SUPPLEMENTARY MATERIALS

Expanded Materials and Methods.

HDL tryptic digestion. HDL was isolated through sequential ultracentrifugation. Due to rotor capacity constraints HDL was isolated in 7 batches. Each batch consisted of 13 randomly selected experimental samples and one sample processing control, a pool of healthy donor plasma (referred to as rotor-control in Online Table III). Following isolation, HDL (10 µg protein as measured by bicinchoninic acid assay) was solubilized with 0.1% RapiGest (Waters, Milford, MA) in 100 mM ammonium bicarbonate, reduced with dithiothreitol, alkylated with iodoacetamide, and digested with trypsin at a 1:10 protease:protein ratio (Promega, Madison, WI) overnight at 37°C. After acidic hydrolysis of RapiGest with 0.5% trifluoroacetic acid, samples were dried and stored at −80°C until MS analysis. [15N]APOA1 was added to the sample before the digestion (10:1 w/w HDL total protein/[15N]APOA1) [39].

DDA analysis of HDL pooled replicates. For data-dependent experiments, after the HDL digestion procedure, pools were created by combining equal quantities by volume of 9-10 samples for each experimental group, producing n=3 pooled non-stroke samples, n=3 pooled 24 h post-intervention stroke samples, and n=2 96 h post-intervention stroke samples. Pooled peptides were dried down and resuspended in 5% acetonitrile, 0.1% formic acid. 1 ug of digested peptides was separated by a 95-minute linear gradient of solvents A (0.1% formic acid in water) and B (0.1% formic acid in acetonitrile) with a nanoACQUITY UPLC (Waters, Milford, MA). Samples were loaded on an Acclaim PepMap nanoLC C18, 75 µm x 25 cm column. Data-dependent, and acquisition by Top 10 method was performed on a Q Exactive HF (Thermo Scientific, Bremen). RAW instrument files were searched with SEQUEST HT search engine with a UniProt Swiss-Prot human protein database downloaded October 2016 (20,120 entries) using Proteome Discoverer (PD) version 1.4.1 (Thermo Scientific). Searches were configured with static modification for carbamidomethyl (+57.021 Da) on cysteines, dynamic modifications for oxidation of methionine residues (+15.9949 Da), parent ion tolerance of 1.25 Da, fragment mass tolerance of 1.0005 Da, monoisotopic masses, and trypsin cleavage (max 2 missed cleavages). Searches used a reversed sequence decoy strategy to control peptide false discovery and identifications were validated by Percolator software. For each experimental group, individual protein relative abundances were estimated from the protein's total PSM count as a fraction of total PSM counts of all proteins (common contaminants were excluded). Results from data-dependent acquisition experiments were compiled and used as a spectral library for validation of the identity of chromatograms extracted from parallel reaction monitoring experiments.

Parallel reaction monitoring assay for HDL peptides. The 42 proteins were primarily selected due to their presence in a previously reported PRM assay for HDL proteins[39] . Specifically, 36 were selected from that assay, and the same two peptides were targeted in our assay. The additional proteins were selected based on the pooled DDA analysis that was performed prior to the targeted assays. For these proteins we performed preliminary PRM analysis on the top 2-5 peptides detected with the most PSMs by DDA. Peptides were then selected based on their intensity and reproducibility across replicate injections. For quantitative PRM analysis an inclusion list for 95 peptide precursors was generated with Skyline (Online table III) [39]. 1 ug digested peptides from each patient isolated HDL fraction was separated by a 95-minute linear gradient as described for the data-dependent acquisition. Patient sample run order was randomized within their ultracentrifugation isolation batch. Peptides were fragmented with normalized collision energy of 26 and parallel reaction monitoring experiments performed with 17,500 resolution on a Q Exactive HF (Thermo Scientific). Target peptide chromatograms were extracted from RAW instrument files, and peptide relative intensity, as area under the curve

values, calculated using Skyline software [39]. Area under the curve for each peptide was calculated by summing the top 5 most intense fragment ion areas with no interference. An average of 7.8 points across the peak were measured across all samples and peptides. For each experimental sample peptide areas were normalized to the total ion current, and to the internal standard [N15]APOA1 peptides.

Relative quantification of APOF by western blot.

For 4 patients, isolated HDL (20µg) was subjected to BOLT® 4–12% Bis-Tris gels for electrophoresis and transferred to Millipore Immobilon ® FL PVDF (0.45 µm) membranes. Membranes were blocked with Odyssey blocking buffer (Li-Cor, NE) in PBS-T (10 mM Na_2HPO_4, 2 mM KH_2PO_4, 2.7 mM KCl, 137 mM NaCl, 0.1% Tween 20, pH 7.6) for 2 h at room temperature. Membranes were probed with goat polyclonal Apolipoprotein F antibody (NBP1-20882, Novus Biologicals) overnight, followed by washes with PBS-T, and incubated with Li-Cor IRDYE 800 conjugated anti-goat secondary antibody for 2 h at room temperature. Western blot was imaged with the Odyssey CLx scanner (Li-Cor, NE) and analyzed using Image J software (NIH). β-actin was used as a loading control.

