Supplemental Figure Legends:

Fig. S1. Expression of FoxOs in mouse brain. IHC analysis on the expression of FoxO1 in cortex (a, b) and SVZ (c, d), FoxO3 (e, f), and Cre (h) in NSC-rich SVZ of 10 day old FoxO WT (upper) and null (lower) mice. Red arrows point to neurons that do not express hGFAP-Cre therefore retain FoxO expression. **(g)** x-gal staining on a brain slice from *hGFAP-cre;ROSA26R* mice shows blue cells in SVZ underwent Cre-mediated recombination. EP, ependymal cells, LV, lateral ventricle. Bars= 40 and 60µm. **(i)** Immunofluorescence analysis on the expression of FoxO1 and FoxO3 in Sox2+ neural progenitors of SVZ in P8 mice. OL, overlap image of two separate channel images of marker co-stained panels. Note lack of FoxO1 and 3 staining in Sox2+ cells in FoxO null brain (right panels). Bar=30µm. Expression of FoxO1 (j) and 3 (k) in the brain of E15.5 embryos. High magnification images of boxed regions are shown on the right. Bars= 200µm, 40µm. **(l)** Expression of FoxO1 and 3 in Nestin+ neural progenitors of VZ from boxed region of panel (j-k) is examined. Note that FoxO 1 and 3 are both nucleocytoplasmically present in the Nestin+ cells of VZ and FoxO3 is more broadly and strongly expressed. Bar=40µm. Expression of FoxO1 (m) and FoxO3 (n) in different anatomic regions of WT mouse brain (8 week old). FoxO1 and 3 IHC used Nova red and DAB chromophore, respectively. 400X microscopic field of indicated regions were taken and boxed areas are shown in the right panels. Identification of cell types expressing FoxO1 or 3 by marker co-staining for cortical neurons (o, NeuN+), astrocytes (p, GFAP+) and oligodendrocytes (q, Olig2+) from corpus callosum. Note all three cell

types express detectable level of FoxO1 and 3 that disappear in FoxO null brain (right panels). Bars= 30µm.

Fig. S2. mRNA (a) and protein (b) expression of FoxO 1,3, and 4 in Rosa26-Cre ERT2 - (black bar) and + (gray bar) NSC 1 week after 4OHT treatment. NSC were derived from E13.5 embryo (a) and P1 pups (b). (c) Expression and localization of FoxO 1 and 3 in NSC (P1) *in vitro*. Both WT and FoxO null NSC grown as neurospheres were dissociated, cytospun, and co-stained for FoxOs (green), Nestin (red), Sox2 (magenta), and DAPI (blue). Bar= 40µm.

Fig. S3. **(a)** FoxO deficiency does not affect overall brain cyotarchitecture and anatomy of young mice. H&E stained coronal brain sections of FoxO WT (right) and null (left) of 3 week old mice. From the top, corpus callosum, SVZ, hippocampus, cerebellum, internal granular layers and Purkinje cell layers are shown. All paired images are under same magnification. Scales bars from top to bottom= 1mm, 1mm, 1mm, 1mm, 200µm. **(b)** Brain weights of indicated genotypes from 3-24 week old mice were plotted after normalizing with average brain weights of their age-matched WT littermate controls. **(c)** Thickness of cortices (upper) and corpus callosum (lower) from 24-32 week old mice were plotted. Numbers are normalized by their age-matched WT littermate controls. n=4, **, p=0.0136, *, p=0.0444

Fig. S4. FoxO deficient brain exhibits signs of degeneration in older mice. **(a)** H&E images of lateral ventricle dilation in 20 week old FoxO null brain (right). Bar=1mm.

600x microscopic field of boxed regions are shown on the side. Arrow points to abnormal thinning of ventricular wall. Bar=60µm. **(b)** Degree of ventricular dilation was measured by unilateral ventricular surface area from 6 pairs of 15-32 week old mice. Y axis displays % difference. WT vs null is 100% vs 751.1%, **, p=0.011. Bar=200µm. **(c)** Decrease in olfactory bulb neurogenesis in 32 weeks old FoxO null mice. Mice received BrdU in drinking water for a week and taken off of BrdU for three weeks before sacrificed. BrdU+ cells (upper panels) and colocalization of BrdU+ and NeuN+ cells (lower panels) are shown. Percent of BrdU+ cells among NeuN+ neurons within olfactory bulb is plotted. WT vs null is 1.76% vs 0.92%, **, p=0.018. Bar=60µm

