

**Additional file 4.** Supplementary tables.

| Gene Name               | Unique peptides | LFQ intensity | Coverage [%] | Molecular Weight [kDa] | Accession |
|-------------------------|-----------------|---------------|--------------|------------------------|-----------|
| Importin-5              | 36              | 3.34E+09      | 45.1         | 123.63                 | O00410    |
| Importin subunit beta-1 | 18              | 1.00E+09      | 26.5         | 97.169                 | Q14974    |
| Annexin A2              | 7               | 2.96E+08      | 25.7         | 38.604                 | P07355    |
| Importin-7              | 20              | 6.80E+08      | 23.7         | 119.52                 | O95373    |
| Importin-9              | 12              | 3.05 E+08     | 13.7         | 115.96                 | Q96P70    |

**Supplementary Table 1.** List of top 5 candidates of RalA-interacting proteins that were identified by co-immunoprecipitation and MS.

| Types                      | Distance | From                     | To                       |
|----------------------------|----------|--------------------------|--------------------------|
| Salt Bridge                | 2.74436  | ANNEXIN<br>A2:ARG37:NH2  | RALA:ASP133:OD2          |
| Salt Bridge                | 3.09922  | ANNEXIN<br>A2:ARG304:NH1 | RALA:GLU141:OE2          |
| Salt Bridge                | 3.19641  | ANNEXIN<br>A2:LYS313:NZ  | RALA:ASP106:OD1          |
| Salt Bridge                | 3.87825  | ANNEXIN<br>A2:LYS313:NZ  | RALA:GLU109:OE1          |
| Salt Bridge                | 3.28976  | RALA:LYS134:NZ           | ANNEXIN<br>A2:ASP52:OD1  |
| Attractive Charge          | 3.07264  | ANNEXIN<br>A2:LYS329:NZ  | RALA:GLU99:OE1           |
| Attractive Charge          | 2.3048   | RALA:ARG145:NH1          | ANNEXIN<br>A2:ASP338:OD1 |
| Attractive Charge          | 4.00128  | RALA:ARG145:NH2          | ANNEXIN<br>A2:ASP338:OD2 |
| Attractive Charge          | 5.48941  | RALA:ARG145:NH2          | ANNEXIN<br>A2:ASP339:OD1 |
| Conventional Hydrogen Bond | 3.3009   | ANNEXIN<br>A2:ASN41:ND2  | RALA:ASP133:OD1          |
| Carbon Hydrogen Bond       | 3.72504  | RALA:SER138:CB           | ANNEXIN<br>A2:ASN62:OD1  |
| Pi-Sulfur                  | 4.74359  | RALA:MET98:SD            | ANNEXIN A2:TYR316        |
| Alkyl                      | 5.32987  | ANNEXIN A2:LEU332        | RALA:MET98               |
| Pi-Alkyl                   | 3.99311  | ANNEXIN A2:TYR333        | RALA:MET98               |

**Supplementary Table 2.** ZDOCK used to predict the binding sites of RalA and ANXA2.

| Gene                            | Sequence (5' to 3')    |
|---------------------------------|------------------------|
| <b>Real time RT-PCR primers</b> |                        |
| FOXD1-F                         | TGAGCACTGAGATGTCCGATG  |
| FOXD1-R                         | CACCACGTCGATGTCTGTTTC  |
| RalA-F                          | ATGGCTGCAAATAAGCCCAAG  |
| RalA-R                          | TGTCTGCTTTGGTAGGCTCATA |
| GAPDH-F                         | CATGGGTGTGAACCATGAGA   |
| GAPDH-R                         | GTCTTCTGGGTGGCAGTGAT   |
| <b>shRNA target sequences</b>   |                        |
| shFOXD1-1                       | tgTCCAGTGTGCGAGAACTTTA |
| shFOXD1-2                       | gcCCTTCTCCATCGAGAGCAT  |
| shFOXD1-3                       | ccTCGCCGAGGAAACAGACAT  |
| <b>siRNA target sequences</b>   |                        |
| si-RalA-1                       | GGACTACGCTGCAATTAGA    |
| si-RalA-2                       | GGAAGAAGGTAGTGCTAGA    |
| si-RalA-3                       | AGATGAGAATGTTCCATTT    |
| si-ANXA2-1                      | ACCAGCTTGCGAATAACAG    |
| si-ANXA2-2                      | TTGACCAACCGCAGCAATG    |
| si-ANXA2-3                      | TTGCTGATCGGCTGTATGA    |
| si-Src-1                        | GTTGTATGCTGTGGTTTCA    |
| si-Src-2                        | CTCGGCTCATTGAAGACAA    |
| si-Src-3                        | GAGAGAACCTGGTGTGCAA    |
| <b>ChIP-qPCR primers</b>        |                        |
| RalA-Fragment3-F                | CATGATCACCTCCTCTCAG    |

RalA-Fragment3-R

CCACAGATCACTTCCGG

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**Supplementary Table 3.** The sequences of the primers.