Supplemental data.

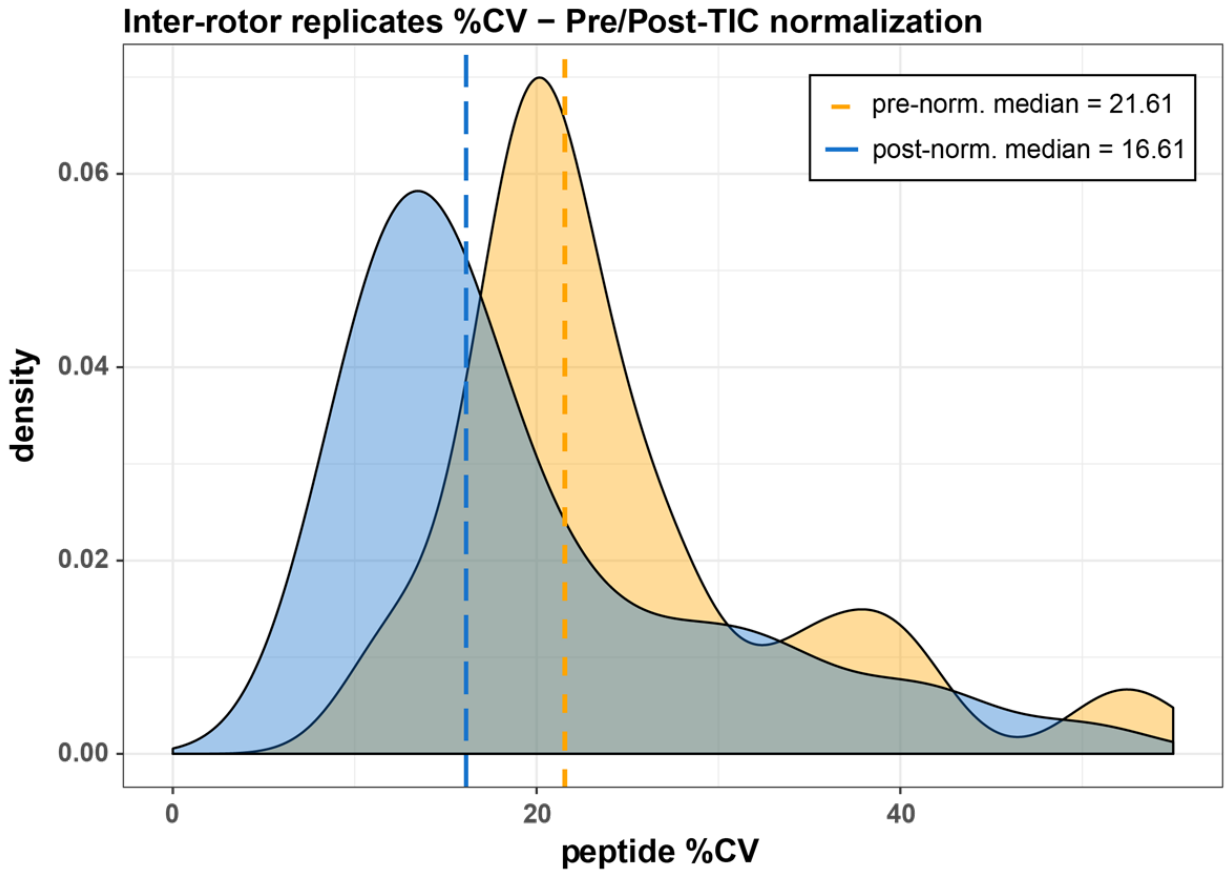
All mass spectrometry .raw files and results files have been deposited in Panorama for PRM & DDA, and are available through either the PRIDE consortium with identifier PXD015001 (Link: http://proteomecentral.proteomexchange.org/cgi/GetDataset?ID=PXD015001) or through the Skyline Panorama environment (Link: https://panoramaweb.org/pamirstroke.url) which provides all Skyline processed results for viewing.

Additional information.

