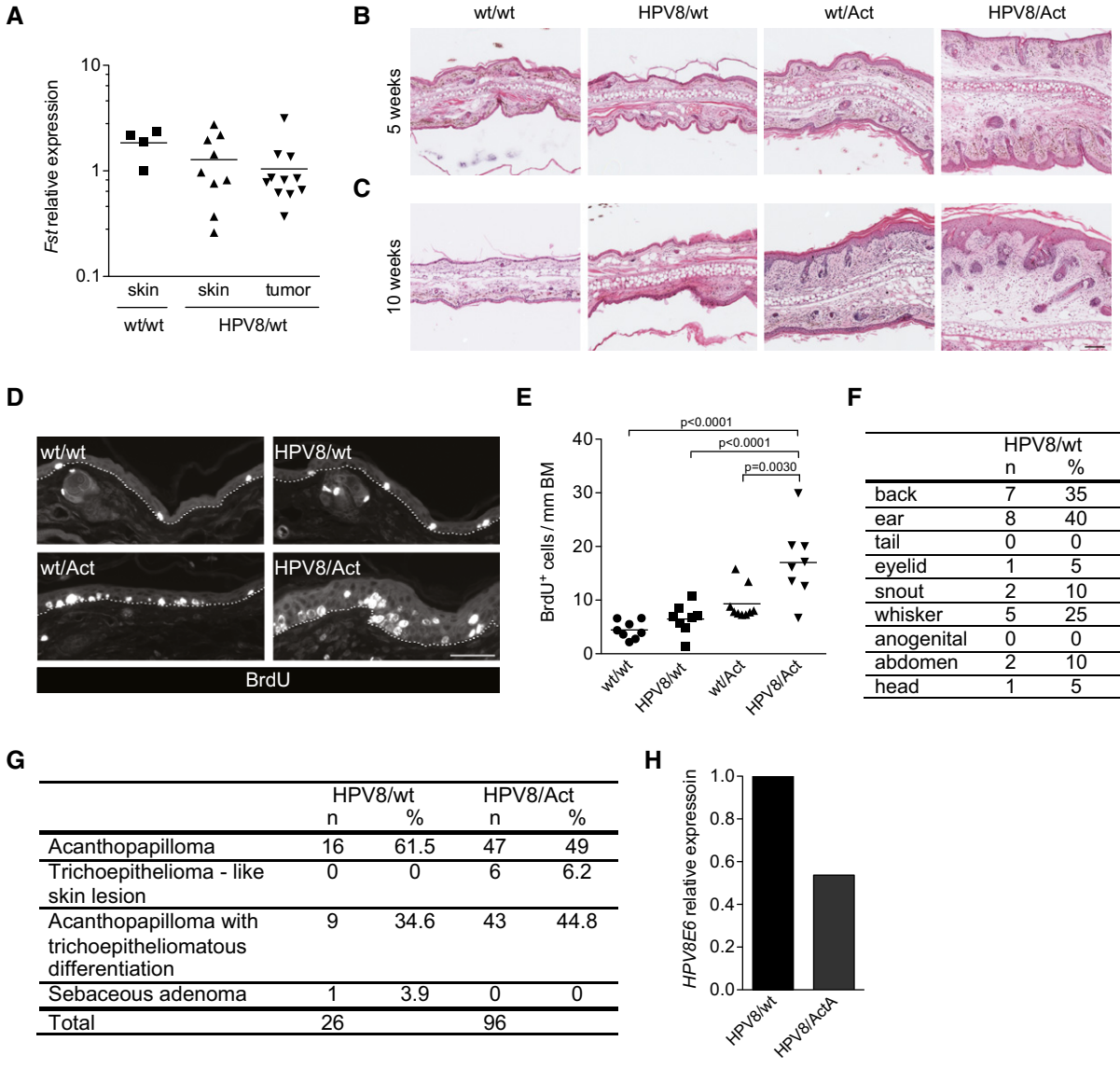
Expanded View Figures



F

	HPV8/wt		HPV8/Act	
	n	%	n	%
back	7	35	14	70
ear	8	40	20	100
tail	0	0	7	35
eyelid	1	5	18	90
snout	2	10	17	85
whisker	5	25	7	35
anogenital	0	0	3	15
abdomen	2	10	2	10
head	1	5	1	5

G

	HPV8/wt		HPV8/Act	
	n	%	n	%
Acanthopapilloma	16	61.5	47	49
Trichoepithelioma - like skin lesion	0	0	6	6.2
Acanthopapilloma with trichoepitheliomatous differentiation	9	34.6	43	44.8
Sebaceous adenoma	1	3.9	0	0
Total	26		96	

Figure EV1. Characterization of skin tumors in HPV8/wt and HPV8/Act mice.

