

# The interleukin 22 pathway interacts with mutant KRAS to promote poor prognosis in colon cancer

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## Supplementary Materials:

**Figure S1.** CONSORT diagram and characteristics of the discovery and verification sets.

**Figure S2.** KRAS mutation promotes poor overall survival in *IL-22RA1*-high CRC.

**Figure S3.** Immune and histotype analysis of tumors based on *IL22RA1* expression, KRAS mutation status, and site (proximal vs distal)

**Figure S4.** KRAS mutation confers poor relapse free and overall survival in CRCs with high expression of *IL10RB*, the second subunit of the heterodimeric IL- 22R.

**Figure S5.** IL-22 induces *MYC* pathway transcription and c-Myc translation in DLD-1 *KRAS* mutant isogenic cells.

**Figure S6.** *IL22RA1*-high tumors preferentially classify as CMS2 and *IL22RA1* and *MYC* expression are correlated in prospectively collected CRC resection tissue.

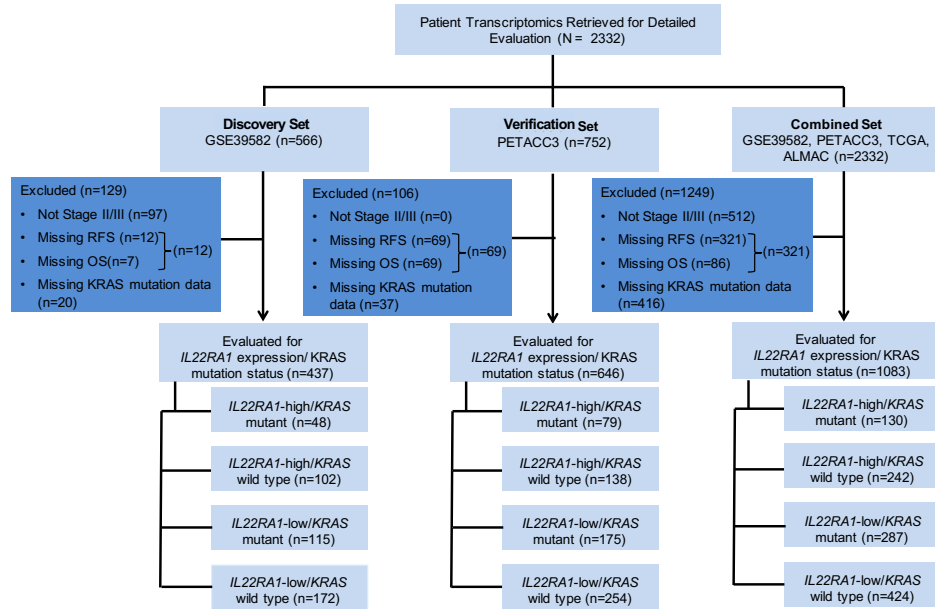
**Table S1.** Univariate Coxph survival analysis of stage II/III CRC patients in discovery (GSE39582), verification (PETACC3), and combined cohorts based on *IL22RA1* expression, KRAS mutation status, and tumor location.

**Table S2.** Multivariate Coxph survival analysis of stage II, III CRC patients in discovery (GSE39582), verification (PETACC3), and combined cohorts based in *IL22RA1* expression, KRAS mutation status, and tumor location.

**Table S3.** Univariate Coxph survival analysis of stage II, III CRC patients in discovery (GSE39582), verification (PETACC3), and combined cohorts based on *IL10RB* and KRAS mutation status.

