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Supplemental Information

A Thymic Epithelial Stem Cell Pool Persists throughout Ontogeny and Is Modulated by TGF-β

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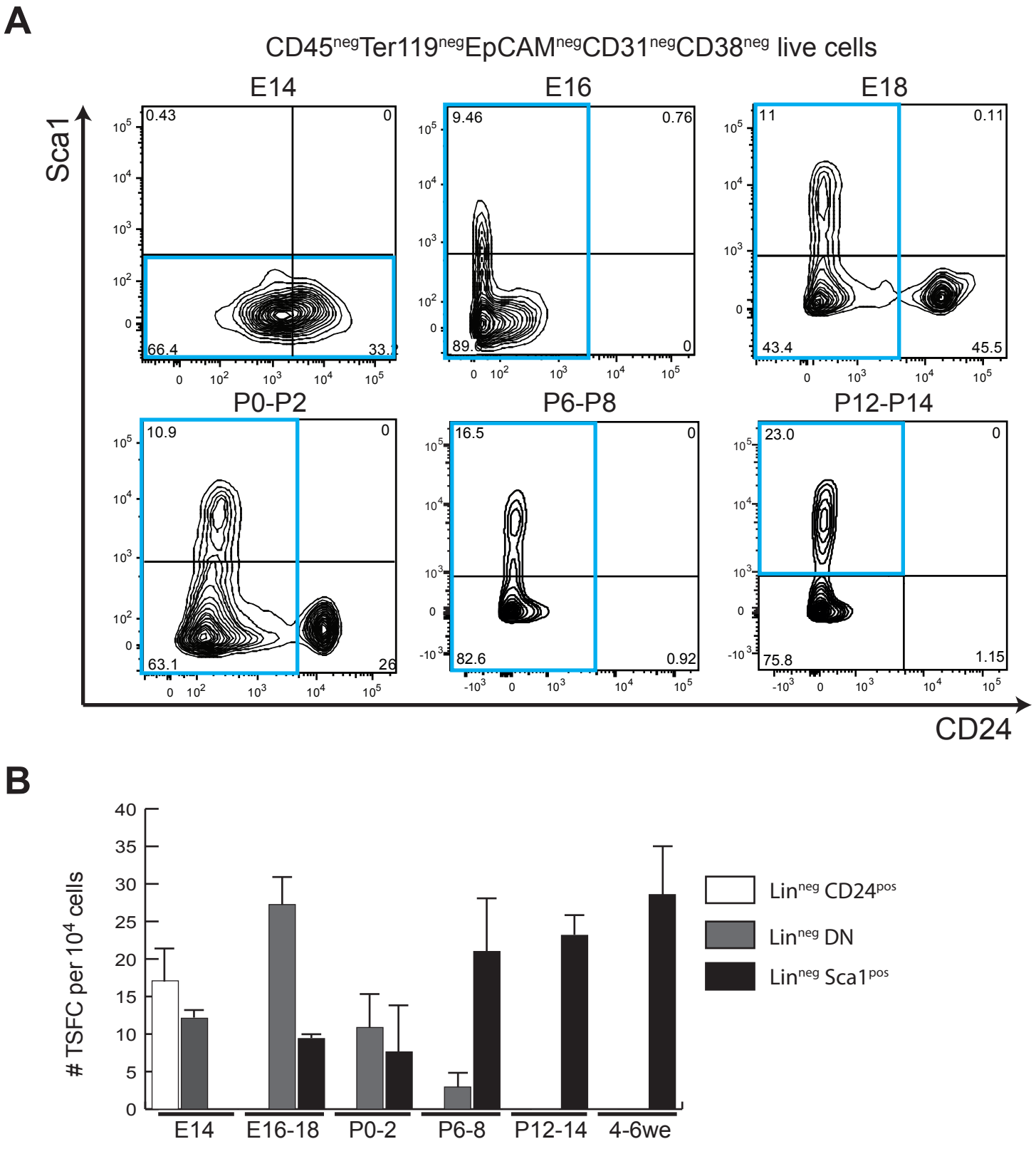

Figure S1, related to Figure 1. Thymosphere-forming cells change their surface marker profile during ontogeny.

