Supplementary Information (SI) for Analytical Methods.

Isolation and Detection of Target Cells in Blood via Immunomagnetic Separation and Atomic Emission Spectroscopy

Supplementary Information.

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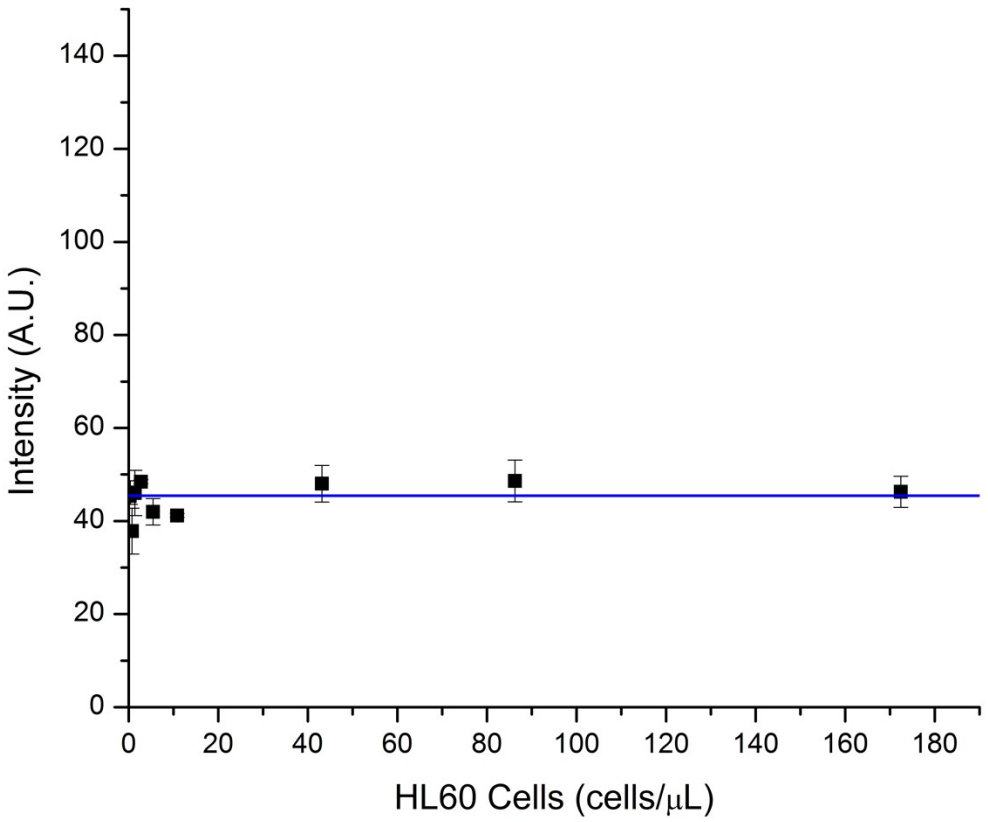
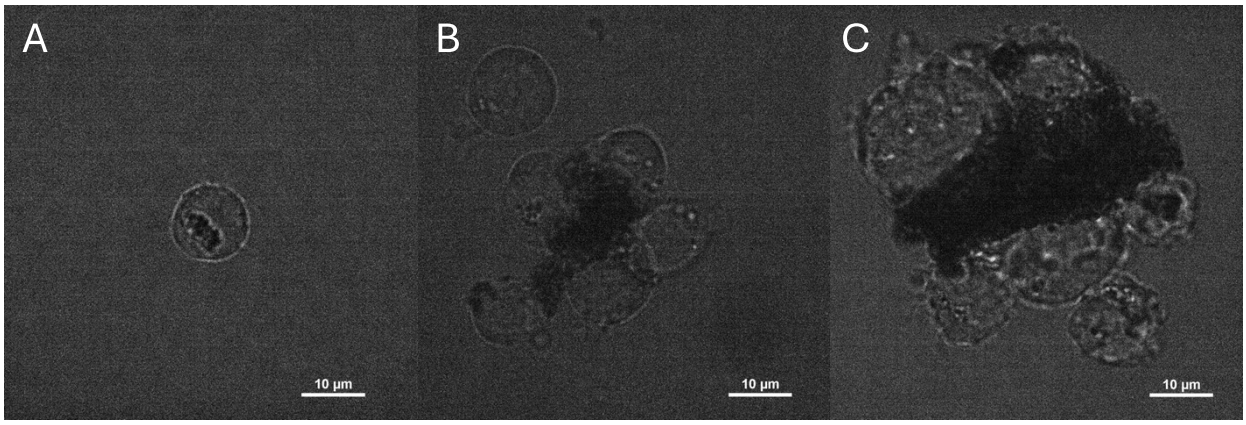


