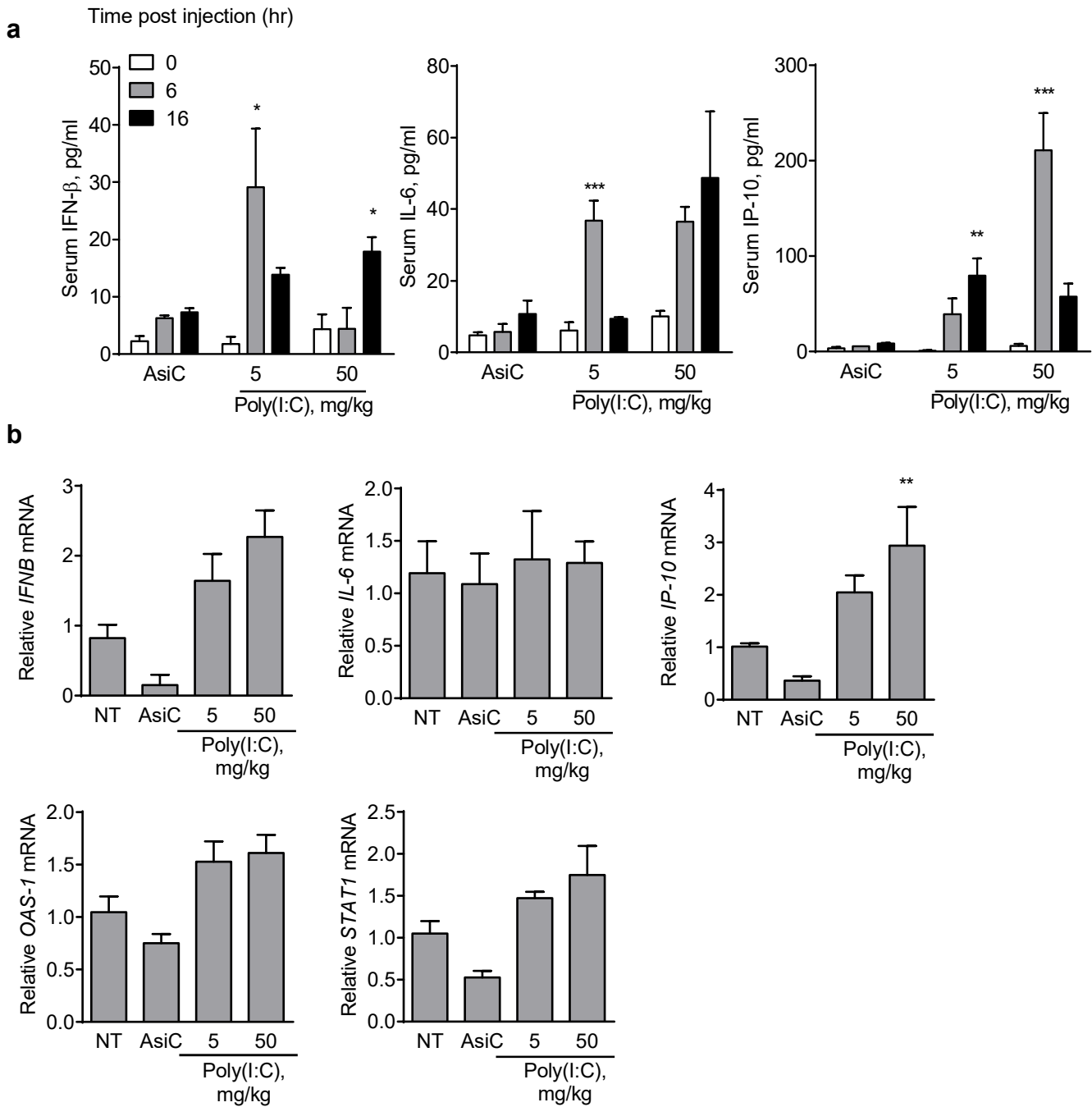


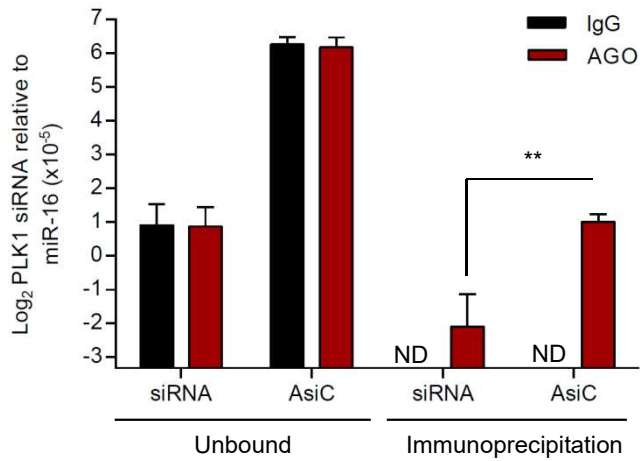
Supplemental Figure 1 – EpCAM-AsiCs are stable in human and mouse serum

eGFP EpCAM-AsiCs, synthesized using 2'-fluoro-pyrimidines, chemically stabilized cholesterol-conjugated *eGFP* siRNAs (chol-siRNA), or unmodified *eGFP* siRNAs were incubated at 37°C in 50% human or mouse serum. Aliquots