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Supplemental Information

Transient Inhibition of PI3Kδ Enhances the Therapeutic Effect of Intravenous Delivery of Oncolytic Vaccinia Virus

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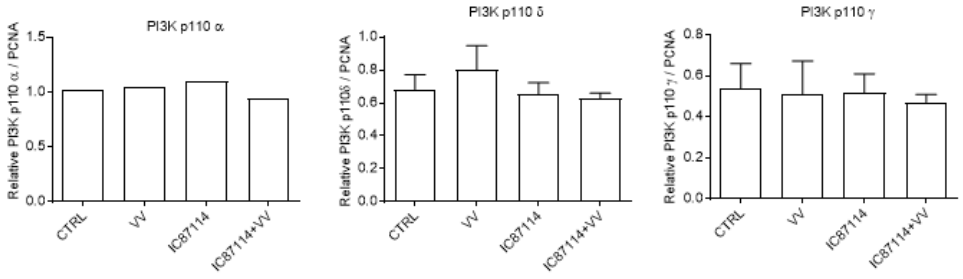
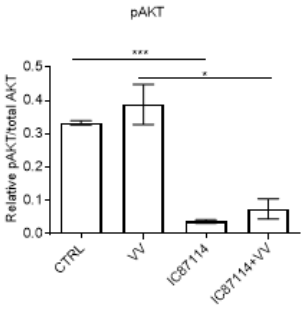
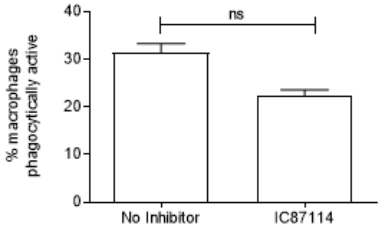
A


PI3K p110 α
Relative PI3K p110 α / PCNA
1.5
1.0
0.5
0.0
CTRL
VV
IC87114
IC87114+VV
PI3K p110 δ
Relative PI3K p110δ / PCNA
1.0
0.8
0.6
0.4
0.2
0.0
CTRL
VV
IC87114
IC87114+VV
PI3K p110 γ
Relative PI3K p110 γ / PCNA
0.8
0.6
0.4
0.2
0.0
CTRL
VV
IC87114
IC87114+VV

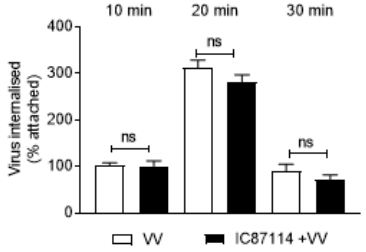
B


pAKT
***
*
Relative pAKT/total AKT
0.5
0.4
0.3
0.2
0.1
0.0
CTRL
VV
IC87114
IC87114+VV

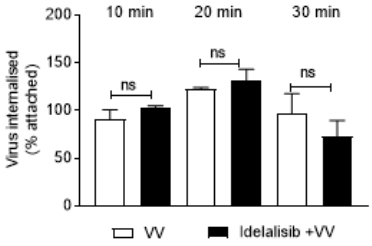
C


ns
% macrophages phagocytically active
40
30
20
10
0
No Inhibitor
IC87114

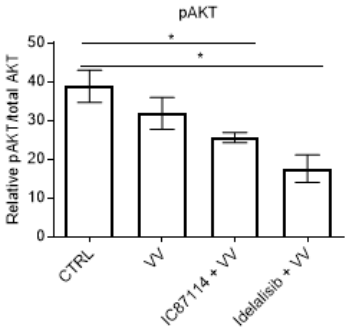
D


10 min
20 min
30 min
Virus internalised (% attached)
400
300
200
100
0
ns
ns
ns
VV
IC87114 +VV

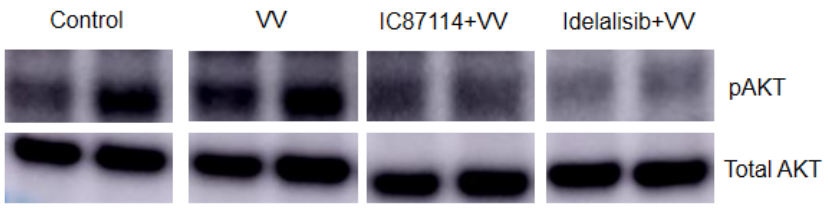
E


10 min
20 min
30 min
Virus internalised (% attached)
200
150
100
50
0
ns
ns
ns
VV
Idelalisib +VV

