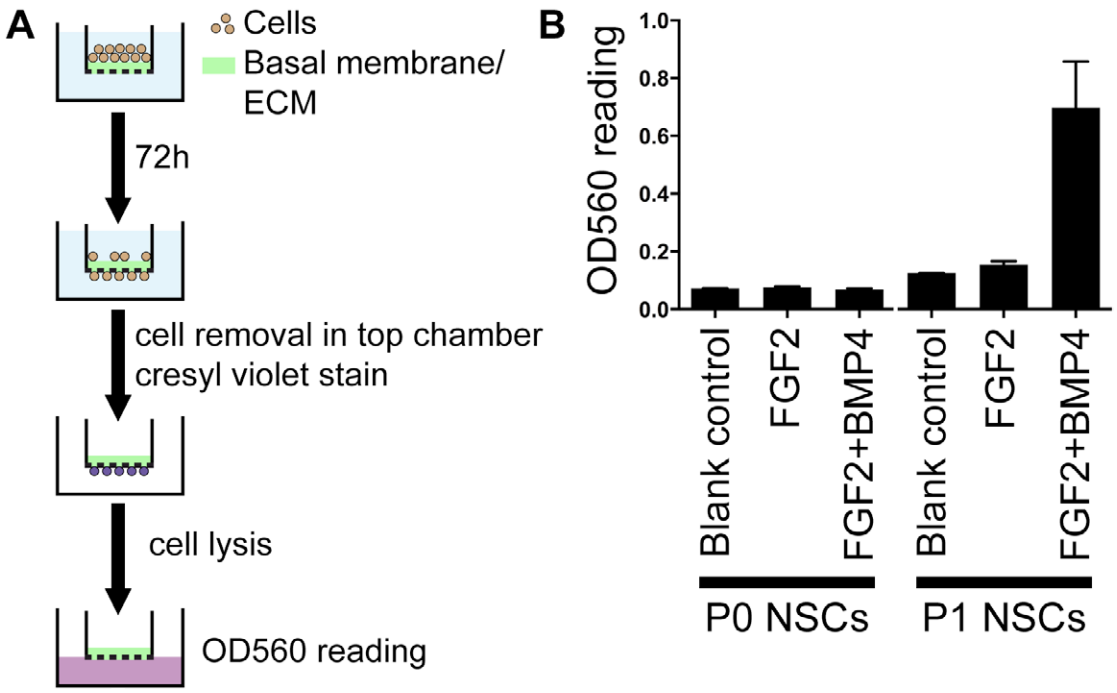

Fig. S1. Freshly isolated NSCs are not invasive. (A) E14.5 rat NSCs from the CVZ were challenged with a optical density Boyden chamber invasion assay directly after isolation (Passage 0, P0) or after 4 days of FGF2 exposure (Passage 1, P1). The amount of cells crossing the basal membrane matrix is quantified via cell stain and optical density reading at 560nm (B) The freshly isolated cells (P0) did not cross the basal membrane matrix and showed a low background reading, whereas the FGF2-exposed cells (P1) did cross the BM/EC matrix (n=3, F(P1) vs FB(P1) *P*=0.0146, no significance between others). Under the same setting (P0, 20'000 cells and 72 hours culture) control (no factor), BMP4 alone, EGF, EGF/BMP4 were tested and did not show any invasive potential (data not shown). Blank control: only cresyl violet stain but no cells/empty chamber. F, FGF2, B, BMP4.