# Supplementary Figures:

A

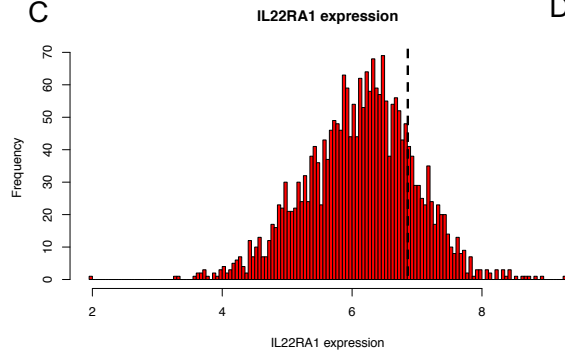


B

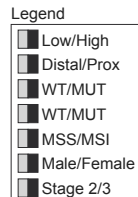
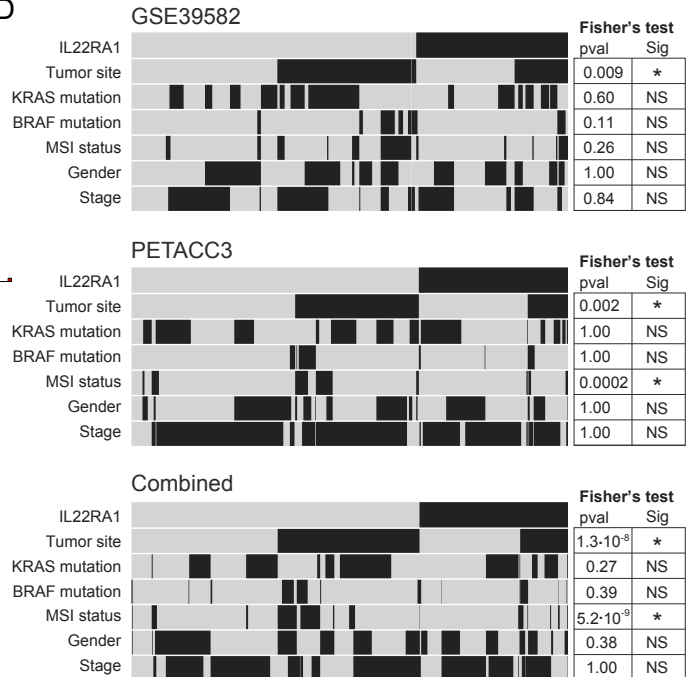
KRAS MUT	G12	G13	A146	Q61	K117	V144	L120+S145	R68	A155	G12/G13	K117/K22	Y71/Q61	NA
GSE39582	55	11	2	4	1	1	1	0	0	0	0	0	94
PETACC3	207	63	0	12	0	0	0	0	0	1	0	0	0
TCGA	54	17	11	3	2	0	0	1	1	1	1	1	0

