

Supplementary Figures and Tables

Supplementary Table 1. MBP-stimulated cytokine / chemokine responses between binary age groups.

Molecule Abbreviation	Molecule Full Name	Age <50 (n=13)	Age ≥50 (n=15)	Unadjusted p-value	FDR
		mean (SD), pg/ml	mean (SD), pg/ml		
BCA-1	B cell-attracting chemokine-1	26.18 (16.65)	19.61 (12.87)	0.25	0.55
EGF	epidermal growth factor	7.04 (6.48)	4.62 (4.81)	0.268	0.57
ENA-78	epithelial-derived neutrophil-activating peptide 78	7858.54 (3051.66)	6029.08 (3872.69)	0.182	0.55
Eotaxin	eotaxin	0.20 (0.38)	0.86 (1.55)	0.149	0.53
Eotaxin.2	eotaxin-2	1058.22 (1562.06)	2167.34 (2144.98)	0.135	0.53
FGF-2	fibroblast growth factor 2	2.52 (2.13)	7.53 (9.85)	0.084	0.53
FLT-3L	fms-like tyrosine kinase receptor 3 ligand	0.39 (0.41)	0.28 (0.20)	0.398	0.6
Fractalkine	fractalkine	3.59 (3.27)	10.22 (11.02)	0.047	0.39
G.CSF	granulocyte colony stimulating factor	7.63 (13.39)	3.59 (5.61)	0.295	0.6
GM-CSF	granulocyte-macrophage colony stimulating factor	29.32 (62.32)	4.13 (8.08)	0.132	0.53
GRO-a	growth related oncogene-alpha	1433.48 (721.58)	1143.09 (797.93)	0.325	0.6
I-309	cytokine I-309	17.74 (38.15)	9.80 (8.77)	0.44	0.63
IFN-a2	interferon alpha 2	2.33 (1.82)	3.42 (5.16)	0.477	0.66
IFN.γ	interferon gamma	8.23 (16.50)	1.27 (1.70)	0.115	0.53
IL-1a	interleukin-1 alpha	2.52 (7.19)	2.90 (4.15)	0.86	0.98
IL-1b	interleukin-1 beta	13.09 (17.89)	17.92 (23.61)	0.552	0.72
IL-1RA	interleukin-1 receptor antagonist	37.84 (89.09)	40.13 (47.67)	0.932	0.98
IL-2	interleukin-2	12.41 (24.20)	6.72 (8.14)	0.398	0.6
IL-3	interleukin-3	2.09 (4.69)	1.21 (1.46)	0.5	0.66
IL-4	interleukin-4	0.28 (0.18)	0.16 (0.12)	0.048	0.39
IL-5	interleukin-5	7.38 (15.77)	1.81 (3.48)	0.194	0.55
IL-6	interleukin-6	686.48 (593.29)	462.24 (549.82)	0.309	0.6
IL-8	interleukin-8	6508.76 (3969.31)	5944.49 (3105.28)	0.677	0.84
IL-9	interleukin-9	2.62 (4.11)	1.11 (1.08)	0.18	0.55
IL-10	interleukin-10	51.96 (46.65)	38.82 (30.34)	0.379	0.6
IL-12p40	interleukin-12 p40 subunit	9.81 (12.40)	9.85 (9.28)	0.992	0.99
IL-12p70	interleukin-12 p70 subunit	0.08 (0.13)	0.30 (0.50)	0.14	0.53
IL-13	interleukin-13	16.96 (29.08)	7.14 (7.61)	0.218	0.55
IL-15	interleukin-15	0.42 (0.50)	0.61 (0.66)	0.413	0.6
IL-16	interleukin-16	33.97 (73.49)	21.02 (52.42)	0.592	0.75
IL-17A	interleukin-17A	9.09 (16.55)	2.60 (2.82)	0.146	0.53
IL-17E (IL-25)	interleukin-17A (also known as interleukin-25)	0.54 (0.44)	1.03 (1.28)	0.205	0.55

