

## **SUPPLEMENTARY INFORMATION**

Untargeted metabolomics reveals N,N,N-trimethyl-L-alanyl-L-proline betaine (TMAP) as a novel biomarker of kidney function

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**Supplemental Table 1.** Chromatographic conditions for RPLC and HILIC separation of metabolites.

	<b>Time (min)</b>	<b>0.1% Formic Acid in Water (%)</b>	<b>0.1% Formic Acid in Acetonitrile (%)</b>	<b>Curve</b>
<b>RPLC</b>	0.00	99	1	6
	2.00	40	60	6
	6.00	15	85	6
	8.00	1	99	6
	10.00	99	1	6
	11.00	99	1	6
<b>HILIC</b>	0.00	1	99	6
	0.50	1	99	6
	6.00	50	50	6
	8.00	70	30	6
	8.51	1	99	6

**Supplemental Table 2.** Goodness of fit (R2Y) and predictive ability (Q2Y) for OPLS-DA and multilevel PLS-DA analysis in this study.

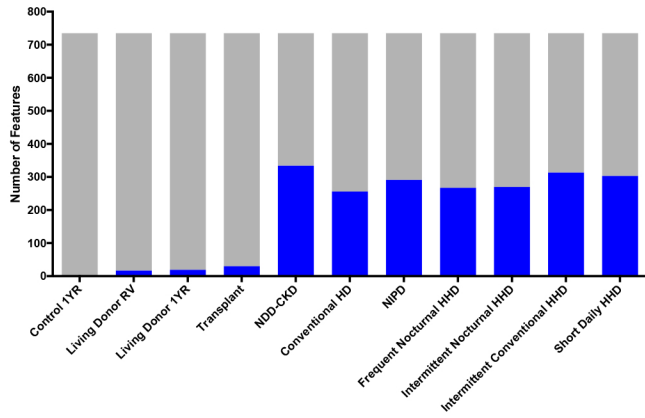
<b>Figure</b>	<b>Comparison</b>	<b>R2Y</b>	<b>Q2Y</b>
<b>Fig. 2A</b>	Living Donor RV vs 1YR Plasma RPLC	0.993	0.664
<b>Fig. 2B</b>	Living Donor RV vs 1YR Plasma HILIC	0.992	0.314
<b>Fig. 2C</b>	Control vs Transplant RPLC	0.616	0.883
<b>Fig. 2D</b>	Control vs Transplant HILIC	0.933	0.592
<b>Suppl. Fig. 1A</b>	Control vs CKD RPLC	0.876	0.653
<b>Suppl. Fig. 1B</b>	Control vs CKD HILIC	0.799	0.634
<b>Suppl. Fig. 1C</b>	Control vs Conv HD Pre RPLC	0.938	0.623
<b>Suppl. Fig. 1D</b>	Control vs Conv HD Pre HILIC	0.875	0.520
<b>Suppl. Fig. 1E</b>	Control vs NIPD Pre RPLC	0.946	0.730
<b>Suppl. Fig. 1F</b>	Control vs NIPD Pre HILIC	0.863	0.629
<b>Suppl. Fig. 1G</b>	Control vs Frequent Nocturnal HHD Pre RPLC	0.974	0.720
<b>Suppl. Fig. 1H</b>	Control vs Frequent Nocturnal HHD Pre HILIC	0.963	0.864
<b>Suppl. Fig. 2A</b>	Transplant vs CKD RPLC	0.870	0.616
<b>Suppl. Fig. 2B</b>	Transplant vs CKD HILIC	0.738	0.513
<b>Suppl. Fig. 2C</b>	Transplant vs Conventional HD Pre RPLC	0.947	0.669
<b>Suppl. Fig. 2D</b>	Transplant vs Conventional HD Pre HILIC	0.819	0.488
<b>Suppl. Fig. 2E</b>	Transplant vs NIPD Pre RPLC	0.948	0.765
<b>Suppl. Fig. 2F</b>	Transplant vs NIPD Pre HILIC	0.819	0.596
<b>Suppl. Fig. 2G</b>	Transplant vs Frequent Nocturnal HHD Pre RPLC	0.983	0.731
<b>Suppl. Fig. 2H</b>	Transplant vs Frequent Nocturnal HHD Pre HILIC	0.945	0.837
<b>Suppl. Fig. 3A</b>	Conventional HD Pre vs NIPD Pre RPLC	0.974	0.251

<b>Suppl. Fig. 3B</b>	Conventional HD Pre vs NIPD Pre HILIC	0.708	0.201
<b>Suppl. Fig. 3C</b>	Conventional HD Pre vs Frequent Nocturnal HHD Pre RPLC	0.816	0.252
<b>Suppl. Fig. 3D</b>	Conventional HD Pre vs Frequent Nocturnal HHD Pre HILIC	0.796	-0.002
<b>Suppl. Fig. 3E</b>	Conventional HD Pre vs NIPD Pre RPLC	0.930	0.594
<b>Suppl. Fig. 3F</b>	Conventional HD Pre vs NIPD Pre HILIC	0.875	0.540
<b>Suppl. Fig. 4A</b>	Conventional HD Pre vs Post RPLC	0.997	0.770
<b>Suppl. Fig. 4B</b>	Conventional HD Pre vs Post HILIC	0.990	0.641
<b>Suppl. Fig. 4C</b>	Frequent Nocturnal HHD Pre vs Post RPLC	0.998	0.914
<b>Suppl. Fig. 4D</b>	Frequent Nocturnal HHD Pre vs Post HILIC	0.981	0.871
<b>Suppl. Fig. 4E</b>	NIPD Pre vs Post RPLC	0.979	0.546
<b>Suppl. Fig. 4F</b>	NIPD Pre vs Post HILIC	0.997	0.181
<b>Suppl. Fig. 5A</b>	Control vs Intermittent Conventional HHD Pre RPLC	0.973	0.744
<b>Suppl. Fig. 5B</b>	Control vs Intermittent Conventional HHD Pre HILIC	0.992	0.908
<b>Suppl. Fig. 5C</b>	Control vs Intermittent Nocturnal HHD Pre RPLC	0.984	0.665
<b>Suppl. Fig. 5D</b>	Control vs Intermittent Nocturnal HHD Pre HILIC	0.959	0.741
<b>Suppl. Fig. 5E</b>	Control vs Short Daily HHD Pre RPLC	0.958	0.759
<b>Suppl. Fig. 5F</b>	Control vs Short Daily HHD Pre HILIC	0.963	0.830
<b>Suppl. Fig. 6A</b>	Intermittent Conventional HHD Pre vs Post RPLC	0.998	0.853
<b>Suppl. Fig. 6B</b>	Intermittent Conventional HHD Pre vs Post HILIC	0.988	0.911
<b>Suppl. Fig. 6C</b>	Intermittent Nocturnal HHD Pre vs Post RPLC	0.999	0.959
<b>Suppl. Fig. 6D</b>	Intermittent Nocturnal HHD Pre vs Post HILIC	0.996	0.795

