Supplementary material

Supplementary Methods

Tumour growth rates calculation

Tumour growth curves were fitted to the Exponential-Linear Model to calculate tumours' growth rates. The model assumes that cells initially proliferate with constant cell cycle duration, T_c, resulting in exponential growth, followed by a linear growth phase, when there is a decrease in actively proliferating cells and these are constrained to the margins of the tumour. The model is described as follows:

$$\frac{\partial V}{\partial t} = \alpha_0 V, t \leq \tau$$
$$\frac{\partial V}{\partial t} = \alpha_1, t > \tau$$

Eq. S1

where V is tumour volume (mm^3), t is time (days), α_0 is the growth rate during the exponential phase, i.e. the fraction of proliferating cells at time $\ln 2/T_c$, α_1 is the growth rate in the linear phase and τ represents the time point at which the growth changes from the exponential to the linear phase. Assuming the solution is continuously differentiable then:

$$\tau = \frac{1}{\alpha_0}\log(\frac{\alpha_1}{\alpha_0 V_0})$$

Eq. S2

where V_0 is the initial volume, in this case 0.1 mm^3, chosen as an arbitrary small initial volume of tumour cells.

The tumour growth fitting was carried out in MATLAB (2013a, 8.1.0.604), using a nonlinear least squared fit to obtain the α_0 and α_1 parameters. Fits were performed on volume data weighted using the measurement uncertainties of each data point, which corresponded to the Verror calculated from Eq. 2, within 95% confidence interval bounds.

Supplementary Tables

Table S1: Summary of slice- and intra-tumour CoVs of the measurements of each animal acquired over 75 minutes, and the average Hb, HbO_2, HbT and sO_2 measured during that time period.

Animal	ROI-averaged Parameter	average±std	Intra-tumour CoV (%)	Average slice-CoV (%)
1	Hb	0.99±0.30	30.1	6.4±2.6
	HbO_2	0.71±0.36	51.5	5.5±1.9
	HbT	1.72±0.68	39.3	4.2±2.2
	sO_2	0.40±0.07	18.7	5.0±2.1
2	Hb	1.12±0.29	25.5	2.4±0.8
	HbO_2	0.66±0.31	47.1	6.5±5.4
	HbT	1.78±0.60	33.5	1.5±1.2
	sO_2	0.35±0.06	18.0	5.6±4.2
3	Hb	1.00±0.18	18.1	2.5±1.2
	HbO_2	0.80±0.16	20.3	4.0±2.4
	HbT	1.81±0.34	19.0	1.7±0.6
	sO_2	0.44±0.01	2.2	3.3±1.5
4	Hb	0.67±0.11	16.2	10.9±2.6
	HbO_2	0.27±0.11	41.9	13.9±7.4
	HbT	0.94±0.23	23.9	9.3±2.5
	sO_2	0.28±0.07	24.9	11.5±7.4
Average intra-tumour CoV (n=4)	Hb	22.5±6.5		
	HbO_2	40.2±13.9		
	HbT	22.9±9.2		
	sO_2	16.0±8.4		

Table S2: Summary of average slice- and intra-tumour CoV of the measurements after re-positioning each animal 3 times, during air-breathing imaging, and the average Hb, HbO_2, HbT and sO_2.

Animal	Parameter	average (±std)	Intra- tumour CoV (%)	Average Slice-CoV (%)
1	Hb	0.86±0.21	24.3	2.6±1.2
	HbO_2	0.46±0.24	52.7	6.9±2.1
	HbT	1.32±0.45	34.0	2.9±1.0
	sO_2	0.33±0.09	27.3	5.3±2.9
2	Hb	0.62±0.15	24.8	3.7±3.4
	HbO_2	0.40±0.15	38.1	7.1±3.6
	HbT	1.02±0.31	30.0	3.7±2.1
	sO_2	0.39±0.03	7.5	4.9±3.2
3	Hb	0.82±0.21	26.0	4.7±3.2
	HbO_2	0.57±0.15	26.4	7.1±3.9
	HbT	1.38±0.36	25.9	2.8±1.7
	sO_2	0.41±0.01	3.4	6.3±3.8
4	Hb	0.47±0.14	29.1	5.6±0.8
	HbO_2	0.18±0.14	78.3	19.4±18.4
	HbT	0.65±0.28	42.5	6.5±3.1
	sO_2	0.24±0.12	49.7	15.4±15.8
4 (excluding position 2)	Hb	0.55±0.07	13.1	5.1±0.2
	HbO_2	0.25±0.11	42.9	9.0±5.8
	HbT	0.80±0.18	22.4	5.1±2.8
	sO_2	0.30±0.07	21.7	6.3±1.3
Average intra-tumour CoV (n=4)	Hb	26.0±2.2		
	HbO_2	48.9±22.4		
	HbT	33.1±7.1		
	sO_2	22.0±21.2		
Average intra-tumour CoV (without position 2, mouse 4)	Hb	22.0±6.0		
	HbO_2	40.0±11.0		
	HbT	28.1±5.1		
	sO_2	15.0±11.3		

Table S3: Rate of change of ROI-averaged Hb, HbO_2, HbT, sO_2 and percentage of black pixels (%BP) signal during the re-positioning study (oxygen-breathing). The goodness of fit, R^2, indicates how much of the signal variability is time dependent and the p-value if the signal change (slope of the linear model) is significantly different from zero. The SSE reflects how similar the predicted values and measured data are, i.e. the closest SSE is to zero, the better the linear fit.

