Fig. S1

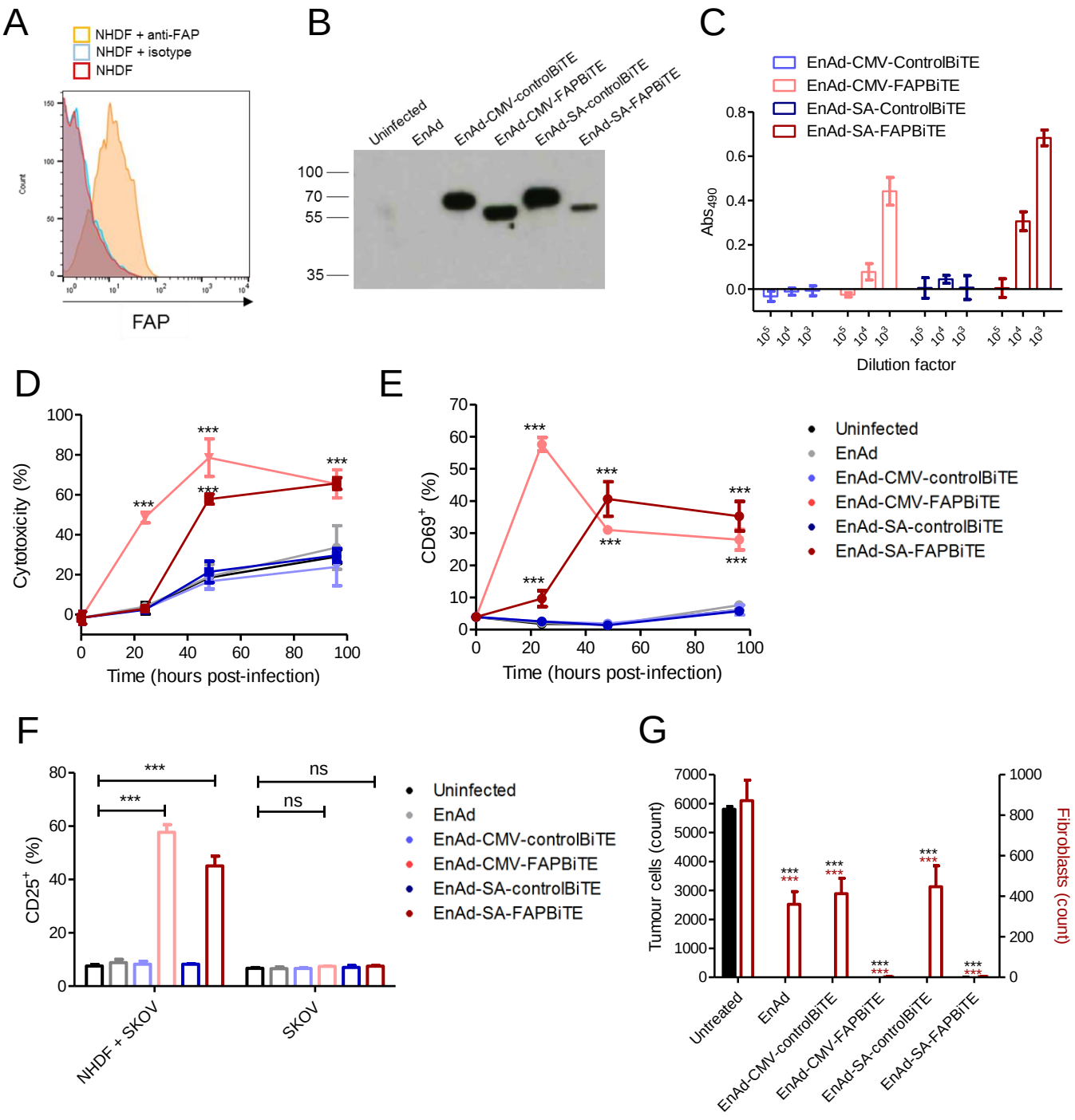

A
NHDF + anti-FAP
NHDF + isotype
NHDF
Count
FAP
B
Uninfected
EnAd
EnAd-CMV-controlBiTE
EnAd-CMV-FAPBiTE
EnAd-SA-controlBiTE
EnAd-SA-FAPBiTE
100
70
55
35
C
EnAd-CMV-ControlBiTE
EnAd-CMV-FAPBiTE
EnAd-SA-ControlBiTE
EnAd-SA-FAPBiTE
Abs490
Dilution factor
D
Cytotoxicity (%)
Time (hours post-infection)
E
CD69+ (%)
Time (hours post-infection)
Uninfected
EnAd
EnAd-CMV-controlBiTE
EnAd-CMV-FAPBiTE
EnAd-SA-controlBiTE
EnAd-SA-FAPBiTE
F
CD25+ (%)
ns
NHDF + SKOV
SKOV
G
Tumour cells (count)
Fibroblasts (count)
Untreated
EnAd
EnAd-CMV-controlBiTE
EnAd-CMV-FAPBiTE
EnAd-SA-controlBiTE
EnAd-SA-FAPBiTE

Fig. S1. EnAd-FAPBiTE-mediated oncolysis induces lysis of NHDF fibroblasts. (A) Flow cytometry spectra assessing the expression levels of surface FAP on NHDF cells. **(B)** Supernatants from uninfected or virus-infected HEK293A cells three days post-infection were assessed for BiTE expression by western blot using an anti-His tag antibody. **(C)** Cytotoxicity (LDH release; Abs_{490}) of NHDF cells in co-culture with T cells for 24 hours. Cells were incubated with diluted supernatants harvested from DLD cells that were infected with the indicated virus for 72 hours. **(D)** Lysis of NHDF and SKOV3 cells infected with EnAd or modified virus and co-cultured with primary T cells (T cell:NHDF:SKOV3 20:4:1). At each time point, supernatants were harvested, and cytotoxicity measured by LDH release. **(E)** CD69 expression on T cells from (C). **(F)** Activation of T cells in co-culture with SKOV3 alone or in combination with NHDF cells. After seeding, cells were infected with virus and CD25 expression on T cells measured at 72 hours post-infection. **(G)** Absolute quantity of viable $EpCAM^+$ DLD cells (black) and FAP^+ NHDF fibroblasts (red) were measured by flow cytometry after 72 hours after co-culture with T cells and the indicated virus. Prior to infection, cells were seeded at a ratio of 25:5:1 for T cells:NHDF:DLD.

(C-F) Data show the mean ± SD of biological triplicates. (C-F) Significance was assessed using one-way ANOVA with Tukey's Post Hoc analysis compared to 'uninfected'; *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$.

