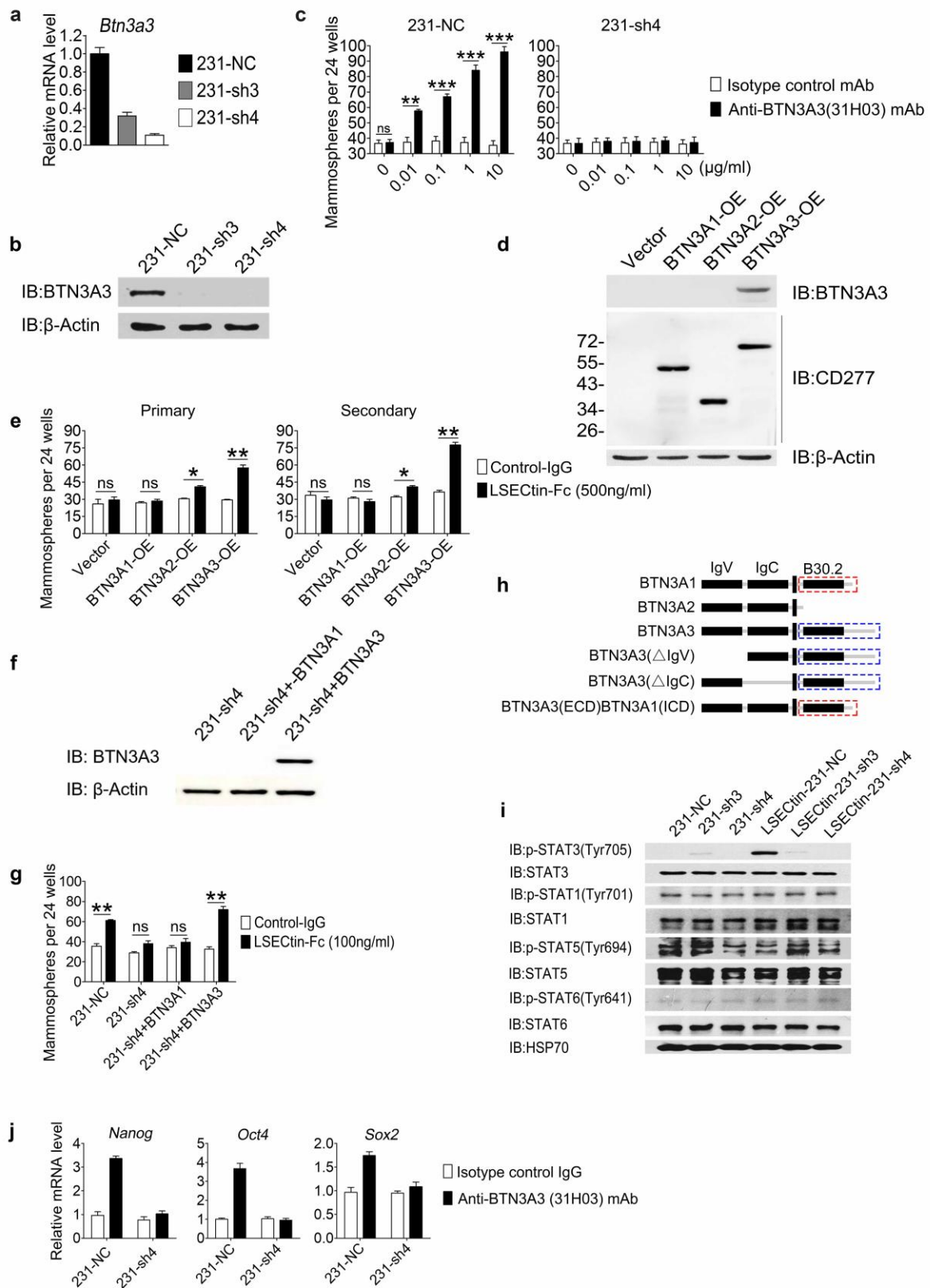


Supplementary information, Fig. S4



**Supplementary information, Fig. S4. The LSEctin-BTN3A3 axis promotes breast cancer stemness *in vitro***

(a,b) Real-time PCR (a) or WB (b) showing expression of BTN3A3 relative to the expression of the housekeeping gene  $\beta$ -actin in BTN3A3-KD cells (231-sh3, 231-sh4) and control cells (231-NC). One of two experiments is shown.

(c) Sphere-forming assay showing the representative mean number of spheres formed by 231-NC or 231-sh4 cells stimulated with anti-BTN3A3 (31H03) mAb or isotype control mAb in a concentration gradient (n = 4 each). One of three experiments is shown.

(d) WB showing the expression of BTN3A relative to that of the housekeeping gene  $\beta$ -actin in BTN3A-OE cells.

(e) Sphere-forming assay showing the representative mean number of spheres of BTN3A-OE BT474 cells in primary and secondary passaged cultures stimulated with LSEctin-Fc recombinant protein or control-IgG are shown (n = 3 each). One of three experiments is shown.

(f) WB showing expression of BTN3A3 relative to the expression of the housekeeping gene  $\beta$ -actin in BTN3A3-knock down cells 231-sh4 , 231-sh4 cells re-overexpressing BTN3A1 (231-sh4+BTN3A1) or BTN3A3 (231-sh4+BTN3A3)

(g) Sphere-forming assay showing the representative mean number of spheres of 231-sh4, 231-sh4+BTN3A1 or 231-sh4+BTN3A3 cells in primary passaged cultures stimulated with LSEctin-Fc recombinant protein or control-IgG are shown (n = 3 each). One of two experiments is shown.

(h) Diagram showing the origin of the BTN3A3 chimeric proteins.

(i) 231-NC, 231-sh3 or 231-sh4 cells stimulated with LSEctin recombinant protein at 10 $\mu$ g/ml were lysed and fractionated to obtain cell extracts for WB. STATs and p-STATs were immunoblotted to demonstrate signal transduction pathway activation. HSP70 served as a control.

(j) Real-time analysis of the expression of *Nanog*, *Oct4* and *Sox2* in 231-NC and 231-sh4 mammosphere cultures with anti-BTN3A3 (31H03) mAb or isotype control IgG (n = 3 each).

Data are presented as the mean  $\pm$ SD. \**P* < 0.05, \*\**P* < 0.01, \*\*\**P* < 0.001 (unpaired Student's t test for c, e, g).