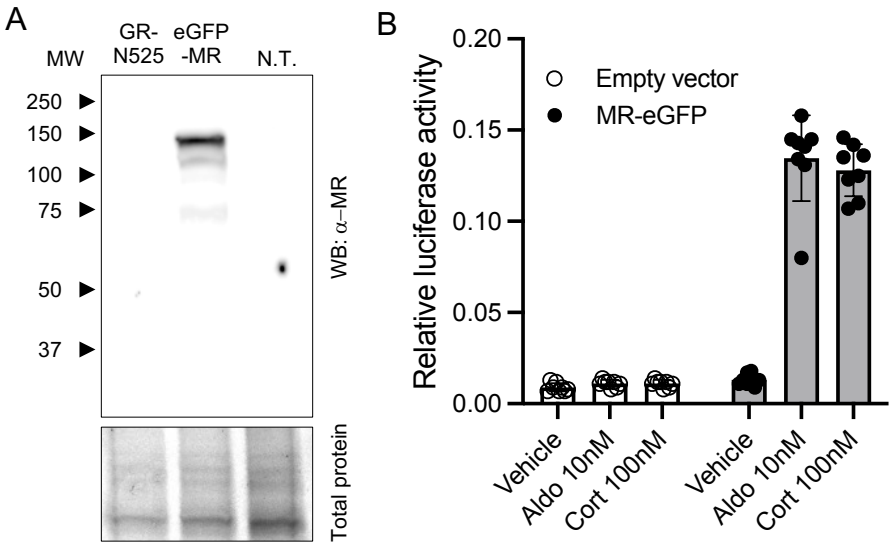
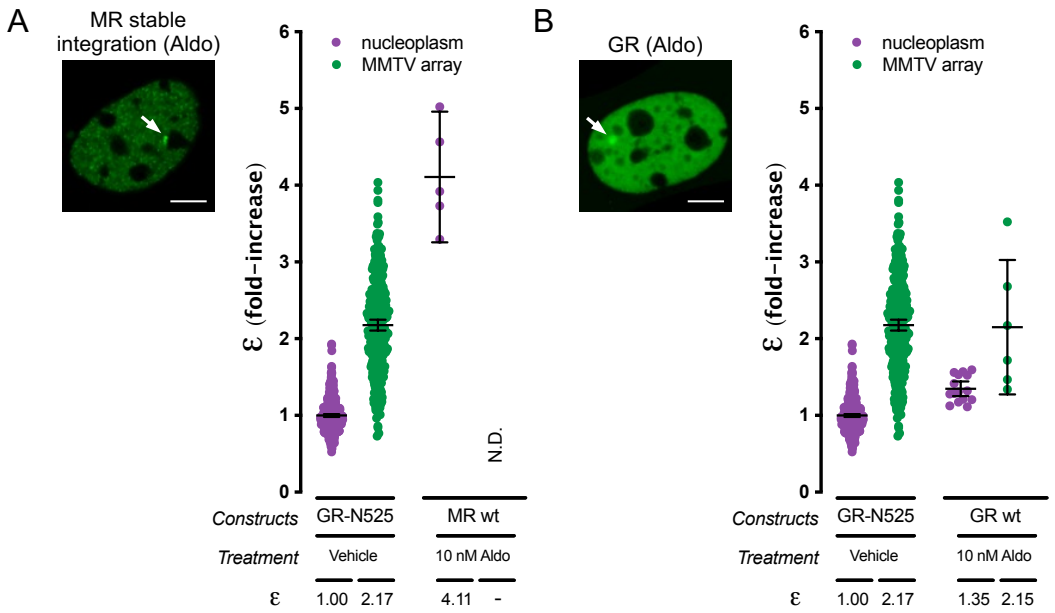
SUPPLEMENTARY FIGURES, Fettweis et al.



Supplementary Figure S1. (A) Representative western blot showing the absence of detectable expression of endogenous MR protein in the cell line used in this study. Cells were transiently transfected with constructs GR-N525, MR-eGFP, or left untransfected (N.T.). Arrows indicate migration of molecular mass markers (MW; values in KDa). The upper panel shows western blot results using an anti-MR antibody. Lower panel shows total protein staining using the Stain-Free reagent (Biorad). (B) Gene reporter assay showing MR-dependent luciferase activity in cells transfected with an empty vector or in cells transfected with a construct expressing MR fused to eGFP. Each dot represents the ratio of firefly and *Renilla* luciferase activities for one well and bars the average ± SD obtained for each condition (n = 8).



