Supplementary material

Table S1

Probes and primers used for microarray hybridization and RTqPCR, respectively, of the *crp1-7* mRNA sequences

probe	accession number	5' nucleotide sequences 3'
crp1	XM_693995	AAACTCCTGGGCGACTTCGAGACGGTGCAGAGTTTCGCCGGTGAACTCACAGACCTGAAC GCCTACCGAACACCCGACT + GCAGGTTGAAAAACGCC
crp2	BC097160	TAGCCAAGCAATGGGGTTCACAGACCTCCAAAGGCAAGTGAAATCTACTTGAAACAAGCT TCTTCTGTTCCCGACTG + GGATGATCTCCCTTTTGG
crp3	BC154042	AGGTTGGAGCTGCTGGTCGATAGGGAGGTCATCTTGTTCGCCTACCGCACATCTGAAGTT ATCCCAGTTATGTTCAAATCG + AGCAGCTCCAACCTG
crp4	BC115188	CGGAGCTGCTGGGATTTAGGGAAGTCATCTTGTTCGCCTACCGCACGCCTGAAGTTGATG GAAAAGTGCTTCTGTTTACAG + CGAACAAGATGACTTCCC
crp5	BC121777	GACGGAGCTGCCGCTCGATAGGGAGGTCATCCTGTTCGCCTATTACACACCTGATGTTGA GTGCTTCAGTTCAAGACG + GATGACCTCCCTATCGAG
crp6	BC162745	CGGCACCGTACTGCTCGGACAGGACCCTGACTCATATGTAGGTTCGTTTGACGCAAATCA GAACTCAATGTGTGGAGAC + AGATAGAAACTTGCTGGATTG
crp7	BC150371	CAACAATATGAAAGGAGACAGTGGCGCTGCTAGAGGCCTGACTGACCCGGTCCTGATCCT CCAAACTGCTACCAGC + AGAATGACTTCCCGCC
ef1a	NM_131263	CTGGAGGCCAGCTCAAACAT + CATTTCCCTCCTTACGCTCAAC
18S	FJ915075	ACCACCCACAGAATCGAGAAA + GCCTGCGGCTTAATTTGACT

The 7 *crp* loci coding for 7 CRP isoform proteins first identified in the CH211-234P6 linkage group 24 of the zebrafish (Danio rerio) genome (Falco et al., 2012) were used to define *crp1-7*/CRP1-7 (meaning from *crp1* /CRP1 to *crp7*/CRP7) transcript/protein sequences, microarray probes and specific primers. The 60-mer *crp* probes used for microarray hybridization are shown above the forward+reverse primers for RTqPCR.

Table S2

Identification of the *crp1-7* genes coding for CRP proteins identified in the UNIPROT data base (UNIPROT accession numbers) by the MASCOT software

UNIPROT accession numbers	Initial CRP protein identification	PubMed gene	confirmative alignment	*crp* gen*	Final *crp* gene identification
A3KPG7	CRP1	XM_693995	***	XM_693995	***crp1***
F6P334	CRP3	BC097160	***	BC097160	***crp2***
X4YKC7	CRP	KJ184329	*		***crp2***
F1QXE4	CRP	-------------	*		***crp2***
L7QIE2	CRP3.1	KC416626	*		***crp2crp3***
L7QIS0	CRP3.2	KC416627	*		***crp2crp3***
A3KPG 5	CRP3	BC154042	***	BC154042	***crp3***
A8KBB0	CRP3	BC154041	***		***crp3***
L7QIE2	CRP3.1	KC416626	*		***crp2crp3***
L7QIS0	CRP3.2	KC416627	*		***crp2crp3***
Q1RM11	CRP4	BC115188	***	BC115188	***crp4***
A3KPH8	CRP	BC093242	***	BC093242	***crp5***
QOIIP8	CRP	BC081513	***		***crp5***
L7QIX7	CRP5.2	KC416624	**		***crp5***
L7QIX8	CRP5.3	KC416629	**		***crp5***
L7QIM0	CRP5.4	KC416625	*		***crp5***
----------	-----------	--------------		BC162745	***crp6***
A3KPH5	CRP7	BC150371	***	BC150371	***crp7***

UNIPROT accession numbers, protein accession numbers identified as CRP in the UNIPROT protein database by their derived tryptic peptides using the MASCOT program. *, confirmative alignement of the protein-derived sequences identified by MASCOT with *crp1-7* genes (Falco et al., 2012). The CRP proteins coded by the *crp2crp3* genes, could not be differentiated by neither MASCOT nor alignement (Clone Manager vs9). **Final *crp* gene identification**, *crp1-7* genes to which the different CRP1-7 UNIPROT accession number were assigned to analyze relative abundance data (Figure 4). To note that for each *crp1-7* gene there were 1 to 5 CRP protein accession numbers identified by their specific tryptic peptides except for *crp6*/CRP6.

Figure S1. Transcript expression levels of *crp1-7* in EPC cells transfected with pMCV1.4-*crp1-7* (A) and presence of CRP1-7 proteins in CRP1-7-enriched supernatants (B).

