

Supplemental Information

HpARI Protein Secreted by a Helminth Parasite

Suppresses Interleukin-33

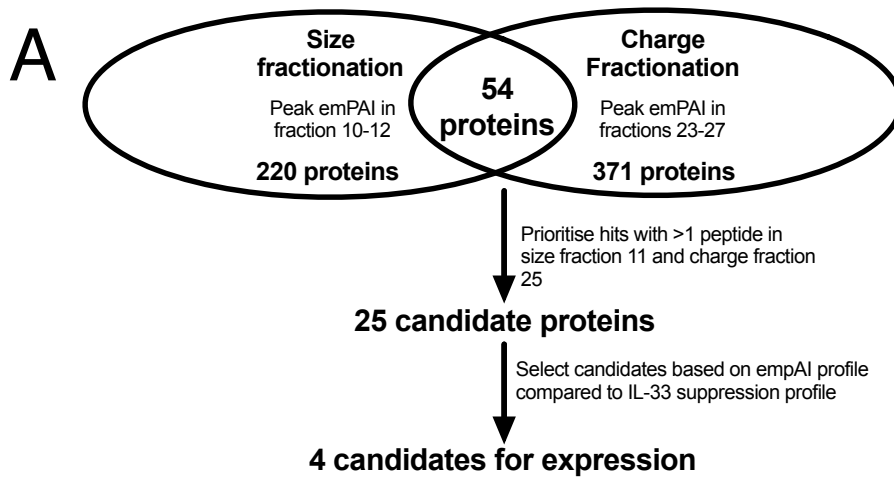
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	Blast hit	Size fraction 11			Charge fraction 25		
		emPAI	Mascot protein score	Rank (of 194) ^a	emPAI	Mascot protein score	Rank (of 262) ^a
Hp_I10793_IG03481_L623	No BLASTX similarities 13330478:13331100 forward	1.35	99	57	0.53	104	77
Hp_I15874_IG07818_L1106	BLASTX similarity to 66.67% ID to CBN-NUC-1 protein [Caenorhabditis brenneri] (376 aa, Accession: gi 341901954 gb EGT57889.1)	1.11	229	19	5.23	786	2
Hp_I46029_IG37973_L313	BLASTX similarity to 55.93% ID to unnamed protein product [Homo sapiens] (304 aa, Accession: gi 189069304 dbj BAG36336.1)	2.01	67	90	0.73	50	163
Hp_I08176_IG02172_L1157	BLASTX similarity to 72.38% ID to PHA domain [Heligmosomoides polygyrus bakeri] (252 aa, Accession: gi 345499002 emb CCC54333.1)	0.13	61	100	1.05	353	18
GSXTT4C07IB13H_length=463	BLASTX similarity to 86.32% ID to venom allergen/ ancylostoma secreted protein-like 1 isoform 4 [Heligmosomoides polygyrus bakeri] (459 aa, Accession: gi 348659354 gb AEP82914.1)	0.53	59	105	2.58	246	1
Hp_I00796_IG00050_L1763	BLASTX similarity to 60.51% ID to metalloprotease 1 precursor [Ancylostoma ceylanicum] (547 aa, Accession: gi 23268453 gb AAN11401.1)	0.37	163	32	0.78	518	3
Hp_I03365_IG00388_L986	BLASTX similarity to 83.05% ID to putative ES protein F7 [Ostertagia ostertagi] (181 aa, Accession: gi 18104159 emb CAD20464.1)	0.2	73	80	3.37	408	14
Hp_I05355_IG00918_L1570	BLASTX similarity to 61.84% ID to astacin-like metalloprotease [Haemonchus contortus] (502 aa, Accession: gi 82653303 emb CAJ43810.1)	0.51	205	20	0.34	137	20
Hp_I07496_IG01832_L2183	BLASTX similarity to 49.47% ID to metalloprotease I [Ostertagia ostertagi] (573 aa, Accession: gi 25005280 emb CAD28559.2)	0.23	184	24	0.52	300	26
Hp_I09193_IG02681_L996	BLASTX similarity to 27.72% ID to Chain A, Caclcium-Bound Ac-Asp-7 (206 aa, Accession: gi 383875397 pdb 3S6U A)	0.2	78	72	1.28	312	24
Hp_I09338_IG02753_L893	BLASTX similarity to 69.57% ID to Chain A, Glutathione Transferase-2, Apo Form, From The Nematode Heligmosomoides Polygyrus (206 aa, Accession: gi 51247756 pdb 1TW9 A)	0.36	81	60	0.23	90	59
