Supplementary Data

Supplementary Table I: List of primary and secondary antibodies used to generate supplementary data

Cat No/Clone	Specificity	Host	Source
Ab6982	Anti-human adenovirus 5	Rabbit	Abcam
E0432	Anti-rabbit biotin	Goat	Dako
628601/C-11	Anti-cytokeratin (pan reactive)	Mouse	Biolegend
A21127	Anti-mouse Alexa 555	Goat	Invitrogen
553239/500A2	Anti-mouse CD3 biotin	Hamster	BD Pharmingen
553090/RA3-6B2	Anti-mouse B220 PE	Rat	BD Pharmingen
120804/MECA-79	Anti-mouse PNAd biotin	Rat	Biolegend
A21434	Anti-rat Alexa 555	Goat	Invitrogen
S32357	Streptavidin Alexa 647		Invitrogen
405503	Anti-hamster DyLight488	Goat	Biolegend
S32355	Streptavidin Alexa 555		Invitrogen

SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure 1: Selective localization of AdV in ductal epithelial cells.

Double immunofluorescence staining for AdV5 (green) and a pan-cytokeratin marker (red) in AdV-treated (A-F) and vehicle-treated salivary glands (G-I). Slides were fixed in PFA 4% and endogenous biotin was blocked with pre incubation with avidin and biotin, samples were then blocked with 10% goat serum, Triton-X 0.2% in PBS and incubated with rabbit anti-human adenovirus 5 antiserum (Abcam) for one hour. Biotinylated anti-rabbit antibody (Dako) was added for 30 minutes followed by streptavidin conjugated fluorophore (Invitrogen). Slides were then incubated for one hour with anti-cytokeratin antibody (Biolegend) followed by appropriate secondary antibody for 30 minutes, counterstained with DAPI (Invitrogen) and mounted. Expression of AdV5 proteins from day 3 pc is exclusively detectable within ductal but not acinar epithelial cells. Original magnification x200 in A-C and G-I and x400 in D-F.

Supplementary Figure 2: Early and late apoptosis following AdV-delivery in the submandibular glands of C57BL/6.

TUNEL assay identifying apoptotic cells (in green with DAPI nuclear counterstaining in blue) in the salivary glands of AdV-treated mice at 1 (**A, B**), 2 (**C, D**) and 3 (**E, F**) weeks pc. TUNEL assay (Trevigen TACS2 TdT Fluo kit) for the detection of apoptotic nuclei was assessed following the manufacturer's instructions. Slides were fixed in PFA 4% and permeabilised with Cytonin for 30 minutes at room temperature. The labeling mixture was prepared using cobalt cation stock. After washing and incubation with provided streptavidin-conjugated fluorophore, slides were counterstained with DAPI and mounted.

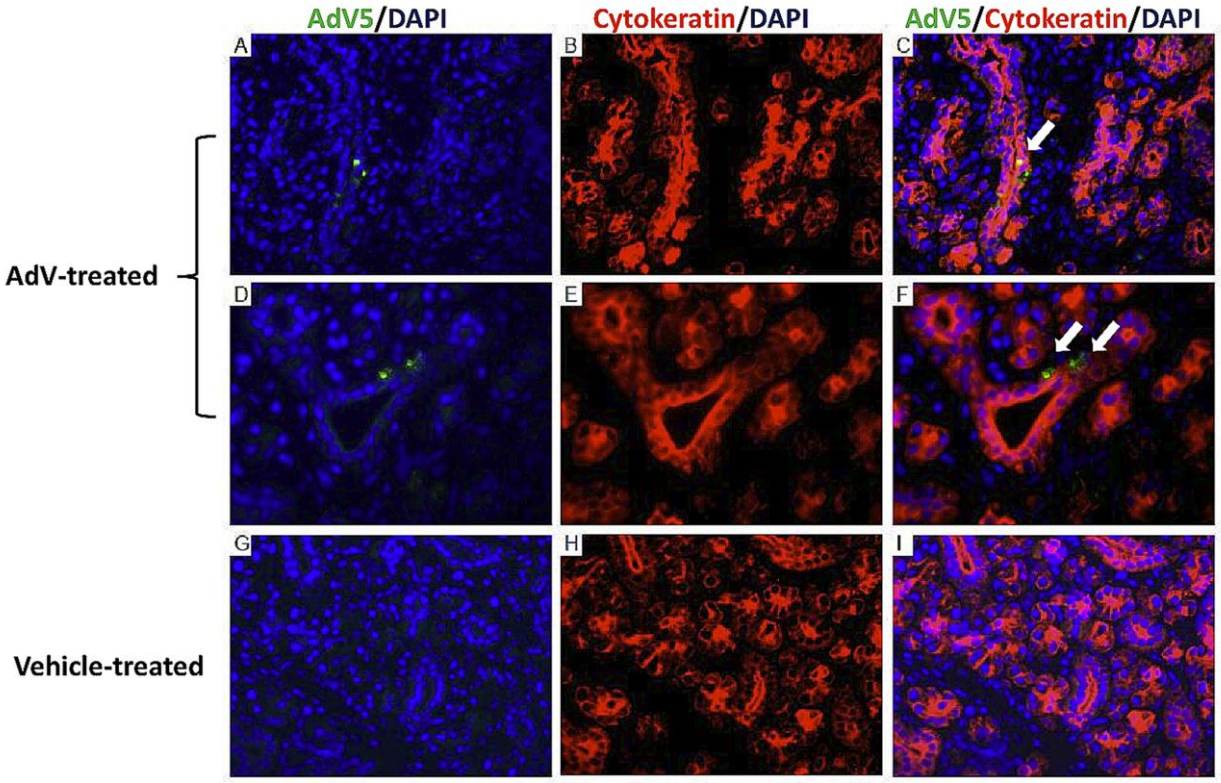
Within the first week apoptotic cells are abundant and diffuse within the parenchyma of the AdV-treated glands (**A**), often within the ductal epithelium (**B**). By week 2 and 3 pc the degree of apoptosis is reduced with TUNEL+ cells almost exclusively localized within periductal foci (**C-F**). Original magnification x100 in A, C, E and x200 in B, D, F.

