

Supplementary materials

Fig. S1. Consort flow diagram of patient collective. The consort flow diagram illustrates how patients with colorectal liver metastases (CLM), who received neoadjuvant treatment, following liver resection between 2005 and 2011 in a curative intent at the Department of Surgery of the Medical University of Vienna, were retrospectively enrolled in this study.

Fig. S2. Representative example of computed areas of whole-slide tissue sections.

Whole-slide tissue section of a CLM stained by IHC for CD45RO (brown color). Nuclei were counterstained (blue color). Two different regions were analyzed within the CLM: (i) tumor (TU) and (ii) invasive margin (IM; tumor/adjacent liver border). The clear distinguishable tumor area was marked manually. On the basis of the tumor area, a standardized invasive margin area with an exact width of 500 μm on each side of the tumor/adjacent liver border was created automatically by the analysis software. Scale bars 1 mm and 200 μm , respectively.

Fig. S3. Representative example for absolute cell quantification of whole-slide tissue sections.

High resolution image (20x magnification) of a CLM stained by IHC for CD45RO (brown color). Nuclei were counterstained (blue color). (i) nuclei mask was programmed, which detects all single cells on the basis of the cell nuclei staining and morphology. This nuclei mask was further used for the generation of an (ii) antibody detection mask, identifying the antibody specific staining pattern (membrane, cytoplasmic or nucleus) of the differently used antibodies. Scale bar 20 μm .

Fig. S4. Representative example of pre- and postoperative therapy response evaluation.

(A) Preoperative therapy response was evaluated by radiographic response, according to the response criteria in solid tumors (RECIST) 1.1 and morphologic response. (B) Contrast-enhanced computed tomography (CT) scan of a CLM (indicated by the black arrow) before neoadjuvant treatment (baseline). (C) Contrast-enhanced CT scan of a CLM

(indicated by the black arrow) after neoadjuvant treatment. According to the RECIST 1.1 criteria the patient was scored as stable disease (SD), whereas by the morphologic criteria as partial response (PR). (D) Postoperative therapy response was evaluated by pathologic response (Vitality), histologic response (TRG) and modified histologic response (mTRG). (E) Representative H&E staining of a CLM illustrating infarct-like necrosis (ILN; black diamonds), which characterizes a therapeutic response to chemotherapy compared to (F) usual necrosis (UN; black asterisks), indicating an insufficient treatment effect, which was considered as part of vital tumor cells. Scale bars 500 μ m.

Fig. S5. DNA damage predicts radiomorphologic therapy response. (A) Comparison of marker panel between good and poor radiomorphologic responder is indicated by box-and-whisker plots and individual values (Mann-Whitney U test). Bolt horizontal line represents the median, top and bottom of the box illustrates the first and third quartiles, whisker represent 1.5 times the interquartile range (IQR). (B) Multivariate logistic regression model (LASSO) for the prediction of radiomorphologic response is illustrated by a receiver operating characteristic (ROC) curve. False positive rate (FPR) is specified at the abscissa and true positive rate (TPR) at the left ordinate. The ROC curve is color-coded according to the threshold values defined on the right ordinate. Black 45° line represents line of identity. Area under the curve (AUC) and corresponding p-values are indicated. (C) Comparison of the multivariate logistic regression model between good and poor radiomorphologic responder. CALR: calreticulin; DDX41: DEAD-box helicase 41; RIG-I: retinoic acid inducible gene I; GBP1: guanylate binding protein 1; cGAS: cyclic guanosine monophosphate adenosine monophosphate (GMP-AMP) synthase; Mx1: interferon-induced GTP-binding protein Mx1; p-eIF2 α : phosphorylated-eukaryotic initiation factor 2 alpha; p-PKR: phosphorylated-protein kinase R; STING: stimulator of interferon genes; γ H2AX: phosphorylated-histone H2AX

