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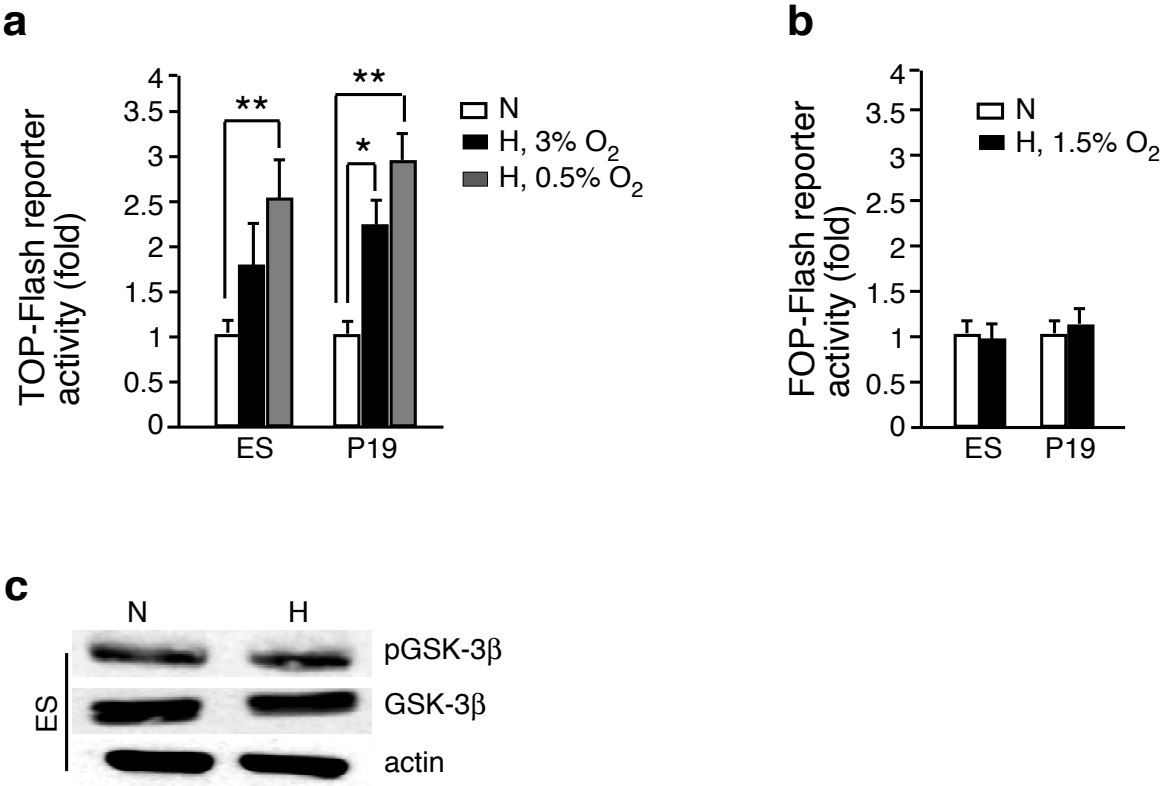


Figure S1 Hypoxia activates Wnt/β-catenin signalling in embryonic cells. **a**, ES and P19 EC cells transiently transfected with TOP-Flash reporter plasmid were cultured under different low O_2 levels (0.5% and 3%) for 16 h (n=6). **b**, No significant enhancement of FOP-Flash reporter activity was observed in hypoxic cells (1.5% O_2 or other low O_2 levels [data not shown]) over cells cultured under normoxia (n=6). Luciferase activity from pRL-SV40 reporter co-transfected with TOP-Flash or FOP-Flash reporter plasmids was used for normalization. **c**, Western blot analysis of whole cell extracts of ES cells cultured under normoxia or hypoxia for phosphorylated GSK-3β, and total GSK-3β. Actin served as the loading control. *= P <0.05, **= P <0.005., Student's t-test. Error bars represent S.D.

