1 **Supplementary Information**
2
3 **Rhinovirus inhibits IL-17A and the downstream immune responses in allergic asthma**

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38 **Supplementary Methods**

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40 **Human study**

41

42 In this study we analysed healthy control children and children with asthma at the age of 4 - 6
43 years. They were participants within the Europe-wide study PreDicta (Post-infectious immune
44 reprogramming and its association with persistence and chronicity of respiratory allergic
45 diseases). This is a multi-centre prospective cohort study carried out in five different centres
46 in Europe across major cultural and climatic regions. The Department of Molecular
47 Pneumology, in collaboration with the Paediatric Pneumology-Allergology, Department of
48 Paediatrics and Adolescent Medicine, Universitätsklinikum Erlangen, Erlangen, Germany is
49 one of these study centres. The underlying hypothesis of PreDicta is that repeated infections
50 may reprogram the immune responses towards a chronic inflammation pattern that translates
51 into disease persistence. Therefore, the host immune responses *in vivo* and *ex vivo* should be
52 evaluated. To this aim, we and the other study centres established and followed up a cohort of
53 pre-school children with asthma for two years. At the baseline visit and at the end of the
54 study, whole blood should be drawn from enrolled children for the isolation of PBMCs.
55 Furthermore, at these two visits also a nasopharyngeal specimen is to be collected in order to
56 determine viral infections. The data which are shown here are based on the baseline visit.

57

58 **Nasopharyngeal fluid collection with Swab and Rhinovirus detection**

59

60 For the detection of the Rhinovirus in the upper airways, a nasopharyngeal specimen from
61 control children and asthmatic children was collected using a per-nasal applicator swab,
62 which has a tip with flocked soft nylon fiber (E-Swab 482CE, Copan, Italy). Swabs were
63 passed through the nostrils until resistance was felt and they were slowly rotated for 5 seconds
64 to allow for mucus absorption. In addition, swabs were also rotated against the mucosa of the
65 anterior nares before exiting the nose. The nylon tip was eluted by turning into the E-Swab´s
66 medium. The nasopharyngeal fluid was then stored at -80°C until further analysis (see
67 **Supplemental Figure S1b**). Rhinovirus detection was performed at the Department of
68 Virology, University of Turku (Finland). Therefore, nucleic acids were extracted using
69 easyMag extractor (BioMeriex, Marcy l'Etoile, France) from 200 µl of the medium according
70 to manufacturer's instruction. An *in house* PCR method was used to detect enteroviruses,
71 rhinoviruses and respiratory syncytial viruses as described earlier[1]. A commercial test kit
72 (Anyplex™ II RV16 Detection, Seegene, Soul, Korea) was used to detect 16 respiratory
73 viruses (adenovirus; influenza A and B viruses; parainfluenza virus 1, 2, 3, and 4; rhinovirus
74 A, B and C; respiratory syncytial virus A and B; bocavirus 1,2,3,4; coronavirus 229E, NL63
75 and OC43; metapneumovirus and enteroviruses)

76

77 **RNA isolation from whole blood**

78

79 Whole blood from each child of the two cohorts was collected into Tempus® Blood RNA
80 Tubes (life Technologies, Carlsbad, CA, USA) and stored at - 80° C. RNA was isolated with
81 MagMAX™ for Stabilized Blood Tubes RNA Isolation Kit (ambion®, life Technologies,
82 Carlsbad, CA, USA) according to the manufacturer`s instructions. Summarized, RNA was
83 isolated and purified using RNA binding beads.

84

85 **Isolation of human Peripheral Blood Mononuclear cells PBMCs**

86

87 Whole blood was collected into 10 ml venous blood collection tubes with lithium heparin.
88 Afterwards, the heparinized blood was transferred to a sterile 15 ml tube and diluted with an
89 equal volume of warm (room temperature) PBS and inverted. After that, the diluted blood was
90 carefully overlaid on top of Ficoll-Hypaque. After density gradient centrifugation, the layer of
91 peripheral blood mononuclear cells (PBMCs), which was found between plasma and Ficoll,
92 was aspirated and transferred to a new sterile 15 ml tube. After washing the cells twice with
93 RPMI 1640 medium, the cells were used for cell culture (see **Supplemental Figure S1c**).

94

95 **Culture of human Peripheral Blood Mononuclear cells PBMCs**

96

97 After isolation, PBMCs were adjusted to a concentration of 1×10^6 viable cells / ml in
98 complete culture medium. Cells were grown in RPMI 1640 medium supplemented with
99 HEPES 25 mM and L-Glutamine (GIBCO, Invitrogen, Darmstadt, Germany) . Furthermore,
100 100 IU/ml Penicillin (Sigma-Aldrich, Steinheim, Germany), 100 µg/ml Streptomycin (Sigma-
101 Aldrich, Steinheim, Germany), β-mercaptoethanol (50 µM; Sigma-Aldrich, Steinheim,
102 Germany), 1 % L-Glutamine 200 mM (Sigma-Aldrich, Steinheim, Germany), 1 % MEM
103 Vitamin (Sigma-Aldrich, Steinheim, Germany), 1% Non Essential aminoacids (Sigma-
104 Aldrich, Steinheim, Germany), 1 % Sodium Pyruvate (Sigma-Aldrich, Steinheim, Germany)
105 and 10 % HI-FBS (Sigma-Aldrich, Steinheim, Germany) were added (complete culture
106 medium). PBMCs were cultured for 24 hours with or for 48 hours without αCD3 (2 µg/ml)

107 and αCD28 (2 µg/ml) antibodies at 37°C and 5 % CO_2. Afer 24 hours,, supernatants were
108 carefully removed and stored at - 80°C until further investigations via ELISA as described
109 below. PBMCs, which were cultured for 48 hours, were diluted in QUIazol® Lysis Reagent
110 (Quiagen Sciences, Maryland, USA) and stored at – 80°C until RNA isolation was performed.

