The chick caudo-lateral epiblast acts as a permissive niche for generating neuromesodermal progenitor behaviours

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Supplementary Figure 1: Tissue Grafts Extend Many Times their Original Length after Transplantation.

The starting diameter of the grafted tissues was measured in the rostro-caudal direction and is plotted on the *x* axis in (A-C). The final distance between the most rostral and most caudal labelled cells (the axial extent) is shown on the *y* axis in (A-C). (A) Points coloured according to the thresholds (dashed lines) used to score short (<500μm), medium (500-1750μm) and long (>1750μm) rostro-caudal contributions. (B) Points coloured according to whether labelled cells were found in neural tissues only (purple), mesodermal tissues only (green) or both tissue compartments (orange). (C) Points coloured according to whether labelled cells were

found in the region associated with the node (red) or not (grey). Grafts that produced medium to long rostro-caudal contributions frequently included both neural and mesodermal tissues and the region around the node.

Supplementary Figure 2: Fate Mapping Defines the Embryonic NMp-Containing Region Caudal and Lateral to the Node.

Small regions of the embryonic tissues were labelled with DiI; the position of each label is represented as absolute axial and lateral positions measured from the caudal limit of the node (at 0,0), as in other figures. The positions of all labels are shown on one plot (top left in (A) and (B)) and separated into different host stages (7.67 represents HH Stage 8^-). (A) Tissue labels coloured by the rostro-caudal length of their final contributions (blue, <500μm; orange, 500-1750μm; red, >1750μm). (B) Tissue labels coloured by the tissues in which labelled cells are scored after incubation. LPM: Lateral Plate Mesoderm, PSM: Pre-Somitic Mesoderm, N: Node, NT: Neural Tube, SM: Somitic Mesoderm.

Supplementary Figure 3: Explanted Chick Node Tissue.

Explants of chick node tissue were labelled with DiI and grafted into host embryos; the position of each graft is represented as absolute axial and lateral positions measured from the caudal limit of the node (at 0,0), as in other figures. The positions of all grafts are shown on one plot (top left in (A) and (B)) and separated into different host stages (7.67 represents HH Stage 8^-). (A) Tissue labels coloured by the rostro-caudal length of their final contributions (blue, <500μm; orange, 500-1750μm; red, >1750μm). (B) Tissue labels coloured by the tissues in which labelled

cells are scored after incubation. LPM: Lateral Plate Mesoderm, PSM: Pre-Somitic Mesoderm, N: Node, NT: Neural Tube, SM: Somitic Mesoderm.

Supplementary Figure 4: Embryonic Stem Cell-Derived NMps, Gouti *et al.* Protocol.

Colonies of ES cells differentiated according to the protocol used by Gouti *et al.* were labelled with DiI and grafted into host embryos; the position of each graft is represented as absolute axial and lateral positions measured from the caudal limit of the node (at 0,0), as in other figures. The positions of all grafts are shown on one plot (top left in (A) and (B)) and separated into different host stages (7.67 represents HH Stage 8^-). (A) Tissue labels coloured by the rostro-caudal length of their final contributions (blue, <500μm; orange, 500-1750μm; red, >1750μm). (B) Tissue labels coloured by the tissues in which labelled cells are scored after incubation. LPM: Lateral Plate Mesoderm, PSM: Pre-Somitic Mesoderm, N: Node, NT: Neural Tube, SM: Somitic Mesoderm.

Supplementary Figure 5: Embryonic Stem Cell-Derived NMps, Turner *et al.* Protocol.

Colonies of ES cells differentiated according to the protocol used by Turner *et al.* were labelled with DiI and grafted into host embryos; the position of each graft is represented as absolute axial and lateral positions measured from the caudal limit of the node (at 0,0), as in other figures. The positions of all grafts are shown on one plot (top left in (A) and (B)) and separated into different host stages (7.67 represents HH Stage 8^-). (A) Tissue labels coloured by the rostro-caudal length of their final contributions (blue, <500μm; orange, 500-1750μm; red, >1750μm). (B) Tissue labels

coloured by the tissues in which labelled cells are scored after incubation. LPM: Lateral Plate Mesoderm, PSM: Pre-Somitic Mesoderm, N: Node, NT: Neural Tube, SM: Somitic Mesoderm.

Supplementary Figure 6: Self-Renewing Embryonic Stem Cells.

Colonies of ES cells from ES+LIF culture (see Materials & Methods) were labelled with DiI and grafted into host embryos; the position of each graft is represented as absolute axial and lateral positions measured from the caudal limit of the node (at 0,0), as in other figures. The positions of all grafts are shown on one plot (top left in (A) and (B)) and separated into different host stages (7.67 represents HH Stage 8^-). (A) Tissue labels coloured by the rostro-caudal length of their final contributions (blue, <500μm; orange, 500-1750μm; red, >1750μm). (B) Tissue labels coloured by the tissues in which labelled cells are scored after incubation. LPM: Lateral Plate Mesoderm, PSM: Pre-Somitic Mesoderm, N: Node, NT: Neural Tube, SM: Somitic Mesoderm.

