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Supplemental information

Longitudinal analysis of the gut microbiota during anti-PD-1 therapy reveals stable microbial features of response in melanoma patients

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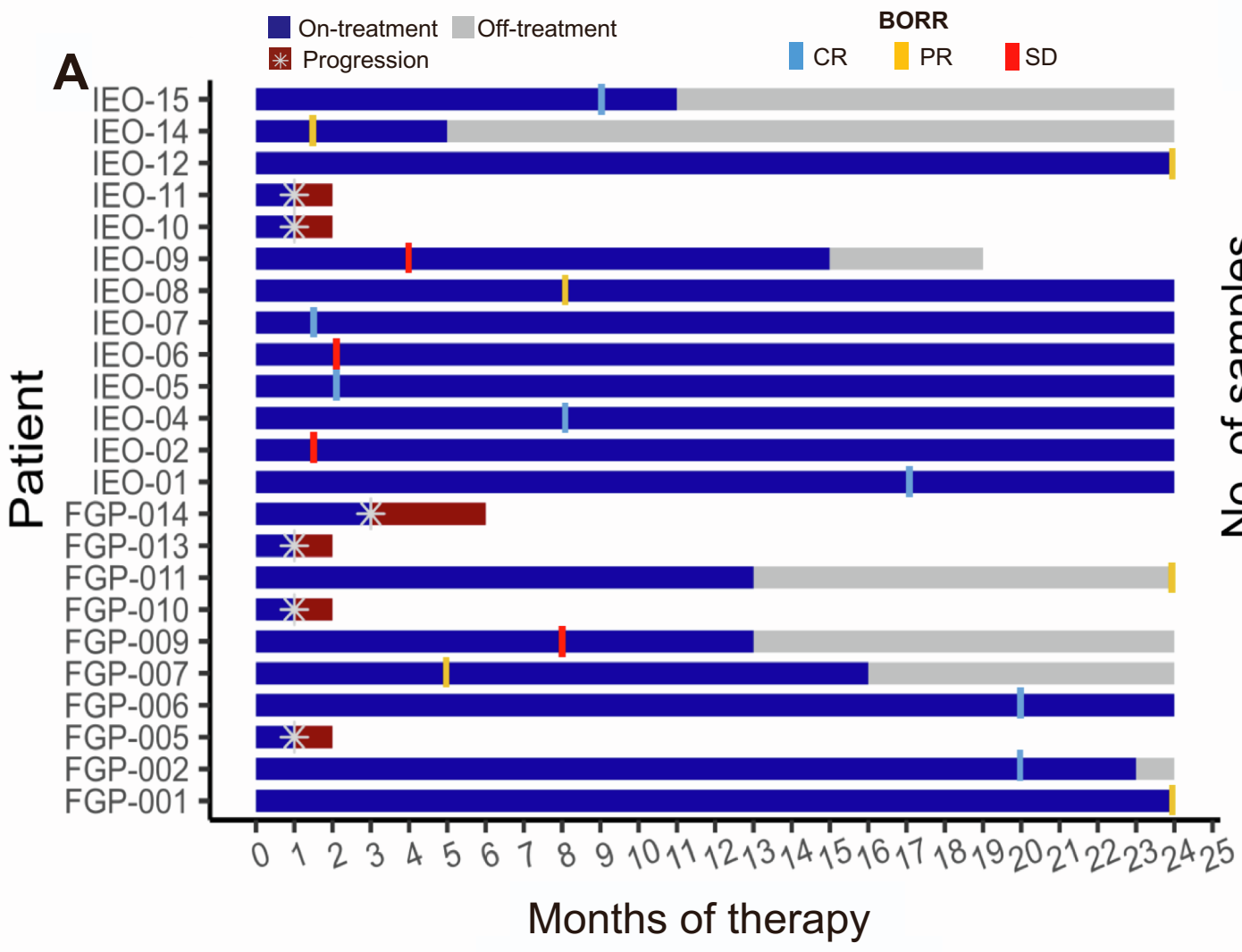
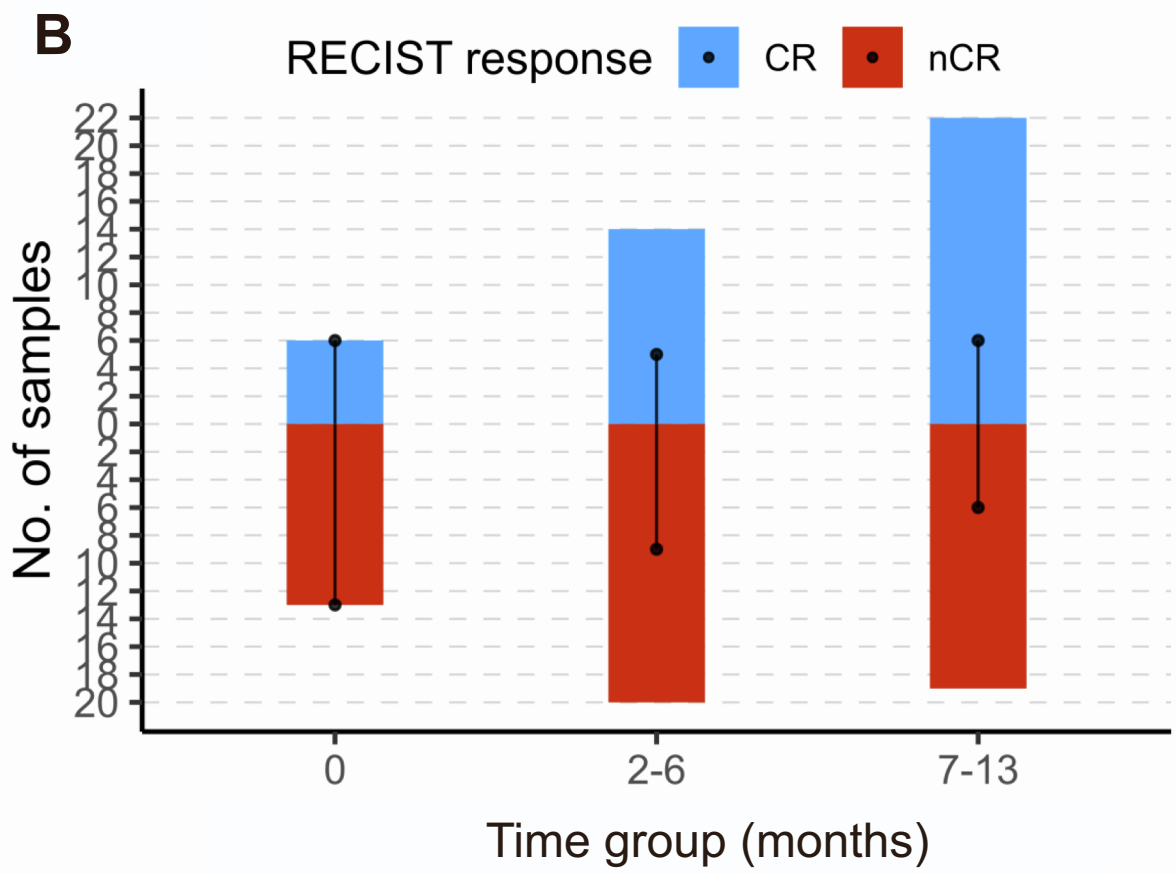
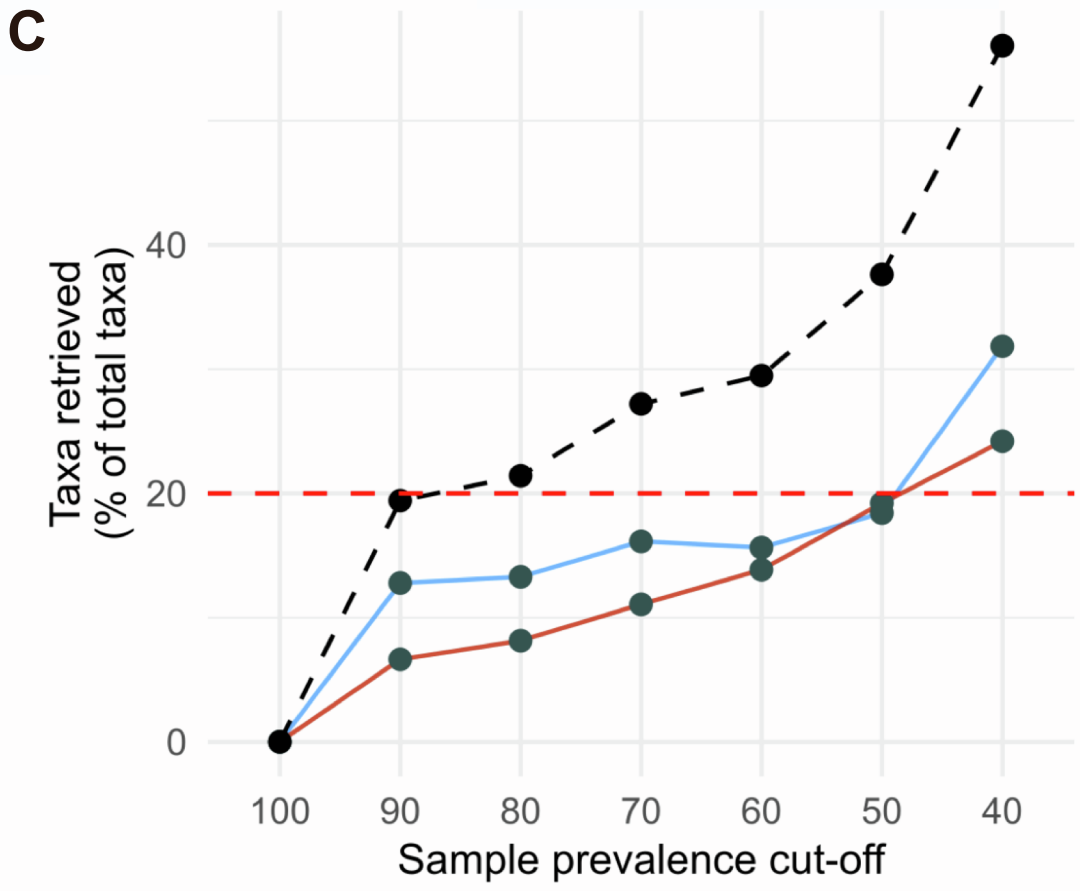
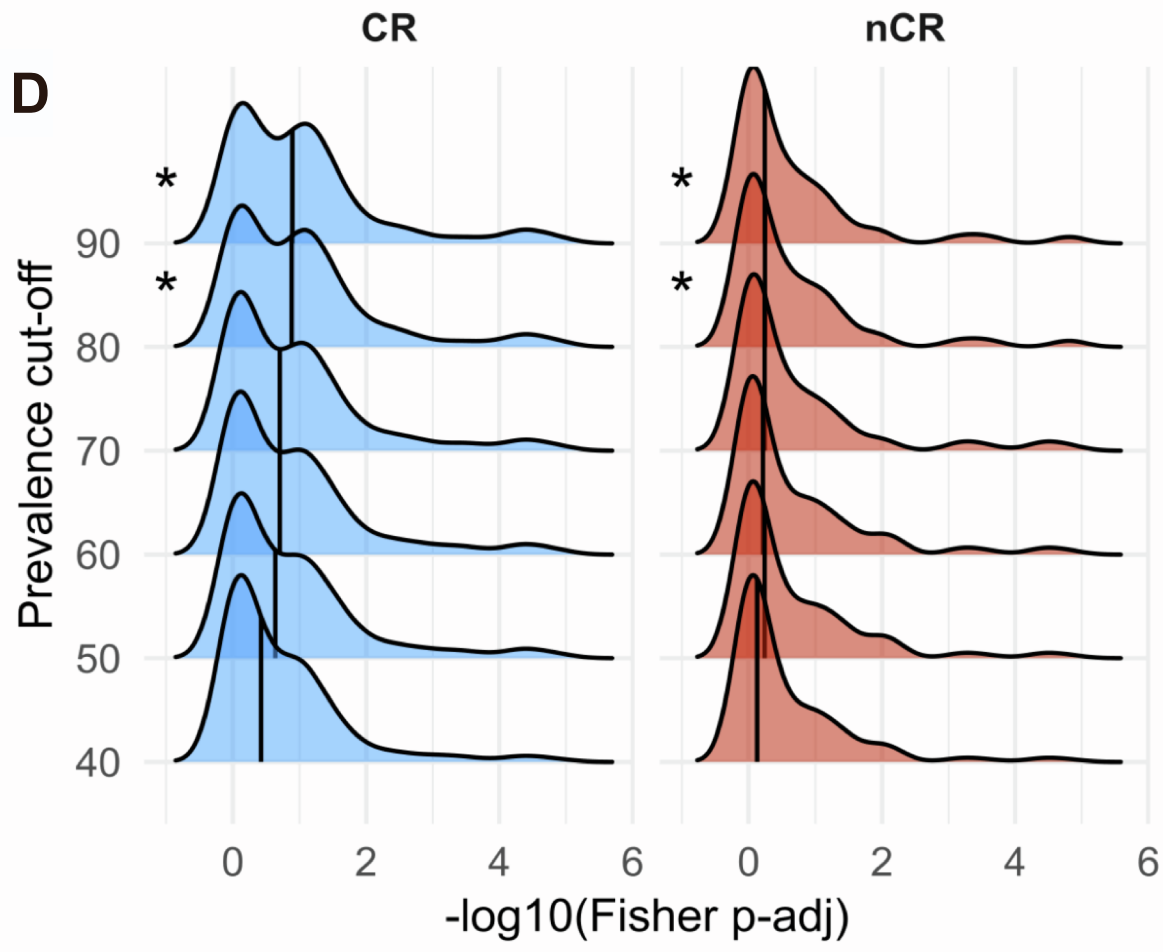
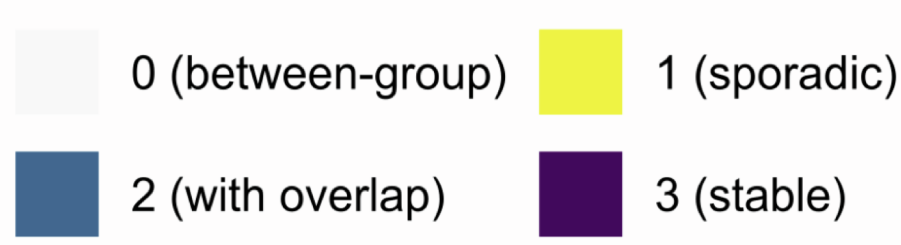
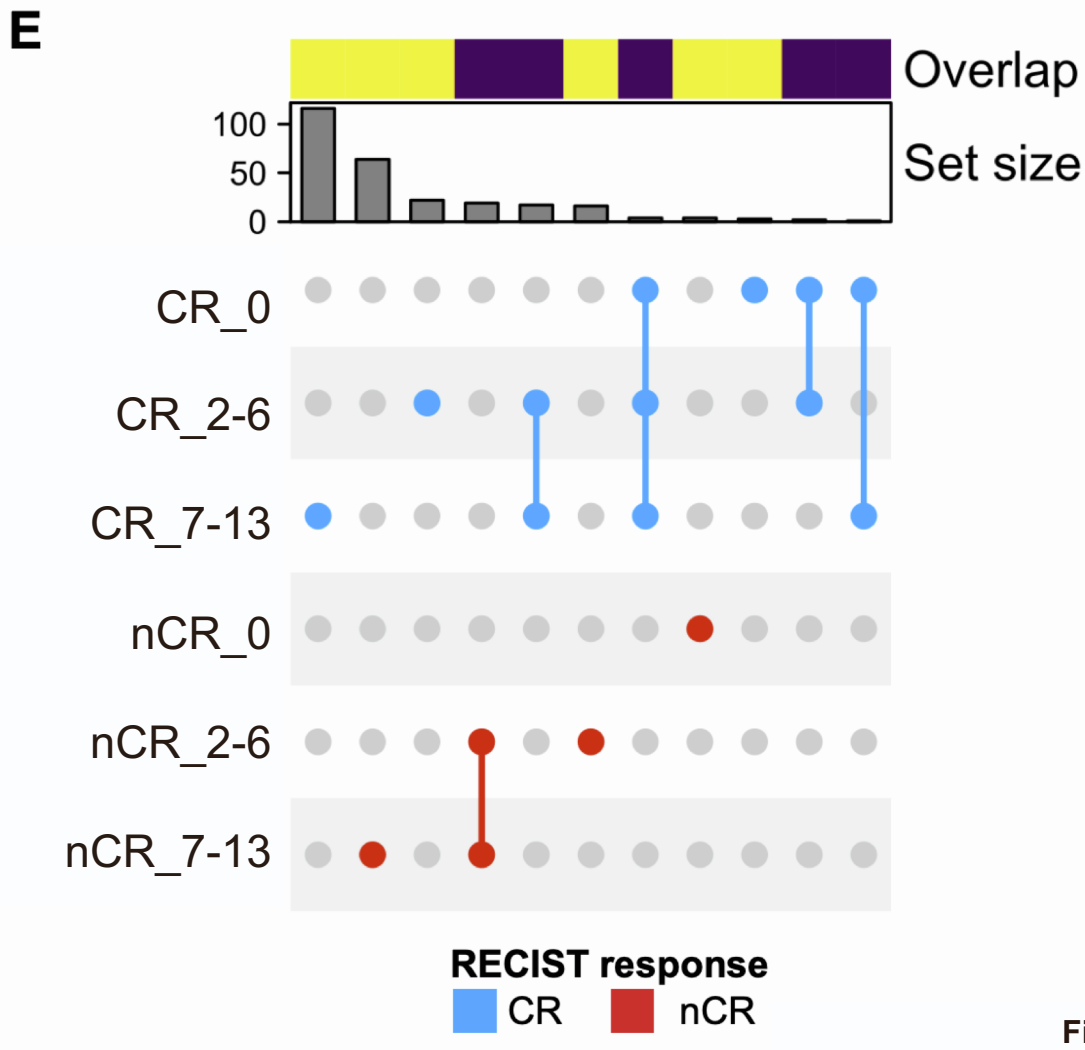

