

# CDX2 and SATB2 loss are associated with myeloid cell infiltration and poor survival in colorectal cancer

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## Supplementary material

**Table S1.** Associations of CDX2 and SATB2 histoscore with tumor infiltrating immune cells in Cohort 1.

| Variable                         | CDX2 |        |       |      |        |        | SATB2 |        |       |      |        |        |
|----------------------------------|------|--------|-------|------|--------|--------|-------|--------|-------|------|--------|--------|
|                                  | dMMR |        |       | pMMR |        |        | dMMR  |        |       | pMMR |        |        |
|                                  | N    | r      | p     | N    | r      | p      | N     | r      | p     | N    | r      | p      |
| T cells overall                  | 161  | -0.053 | 0.506 | 899  | -0.067 | 0.045  | 161   | 0.079  | 0.320 | 899  | 0.078  | 0.020  |
| T cells CT                       | 157  | -0.100 | 0.213 | 881  | -0.016 | 0.627  | 157   | 0.027  | 0.734 | 881  | 0.109  | 0.001  |
| T cells IM                       | 147  | 0.044  | 0.594 | 810  | -0.096 | 0.006  | 147   | 0.076  | 0.357 | 810  | 0.044  | 0.213  |
| B cells overall                  | 165  | 0.012  | 0.879 | 902  | -0.062 | 0.061  | 165   | 0.100  | 0.200 | 902  | 0.022  | 0.513  |
| B cells CT                       | 161  | -0.041 | 0.607 | 888  | 0.027  | 0.427  | 161   | 0.040  | 0.612 | 888  | 0.095  | 0.005  |
| B cells IM                       | 151  | 0.029  | 0.723 | 806  | -0.131 | <0.001 | 151   | 0.079  | 0.338 | 806  | -0.014 | 0.694  |
| Plasma cells overall             | 165  | -0.031 | 0.690 | 902  | -0.031 | 0.350  | 165   | 0.003  | 0.972 | 902  | 0.038  | 0.248  |
| Plasma cells CT                  | 161  | 0.011  | 0.894 | 888  | -0.010 | 0.766  | 161   | -0.023 | 0.070 | 888  | 0.068  | 0.044  |
| Plasma cells IM                  | 151  | -0.036 | 0.657 | 806  | -0.100 | 0.005  | 151   | -0.001 | 0.993 | 806  | -0.004 | 0.908  |
| Macrophages overall              | 161  | -0.076 | 0.337 | 899  | -0.226 | <0.001 | 161   | 0.033  | 0.675 | 899  | -0.096 | 0.004  |
| Macrophages CT                   | 157  | -0.109 | 0.172 | 881  | -0.179 | <0.001 | 157   | 0.011  | 0.890 | 881  | -0.083 | 0.014  |
| Macrophages IM                   | 147  | 0.017  | 0.836 | 810  | -0.212 | <0.001 | 147   | -0.017 | 0.838 | 810  | -0.075 | 0.032  |
| M1-like macrophages overall      | 161  | 0.168  | 0.033 | 899  | -0.007 | 0.834  | 161   | 0.231  | 0.003 | 899  | 0.113  | <0.001 |
| M1-like macrophages CT           | 157  | 0.197  | 0.013 | 881  | -0.010 | 0.770  | 157   | 0.213  | 0.007 | 881  | 0.099  | 0.003  |
| M1-like macrophages IM           | 147  | 0.141  | 0.089 | 810  | 0.004  | 0.899  | 147   | 0.118  | 0.154 | 810  | 0.111  | 0.001  |
| M2-like macrophages overall      | 161  | -0.130 | 0.101 | 899  | -0.197 | <0.001 | 161   | -0.065 | 0.415 | 899  | -0.164 | <0.001 |
| M2-like macrophages CT           | 157  | -0.213 | 0.007 | 881  | -0.163 | <0.001 | 157   | -0.121 | 0.131 | 881  | -0.142 | <0.001 |
| M2-like macrophages IM           | 147  | -0.028 | 0.732 | 810  | -0.197 | <0.001 | 147   | -0.042 | 0.611 | 810  | -0.139 | <0.001 |
| Mature monocytic cells overall   | 158  | -0.153 | 0.056 | 883  | -0.135 | <0.001 | 158   | -0.073 | 0.359 | 883  | -0.039 | 0.248  |
| Mature monocytic cells CT        | 151  | -0.106 | 0.195 | 846  | -0.116 | <0.001 | 151   | -0.057 | 0.489 | 846  | -0.048 | 0.166  |
| Mature monocytic cells IM        | 140  | -0.134 | 0.113 | 762  | -0.153 | <0.001 | 140   | -0.142 | 0.095 | 762  | -0.031 | 0.399  |
| Immature monocytic cells overall | 158  | -0.048 | 0.549 | 883  | -0.168 | <0.001 | 158   | -0.107 | 0.182 | 883  | -0.130 | <0.001 |
| Immature monocytic cells CT      | 151  | 0.026  | 0.752 | 846  | -0.173 | <0.001 | 151   | -0.011 | 0.896 | 846  | -0.171 | <0.001 |
| Immature monocytic cells IM      | 140  | -0.075 | 0.378 | 762  | -0.152 | <0.001 | 140   | -0.124 | 0.144 | 762  | -0.097 | 0.007  |
| Granulocytes overall             | 158  | -0.170 | 0.033 | 883  | -0.042 | 0.214  | 158   | 0.049  | 0.544 | 883  | 0.121  | <0.001 |
| Granulocytes CT                  | 151  | -0.142 | 0.081 | 846  | -0.046 | 0.186  | 151   | 0.021  | 0.801 | 846  | 0.080  | 0.020  |
| Granulocytes IM                  | 140  | -0.131 | 0.124 | 762  | -0.042 | 0.249  | 140   | 0.062  | 0.464 | 762  | 0.142  | <0.001 |
| Mast cells overall               | 158  | 0.196  | 0.014 | 883  | -0.010 | 0.763  | 158   | 0.223  | 0.005 | 883  | 0.069  | 0.039  |
| Mast cells CT                    | 151  | 0.110  | 0.180 | 846  | -0.014 | 0.691  | 151   | 0.116  | 0.156 | 846  | 0.047  | 0.175  |
| Mast cells IM                    | 140  | 0.230  | 0.006 | 762  | -0.037 | 0.311  | 140   | 0.205  | 0.015 | 762  | 0.072  | 0.048  |