SI Figure 1. MP-AES Analysis of Endogenous Fe: HL60 cells in PBS. HL60 cells (0.17-170 cells/μL; $N = 3$) were analyzed for Fe content (Fe 371.993 nm) via MP-AES. Upon performance of a single-tailed Grubb's test (α=0.05), one outlier was removed from analysis (22 cells/μL; -27.43 ± 16.96 A.U.). The remaining samples displayed an average intensity of 45.44 ± 1.05 A.U. (blue), with no linearity between cell concentration and Fe emission signal.

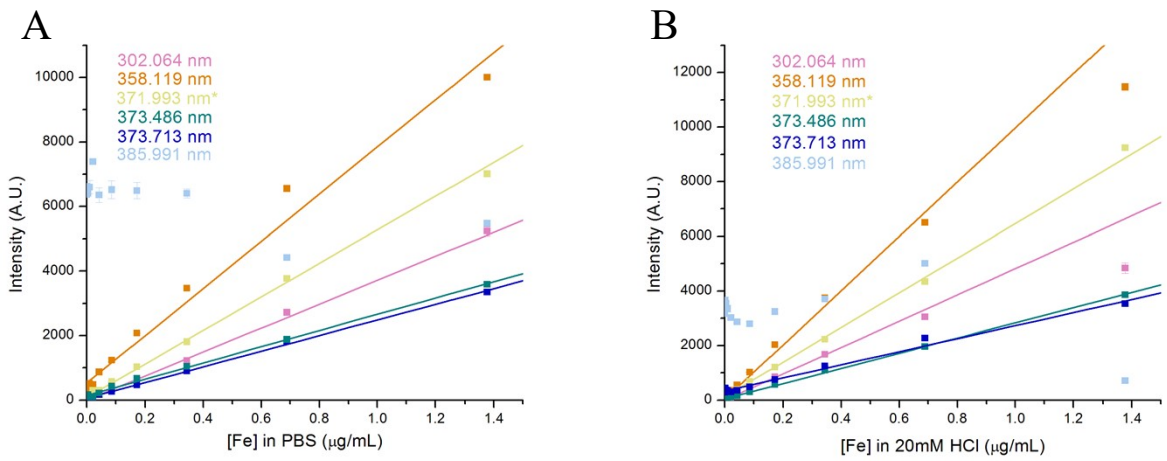


SI Figure 2. Microscopy images of HL60 cells tagged with Fe_2O_3/Fe_3O_4 nanoparticles following an immunomagnetic separation. Imaged HL60 cells incubated with 10% (fig. A), 25% (fig. B), and 50% (fig. C) nanoparticles by volume. Aggregates of HL60 cells increase via aggregation of Fe_2O_3/Fe_3O_4 nanoparticle tags as NP concentration increases.

