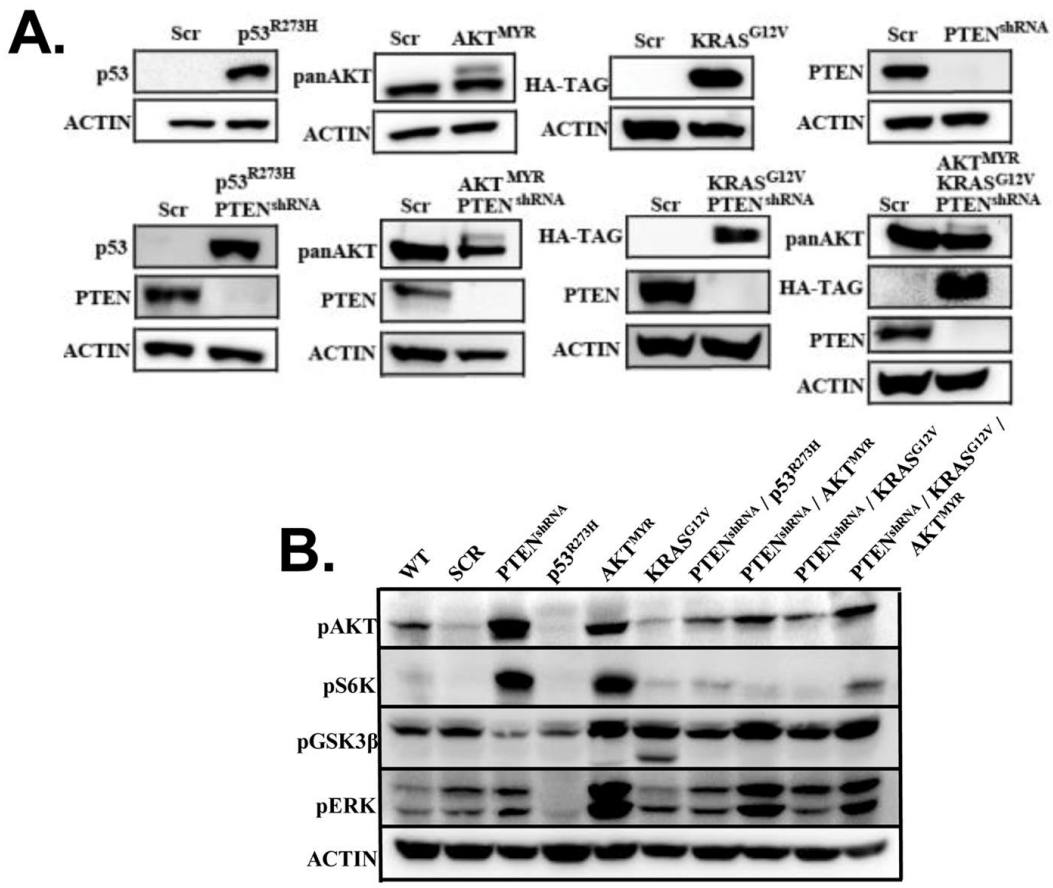
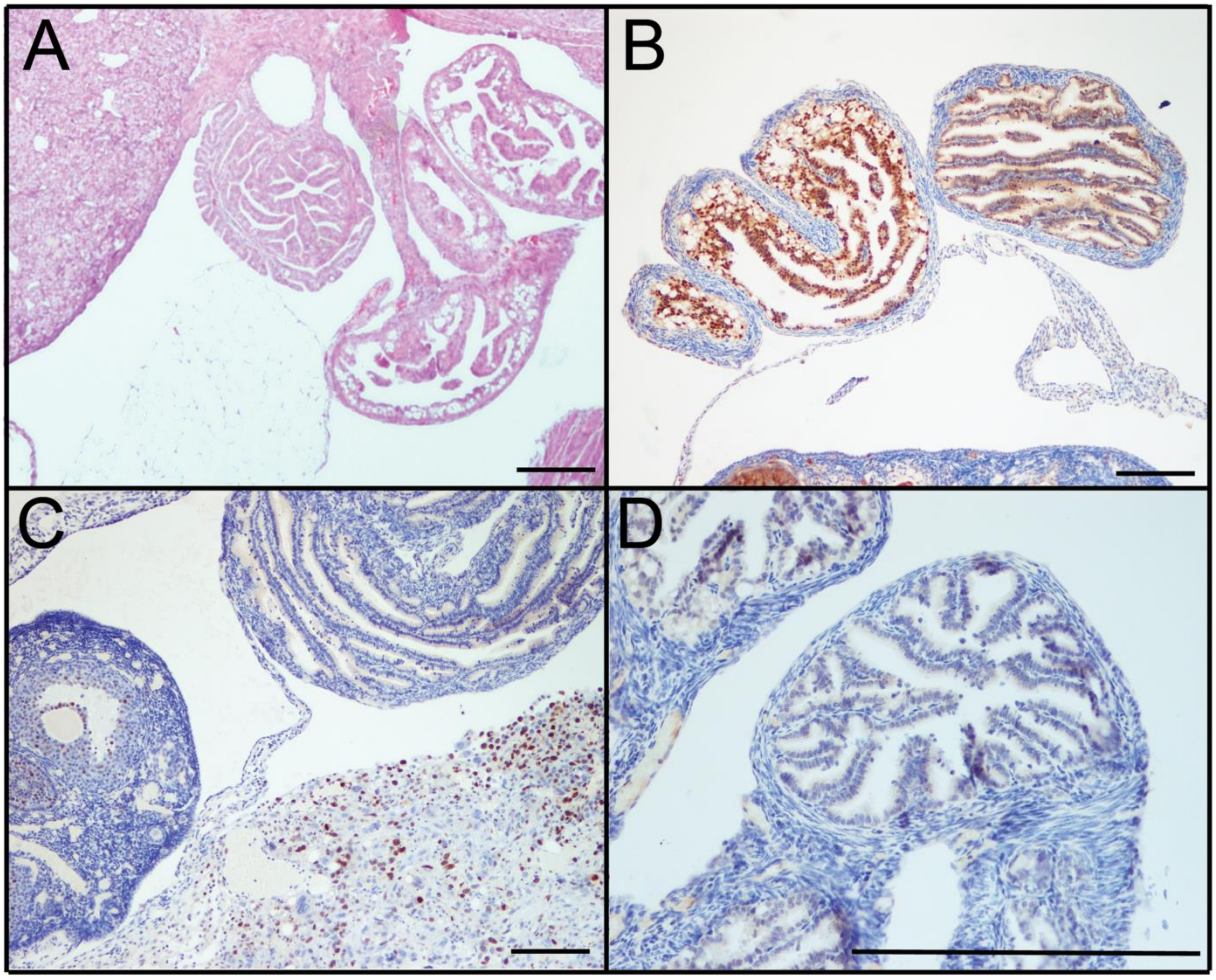
Tumorigenesis and peritoneal colonization from fallopian tube epithelium

