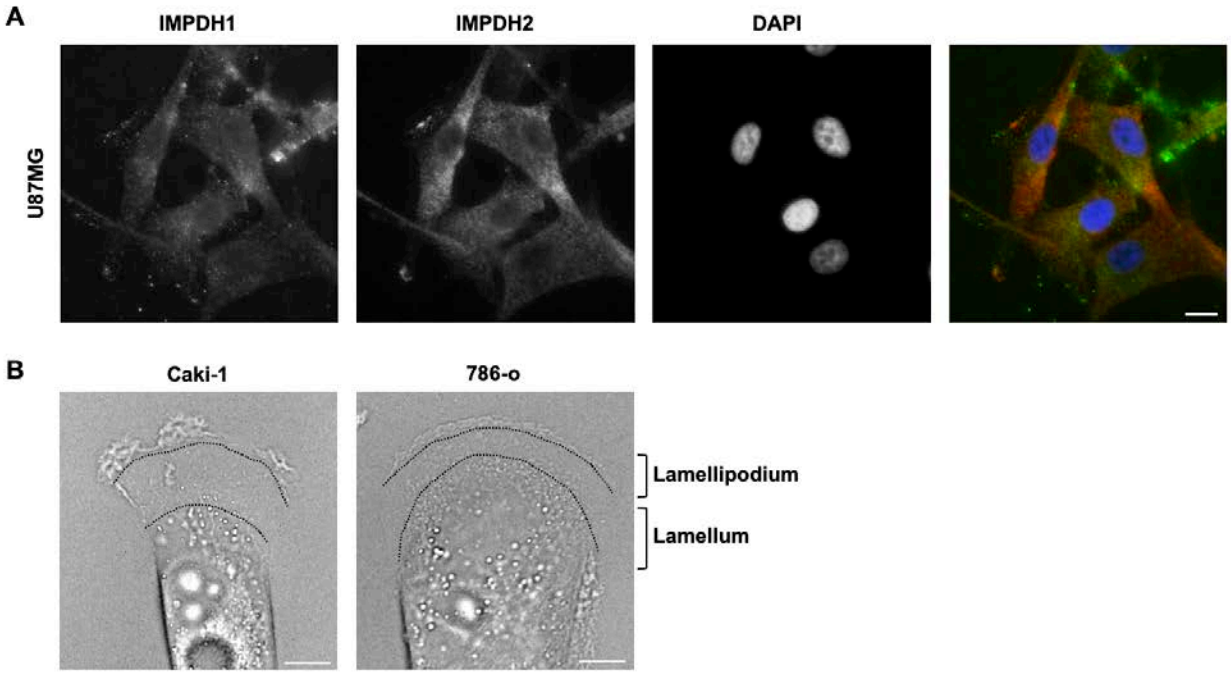
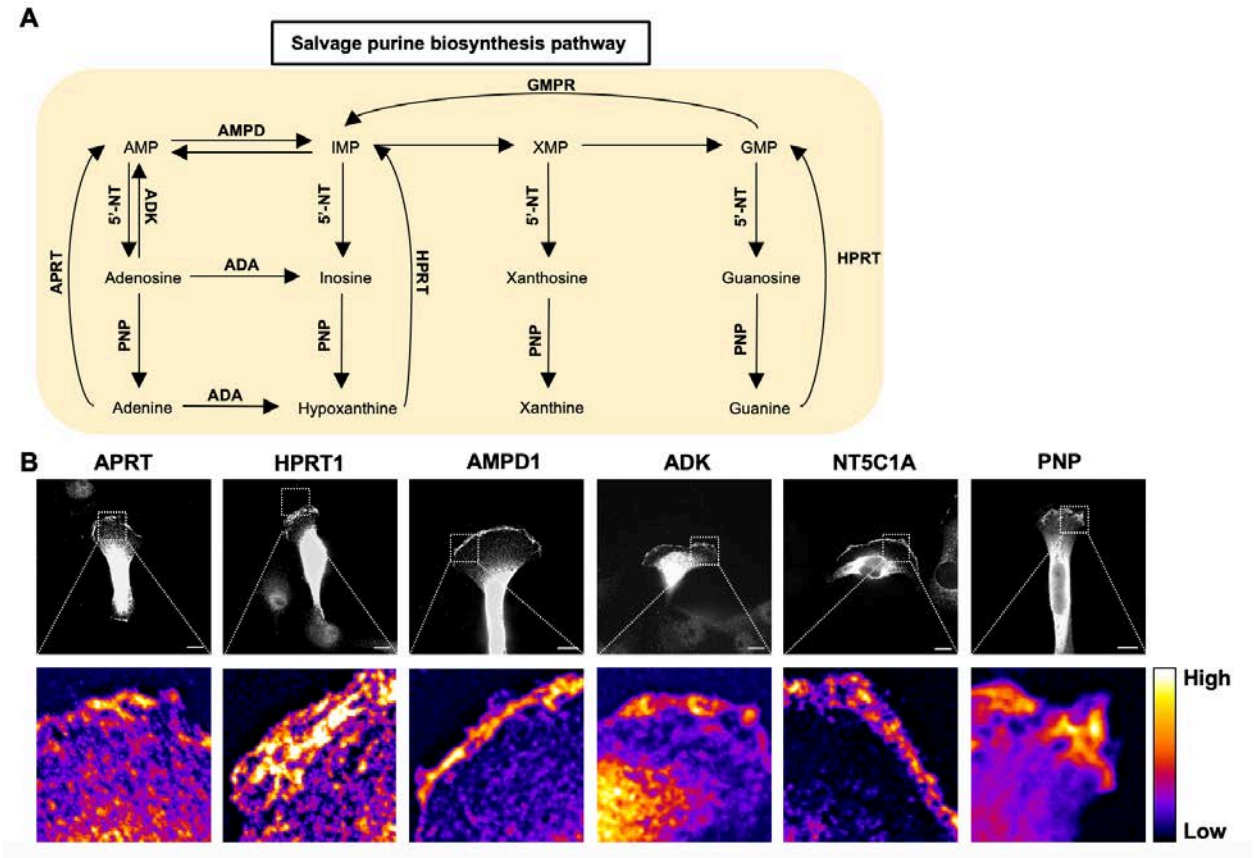
