An algorithm-based meta-analysis of genome- and proteome-wide data identifies a combination of potential plasma biomarkers for colorectal cancer

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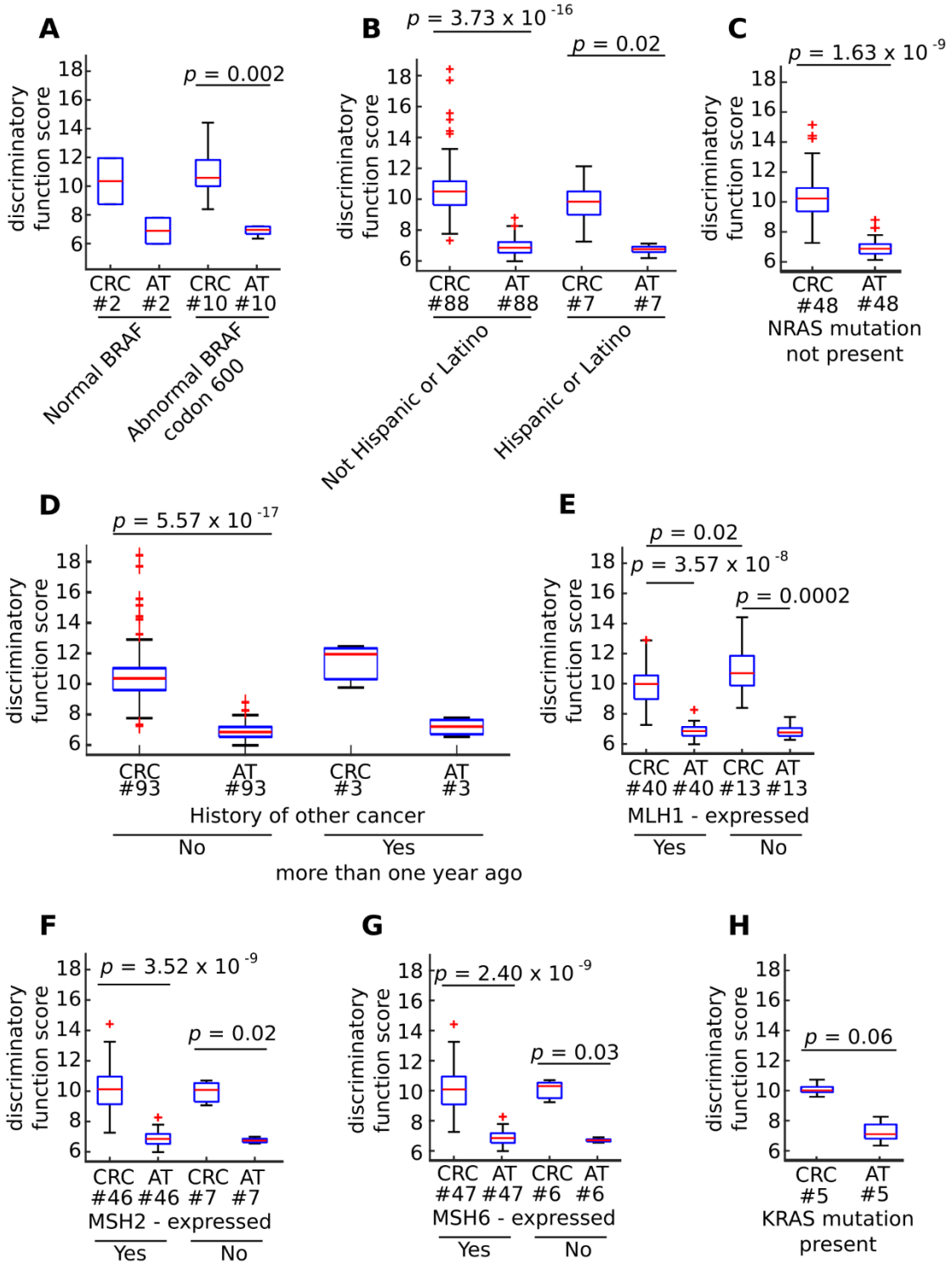
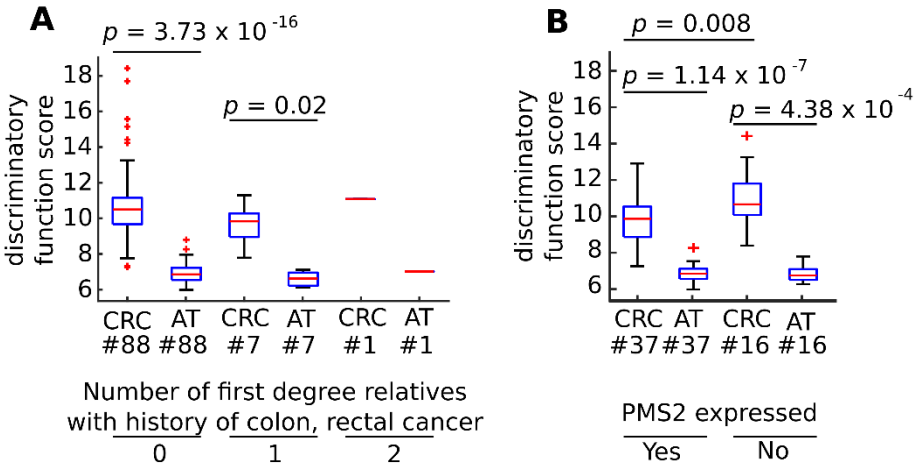
Supplementary Figures

