Supplementary Figure Legends.

Supplementary Figure S1. Serial reconstruction and TUNEL staining of sections from 435S-Tet-CD8/Casp-8 tumors treated with doxycycline via osmotic pumps plus intravenous injection

Note the heterogeneous distribution of apoptotic cells, void spaces and channel-like structures in the serial sections. Apoptosis-rich areas are highlighted by yellow and pink circles. In the same tumor area, while in one section (0 μm) there is almost no apoptosis, in adjacent sections separated by 25 and 30 μm (pink circle) several apoptotic cells and void spaces can be observed.

Supplementary Figure S2. Induction of apoptosis by paclitaxel or TRAIL in breast cancer cells *in vitro*

A, Induction of apoptosis by paclitaxel (100 nM), TRAIL (100 nM), paclitaxel (24 h) followed by TRAIL (24 h) or TRAIL followed by paclitaxel in 435s and W9 cells. The sequential treatment of paclitaxel followed by TRAIL induced significantly more apoptosis. ***B***, Inhibition of apoptosis by caspase-inhibitors in 435s cells. The pan-caspase inhibitor (z-VAD-fmk) blocked the apoptosis induced by paclitaxel or TRAIL. The caspase-8 inhibitor (z-IETD-fmk) also blocked the apoptosis induced by TRAIL and partially blocked paclitaxel-induced apoptosis.

Supplementary Figure S3. Effect of TRAIL pretreatment on the distribution of HSV in tumor spheroids

A, Morphological difference in W9 spheroids treated with PBS or TRAIL (10 nM or 100 nM). Twenty-four hours after the initiation of TRAIL treatment, W9 spheroids showed a loose structure compared with untreated spheroids. ***B***, Viral infection of W9 spheroids with or without TRAIL pretreatment. Twenty-four h after HSV infection, there was an increase in GFP-positive cells in TRAIL-treated spheroids compared to untreated control spheroids. ***C***, Quantification of the number of GFP-positive cells. TRAIL-pretreatment significantly increased the HSV infection in spheroids (*$p<0.001$).

Supplementary Figure S4. Effect of paclitaxel-TRAIL pretreatment and HSV on cell death and void space formation

HSV immunostaining of virus infected areas. In paclitaxel-TRAIL treated tumors there were numerous and large void spaces, whereas in vehicle-treated tumors the virus induced limited cell death and void spaces.

Supplementary Figure S5. HSV infectivity after the cytotoxic treatment of 435S cells *in vitro*

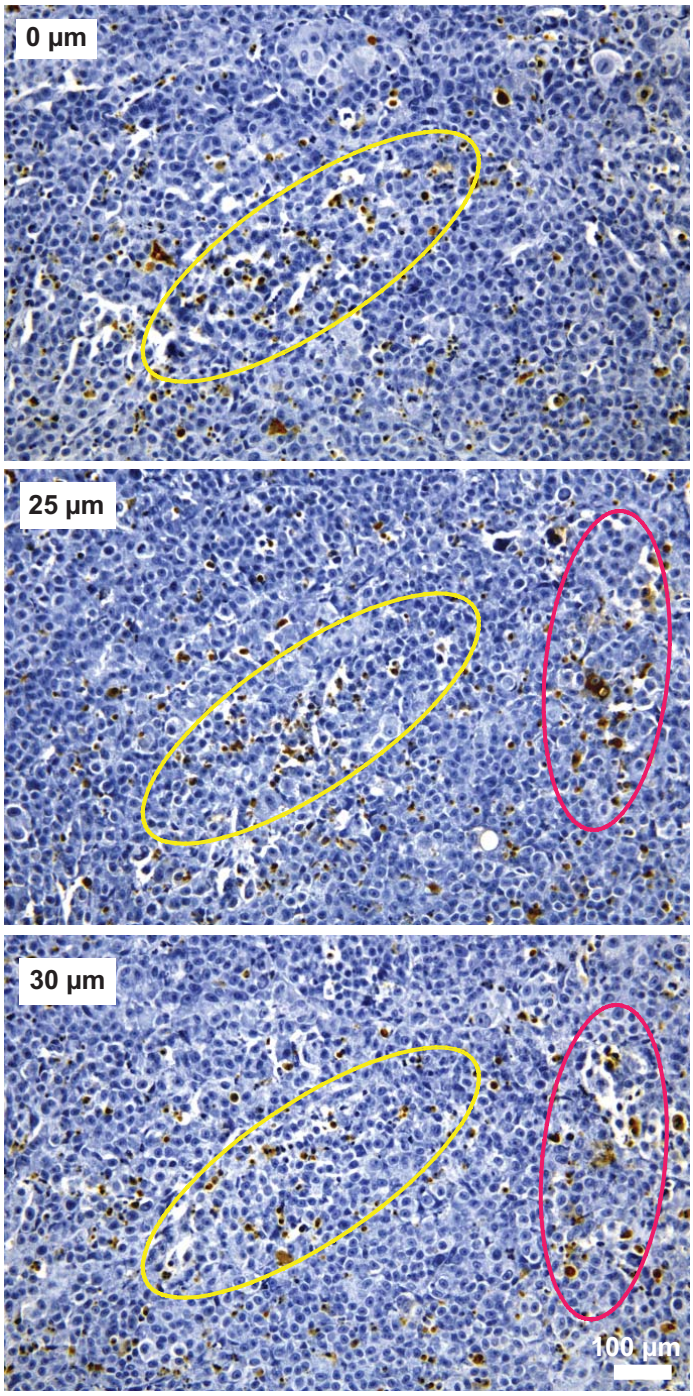
Cells were treated either paclitaxel or TRAIL at different concentrations. One day later, cells

