SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure 1. Hippocampal sections from new-born Pten+/+ and PtenFV/FV pups were stained with haematoxylin and eosin (H&E) and were imaged at (a) low and (b) high magnification. Arrows indicate abnormally positioned neurons in mutant hippocampi. Scale bar = 500 μm (low magnification) or 100 μm (high magnification).

Supplementary Figure 2. (a) Immunoblots of whole cell lysates from Pten+/+, Pten+/Δ or PtenFV/Δ MEFs were probed with specific antibodies for PTEN, TUBULIN was used as a loading control. (b) Immunoblots of whole cell from Pten+/+ and PtenFV/FV MEFs were probed with specific antibodies for PTEN, P-AKT1308, P-AKT5473, AKT, P-GSK3βS9, GSK3β, and the loading control GAPDH lysates (c) cDNA for MEFs with the indicated genotypes were generated as described in Methods. The relative Pten mRNA levels were compared to Gapdh mRNA levels by real-time PCR assays. (d) Examples of the quantitative RT-PCR results used to confirm the microarray data in Figure 2d.

Supplementary Figure 3. (a) Pten+/+ or PtenFV/FV E9.5 embryos were fractionated to isolate lysates from nuclear, cytoplasmic and membrane compartments. Lysates were immunoblotted and probed for PTEN, P-AKT5473, and compartment-specific markers (P-p70, slower migrating band is cytoplasmic and faster migrating band is nuclear; α-TUBULIN, cytoplasmic). (b) Whole cell lysates from Pten+/+, PtenFV/Δ and PtenΔ/Δ MEFs were immunoprecipitated with antibodies against SUMO. Note, in order to compensate for lower PTEN FV protein levels in lysates from PtenFV/Δ MEFs than in Pten+/+ MEFs, we used three times more lysate for immunoprecipitations from PtenFV/Δ MEFs than from Pten+/+ and PtenΔ/Δ MEFs. Samples were then immunoblotted and probed with antibodies against PTEN (left panel). The inputs were immunoblotted and probed for PTEN (right panel). (c) New born pups from PtenFV/+ het-het crosses were sacrificed and fixed in formalin overnight. The cross-linked Pten+/+ and PtenFV/FV tissues were prepared for IHC and stained with antibodies against P-AKT5473. Scale bar = 50 μm.

Supplementary Figure 4. “Lesion” corresponds to the indicated pathological classifications (described in Methods) of the tissues in the table. Total number of mice evaluated for each organ (indicated at the bottom of table) and the numbers of mice with lesions in the specified tissues were noted.

Supplementary Figure 5. (a) Pten was ablated in the endometrium of mice with endometrium specific cre, Spr2f-cre, to generate Pten+/Δ or PtenΔ/Δ females. Mice at the indicated ages were sacrificed and the uteri were harvested. Uterine sections were stained with H & E and histopathological analysis performed. Total number of mice (indicated at the bottom of table) and the number of lesions were noted. (b-d) Sections from uteri of 6 month old Pten+/+, PtenΔ/Δ and PtenFV/Δ females were processed for IHC using antibodies specific for PTEN and AKT5473 (b); P-AKT5473 and FOXO3A (c); or antibodies specific for γH2AX and CC3 (d). Bar scale = 50 μm.

Supplementary Figure 6. (a) Mammary glands from PtenL/L and PtenFVL females were stained with red carmine, and imaged at low magnification (left panel) and at high magnification (right panel). Scale bar = 500 μm. (b) Large palpable mammary tumors were detected in 18 month-old PtenFV/+ female mice (upper panels, white arrows). Histopathological analysis of the H & E-
stained sections from the formalin-fixed tumors revealed adenosquamous carcinoma and adenomyoepithelial carcinoma (bottom panel). Scale bar = 50 μm. (c) Sections from ErbB2 initiated mammary tumors harvested from females with the indicated Pten genotypes were stained with H & E and histopathology was compared. Scale bar = 50 μm.

