Supplementary experimental procedures

Treatment of A2780 and A2780/HPR cells with agonists and antagonists of S1P receptors. Two hundred thousand of A2780 or A2780/HPR cells were plated in 6-well culture plates. After 12 h, A2780 cells were treated with culture medium containing or not 1 μM agonists SEW2871 ($S1P_1$ agonist), or VPC24191 ($S1P_{1/3}$ agonist) or S1P for 96 h. A2780/HPR cells were treated or not with 1 μM VPC23019 ($S1P_{1/3}$ antagonist), or JTE013 ($S1P_2$ antagonist), or CAY10444 ($S1P_3$ antagonist) or 10 μM W146 ($S1P_1$ antagonist), for 95 h followed by 1 h treatment with or not 1 μM S1P. In both treatments the conditioned medium, with agonists or antagonists, were changed every 48 h.

Western blot. After treatments cells were lysed with lysis buffer 1% TX-100 and 1 μM Na3VO4. Cell homogenates were analyzed by SDS-PAGE. After separation, proteins were transferred to PVDF membranes. The presence of Akt and P-Akt was assessed by immunoblotting using Antibodies against Akt 1/2/3 (H-136) and phospho-Akt 1/2/3 (Ser 473) (Santa Cruz, CA, USA). Primary antibodies were visualized by reaction with secondary horseradish peroxidase-conjugated antibodies and enhanced chemiluminescence detection (Pierce Supersignal). The data acquisition was performed using a GS-700 Imaging Densitometer (Bio-Rad). Acquired blots were elaborated using the Quantity One software (Bio-Rad).

Enzyme expression analysis by RT-PC. To detect the expression of SPT1, SPT2, KSDR, ASAH1, ASAH2, ACER1, ACER2, ACER3, SGMS1, SGMS2, DES-1, LASS1, LASS2, LASS3, LASS4, LASS5, LASS6 cDNA from A2780, A2780/HPR, SK transfecant cells and mock cells, were subjected to polymerase chain reaction (PCR) using specific primer listed in Supplementary Table 1. Data were normalized on the housekeeping genes GAPDH and β-ACTIN.

Quantitative Real-Time RT-PCR. To detect the expression of SPT1, SPT2, DES-1, SGMS2, and LASS1, cDNA from A2780, A2780/HPR, SK- and mock-transfected cells, were subjected to Real-Time PCR employing TaqMan Gene Expression Assays. Each measurement was carried out in triplicate, using the automated ABI Prism 7700 Sequence Detector System (Applied Biosystems, Foster City, CA), essentially as previously described (1), by simultaneous amplification of the target sequence (SPT1 Hs00272311_m1, SPT2 Hs00191585_m1, DES-1 Hs00186447_m1, SGMS2 Hs00380453_m1, LASS1 Hs00242151_m1, Applied Biosystems, Foster City, CA) together with the housekeeping gene, 18S rRNA. Results were analysed by ABI Prism Sequence Detection System software (version 1.7) (Applied Biosystems, Foster City, CA). The $2^{-\Delta\Delta Ct}$ method was applied as a comparative method of quantification (2) and data were normalized to ribosomal 18S RNA expression and calibrated against A2780 wt cells.

References

1. Donati,C., Cencetti,F., Nincheri,P., Bernacchioni,C., Brunelli,S., Clementi,E., Cossu,G., Bruni,P. (2007). *Stem Cells* **25**, 1713-1719.

2. Livak,K.J., Schmittgen,T.D. (2001). *Methods* **25**, 402-408.

Legend to supplementary figures

Figure 1: Mass spectrometry analysis of ceramide and dihydroceramide species in A2780/HPR resistant cells treated or not with 10 μM HPR in combinations with SK inhibitor, control cells are treated with vehicle only. X axis reports the long chain base content of sphinganine and sphingosine; Y axis reports the content of each fatty acid species. Data are expressed as pmoles/mg cell protein, and are the means of three different experiments, with S.D. never exceeding 15% of the mean values.

