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Supplemental Material

Quality Control for Single Cell Imaging Analytics Using Endocrine Disruptor-Induced Changes in Estrogen Receptor Expression

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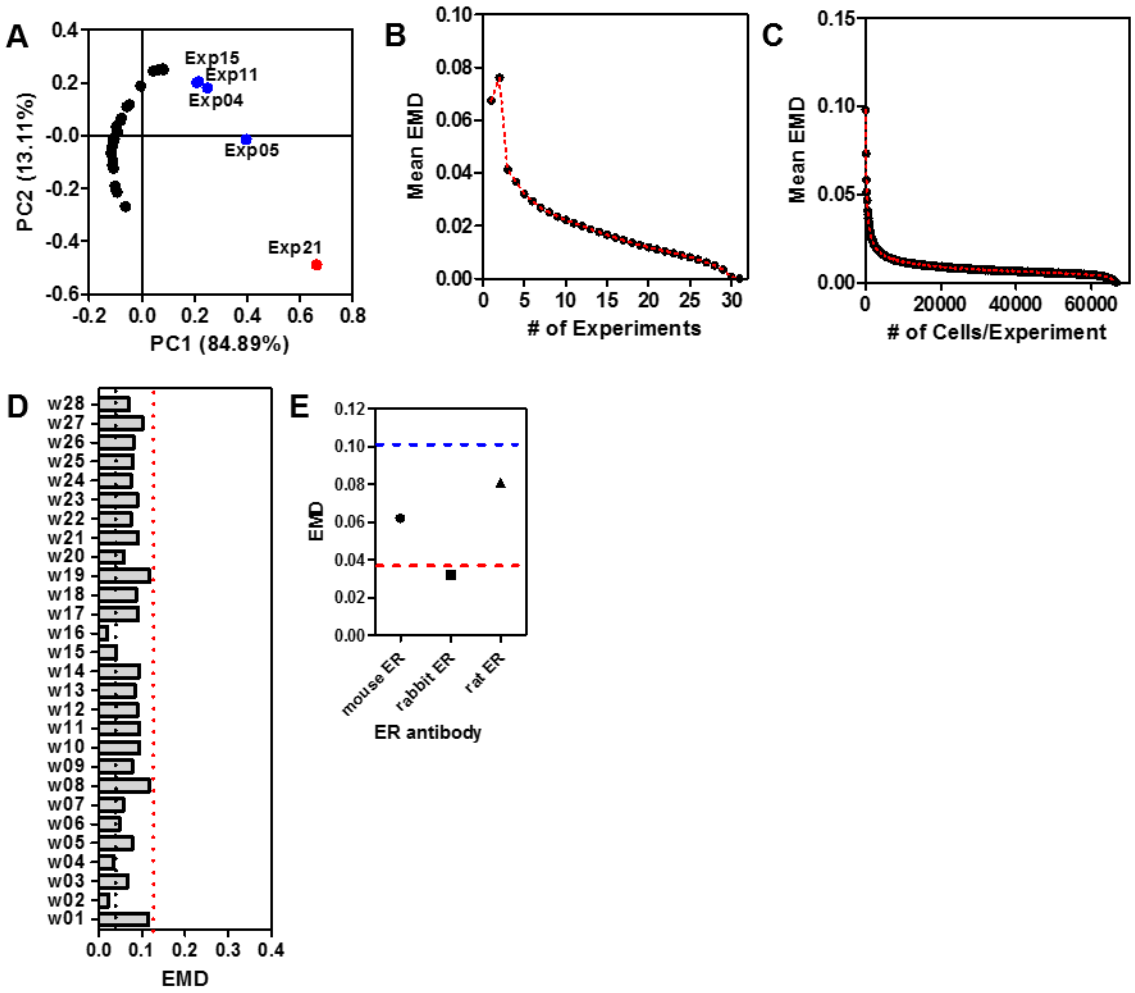
References

Additional File- Excel Document



