

Suppl. Figure legends:

Figure S1: Representative images of H&E stained sections from wt, *Bim*- or *Bmf*-deficient mammary glands on day 0 or day 4 of involution, converted into 8 bit images, used for quantification by ImageJ-Fiji and shown in Fig. 3b. Dark purple staining represents epithelium.

Figure S2: STAT5 binds to the 3' exons of the *Bim* and *Bmf* genes. Genome browser snapshots of ChIP-seq data demonstrate binding of STAT5A to specific GAS sites in the *Bim*, *Bmf*, and *Bid* genes. H3K4me3 ChIP-seq was used to identify the 5' end of the respective genes. For visualization, background signals of less than 2 were eliminated. For *Bim* and *Bmf* gene regions including 5 kbp of upstream sequences and 2kbp 3' flanking sequences are shown. STAT5A binds to sites in the 3' untranslated exon of the *Bcl2l11* (*Bim*) and *Bmf* gene. The *Bid* shown includes 13kb of upstream and 2kb of downstream sequences. STAT5A binds to a GAS motif located approximately 12kb upstream of the transcriptional start site but is unlikely of physiological relevance [1].

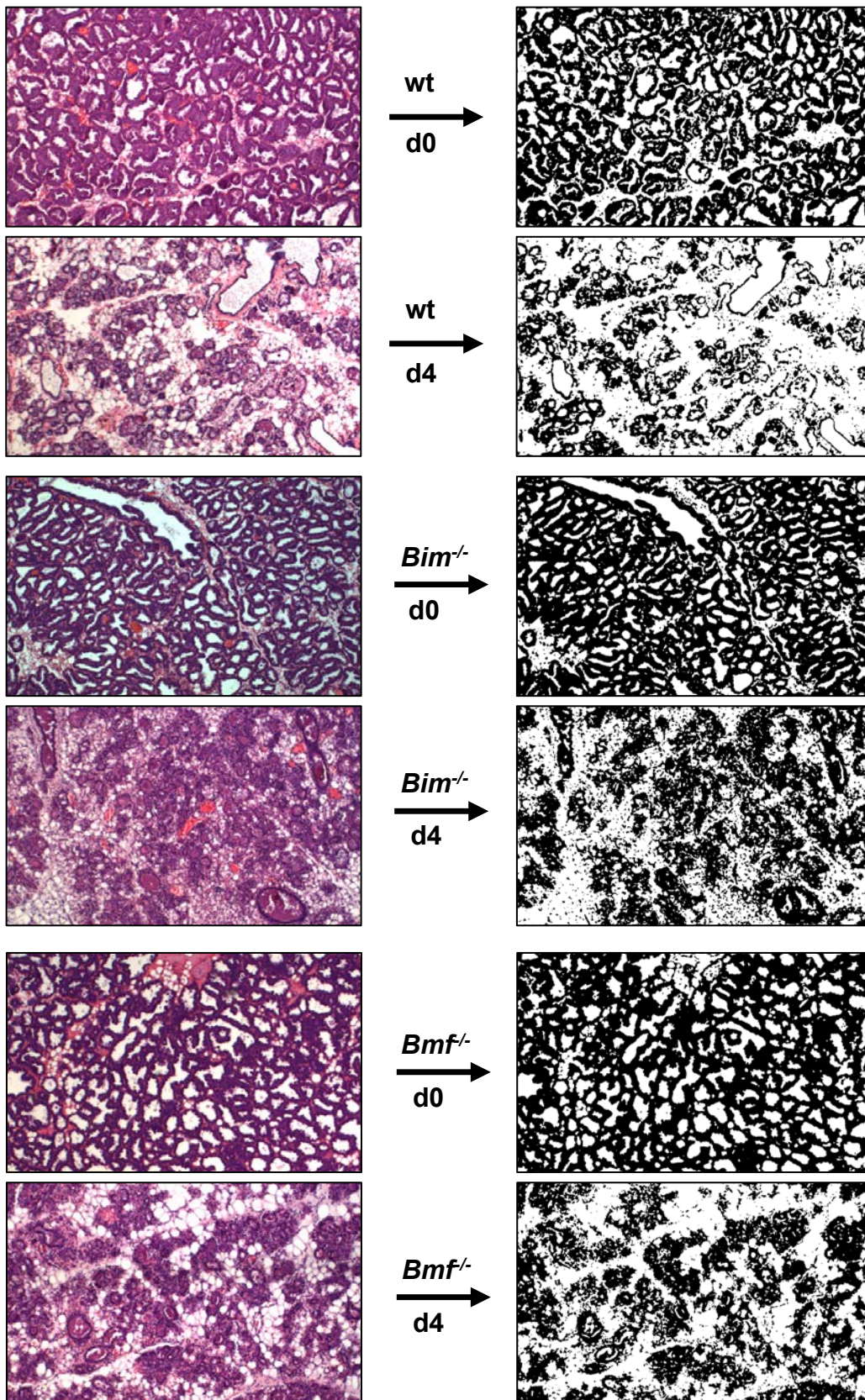
Figure S3: Sorting strategy used to isolate Annexin-V-negative, CD45⁺ hematopoietic or CD49f⁺CD45⁻ mammary epithelial cells from the mammary gland. Cells were lysed in TRIZOL for RNA isolation, used for cDNA synthesis and qRT-PCR analysis shown in Fig. 4b.