Fig. S6. ER stress, DNA sensor cGAS, functional type I and type II IFN system and activated DCs predict modified histologic therapy response. Comparison of marker

panel between good and poor (A) histologic and (D) modified histologic responder is indicated by box-and-whisker plots and individual values (Mann-Whitney U test). Bolt horizontal line represents the median (second quartile), top and bottom of the box illustrates the first and third quartiles, whisker represent 1.5 times the interquartile range (IQR). Multivariate logistic regression model (LASSO) for the prediction of (B) histologic and (E) modified histologic response is illustrated by a receiver operating characteristic (ROC) curve. False positive rate (FPR) is specified at the abscissa and true positive rate (TPR) at the left ordinate. The ROC curve is color-coded according to the threshold values defined on the right ordinate. Black 45° line represents line of identity. Area under the curve (AUC) and corresponding p-values are indicated. Comparison of the multivariate logistic regression model between good and poor (C) histologic and (F) modified histologic responder. CALR: calreticulin; DDX41: DEAD-box helicase 41; RIG-I: retinoic acid inducible gene I; GBP1: guanylate binding protein 1; cGAS: cyclic guanosine monophosphate adenosine monophosphate (GMP-AMP) synthase; Mx1: interferon-induced GTP-binding protein Mx1; p-eIF2 α : phosphorylated-eukaryotic initiation factor 2 alpha; p-PKR: phosphorylated-protein kinase R; STING: stimulator of interferon genes; γ H2AX: phosphorylated-histone H2AX

Table S1. Primer sequences used for mutation analysis.

KRAS: kirsten rat sarcoma viral oncogene homolog; BRAF: V-raf murine sarcoma viral oncogene homolog B1

Gen	Forward sequence	Reverse sequence
<i>KRAS</i> codon 12/13 (exon 2)	ACTGAATATAAACTTGTGGTAGTTGGACCT	TCAAAGAATGGTCCTGGACC
<i>KRAS</i> codon 61 (exon 3)	CTCAGGATTCCTACAGGAAGCAAG	TATCTTCAAATGATTTAGTATTATTTATGG
<i>BRAF</i> (exon 15)	CATAATGCTTGCTCTGATAGGA	CTAGTAACTCAGCAGCATCTC

Table S2. List of antibodies used for immunohistochemistry.

CALR: calreticulin; DDX41: DEAD-box helicase 41; DDX58: DEXD/H-box helicase 58; RIG-I: retinoic acid inducible gene I; GBP1: guanylate binding protein 1; MB21D1: Mab-21 domain

containing 1; cGAS: cyclic guanosine monophosphate-adenosine monophosphate (GMP-AMP) synthase; MLH1: mutL homolog 1; MSH2: mutS protein homolog 2; Mx1: interferon-induced GTP-binding protein Mx1; EIF2S1: eukaryotic translation initiation factor 2 subunit 1; p-eIF2 α : phosphorylated-eukaryotic initiation factor 2 alpha; p-PKR: phosphorylated-protein kinase R; TMEM173: transmembrane Protein 173; STING: stimulator of interferon genes; γ H2AX: phosphorylated-histone H2AX

Antibody	Host	Clone	HIER buffer	Dilution	Company
CALR	Mouse	FMC 75	Citrate pH 6	1:1359	Abcam
CD208	Rat	1010E1.01	Citrate pH 6	1:100	Dendritics
CD3	Rabbit	2GV6	ready to use pH 9	ready to use	Ventana
CD45RO	Mouse	UCHL-1	ready to use pH 9	1:200	BioGenex
CD8	Mouse	C8/144B	ready to use pH 9	1:75	Cell Marque
DDX41	Rabbit	EPR14298	Tris-EDTA pH 9	1:100	Abcam
DDX58 (RIG-I)	Rabbit	Polyclonal	Tris-EDTA pH 9	1:50	Abcam
GBP1	Mouse	1B2	ready to use pH 9	1:150	Abcam
Ki-67	Mouse	MIB-1	ready to use pH 9	1:200	Dako
MB21D1 (cGAS)	Rabbit	Polyclonal	Citrate pH 6	1:700	Sigma-Aldrich
MLH1	Mouse	M1	ready to use pH 8	ready to use	Ventana
MSH2	Mouse	G219-1129	ready to use pH 8	ready to use	Cell Marque
Mx1	Rabbit	Polyclonal	ready to use pH 9	1:150	Abcam
p-EIF2S1 (p-eIF2 α)	Rabbit	E90	Citrate pH 6	1:50	Abcam
p-PKR	Mouse	E120	Tris-EDTA pH 9	1:500	Abcam
TMEM173 (STING)	Mouse	4E12	Citrate pH 6	1:200	Abcam
γ H2AX	Mouse	9F3	Citrate pH 6	1:1000	Abcam
Anti-rat IgG	Rabbit	Polyclonal	not applicable	1:1000	Novus Biologicals

Table S3. Patient characteristics without imputed values estimated by the predictive mean matching method.

