1 **Supplementary Figure Legends**

2 **Supplementary Figure 1. Purification of recombinant VZV gC proteins expressed**
3 **in *Drosophila* S2 cells. (A, C, E, G)** Size-exclusion chromatography (SEC) profiles of
4 purified recombinant $gC_{P23\text{-}V531}$ (A), $gC_{S147\text{-}V531}$ (C), $gC_{Y322\text{-}S523}$ (E) and $gC_{Y419\text{-}S523}$ (G)
5 expressed in *Drosophila* S2 cells. Purification details can be found in Materials and
6 Methods. **(B, D, F, H)** Images taken from SDS-PAGE loaded with purified $gC_{P23\text{-}V531}$
7 (B), $gC_{S147\text{-}V531}$ (D), $gC_{Y322\text{-}S523}$ (F) and $gC_{Y419\text{-}S523}$ (H) and stained for total protein with
8 TCE (B) or Coomassie (D, F, G). In (B) and (F) the analyzed sample was obtained
9 from peak 2. The molecular weight marker (in kDa) is shown on the left side.
10

11 **Supplementary Figure 2. gC binds IFN-γ better than the other IFNs. (A, B)**
12 Sensorgrams showing the results of binding screenings between VZV gC constructs
13 and cytokines using the Biacore S200 (A) or the Creoptix WAVE (B) systems. In (A)
14 the gC constructs were immobilized on a CM5 sensor chip (2853 RU, 1623 RU, and
15 668 RU, respectively) and the cytokines were injected at 100 nM with a flow rate of 30
16 μL/min. In (B) the different gC proteins were immobilised on DXH chips (567 pg/mm³,
17 584 pg/mm³, and 296 pg/mm³, respectively) and cytokines were injected at 200 nM
18 using RAPID in the tight binder mode. Please note the different Y-axis scaling for IFN-γ
19 compared to the other cytokines. Abbreviations: s = seconds, RU = resonance units
20

21 **Supplementary Figure 3. VZV gC modifies the expression of a low number of**
22 **genes, including some modulated by IFN-γ.** HaCaT cells were stimulated with IFN-
23 γ, $gC_{S147\text{-}V531}$, both or mock treated for 4 h. RNA was isolated and further processed
24 for RNAseq. **(A)** Principal component analysis (PCA) of gene counts of stimulated
25 HaCaT cells from three biological replicates. IFN-γ had the strongest effect on variation
26 in the samples, whereas gC only had minor effects on gene expression compared to

27 mock. Similar boundaries of circles indicate samples obtained from the same
28 experiment. **(B-E)** Venn diagrams showing the number of genes, whose expression
29 level was modified at least 1.5-fold (purple) in a statistically significant manner (*P* value
30 < 0.05; yellow) between the two compared conditions. Differential gene expression
31 analysis was performed comparing the different treatment conditions.
32

33 **Supplementary Figure 4. VZV gC induces ISG expression in a biased manner.**
34 HaCaT cells were stimulated with IFN-γ, $gC_{S147\text{-}V531}$, both or mock treated for 4 h. RNA
35 was isolated and further processed for RNAseq. Differential gene expression analysis
36 was performed using DESeq2 comparing the different treatment conditions as
37 indicated. **(A)** Volcano plots; x-axis represents the log2 of the fold change (FC), y-axis
38 represents the negative decade logarithm of the adjusted (adj.) *P* value for the four
39 different comparisons. Each circle represents a gene. Boundaries were set with adj. *P*
40 value of 0.05 and a fold change of 1.5 (equals log2-FC of 0.58). Red circles represent
41 genes with significant change in the adj. *P* value and the fold change, blue circles
42 depict genes with only a significant adj. *P* value, and green circles represent genes
43 with a significant change only in the fold change. In the representation of the IFN-γ-
44 treated versus mock control comparison, the adj. *P* values for *CXCL9*, *HAPLN3*,
45 *NLRC5* and *ICAM1* were too low to be calculated, hence they were set to the lowest
46 calculated adj. *P* value (*CIITA*) in this data set. In the representation of the comparison
47 of HaCaT cells treated with IFN-γ and gC versus mock-treated cells, the adj. *P* values
48 for *CXCL9*, *HAPLN3*, *CIITA*, *NLRC5* and *ICAM1* were too low to be calculated, hence
49 they were set to the lowest calculated adj. *P* value (*WARS1*) in this data set. **(B)** Graphs
50 showing the fold changes (FC) of significantly regulated genes from the indicated
51 comparisons. The grey lines indicate a corridor in which genes are less than 1.5-fold
52 changed between the two groups. Genes below the corridor are more upregulated in

