Supplementary Figure Legends

Supplementary Figure 1. Additional data for main Figures 1 and 2

(**A**) ΔNp63α is the predominant p63 isoform in the adult mammary gland. Immunoblot analysis with pan-p63 4A4 antibody of a representative virgin gland (*lane 1*), along with protein lysates of Phoenix E cells transfected with indicated p63 isoforms (*lanes 2, 3, 5-9*) as positive controls. Protein lysate from p63-/- mouse embryo fibroblasts (MEF, *lane 4*) served as a negative control. Arrowhead, ΔNp63α. Asterisks, non-specific bands. (**B-E**) p63 deficiency has no effect on the architecture of virgin, lactating and completely involuted (restructured) mammary glands. (**B**) Representative images of whole-mount carmine staining (*top*) and H&E staining (*bottom*) of p63+/+ and p63+/- mammary glands from 6 wks old virgin females. (**C-E**) H&E staining of p63+/+ and p63+/- mammary glands at the indicated stages.

Supplementary Figure 2. Additional data for main Figure 3

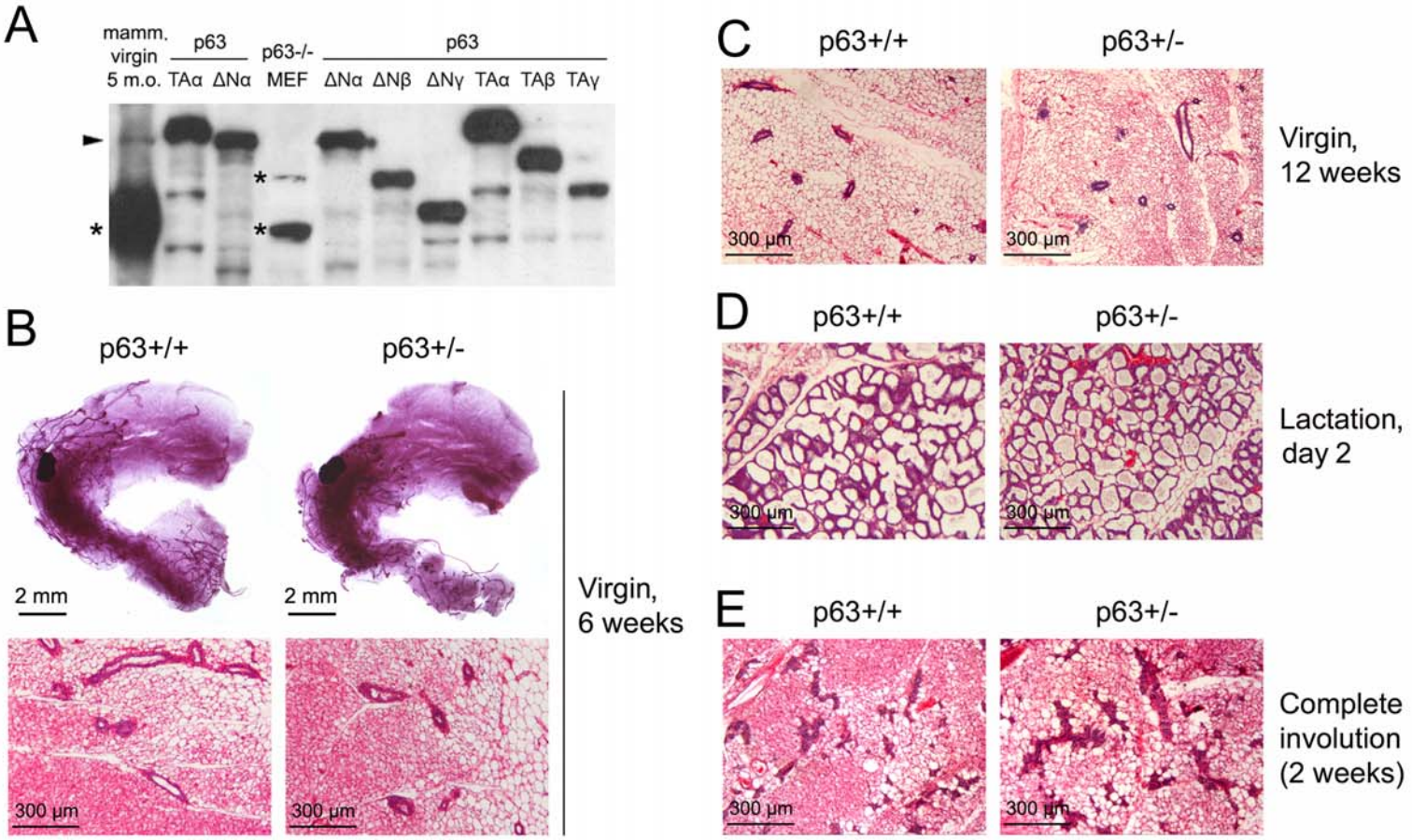
(**A**) Immunoblot analysis of p21, a p53 target gene, in whole WT and matching p63+/- mammary glands at 3 days post involution. L10, lactation day 10. p21 antibody was from BD Biosciences (Cat. No 556431). (**B**) Immunoblot analysis of p53 and MAPK (loading control) in WT and p63+/- mammary glands at indicated stages and in p63-null mouse embryo fibroblasts (p63-/- MEFs). For each stage, one out of two independent experiments is shown, both with similar results. Compl. inv., complete involution.

