

# **Cellular response to persistent foot-and-mouth disease virus infection is linked to specific types of alterations in the host cell transcriptome**

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## List of Supplementary Materials

**Table S1.** The FMDV or FMDV-Op in BHK\_VEC, BHK21 and BHK\_OP54 were detected by qRT-PCR.

**Table S2.** summary statistics of RNA-Sequencing data.

**Table S3.** Primers and probes for quantitative RT-PCR.

**Table S4.** Sequences of primers used for RT-PCR.

**Figure S1.** Indirect immunofluorescence for detection of FMDV or FMDV-Op in BHK\_Op and BHK\_VEC. FMDV 3D-specific antibodies stained negative control BHK-21 (b), BHK\_Op43 (a), BHK\_VEC (d), and positive control BHK-21 cells that were infected with FMDV at 0.06 PFU/cell for 12 hours. The 3D positive is in green (Alexa 555), and the lower row is the cell morphology pictures of each cell line ( $\times 100$ ).

**Figure S2.** Analyses of differentially expressed genes in BHK\_VEC. (a) Differentially expressed genes were determined in RNA levels in BHK\_VEC compared with BHK-21 (FPKM value  $\geq 1$  and  $p\_value \leq 0.05$ ). A total of 4686 genes were differentially expressed including 2168 upregulated genes and 2518 down-regulated genes. (b) GO enrichment analysis of differentially down-regulated expressed genes in BHK\_VEC; based on  $p\_Value$ , we included the top 30 most significant GO categories in the DEGs ( $*p < 0.05$ ). (c) Correlation analysis between qRT-PCR and RNA-seq data. (d) KEGG pathway enrichment analysis of DEGs in BHK-VECs. We included the top 30 most significant KEGG pathway categories of DEGs.

**Figure S3.** GO and KEGG pathway analyses of AS changes in BHK-VEC. (a) GO enrichment analysis of genes with differentially alternative splicing in BHK\_VEC compared with BHK-21. The most significant 20 GO terms of enrichment are shown. (b) KEGG pathway enrichment analysis of genes involved in differentially alternative splicing in BHK\_VEC vs BHK-21. The most significant 10 GO terms of enrichment are shown. (c) Overlap of the genes involved in differentially expressed and differentially alternative splicing in BHK\_VEC.

**Figure S4.** Significantly differentially expressed genes identified by KEGG as involved in MAPK signaling<sup>1</sup>. Red: significantly increased expression (fold-change  $> 2$ ); Blue: significantly decreased expression (fold-change  $< 0.5$ ); Green: genes detected in downregulated groups; Gray: unchanged expression.

**Figure S5.** Effect of MAPK/ERK or p38/MAPK inhibition on the replication of FMDV in BHK-21. BHK-21 cells were pre-incubated (1 h) with DMSO or 20 mM U0126, or 20 mM SB202190, then exposed to  $2.5 \times 10^{-4}$  PFU/cell FMDV in the presence of U0126 (a highly specific ERK inhibitor) or SB202190 (a potent and selective inhibitor of p38/MAPK) or DMSO for different times. At the indicated time maintained, the intracellular RNA was isolated and intracellular virus RNA numbers were detected by QRT-PCR analysis. ( $***p < 0.001$ ,  $*p < 0.05$ ).

**Figure S6.** As a complement, the full-length blot / gel diagram for indicated figures was shown.

**Figure S7.** As a complement, the full-length blot / gel diagram for indicated figures was shown. Among them, we can not always detect the background expression of MAPK13 due its low expression in the host cells (MAPK13 can only be detected when the real-time quantitative PCR cycle number is more than 35 CT values). For example, Figure 6d- ii, we did not succeed in detecting MAPK13 in the pHAGE-mock cell line.