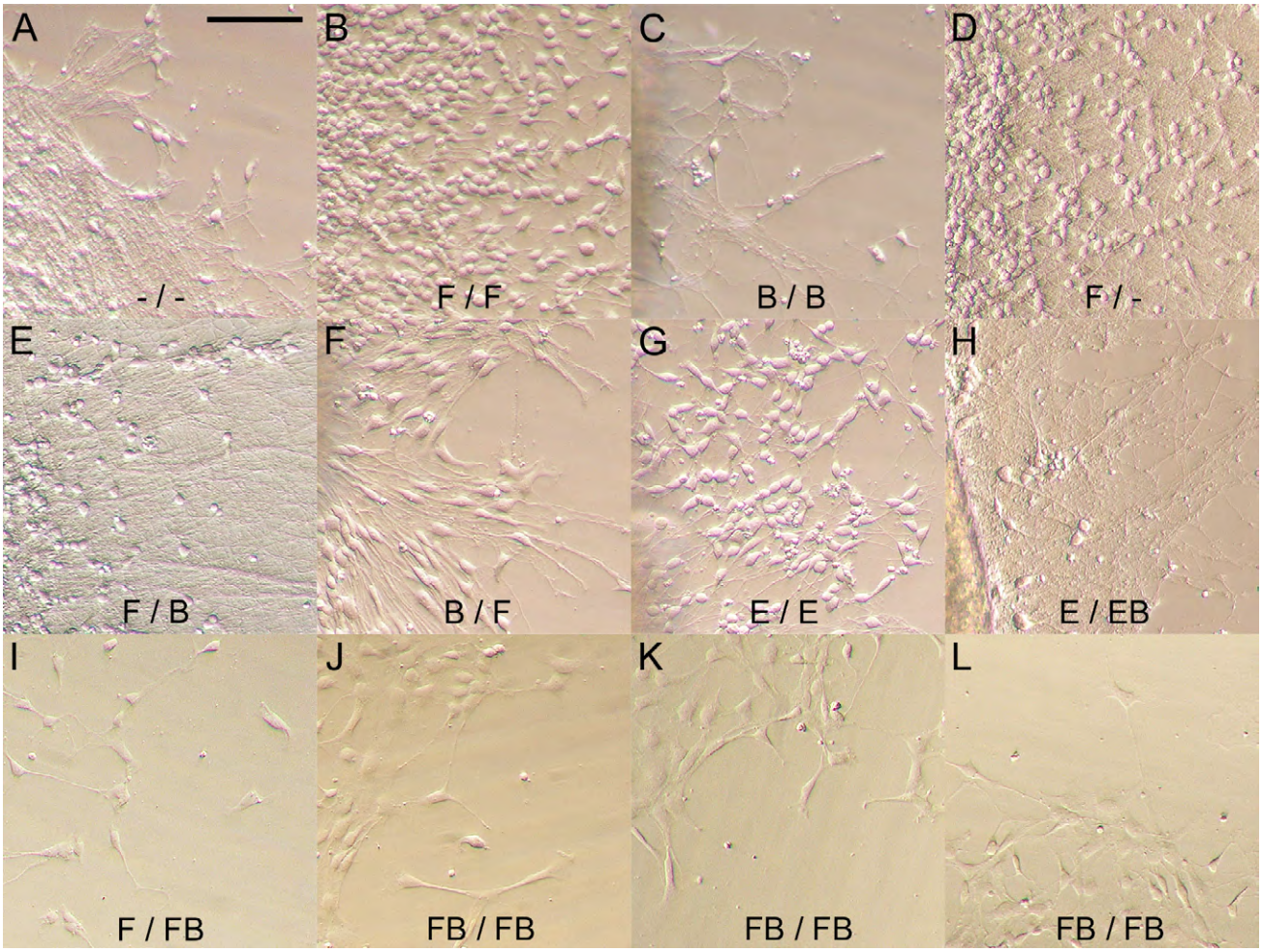

Fig. S2. Only cells exposed to F/FB and FB/FB display a flat migratory phenotype. Representative phase images of cells emigrating from explants at the outermost are shown. Explants were cultured as in Fig. 1. (A) Few small compact cells with axonal outgrowth. (B) Many densely packed, compact cells. Very large number of mitosis figures (MF, cell doublets). (C) Very few compact cells. Some axonal outgrowth. Almost no MF. (D) Compact cells. (E) Tiny compact cells with neuronal morphology. Strong axonal outgrowth. (F) Elongated cells, some MF. (G) Small compact cells, large number of MF. No axonal outgrowth. (H) Very few compact cells. Axonal outgrowth. Very few MF. (I–L) Flat cells, many filopodia, leading and trailing edge. Medium cell density at core, low at peripheral edge. Some interacting cells, many single cells. Few MF. Only the F/FB and FB/FB groups contained flat cells, with many filopodia, and with a migratory phenotype, with leading and trailing edge. Partly forming interacting sheets with cell groups. Few MF. Scale bar: 100 µm.

