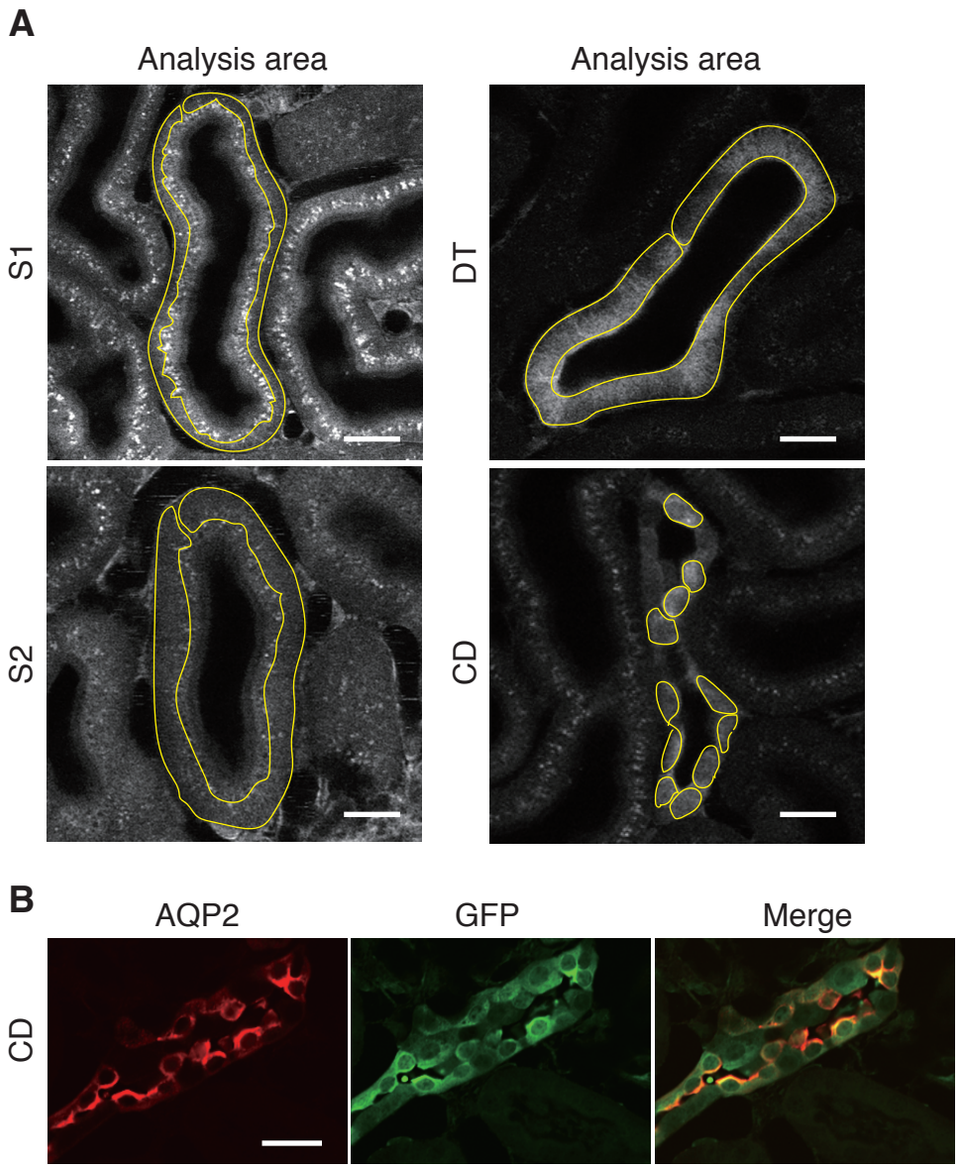
SUPPLEMENTARY APPLEDIX FOR THE STUDY:

Spatiotemporal ATP dynamics during acute kidney injury predicts renal prognosis

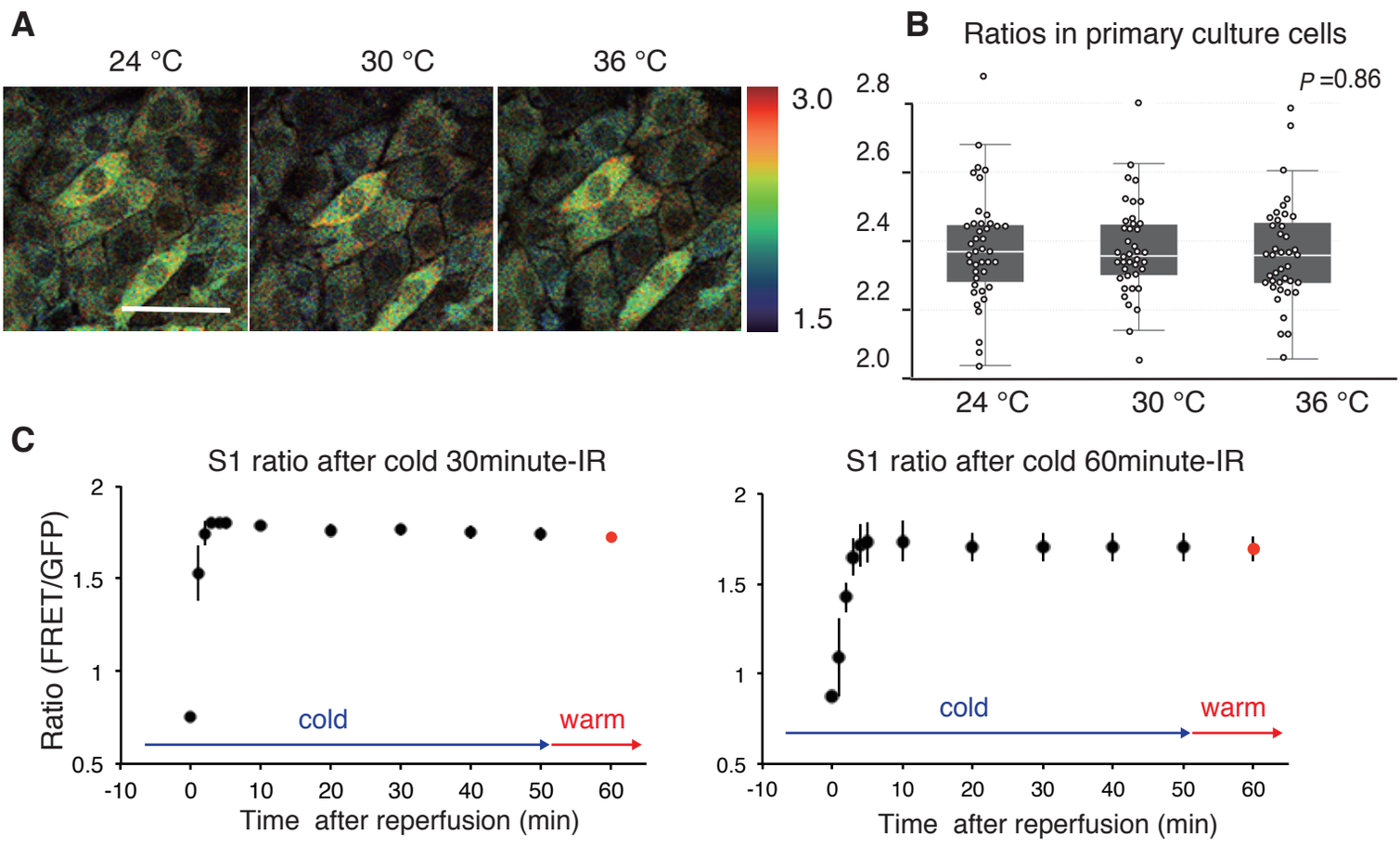
Supplementary material table of contents

