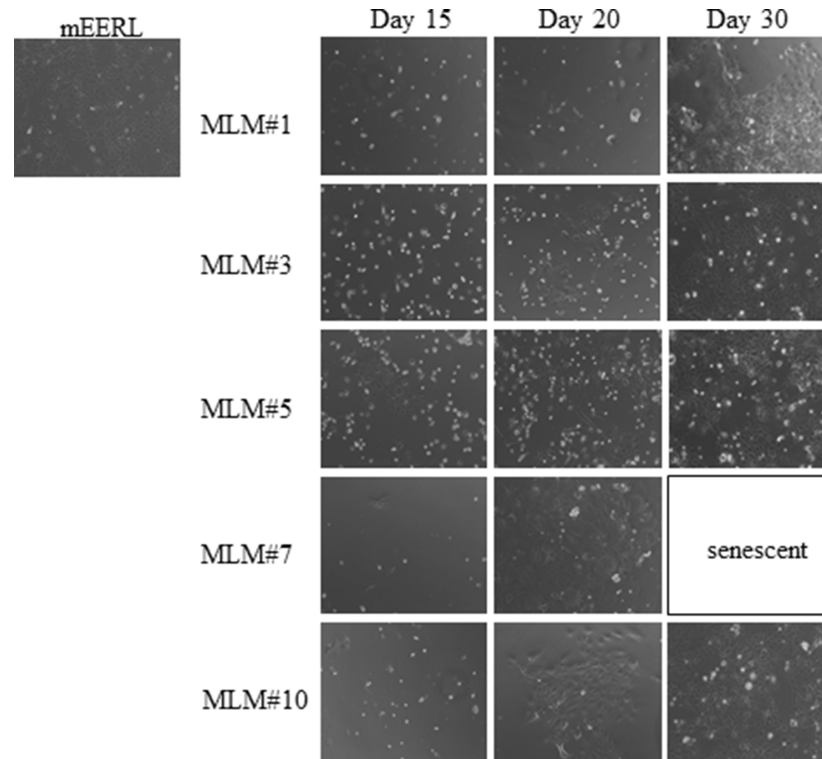
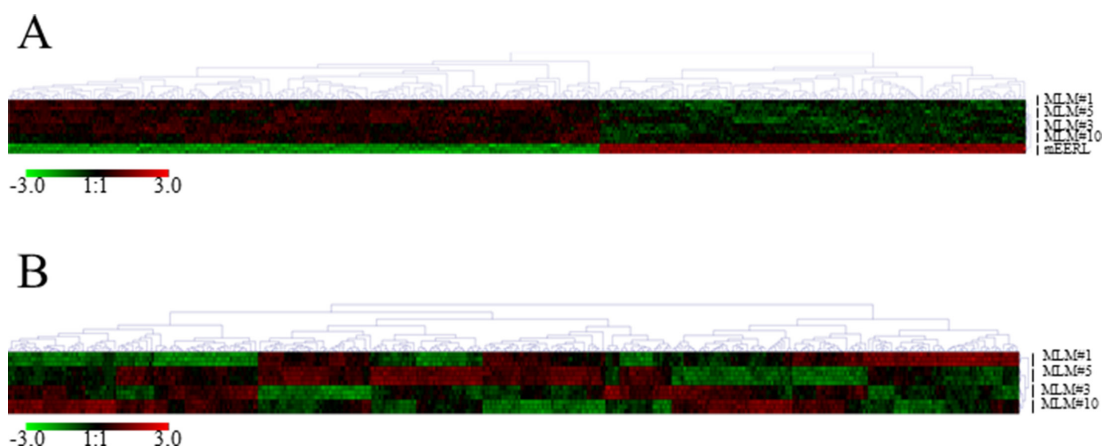


Metastatic model of HPV+ oropharyngeal squamous cell carcinoma demonstrates heterogeneity in tumor metastasis

