

Rhinovirus C15 Induces Airway Hyperresponsiveness Via Calcium Mobilization in Airway Smooth Muscle

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Online Supplemental Data File

Table S1 – Donor Demographics		
hPCLS studies		
<u>Age</u>	<u>Gender</u>	<u>Ethnicity</u>
49	F	Caucasian
20	F	Caucasian
27	M	American Indian or Alaskan Native
38	F	Caucasian
38	M	Caucasian
38	F	Caucasian
HASM/HAEC		
<u>Age</u>	<u>Gender</u>	<u>Ethnicity</u>
28	M	Hispanic
17	F	Caucasian
26	M	African American
22	M	Caucasian
14	M	Caucasian
16	M	Caucasian

Table S1 – Demographics of lung donors from which hPCLS, HAEC, and HASM were derived.

Table S2 – Mediator analysis of Pro-contractile Sensitizers			
<u>Inflammatory Mediator</u>	<u>Donor</u>	<u>Detected at baseline</u>	<u>Detected following RV-C15 infection</u>
TNFα	1	Y	Y (0.72 fold compared to baseline)
	2	N	N
	3	N	N
	4	N	N
IL-33	1	N	N
	2	N	N
	3	N	N
	4	N	N
IL-1β	1	N	N
	2	N	N
	3	N	N
	4	N	N
TGFβ	1	N	N
	2	N	N
	3	N	N
	4	N	N
IL-13	1	N	N
	2	N	N
	3	N	N
	4	N	N

Table S2 – Mediator analysis of “pro-contractile sensitizers” release following RV-C15 stimulation of hPCLS or HAEC.

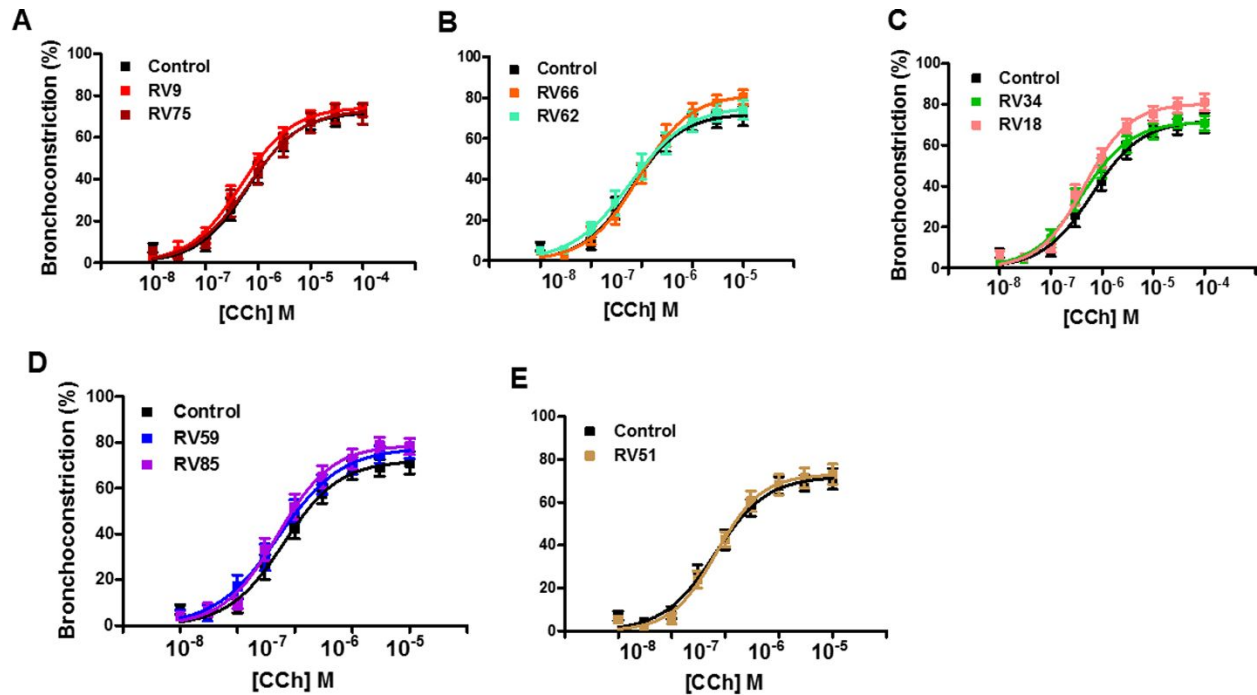


Figure S1 – Exposure of human small airways to RVA strains does not significantly increase airway reactivity to carbachol. hPCLS were infected with RV-A strains (75, 9, 18, 34, 59, 85, 66, 62, or 51; 10^5 Pfu for 48 hr). The slices were subjected to a carbachol dose response (10^{-8} – 10^{-4} M), then bronchoconstriction was plotted as sigmoidal dose response curves of % constriction versus increasing concentration of carbachol (n=8 separate lung donors with 3 slices/condition for each donor). Two-tailed unpaired t-tests comparing each condition to control buffer were performed, as well as a two way ANOVA.