Animal	Signal change, min^{-1} (95% CI)		R^2	p-value	SSE
1	Hb	0.015 (-0.033, 0.062)	0.939	0.158	0.006
	HbO_2	0.023 (-0.022, 0.069)	0.977	0.098	0.006
	HbT	0.038 (-0.055, 0.13)	0.964	0.121	0.024
	sO_2	0.0032 (-0.0049, 0.011)	0.962	0.126	<0.001
	%BP	-0.858 (-5.39, 3.67)	0.853	0.251	57
2	Hb	0.0089 (-0.060, 0.077)	0.732	0.348	0.013
	HbO_2	0.0093 (-0.059, 0.078)	0.752	0.333	0.013
	HbT	0.018 (-0.12, 0.15)	0.742	0.339	0.051
	sO_2	0.0006 (-0.0021, 0.0032)	0.883	0.228	<0.001
	%BP	-0.280 (-2.70, 2.14)	0.685	0.379	16
3	Hb	0.014 (-0.070, 0.098)	0.817	0.281	0.020
	HbO_2	0.017 (-0.023, 0.057)	0.965	0.120	0.004
	HbT	0.031 (-0.093, 0.15)	0.908	0.197	0.043
	sO_2	0.0018 (-0.0044, 0.0079)	0.932	0.160	<0.001
	%BP	0.0038 (-0.194, 0.202)	0.056	0.848	0.109
4	Hb	0.0062 (-0.094, 0.11)	0.380	0.579	0.028
	HbO_2	0.0071 (-0.12, 0.13)	0.346	0.601	0.043
	HbT	0.0084 (-0.20, 0.22)	0.208	0.589	0.141
	sO_2	0.0027 (-0.10, 0.11)	0.101	0.793	0.030
	%BP	-0.349 (-19.1, 18.2)	0.053	0.852	979

Table S4: Summary of inter- and intra-tumour CoVs for the CAL^R tumours (N=10), over a 6 day period, for ROI-averaged Hb, HbO_2, HbT and sO_2, during air-breathing imaging. ΔsO_2 is also included in this table.

Parameter	Intra-tumour CoV (%)	Inter-tumour CoV (%)
Hb	19.3±8.7	27.0±4.1
HbO_2	22.8±15.4	39.7±5.6
HbT	20.6±12.0	32.7±4.7
sO_2	7.5±2.5	13.1±3.2
ΔsO_2	63.5±45.7	74.0±11.5

Table S5: Summary of inter- and intra-tumour CoVs for the CAL^R tumours (N=10), over a 6 day period, for ROI-averaged Hb, HbO_2, HbT and sO_2, during oxygen-breathing imaging.

Parameter	Intra-tumour CoV (%)	Inter-tumour CoV (%)
Hb	41.2±15.2	29.8±5.6
HbO_2	19.5±11.7	35.9±4.5
HbT	22.4±11.7	31.3±4.4
sO_2	6.5±3.2	11.8±3.6

Supplementary Figures

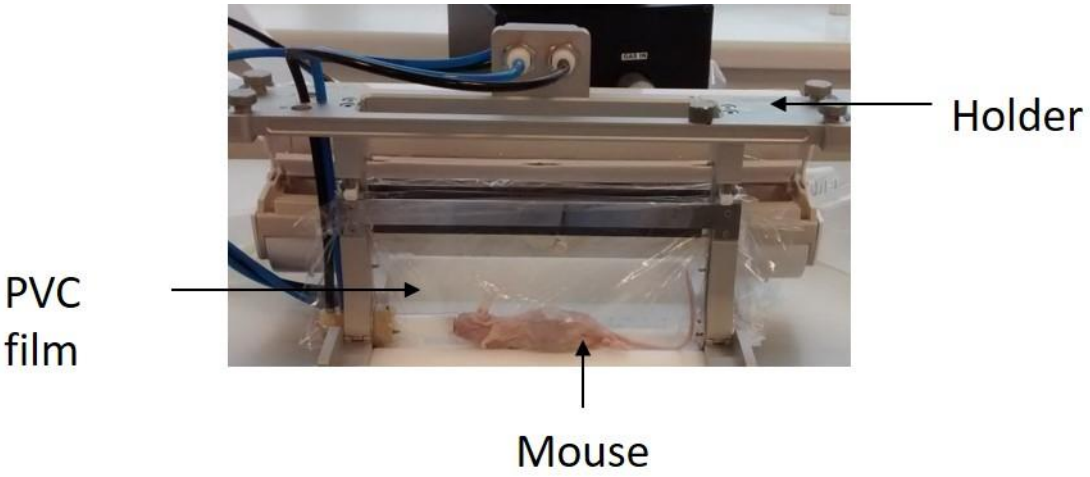


Fig. S1: Photograph showing the experimental setup of the mouse, in the MSOT holder prior to mounting in the imaging system.

Fig. S2: ROI-averaged percentage of black pixels, and Hb, HbO_2, HbT and sO_2 signals, for CAL^R tumours (n=4) imaged after being removed and re-positioned in the tank three times, during oxygen-breathing. Error bars represent the standard deviation over 3 adjacent central tumour slices, 1 mm apart. Solid lines represent a linear regression fitted to the data.

