Supplementary file

Mapping endometrial vascular functional gradients using depth derived ultrasound localization microscopy (D-ULM) for early stratification of post-injury fibrotic remodeling and therapeutic guidance

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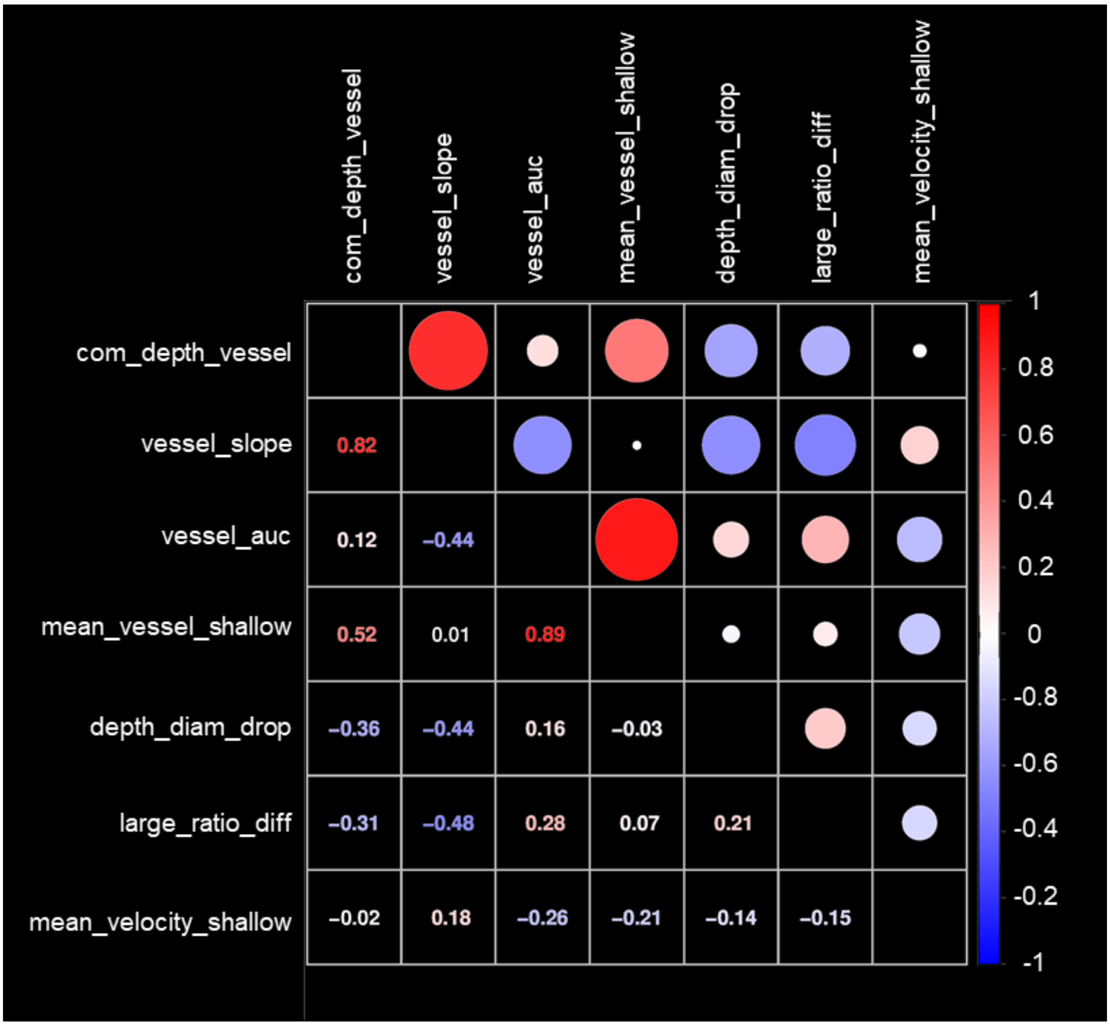

Figure S1. Correlation matrix of D-ULM features (multicollinearity analysis), demonstrating strong positive correlations between com_depth_vessel and vessel_slope, as well as between mean_vessel_shallow and vessel_auc. To assess the impact of collinearity, principal component analysis was performed and the first three principal components, explaining 90% of the total variance, were retained. Hierarchical clustering on the PC scores yielded a cluster assignment with an adjusted Rand index of 0.697 compared to the original clustering, indicating substantial agreement and demonstrating that the original clustering is robust to collinearity among the depth-derived features.

