

Fig S1

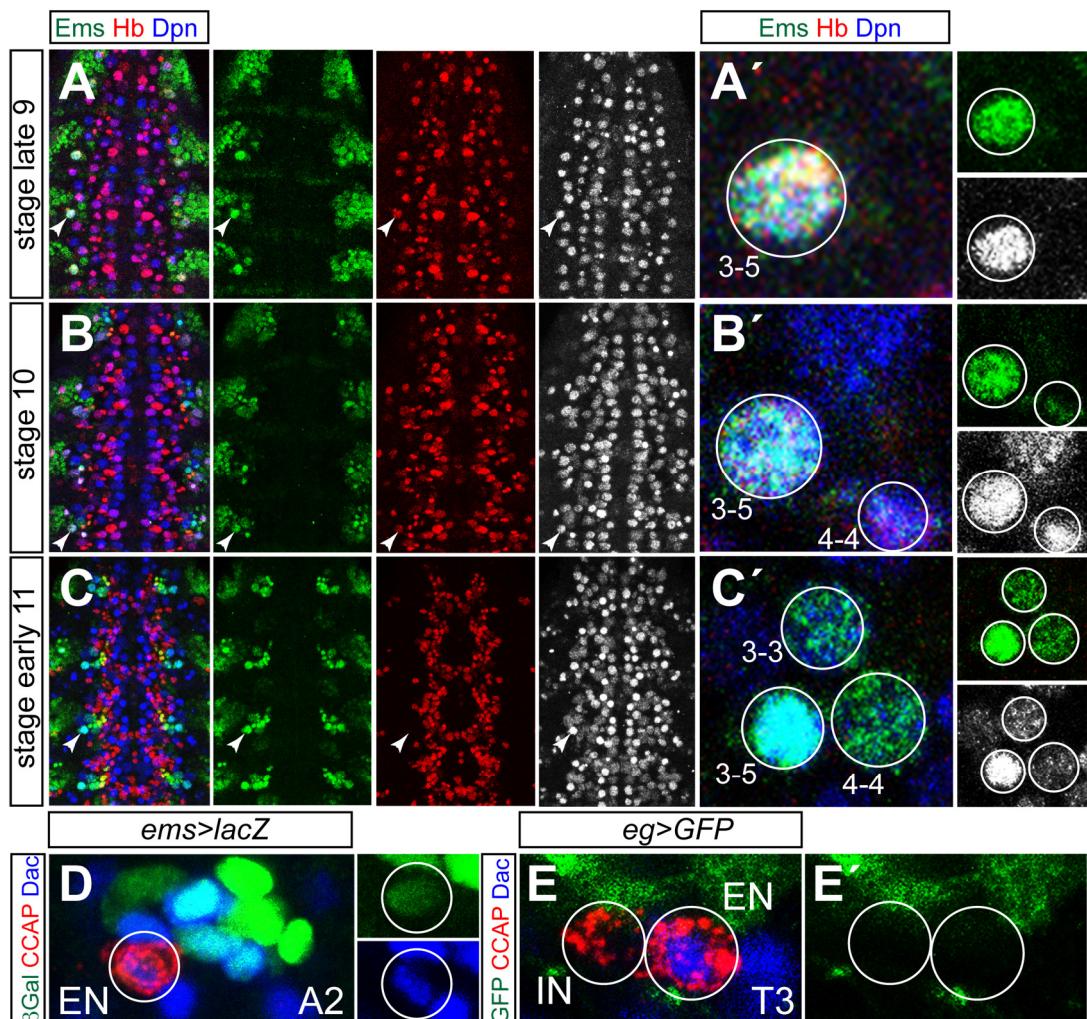


Figure S1. Ems labels the lineage of NB3-5. (A-C) Split panels of embryos at stages late 9 (A), 10 (B) and early 11 (C) showing the expression of Ems (green), Hb (red) and Dpn (blue). (A'-C') Close-up views of selected left hemisegment (arrowheads) of the different stages; NBs are indicated. At stage 9 the unique Ems-expressing NB is NB3-5. At late stage 10 NB3-3 and NB4-4 are starting to delaminate and are in a more external plane than NB3-5. Both NB3-5 and NB4-4 express Hb. At stage 11 all three NBs expressing Ems are fully delaminated and none of them express Hb. To the right the expression of Ems (green) and Dpn (white) is shown separately. (D) Labelling for CCAP (red), β -galactosidase (green) and Dac (blue) in *ems-Gal4 UAS-lacZ* larva 1. The green and blue channels are shown separately on the right. The IN is in a different focal

plane. (E-E') Labelling for CCAP (red), GFP (green) and Dac (blue) in *eg-Gal4 UAS-GFP* larva 1. The green channel is shown separately in (E'). The segment shown in each figure is indicated.

Fig S2

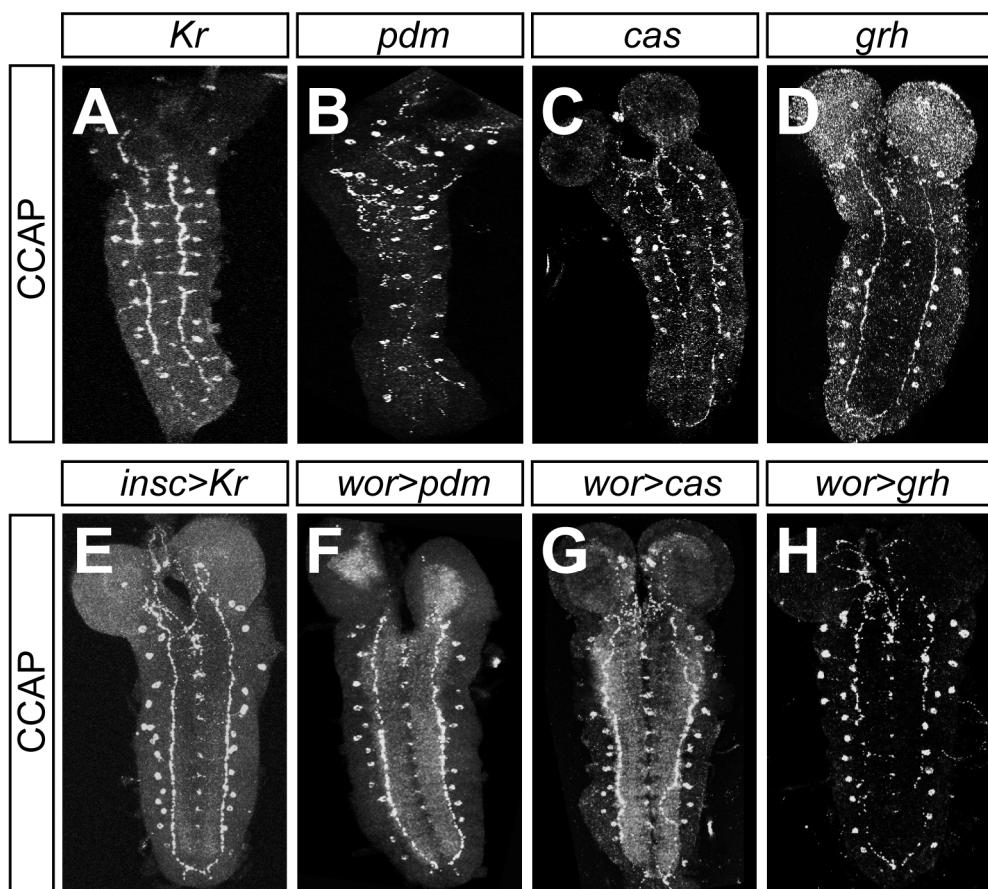


Figure S2. Kr, Pdm, Cas and Grh are not required for the expression of CCAP.
(A-D) Labelling for CCAP in first instar larvae mutant for *Kr^I* *Kr^{CD}* (A), *Df(2L)ED773* (*Pdm⁻*) (B), *cas^{ΔI}* (C) and *grh^{IM}* (D). (E-H) Labelling for CCAP in *insc-Gal4 UAS-Kr* (E), *wor-Gal4 UAS-pdm* (F), *wor-Gal4 UAS-cas* (G) and *wor-Gal4 UAS-grh* (H).

