Supporting Information

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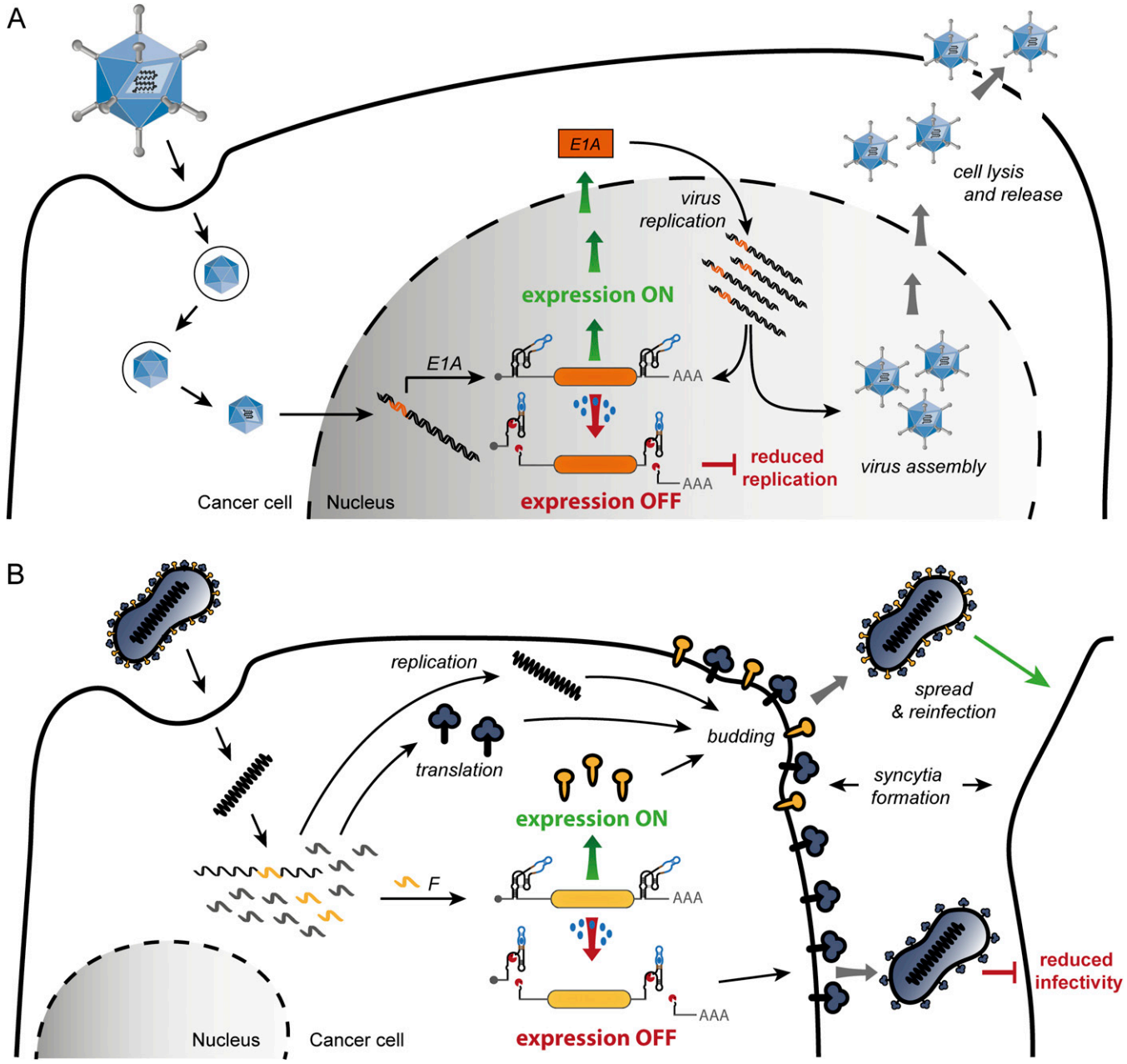

Fig. S1. Schematic outline of the strategy for aptazyme-mediated shutdown of viral genes in adenoviruses and in measles viruses. Regulation of viral gene expression is achieved by insertion of an OFF-switch aptazyme (here P1-F5), a synthetic ligand-dependent self-cleaving ribozyme, into the UTRs. During infection, the viral genes are transcribed in the absence (expression ON), but not in the presence (expression OFF) of the small-molecule ligand (blue dots, here theophylline). (*A*) For adenoviruses, viral replication can be controlled through regulation of the immediate early E1A protein, which triggers expression of further viral genes and modifies the host cell as required for virus genome replication. (*B*) For measles viruses, viral spread can be controlled through regulation of the F protein, which is required for virus-cell fusion, thus regulating the infectivity of progeny viruses.

