Supplementary Information for

Live imaging of remyelination in the adult mouse corpus callosum

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This PDF file includes:

Other supplementary materials for this manuscript include:

Supplementary figures

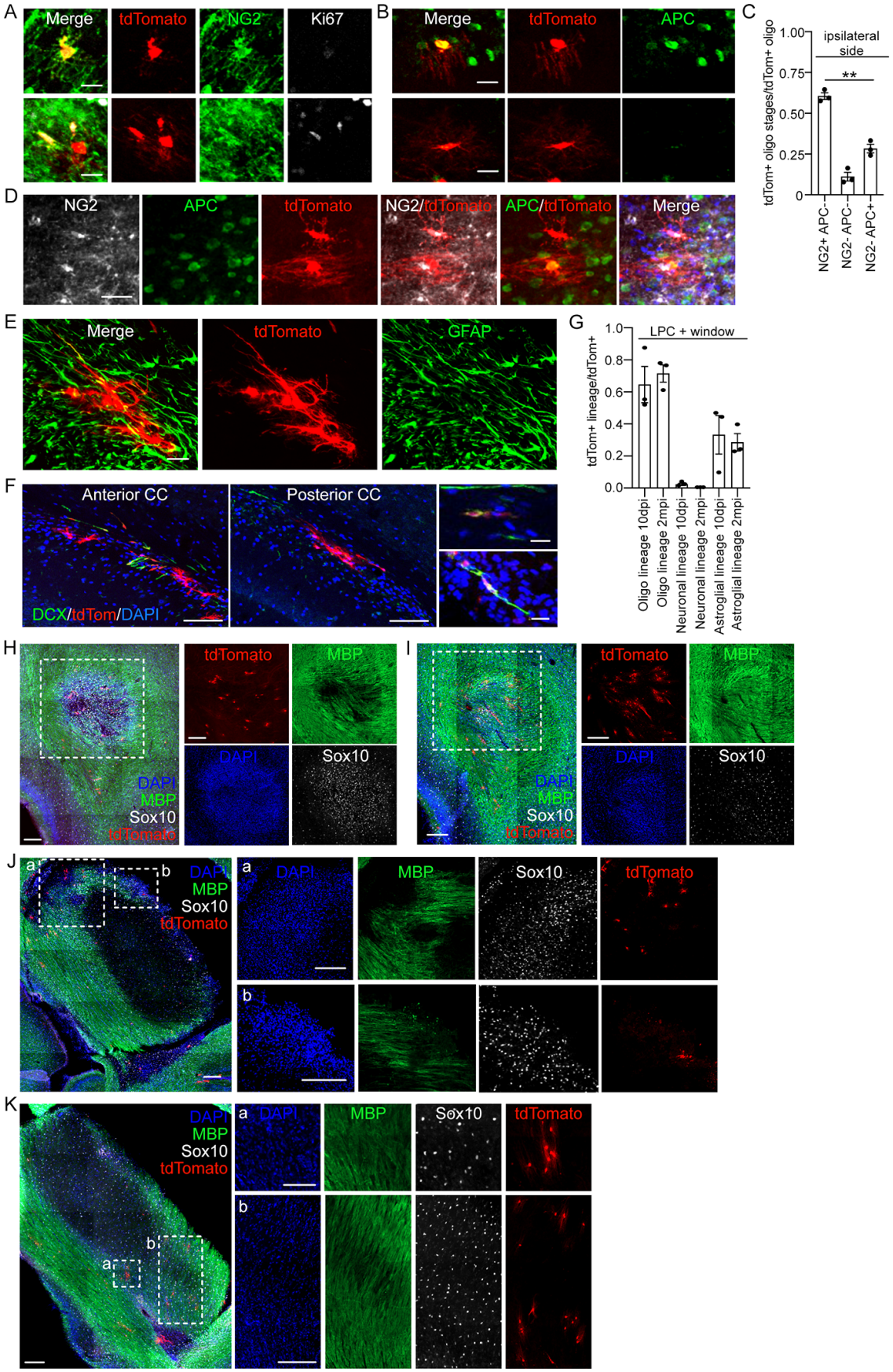

Figure S1. LPC-induced demyelination in the corpus callosum. A, Shown are examples of confocal imaged OPCs in the CC 10 days after Tamoxifen recombination. Ascl1-labeled cells (red) co-label with the OPC marker NG2 (green) and Ki67, a marker for proliferating cell (white). Coronal section. **B,** Shown are examples of confocal imaged pre-myelinating oligodendrocytes in the CC 10 days after Tamoxifen recombination. Ascl1-labeled cells (red) co-label with APC, a marker for pre-myelinating cells (green). Coronal section. **C,** Quantification of ipsilateral tdTomato+ cells belonging to different differentiation stages of oligodendrocytes lineage in LPC treated mice at 10 days after Tamoxifen recombination. Classification of cell stages is performed using immunohistochemical analysis of NG2 and APC markers. Each dot represents a mouse (NG2+ APC-: mean ± sem = 0.6 ± 0.02; n= 3 mice. NG2- APC- and bushy morphology: mean ± sem = 0.11 ± 0.02; n= 3 mice. NG2- APC+: mean ± sem = 0.28 ± 0.02; n= 3 mice. RM one-way ANOVA: F = 68.77; p-value = 0.0012**). **D,** Shown is an example of a confocal imaged OPC and a differentiated cell in the CC 10 days after Tamoxifen recombination. The Ascl1-labeled OPC (red) co-labels with the OPC marker NG2 (white); the differentiated oligodendrocyte co-labels with APC marker (green). DAPI is in blue in the merged image. Horizontal section. **E,** Shown are examples of confocal imaged astrocytes in the CC 10 days after Tamoxifen recombination. Ascl1-labeled cells (red) co-label with GFAP marker for activated astrocytes (green). Coronal section. **F,** Shown are examples of confocal imaged anterior and posterior CC 10 days after Tamoxifen recombination. Some Ascl1-labeled cells (red) co-label with the neuroblast marker DCX (green) in the anterior CC. Right: representative examples of DCX+ cells. Coronal section. **G,** Quantification of ipsilateral tdTomato+ cells belonging to oligodendrocytes, neuronal and astroglial lineages in LPC+window imaged mice at different time points. Each dot represents a mouse (10dpi oligo: mean ± sem = 0.64 ± 0.11; n= 3 mice. 2mpi oligo: mean ± sem = 0.71 ± 0.05; n= 3 mice. 10dpi neuronal: mean ± sem = 0.02 ± 0.007; n= 3 mice. 2mpi neuronal: mean ± sem = 0 ± 0; n= 3 mice. 10dpi astroglial: mean ± sem = 0.33 ± 0.12; n= 3 mice. 2mpi astroglial: mean ± sem = 0.28 ± 0.05; n= 3 mice). **H,** Representative confocal image of CC in LPC treated control mouse 10 days after Tamoxifen injection (tdTomato/red;

MBP/green; DAPI/blue; Sox10/white). Horizontal section. Right: magnification of the damaged area of the CC. tdTomato+ OPCs are present; MBP+ myelin sheaths are disrupted and the area shows DAPI dense labeling and an enrichment in Sox10+ cells. **I,** Representative confocal image of CC in LPC treated control mouse 2 months after Tamoxifen injection (tdTomato/red; MBP/green; DAPI/blue; Sox10/white). Horizontal section. Right: magnification of the remyelinated area of the CC. tdTomato+ mature oligodendrocytes are present; MBP+ myelin sheaths are restored and the area shows less DAPI dense labeling and reduced enrichment of Sox10+ cells compared to (H). **J,** Representative confocal image of LPC injected CC below the window after 10 days of *in vivo* imaging (tdTomato/red; MBP/green; Sox10/white; DAPI/blue). Horizontal section. Right: magnification of the damaged area of the CC. tdTomato+ OPCs are present; MBP+ myelin sheaths are disrupted and the area shows DAPI dense labeling and an enrichment in Sox10+ cells. **K,** Representative confocal image of LPC injected CC below the window after 2 months of *in vivo* imaging (tdTomato/red; MBP/green; Sox10/white; DAPI/blue). Horizontal section. Right: magnification of the remyelinated area of the CC. tdTomato+ mature oligodendrocytes are present; MBP+ myelin sheaths are restored. Values are shown as mean ± s.e.m. Scale bars represent 20 µm (A, B, D, E), 25 µm (F magnification), 100 µm (F, H, I, J overview and magnification B, K), 50 µm (J magnification A).

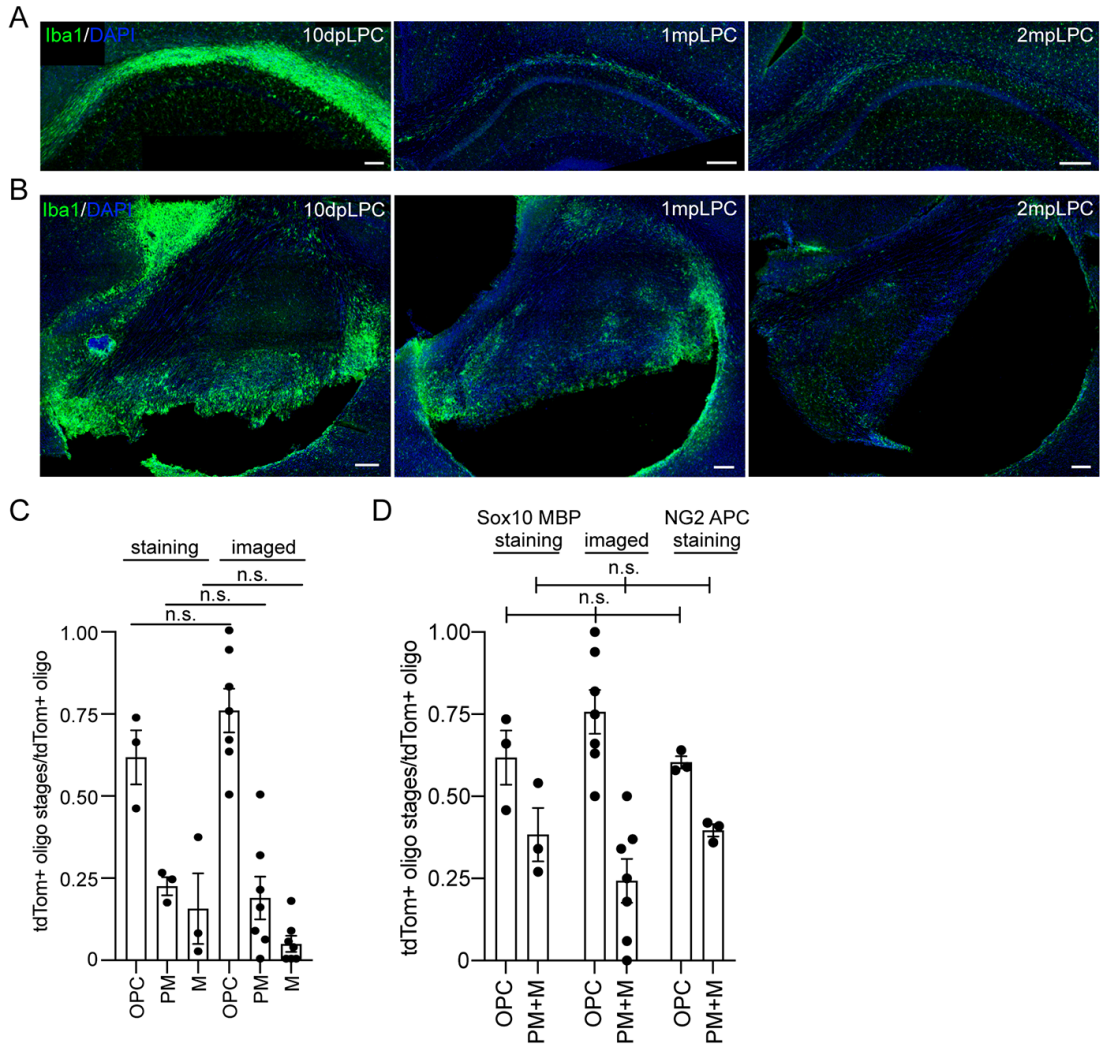

Figure S2. LPC-triggered inflammation reduces over time. A, Representative confocal images of CC in LPC treated control mice 10 days, 1 month and 2 months after LPC injection (Iba1/green; DAPI/blue). Coronal sections. **B,** Representative confocal images of CC in LPC+window imaged mice 10 days, 1 month and 2 months after LPC injection and window implantation (Iba1/green; DAPI/blue). Horizontal sections. **C,** Quantification of ipsilateral tdTomato+ cells belonging to different differentiation stages of oligodendrocytes lineage in LPC+window imaged mice at 10 days after Tamoxifen recombination. Quantification of cell stages using immunohistochemical analysis of Sox10 and MBP markers is displayed under "staining" nomenclature. Quantification of cell stages using *in vivo* imaging data is displayed under "imaged" nomenclature. Each dot represents a mouse (Staining: OPC: mean ± sem = 0.61 ± 0.08; n= 3 mice. PM: mean ± sem = 0.22 ± 0.02; n= 3 mice. M: mean ± sem = 0.15 ± 0.10; n= 3 mice. RM one-way

ANOVA: F = 6.43; p-value = 0.124. Imaged: OPC: mean ± sem = 0.76 ± 0.06; n= 7 mice. PM: mean ± sem = 0.18 ± 0.06; n= 7 mice. M: mean ± sem = 0.04 ± 0.02; n= 7 mice. RM one-way ANOVA: F = 30.46; p-value = 0.0006***. OPC staining vs OPC imaged: Unpaired t test, two tailed: t = 1.22; df = 8; p-value = 0.25. PM staining vs PM imaged: Unpaired t test, two tailed: t = 0.34; df = 8; p-value = 0.74. M staining vs M imaged: Unpaired t test, two tailed: t = 1.42; df = 8; p-value = 0.19). **D,** Quantification of ipsilateral tdTomato+ cells belonging to different differentiation stages of oligodendrocytes lineage in LPC+window imaged mice (Sox10 MBP staining and imaged groups) or LPC treated mice (NG2 APC staining group) at 10 days after Tamoxifen recombination. Quantification of cell stages using immunohistochemical analysis of Sox10 and MBP markers is displayed under “Sox10 MBP staining” nomenclature. Quantification of cell stages using *in vivo* imaging data is displayed under “imaged” nomenclature. Quantification of cell stages using immunohistochemical analysis of NG2 and APC markers is displayed under “NG2 APC staining” nomenclature. Each dot represents a mouse (Sox 10 MBP staining: OPC (Sox 10+ MBP-): mean ± sem = 0.61 ± 0.08; n= 3 mice. PM+M (Sox 10+ MBP+): mean ± sem = 0.38 ± 0.08; n= 3 mice. Imaged: OPC: mean ± sem = 0.75 ± 0.06; n= 7 mice. PM+M: mean ± sem = 0.25 ± 0.06; n= 7 mice. NG2 APC staining: OPC (NG2+ APC-): mean ± sem = 0.6 ± 0.02; n= 3 mice. PM+M (NG2- APC- and NG2- APC+): mean ± sem = 0.4 ± 0.02; n= 3 mice. OPC: One-way ANOVA: F = 1.5; p-value = 0.267. PM+M: One-way ANOVA: F = 1.53; p-value = 0.263). Values are shown as mean ± s.e.m. Scale bars represent 100 μm (A, B)

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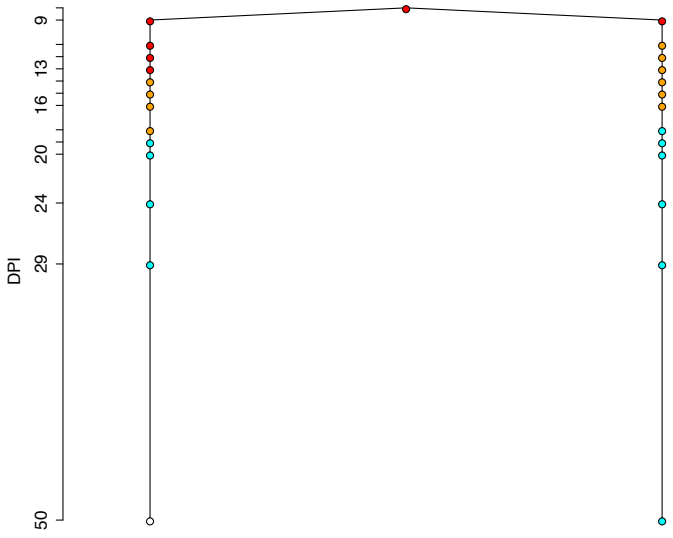

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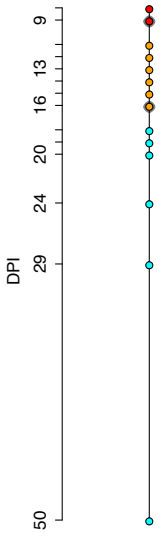

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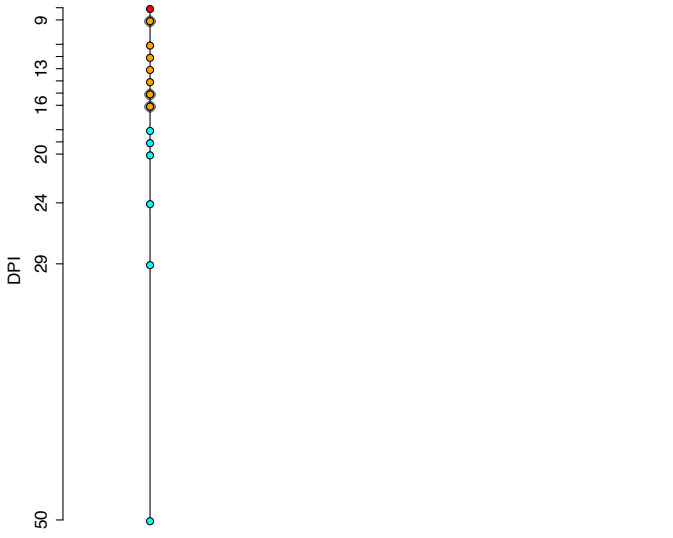

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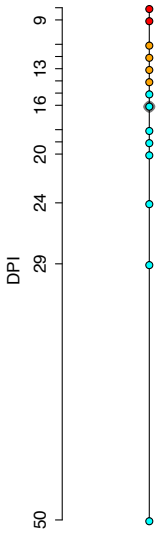

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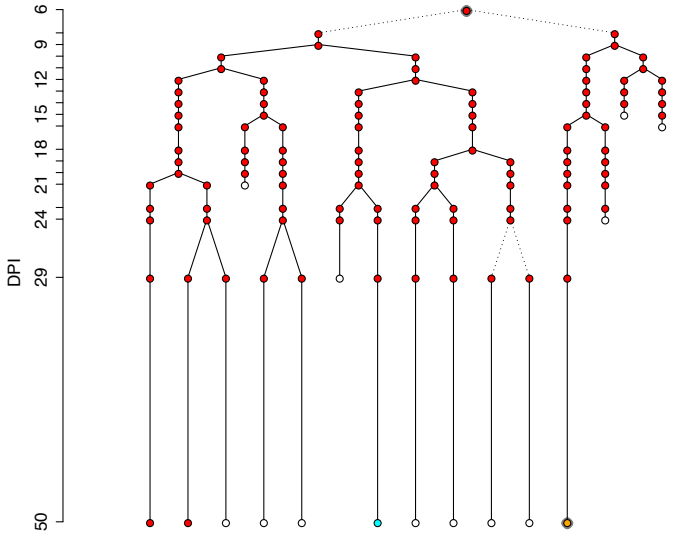

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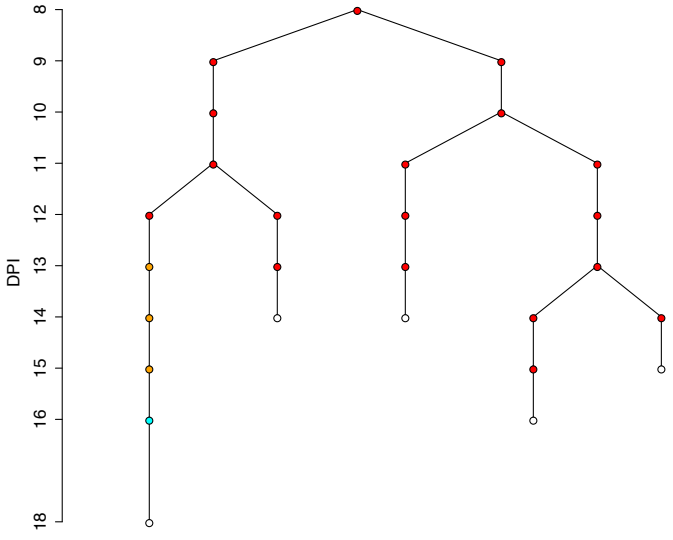

cell types
- OPC
- PM
- M
- cell death

uncertainty cell type
- uncertain

uncertainty lineage
- certain
- semi - certain
- uncertain

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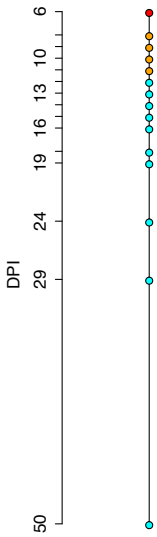

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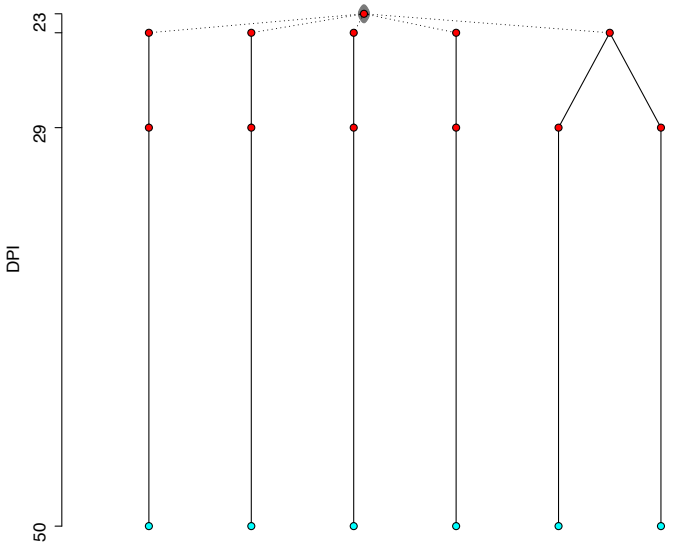

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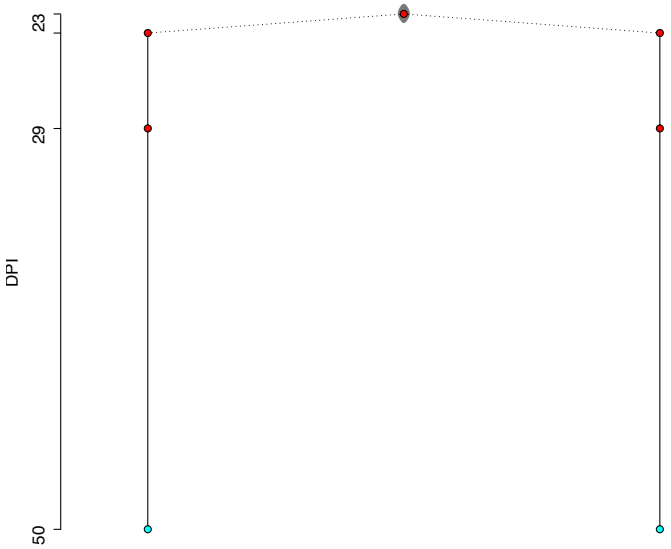

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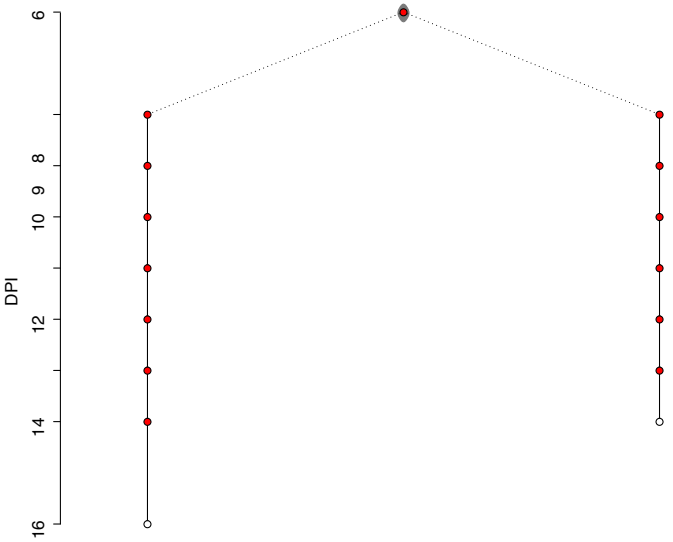

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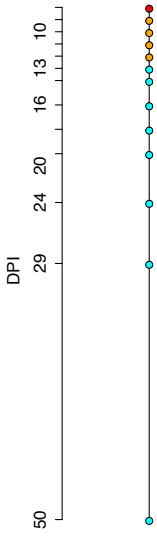

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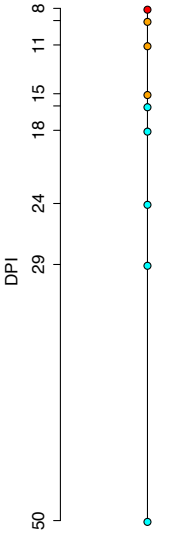

cell types
- OPC
- PM
- M
- cell death

uncertainty cell type
- uncertain

uncertainty lineage
- certain
- semi - certain
- uncertain

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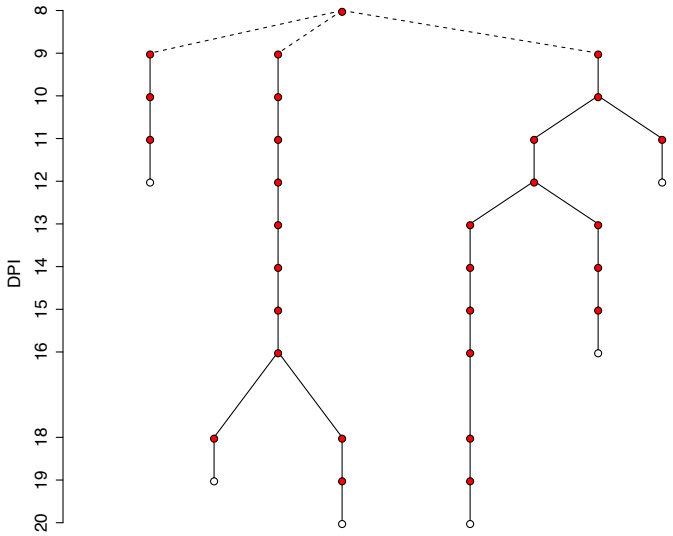

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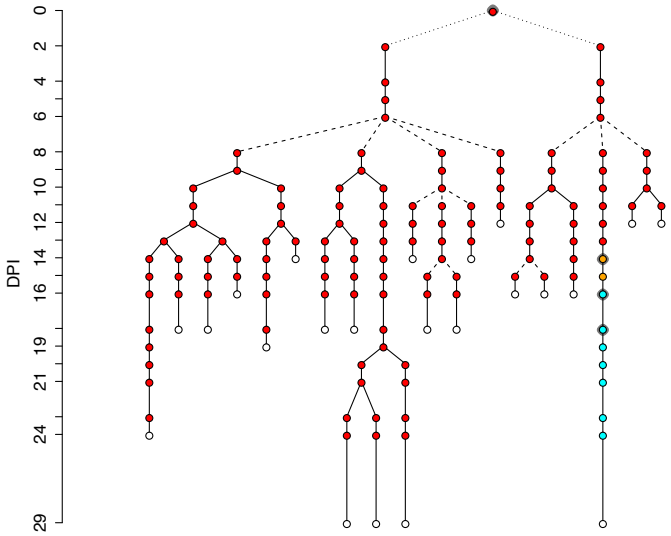

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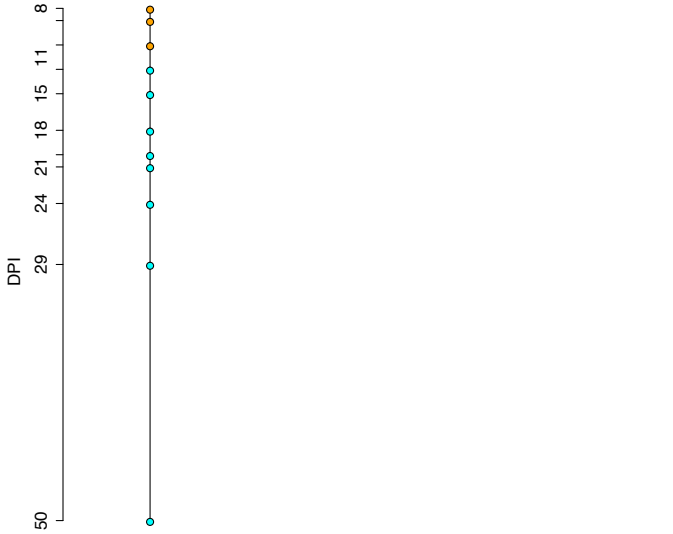

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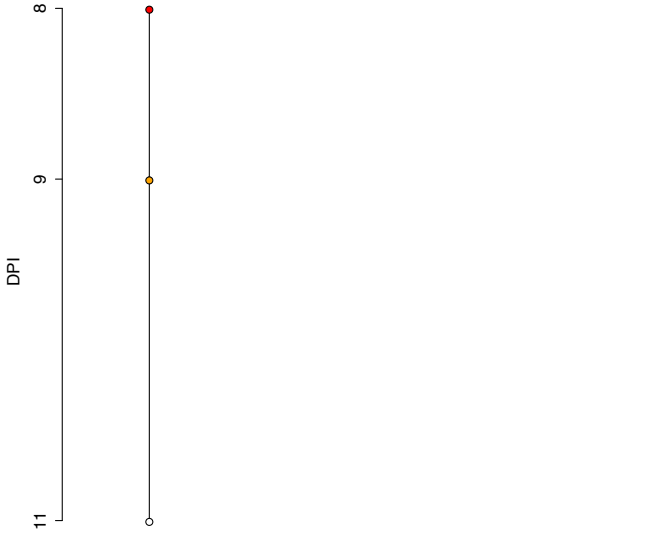

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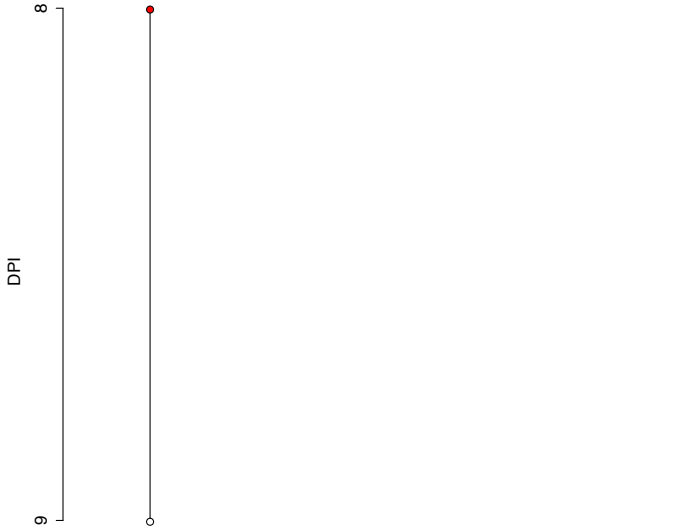