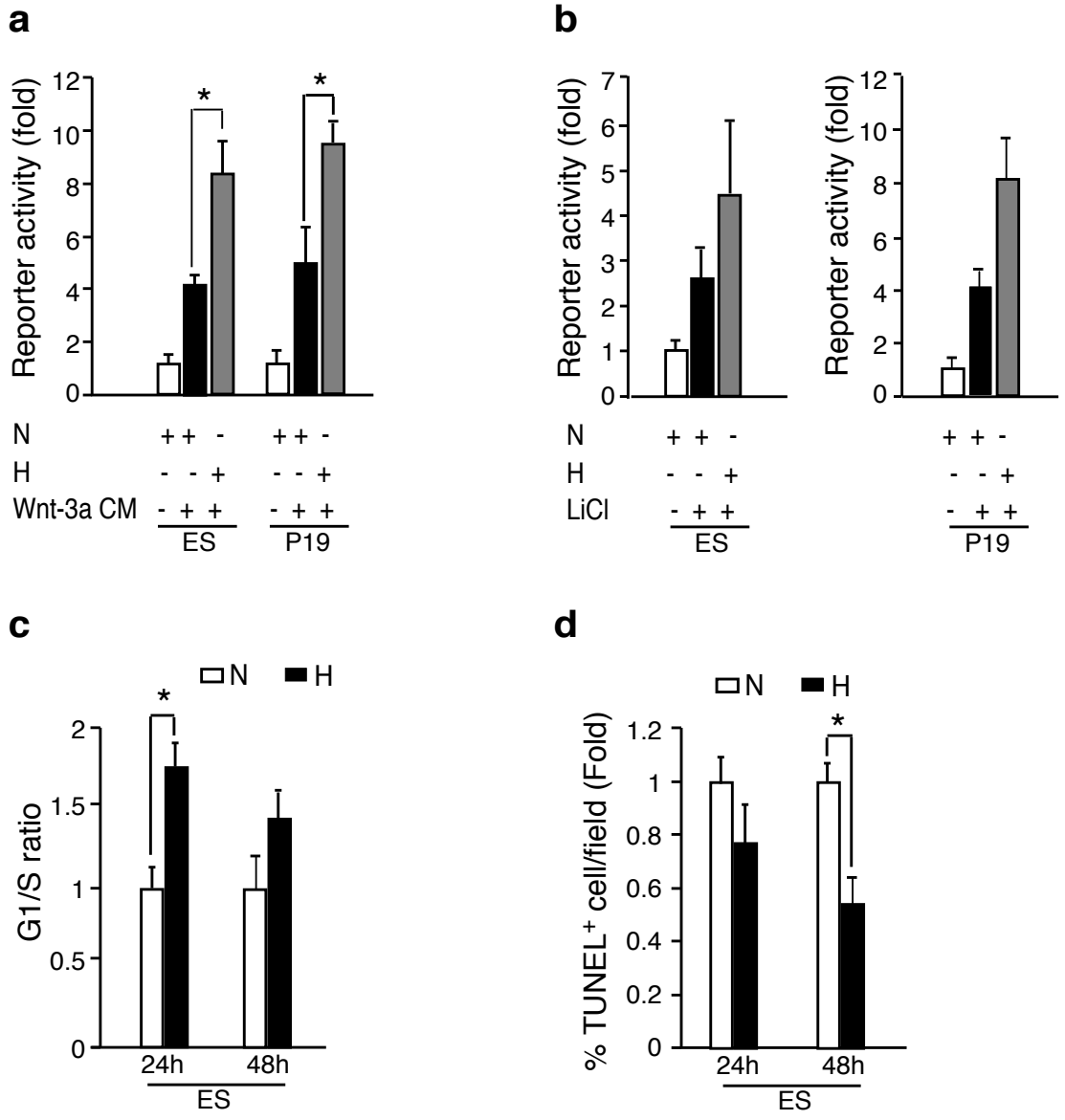


Figure S2 Hypoxia increases Wnt/β-catenin activity in stimulated cells. **a**, **b**, TOP-Flash reporter activity in ES and P19 EC cells treated with either Wnt-3a CM or LiCl and cultured either under 21% or 1.5% O_2 for 16 h (n=9). Whereas Wnt-3a CM and LiCl stimulates TOP-Flash activity, exposure to 1.5% O_2 further increases TOP-Flash activity in stimulated cells. **c,** Cell-cycle analyses for ES cells after exposure to normoxia or hypoxia (1.5% O_2) for 24 h or 48 h. Cells were labelled with BrdU, stained with propidium iodide (PI) and analysed by flow cytometry (n=3 independent experiments). Increased G1/S ratio of hypoxic ES cells indicates increased accumulation in G1 stage, and delayed S-phase entry. Note G1/S phase ratio decreases in ES cells exposed to hypoxia for 48h, as compared to cells cultured under hypoxia for 24 h. **d**, Decreased cell death in ES cells cultured under hypoxia as compared to normoxic control cells (n=3 plates, 9 random fields in each plate) assessed by a TdT mediated dUTP nick end labelling (TUNEL) assay. Increased cell survival likely accounts for ES cell expansion under prolonged hypoxia (Fig. 1g). *= P<0.05., Student's t-test. Error bars represent S.D.

