Appendix

Table A1

PCR primer sequences (5’ to 3’) for Figure A3.

DUSP	Forward	Reverse
1	CGTCCCTGACAGCGCGGAAT	GCCGAACAGTGCGGAGCCAG
2	TCCTGTCTACGACCAGGGTGGC	GGGGAGCCCACCAGTCCACA
4	AAGCCGAGGAGAAAACTCTGGCTG	AACCGGGGGTGGGATGGCT
5	TACTCGCTTGCCTACCCGCC	CGTTGCCACAGGGCTTCTGCT
6	TCCAAGCCGAGTTCTCCCTGCAT	GCTGCTGAGTCCCAGCGTCCT
7	GTGCCACCGAGTCAGACGGC	GCTGCTTAGCCCCAGCGTCC
9	CGGAGAACCGAGCAGAGCGGA	GGGCACTCGGCCTGGAATCT
14	CGGAGAACCGAGCAGAGCGGA	GGGCACTCGGCCTGGAATCT
26	CGACTCGGGCCACCGAATGT	GCTCCCGGCGGTTGTTAGCC

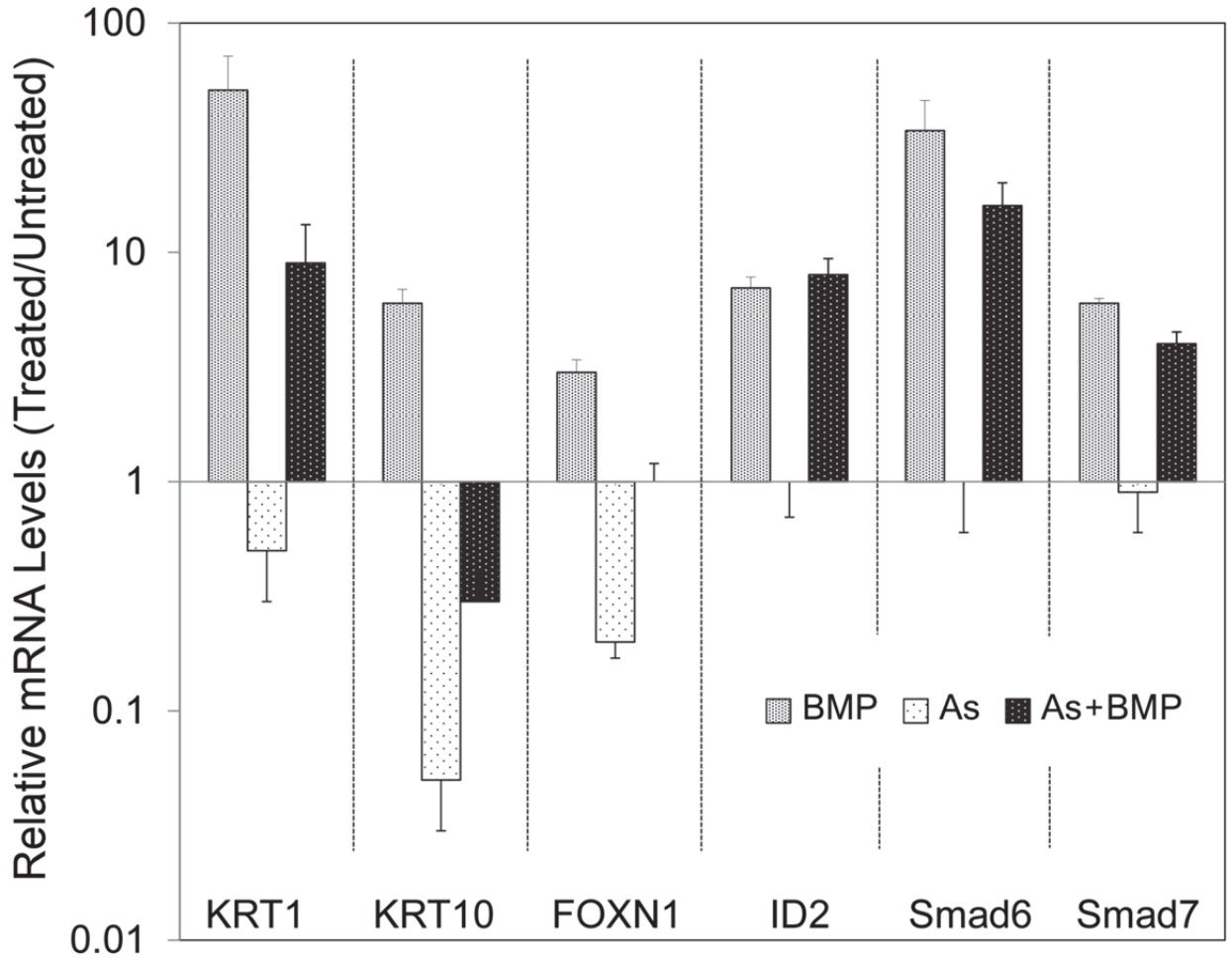

Fig. A1. Arsenite suppression of BMP signaling. Confluent SIK cultures, untreated or treated with BMP6 or arsenite (As) or the combination for 3 days, were harvested and analyzed by real time PCR for the indicated mRNAs. The results are illustrated as the ratio of treated to untreated. The data were used to generate Fig 2A.