**Supplementary Figure 7.** (a) Sections of mammary tumors from female mice with the indicated genotypes were stained for P-AKT5473 (top) or FOXO3A (bottom). Scale bar = 50 μm. (b) Sections of mammary tumors harvested from females with the indicated genotypes were stained with H & E (top), and were processed for IHC using antibodies specific for PTEN and P-AKT (bottom). The pink chromophore corresponds to P-AKT and brown chromophore corresponds to PTEN. Scale bar = 50 μm. (c) Differentially expressed genes in the microarray shown in figure 6e were confirmed by quantitative real-time PCRs. Meis2 and Casp12 were two genes that were similarly expressed in PtenFV/Δ and Pten+/+ or PtenΔ/Δ mammary epithelial cells (MECs). Nupr1 and Mmp9 were two genes exemplifying genes that were similarly expressed in PtenFV/Δ and PtenΔ/Δ MECs. (d) Immunoblots of whole cell lysates derived from MEFs with the indicated genotypes were probed for PTEN, P-AKT308, P-AKT5473, AKT, P-GSK3βS9, GSK3β, P-CHK1S345, CHK1, P-ATM1987, γH2AX, and GAPDH. (e) Mammary tumor sections from females with the indicated genotypes were IHC-stained for γH2AX. Scale bar = 50 μm. (f) The γH2AX-positive nuclei found in the samples represented in (e) were quantified and plotted in a histogram. n, number of mice, light blue line, median. Positive cells were counted as described in Materials and Methods. Double asterisks are indicating p-value < 0.05 with respect to Pten+/+, and PtenΔ/+. One-way ANOVA was used for data analysis. (g) Sections from Pten+/+, PtenΔ/Δ, PtenFV/Δ and PtenΔ/Δ mammary glands of 3 month old female mice were IHC-stained for γH2AX.

**Supplementary Figure 8.** Primers used for the indicated purposes during the generation and characterization of the PtenFV knock-in mice.

**Supplementary Figure 9.** Primers used in rt-PCRs to determine the PTEN mRNA levels in Pten+/+, Pten+/Δ, and PtenFV/Δ MEFs. Gapdh was used as an internal control in these experiments.

**Supplementary Figure 10.** The table shows the parameters used in the setting of the Leica autostainer (BondRX autostainer).

**Supplementary Figure 11.** Primers used in individual rt-PCR reactions to validate expression changes for the indicated target genes observed in the micro-arrays.
Hippocampus and cerebral cortex

**a**

- **Pten**\(^+/+\)
- **Pten**\(^{FV/FV}\)

- Hippocampus and cerebral cortex

  - 4x

**b**

- **Pten**\(^+/+\)
- **Pten**\(^{FV/FV}\)

  - Hippocampus
  - 20x

  - 20x

Supplementary Fig. 1
**Supplementary Fig. 2**

**a**

|         | $Pten^{+/+}$ | $Pten^{FV/FV}$ | $Pten^{Δ/Δ}$ |
|---------|--------------|----------------|--------------|
| PTEN    |              |                |              |
| P-AKT$^{S473}$ |          |                |              |
| TUBULIN |              |                |              |

**b**

- PTEN
- P-AKT$^{T308}$
- P-AKT$^{S473}$
- AKT
- P-GSK3β$^{S9}$
- GSK3β
- GAPDH

**c**

![Graph showing relative gene expression of PTEN mRNA](image)

**d**

**Upregulated genes**
- Fos
- Vav3
- Cd34
- Plk3r3

**Downregulated genes**
- Flt-1
- Wnt-4
- CxcR6
- Tnf11
Supplementary Fig. 3

(a) Western blot analysis of PTEN, P-AKT\(^{S473}\), P-p70, and \(\alpha\)-TUBULIN in Pten\(^{+/+}\) and Pten\(^{FV/FV}\) mice.

(b) Immunoprecipitation (IP) with anti-Sumo and immunoblot (IB) with Pten antibody.