Abbreviations: dMMR, mismatch repair deficient; CT, center of the tumor; IM, invasive margin; pMMR, mismatch repair proficient.

**Table S2.** Multivariable Cox regression models for cancer-specific survival in Cohort 1 and Cohort 2.

|                                                | CDX2 models                  |                              | SATB2 models                 |                              |
|------------------------------------------------|------------------------------|------------------------------|------------------------------|------------------------------|
|                                                | Cohort 1                     | Cohort 2                     | Cohort 1                     | Cohort 2                     |
|                                                | Multivariable HR<br>(95% CI) | Multivariable HR<br>(95% CI) | Multivariable HR<br>(95% CI) | Multivariable HR<br>(95% CI) |
| CDX2                                           |                              |                              | –                            | –                            |
| High                                           | 1 (referent)                 | 1 (referent)                 |                              |                              |
| Low                                            | 1.88 (1.23–2.81)             | 1.51 (0.87–2.60)             |                              |                              |
| Negative                                       | 3.62 (2.08–6.31)             | 3.86 (2.10–7.10)             |                              |                              |
| SATB2                                          | –                            | –                            |                              |                              |
| High                                           |                              |                              | 1 (referent)                 | 1 (referent)                 |
| Low                                            |                              |                              | 1.47 (1.15–1.89)             | 1.45 (1.04–2.02)             |
| Negative                                       |                              |                              | 1.61 (0.97–2.67)             | 1.86 (1.28–2.72)             |
| Year of operation                              |                              |                              |                              |                              |
| 2000–2005                                      | 1 (referent)                 |                              | 1 (referent)                 |                              |
| 2006–2010                                      | 0.64 (0.49–0.83)             | 1 (referent)                 | 0.60 (0.46–0.77)             | 1 (referent)                 |
| 2011–2015                                      | 0.52 (0.40–0.67)             | 0.83 (0.58–1.20)             | 0.52 (0.40–0.67)             | 0.81 (0.57–1.17)             |
| 2016–2020                                      |                              | 0.60 (0.41–0.87)             |                              | 0.62 (0.43–0.90)             |
| Age                                            |                              |                              |                              |                              |
| <65                                            | 1 (referent)                 | 1 (referent)                 | 1 (referent)                 | 1 (referent)                 |
| 65–75                                          | 1.15 (0.88–1.51)             | 1.67 (1.17–2.38)             | 1.22 (0.93–1.59)             | 1.68 (1.17–2.39)             |
| >75                                            | 1.96 (1.50–2.56)             | 2.62 (1.80–3.80)             | 2.06 (1.58–2.70)             | 2.81 (1.92–4.13)             |
| Sex                                            |                              |                              |                              |                              |
| Male                                           | 1 (referent)                 | 1 (referent)                 | 1 (referent)                 | 1 (referent)                 |
| Female                                         | 0.87 (0.70–1.08)             | 0.99 (0.74–1.33)             | 0.88 (0.70–1.09)             | 0.98 (0.73–1.31)             |
| Tumor location                                 |                              |                              |                              |                              |
| Proximal colon                                 | 1 (referent)                 | 1 (referent)                 | 1 (referent)                 | 1 (referent)                 |
| Distal colon                                   | 0.97 (0.74–1.27)             | 1.15 (0.75–1.74)             | 0.93 (0.71–1.22)             | 1.11 (0.73–1.69)             |
| Rectum                                         | 0.87 (0.60–1.26)             | 0.96 (0.63–1.44)             | 0.81 (0.56–1.17)             | 0.91 (0.60–1.37)             |
| Preoperative radiotherapy or chemoradiotherapy |                              |                              |                              |                              |
| No                                             | 1 (referent)                 | 1 (referent)                 | 1 (referent)                 | 1 (referent)                 |
| Yes                                            | 1.11 (0.74–1.67)             | 1.16 (0.78–1.72)             | 1.18 (0.78–1.77)             | 1.26 (0.85–1.90)             |
| Disease stage                                  |                              |                              |                              |                              |
| I                                              | 1 (referent)                 | 1 (referent)                 | 1 (referent)                 | 1 (referent)                 |
| II                                             | 1.67 (0.99–2.82)             | 1.54 (0.71–3.33)             | 1.75 (1.03–2.95)             | 1.53 (0.70–3.32)             |
| III                                            | 4.37 (2.64–7.22)             | 3.92 (1.90–8.11)             | 4.41 (2.67–7.29)             | 3.85 (1.86–7.97)             |
| IV                                             | 29.56 (17.67–49.45)          | 21.65 (10.25–45.75)          | 29.51 (17.63–49.39)          | 22.43 (10.60–47.47)          |
| Tumor grade                                    |                              |                              |                              |                              |
| Low-grade                                      | 1 (referent)                 | 1 (referent)                 | 1 (referent)                 | 1 (referent)                 |
| High-grade                                     | 1.52 (1.14–2.03)             | 1.45 (1.00–2.09)             | 1.65 (1.25–2.18)             | 1.33 (0.92–1.93)             |
| Lymphovascular invasion                        |                              |                              |                              |                              |
| No                                             | 1 (referent)                 | 1 (referent)                 | 1 (referent)                 | 1 (referent)                 |
| Yes                                            | 1.87 (1.49–2.35)             | 2.11 (1.44–3.09)             | 1.88 (1.50–2.36)             | 2.11 (1.44–3.09)             |
| MMR status                                     |                              |                              |                              |                              |
| MMR proficient                                 | 1 (referent)                 | 1 (referent)                 | 1 (referent)                 | 1 (referent)                 |
| MMR deficient                                  | 0.39 (0.24–0.64)             | 0.47 (0.23–0.97)             | 0.47 (0.30–0.77)             | 0.44 (0.21–0.90)             |
| <i>BRAF</i> status                             |                              |                              |                              |                              |
| Wild-type                                      | 1 (referent)                 | 1 (referent)                 | 1 (referent)                 | 1 (referent)                 |
| Mutant                                         | 1.45 (0.98–2.14)             | 1.10 (0.60–2.00)             | 1.31 (0.88–1.95)             | 1.35 (0.79–2.31)             |