Fig. S5. **(a)** No significant differences in cell size of FoxO WT (right) and null (left) 15 week old mice. Left panels show GFAP positive astrocytes and right panels are TuJ1 positive neurons in FoxO WT and null brains. Bar= 40 µm. **(b)** Average volume of dye-loaded cortical neurons isolated from neonatal mice (p1) (n=60, p=0.21 by two tail t-test). Representative z stack view of FoxO WT (right) and null (left) neurons is shown. Heat map shows the distance from the bottom (µm). Bar= 30µm. **(c)** Size of astrocytes isolated from neonatal mice (P1) is measured by flow cytometry. Representative histogram is shown (median values are 128k and 127k for WT and null respectively). **(d)** FoxO null astrocyte from old mice shows signs of activation. *In vitro* differentiated astrocytes from FoxO null NSC derived from adult SVZ shows activated morphology. Bar= 30µm **(e)** Presence of hypertrophic GFAP-positive astrocytes in the cortex of 32 week old FoxO null brain (top). Corresponding region of wild type control lacks GFAP positive cells (bottom). Bar= 40µm.

Fig. S6. Histograms display size distribution of all the neurospheres. **(a, b)** WT/KO 1 and 2 are two independent primary cultures of WT and FoxO null NSC. Cultures are from 18-22 week old (a) and 4 week old (b) mice. NAC (1mM) treated (c) or SESN3-retrovirus-transduced (d) NSC culture. **(e)** NT or shASPM_1 or shASPM_2 lentivirus-infected FoxO null NSC culture.

Fig. S7. (a) Increased mRNA expression of canonical Wnt target genes, CyclinD2 and Myc in FoxO null (gray bar) NSC compared to WT (black bar). **(b)** Knockdown of sFRP1/2 and SOST by individual (200nM) or mixture (mix, each 100nM) siRNAs. mRNA expression was measured by rtqPCR 24hr after the trasfection.

Fig. S8. Molecular targets of FoxO in NSC. **(a)** Regulation on putative FoxO target genes. For mRNA expression of each putative target was confirmed by rtqPCR after enforced expression of CA1 or CA3 in FoxO null NSC. Y axis is fold change over control. Mean ± s.e. is shown. **(b)** ChIP analysis for FoxO1 and FoxO3. Targets bearing conserved FoxO BE were tested for the presence of FoxO binding. Chromatins from FoxO null NSC were used as control. 5% of inputs were used for PCR. '-' and '+' stand for null and WT respectively.

Supplemental Table S1. List of genes differentially expressed in FoxO null embryonic (E13.5) NSC.

Supplemental Table S2. List of genes differentially expressed with SAM>2.0 from Supplemental Table S1.

Supplemental Table S3. FoxO-dependent changes in expression of genes regulating intracellular ROS levels. List is from selected from Supplemental Table S1. Fold changes are calculated using two different statistical methods.

Supplemental Experimental Procedure:

Isolation and culture of NSC. Embryonic NSC were isolated from E13.5 ganglionic eminence as described (Reynolds and Weiss, 1992). Cells were physically dissociated, and propagated in Dulbecco's Modification of Eagle's Medium/F-12 1:1 mix supplemented with B12 and 20ng/ml of bFGF and recombinant human EGF. Primary neurospheres were raised and passaged by enzymatic dissociation using TrypLE™express (Invitrogen). To measure proliferation of NSC 180,000 cells were plated in 6 well plates and then dissociated, counted, and replated every 5 days. Population doubling was calculated as $\log_2$ (cell number counted/plated). Adult NSC were isolated from SVZ of different age of mice. SVZ were dissected out in DMEM/F12 media and mechanically dissociated and filtered through 40 micron mesh. Cells were cultured as above. For differentiation cells were treated with neurobasal media with 1% FBS was added to induce differentiation into neuronal and glial cell types. Cultures were assessed by immunocytochemistry for the presence of different cell lineages after 4-5 days. Embryonic NSC for gene expression profiling study were dissociated and cultured at 100,000 cells/ml with the addition of growth factors every three days. For in vitro deletion of FoxOs freshly isolated *Rosa26-CreERT2*$^{+}$ *or* $^{-}$ *;FoxO1/3/4*$^{L/L}$ NSC were cultured for 1 week then 400nM 4-hydroxy tamoxifen (4OHT) was added to the culture twice in 48hr interval or for adult NSC cells were grown in the presence of 100nM 4OHT

during the formation of primary spheres. All the measurement for mRNA and protein expression was performed on actively growing neurospheres.