were treated with MGH2 (MOI 0.01) and viral infection was observed one day later. TRAIL did not affect the viral infectivity, whereas paclitaxel treatment reduced the HSV infection.

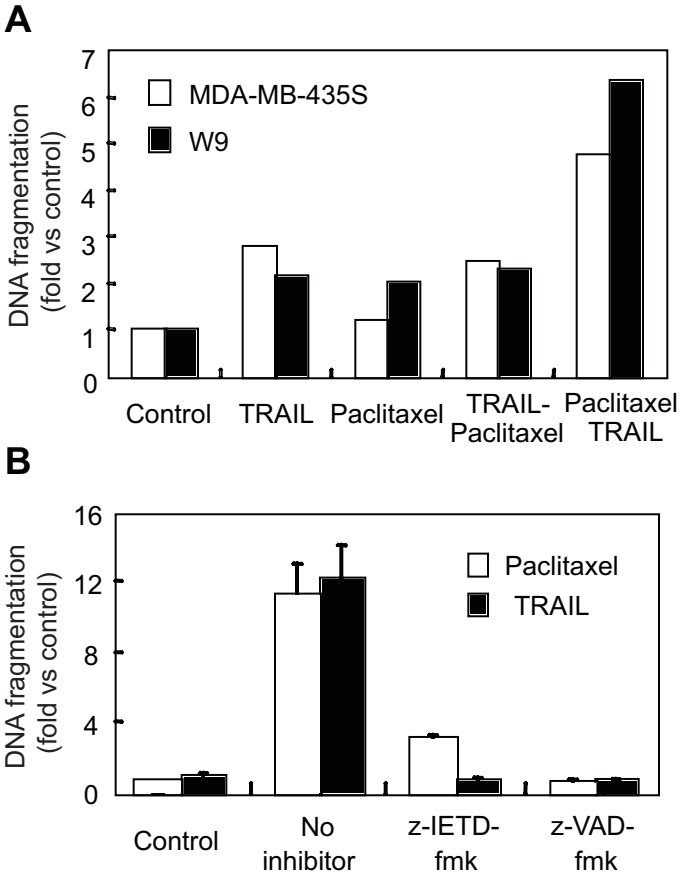
Supplementary Figure S6. Schematic representation of the effect of apoptosis / necrosis induction on the spread of oncolytic HSV in tumors

In the collagen-poor 435S tumors, apoptosis induction (caspase-8 activation, paclitaxel-TRAIL) created void spaces and channel-like structures, which were more or less interconnected. Following an intratumoral infusion of HSV, in the center of control tumors isolated areas of HSV infection were observed, whereas apoptosis induction produced an interconnected and diffuse pattern of HSV infection extending from one tumor edge to another. The widespread HSV spread suggests that apoptosis induction leads to the formation of interconnected interstitial channels, which enhances fluid flow and the penetration of virus during intratumoral infusion. In the collagen-rich 361HK tumor, paclitaxel-treatment produced a large central necrotic region, which correlated with the increase in HSV infection at the interface of necrotic and non-necrotic tissue. Thus similar to the channels formed by apoptosis induction, the necrosis facilitates the fluid movement of virus during intratumoral infusion.