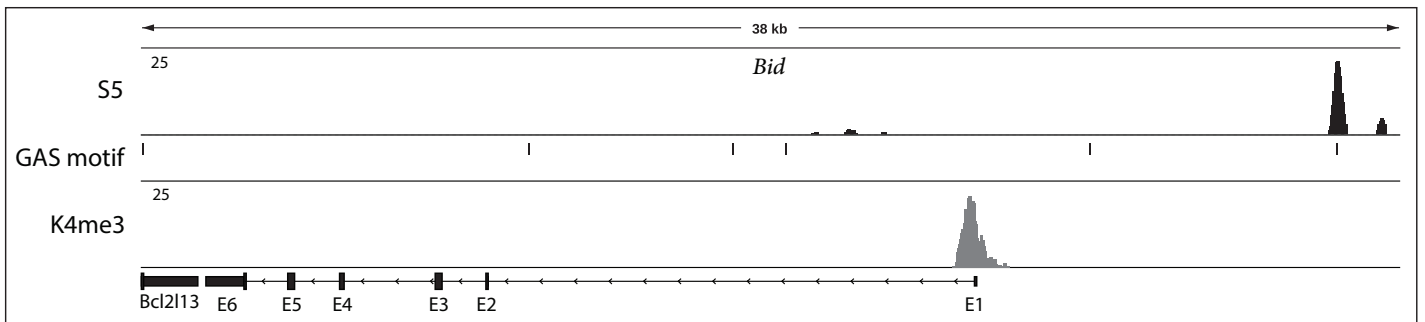
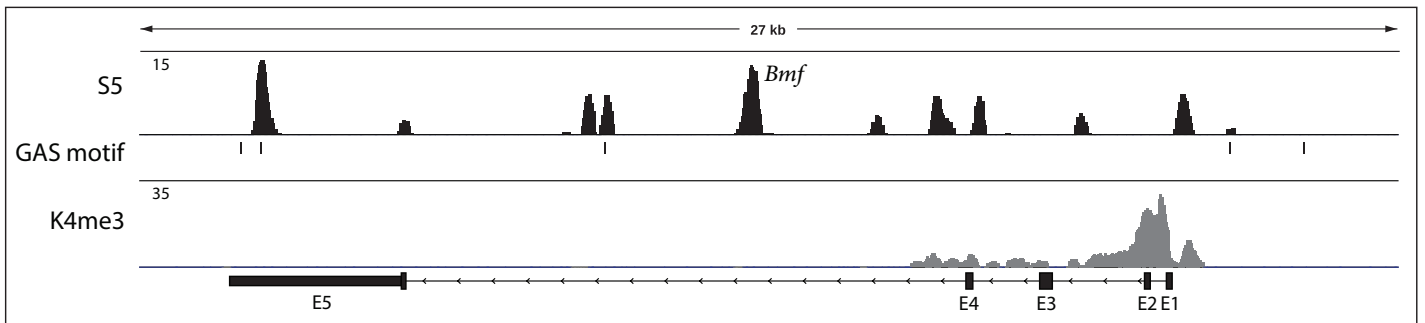
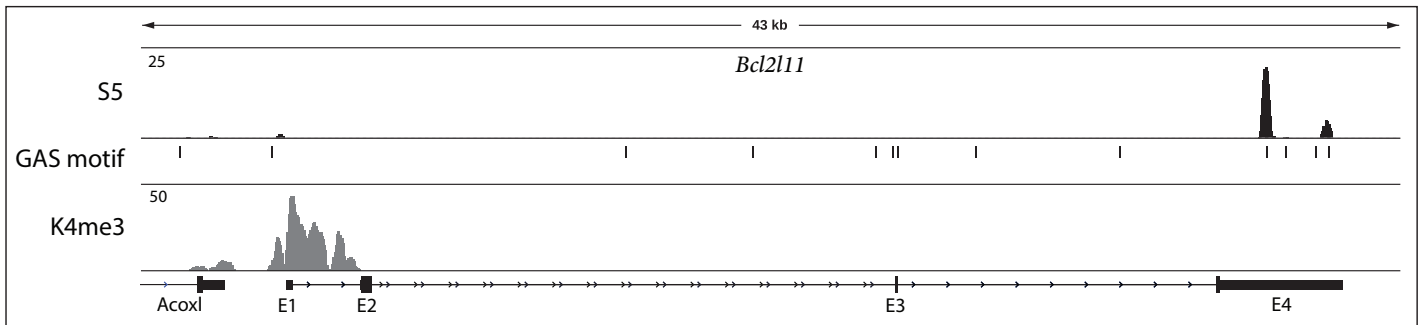
Figure S4: Representative images of the immunofluorescence analysis of *Bim* expression in (A) thymus from wt or *Bim*-deficient mice to confirm specificity of the antibody. (B) BIM expression in F4/80 positive macrophages or (C) Cytokeratin 8 (CK8) positive epithelial cells. Solid arrows show macrophages, open arrows epithelial cells. Scale bar represents 15µm.

