Hypoxia inducible factors are dispensable for myeloid cell migration into the inflamed mouse eye

Peter J Gardner[1*#], Sidath E Liyanage[1#], Enrico Cristante[1], Robert D Sampson[1],

Andrew D Dick[1, 2, 3], Robin R Ali[1, 2] and James W Bainbridge[1, 2]

1 UCL Institute of Ophthalmology, Genetics department (London, (United Kingdom)
2 NIHR Biomedical Research Centre for Ophthalmology, Moorfields Eye Hospital
(London, (United Kingdom)
3 University of Bristol, Academic Unit of Ophthalmology (Bristol, (United Kingdom)
**#** Authors contributed equally
***Corresponding author:** Dr. Peter J Gardner email: p.gardner@ucl.ac.uk
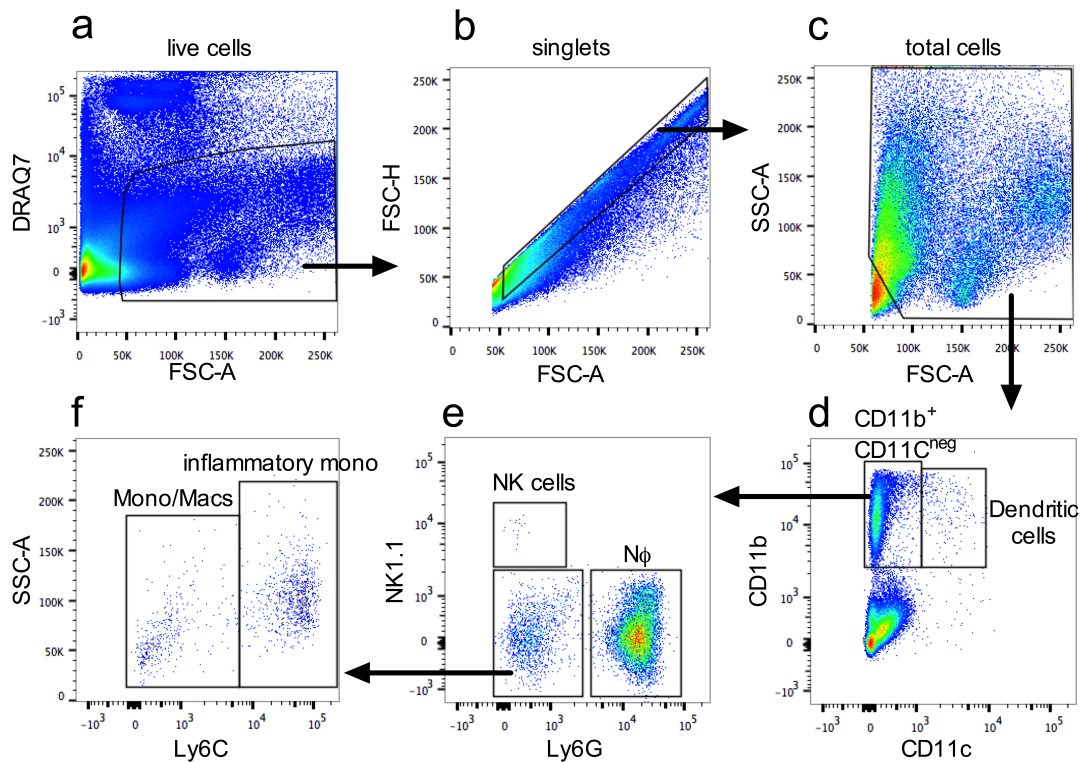
Supplemental Figure 1. Flow cytometry gating strategy. Flow cytometric gating strategy for myeloid cells in the mouse eye 18 hours after EIU induction in WT C57BL/6 mice; (a) live cells are gated using the DRAQ7 dye to stain dead cells (b) singlets are gated, (c) based on event size and granularity debris and non leukocytes are gated out, (d) myeloid cells are gated are gated away from retinal cells using CD11b but excluding CD11c^{+} dendritic cells from analysis, (e) cells are gated for (NK1.1^{+}) Natural killer cells and (Ly6G^{+}) Neutrophils, (f) the remaining cells are gated using expression of Ly6C to discriminate (Ly6C[lo-neg]) monocyte/macrophages and (Ly6C^{hi}) inflammatory monocytes.