Supplementary Figure 3. Additional data for main Figure 4B and for Materials and methods

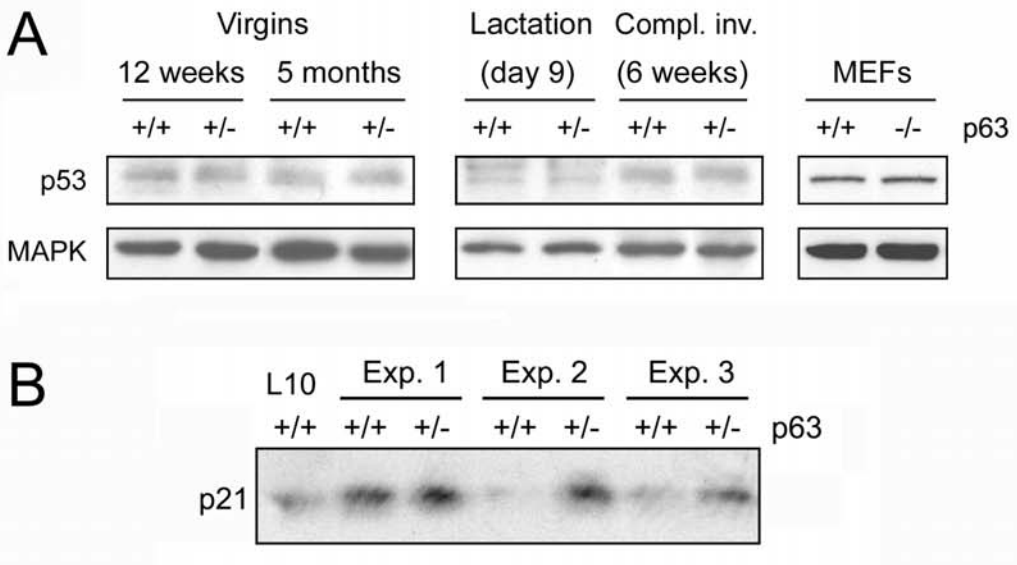
(**A**) Gating on mammary epithelial cells prior to PI/CD24 FACS analysis shown in Figure 4B. FACS gating focused on all live cells (*left*), followed by exclusion of endothelial ($CD31^{positive}$) and hematopoietic ($CD45^{positive}$/$Ter119^{positive}$) cells (*right*). (**B**) Breeding diagram to generate p63;$Rosa^{LSL\text{-}LacZ/+}$;WAP-Cre animals. See Materials and Methods for details.

