

# Supplementary Materials: Exogenous R-Spondin1 Induces Precocious Telogen-to-Anagen Transition in Mouse Hair Follicles

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Table S1. Primers used for qPCR and RT-PCR assays.

Gene	Forward Primer (5'-3')	Reverse Primer (5'-3')
<i>Rspo1</i>	GGGATCAAGGGCAAGAGACAG	CTGGCGGATGTCGTTCCCTC
<i>Rspo2</i>	ATAGAGGCCGCTGCTTTG	TGCCGTGTTCTGGTTTCC
<i>Rspo3</i>	TGGATATTACGGAACTCG	CTAACCCCTTCTGGGCAAC
<i>Rspo4</i>	CTCGCCCTGTACCGAAGGA	CACCTGCCGTAAGTACGGATG
<i>Gapdh</i>	GGTTGTCTCCTGCGACTTCAACAGC	CGAGTTGGGATAGGGCCTCTCTTGC
<i>Ki67</i>	ATCATTGACCGCTCCTTTAGGT	GCTCGCCTTGATGGTTCCT
<i>Ccnd1</i>	GGGATGTGAGGGAAGAGGTGA	GCAGCGAAAACAACGTGAAA
<i>Ccnd2</i>	CTGTGCGCTACCGACTTCAA	CACATCAGTGTGGGTGATCTTG
<i>Ccnd3</i>	TTGCATCTATACGGACCAGGC	AGGAAGTCGTGCGCAATCA
<i>Ccnb1</i>	CTCAGGGTCACTAGGAACACG	AGCTCTTCGCTGACTTTATTACC
<i>Cdkn1a</i>	TCTTGCACCTCTGGTGTCT	CTCTTGACAGAACCAATCT
<i>Cdkn1b</i>	AATTGGGTCTCAGGCAAAC	CCCTTTTGTGTTGCGAAGAAG
<i>Cdkn1c</i>	ACGAGAATCAAGAGCAGC	AGTTCTCTTGCGCTTGG
<i>Tcf7</i>	GCCAGAAGCAAGGAGTTCAC	ACTGGGCCAGCTCACAGTAT
<i>Cd34</i>	AAGGCTGGGTGAAGACCCTTA	TGAATGGCCGTTTCTGGAAGT
<i>Cd44</i>	AGCCCCTCTGAAGAAGACT	ACTCGCCCTTCTTGCTGTAG
<i>Sox7</i>	CCCTTACTCACCAGGAGTTCA	GGGGACATCCAGAAACAGAG
<i>Sox9</i>	ACTCACATCTCTCCTAATGCT	CGTCGGTTTTGGGAGTG
<i>Sox21</i>	AACACTCGCAGGTGGCG	AGATCGCAGGTCTGCTCTG
<i>Krt10</i>	CGAGGACCTTAAGGGGCAG	CAGGGTCACCTCATTCTCGTAT
<i>Krt17</i>	CAGGCTGGGGTCAGCTAGT	CCAAAGTTGCCTCCATAACC
<i>Krt5</i>	GGAGATCGCCACCTACAGGA	TCCGTAGCCAGAAGAGACACT
<i>Lgr4</i>	CCCRACTTCGCATTCACCAA	GCCTGAGGAAATTCATCCAAGTT
<i>Lgr5</i>	CCTACTCGAAGACTTACCCAGT	GCATTGGGGTGAATGATAGCA
<i>Lgr6</i>	GAGGACGGCATCATGCTGTC	GCTCCGTGAGGTTGTTCACT
<i>Axin2</i>	AACCTATGCCCGTTTCCTCTA	GAGTGTAAGACTTGGTCCACC
<i>Myc</i>	GTGTTTCCTGTGTTAATGCCAC	CACAAGTCAGTGGGTTTCC
<i>Lef1</i>	GCCACCGATGAGATGATCCC	TTGATGTCGGCTAAGTCGCC
<i>Wnt1</i>	CAGGGTTCATAGCGATCCATCT	CCCTCAGGATGGCAAAGG
<i>Wnt2</i>	GACTACGGGATCAAGTTTGC	CACACCATGACACTTGCA
<i>Wnt2b</i>	CCCTCCACTTCAAGCCTCTGA	AGCCATAGCAGCCCAGGATT
<i>Wnt3</i>	GCCCGCTCAGCTATGAACAA	CCCGTGGCATTACACTTTAGG
<i>Wnt3a</i>	GCCTCCGCTGGAGTAGCTTT	GGCTGCTGCACCCACAGATA
<i>Wnt4</i>	AGGAGTGCCAATACCAGT	GCTGAAGAGATGGCGTATAC
<i>Wnt5a</i>	GCTTCGCTTGAATTCCTCG	TGGAAGACATGGCACCT
<i>Wnt5b</i>	CCACTGGTGTGCTTTGTCAGA	CCCGTGTGGTGCAGTCACTT
<i>Wnt6</i>	CCAGCAGCTCCCTAGGAAAAGT	GGCTGGTGTAACCCCAAGTTC
<i>Wnt7a</i>	CGCCAAGGTCTTCGTGGAT	CGACCCGCTCGTTATTGT
<i>Wnt7b</i>	CAGCACCAGTTCCGATT	CGTGATGGCATAGGTGAAG
<i>Wnt8a</i>	CCGGCAGATGGGAAATTACC	CTCTGTTGCCAGCCCTTAGCT

