Supp. Fig. 1: Quantification of (Hoescht$^+$, blue) and proliferative (Ki67$^+$, green) nuclei (mean +/- SD).

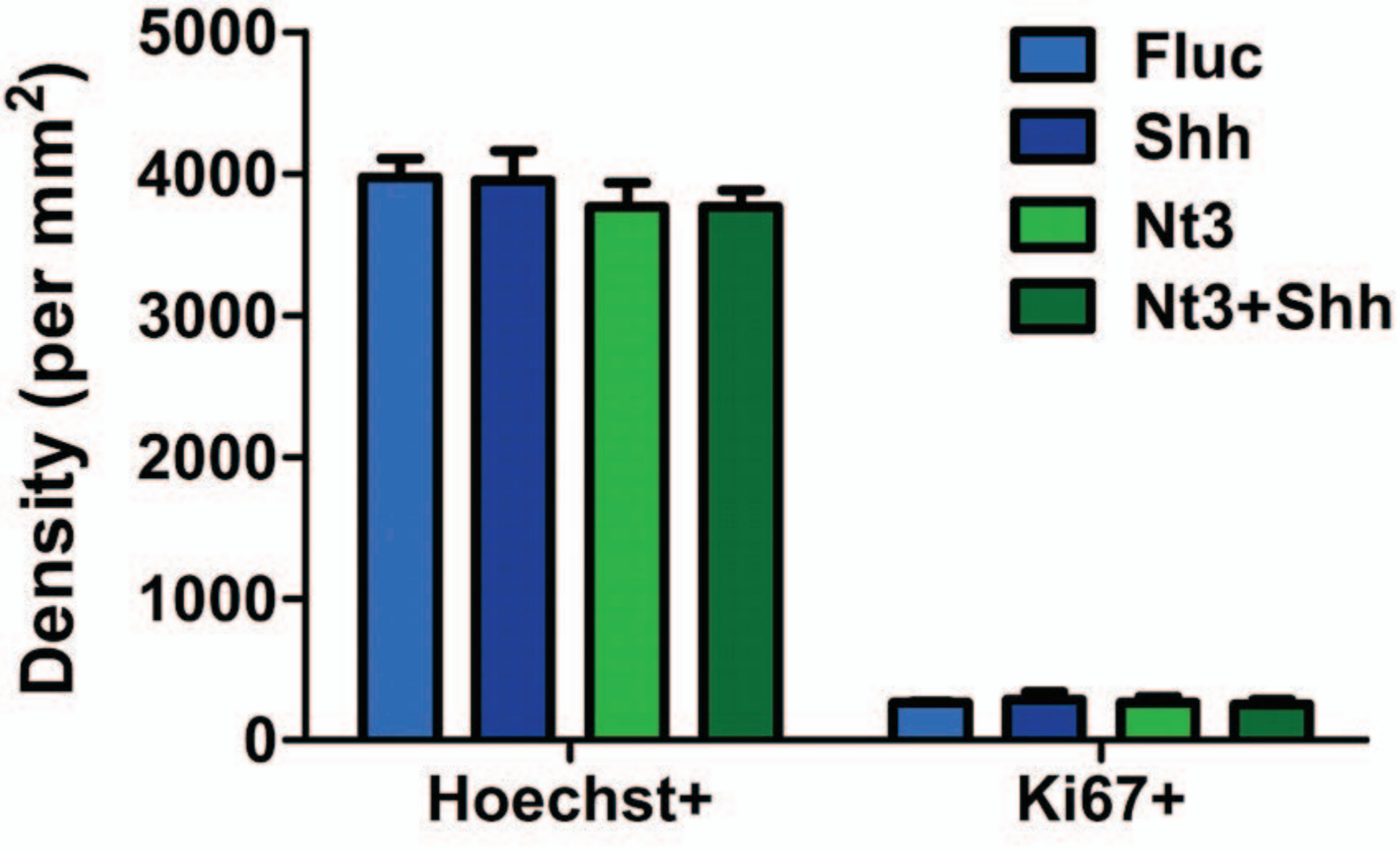
Supp. Fig. 2: Quantification of proliferative (Ki67+) (a) Sox2$^+$, (b) Olig2+ and (c) GFAP+ cells (mean +/- SD) in the bridge area 8 wks after injury when lentiviral particles encoding for either FLuc, SHH, NT3, or NT3 plus SHH were delivered from bridge implants. Ki67$^+$ cells were considered proliferative. White arrowheads indicate Sox2$^+$ cells, whereas yellow arrowheads indicate proliferative Sox2$^+$ cells (double positive for Ki67). Only cells where Hoescht$^+$ pixels overlapped Sox2$^+$, Olig2$^+$ or GFAP$^+$ pixels were counted.

