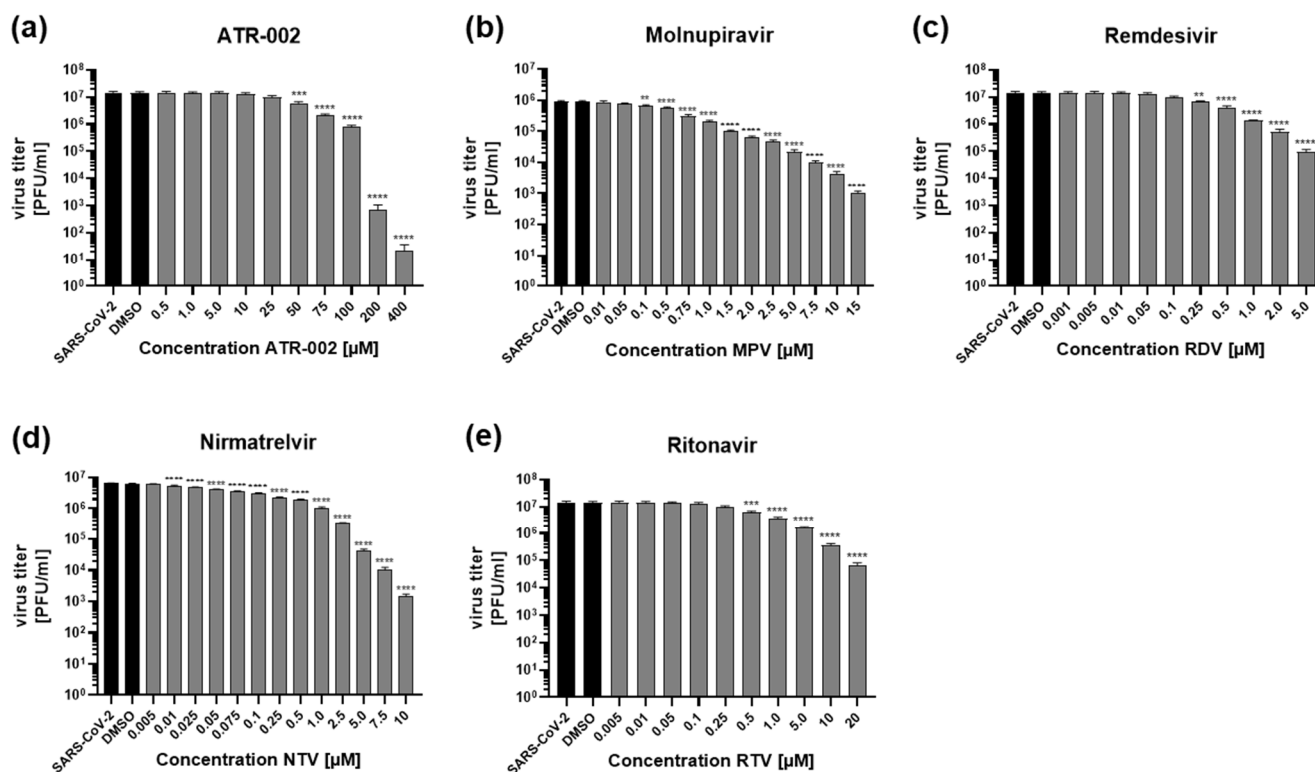


# The MEK1/2 Inhibitor ATR-002 (Zapnometinib) Synergistically Potentiates the Antiviral Effect of Direct-Acting Anti-SARS-CoV-2 Drugs