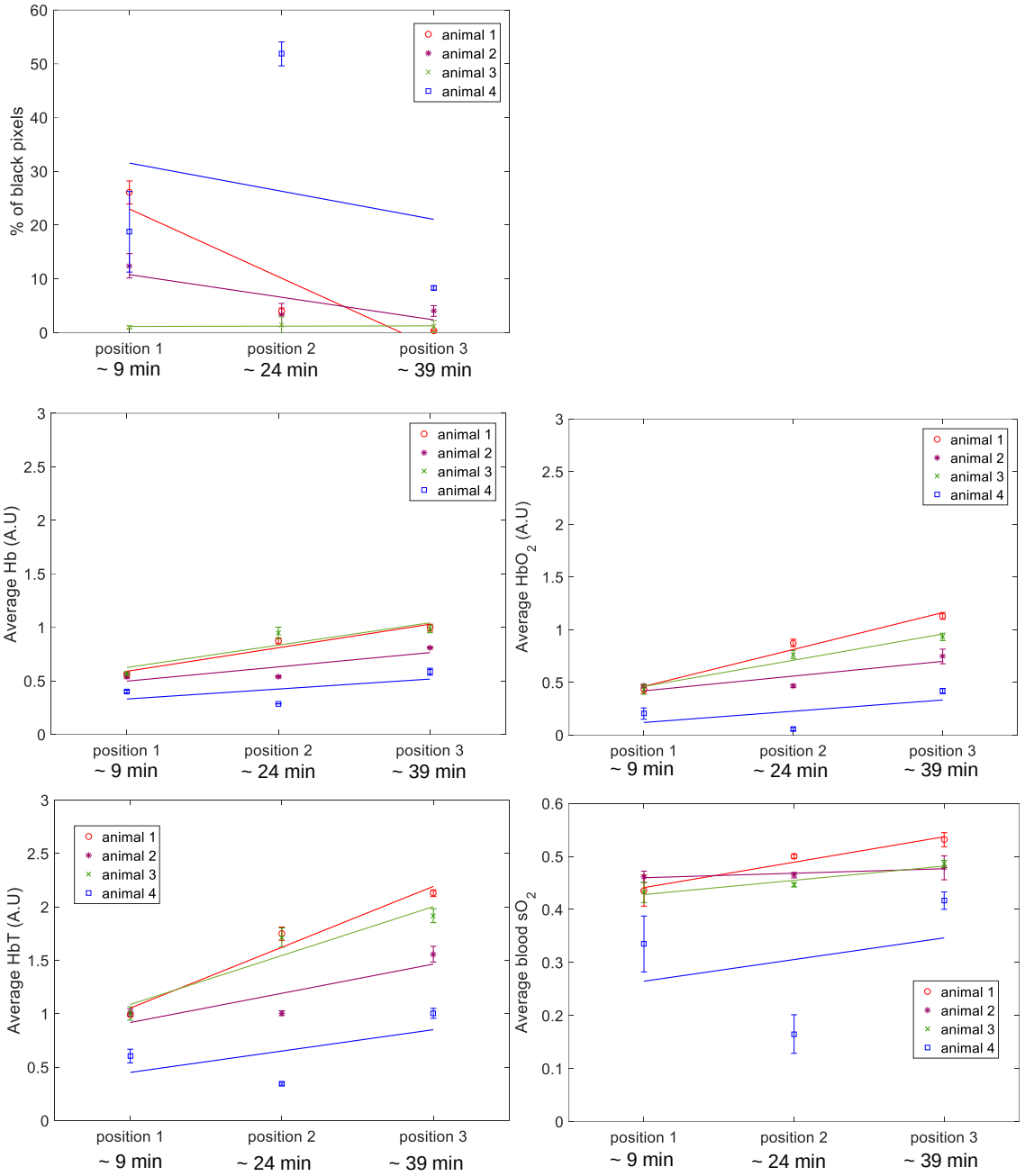


Fig. S3: Comparison of Hb change between longitudinal (75 minute) and re-positioning studies. p-values indicate if the differences between the two slopes resultant from a linear model fitting are statistically significant (if p-value<0.05).