IL-17F	interleukin-17F	34.43 (86.32)	3.55 (5.16)	0.177	0.55
IL-18	interleukin-18	0.61 (1.09)	0.66 (1.44)	0.92	0.98
IL-23	interleukin-23	7.60 (9.28)	6.77 (7.98)	0.803	0.97
IL-27	interleukin-27	14.62 (10.14)	19.18 (17.02)	0.407	0.6
IP-10	Interferon gamma-induced protein 10	1725.41 (1963.95)	1836.38 (2045.87)	0.885	0.98
MCP-1	monocyte chemoattractant protein 1	1170.04 (780.66)	1416.12 (730.53)	0.397	0.6
MCP-2	monocyte chemoattractant protein 2	455.48 (490.72)	97.93 (135.06)	0.012	0.23
MCP-3	monocyte chemoattractant protein 3	906.47 (883.40)	300.90 (220.80)	0.016	0.23
MCP-4	monocyte chemoattractant protein 4	140.84 (253.65)	19.65 (46.88)	0.08	0.53
M-CSF	macrophage colony-stimulating factor	4.22 (11.13)	6.31 (3.23)	0.491	0.66
MDC	macrophage-derived chemokine	48.22 (85.43)	286.27 (232.03)	0.002	0.11
MIG (CXCL9)	monokine induced by gamma (also known as C-X-C motif chemokine ligand 9)	1741.65 (2011.68)	1170.79 (1014.53)	0.342	0.6
MIP-1a	macrophage inflammatory protein 1 alpha	34.89 (49.45)	34.37 (49.23)	0.978	0.99
MIP-1b	macrophage inflammatory protein 1 beta	181.07 (172.08)	190.56 (152.31)	0.878	0.98
PDGF-AA	platelet-derived growth factor AA	41.74 (30.94)	20.72 (14.36)	0.026	0.3
PDGF-AB/BB	platelet-derived growth factor AB/BB	357.92 (194.32)	198.19 (109.85)	0.011	0.23
RANTES	regulated on activation, normal T cell expressed and secreted (also known as C-C motif chemokine ligand 5)	18.57 (49.27)	57.93 (107.24)	0.235	0.55
sCD40L	soluble CD40 ligand	2.02 (3.17)	3.22 (4.17)	0.408	0.6
SDF-1a+b	stromal cell-derived factor 1alpha + beta	17.75 (25.39)	17.60 (18.65)	0.986	0.99
TARC	thymus and activation-regulated chemokine	25.29 (34.49)	10.10 (13.04)	0.125	0.53
TGFa	transforming growth factor alpha	1.40 (1.60)	1.33 (0.80)	0.886	0.98
TNFa	tumor necrosis factor alpha	59.83 (109.28)	63.14 (62.58)	0.921	0.98
TNFb	tumor necrosis factor beta	5.36 (7.87)	2.86 (1.77)	0.24	0.55
TRAIL	tumor necrosis factor-related apoptosis-inducing ligand	0.88 (1.60)	0.35 (0.47)	0.229	0.55
VEGF-A	vascular endothelial growth factor A	49.04 (48.17)	35.23 (36.30)	0.396	0.6

Note: The molecules highlighted in red reached marginal level of significance for association with binary age, but none survived multiple testing correction.

Abbreviation: *FDR*, false discovery rate accounting for multiple testing.