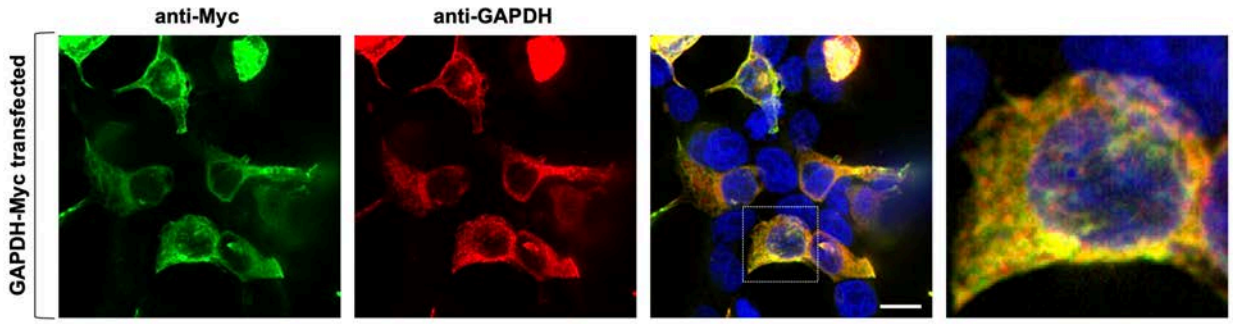


Supplemental Figure 1: IMPDH1 and IMPDH2 in the cytosol of U87MG cells.