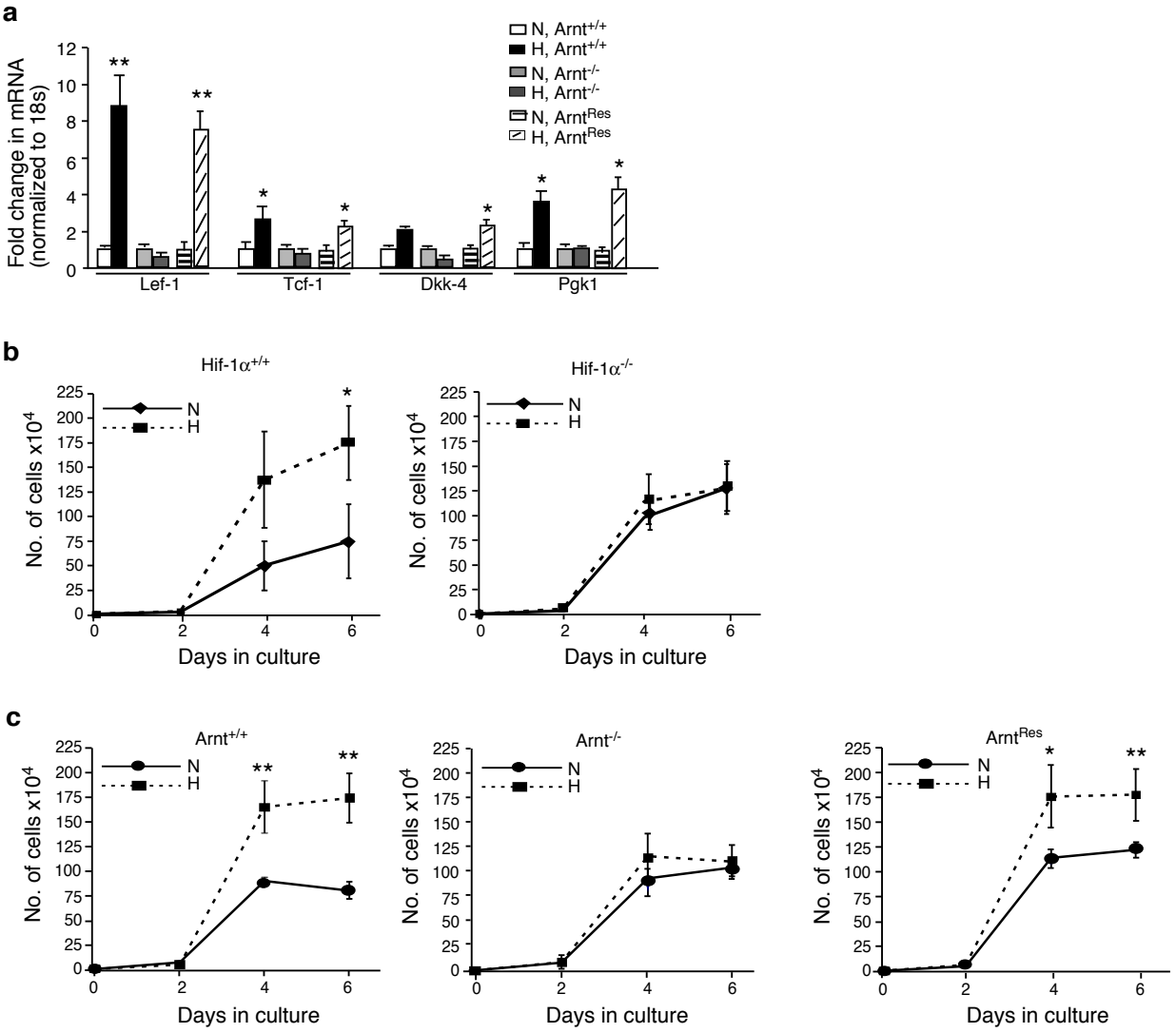


Figure S3 HIF-1α and ARNT are required for hypoxic activation of Wnt/β-catenin signalling in embryonic cells. **a,** qRT-PCR analysis of Wnt-3a target genes in *Arnt* $^{+/+}$, *Arnt* $^{-/-}$ and *Arnt* ^{Res} ES cells show direct dependence of Wnt induction on hypoxic ARNT activity (n=3). **b, c,** Cells were plated at a density of 10^4 cells per 60 mm^2 and cultured under normoxia or 1.5% O_2 for 6 days. Numbers were assessed by cell counts in a hemocytometer at indicated time points. *Hif-1α* $^{+/+}$(**b**), *Arnt* $^{+/+}$ and *Arnt* ^{Res} (**c**) cells displayed cell number expansion under hypoxia. To the contrary, hypoxic *Hif-1α* $^{-/-}$ (**b**) and *Arnt* $^{-/-}$ (**c**) cells grew at rates comparable to normoxic control cells. *= P <0.05, **= P <0.005., Student's t-test. Error bars represent S.D.