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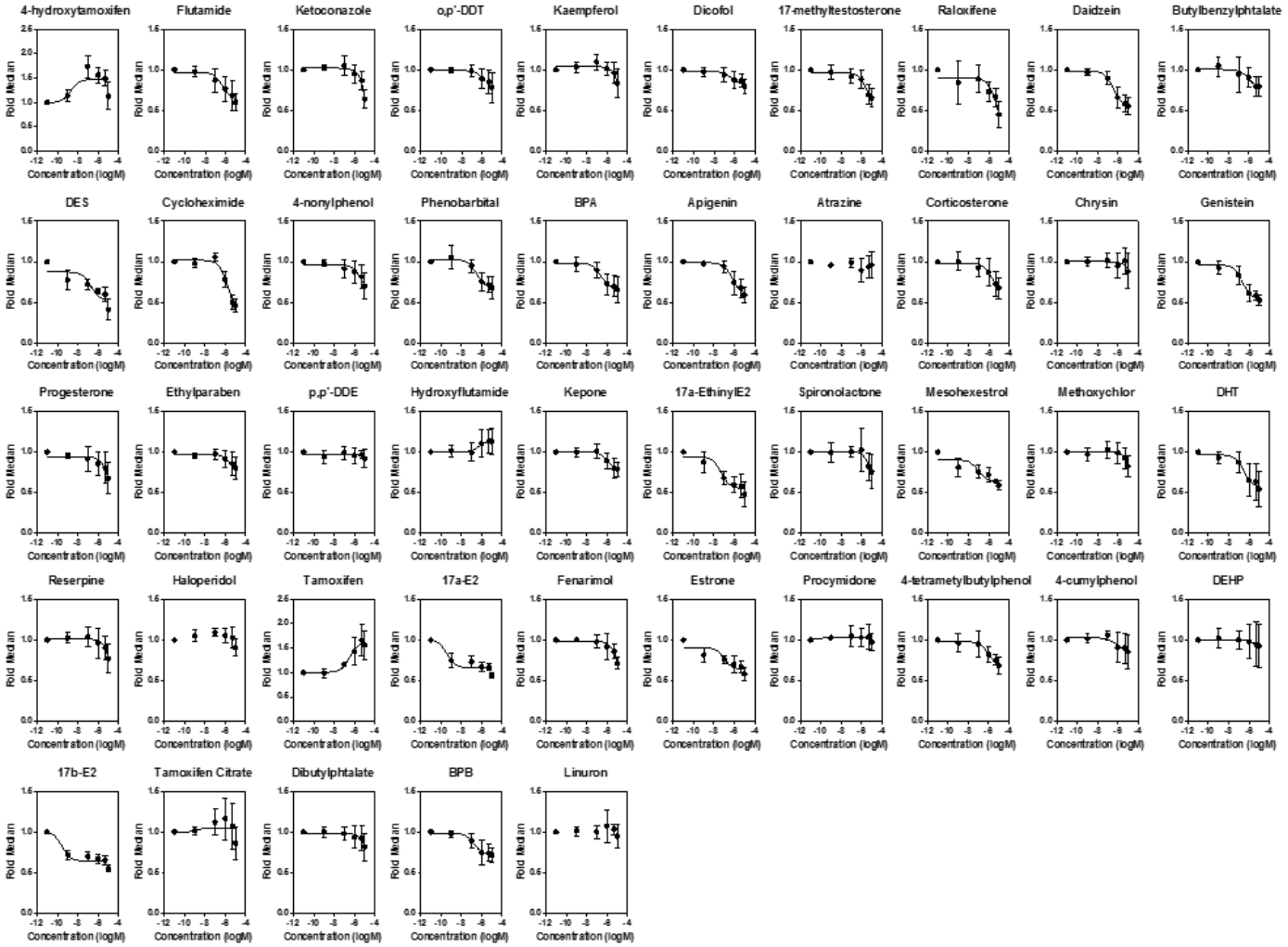

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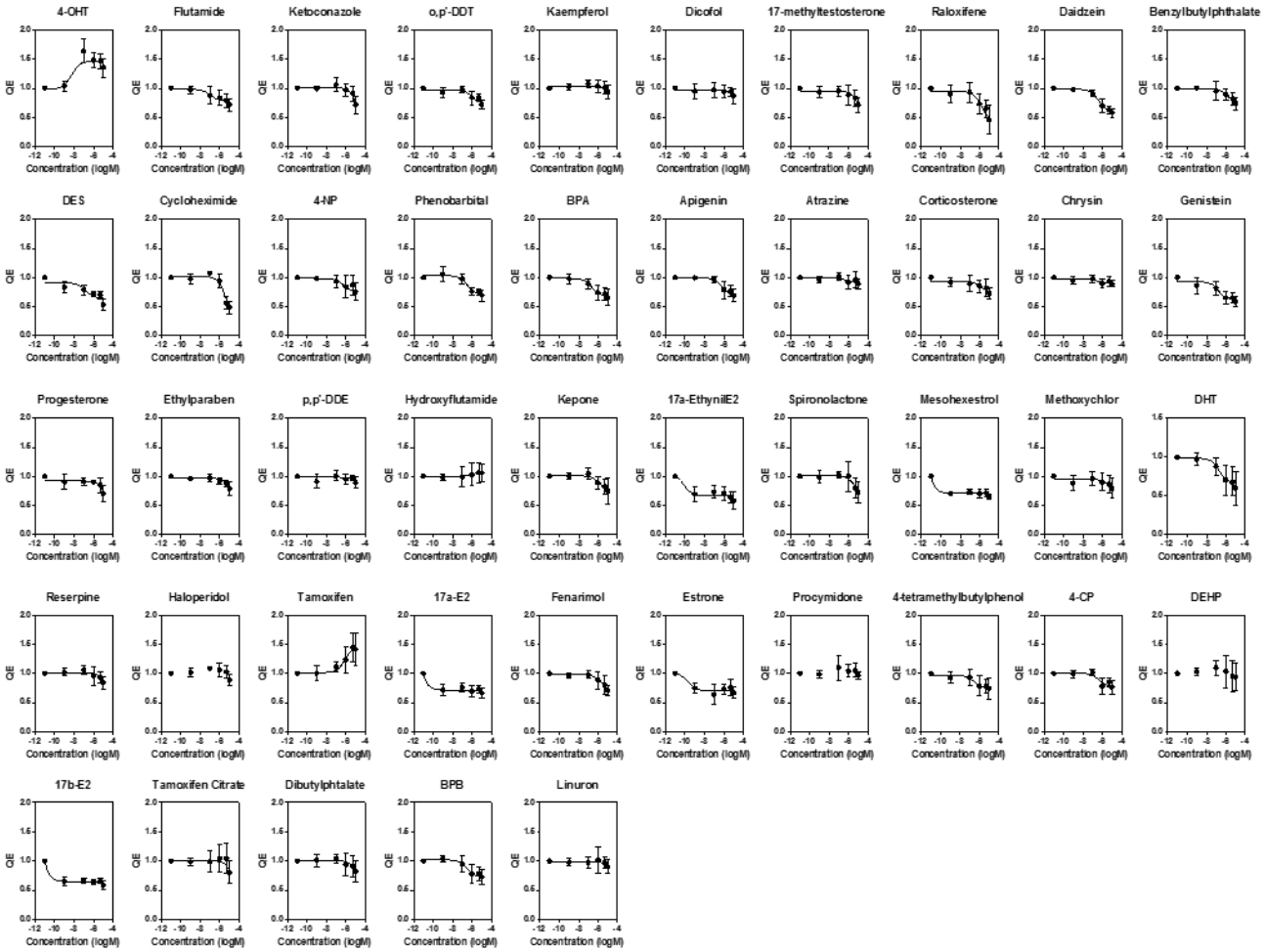

