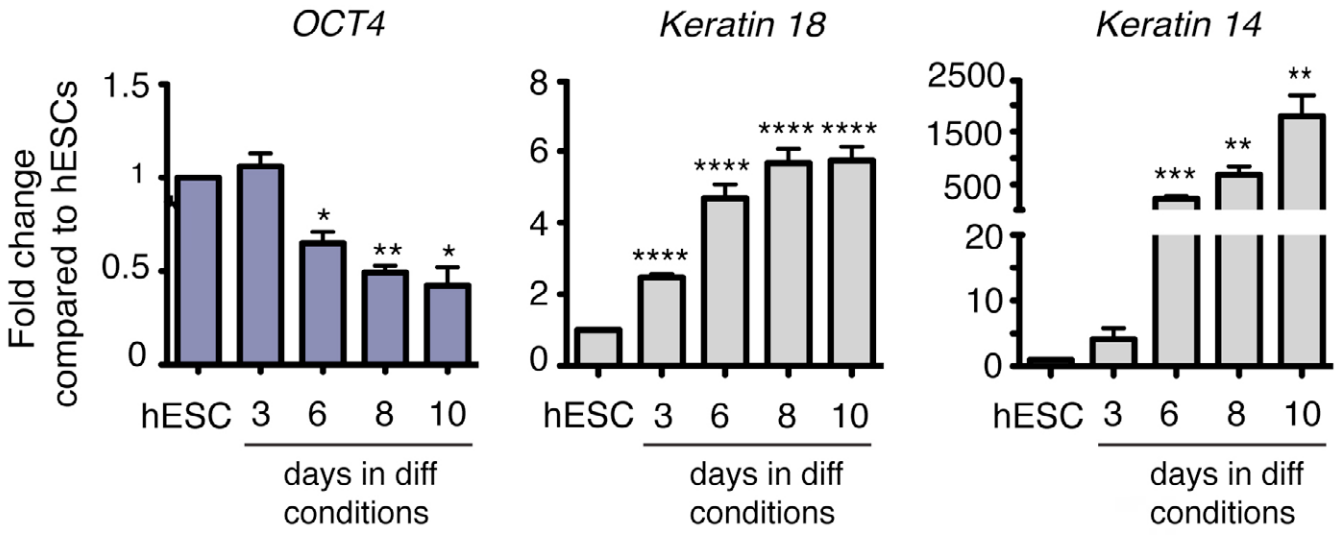

Fig. S1. Upregulation of *K18* and *K14* mRNA levels during ectoderm specification of hESCs. Quantitative real-time PCR analysis of mRNA levels of *OCT4* (*n*=3 independent differentiation experiments for each bar), *K18* and *K14* (*n*=9 independent differentiation experiments for each bar) during hESC ectoderm specification shows a downregulation of the pluripotency marker and an upregulation of keratins.

