Figure S1 – Cultured pre-stasis finite lifespan HMEC at early passage preserve key features of HMEC in vivo. (A) Representative growth curves of HMEC strain 240 from 2nd passage grown in M85+oxytocin (a formulation similar to M87A) versus MCDB170+supplements (commercially MEGM). Growth in M87A stops at stasis (stress-associated senescence) [27, 31]; however MCDB170 induces epigenetic changes leading to abnormal post-stasis HMEC growth [31, 48] (B) Uncultured organoids; (left) FACS analysis for CD227 (Muc-1) and CD10 (CALLA) on cells of dissociated uncultured organoids from 51L mammoplasty tissue shows presence of LEP (CD10−/CD227+) and MEP (CD10+/CD227−) lineages. (right) Epithelial lineages in primary organoid outgrowths in M87A+oxytocin identified by staining with antibodies to K14 (red) and K19 (green); nuclei were stained with DAPI (blue). LEP (K14+/K19−, green), MEP (K14+/K19+, red), and progenitors (K14+/K19+, yellow) are visible. Unstained cells are observed in the organoid core due to incomplete antibody penetration. (C) 4th passage HMEC; (left) Typical FACS and (right) immunofluorescence analyses of a pre-stasis culture at 4th passage.

Figure S2 – Analysis of HMEC lineages in strains derived from reduction mammoplasty (RM) versus peripheral non-tumor regions from mastectomy (P) tissues. (A) Linear regression showing changes in proportions of LEP (filled circles), MEP (filled squares), and cKit+ HMEC (filled triangles) in RM-derived HMEC strains at 4th passage as a function of age (n=21 individuals). (B) Linear regression showing changes in proportions of LEP (open circles), MEP (open squares), and cKit+ HMEC (open triangles) in P-derived HMEC strains at 4th passage as a function of age (n=15 individuals). Associated statistics are shown at the bottom of the regression plots. Dot graphs showing comparisons of the proportions of (C) LEP, (D) MEP, and (E) cKit+ HMEC in 24-29y RM versus 24-30y P and in 41-62y RM versus 45-65y P age groups. Group averages and SE are shown, RM-derived samples are denoted with filled symbols and P-derived with open symbols. (F) A summary of statistics showing the ANOVA and t-tests that were used to compare the groups in C-E.
Figure S3 – Lineage marker distributions in cultured and uncultured HMEC. Seven-parameter flow cytometry analyses of 4th passage HMEC strains (A) 240L and (B) 122L, and of dissociated organoid specimens (C) 53 and (D) 29. Forward and side scatter parameters are not shown; CD10, CD227, CD49f, EpCAM, and CD117/cKit expression parameter plots are shown. Gates demarcating the regions that correspond to CD10⁻/CD227⁺ LEP, CD10⁺/CD227⁻ MEP, and cKit⁺ HMEC were determined using unstained controls (gray-colored shade boxes). For each strain or specimen, CD10/CD227 and CD227/cKit expression profiles are shown as pseudocolor heatmaps with the LEP, MEP, and cKit regions identified. Multicolor overlays show the EpCam/CD49f expression files of the cells that fall within the LEP (green), MEP (red), and cKit (orange) gates. In the multicolor overlay plots, all the cells that are do not fall within the gated regions appear black.