# Tumour-specific metabolic adaptation to acidosis is coupled to epigenetic stability in osteosarcoma cells

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**Supplementary Table S1: Heat map that includes the intracellular metabolites related to the identified pathways involved in the response to acute acidosis.**

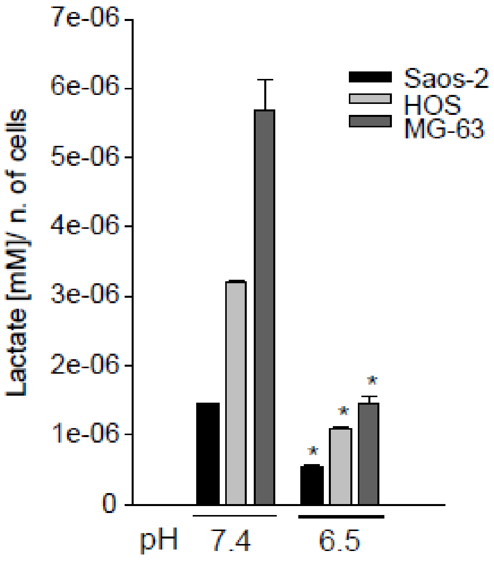
| Compound name | *m/z* | MT | Standardized Relative Area | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Acidic pH | | | | | Neutral pH | | | | |
| | | | Sarcomas (OS) | | | Normals (Fb) | | Sarcomas (OS) | | | Normals (Fb) | |
| | | | HOS | MG-63 | Saos-2 | TIG-121 | TIG-108 | HOS | MG-63 | Saos-2 | TIG-121 | TIG-108 |
| **Dihydroxyacetone phosphate** | 168.991 | 12.79 | -0.411 | -0.665 | -0.355 | 0.026 | 0.057 | -0.022 | 0.149 | -0.666 | -0.277 | 2.492 |
| **Phosphocreatine** | 210.028 | 12.37 | -0.782 | -0.894 | -0.883 | 0.070 | 0.014 | -0.474 | 0.605 | -0.968 | -0.306 | 1.569 |
| **Glu** | 149.063 | 10.93 | -0.577 | -0.644 | -0.169 | 0.094 | 0.352 | -0.228 | -0.050 | -0.518 | 2.407 | 2.717 |
| **Argininosuccinic acid** | 291.128 | 9.42 | -0.634 | -0.587 | -0.697 | -0.278 | -0.184 | -0.297 | -0.144 | -0.532 | 1.548 | 2.820 |
| **ATP** | 505.989 | 11.91 | -0.643 | -0.680 | -0.225 | 0.347 | 0.158 | -0.594 | -0.218 | -0.915 | -0.028 | 2.519 |
| **GTP** | 521.985 | 11.59 | -0.516 | -0.644 | -0.599 | -0.133 | -0.228 | -0.175 | 0.028 | -0.578 | -0.236 | 1.275 |
| **CTP** | 481.977 | 12.48 | -0.232 | 0.205 | -0.543 | 0.802 | 0.577 | 0.115 | -0.386 | -1.134 | 0.551 | 2.756 |
| **UTP** | 482.962 | 12.70 | -0.025 | -0.724 | -0.813 | 1.735 | 1.078 | -0.200 | 0.012 | -0.844 | 0.995 | 1.422 |
| **UDP-glucose, UDP-galactose** | 565.049 | 8.73 | -0.068 | -0.515 | -0.658 | 1.293 | 1.009 | -0.176 | -0.352 | -0.725 | 0.951 | 2.970 |
| **CMP-N-acetylneuraminate** | 613.142 | 8.26 | -0.612 | 0.215 | -0.280 | 1.980 | 2.700 | -0.467 | -0.475 | -0.713 | 1.264 | -0.052 |
| **NAD$^+$** | 662.103 | 6.66 | -0.781 | -1.049 | 0.113 | 1.893 | 1.137 | -0.792 | -0.822 | -0.803 | 0.782 | 1.016 |
| **N$^8$-Acetylspermidine** | 188.175 | 6.32 | -0.391 | -0.217 | -0.370 | 2.665 | 2.808 | -0.416 | -0.394 | -0.415 | -0.421 | -0.421 |
| **Pelargonic acid** | 157.123 | 8.21 | -0.709 | -0.767 | 0.574 | 2.378 | 1.466 | -0.773 | -0.733 | -0.709 | 1.765 | 0.916 |
| **Decanoic acid** | 171.139 | 8.03 | -0.660 | -0.767 | 0.230 | 1.854 | 1.986 | -0.830 | -0.773 | -0.775 | 1.547 | 1.444 |
| Asp | 134.044 | 11.58 | -0.624 | -0.501 | -0.325 | 1.874 | 0.816 | -0.382 | -0.435 | -0.671 | 1.770 | 2.444 |
| Gly | 77.042 | 8.30 | -1.009 | -0.715 | 0.401 | 0.549 | 1.197 | -0.935 | -0.642 | -0.851 | 1.638 | 2.022 |
| His | 156.077 | 7.29 | -1.055 | -0.255 | 0.295 | 0.235 | 0.986 | -0.917 | -0.480 | -1.121 | 2.092 | 1.584 |
| Tyr | 182.081 | 11.36 | -1.151 | -0.367 | 0.761 | 0.247 | 1.087 | -1.033 | -0.608 | -1.179 | 1.646 | 1.283 |
| Phe | 166.086 | 11.09 | -1.131 | -0.418 | 0.818 | 0.423 | 1.376 | -1.030 | -0.592 | -1.175 | 1.654 | 1.211 |
| Trp | 205.096 | 11.02 | -1.134 | -0.254 | 0.807 | 0.581 | 1.603 | -1.142 | -0.771 | -1.277 | 1.505 | 0.930 |
| Val | 118.086 | 10.01 | -1.020 | -0.413 | 0.989 | 0.872 | 1.823 | -1.028 | -0.734 | -1.208 | 1.231 | 0.999 |
| Ile | 132.102 | 10.20 | -1.005 | -0.377 | 0.805 | 0.799 | 1.793 | -0.977 | -0.669 | -1.203 | 1.588 | 1.277 |
| Leu | 132.102 | 10.31 | -1.022 | -0.385 | 0.909 | 0.797 | 1.684 | -0.991 | -0.696 | -1.186 | 1.659 | 1.240 |
| Met | 150.058 | 10.71 | -1.033 | -0.367 | 0.933 | 0.442 | 1.318 | -0.955 | -0.700 | -1.114 | 1.854 | 1.821 |
| Lys | 147.113 | 6.87 | -0.885 | -0.202 | 0.781 | 0.440 | 1.488 | -0.812 | -0.654 | -0.911 | 1.570 | 2.278 |
| Arg | 175.118 | 7.10 | -0.837 | -0.202 | 0.531 | 0.525 | 1.740 | -0.826 | -0.628 | -0.905 | 1.680 | 2.259 |
| Cys | 122.027 | 11.32 | -0.643 | 0.495 | -0.383 | 0.895 | 1.765 | -0.706 | -0.690 | -0.788 | 1.785 | 1.595 |
