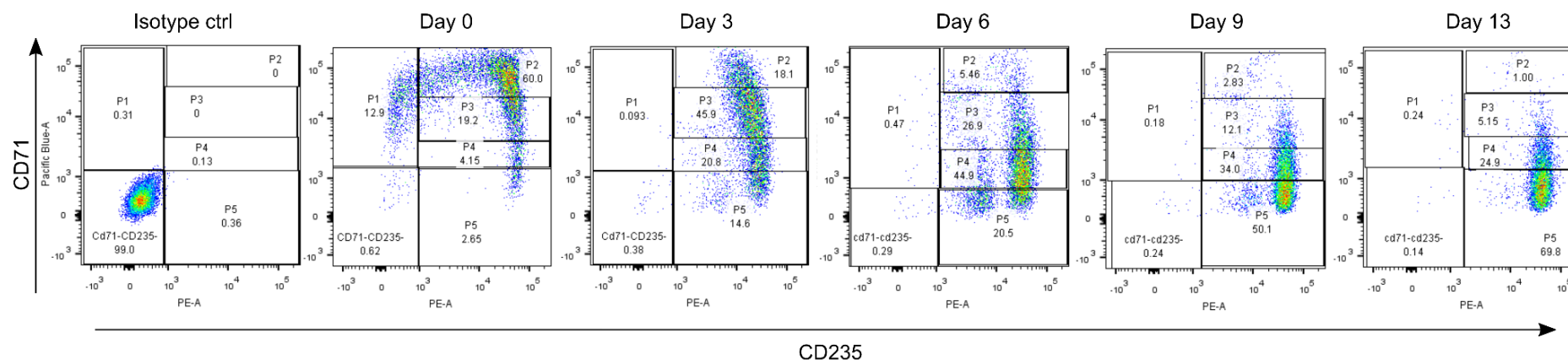


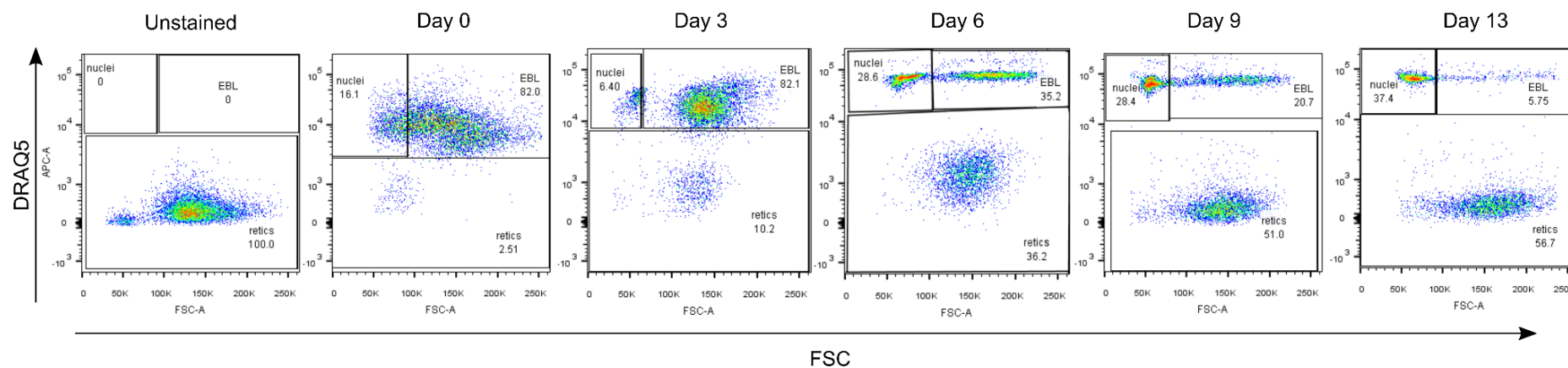
**Figure S1.** Neocytolysis study timeline, divided into three phases: in the pre-altitude phase (day 0) RBC were age-cohort labeled with the isotope  $^{13}\text{C}$ -glycine; a second age-cohort labeling with  $^{15}\text{N}$  was performed at high altitude (day 10). RBC decay was regularly measured by isotope ratio mass spectrometry haem ( $^{13}\text{C}/^{12}\text{C}$  in the pre-altitude phase and  $^{15}\text{N}/^{14}\text{N}$  during high altitude and post-altitude phases) to reveal any difference in RBC lifespan between the two cohorts that could demonstrate the existence of neocytolysis. PBMC collection for *in vitro* erythropoiesis was performed at day 140 pre-altitude and day 19 high altitude (JJ samples). For more information on the study, see Klein et al. [11].