Abbreviations: CI, confidence interval; HR, hazard ratio; MMR, mismatch repair.

**Table S3.** Cancer-specific survival according to combinatory status of CDX2 and SATB2 expression in Cohort 1 and 2.

|                   | Cohort 1     |               |                         |                           | Cohort 2     |               |                         |                           |
|-------------------|--------------|---------------|-------------------------|---------------------------|--------------|---------------|-------------------------|---------------------------|
|                   | No. of cases | No. of events | Univariable HR (95% CI) | Multivariable HR (95% CI) | No. of cases | No. of events | Univariable HR (95% CI) | Multivariable HR (95% CI) |
| <b>CDX2/SATB2</b> |              |               |                         |                           |              |               |                         |                           |
| Both H            | 953          | 232           | 1 (referent)            | 1 (referent)              | 635          | 106           | 1 (referent)            | 1 (referent)              |
| CDX2 H, SATB2 L/N | 215          | 73            | 1.63 (1.25–2.12)        | 1.23 (0.94–1.62)          | 261          | 65            | 1.42 (1.04–1.94)        | 1.27 (0.90–1.78)          |
| CDX2 L/N, SATB2 H | 28           | 9             | 1.55 (0.80–3.02)        | 1.26 (0.63–2.55)          | 10           | 2             | 1.33 (0.33–5.37)        | 0.35 (0.74–1.69)          |
| Both L/N          | 93           | 40            | 2.35 (1.68–3.29)        | 2.94 (1.99–4.33)          | 62           | 23            | 2.49 (1.58–3.90)        | 2.34 (1.37–4.00)          |
| p                 |              |               | <0.001                  | <0.001                    |              |               | <0.001                  | 0.004                     |

Multivariable Cox proportional hazards regression models were adjusted for sex, age (<65, 65–75, >75), year of operation (cohort 1: 2000–2005, 2006–2010, 2011–2015, cohort 2: 2006–2010, 2011–2015, 2016–2020), tumor location (proximal colon, distal colon, rectum), disease stage (I, II, III, IV), tumor grade (well/moderately differentiated, poorly differentiated), lymphovascular invasion (negative, positive), MMR status (proficient, deficient), *BRAF* status (wild-type, mutant) and preoperative radiotherapy or chemoradiotherapy (no, yes). CI confidence interval, H High, HR hazard ratio, L/N Low/Negative.

**Table S4.** Comparison of prognostic power of CDX2 and SATB2 expression and immune cell score using Cox regression models for cancer-specific survival.

