Supplementary information

A Novel Tumor suppressor network in squamous malignancies

Clotilde Costa[1], Mirentxu Santos[1], Carmen Segrelles[1], Marta Dueñas[1], M Fernanda Lara[1,2], Xabier Agirre[3], Felipe Prosper[3], Ramón García-Escudero[1] and Jesús M. Paramio[1,*]

1.-Molecular Oncology Unit, Department of Basic Research, CIEMAT (Ed 70A), Ave Complutense 40. 28040 Madrid (Spain)

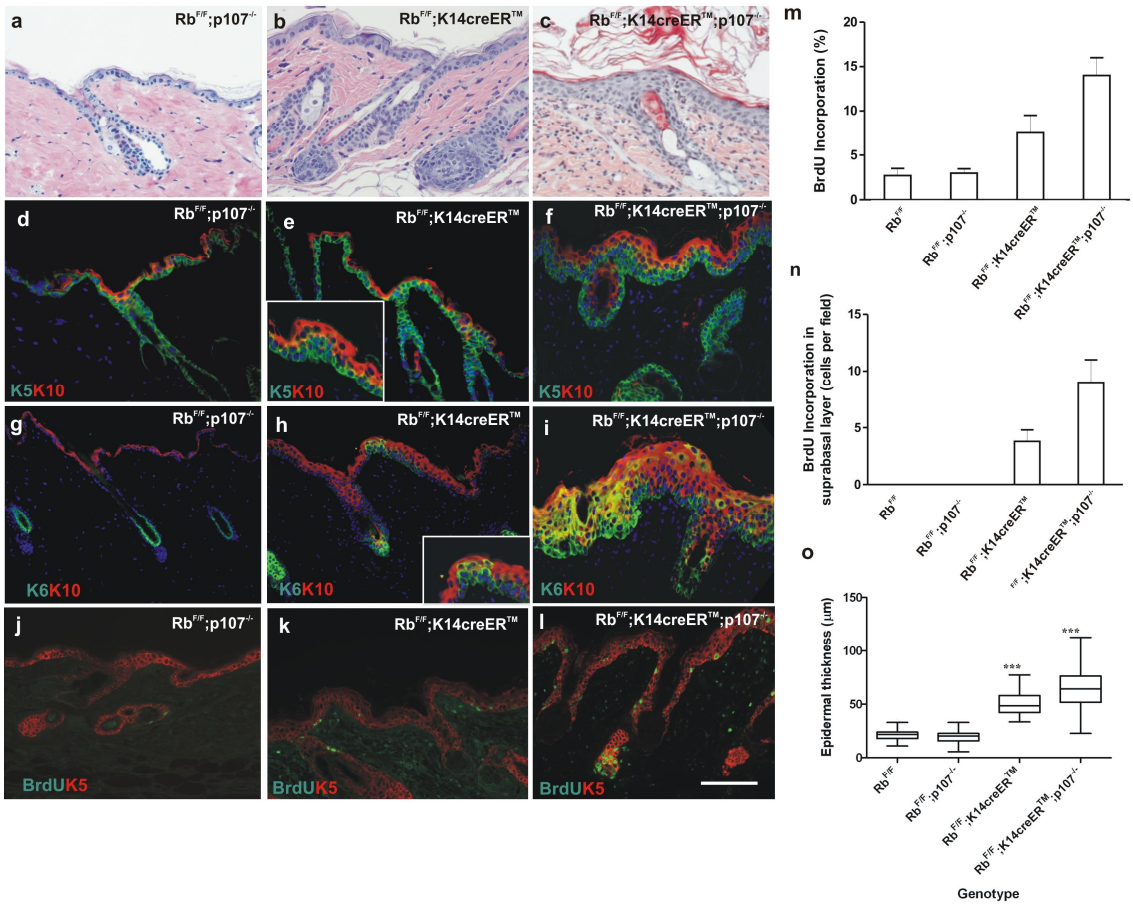
2.- Present Address: Departments of Pediatrics and the Program in Molecular Imaging, Stanford University, Stanford, CA

3.- Center for Applied Medical Research, University of Navarra, Ave. Pío XII, 55 E-31008 Pamplona Spain

