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SUPPLEMENTAL METHODS

605 **Detailed Mass Spectrometry Methods**

606 *Protein Extraction and Digestion*

607 Cells were extracted in lysis buffer (0.3 % SDS in Tris-buffered saline with 1 % protease inhibitor
608 cocktail (P8340 Sigma Aldrich, St. Louis, MO, 6 mM dithiothreitol (DTT)). Chloroform/methanol
609 protein precipitation was performed as described previously.⁵⁹ Precipitated protein was dissolved
610 in 8 M urea in 100 mM NH₄HCO₃ with 3 mM DTT. Proteins were frozen and stored at -80 °C.

611 Cysteines were then subjected to a final reduction with additional DTT at a final total
612 concentration of 5.5 mM, at room temperature for 40 minutes. Proteins were alkylated with
613 iodoacetamide at a concentration of 15 mM in the dark at room temperature for 30 min, and
614 digested with ~1:100 trypsin:protein (Promega, Madison, Wisconsin) for 16 h at 37 °C. The
615 resulting digest was acidified to ~ pH 1 with trifluoroacetic acid, kept on ice for 15 min to
616 precipitate lipids, and centrifuged for 15 min at 20 000 g. Supernatant was transferred to
617 Nestgroup C18 Macrospin columns (Southborough, MA). The peptides were eluted with 40 %
618 acetonitrile, lyophilized in a sample vial, and rehydrated in 3 % acetonitrile with 0.1 % formic acid.
619 A digest of yeast alcohol dehydrogenase at 25 fmol/μL final concentration was added as an internal
620 detection control (Waters Corp, Milford MA).

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622 *Liquid Chromatography and Mass Spectrometry*

623 An UltiMate 3000 RSLCNano (Thermo Scientific) ultrahigh pressure liquid chromatograph with
624 autosampler was used to separate peptides. Peptides were loaded on an Acclaim PepMap 100 75
625 μm ID x 2 cm, 3 μm particle size trap column (Thermo Scientific) at a flow rate of 5 μL/min with 2
626 % acetonitrile in water with 0.05 % TFA for 3 min. The analytical separation was conducted using a
627 75 μm ID x 50 cm, Acclaim PepMap reversed phase C18, 2 μm particle size column (Thermo
628 Scientific). Flow was 300 nL/min with mixtures of buffer A (aqueous 0.1 % formic acid) and buffer

629 B (80 % acetonitrile in water with 0.1 % formic acid). Separation was by isocratic elution for 10
630 min with 2 % B, and was followed by linear gradients to 24 % B for 105 min, and to 40.4 % B for 20
631 min, followed by ramping to 95 % buffer B with an isocratic hold for 3.5 minutes. Final recycle was
632 accomplished by a 1 min ramp and final hold at 2 % B for 20 min before the next sample.

633 Mass spectra were collected with using a Q Exactive HF (Orbitrap) mass spectrometer
634 (Thermo Fisher Scientific, Bremen, Germany) in positive ion mode, using data-dependent
635 acquisition. Settings included top 15 precursors, resolution 120 000 for MS scan, 15 000 for MS/MS
636 scan, dynamic exclusion for 20 s and maximum injection time of 30 ms for MS and 100 ms for
637 MS/MS. NCE was set at 28.0. The Nanospray Flex Ion Source (Thermo Scientific, Bremen, Germany)
638 was operated at 2.2 kV with heated capillary set at 250 °C and the S-Lens RF level at 55.0 %.

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640 ***Protein Data Analysis***

641 For quantitation and identification, the raw data files were imported into the Elucidator Protein
642 Expression Analysis System (V. 4.0.0.2.13, PerkinElmer, Boston, MA,). From within Elucidator,
643 results were searched with the Mascot server (Version 2.5.1, Matrix Science Ltd., London, UK). The
644 search was against a SwissProt human canonical protein sequence database with isoforms
645 (UniprotKB/Swiss-Prot Release 2017_01 containing 84 336 sequences; 48 572 808 residues,
646 www.uniprot.org). Searches were performed with carbamidomethyl (C) as a fixed modification and
647 mass accuracy limits of 10 ppm for MS and 0.02 Da for MS/MS with identification significance
648 threshold of $p < 0.05$. Variable modifications were Gln->pyro-Glu (N-term Q), Oxidation (M), Acetyl
649 (Protein N-term). Elucidator matched accurate mass and retention time values by time alignment of
650 peaks across all chromatographic runs. Precursor mass features (individual components of MS1
651 isotopic series) were annotated by PeptideTeller with a predicted error rate of 0.01. Features
652 detected within 12 and 146 minute retention times and 400-2000 m/z were used for quantitation.
653 Quantitation was based on RT vs. m/z peak volume. Instrument mass accuracy was set at 5 ppm for

654 PeakTeller. Expression ratio and ratio p-values were calculated by Elucidator and based on the
655 calculation error function (xdev) incorporated into the program.

