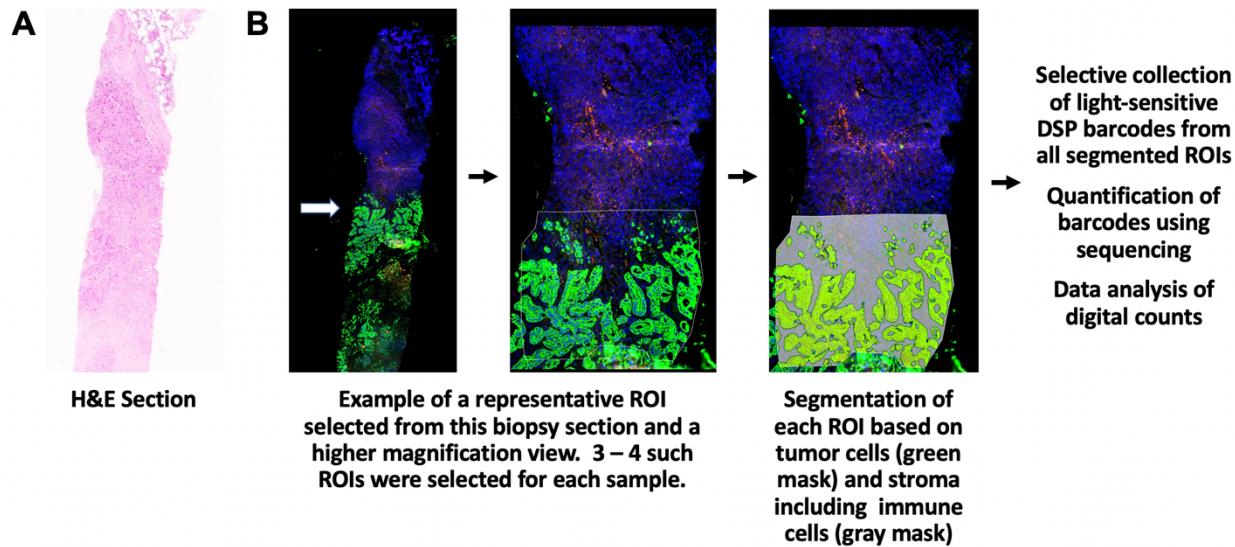


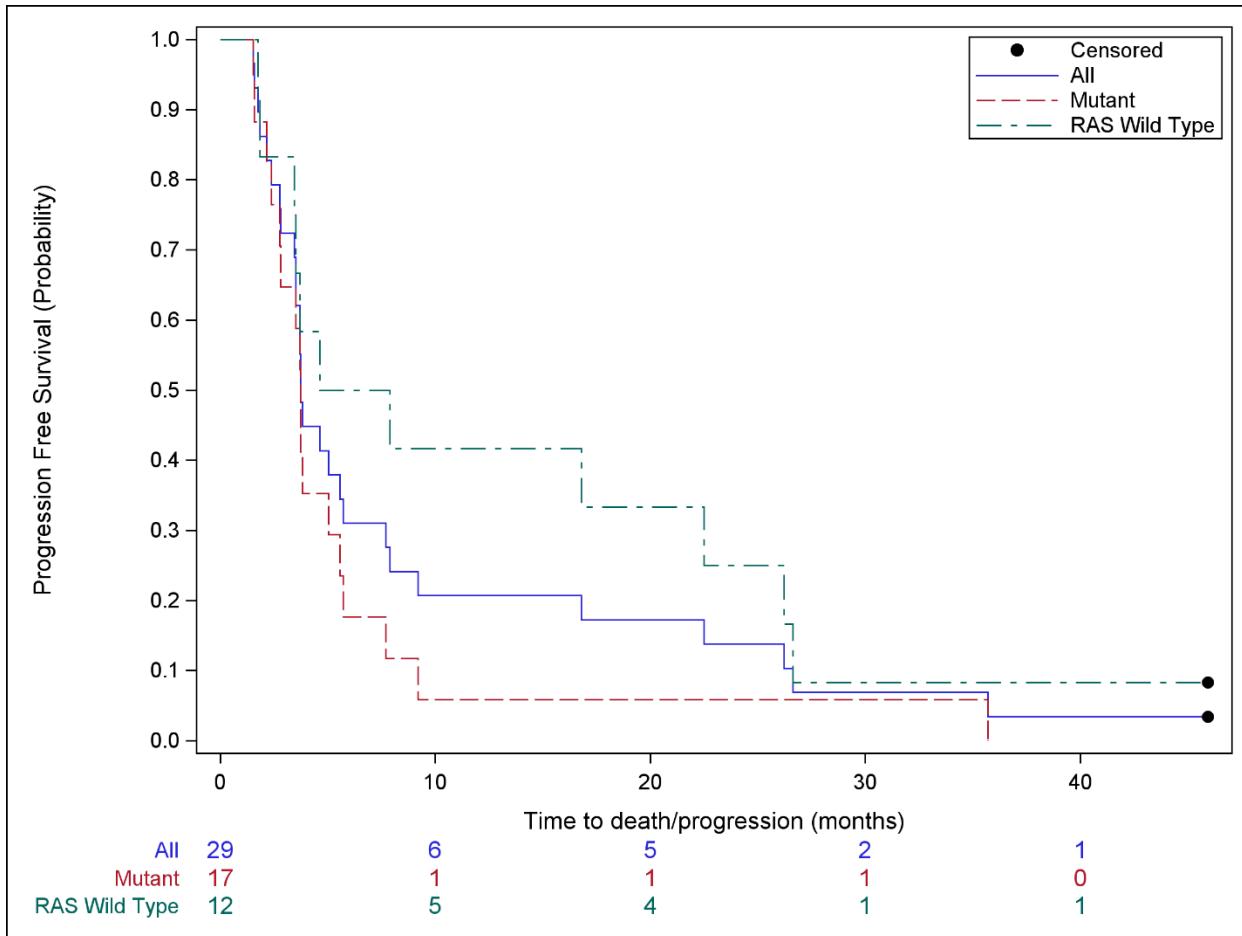
1 **Supplemental Figure 1. Workflow used for digital spatial profiling of clinical samples.** **A)**
2 Pathologist review of an H&E-stained slides used to verify presence of sufficient tumor tissue in
3 the biopsied material. **B)** An unstained serial section was then used for overnight hybridization
4 using ISH probes conjugated to GeoMx DSP barcodes. Fluorescent antibodies against panCK
5 (green) and CD45 (red) were used as morphological markers to identify epithelial cells and
6 immune cells, respectively; Syto 13 dye (blue) was used to label nuclei. Regions of interest (ROIs)
7 were selected using the morphology markers as guides. The ROIs were segmented into tumor cells
8 and surrounding stromal cells including the immune cells followed by selective collection of their
9 corresponding DSP barcodes by the GeoMx instrument. The DSP barcodes were quantified using
10 next generation sequencing and the FASTQ files were processed to yield expression data for each
11 mRNA target in the CTA panel.



