Expanded View Figures

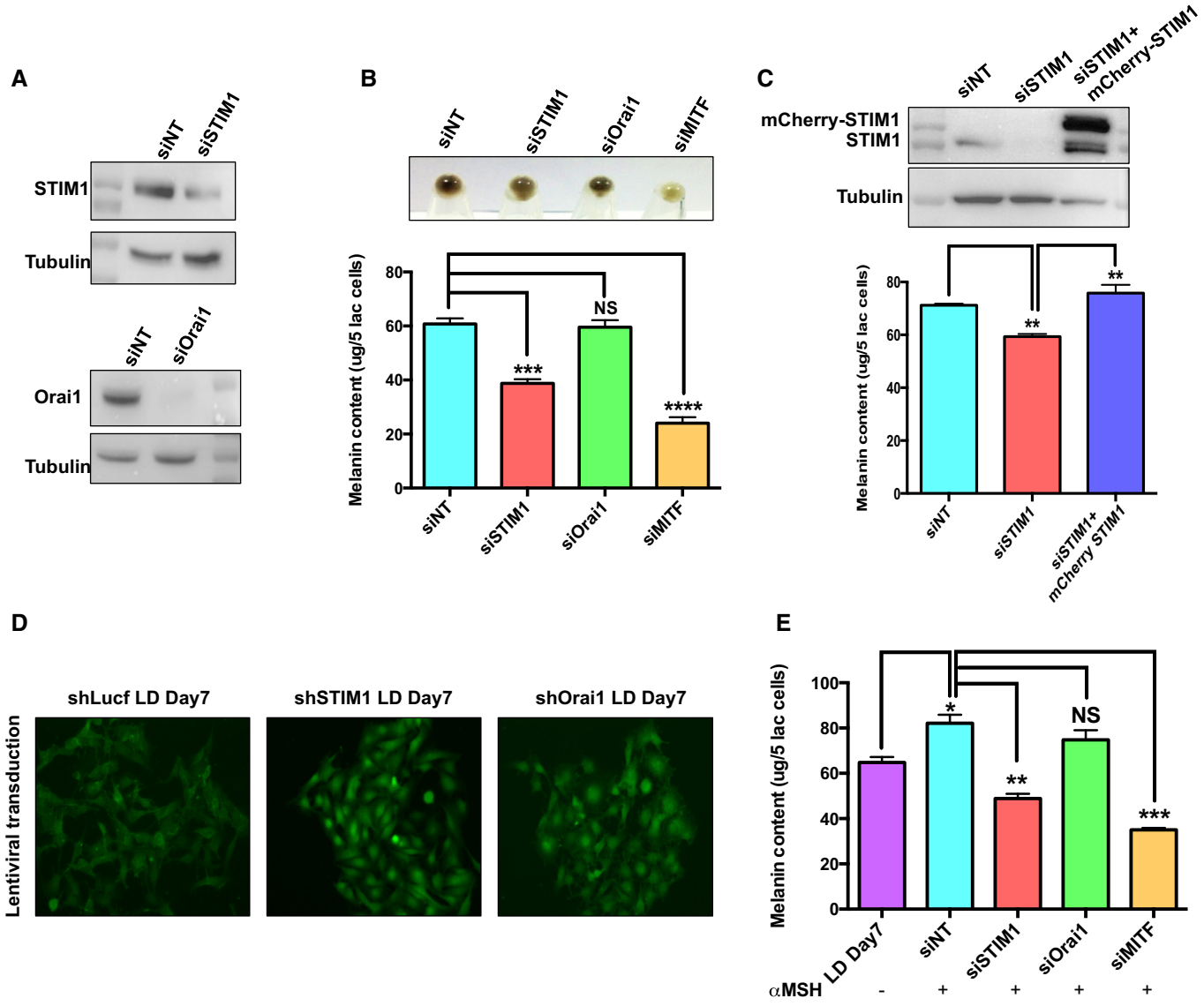

Figure EV1. STIM1 but not Orai1 regulates melanogenesis.

A Western blots demonstrating siRNA-based knockdown of STIM1 and Orai1.