Hp_I12757_IG04701_L2227	BLASTX similarity to 69.27% ID to hypothetical protein CAEBREN_19315 [Caenorhabditis brenneri] (713 aa, Accession: gi 341886485 gb EGT42420.1)	0.9	321	9	0.29	119	67
Hp_I12915_IG04859_L2071	BLASTX similarity to 74.56% ID to hypothetical protein CAEBREN_01953 [Caenorhabditis brenneri] (594 aa, Accession: gi 341889762 gb EGT45697.1)	0.25	146	39	0.43	190	39
Hp_I13075_IG05019_L1949	BLASTX similarity to 25.30% ID to GD14343 [Drosophila simulans] (699 aa, Accession: gi 195589672 ref XP_002084573.1)	0.71	317	10	1.15	478	8
Hp_I13426_IG05370_L1731	BLASTX similarity to 61.51% ID to hexokinase [Haemonchus contortus] (485 aa, Accession: gi 4583627 emb CAB40412.1)	1.51	564	4	0.81	409	13
Hp_I13832_IG05776_L1555	BLASTX similarity to 59.25% ID to metalloprotease III [Ostertagia ostertagi] (507 aa, Accession: gi 21425408 emb CAD19995.2)	0.6	152	38	0.9	340	20
Hp_I14648_IG06592_L1319	No BLASTX similarities 18271778:18273096 forward	1.67	280	15	0.75	180	41
Hp_I14766_IG06710_L1294	BLASTX similarity to 42.09% ID to Hyaluronidase-1, partial [Ascaris suum] (439 aa, Accession: gi 324516157 gb ADY46439.1)	0.15	57	109	0.42	96	86
Hp_I15012_IG06956_L1244	BLASTX similarity to 71.51% ID to hypothetical protein CRE_02222 [Caenorhabditis remanei] (368 aa, Accession: gi 308509410 ref XP_003116888.1)	0.56	167	21	0.25	138	57
Hp_I15720_IG07664_L1128	BLASTX similarity to 83.79% ID to hypothetical protein CRE_30062 [Caenorhabditis remanei] (425 aa, Accession: gi 308473183 ref XP_003098817.1)	0.5	180	26	0.28	88	99
Hp_I15761_IG07705_L1121	BLASTX similarity to 78.44% ID to hypothetical protein CAEBREN_23086 [Caenorhabditis brenneri] (322 aa, Accession: gi 341889746 gb EGT45681.1)	0.18	41	144	1.47	368	16
Hp_I16083_IG08027_L1071	BLASTX similarity to 28.20% ID to Complement factor H [Ascaris suum] (1358 aa, Accession: gi 324499597 gb ADY39830.1)	0.41	72	85	0.19	42	186
Hp_I28418_IG20362_L498	BLASTX similarity to 45.24% ID to hypothetical protein CRE_02231 [Caenorhabditis remanei] (147 aa, Accession: gi 308510374 ref XP_003117370.1)	0.43	42	141	0.43	67	122
Hpb-APY-2	No BLASTX analysis performed; (File Apyrase 124-3 (iso 07051) 081110) 1062 nt	5.9	654	2	0.3	58	137
Hpb-GST-2	No BLASTX analysis performed; partial (no N-terminus) AF128959	1.07	96	60	0.79	138	59