Fig S3

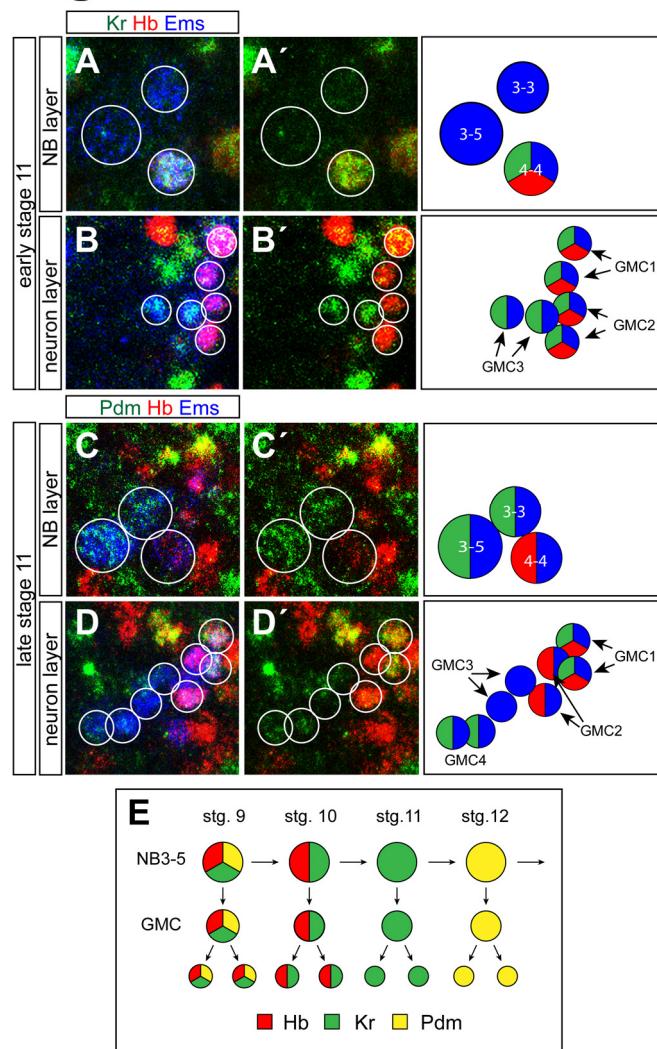


Figure S3. The early lineage of NB3-5. (A-D) Expression of Kr (green in A-B), Pdm (green in C-D), Hb (red) and Ems (blue) in the NB3-5 lineage at early (A-B) and late (C-D) stage 11. NBs expressing Ems and the progeny of the different GMCs are indicated. The green and red channels are shown in (A',B',C' and D'). NB (A and C) and neuron (B and D) layers are shown separately. To the right are graphic representations; circles represent cells expressing the indicated markers; NBs expressing Ems and the progeny of the different GMCs of NB3-5 are indicated; midline to the right, anterior up. (E) Cartoon summarizing the expression of temporal factors in the NB3-5 lineage from its delamination to the stage 12.

