Supplemental Online Content 2

McDermott MM, Ferrucci L, Tian L, et al. Effect of granulocyte-macrophage colony-stimulating factor with or without supervised exercise on walking performance in patients with peripheral artery disease. *JAMA*. doi:10.1001/jama.2017.17437

This supplemental material has been provided by the authors to give readers additional information about their work.

eTable 1. Effect of GM-CSF and Exercise on Study Outcomes at 6-Week Follow-up

	Exercise + GM-CSF	Attention control + GM-CSF	Exercise + placebo	Attention control + placebo
Six-minute walk (meters)				
	N=52	N=53	N=53	N=51
Baseline Mean (SD)	332.5 (106.9)	339.8 (101.0)	338.7 (95.6)	339.1 (92.0)
6-week follow-up Mean (SD)	352.5 (97.7)	343.5 (104.3)	352.2 (95.2)	341.4 (84.6)
Within-group 6-week change Mean (95% CI)	+20.0 (+6.5, +33.5)	+3.6 (-9.8, +17.0)	+13.4 (-0.2, +27.0)	+2.2 (-11.6, +16.1)
6-week change relative to attention control + GM-CSF*	+16.4 (-2.5, +35.3) P=0.090 Adjusted P=0.36	---	---	---
6-week change relative to exercise + placebo*	+6.6 (-12.6, +25.7) P=0.50 Adjusted P=0.89	---	---	---
6-week change relative to placebo + attention control*	---	+1.4 (-17.7, +20.5) P=0.89 Adjusted P=0.89	+11.2 (-7.9, +30.3) P=0.25 Adjusted P=0.89	---
Maximal treadmill walking time (minutes)				
Baseline Mean (SD)	7.7 (5.3)	8.8 (4.7)	6.5 (4.5)	6.9 (3.4)
6-week follow-up Mean (SD)	10.2 (5.2)	8.9 (4.1)	9.7 (4.7)	7.5 (3.8)
Within-group 6-week change Mean (95% CI)	+2.5 (+1.7, +3.4)	+0.1 (-0.7, +1.0)	+3.2 (+2.3, +4.1)	+0.6 (-0.3, +1.5)
6-week change relative to attention control + GM-CSF*	+2.4 (+1.2, +3.6) P<0.001 Adjusted P<0.001	---	---	---
6-week change relative to exercise + placebo*	-0.7 (-1.9, +0.6) P=0.30 Adjusted P=0.47	---	---	---
6-week change relative to placebo + attention control*	---	-0.5 (-1.7, +0.8) P=0.47 Adjusted P=0.47	+2.6 (+1.3, +3.9) P<0.001 Adjusted P<0.001	---

eTable 1 (Continued). Effect of GM-CSF and Exercise on Study Outcomes at 6-Week Follow-up

	Exercise + GM-CSF	Attention control + GM-CSF	Exercise + placebo	Attention control + placebo
Brachial Artery Flow-Mediated Dilation (%)				
	N=52	N=53	N=53	N=51
Baseline Median (IQR)	4.97 (2.71, 7.62)	5.49 (3.83, 7.05)	5.06 (2.98, 7.81)	5.40 (2.67, 7.29)
6-week follow-up Median (IQR)	5.30 (2.86, 7.58)	6.41 (4.15, 8.54)	5.35 (3.23, 7.65)	4.35 (2.60, 7.55)
Within-group 6-week change Median (IQR)	+0.17 (-1.13, +2.13)	+0.23 (-1.17, +1.54)	-0.36 (-1.77, +1.99)	-0.17 (-2.11, +1.68)
6-week change relative to attention control + GM-CSF**	+0.06 (-0.93, +1.05) P=0.91 Adjusted P=0.91	---	---	---
6-week change relative to exercise + placebo**	+0.40 (-0.63, +1.43) P=0.45 Adjusted P=0.91	---	---	---
6-week change relative to placebo + attention control**	---	+0.36 (-0.61, +1.34) P=0.47 Adjusted P=0.91	+0.08 (-1.00, +1.16) P=0.89 Adjusted P=0.91	---

