

SUPPLEMENTARY INFORMATION

Title: Neoadjuvant immune checkpoint blockade in women with mismatch repair deficient endometrial cancer: a phase I study

Anneke L. Eerkens^{1*}, Koen Brummel^{1*}, Annegé Vledder¹, Sterre T. Paijens², Marta Requesens¹, Dominik Loiero³, Nienke van Rooij¹, Annechien Plat¹, Floris-Jan Haan¹, Patty Klok¹, Refika Yigit¹, Thijs Roelofsen¹, Natascha M de Lange⁴, Rie Klomp⁵, David Church^{6,7}, Arja ter Elst⁸, René Wardenaar⁸, Diana Spierings⁸, Floris Foijer⁸, Viktor Hendrik Koelzer³, Tjalling Bosse⁹, Joost Bart¹⁰, Mathilde Jalving¹¹, Anna K.L. Reyners¹¹, Marco de Bruyn^{1‡}, Hans W. Nijman^{1‡}

¹Department of Obstetrics and Gynaecology, University Medical Centre Groningen, Groningen, The Netherlands.

²Department of Radiotherapy, Erasmus University Medical Centre, Rotterdam, The Netherlands.

³Department of Pathology and Molecular Pathology, University Hospital Zurich, University of Zurich, Zurich, Switzerland.

⁴Department of Obstetrics and Gynaecology, Isala, Zwolle, The Netherlands.

⁵Department of Obstetrics and Gynaecology, Treant, Emmen, The Netherlands.

⁶Welcome Centre for Human Genetics, University of Oxford, Oxford, United Kingdom.

⁷National Institute for Health Research (NIHR) Oxford Biomedical Research Centre, Oxford University Hospitals NHS Foundation Trust, John Radcliffe Hospital, Oxford, United Kingdom.

⁸European Research Institute for the Biology of Ageing, University of Groningen, University Medical Centre Groningen, Groningen, The Netherlands.