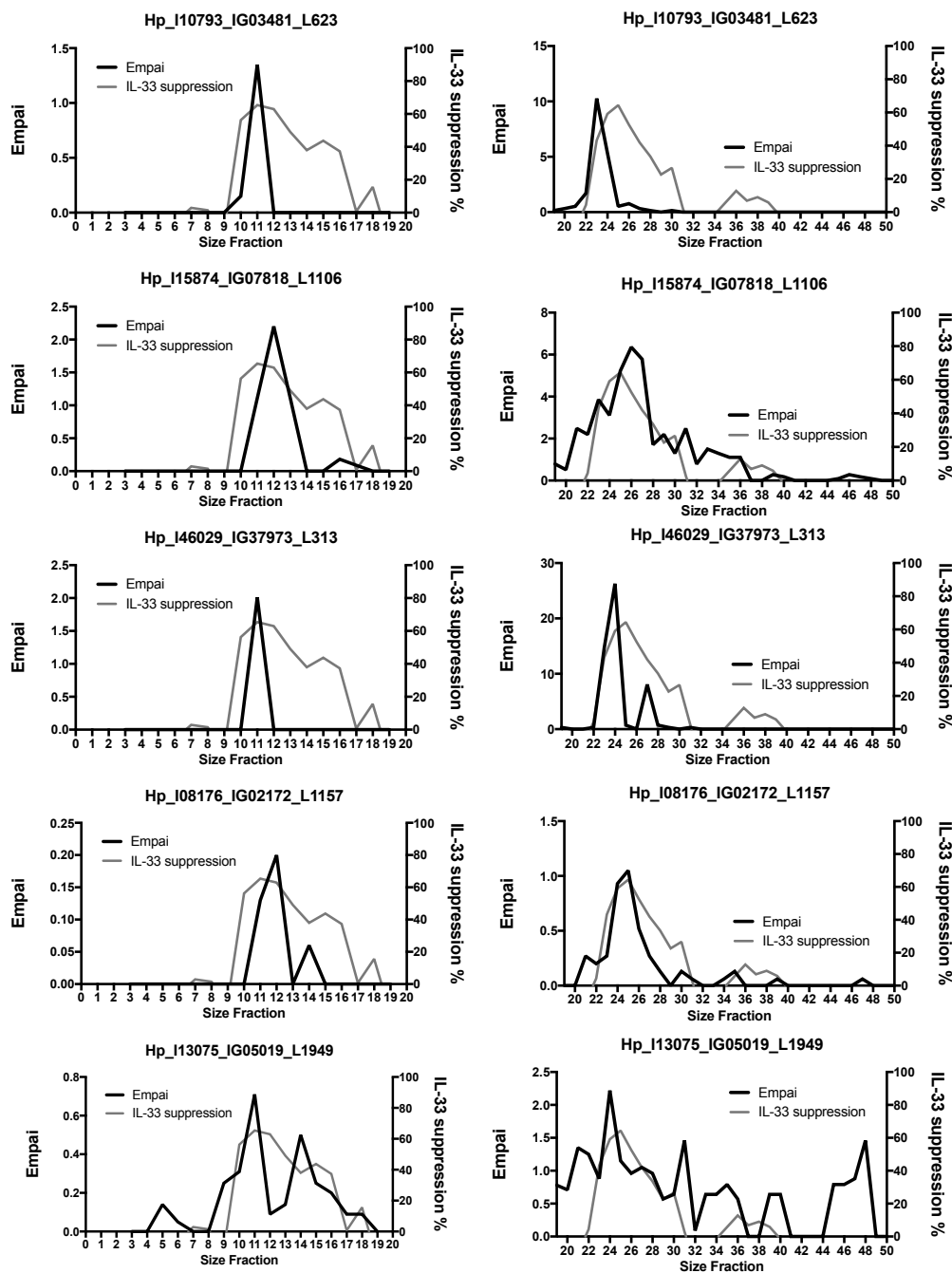
a) Rank of 194 proteins in size fraction 11 or 262 candidates in charge fraction 25 determined by Mascot score, with a minimum score of 20