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657 **Detailed Metabolomics Methods**

658 ***Sample Processing***

659 The individual wells were pooled for each biological replicate. The samples were centrifuged at
660 2,000 x G at 4 °C for 45 minutes and dried under a stream of nitrogen at 35 °C to 1-1.5 mL, then
661 transferred into amber autosampler vials and evaporated to dryness. A 20µL aliquot of myristic
662 acid d27 solution (3 mg Myristic Acid d27 (Sigma, 366889) dissolved in 1mL 2:5:2 Optima
663 Water/Methanol/Isopropanol (Fisher, W6-1, A456-1, A464-1)) were added to the sample vials as
664 an internal retention time locking compound. The samples were again evaporated to dryness under
665 nitrogen at 35 °C, and 0.5mL of methylene chloride (Fisher, Optima D1511) was added 2x to the
666 vials to azeotrope residual water. Free aldehyde groups were protected by derivatization with 30µL
667 of 40 mg/mL of methoxyamine·HCl (Sigma, 226904) in pyridine (Fisher, PI27530). Metabolites
668 were subsequently derivatized by adding 90µL of N-Methyl-N-trimethylsilyltrifluoroacetamide with
669 1% Trimethylchlorosilane (Sigma, 69478).

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671 ***Gas Chromatography Mass Spectrometry***

672 The derivatized samples were analyzed in triplicate GC-MS chromatograms (Agilent 7890B/5977A)
673 using a HP-5ms Ultra Inert GC Column, 30 m, 0.25 mm, 0.25 µm (Agilent J&W 19091S-433UI) The
674 oven temperature gradient was programmed as follows: 60 °C for 1 min; 60 °C to 325 °C at 10
675 °C/min; 325 °C for 10 min and 60 °C for 1 min.

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677 ***Metabolic Data Analysis***

678 Metabolite identification and quantitation was done with the Automated Mass Spectral
679 Deconvolution & Identification System (AMDIS, Version 2.72) (National Institute of Standards and
680 Technology) (NIST) using Retention Index Data type of analysis with the following settings:
681 component width 12; adjacent peak subtraction 1; resolution, sensitivity, and shape requirements
682 were set to medium. Agilent Fiehn Library was chosen as the target compounds library with
683 myristic acid d27 as retention time locking (RTL) compound. Fatty acid methyl esters (FAMES) in
684 the Agilent Fiehn GC/MS Metabolomics Standards Kit were used for system calibration. Student's t-
685 test modified with a false discovery correction for multiple testing with a Q-value R-script (Storey
686 JD and Tibshirani R. 2003. Statistical significance for genome-wide experiments. PNAS, 100: 9440-
687 9445) was used to generate p-values. Metabolite pathways were analyzed with MetaboAnalyst 3.0
688 (<http://www.metaboanalyst.ca/>). (Xia J1, Wishart DS. 2011. Web-based inference of biological
689 patterns, functions and pathways from metabolomic data using MetaboAnalyst. Nat Protoc. 2011.
690 6:743-60).

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692 **Confirmatory Study Methods**

693 ***Flow Cytometry***

694 Differently primed MSCs were collected via 0.25% trypsin-EDTA, washed twice, and then
695 resuspended in 250 µL BD Cytofix reagent in a 1.5 mL Eppendorf tube (cell counts normalized
696 across MSC groups). After 10 minutes fixation at 4 °C, cells were washed twice in BD Cytoperm
697 buffer, after which they were reconstituted in ~95 µL of Cytoperm for cell staining. To this, various
698 antibodies were added based on the target protein (final volume 100 µL). The catalog number,
699 fluorescent conjugate, and test size of these antibodies is shown below.

