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1 FHL1 is a major host factor for chikungunya virus infection

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46 **ABSTRACT**

47 Chikungunya virus (CHIKV) is a re-emerging Old World alphavirus transmitted to humans by mosquito bites which causes musculoskeletal and joint pain^{1–3}. Despite 48 49 intensive investigations, the identity of the human cellular factors critical for CHIKV 50 infection remains elusive, hampering both the understanding of viral pathogenesis and 51 the development of anti-CHIKV therapies. Here, we identified the Four-and-a-Half LIM domain protein 1 (FHL1)⁴ as a host factor required for CHIKV permissiveness and 52 53 pathogenesis. Ablation of FHL1 expression results in massive inhibition of infection by several CHIKV strains and O'nyong-nyong virus, but not by other alphaviruses or 54 55 flaviviruses. Conversely, expression of FHL1 enhances infection of cells that do not 56 express it and are poorly susceptible to CHIKV. We show that FHL1 directly interacts 57 with the hypervariable domain of CHIKV nsP3 protein and is essential for viral RNA 58 replication. FHL1 is highly expressed in CHIKV target cells and particularly abundant in muscles^{4,5}. Significantly, dermal fibroblasts and muscle cells derived from Emery-59 60 Dreifuss muscular dystrophy (EDMD) patients which lack functional FHL1⁶ are 61 resistant to CHIKV infection. Importantly, CHIKV infection is undetectable in mice knocked out for the FHL1 gene. Overall, this study shows that FHL1 is a key host 62 dependency factor for CHIKV infection and identifies nsP3-FHL1 interaction as a 63 64 promising target for the development of anti-CHIKV therapies.

65

66 MAIN TEXT

67 Several host factors implicated in CHIKV infection have been identified, however none of them accounts for CHIKV tropism for joint and muscle tissues^{7–10}. To identify 68 69 key host factors dictating CHIKV cell permissiveness, we performed a genome-wide CRISPR-Cas9 screen in the HAP1 haploid cell line (Fig.1a, Extended Data Fig. 1). 70 HAP1 cells expressing the human GeCKO v2 single guide RNA libraries A and B, 71 which contains each 3 unique sgRNAs targeting 19,050 genes¹¹, were inoculated with 72 73 CHIKV21, a strain isolated from a patient infected during the 2005-2006 CHIKV outbreak in La Reunion Island¹². Genomic DNA from lentivirus-transduced cells that 74 75 survived to CHIKV infection was isolated, amplified and the corresponding integrated sgRNA sequenced. Gene enrichment was assessed using the MAGeCK software¹³ 76 77 (Fig.1a, Extended Data Fig. 1, supplementary Table 1). The top hit of our screen was 78 the gene encoding the Four-and-a-Half LIM protein 1 (FHL1) (Fig.1a, Extended Data Fig. 2a-c), the founding member of the FHL protein family¹⁴. FHL1 is characterized by 79 80 the presence of four and a half highly conserved LIM domains with two zinc fingers 81 arranged in tandem¹⁴. FHL1 is strongly expressed in skeletal muscles and heart^{4,14}. In human, there are three FHL1 splice variants: FHL1A, FHL1B and FHL1C^{4,15,16}. FHL1A 82 is the most abundantly expressed, primarily detected in striated muscles⁴ and 83 84 fibroblasts¹⁷. The two other variants FHL1B and C are expressed in muscles, brain and testis^{15,16}. We functionally validated the requirement of FHL1 in CHIKV21 infection by 85 using two distinct gRNAs targeting all three FHL1 isoforms (Extended Data Fig. 2a). 86 87 We generated HAP1 and 293T knockout *FHL1* clones (Δ FHL1) and confirmed gene 88 editing by sequencing and western blot analysis (Extended Data Fig. 2d, e, f). FHL1 89 knockout did not alter cell proliferation and viability as determined by CellTiter-Glo assay (Extended Data Fig. 2g). CHIKV infection and release of infectious particles was 90

91 drastically inhibited in ∆FHL1 cells (Fig.1b, Extended Data Fig. 3a-d). Trans-92 complementation of \triangle FHL1 cells with a human cDNA encoding FHL1A, but not FHL1B 93 or C, restored both susceptibility to CHIKV21 infection and virus release (Fig. 1c, 94 Extended Data Fig. 4a-b), indicating that FHL1A is a critical factor for CHIKV21 95 infection. Expression of FHL2, a member of the FHL family predominantly expressed in heart¹⁸, restored CHIKV infection in \triangle FHL1 cells, albeit to a lower efficiency than 96 97 FHL1 (Extended Data Fig. 4c). We then assessed FHL1 dependency of CHIKV strains 98 from distinct genotypes. FHL1 is important for infection by strains belonging to the 99 Asian (strain St Martin H20235 2013), the ECSA (East, Central, and South African) 100 strains Ross and Brazza (MRS1 2011) and the Indian Ocean (IOL) (strain M-899) 101 lineages (Fig. 1d). Of note, the requirement for FHL1 was less pronounced with CHIKV 102 37997, a strain from the West African genotype (Fig. 1d). We next tested the 103 requirement of FHL1 for infection by other alphaviruses. Interestingly, O'nyong-nyong virus (ONNV), an Old World alphavirus that is phylogenetically very close to CHIKV¹, 104 105 showed a dramatically reduced infection level in Δ FHL1 cells (Fig.1e, Extended Data 106 Fig. 3e). In sharp contrast, other Old World alphaviruses such as Mayaro virus (MAYV), 107 Sindbis virus (SINV), Semliki Forest Virus (SFV) and Ross River virus (RRV), and New 108 World encephalitic viruses such as Eastern equine encephalitis virus (EEEV), Western 109 equine encephalitis virus (WEEV) or Venezuelan equine encephalitis virus (VEEV) 110 infected HAP1 cells in a FHL1-independent manner (Fig. 1e, f, Extended Data Fig. 3e). 111 No effect of FHL1 was observed for infection by Dengue virus (DENV) or Zika virus 112 (ZIKV), two members of the Flavivirus genus (Fig. 1g, Extended Data Fig. 3f). 113 Consistent with the requirement of FHL1 for CHIKV infection, BeWo or HepG2 cells which are poorly susceptible to CHIKV infection^{20,21} and do not express endogenous 114 FHL1 (Extended Data Fig. 5a) became permissive to the virus upon FHL1A expression 115

(Fig.1h, Extended Data Fig. 5b-d). This highlights the major role played by FHL1A in
human cell permissiveness to CHIKV.

118 To determine which step in CHIKV life cycle requires FHL1, we challenged 119 parental and Δ FHL1 cells with CHIKV particles and guantified the viral RNA at different 120 time points (Fig. 2a). We did not observe any major difference in CHIKV RNA levels in 121 FHL1-deficient cells compared to WT cells at 2h post-infection (Fig. 2a). In contrast, a 122 massive reduction of CHIKV RNA was observed in \triangle FHL1 cells as early as 6h post-123 infection (Fig. 2a) which was even greater 24h post-infection, suggesting that FHL1 expression is involved in an early post-entry step of the CHIKV life cycle. We therefore 124 125 bypassed virus entry and uncoating by transfecting CHIKV RNA into controls or Δ FHL1 126 cells in the presence of NH₄Cl to inhibit further rounds of infection⁹. Upon CHIKV RNA 127 transfection, viral replication was drastically impaired in Δ FHL1 cells compared to WT 128 cells (Fig. 2b, Extended Data Fig 6a). To evaluate the contribution of FHL1 in incoming 129 genome translation versus RNA replication, we generated a replication-deficient 130 CHIKV molecular clone (with the GDD motif of the viral polymerase nsP4 mutated to 131 GAA) encoding a *Renilla* luciferase (Rluc) fused to the nsP3 protein as described ²². 132 Transfection of CHIKV GAA RNA in *AFHL1* or control cells resulted in a similar Rluc 133 activity (Fig. 2c), indicating that FHL1 is dispensable for CHIKV incoming RNA 134 translation. When similar experiments were performed with the WT CHIKV RNA, a 135 massive increase in Rluc activity was observed in control cells but not Δ FHL1 24 hpi 136 (Fig. 2d), demonstrating that FHL1 is essential for viral RNA replication. Furthermore, 137 gRT-PCR experiments showed that ablation of FHL1 resulted in a severely reduced synthesis of CHIKV negative strand RNA (Fig. 2e). We then investigated the impact of 138 139 FHL1 in the production of dsRNA intermediates which are a marker of viral replication complex (vRC) assembly²³. At 6h post-infection, a massive reduction of dsRNA-140

141 containing complexes was observed in Δ FHL1 cells stained with anti-dsRNA mAb 142 when compared to parental cells (Fig. 2f). Consistent with this observation, 143 transmission electron microscopy showed that the formation of plasma membrane-144 associated spherules and cytoplasmic vacuolar membrane structures, which are 145 alphavirus-induced platforms required for viral RNA synthesis²⁴, are absent in Δ FHL1 146 cells (Fig 2g). Altogether, these data show that FHL1 is critical for CHIKV RNA 147 replication and vRC formation in infected cells.

148 We next investigated FHL1 location during infection. Confocal microscopy 149 studies showed that FHL1 displays a diffuse cytoplasmic distribution in uninfected 150 human fibroblasts. In cells infected for 6h, FHL1-containing foci appeared and 151 colocalized with nsP3 (Extended Data Fig. 6b), a CHIKV non-structural protein orchestrating viral replication in the cytoplasm^{25,26}. Indeed, CHIKV nsP3 contains a 152 153 large C-terminal hypervariable domain (HVD)²⁵ known to mediate assembly of protein complexes and regulate RNA amplification^{25,26}. Interestingly, FHL1 and FHL2 have 154 155 been reported as putative nsP3 HVD binding partners in mass spectrometry analyses 156 ^{26,27}. We experimentally validated FHL1-nsP3 interaction (Fig. 2h,I; Extended Data Fig. 157 6c-g) and found that endogenous FHL1 co-immunoprecipitates with nsP3 from CHIKV-158 infected cells (Fig. 2h). Consistent with infection studies, both FHL1A and FHL2 co-159 precipitated with CHIKV nsP3 (Extended Data Fig. 6d). FHL1A-nsP3 interaction is 160 specific for CHIKV as it was not observed with other alphaviruses such as SINV or SFV, which do not depend on FHL1 for infection (Extended Data Fig. 6e). Of note, in 161 162 Δ FHL1 cells, nsP3 retained its ability to bind G3BP1 and 2, two components of the stress granules implicated in CHIKV replication^{22,26} (Extended Data Fig. 6e). We next 163 164 generated chimeric proteins where the HVD region of CHIKV nsP3 is swapped with the corresponding domain of SINV nsP3 and vice versa. Whereas CHIKV-SINV(HVD) 165

166 chimeric protein lost its ability to bind FHL1, the HVD of CHIKV in the context of SINV 167 nsP3 protein conferred binding to FHL1 (Extended Data Fig. 6f). Pull-down 168 experiments with purified proteins showed that FHL1A directly binds to WT nsP3 but 169 not to the HVD-deficient variant (Fig. 2i, Extended Data Fig. 6g). We then mapped the 170 binding region within CHIKV nsP3HVD responsible for FHL1A interaction (Fig. 2j, 171 Extended Data Fig. 7). The FHL1 binding domain, referred as HVD-R4, is found in all 172 CHIKV and ONNV strains and is located upstream of the short repeating peptide 173 corresponding to G3BP1/2 binding sites²⁶ (Fig. 2j, Extended Data Fig. 7a). Deletion of 174 the HVD-R4 region strongly impaired FHL1 interaction with nsP3, without affecting 175 G3BP1/2 binding to the viral protein (Fig. 2j, Extended Data Fig. 7b). To investigate 176 whether FHL1 interaction with the HVD region of nsP3 is required for FHL1 proviral 177 role, we generated two chimeric FHL1A protein either fused to the HVD-R4 peptide 178 (FHL1A-R4) or to a randomized peptide sequence of HVD-R4 (FHL1A-R4*) as a 179 positive control (Fig. 2k, Extended Data Fig. 7c) and assessed their ability to interact 180 with nsP3. Whereas FHL1A-R4 failed to bind nsP3 (Fig. 2k), FHL1A-R4* interacted 181 with nsP3 as efficiently as WT FHL1A protein (Fig. 2k). These results indicate that the 182 fused HVD-R4 peptide likely hides the binding site of FHL1A to nsP3, inhibiting their 183 interaction. Furthermore, trans-complementation of Δ FHL1 cells with a cDNA encoding 184 FHL1A-R4 did not restore CHIKV21 infection when compared to FHL1A-R4* or WT 185 FHL1A (Fig. 2I). Consistent with this, in vitro transcribed RNA from CHIKV molecular 186 clone mutated in FHL1 binding site (Δ R4 or R4^{*}) showed a strong defect in replication 187 after transfection in 293T cells (Extended Data Fig. 7d). Together these data strongly 188 suggest that the interaction between the HVD region of nsP3 with FHL1 is critical for 189 FHL1 proviral function.