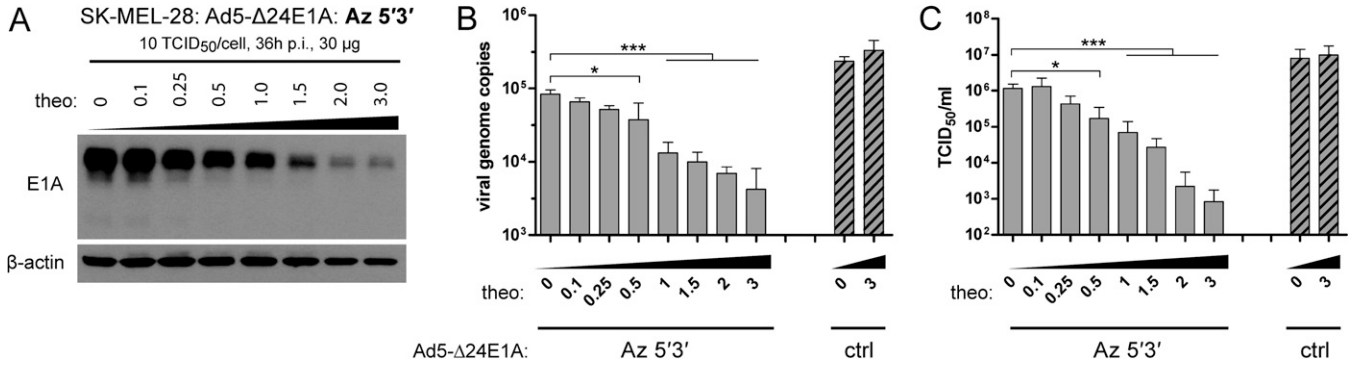

Fig. S2. Aptazyme-mediated control of adenoviral *E1A* gene expression and DNA replication. (*A*–*C*) Dose-dependent aptazyme-mediated regulation of E1A expression (*A*), viral genome copy numbers (*B*), and production of infectious progeny particles (*C*) after infection of SK-MEL-28 cells with Az 5′3′ or ctrl oncolytic adenovirus was assayed by immunoblotting for E1A protein expression, by qPCR for viral genome copy numbers and by titration for produced infectious progenies, respectively. (*A*) Multiple bands for E1A represent multiple splice variants. Human β-actin was used as loading control. (*B*) Viral genome copies were determined 48 h postinfection (p.i.) (1 $TCID_{50}$/cell) by qPCR and are presented relative to cellular DNA content as determined for each sample individually. (*C*) Total infectious progenies were harvested 48 h p.i. (1 $TCID_{50}$/cell) and titrated in HEK293 cells. (*B* and *C*) Columns show mean values, error bars SD of three samples ($n = 3$). Significance for theophylline-dependent regulation for individual Ads is indicated with $^{*}P < 0.05$, $^{***}P < 0.001$.