**Table S1. The FMDV or FMDV-Op in BHK\_VEC, BHK21 and BHK\_OP54 were detected by qRT-PCR.**

| cell     | CT 值  |
|----------|-------|
| BHK21    | NA    |
| BHK_VEC  | NA    |
| BHK_Op54 | 15.04 |

**Table S2. summary statistics of RNA-Sequencing data.**

| Sample name      | BHK-21_1 | BHK-21_2 | BHK-21_3 | VEC_1    | VEC_2    | VEC_3    |
|------------------|----------|----------|----------|----------|----------|----------|
| Raw reads        | 55613610 | 56411208 | 57451398 | 60698628 | 41606510 | 43957326 |
| Clean reads      | 53734426 | 54484692 | 55573216 | 59577214 | 40942086 | 43217992 |
| Q30 (%)          | 94.94    | 94.92    | 94.93    | 94.3     | 94.55    | 94.49    |
| GC content (%)   | 50.17    | 49.98    | 49.85    | 50.93    | 51.45    | 51.74    |
| Total mapped     | 40381924 | 40983864 | 41907283 | 46159447 | 31526450 | 33035916 |
|                  | (75.15%) | (75.22%) | (75.41%) | (77.48%) | (77%)    | (76.44%) |
| Uniquely mapped  | 39973778 | 40546457 | 41487974 | 45683845 | 31210654 | 32691697 |
|                  | (74.39%) | (74.42%) | (74.65%) | (76.68%) | (76.23%) | (75.64%) |
| Reads map to“+”  | 19963997 | 20254923 | 20715860 | 22832938 | 15600284 | 16344676 |
|                  | (37.15%) | (37.18%) | (37.28%) | (38.32%) | (38.1%)  | (37.82%) |
| Reads map to“-”  | 20009781 | 20291534 | 20772114 | 22850907 | 15610370 | 16347021 |
|                  | (37.24%) | (37.24%) | (37.38%) | (38.36%) | (38.13%) | (37.82%) |
| Non-splice reads | 22246041 | 22323855 | 22891087 | 24130499 | 16428437 | 16700228 |
|                  | (41.4%)  | (40.97%) | (41.19%) | (40.5%)  | (40.13%) | (38.64%) |
| Splice reads     | 17727737 | 18222602 | 18596887 | 21553346 | 14782217 | 15991469 |
|                  | (32.99%) | (33.45%) | (33.46%) | (36.18%) | (36.11%) | (37%)    |

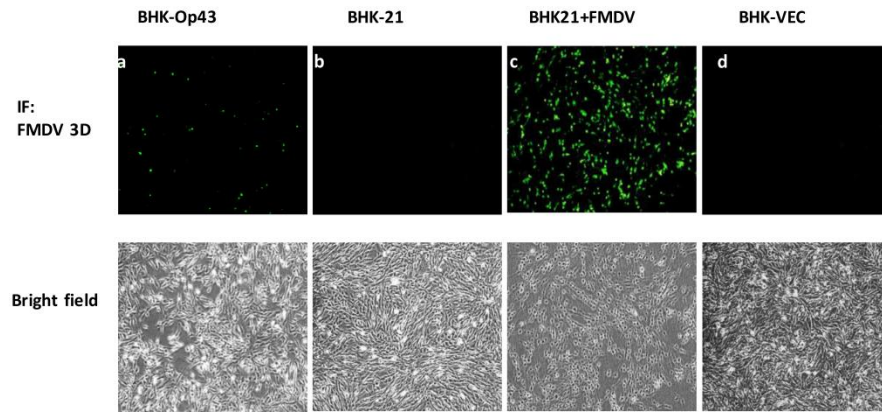
**Table S3. Primers and probes for quantitative RT-PCR.**

| Gene name | Gene ID   | Forward Primer/Probe Sequence (5'-3') | Reverse primer(5'-3') |
|-----------|-----------|---------------------------------------|-----------------------|
| Igfbp7    | 101839936 | TGCTGGTATCTCTCTAAGTAAG                | CTGATGCTGAAGCCTGTCC   |
| Ldb3      | 101829490 | CTACCATCATCCACGCACAG                  | ACAGCCAAGTCTTTCACAGG  |
| Ccl17     | 101828037 | GTCCAGGGCAGGTCCATC                    | ACGGTCACAGGCTAAGGC    |
| Dcn       | 101835832 | TGCCAGTGTCTCTAAGAGTG                  | ATGTTGTGTCAGGTGGAAGG  |
| Ccnd1     | 101837554 | GTATCTACACTGACAACCTCTATCC             | GTTCTCATCCGCCTCTGG    |
| Bmp5      | 101824438 | ACAGTAGACAGAACACAGGAG                 | AAGCCAGAAGCCAGAAGC    |
| Mapk13    | 101824910 | GCTCAGGCTCTTGCTCAC                    | TGCTGTTTCCACTCATCTACG |
| Mras      | 101824687 | TGAAGCACACGGAGATTGAC                  | GGAGTACACGATGAGGAAGC  |
| Slc2a1    | 101823763 | GAGCCCAGCAGCAAGAAAG                   | TGATGACGCCAGTGTATAGC  |
| Klhl13    | 101828313 | ATGCTGGAACCTGTGTATGGAG                | GGTCATTGGTGCCTTCTGG   |
| WNT6      | 101842149 | GTGGAGACGATGTGGACTTC                  | TGCCTGCCTCATTGTTGTG   |
| Pygm      | 101835714 | CGCACACAGCAGCATTAC                    | TCATCGCAGGCATTCTCC    |
| Camk2d    | 101833285 | AGCCATAGAAGTTCAAGGAGAC                | CCACCAGCAAGATGTAGAGG  |

