The PI3K regulatory subunits p55α and p50α regulate cell death *in vivo*

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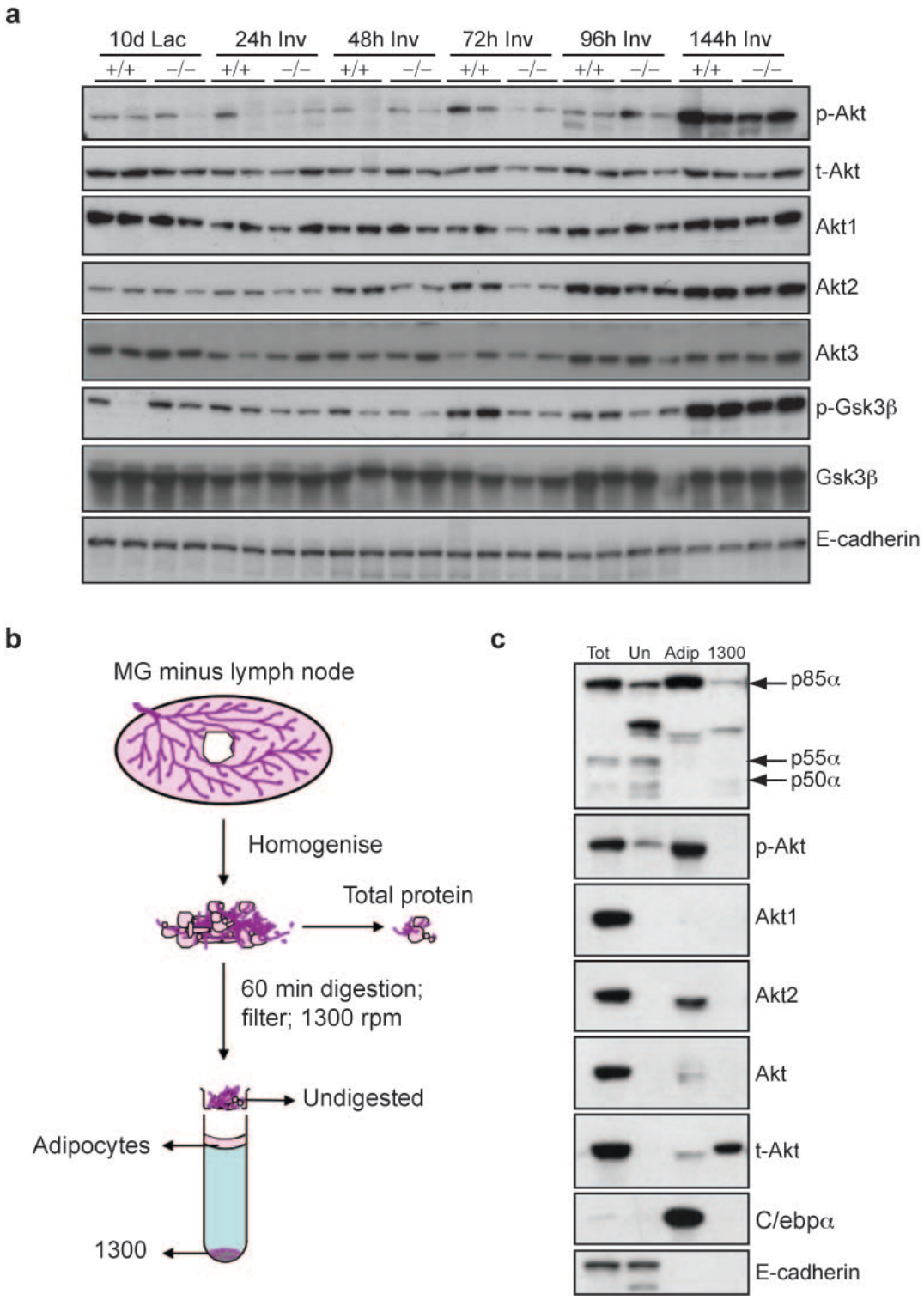
SUPPLEMENTARY FIGURES