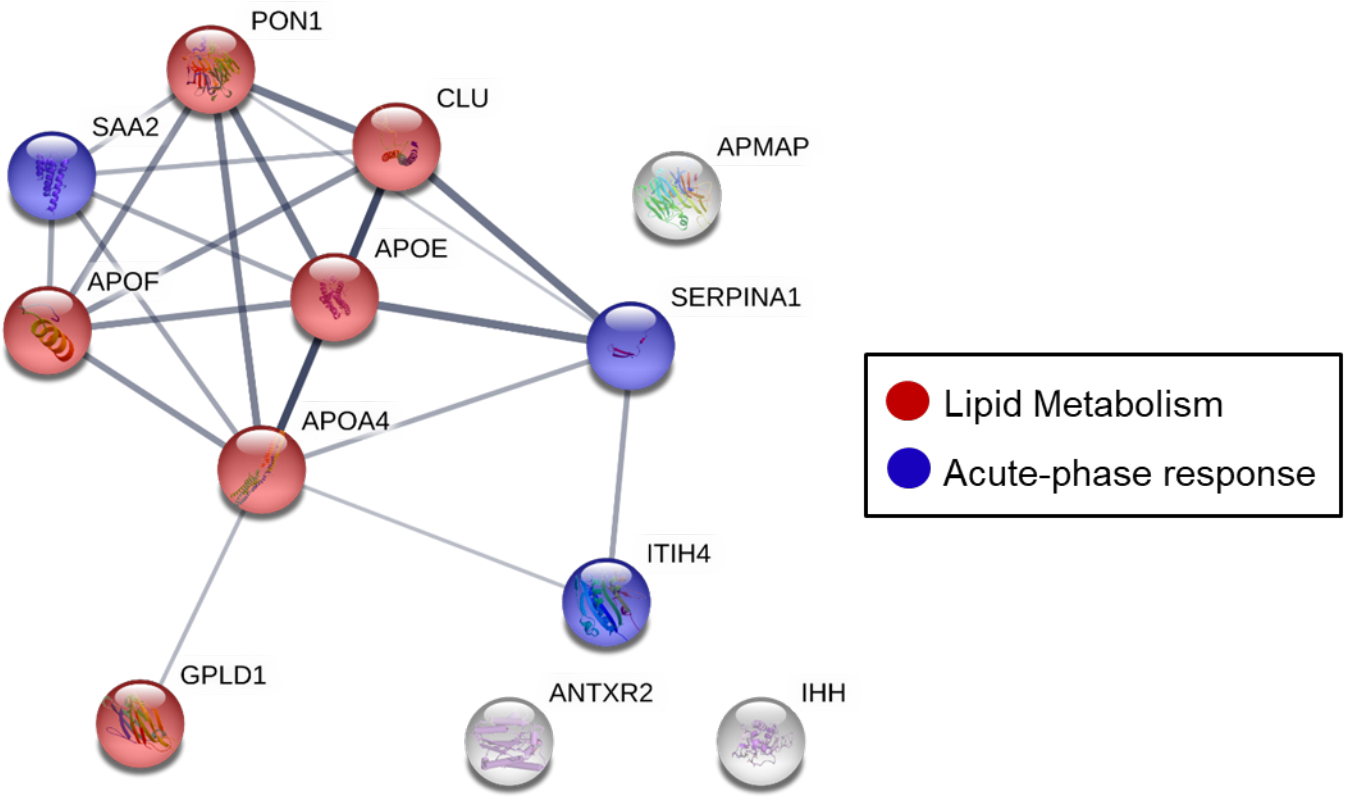
Shotgun HDL proteomic analysis

Post stroke, a distinct time-dependent remodeling was evidenced by coordinated regulation of functional protein groups (Online Table II). The remodeling identified three broad profiles: proteins that increase within 24 hours post stroke intervention and return to normal; proteins that decrease within 24 hours and stay down at 96 hours; and proteins that increase only at the 96-hour time point. We have curated outputs from publicly available enrichment databases such as DAVID, PANTHER, KEGG, and Gene Ontology to classify the proteins according to the biological functions in which they participate .

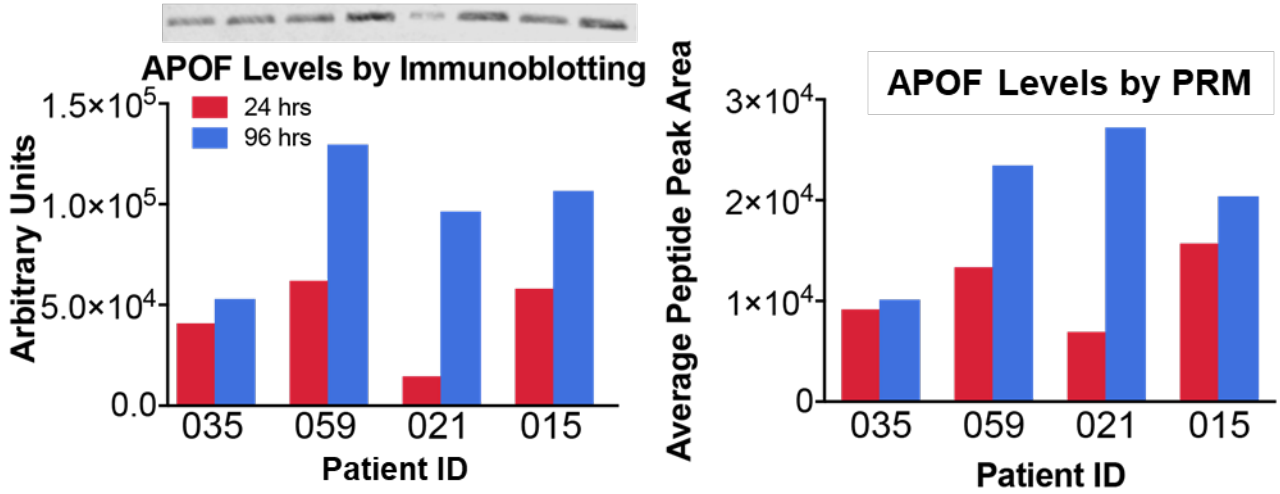
HDL from both non-stroke and stroke subjects harbored proteins associated with lipid metabolism, stress response, acute phase response, platelet activation, and vascular remodeling. Of the 97 proteins 53 are associated with stress response and were significantly increased post stroke (FDR<0.01) (Online Table II). These included: 1. complement cascade proteins (C3, C4A, C4B, C4BPA and C9); 2. acute phase inflammatory response proteins (SAA1, SAA2, APOA2, HP, SERPINA1, SERPINF2, AHSG); 3. platelet granulation and adhesion proteins (PPBP, FGB, AHSG, A2M, CLU, APOH, APOA1, IGF1, SEPP1). Of note, a subset of platelet biology proteins such as PF4, IGF2, FGG, TF, and TUB4A were reduced in stroke samples at both time points.