IQR: interquartile range; Left: descending colon, sigmoid colon or rectum; Right: caecum or ascending colon; Transverse: transverse colon; UICC: union for international cancer control; XELOX: capecitabine, oxaliplatin; XELIRI: capecitabine, irinotecan; FOLFOX: folinic acid, fluorouracil, oxaliplatin; FOLFIRI: folinic acid, fluorouracil, irinotecan; TOMOX: raltitrexed, oxaliplatin; CR: complete remission; PR: partial remission; SD: stable disease; PD: progressive disease; OR: optimal response; PR: partial response; AR: absent response; TRG: tumor regression grade; mTRG: modified tumor regression grade; KRAS: kirsten rat sarcoma viral oncogene homolog; BRAF: V-raf murine sarcoma viral oncogene homolog B1; CI: confidence interval

	Total no
Demographics	(n=33)
<hr/>	
Median age \pm IQR (years)	62 \pm 19
Sex	
Male	17 (52%)
Female	16 (48%)
Primary tumor	
<hr/>	
Tumor location	
Left	26 (12%)
Right	4 (79%)
Transverse	3 (9%)
pT stage	
pT1	2 (8%)
pT2	2 (8%)
pT3	20 (76%)
pT4	2 (8%)
pN stage	
pN0	11 (39%)

pN1	11 (39%)
pN2	6 (22%)

M stage

M0	13 (42%)
M1	18 (58%)

UICC stage

I	1 (4%)
II	4 (15%)
III	4 (15%)
IV	18 (66%)

Tumor differentiation

Well	2 (8%)
Moderate	19 (70%)
Poor	6 (22%)

Residual Tumor classification

R0	26 (100%)
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Liver metastases

Metastases timepoint

Synchronous	19 (58%)
Metachronous	14 (42%)

Distribution

Unilobular	15 (45%)
Bilobular	16 (49%)
Central	2 (6%)

Number of lesions

Solitary	13 (39%)
Multiple	20 (61%)

Neoadjuvant chemotherapy

XELOX	19 (58%)
FOLFIRI	4 (12%)
FOLFOX	4 (12%)
XELIRI	2 (6%)
TOMOX	1 (3%)
Fluorouracil	1 (3%)
Irinotecan	1 (3%)
Raltitrexed + Irinotecan	1 (3%)

Neoadjuvant bevacizumab

Yes	29 (88%)
No	4 (12%)

Neoadjuvant cetuximab

Yes	2 (6%)
No	31 (94%)

Median neoadjuvant cycles \pm IQR 5 \pm 3

Radiographic therapy response

Good response (CR, PR)	15 (45%)
Poor response (SD, PD)	18 (55%)

Radiomorphologic therapy response

Good response (OR, PR)	20 (60%)
Poor response (AR)	13 (40%)

Synchronous primary tumor resection

Yes	2 (6%)
No	31 (94%)

Histology

Adenocarcinoma - Tubular-papillary	27 (82%)
Adenocarcinoma - Mucinous	6 (12%)

Tumor differentiation

Moderate 33 (100%)

Residual Tumor classification

R0 33 (100%)

Pathologic therapy response

Good response (0-25% viable) 9 (27%)

Poor response (\geq 25% viable) 24 (73%)

Histologic therapy response

Good response (TRG 1-3) 4 (12%)

Poor response (TRG 4-5) 29 (88%)

Modified histologic therapy response

Good response (mTRG 1-3) 17 (52%)

Poor response (mTRG 4-5) 16 (48%)

Mismatch repair status

Proficient 33 (100%)

Deficient 0 (0%)

KRAS status

Wild-type 13 (39%)

Mutant 20 (61%)