(A) Distribution of surface markers Sca-1 and CD24 in the lineage-negative ($CD45^{neg}Ter119^{neg}EpCAM^{neg}CD31^{neg}CD38^{neg}$) fraction of thymic digests derived from different ages. Blue rectangles indicate the populations containing thymosphere-forming cells.
(B) Relative numbers of thymospheres derived from different fractions of lineage-negative cells at different ages. Data are shown as mean of n>2 independent experiments ± SD.

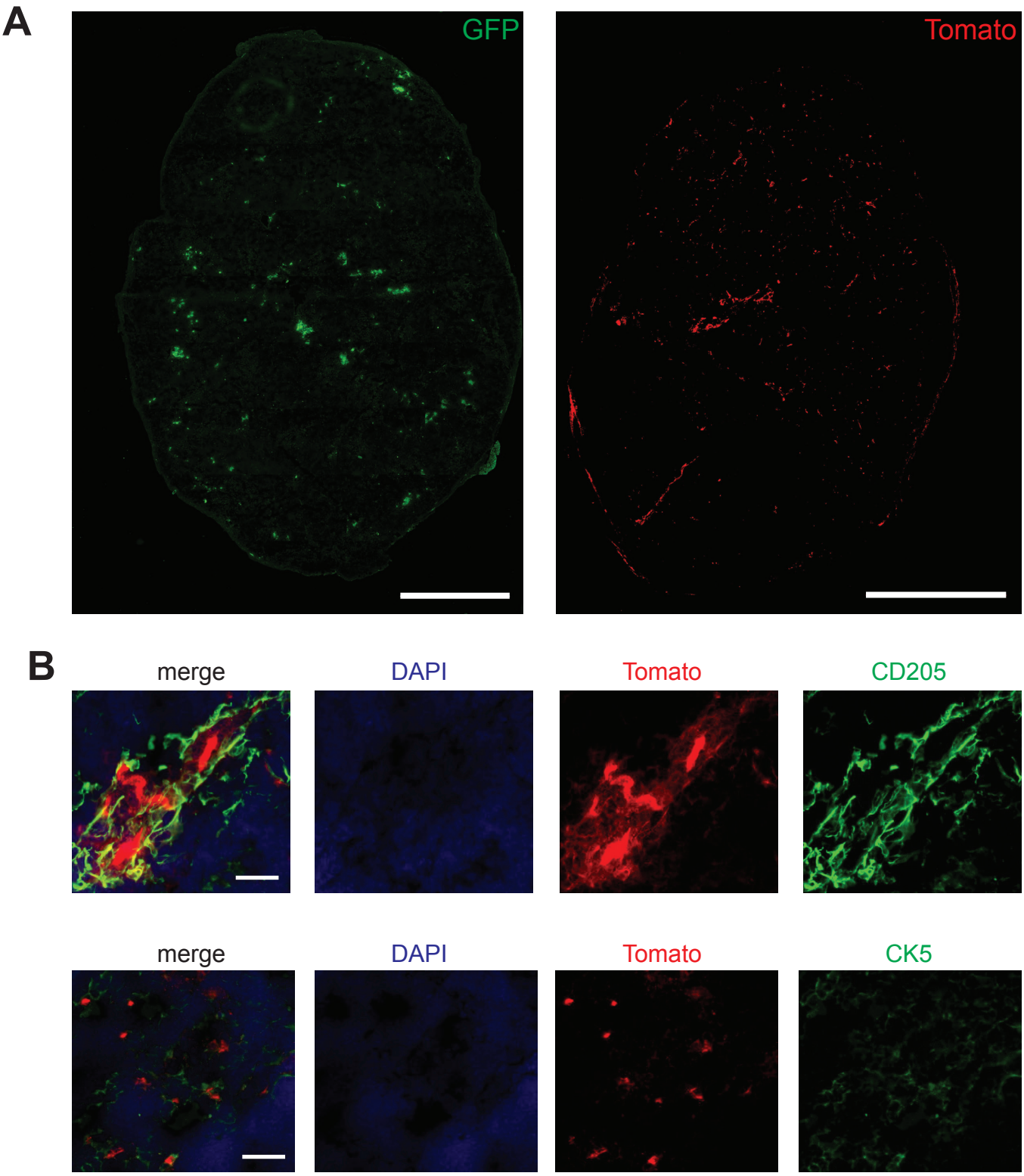

Figure S2, related to Fugure 2. Embryonic thymosphere-derived cells express progenitor markers in the transplant.

(A) Distribution of thymosphere-derived Tomato^{pos} and GFP^{pos} cells in the transplant. Tiled images were acquired and automatically stitched using Zen software and a motorized Zeiss Cell Observer Z1. Scale bars: 1 mm.

(B) Histological analyses of transplanted RTOCs containing FoxN1CrexRosa26-Tomato-GFP thymospheres 5 weeks post-transplantation. Sections of transplants were co-stained for Tomato and either CD205 or CK5. Scale bars: 20 µm