111

112 **Culture of human lung epithelial A549 cells**

113

114 A549 lung epithelial cells were infected with RV1b as described below. Afterwards, cells
115 were re-suspended in F12K-NUT Mix (Gibco®, life technologies, Carlsbad, CA, USA)
116 culture medium supplemented with 10 % fetal calf serum, 100 IU/ml Penicillin, 1 % L-
117 glutamine and 100 µg/ml Streptomycin (1 x10^6cells / ml) and transferred into a culture plate.
118 Subsequently, different doses of recombinant human IL-17A (Peprotech, Rocky Hill, NJ,
119 USA) were added. Cells were cultured for 24 hours at 37°C and 5 % CO_2. Hereafter, cells
120 were harvested for further analysis.

121

122 **Silencing of OAS1 by using siRNA**

123

124 A549 cells were transfected with OAS1 siRNA (Dharmacon™ ON-TARGETplus SMART
125 pool, Dharmacon™, Thermo Fisher Scientific Biosciences GmbH, St. Leon-Rot, Germany)
126 according to the manufacturer`s instructions. Therefore, cells were first cultured for 24 hours
127 (37°C and 5% CO_2) in culture medium without antibiotics. Afterwards, cells were transfected
128 using DharmaFECT™ Transfection Reagents and cultured for further 24 hours (37°C and 5%
129 CO_2). Thereafter, cells were infected with RV1b or UV irradiated RV1b and cultured for 24

130 hours in the presence or absence of different concentrations of rhIL-17A (Peprotech, Rocky
131 Hill, NJ, USA). At the end of the experiment, cells were lysed with PeqGold for following
132 RNA isolation.

133

134 **Mice**

135

136 Il17a $^{(-/-)}$ mice (Yoichiro Iwakura, Center for Experimental Medicine and Systems Biology,
137 The Institute of Medical Science, The University of Tokyo, Tokyo, Japan)[2] and wild-type
138 mice on a Balb/c genetic background were used at the age of 6 to 8 weeks. The mice were
139 bread in the animal facility adjacent to our institute and had free access to food and water.
140 Furthermore, they were maintained under specific pathogen-free conditions with temperature
141 control and all experiments were undertaken with the approved license (54-2532.1-2/10 from
142 the government of Mittelfranken, Bavaria, Germany). One to three independent experiments
143 were performed and one representative experiment or pooled data are shown.

144

145 **OVA sensitization and challenge**

146

147 Mixed gender of Il17a $^{(-/-)}$ and wild-type mice received intraperitoneal (i.p.) injections either
148 of PBS or of 500 µg/ml ovalbumin (OVA; Calbiochem, Gibbstown, NJ) complexed with
149 10 % alum (Sigma-Aldrich, Steinheim, Germany) on days 0 and 7 as described previously[3, 4].
150 On days 18, 19 and 20 the animals were treated intranasally (i.n.) with PBS alone or OVA in
151 PBS (2 mg OVA / ml PBS in solution). Mice were sacrificed either on day 20 after the

152 invasive plathysmography or on day 21. Afterwards, bronchoalveolar lavage fluids (BALF),
153 the whole lung and draining lymph nodes were isolated as described below .

154

155 **Airway hyperresponsiveness (AHR)**

156

157 To measure airway reactivity an invasive method was used on day 20 of the protocol. The
158 invasive plethysmography was performed with a FlexiVent FX1 device (SCIREQ Scientific
159 Repiratory Equipment) using the FlexiWare 7.2.2 software at least 2 hours after the last
160 challenge with OVA. The mice were pentobarbital-anesthetized and breathed spontaneously.
161 After invasive plethysmography, bronchoalveolar lavage of the right lung was performed.

162

163 **Collection and analysis of BALF**

164

165 BALF of the right lung was performed by using 0.8 ml saline twice. The collected BALF was
166 centrifuged at 1500 rpm for 5 min at 4°C. Supernatants were carefully removed and frozen
167 until further analysis by ELISA. Cell pellets were re-suspended in 1 ml PBS and cells were
168 counted by using trypan blue solution and a Neubauer chamber. Subsequently, cells were used
169 to detect eosinophils and neutrophils via fluorescence-activated cell sorting analysis as
170 described below.

171

172 **Histological analysis of murine lung sections**

173

174 Murine lungs were removed, fixed in 10 % formalin-PBS solution, dehydrated and embedded
175 in paraffin. 5 µm thick sections were stained with Periodic acid-Schiff (PAS) for
176 quantification of mucosal cells.

177

178 **Isolation of lung CD4$^+$ T cells**

179

180 Lungs from Il17a $^{(-/-)}$ and wild-type mice were removed to isolate total cells as previously
181 described[5]. Purification of CD4$^+$ T cells was based on positive cell sorting by using magnetic
182 beads (MACS, Miltenyi Biotec, Bergisch-Gladbach, Germany) according to the
183 manufacturer`s protocol. The purity of the isolated cells was verified using flow cytometric
184 analysis as described below. Lung CD4$^+$ T cells were on the one hand diluted in PeqGold
185 RNA Pure™ to isolate RNA for subsequent qPCR analysis or for Gene Array analysis. On the
186 other hand, they were infected with RV1b and cultured for 24 h. Afterwards, cells were
187 collected to isolate RNA.