Supplemental Figure 1



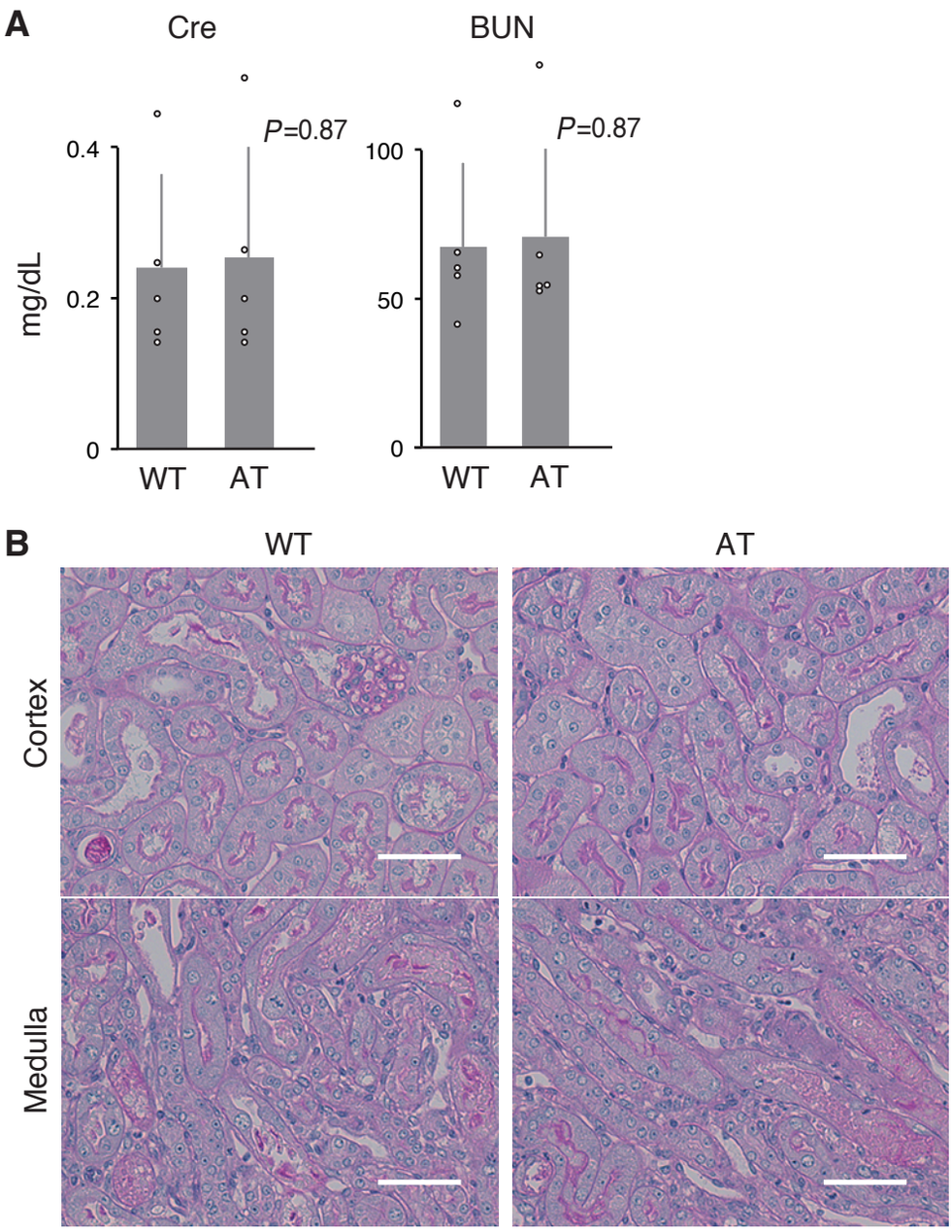
Supplemental Figure 1. (**A**) The areas surrounded by yellow line were analyzed in monitoring ratios in each segment. The apical lumen especially in S1 PTs was omitted to exclude high autofluorescence signals. (**B**) Immunostaining of AQP2 and GFP in the kidneys of GO-ATeam2 mice demonstrating that the cells with strong expression of GO-Ateam in CDs are mainly principal cells. Scale bars: (**A** and **B**) 20 μm.

