

SUPPLEMENTARY TABLE S1A. RESULTS OF SINGLE STEP TANDEM AFFINITY PURIFICATIONS OF BOLA1

| GI | Peptides | Peptides | emPAI | emPAI | emPAI | Background | Protein |
|-----------|----------|----------|-------|-------|-----------|------------|--|
| | neg. | pos. | neg. | pos. | pos./neg. | peptides | |
| 42516576 | 0 | 3 | 0 | 3.64 | 9999 | 0 | Glutaredoxin 5 |
| 4826848 | 0 | 1 | 0 | 0.58 | 9999 | 1 | NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 5 |
| 11024700 | 0 | 1 | 0 | 0.46 | 9999 | 0 | Translocase of inner mitochondrial membrane 13 |
| 4758788 | 0 | 2 | 0 | 0.35 | 9999 | 3 | NADH dehydrogenase (ubiquinone) Fe-S protein 3, 30 kDa (NADH-coenzyme Q reductase) |
| 32483377 | 0 | 1 | 0 | 0.23 | 9999 | 15 | Peroxiredoxin 3 isoform b |
| 153791313 | 0 | 1 | 0 | 0.19 | 9999 | 0 | Cytochrome oxidase deficient homolog 2 |
| 4503607 | 0 | 1 | 0 | 0.16 | 9999 | 2 | Electron transfer flavoprotein, alpha polypeptide isoform a |
| 11321583 | 0 | 1 | 0 | 0.10 | 9999 | 2 | Succinate-CoA ligase, ADP-forming, beta subunit precursor |
| 7705638 | 4 | 11 | 2.72 | 99 | 36.29 | 0 | bolA-like 1 |
| 95113651 | 3 | 10 | 0.50 | 4.81 | 9.60 | 13 | Glutaredoxin 3 |
| 32189392 | 1 | 3 | 0.25 | 0.99 | 3.84 | 17 | Peroxiredoxin 2 isoform a |
| 16596694 | 1 | 2 | 0.46 | 1.15 | 2.46 | 0 | Mitochondrial ribosomal protein L53 |
| 183227678 | 1 | 1 | 0.33 | 0.77 | 2.33 | 31 | Parkinson disease protein 7 |
| 171906593 | 2 | 3 | 0.31 | 0.71 | 2.31 | 4 | Branched chain ketoacid dehydrogenase kinase isoform b |
| 4507231 | 1 | 2 | 0.29 | 0.66 | 2.29 | 20 | Single-stranded DNA binding protein 1 |
| 31542947 | 8 | 13 | 1.27 | 2.72 | 2.13 | 181 | Chaperonin |
| 46593007 | 1 | 2 | 0.11 | 0.24 | 2.11 | 1 | Ubiquinol-cytochrome c reductase core protein I |
| 21735621 | 3 | 5 | 0.43 | 0.83 | 1.89 | 63 | Mitochondrial malate dehydrogenase precursor |
| 155722983 | 5 | 8 | 0.40 | 0.71 | 1.78 | 38 | TNF receptor-associated protein 1 |
| 4885079 | 2 | 3 | 0.42 | 0.70 | 1.64 | 13 | ATP synthase, H ⁺ transporting, mitochondrial F1 complex, gamma subunit isoform H (heart) precursor |
| 4757732 | 4 | 6 | 0.37 | 0.61 | 1.63 | 19 | Programmed cell death 8 isoform 1 |
| 21361114 | 2 | 3 | 0.29 | 0.46 | 1.60 | 27 | Solute carrier family 25 (mitochondrial carrier; oxoglutarate carrier), member 11 |
| 4505773 | 6 | 7 | 1.37 | 2.16 | 1.57 | 77 | Prohibitin |
| 4758638 | 4 | 5 | 1.03 | 1.42 | 1.38 | 48 | Peroxiredoxin 6 |
| 4502491 | 4 | 5 | 0.93 | 1.27 | 1.37 | 39 | Complement component 1, q subcomponent binding protein precursor |
| 47132595 | 4 | 5 | 0.66 | 0.89 | 1.34 | 32 | Solute carrier family 25 member 3 isoform b precursor |
| 11386135 | 14 | 15 | 5.06 | 6.40 | 1.26 | 33 | Branched chain keto acid dehydrogenase E1, alpha polypeptide |
| 70995211 | 5 | 5 | 1.25 | 1.58 | 1.26 | 17 | Peroxisomal enoyl-coenzyme A hydratase-like protein |
| 65506442 | 32 | 33 | 10.93 | 13.25 | 1.21 | 288 | Propionyl-Coenzyme A carboxylase, alpha polypeptide isoform a precursor |
| 32189394 | 8 | 8 | 0.93 | 1.09 | 1.17 | 118 | ATP synthase, H ⁺ transporting, mitochondrial F1 complex, beta subunit precursor |
| 41872631 | 10 | 10 | 0.22 | 0.25 | 1.11 | 160 | Fatty acid synthase |
| 119943100 | 23 | 21 | 5.89 | 5.89 | 1 | 209 | Propionyl Coenzyme A carboxylase, beta polypeptide |
| 4557353 | 8 | 8 | 3.21 | 3.21 | 1 | 12 | Branched chain keto acid dehydrogenase E1 beta polypeptide precursor |
| 24234688 | 17 | 17 | 2.51 | 2.51 | 1 | 137 | Heat shock 70 kDa protein 9 precursor |
| 50345984 | 13 | 13 | 2.30 | 2.30 | 1 | 99 | ATP synthase, H ⁺ transporting, mitochondrial F1 complex, alpha subunit precursor |
| 94538322 | 4 | 4 | 1.27 | 1.27 | 1 | 4 | Hydroxyacyl glutathione hydrolase isoform 1 |
| 4505591 | 3 | 3 | 0.77 | 0.77 | 1 | 78 | Peroxiredoxin 1 |
| 5453607 | 4 | 3 | 0.55 | 0.55 | 1 | 56 | Chaperonin containing TCP1, subunit 7 isoform a |
| 34147630 | 4 | 4 | 0.42 | 0.42 | 1 | 40 | Tu translation elongation factor, mitochondrial precursor |
| 4758504 | 2 | 2 | 0.42 | 0.42 | 1 | 25 | Hydroxysteroid (17-beta) dehydrogenase 10 isoform 1 |
| 50592994 | 1 | 1 | 0.38 | 0.38 | 1 | 26 | Thioredoxin |