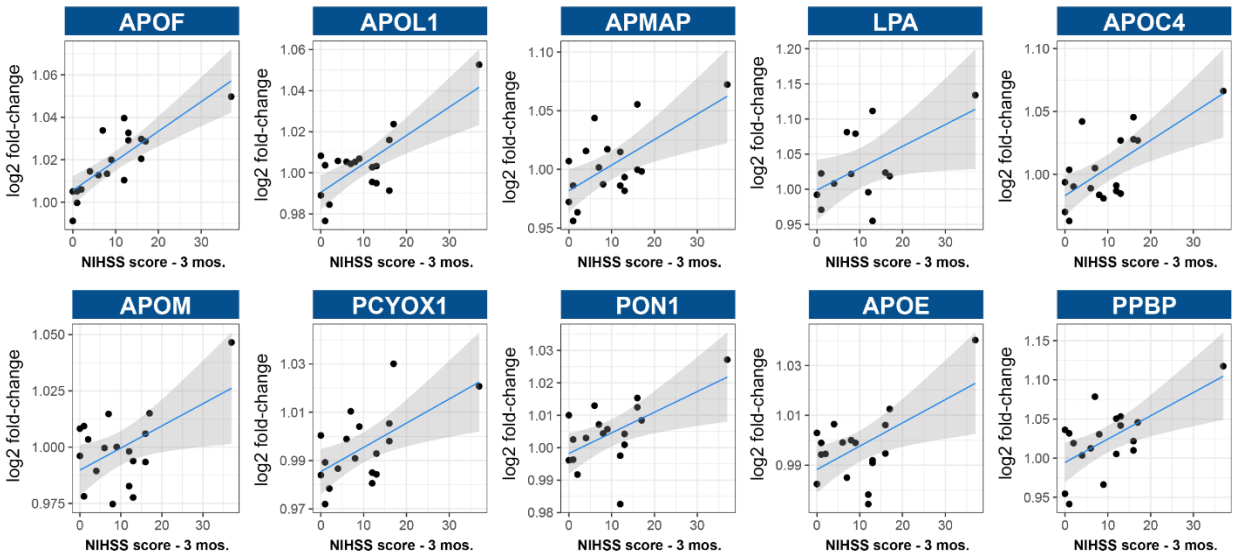
Online Figure I. **Improvement of replicate measure variation after normalization.** The percent coefficient of variation was calculated across the 7 ultracentrifugation rotor control samples for all peptides used for quantitative analysis. Three peptides corresponding to proteins HP, SAA4, and GC were withheld from further analyses due to having %CV >50 post normalization in the rotor replicates.