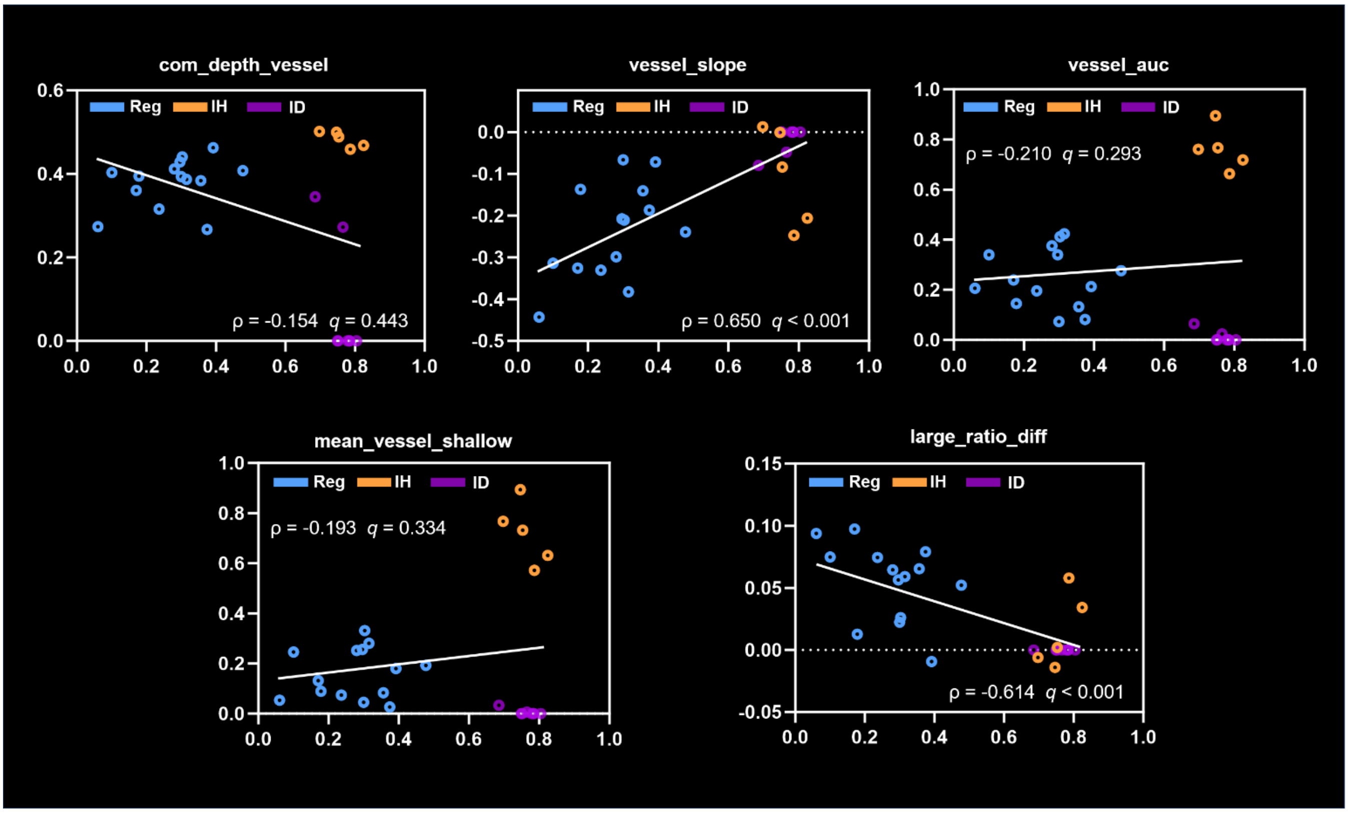

Figure S2. Sensitivity analysis of correlations between day-3 D-ULM features and day-14 CAP including all three phenotypes (Reg, IH, and ID). Reg: regenerative phenotype; IH: inflammatory hyperperfusion phenotype; ID: irreversible destructive phenotype.