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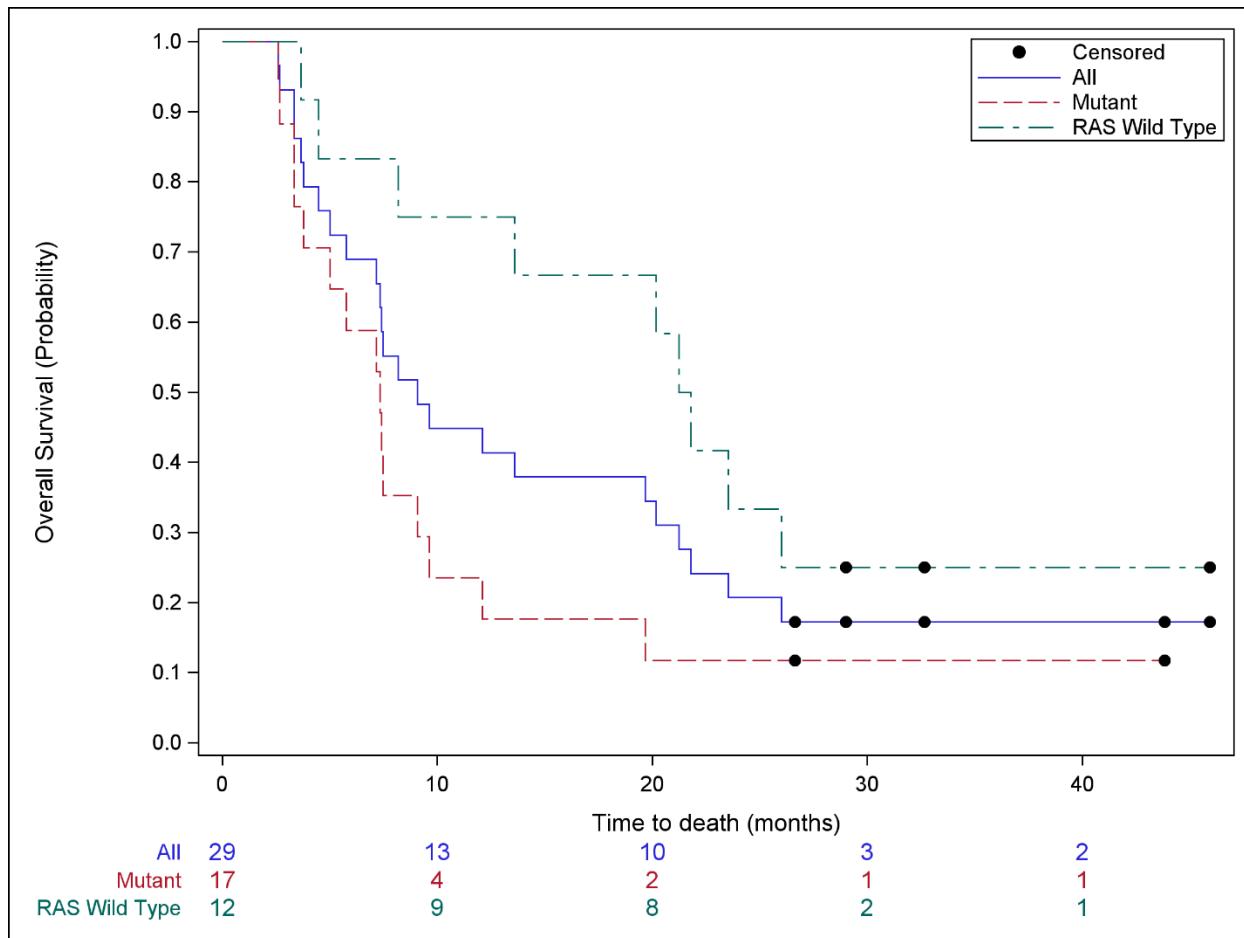
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14 **Supplemental Figure 2. Kaplan-Meier analysis of progression-free survival by *RAS* status.**
15 Blue line represents the overall population. Green (*RAS* wild type) and red (*RAS* mutant) broken
16 lines represent stratification by *RAS* mutation status.



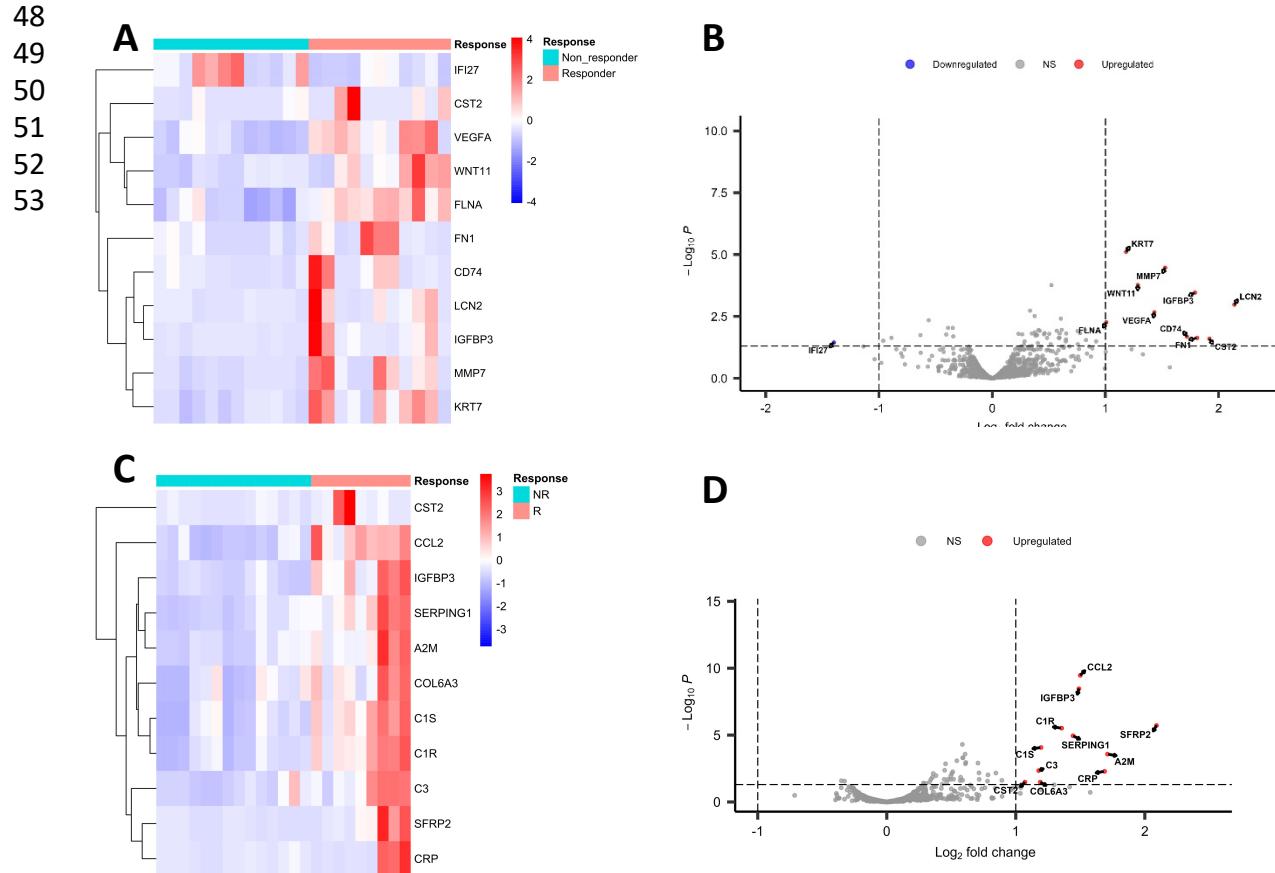
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19 **Supplemental Figure 3. Kaplan-Meier analysis of overall survival by *RAS* status.** Blue line
20 represents the overall population. Green (*RAS* wild type) and red (*RAS* mutant) broken lines
21 represent stratification by *RAS* mutation status.