F

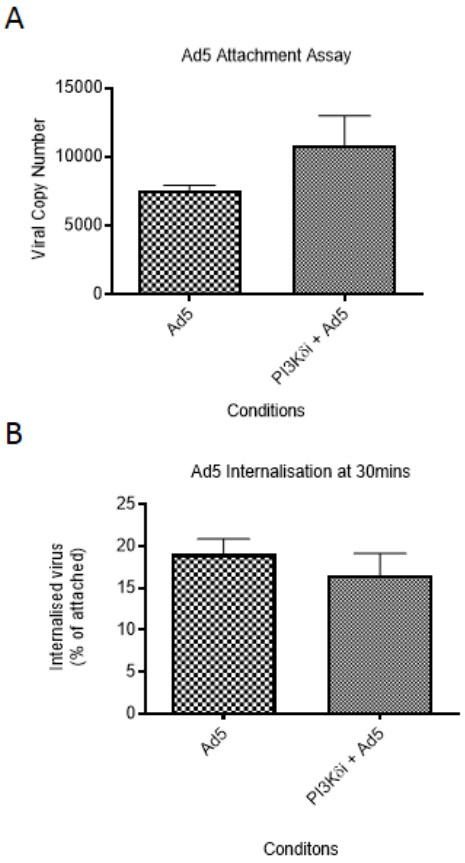

pAKT
*
*
Relative pAKT/total AKT
50
40
30
20
10
0
CTRL
VV
IC87114 + VV
Idelalisib + VV

G


Control
VV
IC87114+VV
Idelalisib+VV
pAKT
Total AKT

Supplementary Figure 1. Pharmacological inhibition of PI3Kδ has no effect on phagocytosis or internalisation of VV in macrophages. (A) Semi-quantification of western blots of p110 isoform expression in macrophages treated with IC87114 (10 μM) ± VVL15 (MOI=5). Analysis was preformed using the ImageJ program and the ratio of p110 isoforms to the PCNA protein used as loading control is shown. **(B)** Semi-quantification of western blots of p-AKT in macrophages treated with IC87114 (1 μM) +/- VVL15 (MOI=5). Analysis was preformed using the ImageJ program and the p-AKT/ t-AKT is shown in the graph (n=3). Data are presented as mean ± SEM. *P < 0.05, **P<0.01, ***P < 0.001 (One-way ANOVA with Newman-Keuls Multiple Comparison Test). **(C)** Phagocytosis of opsonised *Escherichia coli* K-12 bioparticles by wild-type mouse macrophages treated or not with IC87114 (1 μM). cells were pre-treated for 2 h with IC87114 (1 μM) or vehicle before addition of particles. **(D)** Quantitative RT–PCR detection of the amount of VVL15 internalized in macrophages pooled from two wild-type (WT) BALB/c mice pre-treated for 2 h with IC87114 (1 μM) or vehicle before addition of VVL15 at an MOI=5. Data are presented as the mean percentage of the total attached virus that was internalized (n=6). *P < 0.05, **P<0.01, ***P < 0.001(Student's Two-tailed unpaired t-test). **(E)** Quantitative RT–PCR detection of the amount of VVL15 internalized in macrophages pooled from two wild-type (WT) BALB/c mice pre-treated for 2 h with idelalisib (10 μM) or vehicle before addition of VVL15 at an MOI=5. Data are presented as the mean percentage of the total attached virus that was internalized (n=6). *P < 0.05, **P<0.01, ***P < 0.001(Student's Two-tailed unpaired t-test). **(F)** Semi-quantification of western blots of p-AKT in macrophages treated with idelalisib (10 μM) +/- VVL15 (MOI=5). Analysis was preformed using the ImageJ and the p-AKT/t-AKT ratio is shown in the graph (n=3). data are presented as mean ± SEM. *P < 0.05, **P<0.01, ***P < 0.001 (One-Way

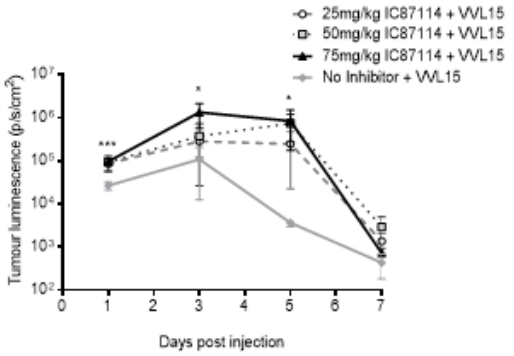
ANOVA with Newman-Keuls Multiple Comparison Test). **(G)** Immunoblot depicting p-AKT and total-AKT in macrophages treated with idelalisib (10 μM) +/- VVL15 (MOI=5).