|                    | No. of cases | No. of events | Model 1 (univariable) HR (95% CI) | Model 2 (multivariable) HR (95% CI) | Model 3 (multivariable) HR (95% CI) |
|--------------------|--------------|---------------|-----------------------------------|-------------------------------------|-------------------------------------|
| <b>Cohort 1</b>    |              |               |                                   |                                     |                                     |
| CDX2               |              |               |                                   |                                     |                                     |
| High               | 1,068        | 285           | 1 (referent)                      | 1 (referent)                        | 1 (referent)                        |
| Low                | 76           | 30            | 1.86 (1.27–2.72)                  | 1.92 (1.31–2.82)                    | 2.35 (1.56–3.55)                    |
| Negative           | 29           | 15            | 2.91 (1.73–4.89)                  | 3.55 (2.10–6.00)                    | 4.61 (2.60–8.18)                    |
| p <sub>trend</sub> |              |               | <0.0001                           | <0.0001                             | <0.0001                             |
| Immune cell score  |              |               |                                   |                                     |                                     |
| 0                  | 232          | 86            | 1 (referent)                      | 1 (referent)                        | 1 (referent)                        |
| 1                  | 692          | 203           | 0.75 (0.58–0.97)                  | 0.75 (0.58–0.97)                    | 0.77 (0.59–1.00)                    |
| 2                  | 249          | 41            | 0.41 (0.28–0.60)                  | 0.38 (0.26–0.55)                    | 0.52 (0.35–0.77)                    |
| p <sub>trend</sub> |              |               | <0.0001                           | <0.0001                             | 0.001                               |
| SATB2              |              |               |                                   |                                     |                                     |
| High               | 900          | 224           | 1 (referent)                      | 1 (referent)                        | 1 (referent)                        |
| Low                | 225          | 88            | 1.85 (1.44–2.38)                  | 1.77 (1.38–2.28)                    | 1.59 (1.22–2.06)                    |
| Negative           | 48           | 18            | 1.99 (1.23–3.21)                  | 2.24 (1.38–3.62)                    | 2.08 (1.25–3.44)                    |
| p <sub>trend</sub> |              |               | <0.0001                           | <0.0001                             | 0.0004                              |
| Immune cell score  |              |               |                                   |                                     |                                     |
| 0                  | 232          | 86            | 1 (referent)                      | 1 (referent)                        | 1 (referent)                        |
| 1                  | 692          | 203           | 0.75 (0.58–0.97)                  | 0.78 (0.60–1.01)                    | 0.78 (0.60–1.01)                    |
| 2                  | 249          | 41            | 0.41 (0.28–0.60)                  | 0.42 (0.29–0.61)                    | 0.56 (0.38–0.83)                    |
| p <sub>trend</sub> |              |               | <0.0001                           | <0.0001                             | 0.003                               |
| CDX2/SATB2         |              |               |                                   |                                     |                                     |
| Both H             | 878          | 214           | 1 (referent)                      | 1 (referent)                        | 1 (referent)                        |
| CDX2 H, SATB2 L/N  | 190          | 63            | 1.57 (1.19–2.08)                  | 1.50 (1.13–1.99)                    | 1.20 (0.90–1.61)                    |
| SATB2 H, CDX2 L/N  | 22           | 5             | 1.06 (0.44–2.57)                  | 1.10 (0.45–2.66)                    | 0.86 (0.34–2.17)                    |
| Both L/N           | 83           | 39            | 2.72 (1.93–3.83)                  | 2.94 (2.08–4.14)                    | 3.48 (2.32–5.20)                    |
| p                  |              |               | <0.0001                           | <0.0001                             | <0.0001                             |
| Immune cell score  |              |               |                                   |                                     |                                     |
| 0                  | 232          | 86            | 1 (referent)                      | 1 (referent)                        | 1 (referent)                        |
| 1                  | 692          | 203           | 0.75 (0.58–0.97)                  | 0.79 (0.61–1.03)                    | 0.80 (0.61–1.04)                    |
| 2                  | 249          | 41            | 0.41 (0.28–0.60)                  | 0.40 (0.28–0.59)                    | 0.53 (0.36–0.79)                    |
| p <sub>trend</sub> |              |               | <0.0001                           | <0.0001                             | 0.002                               |
| <b>Cohort 2</b>    |              |               |                                   |                                     |                                     |
| CDX2               |              |               |                                   |                                     |                                     |
| High               | 878          | 167           | 1 (referent)                      | 1 (referent)                        | 1 (referent)                        |
| Low                | 50           | 14            | 1.51 (0.88–2.61)                  | 1.56 (0.90–2.69)                    | 1.46 (0.78–2.71)                    |
| Negative           | 21           | 11            | 4.14 (2.25–7.63)                  | 5.58 (2.98–10.45)                   | 4.05 (1.95–8.42)                    |
| p <sub>trend</sub> |              |               | <0.0001                           | <0.0001                             | 0.0005                              |
| Immune cell score  |              |               |                                   |                                     |                                     |
| 0                  | 187          | 74            | 1 (referent)                      | 1 (referent)                        | 1 (referent)                        |
| 1                  | 534          | 96            | 0.41 (0.30–0.55)                  | 0.43 (0.32–0.59)                    | 0.67 (0.49–0.93)                    |
| 2                  | 228          | 22            | 0.22 (0.14–0.35)                  | 0.19 (0.12–0.31)                    | 0.45 (0.26–0.78)                    |
| p <sub>trend</sub> |              |               | <0.0001                           | <0.0001                             | 0.002                               |
| SATB2              |              |               |                                   |                                     |                                     |
| High               | 631          | 105           | 1 (referent)                      | 1 (referent)                        | 1 (referent)                        |
| Low                | 200          | 51            | 1.46 (1.04–2.04)                  | 1.32 (0.94–1.85)                    | 1.28 (0.88–1.86)                    |
| Negative           | 118          | 36            | 1.91 (1.31–2.79)                  | 1.75 (1.20–2.56)                    | 1.66 (1.08–2.54)                    |
| p <sub>trend</sub> |              |               | 0.0003                            | 0.003                               | 0.019                               |
| Immune cell score  |              |               |                                   |                                     |                                     |
| 0                  | 187          | 74            | 1 (referent)                      | 1 (referent)                        | 1 (referent)                        |
| 1                  | 534          | 96            | 0.41 (0.30–0.55)                  | 0.42 (0.31–0.58)                    | 0.74 (0.53–1.03)                    |
| 2                  | 228          | 22            | 0.22 (0.14–0.35)                  | 0.23 (0.14–0.37)                    | 0.55 (0.32–0.94)                    |
| p <sub>trend</sub> |              |               | <0.0001                           | <0.0001                             | 0.017                               |
| CDX2/SATB2         |              |               |                                   |                                     |                                     |
| Both H             | 621          | 103           | 1 (referent)                      | 1 (referent)                        | 1 (referent)                        |
| CDX2 H, SATB2 L/N  | 257          | 64            | 1.44 (1.05–1.97)                  | 1.29 (0.94–1.77)                    | 1.21 (0.85–1.72)                    |
| SATB2 H, CDX2 L/N  | 10           | 2             | 1.34 (0.33–5.44)                  | 1.82 (0.45–7.40)                    | 0.47 (0.10–2.72)                    |
| Both L/N           | 61           | 23            | 2.55 (1.62–4.01)                  | 2.55 (1.61–4.03)                    | 2.47 (1.45–4.22)                    |
| p                  |              |               | <0.0001                           | <0.0001                             | 0.004                               |
| Immune cell score  |              |               |                                   |                                     |                                     |
| 0                  | 187          | 74            | 1 (referent)                      | 1 (referent)                        | 1 (referent)                        |