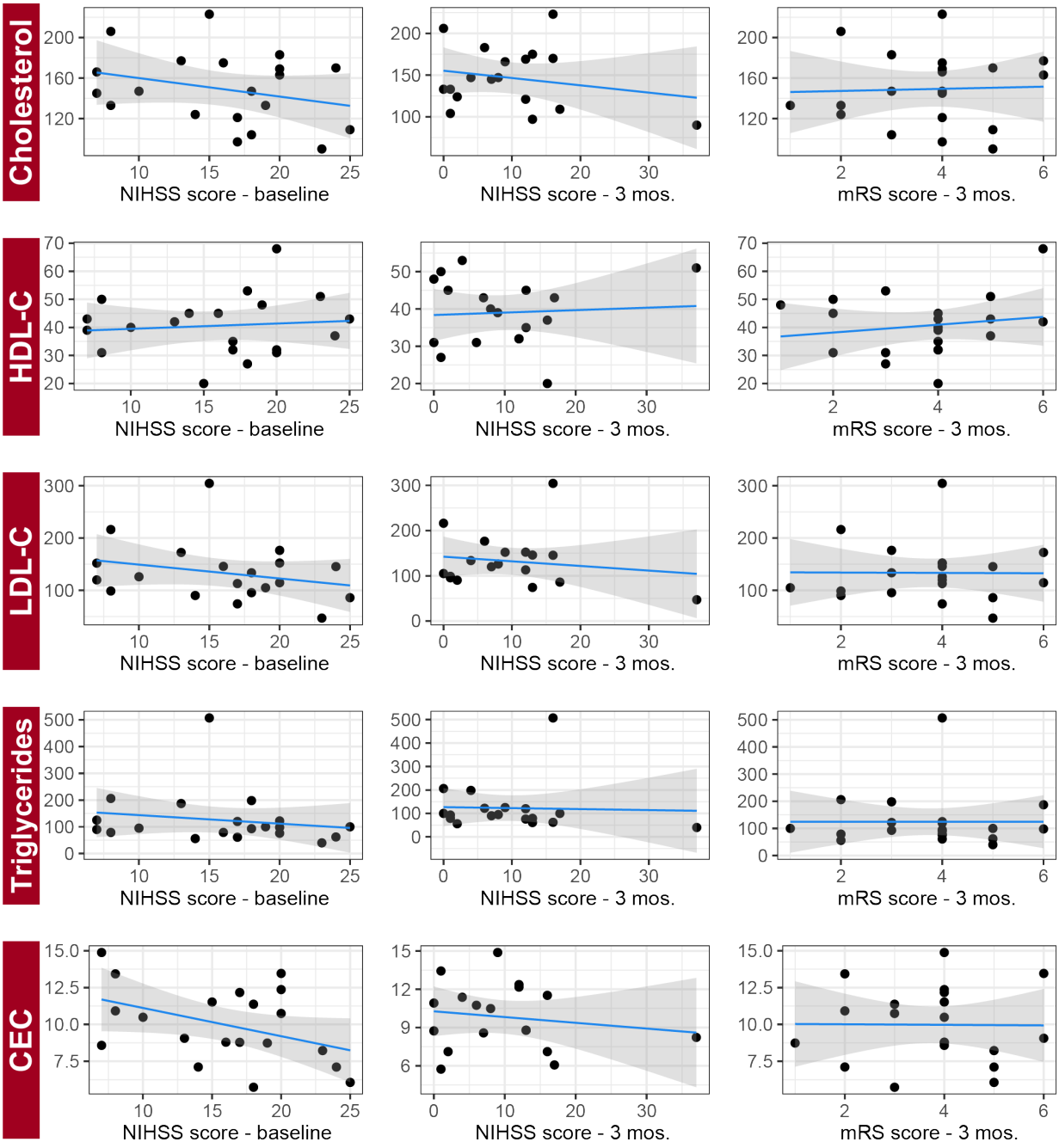
Online Figure II. **Interactions and enrichment of the proteins significantly changing by targeted mass spec analysis.** For the 12 changing proteins between the controls and 96 h, interactions were mapped using StringDB and biological process enrichment analyzed by GO. Enriched terms indicated by corresponding colors for each cluster.



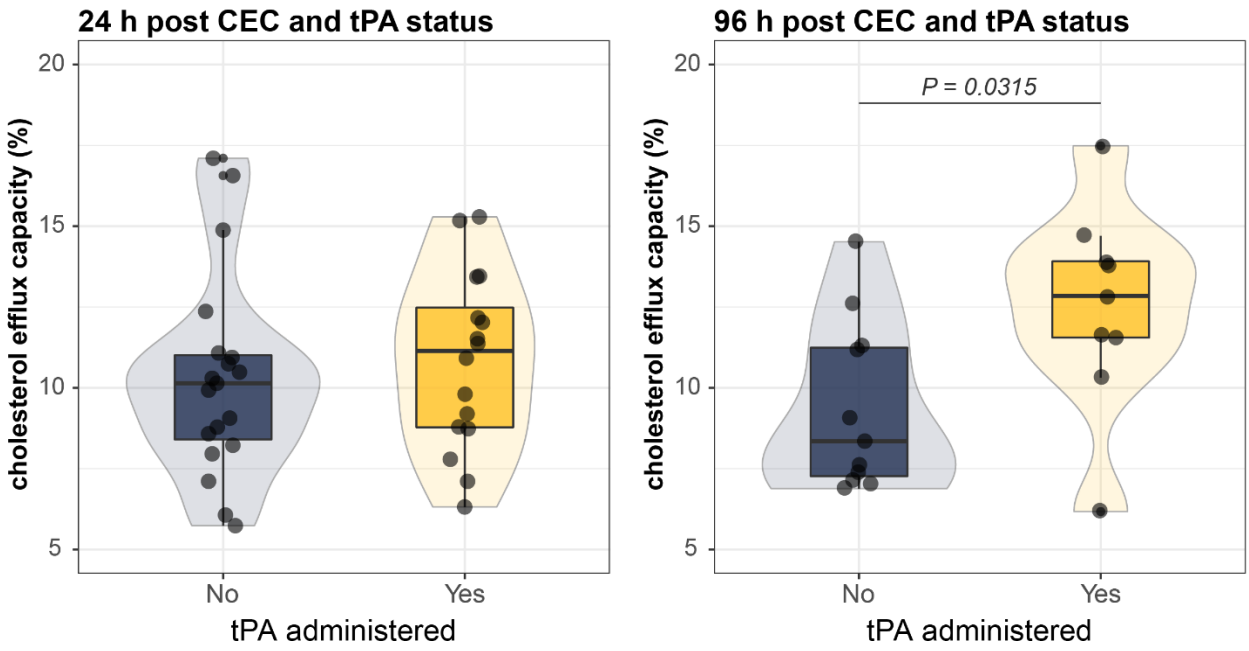
Online Figure III. **Immunostaining of APOF.** (a) Whole HDL isolated from indicated patients was run under reducing conditions on an SDS gel, transferred and blotted for APOF. (b) Average peptide peak intensities for APOF from the corresponding patients as measured by targeted PRM.