Mutations in the FHL1 gene have been associated with X-linked myopathies^{5,28}, 190 191 including the Emery–Dreifuss muscular dystrophy (EDMD)⁶, a rare genetic disease 192 characterized by early joint contractures, muscular wasting and adult-onset cardiac 193 disease²⁹. We studied the permissiveness to CHIKV of dermal fibroblasts and 194 myoblasts from four EDMD male patients carrying FHL1 gene mutations as well as 195 from two healthy donors (Extended Data Fig.8a). A detailed clinical description of P1. 196 P2 and P3 has been reported⁶, and patient P4 presented with EDMD and additional 197 clinical abnormalities (see methods). Analysis of P4 FHL1 gene revealed the insertion 198 of a full-length LINE-1 retrotransposon sequence in exon 4 (Extended Data Fig.8b). 199 FHL1 expression is severely reduced in primary cells from all four EDMD patients as 200 established by immunoblot analysis (Fig. 3a). Infection studies showed that fibroblasts 201 and myoblasts from those EDMD patients are resistant to CHIKV21 and M-899 202 Mauritian strains (Fig. 3b-d, Extended Data Fig.8c), and exhibit a massive defect in the 203 release of infectious particles (Fig. 3d), in contrast to healthy donor cells. Similar results 204 were obtained with the CHIKV strains Brazza, Ross and H20235 (Fig. 3e, Extended 205 Data Fig.8d). FHL1-null myoblasts and fibroblasts remained highly susceptible to 206 MAYV, which does not rely on FHL1 for replication (Fig. 3e). Trans-complementation 207 of EDMD fibroblasts by a lentivirus encoding WT FHL1A restored CHIKV viral antigen 208 synthesis (Fig. 3f, Extended Data Fig.8e) and infectious particle release (Fig. 3g).

To directly assess the role of FHL1 in chikungunya pathogenesis, we conducted *in vivo* experiments in mice expressing or not FHL1. Human and mouse FHL1 orthologues are highly conserved (Extended Data Fig.9a). Murine FHL1 interacts with CHIKV nsP3 and enhances viral infection, albeit less efficiently that its human orthologue (Extended Data Fig.9 b-d). Moreover, CHIKV infection was strongly impaired in the murine muscle cell C2C12 deleted for the *fhl1* gene (Extended Data

215 Fig.9 e-f). Susceptibility to CHIKV infection of young mice deficient or not for FHL1 was 216 then tested. CHIKV actively replicated in tissues of WT littermates, as previously 217 reported²⁰, but virtually no infectious particles were detected in tissues of FHL1-null 218 mice (Fig. 4a). Moreover, necrotizing myositis with massive infiltrates and necrosis of 219 the muscle fibers were observed in skeletal muscle of WT littermates, while FHL-null 220 mouse muscle showed no detectable pathology (Fig. 4b). Immunolabelling with Ab 221 against CHIKV E2 protein, FHL1 and vimentin in muscle revealed that in young WT mice, CHIKV mainly targets muscle fiber expressing FHL1, whereas muscle cells of 222 223 FHL1-null mice show no label for CHIKV nor for FHL1 (Fig. 4c). These experiments demonstrate that FHL1 knock out mice are resistant to CHIKV infection. 224

225 In summary, this study shows that FHL1 is a critical CHIKV host dependency 226 factor for infection and pathogenesis. In vivo, FHL1 expression pattern, which accounts 227 for the clinical presentation of EDMD, also reflects CHIKV tissue tropism for skeletal 228 muscles and joints. This suggests that the hijacking of FHL1 by CHIKV during infection 229 may, on top of allowing viral replication, lead to cellular dysfunctions contributing to 230 muscular and joint pains that are the hallmark of chikungunya disease^{1,2}. 231 Mechanistically, FHL1 interacts with the HVD domain of nsP3 to enable viral RNA 232 synthesis and viral replication complex formation. The alphavirus nsP3 HVD domain is an intrinsically disordered region that binds distinct sets of cellular proteins^{23,26,30} such 233 234 as the G3BP1 and G3PB2, two key components and markers of stress granules that 235 are important for the replication of CHIKV and other alphaviruses^{22,26}. G3BP1/2 nsP3 interactions are thought drive a common alphavirus-specific mechanism that is 236 important for assembly of the replication complex and stabilization of viral G 237 238 RNA^{22,23,26}. FHL1 interacts with a nsP3 HVD region which is located away from 239 G3BP1/2 binding sites. Therefore, FHL1 and G3BP proteins likely play distinct roles

during CHIKV replication. In contrast to G3BPs, FHL1 is selectively used by CHIKV, 240 suggesting that it may accomplish a specific and essential function in CHIKV RNA 241 amplification. Upon interaction with FHL1, CHIKV nsP3 HVD may adopt a unique 242 243 conformation that is critical for the initiation of viral replication. Interestingly, intrinsically disordered domains (IDD) such as the nsP3 HVD have also been shown to induce 244 liquid-liquid phase separations³¹ and negative-stranded RNA viruses use proteins 245 displaying IDDs to form liquid organelles for their replication³². Indeed, in CHIK-infected 246 247 cells, nsP3 forms intracellular granules reminiscent of these virus-induced inclusions 248 ^{33,34}. FHL1 may regulate the formation and/or the dynamic of such granules to create 249 an optimal environment for efficient CHIKV RNA amplification. FHL1 contains four LIM 250 domains arranged in tandem known to function as a modular protein binding interface 251 regulating diverse cellular pathways³⁵. FHL1 has been shown to scaffold MAPK 252 components (Raf-1/MEK2/ERK2) to the stretch sensor Titin N2B to transmit MAPK 253 signals that regulate muscle compliance and cardiac hypertrophy^{36,37}. One may 254 speculate that, during CHIKV infection, FHL1 may be hijacked from its physiological 255 function in sarcomere extensibility and intracellular signaling to act as scaffolding protein promoting CHIKV RNA amplification. 256

257 In conclusion, this study provides major insights into the understanding of 258 CHIKV interactions with its target host cell. Although other host-factors have been 259 identified as required for CHIKV infection, none of them fully account for the specific 260 joint and muscular pathology which is the hallmark of CHIKV and gave its name to its 261 associated disease, chikungunya, which means "that which bends up" in Makonde, to describe the posture of patient with muscle and joint pain. The hijacking of FHL1 by 262 263 nsP3 during CHIKV infection is unique and constitutes a critical clue that paves the 264 way to fully decipher the pathogenesis of chikungunya disease. Targeting FHL1A-

- 265 nsP3 interactions now stands as an attractive therapeutic approach to combat CHIKV
- 266 pathogenesis.

268 **METHODS**

269 Cell culture. HAP1 cells (Horizon Discovery), which are derived from near-haploid 270 chronic myeloid leukemia KBM7 cells, were cultured in IMDM supplemented with 10% 271 FBS, 1% penicillin-streptomycin (P/S) and GlutaMAX (Thermo Fisher Scientific). 272 293FT (Thermo Fisher Scientific), HEK-293T (ATCC), Vero E6 (ATCC), HepG2 (kind 273 gift of Olivier Schwartz, Institut Pasteur, Paris, France), primary myoblasts and primary fibroblasts were cultured in DMEM supplemented with 10% FBS, 1% penicillin-274 275 streptomycin, 1% GlutaMAX and 25 mM Hepes. Human placenta choriocarcinoma Bewo cells were cultured in in DMEM supplemented with 5% FBS, 1% penicillin-276 277 streptomycin, 1% GlutaMAX and 25 mM Hepes. AP61 mosquito (Aedes pseudoscutellaris) cells (gift from Philippe Despres, Institut Pasteur, Paris, France) 278 279 were cultured at 28°C in Leibovitz medium supplemented with 10% FCS, 1% P/S, 1% 280 glutamine, 1X non-essential amino acid, 1X Tryptose phosphate and 10 mM Hepes. 281 All cell lines were cultured at 37°C in presence of 5% CO₂ with the exception of AP61 282 that were maintained at 28°C with no CO₂.

283

284 Virus strains and culture. CHIKV21 (strain 06-21), ZIKV (HD78788) (both are kind 285 gift from Philippe Despres, Institut Pasteur, Paris, France), CHIKV West Africa (strain 286 37997, accession nb AY726732.1) and dengue virus serotype 2 DENV (16681) viruses 287 were propagated in mosquito AP61 cell monolayers with limited cell passages. CHIKV-288 Brazza-MRS1 2011, CHIKV-Ross, CHIKV-St Martin H20235 2013-Asian, RRV (strain 289 528v), MAYV (strain TC 625), ONNV (strain Dakar 234), SINV (strain Egypt 339), EEEV (strain H178/99), VEEV (strain TV83 vaccine), WEEV (strain 47A), SFV (strain 290 291 1745) were obtained from the European Virus Archive (EVA) collection and propagated 292 with limited passage on Vero E6 cells.

293 pCHIKV-M-Gluc (see plasmid sections) and pCHIKV-mCherry molecular clones were 294 derivate of pCHIKV-M constructed from a CHIKV (strain BNI-CHIKV 899) isolated 295 from a patient during Mauritius outbreak in 2006. To generate infectious virus from 296 CHIKV molecular clones, capped viral RNAs were generated from the Notl-linearized 297 CHIKV plasmids using a mMESSAGE mMACHINE SP6 or T7 Transcription Kit 298 (Thermo Fischer Scientific) according to manufacturer's instructions. Resulting RNAs 299 were purified by phenol:chloroform extraction and isopropanol precipitation, 300 resuspended in water, aliquoted and stored at -80°C until use. Thirty µg of purified 301 RNAs were transfected in BHK21 with lipofectamine 3000 reagent and supernatants 302 harvested 72 hours later were used for viral propagation on Vero E6 cells.