Fig. S2

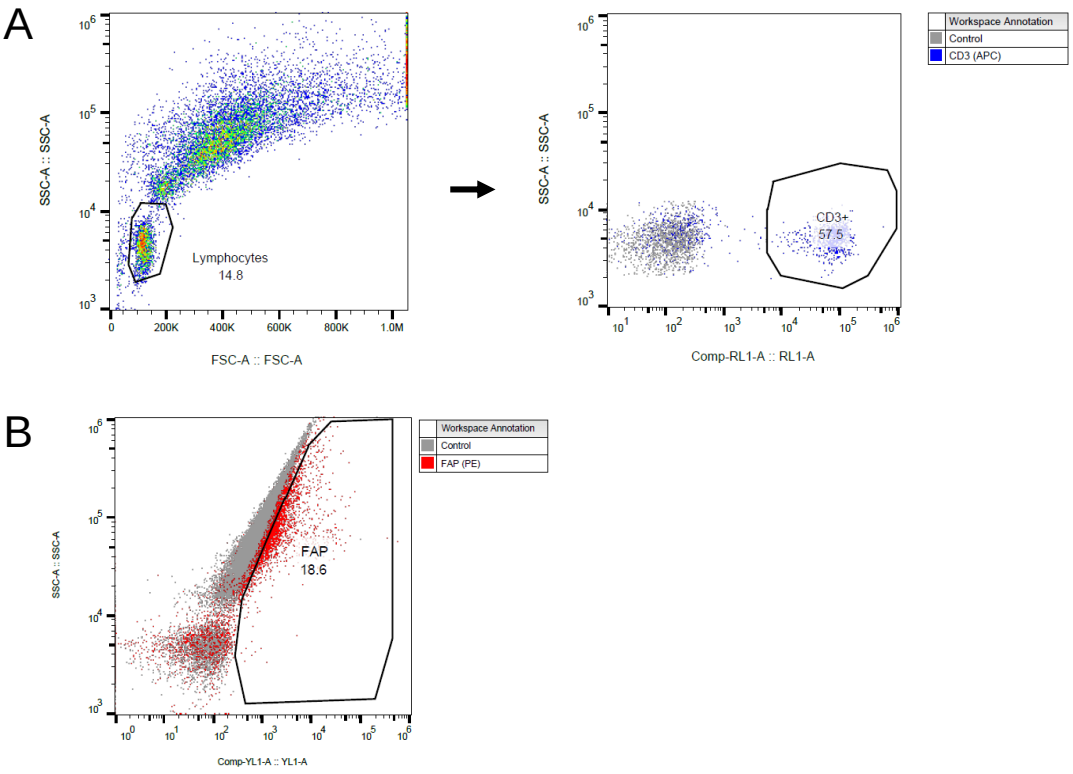


Fig. S2. Representative staining for human ascites used in this study. Representative plots for staining and gating of a human ascites sample are given for (A) CD3 and (C) FAP.