Online Figure IV**. Changes in HDL proteins correlate with recovery from stroke**. The linear relationship for ten protein measures significantly associated with NIHSS scores at 3 months post stroke. Significance was determined by a linear model adjusting for NIHSS scores at baseline. Log2 fold change is calculated as change from 24hr to 96hr.



Online Figure V. **The correlation between HDL function, plasma lipid levels and stroke recovery scores.** Linear regression analysis was performed for total Cholesterol, HDL-C, LDL-C, Triglycerides, and % cholesterol efflux capacity (CEC) in relation to baseline NIHSS scores, 3 mos. NIHSS scores, and mRS.



Online Figure VI. **tPA treated stroke patients have increased CEC at 96 h.** The cholesterol efflux capacity (CEC) of stroke patient apoB-depleted plasma 24 h and 96 h post stroke was tested in relation to patient tPA treatment status. While no significant no difference in means is seen at 24 h, CEC at 96 h patients with tPA treatment is significantly increased compared to patients with no tPA treatment. Significance was determined by two sample t-test.

Online Table V. **Linear model results of stroke recovery prediction adjusting for baseline NIHSS score.** Linear regression analysis for the effect of protein changes on stroke recovery adjusted for baseline stroke severity.

Model: NIHSS-3mos ~NIHSS-baseline + Protein log2FC

Protein	Intercept	(SE)	Adj. R2	p value	F statistic	(DF)	Adj. P value
APOF	-441.22	76.43	0.735	0.0000	24.63	15	0.0007
APOL1	-359.41	86.52	0.604	0.0004	13.95	15	0.0072
APMAP	-151.85	57.23	0.419	0.0067	7.13	15	0.0382
LPA	-128.82	35.30	0.609	0.0037	10.34	10	0.0382
APOC4	-189.47	71.97	0.417	0.0068	7.08	15	0.0382
APOM	-250.51	98.57	0.405	0.0080	6.79	15	0.0382
PCYOX1	-309.69	107.80	0.451	0.0043	7.99	15	0.0382
PON1	-440.11	174.23	0.404	0.0080	6.77	15	0.0382
APOE	-286.90	119.49	0.386	0.0101	6.34	15	0.0427
PPBP	-106.44	45.05	0.377	0.0113	6.14	15	0.0428
PON3	-265.71	121.94	0.354	0.0147	5.67	15	0.0508
APOC3	-238.23	121.94	0.323	0.0211	5.05	15	0.0667
LCAT	-123.63	64.81	0.314	0.0231	4.89	15	0.0676
GPLD1	-113.05	63.97	0.295	0.0285	4.55	15	0.0742
PF4	-78.46	44.56	0.292	0.0293	4.51	15	0.0742
C3	-141.54	85.28	0.281	0.0328	4.33	15	0.0780
SAA1	18.64	21.78	0.299	0.0392	4.20	13	0.0876
SELL	-98.30	69.99	0.248	0.0461	3.80	15	0.0974
IHH	-206.61	155.41	0.241	0.0495	3.70	15	0.0989
APOB	-71.79	56.98	0.230	0.0551	3.54	15	0.1046
SERPINA1	-77.92	68.48	0.217	0.0623	3.36	15	0.1108
ALB	-26.71	49.44	0.167	0.0993	2.70	15	0.1108
AMBP	-84.54	80.29	0.209	0.0675	3.24	15	0.1108
ANTXR2	73.61	107.87	0.181	0.0874	2.88	15	0.1108
APOA1	-109.83	179.16	0.173	0.0937	2.78	15	0.1108
APOA2	-68.36	85.07	0.186	0.0834	2.95	15	0.1108
APOA4	58.11	70.03	0.194	0.0773	3.05	15	0.1108
APOD	-88.59	172.12	0.168	0.0988	2.71	15	0.1108
CAMP	-15.74	95.00	0.155	0.1108	2.56	15	0.1108
CLU	-57.58	80.75	0.179	0.0890	2.86	15	0.1108
AHSG	-13.87	34.05	0.160	0.1058	2.62	15	0.1108
FGA	-39.54	41.05	0.198	0.0749	3.09	15	0.1108
HPR	-23.74	54.94	0.162	0.1039	2.64	15	0.1108
ITIH4	-47.68	41.74	0.222	0.0677	3.28	14	0.1108
PLTP	-82.61	138.09	0.172	0.0947	2.77	15	0.1108
RBP4	-54.00	67.69	0.185	0.0842	2.93	15	0.1108
SAA2	15.69	21.60	0.195	0.0767	3.06	15	0.1108
TTR	-16.88	60.84	0.157	0.1088	2.58	15	0.1108

Online Table VI. **Linear model results of stroke recovery prediction with additional adjustment for tPA status.** Linear regression analysis for the effect of protein changes on stroke recovery adjusted for baseline stroke severity and for tPA treatment status.

