Supplementary Data

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Table S1. Pathways enriched in circulating monocytes following therapy with anti-PD-L1 *in vivo*.

Pathway	**FDR**
IL-1 signaling in melanoma	3.59E-07
Immune response IL-1 signaling pathway	4.81E-07
Cell adhesion_IL-8 family dependent cell migration and adhesion	1.82E-07
IL-1 production in melanoma	4.65E-06
Immune response_IL-18 signaling	2.99E-05
Immune response_IL-17 signaling pathways	2.99E-05
Macrophage and dendritic cell phenotype shift in cancer	6.45E-05

Table S2. Clinical Characteristics.

	Patient 1	**Patient 2**
Age (years)	59	54
Sex	Male	Male
M spike IgH/L isotype	IgA kappa	IgG Lambda
Total number of cycles	7	1
IrAE ($\geq$ grade 2)	Grade 2 adrenal insufficiency Grade 2 hypothyroidism	None
Best Response to therapy	Stable disease	Stable disease

Table S3. Differentially regulated pathways in bone marrow immune cells following therapy with anti-PD-L1.

	Pathway	**FDR q-value**
T cells		
Cluster 0	ALLOGRAFT_REJECTION	3.13E-18
	TNFA_SIGNALING_VIA_NFKB	4.63E-17
	HYPOXIA	2.31E-10
	APOPTOSIS	3.09E-10
	COMPLEMENT	2.55E-09
	INFLAMMATORY_RESPONSE	3.55E-08
	INTERFERON_GAMMA_RESPONSE	4.00E-07
Cluster 1	TNFA_SIGNALING_VIA_NFKB	3.11E-16
	HYPOXIA	1.31E-12
	APOPTOSIS	2.50E-03
	INFLAMMATORY_RESPONSE	3.14E-03
	INTERFERON_GAMMA_RESPONSE	3.14E-03
Cluster 3	TNFA_SIGNALING_VIA_NFKB	1.41E-14
	COMPLEMENT	4.22E-07
	INFLAMMATORY_RESPONSE	4.22E-07
	APOPTOSIS	6.24E-05
	INTERFERON_GAMMA_RESPONSE	1.42E-03
Cluster 4	HYPOXIA	3.37E-13
	TNFA_SIGNALING_VIA_NFKB	3.37E-13
	INTERFERON_GAMMA_RESPONSE	8.76E-04
NK cells		
Cluster 2	TNFA_SIGNALING_VIA_NFKB	3.27E-21
	ALLOGRAFT_REJECTION	1.19E-16
	COMPLEMENT	1.93E-09
	HYPOXIA	6.29E-06
	INTERFERON_GAMMA_RESPONSE	6.29E-06
Myeloid cells		
Cluster 5	COMPLEMENT	2.43E-02
	INFLAMMATORY_RESPONSE	2.43E-02
	INTERFERON_GAMMA_RESPONSE	2.43E-02
	TNFA_SIGNALING_VIA_NFKB	2.43E-02
Cluster 7	INFLAMMATORY_RESPONSE	2.53E-02
Cluster 8	IL2_STAT5_SIGNALING	1.40E-02

Table S4. Differentially regulated pathways in peripheral blood immune cells following therapy with anti-PD-L1.

