In silico modeling of immunotherapy and   
stroma-targeting therapies in human colorectal cancer

**Supplementary Data**

# Table S1

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Parameter** | **Description** | **Default** | **Range** | **Ref** |
| *Tupprol* | Proliferation probability tumor cells | 0.5055 | 0 to 1 | own data |
| *Tupmig*\* | Migration probability tumor cells | 0.35 | 0 to 1 | - |
| *Tupdeath* | Death probability tumor cells | 1-(1-0.0319)^4 | 0 to 1 | own data |
| *Tupmax* | Proliferation capacity tumor cells | 10 | > 0 | (1) |
| *Tups*\* | Symmetric division probability | 0.7 | 0 to 1 | - |
| *Imkmax* | Killing capacity immune cells | 5 | > 0 | (2) |
| *Impmax* | Proliferation capacity immune cells | 10 | > 0 | (1) |
| *Impprol* | Proliferation prob. immune cells | 0.0449 | 0 to 1 | own data |
| *Impmig*\* | Migration probability immune cells | 0.8 | 0 to 1 | - |
| *Impkill*\* | Killing probability immune cells | 0.3 | 0 to 1 | - |
| *Impdeath* | Death probability immune cells | 1-(1-0.0037)^4 | 0 to 1 | own data |
| *Imrwalk*\* | Random influence on immune walk | 0.8 | 0 to 1 | - |
| *Imspeed* | Speed of immune cell movement | 97 | >0 | (3) |
| *engagementDuration* | Duration of immune cell engagement in tumor cell killing | 48 | >0 | (4) |
| *Iminfluxprob*\* | Probability of immune cell influx | 0.3 | 0 to 1 | - |
| *IMinflRate*\* | Number of immune cells per influx | 1 | >0 | - |
| *distMaxNecr* | Max. hypoxia-generating distance | 134 | >0 | own data |
| *probSeedNecr\** | Probability of necrosis seeding | 0.00004 | 0 to 1 | - |
| *probSeedFibr\** | Probability of fibrosis seeding | 0.00025 | 0 to 1 | - |
| *seedFrac\** | Scaling of fibrosis / necrosis seed | 0.3 | 0 to 1 | - |
| *defRadius\** | Radius of fibrosis / necrosis seed | 4 | >0 | - |
| *stromaPerm\** | Stromal permeability | 0.0025 | 0 to 1 | - |

Table 2: List of model parameters.  ***For some parameters, a definite value was available in the literature (corresponding reference given). Other parameters were quantitatively measured in our own experiments (“own data”). All remaining parameters were free parameters (\*).***

# Table S2

|  |  |  |
| --- | --- | --- |
| **Pseudonym** | **Primary tumor / metastasis** | **Sample source** |
| CRC\_UMM\_001 | Liver metastasis | UMM |
| CRC\_UMM\_002 | Liver metastasis | UMM |
| CRC\_UMM\_003 | Liver metastasis | UMM |
| CRC\_UMM\_004 | Liver metastasis | UMM |
| CRC\_UMM\_005 | Liver metastasis | UMM |
| CRC\_UMM\_006 | Primary tumor | UMM |
| CRC\_UMM\_007 | Primary tumor | UMM |
| CRC\_UMM\_008 | Primary tumor | UMM |
| CRC\_UMM\_009 | Primary tumor | UMM |
| CRC\_UMM\_010 | Primary tumor | UMM |
| CRC\_UMM\_011 | Primary tumor | UMM |
| CRC\_UMM\_012 | Primary tumor | UMM |
| CRC\_UMM\_013 | Primary tumor | UMM |
| CRC\_UMM\_014 | Primary tumor | UMM |
| CRC\_UMM\_015 | Primary tumor | UMM |
| CRC\_UMM\_016 | Primary tumor | UMM |
| CRC\_UMM\_017 | Primary tumor | UMM |
| CRC\_UMM\_018 | Primary tumor | UMM |
| CRC\_UMM\_019 | Primary tumor | UMM |
| CRC\_UMM\_020 | Primary tumor | UMM |

**Colorectal cancer tissue samples for measurements of basic histomorphological variables (calibration cohort)**: These N=20 samples were used to measure the fraction of Ki67-positive cells and the fraction of active-Caspase-3-postive cells (in tumor cells and immune cells, three independent regions of interest for each cell types); they were also used to measure the distance from the tumor margin to the necrotic core and the overall cell density used to calibrate the spatial scale. Furthermore, for all samples, a CD3 staining was available that was used as a comparison for spatial patterns generated by the model. UMM = University Medical Center Mannheim, Germany.