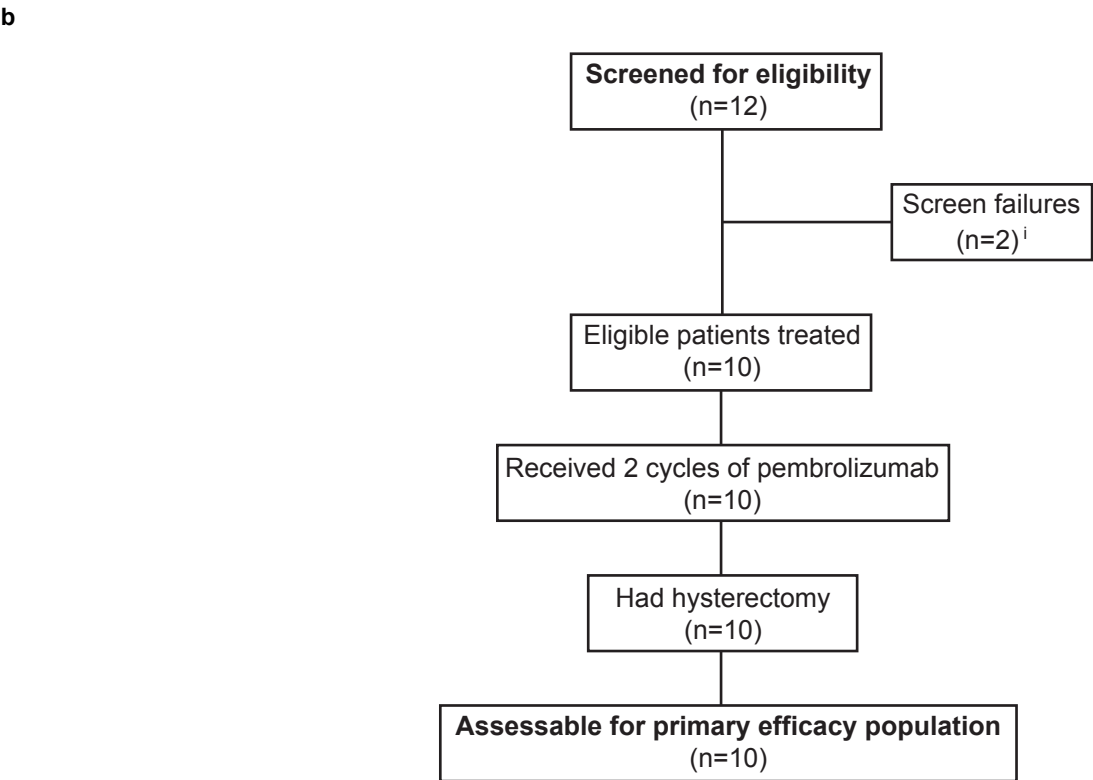
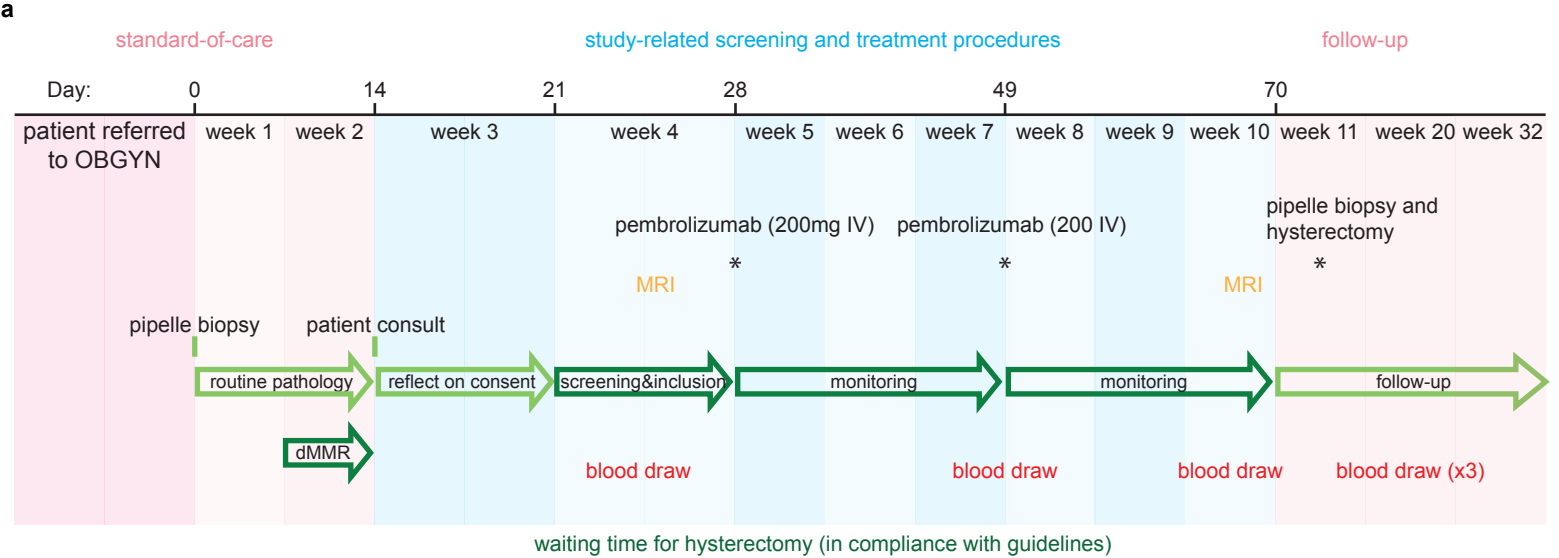
⁹Department of Pathology, Leiden University Medical Centre, Leiden, The Netherlands.

¹⁰Department of Pathology, University Medical Centre Groningen, Groningen, The Netherlands.