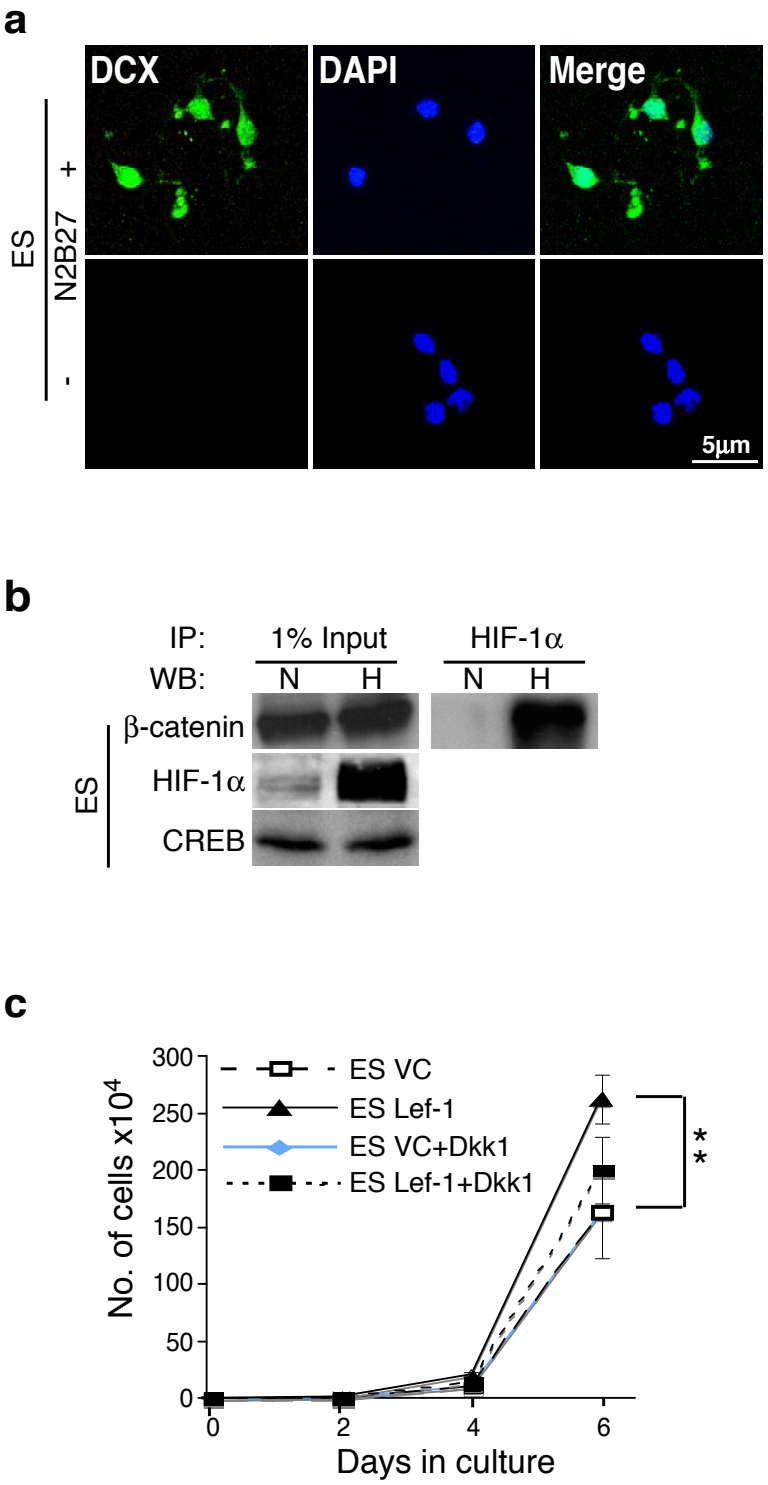


Figure S4 ES cell differentiation, HIF-1α/β-catenin interaction and LEF-1 modulation of ES cell growth. **a**, ES cells treated with (+) or without (-) N2B27 neuronal growth and differentiation supplements differentiate into neurons as indicated by the expression of the neuronal marker doublecortin (DCX) in green. The nuclei are stained with DAPI (blue). **b**, Immunoprecipitation with HIF-1α antibody was performed on nuclear extracts of normoxic or hypoxic (1.5% for 20 h) ES cells. CREB served as the loading control. **c,** ES-*Lef-1* cells were plated at a density of 10^4 cells per 60 mm^2, cultured under normoxia and cell numbers assessed over 6 days. Empty virus transduced (VC) cells served as control. Both cell lines were also treated with DKK-1 (300 ng ml^{-1}). **= P <0.005., Student's *t*-test. Error bars represent S.D.