| Pro | 116.070 | 10.81 | -0.719 | -0.389 | 1.771 | 1.604 | 1.721 | -0.875 | -0.811 | -0.728 | 1.243 | 0.578 |
| Methionine sulfoxide | 166.052 | 11.80 | -0.975 | -0.741 | 0.785 | 0.972 | 1.345 | -0.993 | -0.866 | -0.849 | 2.091 | 1.132 |
| Choline | 104.107 | 6.85 | -0.842 | -0.786 | 1.135 | 0.528 | 1.272 | -1.092 | -0.642 | -0.749 | 2.026 | 1.194 |
| Creatinine | 114.066 | 7.29 | -0.519 | -0.982 | 1.235 | 0.434 | 1.669 | -1.100 | -0.588 | -1.056 | 0.599 | 1.217 |
| Hypotaurine | 110.027 | 18.11 | -0.879 | -0.953 | 1.260 | 0.477 | 1.170 | -0.866 | -0.627 | -0.400 | 0.237 | 1.649 |
| Putrescine | 89.107 | 4.69 | -0.837 | -0.710 | 0.438 | 0.936 | 1.944 | -0.431 | -0.670 | -0.724 | 2.034 | 1.525 |
| *myo*-Inositol 2-phosphate | 259.022 | 10.57 | -0.466 | -0.630 | -0.080 | 1.525 | 1.042 | -0.634 | -0.576 | -0.675 | 0.454 | 3.024 |
| *N*-Acetyllysine | 189.122 | 9.78 | -0.956 | -0.188 | 0.006 | 1.116 | 1.209 | -0.753 | -0.163 | -0.891 | 1.086 | 2.655 |
| Diethanolamine | 106.086 | 7.64 | -0.387 | -0.524 | 0.181 | 0.421 | 2.020 | -0.690 | -0.756 | -0.813 | 2.461 | 1.168 |
| Glycerol | 93.055 | 22.14 | -0.490 | -0.580 | 0.125 | 0.520 | 2.584 | -0.606 | -0.679 | -0.795 | 1.501 | 1.752 |
| 1-Methylnicotinamide | 137.071 | 7.35 | -0.406 | -0.441 | -0.503 | 0.402 | 2.274 | -0.455 | -0.506 | -0.567 | 1.617 | 2.370 |
| XC0132 | 325.159 | 8.68 | -0.518 | -0.415 | -0.386 | 1.472 | 2.753 | -0.525 | -0.477 | -0.500 | 1.322 | 1.419 |
| Lauric acid | 199.170 | 7.76 | -0.503 | -0.639 | 0.847 | 1.377 | 1.789 | -0.781 | -0.718 | -0.590 | 0.669 | 2.424 |
| Carnosine | 227.113 | 6.75 | -0.708 | -0.708 | 0.456 | 1.450 | 2.128 | -0.708 | -0.571 | -0.435 | -0.708 | 2.433 |
| XC0126 | 310.110 | 14.98 | -0.240 | -0.336 | 0.561 | 0.860 | 2.811 | -0.746 | -0.510 | -1.005 | 0.447 | 1.610 |
| Pyridoxine | 170.081 | 8.63 | -0.414 | -0.521 | 0.453 | 0.881 | 2.398 | -0.762 | -0.646 | -0.900 | 0.890 | 1.374 |
| Adenosine 5'-phosphosulfate | 426.013 | 10.22 | -0.111 | -0.051 | 0.901 | 2.170 | 2.007 | -0.962 | -0.770 | -0.688 | 1.012 | -0.221 |
| GABA | 104.070 | 7.69 | -0.501 | -0.467 | 1.388 | 2.345 | 2.150 | -0.738 | -0.284 | -0.198 | 0.154 | 0.692 |
| Morpholine | 88.076 | 6.62 | -0.672 | -0.561 | 0.038 | 2.168 | 2.005 | -0.722 | -0.669 | -0.884 | 0.850 | 1.174 |
| Hexanoic acid | 115.076 | 9.02 | -0.723 | -0.691 | 0.585 | 2.845 | 0.297 | -0.764 | -0.715 | -0.654 | 1.795 | 0.875 |
| *p*-Toluic acid | 135.045 | 9.24 | -0.751 | -0.751 | 0.514 | 3.087 | 0.904 | -0.751 | -0.751 | -0.560 | 1.349 | 0.339 |
| *N*-Acetylneuraminic acid | 308.100 | 7.29 | -0.641 | -0.639 | -0.158 | 2.575 | 1.623 | -0.639 | -0.655 | -0.649 | 1.201 | -0.532 |
| Streptomycin sulfate_+H$_2$O_divalent | 300.644 | 6.86 | -0.384 | -0.372 | -0.259 | 3.580 | 1.398 | -0.431 | -0.433 | -0.441 | 0.210 | 0.184 |
| **Fumaric acid** | 115.004 | 25.85 | -0.582 | -0.816 | -0.591 | -0.245 | -0.329 | 0.080 | 0.030 | -0.214 | -0.816 | 2.110 |
| **Malic acid** | 133.014 | 21.69 | -0.547 | -0.760 | -0.610 | -0.248 | -0.421 | 0.148 | 0.090 | -0.233 | -0.494 | 1.490 |
| **Nicotinamide** | 123.055 | 7.40 | -0.379 | 0.006 | 0.344 | 0.950 | 3.144 | -0.896 | -0.693 | -0.950 | 0.854 | 0.510 |
| **Glycerophosphocholine** | 258.109 | 21.70 | 0.132 | 0.147 | -0.283 | 1.602 | 3.099 | -0.685 | -0.611 | -0.709 | -0.596 | -0.648 |

The heat map represents the fold change of metabolite concentration. Standardization of the heat map was performed as follows: the mean for each metabolite within the group of the values obtained for the different cell models (HOS, MG-63, Saos-2, TIG-121, TIG-108) in the acidic condition or in the neutral condition was calculated and considered as 0. Then, the average deviation was shown as +1 or –1 for increment (red colour) or decrease (blue colour), respectively, compared with the mean.

