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Richard I. Odle,<sup>1,\*</sup>

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### CDK1-Dependent Phosphorylation of Multiple Autophagy Regulators during Mitosis

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Mitotic Phosphorylation of Autophagy Regulators Is Independent of mTORC1

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 $4 - 1 - \delta = 10 - 3$   
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 $474$  ( $\dots, 2007$ ) ( $\dots, 5$ )  
 $620$   
 $8055$  ( $620:8055$ )  
 $(\dots, 2014)$   
 $(\dots, 5)$   $389$   $6$   
 $(\dots, 4)$   
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 $8055$  ( $\dots, 4$ )  
 $\Delta 30$   
 $(\dots, 2013)$   $(\dots, 2012)$   
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 $\Delta 30$  ( $\dots, 5$ )  
 $(\dots, 4)$   
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 $8055$  ( $\dots, 5$ )  
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 $(\dots, 4)$   $\Delta 30$   
 $(\dots, 4)$   $\Delta$



### CDK1-Dependent Mitotic Phosphorylation of Autophagy Regulators Occurs at Known Repressive Sites

13 194 282 ( 259),  
1 706 827 ( 758), 14 348 470 ( 383 440),  
76 160 ( 122 142).  
*in vitro*,  
-3306 (300 L) 6102 (500 L) ( 6 ).  
*in vitro* 13,

(2013, 6; 2013, 4). (2013, 5), (2013, 1) (2013, 2). (2013, 13), (2013, 224), (2013, 258), (2013, 259), (2013, 1), (2013, 259), (2013, 224), (2013, 13), (2013, 8055), (2013, 8055), (2013, 259).

Table 1. ATG13 Is Phosphorylated at Known Repressive Sites during Mitosis

Protein	Site	Reference
ATG13	8055	(Figure 6)
ATG13	8055 <sup>+</sup>	(Figure 7)

