247.3	116.1
ILMN_1668863	<i>LYPD1</i>	2366.7	2320.4	3759.7	2318.7	3823.9	501.0	552.1	414.1	414.3	136.9
ILMN_1679391	<i>MAMDC2</i>	2275.6	7090.7	3018.2	5091.0	11885.4	1219.7	724.8	830.2	537.7	306.4
ILMN_1711331	<i>MAP1LC3C</i>	745.3	1044.2	1240.1	693.2	912.7	232.8	164.4	159.8	188.7	183.3
ILMN_1775170	<i>MT1X</i>	826.1	815.0	684.4	665.2	867.2	4353.5	3349.3	7279.1	3163.5	1727.4
ILMN_1692077	<i>MXRA7</i>	656.4	889.1	641.6	985.9	1003.5	134.4	122.2	143.5	164.4	466.0
ILMN_1776953	<i>MYL9</i>	1618.4	2350.9	1817.3	2307.7	3461.1	263.9	242.4	322.8	304.5	1266.6
ILMN_1656111	<i>MYLIP</i>	304.9	396.3	310.8	238.2	634.0	1968.8	1778.6	1898.0	1849.8	1090.3
ILMN_1713638	<i>NIPAL4</i>	742.5	518.6	453.6	2005.0	617.4	125.5	133.9	126.0	129.0	130.0
ILMN_1763382	<i>NPPB</i>	5187.2	12750.7	7078.6	12244.0	8760.8	133.2	127.8	137.6	220.9	138.7
ILMN_1800160	<i>NR0B1</i>	787.7	634.4	661.0	700.2	202.0	128.3	131.1	134.9	148.0	143.1
ILMN_2094360	<i>NR2F2</i>	4093.8	3408.8	5787.4	3929.4	3501.2	110.9	130.3	121.4	131.6	1406.4
ILMN_1742025	<i>OLFM1</i>	383.8	558.4	234.1	371.7	276.1	1978.4	2418.3	2517.6	1477.4	973.3
ILMN_1710544	<i>PCDH7</i>	948.9	2493.0	777.4	1895.2	1940.1	152.1	148.6	168.6	308.2	242.6
ILMN_1695299	<i>PDLIM3</i>	1028.5	1070.2	1135.8	1254.5	391.7	221.9	301.5	237.2	240.2	145.8
ILMN_2230025	<i>PDLIM3</i>	1718.7	1416.0	1520.2	1834.6	526.6	305.8	442.4	259.1	319.8	156.9
ILMN_1690125	<i>PDLIM7</i>	5842.9	6681.6	4795.1	7360.1	4528.1	1442.8	1312.3	1275.8	1231.5	1995.6
ILMN_2407669	<i>PEAR1</i>	538.2	774.5	788.2	1320.2	624.4	121.4	121.6	124.6	138.6	469.4
ILMN_1805737	<i>PFKP</i>	8674.9	9485.0	10608.3	13887.4	8011.3	3050.3	2716.2	2441.5	2877.2	1687.6
ILMN_1671557	<i>PHLDA2</i>	533.0	837.9	417.0	1027.6	518.0	144.4	134.2	138.1	159.9	251.5
ILMN_1653026	<i>PLAC8</i>	717.6	841.7	1492.8	3325.0	3100.9	136.3	129.6	119.1	115.2	133.0
ILMN_2093343	<i>PLAC8</i>	914.9	1075.2	2724.2	4753.7	4079.4	143.1	138.5	123.8	127.2	154.4
ILMN_1656057	<i>PLAU</i>	11403.7	11522.9	15488.8	18233.0	15034.0	1123.6	960.8	960.2	645.1	1089.4
ILMN_1717706	<i>PLK2</i>	860.4	1025.5	1221.5	1257.8	1509.3	170.5	165.5	174.0	164.7	215.8
ILMN_1795930	<i>PTGER4</i>	1641.8	2359.4	965.1	1080.1	1434.4	182.2	184.0	188.8	179.6	202.4
ILMN_1698766	<i>PYCARD</i>	396.3	492.9	347.6	890.1	288.6	2455.7	2063.6	2211.2	1855.5	1877.0
ILMN_1738558	<i>RGS20</i>	1353.6	2429.1	1132.7	1936.6	3651.0	138.9	125.3	127.6	133.7	265.6
ILMN_1678215	<i>RHOJ</i>	548.5	640.0	436.3	1071.6	2691.9	129.7	120.0	121.4	120.9	203.3
ILMN_1722898	<i>SFRP2</i>	3043.0	1758.5	2424.4	875.0	2014.2	5639.8	5132.9	5456.5	10523.8	8872.8
ILMN_3310065	<i>SFTA1P</i>	1634.5	2337.3	1382.5	3263.1	2306.7	142.8	137.5	139.4	124.2	136.0
