

Supplementary Information

HPV8 Field Cancerization in a Transgenic Mouse Model is due to Lrig1+ Keratinocyte Stem Cell Expansion

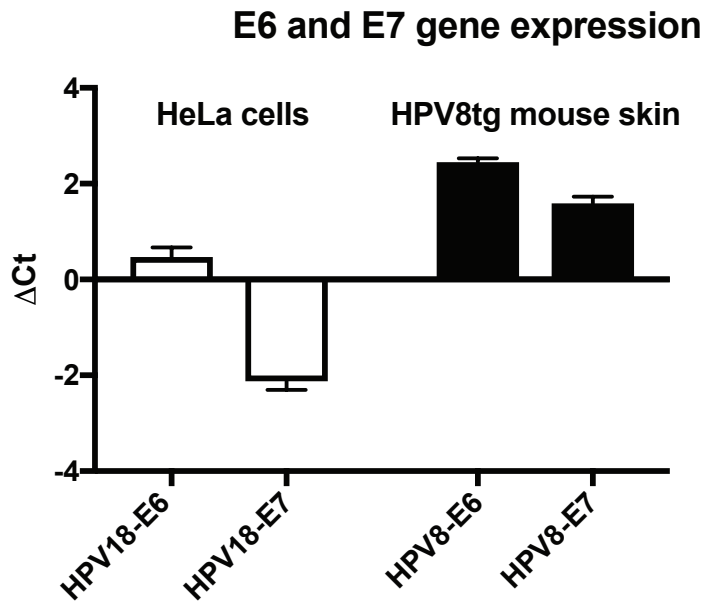
Simone Lanfredini^{1*}, Carlotta Olivero^{2*}, Cinzia Borgogna², Federica Calati², Kathryn Powell¹, Kelli-Jo Davies¹, Marco De Andrea^{2,3}, Sarah Harries¹, Hiu Kwan Carolyn Tang¹, Herbert Pfister⁴, Marisa Gariglio², Girish K Patel¹.

1. European Cancer Stem Cell Research Institute, School of Biosciences, Cardiff University, Maindy Road, Cardiff, UK
2. Virology Unit, Department of Translational Medicine, Novara Medical School, Novara, Italy
3. Viral Pathogenesis Unit, Department of Public Health and Pediatric Sciences, Turin Medical School, Turin, Italy
4. Institute of Virology, University of Cologne, Cologne, Germany

* The first two authors contributed equally to this work.

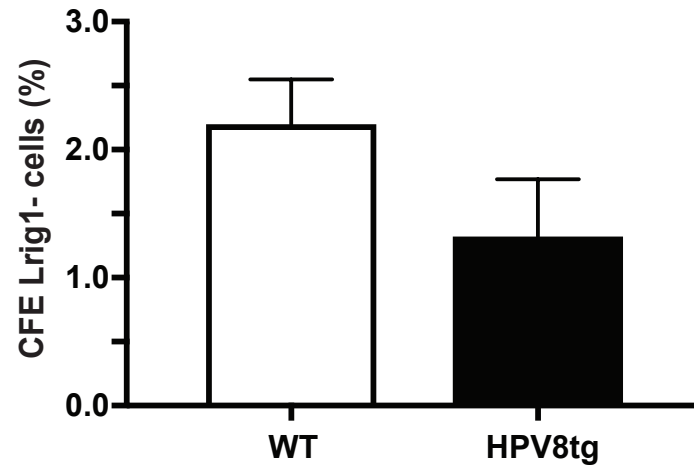
** Marisa Gariglio and Girish K Patel are joint senior authors.

Reprint request and correspondence to: Dr Girish K Patel MD, European Cancer Stem Cell Research Institute, School of Biosciences, Cardiff University, Maindy Road, Cardiff, CF24 4HQ, UK. E-mail: patelgk@cardiff.ac.uk



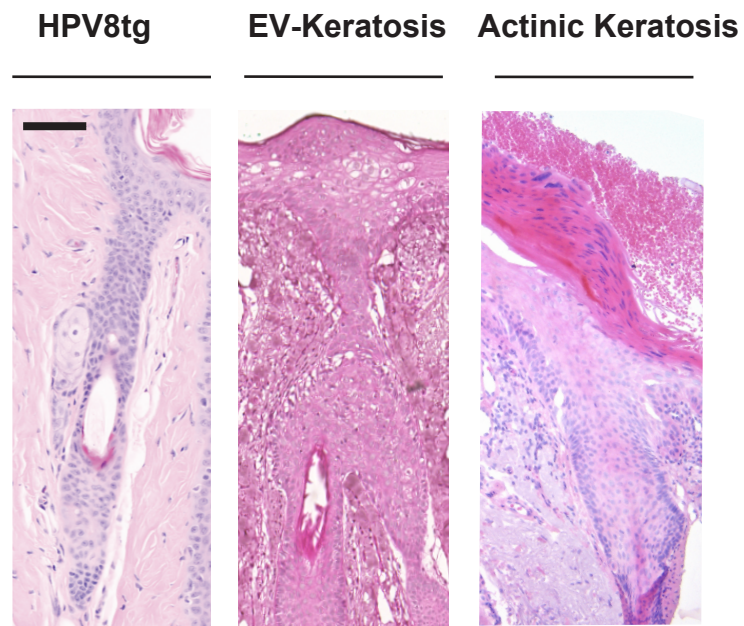
Supplementary Figure S1: Expression of E6 and E7 genes of HPV8 and HPV18 in HPV8tg mouse skin and HeLa cells respectively.