A RNA samples from normal back skin of wt mice (N = 4, n = 4), non-affected back skin from HPV8/wt mice (N = 7, n = 9), and papillomas from HPV8/wt transgenic mice (N = 8, n = 11) were analyzed for the expression follistatin (*Fst*) relative to *Gapdh* by qRT–PCR. Expression level in one of the wild-type back skin samples was set arbitrarily to 1. N, number of mice; n, number of biopsies.

B, C Representative pictures of H&E-stained ear skin sections of 5 (B)- or 10 (C)-week-old mice. Scale bar: 100 μm.

D Representative pictures of BrdU-stained ear sections of 5-week-old mice. Dotted lines indicate the epidermal–dermal border. Scale bar: 50 μm.

E Quantification of BrdU-positive cells per mm of basement membrane (BM). N = 8 wt/wt, HPV8/wt, or HPV8/Act mice; N = 9 wt/Act mice. Statistical significance was determined using one-way ANOVA and Bonferroni's multiple comparisons test.

F Body sites where tumor development in HPV8/wt and HPV8/Act mice was observed; n, number of mice with tumors at the indicated body site; %, percentage of mice with at least one tumor at the respective body site, N = 20 mice per genotype.

G Histopathological analysis of tumors collected from HPV8/wt and HPV8/Act mice by a blinded histopathologist. n, number of tumors; %, percentage of tumors with the respective diagnosis among all tumors.

H RNA samples from MACS-sorted keratinocytes ($CD45^-$ epidermal fraction) were analyzed for the expression of the *HPV8E6* transgene relative to *Rps29* by qRT–PCR. Expression level in keratinocytes from HPV8/wt mice was set arbitrarily to 1. The experiment was performed with cells isolated and pooled from the ears of six mice per genotype.