Supplementary Figure 4. Transcriptional regulation of apoptosis-related genes by ΔNp63 in mammary cells

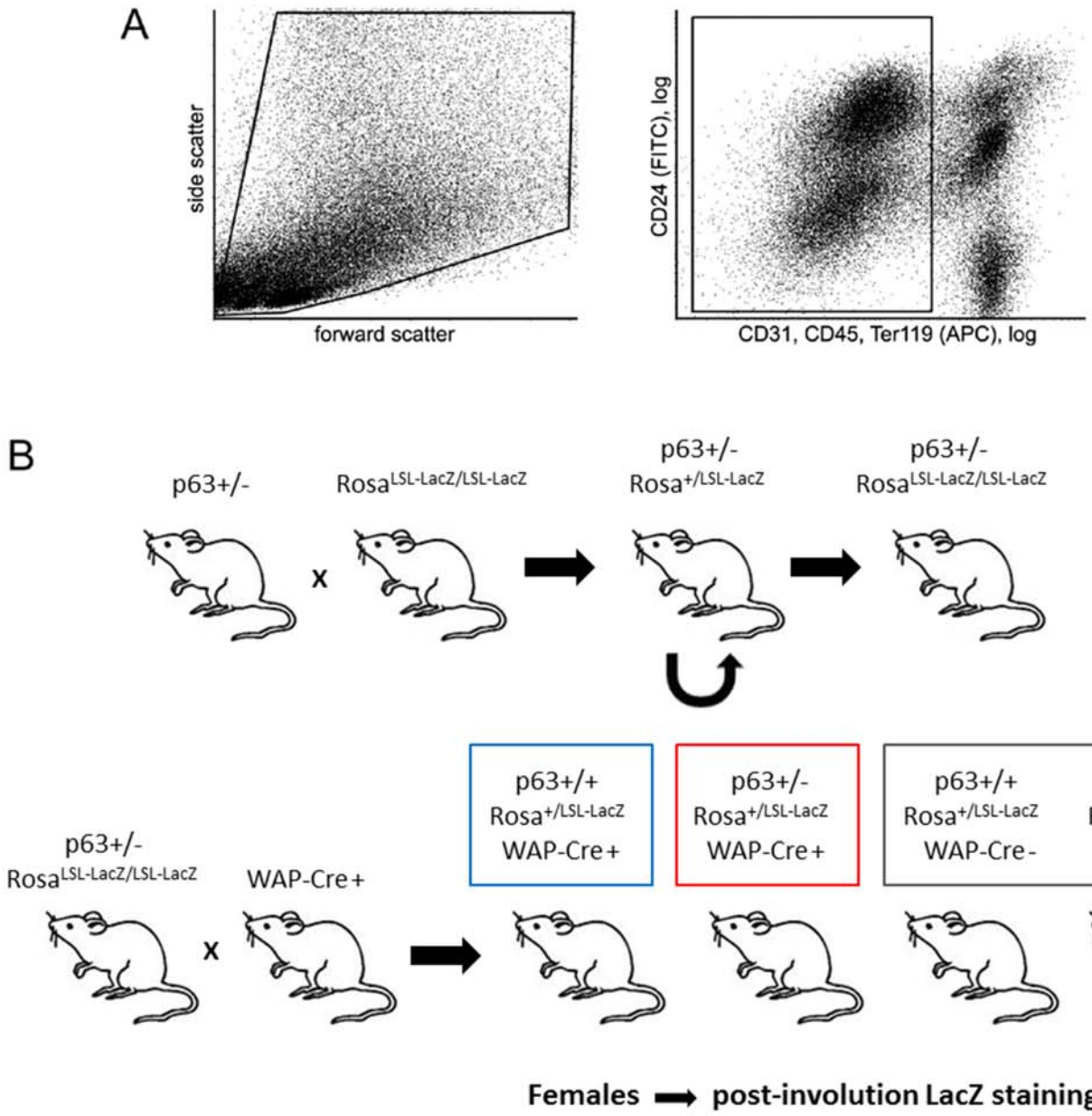
Published microarray data from human non-transformed MCF10A breast epithelial cells (Carroll et al., 2006) were re-analyzed for possible regulation of apoptosis-related genes by ΔNp63. shRNA against the p63 DNA-binding domain (DBD) was used to knock down all p63 isoforms (*top*), whereas shRNA against the p63 TA domain was TAp63-specific (*bottom*). Note that only DBD (but not TA) shRNA shows preferential and strong activation of a pro-apoptotic program, suggesting that ΔNp63, but not TAp63, is a direct transcriptional repressor of the apoptotic program in the mammary gland. Only genes represented at least twice on the microarray were considered. Solid bars represent significant changes (>1.25 fold).