188

189 **Gene Array**

190

191 The gene array was performed at the Core facility of the Institute of Human Genetics in
192 Erlangen. Briefly, RNA from lung CD4$^+$ T cells from asthmatic Il17a $^{(-/-)}$ and wild-type mice
193 was isolated as described above and the RNA underwent quality control on Experion™ (Bio-
194 Rad Laboratories, München, Germany) before hybridization on the GeneChip® Gene 1.0 ST
195 Array (Affymetrix, Santa Clara, USA) as previously described[6].

196

197 **Isolation of total cells from draining lymph nodes**

198

199 Draining lymph nodes from Il17a $^{(-/-)}$ and wild-type mice were removed and transferred to a
200 15 ml tube containing RPMI 1640 medium. Afterwards, the lymph nodes were transferred and
201 pushed through a 40 µm sieve. The sieve was then washed with RPMI 1640 medium. After
202 that, the cell suspension was centrifuged (5 min, 1500 rpm, 4 °C), supernatants were removed
203 and the cells were re-suspended in RPMI 1640 medium and counted using trypan blue
204 solution and a Neubauer chamber. These cells were used for RNA isolation as described
205 below.

206

207 **Infection of lung epithelial cells A549 and murine lung CD4$^+$ T cells with RV1b**

208

209 A549 cells and lung CD4$^+$ T cells were infected with Rhinovirus1b (RV1b) suspension, UV
210 irradiated RV1b or control medium. Specifically, the cells were shook for 1 hour at RT with
211 RV1b suspension (500 µl RV1b / 1x10^6 cells). RV1b was grown as previously described [7] and
212 provided to us by National and Kapodistrian University of Athens (Athens, Greece). After
213 that, medium was added to wash the cells followed by a centrifugation step (300g, 15min,
214 RT). The supernatant was carefully removed and the cells were re-suspended in culture
215 medium (1 x 10^6 cells/ml). Afterwards, the cells were cultured for 24 hours.

216

217 **Cell culture of murine cells**

218

219 Cells were adjusted to a concentration of 1 x 10^6 cells/ml and cultured in RPMI 1640 medium
220 supplemented with 10 % fetal calf serum, 100 IU/ml Penicillin, 1 % L-glutamine and 100 µg/
221 ml Streptomycin for 24 hours at 37 °C and 5 % CO_2. Supernatants were carefully removed
222 and cells were used for RNA isolation.

223

224 **RNA isolation, quantitative real-time PCR and Rhinovirus PCR**

225

226 Total RNA of human PBMCs was extracted by using QUIazol® Lysis Reagent (Quiagen
227 Sciences, Maryland, USA). To isolate RNA from murine cells, PeqGold RNA Pure™
228 (PeqLab, Erlangen, Germany) was utilized. We used for both lysis reagents the same protocol
229 in order to isolate RNA. Cells were lysed by adding PeqGold RNA Pure™ or QUIazol® Lysis
230 Reagent to the cells. Afterwards, chloroform was added to the homogenized samples followed
231 by an incubation phase at 4 °C. After centrifugation (5 min at 12000 x g, 4 °C) the upper
232 aqueous phase was removed and mixed with chloroform. The samples were centrifuged again
233 (5 min, 12000 x g, 4 °C) and the resulting upper aqueous phase was removed and isopropyl
234 alcohol and glycogen (10 mg/ml) were added. After incubation for 15 min at 4 °C, samples
235 were centrifuged (10 min, 12000 x g, 4 °C) and supernatants were removed. RNA pellets
236 were washed with 70 % ethanol twice. After a final centrifugation step (5 min, 12000 x g,
237 4 °C), the excess of ethanol was removed from the RNA pellet by air-drying. Afterwards, the
238 pellet was re-suspended in sterile nuclease free water and the RNA concentration in each
239 sample was determined by using a spectrophotometer (Nanodrop, peqlab, Erlangen,
240 Germany).

