**Supplementary Information Figure Legends**

**Fig. S1.** Fractions 29-60 eluting from size exclusion chromatography of HeLa cytosolic extract were tested for their ability to stimulate the acetylation of MEK by YopJ in the presence of [$^{14}$C]-AcCoA. Lanes marked 'B' indicate reference reactions representing basal YopJ activity towards MEK (in the absence of any added HeLa extract). The numbers indicate fraction numbers from the S75 Superdex gel filtration column. 15 μl of each fraction were included in the acetylation reaction. Lanes marked 'M' contain SDS-PAGE size standards. The migration of gel filtration size standards on the column used is indicated at the top of the figure.

**Fig. S2.** Autoacetylation is not a prerequisite for acetylation of MEK by YopJ. In a 150 μl reaction, 3 μg YopJ was incubated with 50 μM [$^{14}$C]-AcCoA in the absence or presence of 100 nM $IP_6$ at 37°C for 4 hours to obtain unacetylated and (auto)acetylated YopJ, respectively (compare lanes 1 and 6). These preparations were used for subsequent acetylation of MEK that was examined over a time-course of 10 minutes at room temperature (25°C). At t=0, 15 μg MEK was added to autoacetylated YopJ and 15 μg MEK + $IP_6$ (at 100 nM final concentration in the reaction) were added to unacetylated YopJ. Aliquots (25 μl) of the reactions were removed after 1min, 2.5 min, 5 min, 7.5 min and 10 min. Each sample was supplemented with Laemmli sample buffer and plunged in liquid nitrogen pending SDS-PAGE. From the autoradiograph of the resulting gel, and its quantitation presented in the lower panel, it is seen that MEK is acetylated equally well by non-acetylated YopJ (lanes 2-5) as it is by autoacetylated YopJ (lanes 7-10).

**Fig. S3.** $IP_5$ can activate YopJ *in vitro*. The autoradiograph depicts the results of an *in vitro* assay examining the acetylation of 5 μg MEK using 0.3 μg YopJ in the presence of indicated concentrations of $IP_3$ or $IP_5$ or $IP_6$. While $IP_3$ does not support the activation of YopJ, $IP_5$ and $IP_6$ are observed to do so.

**Fig. S4.** (A). Far UV circular dichroism spectra of AvrA (10 μM) were collected in the presence of varying amounts of $IP_6$ (0 μM, 0.5 μM, 1 μM, 2 μM, 3 μM, 4 μM, 5 μM and 10 μM). The resulting spectra are shown superposed in this figure. Judging from the decrease in ellipticity at 208 nm and 222 nm, addition of $IP_6$ to AvrA appears to result in a gain of overall helical content by AvrA. (B) Change in ellipticity at 222 nm in the far UV circular dichroism spectrum of the catalytically inactive C172A AvrA (5 μM) upon addition of $IP_6$ (10 μM).