A) To estimate the amount of *crp1-7* transcripts expressed after transfection of EPC cells, the RNA extracted from EPC cell monolayers transfected with pMCV1.4-*crp1-7* plasmids were amplified by RTqPCR using specific primers (Table S1). The transcript expression levels were calculated by the formula: expression of each *crp1-7* / expression of *ef1a* in each transfection. Results were represented as means and standard deviations (n=3-6). **B)** To detect the presence of CRP1-7 proteins in CRP1-7-enriched supernatants, 500 µl were spotted on nitrocellulose filters. As CRP positive controls, 5 µl of PBS (CRP low-positive control) or oil (CRP high-positive control) were intraperitoneally injected into 12 adult zebrafish and their intraperitoneal liquid (ascites) harvested and separately pooled 3-days later. The CRP content of the dots was estimated using affinity purified anti-CRP rabbit antibodies raised against one of the CRP most conserved among the CRP1-7 isoforms. To detect bound anti-CRP antibodies, horseradish peroxidase labeled goat anti-rabbit immunoglobulins and ECL (BioRad) were finally used. One representative result of three independent determinations was represented in the figure. **CRP1**, supernatants from EPC cells transfected with pMCV1.4-*crp1*. **CRP2**, supernatants from EPC cells transfected with pMCV1.4-*crp2*. **CRP3**, supernatants from EPC cells transfected with pMCV1.4-*crp3*. **CRP4**, supernatants from EPC cells transfected with pMCV1.4-*crp4*. **CRP5**, supernatants from EPC cells transfected with pMCV1.4-*crp5*. **CRP6**, supernatants from EPC cells transfected with pMCV1.4-*crp6*. **CRP7**, supernatants from EPC cells transfected with pMCV1.4-*crp7*. **8**, supernatants from EPC cells transfected with pMCV1.4-*gfp* (CRP negative control). **9,** 10 µl of 20-fold diluted pooled intraperitoneal ascites from PBS-injected adult zebrafish (CRP low-positive control). **10,** 10 µl of 20-fold diluted pooled intraperitoneal ascites from oil-injected adult zebrafish (CRP high-positive control).

Figure S2. **Lack of SVCV neutralization by lipopolysaccharides (LPS) from *E.coli*.**

To discard any influence on SVCV neutralization of residual LPS in the CRP1-7-coding plasmids used for transfection, different concentrations of purified LPS from two different *E.coli* strains were added to EPC cell monolayers, incubated during 24 h, washed and then infected with SVCV. Maximal concentrations of contaminating *E.coli* lipopolysaccharide (LPS) in plasmids purified by Endofree Plasmid Purification Kits were estimated to be < 0.52 ng per 100 ng of plasmid according to their Qiagen (Germany) manufacturer. For simultaneous positive control for SVCV neutralization, EPC cell monolayers were transfected with either pMCV1.4-*crp2* or pMCV1.4-*crp5* in parallel experiments as described in legend of Figure 5A. The resulting number of ffu were then estimated by flow cytometry and means ± standard deviations represented. Two different experiments were performed each using LPS from two strains of *E.coli* (n = 2). *, significatively higher than the neutralization using the pMCV1.4 plasmid at $p < 0.05$ (Student t-test). **Open bars**, LPS from *E.coli* 055B5. **Hatched bars**, LPS from *E.coli* 0111:B4.

Figure S3. ***In vitro* neutralization of SVCV by co-transfected zebrafish CRPs**

To study possible synergistic effects between CRP isoforms, EPC cell monolayers were co-transfected with a mix of 50 ng of pMCV1.4-*crp*^{a} plasmids and 50 ng of other pMCV1.4-*crp*^{b}. Co-transfections of 50 ng of pMCV1.4-*crp* with 50 ng of pMCV1.4-*gfp* were used for the corresponding unique *crp* controls. After transfection, the cells were incubated for 3-days and then infected with SVCV. The resulting viral neutralization levels were calculated as described in the legend of Figure 5. Results were then expressed as means and standard deviations (n=3-6). **A)** Neutralization levels obtained by transfection with 100 ng of pMCV1.4-*gfp,* (no *crp, gfp*) or by co-transfection of 50 ng of pMCV1.4-*crp1* + 50 ng of pMCV1.4-*gfp* (*crp1*). **B)** Neutralization levels obtained by co-transfection of 50 ng of pMCV1.4-*crp2, -crp4* or *-crp7* and 50 ng of pMCV1.4-*gfp* (one *crp,* open bars*)* or of 50 ng of pMCV1.4-*crp2* + 50 ng of pMCV1.4-*crp1, -crp4* or *-crp7* (*crp*^{a} + *crp*^{b}, hatched bars). **C)** neutralization levels obtained by co-transfection with 50 ng of pMCV1.4-*crp5, -crp4* or *-crp7* + 50 ng of pMCV1.4-*gfp* (one *crp,* open bars) or 50 ng of pMCV1.4-*crp5* + 50 ng of pMCV1.4-*crp1, -crp4,* or *-crp7,* (*crp*^{a} + *crp*^{b}, hatched bars). **D)** Neutralization level

obtained by co-transfection with 50 ng of pMCV1.4-*crp2* + 50 ng of pMCV1.4-*crp5,* (crp^a + crp^b, hatched bar). **Black horizontal bars**, neutralization levels theoretically expected if the final neutralization level by crp^a + crp^b would be equal to the sum of each of the individual neutralization levels. **Open bars,** neutralization levels after co-transfection with one pMCV1.4-*crp* and pMCV1.4-*gfp* (*crp* + *gfp*). **Hatched bars**, neutralization levels after co-transfection with two pMCV1.4-*crps* (crp^a + crp^b). *, statistically higher than the neutralization levels obtained after transfection with pMCV1.4-*gfp* at $p < 0.05$ (Student t-test).