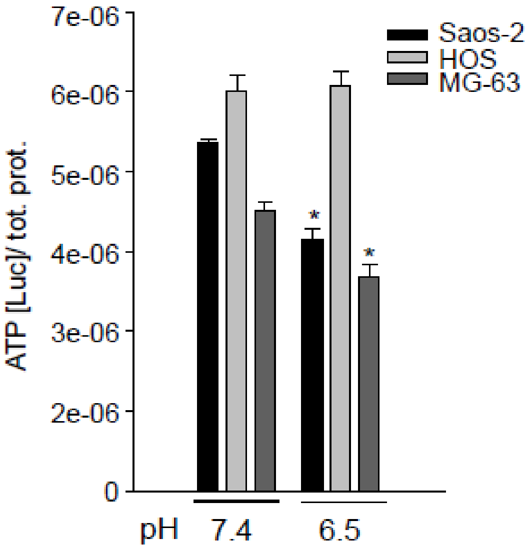
**Supplementary Table S2: Heat map of transcripts of genes related to the metabolic pathways identified by metabolomics analysis and involved in the response to acute acidosis.**

| Gene Symbol | Entrez ID | Ensembl ID | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Acidic pH | | | | | Neutral pH | | | | |
| | | | Sarcomas (OS) | | | Normals (Fb) | | Sarcomas (OS) | | | Normals (Fb) | |
| | | | HOS | MG-63 | Saos-2 | TIG-121 | TIG-108 | HOS | MG-63 | Saos-2 | TIG-121 | TIG-108 |
| *Glycolysis* | | | | | | | | | | | | |
| HK1 | 3098 | ENSG00000156515 | 12.409199 | 11.253214 | 11.562604 | 11.318684 | 11.224559 | 12.044441 | 11.522645 | 11.815693 | 12.082178 | 12.370876 |
| HK2 | 3099 | ENSG00000159399 | 11.342816 | 10.264063 | 11.334791 | 12.502488 | 12.189524 | 10.674194 | 10.743927 | 10.924035 | 10.725984 | 10.640933 |
| HK3 | 3101 | ENSG00000160883 | 0 | 0 | 0 | 0 | 0 | 0 | 1.1116893 | 0 | 0 | 0 |
| PFKL | 5211 | ENSG00000141959 | 9.766287 | 10.308735 | 10.523505 | 10.369005 | 10.582753 | 9.62831 | 10.069268 | 10.940122 | 10.271774 | 10.717787 |
| PFKM | 5213 | ENSG00000152556 | 10.75107 | 10.875085 | 9.812639 | 10.123131 | 9.851434 | 11.006119 | 10.538072 | 10.420568 | 10.633866 | 10.769643 |
| PFKP | 5214 | ENSG00000067057 | 11.692869 | 11.247765 | 10.415174 | 10.299332 | 10.840413 | 11.914386 | 11.091313 | 10.782291 | 11.031064 | 11.509333 |
| ALDOA | 226 | ENSG00000149925 | 12.826196 | 13.137805 | 12.493885 | 12.499638 | 12.47743 | 12.928181 | 12.990125 | 12.531553 | 12.566268 | 12.466882 |
| ALDOB | 229 | ENSG00000136872 | 0 | 2.3689234 | 2.1955543 | 0.76722986 | 2.719281 | 0 | 4.2200866 | 1.6892521 | 1.6951414 | 0 |
| ALDOC | 230 | ENSG00000109107 | 9.843989 | 3.3684845 | 9.716939 | 8.018367 | 8.4635935 | 8.802816 | 4.840587 | 8.633072 | 7.1707773 | 6.682957 |
| TPI1 | 7167 | ENSG00000111669 | 11.35943 | 12.36279 | 11.096159 | 11.006119 | 10.8421335 | 11.458347 | 11.883163 | 11.19116 | 11.548942 | 11.460541 |
| GAPDH | 2597 | ENSG00000111640 | 12.845823 | 13.766205 | 12.8784485 | 12.662054 | 12.797461 | 13.0022955 | 13.440406 | 12.827934 | 12.87597 | 12.857994 |
| GAPDHS | 26330 | ENSG00000105679 | 8.443409 | 7.7832227 | 6.8617463 | 8.138038 | 8.830945 | 7.421616 | 7.9533796 | 6.497021 | 6.9308434 | 7.2491875 |
| PGK1 | 5230 | ENSG00000102144 | 12.153183 | 12.7832575 | 12.823092 | 12.190403 | 12.019995 | 12.367074 | 12.481045 | 12.681366 | 12.703712 | 12.506502 |
| PGK2 | 5232 | ENSG00000170950 | 0 | 0 | 1.2442105 | 0.8200225 | 0 | 0 | 0 | 0 | 0.06469393 | 0 |
| PGAM1 | 5223 | ENSG00000171314 | 8.505315 | 9.618388 | 8.191748 | 8.633442 | 8.458765 | 8.680887 | 8.483391 | 8.056486 | 8.548246 | 8.778131 |
| PGAM2 | 5224 | ENSG00000164708 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| PGAM4 | 441531 | ENSG00000226784 | 0 | 1.2442105 | 0 | 1.0026534 | 1.282275 | 0 | 1.7861416 | 0.11121831 | 1.9980683 | 0 |
| PGAM5 | 192111 | ENSG00000176894 | 10.943783 | 10.763016 | 10.527151 | 9.290789 | 8.598321 | 10.333752 | 10.382821 | 10.873635 | 9.653443 | 9.164069 |
| ENO1 | 2023 | ENSG00000074800 | 13.575606 | 14.060843 | 14.089886 | 12.588876 | 12.216739 | 13.6991415 | 14.116754 | 14.149594 | 13.367027 | 13.3909 |
| ENO2 | 2026 | ENSG00000111674 | 9.656764 | 10.425203 | 8.3362465 | 9.67768 | 9.676269 | 9.597572 | 10.150731 | 8.368593 | 9.470452 | 10.866586 |
| ENO3 | 2027 | ENSG00000108515 | 6.4588904 | 6.6666303 | 4.346933 | 5.2690516 | 6.0184245 | 6.470815 | 6.4919186 | 3.9816904 | 4.917551 | 5.722017 |
| ENO4 | 387712 | ENSG00000188316 | 5.6089706 | 2.2519855 | 4.3200617 | 1.2381275 | 3.536486 | 3.9477217 | 4.2412043 | 2.1342518 | 3.477151 | 4.5170555 |
| PKLR | 5313 | ENSG00000143627 | 0 | 0 | 0.06988551 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| PKM2 | 5315 | ENSG00000067225 | 13.703678 | 14.10248 | 12.863498 | 12.583481 | 13.107946 | 14.040522 | 14.127852 | 13.240816 | 13.668372 | 13.816241 |
| LDHA | 3939 | ENSG00000134333 | 12.797461 | 12.863498 | 12.847786 | 12.75372 | 12.713863 | 13.14687 | 12.856271 | 12.805337 | 13.065542 | 13.232918 |
| LDHAL6A | 160287 | ENSG00000166800 | 1.6134268 | 0 | 0 | 0.5267878 | 0.33389223 | 2.7795272 | 2.3645723 | 3.3218858 | 3.0302413 | 2.9824562 |
| LDHAL6B | 92483 | ENSG00000171989 | 1.8835616 | 3.312686 | 2.600904 | 1.7075523 | 3.377551 | 2.0342543 | 1.7235659 | 2.5791528 | 1.6340747 | 3.8760486 |
| LDHB | 3945 | ENSG00000111716 | 12.810263 | 13.518546 | 12.65571 | 12.417956 | 11.99684 | 13.018645 | 13.2833805 | 12.720022 | 12.469393 | 12.374005 |
| LDHC | 3948 | ENSG00000166796 | 0 | 0 | 0 | 0 | 0 | 0 | 1.3688694 | 0.9233714 | 0 | 1.1581382 |