Fig S4

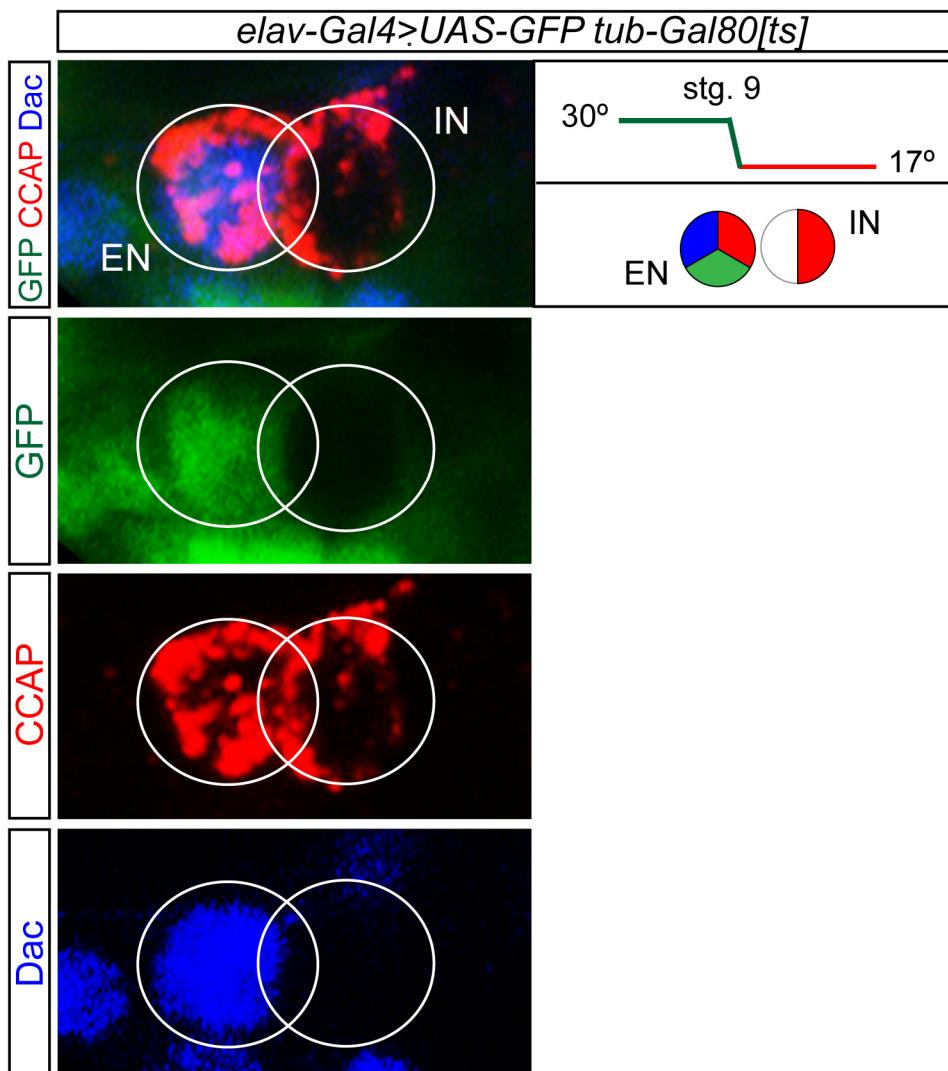
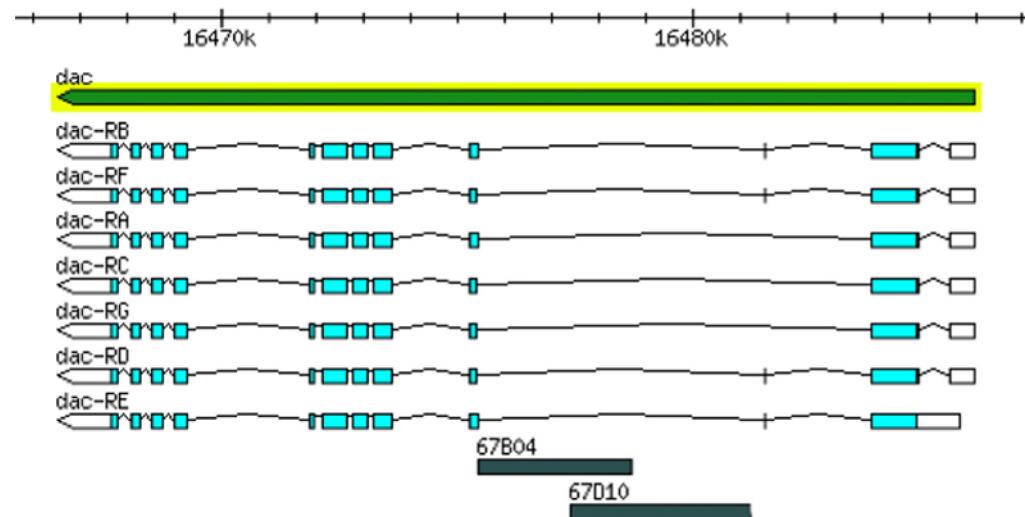