# Table S3

|  |  |  |
| --- | --- | --- |
| **Pseudonym** | **Primary tumor / metastasis** | **Institution / Reference** |
| NCT-Co\_001 | Liver metastasis | described in (5) |
| NCT-Co\_002 | Liver metastasis | described in (5) |
| NCT-Co\_003 | Liver metastasis | described in (5) |
| NCT-Co\_004 | Liver metastasis | described in (5) |
| NCT-Co\_005 | Liver metastasis | described in (5) |
| NCT-Co\_006 | Liver metastasis | described in (5) |
| NCT-Co\_007 | Liver metastasis | described in (5) |
| NCT-Co\_008 | Liver metastasis | described in (5) |
| NCT-Co\_009 | Liver metastasis | described in (5) |
| NCT-Co\_010 | Liver metastasis | described in (5) |
| NCT-Co\_011 | Liver metastasis | described in (5) |
| NCT-Co\_012 | Liver metastasis | described in (5) |
| NCT-Co\_013 | Liver metastasis | described in (5) |
| NCT-Co\_014 | Liver metastasis | described in (5) |
| NCT-Co\_015 | Liver metastasis | described in (5) |
| NCT\_15QWK5 | Liver metastasis | NCT Biobank, #2152 |
| NCT\_17Z30O | Liver metastasis | NCT Biobank, #2152 |
| NCT\_1UUVJF | Liver metastasis | NCT Biobank, #2152 |
| NCT\_1UW7FT | Liver metastasis | NCT Biobank, #2152 |
| NCT\_BZXONL | Liver metastasis | NCT Biobank, #2152 |
| NCT\_GNEY4A | Liver metastasis | NCT Biobank, #2152 |
| NCT\_IR13ZE | Liver metastasis | NCT Biobank, #2152 |
| NCT\_J5Z6D9 | Liver metastasis | NCT Biobank, #2152 |
| NCT\_QNYVRC | Liver metastasis | NCT Biobank, #2152 |
| NCT\_1MDI4V | Liver metastasis | NCT Biobank, #2152 |
| NCT\_16HJHG | Primary tumor | NCT Biobank, #2152 |
| NCT\_16TIQY | Primary tumor | NCT Biobank, #2152 |
| NCT\_18YP5I | Primary tumor | NCT Biobank, #2152 |
| NCT\_1FJEXB | Primary tumor | NCT Biobank, #2152 |
| NCT\_1FKYL9 | Primary tumor | NCT Biobank, #2152 |
| NCT\_1OKS15 | Primary tumor | NCT Biobank, #2152 |
| NCT\_1PUGA6 | Primary tumor | NCT Biobank, #2152 |
| NCT\_1SQOOP | Primary tumor | NCT Biobank, #2152 |
| NCT\_22F7YQ | Primary tumor | NCT Biobank, #2152 |
| NCT\_5QYVXU | Primary tumor | NCT Biobank, #2152 |
| NCT\_LH673K | Primary tumor | NCT Biobank, #2152 |
| NCT\_X1E6XI | Primary tumor | NCT Biobank, #2152 |

