

SUPPORTING INFORMATION

Enhancers of human and rodent oligodendrocyte formation predominantly induce cholesterol precursor accumulation

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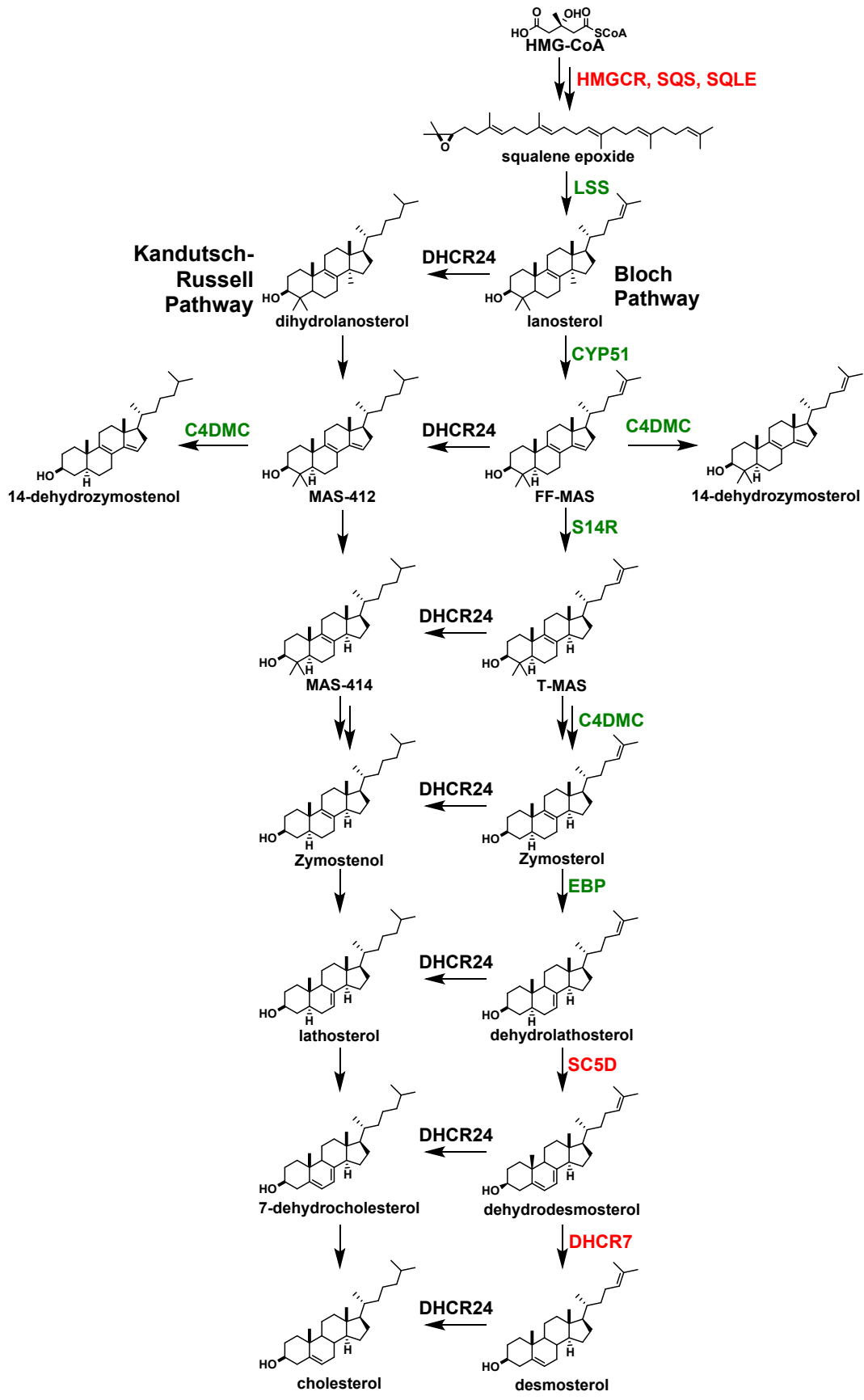


Figure S1| Cholesterol biosynthesis pathway diagram Cholesterol biosynthesis pathway diagram highlighting the Kandutsch-Russel and Bloch pathways. Highlighted in green are enzymes in the pathway that when inhibited lead to enhanced oligodendrocyte formation. Enzymes highlighted in red are enzymes that do not increase oligodendrocyte formation when inhibited.