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Supplier	Catalog #	Product Name	Test Size
eBioscience	329713	Brilliant Violet 421™ anti-human CD274 (B7-H1, PD-L1) Antibody	5

Biolegend	323109	PerCP/Cy5.5 anti-human CD47 Antibody	5
Biolegend	352509	Brilliant Violet 421™ anti-human GARP (LRRC32) Antibody	5
R&D Systems	FAB2463G	Human LAP (TGF-beta 1) Alexa Fluor® 488-conjugated Antibody	5
Biolegend	373203	PE anti-human HLA-F Antibody	5
LSBio	LS-C62879	Anti-CFH / Complement Factor H Antibody (clone OX-24, FITC)	3

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703 ***Lysyl Oxidase Activity Assay***

704 The relative lysyl oxidase activity of cell lysates from differently primed MSCs was determined by
705 using the Abcam Lysyl Oxidase Activity Assay Kit (Fluorometric; ab112139). The assay was run as
706 per the manufacturer's instructions, where RIPA buffer was used to collect cell lysate. Samples
707 from different MSC groups were obtained from similar cell numbers, but to verify similar protein
708 amounts were also used, a BCA assay was conducted. Fluorimetric readings were taken after a 40
709 minute reaction time. The background fluorescence from a blank sample (assay reagent added 1:1
710 with buffer as sample) was subtracted off the recorded fluorescence from experimental samples.
711 Data were analyzed in GraphPad Prism v6.0 (San Diego, CA).

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713 ***Western Blot***

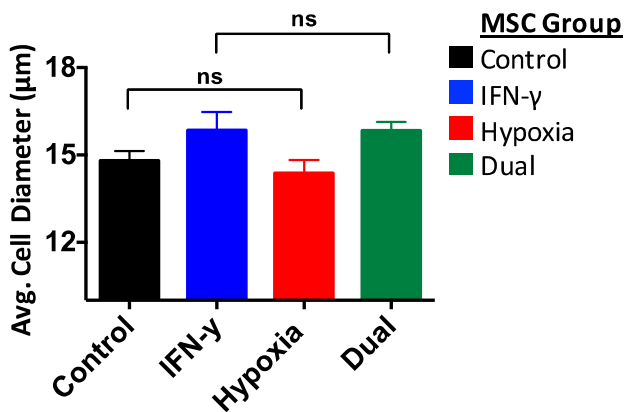
714 MSCs were washed twice with PBS and lysed with RIPA buffer (Thermo Fisher, 89901) containing
715 SIGMAFAST™ Protease Inhibitor Cocktail Tablets (SigmaAldrich, S8830). The cell lysate was diluted
716 1:1 with 2x Laemmli Sample Buffer (Bio-Rad, 610737) and boiled at 70 °C for 10 minutes. The same
717 amount of protein was loaded into each well of a Criterion TGX 4-20% gel (Bio-Rad, 4561094) and
718 were resolved by SDS-PAGE and transferred onto a PVDF membrane. After transfer, the membrane
719 was blocked with SuperBlock Blocking Buffer (ThermoFisher, 37517) for 1 hour and then probed
720 with an anti-Complement Factor H primary antibody (1:250; Atlas Antibodies, HPA049176) at 4 °C
721 overnight. The membrane was then washed and incubated with a goat anti-rabbit IRDye 800CW
722 secondary antibody (1:10,000; Li-cor, 926-32211) for 1 hour.

723 **ELISA**

724 MSCs were cultured as previously described and the supernatant was collected, spun down at
725 10,000x g for 5 min. and stored at -20 C until analysis. The supernatant levels of ANGPTL4 were
726 determined using an ELISA assay (Thermo Fisher, EHANGPTL4).