*Difference in Changes (95% CI). The P value is from the two-sample t-test. ** Hodges-Lehmann estimation of location shift (95% confidence limits), P value from Wilcoxon Rank Sum test. Statistical significance is determined using the Hochberg step-up method to adjust for multiple comparisons. In the Hochberg method, p values for each of the hypotheses are arranged in order from highest to lowest $P_1 < P_2 < P_3 < P_4$. In this analysis plan, the 'hypothesis' refers to the null hypothesis. Rejecting all four hypotheses indicates that all four primary hypotheses are correct and requires that $p_4 \leq 0.05$. If P_4 is not < 0.05, then three hypotheses are rejected if $P_3 < 0.05/2$, two hypotheses are rejected if $P_2 < 0.05/3$, and one hypothesis is rejected if $P_1 < 0.05/4$. IQR = interquartile range. Hochberg adjusted p values can also be calculated as follows: adjusted $P_4 = P_4$, adjusted P_3 = minimum of (adjusted P_4, 2 x the value of P_3), adjusted P_2 = minimum of the value of (adjusted P_3, 3 times P_2), and adjusted P_1= minimum of the value of (adjusted P_2, 4 x P_1). An adjusted Hochberg P value is statistically significant if the adjusted Hochberg P<0.05.

eTable 2. Effects of GM-CSF and Exercise on Study Outcomes at 6-Month Follow-up

	Exercise + GM-CSF	Attention control + GM-CSF	Exercise + placebo	Attention control + placebo
Six-minute walk (meters)				
	N=51	N=52	N=53	N=51
Baseline Mean (SD)	334.1 (107.3)	342.9 (99.5)	338.7 (95.6)	339.1 (92.0)
6-month follow-up Mean (SD)	368.4 (103.4)	342.9 (109.9)	362.0 (91.7)	330.6 (97.7)
Within-group 6-month change Mean (95% CI)	+34.3 (+16.3, +52.2)	+0.1 (-17.8, +18.0)	+23.2 (+5.3, +41.2)	-8.6 (-27.3, +10.1)
6-month change relative to attention control + GM-CSF*	+34.2 (+8.7, +59.7) P=0.009 Adjusted P=0.036	---	---	---
6-month change relative to exercise + placebo*	+11.0 (-14.1, +36.2) P=0.39 Adjusted P=0.39	---	---	---
6-month change relative to placebo + attention control*	---	+8.6 (-17.1, +34.4) P=0.51 Adjusted P=0.51	+31.8 (+6.2, +57.4) P=0.015 Adjusted P=0.045	---
Maximal treadmill walking time (minutes)				
Baseline Mean (SD)	7.8 (5.3)	8.9 (4.8)	6.5 (4.5)	6.9 (3.4)
6-month follow-up Mean (SD)	12.0 (6.1)	9.6 (4.8)	11.0 (5.1)	7.4 (3.9)
Within-group 6-month change Mean (95% CI)	+4.2 (+3.1, +5.3)	0.7 (-0.4, +1.8)	+4.5 (+3.4, +5.6)	+0.5 (-0.6, +1.7)
6-month change relative to attention control + GM-CSF*	+3.5 (+2.0, +5.0) P<0.001 Adjusted P<0.001	---	---	---
6-month change relative to exercise + placebo*	-0.3 (-1.8, +1.2) P=0.70 Adjusted P=0.81	---	---	---
6-month change relative to placebo + attention control*	---	+0.2 (-1.4, +1.7) P=0.81 Adjusted P=0.81	+4.0 (+2.4, +5.6) P<0.001 Adjusted P<0.001	---

eTable 2 (continued). Effects of GM-CSF and Exercise on Study Outcomes at 6-Month Follow-up

	Exercise + GM-CSF	Attention control + GM-CSF	Exercise + placebo	Attention control + placebo
Brachial Artery Flow-Mediated Dilation (%)				
	N=51	N=52	N=53	N=51
Baseline Median (IQR)	4.95 (2.71, 7.62)	5.61 (4.43, 7.13)	5.06 (2.98, 7.81)	5.40 (2.67, 7.29)
6-month follow-up Median (IQR)	4.91 (2.18, 7.91)	5.49 (3.89, 7.54)	5.33 (2.58, 7.15)	4.10 (2.30, 7.17)
Within-group 6-month change Median (IQR)	-0.29 (-1.57, +1.81)	-0.27 (-1.73, +1.15)	-0.36 (-1.75, +1.33)	-0.65 (-2.73, +1.48)
6-month change relative to attention control + GM-CSF**	+0.21 (-0.70, +1.13) P=0.65 Adjusted P=0.71	---	---	---
6-month change relative to exercise + placebo**	+0.19 (-0.83, +1.21) P=0.71 Adjusted P=0.71	---	---	---
6-month change relative to placebo + attention control**	---	+0.36 (-0.76, +1.48) P=0.53 Adjusted P=0.71	+0.41 (-0.80, +1.61) P=0.51 Adjusted P=0.71	---