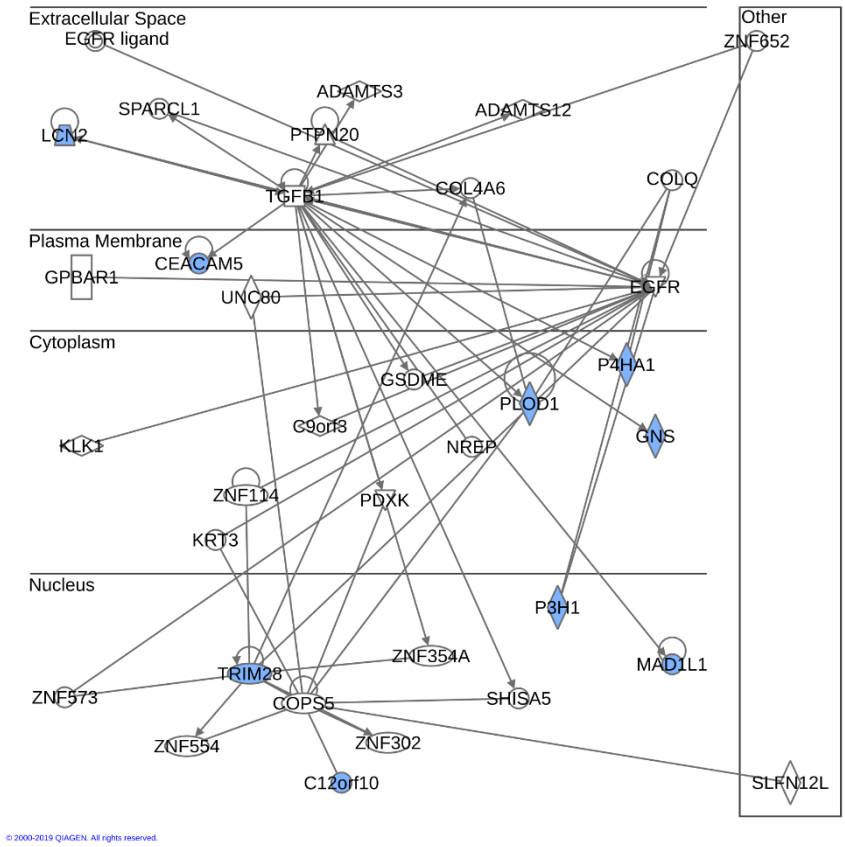


Supplementary Figure 1. Classification accuracy of colorectal cancer tumor samples (CRC) and adjacent tissue (AT). Boxplot presenting discriminatory function score for CRC and AT groups (sum of the 2 to the power of Unshared Log Ratio scores reported in the study for selected proteins: PLOD1, P4HA1, LCN2, GNS, C12orf10, P3H1, TRIM28, CEACAM5, MAD1L1). Significance *p* value was calculated using double-sided Wilcoxon Signed Rank test for paired samples and Wilcoxon Rank Sum test for unpaired samples. The bars in the boxes represent median, 25th and 75th percentiles, while whiskers extend to ± 2.7σ. Numbers below the boxplots denote number of observations per category. Discrimination between CRC and AT samples divided by (**A**) BRAF gene analyses results; (**B**) Ethnicity; (**C**) Presence of NKRAS mutation (only one sample with no NKRAS mutation is

reported in the study, see Supplementary Data 2) (**D**) History of other cancers; (**E**) MLH1 expression; (**F**) MSH2 expression; (**G**) MSH6 expression; (**H**) Presence of KRAS mutation (Only one sample is reported in the study with no KRAS mutation, see Supplementary Data 2).



Supplementary Figure 2. Classification accuracy of colorectal cancer tumor samples (CRC) and adjacent tissue (AT). Boxplot presenting discriminatory function score for CRC and AT groups (sum of the 2 to the power of Unshared Log Ratio scores reported in the study for selected proteins: PLOD1, P4HA1, LCN2, GNS, C12orf10, P3H1, TRIM28, CEACAM5, MAD1L1). Significance *p* value was calculated using double-sided Wilcoxon Signed Rank test for paired samples and Wilcoxon Rank Sum test for unpaired samples. The bars in the boxes represent median, 25th and 75th percentiles, while whiskers extend to ± 2.7σ. Numbers below the boxplots denote number of observations per category. Discrimination between CRC and AT samples divided by (**A**) Number of first degree relatives with history of colon, rectal cancer; (**B**) PMS2 expression.