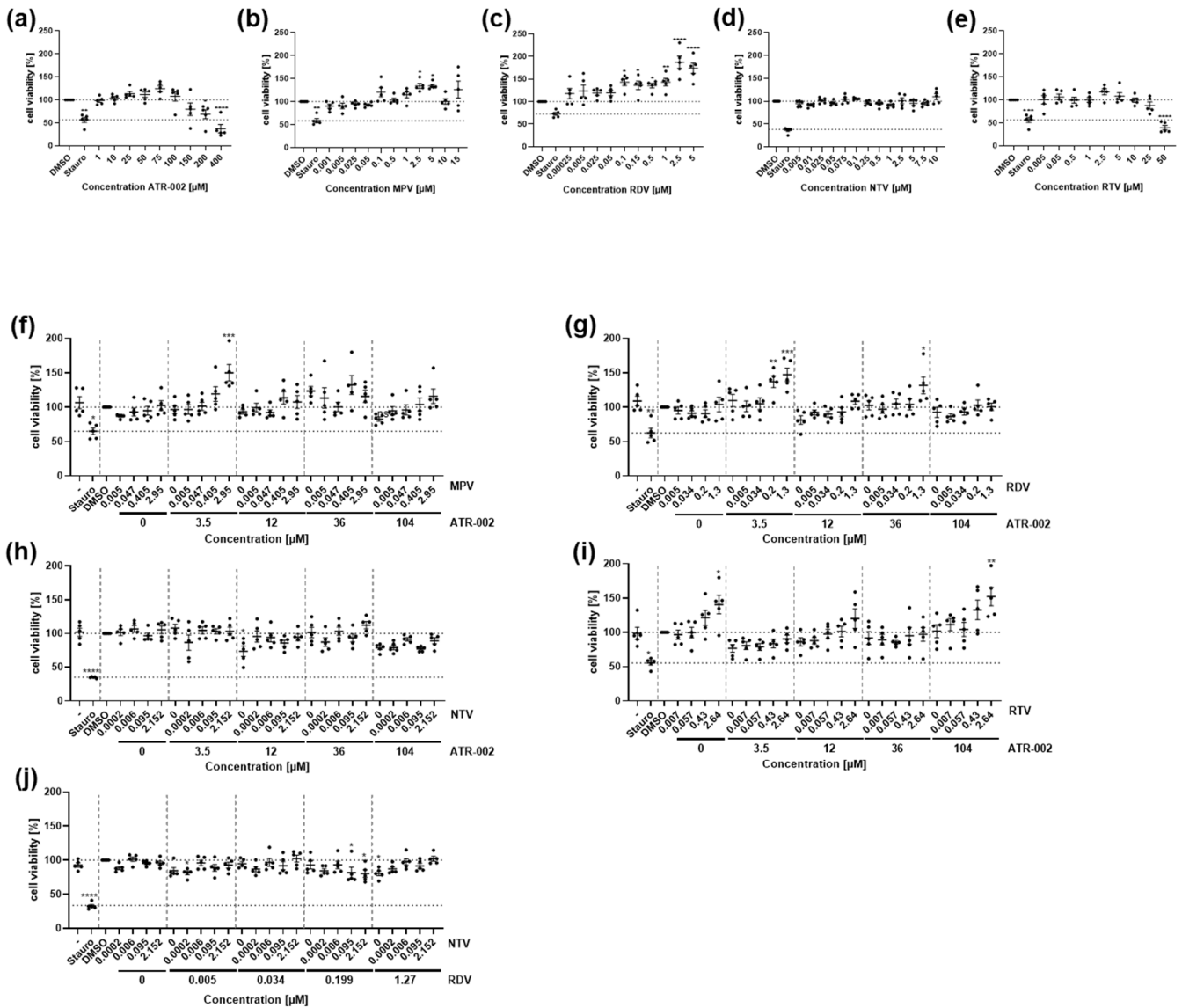
André Schreiber, Benjamin Ambrosy, Oliver Planz, Sebastian Schloer, Ursula Rescher and Stephan Ludwig



**Figure S1. Drug related inhibitory effect on the SARS-CoV-2 replication. Related to Figure 1.**

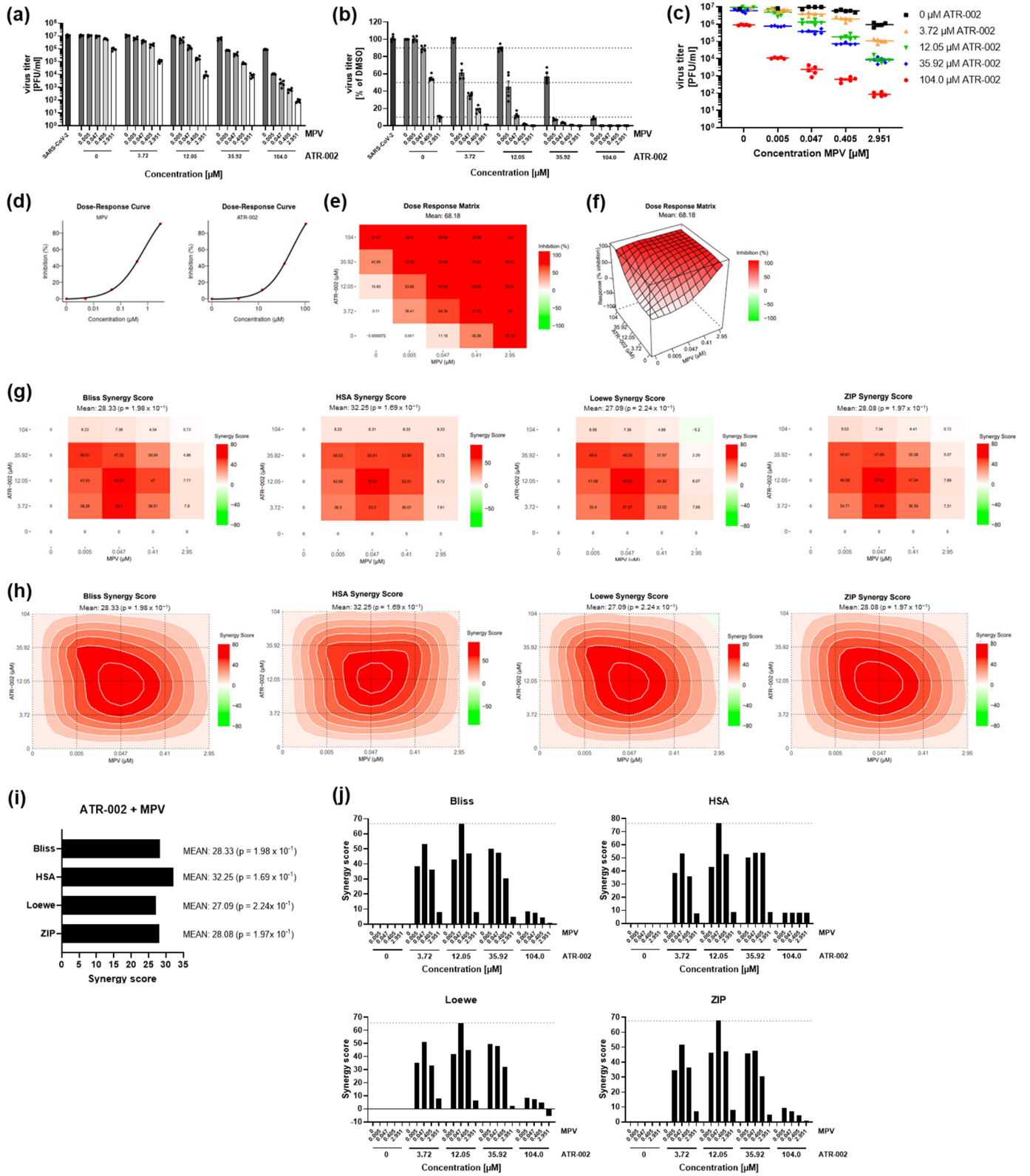
SARS-CoV-2 (MOI: 0.01) was used to infect Calu-3 cells. Drug treatment was initiated 1 h.p.i. Untreated (SARS-CoV-2) and DMSO-treated cells served as negative controls. Viral titers were analyzed 48 h.p.i.