A
On-treatment
Off-treatment
Progression
BORR
CR
PR
SD
Patient
IEO-15
IEO-14
IEO-12
IEO-11
IEO-10
IEO-09
IEO-08
IEO-07
IEO-06
IEO-05
IEO-04
IEO-02
IEO-01
FGP-014
FGP-013
FGP-011
FGP-010
FGP-009
FGP-007
FGP-006
FGP-005
FGP-002
FGP-001
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25
Months of therapy


B
RECIST response
CR
nCR
No. of samples
22 20 18 16 14 12 10 8 6 4 2 0 2 4 6 8 10 12 14 16 18 20
0
2-6
7-13
Time group (months)


C
Taxa retrieved
(% of total taxa)
40
20
0
100
90
80
70
60
50
40
Sample prevalence cut-off


D
CR
nCR
Prevalence cut-off
90
80
70
60
50
40
0 2 4 6 0 2 4 6
-log10(Fisher p-adj)


0 (between-group)
1 (sporadic)
2 (with overlap)
3 (stable)


E
Overlap
Set size
100
50
0
CR_0
CR_2-6
CR_7-13
nCR_0
nCR_2-6
nCR_7-13
RECIST response
CR
nCR

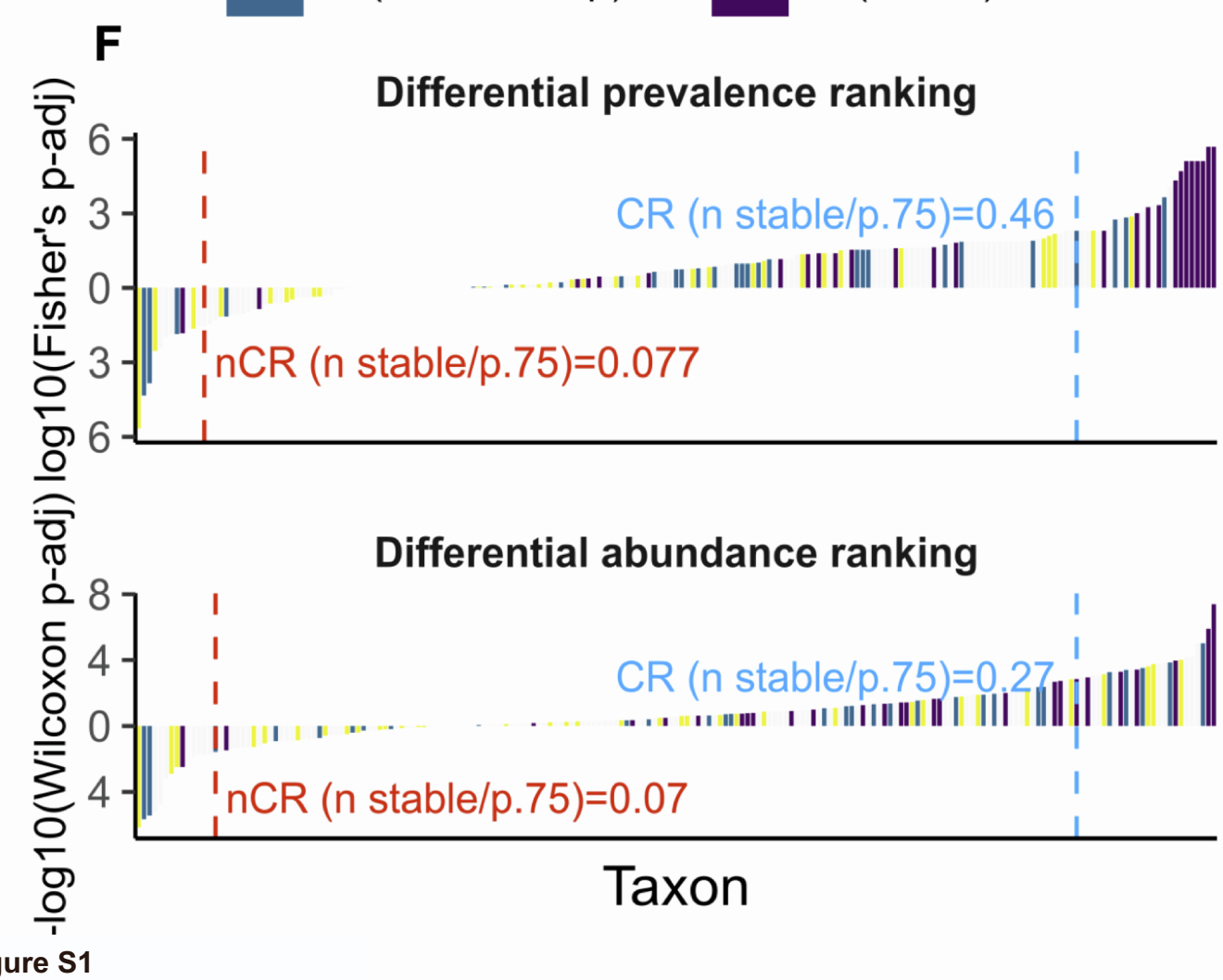

F
Differential prevalence ranking
-log10(Fisher's p-adj)
6
3
0
3
6
CR (n stable/p.75)=0.46
nCR (n stable/p.75)=0.077
Differential abundance ranking
-log10(Wilcoxon p-adj)
8
4
0
4
CR (n stable/p.75)=0.27
nCR (n stable/p.75)=0.07
Taxon

Figure S1

Figure S1. Defining complete response (CR)-associated taxa by within-group prevalence and longitudinal stability, related to Figure 1.
(A) Clinical status of patients during therapy. RECIST 1.1 Best Overall Response (BOR) at two-years is indicated. **(B)** Shotgun sequencing frequency distribution of melanoma CR and nCR samples across time in aggregated months (0, 2–6, and 7–13 months of therapy). Inset line segments (black) indicate number of samples from non-redundant patients. **(C)** Number of taxa retrieved (% of total) at each prevalence cut-off within CR (blue), within nCR (red), and total (dashed), across timepoint groups. Dashed horizontal red line indicates cut-off of 20% taxa retrieved. **(D)** Distribution of Fisher's exact test p-values (-log10(p-adj)) for CR/nCR association of taxa retrieved at increasing within-group sample prevalence cut-off, from 40% (not prevalent) to 80% (highly prevalent). Density plots are grouped into CR- (left) and nCR- (right) associated based on odds ratio. **(E)** Differentially abundant taxa overlap at 0, 2–6, and 7–13 months in CR and nCR. Connecting dots indicate taxa shared between timepoint groups ("with overlap"), whereas non-connected dots indicate taxa detected only in one timepoint group ("spurious"). "Stable" taxa are defined as within-CR and within-nCR prevalent taxa that are present across at least any two timepoints from 0, 2–6, and 7–13 months. **(F)** Ranking of prevalent taxa by differential prevalence (top) and differential abundance (bottom). Colors indicate degree of group-specific overlap, from not group-specific (white), sporadic (yellow), overlap between two timepoints (teal), and overlap across all three timepoints (violet). Annotations indicate the proportion of taxa that are stable (i.e., prevalent within a group across timepoints) among those in the 75th percentile of differential ranking.

