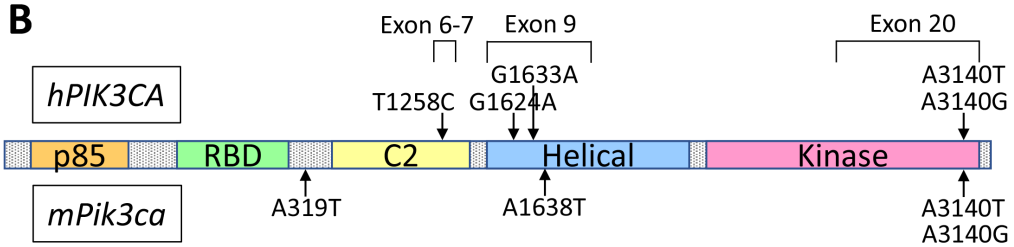
Figure S1. (A) Primers for PCR products used for gene mutations. (B) Mutation hotspots in *hPIK3CA*/*mPik3ca*, which were previously reported in breast/mammary carcinomas in humans and mice. *hPIK3CA*, *PIK3CA* (human); *mPik3ca*, *Pik3ca* (mouse) Ex, exon.

**A** Primers for PCR products used for gene mutations

| Target gene | Primers | Annealing temp. |
|---|---|---|
| *Hras*: | | |
| Ex 2, codon 61 | F; AAACAGGTAGTCATTGATGG<br>R; GCAAATACACAGAGGAAGCC | 61 |
| *Pik3ca*: | | |
| Ex 6 | F; AAGCCACTCTACCCCCAACT<br>R; GGAGCCACAGCTGGTTATGT | 62 |
| Ex 7 | F; TTCTCCTCCCAGAGAAGCAT<br>R; TGGAAACTTCACCACACTGC | 60 |
| Ex 9 | F; CCAAGGAAATCATGGCAGAG<br>R; TGTCCCTGACAGGAAGAAGG | 60 |
| Ex 20 | F; TACCCAGGACAGCTGTGCTA<br>R; CCCAGCTCCCATCTCAGTTC | 65 |

