SUPPLEMENTARY MATERIAL

SUPPLEMENTARY METHODS

Isolation of TEB and duct fragments

Stromal-free terminal end buds (TEB) and ducts were isolated as described previously (Morris and Stein, 2017; Morris et al., 2006). Briefly, C57BL/6 mice were humanely killed at 6-7 weeks (16-18g) and the inguinal mammary glands were dissected and collected in chilled L15 medium. 20 glands were pooled for each preparation, coarsely cut with scalpels and digested with 1mg/ml (w/v) collagenase Type II (Sigma) at 37°C for 20-30 minutes (for TEB) and 30-45 min (ducts) with mild agitation. After incubation, the epithelium was further freed of the stroma by vigorous shaking by hand. The collagenase was diluted and blocked with fresh cold L15 medium with 0.1% FBS and the epithelium spun down at 250g for 5min. The pellet was re-suspended in fresh cold L15 medium with 10% FBS, transferred to a gridded 60 mm dish, and released TEB and ducts were collected under a stereo dissection microscope with a 10μl pipette into 50-100μl TRI-reagent (Sigma) before snap-freezing.

RNA isolation and microarray hybridisation from isolated epithelium

For RNA isolation, frozen samples were thawed and RNA isolated according to manufacturer’s protocol before resuspension in RNase-free water. The RNA was quantified with a Nanodrop ND-1000 spectrophotometer, pooled and subjected to on-column DNase I treatment (Qiagen) and further concentration using a RNeasy-micro kit (Qiagen). RNA quality was finally assessed using a 2100 Bioanalyzer and RNA 6000 Nano kit (both Agilent).

For microarray hybridisation, 1.5μg RNA per sample (from ~300-400 isolated TEBs or ducts) was used in pooled duplicates and analysed at the Henry Wellcome Functional Genomics Facility (Glasgow). rRNA was removed using a RiboMinus Human/MouseTranscriptome Isolation kit and RiboMinus magnetic beads, labelled according to manufacturer’s protocol and finally hybridised to mouse whole-genome exon arrays (GeneChip-Mouse-Exon-1.0-ST-Array, Affymetrix UK Ltd., High Wycombe, UK) using a GeneChip Fluidics Station 450/250. The signals were measured using a GeneChip Scanner 3000 7G. CEL-files were analysed and normalised by RMA using the open-source ‘Altanalyze’ software (Emig et al., 2010). Results of differentially abundant RNAs in TEB and ducts were ranked according to raw p-value (one-way analysis of variance (ANOVA))
(Table S1). Raw data files have been submitted to GEO with the accession number GSE94371.

SUPPLEMENTARY FIGURE LEGENDS

Fig.S1. Flow cytometry gating workflow for isolation of mammary epithelial subpopulations. (A) Gating workflow demonstrating identification of single cells by time-of-flight analysis on forward and then side scatter, followed by exclusion of DAPI+ dead cells and CD45+ white blood cells. Remaining cells are then plotted on a Sca-1 vs CD24 plot and the epithelial cells gated as described (Britt et al., 2009). This sorting strategy isolates all mammary epithelial cells (Britt et al., 2009). The epithelial-only population is then analysed in two ways. First, it is plotted on a Sca-1 vs CD24 dot plot and the luminal ER+ and luminal ER- populations gated, with c-Kit staining of the latter being further assessed to identify the c-Kit+ progenitors. Second, the epithelial population is plotted on a CD24 vs CD49f contour plot (linear density, 5% intervals) to identify the MYOs and MaSCs, as previously described in detail (Britt et al., 2009; Soady et al., 2015). MASCs, MYOs, Luminal ER- and Luminal ER+ cells, as gated, collectively form >90% of the total mammary epithelium (Regan et al., 2012). (B) Control plots showing samples in which either only DAPI was added (left, middle) or in which the anti-c-Kit-PE was omitted (right).

Fig.S2. Ptprb localisation by RNAScope 2.5 HD Duplex in situ hybridisation. Red label indicates a positive signal. ‘Neg’ indicates samples labelled with the negative control probe. ‘Ptprb’ indicates samples labelled with the Ptprb probe. ‘bv’ indicates a blood vessel, ‘ed’ epithelial duct. Bars = 50µm. Top row, liver (left two panels) used as positive control tissue, and sections from 3 week old mammary gland (right two panels). A strong, uniform Ptprb signal was detected on the endothelial cells lining blood vessels (middle right panel) and a positive signal was also detected in a subset of cells lining epithelial ducts (far right panel). Middle row, 6 week old mammary gland, showing a strong Ptprb signal on blood vessels (left two panels, white arrows on inset) and a signal on stromal cells surrounding the epithelial ducts (right panels, white arrows on inset) but no signal could be detected in the epithelium. Note that the positioning of distinctive elongated myoepithelial nuclei (black arrows on inset) demonstrate that the labelled cells are indeed outside the ducts. Bottom row, 12 week old mammary gland, again showing a strong Ptprb signal on blood vessels (left two panels) and a weak positive signal in a subset of cells lining epithelial ducts (right two panels, arrowheads on inset).
Fig. S3. *Ptprb* knockdown does not alter proportions of cell populations *in vitro*. (A) Representative flow cytometry plots form analysis of cells recovered from transplanted mammary fat pads. Only the epithelial populations are shown. Epithelial outgrowths from viral transplants consist of both transduced (GFP positive) and non-transduced cells (GFP negative) and both of these include basal, LumER- and LumER+ populations, defined by CD24 and Sca-1 expression patterns (Soady et al., 2015). Top, analysis of fat pads carrying non-transduced cells. Middle, analysis of shScr-transduced transplants. Bottom, analysis of sh Ptprb 0145-transduced transplants. (B) Proportions of basal, luminal ER negative and luminal ER positive cell populations in GFP+ (viral-transduced) cells harvested from fat pads transplanted with primary mammary epithelial cells transduced with shScr, shPtprb 0145 or shPtprb 3820. Data presented as mean proportion of total epithelial cells ± SD. *n* indicates number of independent transplant experiments, each of which included at least five transplanted fat pads, which contributed to the data. There are no differences between the proportions of the populations in the control compared to the knockdown transplants.