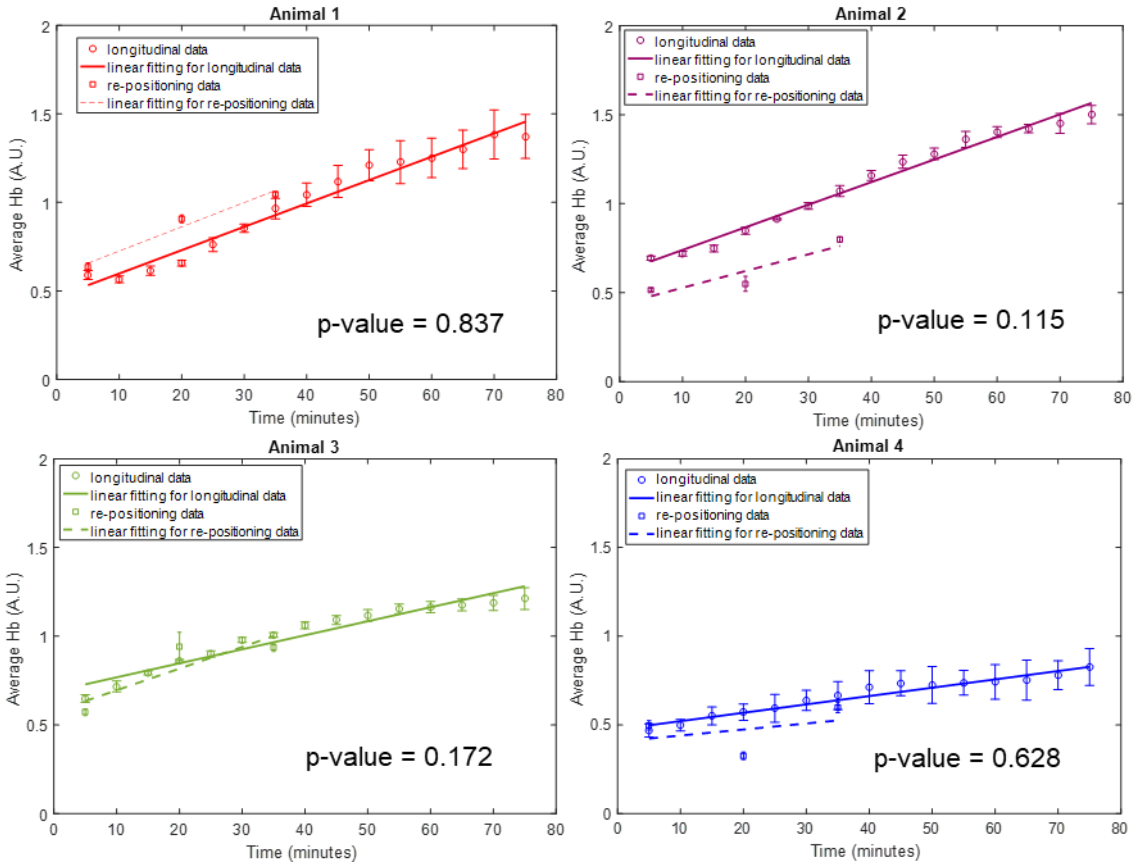

Animal 1
longitudinal data
linear fitting for longitudinal data
re-positioning data
linear fitting for re-positioning data
p-value = 0.837
Average Hb (A.U.)
Time (minutes)
Animal 2
longitudinal data
linear fitting for longitudinal data
re-positioning data
linear fitting for re-positioning data
p-value = 0.115
Average Hb (A.U.)
Time (minutes)
Animal 3
longitudinal data
linear fitting for longitudinal data
re-positioning data
linear fitting for re-positioning data
p-value = 0.172
Average Hb (A.U.)
Time (minutes)
Animal 4
longitudinal data
linear fitting for longitudinal data
re-positioning data
linear fitting for re-positioning data
p-value = 0.628
Average Hb (A.U.)
Time (minutes)

Fig. S4: Comparison of HbO_2 change between longitudinal (75 minute) and re-positioning studies. p-values indicate if the differences between the two slopes resultant from a linear model fitting are statistically significant (if p-value<0.05).