NA - KRAS point mutation not available

C

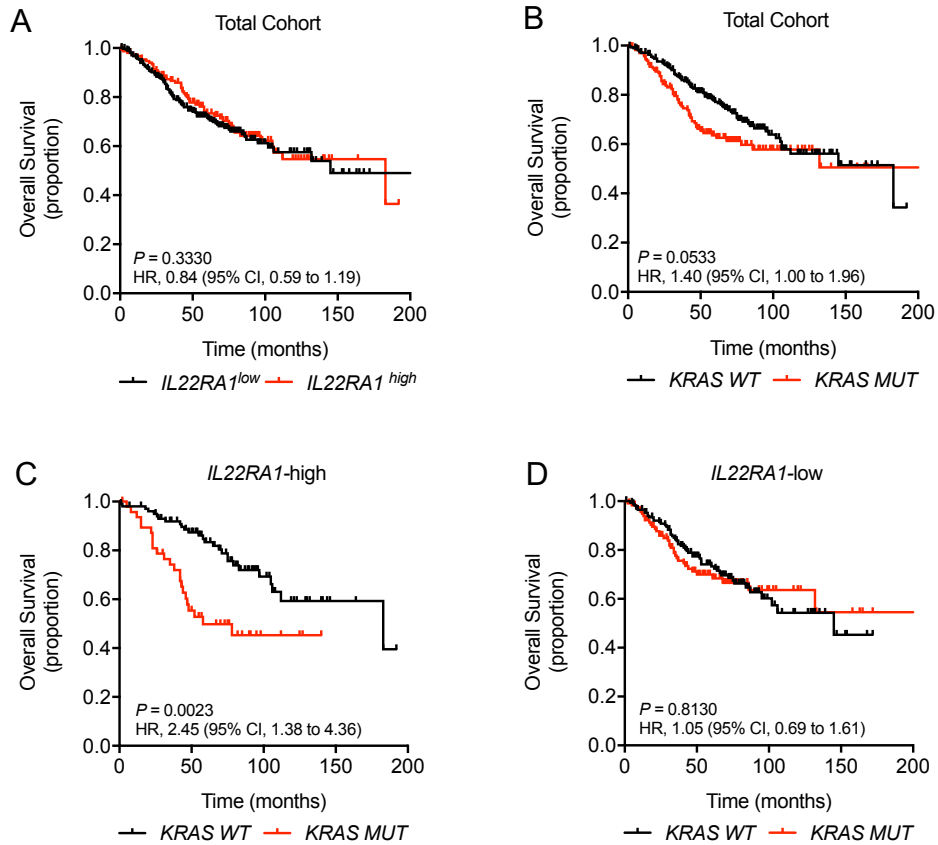


D



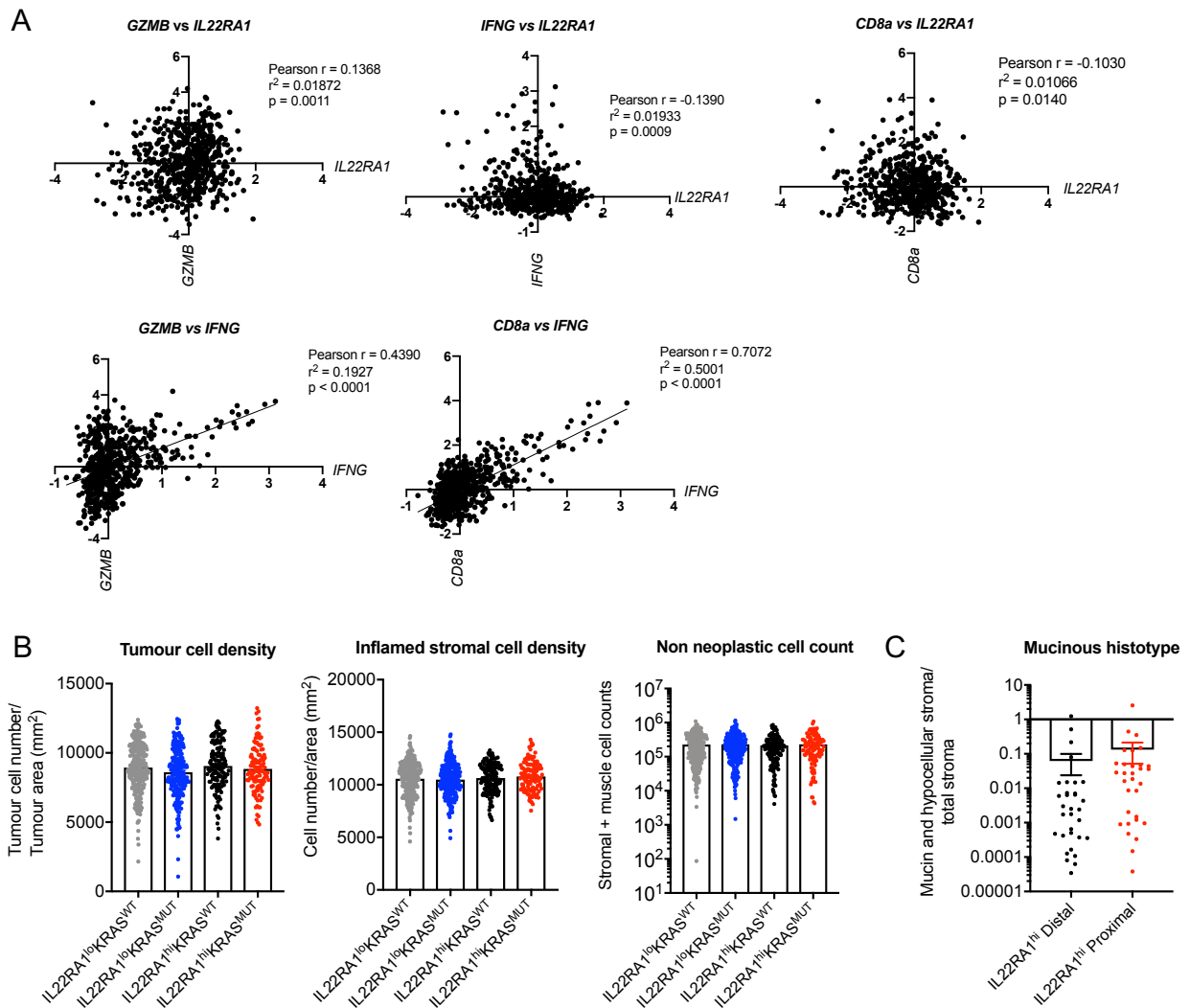
**Figure S1. CONSORT diagram and characteristics of the discovery and verification sets.**

Discovery and verification sets used to assess the prognostic consequence of having high or low expression of *IL22RA1* in the presence or absence of *KRAS* mutation. The total number of patients in each cohort, excluded patients, and number of patients in each molecular subtype of interest are indicated. (RFS data is missing from the TCGA dataset, thus numbers for RFS versus OS analysis in the combined cohort differ significantly.) **(B)** *KRAS* mutations among Stage II/III CRC patients by codon **(C)** Distribution of *IL22RA1* expression in combined GSE39582, PETACC3, and TCGA dataset (N=1083). Patients for whom clinical information was not complete were removed from this analysis. Dashed line represents the cutpoint (67<sup>th</sup> percentile) used with stratify patients by high and low *IL22RA1* expression. **(D)** Fisher's exact tests were used to examine the association of *IL22RA1* expression (high/low) with: tumor location (distal to splenic flexure [distal]/proximal to the splenic flexure [proximal]), *KRAS* mutation status (WT/MUT), BRAF mutation status (WT/MUT), microsatellite stability (microsatellite stable [MSS]/microsatellite instable [MSI]), gender (male/female), and stage (2/3) in the discovery (GSE39582), verification (PETACC3), and combined cohorts. \* $P < 0.05$ .