Fig. S3

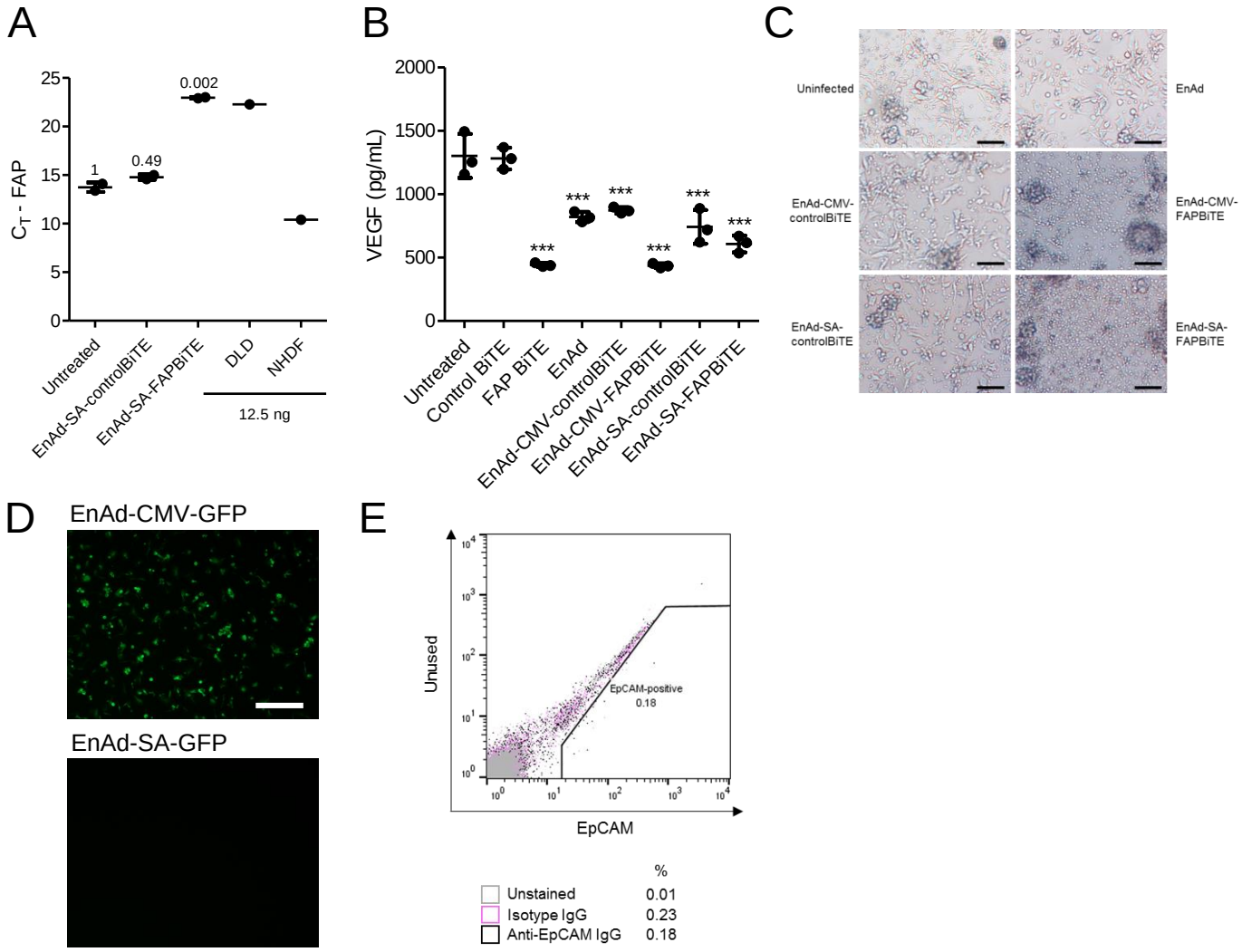

A
B
C
D
E
C_T - FAP
VEGF (pg/mL)
Untreated
EnAd-SA-controlBiTE
EnAd-SA-FAPBiTE
DLD
NHDF
12.5 ng
Control BiTE
FAP BiTE
EnAd
EnAd-CMV-controlBiTE
EnAd-CMV-FAPBiTE
EnAd-SA-controlBiTE
EnAd-SA-FAPBiTE
Uninfected
EnAd
EnAd-CMV-FAPBiTE
EnAd-SA-FAPBiTE
EnAd-CMV-GFP
EnAd-SA-GFP
Unused
EpCAM
EpCAM-positive
0.18
%
Unstained 0.01
Isotype IgG 0.23
Anti-EpCAM IgG 0.18

Fig. S3. EnAd-FAPBiTE induces fibroblast depletion in malignant ascites samples. (A) Representative analysis using RT-qPCR and primers specific for FAP performed on cDNA samples from patient biopsy 4 five days post-infection. cDNA from DLD and NHDF were included as a negative and positive control, respectively. Data show the mean ± SD of biological duplicates. **(B)** The level of VEGF in supernatants from patient biopsy 4. **(C)** Representative microscopy images of an ascites sample (patient biopsy 3) following virus treatment. Original magnification ×10; scale bar, 50 μm. **(D)** Fluorescence microscopy images of patient biopsy sample 1 five days post-infection with EnAd-CMV-GFP or EnAd-SA-GFP. Original magnification ×10; scale bar, 200 μm. **(E)** Proportion of $EpCAM^+$ cells in malignant ascites sample 1 upon receipt, measured using flow cytometry. **(G)** Production of 12 cytokines was evaluated by a multiple human Th cytokine panel.

(C, G) Data show the mean ± SD of biological triplicates. Significance was assessed using one-way ANOVA with Tukey's Post Hoc analysis compared to 'untreated'; *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$.