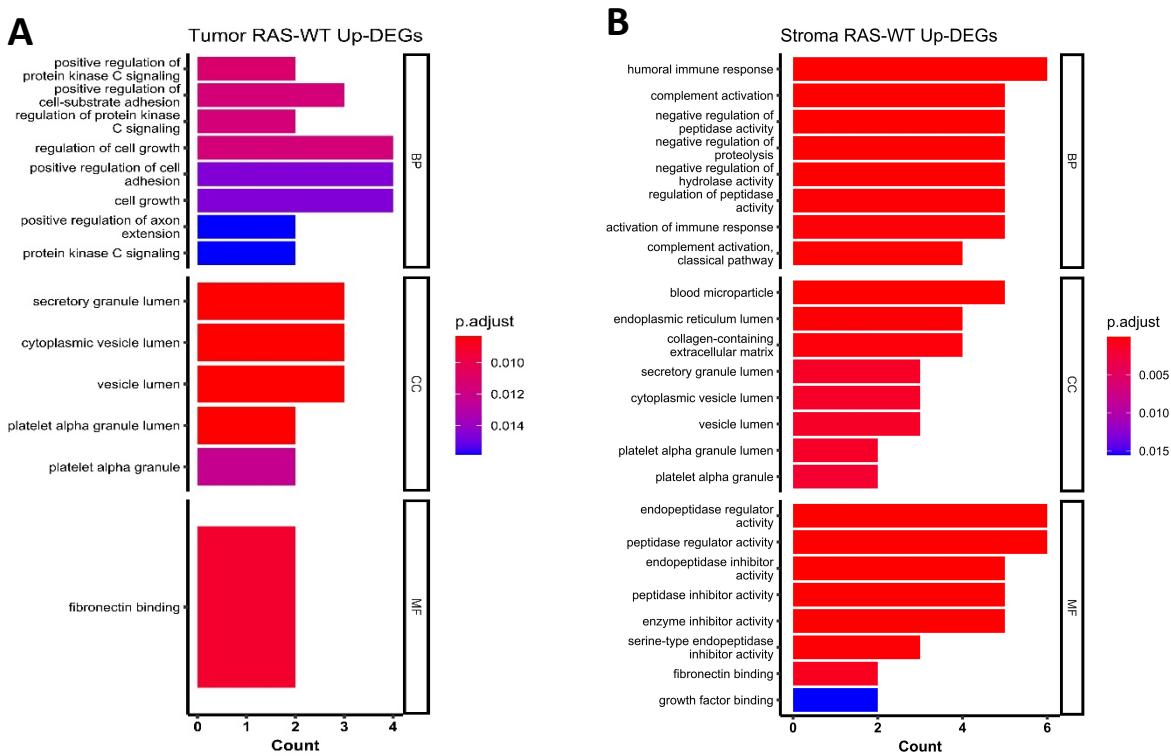


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38 **Supplemental Figure 4. GeoMx spatial transcriptomic analysis results in *RAS* Wild Type**
 39 **(*RAS*-WT) subgroup.** **A)** Heatmap showing the scaled Q3-normalized expression of the
 40 differentially expressed genes (DEGs) between responders (R) and non-responders (NR) in tumor
 41 compartment in *RAS*-WT subgroup. Patients are depicted by key (ID). **B)** Volcano plot for the
 42 DEGs in R versus NR in tumor compartment. Significant DEGs are labeled and shown in red, with
 43 $\log_2 FC > 1$ and adjusted p-value < 0.05 . NS, non-significant. **C)** Heatmap showing the scaled Q3-
 44 normalized expression of the DEGs between R and NR in stroma compartment in *RAS*-WT
 45 subgroup. Patients are depicted by key (ID). **D)** Volcano plot for the DEGs in R versus NR in
 46 stroma compartment in *RAS*-WT subgroup. Significant DEGs are labeled and shown in red, with
 47 $\log_2 FC > 1$ and adjusted p-value < 0.05 . NS, non-significant.