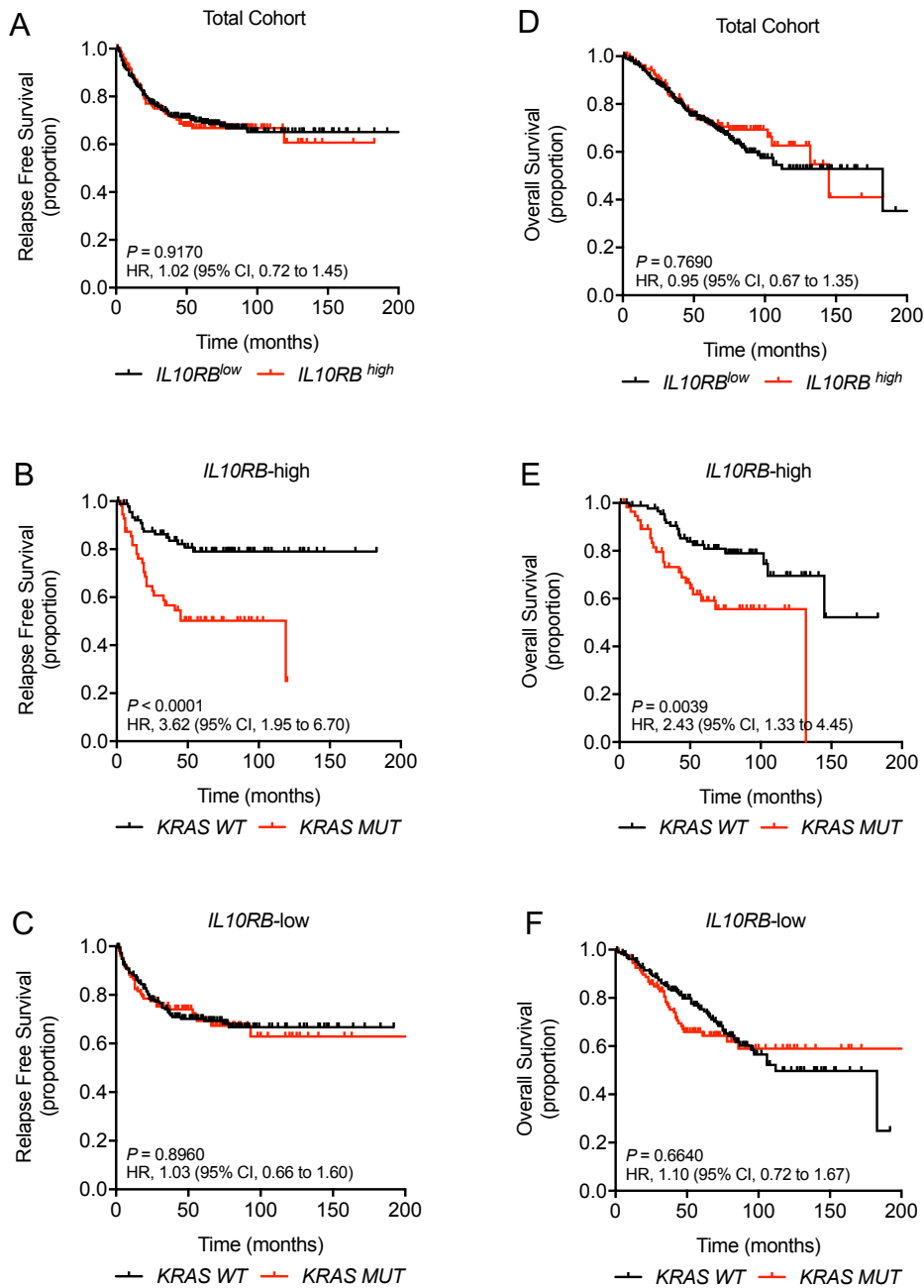
**Figure S2. KRAS mutation promotes poor overall survival in *IL-22RA1*-high CRC. (A to D)**

Kaplan-Meier estimates of overall survival (OS) in stage II/III patients based on (A) *IL22RA1* expression (B) *KRAS* mutation status. OS among (C) *IL22RA1*-high and (D) *IL22RA1*-low patients based on *KRAS* mutation status. *IL22RA1*-high and low is defined by a cutpoint at the 67<sup>th</sup> percentile determined using ROC analysis. *P* values computed using Wald-tests. Hazard ratios (HR) are univariate Cox proportional hazard ratios with 95% confidence intervals for the GSE39582 cohort.



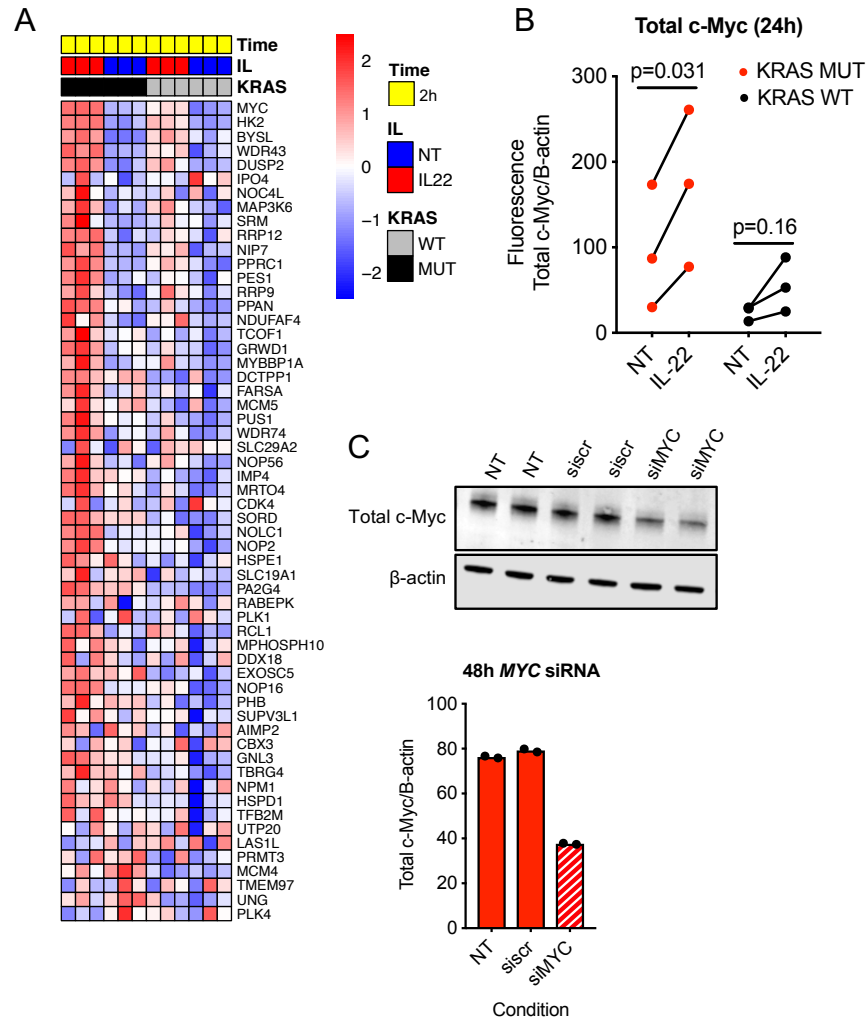
**Figure S3. Immune and histotype analysis of tumors based on *IL22RA1* expression, *KRAS* mutation status, and site (proximal vs distal)** (A) Pearson correlation analysis of *GZMB*, *IFNG*, and *CD8a* versus *IL22RA1* expression in the GSE39582 cohort. *GZMB* and *IFNG* were compared to *CD8a* as controls. (B) Histologic analysis of tissue composition (tumor cell density, inflamed stromal density, non-neoplastic cell count) of tumors in the TCGA and S:CORT cohorts, stratified according to *IL22RA1* expression and *KRAS* mutation status ( $IL22RA1^{lo}KRAS^{WT}$   $N=277$ ;  $IL22RA1^{lo}KRAS^{MUT}$   $N=253$ ;  $IL22RA1^{hi}KRAS^{WT}$   $N=151$ ;  $IL22RA1^{hi}KRAS^{MUT}$   $N=118$ ). Area measurements were normalized by total stromal content. (C) Histologic analysis of mucin and hypocellular stromal content of *IL22RA1*-high tumors in