Fig. S4. *Ptprb* knockdown does not enhance apoptosis. (A) Numbers of transplanted fat pads used to determine the size of outgrowths from knockdown and control cells and the amount of branching. (B) Cleaved Caspase-3 / DAPI staining of shLuc and shPtprb transplants, together with positive control lung tissue sample (Ctx, chemotherapy-treated). Bar = 100 μm. (C) Wholemounts of fat pads from secondary transplants of shScr (top) and shPtprb 0145 (bottom) transduced cells. Bars = 5 mm.

Fig. S5. *In vitro* organoid branching is dependent on FGF2. (A) Transduction with shLuc, shPtprb 0145 or shPtprb 3820 in the absence of FGF2 stimulation does not cause branching. High levels of sh lentiviral transduction are indicated by strong GFP expression. Images are representative of multiple independent wells from three independent experiments. Paired phase contrast and GFP pictures are shown. Scale bars = 30 μm. (B) Examples of untreated organoids and organoids treated for seven days with 50 ng ml⁻¹ FGF2, ANG1 or ANG2. Branching of organoids is only seen in FGF2 stimulated cultures. Images are representative of multiple independent wells from three independent experiments. Scale bars = 30 μm.

Fig. S6. *Ptprb* knockdown enhances FGFR phosphorylation. Raw western blot data of effects of *Ptprb* knockdown on FGFR phosphorylation in response to FGF2 stimulation.
Films of blots probed for phospho FGFR and TUBULIN are shown (blots used for preparing Figure 7A). ‘Sh1’ and ‘1’ indicate sh Ptprb 0145. ‘Sh2’ and ‘2’ indicate sh Ptprb 3820.

**Fig. S7.** Ptprb knockdown does not alter total FGFR levels. (A) Western blot analysis of total FGFR1, 2, 3 and 4 levels in organoid cultures transduced with control, 0145 or 3820 knockdown lentiviruses and either unstimulated or stimulated with FGF2. (B) Quantitation of total FGFR levels (mean ±SD; n=3).

**Fig. S8.** Ptprb knockdown does not alter total FGFR levels. Raw western blot data of total FGFR1, 2, 3 and 4 levels in organoid cultures transduced with control, 0145 or 3820 knockdown lentiviruses and either unstimulated or stimulated with FGF2 (blots used for preparing Supplementary Figure S7).

**Fig. S9.** ERK1/2 inhibition blocks bFGF-induced mammary branching. (A) Western blot analysis of phospho- and total ERK1/2 levels in organoid cultures either unstimulated or stimulated with FGF2 and treated with High (8 nM), Medium (4 nM) or Low (2 nM) concentration of SCH772984. Tubulin was used as loading control. (Bi - Bv) Representative images of organoids at day 8 of culture.

**Fig. S10.** ERK1/2 inhibition blocks bFGF-induced mammary branching. Raw western blot data of effects of SCH772984 treatment on phospho- and total ERK1/2 in response to FGF2 stimulation. Blots probed for pERK1/2, tubulin and pFGFR (top blot, short exposure; middle blot, long exposure) and for total ERK1/2 (bottom blot). Blots used for preparing Supplementary Figure S9.

**Fig. S11.** Ptprb knockdown enhances ERK1/2 phosphorylation. Raw western blot data of effects of Ptprb knockdown on ERK1/2 phosphorylation in response to FGF2 stimulation. Films of representative blots probed for total ERK1/2, phospho ERK1/2 and GAPDH are shown (blots used for preparing Figure 7B).

**SUPPLEMENTARY TABLES**

**Table S1.** Gene expression analysis comparing isolated TEBs vs ductal fragments. Data for 3756 genes with >1.5 fold differential expression between the two structures from two independent replicates each of which analysed TEBs and duct fragments from >100 animals.
Table S2. Sybr green probes and TAQMan assays for quantitative real-time rtrPCR.

SUPPLEMENTARY REFERENCES