| LDHD | 197257 | ENSG00000166816 | 0 | 3.2344809 | 0.7475067 | 1.0792903 | 6.060171 | 0 | 2.6591272 | 0.36865154 | 3.1395311 | 4.222813 |
| PDHA1 | 5160 | ENSG00000131828 | 11.195777 | 11.345575 | 10.405147 | 10.156827 | 10.377528 | 11.248066 | 10.981101 | 10.508164 | 10.494298 | 10.213851 |
| PDHA2 | 5161 | ENSG00000163114 | 6.5416756 | 7.352835 | 6.053615 | 6.0844755 | 5.806089 | 5.7422595 | 6.04653 | 5.7851195 | 5.5564065 | 4.6303153 |
| PDHB | 5162 | ENSG00000168291 | 11.151771 | 10.787737 | 10.01857 | 10.189819 | 10.388985 | 11.545805 | 10.989304 | 10.010316 | 11.223675 | 10.659366 |
| PDHX | 8050 | ENSG00000110435 | 11.469987 | 11.069379 | 10.798909 | 11.0383415 | 10.878394 | 11.018299 | 10.266986 | 9.940872 | 10.120102 | 9.42201 |
| PDK1 | 5163 | ENSG00000152256 | 9.173602 | 11.090658 | 9.899037 | 11.15062 | 10.462985 | 9.06427 | 9.361494 | 9.3715315 | 9.372674 | 8.934001 |
| PDK2 | 5164 | ENSG00000005882 | 6.306005 | 7.9820004 | 8.818203 | 8.370209 | 8.300196 | 7.780081 | 8.296767 | 9.375695 | 9.043879 | 9.461926 |
| PDK3 | 5165 | ENSG00000067992 | 8.446831 | 6.1376824 | 9.52045 | 6.9146137 | 4.635946 | 9.675262 | 5.68029 | 9.095707 | 7.0415764 | 6.6787095 |
| PDK4 | 5166 | ENSG00000004799 | 7.6518774 | 8.032203 | 11.511249 | 11.819046 | 12.233117 | 5.739105 | 5.982508 | 8.313715 | 6.9271545 | 7.760661 |
| *TCA cycle & Glutaminolysis* | | | | | | | | | | | | |
| CS | 1431 | ENSG00000062485 | 12.104154 | 12.021648 | 11.546583 | 10.678837 | 10.871178 | 12.1156435 | 11.830169 | 11.618069 | 11.229054 | 11.537178 |
| ACO1 | 48 | ENSG00000122729 | 11.587562 | 10.381481 | 10.606644 | 11.573494 | 11.407493 | 11.581986 | 10.318593 | 10.8283825 | 11.790245 | 11.535909 |
| ACO2 | 50 | ENSG00000100412 | 10.506633 | 10.818874 | 10.057255 | 10.767778 | 10.978623 | 10.597727 | 10.450865 | 10.106592 | 10.363483 | 10.414825 |
| IDH1 | 3417 | ENSG00000138413 | 10.595347 | 11.01756 | 10.460838 | 10.687077 | 10.902105 | 11.718281 | 11.673391 | 11.204786 | 11.644097 | 11.660861 |
| IDH2 | 3418 | ENSG00000182054 | 11.071193 | 10.961503 | 10.41898 | 9.969523 | 10.418154 | 10.688006 | 10.532778 | 9.9948845 | 9.937695 | 9.433058 |
| IDH3A | 3419 | ENSG00000166411 | 11.032922 | 10.788222 | 10.993995 | 10.726717 | 10.128427 | 11.290143 | 10.7333145 | 10.960981 | 10.555621 | 10.378237 |
| IDH3B | 3420 | ENSG00000101365 | 11.246002 | 11.426627 | 9.633114 | 9.766799 | 9.731436 | 11.145845 | 10.172446 | 9.77124 | 10.073116 | 9.870022 |
| IDH3G | 3421 | ENSG00000067829 | 8.646579 | 9.6368475 | 9.483188 | 9.242747 | 9.570819 | 8.41706 | 7.9897714 | 9.819309 | 8.999907 | 8.99914 |
| OGDH | 4967 | ENSG00000105953 | 11.244468 | 10.544521 | 10.400756 | 10.427435 | 11.404189 | 11.651203 | 11.589277 | 10.963297 | 11.014926 | 11.717787 |
| OGDHL | 55753 | ENSG00000197444 | 7.161757 | 5.225427 | 5.5602875 | 4.660977 | 3.2740405 | 7.903981 | 2.3115678 | 6.4633293 | 2.357908 | 3.3727562 |
| SUCLA2 | 8803 | ENSG00000136143 | 10.113728 | 10.807966 | 10.407981 | 10.005362 | 10.277812 | 10.675802 | 10.400082 | 10.516266 | 10.890263 | 10.650119 |
| SUCLG1 | 8802 | ENSG00000163541 | 10.498468 | 11.132253 | 11.093054 | 9.97737 | 9.808286 | 10.491657 | 10.69309 | 10.95031 | 10.803535 | 10.206687 |
| SUCLG2 | 8801 | ENSG00000172340 | 9.950822 | 11.320187 | 9.990201 | 10.591742 | 10.760133 | 10.025218 | 10.652701 | 8.982019 | 10.740157 | 10.780341 |
| SDHA | 6389 | ENSG00000073578 | 11.6333275 | 10.944166 | 11.536439 | 10.424515 | 10.637836 | 11.3883705 | 10.622279 | 11.294328 | 10.035286 | 10.27693 |
| SDHAF1 | 644096 | ENSG00000205138 | 7.8281655 | 8.214627 | 7.5337143 | 8.502333 | 8.430414 | 6.370119 | 7.297434 | 7.2360277 | 6.83329 | 6.5152802 |
| SDHAF2 | 54949 | ENSG00000167985 | 9.564266 | 8.146522 | 9.127642 | 9.551334 | 9.255624 | 9.358638 | 8.99914 | 9.279241 | 9.578248 | 9.065778 |
| SDHB | 6390 | ENSG00000117118 | 10.841224 | 11.636481 | 11.129066 | 10.4654875 | 10.07992 | 10.830645 | 10.953857 | 11.034346 | 10.471801 | 10.104429 |
| SDHC | 642502 | ENSG00000143252 | 8.821783 | 10.378237 | 9.102445 | 8.874095 | 8.920916 | 9.074144 | 9.313252 | 8.948969 | 9.551334 | 9.134911 |
| SDHD | | ENSG00000204370 | 10.205 | 11.215267 | 10.500593 | 10.576683 | 10.382821 | 10.454075 | 9.94469 | 9.67723 | 10.193846 | 10.058991 |
| FH | 2271 | ENSG00000091483 | 11.337451 | 11.773607 | 10.07992 | 10.934129 | 10.287087 | 11.115621 | 11.463231 | 9.927909 | 11.116081 | 10.61886 |
| MDH1 | 4190 | ENSG00000014641 | 11.485329 | 12.17761 | 12.511225 | 11.288983 | 11.303142 | 11.923249 | 11.82592 | 12.288181 | 11.910987 | 11.740643 |
| MDH1B | 130752 | ENSG00000138400 | 0.64805865 | 6.4000435 | 4.4412627 | 1.8758872 | 0.26045844 | 4.063967 | 5.0481176 | 5.435817 | 3.7565691 | 3.2227116 |