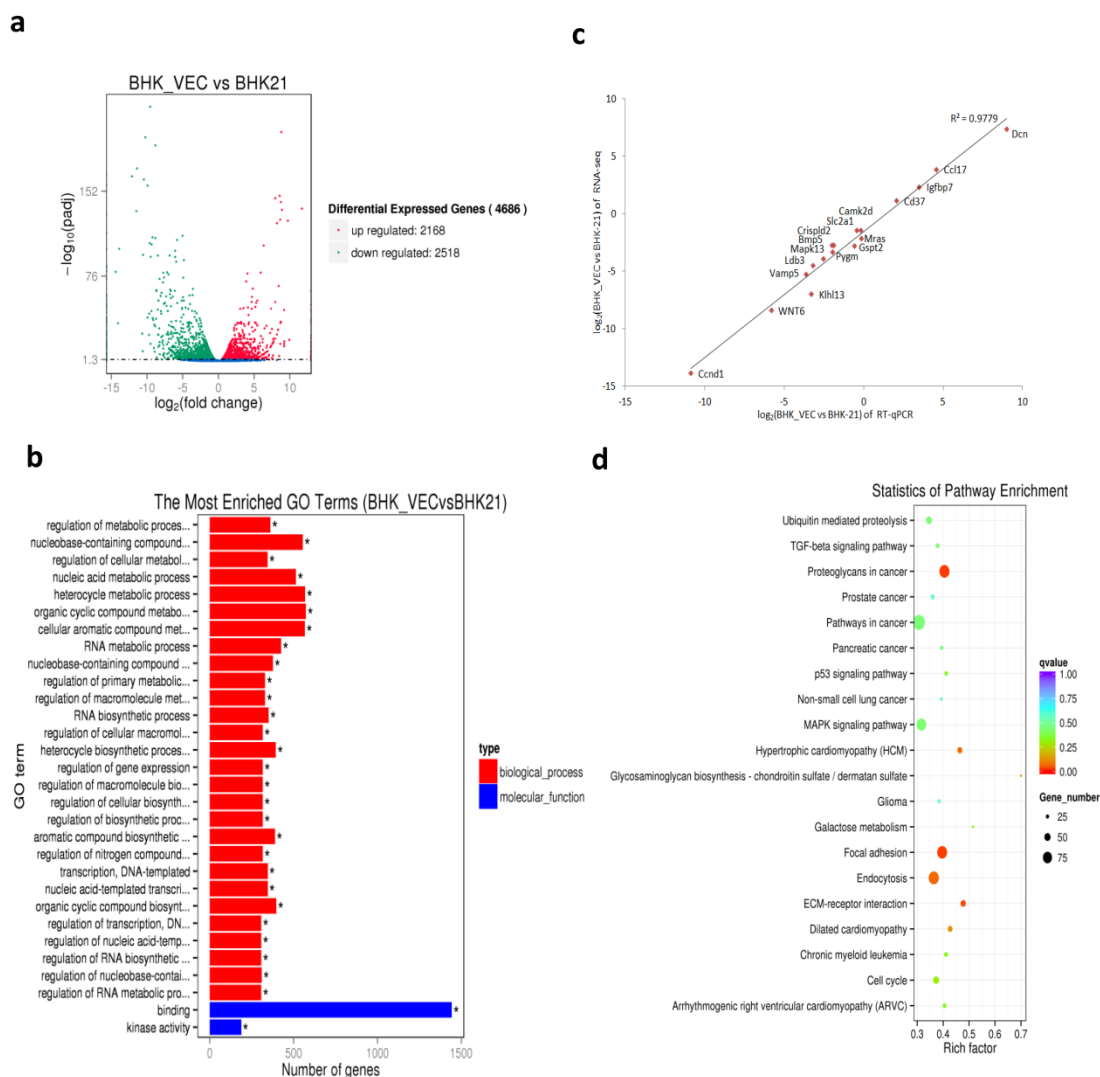
|              |           |                                   |                          |
|--------------|-----------|-----------------------------------|--------------------------|
| Gspt2        | 101825988 | GTAGCAGCGACTCAACTCC               | GCAGAGGCGAATCCATCC       |
| Cd37         | 101824219 | ACGAGTCTGAGGAGCCCTTG              | AGAGGTCAGCGGTCTGTCTC     |
| Vamp5        | 101823478 | TCAGCAAGACAACCAAGACTC             | GACCAGCAGCACAATCAGG      |
| Crispld2     | 101841720 | ACGCCGTGTATCTTGTCTG               | TCTTGGTAGCATAGGTTGTTCC   |
| GAPDH        | 106022412 | AAGGCCATCACCATCTTCCA              | GCCAGTAGACTCCACAACATAC   |
| Tnfrsf9      | 101829103 | CTGCTGCTGTTCTCTGATC               | CCTCCTCCTTCTTCTTCCT      |
| C3           | 101842900 | AGAAGACGGTGCTGACAAG               | TTGACTGGAACCTCCTTACTGG   |
| Fcgrt        | 101825614 | AGGAGATGAACACCATTACC              | CCACCAAGAAGCCAAGAA       |
| Loc101837574 | 101837574 | TCGGCTACTACAACCAGAG               | TCGTAGGCATACTGACTGT      |
| Ccl2         | 101839392 | AACGCTTCTGTGCCTACT                | TGCTGCTGGTGATTCTCT       |
| C1s          | 101823163 | AGAACTACAAGGACTGGATACTG           | GGCTGGCGGTATGAATGG       |
| Rasgrp2      | 101840143 | CCTCTTCAACAGCGTCTCAC              | CCGACAACAGCCATCAGC       |
| Fgfr4        | 101828736 | CCAGCCAGACCAGACCAG                | CTTGTCGCCGACCGATTAGC     |
| Cacna1s      | 101835763 | CTGGAGTCACCTGTCTTCTTG             | GAGGAAGAGCAGGATGAAGG     |
| Cacng6       | 101835632 | ATGATGATGTGGTCTAACTTCTTC          | GCCGAGATTCTGCTGTCC       |
| Cacng1       | 101838313 | ATGAGGCAATCGGTGAAGC               | GAGGAAGAGCAGGATGAAGG     |
| Map2k6       | 101833237 | TGGTGGACTIONCTGTGCTA              | GGCTCTTCTACGACTTGTT      |
| Hspb1        | 101844946 | CTGGTGCTCTCTTCTTGTC               | TGGTGATCTCCGATGATTGTG    |
| Mapk3        | 101841668 | GGAGGTGGAGGTGGTGAAG               | CGTGGTCGTAGGCAGAGC       |
| 3D           |           | GAACACATTCTTTACACCAGGAT           | CATATCTTTGCCAATCAACATCAG |
| 3D-Probe     |           | FAM-ACAACCTACCGCCGAGCCAATTC-TAMRA |                          |