	Pathway	**FDR q-value**
T cells		
Cluster 0	INTERFERON_GAMMA_RESPONSE	1.26E-59
	INTERFERON_ALPHA_RESPONSE	3.38E-53
	INFLAMMATORY_RESPONSE	5.68E-10
	IL2_STAT5_SIGNALING	1.00E-07
	TNFA_SIGNALING_VIA_NFKB	1.00E-07
Cluster 3	INTERFERON_GAMMA_RESPONSE	6.29E-63
	INTERFERON_ALPHA_RESPONSE	1.67E-56
	COMPLEMENT	7.21E-09
	TNFA_SIGNALING_VIA_NFKB	2.35E-06
Cluster 4	INTERFERON_ALPHA_RESPONSE	7.16E-61
	INTERFERON_GAMMA_RESPONSE	1.34E-60
	INFLAMMATORY_RESPONSE	2.70E-06
	TNFA_SIGNALING_VIA_NFKB	2.70E-06
Cluster 6	INTERFERON_GAMMA_RESPONSE	1.53E-16
	INTERFERON_ALPHA_RESPONSE	9.58E-16
	TNFA_SIGNALING_VIA_NFKB	5.42E-06
NK cells		
Cluster 1	INTERFERON_GAMMA_RESPONSE	1.10E-73
	INTERFERON_ALPHA_RESPONSE	3.26E-62
	COMPLEMENT	1.70E-12
	IL2_STAT5_SIGNALING	5.86E-09
	TNFA_SIGNALING_VIA_NFKB	6.88E-08
Cluster 8	INTERFERON_GAMMA_RESPONSE	1.28E-44
	INTERFERON_ALPHA_RESPONSE	3.03E-35
Myeloid cells		
Cluster 2	INTERFERON_GAMMA_RESPONSE	3.75E-118
	INTERFERON_ALPHA_RESPONSE	4.23E-85
	TNFA_SIGNALING_VIA_NFKB	3.44E-32
	INFLAMMATORY_RESPONSE	1.89E-26
	COMPLEMENT	3.43E-11
	REACTIVE_OXYGEN_SPECIES_PATHWAY	2.51E-06
	FATTY_ACID_METABOLISM	2.77E-05
Cluster 7	INTERFERON_GAMMA_RESPONSE	1.28E-44
	INTERFERON_ALPHA_RESPONSE	3.03E-35
B cells		
Cluster 5	INTERFERON_ALPHA_RESPONSE	1.71E-34
	INTERFERON_GAMMA_RESPONSE	6.09E-31
	COMPLEMENT	7.41E-04

Table S5. List of antibody clones used for CyTOF.

	Antibody	Clone
1	41BB	4B4-1
2	B7H3	MIH42
3	BTLA	MIH26
4	CCR7	G043H7
5	CD11b	ICMF44
6	CD11c	Bu15
7	CD123	6H6
8	CD138	M15
9	CD15	W6D3
10	CD155	SKIL4
11	CD16	3G8
12	CD19	HIB19
13	CD20	S2H7
14	CD200	OX-104
15	CD21	BL13
16	CD23	EBVCS-5
17	CD24	ML5
18	CD25	2A3
19	CD27	L128
20	CD28	CD28.2
21	CD3	UCHT1
22	CD33	WM53
23	CD38	HIT2
24	CD4	RPA-T4
25	CD44	IM7
26	CD45RO	UCHL1
27	CD56	HCD56
28	CD57	HCD57

	Antibody	Clone
29	CD69	FN50
30	CD8	RPA-T8
31	CD80	2D10.4
32	CD81	5A6
33	CD95	DX2
34	CTLA4	14D3
35	CXCR4	12G5
36	CXCR5	RF8132
37	DNAM-1	TX25
38	FOXP3	PCH101
39	Granzyme	GB11
40	HLADR	L243
41	HVEM	122
42	ICOS	C398.4A
43	Ig Kappa	MHK-49
44	Ig Lamba	MHK-38
45	IgM	MHM-88
46	Ki67	Ki-67
47	LAG3	11C3C65
48	NKG2D	OW72
49	OX-40	ACT35
50	PD1	EH12.2H7
51	PD-L1	MIH1
52	SLAMF7	162.1
53	SOX2	030-678
54	T-Bet	4B10
55	TIGIT	MBSA43
56	TIM3	F38-2E2