53 the comparison plotted at the x-axis (green). Genes above the corridor are stronger
54 regulated in the comparison plotted at the y-axis (violet).
55
56 **Supplementary Figure 5. Validation of the RNASeq data by RT-qPCR.** Graphs
57 showing expression of *CXCL8*, *CXCL9*, *CXCL10*, *CXCL11* and *IL4I1* relative to *actin*
58 in HaCaT cells stimulated with 5 ng/mL IFN-γ, 300 nM $gC_{S147\text{-}V531}$ or both for the
59 indicated time points. The values were normalized to those obtained from cells
60 stimulated for 4 h with IFN-γ. Each filled circle corresponds to one independent assay.
61 Error bars represent standard deviation of the arithmetic mean from three independent
62 experiments. One-way ANOVA was performed, followed by Šídák's multiple
63 comparisons (comparing each sample to the mock and between IFN-γ stimulated cells
64 vs. co-stimulated cells for each time point). Non-significant comparisons are not
65 indicated. * = $P < 0.033$; ** = $P < 0.002$; *** = $P < 0.001$.
66
67 **Supplementary Figure 6. HaCaT cells co-stimulated with VZV gC and IFN-γ show**
68 **increased mRNA expression and total ICAM1 protein levels. (A)** HaCaT cells were
69 stimulated with 5 ng/mL IFN-γ and/or 300 nM $gC_{S147\text{-}V531}$ for the indicated time points,
70 RNA was isolated and analyzed for *ICAM1* expression by RT-qPCR. Using the ΔΔCt
71 method, *ICAM1* level was normalised to unstimulated cells and to actin as
72 housekeeping gene. Shown are the individual values from three independent assays
73 (filled circles), the resulting mean is shown as solid line with standard deviation (SD)
74 as colored transparent background. The area under the curve (AUC) was calculated,
75 then one-way ANOVA was performed on the AUC values, followed by Dunnett's
76 multiple comparisons. The fold change induced by addition of $gC_{S147\text{-}V531}$ to IFN-γ was
77 calculated for each timepoint and plotted in a bar chart (right panel). Filled circles
78 represent the individual values from each independent experiment, bars represent the

79 mean ± SD. One-way ANOVA, followed by Dunnett's multiple comparisons was
80 performed (comparing to the 1 h stimulation time). **(B)** Immunoblots detecting ICAM1
81 (top panel) and actin (middle panel) and blot showing total protein signal visualised
82 using trichlorethanol (bottom panel) in same samples employed in (A). Quantification
83 of the western blot band intensities is shown in the right panels. Top panel shows the
84 normalization of ICAM1 signal to actin, bottom panel shows ICAM1 signal normalized
85 to total protein. One representative experiment out of three biological repeats is shown.
86 One-way ANOVA, followed by Tukey's multiple comparisons was performed
87 (comparing all samples to each other). ns = not significant; * = P <0.033; ** = P <0.002;
88 *** = P <0.001. Abbreviations: kDa = kilo Dalton; M = marker; h = hours
89
90 **Supplementary Figure 7. Cell lines co-stimulated with VZV gC and IFN-γ have**
91 **increased surface levels of ICAM1. (A)** Schematic representation of the assay. Cells
92 were seeded one day prior to stimulation with 5 ng/mL IFN-γ, 300 nM VZV $gC_{S147-V531}$
93 or both. 24 h after stimulation the cells were detached, stained, and analyzed by flow
94 cytometry to detect ICAM1 (B) of MHCII (C) at the plasma membrane. **(B, C)**. Bar
95 charts showing the fold change of ICAM1 (B) or MHCII (C) surface protein levels
96 compared to unstimulated cells (top row) or induced by $gC_{S147-V531}$ compared to either
97 mock or IFN-γ baseline (bottom row). The median fluorescence intensities were
98 determined after gating on single and alive cells. Bars show the mean ± SD, filled
99 circles represent values from three independent experiments. One-way ANOVA,
100 followed by Šídák's multiple comparisons was performed (comparison IFN-γ to
101 unstimulated and/or condition with gC to baseline without gC). Non-significant
102 comparisons are not indicated. * = P <0.033; ** = P <0.002; *** = P <0.001.
103

