628 the basal layers of OVX control whereas some cells away from basal layer are also
629 positive in the OVX *Pten* CKO mice (G, H). p63 expression area appeared to be
630 extended to the upper layer upon E2 administration in the *Pten* CKO mouse (O, P).
631 Scale bar: 100 μm.
632
633 **Fig. 4.** *Pten* CKO leads to cell proliferation but not contribute to epithelial cell survival
634 in mouse vagina. BrdU immunostaining in control (A, C) and *Pten* CKO mouse vagina
635 (B, D). Note that BrdU positive cells in the upper layer of the OVX K5Cre;$Pten^{f/f}$ mice
636 (red arrowheads in panel B). Black arrowheads indicate normal cell proliferation in the
637 basal layer. Proliferation indices in the OVX *Pten* CKO mouse vagina were higher than
638 those of OVX controls (E). TUNEL assay indicates *Pten* mutation dose not influence
639 cell apoptosis in the vaginal epithelium (F-H). Black arrowheads indicate apoptotic cells.
640 Scale bar: 100 μm.
641
642 **Fig. 5.** mTOR activation is associated with hyperplasia in mouse vagina. Activation
643 status of Akt and mTOR (A). In addition to Akt, mTOR in the mutants show significant
644 increase of phosphorylation when calculated with Image J program (B). Rapamycin
645 administration can partially rescue the hyperplasic phenotype of *Pten* CKO mice, but
646 remains mucous production (C-F; n=5). The level of cell proliferation in the mutant is
647 significant reduced by rapamycin treatment (G). Rapamycin treatment did not influence
648 on the phosphorylation levels of Akt (H). Scale bar: 100 μm.
649
650 **Fig. 6.** Conditional *Pten* deletion results in MAPK and ERα phosphorylation.
651 Phosphorylated MEK and ERK1/2 were detected in the suprabasal cells in the mutants

652 (A-D). Activated MAPK remains in the *Pten* CKO mice administrated with rapamycin
653 (E, F). ERα protein was similarly expressed in both control and *Pten* CKO mouse
654 vagina (G, H), whereas phosphorylated ERα was detected only in the *Pten* CKO mice
655 (I). Expression profiles of growth factor mRNAs in OVX control and *Pten* CKO mouse
656 vagina (J). Scale bar: 100 μm.
657
658 **Fig. 7.** Estrogen-dependent localization of phosphorylated Akt expression in mouse
659 vagina.
660 Pten is mainly expressed in the basal cells in stratified and squamous epithelia in
661 epidermis of the skin (A), tongue (B), esophagus (C) and forestomach (D) of control
662 mice (Note no such signals in the *Pten* CKO mice; A'-D'). In the *Pten* CKO mice,
663 phospho-Akt expression is similarly detected in these tissues (E-H). Phospho-Akt
664 expression in the control mice is not prominent (E'-H'). Phospho-Akt is predominantly
665 expressed in the basal to suprabasal epithelial cells in the E2-administrated control
666 mouse vagina (I). In the *Pten* CKO mice, the phosphorylated Akt expression is
667 augmented (J). Akt is not phosphorylated in the absence of estrogen in the control mice
668 (K). In the OVX *Pten* CKO mouse vagina, phosphorylated Akt is evident in the
669 suprabasal epithelial cells (sbc), but not in the basal epithelial cells (bc) (L). See also
670 Fig. 1 for Pten expression. Dotted lines indicate basal layer of the epithelium. Scale bar:
671 100 μm.
672
673 **Fig. 8.** A possible scheme depicting Pten expression and its regulation of homeostasis
674 for mouse vagina in the absence (A) and presence of estrogen (B).
675

Fig. S1. Phenotypes of stratified and squamous epithelium in the *Pten* CKO mice. In the CKO mice, hyperplasia and keratosis was observed in the epidermis of skin, tongue, esophagus and forestomach.

Fig. S2. Vaginal epithelial cell-specific activation of Cre recombinase is confirmed by Rosa reporter (*K5Cre/+;R26R*$^{eYFP/+}$) mice at 8 weeks of age (A, B). Dotted line indicate basal layer of the epithelium.