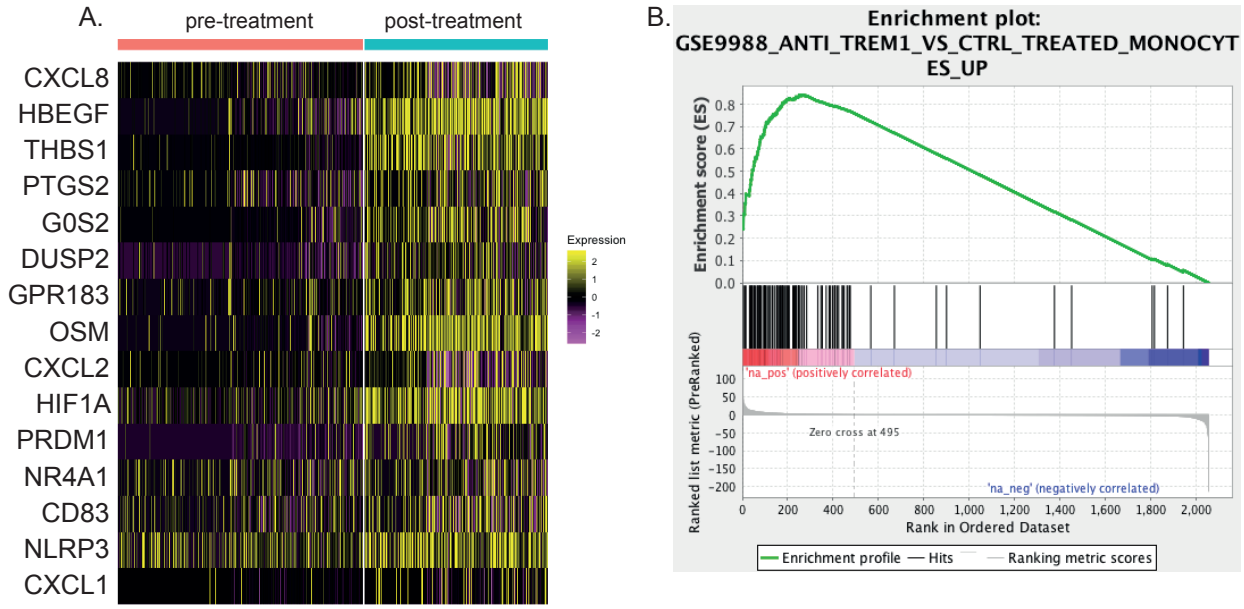


Figure S1: Single-cell RNA sequencing differential gene expression in classical (CD14+ CD16-) monocytes post vs. pre anti-PD-L1 treatment.

A. Heatmap of selected significantly differentially expressed genes (Wilcoxon rank-sum test Bonferroni corrected $p<0.05$) with increased expression in the CD16- monocyte cluster post-treatment relative to the baseline CD16- monocyte cluster. B. Gene Set Enrichment Analysis (GSEA) pathway analysis of all significantly differentially genes in CD16- monocytes post- versus pre-treatment revealed enrichment of gene sets associated with activated/stimulated monocytes after treatment relative to baseline. Representative enriched pathway: anti-TREM1 stimulated monocytes (corresponding to post-treatment monocytes) versus control unstimulated monocytes (corresponding to pre-treatment monocytes).

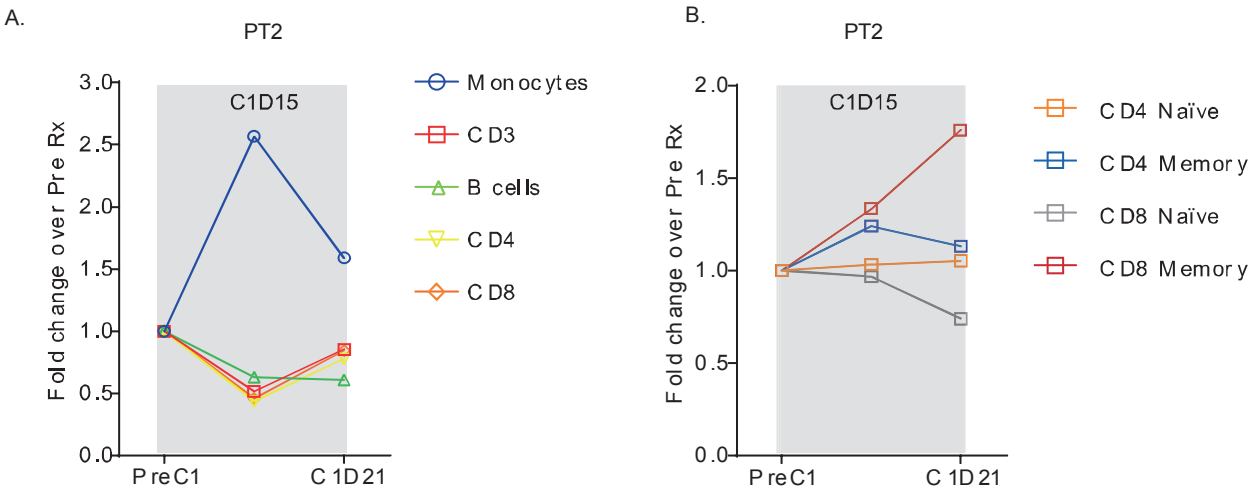


Figure S2: Changes in circulating immune cells of AMM patient following therapy with atezolizumab.