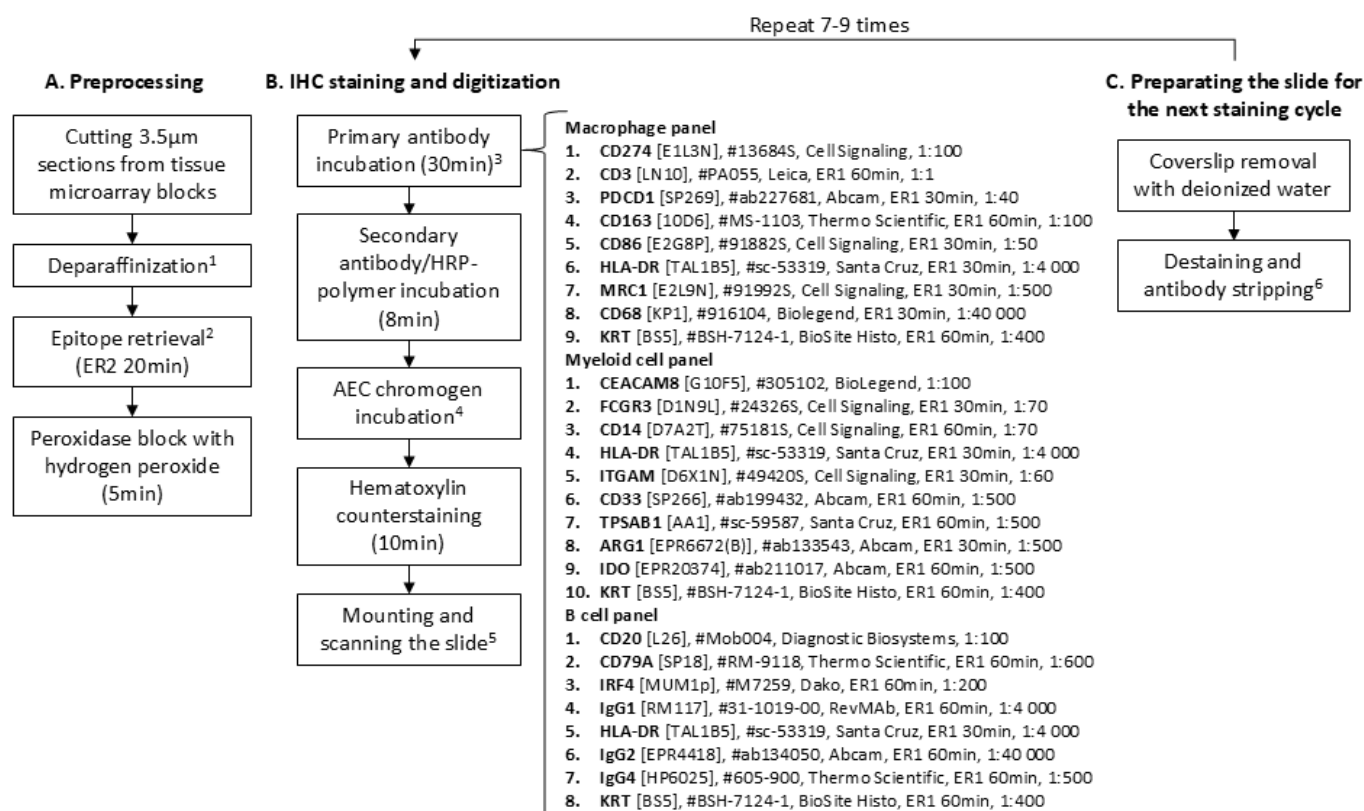
|                    |     |    |                  |                  |                  |
|--------------------|-----|----|------------------|------------------|------------------|
| 1                  | 534 | 96 | 0.41 (0.30–0.55) | 0.43 (0.32–0.59) | 0.71 (0.51–1.00) |
| 2                  | 228 | 22 | 0.22 (0.14–0.35) | 0.22 (0.13–0.35) | 0.54 (0.31–0.94) |
| $P_{\text{trend}}$ |     |    | <0.0001          | <0.0001          | 0.013            |

Model 2: Cox proportional hazards regression model including CDX2 or SATB2 expression and immune cell score.

Model 3: Cox proportional hazards regression model based on Model 2 that was additionally adjusted for sex, age (<65, 65–75, >75), year of operation (cohort 1: 2000–2005, 2006–2010, 2011–2015, cohort 2: 2006–2010, 2011–2015, 2016–2020), tumor location (proximal colon, distal colon, rectum), disease stage (I, II, III, IV), tumor grade (well/moderately differentiated, poorly differentiated), lymphovascular invasion (negative, positive), MMR status (proficient, deficient), *BRAF* status (wild-type, mutant) and preoperative radiotherapy or chemoradiotherapy (no, yes).

$P_{\text{trend}}$  values were calculated by using the three ordinal categories of CDX2 histoscore, SATB2 histoscore and immune cell score as continuous variables in univariable and multivariable Cox proportional hazard regression models.

CI confidence interval, HR hazard ratio, H High, L/N Low/Negative.