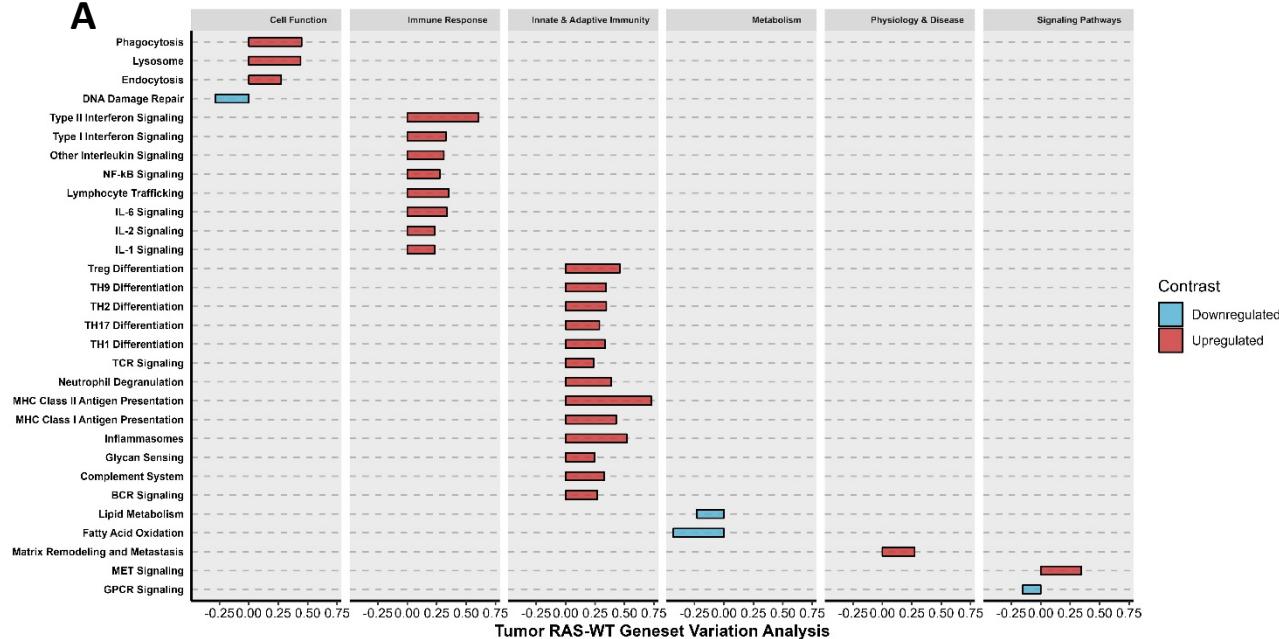


Supplemental Figure 5. Gene ontology analysis results in RAS-WT subgroup. **A)** Bar plots of selected significantly enriched gene ontology (GO) terms including enriched biological processes (BP), cellular components (CC) and molecular function (MF) in responders (R) versus non-responders (NR) in tumor compartment in RAS-WT subgroup. **B)** Bar plots of selected significant GO terms including BP, CC and MF, in R versus NR stroma compartment.

