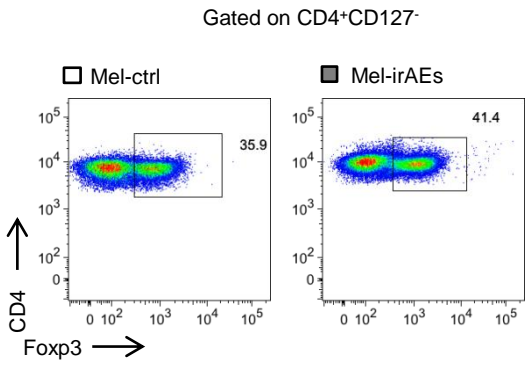
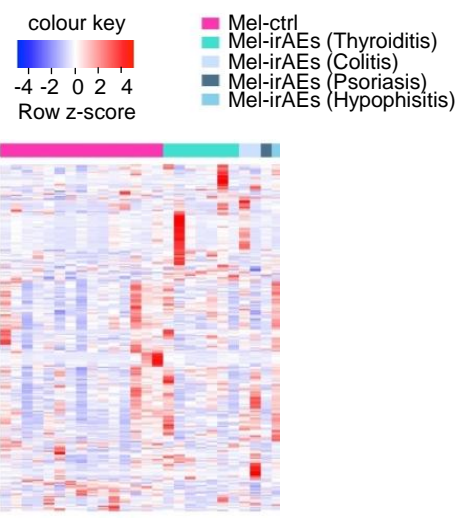


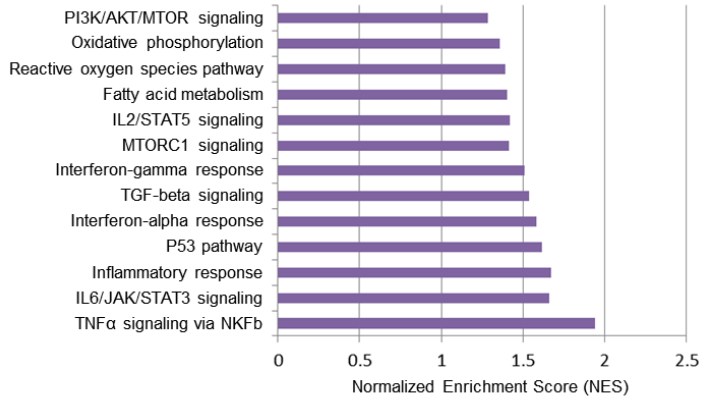
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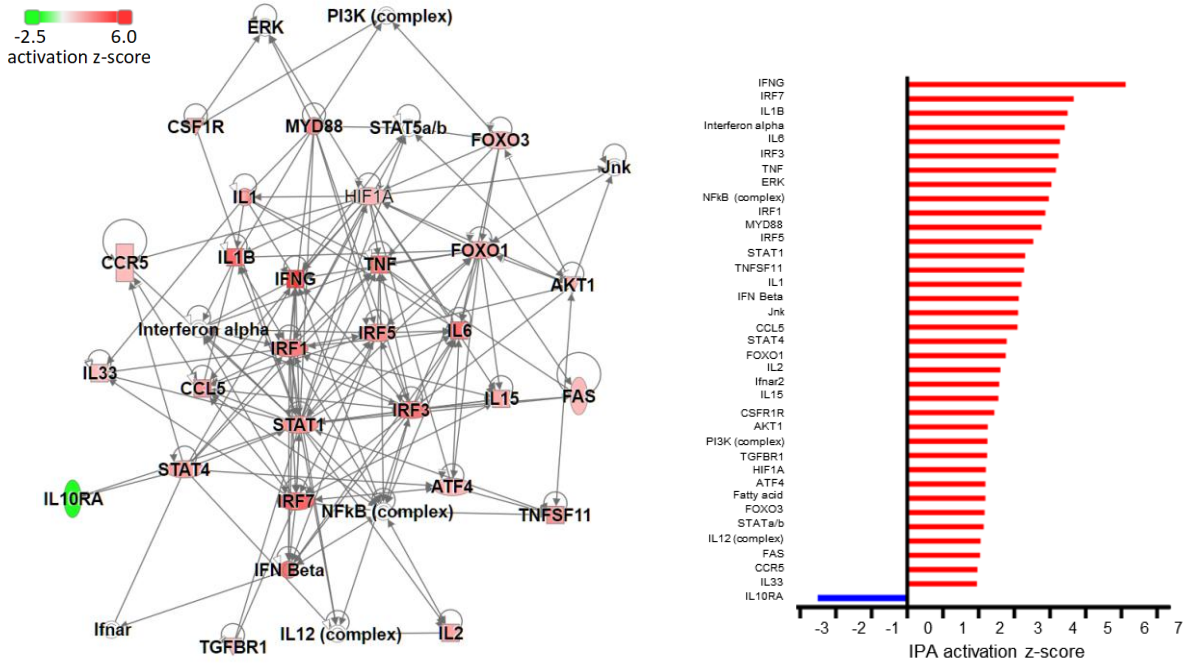
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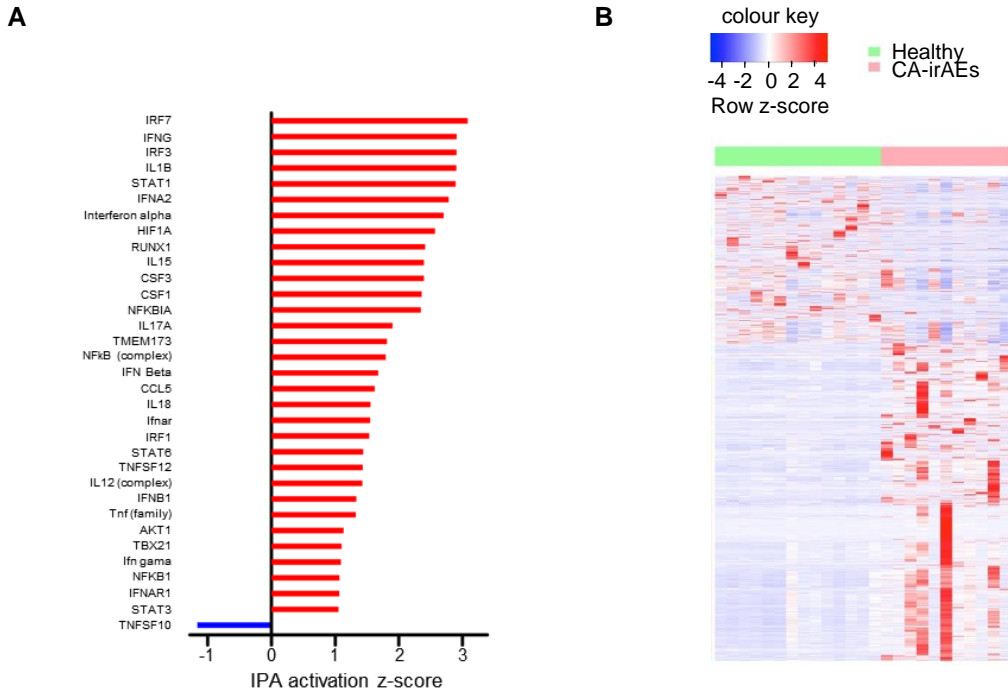
C