**B**

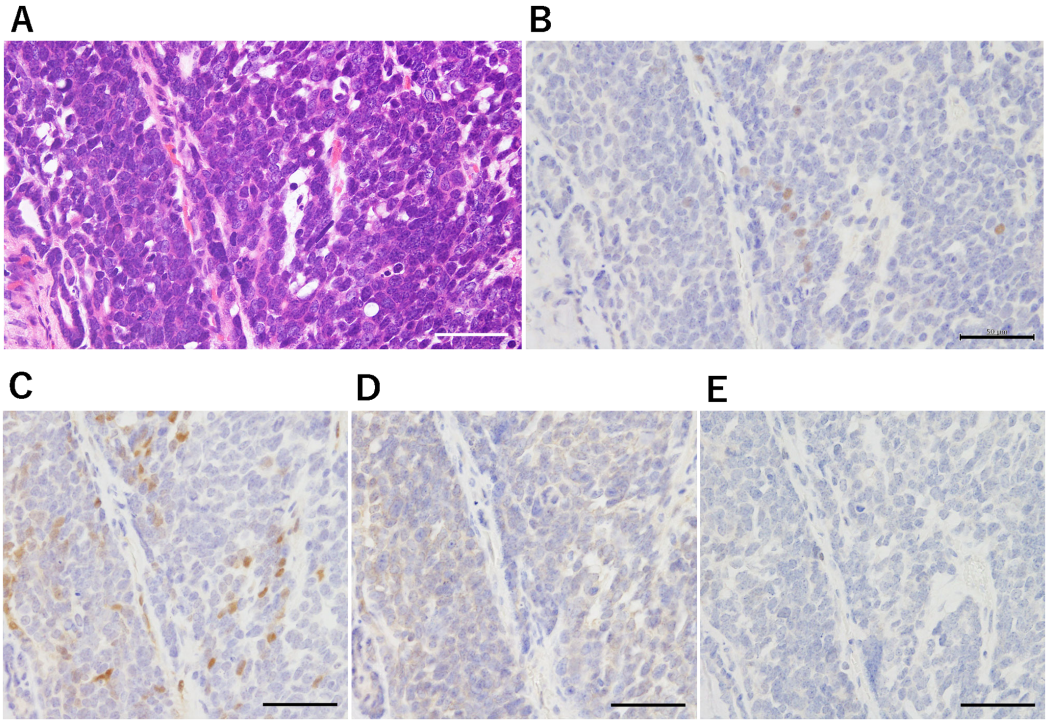



Mutation hotspots in *hPIK3CA*/*mPik3ca*, which were previously reported in breast/mammary adenocarcinomas in humans and mice.

The boxes illustrate functional domains, the p85-binding domain, Ras-binding domain, C2 domain, helical domain, and kinase domain (1). The principle location, and base and amino acid substitution in human breast cancers were T1258C:C420R (2), G1624A:E542K (2, 3), G1633A:E545K (1-3), A3140G:H1047R (1-3), and A3140T:H1047L (2). Those in DMBA-induced mammary adenocarcinomas were A319T:N107Y, A1638T:Q546H (4), A3140G:H1047R (4), and A3140T:H1047L (4).

1. Samuels Y et al., Science 304, 554, 2004.
2. Campbell IG et al., Cancer Res 64, 7678, 2004.
3. Bachman KE et al., Cancer Biol Ther 3, 772-775, 2004
4. Abba MC et al., Oncotarget 7, 64289, 2016.

Figure S2. (A) Mammary carcinoma with solid/microacinar structures in a C3H- crossed with BALB/c-N2 *Trp53* heterozygous knockout mouse; scale bar, 50 $\mu$m) (24). (B) Serial section to (A) immunostained with ERα; nuclear positive cells were scattered. (C) Immunostaining for pERK; cytoplasmic/nuclear positive cells were observed. (D) Immunostaining for β-catenin; membranous positivity was detected. (E) Staining for pAkt was negative. (F) Summary of immunohistochemical and mutation analyses of spontaneous mammary carcinomas in C3H- crossed with BALB/c *Trp53* heterozygous knockout mice. N2, second generation; *Trp53*, tumor protein p53; ERα, estrogen receptor α; N, nuclear; CN, cytoplasmic/nuclear; M, membranous; MC, membranous/cytoplasmic; NT, not tested.




**F** Summary of immunohistochemical and mutation analyses of spontaneous mammary carcinomas in C3H- backcrossed to BALB/c-*Trp53* heterozygous knockout mice

| | F1 | | | N2 | | | |
|---|---|---|---|---|---|---|---|
| Sample # | 1 | 2 | 3 | 1 | 2 | 3 | 4 |
| Immunohistochemistry | | | | | | | |
| ERα | + | – | – | + | – | + | – |
| pERK1/2 | CN | CN | CN | CN | CN/M | CN | CN/M |
| pAKT | N | – | – | – | – | – | – |
| β-catenin | M | M | M | N | M | M | M |
| Gene mutation | | | | | | | |
| *Hras*, codon 61 | – | – | NT | – | NT | – | – |

| | N3 | | | | N4 | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Sample # | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 | 5 | 6 |
| Immunohistochemistry | | | | | | | | | | |
| ERα | – | – | – | – | – | + | – | – | – | + |
| pERK1/2 | CN/M | CN/M | – | – | CN | N | CN | CN | CN/M | – |
| pAKT | – | – | – | – | – | – | – | – | MC | – |
| β-catenin | MC/N | M | M | M | N | MC/N | MC | M | M | M |
| Gene mutation | | | | | | | | | | |
| *Hras*, codon 61 | – | NT | – | – | – | – | – | – | – | – |