Legends for supplementary figures : for Li et al JCI manuscript 46134-RG-RV-3

Supplementary figure 1: Targeted ablation of the Pthrp gene in the mouse mammary epithelium.

(A) Mating strategy to obtain homozygous, heterozygous and control animals for *Pthrp* gene ablation. **(B)**: Top: Southern blot analysis confirming the targeted recombination of the *Pthrp* floxed allele in mammary gland DNA. 6.2 kb: wild-type allele; 5.2 kb: floxed allele. Middle: PCR analysis of mammary gland DNA for concurrent expression of the mT antigen. Bottom: PCR analysis of mammary gland DNA for concurrent expression of the Cre recombinase.

Supplementary figure 2: A small proportion of PTHrP is expressed in myoepithelial cells.

Immunofluorescence confocal image of primary tumor tissues showing co-localization of a proportion of the PTHrP expression with cytokeratin 5 in tumors from homozygous *Pthrp* ablated mice at 18 weeks compared to controls at 13 weeks (same size tumors). PTHrP staining disappears from epithelial cells in homozygous ablated mice. Yellow arrow: myoepithelial cell; white arrow: luminal epithelial cell. DAPI (blue), Ck5 (red), PTHrP (green). Scale bar 50 μm.

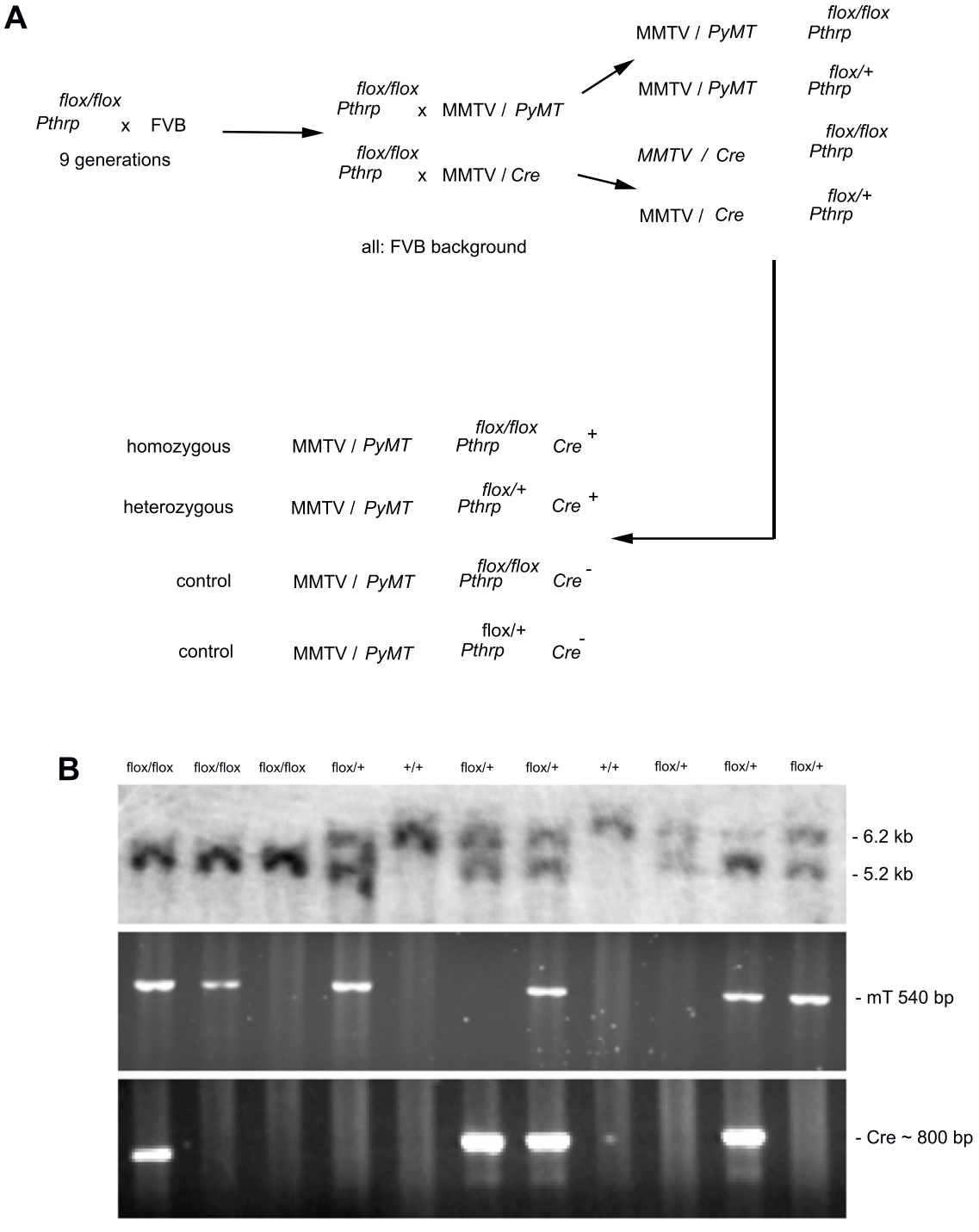
Supplementary figure 3: The PTH1 receptor level is not affected in breast tumors by Pthrp ablation.