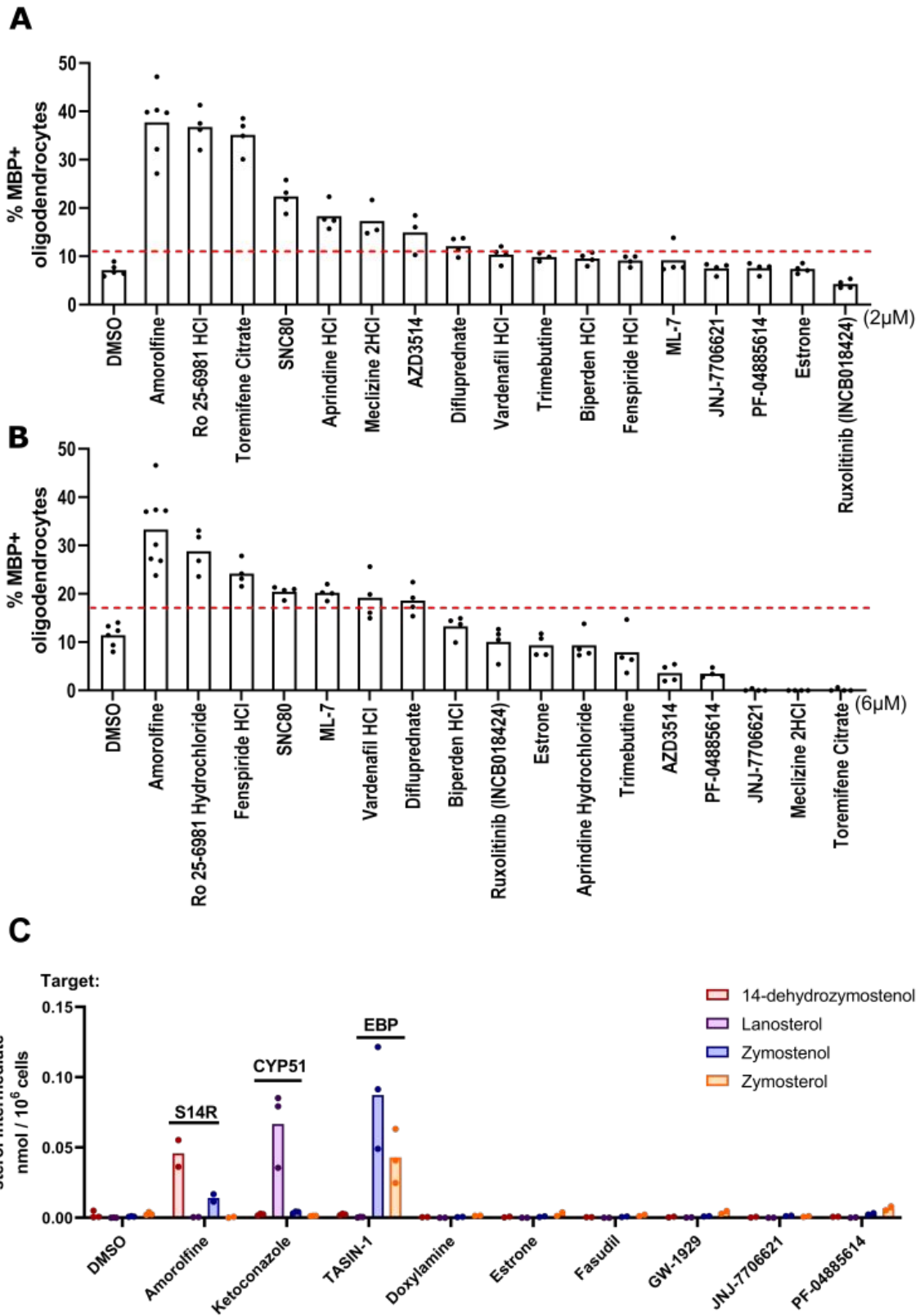


Figure S2| a, b, Percentage of MBP+ oligodendrocytes generated from mouse OPCs following treatment with bioactive small molecules at uniform doses of 2 μ M (**a**) and 6 μ M (**b**). $n=4$ wells per condition, except DMSO and amorolfine, a known S14R inhibitor, $n=8$ wells, with >1,000 cells analyzed per well. Dashed red lines represent the DMSO average of % MBP+ oligodendrocytes multiplied by 1.5 (**a**=10.70, **b**=17.16). **c**, GC-MS based quantification of 14-dehydrozymostenol, lanosterol, zymostenol, and zymosterol in mouse OPCs treated for 24 h with known cholesterol biosynthesis inhibitors and six compounds shown to be inactive in the differentiation assay. Cationic, amphiphilic compounds include fasudil, doxylamine, and PF-04885614. Non-cationic, amphiphilic molecules include JNJ-7706621, GW-1929, and estrone. S14R= sterol-14 reductase.