| Antibody                    | Company     | Catalog no. | Dilution |
|-----------------------------|-------------|-------------|----------|
| <b>Western blotting</b>     |             |             |          |
| FOXD1                       | Abcam       | ab49156     | 1/2000   |
| E-cadherin                  | CST         | 3195        | 1/1000   |
| N-cadherin                  | CST         | 13116       | 1/1000   |
| Vimentin                    | CST         | 5741        | 1/1000   |
| Snail                       | CST         | 3879        | 1/1000   |
| GAPDH                       | Proteintech | 10494-1-AP  | 1/5000   |
| RaIA                        | Proteintech | 13629-1-AP  | 1/1500   |
| p-mek1/2                    | CST         | 9154        | 1/1000   |
| mek1/2                      | CST         | 9122        | 1/1000   |
| p-erk1/2                    | CST         | 4370        | 1/2000   |
| erk1/2                      | CST         | 4695        | 1/1000   |
| β-catenin                   | CST         | 8480        | 1/1000   |
| Src                         | CST         | 2109        | 1/1000   |
| p-ANXA2 (Tyr23)             | Santa Cruz  | sc-135753   | 1/500    |
| ANXA2                       | Abclonal    | A12397      | 1/1000   |
| Mouse                       | Abcam       | ab6789      | 1/5000   |
| Rabbit                      | Abcam       | ab6721      | 1/5000   |
| HA-Tag antibody             | CST         | 3724        | 1/1000   |
| HRP-conjugated Goat         |             |             |          |
| Anti-Mouse IgG Heavy Chain  | Abclonal    | AS064       | 1/5000   |
| HRP-conjugated Goat         |             |             |          |
| Anti-Mouse IgG Light Chain  | Abclonal    | AS062       | 1/5000   |
| <b>Immunohistochemistry</b> |             |             |          |
| FOXD1                       | Abcam       | ab179940    | 1/150    |

|            |             |            |       |
|------------|-------------|------------|-------|
| E-cadherin | CST         | 3195       | 1/400 |
| Vimentin   | CST         | 5741       | 1/100 |
| p-erk1/2   | CST         | 4370       | 1/200 |
| p-mek12    | CST         | 2338       | 1/100 |
| RalA       | Proteintech | 13629-1-AP | 1/300 |

### **Immunofluorescence**

|                                                                                          |            |           |        |
|------------------------------------------------------------------------------------------|------------|-----------|--------|
| E-cadherin                                                                               | CST        | 14472     | 1/100  |
| Vimentin                                                                                 | CST        | 5741      | 1/100  |
| ANXA2                                                                                    | Abclonal   | A12397    | 1/100  |
| p-ANXA2 (Tyr23)                                                                          | Santa Cruz | sc-135753 | 1/50   |
| RalA                                                                                     | Huabio     | EM1707-77 | 1/50   |
| RalA                                                                                     | Abclonal   | A11736    | 1/50   |
| Anti-mouse IgG (H+L),<br>F(ab') <sub>2</sub> Fragment<br>(Alexa Fluor® 647<br>Conjugate) | CST        | 4410      | 1/1000 |
| Goat Anti-Rabbit IgG -<br>H&L (Alexa Fluor® 647)                                         | Abcam      | Ab150079  | 1/2000 |
| Goat Anti-Mouse IgG -<br>H&L (Alexa Fluor® 555)                                          | Abcam      | Ab150114  | 1/2000 |
| Goat Anti-Rabbit IgG<br>H&L (Alexa Fluor® 555)                                           | Abcam      | Ab150078  | 1/2000 |
| DAPI                                                                                     | Solarbio   | C0060     | 1:100  |
| Mounting Medium,<br>antifading                                                           | Solarbio   | S2100     |        |

### **Chromatin**

### **Immunoprecipitation**

|                           |            |           |        |
|---------------------------|------------|-----------|--------|
| FOXD1                     | Santa Cruz | sc-293238 | 5.0 ug |
| Mouse IgG Isotype Control | Invitrogen | 31903     | 5.0 ug |

### **CUT&Tag**

|                                           |             |           |      |
|-------------------------------------------|-------------|-----------|------|
| FOXD1                                     | Santa Cruz  | sc-293238 | 1/50 |
| Mouse IgG Isotype Control                 | Invitrogen  | 31903     | 1/50 |
| NovoNGS® CUT&Tag 2.0 High-Sensitivity Kit | Novoprotein | N259-YH01 |      |

### **GST pull-down assay**

|                             |                 |            |        |
|-----------------------------|-----------------|------------|--------|
| Anti-GST Tag Magnetic Beads | Sino Biological | TB11213    |        |
| GST Tag Polyclonal antibody | Proteintech     | 10000-0-AP | 1:2000 |
| His-Tag Monoclonal antibody | Proteintech     | 66005-1-Ig | 1:5000 |

### **Co-immunoprecipitation (Co-IP)**

|                                      |               |          |  |
|--------------------------------------|---------------|----------|--|
| Anti-FLAG antibody                   | Sigma-Aldrich | F1804    |  |
| Anti-ANXA2 antibody                  | Santa cruz    | Sc-28385 |  |
| Anti-Src antibody                    | Santa cruz    | Sc-8056  |  |
| Mouse IgG Isotype Control            | Invitrogen    | 31903    |  |
| Pierce™Classic Magnetic IP/Co-IP Kit | ThermoFisher  | 88804    |  |

**Supplementary Table 4.** The antibodies applied in this study.