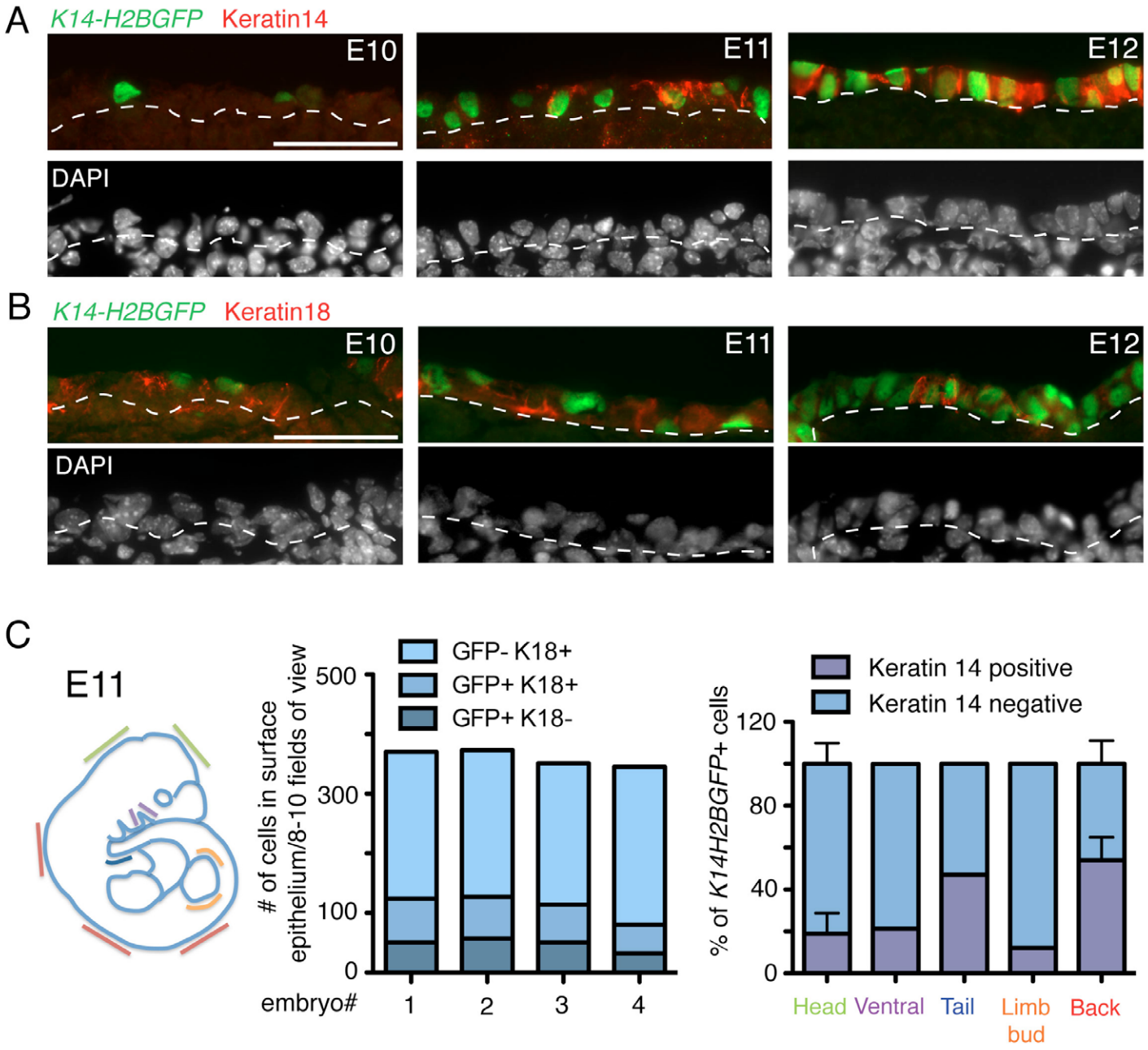

Fig. S2. Keratinocyte specification during embryonic development in *K14-H2BGFP* mice. (**A**) Immunofluorescence analysis of K14 expression during murine epidermal specification in a *K14*H2BGFP reporter mouse. Embryo sections immunostained with an antibody against keratin 14 (red) reveals that the keratin 14 promoter becomes activated (green) in the surface epithelium. (**B**) Immunofluorescence analysis of K18 expression in *K14-H2BGFP* mice during epidermal specification. Sections of *K14H2BGFP* embryos were immunostained with antibodies against keratin 18 (red). K18 expression within the surface epithelium is downregulated from E10 to E12 as keratinocytes are specified. (**C**) Quantification of the total number of surface epithelial cells that are $GFP^{+}K18^{+}$, $GFP^{+}K18^{-}$ or GFP-$K18^{+}$ and percentage of *K14H2BGFP*$^{+}$ cells that are $K14^{+}$ or $K14^{-}$ within the surface epithelium at E11 in several areas of the embryos. *n*=3-4 embryos for each bar. The dotted line indicates surface epithelium boundary. Scale bar: 50 µm.

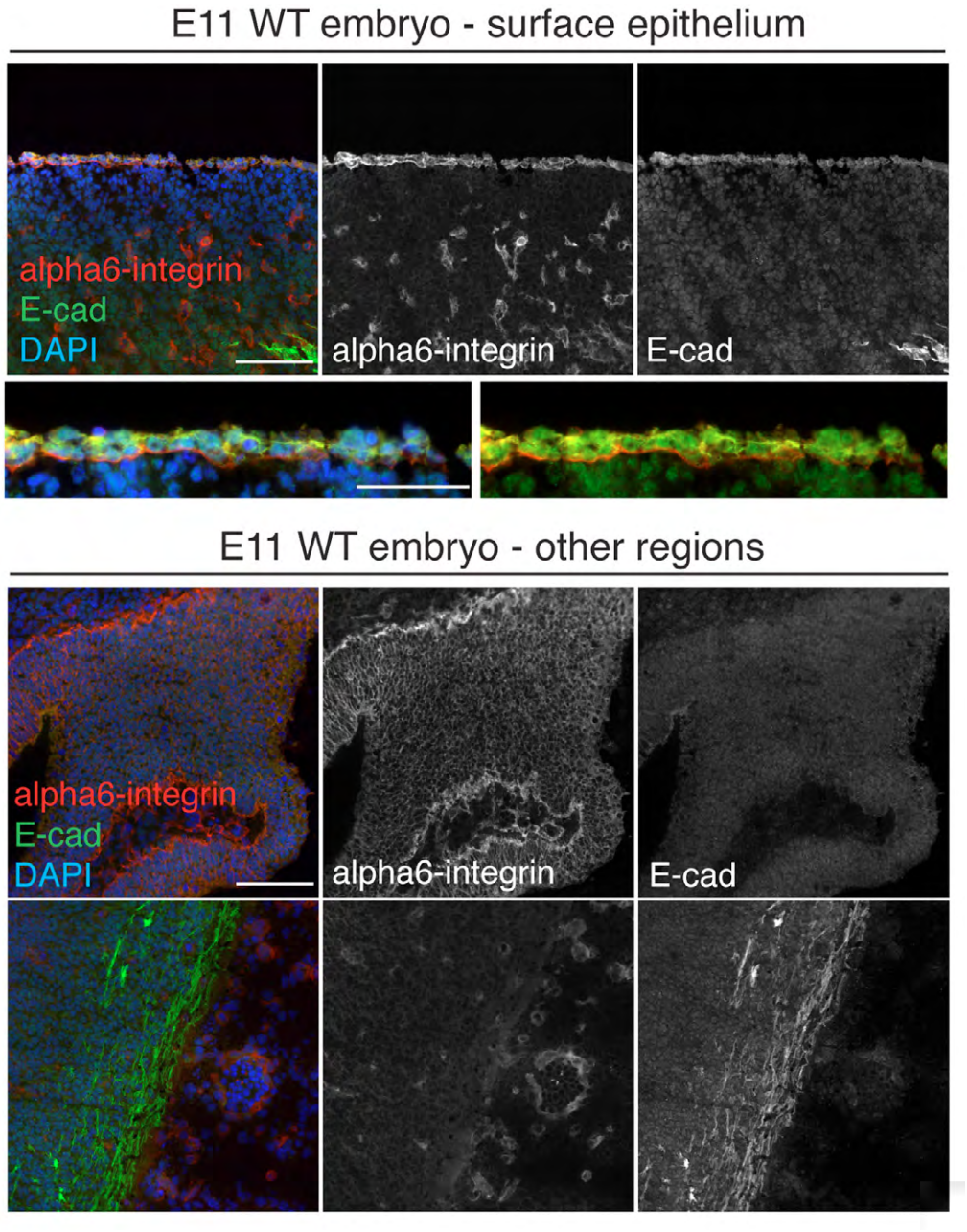

Fig. S3. Characterization of the embryonic murine skin populations isolated by cell sorting. At embryonic day 11 α6 integrin (red) and epidermal cadherin (E-cad, green) colocalize within the surface epithelium but do not colocalize in other areas of the embryo. Scale bar: 100 μm or 50 μm (insets).