Supplementary Figure 7: Chiron-Treated Gastruloid Tissues, Anterior Explants.

Anterior explants of Chiron-treated gastruloid tissues were labelled with DiI and grafted into host embryos; the position of each graft is represented as absolute axial and lateral positions measured from the caudal limit of the node (at 0,0), as in other figures. The positions of all grafts are shown on one plot (top left in (A) and (B)) and separated into different host stages (7.67 represents HH Stage 8^-). (A) Tissue labels coloured by the rostro-caudal length of their final contributions (blue, <500μm; orange, 500-1750μm; red, >1750μm). (B) Tissue labels coloured by the tissues in

which labelled cells are scored after incubation. LPM: Lateral Plate Mesoderm, PSM: Pre-Somitic Mesoderm, N: Node, NT: Neural Tube, SM: Somitic Mesoderm.

Supplementary Figure 8: Chiron-Treated Gastruloid Tissues, Posterior Explants.

Posterior explants of Chiron-treated gastruloid tissues were labelled with DiI and grafted into host embryos; the position of each graft is represented as absolute axial and lateral positions measured from the caudal limit of the node (at 0,0), as in other figures. The positions of all grafts are shown on one plot (top left in (A) and (B)) and separated into different host stages (7.67 represents HH Stage 8^-). (A) Tissue labels coloured by the rostro-caudal length of their final contributions (blue, <500μm; orange, 500-1750μm; red, >1750μm). (B) Tissue labels coloured by the tissues in which labelled cells are scored after incubation. LPM: Lateral Plate Mesoderm, PSM: Pre-Somitic Mesoderm, N: Node, NT: Neural Tube, SM: Somitic Mesoderm.

Supplementary Figure 9: N2B27-Cultured Gastruloid Tissues.

Explants of gastruloids grown continuously in N2B27 were labelled with DiI and grafted into host embryos; the position of each graft is represented as absolute axial and lateral positions measured from the caudal limit of the node (at 0,0), as in other figures. The positions of all grafts are shown on one plot (top left in (A) and (B)) and separated into different host stages (7.67 represents HH Stage 8^-). (A) Tissue labels coloured by the rostro-caudal length of their final contributions (blue, <500μm; orange, 500-1750μm; red, >1750μm). (B) Tissue labels coloured by the tissues in which labelled cells are scored after incubation. LPM: Lateral Plate Mesoderm, PSM: Pre-Somitic Mesoderm, N: Node, NT: Neural Tube, SM: Somitic Mesoderm.

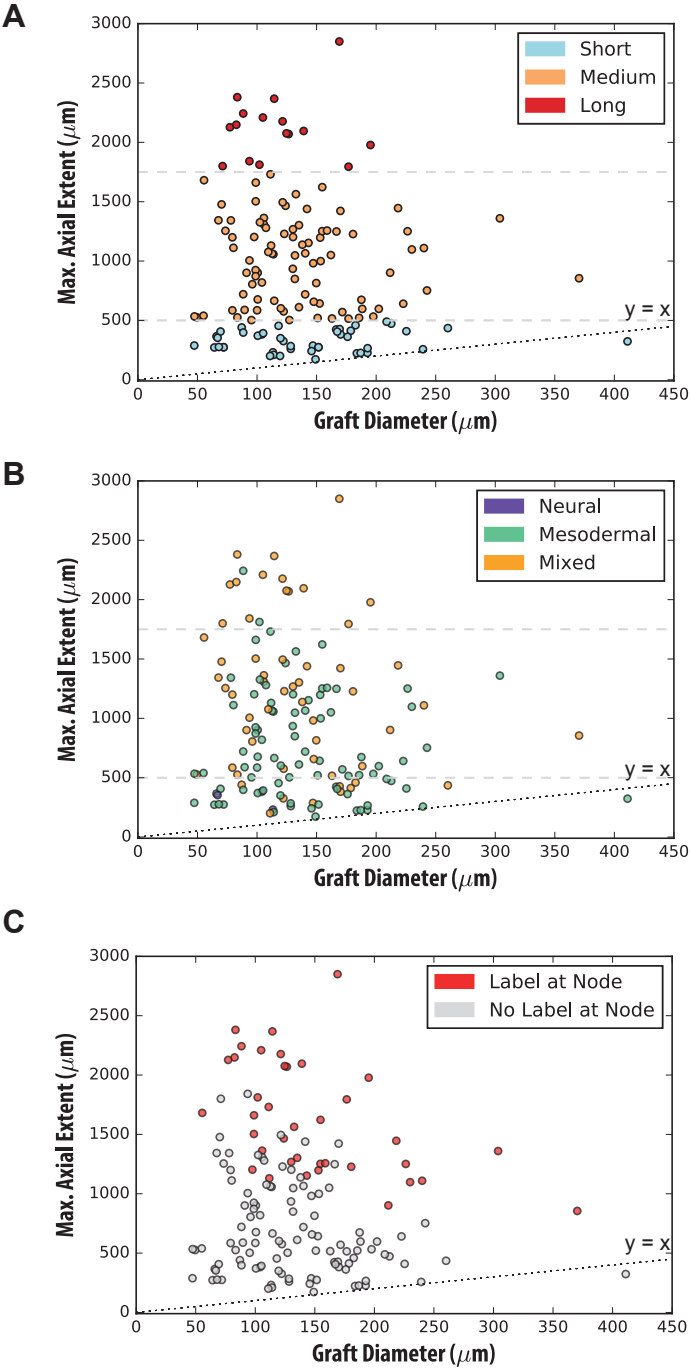
Supplementary Figure 10: All Populations Tested Can Produce Long Neural and Mesodermal Contributions, Except Anterior Chiron-Treated Gastruloid Explants.