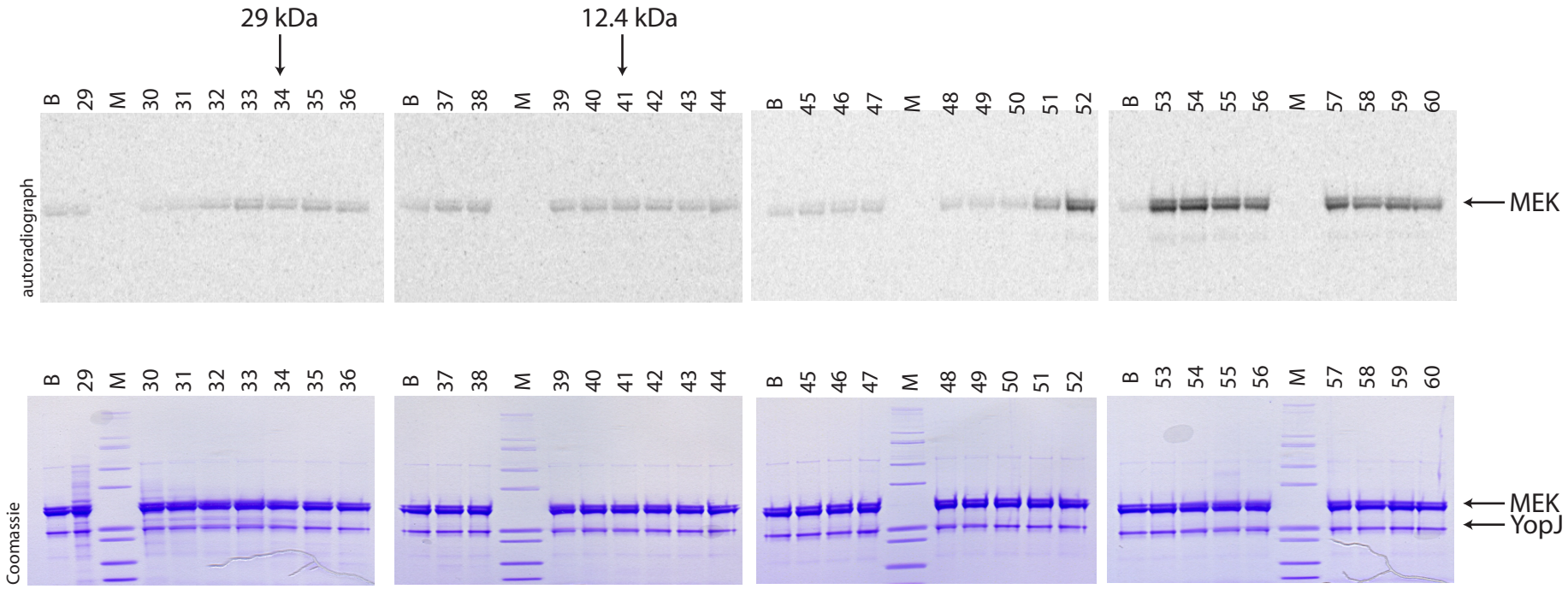
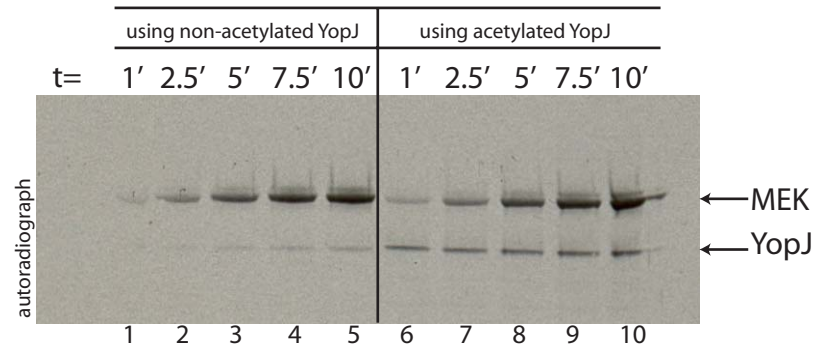

29 kDa
12.4 kDa
B 29 M 30 31 32 33 34 35 36
B 37 38 M 39 40 41 42 43 44
B 45 46 47 M 48 49 50 51 52
B 53 54 55 56 M 57 58 59 60
autoradiograph
MEK
Coomassie
MEK
YopJ

**Figure S1**


using non-acetylated YopJ
using acetylated YopJ
t=
1′ 2.5′ 5′ 7.5′ 10′ 1′ 2.5′ 5′ 7.5′ 10′
autoradiograph
MEK
YopJ
1 2 3 4 5 6 7 8 9 10

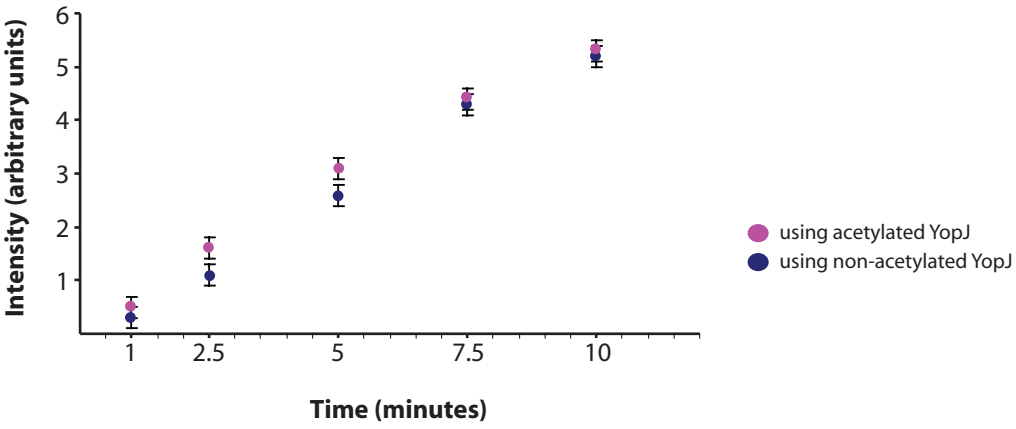

Intensity (arbitrary units)
6
5
4
3
2
1
1
2.5
5
7.5
10
Time (minutes)
using acetylated YopJ
using non-acetylated YopJ

