

**Figure S1:**

A: Expression of IL1RN mRNA levels per individual cell culture.

B: PCR showing IL-1RA variants expressed in NOKs cell cultures.

C-F IL1RN and icIL-1RN transcript levels and tIL-1RA expression after stimulation with 10 ng/ml of rIL-1 $\alpha$  and 10 ng/ml of rIL-1 $\beta$  in OSCC (C, D) and OD (E, F) cell lines.

G: IL-6 and IL-8 response after exposure to rIL-1 $\alpha$  and rIL-1 $\beta$  in same samples from C-F.

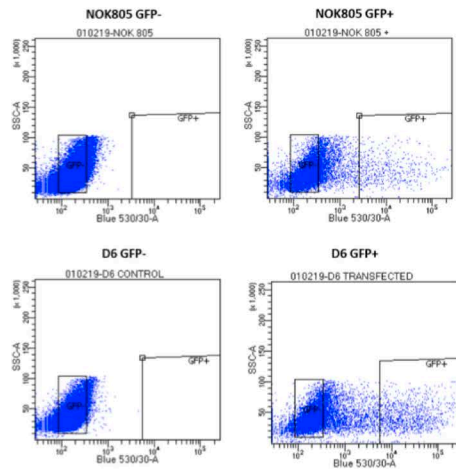
H: rIL-1 $\alpha$  increases icIL-1RN mRNA levels in FNB6 cells

I: IL-1R2 mRNA expression in immortal NOKs (FNB6, OKF4 and OKF6), immortal OD (D19 and D20) and OSCC (b16 and B22) cell lines. Expression is expressed as fold change relative to the reference gene. Data are shown as mean  $\pm$  SEM (N = 3 independent experiments, n = 3 technical replicates). One-way ANOVA with multiple comparisons was used to calculate the exact P value.

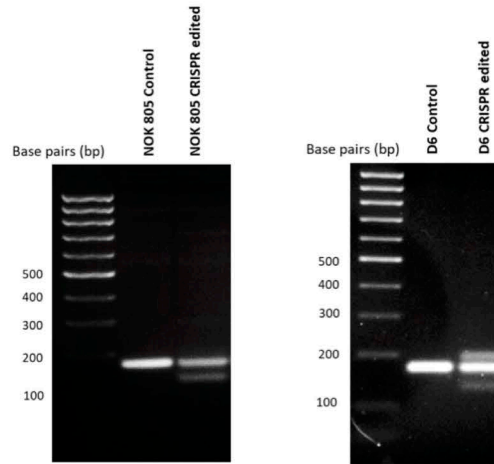
J: IL-1R2 transcript levels after exposure to rIL-1 $\alpha$  and rIL-1 $\beta$  in same samples from C-

Data information: Data are shown as mean  $\pm$  SEM (N = 3 independent experiments, n = 3 technical repeats). \* p < 0.05.

**A**



**B**



**C**

**NOK805 Control**

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forward -----CAACGTATCGTTAGATCTGGGAT 28
expected gctgggcacatggtgctgtgcactacagctgagtccttttctttcagAATCTGGGAT 60
          * * * * *

forward GTTAACCAAGAACCTTCTATCTGAGGAACAACCAACTAGTTCCTGGATACTTGCAGGA 88
expected GTTAACCAGAAGACCTTCTATCTGAGGAACAACCAACTAGTTCCTGGATACTTGCAGGA 120
          * * * * *

forward CCAAATGTC AATTTAGAAGTgagtggtGCCAGGAAGCCAATGATGTGGGCATAAC 147
expected CCAAATGTC AATTTAGAAGTgagtggtGCCAGGAAGCCAATGATGTGGGCATAAC 176
          * * * * *

