SUPPLEMENTAL TABLES:

Supplemental Table S1: Significant differences in metabolomics analyses of stored red blood cells from blood donors with low (20.5±1.0 kg/m2) and high (44.1±5.1 kg/m2) body mass index (BMI). Data were analyzed by MetaboAnalyst and normalized (mean-centered and divided by the standard of each variable) prior to one-way ANOVA.

	Time(F.val)	Time(raw.p)	Time(adj.p)	BMI group(F.val)	BMI group(raw.p)	BMI group(adj.p)	Interaction(F.val)	Interaction(raw.p)	Interaction(adj.p)
BMI	326.89	5.62E-17	2.81E-14	8.4831	0.00060513	0.0031067	29.475	1.80E-09	9.00E-07
Recall.Oxidative	26.819	1.70E-05	0.0042535	26.737	7.05E-09	8.60E-08	8.1378	0.00078977	0.19744
kynurenine	19.745	0.00012672	0.019893	1.8329	0.16942	0.33884	1.1221	0.3328	1
Citrulline uM	19.007	0.00015914	0.019893	4.6604	0.013422	0.046605	1.1018	0.33938	1
LPI204	16.718	0.00033111	0.030077	4.542	0.014859	0.050541	1.4674	0.23926	1
Tyr uM	16.456	0.00036092	0.030077	1.5421	0.22288	0.4097	0.11066	0.89544	1
Arginine	14.467	0.00070966	0.049369	3.7618	0.029314	0.087766	0.27652	0.75944	1
Tyrosine	14.162	0.0007899	0.049369	1.972	0.14872	0.31643	0.58236	0.56193	1
Phe uM	12.973	0.0012086	0.067147	19.309	4.19E-07	3.81E-06	0.25034	0.7794	1
Similar to L-Glutathione oxidized Mass 0.7140 Da	9.6752	0.0042661	0.096957	11.711	5.63E-05	0.00038593	4.5932	0.014219	1
Cystine uM	8.1953	0.0078656	0.12686	5.1832	0.0086044	0.032841	1.4779	0.23687	1
LPS181	6.8984	0.01831	0.2034	9.3488	0.00031381	0.0018245	0.75186	0.47619	1
Drospirenone	6.5772	0.01598	0.21595	7.8407	0.00099514	0.0046941	1.1788	0.31516	1
#NAME?	6.2419	0.01862	0.23778	4.7122	0.01284	0.044895	0.091022	0.91313	1
FA183-GLA	5.6691	0.024307	0.28264	28.398	3.06E-09	4.37E-08	0.77603	0.46511	1
Nonanoic acid pelargonate	5.1733	0.030796	0.32586	12.958	2.37E-05	0.00017431	2.9296	0.061654	1
Arg uM	5.1611	0.030977	0.32586	5.8381	0.0049778	0.019911	0.42461	0.65611	1
GSHNE	4.9817	0.033797	0.33134	4.9172	0.010779	0.038773	1.3116	0.27754	1
GSH4HNone	4.9817	0.033797	0.33134	4.9172	0.010779	0.038773	1.3116	0.27754	1
GSH-dLactone	4.9817	0.033797	0.33134	4.9172	0.010779	0.038773	1.3116	0.27754	1
Allantoate	4.7918	0.037095	0.34348	4.9273	0.010687	0.038773	0.11022	0.89583	1
L-Citrulline	4.543	0.041968	0.37355	8.9144	0.00043545	0.0023926	1.0346	0.36205	1
O-Propanoylcarnitine	4.5139	0.042585	0.37355	16.201	2.81E-06	2.30E-05	0.56796	0.56991	1
R-S-Lactoylglutathione	4.0646	0.053477	0.39321	7.1522	0.001713	0.0079816	1.1025	0.33915	1
Heptanoic acid	3.867	0.059229	0.42306	7.033	0.0018839	0.0086417	2.3934	0.1006	1
Leucine	3.713	0.064194	0.44579	5.4277	0.0070059	0.026946	0.21777	0.80499	1