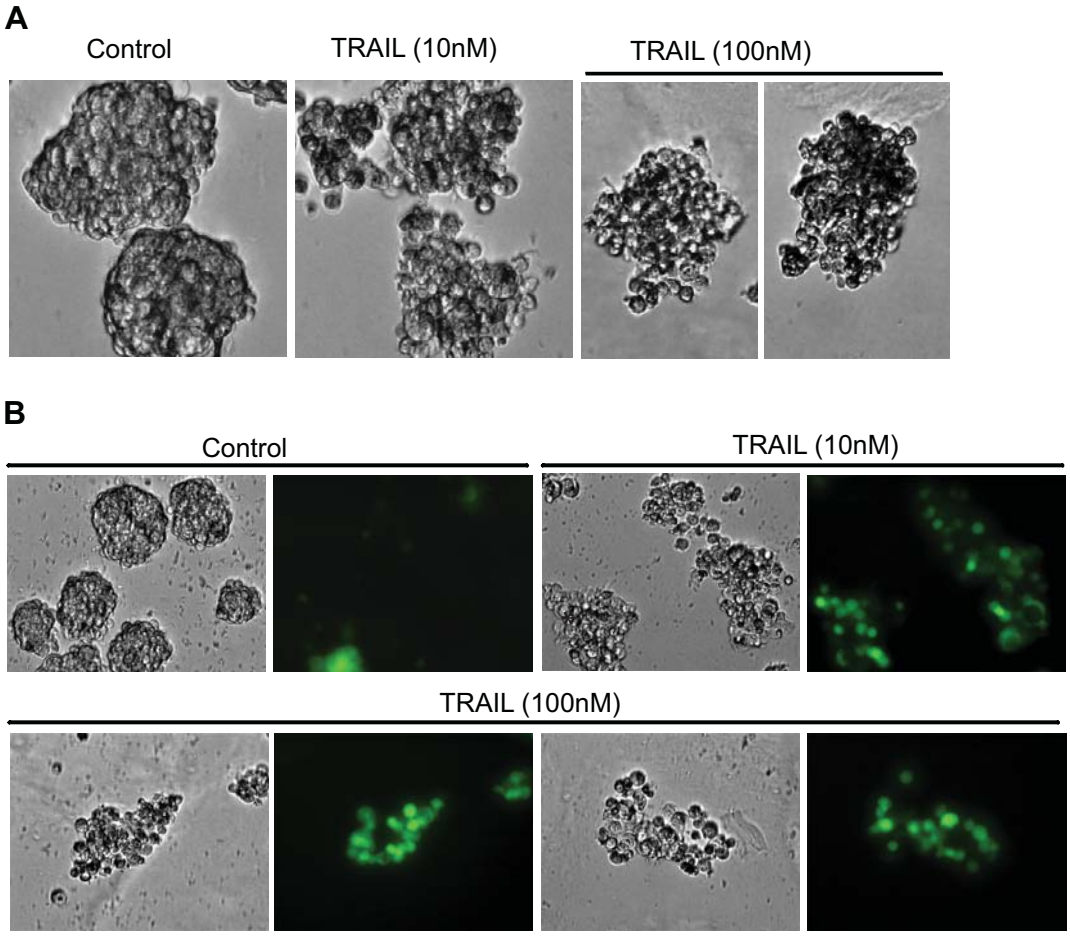
Supplementary Figure S1



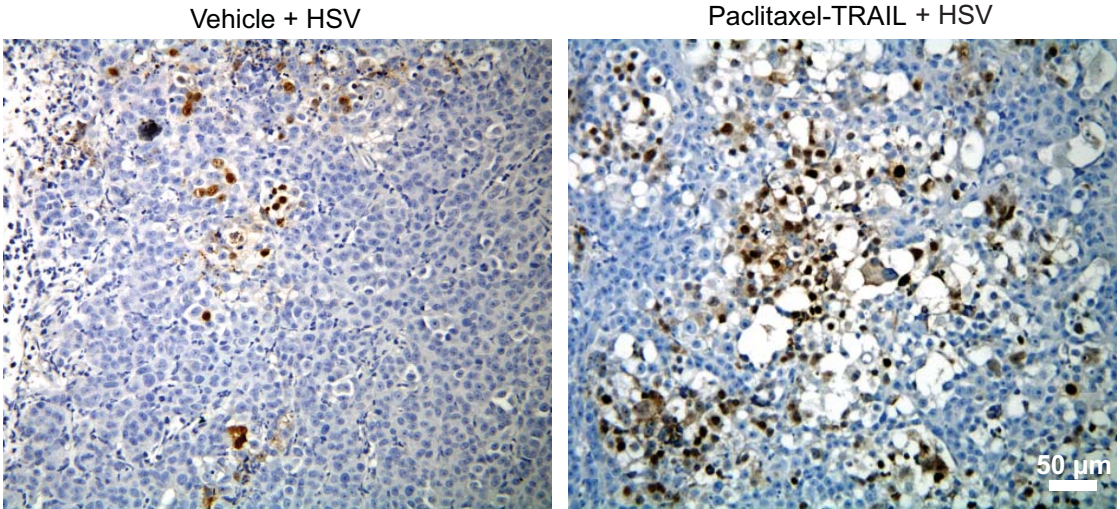
Supplementary Figure S2



