

Neocytolysis study phases

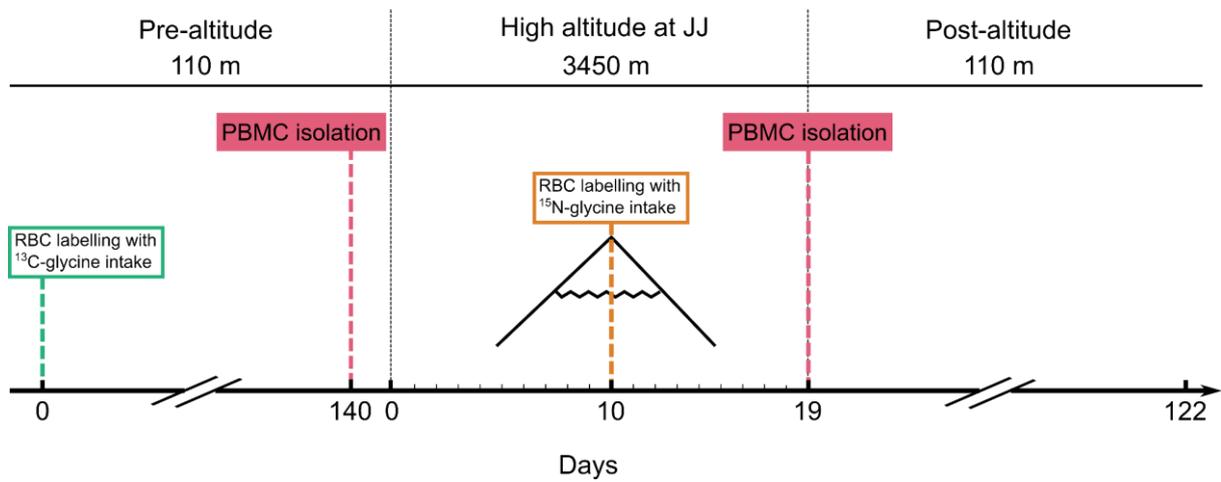
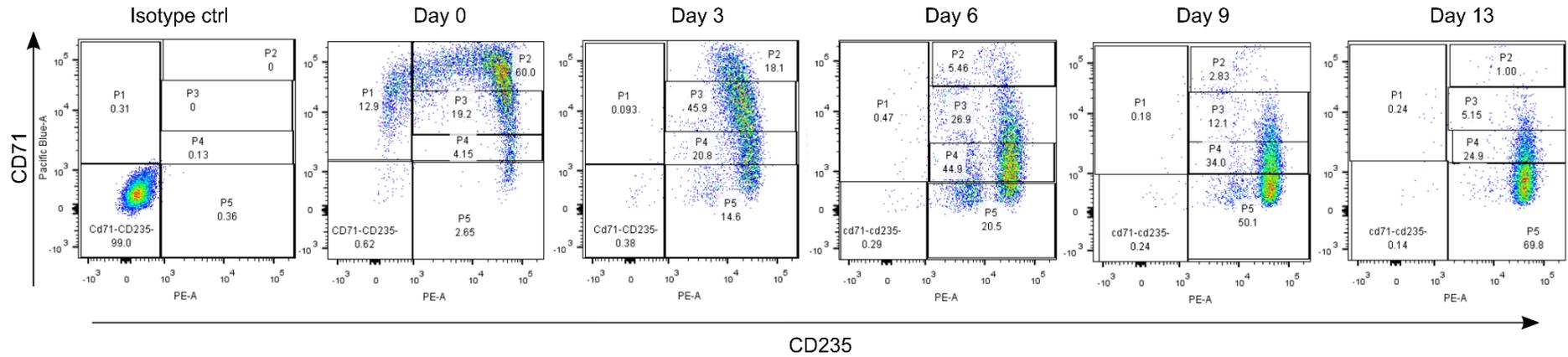


Figure S1. Neocytolysis study timeline, divided into three phases: in the pre-altitude phase (day 0) RBC were age-cohort labeled with the isotope ^{13}C -glycine; a second age-cohort labeling with ^{15}N was performed at high altitude (day 10). RBC decay was regularly measured by isotope ratio mass spectrometry 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^{high} CD235⁻
 P2: CD71^{high} CD235^{high}
 P3: CD71^{med} CD235^{high}
 P4: CD71^{low} CD235^{high}
 P5: CD71⁻ CD235^{high}