241 Total RNA was reverse-transcribed to cDNA using the RevertAid™ First Strand cDNA
242 Synthesis Kit (Fermentas, St. Leon-Rot, Germany) according to the manufacturer`s
243 instructions. The resulting template cDNA was amplified by quantitative real-time PCR
244 (qPCR) using SsoFast EvaGreen Supermix (Bio-Rad Laboratories, München, Germany) and
245 200 nM primers. The qPCR was performed with a cycle of 2 min 98 °C, 50 cycles at 5 sec
246 95 °C, 10 sec 60 °C, followed by 5 sec 65 °C and 5 sec 95 °C in a CFX96 Touch Real-Time
247 PCR Detection System (Bio-Rad Laboratories, Hercules, CA, USA). The primers and
248 sequences used for mouse were as follows: m*Ifnb* (fwd: 5´- CCC TAT GGA GAT GAC GGA
249 GAA G, rev: 5´- GAG CAT CTC TTG GAT GGC AAA), m*Oas1a* (fwd: 5´-TTC CAG CAA
250 GCC TGA TCC CA-3´, rev: 5´- CCC AGC TTC TCC TTA CAC AGT T-3´), m*Oas1g* (fwd:
251 5´- AAT GAT GGT TCC CGA GTG AG-3´, rev: 5´- GGC TGT GAT TGG ACA GGA GT-
252 3´) *mLdlr* (fwd. 5'-TTT GGA GGA TGA GAA CCG GC-3', rev: 5'-TGT CGA CTT CTC
253 TAG GCT GTG-3') and m*Batf* (fwd: 5´-GTT CTG TTT CTC CAG GTC C-3´, rev: 5´-GAA
254 TCG CAT CGC TGC-3´). The mRNA of the genes of interest was normalized using the
255 mRNA levels of the housekeeping gene m*Hprt* (fwd: 5´-GCC CCA AAA TGG TTA AGG
256 TT-3´, rev: 5´-TTG CGC TCA TCT TAG GCT TT-3´). For human analyses the following
257 primers and sequences were used: h*HPRT* (fwd: 5´- TGA CAC TGG CAA AAC AAT GCA-
258 3´, rev: 5´- GGT CCT TTT CAC CAG CAA GCT-3´), hIFNB (fwd: 5´-AGT AGG CGA
259 CAC TGT TCG TG-3´ rev: 5´-AGC CTC CCA TTC AAT TGC CA-3´), h*OAS1* (fwd: 5´-
260 AGC TGG AAG CCT GTC AAA GA-3´, rev: GGT TTA TAG CCG CCA GTC AA-3´) ´)
261 and h*TBX21* (fwd: 5´-CAG AAT GCC GAG ATT ACT CAG-3´, rev: 5´- GGT TGG GTA
262 GGA GAG GAG AG-3´). The primers and sequences were purchased from Eurofins-MWG-
263 Operon (Ebersberg, Germany).

264 To verify that the infection with RV1b was successful a PCR was performed as described
265 previously with some modifications[7, 8]. Therefore, cDNA from infected cells and primers,

266 which are complementary to the antisense RNA at positions 542-557 and 169-185 in the
267 5´noncoding region of RV1b, called *OL26* (5′-GCA CTT CTG TTT CCC C-3′) and *OL27* (5′-
268 CGG ACA CCC AAA GTA G-3′) were used as well as the KAPA2G Fast Ready Mix with
269 dye (Peqlab, Erlangen, Germany). The thermal cycle consisted of 32 cycles with 94°C for 30
270 sec, 50°C for 30 sec and 72°C for 2 min so that a 380-bp amplicon was generated. The
271 samples were analyzed and quantified using the QIAxcel Advanced System (Qiagen GmbH,
272 Hilden, Germany). RV1b was also analysed by using qPCR. Therefore, the following primers
273 were used: RV1b (fwd: 5´- CCA TCG CTC ACT ATT CAG CAC-3´, rev: 5´- TCT ATC
274 CCG AAC ACA CTG TCC-3´)[9]. The mRNA was normalized to *Hprt* expression.

275

276 **ELISA**

277

278 Mouse IL-4, IL-5, IFN-g and total IgE were detected by using OptEIA™ sandwich ELISA
279 kits from BD Bioscience (Heidelberg, Germany). Mouse IL-13 and IL-17A as well as human
280 IL-17A was detected by using a Duoset™ sandwich ELISA kit from R&D Systems
281 (Wiesbaden, Germany).

282

283 **Flow cytometric analysis**

284

285 For surface staining cells were incubated with the particular antibodies for 30 min at 4 °C.
286 Lung CD4$^+$ T cells were stained with anti-CD4-PE (BD Pharming, Heidelberg, Germany) to
287 verify the purity of the isolation. To analyse BAL cells, antibodies against Cd3 (Fitc labeled,
288 eBioscience, Frankfurt, Germany), Ly-6G (PE labeled, BD Bioscience, Heidelberg,

289 Germany), CD45R (PeCy5.5 labeled, eBioscience, Frankfurt, Germany) and CCR-3 (APC-
290 labeled, BD Bioscience, Heidelberg, Germany) were used. To stain IL-13 producing $CD4^+$ T
291 cells, whole lung cells were cultured in RPMI medium at a concentration of 1×10^6 cells / ml
292 over night with OVA (500 µg/ml). On the following day, cells were stimulated with PMA
293 (1 ng/ml, Sigma-Aldrich,Steinheim, Germany), Ionomycin (1 mM, Sigma-Aldrich, Steinheim,
294 Germany) and GolgiStop (BD Bioscience, Wiesbaden, Germany). After centrifugation the
295 cells were incubated with antibodies against Cd4 (Percp conjugated, BD Pharming,
296 Heidelberg, Germany) for 30 min at 4 °C: Subsequently, the cells were fixed with
297 fixation/permeabilization solution (eBioscience, Frankfurt, Germany) for 35 min at 4 °C and
298 then washed with permeabilization buffer (eBioscience, Frankfurt,Germany). Hereafter, the
299 cells were incubated with antibody against IL-13 conjugated with PE (eBioscience, Frankfurt,
300 Germany) for 30 min at 4 °C in permeabilization buffer and washed once. The samples were
301 acquired by using a FACS-Calibur and analysed with FlowJo.
302

303 **Statistical Analysis**

304

305 Differences were evaluated for significance (* $p \leq 0.05$; ** $p \leq 0.01$, *** $p \leq 0.001$) by using
306 1-way ANOVA or the Student-two-tailed t test for independent events (Excel, Microsoft,
307 version 2003; Microsoft, Redmond, Wash), as indicated in the figure legends. Data are given
308 as mean values ± SEM.