Figure S4. Split panels of Fig. 4F. Note the lack of GFP in the IN.

Fig. S5**A****B**

TGGAACTGCGGAATTGCAGAGAGTTACTGTTGGTTGA	75
A tgcca AATGCCTcAG cacagtatcgcaattcagcaaatttacgggtgggatttagaatgtagaacaatgc	150
tattttggcatttgtctgtcattttcacgtttattattagataacacaacttgagagcttaag	225
tggaaaatgaacaaacttggaaattaaatgaaaattttatgtttaagcaagcaaaatggctccagtagatag	300
ctcatcttaaattgttaactcttcacacaaaaccgtggtaagcattcaaaggtaatttatggccctcgccat	375
atttatatatgccagaccgtgtcaatattttaaaaataatattttgtttaattctttgttttattgtct	450
gtaaaagccgagcagttcctcgccaaaaagtgtctataatttttgcattttttatggccgtaaacgcattca	525
tcacg GctTAAC tttCCgagg AATCT tgctgttaataattttccctccaaaaatgcc ATTTTA agccata	600
gtccccaccactcgccgcagttggatt CCTAATTAAGCT tCAaccaacttttagt GAGcaCCCC tttgacttg	675
a GAACAAATAAAAAGCCAAA aaCg AGT Agaaacga AAACGTCTACC ATTTAATTATgt GCCATAAAAAAA tgac	750
aacccgaaacagcaacaacaataaaaggcagccaaacacggagcctaaagaaaaacgtatggaaaaaaagagtt	825
caactattttttaa GtAATT ATGCCt GCAATTGCTG tATT GTaTAaaAAA TATTAAAc AAGt Ct TGGGCA	900
AATACTTGACGCAGGTTaa G CtAAATAGTTAA c CgAAAAG GCAtAAACAc TTG gCGCAGCGGGTGGAA g TTATG	975
ACAAACAA GAGATA aa c cccccaagcagcgcgggggtcgattcaaaaaagccaaaaataataagaataag	1050
aagcaaaaactgactaagtgcagcaaatactacgaaggggagccgggt CTT gtat CAAATAGAGcGACCC cttcca	1125
TcCAGATGG tttcttcc taGCAATTAA tt TATGG a ATCTTCATTC c ATGTTG cc ctGGT ac ACAGGT G tcc	1200
cCTtAGAGCcA aaagacagcgcgc atGGCAAATAAACAc a GGTAGCAAtGATCGT gtgcacgataattat	1275
gccatgcatactccatacacatatacatatgtacatattgtatctaaatgtat ATGGCGAAA	1350
ctct TGAACa ATT TGAA TT TGCGT t ttcGGATAACG tt tttCAAATATT a CAGTTACATATT g CCAcAq	1425
cc AAATATTG g GaCtGG TT AAATTG G CCTca TT AAACGTT taa ccACAAA ca gtcggATTGTT g TCTTg GC	1500
CcCtttG GGTC aa TAAA ga Gact c tttagcca TGAA TCATTCC gagaacgaaagaatagc agcGAAAGT G TTTc	1575
TtagttCTa ATC ggaat cg GAAGGCC gtt gttccccgc ct gTGACGTG CAAAA a Agac ccgagaccgc tg gc gcct	1650
gtcagtcagtcaagttgtatgaatttggaaagt gatgtttggaggtt at gcGg GCAa CACTTT CAT GGAAAGTT	1725
TTcgg ttctat cagg CTGACATGTTG t CT gttt cagaaaatttgc tc caca aaaat gtcttgc ata gt cttt gcata gt	1800
ttcgggaaagattggataattgggtcgaggcgat tacgt aat ggtaat ct gatgt at gcaat at ctacgat ct gc ca	1875
aaat gagctt gt gaaact g actgag ct gtat ct ggtttag ga acat g ctgtat g atgactt at gaaaacactc cg gtaaa	1950
tttagggagttat gtttctgc gacc gaat g ccaagtaacta att tactgg ct ca gt taaggta att tcgca aa	2025
aat cgaaata ag ctaaaaataattatctta att tgatcc gaa atgtt ca gtgtt aa atccaaatt g caat	2100
ct gtcgacc ag gacca CTGCC attt TtGCTA ATT TATG c accGt TGTC ac TCGC gt ga ATGTC ATT Tt g CAT	2175