D



Supplementary Figure 1. Enriched inflammatory pathways characterize Tregs in advanced melanoma individuals developing irAEs. (A) Frequencies of CD4⁺CD25⁺CD127⁻ Foxp3⁺ Tregs in peripheral blood of individuals with advanced melanoma under anti-PD1: without irAEs (Mel-ctrl, n=11) and with irAEs (Mel-irAEs, n=9). For **B-D**, transcriptomic analysis of CD4⁺CD25⁺CD127⁻ Tregs isolated from blood of advanced melanoma patients under anti-PD1 treatment. (B) Heatmap of DEGs of 'Mel-ctrl (n=15) vs Mel-irAEs (n=11)'. (C) Heatmap of DEGs from melanoma individuals 'ORR-ctrl (n=3) vs ORR-irAEs (n=4)' (upper panel); and GSEA analysis showing the positively enriched gene-sets (FDR<0.25; lower panel); Objective Response Rate (ORR) is defined as the proportion of patients with a complete or a partial response to therapy. (D) Upstream regulator network (left) and barplot (right) from the DEGs of 'Mel-ctrl vs Mel-irAEs' using IPA analysis; red represents activation in Mel-irAEs; green represents inactivation in Mel-irAEs; IPA activation z-score is to infer the activation states of predicted transcriptional regulators.



Supplementary Figure 2. Distinct pro-inflammatory transcriptomic profile of irAEs Tregs among different type of cancers. (A) Upstream regulator analysis from the DEGs of ‘CA-ctrl vs CA-irAEs’ treated anti-PD1 using IPA (IPA activation z-score is to infer the activation states of predicted transcriptional regulators). (B) Heatmap of DEGs in Tregs isolated from peripheral blood of ‘Healthy (n=14) vs CA-irAEs (n=11)’.

