

## **Supplementary Information**

### **The thermogenic actions of natriuretic peptide in brown adipocytes: The direct measurement of the intracellular temperature using a fluorescent thermoprobe**

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**Supplementary Table. Fluorescence Ratio at each time point after treatment with ANP**

Control (n)	0	54	60	(minute)
1	0.510±0.0199	0.491±0.0215	0.494±0.0235	
2	0.468±0.0174	0.457±0.0172	0.456±0.0174	
3	0.501±0.0159	0.487±0.0153	0.487±0.0157	
4	0.555±0.0171	0.549±0.0183	0.549±0.0190	
5	0.510±0.0285	0.509±0.0245	0.509±0.0249	
6	0.486±0.0142	0.483±0.0144	0.483±0.0143	

ANP (n)	0	54	60	(minute)
1	0.486±0.0189	0.497±0.0214	0.500±0.0215	
2	0.443±0.0147	0.449±0.0226	0.450±0.0220	
3	0.460±0.0156	0.462±0.0173	0.463±0.0180	
4	0.505±0.0201	0.513±0.0175	0.516±0.0183	
5	0.513±0.0204	0.519±0.0218	0.520±0.0222	
6	0.499±0.0163	0.499±0.0221	0.502±0.0270	

p=0.016, two-way ANOVA

## Supplementary Figure Legend

**Supplementary Figure S1. Representative microscopic images of rat white adipocytes treated with the fluorescent polymeric thermometer.** A differential interference contrast image (a), a fluorescence image (490 nm excitation, 525 nm emission), (b) and a fluorescence image (490 nm excitation, 605 nm emission) (c) of the cellular thermoprobe in rat brown adipocytes on day 8. A merged image of (b) and (c) with the sampling square of the measurement are also shown in (d). Scale bar: 40  $\mu\text{m}$ .

**Supplementary Figure S2. The calibration curve of the fluorescent polymeric thermometer in rat white adipocytes.** The responses of the fluorescence ratio (605nm/525nm) were analyzed (n=4). The data indicate the mean  $\pm$  SEM.

**Supplementary Figure S3. ANP increases the UCP1 levels in rat brown adipocytes.** The quantification of the *Ucp1* gene expression levels in rat brown adipocytes (day 7 or 8) after six hours of incubation with ANP ( $10^{-9}$  M or  $10^{-7}$  M) or isoproterenol ( $10^{-7}$  M) (n=5) at 37°C. The qPCR data were normalized to GAPDH. The data are shown as the fold change normalized to the levels found in untreated cells (control) \*P < 0.05 versus control (unpaired two-tailed Student's t-test). Iso, isoproterenol.

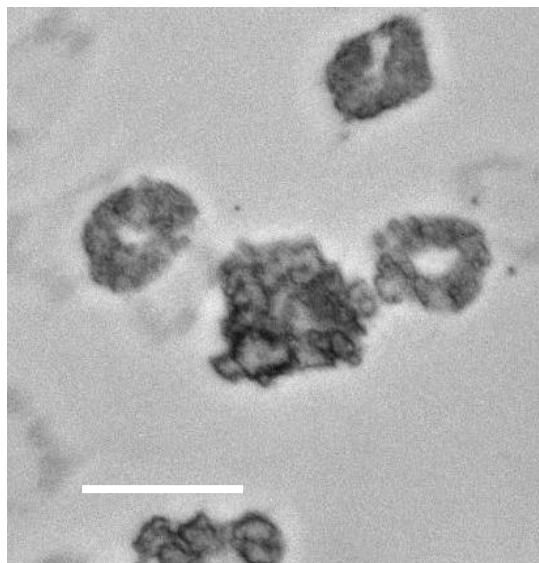
**Supplementary Figure S4. The profile of the intracellular temperature change in rat white adipocytes incubated with ANP.** The intracellular temperature was indicated by the fluorescence ratio (605 nm/525 nm). The changes of the fluorescence ratio in rat white adipocytes (day 8) after treatment with ANP ( $10^{-7}$ M), isoproterenol ( $10^{-7}$ M), or CL316,243 (0.5  $\mu\text{M}$ ) were recorded every 6 minutes at 35°C (n=3 each). The data represent the mean  $\pm$  SEM.