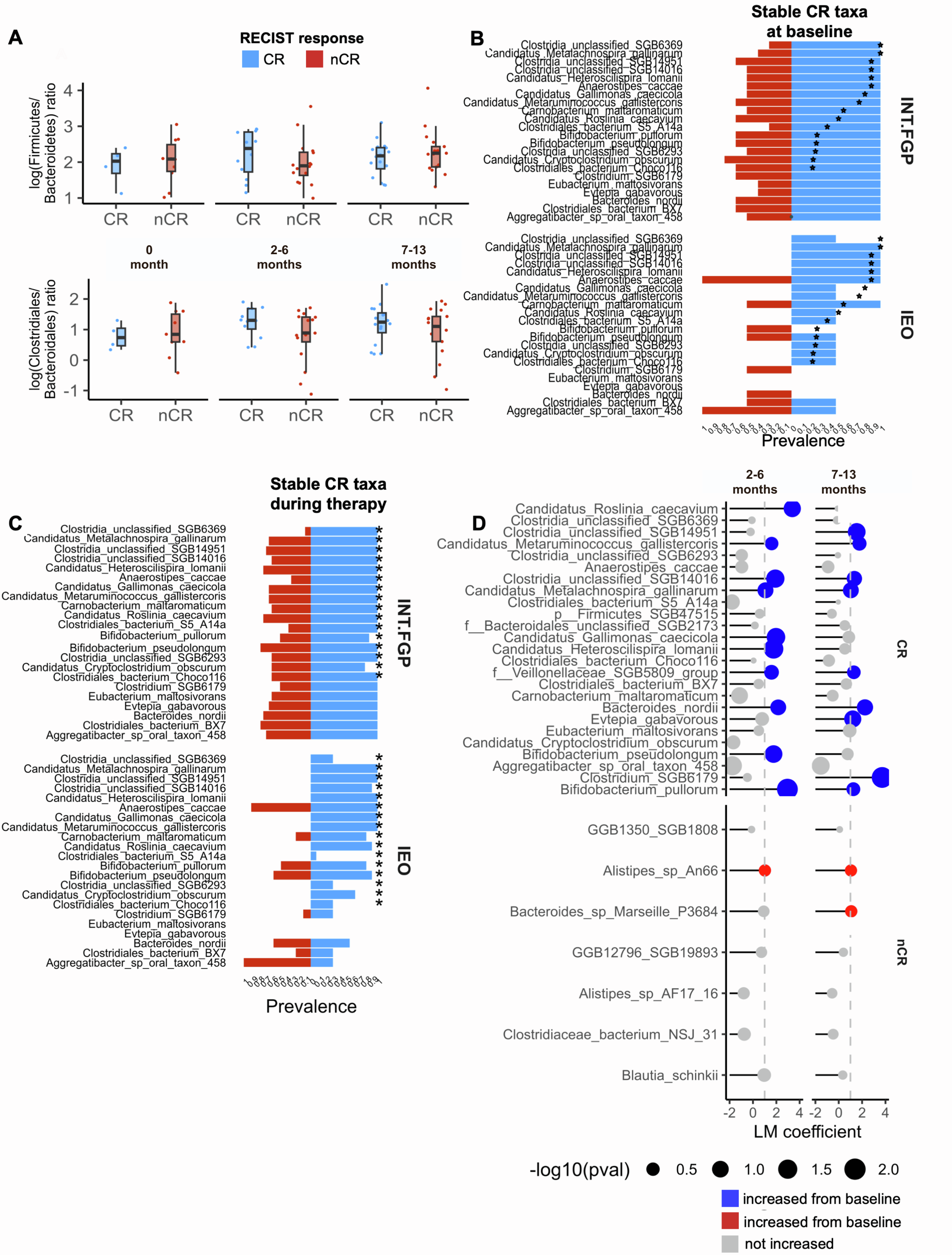

A
RECIST response
CR
nCR
log(Firmicutes/ Bacteroidetes) ratio
log(Clostridiales/ Bacteroidales) ratio
0 month
2-6 months
7-13 months
B
Stable CR taxa at baseline
INT.FGP
IEO
Prevalence
C
Stable CR taxa during therapy
D
2-6 months
7-13 months
Clostridia_unclassified_SGB6369
Candidatus_Metalachnospira_gallinarum
Clostridia_unclassified_SGB14951
Clostridia_unclassified_SGB14016
Candidatus_Heteroscilispira_lomanii
Anaerostipes_caccae
Candidatus_Gallimonas_caecicola
Candidatus_Metaruminococcus_gallistercoris
Carnobacterium_maltaromaticum
Candidatus_Roslinia_caecavium
Clostridiales_bacterium_S5_A14a
Bifidobacterium_pullorum
Bifidobacterium_pseudolongum
Clostridia_unclassified_SGB6293
Candidatus_Cryptoclostridium_obscurum
Clostridiales_bacterium_Choco116
Clostridium_SGB6179
Eubacterium_maltosivorans
Evtepia_gabavorous
Bacteroides_nordii
Clostridiales_bacterium_BX7
Aggregatibacter_sp_oral_taxon_458
p__Firmicutes_SGB47515
f__Bacteroidales_unclassified_SGB2173
f__Veillonellaceae_SGB5809_group
GGB1350_SGB1808
Alistipes_sp_An66
Bacteroides_sp_Marseille_P3684
GGB12796_SGB19893
Alistipes_sp_AF17_16
Clostridiaceae_bacterium_NSJ_31
Blautia_schinkii
CR
nCR
LM coefficient
-log10(pval)
0.5
1.0
1.5
2.0
increased from baseline
increased from baseline
not increased

Figure S2

Figure S2. Assessment of stable taxa in complete response (CR) and non-CR groups by differential prevalence and abundance, related to Figure 1.

(A) Comparison of phylum-level Firmicutes/Bacteroidetes ratio (top), and order-level Clostridiales/Bacteroidales ratio (bottom) between complete responders (CR) and not-complete responders (nCR) at 0, 2–6, and 7–13 months. Ratios are log-transformed for visualization purposes. **(B)** Prevalence barplot of stable CR taxa in CR and nCR groups at baseline, and **(C)** during therapy. Inset points indicate significance in differential prevalence ranking by Fisher's exact test (-log10(p-adj) > 1.3 (*); ns (.)), scaled by the maximum significance. **(D)** Differential abundance of CR (blue) and ncR (red) stable taxa on log-transformed relative abundance, based on Maaslin2 linear model (LM) coefficient, using 'Timepoint group' as fixed effect and 'Patient_number' and 'Study' as random effects. Gray dashed line indicates arbitrary cut-off for increase at LM coefficient >1.0. Colored circles indicate taxa increased from baseline. Size of circle indicates level of significance for increase (–log10(p-value)). Legend: p-adj<0.001 (***), p-adj<0.01 (**), p-adj<0.05 (*), ns (unannotated).