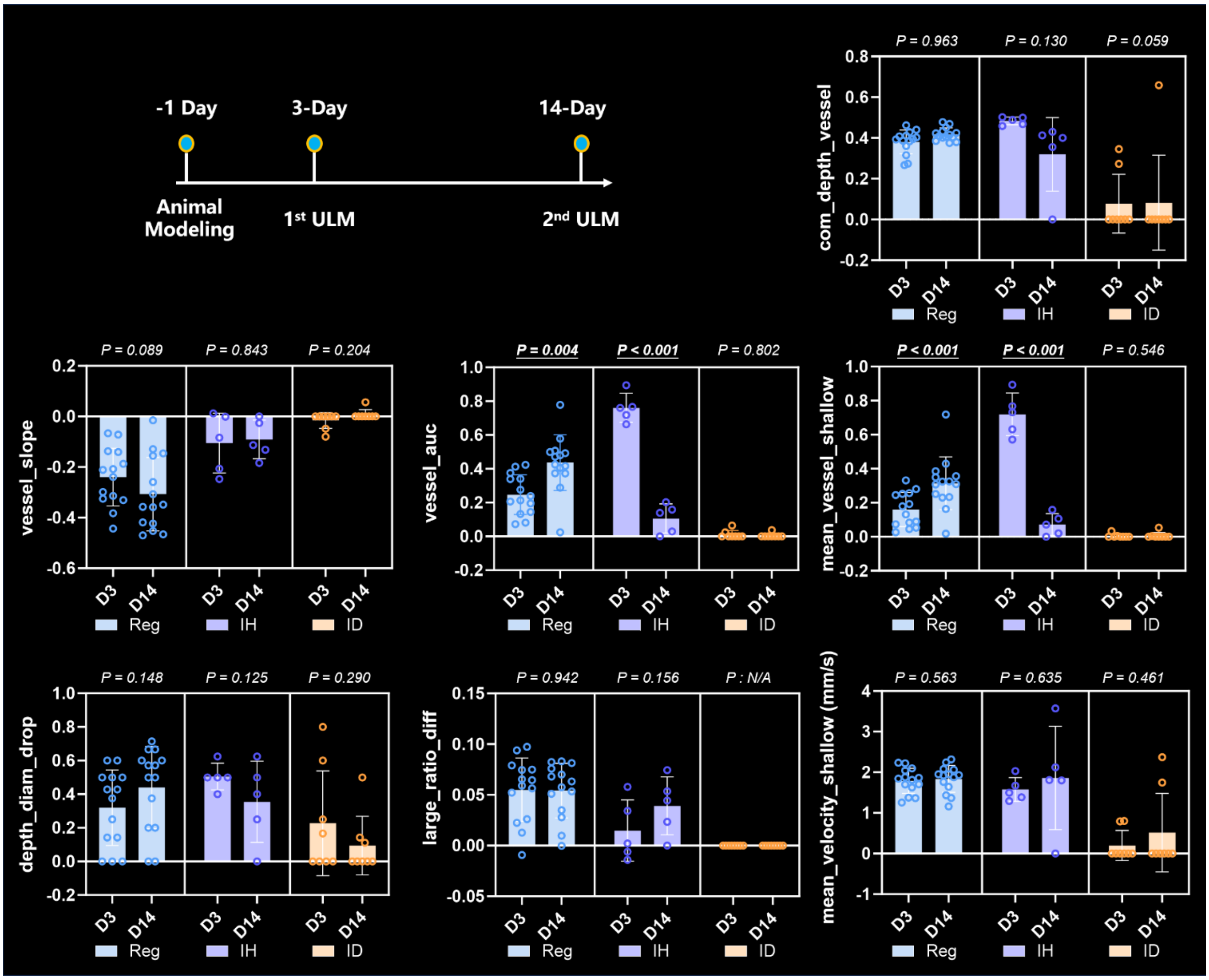

Figure S3. Comparison of day-3 and day-14 D-ULM features within each phenotype. Over time, vessel_auc and mean_vessel_shallow were significantly increased in the reg phenotype, whereas they were markedly decreased in the IH phenotype ($P < 0.01$). Sample sizes: n = 14 (Reg), n = 5 (IH), n = 8 (ID). Reg: regenerative phenotype; IH: inflammatory hyperperfusion phenotype; ID: irreversible destructive phenotype.