Fig. S3. The phenotypes of vaginae in intact (without OVX surgery) *Pten* CKO mice. In control mice, normal estrous cycle is observed (A, B), however, vaginal smears in the *Pten* CKO mice consist of mucus and no keratinocytes (C). Histology of the vagina resembles those of the OVX *Pten* CKO mice (D, see also in Fig. 1B), suggesting a lack or very low levels of E2 production in the mutants. Ovary contains various stages of follicles and corpora lutea in controls (E), however, ovary of *Pten* CKO mouse exhibits no corpora lutea although follicles grow at mature stage (F). We thus suspected defects in the hypothalamus-pituitary axis, and the following experiments were performed by OVX mice.

Fig. 1

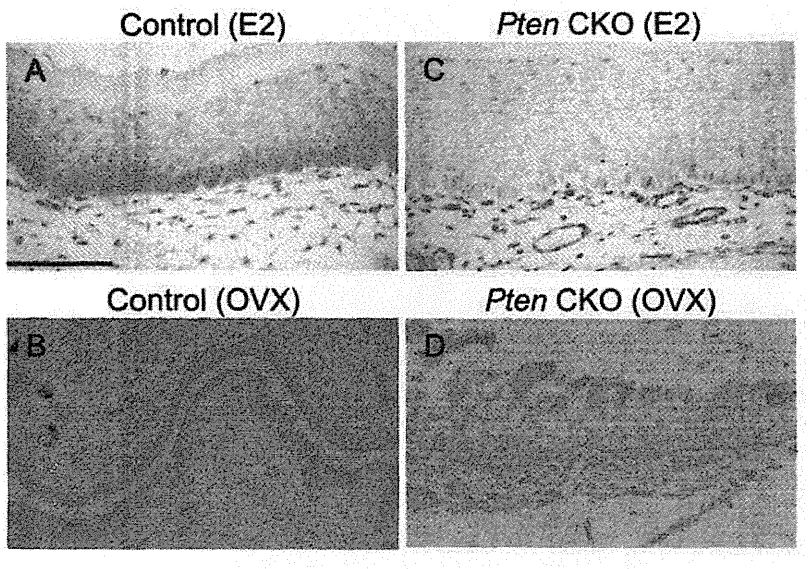


Fig. 2

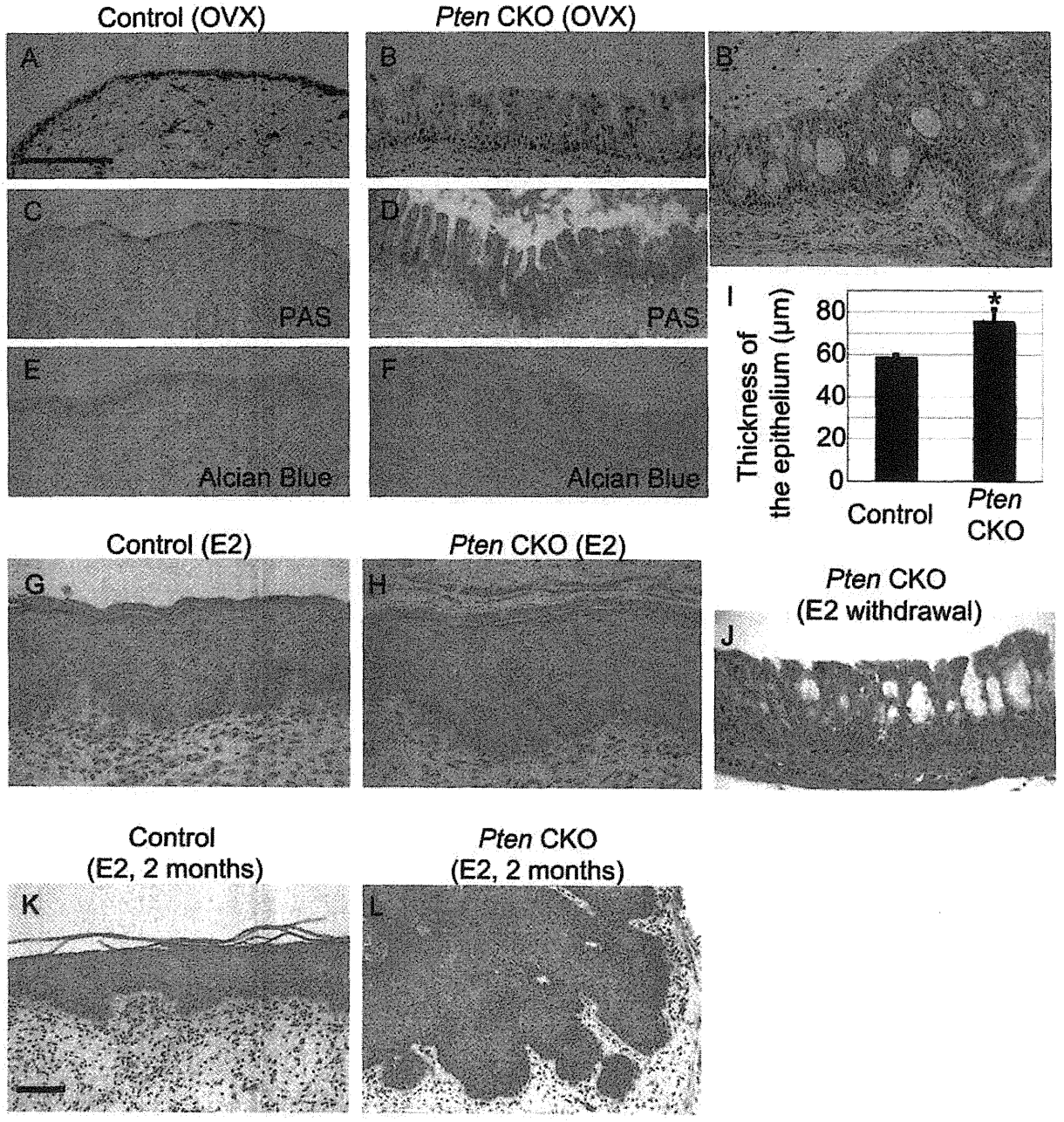

Control (OVX)
Pten CKO (OVX)
A
B
B'
C
PAS
D
PAS
E
Alcian Blue
F
Alcian Blue
I
Thickness of the epithelium (μm)
80
60
40
20
0
*
Control
Pten CKO
Control (E2)
Pten CKO (E2)
G
H
Pten CKO
(E2 withdrawal)
J
Control
(E2, 2 months)
Pten CKO
(E2, 2 months)
K
L