were removed at regular intervals and stored at -80°C before electrophoresis on denaturing PAGE gels. The average intensity (+S.E.M.) of bands from 2 independent experiments quantified by densitometry after ethidium bromide staining is shown.



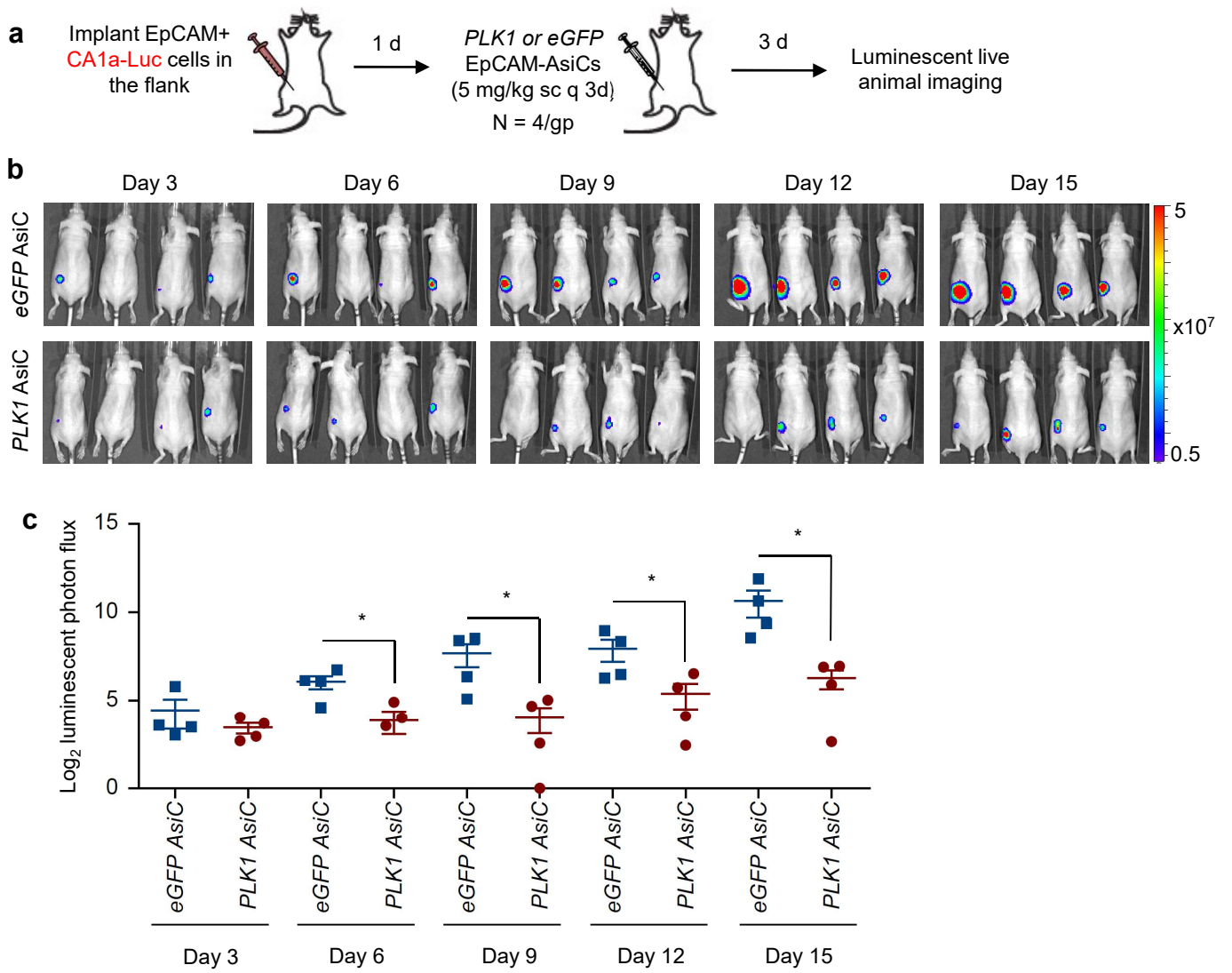
Supplemental Figure 2 - Injection of EpCAM-AsiCs does not stimulate innate immunity