Glutathione disulfide	3.6035	0.068011	0.46518	4.597	0.014173	0.048537	1.8863	0.16115	1
Hexanoic acid caproate	3.5529	0.069858	0.46518	8.5164	0.00058987	0.0031067	4.1538	0.020793	1
Eicosapentaenoic acid	3.4223	0.074901	0.46813	8.3852	0.00065245	0.0031983	1.1993	0.30902	1
5Z-8Z-11Z-14Z-17Z-Icosapentaenoic acid	3.4223	0.074901	0.46813	8.3852	0.00065245	0.0031983	1.1993	0.30902	1
Similar to L-Glutathione reduced Mass 0.9383 Da	3.0323	0.092599	0.52054	6.3877	0.0031704	0.013549	0.8152	0.44773	1
O-glutarylcarnitine	2.6121	0.11727	0.5983	5.0608	0.0095422	0.033605	0.050569	0.95073	1
EPA	2.4429	0.12929	0.61794	9.7476	0.00023309	0.001433	1.6711	0.19727	1
L-Homocysteine	2.3421	0.13714	0.62153	26.066	9.97E-09	1.16E-07	0.61763	0.54285	1
7Z-10Z-13Z-16Z-19Z-Docosa-7-10-13-16-19-pentaenoic acid	2.2863	0.14172	0.62709	32.563	4.15E-10	7.25E-09	0.33932	0.71371	1
16-HDoHE	2.1667	0.15218	0.64786	18.906	5.32E-07	4.75E-06	0.84046	0.43688	1
Lactate	2.1419	0.15446	0.64786	56.739	3.42E-14	1.14E-12	0.44642	0.64218	1
Similar to L-Glutathione reduced Mass -1.0412 Da	2.1278	0.15577	0.64786	21.944	9.18E-08	9.18E-07	2.5928	0.08377	1
11R-HPETE/Leukotriene B4 isobars	2.0986	0.15854	0.64786	6.2502	0.0035471	0.01503	1.4276	0.24848	1
Docosahexaenoic acid	2.0833	0.16002	0.64786	20.697	1.86E-07	1.76E-06	0.90687	0.40963	1
DHA	2.0225	0.16603	0.64786	51.433	2.09E-13	6.15E-12	1.4711	0.23841	1
DPAFA225	1.9858	0.16979	0.64987	46.815	1.12E-12	2.94E-11	1.2946	0.28208	1
Lactate mM uM	1.8855	0.18061	0.66574	124.91	2.27E-21	1.42E-19	0.98802	0.37871	1
Octyl gallate	1.7244	0.19979	0.69544	8.2624	0.00071718	0.0034815	1.7808	0.17792	1
Fumarate	1.7205	0.20029	0.69544	6.8394	0.0022001	0.0095656	0.47121	0.6267	1
Methionine	1.6308	0.21208	0.70351	32.538	4.20E-10	7.25E-09	0.63811	0.53209	1
Leucocyanidin	1.5289	0.22655	0.71386	6.2396	0.0035778	0.015033	1.4823	0.23589	1
D-Ribose	1.5154	0.22856	0.71386	8.4721	0.00061028	0.0031067	0.51803	0.59852	1
5-Oxoproline	1.4576	0.23741	0.71386	105.29	1.06E-19	5.90E-18	0.69275	0.50443	1
GSDHN2	1.4544	0.23792	0.71386	4.7239	0.012711	0.044759	0.23492	0.79141	1
Guanosine	1.42	0.24341	0.71386	29.817	1.52E-09	2.31E-08	1.8254	0.17061	1
LPS160	1.349	0.25526	0.71386	12.237	3.90E-05	0.00027078	0.76423	0.47049	1
Ethyl-Glucoronide	1.3441	0.2561	0.71386	8.9736	0.00041634	0.002313	0.41111	0.6649	1
Arachidonic Acid	1.3366	0.2574	0.71386	20.443	2.16E-07	2.00E-06	0.7454	0.47919	1
FA224	1.2629	0.27065	0.71386	72.504	2.88E-16	1.11E-14	0.64457	0.52874	1
1-3-Bisphosphoglycerate uM	1.2492	0.2732	0.71386	21.608	1.11E-07	1.09E-06	1.5145	0.22878	1
Met uM	1.2119	0.28033	0.71386	40.644	1.25E-11	2.83E-10	0.17795	0.83746	1
