SUPPLEMENTARY FIGURES AND TABLES

Defining trophoblast injury patterns in the transcriptomes of dysfunctional placentas

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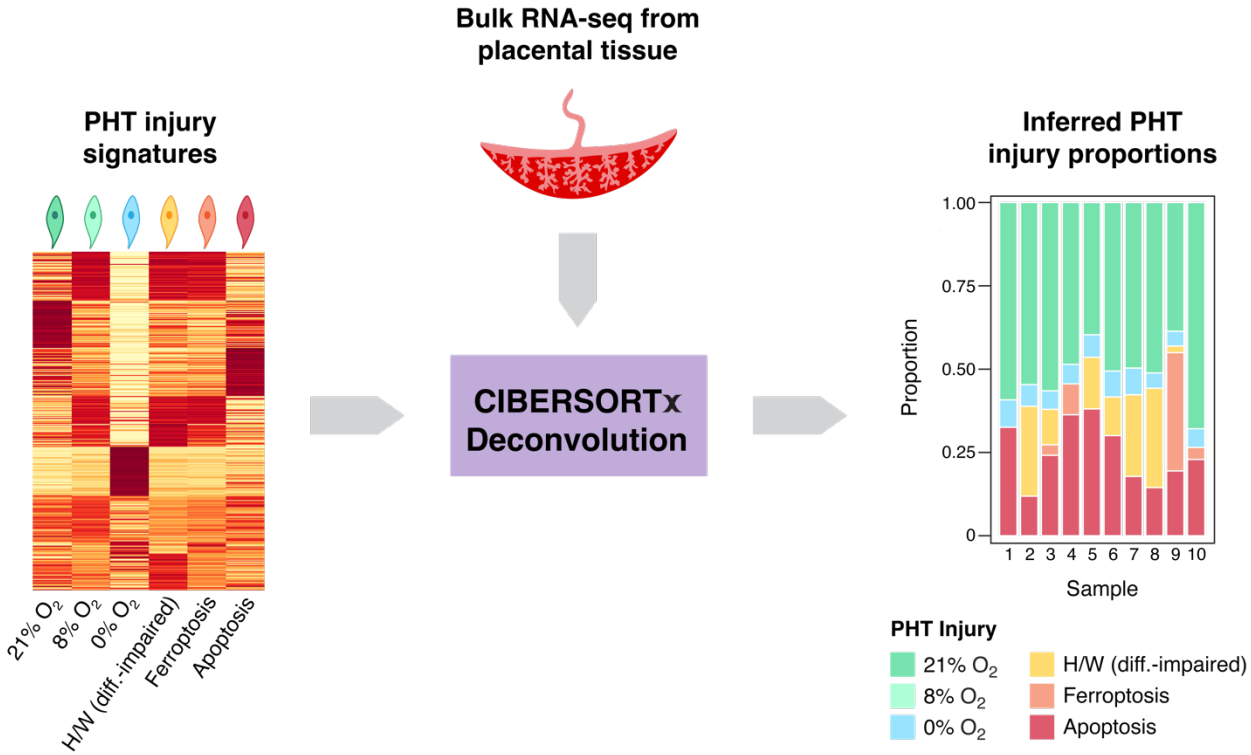

Figure S1. Deconvolution of bulk placental samples based on trophoblast injury signatures. An illustration describing the deconvolution process. The heatmap shows the PHT cell injury-related gene matrix. Using a linear mixed model, we identified transcripts that are significantly upregulated in each cell injury compared to the other injuries. To obtain a better resolution among the 8% O_2, ferroptosis, and differentiation impaired (H/W medium) conditions, we specifically identified genes that were significantly upregulated in each of these three injuries compared to the other two. Using these DEGs, ranked by the log fold change of their differential expression, we constructed a signature matrix with the first 194 differentially expressed genes from each comparison. The number of differentially expressed genes was selected from the range 50-500 to minimize the condition number of the signature matrix, as described in Newman et al (see below). The bar plot on the right depicts the proportion of each PHT injury across ten randomly selected among 271 placental samples.