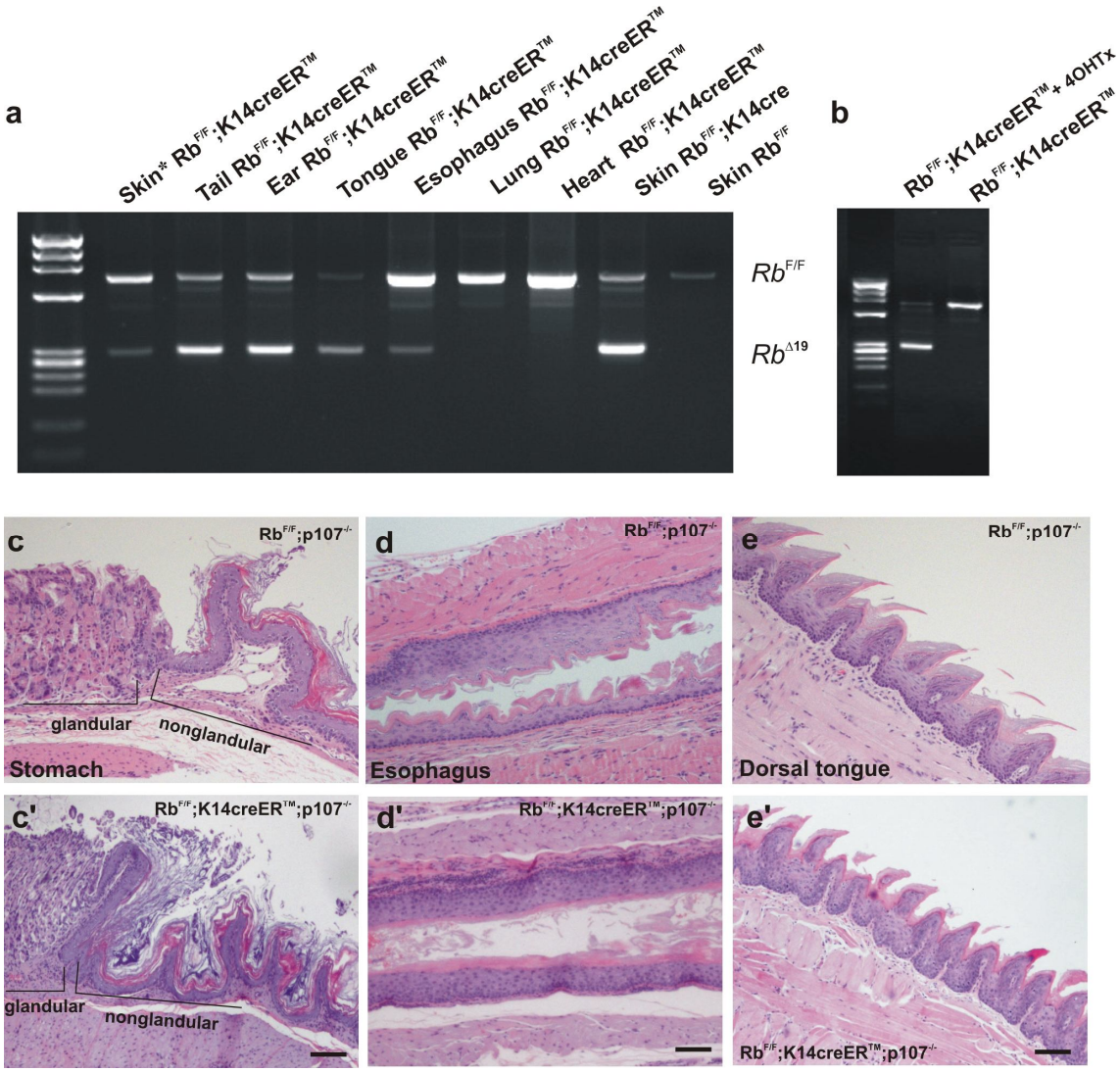
* Corresponding Author jesusm.paramio@ciemat.es

Running Title: Functional connection between p107, pRb, Pten and p53 in squamous tumors