Supp. Fig. 3: Maximum axial projection of confocal slices of the middle of the scaffold (~18 um section thickness). (a) Overlay of GFAP (red), Olig2 (green), Sox2 (magenta), and Hoescht (blue). Grayscale images of (b) GFAP, (c) Olig2, (d) Sox2 and (e) Hoescht (nuclei). White arrows indicate Sox2$^+$/GFAP$^+$/Olig2$^-$ cells, white asterisks indicate Sox2$^+$/GFAP$^-$/Olig2$^-$ cells, red arrows indicate Olig2$^+$/GFAP$^+$/Sox2$^-$ cells, red asterisks indicate Olig2$^+$/GFAP$^-$/Sox2$^-$ cells, blue arrows indicate Sox2$^+$/GFAP$^+$/Olig2$^+$ cells and blue asterisks indicate Sox2$^+$/Olig2$^+$/GFAP$^-$ cells.

Supp. Fig. 4: Markers for oligodendrogenesis 8 wks after injury in the presence of (a,e,i) Fluc, (b,f,j) SHH, (c,g,k) NT3, or (d,h,l) NT3 plus SHH. Tissue sections were stained for (a-d) NG2$^+$ (glial-restricted progenitors, meningeal fibroblasts, non-myelinating Schwann cells, and macrophages: red), (e-h) O4$^+$ (immature oligodendrocytes: green), and (i-l) GalC$^+$ (immature and mature oligodendrocytes: red) in the bridge. Proliferative cells were also Ki67$^+$, as indicated on the image. All images represent areas within the bridge implants. Yellow arrowheads indicate positive staining for the indicated markers, whereas white arrowheads indicate co-localization of the marker with Ki67.

Supp. Fig. 5: Representative images showing overlap of immunofluorescence markers with nuclei. (a-c) Sox2$^+$ cells were identified by co-localization of Sox2$^+$ (red) and Hoescht$^+$ (blue) nuclei in bridge implants. Arrows indicate examples of Sox2$^+$ cells. (d-f) Olig2$^+$ cells were identified by co-localization of Olig2$^+$ (red) and Hoescht$^+$ (blue) nuclei in bridge implants. Arrows indicate examples of Olig2$^+$ cells. (g-i) GFAP$^+$ cells were identified by overlap of GFAP$^+$ (red) cytoplasm and Hosecht$^+$ (blue) nuclei in bridge implants. Arrows indicate examples of GFAP$^+$ cells. Brightness and contrast were adjusted for clarity.

Supp. Fig. 6. Representative mmunofluorescence images demonstrating overlap of neurofilament and myelin markers. (a-c) NFM$^+$ (red) processes were identified as axons. Axonal processes co-labeled with MBP (green) were considered to be myelinated (white arrows). (d-g) NFM$^+$ (blue) processes were identified as axons. Axonal processes co-labeled with MBP (red), but not P0 (green) were considered to be myelinated by oligodendrocytes (yellow arrows), while NFM$^+$ processes positive for both MBP and P0 were considered to be myelinated by Schwann cells (white arrows). Brightness and contrast were adjusted for clarity.