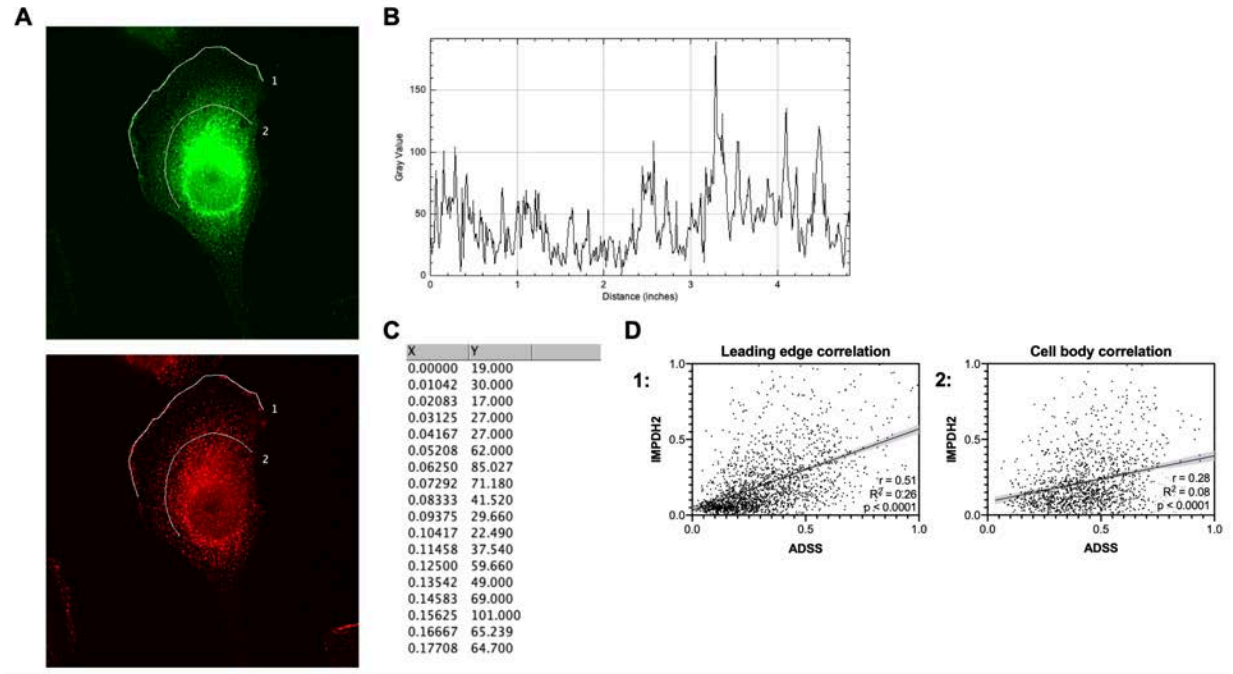
Immunofluorescence staining of IMPDH1 (green) and IMPDH2 (red) in U87MG cells **(A)**. Phase contrast images of Caki-1 (left) and 786-o (right) representing leading edge lamellipodium structure and posterior lamellum **(B).** Scale bars indicate 10 μm.



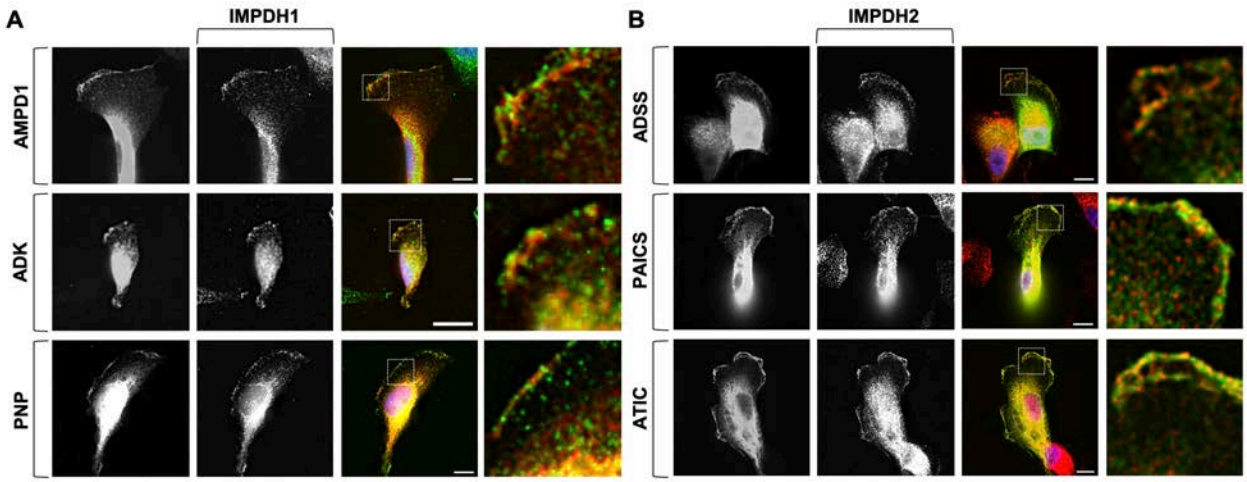
Supplemental Figure 2: Salvage nucleotide pathway enzymes localize to leading-edge. Purine salvage pathway **(A)**. Leading-edge localization of APRT (Myc-tagged, Caki-1), HPRT1 (Myc, Caki-1), AMPD1 (Myc, 786-o), ADK (Myc, Caki-1), NT5C1A (Myc, Caki-1), PNP (Myc, 786-o) **(B)**. Insets of leading-edge in pseudocolor shown below. Scale bars indicate 10 μm. APRT: adenine phosphoribosyltransferase. GMPR: guanosine monophosphate reductase. HPRT: hypoxanthine-guanine phosphoribosyltransferase. AMPD: AMP deaminase. 5'-NT: 5'-nucleotidase. ADK: adenosine kinase. PNP: purine nucleoside phosphorylase. ADA: adenosine deaminase.