Table S1. Cont.

Gene	Forward Primer (5'-3')	Reverse Primer (5'-3')
<i>Wnt8b</i>	ACCCTGCCTTTCTCCGAAGA	GCGGTGAAGGACACAAGTGACT
<i>Wnt9a</i>	GACAACCTCAAGTACAGCA	ACCATGGCATTGCAAGT
<i>Wnt9b</i>	GGCCCTTCCTTAAGTCACACAAG	ACAAACAGAGGGTGGCAGAATG
<i>Wnt10a</i>	TCGGAACAAAGTCCCCTACGA	TGCTATGGCGTAGGCCGAAAG
<i>Wnt10b</i>	TGCTCTCTTTCAGCCCTTTGC	CTCAGGCAGATGCTCTGAGCTT
<i>Wnt11</i>	AAGGTGGTACACCGGCTATG	GAGTCCTTACAGGCCGGATA
<i>Wnt16</i>	GTCATGCAGTGCAGGCAAC	GCCTTCACTTGGTGAGCCAC
<i>Alx4</i>	GGACGGTAGCCTCAAGCTC	CACGGGTTCAAGATCAGGA
<i>Fgf7</i>	ACCTGAGGATTGACAAACGAGG	CCACGGTCCTGATTTCCATGA
<i>Vim</i>	CGGCTGCGAGAGAAATTGC	CCACTTTCCGTTCAAGGTCAAG
<i>Krt14</i>	AGCGGCAAGAGTGAGATTTCT	CCTCCAGGTTATTCTCCAGGG
<i>Kit</i>	TGACGGTACATGGCTGCATT	ACCACCGTAAATGTGTCCCC
<i>Ptprc</i>	GTTTTCGCTACATGACTGCACA	AGGTTGTCCAACACTGACATCTTC
<i>Gsk-3<math>\beta</math></i>	TGGTGTGGATCAGTTGGTGG	GGCCGGAAGACCTTTGTCC
<i><math>\beta</math>-catenin</i>	ATGGAGCCGGACAGAAAAGC	CTTGCCACTCAGGGAAGGA

