Supplementary data

Temperature-sensitive migration dynamics in neutrophil-differentiated HL-60 cells

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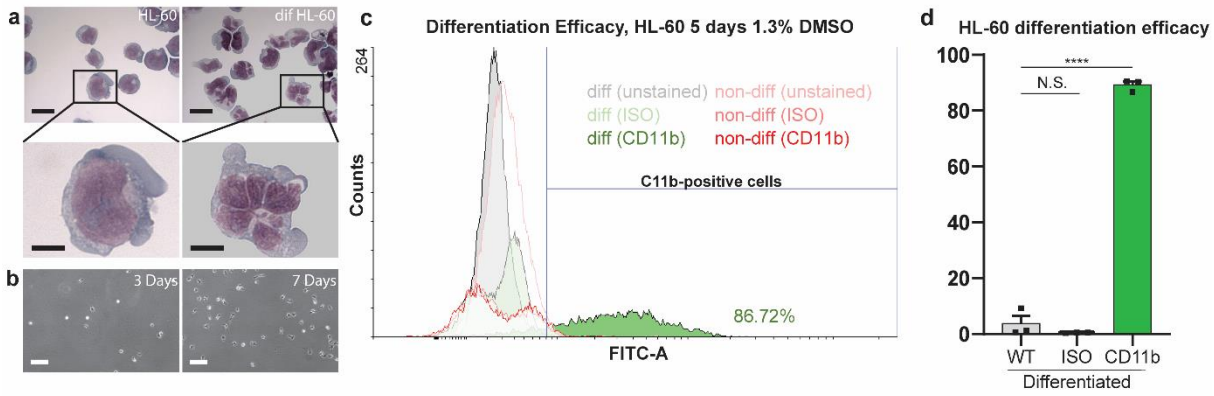
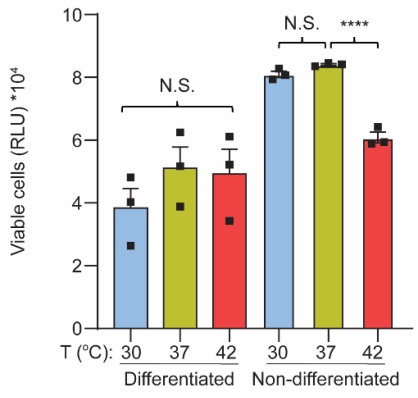
Supplemental Video: Cell detachment during temperature sweep. HL-60 cells plated on glass exposed to a temperature sweep from 37-43 ºC before rapid return to 37 ºC. Images were obtained every 10 seconds for nearly 2.5 hours.

Supplemental Figure 1



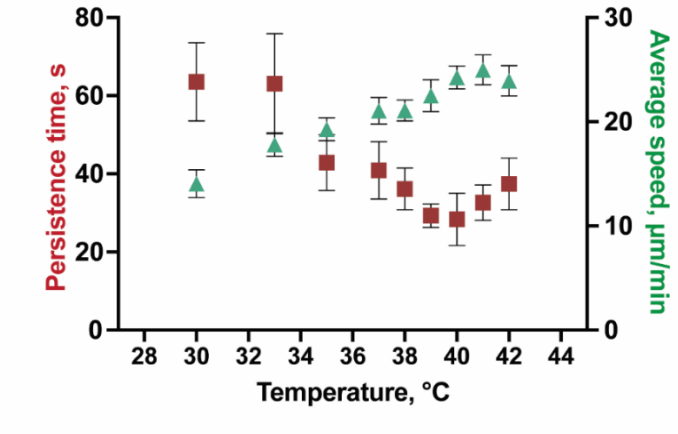
Supplemental Figure 1: Successful differentiation of HL-60 cells into neutrophil-like cells. **a.** Giemsa-Wright staining of HL-60 cells before (left) and 7 days after differentiation (right). Scale bars: 50 µm (top) and 20 µm (bottom). The majority of differentiated HL-60 cells exhibit lobed nuclei characteristic to blood PMN cells. **b.** 10x phase contrast images 3 days and 7 days after the start of differentiation. The amount of attached cells increases up to 10 fold. Scale bars: 50 µm. **c.** Flow cytometry of differentiated HL-60 cells after 5 days of 1.3% DMSO treatment. **d.** Differentiation efficiency of HL-60 cells as measured by CD11b expression.