(c) Immunohistochemical staining of Pten\(^{+/+}\) and Pten\(^{FV/FV}\) in Adrenal Gland, Liver, Lungs, and Intestine.
| Tissue       | Lesion                                | Scored for:                           | Pten<sup>+/+</sup> | Pten<sup>+/-</sup> | Pten<sup>FV/-</sup> |
|--------------|---------------------------------------|---------------------------------------|--------------------|--------------------|---------------------|
| Uterus       | No                                    | nsl, simple endometrial hyperplasia   | 2                  | 18                 | 45                  |
|              | Yes                                   | carcinoma in situ                     | 20                 | 0                  | 3                   |
|              | Total                                 |                                       | 22                 | 18                 | 48                  |
| Thyroid      | No                                    | nsl, follicular hyperplasia           | 35                 | 33                 | 75                  |
|              | Yes                                   | Follicular adenoma                    | 9                  | 0                  | 0                   |
|              | Total                                 |                                       | 44                 | 33                 | 75                  |
| Prostate     | No                                    | nsl, hyperplasia                      | 0                  | 17                 | 48                  |
|              | Yes                                   | PIN I-IV, carcinoma                   | 17                 | 0                  | 0                   |
|              | Total                                 |                                       | 17                 | 17                 | 48                  |
| Mammary Gland| No                                    | nsl, hyperplasia                      | 16                 | 23                 | 21                  |
|              | Yes                                   | LG and HG MIN, Tumor                  | 14                 | 4                  | 34                  |
|              | Total                                 |                                       | 30                 | 27                 | 55                  |
| Adrenal Medulla| No                                   | nsl, medullary hyperplasia            | 8                  | 47                 | 50                  |
|              | Yes                                   | Pheochromocytoma (benign and malignant)| 39                 | 1                  | 47                  |
|              | Total                                 |                                       | 47                 | 48                 | 97                  |
| Thymus       | No                                    | nsl, lymphoid hyperplasia             | 45                 | 27                 | 50                  |
|              | Yes                                   | lymphoma                              | 6                  | 5                  | 27                  |
|              | Total                                 |                                       | 51                 | 32                 | 77                  |
| Stomach      | No                                    | nsl, mucosal hyperplasia              | 44                 | 49                 | 82                  |
|              | Yes                                   | atypical mucosal hyperplasia/dysplasia, carcinoma | 5 | 3 | 16 |
|              | Total                                 |                                       | 49                 | 52                 | 98                  |
### Table

| Tissue                        | Scored for:                      | Pten\(^{+/\Delta}\) | Pten\(^{\Delta/\Delta}\) |
|-------------------------------|----------------------------------|----------------------|---------------------------|
|                               |                                  | 2 mo     | 6 mo     | 2 mo     | 3 mo     | 6 mo     |
| Uterus                        | nsl, simple hyperplasia          | 6        | 0        | 0        | 0        | 0        |
|                               | carcinoma in situ                | 0        | 7        | 7        | 5        | 5        |
|                               | Invasive carcinoma               | 0        | 0        | 2        | 5        | 4        |
|                               | metastasis                       | 0        | 0        | 0        | 2        | 2        |
| **Total**                     |                                  | **6**     | **7**     | **9**     | **10**    | **9**     |

### Figures

**a**

| Tissue | Scored for:                      | Pten\(^{+/\Delta}\) | Pten\(^{\Delta/\Delta}\) |
|--------|----------------------------------|----------------------|---------------------------|
|        |                                  | 2 mo     | 6 mo     | 2 mo     | 3 mo     | 6 mo     |
| Uterus | nsl, simple hyperplasia          | 6        | 0        | 0        | 0        | 0        |
|        | carcinoma in situ                | 0        | 7        | 7        | 5        | 5        |
|        | Invasive carcinoma               | 0        | 0        | 2        | 5        | 4        |
|        | metastasis                       | 0        | 0        | 0        | 2        | 2        |
| **Total** |                                  | **6**     | **7**     | **9**     | **10**    | **9**     |

**b**

**c**

**d**

P-PTEN, P-AKT\(^{847}\), FOXO3A, \(\gamma\)H2AX, CC3.
Adenosquamous carcinoma

Adenomyoepithelioma

PtenL/L PtenFV/L

Pten+/+ PtenFV/

Δ Pten+/Δ

Δ PtenFV/+Δ

Δ
**Supplementary Fig. 7**

**a** ErbB2 Pten+/+ | ErbB2 PtenΔ/Δ | ErbB2 PtenFV/Δ
---|---|---
P-AKT^S473 | | |
FOXO3A | | |
**b** ErbB2 PtenΔ/+ | ErbB2 PtenΔ/Δ | ErbB2 PtenFV/Δ
---|---|---
H & E | | |
P-TEN & P-AKT | | |
**c** Meis2 | Nupr1
---|---
Relative gene expression | |
Casp12 | Mmp9
---|---
+/+ FV/Δ | +/Δ Δ/Δ
**d** Pten+/+ | PtenΔ/+ | PtenΔ/Δ | PtenΔ/Δ
---|---|---|---
PTEN | P-AKT^T308 | AKT | |
P-AKT^S473 | | | |
PTEN | GSK3β | |
AKT | P-GSK3β^S9 | |
GAPDH | |
**e** γ H2AX
---
ErbB2 Pten+/+ | ErbB2 PtenΔ/Δ | ErbB2 PtenΔ/Δ
---|---|---
γ H2AX quantification
---|
ErbB2 PtenFV/Δ | |
**f** γ H2AX quantification
---|---|---|---
ErbB2 Pten+/+ | ErbB2 PtenΔ/Δ | ErbB2 PtenFV/Δ
---|---|---|---
ErbB2 PtenΔ/Δ | ErbB2 PtenΔ/Δ |
---|---|---|---
ErbB2 PtenFV/Δ | ErbB2 PtenFV/Δ |
---|---|---|---
% positive cells | n=10 | n=9 | n=8 | n=7