the TCGA and S:CORT cohorts stratified according to tumor location (distal vs proximal)  
(IL22RA1<sup>hi</sup>distal  $N=35$ , IL22RA1<sup>hi</sup>proximal  $N=32$ ).



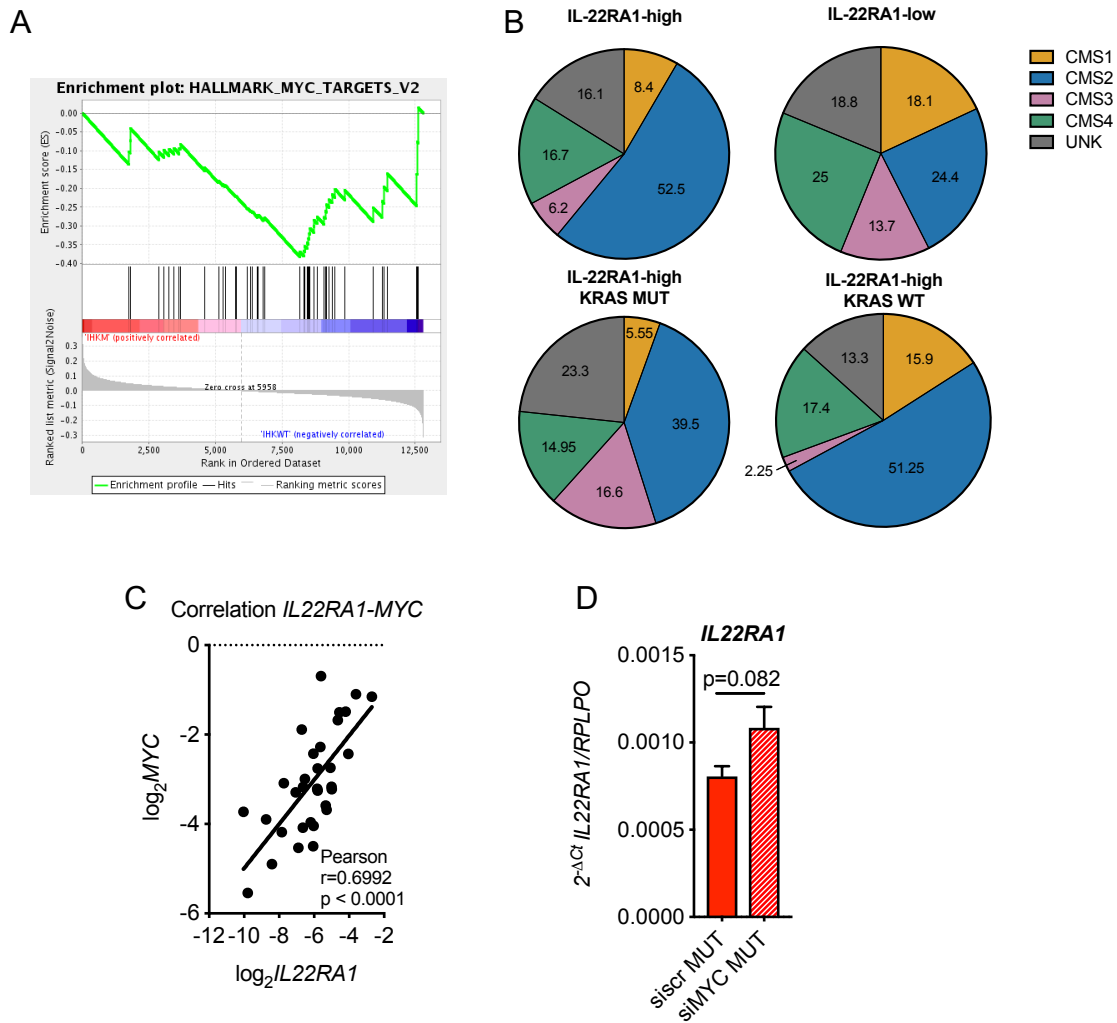
**Figure S4. KRAS mutation confers poor relapse free and overall survival in CRCs with high expression of *IL10RB*, the second subunit of the heterodimeric IL-22R. Kaplan-Meier estimates of (A to C) RFS and (D to F) OS in stage II/III patients based on (A, D) *IL10RB* expression. RFS, OS among (B, E) *IL10RB*-high and (C, F) *IL10RB*-low patients based on *KRAS* mutation status. *IL10RB*-high and low is defined by a cutpoint at the 67<sup>th</sup> percentile. *P* values**