Supplementary Table 1 (related to Figure 1):

Candidate genes with emPAI peaking in size fractions 10-12 and charge fraction 23-27. Data shown for peak IL-33 suppressive fraction (size = 11, charge = 25).



B



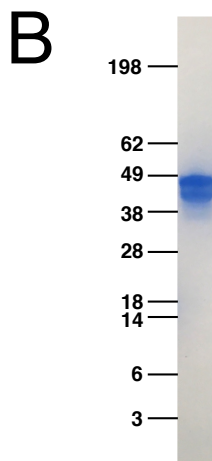
Supplementary Figure 1 (Related to Figure 1):

A. Candidate protein selection strategy

B. Candidate protein Empai profile and IL-33 suppression profile in HES size (left panels) and charge (right panels) fractions. The 4 candidate proteins tested in Fig 2A are shown followed by a candidate not selected for further testing (Hp_I13075_IG05019_L1949), for comparison.

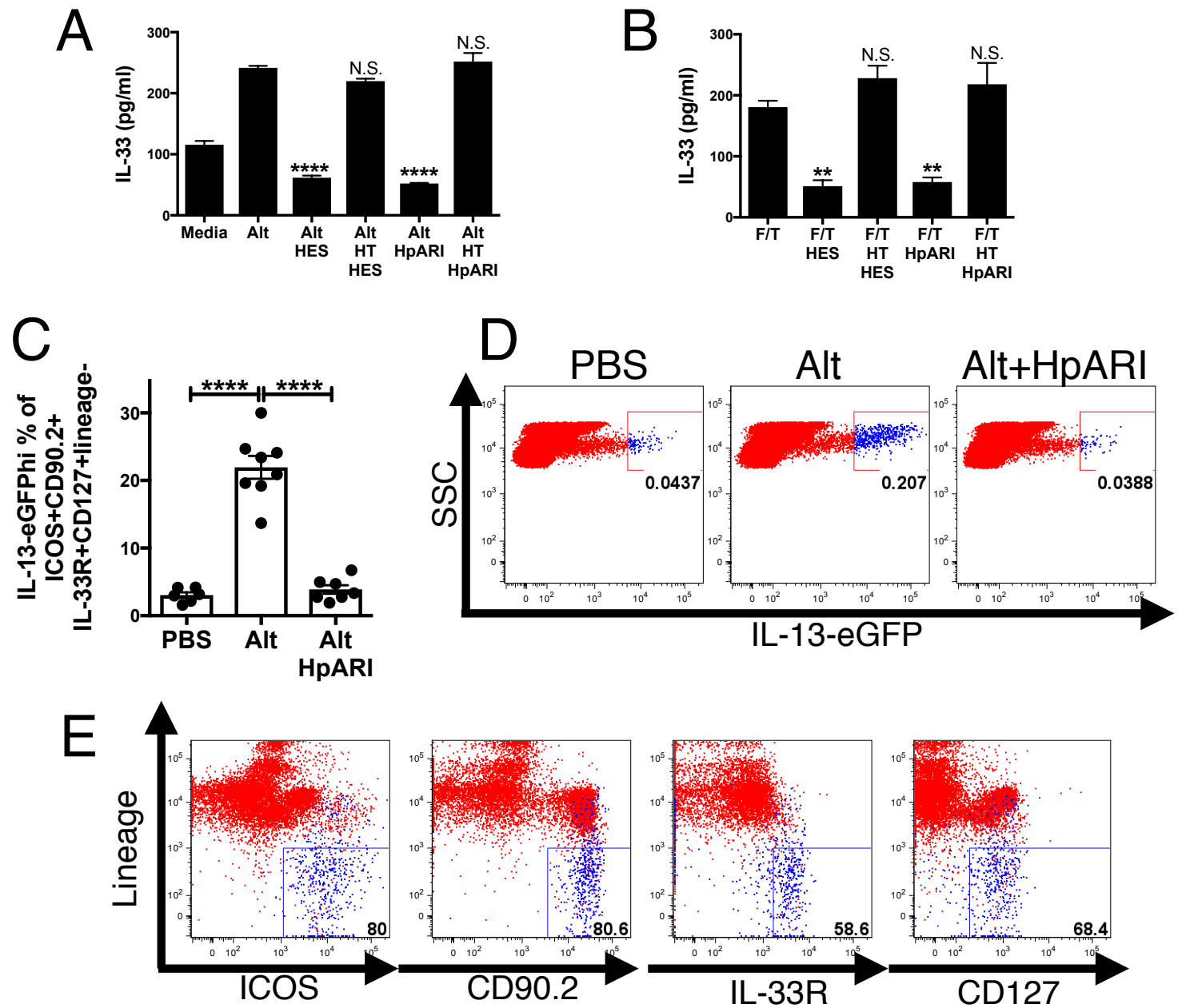
A

0	atgtatcgcccttttctcgcttaggcttcctcacctcatcaacgctgcaggtcagcgc	60
0	M Y R L F L A L G F L T F I N A A G O R	20
61	tgcagattcacccgatgtagaaagagacaaaggctacactggaatgctccttaaggaagc	120
21	C R F T D V E R D K G Y T G M L L K G R	40
121	ttaagaaagacagctggaaacgggaggacggtagaattgatatgtggaagaggaaatcac	180
41	L R K T A G N G R T V E L I C G R G N H	60
181	aactacacctgcgagtcggcgcttttgaaggaaagtctccacggcaagcccgatgtggc	240
61	N Y T C E S G V L K E S S P R O A R C G	80