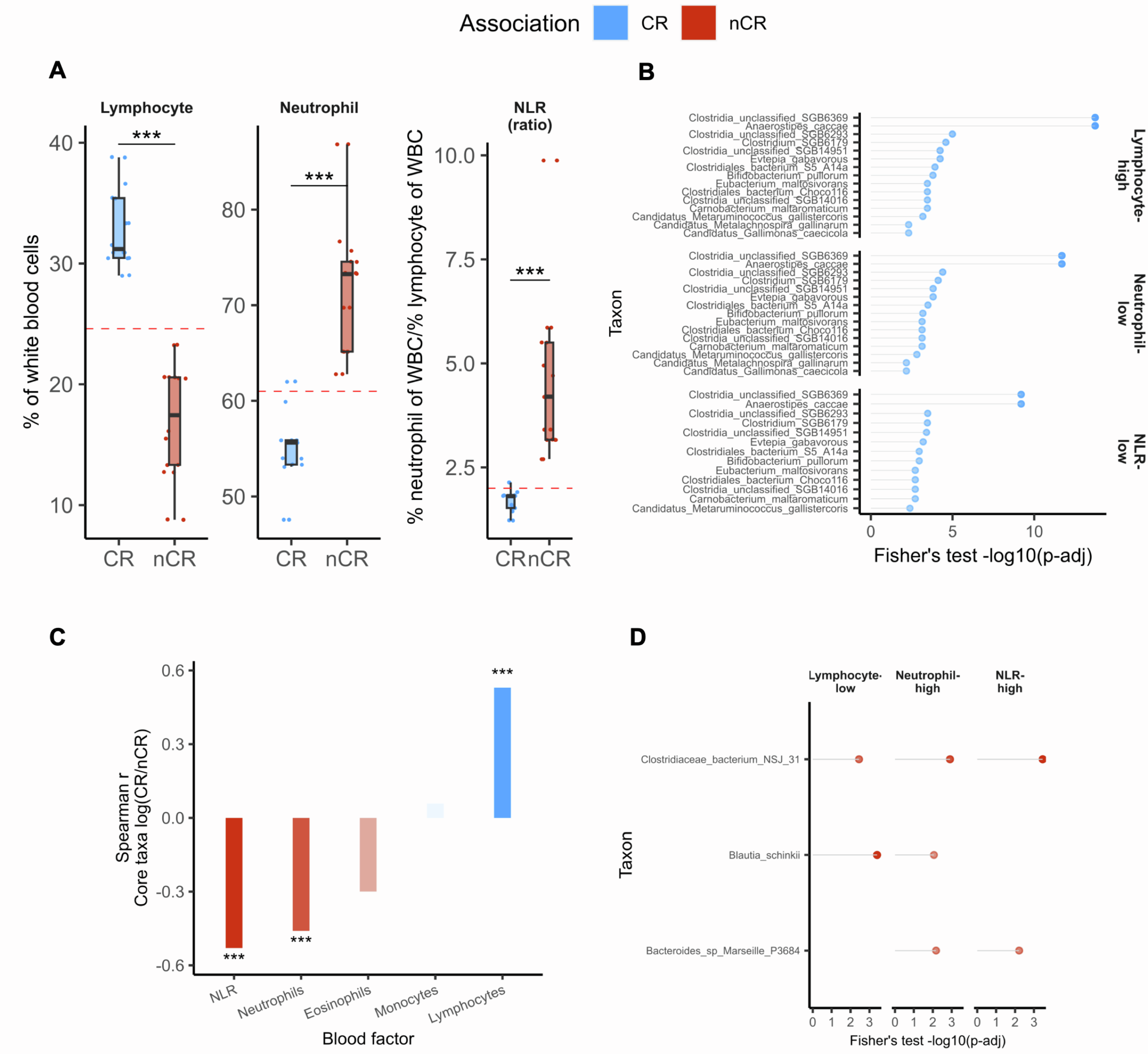
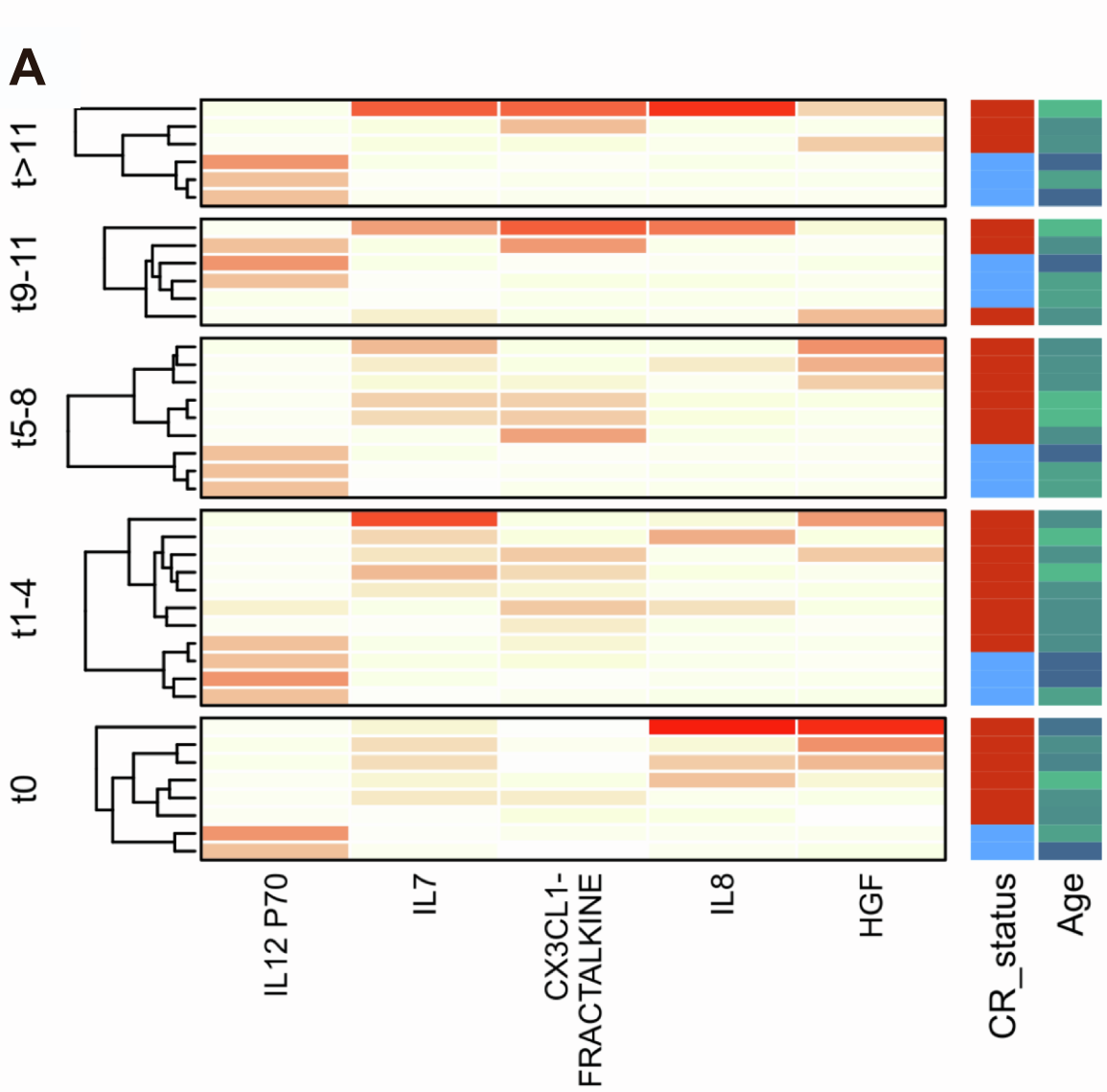
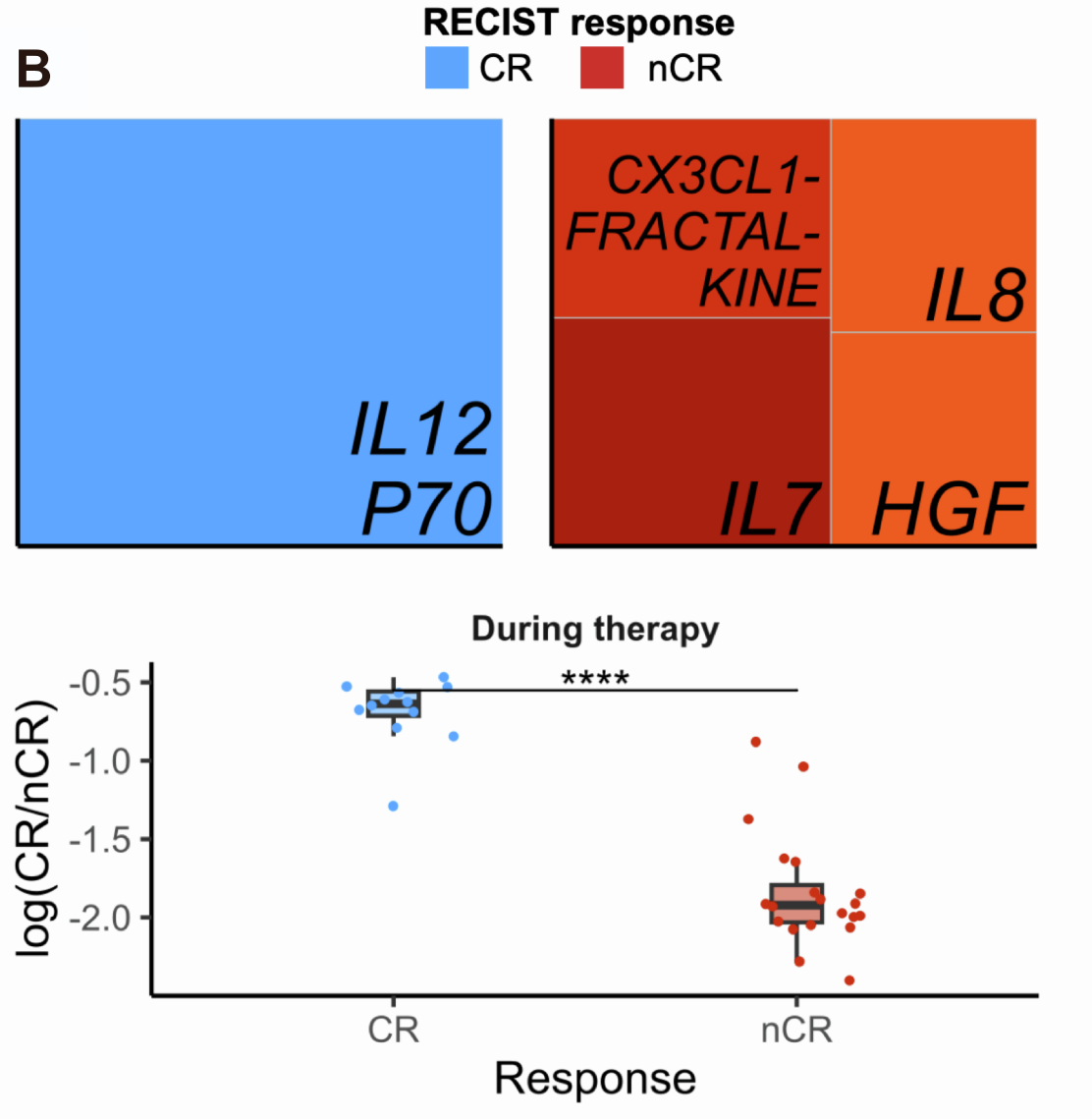
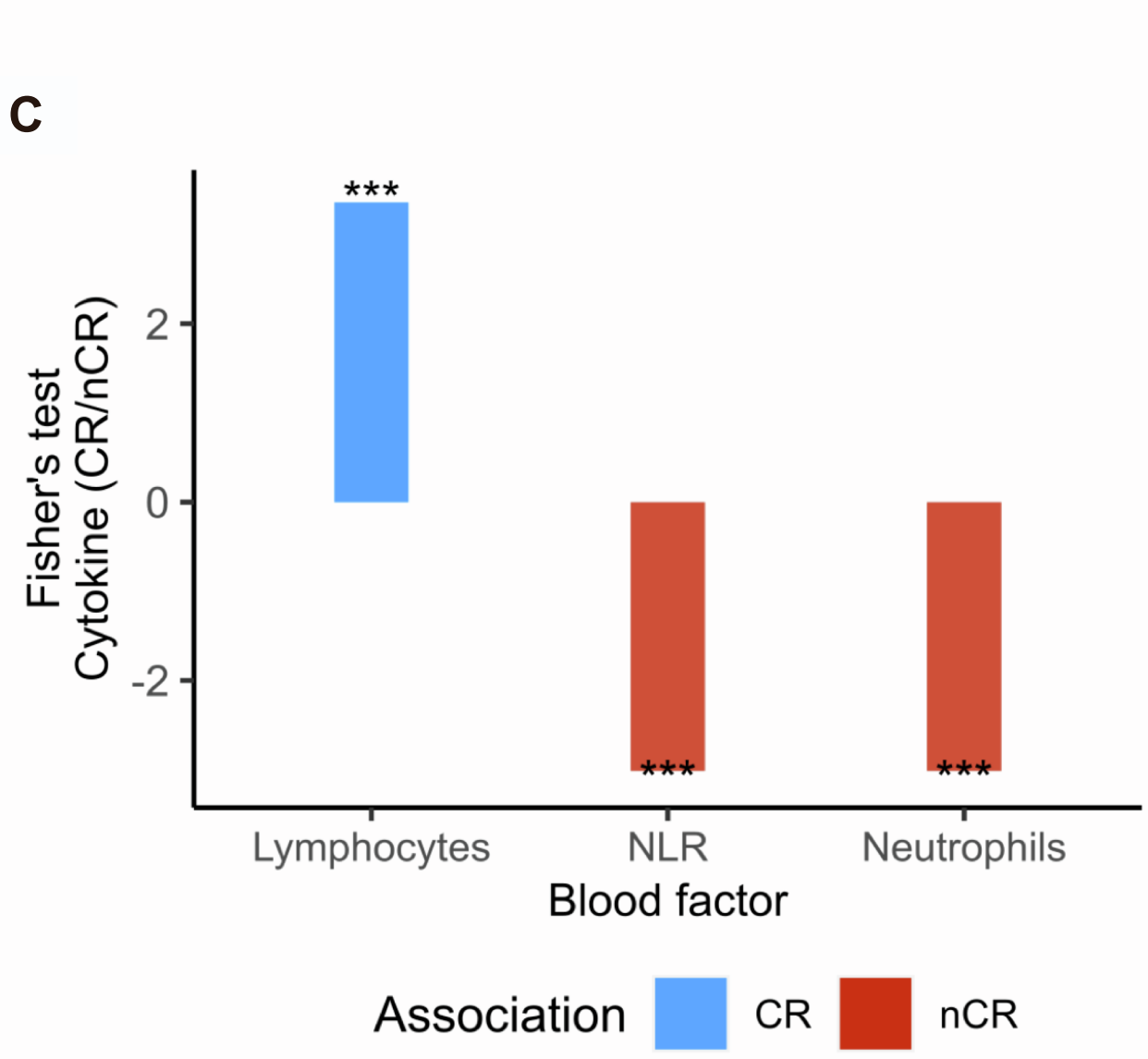
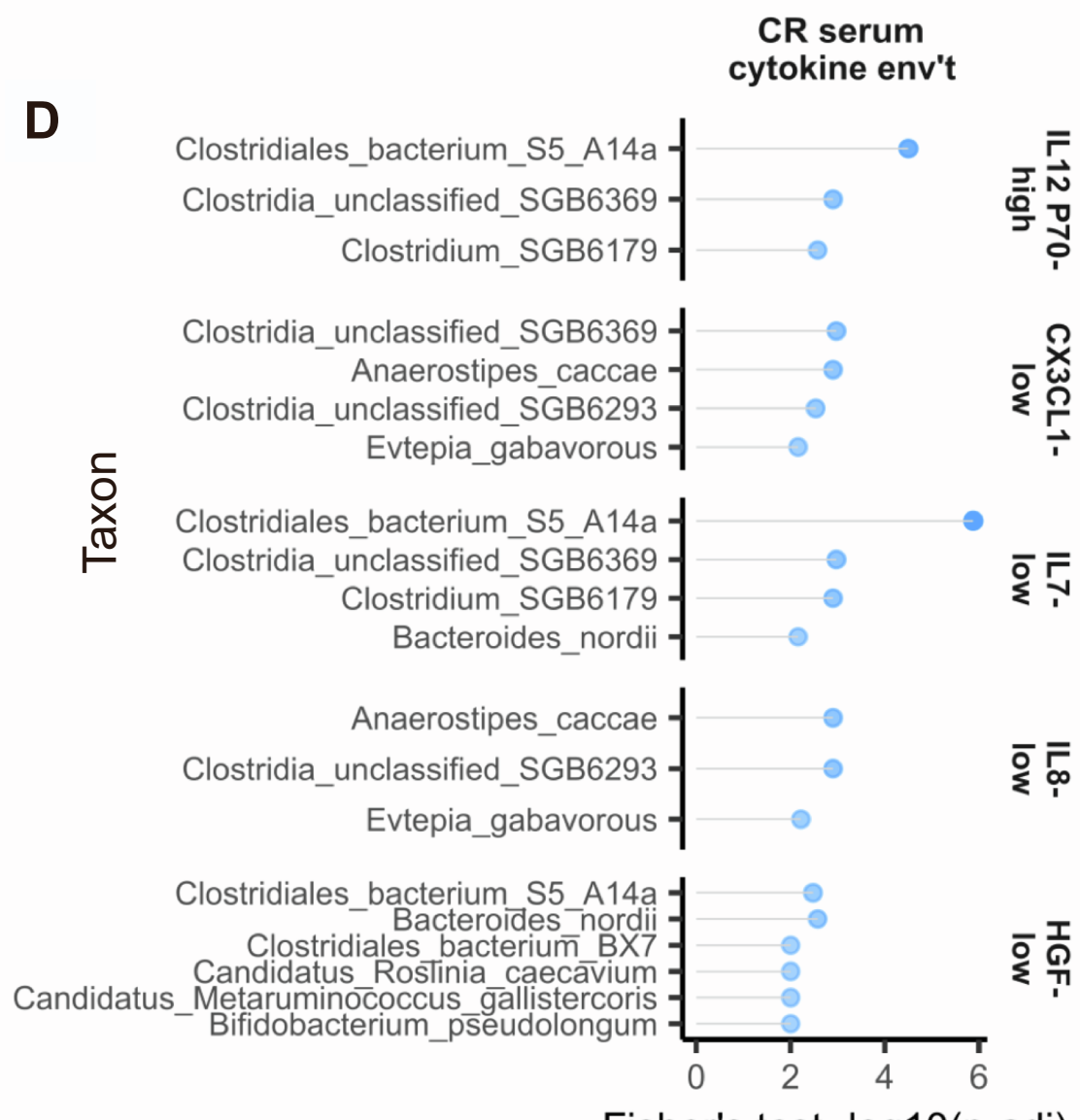