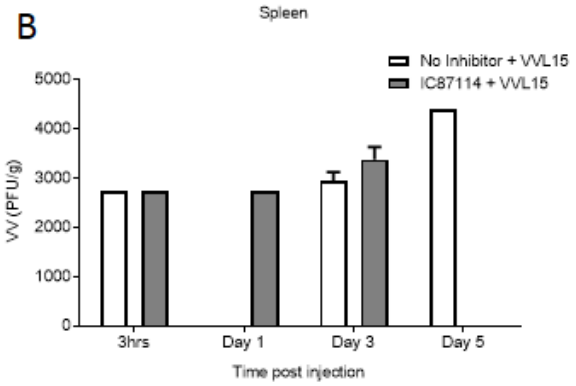
Supplementary Figure 2. Attachment and internalisation of Ad5 to macrophages in vitro. **(A)** Quantitative RT–PCR detection of the amount of Ad5 attached to macrophages pooled from two wild-type (WT) BALB/c mice pre-treated for 2 h with IC87114 (1 μM), a PI3Kδ inhibitor, or vehicle before addition of Ad5 (n=3). A Student's Two-tailed unpaired t-test was used to determine significance. **(B)** Quantitative RT–PCR detection of the amount of Ad5 internalized in macrophages treated as for (**A**) after 30 minutes. Data are presented as the mean percentage of the total attached virus that was internalized (n=3). A Student's Two-tailed unpaired t-test was used to determine significance.

SF3

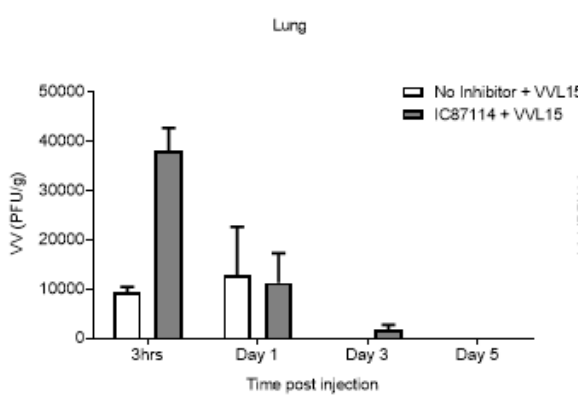
A


25mg/kg IC87114 + VVL15
50mg/kg IC87114 + VVL15
75mg/kg IC87114 + VVL15
No Inhibitor + VVL15
Tumour luminescence (p/s/cm²)
Days post injection

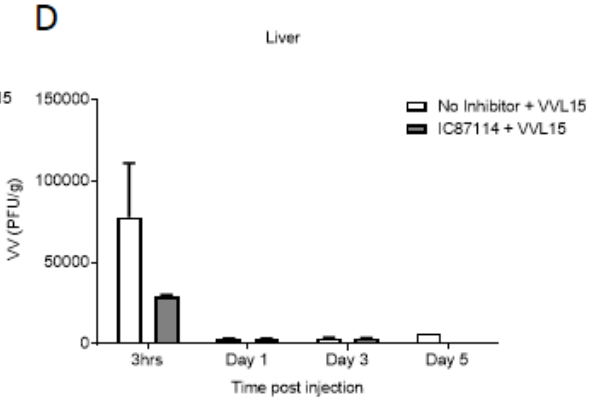
B


Spleen
No Inhibitor + VVL15
IC87114 + VVL15
VV (PFU/g)
3hrs
Day 1
Day 3
Day 5
Time post injection