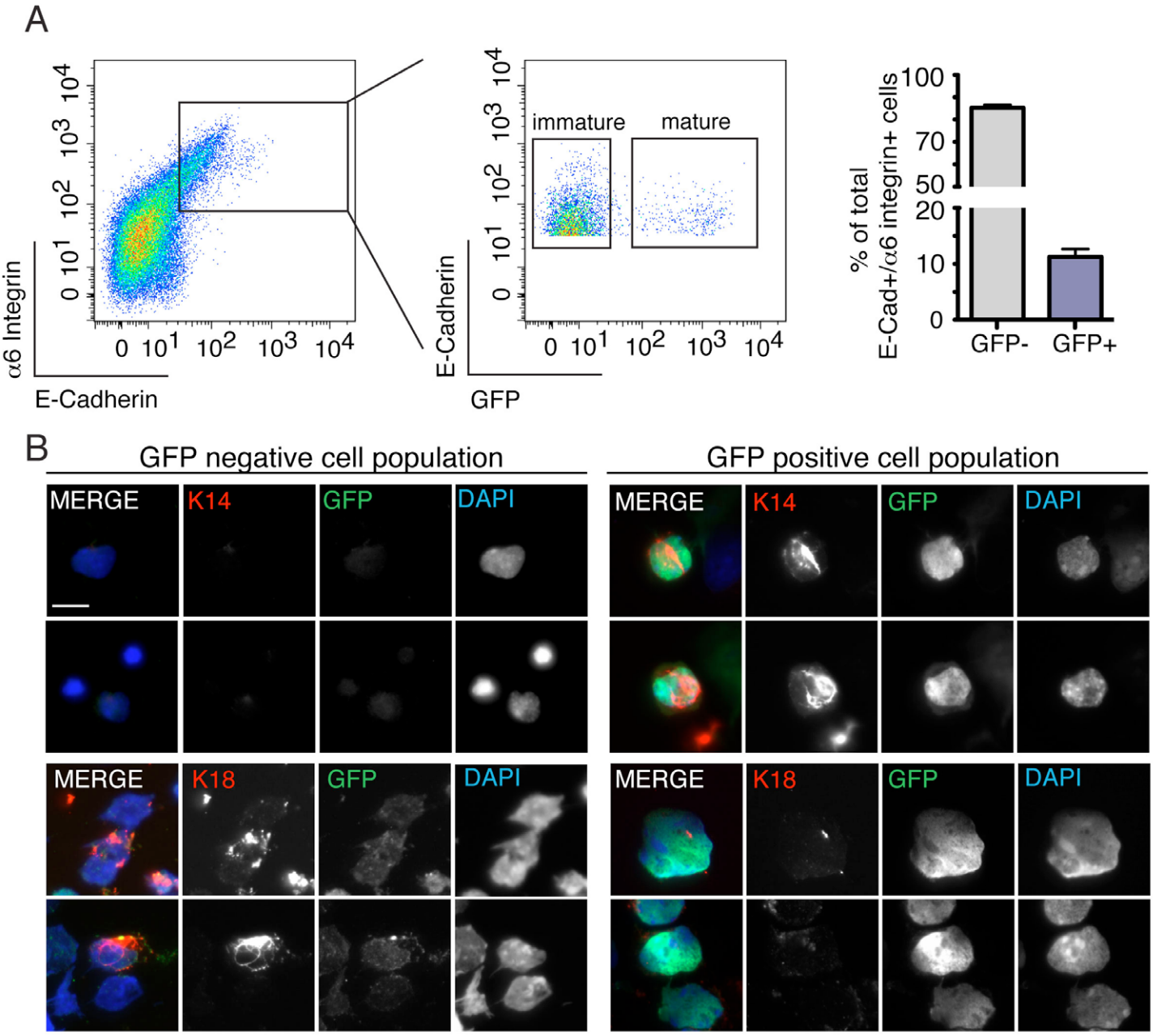

Fig. S4. Characterization of FACS-sorted E11 murine cell populations. (**A**) Dot plots and quantification of dissociated cells from E11 *K14-H2BGFP* embryos stained with antibodies against E-cadherin and α6 integrin (*n*=3 independent FACS-purified cell populations for each bar). (**B**) E11 E-cadherin^{+}, α6 integrin^{+}, GFP^{-} and E11 E-cadherin^{+}, α6 integrin^{+}, GFP^{+} cell populations isolated by cell sorting were characterized by immunofluorescence using antibodies for K14 and K18. Scale bar: 10 μm.

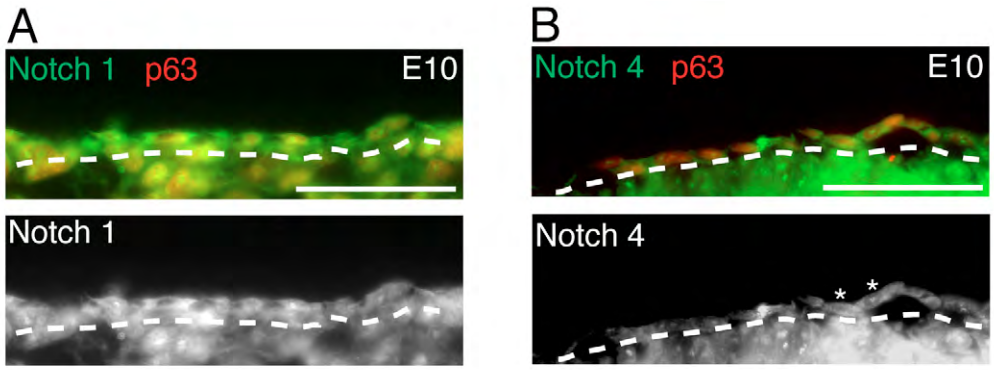

Fig. S5. Cellular localization of the Notch receptors Notch1 and Notch4 in E10 murine embryos. Immunofluorescence analysis of Notch1 (**A**) (green) and Notch4 (**B**) (green) and p63 (red) expression during murine skin development at E10. The dotted line indicates surface epithelium boundary. Scale bars: 50 μm.