Shown are viral titers in PFU/mL. Data represent means  $\pm$  SEM of five independent experiments, each performed in triplicates. Significance was calculated by one-way ANOVA in combination with a Dunnett's multiple comparisons test using DMSO as reference (\*\*  $p \leq 0.0021$ ; \*\*\*  $p \leq 0.0002$ ; \*\*\*\*  $p \leq 0.0001$ ).



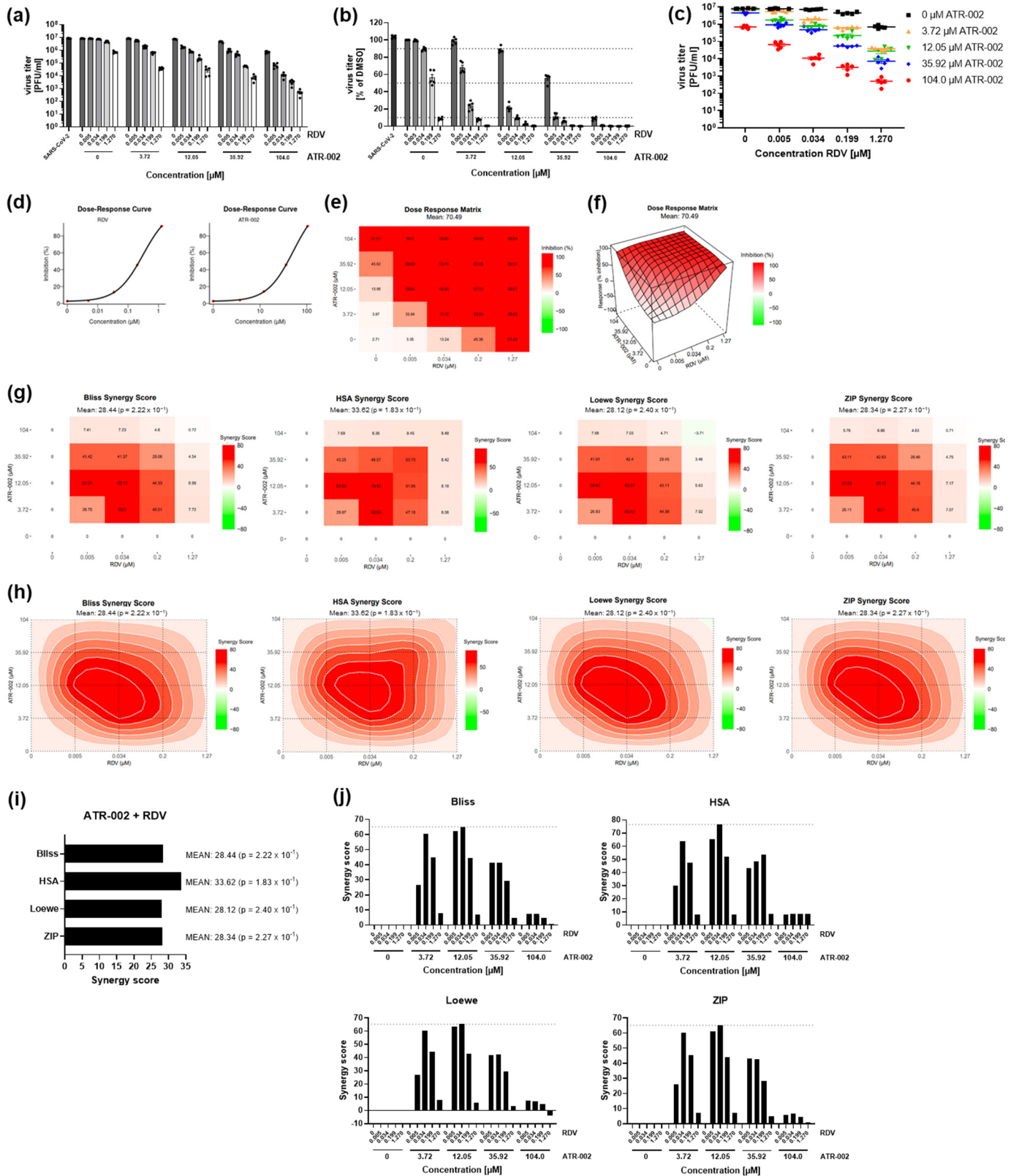
**Figure S2. Determination of the cell viability after single and combinational drug treatment.**