303 For all the viral stock used in flow cytometry analysis experiments, viruses were 304 purified through a 20% sucrose cushion by ultracentrifugation at 80,000xg for 2 hours 305 at 4°C. Pellets were resuspended in HNE1X pH7.4 (Hepes 5 mM, NaCl 150 mM, EDTA 306 0.1 mM), aliquoted and stored at -80°C. Viral stock titers were determined on Vero E6 307 cell by plaque assay and are expressed as PFU per ml. Virus stocks were also 308 determined by flow cytometry as previously described ^{38,39} Briefly, Vero E6 cells were 309 incubated for 1h with 100µl of 10-fold serial dilutions of viral stocks. The inoculum was 310 then replaced with 500µl of culture medium and the percent of E2 expressing cells was 311 quantified by flow cytometry at 8 hpi. Virus titers were calculated using the following 312 formula and expressed as FACS Infectious Units (FIU) per ml. [Titer (FIU/ml) = 313 (average % of infection) x (number of cells in well) x (dilution factor) / (ml of inoculum 314 added to cells)].

315

Reagents. The following antibodies were used: anti-FHL1 mAb (ref MAB5938, R &
D Systems), anti-FHL1 rabbit Ab (ref NBP1-88745, Novus Biologicals), anti-vimentin

antibody (ab24525, abcam), anti-GAPDH mAb (ref SC-47724, Santa Cruz 318 319 Biotechnology), polyclonal rabbit anti-HA (ref 3724, Cell Signaling Technology), anti-320 FLAG M2 mAb (ref F1804, SIGMA), anti-RFP (ref 6G6, Chromotek), anti-CHIKV E2 321 mAb (3E4 and 3E4 conjugated-CY3), anti-alphavirus E2 mAb (CHIK-265 was a kind 322 gift from Michael Diamonds, University school of medicine, St Louis, USA), anti-EEEV 323 E1 mAb (ref MAB8754, Sigma), anti-pan-flavivirus E protein mAb (4G2), anti-dsRNA J2 mAb (Scicons), Alexa Fluor[™] 488-conjugated goat anti-rabbit IgG (A11034, 324 325 Invitrogen), Alexa Fluor[™]-647-conjugated goat anti-chicken IgG (ab150175, abcam), Alexa Fluor[™] 488-conjugated goat anti-mouse IgG (115-545-003, Jackson 326 327 ImmunoResearch), Alexa Fluor[™] 647-conjugated goat anti-mouse IgG (115-606-062, 328 Jackson ImmunoResearch), peroxydase-conjugated donkey anti-rabbit IgG (711-035-329 152, Jackson ImmunoResearch), and anti-mouse/HRP (P0260, Dako Cytomotion). 330 FLAG magnetic beads (ref M8823, SIGMA), HA-magnetic beads (ref 88837, Thermo 331 Fisher Scientific) and anti-RFP coupled to magnetic agarose beads (RFP-Trap MA, 332 Chromotek) were used for immunoprecipitation experiments.

333

CRISPR genetic screen. The GeCKO v2 human CRISPR pooled libraries (A and B) 334 335 encompassing 123,411 different sgRNA targeting 19,050 genes (cloned in the 336 plentiCRISPR v2) were purchased from GenScript. Lentiviral production was prepared 337 independently for each half-library in 293FT cells by co-transfecting sgRNA plasmids 338 with psPAX2 (Kind gift from Nicolas Manel, Institut Curie, Paris, France) and pCMV-339 VSV-G at a ratio of 4:3:1 with lipofectamine 3000 (Thermo Fisher Scientific). 340 Supernatants were harvested 48h after transfection, cleared by centrifugation (750 x 341 g for 10 min), filtered using a 0.45 µM filter and purified through a 20% sucrose cushion 342 by ultracentrifugation (80,000 x g for 2 hours at 4°C). Pellets were resuspended in

343 HNE1X pH7.4, aliquoted and stored at -80°C. HAP1 cells were transduced by 344 spinoculation (750 x g for 2 hours at 32°C) with each CRISPR-sgRNA lentiviral libraries 345 at a multiplicity of infection (MOI) of 0.3 and a coverage of 500 times the sgRNA 346 representation. Cells were selected with puromycin for 8 days and expanded. Sixty 347 million cells from each library were pooled and infected with CHIKV21 using a MOI of 348 1. Simultaneously forty million of non-infected pooled cells were pelleted and kept at -349 80°C to serve as a reference of the library representation at time of infection. 350 Approximately 5 days after infection, cytopathic effect was detectable and surviving 351 cells were collected 2 weeks later. Genomic DNA was extracted from selected cells or 352 non-infected pooled cells using QIAamp DNA column (Qiagen), and inserted gRNA 353 sequences were amplified and subject to next generation sequencing on an Illumina 354 MiSeg (Plateforme MGX, Institut Génomigue Fonctionelle, Montpellier, France), gRNA 355 sequences were analyzed using the MAGeCK software ¹³. Additionaly, gRNA 356 sequences were analyzed using the RIGER software following previously published 357 recommendation⁴⁰.

358

FHL1 editing. FHL1 was validated using two independent sgRNA targeting the exon 359 360 3 and exon 4, which are common to all FHL1 isoforms. sgRNA1, 5'-361 GAGGACTCCCCCAAGTGCAA-3' and sgRNA2, 5'-GCAGTCAAACTTCTCCGCCA-3' 362 were cloned into the plasmid lentiCRISPR v2 according to Zhang lab's 363 recommendation. HAP1 and 293FT cells were transiently transfected with the plasmid 364 expressing individual sgRNA and selected with puromycin until all mock-transfected 365 cells died (approximately 72 hours). Transfected cells were used to ascertain gRNA-366 driven resistance to CHIKV cytopathic effect, and clonal cell lines were isolated by 367 limiting dilution and assessed by immunoblot for FHL1 expression.

368

369 Infection assay. For infection guantification by flow cytometry analysis, cells were 370 plated in 24-well plates. Cells were infected for 24 (293T) or 48 hours (HAP1), 371 trypsinized and fixed with 2% (v/v) paraformaldehyde (PFA) diluted in PBS for 15 min 372 at room temperature. Cells were incubated for 30 min at 4°C with 1µg/ml of either the 373 3E4 anti-E2 mAb for CHIKV strains and ONNV) or the CHIKV 265 anti-E2 mAb for 374 MAYV or the anti-E1 mAb for EEEV or anti-pan-flavivirus E 4G2 for DENV and ZIKV. 375 Ab were diluted in permeabilization flow cytometry buffer (PBS supplemented with 5% 376 FBS, 0.5% (w/v) saponin, 0.1% Sodium azide). After washing, cells were incubated 377 with 1µg/ml of Alexa Fluor 488 or 647-conjugated goat anti-mouse IgG diluted in permeabilization flow cytometry buffer for 30 min at 4°C. Acquisition was performed on 378 379 an Attune NxT Flow Cytometer (Thermo Fisher Scientific) and analysis was done by 380 using FlowJo software (Tree Star). To assess infectious viral particles release during 381 infection, cells were inoculated for 3 hours with viruses, washed once and then 382 maintained in culture medium over a 72-hour period. At indicated time points 383 supernatants were collected and kept at -80°C. Vero E6 cells were incubated with 10-384 fold serial dilution of supernatant for 24 hours and E2 expression was quantified by 385 flow cytometry as described above.

For detection of infected cells by immunofluorescence, control and Δ FHL1 HAP1 cells were plated on Lab-Tek II CC2 glass slide 8 wells (Nunc). Cells were inoculated with CHIKV21 strain (MOI of 20) or CHIKV-nsP3-mCherry (MOI of 20) for 48 hours, then washed thrice with cold PBS and fixed with 4% (v/v) PFA diluted in PBS for 20 min at room temperature. CHIKV E2 protein was stained with the 3E4 mAb at 5 µg/ml, followed by a secondary staining with 1µg/ml of Alexa 488-conjugated goat anti-mouse lgG. Both antibodies were diluted in PBS supplemented with 3% (w/v) BSA and 0.1% 393 saponin. Slides were mounted with ProLong Gold antifade reagent containing 4,6 394 diamidino-2-phenylindole (DAPI) for nuclei staining (Thermo Fisher Scientific).

For colocalization experiments, cells infected with CHIKV-nsP3-mCherry (MOI of 20)
were stained with 10 µg/ml of the anti-FHL1 mAb, followed by a secondary staining
with 1µg/ml of Alexa 488-conjugated goat anti-mouse IgG.

For detection of dsRNA foci, control and Δ FHL1 293T cells were plated on Lab-Tek II CC2 glass slide 8 wells (Nunc) and infected with CHIKV21 strain (MOI of 50) for 4 or 6 hours. After fixation with 4% (v/v) PFA diluted in PBS, cells were stained with 5 µg/ml of the anti-dsRNA mAb, followed by a secondary staining with 1µg/ml of Alexa 488conjugated goat anti-mouse IgG. Both antibodies were diluted in PBS supplemented with 3% (w/v) BSA and 0.1% Triton 100X. Of note, no dsRNA foci were detectable at 404 4hpi.

405 Fluorescence microscopy images were acquired using a LSM 800 confocal406 microscope (Zeiss).

407

408 **Plasmid constructions.** To generate the C-terminal HA-tagged FHL1 isoforms, the cDNAs of FHL1A (NM 001449.4), FHL1B (XM 006724746.2) and FHL1C 409 410 (NM 001159703.1) were purchased from Genscript. Coding sequence (CDS) were 411 amplified with common FHL1 Fwd primer 5'а 412 CCGGAGAATTCGCCGCCATGGCGGAGAAGTTTGACTGCCACTACTGC-3'; and specific FHL1A Rev primer 5'-AATAGTTTAGCGGCCGCTCAAGCGTAATCTGGAA 413 414 CATCGTATGGGTATCCTCCAGCGGCCGA<u>CAGCTTTTTGGCACAGTCGGGACAA</u> 415 TACACTTGCTCC-3'; or FHL1B and С specific Rev primer 5'-416 AATAGTTTA**GCGGCCGC**TCAAGCGTAATCTGGAACATCGTATGGGTATCCTCCA 417 GCGGCCGACGGAGCATTTTTTGCAGTGGAAGCAGTAGTCGTGCC-3' (underline,

418 segment hybridizing with the target sequence; bold, restriction endonuclease site for 419 cloning); and cloned into pLVX-IRES-ZsGreen1 vector (Takara). Using the same 420 approach, coding sequence of murine FHL1 (NM 001077362.2) was amplified with a 421 mFHL1 Fwd primer 5'-CCGGAGAATTCGCCGCCATGGCTTCTCAAAGACACTCAG 422 GTCCCTCC-3' and mFHL1 Rev primer 5'-AATAGTTTAGCGGCCGCTCAAGCGTAA 423 TCTGGAACATCGTATGGGTATCCTCCAGCGGCCGACAGCTTTTTGGCACAGTCA 424 GGGCAATACACCGCTC-3', and cloned into pLVX-IRES-ZsGreen1 vector. C-terminal 425 HA-tagged FHL2 coding sequence was synthesized by Genscript and subcloned into 426 pLVX-IRES-ZsGreen1 vector. The plasmids pCI-neo-3×FLAG plasmids expressing 427 the CHIKV nsP3 and nsP4, the Sindbis virus (SINV) and Semliki Forest virus (SFV) nsP3 proteins were previously described⁴¹. The CHIKV nsP3 Δ HVD, Δ R1 to Δ R4 were 428 429 generated by site-directed mutagenesis (QuickChange XL Site-Directed Mutagenesis 430 Kit, Agilent) using the following sets of primers: ΔHVD -Fwd (5'-431 CGTAAGTCCAAGGGAATATTGATGATCTTCCCAGGAGTCTGC-3') and \triangle HVD-Rev 432 (5'-GCAGACTCCTGGGAAGATCATCAATATTCCCTTGGACTTACG-3'); △R1-F: (5'-433 GTACCTGTCGCGCCGCCCAGAGAGCTGTGTCCGGTCGTACAAGA AAC-3') and ∆R1-R: (5'-GTTTCTTGTACGACCGGACACAGCTCTCTGGGCGGCG 434 435 CGACAGGTAC-3'); ∆R2-F: (5'-GAAACAGCGGAGACGCGTGACAGTACCGCCA 436 CGGAACCGAATC-3') and ∆R2-R: (5'-GATTCGGTTCCGTGGCGGTACTGTCACGC 437 GTCTCCGCTGTTTC-3'); ∆R3-F: (5'-CTTCTTACCAGGAGAAGTGTGATGACTTGA 438 CAGACAGC-3') and ∆R3-R: (5'-GCTGTCTGTCAAGTCATCACACTTCTCCTGGTAA 439 GAAG-3'); ∆R4-F (5'-GACGAGAGAGAGAGGGAATATAACACCGAGTACCGCCACG GAACCGAATC-3') and \triangle R4-R (5'-GATTCGGTTCCGTGGCGGTACTCGGTGTTATA 440 441 TTCCCTTCTCTCTCGTC-3').

