Supplementary Information

Figure S1: Linearity for T308 western blot. Using serially diluted lysates, we probed the linearity of densitometry as described in Materials and Methods.

Figure S2: Linearity for S473 western blot. Using serially diluted lysates, we probed the linearity of densitometry as described in Materials and Methods.

Figure S3: Linearity for Akt kinase activity assay in CHO-EGFR cells. Using serially diluted lysates, we probed the linearity of the Akt kinase activity assay as described in Materials and Methods.

Figure S4. An experimental strategy to quantify and correlate Akt phosphorylation and kinase activity. Two cell types, CHO-EGFR and HT-29, were individually stimulated with EGF (100 ng/ml) or insulin (INS, 500 ng/ml). At each of seven time points distributed throughout two hours, three lysates (biological replicates) per treatment condition were generated. From each lysate, a kinase activity assay and two quantitative western blot against T308 and S473 were performed. Quantification and normalization then allowed comparison between activity and phosphorylation time courses.

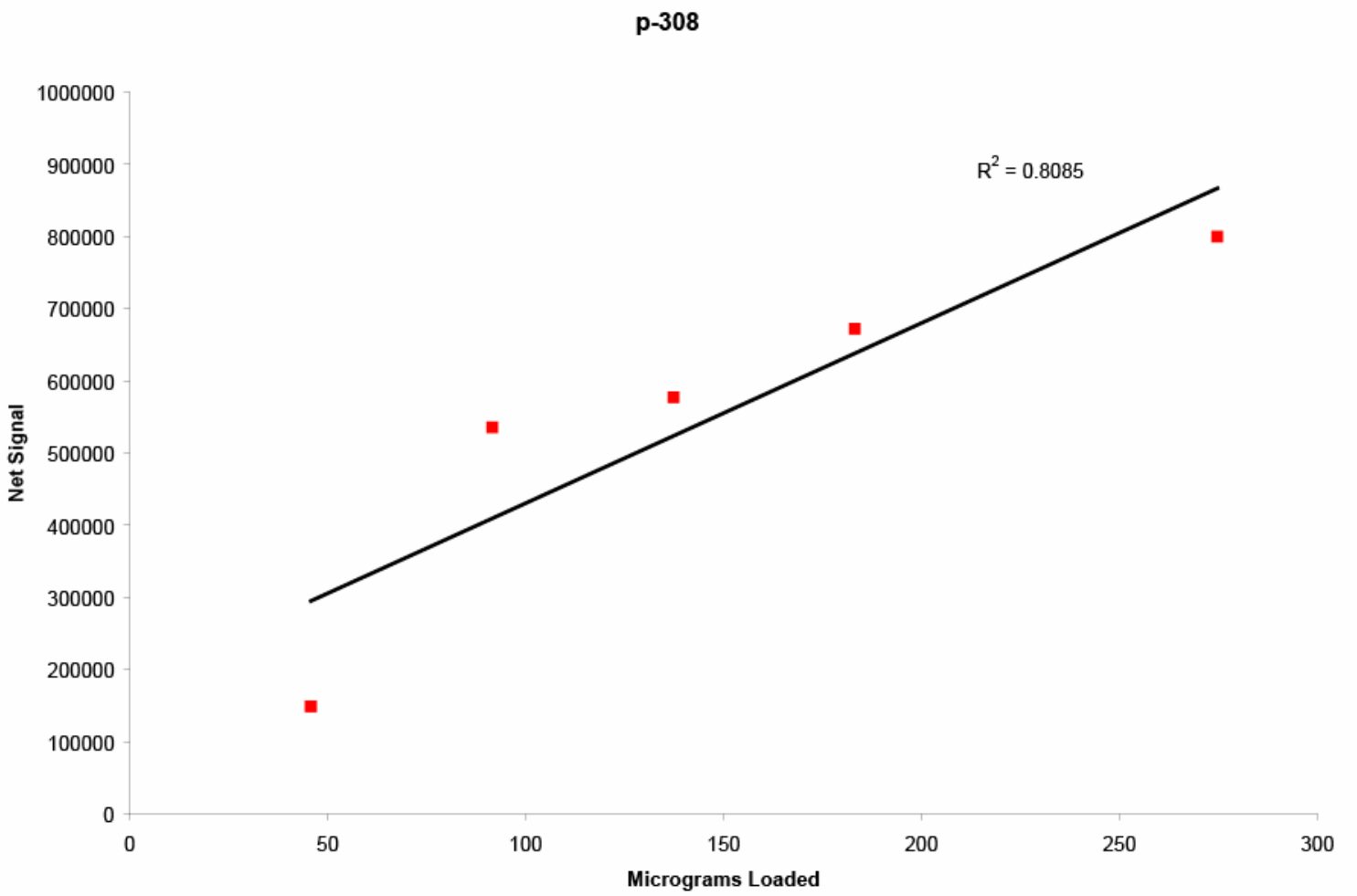
p-308
1000000
900000
800000
700000
600000
500000
400000
300000
200000
100000
0
Net Signal
R^2 = 0.8085
0
50
100
150
200
250
300
Micrograms Loaded