Calu-3 cells were single treated (a–e) or double treated (f–j) with the indicated drug concentrations and combinations. 48 h post-treatment cell viability was evaluated using a MTT-assay. DMSO was used as negative control, Staurosporine (Stauro; 1 μM) as positive control. DMSO and Staurosporine levels are indicated by dashed lines. Shown are means ± SEM of five independent experiments, each performed in triplicates. Significance was calculated using an one-way ANOVA in combination with a Dunnett's multiple comparisons test with DMSO as reference (\*  $p \leq 0.0332$ ; \*\*  $p \leq 0.0021$ ; \*\*\*  $p \leq 0.0002$ ; \*\*\*\*  $p \leq 0.0001$ ).



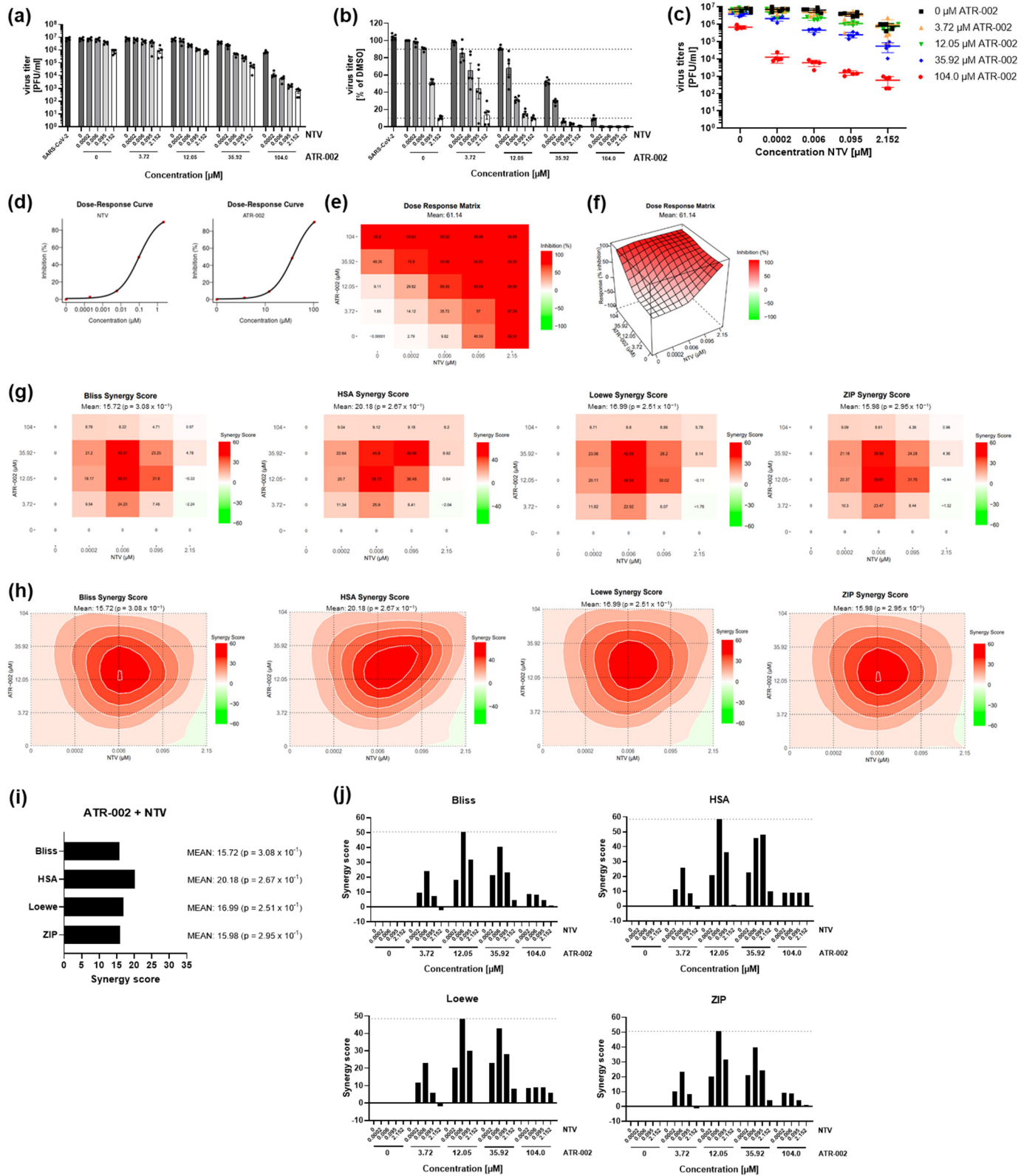
**Figure S3. Determination of the ATR-002 + MPV synergism. Related to Figure 3.**