Density (per mm2)
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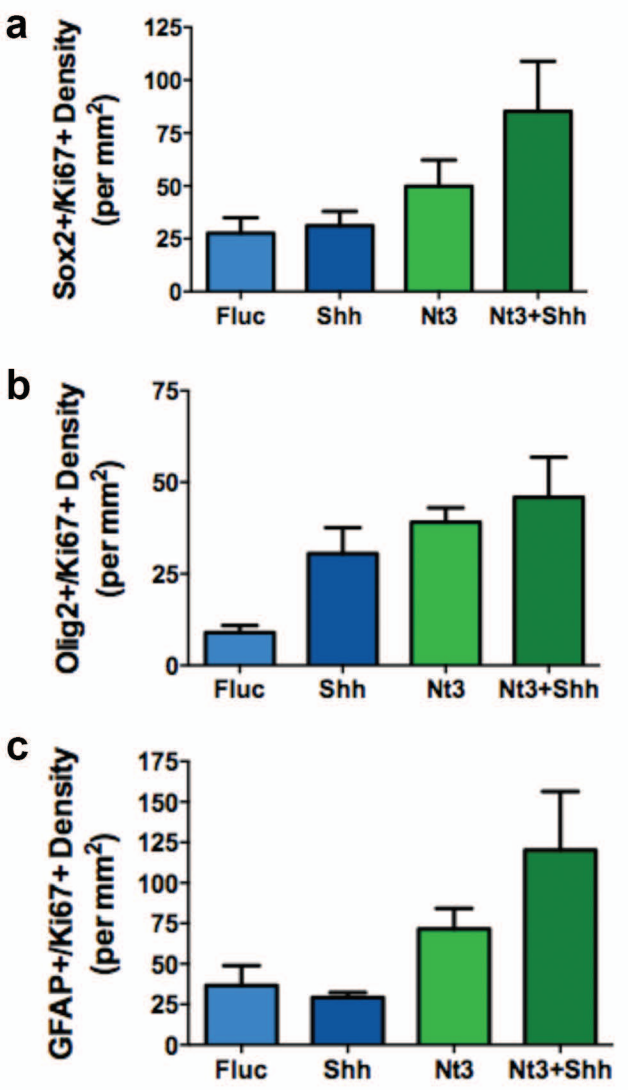
4000
3000
2000
1000
0
Fluc
Shh
Nt3
Nt3+Shh
Hoechst+
Ki67+