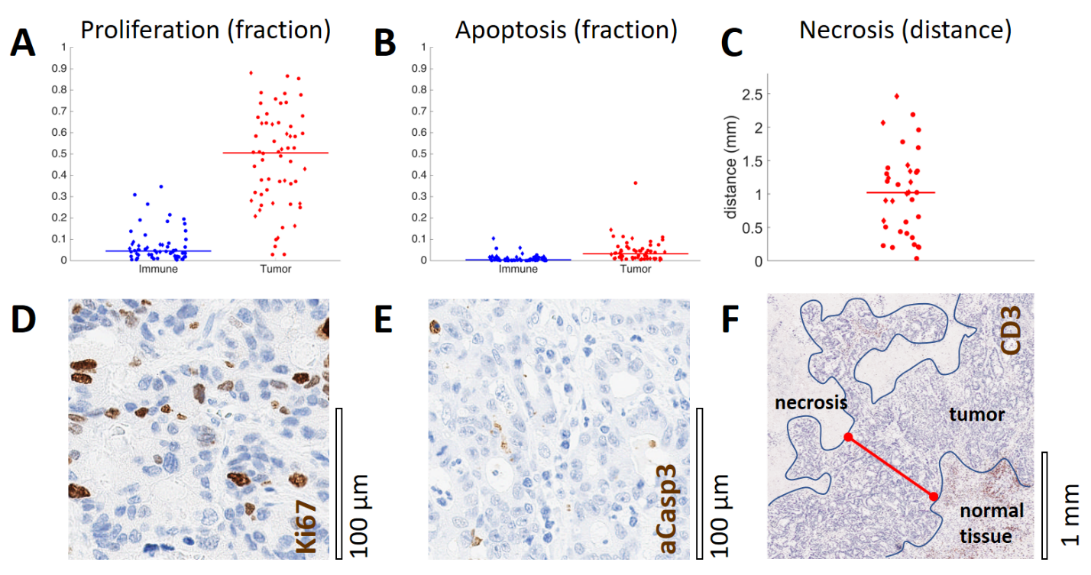
**Colorectal cancer tissue samples for spatial pattern**s **(morphological validation cohort)**: tissue samples that were stained for CD3 and used to compare spatial patterns generated by the model. NCT Biobank = National Center for Tumor Diseases, Heidelberg, Germany

# Table S4

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| 3L-AA1B | 4N-A93T | 4T-AA8H | 5M-AAT4 | 5M-AAT6 | 5M-AATE | A6-2674 |
| A6-2677 | A6-2684 | A6-3809 | A6-3810 | A6-5656 | A6-5656 | A6-5657 |
| A6-5659 | A6-5659 | A6-5660 | A6-5661 | A6-5662 | A6-5664 | A6-5665 |
| A6-5666 | A6-5667 | A6-6137 | A6-6138 | A6-6140 | A6-6141 | A6-6142 |
| A6-6648 | A6-6649 | A6-6650 | A6-6650 | A6-6651 | A6-6652 | A6-6653 |
| A6-6654 | A6-6780 | A6-6780 | A6-6781 | A6-6782 | A6-A565 | A6-A566 |
| A6-A567 | A6-A56B | A6-A5ZU | AA-3489 | AA-3494 | AA-3521 | AA-3524 |
| AA-3526 | AA-3530 | AA-3662 | AA-3664 | AA-3715 | AA-3977 | AA-3979 |
| AA-3980 | AA-3982 | AA-3984 | AA-A004 | AA-A00A | AA-A00D | AA-A00E |
| AA-A00F | AA-A00J | AA-A00K | AA-A00L | AA-A00N | AA-A00O | AA-A00Q |
| AA-A00R | AA-A00U | AA-A00W | AA-A00Z | AA-A010 | AA-A017 | AA-A01C |
| AA-A01D | AA-A01F | AA-A01G | AA-A01I | AA-A01K | AA-A01P | AA-A01Q |
| AA-A01R | AA-A01S | AA-A01T | AA-A01V | AA-A01X | AA-A01Z | AA-A022 |
| AA-A024 | AA-A029 | AA-A02E | AA-A02F | AA-A02H | AA-A02J | AA-A02K |
| AA-A02O | AA-A02R | AA-A02W | AA-A02Y | AA-A03F | AA-A03J | AD-5900 |
| AD-6888 | AD-6889 | AD-6890 | AD-6895 | AD-6899 | AD-6901 | AD-6963 |
