Supplemental Materials

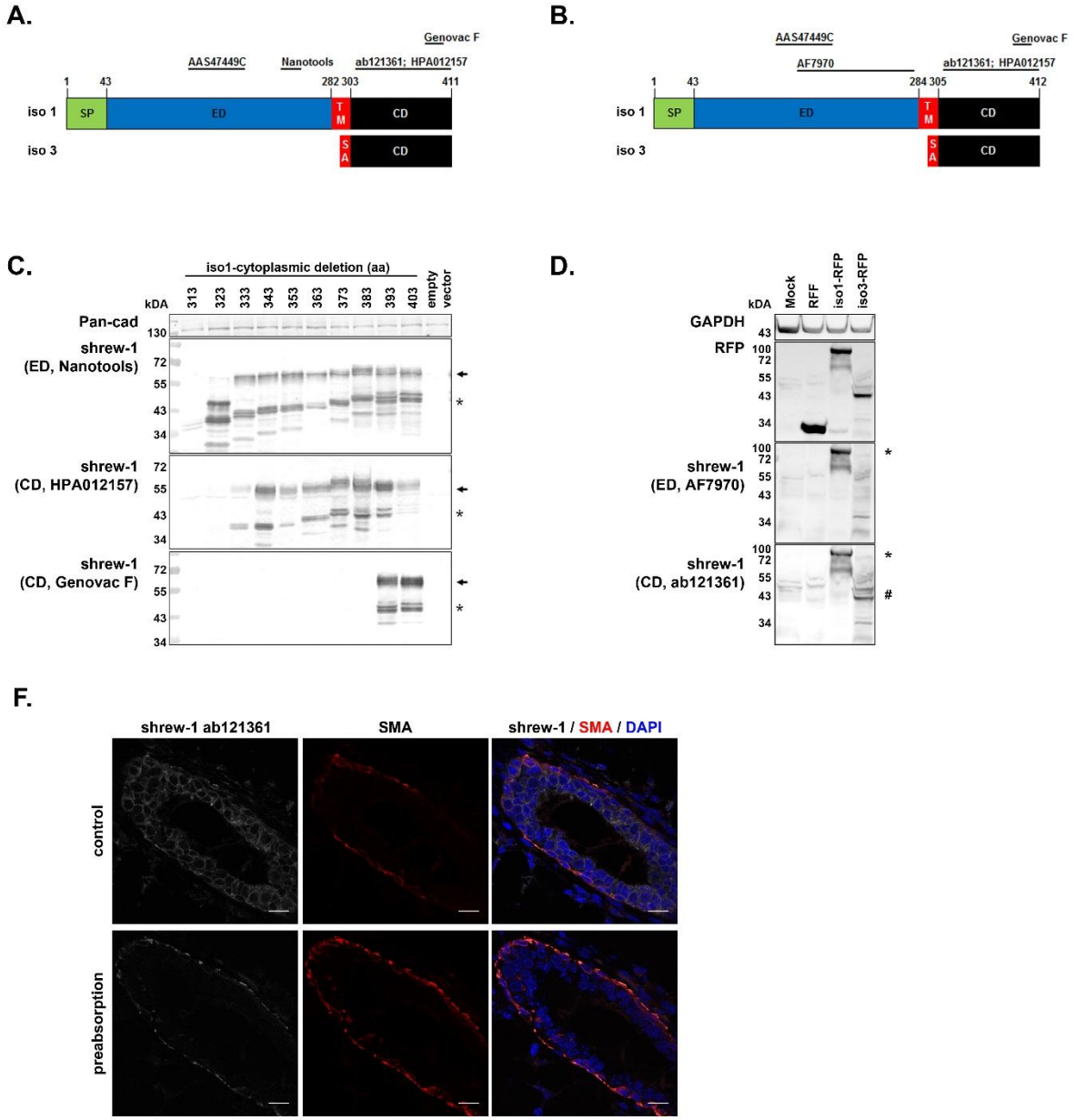

Fig. S1: Mapping of shrew-1 antibody binding sites.

