Supplementary Figure Legends and Table

Fig.S1 Screen and validation of conditional PRMT1 overexpression by inter-crossing male K5-Cre and female PRMT1^{Tg} mice

(A) Western blot analyses of Flag tagged PRMT1 protein expression in the skin and thymus from the cohorts of females with genotypes as indicated at 4 weeks of age. Two founder lines (#4 and #7 positive for the PRMT1^{Tg} transgene by PCR genotyping) were selected for validation of Flag expression. Red dashed rectangle highlighted the expected Flag-tagged PRMT1 protein. (B) IHC analysis of Flag expression in the basal cell layer of epidermis and in the outer root sheath of hair follicles. Note that the basal epidermal cells are positive for Flag staining only in bigenic *K5*-PRMT1^{Tg} females in Founder line #4. (C) Haematoxylin and eosin staining (HE) of the skin of the founder lines.

Fig.S2 Screen and validation of conditional CARM1 overexpression by inter-crossing male K5-Cre and female CARM1^{Tg} mice

(A) Western blot analyses of Flag tagged CARM1 protein expression in skin and thymus from the cohorts of females with genotypes as indicated at 4 weeks of age. Three founder lines (#6, #9 and #11 positive for the CARM1^{Tg} transgene by PCR genotyping) were selected for validation of Flag expression. Shown are the representative images from Founder #6 and #9. Red dashed rectangle highlighted the expected Flag-tagged CARM1 protein, which displays smear signal owing to the glycosylation of CARM1 in vivo; (B) IHC analysis of Flag expression in the epidermis of skin samples. Note the basal epidermal cells are positive for Flag staining in only bigenic *K5*-CARM1^{Tg} females. (C) Haematoxylin and eosin staining (HE) of the skin of the founder lines.

Fig.S3 Screen and validation of conditional PRMT6 overexpression by inter-crossing male K5-Cre and female PRMT6^{Tg} mice

(A) Western blot analyses of Flag tagged PRMT6 protein expression in skin and thymus from cohorts of females with genotypes as indicated at 4 weeks of age. Two founder lines (#2 and #4 positive for the PRMT6^{Tg} transgene by PCR genotyping) were selected for validation of Flag expression. Shown are the representative images from Founder #4. Red dashed rectangle highlighted the expected Flag-tagged PRMT6 protein. (B) IHC analysis of Flag expression in the epidermis of skin samples. Note the basal epidermal cells are positive for Flag staining in bigenic *K5*-PRMT6^{Tg} female skin. (C) Haematoxylin and eosin staining (HE) of the skin of the founder line.

Fig.S4 Spontaneous tumor incidence and immunoprofiling of the mammary tumors in the aged bigenic (*K5-PRMT^{Tg}*) female mammary glands

(A) Statistical comparison of the spontaneous tumor incidence between cohorts of single PRMT^{Tg} and bitransgenic *K5*-PRMT^{Tg} virgin females. The spontaneous tumor incidence is significantly higher in the bitransgenic *K5*-PRMT^{Tg} cohort, as compared with the single PRMT^{Tg} cohort for PRMT1 and CARM1, but not for PRMT6. Of note, these tumors all developed in the aged bigenic females (median onset >20months). *, $p<0.05$ (Chi-square test). ns, not significant. (B) HE staining and immunostaining of the spontaneous carcinoma using a panel of antibody markers as labeled to identify mammary tumor subtypes. For bigenic *K5*-PRMT1^{Tg} tumors, case 1 showed positive staining for K5/K8/K14, while case 2 was positive for K8. For *K5*-CARM1^{Tg} tumors, both cases are positive for K8 rather than K5/K14. Basal cells markers: K5 and K14; Luminal cells: K8; ER, estrogen receptor; PR, progesterone receptor. Note that *K5*-CARM1^{Tg} tumors exhibit luminal subtype feature (K8^{+}), whereas *K5*-PRMT1^{Tg} tumors display a mixture feature for both basal and luminal epithelial markers (K5^{+} & K8^{+}). Scale bar = 30μm. (C) IHC scoring for images in panel (B). The IHC slides by CK5, CK8, CK14 staining were scored for the staining intensity ranging from 0 to 3, for a score value as “0” (no staining), “1” (weakly stained), “2” (moderately stained), and “3” (strongly stained). The ER, PR and HER2 IHC stained slides were scored for intensity as described before (29). Typically, markers with score values of 2 & 3 are considered positive.