**Supplementary Figure S5. ANP does not increase the UCP1 levels in rat white adipocytes.** The quantification of the *Ucp1* gene expression levels in rat white adipocytes (day 7 or 8) after one hour of incubation with ANP ( $10^{-7}$  M), isoproterenol ( $10^{-7}$  M), or CL316,243 (0.5  $\mu\text{M}$ ) (n=3 each). The qPCR data were normalized to GAPDH. The data are shown as the fold change normalized to the levels found in untreated cells (control) Iso, isoproterenol. CL, CL 316,243.

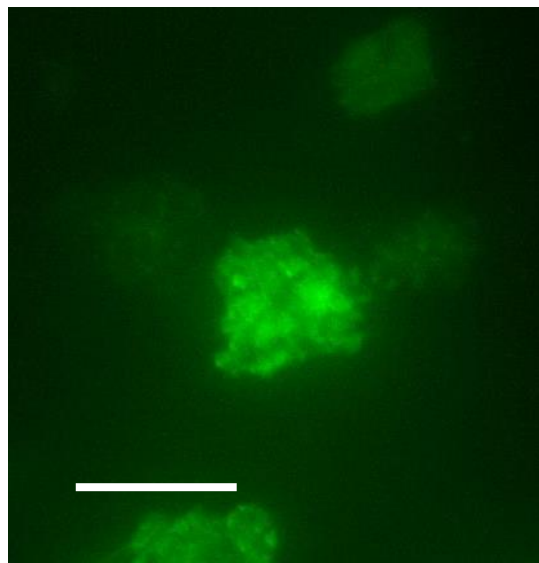
## Supplementary Figure S6. Full-length blots of phospho-p38 and total p38.

Western blotting of phosphorylation of p38MAPK and total p38MAPK in rat brown adipocytes treated with or without ANP ( $10^{-7}$  M) for 60 minutes. M, MagicMark XP Western Protein standard, a molecular weight marker. kDa, kilo Dalton.