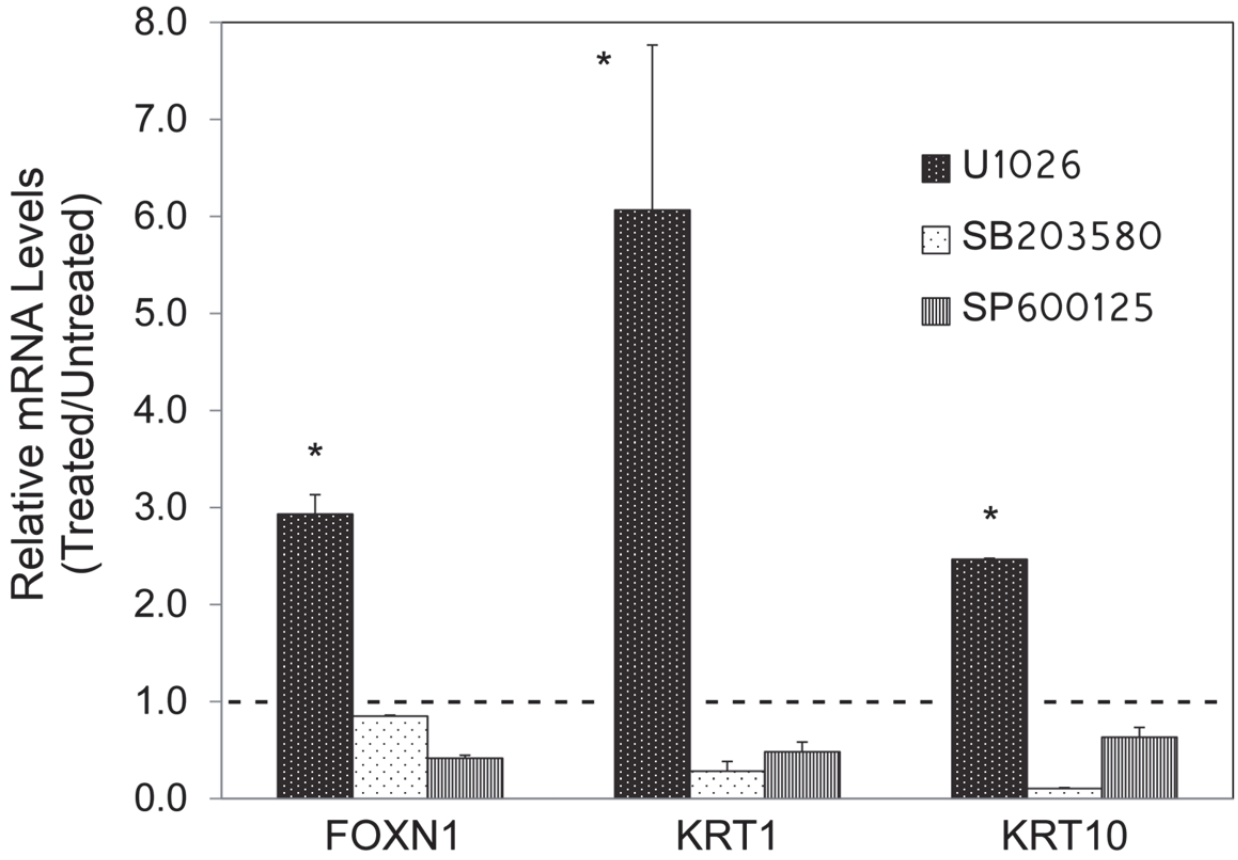

Fig. A2. Effects of MAP kinase inhibitors on keratinocyte differentiation. Confluent SIK cultures were treated with the ERK kinase (MEK1/2) inhibitor U1026, the p38 inhibitor SB203580 or the JNK inhibitor SP600125 in the absence of BMP6 for 3 days. Resulting mRNA levels are shown relative to the untreated control, set at 1. Asterisks indicate significantly different from untreated control as determined by t-test with Bonferroni correction.