Model: NIHSS-3mos ~NIHSS-baseline + tPA + Protein log2FC

Protein	Intercept	(SE)	Adj. R2	p value	F statistic	(DF)	Adj. P value
APOF	-460.26	83.72	0.724	0.0001	15.89	14	0.0033
LPA	-127.71	28.08	0.753	0.0012	13.17	9	0.0196
APOL1	-352.31	90.81	0.580	0.0015	8.83	14	0.0196
APOC4	-204.49	70.14	0.458	0.0087	5.79	14	0.0822
APMAP	-147.63	58.28	0.403	0.0165	4.82	14	0.1046
PCYOX1	-301.28	115.14	0.415	0.0143	5.02	14	0.1046
APOM	-240.88	104.10	0.370	0.0234	4.33	14	0.1113
PON1	-423.65	181.52	0.374	0.0226	4.38	14	0.1113
APOE	-275.99	123.48	0.358	0.0265	4.17	14	0.1117
PPBP	-105.22	50.91	0.333	0.0343	3.82	14	0.1183

Online Table VII. **Linear regression analysis for HDL related lipid and functional metrics and stroke recovery scores.**

	NIHSS - baseline				**NIHSS - 3 mos.**				**mRS - 3 mos.**			
	Adj. R^2	Intercept	Slope	P Value	Adj. R^2	Intercept	Slope	P Value	Adj. R^2	Intercept	Slope	P Value
CEC	0.131	13.032	-0.192	0.065	-0.036	10.270	-0.045	0.531	-0.055	10.049	-0.019	0.967
HDL-C	-0.045	37.585	0.189	0.678	-0.058	38.370	0.065	0.800	-0.024	35.378	1.393	0.467
LDL-C	0.019	175.720	-2.651	0.258	-0.036	142.230	-1.025	0.533	-0.055	134.710	-0.338	0.973
Total Cholesterol	0.031	178.190	-1.824	0.221	-0.015	155.240	-0.876	0.399	-0.054	145.160	1.052	0.870
Triglycerides	-0.022	175.590	-3.190	0.453	-0.061	126.790	-0.421	0.888	-0.056	124.640	0.015	0.999

Online Table VIII. **Linear regression analysis for select HDL proteins and plasma lipid levels and CEC.**

	CEC		HDL-C		LDL-C		Total Cholesterol		Triglycerides	
Protein	Adj. R^2	Adj. p value	Adj. R^2	Adj. p value	Adj. R^2	Adj. p value	Adj. R^2	Adj. p value	Adj. R^2	Adj. p value
APOF	-0.015	0.942	0.078	0.335	-0.056	0.976	-0.044	0.950	-0.055	0.9995
APOL1	-0.047	0.979	0.189	0.335	0.023	0.959	-0.031	0.950	0.001	0.9995
APMAP	-0.055	0.979	-0.026	0.701	0.013	0.959	-0.041	0.950	0.121	0.6378
LPA	-0.076	0.979	-0.076	0.927	-0.070	0.959	-0.077	0.999	0.005	0.9995
APOC4	0.174	0.486	-0.041	0.763	-0.054	0.960	-0.046	0.950	-0.037	0.9995
APOM	-0.055	0.979	0.032	0.375	-0.038	0.959	-0.040	0.950	-0.055	0.9995
PCYOX1	-0.055	0.979	0.046	0.349	-0.053	0.959	-0.055	0.966	-0.056	0.9995
PON1	-0.055	0.979	0.026	0.388	-0.040	0.959	-0.008	0.950	-0.044	0.9995
APOE	-0.015	0.942	0.084	0.335	-0.011	0.959	-0.020	0.950	-0.053	0.9995
PPBP	0.317	0.218	-0.052	0.901	0.152	0.632	0.240	0.314	0.019	0.9995

Online Table IX. **Two-tailed t-test results for stroke patient plasma lipid levels and CEC based on tPA treatment status.**

Lipid measure	24h p-value	96h p-value
Cholesterol	0.48	0.19
Triglycerides	0.14	0.26
HDL-C	0.93	0.40
LDL-C	0.28	0.27
CEC	0.62	0.03

Image ID: 0000051_01
Acquire Time: Mar 2, 2018 8:46:22 AM

Acquisition Information

#	Image ID	Acquire Time	Channels	Resolution	Intensities	Quality	Analysis	Image Name	Comment
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Image Display Values