The distance between the most rostal and most caudal labelled cells from each graft (its axial length) was measured and is plotted as open circles. Box plots show the distribution of axial lengths for each starting population. Centre lines show the medians; crosses denote the sample means. Box limits indicate the 25th and 75th percentiles as determined by the BoxPlotR webtool (see Materials & Methods); whiskers extend 1.5 times the interquartile range (Tukey style). The width of the boxes is proportional to the square root of the sample size.

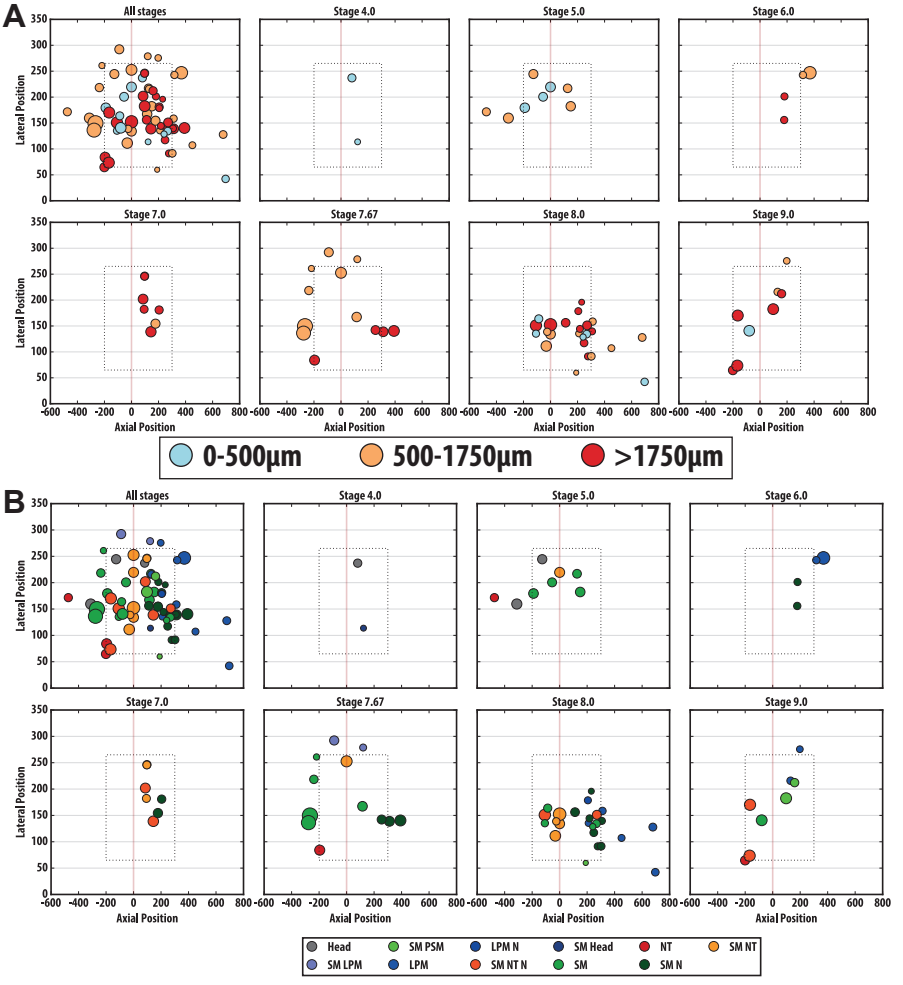
Supplementary Figure 11: All Grafts Analysed Contributed Labelled Cells to the ROI.

The initial size of each labelled graft was measured in the rostro-caudal direction and its position was measured as rostro-caudal and medio-lateral distances from the caudal limit of the node ((0,0) in A-G). Each graft is plotted to scale as a circle centred on the midpoint of the graft. The axial diameter of each circle therefore directly corresponds to the measured rostro-caudal length of each graft. This representation shows that each contributed either wholly or partially to the ROI (dashed box), as defined by the labelling experiment in Figure 1. Any grafts that fell wholly outside the ROI when plotted in this way were excluded from further analysis.