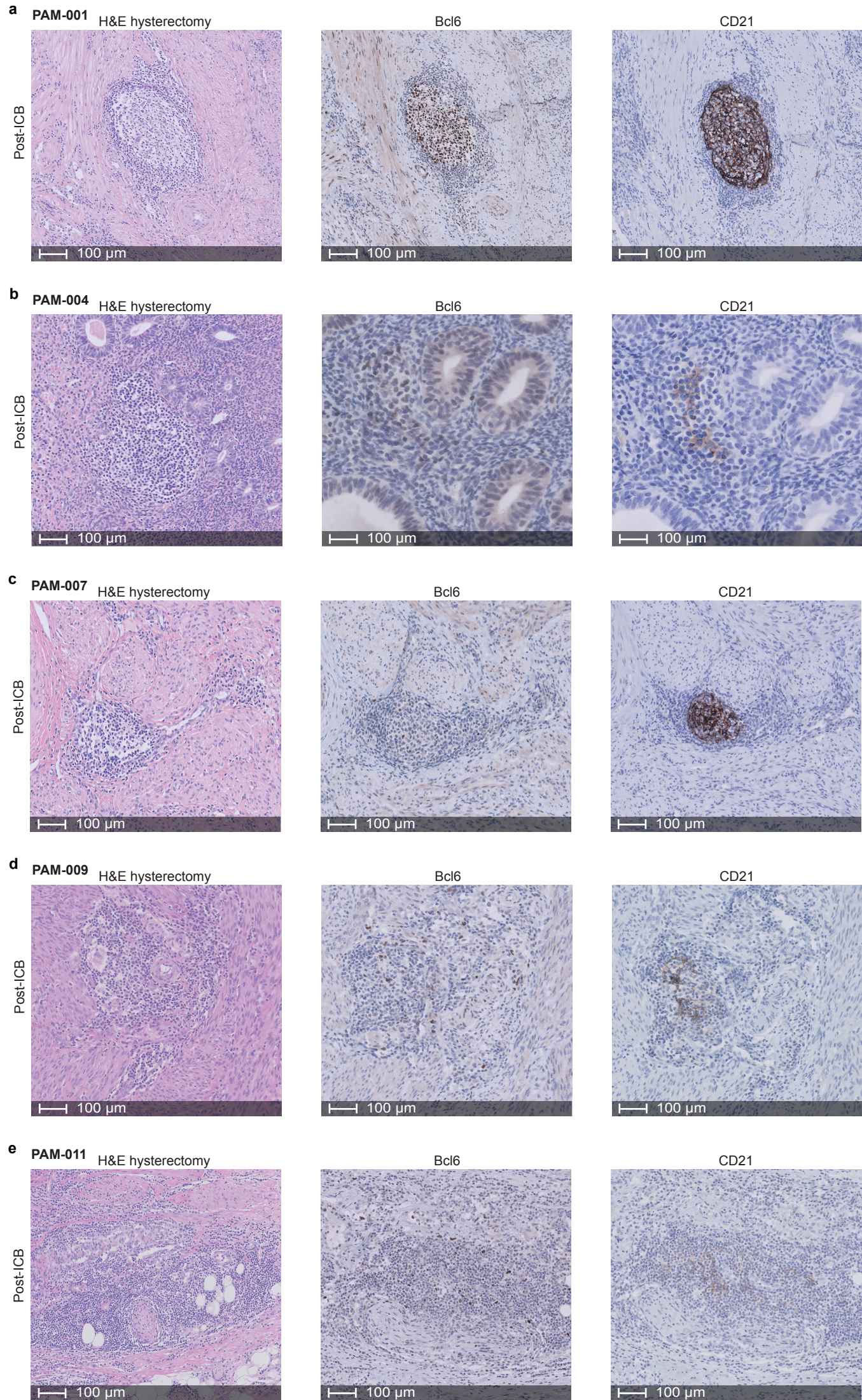
¹¹Department of Medical Oncology, University Medical Centre Groningen, Groningen, The Netherlands.