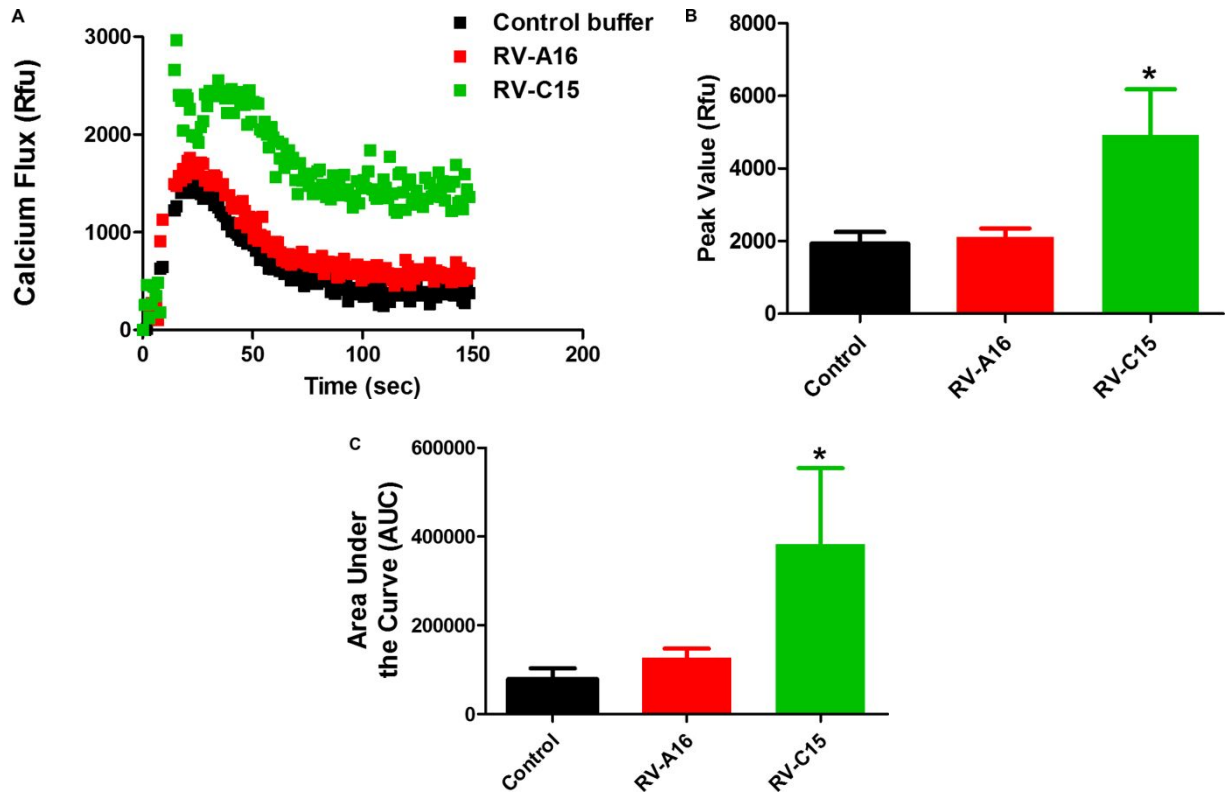


Figure S2 – ALI-differentiated HAEC exposed to RVC, but not RVA, induce a marked increase in $[Ca^{2+}]_i$ in co-cultured HASM. HAEC were exposed to RVA (10^6 Pfu, 48 hr) co-cultured with HASM. (A) $[Ca^{2+}]_i$ flux to Cch was measured using Fluo-8 over 120 sec. (B) Peak $[Ca^{2+}]_i$ and (C) area under the curve were calculated for triplicate wells for 4 separate HASM cell donors and plotted as mean \pm standard error. Two-tailed paired t-tests comparing each condition to control buffer were performed, as was two way ANOVA.

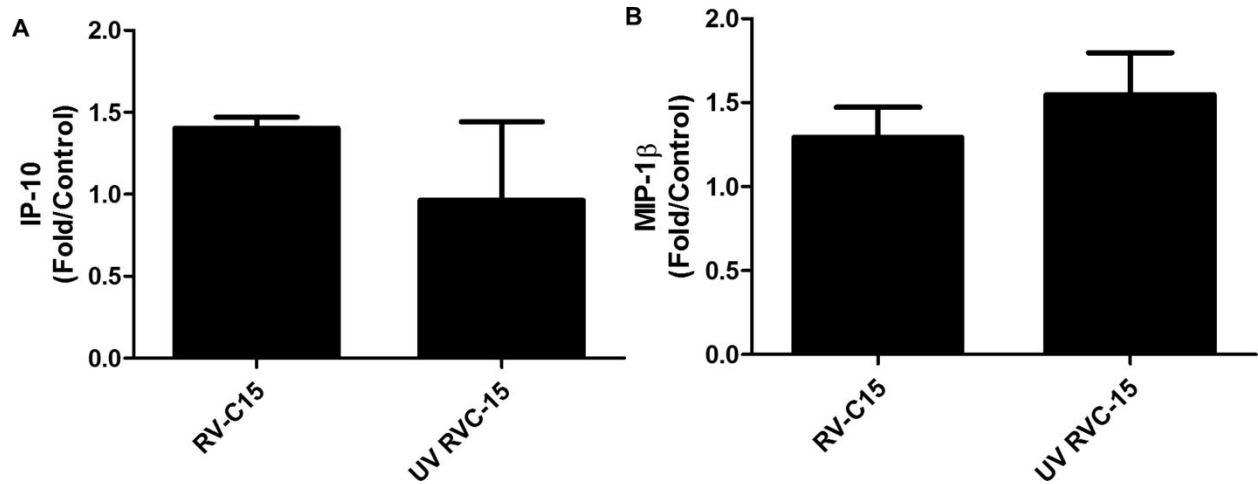


Figure S3 – Exposure of ALI-differentiated HAEC to RV-C15, or UV inactivated RV-C15, induce similar levels of mediator release. Fold induction of IP-10 (A) or MIP1 β (B) release relative to control buffer stimulation was compared following exposure to either RV-C15 or UV inactivated RV-C15 (10^6 Pfu, 48 hr). n=3 donors with data plotted as mean \pm standard error. No statistical significance between RVC treatments was observed. Two-tailed paired t-tests comparing each condition to control buffer were performed, as was two way ANOVA.