Docosapentaenoic Acid	1.1361	0.29559	0.71386	23.284	4.37E-08	4.73E-07	0.12687	0.8811	1
Glutathione mM uM	1.0943	0.30448	0.71386	36.454	7.27E-11	1.45E-09	0.88681	0.41767	1
ADP uM	1.0935	0.30464	0.71386	6.7209	0.0024204	0.010433	0.38746	0.68058	1
Eicosatetraenoic acid	1.0916	0.30506	0.71386	27.724	4.28E-09	5.63E-08	0.34709	0.70825	1
D-Glucose mM uM	1.0809	0.3074	0.71386	11.5	6.54E-05	0.00044191	1.1087	0.33714	1
Adenine	1.0525	0.31371	0.71386	35.616	1.05E-10	1.94E-09	0.44089	0.64568	1
13-HODE	1.041	0.31633	0.71386	30.894	9.09E-10	1.47E-08	1.2841	0.28494	1
1-3-Bisphosphoglycerate	1.0389	0.3168	0.71386	24.692	2.05E-08	2.28E-07	1.3175	0.27597	1
LPE182	1.0237	0.32031	0.71386	10.879	0.00010192	0.00066182	0.20245	0.81732	1
5-oxoproline uM	0.95853	0.33594	0.71386	210.79	8.63E-27	2.16E-24	0.52624	0.59371	1
fluoxetine	0.93655	0.34145	0.71386	6.8845	0.0021218	0.0095576	0.025734	0.97461	1
trans-Homoaconitate	0.92416	0.34461	0.71386	5.9023	0.0047208	0.01919	0.046409	0.95469	1
14-HDoHE	0.89947	0.35104	0.71386	13.835	1.31E-05	9.77E-05	1.8531	0.16624	1
LPE204	0.89877	0.35122	0.71386	22.759	5.83E-08	6.08E-07	0.090395	0.9137	1
12-HETE	0.8674	0.35964	0.72497	23.255	4.44E-08	4.73E-07	0.13224	0.8764	1
S-Glutathionyl-L-cysteine	0.82603	0.37118	0.7394	28.432	3.00E-09	4.37E-08	1.4149	0.2515	1
Cysteine	0.81377	0.3747	0.74346	15.774	3.69E-06	2.93E-05	0.053445	0.94801	1
2-Methyleneglutarate	0.78015	0.38462	0.74475	18.575	6.49E-07	5.54E-06	0.61944	0.54189	1
Oxaloacetate	0.72268	0.40248	0.75104	10.026	0.00018974	0.0001859	0.55796	0.57552	1
AA	0.68782	0.41392	0.76089	94.545	1.12E-18	5.08E-17	0.2938	0.74657	1
MEHP	0.66336	0.42225	0.77053	26.284	8.90E-09	1.06E-07	0.10436	0.90108	1
N-Acetylmethionine	0.64924	0.42717	0.77107	6.0856	0.0040591	0.016913	0.42364	0.65674	1
ADP	0.62046	0.43749	0.77806	37.048	5.62E-11	1.22E-09	0.20275	0.81708	1
Phthalate	0.60962	0.44148	0.77806	26.757	6.98E-09	8.60E-08	0.068958	0.93344	1
9-HETE	0.60894	0.44174	0.77806	14.884	6.55E-06	5.04E-05	0.19309	0.82496	1
LPC204	0.57442	0.45484	0.77806	5.1453	0.0088841	0.033399	0.16277	0.85019	1
Acetyl-L-carnitine	0.56288	0.45936	0.77806	9.4101	0.00029974	0.0017632	0.22937	0.79578	1
acetyl-carnitine	0.56096	0.46012	0.77806	9.4101	0.00029974	0.0017632	0.22799	0.79687	1
Triacanthine	0.56096	0.46012	0.77806	9.4101	0.00029974	0.0017632	0.22799	0.79687	1
Adenosine	0.45972	0.50332	0.80863	20.726	1.83E-07	1.76E-06	0.41191	0.66437	1
9-HODE	0.45935	0.50349	0.80863	29.994	1.40E-09	2.19E-08	0.94914	0.39321	1
D-Glucose	0.45331	0.50629	0.80863	14.111	1.09E-05	8.26E-05	0.38941	0.67927	1