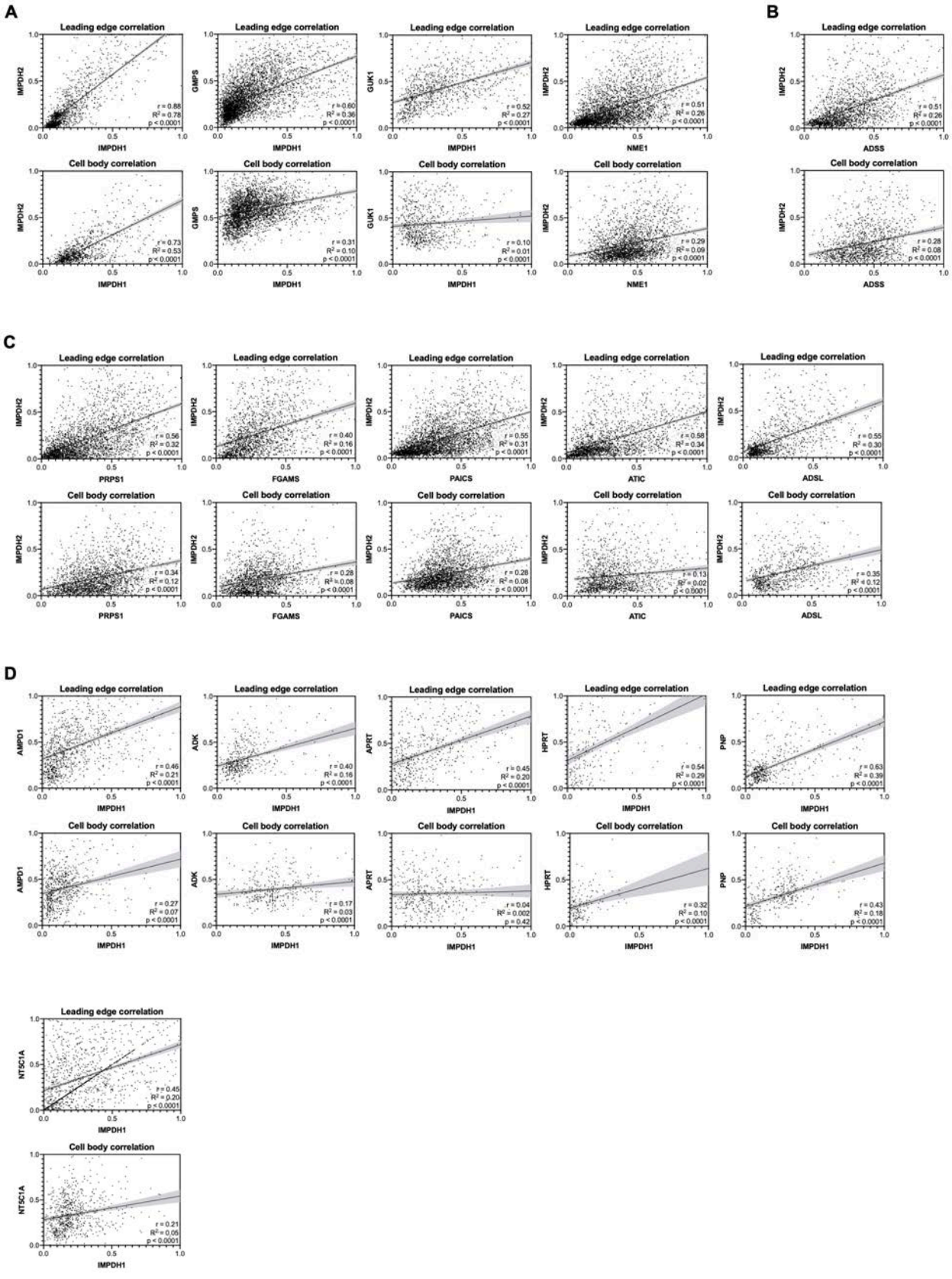
Supplemental Figure 3. GAPDH antibody validation. HEK293T cells transfected with GAPDH-Myc plasmid stained with anti-Myc (green) and anti-GAPDH (red) antibodies. Transfected cells demonstrate increased Myc and GAPDH staining. Insets at left. Scale bars indicate 10 μm.



