SUPPLEMENTARY APPENDIX

Finely-tuned regulation of AMP-activated protein kinase is crucial for human adult erythropoiesis

Meriem Ladli,[1,2,3,4] Cyrielle Richard[1,2,3,4], Lilia Cantero Aguilar,[1,2,3,4] Sarah Ducamp,[1,2,3,4] Sabrina Bondu,[1,2,3,4] Pierre Sujobert,[1,2,3] Jérôme Tamburini,[1,2,3] Catherine Lacombe,[1,2,3,4] Nabih Azar,[5] Marc Foretz,[1,2,3,4] Yael Zermati,[1,2,3,4] Patrick Mayeux,[1,2,3,4] Benoit Viollet[1,2,3,4] and Frédérique Verdier[1,2,3,4]

[1]Institut Cochin, INSERM U1016; [2]CNRS UMR 8104, Paris; [3]Université Paris Descartes, Sorbonne Paris Cité; [4]Labex GREX and [5]Service d'Hémobiologie, Hôpital La Pitié Salpétrière, Paris, France

 doi:10.3324/haematol.2018.191403

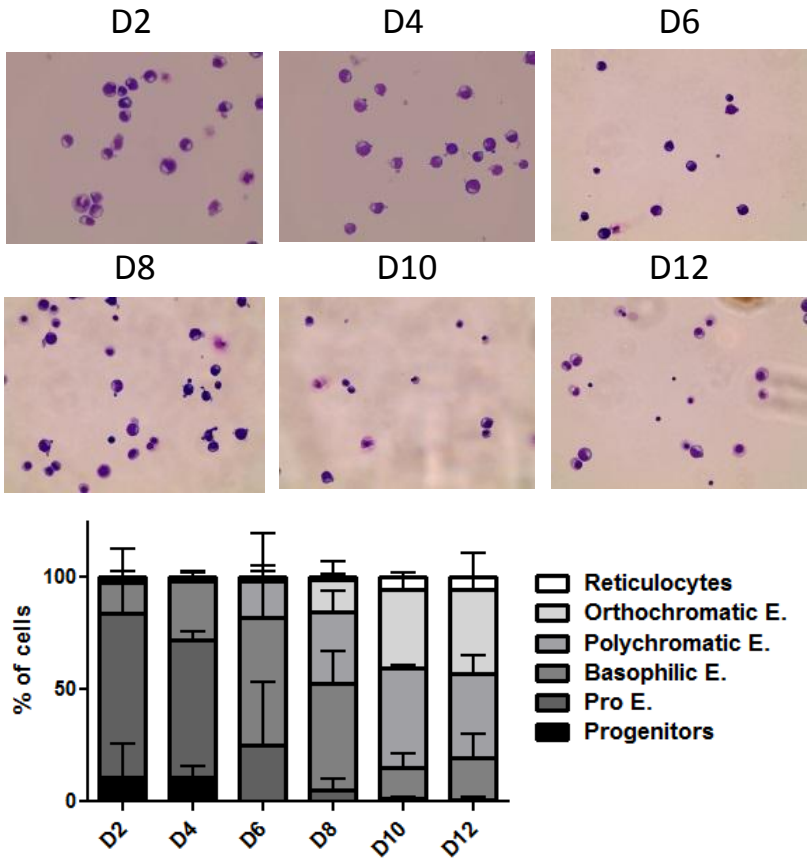
Received: February 18, 2018.

Accepted: October 3, 2018.

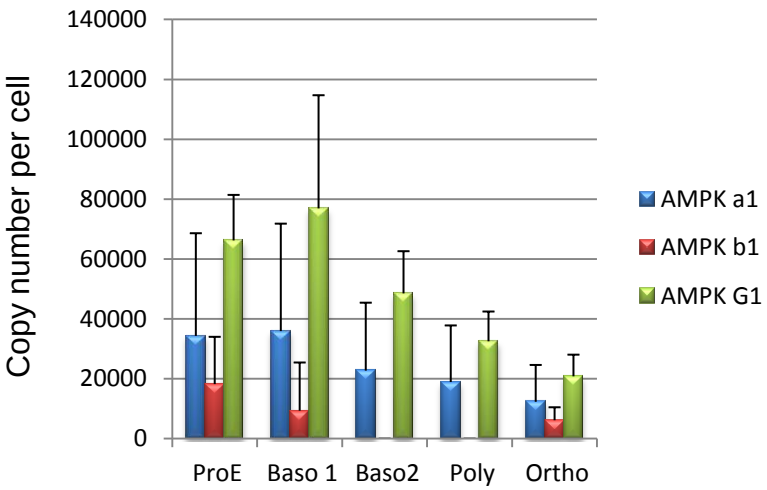
Pre-published: October 11, 2018.