B Representative cell pellets of LD day 7 upon STIM1, Orai1, and MITF silencing along with melanin content analysis ($N = 3$).

C Western blot for examining mCherry-STIM1 expression in B16 cells and melanin content analysis for evaluating rescue of pigmentation loss with mCherry-STIM1 ($N = 3$).

D Lentiviral-transduced B16 stable cell lines used for LD melanogenesis assay showing comparable transduction efficiency.

E Melanin content analysis in the unstimulated LD cells and upon αMSH stimulation in LD cells transfected with siNT, siSTIM1, siOrai1, or siMITF ($N = 3$).

Data information: Data represented are Mean ± SEM (*$P < 0.05$; **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$; unpaired Student's t-test).

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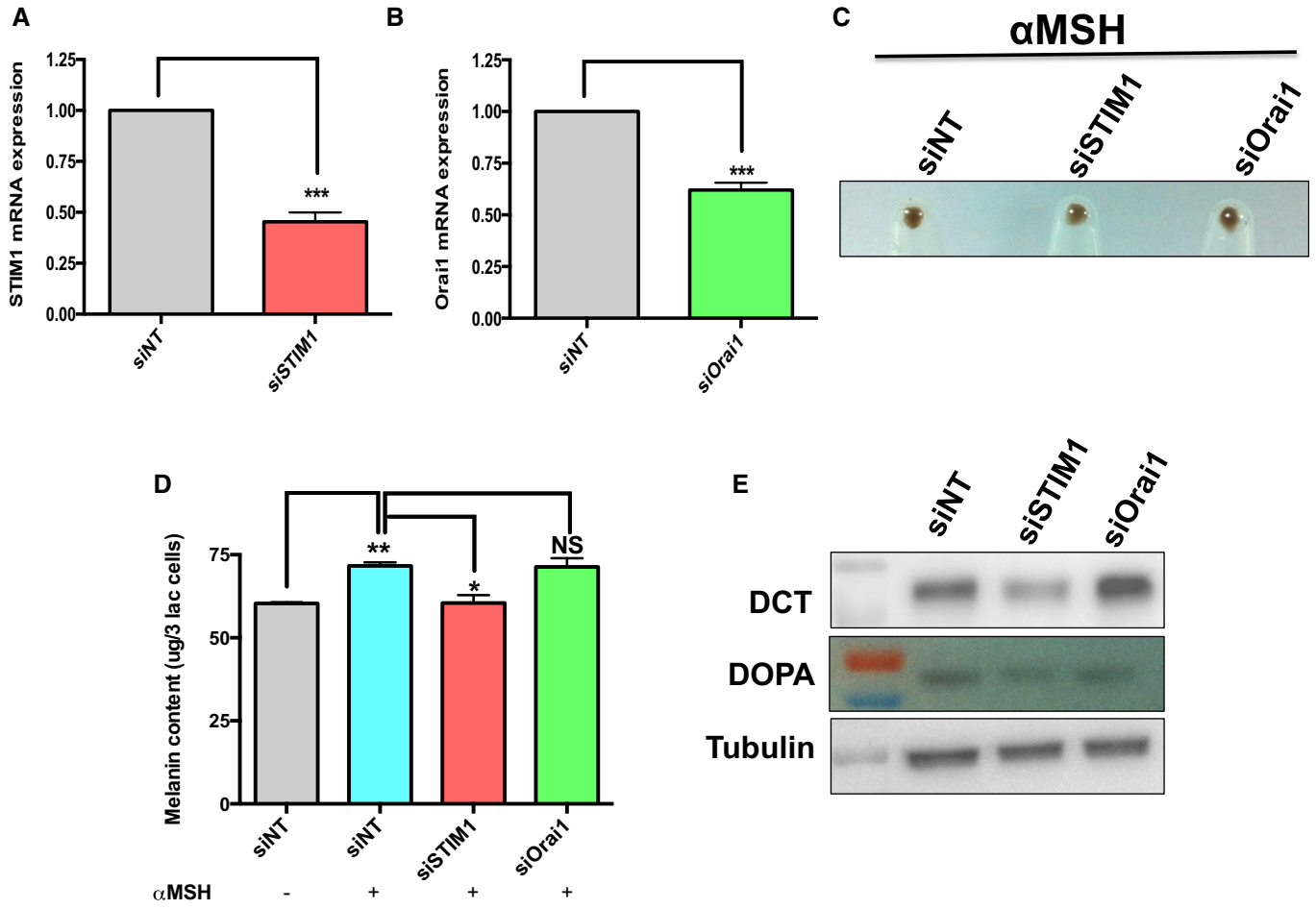

