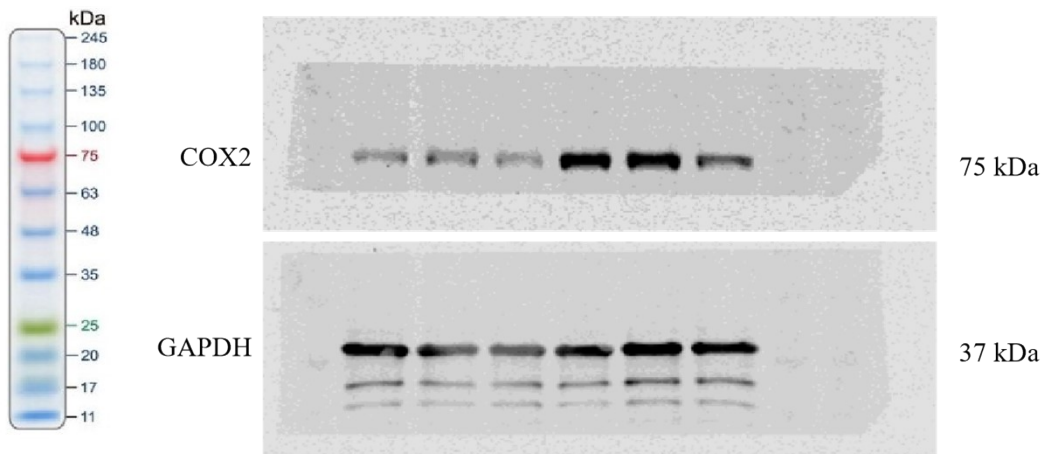


### **Explanation of Western blot analysis**

Sorry We don't have uncropped blots. After blocking in 5% BSA, nitrocellulose membranes were cut based on the protein ladders (ErgggaBig 4~15% TG SDS buffer) on the membranes and the molecular weights of the target proteins. For example, COX2 of 75 kDa, membranes were labelled, cropped around 63 kDa and above 75 kDa and sealed in Seal-0-Bag (plastic film) for primary anti-COX2 antibody incubation at 4°C overnight, while the membranes containing ~37kDa ranges were incubated with primary anti-GAPDH antibody as a reference. In this way, we can save a lot of samples, reagents, and antibodies, avoid nonspecific bindings that influence the development of target proteins, and get clean blots than the whole membrane method. Notably, since we numbered the membranes, finally those membranes could be traced, so that first lane of COX2 on the first membrane can be matched with the corresponding GAPDH for calculation and statistics. Similarly, VCAM-1 protein was also processed using the same method. Sorry, the marker channel is not displayed in the membranes. The original scan file of the membranes has now been deleted from the scanning instrument (Licor Odyssey BioImager, a) by my colleague, but the molecular weight range of the marker protein we used was 11 kDa -245 kDa

We appreciate the significance of uncropped blots in validating data collection. We will consider this and change our method in our future experiments. Your feedback is highly regarded. The blots were shown as follows:

### Membrane-1



### Membrane-2

