

Web Fig. E: IHC levels and corresponding expression levels for Cyclin G2, Smad6, p53, Her2, and Ki-67. The left axis indicates the GeneChip expression level and the right axis indicates the amount of protein staining measured. The error-bars show the standard errors of the mean (S.E.M). The graphs are constructed from 16 Ta tumours, 10 T1 tumours, and 9 T2 tumours where we had expression level results and corresponding IHC staining results. The Ki-67 proliferation index was measured semiquantitatively according to the percentage of positively staining cells as follows: 1+ = 0%-15%; 2+ = 16%-45%; 3+ = 46%-100%. p53 was evaluated according to the following: 0 = no cells positive; 1+ = occasional cells positive; 2+ = frequent positive cells; 3+ = numerous positive cells. Her2 staining was performed using the HercepTest kit (Dako) and evaluated semiquantitatively according to the manufacturer as follows: 0 = no membrane staining or only faint staining in <10% of cells; 1 = faint membrane staining in > 10% of cells; 2 = weak or moderate staining in >10% of cells; 3 = intense membrane staining in >10% of cells. The following positive tissue controls were used: for Ki-67, reactive tonsils (n=2); for p53, two colorectal carcinomas known to harbour p53 mutations; for Her-2, two breast carcinomas showing 3+ Her2-positivity, and positive controls (0, 1+, 2+, 3+) included in the HercepTest (Dako). Negative controls were performed in all experiments and consisted of incubation without specific primary antibody. The tissue arrays included at least one tumour, which was negative for each immunohistochemical target gene product, with the exception of Smad6. These were used as internal negative tissue controls.



