Figure S1

Details on experimental conditions during multiplex qIHC staining. A sequential representation of the major steps during qIHC staining are (a) tissue staining with fluorescent antibodies (b) image acquisition (c) fluorophore unmixing (d) tissue segmentation (e) cell segmentation (f) scoring for marker positivity.

Figure S2

Optimization of protocols for gastric epithelial cell segmentation and cell proliferation. (A) TMA slide (ST1001) comprising of normal gastric tissues (n=8), non-atrophic gastritis (n=9), IM (n=6) and IGC (n=13) were stained with antibodies against **(A)** EpCAM **(B)** pan-Cytokeratin. Plot shows M.F.I for the indicated proteins across cancer stages. **(C)** Normal stomach tissue was stained by mixing EpCAM and pan-Cytokeratin antibodies. (Left) Gastric architecture was revealed by a digitally generated H&E image using autofluorescence and Hoechst 33342 staining as a mask. (Right) Tissue segmentation was performed using combined EpCAM and pan-Cytokeratin (CK) staining. **(D** and **E)** Wild type mice were injected twice with EdU within 24 hours. Tissues harvested after 24 hours were subjected to IHC staining with antibodies against Ki67 and MCM2. EdU signals were detected using click-IT chemistry. **D**-antrum, **E**-corpus. **(F)** Stomach tissue from transgenic *eR1*-CreERT2;LSL-*Kras*^{G12D} mice were subjected to IHC staining with antibodies against MCM2, E-cadherin and Ki67. **(G)** Ki67 and MCM2 nuclear expression in representative cases of normal gastric glands, marked gastritis and marked IM in GCEP samples. Arrows show nuclear expression (200X).

Figure S3 Antibody optimization for DNA damage detection in gastric precancerous lesions. **(A)** HGC-27 cells were exposed to irradiation (2Gy) and cells were harvested after 2 hours. Plot depicts percent the mean fluorescence intensity (M.F.I) of γH2AX. **(B)** A549 cells were subjected to H2AX-siRNA for three days. Q-PCR analysis demonstrates efficient H2AX-knockdown. **(C)** Control or H2AX-knockdown cells were exposed to Etoposide (10 μM) for 6 hours, followed which cells were harvested and processed for quantification of signals using the operetta high-throughput system. γH2AX nuclear fluorescence is shown in the plot. ***p<0.001. Error bars represent S.D.

Figure S4 Immunohistochemical analysis of CDX2 and γH2AX in serial sections of Intestinal metaplasia. (Left) A IM biopsy from the GCEP cohort was subjected to multiplex immunofluorescence staining with EpCAM/pan-Cytokeratin, MCM2 and γH2AX. Scoring map shows the MCM2-positive cells in green. γH2AX-positive cells in magenta, MCM2/γH2AX double-positive (DP) cells in red and MCM2/γH2AX double-negative cells in grey. (Right) A serial section from the same tissue was subjected to conventional DAB-mediated IHC staining. Note: since the serial sections were subjected to different protocols (Multiplex qIHC vs conventional IHC with DAB-based detection), the area of the image and orientation of glands are slightly different between the right and left images. Two representative images labelled as image 1 and image 2 are shown.

Figure S5 Status of DNA damage signaling and DNA hypermethylation in IM lesions and an integrated model summarizing the role of DNA damage signaling in premalignant gastric lesions. (A) Heatmap shows a list of genes that are differentially hypermethylated in genome unstable IM samples. Briefly, DNA methylation data from Huang et al (1) was used compute delta β values for the promoter regions of DNA repair genes in IM samples against 39 normal gastric samples. The student's T-test was used to compare the average delta β value of genome-stable and genome-unstable samples for statistical significance. * p<0.05 **(B)** A schematic representing the pattern of MCM2 and γH2AX staining spanning the premalignant stages. **(C)** Model depicting the relationship between DDR signalling in

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premalignant lesions and GC progression. Left- *Genome-Stable IM*: When the genomes of IM cells encounter chronic inflammation-mediated DNA damage, the activation of the DDR pathway (high γH2AX levels and wild-type p53 status) suppresses mutational accumulation via cell cycle arrest. As long as such IM lesions do not accumulate additional hits that incapacitate their DDR signaling, IM samples with higher DNA damage are predicted to have a lower chance for progression. Right-*Genome-Unstable IM*: When the genomes of IM cells with high CD44v9 expression encounter chronic inflammation-mediated DNA damage, DDR signalling is suppressed in the IM lesions. Such IM lesions have higher ROS defence thus manage to avoid tumor-suppressive networks like the DDR, enabling cells to divide and accumulate mutational/chromosomal aberrations. Epigenetic alterations in DNA repair gene may further fuel genomic instability is such IM lesions. Thus, greater ROS defence in combination with (epi)genomic aberrations in DNA repair pathways can render some IM lesions more permissive to the accumulation of genomic alterations and pose a higher risk for progression.

References

1. Huang KK, Ramnarayanan K, Zhu F, Srivastava S, Xu C, Tan ALK, et al. Genomic and Epigenomic Profiling of High-Risk Intestinal Metaplasia Reveals Molecular Determinants of Progression to Gastric Cancer. Cancer Cell. 2018;33(1):137-50 e5.