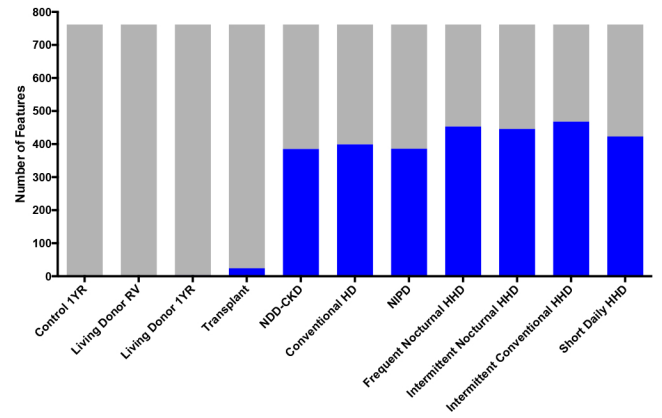
<b>Suppl.</b>	Short Daily HHD Pre vs Post RPLC	0.997	0.927
<b>Fig. 6E</b>			
<b>Suppl.</b>	Short Daily HHD Pre vs Post HILIC	0.992	0.719
<b>Fig. 6F</b>			

# Supplemental Figure 1

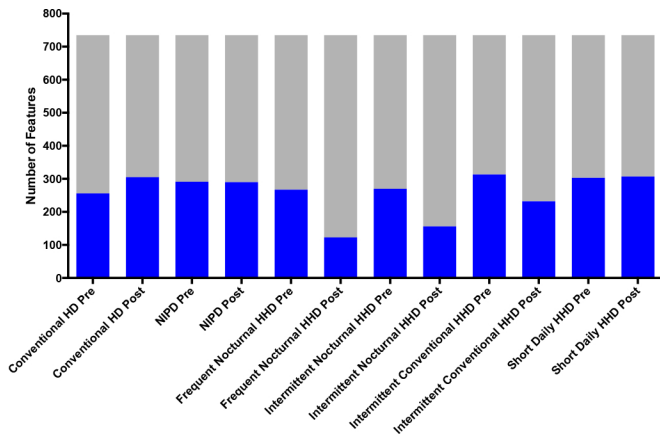
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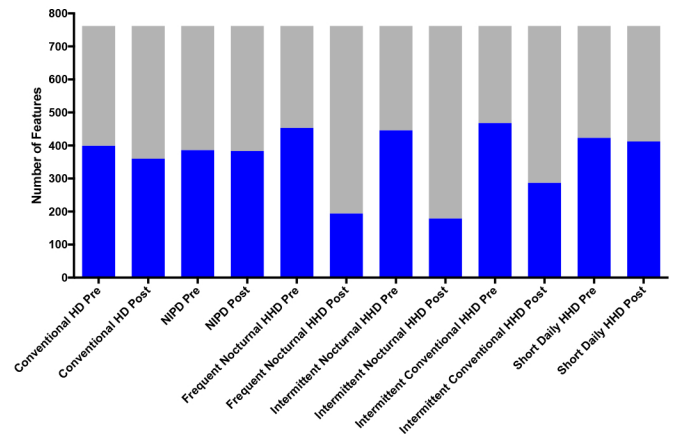
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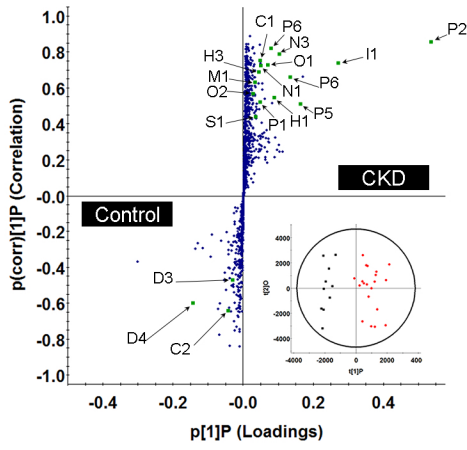
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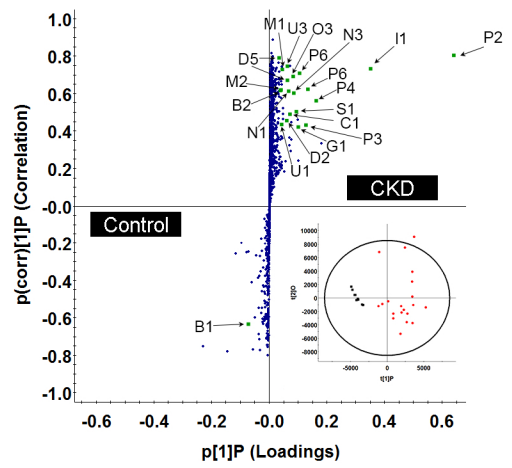
**Supplementary Figure 1.** Number of features significantly different by Kruskal-Wallis ANOVA ( $p < 0.05$  and  $q < 0.05$ ) followed by Dunn's post-hoc test for RPLC (A) and HILIC (B) analysis (grey bars) and features significantly different than control RV samples (blue bars). Features significantly different than control RV for pre- and post-dialysis samples are also presented from RPLC (C) and HILIC (D) metabolomics analysis. Control and living kidney plasma samples were obtained during recruitment visit (RV) and one year follow-up (1YR).