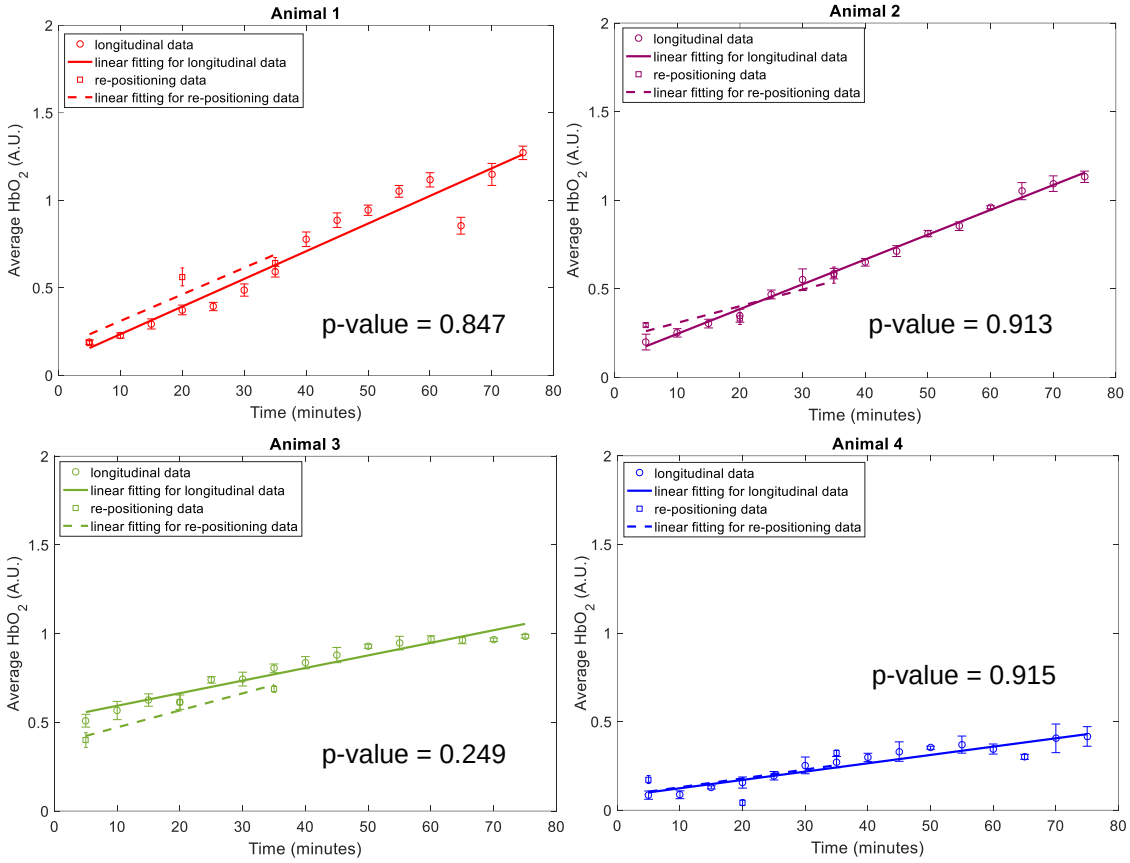


Fig. S5: Comparison of HbT change between longitudinal (75 minute) and re-positioning studies. p-values indicate if the differences between