Supplementary Figure 3: Colocalization of HEV within the T cell rich areas of periductal foci in C57BL/6 submandibular glands following AdV-delivery.

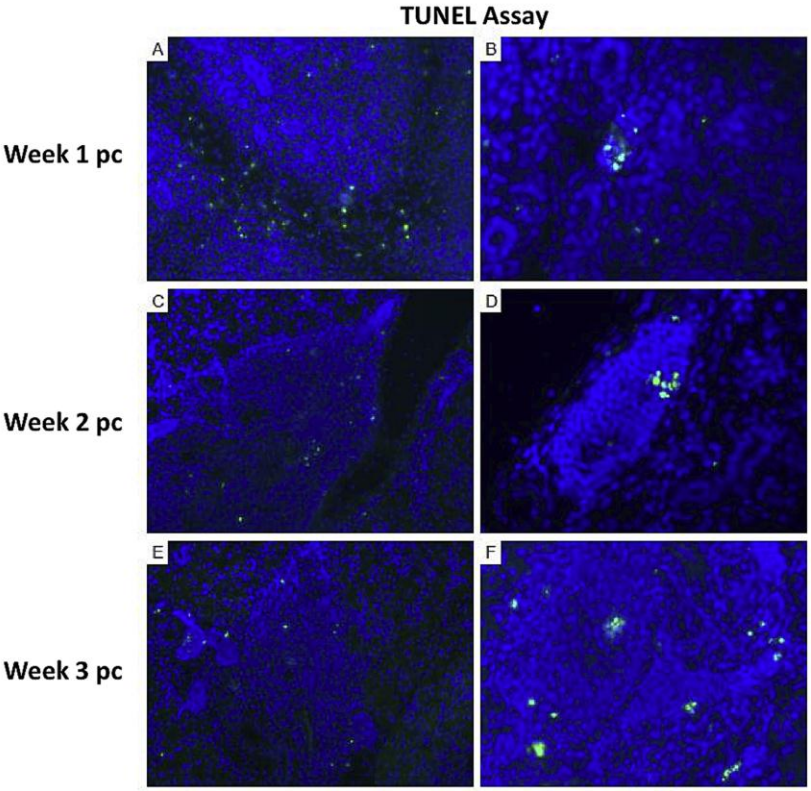
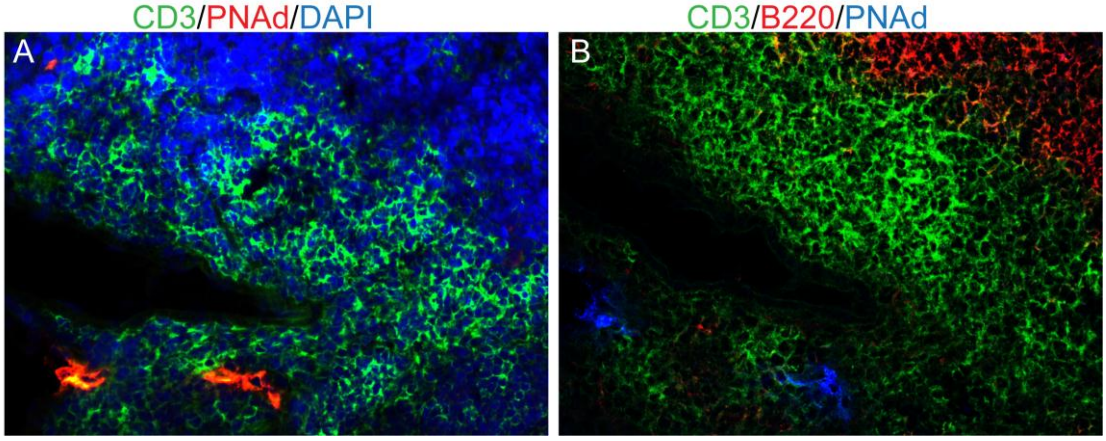
Microphotographs of sequential sections of a C57BL/6 submandibular gland harvested 18 days after cannulation with LucAdV. The presence and localization of HEV in the T cell area of segregated infiltrates in the SG was assessed by double and triple immunofluorescence. Slides were fixed in ice-cold acetone, rehydrated in TBS and incubated with endogenous biotin blocking solution (Vector Labs, UK) followed by protein blocking solution (TBS, 1% BSA, 10% horse serum, 0.1% NaN_3). Slides were then incubated with PNAd and CD3 or PNAd, CD3 and B220 for one hour at room temperature and then incubated with appropriate fluorochrome-conjugated secondary antibodies or streptavidin in case of biotinylated primary antibodies. Slides were counterstained with DAPI (Invitrogen, UK) washed and mounted with MOWIOL solution (Calbiochem, La Jolla, CA). PNAd/CD3 images were taken using an Olimpus BX61 fluorescence microscope; PNAd/CD3/B220 images were taken using a Leica DM 5500 Q confocal microscope.

(A) Double immunofluorescence staining for T cells (CD3, in green) and HEV (PNAd, in red) and **(B)** triple confocal immunofluorescence staining for T cells (CD3, in green), B cells (B220, in red) and HEV (PNAd, in blue) showing that PNAd+ HEV selectively localise in the T cell rich area of segregated aggregates. Original magnification x200.

Supplementary Figure 1



Supplementary Figure 2



Supplementary Figure 3