# Supplemental Figure 2

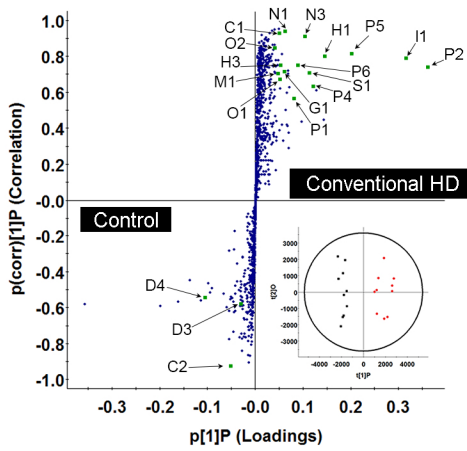
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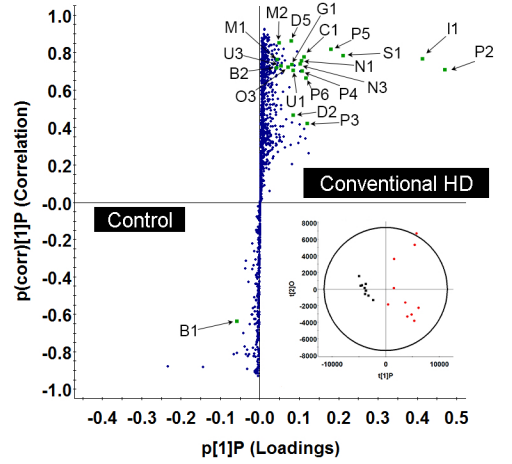
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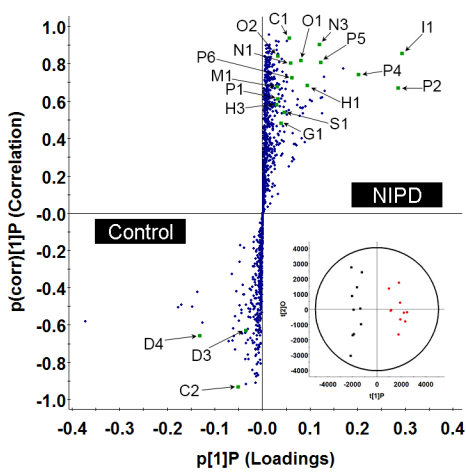
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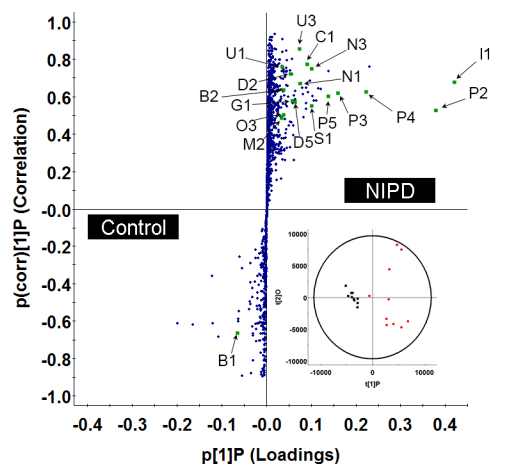
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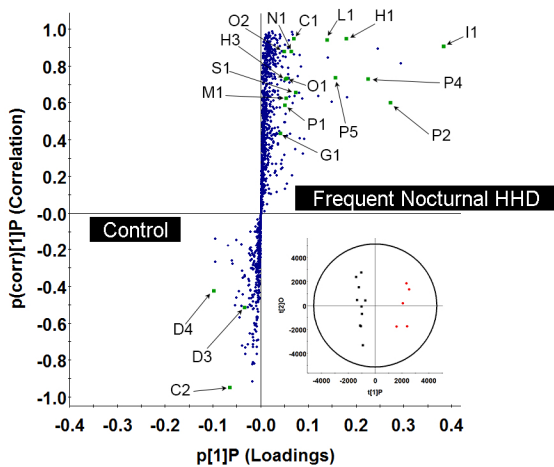
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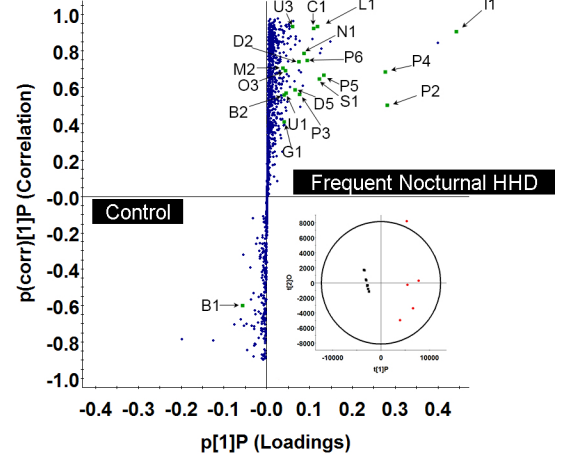
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G



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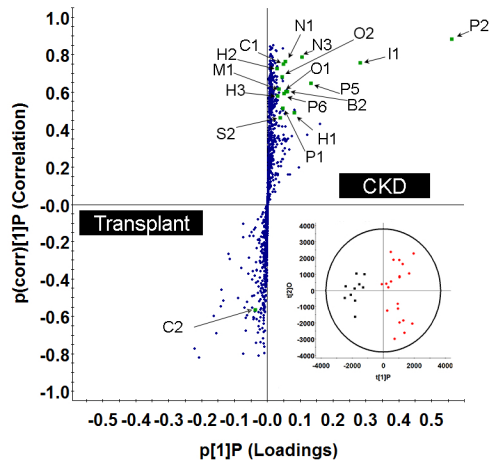




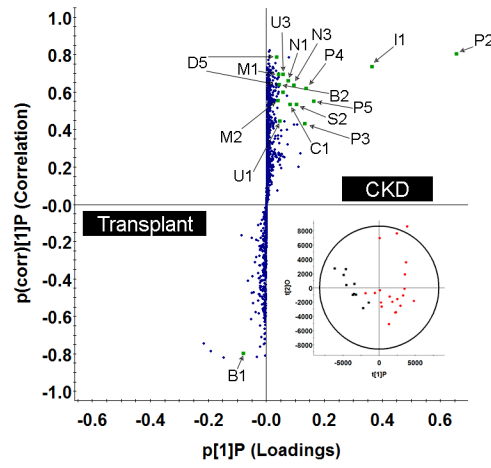
**Supplementary Figure 2.** Orthogonal partial least squares discriminant analysis (OPLS-DA) and S-plot projections comparing plasma metabolic features from control (■, n = 10) subjects with CKD (●, n = 20, A, RPLC; B, HILIC), conventional hemodialysis (conventional HD, ●, n = 10; C, RPLC; D, HILIC), nocturnal intermittent peritoneal dialysis (NIPD, ●, n = 10; E, RPLC; F, HILIC) and frequent nocturnal HHD (●, n = 5; HD E, RPLC; F, HILIC). Features are annotated (see Table 2 for metabolite list) with variable importance in projection (VIP) values > 1 and correlation (pcorr) values > 0.4 or < -0.4.