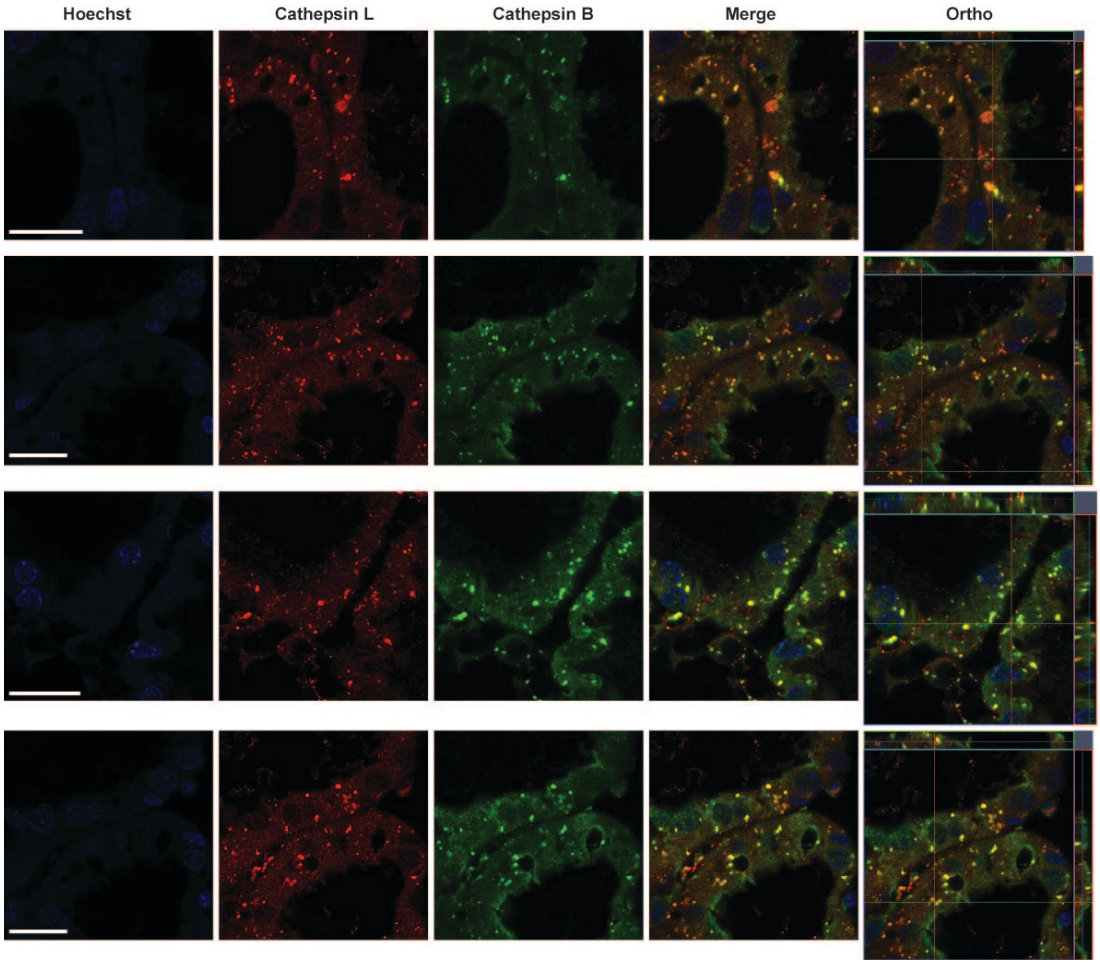
Supplementary Figure 1



Supplementary figure 1. (**a**) Immunoblot showing no significant differences in the phosphorylation of Akt and its downstream target gene Gsk3β in the *p55α*$^{-/-}$*/p50α*$^{-/-}$