The plasmids expressing the chimeric nsP3 CHIKV-HVD SINV and nsP3 SINV-HVD CHIKV were obtained as follows. First, the DNA sequence coding for the N-terminal parts of the CHIKV or SINV nsP3 (MD-AUD region) are obtained by PCR using the pCI-neo-3×FLAG expression plasmids as templates and the following sets of primers: 3xFLAG_NotI-F (5'-ACTGAGCGGCCGCATGGACTACAAAGACCATGAC-3') and Overlap-CHIKV-SINV-R (5'-GCTGTTCTGGCACTTCTATATATTCCCTTGGA

448 CTTACG-3'), or 3xFLAG NotI-F and Overlap-SINV-CHIKV-R (5'-449 CAGACTCCTGGGAAGATCTGTACTTACGGGCGGGAAC-3') for CHIKV and SINV 450 constructs, respectively. HVD coding sequences were also generated by PCR using 451 the following primers: Overlap-CHIKV-SINV-F (5'-452 CGTAAGTCCAAGGGAATATATAGAAGTGCCAGAACAGC-3') and nsP3-453 SINV BamHI-R (5'-ACTGAGGATCCTTAGTATTCAGTCCTCCTGCTC-3') for SINV 454 HVD, and Overlap-SINV-CHIKV-F (5'-GTTCCCGCCCGTAAGTACAGATCTTCCCA

455 GGAGTCTG-3') and nsP3-CHIKV_BamHI-R (5'-ACTGAGGATCCTCATAACTCGT

456 CGTCCGTG-3') for CHIKV HVD. Next, the CHIKV-HVD-SINV and SINV-HVD-CHIKV
457 PCR-fragments were obtained by overlap extension PCR using the previously
458 obtained PCR-products and the following sets of primers: 3XFLAG_NotI-F and nsP3459 SINV_BamHI-R or nsP3-CHIKV_BamHI-R. Finally, the chimeric PCR fragments were
460 cloned into a NotI-BamHI digested pLVX-IRES-ZsGreen1 vector (Takara).

461 The plasmid expressing FHL1A-R4 and FHL1A-R4* fusion proteins were obtained by 462 overlap extension PCR approach as well. First, the FHL1A part which is common to 463 both constructs was amplified from a cDNA template (Genscript, NM_001449.4) using 464 the common FHL1 Fwd primer (5'-465 CCGGAGAATTCGCCGCCATGGCGGAGAAGTTTGACTGCCACTACTGC-3') and 466 the Overlap-FHL1A-Fusion Rev primer (5'- CGCCCTGGAAGTACAGGTTCTCGCCG

467 CCGCCCAGCTTTTTGGCACAGTCGGGACAATAC-3'). Second, nsP3-R4 and –R4*
 468 portions were obtained by PCR using either the pCI-neo-3×FLAG-nsP3 expression
 469 plasmid or the pCHIKV-SG45-R4* plasmid (containing the randomized R4 region) as
 470 templates and the following set of primers: Overlap-FHL1-fusion-Fwd (5' 471 CGAGAACCTGTACTTCCAGGGCGGCGGCGGCCGCCCATGGCTAGCGTCCGATTCT

472 TTAG-3') and FHL1-fusion-Rev (5'-AATAGTTTAGCGGCCGCTCAAGCGTAATCT

473 GGAACATCGTATGGGTAGCCGCCGCCGGTGGTGCCTGAAGAGACATTGCTG-

474 3') for R4 construct, or FHL1-fusion-Rand-Rev primer (5'-

475 AATAGTTTAGCGGCCGCTCAAGCGTAATCTGGAACATCGTATGGGTAGCCGCC

476 GCCCCTCACCTCGGCGCACATGG-3') for the randomized R4* construct. Next, the 477 FHL1A-R4 and FHL1A-R4* PCR-fragments were obtained by PCR using the 478 previously obtained PCR-products and the outer sets of primers: FHL1A Fwd and 479 FHL1-fusion-Rev or FHL1-fusion-Rand-Rev. Amplification fragments were cloned into 480 a NotI-EcoRI digested pLVX-IRES-ZsGreen1 vector (Takara).

481 To obtain pCHIKV-M-Gluc a viral sequence encompassing the CHIKV 26S promoter 482 and a part of the capsid protein sequence was amplified from pCHIKV-M using primers 483 5'-TATGCGTTTAAACCATGGCCACCTTTGCAAGCTCCAGATC-3' and 5'-GCTTCTTATTCTTCCGATTCCTGCGTGG-3', cut with Pmel and BssHII and 484 485 assembled together with an Agel-Pmel fragment from pCHIKVRepl-Gluc⁴² into an 486 Agel-BssHII cut vector. From the resulting plasmid the Agel-BssHII fragment was 487 released and ligated together with a BssHII-SfiI fragment from pCHIKV-M⁴³ into 488 pCHIKV-M cut with Agel and Sfil.

To establish pCHKV-Rluc-GAA two PCR fragments were amplified from pCHIKV-WT
 using primers CHIKV 5590 F (5'-AGACTTCTTACCAGGAGAAGTG-3') and Bo422 (5' CGACTCCATGTATTATGTTacccgctgcGATGAAGGCCGCGCACGCGG-3') or Bo421

492 (5'-CCGCGTGCGCGCGCCTTCATCgcagcgggtAACATAATACATGGAGTCG-3') and
493 CHIKV 8512 R (5'-GAAGTTGTCCTTGGTGCTGC-3'), respectively. The obtained
494 fragments were fused via PCR amplification using the outer primers CHIKV 5590 F
495 and CHIKV 8512 R. The resulting fragment was cut with Agel and Bgll and inserted
496 into pCHIKV-Rluc cut with the same restriction enzymes.

497 For generation of CHIKV-Rluc-ΔR4 and CHIKV-Rluc-R4* first PCR fragments 498 encompassing the desired changes were amplified and assembled as follows: 1) 499 CHIKV-Rluc-AR4: two fragments amplified from CHIKV-Rluc using Bo408 (5'-500 CACCACGTGCTCCTGGTCAGTG-3') Bo1259 (5'and 501 Bo1258 (5'or 502 tgacgagagagagaggaatataacaccgagtaccgccacggaaccgaatc-3') and Bo409 (5'-503 GACTTCCTCCAGGGTGTTCACC-3'), respectively, were fused together using the 504 outer primers Bo408 and Bo409. 2) CHIKV-Rluc-R4*: the randomized sequence 505 cassette was obtained sequentially from three successive PCRs: First PCR fragment 506 generated primers Bo1260 (5'was using 507 AGCACCGTGCCCCTGCCCCTGAGGAGGGCCAGCTTCGCCGACACCATGG 508 AGCAGACC-3') and Bo1261 (5'-509 CCTCACCTCGGCGCACATGGGGAACTGCTCGGCCACGGTCTGCTCCATGGTGT 510 CGGCGAA-3'). Then, it was fused at the 5' end with a PCR fragment amplified from 511 CHIKV-Rluc with Bo408 and Bo1262 (5'-512 TCAGGGCGGGCAGGGGCACGGTGCTtgttatattcccttctctcgtca-3'). Next. the 513 resulting fragment is further fused at the 3' end with a PCR fragment amplified from 514 CHIKV-Rluc with Bo1263 (5'-515 GTTCCCCATGTGCGCCGAGGTGAGGccgagtaccgccacggaaccgaatc-3') and Bo409, 516 using the outer primers Bo408 and Bo409. Finally, the PCR fragments containing the

517 ΔR4 and R4* mutations were cut with SacII and AgeI and fused in each case with a
518 NgoMIV-SacII fragment derived from CHIKV-Rluc (SG45) and were cloned into a
519 NgoMIV-AgeI digested SG45 plasmid.

520

521 Trans-complementation and over expression experiments. The lentiviral plasmids 522 containing FHL1 isoforms were packaged as described above (see 'CRISPR genetic 523 screen' section). Cells of interest were stably transduced by spinoculation (750 x g for 524 2 hours at 32°C) with these lentiviruses and, when necessary, sorted for GFP-positive cells by flow cytometry. For trans-complementation assays cells were inoculated with 525 526 CHIKV21 for 48 hours. Cells were then collected and processed for E2 expression by flow cytometry. For ectopic expression, cells were plated on 24-well plates (5x10⁴) and 527 528 incubated with CHIKV-M-GLuc and CHIKV21, and either processed for E2 expression 529 by flow cytometry or infectious virus yield quantification on Vero E6 cells.

530

531 Kinetic of infection by gPCR assay. Control and \triangle FHL1 HAP1 cells were plated on 532 60 mm dishes (400,000 cells) and inoculated with CHIKV21 (MOI of 5). At indicated 533 time point cells were washed thrice with PBS, incubated with trypsin 0.25% for 5 min at 37°C to remove cells surface bound particles, and total RNA was extracted using 534 535 the RNeasy plus mini kit (Qiagen) according to manufacturer's instruction. cDNAs were 536 generated from 500 ng total RNA by using the Maxima First Strand Synthesis Kit 537 following manufacturer's instruction (Thermo Fisher Scientific). Amplification products 538 were incubated with 1 Unit of RNAse H for 20 min at 37 °C, followed by 10 min at 72°C 539 for enzyme inactivation, and diluted 10-fold in DNAse/RNAse free water. Real time 540 quantitative PCR was performed using a Power Syber green PCR master Mix (Fisher 541 Thermo Scientific) on a Light Cycler 480 (Roche). The primers used for qPCR were:

542 E1-C21_F (5'-ACGCAGTTGAGCGAAGCAC-3'), E1-C21_R (5'-CTGAAGACATTG

543 GCCCCAC-3') for viral RNA quantification, and Quantitect primers for GAPDH were 544 purchased from Qiagen. The relative expression quantification was performed based 545 on the comparative threshold cycle (C_T) method, using GAPDH as endogenous 546 reference control. CHIKV negative strand RNA was quantified as previously described⁴⁴. Briefly, cDNA were generated from 1µg total RNA using a primer 547 548 containing 5' tag sequence CHIKV(-)Tag (5'а 549 GGCAGTATCGTGAATTCGATGCCGCTGTACCGTCCCCATTCC-3') and the 550 SuperScript II reverse transcriptase following the manufacturer's instruction (Thermo 551 Fisher Scientific). Amplifications products were diluted 10-fold and used for real time 552 quantitative PCR with the following primers CHIKV(-)fwd (5'-553 GGCAGTATCGTGAATTCGATGC-3') and CHIKV(-)rev (5'-ACTGCTGAGTCCAAAG 554 TGGG-3'). The 133 bp sequence corresponding to the amplified cDNA was synthesized by Genescript and serially diluted (650 to 6.5x10⁹ genes copies/µl) to 555 556 generate standard curves.