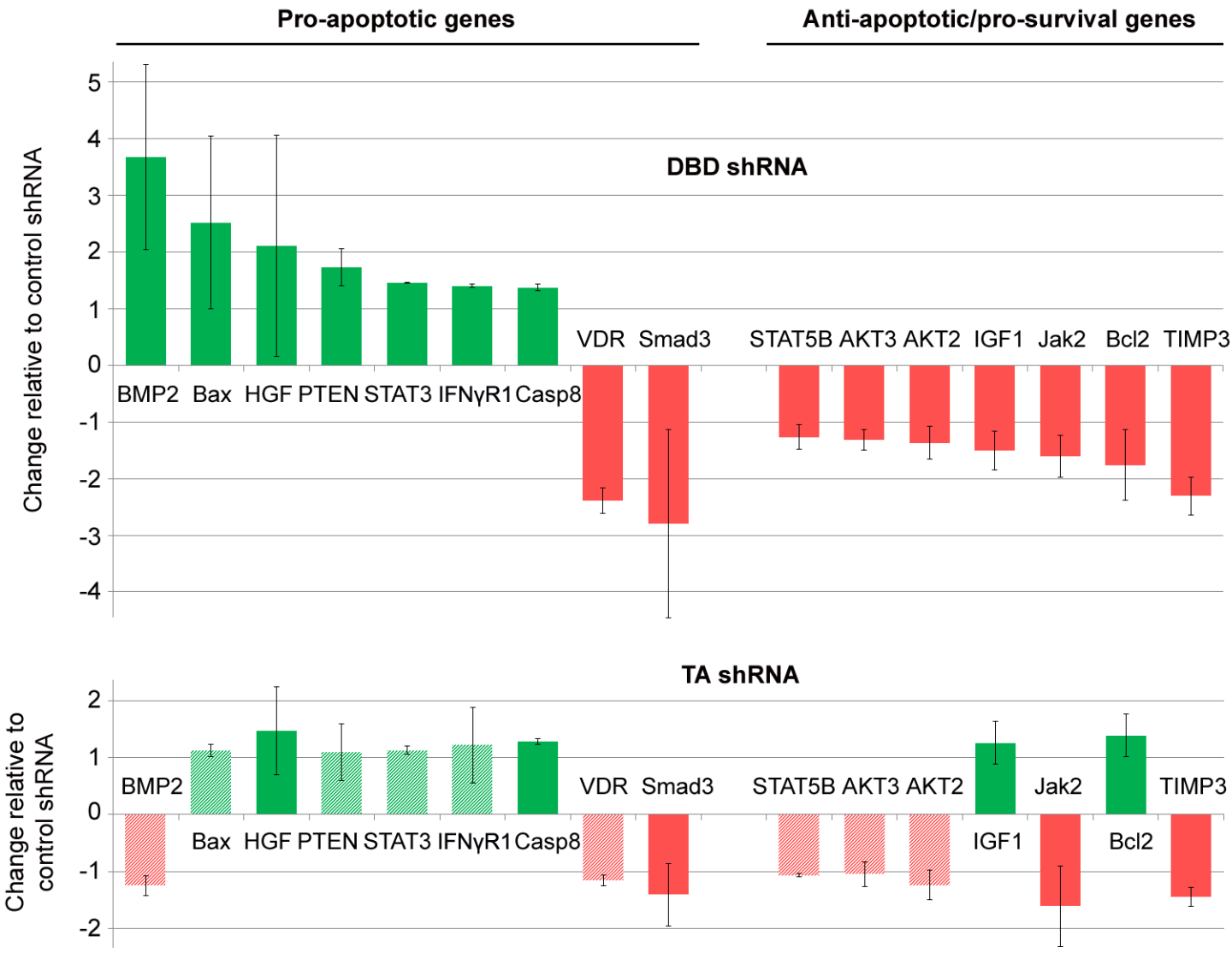
Supplementary Figure 1



Supplementary Figure 2



Supplementary Figure 3



Supplementary Figure 4