Quantitative proteomics reveals that distant recurrence-associated protein R-Ras and Transgelin predict post-surgical survival in patients with Stage III colorectal cancer

SUPPLEMENTARY DATA

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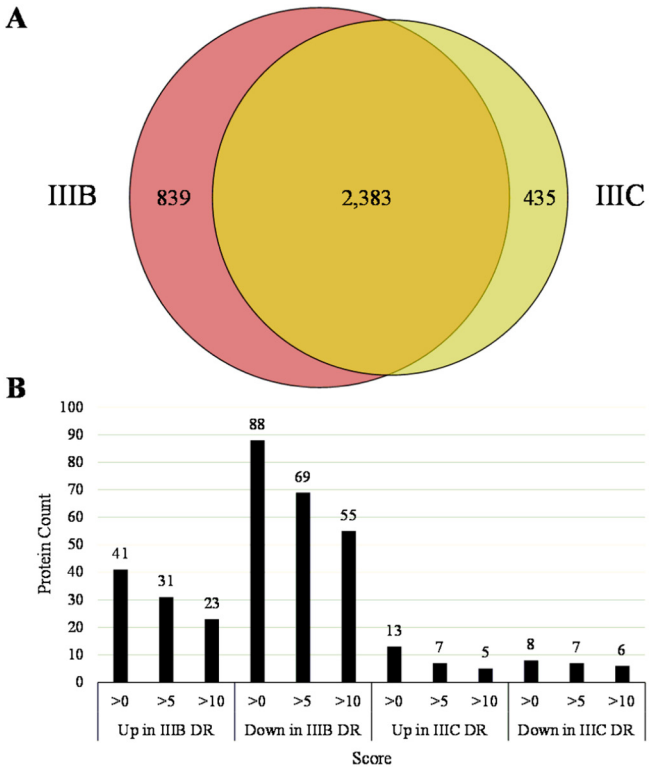
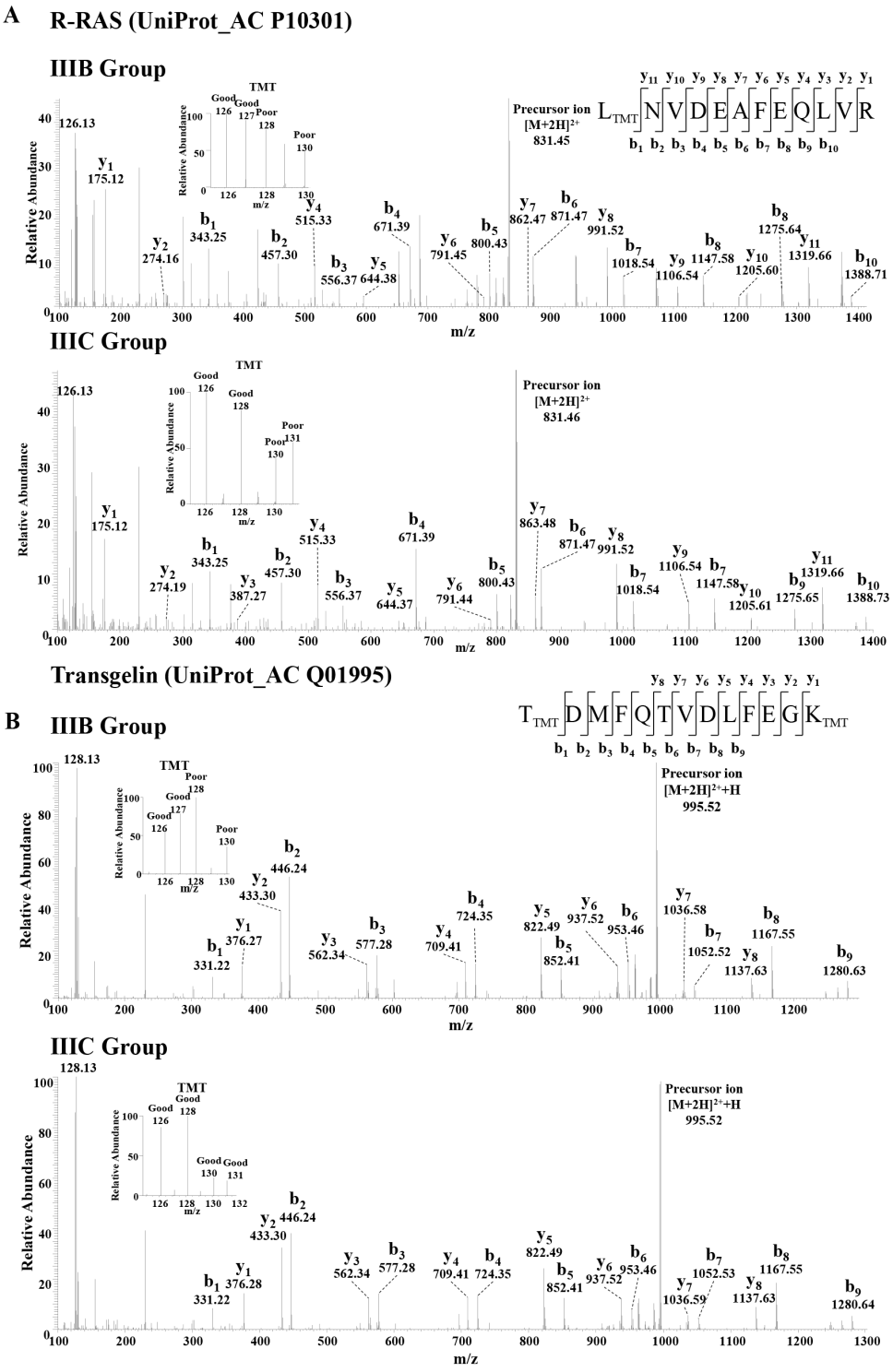
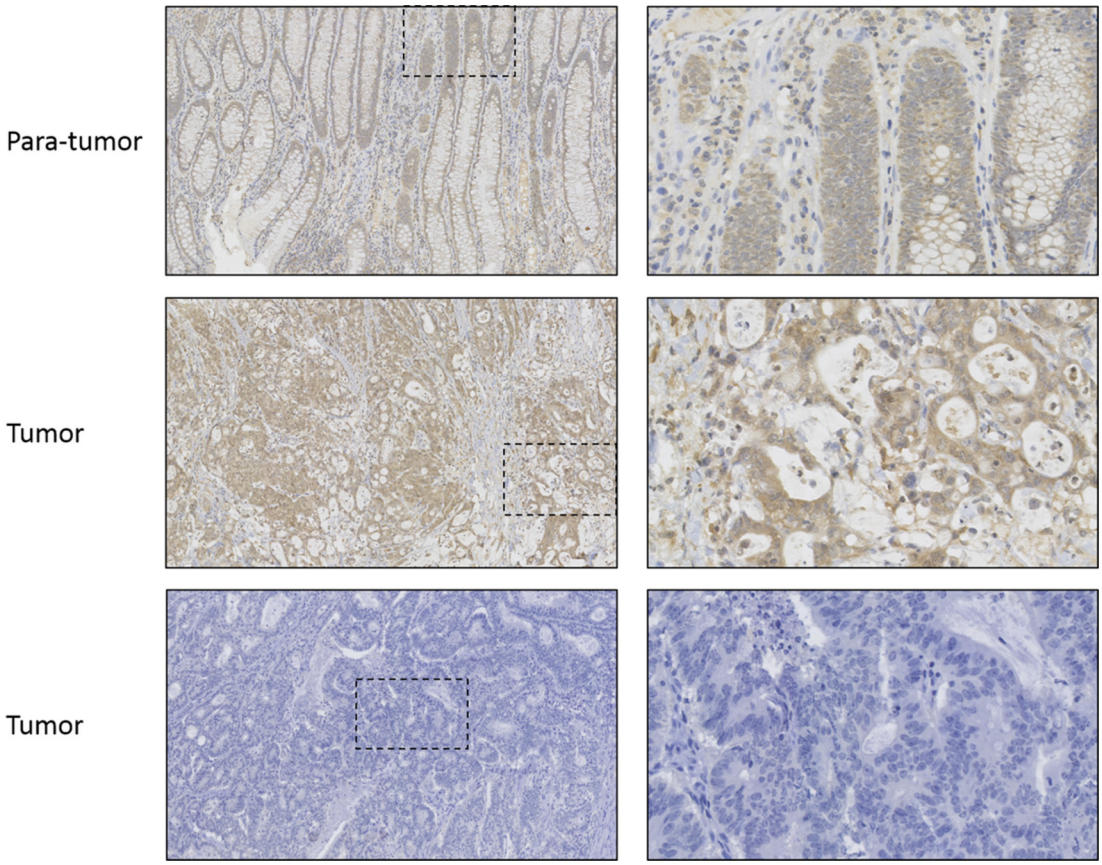