qRT-PCR of E6 and E7 genes from RNA isolated from HeLa cells and HPV8tg mouse skin, relative to β -Actin, with mean \pm SD.



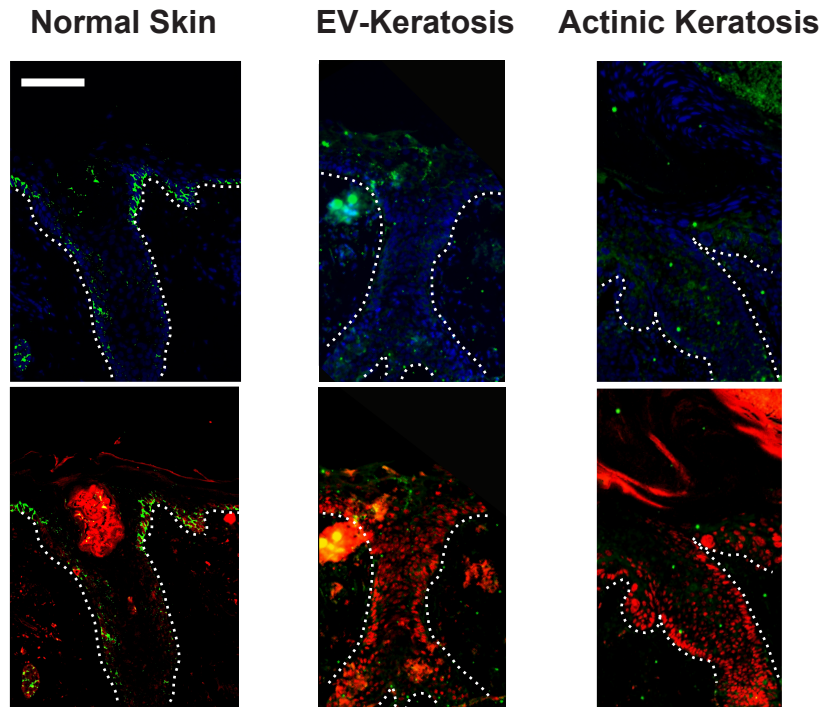
Supplementary Figure S2: Colony forming efficiency from the flow sorted Lrig1 negative keratinocyte populations

Keratinocyte colony forming assays from flow sorted Lrig1 negative cells from WT and HPV8tg (n=6) skin dissociates, with mean \pm SD.



Supplementary Figure S3: H&E staining on HPV8tg mouse skin, EV-Keratosis and Actinic Keratosis

Hematoxylin and eosin staining of paraffin embedded skin sections from HPV8tg mouse, EV-Keratosis and Actinic Keratosis corresponding to figure 5d. Scale bar is 100 μ m.



Supplementary Figure S4: Lrig1 immunolabelling of human skin, EV Keratosis and Actinic Keratosis.

Lrig1 (green) and p63 (red) immunofluorescent immunolabelling of formalin fixed paraffin embedded tissue sections from normal human skin, EV Keratosis and Actinic Keratosis corresponding to figure 5d. The sections were counter stained with DAPI (blue). Scale bar is 100 μ m.