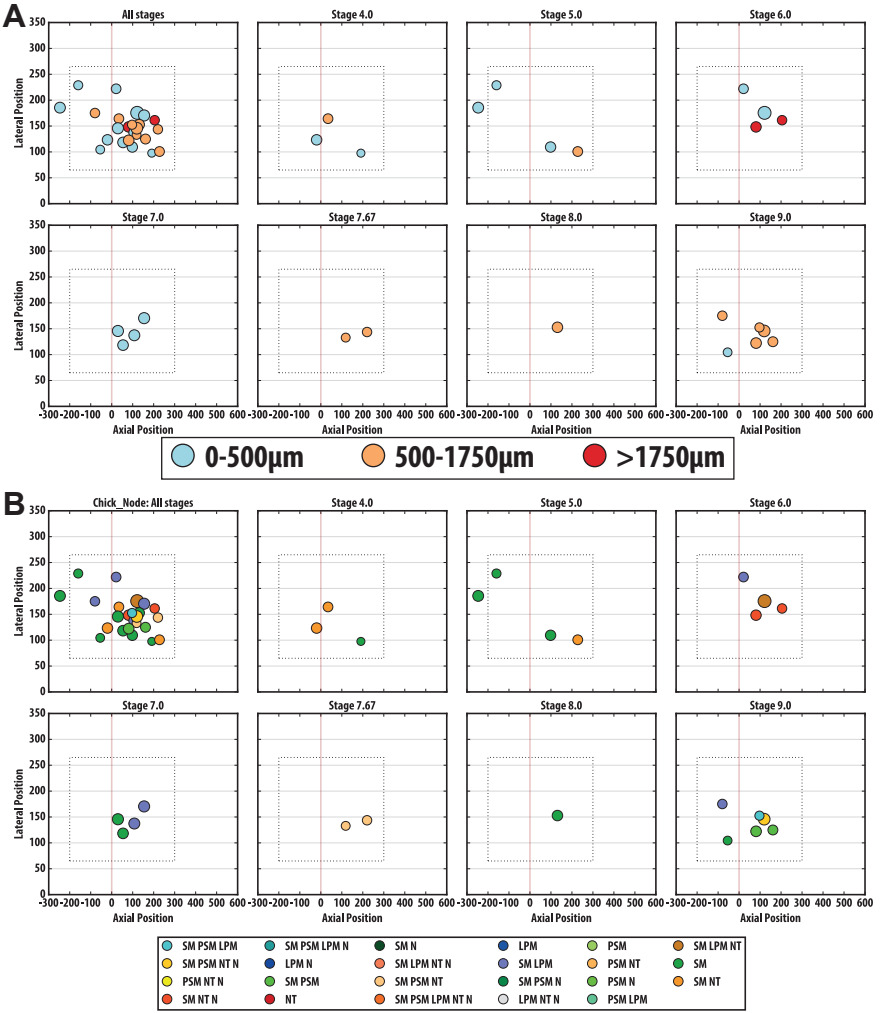
Supplementary Figure 1



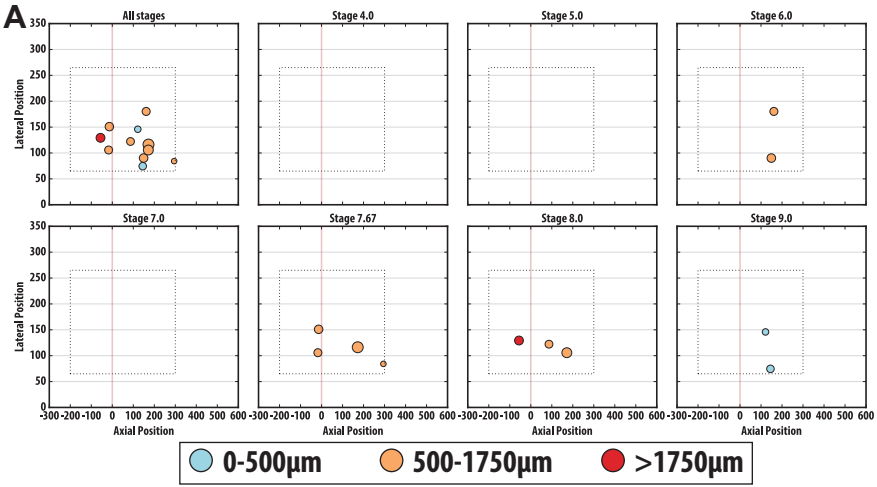
Supplementary Figure 2 - Dil Labelling

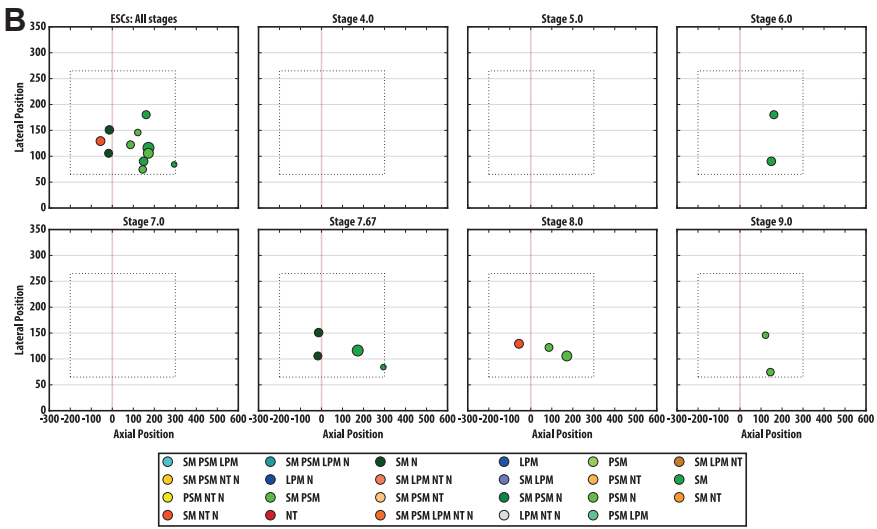


Supplementary Figure 3 - Chick Node Grafts