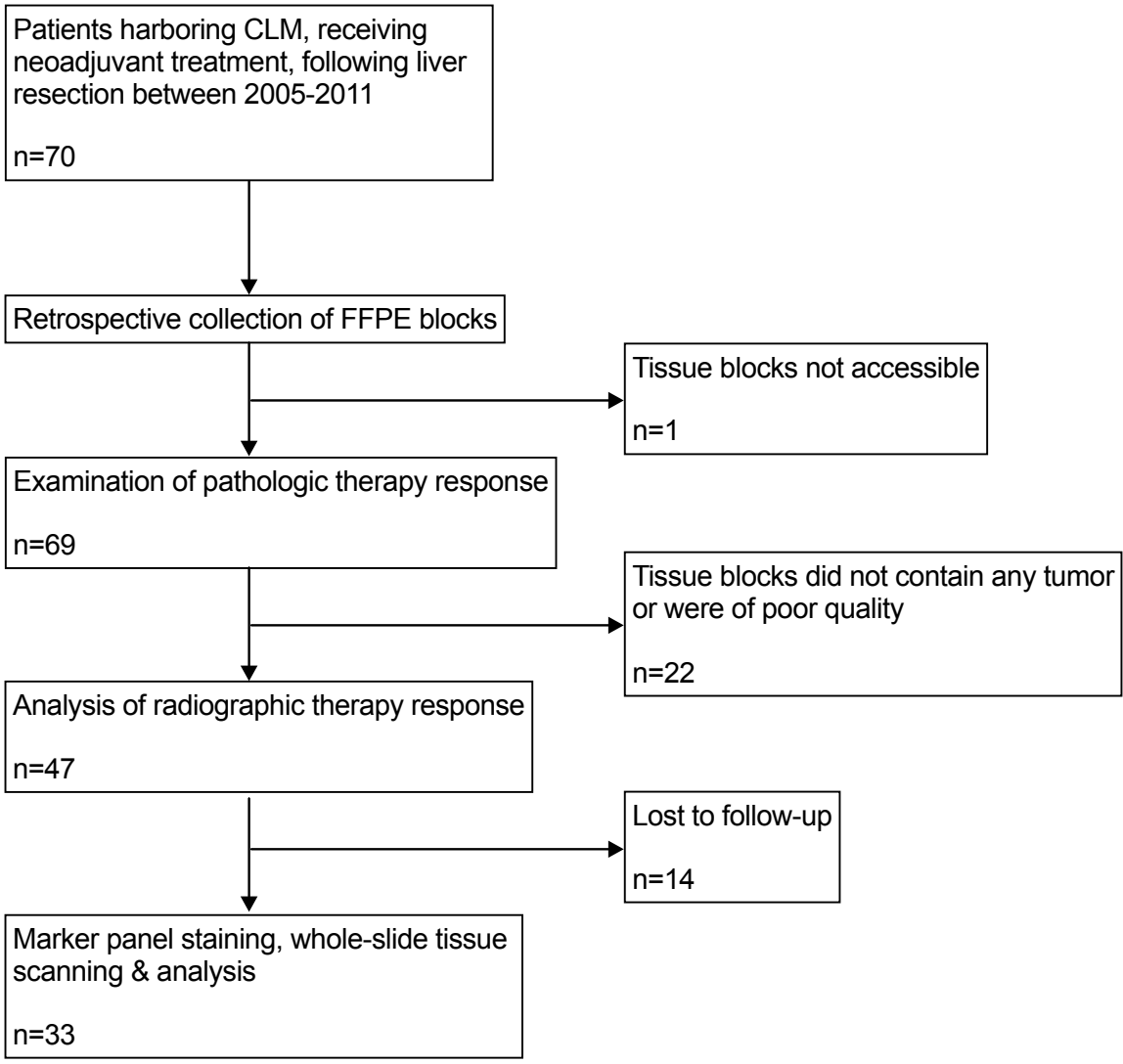
BRAF status

Wild-type 33 (100%)

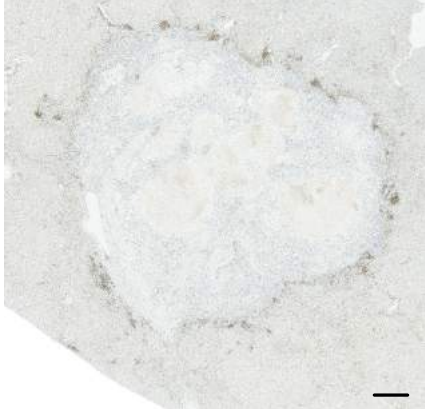
Mutant 0 (0%)

Median recurrence-free survival (months) 10 (95% CI 6.66-13.34)

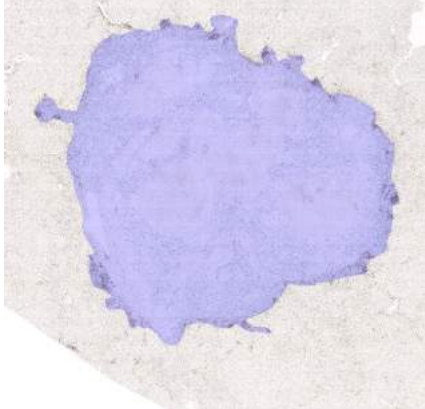
Median overall survival (months) 40 (95% CI 26.50-53.51)