NOK805 CRISPR edited upper band:

forward -----ACAACAGTAATCTTTCATTAGATCTGGGAT 33
expected gctgggcacatggtgctgtgcactacagctgagtccttttctttcagAATCTGGGAT 60
          * * * * *

forward GTTAACCAAGAACCTTCTATCTGAGGAACAACCAACTAGTTCCTGGATACTTGCAGGA 93
expected GTTAACCAGAAGACCTTCTATCTGAGGAACAACCAACTAGTTCCTGGATACTTGCAGGA 120
          * * * * *

forward CCAAATGTC AATTTAGAAGTgagtggtGCCAGGAAGCCAATGATGTGGGCATA 150
expected CCAAATGTC AATTTAGAAGTgagtggtGCCAGGAAGCCAATGATGTGGGCATA 176
          * * * * *

NOK805 CRISPR edited lower band

forward -----CCCGTATCTTTCATTTCGATCTGGGAT 28
expected gctgggcacatggtgctgtgcactacagctgagtccttttctttcagAATCTGGGAT 60
          * * * * *

forward GTTAACCAAGAATCCTTCTATCTGAGGAACAACCAACTAGTTCCTGGATACTTGCAGGA 47
expected GTTAACCAGAAGACCTTCTATCTGAGGAACAACCAACTAGTTCCTGGATACTTGCAGGA 120
          * * * * *

forward CCAAAGGCATTTTAAAGGGGGGGGTTGCCAGGAAGCCAAGGTGGGGGCTAAA 105
expected CCAAATGTC AATTTAGAAGTgagtggtGCCAGGAAGCCAATGATGTGGGCATAAC 176
          * * * * *
    
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**D**

**D6 Control:**

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forward ACTGGCTGGGACAGGGGCTGTGCACTACAGCTGAGTCCTTTTCCTTTT CAGAATCTGGGA 60
expected ----gctgggcacatggtgctgtgcactacagctgagtccttttctttcagAATCTGGGA 56
          * * * * *

forward GGATGTTAAACCAGAAGACCTTCTATCTGAGGAACAACCAACTAGTTCCTGGATACTTGCAGGA 120
expected GGATGTTAAACCAGAAGACCTTCTATCTGAGGAACAACCAACTAGTTCCTGGATACTTGCAGGA 116
          * * * * *

forward AGGACCAAAATGTC AATTTAGAAGTgagtggtGCCAGGAAGCCAATGATGTGGGCATAAC 148
expected AGGACCAAAATGTC AATTTAGAAGTgagtggtGCCAGGAAGCCAATGATGTGGGCATAAC 176
          * * * * *

D6 CRISPR edited upper band:

forward GGCTGGGCACATGCTGGCTGTGCACTACAGCTGAGTCCTTTTCCTTTT CAGAATCTGGGA 60
expected -gctgggcacatggtgctgtgcactacagctgagtccttttctttcagAATCTGGGA 59
          * * * * *

forward GTTAACCAAGAACCTTCTATCTGAGGAACAACCAACTAGTTCCTGGATACTTGCAGGA 120
expected GTTAAACCAGAAGACCTTCTATCTGAGGAACAACCAACTAGTTCCTGGATACTTGCAGGA 119
          * * * * *

forward ACCAAAATGTC AATTTAGAAGTgagtggtGCCAGGAAGCCAATGATGTGGGCATAAC 144
expected ACCAAAATGTC AATTTAGAAGTgagtggtGCCAGGAAGCCAATGATGTGGGCATAAC 176
          * * * * *