**Figure S2**

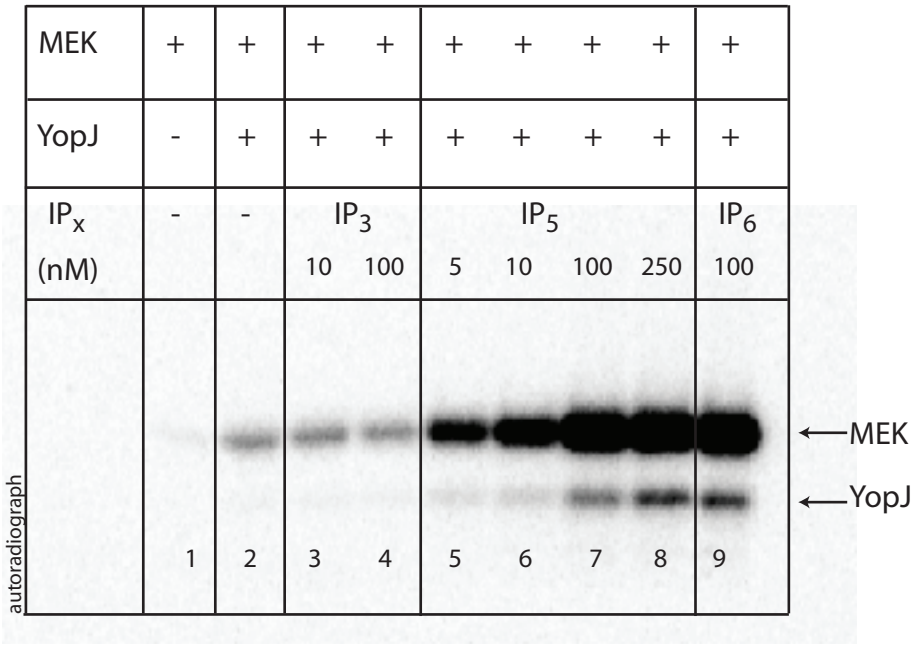
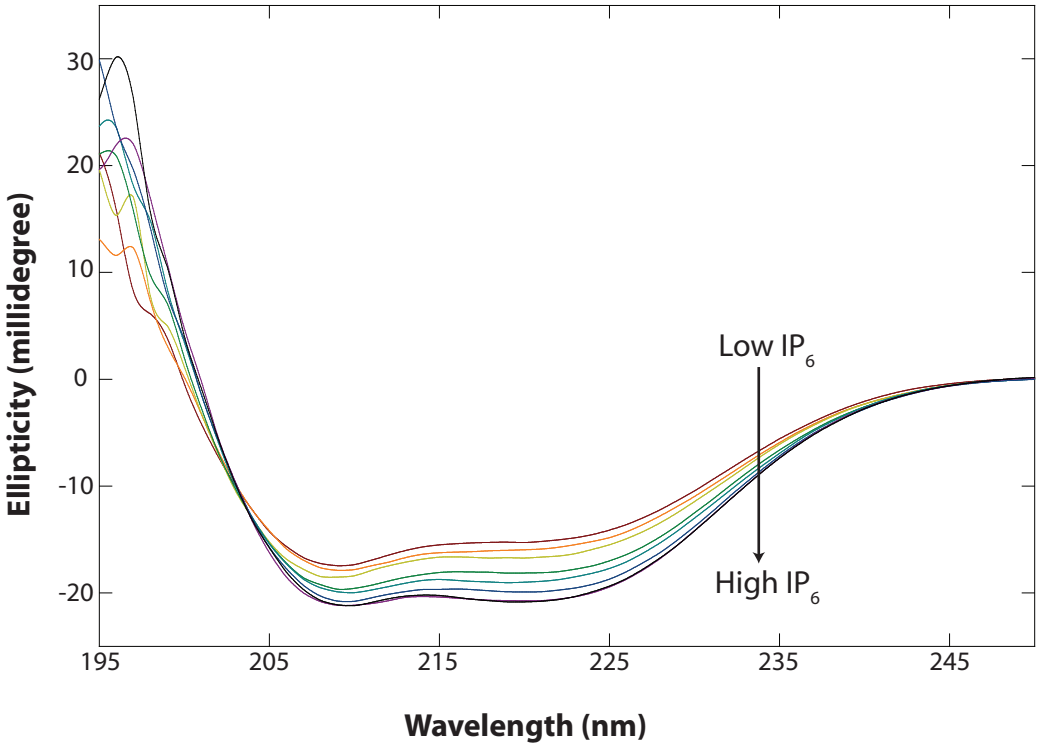