# Supplemental Figure 3

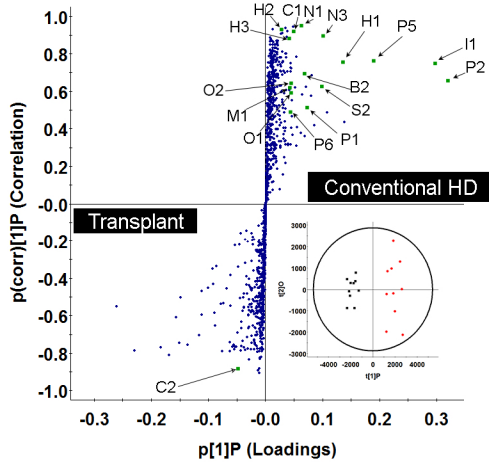
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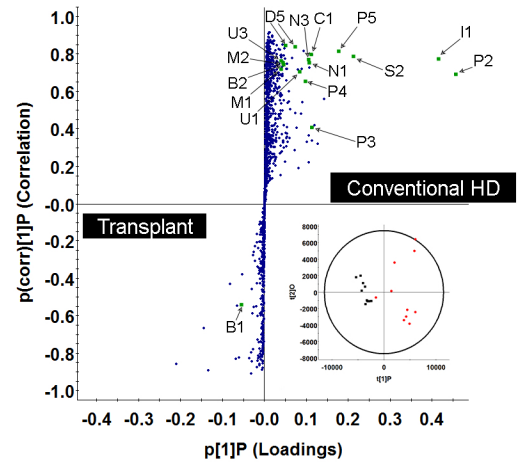
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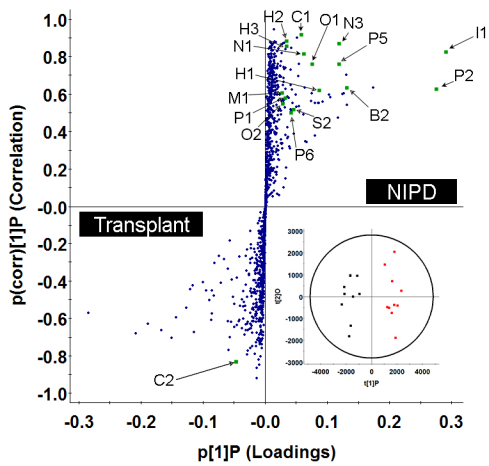
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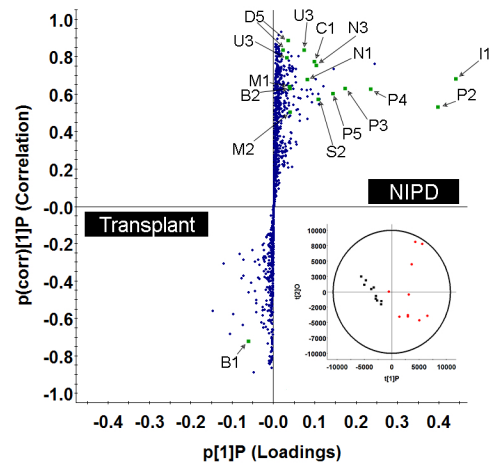
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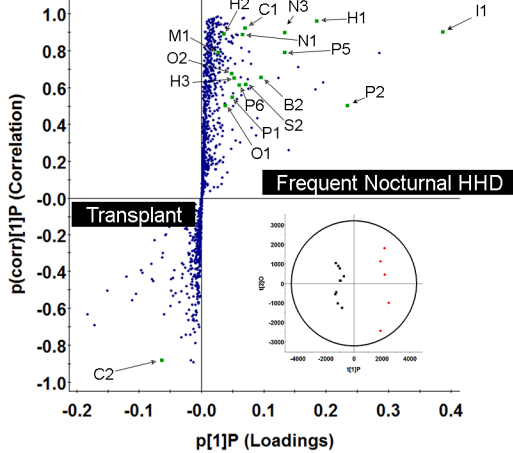
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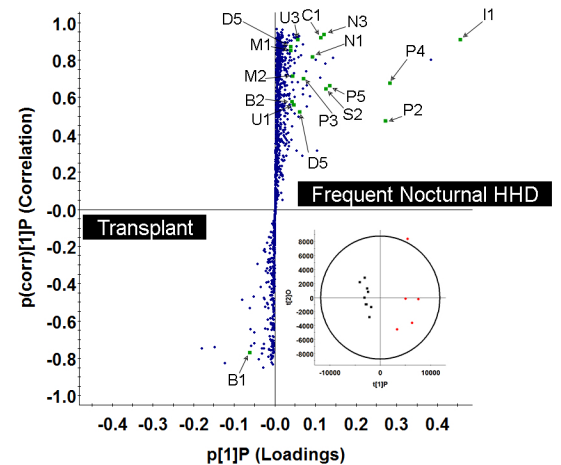
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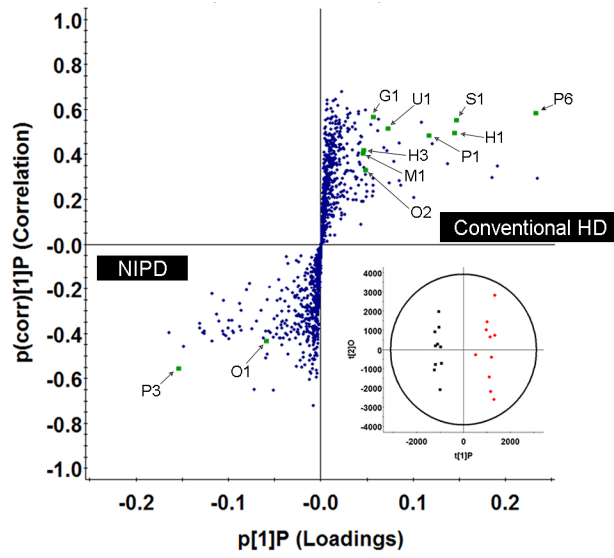
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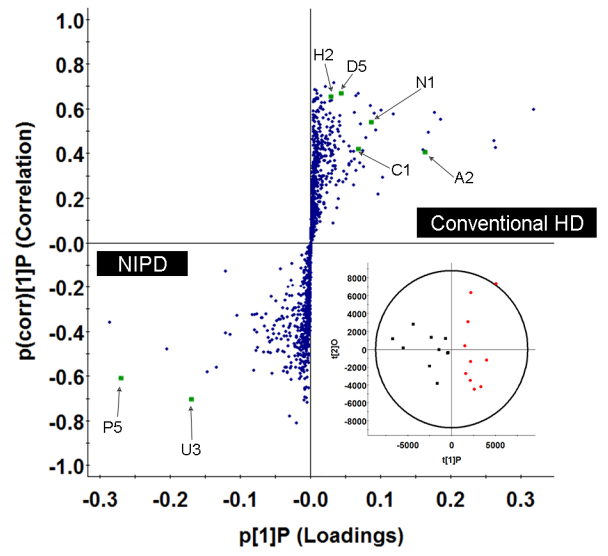
**Supplemental Figure 3.** Orthogonal partial least squares discriminant analysis (OPLS-DA) and S-plot projections comparing plasma metabolic features from kidney transplant (■, n = 10) patients with CKD (●, n = 20, A, RPLC; B, HILIC), conventional hemodialysis (conventional HD, ●, n = 10; C, RPLC; D, HILIC), nocturnal intermittent peritoneal dialysis (NIPD, ●, n = 10; E, RPLC; F, HILIC) and frequent nocturnal HHD (●, n = 5; HD E, RPLC; F, HILIC). Features are annotated (see Table 2 for metabolite list) with variable importance in projection (VIP) values > 1 and correlation (pcorr) values > 0.4 or < -0.4.