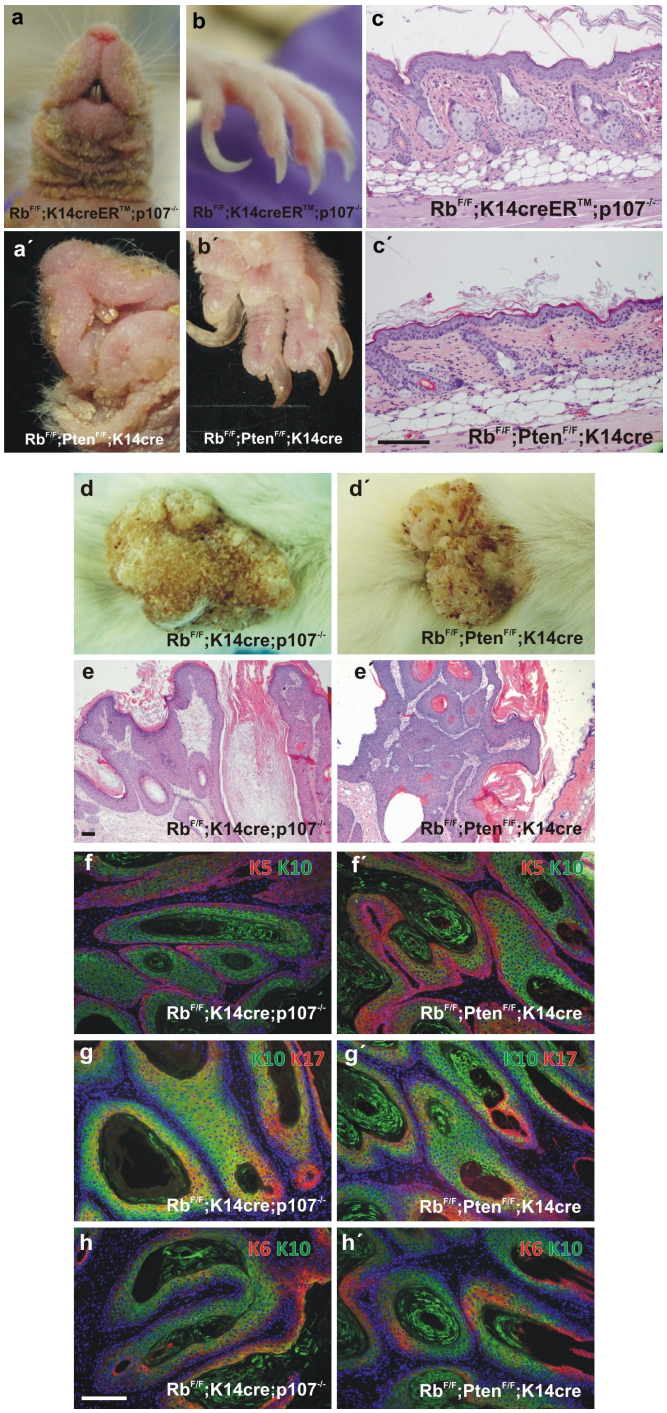
Supplementary figures



Supplementary Figure 1. Differentiation and proliferation analysis in $Rb^{F/F}$;K14creER^{TM}; $p107^{-/-}$ mouse skin. a-c) H&E stained sections of skin of the quoted genotypes. d-l) Representative immunofluorescence of K5 (green) and K10 (red) (d-f); K6 (green) and K10 (red) (g-i); K5 (red) and BrdU incorporation (green) (j-l) of the quoted genotypes. m, n) BrdU incorporation in percentage in basal cells (m) and in suprabasal layer (cells per field) (n) of the quoted genotypes. o) Epidermal thickness (µm) measure in three sections per mice (n=5) of the quoted genotypes; are shown as mean ± s.e.m. Bars= 150µm

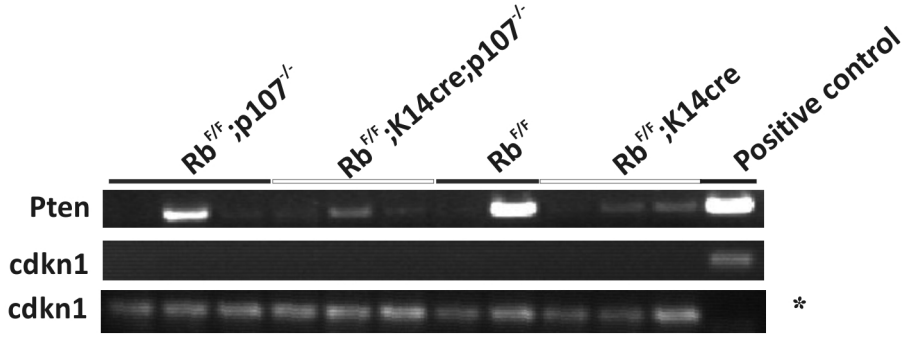


Supplementary Figure 2. Topical tamoxifen treatment induces recombination in untreated areas. a, b) Representative PCR analysis of *Rb1* deletion in different tissues (a) or primary keratinocytes (b) of the quoted genotypes. *Rb1* deletion is represented as $Rb^{\Delta 19}$.* DNA sample from an untreated area of a tamoxifen treated mouse backskin. Of note, K14 promoter is not expressed in lung and heart. c-e') Absence of gross phenotypic changes in specific K14-expressing tissues after recombination. c-e) control (RbF/F;p107-/-) and (c'- e') $Rb^{F/F}$;K14creER^{TM}; $p107^{-/-}$ mouse samples form stomach (c, c'), esophagus (d, d') and dorsal tongue (e-e'). Bars= 150µm