557

558 Genomic viral RNA transfection and kinetic of viral amplification. To assess 559 CHIKV RNA replication within the cells, we transfected control and \triangle FHL1 cells with capped genomic viral RNA generated from pCHIKV-M-Gluc (see 'Virus strains and 560 561 culture' section). Cells were plated on 48 well plate (3x10⁴ cells) and transfected with 100 ng of purified RNA using the Lipofectamine MessengerMax reagent according to 562 563 the manufacturer's instruction (Thermo Fisher Science), and cells were cultured in 564 absence or presence of 15 mM NH₄Cl to prevent subsequent viral propagation. At 565 specific times, cells were washed once with PBS and lyzed with Gaussia lysis buffer. 566 Lysates were kept at -20°C until all samples were collected. Luciferase activity was

567 measured by using the Pierce Gaussia Luciferase Glow assay kit on a TriStar2 LB 942
568 with 20 µl of cell lysate, 20 µl of substrate and 2s integration time.

569 The same experimental approach was used to monitor luciferase activity from capped 570 genomic viral RNA generated from pCHIKV-Rluc WT (SG45), pCHIKV-Rluc-GAA, 571 pCHIKV-Rluc-ΔR4 and pCHIKV-Rluc-R4* mutants. Luciferase activity was measured 572 using the Renilla Luciferase assay system (Promega) on a TriStar2 LB 942 with 20 μl 573 of cell lysate, 20 μl of substrate and 2.5s integration time.

574

Immunoblot. Cell pellet were lysed in Pierce[™] IP Lysis Buffer (Thermo Fisher 575 Scientific) containing Halt[™] Protease and Phosphatase Inhibitor Cocktail (Thermo 576 Fischer Scientific) for 30 min at 4°C. Equal amount of protein, determined by 577 578 DC[™]Protein Assay (BioRad), were prepared in LDS Sample Buffer 4X (Pierce[™]) 579 containing 25 mM dithiothreitol (DTT) and heated at 95°C for 5 min. Samples were 580 separated on Bolt[™] 4-12% Bis-Tris gels in Bolt[®] MOPS SDS Running Buffer (Thermo 581 Scientific), and proteins were transferred onto a PVDF membrane (BioRad) using the 582 Power Blotter system (Thermo Fischer Scientific). Membranes were blocked with PBS containing 0.1% Tween-20 and 5% non-fat dry milk and incubated overnight at 4°C 583 with primary antibody. Staining was revealed with corresponding horseradish 584 585 peroxidase (HRP)-coupled secondary antibodies and developed using SuperSignal[™] 586 West Dura Extended Duration Substrate (Thermo Fisher Scientific) following 587 manufacturer's instructions. The signals were acquired through Fusion Fx camera 588 (VILBERT Lourmat).

589

590 **Co-immunoprecipitation assay.** HEK-293T cells were plated in 10 cm dishes $(5.10^6$ 591 cells/ dish). Twenty-four hours later, the cells were transfected with a total of 15 μ g of

592 DNA expression plasmids (7.5 µg of each plasmid in co-transfection assays). Twenty-593 four hours post-transfection the cells washed once with PBS and collected with a cell 594 scrapper. After 5 min centrifugation (400 x g for 5 min), cells pellets were lysed for 30 min in cold IP lysis buffer supplemented with Halt[™] Protease and Phosphatase 595 596 Inhibitor Cocktail, and then cleared by centrifugation for 15 min at 6,000 x g. 597 Supernatants were incubated overnight at 4°C, with either anti-FLAG magnetic beads 598 or HA magnetic beads (see 'reagent' section above). Beads were washed three times 599 with BO15 buffer (20 mM Tris-HCl pH 7.4, 150 mM NaCl, 5 mM MgCl2, 10% Glycerol, 0.5 mM EDTA, 0.05% Triton, 0.1% Tween-20). The retained complexes were eluted 600 601 twice with either 3xFLAG-peptide (200 µg/ml; SIGMA F4799-4MG) or HA peptide (400 602 µg/ml; Roche# 11666975001) for 30 min at room temperature. Samples were prepared 603 and subjected to immunoblot as described above. For input, 1% of whole cell lysate 604 were loaded on the gel.

605

606 Bacterial expression, purification and GST pull down assay. To express nsP3, 607 $nsP3\Delta HVD$ as glutathione S-transferase fusion proteins, their respective open reading 608 frame (orf) were subcloned into pGEX-4T-1. Similarly, FHL1A cDNA was subcloned into the pET47b (+) and expressed as a 6xHis fusion protein. The following 609 610 oligonucleotides were used to amplify nsP3 and nsP3∆HVD cDNAs (sense: 5'-611 ccccggaattcATGgcaccgtcgtaccgggtaa-3'; 5'antisense: 612 5'ccgctcgagTCAtaactcgtcgtcgtgtctg-3') and FHL1A (sense: 613 ccggaattccATGgcggagaagtttgactgcc-3'; antisense: 5'-614 ccgctcgagTTAcagctttttggcacagtc-3'). E.Coli strain BL21 Star (Invitrogen) was 615 transformed with recombinant expression vectors encoding GST-nsP3, GSTnsP3AHVD or 6xHis-FHL1A recombinant proteins. Transformed bacteria were 616

617 induced with isopropylthio-β-Dgalactoside (IPTG) for 3 hours at 37°C. Cells were 618 collected by centrifugation and the pellets were resuspended in lysis buffer containing 619 lysozyme (1 mg/mL), incubated 30 min at 4°C followed by three subsequent freeze-620 thawed cycles and sonication. The bacterial lysates were centrifuged at 13,000 r.p.m 621 for 20 min and the supernatants were incubated with glutathione-Sepharose beads for 622 GST-nsP3 and GST-nsP3AHVD, or Ni-NTA column (Qiagen) for 6xHis-FHL1A. 623 Column washing and recombinant protein elution were performed according to the 624 manufacturer's instructions. Five µL of eluted GST fusion proteins and 3 µL of Ni-NTA eluted 6xHis-FHL1A were analyzed by SDS-PAGE and proteins were visualized by 625 626 Coomassie staining. For pull-down assay, GST, GST-nsP3 or GST-nsP3∆HVD bound 627 beads were incubated with 6xHis-FHL1A for 1 hour at 4°C in presence of 100 µM 628 ZnSO₄. The resin was washed extensively with a buffer containing 500 mM KCL. The 629 beads were then resuspended in Laemmli buffer, resolved on SDS-PAGE and the 630 presence of 6xHis-FHL1A was assessed by western blot using anti-FHL1 antibody.

631

632 Genetic analysis, fibroblasts and myoblasts from Emery-Dreifuss muscular 633 dystrophy patients. Dermal fibroblasts and myoblasts were taken from 4 patients carrying FHL1 gene mutations. FHL1 gene was analyzed as previously reported ⁶ as 634 635 they had, among other symptoms, features reminiscent of Emery-Dreifuss muscular 636 dystrophy. Patients P1, P2 and P3 were previously reported ⁶ with detailed clinical 637 description (respectively as patient F321-3, F997-8 and F1328-4) while patient P4 was 638 not yet published. Briefly, patient P4 had myopathy with joint contractures, hypertrophic 639 cardiomyopathy, vocal cords palsy, short stature, alopecia, skin abnormalities and 640 facial dysmorphism. In this patient, *FHL1* analysis revealed an insertion of a full-length 641 LINE-1 retrotransposon sequence together with poly A tail of unknown length (i.e.,?

thereafter) after 27 bp of the start of exon 4 (c.183_184ins [LINE1;?; 171_183]) that
results at mRNA level in altered splicing with retention of 108 bp of the inserted LINE
sequence leading to predicted premature termination codon and shorter FHL1A
(Extended Data Fig. 7b).

646

Ethics statement. All materials (skin and/or muscle biopsies) from patients and controls included in this study were taken with the informed consent of the donors and with approval of the local ethical boards. All the procedures were followed alongside the usual molecular diagnostic procedure during patient follow-up, and in accordance with the ethical standards of the responsible national committee on human experimentation.

653

654 In vivo studies. Animals were housed in the Institut Pasteur animal facilities 655 accredited by the French Ministry of Agriculture for performing experiments on live 656 rodents. Work on animals was performed in compliance with French and European 657 regulations on care and protection of laboratory animals (EC Directive 2010/63, French Law 2013-118, February 6th, 2013). All experiments were approved by the Ethics 658 #89 659 Committee (and registered under the reference APAFIS#6954-660 2016091410257906 v2). Male mice either deficient for FHL1 (FHL1-null) or not (WT 661 littermates) were obtained by crossing heterozygous females for FHL1⁴⁵ with WT male 662 Black Swiss mice. Nine day-old male littermates, both FHL1-null and WT mice, were 663 injected with CHIKV21 (10⁵ PFU/20µI) by intradermal route and viral load was determined in tissues by day 7 post infection. Virus titers in tissue samples were 664 665 determined on Vero E6 cells by tissue cytopathic infectious dose 50 (TCID50/g). For 666 histology experiments, muscles were snap frozen in isopentane cooled by liquid

667 nitrogen for cryo-sectioning then processed for histological staining (hematoxylin and668 eosin) or immunolabelling.

669

670 **Transmission electron microscopy.** Cells were scrapped and fixed for 24 h in 1% 671 glutaraldehyde, 4% paraformaldehyde, (Sigma, St-Louis, MO) in 0.1 M phosphate 672 buffer (pH 7.2). Samples were then washed in phosphate-buffered saline (PBS) and 673 post-fixed for 1 h by incubation with 2% osmium tetroxide (Agar Scientific, Stansted, 674 UK). Cells were then fully dehydrated in a graded series of ethanol solutions and propylene oxide. Impregnation step was performed with a mixture of (1:1) propylene 675 676 oxide/Epon resin (Sigma) and then left overnight in pure resin. Samples were then 677 embedded in Epon resin (Sigma), which was allowed to polymerize for 48 hours at 678 60°C. Ultra-thin sections (90 nm) of these blocks were obtained with a Leica EM UC7 679 ultramicrotome (Wetzlar, Germany). Sections were stained with 2% uranyl acetate 680 (Agar Scientific), 5% lead citrate (Sigma) and observations were made with a 681 transmission electron microscope (JEOL 1011, Tokyo, Japan).

682

Cell viability assay. Cell viability and proliferation were assessed using the CellTiter-Glo 2.0 Assay (Promega) according to the manufacturer's protocol. In brief, cells were plated in 48-well plates $(3x10^4)$. At specific times, 100 µl of CellTiter-Glo reagent were added to each well. After 10 min incubation, 200 µl from each well were transferred to an opaque 96-well plate (Cellstar, Greiner bio-one) and luminescence was measured on a TriStar2 LB 942 (Berthold) with 0.1 second integration time.