# Supplemental Figure 4

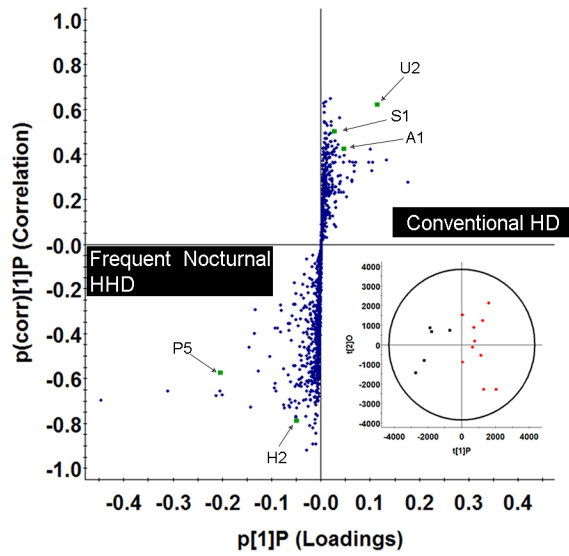
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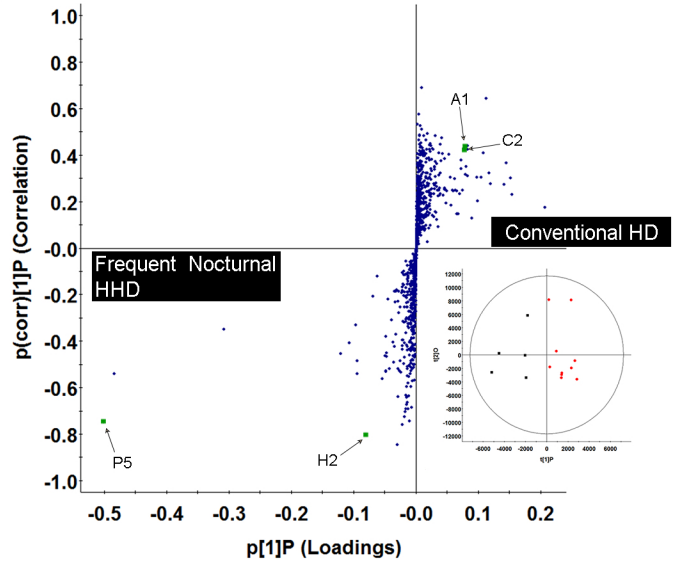
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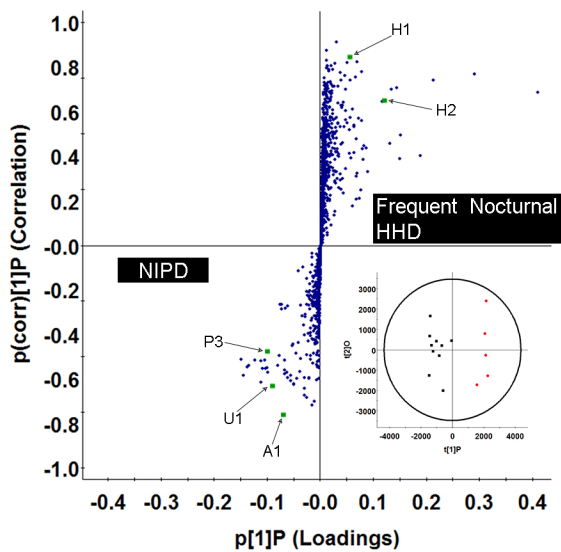
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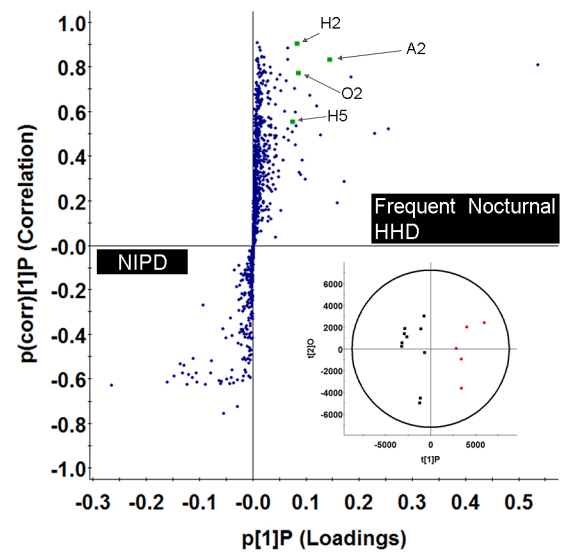
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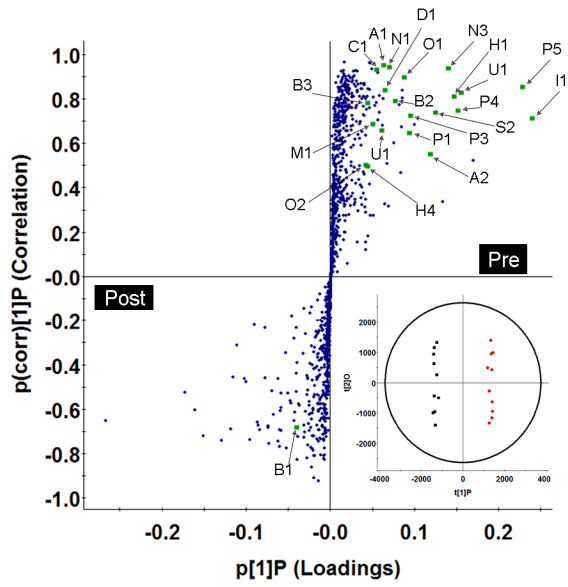
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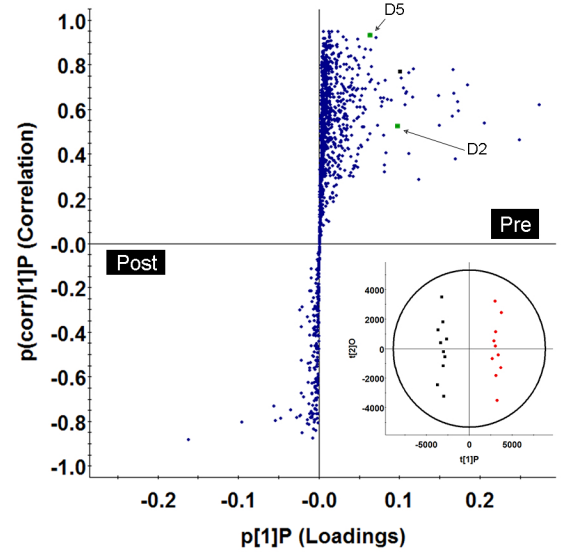
**Supplemental Figure 4.** Orthogonal partial least squares discriminant analysis (OPLS-DA) and S-plot projections comparing pre-dialysis plasma metabolic features between nocturnal intermittent peritoneal dialysis (NIPD, ■, n = 10) and conventional hemodialysis (conventional HD, ●, n = 10; A, RPLC; B, HILIC), frequent nocturnal HHD (■, n = 5) and conventional HD (●, C, RPLC; D, HILIC), and NIPD (■) and frequent nocturnal HHD (●, G, RPLC; H, HILIC). Features are annotated (see Table 2 for metabolite list) with variable importance in projection (VIP) values > 1 and correlation (pcorr) values > 0.4 or < -0.4.