L-Noradrenaline	0.45052	0.50759	0.80863	44.561	2.63E-12	6.59E-11	1.4012	0.2548	1
Malate	0.45002	0.50782	0.80863	11.414	6.95E-05	0.00046358	0.18385	0.83256	1
Sphingosine 1-phosphate	0.3808	0.54216	0.84883	4.6209	0.013885	0.047879	0.34024	0.71306	1
5-HETE	0.36865	0.54863	0.85354	272.7	1.36E-29	6.79E-27	0.40028	0.67203	1
Citrate	0.34836	0.55978	0.85593	8.826	0.0004657	0.002531	0.17102	0.84324	1
D-Glyceraldehyde 3-phosphate/Glycerone phosphate	0.33674	0.56636	0.86029	5.8848	0.0047894	0.019312	0.040917	0.95994	1
13S-HODE	0.33383	0.56803	0.86029	27.086	5.91E-09	7.57E-08	1.0156	0.36875	1
Linoleic Acid	0.33005	0.57022	0.86029	7.9252	0.00093163	0.0044363	1.1679	0.31849	1
9,10,EpOME	0.32367	0.57394	0.86029	9.0649	0.00038857	0.0022078	0.41744	0.66076	1
ATP	0.3217	0.57511	0.86029	43.513	3.96E-12	9.43E-11	0.13776	0.8716	1
Dihomo-g-Linolenic Acid	0.24045	0.6277	0.8917	31.383	7.21E-10	1.20E-08	0.52151	0.59648	1
Adrenic Acid	0.23788	0.62954	0.8917	25.921	1.07E-08	1.22E-07	0.22443	0.79968	1
5-Hydroxyisourate	0.21272	0.64821	0.90785	54.037	8.47E-14	2.65E-12	0.11323	0.89315	1
Hexose phosphate uM	0.20741	0.65232	0.91062	10.344	0.00015027	0.00096329	0.28228	0.75513	1
Hexadecanoic acid	0.1933	0.66356	0.91373	5.7883	0.0051878	0.020586	0.44833	0.64096	1
Glutamine	0.19274	0.66402	0.91373	36.762	6.36E-11	1.32E-09	0.1499	0.86114	1
ATP consider breakdown uM	0.18969	0.66651	0.91373	8.4357	0.00062757	0.0031379	0.12646	0.88146	1
2-Hydroxyglutarate/Citramalate	0.17863	0.67578	0.91569	5.6006	0.0060635	0.023872	0.012099	0.98798	1
Ascorbate	0.17363	0.68008	0.91903	6.854	0.0021744	0.0095656	0.43379	0.65021	1
11-HETE	0.16863	0.68446	0.92137	144.15	8.23E-23	5.88E-21	0.22381	0.80018	1
LA	0.15552	0.6963	0.93088	18.801	5.67E-07	4.97E-06	0.1377	0.87165	1
Gln uM	0.14419	0.70702	0.93769	15.489	4.42E-06	3.46E-05	0.70622	0.49785	1
Asparagine	0.13721	0.71386	0.94246	8.5703	0.00056599	0.003043	0.25859	0.77306	1
N-Acyl-D-mannosaminolactone	0.1291	0.72206	0.94246	4.856	0.011356	0.040269	0.021732	0.97851	1
12,13-diHOME	0.12848	0.7227	0.94246	98.508	4.58E-19	2.29E-17	0.93313	0.39935	1
Malate uM	0.11957	0.73208	0.94246	12.538	3.16E-05	0.00022604	0.65243	0.52469	1
12S-HETE	0.11163	0.74078	0.94246	164.22	3.75E-24	3.75E-22	0.0074525	0.99258	1
15-HETE	0.10641	0.7467	0.94246	193.72	6.89E-26	8.61E-24	1.5812	0.21477	1
UDP-glucose	0.10096	0.75304	0.94246	27.779	4.16E-09	5.62E-08	0.2023	0.81744	1
Similar to L-Glutathione oxidized Mass -0.1044 Da	0.071987	0.79043	0.97105	10.212	0.00016546	0.0010472	0.24887	0.78054	1
N-Acetylneuraminate	0.067351	0.79713	0.97579	18.562	6.54E-07	5.54E-06	0.4631	0.63172	1
Glu uM	0.066628	0.7982	0.97579	5.9229	0.004641	0.01902	0.62239	0.54033	1