Differential Centrifugation

	Fe retention: Supernatant	Fe retention: Cell fraction	Cell retention
0 minutes	N/A	7.66 ± 0.01 µg/mL	450 ± 3 cells/µL
30 seconds	6.38 ± 0.01 µg/mL	0.55 ± 0.01 µg/mL	290 ± 2 cells/µL
1 minute	6.61 ± 0.00 µg/mL	0.36 ± 0.00 µg/mL	310 ± 2 cells/µL
2 minutes	6.09 ± 0.00 µg/mL	0.75 ± 0.01 µg/mL	330 ± 2 cells/µL
3 minutes	5.92 ± 0.01 µg/mL	0.67 ± 0.01 µg/mL	340 ± 2 cells/µL
4 minutes	5.70 ± 0.01 µg/mL	1.21 ± 0.01 µg/mL	320 ± 2 cells/µL
5 minutes	5.30 ± 0.01 µg/mL	1.20 ± 0.01 µg/mL	310 ± 2 cells/µL
	Fe retention: Supernatant	Fe retention: Cell fraction	Cell retention
0 washes	N/A	7.66 ± 0.01 µg/mL	950 ± 5 cells/µL
1 wash	6.38 ± 0.01 µg/mL	0.55 ± 0.01 µg/mL	850 ± 4 cells/µL
2 washes	6.61 ± 0.00 µg/mL	0.36 ± 0.00 µg/mL	700 ± 4 cells/µL
3 washes	6.09 ± 0.00 µg/mL	0.75 ± 0.01 µg/mL	780 ± 6 cells/µL

SI Table 1. Differential Centrifugation: Plotted Data Values and Propagated Error. Data from differential centrifugation protocol development ($N = 3$), shown in figure 3.