Mice were injected sc with eGFP EpCAM-AsiCs (5 mg/kg, n=3) or ip with Poly(I:C) (5 or 50 mg/kg (n=2/dose)). **a**, Serum samples, collected at baseline and 6 and 16 hr after treatment were assessed for IFN β , IL-6 and IP-10 by multiplex immunoassay. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; compared to baseline. **b**, mRNA expression of cytokine and IFN-induced genes, relative to *Gapdh* was assayed by qRT-PCR in total splenocytes harvested 16 hr post treatment. **, $P < 0.01$, compared to untreated (NT, n=3).



Supplemental Figure 3 – *PLK1* siRNA associates with Argonaute (AGO) in cells treated with *PLK1* EpCAM-AsiCs

MB-468 cells, treated with *PLK1* EPCAM-AsiC or siRNA for 2 days, were lysed, and cell lysates were immunoprecipitated with pan-AGO antibody or IgG isotype control. The amount of *PLK1* siRNA in the immunoprecipitates was quantified by Taqman qRT-PCR, presented as \log_2 mean with SEM, relative to miR-16. **, $P < 0.01$ by Student's t-test relative to siRNA-treated cells. ND, not detectable. *PLK1* siRNA was found in the RISC after treatment with *PLK1* EpCAM-AsiCs. However, the Ago immunoprecipitation did not significantly deplete *PLK1* siRNAs from the supernatant. This is likely because most RNAs that are taken up by cells are not released from endosomes to the cytosol (A. Wittrup et al., Visualizing lipid-formulated siRNA release from endosomes and target gene knockdown. Nature Biotechnology 2015, in press).



Supplemental Figure 4 - PLK EpCAM AsiC suppresses MCF10CA1a (CA1a) tumor growth.

a, Experimental scheme. In this experiment the AsiCs were injected sc in the flank near the tumor, but not into the tumor. **b**, Bioluminescent images of treated mice. Heat map indicates photon flux (photon/second/cm²). **c**, Log₂ total luminescent photon flux of the tumors (N = 4); *, P<0.05 by Student's t-test.

Supplemental Table 1. EpCAM-AsiC Sequences

AsiC construct	Sequence
EpCAM PLK1 sense	GCG ACU GGU UAC CCG GUC GUU UUG AAG AAG AUC ACC CUC CUU AdTdT
EpCAM PLK1 anti-sense	UAA GGA GGG UGA UCU UCU UCA dTdT
EpCAM AKT1 sense	GCG ACU GGU UAC CCG GUC GUU GCU GGA GAA CCU CAU GCU GdTdT
EpCAM AKT1 anti-sense	CAG CAU GAG GUU CUC CAG CdTdT
EpCAM GFP sense	GCG ACU GGU UAC CCG GUC GUU UGG CUA CGU CCA GGA GCG CAdTdT
EpCAM GFP anti-sense	UGC GCU CCU GGA CGU AGC CdTdT
siGFP sense	UGG CUA CGU CCA GGA GCG
siGFP antisense	UGC GCU CCU GGA CGU AGC
siAKT1 sense	GCU GGA GAA CCU CAU GCU G
siAKT1 antisense	CAG CAU GAG GUU CUC CAG C
siPLK1 sense	UGA AGA AGA UCA CCC UCC UUA
siPLK1 antisense	UAA GGA GGG UGA UCU UCU UCA