**g** γ H2AX
---
Pten+/+ | |
PtenΔ/+ | |
PtenΔ/Δ | |
PtenΔ/Δ | |
PtenFV/Δ | |
| Name    | Primers                                      |
|---------|----------------------------------------------|
| Short arm-Forward | ctatgttgcaacaagccatgtt               |
| Short arm-Reverse | tggcaactacatttctggccccctgtatc          |
| Long arm-Forward | tagggatcaacttactagggctttctg              |
| Long arm-Reverse | acacgtaaatttgtatctcctcag                |
| primer1  | acacaggcttggtcctcag                      |
| primer2  | gggggaacctctgactagg                      |
| primer3  | atcaggtgtgtctacttcaacaga                 |
| Probe1-Forward | tagggctcccttttaatgtcata            |
| Probe1-Reverse | tctgtatctagaccccaatagca               |
| Probe2-Forward | gagggtcaataaggagctttgata            |
| Probe2-Reverse | ctaattaaggctggggttggaga            |
| Gene | Link UPL   | Left primer     | Right primer       |
|------|------------|-----------------|--------------------|
| Pten | NM_008960  | gactggtgaatgatttg  | ctgactgggaattgactc |
| Gapdh| NM_008084.2| cggtgtgaacggatttggc | tttgatgttagggtctgc  |
| Antibody    | Dilution | Time  | AR  | AR time      | Super block | Post primary |
|-------------|----------|-------|-----|--------------|-------------|--------------|
| PTEN        | 1:150    | 30min | ER2 | 20min (100°C)| Yes 10min   | no           |
| p-AKT (S473)| 1:50     | 60min | ER2 | 20min (100°C)| yes 10min   | no           |
| p-H2AX (S139)| 1:800   | 30min | ER2 | 20min (100°C)| yes 10min   | no           |
| FOXO3A      | 1:400    | 30min | ER2 | 20min (100°C)| yes 10min   | no           |
| Gene    | Link UPL   | Left primer                | Right primer                | Probe# |
|---------|------------|----------------------------|----------------------------|--------|
| Meis2   | NM_010825.2| acacaaggagcagcatctatgg     | gctcgacacttctcAAAacc       | #6     |
| Casp12  | NM_009808.2| gggaattagcacaggcaact       | tlctttctctcagctacagcaaaa  | #48    |
| Nupr1   | NM_019738.1| gaagctgtcgcaccaagtccc     | gtgtgggtgtgtggtggtggtg    | #78    |
| Mmp9    | NM_013599.2| acgacataagacggcatccca     | gctgtggtcagttgtggtggtg    | #19    |
| Fos     | NM_010234.2| gggacagccttctactacc       | agatctgcgcacaaagttctg     | #67    |
| Cd34    | NM_133654.3| gggtagctctctgcctgtatg     | tccggtgtacacagcgatcaaa    | #84    |
| Vav3    | NM_020505  | gcacataaagaatgttgggaag    | gttttgaatggccctgttt        | #53    |
| Pik3r3  | NM_181585  | atccgggatcagcacttt         | gctttcatctgtagcctctttct    | #20    |
| Flt-1   | D88689     | gcgccgggtatttataagac      | ccatccatttaggggaagtcc      | #55    |
| CxcR6   | NM_030712.4| agctactgggtctctttctga     | tcaatagtgcctccatgtcag      | #105   |
| Wnt-4   | NM_009523.2| actggactcccttcctgtct      | tggccttgtctagccta         | #62    |
| Tnsf11  | NM_011613.3| tgaagacacactatcctgtcttg    | cccacaatgttgtggagttc      | #88    |