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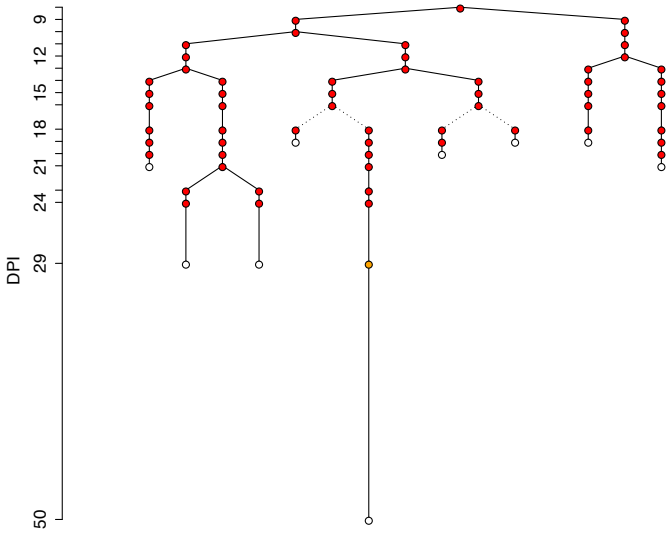

cell types
- OPC
- PM
- M
- cell death

uncertainty cell type
- uncertain

uncertainty lineage
- certain
- semi - certain
- uncertain

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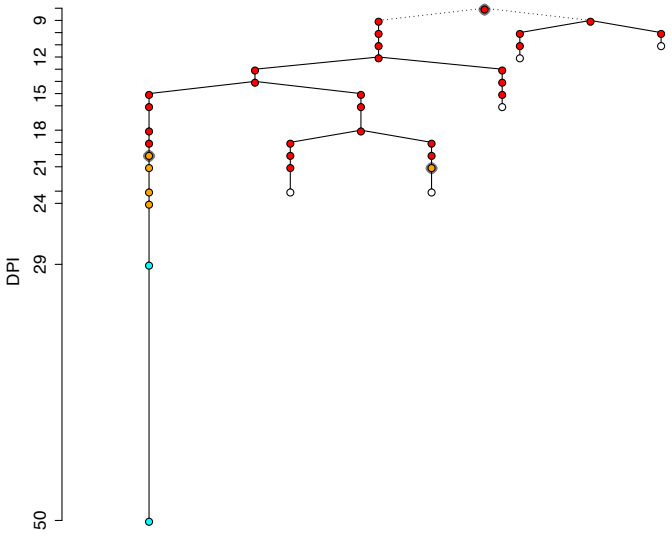

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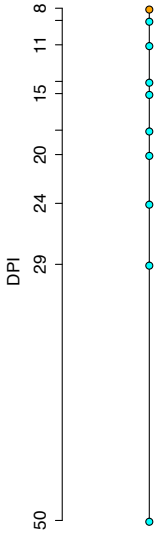

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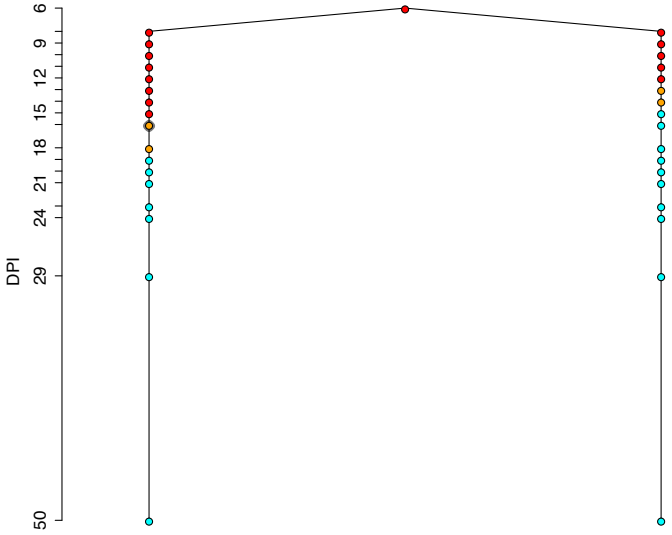

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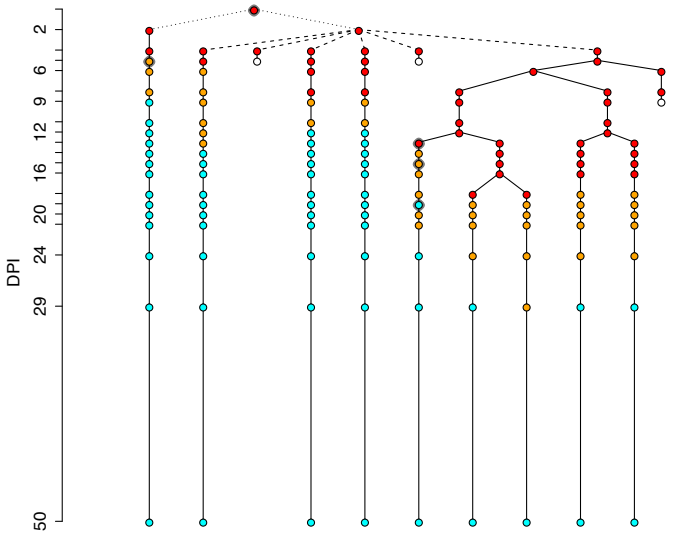

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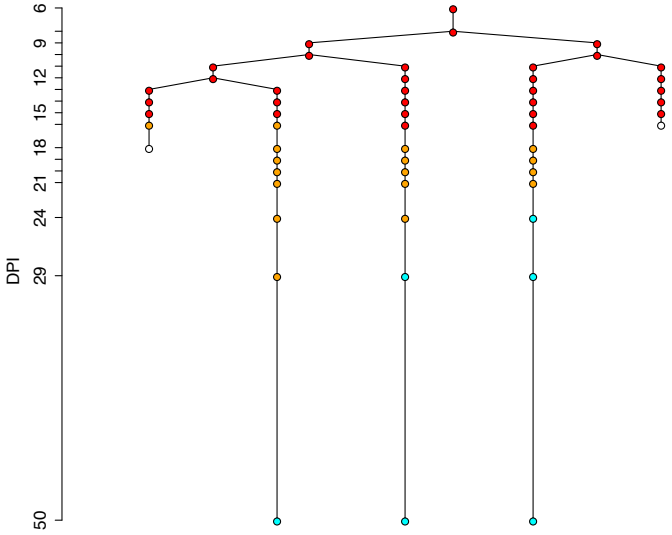

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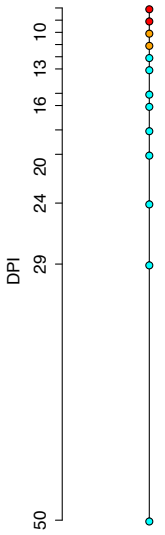

cell types
- OPC
- PM
- M
- cell death

uncertainty cell type
- uncertain

uncertainty lineage
- certain
- semi - certain
- uncertain

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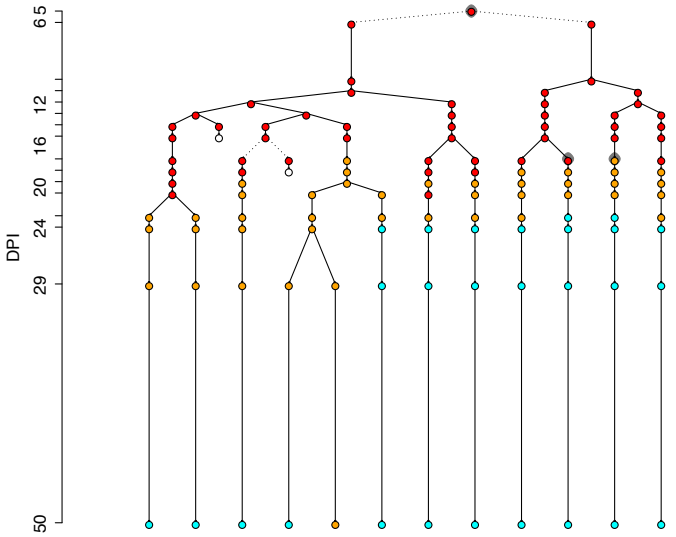

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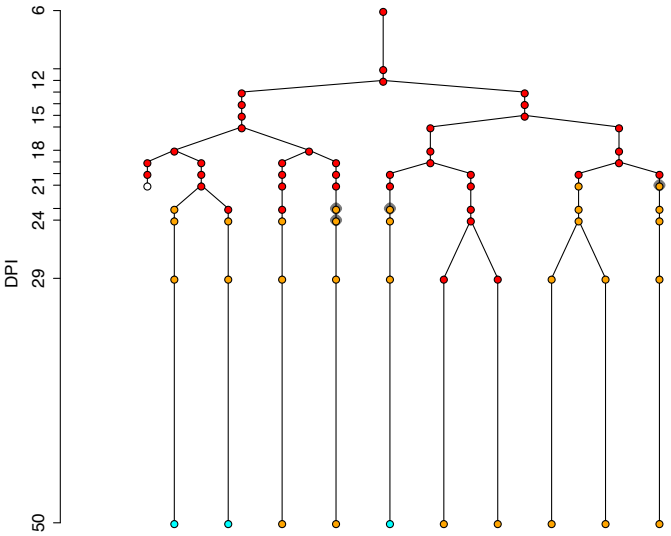

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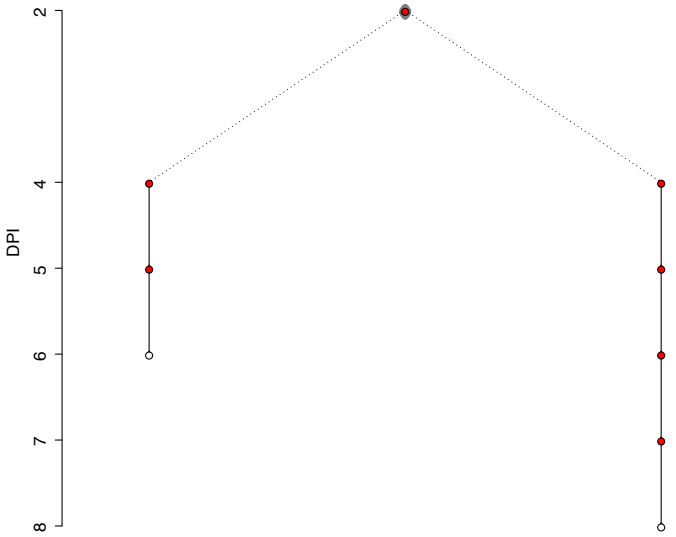

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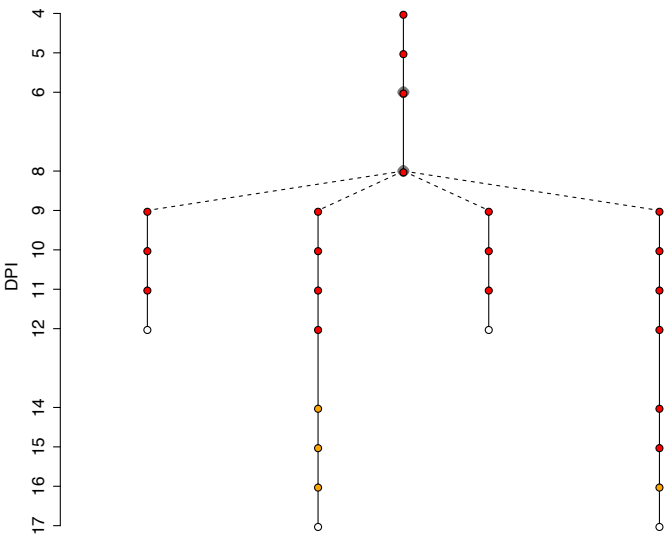

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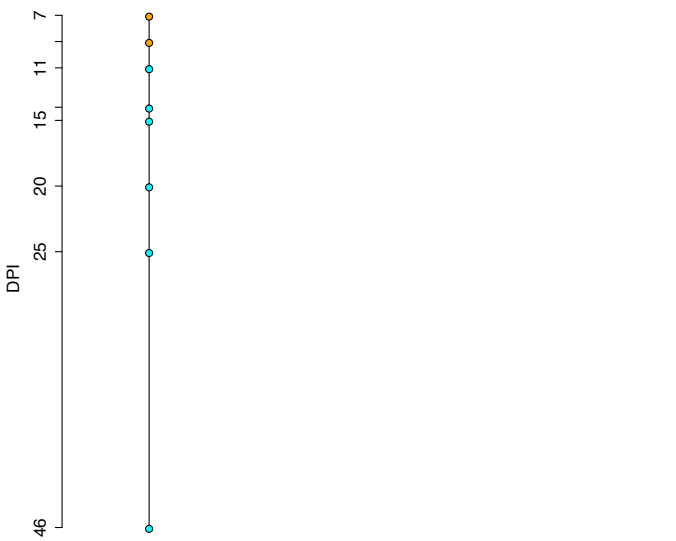

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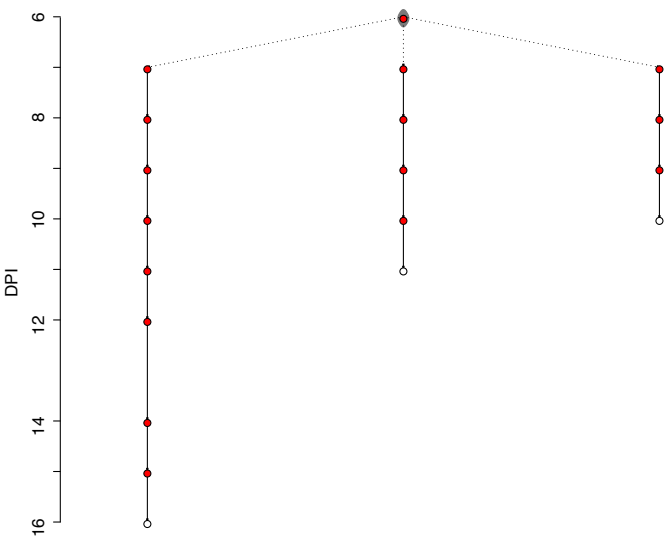

cell types
- OPC
- PM
- M
- cell death

uncertainty cell type
- uncertain

uncertainty lineage
- certain
- semi - certain
- uncertain

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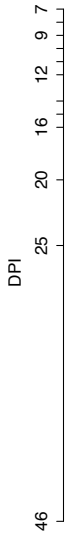

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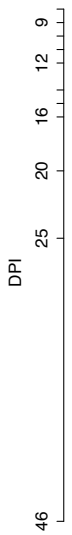

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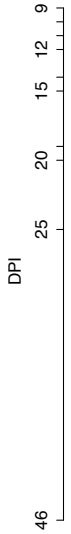

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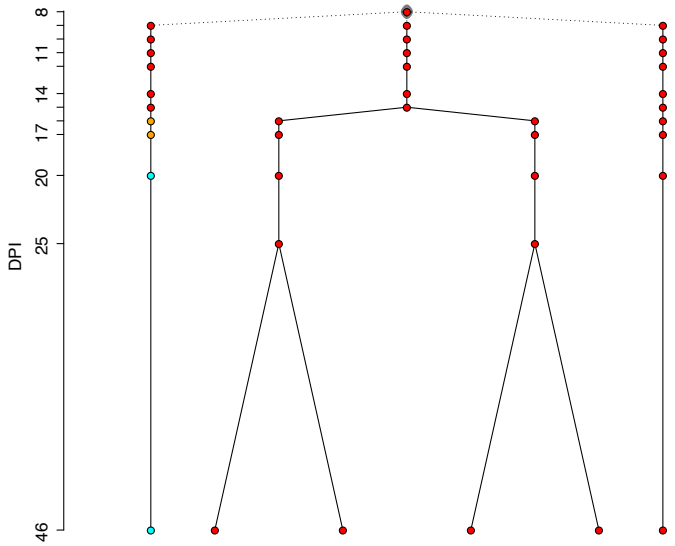
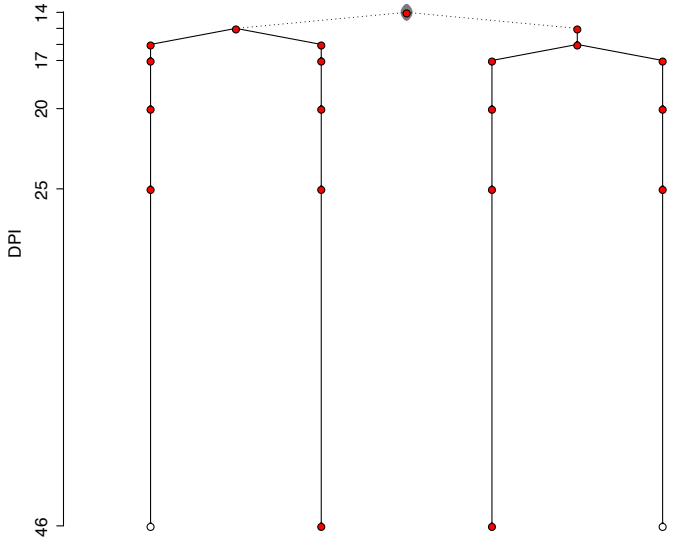
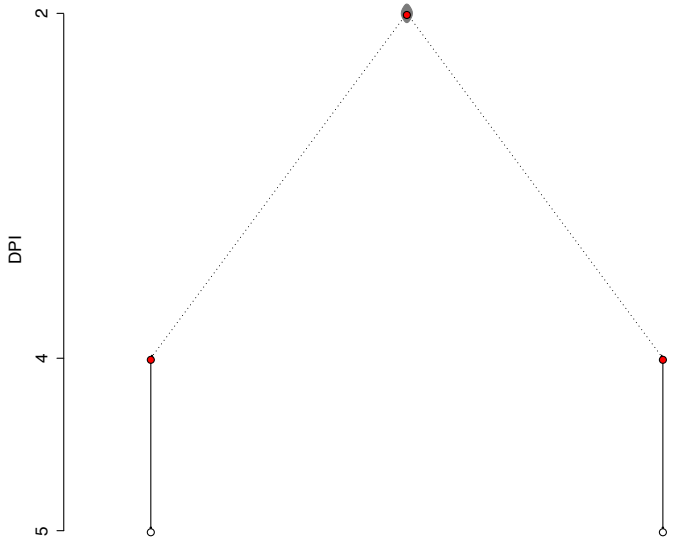


