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**Supplemental Information**

**SLIT/ROBO2 Signaling Promotes Mammary Stem Cell  
Senescence by Inhibiting Wnt Signaling**

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## **MG Transplantation**

Mammary anlage were rescued and tissue fragments from resulting outgrowths were transplanted (Strickland et al., 2006). *Robo2* cells sorted by FACS were manually counted and transplanted at limiting dilution (Shackleton et al., 2006).

## **IHC and SA- $\beta$ -Gal Activity**

IHC was performed as previously described with: anti-SMA (Sigma), anti-P16<sup>INK4a</sup> and anti-p63 (Santa Cruz Biotech), anti-*Robo2* (Abcam), and anti-ABC (Millipore) (Marlow et al., 2010). SA- $\beta$ -Gal activity was detected as previously described (Debacq-Chainiaux et al., 2009). Fluorescent imaging was performed on a Biorevo BZ-9000 Digital Microscope (Keyence), and confocal imaging on a Velocity Spinning Disk Confocal (PerkinElmer).

## **Cell Preparation, Cell Sorting, and Primary Cell Culture**

Cell suspensions for FACS and primary cultures were prepared as described (Shackleton et al., 2006; Vaillant et al., 2008; Zeng and Nusse, 2010). All BC colony 3D Matrigel cultures were generated from FACS-purified BCs. Cultures were treated daily with WNT3A, DKK, (Zeng and Nusse, 2010) or LY294002 (Sigma), or every three days with SLIT2 (Macias et al., 2011). FACS was performed using a FACS Aria (BD Biosciences). Matrigel (BD Biosciences) cultures were fixed with 10% formalin and dehydrated with 70% ethanol prior to paraffin embedding.

## **RNA Extraction, and q-RT-PCR**

Total RNA was isolated using TRIzol (Invitrogen), and then prepared as previously described (Macias et al., 2011). q-RT-PCR was performed using a Bio-rad CFX Connect Real-Time System (Bio-rad) and LightCycler 480 SYBR Green I Master (Roche). Gene expression was determined using CFX Manager software (Biorad). Primers used for q-RT-PCR were: Robo1-F TTATGGTGATGTGGACCTTAGTA, Robo1-R GGTTGTATGGGATGGTTGGAG, Robo2-F CATGGGATTTGGTTATTCGCTAC, Robo2-R GGACCCGATCTTCGTCTTCTT, Myc-F TCTCCATCCTATGTTGCGGTC, Myc-R TCCAAGTAACTCGGTCAT, Jun-F ACTCGGACCTTCTCACGTC, Jun-R GGTCGGTGTAGTGGTGATGT, Tert-F TCTACCGCACTTTGGTTGCC, Tert-R CAGCACGTTTCTCTCGTTGC, P16-F TTTGTGTACCGCTGGGAAC, P16-R TAGCTCTGCTGTTGGGATTG, GAPDH-F CATGGCCTTCCGTGTTTCCTA, GAPDH-R CCTGCTTCACCACCTTCTTGAT.

## **Supplemental References**

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