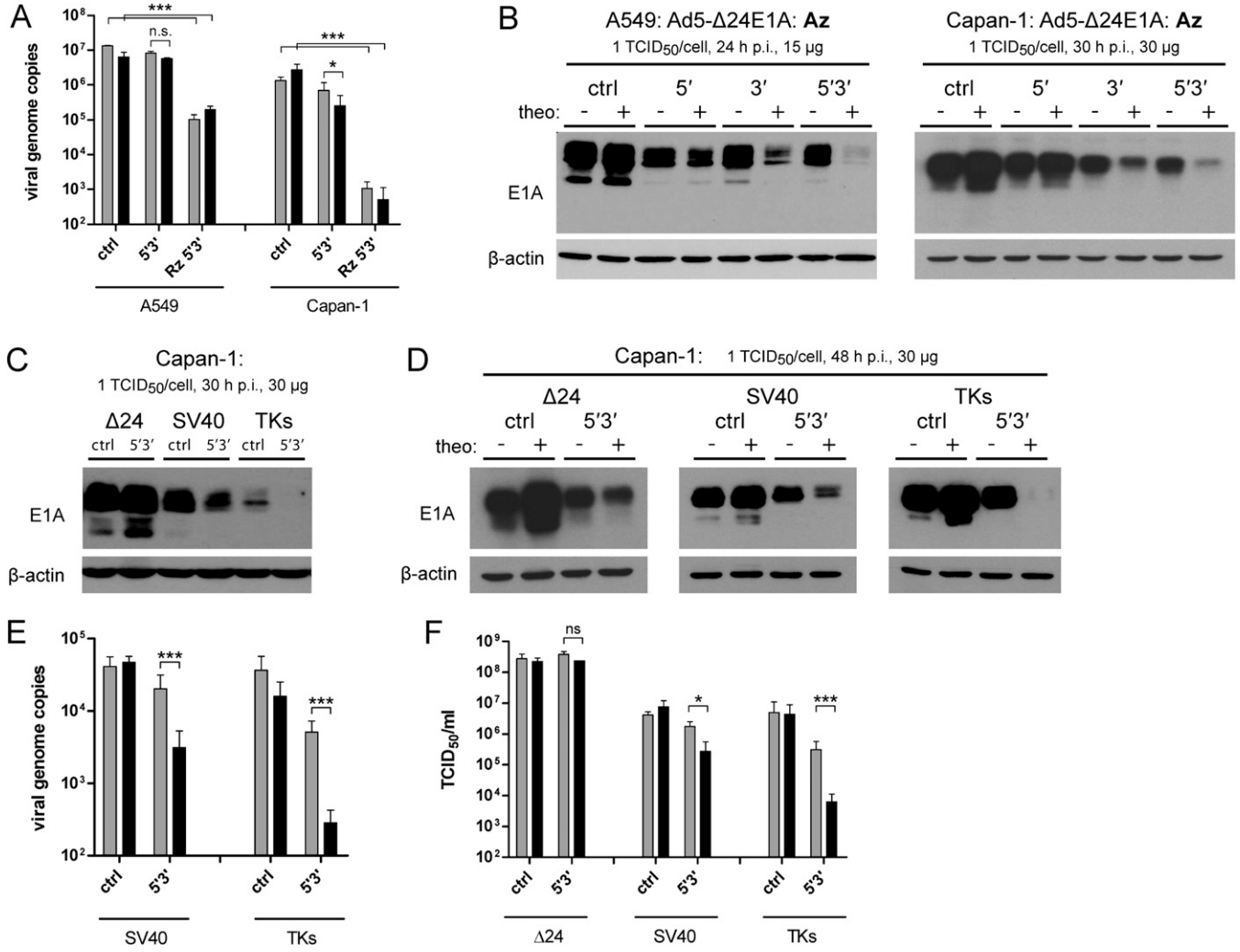

Fig. S3. Restoring aptazyme-mediated regulation of adenovirus replication by reducing the baseline expression of E1A. (*A*) A549 or Capan-1 cells were infected with indicated oncolytic adenoviruses at 1 $TCID_{50}$/cell. Viral genome copy numbers were determined 32 h p.i. by qPCR and are presented as in Fig. S2*B*. No or minimal aptazyme-mediated regulation of virus replication is observed for the Az 5′3′ virus in these cells, whereas the Rz 5′3′ virus shows strongly reduced genome replication. (*B*) E1A expression after infection of A549 and Capan-1 cells with ctrl, Az 5′, Az 3′, or Az 5′3′ adenoviruses in the presence (+) or absence (–) of 3 mM theo. Time points of cell harvest differed between cell types because of differences in adenovirus replication kinetics. Adenovirus replication is faster in A549 than in Capan-1 cells as determined in a pilot time-course experiment. Total lysates were assayed by immunoblotting for E1A protein expression. Theo-dependent inhibition of E1A expression for the Az 5′3′ virus shows that lack of aptazyme-mediated replication control in A549 and Capan-1 cells is not due to failure of the aptazyme to regulate E1A protein expression. (*C*–*F*) Reducing the baseline level of E1A expression restores P1-F5 aptazyme-mediated regulation of adenoviral replication in Capan-1 cells. Oncolytic adenoviruses were generated derived from the Az 5′3′ and ctrl viruses (Fig. 2*A*) by replacing the viral E1A promoter with the SV40 promoter or human thymidine kinase short (TKs) promoter. (*C*) E1A expression levels of adenoviruses with modified (SV40, TKs) or unmodified (Δ24) E1A promoter, each without (ctrl) or with (5′3′) aptazyme