Supplementary Material



Supplementary Figure 1: Validation of genetically engineered MOE. A) Stable MOE cell lines were validated via western blotting to express targets of interest as compared to scrambled shRNA (SCR) transfected control MOE cells. Specifically, stabilized p53 expression, which is indicative of mutation, double-banding of pan-AKT which demonstrates myristolation, HA-Tag expression by the KRAS plasmid, and absence of PTEN expression when transfected with shRNA. **B)** Further, cell lines were evaluated for downstream signaling changes via western blotting, to demonstrate that both select targets and their signaling pathways were functionally altered. WT= wildtype parental MOE cells.



Supplementary Figure 2: Tumorigenic MOE do not metastasize to the oviduct. A) H&E staining demonstrated that although tumors were found on adjacent ovaries, no oviductal involvement was found in any MOE grafted mice. **B)** Oviducts remained normal, as shown by PAX8 staining, **C)** with no infiltration of highly proliferative (Ki67) tumors and **D)** no stabilization of p53. Scale bars equal 200 microns.

Supplementary Table 1: Plasmids utilized for MOE cell development

Target	Vector	Manufacturer	Antibiotic Maintenance
Scrambled non-coding shRNA	pLKO.1 puro	Sigma	0.5 µg/ml puromycin (Sigma)
PTEN shRNA	pLKO.1 puro	Sigma	0.5 µg/ml puromycin (Sigma)
KRAS G12V	pLenti-PGK-Hygro	Addgene (35633)	200 µg/ml hygromycin (Sigma)
myristoylated AKT	pcDNA3 Myr HAtag	Addgene (9008)	300 µg/ml neomycin (Sigma)
p53 R273H	pCMV-Neo-Bam	Addgene (16439)	300 µg/ml neomycin (Sigma)

Supplementary Table 2: Western blotting and immunohistochemical antibodies

Target	Manufacturer	Dilution Factor	Blocking Buffer
Antibodies for western blotting:			
p53	Santa Cruz	1:1000	5% milk-TBST
pan AKT	Cell Signaling	1:500	5% BSA-TBST
HA-Tag	Cell Signaling	1:500	5% milk-TBST
PTEN	Cell Signaling	1:1000	5% BSA-TBST
phospho-AKT	Cell Signaling	1:500	5% BSA-TBST
phospho-S6K	Cell Signaling	1:500	5% BSA-TBST
phospho-GSK3β	Cell Signaling	1:500	5% milk-TBST
phospho-ERK	Cell Signaling	1:1000	5% BSA-TBST
ACTIN	Sigma	1:1000	5% milk-TBST
Antibodies for immunohistochemistry:			
CK8	Iowa Hybridoma Bank	1:200	rabbit serum-TBS/BSA
PAX8	Proteintech	1:100	goat serum-TBS/BSA
WT1	Abcam	1:50	goat serum-TBS/BSA
p53	Santa Cruz	1:50	goat serum-TBS/BSA
Ki-67	Abcam	1:100	goat serum-TBS/BSA