116 (Figure 6).  
 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 264, 265, 266, 267, 268, 269, 270, 271, 272, 273, 274, 275, 276, 277, 278, 279, 280, 281, 282, 283, 284, 285, 286, 287, 288, 289, 290, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300, 301, 302, 303, 304, 305, 306, 307, 308, 309, 310, 311, 312, 313, 314, 315, 316, 317, 318, 319, 320, 321, 322, 323, 324, 325, 326, 327, 328, 329, 330, 331, 332, 333, 334, 335, 336, 337, 338, 339, 340, 341, 342, 343, 344, 345, 346, 347, 348, 349, 350, 351, 352, 353, 354, 355, 356, 357, 358, 359, 360, 361, 362, 363, 364, 365, 366, 367, 368, 369, 370, 371, 372, 373, 374, 375, 376, 377, 378, 379, 380, 381, 382, 383, 384, 385, 386, 387, 388, 389, 390, 391, 392, 393, 394, 395, 396, 397, 398, 399, 400, 401, 402, 403, 404, 405, 406, 407, 408, 409, 410, 411, 412, 413, 414, 415, 416, 417, 418, 419, 420, 421, 422, 423, 424, 425, 426, 427, 428, 429, 430, 431, 432, 433, 434, 435, 436, 437, 438, 439, 440, 441, 442, 443, 444, 445, 446, 447, 448, 449, 450, 451, 452, 453, 454, 455, 456, 457, 458, 459, 460, 461, 462, 463, 464, 465, 466, 467, 468, 469, 470, 471, 472, 473, 474, 475, 476, 477, 478, 479, 480, 481, 482, 483, 484, 485, 486, 487, 488, 489, 490, 491, 492, 493, 494, 495, 496, 497, 498, 499, 500, 501, 502, 503, 504, 505, 506, 507, 508, 509, 510, 511, 512, 513, 514, 515, 516, 517, 518, 519, 520, 521, 522, 523, 524, 525, 526, 527, 528, 529, 530, 531, 532, 533, 534, 535, 536, 537, 538, 539, 540, 541, 542, 543, 544, 545, 546, 547, 548, 549, 550, 551, 552, 553, 554, 555, 556, 557, 558, 559, 560, 561, 562, 563, 564, 565, 566, 567, 568, 569, 570, 571, 572, 573, 574, 575, 576, 577, 578, 579, 580, 581, 582, 583, 584, 585, 586, 587, 588, 589, 590, 591, 592, 593, 594, 595, 596, 597, 598, 599, 600, 601, 602, 603, 604, 605, 606, 607, 608, 609, 610, 611, 612, 613, 614, 615, 616, 617, 618, 619, 620, 621, 622, 623, 624, 625, 626, 627, 628, 629, 630, 631, 632, 633, 634, 635, 636, 637, 638, 639, 640, 641, 642, 643, 644, 645, 646, 647, 648, 649, 650, 651, 652, 653, 654, 655, 656, 657, 658, 659, 660, 661, 662, 663, 664, 665, 666, 667, 668, 669, 670, 671, 672, 673, 674, 675, 676, 677, 678, 679, 680, 681, 682, 683, 684, 685, 686, 687, 688, 689, 690, 691, 692, 693, 694, 695, 696, 697, 698, 699, 700, 701, 702, 703, 704, 705, 706, 707, 708, 709, 710, 711, 712, 713, 714, 715, 716, 717, 718, 719, 720, 721, 722, 723, 724, 725, 726, 727, 728, 729, 730, 731, 732, 733, 734, 735, 736, 737, 738, 739, 740, 741, 742, 743, 744, 745, 746, 747, 748, 749, 750, 751, 752, 753, 754, 755, 756, 757, 758, 759, 760, 761, 762, 763, 764, 765, 766, 767, 768, 769, 770, 771, 772, 773, 774, 775, 776, 777, 778, 779, 780, 781, 782, 783, 784, 785, 786, 787, 788, 789, 790, 791, 792, 793, 794, 795, 796, 797, 798, 799, 800, 801, 802, 803, 804, 805, 806, 807, 808, 809, 810, 811, 812, 813, 814, 815, 816, 817, 818, 819, 820, 821, 822, 823, 824, 825, 826, 827, 828, 829, 830, 831, 832, 833, 834, 835, 836, 837, 838, 839, 840, 841, 842, 843, 844, 845, 846, 847, 848, 849, 850, 851, 852, 853, 854, 855, 856, 857, 858, 859, 860, 861, 862, 863, 864, 865, 866, 867, 868, 869, 870, 871, 872, 873, 874, 875, 876, 877, 878, 879, 880, 881, 882, 883, 884, 885, 886, 887, 888, 889, 890, 891, 892, 893, 894, 895, 896, 897, 898, 899, 900, 901, 902, 903, 904, 905, 906, 907, 908, 909, 910, 911, 912, 913, 914, 915, 916, 917, 918, 919, 920, 921, 922, 923, 924, 925, 926, 927, 928, 929, 930, 931, 932, 933, 934, 935, 936, 937, 938, 939, 940, 941, 942, 943, 944, 945, 946, 947, 948, 949, 950, 951, 952, 953, 954, 955, 956, 957, 958, 959, 960, 961, 962, 963, 964, 965, 966, 967, 968, 969, 970, 971, 972, 973, 974, 975, 976, 977, 978, 979, 980, 981, 982, 983, 984, 985, 986, 987, 988, 989, 990, 991, 992, 993, 994, 995, 996, 997, 998, 999, 1000.