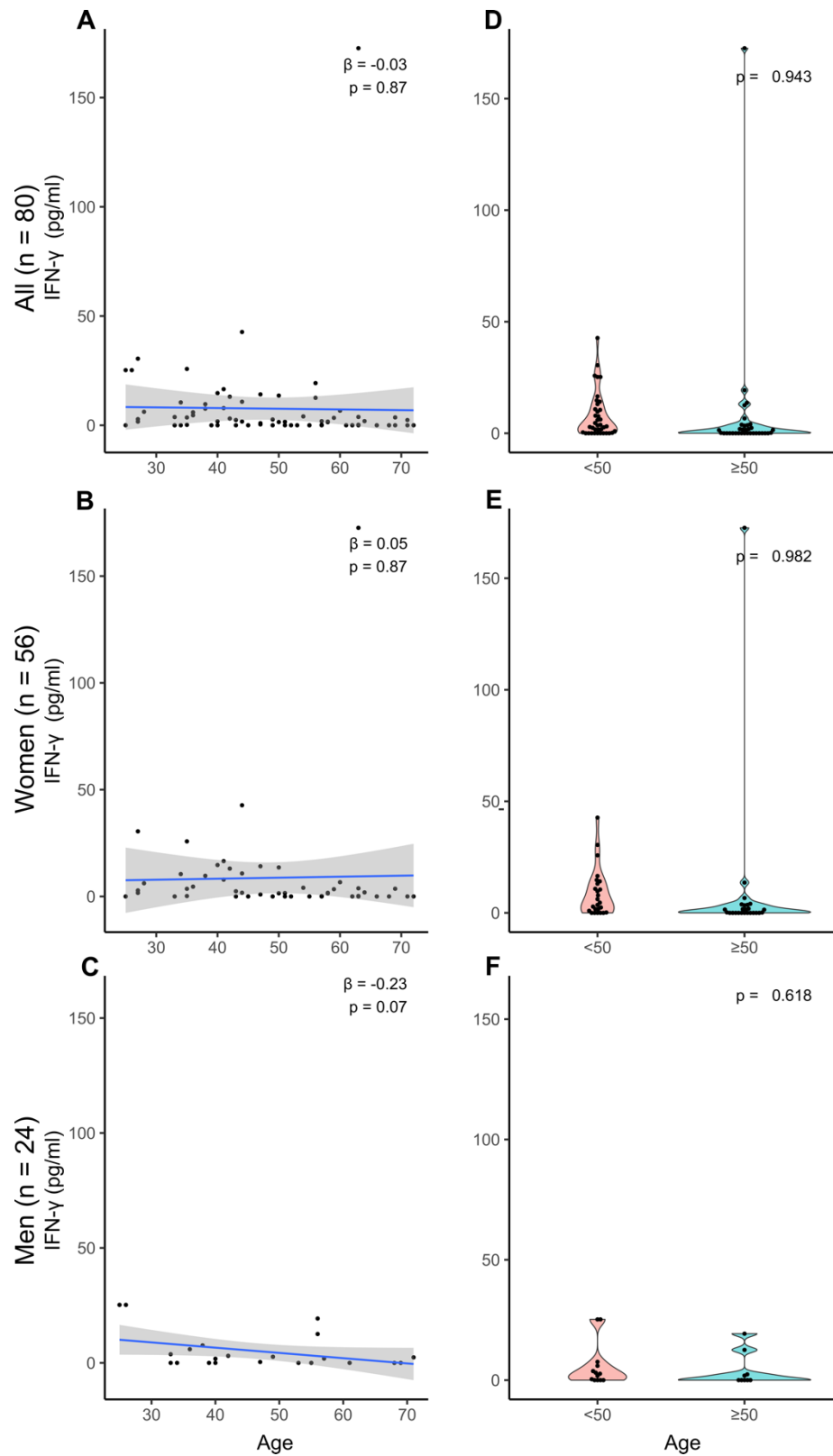
Supplementary Table 2. MBP-driven response of selected molecules (see Supplementary Table 1) as mediators of the association between age and annualized relapse rate.

n=28 (women=20, men=8)		
Fractalkine	Coefficient (95%CI)	p-value
Total effect	-0.265 (-0.410, -0.125)	<0.001
Direct effect	-0.269 (-0.428, -0.079)	0.004
Indirect effect	0.005 (-0.136, 0.046)	0.956
MDC		
Total effect	-0.265 (-0.411, -0.105)	<0.001
Direct effect	-0.254(-0.457, -0.059)	0.016
Indirect effect	-0.012 (-0.113, 0.090)	0.804
IL-4		
Total effect	-0.265 (-0.422, -0.110)	<0.001
Direct effect	-0.310 (-0.460, -0.153)	<0.001
Indirect effect	0.045 (-0.037, 0.134)	0.276
MCP-2		
Total effect	-0.265 (-0.398, -0.097)	<0.001
Direct effect	-0.255 (-0.388, -0.091)	0.004
Indirect effect	-0.011(-0.126, 0.098)	0.956
MCP-3		
Total effect	-0.265 (-0.407, -0.109)	<0.001
Direct effect	-0.278 (-0.450, -0.108)	<0.001
Indirect effect	0.013 (-0.076, 0.078)	0.604
PDGF-AA		
Total effect	-0.265 (-0.415, -0.113)	<0.001
Direct effect	-0.286 (-0.462, -0.114)	<0.001
Indirect effect	0.020 (-0.051, 0.113)	0.616
PDGF-AB/BB		
Total effect	-0.265 (-0.410, -0.119)	<0.001
Direct effect	-0.267 (-0.448, -0.099)	0.008
Indirect effect	0.002 (-0.075, 0.103)	0.984