| AD-6964 | AD-6965 | AD-A5EJ | AD-A5EK | AF-6136 | AF-6655 | AF-A56K |
| AF-A56L | AF-A56N | AG-3593 | AG-3594 | AG-3598 | AG-3882 | AG-A002 |
| AG-A008 | AG-A00C | AG-A00H | AG-A00Y | AG-A011 | AG-A014 | AG-A015 |
| AG-A016 | AG-A01J | AG-A01L | AG-A01N | AG-A01W | AG-A01Y | AG-A020 |
| AG-A023 | AG-A025 | AG-A026 | AG-A02G | AG-A02N | AG-A02X | AG-A032 |
| AG-A036 | AH-6544 | AH-6547 | AH-6897 | AY-A54L | AY-A69D | AY-A71X |
| AY-A8YK | AZ-4308 | AZ-4313 | CA-6715 | CM-5341 | CM-5344 | CM-5348 |
| CM-5349 | CM-5860 | CM-5861 | CM-5862 | CM-5863 | CM-5864 | CM-5868 |
| D5-5538 | D5-5539 | D5-5540 | D5-5541 | D5-6529 | D5-6530 | D5-6531 |
| D5-6532 | D5-6533 | D5-6534 | D5-6535 | D5-6536 | D5-6537 | D5-6538 |
| D5-6539 | D5-6540 | D5-6541 | D5-6920 | D5-6922 | D5-6923 | D5-6924 |
| D5-6926 | D5-6927 | D5-6928 | D5-6930 | D5-6931 | D5-6932 | DC-5337 |
| DC-5869 | DC-6155 | DM-A0X9 | DM-A0XD | DM-A0XF | DM-A1D0 | DM-A1D4 |
| DM-A1D6 | DM-A1D7 | DM-A1D8 | DM-A1D9 | DM-A1DA | DM-A1DB | DM-A1HA |
| DM-A1HB | DM-A280 | DM-A282 | DM-A285 | DM-A288 | DM-A28A | DM-A28C |
| DM-A28E | DM-A28F | DM-A28G | DM-A28H | DM-A28K | DM-A28M | DY-A0XA |
| DY-A1DC | DY-A1DD | DY-A1DE | DY-A1DF | DY-A1DG | DY-A1H8 | EI-6917 |
| F4-6459 | F4-6460 | F4-6461 | F4-6463 | F5-6464 | F5-6465 | F5-6812 |
| G5-6233 | G5-6235 | G5-6641 | NH-A50T | NH-A50U | NH-A50V | NH-A5IV |
| NH-A6GA | NH-A6GB | NH-A6GC | NH-A8F7 | NH-A8F7 | NH-A8F8 | QG-A5YV |
| QG-A5YW | QG-A5YX | QG-A5Z1 | QG-A5Z2 | QL-A97D | RU-A8FL | SS-A7HO |
| T9-A92H | WS-AB45 | - | - | - | - | - |