**Table S4. Sequences of primers used for RT-PCR.**

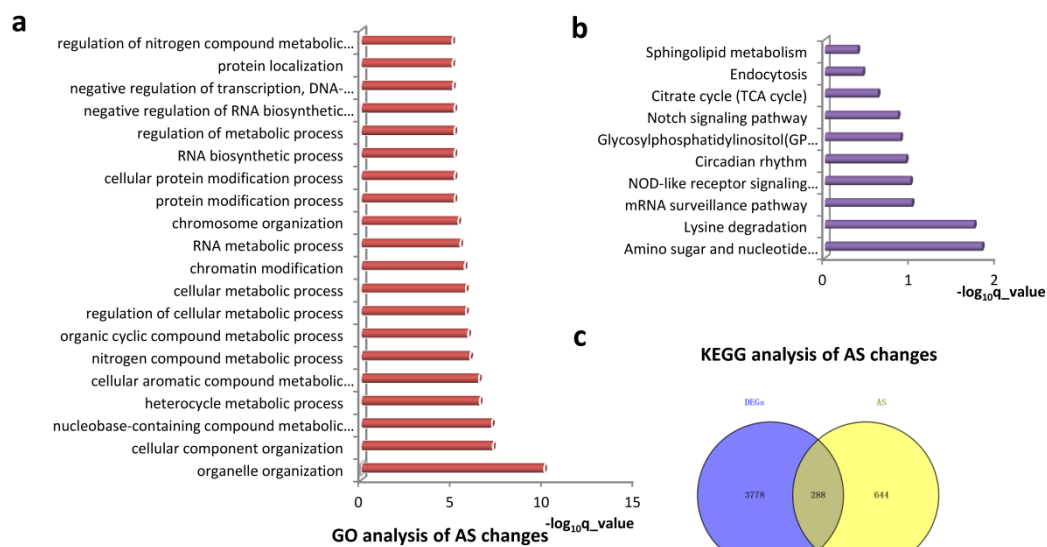
| Primers     | Sequences                  |
|-------------|----------------------------|
| Hnrnpa2b1-F | 5' TGGAAGGAGCCGATACTG 3'   |
| Hnrnpa2b1-R | 5' AACTCATCTGTCTACTCACA 3' |
| Pde4dip-F   | 5' TGAGTCGTTCTGCTGTGT 3'   |
| Pde4dip-R   | 5' CTGTGGGTCTGGAGTTAAG 3'  |
| Syt12-F     | 5' CCTTCACTGCCTGATAAT 3'   |
| Syt12-R     | 5' GCTGCTGCTAAGTCCTTAC 3'  |
| Hypk-F      | 5' AGACGGTCCAGGGAGCAA 3'   |
| Hypk-R      | 5' CATCAGTTGGTTAAGGCAAT 3' |