*Difference in Changes (95% CI). The P value is from the two-sample t-test. ** Hodges-Lehmann estimation of location shift (95% confidence limits), P value from Wilcoxon Rank Sum test. Statistical significance is determined using the Hochberg step-up method to adjust for multiple comparisons. In the Hochberg method, p values for each of the hypotheses are arranged in order from highest to lowest $P_1 < P_2 < P_3 < P_4$. In this analysis plan, the 'hypothesis' refers to the null hypothesis. Rejecting all four hypotheses indicates that all four primary hypotheses are correct and requires that $p_4 \leq 0.05$. If P_4 is not < 0.05, then three hypotheses are rejected if $P_3 < 0.05/2$, two hypotheses are rejected if $P_2 < 0.05/3$, and one hypothesis is rejected if $P_1 < 0.05/4$. IQR = interquartile range. Hochberg adjusted p values can also be calculated as follows: adjusted $P_4 = P_4$, adjusted P_3 = minimum of (adjusted P_4, 2 x the value of P_3), adjusted P_2 = minimum of the value of (adjusted P_3, 3 times P_2), and adjusted P_1= minimum of the value of (adjusted P_2, 4 x P_1). An adjusted Hochberg P value is statistically significant if the adjusted Hochberg P<0.05.

eTable 3. Effect of GM-CSF and Exercise on Changes in Reactive Hyperemia Flow, Reactive Hyperemic Average Peak Velocity, and Brachial Artery Diameter at 12-Week Follow-up

eTable 3a. Changes in reactive hyperemia brachial flow in response to study interventions

	Exercise + GM-CSF	Attention control + GM-CSF	Exercise + placebo	Attention control + placebo
Reactive hyperemia brachial flow (cubic centimeter/minute)				
	N=48	N=47	N=47	N=43
Baseline Mean (SD)	912.93 (384.75)	960.52 (312.38)	863.55 (370.70)	883.62 (301.03)
12-week follow-up Mean (SD)	942.52 (403.01)	957.96 (283.39)	838.05 (353.49)	864.12 (300.31)
Within-group 12-week change Mean (95% CI)	+29.59 (-29.74, +88.94) P=0.3208	-2.56 (-72.30, +67.18) P=0.9413	-25.50 (-93.88, +42.89) P=0.4568	-19.49 (-83.67, +44.69) P=0.5433

eTable 3 (continued). Effect of GM-CSF and Exercise on Changes in Reactive Hyperemia Flow, Reactive Hyperemic Average Peak Velocity, and Brachial Artery Diameter at 12-Week Follow-up

eTable 3b. Changes in reactive hyperemic average peak velocity in response to study interventions

	Exercise + GM-CSF	**Attention control + GM-CSF**	**Exercise + placebo**	**Attention control + placebo**
Reactive hyperemia average peak velocity (centimeter/second)				
	N=48	N=47	N=47	N=43
Baseline Mean (SD)	169.73 (38.35)	165.06 (32.44)	161.57 (33.91)	163.56 (29.79)
12-week follow-up Mean (SD)	173.76 (41.17)	163.47 (24.50)	159.22 (30.08)	163.75 (30.89)
Within-group 12-week change Mean (95% CI)	+4.03 (-4.42, +23.48) P=0.3422	-1.60 (-10.99, +7.80) P=0.7341	-2.35 (-10.78, +6.07) P=0.5766	+0.20 (-7.80, +8.19) P=0.9606

eTable 3 (continued). Effect of GM-CSF and Exercise on Changes in Reactive Hyperemia Flow, Reactive Hyperemic Average Peak Velocity, and Brachial Artery Diameter at 12-Week Follow-up

eTable 3c. Changes in brachial artery diameter in response to study interventions