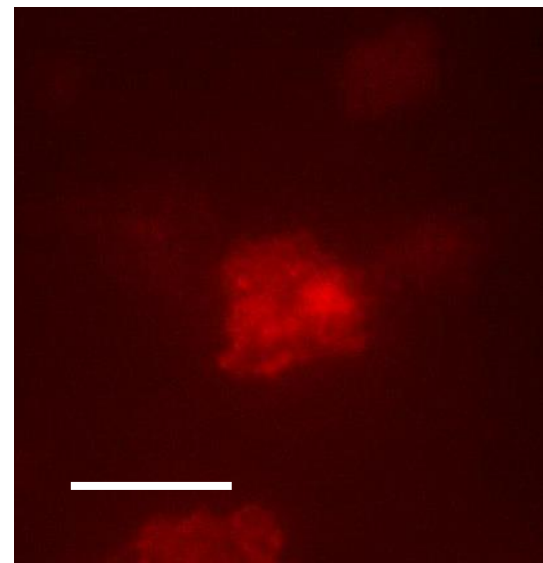
**(a)**



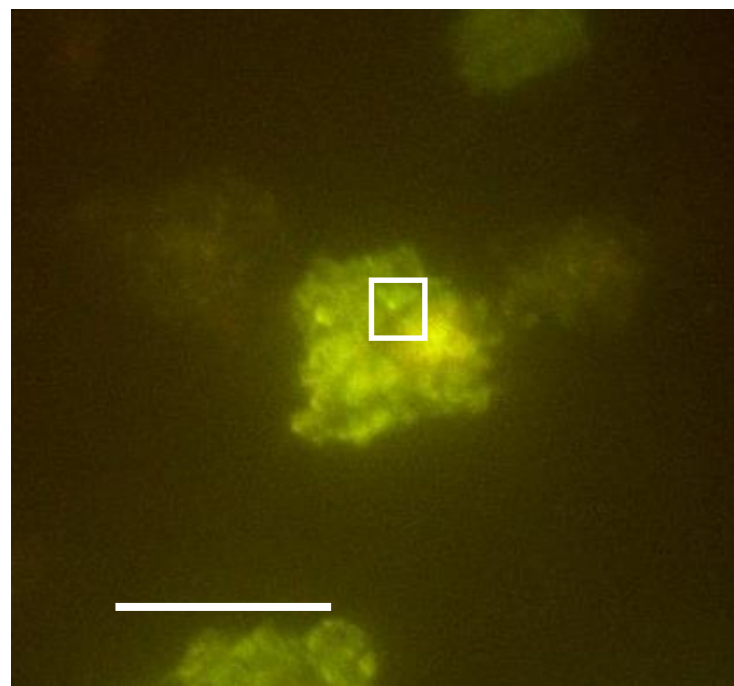
**(b)**

