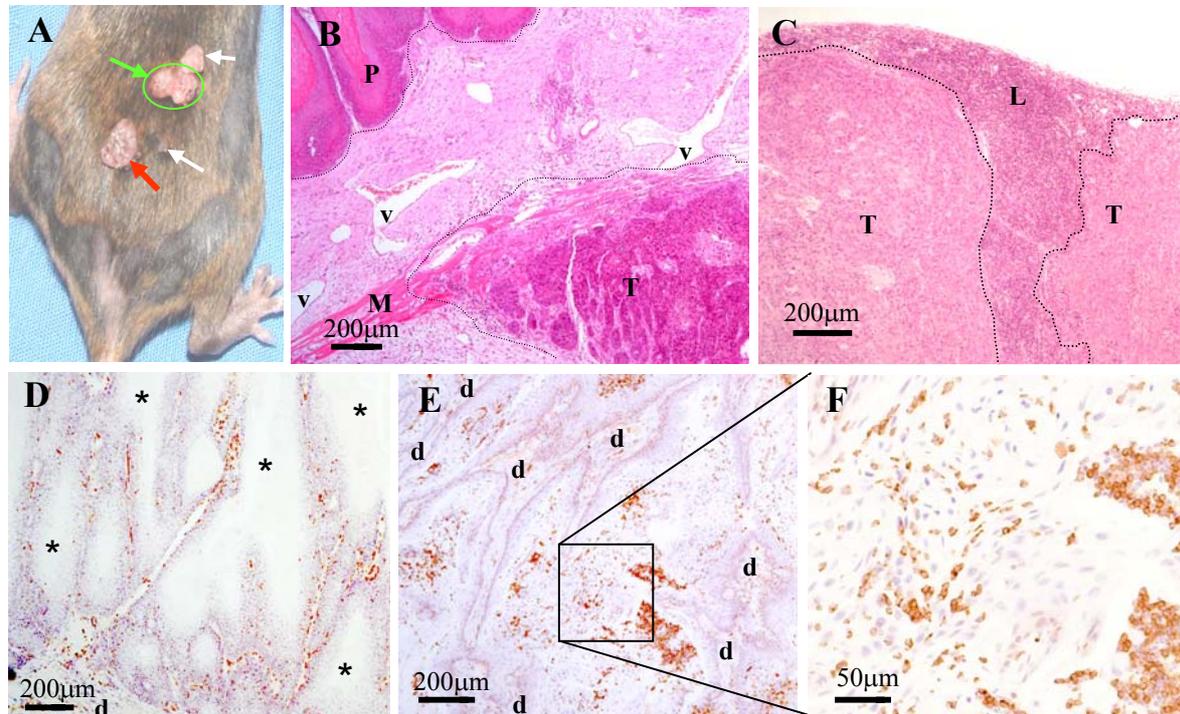
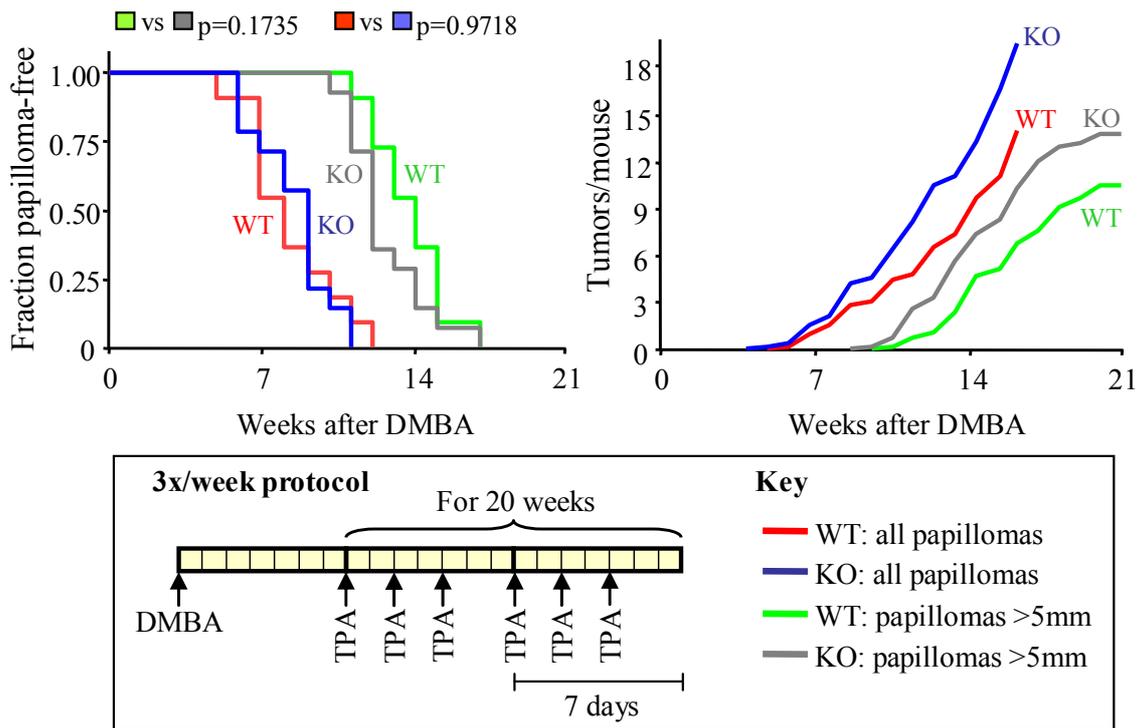