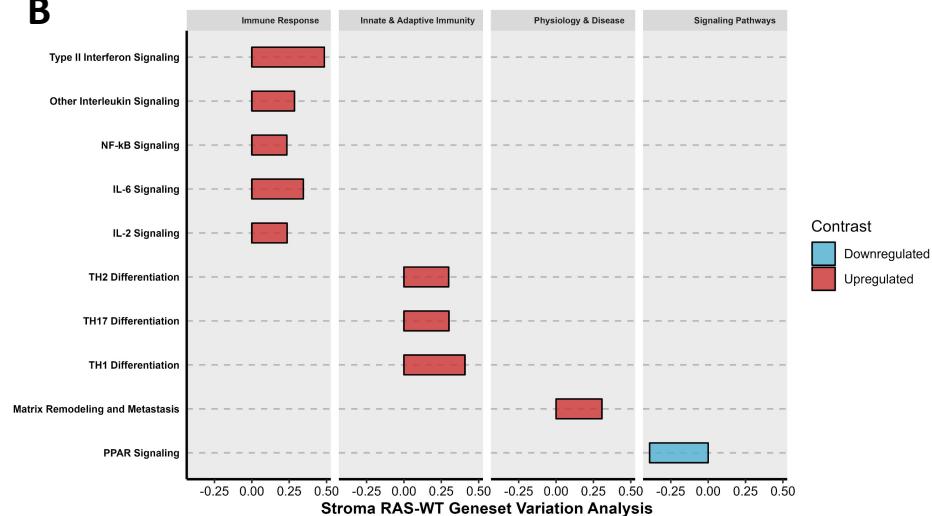


61 **Supplemental Figure 6. Gene Set Variation Analysis (GSVA) of significant differentially**
 62 **enriched pathways in responders versus non-responders in RAS-WT subgroup.** The y-axis
 63 represents annotated gene sets from the Nanostring Cancer Transcriptome Atlas. The pathways are
 64 organized within modules of Cell Function, Metabolism, Immune Response, Innate and Adaptive
 65 Immunity and Signaling Pathways. The x-axis represents the fold change difference of
 66 differentially enriched pathways in responders in comparison to non-responders in RAS-WT
 67 subgroup. Upregulated pathways are tinted in red and downregulated pathways are tinted in blue.
 68 **A)** GSVA of tumor epithelial compartment showing significant differentially enriched pathways
 69 in RAS-WT responders. **B)** GSVA of stroma compartment showing significant differentially
 70 enriched pathways in RAS-WT responders.
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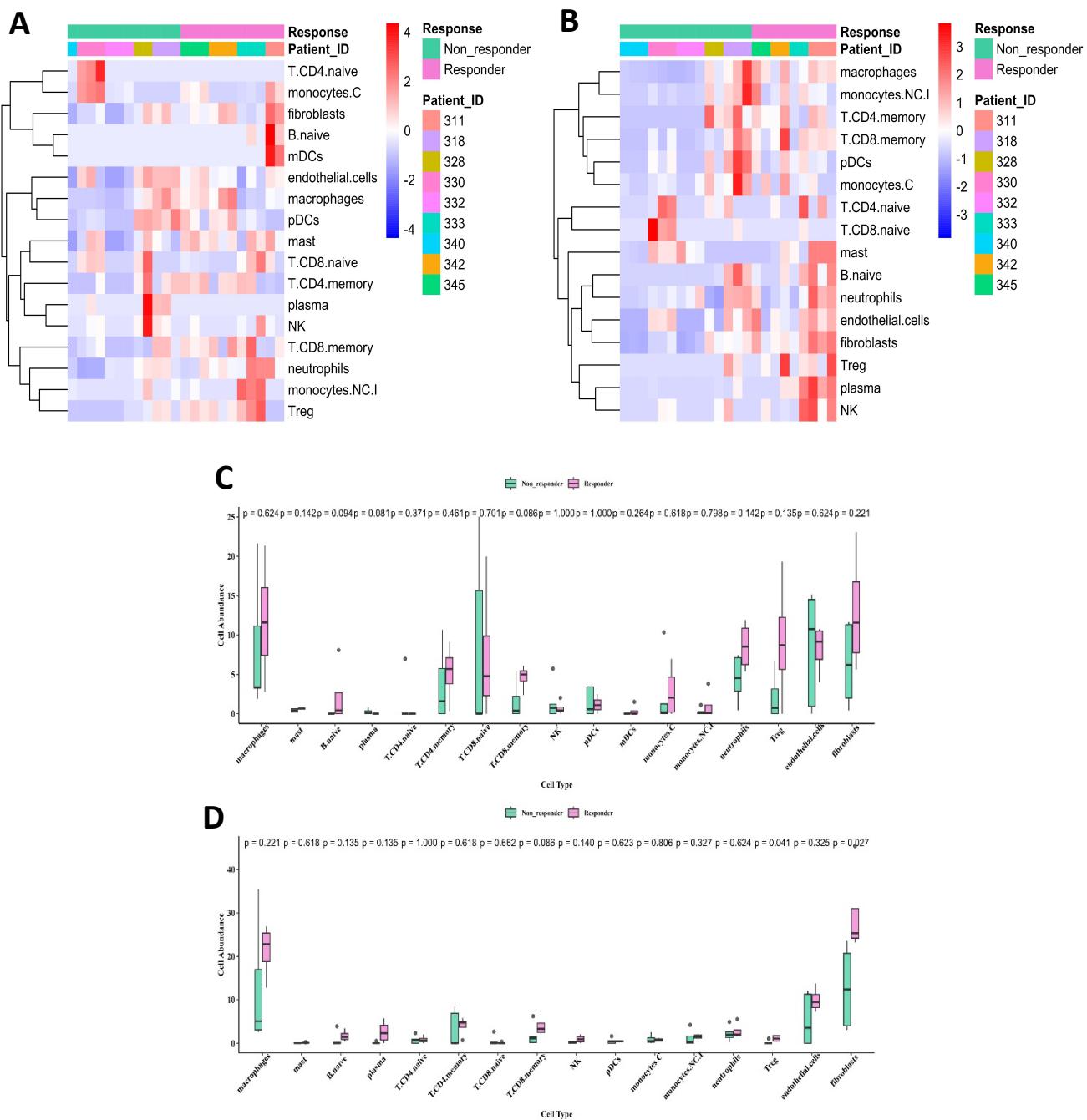
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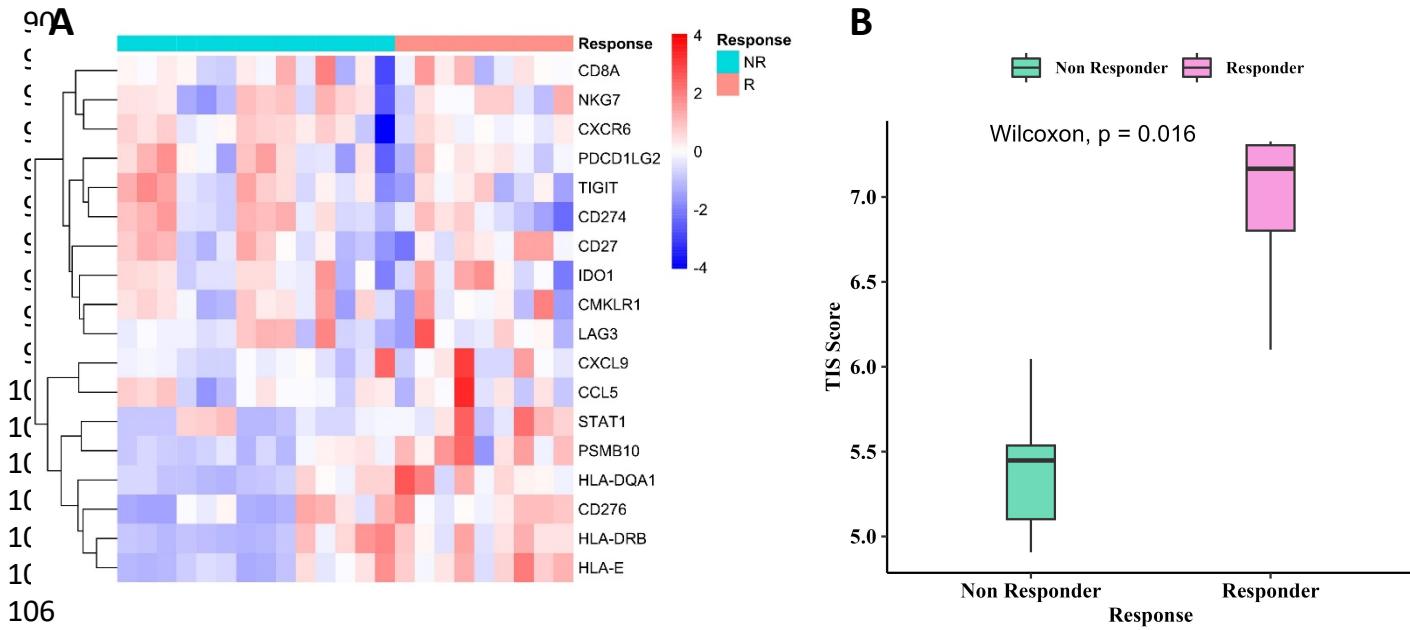


72 **Supplemental Figure 7. Cell type deconvolution by spatialDecon in RAS-WT subgroup. A)**
 73 Heatmap of scaled cell abundance scores (scaled Beta-values) in the tumor epithelial compartment
 74 in *RAS*-WT subgroup. Patients are depicted by key (ID). Plasmacytoid Dendritic cells (pDCs),
 75 myeloid Dendritic cells (mDCs), conventional monocytes (monocytes. C), non-
 76 conventional/intermediate monocytes (monocytes NC.I). **B)** Boxplots showing differences of cell
 77 infiltration between responders (R) and non-responders (NR) in tumor epithelial compartment in
 78 *RAS*-WT subgroup. Statistical significance was tested using Wilcoxon's rank sum test. **C)**
 79 Heatmap of scaled cell abundance scores (scaled Beta-values) in stroma compartment in *RAS*-WT
 80 subgroup. Patients are depicted by Key (ID). **D**) Boxplots showing differences of cell infiltration
 81 between R and NR in stroma compartment in *RAS*-WT subgroup. Statistical significance was
 82 tested using Wilcoxon's rank sum test.