Supplementary Figure S2. (A) Representative image and molecular brightness (ε) in the nucleoplasm of cells stably expressing eGFP-tagged mouse MR (MR). We were unable to record from the MMTV array due to low levels of receptor expression. Individual dots represent values from one cell (n = 490, 307, 5; N.D., not determined). (B) Representative image and molecular brightness (ε) obtained from cells expressing eGFP-tagged wild type mouse GR (GR) and treated with 10 nM aldosterone for 1h (n = 490, 307, 15, 6). White arrows point to the MMTV array. Scale bars: 5 μm. To facilitate comparison, data from Fig.1 showing ε for GR-N525 in the nucleoplasm and MMTV array are shown.

A

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GR DBD   423 PPPKLCLVCSDEASVCHYGVLTCGSCKVFFKRAVEGQHNYLCAGRNDCIIDKIRRKNCPACRYRKCLQ 490
MR DBD   598 RPSKICLVCGDEASGCHYGVVTCGSCKVFFKRAVEGQHNYLCAGRNDCIIDKIRRKNCPACRLQKCLQ 665
             * *:*****.**** *****:************************************ :****
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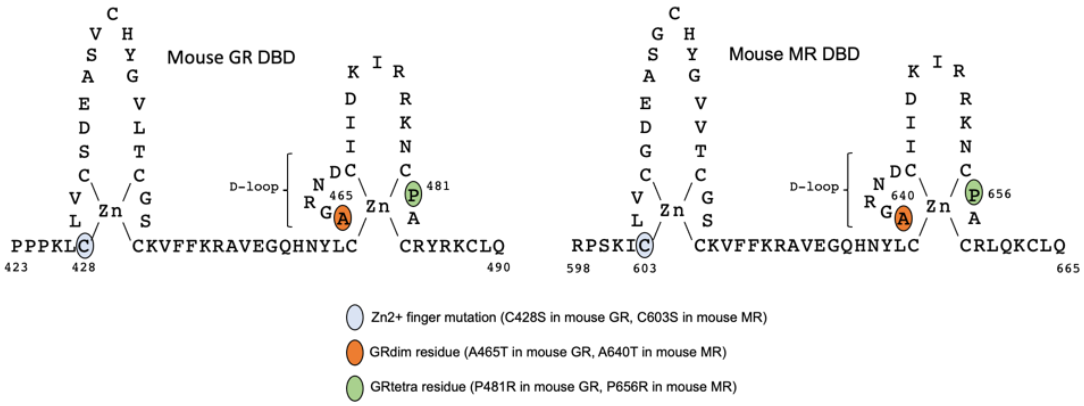
B



C

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GR LBD   526 -----------------IVPAALPQLTPTLVSLLEVIEPEVLYAGYDSSVPDSAWRIMTTLNMLGGRQ 576
MR LBD   705 TYIAPTKEPSVNSALVPQLASITRALTPSPSMILENIEPEIVYAGYDNSKPDTAESLLSTLNRLAGKQ 772
                        : :    ***:   :** ****::*****.* **:*  :::*    ** *.*:*

GR LBD       VIAAVKWAKAIPGFRNLHLDDQMTLLQYSWMFLMAFALGWRSYRQASGNLLCFAPDLIINEQRMTLPC 644
MR LBD       MIQVVKWAKVLPGFKNLPLEDQITLIQYSWMCLSSFALSWRSYKHTNSQFLYFAPDLVFNEEKMHQSA 840
             :* .*****.:***:** *:**:**:***** * :***.****:::..::* *****::**::*   .

GR LBD       MYDQCKHMLFISTELQRLQVSYEEYLCMKTLLLLSSVPKEGLKSQELFDEIRMTYIKELGKAIVKREG 712
MR LBD       MYELCQGMRQISLQFVRLQLTFEEYSIMKVLLLLSTVPKDGLKSQAAFEEMRTNYIKELRKMVTKCPN 908
             **: *: *  ** :: ***:::***  **.*****:***:*****  *:*:* .***** * :.*  .