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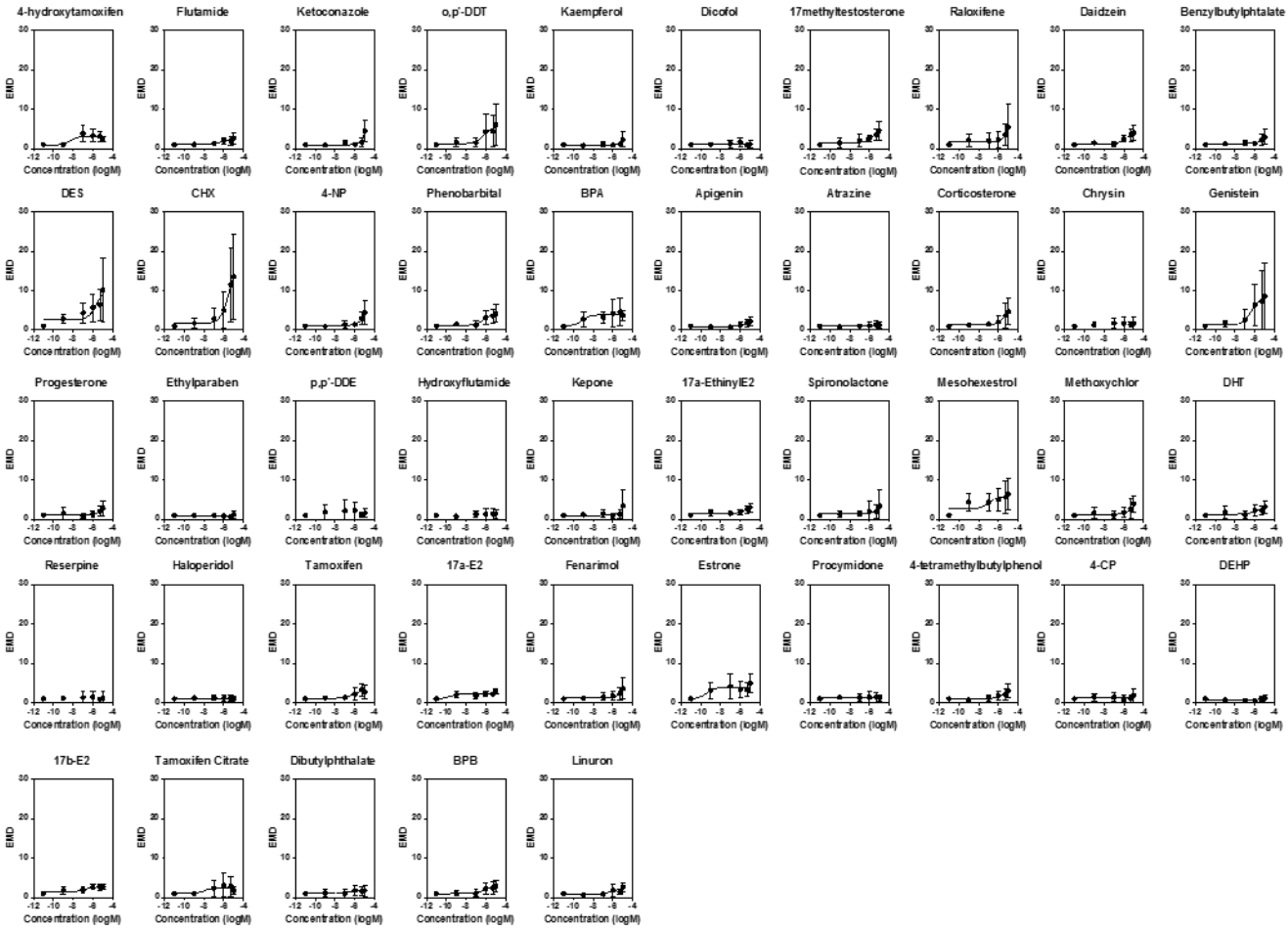

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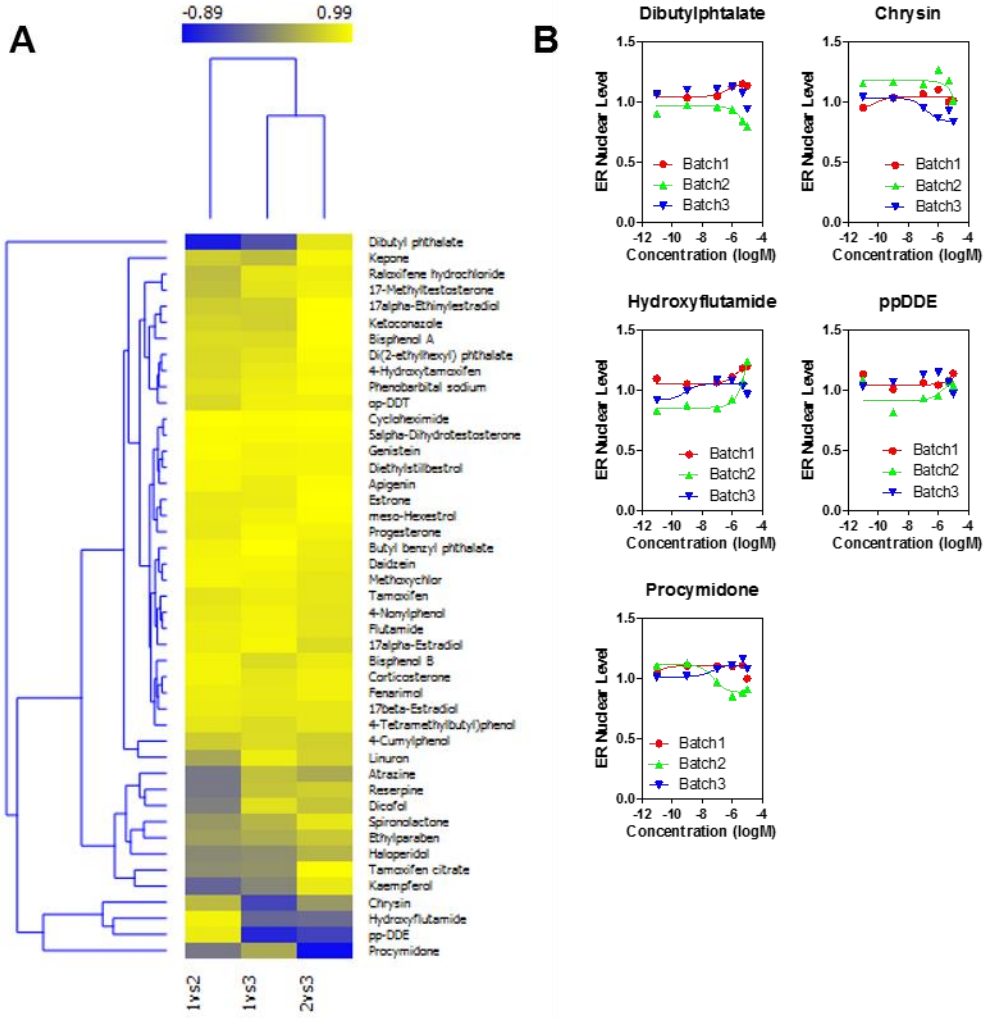