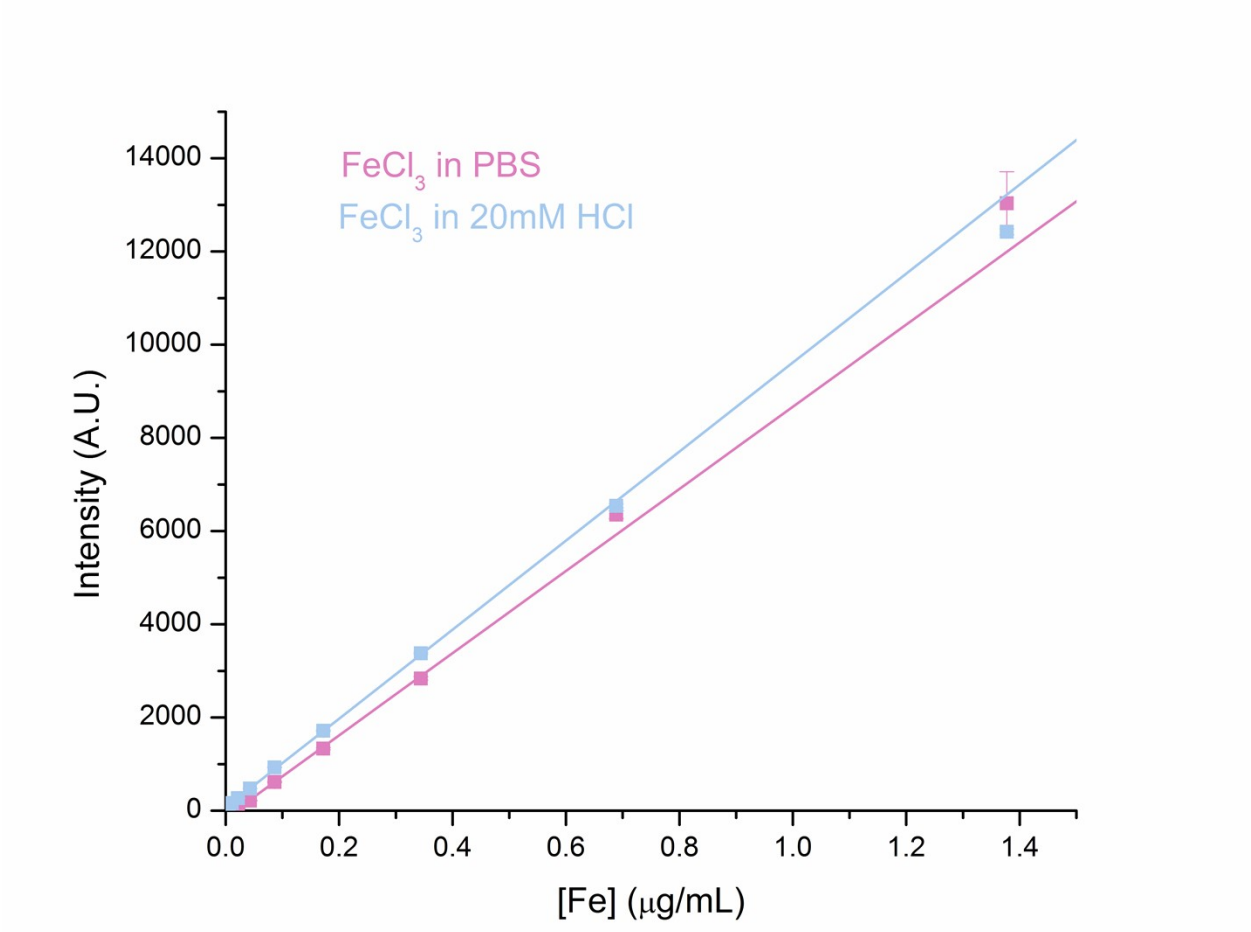


C

in PBS	Fe emission (λ)	Correlation Coefficient (R)	Linear Trend
	302.064 nm	0.99700	y = 3714.49x + 4.39
	358.119 nm	0.99087	y = 7319.95x + 526.87
	371.993 nm	0.99794	y = 5210.53x + 74.02
	373.486 nm	0.99928	y = 2431.39x + 55.81
	373.713 nm	0.99931	y = 2521.99x + 143.19
	385.991 nm	N/A	N/A

in 20mM HCl	Fe emission (λ)	Correlation Coefficient (R)	Linear Trend
	302.064 nm	0.99653	y = 4828.53x - 7.90
	358.119 nm	0.98589	y = 9956.41x + 20.54
	371.993 nm	0.99893	y = 6348.29x + 122.07
	373.486 nm	0.99971	y = 2780.99x + 50.34
	373.713 nm	0.99482	y = 2396.16x + 335.02
	385.991 nm	N/A	N/A

SI Figure 3. MP-AES Analysis of Fe Standards in PBS and 20mM HCl. $FeCl_3$ (0.7 ng/mL-1 µg/mL Fe) was suspended in (A) PBS and (B) 20mM HCl, then evaluated for Fe emission at 6 wavelengths ($N = 3$; 302.064, 358.119, 371.993, 373.486, 373.713, and 385.991 nm). The results were plotted and evaluated for linearity and relative intensity (C).



SI Figure 4. MP-AES Analysis of Fe Standards in PBS and 20mM HCl. $FeCl_3$ was suspended in PBS and acidified solution (20mM HCl) to evaluate the differences Fe emission (371.993 nm) intensity. All intensity values ($N = 3$) were adjusted to account for the blank(s). $FeCl_3$ in PBS (0.7 ng/mL-1 μg/mL Fe) yielded an LoD of 30 ng/mL (Correlation coefficient 0.998; y = 8814.12x - 146.11). $FeCl_3$ in 20mM HCl (0.7 ng/mL-1 μg/mL Fe) yielded an LoD of -5.39 ng/mL (20mM HCl blank applied; Correlation coefficient 0.99961; y = 9552.47x + 63.43). Both samples displayed linear trends and applicable limits of detection in the context of this assay, but PBS was chosen as the more viable matrix due to simplicity of sample preparation and ability to limit operator burden.