the two slopes resultant from a linear model fitting are statistically significant (if p-value<0.05).

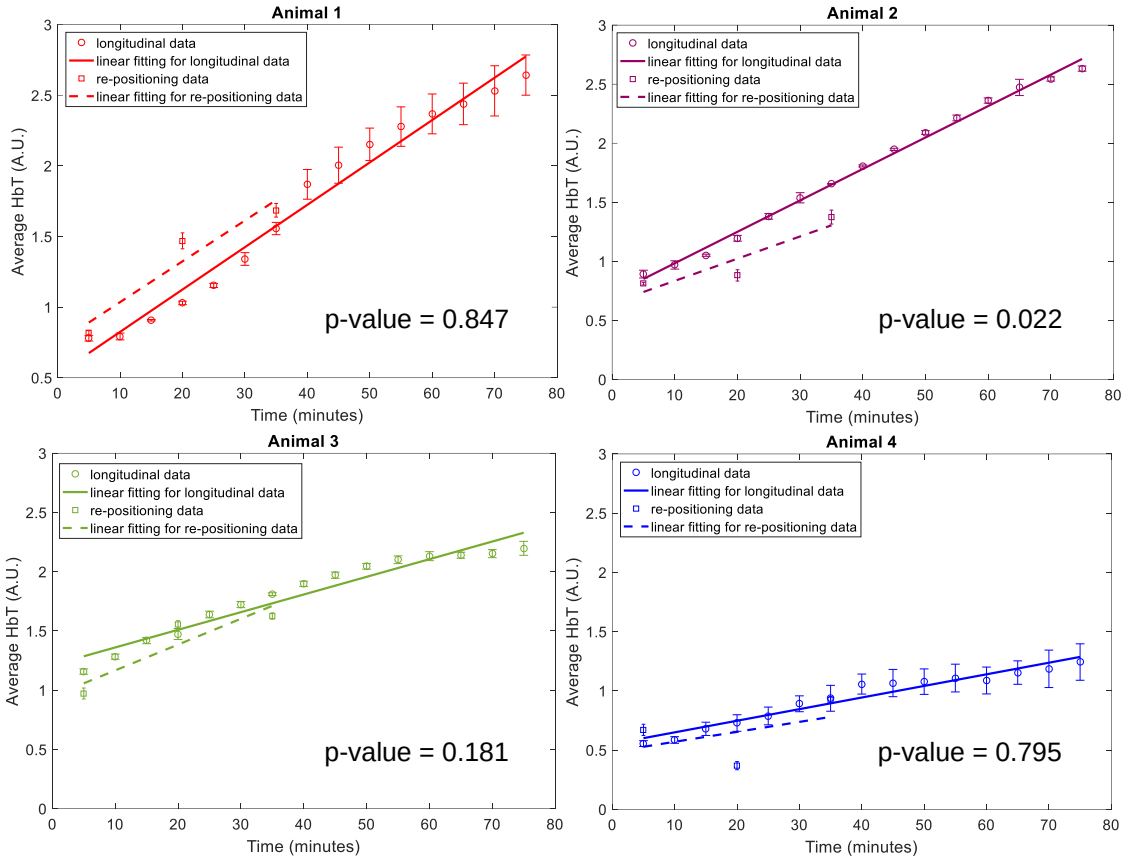
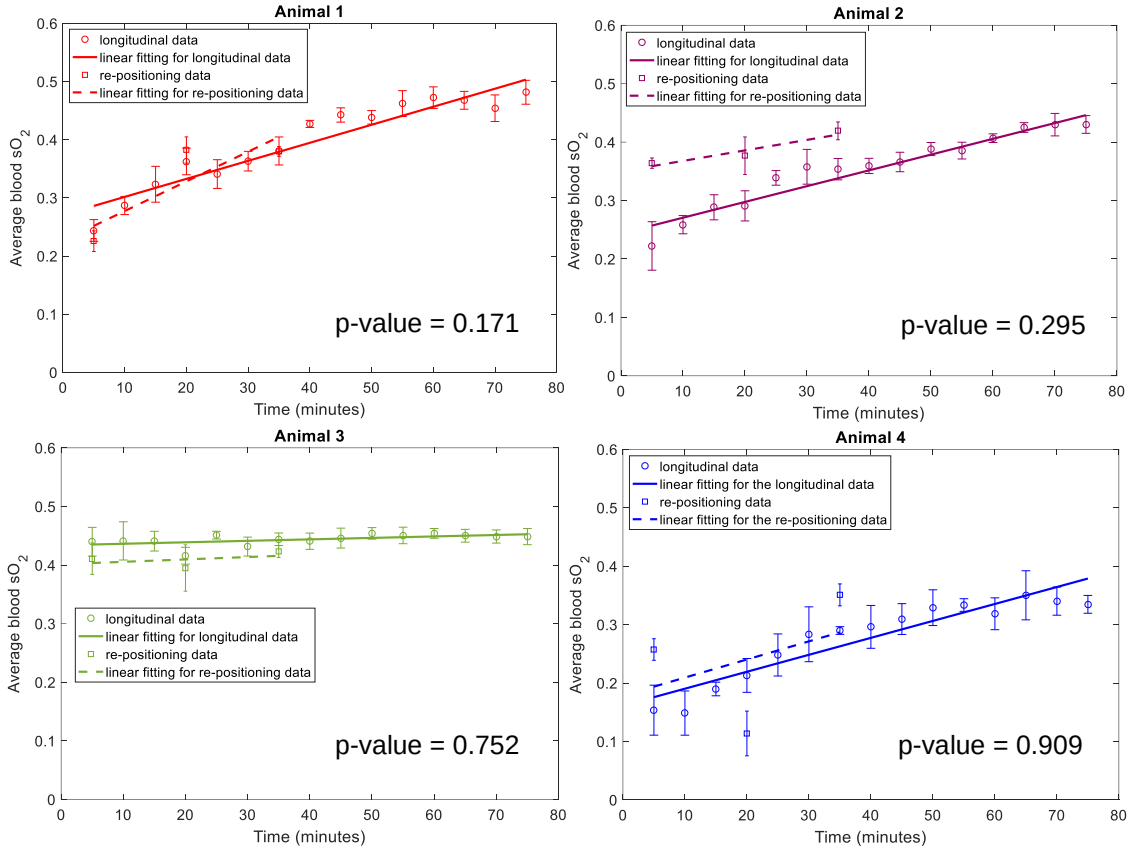