Supplemental Figure 2



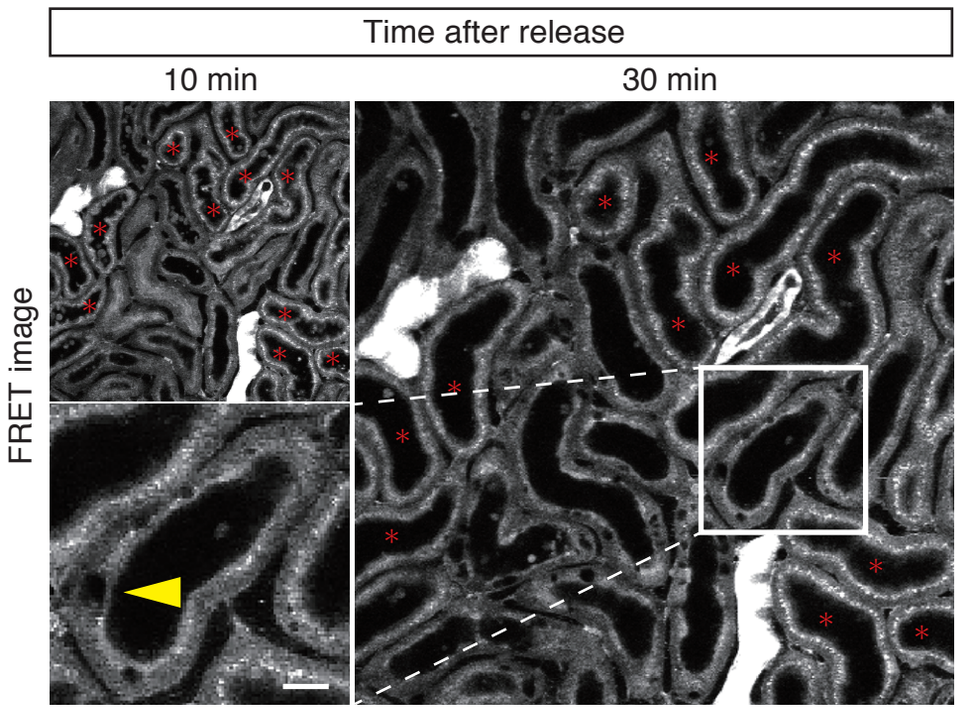
Supplemental Figure 2. (**A**) Ratio images of renal primary culture cells from the kidneys of GO-ATeam2 mice sequentially cultured under 24, 30, and 36 °C, showing similar ratios between groups. (**B**) Ratios were similar between groups, and there was no statistically significant difference. Statistical significance was assessed by ANOVA. $^{*}P = 0.86$ (**C**) Ratio graphs of PT S1 during cold 30 and 60 minute-IR, whose kidneys were warmed 50 minutes after reperfusion, showing that the temperature did not affect the ratio *in vivo* (n = 3 per group).
Scale bars: (**A**) 50 µm.