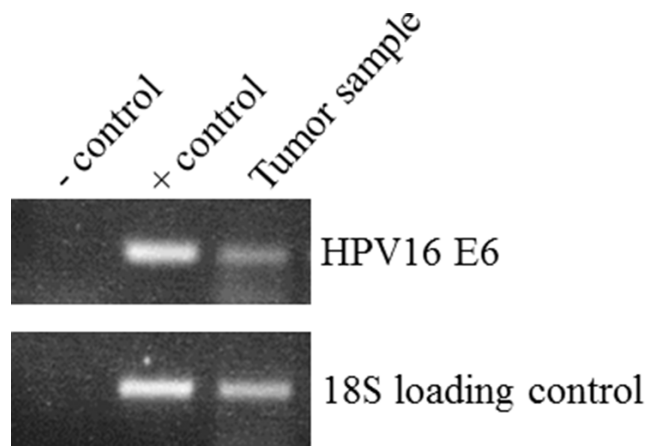
Supplementary Materials



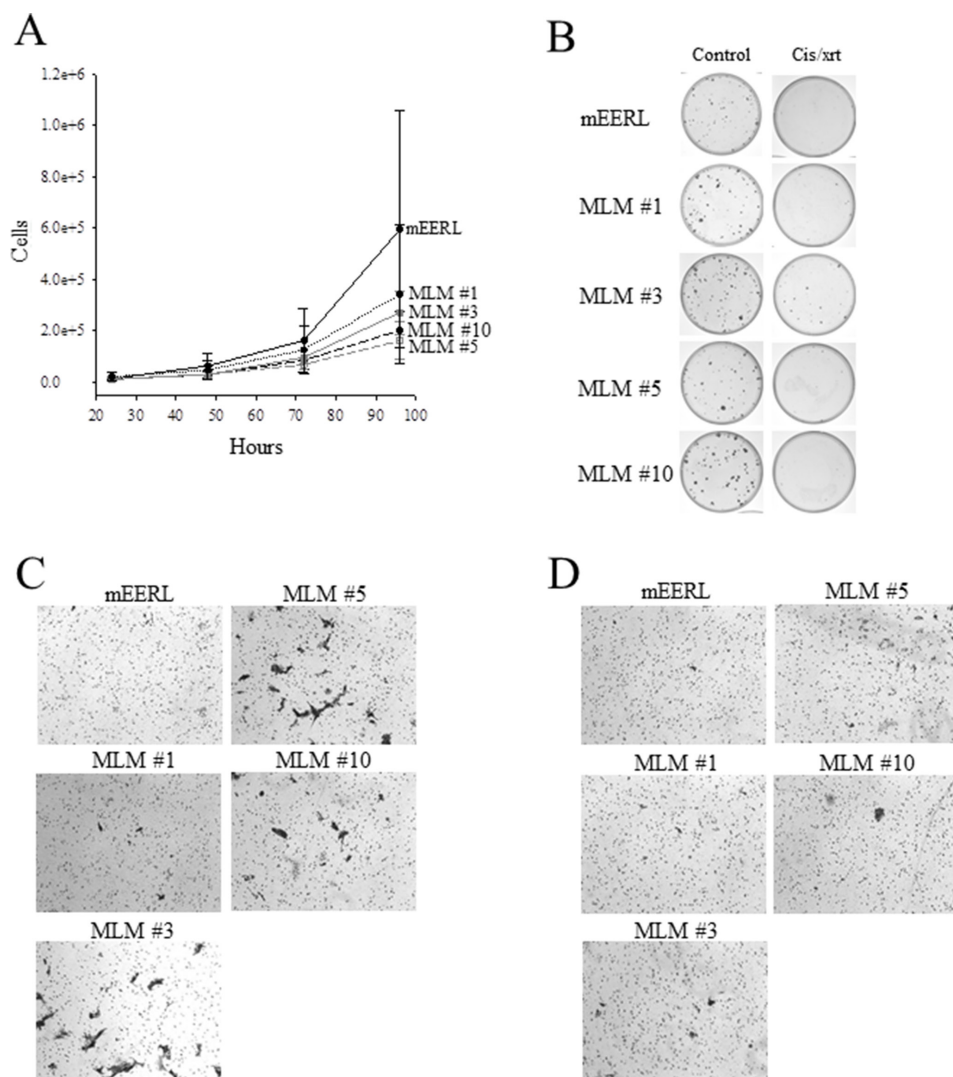
Supplementary Figure S1: Photomicrograph of cultured cells from individual lung tumors compared to parental tumor cells at the indicated day.



Supplementary Figure S2: Hierarchical clustering analysis was performed for two sets of DEGs (Differentially Expressed Genes). Both genes and samples are clustered using an average linkage clustering algorithm. (A) Clustering analysis of DEGs in a comparison between parental mEERL cells and MLM clones. DEGs were determined based on T -test with a False Discover Rate (FDR: Benjamini–Hochberg–Yekutieli procedure) of $p < 0.05$. (B) Clustering analysis of DEGs among MLM clones. DEGs were determined based on ANOVA with a False Discover Rate (FDR: Benjamini–Hochberg–Yekutieli procedure) of $p < 0.05$. Due to instrument error, one of the replicates for MLM5 was run two times.



Supplementary Figure S3: PCR amplification of HPV16 E6 of DNA isolated from an OPSCC patient. Controls included no template (-control) and DNA from a known HPV+ OPSCC cell line (+control). 18S served as a loading control. Tumor from this OPSCC patient was utilized for IHC validation in Figure 3B.