DISCUSSION

293, 306, 319, 330, 343, 356, 369, 382, 395, 408, 421, 434, 447, 460, 473, 486, 499, 512, 525, 538, 551, 564, 577, 590, 603, 616, 629, 642, 655, 668, 681, 694, 707, 720, 733, 746, 759, 772, 785, 798, 811, 824, 837, 850, 863, 876, 889, 902, 915, 928, 941, 954, 967, 980, 993, 1006, 1019, 1032, 1045, 1058, 1071, 1084, 1097, 1110, 1123, 1136, 1149, 1162, 1175, 1188, 1201, 1214, 1227, 1240, 1253, 1266, 1279, 1292, 1305, 1318, 1331, 1344, 1357, 1370, 1383, 1396, 1409, 1422, 1435, 1448, 1461, 1474, 1487, 1500, 1513, 1526, 1539, 1552, 1565, 1578, 1591, 1604, 1617, 1630, 1643, 1656, 1669, 1682, 1695, 1708, 1721, 1734, 1747, 1760, 1773, 1786, 1799, 1812, 1825, 1838, 1851, 1864, 1877, 1890, 1903, 1916, 1929, 1942, 1955, 1968, 1981, 1994, 2007, 2020, 2033, 2046, 2059, 2072, 2085, 2098, 2111, 2124, 2137, 2150, 2163, 2176, 2189, 2202, 2215, 2228, 2241, 2254, 2267, 2280, 2293, 2306, 2319, 2332, 2345, 2358, 2371, 2384, 2397, 2410, 2423, 2436, 2449, 2462, 2475, 2488, 2501, 2514, 2527, 2540, 2553, 2566, 2579, 2592, 2605, 2618, 2631, 2644, 2657, 2670, 2683, 2696, 2709, 2722, 2735, 2748, 2761, 2774, 2787, 2800, 2813, 2826, 2839, 2852, 2865, 2878, 2891, 2904, 2917, 2930, 2943, 2956, 2969, 2982, 2995, 3008, 3021, 3034, 3047, 3060, 3073, 3086, 3099, 3112, 3125, 3138, 3151, 3164, 3177, 3190, 3203, 3216, 3229, 3242, 3255, 3268, 3281, 3294, 3307, 3320, 3333, 3346, 3359, 3372, 3385, 3398, 3411, 3424, 3437, 3450, 3463, 3476, 3489, 3502, 3515, 3528, 3541, 3554, 3567, 3580, 3593, 3606, 3619, 3632, 3645, 3658, 3671, 3684, 3697, 3710, 3723, 3736, 3749, 3762, 3775, 3788, 3801, 3814, 3827, 3840, 3853, 3866, 3879, 3892, 3905, 3918, 3931, 3944, 3957, 3970, 3983, 3996, 4009, 4022, 4035, 4048, 4061, 4074, 4087, 4100, 4113, 4126, 4139, 4152, 4165, 4178, 4191, 4204, 4217, 4230, 4243, 4256, 4269, 4282, 4295, 4308, 4321, 4334, 4347, 4360, 4373, 4386, 4399, 4412, 4425, 4438, 4451, 4464, 4477, 4490, 4503, 4516, 4529, 4542, 4555, 4568, 4581, 4594, 4607, 4620, 4633, 4646, 4659, 4672, 4685, 4698, 4711, 4724, 4737, 4750, 4763, 4776, 4789, 4802, 4815, 4828, 4841, 4854, 4867, 4880, 4893, 4906, 4919, 4932, 4945, 4958, 4971, 4984, 4997, 5010, 5023, 5036, 5049, 5062, 5075, 5088, 5101, 5114, 5127, 5140, 5153, 5166, 5179, 5192, 5205, 5218, 5231, 5244, 5257, 5270, 5283, 5296, 5309, 5322, 5335, 5348, 5361, 5374, 5387, 5400, 5413, 5426, 5439, 5452, 5465, 5478, 5491, 5504, 5517, 5530, 5543, 5556, 5569, 5582, 5595, 5608, 5621, 5634, 5647, 5660, 5673, 5686, 5699, 5712, 5725, 5738, 5751, 5764, 5777, 5790, 5803, 5816, 5829, 5842, 5855, 5868, 5881, 5894, 5907, 5920, 5933, 5946, 5959, 5972, 5985, 5998, 6011, 