GR LBD       NSSQNWQRFYQLTKLLDSMHDVVENLLSYCFQTFLDK-SMSIEFPEMLAEIITNQIPKYSNGNIKKLL 779
MR LBD       SSGQSWQRFYQLTKLLDSMHDLVNDLLEFCFYTFRESQALKVEFPAMLVEIISDQLPKVESGNAKPLY 976
             .*.*.*****************:*::**.:** ** :. ::.:*** **.***::*:** ..** * *

GR LBD       FHQK 783
MR LBD       FHRK 980
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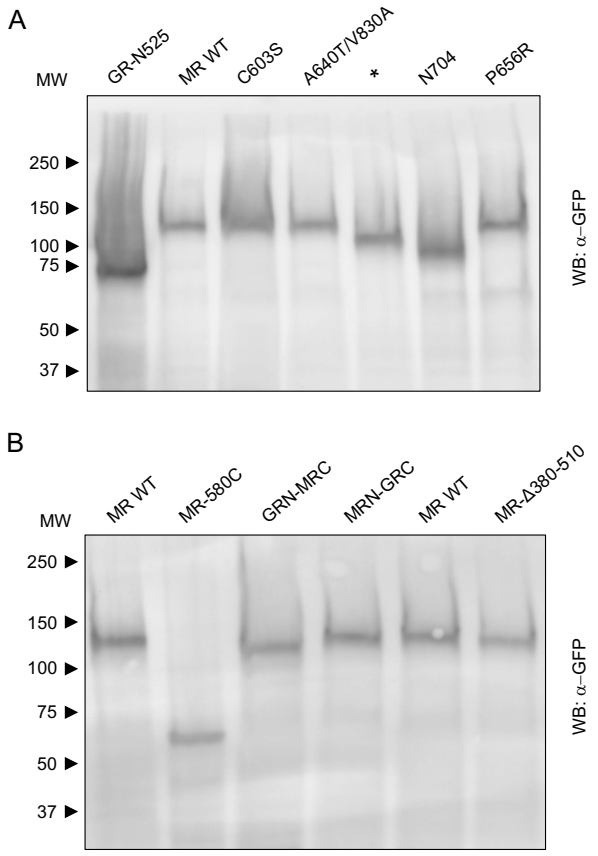
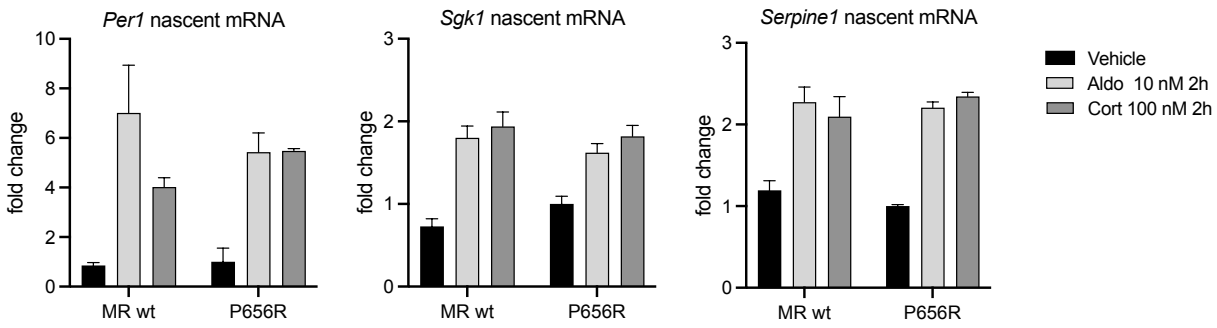
Supplementary Figure S3. (A) Sequence comparison between mouse GR and MR DBDs. Highlighted residues were mutated during this study. (B) Schematic representation of mouse GR and MR DBDs, indicating key residues mutated in MR during this work. (C) Sequence comparison between mouse GR and MR LBDs. Residues GR-I634, part of an LBD-LBD dimerization interface (8) and conservative change MR-V830 are highlighted. Alignments were performed using Clustal Omega (71) and sequences with GenBank accesion numbers AAI29913 and AAI33714 for GR and MR, respectively.



Supplementary Figure S4. Representative western blot showing the expression of GR and MR constructs used in this study (note that all proteins are fusions with eGFP -see *Material and Methods*). Cells were transiently transfected with the indicated constructs and processed for western blot analysis using an anti-GFP primary antibody. Arrows indicate migration of molecular mass markers (MW; values in KDa). (A) Constructs corresponding to Fig. 3. *, construct unrelated to this study. (B) Constructs corresponding to Fig. 4 of the main text. GRN-MRC, construct GR-N408/MR-580C; MRN-GRC, construct MR-N579/GR-407C.



Supplementary Figure S5. RT-qPCR performed on three MR up-regulated genes in cells expressing wild type MR or MR mutant P656R. Cells were treated with vehicle, with 10 nM aldosterone or 100 nM corticosterone for 2h. Plots show fold changes in the indicated nascent mRNA abundance compared to MR-P656R treated with vehicle (n = 2).