Immunohistochemistry and immunofluorescence. Mice were perfused with 4% paraformaldehyde and brains were removed and fixed further and processed for hematoxylin and eosin (H&E) by standard techniques. The entire brain was sectioned in 1–2 mm coronal blocks and submitted in one cassette for paraffin embedding to facilitate analysis of the whole brain. For immunohistochemical analysis, sections were prepared for staining with Paraffin embedded tissues were sectioned at 5 µm thickness. Sections were hydrated in series of ethanol and equilibrated in PBS. Slides were antigen-retrieved using citrate buffer and processed following manufacturer's recommendation (DAKO envision kit). Slides were incubated overnight at 4ºC with the following antibodies: 1:400 Goat-anti-Sox-2 (sc-12, Santa Cruz Biotechnology), 1:200 goat-anti-doublecortin (C-18, Santa Cruz Biotechnology), NeuN (Chemicon), S100 (Neomarkers), FoxO1 (clone C29H4, Cell signaling Technology), FoxO3 (H-144, Santa Cruz Biotechnology), Ki67 (DAKO), GFAP (DAKO), ASPM (Bethyl laboratories), Cre (Covance).. Following secondary antibody incubation, sections were then incubated with DAB and then counterstained with hematoxylin. For immunocytochemistry neurospheres were dissociated and cytospun on a slide in 2% BSA/PBS, fixed in 4% paraformaldehyde and permeabilized with 0.2% TritonX-100 and incubated with the primary antibody. The following antibodies were used: rabbit anti-Ki67(1:100), mouse anti-III-tubulin (Tuj1; 1:400, covance), mouse anti-nestin (1:100, Chemicon), and 8-oxoG (Chemicon). Secondary antibodies (Alexa Fluor-conjegated, Invitrogen) were incubated and

coverslips were mounted with Prolong gold antifade mounting media (Invitrogen). Images were acquired on Yokogawa spinning disk confocal microscope (Andor). For quantitation several random visual fields from each section were viewed. The percentage of positive cells was calculated in relation to the total number of DAPI-stained nuclei present in the culture. All experiments were independently repeated from three separate cultures.

Microarray analysis and identification of putative FoxO BE. Cells were washed and cultured additional 72hr after 4OHT treatment before the harvest of their RNA using Trizol (Invitrogen) and the RNeasy mini kit (Qiagen). Gene expression profiling was performed utilizing the Affymetrix 430 2.0 chips. dChip (Li and Wong, 2001; Li and Wong, 2003) was used to normalize arrays and to compute expression indices. A list of 186 genes significantly differentially expressed between wild type and FoxO null NSC was generated using the SAM statistic. It was required that the absolute value of the SAM statistic be greater than two. Then the 5kb promoter regions of these genes were isolated and scanned for enrichment of the 550 binding motifs in TRANSFAC. Enrichment was assessed by comparing the target regions to matched control regions at the same distance from the transcription start sites of random genes. Promoter analysis on these gene sets for FoxO BE used the CisGenome software (http://www.biostat.jhsph.edu/~hji/cisgenome/).

Gain or loss of function assay. CA or DBD-FoxO1 (Frescas et al., 2005) and CA-FoxO3 mutant (T32A/S253A/S315A) in adenoviral vectors were generously provided by Domenico Accilli (Columbia University) and purchased from Vector Labs respectively.

Viral particles were amplified in NSC medium from HEK293 and titer was determined. shRNAs in pLKO lentiviral vector were acquired from TRC (the RNAi consortium). shRNAs for ASPM are TRCN0000110637-9. Two shRNAs for each gene were selected based on the knockdown efficiency. Vector control or ineffective shRNAs targeting same gene were used as an experimental control. Lentiviral particles were generated in 293T cells by transfecting 1, 6, 6µg of pVSV-G, Δ8,9, and viral vector respectively in 100mm dish using FuGENE6 (Roche) according to the manufacturer's protocol. Supernatant containing viral particles was collected between 48-72hr post transfection and ultracentrifuged (Beckman Coulter) at 23,000rpm for 3hrs. Alternatively On-target plus SMART pool pre designed siRNA mix from Dharmacon were used to knockdown the expression of target genes by Lipofectamine2000 using OptiMEM media for 3hrs. For gain of function experiment human SESN3 ORF was cloned into MSCV-puro-v5 gateway retroviral destination vector and virus was generated by co-transfecting pCL-eco into HEK293T cells. Supernatant was collected and concentrated using centriprep YM-30. NSC were plated on poly-L-ornithine and fibronectin-coated dishes were spin-infected with viral supernatant containing polybrene (8µg/ml). Cells were incubated 96 hrs prior to harvest for verification of knockdown by rtqPCR.