Supplemental Figure 3



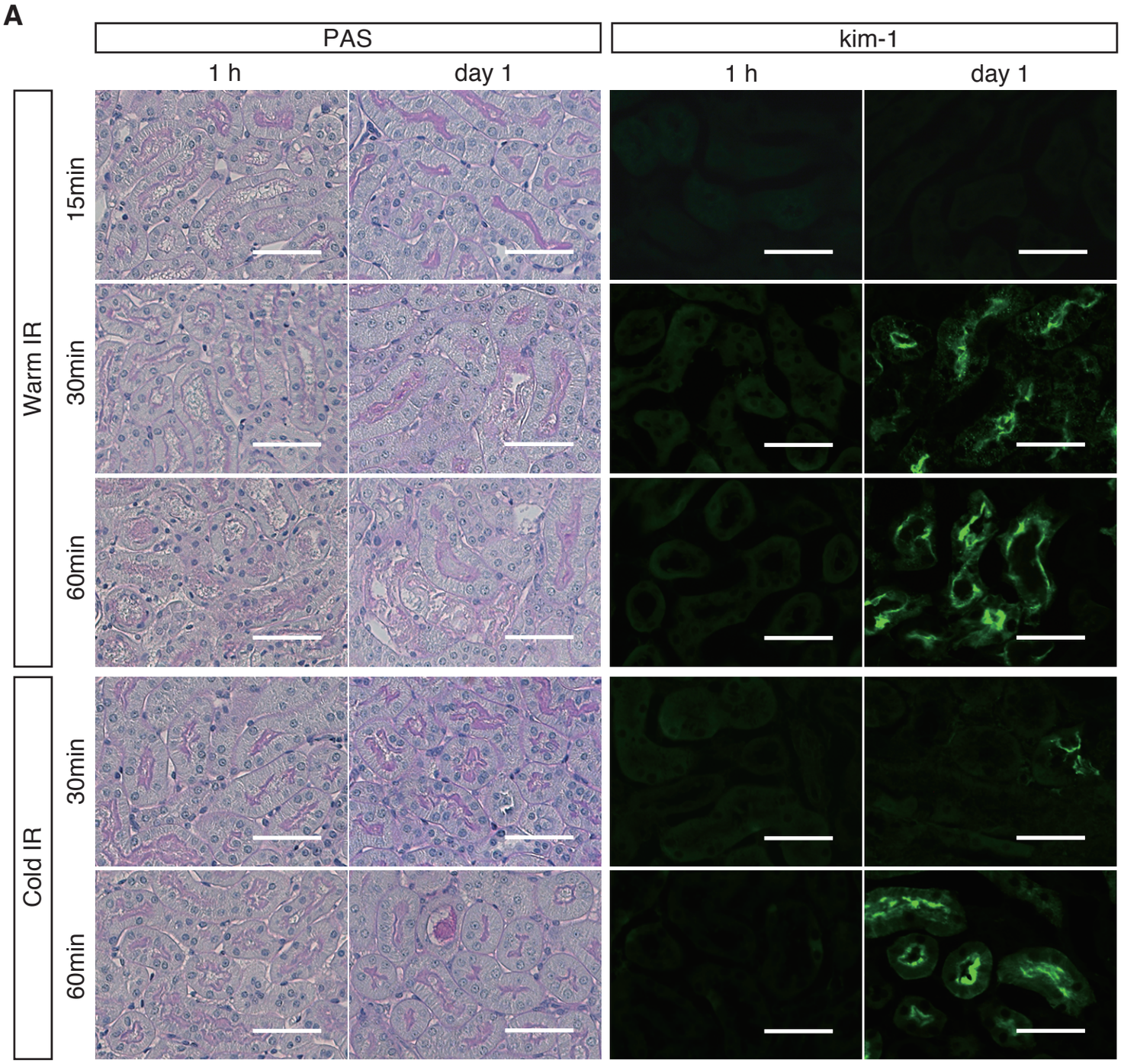
Supplemental Figure 3. (**A**) Serum creatinine and BUN of wild-type (WT) and GO-ATeam2 mice two days after unilateral warm 23 minute-ischemia reperfusion (IR) with contralateral nephrectomy three days before IR (n = 5 mice per group). There was no statistically significant difference between groups. Statistical significance was assessed by Student's *t*-test. (**B**) Representative images of Periodic acid-Schiff (PAS) staining of WT and GO-Ateam2 kidneys harvested at the same time point. The severity of kidney injury was comparable between groups.
Scale bars: (**B**) 50 μm.