(A) The schematic drawing of the identified human shrew-1 protein isoforms indicates the binding site of the Nanotools and the AAS47449C (AntibodyVerify) antibodies in the extracellular domain as well as the binding of the monoclonal antibody Genovac clone F and the polyclonal antibodies against the cytoplasmic domain (Sigma HPA012157 or abcam ab12136; respectively). (B) Schematic illustration of the two identified murine shrew-1 protein isoforms and the binding regions of the available shrew-1 antibodies. (C) Shrew-1 isoform 1 constructs were generated exhibiting successive truncations of the cytoplasmic domain in steps of 10 amino acid residues (313 aa to 403 aa). The constructs were expressed in HEK293T cells and protein

lysates were subjected to immunoblot analysis. Shrew-1 protein was detected using three different anti-shrew-1 antibodies. Pan-Cadherin was detected as a loading control. The Genovac clone F monoclonal antibody detected the protein only if the stretch from aa residue 383 to 393 was present, indicating that the epitope resides in this segment or in the transition to it. The Sigma antibody recognized the protein already, when it was truncated to aa residue 333. The Nanotools antibody recognized as expected all variants of shrew-1. Shrew-1 is an O-glycosylated protein. Exogenously expressed in HEK293T cells, it emerges in the immunoblot in O-glycosylated (arrowhead) and premature (asterisk) protein species. (D) RFP-tagged mouse shrew-1 protein variants isoform 1 and 3 were expressed in HEK293T cells and protein lysates were subjected to immunoblot analysis. The polyclonal antibody from R&D Systems AF7970 raised against the extracellular domain of shrew-1 detected only shrew-1 isoform 1 (asterisk) whereas the polyclonal antibody from abcam ab121361 raised against the cytoplasmic domain recognized both isoforms (asterisk and hash). The abcam ab121361 and Sigma HPA012157 antibody are raised against the identical aa stretch. (F) Staining of virgin murine mammary gland with and without preadsorption of the shrew-1 antibody ab121361 with a mixture of *in vitro* translated shrew-1 protein (human shrew-1: aa 1-411, aa 1-283) and GST-tagged cytoplasmic domain of human shrew-1 (aa 304-411) confirming the antibody specificity for shrew-1 *in vivo*. Preabsorption of the primary antibody mixture did not affect the antibody specificity for the internal control SMA. Single planes, scale bar: 15 µm.