Fig. S4

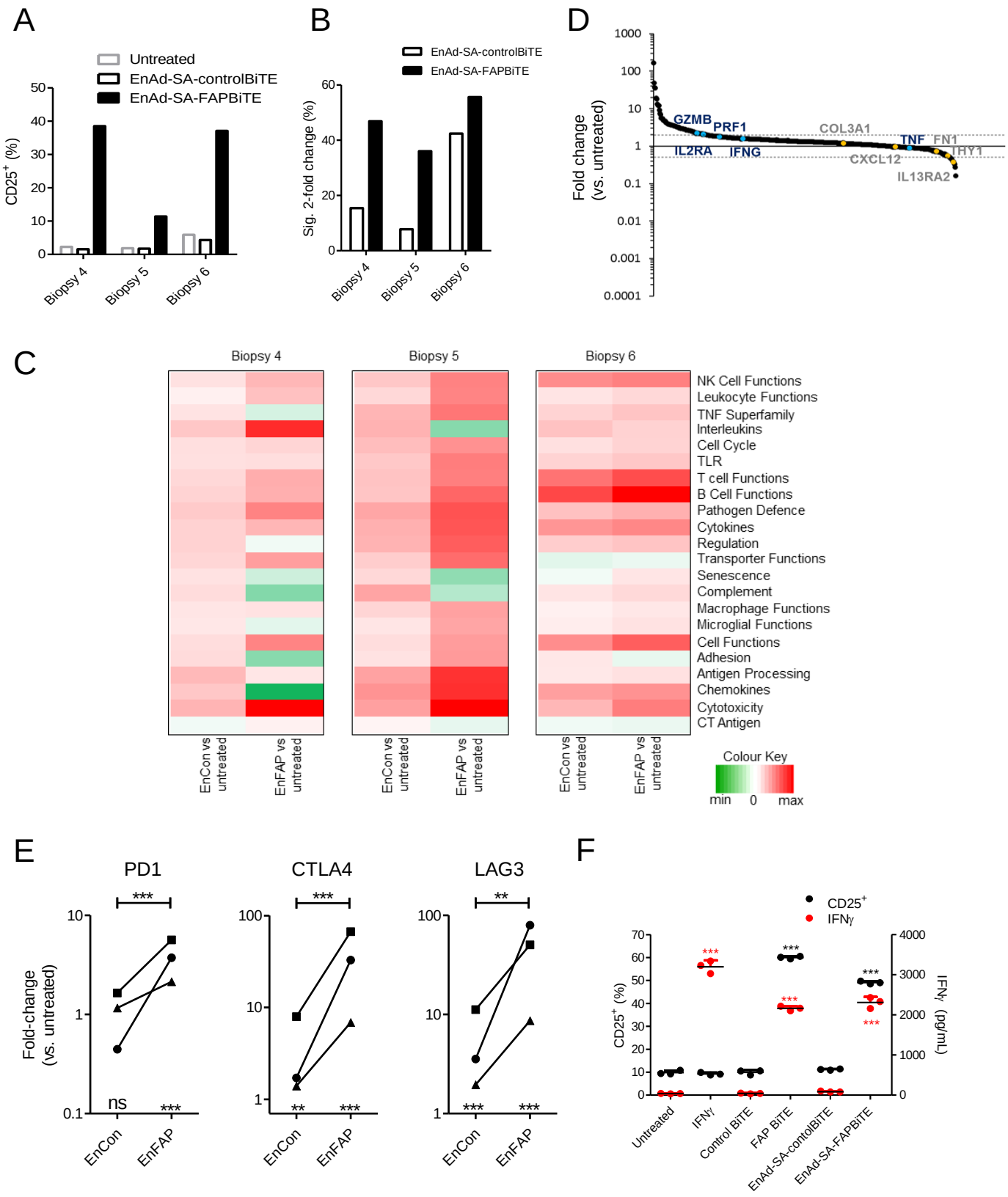
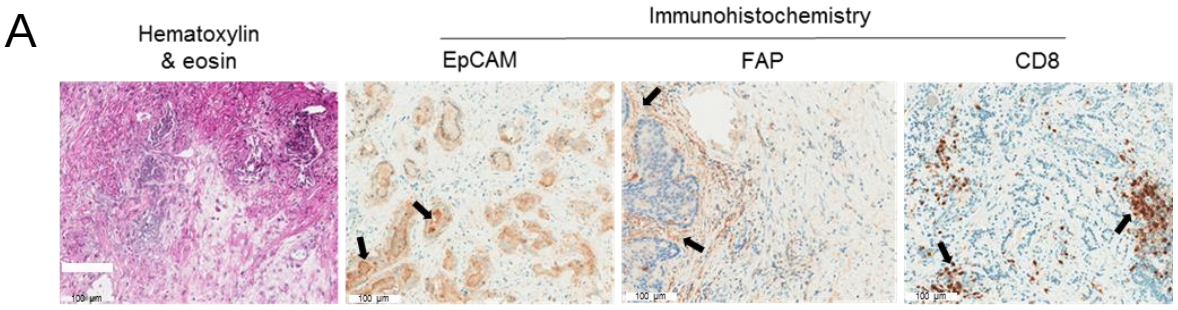
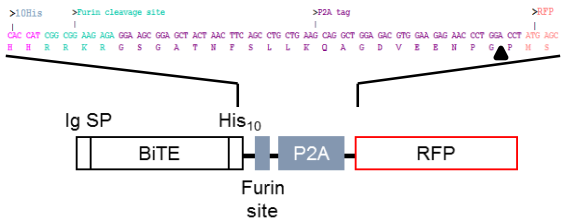