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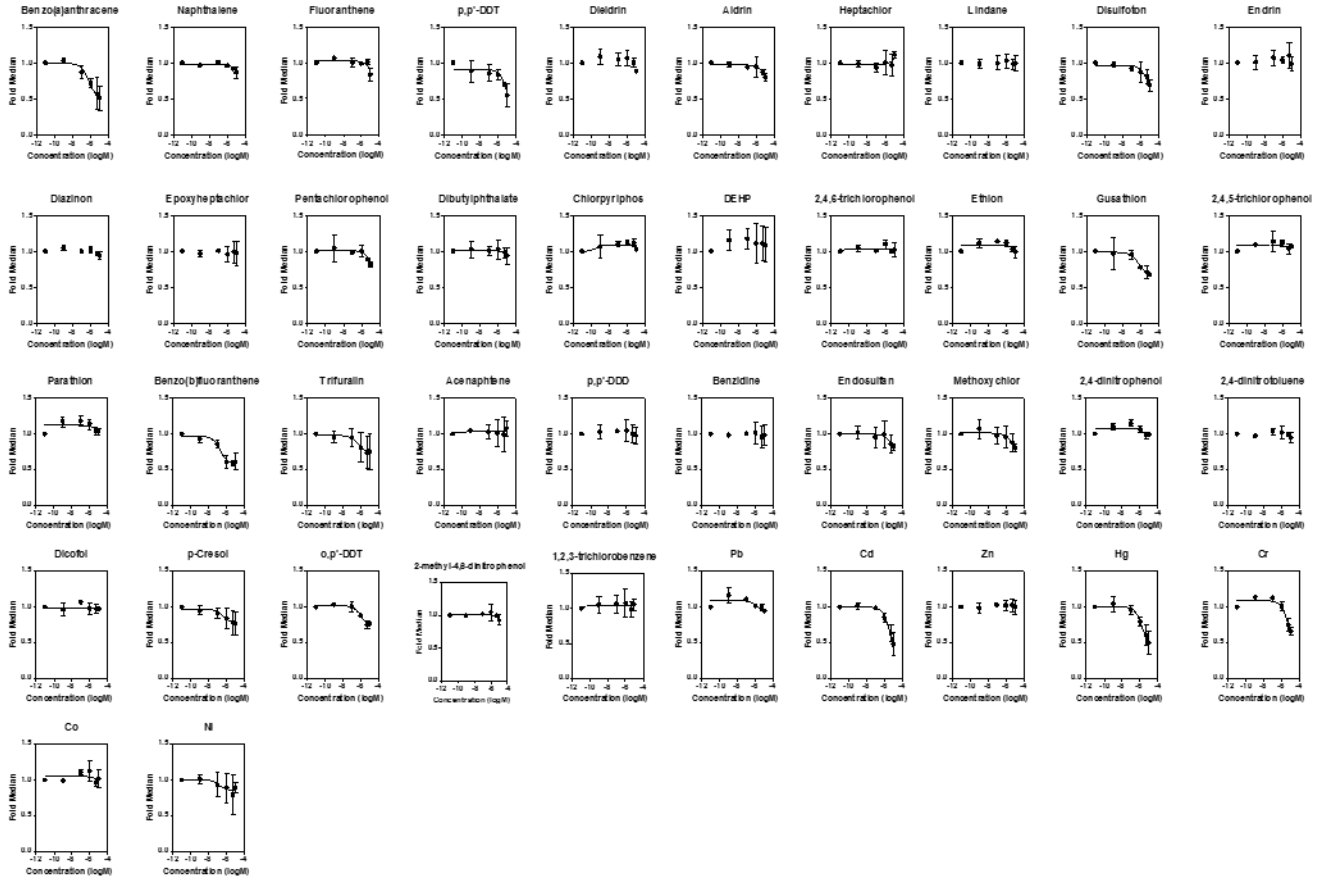

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