	Exercise + GM-CSF	**Attention control + GM-CSF**	**Exercise + placebo**	**Attention control + placebo**
Resting BA diameter (mm)				
	N=48	N=47	N=47	N=43
Baseline Mean (SD)	4.39 (0.67)	4.54 (0.65)	4.33 (0.84)	4.37 (0.73)
12-week follow-up Mean (SD)	4.43 (0.68)	4.53 (0.70)	4.35 (0.86)	4.38 (0.70)
Within-group 12-week change Mean (95% CI)	+0.04 (-0.02, +0.09) P=0.1746	-0.01 (-0.07, +0.05) P=0.7247	+0.02 (-0.04, +0.09) P=0.4985	+0.01 (-0.05, +0.06) P=0.8476

GM-CSF = Granulocyte macrophage colony stimulating factor

eTable 4. Results of Generalized Linear Mixed Models for Effect of GM-CSF and Exercise on Study Outcomes (Longitudinal Analyses) *

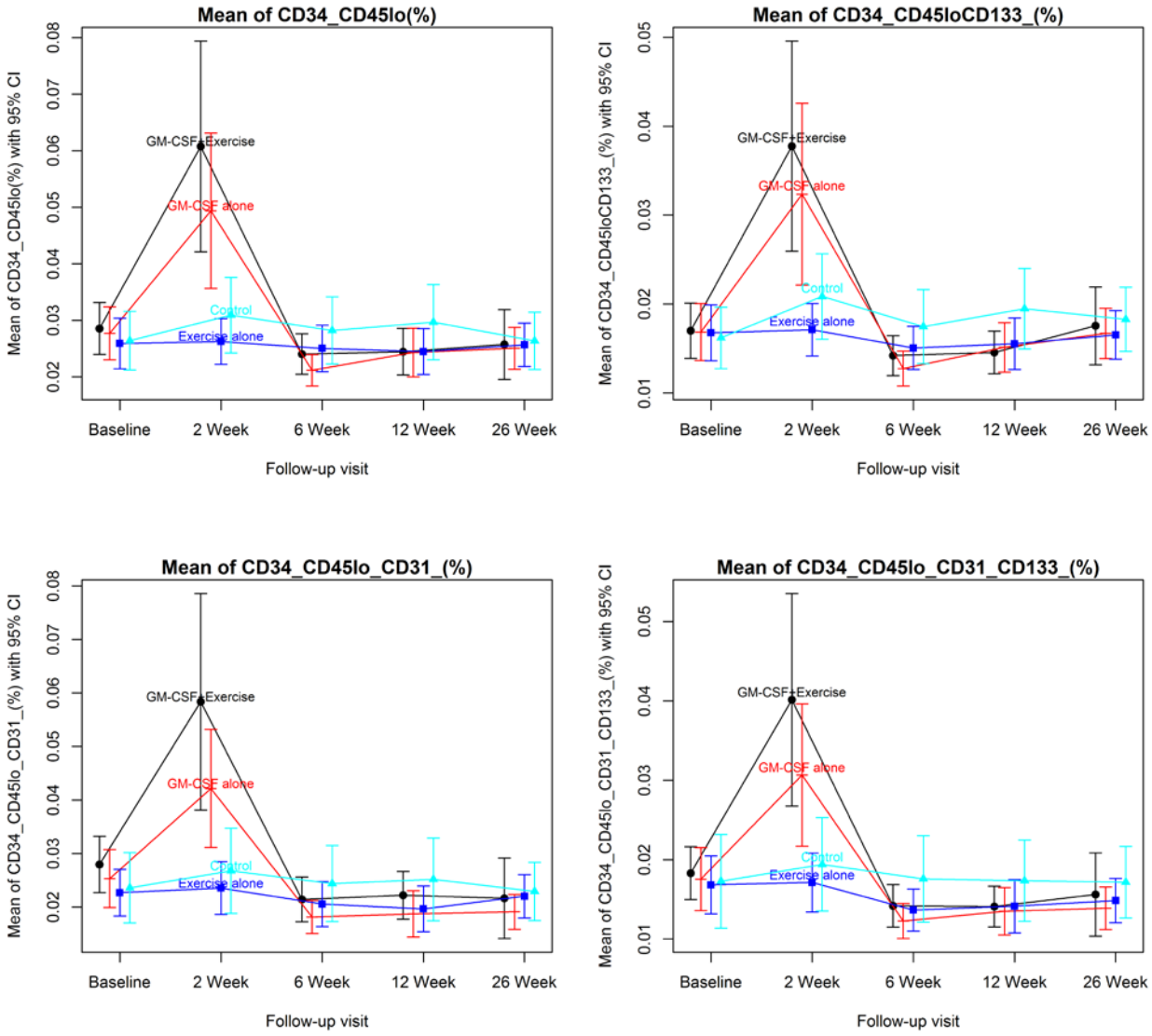
	Exercise + GM-CSF	Attention control + GM-CSF	Exercise + placebo	Attention control + placebo
Six-minute walk (meters)				
	N=51	N=53	N=53	N=51
Relative to attention control + GM-CSF	+25.9 (+4.3, +47.6) P=0.019 Adjusted P=0.057	---	---	---