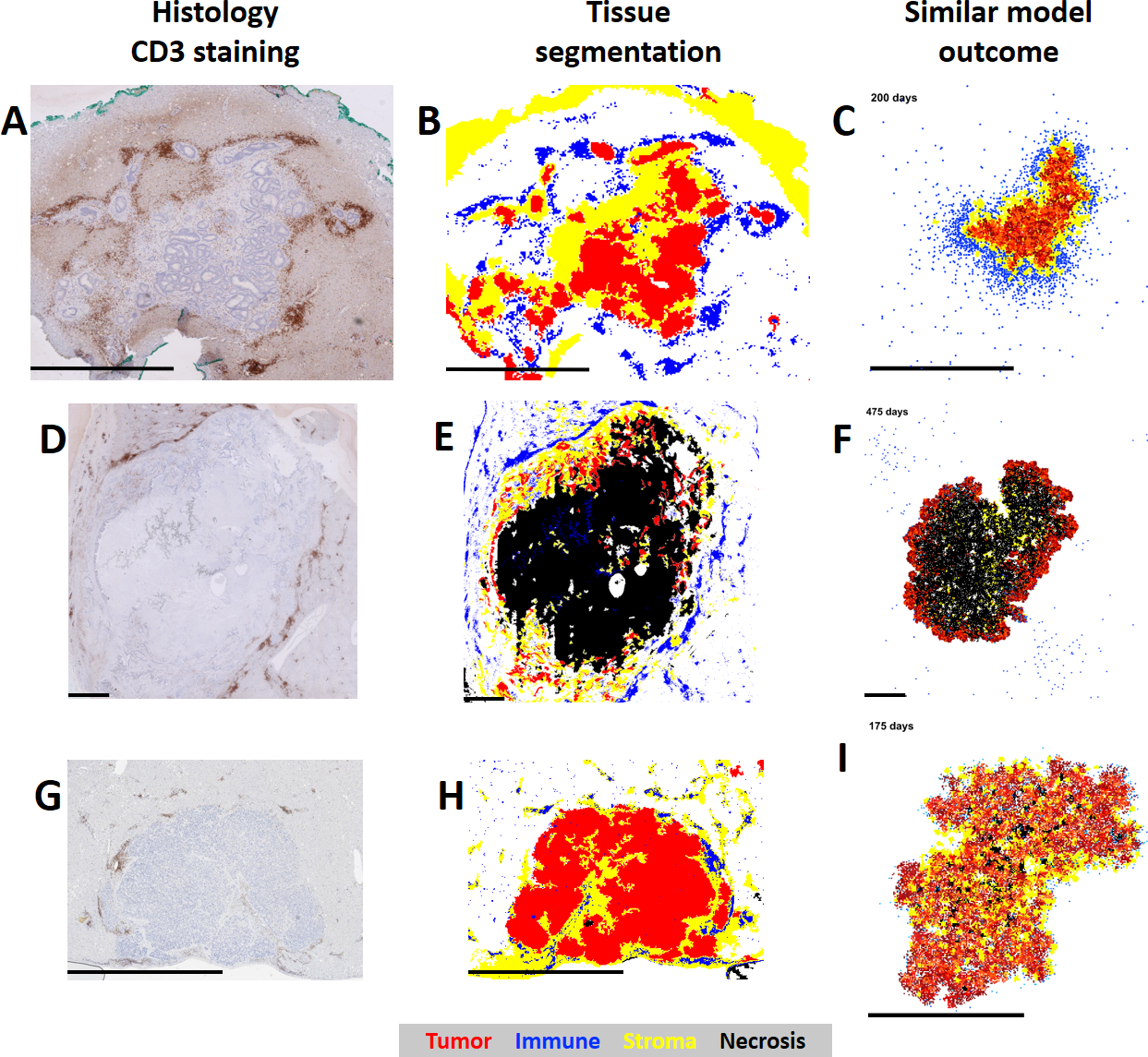
**List of TCGA patient pseudonyms included in the survival analysis (clinical validation cohort)**: A list of all eligible samples from the TCGA (The Cancer Genome Atlas) consortium that were used for survival analyses. Criteria for eligibility were: manual estimation of stromal content and lymphocyte content available and follow-up data available (survival data).