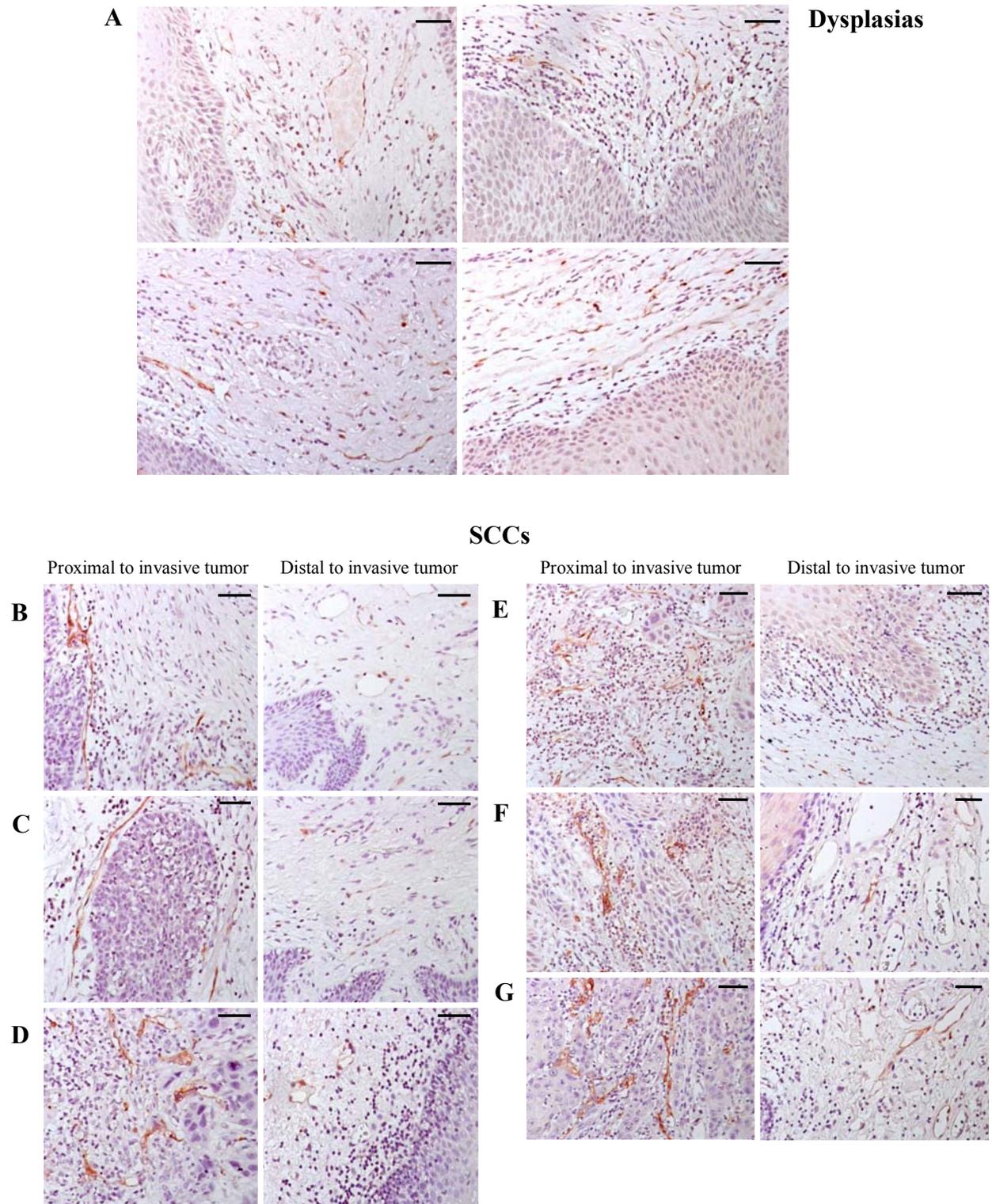
**Supplementary data for Nibbs et al ‘The atypical chemokine receptor D6 suppresses the development of chemically-induced skin tumors.**