a

b
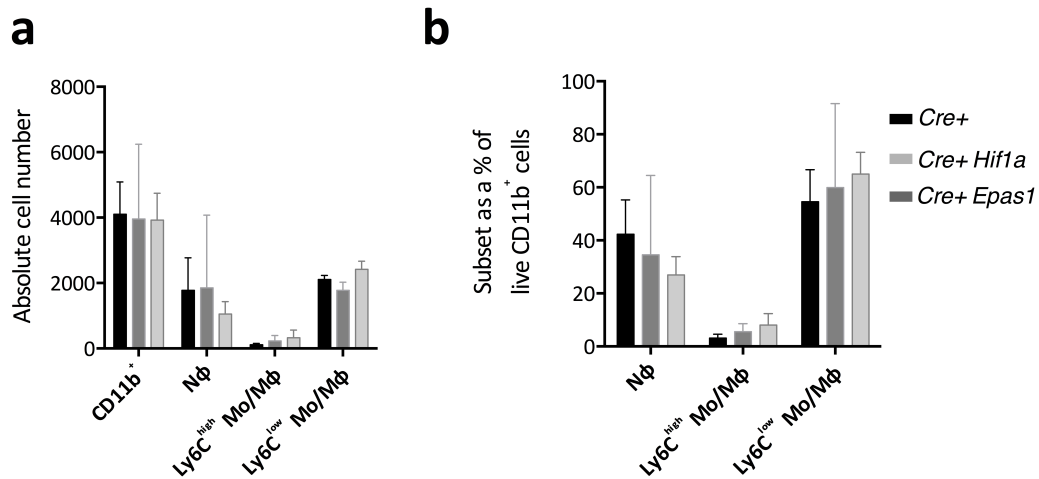
Supplemental Figure 2. Assessment of EIU at 48 hrs post induction. Flow cytometric analyses of (a) absolute cell numbers and (b) proportions of myeloid subsets infiltrated in the eye 48 hours after EIU induction in *Lysm*^Cre/+ animals and mice with myeloid cells deficient in *Hif1a* or *Epas1*: Myeloid cell populations are defined using standard gating strategy. Nφ = neutrophils; Mo/Mφ - monocyte/macrophages. Graphs show mean ± SD; n = 10 - 12 injected eyes per group.