35. **bf3093.SPOT17.clone1**



36. **bf3093.SPOT2.clone1**

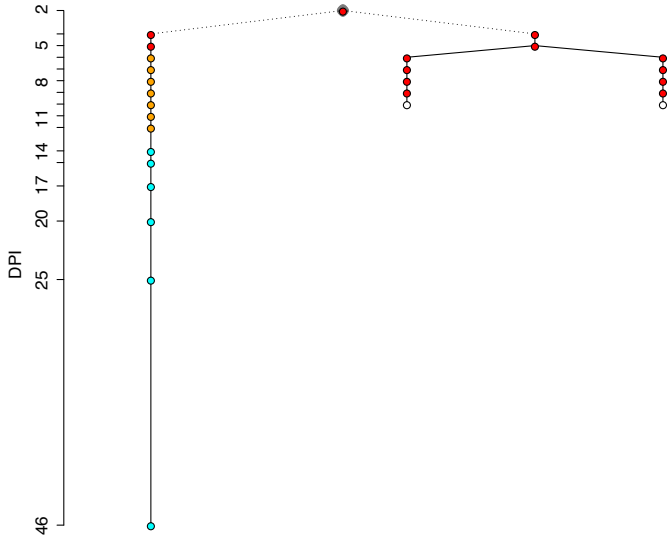


cell types
- OPC
- PM
- M
- cell death

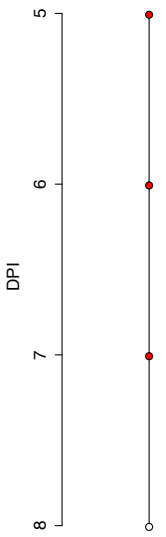
uncertainty cell type
- uncertain

uncertainty lineage
- certain
- semi - certain
- uncertain

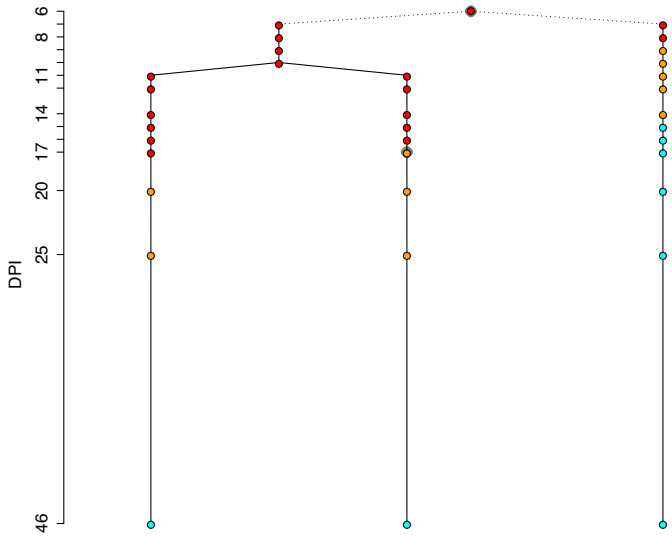
37. **bf3093.SPOT2.clone2**



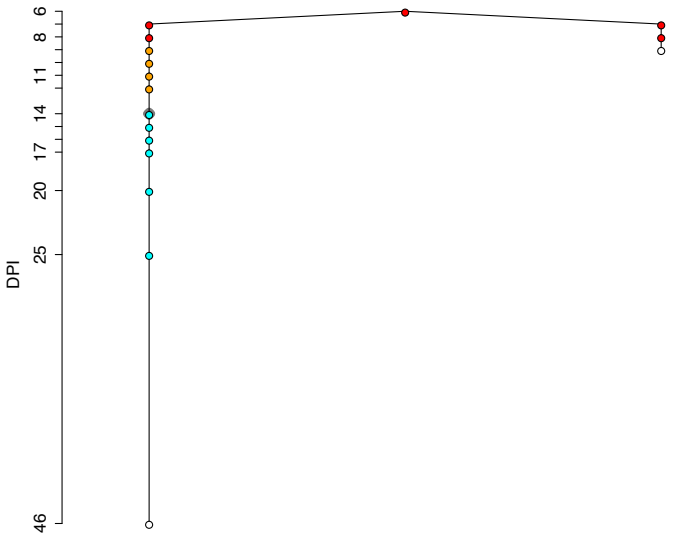
38. **bf3093.SPOT3.clone1**



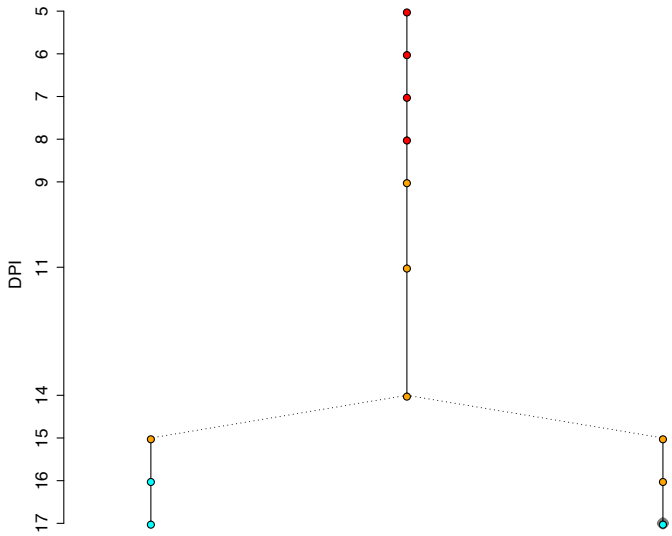
39. **bf3093.SPOT3.clone2**



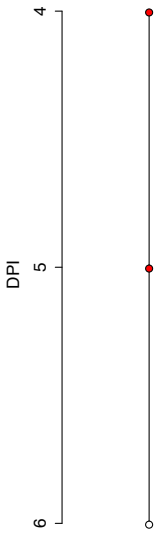
40. **bf3093.SPOT3.clone3**



41. **bf3093.SPOT62.clone1**



42. **bf3093.SPOT7.clone1**

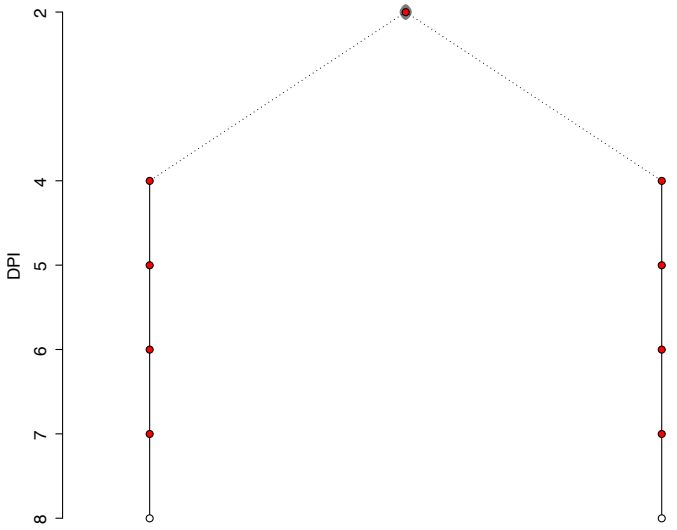


cell types
- OPC
- PM
- M
- cell death

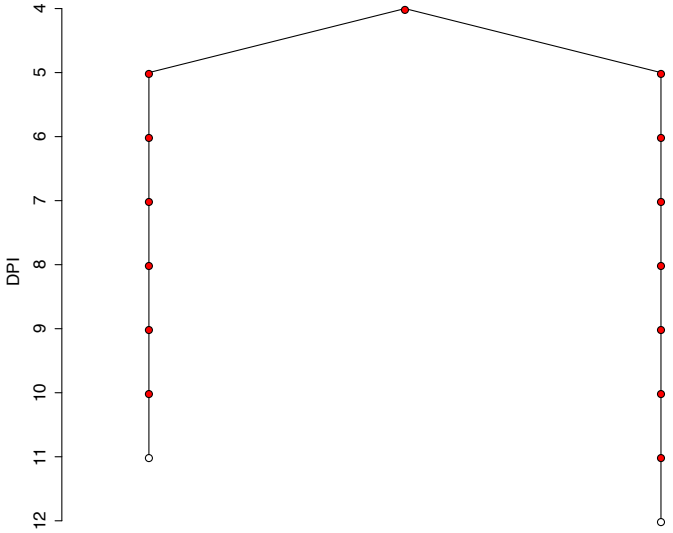
uncertainty cell type
- uncertain

uncertainty lineage
- certain
- semi - certain
- uncertain

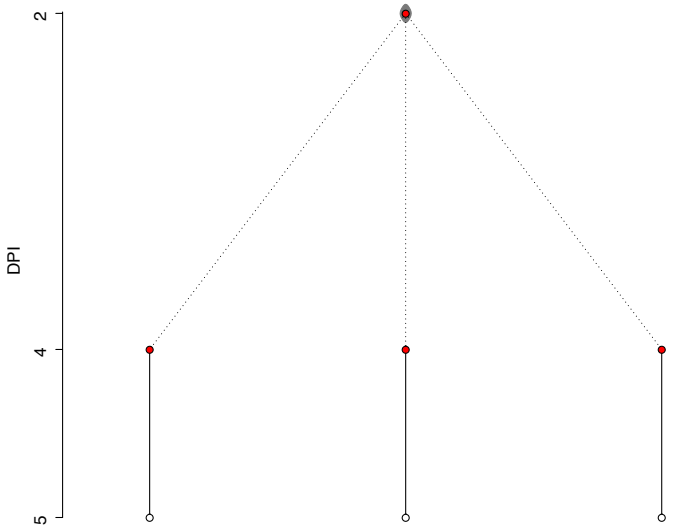
43. **bf3093.SPOT7.clone2**



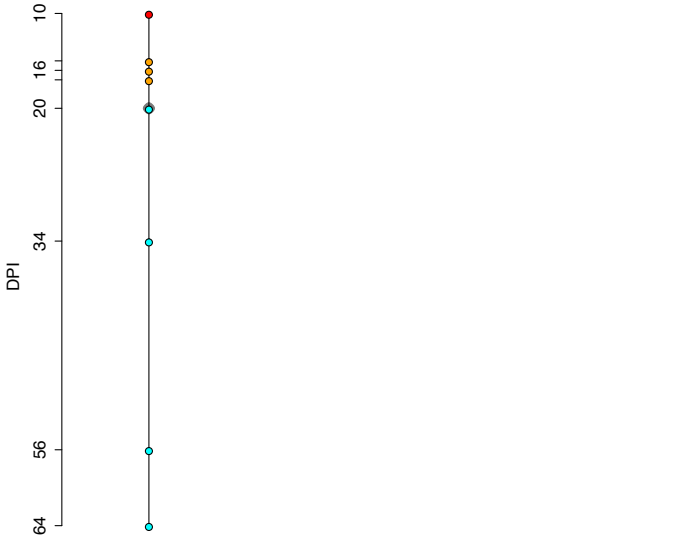
44. **bf3093.SPOT8.clone1**



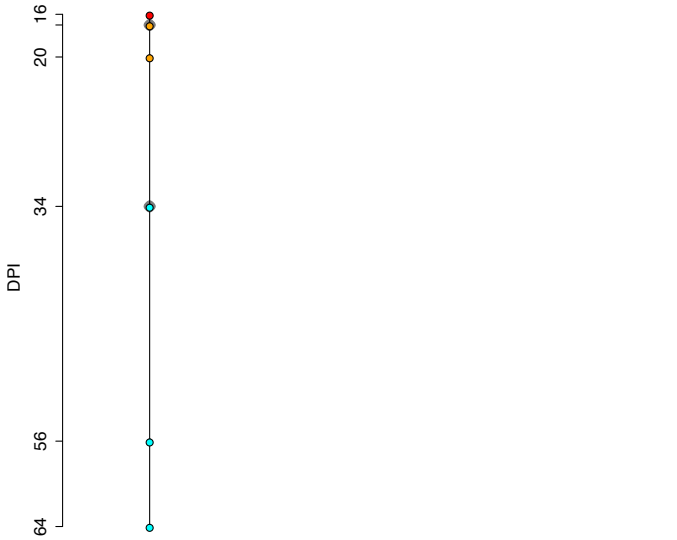
45. **bf3093.SPOT9.clone1**



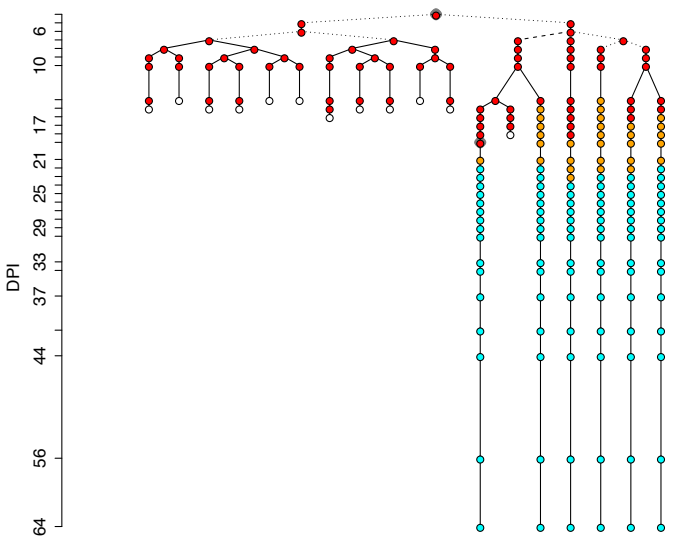
46. **bf3343.SPOT1.clone1**



47. **bf3343.SPOT1.clone2**



48. **bf3343.SPOT41.clone1**

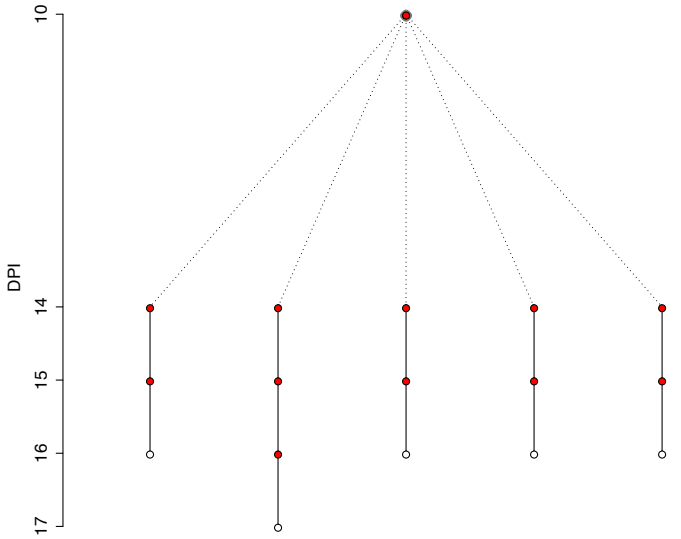


cell types
- OPC
- PM
- M
- cell death

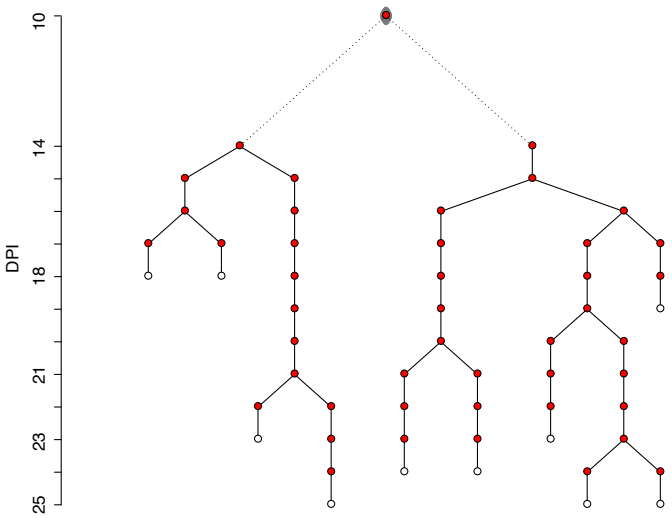
uncertainty cell type
- uncertain

uncertainty lineage
- certain
- semi - certain
- uncertain

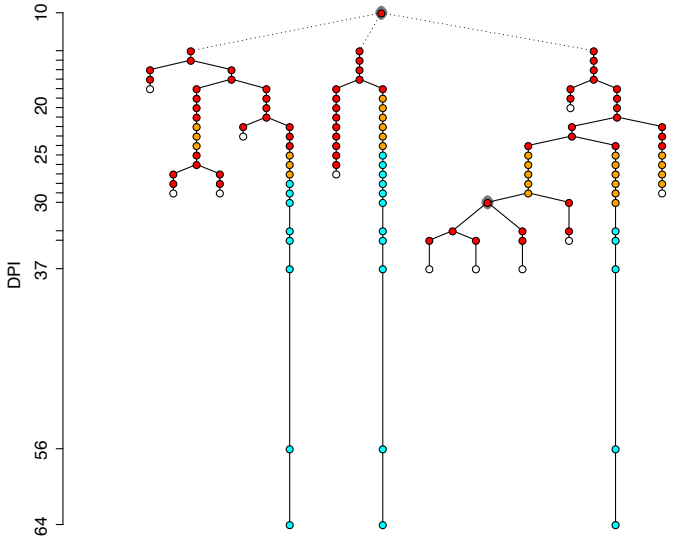
49. **bf3343.SPOT41.clone2**



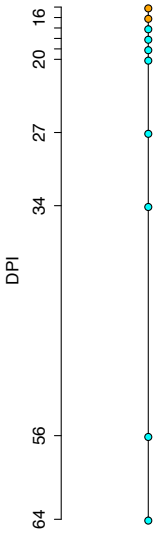
50. **bf3343.SPOT6.clone1**



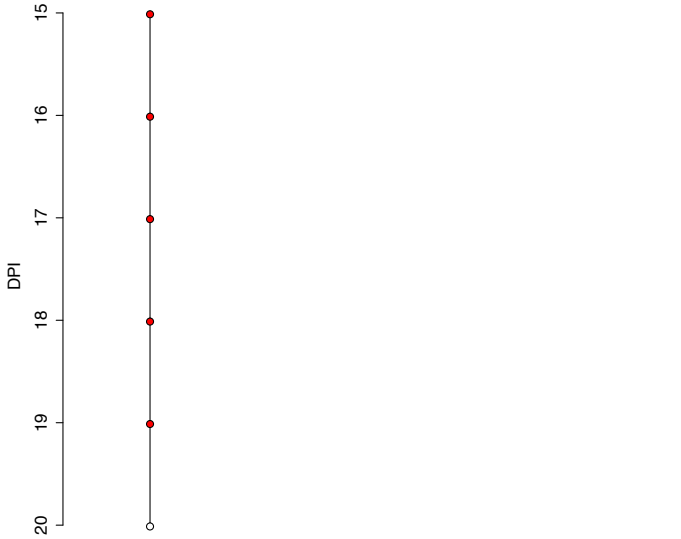
51. **bf3343.SPOT6.clone2**



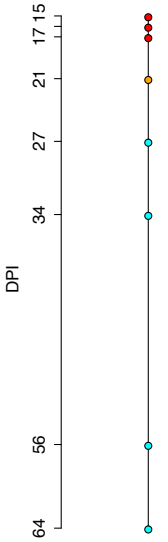
52. **bf3343.SPOT7.clone1**



53. **bf3343.SPOT7.clone2**



54. **bf3343.SPOT8.clone1**

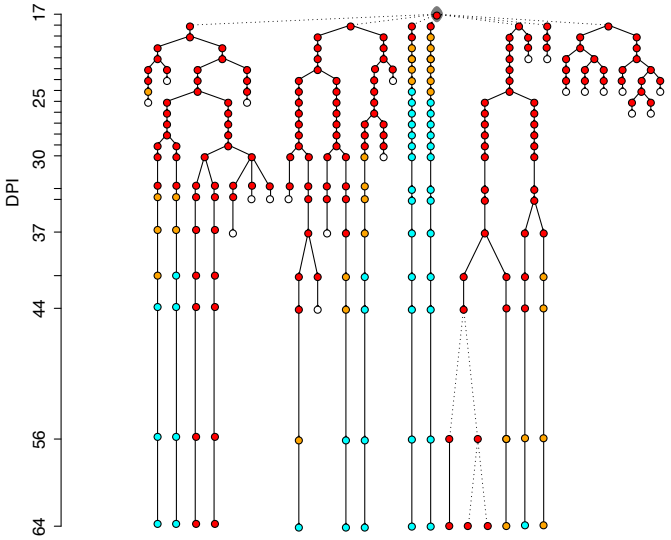


cell types
- OPC
- PM
- M
- cell death

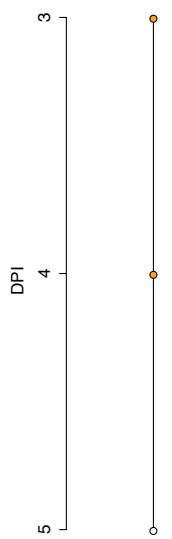
uncertainty cell type
- uncertain

uncertainty lineage
- certain
- semi - certain
- uncertain

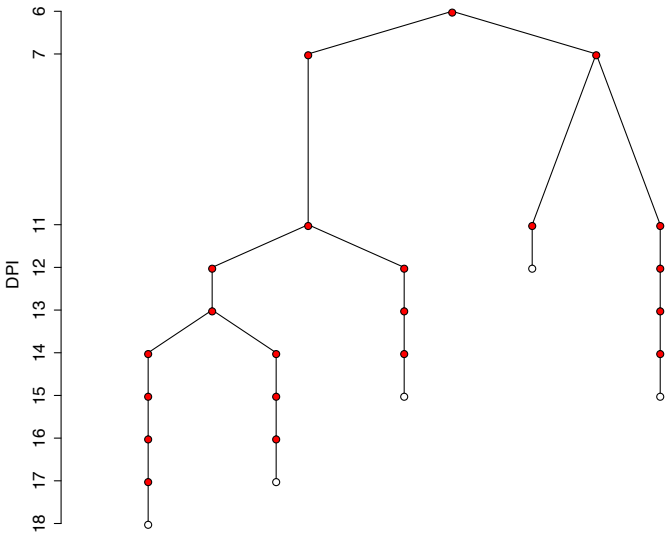
55. **bf3343.SPOT91.clone1**



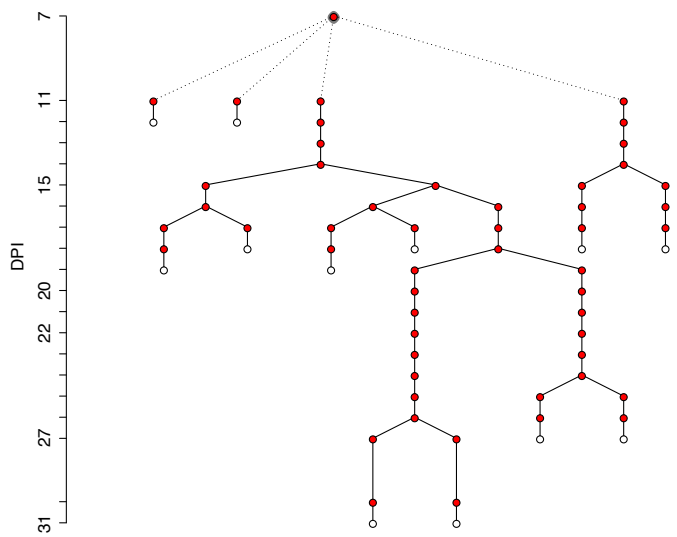
56. **bf3345.SPOT10.clone1**



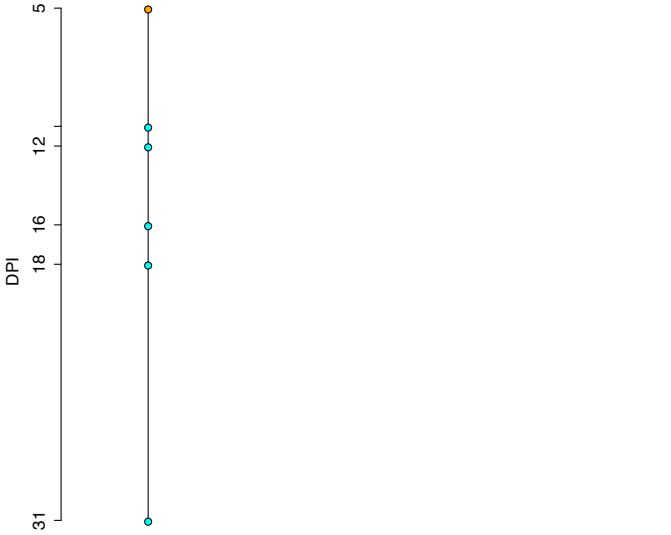
57. **bf3345.SPOT12.clone1**



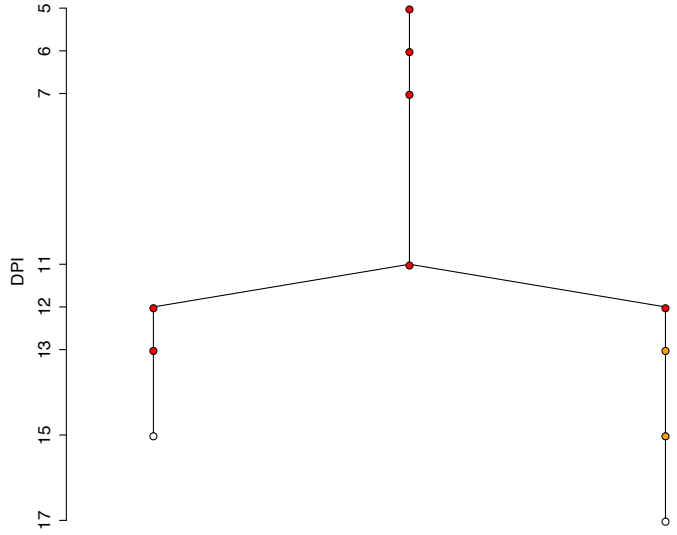
58. **bf3345.SPOT13.clone1**



59. **bf3345.SPOT17.clone1**



60. **bf3345.SPOT18.clone1**

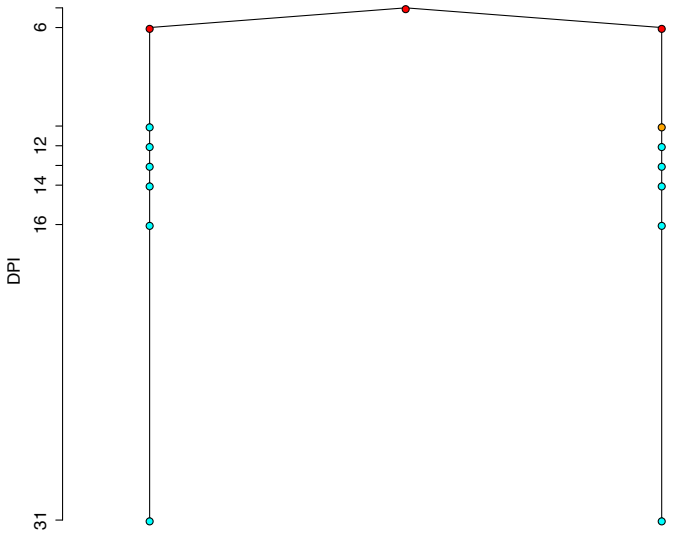


cell types
- OPC
- PM
- M
- cell death

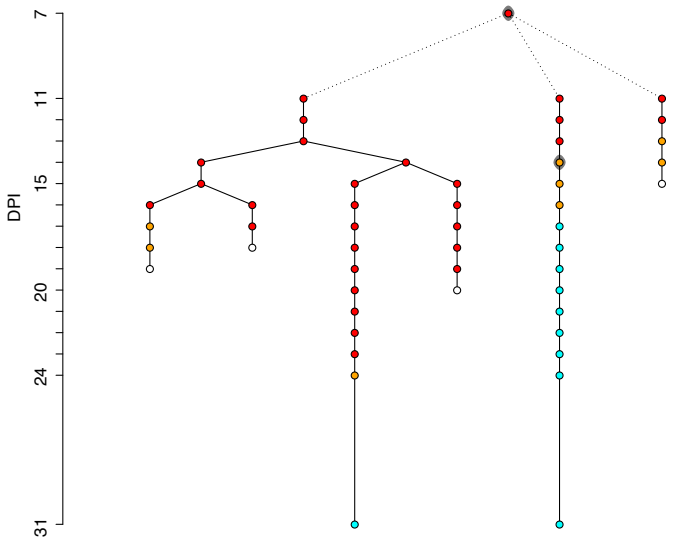
uncertainty cell type
- uncertain

uncertainty lineage
- certain
- semi - certain
- uncertain

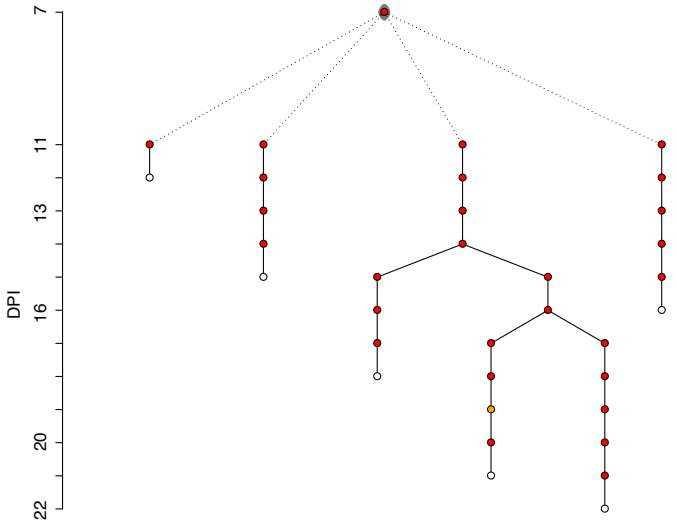
61. **bf3345.SPOT19.clone1**



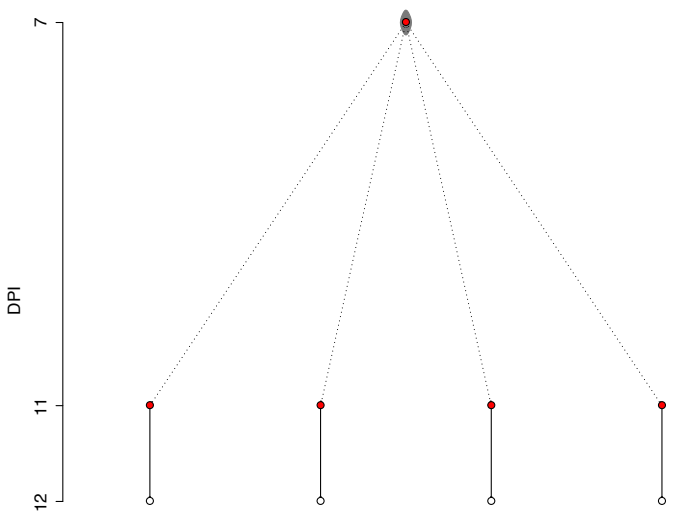
62. **bf3345.SPOT20.clone1**



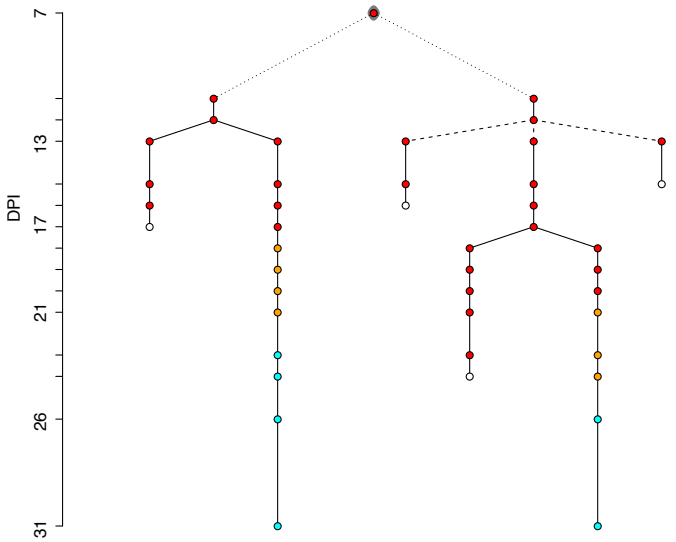
63. **bf3345.SPOT20.clone2**



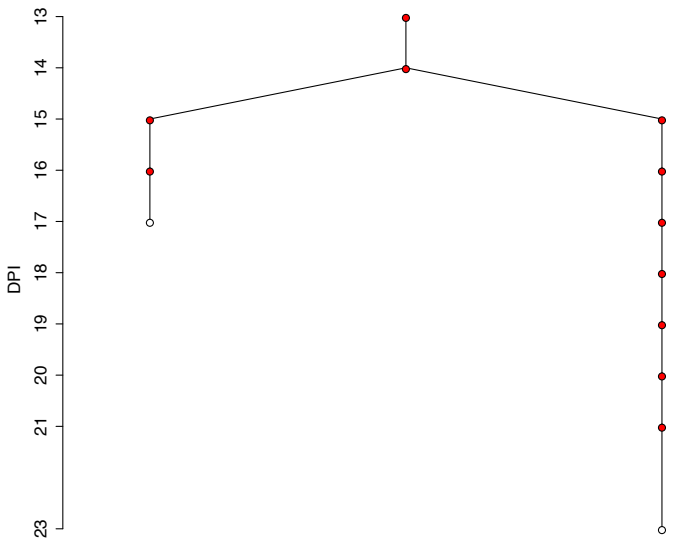
64. **bf3345.SPOT21.clone1**



65. **bf3345.SPOT21.clone2**



66. **bf3345.SPOT21.clone3**

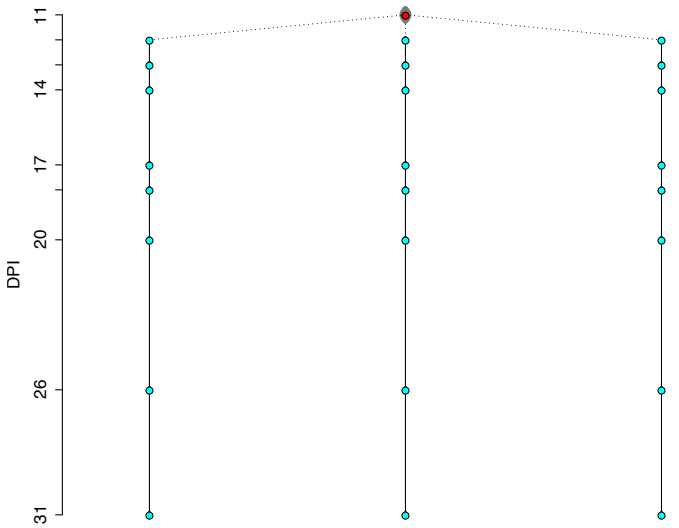


cell types
- OPC
- PM
- M
- cell death

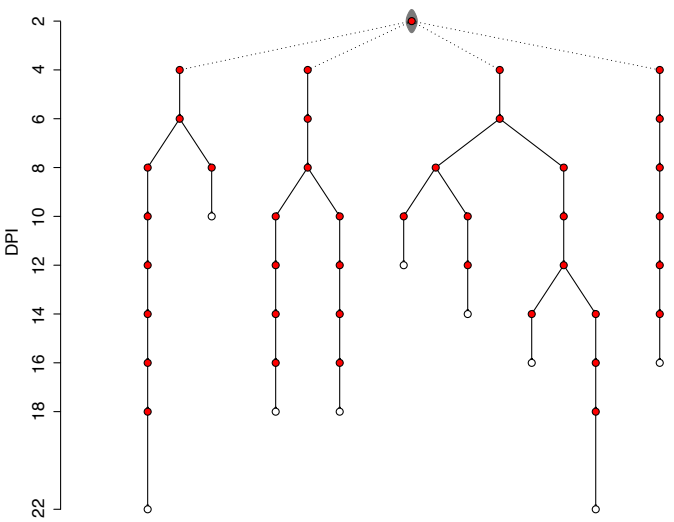
uncertainty cell type
- uncertain

uncertainty lineage
- certain
- semi - certain
- uncertain

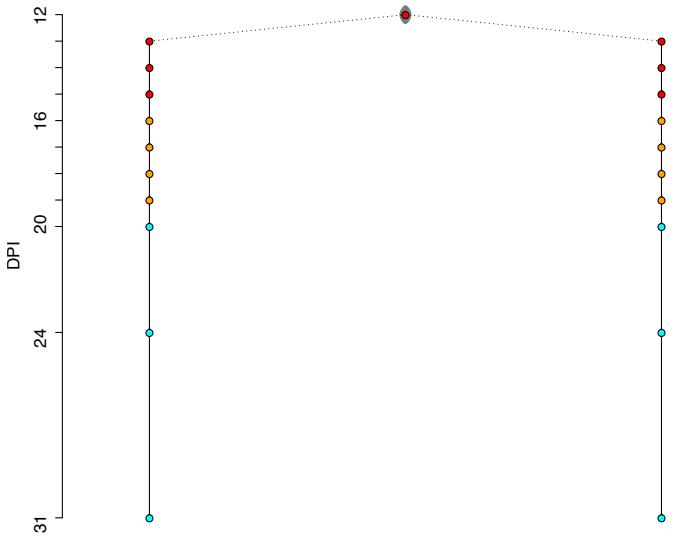
67. **bf3345.SPOT22.clone1**



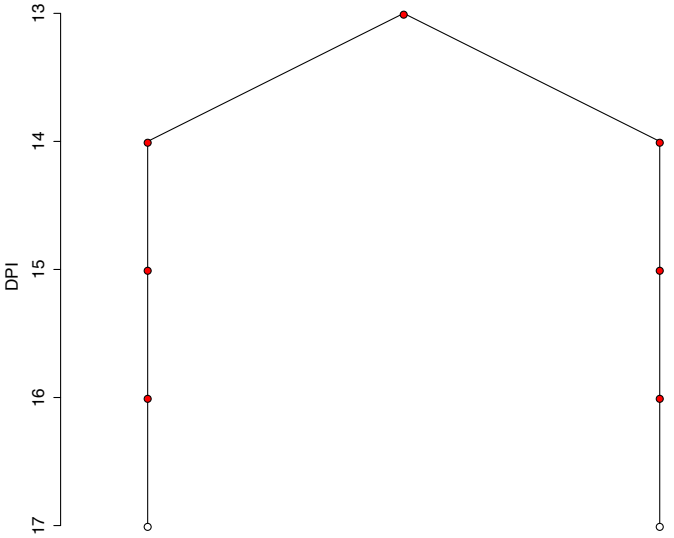
68. **bf3345.SPOT23.clone1**



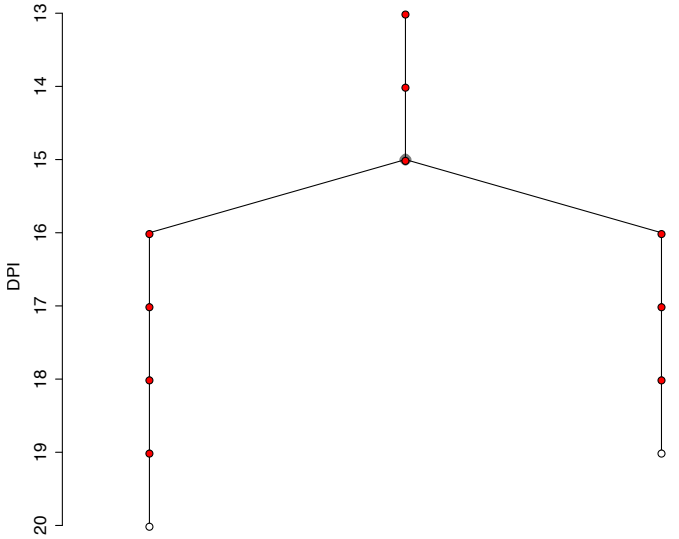
69. **bf3345.SPOT25.clone1**



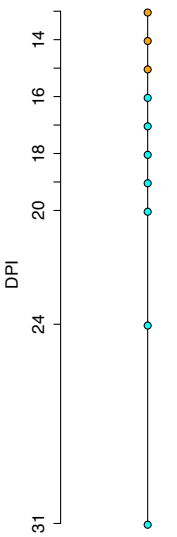
70. **bf3345.SPOT25.clone2**



71. **bf3345.SPOT25.clone3**



72. **bf3345.SPOT25.clone4**