Fig. 3

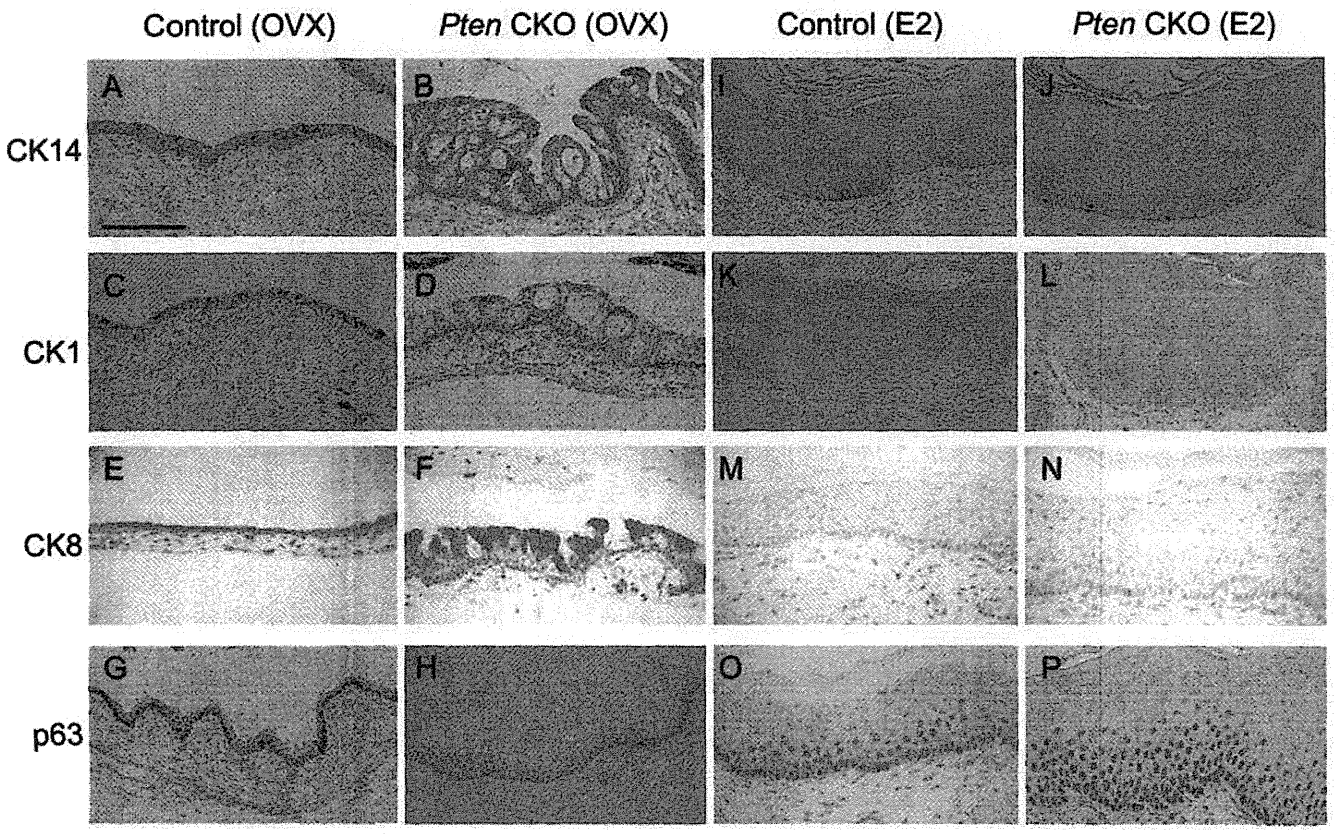

Control (OVX)
Pten CKO (OVX)
Control (E2)
Pten CKO (E2)
CK14
CK1
CK8
p63
A
B
I
J
C
D
K
L
E
F
M
N
G
H
O
P