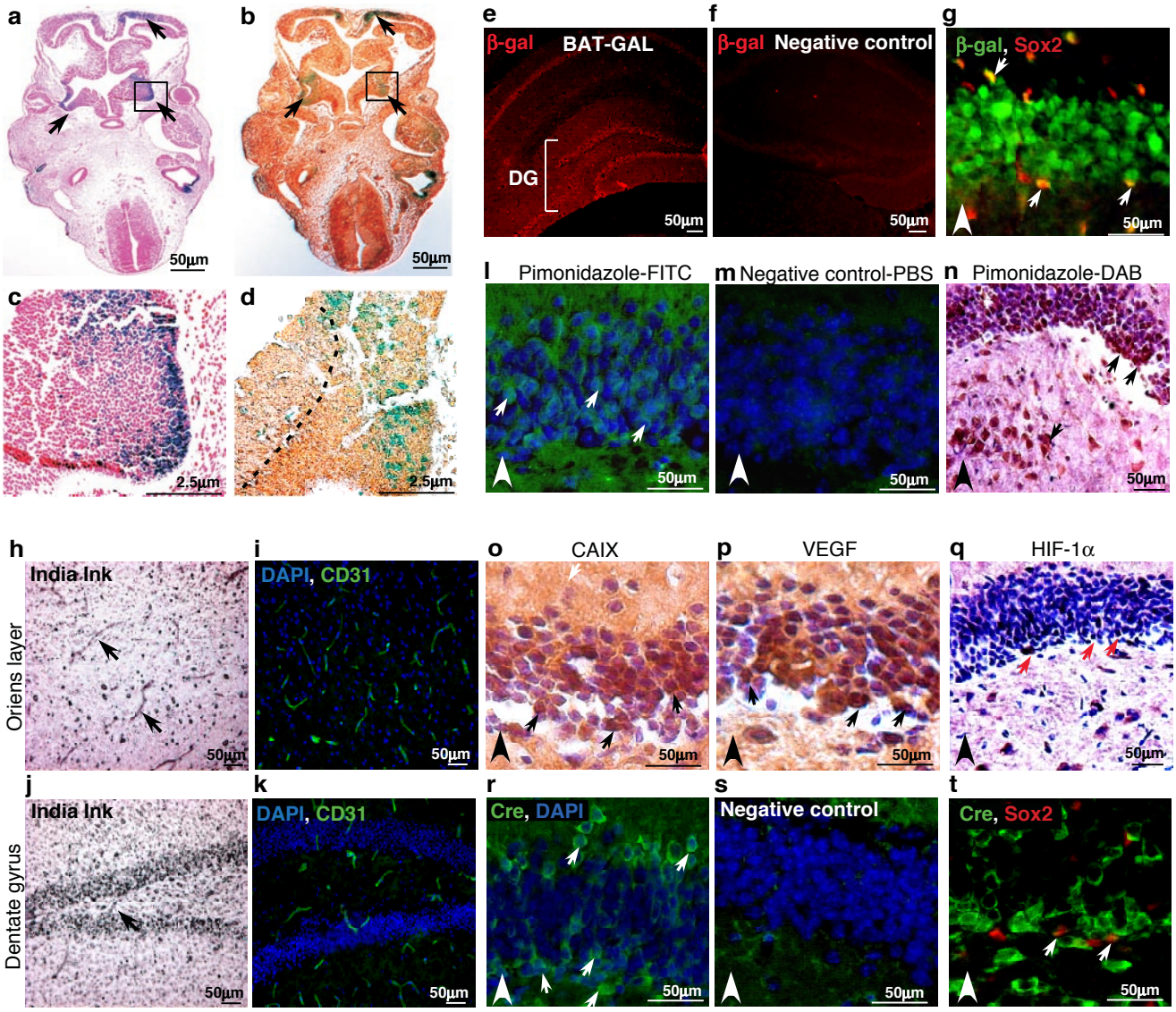


Figure S5 Hypoxic regions in embryonic and adult brain. **a, b,** Wnt /β-catenin activity marked by β-galactosidase enzyme staining (blue) in E11.5 *BAT-GAL* reporter mice (**a**) is closely associated with hypoxic regions marked by pimonidazole staining (brown) in an adjacent β-galactosidase enzyme stained (blue) embryonic section (**b**). (**c, d**) Magnifications of the boxed region in (**a**) and (**b**). Black line in (**d**) demarcates highly hypoxic region from the adjacent light brown area. **e-g**, β-galactosidase (β-gal) immunostaining in *BAT-GAL* reporter line (**e**) and wildtype control (**f**) identifies the GCL as active for Wnt/β-catenin signalling. **g**, Co-expression of β-galactosidase and Sox2 in neural stem cells in the SGZ. **h-k**, India black ink and CD31 mark fewer blood vessels in the GCL of the DG (**j, k**) as compared to the Oriens layer of the hippocampus (**h, i**). **l-n**, Pimonidazole immunofluorescence staining (**l**) and enzymatic immunodetection (**n**) indicates the presence of hypoxic pockets in the GCL. The hippocampus of PBS injected animals served as a negative control (**m**). **o-q**, Expression of CAIX (**o**), VEGF (**p**) and stabilization of nuclear HIF-1α (**q**) within the GCL of the DG. **r-t**, Nuclear (arrows) and cytoplasmic distribution of Cre in the GCL of αCamKII-Cre R1 line (**r**). Cre negative mice served as a negative control (**s**). Colocalization with $Sox2^+$ cells (arrows) indicates the expression of Cre in neural stem and progenitor cells in the SGZ (**t**). In **e-t**, arrowheads point towards the SGZ, and arrows indicate the relevant cells.