# Figure S1



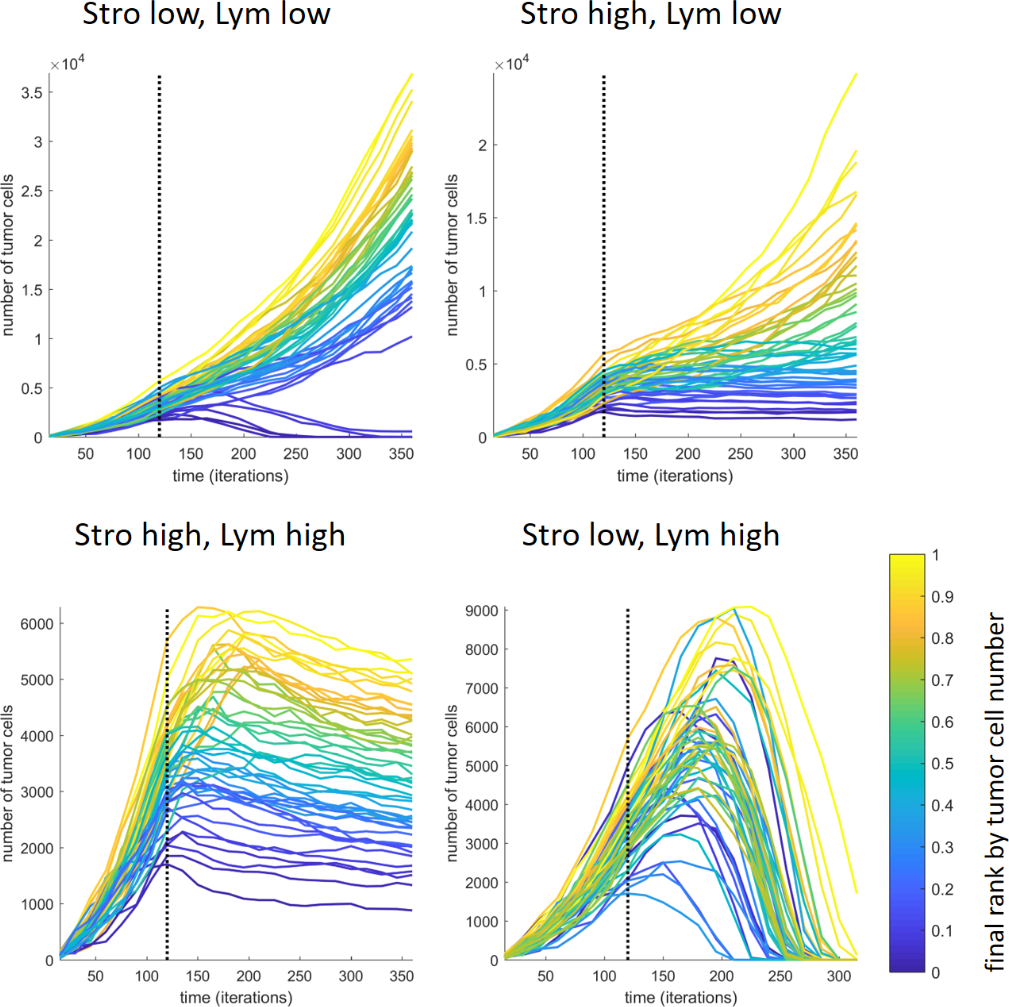
Measurements of proliferation, apoptosis and distance to necrosis in histological samples: a quantitative basis for the agent-based model. Based on morphology, we selected areas of immune cell infiltrates and pure tumor cells in N=20 tissue samples of human colorectal cancer. We quantified Ki67 stained cell fraction (the fraction of proliferating cells, shown in A and active Caspase 3 stained cell fraction (the fraction of dying cells, shown in B. Also, we observed that in colorectal cancer tissue, necrosis occurred at a fixed distance from the surrounding tissue. In 12 of 20 analyzed tissue slides, necrosis was present. The distance between tumor boundary and necrosis in 3 areas in these 12 samples is shown in C. The median is plotted as a straight line for each group. Representative image patches are shown for Ki67 stained tumor tissue (D) and aCasp3 stained tumor tissue (E). In F, the distance to necrosis (red) is demonstrated for a representative CD3 stained section. Median distance of necrosis from the tumor boundary was 1.0215 mm. We used this value as the hypoxia threshold in our model. The boundary between normal tissue (adjacent liver) and tumor tissue as well as the boundary between tumor tissue and necrosis are drawn in blue.