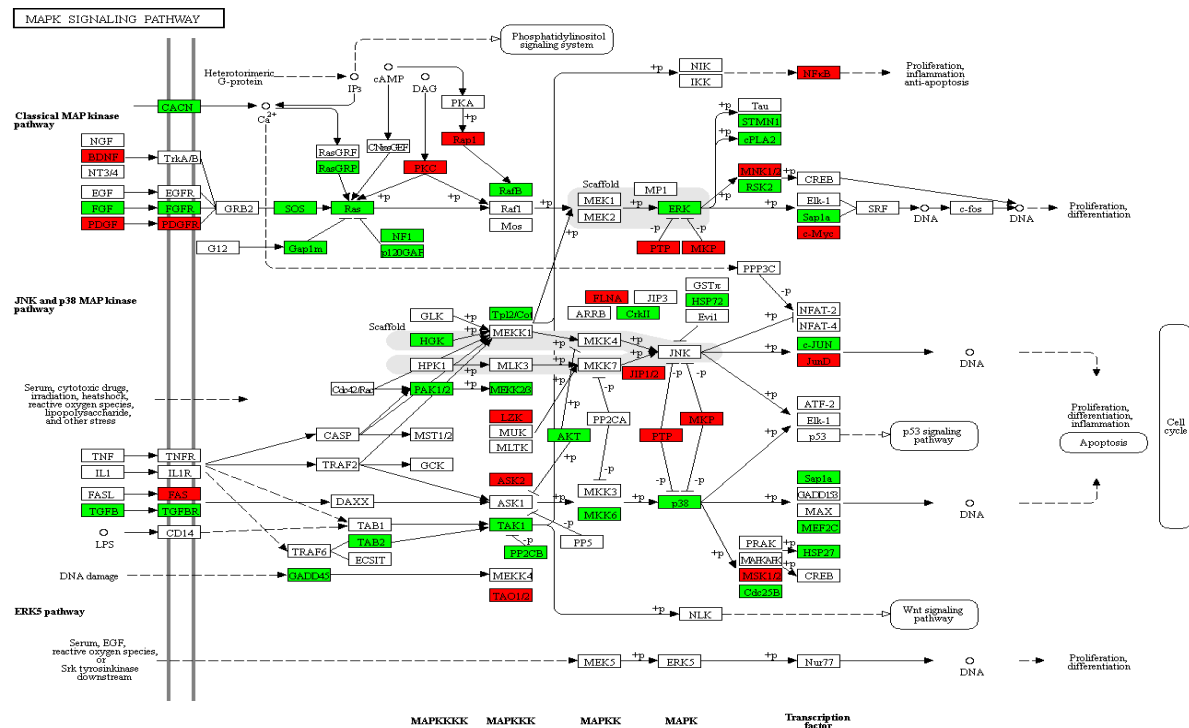
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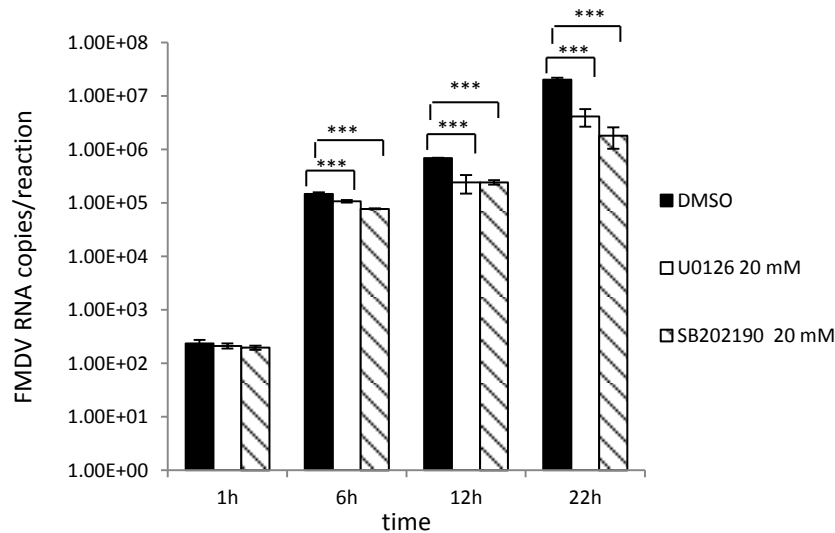
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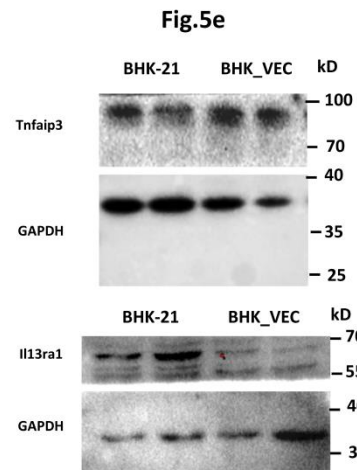