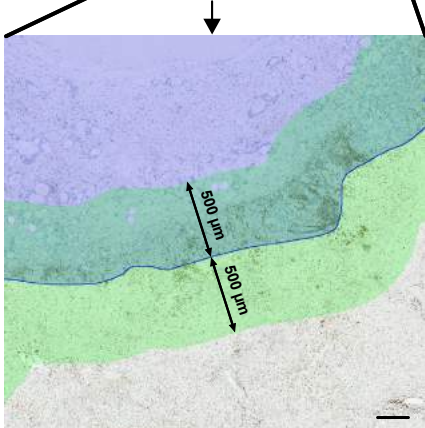
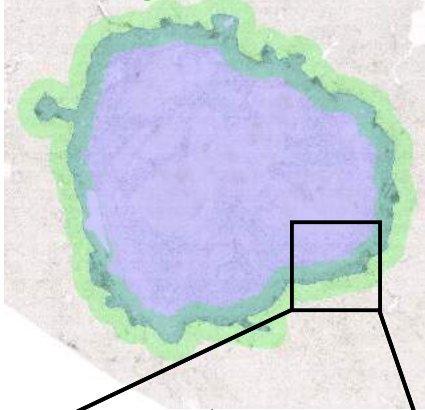
Whole-slide tissue

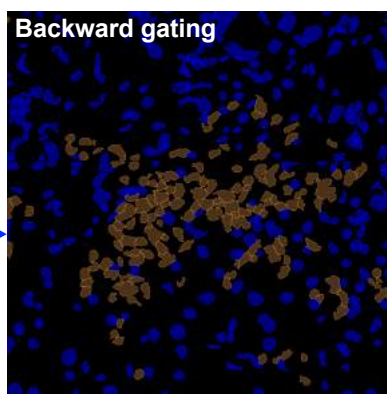
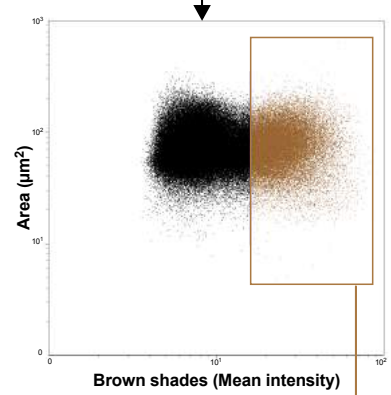
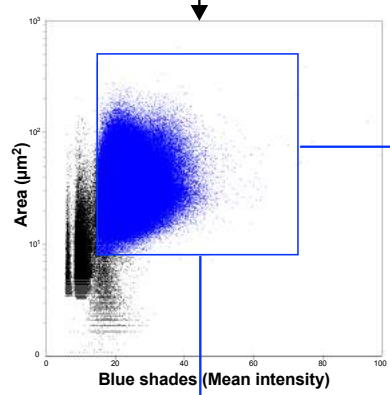
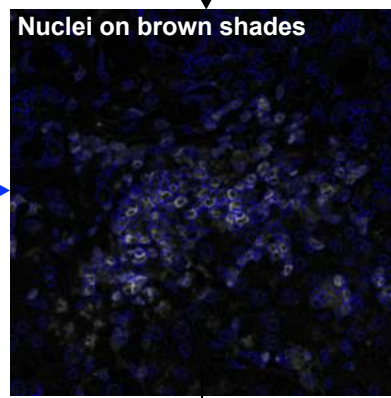
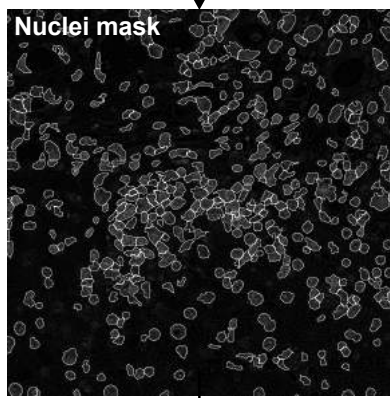
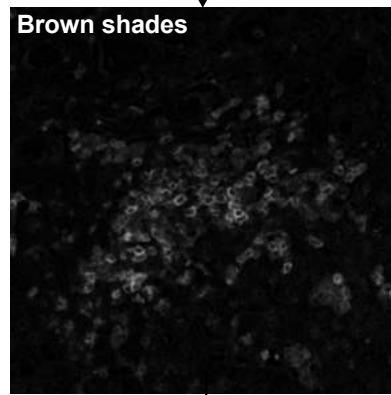
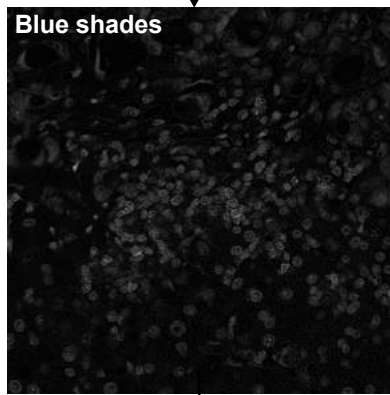
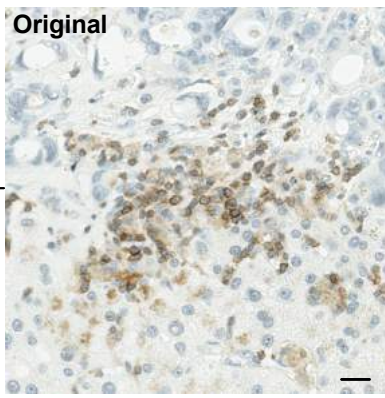