241	tgcaaggaattctcgaatgctcttcgatatgcctaaggaagaacggccctcgccctag	300
81	C K G I L E M L F D M P K E E R P S P M	100
301	tacgactcagtcacctatgaccccacgccaacaccccaacaactgtaggaaaggacggc	360
101	Y D S V T Y D P T P N T P T T V G K D G	120
361	atatggaacgggtagattaccgcaatggctctacagtgaagccttattgcgatactggc	420
121	I W N G V D Y R N G S T V K P Y C D T G	140
421	ccagtcatcaacggatcatcaaagctgtatgtgtgagtggaatgggttcctacgctt	480
141	P V I N G S S K A V C V S G K W V P T L	160
481	ggcgtctgcccaaaaatgtgttcgatcggcagcttgaaggaaaatgggaaattcgtagac	540
161	G V C P K M C S I G S L K E N G K F V D	180
541	gtcacagcaacaactaaaggagacgagctcaacccccgccaaggaacagacattgatt	600
181	V T A T T K G D E L N P P P R E Q T L I	200
601	cctatagtgcgtaaagttgacaaagacaaggtccaacacggcgtcaaagttgttgctctt	660
201	P I V R K V D K D K V O H G V K V V A L	220
661	tgtaaagccgaagattctacaactgccgctgaaggcgtccaggaattcgaatgtgacaac	720
221	C K A E D S T T A A E G V O E F E C D N	240
721	gggaagtggaagccagagcccgtgccctgccctaa	756
241	G K W K P E P V P C P -	251



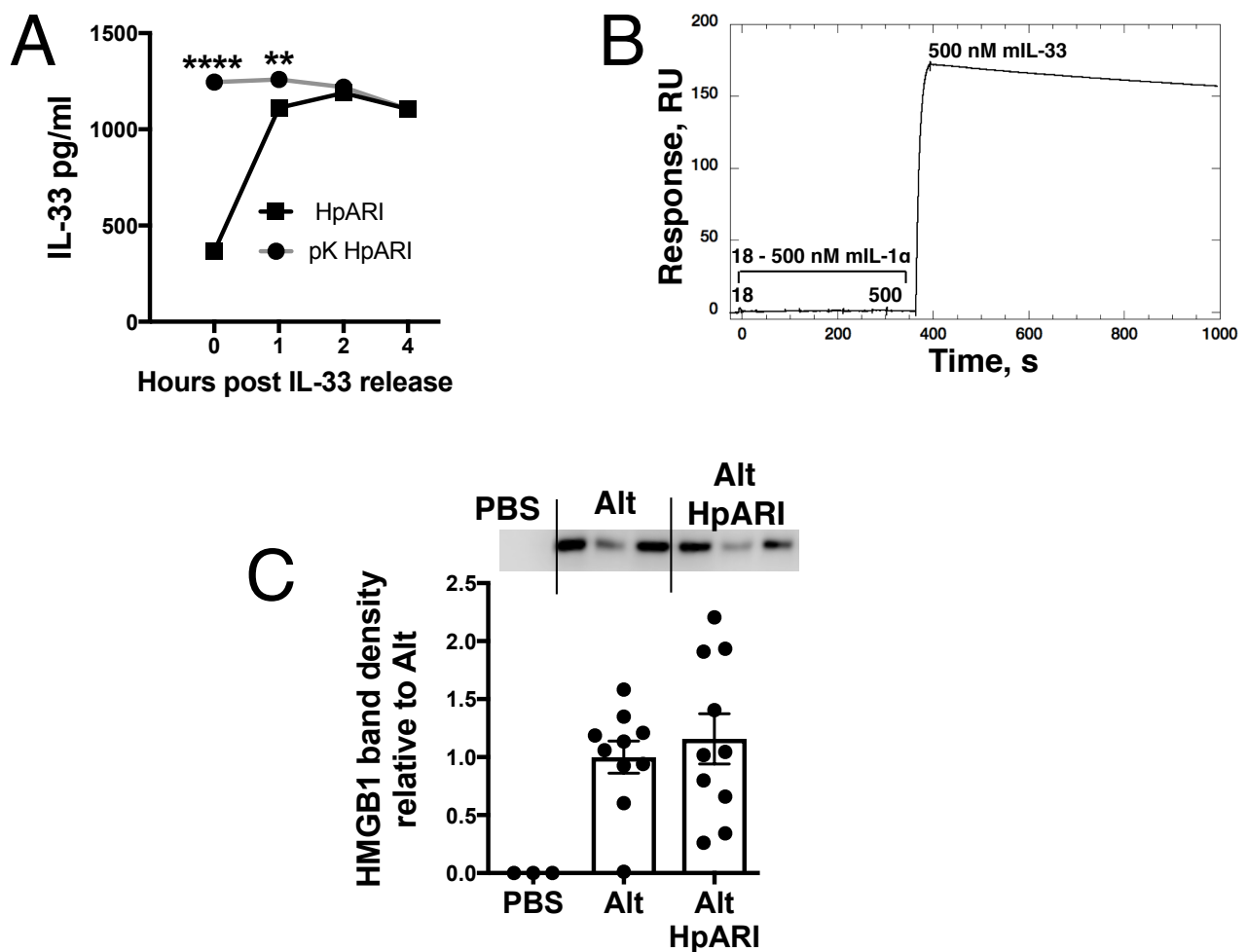
Supplementary Figure 2 (Related to Figure 2):