B Enucleation throughout differentiation

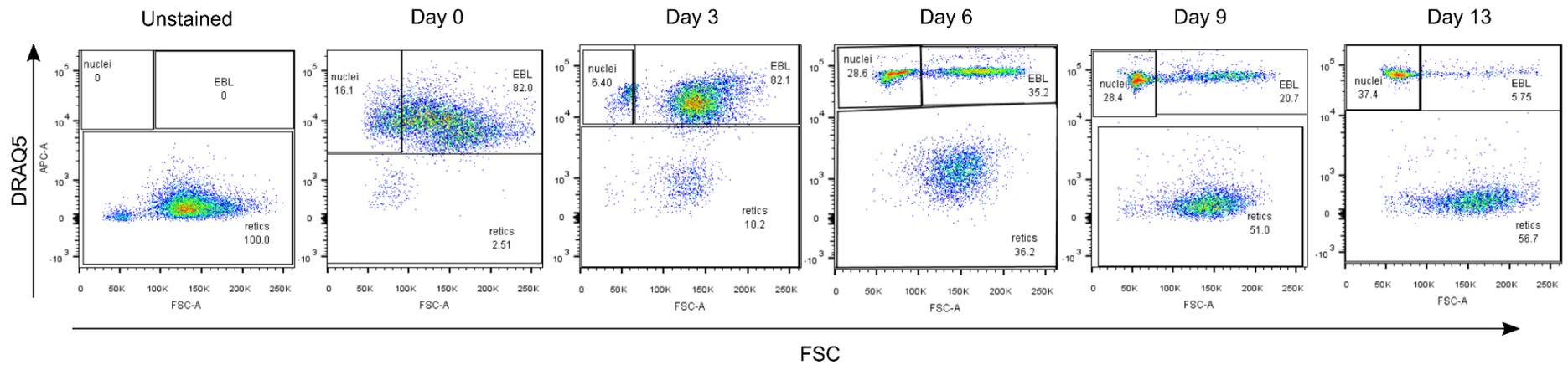
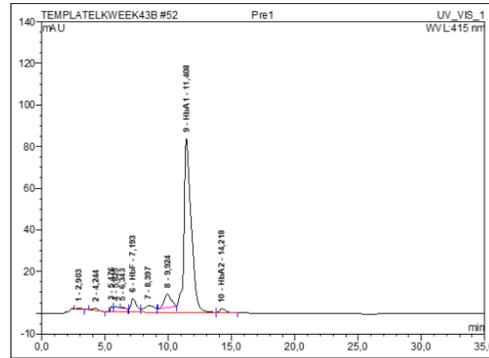


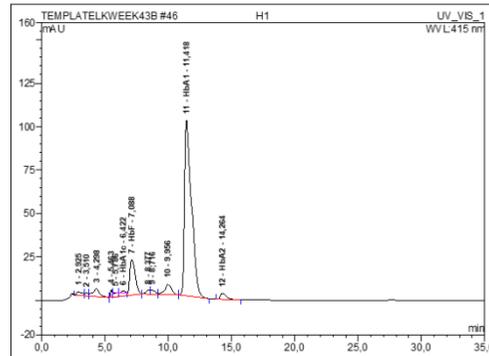
Figure S2. Representative flow cytometry dot plot on one donor over differentiation. (A) Population gating of erythroid precursors at different stages of maturation according to the expression of CD71 and CD235. CD71⁻CD235⁻ indicate non-erythroid cells. (B) DRAQ5 nuclear staining vs FSC. Positive events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

Donor 1 at 20% O₂



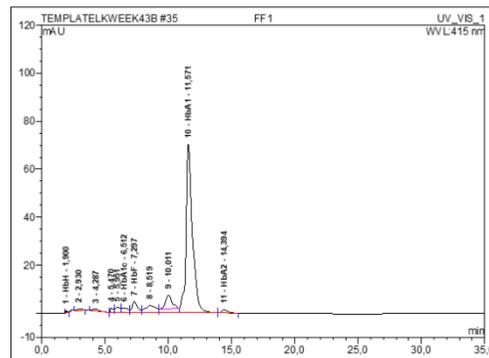
No.	Ret.Time min	Peak Name	Rel.Area %
1	2.90	n.a.	0.46
2	4.24	n.a.	0.76
3	5.48	n.a.	0.78
4	5.83	n.a.	3.35
5	6.34	n.a.	0.20
6	7.19	HbF	4.67
7	8.40	n.a.	4.06
8	9.92	n.a.	5.51
9	11.41	HbA1	78.81
10	14.22	HbA2	1.39
Total:			100.00