Correspondence: FRÉDÉRIQUE VERDIER frederique.verdier@inserm.fr

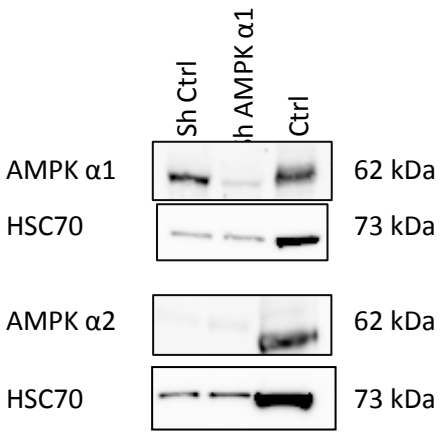
S1


D2
D4
D6
D8
D10
D12
% of cells
100
50
0
D2
D4
D6
D8
D10
D12
Reticulocytes
Orthochromatic E.
Polychromatic E.
Basophilic E.
Pro E.
Progenitors

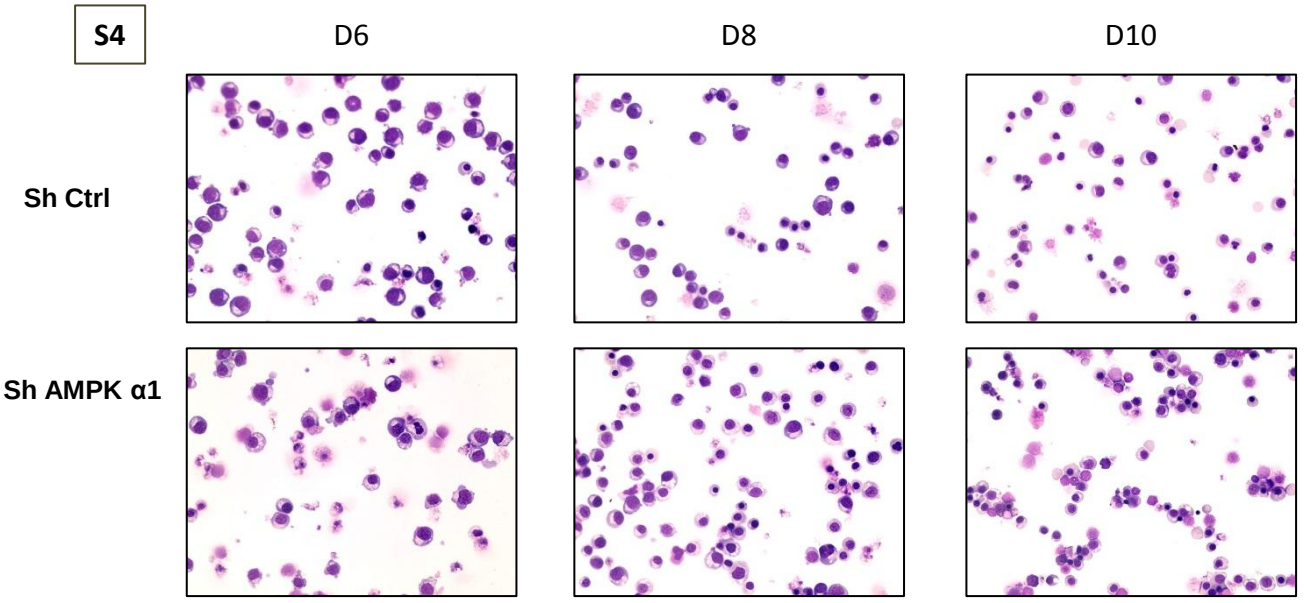
S2


Copy number per cell
140000
120000
100000
80000
60000
40000
20000
0
ProE
Baso 1
Baso2
Poly
Ortho
AMPK a1
AMPK b1
AMPK G1

