

## **Supplementary Material**

### **A universal cell-free DNA approach for response prediction to preoperative chemoradiation in rectal cancer**

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#### **Content:**

- 1. Supplementary Table 1:** Additional clinicopathological characteristics of study population
- 2. Supplementary Figure 1:** Tissue panel of eight colon-specific methylation markers.
- 3. Supplementary Table 2:** Coverage and quality statistics of the targeted bisulfite sequencing analysis. Due to file size, available as separate excel sheet.

**Supplementary Table 1. Additional clinicopathological characteristics of study population**

	No pathCR (n = 30)	PathCR (n = 7)	<i>P</i>
Total mesorectal excision (%)	100%	100%	<i>ns</i>
Low anterior resection/ Abdominoperineal resection/unknown (%)	67%/30%/3%	56%/0/44%	<i>ns</i>
Distal surgical margins involvement <sup>#</sup>	14%		
Circumferential margins involvement <sup>#</sup>	10%		
Lymph node harvest (mean) <sup>§</sup>	19.6	19	<i>ns</i>
Lymphovascular \ perineural invasion (%) <sup>§</sup>	27%	0%	<i>P &lt; 0.05</i>
Mismatch repair deficiency (%) <sup>^</sup>	9.5%		

*ns* – not significant

<sup>#</sup> Data not available for 1 patient

<sup>§</sup> Data not available for 2 patients in non-pathCR group and 3 in pathCR group

<sup>^</sup> Data available for 21 patients (70%)

**Supplementary Figure 1: Tissue panel of eight colon-specific methylation markers.**

The percentage of molecules with colon-specific methylation in genomic DNA was obtained from different cell types and tissues. In these experiments, we determined the fraction of molecules that were largely unmethylated across amplified CpG sites.