689

690 Statistical analysis. Graphical representation and statistical analyses were performed
691 using Prism7 software (GraphPad Software). Unless otherwise stated, results are

shown as means +/- standard deviation (SD) from at least 2 independent experiments
in duplicates. Differences were tested for statistical significance using the unpaired
two-tailed *t* test, One-way or Two-way Anova with multiple comparison post-test.

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823

824 AUTHORS' CONTRIBUTIONS

L.M. and A.A. conceived the study. L.M, M.L.H, T.C, V.K, A.B, L.B.M, C.D., M.L and A.A designed the experiments. L.M. performed the CRISPR-Cas9 screening and infection studies with L.B.M. M.L.H characterized the FHL1 and nsP3 interactions and performed the immunoprecipitation and western blot studies with the help of V.K. A.L. validated the FHL1 gRNA and generated the FHL1 knockout cells described in this study. A.B. performed the immunofluorescence microscopy experiments and infection

831 studies. L.M. and E.S.L. analyzed the gRNA sequencing and identified the hits. J.B.G 832 and P.R. performed the EM experiments. C.D. and M.B performed the GST-pull 833 experiments. B.M.K generated the CHIKV molecular clones described in this study and P.O.V provided key CHIKV reagents. L.P and X.L. provided the alphavirus strains and 834 835 performed infection studies. T.C. and M.L. performed in vivo studies and provided 836 expertise in the design of CHIKV experiments. S.R. performed virus titration assays 837 and mice genotyping. T.G. performed immunofluorescence experiments in mice 838 tissues with T.C. A.T.B, R.B.Y., L.G., R.J.M. and G.B provided myoblasts and 839 fibroblasts from EDMD patients and the description of a new FHL1 mutation in EDMD disease. L.M and AA wrote the initial manuscript draft, and the other authors 840 841 contributed to editing into its final form.

843 **FIGURE LEGENDS**

844 Figure 1. FHL1 is important for infection by CHIKV and ONNV

845 a, Results of the CHIKV screen analyzed by MAGeCK. Each circle represents 846 individual gene. Y-axis represents the significance of sgRNA enrichment of genes in 847 the selected population compared to the non-selected control population. X-axis 848 represents a random distribution of the genes. **b**. E2 protein expression in control or 849 △FHL1 cells infected with the CHIKV 21 strain (MOI of 10). c, △FHL1 HAP1 cells were 850 trans-complemented with FHL1A, B or C isoforms, infected with CHIKV 21 strain (MOI 851 of 10) and stained for E2 protein expression at 48hpi. Data shown in **b** and **c** are mean 852 +/- SD (3 experiments, n=6; one-way ANOVA with Dunnett's multiple comparisons 853 test). **d**, \triangle FHL1 and control cells were inoculated with CHIKV-Ross (MOI of 10), 854 CHIKV-Brazza (MOI of 10), CHIKV-20235 (MOI of 10), CHIKV-M (M-899) (MOI of 10) 855 or CHIKV-37997 (MOI of 10) and analyzed at 24 (293T) or 48hpi (HAP1) for E2 856 expression. Data shown are mean +/- SD (4 experiments, n=8 excepted for CHIKV-857 37997 n=4; one-way ANOVA with Tukey's multiple comparisons test). e-g, Δ FHL1 and 858 control HAP1 cells were inoculated with O'nyong-nyong virus (ONNV) (MOI of 2), 859 Mayaro virus (MAYV) (MOI of 50), Eastern equine encephalitis virus (EEEV) (MOI of 860 2), Sindbis virus (SINV), Semliki Forest Virus (SFV), Ross River virus (RRV), Western 861 equine encephalitis virus (WEEV), Venezuelan equine encephalitis virus (VEEV), 862 Dengue virus (DENV) (MOI of 0.4) or ZIKA virus (ZIKV) (MOI of 50). e, Infection was 863 quantified 48hpi by flow cytometry using the anti-E2 3E4 or 265 CHIKV mAb or the 864 anti-EEEV mAb 1A4B6 (2 experiments, n=4). f, Virus growth was assessed at day 4 865 pi using real-time RT-PCR. Serial dilutions of infected supernatants titrated using the 866 TCID50 method were used as quantification standards for RT-PCR. Accordingly, results were expressed for each virus as "molecular equivalents of TCID50". Data 867

868 shown are representative of two experiments. g, DENV or ZIKV infection were 869 assessed by flow cytometry 48hpi using the anti-E protein 4G2 mAb. (3 experiments, 870 n=6). e-g Data shown are mean +/- SD and significance was calculated using a one-871 way ANOVA statistical test with a Tukey's multiple comparisons test. h, BeWo and 872 HepG2 cells were transduced with FHL1A or a control vector and challenged with 873 CHIKV21 (MOI of 5) or CHIKV- M-899 (MOI of 2). Infection was quantified two days 874 later by flow cytometry using the 3E4 mAb. Data shown are mean +/- SD (2 875 experiments, n=4 excepted for BeWo cells infected with CHIKV21, 3 experiments, n=6; one-way ANOVA with Tukey's multiple comparisons test). n.s non-significant; *** p< 876 877 0.0001.

878

Figure 2. FHL1 interacts with CHIKV nsP3 and is required for CHIKV RNA replication

881 a, Control and △FHL1 HAP1 cells were inoculated with CHIKV 21 (MOI of 10). At the 882 indicated time points, cells were treated with trypsin to remove cell surface bound virus 883 and viral RNA was quantified by qRT-PCR. Data shown are mean +/- SD (3) 884 experiments, n=9; two-tailed t-test). **b**, Control or \triangle FHL1 293T cells were transfected 885 with in vitro transcribed CHIKV-M RNA expressing gaussia luciferase (Gluc) and Gluc 886 activity was monitored at the indicated time points. RLU, relative light units. Data 887 shown are mean +/- SEM (3 experiments, n=12; multiple t-tests). **c**, Control or \triangle FHL1 888 293T cells were transfected with a replication-deficient mutant CHIKV (CHIKV-GAA) 889 RNA expressing renilla luciferase (RLuc) and luc activity was monitored at the 890 indicated time points. Data shown are mean +/- SEM (3 experiments, n=12; multiple t-891 tests). **d**, Control or \triangle FHL1 293T cells were transfected with a replication-competent 892 (CHIKV-GDD) or a replicon-deficient mutant CHIKV (CHIKV-GAA) capped RNA

893 expressing RLuc. The Rluc activity was monitored at described in c. Data shown are 894 mean +/- SEM (3 experiments, n=12; 2-way ANOVA with Tukey's multiple comparison 895 test). e, negative stranded viral RNA quantification by qRT-PCR from samples 896 collected in (a). Data shown are mean +/- SD (2 experiments, n=8; one-way ANOVA 897 with a Tukey's multiple comparisons test). Dashed line represents the experimental 898 background threshold. f. Control and Δ FHL1 293T cells were inoculated with CHIKV 899 21 (MOI of 50). (left panel) Representative images of infected cells stained with anti-900 dsRNA mAb at 6hpi. (right panel) Number of foci per cell was guantified using the Icy 901 software (2 experiments, n=42 cells in control and n=45 cells in Δ FHL1 cells; two-tailed 902 t-test). g, Transmission electron microscopy of control and Δ FHL1 HAP1 cells 903 challenged with CHIKV21 (MOI of 100) at 24h post-infection. Left panel shows CPV-II 904 structures containing attached nucleocapsids at their cytoplasmic side (white arrows) 905 as well as viral particles at the cell surface (thin black arrows). Middle panels show 906 replication spherules (arrowheads) together with viral particles (thin black arrows) at 907 the plasma membrane. PM= Plasma membrane. (Bars, 200nm). h, Co-908 immunoprecipitation of endogenous FHL1 and CHIKV nsP3 from cell lysates of 293T 909 cells infected with a CHIKV nsP3-mCherry reporter virus at MOI 5 or 50. i, In vitro co-910 immunoprecipitation analyzing the direct interaction between CHIKV-nsP3 and FHL1A 911 through the HVD domain. GST-precipitation of GST-nsP3 or GST-nsP3AHVD and 912 immunoblot analysis of 6xHis-FHL1A. j, 293 T were co-transfected with plasmids 913 encoding FHL1A-HA and FLAG-tagged CHIKV nsP3 WT or CHIKV nsP3 ∆HVD or 914 CHIKV lacking the amino acid region 423-454 (AR4). Cellular lysates were subject to immunoprecipitation with anti-FLAG beads followed by immunoblot analysis with anti-915 916 FLAG or anti-HA mAb. k, (left panel) Schematic representation of FHL1A protein in 917 fusion with the nsP3 interacting region (FHL1A-R4) or a similar randomized sequence

918 (FHL1A-R4*). (Right panel) Immunoassay of the interaction between CHIKV nsP3 and 919 FHL1A fusion proteins in 293T cells co-transfected with FLAG-tagged CHIKV nsP3 920 and either a HA-tagged FHL1A, FHL1A-R4 or FHL1A-R4* constructs. Cellular lysates 921 were subject to immunoprecipitation with anti-FLAG followed by immunoblot analysis 922 with anti-FLAG and anti-HA Ab. I, ∆FHL1 293T cells were transfected with an empty 923 vector or plasmids encoding FHL1A, FHL1A-R4 or FHL1A-R4*. Cells were incubated 924 with CHIKV21 (MOI of 5) and infection was quantified 24hpi by flow cytometry. Data 925 shown are mean +/- SD (2 experiments, n=4; one-way ANOVA with Dunnett's multiple 926 comparison test). ** P< 0.01 **** P< 0.0001; ns not significant.

927

Figure 3. Primary myoblasts and fibroblasts from FHL1 deficient patients are resistant to CHIKV infection.

930 a, FHL1 expression in primary myoblasts and fibroblasts from healthy donors or 931 Emery-Dreifuss muscular dystrophy (EDMD) patients. CM: control myoblasts, PM: 932 patient myoblasts; CF: control fibroblast, PF: patient fibroblasts. b, Cells from controls 933 or EMDM patients were inoculated with CHIKV expressing nsP3-mCherry. At 48-hpi, 934 cells were fixed and images were taken on fluorescence microscope. Images are 935 representative of three experiments. c, E2 protein expression in primary cells from 936 healthy controls or EDMD patients infected with CHIKV21 (MOI of 2). Data shown are 937 mean +/- SD (2 experiments, n=4 for myoblast; 4 experiments, n=8 for fibroblast; one-938 way ANOVA with Tukey's multiple comparisons test). d, Quantification of viral particles 939 released in supernatant of infected cells collected at 24, 48- and 72-hpi. FIU, flow 940 cytometry infectious units. Data shown are mean +/- SEM (2 experiments, n=4 for 941 myoblast; 3 experiments, n=6 for fibroblast; multiple t-test). e, Primary fibroblasts from 942 a control (CF1) or two FHL1 null patients (PF2, PF4) were inoculated with CHIKV-

943 Ross, CHIKV-Brazza, CHIKV-H20235 strains or MAYV (MOI of 2) and analyzed for E2 944 expression. Data shown are mean +/- SD (3 experiments n=6, one-way ANOVA with 945 Dunnett's multiple comparisons test). f-g Fibroblasts from control (CF1) or FHL1 null 946 patients (PF2, PF4) were transduced with a lentiviral vector encoding FHL1A or a 947 control vector and then challenged with CHIKV21 (MOI of 2). f, Infection was quantified 948 as described in c. Data shown are mean +/- SD (2 experiments, n=4, one-way ANOVA 949 with Tukey's multiple comparisons test). g, Supernatants were collected from infected 950 cells at indicated time point and viral titers were measured on VeroE6 cells. Data 951 shown are mean +/- SEM (2 experiments, n=4; two-way ANOVA with Dunnett's multiple comparisons test). *P<0.05; **P<0.01; **** P< 0.0001; ns not significant. 952

953

954 **Figure 4. FHL1 is a factor of susceptibility to CHIKV infection in mice.**

a, Viral titers in tissues of nine-day-old mice. WT littermates (n=5) and FHL1-null mice (n=7) were inoculated with 10⁵ PFU of CHIKV via the ID route and sacrificed by 7 days post infection. The amount of infectious virus in tissues was quantified by TCID50. The broken line indicates the detection threshold. **b**, Hematoxylin and eosin staining of transversal section of skeletal muscle in CHIKV-infected mice. **c**, Immunostaining of nuclei, FHL1, vimentin and CHIKV antigens on skeletal muscle of CHIKV-infected mice. ** P<0.01; ns not significant.