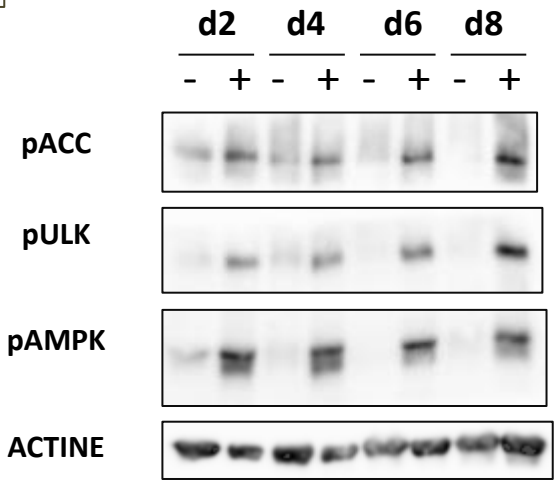
S3


Sh Ctrl
h AMPK α1
Ctrl
AMPK α1
62 kDa
HSC70
73 kDa
AMPK α2
62 kDa
HSC70
73 kDa

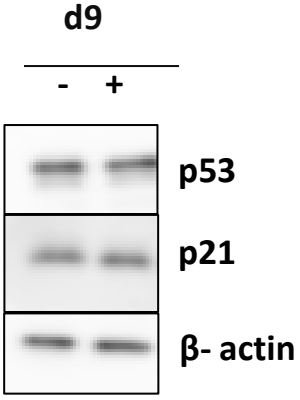
S4


D6
D8
D10
Sh Ctrl
Sh AMPK α1

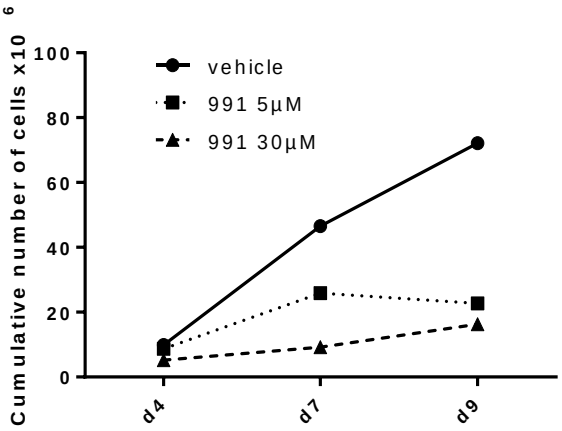
S5

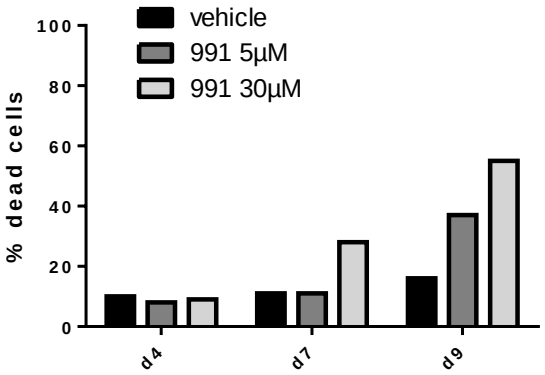

d2
d4
d6
d8
- + - + - + - +
pACC
pULK
pAMPK
ACTINE

S6

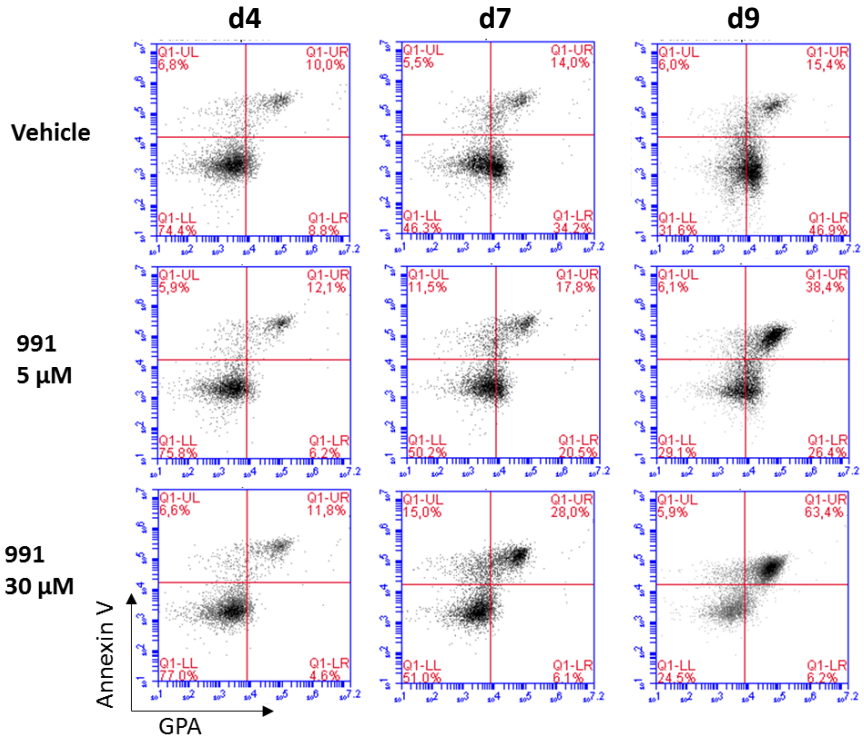

d9
- +
p53
p21
β- actin

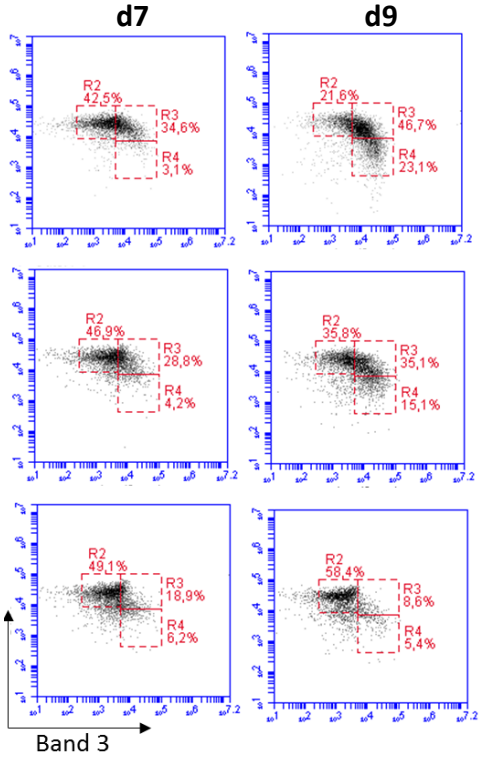
S7A

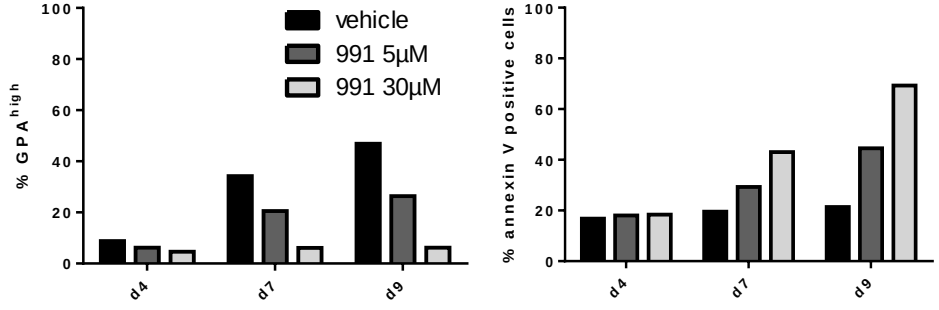

Cumulative number of cells x10 6
vehicle
991 5µM
991 30µM
d4
d7
d9


% dead cells
vehicle
991 5µM
991 30µM
d4
d7
d9

S7B


d4
d7
d9
Vehicle
991 5 µM
991 30 µM
Annexin V
GPA


d7
d9
Band 3


% GPA high
vehicle
991 5µM
991 30µM
d4
d7
d9

% annexin V positive cells
d4
d7
d9

Figure S1: Morphology analysis was performed by May-Grumwald-Giemsa coloration; a representative experiment is shown at d2, d4, d6, d8, d10, d12. The mean cell composition from 3 independent experiments ±SD was calculated according to the erythroblast morphology determined by MGG staining.

Figure S2: Determination of AMPK Isoforms during erythropoiesis by quantitative proteomic. Copy number of proteins in each differentiation stage was determined from data extracted from our work in [11].

Figure S3: Efficiency of the Sh AMPKα1.Protein extracts from Sh AMPKα1 vs. Sh Ctrl cells at d8 of culture were analyzed by WB using anti-AMPKα1, α2 antibodies. Anti-HSC70 was used as a loading control and HEK 293 protein extracts were used as a positive control for the expression of AMPK α2 (Ctrl).