Fig.S5 *PRMT1* and *CARM1* are frequently overexpressed in human breast tumors with elevated HER2/ERBB2 levels

The relative mRNA expression levels of PRMT1, CARM1 and PRMT6 were profiled from microarray data of a cohort of 352 human breast tumors (GSE2109). The data were normalized to Z score and stratified onto two sub-populations: breast tumors expressing high or low *HER2/ERBB2* levels. The box plots indicate the distribution of relative mRNA expression levels for each condition (P values were calculated from the Rank Sum test. *P value*<0.05 was deemed as statistically significant).

Fig.S6 Characterization of focal neoplastic lesions and tumor nodules in a mouse model of NEU-mediated mammary tumor

(A) Gross morphology of focal lesions identified in the carmine-stained mammary gland whole mounts (left panel). Tumor-free mammary glands were isolated at 12 and 15 weeks and were mounted for whole mount analyses. The average number of neoplastic lesions in mammary

glands was determined by counting the total amount of focal lesions under the microscope. Statistical comparison of incidence of focal hyperplasia lesions calculated from the whole mounts in non-palpable virgin female mammary glands with genotypes and ages indicated (n=11) (right panel). Scale bar = 1 mm. (B) Gross morphology (left panel) and quantification (right panel) of the mammary multi-focal tumor nodules between NIC and NIC-CARM1^{Tg} females. Tumor nodules were counted at six weeks following the initiation of palpable tumors. The average total number of focal tumor nodules is higher in the NIC-CARM1^{Tg} females as compared to in the NIC females (n=15). *, $p<0.05$; **, $p<0.01$ (*Student t test*). (C) Growth curves of the mammary tumors in the NIC and NIC-CARM1^{Tg} females. The average cumulative tumor burden was recorded per animal (n=11). Note the tumor onset is delayed in the NIC-CARM1^{Tg} females, but mammary tumors grow faster when initiated in the NIC-CARM1^{Tg} females. Volume values were illustrated as mean±SD. *, $p<0.05$; **, $p<0.01$ (*Student t test*). (D) Comparison of overexpressed PRMTs protein levels using lysates from mammary tumors isolated from the indicated transgenic mice. Western blot (WB) analysis was performed using mouse monoclonal Flag antibody. Long and short exposure times are shown. White dots illustrate the expected protein sizes for each ectopically expressed PRMT. The white asterisk represents the immunoglobulin light chain (~25kD). We used a light chain secondary antibody in this experiment so as not to obscure the PRMT proteins that migrate around 50kD. Equal protein loading among samples was visualized by Actin immunoblotting after stripping the membrane.

Fig.S7 Comparative analyses of PRMT1, CARM1 and PRMT6 in the human breast cancer samples from TCGA database

(A) The frequency of genomic mutation, deletion and amplification for *PRMT1*, *CARM1* and *PRMT6* genes annotated from TCGA database (http://www.cbioportal.org). Green arrows point to the human breast cancer samples. (B) Comparison of relative mRNA expression levels for *PRMT1*, *CARM1* and *PRMT6* in the tumors and adjacent normal breast tissues (TCGA). (C) Comparison of relative mRNA expression levels for *PRMT1*, *CARM1* and *PRMT6* in different subtypes of human breast cancer samples (TCGA). ERBB2 serves as a positive control, which is upregulated in the HER2 patient group.

Table S1. Primer sequences

StopF	AGAAACCACCGTTGCCGTAA
StopR	GGTGGCAAGTGGTATTCCGT
EgfpF	GGACGACGGCAACTACAAGA
EgfpR	CTCGATGTTGTGGCGGATCT
Itga2bF	GAGTTTTCGCGGAGACAAGC
Itga3bR	CACGGCTACCGAATATCCCC
Itga5F	TACCTGGGTGACAAGAACGC
Itga5R	CCTTCATGGGGTTGCCCAG
Itga7F	GCCGGAGACTTGACCTTGAA
Itga7R	ATCCTTGCGCAGAATGACCA
Pik3r3F	GACTTGTACTGGCCGTTGGA
Pik3r3R	GCATCATCACCTCCCTCCAG
Ccnd2F	GAAGGAGGTAAGGGAAGCACT
Ccnd2R	CGCTCCTCGATGGTCAACAG
Creb5F	TAGCCTGCCCTAGTTTGGGT
Creb5R	AGAAAATCCAAAGCCGCTCG