Fig. S6: Comparison of sO_2 change between longitudinal (75 minute) and re-positioning studies. p-values indicate if the differences between the two slopes resultant from a linear model fitting are statistically significant (if p-value<0.05).



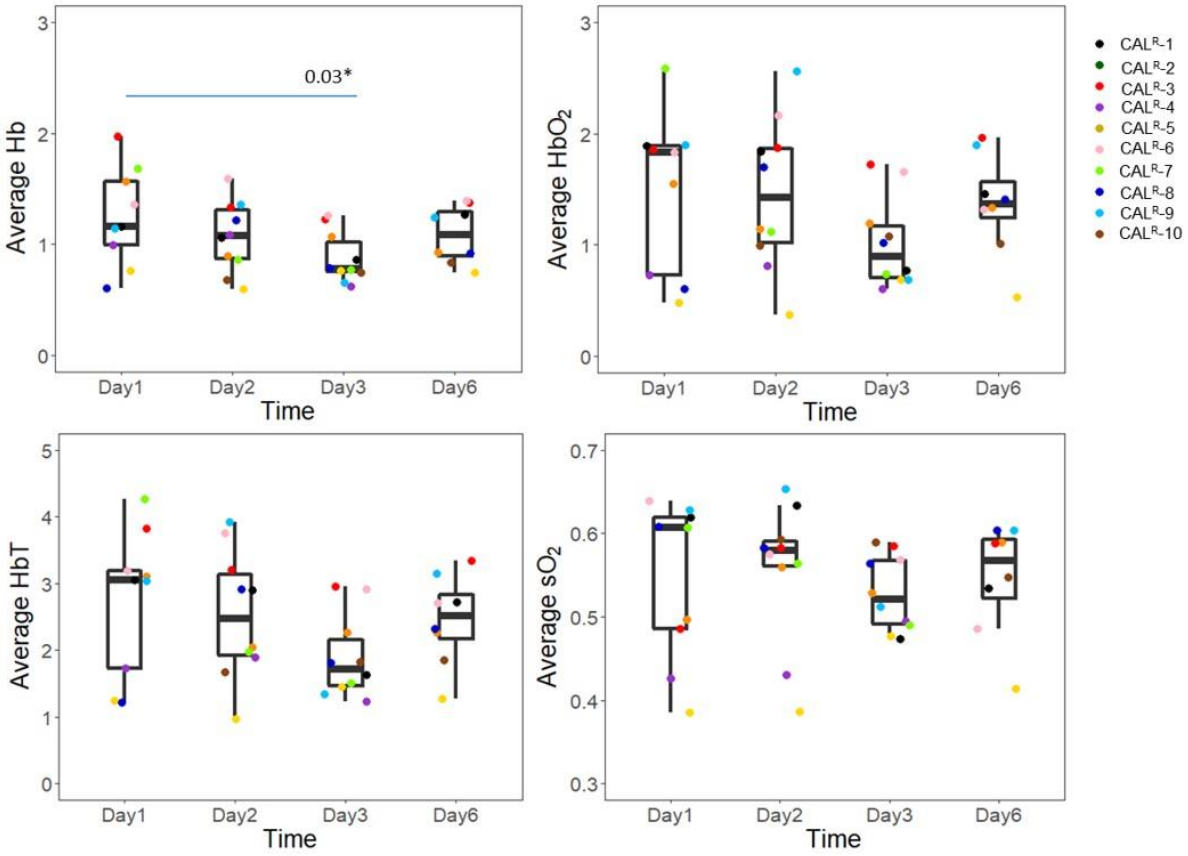


Fig. S7: Average haemoglobin and blood sO_2 values for the 3 central tumour slices of 10 CAL^R tumours, over a 6 day imaging period, for air-breathing imaging. Day 1 corresponds to the time at which tumours reached approximately 200 mm^3. The whisker plots shows the distribution of the measurements; the central black line in each box represents the median of the population; top and bottom horizontal lines in each box represent the upper and lower quartile and the whiskers the minimum and maximum values.

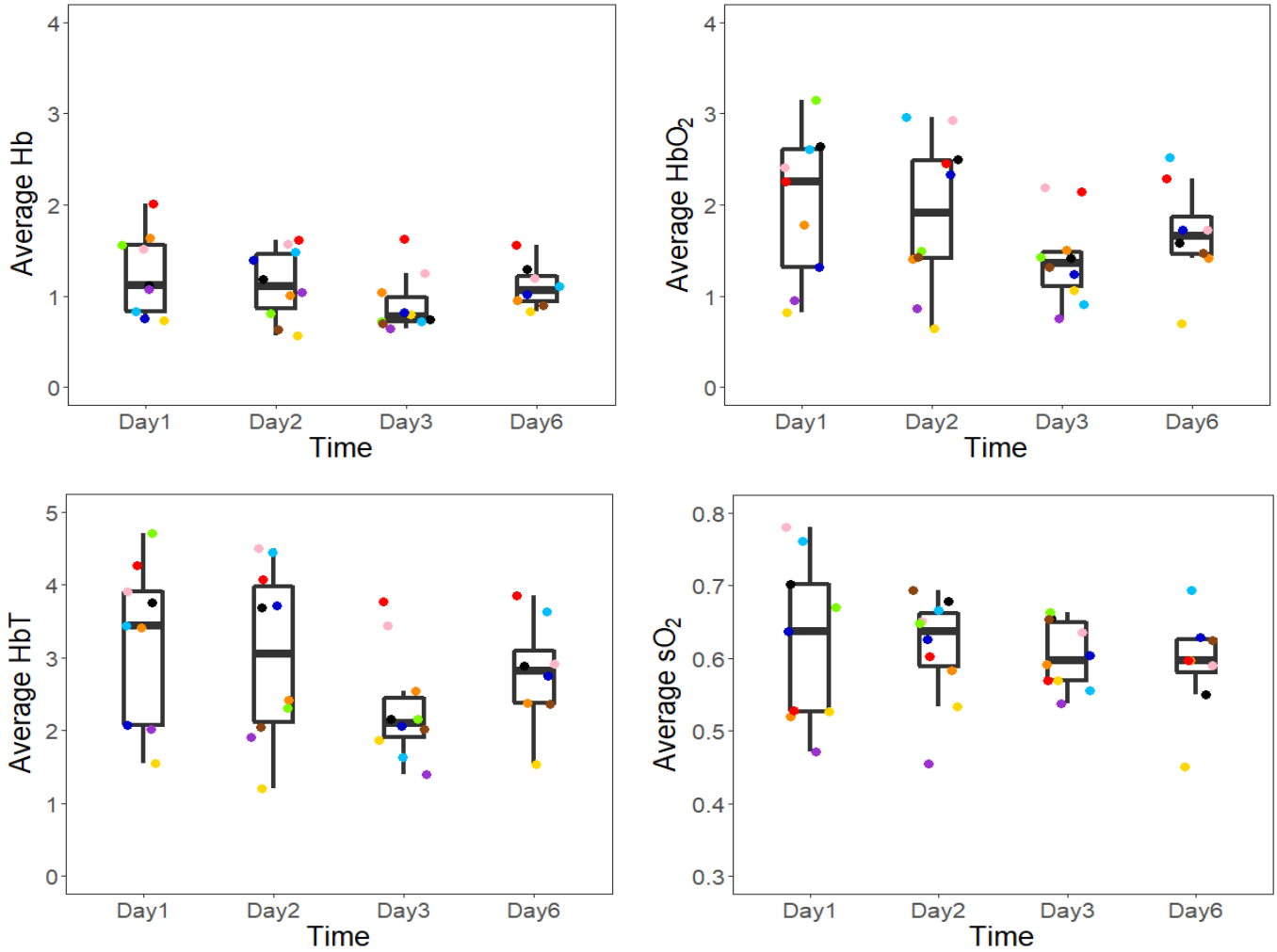


Fig. S8: Average haemoglobin and blood sO_2 values for the 3 central tumour slices of 10 CAL^R tumours, over a 6 day imaging period, for oxygen-breathing imaging. Day 1 corresponds to the time at which tumours reached approximately 200 mm^3. The whisker plots shows the distribution of the measurements; the central black line in each box represents the median of the population; top and bottom horizontal lines in each box represent the upper and lower quartile and the whiskers the minimum and maximum values.