Fig. 4

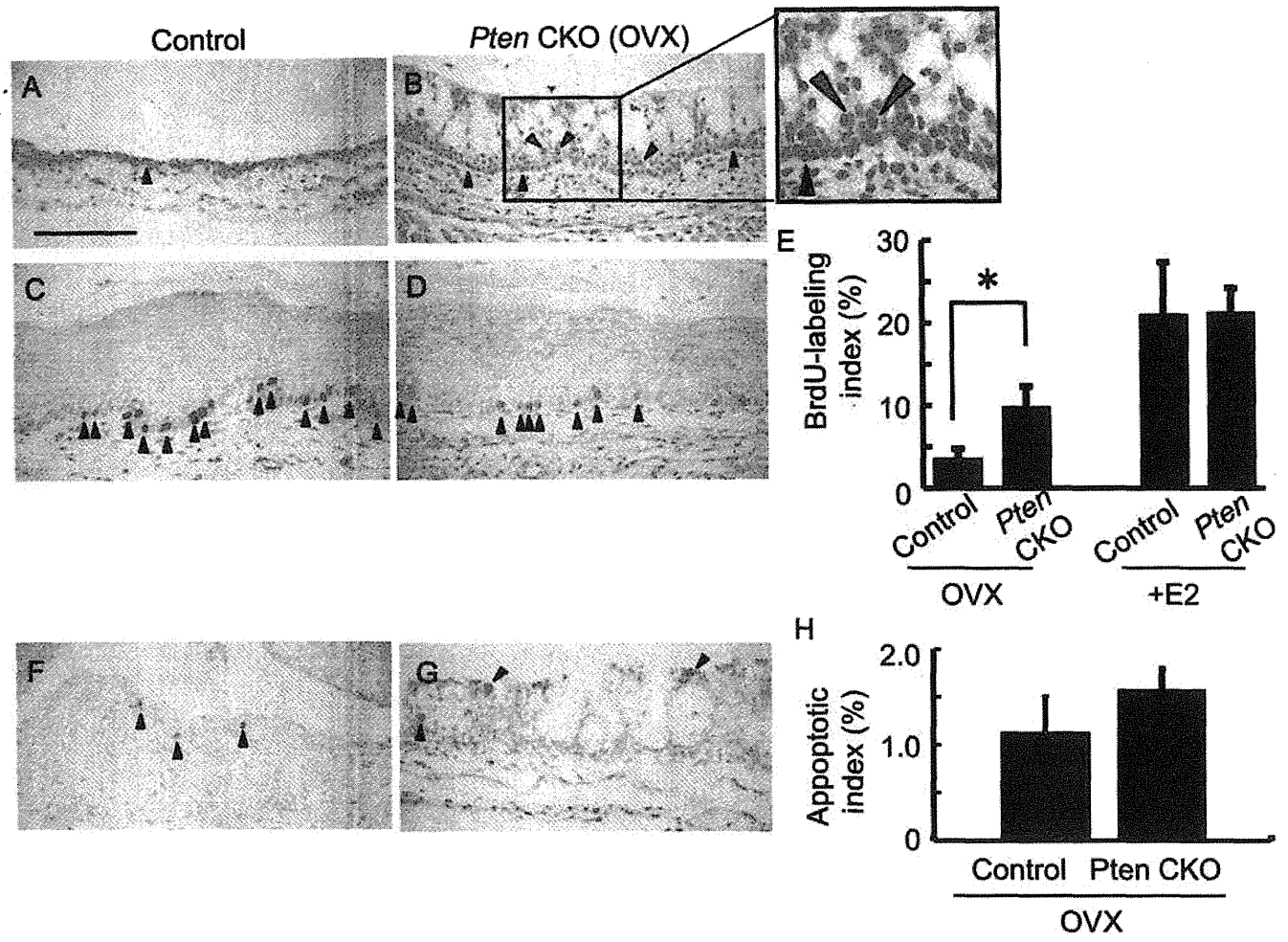

Control
Pten CKO (OVX)
A
B
C
D
E
BrdU-labeling index (%)
30
20
10
0
*
Control
Pten CKO
Control
Pten CKO
OVX
+E2
F
G
H
Appoptotic index (%)
2.0
1.0
0
Control
Pten CKO
OVX