309

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359

360 **Supplementary Figure Legends**

361

362 **Supplementary Figure S1: Determination of Rhinovirus in the Nasal pharyngeal fluid**
363 **and isolation of Peripheral Blood Mononuclear Cells (PBMC) from whole blood**

364 (**a**) Experimental protocol of sample collection within the PreDicta study (**b**) Collection of
365 Nasal pharyngeal Swab samples from control children and asthmatic children and
366 determination of Rhinovirus. (**c**) Isolation of PBMCs from whole blood samples of healthy
367 and asthmatic children. (**d** and **e**) mRNA expression of *IFNB* (**d**) and *TBX21* (**e**) after
368 culturing PBMCs for 48 hours. (**f**) Detection of IL-17A in the supernatants of PBMCs after
369 cell culture for 24 hours in presence of αCD3 and αCD28 antibodies. (**g**) *OAS1* gene
370 expression in PBMCs after cell culture for 48 hours. (n = 8-22)

371

372 **Supplementary Figure S2: IL-17A induces *OAS1* and inhibits *RV1b* mRNA expression**
373 **in RV infected A549 cells**

374 A549 cells were first transfected with OAS1 or non-targeting (NT) siRNA and afterwards
375 infected with RV1b or UV-RV1b. The infected cells were cultured for 24 h with increasing
376 doses (0 / 6 / 12.5 / 25 ng/ml) of rhIL17A (n=3 per group). Data are given as mean values ±
377 SEM. * $p \leq 0.05$, *** $p \leq 0.001$ (Student t test).

378

379 **Supplementary Figure S3: Up-regulation of inflammatory markers in wild-type and**
380 **Il17a $^{(-/-)}$ mice**

381

382 (a) Concentration of IL-17A in SN of lung $CD4^{+}$ T cells (n=3 per group) (b) AHR was
383 measured by using invasive plethysmography in naive and asthmatic mice (n=3). (c-f)
384 Analysis of IL-4 (**c**, n= 14-21), IFN-g (**d**, n=12-20), IL-5 (**e**, n=3-8) and IL-13 (**f**, n=3-5) in
385 the BALF of naïve and OVA-sensitized wild-type and Il17a $^{(-/-)}$ mice (**g**) (**h**) * $p \leq 0.05$, ** $p \leq$
386 0.01, *** $p \leq 0.001$ (a-f: ANOVA).

387

388 **Supplementary Figure S4: Decreased Neutrophilia and mucus production in the airways**
389 **of OVA-sensitized Il17a $^{(-/-)}$ mice.** (**a**) Detection of eosinophils in BALF via fluorescence-
390 activated cell sorting (n=7-13). (**b**) Detection of neutrophils via fluorescence-activated cell
391 sorting analysis (n=10-17 per group). (c) Periodic acid shiff (PAS) staining of murine lung
392 sections. (n=20-42 bronchi per group). (d) IgE concentration in the serum of wild-type and
393 Il17a deficient mice (n= 11-16). (**e**) *Batf* mRNA expression in lung $CD4^{+}$ T cells of naïve

394 mice and after treatment with OVA (n=3-5). (**d**) Data are given as mean values ± SEM. * $p \leq$

395 0.05, *** $p \leq 0.001$ (a, c, d: ANOVA; b,e: Student t test).

396

397 **Supplementary Figure S5: Inhibition of anti-viral Oas1 genes in murine lung $CD4^+$ T**

398 **cells in Il17a $^{(-/-)}$ mice in asthma**

399 (**a, b**) $CD4^+$ T cells were isolate from total lungs of OVA-sensitized wild-type and Il17a $^{(-/-)}$

400 mice 24 hours after the last allergen challenge. After RNA isolation, a gene array was

401 performed. Heat map (**a**) and graphical representation (**b**) of genes associated with anti-viral

402 response (n=3). (**c**) Whole lung cells were analysed by flow cytometry for IL-13^+ $CD4^+$ cells

403 (n=3). (**d-e**) Real time PCR of *Oas1a* (**d**) and *Oas1g* (**e**) from lung $CD4^+$ T cells isolated from

404 naïve and OVA-sensitized wild-type and Il17a $^{(-/-)}$ mice (n=3-5). (**f-g**) Gene expression of

405 *Oas1g* in total lung (**f**, n=7-8) or lymph nodes (**g**, n=3-4) of naïve and asthmatic wt and

406 Il17a $^{(-/-)}$ mice. *Ifnb* mRNA expression in lung $CD4^+$ T cells of wt and Il17a $^{(-/-)}$ mice (n=3).

407 * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$ (ANOVA).

408

409 **Supplementary Figure S6: Successful infection of lung $CD4^+$ T cells with RV1b**

410 Detection of viral infection using PCR analysis and primers, which are complementary to the

411 antisense RNA at positions 542-557 and 169-185 in the 5´noncoding region of RV1b.