Citation (ref 22, main manuscript): A.M. Newman, C.B. Steen, C.L. Liu, A.J. Gentles, A.A. Chaudhuri, F. Scherer, M.S. Khodadoust, M.S. Esfahani, B.A. Luca, D. Steiner, M. Diehn, A.A. Alizadeh, Determining cell type abundance and expression from bulk tissues with digital cytometry, Nat Biotechnol 37(7) (2019) 773-782.

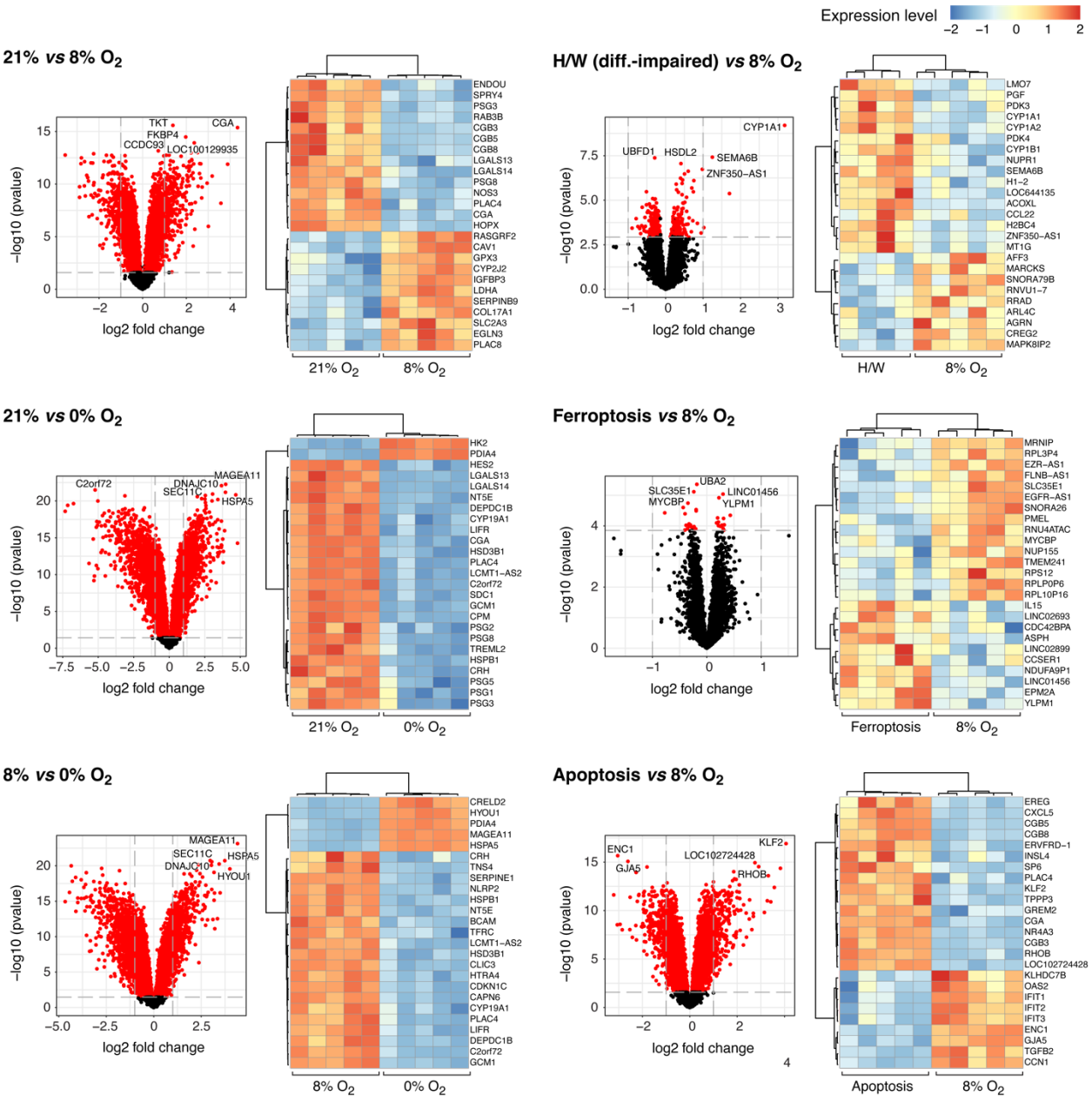

Figure S2. Pairwise comparisons among the different PHT cell injuries. The volcano plots show $\log_2$ fold change (x-axis) and $-\log_{10}$ p-value (y-axis): Each dot represents one mRNA. Each mRNA with FDR <0.05 is depicted in red. The light grey lines represent (vertical) $\log_2$ fold change >1 or <-1, and (horizontal) FDR<0.05. The five mRNAs with the lowest FDR for each comparison are labeled. The hierarchical clustering was performed using the 25 DEGs with the lowest FDR in each comparison: Each row corresponds to one mRNA, and each column represents a placenta. The color scale represents standardized expression levels, where red indicates higher levels and blue indicates lower levels. Note that for the differentiation-impaired (H/W) condition, one culture is missing due to cell contamination.

Supplementary Table S1. Differentially expressed genes in PHT cell culture conditions.

Comparison	Upregulated genes	Downregulated genes	Total DE genes
21% O_2 vs 8% O_2	3520	3501	**7021**
21% O_2 vs 0% O_2	5231	5174	**10405**
8% O_2 vs 0% O_2	4682	4745	**9427**
H/W (diff.-impaired) vs 8% O_2	152	148	**300**
Ferroptosis vs 8% O_2	14	19	**33**
Apoptosis vs 8% O_2	3622	3639	**7261**

Supplementary Table S2. Genes chosen for the CIBERSORTx matrix.

See accompanying Excel file.

Supplementary Table S3. The effect of culture conditions on the representation of PHT cell transcripts among relevant clinical conditions.