insertion, were determined for infected Capan-1 cells by immunoblot. Viruses with SV40 promoter or TKs promoter showed modestly or strongly reduced E1A baseline expression, respectively. (*D*) Aptazyme-mediated shutdown of E1A expression by promoter variants in Capan-1 cells compared with unmodified E1A promoter determined by immunoblot. Exposure time was TKs > SV40 > Δ24 due to differences in baseline expression (see *C*). Later time point of cell harvest compared with *B* showed more stringent aptazyme-mediated regulation by the promoter variants, especially with TKs promoter. (*E*) Aptazyme-mediated shutdown of replication of promoter variants in Capan-1 cells. Cells were infected with the indicated E1A promoter variants at 1 $TCID_{50}$/cell and viral genome copy numbers were determined 32 h p.i. by qPCR and are presented as in Fig. S2*B*. (*F*) Aptazyme-mediated regulation of infectious progeny production by promoter variants compared with unmodified E1A promoter variant after infection of Capan-1 cells at 1 $TCID_{50}$/cell. Infectious progenies were harvested 48 h p.i. and titrated in HEK293 cells. Data are presented as in Fig. S2*C*. Together, the results in *E* and *F* demonstrate that aptazyme-mediated control of adenovirus replication is indeed established for the Az 5′3′ virus with the TKs promoter and to a certain degree also for the SV40 promoter. However, virus genome replication of promoter-modified viruses in the absence of theo was reduced compared with the parental virus with endogenous E1A promoter (compare *A* and *E*). This may be explained by reduced or constitutive expression of E1A.

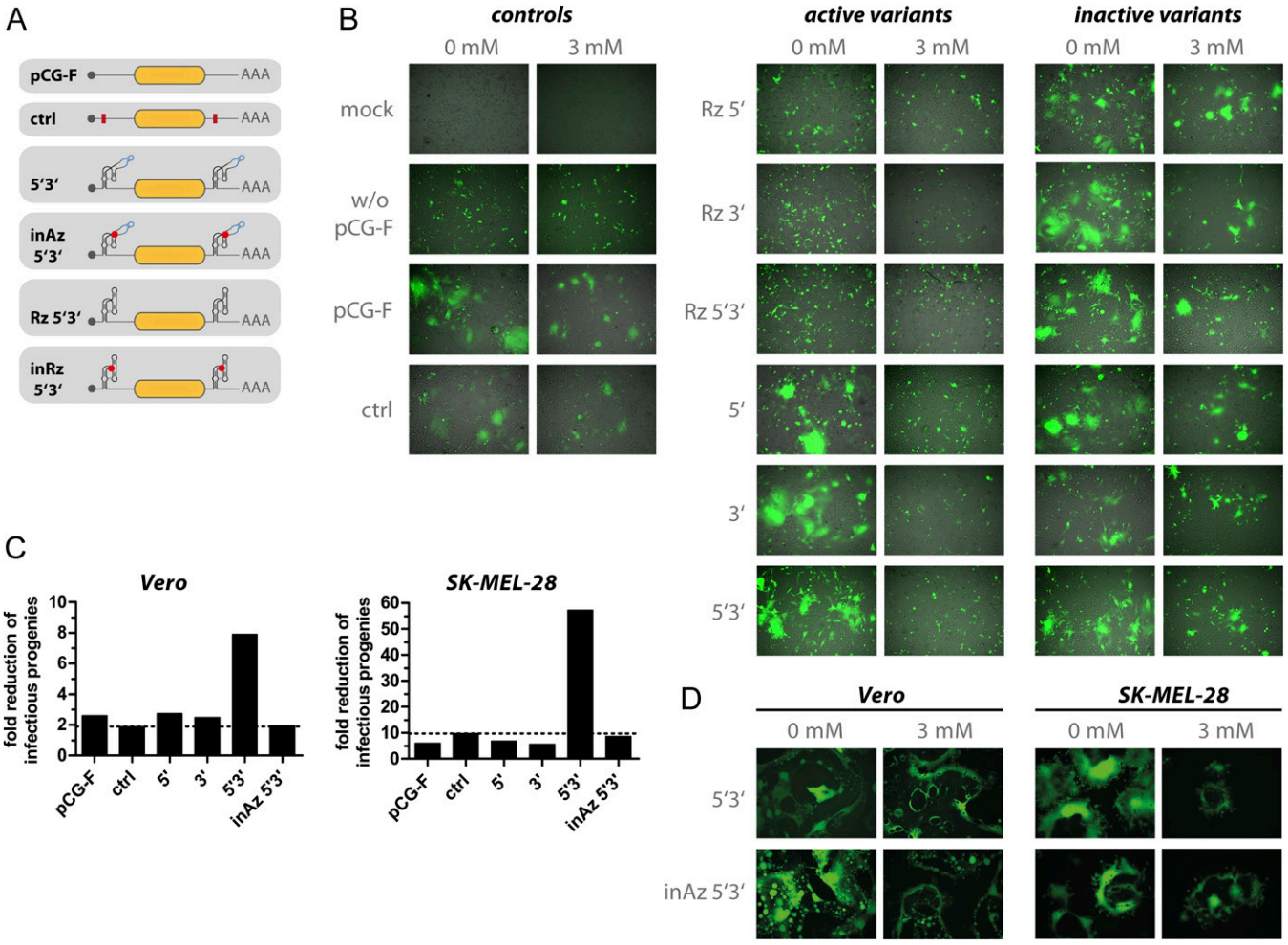