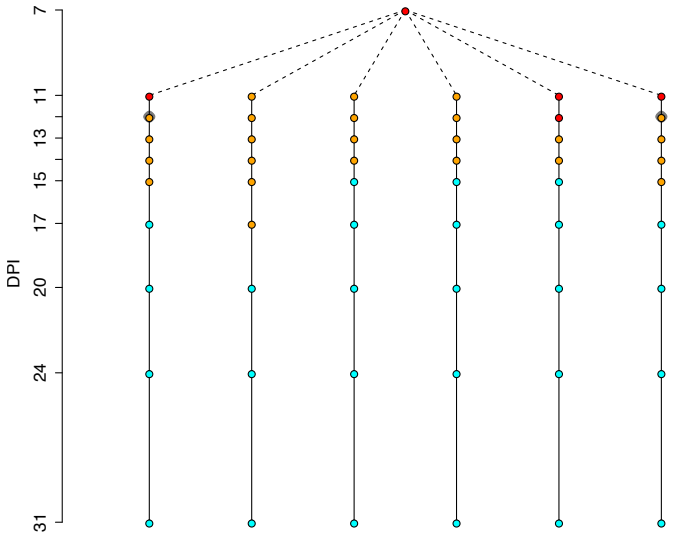


cell types
- OPC
- PM
- M
- cell death

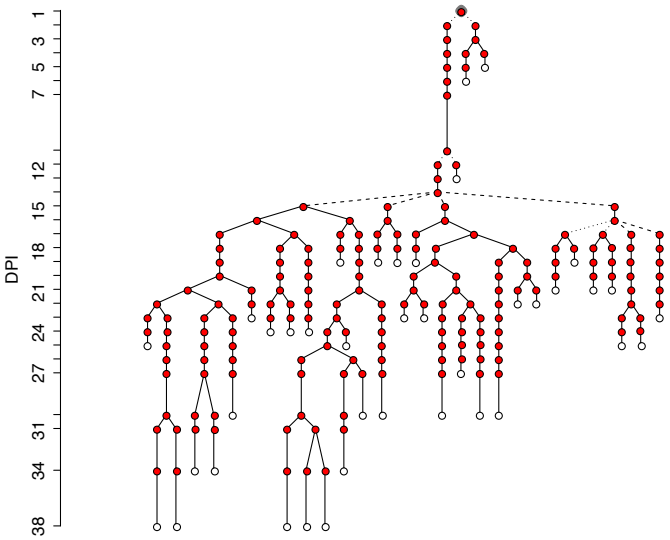
uncertainty cell type
- uncertain

uncertainty lineage
- certain
- semi - certain
- uncertain

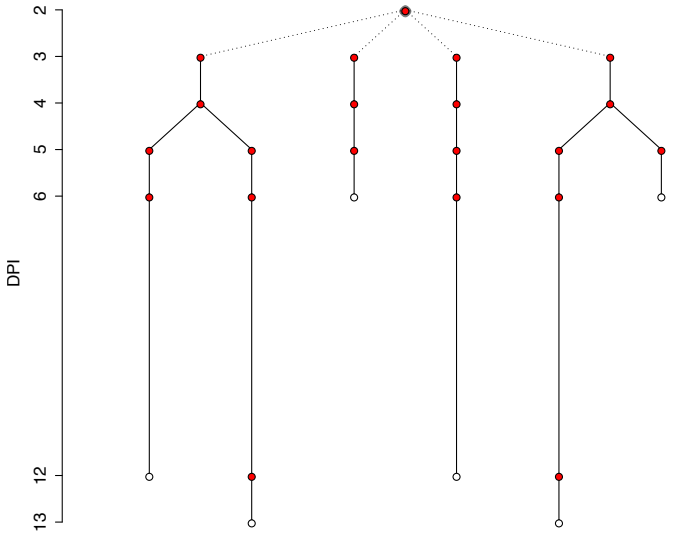
73. **bf3345.SPOT3.clone1**



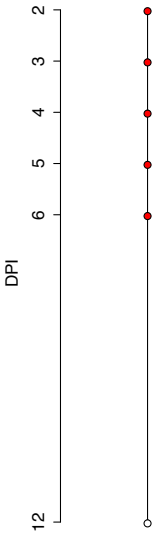
74. **bf3345.SPOT4.clone1**



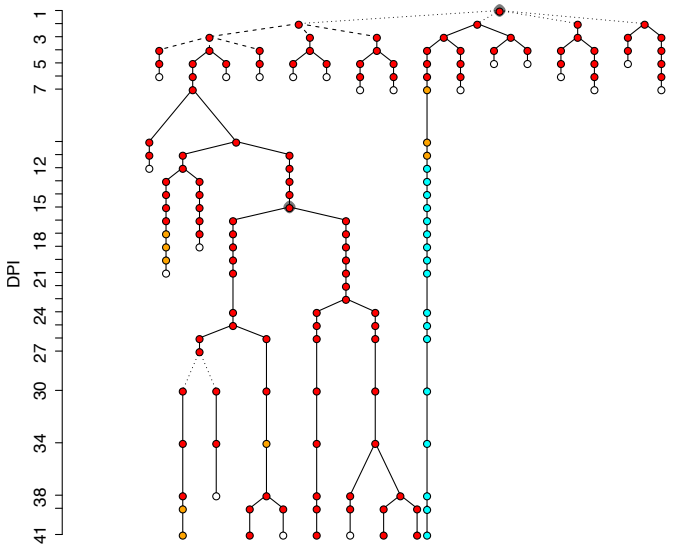
75. **bf3345.SPOT4.clone2**



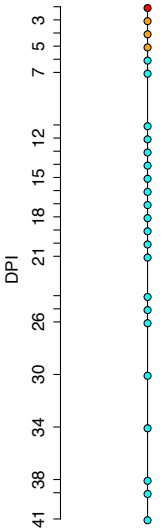
76. **bf3345.SPOT4.clone3**



77. **bf3345.SPOT5.clone1**



78. **bf3345.SPOT5.clone2**

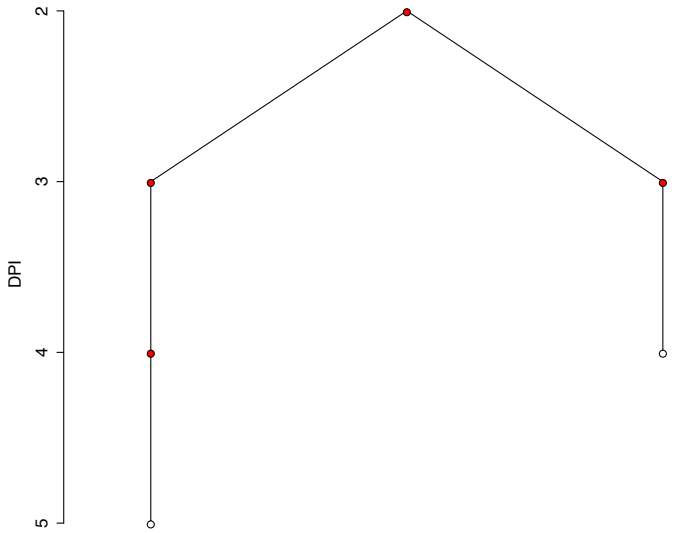


cell types
- OPC
- PM
- M
- cell death

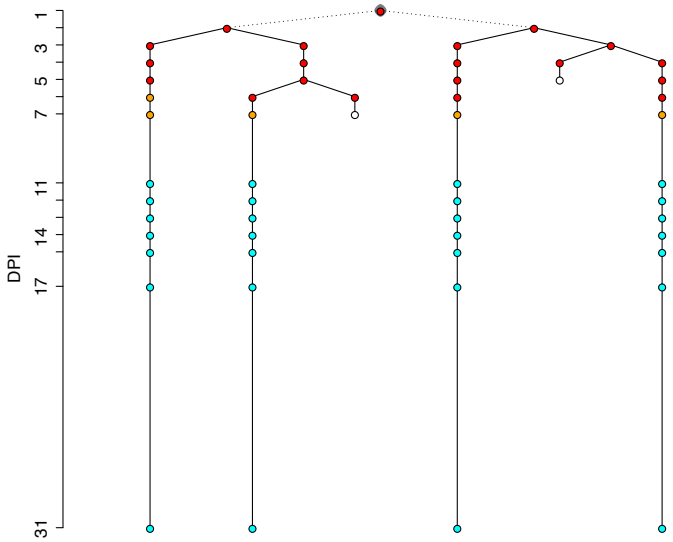
uncertainty cell type
- uncertain

uncertainty lineage
- certain
- semi - certain
- uncertain

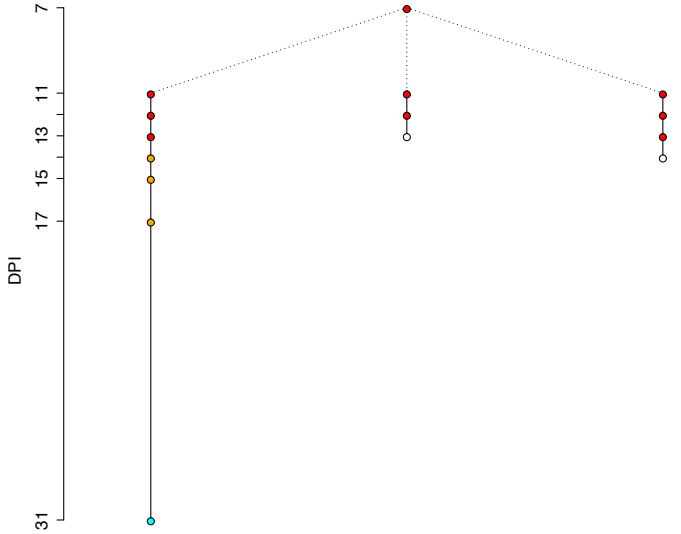
79. **bf3345.SPOT6.clone1**



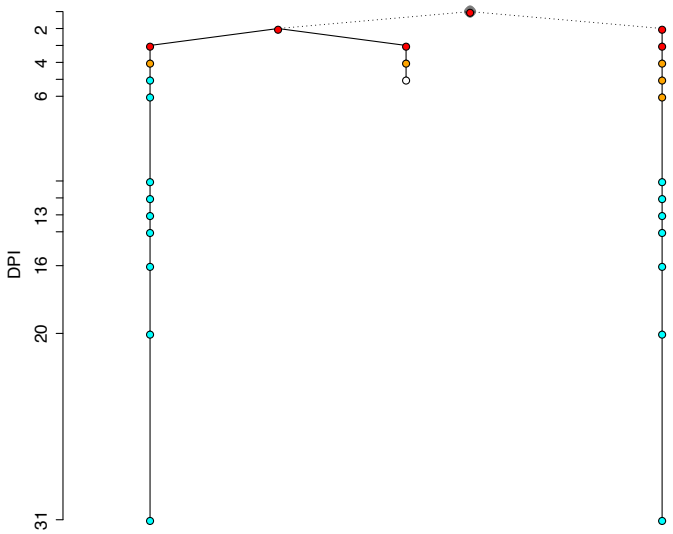
80. **bf3345.SPOT7.clone1**



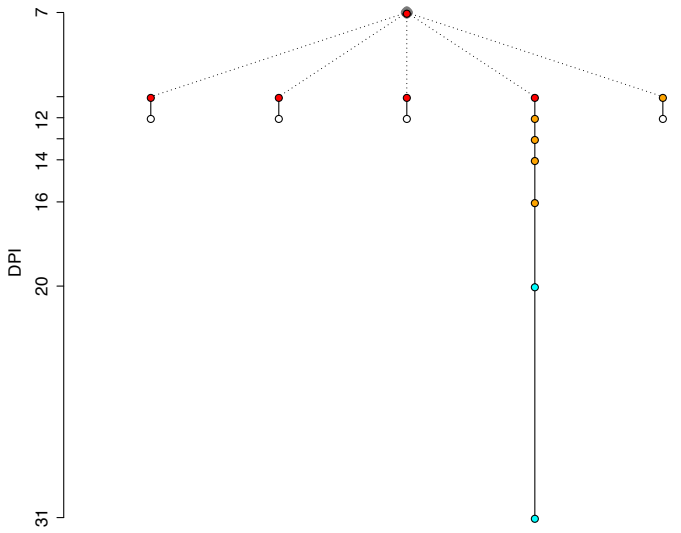
81. **bf3345.SPOT7.clone2**



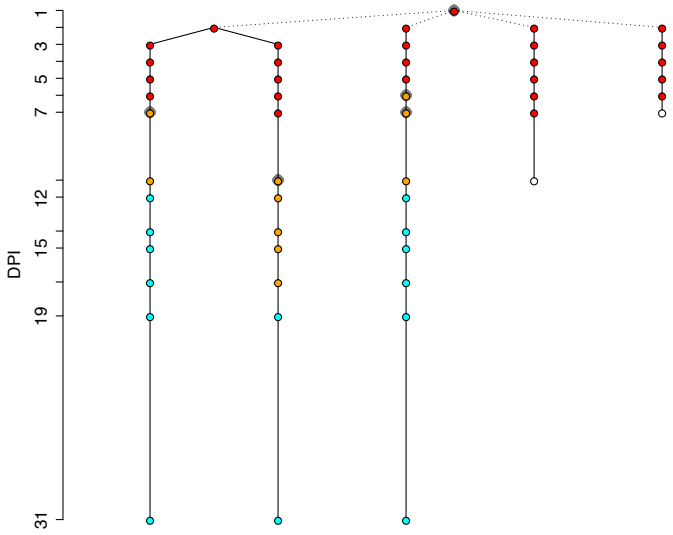
82. **bf3345.SPOT8.clone1**



83. **bf3345.SPOT8.clone2**



84. **bf3345.SPOT9.clone1**

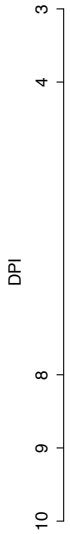


cell types
- OPC
- PM
- M
- cell death

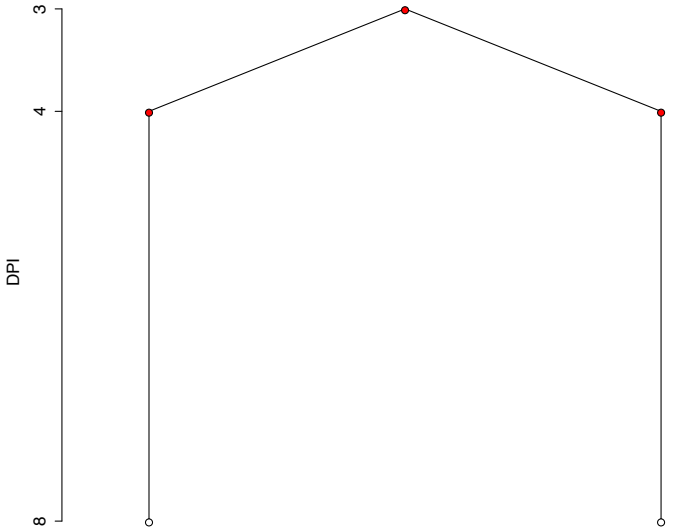
uncertainty cell type
- uncertain

uncertainty lineage
- certain
- semi - certain
- uncertain

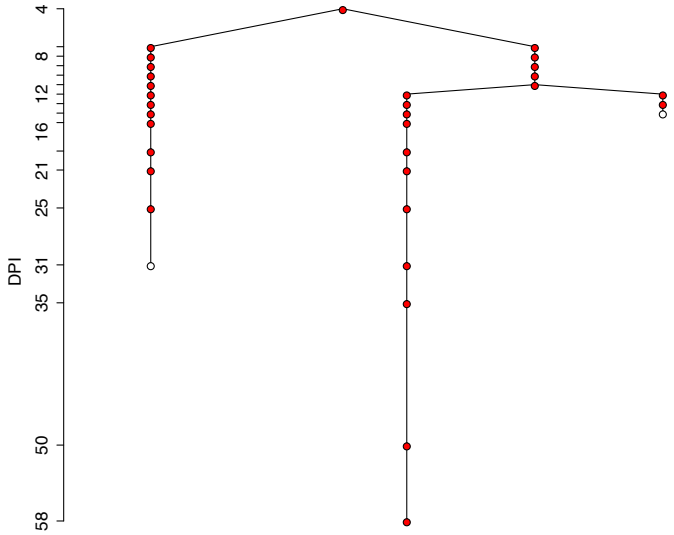
85. **bf3347.SPOT1.clone1**



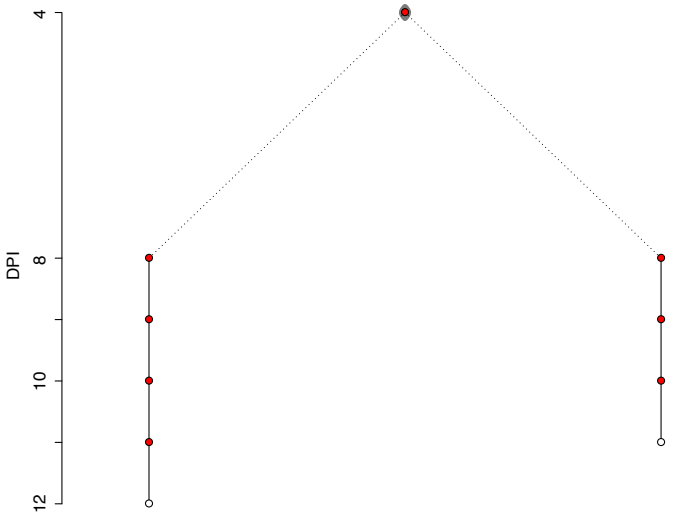
86. **bf3347.SPOT1.clone2**



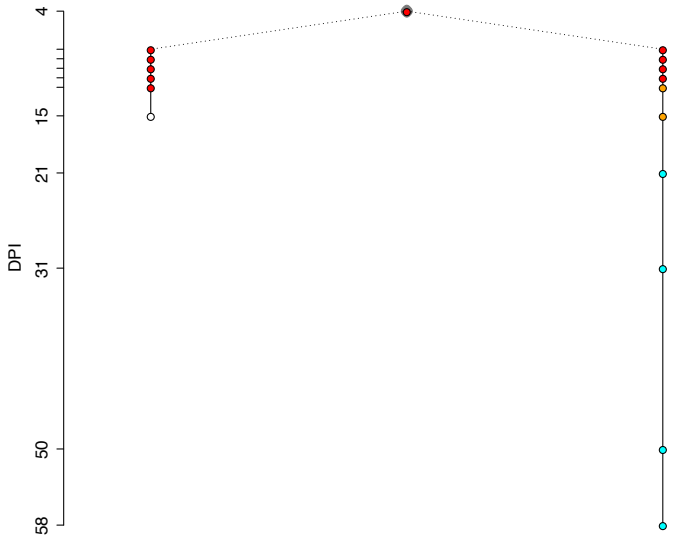
87. **bf3347.SPOT1.clone3**



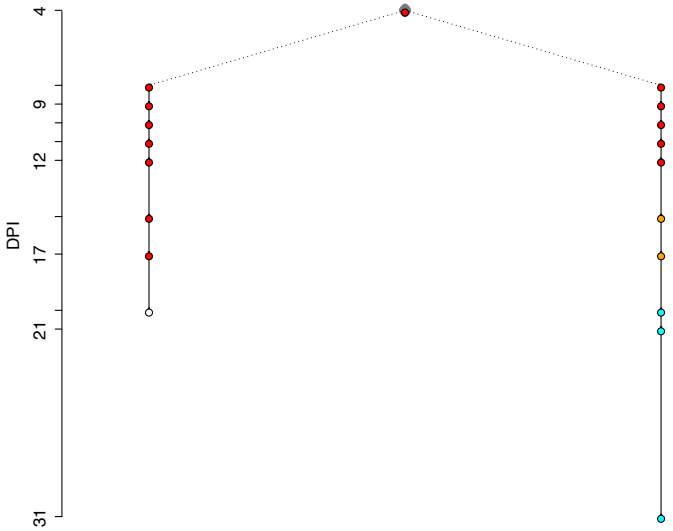
88. **bf3347.SPOT11.clone1**



89. **bf3347.SPOT11.clone2**



90. **bf3347.SPOT12.clone1**

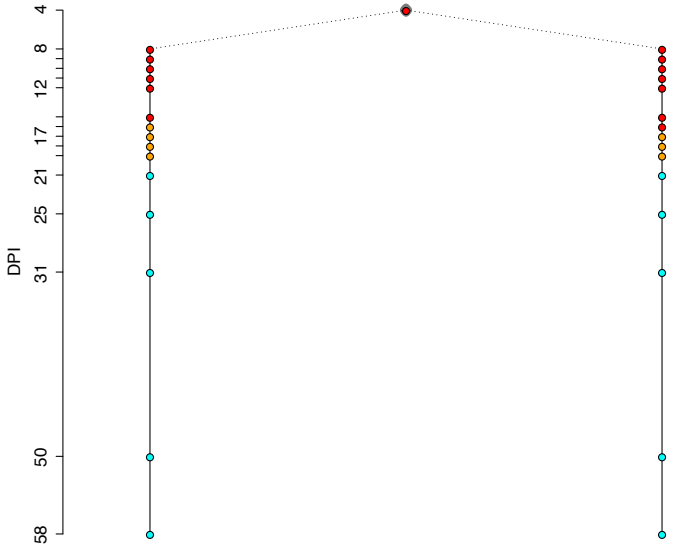


cell types
- OPC
- PM
- M
- cell death

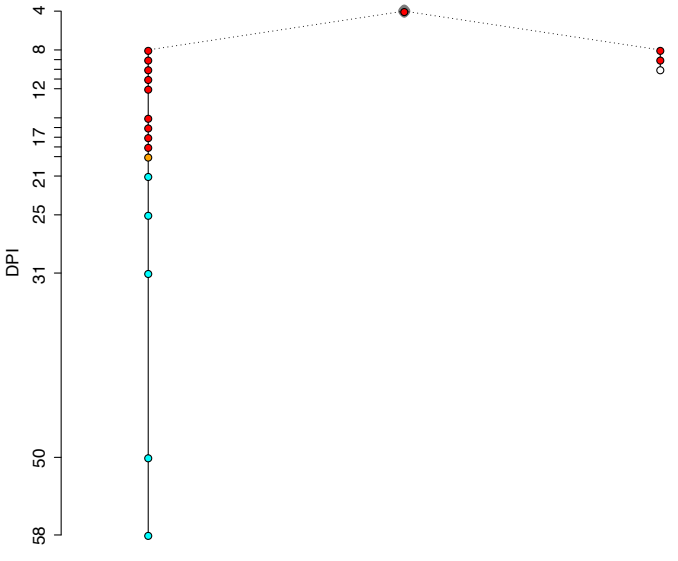
uncertainty cell type
- uncertain

uncertainty lineage
- certain
- semi - certain
- uncertain

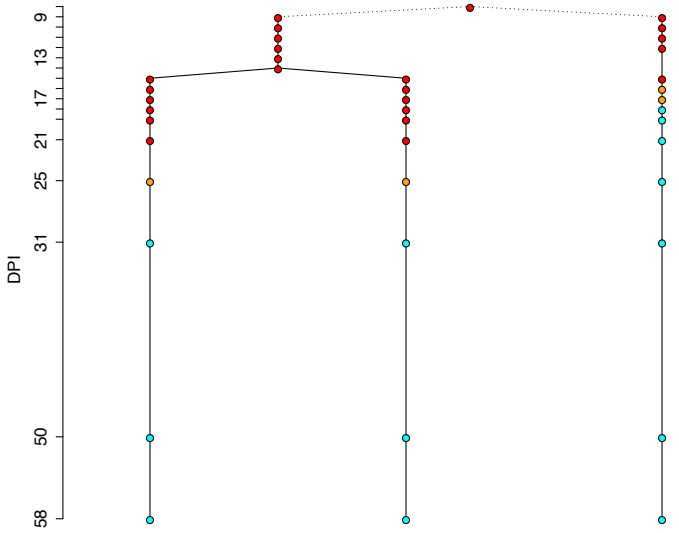
91. **bf3347.SPOT13.clone1**



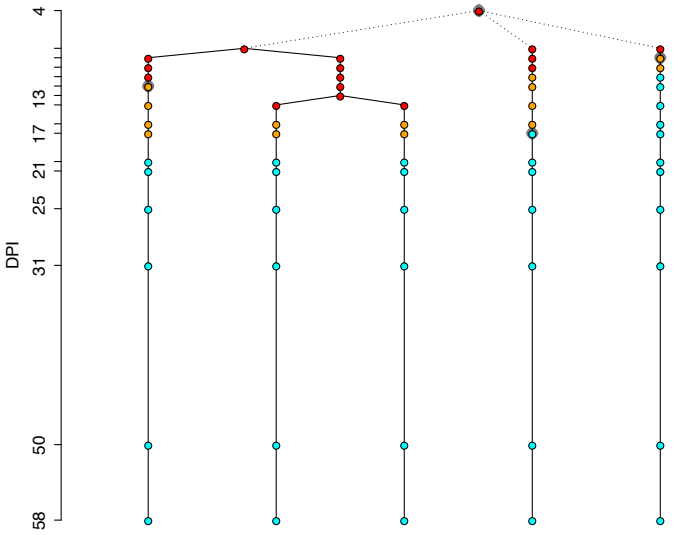
92. **bf3347.SPOT13.clone2**



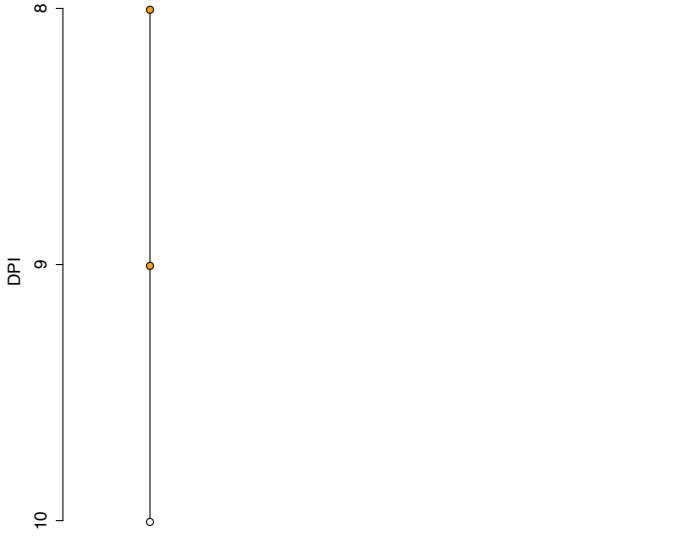
93. **bf3347.SPOT13.clone3**



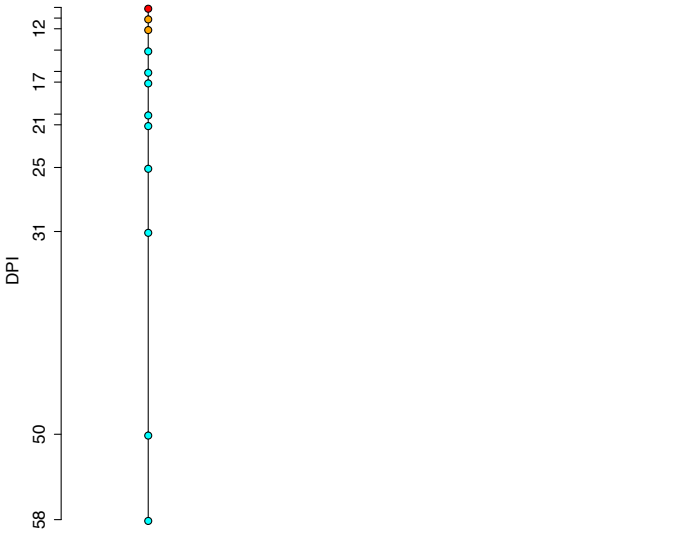
94. **bf3347.SPOT14.clone1**



95. **bf3347.SPOT14.clone2**



96. **bf3347.SPOT14.clone3**

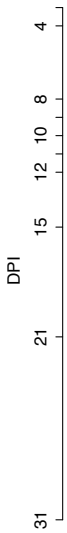


cell types
- OPC
- PM
- M
- cell death

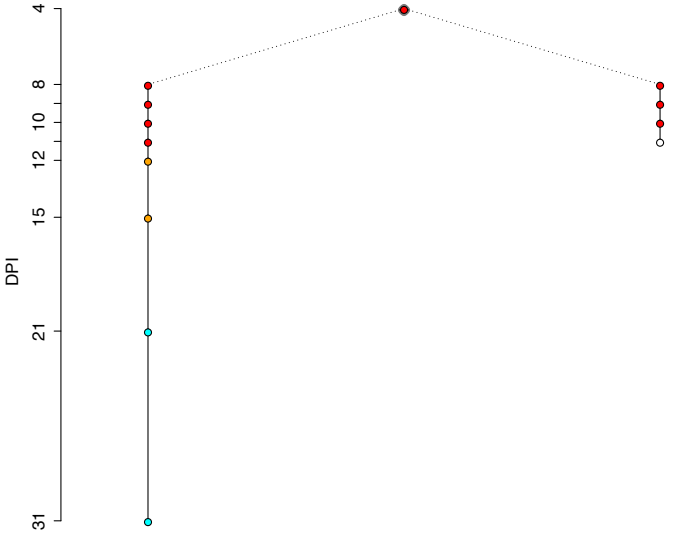
uncertainty cell type
- uncertain

uncertainty lineage
- certain
- semi - certain
- uncertain

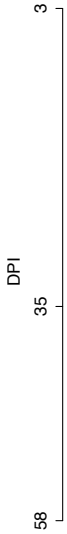
97. **bf3347.SPOT2.clone1**



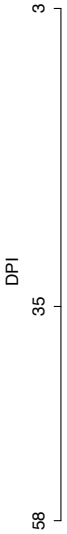
98. **bf3347.SPOT2.clone2**



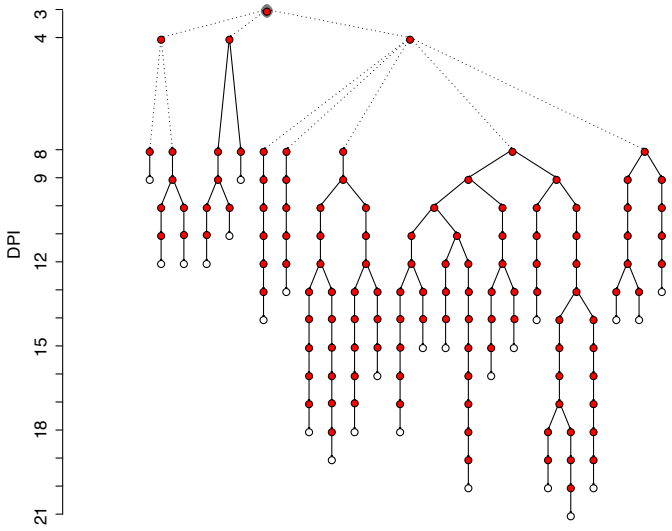
99. **bf3347.SPOT3.clone1**



100. **bf3347.SPOT3.clone2**



101. **bf3347.SPOT8.clone1**



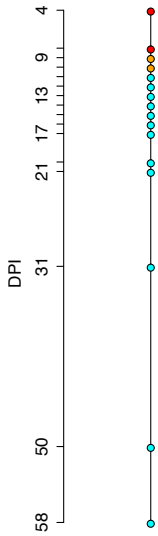
102. **bf3347.SPOT9.clone1**

cell types
- OPC
- PM
- M
- cell death

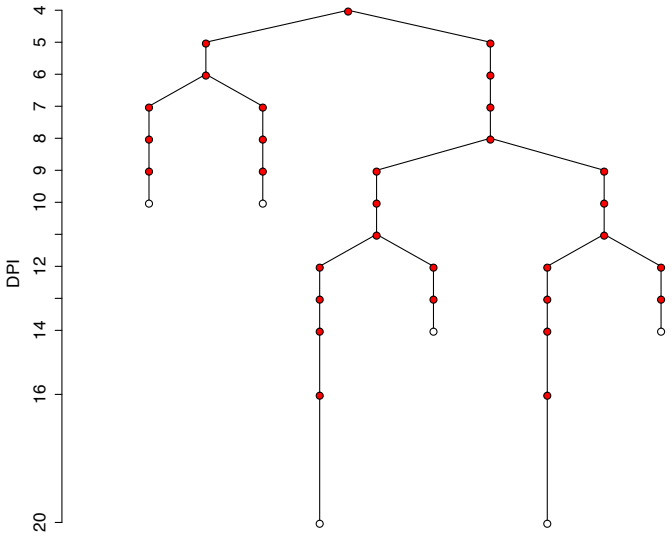
uncertainty cell type
- uncertain

uncertainty lineage
- certain
- semi - certain
- uncertain

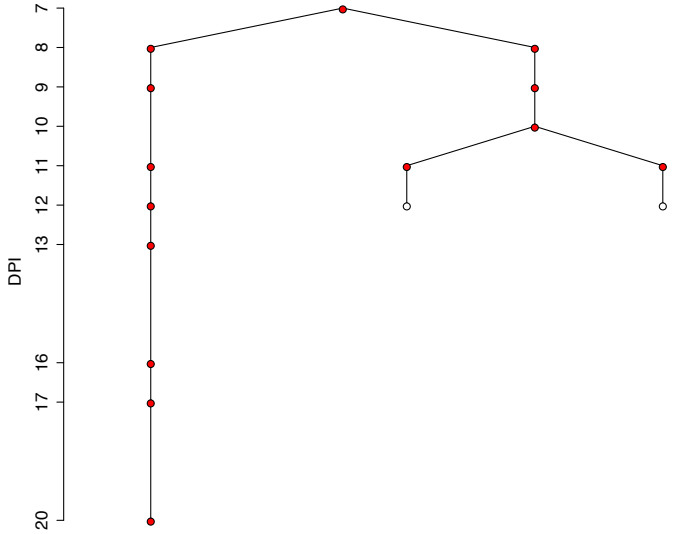
103. **bf3347.SPOT9.clone2**



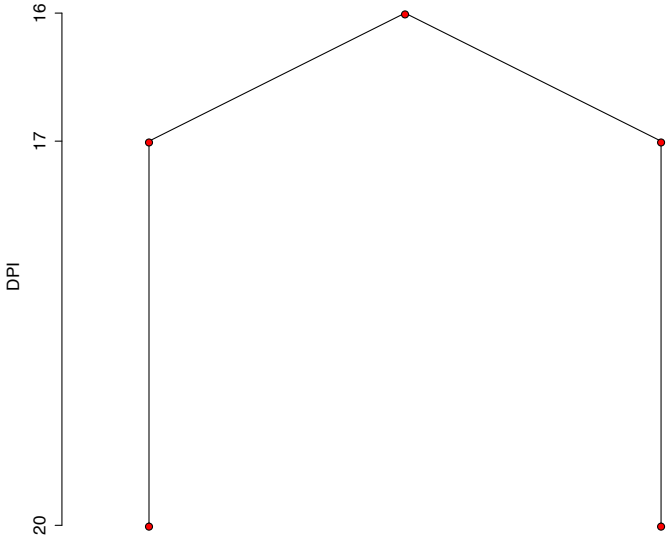
104. **bf3541.SPOT1.clone1**



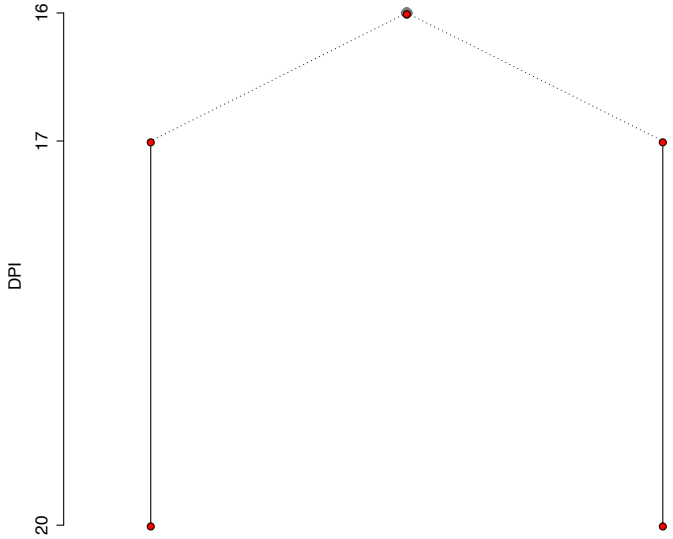
105. **bf3541.SPOT10.clone1**



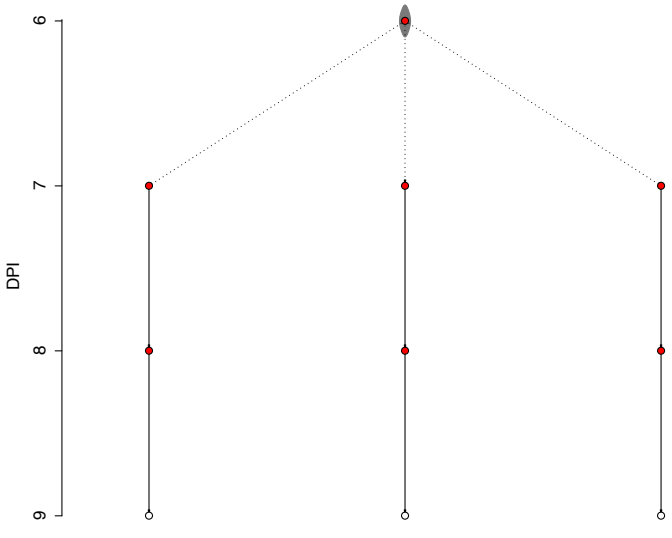
106. **bf3541.SPOT10.clone2**



107. **bf3541.SPOT10.clone3**



108. **bf3541.SPOT11.clone1**

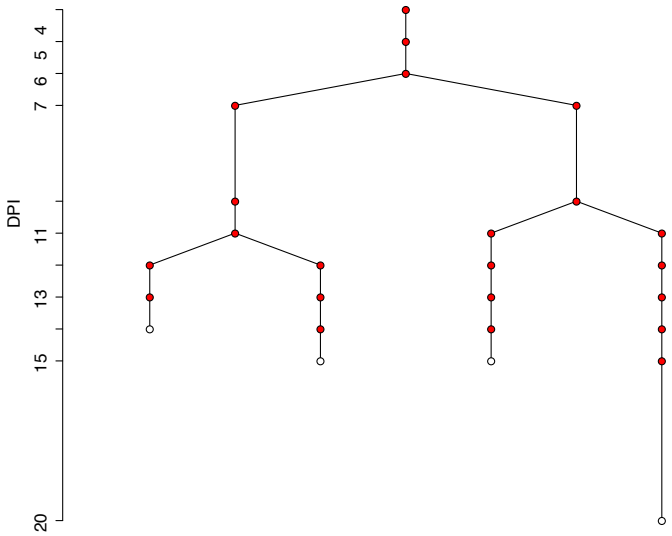


cell types
- OPC
- PM
- M
- cell death