a

total CD11b$^+$

b

CD11b$^+$Ly6G$^+$

c

CD11b$^+$Ly6C^{hi}

d

CD11b$^+$Ly6C$^{lo\_neg}$
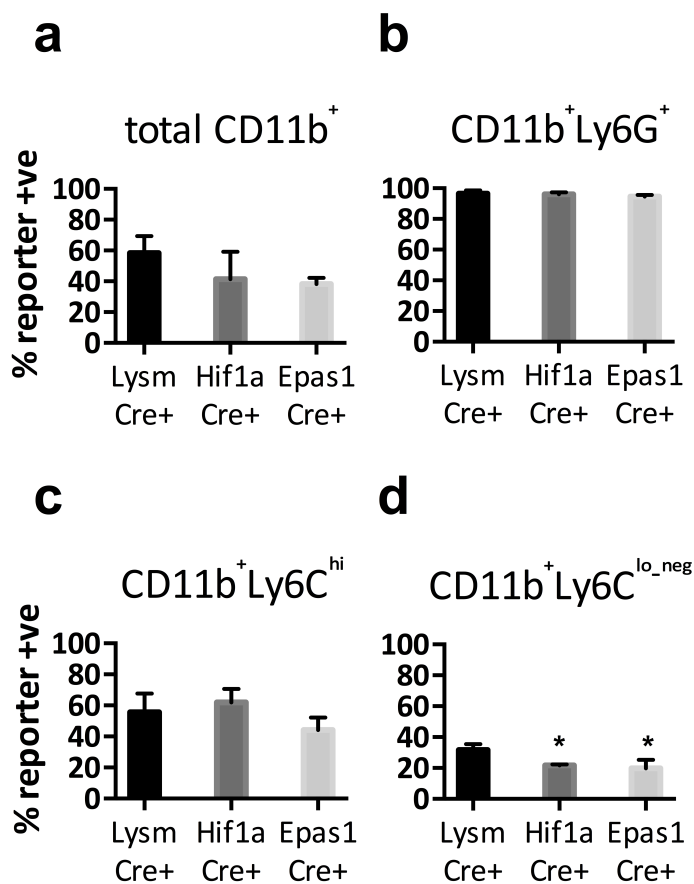
Supplemental Figure 3. Assessment of the presence of HIF knock-out cells in the eye during EIU. Flow cytometric analyses of GFP production driven by Lysm/Cre-mediated deletion of a floxed stop codon in floxed Hif1a and Epas1 mice. Data show the proportion of myeloid subsets positive for GFP infiltrating the eye during EIU as compared to *Lysm*^Cre/+ eYFP reporter animals, a) total CD11b$^+$ myeloid;b) CD11b+Ly6G Neutrophils; c) CD11b+Ly6C^{hi}; d) CD11b+Ly6C^{lo-neg}. Myeloid cell populations are defined using standard gating strategy. Graphs show mean ± SD; n = 3 - 5 injected eyes per group, Kruskal-Wallis one-way ANOVA, * P=0.0165.
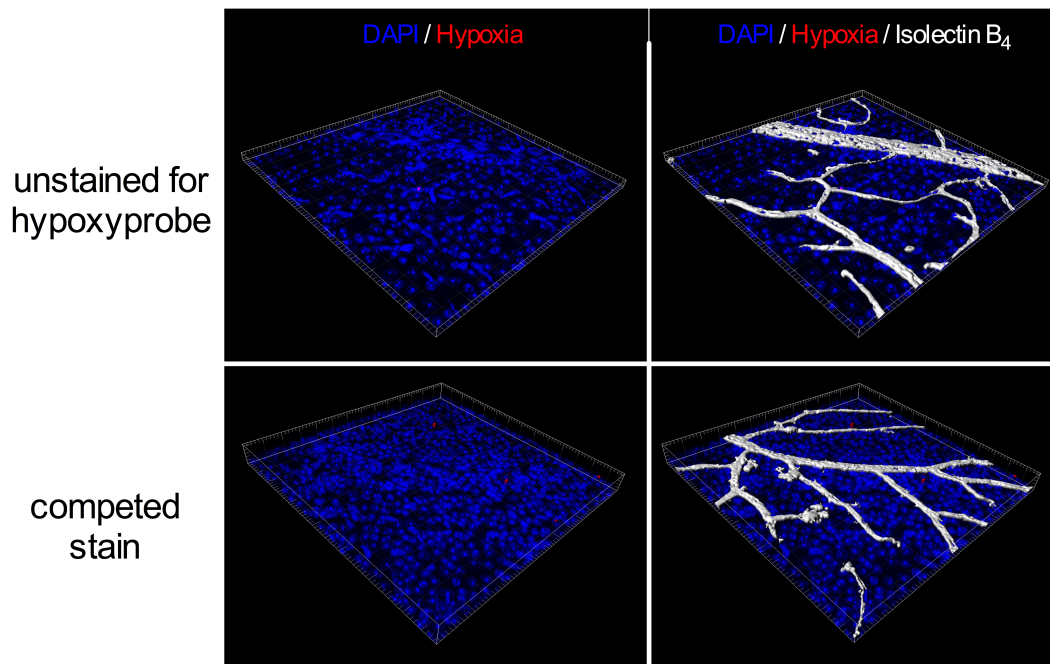
Supplemental Figure 4. Flat mount retina controls for hypoxia staining. 3-dimensional reconstructed imaging of superficial plexus from flat mounted retinae of PHZ treated mice either unstained for hypoxyprobe (no anti-hypoxyprobe antibody) or PHZ mice following two i.p. injections with hypoxiprobe 12 and 2 hrs prior to culling and Hypoxyprobe-competed stain and staining with DAPI, hypoxyprobe and Isolectin.

