Supplemental Figure 1: Targeted fluorescent marking of subpopulations of neural crest cells in vivo within migratory streams using UV-excitation to photoactivate

KikGR. (A) A typical 7-9 somite embryo injected with KikGR was re-incubated for 10-14hrs and shows the hindbrain region, r4, and KikGR-expressing r4 NCCs (green cells within box). (A') The same embryo from (A) was photoactivated (within the box) using 405nm laser light. The photoactivated cells (shown in red) are excited by the 561nm laser. (B) A typical photoactivated embryo after 24hrs of re-incubation showing the r4 region, photoactivated NCCs (arrowhead) and 2nd branchial arch (ba2). (C) A typical 7-9 somite embryo was electroporated with KikGR and re-incubated for 10-14hrs. (D) A line profile though the r4 NCC migratory stream (the yellow line in (C)) shows that the green has higher fluorescence intensity than the red throughout the entire migratory stream, that is, pre-photoactivation. (E) An area of the same r4 NCC migratory stream (in C) was for selected for photoactivation (dotted line box). (F) A line profile (the yellow line in E) shows that in the photoactivated region the red intensity is larger in magnitude than the green fluorescence signal. (G) A typical control embryo in which the entire r4 NCC migratory stream has been photoactivated from green to red and the (H) corresponding distribution of NCCs is shown. (I,J) 24hrs after egg re-incubation, the same embryo shows large number of photoactivated NCCs (red), and includes some green-colored non-photoactivated NCCs that have emigrated into the field of view from the non-photoactivated neural tube region. (K) A control embryo electroporated with KikGR was imaged 10-14hrs after electroporation. No cells were photoactivated (this embryo was never exposed to 405nm laser light). (L) The % of total cell number versus the % length of the r4 NCC migratory stream is measured and shown in bar graphs. (M, N) 24hrs after egg re-incubation, the same embryo shows that the cells have not obtained any red fluorescence over the re-incubation period. The length of the lines in C and E are

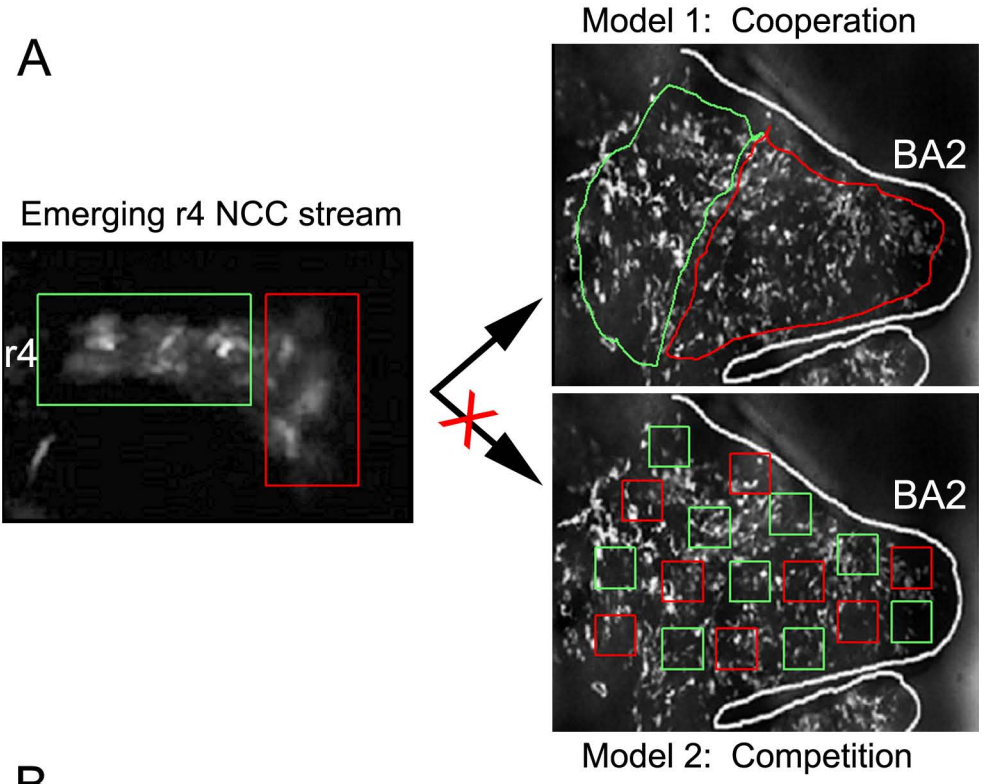
250um. The green and red lines in D and F represent the fluorescence signal detected after 488nm excitation (green) and 561nm excitation (red). r4=rhombomere 4, BA2=branchial arch 2, AFU=arbitrary fluorescence units.