*These authors contributed equally

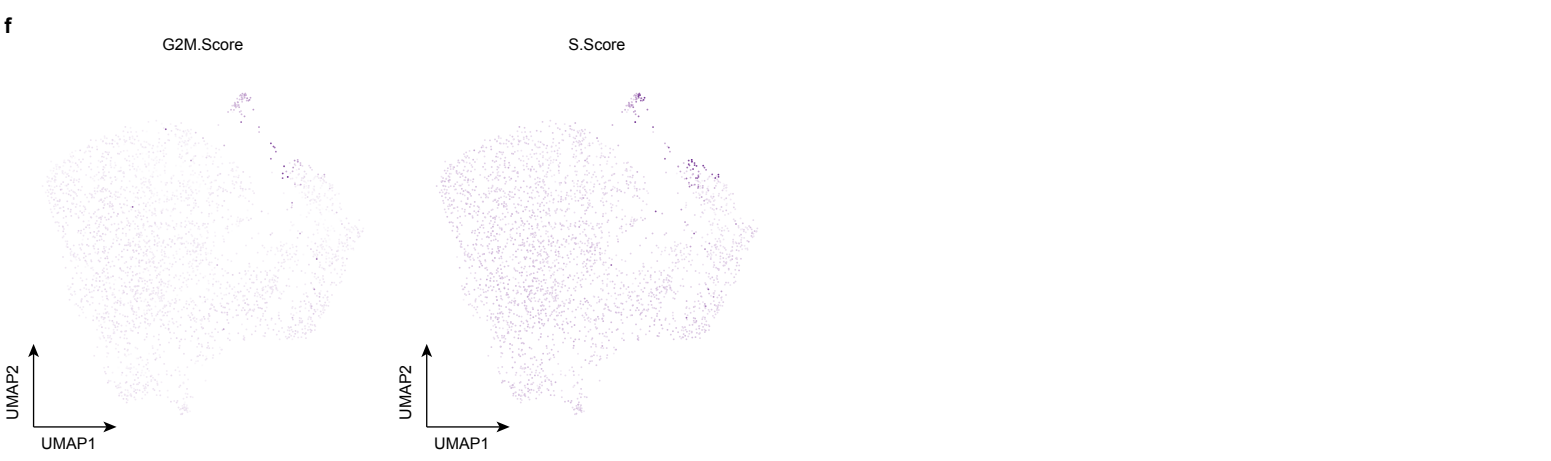
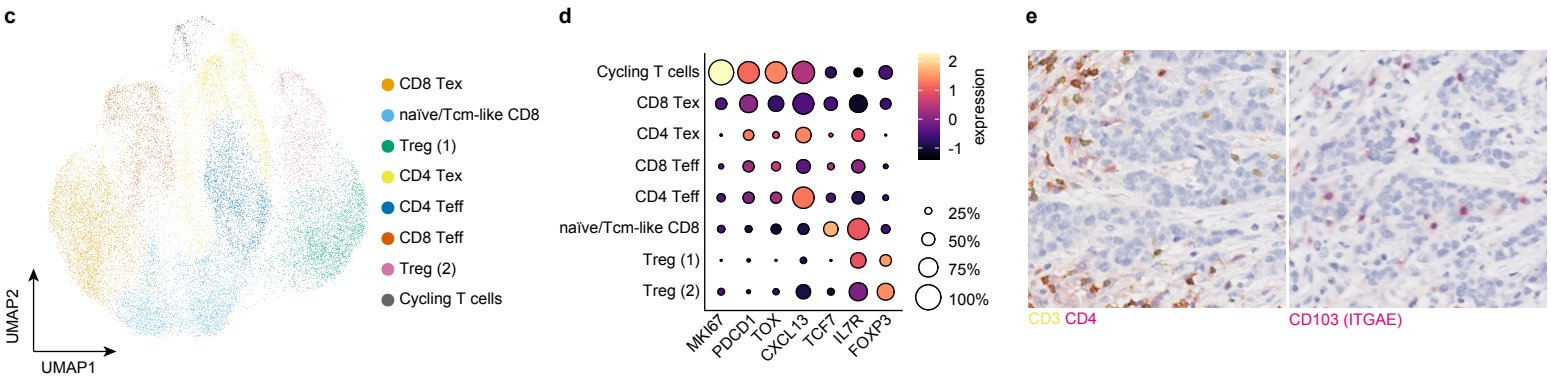