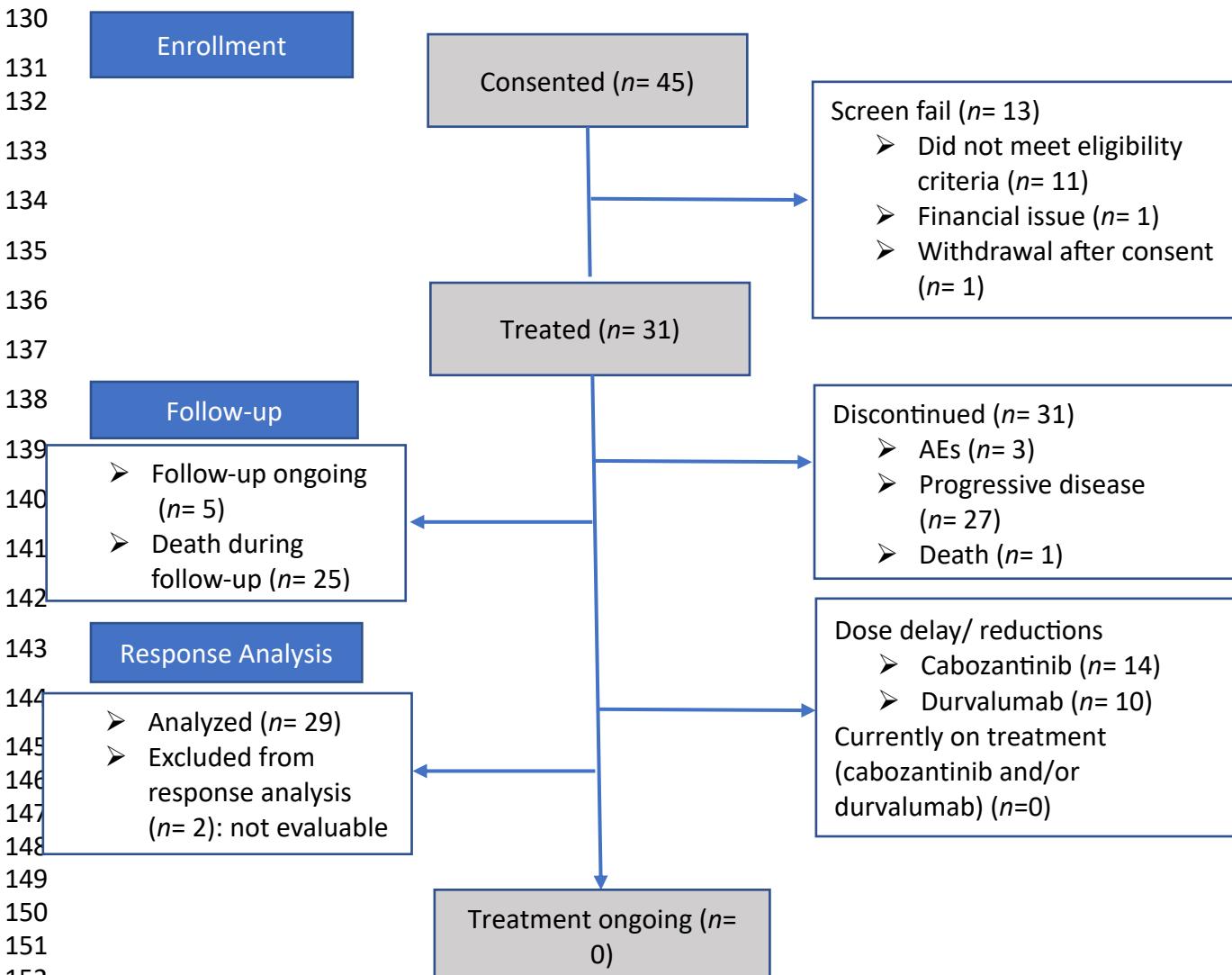


83 **Supplemental Figure 8. T-cell inflamed gene expression signature (TIS) in RAS-WT**
84 **subgroup. A)** Heatmap of genes in T-cell inflamed gene expression signature representing the
85 scaled expression of TIS genes. Patients are depicted by key (ID). **B)** Boxplots showing differences
86 in the TIS scores between responders (R) and non-responders (NR) in RAS-WT subgroup. The y-
87 axis represents the TIS score. Statistical significance was tested using Wilcoxon's rank sum test.
88 Responders (R), non-responders (NR).

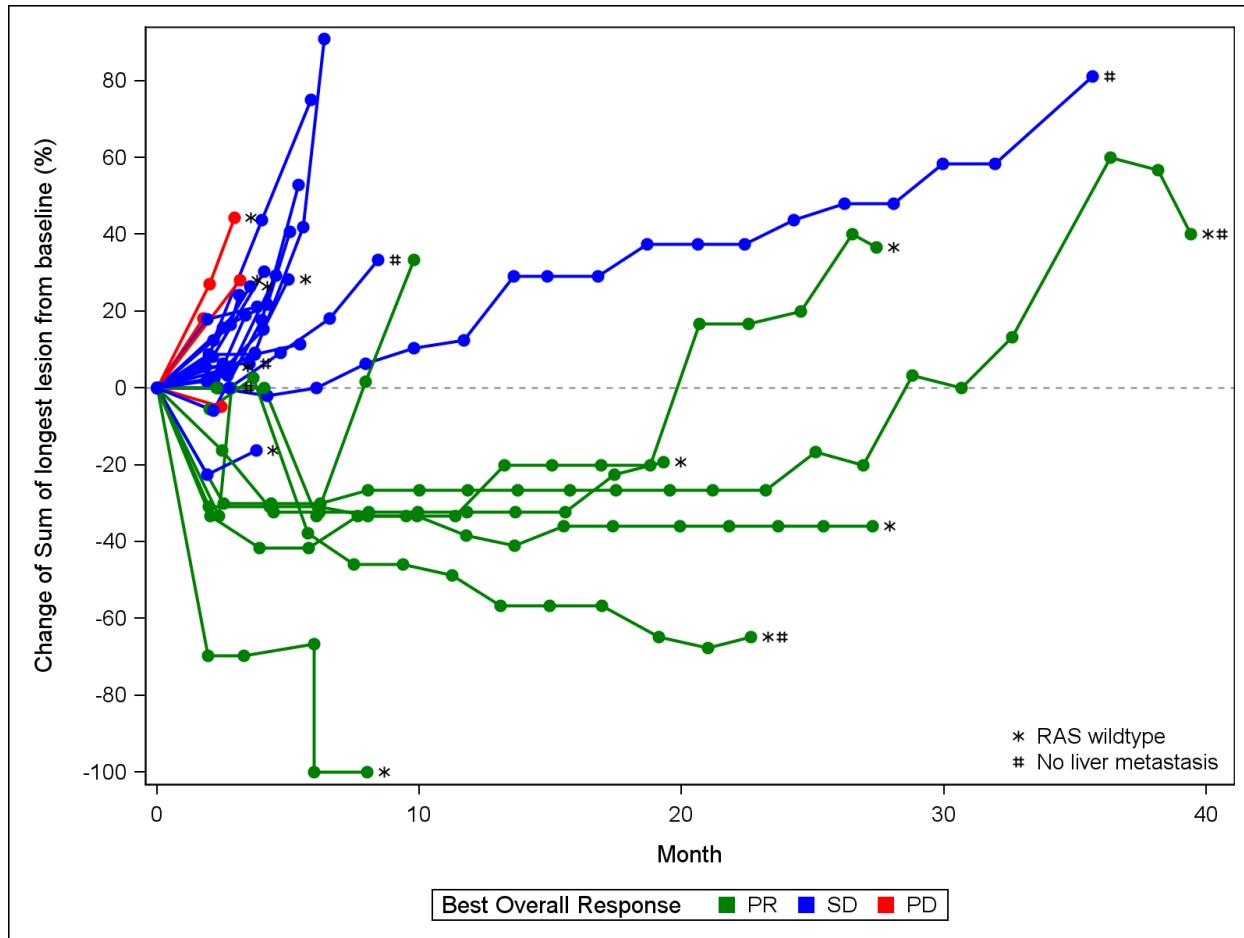
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129 **Supplemental Figure 9. Consort Diagram.** Study Consort Diagram.