2-Oxoglutaramate	0.055734	0.81509	0.98301	15.967	3.26E-06	2.63E-05	0.27987	0.75693	1
FA203-DGLA	0.05076	0.82338	0.98301	50.175	3.27E-13	9.08E-12	0.14607	0.86443	1
5-Hydroxyindoleacetate	0.04917	0.82612	0.98301	5.4367	0.0069531	0.026946	0.9186	0.40501	1
ADP-D-ribose	0.04824	0.82775	0.98301	11.179	8.22E-05	0.00054085	0.87669	0.42179	1
DIALLYL SULFIDE	0.044653	0.83417	0.98301	36.05	8.67E-11	1.67E-09	0.3236	0.72488	1
2/3-Phospho-D-glycerate	0.041628	0.83981	0.98301	7.1441	0.001724	0.0079816	0.5858	0.56003	1
N-Acyl-D-glutamate	0.03633	0.85021	0.98301	12.744	2.75E-05	0.00019891	0.068372	0.93399	1
Glutathione	0.029231	0.86548	0.98301	7.9349	0.00092462	0.0044363	0.79103	0.45838	1
D-Erythrose 4-phosphate	0.026422	0.87204	0.98424	9.7366	0.00023501	0.001433	0.45675	0.63568	1
GSDHN	0.024532	0.87666	0.98501	5.5358	0.0064003	0.025001	0.034875	0.96575	1
Phosphoenolpyruvate	0.02184	0.88357	0.98549	4.8607	0.01131	0.040269	0.20906	0.81198	1
910-DiHOME	0.017629	0.89532	0.98632	60.556	9.96E-15	3.56E-13	0.73359	0.48474	1
Cys-Gly	0.017027	0.89711	0.98632	8.4635	0.00061432	0.0031067	0.44889	0.64062	1
Hypoxanthine	0.013194	0.90937	0.98632	201.29	2.69E-26	4.48E-24	7.25E-05	0.99993	1
Linoleate	0.011063	0.91698	0.98632	5.1634	0.0087492	0.033141	0.26148	0.77084	1
Fructose 1-6-bisphosphate uM	0.0089515	0.9253	0.98749	12.445	3.37E-05	0.00023756	0.33039	0.72004	1
8Z-11Z-14Z-Icosatrienoic acid	0.0072363	0.93281	0.98749	22.542	6.58E-08	6.71E-07	0.075448	0.92742	1
Xanthine uM	0.0071883	0.93304	0.98749	8.9965	0.00040918	0.0022988	0.12819	0.87994	1
AMP uM	0.0069767	0.93403	0.98749	6.8942	0.0021054	0.0095576	0.48396	0.61889	1
D-Fructose 1-6-bisphosphate	0.0062523	0.93754	0.98749	16.79	1.94E-06	1.62E-05	0.30283	0.73993	1
9,10-dHOME	0.0055391	0.9412	0.98866	85.046	1.07E-17	4.46E-16	0.3183	0.72869	1
8-HETE	0.0050183	0.94403	0.98955	152.09	2.33E-23	1.94E-21	0.13674	0.87249	1
Similar to L-Glutathione oxidized Mass -0.1063 Da	0.0045265	0.94684	0.99042	9.1886	0.00035395	0.0020342	0.0611	0.94079	1
Glycerol 3-phosphate	0.0021188	0.96361	0.99577	8.4618	0.00061513	0.0031067	0.022059	0.97819	1
Recall.Storage	0.0014995	0.96939	0.99731	6.8725	0.0021424	0.0095641	0.14702	0.86361	1
12,13-EpOME	0.0012343	0.97222	0.998	6.8418	0.0021958	0.0095656	0.54668	0.58193	1
2-Aminomuconate	0.0007918	0.97775	0.998	8.475	0.0006089	0.0031067	0.15727	0.85485	1
Dopamine	0.00037267	0.98473	0.99848	5.0077	0.0099813	0.036968	0.65596	0.52289	1
Sedoheptulose 1-phosphate	0.00036238	0.98495	0.99848	28.07	3.60E-09	5.00E-08	0.68143	0.51004	1
His uM	1.33E-05	0.99711	0.99848	5.9548	0.0045207	0.01868	0.024838	0.97548	1