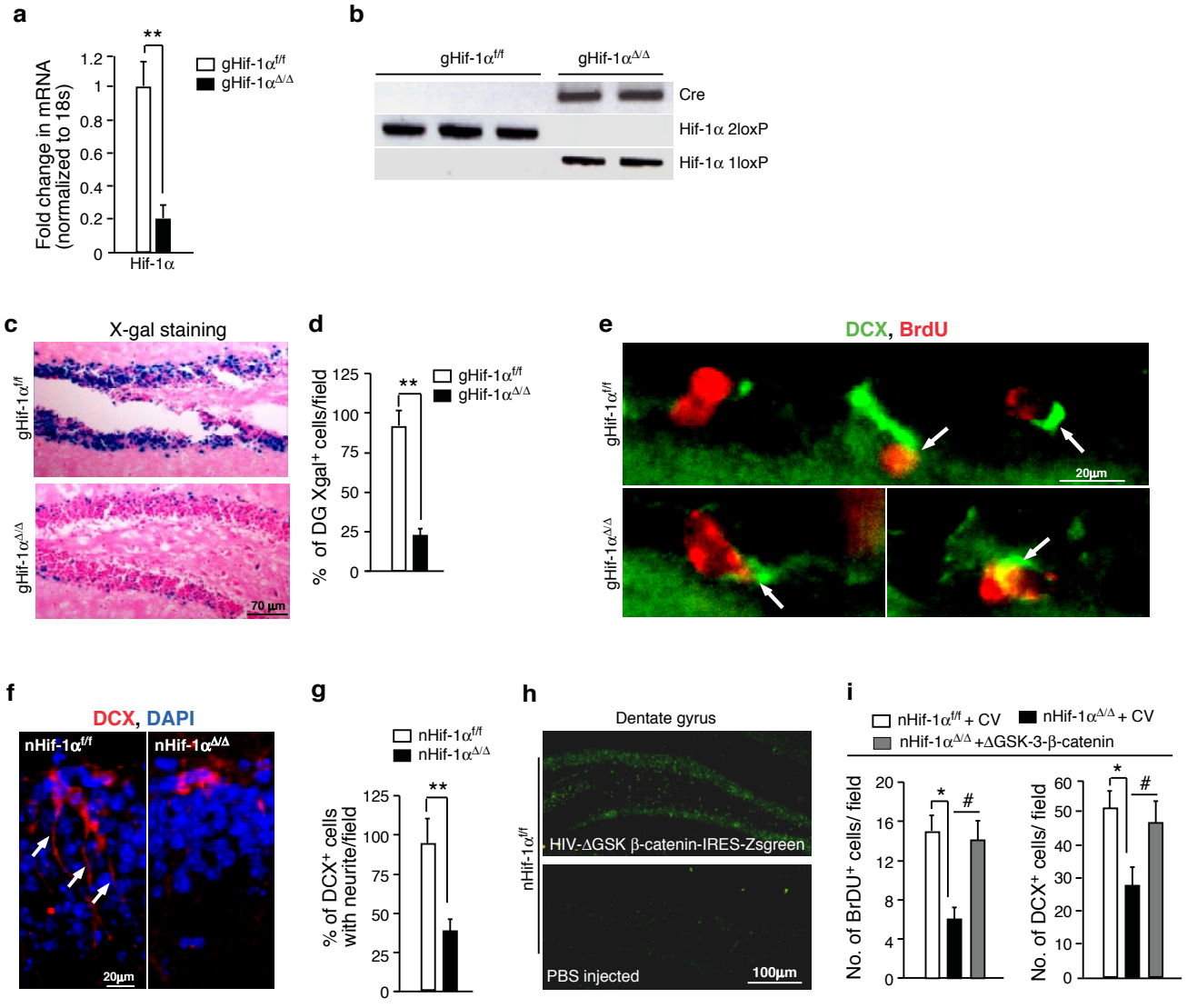
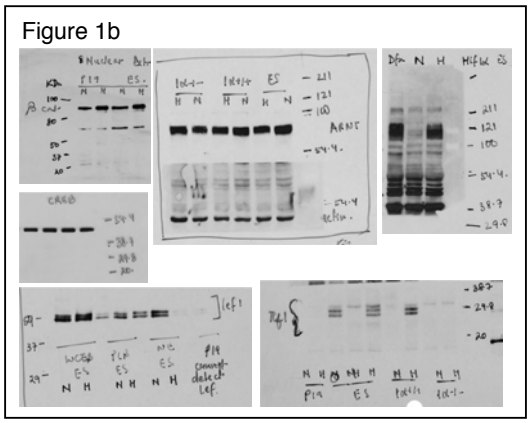
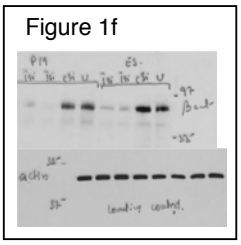