Figure 2: Akt phosphorylation in A2780 or A2780/HPR cells treated with agonists and antagonists of S1P receptors. Panel A: western blot analysis of Akt and its phosphorylated form in A2780 cells treated or not for 96 h with different agonists of S1PRs: 1) control cells, 2) cells treated with S1P, 3) cells treated with VPC24191, 4) cells treated with SEW2871. The graphics report phosphorylation levels of Akt normalized respect to total cell Akt content and expressed as percentage of the control cells treated with only vehicle.

Data are the means ± S.D. of three different experiments.* P<0.01 versus control cells. Panel B: western blot analysis of Akt and its phosphorylated form in A2780/HPR cells treated or not for 95 h with different antagonists of S1PRs followed by 1 h treatment with or not S1P. The graphics report phosphorylation levels of Akt normalized respect to total cell Akt content and expressed as percentage of the control cells treated with only vehicle. Data are the means ± S.D. of three different experiments.* P<0.01 versus control cells.

Figure 3: Sphingolipid metabolism enzyme expression in wild type and mock-transfected A2780, A2780/HPR, SK1overexpressing A2780 cells. RT-PCR of SPT1, SPT2, KSDR, ASAH1, ACER2, ACER3, SGMS1, SGMS2, DES-1, LASS1, LASS2, LASS4, LASS5, LASS6 cDNA from wild type and mock-transfected A2780, A2780/HPR, SK1overexpressing A2780 cells. As loading and reaction control β-ACTIN and GAPDH housekeeping genes were used.

Figure 4: Sphingolipid metabolism enzyme mRNA expression in wild type and mock-transfected A2780, A2780/HPR, and SK1 overexpressing A2780 cells. Quantitative mRNA analysis of SPT1, SPT2, DES-1, SGMS2, LASS1 was performed by Real-Time PCR in total RNA extracted from wild type, mock transfected A2780, A2780/HPR and SK1 overexpressing A2780 cells. Messenger RNA quantitation was based on the $2^{-\Delta\Delta C}{}_{T}$ method, utilizing individual enzyme expression in wild type A2780 as calibrator. Data are the means ± S.D. of the triplicate of three different experiments

Supplementary Table 1.

Gene	Forward primer	Reverse primer
SK1	5'-CCCCAGCAAACCGGACCGAC-3'	5'-CCCCAGCAAACCGGACCGAC-3'
SK2	5'-CCCCTCAGACTCAGCGGCCT-3'	5'-GTGGGCGAGGCAGGTTCCAC-3'
SPT1	5'-CTTGTTCCTCCTGTCCCAAA-3'	5'-CCCCACGCCATACTTCTTT-3'
SPT2	5'-GAATCATGCATCACTGGTTCTGGG-3'	5'-GGCAATCACTTCAGGAAGACGAAC-3'
KSDR	5'-ATTGTGCAGGAATGGCAGTGTC-3'	5'-TCTGTGTCTGGTGGGTAAGCAA-3'
ASAH1	5'-ATATTGGCCCCAGCCTACTT-3'	5'-CCCTGCTTAGCATCGAGTTC-3'
ASAH2	5'-AAACCAGCATTGGGCTACAG-3'	5'-AGATGGCTTTCCCAGGATCT-3'
ACER1	5'-GCCTGGTCTTCATCACCACT-3'	5'-CCTGGCACACGATGTAGAGA-3'
ACER2	5'-CTGATGTGTGCTTTGGCCATGT-3'	5'-TCACACCTCTTTAGCTCTGCGA-3'
ACER3	5'-TCGCCGAGTTCTGGAATACAGT-3'	5'-TCAGAGTCATGTGGAAGCACCA-3'
SGMS1	5'-AGCCCAACTGCGAAGAATAA-3'	5'-GTGTTAGCATGACCGTGTGG-3'
SGMS2	5'-TCAACCTCGTCTTGACAACCGT-3'	5'-ACAGCCACTGGGTGATCCATAA-3'
DES1	5'-GAGCTGATGGCGTCGATGTAGATA-3'	5'-GTTGATGAACAGAGGTCGAAAGGC-3'
LASS1	5'-ATCAGTGACGTGCAGCTTGAGT-3'	5'-TTCATAAGGGTGAGCAGCAGCA-3'
LASS2	5'-ACTGGCTGCCCTCTTGAACATA-3'	5'-TGTGAATCTCCAGCTGGCTTCT-3'
LASS3	5'-AGGAAGCTTGCTGGAGATTTGC-3'	5'-TAATAGCAGCCAGGTGGTGGAT-3'
LASS4	5'-TGGAGGCCTGTAAGATGGTCAA-3'	5'-GCAACATCAGAAGCCCGTTGAA-3'
LASS5	5'-TATTGGCATCGAGGACAGTGGT-3'	5'-GATGGCGAAACCAGCATTGGAT-3'
LASS6	5'-ATCAGCCACTCACAACTGACCT-3'	5'-ACAAAGGACCAGCGTTCCTACT-3'
GAPDH	5'-CGAGATCCCTCCAAAATCAA-3'	5'-GGTGCTAAGCAGTTGGTGGT-3'
β-ACTIN	5'-ACAGAGCCTCGCCTTTGCCG-3'	5'-TGGGCCTCGTCGCCCACATA-3'