A. Changes in circulating CD3, CD4, CD8 T cells, monocytes and B cells at day 15 (C1D15) and 21 (C1D21) following therapy with atezolizumab. Data shows fold change compared to pre therapy (PreC1). B. Change in circulating CD4 and CD8 naïve and memory T cells at day 15 (C1D15) and 21 (C1D21) following therapy with atezolizumab. Data shows fold change compared to pre therapy (PreC1).

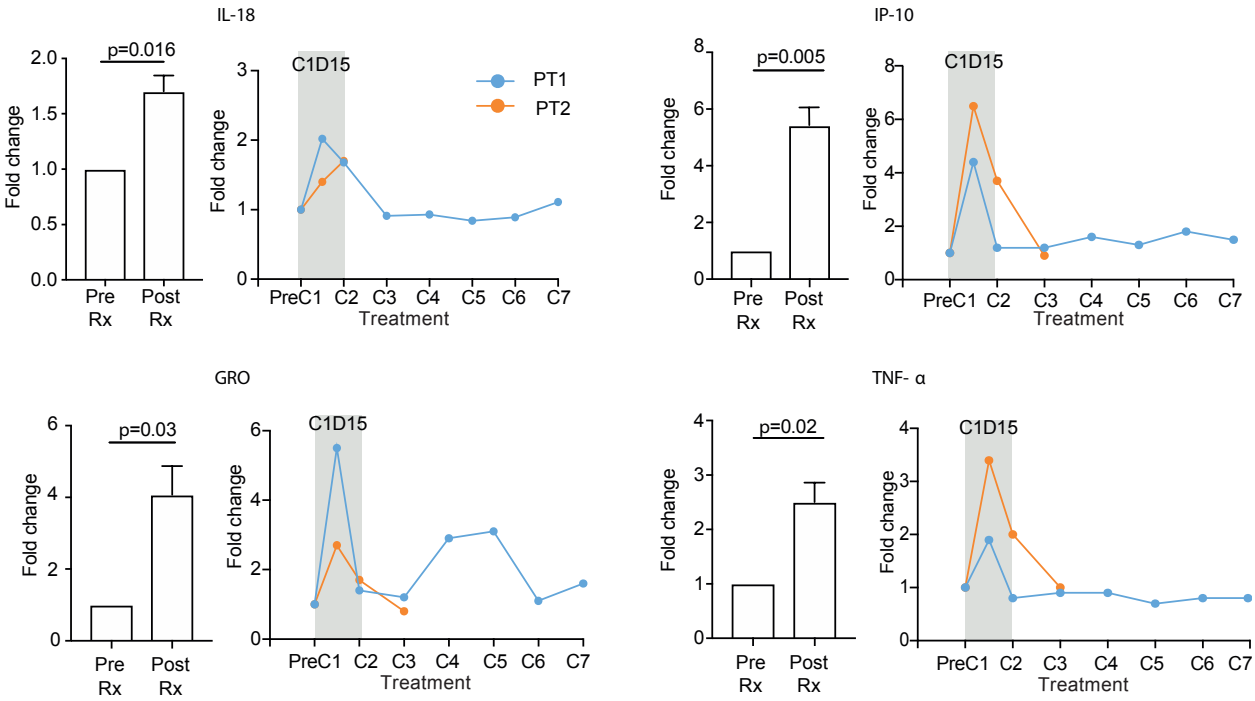


Figure S3: Changes in plasma cytokines following therapy with atezolizumab in AMM patients.

Plasma obtained pre therapy (PreC1), 15 days following first dose of atezolizumab (C1D15) and prior to cycles 2-7 (C2-C7) was analyzed for presence of IL-18, IP-10, GRO and TNF-α. Bar graph shows plasma levels pre (PreRx) and post cycle 1 (PostRx; n=2 patients) as fold change compared to pre therapy. The line graphs show changes in cytokines over time.