Figure EV2. STIM1 but not Orai1 regulates αMSH-induced primary human melanocyte pigmentation.

A qRT–PCR analysis of STIM1 48 h post-transfection with STIM1 siRNA ($N = 3$).

B qRT–PCR analysis of Orai1 48 h post-transfection with Orai1 siRNA ($N = 3$).

C Primary human melanocyte pellets showing the extent of αMSH-driven pigmentation upon STIM1 or Orai1 silencing in comparison with siNT control.

D Melanin content analysis evaluating changes in αMSH-induced pigmentation upon STIM1 and Orai1 silencing ($N = 3$).

E Western blots and gel images of tyrosinase activity (DOPA assay) in primary human melanocytes transfected with either control siRNA or STIM1/Orai1 siRNA.

Data information: Data represented are Mean ± SEM (*$P < 0.05$; **$P < 0.01$, ***$P < 0.001$; unpaired Student's t-test).

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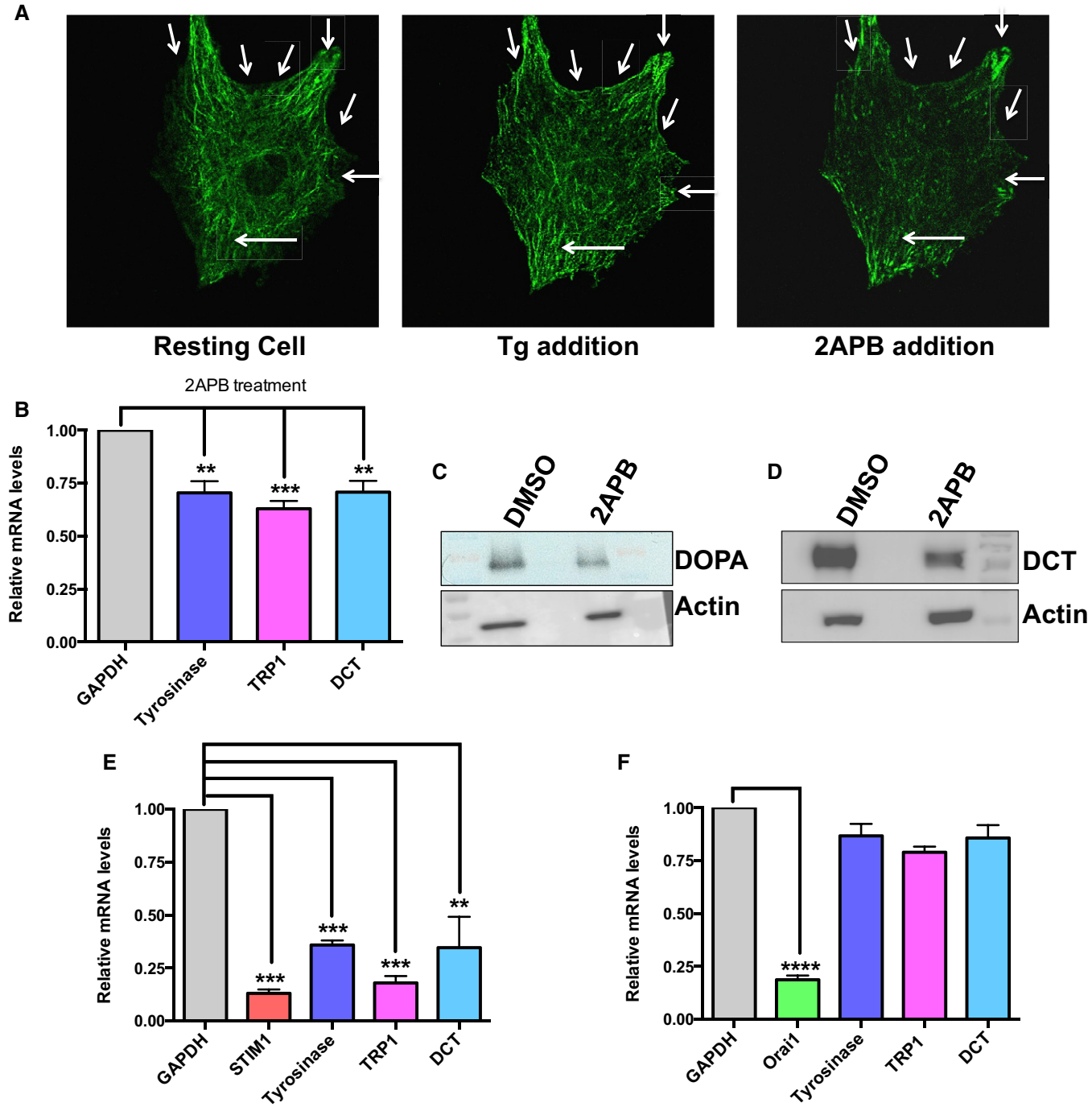