412

413 **Supplementary Tables**

414

415 **Supplementary Table S1: Clinical data of the two cohorts of children analysed in this study**

416

group / patient	age	gender	asthma severity	asthma phenotype	asthma treatment	asthma control
controls						
C1	6	male	-	-	-	-
C2	5	male	-	-	-	-
C3	5	female	-	-	-	-
C4	6	male	-	-	-	-
C5	6	male	-	-	-	-
C6	6	male	-	-	-	-
C7	4	male	-	-	-	-
C8	4	male	-	-	-	-
C9	5	female	-	-	-	-
C10	4	male	-	-	-	-
C11	4	female	-	-	-	-
C12	4	male	-	-	-	-
C13	4	male	-	-	-	-
C14	5	female	-	-	-	-
C15	5	female	-	-	-	-
C16	4	male	-	-	-	-
C17	4	male	-	-	-	-
Mean	4,8					
SEM	0,2					
cases						
A1	4	female	mild persistent	unresolved	steroid + non-steroid	partially controlled
A2	6	male	mild persistent	allergen	steroid + non-steroid	controlled
A3	4	male	intermittent	unresolved	steroid + non-steroid	partially controlled
A4	5	male	intermittent	allergen / virus	steroid + non-steroid	partially controlled
A5	4	female	n.a.	allergen / virus	steroid	partially controlled
A6	6	female	intermittent	virus	non-steroid	partially controlled
A7	5	male	mild persistent	virus / exercise	steroid + non-steroid	controlled
A8	5	female	moderate persistent	allergen / virus	steroid	uncontrolled
A9	6	female	intermittent	allergen / virus / exercise	steroid	controlled
A10	5	male	intermittent	virus	-	controlled
A11	4	male	intermittent	virus	steroid + non-steroid	partially controlled
A12	5	male	mild persistent	unresolved	steroid	partially controlled
A13	5	female	intermittent	unresolved	steroid	partially controlled
A14	4	male	moderate persistent	exercise	steroid + non-steroid	partially controlled
A15	4	male	intermittent	virus	steroid + non-steroid	controlled
A16	4	male	intermittent	virus	non-steroid	controlled
A17	5	male	intermittent	virus	-	controlled
A18	4	male	intermittent	virus	steroid + non-steroid	controlled
A19	5	female	intermittent	exercise	non-steroid	controlled
A20	5	female	mild persistent	virus	steroid + non-steroid	uncontrolled
Mean	4,8					
SEM	0,2					

417

418

419 **Supplementary Table S2**: FEV1 and respiratory infections in the 12 months before
420 recruitment of the two cohorts of children analysed in this study

group / patient	FEV1 [%]	RV detection in NPF	upper respiratory infections during last 12 months [number]	lower respiratory infections during last 12 months [number]
controls				
C1	77	-	3	0
C2	118	-	2	0
C3	84	-	5	0
C4	105	-	1	0
C5	87	-	1	0
C6	111	-	4	0
C7	109	-	7	0
C8	121	-	0	0
C9	121	+	5	0
C10	110	+	5-6	0
C11	111	+	2	1
C12	n.a.	+	3	0
C13	100	+	3	0
C14	112	+	4	0
C15	119	+	3	0
C16	113	+	2	0
C17	123	+	10	0
Mean	107,6			
SEM	3,5			
cases				
A1	95	-	n.a.	0
A2	128	-	4	0
A3	102	-	5	0
A4	143	-	2	10
A5	115	-	7-8	1
A6	98	-	10	2
A7	96	-	12	1
A8	92	-	5	0
A9	111	-	4	0
A10	88	-	2-3	3
A11	71	-	6	0
A12	n.a.	+	5	5
A13	129	+	2	0
A14	115	+	4	1
A15	99	+	3	0
A16	87	+	10	1
A17	101	+	4	0
A18	77	+	6	3
A19	98	+	1	0
A20	69	+	4-6	6
Mean	100,7			
SEM	4,5			

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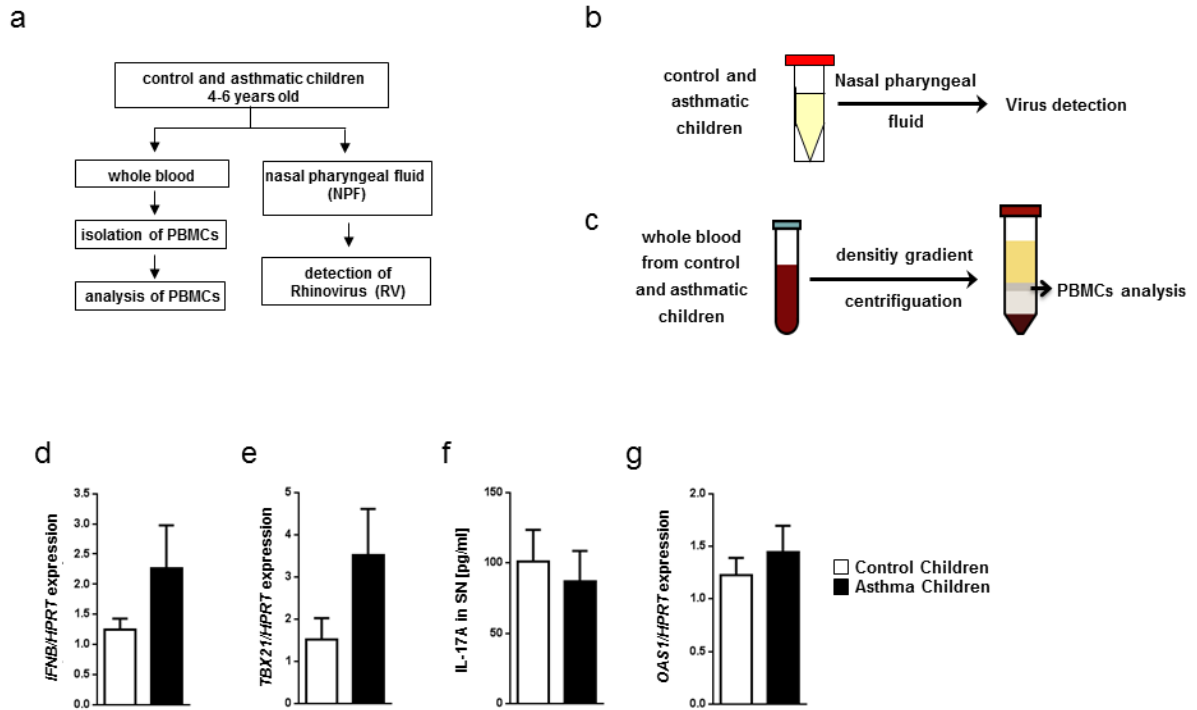
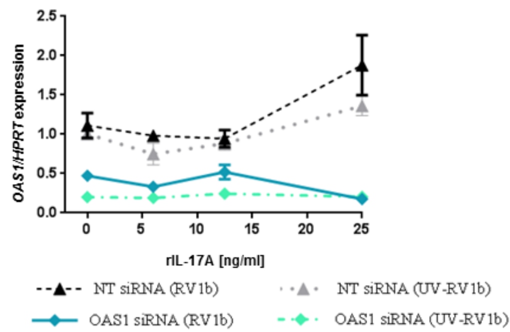
422