Supplementary Figure 3. Ingenuity Pathway Analysis shows that the nine proteins (blue) are highly interconnected and form a network module that regulates cell death and proliferation. In order to test the pathogenic relevance of the nine biomarkers we performed Ingenuity Pathway Analysis, as previously described[1]. Briefly, the background is that proteins that are associated with the same disease tend to be functionally related and interact, forming network modules [1]. Thus, if the proteins did interact, this would support their pathogenic and biomarker relevance. Indeed, we found that the nine proteins did form a network module, which had an overriding function, namely regulating cell death and proliferation.

	Selected cutoff	AUC	PPV	NPV	sensitivity	specificity
LCN2	-3,57	0,52	0,50	--	1,00	0
PLOD1	0,22	0,93	1,00	0,89	0,88	1,00
MAD1L1	-3,19	0,56	0,53	1,00	1,00	0,13
P3H1	-0,43	0,55	0,50	--	1,00	0
CEACAM5	-0,79	0,63	0,50	--	1,00	0
P4HA1	-1,85	0,68	0,62	1,00	1,00	0,25
C12orf10	-3,40	0,56	0,50	--	1,00	0
GNS	-0,05	0,84	0,75	0,75	0,75	0,75
TRIM28	0,30	0,98	0,88	0,88	0,88	0,88

Supplementary Table 1. The nine individual biomarkers were iundividually tersted as classifiers for CRC. Briefly we have created classifiers using 90% of the patient samples (calculate cut-off values) and we have tested the classifier based on the remaining 10% of the samples. We have obtained following cut-off values, positive and negative predictive values, and AUCs. Missing negative predictive values were obtained for classifiers where all samples were classified as "positive" i.e. patient samples.

Description of Additional Supplementary Files

Supplementary Data 1. Randomized elastic net results. 113 ordered by their predictive value (randomized elastic net frequency).

Supplementary Data 2. Covariate impact on the discriminatory function score. We tested if discriminatory function score differs depending on covariates. P values were calculated using Wilcoxon Signed test where applicable or Wilcoxon Rank Sum test. All p values together with the number of samples per group are reported in separate excel sheet.

References

1 Gustafsson, M. *et al.* A validated gene regulatory network and GWAS identifies early regulators of T cell-associated diseases. *Science Translational Medicine* **7** (2015).