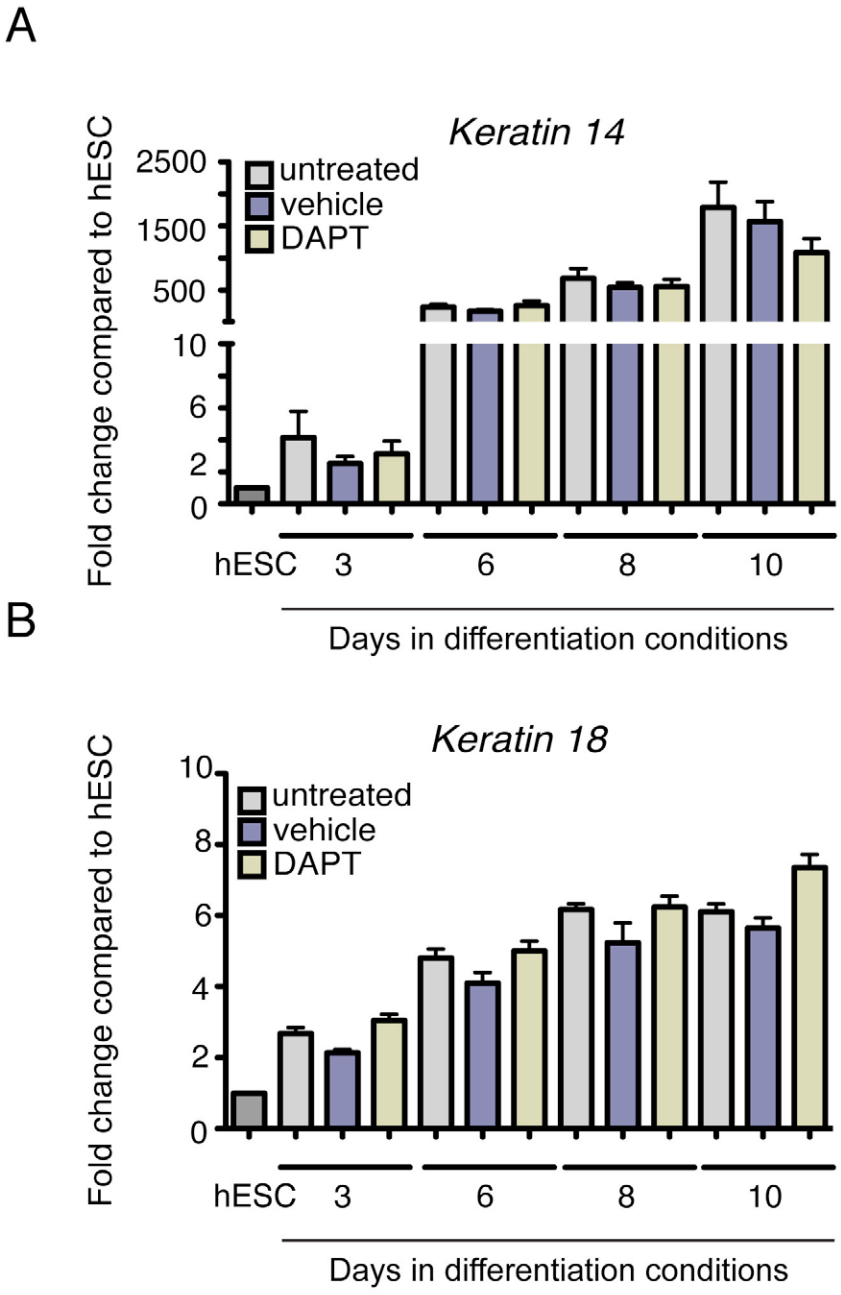

Fig. S6. Notch signaling inhibition does not affect mRNA levels of *K18* or *K14* in differentiated hESCs. Quantitative real-time PCR analysis does not reveal a significant change in mRNA levels for *K14* (**A**) and *K18* (**B**) when hESCs are treated with a Notch signaling inhibitor (DAPT) compared to untreated or vehicle-treated cells throughout the ectoderm differentiation protocol (n=9 independent sorting experiments for each graph bar for *K14* and n=6 independent sorting experiments for each graph bar for *K18*).