A
Untreated
EnAd-SA-controlBiTE
EnAd-SA-FAPBiTE
CD25+ (%)
Biopsy 4
Biopsy 5
Biopsy 6
B
EnAd-SA-controlBiTE
EnAd-SA-FAPBiTE
Sig. 2-fold change (%)
Biopsy 4
Biopsy 5
Biopsy 6
D
Fold change (vs. untreated)
GZMB
PRF1
IL2RA
IFNG
COL3A1
TNF
FN1
THY1
CXCL12
IL13RA2
C
Biopsy 4
Biopsy 5
Biopsy 6
NK Cell Functions
Leukocyte Functions
TNF Superfamily
Interleukins
Cell Cycle
TLR
T cell Functions
B Cell Functions
Pathogen Defence
Cytokines
Regulation
Transporter Functions
Senescence
Complement
Macrophage Functions
Microglial Functions
Cell Functions
Adhesion
Antigen Processing
Chemokines
Cytotoxicity
CT Antigen
EnCon vs untreated
EnFAP vs untreated
Colour Key
min
0
max
E
PD1
CTLA4
LAG3
Fold-change (vs. untreated)
ns
EnCon
EnFAP
F
CD25+
IFNγ
CD25+ (%)
IFNγ (pg/mL)
Untreated
IFNγ
Control BiTE
FAP BiTE
EnAd-SA-contolBiTE
EnAd-SA-FAPBiTE

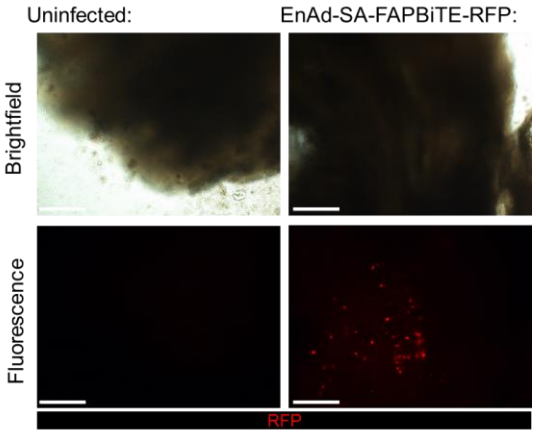
Fig. S4. EnAd expressing FAP BiTE can induce a pro-inflammatory shift in patient biopsy samples. (A) Proportion of $CD25^+$ T cells in three patient samples following 72-hour infection with EnAd-SA-controlBiTE or EnAd-SA-FAPBiTE. **(B)** The proportion of genes demonstrating at least a two-fold change ($p<0.05$) in mRNA counts after virus infection, compared to uninfected. **(C)** A heat map showing directed global significance scores (the cumulative change in mRNA counts) within defined gene sets of biopsy samples treated with EnAd-SA-controlBiTE or EnAd-SA-FAPBiTE, with reference to untreated. **(D)** A fold-change plot showing the differential expression of mRNA counts for all genes following infection of patient biopsy 4 with EnAd-SA-controlBiTE. **(E)** Fold change in mRNA counts (versus untreated) in three patient samples for genes involved in T cell immune checkpoints. **(F)** The induction of CD25 expression on $CD3^+$ cells and levels of IFNγ in supernatants following treatment of an ascites sample with free BiTE or BiTE expressing virus.