# **A CD71 & CD235 expression during differentiation**

P1: CD71<sup>high</sup> CD235<sup>-</sup>  
P2: CD71<sup>high</sup> CD235<sup>high</sup>  
P3: CD71<sup>med</sup> CD235<sup>high</sup>  
P4: CD71<sup>low</sup> CD235<sup>high</sup>  
P5: CD71<sup>-</sup> CD235<sup>high</sup>

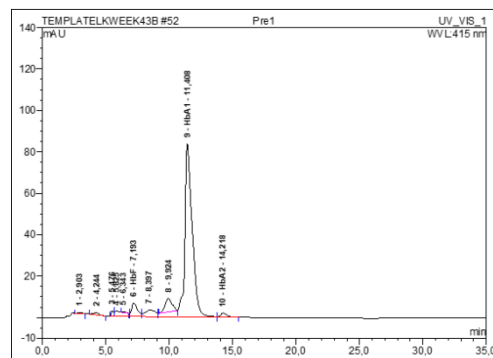


# **B Enucleation throughout differentiation**



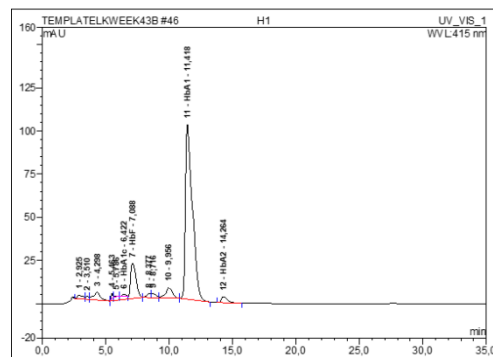
**Figure S2.** Representative flow cytometry dot plot on one donor over differentiation. (A) Population gating of erythroid precursors at different stages of maturation according to the expression of CD71 and CD235. CD71<sup>+</sup>CD235<sup>+</sup> indicate non-erythroid cells. (B) DRAQ5 nuclear staining vs FSC. Positive events refer to nucleated cells (EBL=erythroblasts) and pyrenocytes (nuclei) and negative events to enucleated reticulocytes.

# Donor 1 at 20% O<sub>2</sub>



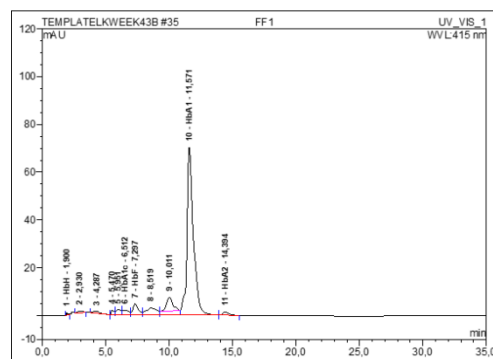
No.	Ret.Time min	Peak Name	Rel.Area %
1	2.90	n.a.	0.46
2	4.24	n.a.	0.76
3	5.48	n.a.	0.78
4	5.83	n.a.	3.35
5	6.34	n.a.	0.20
6	7.19	HbF	4.67
7	8.40	n.a.	4.06
8	9.92	n.a.	5.51
9	11.41	HbA1	78.81
10	14.22	HbA2	1.39
Total:			100.00

# Donor 1 at 3% O<sub>2</sub>



No.	Ret.Time min	Peak Name	Rel.Area %
1	2.92	n.a.	1.18
2	3.51	n.a.	0.59
3	4.30	n.a.	3.16
4	5.46	n.a.	0.49
5	5.79	n.a.	0.12
6	6.42	HbA1c	0.50
7	7.09	HbF	14.03
8	8.38	n.a.	0.92
9	8.72	n.a.	1.12
10	9.96	n.a.	4.10
11	11.42	HbA1	71.70
12	14.26	HbA2	2.08
Total:			100.00

# Donor 1 at 20% O<sub>2</sub> JJ



No.	Ret.Time min	Peak Name	Rel.Area %
1	1.90	HbH	0.20
2	2.93	n.a.	0.59
3	4.29	n.a.	0.80
4	5.47	n.a.	0.84
5	5.95	n.a.	1.93
6	6.51	HbA1c	1.86
7	7.30	HbF	4.02
8	8.52	n.a.	5.00
9	10.01	n.a.	6.02
10	11.57	HbA1	77.46
11	14.39	HbA2	1.26
Total:			100.00

**Figure S3.** Representative examples of primary HPLC data. Donor 1 in the three culture conditions.



**Figure S4.** STRING analysis [59] of significantly differentially abundant proteins between the 3% and 20% O<sub>2</sub> cultures. Clusters are defined in figure 6D. Proteins are highlighted in different colors according to their functional group.