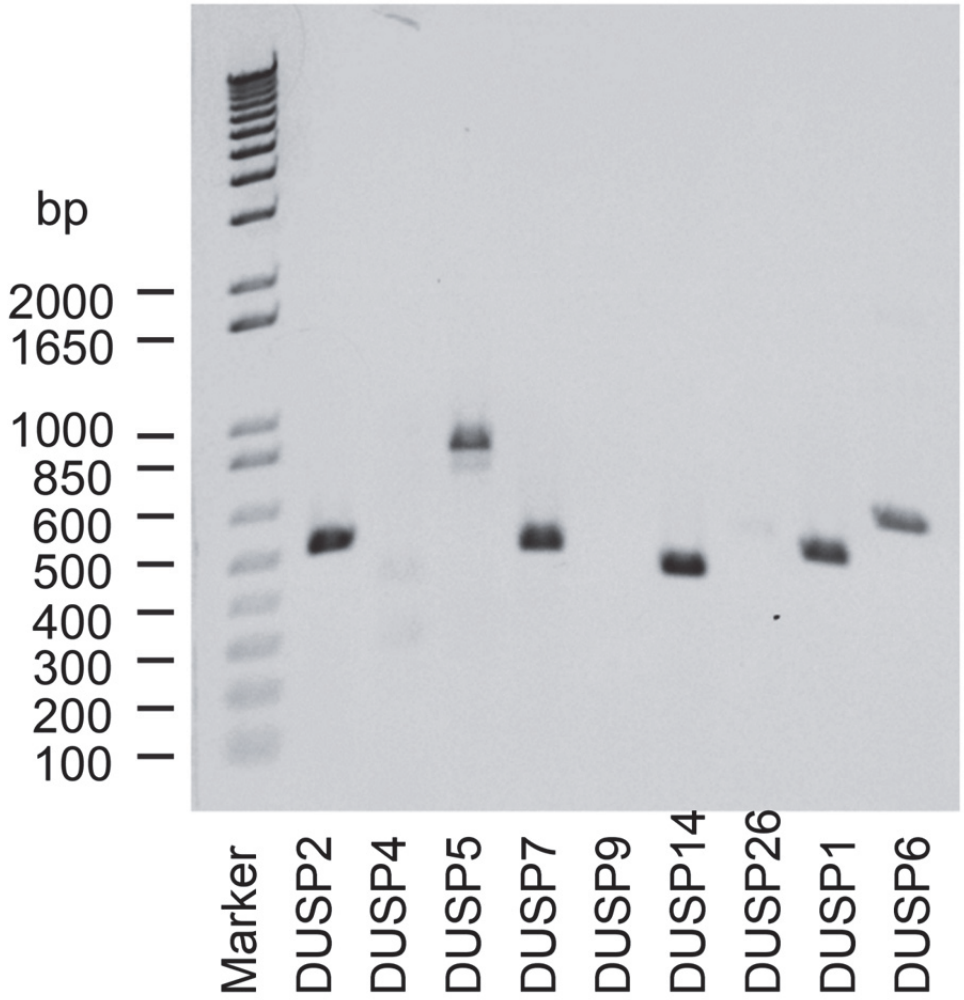

Fig. A3. Expression of DUSP mRNAs in cultured keratinocytes. For experiments to screen for the presence or absence of DUSPs, standard PCR was performed using Phusion High-Fidelity DNA Polymerase (New England Biolabs, Ipswich, MA) according to the manufacturer's protocol with analysis by agarose gel electrophoresis. mRNA prepared from untreated cultures 3 days after confluence was transcribed into cDNA and amplified by PCR with primers for the indicated DUSPs. Ethidium bromide stained gels were viewed using an LAS3000 imaging system (GE Healthcare, Piscataway, NJ). Numbers on the left indicate the sizes in base pairs (bp) of the marker DNA bands. Primer sequences are given in Table A1 (Appendix).