D6 CRISPR edited middle band

forward GGGGTGGCTGGGACATGGTGGCTGTGCACTACAGCTGAGTCCTTTTCCTTTT CAGAATCTGGGA 60
expected ----gctgggcacatggtgctgtgcactacagctgagtccttttctttcagAATCTGGGA 54
          * * * * *

forward TGGGATGTTAACCAAGAACCTTCTATCTGAGGAACAACCAACTAGTTCCTGGATACTTGC 120
expected TGGGATGTTAACCAGAAGACCTTCTATCTGAGGAACAACCAACTAGTTCCTGGATACTTGC 114
          * * * * *

forward AAGGACCAAAATGTC AATTTAGAAGTgagtggtGCCAGGAAGCCAATGATGTGGGCATAAC 149
expected CAAGGACCAAAATGTC AATTTAGAAGTgagtggtGCCAGGAAGCCAATGATGTGGGCATAAC 174
          * * * * *

forward -- 149
expected at 176

D6 CRISPR edited lower band

forward -----AGCCGGTAATGTCCTTTTCGATCTGGGAT 29
expected gctgggcacatggtgctgtgcactacagctgagtccttttctttcagAATCTGGGAT 60
          * * * * *

forward GTTGAAGACCTTCTATCTGAGGAACAACCAACTAGTTCCTGGATACTTGCAGGA 49
expected GTTAACCAGAAGACCTTCTATCTGAGGAACAACCAACTAGTTCCTGGATACTTGCAGGA 120
          * * * * *

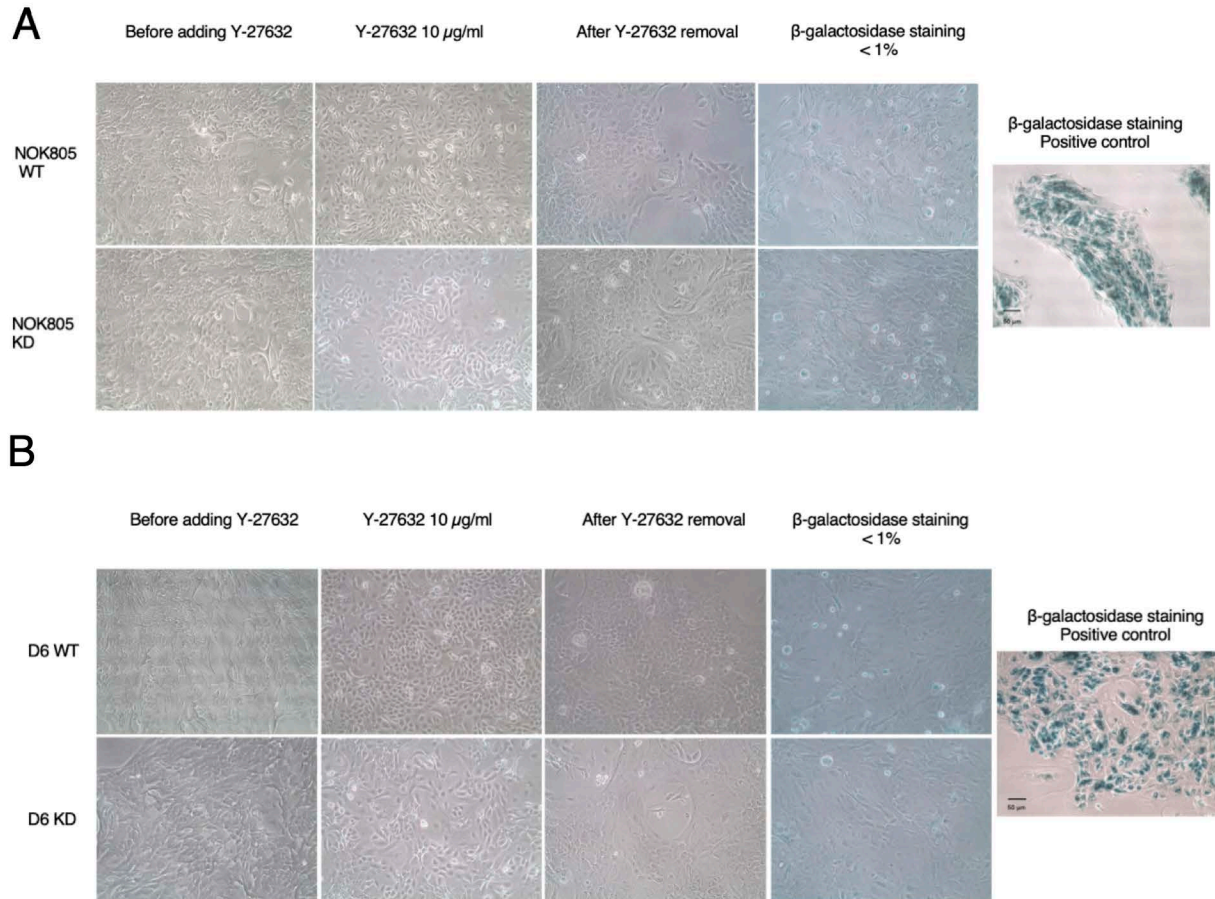
forward CCAAAGGCATTTTAAAGGGGGGGGTTGCCAGGAAGCCAAGGTGGGGGCTAAA 108
expected CCAAATGTC AATTTAGAAGTgagtggtGCCAGGAAGCCAATGATGTGGGCATAAC 176
          * * * * *
    