Supplemental Table S2: Interactions between testosterone replacement therapy (TRT), body mass index (BMI), and day 42 hemolysis in 18 blood donors from Vitalant's cohort.

Hemolysis ~ BMI + TRT + BMI*TRT				
	β TRT	**p value TRT**	**β interaction**	**p value interaction**
Storage hemolysis	-0.51	0.37	0.02	0.30
Osmotic hemolysis	-7.56	0.84	-0.06	0.96
Oxidative hemolysis	8.51	0.81	-0.36	0.77

Supplemental Table S3: RBC posttransfusion recovery (percentage and standard deviation, SD) stratified by blood donor body mass index (BMI). Human leukocyte-reduced RBCs from 14 blood donors were stored for 42 days and later transfused into NSG mice as described under Supplemental Methods.

BMI (kg/m^2)	**<25**			**25-29.9**			**≥30**		
Time after transfusion	**Mean (%)**	**SD**	**n**	**Mean (%)**	**SD**	**n**	**Mean (%)**	**SD**	**n**
10 min	44.8	10.5	3	47.2	11.5	6	34.7	7.3	5
2-h	29.5	10.6	3	30.5	8.2	6	22.0	5.2	5
4-h	35.0	11.9	3	25.1	8.7	6	24.1	7.8	5
8-h	22.1	7.0	3	18.1	5.4	6	15.3	4.9	5
24-h	7.3	7.6	3	4.4	4.1	6	3.8	2.9	5