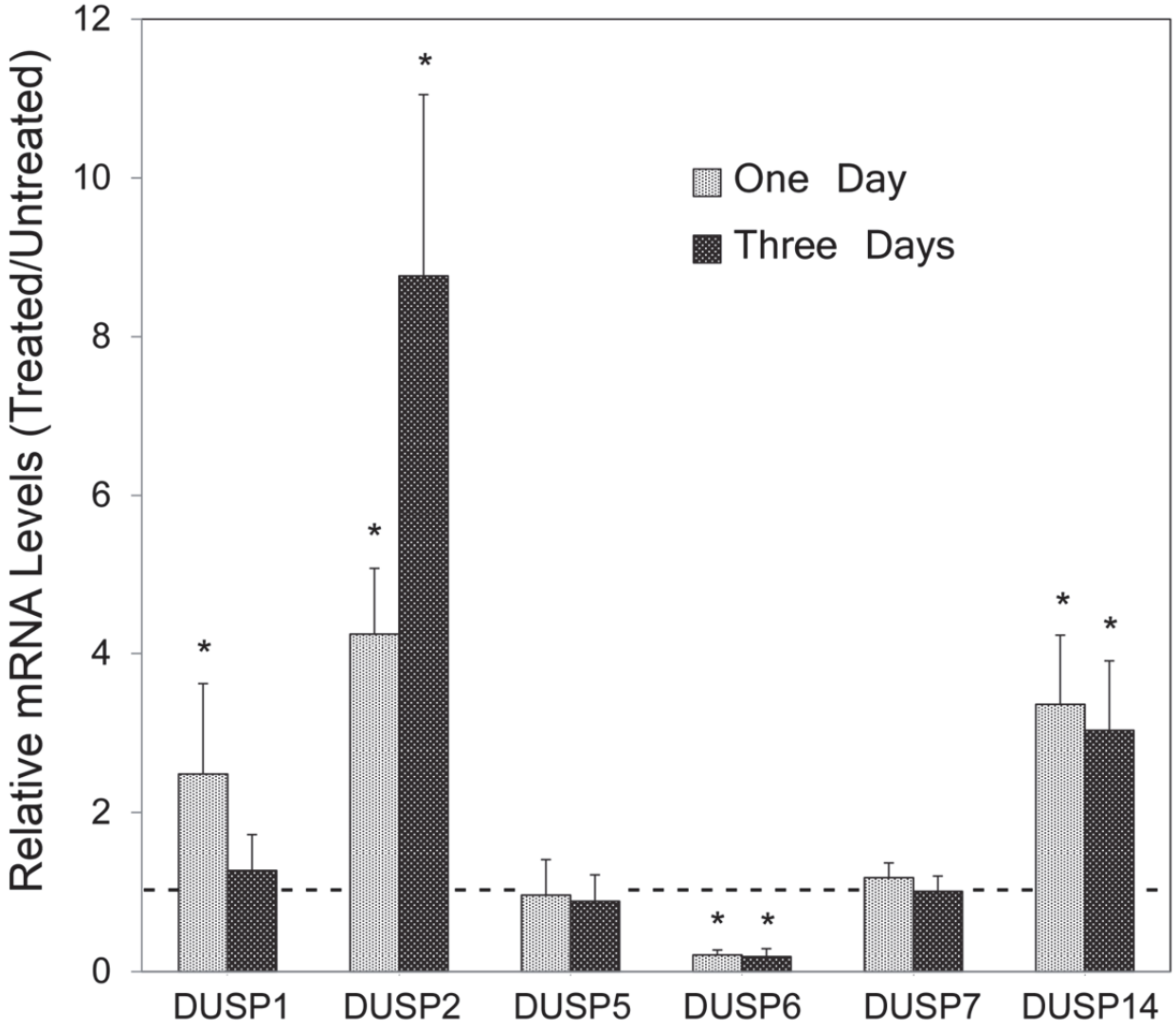

Fig. A4. BMP induction of DUSP2 and DUSP14. Confluent cultures were treated with BMP6 for 1 or 3 days as indicated and analyzed for mRNA levels for the various DUSPs. Asterisks indicate values significantly different from the untreated samples, set at 1.

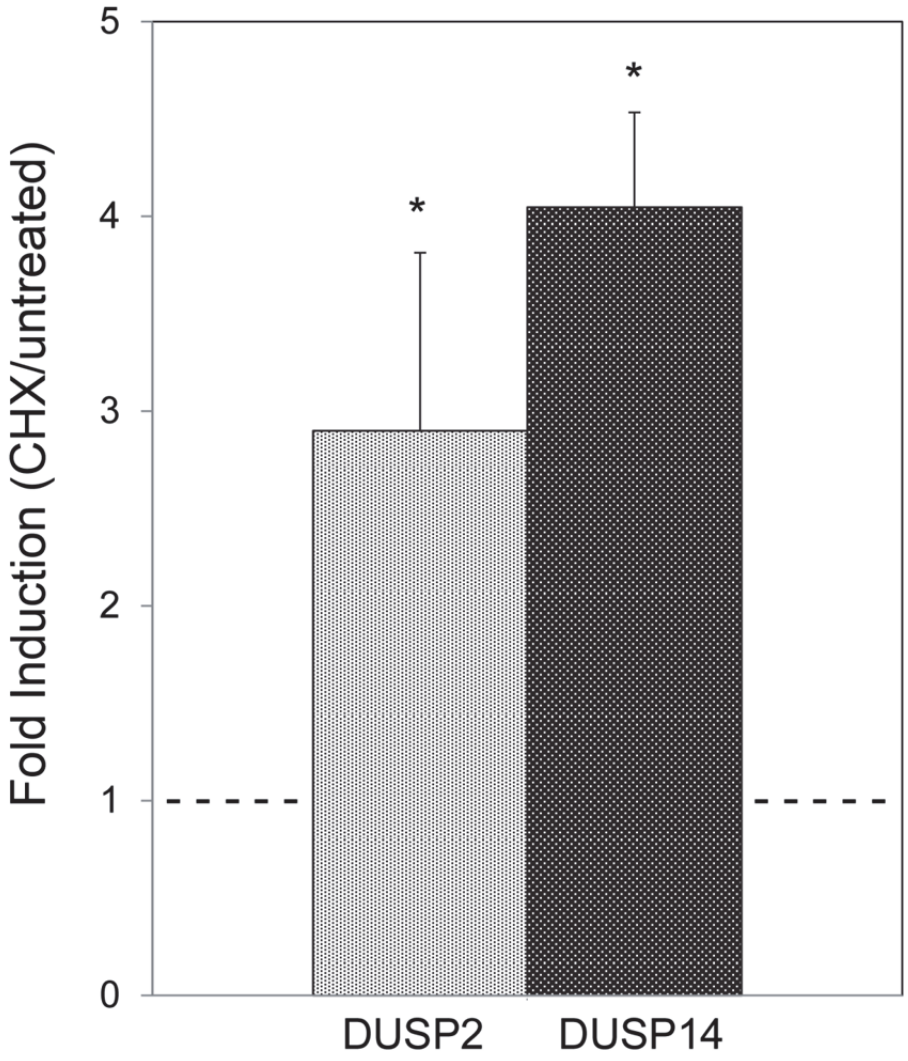

Fig. A5. Cycloheximide induction of DUSP2 and DUSP14. Cultures were treated for 6 h with cycloheximide (CHX, 3 μg/ml) and analyzed for relative levels of DUSP2 and DUPS14 mRNA by real time PCR in comparison to untreated cultures. DUSP14 remained elevated for at least 24 hr (induction 19.7 $\pm$ 4.9 fold), while DUSP2 induction was variable at that time point (not shown). Asterisks indicate the values were significantly different from untreated, set to 1.

