Media	Components	Source	Concentration
AEC basic	DMEM/F-12	Invitrogen Co., Ltd.	
		(Carlsbad, CA)	
	NaHCO ₃	Nakarai Pharmaceutical Co., Ltd.	1.2 mg/ml
	HEPES	Invitrogen Co., Ltd.	3.6 mg/ml
	Penicillin	Nakarai Pharmaceutical Co., Ltd.	100 U/ml
	Streptomycin	Nakarai Pharmaceutical Co., Ltd.	100 µg/ml
	Glutamine	Invitrogen Co., Ltd.	2 mM
	Amphotericin B	Sigma-Aldrich	250 ng/ml
AEC plus	AEC basic		
	Insulin	Sigma-Aldrich	10 µg /ml
	Transferrin	Invitrogen Co., Ltd.	$5 \ \mu g \ /ml$
	Cholera toxin	Calbiochem Co., Ltd	0.1 µg /ml
	Epidermal growth factor	Invitrogen Co., Ltd.	25 ng/ml
	Bovine pituitary extract	Biomedical Technologies Inc.	30 µg/ml
		(Stoughton, MA)	
	Fetal bovine serum	Equitech-Bio Co., Ltd	5% (v/v)
		(Kerrville, TX)	
	Retinoic acid	Wako Chemical Co., Ltd.	0.05 μΜ
		(Osaka, Japan)	
	D-Valine	Tokyo Chemical Industry Co., Ltd.	. 0.1 mg/ml
		(Tokyo, Japan)	

TABLE S1. AEC media components and concentrations

Figure Legend for Supplemental Figures

FIG. S1. Validation of pAEpC isolation from porcine lung by the detection of an epithelial cell marker.

Immunostaining of cytokeratin 19 as an epithelial cell marker and vimentin as a fibroblast marker (negative control) in pAEpC and porcine alveolar fibroblasts. The cells were prepared at the same time. Scale bars, 100 µm.

FIG. S2. Caspase 3 activity in human lung carcinoma cell line A549 at 16 hpi with avian influenza virus.

A549 cells were infected with H5N1, H5N2, or H5N3 at an MOI of 1. Caspase 3 activity (left panel) and cell morphology (right upper panel) at 16 hpi are shown. Virus antigen was detected by western blot analysis (right lower panel). Scale bar, 100 µm.

pAEpC

Porcine alveolar fibroblast

FIG. S1.





FIG. S2.