Supplemental Figure 4



Supplemental Figure 4. Images of FRET signals after warm 30-minute IR showing vacuolization in S2 PTs, but not in S1 PTs with high apical autofluorescence (asterisk). Inset is showing higher magnification of vacuolization (arrowhead). Scale bars: 20 µm.

Supplemental Figure 5



B

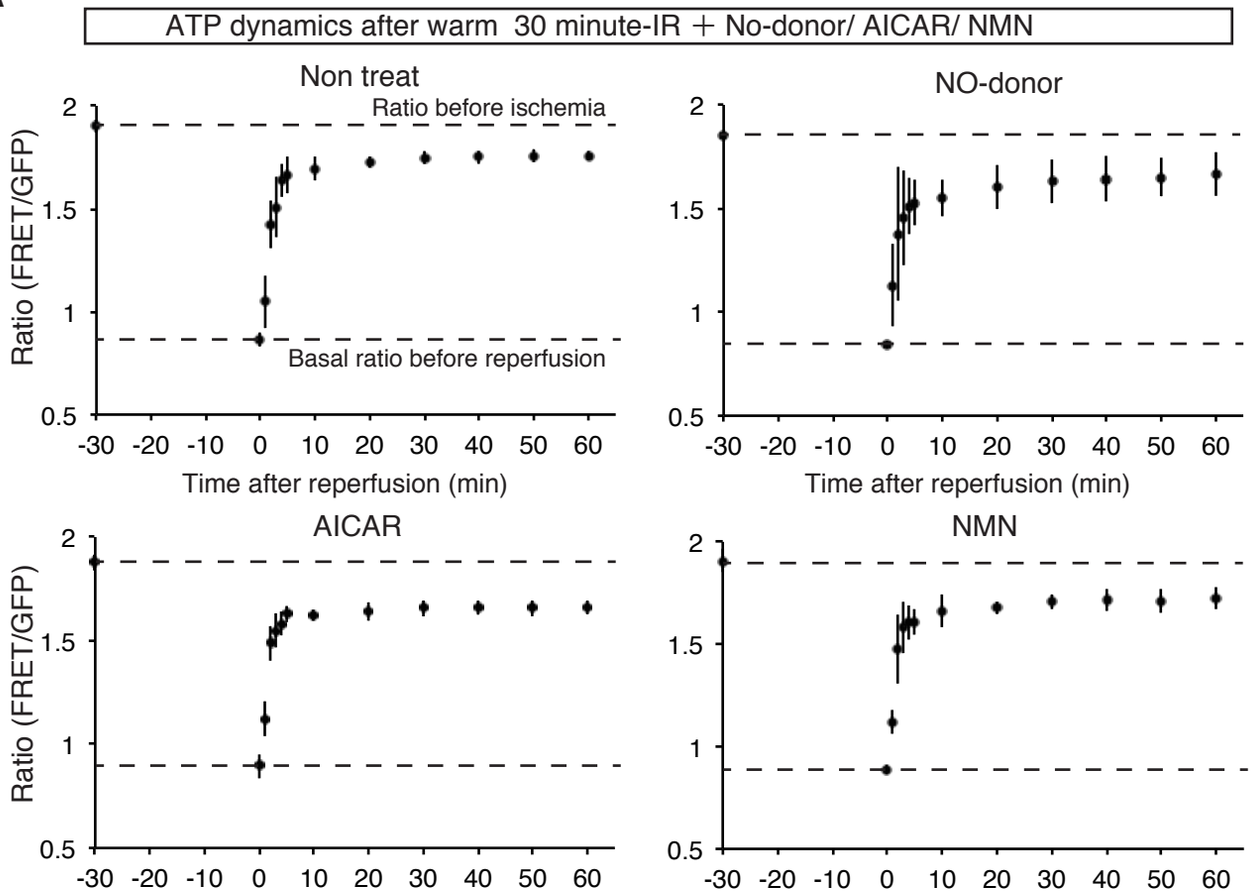
IR day 1 PAS scoring

	Warm			Cold	
	15min IR	30min IR	60min IR	30min IR	60min IR
Grade 0	6	1	0	4	0
Grade 1	4	2	2	7	1
Grade 2	5	9	1	4	12
Grade 3	0	3	1	0	2
Grade 4	0	0	11	0	0

Supplemental Figure 5. (**A**) Histological analysis of acute kidney injury after IR. Periodic acid-Schiff (PAS) staining and immunostaining of Kim-1 of the kidneys harvested one hour, and one day after the induction of reperfusion after warm 15, 30, and 60 minute-ischemia and cold 30 and 60 minute-ischemia. In the kidney harvested one hour after the induction of reperfusion, neither obvious histological injury nor the expression of Kim-1 was observed even in the kidneys treated with warm 60 minute-ischemia. (**B**) Pathological scores of acute kidney injury in the kidneys harvested one day after warm 15, 30, and 60 minute-IR and cold 30 and 60 minute-IR. Five high-power fields in the cortex per each mouse were viewed and graded for tubular injury defined as tubular necrosis, tubular dilation, casts, brush border loss (0 = 0-1%, 1 = > 1-10%, 2 = 10-25%, 3 = > 25-50%, and 4 = > 50%) (n = 3 mice per group). All scoring was performed in a blind fashion by experienced nephrologists. Scale bars: (**A**) 50 μm.

Supplemental Figure 6

A



B

	Ratio before IR/ *P* value (vs NT)
NT	1.90 ± 0.032/ ---
NO-donor	1.85 ± 0.016/ 0.052
AICAR	1.88 ± 0.036/ 0.360
NMN	1.91 ± 0.056/ 0.844
	Recovery slope/ *P* value (vs NT)
NT	0.277 ± 0.050/ ---
NO-donor	0.266 ± 0.155/ 0.884
AICAR	0.297 ± 0.017/ 0.730
NMN	0.295 ± 0.095/ 0.767
	% recovery/ *P* value (vs NT)
NT	86.2 ± 3.8/ ---
NO-donor	82.1 ± 9.5/ 0.461
AICAR	82.0 ± 4.0/ 0.039*
NMN	81.9 ± 10.1/ 0.120

Supplemental figure 6. (**A**) Ratio graphs of PTs S1 during reperfusion after warm 30-minute ischemia with NO-donor (n = 3 mice), AICAR (n = 3 mice), and NMN (n = 3 mice). None of compounds could elevate the ratio before IR and the ratio % recovery. (**B**) Table of each ratio before IR, recover slope, % recover, and *P* value. Differences between the groups (NT vs NO-donor / AICAR / NMN) in the ratio before IR and the % recovery were compared using Student's *t*-test. Those in the recovery slope were compared using ANCOVA. $^{*}P < 0.05$