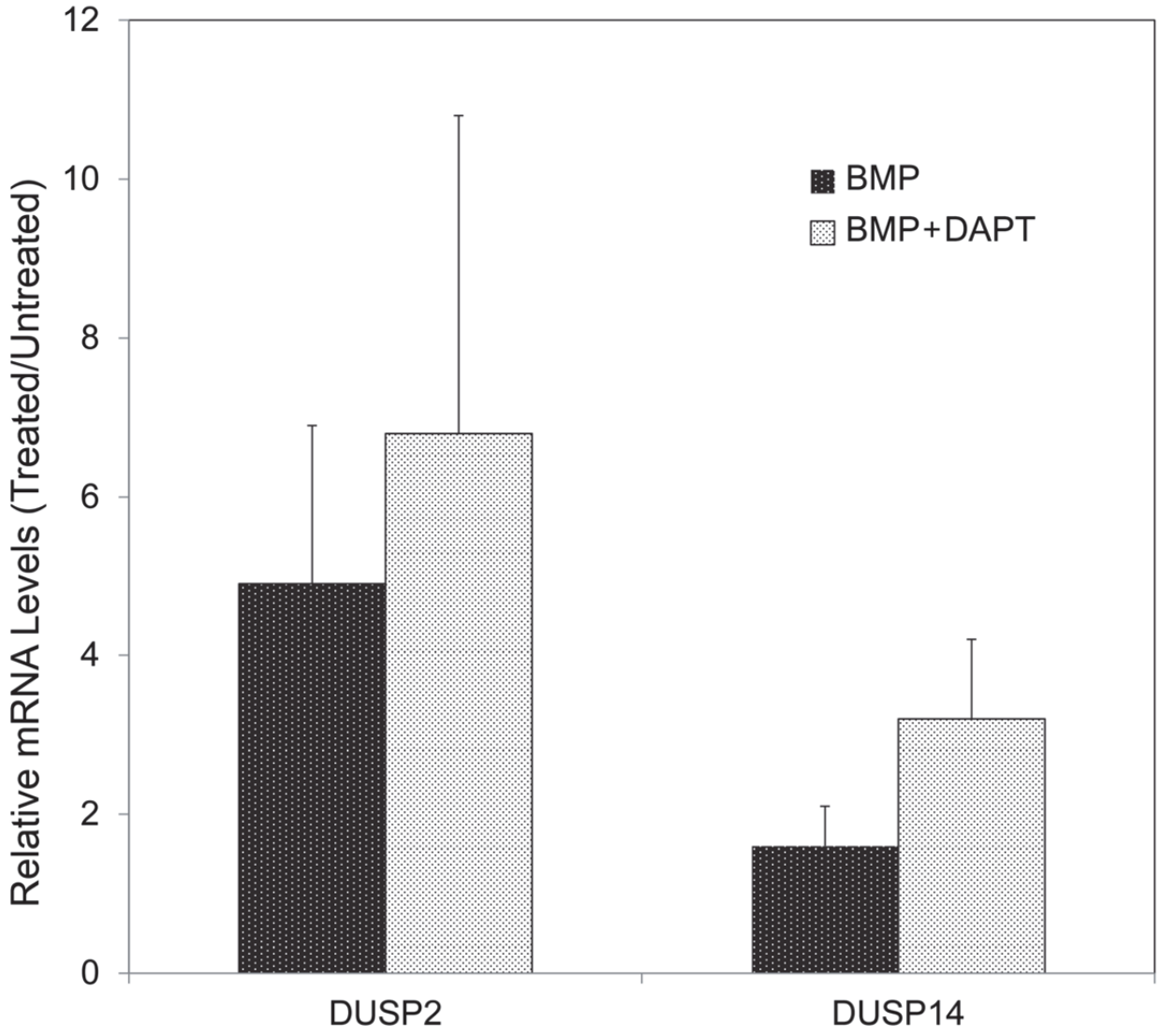

Fig. A6. No suppression of BMP induction of DUSP2 or DUSP14 by inhibition of Notch1 signaling. Cultures were treated with BMP with or without DAPT as indicated for 3 days. Relative mRNA levels are given as ratios to untreated cultures, normalized to 1.