# Figure S2



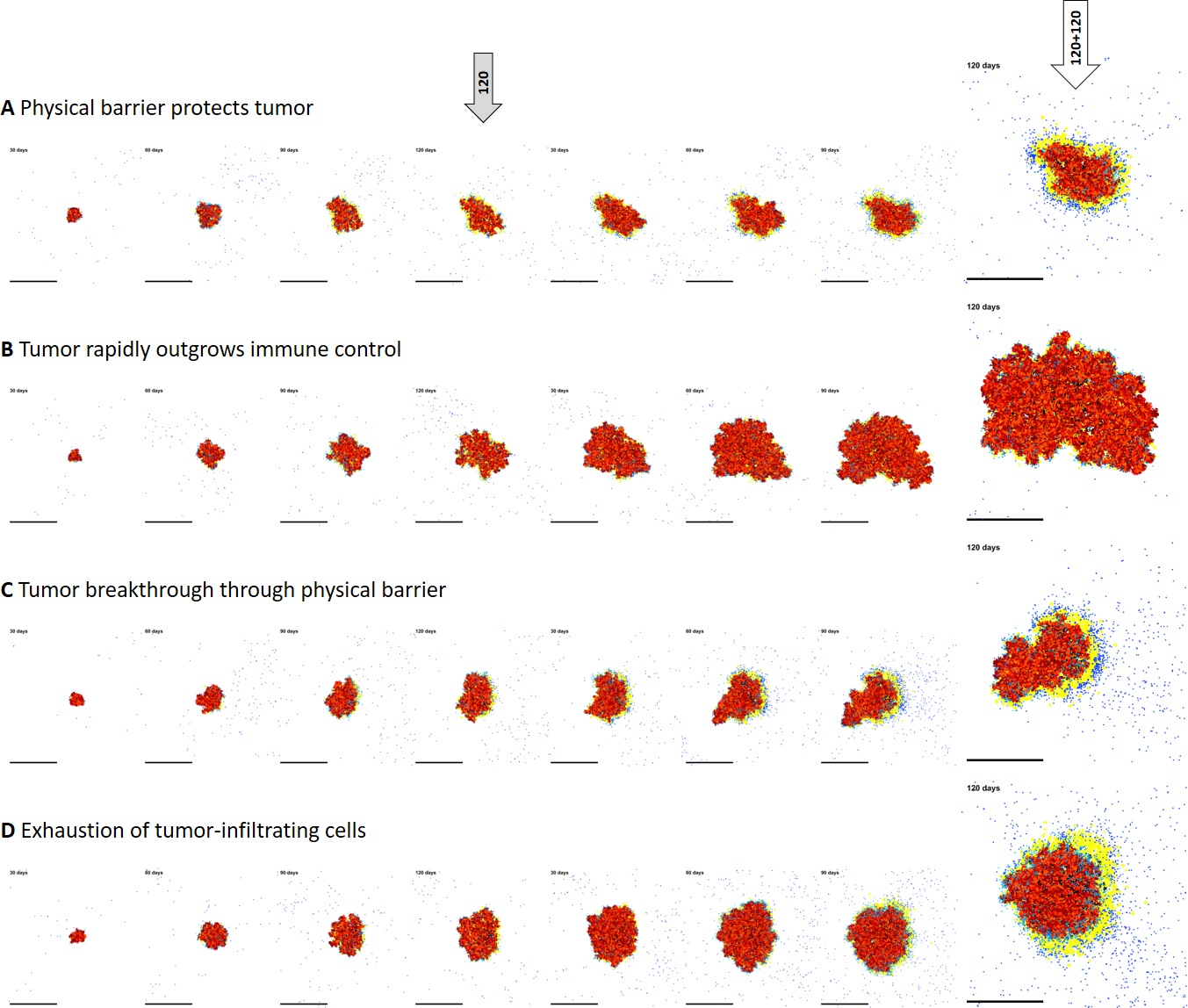
Quantitative histology shows similar patterns in actual tissue samples and simulated spatial patterns on a centimeter scale. (A,D,G) representative histological images of colorectal cancer liver metastases. (B,E,H) Semiautomatic tissue classification of tissue samples. (C,F,I) Results of agent-based simulations are shown. It can be seen that the model can give rise to very different spatial patterns, closely resembling actual histological patterns. All scale bars are 2 mm.

# Figure S3



Tumor growth trajectories for varying degrees of stroma induction and lymphocyte influx. Four tumors (A-D) are shown at different time points (from left to right: 30-60-90-120 days before immune boost and 30-60-90-120 days after immune boost; parameters were A-C: 2x immune boost and 4% stroma permeability, D: 4x immune boost and 16% stroma permeability).

# Figure S4



Phenotypes of immune escape in silico. Four tumors (A-D) are shown at different time points (from left to right: 30-60-90-120 days before immune boost and 30-60-90-120 days after immune boost; parameters were A-C: 2x immune boost and 4% stroma permeability, D: 4x immune boost and 16% stroma permeability).

# References for supplementary data

1. Poleszczuk J, Macklin P, Enderling H. Agent-Based Modeling of Cancer Stem Cell Driven Solid Tumor Growth. Methods Mol Biol. 2016;1516:335-46.

2. Christophe C, Muller S, Rodrigues M, Petit AE, Cattiaux P, Dupre L, et al. A biased competition theory of cytotoxic T lymphocyte interaction with tumor nodules. PLoS One. 2015;10(3):e0120053.

3. Boldajipour B, Nelson A, Krummel MF. Tumor-infiltrating lymphocytes are dynamically desensitized to antigen but are maintained by homeostatic cytokine. JCI Insight. 2016;1(20):e89289.

4. Breart B, Lemaitre F, Celli S, Bousso P. Two-photon imaging of intratumoral CD8+ T cell cytotoxic activity during adoptive T cell therapy in mice. J Clin Invest. 2008;118(4):1390-7.

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