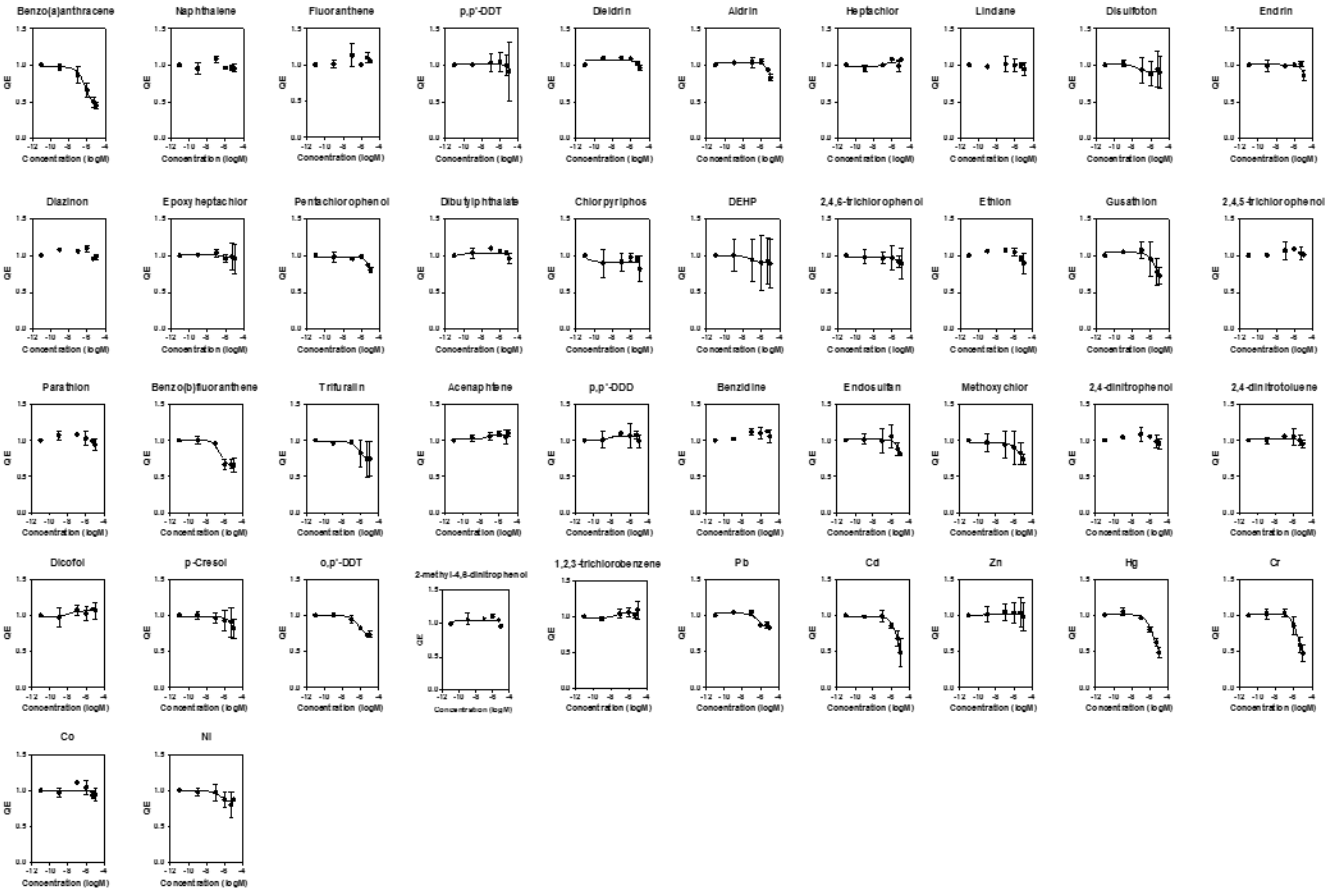

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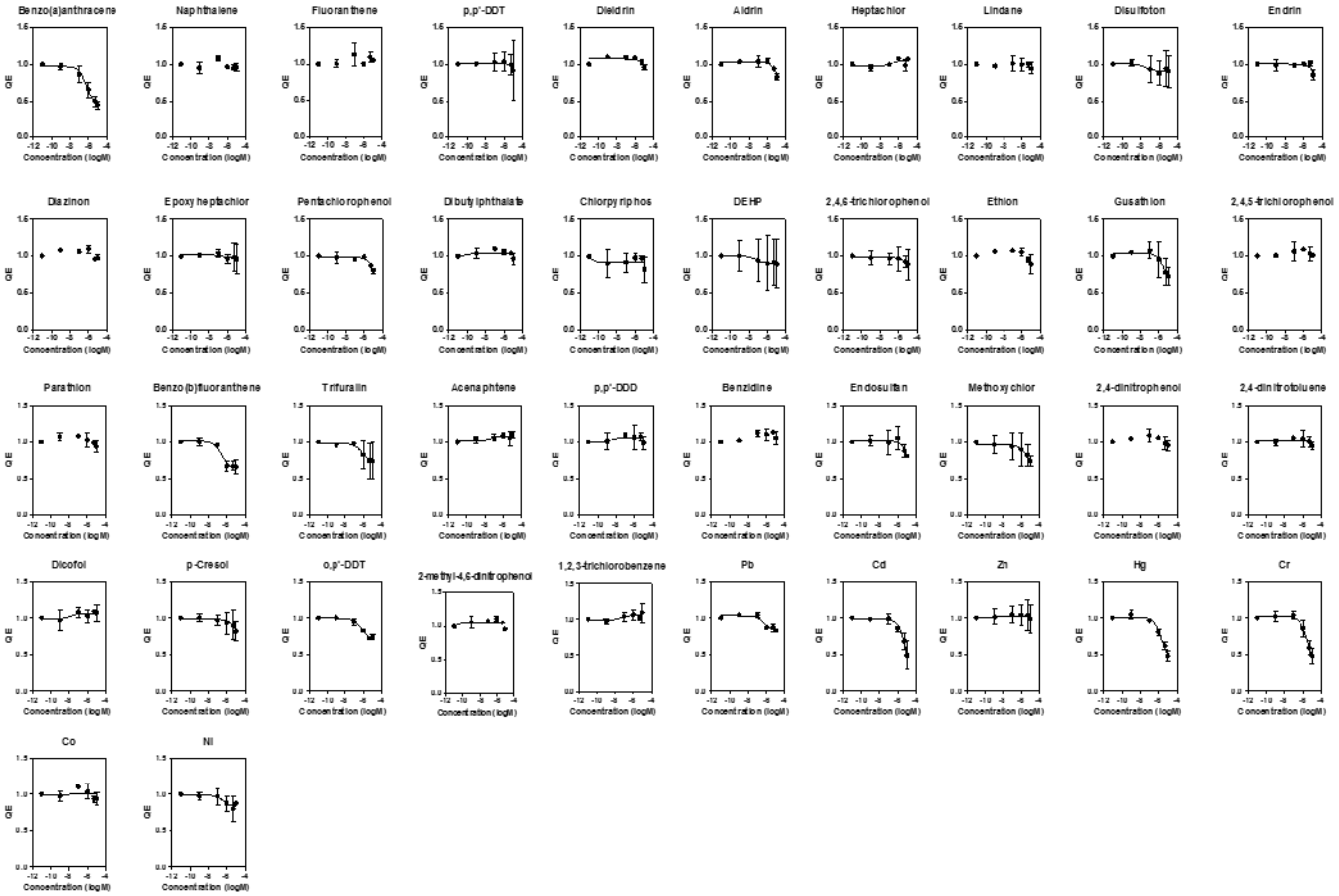

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