

Figure 1 Distortion product otoacoustic emissions (DPOAEs) audiograms (2f1-f2 DPOAE magnitude as function of f2 frequency, mean±standard deviation) from 8 TMC2 (red) and 7 wild type (black) littermates of DPOAEs recorded from the auditory meatus. F1 was set at 70 dB SPL and f2 was set at 60 dB SPL. Noise floor = 0 dB SPL.

 kHz WT mean S.D TMC2 mean S.D

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  2.0142 | 10.83352 | 1.65157 | 11.50778 | 3.036507 |
| 2.9907 | 7.558475 | 5.080759 | 6.451138 | 5.092352 |
| 4.0283 | 5.503892 | 4.117486 | 3.3613 | 3.794762 |
| 5.0049 | 0.766333 | 8.960458 | 3.16342 | 3.745162 |
| 6.0425 | 5.631017 | 6.35406 | -0.21458 | 5.953168 |
| 7.019 | 12.08587 | 4.634316 | 10.37545 | 6.745334 |
| 7.9956 | 12.59805 | 2.307921 | 8.992175 | 6.333361 |
| 9.0332 | 16.73938 | 5.419712 | 14.33375 | 3.431177 |
| 10.01 | 25.72817 | 9.075388 | 22.5965 | 8.130175 |
| 10.986 | 33.87883 | 7.290092 | 29.85175 | 9.271484 |
| 12.024 | 39.399 | 4.597996 | 35.0395 | 9.865544 |
| 13 | 40.80183 | 2.607061 | 35.49525 | 9.122779 |
| 14.038 | 37.57733 | 2.890043 | 27.6559 | 16.97729 |
| 15.015 | 29.29883 | 1.81869 | 28.46025 | 5.029736 |
| 15.991 | 28.76117 | 2.162956 | 24.3615 | 6.466673 |
| 17.029 | 26.39983 | 2.181706 | 26.0225 | 2.900841 |
| 18.005 | 28.063 | 2.880584 | 27.05075 | 4.388949 |
| 19.043 | 23.25667 | 8.145743 | 26.1175 | 6.247722 |
| 20.02 | 23.86217 | 1.794703 | 25.6855 | 2.131697 |
| 20.996 | 23.8245 | 3.995889 | 24.708 | 2.934892 |
| 22.034 | 28.2085 | 5.733079 | 28.1375 | 4.018793 |
| 23.01 | 33.61967 | 7.429585 | 34.7145 | 8.219064 |
| 23.987 | 34.5545 | 10.5395 | 38.38625 | 8.833727 |
| 25.024 | 33.11967 | 7.977593 | 36.1185 | 8.150806 |
| 26.001 | 29.2945 | 5.564569 | 29.57675 | 6.136704 |
| 27.039 | 29.19517 | 7.778862 | 32.02025 | 4.719399 |
| 28.015 | 28.20083 | 6.857428 | 30.1325 | 5.854514 |
| 28.992 | 28.80683 | 6.95572 | 29.9185 | 6.088159 |
| 30.029 | 31.31217 | 6.420901 | 32.1885 | 5.536122 |
| 31.006 | 30.17083 | 5.937178 | 31.45775 | 5.60434 |
| 32.043 | 33.1835 | 6.29935 | 32.62 | 6.345228 |
| 33.02 | 32.162 | 3.224016 | 32.7965 | 3.847981 |
| 33.997 | 29.63067 | 4.145233 | 34.09975 | 1.938482 |
| 35.034 | 26.3735 | 3.809668 | 27.3055 | 2.251547 |
| 36.011 | 24.03383 | 3.989911 | 24.54675 | 4.705433 |
| 36.987 | 25.6125 | 7.602117 | 31.57525 | 4.535048 |
| 38.025 | 24.22417 | 6.696863 | 28.5935 | 4.665596 |
| 39.001 | 25.09417 | 4.063645 | 27.025 | 4.035391 |
| 40.039 | 22.9735 | 5.631001 | 26.28025 | 4.150564 |
| 41.016 | 21.70433 | 5.270372 | 23.8355 | 4.59493 |
| 41.992 | 22.5495 | 3.453311 | 21.45475 | 4.952934 |
| 43.03 | 29.11683 | 4.245068 | 30.30775 | 3.315785 |
| 44.006 | 32.5735 | 4.252599 | 35.18125 | 3.207607 |
| 45.044 | 31.08267 | 5.345395 | 34.44075 | 4.469859 |
| 46.021 | 29.76967 | 2.696677 | 30.208 | 4.208 |
| 46.997 | 29.47967 | 5.605828 | 32.5925 | 3.36702 |
| 48.035 | 25.8805 | 8.141689 | 30.60575 | 4.235731 |
| 49.011 | 23.74252 | 7.849952 | 25.4495 | 4.459612 |
| 49.988 | 27.46367 | 6.263034 | 29.6425 | 4.852226 |
| 51.025 | 32.01467 | 4.410065 | 33.1445 | 3.90135 |
| 52.002 | 32.7415 | 3.457753 | 31.686 | 3.867409 |
| 53.04 | 33.52833 | 2.13443 | 31.54175 | 2.864403 |
| 54.016 | 34.37117 | 4.213609 | 34.75575 | 3.880749 |
| 54.993 | 32.863 | 5.333554 | 34.251 | 4.237895 |
| 56.03 | 31.75483 | 5.169745 | 31.60025 | 4.62362 |
| 57.007 | 28.33317 | 5.452353 | 25.83975 | 7.262012 |
| 58.044 | 22.29295 | 8.13239 | 23.91475 | 6.003967 |
| 59.021 | 19.10692 | 6.159556 | 16.9495 | 5.779061 |
| 59.998 | 21.52783 | 7.643857 | 19.1915 | 5.286353 |
| 61.035 | 25.92135 | 9.676568 | 23.7675 | 6.718516 |
| 62.012 | 22.0647 | 12.0143 | 21.86225 | 8.066857 |
| 62.988 | 21.26025 | 7.90855 | 20.81175 | 7.486721 |
| 64.026 | 17.53142 | 11.93879 | 19.08408 | 10.20958 |
| 65.002 | 15.7587 | 10.60524 | 15.35133 | 11.81356 |
| 66.04 | 12.96877 | 10.36914 | 15.36318 | 8.177074 |
| 67.017 | 14.87433 | 8.910487 | 15.31105 | 8.276213 |
| 67.993 | 14.00315 | 8.932447 | 16.17063 | 7.780071 |
| 69.031 | 13.48407 | 7.536445 | 13.07658 | 8.484157 |
| 70.007 | 9.611617 | 8.087252 | 12.5757 | 5.801459 |
| 70.984 | 12.31412 | 5.360352 | 9.179775 | 7.096063 |