Calu-3 cells were infected with SARS-CoV-2 (MOI: 0.01). 1 h.p.i. drug treatment was initiated. 48 h.p.i. viral titers were determined by plaque titration. (a–c) Viral titers in PFU/mL (a,c) or % of DMSO (b) of the combinational drug treatments. Data represent means  $\pm$  SEM of five independent experiments, each performed in triplicates. (b) Dashed lines indicate 10 %, 50 % and 90 % titer reduction levels. (d) Dose-response curves of the single treatment in combination with DMSO. (e) 2D dose response matrix of the ATR-002 + MPV drug combination. (f) 3D dose response landscape of the ATR-002 + MPV drug combination. (g) 2D heat maps of the 3D landscape visualization and 2D contour shown in Figure 3a and Figure S3h. (h) 2D contour visualization of the synergy calculation. (i) Synergy score for the ATR-002 + MPV drug combination calculated with the indicated synergy reference models. (j) Synergy values of the different drug combinations calculated with the indicated synergy reference models (*see also Table S1*).



**Figure S4. Determination of the ATR-002 + RDV synergism. Related to Figure 3.**

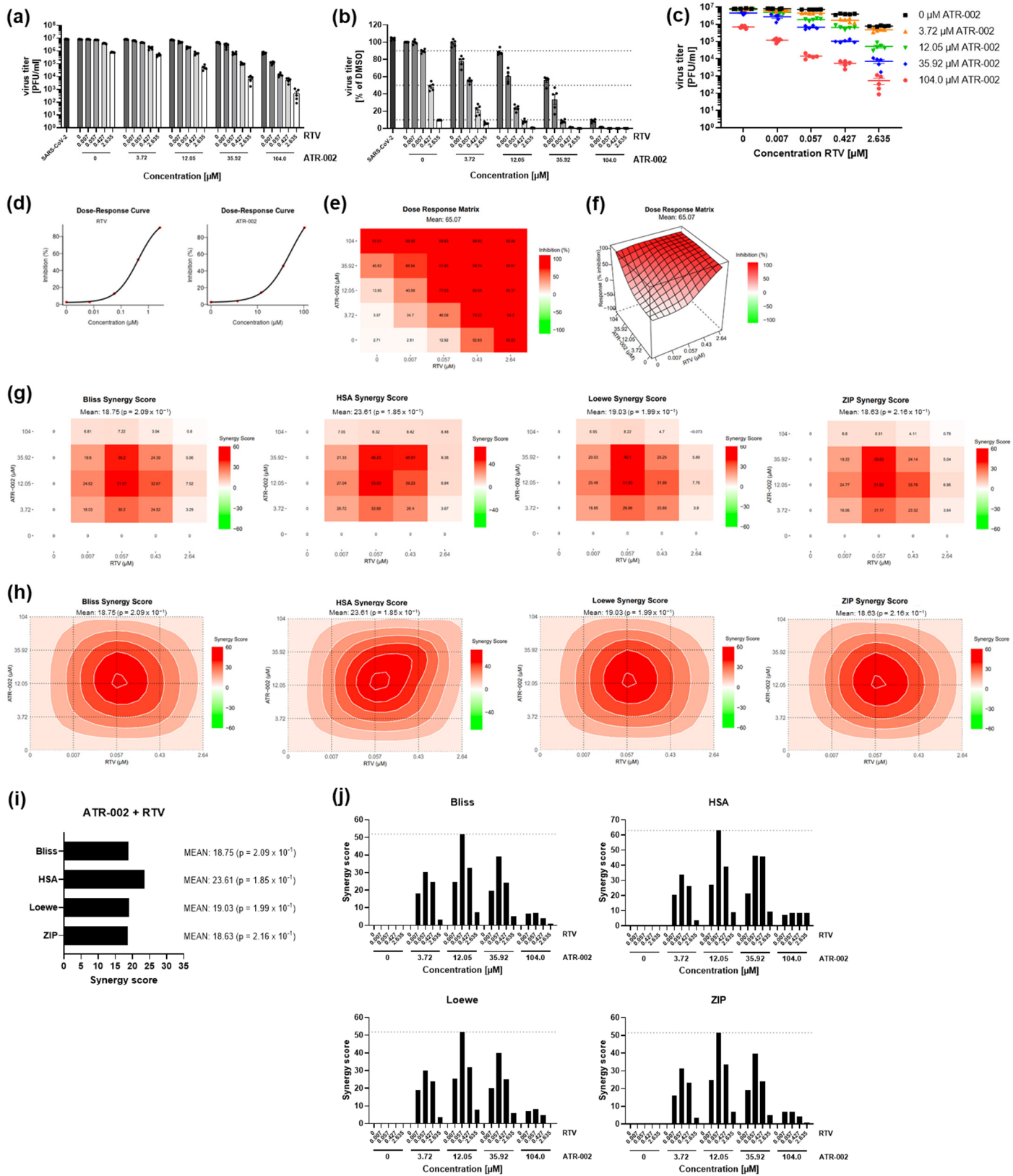
Calu-3 cells were infected with SARS-CoV-2 (MOI: 0.01). 1 h.p.i. drug treatment was initiated. 48 h.p.i. viral titers were determined by plaque titration. (a–c) Viral titers in PFU/ml (a,c) or % of DMSO (b) of the combinational drug treatments. Data represent means  $\pm$  SEM of five independent experiments, each performed in triplicates. (b) Dashed lines indicate 10 %, 50 % and 90 % titer reduction levels. (d) Dose-response curves of the single treatment in combination with DMSO. (e) 2D dose response matrix of the ATR-002 + RDV drug combination. (f) 3D dose response landscape of the ATR-002 + RDV drug combination. (g) 2D heat maps of the 3D landscape visualization and 2D contour shown in Figure 3b and Figure S4h. (h) 2D contour visualization of the synergy calculation. (i) Synergy score for the ATR-002 + RDV drug combination calculated with the indicated synergy reference models. (j) Synergy values of the different drug combinations calculated with the indicated synergy reference models (*see also Table S1*).