Fig. 5

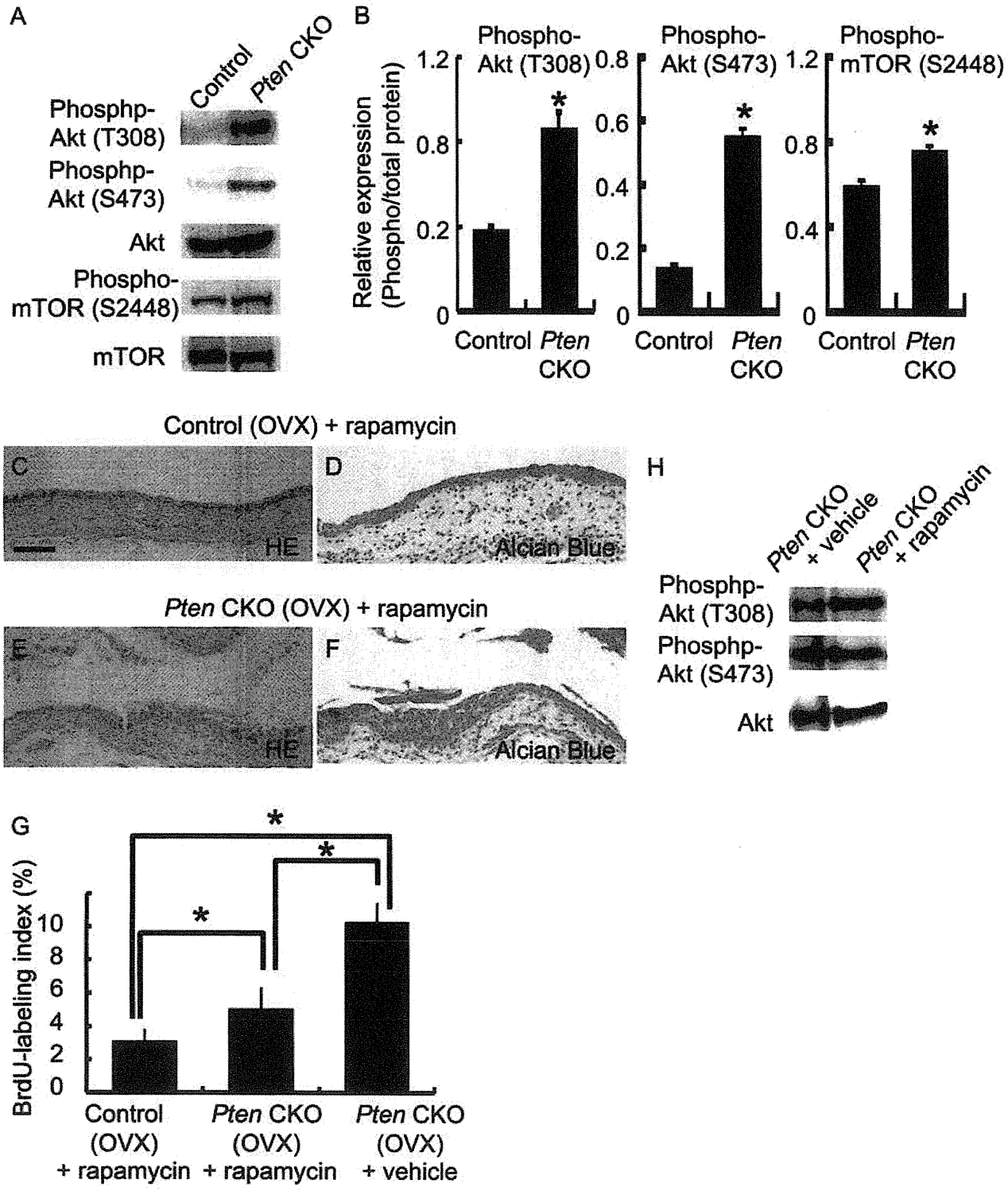

A
Control
Pten CKO
Phosphp-Akt (T308)
Phosphp-Akt (S473)
Akt
Phospho-mTOR (S2448)
mTOR
B
Relative expression (Phospho/total protein)
Phospho-Akt (T308)
Phospho-Akt (S473)
Phospho-mTOR (S2448)
Control
Pten CKO
Control (OVX) + rapamycin
C
HE
D
Alcian Blue
Pten CKO (OVX) + rapamycin
E
HE
F
Alcian Blue
G
BrdU-labeling index (%)
Control (OVX) + rapamycin
Pten CKO (OVX) + rapamycin
Pten CKO (OVX) + vehicle
H
Pten CKO + vehicle
Pten CKO + rapamycin
Phosphp-Akt (T308)
Phosphp-Akt (S473)
Akt