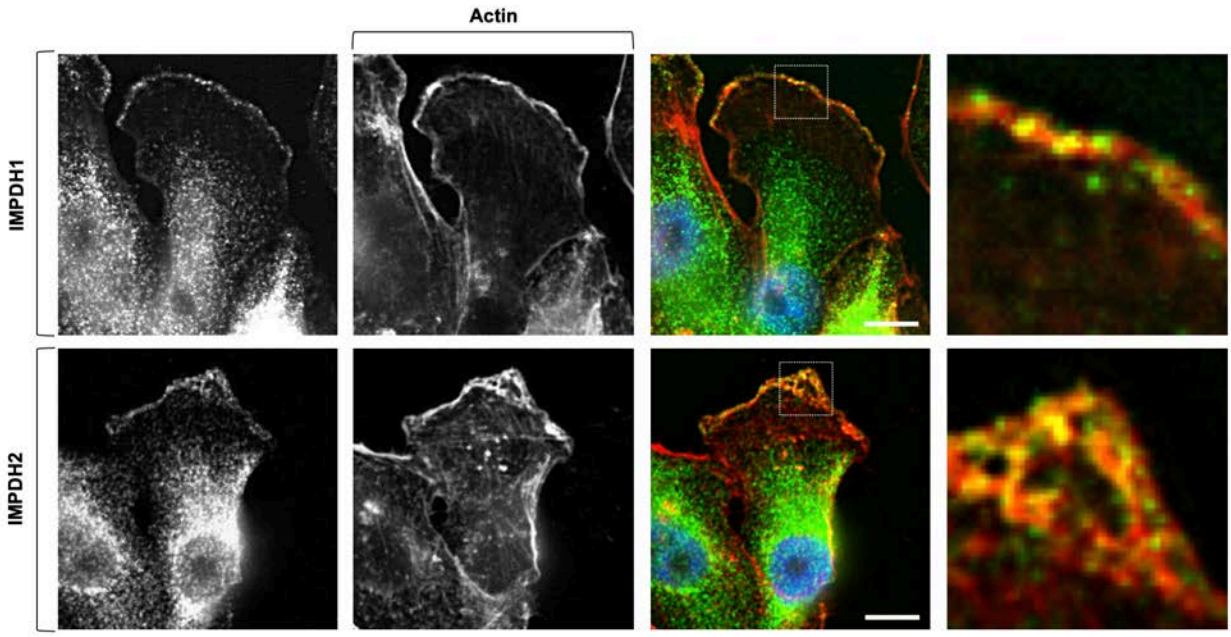
Supplemental Figure 4: ImageJ correlation analysis. ImageJ line profile analysis to quantify the fluorescence intensity of protein A (green) and co-stained protein B (red) followed by plotting the correlation between the location and intensity values at both the leading edge and within the cell body. Manual ImageJ line profiles drawn at leading edge (1) and cell body (2) regions in co-stained cells **(A)**. Line profile plots of immunofluorescence intensity **(2)**. Raw data points taken from line profile plot; x = distance values, y = fluorescence intensity values **(C)**. Correlation plots from all cells measured produced in GraphPad Prism 8 for both leading edge (1) and cell body (2) **(D)**.



Supplemental Figure 5: Purine nucleotide enzymes compartmentalize at the leading edge. The yellow fluorescence overlap illustrates a high degree of co-localization between co-stained enzymes. IMPDH1 (green) **(A)** and IMPDH2 (red) **(B)** co-localize with selected enzymes at the leading edge but not within the lamellum. Insets shown at right. Scale bars indicate 10 μm.