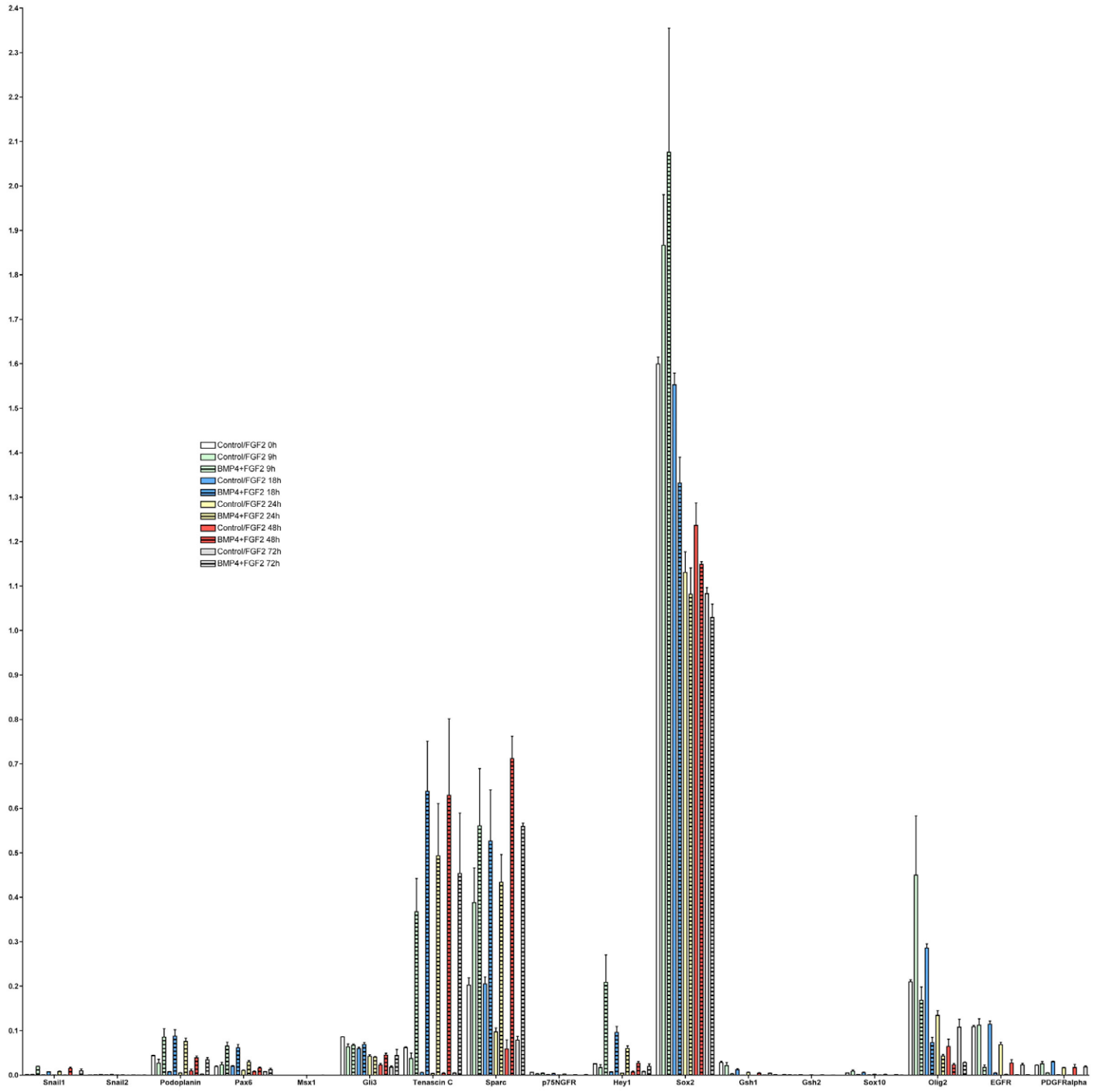

Fig. S3. Absolute mRNA levels after FGF2 or FGF2/BMP4 treatment. Absolute changes in gene expression during FGF2 vs FGF2/BMP4 exposure of E14.5 NSCs as obtained by qRT-PCR after 9, 18, 24, 48 or 72 h, complementary to Fig. 3. Note that Sox2 was expressed at the highest absolute levels during FGF2 exposure and during FGF2/BMP4-exposure but showed rather small relative changes. Genes with the highest absolute RNA expression levels were: *Sox2*, *TNC* and *SPARC*. Note that the large dimensions allow this figure to be ideally viewed on a wide computer screen.