**Figure S1.** Multiplex immunohistochemistry protocol. Tissue microarray slides were stained 8 (B cell panel), 9 (Macrophage panel), or 10 (myeloid cell panel) times sequentially using an automated immunohistochemistry stainer. The incubations were performed in room temperature if not otherwise specified. Abbreviations: IHC, immunohistochemistry; AEC, 3-Amino-9-ethylcarbazole

1 Dewax solution (AR9222, Leica Biosystems), 30min, 60–72 °C.

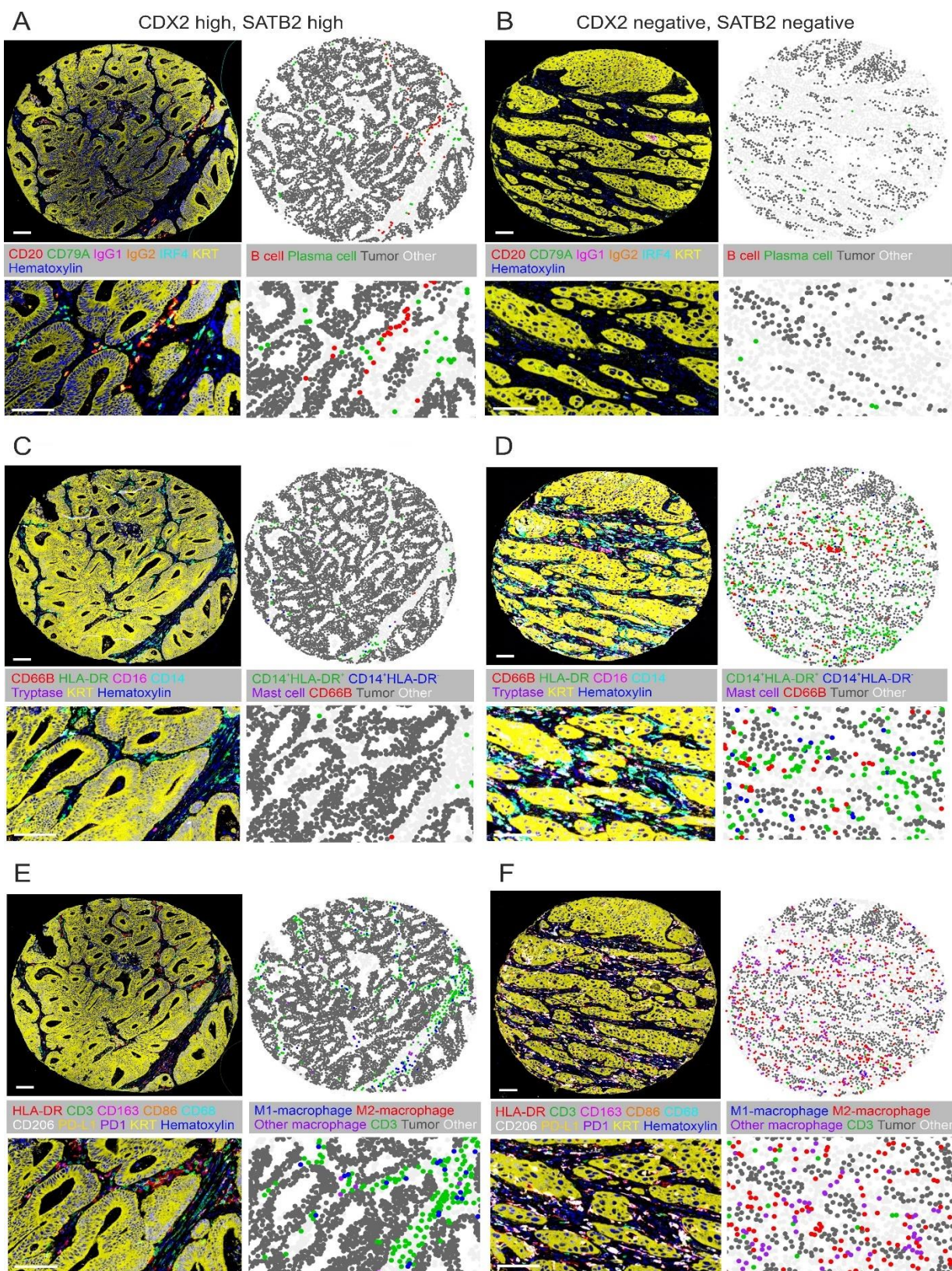
2 Epitope retrieval done with BOND epitope retrieval solution 2 (EDTA based, pH 9, AR9640, Leica Biosystems) with 20min heating time.

3 Primary antibodies (name, clone, catalogue number and manufacturer) together with the selected antigen retrieval conditions and used dilutions are listed in the staining order.