Relative to exercise + placebo	+3.1 (-18.4, +24.7) P=0.775 Adjusted P=0.775	---	---	---
Relative to placebo + attention control	---	+2.7 (-15.6, +21.0) P=0.772 Adjusted P=0.775	+25.5 (+7.3, +43.8) P=0.006 Adjusted P=0.024	---
Maximal treadmill walking time (minutes)				
	N=51	N=53	N=53	N=51
Relative to attention control + GM-CSF	+3.1 (+1.7, +4.5) P<0.001 Adjusted P<0.001	---	---	---
Relative to exercise + placebo	-0.6 (-2.1, +0.9) P=0.431 Adjusted P=0.601	---	---	---
Relative to placebo + attention control	---	-0.3 (-1.4, +0.8) P=0.601 Adjusted P=0.601	+3.4 (+2.3, +4.6) P<0.001 Adjusted P<0.001	---
Brachial Artery Flow-Mediated Dilation (%)				
	N=51	N=53	N=53	N=51
Relative to attention control + GM-CSF	+0.05 (-0.85, +0.95) P=0.918 Adjusted P=0.918	---	---	---
Relative to exercise + placebo	+0.20 (-0.80, +1.20) P=0.696 Adjusted P=0.918	---	---	---
Relative to placebo + attention control	---	+0.31 (-0.48, +1.10) P=0.440 Adjusted P=0.918	+0.16 (-0.74, +1.06) P=0.726 Adjusted P=0.918	---