**Figure S5. Determination of the ATR-002 + NTV synergism. Related to Figure 3.**

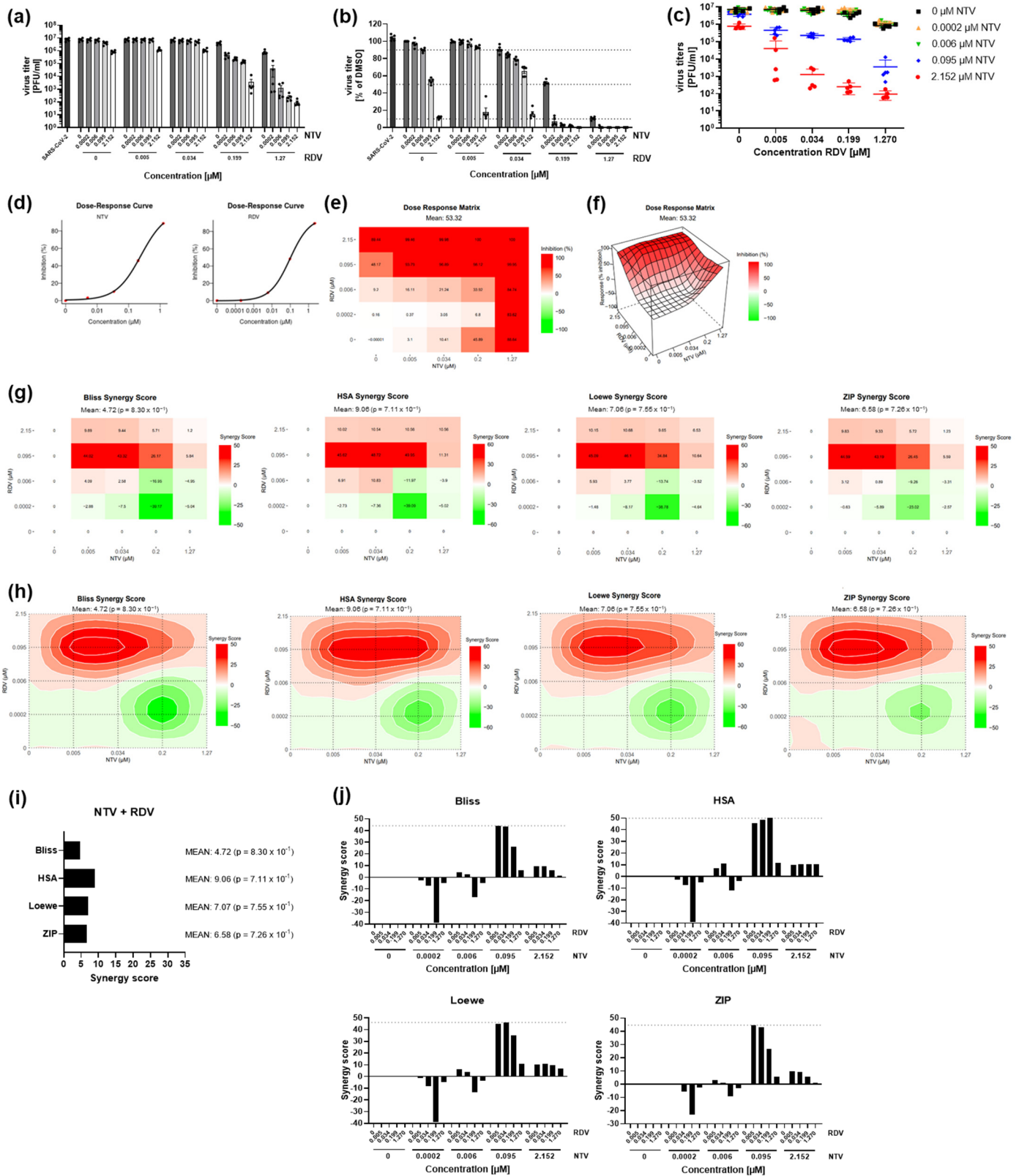
Calu-3 cells were infected with SARS-CoV-2 (MOI: 0.01). 1 h.p.i. drug treatment was initiated. 48 h.p.i. viral titers were determined by plaque titration. **(a–c)** Viral titers in PFU/mL **(a,c)** or % of DMSO **(b)** of the combinational drug treatments. Data represent means  $\pm$  SEM of five independent experiments, each performed in triplicates. **(b)** Dashed lines indicate 10 %, 50 % and 90 % titer reduction levels. **(d)** Dose-response curves of the single treatment in combination with DMSO. **(e)** 2D dose response matrix of the ATR-002 + NTV drug combination. **(f)** 3D dose response landscape of the ATR-002 + NTV drug combination. **(g)** 2D heat maps of the 3D landscape visualization and 2D contour shown in Figure 3c and Figure S5h. **(h)** 2D contour visualization of the synergy calculation. **(i)** Synergy score for the ATR-002 + NTV drug combination calculated with the indicated synergy reference models. **(j)** Synergy values of the different drug combinations calculated with the indicated synergy reference models (*see also Table S1*).