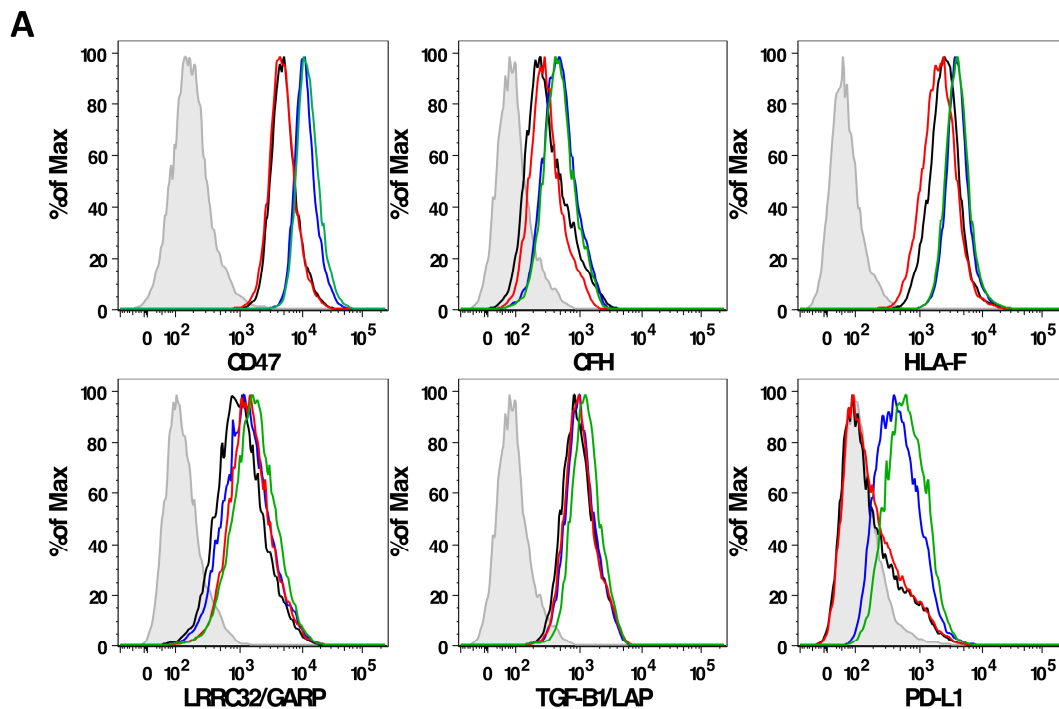
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SUPPLEMENTAL FIGURES

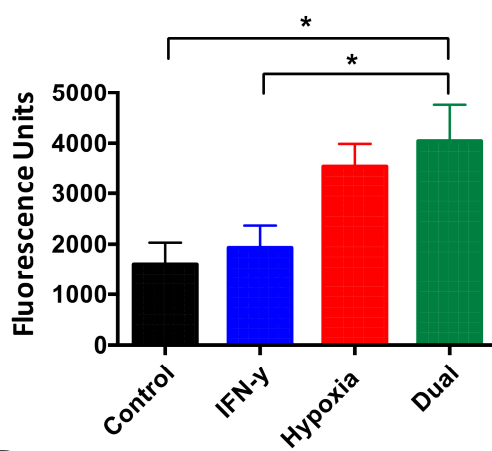


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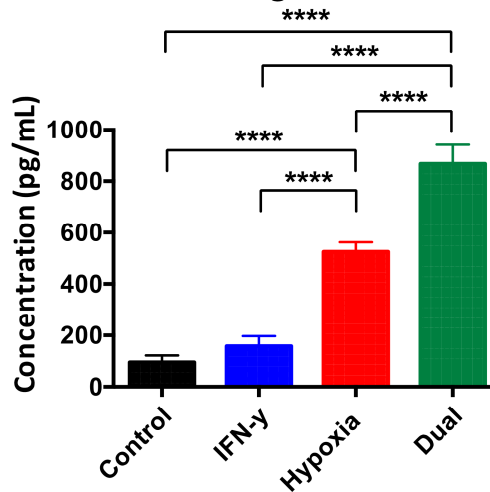
Supplemental Figure 1 The effect of hypoxia and/or IFN-γ priming on the average cell diameter. Cell diameter was determined immediately after passaging using a Countess device. n=5 experiments were averaged. All pairwise comparisons were significant at p<0.05 except where indicated.