- A. HpARI cDNA sequence showing intron/exon boundaries (green/yellow highlight) and amino acid domains (coloured boxes/text, red = signal peptide, green = CCP1, salmon = CCP2, pink = CCP3)
- B. Coomassie-stained purified HpARI (2 µg), purified by nickel affinity chromatography.



Supplementary Figure 3 (Related to Figure 3):

- (A) Naive mouse lung cells were cultured in the presence of *Alternaria* (200 $\mu\text{g/ml}$) and 10 $\mu\text{g/ml}$ HES or HpARI for 1 h or heat-treated (95°C for 30 min) HES or HpARI (HT HES or HT HpARI). IL-33 in supernatants was measured by ELISA.
- (B) Naive murine lung cells were subjected to freeze/thaw treatment (F/T) in the presence of HES, HpARI, HT HES or HT HpARI. IL-33 in supernatants was measured by ELISA.
- **** = $p < 0.0001$, ** = $p < 0.01$, N.S. = Not significant ($p > 0.05$), compared to *Alternaria* or freeze/thaw (F/T) control. Standard error of means of 3 replicates, representative of 2-3 repeat experiments.
- (C) IL-13-eGFP^{hi} proportion of ICOS+CD90.2+IL-33R+CD127+lineage⁻ ILC2s in the lung, 24 h after intranasal administration of *Alternaria* allergen and HpARI to IL-13-eGFP reporter mice. Results pooled from 2 repeat experiments. **** = $p < 0.0001$
- (D) Representative FACS plots of IL-13-eGFP in live CD45+ lung lymphocytes from mouse shown in (C)
- (E) Representative FACS plots of IL-13-eGFP^{hi} cells (blue) and total CD45+ live lung lymphocytes (red) from an *Alternaria*-treated IL-13-eGFP reporter mouse.