Supplementary Table 1

	ID	Disease	Sex	Age	Treatment	irAEs	Adjuvant/Metastatic	Response to ICI
Autoimmune	A1	HT	M	55	Levothyroxine	N/A	N/A	N/A
	A2	HT	F	52	Levothyroxine	N/A	N/A	N/A
	A3	HT	F	63	Levothyroxine	N/A	N/A	N/A
	A4	HT	F	57	None	N/A	N/A	N/A
	A5	RA	F	36	HCQ/AZA/PZ	N/A	N/A	N/A
	A6	RA	F	30	MTx	N/A	N/A	N/A
	A7	UC	M	47	5-ASA STEROIDS	N/A	N/A	N/A
	A8	UC	F	22	Vedolizumab	N/A	N/A	N/A
	A9	CD	F	46	None	N/A	N/A	N/A
	A10	CD	F	66	AZA	N/A	N/A	N/A
	A11	HT	F	49	Levothyroxine	N/A	N/A	N/A
	A12	HT	M	68	Levothyroxine	N/A	N/A	N/A
	A13	HT	M	45	none	N/A	N/A	N/A
	A14	RA	F	60	none	N/A	N/A	N/A
	A15	RA	F	60	HCQ	N/A	N/A	N/A
	A16	RA	F	54	none	N/A	N/A	N/A
	A17	RA	M	68	MTx	N/A	N/A	N/A
	A18	RA	M	78	MTx	N/A	N/A	N/A
	A19	RA	F	77	MTx	N/A	N/A	N/A
RE3	RA	F	63	MTx	N/A	N/A	N/A	
Melanoma irAEs	P14	Melanoma	M	84	aPD1	Thyroiditis	Metastatic	CR
	P24	Melanoma	F	66	aPD1	UC	Metastatic	PD
	P35	Melanoma	M	61	aPD1	Thyroiditis	Metastatic	PD
	P36	Melanoma	F	77	aPD1	Thyroiditis	Metastatic	PD
	P38	Melanoma	F	41	aPD1	thyroiditis	Adjuvant	No recurrence
	P45	Melanoma	F	63	aPD1	Thyroiditis	Metastatic	SD
	P46	Melanoma	M	49	aPD1	Hypophysitis	Metastatic	CR
	P6	Melanoma	M	66	aPD1	UC	Adjuvant	Recurrence
	P62	Melanoma	F	75	aPD1	Psoriasis	Metastatic	CR
	P9	Melanoma	F	53	aPD1	Thyroiditis	Metastatic	CR
	P55	Melanoma	F	71	aPD1	Thyroiditis	Metastatic	PD

Melanoma Control	P4	Melanoma	F	80	aPD1	N/A	Metastatic	CR
	P11	Melanoma	M	69	aPD1	N/A	Metastatic	PD
	P17	Melanoma	F	57	aPD1	N/A	Adjuvant	Recurrence
	P18	Melanoma	F	75	aPD1	N/A	Metastatic	CR
	P41	Melanoma	M	41	aPD1	N/A	Adjuvant	No recurrence
	P42	Melanoma	M	43	aPD1	N/A	Adjuvant	No recurrence
	P47	Melanoma	M	70	aPD1	N/A	Adjuvant	No recurrence
	P49	Melanoma	M	82	aPD1	N/A	Adjuvant	No recurrence
	P52	Melanoma	F	76	aPD1	N/A	Metastatic	PD
	P53	Melanoma	M	49	aPD1	N/A	Metastatic	PD
	P54	Melanoma	M	35	aPD1	N/A	Adjuvant	No recurrence
	P56	Melanoma	F	52	aPD1	N/A	Metastatic	PD
	P57	Melanoma	M	66	aPD1	N/A	Adjuvant	No recurrence
P60	Melanoma	M	62	aPD1	N/A	Adjuvant	Recurrence	
P61	Melanoma	M	84	aPD1	N/A	Metastatic	PR	
Cancer irAEs	P20	CA Kidney	M	36	aPD1	Arthritis	N/A	N/A
	P21	CA Bladder	M	55	aPD1	Thyroiditis	N/A	N/A
	P33	CA Kidney	M	64	aPD1	Pneumonitis	N/A	N/A
	P3	NSCL CA	F	65	aPD1/PZ	Pneumonitis	N/A	N/A
	P39	CA Kidney	M	62	aPD1	Gastritis	N/A	N/A
	PT1	NSCL CA	M	70	aPD1	Pneumonitis	N/A	N/A
	PT4	NSCL CA	M	76	aPD1	Arthritis/Thyroiditis	N/A	N/A
	PT5	NSCL CA	F	64	aPD1	Pneumonitis	N/A	N/A
	PT6	NSCL CA	M	79	aPD1	Thyroiditis/Arthritis	N/A	N/A
	PT7	NSCL CA	M	65	aPD1	Thyroiditis	N/A	N/A
P8	CA Liver	M	67	aPD1	Thyroiditis	N/A	N/A	
Cancer Control	P23	CA Kidney	M	71	aPD1	N/A	N/A	N/A
	P29	CA Kidney	F	78	aPD1	N/A	N/A	N/A
	P30	CA Kidney	F	64	aPD1	N/A	N/A	N/A
	P27	CA Bladder	M	56	aPD1	N/A	N/A	N/A
	P40	CA Bladder	M	54	aPD1	N/A	N/A	N/A

HT: Hashimoto Thyroiditis, RA: Rheumatoid Arthritis, UC: Ulcerative Colitis, CD: Crohn's Disease,
NSCL: Non Small Cell Lung, CA: Cancer, F: Female, M: Male
HCQ: hydroxychloroquine, PZ: Prednisone, AZA: Azathioprine, MTx: Methotrexate
SD: stable disease, PR: partial remission, CR: complete remission, PD: progressive disease

Supplementary Table 1. Demographic and clinical characteristics of human subjects.

Characteristics of human subjects recruited for the study. Individuals with Autoimmunity (n = 20),
Melanoma irAEs (n = 11), Melanoma Control (n = 15), Cancer irAEs (n = 11) and Cancer control (n=5).