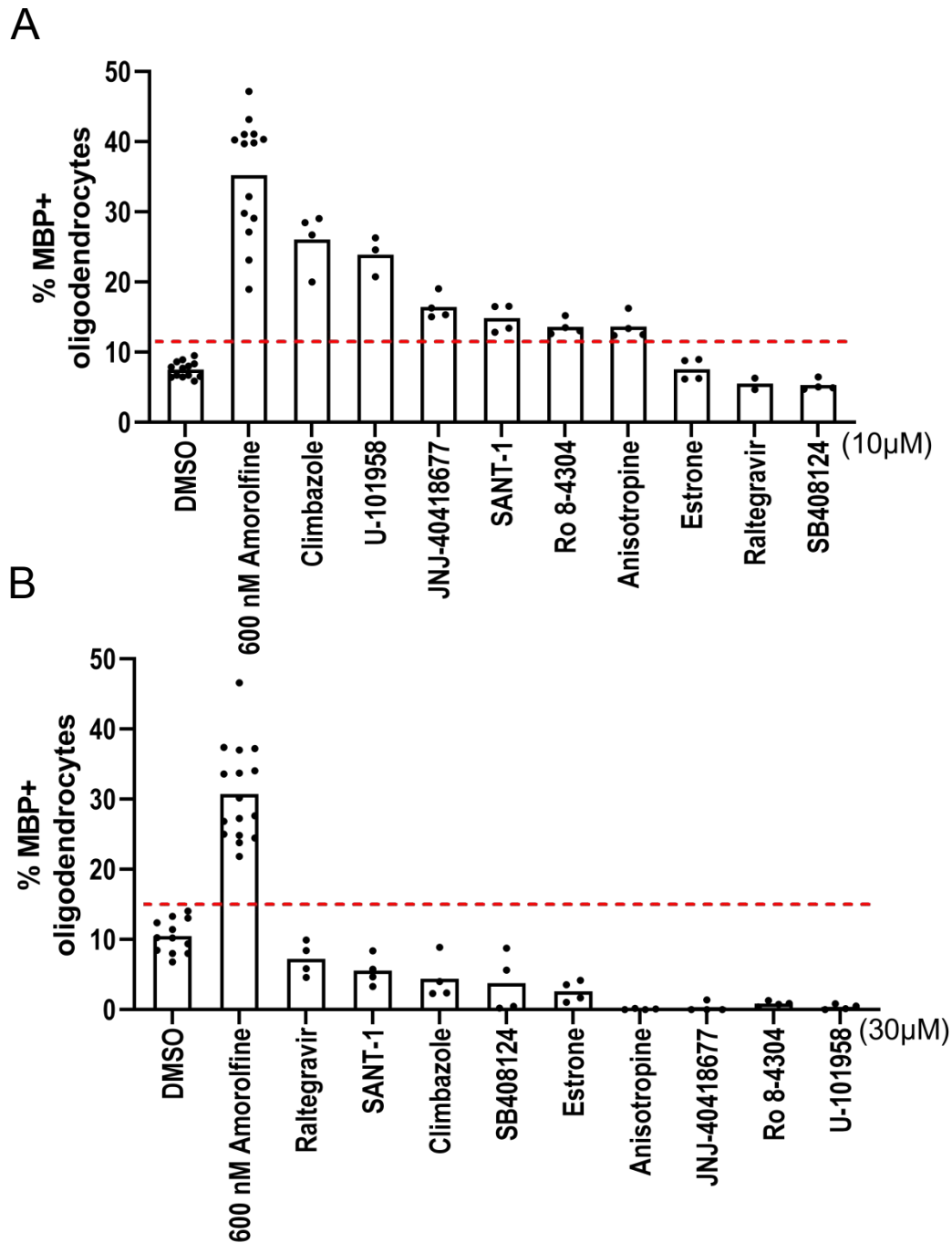


Figure S3| a, b, Percentage of MBP+ oligodendrocytes generated from mouse OPCs following treatment with bioactive small molecules at uniform doses of 10µM (**a**) and 30µM (**b**). $n=4$ wells per condition, except DMSO and amorolfine, a known S14R inhibitor, $n=8$ wells, with >1,000 cells analyzed per well. Dashed red lines represent the DMSO average of % MBP+ oligodendrocytes multiplied by 1.5 (**a**=11.29, **b**=15.69).