Figure S3. Defining complete response (CR) by white blood cell profile, related to Figure 1.
(A) Comparison of peripheral blood factors (percent of total) between CR and nCR samples at therapy timepoints. Dashed red lines indicate cuts-off for “high” or “low” based on distribution (low-lymphocyte <0.246; low-neutrophil < 0.61; low-NLR cut-off <2). (B) Fisher’s test of association between stable CR taxa presence and “lymphocyte-high”, “neutrophil-low”, and “NLR-low” cases among therapy samples. Taxa are filtered to include only significantly associated taxa at p-adj<0.01. **(C)** Spearman correlation of stable taxa measure (log(CR/nCR)) with lymphocytes (r=0.65), neutrophils (r=-0.53) and NLR (r=-0.61). **(D)** Fisher’s test of association between stable nCR gut microbiota taxa (red) and blood environments, “lymphocyte-low, “neutrophil-high”, and “NLR-high” during therapy. Taxa are filtered to include only significantly associated taxa at p-adj<0.01; Color indicates taxa belonging to stable nCR denominator (red).


A
t>11
t9-11
t5-8
t1-4
t0
IL12 P70
IL7
CX3CL1-FRACTALKINE
IL8
HGF
CR_status
Age


B
RECIST response
CR
nCR
IL12 P70
CX3CL1-FRACTAL-KINE
IL8
IL7
HGF
During therapy
****
log(CR/nCR)
-0.5
-1.0
-1.5
-2.0
CR
nCR
Response


C
***
Fisher's test
Cytokine (CR/nCR)
2
0
-2
***
***
Lymphocytes
NLR
Neutrophils
Blood factor
Association
CR
nCR


D
CR serum cytokine env't
Clostridiales_bacterium_S5_A14a
Clostridia_unclassified_SGB6369
Clostridium_SGB6179
IL12 P70-high
Clostridia_unclassified_SGB6369
Anaerostipes_caccae
Clostridia_unclassified_SGB6293
Evtepia_gabavorous
CX3CL1-low
Clostridiales_bacterium_S5_A14a
Clostridia_unclassified_SGB6369
Clostridium_SGB6179
Bacteroides_nordii
IL7-low
Anaerostipes_caccae
Clostridia_unclassified_SGB6293
Evtepia_gabavorous
IL8-low
Clostridiales_bacterium_S5_A14a
Bacteroides_nordii
Clostridiales_bacterium_BX7
Candidatus_Roslinia_caecavium
Candidatus_Metaruminococcus_gallistercoris
Bifidobacterium_pseudolongum
HGF-low
Taxon
0
2
4
6
Fisher's test -log10(p-adj)

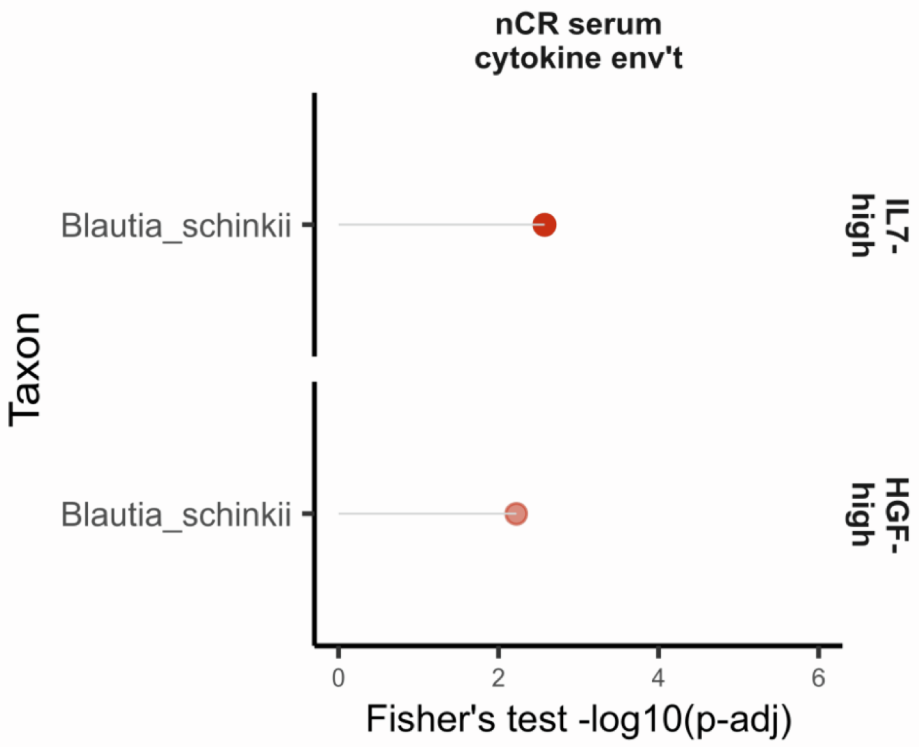

E
nCR serum cytokine env't
Blautia_schinkii
IL7-high
Blautia_schinkii
HGF-high
Taxon
0
2
4
6
Fisher's test -log10(p-adj)

Figure S4

Figure S4. Defining complete response (CR) by serum soluble factor, related to Figure 1.
(A) Heatmap of soluble factor quantities in serum (SD of mean pg/mL) of top CR and nCR-associated cytokines, at 0, 1–4, 5–8, 9–11, and >11 months. **(B)** CR- and nCR-associated cytokines scaled by their significance (-log10(Wilcoxon p-adj)) (top), and their corresponding additive log-ratio (ALR) in CR and nCR groups (bottom). **(C)** Fisher's test of association between CR/ nCR cytokine log-ratio and blood environments, "lymphocyte-high", "neutrophil-low", and "NLR". **(D)** Fisher's test of association between stable CR taxa presence and "IL-12P70-high", "CX3CL1/FRACTALKINE-low", "IL7-low", "IL8-low", and "HGF-low" cases among therapy samples. Features are filtered to include only significantly associated taxa at p-adj<0.01. **(E)** Fisher's test of association between stable nCR gut microbiota taxa (red) and serum cytokine status, "IL7-high" and "HGF-high" in therapy timepoints. Taxa are filtered to include only significantly associated taxa at p-adj<0.01. Continuous values from cytokine data were split into binary states by the median value (high: >median; low: ≤ median).
Legend: p-adj<0.001 (***), p-adj<0.01 (**), p-adj<0.05 (*), ns (unannotated).

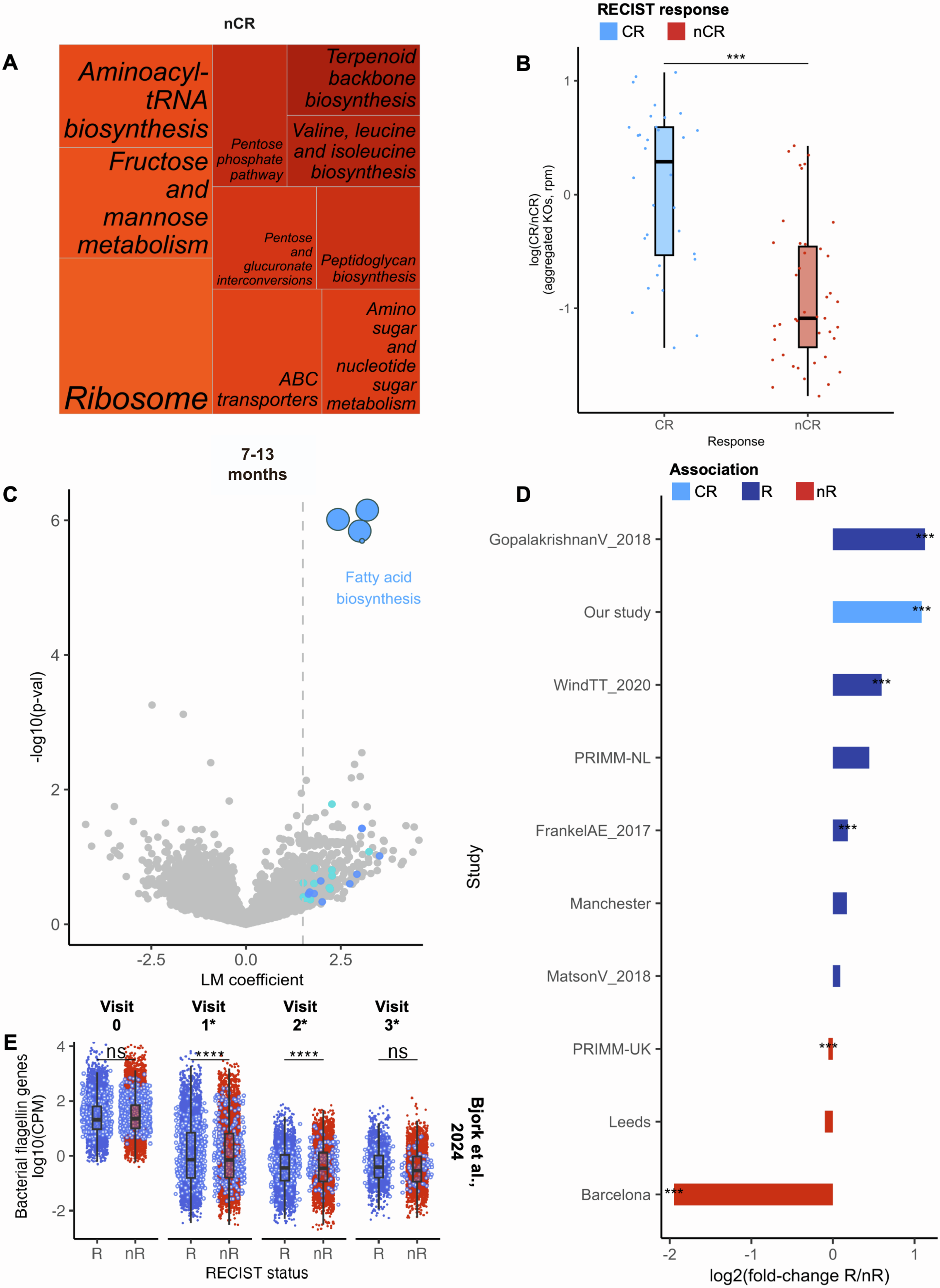

A
nCR
Aminoacyl-tRNA biosynthesis
Fructose and mannose metabolism
Ribosome
Pentose phosphate pathway
Terpenoid backbone biosynthesis
Valine, leucine and isoleucine biosynthesis
Pentose and glucuronate interconversions
Peptidoglycan biosynthesis
ABC transporters
Amino sugar and nucleotide sugar metabolism
B
RECIST response
CR
nCR
***
log(CR/nCR) (aggregated KOs, rpm)
1
0
-1
CR
nCR
Response
C
7-13 months
Fatty acid biosynthesis
-log10(p-val)
6
4
2
0
-2.5
0.0
2.5
LM coefficient
D
Association
CR
R
nR
GopalakrishnanV_2018
Our study
WindTT_2020
PRIMM-NL
FrankelAE_2017
Manchester
MatsonV_2018
PRIMM-UK
Leeds
Barcelona
Study
***
log2(fold-change R/nR)
E
Visit 0
Visit 1*
Visit 2*
Visit 3*
Bacterial flagellin genes log10(CPM)
ns
****
****
ns
R
nR
RECIST status
Bjork et al., 2024
*ranges from 1-4 months of therapy