Supplementary Figure S4: (A) Tumor growth of mEERL and MLM lines over time. Data points are an average of $N = 12$ from three independent experiments. Data points, mean \pm SEM. **(B)** Representative image of a clonogenic assay showing the effects of cisplatin, radiation, or the combined modality on clonogenic survival. **(C)** Representative picture of migration and **(D)** invasion through traditional matrigel inserts.

Supplementary Table S1: Pathway analysis of DEGs: mEERL and MLMs

Ingenuity Canonical Pathways	$-\log(p\text{-value})$
IL-8 Signaling	6.60E + 00
ATM Signaling	6.54E + 00
CXCR4 Signaling	6.26E + 00
STAT3 Pathway	5.65E + 00
Inhibition of Angiogenesis by TSP1	5.33E + 00
HMGB1 Signalling	5.19E + 00
Molecular Mechanisms of Cancer	5.17E + 00
IL-22 Signaling	5.16E + 00
Thrombin Signaling	5.02E + 00
GDNF Family Ligand-Receptor Interactions	5.02E + 00
Pancreatic Adenocarcinoma Signaling	4.96E + 00
Glioma Invasiveness Signaling	4.71E + 00
UVC-Induced MAPK Signaling	4.69E + 00
N-acetylglucosamine Degradation II	4.48E + 00
Cardiac Hypertrophy Signaling	4.23E + 00
Leukocyte Extravasation Signaling	4.22E + 00
Role of NFAT in Cardiac Hypertrophy	4.08E + 00
L-17 Signaling	3.97E + 00
Ephrin B Signaling	3.92E + 00
IL-17A Signaling in Gastric Cells	3.88E + 00

Top twenty significantly enriched pathways. Enriched pathways in the first set of DEGs obtained from a comparison between parental mEERL cells and MLM clones.

Supplementary Table S2: Pathway analysis of DEGs: among MLMs

Ingenuity Canonical Pathways	$-\log(p\text{-value})$
Aryl Hydrocarbon Receptor Signaling	3.96E + 00
Signaling by Rho Family GT Pases	3.35E + 00
Role of NANOG in Mammalian Embryonic Stem Cell Pluripotency	3.30E + 00
Glioblastoma Multiforme Signaling	3.15E + 00
Ascorbate Recycling (Cytosolic)	3.10E + 00
LI-8 Signaling	3.01E + 00
Molecular Mechanisms of Cancer	2.95E + 00
Spermine and Spermidine Degradation 1	2.8GE + 00
Hepatic Fibrosis / Hepatic Stellate Cell Activation	2.78E + 00
Semaphorin Signaling in Neurons	2.76E + 00
Role of Tissue Factor in Cancer	2.64E + 00
Axonal Guidance Signaling	2.64E + 00
NRF2-mediated Oxidative Stress Response	2.52E + 00
CCR3 Signaling in Eosinophils	2.49E + 00
ILK Signaling	2.43E + 00
PC pathway	2.43E + 00
Role of Macrophages, Fibroblasts and Endothelial Cells in Rheumatoid Arthritis	2.40E + 00
Thrombin Signaling	2.35E + 00
Antioxidant Action of Vitamin C	2.25E + 00
Nitric Oxide Signaling in the Cardiovascular System	2.21E + 00

Top twenty significantly enriched pathways. Enriched pathways in the second set of DEGs obtained from a comparison among MLM clones.

Supplementary Table S3: Pathway analysis between the two sets of DEGs

Ingenuity Canonical Pathways	-log (p-value)
Sulfite Oxidation IV	2.95E + 00
Spermine and Spermidine Degradation I	2.34E + 00
Melatonin Degradation II	2.34E + 00
N-acetylglucosamine Degradation II	2.34E + 00
CMP-N-acetylneuraminate Biosynthesis 1 (Eukaryotes)	2.25E + 00
Mineralocorticoid Biosynthesis	1.87E + 00
Bile Acid Biosynthesis, Neutral Pathway	1.84E + 00
Glucocorticoid Biosynthesis	1.84E + 00
Phenylalanine Degradation IV (Mammalian, via Side Chain)	1.80E + 00
Androgen Biosynthesis	1.77E + 00
Putrescine Degradation III	1.72E + 00
Tryptophan Degradation X (Mammalian, via Tryptamine)	1.69E + 00
LPS/IL-1 Mediated Inhibition of RXR Function	1.60E + 00
Estrogen-mediated S-phase Entry	1.57E + 00
Dopamine Degradation	1.57E + 00
Superpathway of Methionine Degradation	1.45E + 00
Xenobiotic Metabolism Signaling	1.43E + 00
Cell Cycle Regulation by BTG Family Proteins	1.41E + 00
Noradrenaline and Adrenaline Degradation	1.41E + 00
Inhibition of Matrix Metalloproteases	1.36E + 00

Top twenty significantly enriched pathways. Enriched pathways in shared genes between the first and the second set of DEGs.