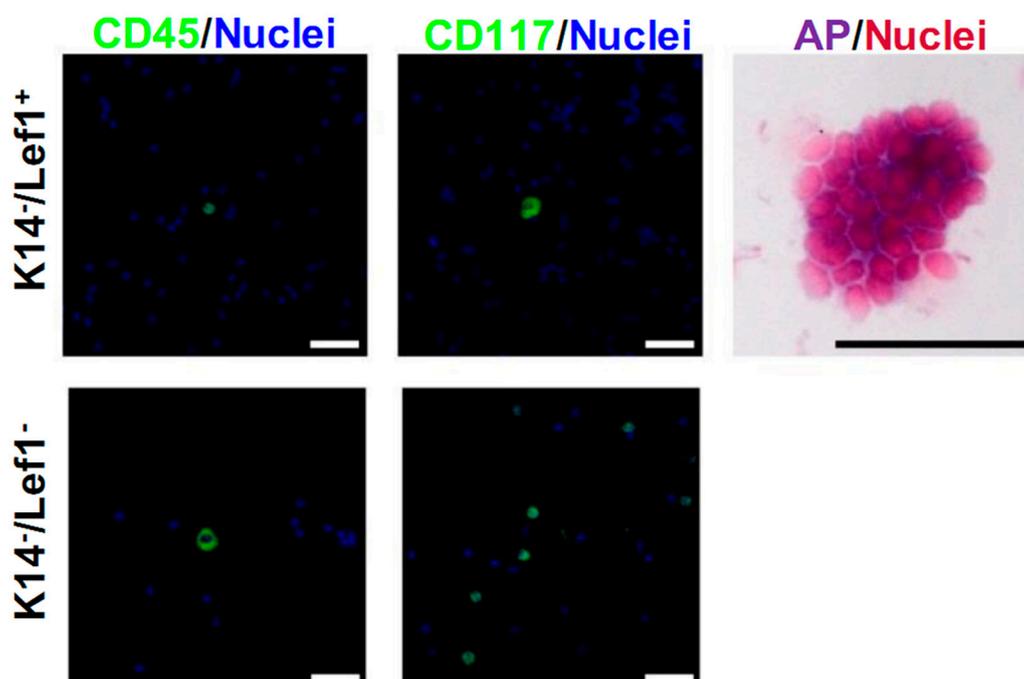
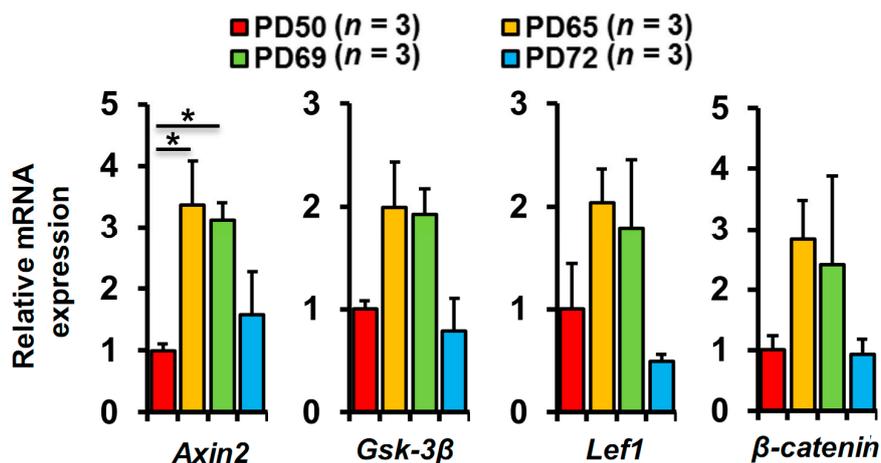
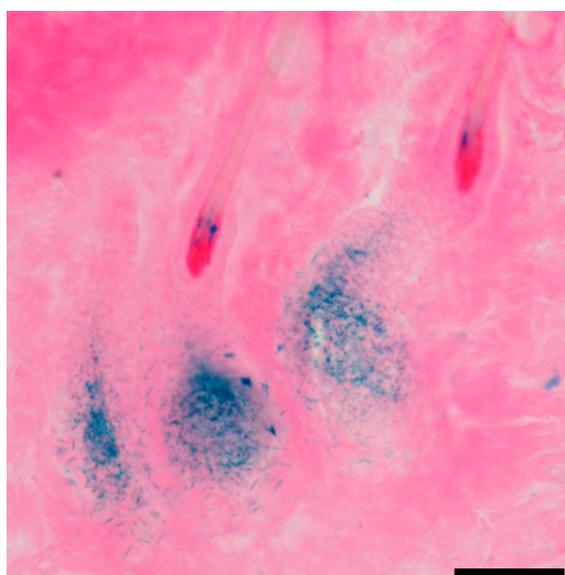


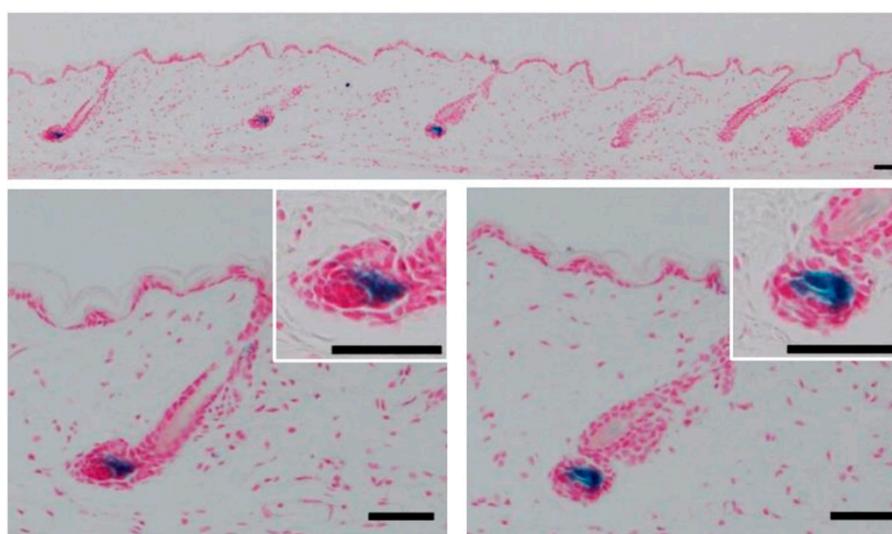
Figure S1. Immunostaining of CD45 and CD117 in the K14<sup>-</sup>/Lef1<sup>+</sup> and K14<sup>-</sup>/Lef1<sup>-</sup> cell populations and AP staining in K14<sup>-</sup>/Lef1<sup>+</sup> cells. AP, alkaline phosphatase. Bar = 50  $\mu$ m.