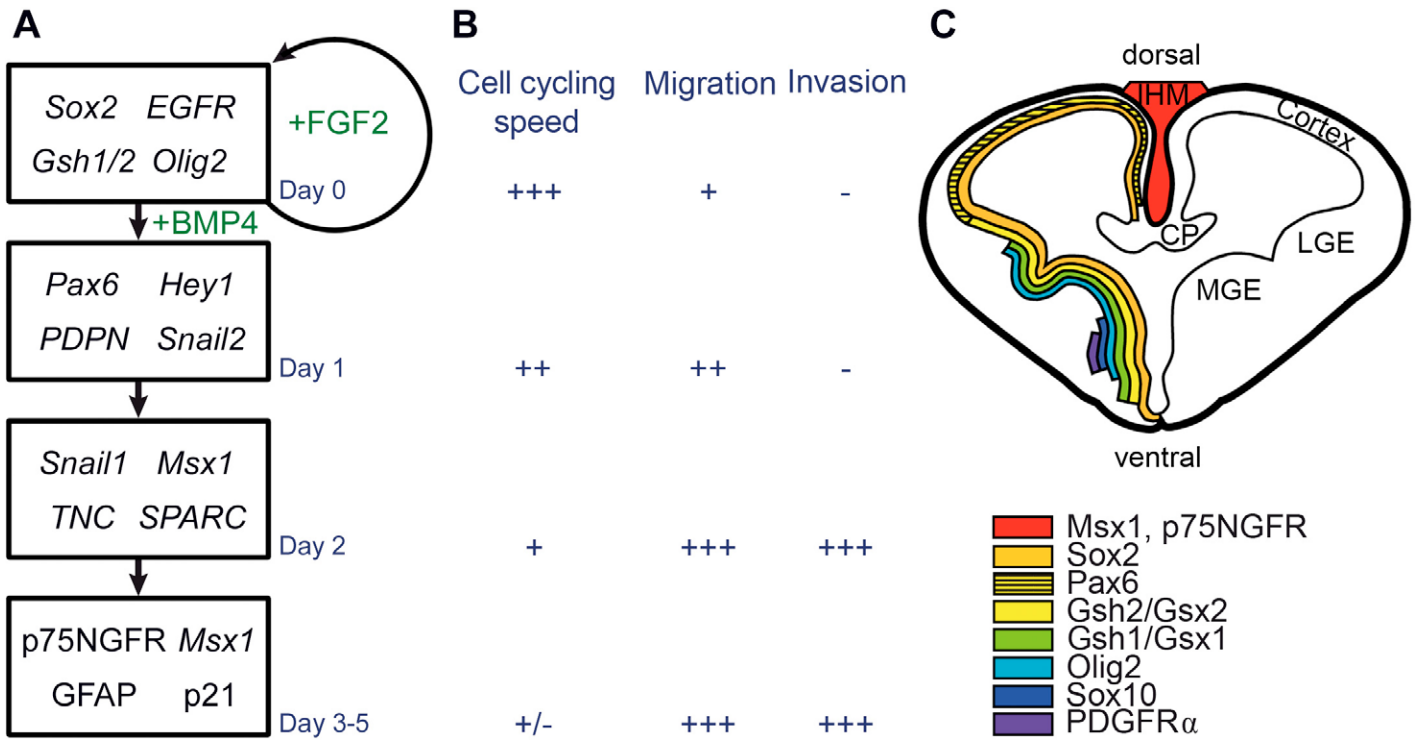

Fig. S4. Neural stem cell invasion model. Summary of gene expression changes (A) and qualitative representation of observed changes in cell cycling speed, migration, and invasion (B). (C) Schematic localization of analyzed genes in the embryonic rat brain. CP, choroid plexus. LGE, lateral ganglionic eminence. MGE, medial ganglionic eminence. TNC, Tenascin C. IHM, interhemispheric mesenchyme.