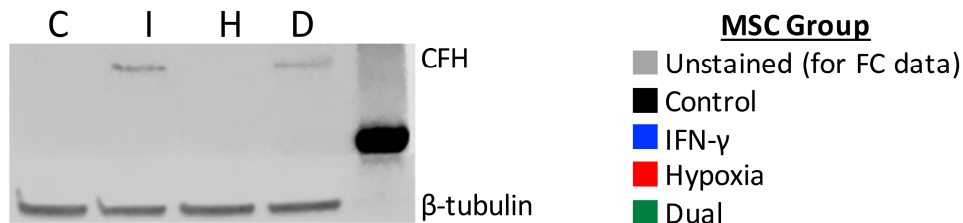
B LOX Activity Assay



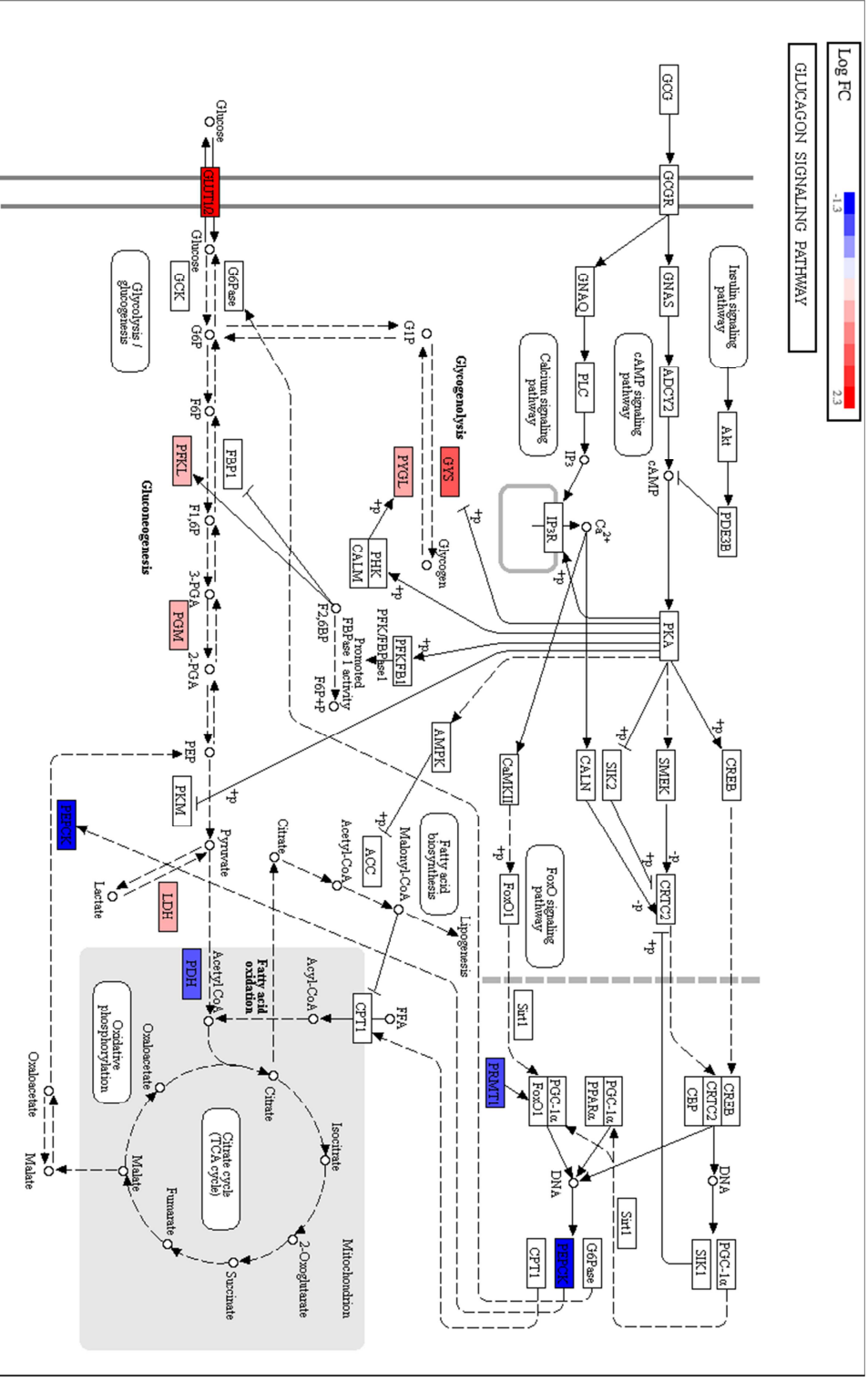
C Ang4 ELISA



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 758 **Supplemental Figure 2 Confirming trends found via mass spectroscopy for select proteins.** A) Flow cytometry was used to assess
 759 how IFN- γ and/or hypoxia affected expression of some proteins that had been detected by a single peptide via mass spectroscopy
 760 (CFH, HLA-F, PD-L1) or had shown a DE of small magnitude on mass spectroscopy (CD47, LRRC32, TGF-B1). B) Lysyl oxidase
 activity assay was used to confirm the increased activity/expression of this family of proteins upon exposure to hypoxia (or dual
 hypoxia/IFN- γ). C) ANGPTL4 ELISA was used to confirm the upregulation of this protein under hypoxia or dual hypoxia/IFN- γ
 priming. D) CFH Western Blot was a second means of confirming the upregulation by IFN- γ and dual priming seen from the mass spec
 data. * $p < 0.05$, *** $p < 0.0001$