| MDH2 | 4191 | ENSG00000146701 | 11.66197 | 11.607603 | 10.77409 | 10.79865 | 10.690191 | 11.504586 | 11.131183 | 10.785915 | 10.97756 | 10.955593 |
| PC | 5091 | ENSG00000173599 | 8.937276 | 8.106986 | 8.480386 | 6.2737064 | 8.171619 | 10.168089 | 8.329315 | 9.256029 | 6.7978544 | 8.089377 |
| GLS | 2744 | ENSG00000115419 | 12.872203 | 12.721584 | 13.473581 | 13.13611 | 12.915356 | 13.236686 | 12.918546 | 13.116251 | 13.542142 | 13.272663 |
| GLS2 | 27165 | ENSG00000135423 | 6.2819786 | 4.7095656 | 5.321238 | 3.579973 | 4.3548245 | 5.7892957 | 6.364008 | 5.875963 | 5.0412135 | 4.8090024 |
| GLUD1 | 2746 | ENSG00000148672 | 11.947237 | 12.342611 | 11.486347 | 11.605608 | 11.831195 | 11.673391 | 11.855499 | 10.993719 | 11.855499 | 11.871166 |
| GLUD2 | 2747 | ENSG00000182890 | 10.615984 | 11.270691 | 10.2407055 | 10.480917 | 10.731118 | 10.455949 | 10.588847 | 9.710762 | 10.556381 | 10.688998 |
| ME1 | 4199 | ENSG00000065833 | 10.930641 | 11.082384 | 11.898049 | 10.697741 | 9.185918 | 10.916459 | 11.037584 | 12.169265 | 11.053282 | 9.932293 |
| ME2 | 4200 | ENSG00000082212 | 10.041449 | 11.026163 | 10.546946 | 10.580257 | 9.87782 | 9.36218 | 10.632639 | 10.660671 | 10.144538 | 9.867981 |
| ME3 | 10873 | ENSG00000151376 | 3.7965806 | 5.0017233 | 8.240245 | 7.6822104 | 7.2118063 | 3.7044168 | 4.144915 | 8.4059105 | 7.3459935 | 8.062891 |
| *HDACs & HATs* | | | | | | | | | | | | |
| **HDAC1** | **3065** | **ENSG00000116478** | 10.845732 | 11.748251 | 10.882499 | 9.059758 | 8.968628 | 11.604327 | 11.380629 | 11.655897 | 11.026163 | 10.721629 |
| HDAC10 | 83933 | ENSG00000100429 | 4.1627007 | 3.837262 | 4.223895 | 3.1271133 | 5.763039 | 2.7646685 | 4.6941094 | 4.2533693 | 4.0389524 | 6.038259 |
| HDAC11 | 79885 | ENSG00000163517 | 6.4516325 | 5.870135 | 7.835693 | 8.265649 | 8.247088 | 5.162749 | 6.3694024 | 7.531474 | 7.8465066 | 7.859753 |
| HDAC2 | 3066 | ENSG00000196591 | 11.853706 | 12.171733 | 12.241243 | 11.290578 | 10.511426 | 12.084416 | 12.216178 | 12.541228 | 11.706302 | 11.296566 |
| HDAC3 | 8841 | ENSG00000171720 | 9.391776 | 10.525565 | 9.342985 | 9.782434 | 9.611809 | 9.755279 | 10.646514 | 10.039416 | 9.94469 | 9.973227 |
| HDAC4 | 9759 | ENSG00000068024 | 8.284027 | 7.2279716 | 8.304613 | 8.869496 | 9.398144 | 9.411644 | 9.506339 | 7.479619 | 8.136078 | 8.921917 |
| HDAC5 | 10014 | ENSG00000108840 | 10.844338 | 10.4477415 | 10.201522 | 10.336226 | 10.821637 | 10.161692 | 9.615221 | 10.099778 | 9.84632 | 10.207613 |
| HDAC6 | 10013 | ENSG00000094631 | 10.768527 | 9.0173235 | 10.310726 | 9.588583 | 10.787426 | 10.153026 | 9.207719 | 9.913226 | 8.830018 | 9.812239 |
| HDAC7 | 51564 | ENSG00000061273 | 10.454075 | 9.481592 | 9.327337 | 10.592936 | 11.102891 | 10.31225 | 10.019533 | 9.753627 | 10.215177 | 10.973004 |
| HDAC8 | 55869 | ENSG00000147099 | 8.232028 | 9.084277 | 8.086519 | 8.206434 | 8.130022 | 8.835331 | 8.60837 | 8.78476 | 8.579402 | 8.300196 |
| HDAC9 | 9734 | ENSG00000048052 | 10.071505 | 9.218938 | 10.755074 | 11.201475 | 11.011009 | 9.625697 | 8.618254 | 10.639841 | 10.326033 | 11.202134 |
| HAT1 | 8520 | ENSG00000128708 | 11.354522 | 11.218137 | 11.428499 | 10.936185 | 10.870205 | 11.3976 | 11.378054 | 11.476685 | 11.280715 | 10.87955 |
| EP300 | 2033 | ENSG00000100393 | 11.966047 | 10.127488 | 10.491086 | 11.267169 | 12.293928 | 12.031978 | 11.511249 | 11.306251 | 10.905961 | 11.746869 |
| KAT2A | 2648 | ENSG00000108773 | 9.066479 | 9.502283 | 8.241745 | 8.139539 | 8.596368 | 9.547913 | 10.135658 | 8.636683 | 8.929302 | 9.285441 |
| KAT2B | 8850 | ENSG00000114166 | 8.253036 | 10.111646 | 10.727945 | 9.206915 | 8.291365 | 9.711062 | 10.159198 | 11.268538 | 9.783195 | 10.201047 |
| KAT5 | 10524 | ENSG00000172977 | 9.4668455 | 10.155611 | 8.852569 | 9.370777 | 9.36218 | 9.045509 | 9.83375 | 8.867645 | 9.138958 | 9.172202 |
| MYST1 | 84148 | ENSG00000103510 | 9.804134 | 10.317613 | 8.652663 | 9.688816 | 9.732168 | 9.327023 | 9.724701 | 8.37596 | 8.855704 | 8.878593 |
| MYST2 | 11143 | ENSG00000136504 | 11.97494 | 9.6439705 | 10.463355 | 10.41898 | 10.183753 | 11.288208 | 10.2507105 | 10.434731 | 9.621223 | 9.723302 |
| MYST3 | 7994 | ENSG00000083168 | 12.081346 | 11.225078 | 11.681289 | 11.301856 | 11.32258 | 11.799946 | 12.1999445 | 11.895291 | 11.26772 | 11.43696 |
| MYST4 | 23522 | ENSG00000156650 | 10.15597 | 10.389736 | 10.046317 | 10.478845 | 11.073472 | 10.704585 | 10.972433 | 10.327886 | 10.794829 | 10.691733 |
| MEAF6 | 64769 | ENSG00000163875 | 9.954605 | 11.286191 | 10.461157 | 9.972485 | 9.739499 | 9.776868 | 10.6517515 | 10.243075 | 10.387928 | 10.209297 |

