Supporting Information

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Transcriptional Repression of Aerobic Glycolysis by OVOL2 in Breast Cancer

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Supplementary Table 1. Potential OVOL2-interacting proteins identified by Co-IP and mass spectrometry

| Protein name | Description                                                   | Mass (Dalton) | Score |
|--------------|---------------------------------------------------------------|---------------|-------|
| NCoR         | Nuclear receptor corepressor                                  | 270212        | 126   |
| β-catenin    | Catenin beta-1                                                | 86069         | 421   |
| α-catenin    | Catenin alpha-1                                               | 100693        | 127   |
| δ-catenin    | Catenin delta-1                                               | 108674        | 38    |
| MDM2         | Murine double minute 2, human homologue                       | 56323         | 89    |
| HDAC1        | Histone deacetylase 1                                         | 55638         | 97    |
| HDAC3        | Histone deacetylase 3                                         | 48848         | 106   |
| SVIL         | Supervillin                                                   | 249417        | 791   |
| ZO1          | Tight junction protein ZO-1                                   | 195682        | 671   |
| LIMA1        | LIM domain and actin-binding protein 1                        | 85630         | 436   |
| PRMT5        | Protein arginine N-methyltransferase 5                        | 73322         | 312   |
| RPS27A       | Ubiquitin-40S ribosomal protein S27a                          | 18296         | 205   |
| FLII         | Protein flightless-1 homolog                                  | 146142        | 182   |
| HSP90AA1     | Heat shock protein HSP 90-alpha                               | 85006         | 153   |
| SCYL2        | SCY1-like protein 2                                           | 104327        | 101   |
| EFHD2        | EF-hand domain-containing protein D2                          | 26794         | 96    |
| MISP         | Mitotic interactor and substrate of PLK1                      | 75482         | 85    |
| TMOD3        | Tropomodulin-3                                                | 39741         | 84    |
| EPSTI1       | Epithelial-stromal interaction protein 1                      | 36942         | 79    |
| SIPA1L1      | Signal-induced proliferation-associated 1-like protein 1      | 201102        | 79    |
| N4BP3        | NEDD4-binding protein 3                                        | 60889         | 72    |
| TRIP11       | Thyroid receptor-interacting protein 11                       | 228131        | 48    |
| THRAP3       | Thyroid hormone receptor-associated protein 3                 | 108658        | 195   |
| BCLAF1       | Bel-2-associated transcription factor 1                       | 106173        | 95    |
| SMARCA5      | SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily A member 5 | 122513 | 79 |
| MEP50        | Methylosome protein 5                                         | 37442         | 71    |
| PPP1R12A     | Protein phosphatase 1 regulatory subunit 12A                  | 115610        | 69    |
| IPO9         | Importin-9                                                    | 116858        | 51    |
| NEB2         | Neurabin-2                                                    | 89309         | 120   |
| IMB1         | Importin subunit beta-1                                       | 98420         | 81    |
| SSRP1        | FACT complex subunit SSRP1                                   | 81367         | 60    |
| UNC45A       | Protein unc-45 homolog A                                      | 104266        | 308   |
| MIC60        | MICOS complex subunit MIC60                                  | 84026         | 239   |
| MCM7         | DNA replication licensing factor MCM7                         | 81884         | 226   |
| NUP93        | Nuclear pore complex protein Nup93                           | 93943         | 196   |
| Symbol | Description                                                                 | SwissProt | EntrezGene |
|--------|-----------------------------------------------------------------------------|-----------|------------|
| XRCC5  | X-ray repair cross-complementing protein 5                                  | 83222     | 190        |
| DHX15  | Pre-mRNA-splicing factor ATP-dependent RNA helicase DHX15                    | 91673     | 174        |
| IQGAP1 | Ras GTPase-activating-like protein IQGAP1                                    | 189761    | 101        |
| MLH1   | DNA mismatch repair protein Mlh1                                             | 85175     | 76         |
| STAT1  | Signal transducer and activator of transcription1-alpha/beta                | 87850     | 74         |
| CAND2  | Cullin-associated NEDD8-dissociated protein 2                               | 136653    | 45         |
| PPM1B  | Protein phosphatase 1B                                                      | 53180     | 44         |
| LSR    | Lipolysis-stimulated lipoprotein receptor                                   | 72534     | 396        |