Supplemental Figure 3 The effect of dual printing on the expression of proteins in the glucagon signaling pathway. The heat map at the top left shows the log2 fold change difference in expression of proteins, where blue indicates a down-regulated protein and red indicates an upregulated protein. Only those proteins that met our criteria for differential expression are shown (log2FC > 0.6 and p < 0.05)

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	IFN-γ	Hypoxia	Dual
% of proteins that are DE	12.10%	8.80%	20.50%
% of DE proteins that are in the mitochondria	12.20%	62.60%	29.30%

	IFN-γ	Hypoxia	Dual
Beta- alanine	6.56	1.63	10.60
hypotaurine	2.27	1.51	3.45
L-kynurenine	1E+99	0.00	1E+99
L-tyrosine	1E+99	0.00	1E+99
D-glucose	1.20	1.45	2.88
O-Phosphoethanolamine	1.15	2.92	2.45
pyruvic acid	0.72	0.44	0.49
L-(+) lactic acid	0.55	1.54	1.83
taurine	1E+99	1E+99	1E+99

	IFN-γ	Hypoxia	Dual	Function
C1QBP	-0.12	-1.2	-1.13	inhibits complement factor 1
C1R	2.55	-0.51	2.66	forms complement factor 1
C1S	2.01	-0.07	1.94	forms complement factor 1
CD47	1.24	0.01	1.19	protection from phagocytosis; transendothelial migration
CD97	1.39	0.17	1.23	chemotaxis
CFH	6.8	0.49	7.38	helps direct complement to pathogens vs. host cells
CSF1	3.41	-0.28	3.18	monocyte maturation
CXCL9	6.51	0.03	6.8	chemotaxis; antimicrobial
CXCL10	2.04	0.12	1.96	chemotaxis; antimicrobial
CXCL11	2.44	-0.13	2.38	chemotaxis; antimicrobial

HLA-E	3.01	-0.03	3.47	protects cells from NK cells
HLA-F	7.16	0.16	6.97	possibly immune tolerance
IDO1	6.64	0.19	6.51	immune tolerance
LGALS3BP	1.54	-0.22	1.32	galectin 3 binding protein
LGALS9B	4.76	-0.88	4.11	analog of galectin 9
LRRC32	0.3	0.35	0.89	promotes surface expression of TGF- β on T-regs
SERPING1	3.61	0.81	3.7	inhibits complement factor 1
PDL1	6.38	-0.2	6.27	immune tolerance
WARS	3.88	-0.08	3.78	tryptophan production

	IFN-γ	Hypoxia	Dual
AGRN*	4.35	1.47	6.16
COL1A1	-1.37	-0.57	-2.22
COL1A2	-1.03	-0.48	-1.61
COL2A1	-1.42	-0.64	-2.67
COL3A1	-1.05	0.13	-1.16
COL4A1	0.65	0.46	1.21
COL4A2	0.51	0.54	4.18
COL5A1	-0.75	-0.14	-0.92
COL5A2	-0.9	-0.74	-2.27
COL6A2	0.18	0.61	0.89
COL6A3	0.1	0.63	0.76
COL12A1	-0.97	-0.96	-2.59
COL15A1	-1.06	-0.56	-1.77
COL16A1	-0.71	-0.2	-0.67
CTGF	-2.25	0.46	-1.94
ELN	-1.27	-0.26	-1.36
FBLN1	-0.73	-0.45	-0.17
FBN1	-0.02	0.26	0.91
LAMA2*	0.67	-0.15	1.34
LAMA4	1.67	0.27	1.83

LAMB1	-1.03	-0.37	-1.11
LAMC1	-0.67	0.05	-0.38
LOX	-0.43	1.05	0.96
LOXL1*	0.22	0.72	2.11
LOXL2	-0.79	0.99	0.27
LOXL3*	0.65	2.72	4.21
PLOD1	0.08	0.68	0.49
PLOD2	0.27	1.74	1.9
SPARC	-1.56	0.21	-1.13
THBS1	-1.25	0.13	-0.76
THBS2	-1.62	0.16	-1.04
TIMP1	0.38	0.91	0.89
TIMP3	-0.56	0.37	-0.86
TNC	0.03	0.01	0.62