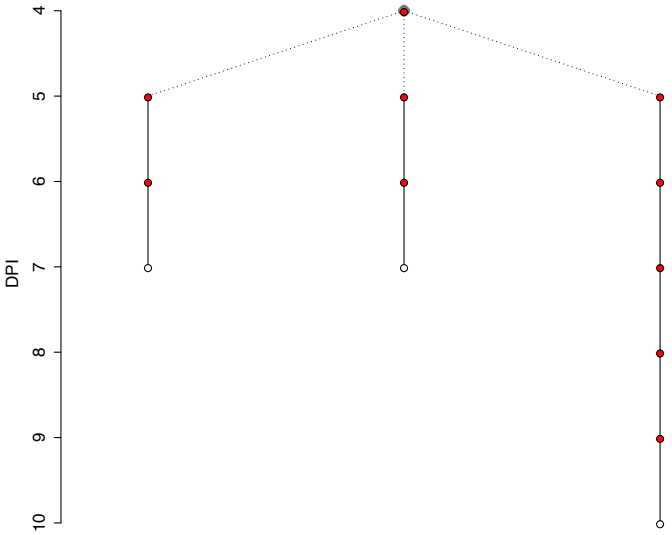
uncertainty cell type
- uncertain

uncertainty lineage
- certain
- semi - certain
- uncertain

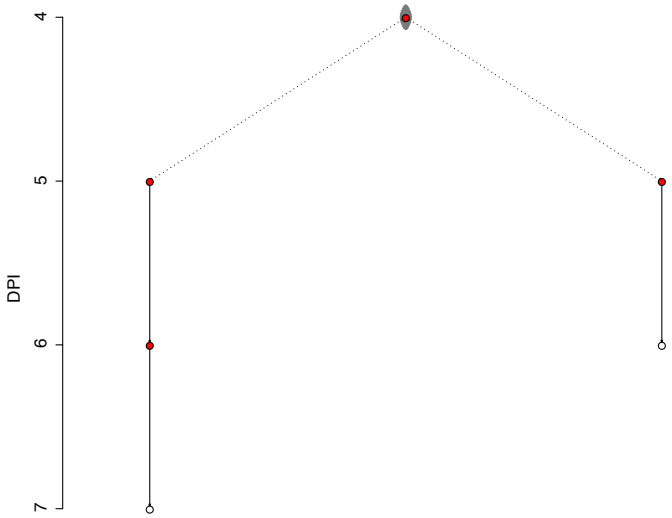
109. **bf3541.SPOT12.clone1**



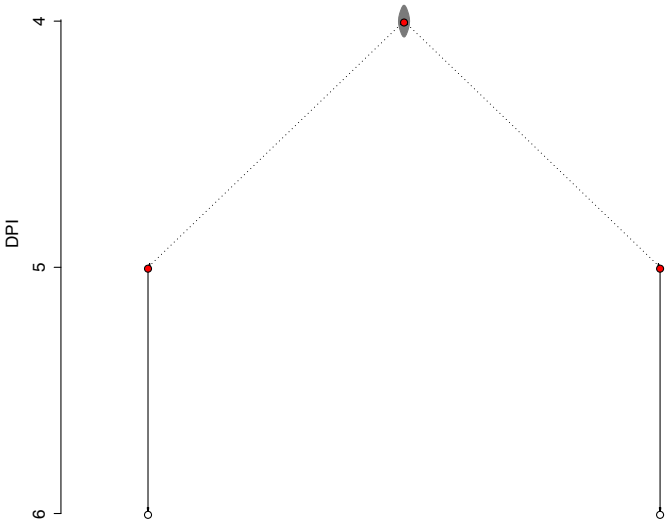
110. **bf3541.SPOT2.clone1**



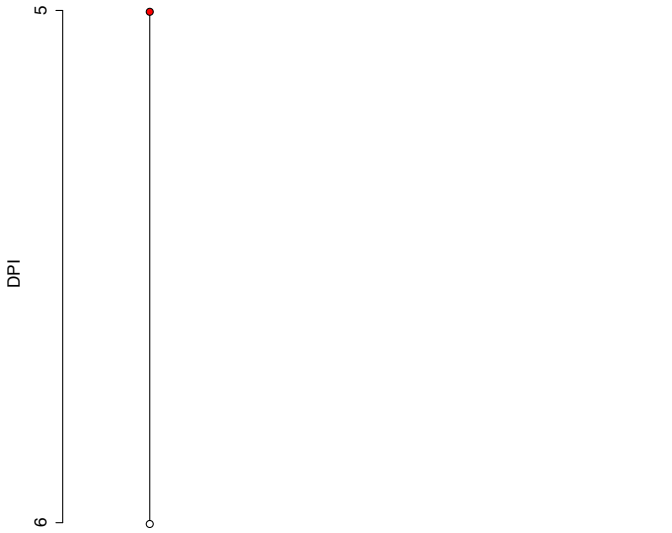
111. **bf3541.SPOT3.clone1**



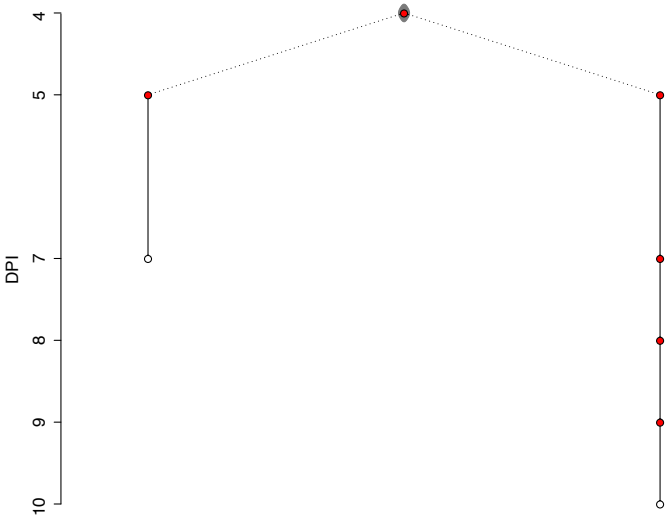
112. **bf3541.SPOT4.clone1**



113. **bf3541.SPOT5.clone1**



114. **bf3541.SPOT6.clone1**

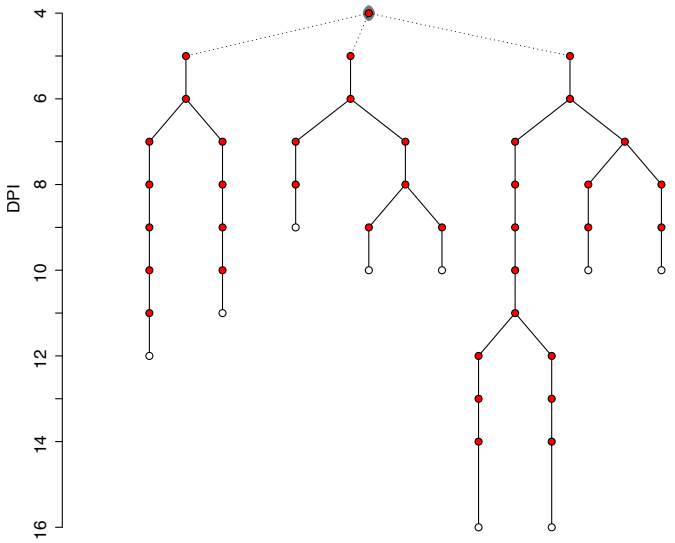


cell types
- OPC
- PM
- M
- cell death

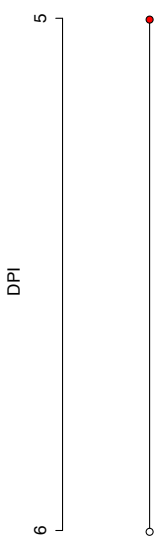
uncertainty cell type
- uncertain

uncertainty lineage
- certain
- semi - certain
- uncertain

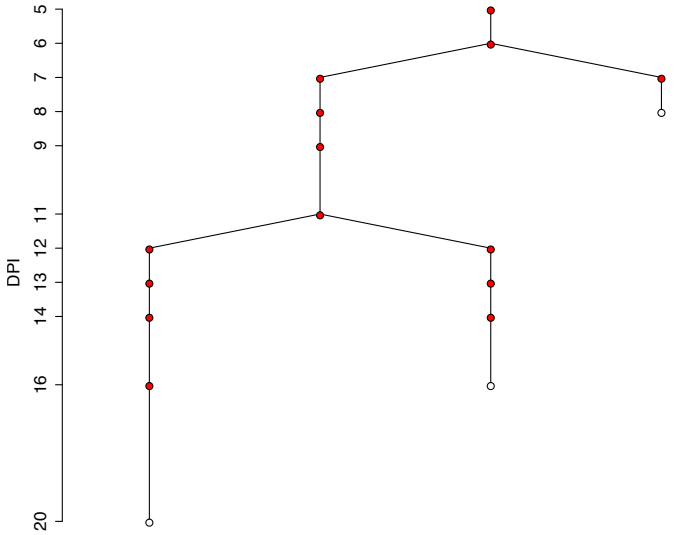
115. **bf3541.SPOT7.clone1**



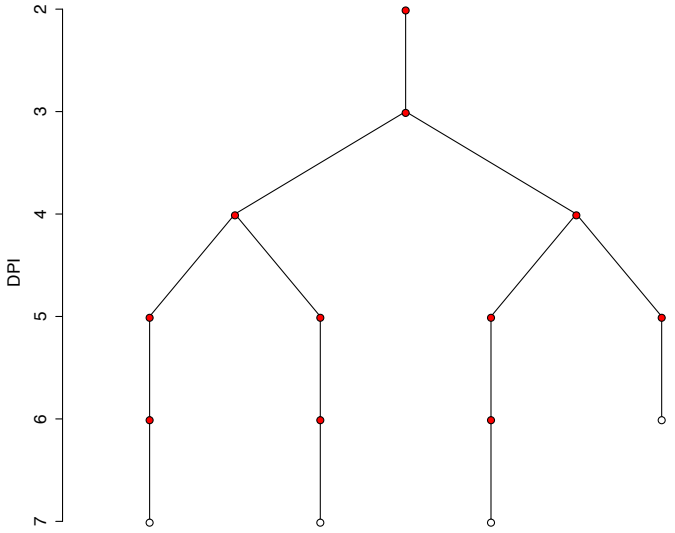
116. **bf3541.SPOT9.clone1**



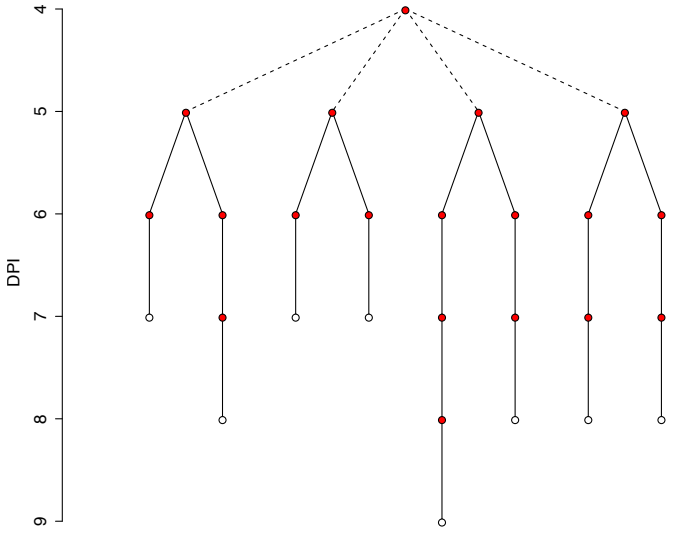
117. **bf3541.SPOT9.clone2**



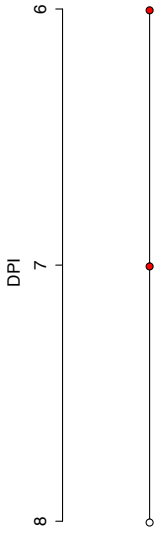
118. **bf3546.SPOT101.clone1**



119. **bf3546.SPOT102.clone1**



120. **bf3546.SPOT15.clone1**

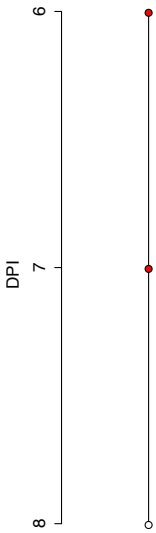


cell types
- OPC
- PM
- M
- cell death

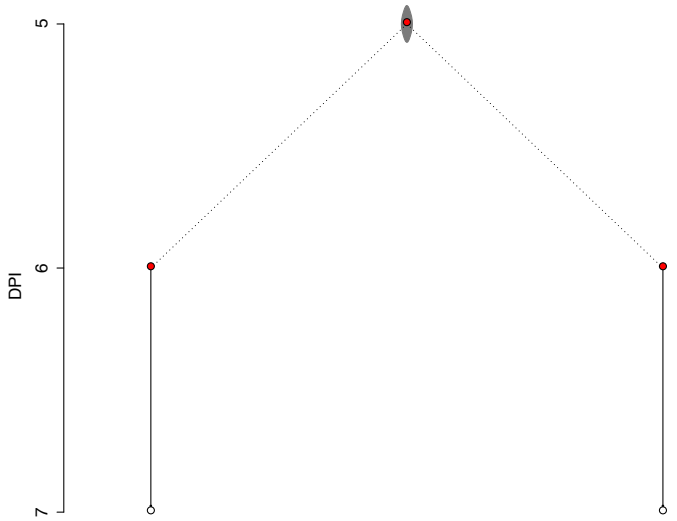
uncertainty cell type
- uncertain

uncertainty lineage
- certain
- semi - certain
- uncertain

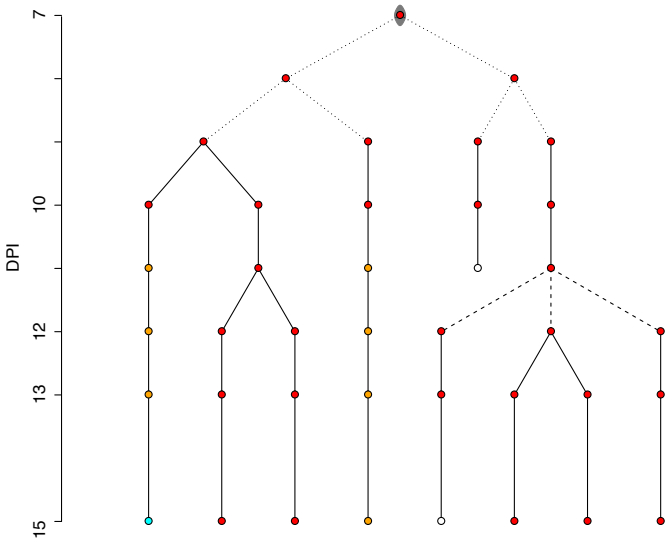
121. **bf3546.SPOT15.clone2**



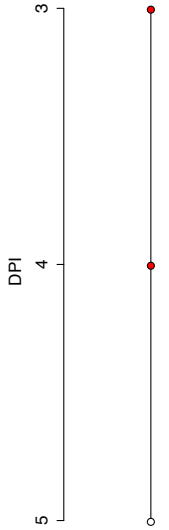
122. **bf3546.SPOT16.clone1**



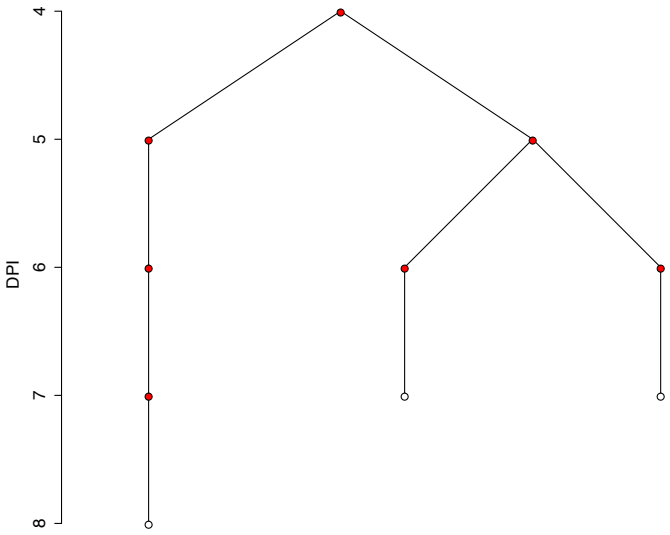
123. **bf3546.SPOT18.clone1**



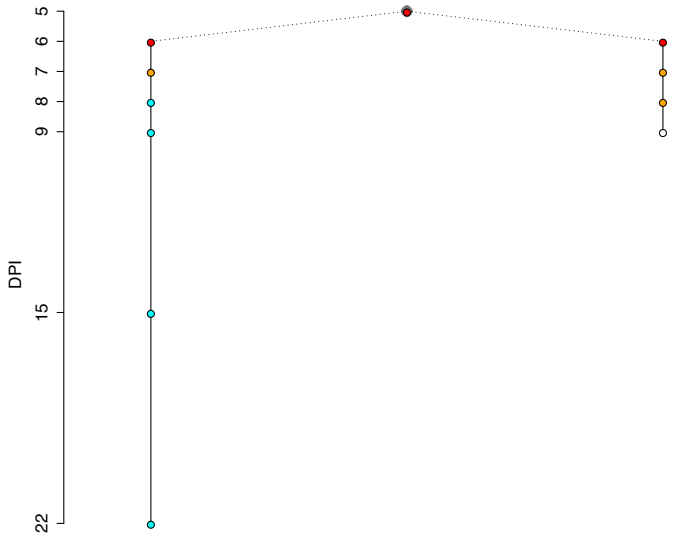
124. **bf3546.SPOT2.clone1**



125. **bf3546.SPOT5.clone1**



126. **bf3546.SPOT5.clone2**

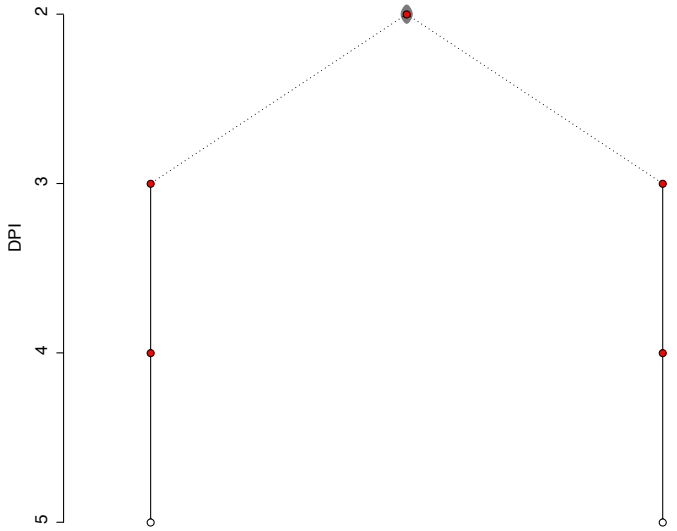


cell types
- OPC
- PM
- M
- cell death

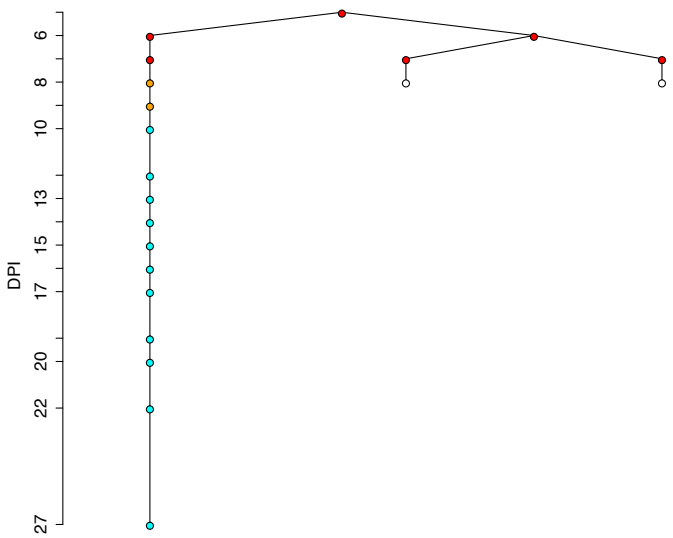
uncertainty cell type
- uncertain

uncertainty lineage
- certain
- semi - certain
- uncertain

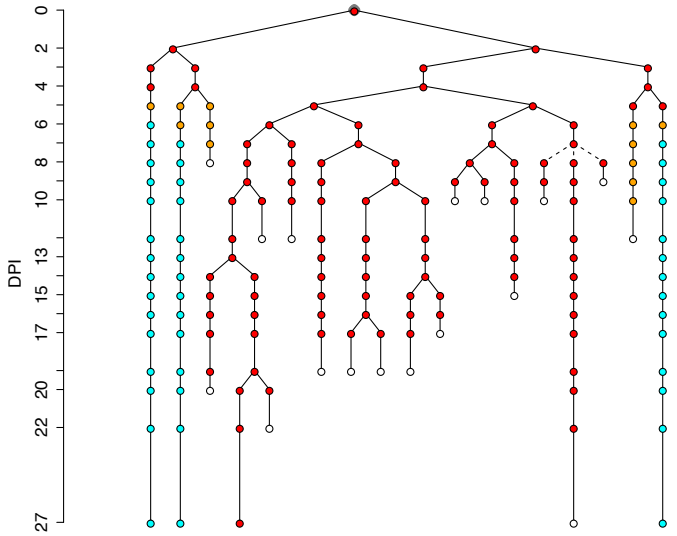
127. **bf3558.SPOT10.clone1**



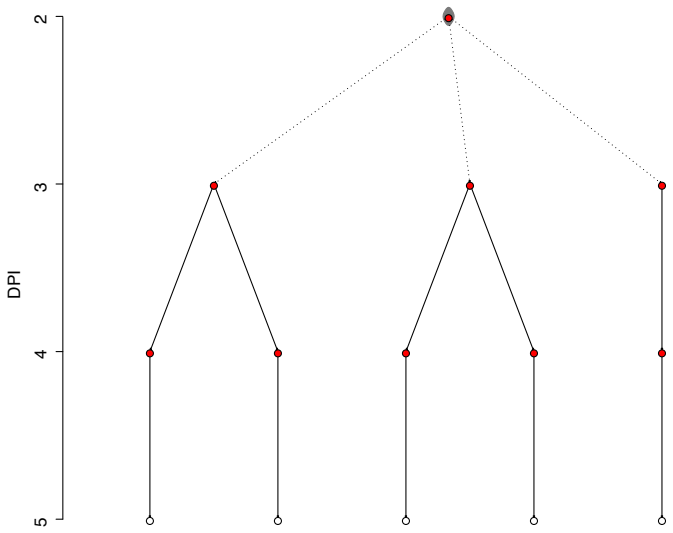
128. **bf3558.SPOT101.clone1**



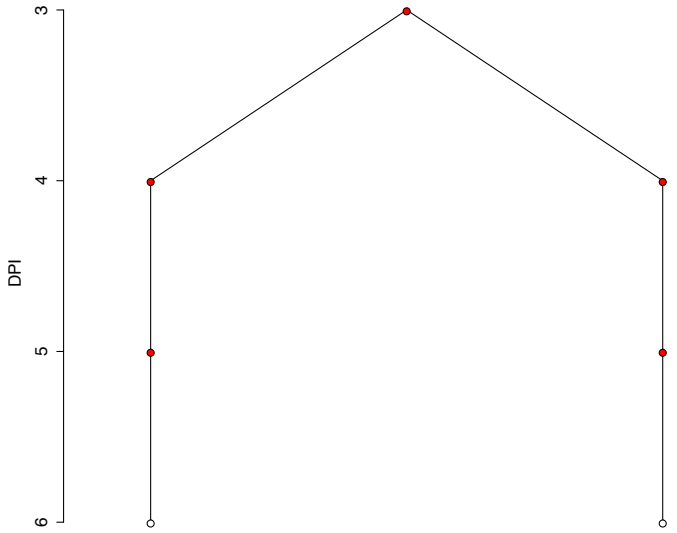
129. **bf3558.SPOT102.clone1**



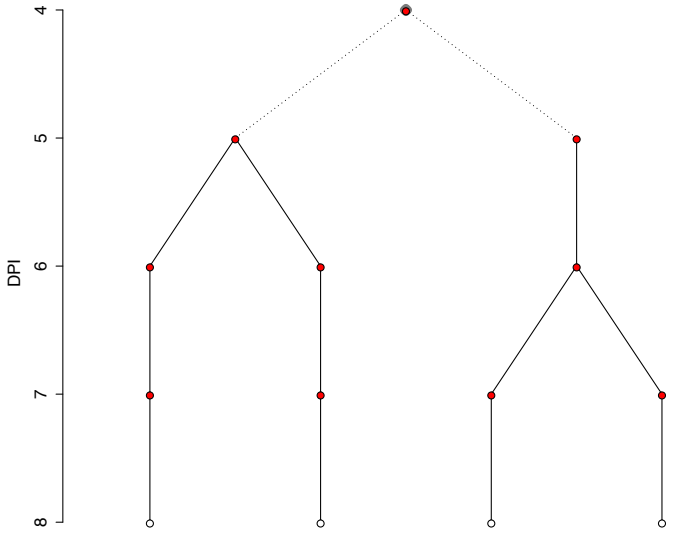
130. **bf3558.SPOT11.clone1**



131. **bf3558.SPOT12.clone1**



132. **bf3558.SPOT12.clone2**

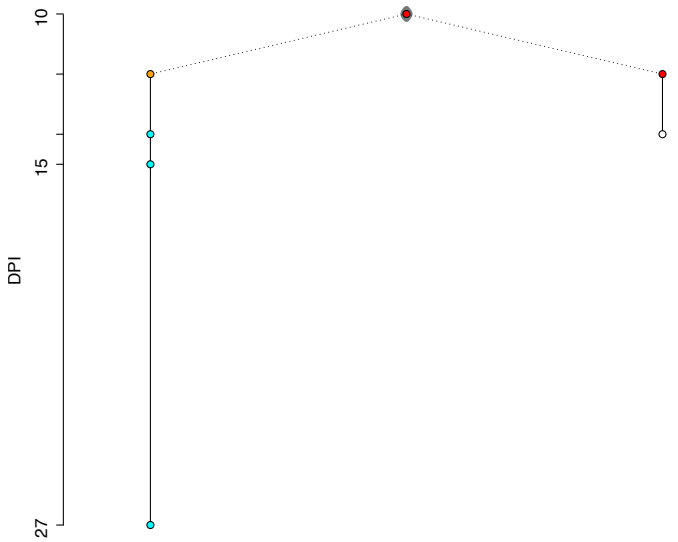


cell types
- OPC
- PM
- M
- cell death

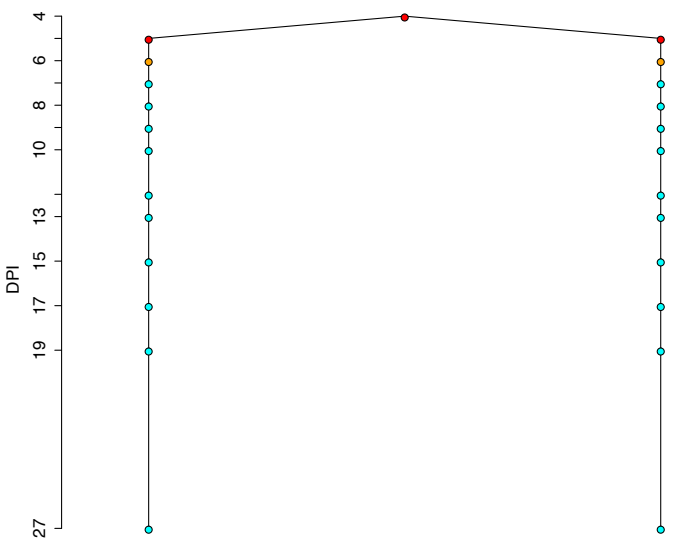
uncertainty cell type
- uncertain

uncertainty lineage
- certain
- semi - certain
- uncertain

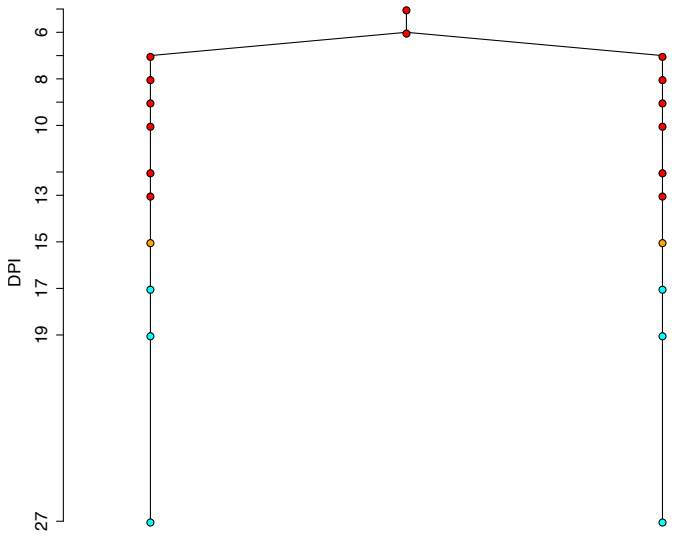
133. **bf3558.SPOT14.clone1**



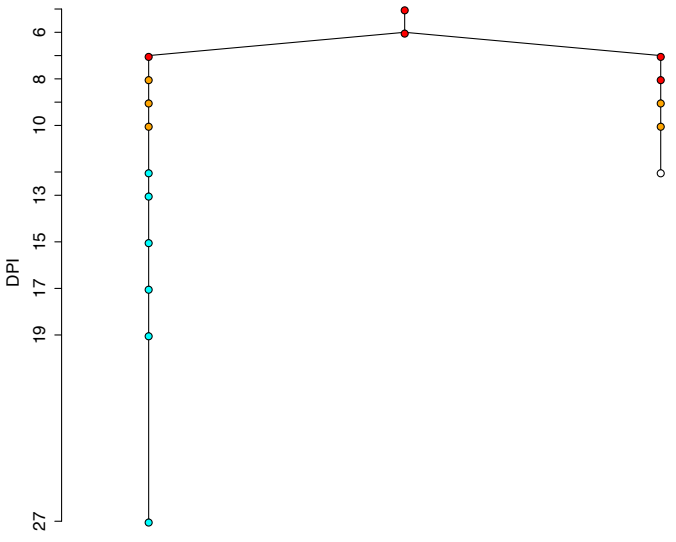
134. **bf3558.SPOT16.clone1**



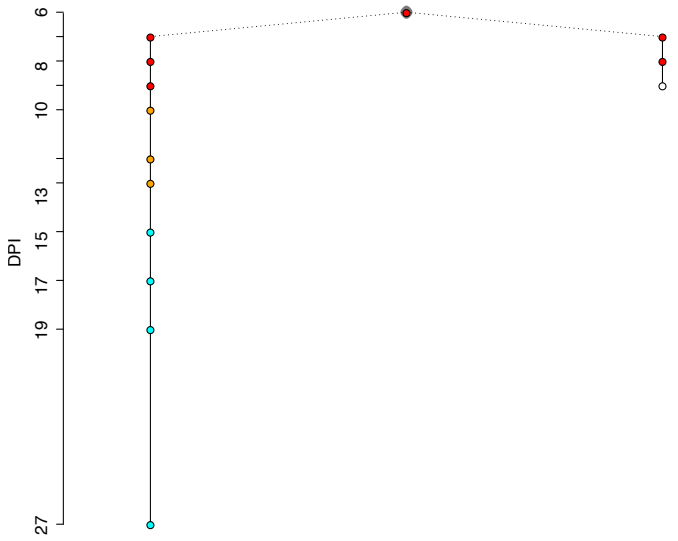
135. **bf3558.SPOT16.clone2**



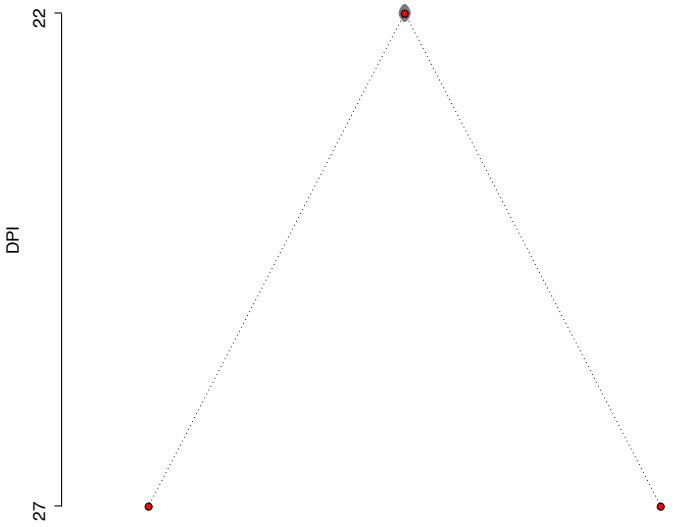
136. **bf3558.SPOT16.clone3**



137. **bf3558.SPOT16.clone4**



138. **bf3558.SPOT16.clone5**

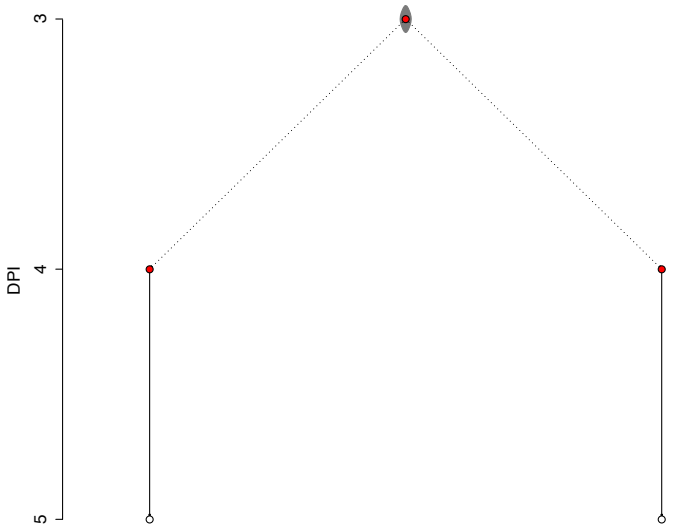


cell types
- OPC
- PM
- M
- cell death

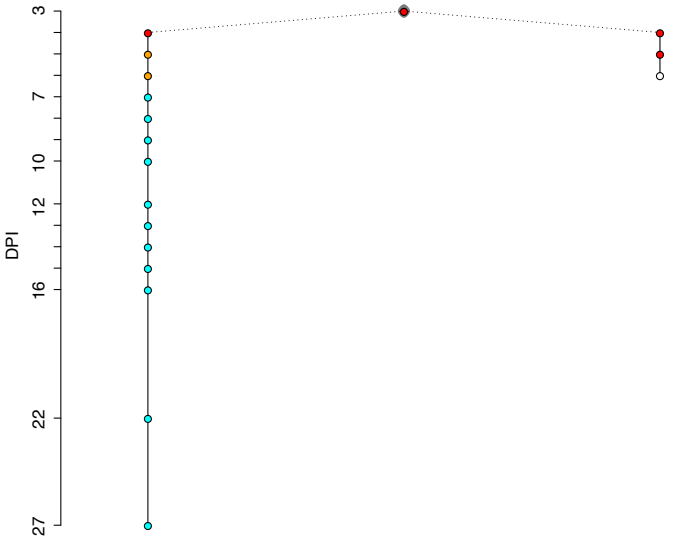
uncertainty cell type
- uncertain

uncertainty lineage
- certain
- semi - certain
- uncertain

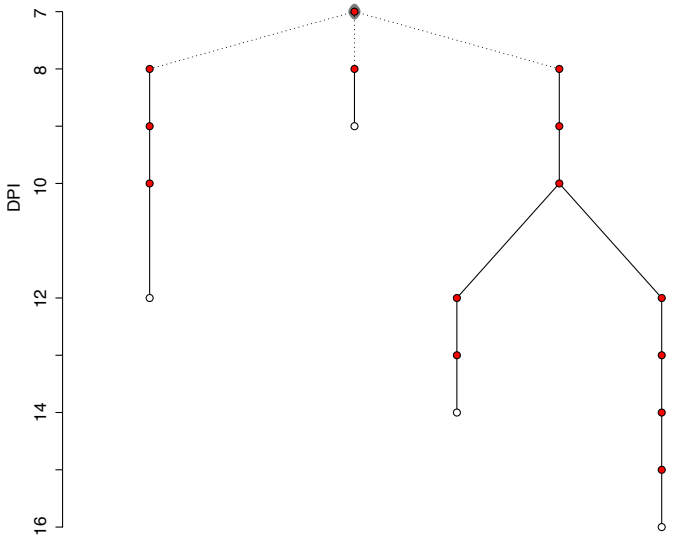
139. **bf3558.SPOT17.clone1**



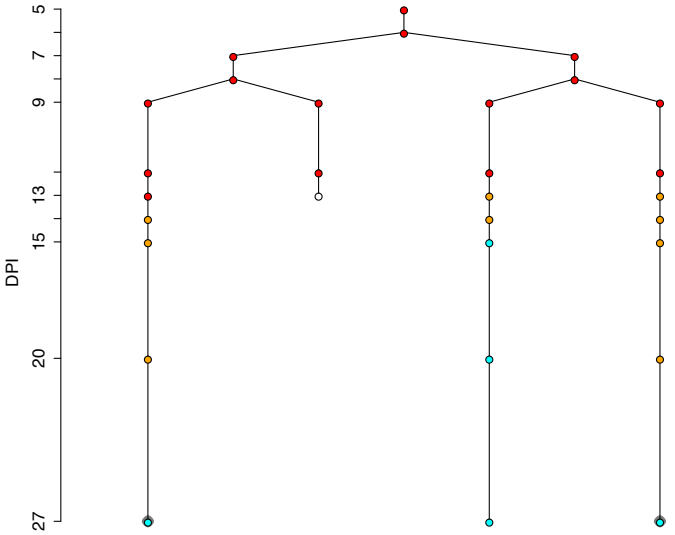
140. **bf3558.SPOT18.clone1**



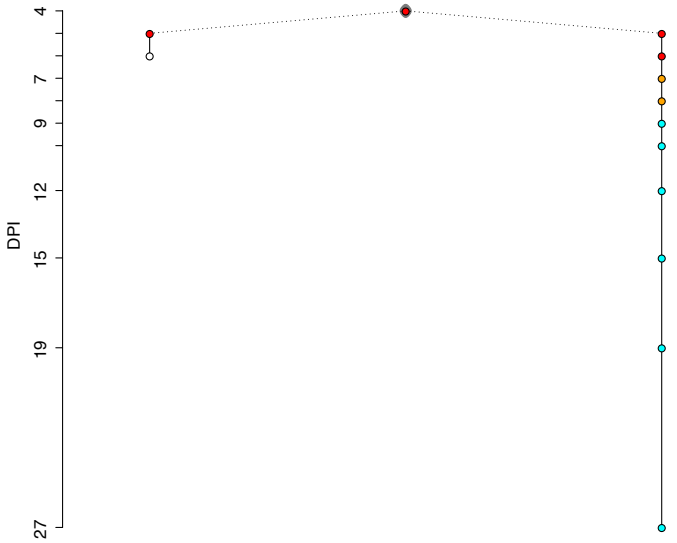
141. **bf3558.SPOT18.clone2**



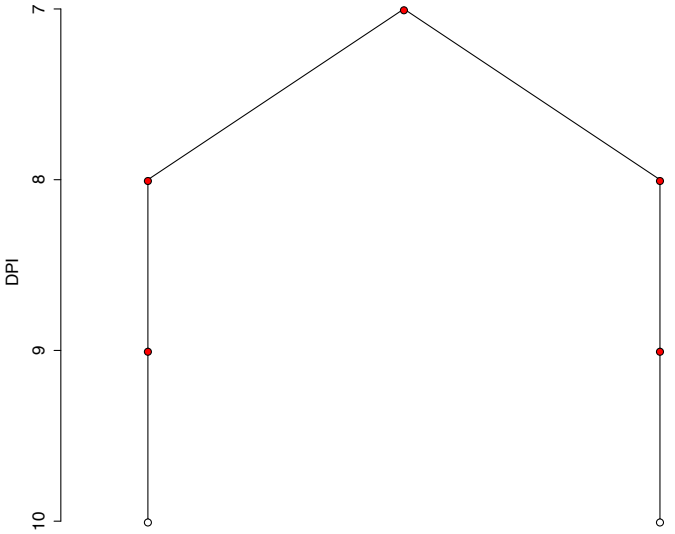
142. **bf3558.SPOT2.clone1**



143. **bf3558.SPOT20.clone1**



144. **bf3558.SPOT20.clone2**

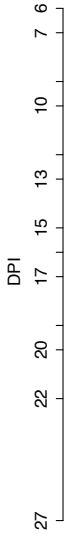


cell types
- OPC
- PM
- M
- cell death

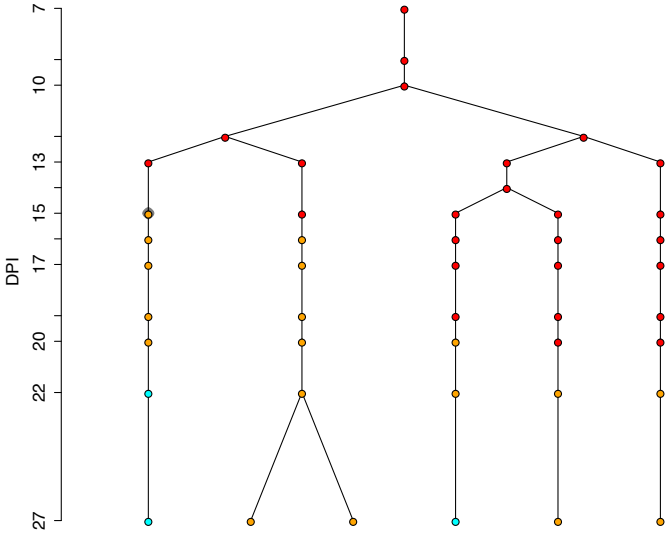