| HSPA9  | Stress-70 protein                                                           | 73920     | 334        |
| AIFM1  | Apoptosis-inducing factor 1                                                 | 67144     | 196        |
| TOM1L2 | TOM1-like protein 2                                                         | 55864     | 174        |
| SRR    | Stress-70 protein                                                           | 73920     | 334        |
| DDX5   | Probable ATP-dependent RNA helicase DDX5                                   | 69618     | 137        |
| DDX17  | Probable ATP-dependent RNA helicase DDX17                                   | 80906     | 85         |
| KARS   | Lysine--tRNA ligase                                                         | 68461     | 79         |
| IVNS1ABP | Influenza virus NS1A-binding protein                                       | 72937     | 73         |
| RUVBL1 | RuvB-like 1                                                                | 50538     | 117        |
| DDX39A | ATP-dependent RNA helicase DDX39A                                           | 49611     | 85         |
| CAMK2D | Calcium/calmodulin-dependent protein kinase type I1-subunit delta           | 56961     | 56         |
| GIPC1  | PDZ domain-containing protein GIPC1                                          | 36141     | 225        |
| HNRP1  | Heterogeneous nuclear ribonucleoprotein H3                                  | 36960     | 126        |
| H2AFY  | Core histone macro-H2A.1                                                    | 39764     | 59         |
| H4     | Histone H4                                                                 | 11360     | 53         |
| DCAF7  | DDB1- and CUL4-associated factor 7                                           | 39528     | 51         |
| PID1   | PTB-containing, cubulin and LRP1-interacting protein                        | 28766     | 41         |
| TRA2A  | Transformer-2 protein homolog alpha                                         | 32726     | 167        |
| HNRP3  | Heterogeneous nuclear ribonucleoprotein A3                                  | 39799     | 149        |
| HNRP1  | Heterogeneous nuclear ribonucleoprotein A1                                  | 38837     | 148        |
| POLDIP2| Polymerase delta-interacting protein 2                                      | 42235     | 119        |
| U2AF1  | Splicing factor U2AF 35 kDa subunit                                         | 28368     | 86         |
| DCD    | Dermcidin                                                                  | 11391     | 66         |
| RALY   | RNA-binding protein Raly                                                    | 32501     | 55         |
| H3F3C  | Histone H3.3C                                                               | 15318     | 46         |
| CDK9   | Cyclin-dependent kinase 9                                                   | 43149     | 45         |
| ARF4   | ADP-riboylsation factor 4                                                   | 20612     | 137        |
| AIF1L  | Allograft inflammatory factor 1-like                                        | 17114     | 132        |
| ARF1   | ADP-riboylsation factor 1                                                   | 20741     | 118        |
| ARF3   | ADP-riboylsation factor 3                                                   | 20645     | 118        |
| JAGN1  | Protein jagunal homolog 1                                                   | 21111     | 97         |
| TAF12  | Transcription initiation factor TFIID subunit 12                            | 18027     | 76         |
| Gene   | Description                                | Molecule | Abundance | Location |
|--------|--------------------------------------------|----------|-----------|----------|
| UBE2V2 | Ubiquitin-conjugating enzyme E2 variant 2  | 16409    | 74        |
| RBM3   | RNA-binding protein 3                      | 17160    | 70        |
| TMED2  | Transmembrane emp24 domain-containing protein 2 | 22860    | 70        |
| H2B1B  | Histone H2B type 1-B                       | 13942    | 69        |
| NME2   | Nucleoside diphosphate kinase B            | 17401    | 65        |
| ARL8B  | ADP-ribosylation factor-like protein 8B    | 21753    | 62        |
| CDC42  | Cell division control protein 42 homolog  | 21587    | 50        |
| CALM1  | Calmodulin                                 | 16827    | 478       |
| ARF5   | ADP-ribosylation factor 5                  | 20631    | 220       |
| SEC11A | Signal peptidase complex catalytic subunit SEC11A | 20612    | 80        |
| REEP5  | Receptor expression-enhancing protein 5    | 21707    | 76        |
| LAMTOR1| Ragulator complex protein LAMTOR1          | 17848    | 69        |
| PDZD11 | PDZ domain-containing protein 11           | 16121    | 69        |
| ARF6   | ADP-ribosylation factor 6                  | 20183    | 66        |
| TCP4   | Activated RNA polymerase II transcriptional coactivatorp15 | 14386    | 63        |
| PDCD6  | Programmed cell death protein 6            | 21912    | 62        |
| NRB2   | Nuclear receptor-binding factor 2          | 32529    | 55        |
| H2AV   | Histone H2A.V                              | 13501    | 49        |
| H2AZ   | Histone H2A.Z                              | 13545    | 49        |
Supplementary Table 2. The cDNA target sequences of shRNAs or siRNAs.