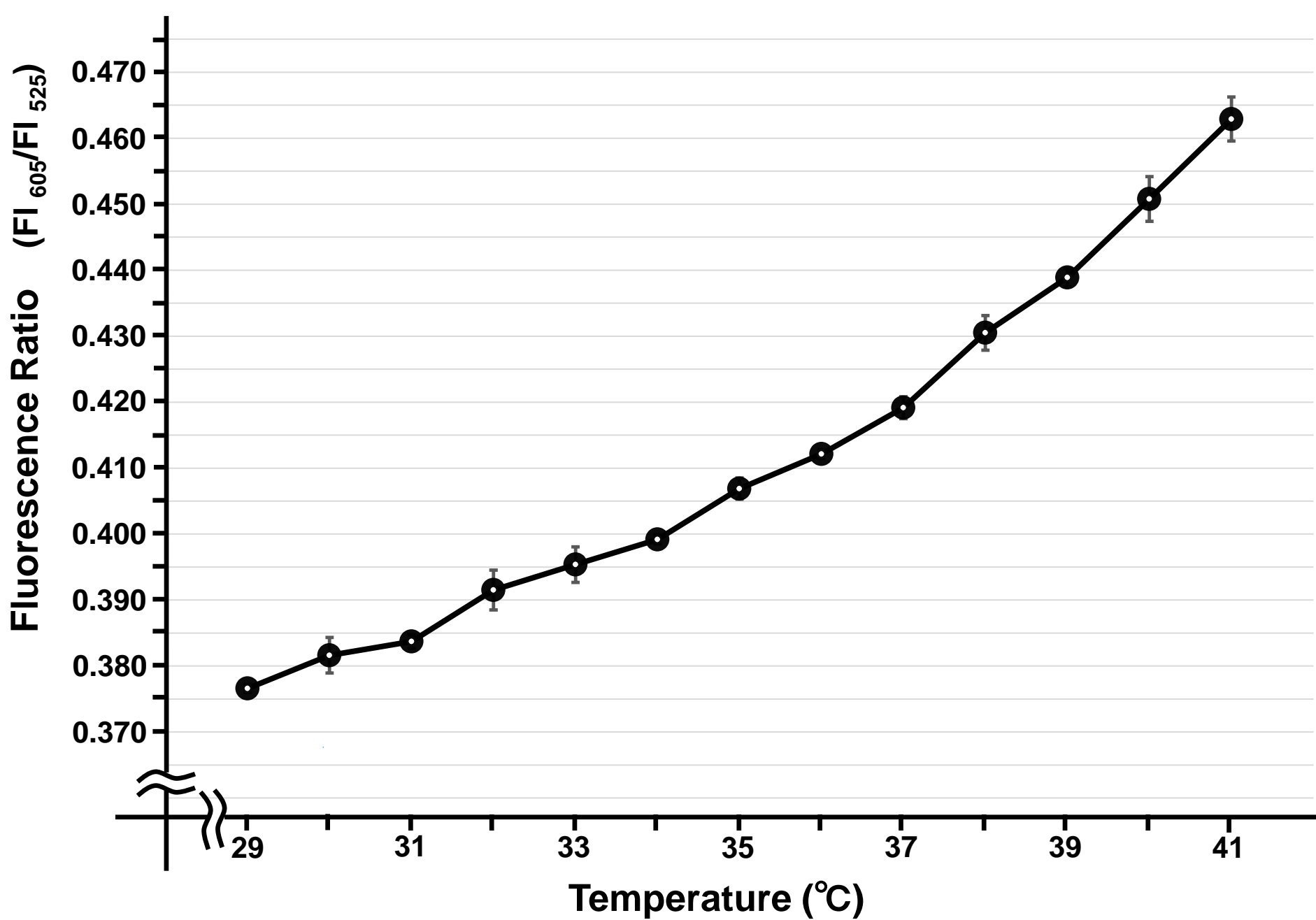


**(c)**

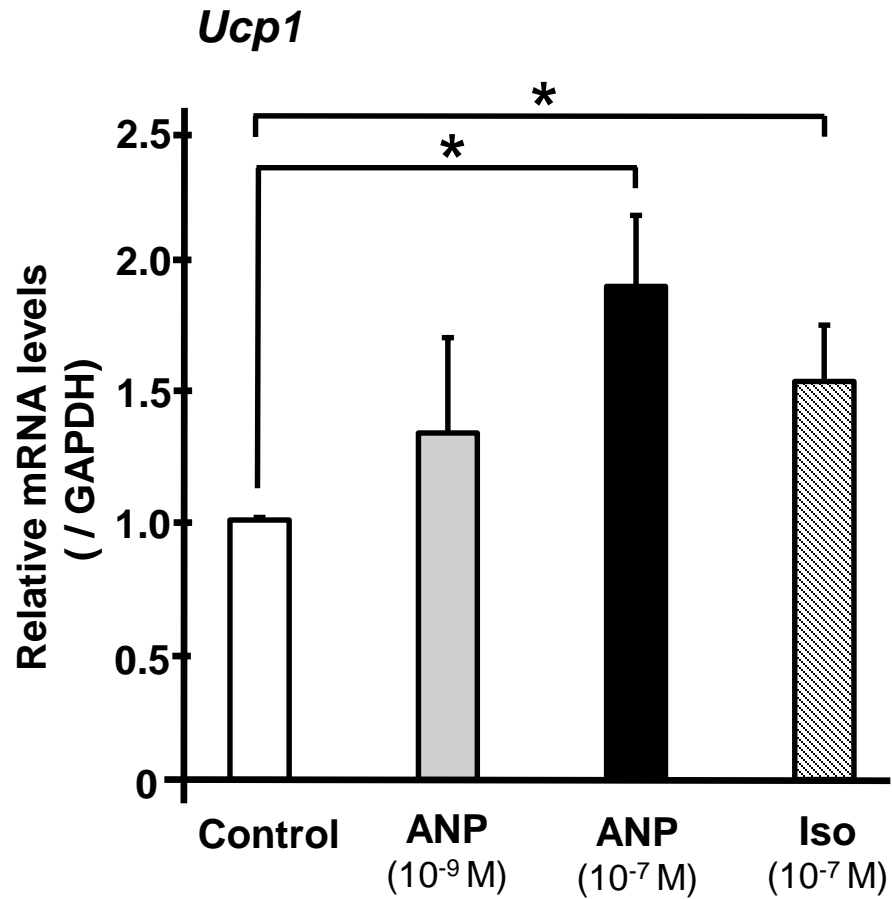


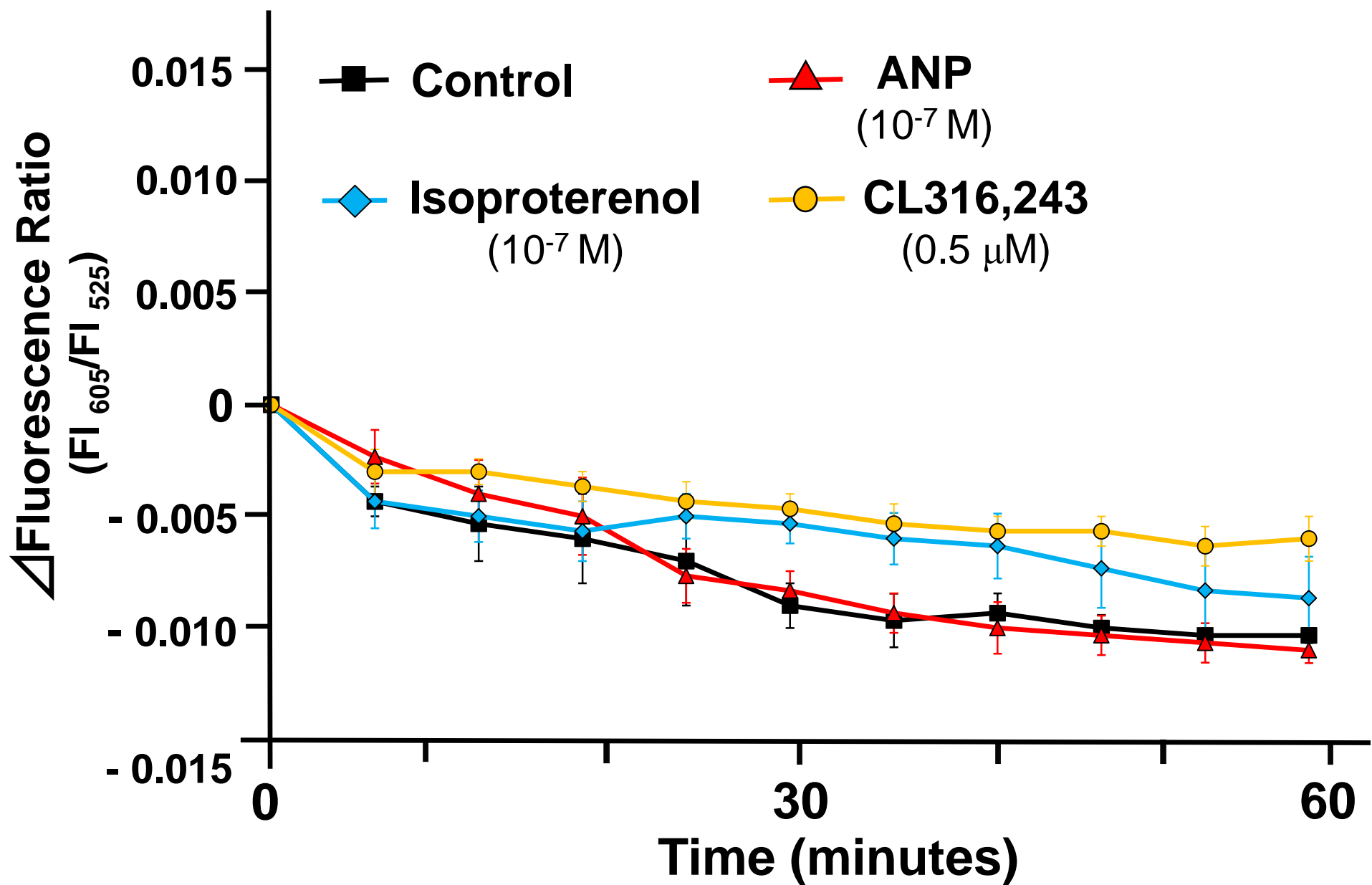