Cell Size Measurement. Primary cortical neurons and astrocytes were isolated from neonatal mice (P1) as described previously (Bachoo et al., 2004). Neurons were cultured in neurobasal media with B27 supplement grown on poly-d-lysine coated coverslips. Neurons were labeled with DiD and scanned though the cell body for collection of Z-axis stacks with 1µm interval using 100x objective on Olympus laser scanning confocal

microscope. Collected images were processed for cellular volume in cubic micronmeter using an in-house semi-automated segmentation algorithm. Astrocytes were lifted by gentle trypsinization and dead cells were gated out by propidium iodide staining. Histogram was created with FSC as X-axis and median values were used for the comparison of the cell size.

Quantitation of Olfactory bulb neurogenesis 32 week old mice received BrdU (1mg/ml) in the drinking water for a week and taken off of BrdU for three weeks before sacrificed. Brain tissues were processed and stained with BrdU and NeuN as above and double positive cells were scored from 5 different areas of olfactory bulb.

Chromatin Immunoprecipitation (ChIP) assay. Two million NSC either from FoxO null or WT embryos were crosslinked by addition of 1% formaldehyde to the medium for 10 min followed by quenching with 125mM glycine. The cells were resuspended in lysis buffer (1% SDS, 10 mM EDTA, and 50 mM Tris (pH 8.1), Protease Inhibitor Cocktail II (Roche)), sonicated 10 times for 30 s with 2 min idle time, the lysates were cleared by centrifugation. One hundred microliters of the sheared DNA was diluted 1:10 in dilution buffer (0.01% SDS, 1.1% Triton X-100, 1.2 mM EDTA, 16.7 mM Tris-HCl (pH 8.1), and 167 mM NaCl). Chromatin solution was precleared for 1 h at 4°C with 60 µl of protein G-agarose/salmon sperm DNA. Ten microliters of the precleared chromatin solutions was saved for assessment of input chromatin, and the rest of the precleared chromatin solutions was incubated with 1ug of anti-Rabbit IgG, anti-FoxO1 IgG, or anti-FoxO3 IgG (Cell signaling, Santa Cruz biotechnology, sc-11350, 11351, respectively) overnight at

4°C. Immune complexes were collected on 60 µl of protein A/G Plus-agarose/salmon sperm beads. Precipitates were washed sequentially for 5 min each in Low salt wash buffer [0.1% SDS, 1% Triton X-100, 2 mM EDTA, 20 mM Tris-HCl (pH 8.1), 150 mM NaCl], High salt wash buffer [0.1% SDS, 1% Triton X-100, 2 mM EDTA, 20 mM Tris-HCl (pH 8.1), 500 mM NaCl], LiCl immune wash buffer. Precipitates were then washed twice with 1X TE (pH. 8.0) and extracted two times with 1% SDS, 0.1 M $NaHCO_3$. Elutes were incubated at 65°C with 0.25 M NaCl overnight to reverse cross-linking followed by another 1 hr incubation at 45°C with 10 µM EDTA, 40 µM Tris-HCl (pH 6.8) and 2µg Proteinase K (Sigma). The DNA was purified using a PCR purification kit (Qiagen) with 60 µl of distilled water. One microliter of immunoprecipitated DNA was used for each PCR reaction. Optimal cycle number was determined by Ct values from rtqPCR. Enrichment was calculated and plotted by % of total input (1:100 diluted).

RNA isolation and real-time PCR. Total RNA was extracted using the PureLink Micro-to-Midi kit (invitrogen) and treated with RQ1 RNase-free DNase Set (Promega). First-strand cDNA was synthesized using 1µg of total RNA and SuperscriptII (Invitrogen). rtqPCR was performed in duplicates with a MxPro3000 and SYBR Greener qPCR mix (Invitrogen). The relative amount of specific mRNA was normalized to beta actin. Primer sequences are available upon request.