Figure S5

Figure S5. Defining complete response (CR) by metagenome functional predictions, related to Figure 1.
(A) Treemap of over-represented pathways in nCR at p-adj < 0.05, based on KOs associated with nCR across all samples (|LM coefficient| > 1.5). Terms in black indicate non-stable pathways. **(B)** Comparison of additive log-ratio (CR/nCR) of aggregated KOs mapping to significantly over-represented pathways (p-adj < 0.05) in CR and nCR groups, compared between CR (left) and nCR (right) pathways. Pathways are further filtered to include only significantly differentially abundant pathways based on aggregated KO abundance (p-adj < 0.1). **(C)** Differentially abundant KOs in CR and nCR at late therapy timepoints. Blue points outlined in gray indicate significant KOs at p-adj < 0.005; positive coefficient refers to CR-associated terms whereas negative coefficient refers to nCR-associated terms. Dashed line indicates cut-off for testing over-represented KOs. Non-outlined blue points indicate KOs that map to over-represented pathways in (B) by the same color. **(D)** Baseline increase in bacteria-associated flagellin genes (log(fold-change in CPM)) in R (right) or nR (left) patients across ten melanoma cohorts, including this study that shows increase in CR (right). Statistics shown are from Wilcoxon rank sum test, whereas magnitude and direction are computed from log2 (fold-change) of counts in R/nR for the nine external studies and CR/nCR for our study. **(E)** Average gene family abundances (log10(CPM)) per patient of bacterial flagellin-related terms in R and nR patients in Bjork et al., 2024, compared at visits 0, 1, 2, and 3, spanning 0 to 1-4 months of therapy, depending on patient. Cut-off of < 4 months of therapy was set for this analysis based on sample size distribution across timepoints of this study. Points outlined in blue indicate top CR-associated flagellin genes from our study.
Legend: p-adj<0.001 (***), p-adj<0.01 (**), p-adj<0.05 (*), ns (unannotated).

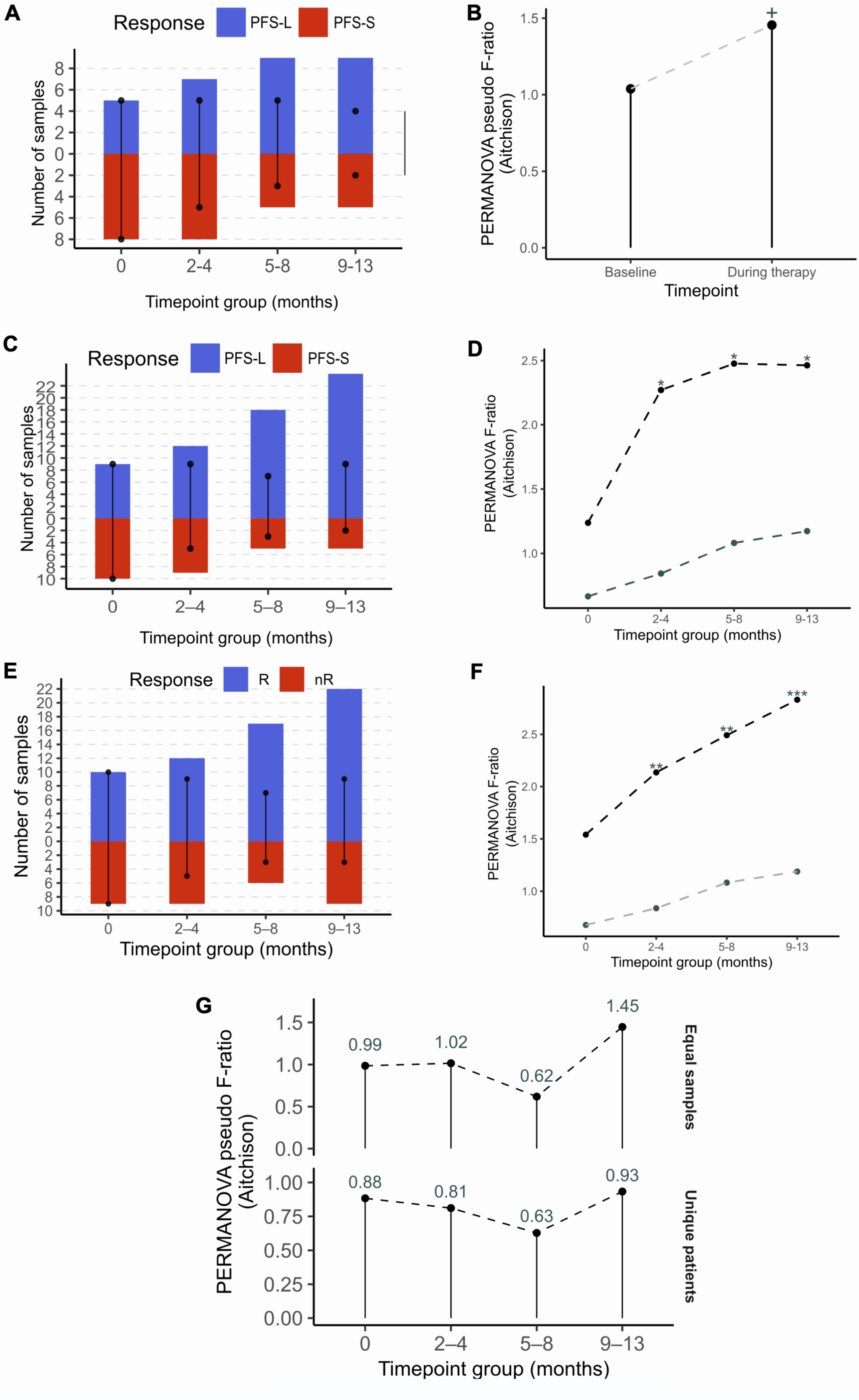

A
Response
PFS-L
PFS-S
Number of samples
Timepoint group (months)
0
2-4
5-8
9-13
B
PERMANOVA pseudo F-ratio (Aitchison)
Baseline
During therapy
Timepoint
C
Response
PFS-L
PFS-S
Number of samples
Timepoint group (months)
0
2–4
5–8
9–13
D
PERMANOVA F-ratio (Aitchison)
Timepoint group (months)
E
Response
R
nR
Number of samples
Timepoint group (months)
F
PERMANOVA F-ratio (Aitchison)
Timepoint group (months)
G
PERMANOVA pseudo F-ratio (Aitchison)
Equal samples
Unique patients
0.99
1.02
0.62
1.45
0.88
0.81
0.63
0.93
Timepoint group (months)

Figure S6

Figure S6. Patients with melanoma responding differently to anti-PD-1 therapy have progressively different gut microbiome diversity, related to Figure 2.

(A) Frequency distribution of melanoma PFS-L and PFS-S samples across time in aggregated months (0, 2–4, 5–8, 9–13 months of therapy). Inset line segments (black) indicate number of samples from non-redundant patients. Sample tallies are that for Shotgun sequencing. **(B)** Shotgun sequencing Aitchison gut composition difference between PFS-L (PFS > 24 months) and PFS-S (PFS < 24 months) groups compared at baseline (using all baseline samples) and therapy (using unique patient samples at 2–4, 5–8, and 9–13 months). '+' indicates PERMANOVA p-adj (distance~group) at the given timepoint group. **(C)** Frequency distribution of melanoma PFS-L and PFS-S samples across time in aggregated months (0, 2–4, 5–8, 9–13 months of therapy). Inset line segments (black) indicate number of samples from non-redundant patients. Sample tallies are that for 16S sequencing. **(D)** 16S sequencing Aitchison gut composition difference between PFS-L and PFS-S (top, in black) and sequencing batch (bottom, in dark gray), compared from 0, 2–4, 5–8, 9–13. Asterisk indicates PERMANOVA p-adj (distance~group) at the given timepoint group. **(E)** 16S sequencing frequency distribution of melanoma R (RECIST1.1 complete and partial response) and nR (RECIST1.1 stable disease and progressive disease) across time in aggregated months (0, 2–4, 5–8, 9–13 months of therapy). Inset line segments (black) indicate number of samples from non-redundant patients. **(F)** 16S sequencing Aitchison gut composition difference between R and nR (top, in black) and sequencing batch (bottom, in dark gray), compared from 0, 2–4, 5–8, to 9–13. Asterisk indicates PERMANOVA p-adj (distance~group) at the given timepoint group. **(G)** 16S sequencing Aitchison gut composition difference between R and nR, using equal samples across timepoint groups (n=6) (top), and using only unique patient samples (bottom), compared from 0, 2–4, 5–8, to 9–13. Asterisk indicates PERMANOVA p-adj (distance~group) at the given timepoint group.

Legend: p-adj<0.001 (***), p-adj<0.01 (**), p-adj<0.05 (*), p-adj<0.10 (+), ns (unannotated).

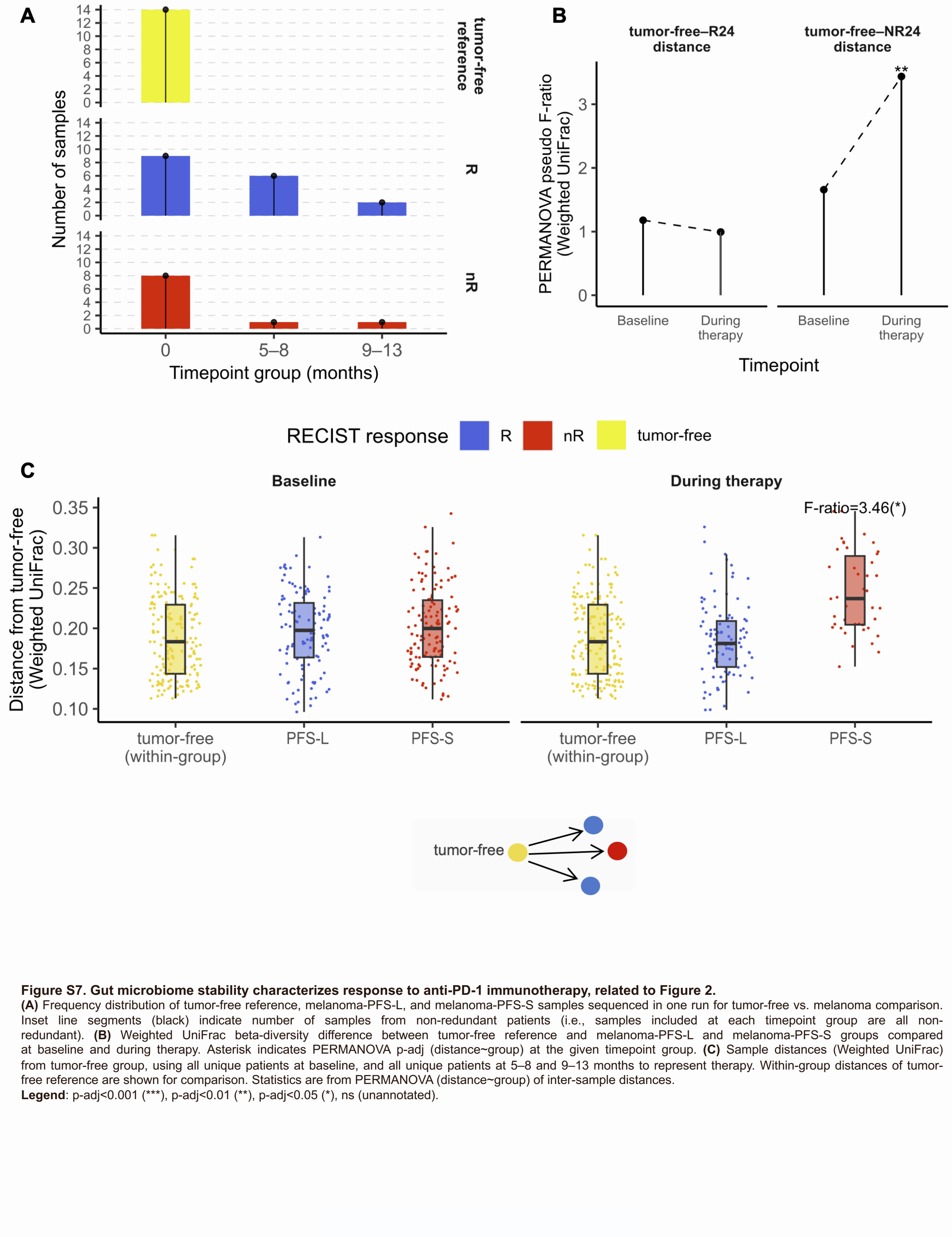


Figure S7. Gut microbiome stability characterizes response to anti-PD-1 immunotherapy, related to Figure 2.
(A) Frequency distribution of tumor-free reference, melanoma-PFS-L, and melanoma-PFS-S samples sequenced in one run for tumor-free vs. melanoma comparison. Inset line segments (black) indicate number of samples from non-redundant patients (i.e., samples included at each timepoint group are all non-redundant). **(B)** Weighted UniFrac beta-diversity difference between tumor-free reference and melanoma-PFS-L and melanoma-PFS-S groups compared at baseline and during therapy. Asterisk indicates PERMANOVA p-adj (distance~group) at the given timepoint group. **(C)** Sample distances (Weighted UniFrac) from tumor-free group, using all unique patients at baseline, and all unique patients at 5–8 and 9–13 months to represent therapy. Within-group distances of tumor-free reference are shown for comparison. Statistics are from PERMANOVA (distance~group) of inter-sample distances.
Legend: p-adj<0.001 (***), p-adj<0.01 (**), p-adj<0.05 (*), ns (unannotated).

