

Supplementary Figure S1. Effect of kinase inhibitors and QBI-139 on the viability of human melanoma cells. Cell viability was measured with a tetrazolium dye-based assay for metabolic activity. EC_{50} values are listed in the table.



Supplementary Figure S2. Expression and purification of biotinylated RI. Biotinylated RI was produced in HEK293T cells. Plasmids that direct the expression of BAP–RI and BirA were transfected transiently into cells. **A**, Immunoblot showing that a 1:1 ratio of Lipofectamine 3000 to DNA plasmids yielded more biotinylated RI than did a 1:2 or 2:1 ratio. **B**, Immunoblot showing that RI production was greater after transfection for 48 h than for 24 or 72 h. **C**, SDS–PAGE gel showing that RI production was greater after transfection for 48 h than for 24 or 72 h. **D**, SDS–PAGE gel showing human-derived RI (which is biotinylated) that was purified by chromatography using monomeric avidin agarose and an RNase A-affinity column. Elution of human-derived RI from an RNase A-affinity column required 3.5 M NaCI, whereas elution of *E. coli*-derived RI is phosphorylated upon incubation with a HEK293T cell lysate and [γ -³²P]ATP. Human angiogenin, which is known to be phosphorylated (Hoang TT, Raines RT. Molecular basis for the autonomous promotion of cell proliferation by angiogenin. Nucleic Acids Res 2017;45:818-31), serves as a positive control.







846.4

717.4

604.3

547.2

432.2

303.2

246.2

175.1

423.7

359.2

302.6

274.1

216.6

152.1

123.6

88.1

828.4

699.3

586.3

529.2

414.2

829.4

700.3

587.2

530.2

415.2

286.2

229.1

158.1

8

7

6

5

4 3

2

1

8

9

10

11

12

13

14

15

895.3

1,008.4

1,065.5

1,180.5

1,309.5

1,366.5

1,437.6

1,611.7

878.3

991.4

1,048.4

1,163.5

1,292.5

1,349.5

1,420.6

1,594.7

877.3

990.4

1,047.4

1,162.5

1,291.5

1,348.5

1,419.6

1,593.7

Ε

L

G

D

Ε

G

A

R

448.2

504.7

533.2

590.7

655.3

683.8

719.3

806.3



| в | B Ions | B+2H | B-NH3 | B-H2O | AA | Y Ions | Y+2H | Y-NH3 | Y-H2O | Y |
|----|---------|---------|---------|---------|------|---------|---------|---------|---------|----|
| 1 | 100.1 | 50.5 | | | ٧ | 3,141.4 | 1,571.2 | 3,124.4 | 3,123.4 | 28 |
| 2 | 213.2 | 107.1 | | | L | 3,042.4 | 1,521.7 | 3,025.3 | 3,024.4 | 27 |
| 3 | 399.2 | 200.1 | | | w | 2,929.3 | 1,465.1 | 2,912.3 | 2,911.3 | 26 |
| 4 | 512.3 | 256.7 | | | L | 2,743.2 | 1,372.1 | 2,726.2 | 2,725.2 | 25 |
| 5 | 583.4 | 292.2 | | | Α | 2,630.1 | 1,315.6 | 2,613.1 | 2,612.1 | 24 |
| 6 | 698.4 | 349.7 | | 680.4 | D | 2,559.1 | 1,280.0 | 2,542.1 | 2,541.1 | 23 |
| 7 | 858.4 | 429.7 | | 840.4 | C+57 | 2,444.1 | 1,222.5 | 2,427.0 | 2,426.0 | 22 |
| 8 | 973.4 | 487.2 | | 955.4 | D | 2,284.0 | 1,142.5 | 2,267.0 | 2,266.0 | 21 |
| 9 | 1,072.5 | 536.8 | | 1,054.5 | ٧ | 2,169.0 | 1,085.0 | 2,152.0 | 2,151.0 | 20 |
| 10 | 1,239.5 | 620.3 | | 1,221.5 | 5+80 | 2,069.9 | 1,035.5 | 2,052.9 | 2,051.9 | 19 |
| 11 | 1,354.5 | 677.8 | | 1,336.5 | D | 1,902.9 | 952.0 | 1,885.9 | 1,884.9 | 18 |
| 12 | 1,441.6 | 721.3 | | 1,423.6 | 5 | 1,787.9 | 894.5 | 1,770.9 | 1,769.9 | 17 |
| 13 | 1,528.6 | 764.8 | | 1,510.6 | 5 | 1,700.9 | 850.9 | 1,683.8 | 1,682.9 | 16 |
| 14 | 1,688.6 | 844.8 | | 1,670.6 | C+57 | 1,613.8 | 807.4 | 1,596.8 | 1,595.8 | 15 |
| 15 | 1,775.7 | 888.3 | | 1,757.7 | 5 | 1,453.8 | 727.4 | 1,436.8 | 1,435.8 | 14 |
| 16 | 1,862.7 | 931.9 | | 1,844.7 | 5 | 1,366.8 | 683.9 | 1,349.8 | 1,348.8 | 13 |
| 17 | 1,975.8 | 988.4 | | 1,957.8 | L | 1,279.7 | 640.4 | 1,262.7 | 1,261.7 | 12 |
| 18 | 2,046.8 | 1,023.9 | | 2,028.8 | Α | 1,166.7 | 583.8 | 1,149.6 | 1,148.7 | 11 |
| 19 | 2,117.9 | 1,059.4 | | 2,099.8 | Α | 1,095.6 | 548.3 | 1,078.6 | 1,077.6 | 10 |
| 20 | 2,218.9 | 1,110.0 | | 2,200.9 | т | 1,024.6 | 512.8 | 1,007.6 | 1,006.6 | 9 |
| 21 | 2,332.0 | 1,166.5 | | 2,314.0 | L | 923.5 | 462.3 | 906.5 | 905.5 | 8 |
| 22 | 2,445.1 | 1,223.0 | | 2,427.1 | L | 810.5 | 405.7 | 793.4 | 792.4 | 7 |
| 23 | 2,516.1 | 1,258.6 | | 2,498.1 | Α | 697.4 | 349.2 | 680.3 | 679.4 | 6 |
| 24 | 2,630.2 | 1,315.6 | 2,613.1 | 2,612.1 | N | 626.3 | 313.7 | 609.3 | 608.3 | 5 |
| 25 | 2,767.2 | 1,384.1 | 2,750.2 | 2,749.2 | н | 512.3 | 256.7 | 495.3 | 494.3 | 4 |
| 26 | 2,854.2 | 1,427.6 | 2,837.2 | 2,836.2 | 5 | 375.2 | 188.1 | 358.2 | 357.2 | 3 |
| 27 | 2,967.3 | 1,484.2 | 2,950.3 | 2,949.3 | L | 288.2 | 144.6 | 271.2 | | 2 |
| 28 | 3,141.4 | 1,571.2 | 3,124.4 | 3,123.4 | R | 175.1 | 88.1 | 158.1 | | 1 |



