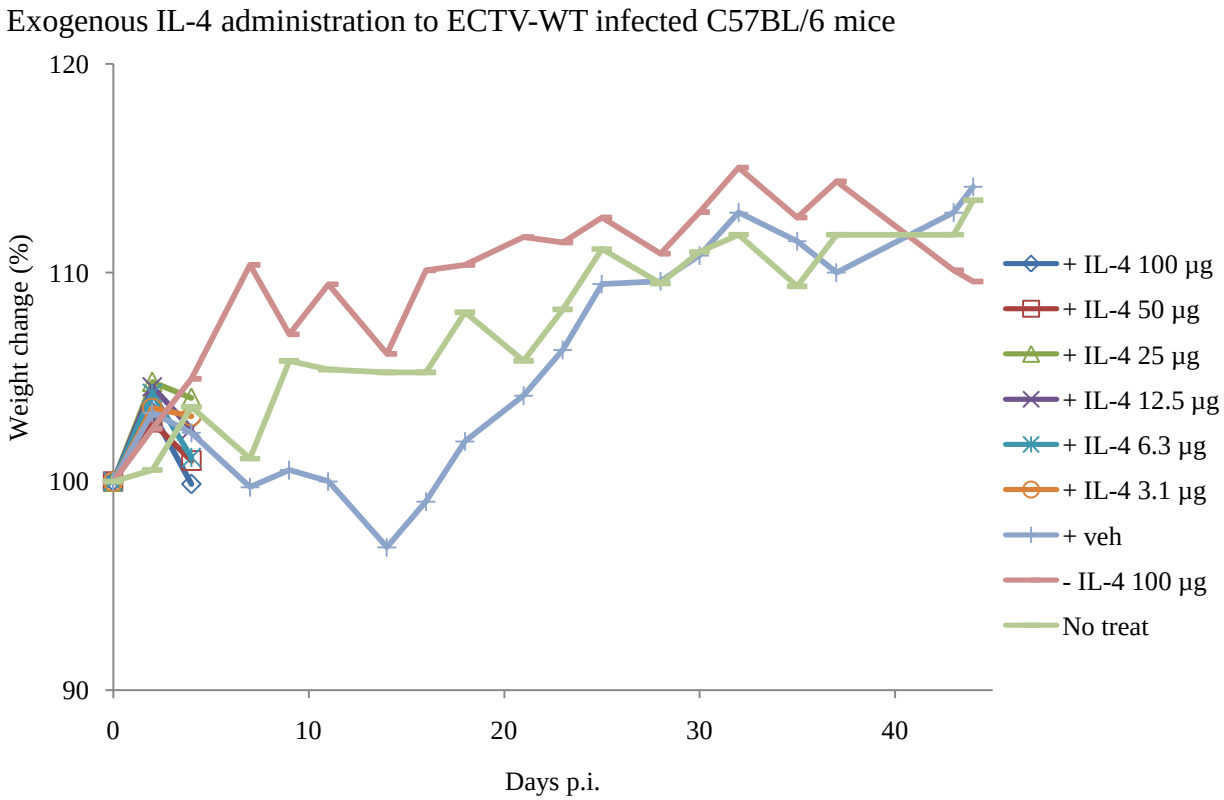
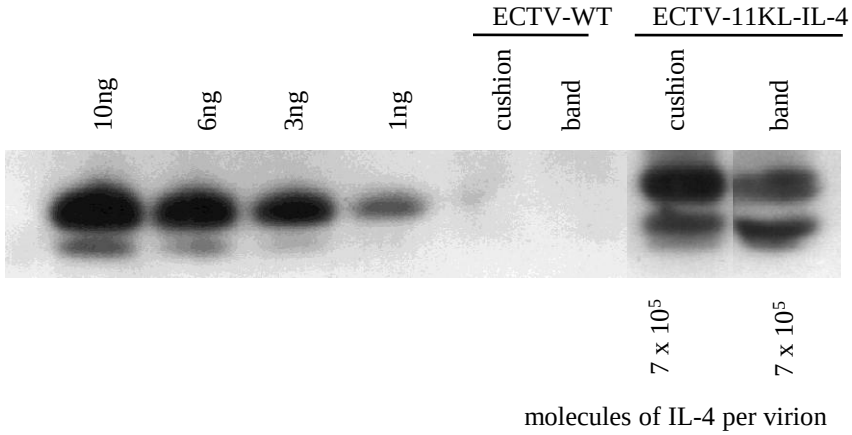