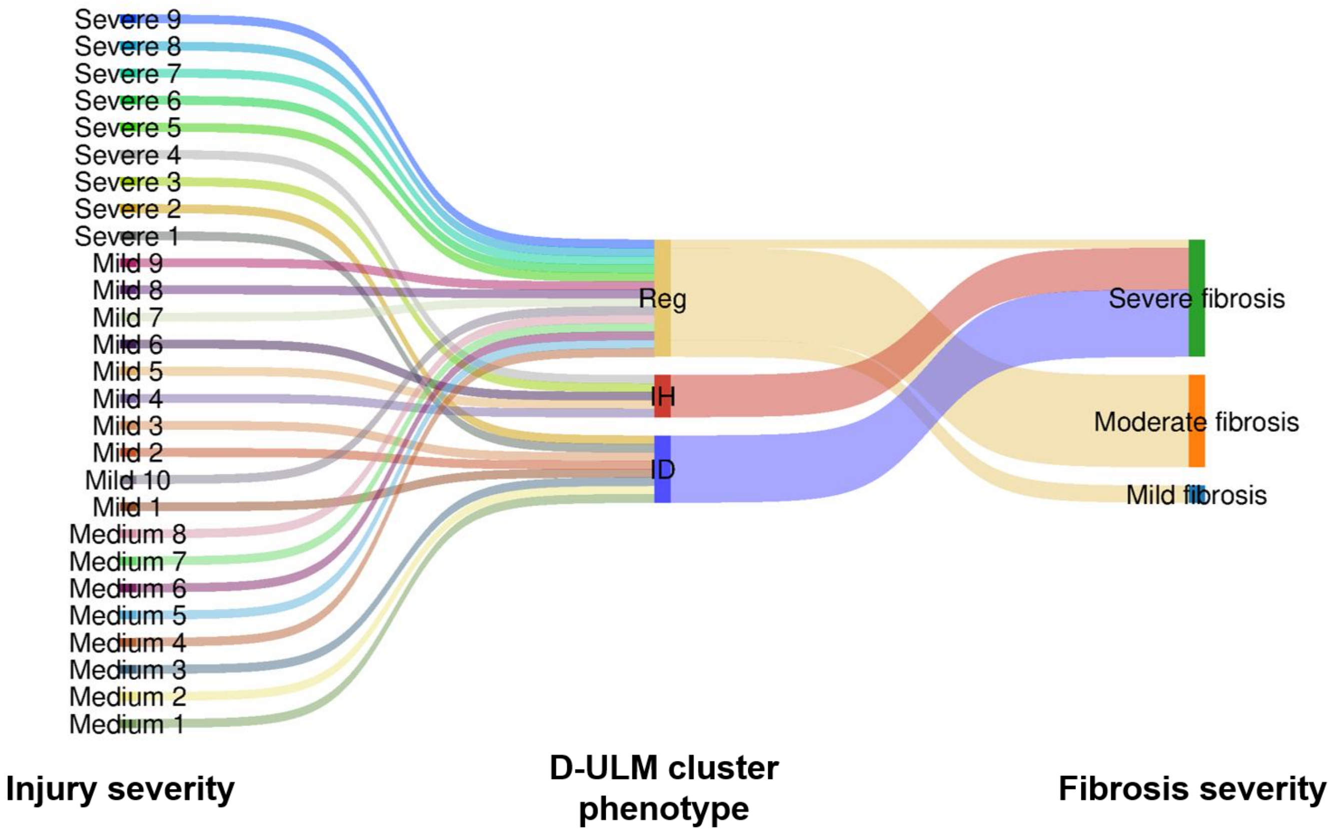

Figure S4. Sankey diagram illustrating the distribution of samples across injury severity (mild, moderate, severe), D-ULM cluster phenotype, and fibrotic severity. Injury severity is categorized based on the residence time of 95% ethanol during modeling: mild, 30 s; medium, 50 s; severe, 90 s. According to the predefined criteria, collagen area percentage ranges for mild, moderate, and severe fibrosis are < 10%, 10–50%, and > 50%, respectively. Among the injuries of the same experimental injury severity, D-ULM phenotypes proved useful in distinguishing rats having different fibrotic outcomes, which implies that the D-ULM system reflects biological heterogeneity of microvascular recovery after injury—not fully determined by injury severity. Reg: regenerative phenotype; IH: inflammatory hyperperfusion phenotype; ID: irreversible destructive phenotype.