uncertainty cell type
- uncertain

uncertainty lineage
- certain
- semi - certain
- uncertain

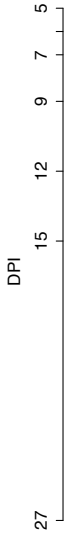
145. **bf3558.SPOT211.clone1**



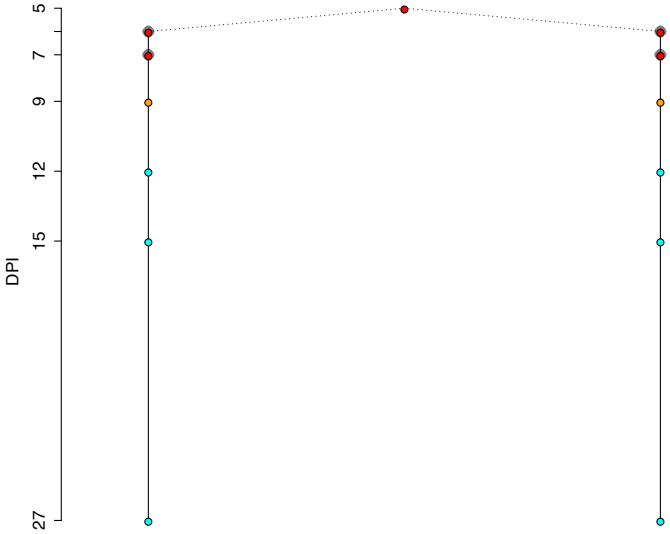
146. **bf3558.SPOT211.clone2**



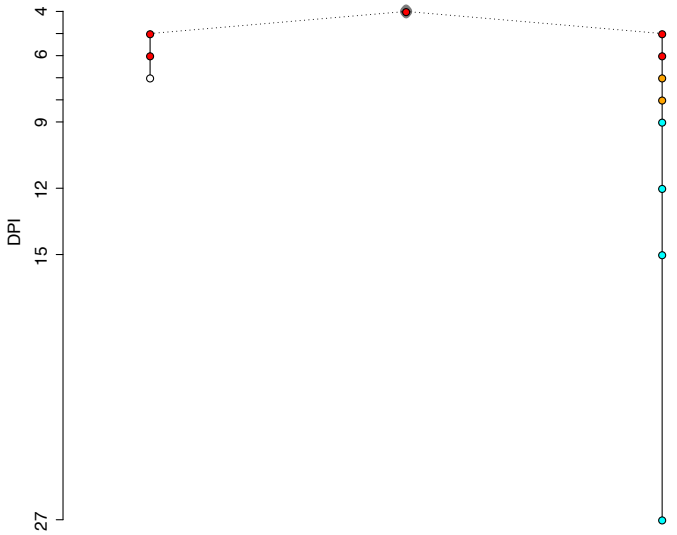
147. **bf3558.SPOT22.clone1**



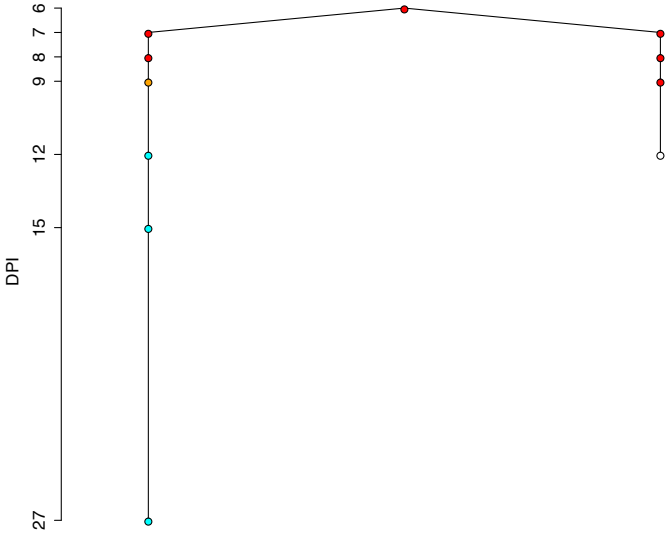
148. **bf3558.SPOT22.clone2**



149. **bf3558.SPOT23.clone1**



150. **bf3558.SPOT23.clone2**

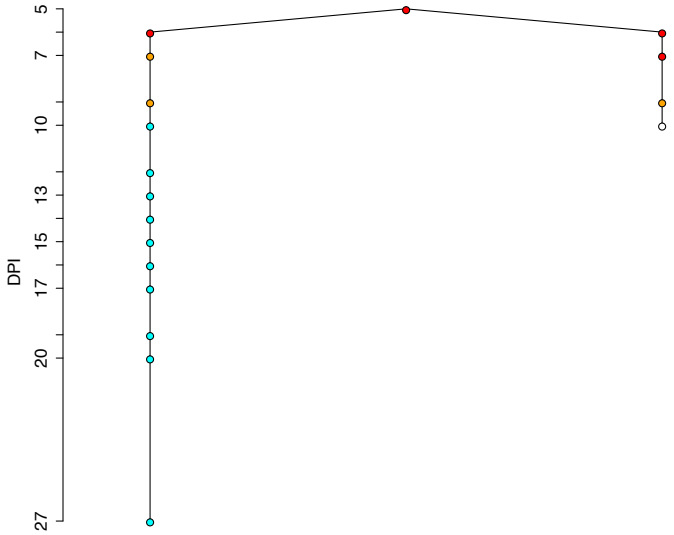


cell types
- OPC
- PM
- M
- cell death

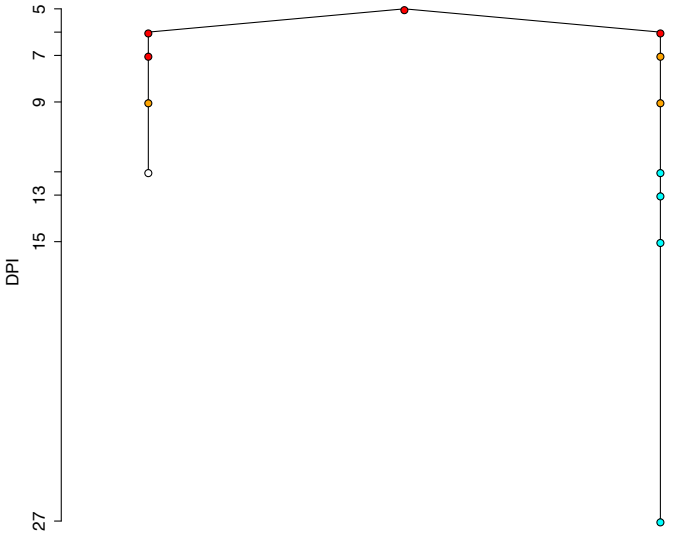
uncertainty cell type
- uncertain

uncertainty lineage
- certain
- semi - certain
- uncertain

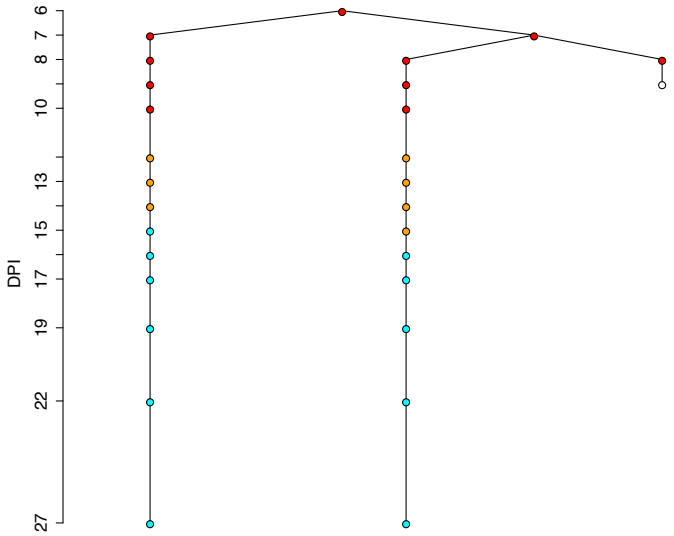
151. **bf3558.SPOT24.clone1**



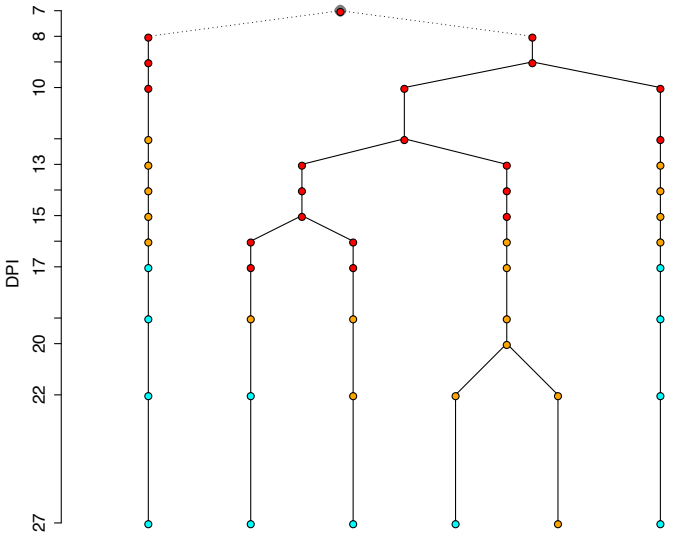
152. **bf3558.SPOT25.clone1**



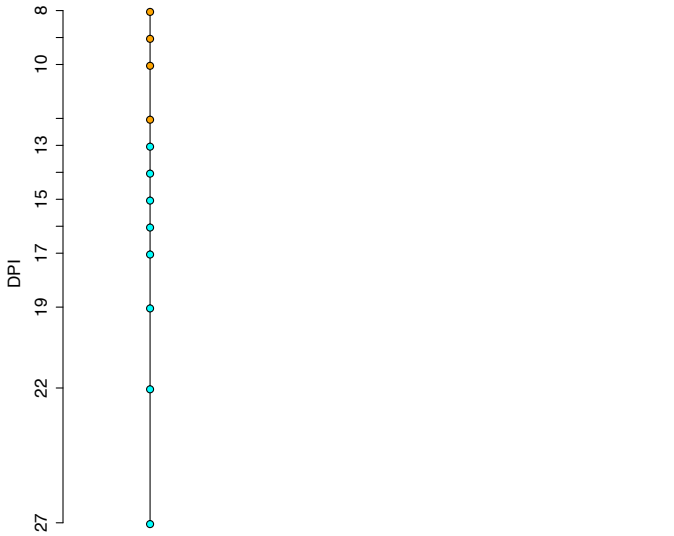
153. **bf3558.SPOT26.clone1**



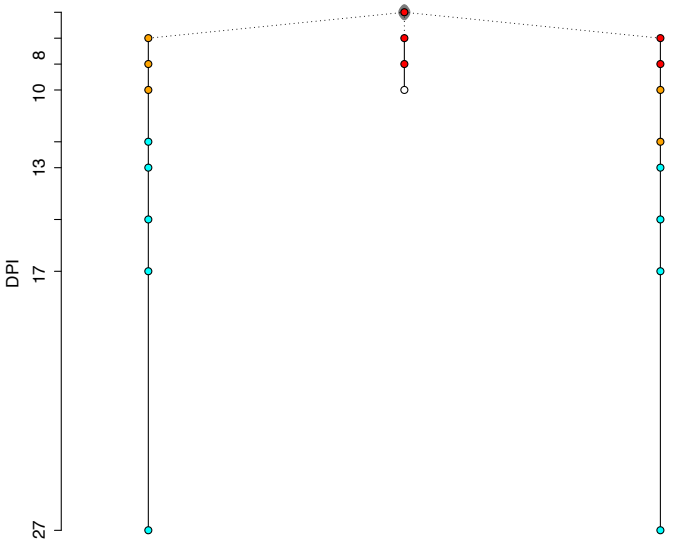
154. **bf3558.SPOT26.clone2**



155. **bf3558.SPOT26.clone3**



156. **bf3558.SPOT28.clone1**

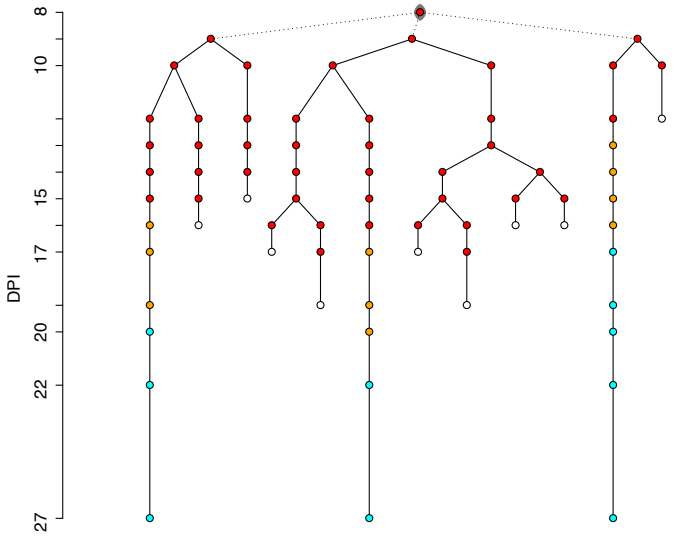


cell types
- OPC
- PM
- M
- cell death

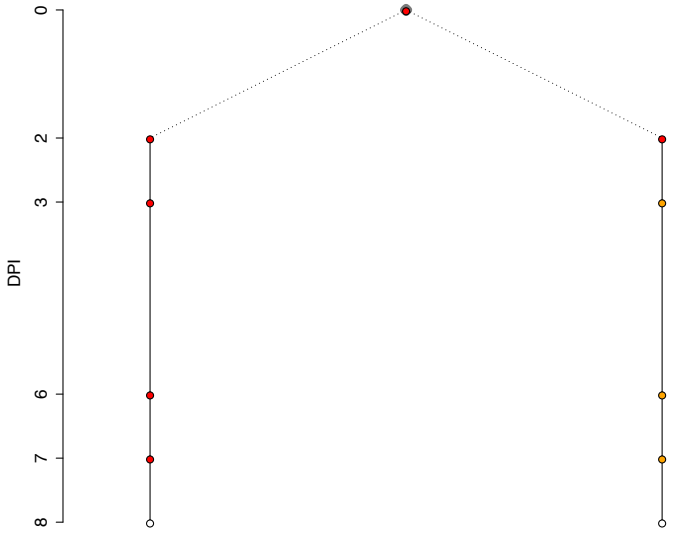
uncertainty cell type
- uncertain

uncertainty lineage
- certain
- semi - certain
- uncertain

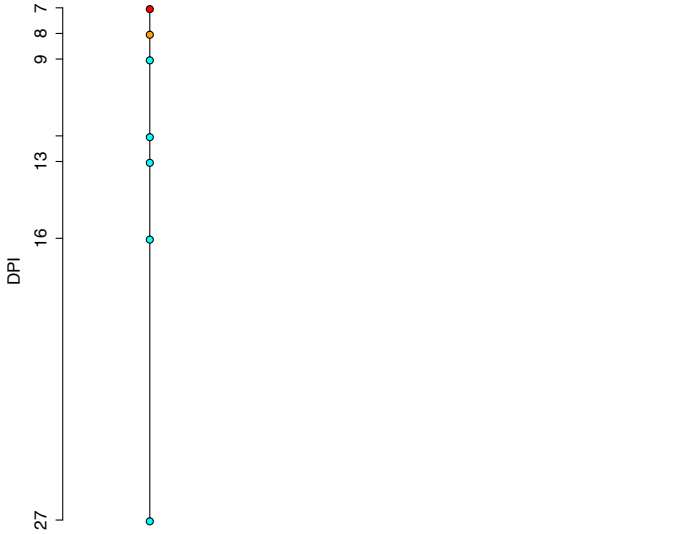
157. **bf3558.SPOT29.clone1**



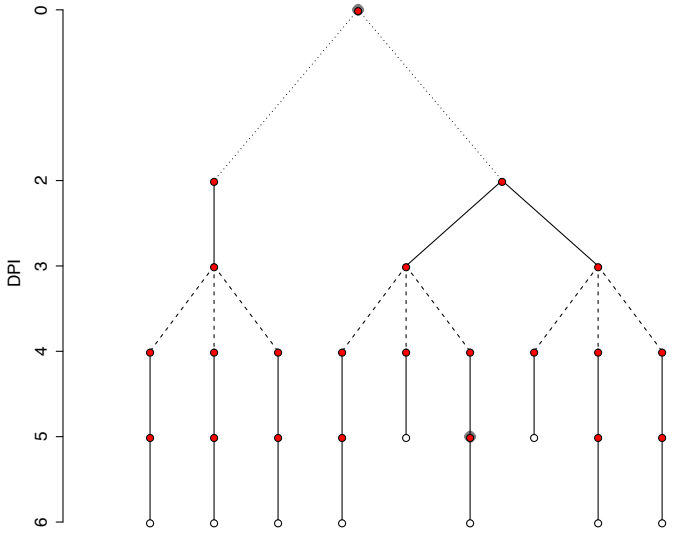
158. **bf3558.SPOT3.clone1**



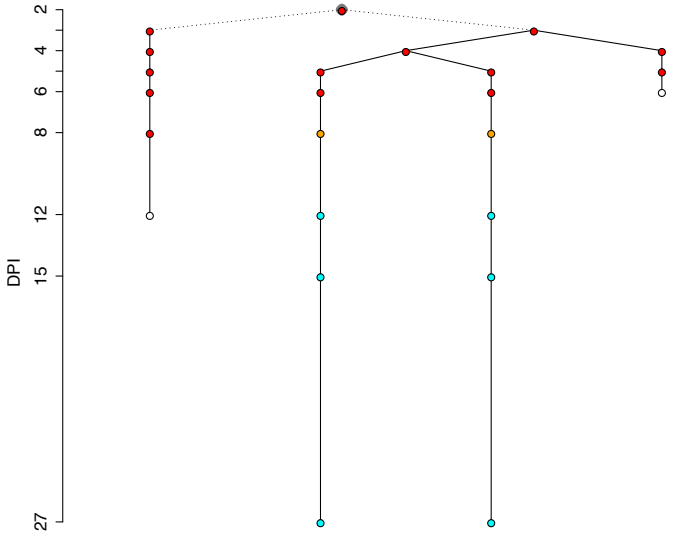
159. **bf3558.SPOT3.clone2**



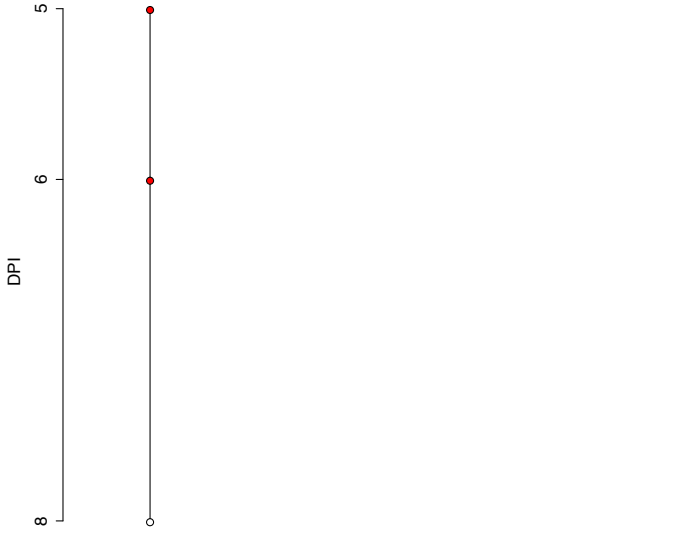
160. **bf3558.SPOT4.clone1**



161. **bf3558.SPOT5.clone1**



162. **bf3558.SPOT5.clone2**

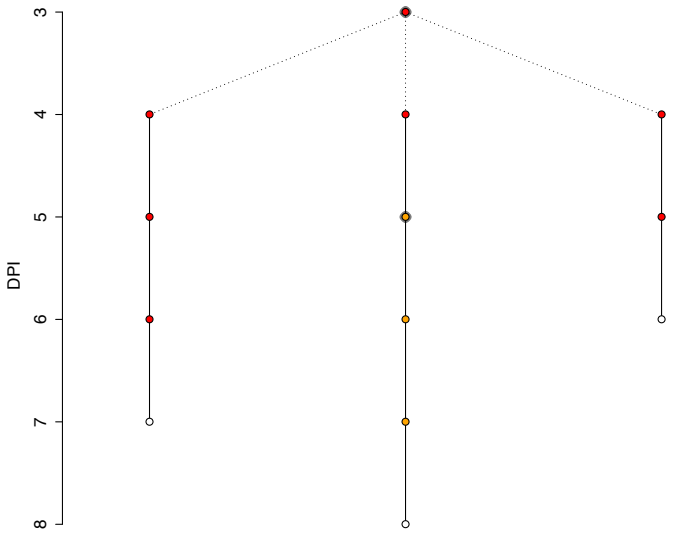


cell types
- OPC
- PM
- M
- cell death

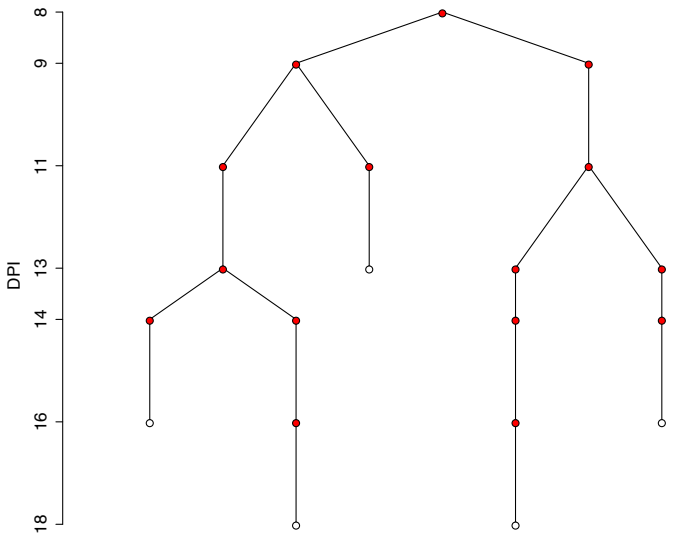
uncertainty cell type
- uncertain

uncertainty lineage
- certain
- semi - certain
- uncertain

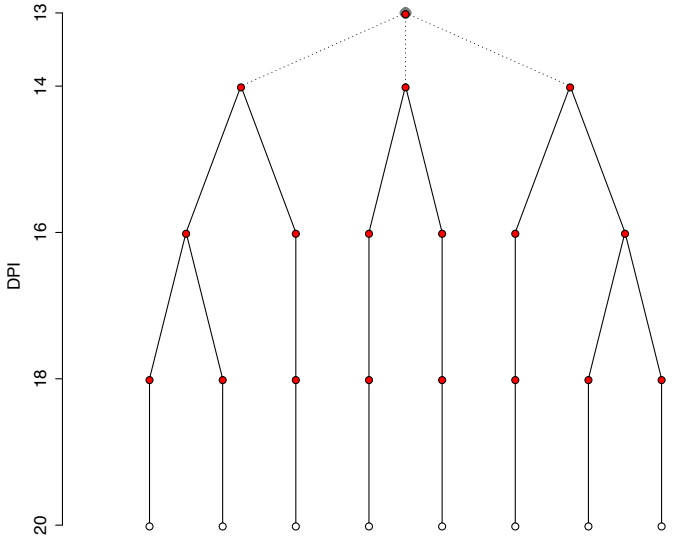
163. **bf3558.SPOT8.clone1**



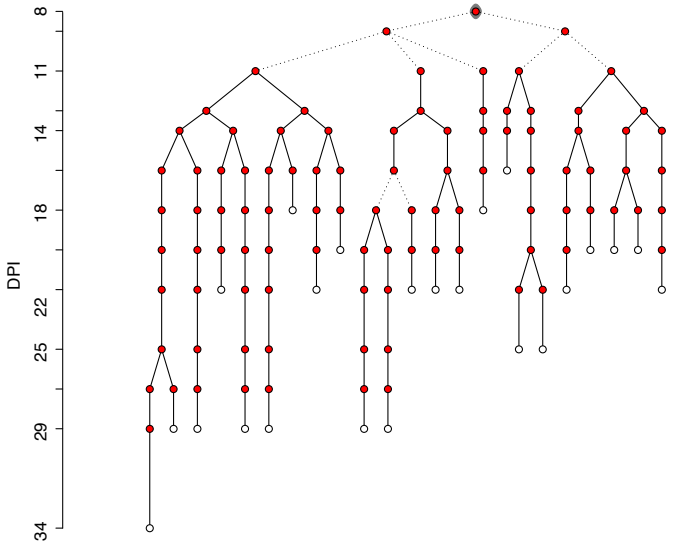
164. **bf4961.SPOT11.clone1**



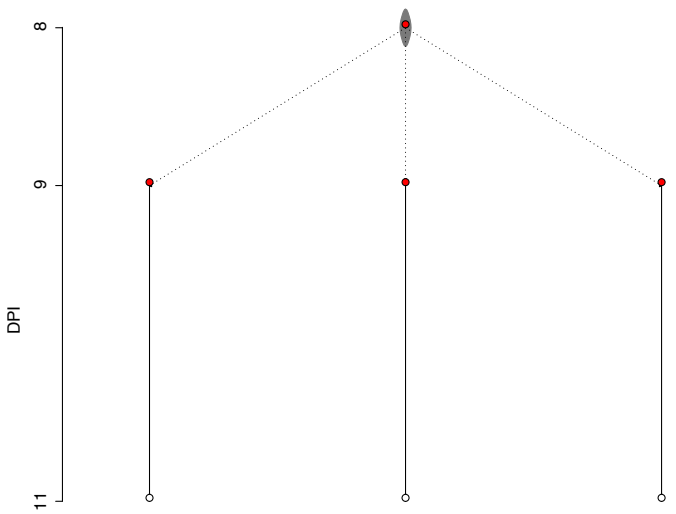
165. **bf4961.SPOT11.clone2**



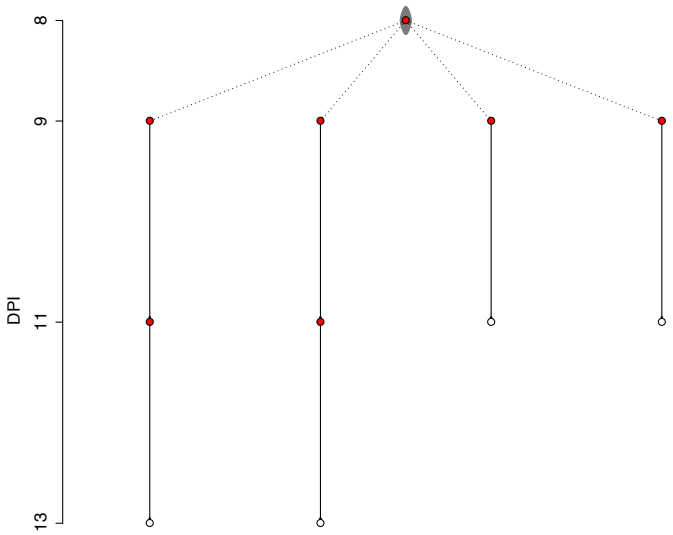
166. **bf4961.SPOT13.clone1**



167. **bf4961.SPOT14.clone1**



168. **bf4961.SPOT14.clone2**

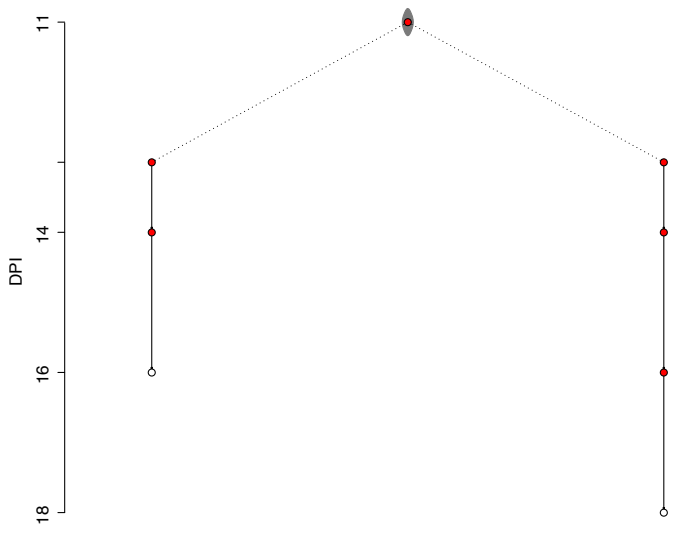


cell types
- OPC
- PM
- M
- cell death

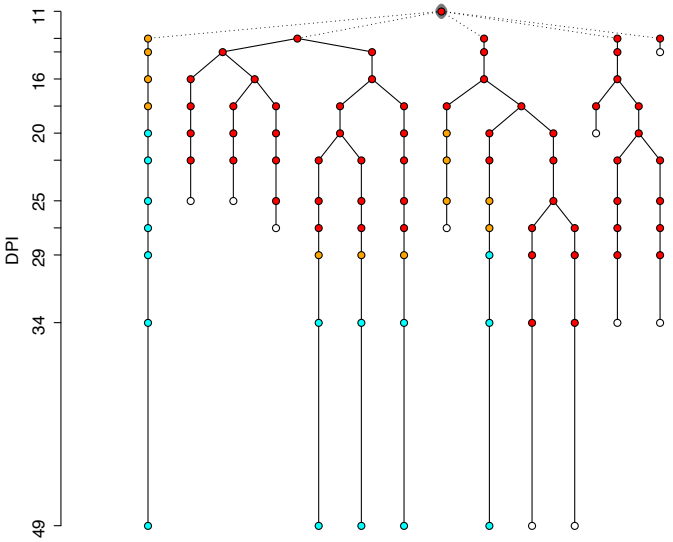
uncertainty cell type
- uncertain

uncertainty lineage
- certain
- semi - certain
- uncertain

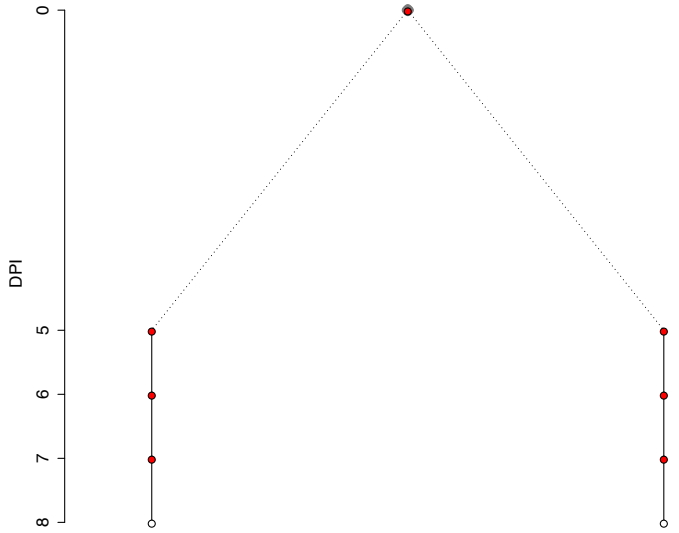
169. **bf4961.SPOT16.clone1**



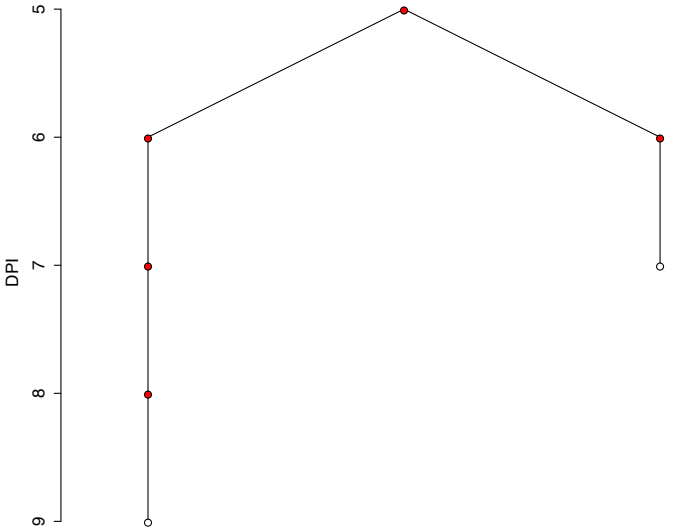
170. **bf4961.SPOT16.clone2**



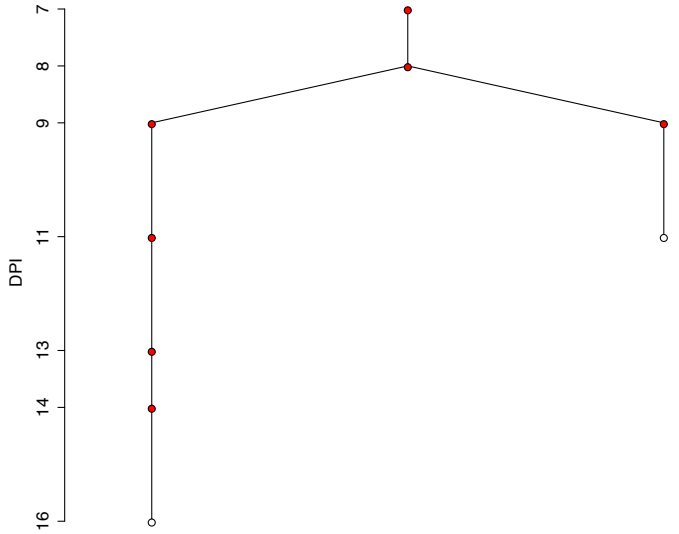
171. **bf4961.SPOT3.clone1**



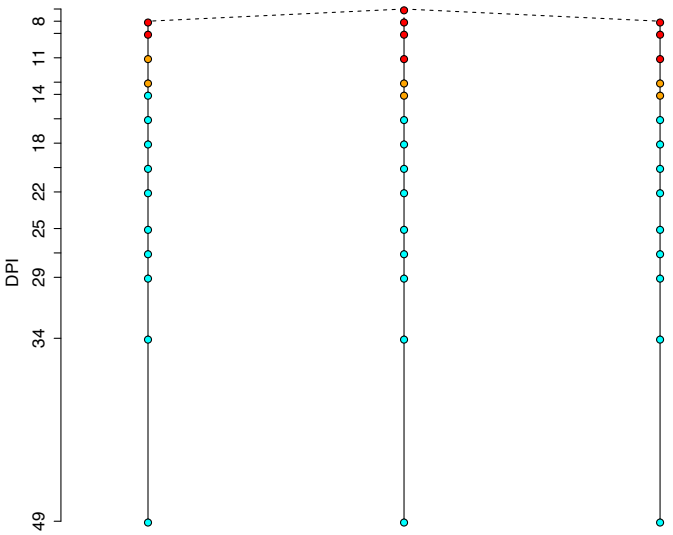
172. **bf4961.SPOT4.clone1**



173. **bf4961.SPOT4.clone2**



174. **bf4961.SPOT4.clone3**

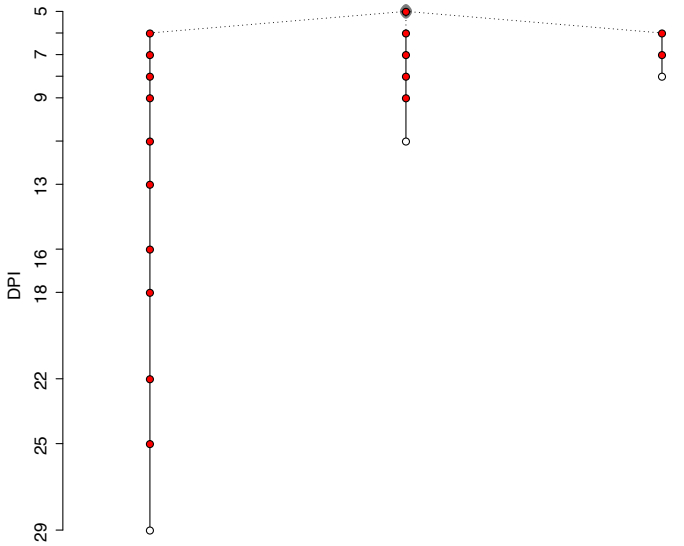


cell types
- OPC
- PM
- M
- cell death

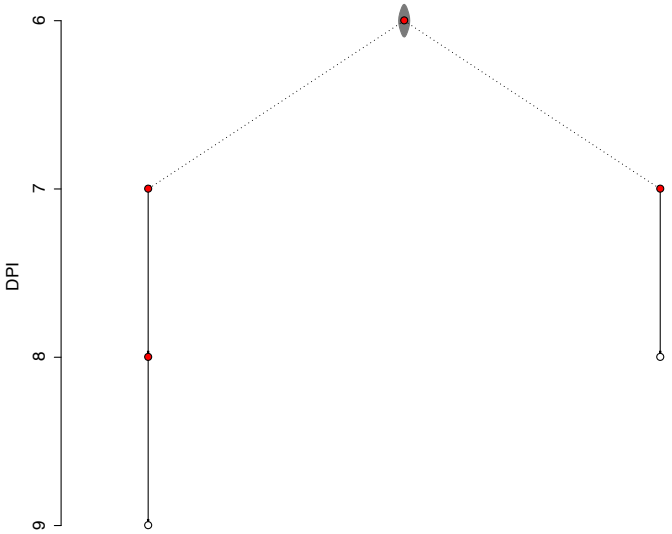
uncertainty cell type
- uncertain

uncertainty lineage
- certain
- semi - certain
- uncertain

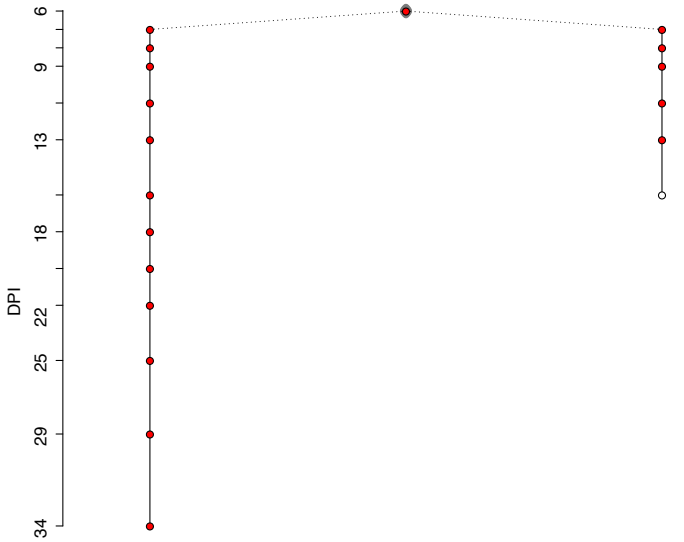
175. **bf4961.SPOT6.clone1**



176. **bf4961.SPOT7.clone1**



177. **bf4961.SPOT7.clone2**



cell types
- OPC
- PM
- M
- cell death

uncertainty cell type
- uncertain

uncertainty lineage
- certain
- semi - certain
- uncertain