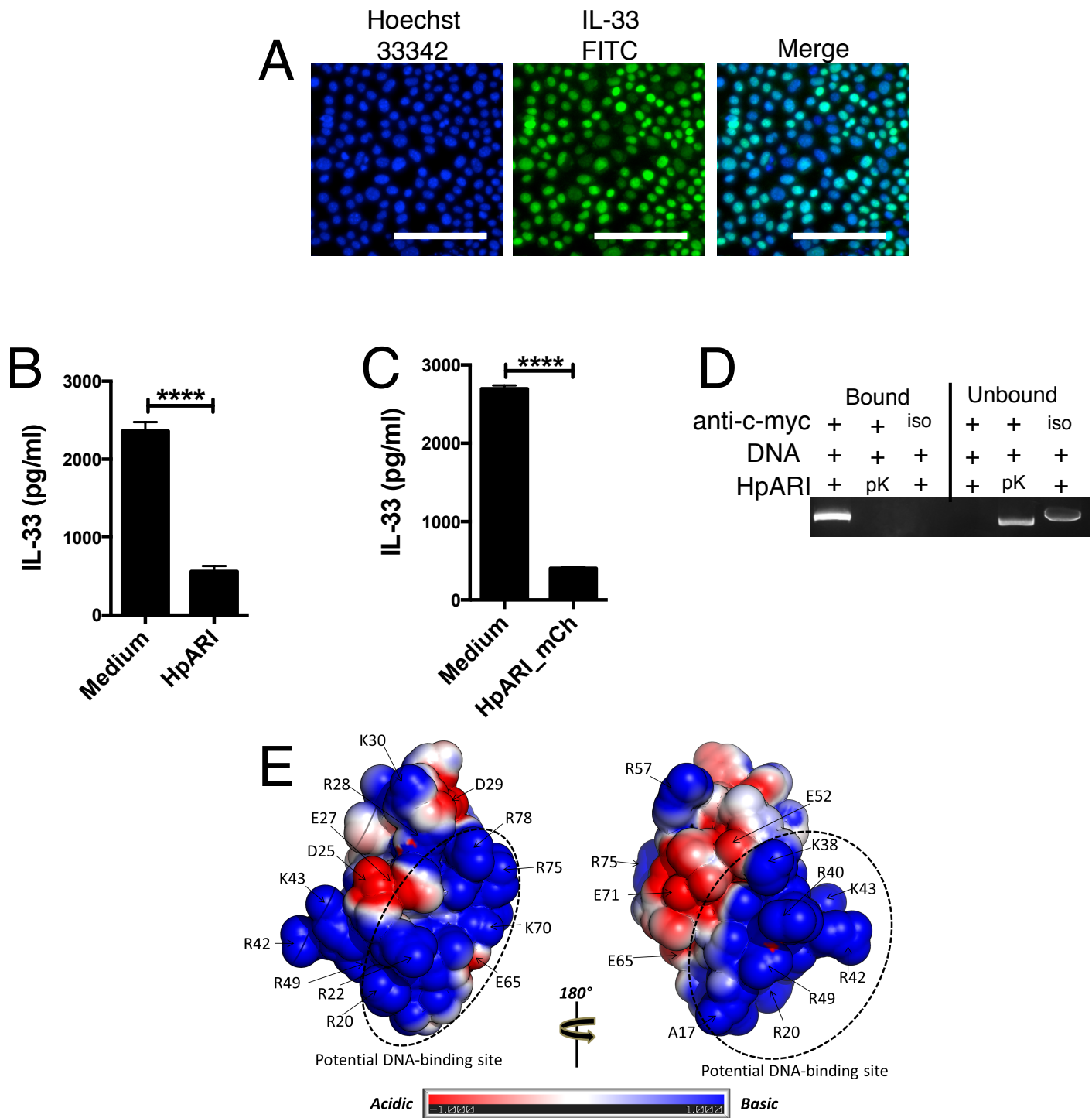


Supplementary Figure 4 (Related to Figure 5):

(A) CMT-64 cell supernatants were taken after freeze-thaw and treated with HpARI or proteinase K-digested and heat-treated HpARI over a timecourse after thaw, as in Figure 5C

(B) Surface plasmon resonance was carried out using mIL-1a or mIL-33 as a ligand for HpARI.

(C) HMGB1 western blots taken 15 min after Alternaria +/- HpARI administration. Pooled from 3 replicate experiments.



Supplementary Figure 5 (Related to Figure 7):

- (A)** CMT-64 cells were fixed and permeabilised, before staining with goat anti-mouse IL-33, followed by rabbit anti-goat IgG FITC secondary and counterstained with Hoechst 33342 nuclear stain. Scale bars indicate 100 μ m.
- (B)** CMT-64 cells were subjected to freeze/thaw necrosis in the presence of 5 μ g/ml HpARI and IL-33 in the supernatant measured by ELISA.
- (C)** CMT-64 cells were subjected to freeze/thaw necrosis in the presence of 5 μ g/ml HpARI_mCherry and IL-33 in the supernatant measured by ELISA. **** = $p < 0.0001$
- (D)** Immunoprecipitation of plasmid DNA by myc-tagged HpARI, but not by proteinase K and heat treated HpARI (pK) nor with isotype control antibody.
- (E)** Two views rotated by 180° along the y-axis depicting the electrostatic surface map of the 3-D model of HpARI CCP1 (calculated using APBS) revealing key residues contributing to the highly basic surface (coloured blue) that may confer DNA-binding properties; acidic surface residues are shown in red. A scaled range of -1kT to +1 kT was used, where k=Boltzman's constant and T= Temperature in Kelvins.