Supplementary Figure 3. Rb$^{F/F}$;K14creER^{TM};p107$^{-/-}$ mouse phenotype resembles Rb$^{F/F}$;Pten$^{F/F}$;K14cre mice. External aspect of ventral neck area (a, a´); abnormal growth of nails (b, b´) and H&E stained skin section (c, c´) showing hyperplasia, hyperqueratosis and inflammatory infiltrate in Rb$^{F/F}$;K14creER^{TM};p107$^{-/-}$ (a, b, c) and Rb$^{F/F}$;Pten$^{F/F}$;K14cre (a´,b´,c´) mice. d, d´) Spontaneous squamous carcinomas from new born skin from Rb$^{F/F}$;K14cre;p107$^{-/-}$ (d) and Rb$^{F/F}$;Pten$^{F/F}$;K14cre (d´) mice transplanted

in immunodeficient mice. e, e´) H&E stained section of the carcinomas from $Rb^{F/F}$;K14cre;$p107^{-/-}$ (e) and $Rb^{F/F}$;$Pten^{F/F}$;K14cre (e´). f-h´) Representative immunofluorescence of K5 (red) and K10 (green) (f, f´); K10 (green) and K17 (red) (g, g´); K6 (red) and K10 (green) (h, h´) of the quoted genotypes. Bars= 150μm



Supplementary Figure 4. Pten gene methylation analysis. Methylation Specific PCR for *Pten* and *cdkn1* gene promoters in DNA samples from new born skin of mice of the quoted genotype. * Control Unmethylated cdkn1 gene.

Supp Table S3

Overlapping between Overexpressed genes (n=855) in RbF/F;K14CreERTM;p107-/- mouse tumors and human SCC Samples under the Concept Over-expression tumor vs. normal tissue from Oncomine

Concept Name	Overlap	p-Value	q-Value	Odds Ratio	Dataset
Squamous Cell Lung Carcinoma vs. Normal - Top 10% Over-expressed (Hou Lung)	276	5.01E-80	2.87E-76	5.1	Hou Lung
Cervical Cancer vs. Normal - Top 10% Over-expressed (Pyeon Multi-cancer)	232	2.02E-51	1.48E-48	3.9	Pyeon Multi-cancer
Nasopharyngeal Carcinoma vs. Normal - Top 10% Over-expressed (Sengupta Head-Neck)	219	5.20E-44	2.28E-41	3.5	Sengupta Head-Neck
Cervical Squamous Cell Carcinoma vs. Normal - Top 5% Over-expressed (Scotto Cervix 2)	118	1.24E-36	3.29E-34	5	Scotto Cervix 2
Floor of the Mouth Carcinoma vs. Normal - Top 10% Over-expressed (Pyeon Multi-cancer)	201	1.41E-34	3.27E-32	3.1	Pyeon Multi-cancer
Oral Cavity Carcinoma vs. Normal - Top 10% Over-expressed (Pyeon Multi-cancer)	200	4.40E-34	9.83E-32	3.1	Pyeon Multi-cancer
Cervical Squamous Cell Carcinoma Epithelia vs. Normal - Top 5% Over-expressed (Zhai Cervix)	114	6.97E-34	1.54E-31	4.7	Zhai Cervix
Squamous Cell Lung Carcinoma vs. Normal - Top 10% Over-expressed (Wachi Lung)	167	1.68E-32	3.44E-30	3.4	Wachi Lung
Squamous Cell Lung Carcinoma vs. Normal - Top 10% Over-expressed (Garber Lung)	145	1.11E-28	1.87E-26	3.5	Garber Lung
Tongue Squamous Cell Carcinoma vs. Normal - Top 10% Over-expressed (Ye Head-Neck)	188	2.18E-28	3.60E-26	2.8	Ye Head-Neck
Squamous Cell Lung Carcinoma vs. Normal - Top 10% Over-expressed (Bhattacharjee Lung)	123	4.94E-28	8.07E-26	3.9	Bhattacharjee Lung
Tongue Squamous Cell Carcinoma vs. Normal - Top 10% Over-expressed (Estilo Head-Neck)	122	2.40E-27	3.69E-25	3.8	Estilo Head-Neck
Tongue Squamous Cell Carcinoma vs. Normal - Top 10% Over-expressed (Talbot Lung)	120	3.49E-26	4.97E-24	3.7	Talbot Lung
Cervical Squamous Cell Carcinoma vs. Normal - Top 10% Over-expressed (Biewenga Cervix)	176	1.57E-22	1.83E-20	2.5	Biewenga Cervix
Thyroid Gland Undifferentiated (Anaplastic) Carcinoma vs. Normal - Top 10% Over-expressed (Giordano Thyroid)	141	7.65E-20	7.65E-18	2.7	Giordano Thyroid
Tongue Carcinoma vs. Normal - Top 10% Over-expressed (Pyeon Multi-cancer)	167	1.48E-19	1.47E-17	2.4	Pyeon Multi-cancer
Oropharyngeal Carcinoma vs. Normal - Top 10% Over-expressed (Pyeon Multi-cancer)	164	2.04E-18	1.88E-16	2.4	Pyeon Multi-cancer
Squamous Cell Lung Carcinoma vs. Normal - Top 10% Over-expressed (Talbot Lung)	103	3.17E-17	2.68E-15	3	Talbot Lung
Tonsillar Carcinoma vs. Normal - Top 10% Over-expressed (Pyeon Multi-cancer)	153	1.64E-14	1.11E-12	2.1	Pyeon Multi-cancer
Thyroid Gland Oncocytic Follicular Carcinoma vs. Normal - Top 10% Over-expressed (Giordano Thyroid)	116	2.03E-10	9.98E-09	2.1	Giordano Thyroid
Tall Cell Variant Thyroid Gland Papillary Carcinoma vs. Normal - Top 10% Over-expressed (Giordano Thyroid)	113	1.74E-09	7.84E-08	2	Giordano Thyroid
Follicular Variant Thyroid Gland Papillary Carcinoma vs. Normal - Top 5% Over-expressed (Giordano Thyroid)	64	1.18E-07	4.29E-06	2.2	Giordano Thyroid
Tongue Squamous Cell Carcinoma vs. Normal - Top 10% Over-expressed (Kuriakose Head-Neck)	77	5.76E-07	1.92E-05	2	Kuriakose Head-Neck
Squamous Cell Lung Carcinoma vs. Normal - Top 10% Over-expressed (Yamagata Lung)	29	6.68E-04	0.012	2.2	Yamagata Lung