(continued)

SUPPLEMENTARY TABLE S1A. (CONTINUED)

| <i>GI</i> | <i>Peptides neg.</i> | <i>Peptides pos.</i> | <i>emPAI neg.</i> | <i>emPAI pos.</i> | <i>emPAI pos./neg.</i> | <i>Background peptides</i> | <i>Protein</i> |
|-----------|--------------------------|--------------------------|-----------------------|-----------------------|----------------------------|--------------------------------|---|
| 13376007 | 1 | 1 | 0.25 | 0.25 | 1 | 0 | Isochorismatase domain containing 2 isoform 2 |
| 13129148 | 1 | 1 | 0.25 | 0.25 | 1 | 0 | Apolipoprotein O |
| 8923390 | 1 | 1 | 0.23 | 0.23 | 1 | 3 | Coiled-coil-helix-coiled-coil-helix domain containing 3 |
| 4557237 | 2 | 2 | 0.22 | 0.22 | 1 | 11 | Acetyl-Coenzyme A acetyltransferase 1 precursor |
| 38569421 | 4 | 4 | 0.18 | 0.18 | 1 | 31 | ATP citrate lyase isoform 1 |
| 4502013 | 1 | 1 | 0.17 | 0.17 | 1 | 1 | Adenylate kinase 2 isoform a |
| 5031815 | 2 | 2 | 0.17 | 0.17 | 1 | 3 | Lysyl-tRNA synthetase isoform 2 |
| 42476028 | 2 | 2 | 0.17 | 0.17 | 1 | 0 | ATPase family, AAA domain containing 3A |
| 21361565 | 1 | 1 | 0.15 | 0.15 | 1 | 8 | ATP synthase, H ⁺ transporting, mitochondrial F0 complex, subunit B1 precursor |

We generated HEK293 T-REx cells that inducibly express the proteins with a C-terminal TAP-tagged BOLA1. Cell lysates were affinity purified and eluates were analyzed using nanospray ionization liquid chromatography tandem mass spectrometry (nLC-MS/MS) to identify interacting proteins. The procedure was carried out without induction (neg.) as well as 24 h after antibiotic induction (pos.) of TAP-tagged protein. The list is limited to proteins in mitochondrial compendium (11). Background peptides denote number of peptides of the protein copurified with TAP-tagged control proteins. Proteins that were not copurified with control proteins and only copurified after antibiotic induction are marked with bold typeface. GI, Protein Genbank Identifier Accession; emPAI, exponentially modified Protein Abundance Index.