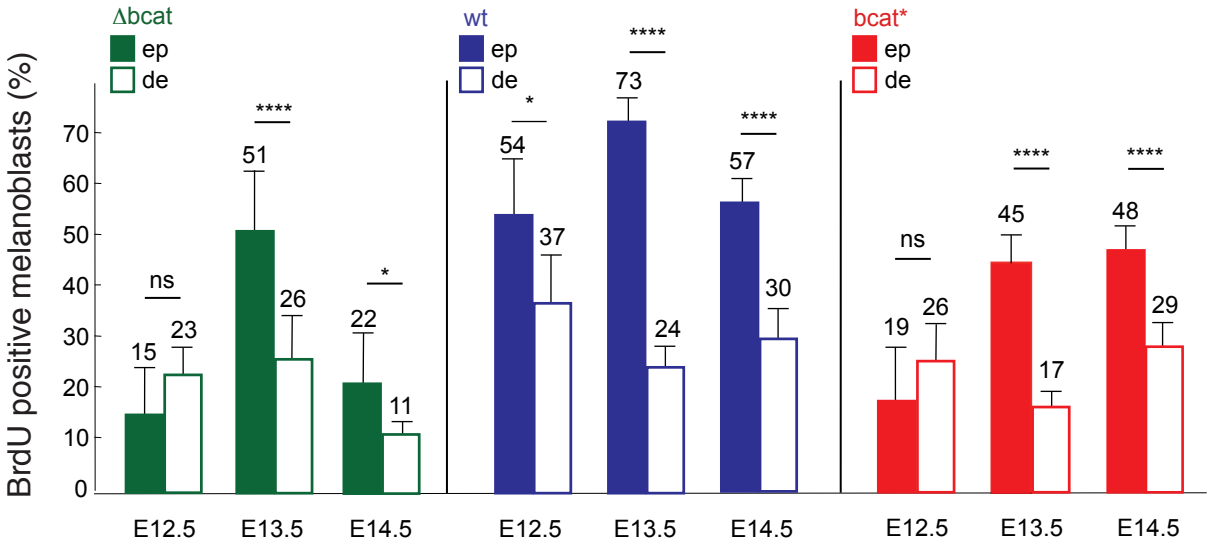
Δbcat
ep
de
wt
ep
de
bcat*
ep
de
BrdU positive melanoblasts (%)
0
10
20
30
40
50
60
70
ns
23
15
****
51
26
*
22
11
*
54
37
****
73
24
****
57
30
ns
19
26
****
45
17
****
48
29
E12.5
E13.5
E14.5
E12.5
E13.5
E14.5
E12.5
E13.5
E14.5

Figure S4. Establishing bounds to evaluate $\kappa_\theta(t)$

The proliferation rate of melanoblasts between E12.5 and E14.5 in Δbcat, wt and bcat* embryos was evaluated from BrdU incorporation experiments (see Figure 5). Statistical significance was calculated by comparing the proliferation rate of epidermal and dermal melanoblasts for each genotype at each stage with the Mann-Whitney test (StatEL) and is indicated, **** : p-value < 10^{-5}, * : p-value < 10^{-2}, ns=non significant. The proliferation rate of melanoblasts in the epidermis and that in the dermis were not significantly different at E12.5 for Δbcat and bcat* ($\tau_e = \tau_d$). With this exception, the proliferation rates of melanoblasts differed significantly between the dermis and epidermis on all developmental days and for each genotype (at least p < 10^{-2}). The proliferation rate is inversely correlated to the doubling time, so the doubling times of melanoblasts in epidermis are shorter than or similar to those in the dermis ($\tau_e \leq \tau_d$). An upper limit was established from biological findings. At this stage, cells are expected to be uniformly distributed around the cell cycle. BrdU labels cells in the S phase, such that the proportion of BrdU-positive cells indicates the proportion of cells in S phase. Assuming that the S phase is of similar length in the dermis and epidermis, then the relative lengths of the cell cycles can be estimated (%BrdU-positive in epidermis / %BrdU-positive in dermis). Experimentally, we found that %BrdU-positive in epidermis / %BrdU-positive in dermis was never greater than 3, such that $3\mu_d \geq \mu_e$. Indeed, in every case, τ_d was found to be lower than $3\tau_e$ (Δbcat : 74 h < 147 h [3*49], for wt 28h < 54 h [3*18] and for bcat* 31h < 69 h [3*23]).

These limits allow for bounds to be established for the unknown function $\kappa_\theta(t)$ by comparing the proliferation rate of melanoblasts between E12.5 and E14.5 in Δbcat, wt and bcat* embryos. Because of the uncertainty on the κ function, each $\kappa(t^i)$ at development day $E(t^i)$ was modeled as a random variable. As a first assessment, we decided to use Gaussian variables only involving expectation (E) values of $\kappa(t^i)$ and standard deviations of $\kappa(t^i)$. Both expectation values and standard deviations were roughly estimated from the bounds on

the κ function $E\left(\kappa\left(t_i\right)\right)=\frac{\kappa_{\min}\left(t_i\right)+\kappa_{\max}\left(t_i\right)}{2}$ and $\sigma_i=\frac{\kappa_{\max}\left(t_i\right)-\kappa_{\min}\left(t_i\right)}{2}$. The values obtained were reproducible and were biologically sound. This allowed us to design $\kappa(t)$ as random variable, Kmin ≤ K ≤ Kmax, which is $\max\left(0,\hat{c}(t)-\frac{2}{3-3y_\theta(t)}\hat{\mu}(t)\right)\le\kappa_\theta(t)\le\hat{c}(t)$. Bars represent standard deviation. Therefore $\kappa_\theta\min(t)=\max\left(0,\,c_\theta(t)-2\mu_\theta(t)/3-3y_\theta(t)\right)$ and $\kappa_\theta\max(t)=c_\theta(t)$. These two equations are derived from the following steps : starting from $3\mu_{d,\theta}\ge\mu_\theta$, then $\mu_{d,\theta}-\mu_\theta\ge-2\mu_\theta/3$, then $\left(\mu_{d,\theta}-\mu_\theta\right)/\left(1-y_\theta\right)\ge-2\mu_\theta/\left(3-3y_\theta\right)$, and $\mu_{d,\theta}-\mu_\theta=-\left(1-y_\theta\right)\left(c_\theta-\kappa_\theta\right)$ [9], then $\kappa_\theta\ge c_\theta-2\mu_\theta/\left(3-3y_\theta\right)$, therefore $\kappa_\theta\min=c_\theta-2\mu_\theta/\left(3-3y_\theta\right)$. Starting from $\mu_{d,\theta}=\mu_\theta-\left(1-y_\theta\right)\left(c_\theta-\kappa_\theta\right)$ [9] and $\mu_{\theta,d}\le\mu_\theta$, then $\kappa_\theta=c_\theta+\left(\mu_{d,\theta}-\mu_\theta\right)/\left(1-y_\theta\right)$, and then $\kappa_\theta\max(t)=c_\theta(t)$.