*Statistical significance is determined using the Hochberg step-up method to adjust for multiple comparisons. In the Hochberg method, p values for each of the hypotheses are arranged in order from highest to lowest $P_1 < P_2 < P_3 < P_4$. In this analysis plan, the 'hypothesis' refers to the null hypothesis. Rejecting all four hypotheses indicates that all four primary hypotheses are correct and requires that $p_4 \leq 0.05$. If P_4 is not < 0.05, then three hypotheses are rejected if $P_3 < 0.05/2$, two hypotheses are rejected if $P_2 < 0.05/3$, and one hypothesis is rejected if $P_1 < 0.05/4$. IQR = interquartile range. Hochberg adjusted p values can also be calculated as follows: adjusted $P_4 = P_4$, adjusted P_3 = minimum of (adjusted P_4, 2 x the value of P_3), adjusted P_2 = minimum of the value of (adjusted P_3, 3 times P_2), and adjusted P_1= minimum of the value of (adjusted P_2, 4 x P_1). An adjusted Hochberg P value is statistically significant if the adjusted Hochberg $P<0.05$.

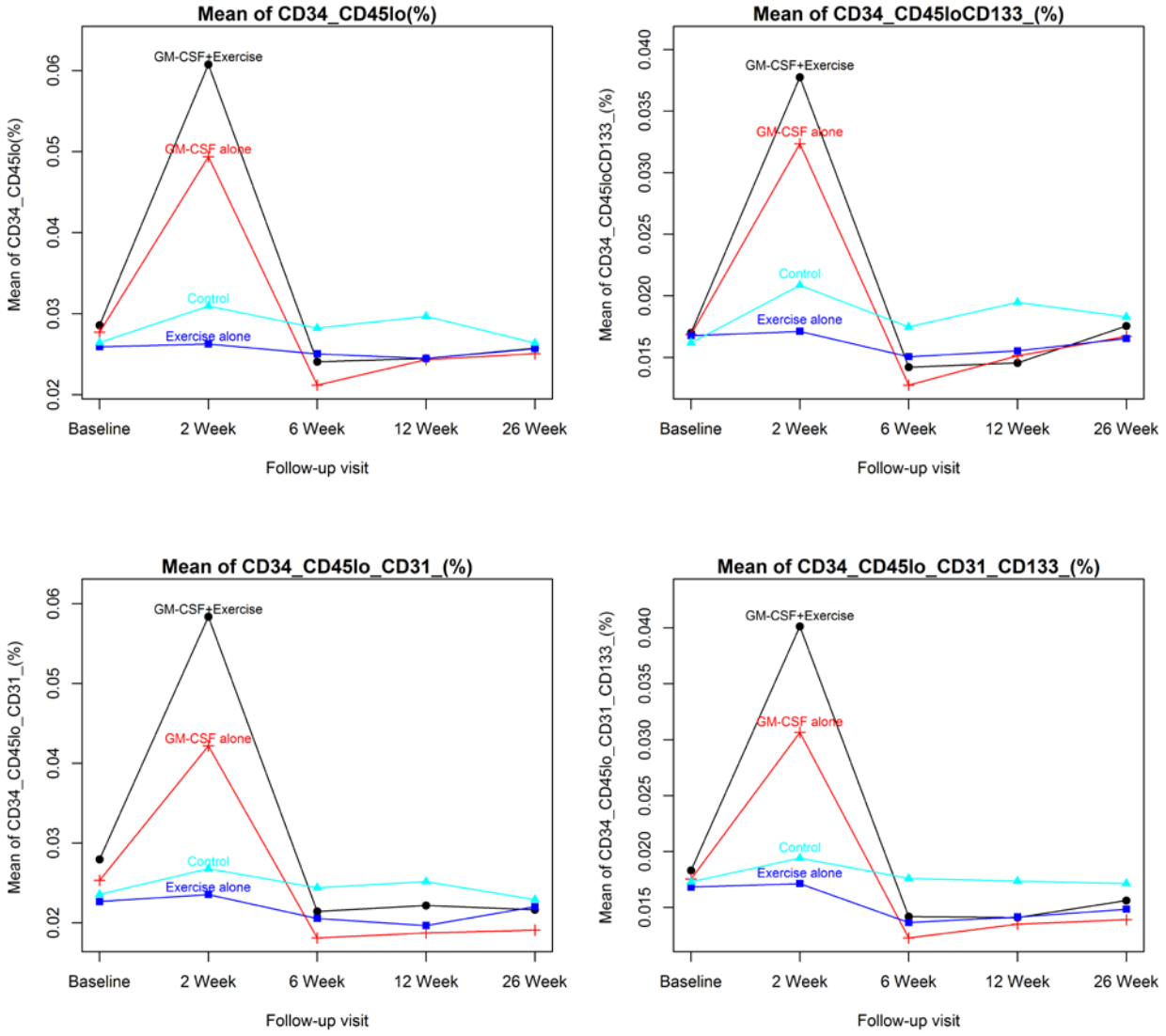
eMethods. Detailed Methods for Measurement of Progenitor Cells

Blood from peripheral venipuncture was washed to lyse red blood cells. Remaining cell counts were obtained using the Countess® Automated Cell Counter (Life Technologies, NY). White cells were stained with LIVE/DEAD® Fixable Dead Cell Stains (Life Technologies, NY), preincubated with human Fc block (Miltenyi Biotec), and stained with antibodies: anti-CD34 VioBlue (Miltenyi, CA), anti-CD133-APC (Miltenyi, CA), anti-CD45 AlexaFluor 700 (BD biosciences**,** CA), and anti-CD31 (PECAM-1) APC-eFluor® 780 (Ebiosciences, CA) antibodies (11). Stained samples were acquired on a BD LSRII Flow Cytometer (BD Biosciences, CA) and analyzed using Flowjo software (Treestar, OR). Dead cells were excluded. Cell populations were gated and analyzed based on an anti-CD133-APC FMO control.