computed using Wald-tests. Hazard ratios (HR) are univariate Cox proportional hazard ratios with 95% confidence intervals for the GSE39582 cohort.



**Figure S5. IL-22 induces *MYC* pathway transcription and c-Myc translation in DLD-1 KRAS mutant isogenic cells.** (A) Expression of genes in “MYC\_TARGETS\_V2” MSigDB Hallmark signature in 2h IL-22 stimulated DLD-1 isogenic cells. Heatmap represents row normalized  $\log_2(\text{RPKM}+1)$  values. (B) Quantification of c-Myc protein relative to  $\beta$ -actin in Western blot analysis of DLD-1 isogenic cells stimulated for 24h with 10ng/mL IL-22. N=3 independent experiments. Statistical significance assessed using paired T-tests. (C) DLD-1 KRAS

MUT cells were transfected with 40nM *MYC* siRNA (siMYC) or control siRNA (siscr) for 48h. Representative western blot for total c-MYC and quantification using LI-COR fluorescence detection (n=2).



**Figure S6. *IL22RA1*-high tumors preferentially classify as CMS2 and *IL22RA1* and *MYC* expression are correlated in prospectively collected CRC resection tissue. (A)** Gene set enrichment analysis (GSEA) comparing enrichment of Myc targets (“MYC\_TARGETS\_V2”) in *IL22RA1*-high, KRAS MUT (IHKM) versus *IL22RA1*-high, KRAS WT (IHKWT) patients in the combined cohort. **(B)** Proportion of tumors (%) in the combined cohort that classify into each of the Consensus Molecular Subtypes (CMS) of colorectal cancer. Each pie chart displays the CMS classifications within an *IL22RA1*/KRAS subgroup. **(C)** Pearson correlation analysis of *IL22RA1* and *MYC* expression assessed by qPCR in prospectively collected CRC biopsies (N=32) from patients undergoing colectomies at the Churchill Hospital, Oxford. **(D)** *IL22RA1* expression assessed by qPCR in DLD-1 KRAS MUT cells transfected with *MYC* siRNA (siMYC) or control

siRNA (siscr) for 48h. Data represent the mean  $\pm$  SEM for n=4 independent experiments.

Statistical significance was assessed using unpaired T test.

## Supplementary Tables:

<b>Table S1.</b> Univariate Coxph survival analysis of stage II/III CRC patients in training (GSE39582), verification (PETACC3), and combined cohorts based on <i>IL22RA1</i> expression, <i>KRAS</i> mutation status, and tumour location.									
		RFS				OS			
		No.	<i>P</i>	HR	95% CI	No.	<i>P</i>	HR	95% CI
<b>GSE39582</b>									
<i>IL22RA1</i> <sup>low</sup>	Proximal <i>KRAS</i> mut / <i>KRAS</i> WT	61 / 67	0.414	0.73	0.35 to 1.54	63 / 67	0.871	0.95	0.50 to 1.80
	Distal <i>KRAS</i> mut / <i>KRAS</i> WT	54 / 105	<b>0.031</b>	<b>1.77</b>	<b>1.05 to 2.97</b>	54 / 107	0.531	1.20	0.67 to 2.15
<i>IL22RA1</i> <sup>high</sup>	Proximal <i>KRAS</i> mut / <i>KRAS</i> WT	23 / 23	<b>0.012</b>	<b>4.23</b>	<b>1.38 to 13.01</b>	23 / 23	<b>0.003</b>	<b>9.41</b>	<b>2.13 to 41.60</b>
	Distal <i>KRAS</i> mut / <i>KRAS</i> WT	25 / 79	0.073	2.13	0.93 to 4.87	25 / 80	0.450	1.37	0.61 to 3.06
<b>PETACC3</b>									
<i>IL22RA1</i> <sup>low</sup>	Proximal <i>KRAS</i> mut / <i>KRAS</i> WT	81 / 99	0.252	1.32	0.82 to 2.14	81 / 99	0.404	1.26	0.73 to 2.17
	Distal <i>KRAS</i> mut / <i>KRAS</i> WT	94 / 155	0.635	1.11	0.73 to 1.67	94 / 155	0.204	1.36	0.85 to 2.18
<i>IL22RA1</i> <sup>high</sup>	Proximal <i>KRAS</i> mut / <i>KRAS</i> WT	24 / 36	<b>0.008</b>	<b>2.84</b>	<b>1.31 to 6.12</b>	24 / 36	<b>0.033</b>	<b>2.52</b>	<b>1.08 to 5.90</b>
	Distal <i>KRAS</i> mut / <i>KRAS</i> WT	55 / 102	0.281	1.32	0.79 to 2.21	55 / 102	0.088	1.73	0.92 to 3.25
<b>Combined</b>									
<i>IL22RA1</i> <sup>low</sup>	Proximal <i>KRAS</i> mut / <i>KRAS</i> WT	140 / 168	0.572	1.12	0.75 to 1.68	177 / 247	0.705	0.93	0.63 to 1.36
	Distal <i>KRAS</i> mut / <i>KRAS</i> WT	147 / 256	0.129	1.28	0.93 to 1.77	160 / 293	0.222	1.25	0.87 to 1.79
<i>IL22RA1</i> <sup>high</sup>	Proximal <i>KRAS</i> mut / <i>KRAS</i> WT	49 / 57	<b>0.001</b>	<b>2.93</b>	<b>1.57 to 5.48</b>	55 / 82	<b>0.000</b>	<b>3.70</b>	<b>1.93 to 7.07</b>
	Distal <i>KRAS</i> mut / <i>KRAS</i> WT	81 / 185	<b>0.025</b>	<b>1.64</b>	<b>1.07 to 2.54</b>	88 / 213	0.167	1.40	0.87 to 2.25