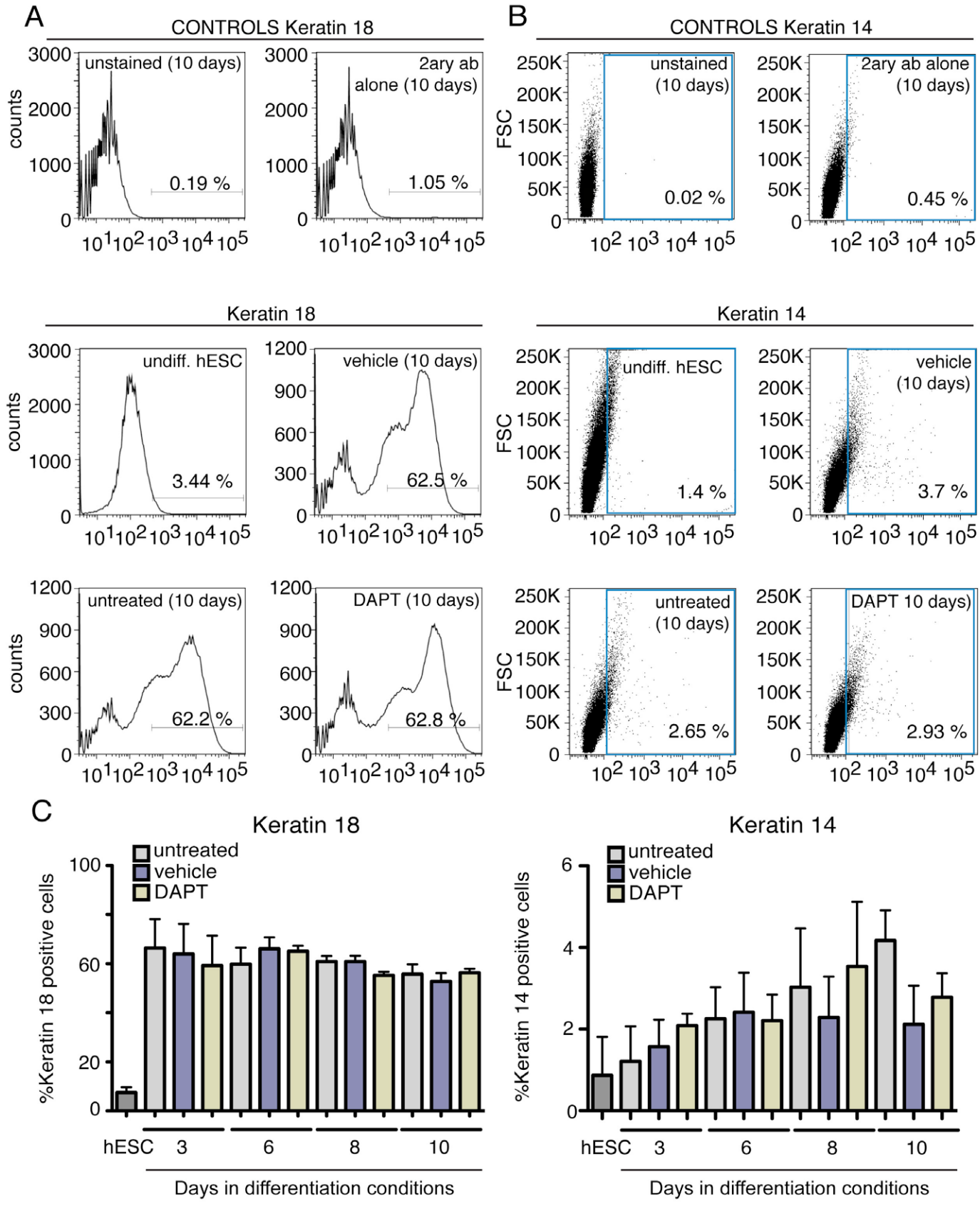

Fig. S7. Notch signaling inhibition does not affect the percentage of K14$^+$ and K18$^+$ cells after differentiation. (**A**) FACS analysis of hESCs reveals that upon differentiation there is a significant increase in the percentage of K18$^+$ cells, but this percentage is not affected when Notch signaling is inhibited by DAPT. (**B**) Similarly, there is a small increase in the levels of K14 at the end of the differentiation protocol, but this change is not affected by DAPT treatment throughout the differentiation protocol. Percentages of K18 or K14 are indicated on each plot. (**C**) Quantification of K14 and K18 FACS results shows that there are no significant changes in DAPT-treated cells when compared with untreated or vehicle treated cells throughout the entire differentiation protocol. All data are ± s.e.m. (*n*=3 independent differentiation experiments for each bar).

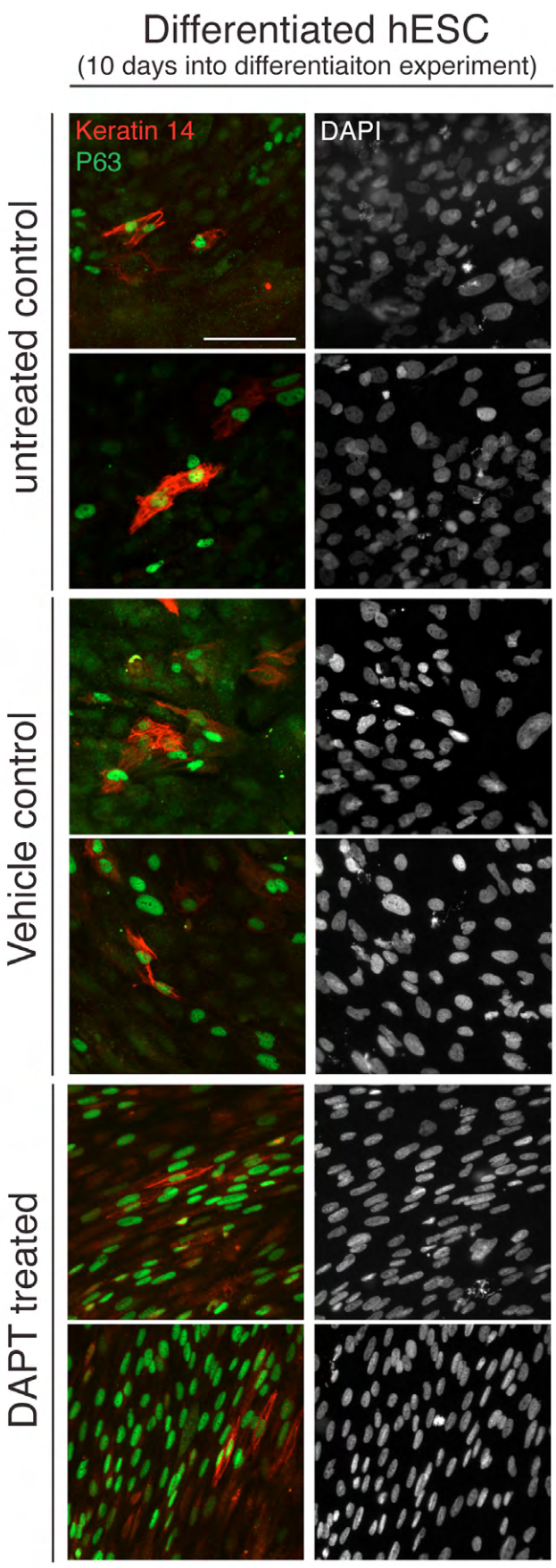

Fig. S8. Notch inhibition does not affect the numbers of Keratin 14 and P63 double positive cells after differentiation. Immunofluorescence analysis of differentiated hESCs using antibodies for K14 (red) and P63 (green) reveals that Notch signaling inhibition by DAPT does not affect K14/P63 double positive cells when compared to untreated or vehicle-treated cells. Scale bar: 100 μm.