MEK + + + + + + + + +
YopJ - + + + + + + + +
IPx - - IP3 IP5 IP6
(nM) 10 100 5 10 100 250 100
MEK
YopJ
autoradiograph
1 2 3 4 5 6 7 8 9

**Figure S3**

**A**


30
20
10
0
-10
-20
195
205
215
225
235
245
Ellipticity (millidegree)
Wavelength (nm)
Low IP6
High IP6

**B**

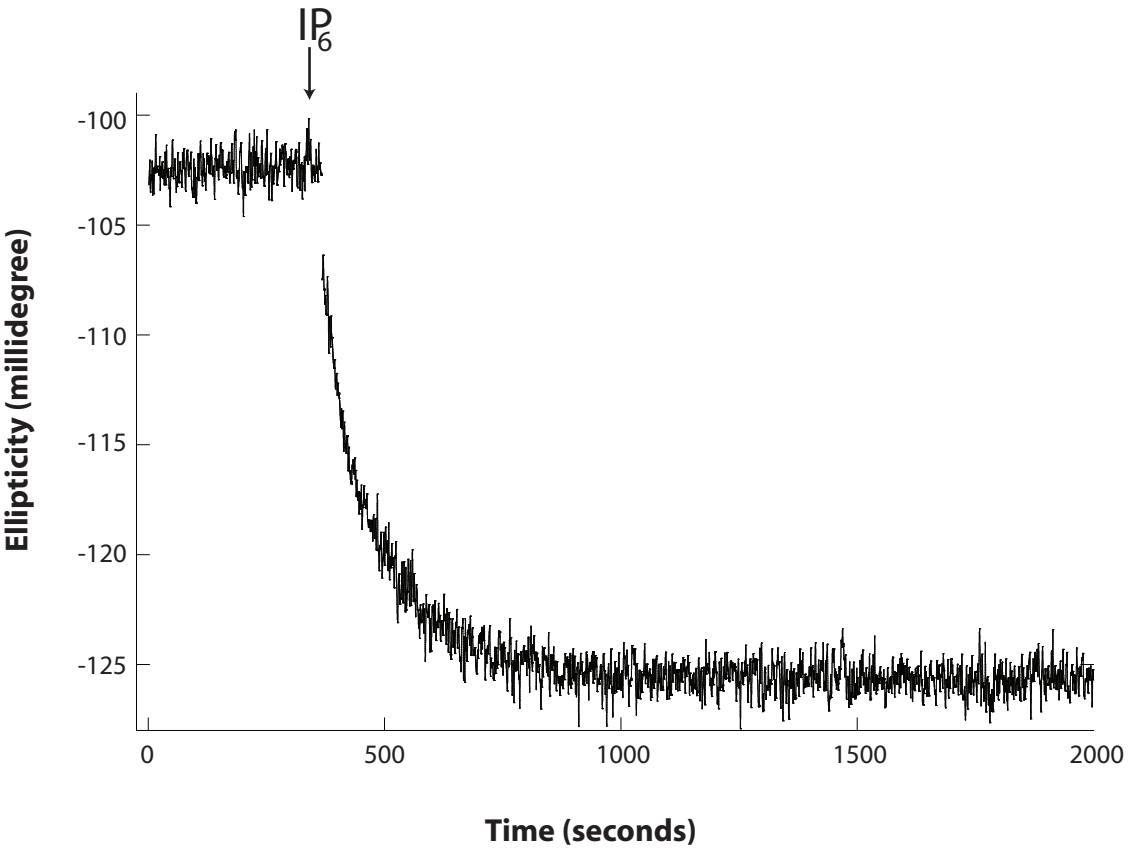

IP6
-100
-105
-110
-115
-120
-125
0
500
1000
1500
2000
Ellipticity (millidegree)
Time (seconds)

**Figure S4**