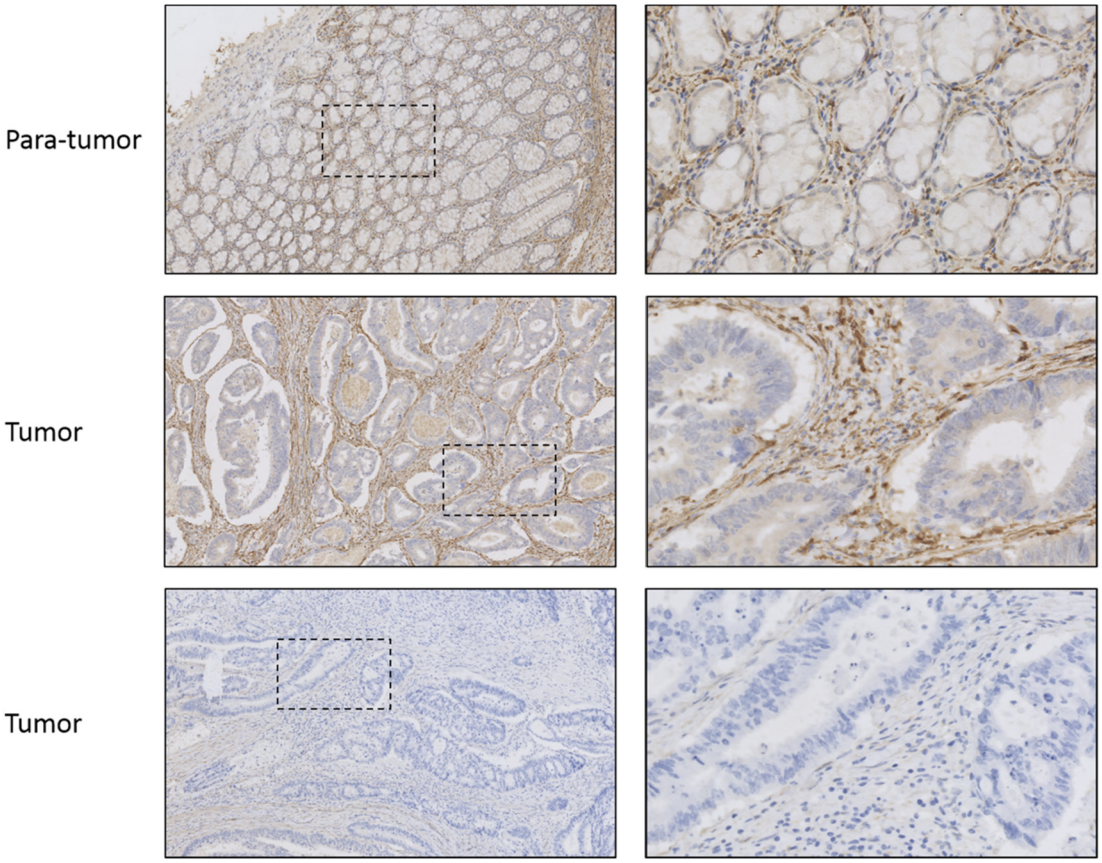
Supplementary Figure S1: Quantitative MS identified 146 DEPs associated with distant recurrence. A. 2,383 proteins were identified in both IIIB and IIIC groups. Additionally, 839 IIIB-specific proteins and 435 IIIC-specific proteins were also identified. **B.** Out of the total 3,657 proteins, 146 proteins were differentially expressed between good outcome and distant recurrence. About half of them had a score more than 10. (DR, distant recurrence).