**Figure S2.** Relative expression of Wnt signaling related genes in the DP in different periods of telogen. PD, postnatal day. \*  $p < 0.05$ .



**Figure S3.** X-gal staining of dorsal skin of TOP-Gal mice injected with R-spondin1-Fc one week after the initial injection with eosin counterstaining. Bar = 50  $\mu\text{m}$ .



**Figure S4.** X-gal staining of dorsal skin of TOP-Gal mice injected with BSA on PD76 with nuclear fast red counterstaining. Bar = 50  $\mu\text{m}$ .

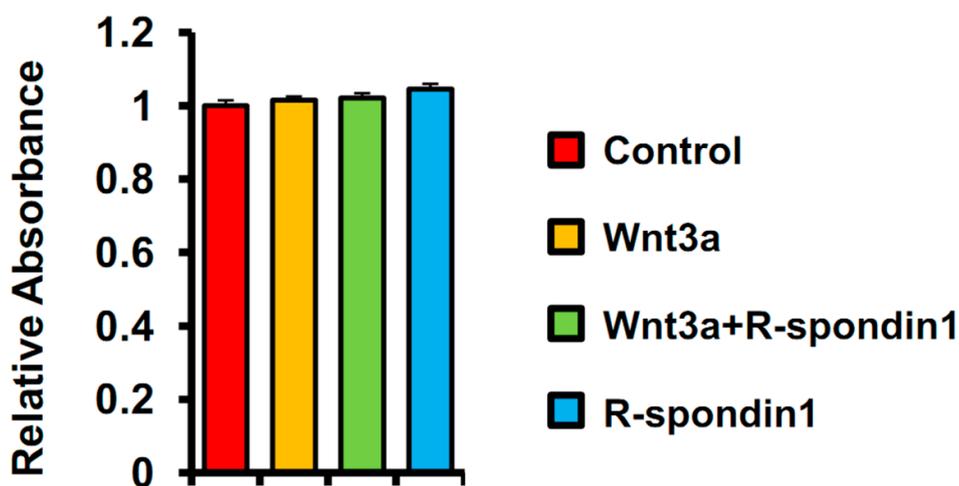


Figure S5. MTS assay of bulge stem cells treated with Wnt3a or/and R-spondin1 for 24 h following an 8 h starvation.

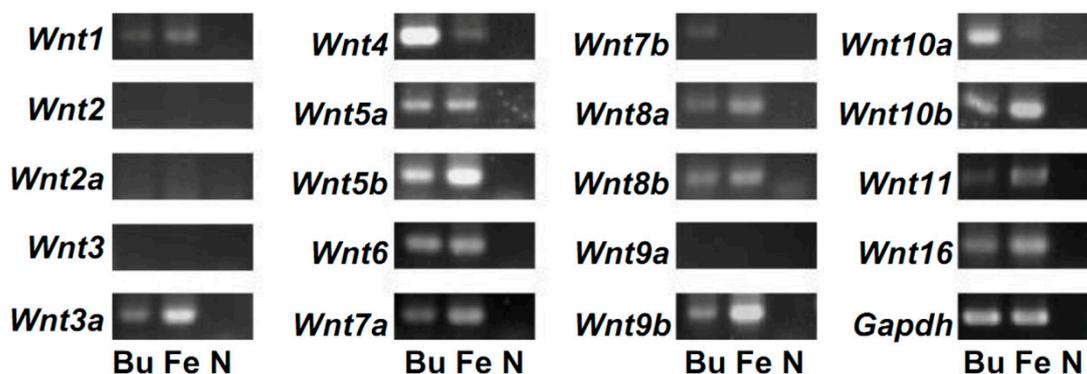


Figure S6. RT-PCR showing the expression of different Wnt ligands in bulge stem cells (Bu) and NIH-3T3 feeder cells (Fe). H<sub>2</sub>O was used instead of cDNA as a negative control (N) and *Gapdh* expression was assessed as an internal control.