# Supplementary Figure 5

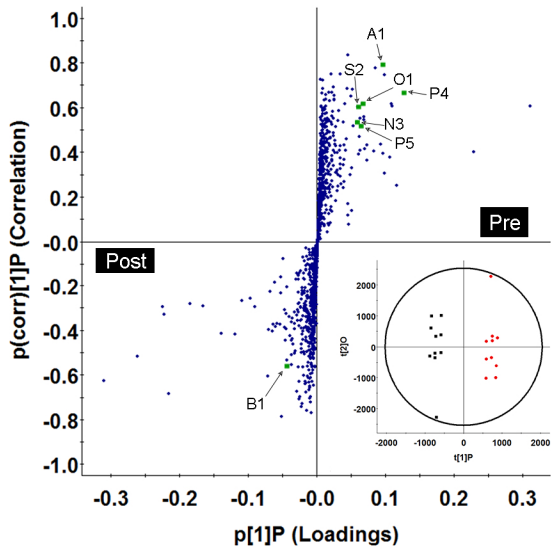
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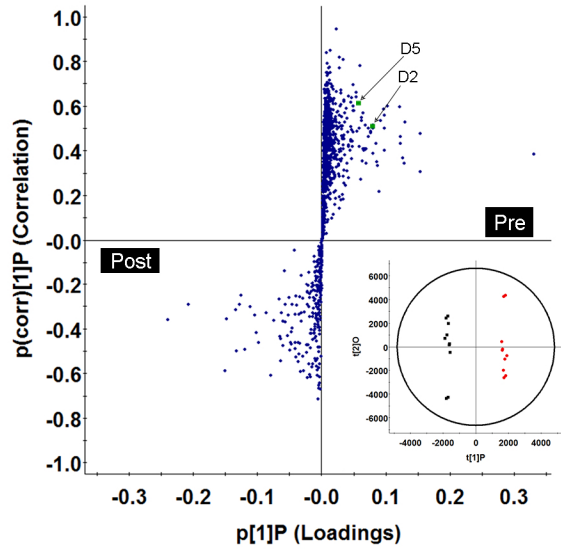
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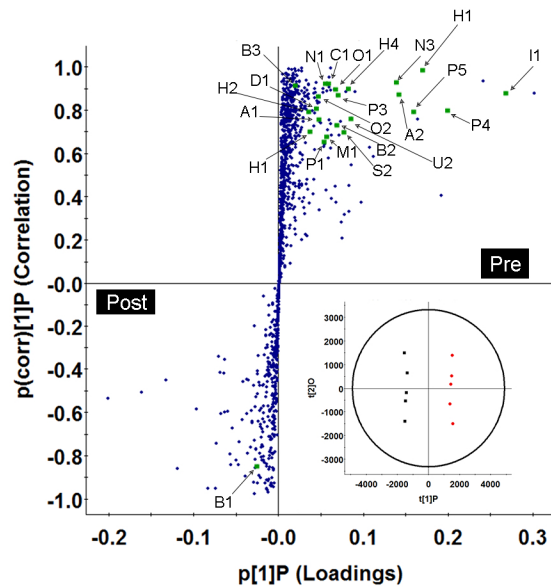
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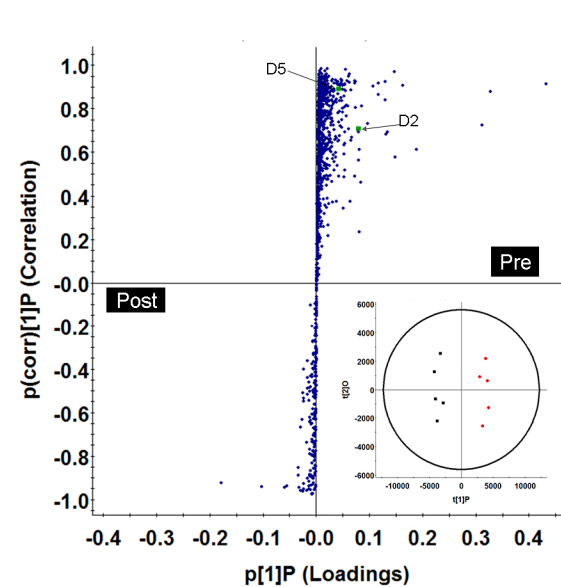
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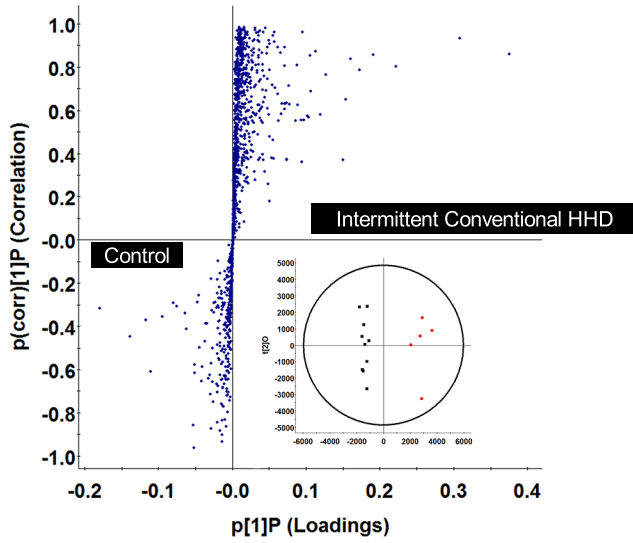
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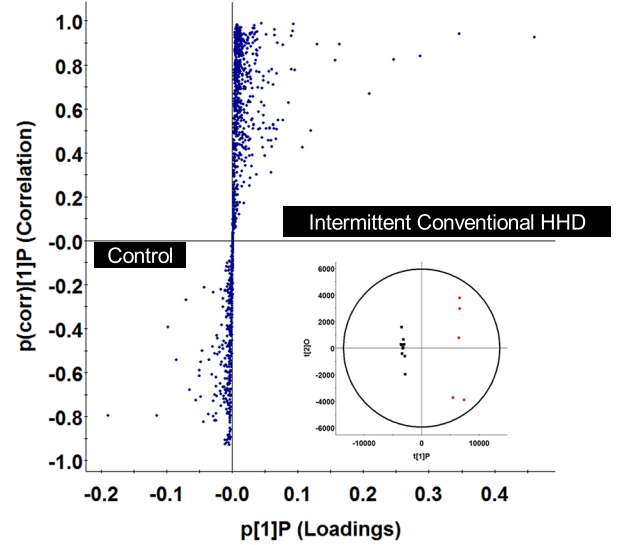
**Supplemental Figure 5.** Multilevel partial least squares discriminant analysis and S-plot projections of pre-(●) and post(■)-dialysis plasma samples from conventional hemodialysis (n = 10; A, RPLC; B, HILIC), nocturnal intermittent peritoneal dialysis (n = 10; C, RPLC; D, HILIC) and frequent nocturnal HHD (n = 5; E, RPLC; F, HILIC). Features are annotated (see Table 2 for metabolite list) with variable importance in projection (VIP) values > 1 and correlation (pcorr) values > 0.4 or < -0.4.