Figure 2 Compound action potential (CAP) audiograms (CAP threshold as function of stimulus frequency, mean±standard deviation) from the 8 TMC2 (red) and 7 wild type (black) littermates in Fig 1. CAP was recorded from the round window. High frequency hearing loss in the CAP response appears to be strain-related rather than due to the genetic modification.

 kHz WT mean S.D TMC2 mean S.D

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| 1 | 66.35 | 4.398863 | 63.275 | 18.26607 |
| 2 | 61.95 | 9.952387 | 51.975 | 18.64893 |
| 3 | 60.85 | 16.57317 | 49.05 | 21.14088 |
| 4 | 46.2 | 13.75209 | 34.3725 | 24.67145 |
| 5 | 38.775 | 15.01341 | 12.295 | 18.39142 |
| 6 | 23.475 | 25.17265 | -4.03 | 13.51985 |
| 7 | 18.68 | 18.01656 | 1.3125 | 8.667877 |
| 8 | 20.085 | 21.5636 | 6.905 | 11.45117 |
| 9 | 26.8 | 17.05227 | 13.705 | 13.44713 |
| 10 | 17.525 | 28.62148 | 9.745 | 19.62914 |
| 11 | 11.2325 | 26.86076 | 6.525 | 10.90344 |
| 12 | 11.275 | 25.18285 | 7.5515 | 18.40359 |
| 13 | 7.6 | 23.00014 | 2.475 | 23.52508 |
| 14 | 7.325 | 17.74211 | -0.225 | 24.12749 |
| 15 | 10.325 | 18.96846 | 3.075 | 27.98111 |
| 16 | 10.325 | 20.4588 | 7.0575 | 19.42484 |
| 17 | 12.25 | 19.2656 | 4.725 | 19.85873 |
| 18 | 7.975 | 17.2753 | 0.7575 | 13.80797 |
| 19 | 10.125 | 15.73687 | -4.2925 | 15.68072 |
| 20 | 12.46 | 18.35966 | 3.395 | 13.61232 |
| 21 | 17.275 | 20.13428 | 8.1675 | 13.41347 |
| 22 | 15.95 | 20.70885 | 8.38 | 10.35449 |
| 23 | 13.5 | 21.25057 | 9.875 | 14.16577 |
| 24 | 17.8 | 21.54886 | 11.2125 | 11.6786 |
| 25 | 20.445 | 16.20025 | 13.0925 | 9.65899 |
| 26 | 26.8025 | 21.14628 | 13.6625 | 5.503541 |
| 27 | 33.38 | 22.35246 | 18.6 | 4.583303 |
| 28 | 39.75 | 18.9134 | 34.2 | 12.88953 |
| 29 | 50.675 | 22.58825 | 39.975 | 14.40634 |
| 30 | 41.71 | 28.60614 | 40.25 | 21.49333 |
| 31 | 58.15 | 15.00811 | 57.625 | 27.64108 |
| 32 | 62.75 | 11.22809 | 63.35 | 33.02872 |
| 33 | 68.075 | 10.06094 | 72.025 | 24.56588 |
| 34 | 70.05 | 11.61221 | 76 | 11.94934 |
| 35 | 71.475 | 13.60083 | 77.375 | 14.26239 |
| 36 | 78.8 | 17.42642 | 77.95 | 17.43588 |
| 37 | 79.575 | 19.86897 | 64.025 | 9.911735 |
| 38 | 78 | 25.43698 | 58.9 | 18.09199 |
| 39 | 77.35 | 26.26093 | 67.175 | 7.786045 |
| 40 | 81.775 | 21.06187 | 72.625 | 5.694076 |
| 41 | 84.275 | 15.15198 | 79.325 | 12.11703 |