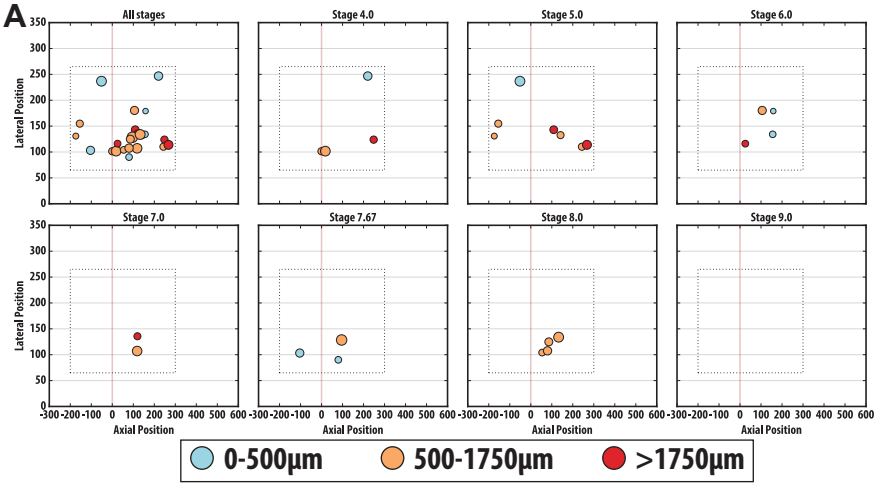


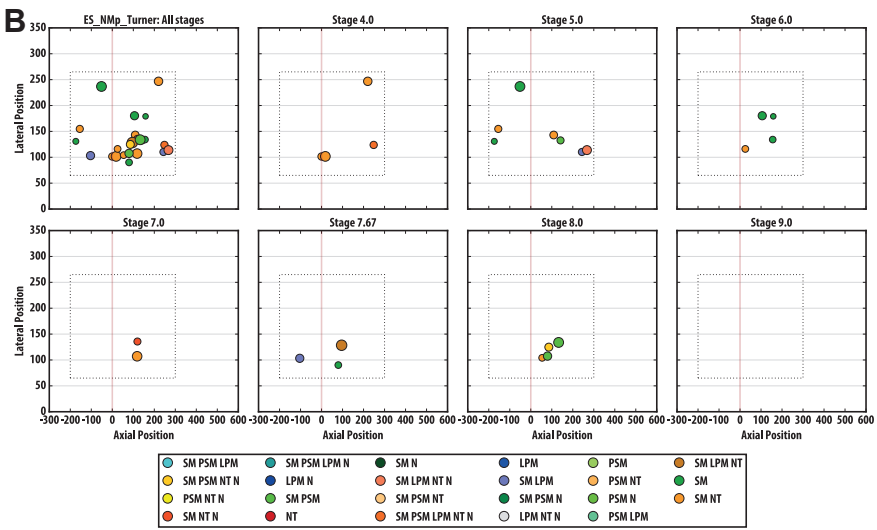
Supplementary Figure 4 - ESC Grafts



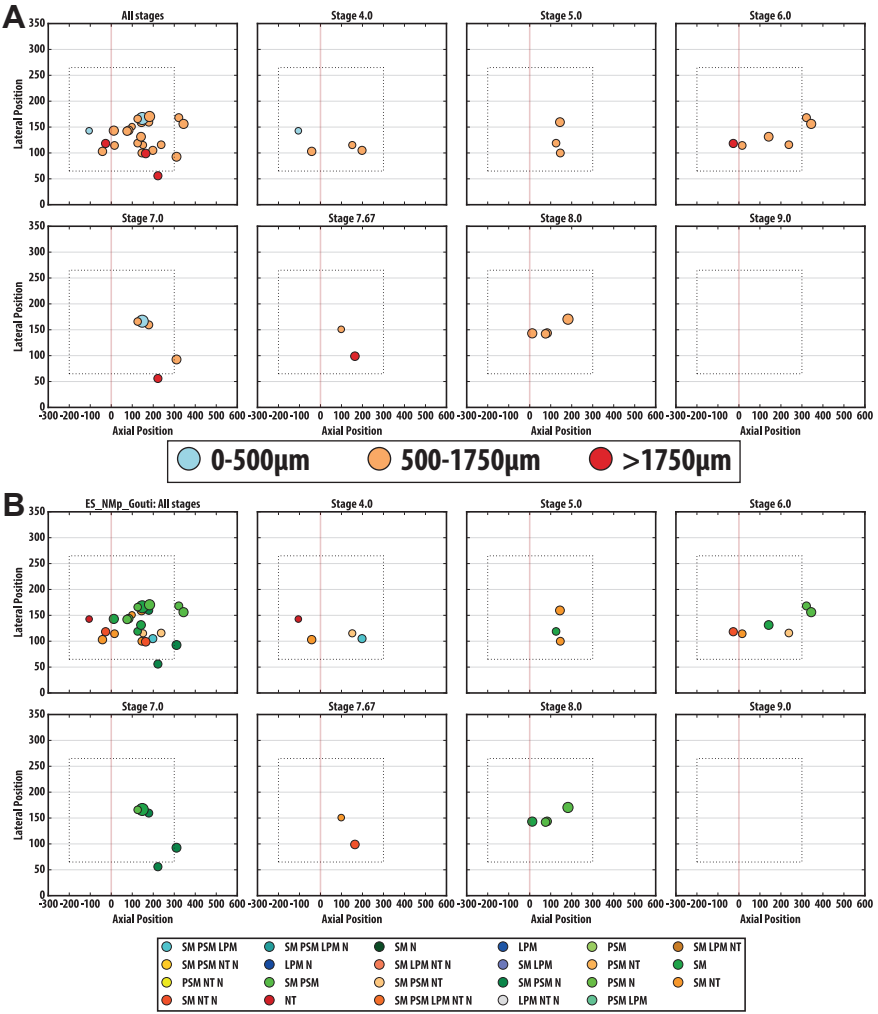


Supplementary Figure 5 - ES NMps (Turner)