Table S1. The unsupervised hierarchical clustering results, priori injury categories and related D-ULM feature values.

No.	Expert-based visual category	D-ULM Cluster phenotype	CAP	com_depth_vessel	vessel_slope	vessel_auc	mean_vessel_shallow	depth_diam_drop	large_ratio_diff	mean_velocity_shallow
1	ID	ID	0.751	0	0	0	0	0.167	0	0
2	ID	ID	0.749	0	0	0	0	0	0	0
3	ID	ID	0.784	0	0	0	0	0	0	0
4	ID	ID	0.804	0	0	0	0	0.8	0	0
5	ID	ID	0.685	0.345	-0.08	0.065	0.034	0.25	0	0.802
6	ID	ID	0.765	0.273	-0.048	0.024	0.006	0.6	0	0.794
7	ID	ID	0.784	0	0	0	0	0	0	0
8	ID	ID	0.778	0	0	0	0	0	0	0
9	IH	IH	0.753	0.489	-0.084	0.766	0.732	0.625	0.002	2.031
10	IH	IH	0.697	0.502	0.013	0.761	0.768	0.5	-0.006	1.674
11	**IH**	**Reg**	0.477	0.408	-0.239	0.277	0.193	0.429	0.052	2.043
12	IH	IH	0.824	0.468	-0.206	0.719	0.631	0.500	0.034	1.386
13	IH	IH	0.786	0.459	-0.247	0.663	0.572	0.500	0.058	1.498
14	IH	IH	0.746	0.5	-0.001	0.895	0.893	0.400	-0.014	1.292
15	Reg	Reg	0.303	0.441	-0.211	0.412	0.331	0.600	0.026	1.707
16	Reg	Reg	0.296	0.429	-0.208	0.34	0.256	0.600	0.056	1.701
17	Reg	Reg	0.300	0.393	-0.066	0.073	0.046	0	0.022	1.842
18	Reg	Reg	0.236	0.316	-0.33	0.196	0.074	0.500	0.075	2.099
19	Reg	Reg	0.170	0.360	-0.325	0.240	0.132	0.375	0.097	2.221
20	Reg	Reg	0.100	0.403	-0.314	0.340	0.246	0.143	0.075	1.864
21	Reg	Reg	0.374	0.267	-0.187	0.082	0.027	0.143	0.079	2.233
22	Reg	Reg	0.178	0.395	-0.137	0.145	0.089	0	0.013	1.816
23	Reg	Reg	0.392	0.462	-0.072	0.213	0.180	0	-0.009	1.377
24	Reg	Reg	0.315	0.387	-0.382	0.424	0.281	0.250	0.059	1.880
25	Reg	Reg	0.280	0.412	-0.298	0.375	0.252	0.500	0.065	1.255
26	Reg	Reg	0.060	0.274	-0.443	0.206	0.054	0.429	0.094	1.366
27	Reg	Reg	0.356	0.384	-0.141	0.132	0.084	0.500	0.065	1.626

* The expert-based visual categories served as an external validation for the unsupervised clustering. Of note, one rat (No. 11) visually classified as IH was assigned to the Reg phenotype by D-ULM clustering. D-ULM revealed relatively preserved deep vascular signals that may not have been appreciable on visual inspection, highlighting the ability of depth-derived feature analysis to refine phenotype identification. ID: irreversible destructive phenotype; IH: inflammatory hyperperfusion phenotype; Reg: regenerative phenotype; CAP: collagen area percentage.

Table S2. Spearman correlation analysis between CAP and conventional ROI-based ULM parameters (as a comparison with D-ULM features).

Conventional ROI-based ULM parameters	ρ	q value
Fractal Dimension	0.354	0.137
Vessel Proportion	0.568	0.011
Max Velocity (mm/s)	0.314	0.190
Mean Velocity (mm/s)	-0.147	0.547
Tortuosity	0.509	0.026
Mean Diameter (μm)	0.465	0.045
Large Vessel Diameter (μm)	0.467	0.044
Tiny Vessel Diameter (μm)	0.577	0.010
Junction Count	0.049	0.842
Branching Index (mm^{-2})	0.456	0.050