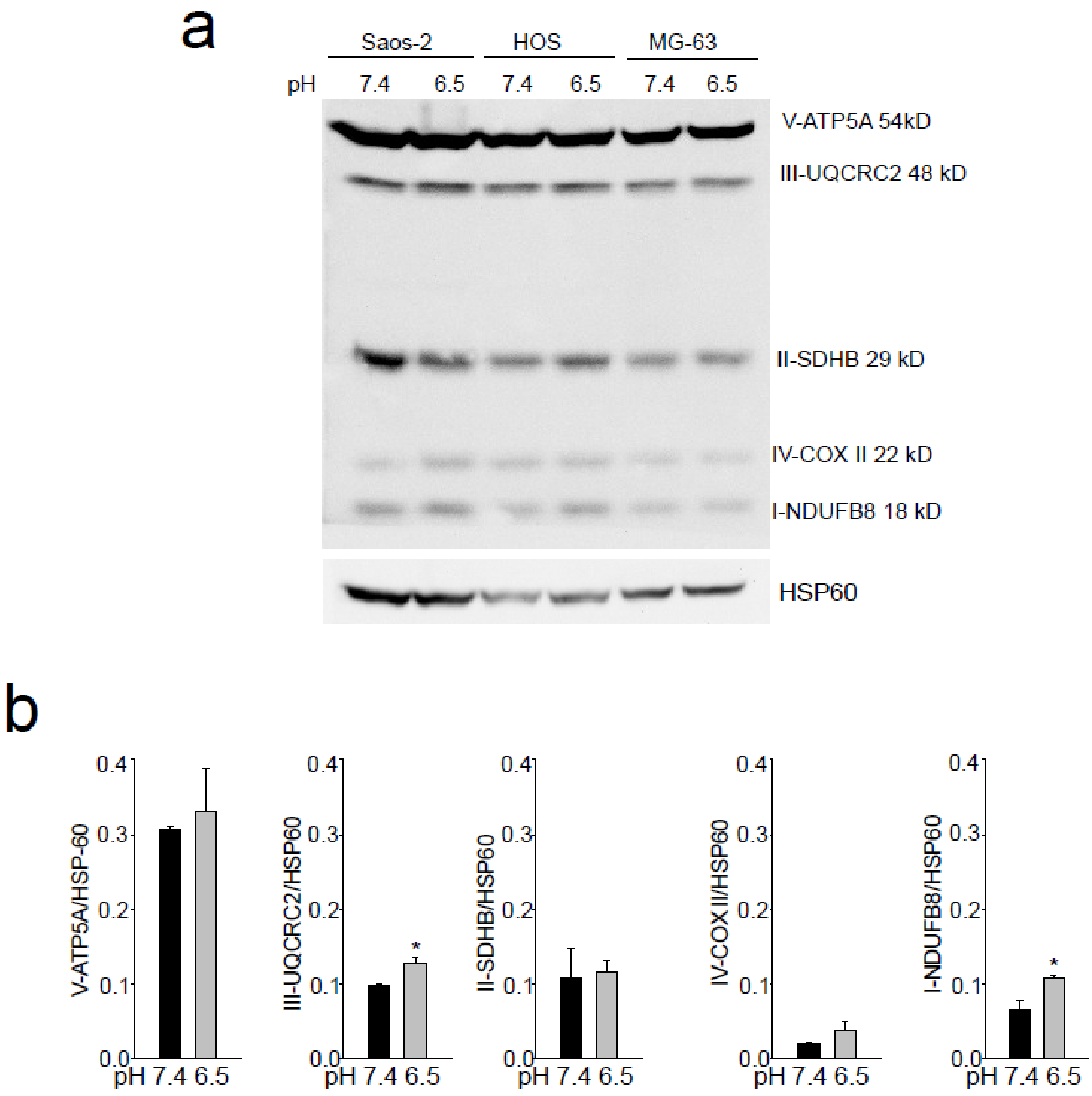
All the enzymes associated with the glycolysis, TCA cycles & glutaminolysis and with epigenetic modification (HDACs & HATs) were considered. Values relative to the specific transcript expression are shown as exponential numbers of 2. Increased values are shown as red scale colours, reduces values are shown as blue scale colours.