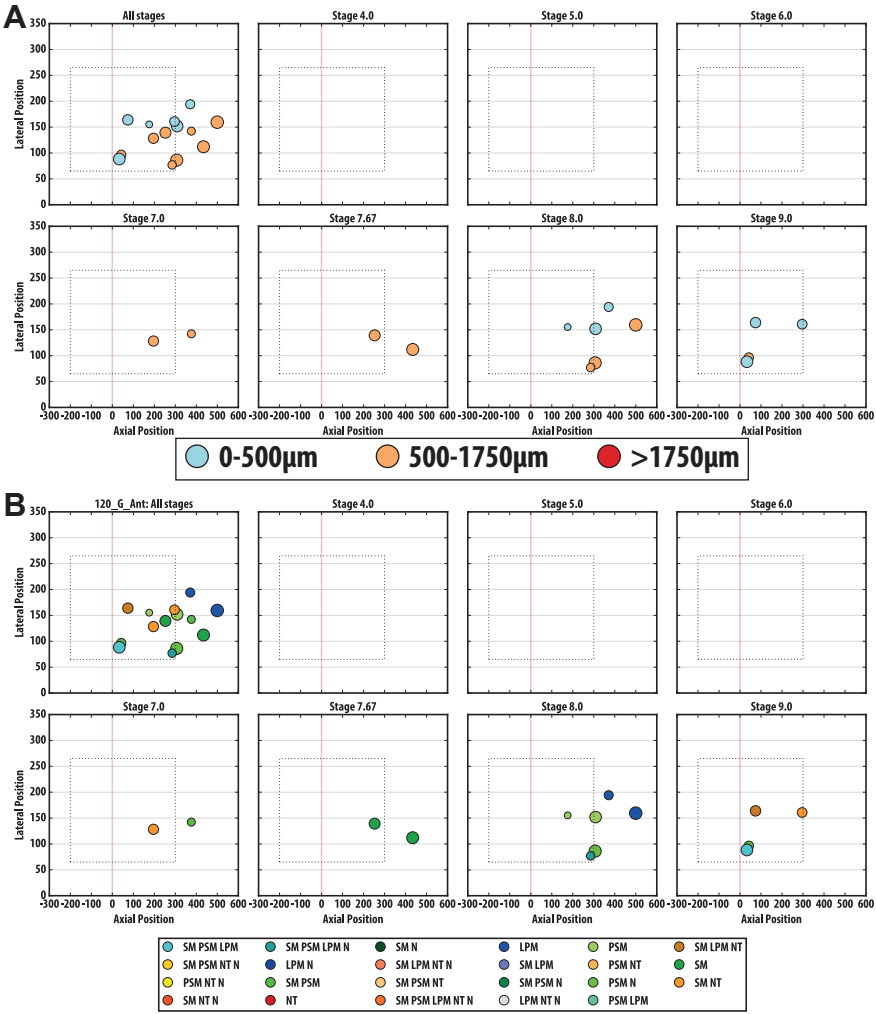




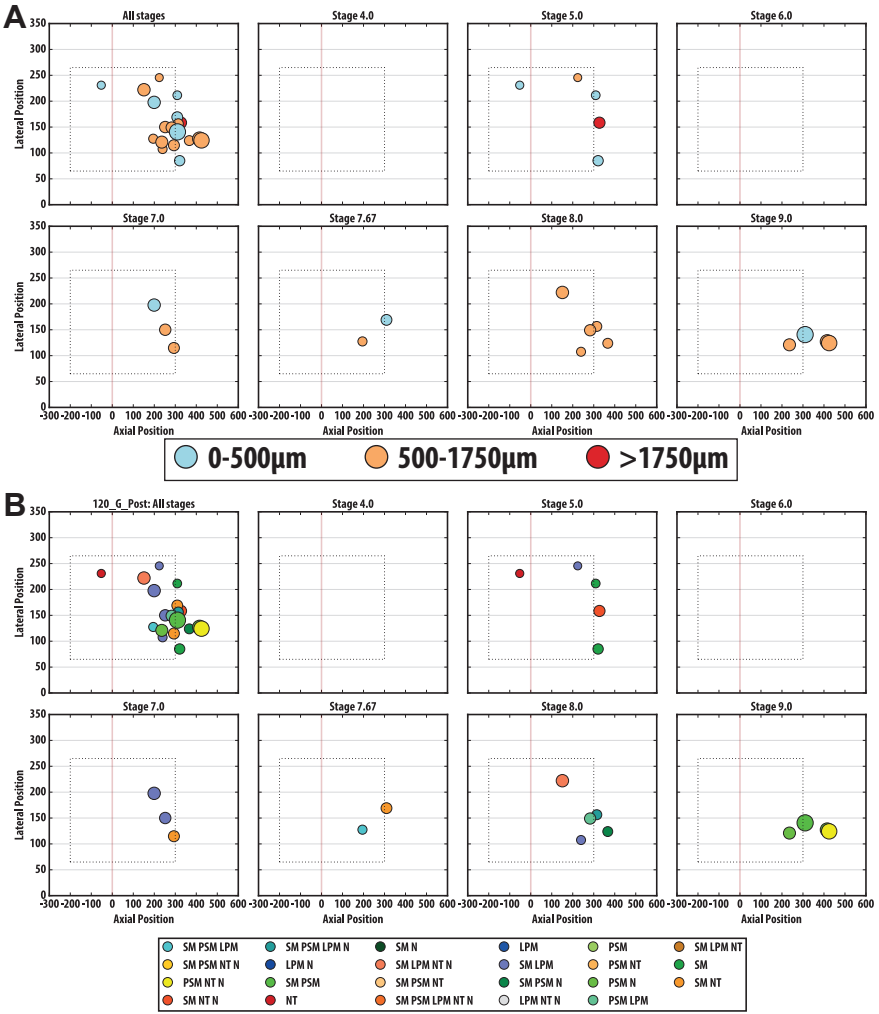
Supplementary Figure 6 - ES