Immunofluorescence confocal image of breast tumor tissue showing no difference in expression of the PTH1 receptor with *Pthrp* ablation in same size primary tumor tissues from control (13 weeks) and homozygous mice (18 weeks). DAPI (blue), PTH1R (red). Scale bar 50 μm.

Supplementary figure 4: The expression of the Cre recombinase enzyme by itself does not affect the levels of signaling molecules. Western blots show that the levels of expression of PTHrP, AKT1 and 2, CXCR4, Factor VIII, cyclin D1, and Bcl-2 do not vary in tumors from the *Cre* positive control animals if

the *Pthrp* gene is not ablated, and that their levels correspond to those observed in the *Pthrp*$^{flox/flox}$; *Cre*$^{-}$ control.

Supplementary figure 5: Cells presenting expression of the Cre recombinase show no PTHrP expression. Immunofluorescence confocal images of cultured cells isolated from breast tumor tissues showing PTHrP expression only in cells without Cre staining (arrow). DAPI (blue), Cre (green), PTHrP (red). Scale bar 50 μm.


A
flox/flox
Pthrp
x FVB
9 generations
flox/flox
Pthrp
x MMTV / PyMT
flox/flox
Pthrp
x MMTV / Cre
MMTV / PyMT
flox/flox
Pthrp
MMTV / PyMT
flox/+
Pthrp
MMTV / Cre
flox/flox
Pthrp
MMTV / Cre
flox/+
Pthrp
all: FVB background
homozygous
MMTV / PyMT
flox/flox
Pthrp
Cre +
heterozygous
MMTV / PyMT
flox/+
Pthrp
Cre +
control
MMTV / PyMT
flox/flox
Pthrp
Cre -
control
MMTV / PyMT
flox/+
Pthrp
Cre -
B
flox/flox
flox/flox
flox/flox
flox/+
+/+
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flox/+
+/+
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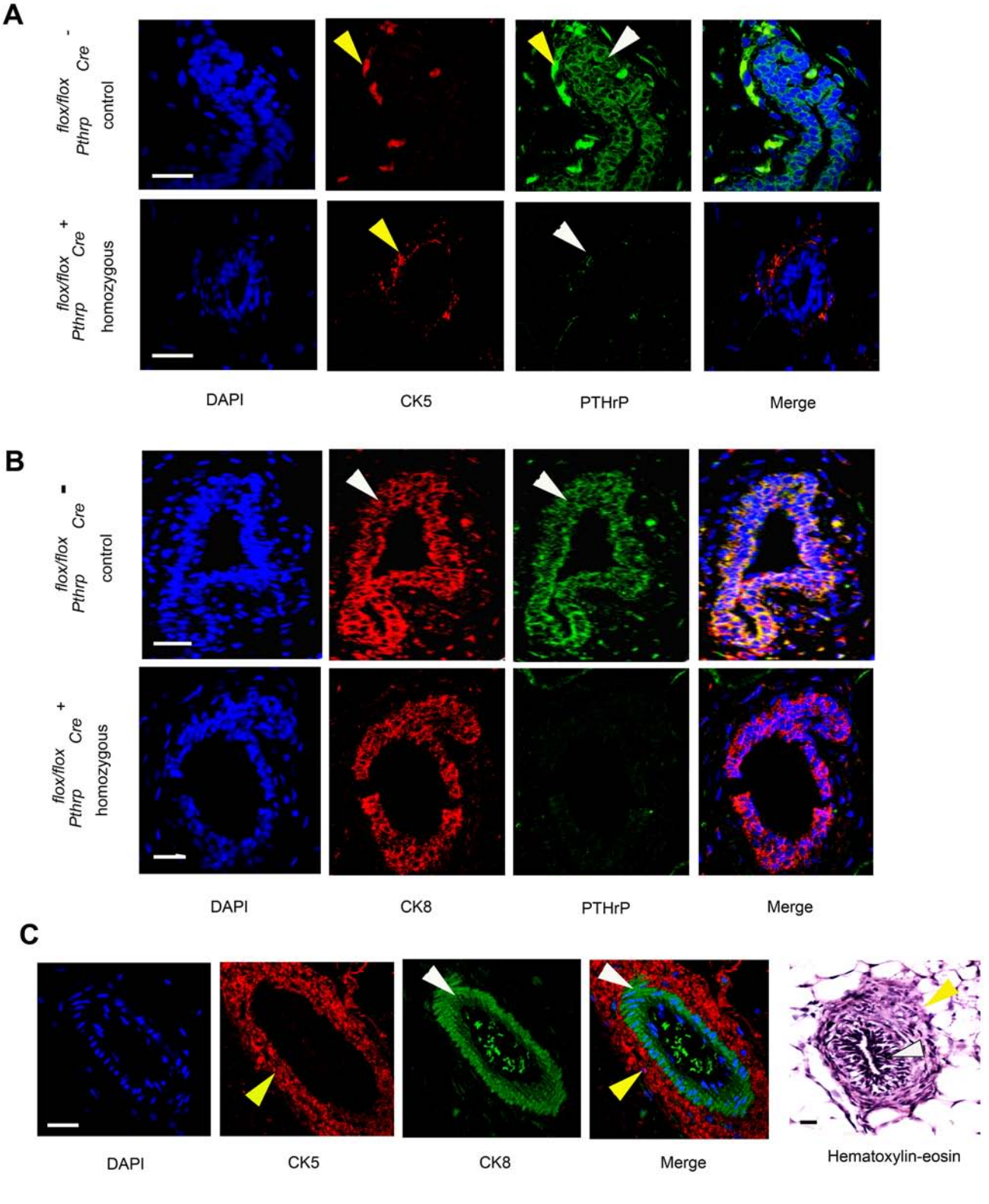
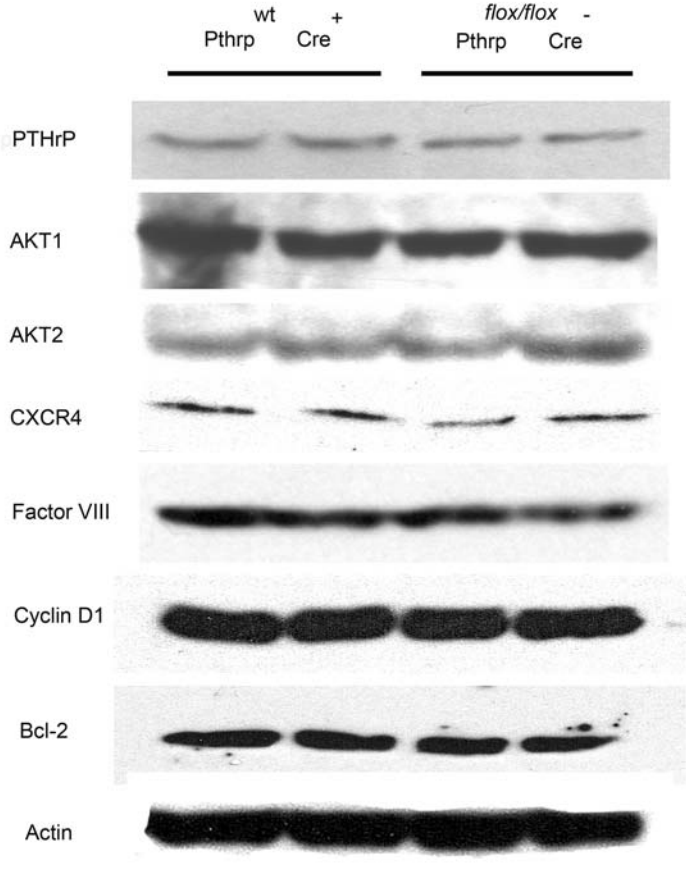
flox/+
flox/+
- 6.2 kb
- 5.2 kb
- mT 540 bp
- Cre ~ 800 bp