Figure S3. Lineage trees deduced from longitudinal *in vivo* imaging of OPCs clones. Lineage trees obtained from tracking OPC clones (n = 177 from 9 experimental mice. Mouse bf3092: 26 clones; mouse bf3093: 19 clones; mouse bf3343: 10 clones; mouse bf3345: 29 clones; mouse bf3347: 19 clones; mouse bf3541: 14 clones; mouse bf3546: 9 clones; mouse bf3558: 37 clones; mouse bf4961: 14 clones). Different colors indicate different cell types. Cell type and lineage uncertainties are annotated. The scale is reported in days after first Tamoxifen injection (DPI).

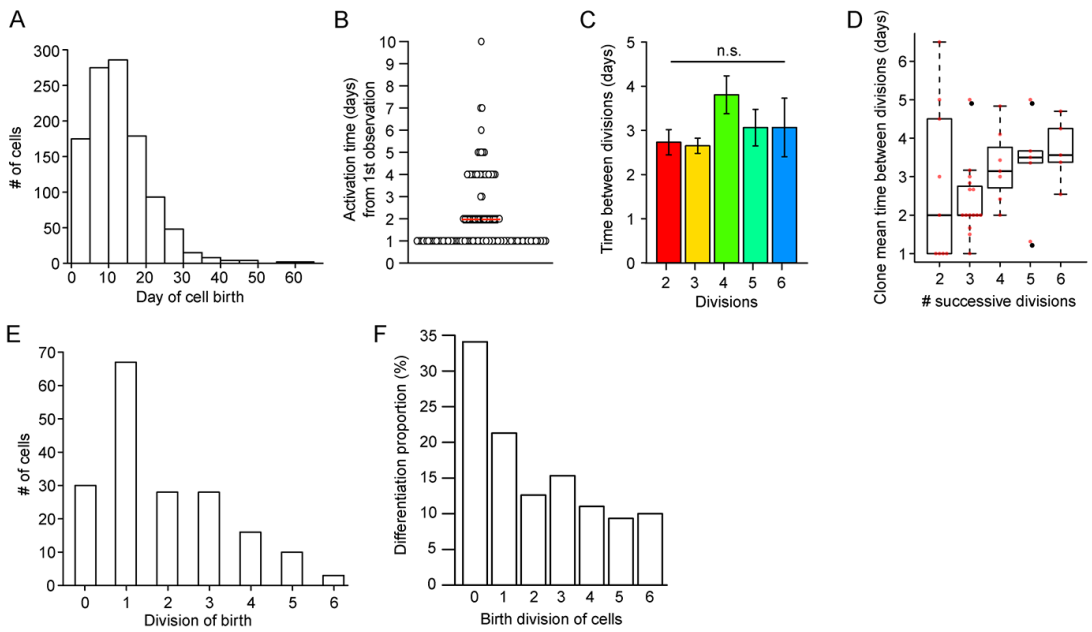


Figure S4. Dynamics of OPC activation and differentiation in the corpus callosum. A, Measure of proliferation expressed by number of cells born per day (Mean = 13.4; n = 1091 cells). **B,** Time until the first division (in days) of the first OPC of the clone calculated from the first observation of the cell. Each circle represents a clone (Mean ± sem = 1.96 ± 0.13; n = 131 cells) **C,** Time between division (days) of OPCs for different divisions (Div2: mean ± sem = 2.73 ± 0.28; n = 28 div. Div3: mean ± sem = 2.65 ± 0.17; n = 66 div. Div4: mean ± sem = 3.8 ± 0.42; n = 54 div. Div5: mean ± sem = 3.06 ± 0.41; n = 32 div. Div6: mean ± sem = 3.06 ± 0.66; n = 15 div. One-way ANOVA: F = 2.134; p-value = 0.07). **D,** Mean division time (days) per clone. Each red dot represents a clone. Clones are binned according to their maximum number of consecutive divisions. Values are shown as median and quartiles (Div2 clones: mean ± sem = 2.77 ± 0.69; n = 9 clones. Div3 clones: mean ± sem = 2.36 ± 0.24; n = 15 clones. Div4 clones: mean ± sem = 3.27 ± 0.36; n = 7 clones. Div5 clones: mean ± sem = 3.36 ± 0.59; n = 5 clones. Div6 clones: mean ± sem = 3.68 ± 0.37; n = 5 clones). **E,** Birth division of differentiating cells. Cells depicted in “Division 0” correspond to the first single cells of the tree found during imaging sessions (Mean = 2.9; n = 182 cells). **F,** Differentiation proportion of cells according to their birth division in the clone. It is calculated as the percentage of differentiating cells born at the considered division over the total amount of cells born at that division. Cells depicted in

“Division 0” correspond to the first single cells of the tree found during imaging sessions. Clones with OPCs at last imaging time point that were born less than 10 days before are excluded (n = 993 cells). Values are shown as mean ± s.e.m.

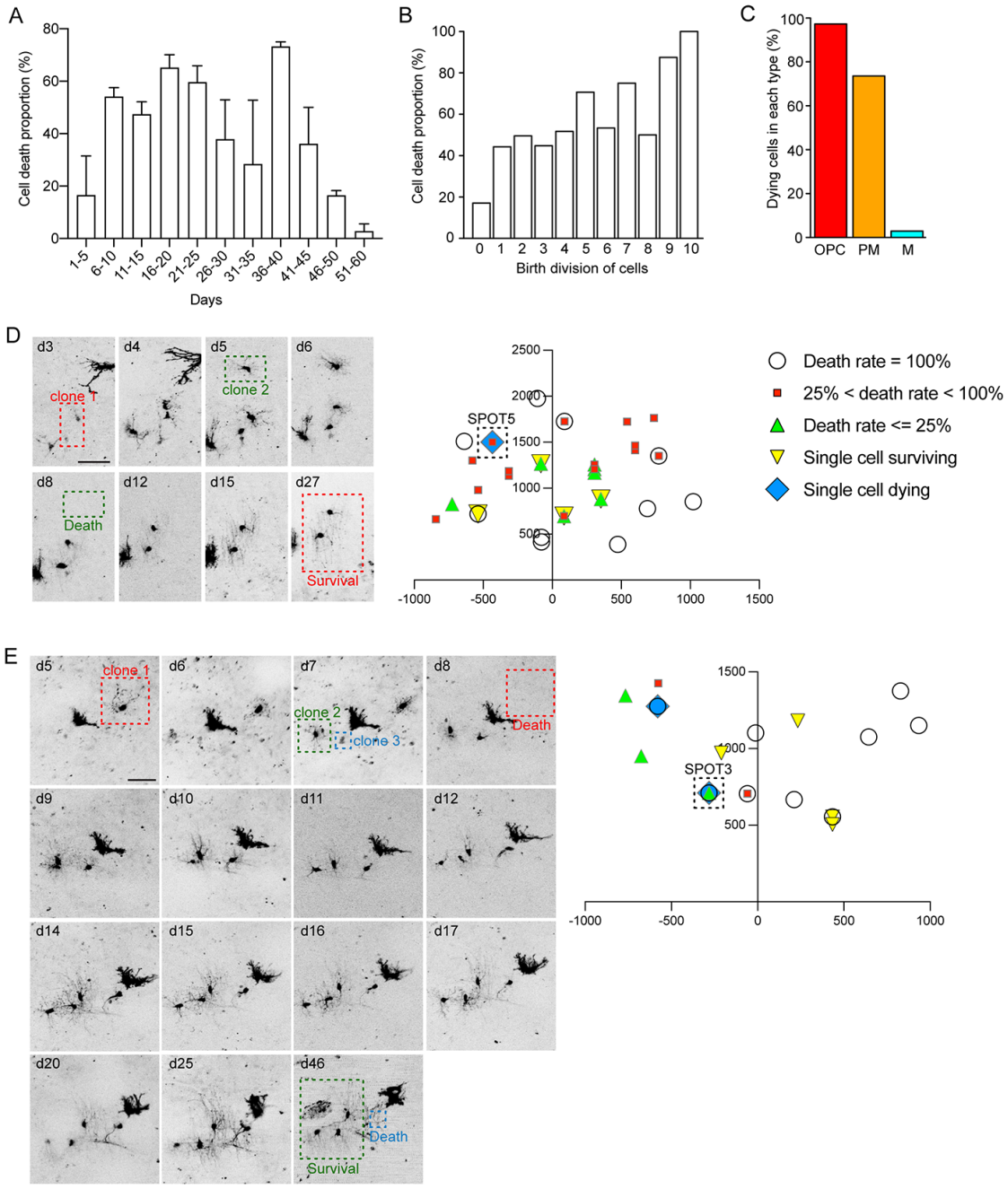


Figure S5. Cell death in OPC clones. A, Cell death proportion binning cells according to their birthday (dpi). It is calculated as the percentage of dying cell born at the considered bin over the total amount of cell born at that bin. **B,** Cell death proportion according to their birth division in the clone. It is calculated as the percentage of dying cells born at the considered division over the total amount of cells born at that division. Cells depicted in "Division 0" correspond to the first single cells of the tree found during imaging sessions. Clones with OPCs at last imaging time point that were born less than 10 days before are excluded (n = 1033 cells). **C,** Percentage of dying cells in each cell

type group calculated considering only cells after last division. Clones with OPCs at last imaging time point that were born less than 10 days before are excluded (n = 678 cells). **D and E,** Representative examples of the position of *in vivo* imaged clones below the transcortical window with example pictures (collapsed z-stacks) of clones in the same SPOT (SPOT5 in D and SPOT3 in E) which show different cellular outcomes. Scale bars represent 50 µm (D-E).