Channel	Color	Minimum	Maximum	K
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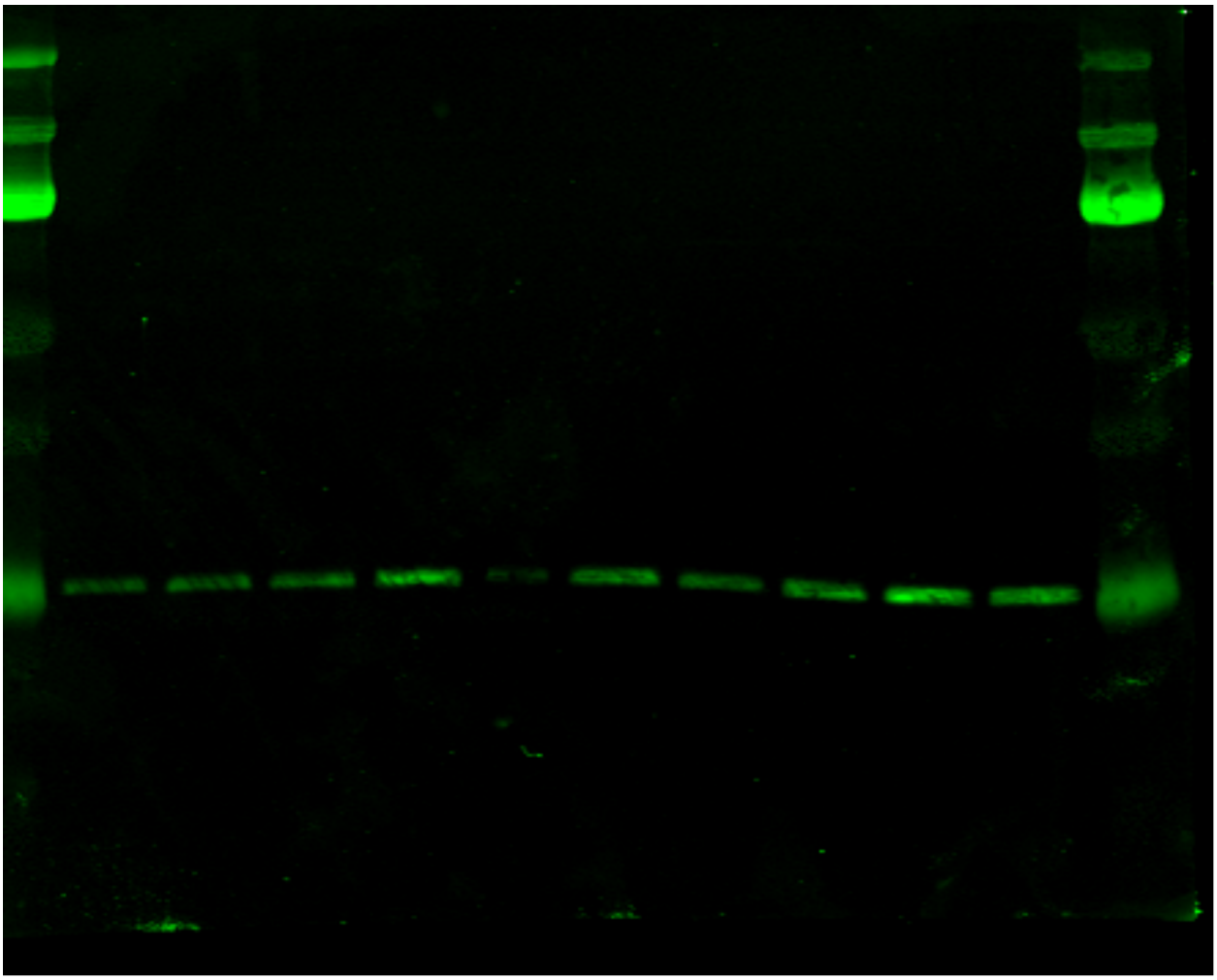

From left to right:
Lane 1 the molecular wieght mark.(BioRad,Precision Plus Protein™ Dual Color Standards, #1610374)
Lanes 2 and 3 were plasma from patient 035-RVD at two time points, 24 and 96 hours respectively.
Lanes 4 and 5 were plasma from patient 0059-JMF at 24 and 96 hours respectively.
Lane 6 was from patient 021-D-V at 24 hours and lane 7 was from the same patient at 96 hours.
Lanes 8 and 9 were plasma from patient 015-D-S at 24 and 96 hours respectively.
Lanes 10 and 11 were plasma from a healthy patient.
ApoF was separated on a 12% Bis-tris gel, transferred to a nitrocellusoe membrane and incubated with ApoF anti-Gt (Novus NBP1-20882) primary antibody and IRDye 800CW Donkey anti-goat (Li-cor, Lot: C61207-04) secondary.

Acquisition Information (continued)

#	Image Modifications
1	