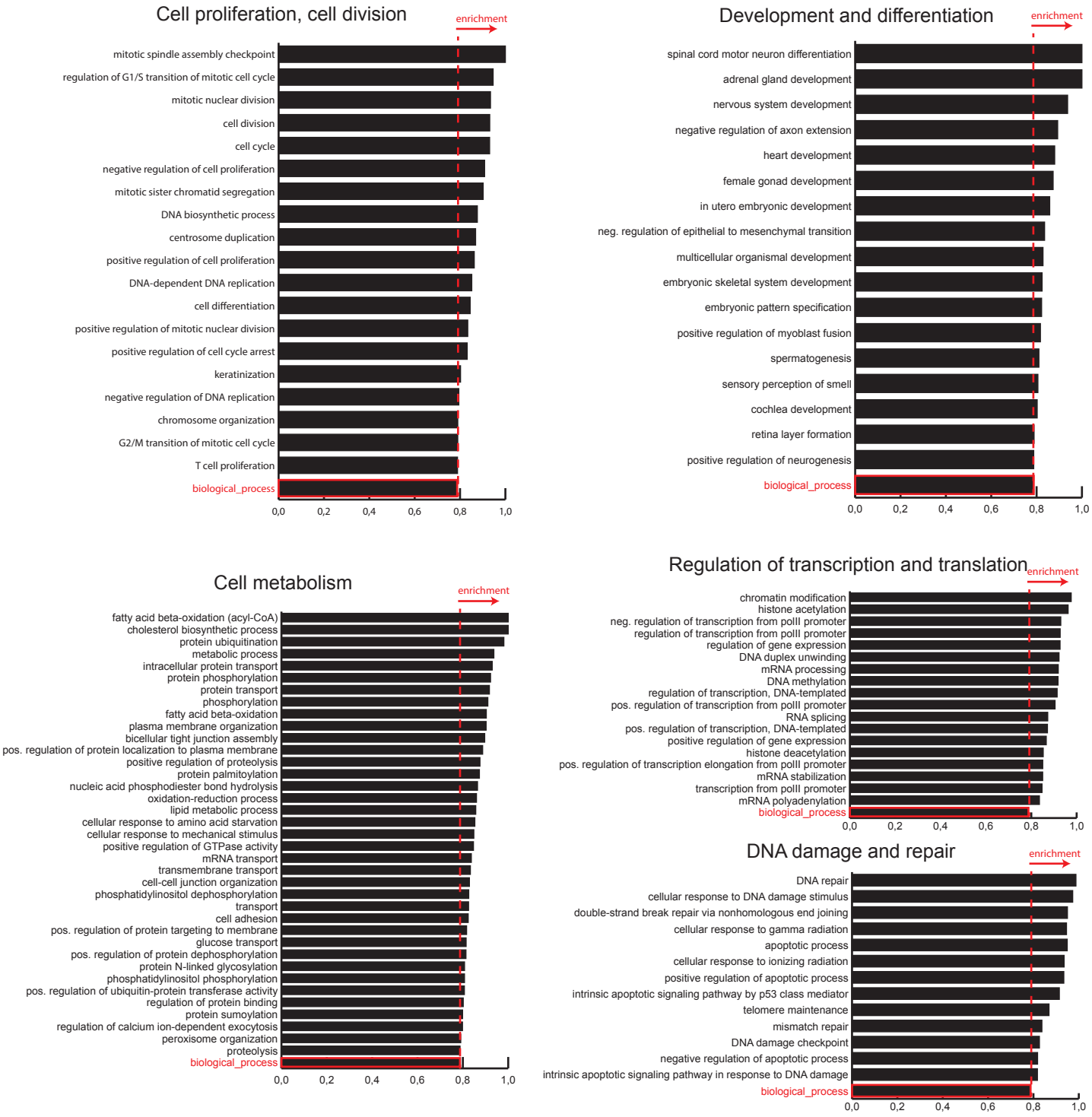

Figure S3, related to Figure 3. Thymospheres change their expression profile during ontogeny.

Top gene sets significantly changed between embryonic and adult thymospheres were grouped in categories related to cell proliferation, development and differentiation, cell metabolism, regulation of transcription and translation, and DNA damage and repair. The bars show the estimated fraction of regulated genes for each gene set. Gene