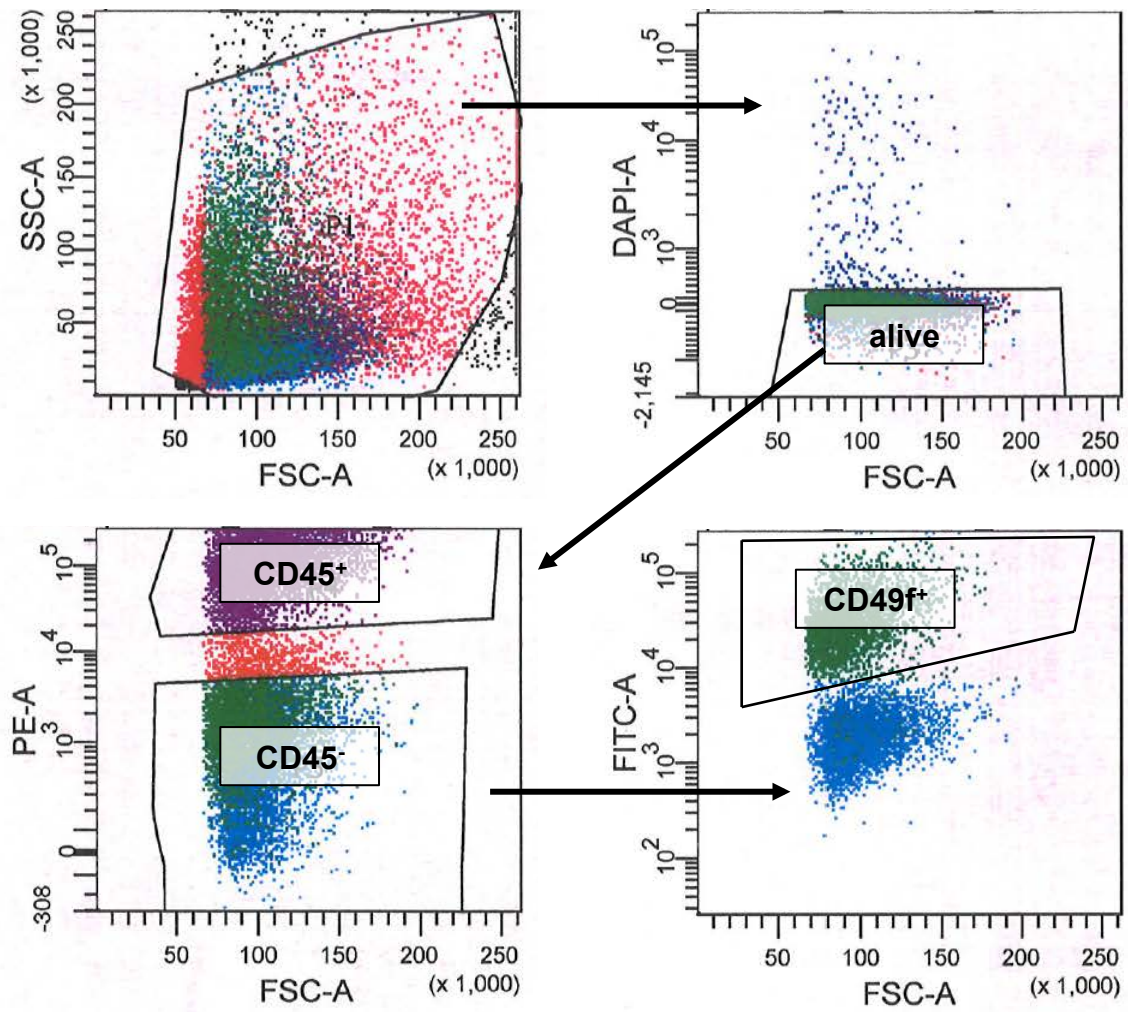
Figure S5: Handling of immunofluorescence microscopy data for measurement of the BIM signal in F4/80⁺ (A) and CK8⁺ (B) cells in the lactating vs. involuting mammary gland. In brief, CK8 and F4/80 images were background- and contrast-corrected and converted into binary masks. Such masks were subtracted from the background- and contrast-corrected image of the *Bim* signal. Representative photos of a day 0 mammary gland are presented.