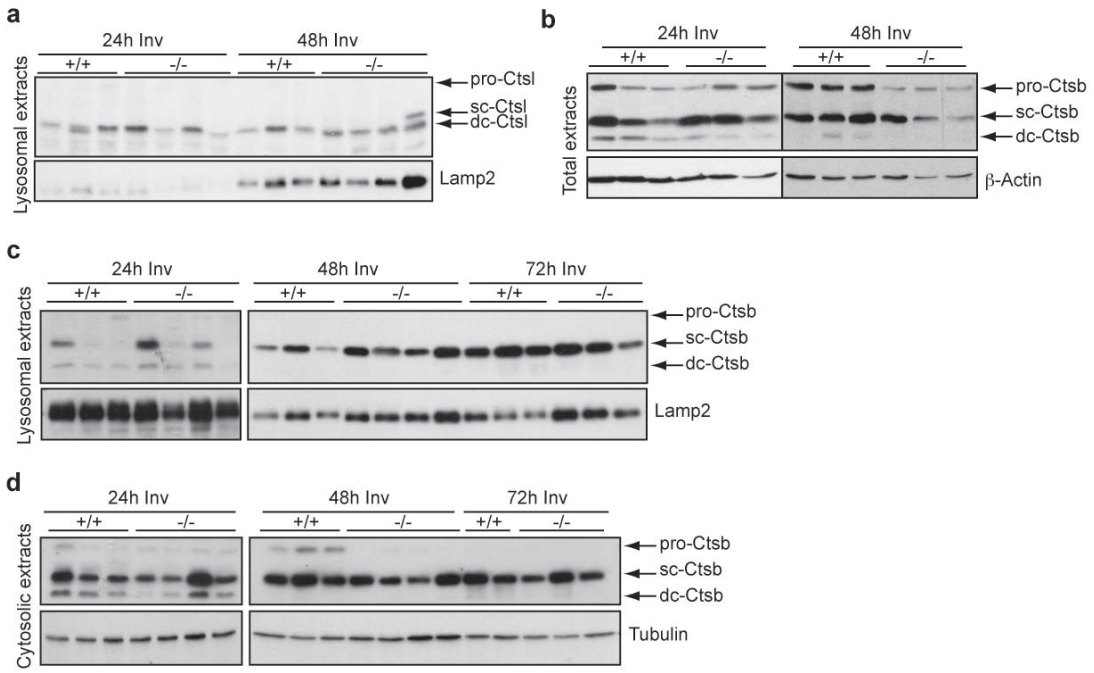
glands (-/-) compared to control glands (+/+). 10d Lac, 10 days lactation; h Inv, hours of involution. E-cadherin is the loading control. (**b,c**) Isolation of the adipocytic fraction of the mammary glands reveals partitioning of the Akt isoforms. (**b**) Diagram of the fractionation protocol. (**c**) Fractionation of a representative control gland at 72h involution and immunoblot of the indicated proteins, showing the presence of the Akt isoforms, Akt2 in particular, in the adipocytes of the gland. Tot, total gland; Un, undigested gland; Adip, adipocytes; 1300, 1300 rpm pellet, which should contain pre-adipocytes. Of note, the small subunits are absent from the adipocyte fractions. C/ebpα and E-cadherin are shown as adipose and epithelial markers, respectively.