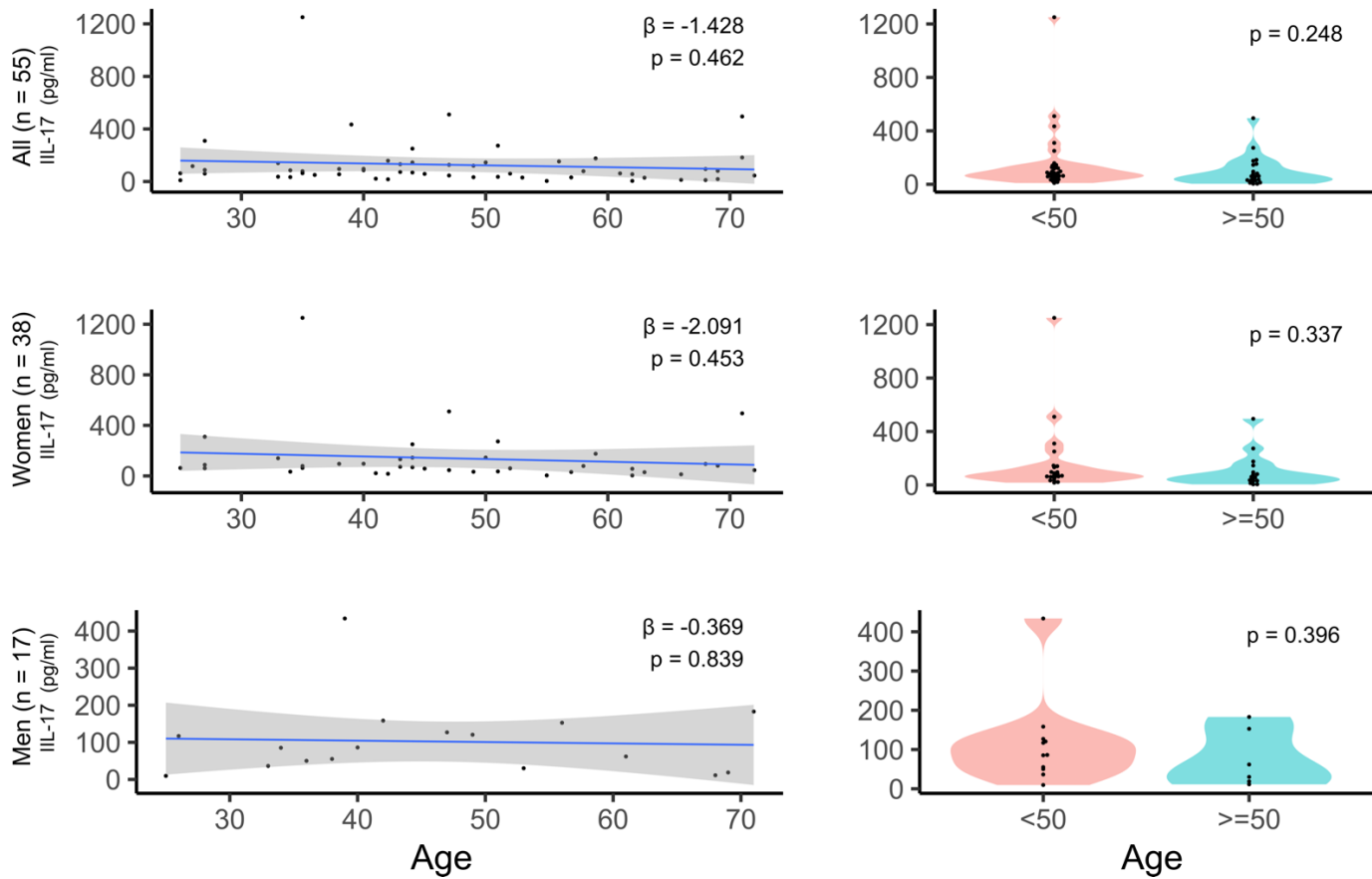
Note: We conducted mediation analyses to assess the extent to which MBP-driven cytokine or chemokine response (selected from the list in Supplementary Table 1) explains the association between age and annualized relapse rate in people with multiple sclerosis. We compared the group ≥ 50 years of age to the group < 50 years of age as the reference group. In each mediation analysis, the independent variable in the total effect model is age. Independent variables in the direct effect model are age, and MBP-driven cytokine or chemokine level. Indirect effect was calculated by subtracting direct effect from total effect. We consider a negation when the indirect effect is in the opposite direction as the direct or total effect.