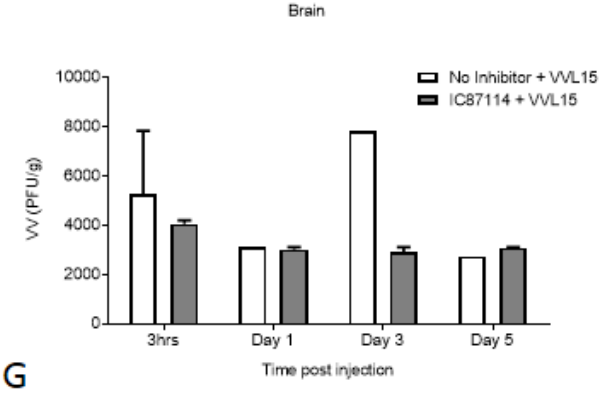
C


Lung
No Inhibitor + VVL15
IC87114 + VVL15
VV (PFU/g)
3hrs
Day 1
Day 3
Day 5
Time post injection

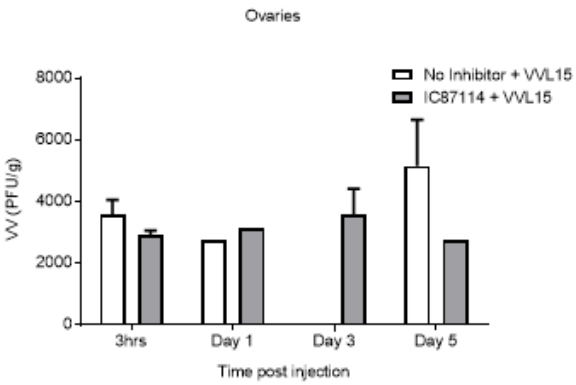
D


Liver
No Inhibitor + VVL15
IC87114 + VVL15
VV (PFU/g)
3hrs
Day 1
Day 3
Day 5
Time post injection

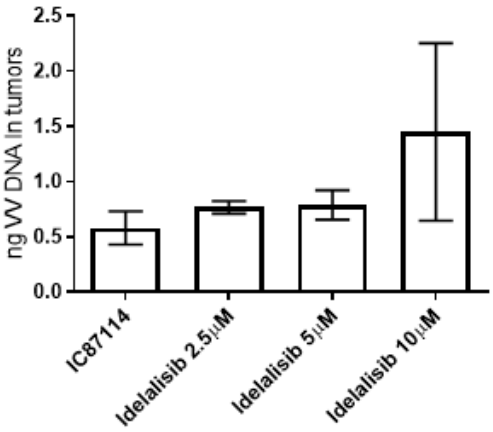
E


Brain
No Inhibitor + VVL15
IC87114 + VVL15
VV (PFU/g)
3hrs
Day 1
Day 3
Day 5
Time post injection