6024, 6037, 6050, 6063, 6076, 6089, 6102, 6115, 6128, 6141, 6154, 6167, 6180, 6193, 6206, 6219, 6232, 6245, 6258, 6271, 6284, 6297, 6310, 6323, 6336, 6349, 6362, 6375, 6388, 6401, 6414, 6427, 6440, 6453, 6466, 6479, 6492, 6505, 6518, 6531, 6544, 6557, 6570, 6583, 6596, 6609, 6622, 6635, 6648, 6661, 6674, 6687, 6700, 6713, 6726, 6739, 6752, 6765, 6778, 6791, 6804, 6817, 6830, 6843, 6856, 6869, 6882, 6895, 6908, 6921, 6934, 6947, 6960, 6973, 6986, 6999, 7012, 7025, 7038, 7051, 7064, 7077, 7090, 7103, 7116, 7129, 7142, 7155, 7168, 7181, 7194, 7207, 7220, 7233, 7246, 7259, 7272, 7285, 7298, 7311, 7324, 7337, 7350, 7363, 7376, 7389, 7402, 7415, 7428, 7441, 7454, 7467, 7480, 7493, 7506, 7519, 7532, 7545, 7558, 7571, 7584, 7597, 7610, 7623, 7636, 7649, 7662, 7675, 7688, 7701, 7714, 7727, 7740, 7753, 7766, 7779, 7792, 7805, 7818, 7831, 7844, 7857, 7870, 7883, 7896, 7909, 7922, 7935, 7948, 7961, 7974, 7987, 7999, 8012, 8025, 8038, 8051, 8064, 8077, 8090, 8103, 8116, 8129, 8142, 8155, 8168, 8181, 8194, 8207, 8220, 8233, 8246, 8259, 8272, 8285, 8298, 8311, 8324, 8337, 8350, 8363, 8376, 8389, 8402, 8415, 8428, 8441, 8454, 8467, 8480, 8493, 8506, 8519, 8532, 8545, 8558, 8571, 8584, 8597, 8610, 8623, 8636, 8649, 8662, 8675, 8688, 8701, 8714, 8727, 8740, 8753, 8766, 8779, 8792, 8805, 8818, 8831, 8844, 8857, 8870, 8883, 8896, 8909, 8922, 8935, 8948, 8961, 8974, 8987, 9000, 9013, 9026, 9039, 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12900, 12913, 12926, 12939, 12952, 12965, 12978, 12991, 13004, 13017, 13030, 13043, 13056, 13069, 13082, 13095, 13108, 13121, 13134, 13147, 13160, 13173, 13186, 13199, 13212, 13225, 13238, 13251, 13264, 13277, 13290, 13303, 13316, 13329, 13342, 13355, 13368, 13381, 13394, 13407, 13420, 13433, 13446, 13459, 13472, 13485, 13498, 13511, 13524, 13537, 13550, 13563, 13576, 13589, 13602, 13615, 13628, 13641, 13654, 13667, 13680, 13693, 13706, 13719, 13732, 13745, 13758, 13771, 13784, 13797, 13810, 13823, 13836, 13849, 13862, 13875, 13888, 13901, 13914, 13927, 13940, 13953, 13966, 13979, 13992, 14005, 14018, 14031, 14044, 14057, 14070, 14083, 14096, 14109, 14122, 14135, 14148, 14161, 14174, 14187, 14200, 14213, 14226, 14239, 14252, 14265, 14278, 14291, 14304, 14317, 14330, 14343, 14356, 14369, 14382, 14395, 14408, 14421, 14434, 14447, 14460, 14473, 14486, 14499, 14512, 14525, 14538, 14551, 14564, 14577, 14590, 14603, 14616, 14629, 14642, 14655, 14668, 14681, 14694, 14707, 14720, 14733, 14746, 14759, 14772, 14785, 14798, 14811, 14824, 14837, 14850, 14863, 14876, 14889, 14902, 14915, 14928, 14941, 14954, 14967, 14980, 14993, 15006, 15019,