Figure EV3. STIM1 oligomerization regulates expression of key melanogenic genes.

A Confocal images of B16 cells transfected with eYFP-STIM1 showing that Tg activates STIM1 oligomerization and 2APB disrupts STIM1 puncta. Arrows point to oligomeric STIM1 punta.

B qRT–PCR data demonstrating that 2APB application for 48 h reduces mRNA expression of tyrosinase, TyRP1, and DCT ($N = 3$).

C, D Gel images of tyrosinase activity and Western blots for DCT on LD day 7 treated with either vehicle control (DMSO) or 2-APB.

E qRT–PCR analysis showing that STIM1 silencing not only decreases mRNA expression of STIM1 but also that of tyrosinase, TyRP1, and DCT ($N = 3$).

F qRT–PCR analysis showing that Orai1 knockdown decreases Orai1 mRNA levels, but expression of melanogenic genes remains largely unaffected ($N = 3$).

Data information: Data represented are Mean ± SEM (**$P < 0.01$, ***$P < 0.001$; unpaired Student's t-test).

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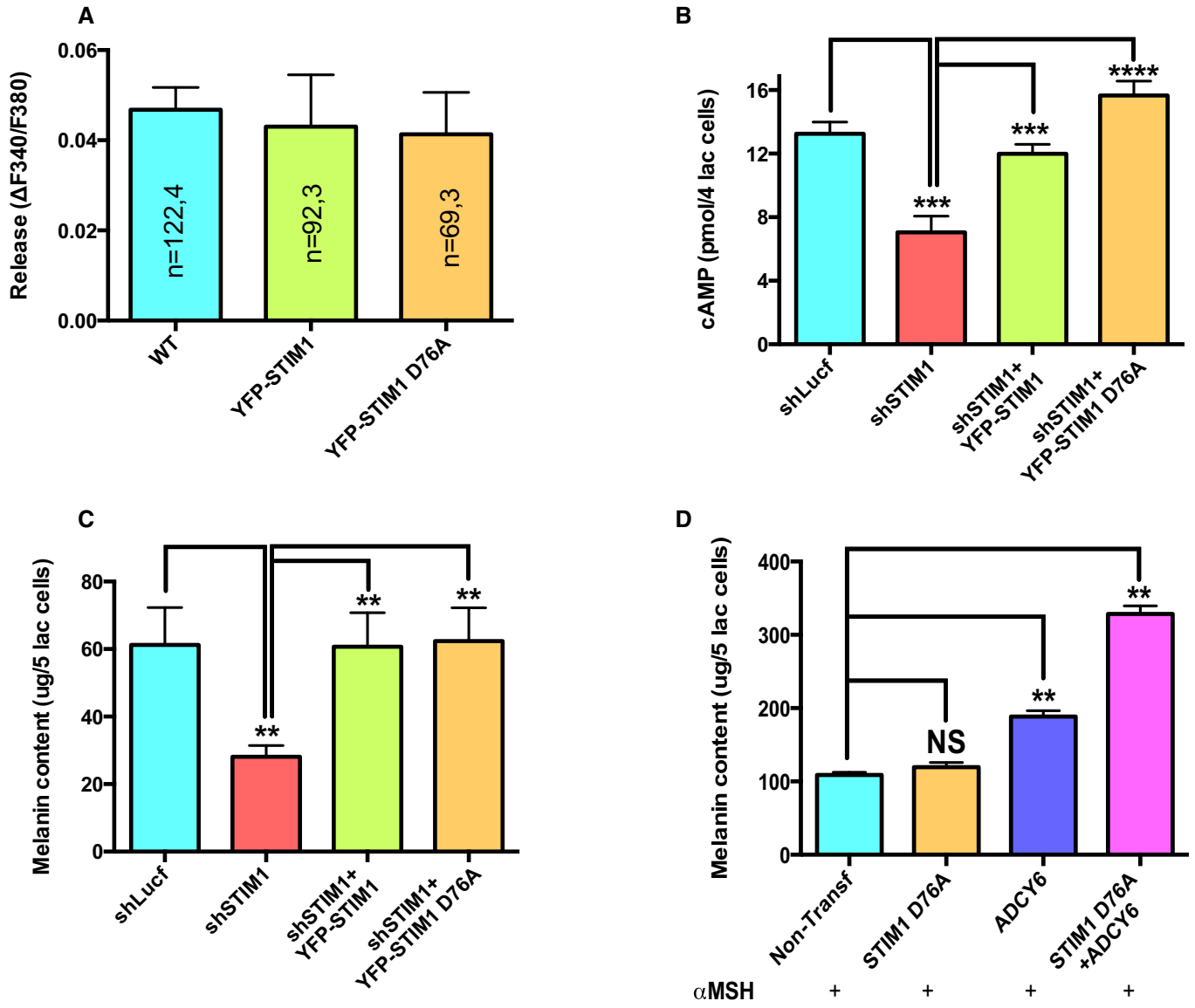

Figure EV4. Constitutively active STIM1 rescues cAMP generation and melanogenesis.

A Amplitude of αMSH-induced Ca^{2+} release in WT cells and in cells overexpressing either wild-type STIM1 (YFP-STIM) or constitutively active STIM1 YFP-STIM1 D76A.

B αMSH-stimulated cAMP generation upon STIM1 silencing and its rescue with YFP-STIM or YFP-STIM1 D76A ($N = 4$).

C LD day 7 melanin content analysis in the B16 shSTIM1 stables and rescue with YFP-STIM or YFP-STIM1 D76A ($N = 6$).

D Melanin content analysis of B16 cells stimulated with αMSH in non-transfected control cells and in cells overexpressing either YFP-STIM1 D76A, ADCY6-CFP, or YFP-STIM1 D76A along with ADCY6-CFP ($N = 2$).

Data information: Data represented are Mean ± SD (**$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$; unpaired Student's t-test). The number of cells in (A) is reported as "$n = x$, y" where "x" denotes the total number of cells imaged and "y" denotes number of traces recorded for imaging "x" cells.