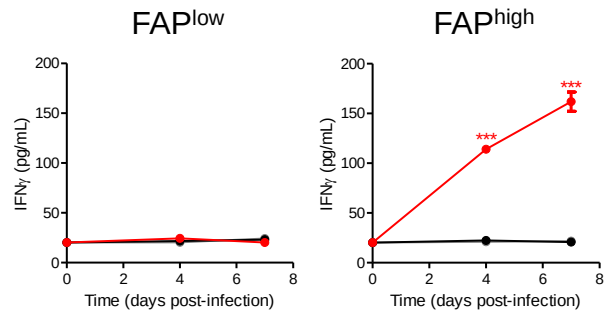
(C-E) Data show the mean of biological duplicate wells. Significant changes between treatments were assessed using a multivariate linear regression algorithm using three patient biopsy samples. For E, significance of changes in gene expression induced by each virus versus uninfected is displayed adjacent the x-axis, and between EnAd-SA-controlBiTE or EnAd-SA-FAPBiTE displayed above the plot; (F) Data show the mean ± SD of biological triplicates. Significance was assessed using one-way ANOVA with Tukey's Post Hoc analysis compared to 'untreated'; *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$.

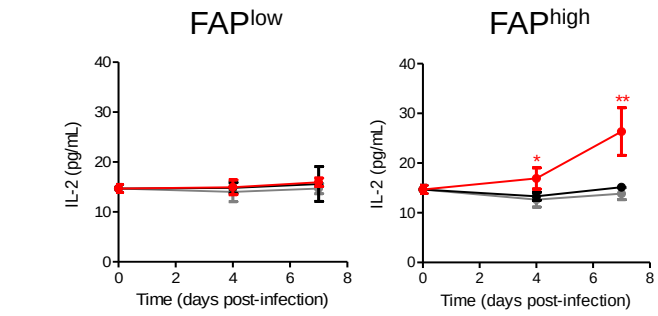
Fig. S5


A
Hematoxylin & eosin
Immunohistochemistry
EpCAM
FAP
CD8


B
>10His
>Furin cleavage site
>P2A tag
>RFP
Ig SP
His10
BiTE
P2A
RFP
Furin site


C
Uninfected:
EnAd-SA-FAPBiTE-RFP:
Brightfield
Fluorescence
RFP


D
FAPlow
FAPhigh
IFNγ (pg/mL)
Time (days post-infection)


E
FAPlow
FAPhigh
IL-2 (pg/mL)
Time (days post-infection)

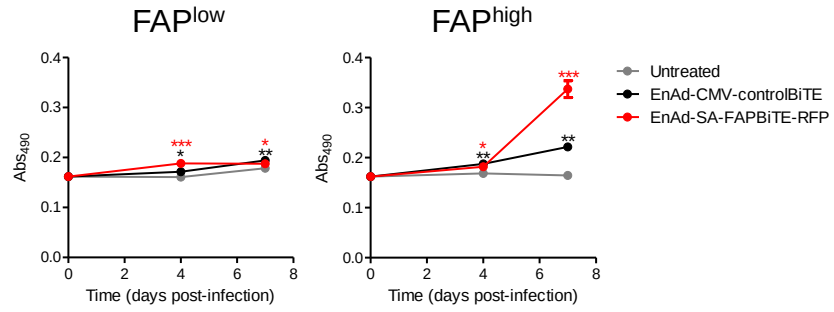

F
FAPlow
FAPhigh
Abs490
Time (days post-infection)
Untreated
EnAd-CMV-controlBiTE
EnAd-SA-FAPBiTE-RFP