| Gene        | Target sequence (5’→3’)          |
|-------------|----------------------------------|
| p53 (siRNA/shRNA) | AAGACUCCAGUUGAUAAUCUAC           |
| MDM2 (siRNA/shRNA) | GCUUCGGAACAAGACUC                 |
| PKM2 (shRNA)   | CCAUAAUCGUCCACACAA               |
| LDHA (shRNA)   | GGAGAAAGCCGUCUAAUU               |
| NCoR (shRNA)   | GCUCCUCAGCAGCAGUAU               |
| HDAC3 (shRNA)  | CCAAGAGUCUUAUGCCUU               |
Supplementary Table 3. Primers used for real-time PCR.

| Gene     | Species | Forward (5’→3’) | Reverse (5’→3’) |
|----------|---------|-----------------|-----------------|
| GLUT1    | Human   | CATCCCATGTTTCCATCGTGATGCTGAACT | GAAGTAGGTTGAGATGGAAGAAGAAGAACAGAAGAC |
| HK2      | Human   | GCCATGCTGCAACACTTATGGGCTTAGGAG | GTGAGGATGTAAGGCTTGGAGAGGAGTCC |
| GPI      | Human   | TATTGTGTTCCAAACAGTCTACACC | TGTTAGGAAGCTGCTGAGAGGGTCGTC |
| PFKL     | Human   | GGAGAACTGCTGAGGATTTTAC | ATTTGTTGCTGAGATTCGAGTACGAG |
| ALDOA    | Human   | AGGCCATGCTTGGATGACGAGTGCAG | AGGGCCAGGAGCCTGACGAGAG |
| GAPDH    | Human   | TTTCTGCTACCAGCTACGAGGATGAC | CAAAAGTGGAGAAGATGGTGTCGAG |
| PGK1     | Human   | ATGTGCTTTTCTAACAGGACTGTA | GCAGGAGTTCCTGCAGCA |
| PGAM1    | Human   | GAAGACCTGTACTGATGACGAGTGCAG | TTCCATGCTTGGCAGCAGGAGT |
| ENO1     | Human   | GACTTGGCTGCAACATCGTACGAG | GGCTATCGGAGACGTGAA |
| PKM2     | Mouse   | GCCACGTGAGGCTGTTAGGAGTGCAG | TGGTACAGAGATGACGAGTAG |
| LDHA     | Mouse   | ATGGCAACTCTAAGAGGATCA | GCAACTTGCAAGTGTCGAG |
| α-Tubulin| Mouse   | CCAGGAGCTACACTTCAGTCCAG | CATAGAAGCTGAGTCCAG |
| ASCL1    | Human   | CGCGTCAAGTTGTTGACGCTTGCAG | GCCTCCAGCTACGAGTCC |
| CREB3L1  | Human   | TTGATGACCTGTGCTGATGGCTGAG | GCTTCTGCCACATGATGAG |
| Ovol2    | Human   | TCACCTCAAGTGTTCCACAACCAAG | CCGCAGTTGACAGCAGTCC |
| MSX1     | Mouse   | TCAAGCTGCAAGATAGCAGCAG | GCCTTCCAGATGACGAGT |
| MSX2     | Mouse   | CTATGGCTCCAGAAAGAGACAGCG | CAGCTTCCAGATGACGAG |
| ATF3     | Human   | TCTGCGCGGAACTCTAGTCATCCT | GAGATCGTGGTTGAGGAGT |
| KLF7     | Human   | CCAGGACGCTAAGCTTTCAGTCCAC | TCTTCTACAGATGAGCCGTCCT |
| GLUT1    | Mouse   | CATGTTCTATGTTGCGACCAGGGTACG | TCGGATGCTGTCGAGGACTT |
| HK2      | Mouse   | CCTGCTACAGGTTCTGAGCAGCCTCTT | TGGGAGCTAGGCTGAGGAGT |
| GPI      | Mouse   | AACGGGAGCCACCAAATCTTATGGT | TGGCAGCTGAGCAGGAGGAG |
| PFKL     | Mouse   | AAAGTGGTGCCACGACTGACGAGCAG | GAACTGGGCTCCAGACACCTC |
| ALDOA    | Mouse   | ATGAGGAGATTGCATGGAACCGCAG | TTAGAGCAGAGGCTGACGAG |
| GAPDH    | Mouse   | GGAGAAGAGAAATGCTATTTTTG | TGCGATGAGGCAATGAGCAG |
| PGK1     | Mouse   | AGACTGCTCAGCTGCTGAGGAGG | GAACTGCTGTTTCCCAGAGGAGC |
| PGAM1    | Mouse   | TACGCAGACCTCTAGTGAAGACGAG | AGCTTACAGATGACGAGTACG |
| ENO1     | Mouse   | ACCACCTTAAAGCGGATTTGCAAGGA | AGTTCTGATCGGCAATGAG |
| PKM2     | Mouse   | TCAGCTGACGACTGATT | CCTGGAATAGCTGCAAGT |
| LDHA     | Mouse   | GCAAGTGGTGAAGATTGAGAGGAG | ACATGAGAAGACATGAGGAG |
| α-Tubulin| Mouse   | AACAGAGATGCTGAATGTTGACCTT | CACAGTTGGAGGCTGAGGAG |