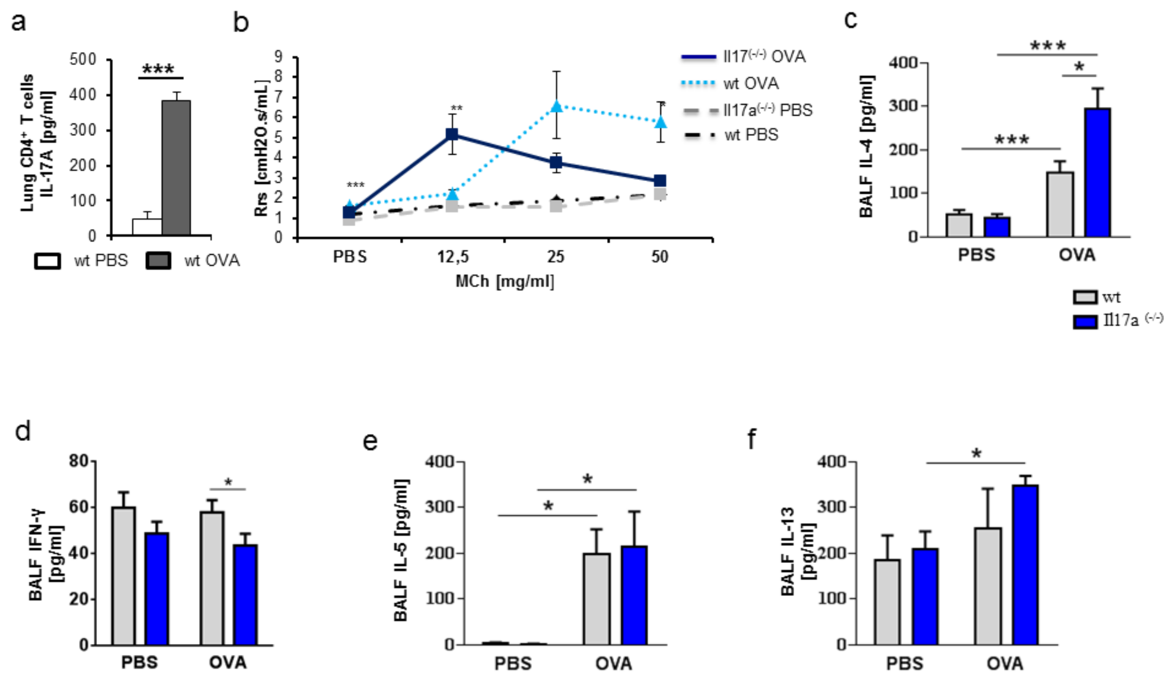
423 Supplementary Figure S1

424



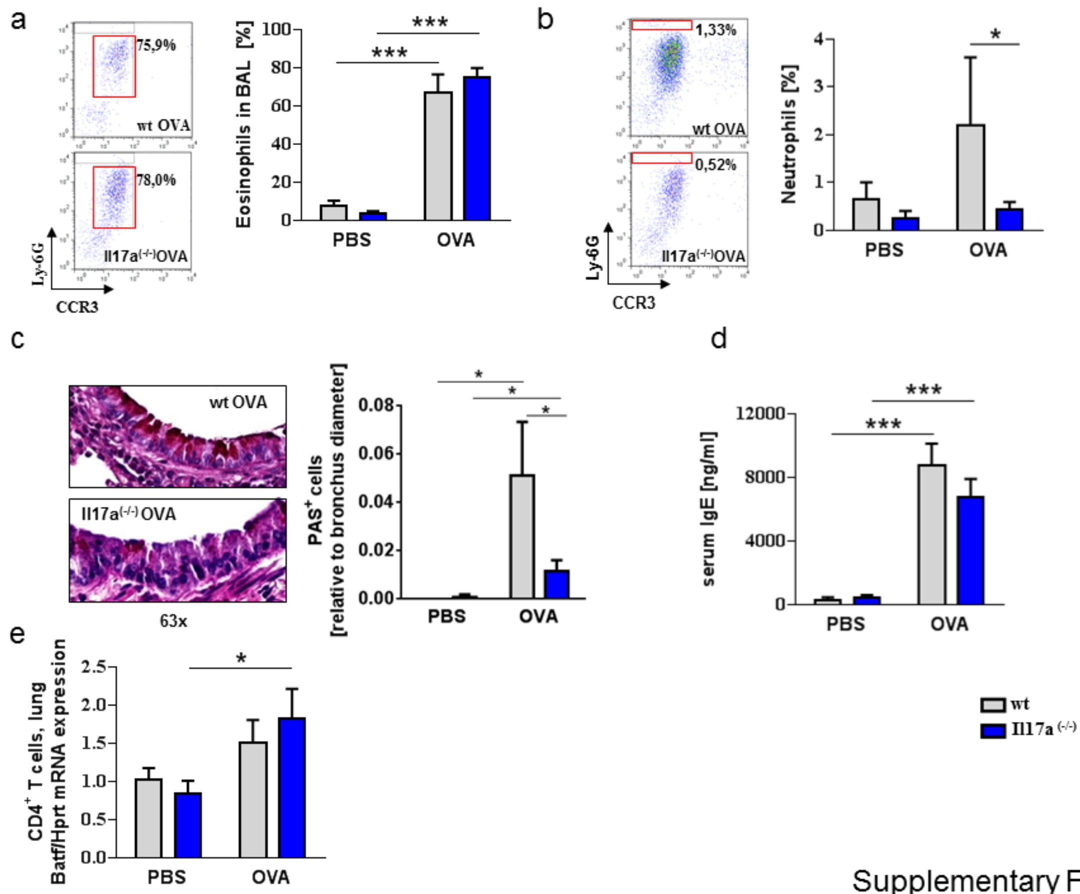
425 Supplementary Figure S2

426



427 Supplementary Figure S3

428



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Supplementary