A
flox/flox Pthrp Cre -
control
flox/flox Pthrp Cre +
homozygous
DAPI
CK5
PTHrP
Merge
B
flox/flox Pthrp Cre -
control
flox/flox Pthrp Cre +
homozygous
DAPI
CK8
PTHrP
Merge
C
DAPI
CK5
CK8
Merge
Hematoxylin-eosin

breast tumor
flox/flox Pthrp Cre - control
flox/flox Pthrp Cre + homozygous
DAPI
PTH 1 receptor
Merge


wt
+
Pthrp
Cre
flox/flox
-
Pthrp
Cre
PTHrP
AKT1
AKT2
CXCR4
Factor VIII
Cyclin D1
Bcl-2
Actin

flox/flox Pthrp Cre - control
flox/flox Pthrp Cre + homozygous
DAPI
Cre
PTHrP
Merge

Supplementary table 1

	Pthrp $^{flox/flox}$ *Cre*$^{-}$ (n = 20) Control 1	*Pthrp* $^{flox/+}$ *Cre*$^{-}$ (n = 18) Control 2	*Pthrp* ^{wt} *Cre* + (n = 22) *Control 3*	*Pthrp* $^{flox/+}$ *Cre+* (n = 12) Heterozygous	*Pthrp* $^{flox/flox}$ *Cre*$^{+}$ (n = 30) Homozygous
Average weight of tumor load per mouse (g)	5.87 ± 1.38	5.90 ± 1.67	6.03 ± 1.0	3.03 ± 0.50 *^{o}	1.59 ± 0.54 *
Average weight of primary tumors (g)	1.66 ± 0.45	1.75 ± 0.52	1.71 ± 0.63	1.03 ± 0.19 *^{o}	0.59± 0.19 *
Average number of tumors per mouse	12.30 ± 1.70	12.50 ± 2.07	13.7 ± 1.50	8.33 ± 0.78 *^{o}	5.05 ± 1.05 *

Supplementary table 1: Variation in tumor load, weight and numbers with Pthrp ablation.

Partial or total *Pthrp* ablation is accompanied by a significant reduction in tumor load, primary tumor weight and number of tumors per mouse. Figures marked with * have p values < 0.01 with respect to controls. Figures marked with ^{o} have p values < 0.01 with respect to homozygous values.