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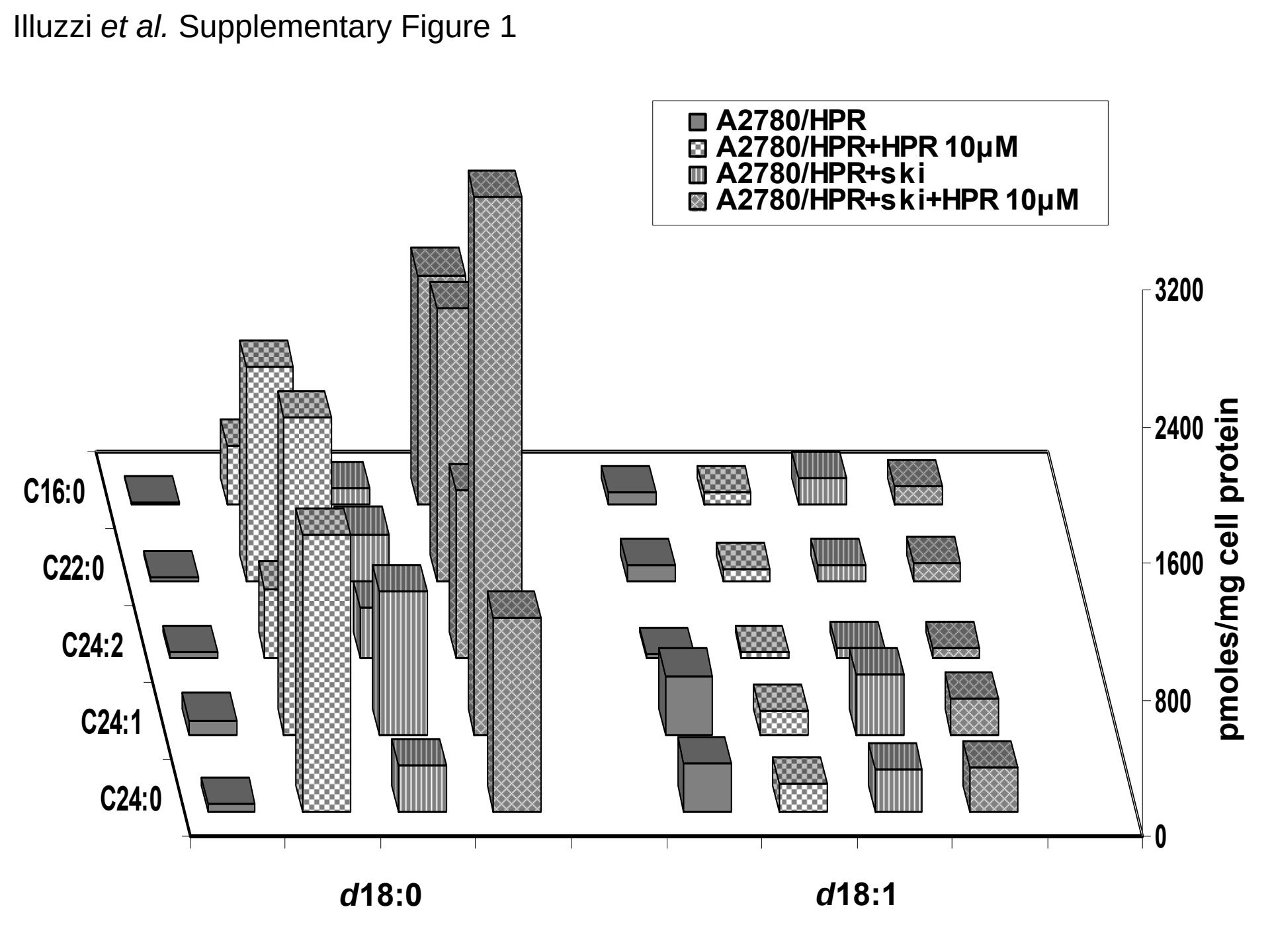