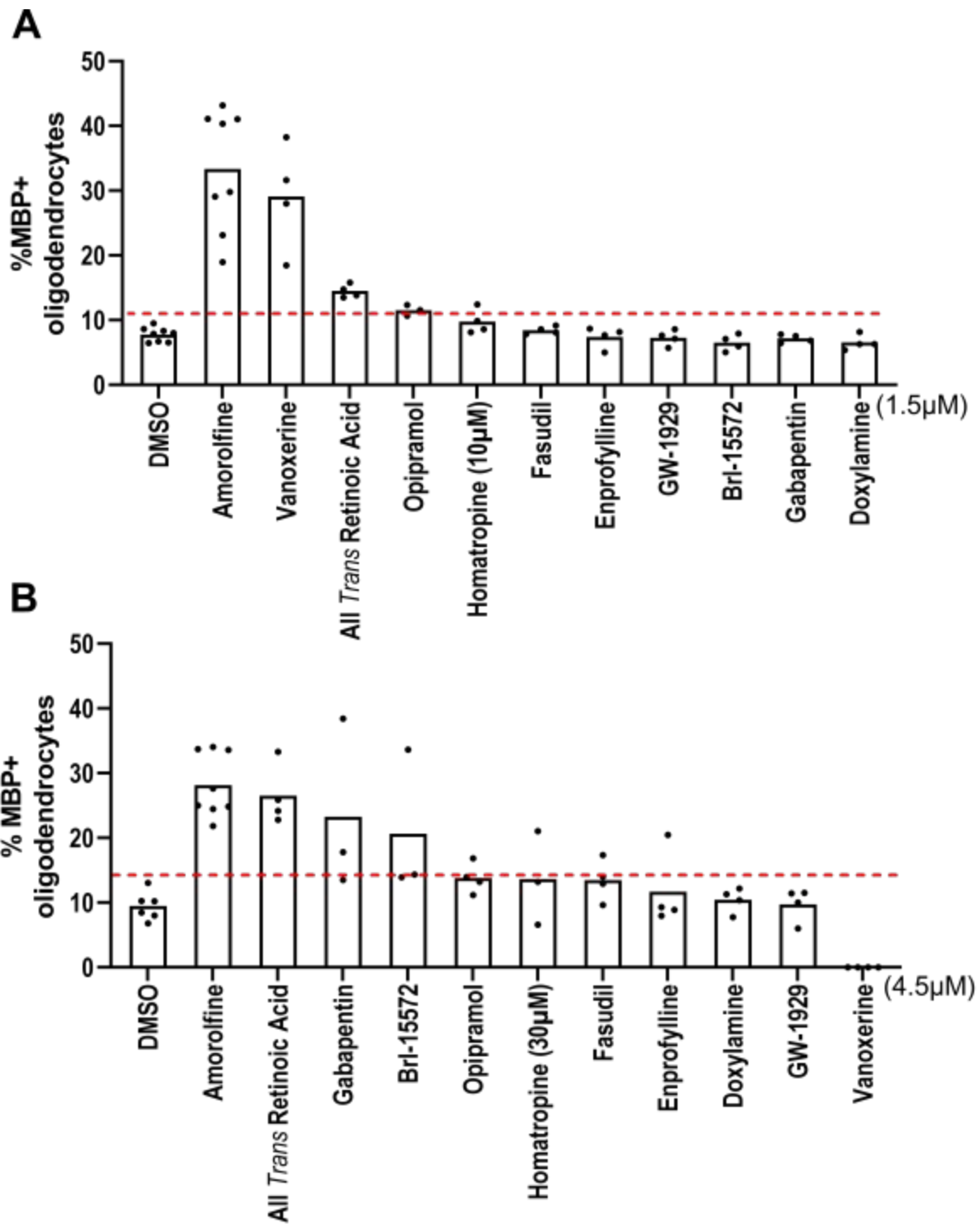


Figure S4| a, b, Percentage of MBP+ oligodendrocytes generated from mouse OPCs following treatment with bioactive small molecules at uniform doses of 1.5µM (**a**) and 4.5µM (**b**). $n=4$ wells per condition, except DMSO and amorolfine, a known S14R inhibitor, $n=8$ wells, with >1,000 cells analyzed per well. Dashed red lines represent the DMSO average of % MBP+ oligodendrocytes multiplied by 1.5. (**a**=11.66, **b**=14.22).