Supplementary Table 4. Primers used for ChIP.

| Gene          | Forward (5’→3’)                         | Reverse (5’→3’)                      |
|---------------|----------------------------------------|--------------------------------------|
| GLUT1/2       | GTGAAACCCCGTCTCTACTAA                   | TCTCAGACAGAGTCTCGCTCG               |
| GLUT3         | TTGGTCTTCTACAACCTCAGG                  | CTTCCTAGAAAAAGGTGGAGCC              |
| HK2-1         | GAAATAGCCTGCACTCCAGA                   | GAGTCCCTGTTGCTCTACTTTGG            |
| HK2-2         | AGAGGGTGAGAGGACAGCAGCCCAAT             | GGAGATGGCTGAGGAATATACAC             |
| GPI           | GACAAGGATGCTGCTGCTGCA                  | AGGTGGGATACGCTGAGC                 |
| PFKL-1        | GCCATCCCTGTTTCCCACAGAGCT              | AGGCAGAGGTTGAGCTGAGTTGAG           |
| PFKL-2        | TGGCTCAGCCCTCCTCGATAGCT                | ATATCCCGAAGATCTGG                  |
| ALDOA-1       | TCATGCCGACTAACCTACTGAC                 | CTTCTGGGTTGCAAGCTG                  |
| ALDOA-2       | AGACCTTTGGAGAAGCTGAG                  | TGGTCAAGGTGCTTCAAGC                |
| ALDOA-3       | GACAGAGGGAGACTCGTCACG                 | GACAGAGGGAGACTCGTCAC               |
| GAPDH         | GTCCCCAGCTTATGCTCATAGGT                | TGGCCAGAGAGCCTGGAAAGG              |
| PGK1-1        | TGAAACTCTGCTCTCCGC                   | ACGGAAGTAAACCCGCGG                 |
| PGK1-2        | AGCGGCGGACCCCTGGGTCT                | CAGAAGACGCCGAGCTGAGCTG             |
| PGK1-3        | CCGACGCTCACCGGAGATCTCGCG             | TTTGTCAGCCGGCACGCGCG              |
| PGK1-4        | AAGTTGCTCAGCTGCTCGC                  | TCGTAGACCGCCGTATTG                 |
| PGAM1-1       | GGCACCTGCTGCTACGCTGTAAAT              | GTTAAAGCAATTTGCTGCTCG              |
| PGAM1-2       | AGATCGGACACACTGACCTCCAGTCT             | CTTCACACAGTGAAGTCAGTGT             |
| ENO1-1        | TCCAGCCGCCCACCCCCCGGAGT              | CAGCTGCTTCCAGCTTGG                |
| ENO1-2        | CAGCGCCGCCAACCCCCCGGAGT              | CCCTGACCTTGTGACACACTTGG            |
| PKM2-1        | TTATTCATGCGCCCCCCGCGCGG             | ATGAGAGAGAATAGGAGGAGGTGGCG        |
| PKM2-2        | TCGGAAGGCGGCCAGAGCCTCGA               | ACTGGCCGCTGCTGCTGCT                |
| LDHA-1        | TGGAATCACGAAGAATACAGGGC              | CTGAGAAATAGGCCGAGTTAGC             |
| LDHA-2        | TAGGAGGACAGAGATCGTGGTAAAC             | TCTGATCGCCGAGTTAGGAGG              |
| GLUT1-Upstream| TGCATTGACAGAGGGTGCA                  | ACAAGAGGGAGATAGCAGAGG             |
| HK2-Upstream  | TGATATTTGCTTCCAGCTGCTGAGA            | AAATGAAGAGAGGAGGATGCTTCG           |
| GPI-Upstream  | ACCGTGACCGGCTCTACTTCAC                | CTACCAACGCGCCGACTCCTCC             |
| PFKL-Upstream | TCACTCTGCTCCGACGAC                   | GCTTGTTACAGCCGACGACT               |
| ALDOA-Upstream| TCAACCTGTAACTCCAGA                   | GTGATGCTACGTCATGCG                 |
| PGK1-Upstream | GCCAAACAGGGTGCTCTCTAGAG              | CAGAAGAACCACCTGACATCCAC            |
| PGAM1-Upstream| AGGCAAGTTCTCCCCTACCA                | GAGGAGGGGTTGAGCTGAGG               |
| ENO1-Upstream | AGGGGAGTGGTGACAGAC                   | TGGTGAGAGAGGATTCGTCAGC             |
| PKM2-Upstream | CTTCTGAGAGACGGGACTCAGCA              | AGCAGGGCTCACGAGGAAGGGAATTGCC       |
| LDHA-Upstream | GTAGTCCCCAGTGCTCTCGG                  | CTGCACTTTCTACTGCGCAG              |
Supplementary Table 5. Clinicopathological data of breast cancer samples.