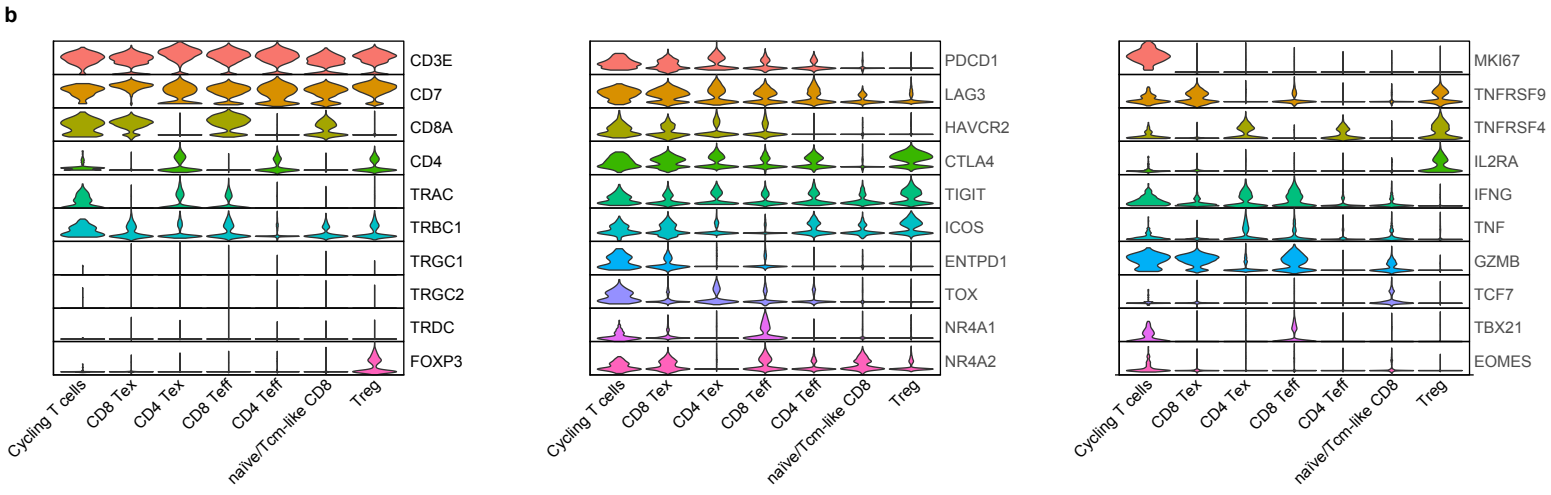
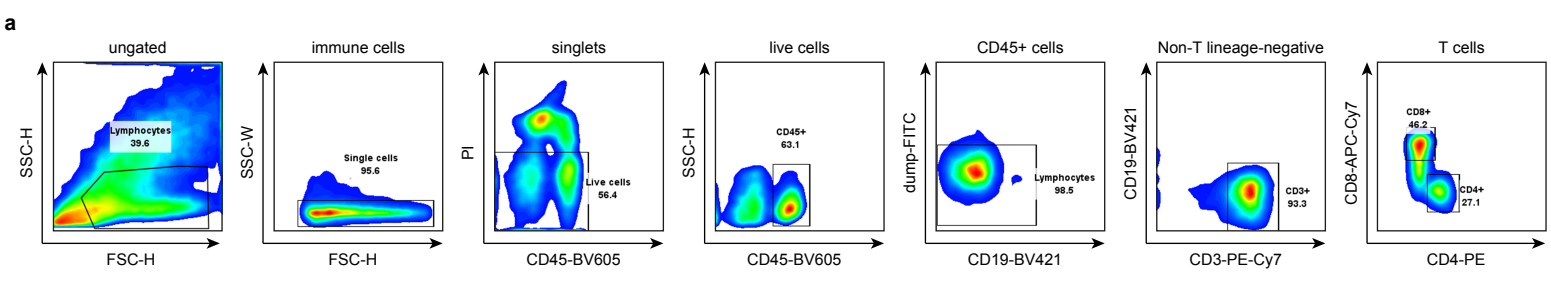
‡ Corresponding authors: h.w.nijman@umcg.nl; m.de.bruyn@umcg.nl