| 42 | 78.85 | 17.26818 | 83.6 | 13.07211 |
| 43 | 79.275 | 24.12625 | 73.725 | 6.962938 |
| 44 | 70.525 | 20.19924 | 73.875 | 6.247866 |
| 45 | 74.075 | 18.80769 | 76.75 | 3.525621 |
| 46 | 75.65 | 18.54463 | 69.95 | 7.328711 |
| 47 | 74.875 | 19.61247 | 70.175 | 5.727347 |
| 48 | 69.425 | 23.96475 | 65.325 | 6.277672 |
| 49 | 74.55 | 29.38996 | 68.3 | 6.450323 |
| 50 | 76.975 | 26.65613 | 74.15 | 12.18756 |
| 51 | 76.9 | 26.67671 | 69.85 | 8.490583 |
| 52 | 59.775 | 23.90807 | 56.2 | 6.069596 |
| 53 | 67.55 | 23.05421 | 46.675 | 10.81893 |
| 54 | 64.475 | 20.7172 | 39.925 | 14.72693 |
| 55 | 68.725 | 27.1446 | 47.725 | 7.047636 |
| 56 | 72.05 | 22.94435 | 43.8 | 6.376519 |
| 57 | 80.975 | 22.20366 | 54.35 | 4.413238 |
| 58 | 75.6 | 16.472 | 58.175 | 5.937662 |
| 59 | 73.125 | 3.587362 | 60.45 | 11.84947 |
| 60 | 75.175 | 7.589631 | 48.2 | 20.22853 |
| 61 | 89.475 | 19.0395 | 57.875 | 11.07441 |
| 62 | 90.05 | 19.9 | 50.55 | 14.1432 |
| 63 | 92.575 | 14.85 | 71.4 | 24.54818 |
| 64 | 88.825 | 22.35 | 58.6 | 7.842194 |
| 65 | 64.85 | 28.45025 | 43.925 | 12.1785 |
| 66 | 65.95 | 10.76739 | 48.975 | 11.12726 |
| 67 | 64.65 | 20.45002 | 42.625 | 17.29863 |
| 68 | 77.85 | 16.99461 | 58.45 | 7.643952 |
| 69 | 63.25 | 22.72568 | 59.675 | 8.534782 |
| 70 | 62.8 | 24.90315 | 50.2 | 11.4987 |



Figure 3 Cochlear microphonic potential (CM) as a function of the level of the 5 kHz stimulus tone (mean±standard deviation) recorded from the round windows of the 8 TMC2 (red) and 7 wild type (black) littermates in Fig 1.

 dB SPL WT mean S.D TMC2 mean S.D

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| 20 | 0.007201 | 0.003207 | 0.005763 | 0.001844 |
| 25 | 0.007037 | 0.004105 | 0.006806 | 0.002949 |
| 30 | 0.007465 | 0.001226 | 0.00778 | 0.005355 |
| 35 | 0.015095 | 0.001501 | 0.015602 | 0.009788 |
| 40 | 0.024165 | 0.002708 | 0.028657 | 0.017858 |
| 45 | 0.040458 | 0.006039 | 0.045554 | 0.031732 |
| 50 | 0.075318 | 0.009259 | 0.080193 | 0.05552 |
| 55 | 0.125849 | 0.015067 | 0.134091 | 0.095366 |
| 60 | 0.221518 | 0.031951 | 0.217861 | 0.150325 |
| 65 | 0.320273 | 0.048037 | 0.334247 | 0.221142 |
| 70 | 0.434208 | 0.086271 | 0.476513 | 0.275882 |
| 75 | 0.552875 | 0.148061 | 0.620693 | 0.30781 |
| 80 | 0.86205 | 0.267781 | 0.731737 | 0.320322 |