MP-AES Analysis of $FeCl_3$ and Fe_2O_3/Fe_3O_4 Nanoparticles in PBS

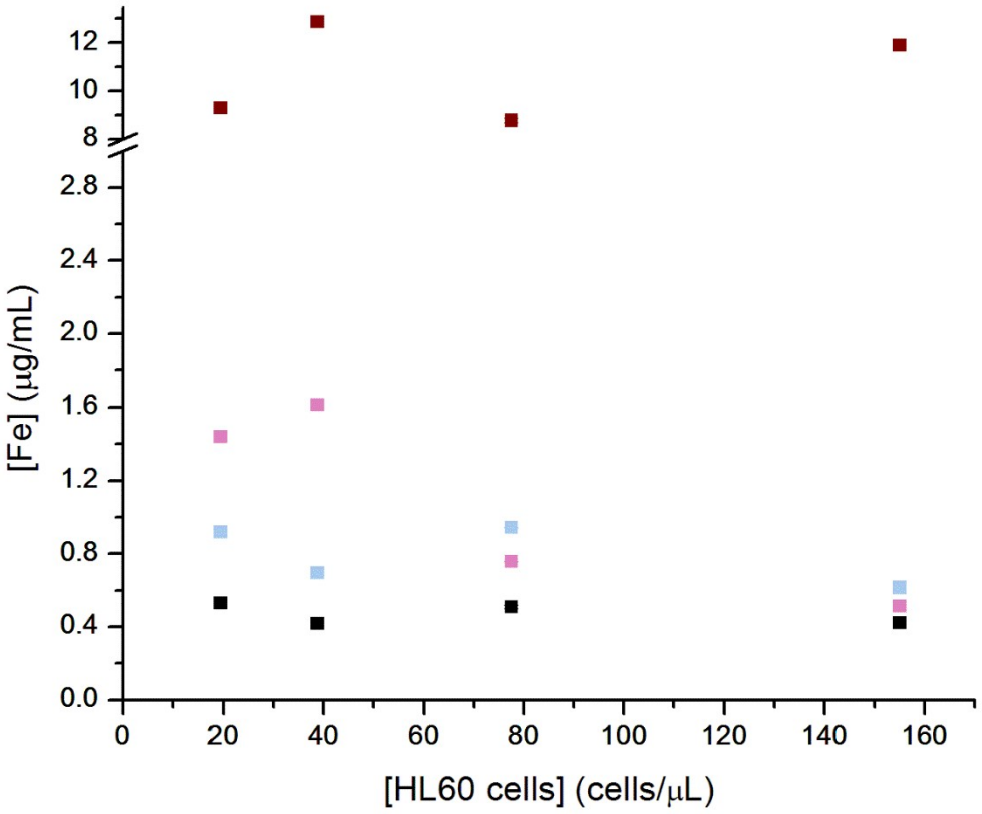
[$FeCl_3$]	Intensity (*N*=3)	[$FeCl_3$]	Intensity (*N*=3)
20 µg/mL	98083.31 ± 5512.28 A.U.	19 ng/mL	31.41 ± 8.20 A.U.
10 µg/mL	47815.67 ± 1444.03 A.U.	9.4 ng/mL	-2.85 ± -0.75 A.U.
5 µg/mL	23020.71 ± 168.05 A.U.	4.7 ng/mL	-22.88 ± -9.40 A.U.
2.4 µg/mL	10842.79 ± 685.26 A.U.	2.4 ng/mL	-60.36 ± -186.34 A.U.
1.2 µg/mL	5593.35 ± 192.97 A.U.	1.2 ng/mL	-41.14 ± -76.07 A.U.
0.60 µg/mL	2794.85 ± 58.13 A.U.	0.59 ng/mL	-255.93 ± -20034.07 A.U.
0.30 µg/mL	1426.97 ± 17.41 A.U.	0.29 ng/mL	-34.05 ± -18.98 A.U.
0.15 µg/mL	761.19 ± 78.63 A.U.	0.15 ng/mL	-72.28 ± -3678.15 A.U.
75 ng/mL	366.61 ± 42.05 A.U.	74 pg/mL	-58.96 ± -3818.69 A.U.
38 ng/mL	171.79 ± 23.36 A.U.	37 pg/mL	-63.93 ± -9996.84 A.U.
[Fe_2O_3/Fe_3O_4 NPs]	Intensity (*N*=3)	[Fe_2O_3/Fe_3O_4 NPs]	Intensity (*N*=3)
0.01 mg/mL	13035.95 ± 1652.96 A.U.	0.2 µg/mL	53.78 ± 1.20 A.U.
5 µg/mL	6351.39 ± 494.77 A.U.	0.08 µg/mL	-18.2 ± 5.07 A.U.
3 µg/mL	2837.78 ± 29.80 A.U.	0.04 µg/mL	-93.39 ± 4.01 A.U.
1 µg/mL	1331.87 ± 29.83 A.U.	0.02 µg/mL	-88.1 ± 100.33 A.U.
0.6 µg/mL	615.87 ± 1.91 A.U.	10 ng/mL	-120.92 ± 58.83 A.U.
0.3 µg/mL	215.05 ± 12.88 A.U.	5 ng/mL	-149.14 ± 421.37 A.U.