Donor 1 at 3% O₂



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

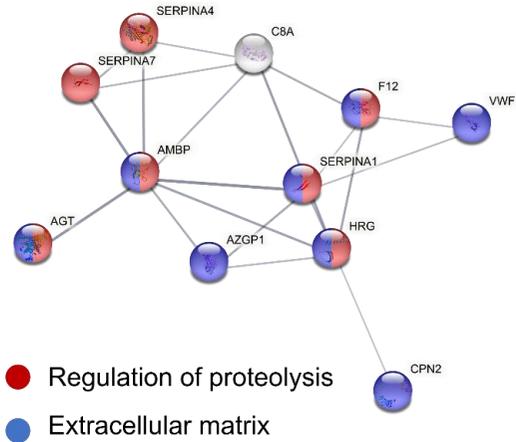
Donor 1 at 20% O₂ JJ



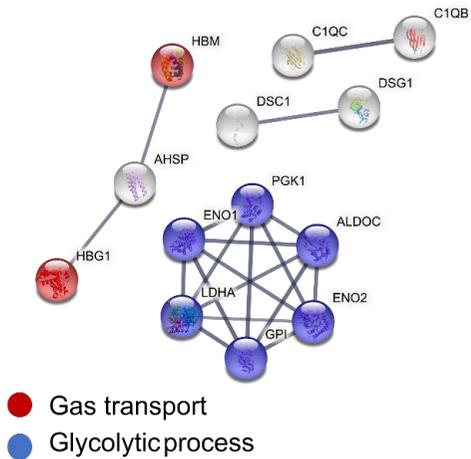
No.	Ret.Time min	Peak Name	Rel.Area %
1	1.90	HbH	0.20
2	2.93	n.a.	0.59
3	4.29	n.a.	0.80
4	5.47	n.a.	0.84
5	5.95	n.a.	1.93
6	6.51	HbA1c	1.86
7	7.30	HbF	4.02
8	8.52	n.a.	5.00
9	10.01	n.a.	6.02
10	11.57	HbA1	77.46
11	14.39	HbA2	1.26
Total:			100.00

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

Cluster 1



Cluster 3



Cluster 2

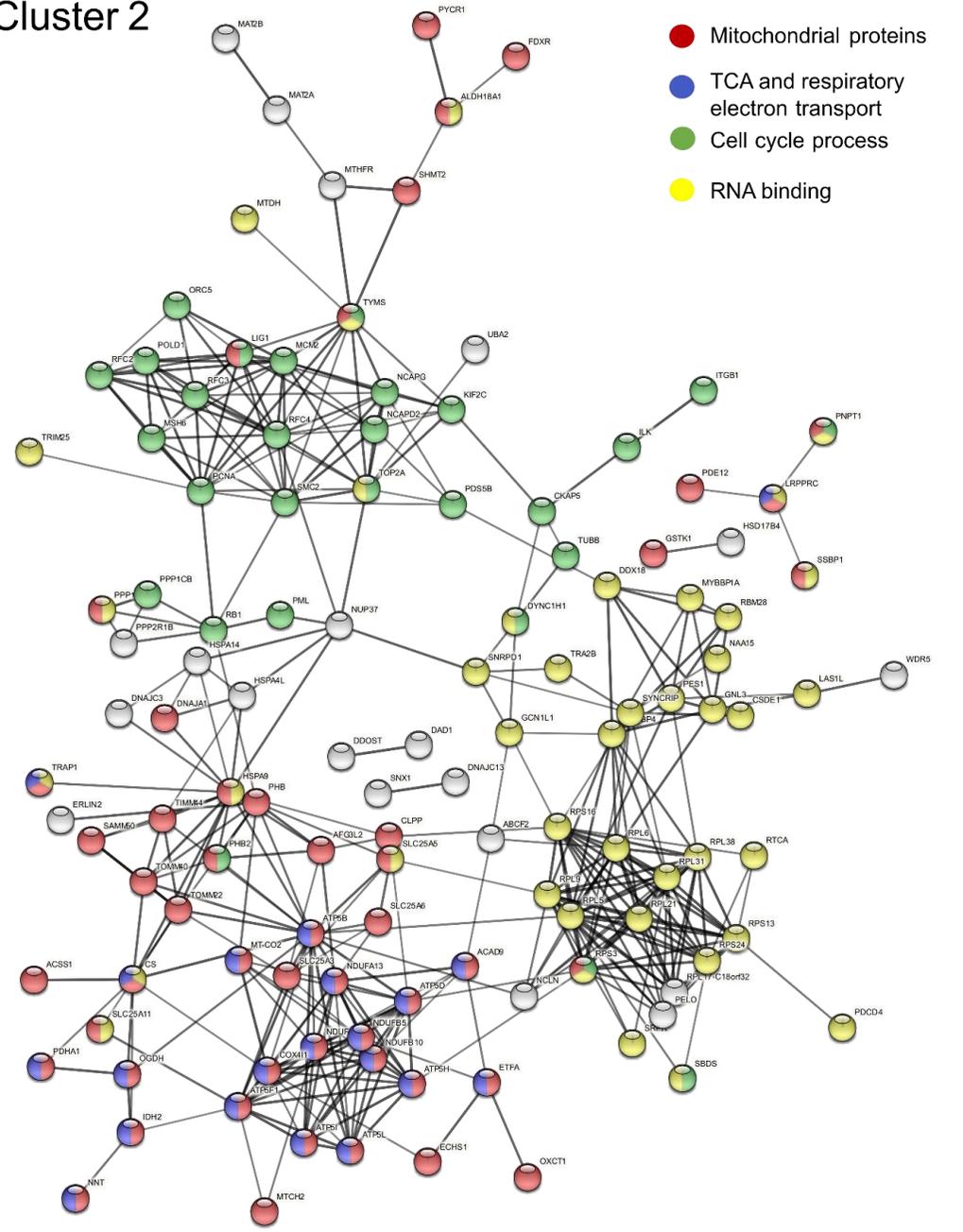


Figure S4. STRING analysis [59] of significantly differentially abundant proteins between the 3% and 20% O₂ cultures. Clusters are defined in figure 6D. Proteins are highlighted in different colors according to their functional group.