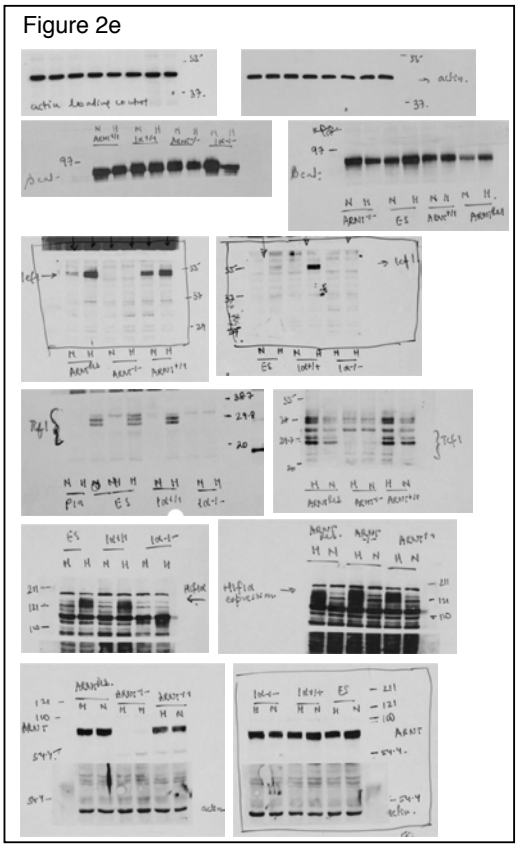
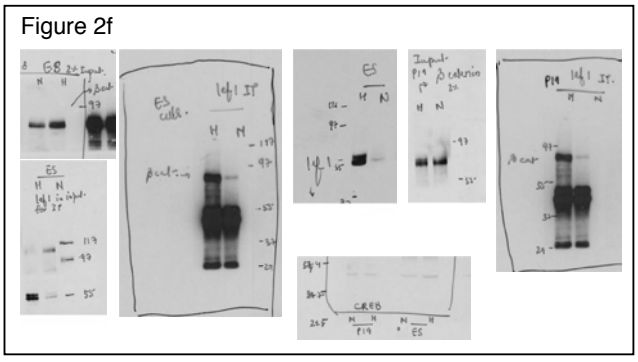