Figure 1. Schematic diagram of the STAR method.

### STAR★METHODS

Figure 2. Schematic diagram of the STAR method.

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התאחדות המורים (2009). *התאחדות המורים*. תל אביב: הוצאת המורים, 10.





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LEAD CONTACT AND MATERIALS AVAILABILITY

Treatments

(2<sup>-1</sup>) 2

Western blot

(50 L, 8, 150 L, 1%, -100, 1%, 0.1%, 50 L, 1 L, 3, 4, 1 L, 5, 10, 13,000, 10, 4°)

(20 L, 7.6; 137 L, 1%; -100; 10% ; 1 L ; 1.5 L L ; 2; 50 L ; 1 L ; 3, 4; 1 L L ; 10 ; 5)

300 100 5% L

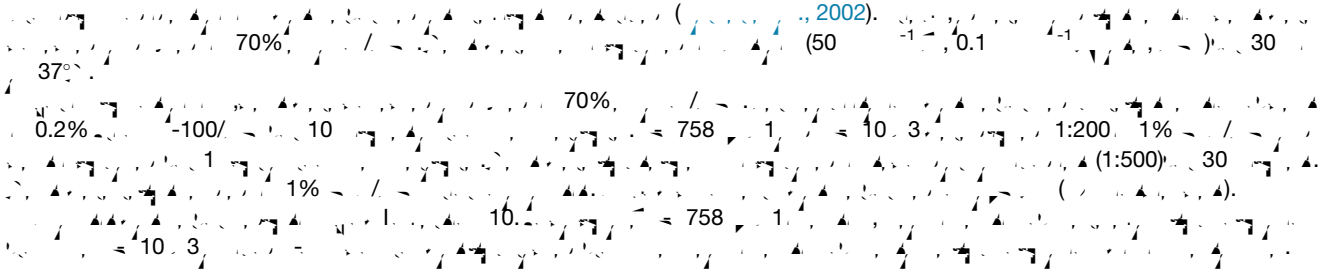
6

Immunoprecipitation

5% 10%

4682992 7240 ( ) 1 ; ( 256 8

## Flow cytometry



## Recombinant protein expression

Recombinant protein expression of TFEB (76-160), ATG13 (194-282), ULK1 (706-827), and ATG14 (348-470) was performed in *E. coli* using the pET system. The proteins were purified by Ni-NTA affinity chromatography and quantified by Bradford assay. The protein concentrations were 10, 13, 14, and 1 mg/ml, respectively.

### TFEB (76-160)

TFEB (76-160) was expressed in *E. coli* using the pET system. The protein was purified by Ni-NTA affinity chromatography and quantified by Bradford assay. The protein concentration was 10 mg/ml.

### ATG13 194-282

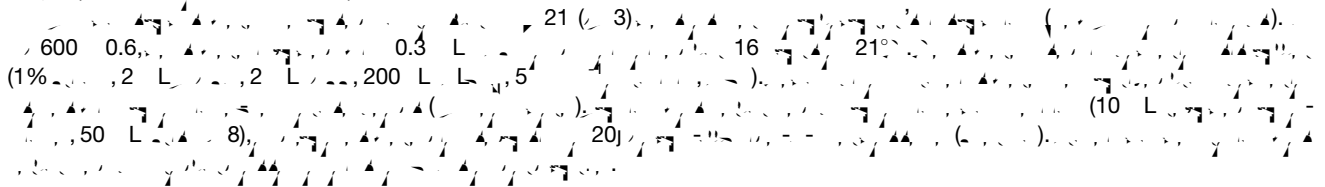
ATG13 194-282 was expressed in *E. coli* using the pET system. The protein was purified by Ni-NTA affinity chromatography and quantified by Bradford assay. The protein concentration was 13 mg/ml.

### ULK1 (706-827)

ULK1 (706-827) was expressed in *E. coli* using the pET system. The protein was purified by Ni-NTA affinity chromatography and quantified by Bradford assay. The protein concentration was 14 mg/ml.

### ATG14 (348-470)

ATG14 (348-470) was expressed in *E. coli* using the pET system. The protein was purified by Ni-NTA affinity chromatography and quantified by Bradford assay. The protein concentration was 1 mg/ml.



## CCNB1-CDK1 kinase assay



## QUANTIFICATION AND STATISTICAL ANALYSIS

Quantification and statistical analysis were performed using ImageJ (v1.52r) and GraphPad Prism (v8.0.2). Statistical significance was determined using a two-tailed t-test. Error bars represent standard deviation. Statistical significance is indicated by asterisks: \*  $p < 0.05$  (\*), \*\*  $p < 0.01$  (\*\*), \*\*\*  $p < 0.001$  (\*\*\*), \*\*\*\*  $p < 0.0001$  (\*\*\*\*).

## DATA AND CODE AVAILABILITY

Source data and code are available at <https://doi.org/10.17632/9kx9-1>.