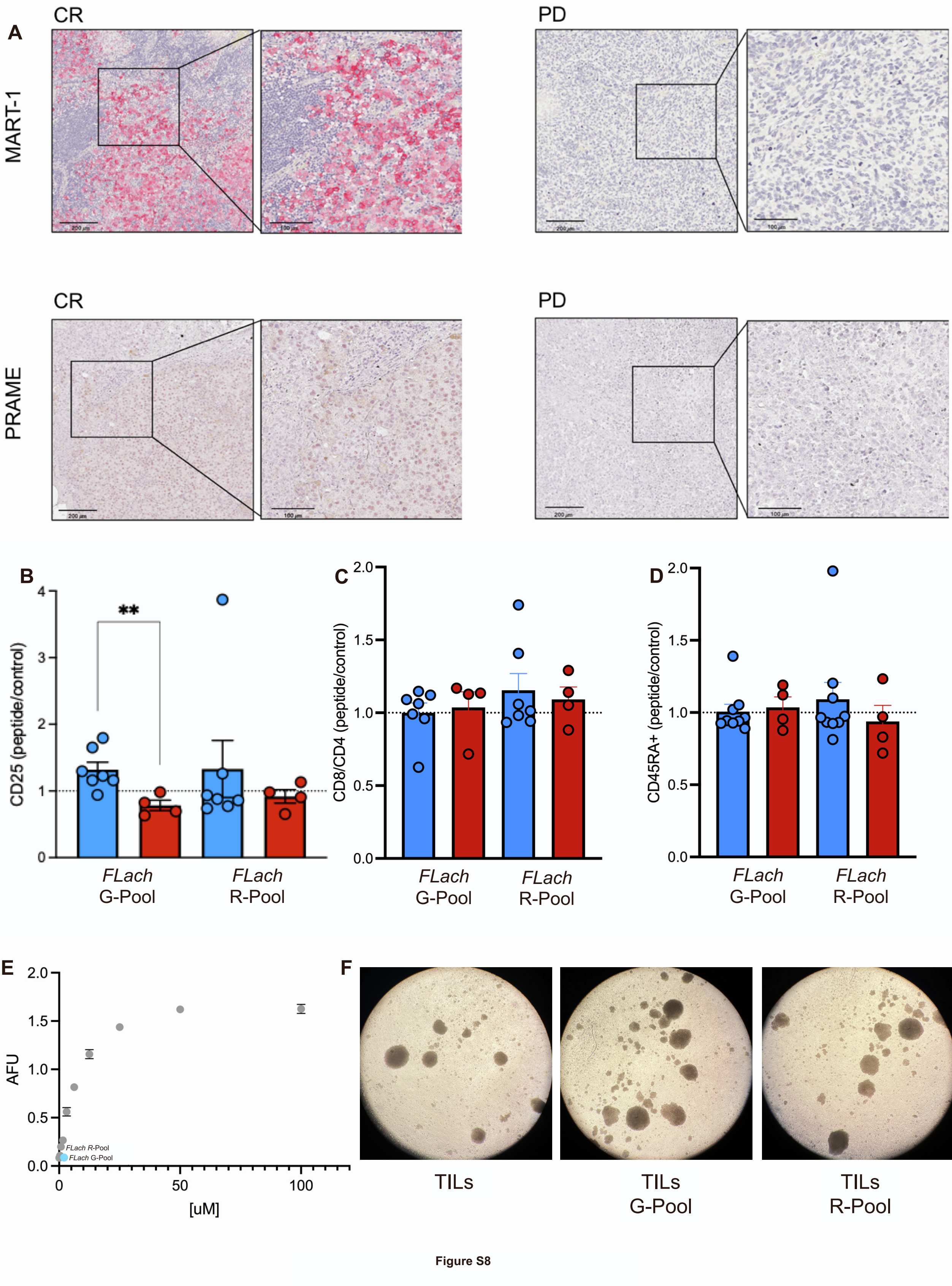

A
CR
PD
MART-1
PRAME
200 µm
100 µm
B
CD25 (peptide/control)
FLach G-Pool
FLach R-Pool
**
C
CD8/CD4 (peptide/control)
D
CD45RA+ (peptide/control)
E
AFU
[uM]
FLach R-Pool
FLach G-Pool
F
TILs
TILs G-Pool
TILs R-Pool

Figure S8

Figure S8. Tumor and peripheral immune characterization of melanoma patients with different response, related to Figures 3 and 4. **(A)** Immunohistochemistry staining of representative CR and PD tumor samples using anti-Melan-A (top) and anti-PRAME (bottom) specific antibodies. **(B-E)** Flow cytometry analysis of T cell populations in peripheral blood monocytic cells (PBMC) from patients with melanoma grouped by response to ICI (complete response=4, progressive disease= 2). **(B)** MFI measurements of CD25 on CD8+ T cells, **(C)** CD4/CD8 ratio and **(D)** CD45RA+ CD8+ T cells (normalized on untreated controls) in peripheral blood monocytic cells (PBMC) from patients with melanoma grouped by response to ICI (responsive=7, non-responsive= 4). **(E)** Quantification of biologically active flagellin, based on the activation of Toll-like receptor 5 (TLR5) in engineered HEK-Blue™ hTLR5 cells. Absorbance values for flagellin standard curve and *FLach* peptide pools are shown. **(F)** Representative pictures of tumor infiltrating lymphocytes (TILs) from human melanoma tumors expanded with or without adding the indicated *FLach* peptide pools.
Legend: p-adj<0.001 (***), p-adj<0.01 (**), p-adj<0.05 (*), ns (unannotated).

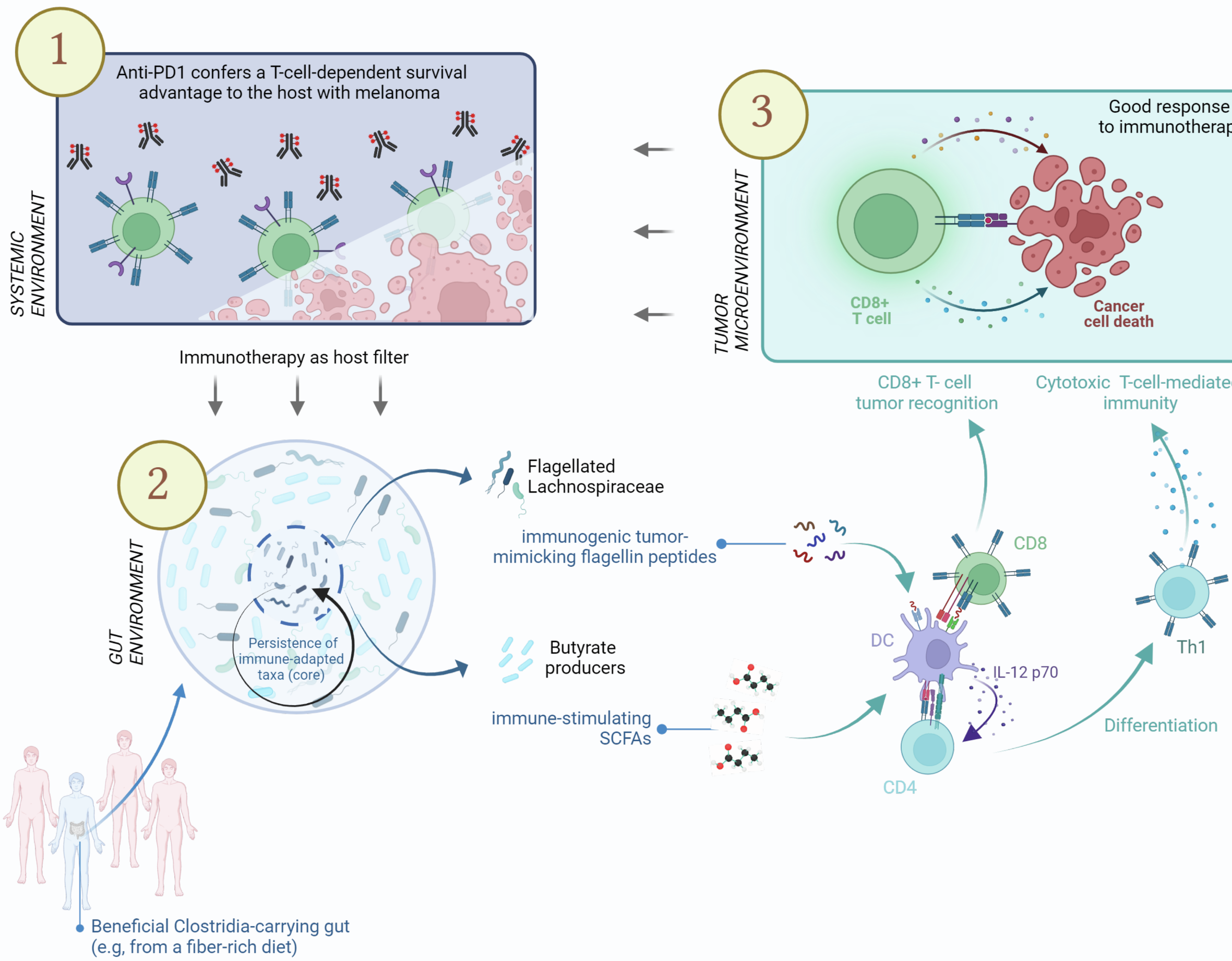


Figure S9. Clostridia-carrying responder gut, related to Figures 1–4.
Proposed model by which the gut of immunotherapy-responsive melanoma patients, through the presence of beneficial Clostridia taxa, mediates a T-cell response synergistic to ICI therapy.