	Control (n = 95)	Control PT (n = 13)	PTD (n = 55)	FGR (n = 26)	FGR+HDP (n = 27)	Severe PE (n = 55)	padj
21% O_2	0.582 (0.109)	0.605 (0.112)	0.574 (0.153)	0.588 (0.102)	0.549 (0.158)	0.602 (0.139)	ns
8% O_2	0.000961 (0.00728)	0 (0)	0.00508 (0.0336)	0.00393 (0.0159)	0.00712 (0.0334)	0.000283 (0.0021)	ns
0% O2	0.0646 (0.0252)	0.0484 (0.0162)	0.0574 (0.0203)	0.0689 (0.0248)	0.0512 [a] (0.0251)	0.0591 (0.0255)	<0.05
H/W (diff. - impaired)	0.114 (0.118)	0.186 (0.122)	0.135 (0.166)	0.072 (0.12)	0.0152 [b] (0.0377)	0.0902 (0.142)	<0.0001
Ferroptosis	0.0444 (0.0708)	0.0391 (0.0641)	0.047 (0.105)	0.0447 (0.0721)	0.18 [b] (0.189)	0.077 (0.0894)	<0.0001
Apoptosis	0.194 (0.0896)	0.121 (0.0666)	0.182 (0.0975)	0.223 [a] (0.0941)	0.197 (0.0899)	0.171 (0.0846)	<0.05

[a] Significant compared to Control PT.

[b] Significant compared to both controls.

Supplementary Table S4. The effect of culture conditions on the representation of PHT cell transcripts among SNF-defined clusters.

	Cluster 1 (n = 126)	**Cluster 2 (n = 66)**	**Cluster 3 (n = 49)**	**Cluster 4 (n = 30)**	**padj**
21% O_2	0.592 (0.108)	0.617 (0.0935)	0.589 (0.127)	0.457 (0.201) [a]	<0.0001
8% O_2	0.000976 (0.0069)	0.000236 (0.00192)	0.00392 (0.0248)	0.0117 (0.047)	ns
0% O_2	0.0657 (0.0232) [c]	0.0529 (0.0217)	0.0523 (0.0261)	0.0674 (0.0245) [c]	<0.0001
H/W (diff. - impaired)	0.0919 (0.107)	0.159 (0.123) [a]	0.0316 (0.0747) [a]	0.143 (0.241)	<0.0001
Ferroptosis	0.0387 (0.0644)	0.0434 (0.0599)	0.134 (0.126) [b]	0.109 (0.197)	<0.0001
Apoptosis	0.21 (0.0846)	0.127 (0.0678) [a]	0.189 (0.0785)	0.212 (0.123)	<0.0001

[a] Significant compared to all others.
[b] Significant compared to Clusters 1 and 2.
[c] Significant compared to Clusters 2 and 3.