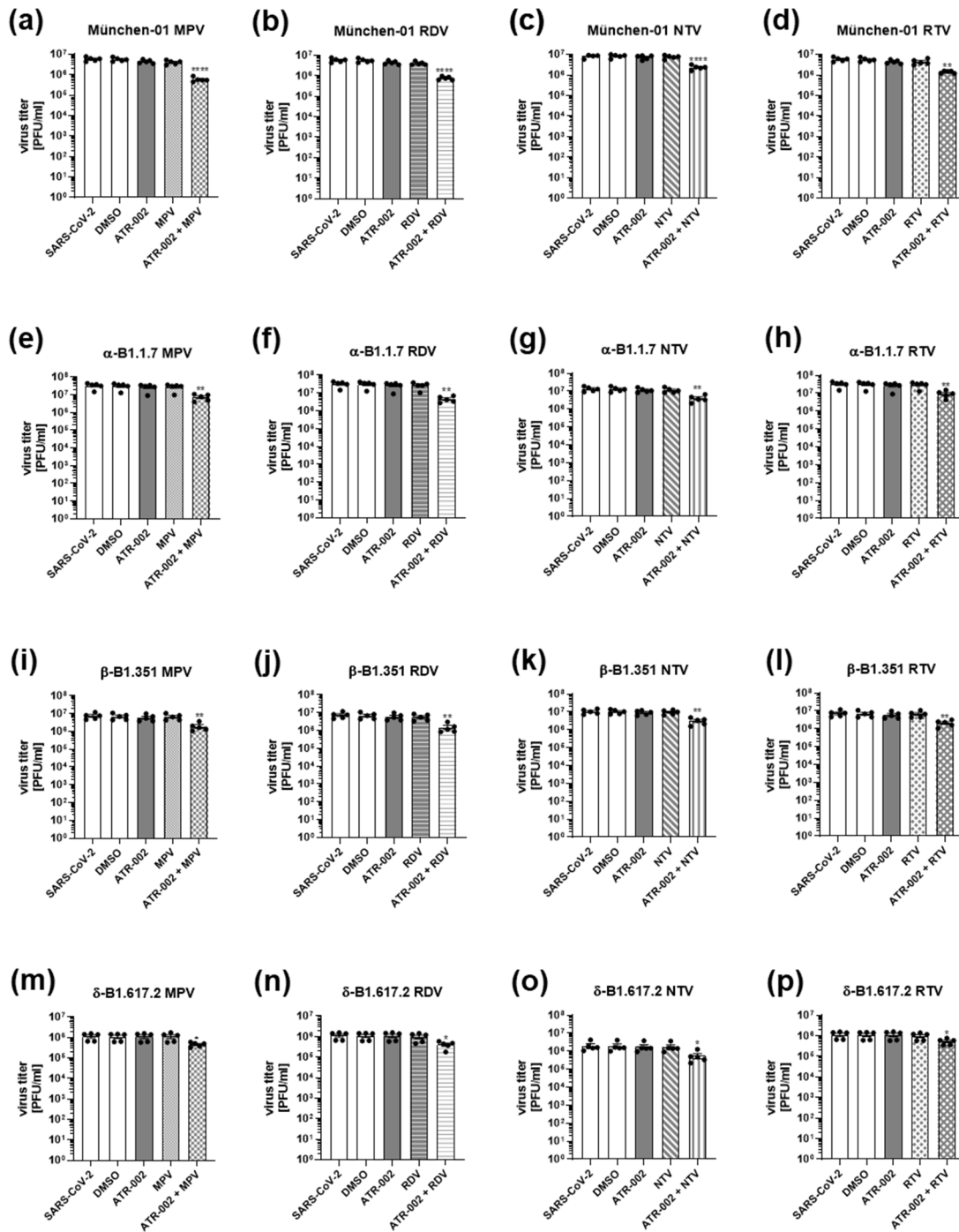
**Figure S6. Determination of the ATR-002 + RTV synergism. Related to Figure 3.**