**Figure S1: DMBA/TPA-induced papillomas and invasive SCC on *D6* deficient B6/129 mice.** **A.** Four papillomas, indicated by arrows, on a *D6* deficient mouse. The tumor labelled in green shows two papillomas that have grown together. The red arrow indicates a papilloma >5mm in diameter. **B.** H&E-stained section of an SCC from a *D6* deficient mouse. Top left, delineated by the dotted line, is a region typical of a non-invasive papilloma (P); bottom right is an area where the tumor (T) has invaded into, and under, the cutaneous muscle layer (M). Large blood vessels are indicated (V). **C.** H&E-stained section of an inguinal lymph node from a *D6* deficient mouse. The boundaries of two tumor deposits (T) are indicated with dotted lines, with an intervening region containing lymphocytes (L). **D.** Section of a papilloma immunostained with anti-CD3, visualised with DAB (red/brown), and counterstained with haematoxylin (blue). \* denote regions in the epidermis full of squames, and the underlying dermis (d) is indicated. **E-F.** SCC section immunostained with anti-CD3, visualised with DAB (red/brown), and counterstained with haematoxylin (blue). The dermis (d), with numerous blood vessels, is labelled. Panel F shows a higher magnification of the region indicated by the box in D. B-E: original magnification 5x, black bar represents 200 $\mu$ m; in F, original magnification 20x, black bar is equivalent to 50 $\mu$ m.



**Figure S2: DMBA/TPA induced tumors on FVB/N WT and D6 deficient mice.** 15 DMBA-treated *D6* deficient (KO) or WT mice received TPA three times a week for 20wks as shown. All papillomas, or just those >5mm in diameter, were counted once a week. The fraction of mice papilloma-free (left panel) and mean tumor burden per mouse (right panels) are shown. Logrank tests were applied to data in the left panels. Differences in tumor burden became statistical significance on week 12 for all tumors ( $p < 0.05$ ) or week 18 if only those >5mm were scored ( $p < 0.05$ ), but at later time-points, this level of significance was not always achieved.



**Figure S3: D6 immunoreactivity in human oral SCCs and dysplasias.** Tissue sections were stained according to previous protocols (20). D6 immunoreactivity was visualised using DAB (red-brown) and nuclei counterstained with haematoxylin (blue). Original magnification: 20x. The black bars are equivalent to 50µm. **A.** Four representative images taken at the dermal/epidermal junction of 3 stained sections of oral dysplasia. **B-G.** Pairs of

representative images taken from six individual immunostained oral SCC sections. Images were taken from regions of each section in which invasive tumor cells were either present (left) or absent (right). Epithelial/tumor cells were identified by their epidermal location, and/or their large size and large nucleus, on the immunostained sections and/or adjacent H&E-stained sections.