Supplemental Figure 2



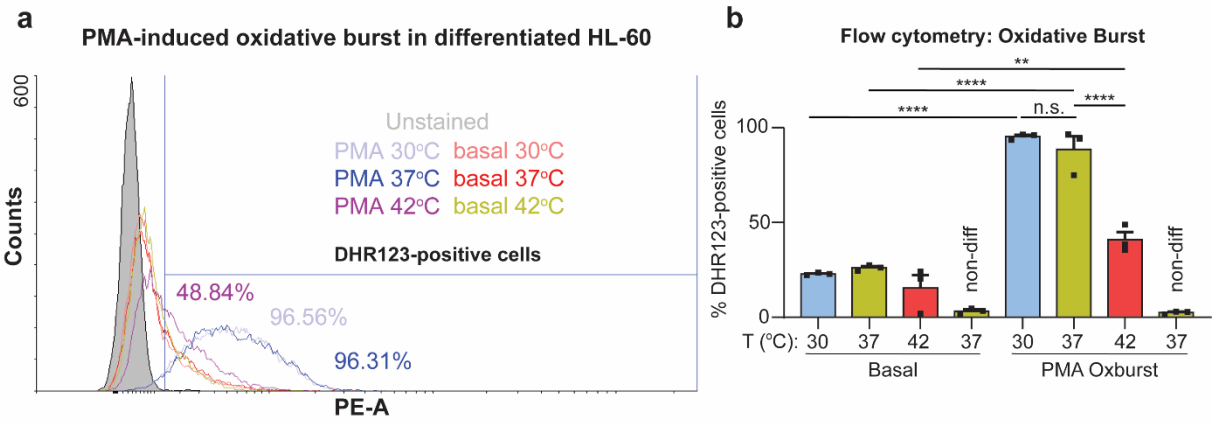
Supplemental Figure 2: HL-60 cell survival as a function of temperature. ATP-based cell viability determined with CellTiter-Glo assay to assess the relative level of viable cells at 30, 37, and 42 °C.

Supplemental Figure 3



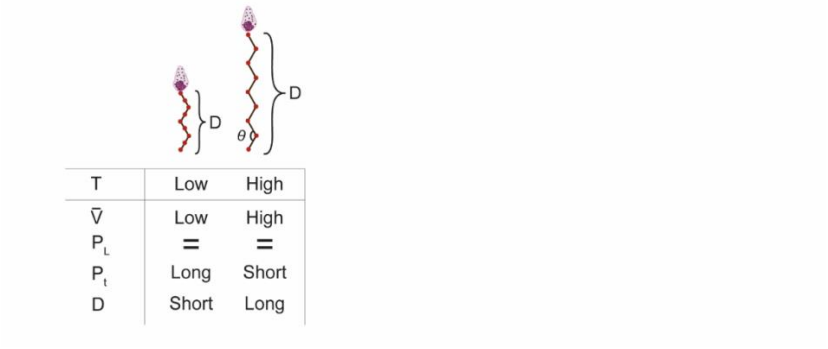
Supplemental Figure 3: Relationship between average cell speed and migration persistence time for differentiated HL-60 cells at control conditions. With increasing temperature cell average speed increases (right axis, green), but migration persistence time decreases (left axis, red).

Supplemental Figure 4



Supplemental Figure 4: Oxidative burst in differentiated HL-60 cells as a function of temperature. **a)** Flow cytometry of PMA-induced oxidative burst in differentiated and non-differentiated HL-60 cells. **b)** Quantitative analysis of the percentage of DHR123-positive cells with and without PMA induction.

Supplemental Figure 5



T	Low	High
$\bar{V}$	Low	High
P_L	=	=
P_t	Long	Short
D	Short	Long

Supplemental Figure 5: Higher speeds, lower persistence times, and equal persistence lengths allow cells to migrate more effectively at higher temperatures. An illustration of cell tracks at low and high temperatures. V is the average velocity, P_L is the persistence length, P_t is the persistence time and D is the length of the cell track. At low temperatures, cells have a long persistence time and short track lengths. At higher temperatures, cells have a short persistence time but long track lengths. As a result, persistence length stays the same at both low and high temperatures.