Supplemental Figure 2: Model representation of the r4 neural crest cell migratory stream invasion order into the 2nd branchial arch and comparison of the migratory order between experimental and computer simulation data. (A) Two possible r4 NCC migration models under investigation. A newly emerging r4 NCC stream population can migrate and invade a distal target in at least two ways. First, NCCs may respect their order of emergence from the neural tube and maintain their spatial order (Model 1: Cooperation). Alternatively, NCCs may compete for lead positions within the r4 stream and invade BA2 in a salt and pepper mix (Model 2: Competition). Our analysis supports Model 1. Cell proliferation, cell mixing and cell death are not considered in these basic models. (B) A comparison of the final order of lead and trailing NCCs along the r4 migratory route from experimental and hypothetical computer simulations. Green refers to the area containing only trailing NCCs, yellow refers to the area containing a mixed population of both lead and trailing NCCs, and red refers to the area containing only lead NCCs. r4= rhombomere 4, BA2= branchial arch 2.

Movie 1: Typical cell division of KikGR-photoactivated NCCs along the r4 NCC migratory route, with a time interval of 2min between frames for a total of approximately 40min.

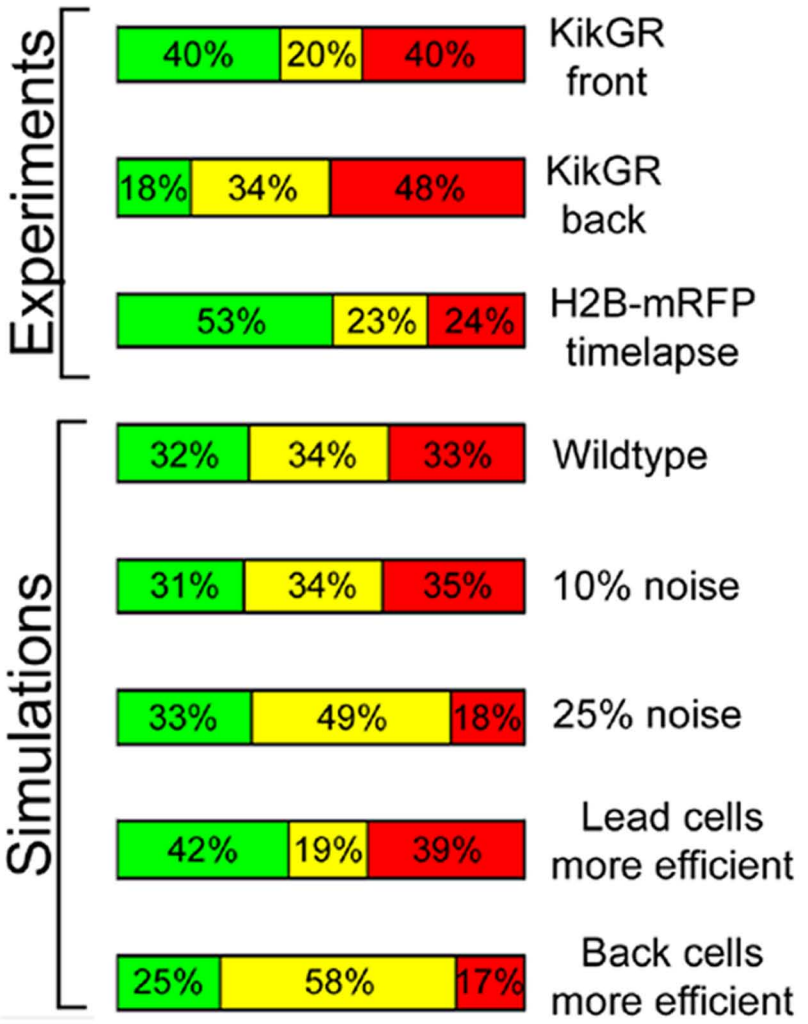
Movie 2: Time-lapse confocal session of H2B-mRFP1 transfected NCCs migrating with the r4 NCC migratory stream in a whole chick embryo explant, with a 5min interval between frames for a total time of approximately 8hrs.