Figure 4. Threshold frequency tuning curves of 0.2 nm displacements recorded from the cochlea basal turns of TMC2 (red) and wild type (black) littermates with good high frequency hearing based on their DPOAE audiograms

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| kHz | TMC19 | TMC17 |  | TMC13WT | TMC7WT |  | TMC20 |  | TMCwt1 |  | TMC2 |  | TMC21 |
| 10 | 76 | 78 | 10 | 80 | 10 | 83 | 10 | 66 | 10 | 70 | 10 | 83 | 10 | 70 |
| 15 | 72 | 76 | 15 | 76 | 15 | 77 | 15 | 66 | 15 | 68 | 15 | 81 | 15 | 80 |
| 20 | 67 | 68 | 20 | 67 | 20 | 75 | 20 | 63 | 20 | 67 | 20 | 76 | 20 | 64 |
| 25 | 65 | 57 | 25 | 60 | 25 | 52 | 25 | 56 | 25 | 54 | 25 | 71 | 25 | 56 |
| 30 | 65 | 58 | 30 | 56 | 30 | 62 | 30 | 62 | 30 | 58 | 30 | 70 | 29 | 60 |
| 35 | 58 | 53 | 35 | 56 | 35 | 56 | 35 | 66 | 35 | 56 | 35 | 66 | 35 | 62 |
| 40 | 60 | 51 | 40 | 46 | 40 | 56 | 40 | 52 | 40 | 52 | 40 | 61 | 40 | 58 |
| 45 | 47 | 48 | 45 | 47 | 45 | 55 | 42 | 42 | 42 | 57 | 42 | 56 | 45 | 56 |
| 46 | 48 | 44 | 46 | 46 | 46 | 55 | 44 | 44 | 44 | 57 | 44 | 55 | 46 | 57 |
| 47 | 48 | 43 | 47 | 41 | 47 | 47 | 45 | 47 | 46 | 57 | 46 | 55 | 47 | 56 |
| 48 | 46 | 28 | 48 | 32 | 48 | 47 | 46 | 50 | 47 | 47 | 48 | 56 | 48 | 50 |
| 49 | 43 | 31 | 49 | 30 | 49 | 46 | 47 | 46 | 48 | 47 | 50 | 56 | 49 | 47 |
| 50 | 41 | 31 | 50 | 26 | 50 | 36 | 48 | 46 | 49 | 46 | 51 | 56 | 50 | 45 |
| 51 | 36 | 22 | 51 | 23 | 51 | 38 | 49 | 46 | 50 | 46 | 52 | 46 | 51 | 45 |
| 52 | 34 | 24 | 52 | 32 | 52 | 41 | 50 | 45 | 51 | 45 | 53 | 45 | 52 | 52 |
| 53 | 33 | 36 | 53 | 46 | 53 | 36 | 51 | 50 | 52 | 39 | 54 | 41 | 53 | 51 |
| 54 | 32 | 46 | 54 | 40 | 54 | 28 | 52 | 51 | 53 | 38 | 55 | 39 | 54 | 45 |
| 55 | 41 | 47 | 55 | 40 | 55 | 30 | 53 | 41 | 54 | 33 | 55.5 | 39 | 55 | 46 |
| 56 | 41 | 47 | 56 | 42 | 56 | 34 | 54 | 43 | 55 | 39 | 56 | 40 | 56 | 46 |
| 57 | 50 | 41 | 57 | 46 | 57 | 53 | 55 | 51 | 56 | 41 | 57 | 51 | 57 | 52 |
| 58 | 51 | 51 | 58 | 42 | 58 | 57 | 56 | 52 | 57 | 46 | 58 | 56 | 58 | 47 |
| 60 | 52 | 51 | 60 | 42 | 59 | 56 | 57 | 53 | 58 | 47 | 60 | 57 | 60 | 51 |
| 62 | 53 | 51 | 62 | 47 | 60 | 52 | 58 | 62 | 59 | 53 | 62 | 75 | 62 | 57 |
| 64 | 54 | 52 | 64 | 52 | 61 | 53 | 60 | 63 | 60 | 50 |  |  | 64 | 66 |
| 66 | 57 | 63 | 66 | 56 | 62 | 60 | 62 | 64 | 62 | 55 |  |  | 66 | 70 |
| 68 | 58 | 62 | 68 | 56 | 63 | 60 | 64 | 56 | 64 | 55 |  |  | 68 | 67 |
| 70 | 63 | 61 | 70 | 58 | 64 | 63 | 66 | 56 | 66 | 61 |  |  | 70 | 78 |