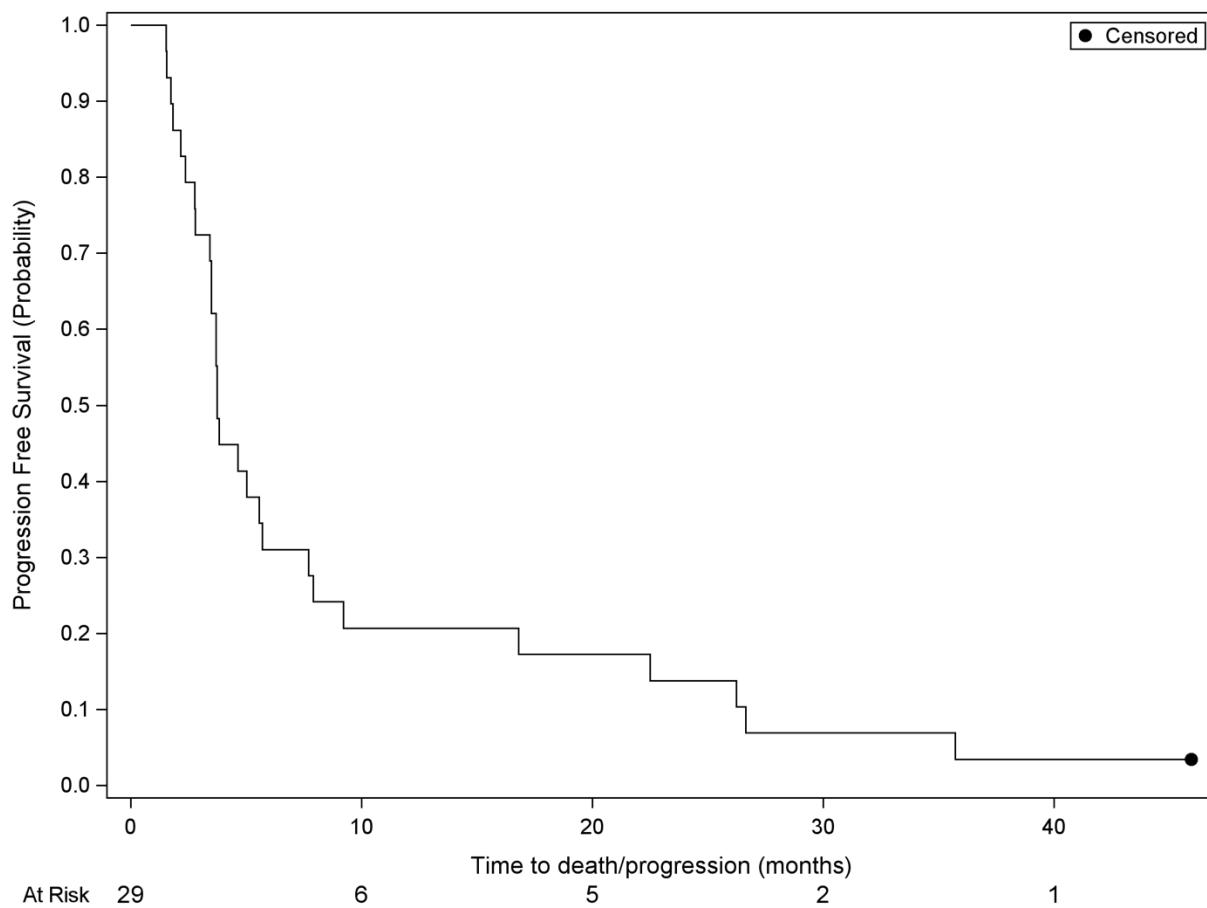


162 **Supplemental Figure 10. Spider plot of individual patient responses.** Colors of lines represent
163 best overall response (green, partial response; blue, stable disease; red, progressive disease). A star
164 denotes a *RAS* wild type patient and # represents a patient with no liver metastasis.