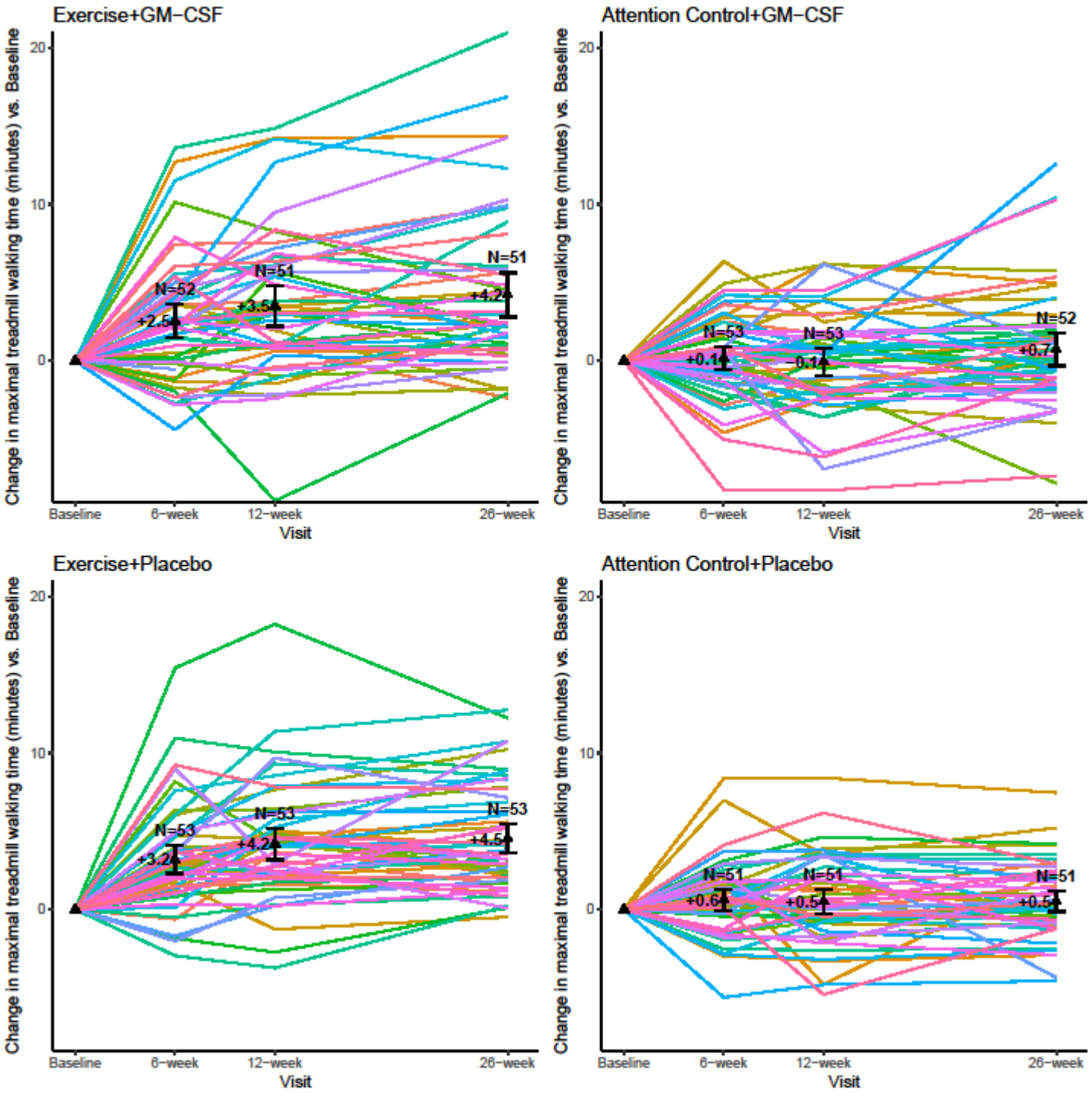
Supplementary Figure 1. Temporal changes in endothelial progenitor cells according to study group assignment with error bars



Supplementary Figure 2. Temporal changes in endothelial progenitor cells according to study group assignment without error bars



eFigure 3. Changes in Treadmill Walking Time by Study Intervention (N=208)



eFigure 4. Changes in Brachial Artery Flow-Mediated Dilation by Study Intervention (N=208)