Figure S1

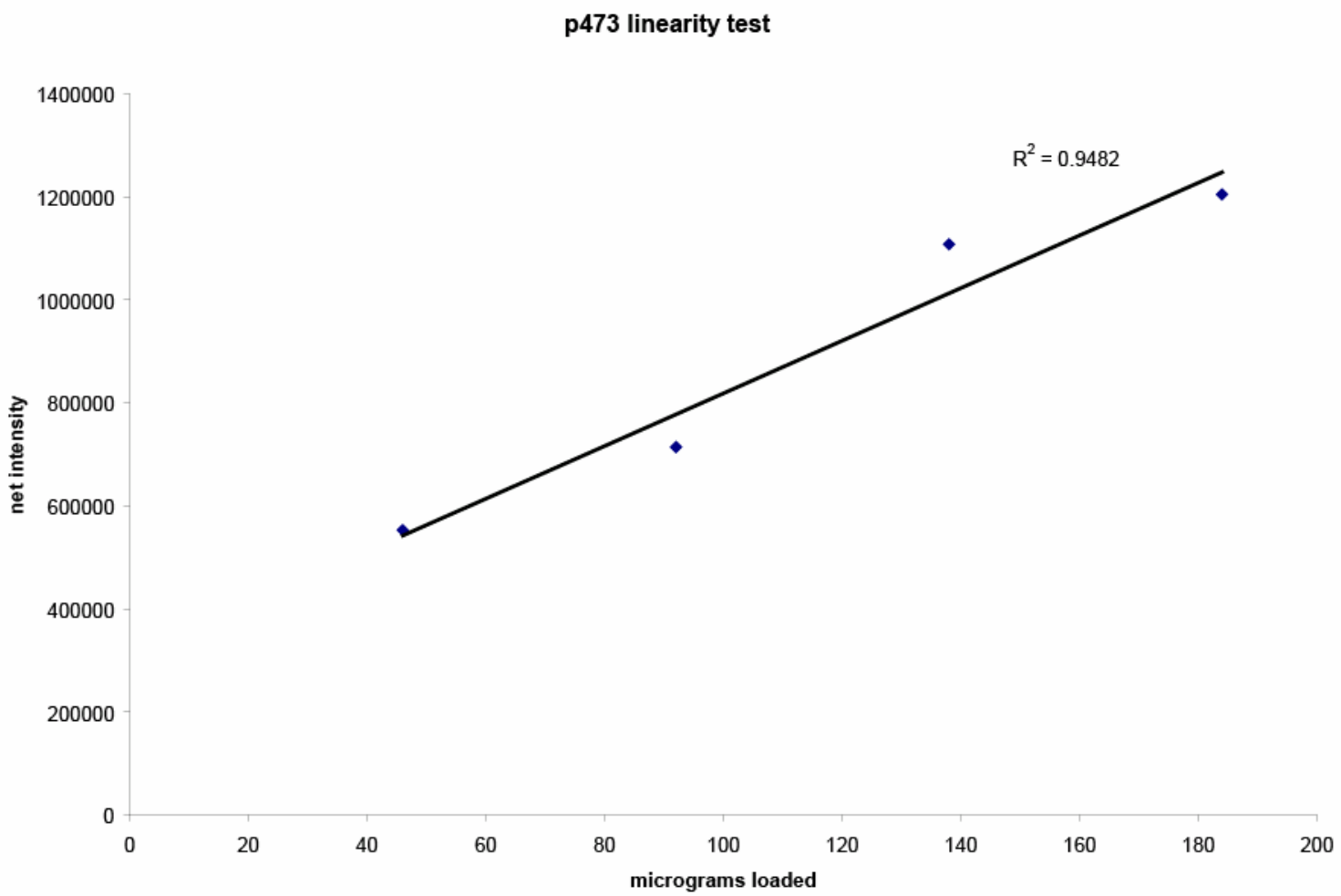
p473 linearity test
1400000
1200000
1000000
800000
600000
400000
200000
0
net intensity
R^2 = 0.9482
0
20
40
60
80
100
120
140
160
180
200
micrograms loaded