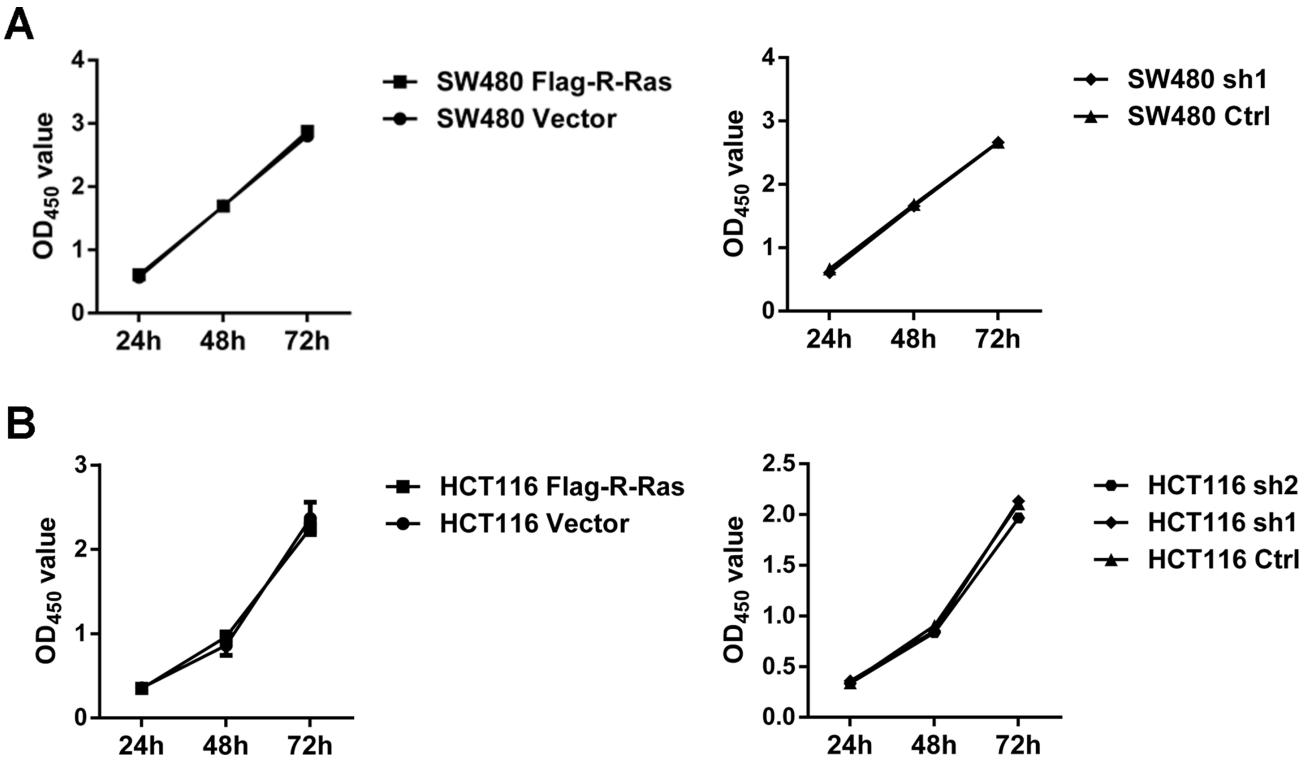
Supplementary Figure S2: MS/MS spectra of R-Ras and Transgelin unique peptides. A. MS/MS spectra of R-Ras aa177-188 (m/z = 831.45) in the MS experiments of IIIB and IIIC groups. **B.** MS/MS spectra of Transgelin aa109-121 (m/z = 995.52) in the MS experiments of IIIB and IIIC groups. The TMT reporter signals were also shown.



Supplementary Figure S3: Representative photos (amplification: 100) of anti-R-Ras IHC with regions zoomed in (amplification: 400). Top panel: R-Ras dense staining is observed in epithelial cells of longitudinally cut mucosa crypts of para-tumor tissue. Medium panel: positive R-Ras staining of poorly differentiated adenocarcinoma. Bottom panel: well differentiated adenocarcinoma with negative R-Ras staining.



Supplementary Figure S4: Representative photos (amplification: 100) of anti-Transgelin IHC with regions zoomed in (amplification: 400). Top panel: Transgelin dense staining is observed around tangentially cut mucosa crypts of para-tumor tissue. Medium panel: moderately differentiated tumor tissue of positive Transgelin staining. Bottom panel: Transgelin-negative tumor tissue shows desmoplasia. Necrotic debris can be observed in glandular lumina.



Supplementary Figure S5: R-Ras has no effect on CRC cells proliferation. (A) SW480 and (B) HCT116 were stably transfected with pLv-CP06 vector and pLv-CP06-Flag-RRAS (left panel); pLv-shRNA-KP Ctrl, pLv-shRNA-KP-sh1, and pLv-shRNA-KP-sh2 (right panel), respectively. Monolayer growth rates of cells were determined by CCK8 assay. Results are presented as means±SD (n=4).

Supplementary Table S1:

See Supplementary File 1

Supplementary Table S2: Manual search in literature databases found that the relevance of 66 DEPs with CRC has been reported previously

See Supplementary File 2

Supplementary Table S3: R-Ras and Transgelin expression in tumor and para-tumor tissue of Stage III CRC

Protein	Tissue type	Number	Staining		*p*-value	χ2 value
			Positive	Negative		
R-Ras	Tumor	192	83	109	<0.001	59.21
	Para-tumor	192	157	35		
Transgelin	Tumor	192	84	108	<0.001	75.21
	Para-tumor	192	166	26		

Supplementary Table S4: DEPs expression measurement information IIIB

See Supplementary File 3