SI Table 2. MP-AES Analysis of Fe standards and Iron Oxide Nanoparticles: Plotted Data Values and Propagated Error. Background corrected data from MP-AES analysis of Iron (III) Chloride in PBS and Iron Oxide Nanoparticles in PBS ($N = 3$).

MP-AES Analysis of Fe_2O_3/Fe_3O_4 Nanoparticle-labeled Cells in PBS

[HL60 cells]	Intensity (*N*=3)	Calculated [Fe]
170 ± 1 cells/μL	2328.58 ± 71.52 A.U.	0.26 ± 0.01 μg/mL
86 ± 0 cells/μL	2076.61± 27.34 A.U.	0.24 ± 0.00 μg/mL
43 ± 0 cells/μL	1846.41 ± 20.07 A.U.	0.21 ± 0.00 μg/mL
22 ± 0 cells/μL	1194.60 ± 51.30 A.U.	0.15 ± 0.01 μg/mL
11 ± 0 cells/μL	651.87 ± 15.91 A.U.	0.088 ± 0.002 μg/mL
5.4 ± 0 cells/μL	360.49 ± 12.82 A.U.	0.058 ± 0.002 μg/mL
2.7 ± 0 cells/μL	365.24 ± 17.66 A.U.	0.058 ± 0.003 μg/mL
1.3 ± 0 cells/μL	301.14 ± 12.67 A.U.	0.052 ± 0.002 μg/mL
0.67 ± 0 cells/μL	293.38 ± 4.76 A.U.	0.051 ± 0.001 μg/mL
0.34 ± 0 cells/μL	220.95 ± 3.04 A.U.	0.043 ± 0.001 μg/mL
0.17 ± 0 cells/μL	345.12 ± 7.26 A.U.	0.056 ± 0.001 μg/mL

SI Table 3. MP-AES Analysis of Cells Detected in Buffer: Plotted Data Values and Propagated Error. Background corrected data from MP-AES analysis of cells labeled with iron oxide nanoparticles in PBS ($N = 3$).



SI Figure 5. AES analysis of HL60 cells in lysed blood. [Fe] in iron oxide-labeled HL60 cells blood following lysis of erythrocytes; isolated cell samples (black), supernatant from two subsequent washes with a magnetic rack (maroon and pink, respectively), and supernatant following the differential centrifugation protocol (blue) were all analyzed, and no distinguishable trend was observed in the isolated cell samples due to contamination by Fe from lysed erythrocytes ($N = 3$).