# Supplemental Figure 6

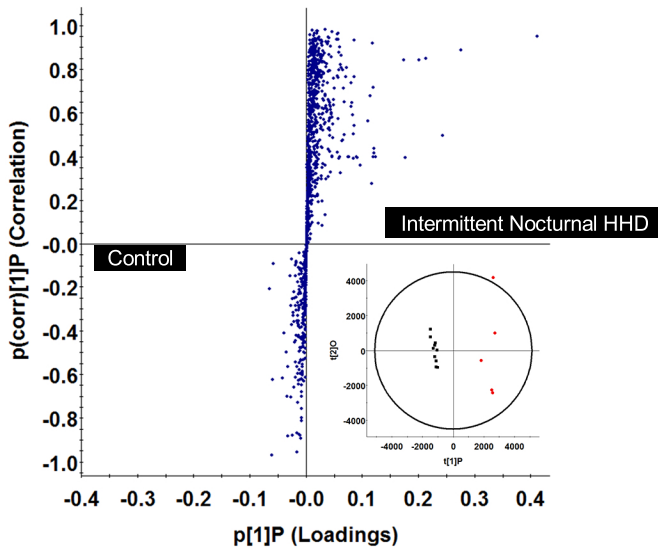
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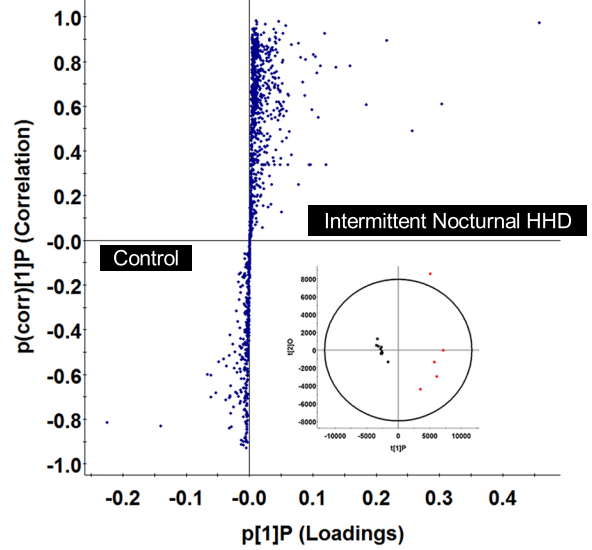
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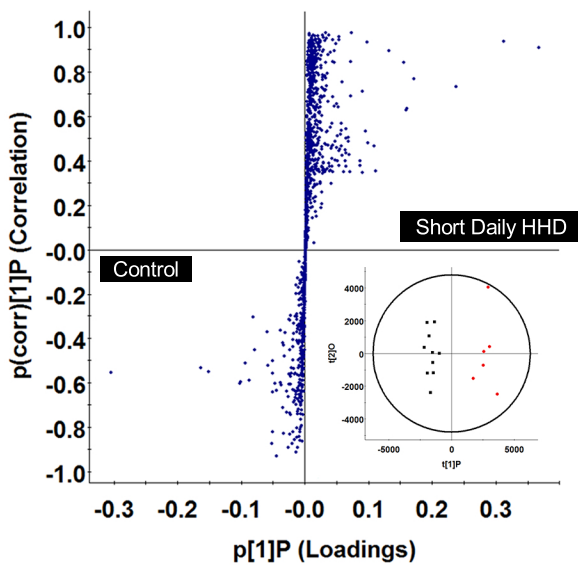
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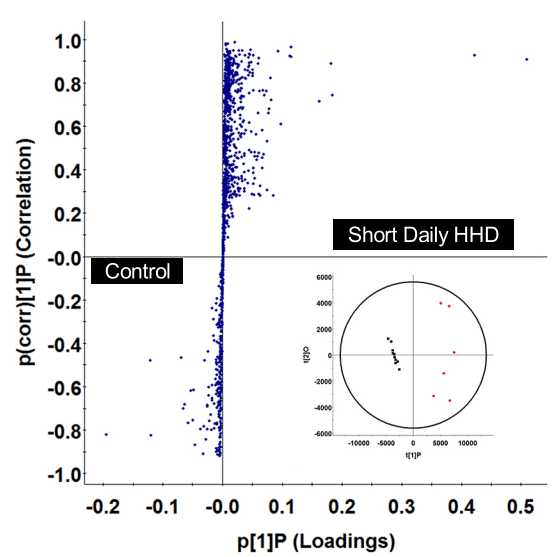
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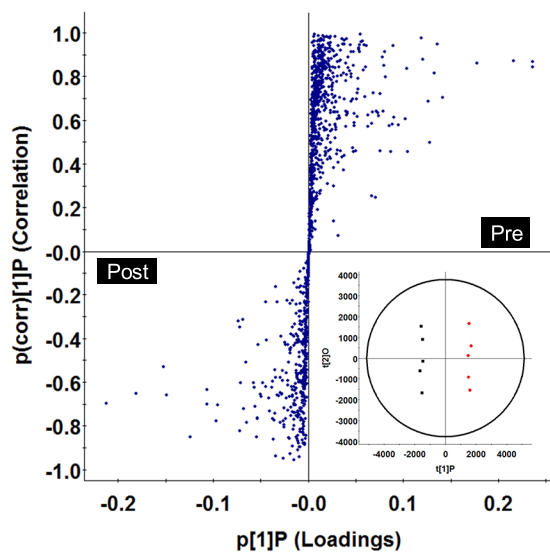