NMps (Gouti)



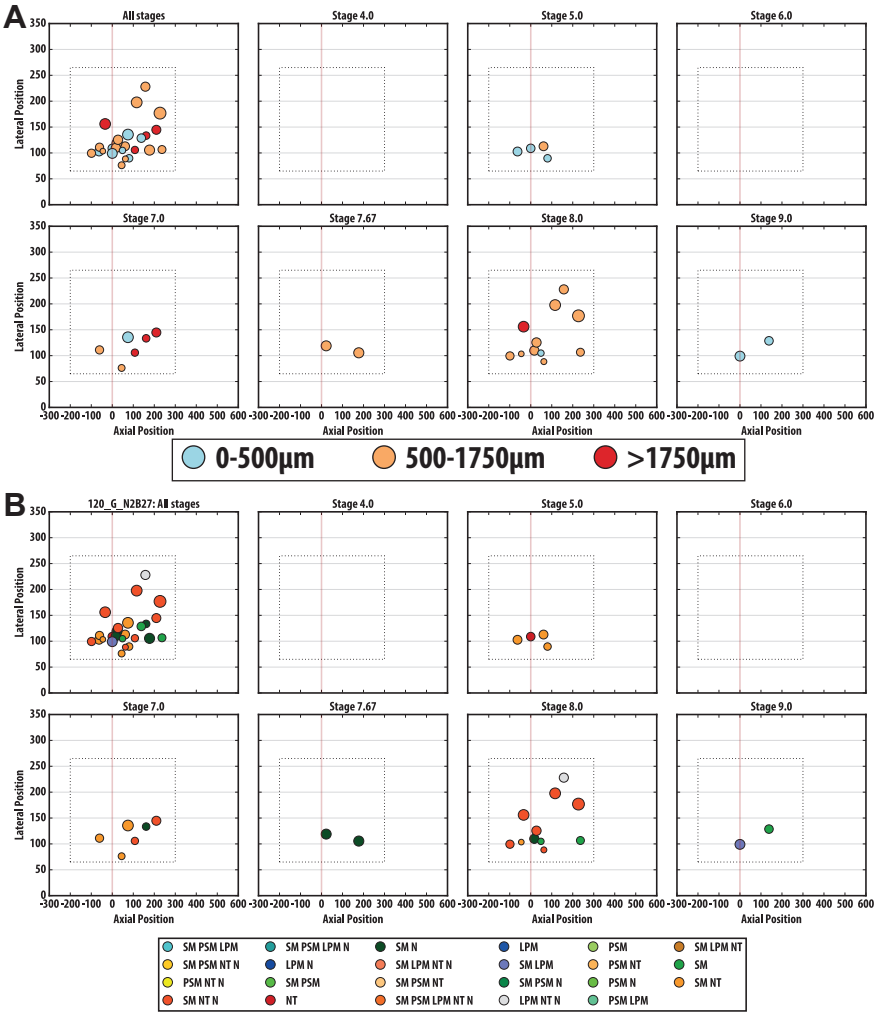
Supplementary Figure 7 - Anterior Gastruloid Grafts