figure 1

Cre		cell numbers		percentages
Cre	CD11b$^+$	0.697424		
	Nφ	0.750487	Nφ	0.696097
	Ly6C^{high} Mo/Mφ	0.970296	Ly6C^{high} Mo/Mφ	0.837179
	Ly6C^{low} Mo/Mφ	0.671288	Ly6C^{low} Mo/Mφ	0.687397
	NK	0.977758	NK	0.947649

figure 2

		cell numbers		percentages
Hif1a	CD11b$^+$	0.603547		
	Nφ	0.376868	Nφ	0.0217093
	Ly6C^{high} Mo/Mφ	0.521054	Ly6C^{high} Mo/Mφ	0.504333
	Ly6C^{low} Mo/Mφ	0.223981	Ly6C^{low} Mo/Mφ	0.0386536
	NK	0.349505	NK	0.50572

		cell numbers		percentages
Epas1	CD11b$^+$	0.0512081		
	Nφ	0.0605257	Nφ	0.231222
	Ly6C^{high} Mo/Mφ	0.0385813	Ly6C^{high} Mo/Mφ	0.905162
	Ly6C^{low} Mo/Mφ	0.774895	Ly6C^{low} Mo/Mφ	0.16498
	NK	0.977471	NK	0.175579

		cell numbers		percentages
Hif1a / Epas1	CD11b$^+$	0.718826		
	Nφ	0.654876	Nφ	0.0859047
	Ly6C^{high} Mo/Mφ	0.916377	Ly6C^{high} Mo/Mφ	0.428685
	Ly6C^{low} Mo/Mφ	0.597299	Ly6C^{low} Mo/Mφ	0.0588428
	NK	0.528388	NK	0.257931

figure 3

		cell numbers		percentages
Vhl	CD11b$^+$	0.815316		
	Nφ	0.870075	Nφ	0.990025
	Ly6C^{high} Mo/Mφ	0.820353	Ly6C^{high} Mo/Mφ	0.737409
	Ly6C^{low} Mo/Mφ	0.884046	Ly6C^{low} Mo/Mφ	0.435415
	NK	0.949043	NK	0.865256

		cell numbers		percentages
Hif1a / Vhl	CD11b+	0.873441		
	Nφ	0.881888	Nφ	0.467648
	Ly6C^{high} Mo/Mφ	0.940761	Ly6C^{high} Mo/Mφ	0.602551
	Ly6C^{low} Mo/Mφ	0.582693	Ly6C^{low} Mo/Mφ	0.595893
	NK	0.926561	NK	0.661085

		cell numbers		percentages
Epas1 / Vhl	CD11b$^+$	0.286565		
	Nφ	0.357838	Nφ	0.664635
	Ly6C^{high} Mo/Mφ	0.391069	Ly6C^{high} Mo/Mφ	0.120752
	Ly6C^{low} Mo/Mφ	0.0570904	Ly6C^{low} Mo/Mφ	0.557369
	NK	0.162802	NK	0.653947

figure 6

		cell numbers		percentages
Hif1a	CD11b$^+$	0.513827		
	Nφ	0.493108	Nφ	0.509409
	Ly6C^{high} Mo/Mφ	0.782881	Ly6C^{high} Mo/Mφ	0.946335
	Ly6C^{low} Mo/Mφ	0.923466	Ly6C^{low} Mo/Mφ	0.209387
	NK	0.387515	NK	0.928142

Epas1	CD11b+	0.071339		
	Nφ	0.0612485	Nφ	0.0313879
	Ly6C^{high} Mo/Mφ	0.227254	Ly6C^{high} Mo/Mφ	0.373304
	Ly6C^{low} Mo/Mφ	0.124648	Ly6C^{low} Mo/Mφ	0.0131476
	NK	0.685331	NK	0.0296188

Hif1a / Epas1	CD11b$^+$	0.164664		
	Nφ	0.172859	Nφ	0.074792
	Ly6C^{high} Mo/Mφ	0.0777661	Ly6C^{high} Mo/Mφ	0.123479
	Ly6C^{low} Mo/Mφ	0.827501	Ly6C^{low} Mo/Mφ	0.0671834
	NK	0.913782	NK	0.165067

Supplemental Table S1. P values from statistical analyses carried out on EIU infiltrate data. Absolute counts and myeloid subset percentages of total CD11b$^+$ cells were compared between mutant and floxed control mice as shown in Fig. 1, 2, 3 and 6, using multiple comparison t tests with statistical significance determined using the Holm-Sidak method with alpha =5.0%