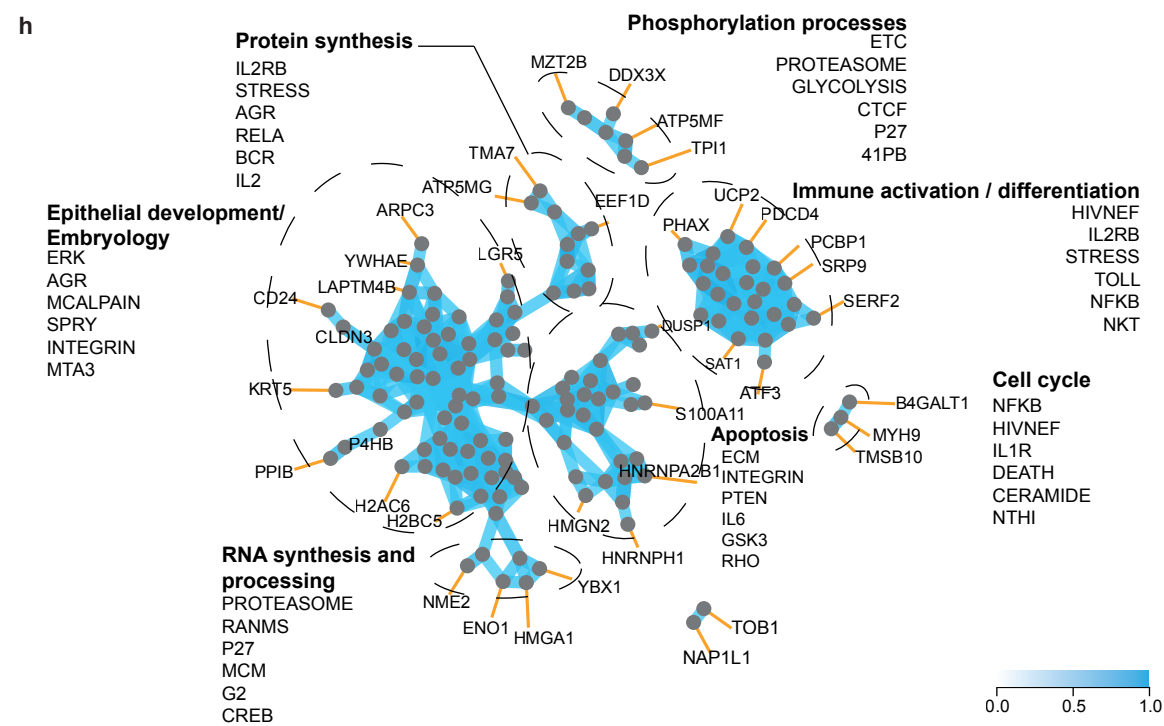
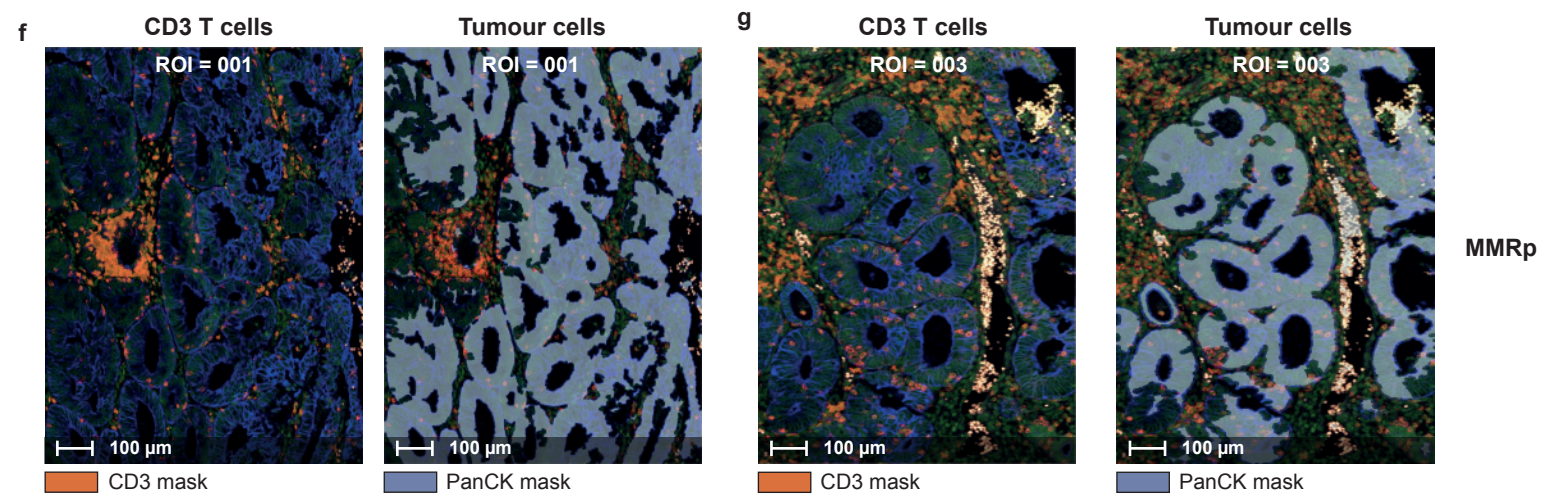
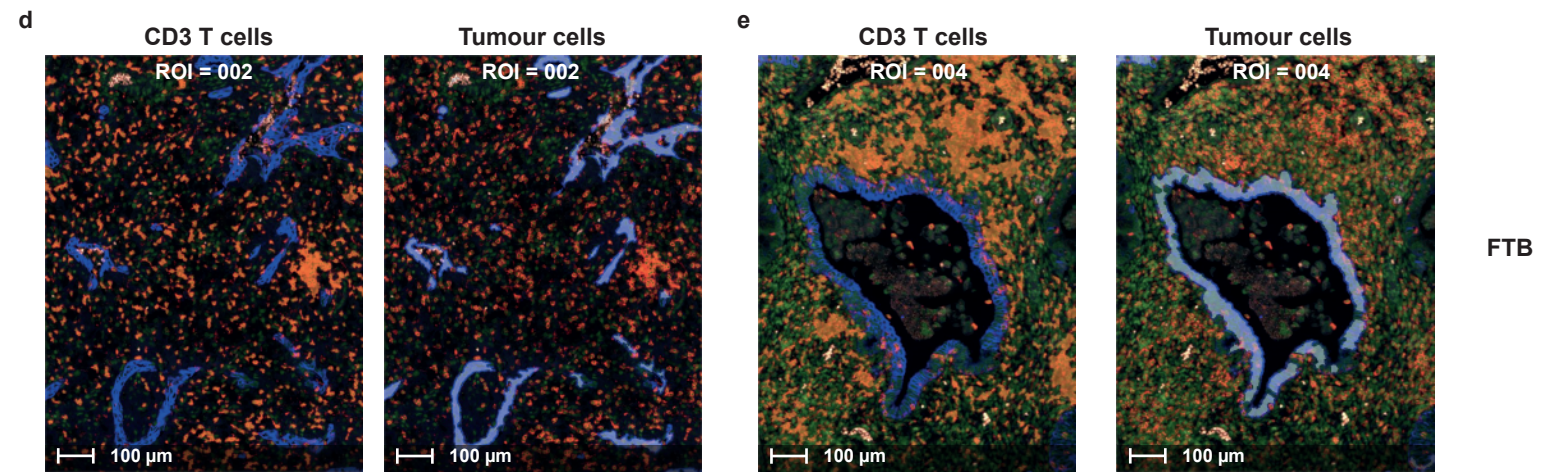
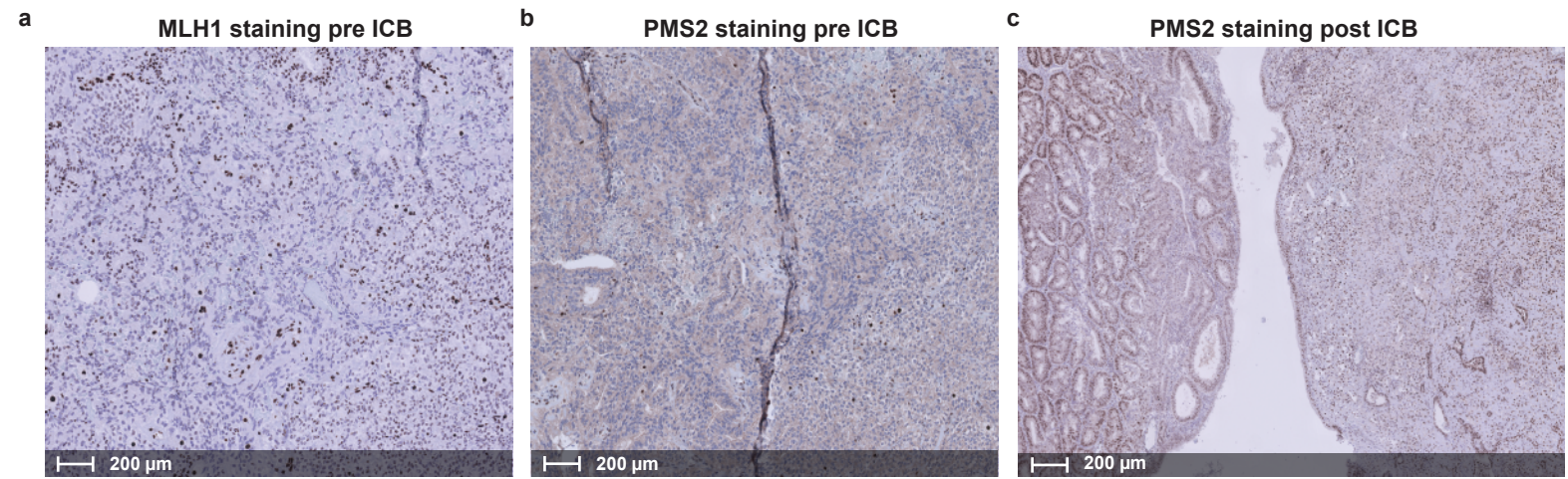
Supplementary Fig. 1 Study design and consort diagram | a, Study design. Women with resectable pathologically confirmed MMRd endometrial cancer grade 1-3 were enrolled. Patients were treated with two cycles of neoadjuvant pembrolizumab 200 mg IV every 3 weeks, followed by standard-of-care hysterectomy. Maximum time from informed consent to surgery was 6-7 weeks. OBGYN: obstetrics and gynaecology; MMRd: MisMatch Repair Deficiency; MRI: Magnetic Resonance Imaging; *: pembrolizumab cycle. **b,** Consort diagram depicting the disposition of patients throughout the study, including screening, neoadjuvant treatment, and surgery. 12 patients were recruited to this study of whom 10 received treatment and were assessable for the primary endpoint analysis. (i) Reasons for screen failure were secondary malignancy and abnormal liver values found at screening.