Exogenous IL-4 administration to ECTV-WT infected C57BL/6 mice
Weight change (%)
120
110
100
90
0
10
20
30
40
Days p.i.
+ IL-4 100 µg
+ IL-4 50 µg
+ IL-4 25 µg
+ IL-4 12.5 µg
+ IL-4 6.3 µg
+ IL-4 3.1 µg
+ veh
- IL-4 100 µg
No treat

Supp. Fig 1


ECTV-WT
ECTV-11KL-IL-4
10ng
6ng
3ng
1ng
cushion
band
cushion
band
7 x 10^5
7 x 10^5
molecules of IL-4 per virion

Supp. Fig. 2

Supplemental Table 1. ECTV-11KM-IL-4 severe disease is mediated through the IL-4 receptor

Strain[1]	Challenge virus	Mean time to death	Mortality (%[3])
BALB/cJ	-WT	NA[2]	0/4 (0)
BALB/cJ	-SSE-IL-4	8.5 ± 1.0[4]	4/4 (100)[4]
BALB/c-IL-4R$^{-/-}$	-WT	NA	0/4 (0)
BALB/c-IL-4R$^{-/-}$	-SSE-IL-4	NA	0/4 (0)

[1]Wild type (BALB/cJ) or IL-4 receptor knockout mice (BALB/c-IL-4R$^{-/-}$) were vaccinated by tail scarification with 2 x 10^5 PFU of Dryvax™. Four weeks later mice were challenged in the right-rear FP with 1.5 x 10^4 PFU of the indicated virus.
[2]NA – not applicable.
[3]Percent morality.
[4]$P<0.05$ compared to ECTV-WT control.

Supplemental Table 2. Exogenous administration of IL-4 in combination with FP ECTV-WT infection results in lethality and recapitulates ECTV-IL-4 infections in C57BL/6 mice.

Virus[1]	IL-4	Vehicle	BID dosing for 5 days[2]						Mean time to death	Mortality (%)
			100	50	25	12.5	6.3	3.1		
WT	+	-	+						7±0	4/4 (100)[3]
WT	+	-		+					7±0	4/4 (100)[3]
WT	+	-			+				7±0	4/4 (100)[3]
WT	+	-				+			7±0	4/4 (100)[3]
WT	+	-					+		7±0	4/4 (100)[3]
WT	+	-						+	7±0	2/4 (50)[4]
WT	-	+								0
-	+	-	+							0
-	-	-	-	-	-	-	-	-		0

[1]C57BL/6 mice were infected with 3 x 10^4 PFU of wild type ECTV in the FP.
[2]IP bolus injections ranging from 100 -3.1 µg/mouse of IL-4 were administered twice daily for the first 5 days of infection. Mice were monitored for weight change and mortality.
[3]$P<0.05$ compared to non infected controls.
[4]$P>0.05$ compared to non infected controls.

Supplemental Table 3. A/NCR and C57BL/6 mice vaccinated with Dryvax™ 7 days before infection are not protected against an ECTV-IL-4 infection.

Challenge virus[1]	Mouse Strain	Dryvax™[2]	Mean time to death	Mortality at 21 days p.i. (%)
-11KL-IL-4	A/NCR	-	7±0	8/8 (100)
	A/NCR	+	7.4±0.5	8/8 (100)
	C57BL/6	-	7.0±0	8/8 (100)
	C57BL/6	+	10.6±1.4	7/7 (100)
WT	A/NCR	-	7.4±0.2	8/8 (100)
	A/NCR	+[3]	NA[4]	0/8 (0)

[1]Mice were infected in the left-rear FP with $2.4x10^3$ PFU of ECTV-IL-4.
[2]Mice were vaccinated in the right-rear FP with $2.4x10^4$ PFU Dryvax™ 7 days before infection with ECTV-11K-IL-4.
[3]This group of mice was immunized by scarification 4 days pre infection.
[4]Not applicable.