104 **Supplementary Figure 8. Effect of gC on ICAM1 protein level iPSC-derived**
105 **macrophages. (A, B)** iPSC-derived macrophages from a healthy donor (A, B) and
106 from and IFNGR2-deficient donor (B) were mock-stimulated or stimulated with 5 ng/mL
107 IFN-γ, 300 nM $gC_{S147\text{-}V531}$ or both for the indicated time points and then labelled with
108 antibodies to ICAM1, MHCII, and stained with Zombie-NIR dye. **(A)** Graphs showing
109 ICAM1 (left) and MHCII (right) protein levels on the plasma membrane. Cells were
110 analyzed by flow cytometry and median fluorescence intensities (MFI) were
111 determined after gating on alive single cells. **(B)** Graphs showing ICAM1 (left) and
112 MHCII (right) protein levels at the plasma membrane at 8 and 24 hours post-
113 stimulation. MFI was normalized to the mock-treated cells for each donor within each
114 experiment. Circles represent results of biological replicates, bars show mean values
115 ± SD. Two-way ANOVA, followed by Tukey's multiple comparisons was performed
116 (comparison between all treatments within each donor). Non-significant comparisons
117 are not depicted. * = P <0.033; ** = P <0.002; *** = P <0.001.

118

119 **Supplementary Figure 9. GAGs compete with the gC – IFN-γ interaction. (A-D)**
120 Bar graphs showing the effect of heparin (A), heparan sulphate (B), chondroitin A
121 sulphate (C) and chondroitin B sulphate (D) on binding of IFN-γ to gC. Purified $gC_{S147\text{-}}$
122 $_{V531}$ was immobilized on a CM5 sensor chip (9,800 RU) and IFN-γ was injected at 100
123 nM either alone or together with increasing amounts of different GAGs (weight ratio).
124 The response levels at the binding report point were normalized to the response
125 obtained for IFN-γ alone and plotted for the different weight ratios. The bars represent
126 the mean ± SD, the filled circles represent the values from three independent
127 experiments. One-way ANOVA was performed to test for statistical significance
128 (comparing to control without GAGs), followed by Dunnett's multiple comparisons test.
129 ns = not significant; * = P <0.033; ** = P <0.002; *** = P <0.001

130

131 **Supplementary Figure 10. The interaction between gC and IFN-γ contains a**
132 **stable and a transient component**

133 **(A)** Sensorgrams showing the results of assays to determine the kinetics of the
134 interactions between VZV gC constructs and IFN-γ using the Biacore S200. The gC
135 constructs were immobilized on a CM4 sensor chip (877 RU, 399 RU, and 293 RU,
136 respectively) and IFN-γ was injected in a 1:2 dilution series starting at 50 nM with a
137 flow rate of 30 μL/min. Black lines indicate the heterogenous ligand fit or 1:1 binding
138 model (as indicated) determined with the Biacore S200 Evaluation software. **(B)**
139 Sensorgrams showing the contribution of the transient and stable interactions upon
140 injection of a low and high IFN-γ concentration onto the $gC_{S147\text{-}V531}$ chip. **(C)**
141 Sensorgram showing results of binding experiments between $gC_{S147\text{-}V531}$ immobilised
142 on a CM5 sensor chip (877 RU) and IFN-γ. The A-B-A injection scheme of the Biacore
143 S200 system to test different buffer conditions was used. Pre-sample contact time was
144 set to 360 s. The association time was 90 s, followed by 240 s of post-sample contact
145 time. IFN-γ was injected at 100 nM with a flow rate of 30 μL/min. **(D)** Sensorgrams
146 showing the results of assays to determine the kinetics of the interactions between
147 VZV $gC_{S147\text{-}V531}$ and $gC_{Y322\text{-}V523}$ using the Creoptix WAVE system. The gC proteins
148 were immobilised on a DXH chip (567 pg/mm^3 and 584 pg/mm^3, respectively) and
149 IFN-γ was injected at 200 nM using RAPID in the tight binder mode. Black line indicates
150 the fit using a heterogenous ligand model and traditional fitting.