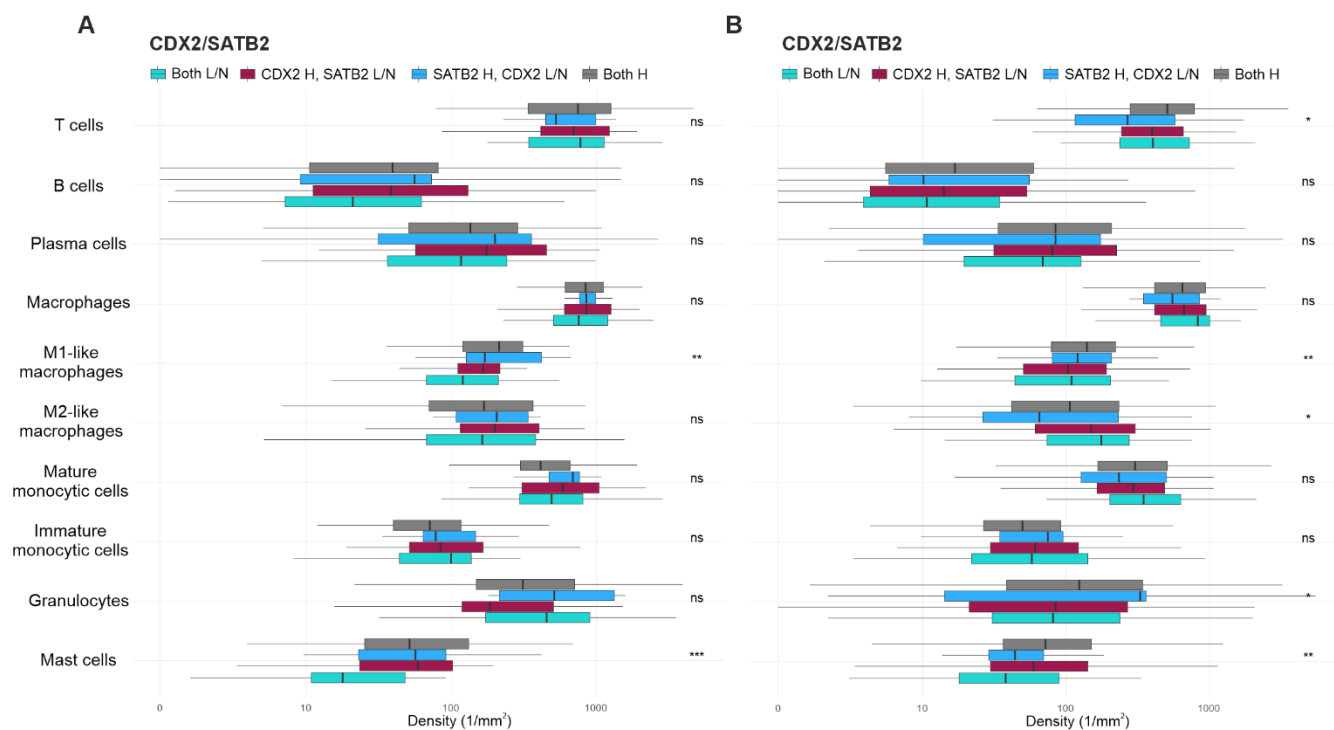
4 AEC + high sensitivity substrate (K3469, Dako).

5 VectaMount AQ Aqueous Mounting Medium (H-5501, Vector Laboratories), digitized with a 20x objective using NanoZoomer XR (Hamamatsu) slide scanner.

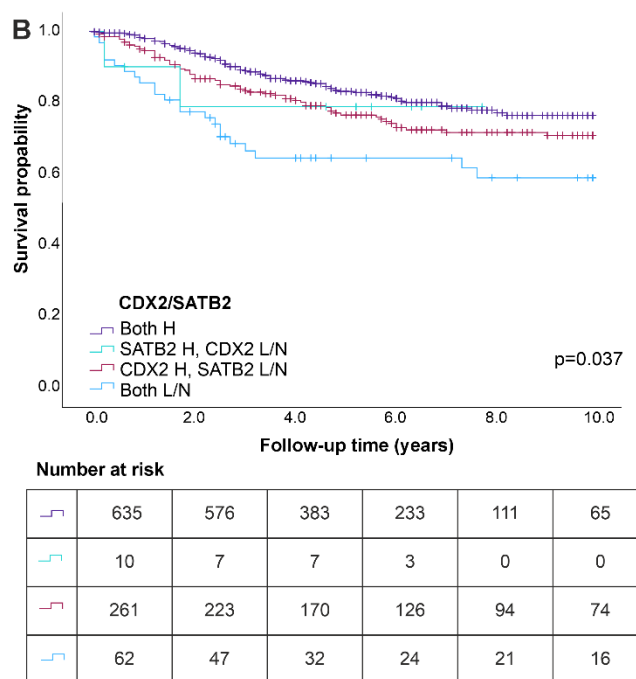
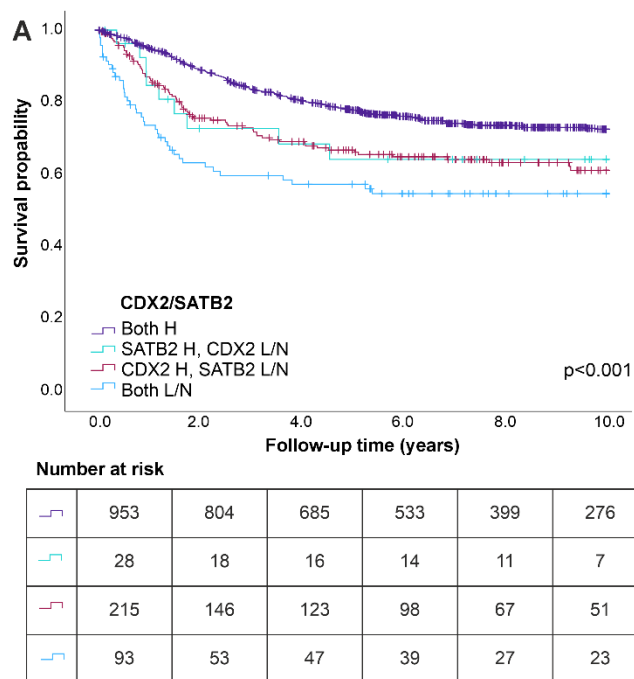
6 The sections were destained with ethanol and antibody stripping was done with heat-induced epitope retrieval using BOND epitope retrieval solution 1 (citrate based, pH 6, AR9961, Leica Biosystems) or 2. Suitable epitope retrieval conditions were optimized for each antibody.



**Figure S2.** Example multiplex immunohistochemistry images of CDX2 high/SATB2 high (A,C,E) and CDX2 negative/SATB2 negative (B,D,F) tumors, their corresponding cell maps, and close-up views.