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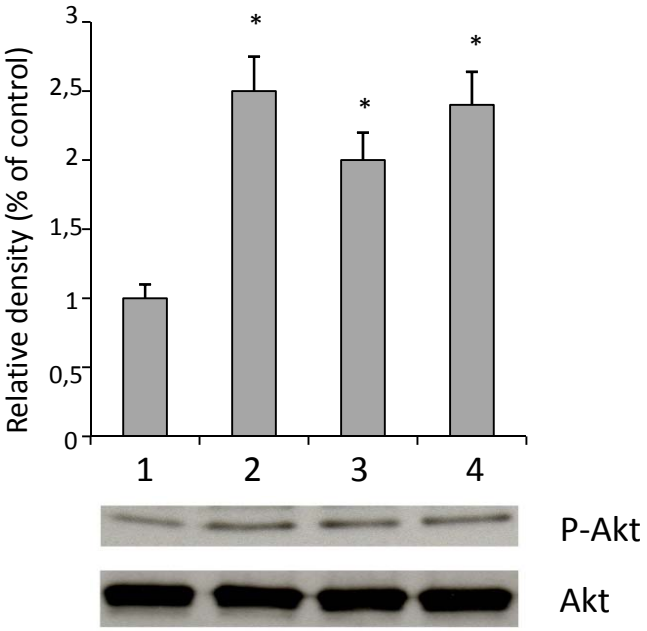
A



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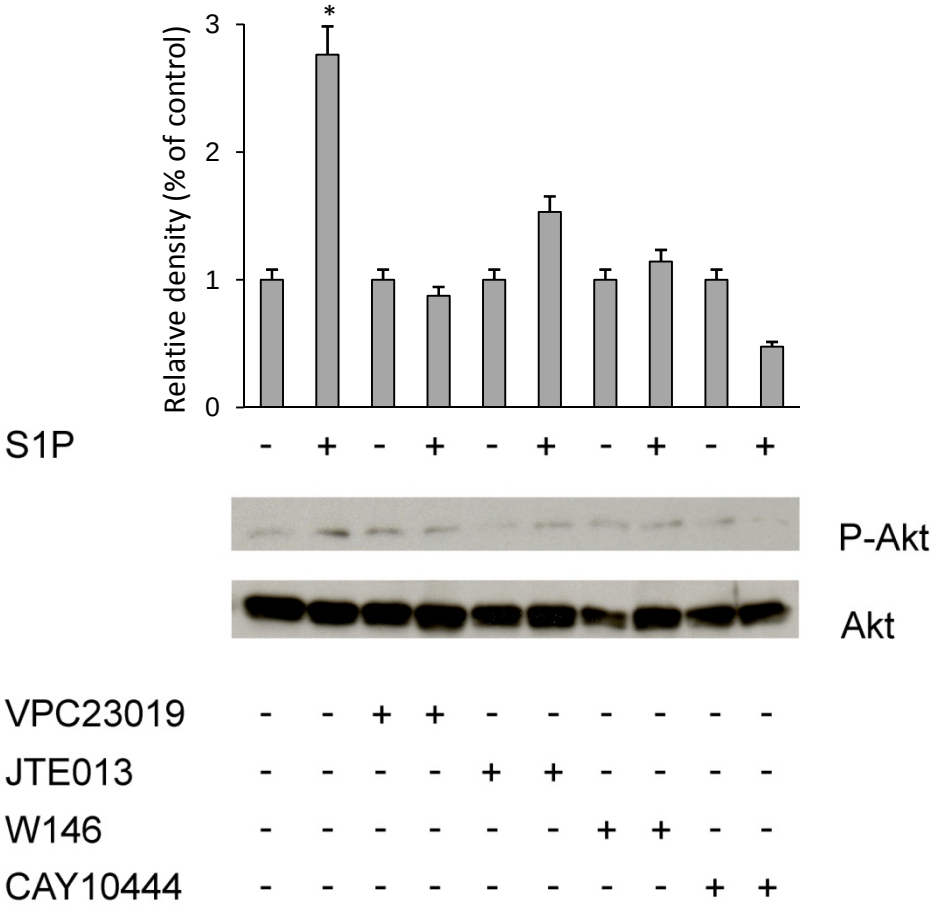