Figure S2

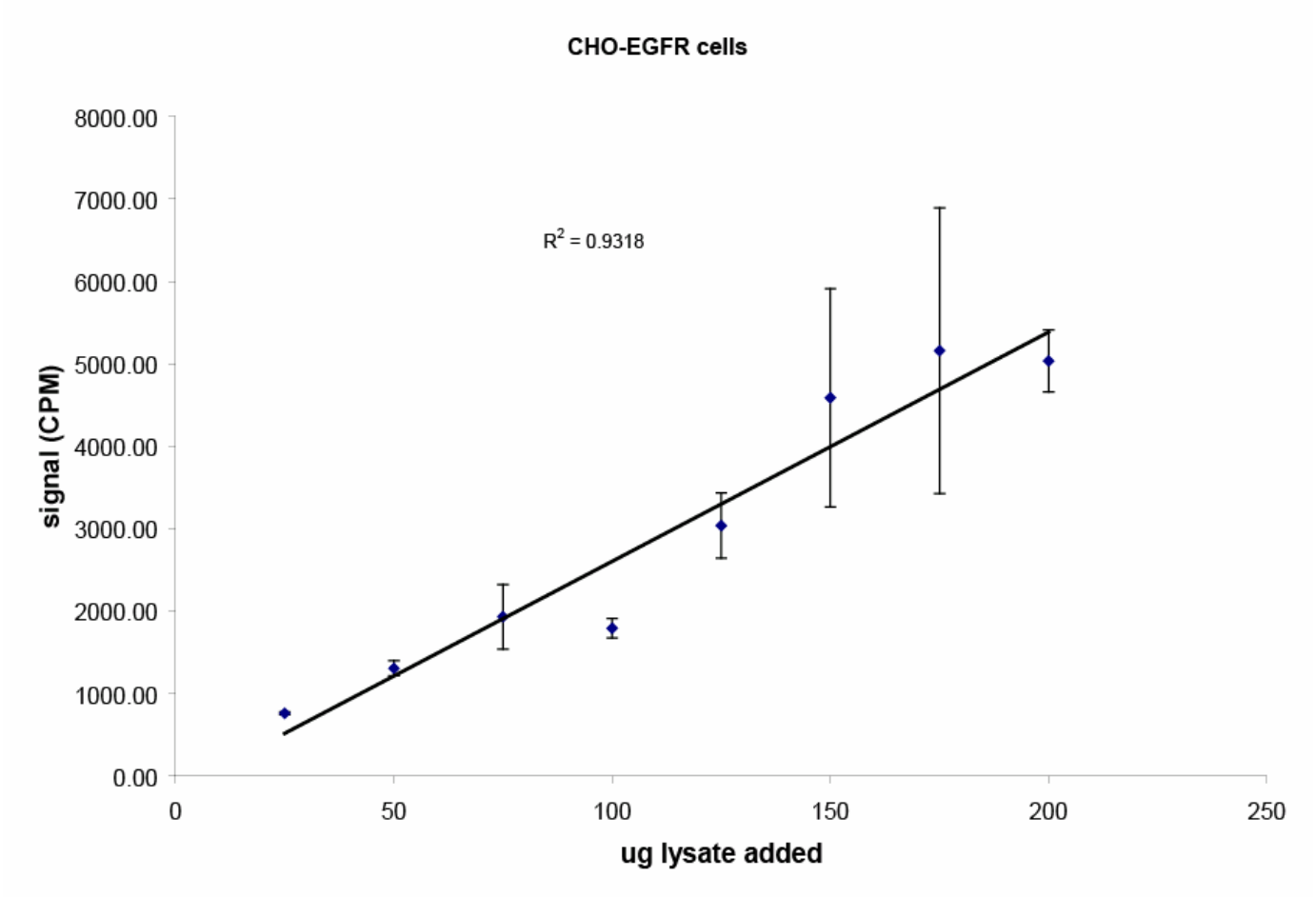
CHO-EGFR cells
8000.00
7000.00
6000.00
5000.00
4000.00
3000.00
2000.00
1000.00
0.00
signal (CPM)
R2 = 0.9318
0
50
100
150
200
250
ug lysate added

