Supplemental video 1: MACs localised within the retina. 3D reconstruction of confocal images taken from representative ischaemic retina injected with MACs. Retinal vasculature appears in green by isolectin staining and MACs were labelled with red fluorescent Qdots prior to delivery. Note spherical shape of MAC, lack of integration into the resident vascular network, and specific location to the outer retinal layers in close proximity to the vitreal side where they were injected.
Supplementary Figure 1. MACs delivered into the ischaemic retina constitute amoeboid retinal phagocytes. Human MACs labelled with red Qdots were injected into the ischemic retina of C57/B6 mice at P12. At P15, retinas were flat mounted and imaged. Retinal vasculature is labelled in green with isolectin. MACs (labelled in red) do not adapt a ramified morphology but contribute to the resident population of amoeboid retinal phagocytes. MACs significantly increased the total number of amoeboid cells by more than two fold (p<0.05).
Supplementary Figure 2. MACs and monocytes have distinctive transcriptomes. Normalised data from Illumina WG-6 v3.0 Expression Beadchip were imported into NIA array for analysis. Upregulated transcripts for MACs are shown in red and identify genes associated with angiogenesis while upregulated transcripts for monocytes, shown in green, identify genes associated with inflammation and immune response.
Supplementary Figure 3. Transcriptome-based interactome analysis of MACs vs. circulating monocytes. Differentially expressed transcripts were imported into visANT software to create gene networks. Diagrams show nodes representing genes, lines indicate interactions between proteins, and boxes denote functional categories. (A) Upregulated networks in MACs, nodes shown in green are predominantly angiogenic, anti-inflammatory and linked to endocytosis and cathepsins. (B) Downregulated gene network in MACs, nodes in green are mainly associated with inflammation and apoptosis.
Supplementary Figure 4. Gene microarray validation by real time qRT-PCR. Primers were designed for M2, M1, and angiogenic genes. Real time qRT-PCR results confirmed that MACs highly express M2 and angiogenic genes, while lowly express M1 markers, when compared to CD14+ circulating monocytes.
Supplementary Figure 5. Immunophenotyping of MACs confirmed myeloid and M2 macrophage characteristics. (A) Histograms showing MAC immunophenotype characterisation. MACs expressed myeloid markers CD45, CD14 and CD68 (red) and M2 markers CD163 and CD206 (green). They were negative for dendritic markers CD83, CD209 (blue). Respective isotype controls are shown as grey histograms and the percentage of positive cells is shown in the top right corner.
Supplementary Figure 6. CFU-Hill phenotype is also consistent with alternatively activated M2 macrophages. (A) Phase-contrast representative image of a CFU-Hill colony. (B) Flow cytometry analysis highlighting that CFU-Hill main population contains 79% of the total cell number. (C) Flow cytometry immunphenotyping of CFU-Hill shows expression of M2 markers CD163 and CD206.
Supplementary Figure 7. MACs have significant phagocytic activity. MACs phagocytic index was comparable to M1 macrophages, however morphology was strikingly dissimilar. While MACs are spindle-shaped, macrophages are flat polygonal-shaped cells. Scale bars: 120µm.
Supplementary Figure 8. Induction of RMECs tube formation by MACs requires an active VEGF signaling pathway. (A) Co-culture of MACs labelled in red with RMECs labelled in green exposed to goat IgG. (B) Co-culture of MACs labelled in red with RMECs labelled in green exposed to goat anti-human VEGFR2. Scale bars for A, B: 500µm. (C) Quantification of tube length. Data expressed as mean ± SEM. N=4. ***p<0.001 versus control IgG.
Supplementary Figure 9. IL8 increases endothelial permeability. Confluent RMEC monolayers in triplicates were treated with 100ng/ml VEGF of 100ng/ml IL8 at the time indicated with the red arrow. IL8 decreased impedance across RMECs to the same extent as VEGF.