Figure S5. Hunchback putative binding sites in the *dachshund* gene. (A) Schematic depiction of the *dachshund* gene showing the fragments inserted in the *GMR67B04-GAL4* and the *GMR67D10-GAL4* lines (Janelia Farm GAL4 stock collection (http://flystocks.bio.indiana.edu/Browse/gal4/gal4_JaneliaD_M.php)). (B) Sequence of the fragment inserted in the *GMR67B04-GAL4* and *GMR67D10-GAL4* lines (2L:16475407,16481194). From the whole set of *dac-Gal4* fly stocks generated by Pfeiffer et al. for enhancer activity (29 lines) (Pfeiffer et al., 2008), these two lines show expression in large subsets of neurons in embryos of stage 16. Black capital letters represent bases in the *Drosophila melanogaster dachshund* reference sequence that are conserved in the *D. simulans*, *D. yakuba*, *D. erecta*, *D. ananassae* and *D. persimilis* orthologous DNAs (EvoPrinterHD). Red letters represent putative Hunchback binding sites in conserved domains (Jaspar database).

Table S1. Expression of CCAP and Burs α in different genetic combinations. Mean number of cells expressing CCAP or Burs α (#) per hemi-subesophage (SE), hemithorax (Th) and hemi-abdomen (Abd) in the different genotypes used in this work of first instar ventral ganglia. N: number of individual scored; SD: standard deviation; (*) significant differences from the *wild-type* (Student two-tailed t-test; P<0.01). Note that in *wild-type* abdomen the number of neurons expressing Burs α is higher than those expressing CCAP, this is because the onset of CCAP expression in the ENs happens later in larval development.