Figure S3

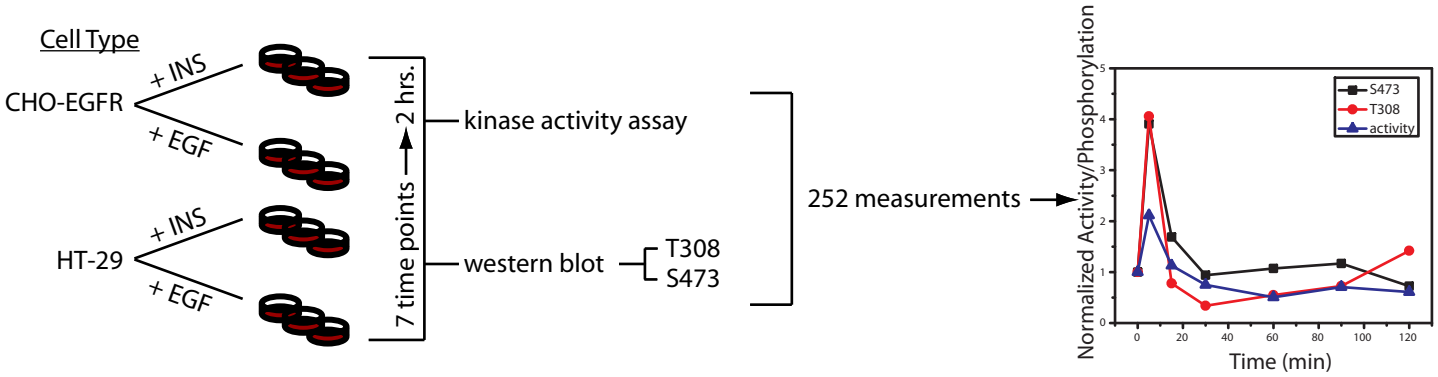
Cell Type
CHO-EGFR
+ INS
+ EGF
HT-29
+ INS
+ EGF
7 time points — 2 hrs.
kinase activity assay
western blot
T308
S473
252 measurements
Normalized Activity/Phosphorylation
Time (min)
S473
T308
activity

Figure S4