A
Leading edge correlation
IMPDH2
IMPDH1
r = 0.88
R² = 0.78
p < 0.0001
Leading edge correlation
GMPS
IMPDH1
r = 0.60
R² = 0.36
p < 0.0001
Leading edge correlation
GUK1
IMPDH1
r = 0.52
R² = 0.27
p < 0.0001
Leading edge correlation
IMPDH2
NME1
r = 0.51
R² = 0.26
p < 0.0001
Cell body correlation
IMPDH2
IMPDH1
r = 0.73
R² = 0.53
p < 0.0001
Cell body correlation
GMPS
IMPDH1
r = 0.31
R² = 0.10
p < 0.0001
Cell body correlation
GUK1
IMPDH1
r = 0.10
R² = 0.01
p < 0.0001
Cell body correlation
IMPDH2
NME1
r = 0.29
R² = 0.09
p < 0.0001
B
Leading edge correlation
IMPDH2
ADSS
r = 0.51
R² = 0.26
p < 0.0001
Cell body correlation
IMPDH2
ADSS
r = 0.28
R² = 0.08
p < 0.0001
C
Leading edge correlation
IMPDH2
PRPS1
r = 0.56
R² = 0.32
p < 0.0001
Leading edge correlation
IMPDH2
FGAMS
r = 0.40
R² = 0.16
p < 0.0001
Leading edge correlation
IMPDH2
PAICS
r = 0.55
R² = 0.31
p < 0.0001
Leading edge correlation
IMPDH2
ATIC
r = 0.58
R² = 0.34
p < 0.0001
Leading edge correlation
IMPDH2
ADSL
r = 0.55
R² = 0.30
p < 0.0001
Cell body correlation
IMPDH2
PRPS1
r = 0.34
R² = 0.12
p < 0.0001
Cell body correlation
IMPDH2
FGAMS
r = 0.28
R² = 0.08
p < 0.0001
Cell body correlation
IMPDH2
PAICS
r = 0.28
R² = 0.08
p < 0.0001
Cell body correlation
IMPDH2
ATIC
r = 0.13
R² = 0.02
p < 0.0001
Cell body correlation
IMPDH2
ADSL
r = 0.35
R² = 0.12
p < 0.0001
D
Leading edge correlation
AMPD1
IMPDH1
r = 0.46
R² = 0.21
p < 0.0001
Leading edge correlation
ADK
IMPDH1
r = 0.40
R² = 0.16
p < 0.0001
Leading edge correlation
APRT
IMPDH1
r = 0.45
R² = 0.20
p < 0.0001
Leading edge correlation
HPRT
IMPDH1
r = 0.54
R² = 0.29
p < 0.0001
Leading edge correlation
PNP
IMPDH1
r = 0.63
R² = 0.39
p < 0.0001
Cell body correlation
AMPD1
IMPDH1
r = 0.27
R² = 0.07
p < 0.0001
Cell body correlation
ADK
IMPDH1
r = 0.17
R² = 0.03
p < 0.0001
Cell body correlation
APRT
IMPDH1
r = 0.04
R² = 0.002
p = 0.42
Cell body correlation
HPRT
IMPDH1
r = 0.32
R² = 0.10
p < 0.0001
Cell body correlation
PNP
IMPDH1
r = 0.43
R² = 0.18
p < 0.0001
Leading edge correlation
NT5C1A
IMPDH1
r = 0.45
R² = 0.20
p < 0.0001
Cell body correlation
NT5C1A
IMPDH1
r = 0.21
R² = 0.05
p < 0.0001

Supplemental Figure 6: Purine nucleotide enzyme correlations in leading edge and cell body. Correlation plots at leading edge (top) and cell body (bottom) for GTP-related enzymes **(A)**, ATP-related enzymes **(B)**, *de novo* synthesis enzymes **(C)**, and salvage enzymes **(D)**. r = coefficient of correlation; R^2 = coefficient of determination; p = significance value.



Supplemental figure 7: IMPDH1 and IMPDH2 localize with actin ruffling at the leading edge. IMPDH1 (top, green) and IMPDH2 (bottom, green) localize with actin ruffles (phalloidin, red) in Caki-1 cells. Insets shown at right. Scale bars indicate 10 μm.