Supplementary Figure S3. MS/MS mass spectra of tryptic peptides from RI used to identify phosphorylation sites. Biotinylated RI was produced in HEK293T cells and purified with RNase A-affinity chromatography. Purified RI was digested with trypsin, and the ensuing peptides were analyzed to identify phosphorylation sites. Five sites were identified: Thr81, Ser177, Ser289, Ser382, and Ser405.

| | Ser177 | Ser289 | Ser405 |
|-------|------------------------------|------------------------------|-----------------------------|
| Human | KELTV <mark>S</mark> NNDINE. | SLKEL <mark>S</mark> LAGNEL. | RELDL <mark>S</mark> NNCLGD |
| Mouse | KELVL <mark>S</mark> NNDLHE. | SLKEL <mark>S</mark> LASNEL. | RELDL <mark>S</mark> NNCMGG |
| Rat | KELVL <mark>S</mark> NNDFHE. | SLKEL <mark>S</mark> LAGNEL. | RELDL <mark>S</mark> NNCMGD |
| Pig | KELTV <mark>S</mark> NNDIGE. | TLKEL <mark>S</mark> LAGNKL. | RELDL <mark>S</mark> NNCVGD |

Supplementary Figure S4. Amino acid sequences near phosphorylated residues of mammalian RIs. Phosphoryl groups on these residues interact favorably with a ptRNase (Fig. 5E).



Supplementary Figure S5. Proximity of a phosphoryl group on Ser405 of RI to the phosphoryl group-binding subsites in an RI·ptRNase complex. The image shows the superposition of the RNase A·d(ATAAG) complex (PDB entry 1rcn) on the RNase 1·RI complex (PDB entry 1z7x). RI is depicted as a gray ribbon; RNase 1 is depicted as lines with its three active-site residues (His12, Lys41, and His119) depicted as sticks. Only the phosphoryl groups from PDB entry 1rcn are shown. A phosphoryl group was installed computationally on O^{γ} of Ser405; for clarity, only its phosphorus atom is shown. Phosphorus atoms are shown as orange spheres. Dashed lines depict phosphorus–phosphorus distances, which are 8.5–11.5 Å. The image was made with the program PyMOL from Schrödinger (New York, NY).



Supplementary Figure S6. Effect of RI phosphorylation on its affinity for ANG. **A** and **B**, Images showing the proximity of a phosphoryl group on Ser289 of RI (panel **A**) or Ser405 of RI (panel **B**) to key residues of ANG in the RI·ANG complex. RI is depicted as a gray ribbon; ANG is depicted as a green ribbon. Ser87 is phosphorylated in ANG(1). A phosphoryl group was installed computationally on O^{Y} of the two serine residues of RI. The image was made with PDB entry 1a4y and the program PyMOL.



Supplementary Figure S7. Effect of RI phosphorylation on its oxidative stability. **A** and **B**, Images showing the proximity of a phosphoryl group on Thr81 or Ser382 to cysteine residues of RI. A phosphoryl group was installed computationally on O^v of Thr81 and Ser382. RI is depicted as a gray ribbon; ANG is depicted as a green ribbon. The image was made with PDB entry 1z7x and the program PyMOL. **C**, Heat map of the change in Coulombic interaction energy between each cysteine thiolate on RI and the rest of the protein upon phosphorylation at Thr81, Ser177, Ser289, Ser382, or Ser405. In general, phosphorylation hinders thiolate formation at cysteine residues, especially those proximal to the phosphorylation site.