Supplementary Figure Legends

Figure S1

H_v1 amino acid sequence and expression profile.

a) Alignment of orthologous protein translations from human (BC032672), mouse (BC021548), chicken (NM_001030663) and zebrafish (BC075916) mRNAs. Identical residues are boxed; an asterisk indicates amino acids mutated in this study. **b**) Kyte-Doolittle hydropathy plot (window = 8) indicates 4 transmembrane segments designated S1-S4. The y-axis represents hydrophilicity (± 4.5 units) and the x-axis represents the 273 a.a. linear polypeptide sequence. **c)** Diagram of Hv1 protein with putative transmembrane segments in boxes. N and C termini are cytoplasmic. Mutated residues are indicated. Green circles, polar amino acids (N, Q, S, T); red circles, amino acids (H, K, R); orange circles, amino acids (D, E); lavender circles, amino acids (C, G, P); blue circles, amino acids (A, I, L, M, F, W, V, Y). **d**) Hybridization of a ^{33}P-labeled H_v1 RNA probe to a human RNA dot blot indicates strong expression in lymph node (spot F7). **e**) Human RNA dot blot was quantified by measuring the integrated pixel intensity over a fixed area for each spot. X-axis is arranged (left to right) A1-H1, A2-H2, etc. and spot intesnity is plotted as arbitrary units (A.U.) normalized to the intensity of spot F7. Key: A1, whole brain; B1 cerebral cortex; C1 frontal lobe; D1, parietal lobe; E1, occipital lobe; F1, temporal lobe; G1, postcentral gyrus of cerebral cortex; H1, pons; A2, left cerebellum; B2, right cerebellum; C2, corpus callosum; D2, amygdala; E2, caudate nucleus; F2, hippocampus; G2, medulla oblongata; H2, putamen; A3, substantia nigra; B3, nucleus accumbens; C3, thalamus; D3, pituitary; E3, spinal cord; F3-H3, blank; A4, heart; B4, aorta; C4, left atrium; D4, right atrium; E4, left ventricle; F4, right ventricle;

G4, interventricular septum; H4, apex of heart; A5, esophagus, B5, stomach; C5, duodenum; D5, jejunum; E5, ileum; F5, ileocecum; G5, appendix; H5, ascending colon; A6, transverse colon; B6, descending colon; C6, rectum; D6-H6, blank; A7, kidney; B7, skeletal muscle; C7, spleen; D7, thymus; E7, peripheral blood leukocyte; F7, lymph node; G7, blank; H7, trachea; A8, lung; B8, placenta; C8, bladder; D8, uterus; E8, prostate; F8, testis; G8, ovary; H8, blank; A9, liver; B9, pancreas; C9, adrenal gland; D9, thyroid gland; E9, salivary gland; F9, mammary gland; G9-H9, blank; A10, HL-60; B10, HeLa S3; C10, K-562; D10, MOLT-4; E10, Raji; F10, Daudi; G10, SW 480; H10, A549; A11, fetal brain; B11, fetal heart; C11, fetal kidney; D11, fetal liver; E11, fetal spleen; F11, fetal thymus; G11, fetal lung; H11, blank; A12, yeast total RNA; B12, yeast tRNA; C12, *E. coli* rRNA; D12, E. coli DNA; E12, poly r(A); F12, human C_0t1 DNA; G12, human DNA 100 ng; H12, human DNA 500 ng.

Figure S2

a) Human tissue Western blot probed with 4234 antibody (5 μg/ml) demonstrates expression of native H_{v1} protein (~32 kDa) in immune tissues. Lane: 1, appendix; 2, kidney; 3, liver; 4, lymph node; 5, peripheral blood leukocytes (PBL); 6, spleen; 7, tonsil; 8, thymus; 9, thyroid. We detected H_v1 protein in freshly isolated human polymorphonuclear leukocytes (data not shown); high protease activity in PBL could account for the anomalously low density of H_v1 protein in PBLs on the commercial Western blot. **b**) Western blot of total cell lysates prepared from Jurkat (lane 1) or HEK-293T cells transfected with the indicated cDNA (lanes 3-5); lane 2, protein marker. Native H_v1 protein in Jurkat cells migrates with an apparent molecular mass of ~32 kDa

(left arrow). An immunoreactive band in 293T cell extracts that migrates slightly faster than H_v1 is nonspecific; GFP- H_v1 and H_v1-HA migrate more slowly (~59 kDa and ~34 kDa, respectively). **c**) In HL-60 cells, H_v1 protein appeared to be increased when cells were cultured in the presence of 1.3% DMSO (lane 2); G_{vH+} is reported to increase in parallel with components of the NADPH oxidase complex during HL-60 differentiation[29]. GFP- H_v1 is detectable in transfected non-differentiated HL-60 cells (lane 4). Preincubation with antigenic peptide abolished H_v1 immunoreactivity in HL-60 cells (lanes 6, 7) and transfected HEK-293T cells (data not shown); lane 3, blank; lane 5, protein marker.

Figure S3

a) H_v1-like currents were not detectable in HM1 cells. This representative cell was transfected with pEGFP-N1 and recorded in symmetrical TMA6.5 solution. Voltage was stepped from -80 mV to +140 mV in 20 mV increments (V_h = -70 mV). Scale bars: 50 pA, 1 s. **b)** Native G_{vH^+} in a DMSO-differentiated HL-60 cell. This representative cell was transfected with maxGFP using the nucleofection technique (Amaxa) and recorded in symmetrical TMA6.5 solution. Voltage was stepped from -80 mV to +100 mV in 20 mV increments (V_h = -40 mV). Scale bars: 50 pA, 1 s. **c)** Temperature dependence of H_v1 currents. This representative HM1 cell was transfected with GFP- H_v1 and recorded in symmetrical Na6.5 solution. Temperature was controlled by continuous superfusion through a Warner SH-27B inline solution heater and subjected to slow (0.2 – 0.9°C·s^{-1}) thermal cycling between 24°C and 33°C while applying voltage steps (+25 mV, 750 ms then -20 mV, 500 ms; 0.1 Hz). Selected sweeps are displayed: red,

33.2°C; orange, 31.5°C; olive, 28.2°C; green, 27.0°C; blue, 26.5°C; violet, 24°C. Scale bars: 0.5 nA, 0.5 s. **d)** Monoexponential fits of τ_{ACT} from the same cell as shown in panel c illustrate strong temperature-dependence of H_v1 kinetics. Inset shows an Arrhenius plot of the data; Q_{10} = 32.6. τ_{DEACT} was less temperature-dependent (Q_{10} = 11.1, data not shown). **e)** R205A currents (-80 mV to +120 mV, V_h = -70 mV). Scale bars: 0.5 nA, 0.2 s. τ_{act} = 3.8 ± 0.6 ms, n = 3 and τ_{deact} = 5.6 ± 3.1 ms, n = 4. **f)** R208A currents (-80 mV to +120 mV, V_h = -70 mV). Scale bars: 4 nA, 0.2 s. τ_{act} = 69 ± 22 ms, n = 4 and τ_{deact} = 4.9 ± 0.6 ms, n = 5. The difference in current density between example traces of R205A and R208A reflects variable expression levels resulting from transient transfection and is not representative. **g)** R211A currents (-40 mV to +120 mV, V_h = -40 mV, $\tau_{act} \approx$ 1.6 s, $\tau_{deact} \approx$ 6 ms). Scale bars: 0.2 nA, 1 s. Currents and average data in **e-g** were measured in Na6.5 solution ($\rho H^+_{i/o}$ = 1).