Fig. S4. Aptazyme-mediated control of measles virus (MV) syncytia formation upon regulation of *F* gene expression. (*A*) Schematic outline of MV F mRNA variants cloned into pCG-F expression plasmids (shown) or MV-EGFP-F virus genomes (Fig. 4*A*). Insertion sites for the P1-F5 aptazyme (Az), the parental ribozyme (Rz), or the inactive mutants thereof (inAz and inRz) were positioned in the 5′- and/or 3′-UTR of the *F* gene (yellow box, shown for 5′3′ insertions). ctrl, modified pCG-F plasmid with restriction sites for Az/Rz insertion (red bar); red dot in inAz/inRz 5′3′, inactivating point mutation. (*B*) Fusion assay in SK-MEL-28 cells. Cells were cotransfected with plasmids encoding EGFP, MV H protein, and with indicated pCG-F variants or were mock transfected. Subsequently, cells were cultured in the absence or presence of 3 mM theophylline. Phase contrast and fluorescence photos were taken 50 h posttransfection when syncytia formation was established for the positive controls and merged. (*C*) Vero and SK-MEL-28 cells were infected with indicated MV variants at 0.03 cell infectious units (ciu)/cell and cultured in the absence or presence of 3 mM theophylline. Cells were harvested 48 h p.i. and produced infectious progeny particles in cells and supernatant were determined by titration on Vero cells. Fold reduction of infectious progenies was calculated as ratio between infectious progenies in the absence and presence of theophylline. Dotted line, theophylline-dependent reduction of ctrl. (*D*) Syncytia formation in SK-MEL-28 and Vero cells upon infection (0.03 ciu/cell) with indicated MV variants in the absence or presence of 3 mM theophylline. Fluorescence photos were taken 48 h p.i. when syncytia formation was almost 100% for the inAZ virus.