Abbreviations: CI, confidence interval

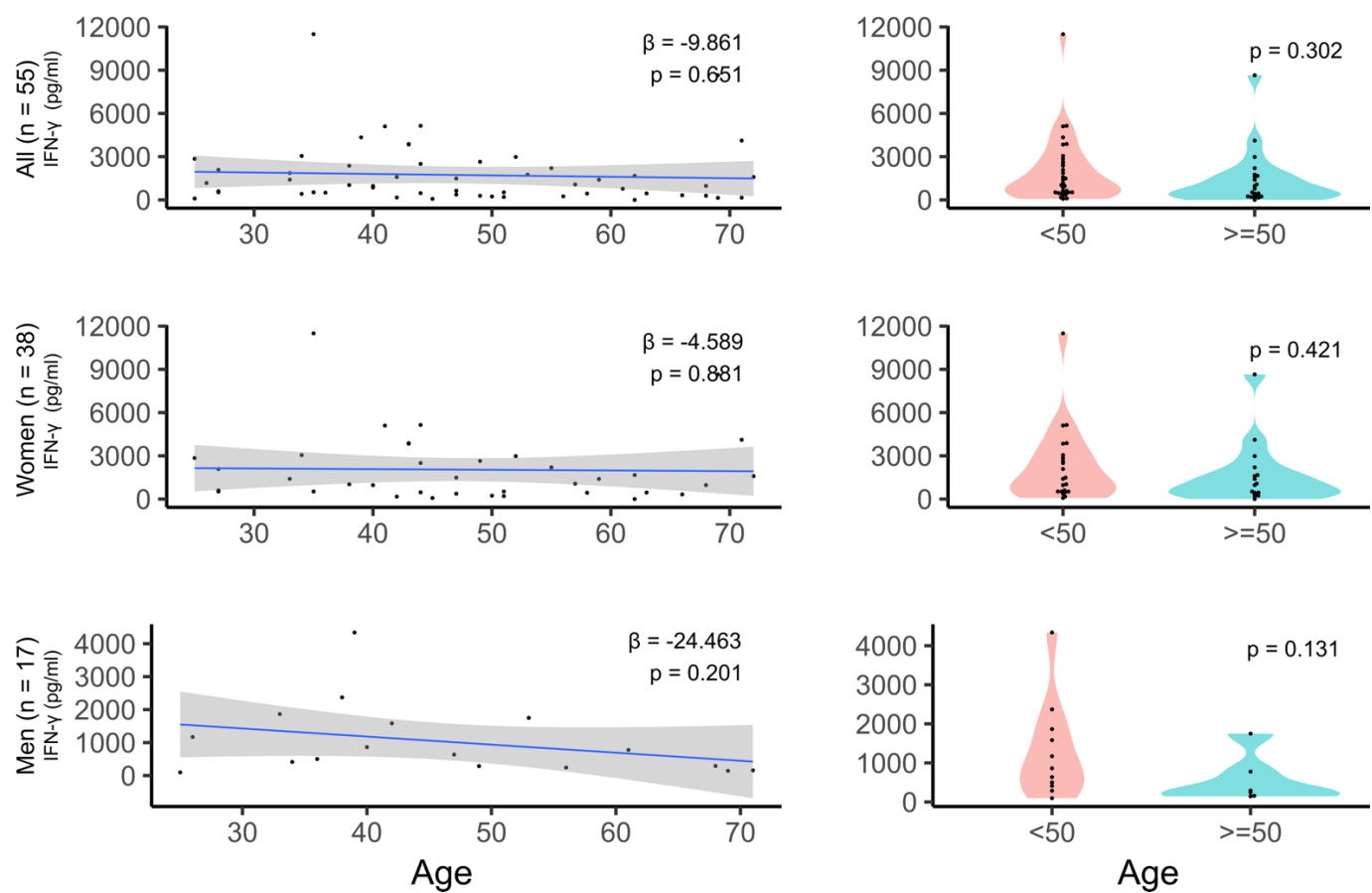
Supplementary Figure 1. MBP-driven IFN- γ response was not associated with age as either a continuous variable (A-C) or binary variable (D-F).



Supplementary Figure 2. HKC-driven IL-17 response was not associated with age either as a continuous variable (A-C) or binary variable (D-F).