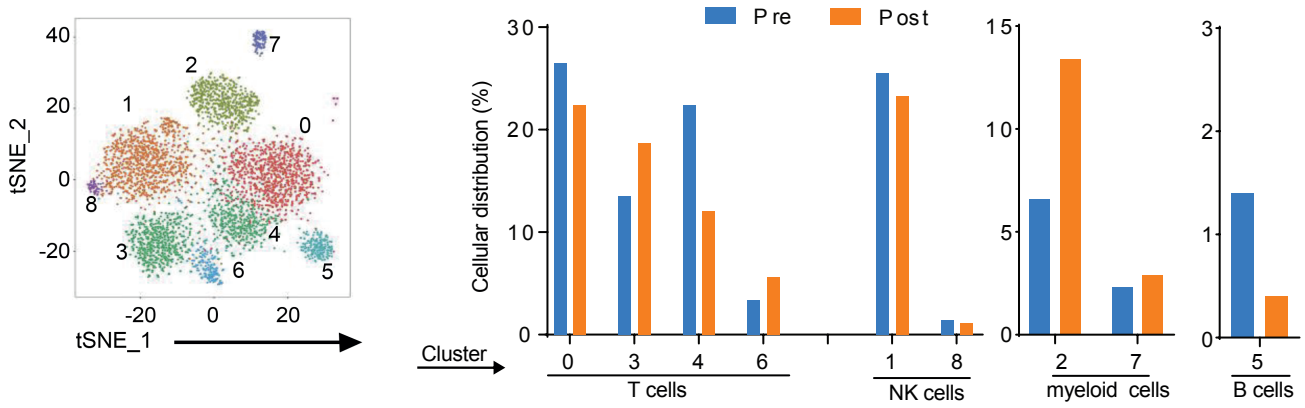


Figure S4: Changes in transcriptome profile in PBMCs following therapy with atezolizumab.

Single cell RNA sequencing analysis was performed on PBMCs obtained prior to therapy and 15 days following start of atezolizumab (Patient 1 as in figure 3E). TSNE plot shows distinct cell clusters as determined by unsupervised cluster analysis. Bar graph shows the percent of cells in each of these distinct populations pre- and post-treatment.