151

152 **Supplementary Figure 11. Induction of ICAM1 by IFN-γ is higher in VZV**
153 **inoculated cultures. (A)** Schematic representation of the assay. HaCaT cells were
154 seeded 24 h prior to infection with pOka-Δ57-GFP. 48 h after infection, the cells were
155 stimulated with IFN-γ or mock treated. The next day, cells were detached and labelled

156 with anti-ICAM1-APC and stained with Zombie-NIR dye, fixed, and analyzed by flow
157 cytometry. **(B)** Bar chart showing the median fluorescence intensities (MFI) for ICAM1
158 in the four treatment conditions after gating on alive cells. **(C)** Bar chart showing the
159 percentage of infected HaCaT cells in mock- and IFN-γ-treated cultures after gating on
160 alive cells. The different colors discriminate the proportion of GFP^{high} and GFP^{low}
161 expressing cells. Ordinary two-way ANOVA analysis showed no significant
162 differences. **(D,E)** Bar charts showing the calculated fold-change of ICAM1 levels in
163 mock and VZV-infected cells after gating on uninfected or infected cells (D) and after
164 differentiating between GFP^{high} and GFP^{low} cells (E). As reference in E the fold change
165 by IFN-γ of uninfected cells (from D) is indicated with a grey line. Ordinary two-way
166 ANOVA analysis with main effects only followed by Dunett's multiple comparison was
167 performed (D). Bars in (B-E) represent the mean ± SD and filled circles represent the
168 individual values from three independent experiments. ns = not significant; * = *P*
169 <0.033; ** = *P* <0.002; *** = *P* <0.001.

170

171 **Supplementary Figure 12. VZV-gC-GFP and VZV-ΔgC-GFP show similar**
172 **replication kinetics in HaCaT cells. (A)** Schematic representation of the BAC-derived
173 VZV pOka strains. The repeat and unique regions of the parental, BAC-derived, VZV
174 genome are shown. *ORF14*, encoding gC is highlighted in magenta. The arrow above
175 *ORF14* indicates that this gene is located in the reverse DNA strand. Monomeric GFP
176 was inserted instead of the *ORF14* locus and at the 3' end of *ORF14* to generate VZV-
177 ΔgC-GFP and VZV-gC-GFP recombinant viruses, respectively. In both cases, the
178 *ORF14* promoter drives expression of GFP. **(B)** HaCaT cells were infected with 100
179 PFU of the two indicated BAC-derived virus strains. At the different time points post-
180 infection, cells were imaged and collected for titration. Assays were performed in
181 triplicates. Collected cells were titrated and the $TCID_{50}$ was determined on HaCaT

182 cells. $TCID_{50}$ values are plotted over time in the left panel. The right panel shows the
183 mean GFP fluorescence over time measured with the Cytation3 (BioTek).
184 Abbreviations: TR_l = terminal repeat long; TR_s = terminal repeat short; U_L = unique
185 long region; IR_L = internal repeat long; IR_s = internal repeat short; U_S = unique short
186 region; ORF = open reading frame; GFP = green fluorescent protein; $TCID_{50}$ = tissue
187 culture infectious dose 50; hpi = hours post-infection.
188
189