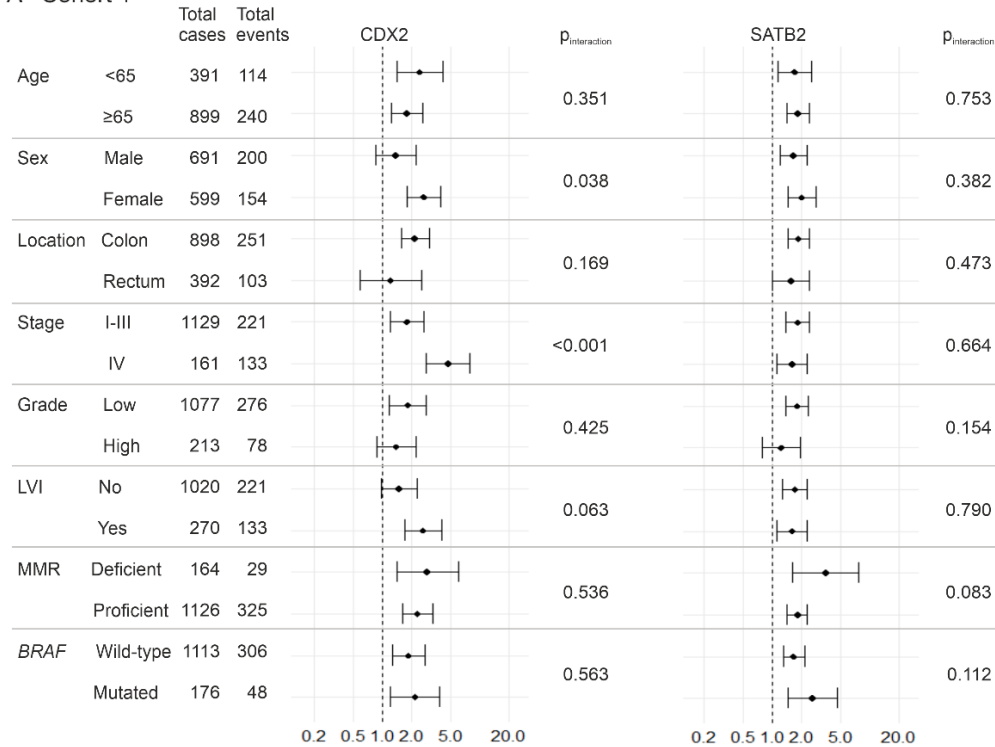


**Figure S3.** Boxplots of distributions of immune cell densities in the mismatch repair deficient tumors (A) and mismatch repair proficient tumors (B) according to combinatory status of CDX2 and SATB2 expression in Cohort 1. \*p value<0.05, \*\*p value<0.01, \*\*\*p value<0.001. H High, L/N Low/Negative.

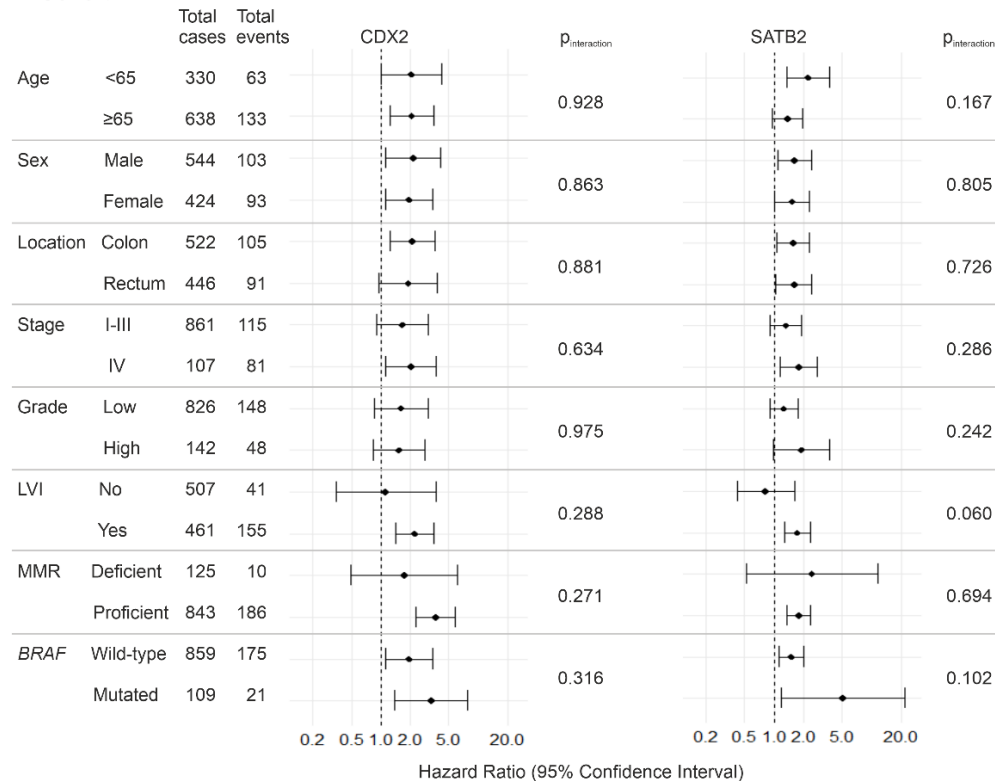


**Figure S4.** Kaplan Meier analysis and log rank test of colorectal cancer specific survival according to the combined CDX2/SATB2 expression variable in Cohort 1 (A) and Cohort 2 (B). H High, L/N Low/Negative.

### A Cohort 1



### B Cohort 2



**Figure S5.** Subgroup analysis of cancer-specific survival by CDX2 and SATB2 expression in Cohort 1 (A) and Cohort 2 (B). The plots present hazard ratios for negative/low (vs. high) CDX2 and SATB2 expression in various patient subgroups. Abbreviations: LVI, lymphovascular invasion; MMR, mismatch repair.