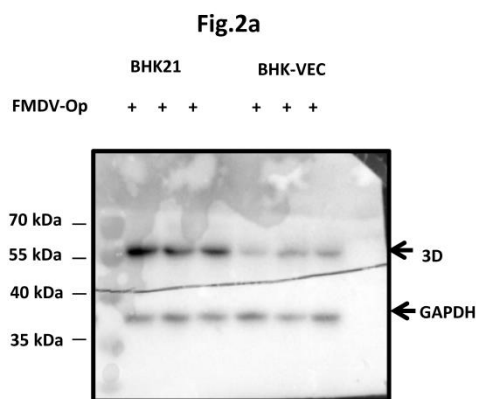
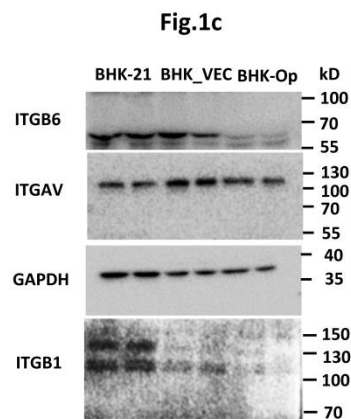
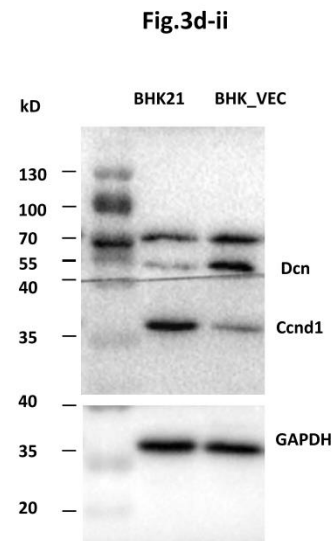
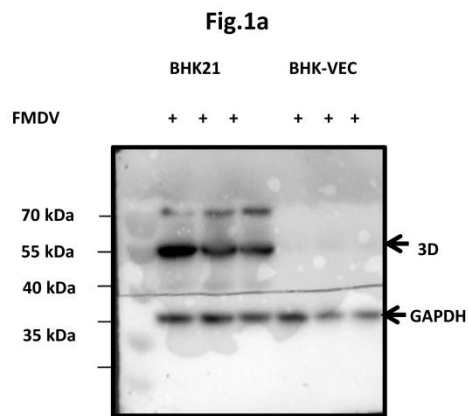
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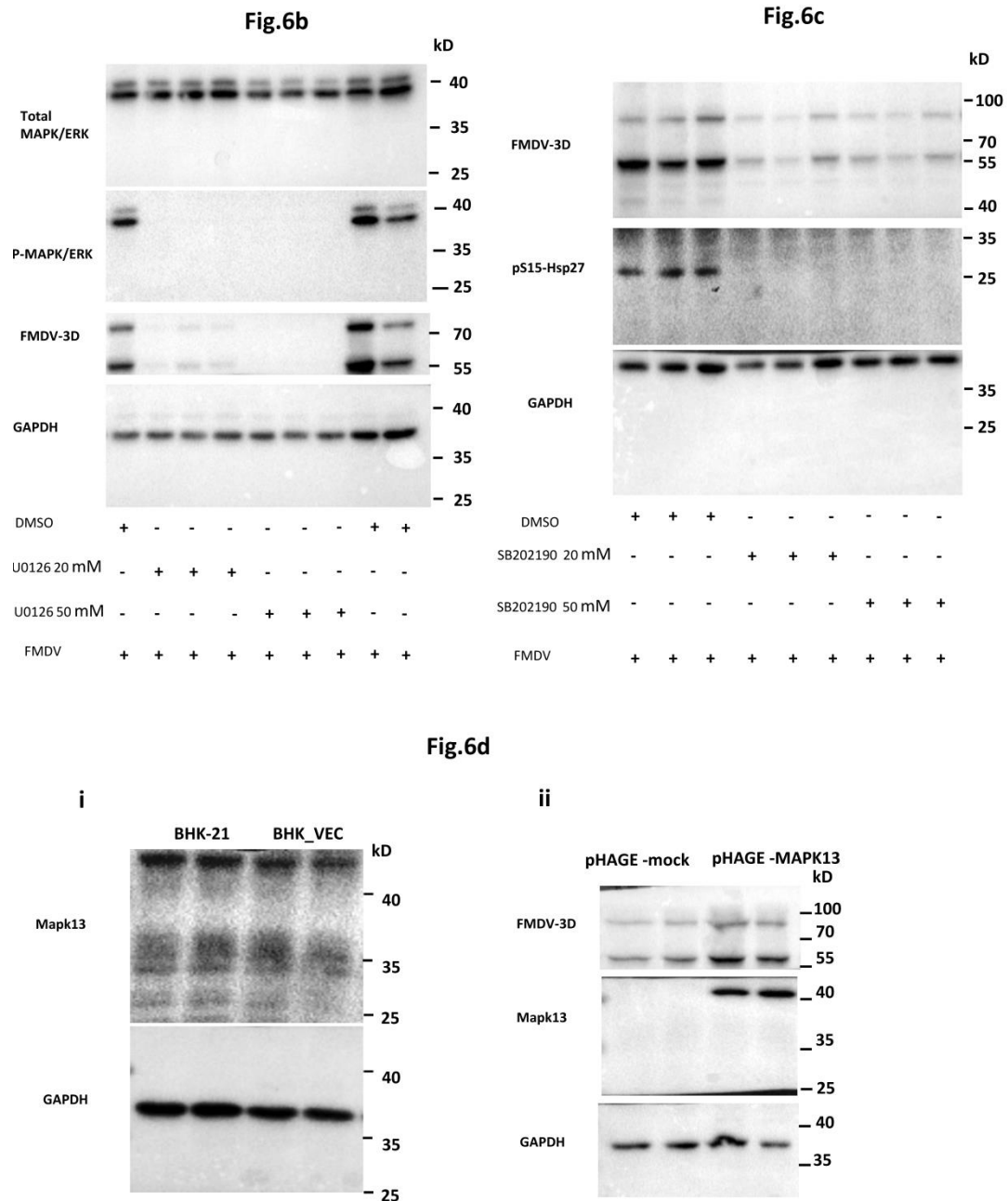
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## References

- 1 Kanehisa, M., Sato, Y., Kawashima, M., Furumichi, M. & Tanabe, M. KEGG as a reference resource for gene and protein annotation. *Nucleic Acids Res* **44**, D457-462 (2016).