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964 **Extended Data Figures**

965 Extended Data Fig. 1. CRISPR-Cas9 genetic screen identified essential host
 966 factors of CHIKV infection

a, Schematic of CRISPR-Cas9 genome-wide screen in HAP1 haploid cells. b, Ranked
list of the top 30 genes identified using MAGeCKs algorithm and their corresponding
rank in RIGER analysis. c, Venn diagram comparing the top 200 hits from our screen
and previous CRISPR and haploid screens for CHIKV host factors.

971

972 Extended Data Fig. 2. Validation of FHL1 gene edition by CRISPR-Cas9

973 Schematic of the genomic organization of FHL1 (a), alternative splicing of the isoforms 974 FHL1A, FHL1B and FHL1C (b) and their corresponding proteins (c). Initiation and stop 975 codon are indicated in red and relative positions of the sequence targeted by the 976 sgRNA are indicated in blue. **d**, Sanger sequencing of FHL1 in control and Δ FHL1 977 HAP1 cells. e, Genomic DNA was used for PCR amplification using primers flanking 978 the sequence targeted by FHL1 sgRNA2. The absence of an amplification product of 979 3.9 kb (black arrow) in HAP1 clone suggests that a large indel is responsible for the 980 absence of FHL1 expression. Asterisk: unspecific PCR products. f, Immunoblot of 981 FHL1 in control and \triangle FHL1 cells. One representative of three experiments is shown. 982 **e**, Control and \triangle FHL1 cells were plated and viability was assessed over a 72 hours 983 period using the CellTiter-Glo assay. Data shown are mean +/- SEM (2 experiments, 984 n=8; two-way ANOVA with Dunnett's multiple comparisons test). *P<0.05; ns not 985 significant.

986

988 Extended Data Fig. 3. FHL1 is an essential host factor for CHIKV and ONNV 989 infection

990 a, Immunofluorescence images of control and \triangle FHL1 HAP1 cells inoculated with 991 CHIKV21 (MOI of 10), fixed 48 hpi and stained for E2 expression. b, 992 Immunofluorescence images of control and \triangle FHL1 HAP1 cells inoculated with CHIKV 993 expressing nsP3-mCherry (MOI of 10) and fixed 48 hpi. a, b, Images were taken on 994 fluorescence microscope and are representative of three experiments. c, Control and 995 Δ FHL1 HAP1 cells were inoculated with increasing MOI of CHIKV21, and infection was 996 quantified 48hpi by flow cytometry using the anti-E2 3E4 mAb. Data shown are mean 997 +/- SD (3 experiments, n=6; two-way ANOVA with Tukey's multiple comparison test). 998 d, Multi-step growth curves with CHIKV 21 strain in control or ∆FHL1 cells. Data shown 999 are mean +/- SEM (2 experiments, n=4; multiple t-tests). e, Control and ∆FHL1 HAP1 1000 cells were inoculated with increasing MOI of ONNV or MAYV, and Infection was 1001 guantified 48hpi by flow cytometry using anti-E2 3E4 and 265 mAbs. Data shown are 1002 mean +/- SEM (2 experiments, n=4; two-way ANOVA with Tukey's multiple 1003 comparisons test). **f**, Control and \triangle FHL1 HAP1 cells were inoculated with increasing 1004 MOI of DENV or ZIKV, and infection was quantified 48hpi by flow cytometry using the 1005 anti-E 4G2 mAb. Data shown are mean +/- SEM (3 experiments, n=6; two-way ANOVA 1006 with Tukey's multiple comparisons test). * P< 0.05; **** P< 0.0001; ns not significant.

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- 1008

1009 Extended Data Fig. 4. FHL1A and FHL2 ectopic expression in ∆FHL1 cells 1010 restores CHIKV infection

a, Immunoblot of ectopic FHL1 expression in HAP1 cells stably transduced with an
 empty vector or FHL1A, FHL1B or FHL1C isoform. b, Quantification in the supernatant

1013 of infected HAP1 cells of viral particles released by measuring viral titer on Vero E6 1014 cells. Data shown are representative of 3 experiments, mean +/- SEM. **c**, Δ FHL1 293T 1015 cells transfected with an empty vector or HA-tagged plasmids encoding FHL1A and 1016 FHL2 were subjected to infection with increasing MOI of CHIKV21. Infection was 1017 quantified 24hpi by flow cytometry. Data shown are mean +/- SD (3 experiments, n=6; 1018 two-way ANOVA with Dunnett 's multiple comparison test) **P<0.01; **** P< 0.0001; 1019 ns not significant.

1020

1021 Extended Data Fig. 5. FHL1A overexpression in BeWo and HepG2 cells enhances 1022 CHIKV infection

1023 **a**, Expression of endogenous FHL1 in HAP1, 293T, BeWo and HepG2. **b**, Immunoblot 1024 of ectopic FHL1 expression in Bewo and HepG2 cells stably transduced with an empty 1025 vector or HA-tagged FHL1A. c and d, Bewo cells stably transduced with an empty 1026 vector or HA-tagged FHL1A were inoculated with increasing MOI of CHIKV21. c, 1027 Infection was quantified 48hpi by flow cytometry using the anti-E2 3E4 mAb. Data 1028 shown are mean +/- SEM (3 experiments, n=6; Two-way ANOVA with Tukey's multiple comparisons test). d, Quantification in the supernatants of infected cells of viral 1029 1030 particles released by measuring viral titer on Vero E6 cells. Data shown are mean +/-1031 SD (2 experiments, n=4; two-tailed t-test). e, HepG2 cells stably transduced with an 1032 empty vector or FHL1A were inoculated with increasing MOI of CHIKV-M-Gluc. 1033 Infection was guantified 48hpi as indicated in c. Data shown are mean +/- SEM (2 1034 experiments, n=4; Two-way ANOVA with Tukey's multiple comparisons test). ** P<0.001; **** P< 0.0001; ns not significant. 1035

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1037 Extended Data Fig. 6. CHIKV nsP3 directly interacts with FHL1A and FHL2

1038 a, Control or \triangle FHL1 HAP1 cells were transfected with CHIKV-M-Gluc capped genomic 1039 RNA expressing Gaussia luciferase (Gluc). Gluc activity was monitored at indicated time point. RLU, relative light units. Data shown are mean +/- SEM (3 experiments, 1040 1041 n=12; multiple t-tests). b, Confocal microscopy of the colocalization of CHIKV nsP3 1042 with FHL1 protein in fibroblasts inoculated with CHIKV-nsP3-mCherry (MOI of 2), fixed 1043 48 hpi and stained with anti-FHL1. Images are representative of three experiments. c. 1044 Immunoassay of the interaction between CHIKV nsP3 and FHL1 isoforms in 293T cells 1045 transfected with FLAG-tagged CHIKV nsP3 and either an empty vector or plasmids 1046 encoding the three HA-tagged FHL1 isoforms. Cellular lysates were subject to 1047 immunoprecipitation with anti-FLAG followed by immunoblot analysis with anti-FLAG 1048 and anti-HA. d, Immunoassay of the interaction between CHIKV nsP3 and FHL2 in 1049 293Tcells transfected with FLAG-tagged CHIKV nsP3 and either an empty vector or 1050 plasmids encoding HA-tagged FHL1 and FHL2. Cellular lysates were subject to 1051 immunoprecipitation with anti-FLAG followed by immunoblot analysis with anti-FLAG 1052 and anti-HA. e, Endogenous FHL1, G3BP1 or G3BP2 immunoprecipitation from 1053 control and Δ FHL1 293T cells transfected with plasmids encoding FLAG-tagged 1054 CHIKV, Sindbis (SINV) or Semliki forest virus (SFV) nsP3. Cellular lysates were 1055 subject to immunoprecipitation with anti-FLAG followed by immunoblot analysis with 1056 anti-FLAG, anti-FHL1, anti-G3BP1 and anti-G3BP2. f, Endogenous FHL1 1057 immunoprecipitation from 293T cells transfected with plasmids encoding FLAG-tagged 1058 full length CHIKV nsP3, CHIKV nsP3 carrying the SINV HVD (CHIKV/HVD-SIV) or 1059 Sindbis nsP3 carrying CHIKV HVD (SINV/HVD-CHIKV). Cellular lysates were 1060 subjected to immunoprecipitation with anti-FLAG followed by immunoblot analysis with 1061 anti-FLAG and anti-FHL1. g, Purified GST-tagged nsP3 constructs and HA-tagged

FHL1A detected by coomassie blue staining. c-i, One experiment representative of
three is shown. *P<0.05; **P<0.01; **** P< 0.0001; ns not significant.

1064

1065 Extended Data Fig. 7. Mapping the FHL1–nsP3 interaction

1066 **a**, The sequence alignment of nsP3 protein HVD domains of representative members 1067 of New and Old World alphaviruses. Sequence alignment was performed with Clustall 1068 Omega and edited with Jalview. R1, R2 and R3 sequences of high homology between 1069 CHIKV strains and ONNV are defined by colored lines. CHIKV06-21 (GenBank 1070 accession number AM258992.1); CHIKV Ross (GenBank accession number 1071 MG280943.1); CHIKV H20235 (GenBank accession number MG208125.1); CHIKV 1072 37997 (GenBank accession number AY726732.1); ONNV (GenBank accession 1073 number MF409176.1); SFV (GenBank accession number HQ848388.1); MAYV 1074 (GenBank accession number KY618137.1); SINV (GenBank accession number 1075 MF409178.1); EEEV (GenBank accession number Q4QXJ8.2); VEEV (GenBank 1076 accession number P27282.2). b, (top panel) Schematic representation of CHIKV nsP3 1077 constructs deleted for the R1, R2, R3 or R4 sequences. (bottom panel) 293T cells were transfected with FHL1A-HA and either an empty vector or plasmids encoding FLAG-1078 1079 tagged nsP3 constructs. Cell lysates were immunoprecipitated with anti-FLAG 1080 followed by immunoblot analysis with anti-HA or anti-FLAG Ab. One experiment 1081 representative of three is shown. c, Alignment of nsP3 regions containing the WT R4 1082 sequence or the corresponding randomized sequence. Dashes represents identical 1083 aa. d, Control 293T cells were transfected with the indicated CHIKV capped in vitro 1084 transcribed RNA expressing renilla luciferase (Rluc). Rluc activity was monitored at 1085 indicated time points. RLU, relative light units. Data shown are mean +/- SEM (2)

experiments, n=8; Two-way ANOVA with Tukey's multiple comparisons test). **** P<
0.0001; ns not significant.