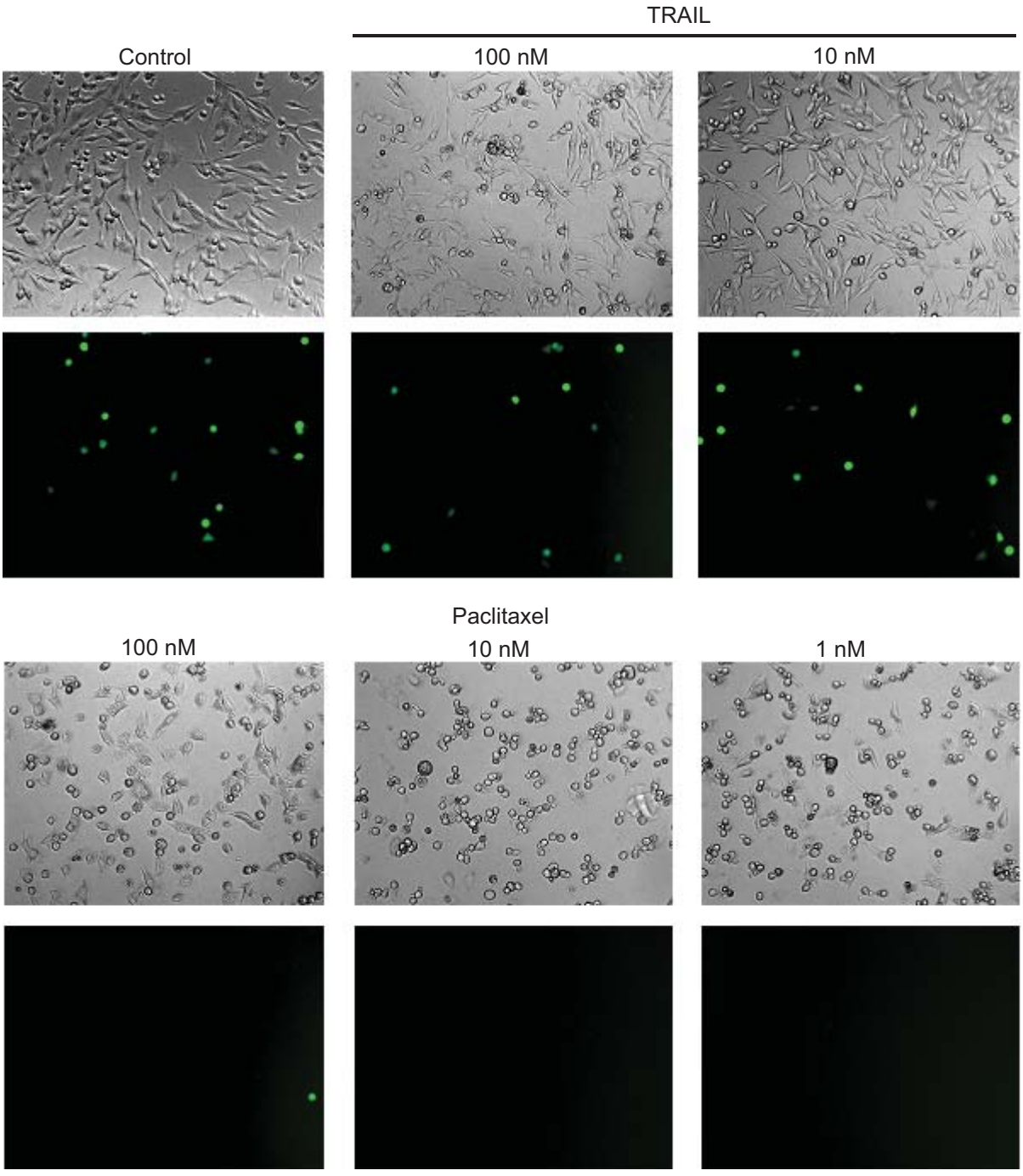
Supplementary Figure S3



Supplementary Figure S4



Supplementary Figure S5



Supplementary Figure S6