Calu-3 cells were infected with SARS-CoV-2 (MOI: 0.01). 1 h.p.i. drug treatment was initiated. 48 h.p.i. viral titers were determined by plaque titration. **(a–c)** Viral titers in PFU/mL **(a,c)** or % of DMSO **(b)** of the combinational drug treatments. Data represent means  $\pm$  SEM of five independent experiments, each performed in triplicates. **(b)** Dashed lines indicate 10 %, 50 % and 90 % titer reduction levels. **(d)** Dose-response curves of the single treatment in combination with DMSO. **(e)** 2D dose response matrix of the ATR-002 + RTV drug combination. **(f)** 3D dose response landscape of the ATR-002 + RTV drug combination. **(g)** 2D heat maps of the 3D landscape visualization and 2D contour shown in Figure 3d and Figure S6h. **(h)** 2D contour visualization of the synergy calculation. **(i)** Synergy score for the ATR-002 + RTV drug combination calculated with the indicated synergy reference models. **(j)** Synergy values of the different drug combinations calculated with the indicated synergy reference models (*see also Table S1*).



**Figure S7. Determination of the RDV + NTV synergism. Related to Figure 3.**

Calu-3 cells were infected with SARS-CoV-2 (MOI: 0.01). 1 h.p.i. drug treatment was initiated. 48 h.p.i. viral titers were determined by plaque titration. **(a–c)** Viral titers in PFU/mL **(a,c)** or % of DMSO **(b)** of the combinational drug treatments. Data represent means  $\pm$  SEM of five independent experiments, each performed in triplicates. **(b)** Dashed lines indicate 10 %, 50 % and 90 % titer reduction levels. **(d)** Dose-response curves of the single treatment in combination with DMSO. **(e)** 2D dose response matrix of the RDV + NTV drug combination. **(f)** 3D dose response landscape of the RDV + NTV drug combination. **(g)** 2D heat maps of the 3D landscape visualization and 2D contour shown in Figure 3e and Figure S7h. **(h)** 2D contour visualization of the synergy calculation. **(i)** Synergy score for the RDV + NTV drug combination calculated with the indicated synergy reference models. **(j)** Synergy values of the different drug combinations calculated with the indicated synergy reference models (*see also Table S1*).



**Figure S8. Synergistic effects of different drug combinations on the replication of SARS-CoV-2 variants. Related to Figure 4.**

Calu-3 cells were infected with SARS-CoV-2 (MOI: 0.01). 1 h.p.i. drug treatment was initiated. MPV (0.047  $\mu$ M) (a,e,i,m), RDV (0.034  $\mu$ M) (b,f,j,n), NTV (0.006  $\mu$ M) (c,g,k,o) or RTV (0.057  $\mu$ M) (d,h,l,p) were combined with ATR-002 (12.05  $\mu$ M). Untreated (SARS-CoV-2), DMSO treated and single treated (drug + DMSO) cells served as controls. 48 h.p.i. viral titers were determined by plaque titration. Shown are means  $\pm$  SEM five independent experiments, each performed in triplicates. Significance was calculated using an unpaired *t*-test for each inhibitor individually, comparing the single treated (MPV, RDV, NTV, RTV) vs. the double treated results (\*  $p \leq 0.0332$ ; \*\*  $p \leq 0.0021$ ; \*\*\*  $p \leq 0.0001$ ).

**Table S1. Highest synergy score values.**

Highest synergy score values, calculated for the drug combinations MPV 0.047  $\mu$ M / ATR-002 12.05  $\mu$ M, RDV 0.034  $\mu$ M / ATR-002 12.05  $\mu$ M, NTV 0.006  $\mu$ M / ATR-002 12.05  $\mu$ M, RTV 0.057  $\mu$ M / ATR-002 12.05  $\mu$ M and RDV / NTV (Bliss, Loewe, ZIP: RDV 0.034  $\mu$ M + NTV 0.095  $\mu$ M; HSA: RDV 0.199  $\mu$ M + NTV 0.095  $\mu$ M) *Related to Figure S3–S7j.*

Drug combination	Reference model	Synergy score
MPV / ATR-002	Bliss	66.83
	HSA	76.45
	Loewe	65.69
	ZIP	67.63
RDV / ATR-002	Bliss	65.15
	HSA	76.55
	Loewe	65.27
	ZIP	65.15
NTV / ATR-002	Bliss	50.51
	HSA	58.73
	Loewe	48.54
	ZIP	50.61
RTV / ATR-002	Bliss	51.97
	HSA	63.09
	Loewe	51.86
	ZIP	51.52
RDV / NTV	Bliss	44.01
	HSA	49.95
	Loewe	46.10
	ZIP	44.59

**Table S2. Drug combination sensitivity score (CSS).**

	ATR-002 + MPV	ATR-002 + RDV	ATR-002 + NTV	ATR-002 + RTV	NTV + RDV
CSS	98.25	98.12	90.01	94.63	80.1