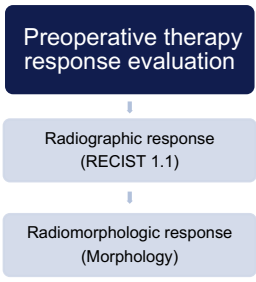
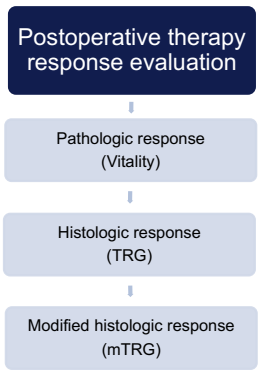
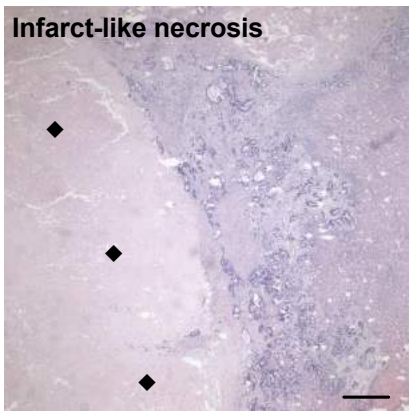
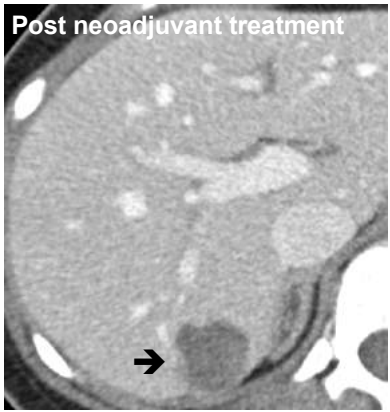
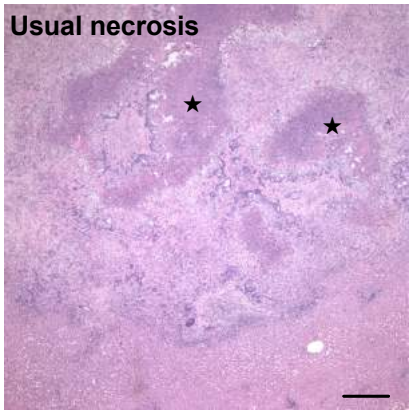
Tumor