*IL22RA1*-high/low is defined by a cutpoint at the 67<sup>th</sup> percentile for each gene. Proximal is defined as tumors proximal to the splenic flexure. Cox proportional hazard ratios (HR) for relapse free survival (RFS) and overall survival (OS) are displayed with 95% confidence intervals (95% CI). Significant results are bolded. Patient numbers (No.) in each subgroup are indicated.

**Table S2.** Multivariate Coxph survival analysis of stage II, III CRC patients in training (GSE39582), verification (PETACC3), and combined cohorts based in *IL22RA1* expression, *KRAS* mutation status, and tumour location.

		RFS			OS		
		<i>P</i>	HR	95% CI	<i>P</i>	HR	95% CI
<b>GSE39582</b>							
<i>IL22RA1</i> <sup>low</sup>	Proximal <i>KRAS</i> mut / <i>KRAS</i> WT	0.466	0.71	0.28 to 1.78	0.617	1.24	0.53 to 2.91
	Distal <i>KRAS</i> mut / <i>KRAS</i> WT	0.375	1.33	0.71 to 2.47	0.820	0.92	0.43 to 1.96
<i>IL22RA1</i> <sup>high</sup>	Proximal <i>KRAS</i> mut / <i>KRAS</i> WT	<b>0.032</b>	<b>5.13</b>	<b>1.15 to 22.84</b>	<b>0.015</b>	<b>12.74</b>	<b>1.66 to 97.98</b>
	Distal <i>KRAS</i> mut / <i>KRAS</i> WT	0.068	2.43	0.94 to 6.32	0.740	1.18	0.44 to 3.17
<b>PETACC3</b>							
<i>IL22RA1</i> <sup>low</sup>	Proximal <i>KRAS</i> mut / <i>KRAS</i> WT	0.205	1.42	0.83 to 2.42	0.195	1.51	0.81 to 2.83
	Distal <i>KRAS</i> mut / <i>KRAS</i> WT	0.071	1.51	0.97 to 2.36	<b>0.010</b>	<b>1.98</b>	<b>1.18 to 3.33</b>
<i>IL22RA1</i> <sup>high</sup>	Proximal <i>KRAS</i> mut / <i>KRAS</i> WT	<b>0.003</b>	<b>4.87</b>	<b>1.70 to 13.96</b>	<b>0.002</b>	<b>10.75</b>	<b>2.46 to 46.89</b>
	Distal <i>KRAS</i> mut / <i>KRAS</i> WT	0.386	1.26	0.75 to 2.13	0.092	1.77	0.91 to 3.42
<b>Combined</b>							
<i>IL22RA1</i> <sup>low</sup>	Proximal <i>KRAS</i> mut / <i>KRAS</i> WT	0.581	1.14	0.72 to 1.81	0.575	1.14	0.71 to 1.84
	Distal <i>KRAS</i> mut / <i>KRAS</i> WT	0.185	1.27	0.89 to 1.81	0.124	1.37	0.92 to 2.05
<i>IL22RA1</i> <sup>high</sup>	Proximal <i>KRAS</i> mut / <i>KRAS</i> WT	<b>0.000</b>	<b>4.18</b>	<b>1.90 to 9.19</b>	<b>0.000</b>	<b>6.74</b>	<b>2.60 to 17.43</b>
	Distal <i>KRAS</i> mut / <i>KRAS</i> WT	<b>0.018</b>	<b>1.75</b>	<b>1.10 to 2.78</b>	0.098	1.55	0.92 to 2.60

*IL22RA1*-high/low is defined by a cutpoint at the 67<sup>th</sup> percentile for each gene. Proximal is defined as tumors proximal to the splenic flexure. Cox proportional hazard ratios (HR) for relapse free survival (RFS) and overall survival (OS) are displayed with 95% confidence intervals (95% CI). Significant results are bolded. Covariates included in the analysis were: microsatellite stability/instability (MSI) status, BRAF mutation status, and stage.