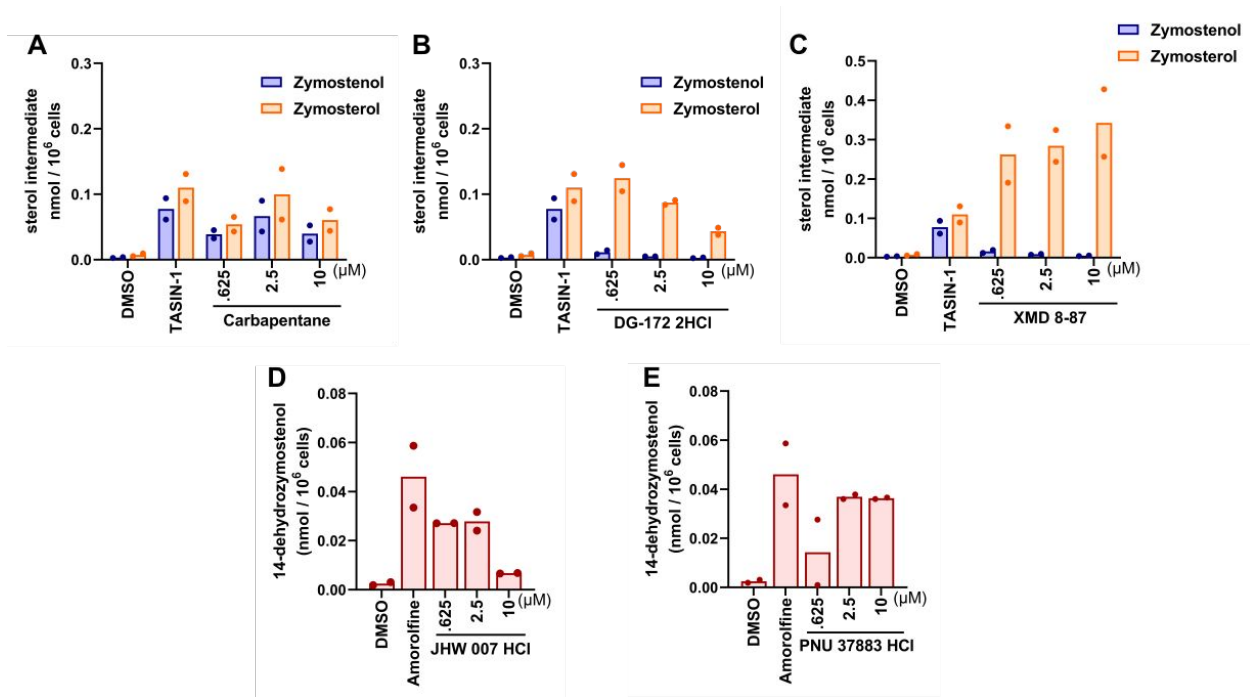


Figure S5| a, b, c, GC-MS based quantification of zymostenol and zymosterol levels in mouse OPCs treated for 24 h with the indicated screening hits in dose response as shown. *n*=2 wells per condition. **d, e,** GC-MS based quantification of 14-dehydrozymostenol levels in mouse OPCs treated for 24 h with the indicated screening hits in dose response as shown. *n*=2 wells per condition