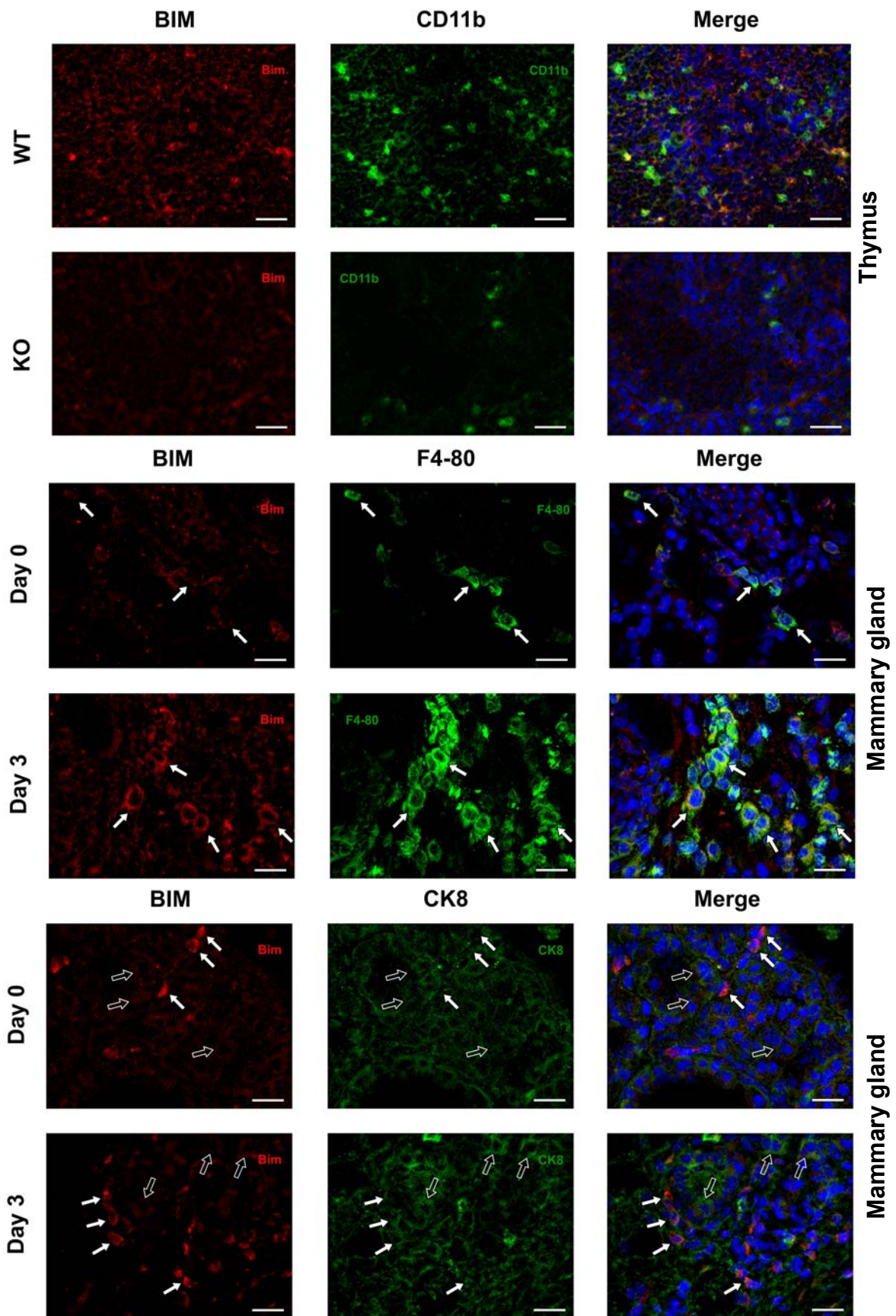
Reference

1. Kang K, Yamaji D, Yoo KH, Robinson GW, Hennighausen L: **Mammary-specific gene activation is defined by progressive recruitment of STAT5 during pregnancy and the establishment of H3K4me3 marks.** *Mol Cell Biol* 2014, **34**:464-473.