Fig. 6

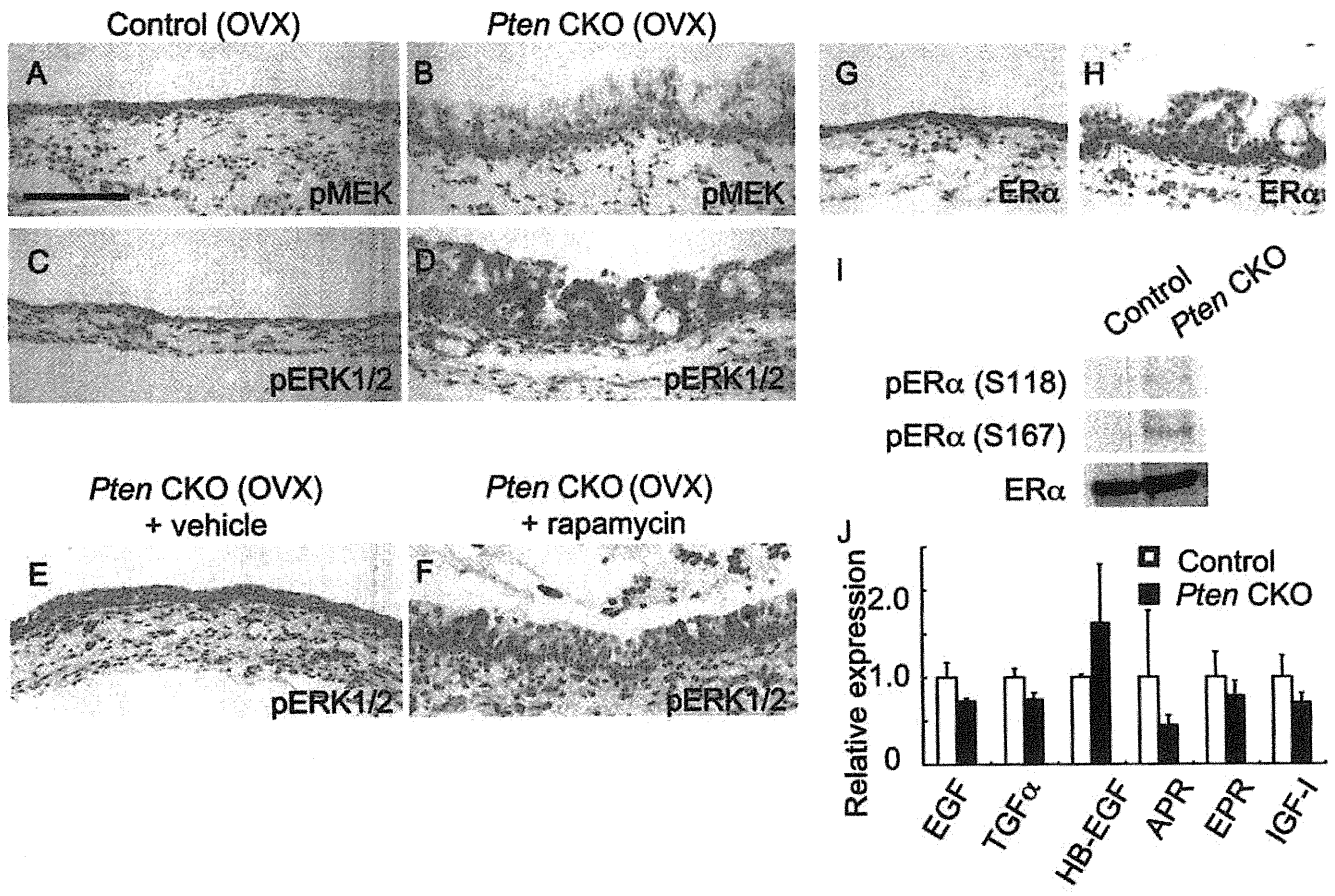
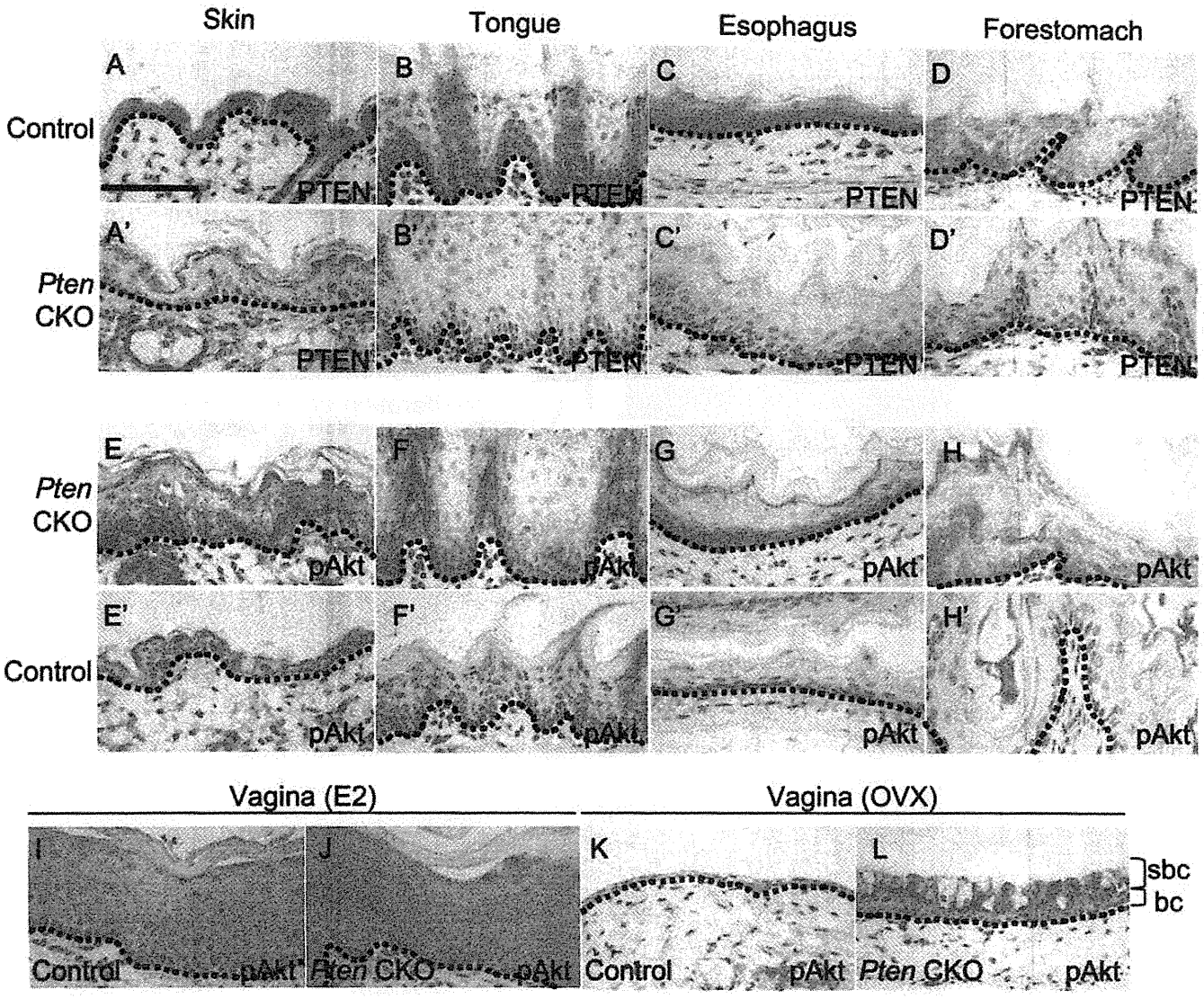

Control (OVX)
Pten CKO (OVX)
A
pMEK
B
pMEK
C
pERK1/2
D
pERK1/2
Pten CKO (OVX)
+ vehicle
Pten CKO (OVX)
+ rapamycin
E
pERK1/2
F
pERK1/2
G
ERα
H
ERα
I
Control
Pten CKO
pERα (S118)
pERα (S167)
ERα
J
Relative expression
2.0
1.0
0
Control
Pten CKO
EGF
TGFα
HB-EGF
APR
EPR
IGF-I

Fig. 7


Skin
Tongue
Esophagus
Forestomach
A
B
C
D
Control
PTEN
A'
B'
C'
D'
Pten CKO
PTEN
E
F
G
H
Pten CKO
pAkt
E'
F'
G'
H'
Control
pAkt
Vagina (E2)
Vagina (OVX)
I
J
K
L
sbc
bc
Control
pAkt
Pten CKO
pAkt
Control
pAkt
Pten CKO
pAkt