Supplementary discussion:

**Important negative results:**

A multitude of associations of gene-specific DNA methylation and the different molecular and clinical parameters of the analyzed samples were found, some of them confirming previous studies while other ones are reported here for the first time. We did not find any methylation in the *ATM* promoter region which is in concordance with another recent report showing that previous positive findings for the presence of methylation were caused by a suboptimal design of the primers used for methylation-specific PCR [1, 2]. No DNA methylation was also detected in the promoter region of the chemokine receptor *CXCR4*, an important regulator of breast cancer metastasis [3]. So far the presence of DNA methylation had not been described for primary breast tumors or breast cancer cell lines. We did not observe any methylation in the promoter region of *FBXW7*, a *TP53* dependent tumor suppressor gene acting as ubiquitin ligase involved in the degradation of phosphorylated cyclin E and mTOR [4]. Extensive genetic screens have established its role as a tumor suppressor gene and somatic alterations have been found in various cancers including breast cancer [5, 6]. No inactivation by epigenetic mechanisms has so far been reported and our data supports the hypothesis of solely genetic mechanisms for its inactivation. *CDH3 (P-cadherin)* is a marker of basal-like breast carcinomas [7] and its overexpression is at least partially caused by loss of DNA methylation in the promoter CpG island, which has been proposed as a marker for poor prognosis in invasive breast carcinomas [8]. Analyzing three different regions of the promoter CpG island including the previously reported one we were not able to detect methylation, neither in the cell lines nor in the normal breast tissues or the primary tumors which questions both the methylation of its promoter in normal tissue and its demethylation during carcinogenesis. Loss of the negative regulator of the Akt pathway *PTEN* is either caused by mutations but occurs also frequently through promoter hypermethylation
particularly in estrogen positive breast tumors [9]. These results were confirmed in our study, however, neither of the previously reported associations of PTEN methylation with the absence [9] or the presence [10] of ERBB2 amplification was confirmed in the present study possibly due to the low number of ERBB2 positive tumors.