Supplementary Fig. 2 | Mature tertiary lymphoid structures characterized by Bcl6⁺ germinal centre and CD21⁺ follicular dendritic cells. a-e, Representative H&E, Bcl6, and CD21 IHC images of hysterectomy specimen of PAM-001, PAM-004, PAM-007, PAM-009, PAM-011, respectively.



Supplementary Fig. 3 | Single cell analysis of T cells from patients treated with neoadjuvant ICB. a, Flow cytometry plots showing the gating strategy used for the isolation of T cells used in the scRNA-seq analysis. **b,** Violin plots showing the expression and abundance of canonical marker genes used in the identification of T cell clusters. **c,** All high-quality T cells from post-treatment tissue were clustered. UMAP visualization of 21,765 T cells after clustering. Clusters are colour coded by their inferred identity. **d,** Bubble blot showing the expression and abundance of key canonical marker genes used in the identification of the T cell clusters. **e,** CD3, CD4, and CD103 staining images of post-treatment tissue showing the intra-epithelial location of CD103 (ITGAE) positive T-cells. **f,** Cell cycle phase was inferred for individual expanded CD8 T cells. UMAP of 3014 expanded CD8 T cells. Left: cells are colour coded by G2M cycle score. Right: cells are colour coded by S cycle score.



Supplementary Fig. 4 | Spatial transcriptomics of tumour tissue from patient PAM-004. a-b, MLH1 and PMS2 staining on pre-treatment biopsy of PAM-004 showing a tumour that is MMR deficient for MLH1 and PMS2. **c,** PMS2 staining on hysterectomy sample of PAM-004 showing viable tumour cells that are MMR proficient (left) and a fibrotic tumour bed (FTB) that is MMR deficient (right). **d-e,** Images of region of interest 002 and 004 of the FTB. From left to right: CD3 mask for T cells (orange) and PanCK mask for tumour cells (blue). **f-g,** Images of region of interest 001 and 003 of the MMRp region. From left to right: CD3 mask for T cells (orange) and PanCK mask for tumour cells (blue). **h,** Co-functionality network showing the top 250 genes expressed in PanCk positive areas of the pooled regions of interest within both the FTB and MMRp regions. Ribosomal genes were excluded. *ICB: immune checkpoint blockade, FTB: fibrotic tumour bed, MMRp: mismatch repair proficient.*