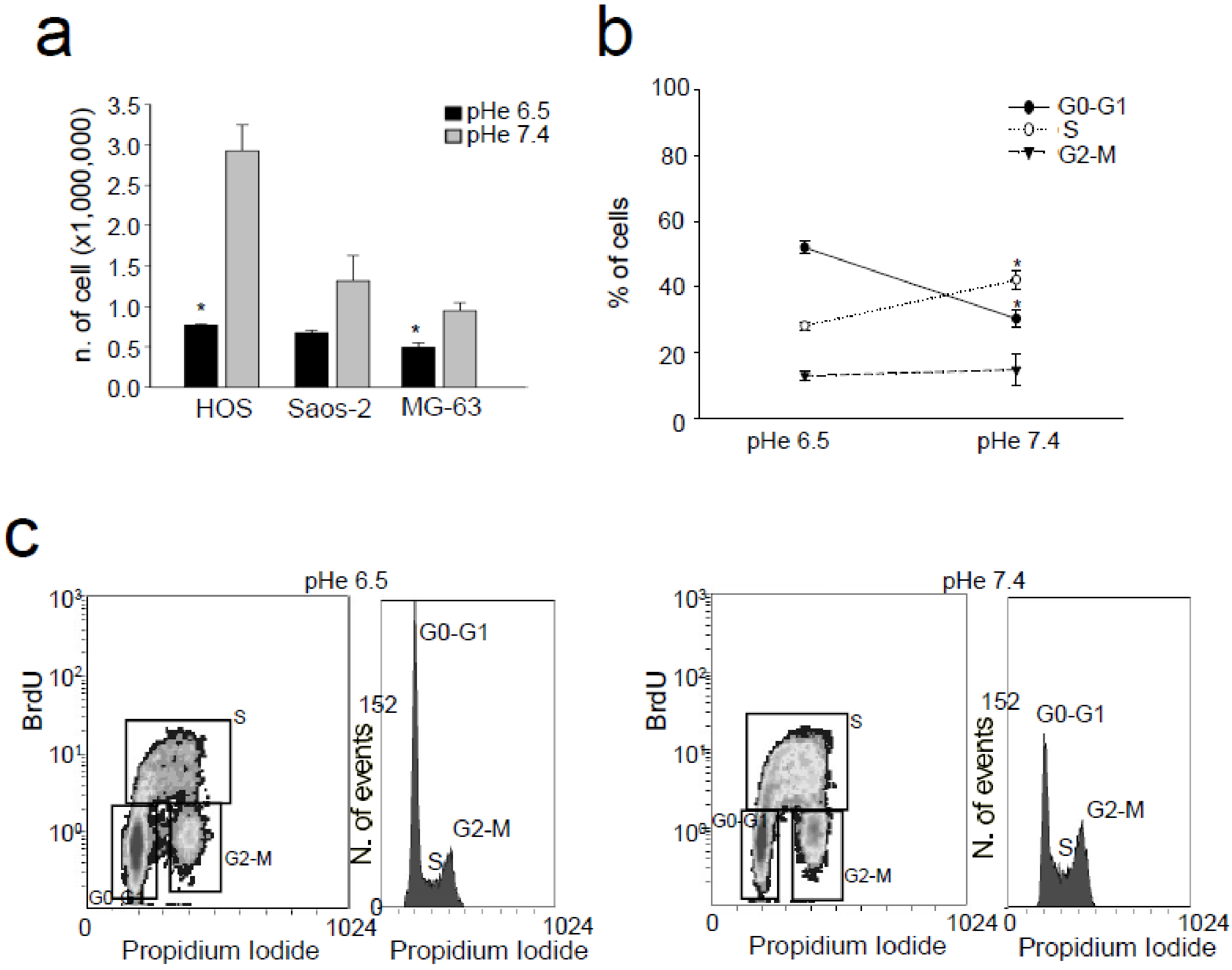
**Supplementary Figure S1: Lactate concentration in the supernatant of OS cells maintained in neutral and acidic medium.** Lactate concentration is significantly decreased under acidity in all the OS cell lines supernatants, mean ± SE (*p < 0.05, n = 3).