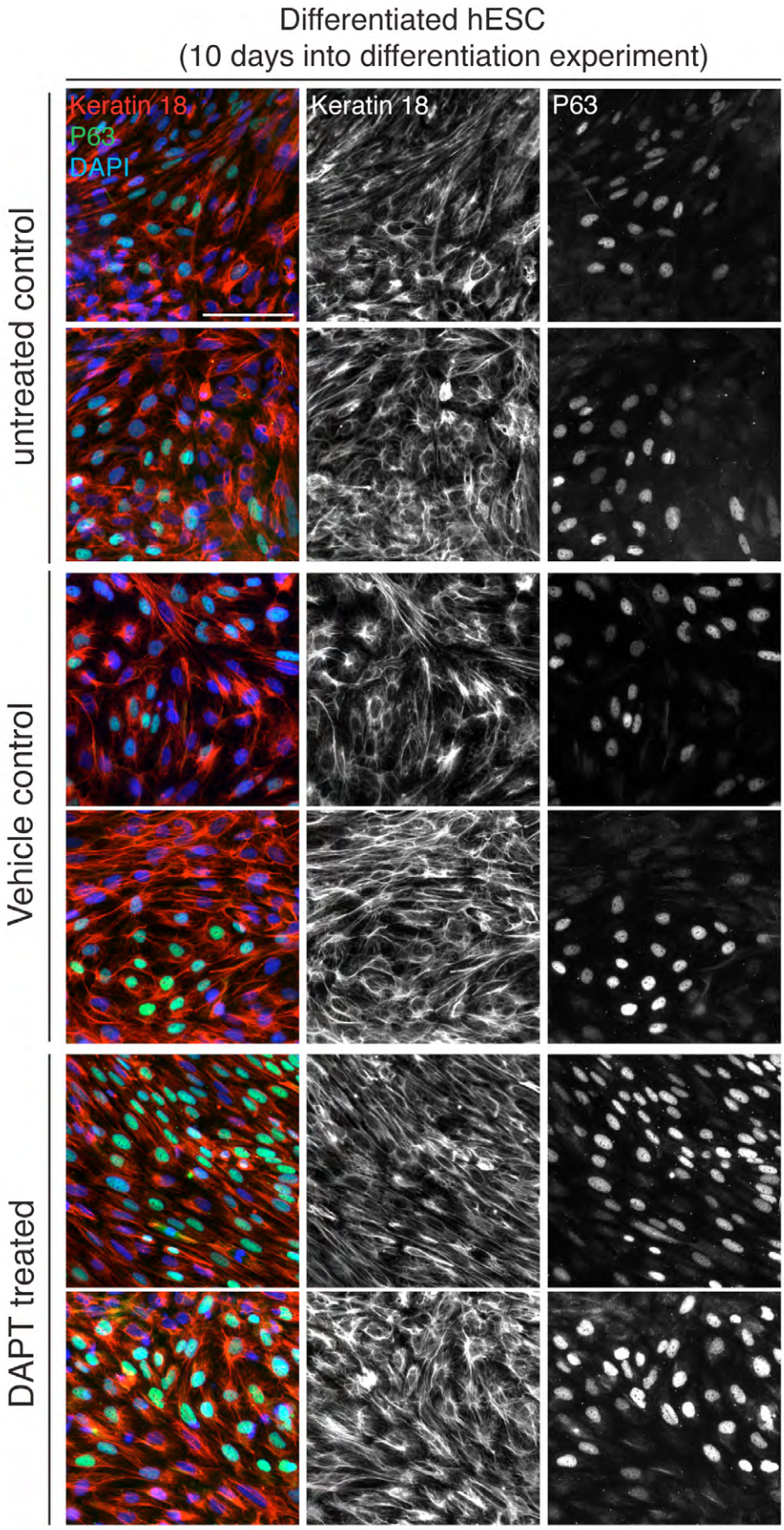

Fig. S9. P63 is expressed in K18^{+} ectoderm cells in the presence and absence of Notch signaling. Immunofluorescence analysis of differentiated hESCs using antibodies for keratin 18 (red) and P63 (green) reveals that P63^{+} keratinocyte progenitors express keratin 18 and that this expression pattern is not affected when cells are treated with the Notch signaling inhibitor DAPT throughout the differentiation protocol. Scale bar: 100 μm.