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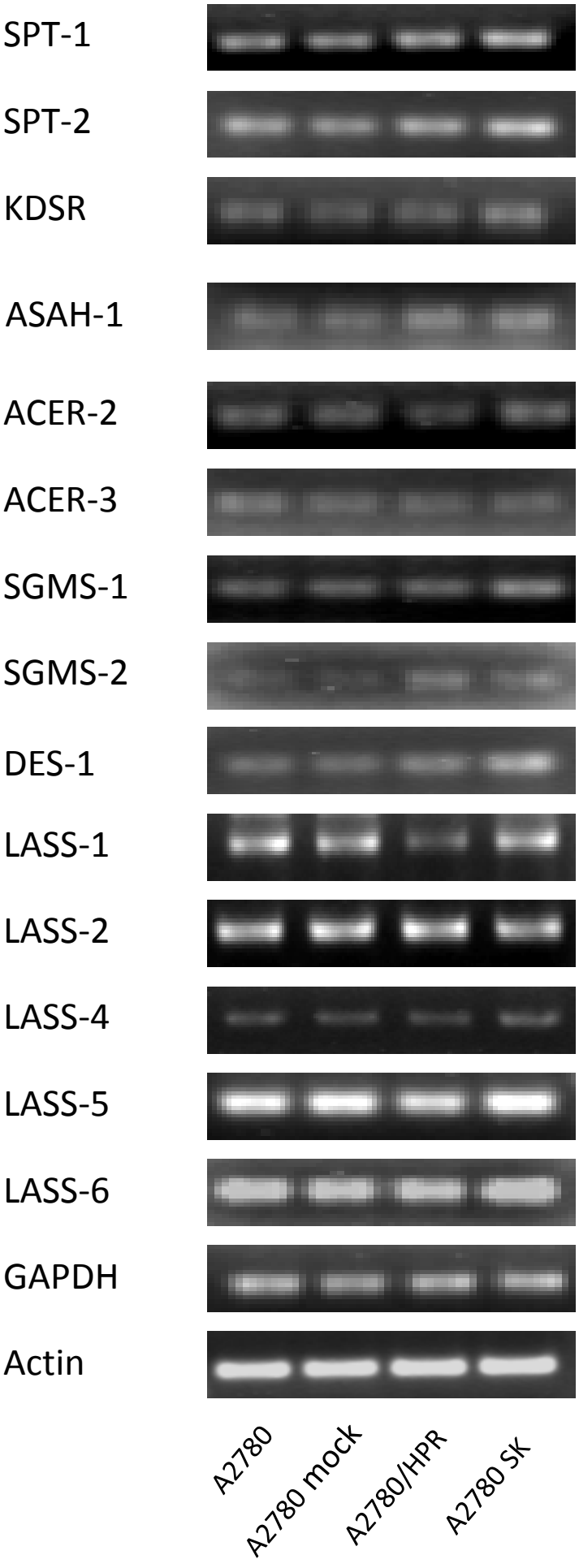

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