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**Figure S2:**

A: Transfected NOK805 and D6 cells were sorted into GFP+ and GFP- populations using a cell sorter. Same amount of GFP+ and GFP- cells per cell type were collected and expanded for further analysis.

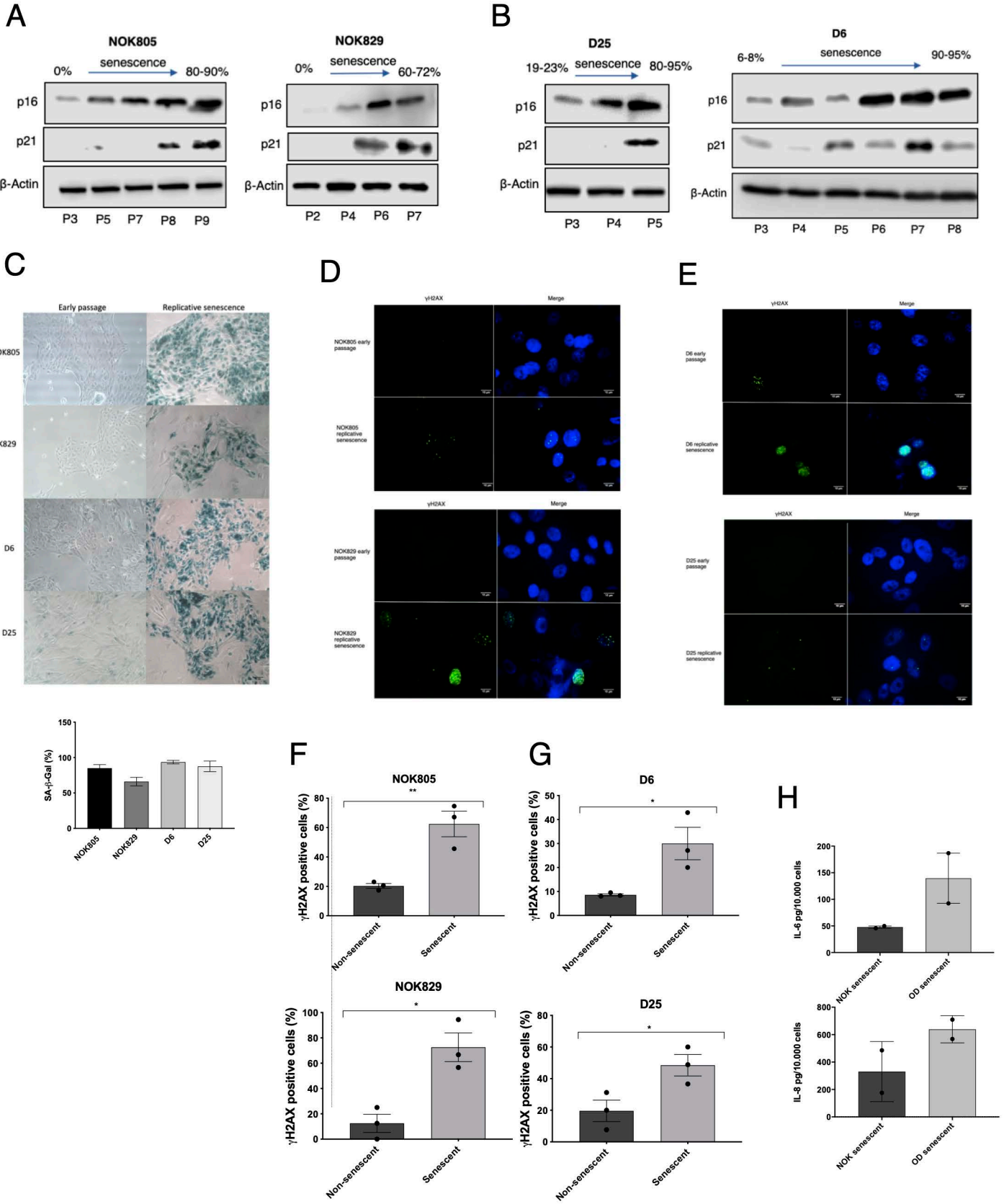
B: PCR products of NOK805 and D6 CRISPR edited and control cells using primers targeting the CRISPR target, resolved on a 2% agarose gel. Expected band size was of □ 176 bp for non-edited cells and of □ 130 bp for CRISPR edited cells. The presence of both bands in the CRISPR edited cells suggests a heterozygous population.