Invasive margin



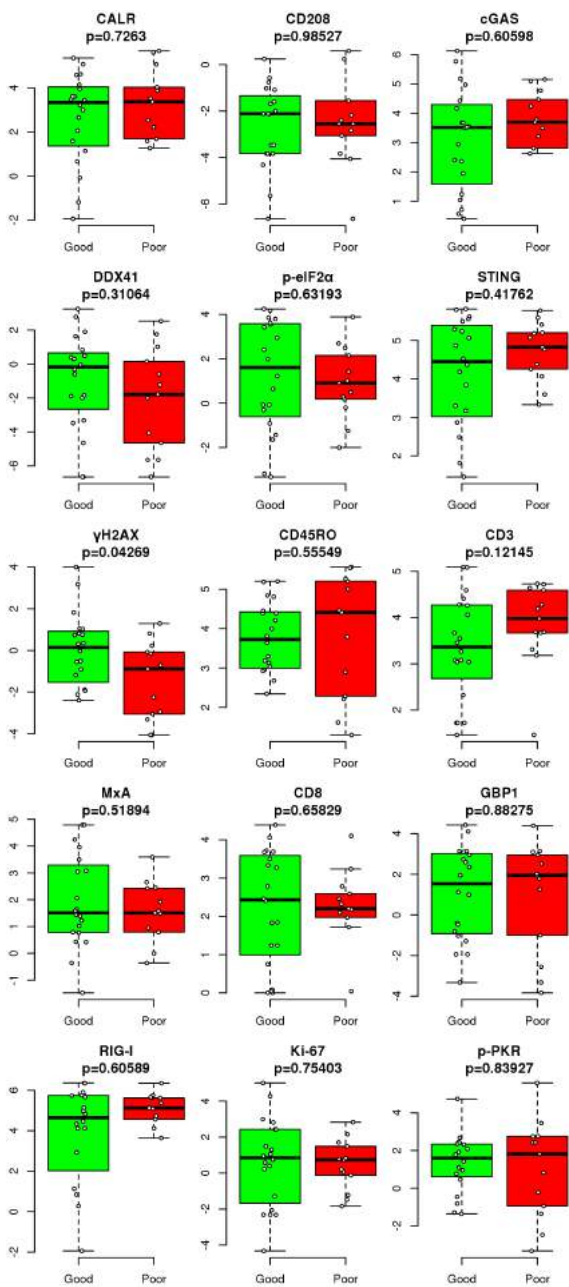
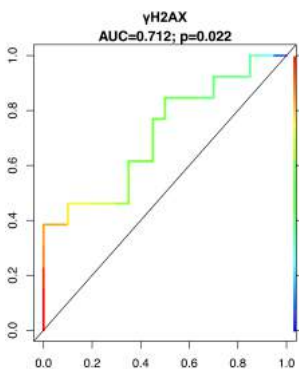
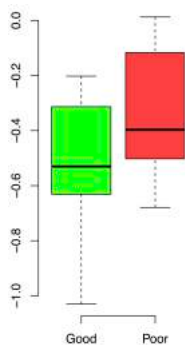


A**D****B****E****C****F**

A

Preoperative therapy
response evaluation

Radiomorphologic response
(Morphology)