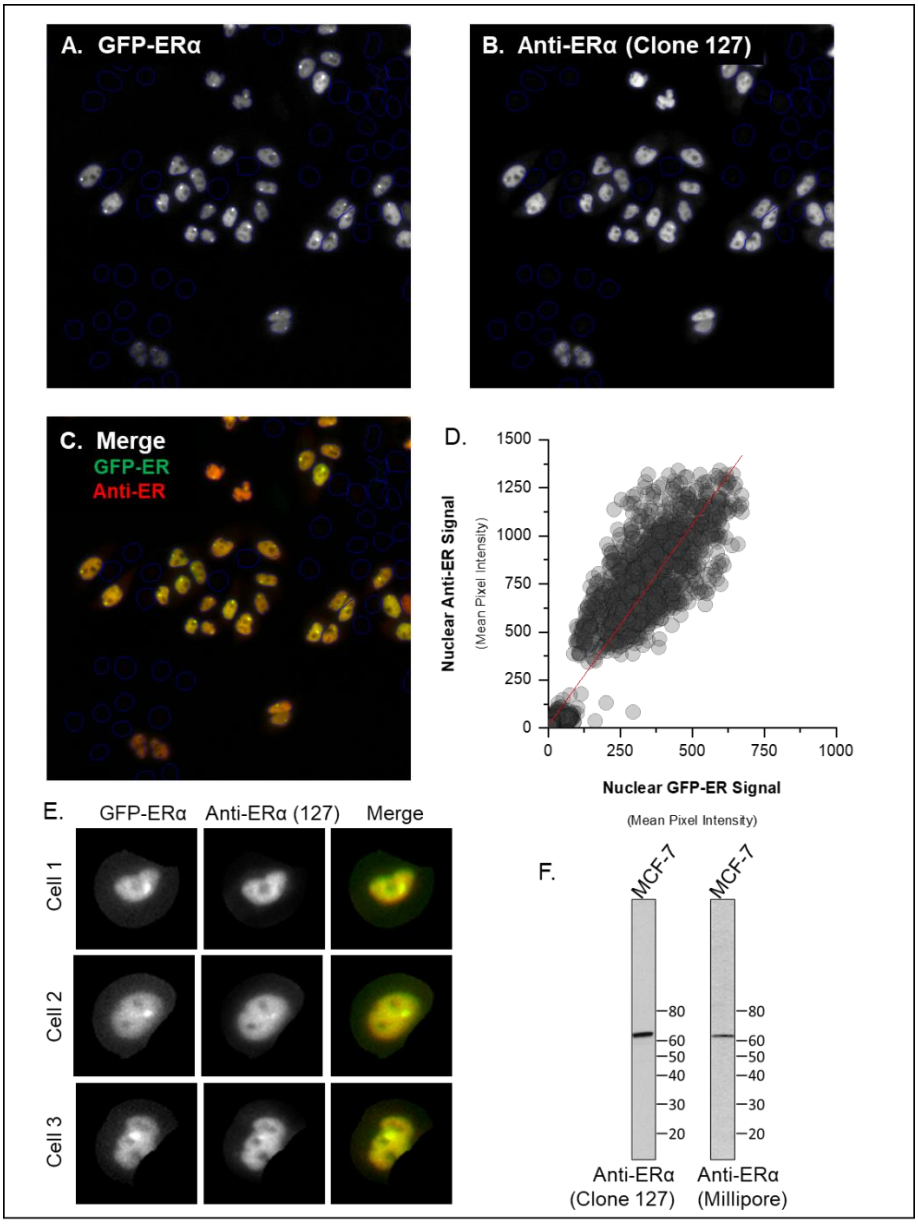

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References:

Ashcroft FJ, Newberg JY, Jones ED, Mikic I, Mancini MA. 2011. High content imaging-based assay to classify estrogen receptor-α ligands based on defined mechanistic outcomes. Gene. 15;477(1-2):42-52. doi: 10.1016/j.gene.2011.01.009.