Fig. S5. Infection and replication of armed EnAd viruses in *ex vivo* prostate tissue slice cultures. (A) Haematoxylin and eosin stained section of prostate tumour tissue slice, alongside immunohistochemistry staining for cellular markers of epithelial cells (EpCAM), activated fibroblasts (FAP) and cytotoxic T cells (CD8). **(B)** Schematic of the FAP BiTE-RFP expression cassette. The FAP BiTE and RFP are joined by a P2A sequence with an upstream furin cleavage site. A GSG spacer was included at the N-terminus of the P2A site to improve cleavage efficiency. Black triangle; P2A self-cleavage site. Ig SP, signal peptide; His_{10}, decahistidine tag. **(C)** Representative bright-field and fluorescence images showing prostate tissue slices infected with EnAd-SA-FAPBiTE-RFP. Scale bar, 200 μm. **(D, E)** IFNγ (D) and IL-2 (E) produced from FAP^{low} (benign) and FAP^{high} (malignant) prostate tissue slices infected with BiTE expressing EnAd. **(F)** LDH released from FAP^{low} (benign) and FAP^{high} (malignant) prostate tissue slices following BiTE-armed EnAd infection.

(D-F) Data show the mean ± SD of technical triplicates. Significance for each sample was assessed using one-way ANOVA with Tukey's Post Hoc analysis compared to 'uninfected'; *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$.

Table S1. Viral titre and TCID50 for purified virus stocks.

	Virus titre (vp/mL)	Infectious particles (PFU/mL)	vp:PFU ratio
EnAd	4.64×10^{12}	2.91×10^{11}	15.9
EnAd-CMV-FAPBiTE	1.02×10^{12}	7.94×10^{9}	128.6
EnAd-CMV-ControlBiTE	4.81×10^{11}	1.00×10^{10}	48.1
EnAd-SA-FAPBiTE	6.64×10^{11}	7.94×10^{9}	83.6
EnAd-SA-ControlBiTE	1.61×10^{12}	3.98×10^{10}	40.6

Table S2. Cellular composition of human ascites samples used in Figure 4.

Sample	Cancer type	Cell type (%)							
		EpCAM+	FAP+	EGFR+	CD11b+	CD3+	CD4+	CD8+	CD56+
1	Breast	0	1	1	5.4	79.4	63.1	16.5	3.92
2	Pancreatic	0.38	9.7	-	28.2	22.1	-	-	-
3	Ovarian	22.2	1.2	-	34.1	37.9	27.4	10.7	-
4	Ovarian	0.2	18.9	-	20.1	46.2	13.2	22.8	-

Table S3. Proportion of CD3+, CD11b+, EpCAM+ and FAP+ cells in malignant ascites biopsy samples used in the Nanostring analysis illustrated in Figure 5.

	Proportion of total cells in biopsy (%)			
	$EpCAM^+$	FAP^+	$CD3^+$	$CD11b^+$
Biopsy 4	3.3	19	46.2	20.2
Biopsy 5	14.6	9	55.9	22.3
Biopsy 6	47.3	4.7	32.8	19.6

Movie S1. **Time-lapse sequence of uninfected control cultures of DLD, NHDF and T cells.** Time-lapse sequences showing co-cultures of DLD carcinoma cells (unstained), NHDF fibroblasts (red) and CD3-purified PBMC (blue) infected with EnAd. Apoptosis was visualised using CellEvent Caspase 3/7 detection reagent (green). Images were collected at intervals of 15 minutes covering a period of 72 hours; original magnification ×10.

Movie S2. Time-lapse sequence of EnAd-mediated cytotoxicity of DLD cells. Time-lapse sequences showing co-cultures of DLD carcinoma cells (unstained), NHDF fibroblasts (red) and CD3-purified PBMC (blue) infected with EnAd. Apoptosis was visualised using CellEvent Caspase 3/7 detection reagent (green). Images were collected at intervals of 15 minutes covering a period of 72 hours; original magnification ×10.

Movie S3. Time-lapse sequence of EnAd-CMV-FAPBiTE-mediated cytotoxicity of DLD and NHDF cells. Time-lapse sequences showing co-cultures of DLD carcinoma cells (unstained), NHDF fibroblasts (red) and CD3-purified PBMC (blue) infected with EnAd. Apoptosis was visualised using CellEvent Caspase 3/7 detection reagent (green). Images were collected at intervals of 15 minutes covering a period of 72 hours; original magnification ×10.