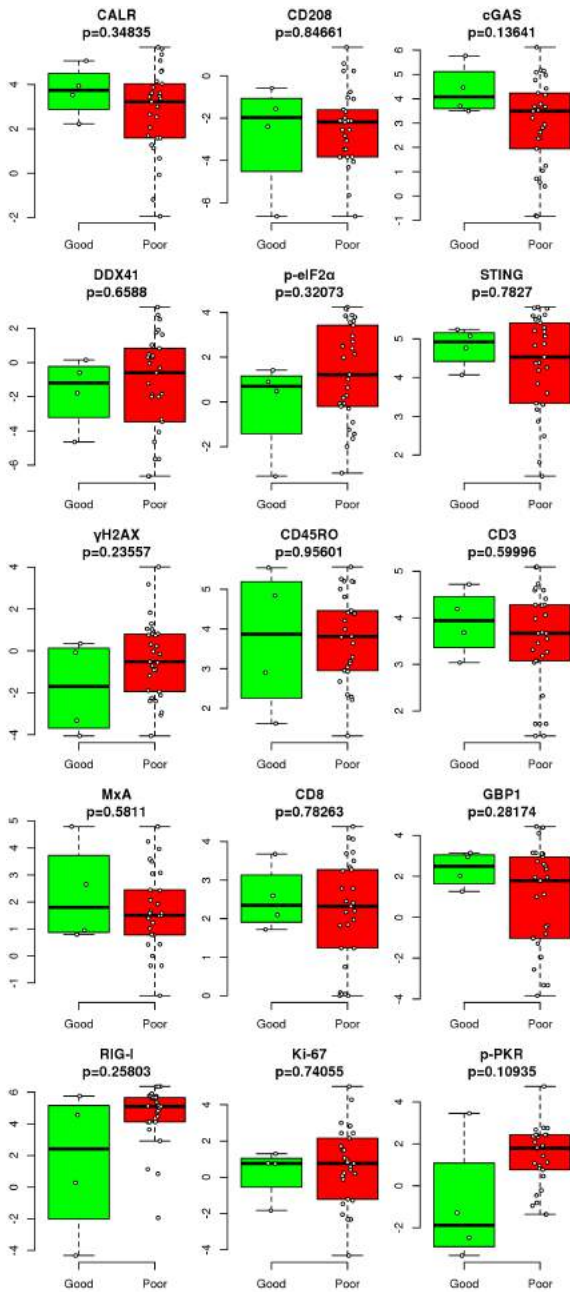
**B****C**

Postoperative therapy response evaluation

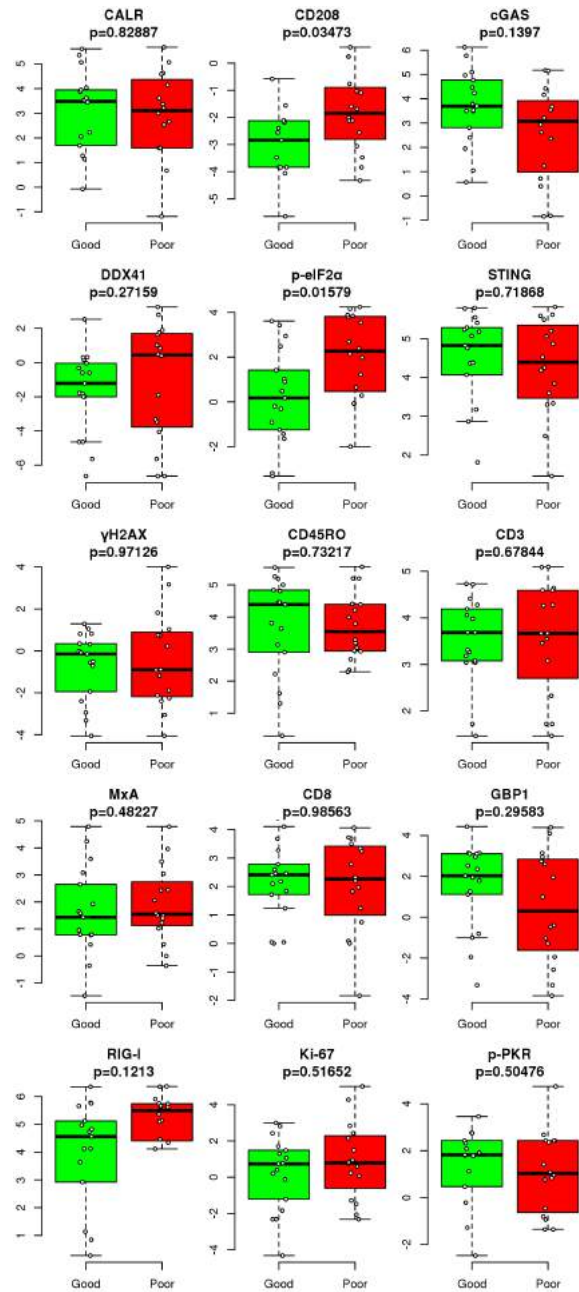
Histologic response (TRG)

Modified histologic response (mTRG)

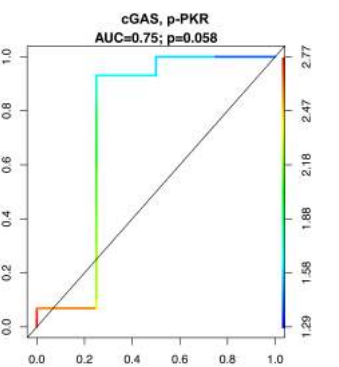
A



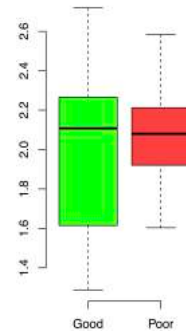
D



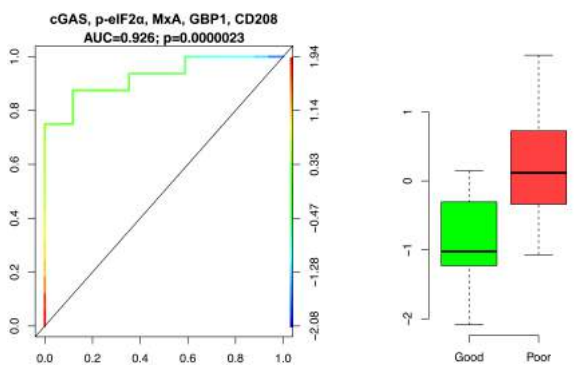
B



C



E



F