1088

Extended Data Fig. 8. CHIKV Infection of myoblasts and fibroblasts derived from EDMD patients

1091 a, Schematic of FHL1A protein in three EDMD patient (P1, P2 and P3). b, Schematic 1092 of FHL1 genomic organization in newly described patient with a LINE1 insertion within 1093 exon 4 (P4). c, Myoblasts and fibroblasts from EDMD patients or healthy donors were 1094 infected with increasing MOI of CHIKV21, and infection was quantified 24hpi by flow 1095 cytometry using the anti-E2 3E4 mAb. Data shown are mean +/- SEM (2 experiments, 1096 n=4 for myoblast; 3 experiments, n=6 for fibroblast; Two-way ANOVA with Dunnett's 1097 multiple comparisons test). d, Fibroblasts from EDMD patients or healthy donors were 1098 inoculated with increasing MOI of CHIKV-Ross, CHIKV-Brazza, CHIKV-H20235, and 1099 infection was guantified 24hpi by flow cytometry using the anti-E2 3E4 mAb. Data 1100 shown are mean +/- SEM (3 experiments, n=6; two-way ANOVA with Dunnett's 1101 multiple comparisons test). e, Immunoblot of ectopic FHL1 expression in patient primary fibroblast (PF2 and PF4) cells stably transduced with an empty vector or a 1102 1103 plasmid encoding HA-FHL1A. One representative of two experiments is shown. **** 1104 P< 0.0001; ns not significant.

1105

1106 Extended Data Fig.9. Mouse FHL1 interacts with CHIKV nsP3 and restores 1107 infection in Δ FHL1 cells

a, Sequence alignment of murine and human FHL1A proteins. b, 293Tcells were co transfected with FLAG-tagged CHIKV nsP3 and plasmids encoding HA-tagged mFHL1
 or hFHL1A. Cellular lysates were subject to immunoprecipitation with anti-HA followed

1111 by immunoblot analysis with anti-FLAG (nsP3) and anti-HA (FHL1). c, Immunoblot of FHL1 ectopic expression in \triangle FHL1 293T stably transduced with plasmid encoding 1112 1113 murine FHL1 (mFHL1) or human FHL1A (hFHL1A). d, Cells showed in c were 1114 inoculated with increasing MOI of CHIKV21. Infection was quantified by flow cytometry 1115 at 24 hpi using anti-E2 3E4 mAb. Data shown are mean +/-SD (3 experiments, n=6; 1116 two-way ANOVA with Dunnett's multiple comparisons test). e, (left panel) Immunoblot 1117 of endogenous FHL1 in control and \triangle FHL1 C2C12 murine cells. (right panel) Control 1118 and \triangle FHL1 cells were inoculated with CHIKV21 or MAYV (MOI of 2) and infection was 1119 quantified at 24hpi by flow cytometry using anti-E2 3E4 or anti-E2 265 mAb. One representative of three experiments is shown. ***P<0.001; ****P<0.0001; ns not 1120 1121 significant.



BeWo HepG2







b







С

Tanaka *et al.* Loss-of-function genetic screen in HAP1 human haploid cells This study CRISPR/Cas9 genetic screen in HAP1 human haploid cells



in 3T3 mouse fibroblast

Ranked Genes	MAGeCK rank	RIGER Rank
FHL1	1	1
SLC35B2	2	2
PAPSS1	3	3
ELAVL1	4	4
FURIN	5	7
YBX1	6	6
GATAD1	7	5
B3GAT3	8	9
OR10W1	9	217
MTRNR2L5	11	18
COA5	12	436
POMT2	13	641
ELP5	14	47
PRIM2	16	337
NRCAM	17	238
PDE8B	18	78
EXT1	19	26
ELF2	20	10
MYT1L	21	381
CUL5	22	956
MAP4K3	23	37
ALDH18A1	24	107
ACTR10	25	13544
SLC7A6OS	26	2704
C11orf30	27	11
BID	28	8
SMOC1	30	507
SCGB1D1	32	84
RPE65	33	75
FAM124B	34	459



d

sgRNA1 PAM sgControl CCTGTGCAACAAGTGCACCACTCGGGAGGACTCCCCCAAGTGCAAGGGGTGCTTCAAGGCCATTGTGGCAGGTACTGCCTCC FHL1 sgRNA1 CCTGTGCAACAAGTGC-----CATTGTGGCAGGTACTGCCTCC



g

















b

b



■ BeWo + vector

∆ BeWo + FHL1A

а

d







Extended Figure 5







С

f



d

b













CHIKV(06-21) CHIKV(RQSS) CHIKV(120235) CHIKV(37997) ONNV SFV MAYV SINV VEEV EEEV	325 RSSQESAQEASTITSLTH:SQFDLGVOGETLEVPSDLDADAP 325 RSSQESVREVSHITSLTH:SQFDLGVOGETLEVPSDLDADAP 326 RSSQESVREVSHITSLTH:SQFDLGADACP 327 RSSQESVREVSHITSLTH:SQFDLGVOGETLEVPSDLDADAP 328 RSQESVREVSHITSLTH:SQFDLGVOGETLEVPSDLDADAP 329 RSADETLITTQ:DTFLGCUQADLUGSINDEAVPVPSDLEACDAT 326 RAPAELTITTO:DTFLGCUQADLUGSINDEAVPVPSDLEACDAT 325 RSADETT-VSLSS 325 LSTSHETT-VSLSS 326 LEVPEOPTAPPAQAEEA 327 LEVPEOPTAPPAQAEEA 328 NEVTEPVOETPEPSARA 329 ASTTUEDETRITTS-LEVEOPTAPPAQAEEA	LEP	DOGATHTLPSTTGNLAAVSDWVIS DOGATHTLPSTTGNLAAVSDWVIS DOGATHTLPSTTGNLAAVSDWVMS DOGATHTTGNLAAVSDWMS GOSMLSISSEPAQNUTRPTDNFSAVSDWVM GGSMLSISSEPAQNUTRPTDOS DOSDTSTSV EPAGTADLAQVHPEPADHVDL DPOSM-QNTSVEPV DPOSMSDPOSSLETVOR-RQVVADVHAVQE SVSSSWSIS PHASOFDVOSLSTLOTLEGASVTSGATSAETNSYF VDQVSLWSI	TVPVAPPRRR 405 TVPVAPPRRR 405 TVPVAPPRRR 405 TVPVAPPRRR 405 TAPVAPPRRR 405 TAPVAPPRRR 405 TAPVAPPRRR 405 TAPVAPPRRR 405 TAPVAPPRRR 405 TAPVAPPRRR 405 TAPVAPPRRK 303 TAPVAPPRRK 428 TAPVAPPRRK 405 TAPVAPPRRK 405 TAPVAPPRRK 405 TAPVAPPRRK 405 TAPVAPPRRK 405 TAPVAPPRRK 405 TAPVAPPRRK 405
	R1	R2	R3	
	423	R4 454		
CHIKV(06-21)	406RGRNLTVTCDEREGNIT PMAS VRFF	RAELCPVVQETAETRDTAMSLQAPPSTATEPNHPPISFGASSE	T-FPITFGDFNEGEIESLSSELLTFGDFLPGEVDDLTDSDWSTCSDTDDEL	523
CHIKV(ROSS)	406RGRNLTVTCDEREGNIT PMAS VRFF	RAELCPVVQETAETRDTAMSLQAPPSTATELSHPPISFGAPSE	T-FPITFGDFNEGEIESLSSELLTFGDFLPGEVDDLTDSDWSTCSDTDDEL	523
CH1KV(H20235)	402RGRNLIVICDEREGNIIPMASVRFF	RAEQCPTVQETAETRDTATSFRAPPSITVELSHPPISFGAPSE		519
CHIKV(3/99/)	400RGNNLINVICDEREGNVLIPHASVHFF			523
	456LGRTINVICDEREGRIL			(AGG 569
	410RARLAS		A_VDTEFCAPTFEFSE_TTFCDFSASEWETTSNSS	482
STNV	429KMARI AAARKEPTPPASNSSESI HISEG	GVSMSI GSTEDGETAROAAVOPI ATGPT	D-VPMSEGSESDGETDELSRRVTESEPVLEGSEEPGEVNSTTSSRSAVSEPLRKORRRP	SRRTFY 549
VEEV	466 FRNPPHPAPRTRTPSLAPSRACSRTSLVSTPPGVNRVITRE	ELEALTPSRTPSRSVSRT-SLVSNPPG	VNRVITREEFEAFVA000	550
EEEV	447 APSPPSQDSRPSTPSASGSHTSVDLITFDSVAEILEDFSRSPF	QFLSEIKPIPAPRTRVNNMSRSADTIKPIPKPRKCQ-VKYTQPPG	VARVISAAEFDEFVRRHSN	552
	Bin1 binding motif G3BP1/2 bindin; G3BP1/2 noncat FHL1 binding region G3BP1/2 noncat binding-site	g motifs CD2AP binding motif ionical FXR binding-site		





С

b



d



Extended Figure 7



C276Y

b









FHL1-A
Control LIM1 LIM2 LIM3 LIM4

FHL1-AX281E P2 _____LIM1 | LIM2 | LIM3 | LIM4 |

P3 LIM1 LIM2 LIM3 LIM4

FHL1-A Del2bp P1 _____ LIM1 | LIM2 | _____

FHL1-A













Human FHL1A	MAEKFDCHYCRDPLQGKKYVQKDGHHCCLKCFDKFCANTCVECR	44
Murine FHL1	MASQRHSGPSSYKVGTMSEKFDCHYCRDPLQGKKYVQKDGRHCCLKCFDKFCANTCVDCR	60
Human FHL1A	KPIGADSKEVHYKNRFWHDTCFRCAKCLHPLANETFVAKDNKILCNKCTTREDSPKCKGC	104
Murine FHL1	KPISADAKEVHYKNRYWHDNCFRCAKCLHPLASETFVSKDGKILCNKCATREDSPRCKGC	120
Human FHL1A	FKAIVAGDQNVEYKGTVWHKDCFTCSNCKQVIGTGSFFPKGEDFYCVTCHETKFAKHCVK	164
Murine FHL1	FKAIVAGDQNVEYKGTVWHKDCFTCSNCKQVIGTGSFFPKGEDFYCVTCHETKFAKHCVK	180
Human FHL1A	CNKAITSGGITYQDQPWHA <mark>D</mark> CFVCVTCSKKLAGQRFTAVEDQYYCVDCYKNFVAKKCAGC	224
Murine FHL1	CNKAITSGGITYQDQPWHA <mark>E</mark> CFVCVTCSKKLAGQRFTAVEDQYYCVDCYKNFVAKKCAGC	240
Human FHL1A	KNPITGFGKGSSVVAYEGQSWHDYCFHCKKCSVNLANKRFVFH <mark>Q</mark> EQVYCPDCAKKL	280
Murine FHL1	KNPITGFGKGSSVVAYEGQSWHDYCFHCKKCSVNLANKRFVFH <mark>N</mark> EQVYCPDCAKKL	297

d

b





С



е