Supp Table S4

Overlapping between Underexpressed genes (n=753) in RbF/F;K14CreERTM;p107-/- mouse tumors and human SCC Samples under the Concept Under-expression tumor vs. normal tissue from Oncomine

Concept Name	Overlap	P-Value	Q-Value	Odds Ratio	Dataset
Squamous Cell Lung Carcinoma vs. Normal - Top 10% Under-expressed (Hou Lung)	162	2.14E-22	1.58E-19	2.7	Hou Lung
Tongue Squamous Cell Carcinoma vs. Normal - Top 10% Under-expressed (Estilo Head-Neck)	111	3.64E-20	2.08E-17	3.2	Estilo Head-Neck
Cervical Squamous Cell Carcinoma vs. Normal - Top 10% Under-expressed (Biewenga Cervix)	145	1.47E-15	4.31E-13	2.3	Biewenga Cervix
Squamous Cell Lung Carcinoma vs. Normal - Top 10% Under-expressed (Wachi Lung)	116	1.11E-13	2.47E-11	2.4	Wachi Lung
Tongue Squamous Cell Carcinoma vs. Normal - Top 10% Under-expressed (Ye Head-Neck)	138	3.01E-13	6.32E-11	2.1	Ye Head-Neck
Tongue Squamous Cell Carcinoma vs. Normal - Top 10% Under-expressed (Talbot Lung)	91	3.27E-11	4.60E-09	2.4	Talbot Lung
Squamous Cell Lung Carcinoma vs. Normal - Top 10% Under-expressed (Garber Lung)	102	3.09E-10	3.53E-08	2.2	Garber Lung
Squamous Cell Lung Carcinoma vs. Normal - Top 10% Under-expressed (Bhattacharjee Lung)	88	3.89E-10	4.35E-08	2.3	Bhattacharjee Lung
Squamous Cell Lung Carcinoma vs. Normal - Top 10% Under-expressed (Talbot Lung)	82	5.28E-08	3.58E-06	2.1	Talbot Lung
Tongue Squamous Cell Carcinoma vs. Normal - Top 5% Under-expressed (Kuriakose Head-Neck)	48	5.46E-07	2.89E-05	2.4	Kuriakose Head-Neck