set “biological process” (highlighted) at the bottom of each category has been included as a reference set.

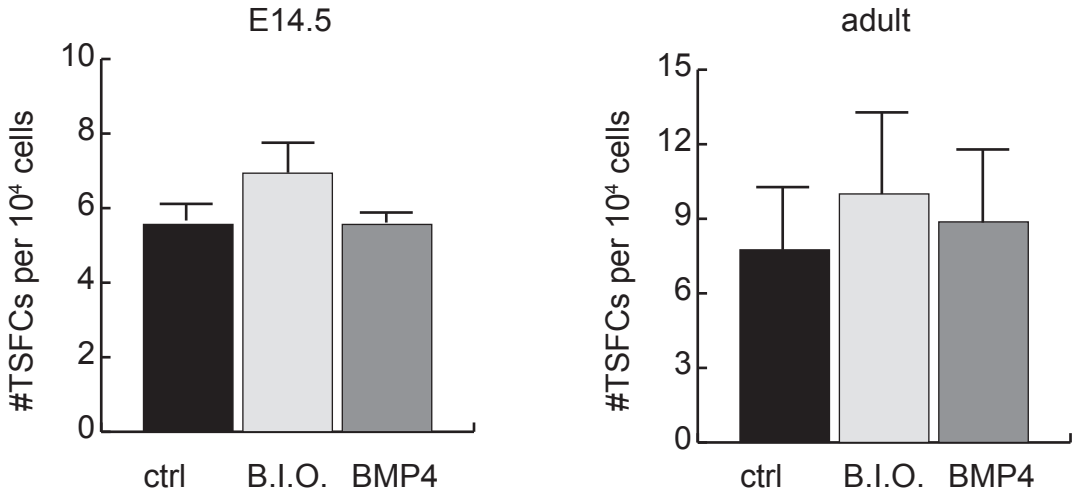

Figure S4, related to Figure 4. Embryonic and adult thymosphere formation is not affected by the activation of Wnt or BMP4 signalling.

E14.5 thymus-derived (left) and adult (right) thymosphere formation rate does not change in the presense of B.I.O. (GSK3b inhibitor) or recombinant BMP4 in the sphere culture medium.