**Table S3.** Univariate Coxph survival analysis of stage II, III CRC patients in training (GSE39582), verification (PETACC3), and combined cohorts based on *IL10RB* and KRAS mutation status.

	No.	RFS			No.	OS		
		<i>P</i>	HR	95% CI		<i>P</i>	HR	95% CI
<b>GSE39582</b>								
IL10RB <sup>high</sup> / IL10RB <sup>low</sup>	148 / 289	0.9170	1.02	0.72 to 1.45	148 / 294	0.7690	0.95	0.67 to 1.35
KRAS mut / KRAS WT	163 / 274	<b>0.0103</b>	<b>1.57</b>	<b>1.11 to 2.23</b>	165 / 277	0.0533	1.40	1.00 to 1.96
Within IL10RB <sup>low</sup> : KRAS mut / KRAS WT	107 / 182	0.8960	1.03	0.66 to 1.60	109 / 185	0.6640	1.10	0.72 to 1.67
Within IL10RB <sup>high</sup> : KRAS mut / KRAS WT	56 / 92	<b>0.0000</b>	<b>3.62</b>	<b>1.95 to 6.70</b>	56 / 92	<b>0.0039</b>	<b>2.43</b>	<b>1.33 to 4.45</b>
Within KRAS WT: IL10RB <sup>high</sup> / IL10RB <sup>low</sup>	92 / 182	<b>0.0256</b>	<b>0.53</b>	<b>0.30 to 0.93</b>	92 / 185	0.0938	0.65	0.40 to 1.08
Within KRAS mut: IL10RB <sup>high</sup> / IL10RB <sup>low</sup>	56 / 107	<b>0.0196</b>	<b>1.84</b>	<b>1.10 to 3.07</b>	56 / 109	0.2840	1.34	0.79 to 2.27
<b>PETACC3</b>								
IL10RB <sup>high</sup> / IL10RB <sup>low</sup>	225 / 421	<b>0.0131</b>	<b>1.36</b>	<b>1.07 to 1.74</b>	225 / 421	0.2820	1.17	0.88 to 1.57
KRAS mut / KRAS WT	254 / 392	<b>0.0215</b>	<b>1.34</b>	<b>1.04 to 1.72</b>	254 / 392	<b>0.0051</b>	<b>1.51</b>	<b>1.13 to 2.02</b>
Within IL10RB <sup>low</sup> : KRAS mut / KRAS WT	169 / 252	0.0856	1.33	0.96 to 1.84	169 / 252	<b>0.0302</b>	<b>1.51</b>	<b>1.04 to 2.18</b>
Within IL10RB <sup>high</sup> : KRAS mut / KRAS WT	85 / 140	0.0981	1.39	0.94 to 2.05	85 / 140	0.0577	1.57	0.99 to 2.51
Within KRAS WT: IL10RB <sup>high</sup> / IL10RB <sup>low</sup>	140 / 252	0.0520	1.40	1.00 to 1.96	140 / 252	0.3140	1.23	0.82 to 1.86
Within KRAS mut: IL10RB <sup>high</sup> / IL10RB <sup>low</sup>	85 / 169	0.0580	1.44	0.99 to 2.10	85 / 169	0.2920	1.26	0.82 to 1.94
<b>Combined</b>								
IL10RB <sup>high</sup> / IL10RB <sup>low</sup>	373 / 710	0.1190	1.15	0.96 to 1.37	445 / 873	0.4830	1.07	0.89 to 1.28
KRAS mut / KRAS WT	417 / 666	<b>0.0006</b>	<b>1.43</b>	<b>1.16 to 1.75</b>	481 / 837	<b>0.0054</b>	<b>1.35</b>	<b>1.09 to 1.66</b>
Within IL10RB <sup>low</sup> : KRAS mut / KRAS WT	277 / 433	0.1250	1.22	0.95 to 1.58	322 / 551	0.1710	1.20	0.92 to 1.56
Within IL10RB <sup>high</sup> : KRAS mut / KRAS WT	140 / 233	<b>0.0002</b>	<b>1.89</b>	<b>1.36 to 2.63</b>	159 / 286	<b>0.0027</b>	<b>1.73</b>	<b>1.21 to 2.48</b>
Within KRAS WT: IL10RB <sup>high</sup> / IL10RB <sup>low</sup>	233 / 433	0.7310	0.95	0.71 to 1.27	286 / 551	0.4440	0.89	0.66 to 1.20
Within KRAS mut: IL10RB <sup>high</sup> / IL10RB <sup>low</sup>	140 / 277	<b>0.0172</b>	<b>1.45</b>	<b>1.07 to 1.97</b>	159 / 322	0.1680	1.26	0.91 to 1.75

*IL10RB*-high is defined by a cutpoint at the 67<sup>th</sup> percentile. Cox proportional hazard ratios (HR) for relapse free survival (RFS) and overall survival (OS) are displayed with 95% confidence intervals (95% CI). Significant results are bolded. Patient numbers (No.) in each subgroup are indicated.