Supplemental Table 4. Vaccination with ECTV provides more efficacious protection against ECTV-IL-4 infection than vaccination with Dryvax™.

ECTV-IL-4 dose (PFU)[1]	Immunization			Mean time to death	Mortality (%)
	Dryvax™ [2]	ECTV[3]	Placebo		
16	+	-	-	23±5.0[4]	6/6 (100)[5]
160	+	-	-	19.8±6.1[4]	4/6 (67)[5]
1600	+	-	-	6.6±1.7[5]	5/6 (83)[5]
16	-	+	-	NA[6]	0/2 (0)[4]
160	-	+	-	NA	0/5 (0)[4]
1600	-	+	-	NA	0/4 (0)[4]
16	-	-	+	8.4±0.2	6/6 (100)
160	-	-	+	8±0	6/6 (100)
1600	-	-	+	8±0	6/6 (100)
N/A	-	-	-	NA	0/6 (0)[4]

[1]C57BL/6 mice were IN infected with the indicated dose of ECTV-11KL-IL-4 at 49 days post vaccination with Dryvax™ or ECTV or saline.
[2]Mice were vaccinated in the right-rear FP with $7.6x10^4$ PFU of Dryvax™ 49 days prior to infection.
[3] Mice were vaccinated in the right-rear FP with $6.1x10^3$ PFU of ECTV 49 days prior to infection.
[4]$P<0.05$ compared to infected and placebo treated controls.
[5]$P>0.05$ compared to infected and placebo treated controls.
[6]Not applicable.

Supplemental Table 5. VIG treatment does not protect A/NCR mice against a FP infection with ECTV-11KL-IL-4.

Virus[1]	VIG[2] T=-4	Placebo T=-4	Mean time to death	Mortality (%)
-WT	-	+	8.0±0.4	4/4 (100)
-WT	+	-	7.5±0.5[3]	4/4 (100)[4]
-11KL-IL-4	-	+	6±0	4/4 (100)
-11KL-IL-4	+	-	7±0	4/4 (100)[4]

[1]A/NCR mice were inoculated in the FP with 130 PFU of ECTV-WT or ECTV-11KL-IL-4.
[2]Indicated mice were treated with VIG (2g/Kg; 40 mg/mouse in 0.75 ml by IP route) at 4 days prior to infection or with vehicle.
[3]P=0.6 compared to ECTV-WT mice receiving vehicle.
[4]P=1.0 compared to vehicle treated controls.

Supplemental Table 6. Combination of an antiviral and neutralizing IL-4 mAB protects against ECTV-11KL-IL-4 disease in A/NCR and C57BL/6 mice.

Challenge virus	Mouse Strain	Dose (PFU)	Treatments			Mean time to death	Mortality at 21 days p.i. (%)
			Anti IL-4 mAB	Control mAB	CDV		
-11KL-IL-4	A/NCR[1]	1000	+	-	-	7 ± 0[3]	8/8 (100)[3]
	A/NCR[1]	1000	+	-	+	-	0/8 (0)[4]
	A/NCR[1]	1000	-	+	-	5.9 ± 0.4[3]	8/8 (100)[3]
	A/NCR[1]	1000	-	+	+	12.8 ± 1.9[4]	8/8 (100)[3]
	A/NCR[1]	1000	-	-	-	6 ± 0	8/8 (100)[3]
	C57BL/6[2]	15	+	-	-	11.6 ± 3.2[4]	5/8 (62)[3]
	C57BL/6[2]	15	+	-	+	-	0/7 (0)[4]
	C57BL/6[2]	15	-	+	-	7.5 ± 0.53[3]	8/8 (100)[3]
	C57BL/6[2]	15	-	+	+	13.8 ±0.71[4]	7/7 (100)[3]
	C57BL/6[2]	15	-	-	-	7.4 ± 0.52	8/8 (100)