**(d)**



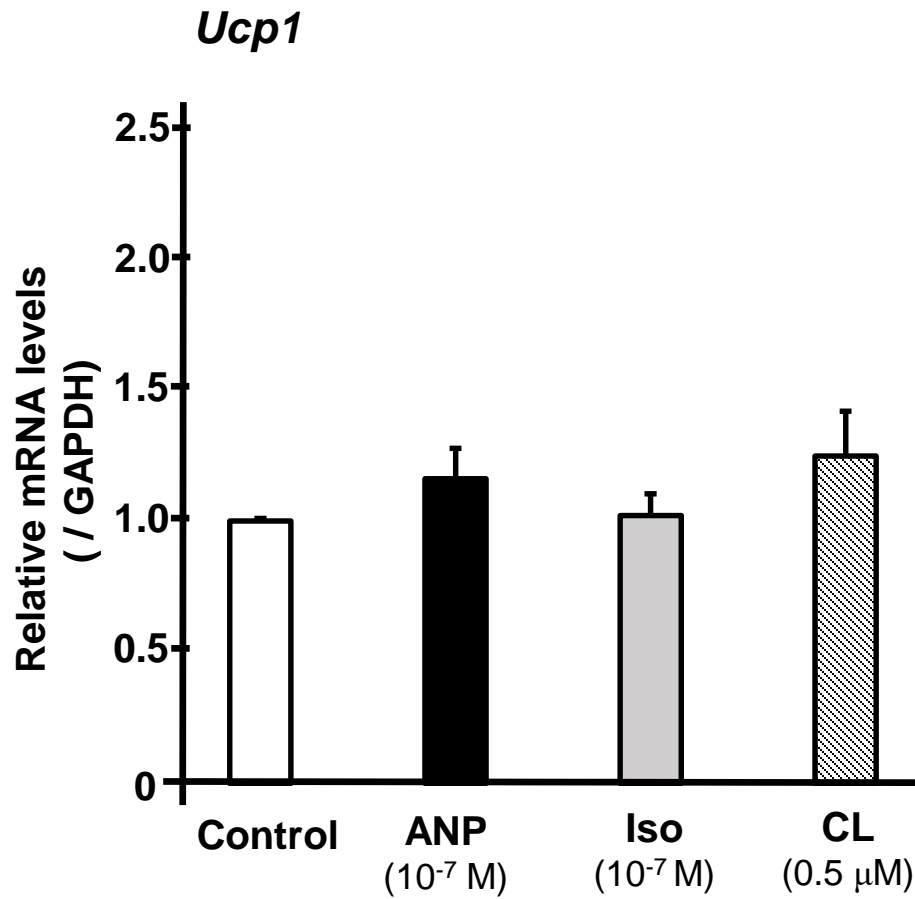


***Supplementary Fig. S2***