			Timepoint (months)														
n	Patient ID	Sample	0	1	2	3	4	5	6	7	8	9	10	11	12	13	Total n
1	IEO.01	16S	x	-	-	x	x	x	x	x	x	-	x	x	-	-	9
		WGS	x	-	-	x	x	x	x	x	x	-	x	x	-	-	9
2	IEO.02	16S	x	-	-	x	x	-	x	x	-	-	x	x	x	-	8
		WGS	x	-	-	x	x	-	x	x	-	-	x	x	x	-	8
3	IEO.04	16S	x	-	x	x	x	x	x	x	x	x	-	-	-	-	9
		WGS	x	-	x	x	x	x	-	-	-	-	-	-	-	-	5
4	IEO.05	16S	-	-	-	-	-	-	-	-	-	-	x	x	x	x	4
		WGS	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
5	IEO.06	16S	-	-	-	-	x	-	x	x	-	x	x	x	x	-	7
		WGS	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
6	IEO.07	16S	-	-	-	-	-	-	-	-	-	x	x	x	x	x	5
		WGS	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
7	IEO.08	16S	x	-	-	-	-	-	-	-	-	-	-	-	-	-	1
		WGS	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
8	IEO.09	16S	x	-	x	x	x	-	-	-	-	-	-	-	-	-	4
		WGS	x	-	-	x	x	-	-	-	-	-	-	-	-	-	3
9	IEO.10	16S	x	-	-	-	-	-	-	-	-	-	-	-	-	-	1
		WGS	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
10	IEO.11	16S	x	-	-	-	-	-	-	-	-	-	-	-	-	-	1
		WGS	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
11	IEO.12	16S	-	-	-	-	-	-	-	-	-	-	-	-	x	-	1
		WGS	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
12	IEO.14	16S	x	-	x	-	-	-	-	-	-	-	-	-	-	-	2
		WGS	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
13	IEO.15	16S	x	-	x	-	-	-	-	-	-	-	-	-	-	-	2
		WGS	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
14	INT.FGP-001	16S	x	-	-	x	-	-	x	-	x	-	-	x	-	x	6
		WGS	x	-	-	x	-	-	x	-	x	-	-	x	-	x	6
		white blood cells	x	-	-	x	-	-	x	-	x	-	-	x	-	x	6
		serum	-	-	x	-	x	-	x	-	-	-	-	x	-	-	4
15	INT.FGP-002	16S	x	-	-	x	-	x	-	-	x	-	x	-	x	-	6
		WGS	x	-	-	x	-	x	-	-	x	-	x	-	x	-	6
		white blood cells	x	-	-	x	-	x	-	-	x	-	x	-	x	-	6
		serum	x	-	x	-	x	-	-	x	-	x	-	-	-	-	5
16	INT.FGP-005	16S	x	-	-	-	-	-	-	-	-	-	-	-	-	-	1
		WGS	x	-	-	-	-	-	-	-	-	-	-	-	-	-	1
		white blood cells	x	-	-	-	-	-	-	-	-	-	-	-	-	-	1
		serum	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
17	INT.FGP-006	16S	x	-	-	x	-	-	x	-	x	-	x	-	x	-	6
		WGS	x	-	-	x	-	-	x	-	x	-	x	-	x	-	6
		white blood cells	x	-	-	x	-	-	x	-	x	-	x	-	x	-	6
		serum	x	-	x	-	-	x	-	x	-	x	-	x	-	-	6
18	INT.FGP-007	16S	x	-	-	-	x	-	x	-	-	-	-	-	-	-	3
		WGS	x	-	-	-	x	-	x	-	-	-	-	-	-	-	3
		white blood cells	x	-	-	-	-	-	x	-	-	-	-	-	-	-	2
		serum	x	-	-	x	-	x	-	-	-	-	-	-	-	-	3
19	INT.FGP-009	16S	x	-	x	-	x	-	x	-	x	-	-	x	-	x	7
		WGS	x	-	x	-	x	-	x	-	x	-	-	x	-	x	7
		white blood cells	x	-	x	-	x	-	x	-	x	-	-	-	-	-	5
		serum	x	x	-	x	-	x	-	x	-	-	x	-	x	-	7
20	INT.FGP-010	16S	x	-	-	-	-	-	-	-	-	-	-	-	-	-	1
		WGS	x	-	-	-	-	-	-	-	-	-	-	-	-	-	1
		white blood cells	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
		serum	x	-	-	-	-	-	-	-	-	-	-	-	-	-	1
21	INT.FGP-011	16S	x	-	x	-	x	-	x	-	-	x	-	x	-	x	7
		WGS	x	-	x	-	x	-	x	-	-	x	-	x	-	x	7
		white blood cells	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
		serum	x	x	-	x	-	x	-	-	x	-	x	-	x	-	7
22	INT.FGP-013	16S	x	-	-	-	-	-	-	-	-	-	-	-	-	-	1
		WGS	x	-	-	-	-	-	-	-	-	-	-	-	-	-	1
		white blood cells	x	-	-	-	-	-	-	-	-	-	-	-	-	-	1
		serum	x	-	-	-	-	-	-	-	-	-	-	-	-	-	1
23	INT.FGP-014	16S	x	-	x	-	-	-	-	-	-	-	-	-	-	-	2
		WGS	x	-	x	-	-	-	-	-	-	-	-	-	-	-	2
		white blood cells	x	-	x	-	-	-	-	-	-	-	-	-	-	-	2
		serum	x	x	-	-	-	-	-	-	-	-	-	-	-	-	2
	Total	16S	19	0	7	7	8	3	9	4	6	4	7	8	7	5	94
		WGS	14	0	4	7	7	3	7	2	5	1	4	5	3	3	65
		white blood cells	8	0	2	3	1	1	4	0	4	0	2	2	2	2	31
		serum	8	3	3	3	2	4	1	3	1	2	2	2	2	-	36

Table S1. Breakdown of samples collected from each patient across timepoints, related to Figures 1 and 2.
Fecal samples were processed for 16S and whole genome sequencing (WGS), whole blood samples for white blood cell count, and serum samples for soluble factor analysis.

Demographics	n (tot=14)	%
Age, years (median)	42 (29-54)	
Sex		
Male	7	50%
Female	7	50%
BMI, kg/m2 (median)	22.21 (18.96-32.74)	

Table S2. Tumor-free summary demographics, related to Figure 2.
Tumor-free cohort used for longitudinal comparison with melanoma patients from this study.

n	KO ID	Annotation	Associated group	LM coefficient	p-adj
1	K08168	MFS transporter	CR	3.00	0.00188
2	K11263	acetyl-CoA/propionyl-CoA carboxylase	CR	3.20	0.00188
3	K18120	4-hydroxybutyrate dehydrogenase	CR	2.43	0.00188
4	K09705	hypothetical protein	CR	3.06	0.00197

Table S3. Response-associated metagenome functions, related to Figure 1.
Significant KEGG orthologs (KOs) associated with complete response (CR) at late therapy (p-adj < 0.05).

n	Bacterial flagellin marker	log2FC	Percentile rank	Organism
1	UniRef90_A0A0M6WP36	19.58	100	Roseburia inulinivorans
2	UniRef90_C0FQ31	19.56	99	Roseburia inulinivorans DSM 16841
3	UniRef90_A0A127SWS0	19.26	98	uncultured bacterium
4	UniRef90_C0FQ36	19,093	97	Roseburia inulinivorans DSM 16841
5	UniRef90_A0A396AI63	19,092	96	Roseburia inulinivorans
6	UniRef90_A0A0M6WMV3	19,087	95	Roseburia inulinivorans
7	UniRef90_A0A0M6WD36	18.69	94	Roseburia inulinivorans
8	UniRef90_D4KYR7	18.66	93	Roseburia intestinalis XB6B4
9	UniRef90_R6VY20	18.62	92	Clostridium sp. CAG:91
10	UniRef90_A0A351R0D5	18.57	91	Roseburia sp.
11	UniRef90_A0A0M6WBR8	18.49	90	Roseburia inulinivorans
12	UniRef90_A0A0M6WYS8	18.34	89	Roseburia inulinivorans
13	UniRef90_R6EJ07	18.25	88	Firmicutes bacterium CAG:65
14	UniRef90_R6VQ94	18.17	87	Clostridium sp. CAG:91

Table S4. Candidate complete response (CR) markers, related to Figure 1.
Top CR-associated flagellin gene families (UniRef90).

n	Identity (UniRef90)	Peptide	HLA	Affinity	TAA match	Peptide	HLA	Affinity	Strong binding (SB)	Pool
1	tr_A0A0M6WD36_A	KMSYEDIEL	HLA-A*02:01	71.69	secernin 1	KMDAEHPEL	HLA-A*02:01	33.52	Yes	FLach-G
2	tr_A0A0M6WMV3_A	MPKDGAAFI	HLA-B*07:02	93.13	Melan-A/MART-1	MPREDAHFI	HLA-B*07:02	64.99	Yes	
3	tr_A0A0M6WP36	GLDALNNLL	HLA-A*02:01	51.79	PRAME	VLDGLDVLL	HLA-A*02:01	24.52	Yes	
	tr_A0A0M6WP36	GLDALNNLL	HLA-A*02:01	51.79	PAP	ALDVYNGLL	HLA-A*02:01	241.11	Yes	
1	tr_A0A0M6WYS8_A	ALNETSAIL	HLA-A*02:01	67.47	MAGE-A1	ALAETSYVK	HLA-A*02:01	10808.84	No	FLach-R
2	tr_A0A351R0D5_A	ILAQAGQSM	HLA-B*15:01	32.57	N-ras	ILDTAGREE	HLA-B*15:01	17909.95	No	
3	tr_A0A396AI63_A	NEFNADLLM	HLA-B*40:01	55.77	CEA	IYPNASLLI	HLA-B*40:01	29590.65	No	
4	tr_R6EJ07_R6EJ0	TEFNGQKLL	HLA-B*40:01	24.94	alpha-actinin-4	IASNGVKLV	HLA-B*40:01	32748.64	No	
5	tr_R6EJ07_R6EJ0/tr_R6VY20_R6VY2	SVRGRLGAF	HLA-B*15:01	66.44	NY-ESO-1/LAGE-2	GGRGPRGAG	HLA-B*15:01	29085.32	No	
6	tr_R6VQ94_R6VQ9	FPELKHFTM	HLA-B*08:01	41.6	NY-ESO-1/LAGE-2	GVLLKEFTV	HLA-B*08:01	7887.94	No	
	tr_R6VQ94_R6VQ9	FPELKHFTM	HLA-B*08:01	41.6	MAGE-A3	VAELVHFLL	HLA-B*08:01	10188	No	
7	tr_A0A351R0D5_A	ILAQAGQSM	HLA-B*15:01	32.57	N-ras	ILDTAGREE	HLA-B*15:01	32728.81	No	

Table S7. Breakdown of *FLach* peptide pool, related to Figures 3 and 4.
List of peptides that comprise the *FLach*-G (green) and *FLach*-R (red) pool that were used for the functional validation experiments.

Cohort details	Baruch et al., 2021	Davar et al., 2021	Routy et al., 2023	Bjork et al., 2024
Treatment description	FMT+anti-PD1 on anti-PD1 refractory patients	FMT+anti-PD1 on anti-PD1 refractory patients	FMT+anti-PD1 on anti-PD1 naive patients	anti-PD1 on anti-PD1 naive patients
Disease type	melanoma	melanoma	melanoma	melanoma
Donor	CR (≥ 12 m.) (n=2)	Durable PR (≥ 24 m.) (n=3) /CR (≥ 12 m.) (n=4)	Healthy male (BMI 18.5-30, w/ exclusion criteria for disease, risk, or infection) (n=3)	NA
Recipient	Progressor to anti-PD1 (n=10) *3/10 success rate rate (30%)	Progressive disease to anti-PD1/ anti-PD1 + anti-CTLA-4 (n=15) *randomly assigned donor *6/15 success rate (40%)	anti-PD1-naive melanoma patients (48-90 y/o, 60% male) (n=20) *57-75% success rate	NA
Preparation	•colonoscopy (day 0): 50 g feces --> normal saline suspension -> sieve -> 3x concentrate -> NSS + 10% glycerol •oral capsule (from day 1, every 2 weeks): 15 g feces -> concentrate -> NSS + 10% glycerol -> hypromellose capsule (x12)	•endoscopy	single FMT by oral capsules (day 1): 80-100 g feces -> > 0.9% normal saline + glygcerol -> filter à sediment (10^13 CFU/mL) -> gelatin capsule -> flash freeze	NA
ICI treatment	microbiota depletion -> colonoscopy FMT -> capsule FMT -> ICI on day 14 of FMT -> ICI every 2 weeks	endoscopy FMT + ICI on day 0 of FMT -> ICI every 3 weeks	PEG laxative -> capsule FMT (x36-40) -> ICI on day 7 of FMT -> ICI every 3-4 weeks	NA
Baseline timepoint/ FMT initiation	day 0 (pre-FMT)	day 0 (pre-FMT)	day 0 (pre-FMT, day 1 post-laxative)	NA
Anti-PD1 initiation timepoint	day 14 of FMT	day 0	day 7 of FMT	day 0
Study design	1 sample per patient at each fixed timepoint (day 0, 7, 31, 65)	multiple samples from patients at variable timepoints (day 0 to 558)	1 sample per patient at each fixed timepoint (day 0, 7, 28, 56)	multiple samples from patients at variable timepoints (day 0 to 277)*

Table S8. Description of external studies analyzed, related to Figure 2.
Cohort details of the external melanoma (Bjork et al., 2023) and fecal microbiota transplant (Baruch et al., 2021; Davar et al., 2021; Routy et al., 2023) longitudinal anti-PD-1 datasets used for comparison against our melanoma anti-PD-1 cohort.
*In analyses done in our study, only timepoints 0 to 4 months (day 120) were included, where the bulk of the samples were