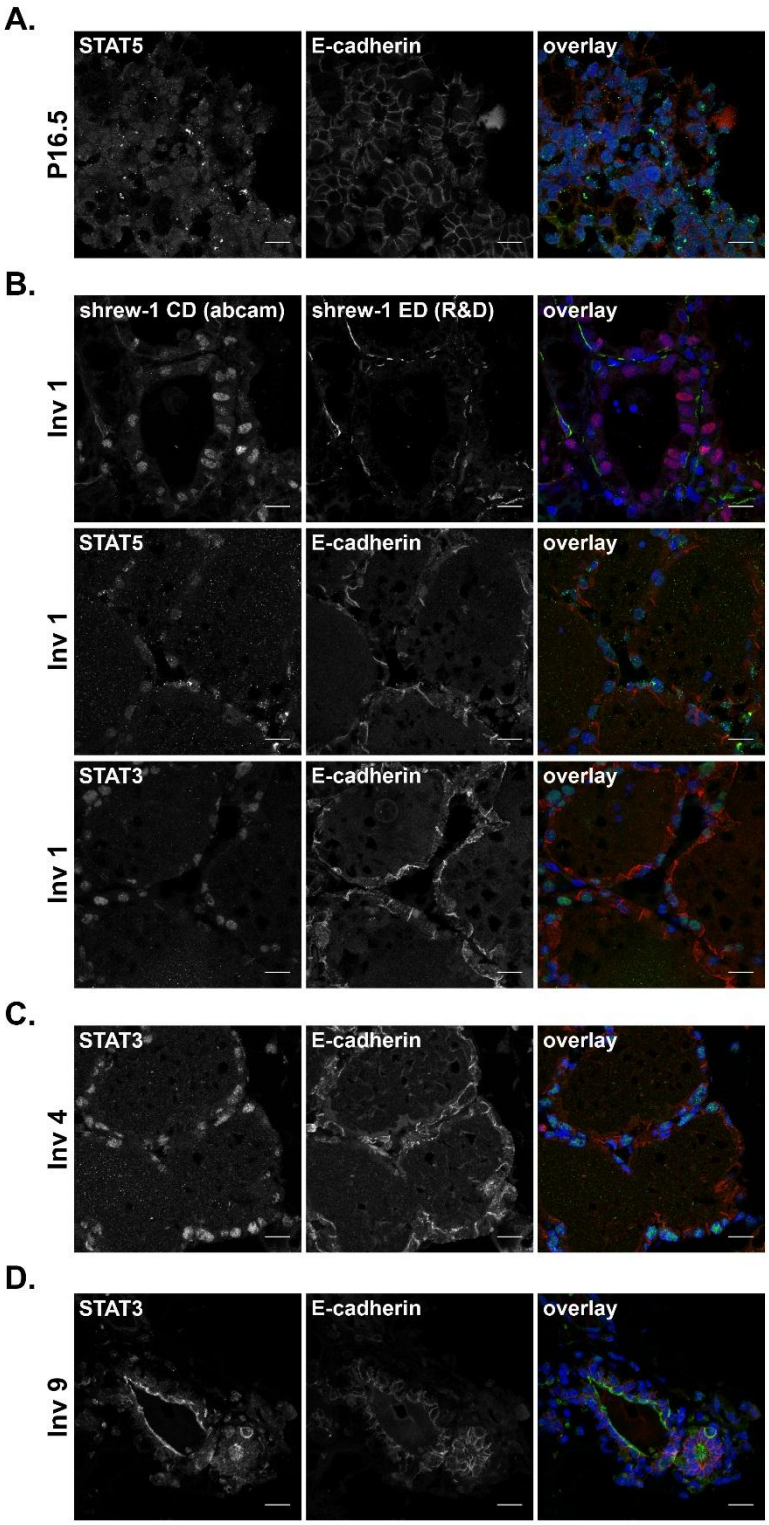

Fig. S2: shrew-1 splice variants are differentially expressed in mammary gland *in vivo*.

Histological analysis of mouse mammary gland tissue obtained at different developmental stages with antibodies against either STAT5 or STAT3 in combination with E-cadherin or a double staining with antibodies against the cytoplasmic (CD; abcam) or extracellular (ED; R&D Systems) domain of shrew-1. Nuclear STAT5 is a

marker for lactogenic differentiation and nuclear STAT3 is marker for involution in mammary gland differentiation and remodeling. (A) The nuclear translocation of shrew-1 in luminal cells during late pregnancy (P16.5) occurs alongside nuclear appearance of STAT5, which drives the expression of milk genes. (B) The nuclear localization of shrew-1 and STAT5 persists throughout early involution events. (B, C) Nuclear translocation of STAT3 marks the onset of tissue remodeling, occurs one day after cessation of suckling (Inv 1), and remains nuclear during reversible stages of remodeling (Inv4). (D) Nuclear STAT3 shifts back to the cytoplasm during later and irreversible stages of involution (Inv 9). Microscope: Zeiss LSM780; objective lens: 63x/1.4 oil DIC M27 Pl Apo; Max Z projection, scale bar 15 µm.

A.

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  1  GTGACACCAG AGAGTCCACT GGTCCAGAGG CGACCCCAGA CCTGGTGACA AAGCCGCTCC ATGTGAACTG GGGAGGGGCC
 81  GCCCCGCCAG CCCTGACTTC TCCCCTGCAG CCCCGCTGCG TGGAGAGGCA GCCCCGCCCT CCTCAGGGTT GCGCAGGATT
161  TGCCCTTTCT GCAGCCCTCC GGGGAGCTGA GGAGGTGGAT AACAGGGTTT CAAAGGGCAC CGGCACTGCA GATAAACAGG
241  GGGAGCGGAA ATGTCAACAG AACAGACACT TCGCGTCAAT AAACTTTAGG TTTATCCGGA GCAGATCTCA TTTCCCTGAG
321  TACGATCCAG AGGAGGCCAG AA
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B.

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          F_exon1_27mer >
    TCTGAGGCCC CGCTCCCCGA AACGTGA
  1 TCTGAGGCCC CGCTCCCCGA AACGTGACCA TGTGGATTCA ACAGCTTTTA GGACTCAGGT CTGGCTGTCC ATCAGATCAT

 81 CACCATCACC GTCTCCCTCA TCATGGTCAT AGCTGCTCTC ATCACAACTC TTGTCTTAAA AAATTGCTGT GCCCAAAGCG
                         >>.........................Isoform_3.........................>
                           m  v  i  a  a  l  i  t  t  l  v  l  k  n  c  c  a  q  s

161 GGAACACTCG TCGGAACAGC CACCAGCGGA AGACCAACCA GCAGGAGGAG AGCTGCCAGA ACCTCACGGA CTTCCCCTCG
    >.....................................Isoform_3.....................................>
     g  n  t  r  r  n  s  h  q  r  k  t  n  q  q  e  e  s  c  q  n  l  t  d  f  p  s

241 GCCCGGGTGC CCAGCAGCCT GGACATATTC ACGGCCTATA ACGAGACCCT GCAGTGTTCT CACGAGTGCG TCAGGGCATC
    >.....................................Isoform_3.....................................>
      a  r  v  p  s  s  l  d  i  f  t  a  y  n  e  t  l  q  c  s  h  e  c  v  r  a