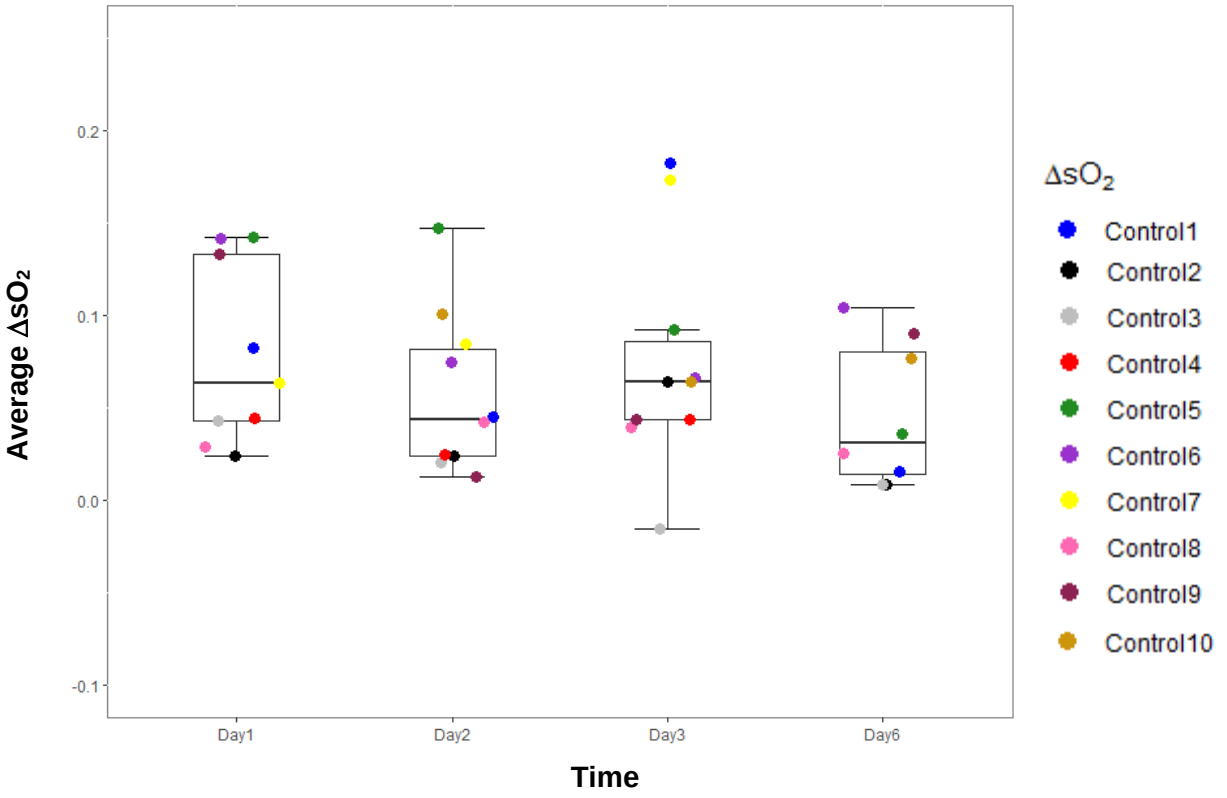


Fig. S9: Average blood ΔsO_2 values for the 3 central tumour slices of 10 CAL^R tumours, over a 6 day imaging period. Day 1 corresponds to the time at which tumours reached approximately 200 mm^3. The whisker plots shows the distribution of the measurements; the central black line in each box represents the median of the population; top and bottom horizontal lines in each box represent the upper and lower quartile and the whiskers the minimum and maximum values.

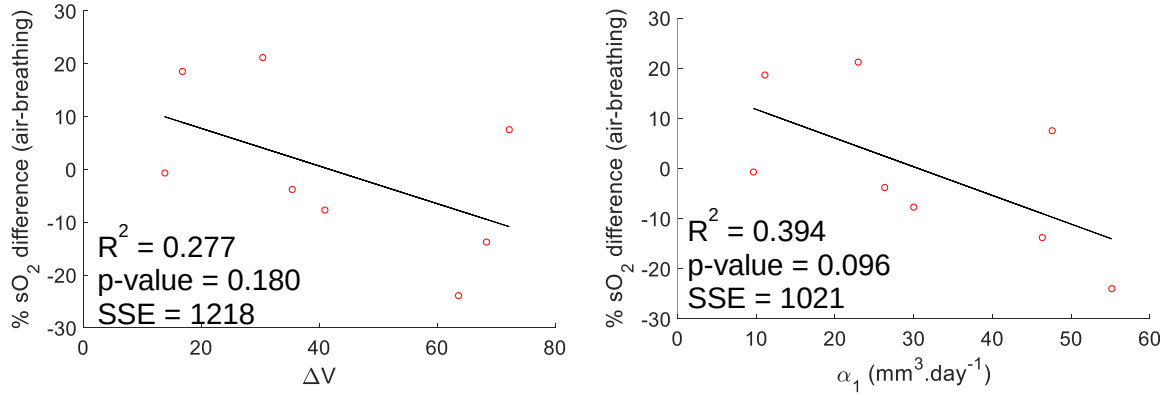


Fig. S10: Correlation between rate of change in volume between day 1 and 6 of imaging, ΔV, or linear growth rate, $\alpha 1$, and the percentage difference in sO_2 between days 1 and 6 of imaging for 8 CAL^R tumours, for air-breathing measurements.