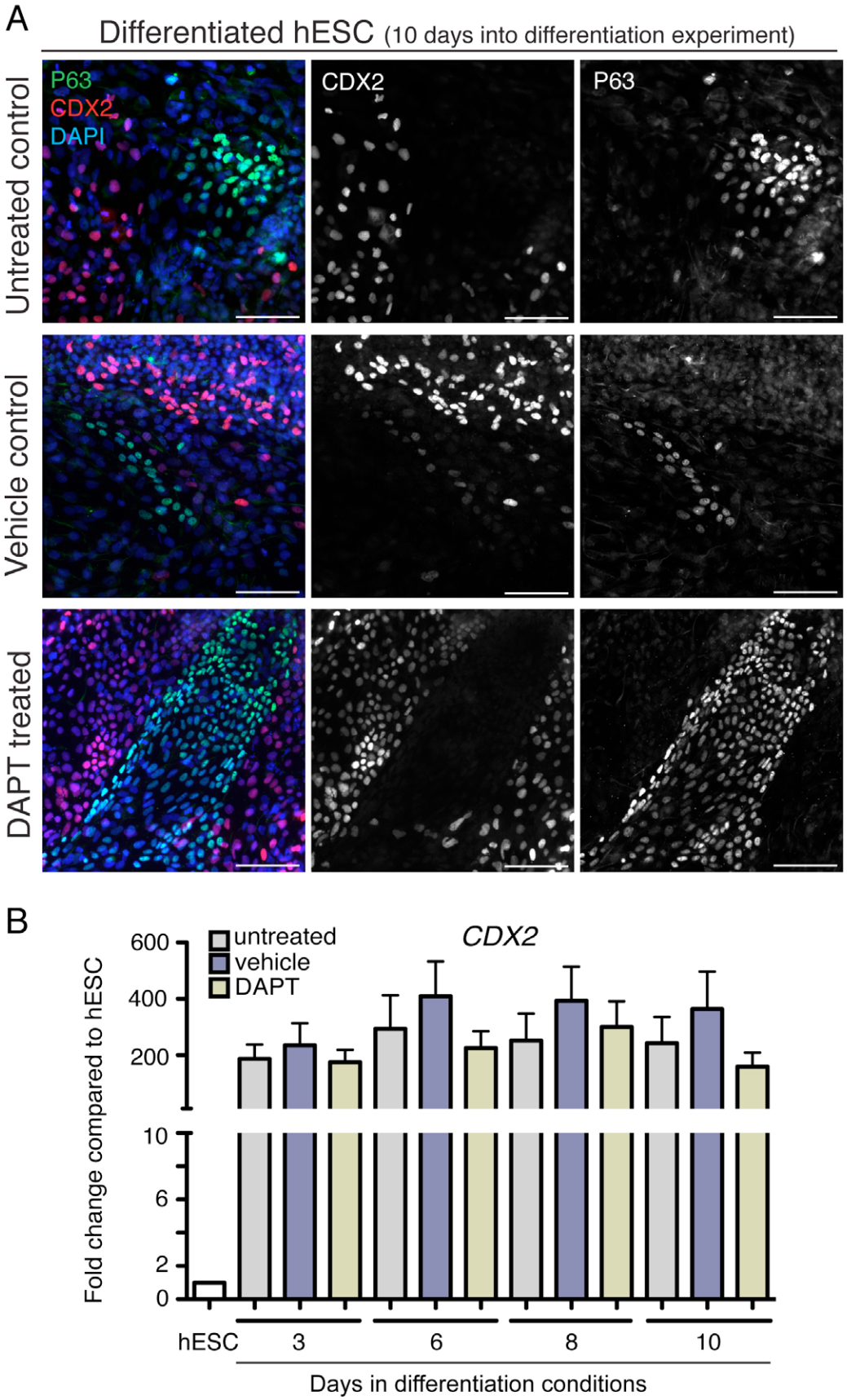

Fig. S10. Notch signaling inhibition does not alter trophoblast formation during hESC differentiation. (**A**) Treatment of hESCs throughout the entire differentiation protocol with the γ-secretase inhibitor DAPT leads to an increase in the numbers of P63-positive cells (green) that are CDX2 (red) negative. (**B**) Quantitative real-time PCR reveals that the global levels of *Cdx2* mRNA do not significantly change when Notch signaling is inhibited by DAPT compared to untreated or vehicle-treated control experiments. All data are ± s.e.m. (*n*=9 independent differentiation experiments for each graph bar). Scale bar: 100 μm.

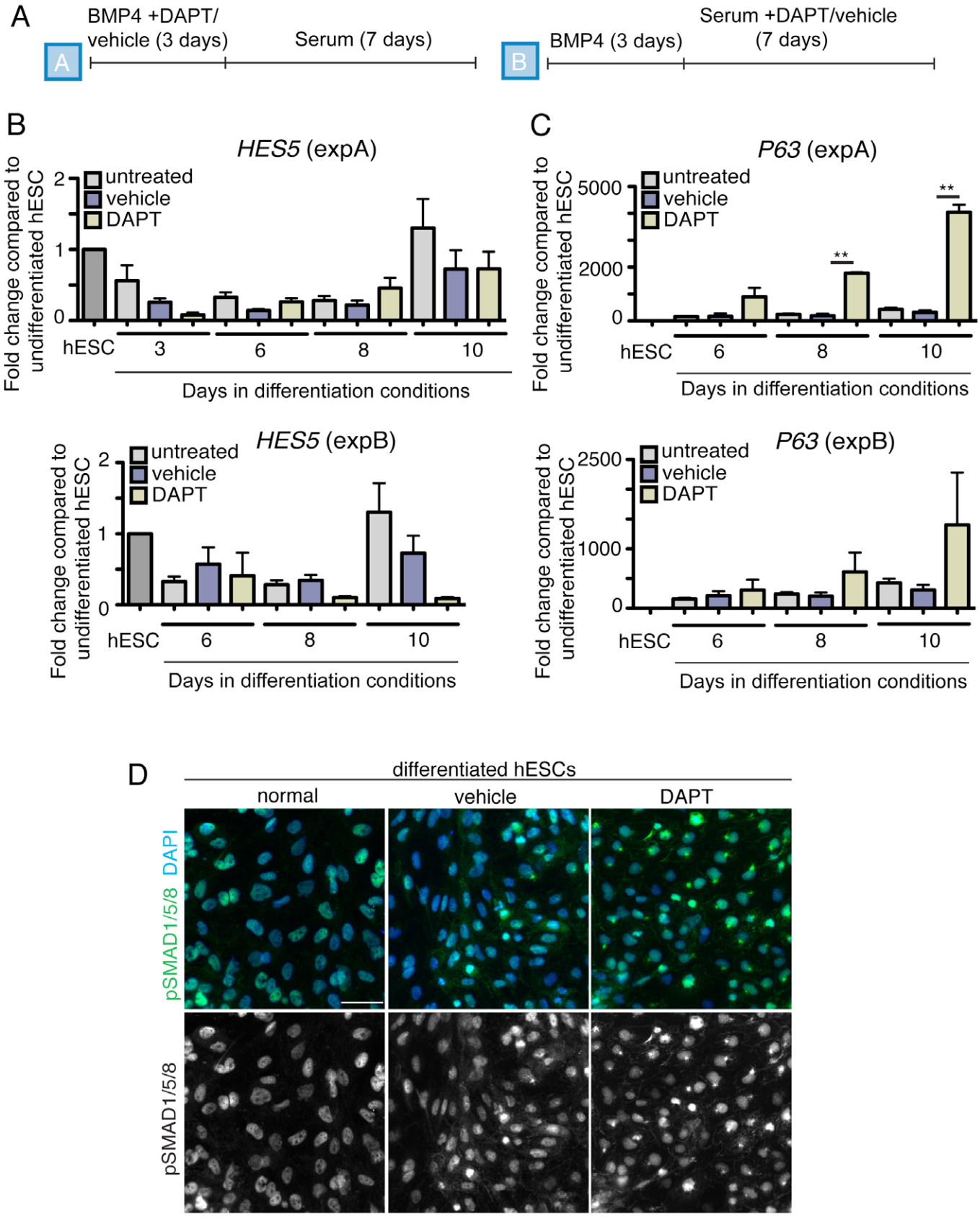

Fig. S11. Inhibition of Notch signaling during BMP treatment is required to increase P63 expression in hESCs. (**A**) hESCs were treated with vehicle or DAPT either during the BMP4 treatment (first 3 days) or after BMP4 treatment (days 4-10). (**B**,**C**) Quantitative real-time PCR analysis of mRNA levels of *HES5* and *P63* shows that inhibition of Notch signaling during BMP4 incubation was sufficient to induce an increase in the levels of P63 mRNA. All data are ± s.e.m. (**0.001<*P*<0.01) (*n*=3 independent differentiation experiments for each bar). (**D**) Immunofluorescence analysis of pSMAD1/5/8 (green) expression in ectoderm specified hESCs reveals that inactivation of Notch signaling with DAPT does not affect the levels of pSMAD1/5/8. Scale bars: 50 µm.