Data are shown as mean of n=3 independent experiments ± SD.

Supplemental Experimental Procedures

FACS staining, whole mount sphere staining and immunohistochemistry

For FACS sorting experiments thymic stroma-enriched single cell suspensions were blocked with anti-FcR mAb 2.4G2 and stained for various surface markers with the following antibodies: CD45 (clone 30F11, BD Pharmingen), Ter119 (clone Ter119, Invitrogen), EpCAM (clone G8.8), MHCII (clone M5/114.15.2, BD Pharmingen), Sca1 (clone D7, BD Biosciences), CD24 (clone M1/69, eBioscience), CD31 (clone 390, BD Pharmingen), CD38 (clone 90, BD Pharmingen), and Sav-PE-Cy7 (BD Biosciences). DAPI was used for dead cell exclusion.

For whole mount staining, spheres were fixed in 2% paraformaldehyde, 100 mM Hepes, 50 mM EGTA, 10 mM $MgSO_4$, 0.05% glutaraldehyde, washed in PBS/BSA and permeabilized in 0.2% Triton-X100 in PBS/BSA overnight. After blocking in 3% BSA / PBS, spheres were stained at RT with antibodies against cytkeratin-8 and -14 in PBS/BSA and mounted using ProLong gold antifade reagent with DAPI (Life Technologies). Confocal imaging was performed using an Olympus FluoView FW1000 motorized inverted microscope.

Antibodies directed against the following markers were used for immunohistochemistry: GFP (rabbit, Life Technologies), mCherry (rabbit, Clontech), CK8 (rat, Troma1c, DSHB), CK14 (rabbit, Covance), CD205 (clone NLDC-145, a kind gift of J. Dooley, VIB, Belgium), UEA1 (rat, Vector Labs), Aire (clone B1/02-5H12-2; a kind gift of H. Scott, Center for Cancer Biology, Australia), Cy3-donkey-anti-rat, Cy3-goat-anti-rabbit, Cy3-Sav, FITC-rabbit-anti-guinea pig (all from Jackson ImmunoResearch), AF488-goat-anti-rat and AF488-goat-anti-rabbit (both from Molecular Probes).

TGF-β signalling detection in protein lysates

Whole cell protein lysates were prepared with 1× RIPA buffer (1% NP40, 0.5% DOC, 0.1% SDS, 250 mM NaCl, 2.5 mM EDTA, 50 mM Tris, pH 7.2) supplemented with protease and phosphatase inhibitors. Lysates were kept on ice for 15 min and then sonicated for 25 seconds (amplitude 75%, 0.1 s “on”, 0.5 s “off” on Sonopuls, Bandelin). Following centrifugation for 10 min at 21,000 rcf and 4°C, supernatants were collected into the new tubes, and BCA reaction was performed to measure protein concentration. Equal amounts of protein from each cell population were taken for analysis.

Briefly, 25 µl of a magnetic beads mixture was combined with 25 µl of diluted lysates on a pre-wetted 96-well plate followed by overnight incubation at 4º C with shaking at 700 rpm by using a IKA MTS 2/4 digital shaker (IKA WERKE). Afterwards wells were washed twice with 100 µl of Assay buffer (Millipore) and 25 µl of detection antibodies was added per well. After 1 hour of incubation at RT, detection antibodies were removed and 25 µl of Streptavidin-Phycoerythrin (SAPE) was added per well. Plates were incubated for 15 min at RT with vertical shaking at 300 rpm. Without removing SAPE, 25 µl of Amplification buffer was added per well and incubate at RT with vertical shaking 300 rpm for 15 min. Afterwards the SAPE/Amplification buffer mixture was removed and the magnetic beads were reconstituted in 150 µl of Assay buffer. Data acquisition was performed by using the BioPlex 200 Systerm (BioRad) and data analysis by using BioPlex Manager (BioRad) software.