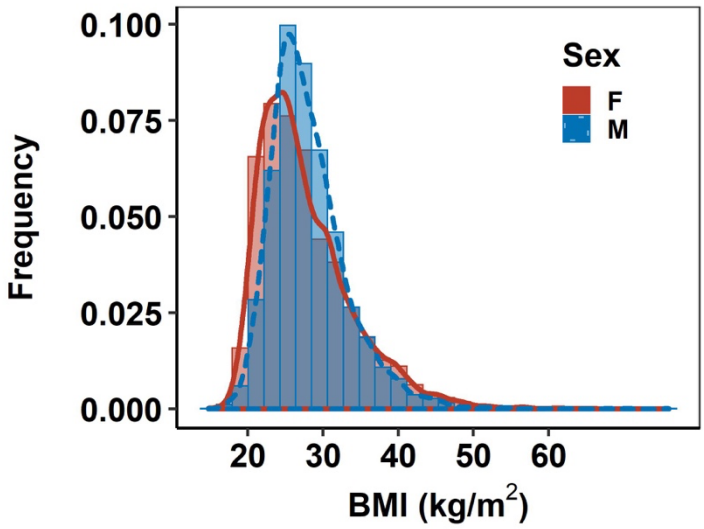
Supplemental Table S4 for Figure 2: Linear regression analysis of blood donor body mass index (BMI) with each of the three day-42 hemolysis measures and ferritin. Data derived from 13,317 blood donors who participated in the National Heart, Lung, and Blood Institute Red Blood Cell Omics (RBC-Omics) study between 2013-2015. Parameter estimates represent the change in each variable per each BMI unit in male and female donors. p values obtained by multivariable linear regression.

	Measure	**Males**		**Females**	
		Parameter estimate	**P**	**Parameter estimate**	**P**
All donors	Storage hemolysis	**0.0026**	**<0.001**	**0.00095**	**0.13**
	Osmotic hemolysis	**0.27**	**<0.001**	**0.25**	**<0.001**
	Oxidative hemolysis	**0.36**	**<0.001**	**0.30**	**<0.001**
	Ferritin	**1.43**	**<0.001**	**0.60**	**<0.001**
First-time donors	Oxidative hemolysis	**0.34**	**<0.001**	**0.26**	**<0.001**
	Ferritin	**3.67**	**<0.001**	**1.17**	**<0.001**

Supplemental Table S5 for Figure 3: Demographic and hemolysis data for the 15 blood donors with low and high BMI groups (20.5±1.0 kg/m^2 defined as low BMI versus 44.1±5.1 kg/m^2 defined as high BMI). p-values were calculated for continuous variables using rank test.

	BMI low group	BMI high group	p-value
Sex (Males/Females)	6/9	8/7	
Age (Mean±SD)	50.7±15.2	52.2±14.4	0.42
Race (White/African American/Asian/Hispanic)	12/0/2/1	8/3/2/2	
Storage hemolysis (Mean±SD)	0.40±0.61	0.42±0.31	0.13
Osmotic hemolysis (Mean±SD)	27.6±14.5	34.4±12.5	0.22
Oxidative hemolysis (Mean±SD)	18.2±9.3	37.1±9.7	<0.001
Ferritin hemolysis (Mean±SD)	18.9±10.2	92.5±103.5	<0.001

Supplemental Figure S1 for Figure 2: Distribution of blood donor body mass index (BMI) by sex in the RBC-Omics cohort.



SUPPLEMENTAL METHODS:

Hemolysis assays:

Hemolysis was determined on a weekly basis in Vitalant's cohort and on day 39-42 in RBC-Omics. In each testing day, two aliquots (1mL each) were collected from each bag. One aliquot was used for spontaneous storage hemolysis and the other for the stress hemolysis assays. Hemoglobin levels were determined using the Drabkin's method.[1] Spontaneous storage hemolysis was determined by $\frac{(100-HCT)\times Hb_{supernatant}}{Hb_{total}}$. HCT is the sample hematocrit, $Hb_{supernatant}$ corresponds to the levels of free hemoglobin obtained after centrifugation (1500G, 10 minutes, 18°C) measured in the supernatant, and Hb_{total} refers to the total amount of sample hemoglobin before centrifugation. The other aliquot was washed three times with phosphate-buffered saline and packed RBCs were subjected to osmotic and oxidative hemolysis as described before.[2,3] In brief, osmotic stress was induced by incubating RBCs in pink test buffer (a hypotonic solution containing 70mM Bis-Tris, 25mM sodium chloride, and 135mM glycerol; pH 6.6) at room temperature for four hours and hemolysis was calculated using $\frac{Hb_{osmotic}}{Hb_{total}} x100$, where $Hb_{osmotic}$ equals the level of free hemoglobin in the supernatant after centrifugation (1500G, 10 minutes, 18°C) and Hb_{total} is the total level of hemoglobin in the sample. Oxidative hemolysis was determined by incubating a suspension of washed RBCs with 150mM AAPH at 37°C for three hours. Percent hemolysis was calculated using $\frac{Hb_{AAPH}-Hb_{control}}{Hb_{total}} x100$, where Hb_{AAPH} corresponds to the free hemoglobin in treated samples after centrifugation (1500G, 10 minutes, 18°C), $Hb_{control}$ refers to the free hemoglobin in untreated samples, and Hb_{total} corresponds to the total amount of hemoglobin in each sample.

Quantification of RBC posttransfusion recovery in NSG mice:

Male and female (5-12 weeks old) immunodeficient NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl}/SzJ (NSG) mice[4] (The Jackson Laboratory) were bred, housed and maintained in a specific pathogen free vivarium under barrier conditions at Vitalant Research Institute (San Francisco, CA). These mice have been used for engraftment of human hematopoietic tissues as they lack murine lymphoid cells.[4] The data reported in this study were from 14 blood donors (Vitalant cohort), for whom we quantified RBC posttransfusion recovery over time after infusion into 8-10 NSG mice per donor sample. In all cases, LR-RBCs stored for 39-42 days were acclimated to room temperature for a minimum of 30 minutes and each sample hematocrit was adjusted to 60±6% with sterile PBS prior to transfusion. NSG mice were gently warmed under heat lamps prior to intravenous lateral tail vein injection of 200 µL of human RBCs. Blood was collected from mice pre-injection and at 10 minutes, 2, 4, 8, and 24 hours post infusion by tail tip excision and collection into Sarstedt Minivette tubes coated with anticoagulant ethylenediaminetetraacetic acid. A 5 µL aliquot of this collection was promptly aliquoted and resuspended in sterile filtered PBS with 5% mouse serum, 0.01% sodium azide and 14% citrate phosphate dextrose adenine-1 solution for staining and flow cytometry. Quantification of RBC posttransfusion recovery was determined by

flow cytometric analysis using the human-specific marker for erythrocyte membrane glycophorin A (CD235a-phycoerythrin; Biolegend, HI264).

RBC posttransfusion recovery was determined by staining murine whole blood samples with a human-specific marker for erythrocyte membrane glycophorin A (CD235A-phycoerythrin; Biolegend, HI264). Samples were incubated for 30 minutes at 4°C, washed in PBS, resuspended in a 1:500 dilution of propidium iodide and run on a BD FACS LSR II (BD Biosciences) with acquisition by BD FACSDiva software. Analysis of cytometry data was performed on FlowJo software version 10 (FlowJo, LLC). Data normalization was generated according to the National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs).[5] Baseline mouse total blood volume was estimated by multiplying mouse weight by the practical blood sample volume value of 58.5 mL/kg. Total mouse recipient RBCs prior to transfusion was estimated by multiplying the estimated total blood volume by the mouse pre-transfusion RBC count. The estimated expected total recovery of human RBCs from mouse recipients after transfusion was calculated by dividing the total number of human RBCs used for transfusion by the estimated total mouse recipient RBCs. This was set as the maximum human RBC recovery value and the flow cytometry readings of human RBC recovery at the subsequent timepoints was a fraction thereof. Analysis and plots were generated on Prism version 7 (GraphPad Software, San Diego, CA) using matched two-way analysis of variance (ANOVA) followed by Tukey's multiple comparison post-test to compare each group to every other group. Additional information is referenced below.[6]

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