Figure S4: Morphology analysis of Sh AMPKα1 vs. Sh Ctrl cells was performed by May-Grumwald-Giemsa coloration; a representative experiment is shown at d6, d8 and d10.

Figure S5: **GSK621-induced activation of AMPKα1 along erythroid maturation**. 20µM GSK621 was added at d1, Protein samples from primary erythroblasts at d2, d4, d6 and d8 were analysed by Western Blot using pT172 AMPKα1, pS79 ACC and pS555 ULK1 antibodies. Anti-β ACTIN antibodies were used as loading control.

Figure S6: Expression of proteins regulating the cell cycle. P53 and P21 were detected by WB experiments using specific antibodies. β-ACTIN was used as a loading control.

Figure S7: 991-mediated AMPK activation leads to apoptosis of mature GPA^{High} erythroblasts. (A) Inhibition of cell proliferation and increased cell death in the presence of 991. Erythroid cells were incubated in the absence (vehicle) or presence of 5 or 30µM 991 from d0 to the indicated days of culture. Cumulative cell number (left panel) and proportion of dead cells (right panel) were determined by counting cells with Trypan blue exclusion method. **(B)** Expression of cell surface markers and Annexin V in the presence of 991. Cells were labeled with anti-GPA and for Annexin V binding and Band3/α4 integrin, then analyzed by flow cytometry. A representative

experiment and histogram with the percentage of GPA^{high} and Annexin V positive cells are shown.