a
Sox2+/Ki67+ Density (per mm2)
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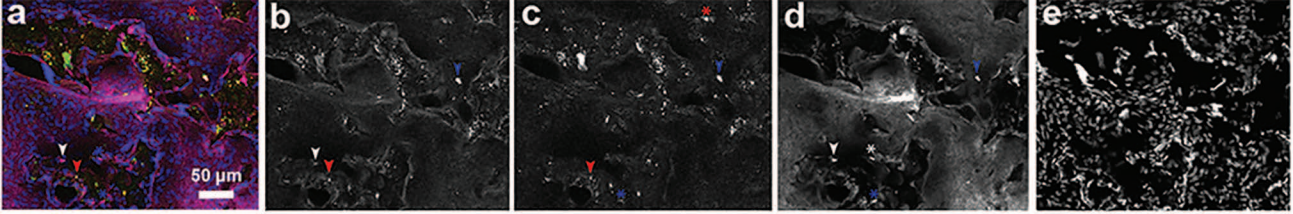
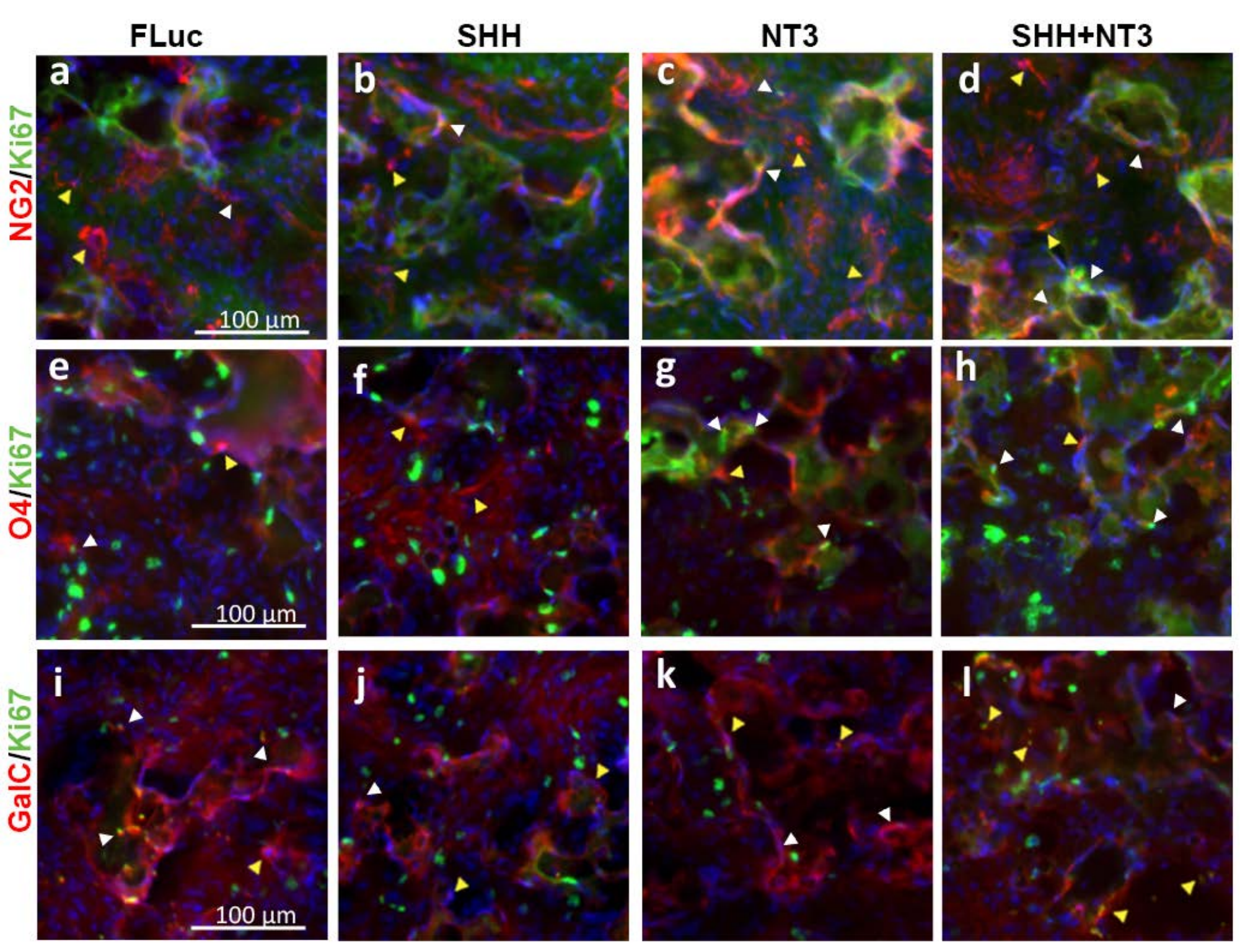
100
75
50
25
0
Fluc
Shh
Nt3
Nt3+Shh
b
Olig2+/Ki67+ Density (per mm2)
75
50
25
0
Fluc
Shh
Nt3
Nt3+Shh
c
GFAP+/Ki67+ Density (per mm2)
175
150
125
100
75
50
25
0
Fluc
Shh
Nt3
Nt3+Shh



GFAP/Olig2/Sox2/Hoescht


FLuc
SHH
NT3
SHH+NT3
NG2/Ki67
O4/Ki67
GalC/Ki67
a
b
c
d
e
f
g
h
i
j
k
l
100 µm
100 µm
100 µm

a
20 μm
b
c
Sox2/Hoescht
d
20 μm
e
f
Olig2/Hoescht
g
20 μm
h
i
GFAP/Hoescht