C-D: Sequencing results of individual PCR products obtained after agarose electrophoresis of NOK805 and D6 edited cells. Expected deletions are highlighted in yellow. Acquired deletions are highlighted in green. Red letters correspond to each sgRNA target site. \* means perfect match.



**Figure S3:**

A-B: Shows cells morphology before and after adding Y-27632 to NOK805 (A) and D6 (B) cell cultures and SA- $\beta$ -GAL activity after withdrawal.



**Figure S4:**

A-B: Immunoblotting showing p16<sup>Ink4a</sup> and p21<sup>Waf1/Cip1</sup> expression in normal (A) and dysplastic (B) oral keratinocytes during replicative senescence.

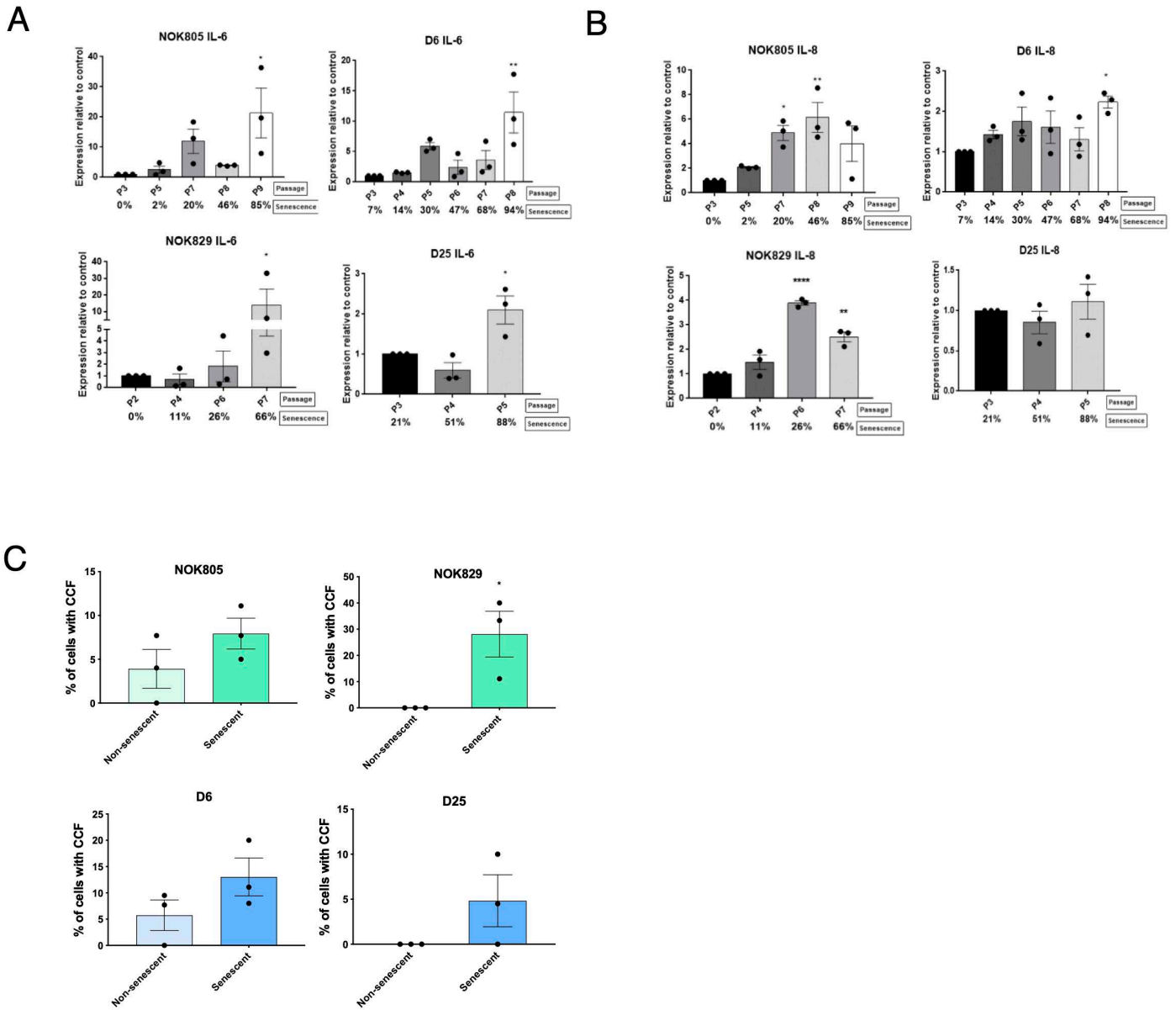
C: Representative images of SA-β-GAL activity in non-senescent and senescent normal and dysplastic oral keratinocytes. Scale bar is 50 μm.

D-G: Immunofluorescence and quantification of γH2AX expression in early passage and senescent normal (D, F) and dysplastic (E, G) oral keratinocytes. Data are shown as mean ± SEM (N = 3 independent experiments). Two-tailed T-test was used to calculate the exact P value. Scale bar is 10 μm.

H: IL-6 and IL-8 secretion levels of senescent NOKs (NOK805 and NOK829 combined) and ODs (D6 and D25 combined).

Senescence % is based on the % of cells stained positive for SA-β-GAL

Data information: \**P* < 0.05, \*\**P* < 0.005.



**Figure S5:**

A-B: IL-6 and IL-8 mRNA transcript levels of normal and dysplastic oral keratinocytes during replicative senescence. Data are shown as mean fold change relative to control  $\pm$  SEM (N = 3 independent experiments, n = 3 technical repeats). Statistical analysis was done using one-way ANOVA with multiple comparisons.

C: Quantification of non-senescent and senescent cells presenting with CCF. Data are shown as mean  $\pm$  SEM (N = 3 independent experiments). Two-tailed T-test was used to calculate the exact P value.

Senescence % is based on the % of cells stained positive for SA- $\beta$ -GAL

Data information: \* $P < 0.05$ , \*\* $P < 0.005$ , \*\*\*  $P < 0.0005$ , \*\*\*\*  $P < 0.00001$