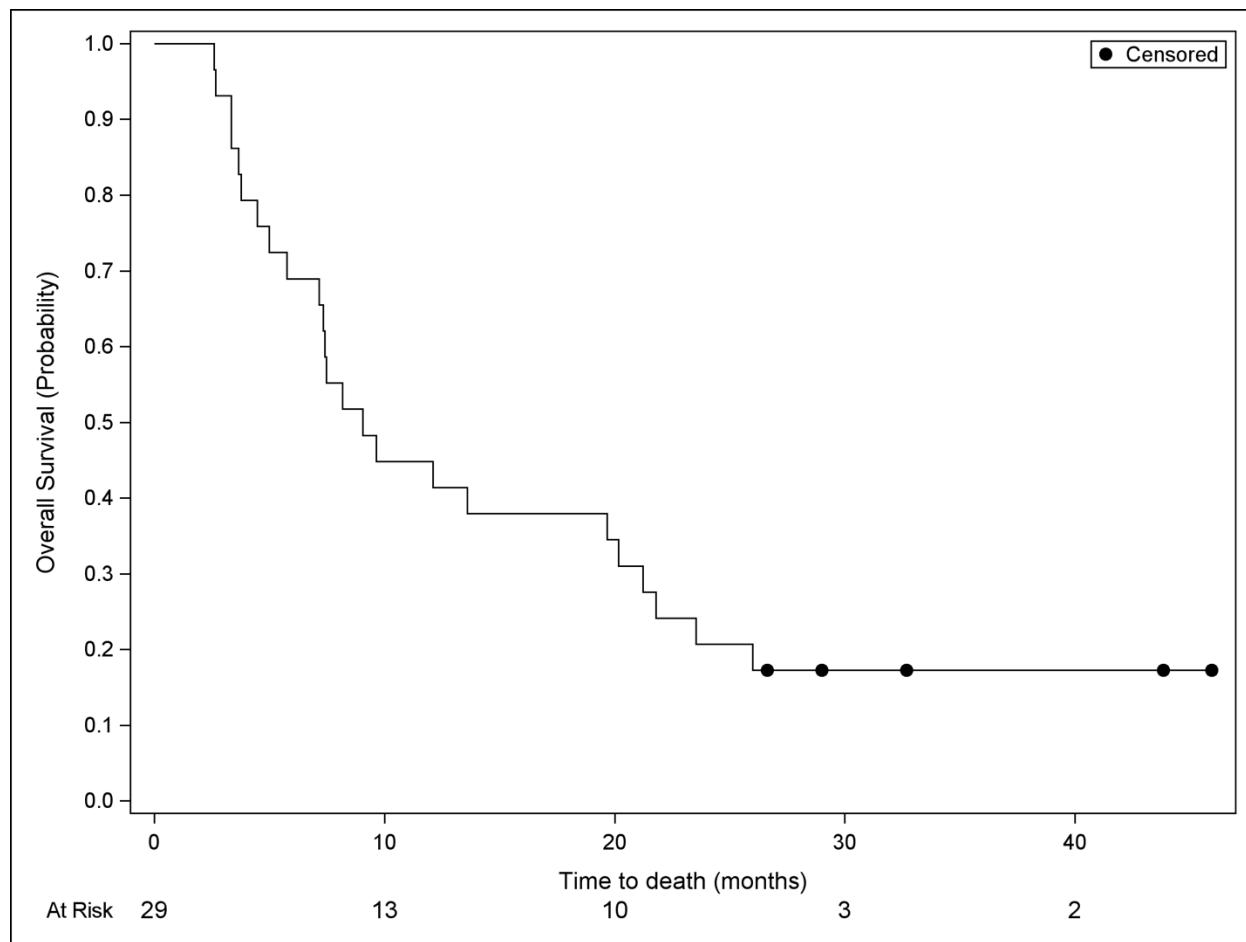
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167 **Supplemental Figure 11. Kaplan-Meier analysis of progression-free survival.** X axis depicts
168 time to death or progression in months. Y axis depicts the probability of progression free survival.



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171 **Supplemental Figure 12. Kaplan-Meier analysis of overall survival.** X axis depicts time to
172 death in months. Y axis depicts the probability of overall survival.



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175 **Supplemental Table S1. RAS wild type subgroup summary of efficacy data.**

Variable	RAS Wild Type (N=12)
Overall objective responses (ORR, 95% CI)	6 (50%, 21.1-78.9)
Confirmed partial response (ORR, 95% CI)	6 (50%, 21.1-78.9)
Best overall response	
Complete response	0
Partial response	6 (50 %)
Stable disease	4 (33.3%)
Progressive disease	2 (16.6%)
Disease control rate	10/12 (83.3%, 51.6-97.9)

Median progression-free survival	6.3 months (1.8-26.2 months)
Median overall survival	21.5 months(4.5-not estimable)
4-month PFS rate	n/a
6-month PFS rate	n/a

176 Note. Data area presented as No. (%), lower and upper limits of 95% confidence interval) unless
 177 otherwise noted.

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179 **Supplemental Table S2. Clinical and molecular baseline characteristics and tumor biopsy**
 180 **sites for patient samples tested in the DSP study.**

Study ID	Gender	Age	Primary colon tumor Side	RAS gene status	Number of Prior Lines of therapy	Prior EGFR inhibitor	Number of Metastatic Sites <3 / ≥3	Liver metastasis	Best response	Progression free time (in days)	Survival time (in days)	Biopsy Site	Response
311	F	76	Right	Wild type	3	yes	>3	no	PR	1149	1149	lung	Responder
312	M	53	Left/ rectum	KRAS mutant	3	no	>3	yes	SD	111	215	liver	Non responder
314	M	66	Left	KRAS mutant	2	no	>3	yes	SD	112	222	liver	Non responder
315	M	62	Left	KRAS mutant	2	no	>3	yes	SD	105	272	liver	Non responder
316	F	57	Left	KRAS mutant	2	no	>3	no	SD	231	590	lung	Non responder
318	F	51	Left/ rectum	Wild type	3	yes	>3	yes	SD	139	654	liver	Non responder
321	F	51	Right	KRAS mutant	2	no	>3	yes	PD	47	80	lung	Non responder
327	F	52	Left/ rectum	NRAS mutant	3	yes	>3	yes	SD	112	224	liver	Non responder
328	M	27	Left/ rectum	Wild type	2	yes	>3	yes	SD	105	245	liver	Non responder
330	F	61	Left	Wild type	3	yes	>3	yes	PD	55	605	adnexal mass	Non responder
332	F	36	Left	Wild type	3	no	>3	yes	SD	103	110	liver	Non responder
333	F	64	Left/ rectum	Wild type	3	yes	>3	no	PR	675	704	retroperitoneal mass	Responder
338	F	50	Left/ rectum	KRAS mutant	3	no	>3	no	SD	83	113	lymph node	Non responder
340	F	57	Left	Wild type	3	yes	>3	yes	PD	52	134	liver	Non responder
341	M	51	Left/ rectum	KRAS mutant	2	no	>3	yes	SD	46	100	liver	Non responder
342	F	70	Left/ rectum	Wild type	2	yes	>3	yes	PR	612	641	liver	Responder
343	M	62	Left/ rectum	NRAS mutant	2	yes	>3	yes	SD	171	173	liver	Non responder
344	F	70	Left	KRAS mutant	2	no	>3	yes	SD	84	150	liver	Non responder
345	M	65	Left/ rectum	Wild type	3	yes	>3	yes	PR	504	619	liver	Responder
353	M	63	Left/ rectum	KRAS mutant	2	no	>3	yes	SD	167	220	liver	Non responder

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183 **Supplemental Table S3. Characteristics of patients with confirmed partial response to the**
 184 **investigational regimen.**

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	Tumor location	Type of Overall Tumor Response	Progression free time (months)	Presence of Liver Metastasis	Best response in liver metastatic lesions	RAS mutational status	Prior EGFR targeted therapy
Patient #1	Right sided (Cecum)	PR	38	No	N/A	Wild type	Yes
Patient #2	Left sided (Rectum)	PR	25	Yes	SD	Wild type	Yes
Patient #3	Left sided (Rectum)	PR	22	No	N/A	Wild type	Yes
Patient #4	Left sided (sigmoid)	PR	8	Yes	SD	Wild type	Yes

Patient #5	Left sided (Rectum)	PR	20	Yes	PR	Wild type	Yes
Patient #6	Left sided (Rectum)	PR	17	Yes	SD	Wild type	Yes

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