Figure S4

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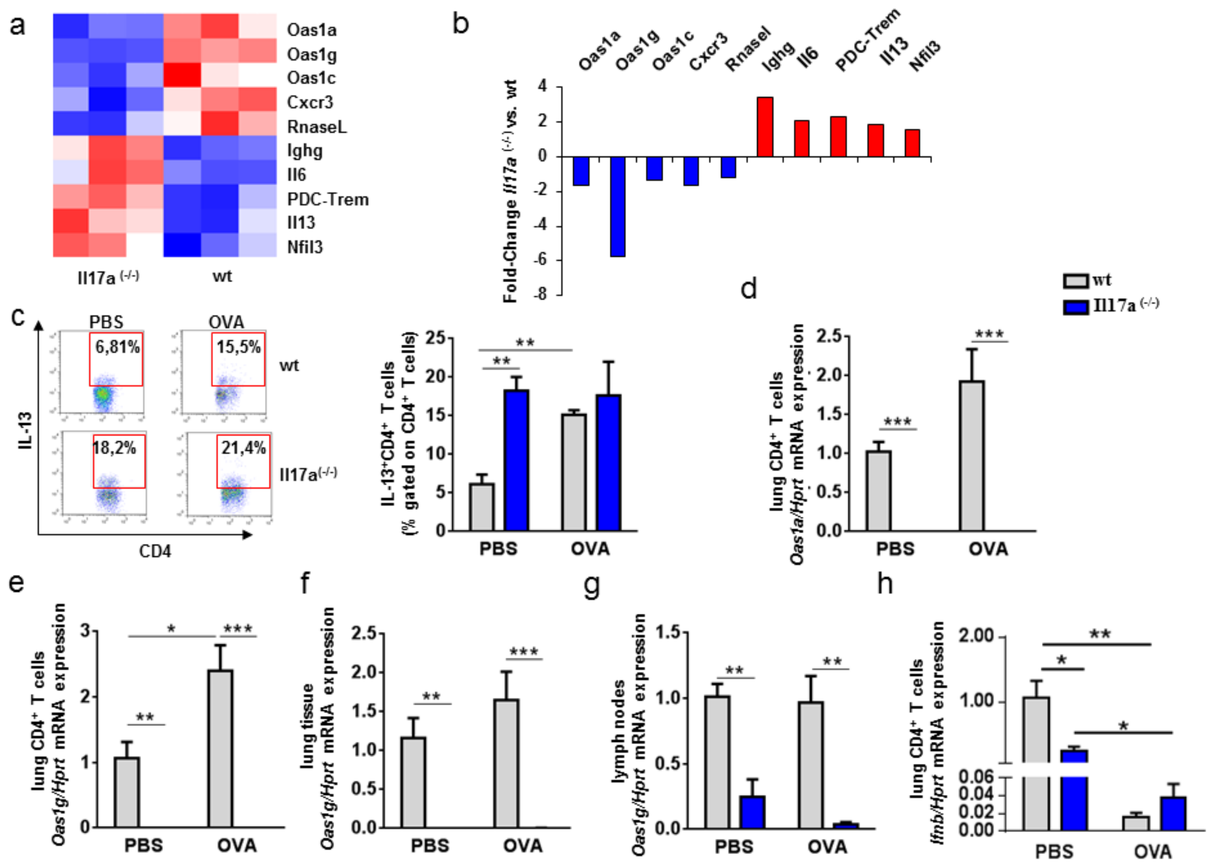
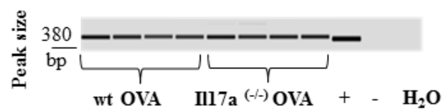
431



Supplementary Figure S5

432



Supplementary Figure S6