321 TGTGCCCGTG TACACCGATG AGACGCTGCA CTCGACGACG GGGGAGTACA AATCCACATT TAATGGAAAC CGACCCTCCT
    >.....................................Isoform_3.....................................>
    s  v  p  v  y  t  d  e  t  l  h  s  t  t  g  e  y  k  s  t  f  n  g  n  r  p  s

401 CTTCTGATCG GCATCTTATT CCTGTGGCCT TCGTGTCTGA GAAATGGTTT GAAATCTCCT GCTGACTGGC CGAAGTCTTT
    >...............................Isoform_3...............................>>
     s  s  d  r  h  l  i  p  v  a  f  v  s  e  k  w  f  e  i  s  c  -

          < R_3UTR_28mer
    AAATGGAGGA CCCCCGTCCC GTCTGCGG
481 TTTACCTCCT GGGGGCAGGG CAGACGCC
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Fig. S3: Shrew-1 sequences.

(A) Identified genomic sequence of E1a. (B) Sequence of the 508 bp PCR product from kidney, representing the novel transcript variant encoding the putative shrew-1 protein isoform 3

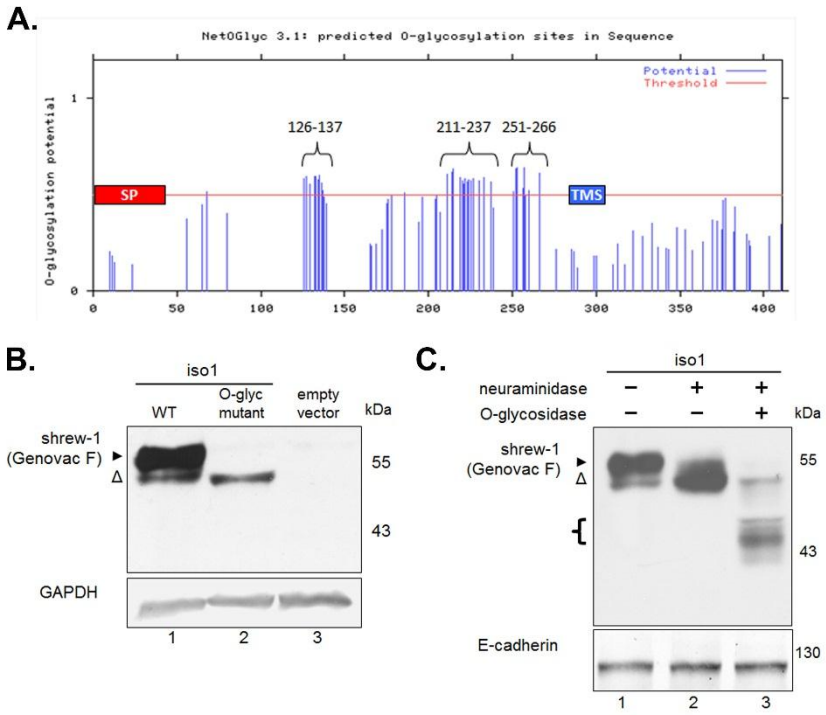

Fig. S4: Validation of the predicted O-glycosylation of shrew-1.

(A) Graphical output of the NetOGlyc v3.1 analysis of the human shrew-1 protein sequence (NP_061324.1) showing the identified 34 putative O-glycosylation sites of the mucin type in the extracellular domain of shrew-1. On the x-axis the protein sequence of shrew-1 is plotted (position 1 to 411). The red and blue boxes depict the position of the SP and the TMS, respectively. The red horizontal line indicates the threshold. The potential of each aa residue being O-glycosylated is depicted as a blue vertical line. Residues exhibiting a potential that exceeds the threshold are predicted to be O-glycosylated. The predicted O-glycosylation sites are arranged in three clusters encompassing the aa residues 126 to 137, 211 to 237, and 251 to 266. (B) Shrew-1 (isoform 1) wild type, the shrew-1 O-glycosylation mutant, and the empty vector were stably expressed in MDCK cells. Lysates of the cells were analyzed by immunoblot using the anti-shrew-1 antibody Genovac F. GAPDH was visualized as a loading control. The O-glyc mutant emerged at a smaller size (delta) compared to the wild type shrew-1 protein (arrowhead). (C) Lysates of MDCK cells stably expressing shrew-1 were treated, as indicated (+), with neuraminidase alone or additionally with O-glycosidase, and subsequently separated by SDS-PAGE (8% gels) and analyzed by immunoblot using the anti-shrew-1 antibody Genovac F. As a loading control E-cadherin was visualized.