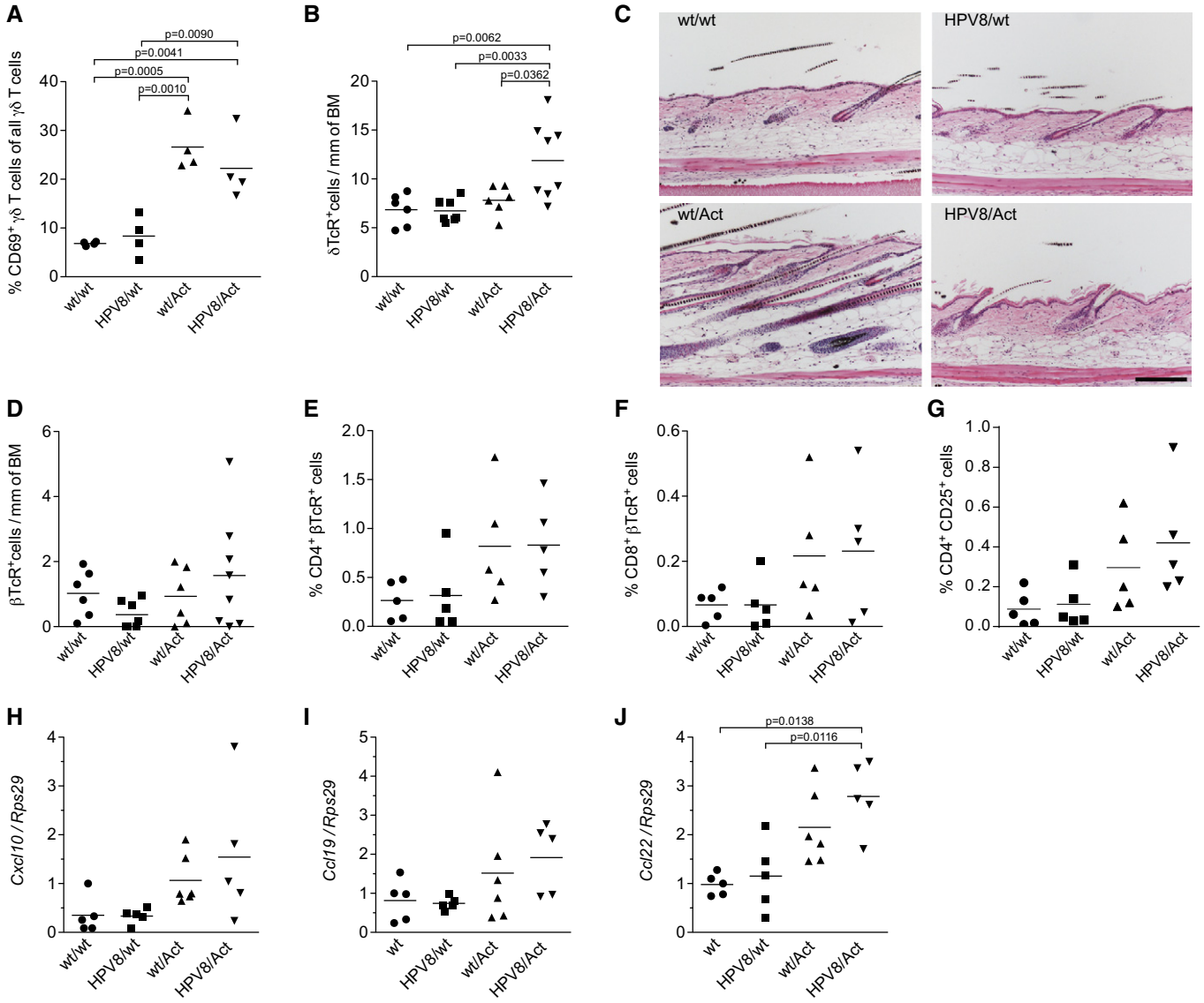


Figure EV2. Activin affects T cells and expression of T-cell chemokines in the skin.

A Percentage of activated CD69$^+$ γδ T cells in the ear skin epidermis of 14-week-old mice, analyzed by flow cytometry. wt/wt mice (N = 4); HPV8/wt mice (N = 4); wt/Act mice (N = 4); HPV8/Act mice (N = 4). N, number of mice.

B Quantification of δTcR$^+$ cells in cryosections of back skin from 10-week-old wt/wt mice (N = 6), HPV8/wt mice (N = 7), wt/Act mice (N = 6), and HPV8/Act mice (N = 8).

C Representative pictures of H&E-stained sections of back skin at 10 weeks of age. Scale bar: 100 μm.

D Quantification of βTcR$^+$ cells in cryosections of back skin from 10-week-old wt/wt mice (N = 6), HPV8/wt mice (N = 7), wt/Act mice (N = 6), and HPV8/Act mice (N = 8).

E–G Quantification of CD4$^+$ (E), CD8$^+$ (F), and CD4$^+$CD25$^+$ (G) T cells in the ear skin dermis of 14-week-old mice by flow cytometry. Results from five experiments with ears pooled from at least three mice per genotype are shown.

H–J RNA samples from total ear skin were analyzed for the expression of chemokines relative to *Rps29* by qRT–PCR. Expression in one of the wild-type ear skin samples was set to 1. Log_2-transformed data were used for statistical analysis. wt/wt mice (N = 5); HPV8/wt mice (N = 5); wt/Act mice (N = 6); HPV8/Act mice (N = 5).

Data information: Statistical significance was determined using one-way ANOVA and Bonferroni's multiple comparisons test of original (A–G) or log-transformed (H–J) data.