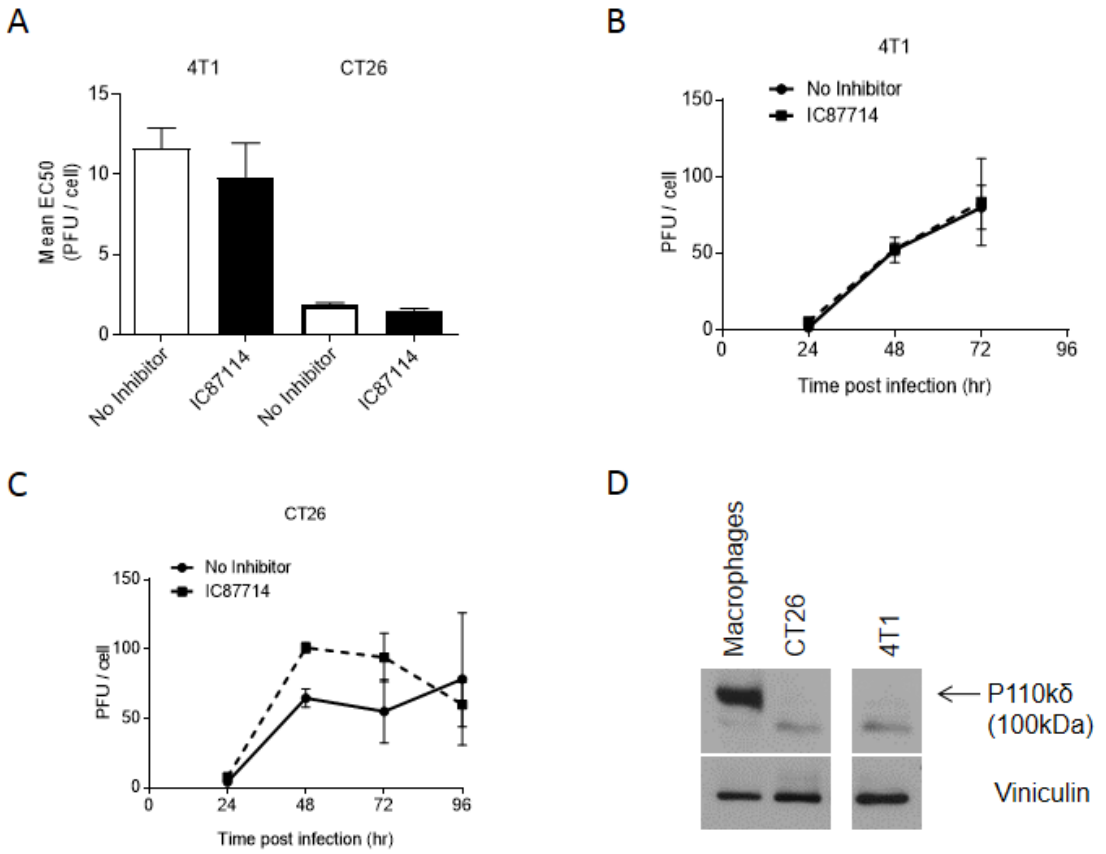
F


Ovaries
No Inhibitor + VVL15
IC87114 + VVL15
VV (PFU/g)
3hrs
Day 1
Day 3
Day 5
Time post injection

G


ng VV DNA in tumors
IC87114
Idelalisib 2.5μM
Idelalisib 5μM
Idelalisib 10μM

Supplementary Figure 3. Pharmacological inhibition of PI3Kδ has no effect on off-target uptake of VVL15. (A) Four BALB/c mice bearing CT26 flank tumours received either one of the various doses of IC87714 or vehicle buffer three hours before one intravenous injection of $1x10^8$ PFU VVL15. Biodistribution of VVL15 was ascertained by In Vivo Imaging System (IVIS) under inhalation anesthesia from 5 minutes following intra-peritoneal (IP) injection of D-Luciferin (15mg ml^{-1}). Mean luminescence values of tumours ± SEM are displayed. There was no significant difference between the group pretreated with 25mg kg^{-1} and the no inhibitor group. The group pretreated with 50mg kg^{-1} IC87114 and the no inhibitor group; there was significantly more signal detected from the group pretreated with 50mg kg^{-1} IC87114 at day 5 ($P<0.01$). The group pretreated with 75mg kg^{-1} IC87114 and the no inhibitor group; significance is depicted on the graph. There was significantly more signal detected from the group pretreated with 75mg kg^{-1} IC87114 at days 1, 3 and 5 ($P<0.001$ at day 1 and $P<0.05$ at days 3 & 5). (**B-F**) Immunocompetent mice bearing CT26 tumors were inected once i.v with 1x108P PFU VVL15. Organs were isolated at the indicated timepoints and the amount of virus present determined using qPCR (n=3/group). Virus accumulation in the spleen (**B**), lungs (**C**), liver (**D**), brain (**E**) and ovaries (**F**) was examined. **(G)** CT26 tumor-bearing immunocompetent mice were treated with IC87114 (75 mg/kg) or varying doses idelalisib 3 h prior to i.v. delivery of $1x10^8$ PFU VVL15. 3 days post treatment, tumors were analysed for the presence of VV using quantitative RT–PCR.



Supplementary Figure 4. Pharmacological inhibition of PI3Kδ has no effect on virus replication and cytotoxicity *in vitro*. (A) Direct cytotoxicity of VVL15 in CT26 and 4T1 cancer cell lines upon addition of IC87114. The mean EC50 value/cell is shown. All experiments were performed in duplicate (n=4). There are no statistical differences between any of the groups. **(B-C)** $TCID_{50}$ assay for the replication of VVL15 after the addition of IC87114 to cultures of CT26 (**B**) and 4T1 (**C**) cell lines (n=3). There is no significant difference between any of the groups in any of the cell lines. **(D)** Western blot assay of p110δ in CT26 and 4T1 lysates. Viniculin is shown as a loading control.