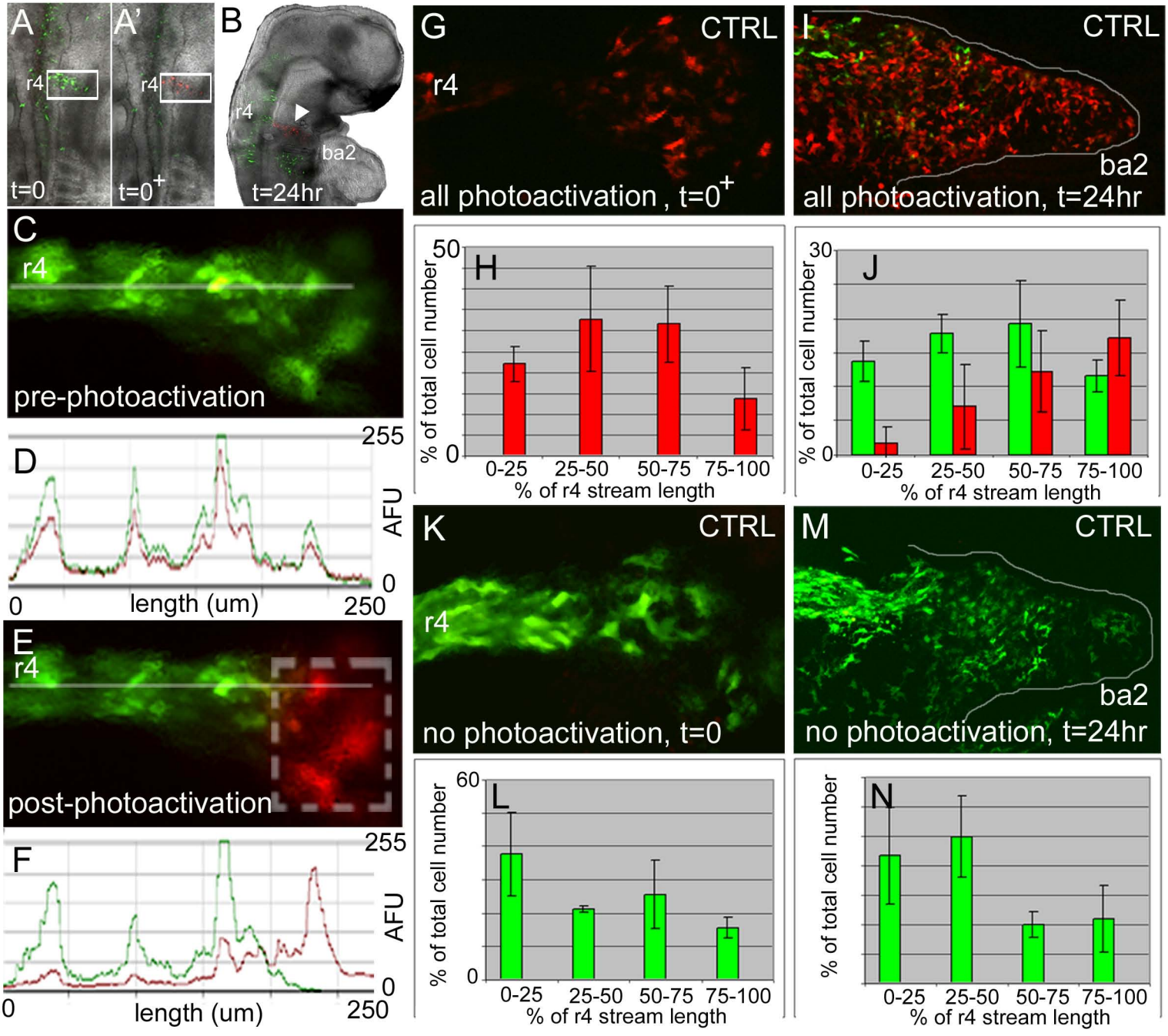
Movie 3: Cell tracking analysis of Movie 2, with the H2B-mRFP1 transfected NCCs identified and tracked with spot identification.



B

From the neural tube to distal BA2 (r4 NCC migratory route)




A
A'
B
r4
r4
r4
ba2
t=0
t=0+
t=24hr
C
r4
pre-photoactivation
D
255
AFU
0
0
length (um)
250
E
r4
post-photoactivation
F
255
AFU
0
0
length (um)
250
G
CTRL
r4
all photoactivation , t=0+
I
CTRL
ba2
all photoactivation, t=24hr
H
50
0
% of total cell number
0-25
25-50
50-75
75-100
% of r4 stream length
J
30
0
% of total cell number
0-25
25-50
50-75
75-100
% of r4 stream length
K
CTRL
r4
no photoactivation, t=0
M
CTRL
ba2
no photoactivation, t=24hr
L
60
0
% of total cell number
0-25
25-50
50-75
75-100
% of r4 stream length
N
% of total cell number
0-25
25-50
50-75
75-100
% of r4 stream length