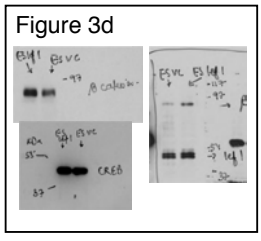
Figure S6 *In vivo* deletion of *Hif-1α* impairs adult hippocampal neurogenesis and Wnt/β-catenin signalling. **a**, qRT-PCR confirmation of *Hif-1α* deletion in the adult hippocampal extracts of *gHif-1α*$^{\Delta/\Delta}$ mice (*n=3-4* in each group). **b**, PCR genotyping indicating *Cre* mediated deletion of *Hif-1α* marked by the absence of *2loxP* band and the presence of *Cre* and *1loxP* bands (*gHif-1α*$^{\Delta/\Delta}$ mice). The recombination efficiency of Cre is variable. Weak *2loxP* band was detected in some *Hif-1α*$^{\Delta/\Delta}$ mice (data not shown). **c**, β-galactosidase enzyme (X-gal) staining of the dentate gyrus of *Hif-1α*$^{f/f}$, *BAT-GAL*^{Tg} (c upper panel) and *gHif-1α*$^{\Delta/\Delta}$, *BAT-GAL*^{Tg} (c lower panel). **d**, 4 fold reduction in Wnt activity as assessed by X-gal staining in the dentate gyrus of adult *gHif-1α*$^{\Delta/\Delta}$ compared to *gHif-1α*$^{f/f}$ control animals (*n=3,* 5 sections per animal). **e**, DCX (green) and BrdU (red) double positive cells (white arrows) reveal post-mitotic neurons in the SGZ and GCL of *gHif-1α*$^{f/f}$ (upper) and *gHif-1α*$^{\Delta/\Delta}$ mice (lower). **f**, **g**, *In vivo* deletion of neuronal Hif-1α impairs adult hippocampal neuronal morphology (n=*3*). *= *P* <0.05, **= *P* <0.005., Student's *t*-test. Error bars represent S.E.M.. **h**, Stereotactic injection of Δ-GSK3-β-catenin lentivirus (HIV- ΔGSK3-β-catenin-IRES-Zsgreen) into the adult DG (12-16 weeks) is detected by green fluorescence, which is absent in PBS injected adult DG. **i,** Quantification of BrdU$^+$ (i left panel) and DCX$^+$ (i right panel) cells in *nHif-1α*$^{\Delta/\Delta}$ DG transduced with high titer Δ-GSK3-β-catenin lentivirus. Following 2 weeks of recovery, mice were injected with BrdU (100 mg Kg^{-1}) i.p daily for 4 days. Control virus treated *nHif-1α*$^{\Delta/\Delta}$ and *nHif-1α*$^{f/f}$ mice served as controls (*n=3-5* per group). Statistical significance (**i**) was computed using one-way ANOVA. *nHif-1α*$^{\Delta/\Delta}$ animals transduced with high titer Δ-GSK3-β-catenin lentivirus displayed remarkable increase in BrdU$^+$ and DCX$^+$ cell counts as compared to control virus treated *nHif-1α*$^{\Delta/\Delta}$ animals, and is approaching significance (# indicates *P*=0.06). Error bars represent S.E.M.

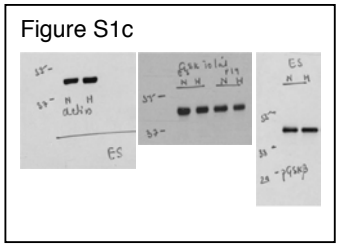


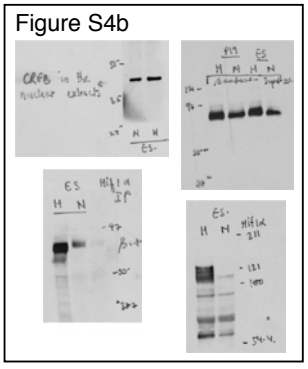












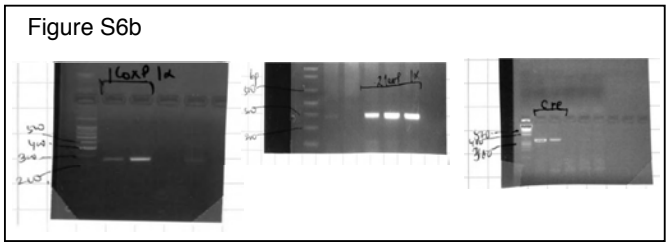


Figure S7 Uncropped blots