**Supplementary Results and Discussion**

***Defect in DNA damage repair drives chemokine-induced immune response in breast cancer***

DDIR-positive tumours, as previously derived in the BC study (1), are defined by an impairment in HR and FA DNA damage repair machinery, which in turn is associated with increased levels of chemokine signalling and lymphocytic infiltration. Here, we utilise transcriptional profiles from the TRANSBIG BC cohort (2), originally used in the development of the DDIR assay, to both validate and further characterise the signalling associated with DDIR biology in BC. Using DDIR continuous scores alongside ssGSEA scores for both KEGG HR and REACTOME FA pathways, we observe a positive (>3) correlation with DDIR in BC (Supplementary Figure 3A; Pearson r = 0.3457 and Figure 3B; Pearson r = 0.3209).

We next tested the association between DDIR and HR and FA pathways in CRC, using a subset of the FOCUS and FOxTROT clinical trial cohorts, applying a CRC-specific DDIR threshold. Unlike in BC, in the FOCUS CRC cohort we observe no clear correlation (<3) between DDIR score and either of the DNA damage repair pathways (Supplementary Figure 3C; Pearson r = 0.1225 and Figure 3D; Pearson r = -0.0203). Similarly, no correlation between DDIR score and ssGSEA scores for these DNA damage repair pathways was found in the FOxTROT clinical trial cohort (Supplementary Figure 3E; Pearson r = 0.085 and Figure 3F; Pearson r = 0.1114), implying different mechanisms underlie the gene expression captured by the DDIR assay in CRC.

DDIR signature was developed on the foundation of BC biology where 25-40% of BC patients display disruption in HR and FA/BRCA pathways through germline or somatic BRCA mutations (3,4). Pan-cancer analyses have revealed various DDR signalling pathway alterations according to tumour type, with gene mutations in FA/BRCA enriched within BC and OC, whereas defects in the MMR machinery are observed in CRC (5,6). Although some studies have highlighted mutations in BRCA1, BRCA2 and other HR components within CRC, these mutations are observed at very low frequency compared to deficient MMR prevalence. Also, the FA pathway is very rarely linked with hereditary CRC compared to sporadic CRC (7). This is further consolidated with our finding where DDIR-positivity is strongly associated with HR and FA pathways in BC but no such correlation was seen for CRC

***Activation of immune response pathways upregulated in DDIR-positives***

In addition to DNA repair and general immune signalling, Parkes and colleagues previously demonstrated that DDIR-positivity in BC was specifically associated with activation of the cGAS/STING/TBK1 innate immune response axis (8). Assessment of the expression levels of cGAS (Supplementary Figure 4A) and STING (Supplementary Figure 4B) was performed using IHC in the CRC FOCUS cohort, but did not reveal any significant associations with DDIR-positivity (Supplementary Figure 4C; Student’s t-test, p = 0.2187, and Figure 4D; Pearson r = 0.0525. Figure 4E; Student’s t-test, p = 0.0647, and Figure 4F; Pearson r = 0.0415). While our observation shows no evidence of involvement for the STING pathway in DDIR in our CRC cohorts, our data cannot conclusively rule out activation of specific STING-related pathways events, as such dynamic signalling may not be detectable by the applied methodologies in our study, and would require further investigations (9).

**References**

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