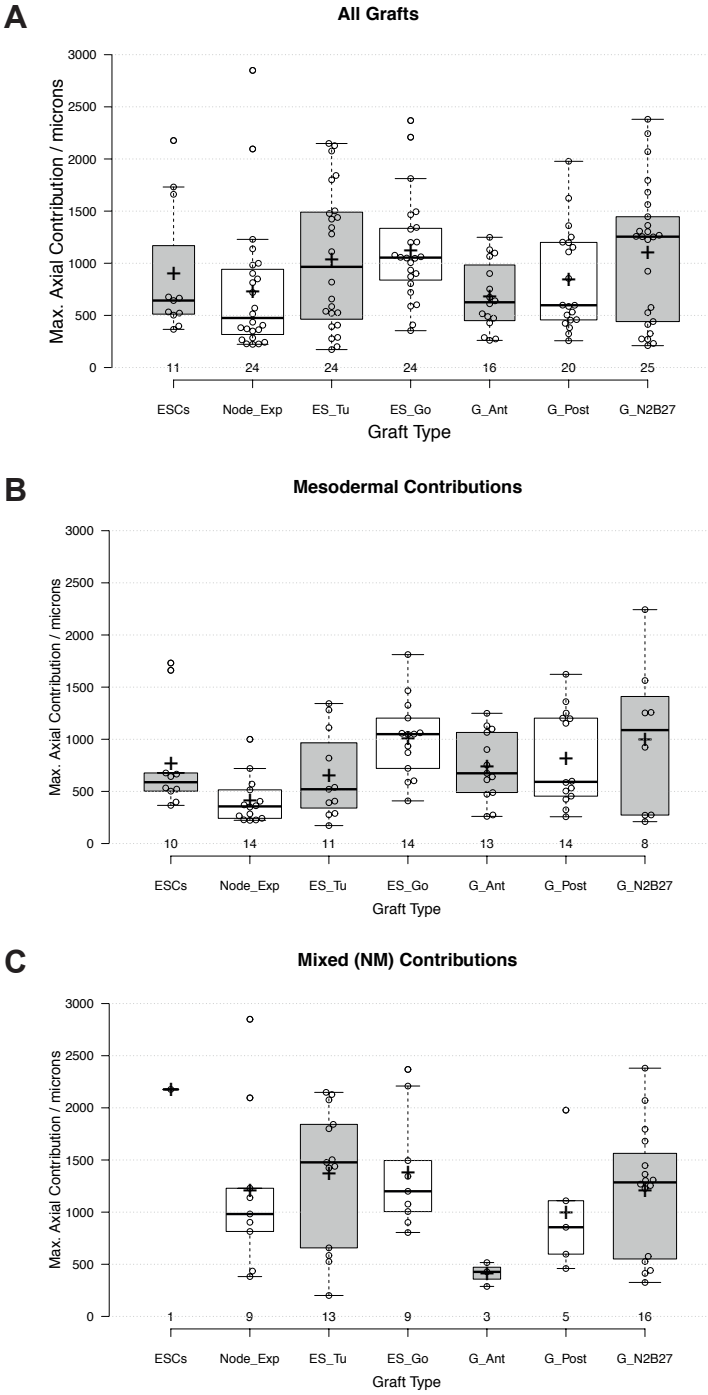
Supplementary Figure 8 - Posterior Gastruloid Grafts



Supplementary Figure 9 - N2B27 Gastruloid Grafts



Supplementary Figure 10



Supplementary Figure 11 - Grafts Represented to Scale