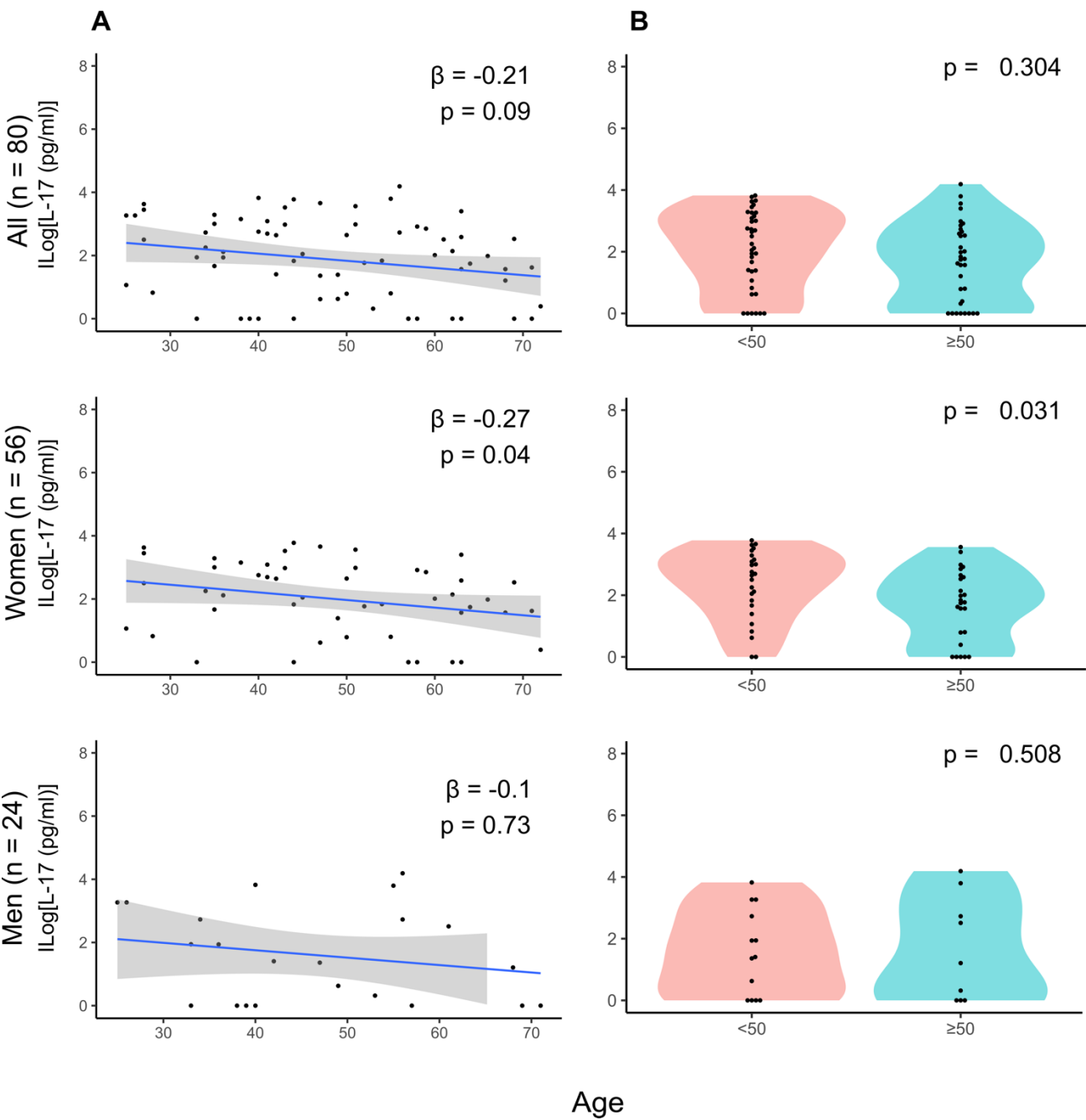


Supplementary Figure 3. HKC-driven IFN- γ response was not associated with age either as a continuous variable (A-C) or binary variable (D-F).



Supplementary Figure 4. MBP-driven (A-B) and HKC-driven (C-D) IL-17 response as shown in log scale with age either as a continuous variable (A, C) or binary variable (B, D).

MBP-Driven IL-17 Repsonse



HKC-Driven IL-17 Repsonse