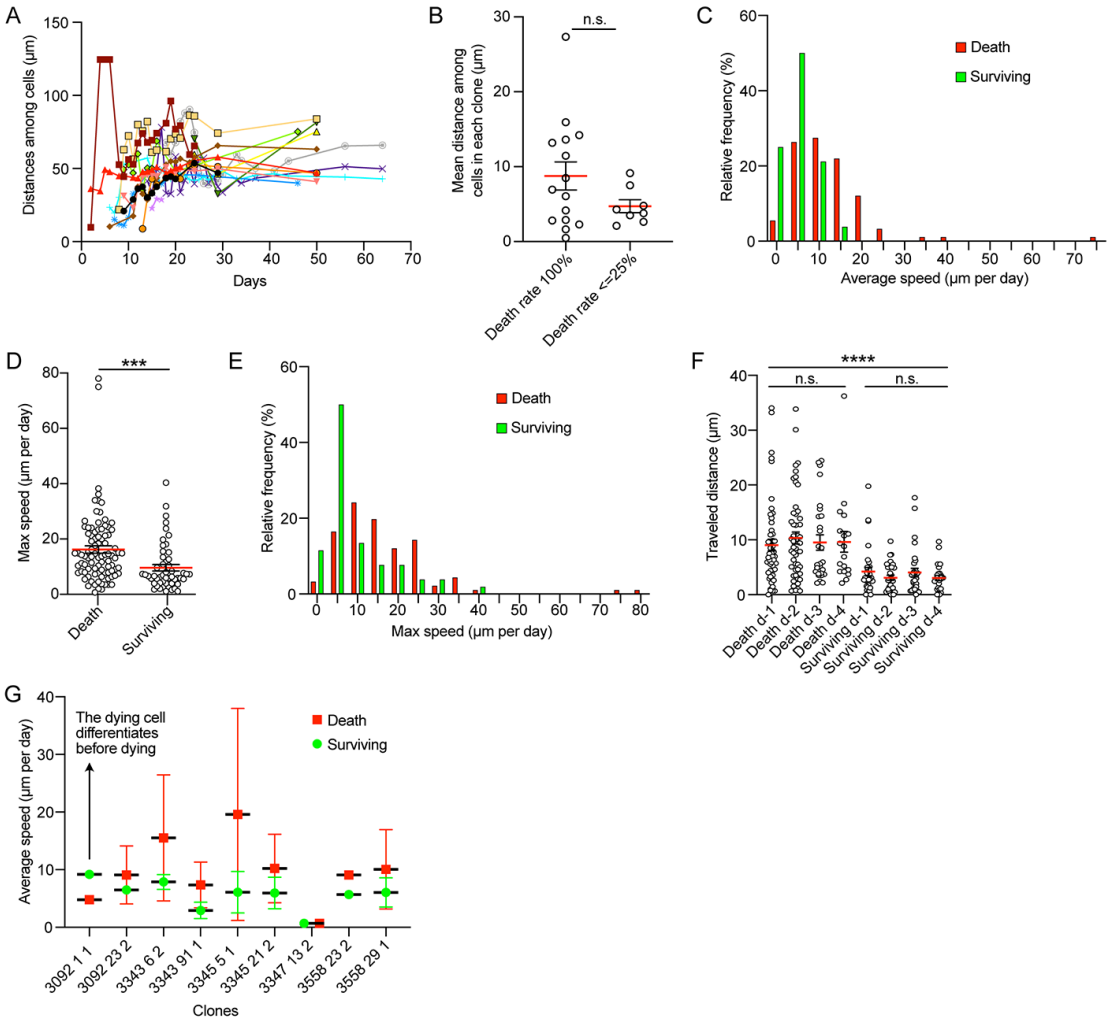


Figure S6. Migration of OPCs and their progeny in the corpus callosum. **A,** Median distance (µm) among cells in the clones over time (days). Each line depicts a single clone where at least 2 cells terminally differentiate to remyelinating oligodendrocytes (n = 15 clones from 3 mice). **B,** Mean distance change (µm) among cells in clones showing 100% or less than 25% death rate. Clones are tracked until at least one OPC is present in the clone. Each circle represents a single clone (100% death rate: mean ± sem = 8.76 ± 1.87; n = 15 clones. <25% death rate: mean ± sem = 4.72 ± 0.85; n = 8 clones. Unpaired t test, two tailed: t = 1.51; df = 21; p-value = 0.14). **C,** Relative frequencies of different average speeds (µm per day) in dying and surviving OPCs. The speed of the cells is calculated tracking the actual migration of single cells over time (minimum of 3 days tracking per cell. Death n = 91 cells; Surviving n = 58 cells). **D,** Maximum speed (µm per day) of dying and surviving OPCs calculated tracking the actual migration of single cells over

time (minimum of 3 days tracking per cell). Each circle represents a single cell (Death: mean ± sem = 16.23 ± 1.32; n = 91 cells. Surviving: mean ± sem = 9.66 ± 1.06; n = 58 cells. Unpaired t test, two tailed: t = 3.52; df = 147; p-value < 0.0006***). **E,** Relative frequencies of different maximum speeds (μm per day) in dying and surviving OPCs (Death n = 91 cells; Surviving n = 58 cells). **F,** Traveled distance (μm) at each day before death or differentiation in dying and surviving cells. Each circle represents a single cell (Death d-1: mean ± sem = 9.04 ± 1.07; n = 52 cells. Death d-2: mean ± sem = 10.34 ± 1.08; n = 52 cells. Death d-3: mean ± sem = 9.51 ± 1.41; n = 27 cells. Death d-4: mean ± sem = 9.63 ± 1.88; n = 18 cells. Surviving d-1: mean ± sem = 4.22 ± 0.76; n = 32 cells. Surviving d-2: mean ± sem = 3.09 ± 0.44; n = 32 cells. Surviving d-3: mean ± sem = 4.04 ± 0.78; n = 30 cells. Surviving d-4: mean ± sem = 3.04 ± 0.46; n = 26 cells. Kruskal-Wallis test: p-value < 0.0001****. Dying cells: Kruskal-Wallis test: p-value = 0.86. Surviving cells: Kruskal-Wallis test: p-value = 0.79. Dying d-1 vs surviving d-1: Mann-Whitney test, two tailed: p-value = 0.0001***. Dying d-2 vs surviving d-2: Mann-Whitney test, two tailed: p-value < 0.0001****. Dying d-3 vs surviving d-3: Mann-Whitney test, two tailed: p-value = 0.0003***. Dying d-4 vs surviving d-4: Mann-Whitney test, two tailed: p-value < 0.0001****). **G,** Comparison of the average speed (μm per day) of dying and surviving cells belonging to the same clone. Mean and sd are shown. Only clones with mixed fate outcome. The tracking of the cells ends when they differentiate. Values are shown as mean ± s.e.m.

Supplementary Materials & Methods

Transgenic animals and labeling of OPCs

Ascl1CreER^{T2} mice (Ascl1Cre ER^{T2}; Ascl1$^{tm1.1(Cre/ERT2)Jejo}$; JacksonLab strain 012882) were bred with the CAG tdTomato line (Ai14; B6.Cg-Gt(ROSA)$^{26Sortm14(CAG-tdTomato)Hze}$; JacksonLab strain 007914) to obtain Ascl1CreER^{T2}/tdTom mice for imaging. At the age of 9-12 weeks mice were injected with lysolecithin in the corpus callosum and the transcortical window was implanted. Testing different timings and doses of Tamoxifen administration before and after surgery, we identified an immediate strong reaction of Ascl1-targeted astrocytes to demyelination followed by Ascl1-targeted OPCs response. The paradigm which allowed to better combine the labeling of the first reacting OPCs with the exclusion of the majority of the astrocytes was chosen for all the experiments: two and three days after surgery, sparse labeling of OPCs was achieved by i.p. injection of Tamoxifen (Tam; 180mg/kg bodyweight; Sigma). Imaging fields of view (SPOTs) containing identified OPCs were selected to obtain 10-20 SPOTs per mouse.

In vivo 2-photon imaging in the demyelinated corpus callosum

Mice at the age of 9-12 weeks were injected with lysophospatidylcholin (LPC; Sigma) in the corpus callosum and the transcortical window was implanted (1, 2). Chronic 2-photon imaging was performed from 3 days after surgery (1 day after the first Tamoxifen injection), as described previously (2). In brief, after installation of an aluminum headpost on the contralateral side of the mouse head for repeated placement of mice in a frame, mice were anesthetized with isofluorane (1.5-2% in O2) and the body temperature was measured and kept at 37°C with a heating pad. The imaging was performed on custom-built 2-photon microscope (Sutter Instrument Movable Objective Microscope type) using a long-working distance objective (water immersion, 16x magnification, 0.8NA, Nikon) and equipped with a ytterbium-doped laser system at 1045nm and 200fs (Femtotrain, High-Q lasers) or a fiber oscillator laser at 1070nm

(Fidelity-2, Coherent) to excite tdTomato labeled cells in the CC. Emission light was detected using a photomultiplier tube (Hamamatsu) after passing a red emission filter (610/75 nm; AHF). Individual fields of view (SPOTs) containing OPCs were selected and could be revisited in subsequent sessions using a coordinate system with the windows cannula being the reference (zero position, x=0, y=0, z=0). Every SPOT was imaged repeatedly by acquisition of a z-stack (512x512 pixel resolution, 2x zoom, 5µm step-size) carefully considering and checking for all cells of the clone. All SPOTs were checked on a daily basis and no z-stack was acquired if no changes occurred. The time spent under the microscope for the mouse was kept minimal (<1h per day).

Processing imaging raw data and coding of lineages

The first step of the analysis of the images was carried out using FIJI. All timepoints and z-levels of a chronically imaged SPOTs were compiled into a single file. The ROI manager in FIJI was used to code each individual cell at every timepoint throughout the compiled imaging file. The coding parameters were: Cell ID, Cell Type, Uncertainty Cell Type, Time point, Mother ID, Uncertainty Mother Cell, Sister ID, Uncertainty Sister Cell and Cell Death. See Supplementary Table 1 for detailed code explanation and criteria used for the categorization of different cell types. The structural information from all z-levels were considered and carefully analyzed during the categorization of cell types and lineage relationships. In addition, the information taken from all z-plans was crucial to distinguish neighboring cells right after cell division. Importantly, information about the behavior and morphological changes of the cells taken from consecutive time points were essential to exclude tdTomato labeled astrocytes. Every lineage was annotated with the above-mentioned code two times by a blinded researcher. The two independent annotations are then compared and a final revised version is compiled. Only if both times the coding of cell type characterization or lineage transitions was classified identically and as certain, this was finally registered as such. Otherwise uncertain annotation was used. Finally, the assembling of lineage trees was performed using R software using a custom-made script and igraph package

was used for trees visualization (3, 4). The resulting lineage trees were again double checked for inconsistencies with the imaging raw data. Raw data was deposited to the Image Data Resource (https://idr.openmicroscopy.org/search/?query=Name:113) under accession number idr0113.

Analysis of the lineage trees

Part of the data analysis was performed in R software using a custom-made script (3, 4). The dataset consists of 177 lineages. In total 9 animals have been used. 3 animals were imaged for 1 month, 4 animals were imaged between 41 and 50 days and 2 animals were imaged for 2 months. Clones imaged and tracked per mouse: mouse bf3092: 26 clones; mouse bf3093: 19 clones; mouse bf3343: 10 clones; mouse bf3345: 29 clones; mouse bf3347: 19 clones; mouse bf3541: 14 clones; mouse bf3546: 9 clones; mouse bf3558: 37 clones; mouse bf4961: 14 clones. Multiple parameters (listed below) were used to describe the behavior of the imaged clones and cells. The value of the parameters was calculated using the R script and then double-checked and eventually corrected manually after inspection of the lineage trees.

Parameters:

- Number of successive divisions: it represents the maximum number of successive divisions in the clone. Note that, due to lineage transition uncertainties, this value could be slightly underestimate in some cases.
- Activity duration of the clone: it represents the time (in days) from the first division until the last division of any cell in the clone. If the clone does only 1 division the activity duration is 0.
- Maximum cell number: it represents the maximum cell number reached in the clone at one point (the time point can be any).
- Size of clonal output: it represents the number of cells present in the clone at the last time point of the imaging.
- Activation time: it represents the time (in days) from the first observation of the cell under the window to the first time the daughter cells are seen. If

the clone is observed with 2 or more cells at its first observation the value 1 is assigned.

- Time between OPC divisions: it is measured only for cells for which transitions from mother to daughters are certain. The first division of the clone (activation time) is excluded. Each division happens during a time window: the last time point the mother is seen and the first time point the daughters are seen. If this timing uncertainty is greater than 6 days, the value of the time between divisions is discarded. To measure the time between divisions, a minimum time is calculated as the time (in days) between the day the cell is seen for the first time and the day the cell is seen last time before division; a maximum time is calculated as the time (in days) between the day the mother of the cell is seen for the last time and the day the daughter cells are seen the first time. Finally, the mean between these 2 values is taken as the time between divisions.
- Time between divisions in successive divisions: it is measured only for cells for which transitions from mother to daughters are certain. It represents the time (in days) between OPC divisions for different consecutive divisions. The first division of the clone (activation time) is excluded.
- Clone mean time between divisions: it is measured only for cells for which transitions from mother to daughters are certain. It represents the average of all the times between OPC divisions in each clone.
- Differentiation time: it represents the time (in days) between the day the cell is seen for the first time and the first day it shows a mature remyelinating oligodendrocyte morphology.
- Differentiation proportion: it represents the percentage of differentiating cells calculated on the total amount of cells produced by the clone. Clones with OPCs at last imaging time point that were born less than 10 days before are excluded.
- Survival time: it represents the time (in days) between the day the cell is seen for the first time and the day the cell is seen for the last time before dying. Only dying cells are considered.

- Cell death proportion: it represents the percentage of dying cells calculated on the total amount of cells produced. Clones with OPCs at last imaging time point that were born less than 10 days before are excluded.
- Cell death rate of the clone: it represents the percentage of dying cells in each clone calculated considering the number of cells after last cell division (dividing cells excluded).

For the analysis of the modes of cell divisions (Figure 2I-J) only certain mothers and daughter cells and certain transitions were considered. Lattice, pheatmap and ggplot2 packages of R and Graph pad Prism (version 8) were used for data visualization (5-7).

Distances among cells in the clones and migration analysis

The quantification of the distances between couple of cells in the clone were computed using a custom-made macro in Fiji. The value corresponding to the distance among cells in the clone was computed measuring the distance (µm) between all possible couple of cells per time point and calculating the median of these distances to get one value per clone at each imaged time point. This value gives an idea of how close to each other cells are in the clone. The mean distance change among cell cells in each clone was computed as the sum of the absolute values of the differences of the distance among cells at consecutive time points, normalized per days of imaging. Since we observed that cells stop moving once they differentiate to PM and mature remyelinating oligodendrocytes, each clone was tracked only until last cell at OPC stage was present in the clone. The maximum distance between sister cells represents the biggest distance that two sisters reach during the imaging period. If both sisters differentiated in mature oligodendrocytes, cells were tracked only until they were in the undifferentiated OPC stage.

To measure the actual migration of single cells, the pictures of the same SPOT taken at different time points were manually aligned using a reference point inside the image (stable astrocyte, blood vessel). The manual tracking pluggin in Fiji was used (Figure 4E and Movie S3). Cells were considered only until they were in OPC stage. The average speed (per day) of a cell is the

mean of the distances (μm) that the cell travels in different single days. The maximum speed (per day) of a cell is the longest traveled distance (μm) by the cell in one day. For these analysis surviving cells were followed only for the first 4 days after birth (corresponding to the average time that dying progenitors were tracked), with the intent to make them more comparable to the dying cells, not only from the morphological point of view but also considering the timing. Quantification graphs were visualized using Graph pad Prism (version 8).

Immunohistochemical stainings and confocal microscopy

Animals were put to deep anesthesia (Burprenophine) and then perfused with first cold saline and then paraformaldehyde (4% PFA in phosphate buffer). Brains were post-fixed overnight and cryo-protected (30% sucrose) before being cut horizontally or coronally in 40μm thickness on a cryotom (Leica SM2010R). Immunohistochemical stainings were performed with antibodies against Sox10 (1:300, goat, R&D), GFAP (1:500, mouse, Sigma), MBP (1:500, rat, Bio-Rad) and Ki67 (1:250, rabbit, Abcam), NG2 (1:250, Rabbit, Millipore), APC (1:500, mouse, Millipore), Iba1 (1.500, rabbit, WAKO). Images were taken on confocal laser scanning microscope (Zeiss LSM800). CC overviews in Fig. 1, S1 and S2 were acquired with the Blue Zen (Zeiss) software tiling function. 20X objective and digital zoom 0.5X were used.

Distribution of imaged clones within the CC

For the analysis of the distribution of imaged clones within the damaged CC under the window, coordinates were assigned to each clone taking into account its center. The window cannula was the reference point (zero position, x=0, y=0, z=0, Supplementary Figure 3h). The distance between each clone whose death rate was >75% and all the other clones fulfilling the same death rate criterium was measured. In the same way, the distance between each clone whose death rate was <25% and all the other clones fulfilling the same death rate criterium was measured. Finally, the distance between clones whose death rate was <25% and clones whose death rate

was >75% was measured. Mice with at least 3 clones per condition were analyzed.

Statistical analysis and reproducibility

The quantifications of the immunohistochemical stainings were performed on a minimum of 3 animals, using 6 CC horizontal sections per brain. The demyelinated area was measured considering the MBP- area surrounded by MBP+ tissue within the CC and normalized using the total CC area. The analysis of the distribution of the imaged clones were performed using 5 mice. The analysis of the distance among cells in the clones was performed using clones from 3 animals. The data for the migration analysis were taken from 7 mice. The statistical analysis of the data was performed using Graph pad Prism (version 8). A detailed report of statistical tests used, test results, sample sizes and *p*-values can be found in Supplementary Tables 2-5 and in the figure legends for Supplementary Figures. First, normality was tested and when data did not follow a normal distribution according to the Shapiro-Wilk test, the non-parametric Mann Whitney test or Wilcoxon matched-pairs test were performed. Alternatively, a paired or an unpaired two-tailed t-test was used for mean comparison depending on the nature of the analysis (within or between subjects). In case of comparison of 3 or more groups, One-way ANOVA or Kruskal-Wallis tests were performed. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 were considered significant. All the applied statistics were conducted on data from three or more biologically independent experimental replicates.

Table S1

Parameters of the code	Value	Description
Cell ID	Unique identifier (e.g. 2-1)	A series of two digits uniquely identifying the cell
Cell type	1	OPC: oligodendrocyte precursor cells (OPCs) and committed oligodendrocyte precursors (COPs). Cells with some processes of similar length that radially extends from the cell body. These processes have no extensions along axons. Identified in post imaging immunostaining as Sox10 positive MBP negative. Note: we cannot definitely exclude the possibility that very early NFOL would be, in some cases, classified as progenitors.
	2	PM: pre-myelinating oligodendrocytes (or NFOL). Cells with many processes of similar length that radially extends from the cell body. One or few processes initiate remyelination extending along axons. Identified in post imaging immunostaining as Sox10 positive with few MBP positive processes (8-10).
	3	M: myelinating oligodendrocytes. Cells in which all processes ensheath axons and are parallel among each other. Identified in post imaging immunostaining as Sox10 positive with MBP positive processes.
Uncertainty type	1	Certain cell type: cell fulfills all the criteria mentioned above.
	0	Uncertain cell type: no clear indications from morphology; the cell is assigned to the most likely cell type with uncertainty. In case the first mother root cell of the clone was not visible before a potential first division, an OPC cell type is assigned but it is classified as uncertain.
Time point		Number of the imaging time point in which the cell has been annotated.
Mother ID	Unique identifier (e.g. 1-1)	Cell ID of the mother of the current cell. Not Available (NA) if the cell is the first root cell of the clone.
Uncertainty mother	1	Lineage transition is certain: location of the mother in the previous time point and location and morphology of the two sister cells are clearly identifying the transition from mother to daughters. Only possible when exactly two daughter cells are generated.
	0.5	Lineage transition is semi-certain: location of the mother in the previous time point and location and morphology of the sister cells are identifying the transition from that mother to the daughters. However, more than two daughters are present indicating that at least one division has been lost.
	0	Lineage transition is uncertain: impossible to determine the mother cell because several potential cells in the previous time point could have generated the daughters. The most probable cell is chosen as mother and label as uncertain.
Sister ID	Unique identifier (e.g. 2-2)	Cell ID of the second daughter cell that originates from a common mother cell. Not Available (NA) if the daughter cells generated are odd and no sister can be associated to the cell.
Uncertainty sister	1	Certain sister: sister relationship is certain because lineage transition is certain.
	0.5	Semi-certain sister: lineage transition is semi-certain. The most probable sister among the multiple daughter cells generated according to the location and morphology of the cells is chosen.
	0	Uncertain sister: lineage transition is uncertain. The most probable sister among the cells generated is chosen.
Cell death	0 added to the code	Cell disappears from its previous location (without movement to another location) or apoptotic body is detected. Importantly, during each imaging session all SPOTs are checked and their surrounding area (on x, y and z axis) is monitored to identify migrating cells. When cells disappear from a SPOT, they were not detected anywhere else in the surrounding tissue under the window.

Table S1: List of the parameters of the code, showing how cells were coded to generate clonal lineage trees.

Table S2

Figure number	n number	Descripive stats (mean±sem)	Test used	P-value
Figure 1B	10dpi LPC: n= 3 mice 2mpi LPC: n= 3 mice 10dpi LPC + window: n= 3 mice 2mpi LPC + window: n= 3 mice	10dpi LPC: mean ± sem = 20.63 ± 0.74 2mpi LPC: mean ± sem = 0.4 ± 0.2 10dpi LPC + window: mean ± sem = 30.58 ± 5.69 2mpi LPC + window: mean ± sem = 8.44 ± 3.28	i) One-way ANOVA: F = 16.15 ii) 10dpi LPC vs 2mpi LPC: Unpaired t test, two tailed: t = 26.03; df = 4 iii) 10dpi LPC + window vs 2mpi LPC + window: Unpaired t test, two tailed: t = 3.36; df = 4 iv) 10dpi LPC vs 10dpi LPC + window: Unpaired t test, two tailed: t = 1.73; df = 4 v) 2mpi LPC vs 2mpi LPC + window: Unpaired t test, two tailed: t = 2.44; df = 4	i) p-value = 0.0009 ii) p-value < 0.0001 iii) p-value = 0.028 iv) p-value = 0.158 v) p-value = 0.071
Figure 1C	10dpi LPC contra: n= 3 mice 10dpi LPC ipsi: n= 3 mice 2mpi LPC contra: n= 3 mice 2mpi LPC ipsi: n= 3 mice 10dpi LPC + window contra: n= 3 mice 10dpi LPC + window ipsi: n= 3 mice 2mpi LPC + window contra: n= 3 mice 2mpi LPC + window ipsi: n= 3 mice	10dpi LPC contra: mean ± sem = 11.66 ± 6.06 10dpi LPC ipsi: mean ± sem = 56.21 ± 15.19 2mpi LPC contra: mean ± sem = 22.56 ± 8.47 2mpi LPC ipsi: mean ± sem = 80.11 ± 36.51 10dpi LPC + window contra: mean ± sem = 5.57 ± 3.04 10dpi LPC + window ipsi: mean ± sem = 64.35 ± 20.33 2mpi LPC + window contra: mean ± sem = 9.64 ± 2.22 2mpi LPC + window ipsi: mean ± sem = 45.86 ± 11.38	i) 10dpi LPC contra vs ipsi: Paired t test, two tailed: t = 4.75; df = 2 ii) 2mpi LPC contra vs ipsi: Paired t test, two tailed: t = 3.31; df = 2 iii) 10dpi LPC + window contra vs ipsi: Paired t test, two tailed: t = 6.04; df = 2 iv) 2mpi LPC + window contra vs ipsi: Paired t test, two tailed: t = 21.8; df = 2 v) 10dpi contra LPC vs LPC + window: Unpaired t test, two tailed: t = 0.89; df = 4 vi) 10dpi ipsi LPC vs LPC + window: Unpaired t test, two tailed: t = 0.32; df = 4 vii) 2mpi contra LPC vs LPC + window: Unpaired t test, two tailed: t = 1.47; df = 4 viii) 2mpi ipsi LPC vs LPC + window: Unpaired t test, two tailed: t = 0.89; df = 4	i) p-value = 0.04 ii) p-value = 0.08 iii) p-value = 0.02 iv) p-value = 0.0021 v) p-value = 0.42 vi) p-value = 0.76 vii) p-value = 0.21 viii) p-value = 0.42

Table S2. Statistical analysis, numbers, sample size and *p* values for Fig. 1.

Table S3

Figure number	n number	Descripive stats (mean±sem)	Test used	P-value
Figure 2H	Low dividing: n = 24 clones High dividing: n = 17 clones	Low dividing: mean ± sem: 2.52 ± 0.29 High dividing: mean ± sem: 3.42 ± 0.24	Unpaired t test, two tailed: t = 2.21; df = 39	p-value = 0.03

Table S3. Statistical analysis, numbers, sample size and *p* values for Fig. 2.

Table S4

Figure number	n number	Descripive stats (mean±sem)	Test used	P-value
Figure 3H	>75% death rate clones: n = 5 mice <25% death rate clones: n = 5 mice <25% - >75% death rate clones: n = 5 mice	>75% death rate clones: mean ± sem = 776.4 ± 117.6 <25% death rate clones: mean ± sem = 583.2 ± 98.51 <25% - >75% death rate clones: mean ± sem = 793.4 ± 105.1	i) >75% vs <25% death rate clones: paired t test, two tailed: t = 2.27; df = 4 ii) >75% vs <25% - >75% death rate clones: Wilcoxon matched-pairs test, two tailed iii) <25% vs <25% - >75% death rate clones: Wilcoxon matched-pairs test, two tailed	i) p-value: 0.08 ii) p-value: 0.62 iii) p-value: 0.12

Table S4. Statistical analysis, numbers, sample size and *p* values for Fig. 3.

Table S5

Figure number	n number	Descripive stats (mean±sem)	Test used	P-value
Figure 4D	Max 4 days tracked: n = 70 cells pairs 5 to 7 days tracked: n = 37 cells pairs More 8 days tracked: n = 12 cells pairs	Max 4 days tracked: mean ± sem = 30.77 ± 2.09 5 to 7 days tracked: mean ± sem = 42.27 ± 3.91 More 8 days tracked: mean ± sem = 48.1 ± 7.78	Kruskal-Wallis test	p-value = 0.005
Figure 4F	Death: n = 91 cells Surviving: n = 58 cells	Death: mean ± sem = 12.06 ± 1.01 Surviving: mean ± sem = 4.2 ± 0.35	Unpaired t test, two tailed: t = 6; df = 147	p-value < 0.0001
Figure 4G	Death d1: n = 50 cells Death d2: n = 51 cells Death d3: n = 28 cells Death d4: n = 14 cells Surviving d1: n = 39 cells Surviving d2: n = 28 cells Surviving d3: n = 30 cells Surviving d4: n = 29 cells	Death d1: mean ± sem = 12.68 ± 1.1 Death d2: mean ± sem = 12.02 ± 1.18 Death d3: mean ± sem = 9.01 ± 1.15 Death d4: mean ± sem = 7.87 ± 1.88 Surviving d1: mean ± sem = 6.82 ± 1.29 Surviving d2: mean ± sem = 6.39 ± 1.45 Surviving d3: mean ± sem = 6.37 ± 1.08 Surviving d4: mean ± sem = 4.15 ± 0.62	i) Kruskal-Wallis test ii) Dying cells: Kruskal-Wallis test iii) Surviving cells: Kruskal-Wallis test iv) Dying d1 vs surviving d1: Mann-Whitney test, two tailed v) Dying d2 vs surviving d2: Mann-Whitney test, two tailed vi) Dying d3 vs surviving d3: Mann-Whitney test, two tailed vii) Dying d4 vs surviving d4: Mann-Whitney test, two tailed	i) p-value < 0.0001 ii) p-value = 0.042 iii) p-value = 0.617 iv) p-value < 0.0001 v) p-value = 0.0001 vi) p-value = 0.036 vii) p-value = 0.014

Table S5. Statistical analysis, numbers, sample size and *p* values for Fig. 4.

Supplementary movie legends

Movie S1. Surviving OPC clone. Representative movie (corresponding to the lineage shown in Fig. 1G) of an OPC and its progeny imaged over the course of 27 days exhibiting survival and differentiation to remyelinating oligodendrocytes. Arrowheads with cell type indications highlight the time point cells change their differentiation stage. Scale bar represents 50 μm.

Movie S2. Dying OPC clone. Representative movie (corresponding to the lineage shown in Fig. 1H) of an OPC and its progeny imaged over the course of 34 days showing the failure of that clone to generate surviving progeny. Note: all cells generated remain in OPC stage. Scale bar represents 50 μm.

Movie S3. Tracking of migration. Representative movie (corresponding to Fig. 4E) of cell movements tracking over the course of 27 days. Colored lines highlight the cumulative distance traveled by single cells over time. Some cells die and some cells terminally differentiate to remyelinating oligodendrocytes. Arrowheads with cell type indications highlight the time point cells change their differentiation stage. Scale bar represents 50 μm.

Supplementary References

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