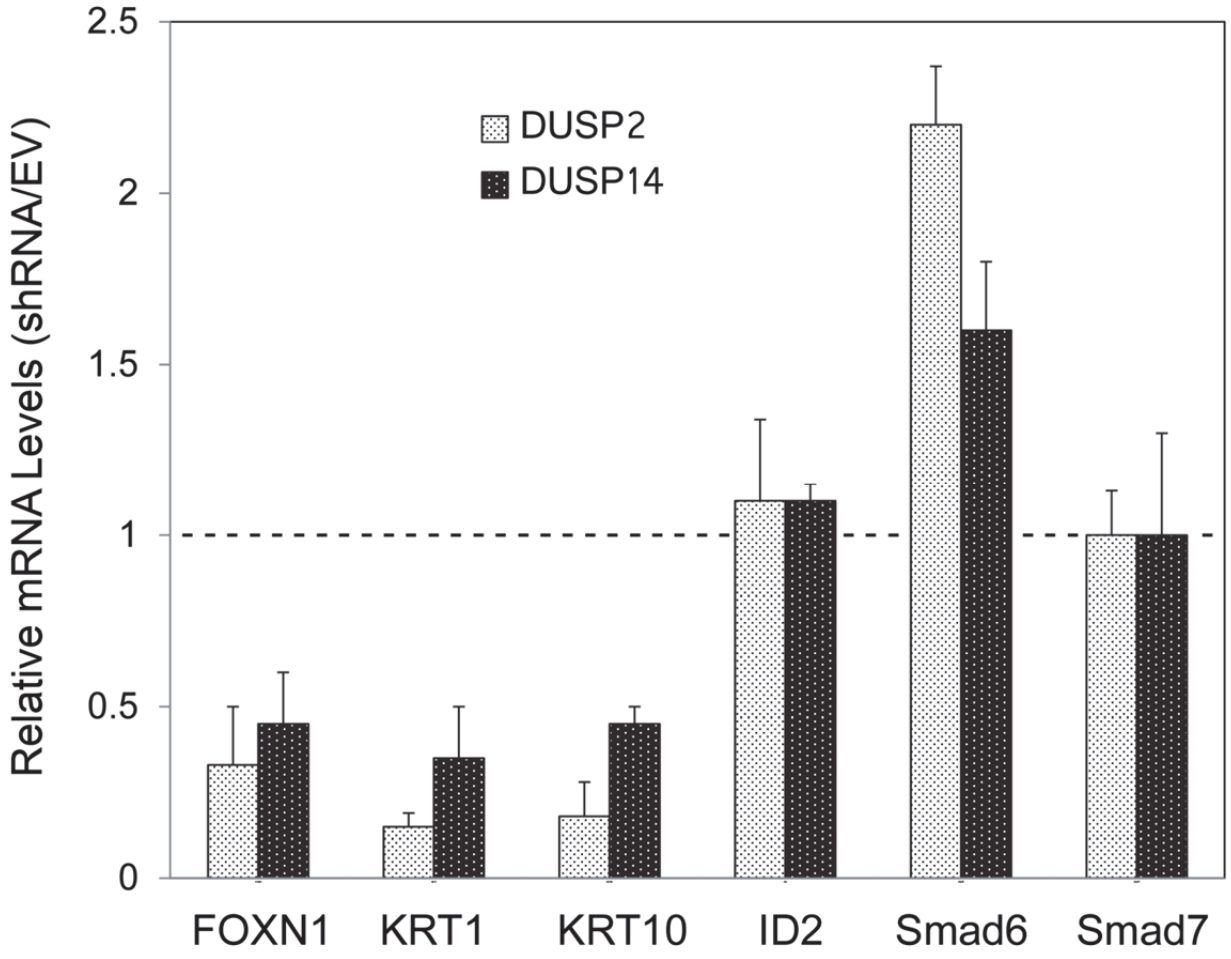

Fig. A7. Suppression of KRT1, KRT10 and FOXN1 expression by DUSP knockdown without added BMP. Other conditions as in Fig 9.