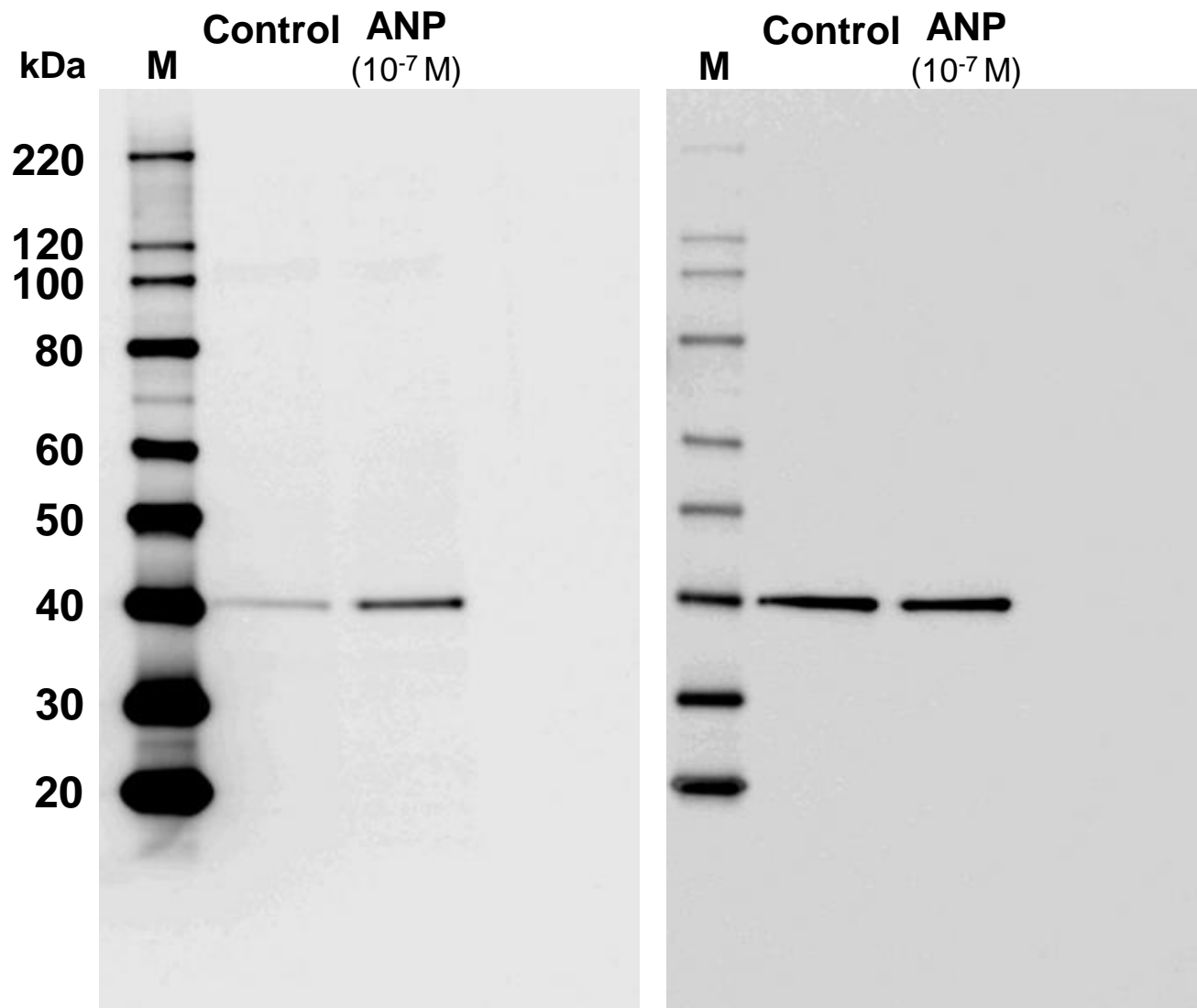
*Supplementary Fig. S4*





**p-p38**

**total p38**



***Supplementary Fig. S6***