Supplementary Figure 2



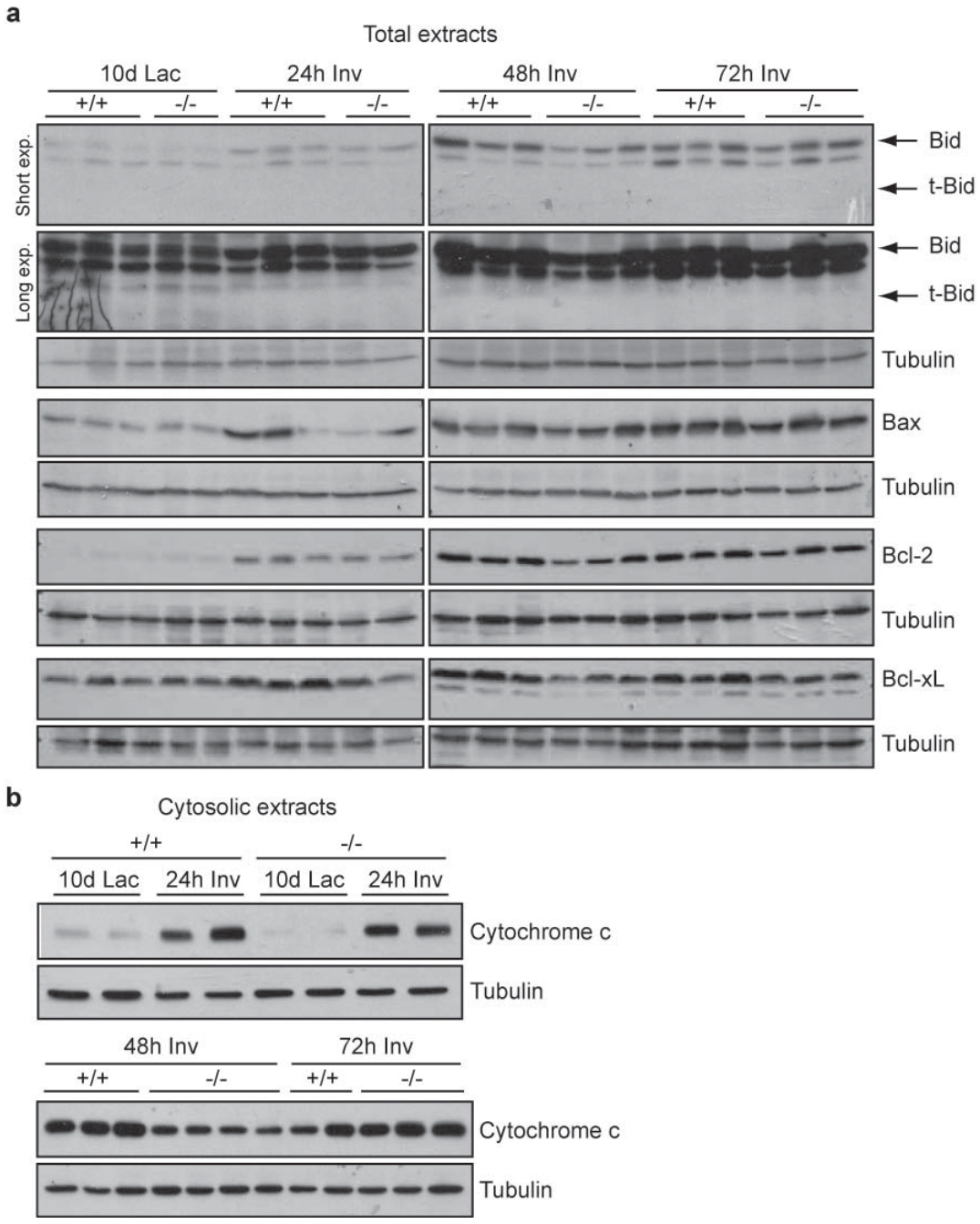
Supplementary figure 2. Vesicles containing cathepsin L (red) but not cathpsin B (green) are shown by immunohistochemistry of 12 hours involution samples. Nuclei are stained with Hoechst. Ortho, orthographic projection. Scale bar, 20 μm.

Supplementary Figure 3



Supplementary figure 3. Cathepsin B is unaffected in the $p55\alpha^{-/-}/p50\alpha^{-/-}$ mice. (**a**) Western blot analysis showing no differences in the expression of cathepsin L (Ctsl) in the lysosomal fraction of glands of $p55\alpha^{-/-}/p50\alpha^{-/-}$ mice when compared to controls. Total (**b**), lysosomal (**c**) and cytosolic (**d**) fractions display no major differences in the levels of cathepsin B (Ctsb) in the glands of $p55\alpha^{-/-}/p50\alpha^{-/-}$ mice when compared to controls at the indicated hours of involution. -/-, $p55\alpha^{-/-}/p50\alpha^{-/-}$ glands; +/+, $p55\alpha^{+/+}/p50\alpha^{+/+}$ glands. Lamp2 and Tubulin are shown as lysosomal and cytosolic markers, respectively; β-Actin is shown as loading control for the total extracts.