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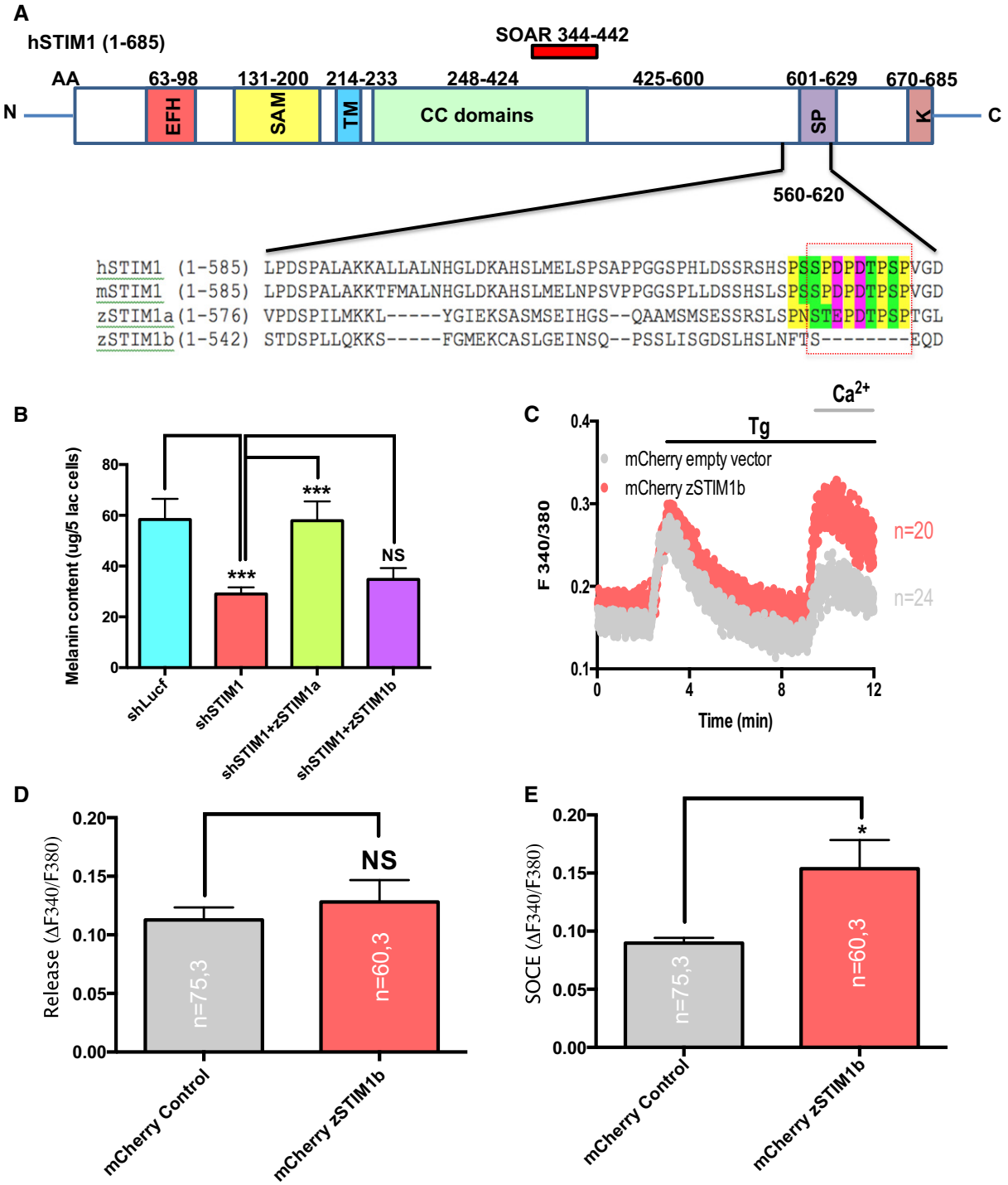

Figure EV5. STIM1 C-terminus S/P-rich domain regulates STIM1-mediated melanogenesis.

A Domain architecture of hSTIM1 and the protein sequence analysis of C-terminus of hSTIM1, mSTIM1, zSTIM1a, and zSTIM1b.

B Melanin content analysis in B16 shSTIM1 stables and its rescue with zSTIM1a and zSTIM1b ($N = 5$).

C Representative SOCE traces of B16 cells expressing either empty mCherry vector or mCherry-zSTIM1b plasmid.

D The quantitative analysis of ER Ca^{2+} release in vector control and zSTIM1b-expressing cells.

E The analysis of SOCE amplitude upon ectopic expression of zSTIM1b.

Data information: Data represented are Mean ± SEM (*$P < 0.05$; ***$P < 0.001$; unpaired Student's t-test). (D, E) The number of cells are reported as "$n = x, y$" where "x" denotes the total number of cells imaged and "y" denotes number of traces recorded for imaging "x" cells.

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