| Age years | No. of cases | No. of disease-free survival (%) | No. of overall survival (%) |
|-----------|--------------|----------------------------------|-----------------------------|
| <40       | 14           | 6 (18.2%)                        | 8 (9.0%)                    |
| 40-49     | 47           | 6 (18.2%)                        | 41 (46.1%)                  |
| 50-59     | 20           | 7 (21.2%)                        | 13 (14.6%)                  |
| 60-69     | 20           | 5 (15.1%)                        | 15 (16.8%)                  |
| ≥70       | 21           | 9 (27.3%)                        | 12 (13.5%)                  |
| Total     | 122          | 33 (100.0%)                      | 89 (100.0%)                 |

ER

| Status    | No. of cases | No. of disease-free survival (%) | No. of overall survival (%) |
|-----------|--------------|----------------------------------|-----------------------------|
| Negative  | 38           | 12 (36.4%)                       | 26 (29.2%)                  |
| Positive  | 84           | 21 (63.6%)                       | 63 (70.8%)                  |
| Total     | 122          | 33 (100.0%)                      | 89 (100.0%)                 |

PR

| Status    | No. of cases | No. of disease-free survival (%) | No. of overall survival (%) |
|-----------|--------------|----------------------------------|-----------------------------|
| Negative  | 55           | 19 (57.6%)                       | 36 (40.4%)                  |
| Positive  | 67           | 14 (42.4%)                       | 53 (59.6%)                  |
| Total     | 122          | 33 (100.0%)                      | 89 (100.0%)                 |

HER2

| Status    | No. of cases | No. of disease-free survival (%) | No. of overall survival (%) |
|-----------|--------------|----------------------------------|-----------------------------|
| Negative  | 97           | 22 (66.7%)                       | 75 (84.3%)                  |
| Positive  | 25           | 11 (33.3%)                       | 14 (15.7%)                  |
| Total     | 122          | 33 (100.0%)                      | 89 (100.0%)                 |