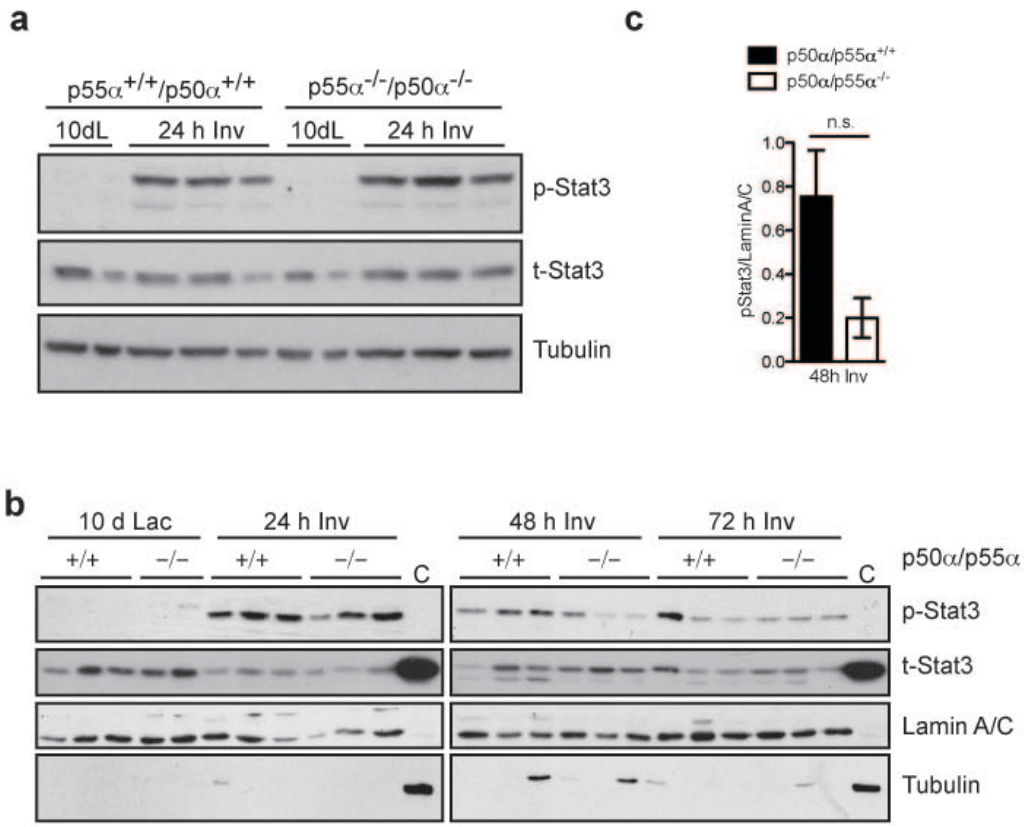
Supplementary Figure 4



Supplementary figure 4. The mitochondrial pathway of cell death is mostly unaffected in the *p55α*$^{-/-}$*/p50α*$^{-/-}$ mice. (**a**) Immunoblot of total mammary gland extracts showing no significant differences in the pro-apoptotic Bcl-2 family members Bax and Bid and the anti-apoptotic factors Bcl-2 and Bcl-xL in the *p55α*$^{-/-}$*/p50α*$^{-/-}$ mice as compared to controls. (**b**) Immunoblot showing cytochrome c release in the

cytosol during involution in the *p55α*$^{-/-}$*/p50α*$^{-/-}$ and control mice. 10d Lac, 10 days lactation; h Inv, hours of involution. Tubulin is shown as loading control.

Supplementary Figure 5



Supplementary figure 5. Stat3 phosphorylation and nuclear localisation is unaffected in the *p55α*$^{-/-}$*/p50α*$^{-/-}$ mice. (**a**) Immunoblot of control and *p55α*$^{-/-}$*/p50α*$^{-/-}$ glands total extracts showing phosphorylation levels of Stat3. t-Stat3, total Stat3. 10dL, 10 days lactation; 24 h Inv, 24 hours involution. Tubulin is shown as loading control. (**b**) Immunoblot of nuclear fractions of control (+/+) and *p55α*$^{-/-}$*/p50α*$^{-/-}$ (-/-) glands showing no differences in p-Stat3 nuclear localisation. Lamin A/C and Tubulin are shown as loading and purity controls for the nuclear and cytosolic fraction, respectively. Lanes represent individual mice. 10 d Lac, 10 days lactation; 24, 48, 72, 96 h Inv, hours of involution. (**c**) Quantification of the 48h involution time point from (**b**) showing no significant difference in the amount of nuclear p-Stat3, although a trend is present. Results are means ± s.e.m.