

b





Chi-sq (yates) p-value < 0.0001

Illumina probes

| | MITF occupied | Not occupied | Total |
|---------------------------|------------------|-----------------|-------|
| sig changed with siCITED1 | 128 | 184 | 312 |
| not signif | 8144 | 38876 | 47020 |
| Total | 8272 | 39060 | 47332 |





b

A2058 % change siCITED1





HT144 % change siCITED1

С









TMEGC etc.

Supplementary Figure Legends S1-5

S1: A scatter plot of CITED1 expression versus MITF expression in our in-house cell lines subjected to gene expression analysis in agreement with the observation in the public data set (see Fig 2a).

S2: (a) Of the 5578 unique occupied genes (those associated with genomic regions bound by MITF) identified by Strub et al., 5169 could be found in the Illumina HT12 gene set, mapping to 8272 probes by searching on gene symbol ¹. Of the 312 probes significantly changed by siCITED1, 41% or 128 are found in this list. (b) A Venn diagram indicating the number of genes significantly up or down regulated by siCITED1 represented in the MITF occupied gene list.

S3: (a) Western blot showing the effect of silencing MITF using two siRNAs (siM1, siM3) on both MITF and CITED1 levels in WM239A cells relative to a negative control siRNA (N) at 48 hours post-transfection. β -Actin is used as a loading control. (b) Western blot showing the effect of silencing MITF using the siRNA siM3 on both MITF and CITED1 levels in SKMEL5 cells relative to a negative control siRNA (N) at 48 hours post-transfection.

S4: (a) A bar chart showing the % change in cell cycle distribution in both #1 and #3 siCITED1 treated HT144 cells relative to siNEG treated HT144 cells. The reduction in total S-phase is shown at 33 hours, 48 hours and 72 hours post-transfection in addition to the corresponding increase in the diploid G1 fraction. (b) A bar chart showing the % change in cell cycle distribution in both #1 and #3 siCITED1 treated relative to siNEG treated A2058 cells. The reduction in total S-phase and corresponding increase in G1 is shown at 48 hours post-transfection. (c) A bar chart

showing the % change in cell cycle distribution in both #1 and #3 siCITED1 treated relative to siNEG treated A375 cells. The reduction in total S-phase and corresponding increase in G1 is shown at 48 hours post-transfection.

S5: Western blot of melanoma cells lines indicating the differential expression of the cell cycle regulator CDKN1A/P21 and CDKN1C/P57 in addition to MITF and CITED1. β -Actin is used as a loading control.

S6: The rheostat model of MITF action in melanoma indicating the hypothesised role of CITED1 in the regulation of MITF expression and melanoma cell behaviour. The model is an original adaptation of those previously proposed by others, now incorporating our data ²⁻⁴.

References

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- 3. Carreira, S. *et al.* Mitf regulation of Dia1 controls melanoma proliferation and invasiveness. *Genes Dev* **20**, 3426–3439 (2006).
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