[1]1000 PFU infected A/NCR mice were injected IP with 2 mg/mouse of rat anti-IL-4 (clone11B11, IgG1) or rat anti-p30 gag capsid protein (clone R187) on days T=-1, +1, +3, and +5. Mice were challenged in the presence or absence of 12.5mg/kg of cidofovir (CDV) injected IP on days T=0, 1, 2, 3, 4, and 5.
[2]15 PFU infected C57BL/6 mice were injected IP with 2 mg/mouse of the indicated mAB on days T=-1, +0, +1, +2, +4 and +6. Mice were challenged in the presence or absence of an optimal course of CDV. Data from A/NCR and C57BL/6 experiments were reported from independent experiments.
[3]$P>0.05$ compared to non-treated infected controls.
[4]$P<0.05$ compared to non-treated infected controls.

Supplemental Table 7. The effect of prime boost vaccination and antiviral treatment on the course of ECTV IL-4 lethally in infected A/NCR mice.

Challenge virus[1]	Dose (PFU)	Treatments			Mean time to death	Mortality at 21 days p.i. (%)
		Dryvax™ prime[1]	Dryvax™ boost[2]	CDV		
-11KL-IL-4	25	+	-	-	10.8 ± 1.9	8/8 (100)
		+	-	+	20.3 ± 5.3[4]	7/8 (87.5)[5]
		+	+	-	14.7 ± 5.4[5]	6/8 (75)[5]
		+	+	+	NA[3]	0/8 (0)[4]
	250	+	-	-	8.6 ± 1.7[4]	8/8 (100)[5]
		+	-	+	28 ± 15[4]	6/8 (75)[5]
		+	+	-	12.8 ± 5.2[5]	8/8 (100)[5]
		+	+	+	50.8 ± 25.7[4]	4/8 (50)[5]
	2500	+	-	-	8.8 ± 1.9[5]	8/8 (100)[5]
		+	-	+	37.5 ± 0.7[4]	2/8 (25)[4]
		+	+	-	17.3 ± 11.5[5]	8/8 (100)[5]
		+	+	+	29 ± 16.3[4]	2/8 (25)[4]

[1]A/NCR mice were vaccinated with $2.4x10^4$ PFU/mouse of Dryvax™ smallpox vaccine at the base of the tail.
[2]At 4 weeks post-vaccination the mice were boosted with $2.4x10^4$ PFU of Dryvax™ vaccine and 7 days later were challenged with the indicated dose of virus in the presence of an optimal course of CDV (see supplemental table 5).
[3]Not applicable.
[4]$P<0.05$ compared to mice receiving Dryvax™ primed and challenged with 25 PFU of virus.
[5]$P>0.05$ compared to mice receiving Dryvax™ primed and challenged with 25 PFU of virus.

Supplemental Table 8. Combination therapy with ST-246 and CDV protects A/NCR mice against intranasal infection with ECTV-11KM-IL-4[1].

Virus[2]	ST-246[3]	CDV[4]	ST-246 veh[5]	CDV veh[6]	Mean time to death	Mortality (%)
-11KL-IL-4	-	-	-	-	5.6±0.2	16/16 (100)
	-	-	+	+	5.6±0.2[8]	16/16 (100)[8]
	+	-	-	+	12.9±1.2[7]	14/16 (87.5)[8]
	-	+	+	-	18.2±4.1[7]	11/16 (68.8)[7]
	+	+	-	-	NA	0/16 (0)[7]
Mock	-	-	+	+	NA	0/16 (0)
	-	-	-	-	NA	0/16 (0)

[1]Pooled data from 2 independent experiments.
[2]Mice were infected IN with 24 or 600 PFU of ECTV-11KL-IL-4 or were mock infected.
[3]Mice were dosed with 100 mg/kg of ST-246 for 10 days starting 6 hours p.i.
[4]Mice were dosed with 100 mg/kg of CDV on T=-1, 3, 7, 11, and 15.
[5]ST-246 vehicle controls received 0.75% methylcellulose and 1% tween.
[6] CDV vehicle controls received USP sterile saline.
[7]$P<0.05$ compared to untreated and infected mice.
[8]$P>0.05$ compared to untreated and infected mice.
[9] Not applicable.