	SE			Th			Abd		
	N	Mean	SD	N	Mean	SD	N	Mean	SD
<i>wild-type</i>	70	2.8	0.5	73	3.9	0.2	74	7.7	1.64
<i>wild-type</i> (#)	20	3	0	20	4	0	20	10.4	1.9
<i>hb</i> ^{P1} <i>hb</i> ^{FB}	3	0*	0	3	0*	0	3	0*	0
<i>hb</i> ^I / <i>hb</i> ^{P1} <i>hb</i> ^{FB} (#)	21	0*	0	21	1.6*	0.66	21	6.08*	1.08
<i>Kr</i> ^I <i>Kr</i> ^{CD}	18	3	0.3	20	3.6	0.7	20	8.05	2.3
<i>Df(2L)ED773</i> (<i>pdm</i> ⁻)	37	3	0	38	4	0	38	6.75	2.3
<i>cas</i> ^{D1}	13	3	0.5	13	3.6	0.5	13	6.6	1.3
<i>cas</i> ^{D3}	24	2.8	0.6	24	2.7*	0.9	24	5.7*	1.8
<i>cas</i> ^{D3} (#)	20	3.1	0.4	20	4	0	20	10.9	0.2
<i>grh</i> ^{IM}	14	2.9	0.3	16	3.8	0.4	16	7.25	1
<i>ins-Gal4 UAS-hb</i>	20	7.2*	1.6	20	6.5*	0.8	20	14.2*	1.6
<i>ins-Gal4 UAS-hb</i> (#)	11	6.5*	0.8	12	6.5*	0.5	12	16*	3.1
<i>elav-Gal4 UAS-hb</i>	17	4.1*	0.6	17	4.75*	0.6	17	7.9	1.3
<i>elav-Gal4 UAS-hb</i> (#)	16	4.7*	0.5	16	5.4*	0.6	16	12.6*	1
<i>svp</i> ^I	35	3.9*	1.1	35	5.1*	0.9	26	10.1*	2.3
<i>svp</i> ^I (#)	14	5.5*	1.5	14	6.1*	0.5	13	13.8*	1.4
<i>insG4-Gal4 UAS-Kr</i>	20	2.9	0.2	20	3.7	0.6	20	9.6	2.5
<i>wor-Gal4 UAS-pdm</i>	20	2.8	0.3	20	3.9	0.4	20	7.2	0.9
<i>wor-Gal4 UAS-cas</i>	17	3.1	0.7	17	3.9	0.9	17	10	1.1
<i>wor-Gal4 UAS-grh</i>	21	2.9	0.3	21	4.2	0.6	21	8	1.1
<i>dac</i> ⁴	21	3	1	21	3.7	0.7	21	7.6	1.1
<i>spdo</i> ^{G104}	12	2.25	1.3	11	3	1.5	12	2.5*	2
<i>spdo</i> ^{G104} (#)	9	3	1.8	9	5.2	2.5	10	9.25	2.2
<i>Df(3L)H99</i>	9	5.4*	1.8	10	4.2*	1.2	8	6.5	1.5
<i>Df(3L)H99</i> (#)	9	4.5*	0.5	11	5.8*	0.6	11	9.9	1.8
<i>ems</i> ^I	16	1.2*	0.9	22	1.3*	1.3	24	2.04*	2.1

Table S2. Levels of Hb expression in the NB3-5 lineage. Levels of Hb expression in the NB3-5 at stages 8-11. MGV: Mean Gray Values (average gray values within selected areas); IntDen: Integrated Density (area times Mean Gray Value); N = 6 in each stage; SD: standard deviation.

Stage 8	Area	MGV	IntDen
1	61.57	1355.32	83443.69
2	57.3	1414.99	81072.32
3	62.15	1178.05	73211.39
4	70.27	1062.91	74685.95
5	60.95	1481.41	90299.1
6	59.37	1215.19	72148.36
Mean	61.935	1284.645	79143.46833
SD		158.7791807	7080.269157
Stage 9	Area	MGV	IntDen
1	51.27	1304.11	66861.31
2	45.74	1733.94	79306.01
3	61.77	1665.8	102899.48
4	59.51	1856.26	110462.57
5	74.21	1182.22	87737.99
6	41.38	2536.9	104976.39
Mean	55.64666667	1713.205	92040.625
SD		479.77477	16966.42447
Stage 10	Area	MGV	IntDen
1	43.76	2141.86	93733.93
2	57.98	1999.16	115903.33
3	58.52	2293	134188.24
4	64.65	2357.71	152422.48
5	65.69	2016.36	132448.52
6	68.43	1932.09	132207.78
Mean	59.83833333	2123.363333	126817.38
SD		171.7549155	19921.5864
Stage 11	Area	MGV	IntDen
1	45.53	276.19	12575.75
2	29.29	246.97	7234.86
3	40.05	265.87	10648.53
4	33.79	236.79	8000.6
5	59.05	308.5	18216.71
6	30.79	322.54	9931.9
Mean	39.75	276.1433333	11101.39167
SD		33.77844382	3973.392959