MP-AES Analysis of Fe_2O_3/Fe_3O_4 Nanoparticle-labeled Cells in Blood

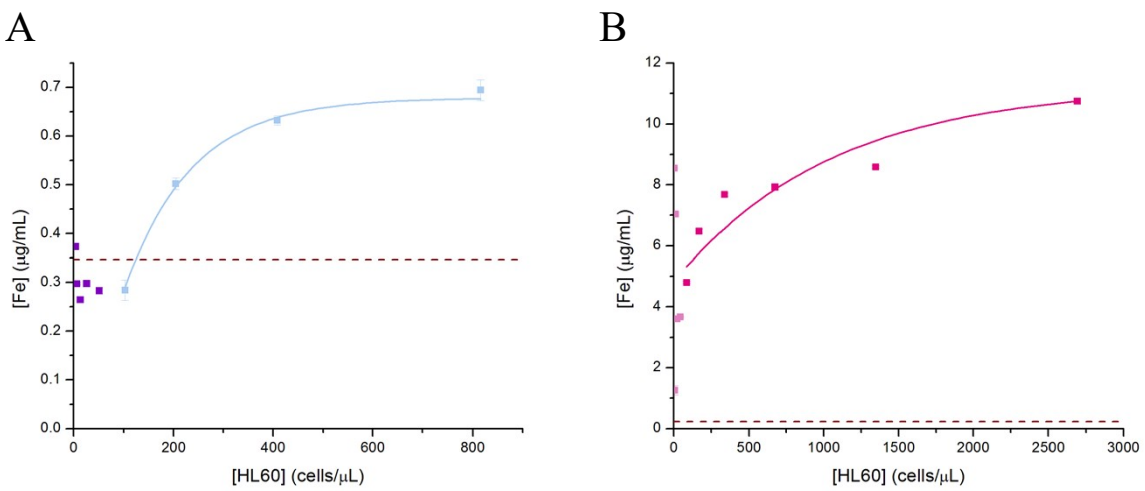
Old Control Blood

[HL60 cells]	Intensity (*N*=3)	Calculated [Fe]
820 ± 3 cells/µL	3349.09 ± 73.90 A.U.	0.71 ± 0.02 µg/mL
410 ± 2 cells/µL	3118.83 ± 31.49 A.U.	0.66 ± 0.01 µg/mL
200 ± 1 cells/µL	2635.67 ± 40.98 A.U.	0.57 ± 0.01 µg/mL
100 ± 0 cells/µL	1826.85 ± 68.02 A.U.	0.41 ± 0.02 µg/mL
51 ± 0 cells/µL	1195.26 ± 28.14 A.U.	0.28 ± 0.01 µg/mL
25 ± 0 cells/µL	1267.22 ± 18.99 A.U.	0.30 ± 0.00 µg/mL
13 ± 0 cells/µL	1099.04 ± 13.88 A.U.	0.26 ± 0.00 µg/mL
6.4 ± 0 cells/µL	1264.29 ± 7.96 A.U.	0.30 ± 0.00 µg/mL
3.2 ± 0 cells/µL	1648.07 ± 15.45 A.U.	0.37 ± 0.00 µg/mL

Fresh Control Blood

[HL60 cells]	Intensity (*N*=3)	Calculated [Fe]
2700 ± 5 cells/µL	54096.87 ± 11.04 A.U.	11 ± 0 µg/mL
1300 ± 2 cells/µL	43180.16 ± 218.45 A.U.	8.6 ± 0.0 µg/mL
670 ± 1 cells/µL	39821.03 ± 105.70 A.U.	7.9 ± 0.0 µg/mL
340 ± 1 cells/µL	38575.81 ± 286.40 A.U.	7.7 ± 0.1 µg/mL
170 ± 0 cells/µL	32523.90 ± 336.83 A.U.	6.5 ± 0.1 µg/mL
84 ± 0 cells/µL	24043.61 ± 181.93 A.U.	4.8 ± 0.0 µg/mL
42 ± 0 cells/µL	18302.80 ± 97.11 A.U.	3.7 ± 0.0 µg/mL
21 ± 0 cells/µL	18011.92 ± 79.72 A.U.	3.6 ± 0.0 µg/mL
11 ± 0 cells/µL	35372.55 ± 171.86 A.U.	7.0 ± 0.0 µg/mL
5.3 ± 0 cells/µL	6121.83 ± 739.16 A.U.	1.3 ± 0.2 µg/mL
2.6 ± 0 cells/µL	42971.42 ± 123.91 A.U.	8.5 ± 0.0 µg/mL

SI Table 4. MP-AES Analysis of Target Cells in Blood: Plotted Data Values and Propagated Error. Background corrected data from MP-AES analysis of target cells labeled with iron oxide nanomaterial in old control blood and fresh control blood ($N = 3$).



SI Figure 6. MP-AES Analysis of Old and Fresh Blood Samples: Endogenous Fe as a Baseline. The blood samples were processed and the endogenous Fe content was calculated via MP-AES analysis. As indicated by the dashed lines, the older sample (A) displayed 0.35 ± 0.07 µg/mL Fe after processing ($N = 6$) and the fresh sample (B) displayed 0.23 ± 0.05 µg/mL Fe after processing ($N = 6$). While the end of the linear range is within the error of detected endogenous Fe in the old sample, it is outside the range of error in the fresh sample. Calculated LoDs using the endogenous [Fe] to inform LoB yielded values of 0.45 µg/mL and 0.66 µg/mL for the older and fresh sample, respectively. Dark purple and light pink data points indicate values below the cellular limit of detection informed by the linear range (Figure 6; 100 and 84 cells/µL, respectively).