ILMN_1766261	<i>SLC2A12</i>	928.7	1124.5	695.5	886.8	781.8	284.2	327.0	282.5	215.9	122.5

ILMN_2407879	<i>SORBS2</i>	829.9	1913.2	301.4	992.1	1696.5	168.4	170.5	188.8	167.8	184.3
ILMN_1651354	<i>SPP1</i>	4229.6	5784.2	6404.3	3115.1	4009.8	303.8	381.3	806.1	402.8	169.3
ILMN_2374449	<i>SPP1</i>	2936.6	4594.4	3751.1	2211.8	2791.2	238.2	235.0	665.6	304.8	170.8
ILMN_2086105	<i>SPRY4</i>	317.2	329.5	220.5	293.3	195.7	2965.1	3106.1	2462.8	3533.8	494.8
ILMN_1667460	<i>SULF2</i>	2099.8	1850.7	3194.4	2147.2	1709.4	489.5	504.9	453.7	414.8	1226.8
ILMN_1686981	<i>SULF2</i>	680.3	676.6	953.8	916.3	935.4	155.4	156.4	139.2	156.9	538.0
ILMN_2345142	<i>SULF2</i>	1326.7	1478.3	1616.6	1525.0	1872.5	338.0	258.5	265.0	240.4	852.4
ILMN_2400935	<i>TAGLN</i>	8903.3	13503.2	8369.1	13263.2	12888.8	1049.9	641.6	1113.6	1025.8	4064.0
ILMN_1662619	<i>TFPI</i>	1359.5	2360.4	1552.8	1614.8	5130.7	150.7	131.4	159.2	277.7	716.3
ILMN_1812526	<i>TGFB2</i>	340.4	1096.8	472.3	834.1	2695.1	118.1	113.8	118.2	112.9	115.2
ILMN_1679267	<i>TGM2</i>	458.8	668.9	736.6	1090.2	768.0	157.6	155.4	127.4	144.5	247.0
ILMN_1705750	<i>TGM2</i>	3031.6	3621.6	6073.6	15707.0	10241.9	160.2	181.2	147.5	178.8	751.6
ILMN_1804663	<i>THBS3</i>	2661.0	2209.7	3625.5	2587.5	1815.5	233.6	266.3	287.2	252.5	1294.8
ILMN_1736078	<i>THBS4</i>	320.4	165.8	214.7	252.7	135.3	879.2	843.6	799.2	499.1	944.4
ILMN_1779875	<i>THY1</i>	1079.1	1636.7	1273.6	2810.6	1118.2	6743.1	7021.1	6430.4	6884.4	12613.6
ILMN_1770338	<i>TM4SF1</i>	3151.1	2595.4	1442.7	4095.7	4456.1	142.9	128.8	133.5	186.2	342.4
ILMN_1678403	<i>TMEM178</i>	730.2	1266.0	546.8	759.4	927.8	253.3	215.2	371.5	256.6	115.3
ILMN_1757129	<i>TMEM88</i>	1544.3	1689.2	1978.5	2553.6	910.3	282.7	209.8	160.4	250.0	120.5
ILMN_1699695	<i>TNFRSF21</i>	10778.0	11540.3	12188.4	13621.3	6880.1	3061.5	3023.0	2998.7	3452.4	967.6
ILMN_2331231	<i>TNFRSF6B</i>	415.9	468.2	575.9	1374.4	550.4	139.8	139.8	135.4	149.1	299.4
ILMN_1699489	<i>TUBB6</i>	5562.1	5894.3	5388.3	7773.9	5195.4	1156.7	1166.1	1236.3	836.2	3929.7
ILMN_1702636	<i>TUBB6</i>	2811.8	4374.7	2407.1	3746.3	4410.1	511.0	459.3	687.2	437.2	2125.2
ILMN_1678841	<i>UBD</i>	923.8	1281.3	344.6	729.5	690.4	121.8	136.4	141.3	131.4	150.9
ILMN_1671969	<i>UGP2</i>	276.4	321.0	283.1	150.9	447.3	1444.6	1587.7	1824.9	2311.1	815.9
ILMN_2389155	<i>UGP2</i>	520.8	574.3	574.4	509.8	933.7	3017.8	3415.4	2686.0	3646.7	1546.0
ILMN_2307903	<i>VCAM1</i>	1023.4	498.9	577.8	598.4	286.0	118.0	100.7	120.4	122.6	258.2
ILMN_1760315	<i>VWCE</i>	1247.7	1776.0	1025.5	1601.5	564.3	269.3	361.8	342.8	179.4	231.3
ILMN_1740269	<i>WNT2B</i>	1943.1	1711.3	981.8	1639.9	1088.8	121.8	120.1	124.0	115.9	225.2
ILMN_1779015	<i>ZNF467</i>	810.0	422.2	968.5	587.3	357.5	119.4	137.1	122.1	209.0	182.0