

A tryptophan in culture media of cell lines - time course


TCDD 10 nM




[^0]Supplementary Figure 2. Tryptophan effects in culture medium on AhR activity. (A) Time-course of Trp levels in culture media from hepatic (AZ-AHR), instestinal (LS174T) and skin (HaCaT) cells. Determined by HPLC. (B) Induction of AhR-dependent luciferase activity in AZ-AHR cells incubated for 24 h with TCDD, Indole and Trp in Trp/serum-free culture medium. (C) Induction of AhR-dependent luciferase activity in AZ-AHR cells incubated for 24 h with TCDD and Indole in standard (+Trp/+FBS), serum-free (+Trp/-FBS), Trp-free (-Trp/+FBS) and Trp/serum-free (-Trp/-FBS) culture medium.

| incubation |  | \# fields | ¿cells | \# positive AhR nude | \% positive <br> i AhR nuclei | \% relative AhR nuclei | $\emptyset$ AhR positive nuclei |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Vehicle (DMSO) | $\exp 1$ | 4 | 316 | 17 | 5 | 9 |  |
|  | $\exp 2$ | 11 | 545 | 19 | 3 | 4 | $13 \pm 11.6$ |
|  | exp 3 | 11 | 553 | 90 | 16 | 26 |  |
| 10 nM TCDD | exp 1 | 7 | 318 | 171 | 54 | 100 |  |
|  | $\exp 2$ | 14 | 555 | 430 | 77 | 100 | $100 \pm 11.8$ |
|  | exp 3 | 11 | 538 | 330 | 61 | 100 |  |
| 1 mM Indole | $\exp 1$ | 6 | 335 | 203 | 61 | 113 |  |
|  | $\exp 2$ | 9 | 338 | 248 | 73 | 95 | $105 \pm 9.2$ |
|  | $\exp 3$ | 12 | 500 | 323 | 65 | 107 |  |
| $200 \mu \mathrm{M}$ IPY | $\exp 1$ | 7 | 326 | 136 | 42 | 78 |  |
|  | $\exp 2$ | 9 | 295 | 176 | 60 | 78 | $73 \pm 9.0$ |
|  | $\exp 3$ | 11 | 514 | 194 | 38 | 62 |  |
| $200 \mu \mathrm{M} \mathrm{3-MI}$ | $\exp 1$ | 6 | 407 | 264 | 65 | 120 |  |
|  | $\exp 2$ | 9 | 329 | 236 | 72 | 94 | $105 \pm 13.8$ |
|  | exp 3 | 13 | 603 | 375 | 62 | 102 |  |
| $200 \mu \mathrm{MTA}$ | $\exp 1$ | 6 | 421 | 187 | 44 | 81 |  |
|  | $\exp 2$ | 11 | 319 | 208 | 65 | 84 | $91 \pm 14.6$ |
|  | $\exp 3$ | 12 | 527 | 346 | 66 | 108 |  |
| $100 \mu \mathrm{MIAC}$ | exp 1 | 6 | 393 | 199 | 51 | 94 |  |
|  | $\exp 2$ | 9 | 356 | 218 | 61 | 79 | $98 \pm 20.4$ |
|  | exp 3 | 11 | 499 | 366 | 73 | 120 |  |
| $200 \mu \mathrm{MIAD}$ | $\exp 1$ | 7 | 377 | 175 | 46 | 85 |  |
|  | exp 2 | 9 | 344 | 252 | 73 | 95 | $83 \pm 13.1$ |
|  | exp 3 | 11 | 538 | 228 | 42 | 69 |  |

Suppl. Table 1. Nuclear translocation of AhR - quantification of immunofluorescence.
LS180 cells were incubated for 90 min with MICT, vehicle or TCDD. Intracellular AhR was visualized with Alexa Fluor 488 labelled primary antibody. The whole staining protocol was performed in three independent experiments in technical duplicates. The AhR translocation was evaluated visually depending on the distinct signal intensity of AhR antibody in the nucleus and cytosol. For percentage calculation, approximately one hundred cells from at least four randomly selected fields of view in each replicate were used.


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