Supplementary Table S1: Summary of EV patient lesions

EV patient	EVER 1/2 genetics	T-Cell phenotype ¹	Year of birth	Nature of lesion ²	Anatomic site	Year of lesion excision	References
1	WT	Slightly low CD3 Normal CD4 Low CD8	1967	Wart-like lesion	forearm	2003	1,2
				SCC	temple	2003	
				SCC	forehead	2003	
				AK	elbow	2003	
				Bowenoid lesion	forehead	2003	
2	WT	Normal CD3 Slightly low CD4 Normal CD8	1947	Wart-like lesion	forearm	2005	2,3
3	WT	Low CD3 Low CD4 Normal CD8	1969	SCC	temple	1999	2
				Bowenoid lesion	temple	1999	
				Wart-like lesion	forearm	1999	
4	EVER 2 mutation (p.Y109fsX118)	Normal CD3 Normal CD4 Normal CD8	1948	Bowenoid lesion	back	1990	2,4,5
				SCC	eyelid	1993	
				BCC	eyelid	2007	
				SCC/AK	cheek bone	2007	
				SCC	temple	2008	
				SCC	eyelid	2008	
				SCC	nose	2008	
				SCC	nose	2008	
				AK	eyelid	2008	
SCC	nose	2008					
5	EVER 2 mutation (p.V191fsX226)	Normal CD3 Normal CD4 Normal CD8	1952	Bowenoid lesion	temple	2005	4,5
				Bowenoid lesion	ear	2005	
				Bowenoid lesion	forehead	2007	
				Keratosis	lower lip	2007	
				SK	back	2007	
				Keratosis	back	2007	
				AK	scalp	2007	
				SCC	scalp	2008	
				Bowenoid lesion	neck	2008	
				SCC	scalp	2008	
				SCC	nose	2008	
				SCC	ear	2008	
				Bowenoid lesion	lower lip	2008	
6	WT	Normal CD3 Low CD4 Normal CD8	1958	Wart-like lesion	leg	1991	6
				Wart-like lesion	dorsum of hand	1991	
				Wart-like lesion	leg	1991	
				Skin fibroma	armpit	1997	
				Wart-like lesion	elbow	2003	
				Wart-like lesion	elbow	2005	
				AK	temple	2006	
				Bowenoid lesion	scapula	2008	
				SCC	forehead	2010	
				SCC	forehead	2010	
				Bowenoid lesion	temple	2010	
AK	temple	2010					

¹Slightly low = greater than 20% but less than 50% reduction in total count; low = less than 50% reduction in total count

²SCC (Squamous Cell Carcinoma), BCC (Basal Cell Carcinoma), AK (Actinic Keratosis), SK (Seborrheic Keratosis).

Supplementary Table S2: Antibody list:

Antibody	Cat.No	Company	Technique
K15	MA1-90929	ThermoFisher Scientific	WM
Ki67	ab16667	Abcam	WM
LGR6	ab12747	Abcam	WM
LGR5	NLS1236	Novus Biologicals	WM
CD34 (RAM34)	553731	BD Pharmingen	WM
CD34	560230	BD Pharmingen	FACS
Lrig1 (C2C3)	GTX119485	GeneTex	IF
Lrig1	AF3688	R&D Systems	WM
Lrig1	FAB3688G	R&D Systems	FACS
p63 (BC4A4)	ab735	Abcam	IF
K14	ab130102	Abcam	IF
β -HPV E4		*	IF
p63 (Δ N)	619002	Biolegend	WB
p63 (TA)	618902	Biolegend	WB
GAPDH	MAB374	Millipore	WB

*E4 β -HPV antibody was kindly provided by John Doorbar, University of Cambridge-UK.

Supplementary Table S3: RT-PCR primers:

mΔNp63 fw	5' ATGTTGTACCTGGAAAACAATG 3'
mΔNp63 rev	5' GATGGAGAGAGGGGCATCAAA 3'
mTAp63 fw	5' AGACAAGCGAGTTCCTCAGC 3'
mTAp63 rev	5' TGCGGATACAATCCATGCTA 3'
HPV8-E6 fw	5' GCGGCTTTAGGTATTCCATTGC 3'
HPV8-E6 rev	5' GCTACACAACAACAACGACAACACG 3'
HPV8-E7 fw	5' CCTGAAGTGTTACCAGTTGACCTGC 3'
HPV8-E7 rev	5' CAGTTGCGTTGACAAAAAGACG 3'
mβ-actin fw	5' CCAGAGCAAGAGAGGTATCCTGAC 3'
mβ-actin rev	5' CATTGTAGAAGGTGTGGTGCCAG 3'
mGAPDH fw	5' TGTCAGCAATGCATCCTGCA 3'
mGAPDH rev	5' TGTATGCAGGGATGATGTTC 3'
HPV18-E6 fw*	5' GTGCCAGAAACCGTTGAATC 3'
HPV18-E6 rev*	5' TTGTGTTTCTCTGCGTCGTT 3'
HPV18-E7 fw*	5' TGAAATTCCGGTTGACCTTC 3'
HPV18-E7 rev*	5' CACGGACACACAAAGGACAG 3'

*Lo Cigno I, De Andrea M, Borgogna C, Albertini S, Landini MM, Peretti A, Johnson KE, Chandran B, Landolfo S, Gariglio M. The Nuclear DNA Sensor IFI16 Acts as a Restriction Factor for Human Papillomavirus Replication through Epigenetic Modifications of the Viral Promoters. J Virol. 2015 Aug;89(15):7506-20.

Supplementary Table S4: Taqman primers:

ΔNp63	Mm01169470_m1
TAp63	Mm01150797_m1
Tmc6- EVER1	Mm00520751_m1
Tmc8- EVER2	Mm01241000_m1
β-actin	Mm02619580_g1
GAPDH	Mm99999915_g1