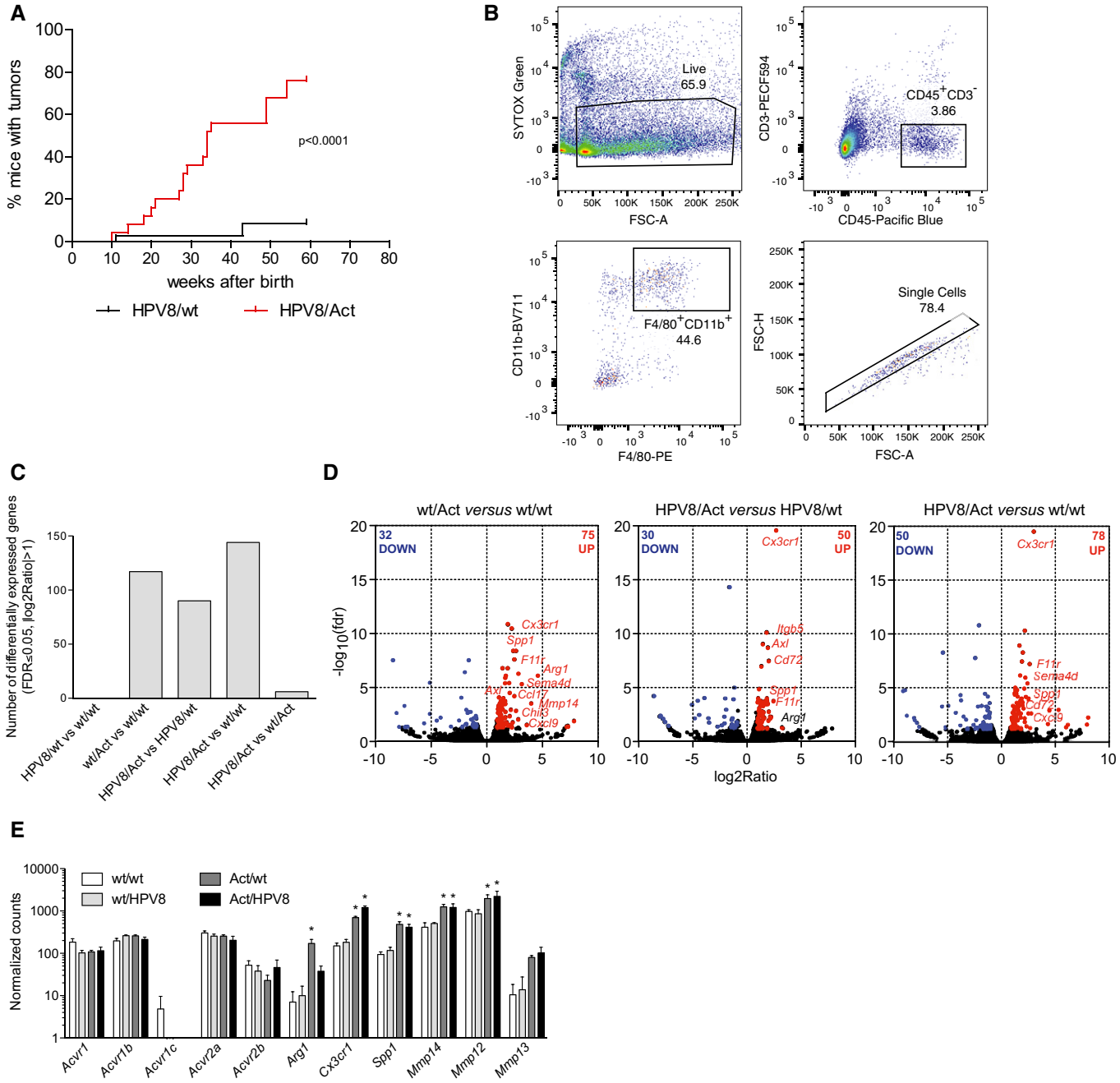


Figure EV3. Activin induces a pro-tumorigenic gene expression pattern in macrophages.

A Kinetics of tumor incidence in HPV8/wt and HPV8/Act mice after transfer of the mice into a new animal facility; N = 32 for HPV/wt, N = 23 for HPV8/Act mice. Statistical significance was determined using the log-rank (Mantel–Cox) test. N, number of mice.

B Gating strategy to enrich macrophages from ear skin of 13- to 15-week-old female mice by FACS. Representative pseudo-color plots of one of the wt/wt samples are shown. Single F4/80$^+$CD11b$^+$ cells were sorted after gating on single viable (SYTOX Green-negative) CD45$^+$CD3$^-$ cells.

C Number of differentially expressed genes (FDR < 0.05, $|\log_2 FC| > 1$) identified by RNA sequencing of F4/80$^+$CD11b$^+$ cells isolated from ear skin of 13- to 15-week-old female mice by FACS. Each replicate represents cells sorted from a pool of 3–6 mice per genotype in an independent experiment.

D Differential expression of genes was analyzed using the edgeR software package. Volcano plots show comparisons between wt/Act and wt/wt mice, HPV8/Act and HPV8/wt mice, or HPV8/Act and wt/wt mice. Significantly upregulated genes (FDR ≤ 0.05, $\log_2$ratio > 1) are highlighted in red, downregulated genes (FDR ≤ 0.05, $\log_2$ratio < −1) in blue.

E Normalized counts of transcripts for activin receptors and several tumor-promoting genes identified by RNA sequencing. N = 3. *FDR < 0.05 for comparison with wt/wt mice. Statistical significance was determined using exact test adapted for over-dispersed data. Exact P-values for different comparisons can be found in Dataset EV2. N = number of pools of 3–6 mice, mean ± SEM.