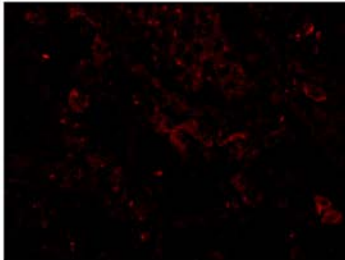








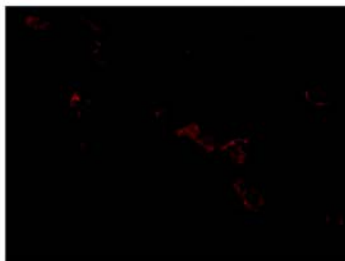
Background-subtracted, contrast adjusted
8 bit photos of the Bim staining



subtract

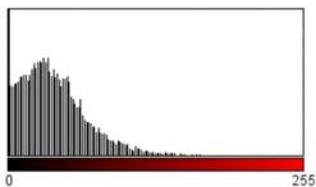


equals



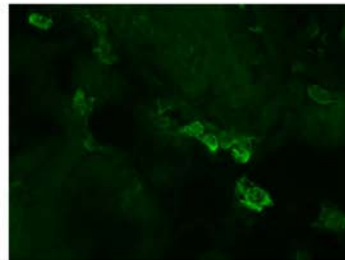
Bim signal in F4/80+ cells

Measure:
Area, Mean Signal, Integrated Density
Histogram

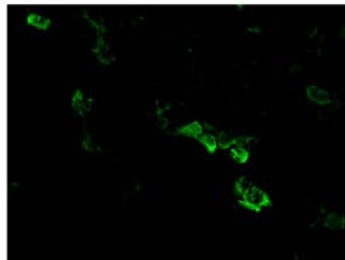


Count: 1392640 Min: 0
Mean: 0.760 Max: 176
StdDev: 6.797 Mode: 0 (1367025)

Raw-signal photos
of the F4/80 staining

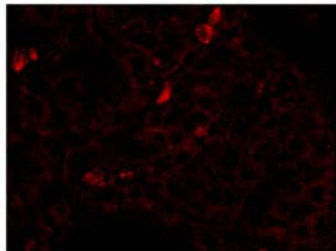


conversion into 8 bit image
background subtraction (Rolling Ball, r=50)
contrast adjustment (l=10, h=168)

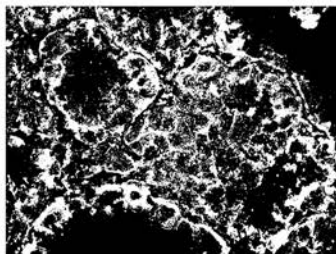


thresholding (IsoData)
binarization
white=0, black = 255

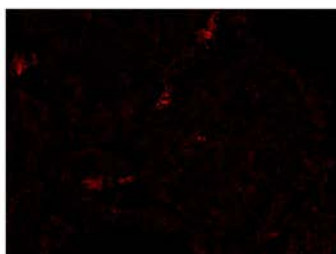
Background-subtracted, contrast adjusted
8 bit photos of the Bim staining



subtract



equals



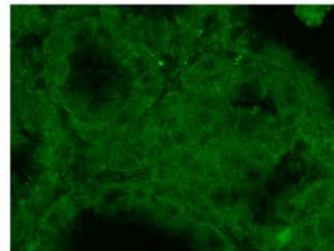
Bim signal in CK8⁺ cells

Measure:
Area, Mean Signal, Integrated Density
Histogram

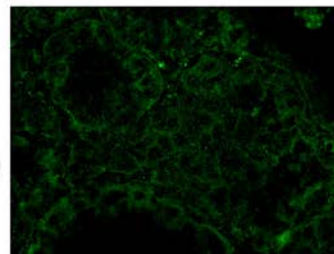


Count: 1392640 Min: 0
Mean: 7.175 Max: 255
StdDev: 15.127 Mode: 0 (865470)

Raw-signal photos
of the CK8 staining



conversion into 8 bit image
background subtraction (Rolling Ball, r=50)
contrast adjustment (l=10, h=168)



thresholding (IsoData)
binarization
white=0, black = 255