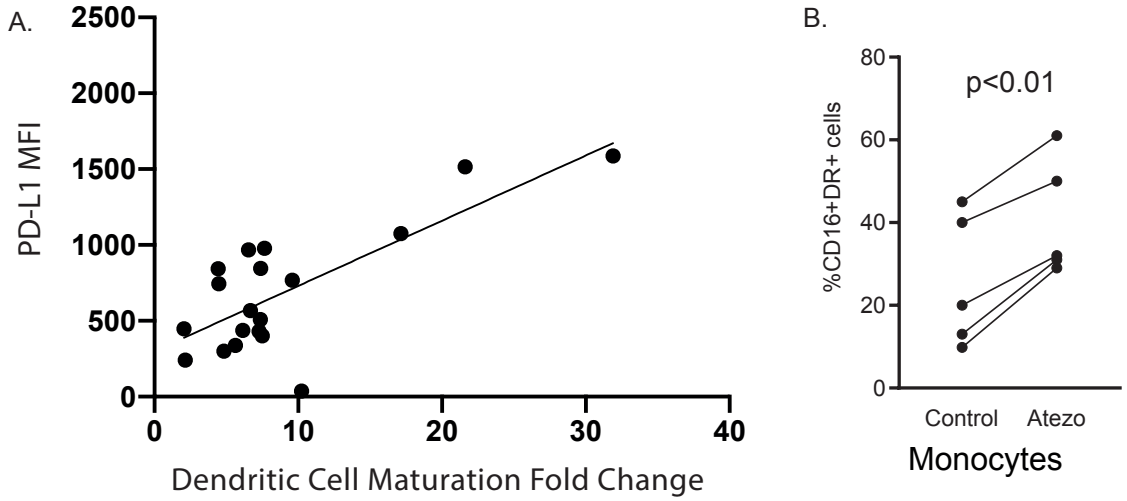
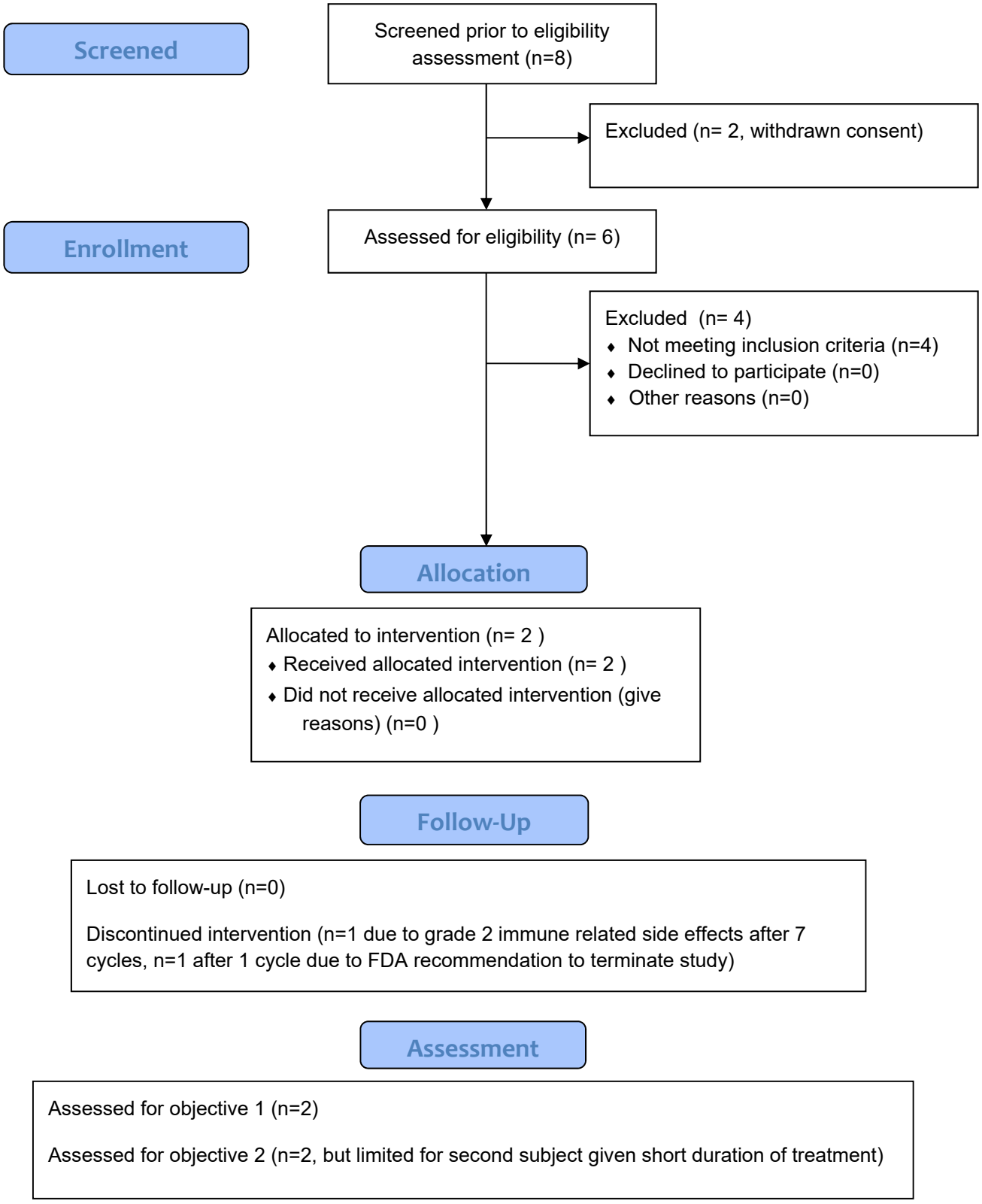


Figure S5: Atezolizumab-induced changes in myeloid cells in culture

A. Correlation of PD-L1 expession and changes in dentritic cell maturation markers (CD83, CD80) following exposure of monocyte-derived dendritic cells to CD40L + atezolizumab compared to untreated control. B. Changes in CD14+ CD16+ HLA-DR+ myeloid cells in bone marrow mononuclear cells cultured with atezolizumab versus control.



Citation: Eldridge SM, Chan CL, Campbell MJ, Bond CM, Hopewell S, Thabane L, et al. CONSORT 2010 statement: extension to randomised pilot and feasibility trials. BMJ. 2016;355.