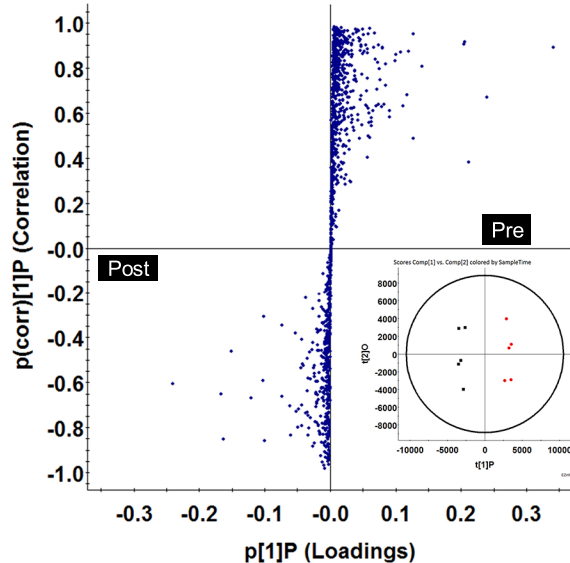
**Supplemental Figure 6.** Orthogonal partial least squares discriminant analysis (OPLS-DA) and S-plot projections comparing plasma metabolic features from control (■, n = 10) subjects with intermittent conventional HHD (●, n = 5; C, RPLC; D, HILIC), intermittent nocturnal HHD (●, n = 10; E, RPLC; F, HILIC) and short daily HHD (●, n = 6; HD E, RPLC; F, HILIC). Features are annotated (see Table 2 for metabolite list) with variable importance in projection (VIP) values > 1 and correlation (pcorr) values > 0.4 or < -0.4.

# Supplemental Figure 7

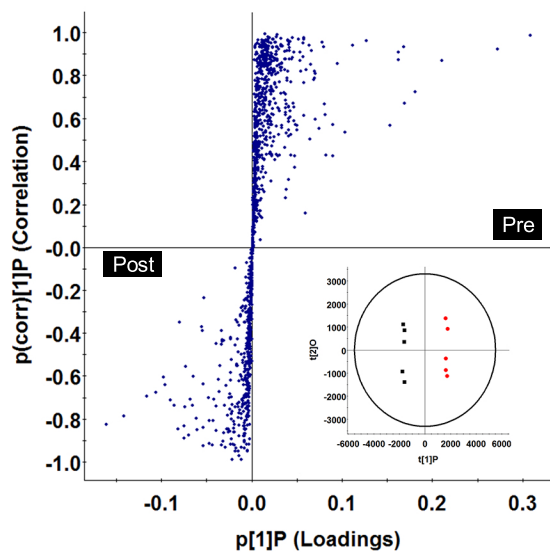
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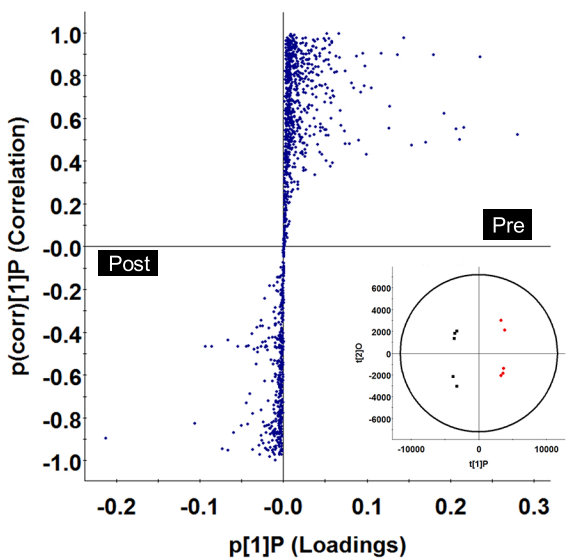
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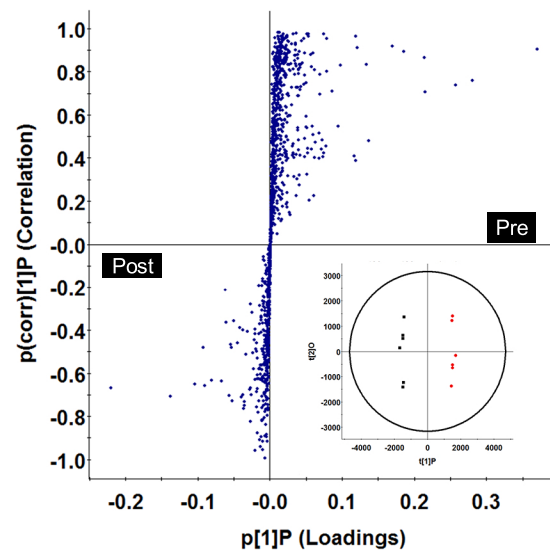
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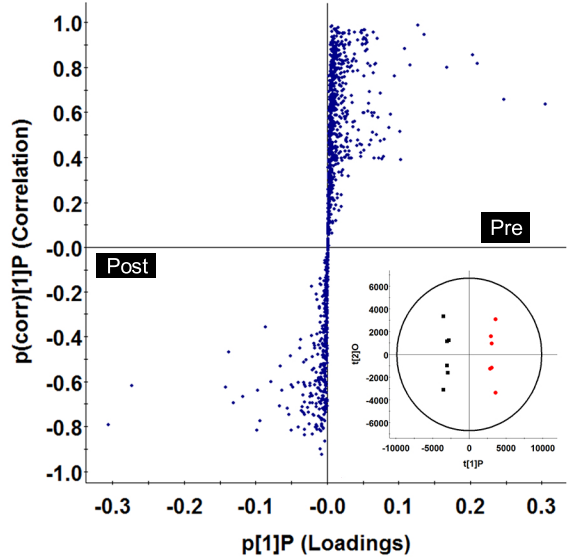
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F



**Supplemental Figure 7.** Multilevel partial least squares discriminant analysis and S-plot projections of pre-(●) and post(■)-dialysis plasma samples from intermittent conventional HHD (n = 5; C, RPLC; D, HILIC), intermittent nocturnal HHD (n = 10; E, RPLC; F, HILIC) and short hours HHD (n = 6; HD E, RPLC; F, HILIC). Features are annotated (see Table 2 for metabolite list) with variable importance in projection (VIP) values > 1 and correlation (pcorr) values > 0.4 or < -0.4.