**Supplementary Figure S2: Intracellular ATP content in OS cells cultured under acidic medium.** Mean ± SE (*p < 0.05, n = 3). ATP values ware significantly reduced only up to at 80-100% in Saos-2 and MG-63 cells and unchanged in HOS cells.



**Supplementary Figure S3: Protein expressions of complexes of the respiratory chain (OXPHOS) in OS cell lines under both neutral and acidic medium.** **(a)** Western blot analysis of the expression of OXPHOS complexes under different pHs. Representative image. **(b)** Bar graph of the quantification of the expression of OXPHOS complexes showed in panel A, mean ± SE (*$p < 0.05$, $n = 3$).



**Supplementary Figure S4: Proliferation under different pH.** **(a)** Cell count of OS cells cultured at different pHs, soon after the time population doubling; **(b)** cell cycle analysis of OS cells cultured at different pH; **(c)** representative graph of the cell cycle analysis showed in panel B.

## Supplementary methods

***Lactate assay.*** Two × $10^5$ cells of OS were seeded into 6-well plates and allowed to attach overnight. After 24 h the media were replaced with 3 ml of freshly neutral (pH 7.3) or acidic (pH 6.5) DMEM containing 2% NuSerum, a low protein alternative to FBS (BD Biosciences). After additional 24h, lactate concentration in the supernatant was measured by lactate Assay kit (EnzyChrome, BioAssay Systems), according to the manufacturer's instruction. The assay was repeated three times in quadruplicate. Lactate concentration was normalized to total cell number.

***ATP assay.*** OS cells were seeded into 6-well plates at a density of 2 × $10^5$ cells/well. After 24 h media were replaced with 2 ml of freshly neutral (pH 7.4) or acidic (pH 6.5) DMEM high glucose (4.5 g/L) containing 10% FBS. The cells were incubated at 37 °C and 5% $CO_2$ for 24 h. For the analysis of ATP content, cells were lysed by using boiling ATP lysis buffer [0.1 M Tris (hydroxymethyl) aminomethane (Tris base) (Sigma) and 2 mM EDTA adjusted to pH 7.75 with acetic acid (Sigma) and 2.5% Dodecyltrimethylammonium bromide (Sigma)]. The ATP content was measured with the ATP determination kit (Molecular Probes). The luciferase signal was normalized with the total protein concentration measured by Bradford assay (Bio-Rad Laboratories). All experiments were performed in triplicates.

***Western Blotting.*** To detect ATP synthase subunit-α, Complex II subunit 30 kDa, Complex III subunit Core 2, V0c and V1B2 subunits of the V-ATPase protein, and heat shock protein 60 (HSP60) in total lysates, the cells were harvested in lysis buffer (RIPA buffer) containing protease and phosphatase inhibitors and centrifuged at 13,000x g for 10 min at 4°C to remove insoluble debris. Protein concentrations were analyzed using Bradford assay (Biorad). 30 µg of proteins were loaded and separated by SDS-PAGE and transferred to a 0.2 µm nitrocellulose membrane (Fisher Scientific). After blocking for 1 h in TBST (10 mM Tris-HCl pH 8.0, 150 mM NaCl, 0.5% Tween-20) with 5% non-fat dry milk, membranes were incubated with the primary antibody for 1 h or overnight, washed and incubated for 1 h with horseradish peroxidase-conjugated secondary antibodies. The membranes were washed and incubated with an enhanced chemi-luminescence substrate (ECL; Thermo Scientific). Blots were probed with MitoProfile total oxidative phosphorylation (OXPHOS) human antibody Cocktail 1:300 (Abcam, Cambridge, UK), anti-HSP60 (1:2000, Sigma) polyclonal antibodies.

***Cell cycle analysis.*** In order to analyze cell cycle distribution after exposure to acidic conditions, DNA content and bromodeoxyuridine (BrdU) incorporation were determined by simultaneous analysis of propidium iodide and fluorescein isothiocyanate (FITC)-conjugated anti-BrdU fluorescence. Cells were seeded at low density in complete medium, and after 24 h the medium was changed with complete medium at pH 6.5 or 7.4. After additional 48 h, cells were incubated with 10 mM BrdU (Sigma) for 60 min before harvesting. Cells were collected by trypsinization, followed by fixation in 40% ethanol for 20 min. Partial DNA denaturation was performed by incubating cells in HCl, followed by neutralization with sodium tetraborate. Samples were then exposed to a monoclonal anti-BrdU FITC antibody (BD Biosciences, Erembodegem, Belgium), washed, and finally stained with 2.5 mg/ml propidium iodide (Sigma). Flow cytometric analysis was performed with a Coulter EPICS1 XL Flow Cytometer (Coulter Corporation, Beckman Coulter, Milan, Italy). Monoparametric and biparametric analyses were performed using the WinMDI 2.7 software. The experiment was repeated three times.

***RNA extraction and RNA-seq. analysis.*** Total RNA from each cell was extracted using guanidinium thiocyanate-phenol-chloroform. The total RNA was quantified with Bioanalyzer (Agilent, Santa Clara, CA) following the manufacturer's instructions. RIN (RNA Integrity Number) and A260/A280 ratio of the prepared total RNA were all 10, and over 1.8, respectively.

The library of template molecules for high throughput DNA sequencing was converted from the total RNA using TruSeq RNA Sample Prep Kit v2 (Illumina, San Diego, CA) following the manufacturer's protocol. The library was quantified with Bioanalyzer (Agilent) following the manufacturer's instruction. Library (7 pM) was subjected to cluster amplification to cluster generation on a Single Read Flow Cell v4 with a cluster generation instrument (Illumina). Sequencing was performed on a Genome Analyzer GAIIx for 76 cycles using Cycle Sequencing v4 regents (Illumina)..

Image analysis and base calling were performed using Off-Line Basecaller Software 1.6 (Illumina). Reads were aligned using ELAND v2 of CASAVA Software 1.7 with the sequence data sets. Human genome build 19 (hg19) were downloaded from University of California, Santa Cruz genome browser (http://genome.ucsc.edu/) as the analytic reference. Transcript coverage for every gene locus was calculated from the total number passing filter reads that mapped, by ELAND-RNA, to exons. These analyses were performed using default parameters. The advanced analysis for quantification with Quantile normalization algorithm was performed using Avadis NGS software (version1.5, Strand Scientific Intelligence Inc., San Francisco, CA). The filtering was performed using default parameters. All new data has been deposited in DDBJ/EMBL/GenBank under DRA004087.