Table S1. Mouse (*Mus musculus*) primers used for quantitative real-time PCR analysis

Gene	Fw primer	Rev primer
keratin 18	GACGCTGAGACCACACT	TCCATCTGTGCCTTGTAT
keratin 14	AGGGAGAGGACGCCCACCTT	CCTTGGTGCGGATCTGGCGG
Trp63	ACGCCCCGCCTCTTTGCAAAT	TGAGCTGGGGTTTCTATGAAACGCT
Eya4	ACAGCTGTACCCCTCCAAGCCC	TAGACGGCCGGCTGCTGCAT
Gata2	CCGCCTCCAGCTTCACCCCTA	TGCACAGGTAGTGGCCCGTG
Irf8	GCAACGCGGTGGTGTGCAAG	ACAGCTGCTCTACCTGCACCAGA
Gbx2	GCAAGTTCGCTCCACAGCCAC	AGCTCTCCTCCTTGCCCTTCGG
Six1	GGCCAAGGAAAGGGAGAACACCG	TGAGCTGGACATGAGCGGCTTG
Notch1	GGCTGCACAGAAGCGAGGCAT	CTGCCCGTGTAGCCTGCCTG
Notch2	TTCGTGTCCCCCAGGCACCC	AATCCGGTCCACGCACTGGC
Notch3	GCACCCCCTTGTCTGGATGGA	GTGCCCGCCACCACTGAACTC
Notch4	ACCTGTGTGCCTCAGCCCAGT	GGGCTGGGACTGACAAGCGTC
Jag1	TGGACTGGCCCCACGTGTTC	GGGCGGGCACACACACTTGAA
Jag2	ACCCGGGCCTCGTCGTCAT	TGCAGGCTCTTCCAGCGGTC
Dll1	CGGGCCAGGGGAGCTACACA	AGCTGTCCTCAAGGTCCGTGC
Dll3	TGCCCTTCCGCGATGCTTGG	CTCCCATGTGCCTGTGCGCT
Dll4	CAGCATCCCCTGGCAGTGTGC	GCTGGCACACTTGCTGAGTCCC
Dlk1	CCCCCTGCGCCAACAATGGA	CCGTGCTGGCAGGGAGAACCAT
Dlk2	CCTGCCAGAGCGGATGACTGC	CTCACAGTGCAGCCCCTCCCA
Dner	GCCCAGCTGGTGGACTTCTGC	GGCCATGGTAACCTGGATCGC
Hey1	GCGCCGACGAGACCGAATCAA	CAGGGCGTGCGCGTCAAAAT
Hes5	GCTCCGCTCGCTAATCGCCT	CCGGCTTCCGCAGTCGGTTTTT

Table S2. Human (*Homo sapiens*) primers used for quantitative real-time PCR analysis

Gene	Fw primer	Rev primer
keratin 18	TGAGACGTACAGTCCAGTCCTT	GCTCCATCTGTAGGGCGTAG
keratin 14	TCAGCATGAAAGCATCCCTGGAGAA	ATTTGGCGGCTGGAGGAGGTCA
TRP63	AGCCAGAAGAAAGGACAGCAGCATT	CTGTGCGGGCCTGGGTAGTC
OCT4	CCCCTGGTGCCGTGAAGCTG	CCCCAGGGTGAGCCCCACAT
CDX2	CGGCGGAACCTGTGCGAGT	TGGCGGCTAGCTCGGCTTT
SOX1	TCTATGCTCCAGGCCCTCTCCTCG	GGACCACACCATGAAGGCGTTCA
FOXA2	GAGCAGCAGCGGGCGAGTTA	CCCAGGCCGGCGTTCATGTT
NOTCH1	CTACGTGTGCACCTGCCGGG	CGTTTCTGCAGGGGCTGGGG
NOTCH2	GCACTCGGGGCCTACTCTGTGAAGA	AGGGGTTGGAGAGGCACTCGT
NOTCH3	GTGGACGAGTGTGCTGGCCC	CGGCGAAACCAGGGAGGCAG
NOTCH4	TCCCCAGCTCTCCCTCTCCATTG	CAGAAGTCCCGAAGCTGGCACT
JAG1	TGCGAGCCAAGGTGTGTGGG	CGTGGACCCTGAGCCGAAGC
JAG2	ATCAACGTCAACGACTGTCGCGGG	TATAGCAGCGAGCGCCGTTCC
DLL1	GCAGCCCTGGCAGTGCAACT	CGAGATCCGTGCAGCTCCCT
DLL3	ATCTACGCTCGGGAGGCCTGAC	AGACTGGGCACCACCGAGCAA
DLL4	ACCTTGAGCTGCGCCGACTC	CACTGTCCCCCGTTGGCACA
DLK1	ACCTGCGTGAGCCTGGACGA	GCAGGGGGAGCCGTTGATCAC
DLK2	GAGGTGTCCACGCGTCCGGC	CGCTCACAGTGCAGCCCCTC
DNER	GGGATCTCCGGCGCCAACTG	AAGCTGTCGGGGTGCCATGG
HES1	ATGACGGCTGCGCTGAGCAC	TAACGCCCTCGCACGTGGAC
HES5	CCGGTGGTGGAGAAGATG	GACAGCCATCTCCAGGATGT
HEY1	TGAGCTGAGAAGGCTGGTACCCA	TGCGCGTCAAAGTAACCTTTCCC